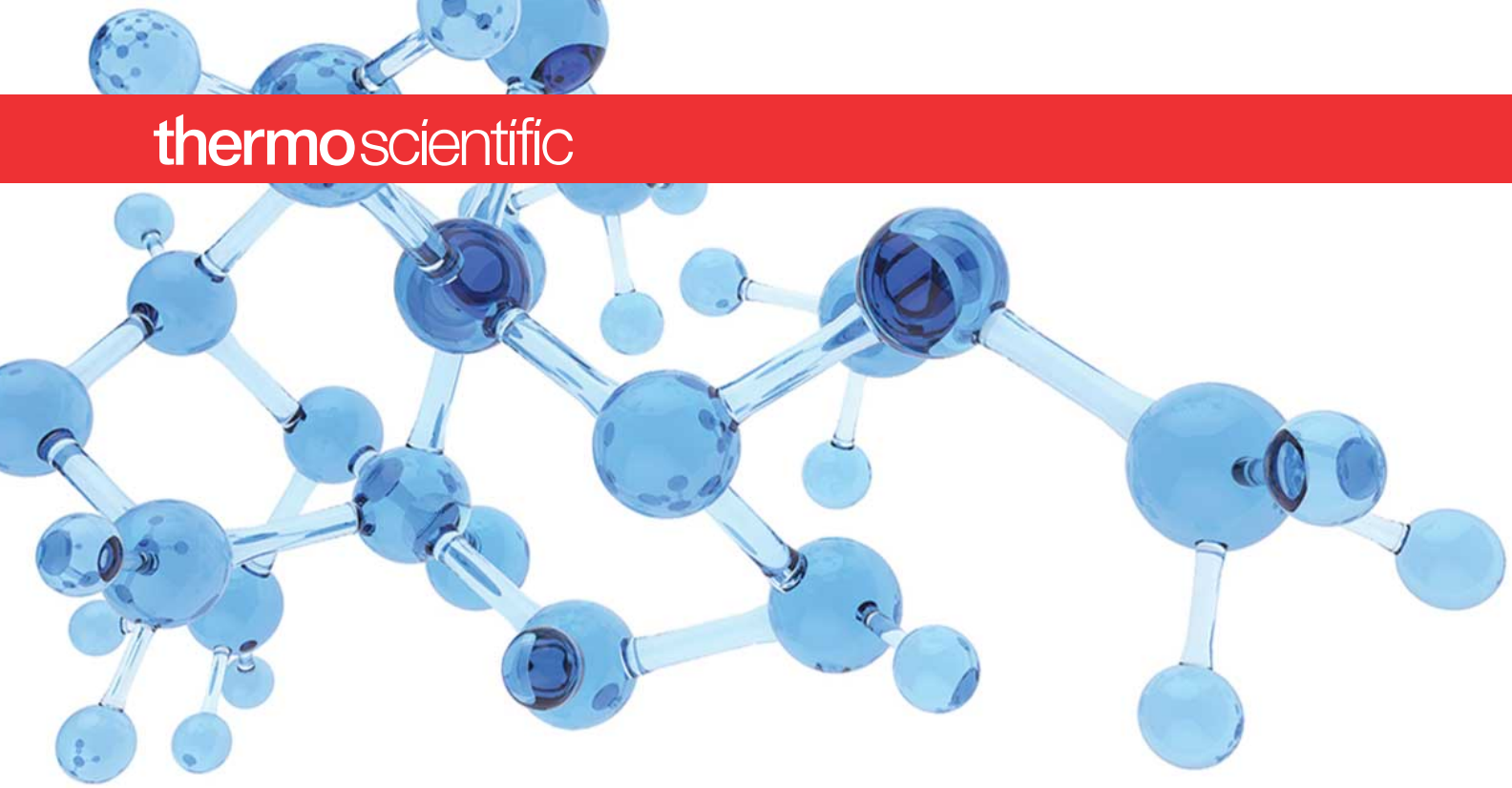
thermo scientific



BioPharma Finder

USER GUIDE

Software Version 4.0

Publication Number XCALI-98190 **Revision** A March 2020

ThermoFisher SCIENTIFIC

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Software version: Software version: Thermo BioPharma Finder™ 4.0 and later, Microsoft™ Windows™ 7 Professional (English) (64-bit), Microsoft Windows 10 (English) (64-bit)

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Revision	Date	Description
A	March 2020	Initial release

Intended Use: For Research Use Only. Not for use in diagnostic procedures.

Contents

Preface

This guide describes how to use the Thermo BioPharma Finder™ 4.0 application to characterize the following:

- Biotherapeutic proteins by using three key workflows—Peptide Mapping Analysis, Intact Mass Analysis, or Top Down Analysis.
- Nucleotide-based pharmaceuticals by using two key workflows—Oligonucleotide Analysis and Intact Mass Analysis.

Accessing documentation

In addition to this guide, the BioPharma Finder application includes Help, animations, and the *Thermo BioPharma Finder Installation Instructions*, available on the software DVD.

For more details about how to access the documentation, see the following topics:

- Viewing the product manual (page 20)
- Viewing the help and animations (page 21)
- Help menu commands (page 21)

Viewing the product manual

You can view the product manual either from the BioPharma Finder application or from the Thermo Fisher Scientific website.

Table 1 Viewing the product manual

To view from …	Do this …
The application	From the BioPharma Finder window, choose **Help ▸ Manual ▸ BioPharma Finder User Guide**.
The website	**1.** Go to **thermofisher.com**. **2.** Point to **Services & Support** and click **Manuals** on the left. **3.** In the Refine Your Search box, search by the product name. **4.** From the results list, click the title to open the document in your web browser, save it, or print it. To return to the document list, click the browser **Back** button.

Viewing the help and animations

You can view both the Help and animations from the BioPharma Finder application.

Table 2 Viewing the Help and animations

To view …	Do this …
The Help	From the BioPharma Finder window, choose **Help ▸ BioPharma Finder Help**. To locate a particular topic, use the Help Contents, Index, or Search panes. In applications that have a Communicator bar, click the field or parameter to display definitions, required actions, ranges, defaults, and warnings, if they are available. **Note:** Messages in the Communicator bar sometimes do not synchronize with the current field after you move to another user interface area and then move back.
The animations	From the BioPharma Finder window, choose **Help ▸ Animations**. Then, view a specific animation by clicking its corresponding link.

Help menu commands

The Help menu in the BioPharma Finder application provides the following commands.

Help menu commands	Description
Snapshot	Creates and restores backup of the application files and databases.
BioPharma Finder Help	Displays the BioPharma Finder Help.
How to Use Help	Displays the BioPharma Finder Help topic that explains how to use the Help.
Glossary	Displays pages of glossary terms in alphabetical order and their related definitions.
Manual	Provides access to the BioPharma Finder User guide in PDF format.
Animations	Displays links to animations that demonstrate typical user interactions in various views.
About BioPharma Finder	Displays the release and copyright information. Provides access to the license activation/deactivation dialog box, the version information, and the user license agreement for the BioPharma Finder application.
Report an Issue	Provides access to the submission of defects or enhancement requests.

System requirements

The following are the minimum and recommended system requirements for installing BioPharma Finder 4.0:

System	Minimum requirements	Recommended requirements
Hardware	• Intel™ Core™ i7-4770 CPU@3.40 GHz • 8 GB registered RAM • 100 GB storage hard drive—ST1000DM-003 SCSI disk device • DVD/CD-ROM drives • Resolution display 1280 × 1024 (SXGA)	• Quad-core Intel™ Xeon™ CPU (E5-1630 v3 3.7 GHz 10 MB 2133 4C) • 32 GB DDR4-2133 (4 × 8 GB) registered RAM • 2 TB storage hard drive (SATA, 7200 rpm)—512 GB solid state boot drive (SATA) • DVD-RW optical drive • Resolution display 1920 × 1080 (WUXGA)
Software	• Microsoft™ Windows™ 7 Professional (English) SP1 (64-bit) • Microsoft™ .NET Framework 4.7.2 • Microsoft™ Office 2010 • Adobe™ Acrobat Reader™ DC	• Microsoft™ Windows™ 10 Professional (English) (64-bit)[1] • Microsoft™ .NET Framework 4.7.2 • Microsoft™ Office 2016 • Adobe Acrobat™ Pro DC

[1] Windows™ 10 Enterprise LTSB and LTSC editions are supported.

Note: If you are not able to see the entire interface of the BioPharma Finder application, make sure that your computer resolution is set to at least 1280 × 1024.

Activating and deactivating a license

Use the Thermo Scientific™ Product Licensing wizard to activate or deactivate the license for the BioPharma Finder application. To activate the license, you must have an activation code from Thermo Fisher Scientific. You must deactivate the license before you transfer it to another computer.

To start the license activation or deactivation process

1. Open the BioPharma Finder application.
2. Choose **Help ▸ About BioPharma Finder** to display the About dialog box.
3. Click **Activate (Deactivate)** to start the activation (or deactivation) process, as applicable.
4. Follow the instructions in the License Activation wizard.

For additional instructions, click **Help** in the wizard.

Special notices

This guide uses the following types of special notices:

IMPORTANT! Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.

Tip: Highlights helpful information that can make a task easier.

Note: Highlights information of general interest.

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Part I

Introduction to BioPharma Finder

1 Introduction to BioPharma Finder

This introduction describes the features and types of analyses in the BioPharma Finder™ application, how to start and exit this application, how to specify global settings and interact with the user interface, and changes that occur when you convert your results from a legacy application - Protein Deconvolution.

BioPharma Finder features

The BioPharma Finder application provides in-depth characterization of biotherapeutics including both proteins and oligonucleotides. Characterization can be challenging, whether you are performing intact mass analysis, top- and middle-down protein analysis, peptide mapping, oligonucleotide analysis or multi-attribute method (MAM) workflows. BioPharma Finder workflow driven software facilitates comprehensive interpretation and data visualization, allowing you to confidently characterize your biologics with speed and ease. From novel deconvolution algorithms generating complete results to easy-to-understand data visualization tools, Thermo Scientific BioPharma Finder software helps you choose the right path for confident protein characterization.

The BioPharma Finder application consolidates the Thermo Scientific™ PepFinder™ and Protein Deconvolution applications into one platform. It also includes various features from the ProSight Lite and Pinpoint™ applications.

Note: ProSight Lite only: Available from Proteomics™ Center of Excellence, Northwestern University.

Types of analyses

There are four main types of analyses in this application - Peptide Mapping Analaysis, Oligonucleotide Analysis, Intact Mass Analysis and Top Down Analysis.

- Peptide Mapping Analysis
  - Identifies peptides by using a new prediction algorithm (unique differentiator).
  - Performs relative quantitation of post-translational modifications (PTMs).
  - Provides in-depth identification using error-tolerant searching, amino acid substitution, and de novo sequencing (unique differentiator).
  - Performs a targeted peptide mapping search by using a saved peptide workbook as a protein sequence.
  - Hydrogen Deuterium Exchange (HDX) workflow is a highly automated process and the output is the protection factor plot. This is so the used to quickly assess and compare the protein folding across batches of biopharmaceuticals.
  - Identification and relative quantitation of both O linked and N linked glycopeptides.
  - Multi-attribute method (MAM) method development and non QC MAM applications for high throughput analytics.
  - Host cell proteins (HCP) determination can be completed with combining BioPharma Finder with Proteome Discoverer.
- Oligonucleotide Analysis
  - Performs a complete characterization of nucleotide-based pharmaceuticals.
  - Sequence manager supports DNA and RNA for creation of oligonucleotide sequences.
  - Create customized building blocks subunits including: base, backbone linker, 2’ ribose, 3’ terminal and 5’ terminal.
  - Editing sequences with customized building blocks is achieved using a simple user interface allowing for maximum editing of all key components.
  - Confirmation of oligonucleotide sequence with a novel MS2 prediction algorithm, providing extra confidence in your oligonucleotide sequence.
  - Comparative analysis of multiple samples an ideal workflow for bioanalysis.
  - Automatic identification and annotation using MS2 data utilizing both HCD and CID fragmentation modes.
  - New peak detection which allows for monitoring of expected impurities but also provides results for detection of unexpected impurities or metabolites.
  - MS2 confirmation provides site specific localization of modifications or failure sequence.
  - Confirmation of expected and unexpected modifications.

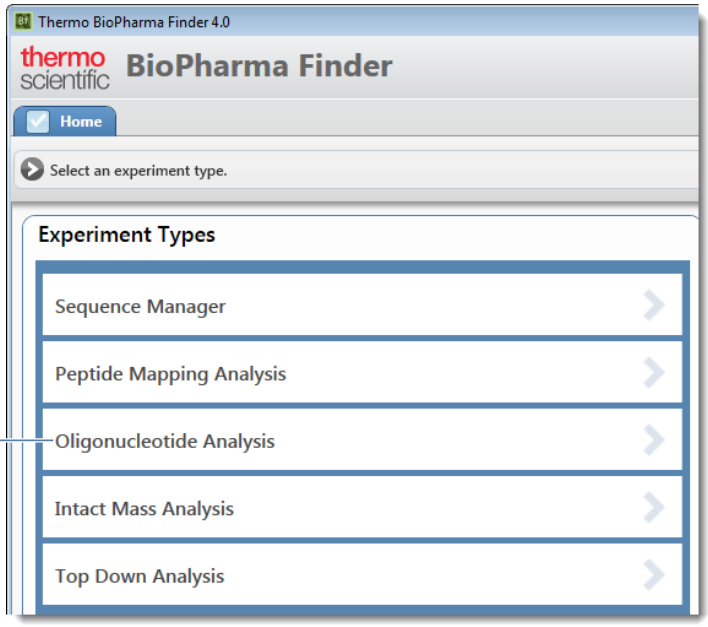
- Intact Mass Analysis
  - Provides molecular weight values of the biotherapeutic drugs and is necessary for confirmation.
  - Ensures high-quality results using two complementary deconvolution algorithms optimized for isotopically resolved (Xtract) and unresolved data (ReSpect). These algorithms produce highly accurate results, even for low-abundance molecules, and enable detection of protein modifications with mass shifts of just a few daltons.
  - Mass confirmation of target sequence including both proteins and oligonucleotides.
  - Confident deconvoluted molecular weight of proteins in both acidic and native conditions.
  - Batch-to-batch analysis for sample comparison.
  - Novel sliding window algorithm to improve detection on low abundant species.
  - Two deconvolution algorithms supports both high resolution data (isotopically resolved data Xtract) and low resolution data (isotopically unresolved data ReSpect).
  - Target protein sequence matching, which identifies n-linked glycosylations and other common modifications using the intact mass.
  - Identification of Antibody Drug Conjugates (ADC) using sliding window algorithm.
  - Default processing method for oligonucleotides.
  - Added support for TSQ data files including a default method processing methods.
  - Protein or oligonucleotide sequence is not required to annotate a deconvoluted mass. Sequences can be created by using only the mass or chemical formula.
  - Improved mass accuracy for deconvolution for modified oligonucleotides for isotopically resolved data using a new feature called sequence-specific isotope table.
- Top Down Analysis
  - Identifies and characterizes intact proteoforms, providing precise data of molecular composition.
  - Processes peak-specific parameters for component detection.
  - Uses the Xtract and ReSpect deconvolution algorithms, similar to Intact Mass Analysis.

    Note: Top Down Analysis currently supports the processing of MS2 spectra using only Xtract deconvolution. For processing Full MS spectra, it supports both the Xtract and ReSpect algorithms.
  - Provides separate results tables for Full MS and MS2 scans.
  - Provides a simple workflow for sequencing intact protein molecules using ProSightBP as the core algorithm.

- Does not require MS scan information but encourages targeted MS2 experiments.
- Compares multiple raw data files with combined interactive fragmentation coverage maps.
- Supports multiple modes of fragmentation—CID, HCD, ETD, EThcD, and UVPD—enabling maximum protein coverage.

You start each type of experiment from the Home page.

New experiment type in BioPharma Finder 4.0



For more information specific to these key analyses, see the following:

- Chapter 9, “Peptide mapping analysis features”
- Chapter 16, “Intact mass analysis features”
- Chapter 24, “Top down analysis features”
- Chapter 29, “Oligonucleotide Analysis features”

Sequence Manager

The Sequence Manager is the starting point for using the BioPharma Finder™ application. It contains two main pages: Sequence Manager and Theoretical Protein/Peptide Manager.

The Sequence Manager page provides access to a central database of protein and oligonucleotide sequences that you can use for your data processing experiments. See Chapter 2, “Sequence Manager”. This page also provides access to the following editors:

- The Protein Sequence Editor where you can edit existing protein or peptide sequences and create new protein or peptide sequences. See Chapter 3, “Protein Sequence Editor”.
- The Oligonucleotide Sequence Editor where you can edit existing oligonucleotide sequences or create new oligonucleotide sequence. See Chapter 6, “Oligonucleotide Sequence Editor”.

You can use the Protein Sequence Editor to do the following:

- Import a sequence from a FASTA file.
- Enter a new sequence or paste a sequence from a copied source.
- Define multiple chains—for example, two light chains and two heavy chains—for intact analysis of unreduced antibodies, enabling the deconvolution of the ~150 kDa protein.
- Specify a mass only sequence.
- Add fixed or variable modifications and disulfide bonds.
- Define a list of default modifications.
- Create custom modifications.
- Select a list of possible glycosylation structures.
- Generate a list of proteoforms.

You can use the Oligonucleotide Sequence Editor to do the following:

- Import a sequence from a FASTA file.
- Enter a new sequence or paste a sequence from a copied source.
- Specify a mass only sequence.
- Add fixed or variable modifications
- Define a list of default modifications.
- Create custom varible modifications.
- Create custom oligo building blocks

Use the Theoretical Protein/Peptide Manager page to process theoretical proteins and peptides and increase your confidence in the validity of the processed targeted data. See Managing theoretical proteins and peptides (page 87).

Using common features for all types of analyses

Peptide Mapping Analysis, Intact Mass Analysis, Oligonucleotide Analysis, and Top Down Analysis share these common features:

- Setting up an experiment (page 30)
- Method editor (page 31)
- Run queue (page 31)
- Real-time optimization (page 31)

Setting up an experiment

Before you set up an experiment, you must define the target sequences to perform identification. See Chapter 2, “Sequence Manager”.

You set up and run an experiment the same way for all types of analyses:

1. Enter an experiment name.
2. Load one or more raw data files.

   Note: For Peptide Mapping Analysis and Oligonucleotide Analysis, when you load multiple raw data files, you must specify the conditions. For Intact Mass Analysis and Top Down Analysis, the conditions are optional.
3. Depending on the analysis, select one or more sequences that you want to identify:

   - For non-targeted Peptide Mapping Analysis select one sequence.
   - For targeted Peptide Mapping Analysis, select one targeted peptide sequence.
   - For Intact Mass Analysis or a Top Down Analysis, select up to 10 sequences.
   - For Oligonucleotide Analysis, select one sequence.

   Note: You must select the target sequence for a targeted Peptide Mapping Analysis. Selecting a sequence is optional for all other analysis types; however, if you do not select a sequence, the application only performs component detection and deconvolution.
4. Depending on the analysis type and whether you want to use information from the raw data to customize the processing method, select a processing method, edit the method, and start processing.

   IMPORTANT! For Top Down Analysis, you must edit the processing method before submitting the job to the queue. For other analysis types, editing the method is optional but recommended, as it lets you customize the method for the raw data that you are processing.

   Using a new feature in BioPharma Finder 4.0 for Peptide Mapping Analysis and Oligonucleotide Analysis, you can run multiple raw files in batch mode or multiconsensus mode.

   The application submits the job to the job queue.

Method editor

The method editor guides you through the different steps in creating or editing a processing method including:

- Entering component detection parameters
- Identification parameters
- HDX parameters (Peptide Mapping Analysis only)
- Report parameters (Intact Mass Analysis only)
- Saving the method

For Peptide Mapping Analysis, the editor includes interactive graphics for viewing the absolute MS signal threshold. As you change it to this threshold, a red line that indicates it moves up and down accordingly.

You can view or save a summary of the method settings on the Save Method or Save Experiment page before you begin processing.

Run queue

The application features a run queue, where you can monitor the processing, manage the items in the queue, and open the results of an experiment. You can set up multiple experiments at one time and let them run overnight to increase your productivity. While the application processes experiments, you can simultaneously review the results of other experiments or perform other data processing.

Real-time optimization

The BioPharma Finder application also offers a real-time optimization feature so that you can adjust the processing parameters while viewing the potential impact on your results. You can quickly adjust the parameters and then reprocess the experiment by using the new values.

For Peptide Mapping Analysis and Oligonucleotide Analysis, real-time optimization lets you view the absolute MS signal threshold for multiple files at one time. Use the Select Chromatogram feature to display the base peak chromatogram (BPC) for multiple data files, adjust the absolute MS signal threshold (by changing the MS noise threshold, the signal-to-noise threshold, or both), and watch the red line move up and down in all of the different BPCs. This feature helps you optimize the parameters for the specific data set in the experiment.

Starting the BioPharma Finder application

To start the BioPharma Finder™ application

Choose **Start ▸ All Programs ▸ Thermo BioPharma Finder ▸ Thermo BioPharma Finder** or double-click the **BioPharma Finder** icon, .

The BioPharma Finder window opens showing the Home page.

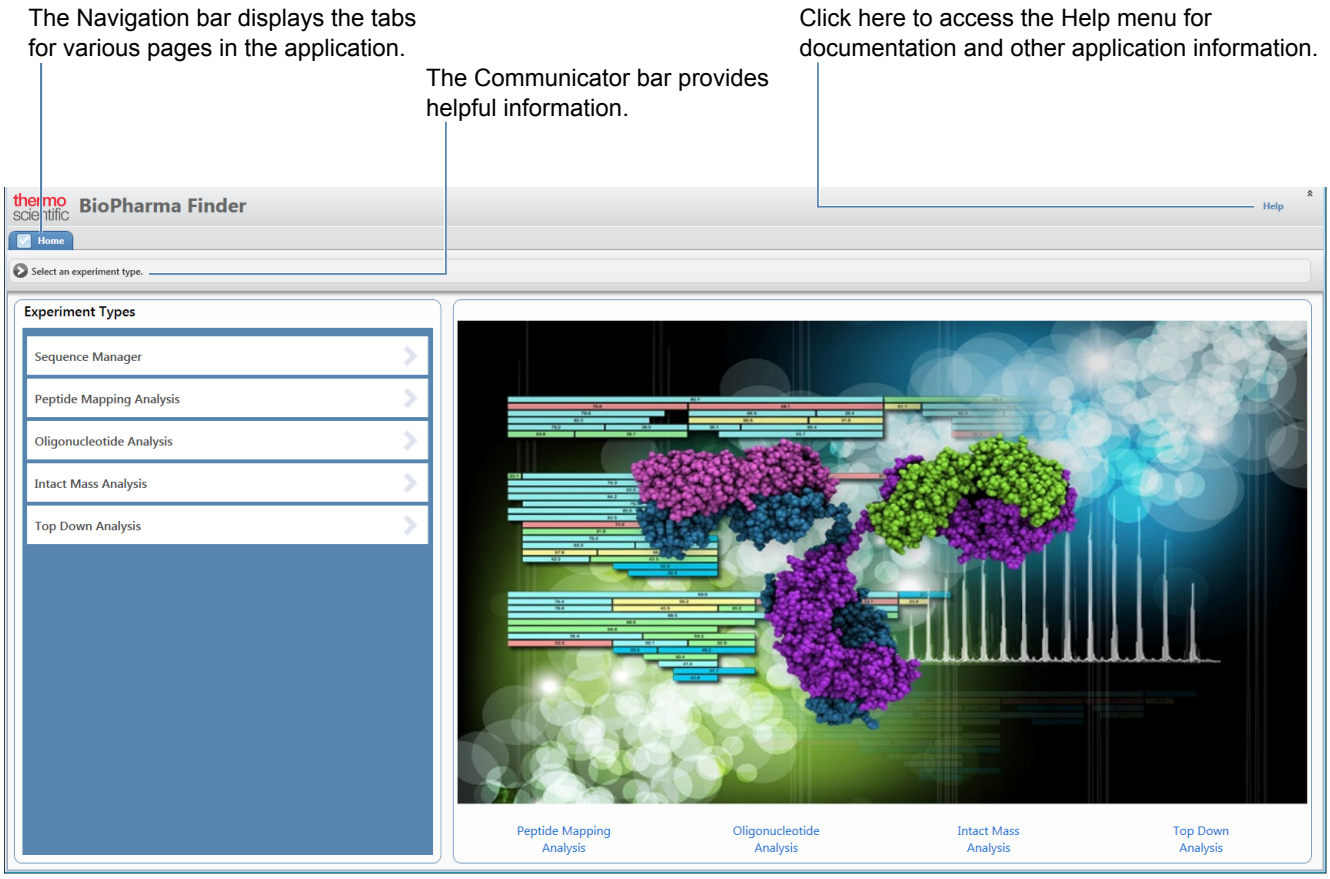


Figure 1 Home page of the BioPharma Finder application

Note: If the BioPharma Finder software is installed on a computer system with multiple users and you start the application, you must close it before another user can open it.

Handling database service error

If the database service is not installed properly, the following Application Error message appears when you start the BioPharma Finder application: Unable to connect to database service.

To restart the database service

1. At the bottom right of the error message, click **Exit** to exit the BioPharma Finder application.
2. Right-click the *C*:\Program Files\Thermo\BioPharma Finder\RepairDatabase-RunAsAdmin.bat file and choose **Run as Administrator**.

   This utility stops the database service and restarts it.

Handling other errors

If you cannot run the application or you encounter errors when loading the raw data files, run the Repair utility.

To run the installer Repair utility

1. With the BioPharma Finder application still installed on your system, run the **BioPharmaFinderSetup.exe** installer again.

   The Thermo BioPharma Finder Suite dialog box opens.

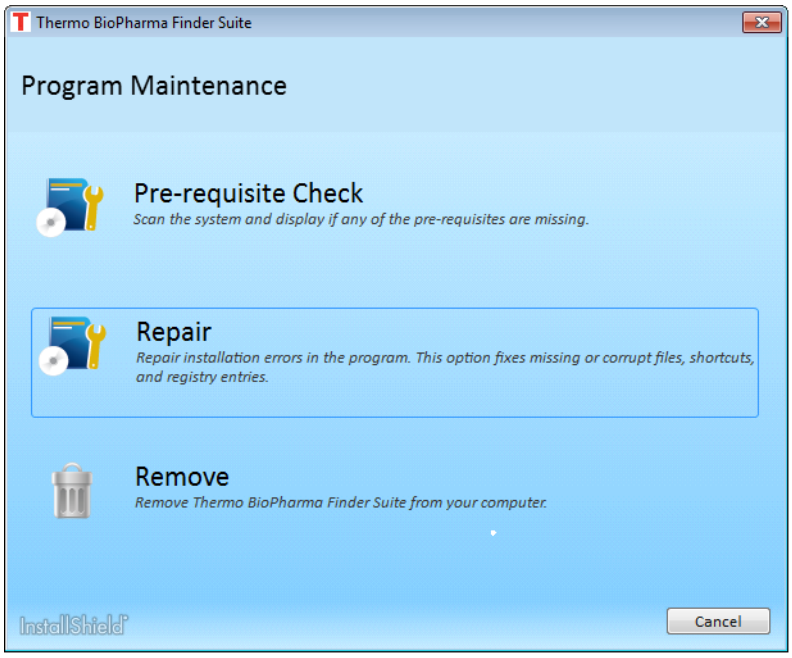


Figure 2 Thermo BioPharma Finder Suite dialog box

2. Click **Repair**.

   This utility repairs missing or corrupt files, shortcuts, and registry entries. During the repair process, it also checks for missing prerequisite applications and installs them. It does not overwrite your database unless the repair process finds that the database is corrupted.

3. When repairs are complete, click **Finish** to exit the installer.

You can also run the **Pre-requisite Check** utility if needed. It scans your system and prompts you to install any missing required applications.

To uninstall the BioPharma Finder software, run the **Remove** utility.

Specifying the global setting for Peptide Mapping Analysis or Oligonucleotide Analysis

The only global setting for a peptide mapping analysis or an oligonucleotide analysis is the default location of the folder for the raw data files.

To specify the default folder for the raw data files

1. On the Home page, click **Peptide Mapping Analysis** or **Oligonucleotide Analysis**, in the Experiment Types pane or below the splash graphic.

   The Peptide Mapping Analysis page or the Oligonucleotide Analysis page opens. When you open an analysis page, the Settings icon, ⚙, appears in the upper right of the page.

   Tip: Clicking the Settings icon opens a settings dialog box for the specific analysis type.

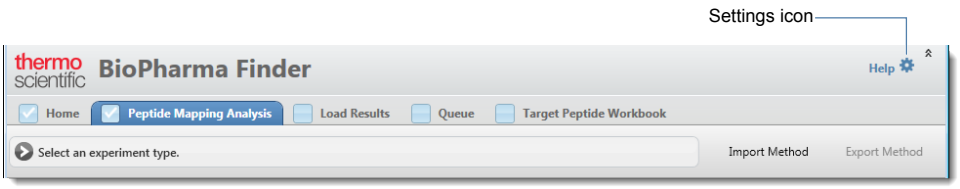


Figure 3 Settings icon

2. Click the **Settings** icon, ⚙, in the upper right of the Peptide Mapping Analysis page or the Oligonucleotide Analysis page.

   The Peptide Mapping Analysis Settings dialog box or the Oligonucleotide Analysis Settings dialog box opens.

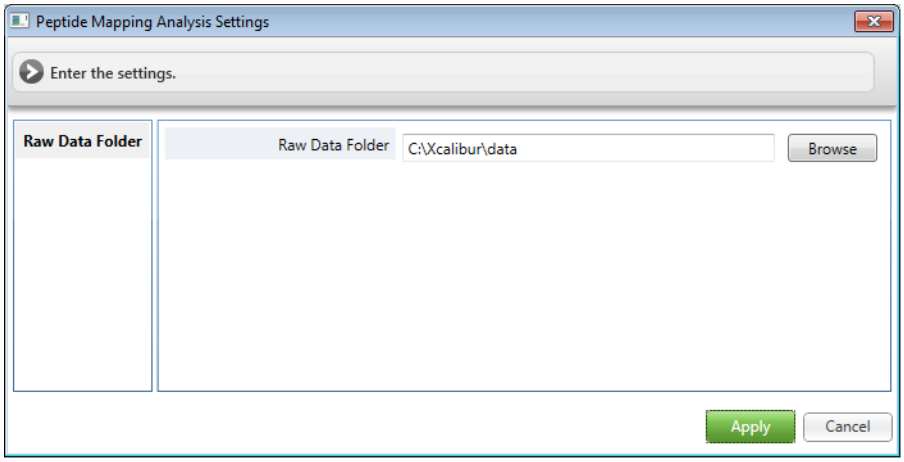


Figure 4 Peptide Mapping Analysis Settings dialog box

3. Browse to and select the appropriate raw data folder.

   By default, the Browse dialog box opens to the *C*:\Xcalibur\data\'folder_name'.

4. In the Peptide Mapping Analysis Settings dialog box or the Oligonucleotide Analysis Settings dialog box, click **Apply.**

Your selected folder is the default folder for the Select Raw Data box on the Peptide Mapping Analysis page or on the Oligonucleotide Analysis page. Use this page to load the raw data files for the experiment.

Specifying global settings for Intact Mass Analysis or Top Down Analysis

You can specify these three global settings for an Intact Mass Analysis or a Top Down Analysis: the default folder for the raw data files, the dimensions for exporting images, and the mass precision.

To specify the global settings

1. On the Home page, click either **Intact Mass Analysis** or **Top Down Analysis** in the Experiment Types pane or below the splash graphic.

   The Intact Mass Analysis page or the Top Down Analysis page opens. When you open an analysis page, the Settings icon, ⚙, appears in the upper right of the page.

   Tip: Clicking the Settings icon opens a settings dialog box for the specifc analysis type.

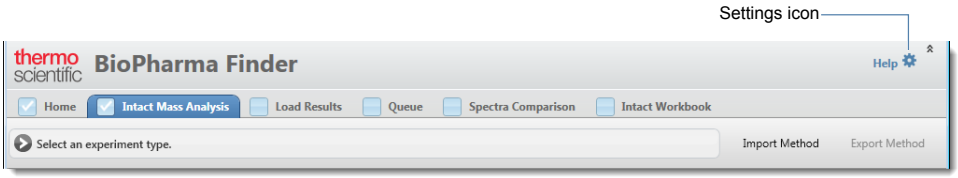


Figure 5 Settings icon

2. Click the **Settings** icon, , in the upper right of the page.

   The Intact Mass Analysis Settings dialog box or the Top Down Analysis Settings dialog box opens.

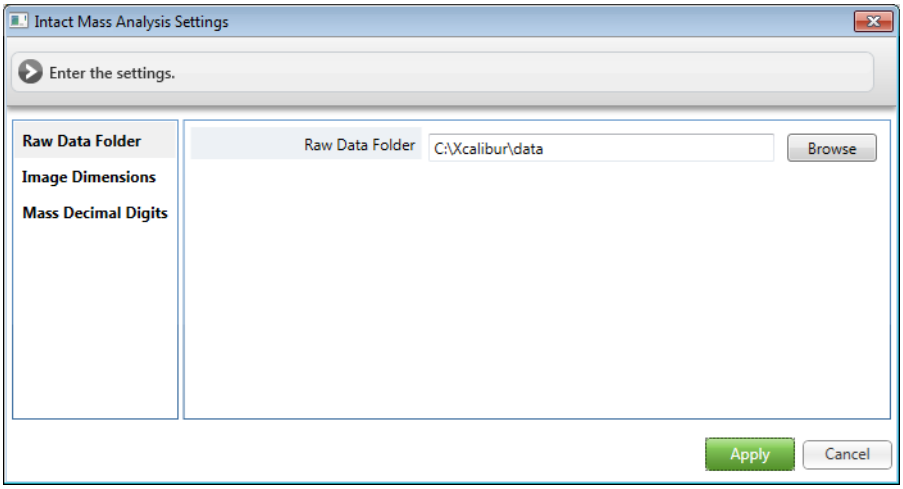


Figure 6 Intact Mass Analysis Settings dialog box

To specify the global settings for an Intact Mass Analysis or a Top Down Analysis, see the following topics:

- Specifying the default raw data file folder for each analysis type (page 36)
- Specifying the image dimensions (page 37)
- Specifying the precision for mass values (page 38)

Specifying the default raw data file folder for each analysis type

You can specify a separate default folder where you store the raw data files for each analysis type.

To specify the default folder for your raw data files

1. Open the settings dialog box for a specific analysis type.
2. In the settings dialog box, select **Raw Data Folder** in the left pane.
3. Browse to and select the appropriate folder.

   By default, the Browse dialog box opens to the *C*:\Xcalibur\data\ folder.
4. In the dialog box, click **Apply**.

The selected folder is the default folder for the Select Raw Data box in the Load Raw Data area on an analysis page. You use the Load Raw Data area of an analysis page to load the raw data files for your processing experiments.

Specifying the image dimensions

You can specify the global dimensions—width and height in either millimeters or inches—for various plots that you want to copy to the Clipboard. Then, select the Copy Per Global Settings command from the shortcut menu for each image.

To specify the image dimensions

1. In the Intact Mass Analysis Settings dialog box or the Top Down Analysis Settings dialog box, click **Image Dimensions** in the left pane.

2. In the Images area, select one of the image options.

For Intact Protein	For Top Down
• Chromatogram • Deconvoluted Spectrum • Source Spectrum • Spectra Comparison	• Chromatogram • Intact Fragmentation Source Spectrum • Intact Fragmentation Deconvoluted Spectrum • Intact Deconvolution Source Spectrum • Intact Deconvolution Deconvoluted Spectrum

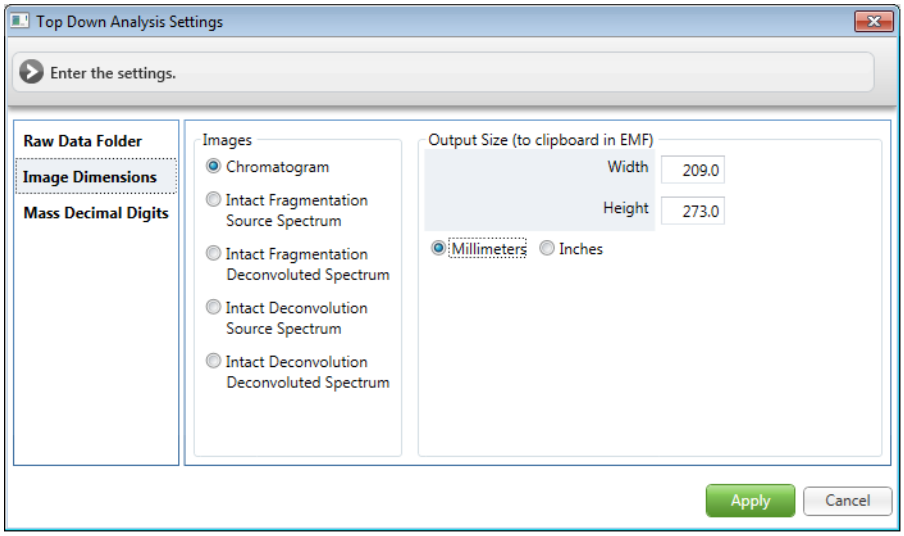


Figure 7 Image options for a Top Down Analysis

3. In the Output Size (To clipboard in EMF) area, type the output size for the Width and Height, and then select Millimeters or Inches, for this size.

   The default width and height for images that you copy to the Clipboard is 209 mm by 273 mm (8.23 in. by 10.75 in.).

4. Click **Apply**.

Specifying the precision for mass values

You can specify the precision—or number of decimals—that Intact Mass Analysis or Top Down Analysis displays for mass values in the results.

To specify the number of decimals displayed for mass values

1. In the Intact Mass Analysis Settings dialog box or the Top Down Analysis Settings dialog box, click **Mass Decimal Digits** in the left pane.

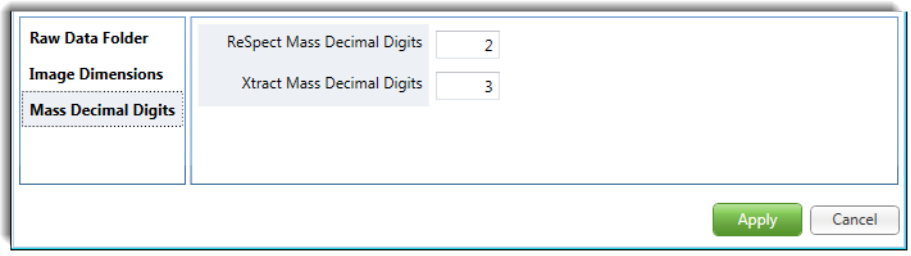


Figure 8 Mass Decimal Digits settings

2. In the ReSpect Mass Decimal Digits box, specify the number of decimals used to display the mass values for the ReSpect algorithm.
3. In the Xtract Mass Decimal Digits box, specify the number of decimals used to display the mass values for the Xtract algorithm.
4. Click **Apply**.

Note: For Intact Mass Analysis, changes to these settings affect the mass values in the reports as well as the labels in the deconvoluted spectra. For all peaks processed in Top Down Analysis, changes to these settings affect the mass values in both the full scan and MS2 deconvoluted spectra, the Intact Fragmentation Results, Intact Deconvolution Results, and ProSightBP Output tables.

Interacting with the user interface

To interact with the various panes, chromatograms, spectra, and results tables in the BioPharma Finder application, use the procedures in the following table.

Table 3 User interface interactions

To change the display of …	Do this …
Panes	Expand the pane. **Note:** To enlarge the pane to view more details, detach the pane into a floating window and increase the size of the window.
Chromatograms	Reset the scale, zoom in on, or zoom out of the chromatogram plots.
Spectra	Reset the scale, zoom in on, or zoom out of the spectral plots.
Tables	Sort the columns or filter the data in any of the tables in the various pages and panes in the application, when available.

You can also copy the graphical information in a pane, when available.

Snapshots of the BioPharma Finder database

Generate a backup copy of the BioPharma Finder application using the Snapshot command in the Help menu. To restore a backup copy, use the Restore command in the Snapshot menu. This action restores the complete backup and replaces your current files and databases.

For details, see these topics:

- “Creating application backups using the Snapshot command” on page 39
- “Restoring a snapshot” on page 40

Creating application backups using the Snapshot command

You can create and save a snapshot file to any location where you have write permissions; however, the location must have enough space to save the file. The application displays a message if there is not enough disk space for the file.

To generate a snapshot of the application files and databases

1. From the BioPharma Finder window, choose **Help ▸ Snapshot ▸ Create**.
   The Create a Snapshot dialog box opens.

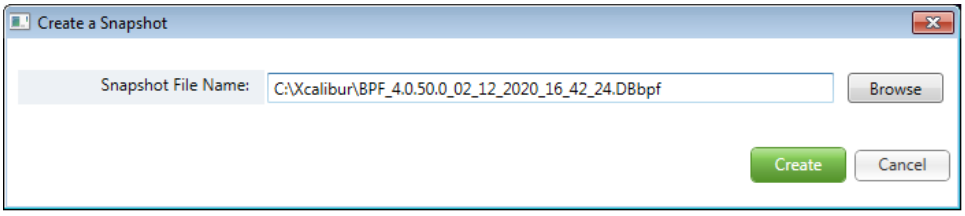


Figure 9 Create a Snapshot dialog box

2. In the Create a Snapshot dialog box, browse to the folder where you want to store the backup, and then click **Create**.

   The application saves the BioPharma Finder snapshot file with the .DBbpf extension to the selected folder. The default folder is *C*:\Xcalibur\.

   Note: If you attempt to generate a snapshot when there are active experiments, the application displays an error message.

   After the application creates the backup file, a message appears indicating the confirmation of the backup.

3. At the prompt, click **OK**.

Restoring a snapshot

To restore a snapshot of the application files and databases

1. From the BioPharma Finder window, choose **Help ▸ Snapshot ▸ Restore**.

   The Restore Snapshot dialog box opens.

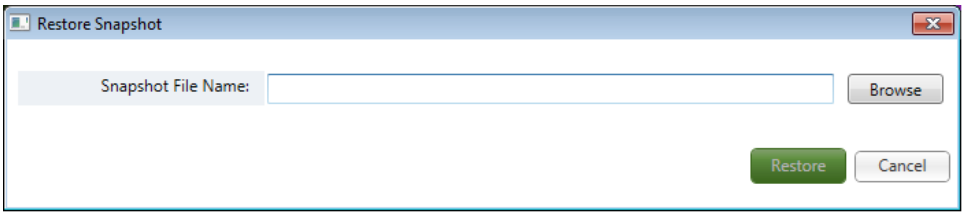


Figure 10 Restore Snapshot dialog box

2. In the Restore Snapshot dialog box, browse to and select the Snapshot file (*filename*.DBbpf), and then click **Open**.

   Because this action erases the current database and any processing results generated, the application prompts you to confirm this action.

3. At the prompt, click **Yes**. Then, at the next prompt, click **OK**.

Exiting the BioPharma Finder application

To exit the BioPharma Finder application

1. Click the **Close** button,  .

   If the application has already started to process an experiment in the queue, a message box opens that is specific to the type of analysis currently in use.

2. If a message box opens, click **OK** to close it, and then do one of the following:

   - For Peptide Mapping Analysis, Oligonucleotide Analysis or Top Down Analysis experiments, you can either wait until processing is completed or stop the queue to cancel the current job.
   - For Intact Mass Analysis experiments, there are two possibilities:
     - In automatic mode, you must wait for the processing of the current experiment to be completed, even when you pause the queue.
     - In manual mode, when you are running a sliding windows experiment, you can stop the processing of the current experiment by clicking the **Cancel** icon,  , on the Process and Review page. If you are running another type of experiment, you must wait for the processing of the current experiment to be completed.

3. Click the **Close** button again to exit the application.

   Exiting the BioPharma Finder application retains the state of the run queue, even if experiments remain in the queue.

System backups

Thermo Fisher Scientific recommends that you regularly back up your current database and other files related to the BioPharma Finder application, using the Snapshot feature instead of manual back up.

Backing up the database and files

IMPORTANT! Thermo Fisher Scientific recommends using the Snapshot feature instead of manual back up.

To manually back up a database and other application files

1. Choose **Start ▸ Control Panel ▸ System and Security ▸ Administrative Tools ▸ Services** to open the Services window.
2. Stop the service **Thermo BioPharma Data Service** if it is running.
3. Copy the *C*:\ProgramData\ThermoScientific folder and paste it to an archive location on a different drive.

   The ProgramData\ThermoScientific folder contains two subfolders: BioPharma and Databases.
4. Start the service **Thermo BioPharma Data Service**.

Retrieving the database and files

IMPORTANT! We highly recommend using the Snapshot feature instead of manual retrieving of database.

To retrieve data from your archived database and application files

1. Choose **Start ▸ Control Panel ▸ System and Security ▸ Administrative Tools ▸ Services** to open the Services window.
2. Stop the service **Thermo BioPharma Data Service** if it is running.
3. Delete the *C*:\ProgramData\ThermoScientific folder.
4. Copy the ThermoScientific folder from your archive location and paste it under the *drive*:\ProgramData\.
5. Start the service **Thermo BioPharma Data Service**.

Data conversion from legacy applications

By using either the installer for BioPharma Finder version 4.0 or the Convert Legacy Results command on the Load Results page for Intact Mass Analysis, you can convert saved data from previous versions of the BioPharma Finder and Protein Deconvolution applications to a format compatible with version 4.0 of the BioPharma Finder application.

The installer automatically converts the SQLite™ results files that are stored in the default folder specified in either the BioPharma.exe.config or ProteinDeconvolution.exe.config file. The following folders contain these configuration files:

C:\ProgramData\ThermoScientific\BioPharma\

C:\ProgramData\ThermoScientific\ProteinDeconvolution\

The installer renames these SQLite files to *File Name*.SQLite.Backup. To load these files again using a legacy application, rename them back to *File Name*.SQLite.

For Peptide Mapping Analysis, Thermo Fisher Scientific recommends that you do the following:

- Update legacy results from versions 3.1 or earlier because the BioPharma Finder 4.0 application provides enhancements with the addition of the Best Overall Average Structural Resolution parameter and possible changes to the ID Type parameter.
- When you open a legacy results file from the Load Results page, the application prompts you to proceed with the automatic update.
- After the update is completed, the Queue page shows two experiments with the same name and containing the same upgraded results.
- The first experiment displays the original date and time of completion and the second experiment displays the current date and time of the update.

In addition, for the Ratio (Condition/Reference Condition) parameter and the Trend Ratio plot, the application automatically uses the first raw data file condition in the legacy results as the reference condition.

For Intact Mass Analysis, you must manually convert other results not stored in the default folder by using the Convert Legacy Results command on the Load Results page. All converted results appear in the table on this page. The Total Processing Time column displays 0 for all converted results because the start and complete times are not available.

Converted protein sequences

When you convert legacy data from a prior version of the BioPharma Finder application, the following occur for protein sequences:

- The BioPharma Finder 4.0 application migrates all protein sequences available on the Sequence Manager page of the legacy application.
- The Last Modified Time column on the Sequence Manager page displays the conversion time, not the original modified time.
- When you add a protein sequence to an experiment for Intact Mass Analysis, the sequence name does *not* appear on the Load Results page. However, it appears on the Process and Review page in these areas:
  - The Sequences Added to Experiment table under the Real Time Optimization > Identification subtab
  - The Results table for a target sequence matching experiment
  - The Matched Sequence pane

When you convert legacy data from the Protein Deconvolution application, the following occur for protein sequences:

- When you add a protein sequence to the method, the sequence name does not appear on the Load Results page or on the Process and Review page in the Sequences Added to Experiment table for real-time optimization. However, it appears in the Results table and the Matched Sequence pane.
- The BioPharma Finder application does *not* extract the sequence information from the results file and does *not* display this information on the Sequence Manager page.

Converted processing methods

When you convert the legacy data, the following occur for the processing methods:

- The BioPharma Finder 4.0 application does not migrate legacy default methods, only custom methods. The application automatically installs new default methods. It displays the migrated methods in the Processing Methods pane on the Peptide Mapping Analysis page, the Intact Mass Analysis page, or the Top Down Analysis page.
- Versions 1.0 and 2.0 of the Protein Deconvolution application did not include the Rel. Intensity Threshold (%) parameter, so a method created from these applications did not store this value. To use this legacy method for processing, you must first manually enter the appropriate Rel. Intensity Threshold (%) value on the Parameters > Component Detection page.
- The application does not migrate the values for the RT Range parameter. The values displayed in the header of the source spectrum plot and the RT Range column of the results tables differ from these values displayed in the legacy application. You must manually specify the appropriate RT Range values in the method. Save the method and then use it for processing.

Part II

Sequence Manager and editors

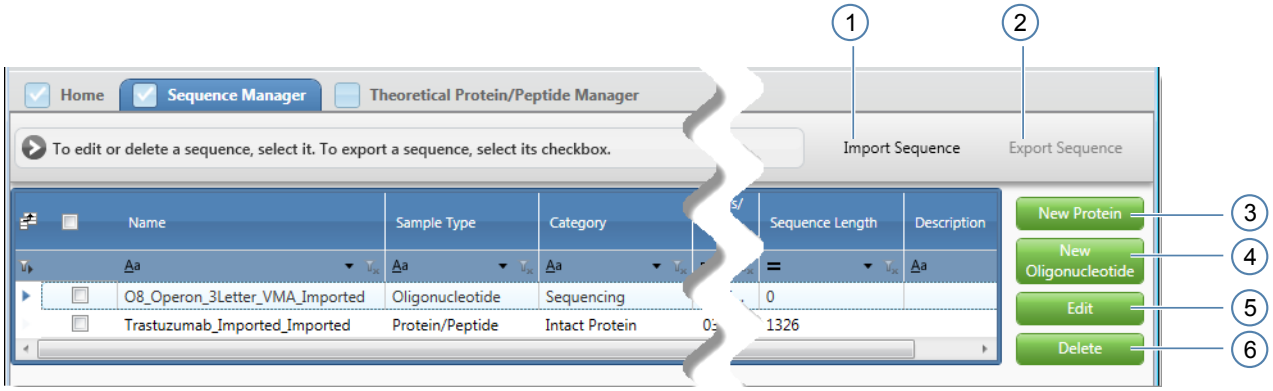
2 Sequence Manager

Use the Sequence Manager to manage your protein sequences and oligonucleotide sequences.

The BioPharma Finder application matches detected components within a mass tolerance to user-defined sequences. The sequences are divided by sample type (protein/peptide or oligonucleotide), and then further divided by category (experiment type). The sequence is optional for most of the workflows (except for Top Down Analysis).

Sequence Manager tasks

Use the Sequence Manager to open one of the sequence editors or to import, export, or delete sequences.



Item	Description
1.	Opens a file browser for selecting a BPF sequence to import.
2.	Exports the selected sequences as BPF sequence files.
3.	Opens the Protein Sequence Editor.
4.	Opens the Oligonucleotide Sequence Editor.
5.	Opens the editor for the selected sample type (Oligonucleotide or Protein/Peptide).
6.	Deletes the selected sequences.

Table 4 Tasks that you can perform from the Sequence Manager

To do this	Do the following...
Import a BioPharma Finder sequence	Click **Import Sequence** at the upper right of the page, and then browse to the folder where you store BioPharma Finder sequence files, select the file, and click **Open**. Then, at the prompt, click **OK**. The sequence files have a .sequencebpf extension. Other file types are not visible from the sequence browser dialog box.
Delete sequences from the Sequence Manager	1. In the sequence table, select one or more sequence rows. 2. Click **Delete** or press the DELETE key. 3. In the confirmation box, click **Yes**. The application removes the selected sequences from the table.
Submit an existing protein or peptide sequence to the Protein Sequence Editor for editing	1. In the sequence table, select the protein or peptide sequence that you want to edit. **Note:** The sample type for protein or peptide sequences is Protein/Peptide. 2. Click **Edit**. The Protein Sequence Editor opens with editable information about the selected sequence.
Open the Protein Sequence Editor to create a new sequence	Click **New Protein** to the right of the sequence table. The Protein Sequence Editor opens. See Chapter 3, “Protein Sequence Editor”.
Submit an existing oligonucleotide sequence to the Oligonucleotide Sequence Editor for editing	1. In the sequence table, select the oligonucleotide sequence that you want to edit. **Note:** The sample type for oligonucleotide sequences is Oligonucleotide. 2. Click **Edit**. The Oligonucleotide Sequence Editor opens with editable information about the selected sequence.
Open the Oligonucleotide Sequence Editor to create a new sequence	Click **New Oligonucleotide** to the right of the sequence table. The Oligonucleotide Sequence Editor opens. See Chapter 6, “Oligonucleotide Sequence Editor”.
Export BioPharma Finder sequences	1. For each sequence that you want to export, select the check box to the left of the Name column. Or, to select all the sequences in the table, select the check box to the left of the Name heading. 2. Click **Export Sequence**. 3. For a single sequence, browse to the folder where you want to store the file, and then click **Save**. For multiple sequences, select the storage folder or create a new folder, and then click **OK**. 4. At the successful export prompt, click **OK**.

Sequence Manager page parameters

The following table describes the table columns and the command buttons on the Sequence Manager page. To open the Sequence Manager page, click **Sequence Manager** on the Home page.

Table 5 Parameters on the Sequence Manager page

Column	Description
Sequence table	Displays information about the existing sequences.
Name	Displays the name of the sequence.
Category	Displays the category of the sequence.
Last Modified Time	Displays the date and time that you last modified the sequence.
Average Mass	Displays the average mass of the sequence.
Monoisotopic Mass	Displays the monoisotopic mass of the sequence.
Num. of Chains	Displays the number of chains in the sequence.
Max. Num. of Modifications	Displays the maximum number of modifications for the sequence. This number is specified by the Intact Protein or Peptide Mapping value in the Variable Modifications for Intact and Peptide Analysis pane or the Number of Modifications per Proteoform value in the Site Specific Variable Modifications for Top Down Analysis pane.
Glycosylation	For protein sequences, displays the selected glycosylation in the protein sequence or None. For oligonucleotide sequences, displays None.
Num. of Proteoforms	Displays the total number of generated proteoforms in protein sequences used for Top Down Analysis.
Variable Modifications	Displays all of the variable modifications in the sequence.
Protein Static Modifications/Oligonucleotide Building Blocks	Displays all of the static modifications in protein sequences and the oligonucleotide building blocks in oligonucleotide sequences.
Sequence Length	Displays the total number of amino acids in protein sequences.
Description	Displays a description for each sequence.
Buttons	
New Protein	Opens the Protein Sequence Editor for creating a new protein sequence.
New Oligonucleotide	Opens the Oligonucleotide Sequence Editor for creating a new oligonucleotide sequence.
Edit	Opens the selected sequence in the appropriate editor for editing.

Column	Description
Delete	Deletes the selected sequence from the sequence table. To make this button available, you must select one or more sequence rows.
Import Sequence	Imports a sequence and adds it to the sequence table.
Export Sequence	Exports a sequence to a BioPharma Finder sequence file to share with other users. To make this button available, you must select the check boxes for one or more sequences. The application exports each sequence as a separate BioPharma Finder sequence file.

For information about using the sequence editors, see these topics: “Creating and editing oligonucleotide sequences” on page 105 and “Creating and editing protein sequences” on page 50.

3 Protein Sequence Editor

Use the Protein Sequence Editor to create and edit protein and peptide sequences. You can open the Protein Sequence Editor from the Sequence Manager page.

Creating and editing protein sequences

To create a new protein sequence or edit an existing protein sequence, you can do any of the following:

- Import a BioPharma Finder file from another machine or the local database on the current machine into the Sequence Manager, and then send the sequence file to the Protein Sequence editor for editing. A BioPharm Finder file can include a protein sequence and its modifications.
- Open the Protein Sequence Editor from the Sequence Manager, and then import a FASTA file that contains the protein sequence.
- Open the Protein Sequence Editor from the Sequence Manager, and then manually enter the sequence.

Importing a FASTA file with a protein sequence

To import a FASTA file in the Protein Sequence Editor

1. Open the Sequence Manager by clicking **Sequence Manager** on the Home page.
2. Click **New Protein** to the right of the sequence table.

   The Protein Sequence Editor opens with the the Manual Input Protein Sequence pane expanded.

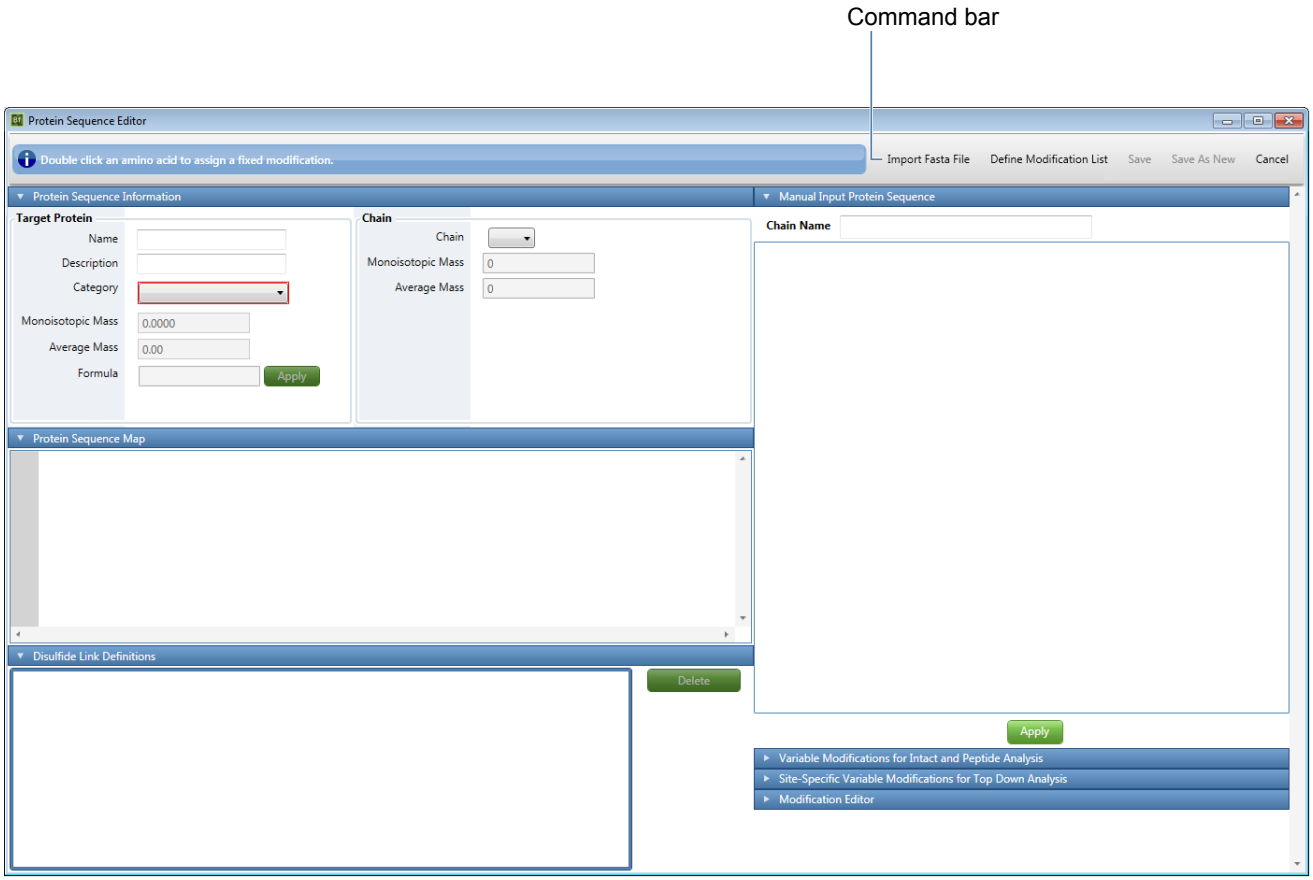


Figure 11 Protein Sequence Editor

Tip: If the resolution and text size of you computer screen is not set properly, you might not be able to see some of the features on the Protein Sequence Editor, specifically the Variable Modifications for Intact and Peptide Analysis pane, the Site-Specific Variable Modifications for Top Down Analysis pane, and the Modification Editor pane. To correct this problem, use the recommended screen resolution of 1920 × 1080 pixels and consider changing the text size.

3. To import a FASTA file, click **Import FASTA File** in the command bar at the top, and then browse to the folder containing the FASTA files.

   The FASTA file must have the .fasta extension for the application to be able to find the file.

   The dialog box displays all of the FASTA files in the selected folder.

4. Select a FASTA file name to import, and then click **Open**.

   Note: You can import FASTA files of 1 MB or less. If a FASTA file contains invalid amino acids or an invalid format, an error message appears.

   The application displays the protein sequence information from the FASTA file in the Protein Sequence Map pane of the Protein Sequence Editor. It highlights the cysteines in yellow. In addition, the Protein Sequence Information pane displays both the monoisotopic and average masses of the sequence in the Target Protein area and the monoisotopic and average masses of the first chain in the

Chain area. To view the masses of a different chain, select the chain number from the Chain list.

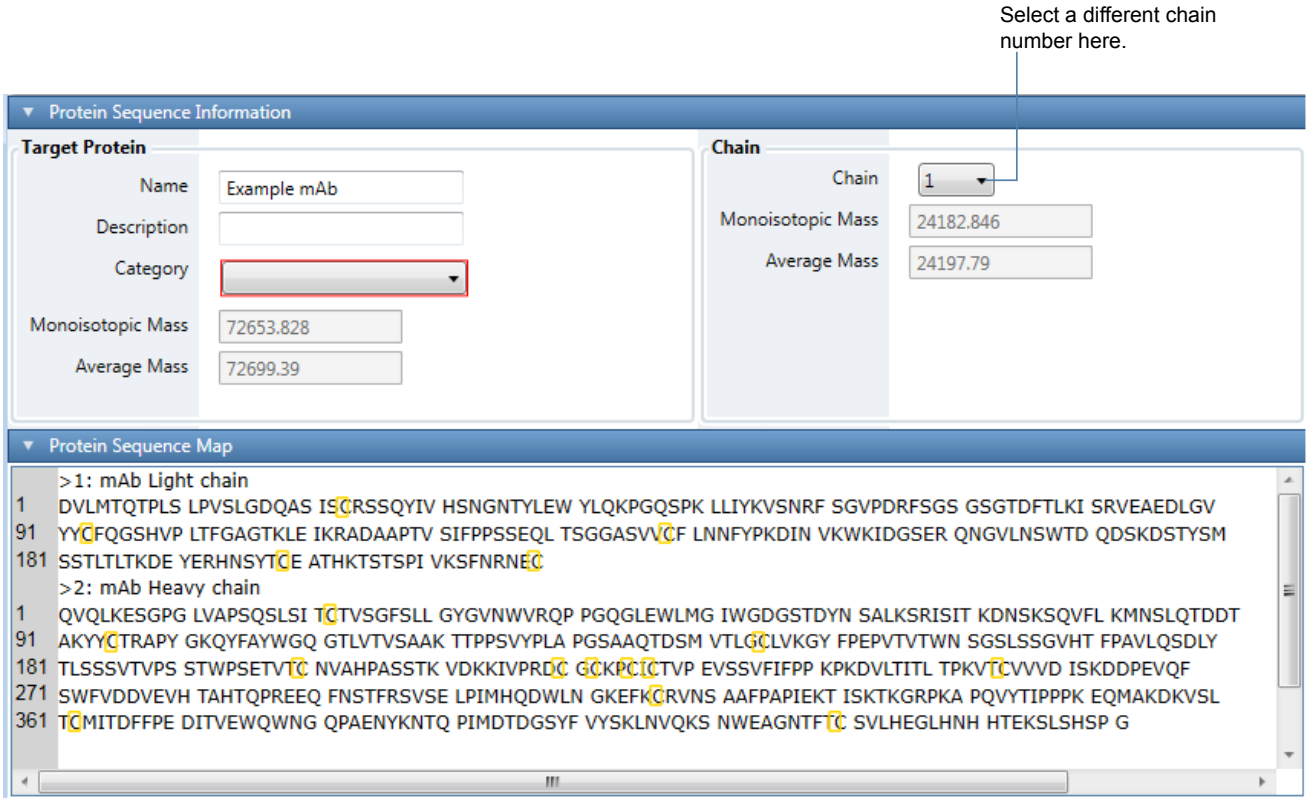


Figure 12 Imported sequence in the Protein Sequence Map pane

Note: For intact analysis of unreduced proteins, you must include two copies of each chain if the molecule is a homodimer. For example, if your sample is a monoclonal antibody, include two copies of both the light chain and the heavy chain. Right-click in the Protein Sequence Map pane to connect the cysteines to form disulfide linkages. Watch the target protein monoisotopic and average masses change as you link the bonds. Verify that these masses match the masses of the total protein being analyzed. This procedure is required only for intact molecular weight determination, not for peptide mapping disulfide bond characterization.

For intact analysis of protein subunits, you must create an individual sequence for each subunit for the application to match the masses correctly. For example, if your sample is a monoclonal antibody that you have cleaved into its light chain, Fc, and Fd subunits, you must create individual sequences for each subunit. When you create the intact experiment, you can select all three individual sequence files and add them to the experiment.

After you specify the protein sequence and select its category, you can add modifications and save the sequence.

Manually creating a new protein sequence

This procedure describes how to manually enter the chains for a protein sequence by typing the information in the Manual Input Protein Sequence pane or by copying the information from a FASTA file that is open in a text editor.

To manually create a new protein sequence

1. On the Home page, click **Sequence Manager** in the left pane.

   The Sequence Manager page opens showing the protein sequence table, which contains a list of existing sequences. The experiment itself uses the sequences that you select from this list when you create the experiment.

2. To add a new sequence, click **New** on the right side of the page.

   The application displays the Protein Sequence Editor and by default expands the Manual Input Protein Sequence pane to the right.

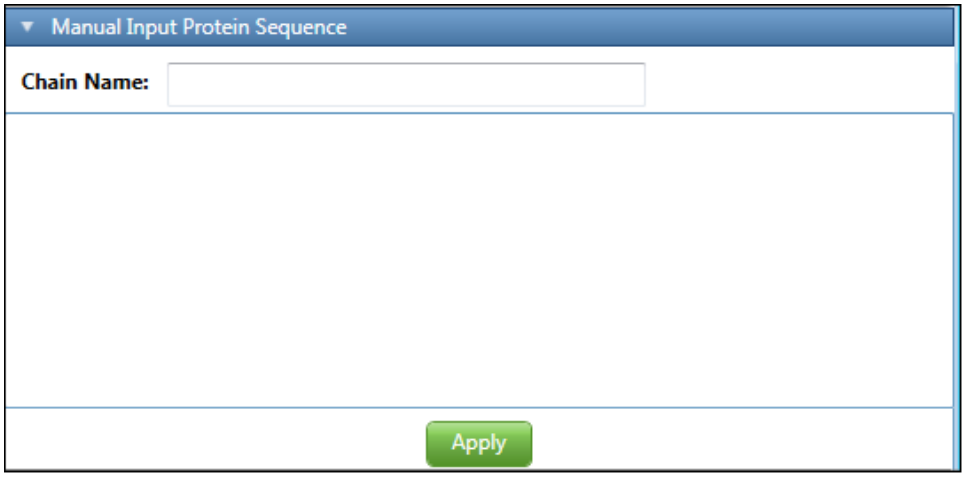


Figure 13 Manual Input Protein Sequence pane

To add a new protein sequence manually, enter one chain in the sequence at a time.

3. For each chain that you want to add, do the following:

   a. In the Chain Name box, type the name of a chain in the new sequence.

   Tip: To add a new protein sequence manually, enter one chain in the sequence at a time.

b. (Optional) Copy each chain that you want to add from a FASTA file, using Notepad or another text editing tool.

IMPORTANT! The FASTA file might contain comment lines that begin with the greater-than sign (>) to distinguish each chain, as shown in the following figure. When you copy the chain information, do not include the comment lines or use them to name the chain. The application interprets pasted comment lines as amino acid sequences.

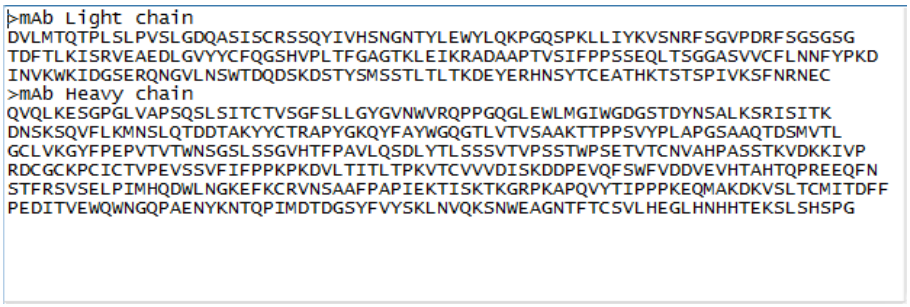


Figure 14 FASTA file format

c. In the large area below the Chain Name box, type or paste the new chain information.

d. Click **Apply**.

Note: If the entered information contains invalid amino acids or an invalid format, an error message appears.

The application displays the entered sequence information in the Protein Sequence Map pane of the Protein Sequence Editor. In addition, it displays the monoisotopic and average masses of the sequence and the first chain in the Protein Sequence Information pane.

4. Save the sequence.

The application adds the saved sequence to the table on the Sequence Manager page.

Selecting the category for a protein sequence

To select the category for a protein sequence

1. Open the Protein Sequence Editor by doing one of the following:

- On the Sequence Manager page, select a sequence, and then click **Edit**.
The Protein Sequence Editor opens with information for the selected sequence.
- On the Sequence Manager page, click **New Protein**.
The Protein Sequence Editor opens with unpopulated data entry boxes.

2. In the Description box, type a description for the sequence or edit the existing description.

   Note: When a previously saved protein sequence contains a Category value other than the default selections of Peptide Mapping, Intact Protein, Top Down, and Unknown, that value moves to the Description box automatically.

3. In the Category list, select one of these categories to assign to the sequence:
   - Peptide Mapping
   - Top Down
   - Intact Protein

   You must assign a category to a sequence before saving it and before editing variable modifications. For example, if you have a sequence frequently used for Intact Mass Analysis, you can assign the Intact Protein category to the sequence. If the list of sequences is long, the appropriate category helps you to easily find the sequence at a later time by using the category to filter the tables.

   Note: In earlier versions of the BioPharm Finder application, you could add custom text to the Category box, and this text was optional. When you import a sequence created in BioPharma Finder 2.0 or earlier, the application assigns the

Unknown category to it and adds any custom category text to the Description box.

IMPORTANT! You must select the appropriate category for the sequence to be visible in the Protein Sequence table on the corresponding analysis page. For example, for a protein sequence to be visible on the Top Down Analysis page, assign the Top Down category to this sequence.

For sequences saved in previous versions of the BioPharma Finder application without a category value, their categories automatically default to Unknown.

The category controls the functionality of some of the panes in the Protein Sequence Editor, as follows:

- A blank category deactivates all panes except for the Manual Input Protein Sequence and Modification Editor panes.
- The Peptide Mapping, Intact Protein, or Unknown category activates the Variable Modifications for Intact and Peptide Analysis pane and deactivates the Site-Specific Variable Modifications for Top Down Analysis pane. All parameters in the inactive pane are not editable.
- The Top Down category activates the Site-Specific Variable Modifications for Top Down Analysis pane and deactivates the Variable Modifications for Intact and Peptide Analysis pane. All parameters in the inactive pane are not editable.

When your change from one category to another affects the functionality of a pane, you receive a prompt to confirm this action. Once it is confirmed, the application automatically clears all of your currently specified variable modifications, maximum number of modifications, glycan, and proteoform settings. The application resets these parameters to the default values before deactivating the relevant pane.

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4. Define or edit the protein sequence, add the appropriate modifications to the sequence, and then save the sequence.

   See Saving a protein or peptide sequence (page 64).

Editing the amino acids in an existing sequence

To edit the amino acids in an existing sequence

1. On the Home page, click **Sequence Manager** in the left pane.

   The Sequence Manager page opens.

2. In the table, select the row for a protein sequence and click **Edit**, or double-click the row.

   The Protein Sequence Editor opens.

3. Copy the entire sequence of interest in the Protein Sequence Map pane.

4. Paste the sequence into Wordpad or another editing tool.

5. Edit the sequence and save it under the same FASTA file name and folder as the original sequence.

   IMPORTANT! Make sure that the edited FASTA information contains comment lines that begin with the greater-than sign (>) to distinguish each chain, separate from the amino acid lines.

6. In the Protein Sequence Editor, click **Import Fasta File** in the command bar.

7. In the dialog box, locate the saved file and click **Open**.

   The application displays the edited sequence information from the FASTA file in the Protein Sequence Map pane of the Protein Sequence Editor.

8. Save the sequence.

   Because the name of the edited import file is the same as an existing protein sequence in the table on the Sequence Manager page, a message box opens with a warning that the sequence name already exists and will be overwritten.

9. Click **Yes** to overwrite the existing protein sequence.

Modification assignments for a protein sequence

Modifications to protein sequences include static and variable modifications, disulfide bonds, glycosylations, and proteoforms. You can define and assign the various modifications to the target protein sequences.

For information about assigning the modifications, see Assigning modifications to a protein sequence (page 67).

Sequence matching components

To aid in component identification, the BioPharma Finder application can match the measured masses of detected components to the fragment masses and modified fragment masses of components in user-specified sequences. If the measured mass of some components lies within a user-supplied tolerance of the mass in the associated target sequence, the application displays the matched target sequence in an identification column in the component list results.

The protein sequences can include static modifications, variable modifications, glycosylations, and disulfide links. If you enter these modifications, the application applies them in the following order:

1. Disulfide links, which provide the bonds between heavy chains and light chains or bonds within the same chain.

2. Static modifications, which are modifications that you can apply to a single site or all sites for that residue. There are three types of static modifications: side chain, C-terminus, and N-terminus.

3. Glycosylation, which is a process in which chains of saccharides are linked to produce glycans that can be attached to glycosylation sites on the target sequence. The application applies all possible glycosylations of the user-specified type to one or more of the available glycosylation sites.

   The "Glycans" appendix lists all of the N-linked glycans included in the default modifications list, the N-linked glycans included in the N-glycan-specific search and sorted by host cell-line type, and the O-linked glycans that are supported by Peptide Mapping Analysis.

   Note: The algorithm that detects possible glycosylation sites was designed for use on intact proteins. If it is applied to peptides, it might fail to identify motifs that have been truncated by a cleavage. To address this issue when using a peptide as a target sequence, append an amino acid to the sequence to complete the motif, and then define and apply a custom modification that subtracts the mass of that amino acid.

4. Variable modifications: The possible side-chain or terminal modifications whose specific sites and number of occurrences might not be known. The application applies all possible combinations between zero and a user-specified maximum number of variable modifications to the target sequence.

When you select glycosylations and variable modifications, the application first searches for matches to base masses that include static or disulfide bonds, and then tries to match masses with various glycosylations and variable modification combinations. The combinations of variable modifications are limited to the specified maximum number of modifications. Setting the maximum number of modifications to a high value can greatly increase the number of combinations and the search time. The maximum number of modifications does not include glycosylations.

For a list of Glycans, see Glycans (page 681).

Changing the default modifications for protein sequences

Upon installation, the BioPharma Finder application provides a default list of variable modifications, including N-glycans as side chain modifications. You can access this list from the Protein Sequence Editor and set a sublist for quick loading into a protein sequence. You can also select which modifications will be visible in the modification editing panes.

For details, see the following topics:

- Default sublist of modifications for quick loading (page 59)
- Changing the default and visible sublist of modifications for protein sequences (page 59)

Default sublist of modifications for quick loading

Within the default list of modifications, the BioPharma Finder application sets the modifications shown in the following table as a default sublist that you can quickly load to assign as C-terminal or side chain modifications to a protein sequence.

Table 6 Default sublist of modifications

Modification name	Modification type
Lys	C-terminal
Deamidation (N)	Side chain
Double Oxidation	Side chain
Glycation	Side chain
H2O loss	Side chain
Hydroxylation	Side chain
Mannosylation (S)	Side chain
NH3 loss	Side chain
Oxidation (MW)	Side chain

Changing the default and visible sublist of modifications for protein sequences

From the default list of modifications, you can select which modifications/N-glycans will be visible in the Variable Modifications for Intact and Peptide Analysis and Modification Editor pane and the Site-Specific Variable Modifications for Top Down Analysis pane of the Protein Sequence Editor. You can also select the items to include in the sublist for quick loading.

The "Glycans" appendix lists all of the N-glycans in the default list.

To change the visible default list and sublist of modifications/N-glycans

1. In the Protein Sequence Editor, click **Define Modification List** in the command bar.

   The default list table opens in the Define Modification List window.

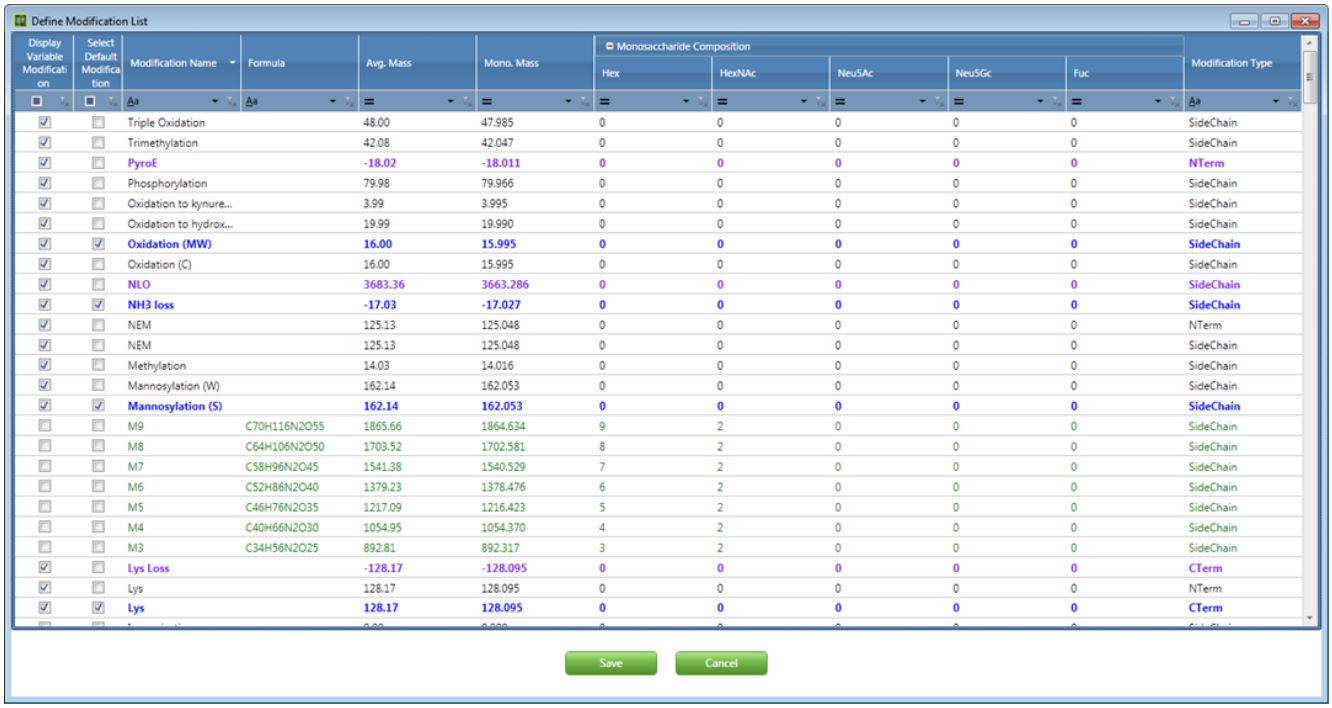


Figure 15 Table in the Define Modification List window

The modifications are color-coded as follows:

- Blue—Recommended default peptide mapping modifications.
- Black—Original modifications that are not set for quick loading. These modifications are not selected.
- Green—N-glycans. These modifications are not selected.
- Purple—Modifications created by the user.

The table shows the formula, average mass, monoisotopic mass, and modification type for each item in the list. The table also displays the monosaccharide composition of the N-glycans, consisting of the Hex, HexNAc, Neu5Ac, Neu5Gc, and Fuc data.

Tip: You can click the collapse icon, ⊟, to the left of the Monosaccharide Composition column header to hide this information, and then click the expand icon, ⊞, to show this information again.

You can sort and filter the table to shorten the list.

---

2. In the Display Variable Modification column, select the check boxes for the modifications/N-glycans that you want visible in the following panes:

   - Variable Modifications for Intact and Peptide Analysis—default list of variable modifications
   - Site-Specific Variable Modifications for Top Down Analysis—Select Type of Modification table
   - Modification Editor—default list of variable modifications

Or, clear the check boxes for the items that you want to remove from these areas.

Note: When you add a new custom modification, the application automatically adds it to the table and selects the check box in the Display Variable Modification column for that item. Changes to the selections in the Display Variable Modification column persist when you close the application.

IMPORTANT! You cannot clear the check box in the Display Variable Modification column if the check box in the Select Default Modification column is selected for a particular item in the list.

---

3. In the Select Default Modification column, select the check boxes for the modifications/N-glycans that you want to include in the quick loading sublist, and clear the check boxes for the items that you want to remove from this sublist.

   Note: When you select the check box in the Select Default Modification column, the application automatically selects the check box in the Display Variable Modification column to add the selected item to the visible default list.

4. Click **Save**.

For more details, see these topics:

- Glycans (page 681)
- Managing custom modifications for protein sequences (page 61)
- Managing variable modifications (page 76)
- Filtering data in a table (page 673)

Managing custom modifications for protein sequences

In addition to the default modifications provided with the BioPharma Finder application, you can create custom modifications to assign to a side chain, an N-terminus, and a C-terminus. Define these custom modifications before you begin assigning them to the target protein sequences.

For details, see the following topics:

- Creating custom modifications and oligo building blocks for oligonucleotide sequences (page 120)
- Modifying custom modifications (page 62)
- Deleting custom modifications (page 62)
- Modification Editor pane parameters (page 63)

Modifying custom modifications

To modify an existing custom modification

1. In the Protein Sequence Editor, click the title bar for the Modification Editor to open its pane.

   Existing custom modifications appear in purple in the modification lists on the left.

2. Add a custom modification with the same name as an existing modification.

   The application overwrites the existing modification parameters with the new information.

For details, see the following topics:

- Importing a FASTA file with a protein sequence (page 50)
- Creating custom modifications and oligo building blocks for oligonucleotide sequences (page 120)

Deleting custom modifications

To delete custom modifications

1. In the Protein Sequence Editor, click the title bar for Modification Editor to open its pane.

2. In the modification lists on the left side, select the custom modification (in purple) that you want to delete.

   Note: You cannot delete any of the default modifications. In addition, you cannot delete any custom modification that an experiment is currently using.

3. Click **Delete** in the N Terminal, C Terminal, or Side Chain area, as appropriate.

For details, see the following topics:

- Importing a FASTA file with a protein sequence (page 50)
- Creating custom modifications and oligo building blocks for oligonucleotide sequences (page 120)

Modification Editor pane parameters

The following table describes the parameters of the Modification Editor pane of the Protein Sequence Editor.

Table 7 Modification Editor pane parameters

Parameter	Description
Modifications	Lists the modifications available to apply to an N terminus, C terminus, or side chain.
N Terminal	Displays information about the N-terminal modification that you selected from the list.
Mono. Mass	Displays the monoisotopic mass of the selected N-terminal modification.
Avg. Mass	Displays the average mass of the selected N-terminal modification.
Add	Opens the Add New Modification dialog box, so that you can add a custom N-terminal modification.
Delete	Permanently removes the selected custom modification from the list of N-terminal modifications.
C Terminal	Displays information about the C-terminal modification that you selected from the list.
Mono. Mass	Displays the monoisotopic mass of the selected C-terminal modification.
Avg. Mass	Displays the average mass of the selected C-terminal modification.
Add	Opens the Add New Modification dialog box, so that you can add a custom C-terminal modification.
Delete	Permanently removes the selected custom modification from the list of C-terminal modifications.
Side Chain	Displays information about the side-chain modification that you selected from the list.
Mono. Mass	Displays the monoisotopic mass of the selected side-chain modification.
Avg. Mass	Displays the average mass of the selected side-chain modification.
Residues	Displays the amino acid residue or residues for the selected side-chain modification.
Add	Opens the Add New Modification dialog box, so that you can add a custom side-chain modification. In this version of the dialog box, the Residues box is enabled.
Delete	Permanently removes the selected custom modification from the list of side-chain modifications.

For details, see the following topic:

- Managing custom modifications for protein sequences (page 61)

Saving a protein or peptide sequence

If you make any changes to a sequence, you can save it under the same name to overwrite an existing sequence or under a different name to create a new sequence. The information saved includes all of the items listed in the table on the Sequence Manager page.

For details, see the following topics:

- Saving a protein or peptide sequence with the same name (page 64)
- Saving a protein or peptide sequence with a different name (page 64)

Saving a protein or peptide sequence with the same name

To save a protein/peptide sequence with the same name

1. Click **Save** in the command bar of the Protein Sequence Editor to save the sequence under the same name.

   If this sequence already exists, the following warning prompt appears: The sequence name already exists and will be overwritten.
2. At the prompt, click **Yes** to have the current sequence overwrite previously saved data.

Information from the saved sequence populates the columns of the sequence table on the Sequence Manager page.

Saving a protein or peptide sequence with a different name

To save a protein/peptide sequence with a different sequence name

1. Click **Save As New** in the command bar of the Protein Sequence Editor.

   The Save As New dialog box opens.

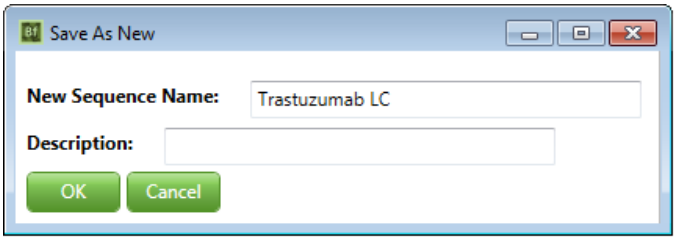


Figure 16 Save As New dialog box

2. Do the following:
   a. In the New Sequence Name box, type the new name of the protein sequence.

b. (Optional) In the Description box, type a description for the protein sequence.

3. Click **OK**.

   Information from the saved sequence populates the columns of the sequence table on the Sequence Manager page.

Protein Sequence Editor parameters

The following table describes the parameters in the Protein Sequence Editor.

Table 8 Parameters in the Protein Sequence Editor

Parameter	Description
Command Bar	
Import Fasta File	Imports a protein sequence from a FASTA file.
Define Modification List	Opens a window for you to edit the default list of modifications that appear in the Variable Modifications for Intact and Peptide Analysis pane, the Site-Specific Variable Modifications for Top Down Analysis pane, the Modification Editor pane, and the sublist of modifications used for quick loading into the protein sequence.
Save	Saves the changes to a protein sequence under its existing name.
Save As New	Opens the Save As New dialog box, so that you can save a protein sequence under a new name and assign to it a category.
Cancel	Closes the Protein Sequence Editor if you already saved your changes. Otherwise, in the save changes message box, click Yes or No.
Protein Sequence Information pane	Displays the protein sequence and chain information.
Target Protein	Displays information about the current protein sequence.
Name	Displays the name of the protein sequence.
Description	Displays the description of the protein sequence.
Category	Displays the category of the protein sequence.
Monoisotopic Mass	Displays the monoisotopic mass of the protein sequence.
Average Mass	Displays the average mass of the protein sequence.
Chain	Displays information about a selected chain.
Chain	Lists the number of each chain in the protein sequence.
Monoisotopic Mass	Displays the monoisotopic mass of the chain that you selected in the Chain list.
Average Mass	Displays the average mass of the chain that you selected in the Chain list.

Parameter	Description
Protein Sequence Map pane	Displays the amino acids from the chains in the protein sequence.
Disulfide Link Definitions pane	Displays dilsulfide bonds for you to edit.
Manual Input Protein Sequence pane	Displays information about manually added protein sequence chains.
Chain Name	Specifies the name of an added chain in the protein sequence.
(Editor box)	Provides an area for you to type or paste the new chain information.
Apply	Adds a new chain to the Protein Sequence Map pane.
Residue Properties and Modifications dialog box	Displays static modifications for you to edit.
Variable Modifications for Intact and Peptide Analysis pane	Displays variable modifications for you to edit for Intact Mass Analysis or Peptide Mapping Analysis.
Site-Specific Variable Modifications for Top Down Analysis pane	Displays variable modifications for you to edit for Top Down Analysis.
Modification Editor pane	Displays boxes for you to add or delete custom modifications.

For information about using the Protein Sequence Editor, see these topics: “Creating and editing protein sequences” on page 50 and Chapter 4, “Assigning modifications to a protein sequence”.

4 Assigning modifications to a protein sequence

You can assign modifications to the protein sequence for automated searching of modified peptides. These modifications include disulfide links, glycosylations, static modifications, and variable modifications.

Order of modification assignments

For Intact Mass Analysis and Peptide Mapping Analysis, you assign the variable modifications to the N-terminal, C-terminal, and side chains in a protein sequence. For example, you can use PTMs (such as phosphorylation) or modifications due to artifacts from sample handling or digestion (such as overalkylation, oxidation, or deamidation). For Top Down Analysis, you select a sublist of variable modifications to generate a list of proteoforms for searching to identify fragment ions.

You can edit the sublist of default modifications for quick loading before assigning them to the sequences. You can also create custom modifications before assigning them to the sequences. Thermo Fisher Scientific recommends this step if you expect to use the custom modifications in subsequent analyses.

You can specify the modifications in any order, but the application always applies them in this order:

1. Disulfide links
2. Static modifications
3. Glycosylations

   Glycosylations take precedence over variable modifications.
4. Variable modifications

After the application applies a static modification or disulfide link to a site, you can make no other changes to the site, except to apply two modifications to an end terminal. For example, when you link a particular cysteine in a disulfide bond to a site, the site cannot be a candidate for any other static or variable modifications.

For more details, see the following:

- Changing the default modifications for protein sequences (page 58)
- Managing custom modifications for protein sequences (page 61)
- Sequence matching components (page 57)
- Managing disulfide links (page 68)
- Managing static modifications (page 70)
- Managing glycosylations (page 74)
- Managing variable modifications (page 76)

Managing disulfide links

To manage disulfide links in a target protein sequence, see the following topics:

- Assigning disulfide links (page 68)
- Removing disulfide links (page 70)
- Disulfide link definitions pane parameters (page 70)

Note: Disulfide bond links are required only for intact protein experiments, not for disulfide bond identification in peptide mapping and top-down experiments.

Assigning disulfide links

You can assign disulfide links only to protein sequences with the Intact Protein or Unknown category. The application disables the disulfide linking capability for all other categories.

To assign disulfide links

1. Load or create a target protein sequence.
2. In the Protein Sequence Map pane of the Protein Sequence Editor, position the cursor before the unmodified cysteine of interest (letter C), right-click, and choose **Create Link**.
3. Right-click the unmodified cysteine (letter C) to link it to, and choose **Bridge Link**.
4. Repeat step 2 to step 3 to add more disulfide links, but do not link a cysteine to more than one cysteine.

   The Protein Sequence Map pane displays orange lines connecting the linked cysteines, and the Disulfide Link Definitions pane displays the numbers of the chains that they belong to and their locations within those chains.

When you select a row in the Disulfide Link Definitions table, the application uses green to highlight the corresponding link in the Protein Sequence Map pane.

You cannot link a cysteine to more than one cysteine. You cannot statically modify a linked cysteine.

Note: For peptide mapping and top-down experiments, you are not required to link the disulfide bonds to perform a disulfide bond mapping experiment. However, you must link disulfide bonds when you process intact data from an unreduced molecule. For example, when you process data for a homodimer unreduced antibody, add two copies of each chain so that the protein sequence map shows four sequences. Then, connect all of the linkages to ensure that you obtain the correct molecule mass of the molecule.

The example protein sequence in the following figure links the cysteines that are in the table in the Disulfide Link Definitions pane. The first two columns in the table indicate the starting point of the link and the last two columns indicate the ending point.

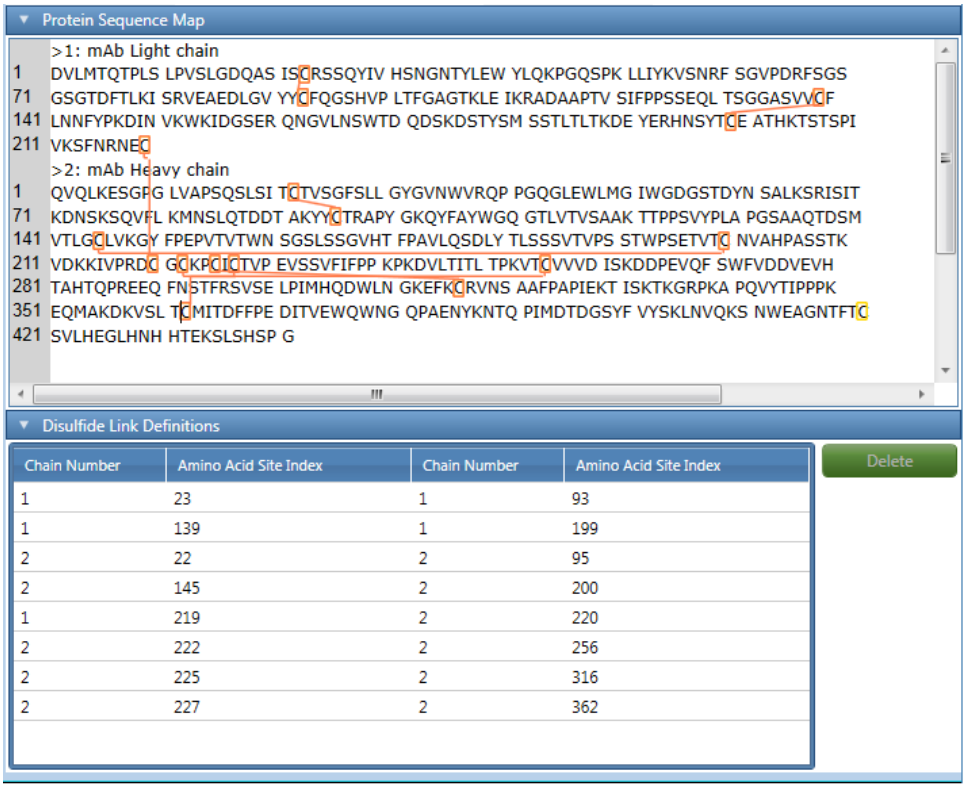


Figure 17 Disulfide links in the Protein Sequence Map and Disulfide Link Definitions panes

5. If you do not want to save these modifications to the sequence, click **Cancel** in the command bar of the Protein Sequence Editor to leave this window.

   Otherwise, click **Save** or **Save as New** to save the modifications.

For information about creating and editing protein sequences and saving sequences, see these topics: “Creating and editing protein sequences” on page 50 and “Saving a protein or peptide sequence” on page 64.

Removing disulfide links

To remove disulfide links

- In the table in the Disulfide Link Definitions pane, select the row for the disulfide link that you want to delete and click **Delete**.

–or–

- In the Protein Sequence Map pane, right-click the cysteine with the disulfide link and choose **Remove Link**.

Disulfide link definitions pane parameters

The following table describes the columns in the table in the Disulfide Link Definitions pane of the Protein Sequence Editor.

Table 9 Disulfide Link Definitions pane parameters

Column	Description
Chain Number	Displays the number of the chain that includes the first cysteine in the disulfide bond.
Amino Acid Site Index	Displays the location within the chain of the first cysteine in the disulfide bond.
Chain Number	Displays the number of the chain that includes the second cysteine in the disulfide bond.
Amino Acid Site Index	Displays the location within the chain of the second cysteine in the disulfide bond.
Button	
Delete	Deletes the selected row from the table in the Disulfide Link Definitions pane.

Managing static modifications

To manage static modification in a target protein sequence, see the following topics:

- Assigning static modifications (page 71)
- Removing static modifications (page 73)
- Residue properties and modifications dialog box parameters (page 73)

Assigning static modifications

To assign static N-terminal, C-terminal, and side chain modifications to the sequence

1. In the Protein Sequence Map pane of the Protein Sequence Editor, place the cursor to the left of one of these letters:
   - The side-chain letter of interest (for a side chain modification)
   - The first letter of the chain (for an N-terminal modification)

   –or–
   - The last letter of the chain (for a C-terminal modification)

   Then, double-click.

   The Residue Properties and Modifications dialog box opens.

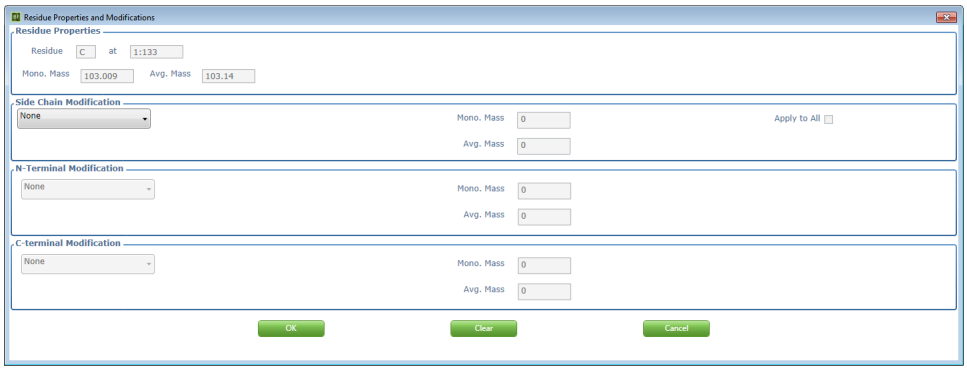


Figure 18 Residue Properties and Modifications dialog box

   The properties of the selected amino acid appear in the Residue Properties area. Check these properties to make sure that you selected the appropriate amino acid.

2. (Optional) Assign any side chain modifications:
   a. In the Side Chain Modification area, select the modification from the list to assign to the side chain.

      The monoisotopic mass of the selected modification appears in the Mono. Mass box. The average mass of the selected modification appears in the Avg. Mass box.

   b. (Optional) To apply the side chain modification to all residues of the selected amino acid, select the **Apply to All** check box.

      Tip: If you want to apply a modification to most of the instances of an amino acid in a side chain, select the **Apply to All** check box and then click **OK**. Then, repeat step 1 through step 2 and select **None** from the list in the Side Chain Modification area or **Clear** for instances when you do not want to have this modification.

3. (Optional) Assign any N- or C-terminal modifications:

   In the N- (or C-) Terminal Modification area, select the modification from the list to assign to the chain's N- or C-terminal.

   The monoisotopic mass of the new modification appears in the Mono. Mass box. The average mass of the new modification appears in the Avg. Mass box.

4. Click **OK**.

5. (Optional) Repeat the previous steps to assign the side chain, N-terminal, or C-terminal modifications to any other chains of interest.

6. If you do not want to save these modifications to the sequence, click **Cancel** in the command bar of the Protein Sequence Editor to leave this window.

   Otherwise, click **Save** or **Save as New** to save the modifications.

When you add a static modification to a side chain, N-terminal, or C-terminal, the modified amino acid turns blue in the sequence map. The BioPharma Finder application assumes the new *m/z* value for this amino acid for the search. If you selected the Apply to All option for a side chain modification, all of the instances of the modified amino acid turn blue.

The following figure shows all of the "C" amino acids in the chains highlighted in blue from the global application of a side chain modification.

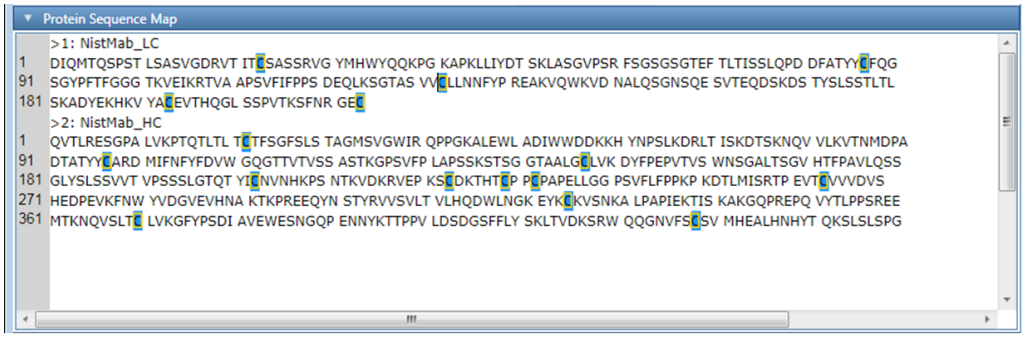


Figure 19 Modified amino acids highlighted in blue

Note: A static modification does not appear as a modification in the results table after you process an experiment.

For details, see the following topics:

- Importing a FASTA file with a protein sequence (page 50)
- Saving a protein or peptide sequence (page 64)
- Viewing the peptide results table (page 246)
- Viewing the intact results table (page 423)
- Viewing the intact deconvolution results table (page 537)

Removing static modifications

To remove static N-terminal, C-terminal, and side chain modifications

1. In the Protein Sequence Map pane of the Protein Sequence Editor, place the cursor to the left of one of these letters:
   - The side-chain letter of interest (for a side chain modification)
   - The first letter of the chain (for an N-terminal modification)

   –or–
   - The last letter of the chain (for a C-terminal modification)

   Then, double-click.
   The Residue Properties and Modifications dialog box opens.

2. (Optional) To globally remove a side chain modification applied to the selected amino acid letter, select the **Apply to All** check box in the Side Chain Modification area and then click **Clear**.

   –or–

   Click **None** in the N-Terminal, C-Terminal, or Side Chain Modification area or in all three areas.

   Tip: You can use the Apply to All check box to globally make changes only for side chain modifications. If you want to modify N-terminal or C-terminal modifications, you must modify each one individually.

When you remove a static modification from a side chain, N-terminal, or C-terminal, the blue highlight for the modified amino acid disappears in the sequence map. If you selected the Apply to All option for a side chain modification, all of the blue highlights for the modified amino acids disappear. These amino acids are no longer modified.

Residue properties and modifications dialog box parameters

The following table describes the parameters of the Residue Properties and Modifications dialog box of the Protein Sequence Editor.

Table 10 Residue Properties and Modifications dialog box parameters

Parameter	Description
Residue Properties	Displays information about the amino acid that you selected in the Protein Sequence Map pane.
Residue	Displays the letter of the amino acid that you selected in the Protein Sequence Map pane.
At	Displays the number of the chain that includes the selected amino acid, followed by ":", and then the location of the amino acid within that chain.
Mono. Mass	Displays the monoisotopic mass of the amino acid.
Avg. Mass	Displays the average mass of the amino acid.

Parameter	Description
Side Chain Modification	Displays the fields related to a side chain modification.
(list)	Displays a list of the available side chain modifications.
Mono. Mass	Displays the monoisotopic mass of the modification that you selected from the list.
Avg. Mass	Displays the average mass of the modification that you selected from the list.
Apply to All	Applies the selected modification to all instances of the selected amino acid type in the protein sequence.
N-Terminal Modification	Displays the fields related to an N-terminal modification.
(list)	Displays a list of available modifications for the N-terminus.
Mono. Mass	Displays the monoisotopic mass of the modification that you selected from the list.
Avg. Mass	Displays the average mass of the modification that you selected from the list.
C-Terminal Modification	Displays the fields related to a C-terminal modification.
(list)	Displays a list of available modifications for the C-terminus.
Mono. Mass	Displays the monoisotopic mass of the modification that you selected from the list.
Avg. Mass	Displays the average mass of the modification that you selected from the list.
Buttons	
OK	Applies the selected modifications to the selected amino acid.
Clear	Removes the modification from the selected instance of the amino acid and closes the dialog box. If you select the Apply to All check box before you click Clear, the BioPharma Finder application removes the modification from all instances of the selected amino acid.
Cancel	Closes the dialog box without applying any modification to the selected amino acid.

Managing glycosylations

Follow this procedure to assign a type of glycosylation to a target protein sequence.

To add a search for a specific glycosylation to a protein sequence

1. In the Protein Sequence Editor, click the title bar for Variable Modifications for Intact and Peptide Analysis.

   The following figure shows the open pane.

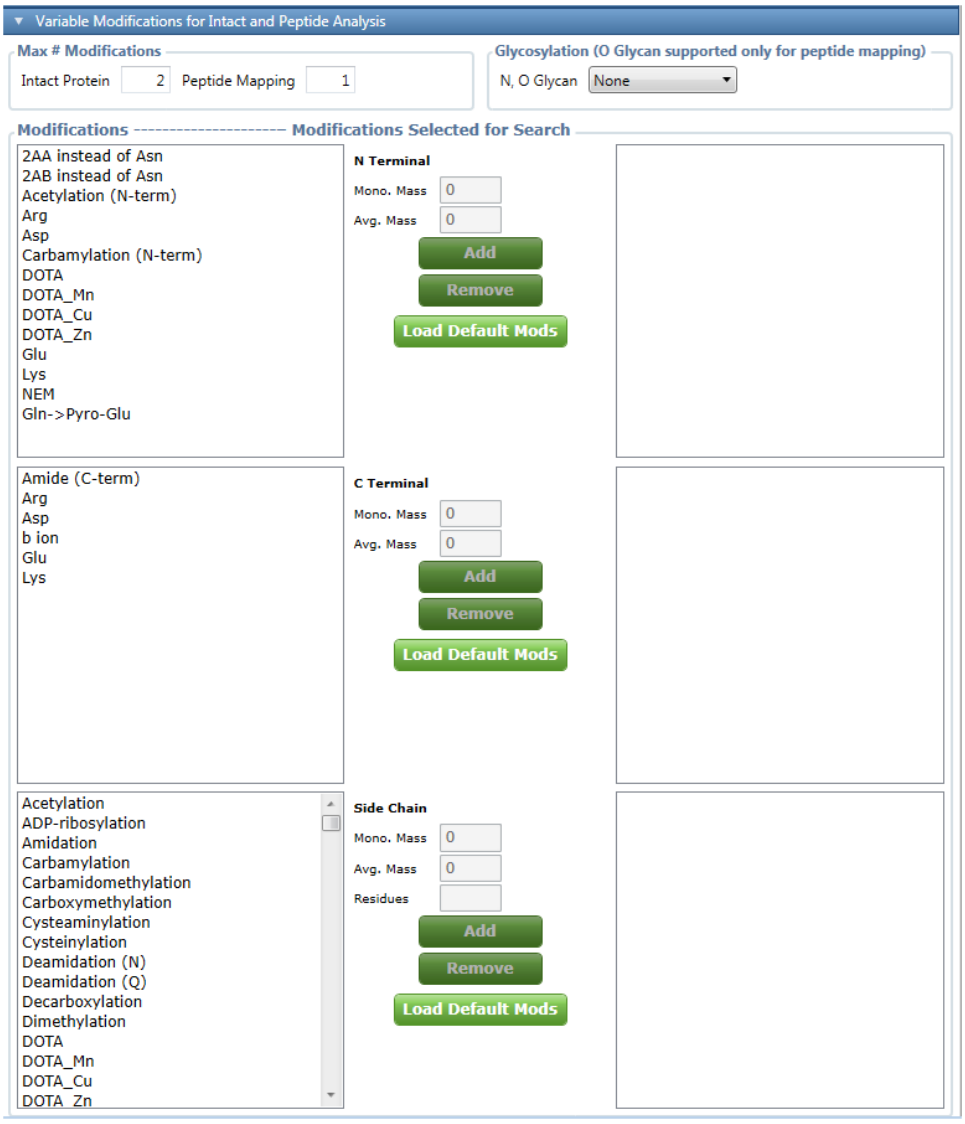


Figure 20 Variable Modifications for Intact and Peptide Analysis pane

Note: To edit the parameters in this pane, you must set the Category for the protein sequence to Peptide Mapping, Intact Protein, or Unknown.

If the resolution and text size of you computer screen is not set properly, you might not be able to see some of the features at the bottom of the pane. To correct this problem, use a screen with the recommended resolution of 1920 × 1080 pixels and consider changing the text size.

2. In the N, O Glycan list in the Glycosylation area, select the type of glycosylation (or none) to apply to the N-linked glycans and O-linked glycans:
   - **None:** Does not add any search for glycosylations.
   - **CHO:** Adds a search for glycosylations to the Chinese hamster ovary (CHO) glycans.
   - **Human:** Adds a search for glycosylations to the human glycans.

Note: The application supports O-linked glycans only for Peptide Mapping Analysis.

The N-linked glycans are the linkage between N-Acetylglucosamine and the asparagine (Asn) side chain that is part of the amino acid sequence motif Asn_*Xxx*_Ser/Thr/Cys. The O-linked glycans are usually the linkage between N-Acetylgalactosamine and the amino acids serine (S) or threonine (T).

3. If you do not want to save these modifications to the sequence, click **Cancel** in the command bar of the Protein Sequence Editor to leave this window.

   Otherwise, click **Save** or **Save as New** to save the modifications.

Managing variable modifications

To manage variable modifications in a target protein sequence for Intact Mass Analysis or Peptide Mapping Analysis, see the following topics.

- Assigning variable modifications (page 76)
- Variable modifications for intact and peptide analysis pane parameters (page 80)
- "Variable modification for oligo sequences for intact deconvolution category" on page 78

Assigning variable modifications

To assign variable N-terminal, C-terminal, and side chain modifications

1. In the Protein Sequence Editor, click the title bar for Variable Modifications for Intact and Peptide Analysis to open this pane.

   Note: To edit the parameters in this pane, you must set the Category for the protein sequence to Peptide Mapping, Intact Protein, or Unknown.

   If the resolution and text size of you computer screen is not set properly, you might not be able to see some of the features at the bottom of the pane. To correct this problem, use a screen with the recommended resolution of 1920 × 1080 pixels and consider changing the text size.

   The lists at the left side of the pane, under Modifications, show all of the available default variable modifications that you want to display in the pane. You can modify the displayed lists of default modifications as appropriate.

2. In the Max # Modifications area, do the following:
   - In the Intact Protein box, type a value for the maximum number of modifications to assign to the sequence for Intact Mass Analysis.
   - In the Peptide Mapping box, type a value for the maximum number of modifications to assign to the sequence for Peptide Mapping Analysis.

   Tip: Increasing these numbers might substantially increase processing time.

3. In the N Terminal, C Terminal, or Side Chain area, do the following:
   a. To quickly load the modifications from the default sublist, click **Load Default Mods**.

      Tip: You can edit the default sublist of variable modifications used for quick loading.

   b. To assign another modification, select it from the Modifications lists on the left, and then click **Add**.

Tip: You can assign more than one modification but select only one at a time.

The following figure shows the Variable Modifications for Intact and Peptide Analysis pane after quick loading from the default sublists.

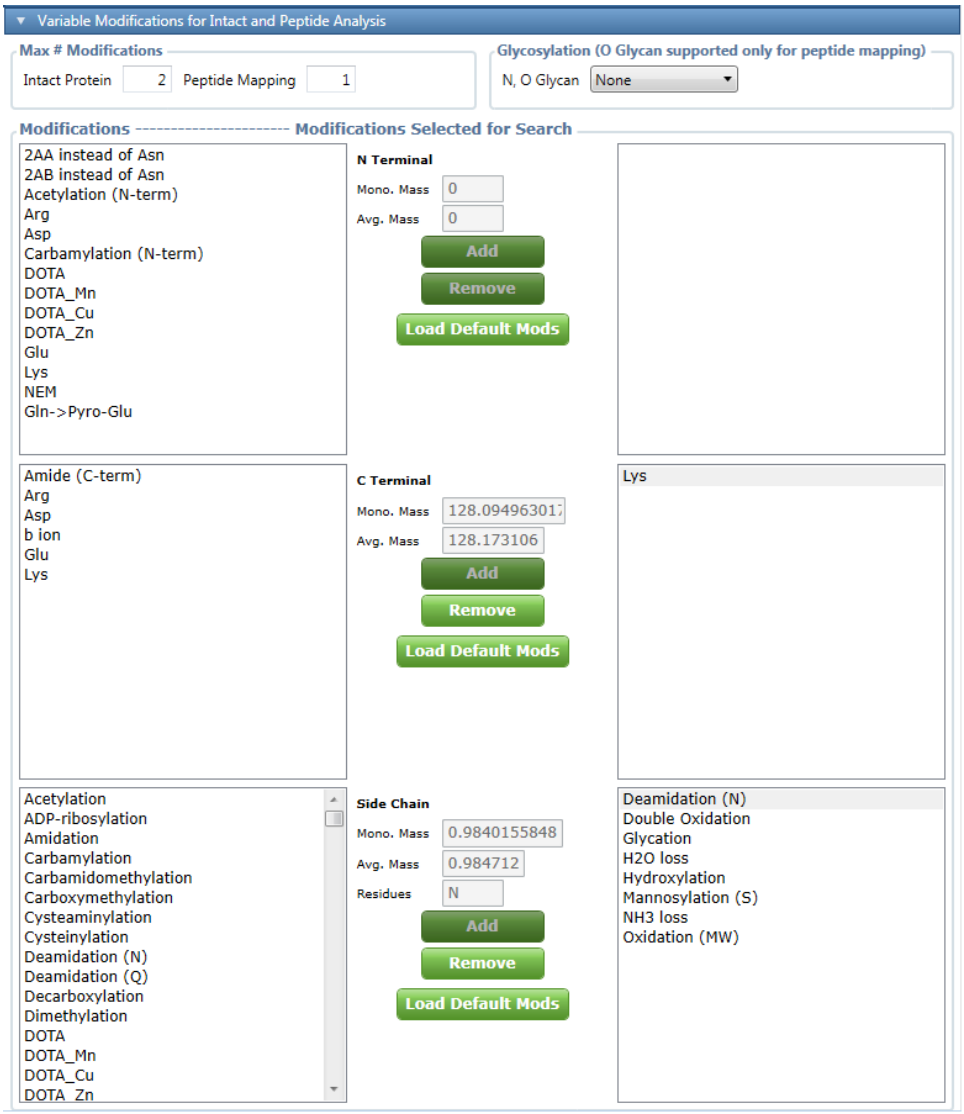


Figure 21 Variable Modifications for Intact and Peptide Analysis pane with loaded default modifications

The loaded and selected modifications appear in the lists on the right. They are used as variable modifications during the identification step of the experiment. To identify modification sites automatically, make sure to select the appropriate variable modifications. The application can detect most unspecified modifications for Peptide Mapping Analysis if enough information is available.

4. If you do not want to save these modifications to the sequence, click **Cancel** in the command bar of the Protein Sequence Editor to leave this window.

   Otherwise, click **Save** or **Save as New** to save the modifications.

Variable modification for oligo sequences for intact deconvolution category

1. On the Home page, click Sequence Manager in the left pane. The Sequence Manager page opens showing the sequence table, which contains a list of existing sequences. The experiment itself uses the sequences that you select from this list when you create the experiment.

2. To add a new Oligo sequence, click New Oligonucleotide on the right side of the page. The application displays the Oligonucleotide Sequence Editor and by default expands the Manual Input Sequence pane to the right.

3. Click on the **Assign Variable Modifications** pane to expand it and select any modification under Oligo Variable Modification sub section. Click **Add**.

4. Click on the **Import Fasta File** button. Copy each chain that you want to add from a FASTA file, using Notepad or another text editing tool.

5. Select and type the **Name** of the Target Oligonucleotide.

6. Select Intact Deconvolution in the **Category** list.

7. Click **Save** or **Save as New** to save the modifications.

   You can see the new Intact Deconvolution Nucleotide name in the list of sequences in the Sequence Manager page.

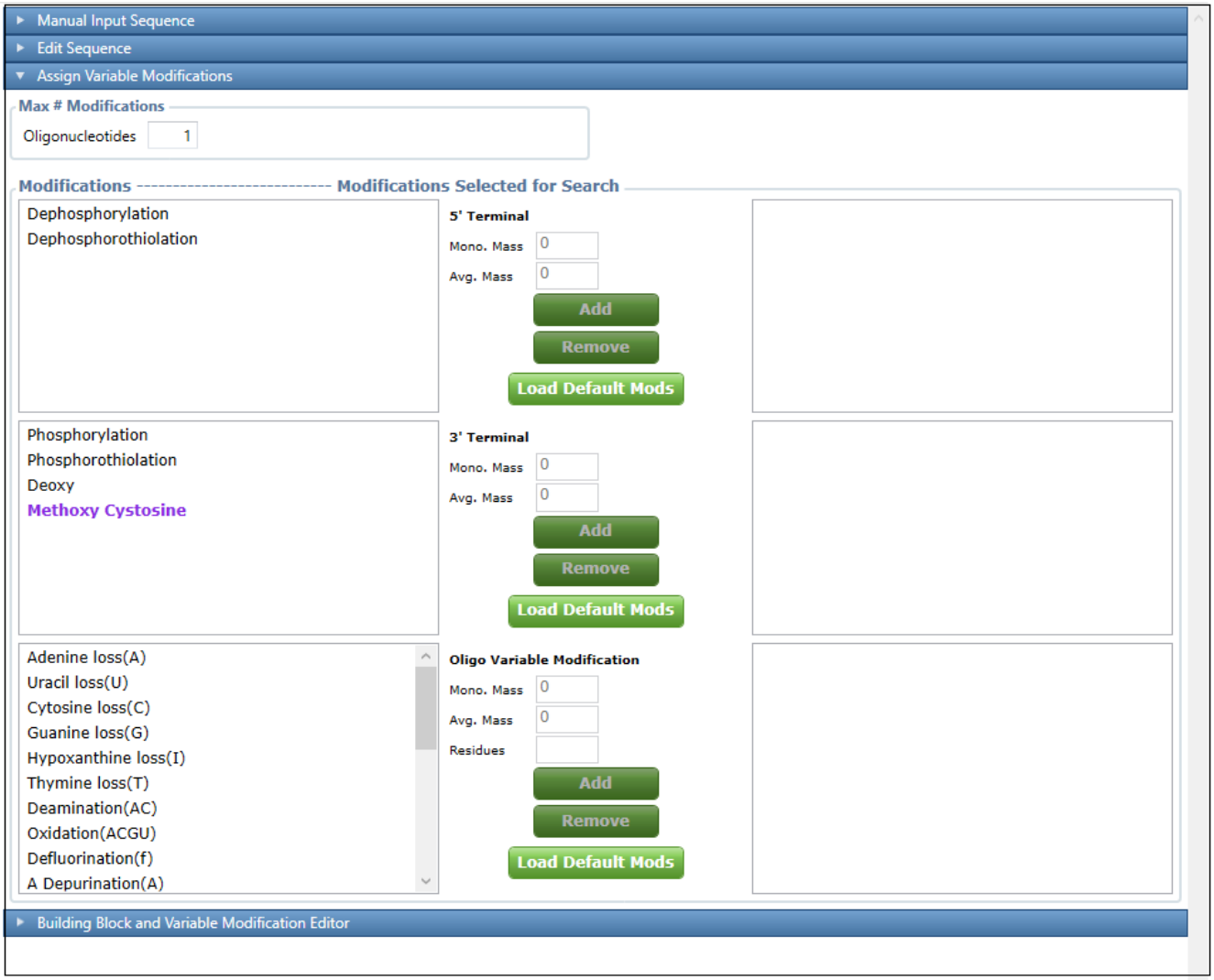


Figure 22 Variable Modification for Oligo sequences for Intact Deconvolution Category

Variable modifications for intact and peptide analysis pane parameters

The following table describes the parameters of the Variable Modifications for Intact and Peptide Analysis pane in the Protein Sequence Editor.

Table 11 Variable Modifications for Intact and Peptide Analysis pane parameters

Parameter	Description
Max # Modifications	Displays the maximum number of variable modifications that you want to assign to the target protein sequence.
Intact Protein	Displays the maximum number of variable modifications for Intact Mass Analysis.
Peptide Mapping	Displays the maximum number of variable modifications for Peptide Mapping Analysis.
Glycosylation	Specifies the type of glycosylation that you want to assign to the target protein sequence.
N, O Glycan	Lists the types of glycosylations that you can apply to the N-linked and O-linked glycans. **Note:** The application supports O-glycans only for Peptide Mapping Analysis.
Modifications	Lists the modifications available to apply to an N terminus, C terminus, or side chain.
N Terminal	Displays information about the N-terminal modification that you selected from the list.
Fields	
Mono. Mass	Displays the monoisotopic mass of the selected N-terminal modification.
Avg. Mass	Displays the average mass of the selected N-terminal modification.
Buttons	
Add	Adds the selected modification to the Modifications Selected for Search list.
Remove	Removes the selected modification from the Modifications Selected for Search list.
Load Default Mods	Quickly loads the sublist of default modifications into the Modifications Selected for Search list.
C Terminal	Displays information about the C-terminal modification that you selected from the list.
Fields	
Mono. Mass	Displays the monoisotopic mass of the selected C-terminal modification.
Avg. Mass	Displays the average mass of the selected C-terminal modification.
Buttons	
Add	Adds the selected modification to the Modifications Selected for Search list.
Remove	Removes the selected modification from the Modifications Selected for Search list.

Parameter	Description
Load Default Mods	Quickly loads the sublist of default modifications into the Modifications Selected for Search list.
Side Chain	Displays information about the side-chain modification that you selected from the list.
Fields	
Mono. Mass	Displays the monoisotopic mass of the selected side-chain modification.
Avg. Mass	Displays the average mass of the selected side-chain modification.
Residues	Displays the amino acid residue for the selected modification.
Buttons	
Add	Adds the selected modification to the Modifications Selected for Search list.
Remove	Removes the selected modification from the Modifications Selected for Search list.
Load Default Mods	Quickly loads the sublist of default modifications into the Modifications Selected for Search list.

Managing proteoforms

A protein might have several sites of modification—that is, sites where you can observe or predict particular residues to be modified in some way. In a particular instance of a given protein, the modified sites are active and the unmodified sites are inactive. This instance is called a proteoform, a distinct molecular form of a protein product that arises from a single gene. It is defined by its exact amino acid sequence combined with any PTMs on that sequence.

Because you might not know which sites are simultaneously active in a living organism, the BioPharma Finder application precomputes the masses and identities of the possible proteoforms of a given protein. The result is up to 2^n combinations of proteoforms, where n is the maximum number of sites of modifications on the protein. Some of these generated proteoforms might not exist in nature or in living organisms.

For example, for a protein that has only three phosphorylation sites and no other modifications, the application generates up to the following eight (2^3) records in the protein sequence:

- One record for the unmodified sequence with no variable modifications
- Three records for the three proteoforms, each containing one modification
- Three records for the possible combinations of two phosphorylations
- One record for the proteoform with all three phosphorylations

The actual number of generated proteoforms depends on the minimum and maximum number of modification sites that you set. For this example, if you set the minimum to two and the maximum to three, the total number of generated proteoforms is four if you do not want to use the unmodified sequence: 3 (for three

combinations of two modifications) + 1 (for one combination of three modifications). If you want to save the unmodified sequence, then the total number of generated proteoforms is five. From this generated list of proteoforms for this protein, you then select which proteoforms you want to save with the protein sequence for a search.

The BioPharma Finder application processes the saved proteoforms to identify those observed in top-down experiments. For details, see the following topics:

- Defining the modification list for proteoforms (page 82)
- Generating and saving the proteoforms (page 85)
- All possible proteoforms table (page 86)

Defining the modification list for proteoforms

Use the following procedure to define the modifications list used for generating the proteoforms for the target protein sequences.

To define the modification list

1. Create a new protein sequence or edit an existing one.

   IMPORTANT! For Top Down Analysis, the protein sequence can have only one chain.

2. In the Protein Sequence Editor, click the title bar for Site-Specific Variable Modifications for Top Down Analysis.

   The following figure shows the open pane.

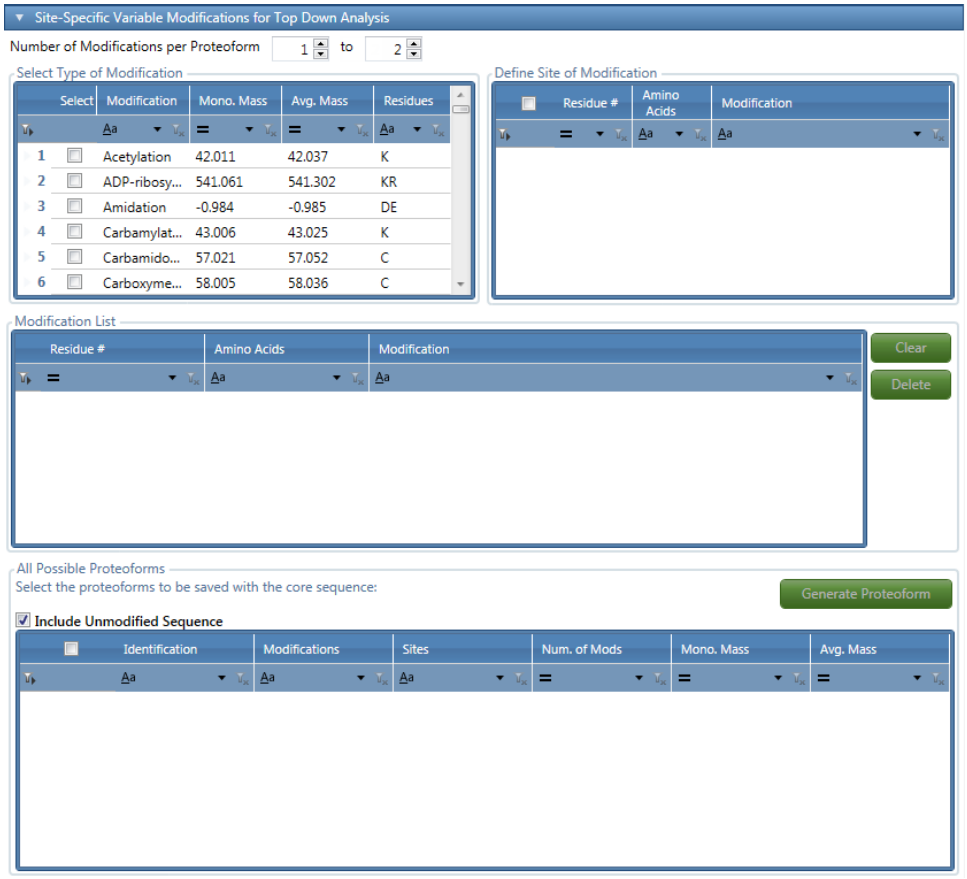


Figure 23 Site-Specific Variable Modifications for Top Down Analysis pane

Note: To edit the parameters in this pane, you must set the Category for the protein sequence to Top Down. If the sequence contains parameters previously set for Intact Mass Analysis or Peptide Mapping Analysis, the application automatically clears them, including variable modifications, disulfide bonds, glycan, and maximum number of modifications.

If the resolution and text size of you computer screen is not set properly, you might not be able to see some of the features at the bottom of the pane. To correct this problem, use a screen with the recommended resolution of 1920 × 1080 pixels and consider changing the text size.

The Select Type of Modification table in the top left shows all of the default side chain variable modifications, along with their monoisotopic/average masses and residues information. You can add more side chain variable modifications to this table as needed.

3. In the Number of Modifications per Proteoform boxes, enter the minimum and maximum number of modification sites for each proteoform.

   Note: The highest number that you can set for the maximum value is three.

   The range that you enter determines how many proteoforms the application generates for the total list of proteoforms. You can then select from this total list which proteoforms you want to save with the protein sequence for a search.

4. In the Select Type of Modification table, select the check box for one variable modification.

   The Define Site of Modification table to the right automatically displays the residue and amino acid information for all of the sites in the protein sequence that can have the selected variable modification.

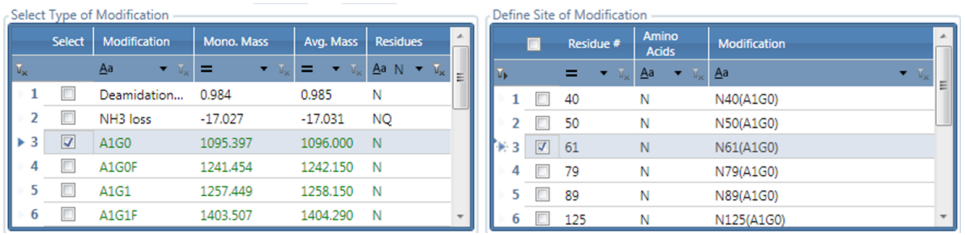


Figure 24 Select Type of Modification and Define Site of Modification tables

5. In the Define Site of Modification table, select the check boxes for all of the site-specific modifications that you want to include in the Modification List table.

   The Modification List table (in the center) is a working list of modifications used as input for generating the proteoforms. This table automatically appends a new selected modification above the top existing row, along with its residue and amino acid information. Scroll down as needed to view the entire list. To clear all entries in this table, click **Clear**. To delete a particular entry in this table, select that row, and then click **Delete** or press the DELETE key.

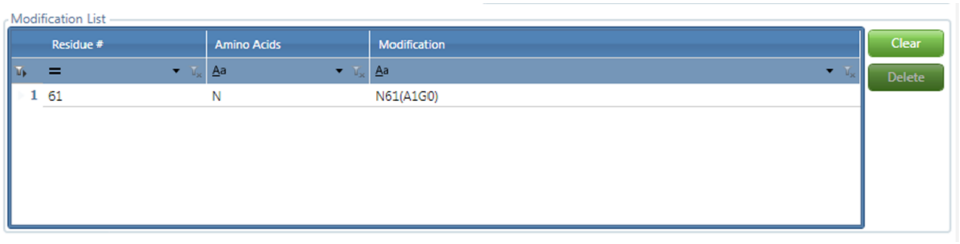


Figure 25 Modification List table

6. Repeat step 4 on page 84 and step 5 on page 84 to keep adding modifications as needed.

   The appended modifications appear at the top of the table.

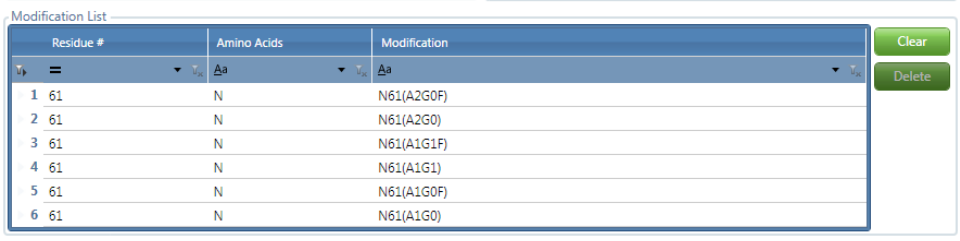


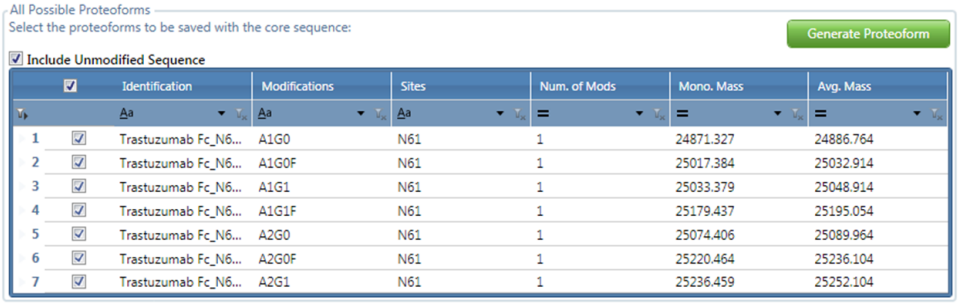
Figure 26 Modification List table appended

Generating and saving the proteoforms

To generate and save the proteoforms

1. When you are done defining the modification list, click **Generate Proteoform**.

   The All Possible Proteoforms table at the bottom of the pane displays all of the combinations of proteoforms generated from the chosen list of modifications, along with the monoisotopic and average masses for each proteoform. The total number of possible combinations is based on the entered Number of Modifications per Proteoform range.



		Identification	Modifications	Sites	Num. of Mods	Mono. Mass	Avg. Mass
		Aa	Aa	Aa	=	=	=
1	☑	Trastuzumab Fc_N6...	A1G0	N61	1	24871.327	24886.764
2	☑	Trastuzumab Fc_N6...	A1G0F	N61	1	25017.384	25032.914
3	☑	Trastuzumab Fc_N6...	A1G1	N61	1	25033.379	25048.914
4	☑	Trastuzumab Fc_N6...	A1G1F	N61	1	25179.437	25195.054
5	☑	Trastuzumab Fc_N6...	A2G0	N61	1	25074.406	25089.964
6	☑	Trastuzumab Fc_N6...	A2G0F	N61	1	25220.464	25236.104
7	☑	Trastuzumab Fc_N6...	A2G1	N61	1	25236.459	25252.104

Figure 27 All Possible Proteoforms table

   Whenever you make a change to the Number of Modifications per Proteoform entries or the Modification List table, the application automatically clears the All Possible Proteoforms table.

   You can repeat the previous steps on page 85 to make changes and then click **Generate Proteoform** again to update the All Possible Proteoforms table, as needed.

2. To save the unmodified sequence as a proteoform in the protein sequence, select the **Include Unmodified Sequence** check box above the All Possible Proteoforms table.

   Note: The unmodified sequence does not contain any variable modifications; however, it can contain already assigned static modifications. The unmodified sequence does not appear in the All Possible Proteoforms table.

   If you define a variable modification but then you assign it as a static modification, the application removes it from the list of variable modifications.

3. In the All Possible Proteoforms table, select the check boxes for the proteoforms that you want to save with the protein sequence for a search.

   When you select a particular proteoform, the application highlights in green the corresponding modified amino acids in the Protein Sequence Map pane.

4. Save the current protein sequence along with the selected proteoforms.

All possible proteoforms table

The following table describes the data in the All Possible Proteoforms table.

Table 12 All Possible Proteoforms table columns

Column	Description
Row number	The number assigned to each visible proteoform row in the table. This sequential numbering does not change when you sort or filter the table.
☐	Select this check box if you want to save the proteoforms in the selected rows with the protein sequence for a search. **Note:** To select or clear all of the check boxes at once, select or clear the check box in the column header. If you filter the table, the following occurs: • Clearing all check boxes affects all of the original rows in the table, before any filtering. • Selecting all check boxes affects only the filtered and currently visible rows.
Identification	Displays the protein sequence name, followed by an underscore, and then "*site*(*modification*)" for each modification separated by a comma. For example, in the identification of "Protein_C4(Oxidation), N35(Deamidation)": • "Protein" represents the protein sequence name. • "C4" represents the site of the first modification. • "Oxidation" represents the first modification. • "N35" represents the site of the second modification. • "Deamidation" represents the second modification.
Modifications	Displays the modifications listed in the Identification column.
Sites	Displays the sites listed in the Identification column.
Num of Mods	Displays the number of modifications occurring in the proteoform. This number is within the range of the Number of Modifications per Proteoform values.
Mono. Mass	Displays the monoisotopic mass of the proteoform.
Avg. Mass	Displays the average mass of the proteoform.

For details, see the following topics:

- Filtering data in a table (page 673)
- Defining the modification list for proteoforms (page 82)

5 Managing theoretical proteins and peptides

The BioPharma Finder application includes some features from the Pinpoint application for managing targeted proteins and peptides. These features help you perform the following tasks:

- Enter targeted proteins and proteotypic peptides.
- For targeted proteins, perform digestion and predict the theoretical peptide fragments.
- Create a mass list of the peptides (unmodified native peptides and modified peptides) using High Resolution Accurate Mass (HRAM) transitions for processing.
- Modify any of the amino acids in the protein or peptide sequence.
- Export a targeted mass list to a BPF file format that is compatible with the Thermo Scientific™ Chromeleon™ Chromatography Data System.

These features provide a direct connectivity between the BioPharma Finder application and the Chromeleon data system for targeted monitoring of peptides or critical quality attributes. In addition, you can save all or selected peptides to a workbook to use as a protein sequence for a targeted peptide mapping experiment.

Creating or importing a protein or peptide sequence

You can quickly import a protein or peptide sequence from a FASTA file or manually enter it.

To import or enter a protein or peptide sequence

1. On the Home page, click **Sequence Manager**.
   The Sequence Manager page opens, showing the protein sequence table.
2. Click the **Theoretical Protein/Peptide Manager** tab.
   The Theoretical Protein/Peptide Manager page opens.

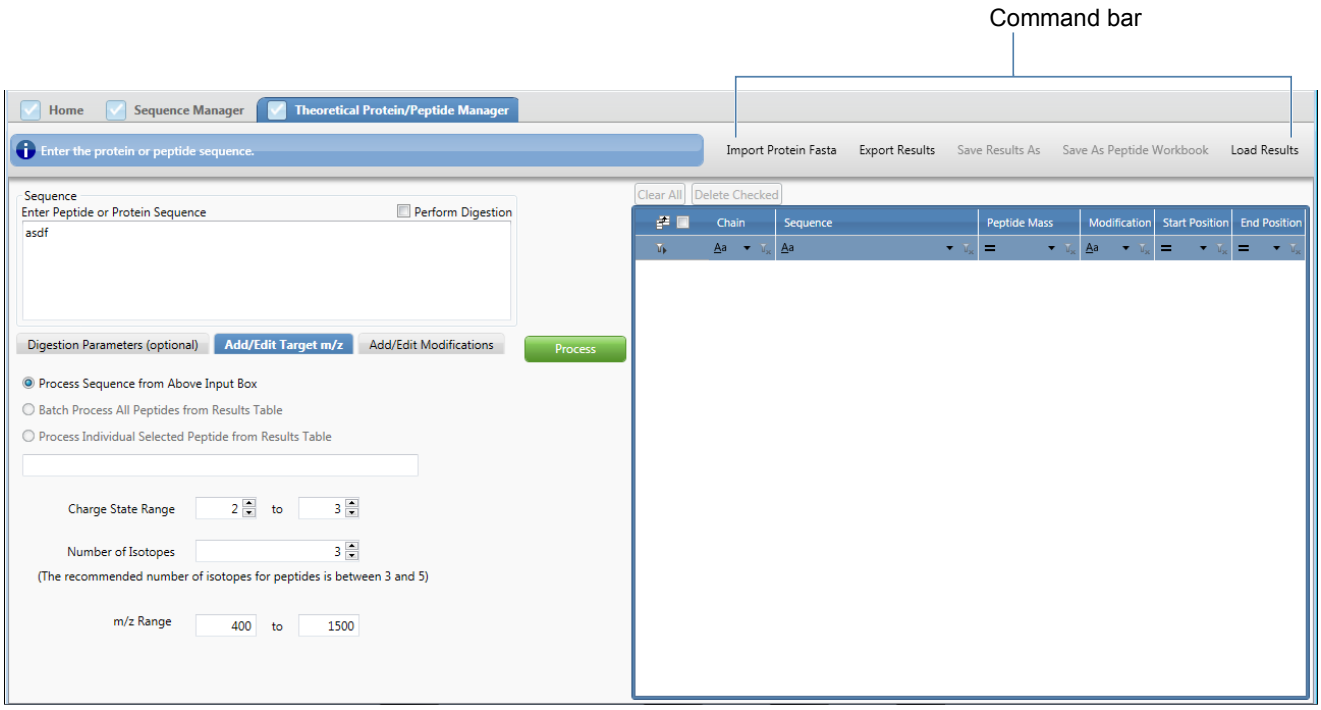


Figure 28 Theoretical Protein/Peptide Manager page

3. Do one of the following:

   - Click **Import Protein Sequence** in the command bar, and then browse to the folder containing the FASTA files.

     Each FASTA file must have the .fasta extension for the application to be able to find it. The dialog box displays all of the FASTA files in the selected folder. Select a FASTA file name to import a protein sequence, and then click **Open**.

     Note: You can import FASTA files of 1 MB or less. If a FASTA file contains invalid amino acids or a bad format, an error message informs you.

     The application displays the sequence information from the FASTA file in the Enter Peptide or Protein Sequence box, overwriting any previous content in this box.

   - In the Sequence area, in the Enter Peptide or Protein Sequence box, type the protein or peptide sequence.

     Note: You cannot specify both proteins and peptides at the same time; however, you can enter them in two separate sessions.

     Follow these FASTA rules for specifying proteins:

     – Use the approved amino acid alphabet.

     – Begin each chain with a single-line description. Use a greater-than (>) sign at the start of this line to distinguish the chain description from the sequence data. Use no space between the > and the first letter of the identifier in the description, for example:

       ```
       >Rituximab -LC
       ```

     – Follow the description with lines of sequence data, for example:

       ```
       QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYAT
       SNLASGVPVRFSG SGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGG
       GTKLEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWK
       VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTH
       QGLSSPVTKSFNRGECHHEAELKPLAQSHATKHKIPIKYLEFISDAIIHV
       LHSKH PGNFGADAQGAMTKALELFRNDIAAKYKELGFQG
       ```

     – The chain ends when another line starting with > appears, indicating the start of another chain.

     Follow these rules for specifying peptides:

     – Use the approved amino acid alphabet.

     – Use a contiguous sequence of characters to define each peptide.

     – Enter multiple peptides on separate lines.

   –or–

   - In Windows™ Explorer, open a file containing a protein or peptide sequence and paste the sequence into the Enter Peptide or Protein Sequence box.

   Note: You can manually edit the imported or entered sequence.

4. If you want to perform digestion on the protein, select the **Perform Digestion** check box.

Choosing digestion parameters

When you select the Perform Digestion check box at the top right of the Sequence area of the Theoretical Protein/Peptide Manager page for a sequence, the application activates the Digestion Parameters (optional) tab for you to choose the protease to apply to the digestion, along with other peptide constraints. The application uses these parameters to predict the theoretical peptide fragments when you process the sequence.

To choose the digestion parameters

1. On the Theoretical Protein/Peptide Manager page, input a protein or peptide sequence.
2. Select the **Perform Digestion** check box.
3. Click the **Digestion Parameters (optional)** tab.
   The Digestion Parameters (optional) pane opens.

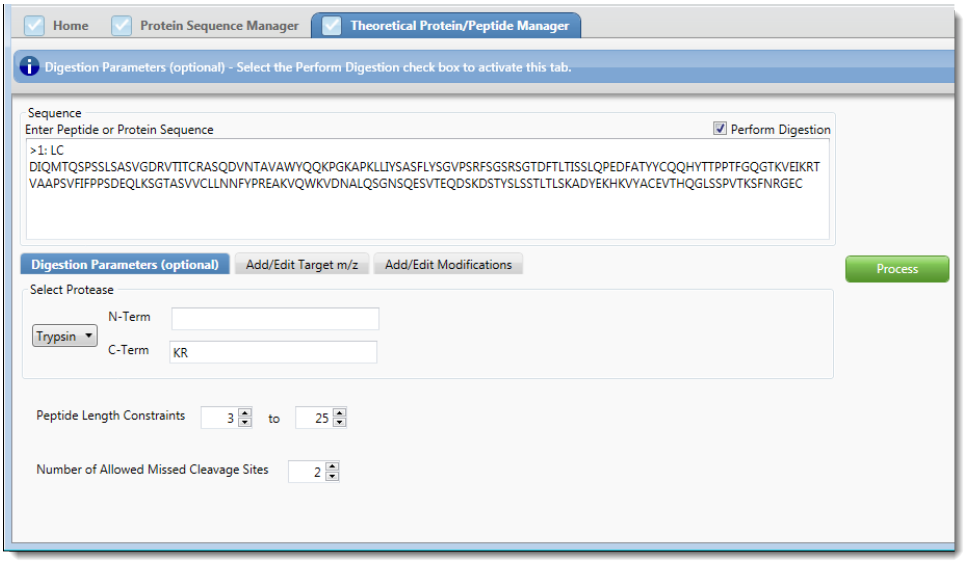


Figure 29 Digestion Parameters (optional) pane

4. In the Select Protease area, select a protease to apply to the digestion.
   The activities at the N-terminus and C-Terminus for the selected protease appear in the N-Term and C-Term boxes but are not editable. This information indicates where the protease will cleave during digestion.
   To add a new protease or edit the protease information, use the Parameters > Identification page for Peptide Mapping Analysis.

5. In the Peptide Length Constraints boxes, specify the lower and upper values for the range. The application displays in the Results table only the peptides that fit the specified length.

6. In the Number of Allowed Missed Cleavage Sites box, specify the maximum number of internal cleavage sites within a peptide fragment that a protease enzyme can miss during the digestion process.

   The enzymatic digestion process does not always result in all of the available cleavage sites in a protein being cleaved; therefore, it is important to specify the number of missed cleavage sites that can be present in a peptide fragment where the enzyme could have cleaved but did not.

   Specifying 0 means that the application considers the enzyme to have efficiently cleaved at all the possible cleavage sites in a protein with 100 percent specificity.

Editing target *m/z* parameters

To apply the transitions to the mass values for predicting theoretical peptides, you can specify the charge state and *m/z* ranges, as well as the maximum number of isotopes.

To edit the target *m/z* parameters

1. On the Theoretical Protein/Peptide Manager page, click the **Add/Edit Target m/z** tab.

   The Add/Edit Target m/z pane opens.

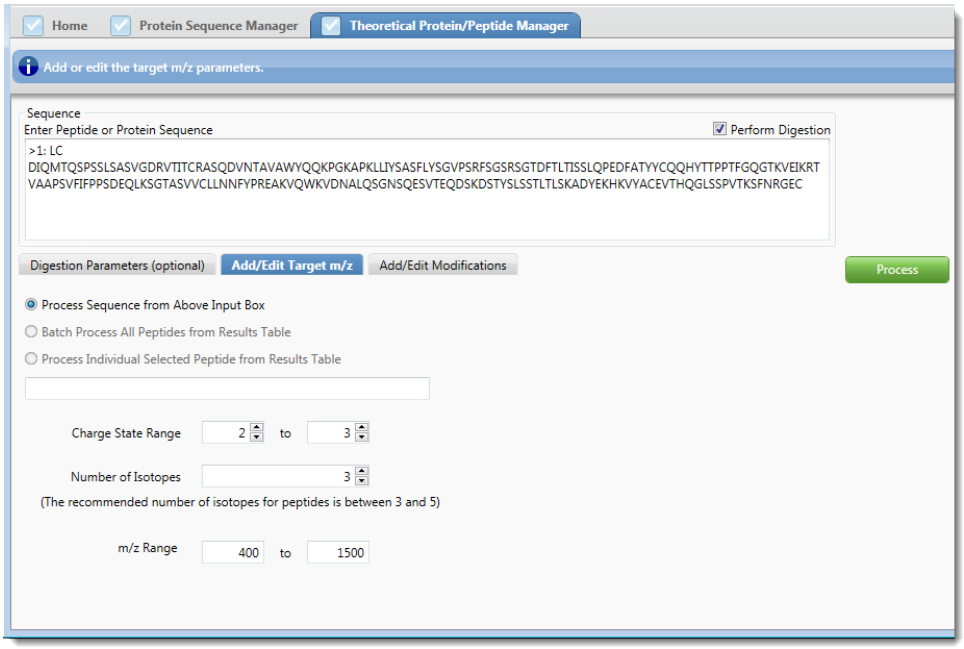


Figure 30 Add/Edit Target m/z pane

2. Select one of the processing mode options:
   - (default) **Process Sequence from Above Input Box**

     For this option, when you process the transitions, all of the target *m/z* parameters apply *globally* to the original protein or peptide sequence in the Sequence area of the Theoretical Protein/Peptide Manager page.
   - **Batch Process All Peptides from Results Table**

     IMPORTANT! To enable this option, process the transitions at least once to generate the peptides in the Results table on the right side of the Theoretical Protein/Peptide Manager page.

     For this option, when you process the transitions again, all of the target *m/z* parameters apply *globally* to all of the processed peptides in the Results table.

   –or–
   - **Process Individual Selected Peptide from Results Table**

     IMPORTANT! To enable this option, after processing the transitions at least once, select one peptide row in the Results table on the right side of the Theoretical Protein/Peptide Manager page.

     This action switches from the other options to the Process Individual Selected Peptide from Results Table option and displays the selected peptide in the adjacent box (see the following figure). This box is not editable.

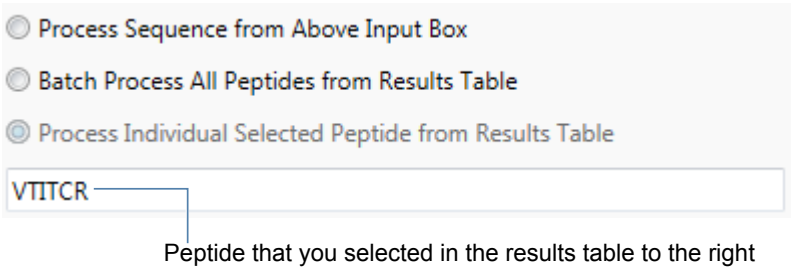


Figure 31 Process individual selected peptide mode

For this option, when you process the transitions again, all of the target *m/z* parameters apply *locally* to only the selected peptide.

3. In the Charge State Range boxes, specify the lower and upper values for the range of charge states to be used for the prediction.

4. In the Number of Isotopes box, specify the number of isotopes that you want returned from the prediction of each charge state.

   Tip: To ensure that the processed results are compatible with the Chromeleon data system, limit the range of isotopes to between three and five.

5. In the m/z Range boxes, type the lower and upper values for the *m/z* range for the prediction.

Adding and editing modifications

You can process transitions either globally to all peptides or locally to one. To do so, add new modifications to the amino acids in the protein or peptide, or edit an existing modification.

To specify global or local modifications

1. On the Theoretical Protein/Peptide Manager page, click the **Add/Edit Modifications** tab.

   The Add/Edit Modifications pane opens.

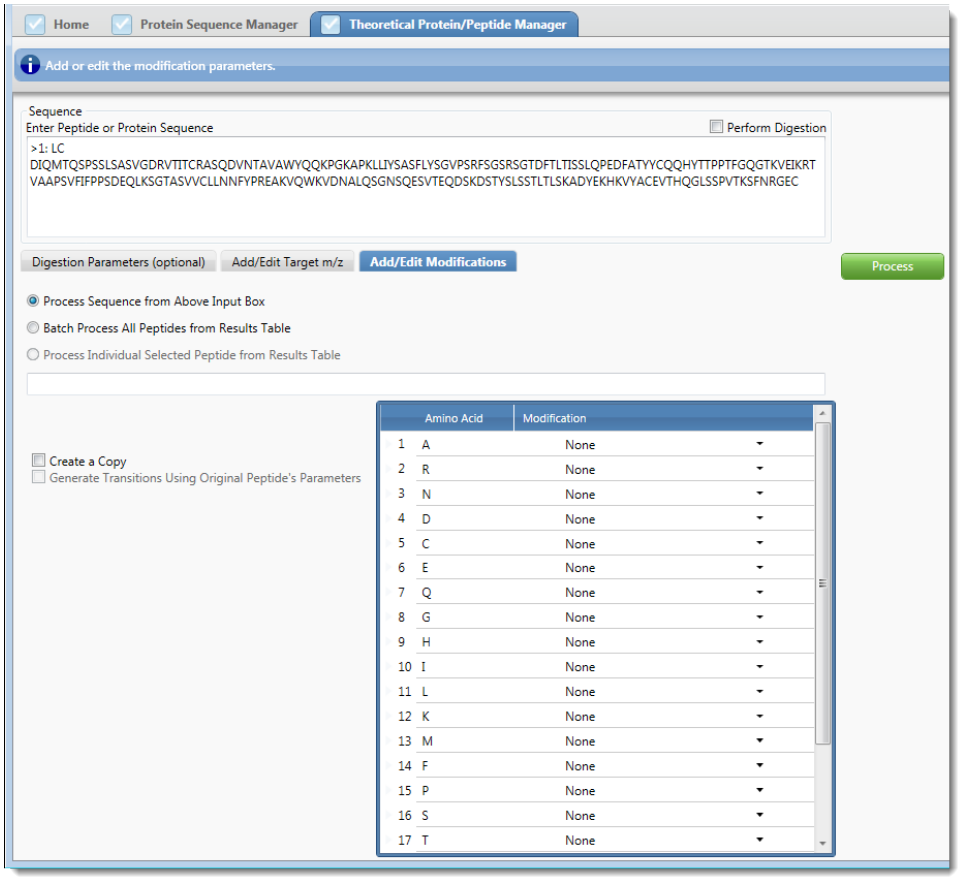


Figure 32 Add/Edit Modifications pane

2. Select a processing mode option: Process Sequence from Above Input Box, Batch Process All Peptides from Results Table, or Process Individual Selected Peptide from Results Table.

   When you process the transitions, the modification parameters apply *globally* to the original protein/peptide sequence that was entered, *globally* to all of the processed peptides in the Results table, or *locally* to one selected peptide in the Results table, depending on the selected mode.

Note: Local modifications overwrite previous global modifications. Global modifications do *not* overwrite previous local modifications.

3. In the modifications table, select a modification for terminals or amino acid letters, as needed.

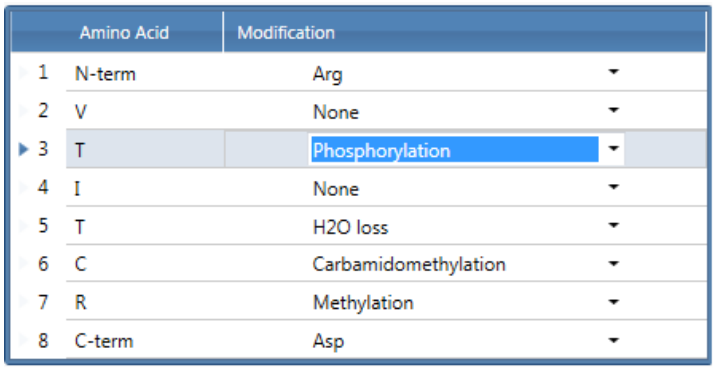
   By convention, the peptides are written from the N-terminal to the C-terminal. The N-terminal refers to the free amine group of the first amino acid. The peptide terminates with a free carboxylic group of the last amino acid, which is the C-terminal.

   For the first two processing modes (Process Sequence from Above Input Box or Batch Process All Peptides from Results Table), the modifications table displays all amino acid letters and then the N-terminal and C-terminal. The letters appear in the order listed in the amino acids table.

	Amino Acid	Modification
1	A	None
2	R	Methylation
3	N	None
4	D	Amidation
5	C	None
6	E	None
7	Q	None
8	G	None
9	H	None
10	I	None
11	L	None
12	K	Acetylation
13	M	None
14	F	None
15	P	None
16	S	H2O loss
17	T	None
18	W	None
19	Y	None
20	V	None
21	N-term	Lys
22	C-term	Glu

Figure 33 Modifications table for the first two processing modes

For the third mode (Process Individual Selected Peptide from Results Table), the modifications table displays the N-terminal, all amino acid letters in the selected peptide, and then the C-terminal. The letters appear in the order that they show up in the selected peptide. If the selected peptide already contains some modifications, these modification appear in the modifications table.



	Amino Acid	Modification
1	N-term	Arg
2	V	None
3	T	Phosphorylation
4	I	None
5	T	H2O loss
6	C	Carbamidomethylation
7	R	Methylation
8	C-term	Asp

Figure 34 Modifications table for the last processing mode for peptide VTITCR

In the Modification column of the table, a dropdown list displays all available modifications for each terminal or letter.

For the first two modes, each selected modification applies to *all* instances of the corresponding letter in the original sequence or processed peptides. For the third mode, each specified modification applies to only *one* instance of the corresponding letter in the selected peptide.

4. Select the **Create a Copy** check box, the **Generate Transitions Using Original Peptide's Parameters** check box (third mode only), or both (third mode only).
   - For the first two modes
     - Select the **Create a Copy** check box to create and retain an original, unmodified version for all peptides, and then apply the specified modifications to a new copy of each of these peptides.
     - Clear the **Create a Copy** check box to overwrite all current peptides with the specified modifications.
   - For the third mode
     - Select the **Create a Copy** check box to save a version of the currently selected peptide and then apply the specified modifications to a new copy of this peptide.
     - Clear the **Create a Copy** box to overwrite the currently selected peptide with the specified modifications.
     - Select the **Generate Transitions Using Original Peptide's Parameters** check box to generate the transitions for the selected peptide using the parameter settings applied to its original version, not from any current parameter settings.
     - Clear the **Generate Transitions Using Original Peptide's Parameters** check box to generate the transitions for the selected peptide using the current parameter settings.

Managing the processed results

When you are done specifying the digestion, target *m/z*, and modification parameters, you can then process the digestion using the specified protease and constraint information, apply the target *m/z* settings for the transitions, apply the specified modifications, and view the processed peptides in the Results table to the right of the Theoretical Protein/Peptide Manager page.

For proteins, the Results table shows the predicted list of peptides generated by the enzymatic cleavage process. For peptides, the Results table displays all peptide sequences as user-created.

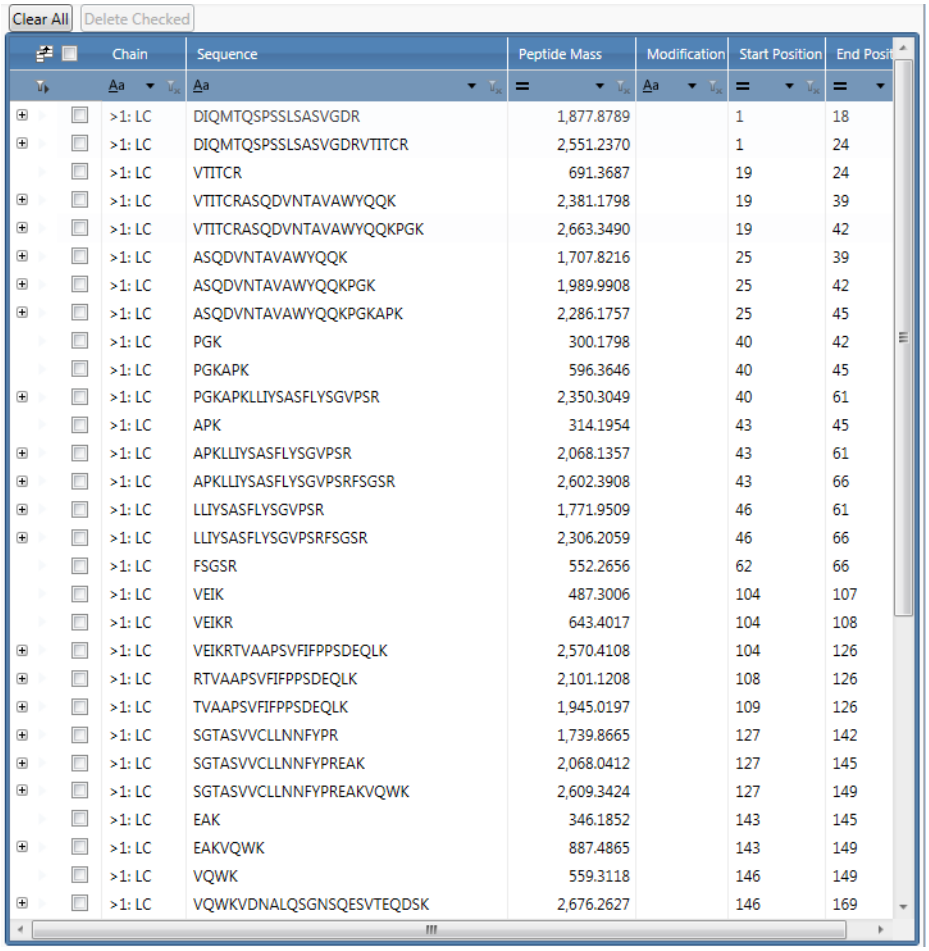
For more details, see the following topics:

- Viewing the processed results (page 96)
- Modifying the results display (page 99)
- Saving the processed results (page 99)
- Opening previously saved results (page 99)
- Exporting the processed results (page 100)
- Saving the processed results to a workbook (page 100)

Viewing the processed results

To view the processed results

1. On the Theoretical Protein/Peptide Manager page, click **Process**.
   The Results table displays the processed peptide information.



Clear All | Delete Checked

Chain	Sequence	Peptide Mass	Modification	Start Position	End Position
>1: LC	DIQMTQSPSSLSASVGDR	1,877.8789		1	18
>1: LC	DIQMTQSPSSLSASVGDRVTITCR	2,551.2370		1	24
>1: LC	VTITCR	691.3687		19	24
>1: LC	VTITCRASQDVNTAVAWYQQK	2,381.1798		19	39
>1: LC	VTITCRASQDVNTAVAWYQQKPGK	2,663.3490		19	42
>1: LC	ASQDVNTAVAWYQQK	1,707.8216		25	39
>1: LC	ASQDVNTAVAWYQQKPGK	1,989.9908		25	42
>1: LC	ASQDVNTAVAWYQQKPGKAPK	2,286.1757		25	45
>1: LC	PGK	300.1798		40	42
>1: LC	PGKAPK	596.3646		40	45
>1: LC	PGKAPKLLIYSASFLYSGVPSR	2,350.3049		40	61
>1: LC	APK	314.1954		43	45
>1: LC	APKLLIYSASFLYSGVPSR	2,068.1357		43	61
>1: LC	APKLLIYSASFLYSGVPSRFSGSR	2,602.3908		43	66
>1: LC	LLIYSASFLYSGVPSR	1,771.9509		46	61
>1: LC	LLIYSASFLYSGVPSRFSGSR	2,306.2059		46	66
>1: LC	FSGSR	552.2656		62	66
>1: LC	VEIK	487.3006		104	107
>1: LC	VEIKR	643.4017		104	108
>1: LC	VEIKRTVAAPSVFIFPPSDEQLK	2,570.4108		104	126
>1: LC	RTVAAPSVFIFPPSDEQLK	2,101.1208		108	126
>1: LC	TVAAPSVFIFPPSDEQLK	1,945.0197		109	126
>1: LC	SGTASVVCLLNNFYPR	1,739.8665		127	142
>1: LC	SGTASVVCLLNNFYPREAK	2,068.0412		127	145
>1: LC	SGTASVVCLLNNFYPREAKVQWK	2,609.3424		127	149
>1: LC	EAK	346.1852		143	145
>1: LC	EAKVQWK	887.4865		143	149
>1: LC	VQWK	559.3118		146	149
>1: LC	VQWKVDNALQSGNSQESVTEQDSK	2,676.2627		146	169

Figure 35 Results table

You can access three levels of resulting data:

- The top level shows the peptide sequence information.
- Click the expand icon, ⊞, if available to the left of a top-level sequence row, to view the second level with precursor information related to the selected sequence.
- Click the expand icon, ⊞, if available to the left of a second-level precursor row, to view the third level with isotope information related to the selected precursor.

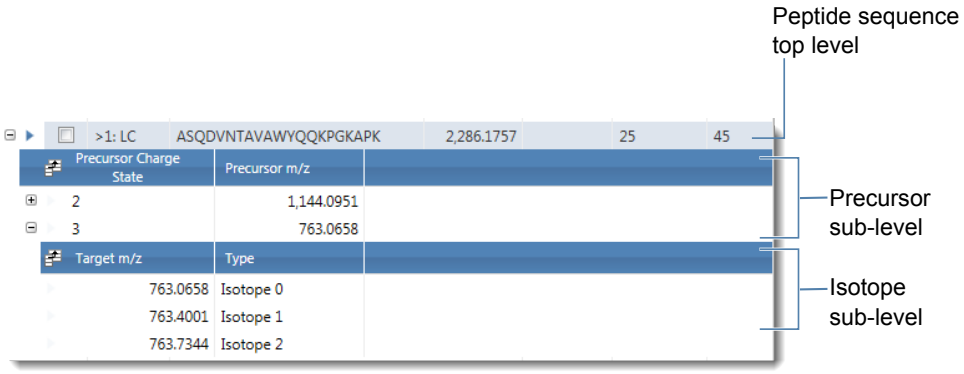


Figure 36 Results table showing the three levels

2. Select a sequence row at the top level to switch the processing mode to the Process Individual Selected Peptide from Results Table option. This mode applies the specified parameters only to the selected peptide sequence when processing.

3. Edit the digestion, target *m/z*, and modification parameters as needed and then click **Process** again.

The following occurs in the Results table, depending on the processing mode you select:

- Process Sequence from Above Input Box: The new processed results appear at the bottom of the Results table.
- Batch Process All Peptides from Results Table: Newly processed data replaces all of the data in the Results table.
- Process Individual Selected Peptide from Results Table:
  - If you change the target *m/z* parameters, newly processed data replaces the selected peptide in the Results table.
  - If you change the modification parameters, the application processes the selected peptide in the Results table as follows:
    - If you do *not* select the Create a Copy option, the modified peptide replaces the original peptide.
    - If you select the Create a Copy option, the modified peptide appears right below the copy of the original peptide.

Modifying the results display

To modify the display of the results

- Click **Clear All** above the Results table of the Theoretical Protein/Peptide Manager page to clear the entire table.

–or–

- Select the check box for one or more top-level rows in the Results table and then click **Delete Checked** above the table to delete the selected rows.

  To select all of the rows, select the check box in the column header.

Saving the processed results

To save the processed results

1. Click **Save Results As** in the command bar of the Theoretical Protein/Peptide Manager page.

   Note: The application deactivates this command until processed results are available in the Results table.

2. In the Save As dialog box, enter the name for the file to be saved, browse to the appropriate folder location as needed, and then click **Save**.

   The application saves the processed results in the table to an XML file with the .msqc extension. The saved data retains your check box selections for export/deletion but does not retain any filtering options. If a file already exists and you use the same file name, the current results overwrite the previously saved results in that file.

Opening previously saved results

To open previously saved results

1. Click **Load Results** in the command bar of the Theoretical Protein/Peptide Manager page.

2. In the Open dialog box, browse to the .msqc file containing the previously saved results that you want to view, and then click **Open**.

   If the Results table is currently empty, the application retains all current parameter settings and displays the saved results from the file in the Results table.

   Otherwise, if the Results table contains data, the application prompts you to confirm overwriting the current data with previously saved data from the file. If you confirm this action, the application clears the Enter Peptide or Protein Sequence box and resets all parameters on the Theoretical Protein/Peptide Manager page to their default values before displaying the saved results in the Results table.

Exporting the processed results

To export the processed results

Click **Export Results** in the command bar of the Theoretical Protein/Peptide Manager page and then choose from these options:

- **Export All** to export all results in the Results table to a BPF file.

–or–

- **Export Checked** to export only the *selected* results in the table to a BPF file.
  To select a row of results to export, select the check box in that row.
  To select all of the rows, select the check box in the column header.

The exported information is in a format that is compatible with the Chromeleon data system.

Note: You can change the extension of the exported file to .csv to open it in a Microsoft™ Excel™ spreadsheet.

Saving the processed results to a workbook

You can save the results to a peptide workbook to use as a protein sequence for a targeted peptide search. You can also export the workbook data to a file compatible with the Chromeleon data system.

To save the processed results to a workbook

1. (Optional) On the Theoretical Protein/Peptide Manager page, in the Results table, select the check box in the row of each peptide that you want to save to the workbook.
   To select/deselect all of the rows, select/clear the check box in the column header.

2. Click **Save as Peptide Workbook** in the command bar of the Theoretical Protein/Peptide Manager page and then choose from these options:
   - **All** to save all of the rows in the table to a workbook.

   –or–

   - **Checked** to save only the *selected* rows in the table to a workbook.

   IMPORTANT! The application does not support saving peptides with unspecified modifications, dimers, adducts, disulfide bonds, or any type of gas phase modifications to the workbook. You must clear the check boxes for these peptides to save the workbook.

   ---

3. In the Save Peptide Workbook As dialog box, do the following:
   a. Select one of these options: **Create a New Peptide Workbook** or **Select an Existing Workbook**.

b. (For the Create a New Peptide Workbook option) In the Workbook Name box, type a new name to create a new workbook or keep the same name to overwrite the workbook if it already exists.

   The default workbook name is the same as the experiment name.

   Note: Use only alphanumeric, space, underscore "_", and period "." characters in the workbook name.

   –or–

   (For the Select an Existing Workbook option) Select the check box for an existing workbook in the table.

   The application adds data from your selection of peptides to that workbook.

c. (Optional) In the Description box, type a description for the workbook.

The number of isotopes for the saved results appears in the dialog box but is not editable.

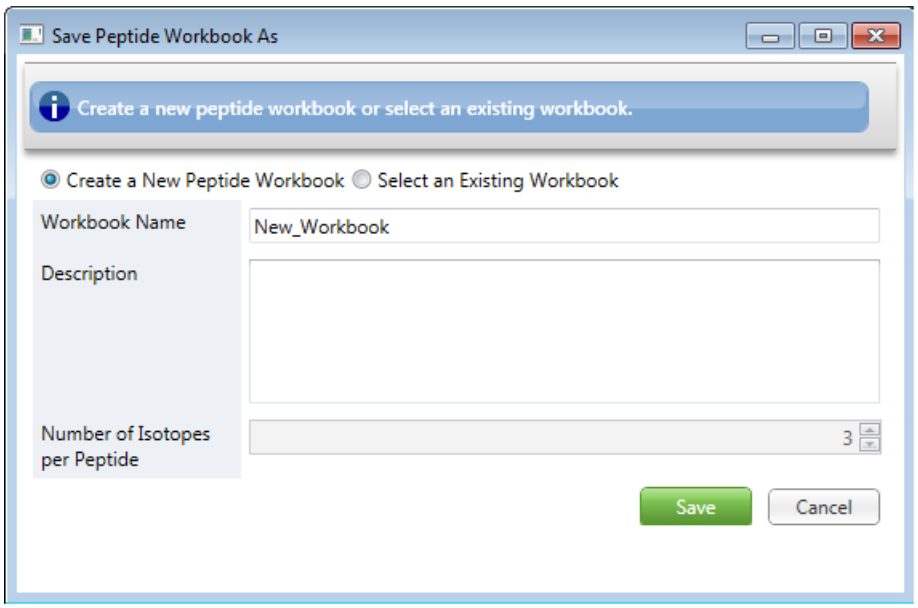


Figure 37 Save Peptide Workbook As dialog box

4. Click **Save**.

   Note: If the workbook is currently open on the Workbook Editor page for editing, indicate whether you want the application to automatically save and close the open workbook before proceeding.

   The application does not save peptides without charge states to the workbook.

   If duplicate peptides are present in the Results table, the application saves only one copy to the workbook. The application automatically determines the data to save for the identification, peptide sequence, site of modification, and relative quantitation group number from the available results data.

If you are creating a new workbook, the application automatically assigns a relative quantitation group number to all workbook entries with the same protein and sequence, starting with group number "1" and incrementing by one for each new group.

If you are adding to an existing workbook, the added entries with the same protein and sequence as existing entries in the workbook receive the same group number as the existing entries. All other entries receive incremented group numbers.

After saving, you can manage the workbook using the Target Peptide Workbook page.

Amino acid letter codes

The following table lists the single-letter codes for amino acids. You can also enter lowercase characters.

Table 13 Amino acid letter codes

Amino acid	Description
A	alanine
R	arginine
N	asparagine
D	aspartic acid
C	cysteine
E	glutamic acid
Q	glutamine
G	glycine
H	histidine
I	isoleucine
L	leucine
K	lysine
M	methionine
F	phenylalanine
P	proline
S	serine
T	threonine

Amino acid	Description
W	tryptophan
Y	tyrosine
V	valine

Results table parameters

The following table describes the columns in the Results table of the Theoretical Protein/Peptide Manager page.

Table 14 Results table parameters

Column	Description
Sequence level	
+/–	Click to show or hide the lower level of precursor information related to the current sequence row.
☐	Select this check box to delete or export only the selected rows in the table.
Chain	Displays the chain identifier for the peptide sequence.
Sequence	Displays the amino acid letters and modifications in the peptide sequence. The modifications are surrounded by brackets, "[" and "]", and appear to the right of the affected amino acid letter. If there are multiple modifications for one letter, the N-terminal or C-terminal modification appears first, followed by the side chain modification.
Peptide Mass	Displays the mass of the peptide.
Modification	Displays the modifications applied to the peptide. If there is no modification, this cell is empty.
Start Position	Displays the start position of the peptide in the protein sequence where digestion is performed. **Note:** If you select the Create a Copy check box to create a copy of the peptide, the application copies the start position from the original unmodified peptide to display here.
End Position	Displays the end position of the peptide in the protein sequence where digestion is performed. **Note:** If you select the Create a Copy check box to create a copy of the peptide, the application copies the end position from the original unmodified peptide to display here.
Precursor level	
+/–	Click to show or hide the lower level of isotope information related to the current precursor row.

Column	Description
Precursor Charge State	Displays the charge state number for the precursor. The table displays only the charge states within the range specified in the Charge State Range boxes.
Precursor m/z	Displays the precursor mass-to-charge ratio. The table displays only the m/z values within the range specified in the m/z Range boxes.
Isotope level **The maximum number of isotopes is from the Number of Isotopes parameter setting.**	
Target m/z	Displays the target mass-to-charge ratio of an isotope.
Type	Displays the isotope type.

6 Oligonucleotide Sequence Editor

Use the Oligonucleotide Sequence Editor to create and edit oligonucleotide sequences. You can open this editor from the Sequence Manager page.

Creating and editing oligonucleotide sequences

In the Sequence Manager, you can import an existing BioPharma sequence file (.sequencebpf), and then open the sequence in the Oligonucleotide Sequence Editor for editing.

In the Oligonucleotide Sequence Editor, you can edit the existing sequence that you selected in the Sequence Manager. Or, you can create a new sequence in two ways:

- By importing a FASTA file that contains the sequence chains of interest

  Tip: The application cannot import the contents of a FASTA file that contains undefined oligo building blocks or an invalid format. Before you open an unknown FASTA file, review its contents in a text editor such as Notepad. If the FASTA file includes undefined building blocks, use the Oligo Building Block editor to create them before you import the file.

- By manually entering the sequence

After you enter the sequence and select its category, you can add static modifications to it and assign variable modifications to search against.

For details, see these topics:

- “Creating a new oligonucleotide sequence” on page 106
- “Opening a FASTA file to verify or copy its contents” on page 109
- “Importing an oligonucleotide sequence from a FASTA file” on page 110
- “Creating mass-only or formula-only oligo sequences” on page 112
- “Assigning custom building blocks to an oligo sequence by using the Edit Sequence pane” on page 114

- “Assigning variable modifications for oligonucleotide sequences” on page 124
- “Oligonucleotide Sequence Editor parameters” on page 129

Creating a new oligonucleotide sequence

The following procedure describes how to create a new oligonucletide sequence by populating the Oligo Sequence Map area with manual input (by typing the text) or the sequence information from a FASTA file. For information about creating mass-only or formula-only sequences, see “Creating mass-only or formula-only oligo sequences” on page 112.

To create a new oligonuleotide sequence

1. On the Home page, click **Sequence Manager** in the left pane.

   The Sequence Manager page opens showing the sequence table, which contains a list of existing sequences that you can use for various experiments depending on the sample type and category.

2. To add a new sequence, click **New Oligonuceotide** to the right of the sequence table.

   The Oligonucleotide Sequence Editor opens with the Manual Input Sequence pane expanded for input at the right.

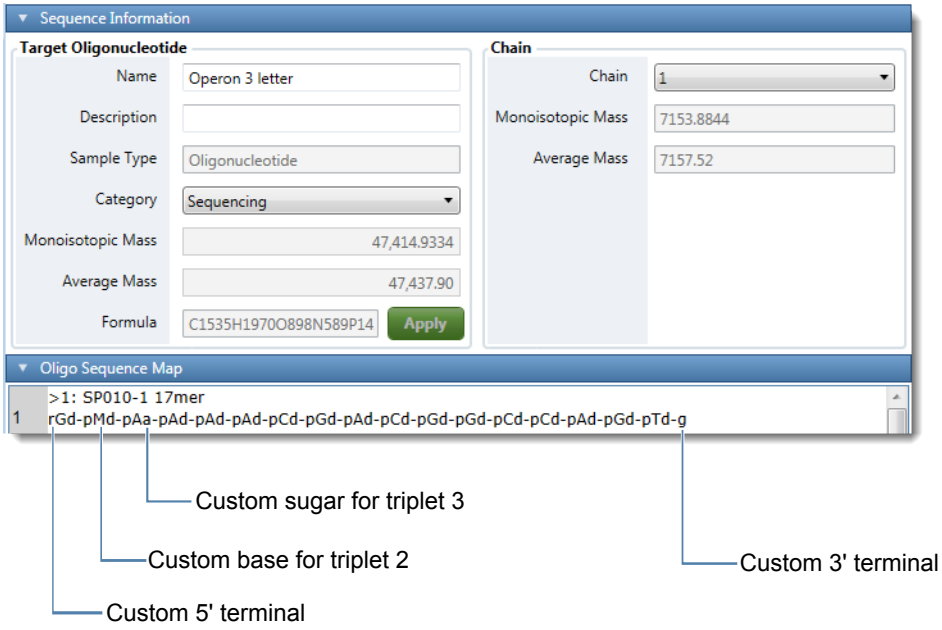


Figure 38 Oligonucleotide Sequence Editor

3. (Optional) To populate the Oligo Sequence Map area with the sequence information in a FASTA file, follow the instructions in "Importing an oligonucleotide sequence from a FASTA file" on page 110. Then, go to the next step.

   IMPORTANT! Importing a sequence from a FASTA file overwrites the current sequence information in the Sequence Information area of the Oligonucleotide Sequence Editor. After you import the contents of a FASTA file, you can rename the sequence, select a different category, add more chains to the sequence map, and so on.

4. In the Sequence Information pane, name the sequence and select its category—**Sequencing** or **Intact Deconvolution**—from the dropdown Category list.

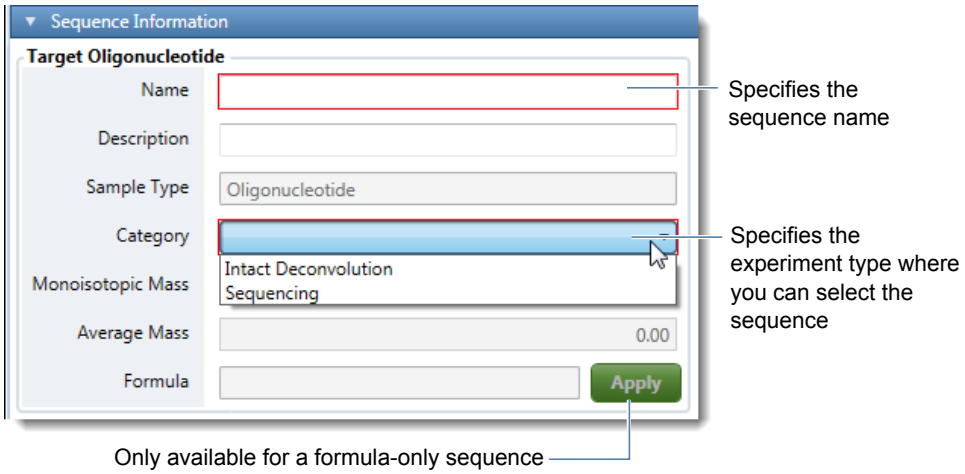


Figure 39 Sequence Information pane

Oligo sequences in the Intact Deconvolution category appear in the Sequence table on the Intact Mass Analysis page and oligo sequences in the Sequencing category appear in the Oligonucleotide Sequence table on the Oligonucleotide Analysis page.

5. In the Manual Input Sequence pane, do the following for each chain that you want to add to the sequence:
   a. In the Chain Name box, type a name for the chain.

b. Type or paste the chain information in the box below the Chain Name box. For instructions on how to copy the chains from a FASTA file, see “Opening a FASTA file to verify or copy its contents” on page 109.

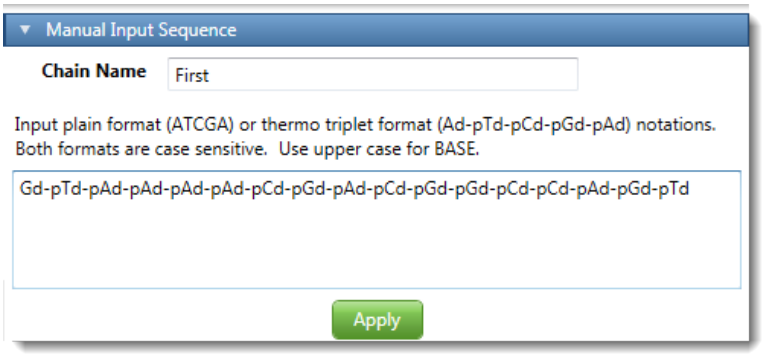


Figure 40 Manual Input Sequence pane

c. Click **Apply**.

The application adds the chain to the Oligo Sequence Map pane.

Figure 41 Oligo Sequence Map pane

6. (Optional) To edit the oligo building blocks in each sequence chain, follow the instructions in “Assigning custom building blocks to an oligo sequence by using the Edit Sequence pane” on page 114.

Tip: To define custom building blocks for an oligo sequence, see “Creating custom oligo building blocks” on page 121.

7. (Optional) To assign variable modifications to the sequence, follow the instructions in “Assigning variable modifications for oligonucleotide sequences” on page 124.

Tip: To define custom variable modifications for an oligonucleotide sequence, see “Creating custom oligo variable modifications” on page 120.

8. To save the sequence, click **Save**.

Opening a FASTA file to verify or copy its contents

You can copy the oligonucleotide chains in a FASTA file into the Manual Input Sequence area of the Oligonucleotide Sequence Editor one-by-one, or you can import the entire contents of the file into this area.

This procedure describes how to open a FASTA file for an oligo sequence, verify its content, and copy the chains of interest into the Manual Input Sequence area.

To open a FASTA file and copy all or part of its content into the Oligonucleotide Sequence Editor

1. Using Windows Explorer, browse to the FASTA file that contains the oligonucleotide sequence of interest.

2. Right-click the file and open it with Notepad or another text editor.

   IMPORTANT! The FASTA file might contain comment lines that begin with the greater-than sign (>) to distinguish each chain, as shown in the following figure. When you copy the chain information, do not include the comment lines.

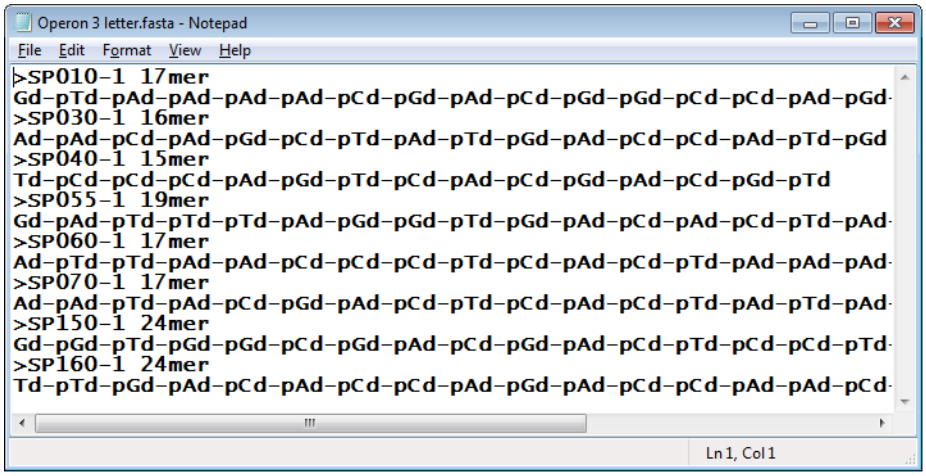


Figure 42 FASTA file format

3. Check whether any of the chains that you want to copy include undefined oligo building blocks.

   If a chain contains undefined building blocks, define them as described in “Creating custom oligo building blocks” on page 121.

   IMPORTANT! Before you can copy a chain with an undefined oligo building block to the editor, you must first define the building block by using the Oligo Building Block editor.

4. If the Oligonucleotide Sequence Editor is not open, open it as follows:
   a. On the Home page, click **Sequence Manager**.
   b. At the right of the sequence table, click **New Oligonucleotide**.

5. For each chain that you want to add to the sequence, do the following:
   a. In the Manual Input Sequence area on the right side of the editor, type a name in the Chain Name box.
   b. In the text editor, copy the chain to the Clipboard.
   c. In the Manual Input Sequence area, place the cursor in the box below the Chain Name box, and then paste the chain to the box.
   d. Click **Apply**.

   The chain appears in the Oligo Map Sequence pane.
6. Name the sequence and select its category.
7. (Optional) Customize the sequence as follows:
   a. Using the Edit Sequence pane, add custom building blocks to the 5' and 3' terminals or replace any of the internal subunits with custom building blocks.“Assigning custom building blocks to an oligo sequence by using the Edit Sequence pane” on page 114
   b. Using the Assign Variable Modifications pane, assign variable modifications to specific subunit sites. See “Assigning variable modifications for oligonucleotide sequences” on page 124

Importing an oligonucleotide sequence from a FASTA file

This procedure describes how to create a new oligonucleotide sequence by importing it from a FASTA file. Importing a sequence from a FASTA file overwrites the current sequence information in the Sequence Information area of the Oligonucleotide Sequence Editor.

To create a new oligo sequence by importing a FASTA file

1. On the Home page, click **Sequence Manager**.

   The Sequence Manager page opens.
2. Click **New Oligonucleotide** on the right side of the page.

   The application displays the Oligonucleotide Sequence Editor and expands the Manual Input Sequence pane to the right.

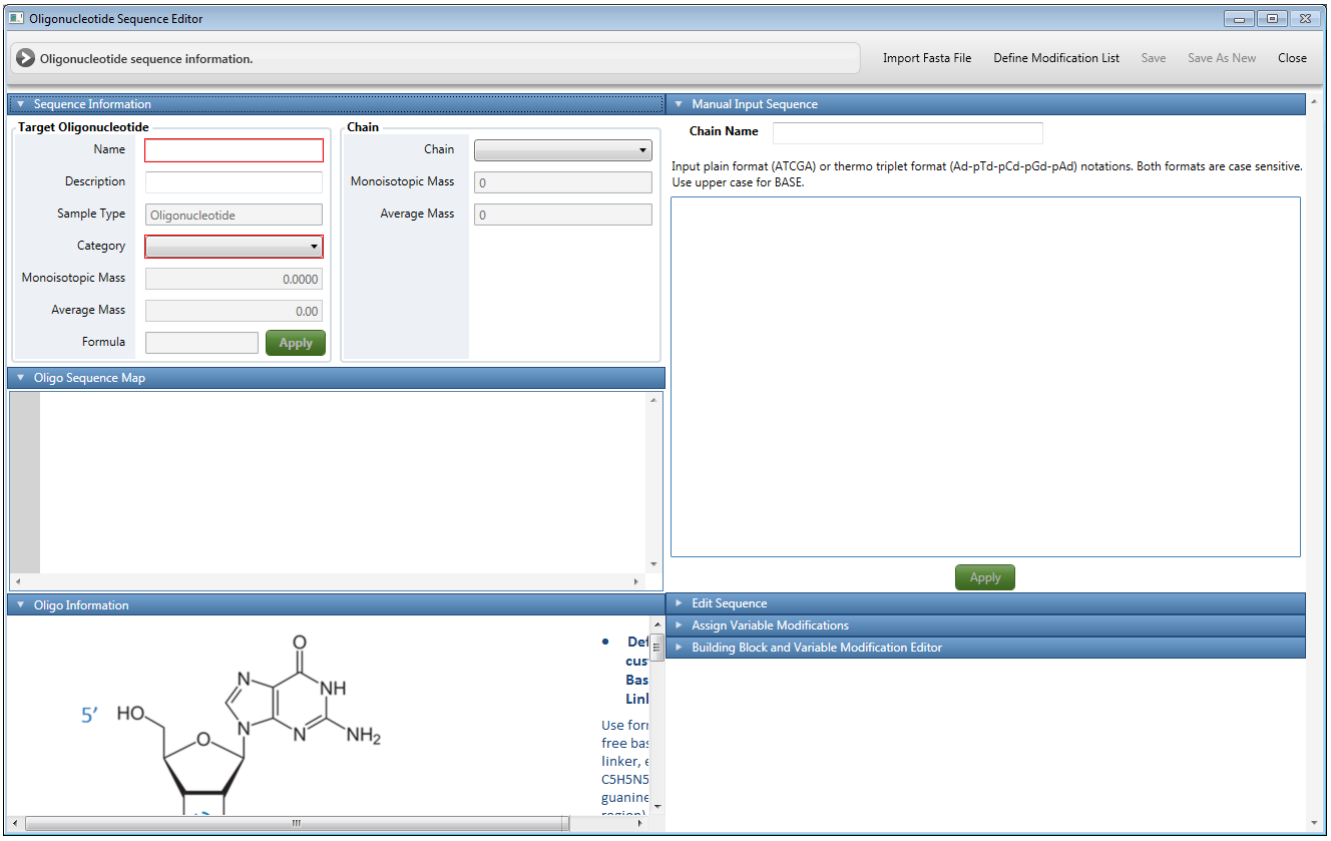


Figure 43 Oligonucleotide Sequence Editor

Note: If the resolution and text size of you computer screen is not set properly, you might not be able to see some of the features on the Oligonucleotide Sequence Editor. To correct this problem, set the screen resolution to 1920 × 1080 pixels and consider changing the text size.

3. To import the sequence information in a FASTA file, click **Import Fasta File** in the command bar at the top, and then browse to the folder containing the FASTA files.

   The FASTA file must have the .fasta extension for the application to be able to find the file.

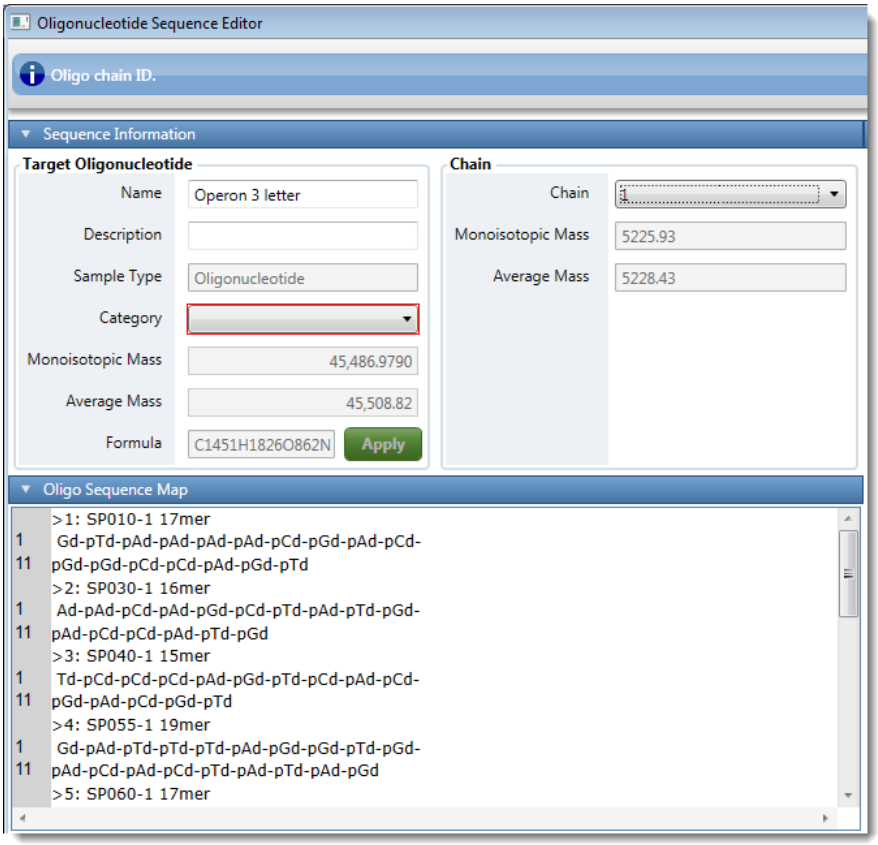
   The dialog box displays all of the FASTA files in the selected folder.

4. Select a FASTA file name to import, and then click **Open**.

   Note: You can import FASTA files of 1 MB or less. If a FASTA file contains undefined oligo building blocks or an invalid format, an error message appears.

   Tip: If an error message appears during the import operation, open the FASTA file in a text editor such as Notepad and verify the format of each chain. If the FASTA file contains undefined oligo building blocks, use the Oligo Building Block editor to define them.

The application displays the oligo sequence information from the FASTA file in the Oligo Sequence Map pane of the Oligonucleotide Sequence Editor. In addition, the Sequence Information pane displays both the monoisotopic and average masses of the sequence in the Target Oligonucleotide area and the monoisotopic and average masses of the first chain in the Chain area. To view the masses of a different chain, select the chain number from the Chain list.



5. Finish editing the sequence as described in “Creating a new oligonucleotide sequence” on page 106.

Creating mass-only or formula-only oligo sequences

You can create mass-only or formula-only oligonucleotide sequences for Intact Mass Analysis. Mass-only sequences are useful when you want the application to identify detected components—for example, impurities— with a known mass. To create a mass-only or formula-only oligo sequence, you must select the Intact Deconvolution category for the sequence.

Note: You cannot assign variable modifications to a mass or formula only sequence.

To create mass or formula only oligonucleotide sequences

1. On the Home page, click **Sequence Manager** in the left pane.

   The Sequence Manager page opens showing the sequence table, which contains a list of existing sequences.

When you start an experiment, the sequences that are compatible with the experiment type appear in the Sequence table for the experiment.

2. To create a new oligo sequence, click **New Oligonucleotide** on the right side of the page.

   The application displays the Oligonucleotide Sequence Editor and by default expands the Sequence Information pane to the left.

3. In the **Name** box, enter a name for the user-specified nucleotide sequence.

4. In the dropdown Category list, select **Intact Deconvolution**.

   Note: The Sample Type field is by default populated with Oligonucleotide.

   Optional: The Monoisotopic Mass and Average Mass fields are editable and new or modified values can be added in these fields.

5. Do one of the following:

   - To create a formula-only sequence, enter the chemical formula of the sequence in the Formula box, and then click **Apply**.
   - To create a mass-only sequence, enter the monoisotopic mass and the average mass of the sequence in the respective boxes.

     Note: The Apply button is not available for a mass-only sequence.

   For a formula-only sequence, the application automatically calculates the monoisotopic mass and average mass of the sequence from the user-specified formula.

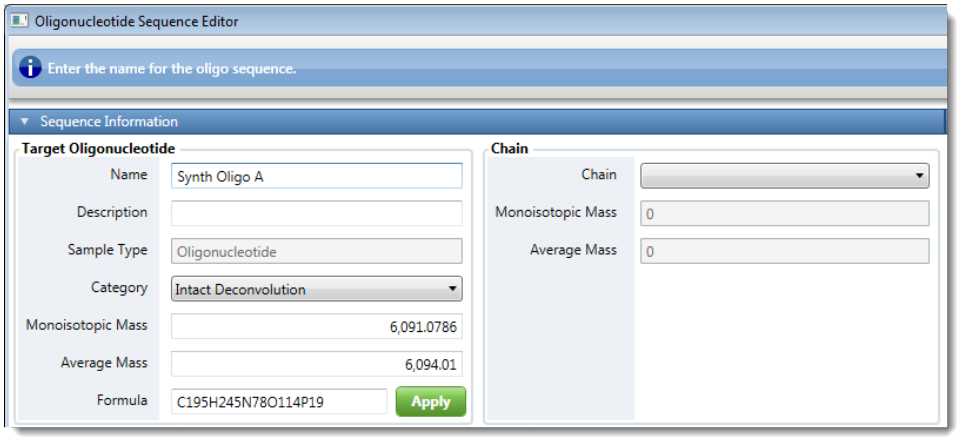


Figure 44 Mass-only sequence

6. To save the sequence, click **Save** in the top right corner of the Oligonucleotide Sequence Editor.

   The new sequence appears on the Sequence Manager page.

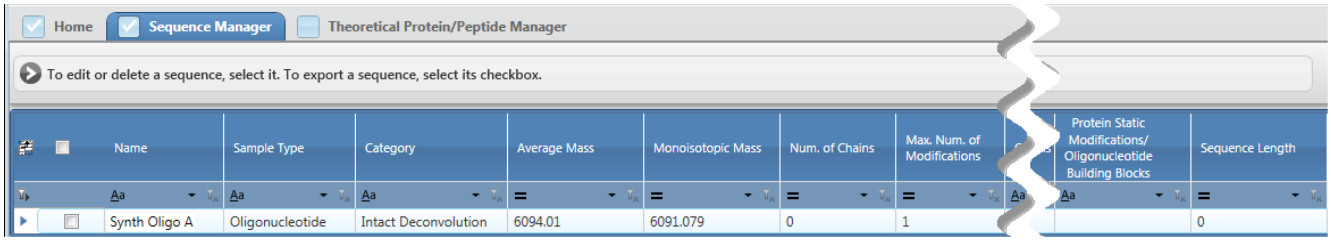


Figure 45 Sequence Manager with a formula-only sequence

When you start an Intact Mass Analysis experiment, the sequence is available in the Sequence Manager table on the Intact Mass Analysis page.

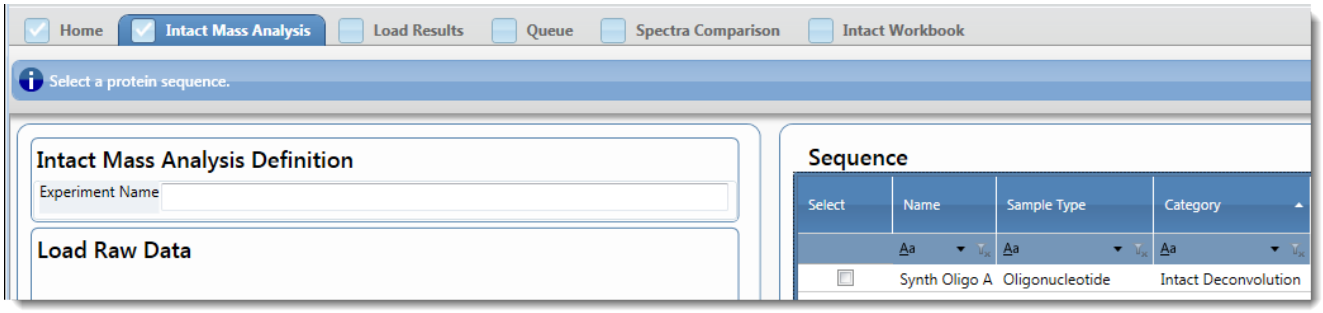


Figure 46 Intact Mass Analysis page

Assigning custom building blocks to an oligo sequence by using the Edit Sequence pane

This procedure describes how to use the Edit Sequence pane of the Oligonucleotide Sequence Editor to replace the bases, backbone linkers, and 2' ribose subunits of an oligonucleotide sequence with custom building blocks or add custom building blocks to the 5' and 3' terminals of the sequence chains. The custom building blocks for the 5' and 3' terminals can add or remove chemical groups from these terminals.

To edit the building blocks of an oligonucleotide sequence

1. Open an existing sequence or create a new sequence in the Oligonucleotide Sequence Editor as follows:
   - To open an existing sequence, select the sequence on the Sequence Manager page, and then click **Edit**.
   - To create a new sequence, follow the instructions in “Creating a new oligonucleotide sequence” on page 106.

   The Oligo Sequence Map area displays all the oligo building blocks for the sequence in read-only mode.

2. On the right side of the Oligonucleotide Sequence Editor, click the **Edit Sequence** title bar to open the Edit Sequence pane.

3. For each chain that you want to modify, do the following:
   a. Select the chain from the Select Chain dropdown list.

b. Select the oligo building blocks that you want to apply and where you want to apply them.

Select a 5' terminal from the dropdown list above the triplet table. Select oligonucleotide building blocks for the triplets by using the dropdown lists in the table. Select a 3' terminal from the dropdown list below the triplet table.

This figure shows the selection of custom building blocks for the 5' terminal of chain 1, the base for triplet 1, the 2' ribose for triplet 3, and the 3' terminal of the chain.

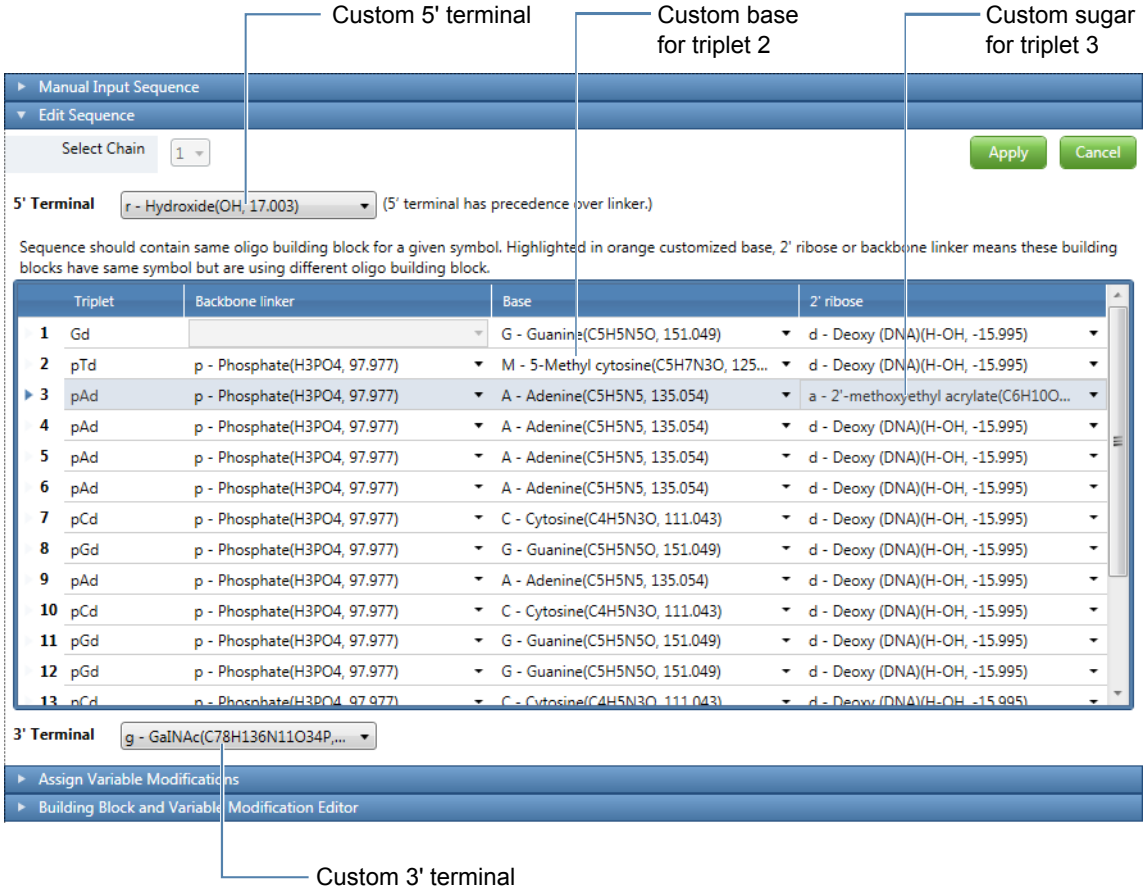


Figure 47 Selections for custom building blocks

c. Click **Apply**.
The updated oligo building blocks appear on the chain in the Oligo Sequence Map pane. The application displays the recalculated masses and the revised formula in the Sequence Information pane.

This figure shows the updated sequence chain in the Oligo Sequence Map pane and its modified mass and formula in the Sequence Information pane.

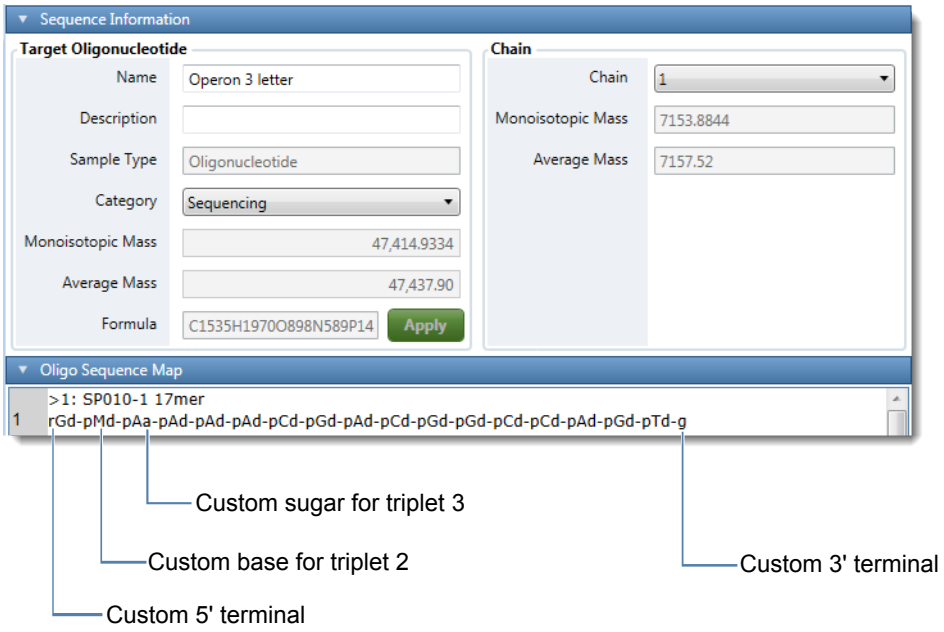


Figure 48 Modified sequence chain

4. Save the modified sequence.

Defining the default modifications for oligonucleotide sequences

Upon installation, the BioPharma Finder™ application provides a default list of variable modifications for oligonucleotide sequences. You can access this list from the Oligonucleotide Sequence Editor and define which modifications appear in the lists of variable modifications in the Assign Variable Modifications pane and which modifications are available for quick loading.

You can add modifications to this list by using the Oligo Variable Modification editor in the Building Block and Variable Modification Editor pane of the Oligonucleotide Sequence Editor.

For details, see the following topics:

- Default sublist of modifications for quick loading (page 59)
- Changing the default and visible sublist of modifications for protein sequences (page 59)

Default sublist of modifications for quick loading in oligonucleotide analysis

Within the default list of modifications, the BioPharma Finder™ application sets the modifications shown in the following table as a default sublist that you can quickly load to assign as 5' terminal, 3' terminal, or oligo variable modifications to an oligonucleotide sequence. You use the Assign Variable Modifications pane to assign these modification.

Table 15 Default sublist of modifications

Modification name	Modification type
Dephosphorylation	5' Terminal (Modification)
Phosphorylation	3' Terminal (Modification)
Adenine loss(A)	Base (Modification)
Uracil loss(U)	Base (Modification)
Cytosine loss(C)	Base (Modification)
Guanine loss(G)	Base (Modification)
Thymine loss(T)	Base (Modification)
Deamination(AC)	Base (Modification)
Oxidation(ACGU)	Base (Modification)
G Depurination(G)	Base (Modification)
U Depyrimidination(U)	Base (Modification)
C Depyrimidination(C)	Base (Modification)
T Depyrimidination(T)	Base (Modification)
I Depurination(I)	Base (Modification)
Acetyl group(AGC)	Base (Modification)
Benzoyl group(AC)	Base (Modification)
Isobutyl group(G)	Base (Modification)
Cnet (ps)	Backbone linker (Modification)
HF loss(f)	2' ribose (Modification)
Sulfur loss(s)	Backbone linker (Modification)
Phosphorothioate conversions(s)	Backbone linker (Modification)

Changing the default and visible sublist of variable modifications for oligo sequences

From the default list of modifications, you can select which modifications will be visible in the Assign Variable Modifications pane. You can also select the items to include in the sublist for quick loading.

To change the visible default list and sublist of variable modifications

1. In the Oligonucleotide Sequence Editor, click **Define Modification List** in the command bar.

   The default list table opens in the Define Modification List dialog box.

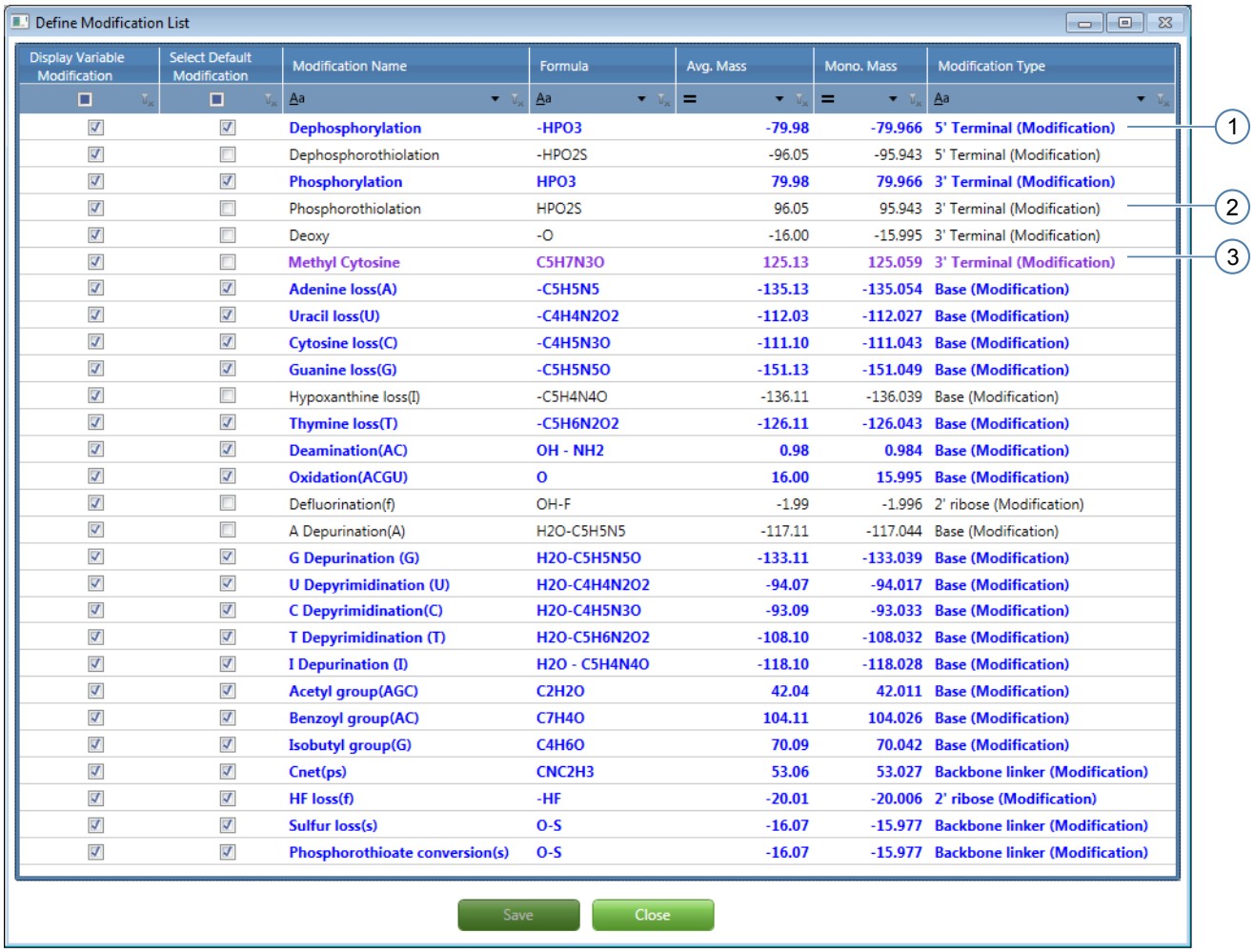


Figure 49 Table in the Define Modification List dialog box

The variable modifications are color-coded as follows:

- 1. Blue—Default modifications that are set for quick loading. These modifications are selected.
- 2. Black—Default modifications that are not set for quick loading. These modifications are not selected.
- 3. Purple—Custom modifications that are not set for quick loading. These modifications are not selected.

The table shows the modification name, formula, average mass, monoisotopic mass, and modification type for each item in the list.

Tip: You can sort and filter the table to shorten the list.

2. In the Display Variable Modification column, select the check boxes for the modifications that you want to be visible in the Assign Variable Modifications pane.

   Note: When you add a new custom modification, the application automatically adds it to the table and selects the check box in the Display Variable Modification column for that item. Changes to the selections in the Display Variable Modification column persist when you close the application.

   IMPORTANT! You cannot clear the check box in the Display Variable Modification column if the check box in the Select Default Modification column is selected for a particular item in the list.

3. In the Select Default Modification column, select the check boxes for the modifications that you want to include in the quick loading sublist, and clear the check boxes for the items that you want to remove from this sublist.

   Note: When you select the check box in the Select Default Modification column, the application automatically selects the check box in the Display Variable Modification column to add the selected item to the visible default list.

4. Click **Save** to save your modifications. Or, click **Close** to close this dialog box without saving any changes.

For instructions on assigning variable modifications to a sequence, see “Assigning variable modifications for oligonucleotide sequences” on page 124.

Creating custom modifications and oligo building blocks for oligonucleotide sequences

In addition to the default modifications and default oligo building blocks provided with the BioPharma Finder application, you can create custom building blocks and custom variable modifications for an oligonucleotide sequence. You use the Building Block and Variable Modification Editor pane to create custom modifications.

For details, see these topics:

- “Creating custom oligo building blocks” on page 121.
- “Creating custom oligo variable modifications” on page 120.

Creating custom oligo variable modifications

This procedure describes how to create custom oligo variable modifications by using the Oligo Variable Modification editor in the Building Block and Variable Modification pane of the Oligonucleotide Sequence Editor. This editor is located on the right side of the pane.

To create a custom oligo variable modification

1. Open the Oligonucleotide Sequence Editor, and then at the lower right of the page, click the **Building Block and Variable Modification Editor** title bar.

   For instructions on opening the Oligonucleotide Sequence Editor, see “Sequence Manager tasks” on page 46.

2. In the Oligo Variable Modification area, select the subunit for the modification from the Subunit list.

   The selections are Base, Backbone Linker, 2' ribose, 3' Terminal, and 5' Terminal.

3. Name the modification.

4. For the Base, Backbone Linker, or 2' ribose subunit, in the Site list, select the check box for each available site where you want to apply the modification.
   - For a Base subunit, the list includes check boxes for all the default bases and the oligo building blocks that you have defined for this subunit.
   - For a Backbone Linker subunit, the list includes check boxes for Phosphate (p), Phosphorothioate (s), and the oligo building blocks that you have defined for this subunit.
   - For a 2' ribose subunit, the list includes check boxes for Deoxy (DNA) (d), Hydroxy (RNA) (r), Methoxy (m), Fluorine (f), Defluorination (f), HF loss (f), and the building blocks that you have defined for this subunit.

5. Enter the monoisotopic mass and the average mass for the modification.

   The editor accepts positive or negative values.

6. (Optional) In the formula box, enter the elemental composition of the modification.

   The formula is optional; however, if you enter a formula, it must match the specified mass. To enter a formula as a loss of mass, use a hyphen.

7. Click **Apply**.
   The editor error verifies the entries. You must enter a name, and the name must be unique for the selected subunit. If you enter a formula, it must match the specified masses. In addition, you must select the site for the modification. If the entries pass the error check, the modification appears in the list at the bottom of the pane.

8. (Optional) To define this modification as a default modification for quick loading in the Assign Variable Modifications pane, do the following:
   a. Click **Define Modification List** in the command bar of the Oligonucleotide Sequence Editor.
   b. Select the check box in the Select Default Modification column for the custom variable modification, and then click **Save**.

For information about assigning variable modifications, see “Assigning variable modifications for oligonucleotide sequences” on page 124.

Creating custom oligo building blocks

This procedure describes how to use the Oligo Building Block editor to create custom building blocks for an oligonucleotide sequence. This editor is located on the left side of the Building Block and Variable Modification Editor pane of the Oligonucleotide Sequence Editor.

To create a custom oligo building block

1. Open the Oligonucleotide Sequence Editor, and then at the lower right of the page, click the **Building Block and Variable Modification Editor** title bar. For instructions on opening the Oligonucleotide Sequence Editor, see“Sequence Manager tasks” on page 46.

2. In the Oligo Building Block area, select the subunit for the building block from the Subunit list.

   The selections are Base, Backbone linker, 2' ribose, 3' Terminal, and 5' Terminal.

3. In the Name box, name the building block.

   The name is limited to 50 characters and can include alphanumeric and special characters.

4. Select a symbol for the building block from the Symbol list.

   For a custom base, the application does not accept the following symbols: A, G, U, T, C, I, or H. These symbols are in the list, but are reserved for the default bases.

5. In the Formula box, enter the formula of the building block. To define a loss for a 2' ribose modification, type a hyphen at the beginning of the formula.

   Note: The application does not accept losses (-) for bases, backbone linkers, the 5' terminal, or the 3' terminal. To define a loss for a 2' ribose modification, type a hyphen at the beginning of the formula.

   This figure shows the entries for a custom 3' terminal.

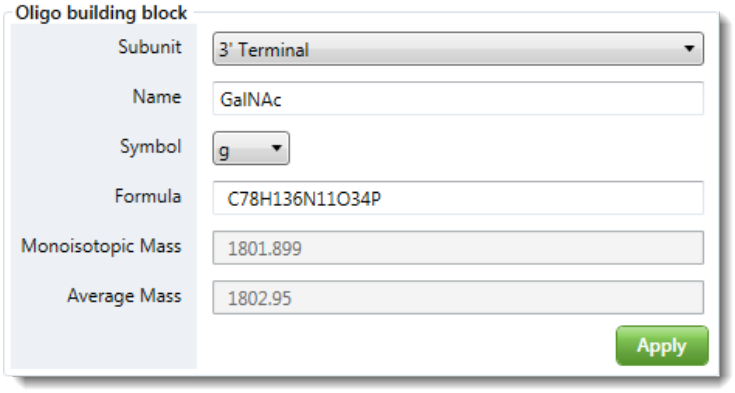


Figure 50 Example for a custom 3' terminal .

This figure shows the entries for a custom backbone linker.

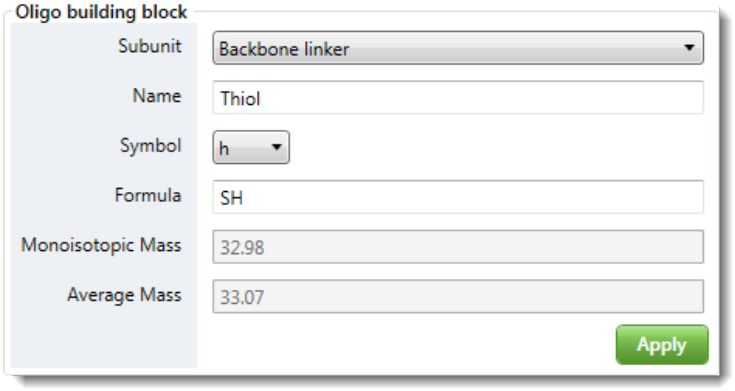


Figure 51 Example for a custom backbone linker .

This figure shows the entries for a custom base.

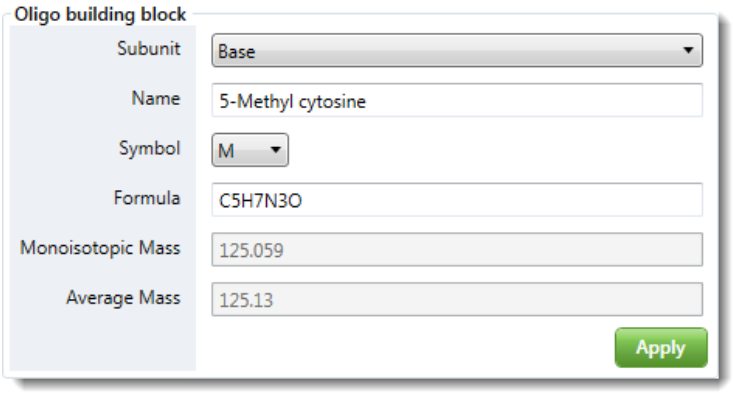


Figure 52 Example for a custom base .
This figure shows the entries for a custom 2' ribose.

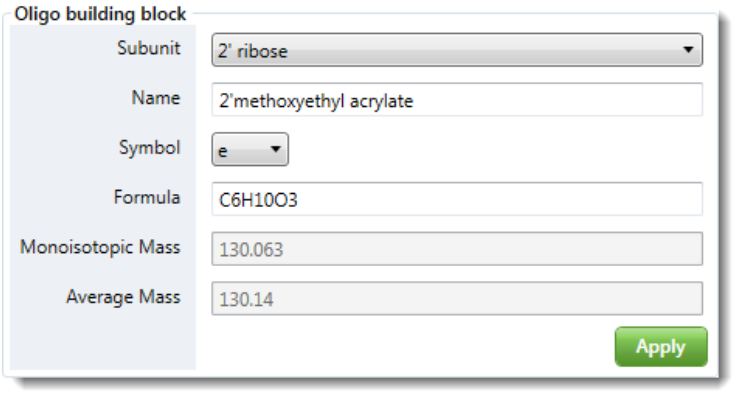


Figure 53 Example for a 2' ribose.
This figure shows the entries for a custom 5' terminal.

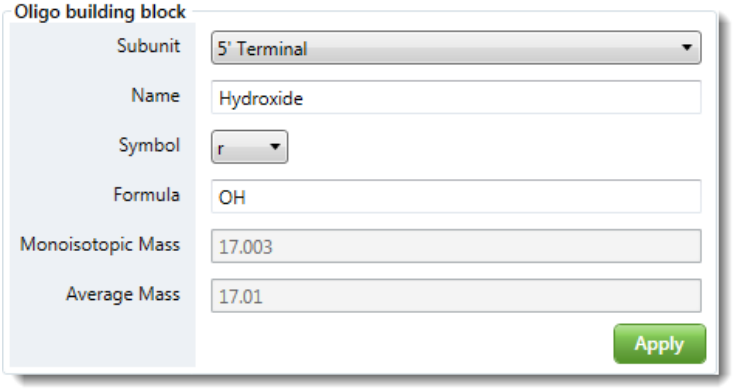


Figure 54 Example for a 5' terminal.

6. To add the modification to the list at the bottom of the pane, click **Apply**.
   The custom modification appears in purple text in the list.

   Note: To delete one or more custom building blocks, select them in the list, and then click **Delete**. If a sequence in the Sequence Manager includes the custom building block, a message box opens to let you know that the building block is

in use and that the application cannot delete it. You can delete the building block from the list only after you delete the modified sequence from the Sequence Manager.

Note: To edit a custom building block, select it in the list, and then click **Edit**. The specifications for the building block appear in the Oligo Building Block area. Modify the entries as appropriate, and then click **Apply**.

Assigning variable modifications for oligonucleotide sequences

You can assign multiple modifications to an oligonucleotide sequence. During data processing, the application looks for sequences that match any combination of the assigned modifications up to the maximum number of modifications.

For information about the parameters in the Assigning Variable Modifications pane, see “Assign Variable Modifications pane parameters” on page 126.

To assign variable modifications to oligonucleotide sequence

1. In the Sequence Manager, select an existing oligonucleotide sequence and click **Edit**. Or, click **New Oligonucleotide** to assign the variable modifications to a new oligonucleotide sequence.

   The application displays the Oligonucleotide Sequence Editor and by default expands the Manual Input Sequence pane to the right.

2. If you are creating a new sequence, do any of the following:

   - Click **Import Fasta File** to import any FASTA file.
   - Open a Fasta file in Notepad or another text editing tool, and then copy and paste each chain that you want to add to the sequence to the Manual Input Sequence pane.
   - Type the sequence in the Manual Input Sequence pane.

3. To open the Assign Variable Modifications pane, click the **Assign Variable Modifications** title bar on the right side of the Oligonucleotide Sequence Editor page.
   The lists show all the available default variable modifications that you want to display in the pane. You can modify the displayed lists of variable modifications as appropriate. See “Changing the default and visible sublist of variable modifications for oligo sequences” on page 118.

4. In the Max # Modifications area, type a value for the maximum number of modifications to assign to the sequence.

   Note: Increasing these numbers might substantially increase processing time. You can assign up to eight modifications to a sequence.

5. In the 5' Terminal, 3' Terminal, or Oligo Variable Modification areas, do the following:
   a. To quickly load the modifications from the default sublist, click **Load Default Mods**.

      Note: You can edit the default sublist of variable modifications used for quick loading.

   b. To assign another modification, select it from the Modifications list on the left for the respective modification type, and then click **Add**.

Note: You can assign more than one modification but select only one at a time.

The following figure shows the assignment of the default modifications for the 5' terminal and the 3' terminal.

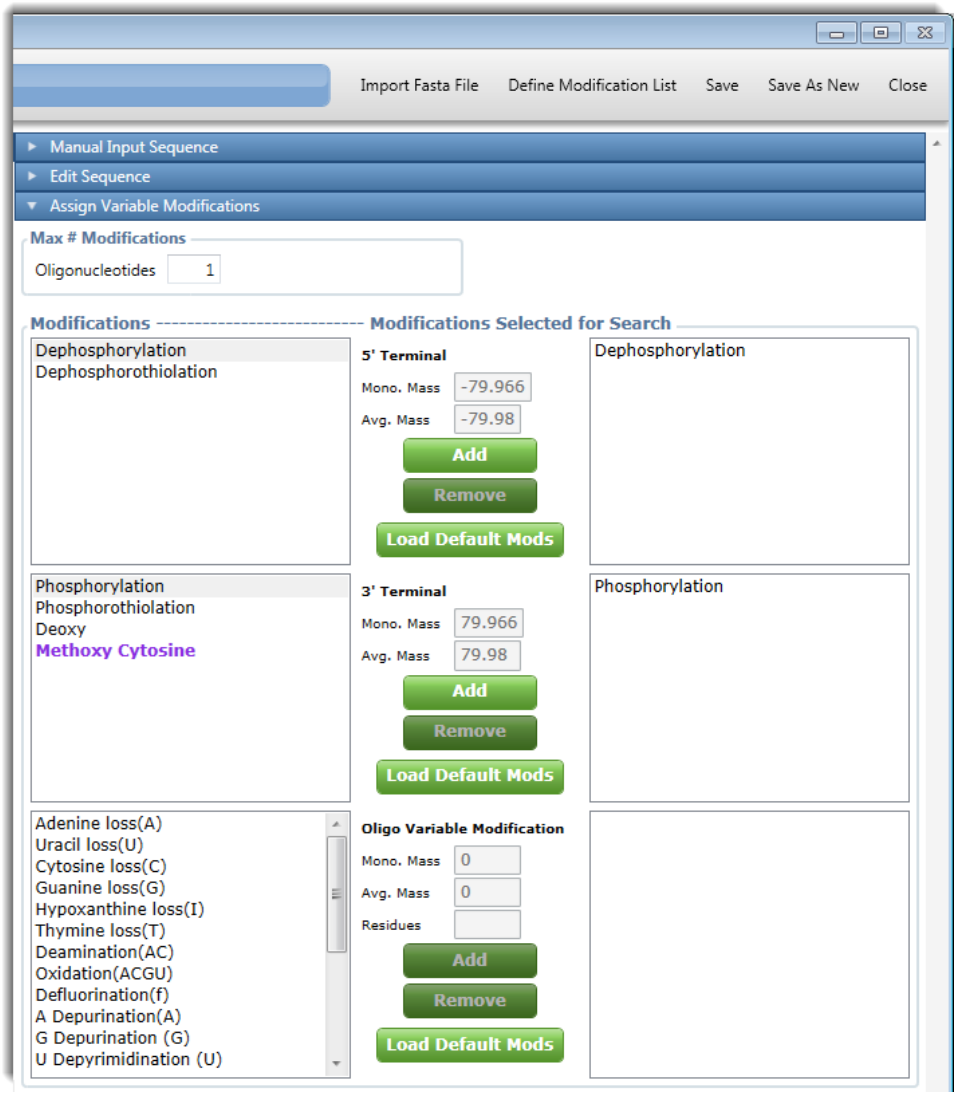


Figure 55 Assign Variable Modifications pane with assignments for the 5' and 3' terminals

The loaded and selected modifications appear in the lists. They are used as variable modifications during the identification step of the experiment.

6. Click **Save** or **Save as New** to save the modifications to the sequence.
   If you do not want to save these modifications to the sequence, click **Close** in the command bar.

The following figure shows the sequence and its assigned variable modifications in the Sequence Manager.

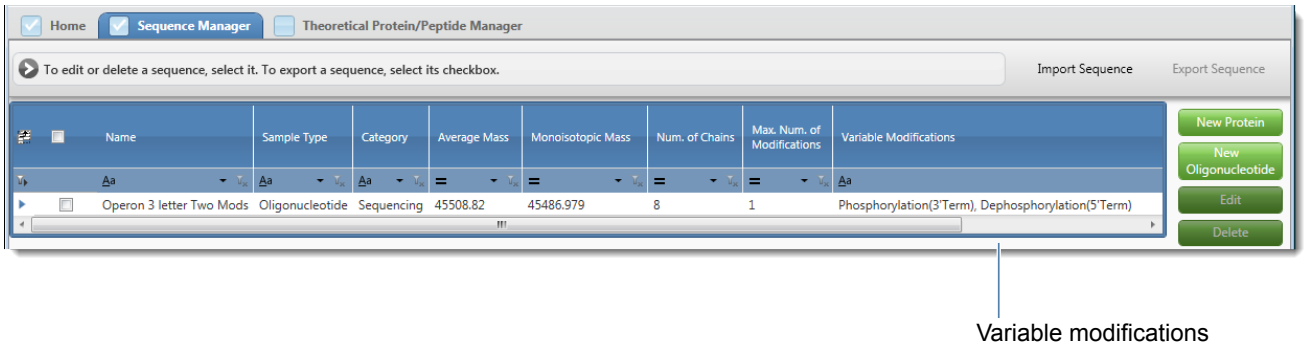


Figure 56 Sequence Manager

Assign Variable Modifications pane parameters

The following table describes the parameters in the Assign Variable Modifications pane in the Oligonucleotide Sequence Editor.

Table 16 Parameters descriptions for the Assign Variable Modifications pane

Parameter	Description
Max # Modifications	Specifies the maximum number of variable modifications that you want to assign to the target oligonucleotide sequence. Range: 0 to 8; default: 1
Modifications	Lists the modifications available to apply to a 5' terminal, 3' terminal, and oligo variable modification.
5' Terminal	Displays information about the 5' terminal modification that you selected from the list.
Read-only fields	
Mono. Mass	Displays the monoisotopic mass of the selected 5' terminal modification to three decimal places.
Avg. Mass	Displays the average mass of the selected 5' terminal modification to two decimal places.
Buttons	
Add	Adds the selected modification to the Modifications Selected for Search list.
Remove	Removes the selected modification from the Modifications Selected for Search list.

Parameter	Description
Load Default Mods	Quickly loads the sublist of default modifications into the Modifications Selected for Search list.
3' Terminal	Displays information about the 3' terminal modification that you selected from the list.
Read-only fields	
Mono. Mass	Displays the monoisotopic mass of the selected 3' terminal modification to three decimal places.
Avg. Mass	Displays the average mass of the selected 3' terminal modification to two decimal places.
Buttons	
Add	Adds the selected modification to the Modifications Selected for Search list.
Remove	Removes the selected modification from the Modifications Selected for Search list.
Load Default Mods	Quickly loads the sublist of default modifications into the Modifications Selected for Search list.
Oligo Variable Modifications	Displays information about the oligo variable modification that you selected from the list.
Read-only fields	
Mono. Mass	Displays the monoisotopic mass of the selected oligo variable modification to three decimal places.
Avg. Mass	Displays the average mass of the selected oligo variable modification to two decimal places.
Residues	Displays residue for the selected oligo variable modification.
Buttons	
Add	Adds the selected modification to the Modifications Selected for Search list.
Remove	Removes the selected modification from the Modifications Selected for Search list.
Load Default Mods	Quickly loads the sublist of default modifications into the Modifications Selected for Search list.

Saving an oligo sequence

If you make any changes to an oligonucleotide sequence, you can save it under the same name to overwrite an existing sequence or under a different name to create a new sequence. The information saved includes all of the items listed in the table on the Sequence Manager page.

For details, see these topics:

- "Saving an oligo sequence with the same name" on page 128
- "Saving an oligo sequence with a different name" on page 128

Saving an oligo sequence with the same name

To save an oligonucleotide sequence with the same name

1. Click **Save** in the command bar of the Oligonucleotide Sequence Editor to save the sequence under the same name.
   If this sequence already exists, the following warning prompt appears: The sequence name already exists and will be overwritten.
2. At the prompt, click **Yes** to have the current sequence overwrite previously saved data.
   Information from the saved sequence populates the columns of the sequence table on the Sequence Manager page.

Saving an oligo sequence with a different name

To save an oligonucleotide sequence with a different name

1. Click **Save As New** in the command bar of the Oligonucleotide Sequence Editor.
   The Save As New dialog box opens.
2. Do the following:
   a. In the New Sequence Name box, type the new name of the oligonucleotide sequence.
   b. (Optional) In the Description box, type a description for the oligonucleotide sequence.
3. Click **OK**.
   Information from the saved sequence populates the columns of the sequence table on the Sequence Manager page.

Oligonucleotide Sequence Editor parameters

The following table describes the parameters in the Oligonucleotide Sequence Editor.

For information about opening the Oligonucleotide Sequence Editor, see “Sequence Manager tasks” on page 46.

Table 17 Parameters in the Oligonucleotide Sequence Editor

Parameter	Description
Command Bar	
Import Fasta File	Imports a sequence from a FASTA file. See “Importing an oligonucleotide sequence from a FASTA file” on page 110 and “Opening a FASTA file to verify or copy its contents” on page 109.
Define Modification List	Opens the Define Modification List dialog box where you specify which modications the application is to display in the Modifications lists on the left side of the Assign Variable Modifications pane and the sublist of these modifications that are available for quick loading to the Modifications Selected for Search lists on the right side of the pane.
Save	Saves the changes to a sequence under its existing name.
Save As New	Opens the Save As New dialog box, so that you can save a sequence under a new name.
Close	Closes the Oligonucleotide Sequence Editor if you already saved your changes. Otherwise, in the save changes message box, click Yes or No. **Note:** The **Close** icon,  , is unavailable.
Sequence Information pane	Displays the sequence and chain information.
Target Oligonucleotide	Displays information about the current oligonucleotide sequence.
Name	Specifies the name of the oligonucleotide sequence.
Description	Provides a description of the oligonucleotide sequence.
Sample Type	Displays the sample type of the sequence—Oligonucleotide.
Category	Specifies the category of the oligonucleotide sequence. Selections: Sequencing and Intact Deconvolution
Monoisotopic Mass	Displays or specifies the monoisotopic mass of the oligonucleotide sequence. After you enter the chain information, this box becomes a read-only field. To create a mass-only sequence, see “Creating mass-only or formula-only oligo sequences” on page 112.
Average Mass	Displays or specifies the average mass of the oligonucleotide sequence. After you enter the chain information, this box becomes a read-only field.

Parameter	Description
Chain	Displays information about a selected chain.
Chain	Lists the number of each chain in the oligonucleotide sequence.
Monoisotopic Mass	Displays the monoisotopic mass of the chain that you selected in the Chain list.
Average Mass	Displays the average mass of the chain that you selected in the Chain list.
Oligo Sequence Map pane	Displays the nucleotides in the chains in the oligonucleotide sequence.
Oligo Information pane	Displays a graphical depiction of two nucleotides with a phosphate linker. The text to the right of the graphic describes how to define customized bases, linkers, and 2' ribose groups.
Manual Input Sequence pane	Use this pane to manually specify the chains in an oligonucleotide sequence. “Creating a new oligonucleotide sequence” on page 106.
Chain Name	Specifies the name of an added chain in the oligo sequence.
(Editor box)	Provides an area for you to type or paste the new chain information.
Apply	Applies the changes.
Edit Sequence pane	Use this pane to edit the building blocks of the selected chain and adjust the 3' terminal and 5' terminals. See “Assigning custom building blocks to an oligo sequence by using the Edit Sequence pane” on page 114.
Select Chain	Lists all the chain numbers. To modify a chain, select the chain number from the list. The chain information appears below the list box.
5' Terminal	Displays the 5' terminal building blocks in a dropdown list.
Building Block table	Use to change base, linker, and 2' ribose.
3' Terminal	Displays the 3' terminal building blocks in a dropdown list.
Apply	Applies the selected changes to the chain.
Cancel	Discards the changes made in the Edit Sequence pane.
Assign Variable Modifications pane	Use this pane to assign variable modifications to the sequence. The application searches for these modified sequences to identify detected components. “Assigning variable modifications for oligonucleotide sequences” on page 124.

Parameter	Description
Max # Modifications	Specifies the maximum number of modifications for the sequence. The application searches for any combination of the selected modifications up the maximum number of modifications per sequence. For example, if you select three modifications and a maximum of one modification per sequence, the application searches for four defined sequences— the unmodified sequence, and an additional sequence for each modification. If you select three modifications and a maximum of two modifications per sequence, the application searches for seven defined sequences, and so on. Range: 0 to 8; default: 1
Mono. Mass	Displays the monoisotopic mass for the 5' terminal, 3' terminal, and oligo variable modifications to three decimal places.
Avg Mass	Displays the average mass for the 5' terminal, 3' terminal, and oligo variable modifications to two decimal places.
Add	Adds the specified custom modifications to the 5' Terminal, 3' Terminal, or Oligo Variable Modifications list.
Remove	Removes the selected custom modifications from the 5' Terminal, 3' Terminal, or Oligo Variable Modifications list.
Load Default Modification	Displays the default modification list.
Residues	Displays residue for the selected oligonucleotide variable modification.
Building Block and Variable Modification Editor pane	Displays oligo building blocks and oligonucleotide variable modifications for you to manage. See “Creating custom oligo building blocks” on page 121 and “Creating custom oligo variable modifications” on page 120
Subunit	Specifies the subunit. Selections: Base, Backbone linker, 2' ribose, 3' Terminal, and 5' Terminal Default: Base
Name	Specifies the name of the building block. You can enter up to 50 characters.
Symbol	The list of symbols is unique for each subunit. Depending on the selected subunit, the list of symbols includes all the lowercase or uppercase letters in the alphabet. The lists do not exclude letters that are reserved or in use. Instead, if you select a reserved letter, the communication bar prompts you to modify your selection. For example, if you select Base in the Subunit list, and then select A, G, U, T, or C in the Symbol list, the communication bar states that these symbols are already reserved for the default bases.

Parameter	Description
Formula	Specifies the chemical formula. The application only recognizes the symbols for the elements in the periodic table and integers for the number of atoms. For the base, backbone linker, and 2' ribose subunits, the application supports only these elements: C, H, O, N, S, P, and F. Hyphens specify the loss of an element or chemical group. For example, F-OH specifies the addition of a fluorine atom (F) and the loss of a hydroxyl group (OH).
Monoisotopic Mass	The monoisotopic mass is automatically populated based on the formula. Displays the value up to four decimal places.
Average Mass	The average mass is automatically populated based on the formula. Displays the value up to two decimal places.
Base, backbone linker, 2' ribose, and variable modification list	Lists the default and custom building blocks and variable modifications.
Edit	Populates the Oligo Building Block area or the Oligo Variable Modification area with editable information about the selected custom modification. To edit a custom modification, select it from the table, click **Edit** to make the information available in the applicable area, make your changes, and then click **Apply**. **Note:** You cannot edit the default modifications, which are displayed in black text.
Delete	Deletes the selected custom variable modifications.or custom building blocks. You can delete mulitple items at a time. **Note:** You cannot delete the default modifications or default building blocks.
Apply	Saves the custom variable modification or custom building block. The information is displayed in the table at the bottom of the pane.

Part III

Common tasks

7 Common features for different analyses

Peptide Mapping Analysis, Intact Mass Analysis, Oligonucleotide Analysis, and Top Down Analysis have similar workflows for creating experiments, saving processing methods, and working with workbooks.

Creating a new experiment

To create a new experiment for Peptide Mapping Analysis, Oligonucleotide Analysis, Intact Mass Analysis, or Top Down Analysis, see the following topics:

- Raw data files and sequences (page 134)
- Loading the raw data files (page 135)
- Deleting the raw data files (page 138)
- Changing the location of a raw data file (page 138)
- Selecting one or more sequences (page 139)
- Importing a processing method (page 140)
- Exporting a method (page 143)
- “Selecting a processing method” on page 141
- Deleting a method (page 143)

Raw data files and sequences

A new experiment requires one or more raw data files as input. Before loading the raw data files, you can first import a sequence into the Sequence Manager or create a sequence by using the Protein Sequence Editor or the Oligonucleotide Sequence Editor. The sequence establishes the user-specified protein, peptide, or oligonucleotide that you want the BioPharma Finder application to use to match detected components to potential identifications.

Without this sequence, the application still performs component detection, but entries in the Results tables have no identification information. If you perform the initial ion detection without selecting a sequence, you can identify components by importing or creating a sequence.

Loading the raw data files

You must load one or more raw data files for your experiment. When you load multiple files for Peptide Mapping Analysis or Oligonucleotide Analysis, the application requires that you assign conditions to them. When you load multiple files for Intact Mass Analysis or Top Down Analysis, assigning conditions to them is optional.

Note: The more raw data files you load and the larger they are, the longer the application might take to process the results.
Because the application repeatedly accesses the raw data files during data processing, your processing time is very slow if the application accesses the raw data files through a network. Copy the raw data files to your local computer for faster processing speed.

To select and load the raw data files

1. On the Home page, click **Peptide Mapping Analysis, Oligonucleotide Analysis, Intact Mass Analysis**, or **Top Down Analysis** in the Experiment Types pane or below the splash graphic.

2. In the Load Raw Data pane for the applicable page, do the following:
   a. Next to the Select Raw Data box, browse to the location of your files if necessary.

      You can specify the default folder from which to load your raw data files. Then, the Browse dialog box automatically opens to this folder location.

   b. Select one or more raw data files for processing.

      To run a set of raw data files (for example replicates of the same sample or samples with various conditions) with a given method, select either contiguous file names using the SHIFT key or noncontiguous file names using the CTRL key.

   c. Click **Open**.

   The application displays the selected raw data files in the table in the Load Raw Data area. The order of the files in this table defines the order that they appear in the Results tables when you view the processed results.

3. If you load multiple raw data files, do the following:
   a. In the Condition box, type the conditions to assign to the files.

      Note: For Peptide Mapping Analysis, you must specify the conditions for all of the raw data files. The Start Processing button is not enabled until you specify the conditions. If you run an HDX experiment, use specific conditions. The application adds the entered conditions to the Reference Condition list, as well as the list in the Condition column in the raw data file table.

For Intact Mass Analysis and Top Down Analysis, conditions are optional. For Oligonucleotide Analysis, conditions are required for loading multiple raw data files

Use the smallest identifiable condition for each group of assigned raw data files. Separate multiple conditions with a space.

If your experiment uses a blank file, type the word "blank" as a condition to identify a blank group for background subtraction.

If an entered condition matches a portion of a raw data file name, the application automatically assigns that condition to the matching raw data file. It is possible that the application assigns one condition to multiple matching raw data files.

b. (For Peptide Mapping Analysis only) In the Reference Condition list, select the reference condition from the list of entered conditions.

The default reference condition is the first entered condition in the Condition box.

Upon processing, the application calculates the ratio between the average component areas for the different conditions and the area for reference condition, and then displays these ratios in the Results table on the Process and Review page.

c. In the Condition column in the table, select a condition from the list to assign to each raw data file if necessary.

The application automatically groups together all files assigned to the same condition.

If you run an HDX experiment, you must assign the condition "ref" or "Ref" to one of the raw data files to designate it as the reference file.

If your experiment has a blank sample file, ensure that it is not the first raw data file in the table. For best results, make the first raw data file the most complex file, as close as possible to 100 percent of all components in all samples. By creating a pooled sample, your results are more satisfactory.

Note: You can reorder the list of files and load them again (so that the blank file is not listed as the first file, for example). However, you must reenter the conditions in the Condition box and then reassign them to the raw data files, as needed.

To change the order of the list of loaded raw data files, click ... and browse to the list of raw data files. You can reorder by name, date, type, size, and so on. The order of the files in the Add Analysis File(s) dialog box is the loaded order of the files in the application after you click Open.

The following figure shows a set of raw data files loaded for a Peptide Mapping Analysis experiment and their assigned conditions.

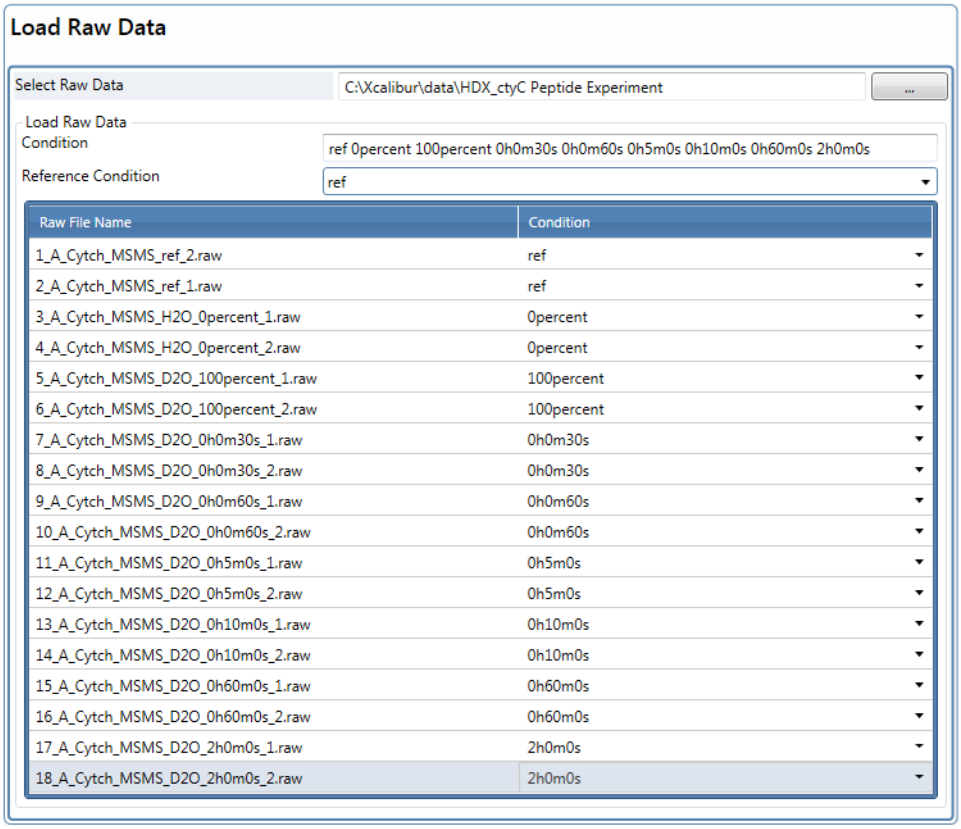


Figure 57 Loaded raw data files and conditions for Peptide Mapping Analysis

The following figure shows a set of raw data files loaded for an Intact Mass Analysis or Top Down Analysis experiment and their assigned conditions.

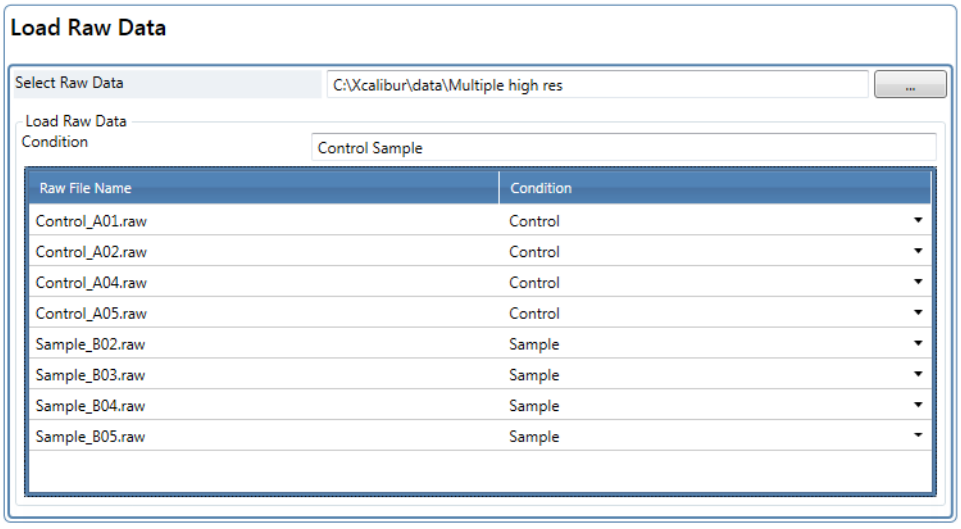


Figure 58 Loaded raw data files and conditions for Intact Mass Analysis or Top Down Analysis

Changing the location of a raw data file

The location of a raw data file is not always the same from one computer to another. So, there should be a way to change the location of the raw file. Besides, the raw file may have been moved to a new location on the computer, thereby rendering the experiment and its results inaccessible.

When you restore the application, and an open an experiment, the application will have the original raw file path. It will look for the files in this location. If raw files are not present, you will be prompted to browse to a different location to find the files. The application needs the raw files to display the spectrum and traces.

The following steps are the same for all work-flows (Peptide Mapping Analysis, Oligonucleotide Analysis, Intact Mass Analysis, or Top Down Analysis).

To change the location of the raw data file

1. Open the Results pane from the Load Results page or the Queue page.
2. Verify that the raw data files are in the correct location designated in the experimental results.

   If the correct raw data files are in the most recent location matching the database and the .PMF file, the application opens the experiment.
3. If the raw data files are not in the most recent location, the application opens a dialog box where you can browse for the raw data files from a new location.

   The default directory is C:\Xcalibur\data.
4. After you select the raw data files, click **Open**.

   The application checks to see if the selected files match the files used in the experiment.
   - If they match, the application opens the experiment.
   - If they do not match, the application displays a message that the data file is missing or does not match.

Deleting the raw data files

To delete raw data files from the Load Raw Data pane

1. On the Home page, click **Peptide Mapping Analysis**, **Oligonucleotide Analysis, Intact Mass Analysis**, or **Top Down Analysis** in the Experiment Types pane or below the splash graphic.
2. In the list of raw data files in the Load Raw Data pane, select the name of the raw data file to remove.

   The application highlights the selected file in blue.

   Tip: You can select only one raw data file at a time to delete.
3. Press the DELETE key.
4. In the confirmation box, click **Yes**.

Selecting one or more sequences

The BioPharma Finder application automatically displays the available protein sequences and their data in the Protein Sequence table of the Peptide Mapping Analysis page, the Intact Mass Analysis page, or the Top Down Analysis page and the available oligonucleotide sequences and their data in the Oligonucleotide Sequence table of the Oligonucleotide Analysis page. The list of sequences comes from the table on the Sequence Manager page. For a targeted peptide mapping search, some protein sequences are saved workbooks from the Target Peptide Workbook > Workbook Manager page.

IMPORTANT! You must select the appropriate category for the sequence to be visible in the Protein Sequence table or the Oligonucleotide Sequence table on the corresponding analysis page. For example, for a protein sequence to be visible on the Intact Mass Analysis page, assign the Intact Protein category to this sequence before saving it.

Saved workbooks that you use as protein sequences for a targeted search automatically receive the Targeted Peptide category.

You cannot edit the information in the Protein Sequence table or the Oligonucleotide Sequence table. However, you can click the column headers to sort the order of the displayed data and filter the rows by using the filter options in the column headers.

To select a sequence for an experiment

1. On the Home page, click **Peptide Mapping Analysis, Oligonucleotide Analysis, Intact Mass Analysis**, or **Top Down Analysis** in the Experiment Types pane or below the splash graphic.

2. (Optional for Peptide Mapping Analysis non-targeted experiment and Intact Mass Analysis experiment, required for Peptide Mapping Analysis targeted experiment and Top Down Analysis experiment) In the Protein Sequence table, select the check box in the sequence row for the protein sequence.

   Note: For Peptide Mapping Analysis, you can select only one protein sequence to be searched in an experiment. For Intact Mass Analysis and Top Down Analysis, you can select up to 10 protein sequences.

The following figure shows the selection of a peptide mapping protein sequence for the experiment.

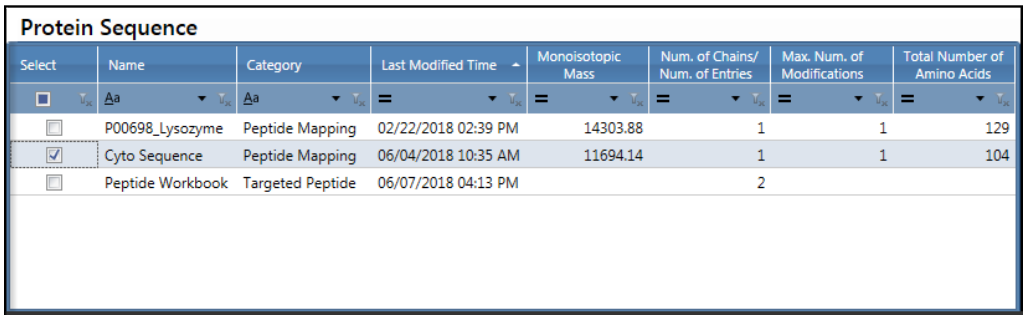


Figure 59 Selected protein sequence

Importing a processing method

You can import a processing method for all types of analyses:

To import a processing method

1. On the Home page, click **Peptide Mapping Analysis**, **Oligonucleotide Analysis**, **Intact Mass Analysis**, or **Top Down Analysis** in the Experiment Types pane.

   The analysis definition page for the selected experiment type opens.

2. In the top-right corner of the page, click **Import Method**.

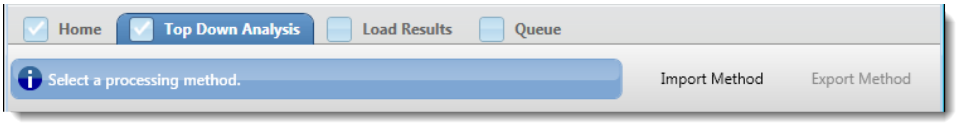


Figure 60 Import Method button

3. In the dialog box, locate and select one or more method files for importing:

   - For Peptide Mapping Analysis, select *filename*_peptide.methodbpf.
   - For Oligonucleotide Analysis, select *filename*_oligo.methodbpf.
   - For Intact Mass Analysis, select *filename*_intact.methodbpf.
   - For Top Down Analysis, select *filename*_topdown.methodbpf.

   To import multiple files at once, the files must be located on the same folder.

4. Click **Open**.

   A message appears indicating the confirmation of the import.

   The application adds the imported processing method in the Processing Method table.

Tip: If the imported method has the same name as an existing method, the applications appends _imported to the file name of the new method.

Selecting a processing method

The application automatically displays the available default methods and their descriptions in the Processing Method table on the Peptide Mapping Analysis page, the Intact Mass Analysis page, the Top Down Analysis page, or the Oligonucleotide Analysis page. If you create new custom methods (see Chapter 11, “Working with a peptide mapping processing method”, Chapter 18, “Working with an Intact Mass processing method”, Chapter 31, “Working with an Oligonucleotide Processing Method”, or Chapter 26, “Working with a top down processing method”), the application displays them here as well.

You cannot edit the information in the Processing Method table. However, you can click the column headers to sort the order of the displayed data and filter the rows by using the filter options in the column headers (see “Using basic table functions” on page 670 and “Filtering data in a table” on page 673). From this table, select a processing method for the current experiment.

To select a processing method for an experiment

1. On the Home page, click **Peptide Mapping Analysis, Oligonucleotide Analysis, Intact Mass Analysis,** or **Top Down Analysis** in the Experiment Types pane or below the splash graphic.

2. In the Processing Method table, select the check box for a method row.

   Note: You can select only one method for an experiment.

The following figures show the selection of a processing method for the various experiment types.

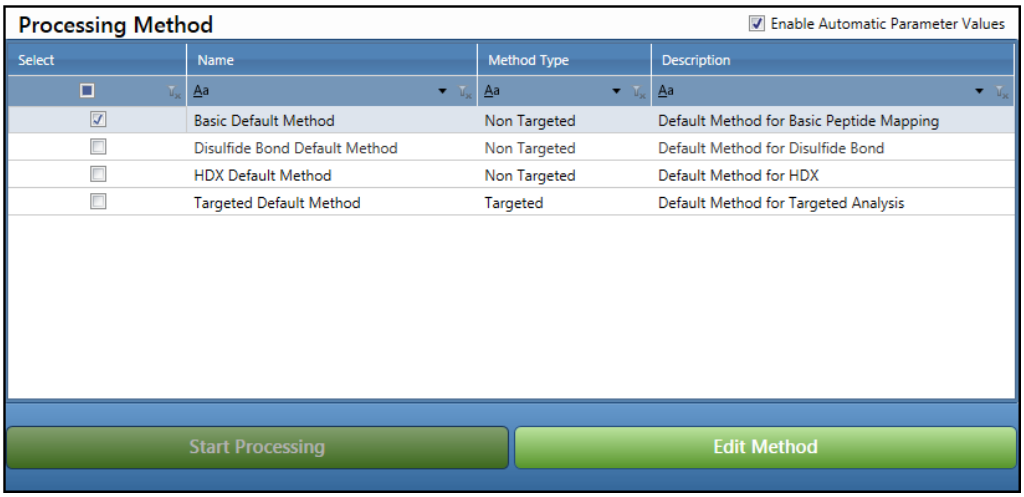


Figure 61 Selected processing method for Peptide Mapping Analysis

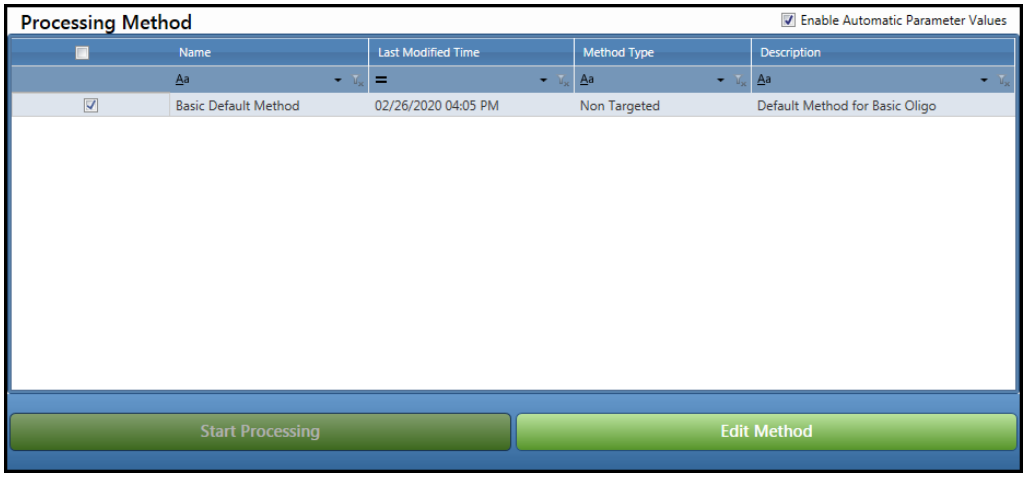


Figure 62 Selected processing method for Oligonucleotide Analysis

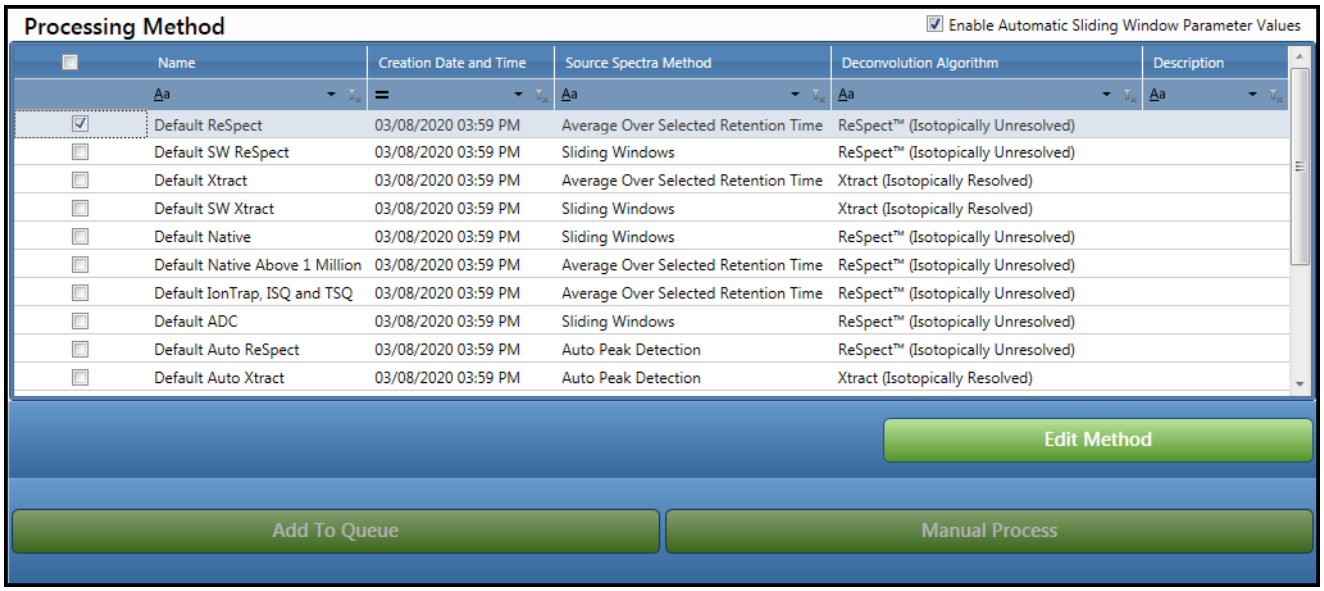


Figure 63 Selected processing method for Intact Mass Analysis

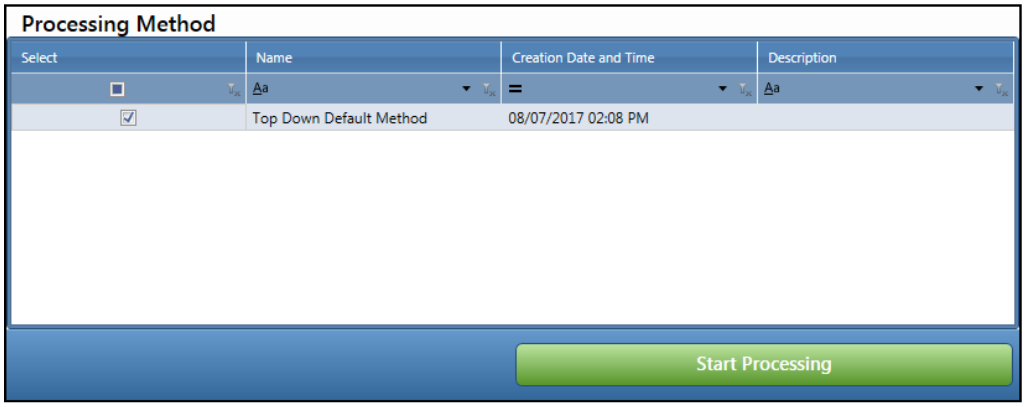


Figure 64 Selected processing method for Top Down Analysis

Exporting a method

You can export a processing method for all types of analyses.

To export a processing method

1. On the Home page, click **Peptide Mapping Analysis, Oligonucleotide Analysis, Intact Mass Analysis**, or **Top Down Analysis** in the Experiment Types pane or below the splash graphic.
2. In the Processing Method table, select one or more check boxes corresponding to the method that you want to export.

   The Export Method button becomes available.

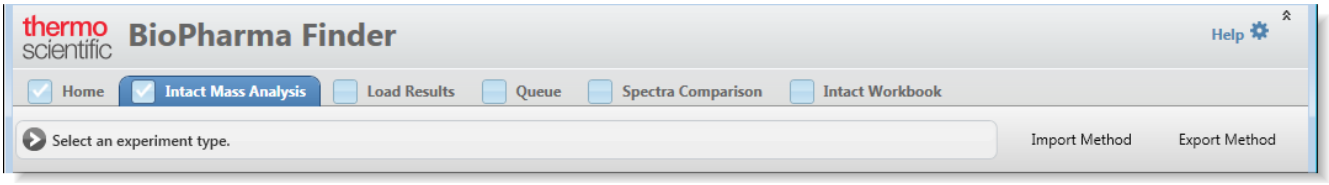


Figure 65 Export Method button

3. Click **Export Method**.
4. In the dialog box, browse to the appropriate folder, and click **OK**.

   A message appears indicating the confirmation of the export, and the exported processing method is saved on the specified folder.

Deleting a method

To delete an existing method

1. On the Home page, click **Peptide Mapping Analysis**, **Oligonucleotide Analysis**, **Intact Mass Analysis**, or **Top Down Analysis** in the Experiment Types pane or below the splash graphic.
2. In the table in the Processing Method area, select a method row.

   The application highlights the selected method in blue.

   IMPORTANT! You can select only one method at a time to delete. You cannot delete any default method.

   To select the row for deletion, click any column in that row except for the first column. The first column contains the check box to select the row for editing.
3. Press the DELETE key and click **Yes** in the confirmation box.

Saving a processing method

After you modify the processing method parameters, save the method to store the modified values for processing. If you modified the parameters in a default method, you must save them to a new method with a different name. All experiment names, sequence names, and method names are case-insensitive.

For more details, see the following topics:

- Navigating to the method summary (page 144)
- Method summary display (page 144)
- Exporting the method summary and saving the method (page 148)
- Effects after saving the method (page 149)

Navigating to the method summary

To navigate to the method summary

1. On the Home page, click **Peptide Mapping Analysis, Oligonucleotide Analysis, Intact Mass Analysis** or **Top Down Analysis** in the Experiment Types pane or below the splash graphic.
2. Select a processing method and then click **Edit Method**.
3. In the navigation bar, click the **Parameters** tab, and then click the **Save Method** subtab (for Peptide Mapping Analysis and Intact Mass Analysis) or the **Save Experiment** subtab (for Top Down Analysis).

Method summary display

The Save Method or Save Experiment page displays the method name and description on the left side.

The following figure shows the left side of the page for Peptide Mapping Analysis (similar to Intact Mass Analysis).

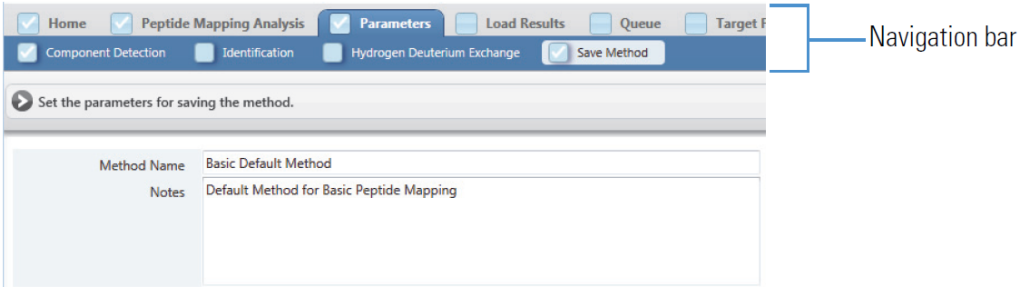


Figure 66 Save Method page for Peptide Mapping Analysis (left side)

The following figure shows the left side of the Save Method page for Oligonucleotide Analysis.

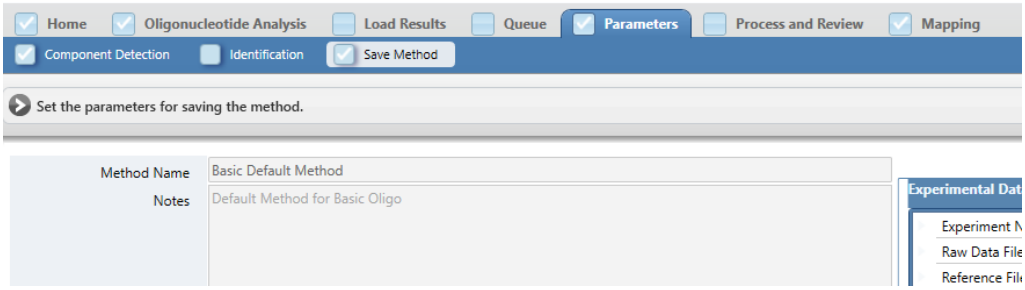


Figure 67 Figure 2. Save Method page for Oligonucleotide Analysis

The following figure shows the left side of the page for Top Down Analysis.

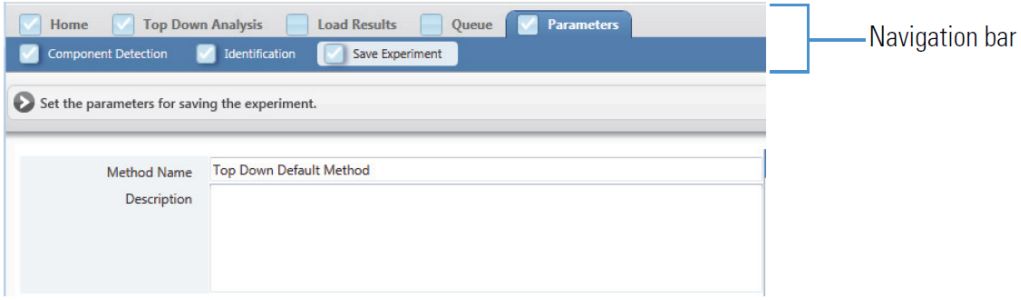


Figure 68 Save Experiment page for Top Down Analysis (left side)

The Method Summary table on the right side of the page lists the current method parameters, including those that you might have changed on the previous method parameter pages.

The following figure shows the right side of the page for Peptide Mapping Analysis (similar to Intact Mass Analysis).

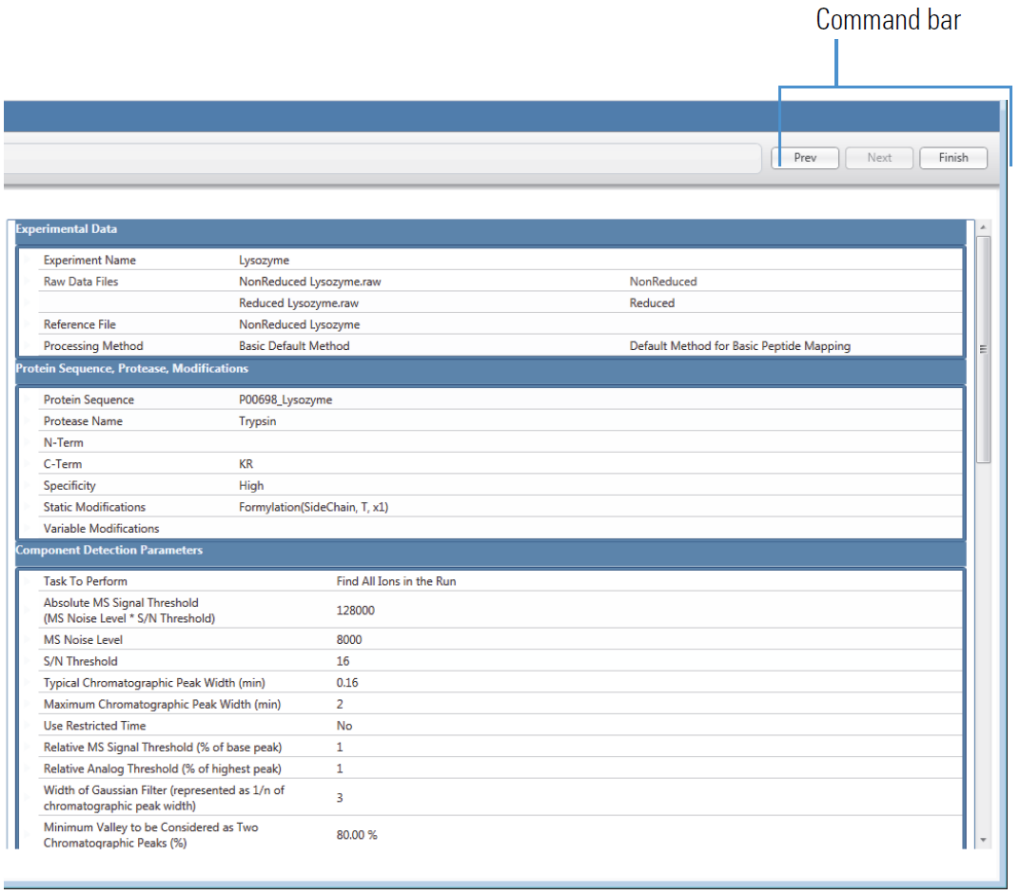


Figure 69 Save Method page for Peptide Mapping Analysis (partial right side)

The following two figures show the right side of the page for Top Down Analysis.

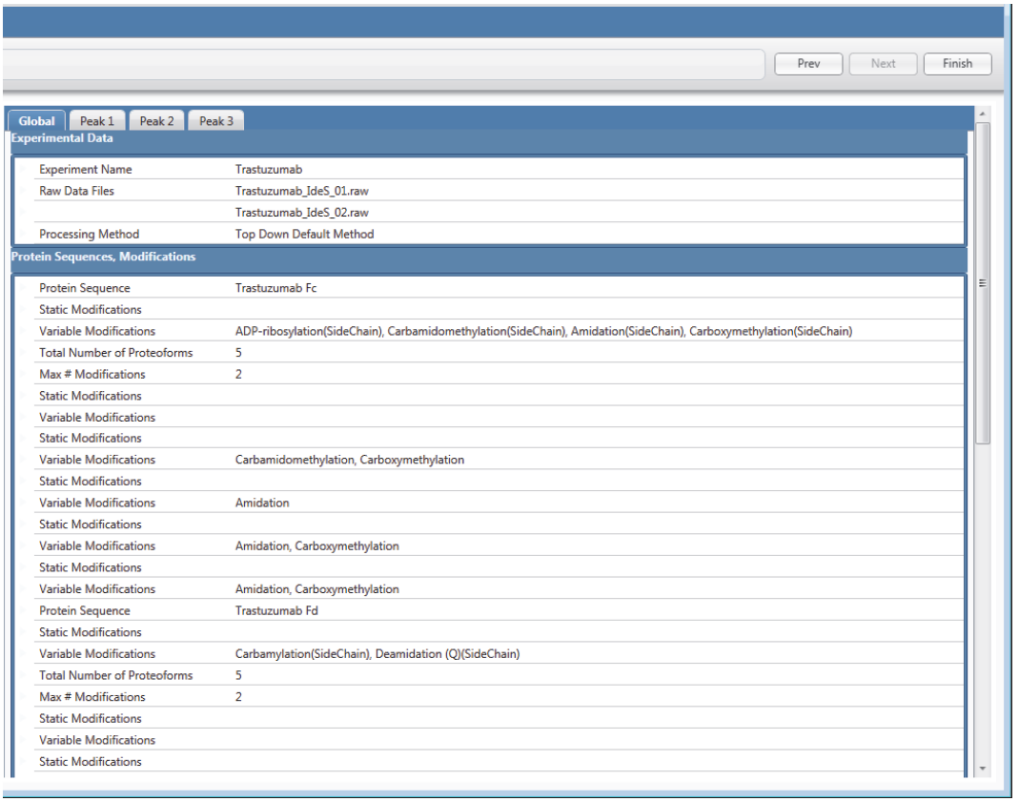


Figure 70 Save Experiment page for Top Down Analysis–Global subtab (partial right side)

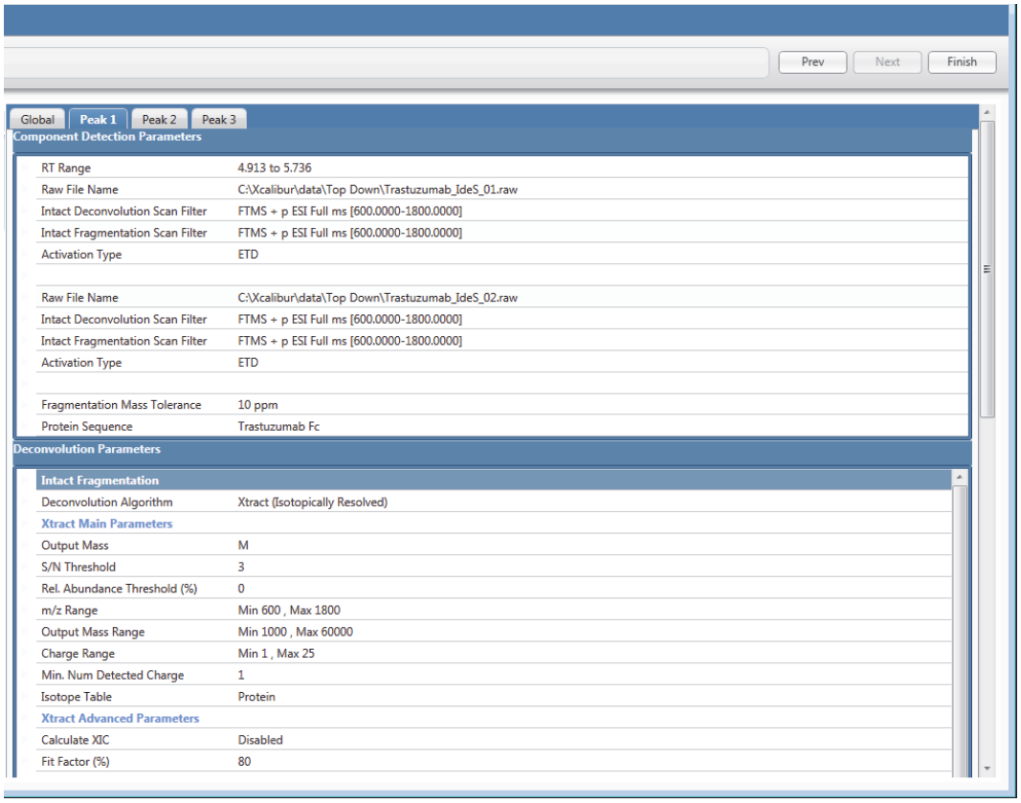


Figure 71 Save Experiment page for Top Down Analysis–Peak 1 subtab (partial right side)

The Method Summary information is not editable. However, you can export the data in the table to a Microsoft™ Excel™ or Word™ file.

Exporting the method summary and saving the method

To export the method summary and save the processing method

1. Navigate to the Method Summary page.
2. (For Top Down Analysis only) Click the **Global** or **Peak #** subtab to view the corresponding parameters in the summary.

   Tip: Click the **Global** subtab to view the global data for the experiment, protein sequences (including proteoforms), and Identification parameters. Click each **Peak #** subtab to view the Component Detection parameters specific to each peak.
3. To export the summary information, right-click the table and choose **Export Parameters to Microsoft™ Excel™** or **Export Parameters to Word™**.

   In the dialog box, browse to the appropriate folder and enter a file name, and then click **Save**.

For Peptide Mapping Analysis and Intact Mass Analysis, the BioPharma Finder application exports all summary information in the table to a Microsoft™ Excel™ or a Word™ file to your chosen folder location. For Top Down Analysis, the application exports only the parameters listed under the selected tab to the file.

4. (Optional) To change any of the parameters listed in the Method Summary, click **Prev** to go back to a previous parameter page and make your changes there. Then, click **Next** until you advance to the Save Method or Save Experiment page.

5. In the Method Name box, retain the current name or type a new name for the method.

   If you retain the current method name, the application saves all current parameter values to the existing method and overwrites any previous values.

   If you change the method name, the application saves all current parameter values to a new method.

   Note: You cannot overwrite a default method. If you try, the application automatically prompts you to enter a different method name to save to a new method.

   Use only alphanumeric, space, underscore "_", and period "." characters in the method name.

6. (Optional) In the Notes or Description box, briefly describe the method. For example, you might want to describe the experiment and the analyzed proteins.

7. Click **Finish**.

Effects after saving the method

For Peptide Mapping Analysis, the following occurs after you save the processing method:

- If you previously entered the experiment name and uploaded one or more raw data files for the experiment on the Peptide Mapping Analysis page, the application saves the method, opens the Queue page, and places your job in the run queue.

  If the queue is not paused, and the queue is empty or all queued jobs are already completed, the application immediately processes the experiment using the saved method information. Otherwise, your job waits in the run queue until you resume the queue processing or your job moves to the top of the queue.

- If you did not previously specify the experiment name and raw data files, the application saves the method and transfers you back to the Peptide Mapping Analysis page. This page displays the saved method in the Processing Method area, and you can select this method to use for processing a new experiment.

For Intact Mass Analysis, the application saves the method and transfers you back to the Intact Mass Analysis page. This page displays the saved method in the Processing Method area, and you can select this method to use for processing a new experiment.

For Top Down Analysis, the application saves the experiment parameters to the database. If you use the same name as an existing experiment, it overwrites that experiment. It also saves the method parameters to a custom method, except for the protein sequence information, and then transfers you to the Queue page to start processing the experiment. The next time you enter the Top Down Analysis page, it displays the saved method in the Processing Method area, and you can select this method to use for processing a new experiment. Before processing, you must reselect a protein sequence for each peak in the method from the list of sequences that you select on the Top Down Analysis page.

Note: The application saves all methods that you create to a database. You cannot save individual methods to a folder that you choose.

Using a Chromeleon-Compatible workbook

A workbook is a saved set of data containing the results from an experiment. You can export this data to a file compatible with the Chromeleon data system.

For Peptide Mapping Analysis, the peptide workbook contains information about the targeted peptides that you can use to run a targeted peptide mapping analysis in either the BioPharma Finder application or in the Chromeleon data system.

For Intact Mass Analysis, the intact workbook contains the processing method parameters and results that you can use to run a targeted intact mass analysis, in the Chromeleon data system as part of the intact deconvolution workflow.

After saving a workbook, you can edit and export it for the Chromeleon data system to import, for both Peptide Mapping Analysis and Intact Mass Analysis.

For more details, see the following topics:

- Managing a workbook (page 150)
- Workbook manager page parameters (page 152)
- Editing a workbook (page 154)
- Workbook editor page parameters (page 157)

Managing a workbook

Use the Workbook Manager page to view a table containing workbook-related information. On this page, you can select a workbook to edit, and also select one or more workbooks to delete or export to a Chromeleon-compatible file.

To manage a workbook

1. On the Home page, click **Peptide Mapping Analysis** or **Intact Mass Analysis** in the left pane or below the splash graphic.
2. Click the **Target Peptide Workbook** or **Intact Workbook** tab.
3. Click the **Workbook Manager** subtab.

   The Workbook Manager page opens showing the saved workbooks in a table.

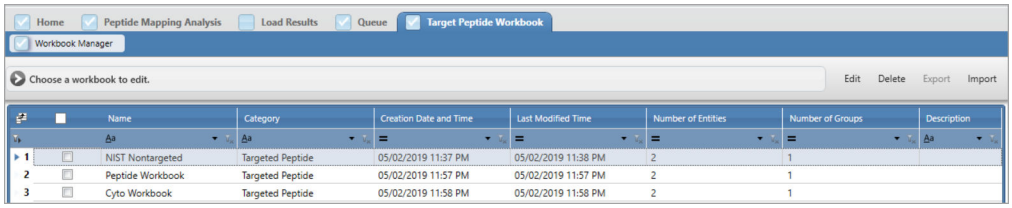


Figure 72 Workbook Manager page for Peptide Mapping workbooks

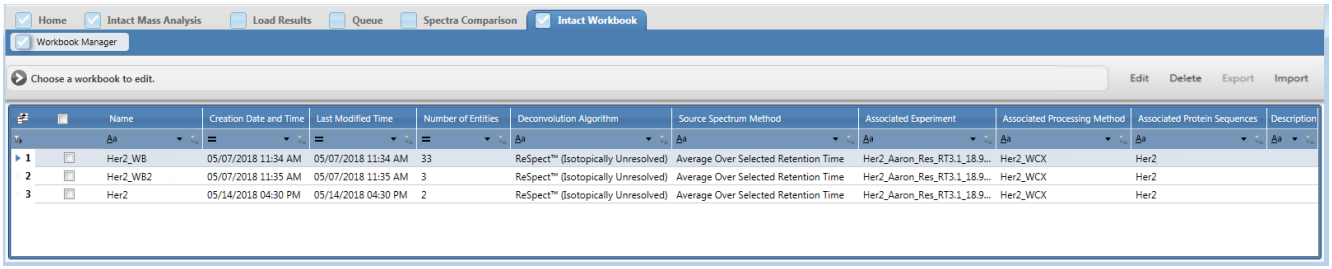


Figure 73 Workbook Manager page for Intact Protein workbooks

4. Do any of the following:

   a. To edit a workbook, select its row in the table and then click **Edit** or double-click the workbook row.

      The applications automatically opens the Workbook Editor page.

   b. To delete one or more workbooks, select their rows (*not* the check box) in the table and then click **Delete** or press the DELETE key.

      If you select one workbook and it is already open on the Workbook Editor page, the application asks you whether you want to close that workbook and then delete it.

      If you select multiple workbooks and one of them is already open on the Workbook Editor page, the application asks you whether you want to close that workbook and then delete it. The application automatically deletes all of the other selected workbooks that are not open.

   c. To export one or more workbooks, select the check box for those workbooks in the table and then click **Export**.

      A dialog box opens at the default folder *drive*:\xcalibur\data\. If this folder does not exist, for the export of a single workbook, the dialog box opens by default at the last accessed folder. For the export of multiple workbooks, the dialog box opens by default at the desktop. You can browse to another folder if necessary.

      The application exports the workbooks to files with the .wbpf extension that are compatible with the Chromeleon data system. The file names are the same as the workbook names. If the folder selected for export already contains files with these names, the application prompts you to confirm overwriting them.

Workbook manager page parameters

The following table describes the types of information in the table on the Workbook Manager page.

Table 18 Workbook Manager table parameters

Column	Description
Row number	The number assigned to each visible workbook row in the table. This sequential numbering does not change when you sort or filter the table.
	Select this check box if you want to export the workbooks in the selected rows to a Chromeleon-compatible file. **Note:** To select or clear all of the check boxes at once, select or clear the check box in the column header. If you filter the table, the following occurs: • Clearing all check boxes affects all of the original rows in the table, before any filtering. • Selecting all check boxes affects only the filtered and currently visible rows.
Name	Displays the name of the saved workbook.
Category	(For Peptide Mapping Analysis only) Displays the category of the protein sequence used to generate the results saved into the workbook.
Creation Date and Time	Displays the date and time for when you created the workbook.
Last Modified Time	Displays the date and time for when you last modified the workbook.
Number of Entities	Displays the number component masses saved into the workbook.
Number of Groups	(For Peptide Mapping Analysis only) Displays the number of component groups saved into the workbook (see the Relative Quantitation Group Number parameter on page 158).
Deconvolution Algorithm	(For Intact Mass Analysis only) Displays the deconvolution algorithm (ReSpect or Xtract) used to generate the results saved into the workbook.
Source Spectrum Method	(For Intact Mass Analysis only) Displays the method used to generate the source spectrum (Average Over RT or Sliding Windows) for the results saved into the workbook.
Associated Experiment	(For Intact Mass Analysis only) Displays the name of the experiment that generated the results saved into the workbook.
Associated Processing Method	(For Intact Mass Analysis only) Displays the name of the processing method used to generate the results saved into the workbook.
Associated Protein Sequences	(For Intact Mass Analysis only) Displays the name of the protein sequence or sequences used to generate the results saved into the workbook.
Description	Displays the description you entered when you saved the workbook.

Importing and exporting a workbook

This feature allows the user to import and/or export a workbook back into the BioPharma Finder. It is available in Intact Mass Analysis and Peptide Mapping Analysis only.

To export a workbook

1. Go to Target Peptide Workbook or Intact Workbook tab
2. Select **Workbook Manager**
3. In the Workbook Manager page select the **Export Workbook** button
4. In the dialog box that appears, browse to the folder. The default folder is c:\xcalibur\data, but user can browse to any folder.The default folder is c:\xcalibur\data, but user can browse to any folder.
5. In the dialog box, browse to the appropriate folder, and click **OK**.
6. A message appears indicating the confirmation of the export
7. In the dialog box, browse to the appropriate folder, and click **OK**.
8. A message appears indicating the confirmation of the export

To import or export a workbook

1. Go the Target Peptide Workbook page or the Intact Workbook page.
2. Click **Workbook Manager**.
3. On the Workbook Manager page, click **Import**.

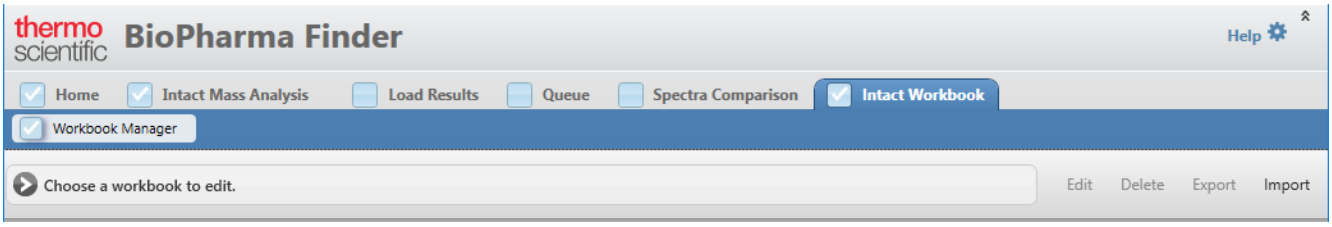


Figure 74 Import/Export a Workbook button

4. In the dialog box that appears, browse to the folder and select the workbook.
5. Select one or multiple files and click **Import**.

   Note: If an existing workbook has the same name as the newly imported workbook, the software tags the new workbook with _imported. Importing a workbook never overwrites an existing workbook.

Editing a workbook

Use the Workbook Editor page to edit workbook-related information. On this page, you can view and modify some of the data saved into the workbook.

To edit a workbook

1. Go to the Workbook Manager page.
2. Select a workbook row in the table on the Workbook Manager page and then click **Edit**, or double-click the workbook row.

   The applications automatically opens the Workbook Editor page and displays information related to the selected workbook in a table.

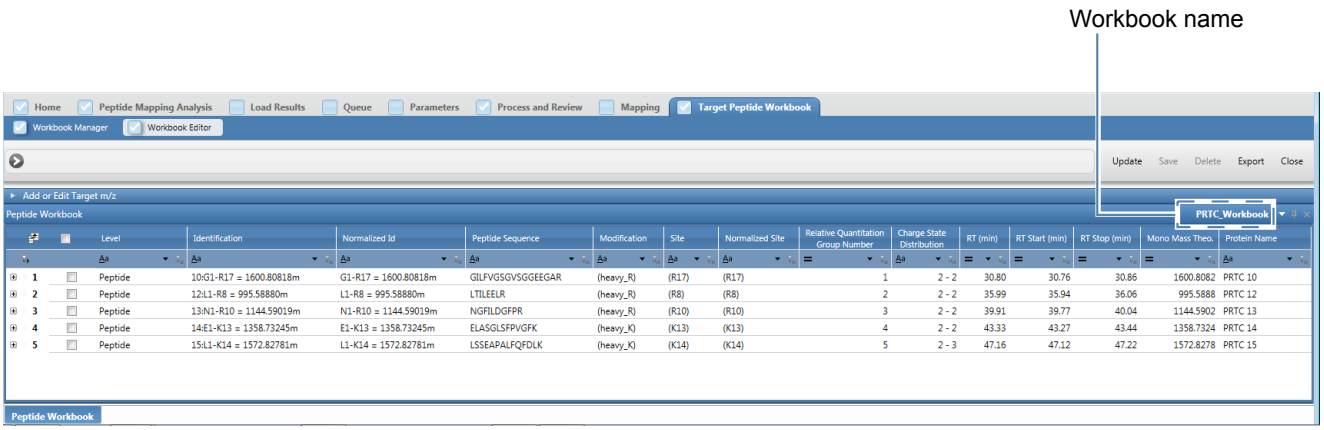


Figure 75 Workbook Editor page for a Peptide Mapping workbook

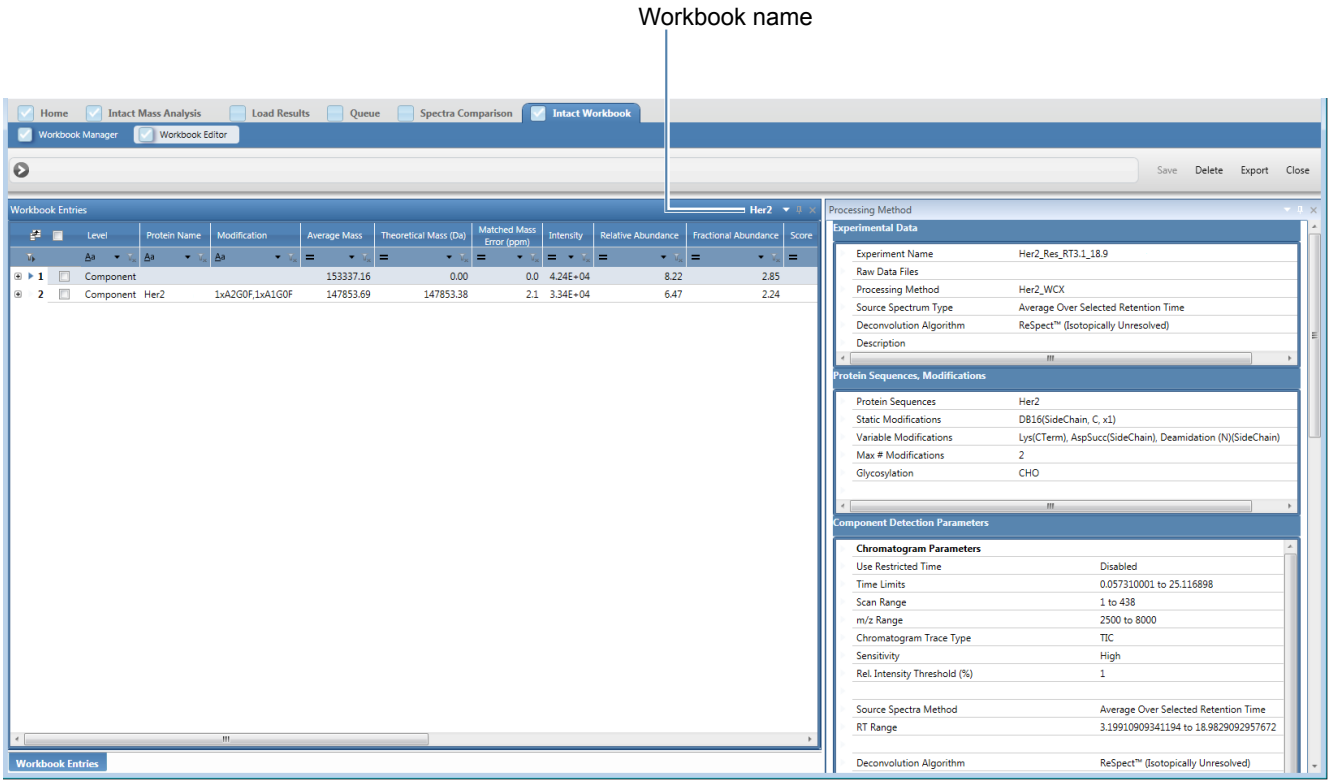


Figure 76 Workbook Editor page for an Intact Protein workbook

The Workbook Editor page for an Intact Protein workbook automatically sorts the components in descending order of Relative Abundance values. The page also displays the processing method summary at the right side because the workbook contains the method parameters except for the reporting parameters.

3. (For Peptide Mapping Analysis only) Do any of the following:
   - Click the **Add or Edit Target m/z** title bar to show the real-time optimization options.

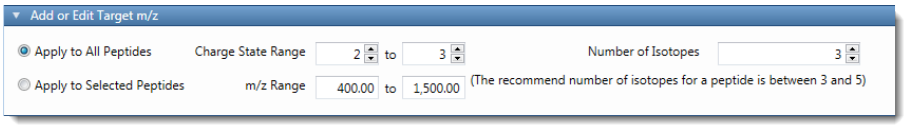


Figure 77 Add or Edit Target m/z pane

You can change the charge state range, *m/z* range, and/or number of isotopes parameters, and then update the workbook without leaving this page.

Select the **Apply to All Peptides** option to apply your changes to all of the peptides in the workbook. Or, select the check boxes for specific peptides in the table, and then select the **Apply to Selected Peptides** option to apply your changes to only the selected peptides. Then, click **Update** in the command bar to view the updated data in the table.

- Edit the values in the following table columns as needed for your targeted search:
  - Relative Quantitation Group Number
  - RT (min)
  - RT Start (min)
  - RT Stop (min)
  - Protein Name

    Note: Use only alphanumeric, space, underscore "_", and period "." characters in the protein name.

4. Do any of the following:
   - To delete one or more entries in the workbook, select their rows in the table and then click **Delete** or press the DELETE key.

     IMPORTANT! For a Peptide Mapping workbook, you cannot delete the last remaining charge state for a particular component or the last remaining isotope for a particular charge state.

     For an Intact Protein workbook, you can only delete a row at the component level.

   - To save your changes to the workbook, click **Save** and then enter the workbook name and description.

     You can enter a new name or retain the current name to overwrite. The saved workbook remains open until you edit another workbook or exit the application.

     Note: The maximum number of components that you can save to a workbook is 250. You cannot add extra items to an existing workbook if it already contains this maximum number of components.
   - To export entries in the workbook, choose from these options:
     - **Export ▸ Export All** to export all entries in the workbook.
     - **Export ▸ Export Checked** to export only the selected entries.

       To select/deselect an entry to export, select/clear the check box in that row.

       To select/deselect all of the entries, select/clear the check box in the column header.

     A dialog box opens at the default folder *drive*:\xcalibur\data\. If this folder does not exist, for the export of a single entry, the dialog box opens by default at the last accessed folder. For the export of multiple entries, the dialog box opens by default at the desktop. You can browse to another folder if necessary.

     The application exports the entries to a file with the .wbpf extension that is compatible with the Chromeleon data system. The file name is the same as the workbook name by default. If the folder selected for export already contains a file with this name, the application prompts you to confirm

overwriting it. If you do not want to overwrite this file, you can enter a different file name.

- To close the workbook, click **Close**.

  If you made changes to the workbook, a prompt asks whether you want to save them.

  The application returns to the Workbook Manager page.

Workbook editor page parameters

The following table describes the types of information in the table on the Workbook Editor page for a Peptide Mapping workbook.

Table 19 Workbook Editor table parameters for a Peptide Mapping workbook

Column	Description
Component level	
+/–	Click to show or hide the lower level of charge state information related to the current component row.
Row number	The number assigned to each visible workbook row in the table. This sequential numbering does not change when you sort or filter the table.
	Select this check box to export a workbook entry. **Note:** To select or clear all of the check boxes at once, select or clear the check box in the column header. If you filter the table, the following occurs: • Clearing all check boxes affects all of the original rows in the table, before any filtering. • Selecting all check boxes affects only the filtered and currently visible rows.
Level	Indicates that the row is displaying peptide information (top level).
Identification, Peptide Sequence, Modification, Site, and Mono Mass Theo.	For more details, see “Results table parameters” on page 250.
Normalized Id	Displays the same information as in the Identification column except that it is specific to an individual peptide in the sequence, derived from the conversion from protein to peptide form. This identification value always starts at position 1.
Normalized Site	Displays the same information as in the Site column except that it is specific to an individual peptide in the sequence, derived from the conversion from protein to peptide form. This site value is derived from the value in the Normalized Id column, not from the value in the Identification column.

Column	Description
Relative Quantitation Group Number	(Editable) Displays the group number that the application automatically assigns to each group of workbook entries with the same sequence and protein values, when you save the workbook. This number groups together the peptides used in the abundance percentage calculation in the modification summary of a targeted peptide mapping experiment. You can edit this value to have full control of which peptides and charge states are part of this calculation. This number starts at 1 for the first group and increases consecutively for each subsequent group.
Charge State Distribution	Displays the range of charge states detected for the component, from the lowest to the highest charge state. See the Charge State column at the lower charge state level.
RT (min)	(Editable) Displays the retention time range for the component from the raw data file with the most abundant MS area. **Note:** If you save the workbook from the Theoretical Protein/Peptide Manager page, this cell displays 0.00 because the retention time is not available.
RT Start (min)	(Editable) Displays the start of the retention time range for the component from the raw data file with the most abundant MS area. **Note:** If you save the workbook from the Theoretical Protein/Peptide Manager page, this cell displays 0.00 because the retention time is not available.
RT Stop (min)	(Editable) Displays the end of the retention time range for the component from the raw data file with the most abundant MS area. **Note:** If you save the workbook from the Theoretical Protein/Peptide Manager page, this cell displays 0.00 because the retention time is not available.
Protein Name	(Editable) Displays the identified protein for the component.
Charge State level	
+/–	Click to show or hide the lower level of isotope information related to the current component row.
Row number	The number assigned to each visible charge state row in the table. This sequential numbering does not change when you sort or filter the table.
Level	Indicates that the row is displaying charge state information (lower level).
M/Z	Displays the mass-to-charge ratio of the calculated monoisotopic mass for each charge state from the raw data file with the most abundant MS area.
Charge State	Displays each charge state for the component from the first raw data file.
Mono Mass	Displays the monoisotopic mass for each charge state from the first raw data file.
Avg. Mass	Displays the average mass for each charge state from the first raw data file. **Note:** If you save the workbook from the Theoretical Protein/Peptide Manager page, this cell displays 0.00 because the average mass is not available.

Column	Description
MS Area	Displays the area for each charge state from the raw data file with the most abundant MS area. **Note:** If you save the workbook from the Theoretical Protein/Peptide Manager page, this cell displays 0.00 because the MS area is not available.
Number of Isotopes	Displays the number of isotopes for each charge state.
Isotope level	
Row number	The number assigned to each visible isotope row in the table. This sequential numbering does not change when you sort or filter the table.
Target m/z	Displays the target mass-to-charge ratio of an isotope.
Type	Displays the isotope type.

The following table describes the types of information in the table on the Workbook Editor page for an Intact Protein workbook.

Table 20 Workbook Editor table parameters for an Intact Protein workbook

Column	Description
Component level	
+/–	Shows or hides the lower level of charge state information related to the current component row.
Row number	The number assigned to each visible workbook row in the table. This sequential numbering does not change when you sort or filter the table.
	Selected check box indicates workbook entry for export. **Note:** To select or clear all of the check boxes at once, select or clear the check box in the column header. If you filter the table, the following occurs: • Clearing all check boxes affects all of the original rows in the table, before any filtering. • Selecting all check boxes affects only the filtered and currently visible rows.
Level	Indicates that the row is displaying component information (top level).
Various columns for different component parameters	For more details on these parameters, depending on the type of experiment (batch, multiconsensus, target sequence matching, or DAR-enabled), the deconvolution algorithm, and the source spectrum method, see Chapter 22, “Various results tables for intact mass analysis”.
Charge State level	
Row number	The number assigned to each visible charge state row in the table. This sequential numbering does not change when you sort or filter the table.

Column	Description
Level	Indicates that the row is displaying charge state information (lower level).
Various columns for different charge state parameters	For more details on these parameters, depending on the type of experiment (batch, multiconsensus, target sequence matching, or DAR-enabled), the deconvolution algorithm, and the source spectrum method, see Chapter 22, “Various results tables for intact mass analysis”.

8 Using the run queue

The BioPharma Finder application processes one submitted job at a time. When you submit multiple jobs for processing, the jobs wait for their turn to be processed in the run queue on the Queue page for Peptide Mapping Analysis, Oligonucleotide Analysis, Intact Mass Analysis in automatic mode, and Top Down Analysis.

While the application is processing experimental jobs in the run queue, you can still create new methods or edit existing methods (even if the submitted jobs use those methods). This is because the application saves a copy of the method parameters when it saves the experiment, so subsequent method changes do not affect the processing.

Managing the run queue for peptide mapping analysis

For Peptide Mapping Analysis, the BioPharma Finder application processes multiple jobs in the queue in the *reverse* order that you submitted them; that is, it processes the *most recently* submitted job first and places it at the top of the queue.

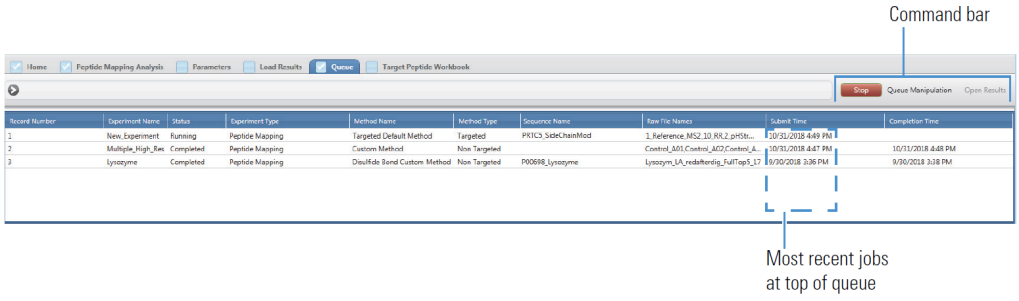


Figure 78 Queue page for Peptide Mapping Analysis

By default, the BioPharma Finder application processes the jobs in the queue as follows:

- If the queue is currently empty, it places your new submitted job in the first row of the queue. Unless you pause the queue, the application runs the new job immediately and sets its status to Running. Otherwise, the job waits until you resume processing and its status is Submitted.
- If a job is currently processing in the queue, it places your new submitted job with a Submitted status one row below the currently running job, above all other waiting jobs. Other previously queued jobs move down one row and wait for processing after the new job.
- If all of the jobs in the queue have already completed processing or are canceled, it places your new submitted job in the first row of the queue. All of the completed or canceled jobs move down one row. Unless you pause the queue, the application runs the new job immediately and sets its status to Running. Otherwise, the job waits until you resume processing and its status is Submitted.
- If no jobs are running because you paused the queue and other jobs are waiting for processing, it places the new submitted job with a Submitted status in the highest row among the waiting jobs. Other previously queued jobs move down one row and wait for processing after the new job.

When the application finishes processing the currently running job, the table on the Queue page displays the following changes for this job:

- The Status column changes to Completed.
- The Completion Time column displays the date and time when the run finished.

For more details, see the following topics:

- Pausing the run queue (page 162)
- Resuming the paused queue (page 163)

Once the status displayed in the Status column shows Completed, you can open the results and view the processed data.

Pausing the run queue

If you are running a job and realize that you need to change parameters, you can click Stop to cancel the processing of the experiment. The application pauses the run queue until you click Run again. Use the method editor to make those parameter changes and save them to a different method. Resubmit this job with that method and the application places it below all other already submitted jobs.

To stop the processing of the currently running job and pause the run queue

1. Click the **Queue** tab to open the Queue page if necessary.
2. Click **Stop** in the command bar.

   Note: The Stop button is enabled only when the queue contains one or more jobs and the Status column shows Running for the currently running job.

The application cancels the processing of the currently running job. That job's processing status changes to Cancelled. The application then pauses the queue. The rest of the jobs remain in the run queue in the Submitted state. The Stop button changes to a Run button.

If you want to rerun the canceled job, you must resubmit it by creating a new experiment.

While the queue is paused, you can add new jobs to the queue as needed. These jobs all have the Submitted status.

Resuming the paused queue

To resume processing jobs in the paused queue

1. Click the **Queue** tab to open the Queue page if necessary.

   The paused queue shows jobs with the Submitted status that await processing.

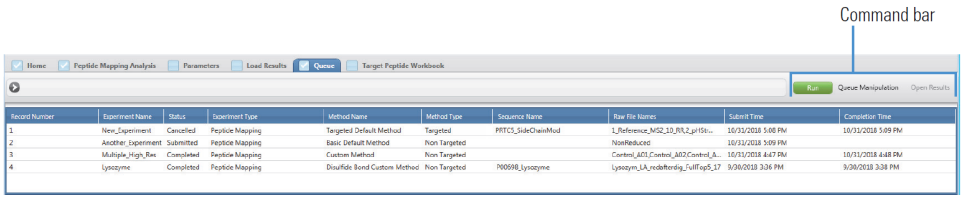


Figure 79 Paused queue for Peptide Mapping Analysis

2. Click **Run** in the command bar.

   The Run button changes to a Stop button. The application starts processing the first job in the queue with the Submitted status. After a few moments, the status of that job changes to Running. When the processing is completed, the application automatically continues to process subsequent jobs in the queue with the Submitted status.

If you want to close the application while a job is actively processing, you can stop the queue to cancel the processing of the current job. The application does not save any results for that job and the Status column for this job changes to Cancelled. If you want to rerun this job, you must resubmit it by creating a new experiment.

When you restart the application and open the Queue page, if there are submitted experiments, the queue remains in a paused state. Click Run to begin processing the job at the top of the queue with a Submitted status and to continue to process other queued jobs in order.

Managing the run queue for Oligonucleotide Analysis

For Oligonucleotide Analysis, the BioPharma Finder application processes multiple jobs in the queue in the *reverse* order that you submitted them; that is, it processes the *most recently* submitted job first and places it at the top of the queue.

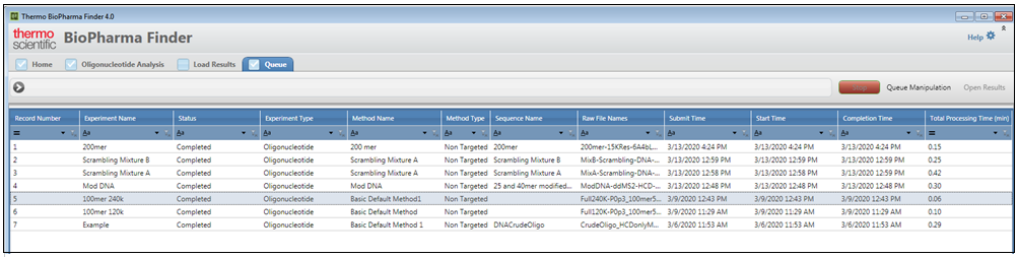


Figure 80 Queue page for Oligonucleotide Analysis

By default, the BioPharma Finder application processes the jobs in the queue as follows:

- If the queue is currently empty, it places your new submitted job in the first row of the queue. Unless you pause the queue, the application runs the new job immediately and sets its status to Running. Otherwise, the job waits until you resume processing and its status is Submitted.
- If a job is currently processing in the queue, it places your new submitted job with a Submitted status one row below the currently running job, above all other waiting jobs. Other previously queued jobs move down one row and wait for processing after the new job.
- If all of the jobs in the queue have already completed processing or are canceled, it places your new submitted job in the first row of the queue. All of the completed or canceled jobs move down one row. Unless you pause the queue, the application runs the new job immediately and sets its status to Running. Otherwise, the job waits until you resume processing and its status is Submitted.
- If no jobs are running because you paused the queue and other jobs are waiting for processing, it places the new submitted job with a Submitted status in the highest row among the waiting jobs. Other previously queued jobs move down one row and wait for processing after the new job.

When the application finishes processing the currently running job, the table on the Queue page displays the following changes for this job:

- The Status column changes to Completed.
- The Completion Time column displays the date and time when the run finished.

For more details, see the following topics:

- “Pausing the run queue” on page 165
- “Resuming the paused queue” on page 165

Once the status displayed in the Status column shows Completed, you can open the results and view the processed data.

Pausing the run queue

If you are running a job and realize that you need to change parameters, you can click Stop to cancel the processing of the experiment. The application pauses the run queue until you click Run again. Use the method editor to make those parameter changes and save them to a different method. Resubmit this job with that method and the application places it below all other already submitted jobs.

To stop the processing of the currently running job and pause the run queue

1. Click the **Queue** tab to open the Queue page if necessary.
2. Click **Stop** in the command bar.

   Note: The Stop button is enabled only when the queue contains one or more jobs and the Status column shows Running for the currently running job.

   The application cancels the processing of the currently running job. That job's processing status changes to Cancelled. The application then pauses the queue. The rest of the jobs remain in the run queue in the Submitted state. The Stop button changes to a Run button.

   If you want to rerun the canceled job, you must resubmit it by creating a new experiment.

   While the queue is paused, you can add new jobs to the queue as needed. These jobs all have the Submitted status.

Resuming the paused queue

To resume processing jobs in the paused queue

1. Click the **Queue** tab to open the Queue page if necessary.

   The paused queue shows jobs with the Submitted status that await processing.
2. Click **Run** in the command bar.

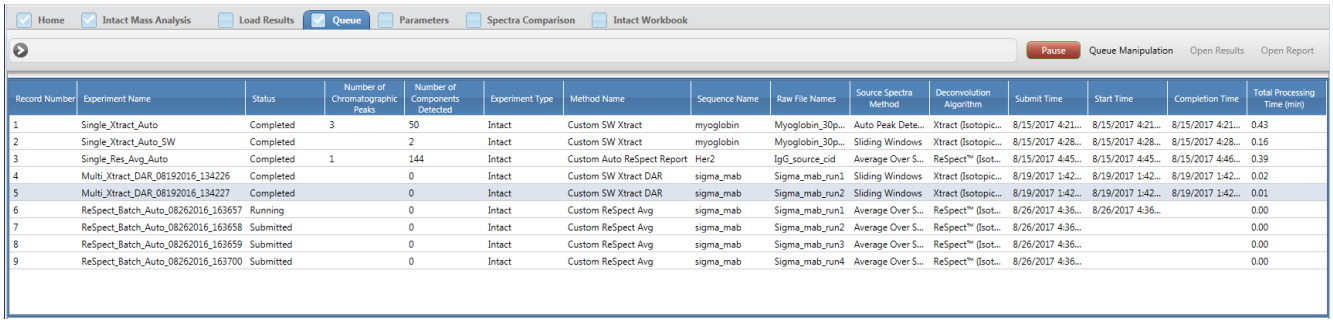
   The Run button changes to a Stop button. The application starts processing the first job in the queue with the Submitted status. After a few moments, the status of that job changes to Running. When the processing is completed, the application automatically continues to process subsequent jobs in the queue with the Submitted status.

If you want to close the application while a job is actively processing, you can stop the queue to cancel the processing of the current job. The application does not save any results for that job and the Status column for this job changes to Cancelled. If you want to rerun this job, you must resubmit it by creating a new experiment.

When you restart the application and open the Queue page, if there are submitted experiments, the queue remains in a paused state. Click Run to begin processing the job at the top of the queue with a Submitted status and to continue to process other queued jobs in order.

Managing the run queue for intact mass analysis

The BioPharma Finder application processes multiple jobs in the queue differently for Intact Mass Analysis than it does for Peptide Mapping Analysis—that is, it processes jobs in the order in which they were received, rather than the most recent submissions first. The application continues to process other submitted jobs after the completion of the top rows in the queue.



Record Number	Experiment Name	Status	Number of Chromatographic Peaks	Number of Components Detected	Experiment Type	Method Name	Sequence Name	Raw File Names	Source Spectra Method	Deconvolution Algorithm	Submit Time	Start Time	Completion Time	Total Processing Time (min)
1	Single_Xtract_Auto	Completed	3	50	Intact	Custom SW Xtract	myoglobin	Myoglobin_30p...	Auto Peak Dete...	Xtract (Isotopic...	8/15/2017 4:21...	8/15/2017 4:21...	8/15/2017 4:21...	0.43
2	Single_Xtract_Auto_SW	Completed		2	Intact	Custom SW Xtract	myoglobin	Myoglobin_30p...	Sliding Windows	Xtract (Isotopic...	8/15/2017 4:28...	8/15/2017 4:28...	8/15/2017 4:28...	0.16
3	Single_Res_Avg_Auto	Completed	1	144	Intact	Custom Auto ReSpect Report	Her2	IgG_source_cid	Average Over S...	ReSpect™ (Isot...	8/15/2017 4:45...	8/15/2017 4:45...	8/15/2017 4:46...	0.39
4	Multi_Xtract_DAR_08192016_134226	Completed		0	Intact	Custom SW Xtract DAR	sigma_mab	Sigma_mab_run1	Sliding Windows	Xtract (Isotopic...	8/19/2017 1:42...	8/19/2017 1:42...	8/19/2017 1:42...	0.02
5	Multi_Xtract_DAR_08192016_134227	Completed		0	Intact	Custom SW Xtract DAR	sigma_mab	Sigma_mab_run2	Sliding Windows	Xtract (Isotopic...	8/19/2017 1:42...	8/19/2017 1:42...	8/19/2017 1:42...	0.01
6	ReSpect_Batch_Auto_08262016_163657	Running		0	Intact	Custom ReSpect Avg	sigma_mab	Sigma_mab_run1	Average Over S...	ReSpect™ (Isot...	8/26/2017 4:36...	8/26/2017 4:36...		0.00
7	ReSpect_Batch_Auto_08262016_163658	Submitted		0	Intact	Custom ReSpect Avg	sigma_mab	Sigma_mab_run2	Average Over S...	ReSpect™ (Isot...	8/26/2017 4:36...			0.00
8	ReSpect_Batch_Auto_08262016_163659	Submitted		0	Intact	Custom ReSpect Avg	sigma_mab	Sigma_mab_run3	Average Over S...	ReSpect™ (Isot...	8/26/2017 4:36...			0.00
9	ReSpect_Batch_Auto_08262016_163700	Submitted		0	Intact	Custom ReSpect Avg	sigma_mab	Sigma_mab_run4	Average Over S...	ReSpect™ (Isot...	8/26/2017 4:36...			0.00

Figure 81 Queue page for Intact Mass Analysis in automatic mode

By default, the application processes the jobs in the queue as follows:

- If the queue is currently empty, it places your new submitted job in the first row of the queue. Unless you pause the queue, the application runs the new job immediately and sets its status to Running. Otherwise, the job waits until you resume processing and its status is Submitted.
- If a job is currently processing in the queue, it places your new submitted job with a Submitted status below all other waiting jobs.
- If all of the jobs in the queue have already completed processing or are canceled, it places the new submitted job at the bottom of the queue. Unless you pause the queue, the application runs the new job immediately and sets its status to Running. Otherwise, the job waits until you resume processing and its status is Submitted.
- If no jobs are running because you paused the queue and other jobs are waiting for processing, it places your new job with a Submitted status below all of the waiting jobs.
- For an experiment using the Batch Processing result format, it processes the first experiment in the batch, and the remaining experiments wait for processing with a Submitted status. When the application completes the first experiment, it continues to process the remaining experiments in the batch in order.

When the application finishes processing the currently running job, the table on the Queue page displays the following changes for this job:

- The Status column changes to Completed.
- The Completion Time column displays the date and time when the run finished.
- The Total Processing Time column displays the total time used to process the experiment.

Note: If you process an experiment in manual mode or use real-time optimization to reprocess an experiment, the Total Processing Time cell displays 0.00. This cell displays the actual total processing time only when you process an experiment in the run queue.

For more details, see the following topics:

- Pausing the run queue (page 167)
- Resuming the paused queue (page 167)

Once the status displayed in the Status column shows Completed, you can open the results or a report and view the processed data.

Pausing the run queue

If you are running a job and realize that you need to change parameters, you can still use the manual mode or the method editor to make those parameter changes and save them to a different method. Just resubmit this job with that method, and the application places it below all other already submitted jobs.

To pause the run queue

1. Click the **Queue** tab to open the Queue page if necessary.
2. Click **Pause** in the command bar.

   Note: The Pause button is enabled only when the queue contains one or more jobs and the Status column shows Running for the currently running job.

   The application continues the processing of the currently running job until that job's processing status changes to Completed. The application then pauses the queue. The rest of the jobs remain in the run queue in the Submitted state. The Pause button changes to a Run button.

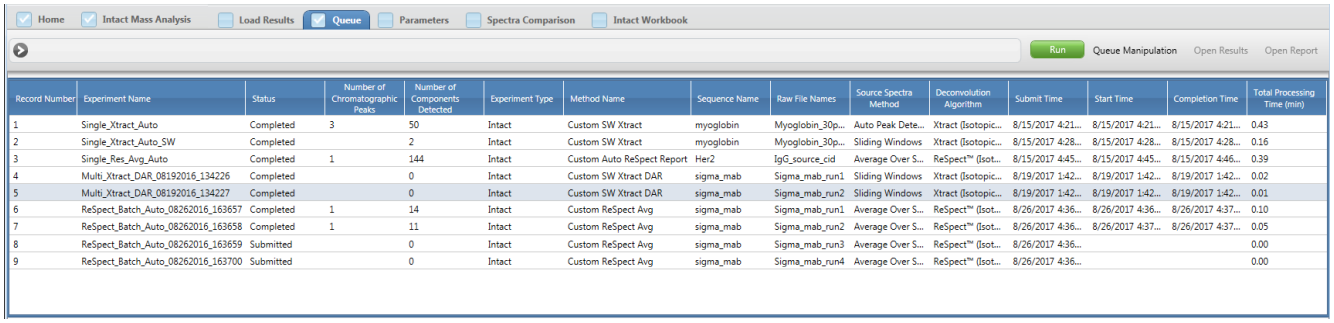
   While the queue is paused, you can add new jobs to the queue as needed. These jobs all have the Submitted status.

Resuming the paused queue

To resume processing jobs in the paused queue

1. Click the **Queue** tab to open the Queue page if necessary.

   The paused queue shows jobs with the Submitted status that await processing.



Record Number	Experiment Name	Status	Number of Chromatographic Peaks	Number of Components Detected	Experiment Type	Method Name	Sequence Name	Raw File Names	Source Spectra Method	Deconvolution Algorithm	Submit Time	Start Time	Completion Time	Total Processing Time (min)
1	Single_Xtract_Auto	Completed	3	50	Intact	Custom SW Xtract	myoglobin	Myoglobin_30p...	Auto Peak Dete...	Xtract (Isotopic...	8/15/2017 4:21...	8/15/2017 4:21...	8/15/2017 4:21...	0.43
2	Single_Xtract_Auto_SW	Completed		2	Intact	Custom SW Xtract	myoglobin	Myoglobin_30p...	Sliding Windows	Xtract (Isotopic...	8/15/2017 4:28...	8/15/2017 4:28...	8/15/2017 4:28...	0.16
3	Single_Res_Avg_Auto	Completed	1	144	Intact	Custom Auto ReSpect Report	Her2	IgG_source_cid	Average Over S...	ReSpect™ (Isot...	8/15/2017 4:45...	8/15/2017 4:45...	8/15/2017 4:46...	0.39
4	Multi_Xtract_DAR_08192016_134226	Completed		0	Intact	Custom SW Xtract DAR	sigma_mab	Sigma_mab_run1	Sliding Windows	Xtract (Isotopic...	8/19/2017 1:42...	8/19/2017 1:42...	8/19/2017 1:42...	0.02
5	Multi_Xtract_DAR_08192016_134227	Completed		0	Intact	Custom SW Xtract DAR	sigma_mab	Sigma_mab_run2	Sliding Windows	Xtract (Isotopic...	8/19/2017 1:42...	8/19/2017 1:42...	8/19/2017 1:42...	0.01
6	ReSpect_Batch_Auto_08262016_163657	Completed	1	14	Intact	Custom ReSpect Avg	sigma_mab	Sigma_mab_run1	Average Over S...	ReSpect™ (Isot...	8/26/2017 4:36...	8/26/2017 4:36...	8/26/2017 4:37...	0.10
7	ReSpect_Batch_Auto_08262016_163658	Completed	1	11	Intact	Custom ReSpect Avg	sigma_mab	Sigma_mab_run2	Average Over S...	ReSpect™ (Isot...	8/26/2017 4:36...	8/26/2017 4:37...	8/26/2017 4:37...	0.05
8	ReSpect_Batch_Auto_08262016_163659	Submitted		0	Intact	Custom ReSpect Avg	sigma_mab	Sigma_mab_run3	Average Over S...	ReSpect™ (Isot...	8/26/2017 4:36...			0.00
9	ReSpect_Batch_Auto_08262016_163700	Submitted		0	Intact	Custom ReSpect Avg	sigma_mab	Sigma_mab_run4	Average Over S...	ReSpect™ (Isot...	8/26/2017 4:36...			0.00

Figure 82 Paused queue for Intact Mass Analysis

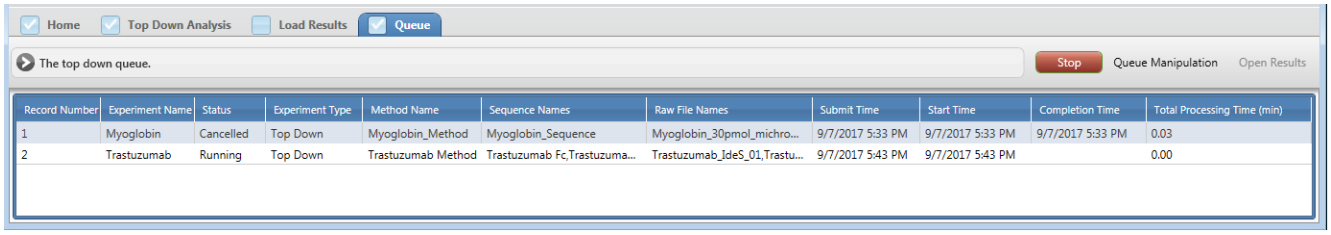
2. Click **Run** in the command bar.

   The Run button changes to a Pause button. The application starts processing the first job in the queue with the Submitted status. After a few moments, the status of that job changes to Running. When the processing is completed, the application automatically continues to process subsequent jobs in the queue with the Submitted status.

If you want to close the application while a job is actively processing, you can pause the queue and wait for the processing of the current job to complete. When you restart the application in this case and you choose to continue processing when prompted, the application opens the Queue page, begins processing the job at the top of the queue with a Submitted status, and continues to process other queued jobs in order. Otherwise, the queue remains in a paused state.

Managing the run queue for top down analysis

For Intact Mass Analysis and Top Down Analysis, jobs are processed in the order in which they are received, rather than the most recent submissions first. The application continues to process other submitted jobs after completing those in the top rows of the queue.



Record Number	Experiment Name	Status	Experiment Type	Method Name	Sequence Names	Raw File Names	Submit Time	Start Time	Completion Time	Total Processing Time (min)
1	Myoglobin	Cancelled	Top Down	Myoglobin_Method	Myoglobin_Sequence	Myoglobin_30pmol_michro...	9/7/2017 5:33 PM	9/7/2017 5:33 PM	9/7/2017 5:33 PM	0.03
2	Trastuzumab	Running	Top Down	Trastuzumab Method	Trastuzumab Fc,Trastuzuma...	Trastuzumab_IdeS_01,Trastu...	9/7/2017 5:43 PM	9/7/2017 5:43 PM		0.00

Figure 83 Queue page for Top Down Analysis

By default, the application processes the jobs in the queue as follows:

- If the queue is currently empty, it places your new submitted job in the first row of the queue. Unless you pause the queue, the application runs the new job immediately and sets its status to Running. Otherwise, the job waits until you resume processing and its status is Submitted.
- If a job is currently processing in the queue, it places your new submitted job with a Submitted status below all other waiting jobs.
- If all of the jobs in the queue have already completed processing or are canceled, it places the new submitted job at the bottom of the queue. Unless you pause the queue, the application runs the new job immediately and sets its status to Running. Otherwise, the job waits until you resume processing and its status is Submitted.
- If no jobs are running because you paused the queue and other jobs are waiting for processing, it places your new job with a Submitted status below all of the waiting jobs.
- For an experiment using the Batch Processing result format, it processes the first experiment in the batch, and the remaining experiments wait for processing with a Submitted status. When the application completes the first experiment, it continues to process the remaining experiments in the batch in order.

When the application finishes processing the currently running job, the table on the Queue page displays the following changes for this job:

- The Status column changes to Completed.
- The Completion Time column displays the date and time when the run finished.
- The Total Processing Time column displays the total time used to process the experiment.

Note: If you use real-time optimization to reprocess an experiment, the Total Processing Time cell displays 0.00. This cell displays the actual total processing time only when you process an experiment in the run queue.

For more details, see the following topics:

- Pausing the run queue (page 170)
- Resuming the paused queue (page 170)

Once the status displayed in the Status column shows Completed, you can open the results and view the processed data.

Pausing the run queue

If you must change parameters while a job is running, you can click Stop to cancel the processing of the experiment. The run queue pauses until you click Run again. Use the method editor to make those parameter changes and save them to a different method. Resubmit this job with that method and it appears below all other already submitted jobs.

To stop the processing of the currently running job and pause the run queue

1. Click the **Queue** tab to open the Queue page if necessary.
2. Click **Stop** in the command bar.

   Note: The Stop button is enabled only when the queue contains one or more jobs and the Status column shows Running for the currently running job.

   The Stop button changes to a Run button. The application cancels the processing of the currently running job, changes its status to Cancelled, and then pauses the queue. The rest of the jobs remain in the run queue in the Submitted state.

   If you want to rerun the canceled job, you must resubmit it by creating a new experiment.

   While the queue is paused, you can add new jobs as needed. These jobs have the Submitted status.

Resuming the paused queue

To resume processing jobs in the paused queue

1. Click the **Queue** tab to open the Queue page if necessary.

   The paused queue shows jobs with the Submitted status that await processing.

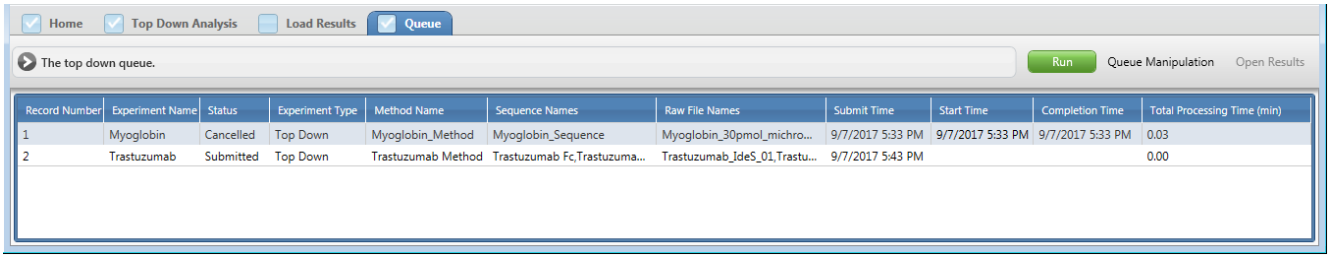


Figure 84 Paused queue for Top Down Analysis

2. Click **Run** in the command bar.

   The Run button changes to a Stop button. The application starts processing the first job in the queue with the Submitted status. After a few moments, the status of that job changes to Running. When the processing is completed, the application automatically continues to process subsequent jobs in the queue with the Submitted status.

You can close the application even when a job is actively processing by clicking Stop. Results are not saved, and Cancelled appears in the Status column. To rerun the job, you must resubmit it as a new experiment.

If there are submitted experiments on the Queue page when you restart the application, they remain in a paused state. Click Run to begin processing them, starting from the top.

Using common run queue features

The following features function the same way in the run queue for Peptide Mapping Analysis, Intact Mass Analysis, and Top Down Analysis:

- Removing selected jobs (page 171)
- Removing completed jobs (page 171)
- Removing all jobs (page 171)

Removing selected jobs

To remove a selected job or jobs from the queue

1. In the table on the Queue page, select the job or jobs to remove from the queue. Remove multiple jobs by selecting either contiguous job names using the SHIFT key or noncontiguous job names using the CTRL key.
2. In the command bar, choose **Queue Manipulation ▸ Remove Selected**.

   Note: You cannot remove a job when it is still processing and its status shows "Running".
3. In the confirmation dialog box, click **Yes**.

Removing completed jobs

To remove all completed jobs from the queue

1. In the command bar on the Queue page, choose **Queue Manipulation ▸ Remove Completed**.
2. In the confirmation dialog box, click **Yes**.

Removing all jobs

To remove all jobs from the queue

1. In the command bar on the Queue page, choose **Queue Manipulation ▸ Remove All**.

   Note: You cannot remove all jobs when a job is still processing and its status shows "Running."
2. In the confirmation dialog box, click **Yes**.

Queue page parameters

The following table describes the commands and parameters on the Queue page.

Table 21 Queue page parameters

Parameter	Description
Command bar	
Stop/Pause/Run	(For Peptide Mapping Analysis, Oligonucleotide Analysis and Top Down Analysis only) Stop cancels the processing of the currently running job and pauses the queue. The button changes to Run. (For Intact Mass Analysis only) Pause completes the processing of the currently running job and pauses the queue. The button changes to Run. Run resumes the processing of the remaining jobs in the queue. The button changes to Stop/Pause.
Queue Manipulation	Contains commands that you use to remove jobs from the queue.
Remove Selected	Removes the selected job or jobs from the queue.
Remove Completed	Removes all completed jobs from the queue.
Remove All	Removes all jobs from the queue.
Open Results	Transfers you to the Process and Review page that displays the results after the application finishes processing a job.
Open Report	(For Intact Mass Analysis only) Transfers you to the Reporting page to view the resulting report after the application finishes processing an experiment.
Queue table	
Record Number	Displays a sequential number that identifies each experiment in the queue.
Experiment Name	Displays the name of the experiment.

Parameter	Description
Status	Displays the real-time status of the analysis: • Submitted: The experiment is waiting for processing. • Running: The application is analyzing the experiment. • Completed: The application has finished analyzing the experiment and has generated the results (and reports for Intact Mass Analysis). • (For Intact Mass Analysis only) Completed, Report Not Concatenated: The application has finished analyzing the experiment but has not generated the reports because of size constraints. • Cancelled: You stopped the processing of the experiment, so the application did not generate the results (or reports for Intact Mass Analysis). • Aborted: The application crashed or stopped during the processing and cannot generate the results (or reports for Intact Mass Analysis). • Error, possible memory issue, cannot continue the execution of current operation: There is not enough memory to process the job. If the application cannot process the job, be sure that the type of data being processed is appropriate. Reduce the amount of data by adjusting the retention time range, S/N threshold, or *m/z* range. Then restart the application if needed and resubmit the experiment for processing.
Experiment Type	Displays the type of experiment: Peptide Mapping, Oligonycleotide, Intact, or Top Down.
Method Name	Displays the processing method used to run the experiment.
Method Type	(For Peptide Mapping Analysis only) Displays the type of peptide mapping method: Non Targeted or Targeted.
Sequence Names	(Optional) Displays the name of the protein sequence or sequences assigned to the experiment.
Raw File Names	Displays the name of the raw data file or files loaded for the experiment.
Source Spectra Method	(For Intact Mass Analysis only) Displays the method used to select the source spectra for deconvolution: Sliding Windows, Auto Peak Detection, or Average Over Selected Retention Time.
Deconvolution Algorithm	(For Intact Mass Analysis only) Displays the algorithm used for deconvolution: Xtract (Isotopically Resolved) or ReSpect (Isotopically Unresolved).
Number of Chromatographic Peaks	(For Intact Mass Analysis average over RT deconvolution or auto peak detection experiments only) Displays the number of chromatographic peaks detected in the spectrum. If the application finds no chromatographic peaks or proteins, the value is 0.
Number of Components Detected	(For Intact Mass Analysis only) Displays the number of components detected from the deconvolution of each averaged spectrum from all found chromatographic peaks. If the application finds no chromatographic peaks or proteins, the value is 0.
Submit Time	Displays the date and time that you placed the experiment into the run queue.
Start Time	(For Intact Mass Analysis and Top Down Analysis only) Displays the date and time that the processing started for the given experiment.

Parameter	Description
Completion Time	Displays the date and time that the processing finished for the given experiment.
Total Processing Time (min)	(For Intact Mass Analysis and Top Down Analysis only) Displays the total time used to process the given experiment.

Part IV

Peptide Mapping Analysis

9 Peptide mapping analysis features

Use the Peptide Mapping Analysis features in the BioPharma Finder application to analyze data-dependent LC/MS2 or full-scan LC/MS data from a proteolytic digestion of a few relatively pure proteins, such as therapeutic proteins. This type of analysis provides a high-confidence platform for fully automated characterization of these proteins. It performs component detection, peptide identification, and identification and quantification of post-translational modifications (PTMs) using the data from Thermo Scientific™ mass spectrometers.

Experiment results display

Peptide Mapping Analysis displays the results of a completed experiment in a Results table that is organized by components at the upper level and by raw data files at the lower level. You can export the data in the Results table to a Microsoft™ Excel™, a CSV, or a Mascot™ Generic Format (MGF) file. You can also save the data to a peptide workbook for exporting to a file compatible with the Chromeleon data system or for running a targeted peptide mapping analysis using the workbook as a protein sequence.

The analysis output also includes the Modification Summary page, which shows the recovery status and abundance of all detected modifications, and the Components table displaying the components of a completed experiment related to a particular modification.

Peptide Mapping Analysis provides the results in chromatograms, trend ratio and trend MS area plots, HDX plot, peptide fragment coverage maps, protein sequence maps, full-scan spectra, and MS2 spectra, including predicted kinetic MS2 spectra. You can view MS2 spectra for each fragmentation type and resolution combination.

The BioPharma Finder application generates both a BPC plot, which shows only the most intense peak in each spectrum, and the selected ion chromatogram (SIC) plot, which plots the intensity of the signal observed at a chosen mass-to-charge ratio (*m/z*). You can view the BPC in different shades of color to indicate unidentified proteins and identified proteins by type.

Quantification of modifications

You can use the Modification Summary page to see the amount of a specific modification in the sample compared to the unmodified material. For example, if the amino acid at position 32 in an antibody becomes oxidized as you create the molecule, you must know how much of this oxidized amino acid is present in the sample and determine if the level is too high. The BioPharma Finder application determines this level automatically.

Sequence variant analysis with Error-Tolerant search

Peptide Mapping Analysis offers a specific feature for detecting and characterizing unanticipated sequence variants using the specialized amino acid substitution search. Not only does the BioPharma Finder application identify low-level modification using MS2 spectra, it also automatically provides you with the abundance percentage in the sample. It achieves deeper characterization by using an error-tolerant search to identify unknown modifications. It searches user-definable mass ranges against identified peptides and easily identifies unexpected modifications.

De novo sequencing

You can use de novo sequencing to identify components that contain MS2 spectra that were not associated with the protein sequence imported into the processing method. Through de novo sequencing, you can identify unexpected containments or product-related impurities that can pose concerns regarding bioactivity, stability, and immunogenicity of the biotherapeutics.

Disulfide mapping

Peptide Mapping Analysis provides you with a specific processing method for characterization of disulfide bond linkage. You can perform disulfide mapping by processing an unreduced sample. It provides even more confidence when it processes both unreduced and reduced samples together in the same experiment. You can use new plotting features to display the SIC for individual peptides in multiple files. This chromatogram gives you a visual aid to identify disulfide bond peptides. (Disulfide bond peptides appear in the unreduced samples but are not present in the reduced sample.) No previous knowledge of linkage sites is required, so MS/MS can identify and confirm new linkages and potential disulfide-bond scrambling.

Localization of glycosylation sites on glycopeptides

You can use Peptide Mapping Analysis to characterize the presence of common and uncommon N-linked/O-linked glycosylation in biotherapeutics.

For example, monoclonal antibodies (mAb) are glycoproteins and contain a conserved N-linked glycosylation site on the Fc region in the CH2 domain. This modification has many known physical functions in the molecule, and characterization is extremely important.

N-linked glycosylation occurs as a post-translational modification and as a co-translational process through which carbohydrates (glycans) are added to an asparagine (N) at the consensus motif asparagine-*X*-serine/threonine (NXS/T), where *X* is any amino acid except proline. The BioPharma Finder search algorithm uses this consensus motif when identifying this specific type of modification.

O-linked glycosylation usually occurs as the linkage between N-Acetylgalactosamine and the amino acids serine (S) or threonine (T).

The application has two different N-linked glycan databases (the CHO cell line or the human cell line) that you can select. They contain over 200 different glycan structures so that you can potentially identify unexpected modifications.

The appendix lists all of the N-linked glycans included in the default modifications list, the N-linked glycans included in the N-glycan-specific search and sorted by host cell-line type, and the O-linked glycans that are supported by Peptide Mapping Analysis.

Running a hydrogen deuterium exchange experiment

A typical Hydrogen Deuterium Exchange (HDX) experiment involves comparing conformation of the same protein sequence at different conditions, for example, when free or bound to another molecule. You specify the individual conformation conditions with unique names, such as A, B, C, and so on. You can compare up to 26 different conformation conditions from A to Z. If you have a control condition, name it A.

Unless you know that the residue is heavily modified, Thermo Fisher Scientific recommends that you do *not* use modified peptides for HDX modeling. The HDX modeling assumes that only one major form exists in the sample—that is, each residue has only one protection factor, regardless of whether nearby residues are modified or not. Therefore, the sample is assumed to be pure; for example, a methionine is either not oxidized or 100 percent oxidized. The exception is glycosylation, due to its variable nature.

For more details, see the following topics:

- HDX deuterium labeling (page 179)
- Collecting HDX data (page 180)
- Processing an HDX experiment (page 180)
- HDX output (page 181)

HDX deuterium labeling

The BioPharma Finder application performs deuterium labeling at different time intervals to obtain time courses for each conformation condition. It uses a 5-parameter equation to fit these curves to calculate the variance in the data. To get a good estimate of the variance, Thermo Fisher Scientific suggests that you collect a minimum of 12 data points (six time points with duplicate measurements) for each conformation condition.

You must analyze unlabeled protein samples with data-dependent MS/MS for peptide identification purposes. Repeat the run several times to maximize peptide identification. Separate precursor selections by mass range or charge state in these runs to maximize peptide identification. For example, perform MS/MS of doubly charged precursors in the first run, triply charged in the second run, all others in the third run, and so on.

Ideally, perform a 0% deuteration control and a 100% deuteration control for appropriate back exchange modeling:

- Obtain the 0% deuteration control by quenching the protein/H_2O sample directly into a buffer with exactly the same composition as the real sample, followed by digestion and analysis. If the 0% deuteration control is not available, treat the unlabeled runs as the control. With on-column digestion, the 0% control is very similar to the unlabeled sample; however, for in-solution digestion, there is usually a small difference between the two.
- Obtain the 100% deuteration control by labeling the protein condition as denatured for an extended period of time. Carefully design the procedure for this control to reduce the difference in the digestion condition between the control and the samples. Ideally, analyze the controls in triplicate or more instances, for assessment of variance.

Use short peptides as internal standards to correct run-to-run variations. When using these internal standards, the application adds them to the protein sample as well as the labeling buffer at equal concentrations. When using the tetrapeptide (PPPI) to model the intrinsic exchange rate, add it to the protein sample.

Collecting HDX data

To collect the HDX MS data in a fully automated fashion

- Set up a few data-dependent MS/MS instrument methods to collect LC/MS2 data with doubly charged precursors, triply charged precursors, and so on.
- Do not perform MS/MS for deuterated samples.
- Collect all data in centroid mode (profile mode data is fine but not necessary).

Processing an HDX experiment

Process an HDX experiment as follows:

1. Create a text file containing the protein sequence in FASTA format, including all chains of the protein, short peptides used as back exchange standards, and PPPI used as an intrinsic exchange internal standard.
2. Use the Protein Sequence Editor to import the FASTA file and specify the appropriate modifications.
3. Set the S/N threshold in the processing method if necessary.
4. Specify the HDX parameters in the processing method.

5. Start a Peptide Mapping Analysis experiment by entering the experiment name, loading all of the raw data files, and selecting the appropriate protein sequence and processing method.

   Specify the following conditions for the raw data files:

   - For the reference file: Use "ref" or "Ref".
   - For 0% deuteration controls: For one conformation condition, use "0%". For multiple conformation conditions, use "A:0%", "B:0%", and so on.
   - For 100% deuteration controls: For one conformation condition, use "100%". For multiple conformation conditions, use "A:100%", "B:100%", and so on.
   - For labeling time points: Use the format "A:2h15m30s", for example, condition A with labeling time 2 hr 15 min 30 sec.

   Load the reference raw data file as the first file. Assign the appropriate condition to each loaded raw data file.

6. Process the HDX experiment using the run queue.

HDX output

HDX processing might take some time to complete, depending on the complexity of the data. After completion, you can view the HDX plot that shows the protection factor results.

The application also generates a series of CSV files and automatically stores them in a folder named "*ExperimentName*HDXResults" under the raw data file folder. You can open these CSV files in an Microsoft™ Excel™ spreadsheet and plot the data as appropriate.

The output CSV files include the following (in alphabetical order by file name):

- (For multiple conformation conditions only) DifferentialProtectionPlot.csv: Differences in the average possible log (protection factors) compared to the control condition A.
- HDX100ControlData.csv: Deuterium contents in 100% deuteration control.
- HDX100ControlModel.csv: Deuterium contents in 100% deuteration control compared to the values predicted by the back exchange model.
- HDXData*x*of*y*.csv: Raw deuterium content data.

  Note: where *x* and *y* = 1 to the number of conformation conditions; for example, "*x*of*y*"is "1of1" when there is only one conformation condition, "1of2" and "2of2" when there are two conformation conditions, and so on.
- HDXData1stApproximationion*x*of*y*.csv: Time course data with internal standard first approximation ion but without back exchange ion.
- HDXSimulated*x*of*y*.csv: Raw deuterium content data compared to the values predicted by the best HDX model.
- OptimizedAverageSolution.csv: The average value of the top 20 solutions optimized. This solution is often better than any of the top 20 solutions.

- (For multiple conformation conditions only) ProtectionFactorDifferential*x*of*y*.csv: Top 20 differential log (protection factors), as well as their average values, of each condition as compared to the control condition A.
- ProtectionFactorPlot.csv: Average of the top 20 possible solutions for all conditions.
- Solution*x*of*y*.csv: Top 20 best solutions for each condition.
- TimeCourses.csv: Back exchange corrected time course data.

Glycan structures

The following figure shows the four largest glycan structures that the kinetic model considers: the complex type, the hybrid type, the high-mannose type, and the trimannosylated core structure.

Note: Zhang, Zhongqi; Shah, Bhavana. Prediction of Collision-Induced Dissociation Spectra of Common N-Glycopeptides for Glycoform Identification. *Anal. Chem*. **2010,** *82*, 10194-10202.

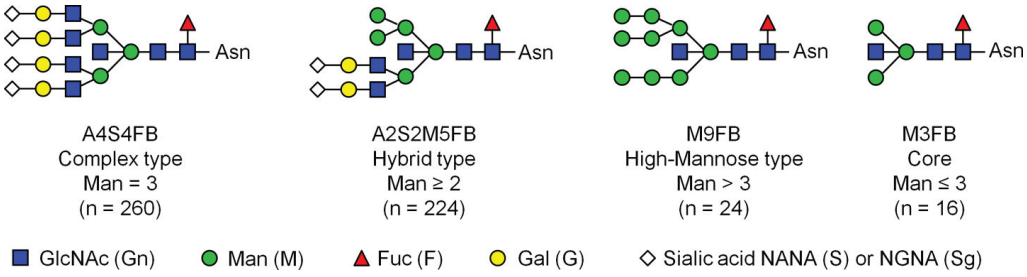


Figure 85 Glycan structures

Complex N-glycans are represented in the following form:

A*a*[Sg*s1*|S*s2*|Ga*g1*|G*g2*|F|B]

Hybrid N-glycans are represented in the following form:

A*a*[Sg*s1*|S*s2*|Ga*g1*|G*g2*|M*m*|F|B]

High-mannose N-glycans are represented in the following form:

M*m*

where:

- Any portion separated by | is optional.
- *a* represents the total number of antennas.
- *s1* represents the number of antennas terminating with N-Glycolyl Neuraminic Acid (NGNA).
- *s2* represents the number of antennas terminating with N-Acetyl Neuraminic Acid (NANA).

- *g1* represents the number of antennas terminating with alpha-galactose.

  Note: This is a galactose linked directly to the beta-galactose through alpha linkage and rarely appears when you select the Chinese hamster ovary (CHO) glycosylation option in the protein sequence.

- *g2* represents the number of antennas terminating with beta-galactose.

  Note: This type of galactose is more common.

- F represents the presence of core fucose.
- B represents the presence of bisecting GlcNAc.

  Note: It is sometimes difficult to distinguish bisecting GlcNAc from antenna GlcNAc based on CID or HCD data. When you select the CHO glycosylation option in the protein sequence, the application does *not* search for bisecting GlcNAc because CHO cells do not produce these glycans. However, when you select the Human glycosylation option, the application does search for bisecting GlcNAc. The application might interpret bisecting GlcNAc as antenna GlcNAc (for example, the results can display A1G0FB as A2G0F). To make the correct determination might require your judgment.

- *m* represents the number of mannose residues.

For example, A2G0F represents a glycan with two antennas, both terminating with antenna GlcNAc (zero galactose) and with a fucose core.

The following figure represents the nomenclature of O-glycans.

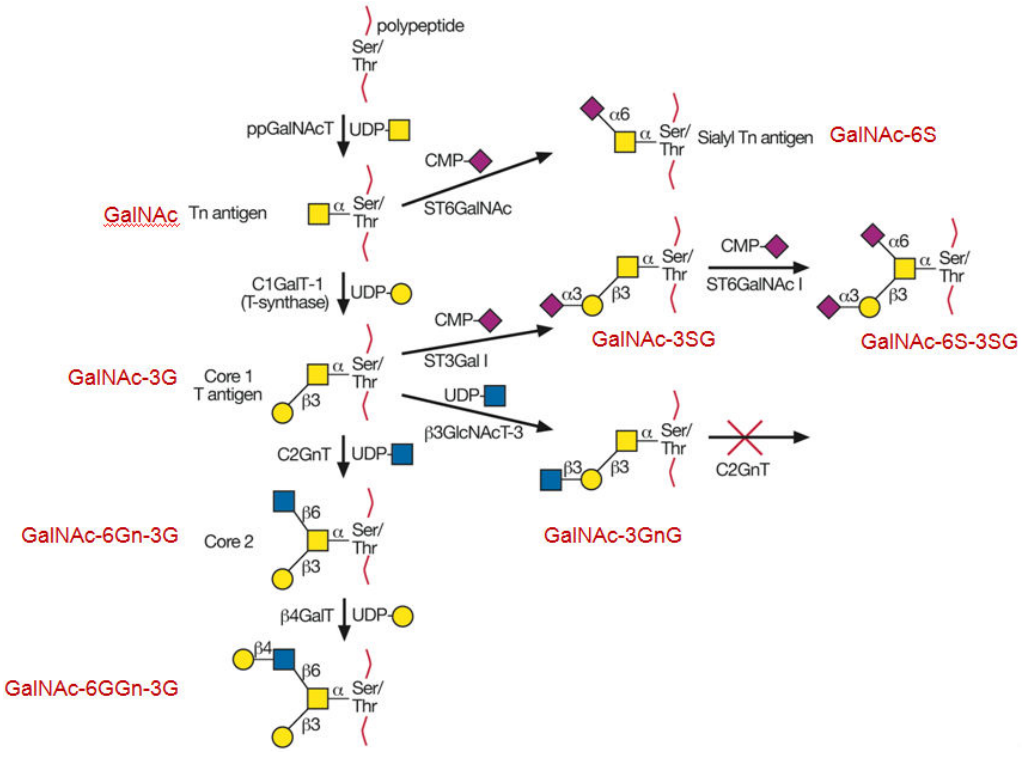


Figure 86 O-glycan nomenclature

The following figure illustrates glycan fragment ion nomenclature. For glycosidic bond cleavage, the reducing end fragments (together with the peptide moiety) are labeled either with the abbreviation of the remaining glycan or with the loss of the nonreducing end (that is, –M represents the loss of a mannose from the nonreducing end), depending on which one is more concise. Fragments of the nonreducing end are labeled with its residue composition enclosed in parentheses. Cleavages of the chitobiose core generates Y1, Y2, Bn, and Bn–1 ions.

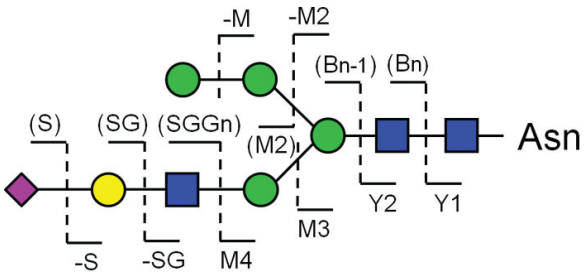


Figure 87 Glycan fragment ion nomenclature

The following figure displays the names of glycan structures commonly observed on antibodies.

Note: Shah, Bhavana; Jiang, Xinzhao Grace; Chen, Louise; Zhang, Zhongqi. LC-MS/MS Peptide Mapping with Automated Data Processing for Routine Profiling of N-Glycans in Immunoglobulins. *J. Am. Soc. Mass Spectrum*. **2014,** 25:999Y1011.

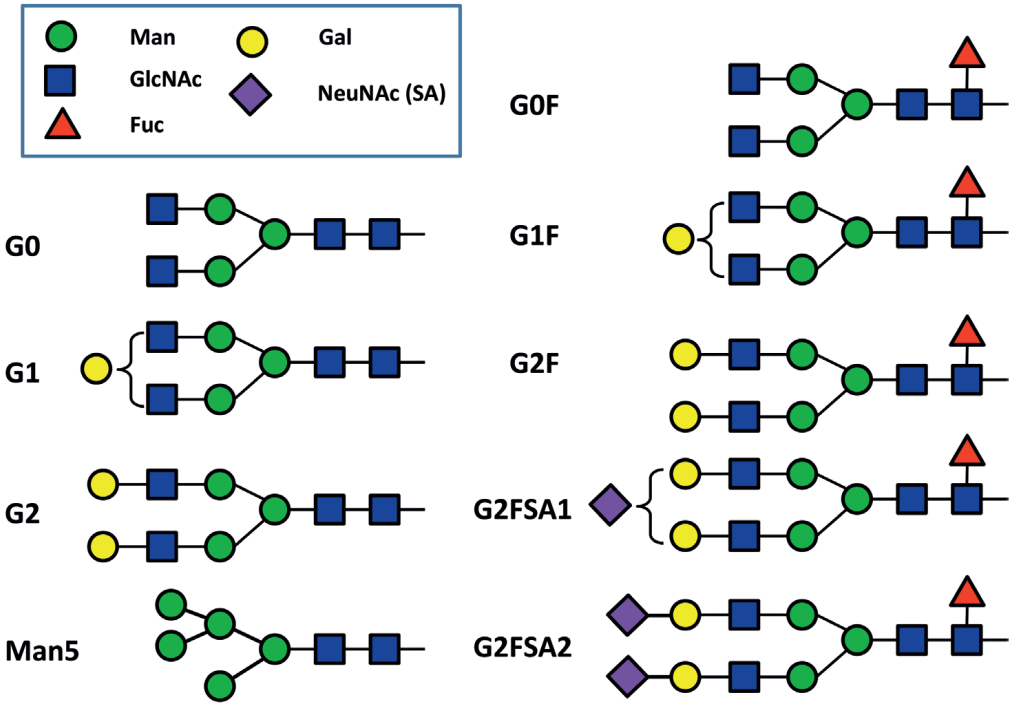


Figure 88 Glycan structures commonly observed on antibodies

Fragmentation

Peptide tandem mass spectra generated from fragmentation techniques such as ETD provide sequence information by cleaving a peptide backbone in a less selective way, potentially providing more peptide sequence information.

The radical-induced backbone cleavage produces c and z· ions, as well as b· and y ions. The c ion is not a radical.

- An H· transfer from c to z· produces c· (c–1) and z' (z·+1) fragments.
- An H· transfer from y to b· produces y· (y–1) and b' (b+1) fragments.
- The loss of an H· from z· or b· forms z (z·–1) or b (b·+1), respectively.
- The loss of H2 from a z· ion forms a z·' (z·–2) ion.
- The loss of CO from a b· ion produces an a· ion.

When you select a different type of MS2 scan in the MS2 Spectra pane—that is, a different combination of fragmentation type and resolution type—the fragmentation overage map automatically updates to display the results for that selection.

When you select a component identified as a disulfide bond, the application displays an MS2 Spectra pane for the selected peptide in the bond.

For more information and examples of fragmentation, visit the Matrix Science™ website:

http://www.matrixscience.com/help/fragmentation_help.html

Peptide mapping analysis input

The type of input file used in Peptide Mapping Analysis is the raw data file from a mass spectrometry experiment. The raw data file can contain LC/MS data or an individual mass spectrum.

You can use Peptide Mapping Analysis with data from various mass spectrometry systems: ion trap, Thermo Scientific™ Exactive™ Series and Orbitrap™ Series, and Fourier transform mass spectrometry (FTMS) series.

Peptide mapping analysis output

As output, Peptide Mapping Analysis produces peak and protein information. It generates a Peptide Mapping File (PMF) that contains both the parameter settings that you applied to the raw data file and the results of the analysis. These results appear in the Results table of the Process and Review page.

When you want to view the results from another Peptide Mapping session, you can go to the Load Results page to load the PMF and display the results of that analysis.

Performing a Non-Targeted peptide mapping analysis experiment

Follow this workflow to perform a non-targeted Peptide Mapping Analysis experiment:

1. (Optional) Create a new protein sequence or edit the parameters in an existing sequence.
2. (Optional) Create a new processing method or edit the parameters in an existing method.
3. Create a new experiment by naming it, selecting the raw data files, and choosing the protein sequence (optional) and processing method.
4. Submit the experiment for processing and monitor the status of the experiment by using the queue.
5. View the results of the analysis.
6. (Optional) Use real-time optimization to change the parameters and reprocess the results.

The following figure shows the workflow involved in using the Protein Sequence Editor and Peptide Mapping Analysis features for a non-targeted peptide mapping experiment.

Figure 89 Workflow in non-targeted Peptide Mapping Analysis

Performing a targeted peptide mapping analysis experiment

Follow this workflow to perform a targeted Peptide Mapping Analysis experiment:

1. Use the steps in the previous procedure to run a non-targeted peptide mapping experiment.

2. After viewing the results of the experiment, select and save the targeted data to a peptide workbook.

   You can also save targeted peptides to a workbook from the Theoretical Protein/Peptide Manager page.

3. Edit the workbook as necessary and save it.
4. Run a targeted peptide mapping experiment by using the saved workbook (as the protein sequence) and a targeted processing method.
5. Submit the experiment for processing and monitor the status of the experiment by using the queue.
6. View the results of the analysis.
7. (Optional) Use real-time optimization to change the parameters and reprocess the results.

10 Running a peptide mapping analysis

After you add a sequence for peptide mapping to the Sequence Manager, you can start a peptide mapping experiment by clicking Peptide Mapping Analysis on the Home page.

The Sequence Manager lists your peptide and protein sequences by sample type (peptide/protein) and category (Intact Protein, Peptide Mapping, or Top Down).

Data acquisition and peptide identification

The BioPharma Finder application can interpret different kinds of MS2 data from Thermo Scientific™ instruments, including CID (collision-induced dissociation), ETD (electron transfer dissociation), and HCD (higher energy collision-induced dissociation). You can process LC/MS/MS runs using one of these methods:

- For an Orbitrap™ or LTQ™ FT instrument, acquire data using a high-resolution scan followed by several MS2 scans, either in high resolution or low resolution. The BioPharma Finder application can also process full-scan data (MS-only data).
- For an LCQ™/LTQ™ Series instrument, acquire the data in triple-play mode, for example, a full-scan followed by a data-dependent zoom scan or ultra-zoom scan, followed by a data-dependent MS2 scan in centroid mode.

The application provides peptide identification using a novel prediction algorithm (unique differentiator), relative quantitation of post-translational modifications (PTMs), and in-depth identification using error-tolerant searching, amino acid substitution, and de novo sequencing (unique differentiator).

Starting a new peptide mapping experiment

Use the Peptide Mapping Analysis page to create a new peptide mapping experiment. Enter the experiment name, load the raw data file or files, select a protein sequence (optional for a non-targeted experiment, required for a targeted experiment), and select a processing method to start processing.

You can specify the default folder from which you want to load your raw data files for the peptide mapping experiments.

To start a new experiment for Peptide Mapping Analysis

1. On the Home page, click **Peptide Mapping Analysis**.

   The Peptide Mapping Analysis page opens.

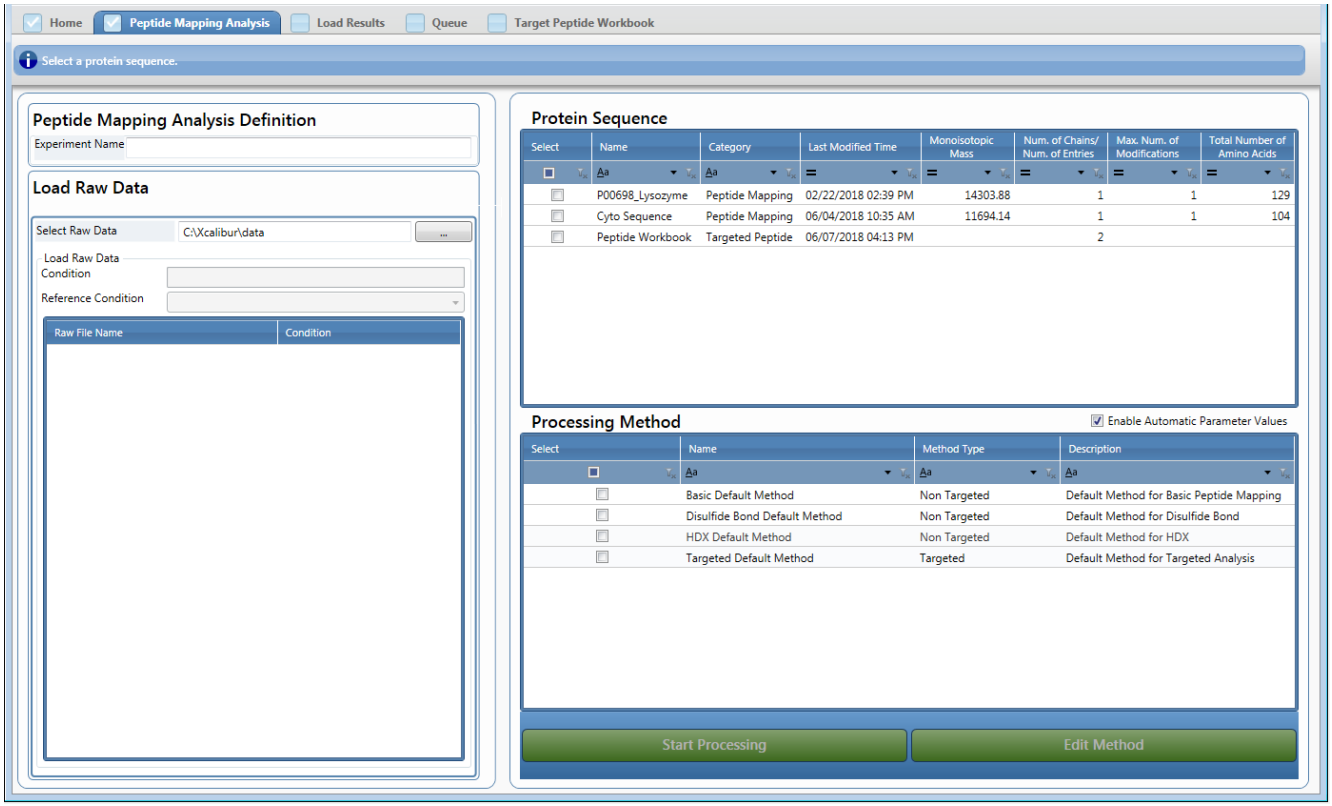


Figure 90 Peptide Mapping Analysis page

2. In the Peptide Mapping Analysis Definition area, in the Experiment Name box, type the name of the experiment.

   Note: Use only alphanumeric, space, underscore "_", and period "." characters, up to 50 characters maximum, in the experiment name.

   If an existing experiment is already completed or canceled, you can overwrite it by entering the same experiment name. You cannot overwrite an experiment that is currently submitted to the run queue or is actively processing or canceling.

3. In the Load Raw Data area, load the raw data file or files for the experiment.

   If you load multiple files, enter the required condition information. The application automatically groups together all files assigned to the same condition for processing the results related to conditions.

4. In the table in the Protein Sequence area, select the check box for a protein sequence for the experiment.

   Note: You must select a protein sequence when you run an HDX experiment or a targeted peptide mapping experiment (selected sequence must have the Targeted Peptide category). For a non-targeted peptide mapping experiment, this selection is optional.

   IMPORTANT! For Peptide Mapping Analysis, only the protein sequences with a category of Peptide Mapping, Targeted Peptide, or Unknown appear in the table. If you want to use a sequence for Peptide Mapping Analysis and you do not see it in the table, change its category value to Peptide Mapping in the Protein Sequence Editor.

   The application automatically assigns the Targeted Peptide category to peptide workbooks used as protein sequences. The application automatically assigns the Unknown category to protein sequences saved in previous versions of the BioPharma Finder application without a category value.

   ---

5. In the table in the Processing Method area, select the check box for a processing method for the experiment.

   The application provides four default processing methods:

   - To use the default method for non-targeted peptide processing, select **Basic Default Method**.
   - To use the default method for disulfide bonds processing, select **Disulfide Bond Default Method**.
   - To use the default method for HDX processing, select **HDX Default Method**.
   - To use the default method for targeted peptide processing, select **Targeted Default Method**.

6. If you load one or more raw data files and you want to use certain peak detection or identification parameters stored in the loaded files for processing, select the **Enable Automatic Parameters Values** check box above the table in the Processing Method area.

   The application determines the component detection or identification parameters from the loaded raw data files and displays these parameters when you want to edit the method.

   Otherwise, to use the parameters stored in the processing method, clear this check box.

The *component detection* parameters from the loaded raw data file or files include the following:

- Absolute MS signal threshold (10^3 counts, default S/N = 20)
- Typical chromatographic peak width (min)
- Maximum MS peak width (Da)
- Restricted retention time range (start and stop times)
- Mass tolerance (ppm for high-resolution or Da for low-resolution)
- Maximum retention time shift (min)

The *identification* parameter from the loaded file or files includes the mass accuracy in ppm.

7. Click **Start Processing** to process the experiment without editing the method parameters.

   If you load multiple raw data files, the Start Processing button is not enabled until you assign conditions to all raw data files.

   –or–

   Click **Edit Method** to review the method parameter information, make adjustments to the threshold and other method parameters, and then save the method before processing.

   You can create a new custom method by editing the parameters in an existing method and then saving it to a different name. You cannot overwrite a default method. You can also import a method file from your computer. The application appends _imported to the file name of the new method.

   Note: Thermo Fisher Scientific recommends that you review the method parameters before processing the experiment, because the default threshold values might be too low, causing the experiment to take a long time to finish processing.

   For example, to process an experiment using an Absolute MS Signal Threshold of 1E6, regardless of the raw data files used, create a processing method with this threshold set to 1E6, and save this method. When you create a new experiment, select the saved method to run the experiment with the specified threshold value.

   The application requires the experiment name, the raw data files, the conditions if there are multiple files, a protein sequence (only for HDX and targeted peptide experiments), and a processing method to start processing a new experiment. If you adjust the method parameters and the experiment meets all of these requirements, the application begins processing. If the experiment does not meet all of the requirements, the application returns to the Peptide Mapping Analysis page.

Batch and multiconsensus result formats for peptide mapping analysis

For Peptide Mapping Analysis experiments with multiple loaded raw data files, you can run the experiment using either of these result formats:

- The **Batch Processing** format maintains separate results from each of the multiple raw data files.

  In this format, the BioPharma Finder application processes one experiment individually for each loaded raw data file, so you can process multiple experiments at one time but retain individual results. For the name for each batch experiment, the application concatenates the specified experiment name on the Peptide Mapping Analysis page with a unique date-and-time stamp. The results for each batch experiment are the same as if you ran an experiment with just a single raw data file.

–or–

- The **Multiconsensus** format merges the results from the multiple raw data files together.

  In this format, the application processes one experiment and merges the deconvolution results from all of the loaded raw data files together. By default, the name of this multiconsensus experiment uses the same name as the specified experiment name on the Peptide Mapping Analysis page.

Peptide mapping experiment processing on the queue page

When all requirements are met for processing, the application saves all entered information for the new job and then opens the Queue page. If another job is already processing in the run queue, this experiment waits in line on the Queue page with a status of Submitted. Otherwise, the application immediately starts to process it with a status of Running. When the experiment is completed, its status shows Completed.

If you are loading multiple raw data files, the application determines the component detection parameters from the whole set of raw data files for processing.

Select a completed job on the Queue page to view the processed results.

11 Working with a peptide mapping processing method

The BioPharma Finder application provides several default processing methods for Peptide Mapping Analysis. If needed, you can use the editing wizard to edit the parameters in one of these methods and save them to a new file to create a custom method for your experiment.

Using a processing method for peptide mapping analysis

To create a new method or edit a current method

1. On the Home page, click **Peptide Mapping Analysis** in the left pane or below the splash graphic.

   The Peptide Mapping Analysis page opens.

2. (Optional) Enter the experiment name, load the raw data file or files and enter the conditions if needed, and select a protein sequence.

3. In the Processing Method area, select a processing method in the table to edit, and then click **Edit Method**.

   Tip: To create a custom method, you modify the parameters in a default method, and then save them to a custom method using a different name.

   The editing wizard opens the Component Detection page under the Parameters tab, showing the component detection parameters in the current method.

   Note: If you create a new experiment and load multiple raw data files, the application determines the parameters for the whole group and displays them on the Component Detection and Identification pages under the Parameters tab.

4. Use the editing wizard on the Component Detection, Identification, and Hydrogen Deuterium Exchange pages to specify the parameters for the following:
   - Peak detection
   - Retention time alignment
   - Monoisotopic and average mass determination
   - Peptide identification
   - Hydrogen Deuterium Exchange (HDX) conditions and modeling parameters

   If you load raw data files for an experiment, the application uses them to automatically determine some of the parameters, such as the absolute signal threshold and retention time shift. You must manually set other parameters that are appropriate for the experiment, such as parameters for peptide identification.

When you are done editing the parameters on each of these pages, click **Next** in the command bar. When you get to the Save Method page, save all of the modified parameters to a method. You can then select that method to use for processing another experiment.

Editing component detection parameters for peptide mapping analysis

When you want to create a new processing method or edit an existing method for Peptide Mapping Analysis, go to first page of the editing wizard, the Component Detection page.

For more details, see the following topics:

- Editing the component detection page (page 195)
- Component detection page layout (page 197)
- Component detection page parameters (page 199)
- Viewing the signal threshold (page 202)

Editing the component detection page

To edit the Component Detection page

1. (Optional) On the Peptide Mapping Analysis page, enter an experiment name, load the raw data files, and select a protein sequence.

2. Select a method and then click **Edit Method**.

   The Component Detection page opens. If you navigate away from this page and want to get back to it, in the navigation bar, click the **Parameters** tab, and then click the **Component Detection** subtab.

If you selected the **Enable Automatic Parameters Values** check box above the Processing Method table, the application uses certain peak detection parameters stored in the loaded files for processing instead of the same parameters stored in the method.

3. Enter the appropriate values on the Component Detection page.

4. When you are done editing the parameters on this page, click **Next** in the command bar to advance to the Identification page.

Component detection page layout

The Component Detection page includes five different areas: Select Task to Be Performed, Peak Detection, Ion Alignment, and Mass Measurement on the left side, and Base Peak Chromatogram Display on the right side.

Note: Select the **Show Advanced Parameters** check box to edit advanced options that are hidden by default. Normally, these advanced parameters do not need to be edited.

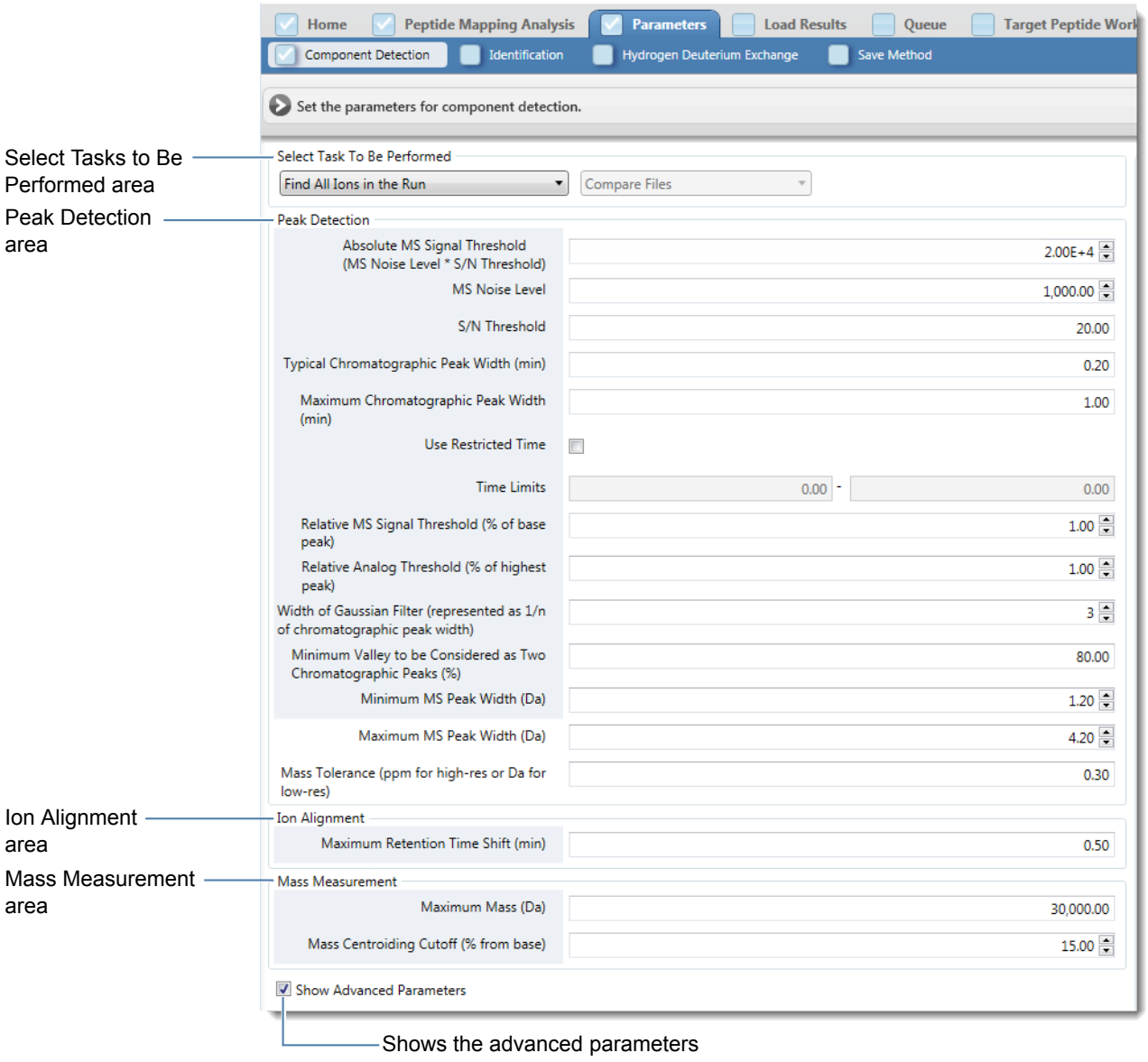


Figure 91 Component Detection page areas (left side)

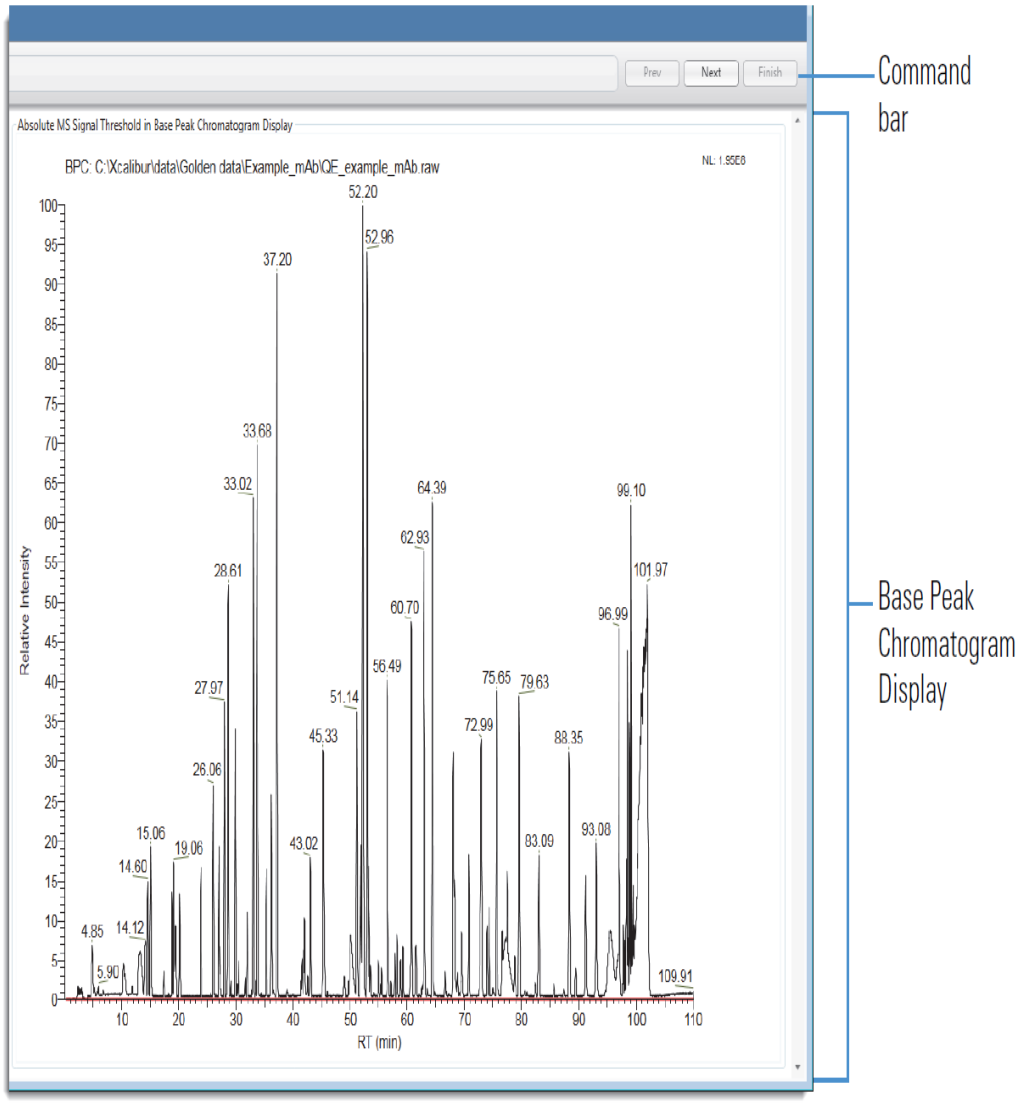


Figure 92 Component Detection page (right side)

Component detection page parameters

The following table describes the parameters available on the Component Detection page under the Parameters tab.

Table 22 Component Detection page parameters

Parameter	Description
Select Task to Be Performed	
(task list)	Specifies the type of processing to perform. • Find All Ions in the Run: Displays information about each ion in the Results table, including the peptide sequence, retention time, *m/z*, MS peak area, charge state, and so forth. • Find All Masses in the Run: Combines multiple charge states into a single entry. • Find Peaks in the Total Ion Chromatogram: Detects peaks in the TIC, deconvolves the spectra, and identifies MS2 spectra associated with the major ions under these peaks. • Find Peaks in the Base Peak Chromatogram: Detects peaks in the BPC, deconvolves the spectra, and identifies MS2 spectra associated with the major ions under these peaks. • Find Peaks in the Analog Chromatogram: Detects peaks in the analog chromatogram. • Find All Ions with MS/MS: Detects ions that have an associated MS/MS.
Peak Detection	
Absolute MS Signal Threshold	(Not editable) Specifies the absolute MS signal threshold. Absolute MS Signal Threshold = MS Noise Level × S/N Threshold Adjust the value of this parameter by changing the MS Noise Level parameter or the S/N Threshold parameter, or both. The application provides a visual aid for this signal threshold by displaying a thin red horizontal line on the BPC displayed on the right side of the Component Detection page. In general, processing takes much more time if this red line is well below the background noise level in the BPC or total ion chromatogram (TIC).
MS Noise Level	Specifies the noise level in the raw data files.
S/N Threshold	Specifies the signal-to-noise threshold in the raw data files.
Typical Chromatographic Peak Width (min)	Specifies the typical chromatographic peak width in the LC/MS run, in minutes. The application automatically determines the typical chromatographic peak width of the experimental LC/MS data file in minutes and sets the value to the width of the highest peak in the chromatogram.
Maximum Chromatographic Peak Width (min)	Specifies the maximum chromatographic peak width in the LC/MS run, in minutes. The application automatically sets the maximum chromatographic peak width in the LC/MS run. It sets this initial value to the geometric mean of the width of the highest peak and the range of the chromatogram. The application considers any peak wider than this value to be part of the background and does not include it in the results.

Parameter	Description
Use Restricted Time	Activates the Time Limits boxes so that you can enter a restricted time range.
Time Limits	Specifies the retention time range used to truncate the chromatogram and reduce the results to an appropriate area.
Relative MS Signal Threshold (% of highest peak)	Specifies the relative MS signal threshold value as a percentage so that the application can detect an ion at a given point in the chromatogram. The threshold specified by the Absolute MS Signal Threshold parameter sets the minimum MS signal for ion detection. This parameter defines the relative threshold abundance, as a percentage of the base peak, for the detection of a component that coelutes with a larger component in the same experiment.
Relative Analog Threshold (% of highest peak)	Specifies the relative analog threshold value as a percentage that the application can detect in relation to the strongest signal in the chromatogram. Use this parameter as a percentage of the highest peak only when setting up a task to find peaks by using the Find Peaks in the Analog Chromatogram task.
Width of Gaussian Filter (represented as *1/n* of chromatographic peak width)	Specifies the width of the Gaussian filter as a percentage of the typical chromatographic peak width. Using a Gaussian filter to read the LC/MS file, the application averages nearby full MS scans by applying a moving Gaussian function to improve the signal-to-noise ratio (S/N) of each scan. Setting the width of the Gaussian filter is an important step when optimizing the S/N of each scan. For example, a value of 4 means the filter width is 40 percent of the chromatographic peak width. Lower the number to optimize sensitivity and increase the number to optimize chromatographic resolution. A value of 1 represents maximum sensitivity for a Gaussian filter.
Minimum Valley to Be Considered as Two Chromatographic Peaks (%)	Specifies the size, as a percentage value, for the minimum valley that the application considers as two chromatographic peaks.
Minimum MS Peak Width (Da)	Specifies the minimum MS peak width, in daltons. The application uses the Minimum MS Peak Width and the Maximum MS Peak Width parameters to generate a range for the isotopic envelope of the ion.
Maximum MS Peak Width	Specifies the maximum MS peak width, in daltons. The application uses the Maximum MS Peak Width and the Minimum MS Peak Width parameters to generate a range for the isotopic envelope of the ion.
Mass Tolerance (ppm for high-rest or Da for low-res)	Specifies the maximum mass difference of the same ion in different scans. The application measures this value in ppm for high resolution and Da for low resolution.
Ion Alignment	
Maximum Retention Time Shift (min)	Specifies the maximum retention time shift, in minutes, when the application compares two or more LC/MS runs.

Parameter	Description
Mass Measurement	
Maximum Mass (Da)	Specifies a value to change the calculation for the average mass of an ion, in daltons. You can set the maximum mass of the peptide or protein in the LC/MS run.
Mass Centroiding Cutoff (% from base)	Specifies a percentage value to change the calculation of the average mass of an ion.
Check Box	
Show Advanced Parameters	Determines whether the application displays advanced options that are hidden by default. Selected: Displays advanced options. Cleared: Hides advanced options.
Absolute MS Signal Threshold in Base Peak Chromatogram Display	Displays the red line representing the value shown in the Absolute MS Signal Threshold box. The value of the read-only Absolute MS Signal Threshold parameter changes when you adjust the MS Noise Level value, the S/N Threshold value, or both in the Peak Detection area. As the value of Absolute MS Signal Threshold changes, the red line on the BPC on the right side of the page moves to reflect this change. If you are a user of the Thermo PepFinder application, you can mimic results in the BioPharma Finder application by setting the MS Noise Level parameter to a value of 1000. Then, set the S/N Threshold parameter to the Absolute MS Signal Threshold value provided by the PepFinder application. **Note:** To see the BPC with the red line, you must first load one or more raw data files on the Peptide Mapping Analysis page. If you load multiple raw data files, the application displays the BPC for the first raw data file.

Viewing the signal threshold

Use the Peak Detection area of the Component Detection page to define the Absolute MS Signal Threshold value.

As you change the MS Noise Level parameter, the S/N Threshold parameter, or both, the red line on the BPC on the right side of the page moves to reflect this change. You can zoom in to see the red line movement more clearly.

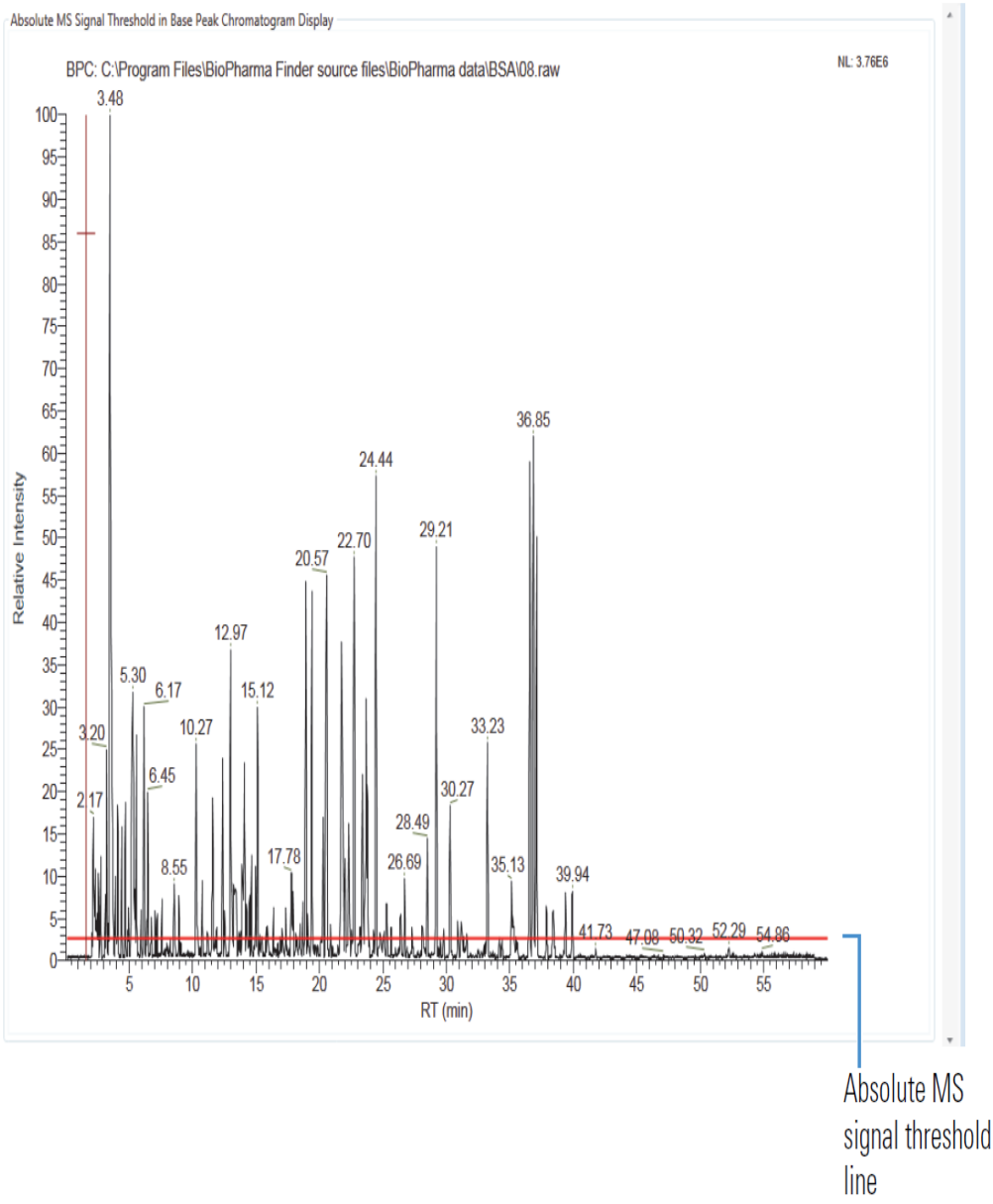


Figure 93 Absolute MS signal threshold line

To see detailed information about the retention time along the *x* axis in a certain area —for example, between the retention times of 10 and 15—drag the mouse horizontally along the bottom of the chromatogram from the lowest retention time to the highest. The following figure shows the magnified area between retention times 10 and 15 of the BPC.

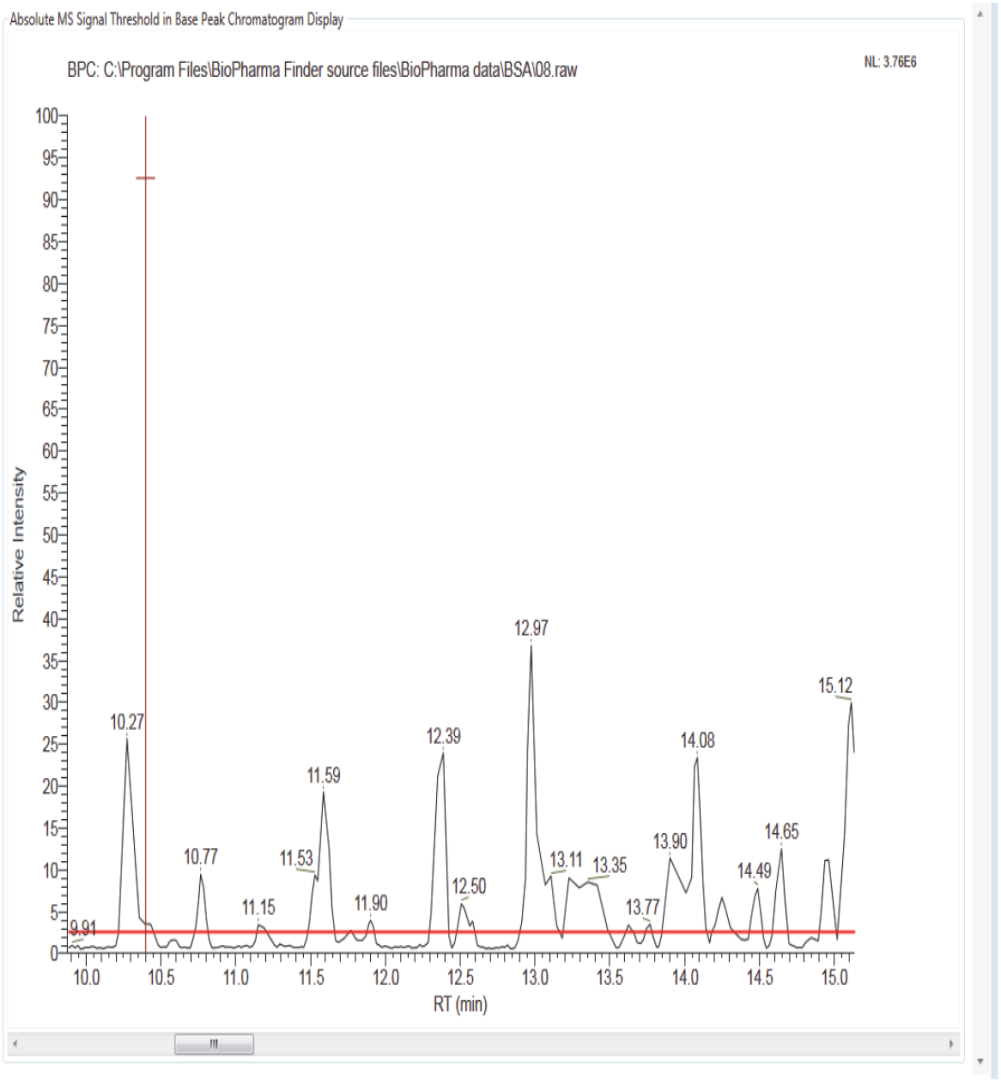


Figure 94 Magnified area of retention time

To see detailed information about the relative intensity along the *y* axis in a certain area—for example, between the relative intensities of 0 and 15—drag the mouse vertically along the left side of the chromatogram from the lowest intensity to the highest. The following figure shows the magnified area between the relative intensities of 0 and 15 of the BPC.

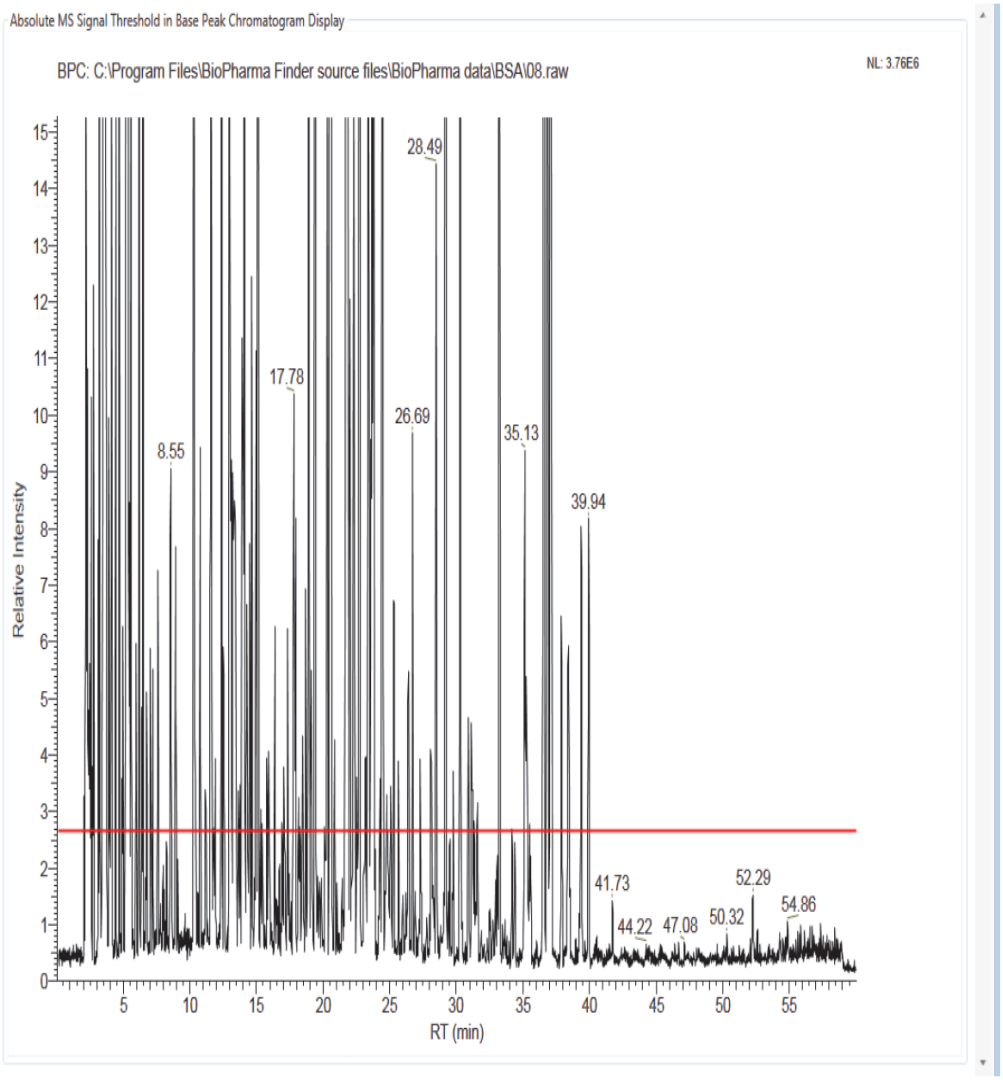


Figure 95 Magnified area of relative intensity

To return the BPC to its original scale, right-click and choose **Reset Scale**.

Editing identification parameters for peptide mapping analysis

When you want to create a new method or edit an existing method for Peptide Mapping Analysis, first go to the Component Detection page, the first page of the editing wizard. Next, advance to the Identification page, the second page of the editing wizard.

For more details, see the following topics:

- Editing the identification page (page 205)
- Identification page layout (page 206)
- Identification page parameters (page 207)

Editing the identification page

To edit the Identification page

1. On the Component Detection page, click **Next** in the command bar.

   –or–

   In the navigation bar, click the **Parameters** tab, and then click the **Identification** subtab.

   If you selected the **Enable Automatic Parameters Values** check box above the Processing Method table on the Peptide Mapping Analysis page, the application uses mass accuracy determined by the algorithm automatically based on the loaded raw data.

2. Enter the appropriate parameter values on the Identification page.

3. When you are done editing the parameters on this page, click **Next** in the command bar to advance to the Hydrogen Deuterium Exchange page.

Identification page layout

The Identification page includes six different areas: Peptide Identification, Advanced Search, Disulfide Search, and Reduced LC/MS Run on the left side, and Select Protease and Delete or Add New Protease on the right side.

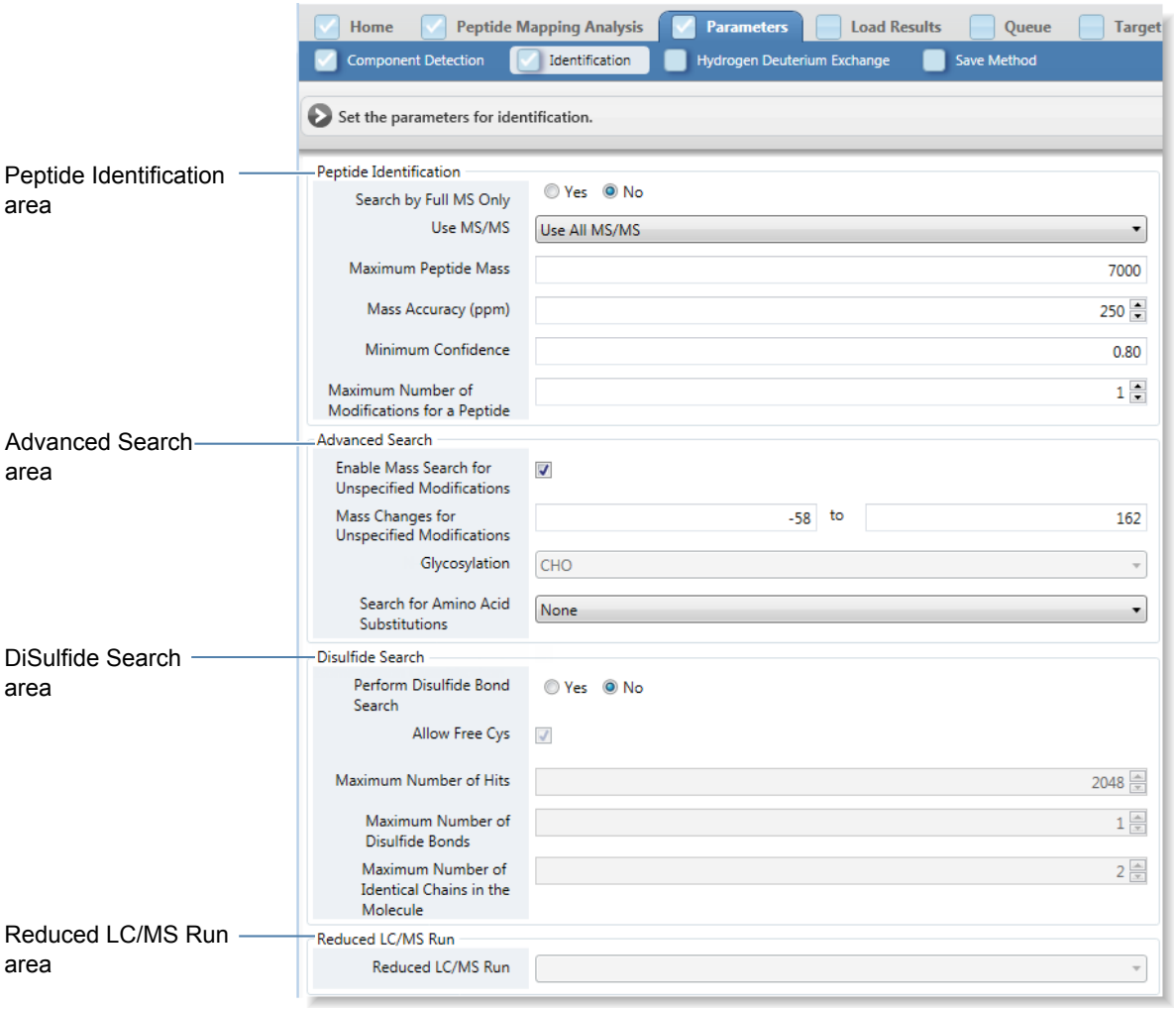


Figure 96 Identification page areas (left side)

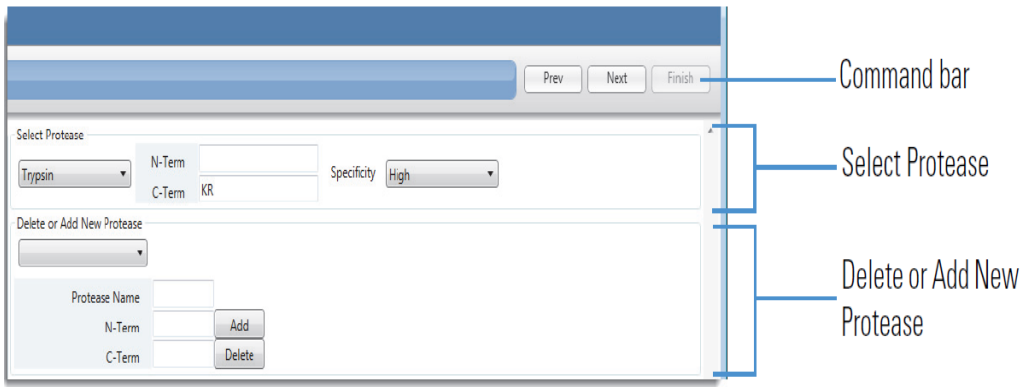


Figure 97 Identification page areas (right side)

Identification page parameters

The following table describes the parameters on the Identification page under the Parameters tab.

Table 23 Identification page parameters

Parameter	Description
Peptide Identification	
Search by Full MS Only	Indicates the type of data that the raw data file contains. • Yes: Indicates that the raw data file contains only full-scan data. • No: Indicates that the raw data file contains MS2 data.
Use MS/MS	Specifies the type of data to process. • Use All MS/MS: Uses all the MS2 spectra in the raw data file to identify the peptides. • Ignore MS/MS: Uses only the full-scan spectra in the raw data file to identify the peptides. • Use CID/HCD Only: Uses only the CID/HCD spectra in the raw data file to identify the peptides. • Use ETD/ECD Only: Uses only the ETD/ECD spectra in the raw data file to identify the peptides.
Maximum Peptide Mass	Specifies the maximum peptide mass to be identified. Increase this value to look for disulfide bonds in non-reduced samples.
Mass Accuracy (ppm)	Specifies the maximum mass deviation (ppm) when comparing the theoretical peptide mass to the calculated mass of a particular ion to determine identification. **IMPORTANT!** For non-targeted experiments, the application does *not* strictly enforce this parameter so it identifies more peptides. For targeted experiments, the application strictly enforces this parameter so it does not identify peptides outside of the mass tolerance.
Minimum Confidence	Specifies the minimum confidence level to be reported for a peptide assignment on a 0-to-1 scale, with 1 having the highest confidence. **Note:** If you create custom glycans and assign them as side chain modifications to a protein sequence used for the experiment, you must set this parameter to 0; otherwise, the application will not identify the components.
Maximum Number of Modifications for a Peptide	(Read-only) Specifies the maximum number of modifications for each peptide. This value comes from the Peptide Mapping parameter in the Max # Modifications area of the protein sequence that you assign to the experiment.

Parameter	Description
Advanced Search	
Enable Mass Search for Unspecified Modifications	Determines whether the application performs a mass search for unspecified modifications. • Selected: Performs a mass search for unspecified modifications. • Cleared: Does not perform a mass search for unspecified modifications.
Mass Changes for Unspecified Modifications	Specifies a mass range to use in the search for unspecified modifications. For full characterization of a target protein, you must identify unspecified modifications. To identify an unspecified modification, the application applies a mass change within the defined range to the mass of an unknown peptide, and then attempts to match that modified mass to the mass of an identified peptide. If the application finds a match but it cannot determine the exact modification site, it places a tilde (~) mark in front of the modification site to indicate the approximate location of an unspecified modification. For example, an unspecific modification on a peptide, ~C310–57.0212, stands for the loss of 57.0212 Da near Cys-310, which indicates an incomplete alkylation.
Glycosylation	(Read-only) Specifies the type of glycosylation to apply to the N-linked/O-linked glycans. This value comes from the N, O Glycan selection in the Glycosylation area of the protein sequence chosen for the experiment. Valid values: • None: Adds no glycosylations. • CHO: Adds glycosylations from the Chinese hamster ovary (CHO) cell line. • Human: Adds glycosylations from the human cell line.
Search for Amino Acid Substitutions	Determines how the application searches for amino acid substitutions. • None: Does not search for amino acid substitutions. • Single Base Change: Finds amino acid substitutions involving only one base change in their codons. Use this setting to search for DNA mutations, because amino acid substitutions caused by DNA mutations rarely have more than one base change in the codons of the two amino acids. • All Substitutions: Finds all amino acid substitutions.
Disulfide Search	
Perform Disulfide Bond Search	Determines whether the application performs a search for disulfide bonds. • No: Does not search for disulfide bonds. • Yes: Searches for disulfide bonds. **Note:** Selecting No deactivates the rest of the disulfide search parameters.
Allow Free Cys	Determines whether the application allows free cysteine residues in the molecule. • Selected: Allows free cysteine residues in the molecule. • Cleared: Does not allow free cysteine residues in the molecule.

Parameter	Description
Maximum Number of Hits	Specifies the maximum number of search results before the application stops searching for more disulfide-linked peptides.
Maximum Number of Disulfide Bonds	Specifies the maximum number of disulfide bonds.
Maximum Number of Identical Chains in the Molecule	Specifies the maximum number of identical chains in the molecule. For example, if the molecule is a disulfide-linked homodimer, then set this parameter to 2.
Reduced LC/MS Run	
Reduced LC/MS Run	Specifies the name of the reduced raw data file for the LC/MS run. If you select to perform a disulfide search, use the Reduced LC/MS Run area of the Identification page to select the reduced raw data file to use for the LC/MS run. Selecting the reduced raw data file helps identify the disulfide-linked peptides. When the application searches for matches of disulfide-linked peptides, it generates a score for each match. The application primarily determines the score from how close the match is between the experimental MS2 spectra and the predicted MS2 spectra. However, if the application observes peptide chains in the reduced run, it adds an award to the score. Specifically, it adds an award of 0.1 if it observes that all of the chains are in the reduced run, and no award if it does not observe any chain. If you have both reduced and non-reduced data files, you can process both types in the same experiment to generate more reliable disulfide assignments. When loading your data files (.raw extension), use the CTRL or SHIFT key to select both the reduced and non-reduced files at the same time. Then, select the name for the reduced raw data file in this list.
Select Protease	
(list of proteases)	Lists the names of the proteases available to use in the digestion of the target protein. The application identifies proteolytic peptides by searching the mass of the ion against the known protein sequence, following the rules of the protease that you assign. At least one of the two cleavage sites must match the protease specificity to be considered a peptide candidate. No limitation is applied to the maximum number of missed cleavages inside a peptide when the application is identifying peptide candidates.[1] **Note:** If you assign a protein sequence to the experiment, you must select a protease. You can select only one protease for each experiment.
Protease Name	Specifies the protease to assign to the currently open processing method. The application provides a list of default proteases. If your protease does not appear in this list, you can add custom proteases before assigning them to the method. Thermo Fisher Scientific recommends that you add custom proteases if you expect to use them in subsequent analyses. After you select a protease other than Nonspecific, the application displays the activity at the N- and C-terminus in the form of 1-letter amino acids. This activity indicates the residues where the protease cleavages.

Parameter	Description
N-Term	Specifies the activity at the N-terminus. If you select the Nonspecific protease, this cell is empty.
C-Term	Specifies the activity at the C-terminus. If you select the Nonspecific protease, this cell is empty.
Specificity	Specifies the level of protease specificity. For the application to identify a peptide, at least one of the two cleavage sites must meet the specificity level that you defined for the protease. The High, Medium, and Low levels are confidence factors that the application uses to determine the final confidence score. The application only applies the factors to peptides that have both ends meeting the specificity requirements of the protease (it does not affect a half-tryptic peptide). Therefore, if you set the level to High, the factor is larger for the same peptide than if you set it to Medium. This difference can impact your results when two or more peptides match a spectrum and only one of these peptides follows the protease specificity requirements, because the application gives it a higher confidence score. If your sample has a large number of missed cleavages, you might want to use Medium or Low. If you set the level to Strict, the application only looks for peptides that match the theoretical peptide and that follow the protease rules 100 percent, with cuts only at the specified sites at the N- and C-terminus. Both ends of the peptide must match the selected protease specificity. There is no limitation on the maximum number of missed cleavages inside the peptide.
Delete or Add New Protease	
(list of proteases)	Lists the names of the proteases that you can add, edit, or delete.
Protease Name	Specifies the name of the protease to add, edit, or delete.
N-Term	Specifies the activity at the N-terminus.
C-Term	Specifies the activity at the C-terminus.

Parameter	Description
Buttons	
Add	Adds the specified protease to the Select Protease list. **To add a new custom protease** 1. Set the following parameters: • Protease Name: Type the name of the new custom protease. • N-Term and C-Term: Type the activity at the N- and C-Terminus in the form of 1-letter amino acids. This activity indicates the residues where the protease cleavages. 2. Click **Add**. The new custom protease appears in the list of proteases. **To edit an existing custom protease** Edit an existing custom protease by deleting it and then adding a new custom protease with the same name but with different terminal information. The new information overwrites the previous protease information. **Note:** You cannot edit any custom protease that a method is currently using for processing.
Delete	Removes the specified protease from the Select Protease list. **To delete an existing custom protease** 1. From the Protease list, select the custom protease that you want to delete. 2. Click **Delete**. **Note:** You cannot delete any of the default proteases or any custom protease that a method is currently using.

[1] Zhang, Z. Large-scale Identification and Quantification of Covalent Modifications in Therapeutic Proteins. *Anal. Chem*. **2009,** *81*(20), 8354-8364.

Editing HDX parameters for peptide mapping analysis

When you want to create a new method or edit an existing method, first go to the Component Detection and the Identification pages. Next, advance to the Hydrogen Deuterium Exchange page, the third page of the editing wizard.

The BioPharma Finder application performs HDX modeling for Peptide Mapping Analysis by minimizing the Chi^2 difference between the simulated data and the experimental data. Protection factors are the parameters in the model. Minimizing the Chi^2 difference generates a protection factor for each amide hydrogen. To avoid over-interpretation of the data, the application also tries to maximize the smoothness of the protection factor plot and minimize the difference between labeling conditions.

Similar to the protection factor Chi^2 modeling, the back exchange Chi^2 modeling produces a model with a minimal Chi^2 difference between the simulated data and the experimental data of the fully deuterated controls. The modeling process generates effective times for the back exchange during digestion and during the high-

performance liquid chromatography (HPLC) separation. Again, to avoid over-interpretation of the data, the application sets the effective times during digestion as close as possible for each peptide, and correlates the effective times during separation to the elution times as much as possible.

For more details, see the following topics:

- Editing the hydrogen deuterium exchange page (page 212)
- Hydrogen deuterium exchange page layout (page 213)
- Hydrogen deuterium exchange page parameters (page 214)

Editing the hydrogen deuterium exchange page

To edit the Hydrogen Deuterium Exchange page

1. On the Identification page, click **Next** in the command bar.

   –or–

   In the navigation bar, click the **Parameters** tab, and then click the **Hydrogen Deuterium Exchange** subtab.

2. Enter the appropriate parameter values on the Hydrogen Deuterium Exchange page.

3. When you are done editing the parameters on this page, click **Next** in the command bar to advance to the Save Method page.

Hydrogen deuterium exchange page layout

The Hydrogen Deuterium Exchange page includes six different areas: Basic Parameters, Labeling Conditions, Quench/Digest Conditions, and LC Conditions on the left side, and Protection Factor Chi^2 Modeling and Back Exchange Chi^2 Modeling on the right side.

Note: Select the **Show Advanced Parameters** check box to edit advanced options on the right side of the page that are hidden by default. Normally, these advanced parameters do not need to be edited.

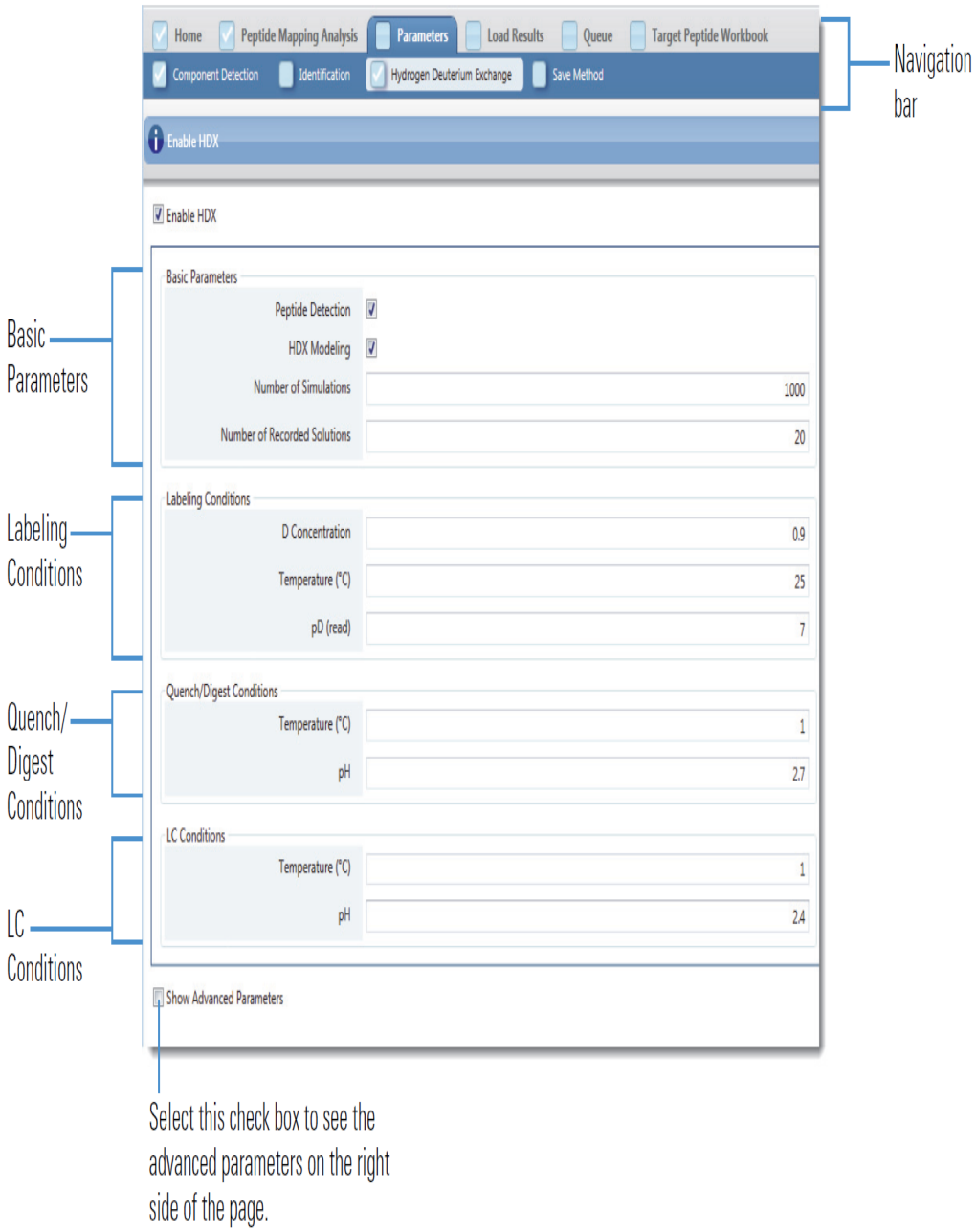


Figure 98 Hydrogen Deuterium Exchange page areas (left side)

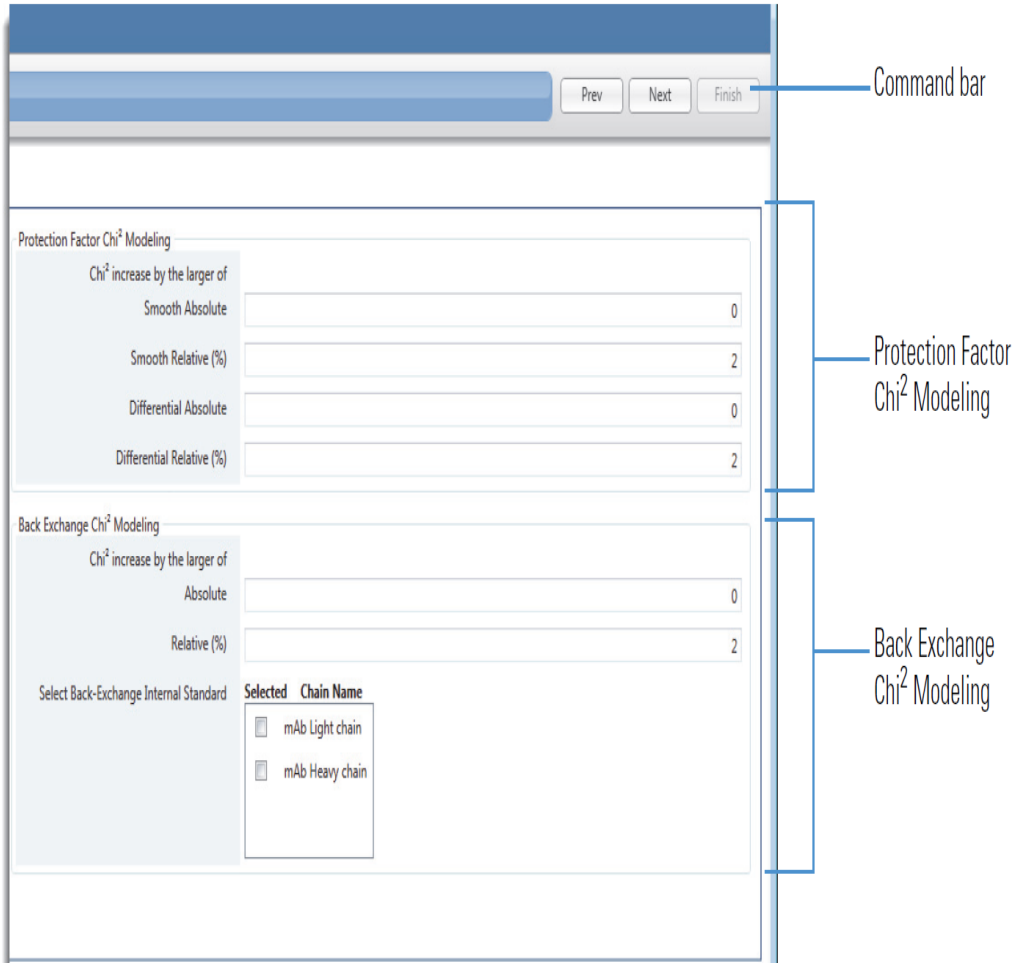


Figure 99 Hydrogen Deuterium Exchange page areas (right side)

Hydrogen deuterium exchange page parameters

The following table describes the parameters on the Hydrogen Deuterium Exchange page under the Parameters tab.

Table 24 Hydrogen Deuterium Exchange page parameters

Parameter	Description
Enable HDX	Select this check box to edit the HDX parameters and enable HDX processing.

Parameter	Description
Basic Parameters	
Peptide Detection	Select this check box to perform peptide detection. **Note:** You can choose to perform peptide detection at the same time as HDX modeling. Or, you can select to perform peptide detection first, manually edit the peptide list, and then perform HDX data processing separately by selecting only the HDX Modeling option. If you select this check box, the application does *not* display the modifications in the Identification column of the Results table for HDX experiments. However, labels for the peptide ions *do* appear on the experimental MS2 spectra. If you clear this check box, the application displays the modifications but not the labels.
HDX Modeling	Select this check box to perform HDX modeling.
Number of Simulations	Specify the number of Monte Carlo simulations to perform.
Number of Recorded Solutions	Specify the number of top solutions to record. The application discards the remaining solutions.
Labeling Conditions	
D Concentration	Specify the concentration of deuterium in the labeling solution. For example, if you initiate the HDX by a 10-fold dilution of the sample into a D_2O buffer, then the D Concentration is 0.9.
Temperature (°C)	Specify the temperature at which the application performs the HDX. The application uses this value to calculate the theoretical intrinsic rate of exchange to calculate the protection factor.
pD (read)	Specify the pH of the labeling solution as read from a pH meter. The application uses this value to calculate the theoretical intrinsic rate of exchange to calculate the protection factor.
Quench/Digest Conditions	
Temperature (°C)	Specify the temperature during digestion. The application uses this value to calculate the theoretical back exchange rate of each backbone amide hydrogen during digestion.
pH	Specify the pH value during digestion. The application uses this value to calculate the theoretical back exchange rate of each backbone amide hydrogen during digestion.
LC Conditions	
Temperature (°C)	Specify the temperature during liquid chromatography. The application uses this value to calculate the theoretical back exchange rate of each backbone amide hydrogen during peptide separation.

Parameter	Description
pH	Specify the pH value during liquid chromatography. The application uses this value to calculate the theoretical back exchange rate of each backbone amide hydrogen during peptide separation.
Check Box	
Show Advanced Parameters	Select this check box to display advanced options that are hidden by default.
Protection Factor Chi^2 Modeling **Chi^2 increase by the larger of**	
Smooth Absolute	Specify the absolute increase of the Chi^2 value for determining the weight of the smoothness function.
Smooth Relative (%)	Specify the relative percentage of increase of the Chi^2 value for determining the weight of the smoothness function.
Differential Absolute	Specify the absolute increase of the Chi^2 value for determining the weight of the differential function.
Differential Relative (%)	Specify the relative percentage of increase of the Chi^2 value for determining the weight of the differential function.
Back Exchange Chi^2 Modeling **Chi^2 increase by the larger of**	
Absolute	Specify the absolute increase of the Chi^2 value for determining the Lagrange multiplier. To minimize Chi^2 and maximize smoothness at the same time, the application applies the Lagrange multiplier, which is a weight, to the smoothness function.
Relative (%)	Specify the relative percentage of increase of the Chi^2 value for determining the Lagrange multiplier.
Select Back-Exchange Internal Standard	(Optional) Select the check box for one or more chains to designate as internal back exchange standards (short peptides that exchange very quickly) to help reduce run-to-run variations. These chains come from the protein sequence that you selected for the experiment. If you use the tetrapeptide (PPPI) as an internal standard for the intrinsic rate, select it here. **Note:** The processing method does not save your internal standard selections because these vary depending on the selected protein sequence or sequences. The next time you use the same method, you must make these selections again.

12 Viewing the peptide mapping analysis results

You can view the Peptide Mapping Analysis results from multiple pages in the BioPharma Finder application.

Opening the results from the queue page

When you run a Peptide Mapping Analysis experiment, the Queue page displays the run queue containing all of the completed, running, submitted, or canceled jobs. After a job is completed, you can open its results from the Queue page and view the processed data.

Note: A completed job displays "Completed" in the Status column.
You cannot open the results if the application has not yet analyzed the experiment or is in the process of analyzing it. In this case, the Open Results button is inactive until processing of the selected job is completed.
Opening the results does not stop the application from analyzing subsequent experiments in the queue.

To view the results of an experiment from the Queue page

1. On the Home page, click **Peptide Mapping Analysis** in the left pane or below the splash graphic.
2. Click the **Queue** tab.
   The Queue page opens showing the queued jobs in a table.
3. In the table, click a job row to select the completed job whose results you want displayed.

4. In the command bar, choose **Open Results**.

   The application transfers you to the Process and Review page, which displays the following:

   - Chromatograms in the Chromatogram pane
   - Visible only for experiments with multiple raw data files:
     - Trend ratios plot in the Trend Ratio pane
     - Trend MS areas plot in the Trend MS Area pane
   - Fragment coverage map in the Peptide Sequence Coverage pane
   - Protein sequence in the Protein Sequence Coverage pane
   - Output spectra in the Full Scan Spectra and MS2 Spectra panes
   - Components in the Results table

   You can adjust the size or location of the panes on this page as needed.

   In addition to the Process and Review page, you can also click the Mapping tab to view the Coverage page, Modification Summary page, and optionally the Hydrogen Deuterium Exchange page.

Opening the results from the load results page

Because you can delete jobs in the run queue on the Queue page, after deletion, you can no longer view the related results. However, the BioPharma Finder application saves all of the completed jobs on the Load Results page so that you can view any previously saved experimental results. You can also import or export the results of an experiment.

To view or delete the results of an experiment from the Load Results page

1. On the Home page, click **Peptide Mapping Analysis** in the left pane or below the splash graphic.

2. Click the **Load Results** tab.

   The table on the Load Results page displays all of the previously saved Peptide Mapping Analysis results, in order of completion time.

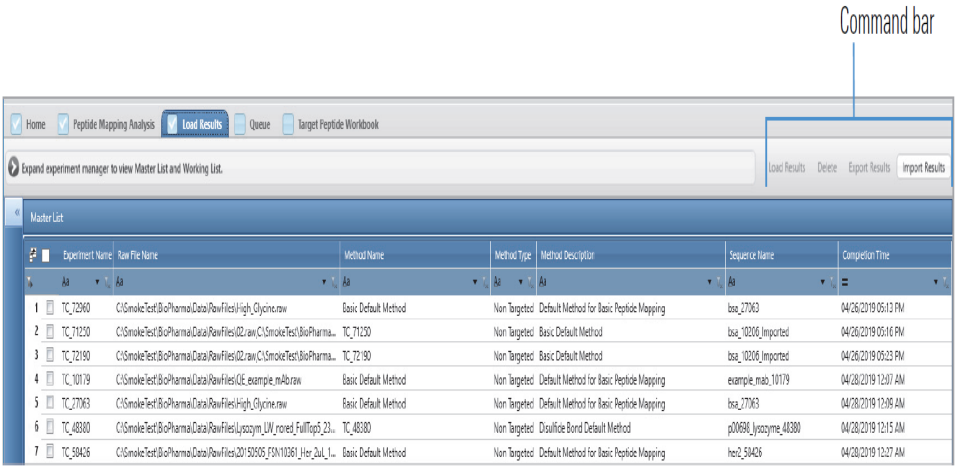


Figure 100 Load Results page

The table provides information including the following:

- Experiment name
- Raw data file names
- Each processing method and the protein sequences (optional) assigned to that analysis
- Source spectra method
- Deconvolution algorithm

You can sort the columns or filter the data in the table as needed.

3. In the table on the Load Results page, do any of the following:
   - Double-click a row to select an experiment and view its results, or click a row and then choose **Load Results** in the command bar.

     The application transfers you to the Process and Review page.

     In addition to the Process and Review page, you can also click the Mapping tab to view the Coverage page, Modification Summary page, and optionally the Hydrogen Deuterium Exchange plot.
   - Select one or more rows and then choose **Delete** in the command bar.

     Select multiple rows by using the SHIFT key for contiguous rows or the CTRL key for noncontiguous rows.

     The application deletes the selected set of results from the database. If the selected experiments still exist in the run queue on the Queue page, the application removes them from the queue as well.

To import the results of an experiment from the Load Results page

1. On the Home page, click **Peptide Mapping Analysis** in the left pane or below the splash graphic.
2. Click the **Load Results** tab.

   The table on the Load Results page displays all of the previously saved Peptide Mapping Analysis results in the order of completion time.
3. Click **Import Results**.
4. In the dialog box, locate and select one or more Protein Mapping Analysis result file (*Filename*_peptide.resultsbpf) that you want to import.

   The files must be in the same folder when importing multiple files simultaneously.
5. Click **Open**.

   A message appears indicating the confirmation of the import.

   The application adds the imported result in the experiment result table of the Master List and Working List folders.

   Note: If an existing method has the same name as the imported method, the application appends _imported to the file name of the new method.

To export the results of an experiment from the Load Results page

1. On the Home page, click **Peptide Mapping Analysis** in the left pane or below the splash graphic.
2. Click the **Load Results** tab.

   The table on the Load Results page displays all of the previously saved Peptide Mapping Analysis results in order of completion time.
3. Select one or more check boxes corresponding to the results of the experiments that you want to export, and do one of the following:
   - Choose **Export Results ▸ Export Experiment with Raw Files** in the command bar.

     –or–
   - Choose **Export Results ▸ Export Experiment without Raw Files** in the command bar.

   Note: You can only view the result when you include the raw files in the export of the experiment result file. To display the spectra when you import the exported experiment result file, you must store the raw file in the same directory as processed.

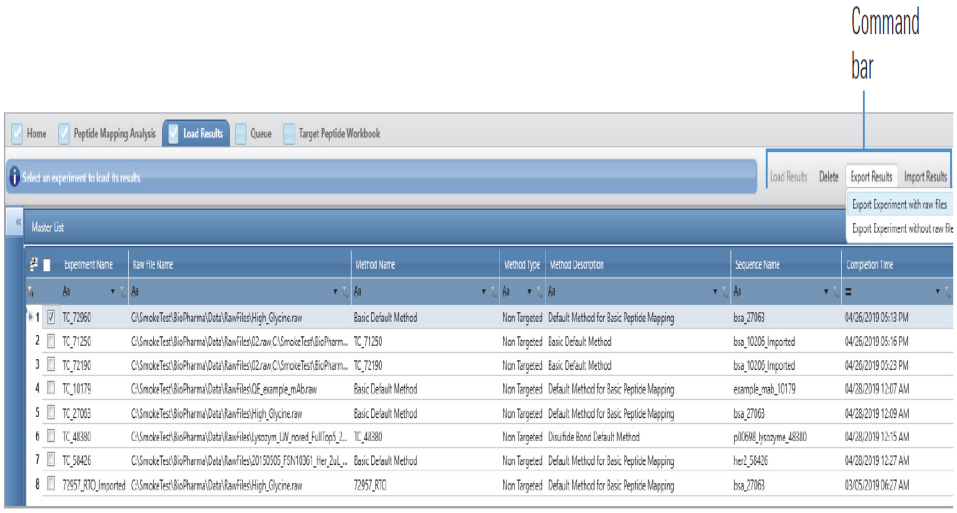


Figure 101 Export Results options in the Load Results page

4. In the dialog box, browse to the appropriate folder, and click **OK**.

   For export of a single experiment result, the Browse dialog box opens to the *drive*:\Xcalibur™\data\ folder by default.

   A message appears indicating the confirmation of the export. The application saves the exported experiment result (*Filename*_peptide.resultsbpf) on the specified folder.

Using Real-Time optimization for peptide mapping analysis

After viewing the results on the Process and Review page, you can change the parameters in the protein sequence, the parameters in the processing method, or both sets of parameters, and then reprocess the experiment without leaving this page, for real-time optimization.

To reprocess the experiment with modified sequence or method parameters

1. Click the **Process and Review** tab if necessary.

2. Click the **Real Time Optimization** title bar to see the Sequence, Component Detection, and Identification subtabs.

   The Sequence pane expands automatically and displays the sequence information including the amino acids in the chains, the protease information, and the assigned modifications/glycosylation, for the protein sequence assigned to the currently open experiment (see the following figure for a non-targeted experiment).

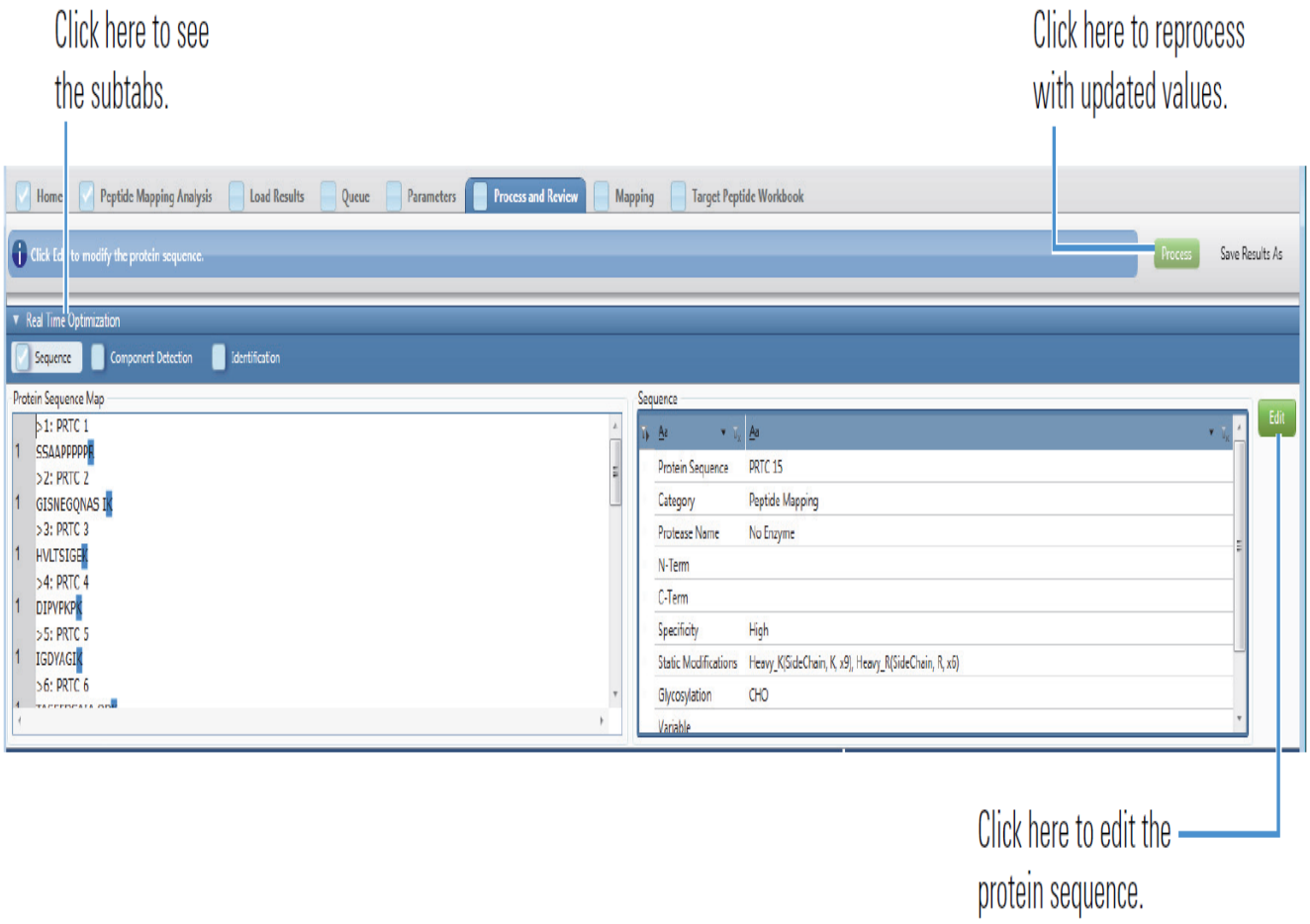


Figure 102 Sequence pane for real-time optimization of non-targeted experiment

The following figure shows the Sequence pane for a targeted peptide mapping experiment that used a saved peptide workbook. The application automatically converts the workbook into a protein sequence and names each chain as "*x*: Sequence *x*" (where *x* is a consecutive number). It also consolidates the sequences to avoid multiple entries for the same peptide. The order of the chains matches the order of the Relative Quantitative Group Number in the workbook.

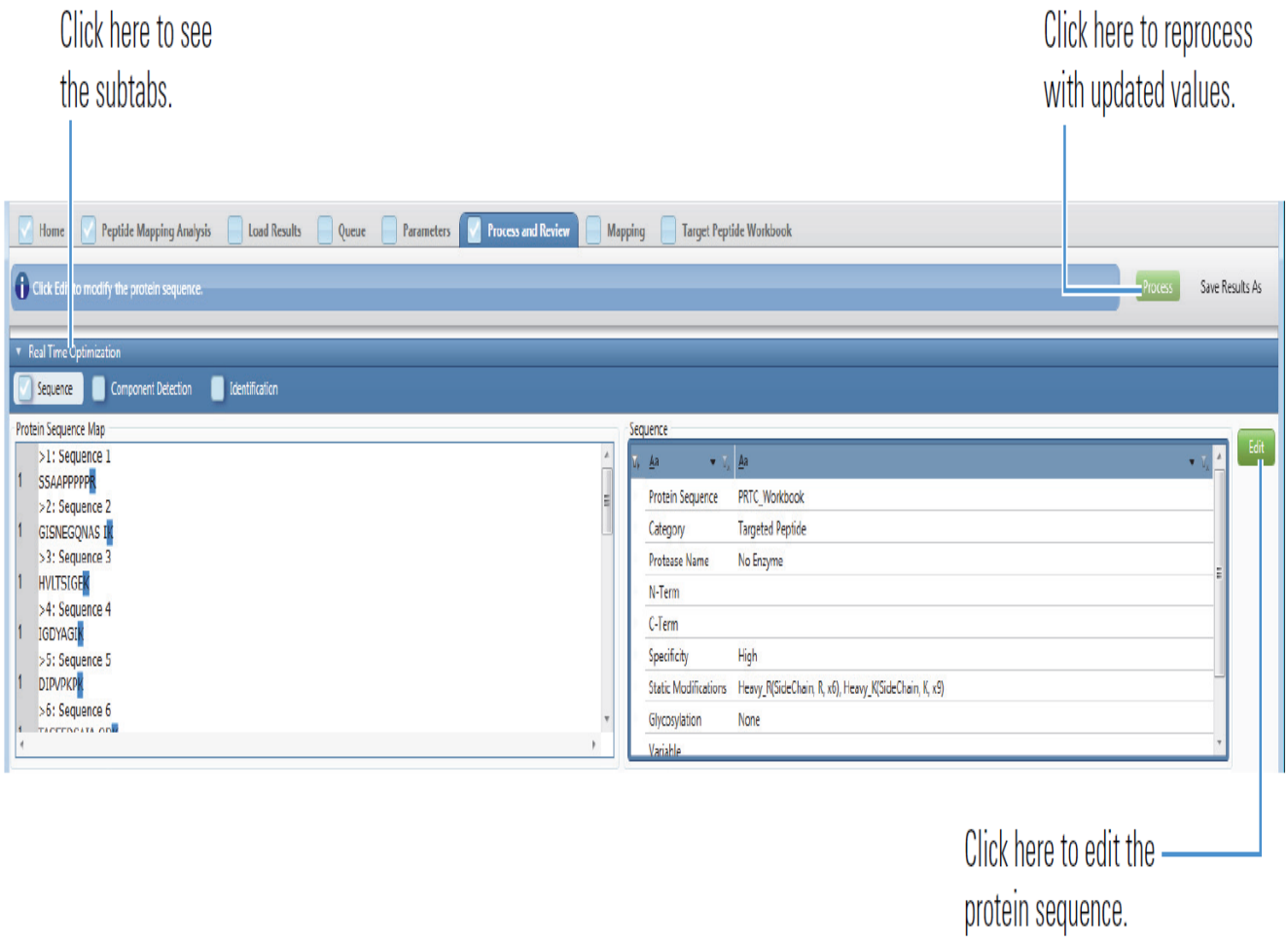


Figure 103 Sequence pane for real-time optimization of targeted peptide experiment

When you click the other subtabs, the Component Detection and Identification panes display the parameter values in the method that are used for processing the currently open experiment.

3. For a non-targeted peptide mapping experiment, in the Sequence pane, click **Edit** to open the Protein Sequence Editor, change the protein sequence parameters as needed, and then save your changes to a protein sequence.

   Note: You can only change the modifications or glycosylation information for the current protein sequence. You cannot edit the chains or select a different protein sequence.

   For a targeted peptide mapping experiment, in the Sequence pane, click **Edit** to open the workbook in a window, change the parameters as needed, and then save your changes to the open peptide workbook. The application uses this workbook as a protein sequence when you reprocess the targeted experiment. Your saved changes appear in the Sequence pane.

4. Click the **Component Detection** tab. Change the parameters, and then click the **Basic** or **Advanced** subtab to update the basic or advanced parameters as needed.

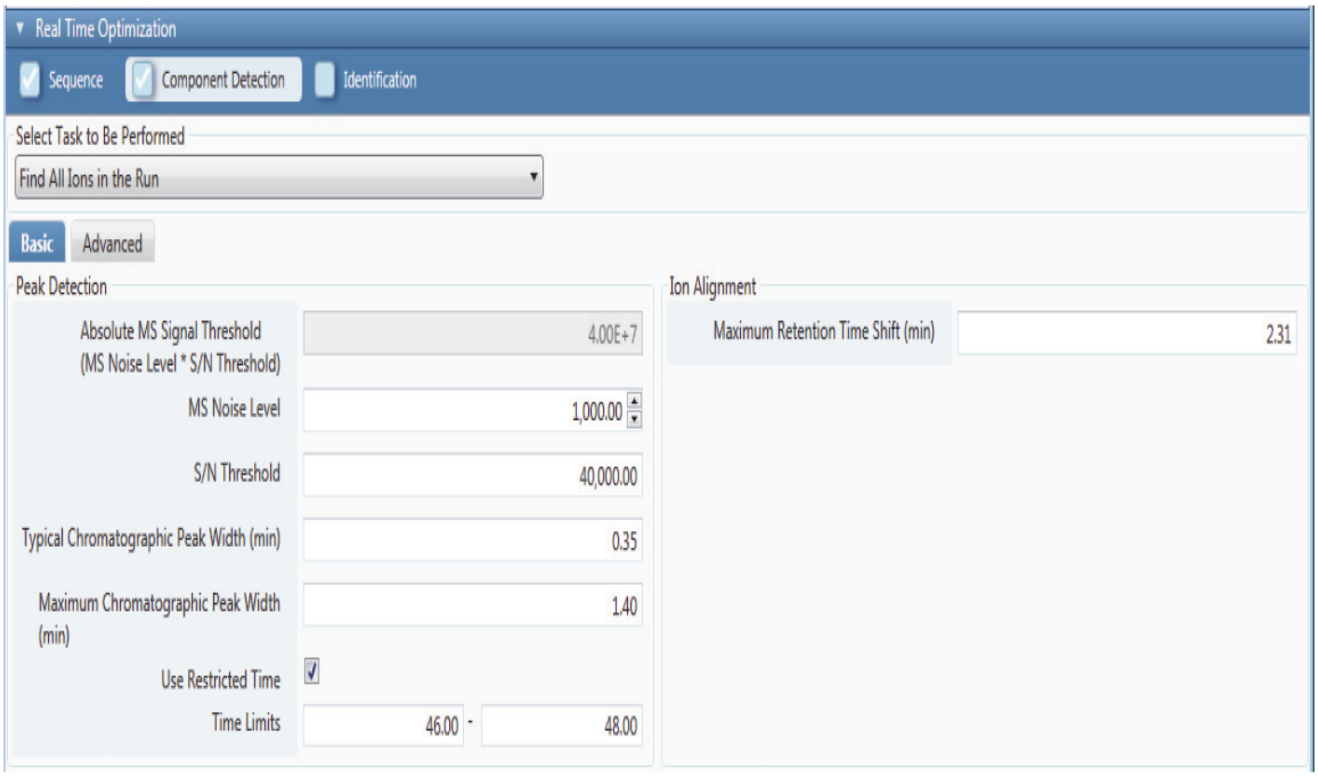


Figure 104 Component Detection pane for real-time optimization

5. Click the **Identification** tab, and then click the **Peptide Identification, Advanced Search, Disulfide Search**, or **Protease** subtab to update the corresponding parameters as needed.

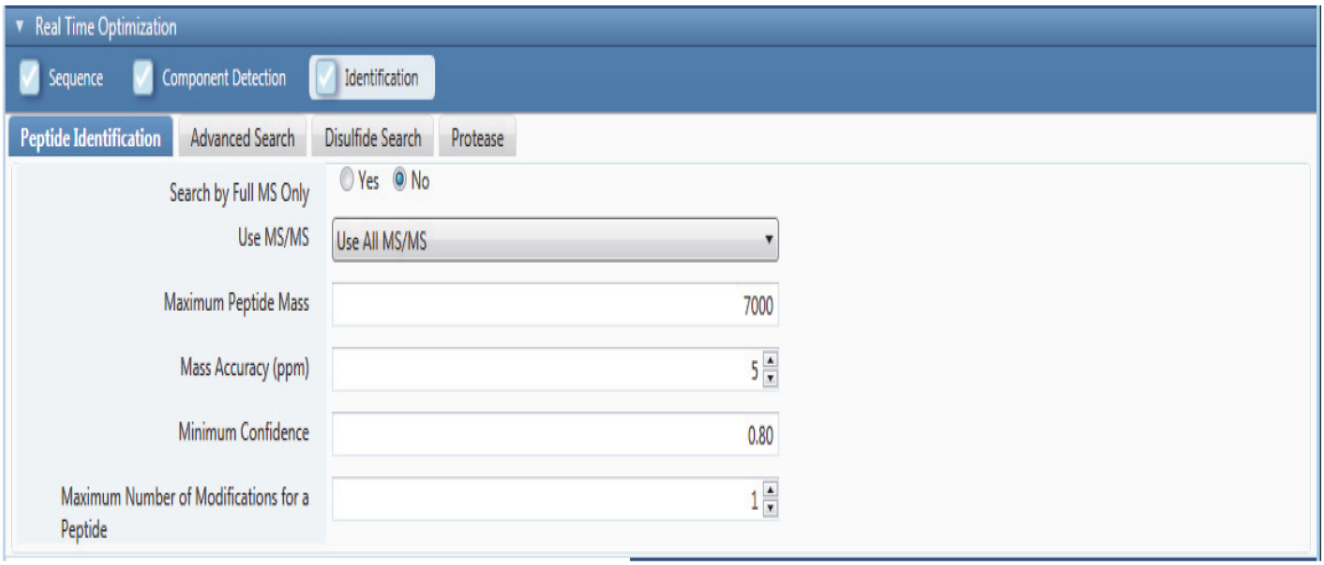


Figure 105 Identification pane for real-time optimization

Note: You cannot change the Glycosylation parameter value under the Advanced Search subtab. This value comes from the N, O Glycan selection in the protein sequence that you assign to the experiment. To change this value, you must change it in the protein sequence (see step 3 on page 223).

6. Click **Process** in the command bar of the Process and Review page.

   If the application finds invalid parameter entries, it displays an error dialog box to inform you. To continue, enter all required parameter values within the appropriate ranges.

7. If all of the entered parameters are valid, enter the experiment and method names in the Reprocess Experiment dialog box, and then click **Reprocess**.

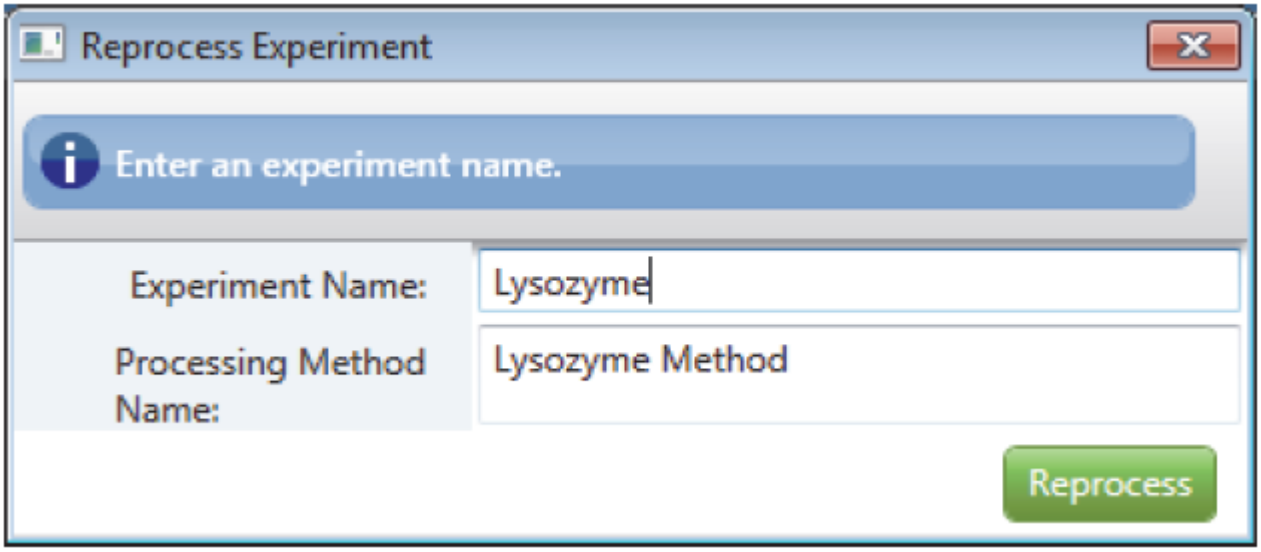


Figure 106 Reprocess Experiment dialog box

Note: If an existing experiment is already completed or is canceled, you can overwrite it by using the same experiment name. You cannot overwrite an experiment that is currently submitted to the run queue or is actively processing or canceling.
If the method you are using is not a default method, you can overwrite it by using the same method name. Otherwise, if you change the parameters in a default method, save the method to a new name.
Use only alphanumeric, space, underscore "_", and period "." characters in the experiment and method names.
The entry of new experiment and method names saves your changes to new files to ensure that you do not overwrite the previous experiment results and method parameters.

The application clears all of the previous results on the Process and Review page and submits a new processing job with your changes to the run queue. The job moves to the top of the queue, behind the currently running job. If no job is currently running, the application reprocesses the new job immediately. During this time, you remain on the Process and Review page and can navigate to other areas or open other results if you want. When the reprocessing is completed, the Process and Review page automatically displays the new results, except when you open another results file while the application is reprocessing. In this case, it does not update the display of the reprocessing results when they are completed.

You can open the saved experiment results file to view later.

Viewing the hydrogen deuterium exchange plot

After the application completes the analysis of an HDX peptide mapping experiment, you can open the results and view the HDX plot that shows the protection factor results on the Hydrogen Deuterium Exchange page.

For more details, see the following topics:

- Opening the hydrogen deuterium exchange page (page 227)
- Hydrogen deuterium exchange page display (page 227)
- Hydrogen deuterium exchange page commands (page 228)

Opening the hydrogen deuterium exchange page

To view the results on the Hydrogen Deuterium Exchange page

1. Open the results of the HDX experiment from the Queue page or from the Load Results page.

   The application transfers you to the Process and Review page. The current experiment name appears in the upper right of the page. If you load only one raw data file for the experiment, its name also appears in this area; otherwise, "(multiple files)" appears.

2. Click the **Mapping** tab and then click the **Hydrogen Deuterium Exchange** subtab.

   Note: This subtab appears only when you run an HDX experiment.

Hydrogen deuterium exchange page display

The Hydrogen Deuterium Exchange page displays one plot for each chain in the protein sequence used for the HDX experiment, with the title of each chain in red above the plot. For an experiment with only one raw data file, the plot shows only one color and symbol.

For an experiment with multiple raw data files, you must enter different conditions for these files. The plot shows a different color and symbol for each condition, with the color/symbol legend at the upper right of the plot.

The numbers at the top of each plot represent the residue numbers in the sequence. The letters at the bottom represent the amino acids in the sequence. The left side of the plot displays the protection factor range. Each dot shows the protection factor value for a particular amino acid and residue.

You can zoom in to a particular area of the plot by drawing a box for that area. Double-click to zoom out.

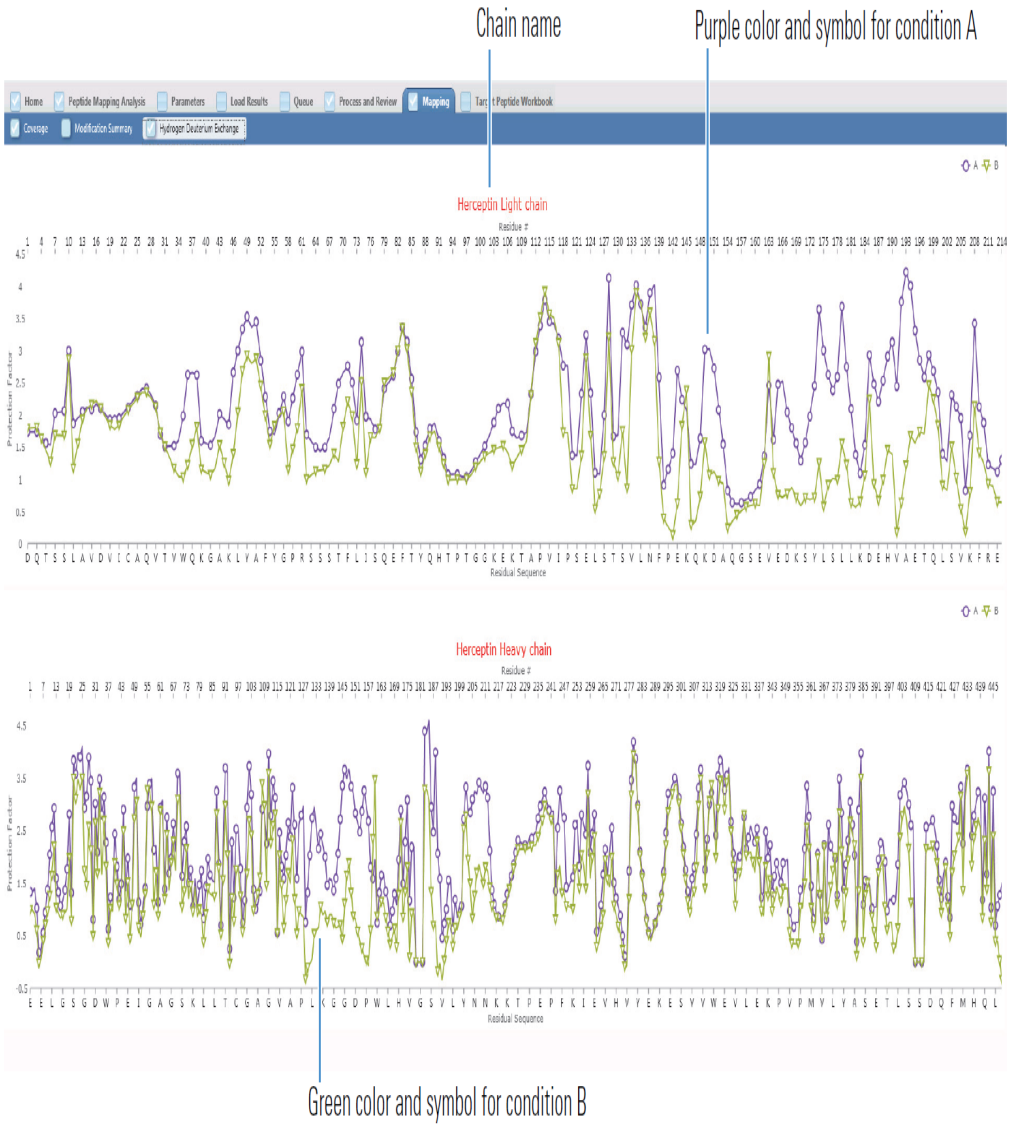


Figure 107 Hydrogen Deuterium Exchange page

Hydrogen deuterium exchange page commands

Right-clicking the plots on the Hydrogen Deuterium Exchange page opens a shortcut menu with the commands listed in the following table.

Table 25 Hydrogen Deuterium Exchange page shortcut menu

Command	Description
Reset Scale	Restores the original scale that first appeared on the page.
Copy	Copies the image on the page to the Clipboard.

Performing the kinetic MS/MS model prediction

The BioPharma Finder application uses the Kinetic model to predict peptide MS2 spectra. In the MS2 Spectra pane, you can change the parameters to regenerate the predicted spectrum using this Kinetic model.

To perform spectral prediction for a peptide dataset using the Kinetic model

1. On the Process and Review page, click the **MS2 Spectra** subtab.
2. Click a row in the Results table that displays "MS2" in the ID Type column.
3. Right-click the MS2 Spectra pane and choose **Predict Peptide MS/MS (Kinetic Model)**.

   The application opens the Predict Peptide MS/MS (Kinetic Model) dialog box.

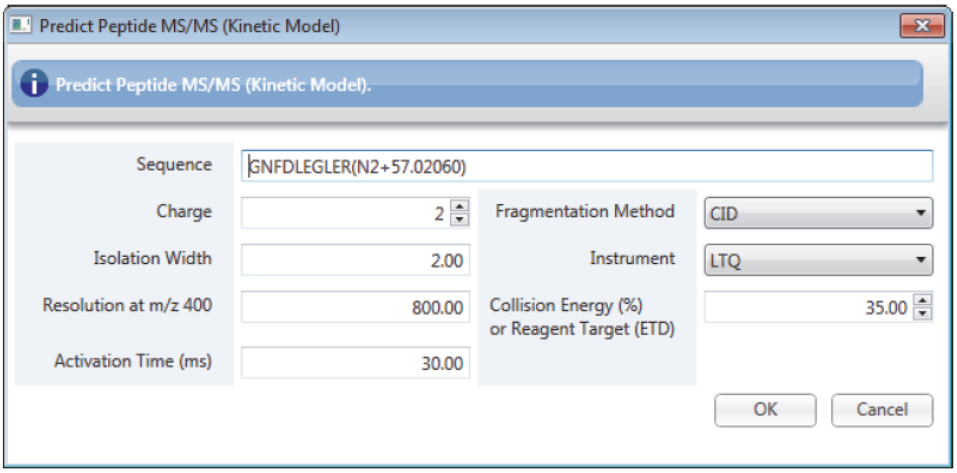


Figure 108 Predict Peptide MS/MS (Kinetic Model) dialog box

4. In the Sequence box, type the one-letter codes for a peptide sequence.

   Note: Use uppercase for all one-letter codes.

   The following table shows some extra sample sequence codes for modification letters in addition to the original 20 amino acid letters.

Table 26 Sample codes for a peptide sequence

Code	Definition
D	deamidated asparagine
J	carboxymethylated cysteine
U	carboxyamidomethylated cysteine
O	oxidized methionine
S	phosphorylated serine

You can also use the following special modification codes:

- For a modification, put the modification in parentheses after the sequence. For example, AADECFGHK(C5+250)(H8–9) means Cys at position 5 is modified by +250 u, and His at position 8 is modified by –9 u. AANASAA(N3+A2G0F) means Asn at position 3 is glycosylates with A2G0F.
- To define a disulfide bond, put the bond in parenthesis. For example, ADCAGHTYCHPEK(C3-C9) means Cys at position 3 and Cys at position 9 are linked by a disulfide bond.

5. Enter values for the following parameters:
   a. To set the charge state, the isolation width used for fragmentation, the resolution at *m/z* 400, and the activation time (ms), enter a value in their boxes.
   b. To define a fragmentation method, select from the available options in the list.

      Valid values: CID, CID with WB activation, ETD, ETD with supplemental activation, ECD, and HCD
   c. To choose an instrument, select an instrument name from the list.

      Valid values: LCQ, LTQ, Orbitrap™, LTQ FT, LTQ Velos, Velos Orbitrap™, QExactive, Orbitrap™ Fusion™, Fusion™ Ion Trap
   d. To define the collision energy or reagent target, enter a value in the box.

      Specify normalized collision energy as a percentage value (%) or specify the reagent ion target value for the ETD fragmentation method.
6. Click **OK** to see the MS2 predicted spectrum.

The application displays the changes in the predicted spectrum in the MS2 Spectra pane, as shown in the following figure.

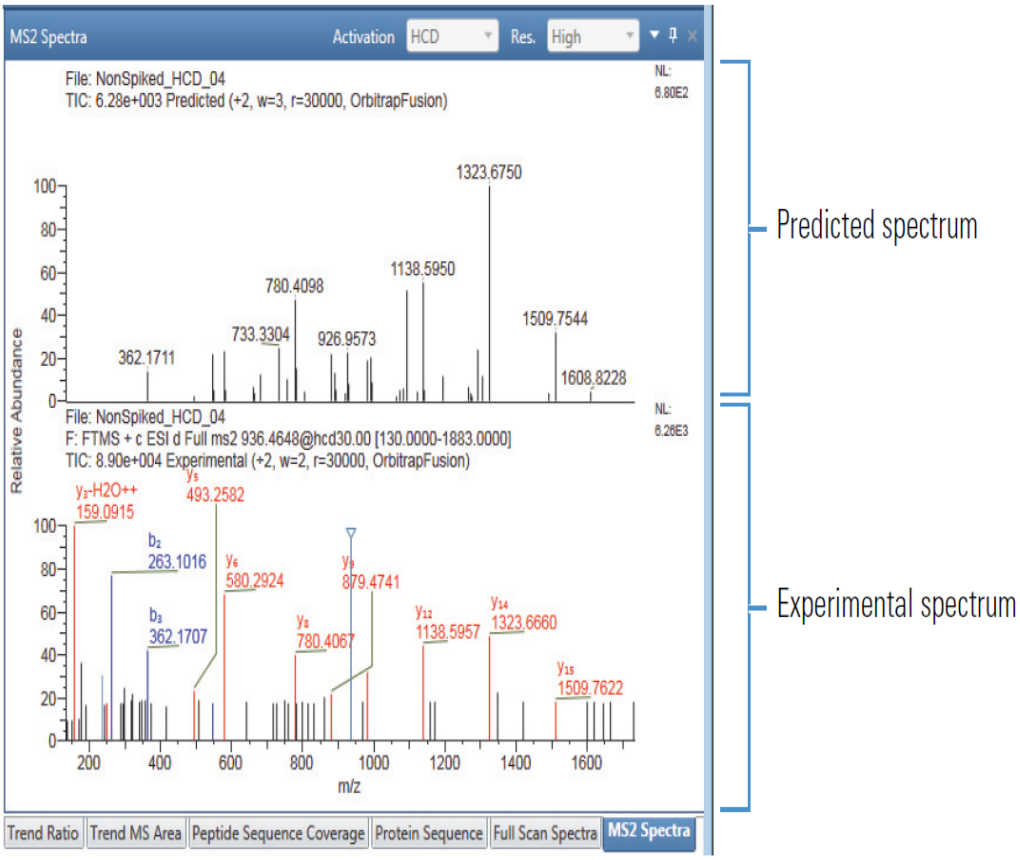


Figure 109 Predicted spectrum after applying the Kinetic model

Note: For components identified as dimers, the resulting predicted spectrum using the Kinetic model will not be correct.

Identifying components using de novo sequencing

If you have components that the BioPharma Finder application did not identify during peptide mapping processing or that might not be in your FASTA file, you can use de novo sequencing to potentially identify them.

The application performs de novo sequencing at the component level. It verifies that the first raw data file is of type MS2. If it is not, the application does not perform de novo sequencing.

Note: De novo sequencing works only when MS2 spectra are available in the results. It does not work with ETD data. It also does not work on an unidentified component from a multi-file experiment, unless the first file contains MS2 spectra.
You can perform de novo sequencing on only one component at a time. The de novo identification results automatically overwrite all previous results, including any identification results from peptide mapping processing.

For more details, see the following topics:

- Performing de novo sequencing (page 232)
- Canceling de novo sequencing (page 232)
- Setting up the de novo sequencing parameters (page 233)
- Defining the amino acids for de novo sequencing (page 234)

Performing de novo sequencing

To perform de novo sequencing on a single component in the Results table

1. Right-click a component row in the Results table on the Process and Review page and choose **Run De Novo Processing**.

2. Define the processing parameters for the de novo sequencing and click **OK** to begin the search.

   If the experiment contains data from multiple raw data files, the application uses the first raw data file (in the order listed in the Results table) that provides MS2 data for the de novo search.

   The application searches for the best identification for the selected component and, if it is found, displays the results in the Results table in the following columns: Identification, Peptide Sequence, Delta (ppm), Confidence Score, ID Type, and Mono Mass Exp., and overwrites previous data. The application also displays "De Novo" in the Protein column.

   The application also updates the fragment coverage map and the predicted and experimental spectra to display the found identification information, and saves all of the de novo results.

   The application displays a progress indicator, as some searches might take longer to complete. You can perform other actions while the search continues.

3. When the search is completed, right-click the component row in the Results table and choose **Show Component Information**.

   Other identification possibilities appear in a dialog box, listed in descending order of confidence score.

   These other possibilities have lower confidence scores than the identified component originally displayed in the Results table, which had the best score and is displayed at the top of the list in the dialog box.

Canceling de novo sequencing

To cancel de novo sequencing on a single component in the Results table

While the de novo processing is in progress, right-click the Results table on the Process and Review page and choose **Cancel De Novo Processing**.

The application cancels the search for the component that you previously selected for the de novo processing and does not save any de novo results.

Setting up the de novo sequencing parameters

To run de novo sequencing, set up the processing parameters in the De Novo Sequencing dialog box.

To define processing parameters for de novo sequencing

1. Right-click a component row in the Results table and choose **Run De Novo Processing**.

   The De Novo Sequencing dialog box opens, as shown in the following figure.

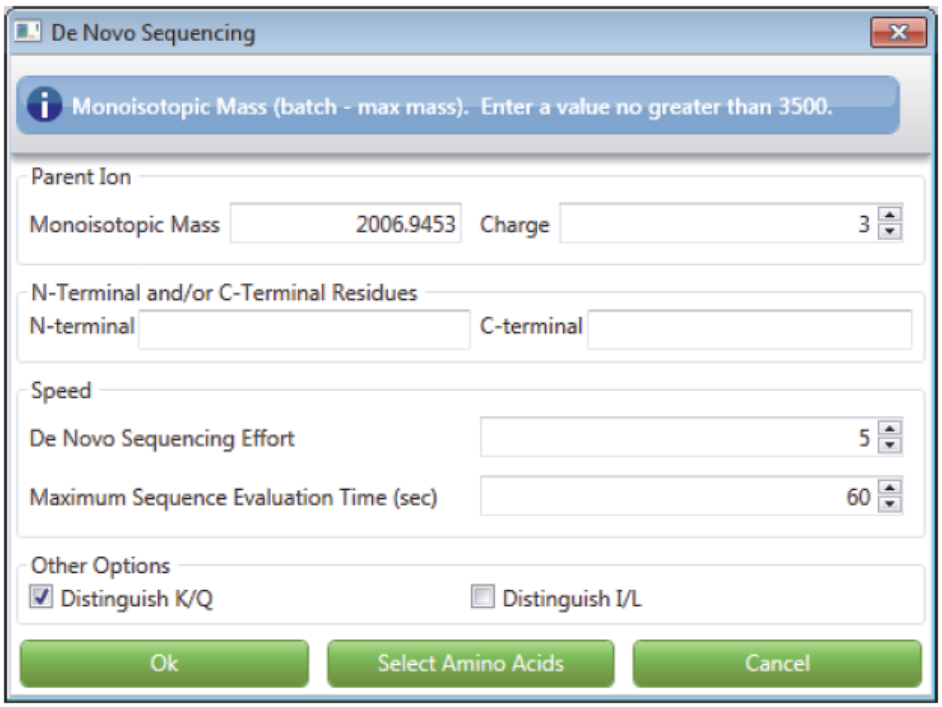


Figure 110 De Novo Sequencing dialog box

2. To specify the size of the monoisotopic mass of the precursor ion, type a value in the Monoisotopic Mass box.

   For reliable sequencing, set the value within 0.5 Da of the real mass. The application usually provides the value. When performing de novo sequencing to identify multiple peptides, the application uses this value to define the heaviest peptide for sequencing.

3. To specify the charge of the peptide, type a value in the Charge box.

4. To specify possible N-terminal and C-terminal residues, specify them in the appropriate boxes.

   For example, if the peptide is generated from a tryptic digest of a protein, set the C-Terminal as KR; otherwise, leave it blank.

5. To specify how much effort to spend on de novo sequencing, type a value in the De Novo Sequencing Effort box. Select **5** as a good starting point.

6. To specify the maximum time you want to spend on each sequencing task, type a value in the Maximum Sequence Evaluation Time box. Select a time of **60** seconds for most tasks.

   For large peptides (greater than1500 Da), you can set a longer time.

7. In the Other Options area, select the check boxes to specify if you want the algorithm to distinguish K/Q, I/L, or both.

   The algorithm can distinguish I/L amino acids to some extent, but not reliably. The distinction of K/Q amino acids is more reliable.

8. To define the amino acids to include in the de novo sequencing, click **Select Amino Acids**, or to start de novo sequencing, click **OK**.

Defining the amino acids for de novo sequencing

After setting up the processing parameters in the De Novo Sequencing dialog box, define the amino acids to include in the de novo sequencing.

To define the amino acids to include in the de novo sequencing

1. At the bottom of the De Novo Sequencing dialog box, click **Select Amino Acids** to open the Select Amino Acids dialog box.

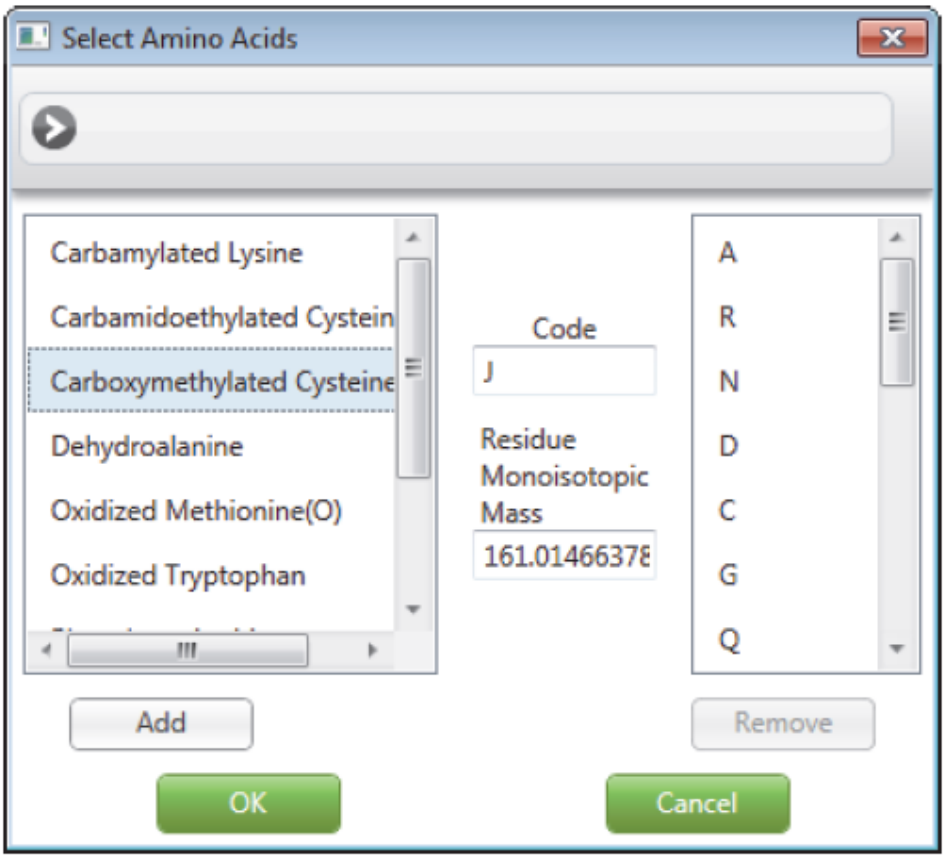


Figure 111 Select Amino Acids dialog box

The list on the left shows the default modified amino acids and the list on the right shows the 20 natural amino acids. The de novo search uses all of the amino acids in the list on the right.

2. To add an amino acid to the list on the right, select a name in the list on the left and click **Add**.

   The selected amino acid appears in the list on the right for inclusion in the de novo sequencing.

3. To exclude an amino acid from the sequencing, select a name in the list on the right and click **Remove**.

   The selected amino acid disappears from the list on the right and is no longer included in the de novo sequencing.

   When you select an item from these lists, the Code box displays the code related to the selected item that the BioPharma Finder application uses for de novo sequencing, and the Residue Monoisotopic Mass box displays the monoisotopic mass of that item if it is available. If no value is displayed, enter the code and mass values in the corresponding fields.

4. Click **OK** to apply your changes.

Organizing the experimental results

Use the Experiment Management pane to organize the experimental results in different folders. The Master List contains all of the previously saved Peptide Mapping Analysis experimental results. In the Working List, you can create folders or subfolders and drag and drop results from the Working List to any folder that you created. The Master List folder shows all the experimental results regardless of the folder in which they reside.

To access the Experiment Management pane

1. On the Home page, click **Peptide Mapping Analysis** in the left pane or below the BioPharma Finder splash graphic.

   The Peptide Mapping Analysis page opens.

2. Click the **Load Results** tab.

   The Load Results page opens to the Master List by default. The BioPharma Finder application displays all of the previously saved Intact Mass Analysis experimental results in the Master List.

3. Do one of the following to access the Experiment Management pane.

   - Click « to expand the Experiment Management pane.

     The Experiment Management pane opens to display the Master List and the Working List.

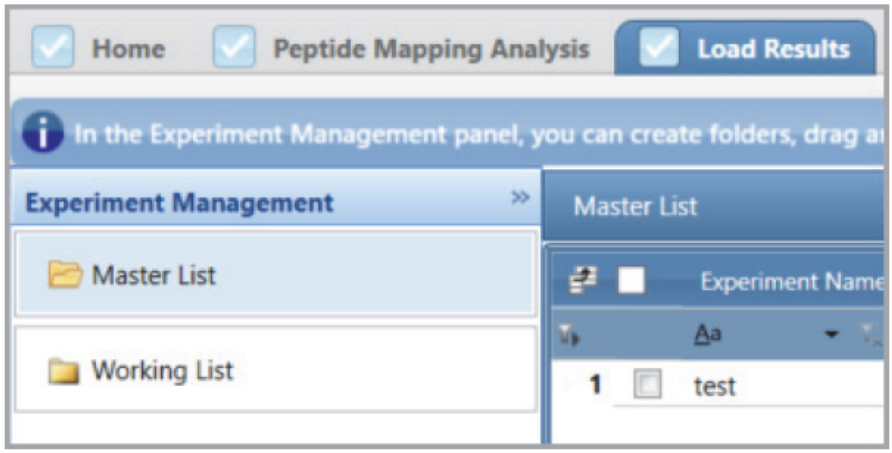


Figure 112 Experiment Management pane

–or–

- Click the **Experiment Management** pane on the left.
  A window appears showing the Master List and the Working List.

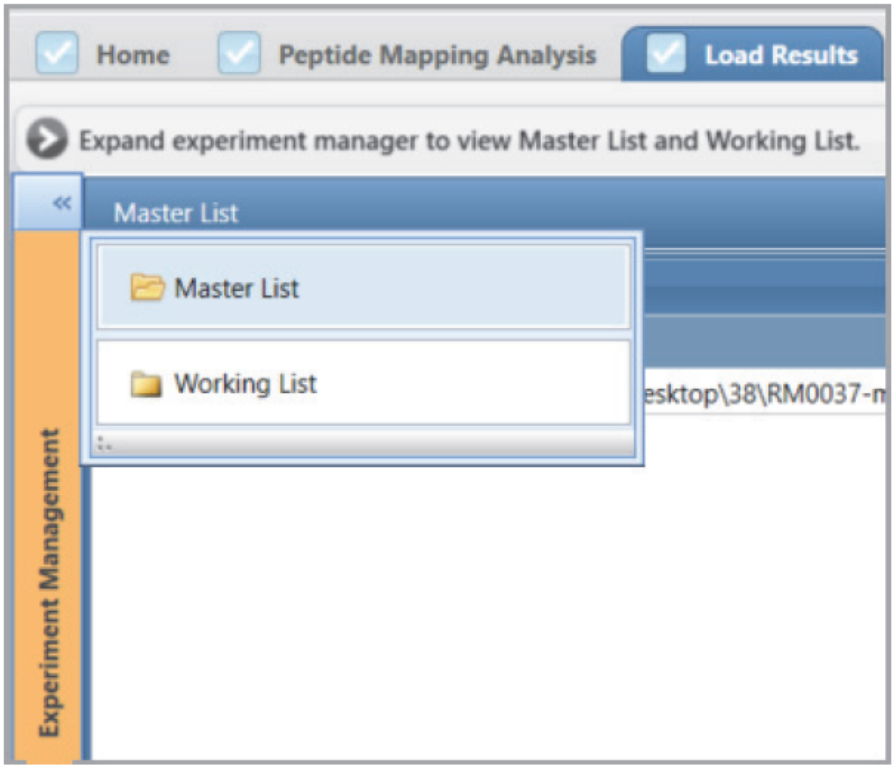


Figure 113 Experiment Management window

To create a folder in the Working List

1. In the Experiment Management pane of the Load Results page, select the **Working List**.
   By default, the Working List contains the Home folder.
2. Click the **Home** folder, and then click **Add**.

   Note: You can only create a new folder under the Home folder of the Working List. You cannot create a folder in the Master List.
3. In the Add New Folder dialog box, type the Folder Name, and then click **Add**.
   A new folder appears under the Home folder in the Working List.
4. To create a subfolder, select the folder to which you want to add a subfolder, and then click **Add**.
   A new subfolder appears under the folder.
   Each folder can contain subfolders, and each subfolder, in turn, can contain more subfolders, and so on.

To rename a folder

1. In the Experiment Management pane of the Load Results page, select the **Working List** to view its folder structure.
   When the Working List opens, only the Home folder appears in the pane.
2. To navigate through the folder structure, click the arrow next to a folder.
3. To rename a folder, select the folder of interest, and then click **Edit**.
4. In the dialog box, type the new name.

   Note: You can only rename a user-created folder.

To move an experiment result to a folder

1. In the Experiment Management pane of the Load Results page, select the **Working List** to view its folder structure.
   The right pane shows the contents of the Working List.
2. From the Working List, select an experiment result or use the CTRL key to select multiple results, and then drag and drop to the destination folder of interest.
   The moved experimental result or results appear in the new destination folder.

To delete an experiment result from a folder

1. In the Experiment Management pane of the Load Results page, select the **Working List** to view its folder structure.
   When the Working List opens, only the Home folder appears in the pane.
2. To navigate through the folder structure, click the arrow next to a folder.
3. Select the folder that contains the result to delete.
   The right pane shows the contents of the selected folder.
4. To delete an experiment result, select the check box corresponding to the result.
   You can also select multiple check boxes to delete more than one result.
   In the command bar, click **Delete**, and do one of the following:
   - In the Delete Selected Results from Folder dialog box, select **Delete Results from Folder**.
     The application deletes the experiment result from the folder and moves it to the Working List.
     –or–
   - In the Delete Selected Results from Folder dialog box, select **Delete Results from Folder and Master List**.
     The application deletes the experiment result from both the folder and the Master List.

To delete a folder

1. In the Experiment Management pane of the Load Results page, select the **Working List** to view its folder structure.
   When the Working List opens, only the Home folder appears in the pane.
2. To navigate through the folder structure, click the arrow next to a folder.
3. To delete a folder, select the folder.
4. In the Experiment Management pane, click **Delete**.

5. In the Delete Selected Folder dialog box, do one of the following:
   - Select **Delete Folder**.
     The application deletes the folder and moves its experiment result content (if any) to the Working List.
     –or–
   - Select **Delete Folder and Results from Master List**.
     The application deletes both the folder and its experiment result content from the Master List.

Viewing the process and review page for peptide mapping analysis

After the application completes the analysis of an experiment, you can open the results of that analysis and view the chromatograms, trend plots, sequence and fragment coverage maps, MS spectra, and Results table on the Process and Review page.

You can open the results from the Queue page or from the Load Results page.

The current experiment name appears in the upper right. If you load only one raw data file for the experiment, its name also appears below the experiment name; otherwise, "(multiple files)" appears.

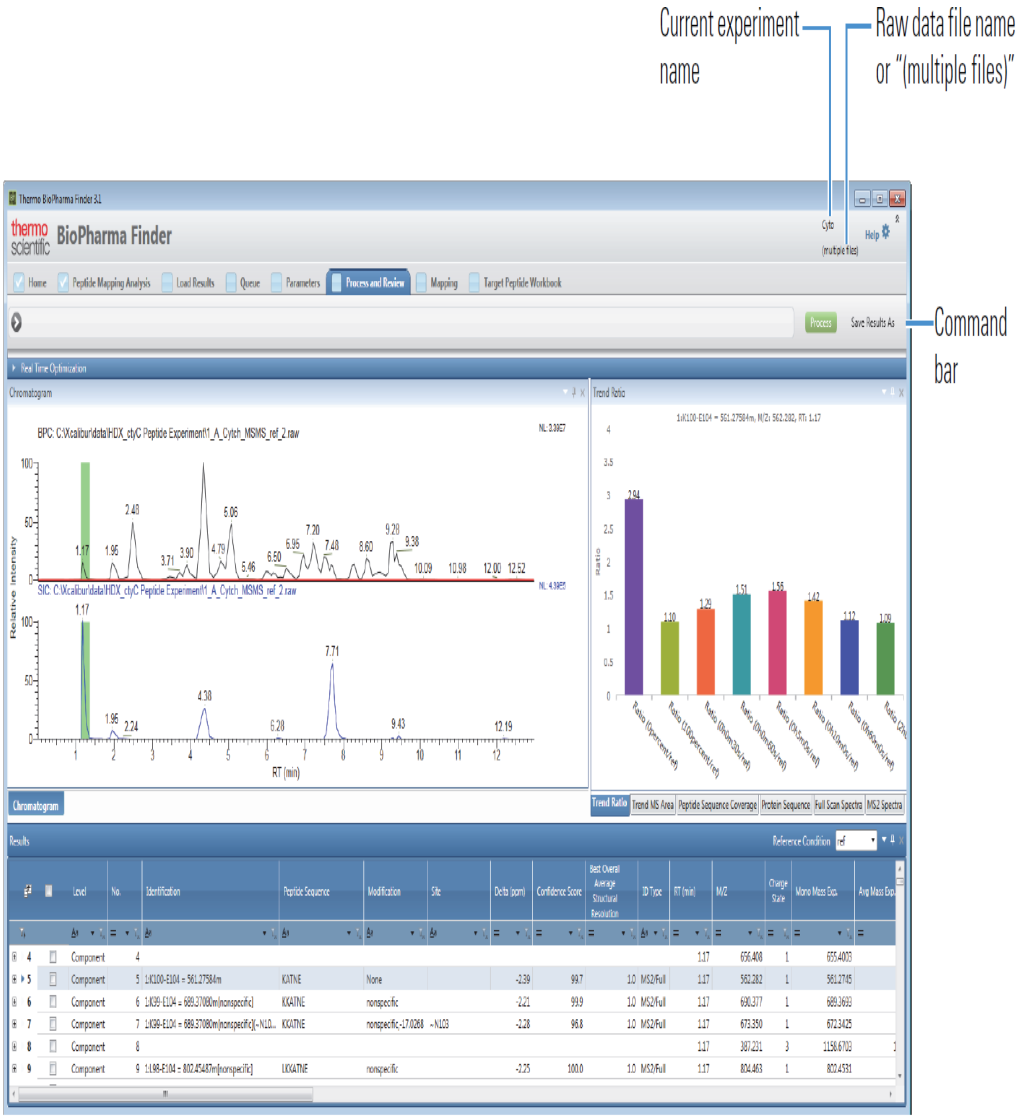


Figure 114 Process and Review page for Peptide Mapping Analysis

Process and review page parameters for peptide mapping analysis

The following table describes the types of information on the Process and Review page. To display the content of a pane that is not currently visible, click its subtab. You can adjust the size or location of the panes on this page as needed.

Table 27 Process and Review page parameters

Parameter	Description
Results table	Displays at the upper level the components and their peptide sequences, retention times, modifications, and detected masses, along with their confidence scores. At the lower level, the table displays all of the raw data files that are loaded for the experiment and their information.
Chromatogram pane	Displays the chromatograms for the component or raw data file that you select in the Results table.
Relative Intensity (*y* axis)	Displays the ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity.
RT (min) (*x* axis)	Displays the retention time of the spectrum, which is the time after injection at which a compound elutes. Retention time can also refer to the total time that the compound is retained on the chromatograph column.
Trend Ratio pane	Displays the bar plots for the ratios between various conditions and the reference condition, for the component that you select in the Results table.
Ratio value (*y* axis)	Displays the Avg MS Area value for a particular condition ÷ Avg MS Area value for the reference condition.
Ratio conditions (*x* axis)	Displays the names of the various conditions and the name of the reference condition.
Trend MS Area pane	Displays the bar plots for the MS Area values for the component that you select in the Results table, for each raw data file used in the experiment.
MS Area value (*y* axis)	Displays the MS Area values from each of the individual raw data files assigned to a particular condition.
Condition-Raw data file (*x* axis)	Displays the names of the various conditions and their assigned raw data files used in the experiment.
Peptide Sequence Coverage pane	Displays the fragment coverage map, including the peptide sequence information and the color-coded fragment ions.
Protein Sequence pane	Displays the protein sequence assigned to the experiment, which shows the highlighted peptide sequence that you select from the Results table.
Full Scan Spectra pane	Displays the deconvoluted and full-scan spectra with mass and *m/z* information.
Relative Abundance (*y* axis)	Displays the ratio of the abundance of a specific peak to the abundance of the peak with the highest abundance.

Parameter	Description
Mass or *m/z* (*x* axis)	Displays the mass or mass-to-charge ratio of the ions formed from molecules. This ratio is the quantity formed by dividing the mass of an ion, in daltons, by the number of charges that the ion carries.
MS2 Spectra pane	Displays the predicted spectrum stacked on top of the experimental spectrum.
Relative Abundance (*y* axis)	Displays the ratio of the abundance of a specific peak to the abundance of the peak with the highest abundance.
m/z (*x* axis)	Displays the mass-to-charge ratio of ions formed from molecules. This ratio is the quantity formed by dividing the mass of an ion, in daltons, by the number of charges that the ion carries.
Real Time Optimization pane	Displays the same parameters as those on the Parameters > Component Detection and Identification pages, so that you can adjust these parameters and perform real-time optimization.

Note: If the *x*- or *y*-axis label in a pane is not visible, enlarge the pane or change the pane to a larger floating window.

Process and review page commands for peptide mapping analysis

The following table describes the commands on the Process and Review page.

Table 28 Commands on the Process and Review page

Command	Description
Process	Processes the peptide mapping experiment and deconvolves the source spectra with any or all of these parameters: modified protein sequence, component detection, and identification. Also, displays the new results. Saves the latest results in a database after you process an experiment. Click this button to open a dialog box where you can enter a new experiment/method name or retain the same name to overwrite previously saved results/parameters in the current experiment with the new data. **Note:** To activate the Process button, you must modify the experiment parameters. Use only alphanumeric, space, underscore "_", and period "." characters, up to 50 maximum, in the experiment and method names. You cannot overwrite an experiment that is currently submitted to the run queue or is actively processing or canceling. You also cannot overwrite a default method.
Save Results As	Saves the latest results in a database. Click this button to open a dialog box where you can enter a new experiment name or retain the same name to overwrite previously saved results in the current experiment with the new results. You can also enter a description for the experiment. This button is inactive if you modified any processing method parameter for real-time optimization. In this case, click **Process** to reprocess the experiment and reactivate this button. **Note:** Use only alphanumeric, space, underscore "_", and period "." characters, up to 50 maximum, in the experiment name. You cannot overwrite an experiment that is currently submitted to the run queue or is actively processing or canceling.

Viewing the results table for peptide mapping analysis

The Results table on the Process and Review page displays the results of a completed Peptide Mapping Analysis experiment, organized by the components at the upper level and by the raw data files at the lower level.

When you select the row for a component or one of the raw data files in the Results table, you can view related information in the various other panes of the Process and Review page.

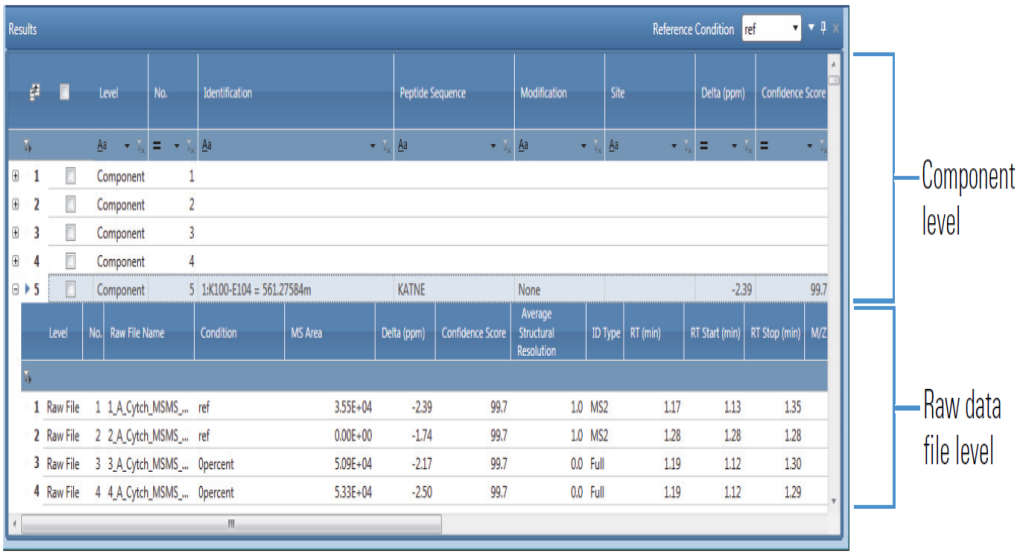


Figure 115 Results table on the Process and Review page

For more details, see the following topics:

- Viewing the peptide results table (page 246)
- Changing the reference condition (page 246)
- Exporting the results table (page 247)
- Saving a peptide workbook from the process and review page (page 248)
- Results table parameters (page 250)
- Modification parameters (page 257)
- Results table commands (page 258)

Viewing the peptide results table

To view data in the Results table

1. Click the **Process and Review** tab if necessary.

   The Process and Review page displays the component results for Peptide Mapping Analysis in the Results table.

2. Click a component row in the table to view information that is related to that component in the other panes on this page.

3. Click the plus icon, ⊞, at the left side of a component row to view information that is related to that component and specific to each raw data file loaded for the experiment.

Changing the reference condition

For Peptide Mapping analysis with multiple raw data files, you must enter conditions to associate with these files, by including a reference condition. After processing, you can change this reference condition to a different condition and the application automatically recalculates and redisplays the updated ratio values.

To change the reference condition

At the far right of the title bar of the Results pane on the Process and Review page, select a different reference condition from the list of conditions for the raw data files used in the experiment.

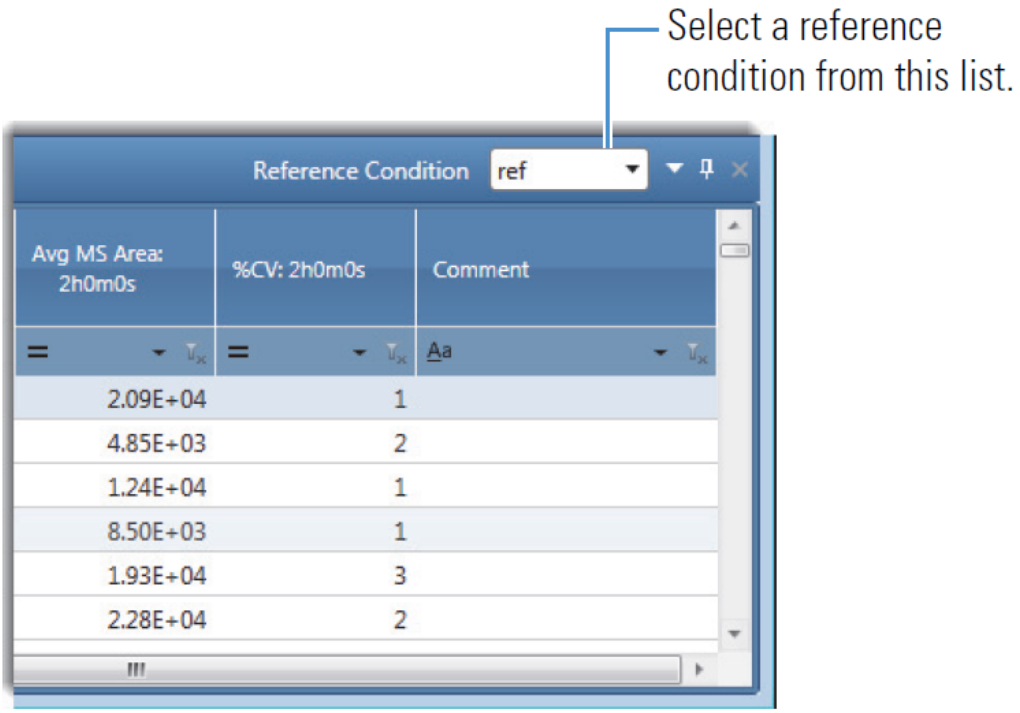


Figure 116 Reference Condition list in title bar of Results pane

The application automatically recalculates the ratio between the average component area for each condition and the average component area for the selected reference condition, and then displays this value in the Ratio (Condition/Reference Condition) column in the Results table as well as in the Components table on the Modification Summary page. The Max Condition and Min Condition cells also update to show the conditions with the highest and lowest ratio values, respectively.

Exporting the results table

You can export all or selected results to external files to save the data to a spreadsheet, to a file compatible with the Chromeleon data system, or to a file that the Mascot™ search engine can read.

Before exporting, you must run a new peptide mapping experiment or load an existing experiment.

To export the data in the Results table

1. On the Process and Review page, right-click anywhere in the Results table for Peptide Mapping Analysis and choose one of the following menu commands:
   - **Export All Components** to export data at all levels for *all* components in the table
   - **Export Checked Components** to export data at all levels for only the *selected* components in the table

     To select a row of results to export, select the check box in that row.

     To select all of the rows, select the check box in the column header.

   –or–

   - **Create .mgf File** to store the mass and charge state information in the Results table in an MGF file that the Mascot™ search engine can read

     You can use this file to expand the search by using the Mascot™ search engine and providing a means for identifying host cell proteins or contaminants.

2. For the **Export All Components** and the **Export Checked Components** commands, choose one of these submenu commands:
   - **As Displayed** to export to a Microsoft™ Excel™ file exactly as currently displayed in the table
   - **Microsoft™ Excel™ Workbook** to export to an Excel file in the default format
   - **CSV** to export data to a CSV file in the default format
   - **Chromeleon** to export to a BioPharma Finder (BPF) file in a format that is compatible with the Chromeleon data system

     The Isotope Count dialog box opens. Enter the isotope count to indicate the number of isotopes to export.

     Note: For the Chromeleon option, the BioPharma Finder application does not export components that do not have a sequence identification.

The exported data reflects the filtering, sorting, and reordering of columns of the Results table only for the As Displayed option. The application exports the table rows based on the sequential order of the numbers in the No. column.

3. In the Save As dialog box, browse to or type the name of the file to store the exported results in.

   By default, the file name is the same name as the experiment.

4. Click **Save**.

The application stores the exported data in the specified file. If you do not browse to a different folder, the application places the exported file by default in the folder with the raw data files for the experiment.

Saving a peptide workbook from the process and review page

A peptide workbook is a list of peptides selected from the results of a Peptide Mapping Analysis experiment (or from the results on the Theoretical Protein/Peptide Manager page), but not the protein sequences and the method parameters used to process those results. You can use a saved peptide workbook as a sequence for a targeted peptide search and export data from a workbook to a file compatible with the Chromeleon data system.

To save the results for Peptide Mapping Analysis to a workbook

1. Load an experiment to open the Process and Review page and view the results.

2. In the Results table, select the check box in the row of each component that you want to save to the workbook.

   To select/deselect all of the rows, select/clear the check box in the column header.

3. Right-click anywhere in the Results table and choose **Save As Peptide Workbook ▸ Checked** to save the results of the selected rows in the table to a workbook.

   IMPORTANT! The application does not support saving components with unspecified modifications, dimers, adducts, disulfide bonds, or any type of gas phase modifications to the workbook. You must clear the check boxes for these components to save the workbook.

4. In the Save Peptide Workbook As dialog box, do the following:
   a. Select one of these options: **Create a New Peptide Workbook** or **Select an Existing Workbook**.

b. (For the Create a New Peptide Workbook option) In the Workbook Name box, type a new name to create a new workbook or keep the same name to overwrite the workbook if it already exists.

   The default workbook name is the same as the experiment name.

   Note: Use only alphanumeric, space, underscore "_", and period "." characters in the workbook name.

   –or–

   (For the Select an Existing Workbook option) Select the check box for an existing workbook in the table.

   The application adds data from your selection of components to that workbook. A peptide workbook can contain added data from multiple different experiments.

c. (Optional) In the Description box, type a description for the workbook.

d. In the Number of Isotopes per Peptide box, specify the number of isotopes for the selected components.

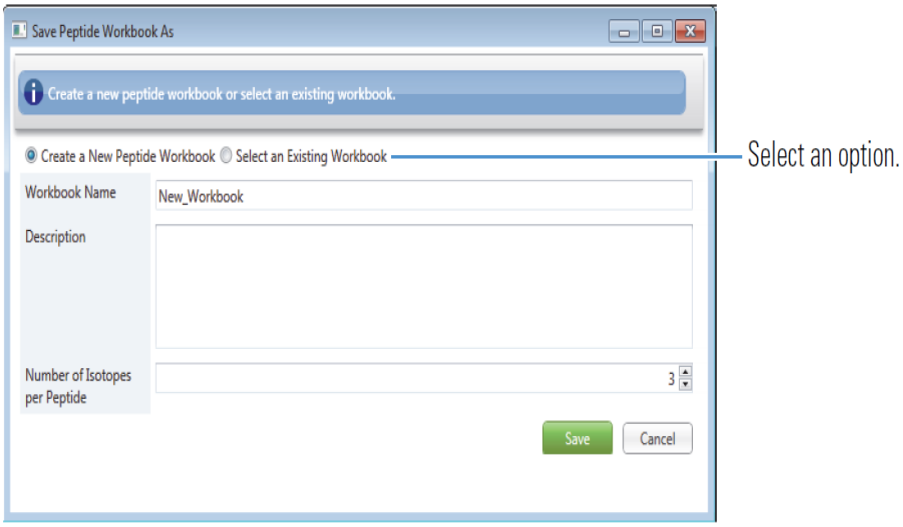


Figure 117 Save Peptide Workbook As dialog box

5. Click **Save**.

   Note: If the workbook is currently open for editing on the Workbook Editor page, indicate whether you want the application to automatically close the open workbook before saving.

   The application saves all selected and identified components (but not the unidentified components) to the indicated workbook.

If you are creating a new workbook, the following occur:

- Multiple peptide components with the same set of data—identification, sequence, modification, site, theoretical monoisotopic mass (within a tolerance that is ± 10 ppm of each other), protein name, and RT (within a tolerance that is ± half of the typical chromatographic peak width for the experiment)—merge into one entry in the workbook. However, the merged entry saves the charge state distribution from the individual components.
- The application automatically assigns a relative quantitation group number to all workbook entries with the same protein and sequence, starting with group number "1" and incrementing by one for each new group.

If you are adding to an existing workbook, the following occur:

- The application does not merge the added components that have the same data as the components already existing in the workbook. In this case, application inserts the added components to the workbook as duplicates.
- The application automatically adjusts the relative quantitation group number. The added entries with the same protein and sequence as some existing entries in the workbook receive the same relative quantitation group number as the existing entries. All other added entries receive incremented group numbers.

After saving, you can manage the workbook using the Target Peptide Workbook page.

Results table parameters

The following table describes the types of information in the Results table for Peptide Mapping Analysis on the Process and Review page.

IMPORTANT! The calculated Mono Mass Exp. and Avg Mass Exp. values from the BioPharma Finder application might be slightly different from the calculated masses from the PepFinder application. The BioPharma Finder application uses an updated algorithm.

Table 29 Results table parameters

Column	Description
Component level	
+/–	Click to show or hide the lower level of raw data file information related to the current component row.
Row number	The number assigned to each visible component row in the table. This sequential numbering does not change when you sort or filter the table.

Column	Description
	Select this check box if you want to export the results for the components in the selected rows to an Excel file, using the shortcut menu. **Note:** To select or clear all of the check boxes at once, select or clear the check box in the column header. If you filter the table, the following occurs: • Clearing all check boxes affects all of the original rows in the table, before any filtering. • Selecting all check boxes affects only the filtered and currently visible rows.
Level	Indicates that the row is displaying component information (top level).
No.	Displays a number for each component in the Results table.
Identification	Displays the identification associated with the component, including optional modifications information. For example, 7:T95-R119 = 2544.30062m(C98+Carbamidomethylation) that shows • 7 = the protein ID number • T95 = the first amino acid in the peptide sequence and its position number • R119 = the last amino acid in the peptide sequence and its position number • 2544.30062m = the mass of the unmodified peptide (this is neutral and not a charged mass) • (optional) (C98+Carbamidomethylation) = the modification information. If the component is not identified, this cell is empty. **IMPORTANT!** In some situations, a combination of low resolution, high mass, or high charge causes the application to not isotopically resolve the mass in the results. In this situation, the mass in this cell is an average mass and the cell shows the letter "a" after the mass value. Conversely, the cell shows the letter "m" after the mass value to denote a monoisotopic mass. For example, "1128.27a" is an average mass. The application does not calculate the Mono Mass Exp. and displays a zero value in that cell. The Delta (ppm) value is generally larger from using the Avg Mass Exp. value instead of the Mono Mass Exp. value for the Delta (ppm) calculation. **Note:** For a targeted peptide mapping experiment, even when this cell is empty, the Comment cell might show a possible suggested identification.
Peptide Sequence	Displays the peptide sequence for the identified component. If the component includes a disulfide bond, this cell lists each peptide sequence in the bond, including the protein number. If the component is not identified, this cell is empty.

Column	Description
Modification	Displays the type of modification that the application identified. This list might include all of the variable modifications used during processing and other modifications that the application determines automatically. If modifications exist, this cell lists the variable modifications first, followed by the static modifications in parentheses. Commas (",") separate multiple modifications. If there is no modification, this cell displays "None". If the peptide does not follow the rules of the protease, this cell displays "nonspecific". This cell might also list masses using a format similar to the Identification column. If the component is not identified or the Sequence Variant column displays the amino acid substitution, this cell is empty.
Site	Displays the position of the modification if it is listed in the Identification column, or of the amino acid substitution if it is listed in the Sequence Variant column. For example, if the Identification column lists 7:T95-R119 = 2544.30062m(C98+Carbamidomethylation), then C98 is the site position. If modifications exist, this cell lists the sites for the variable modifications first, followed by the sites for the static modifications in parentheses. Commas (",") separate multiple sites. For disulfide bonds, this cell displays "/" to separate each peptide in the bond and provides the site information for each peptide in this format: • Protein number of the peptide; for example, "1:" • "C" followed by the position of the cysteine in the peptide; for example, "C64" For example, if the Identification column lists 1:C6-R14/N46-R68 = 3662.03a[1ss] and there is a cysteine at position 64, then 1:C6/1:C64 is the site value. If the application uses full-scan information to identify the component, or there are multiple cysteines in the bond and the application cannot provide the exact site, this cell displays commas (",") to separate the multiple cysteines and lists "∼" in front of the position to indicate an approximate site. If the component is not identified, this cell is empty.
Sequence Variant	(Visible only when you set the Search for Amino Acid Substitutions option on the Identification page for the processing method) Displays the amino acid substitution for an identified component that contains a sequence variant. If the component is not identified or does not contain a sequence variant, this cell is empty.
Delta (ppm)	Displays the mass difference between the theoretical mass of the peptide and the experimental measured mass. Delta (ppm) = 1 000 000 × ([Mono Mass Exp. – Mono Mass Theo.] ÷ Mono Mass Theo.) If the component is not identified, this cell is empty. If the application cannot confidently determine the Mono Mass Exp. value, it uses the Avg Mass Exp. value instead.

Column	Description
Confidence Score	Displays the quality of the match between the predicted spectra and the experimental spectra. This cell displays a value between 0 and 100%. • 0% indicates only a poor fit between the predicted spectra and the experimental spectra. • 100% indicates a very good (though not exact) fit between the predicted spectra and the experimental spectra. A fit factor of 100% means that the observed peaks in the predicted spectrum are absolutely identical to those in an experimental spectrum and that any missing peaks fall below a restrictive threshold. If the component is not identified, this cell is empty.
Best Overall Average Structural Resolution	Displays the average structural resolution value, which is the value found on the fragment coverage map. This cell displays the resolution value that is best for the component (closest to 1.0), from all of the resolution values for all of the raw data files loaded for the experiment, instead of the value from the first raw data file by default. The value shown in the fragment coverage map in the Peptide Sequence Coverage pane is from the first raw data file, therefore it might not match the value in this cell.
ID Type	Indicates the type of peptide identification. When the experiment uses only one raw data file, this cell displays the same identification type as shown at the raw data file level. For details about each identification type from an individual raw file, see the ID Type column at the raw data file level. When the experiment uses multiple files, this cell displays the following: • MS2: The raw data files contain only MS2 scans. • Full: The raw data files contain only Full MS scans. • MS2/Full: The raw data files contain a mixture of MS2 and Full MS scans. The MS2 scan provides more confidence in identifying the component than a Full MS scan. If the peptide is unidentified, this cell is empty.
RT (min)	Displays the retention time for the component from the first raw data file.
M/Z	Displays the mass-to-charge ratio of the calculated monoisotopic mass for the component.
Charge State	Displays the charge state for the component. This is the imbalance between the number of protons (in the nuclei of the atoms) and the number of electrons that a molecular species (or adduct ion) possesses. If the species possesses more protons than electrons, its charge state is positive. If it possesses more electrons than protons, its charge state is negative.
Mono Mass Exp.	Displays the experimental monoisotopic mass for the component. If the application cannot confidently determine the Mono Mass Exp. value, this cell displays a zero value.

Column	Description
Avg Mass Exp.	Displays the experimental average mass for the component. If the application cannot confidently determine the Avg Mass Exp. value, this cell displays a zero value.
Mono Mass Theo.	Displays the theoretical monoisotopic mass for the component. If the component is not identified, this cell is empty.
Ratio (*Condition*/*Reference Condition*)	(Visible only for an experiment with multiple raw data files and conditions) Displays this ratio: Avg MS Area value for a particular condition ÷ Avg MS Area value for the reference condition **Note:** The Results table displays this column for each condition specified for the experiment that is not the reference condition. If the denominator for the ratio is 0, this cell displays "9999.99".
Max Condition	(Visible only for an experiment with multiple raw data files and conditions) Displays the condition with the highest ratio value. See the Ratio (*Condition*/*Reference Condition*) on page 254 column.
Min Condition	(Visible only for an experiment with multiple raw data files and conditions) Displays the condition with the lowest ratio value. See the Ratio (*Condition*/*Reference Condition*) on page 254 column.
MS Area or Avg MS Area: *Condition*	Displays the area (for an experiment with a single raw data file) or the average area for a particular condition (for an experiment with multiple raw data files). If you load only one raw data file for the experiment, MS Area = value in MS Area column for the loaded raw data file. Otherwise, if you load multiple raw data files, Avg MS Area = average of the MS Area values from all of the individual raw data files that belong to the group that is assigned to a particular condition. **Note:** For an experiment with multiple raw data files, the Results table displays this column for each condition specified for the experiment.
%CV: *Condition*	(Visible only if the experiment contains one or more conditions for grouping the raw data files) Displays the coefficient of variation (CV) for a particular condition. %CV = 100 × (standard deviation of the MS Area values from all of the individual raw data files that belong to the group assigned to this condition ÷ the average of these areas). **Note:** The Results table displays this column for each condition specified for the experiment.
Protein	Displays the identified protein for the component. If the component is not identified, this cell is empty. If you use de novo sequencing to identify the component, this cell displays "De Novo."

Column	Description
Comment	Enter a comment for the component. Any comment you enter here automatically appears in the Comment column of the Components table on the Modification Summary page and vice versa. You can enter up to 128 alphanumeric and symbolic characters. **Note:** For a targeted peptide mapping experiment, you might create a peptide workbook from the Theoretical Protein/Peptide Manager page and use it as a protein sequence. In the case when this workbook contains peptides with multiple amino acids that are the same, or even when there is no value in the Identification column, this cell might show a suggested identification. Because the retention time and MS2 spectra information are not available in this type of workbook, the suggested identification is based on the M/Z and Mono Mass Exp. values, showing an approximate site determination.
Raw data file level	
Row number	The number assigned to each visible raw data file row in the table. This sequential numbering does not change when you sort or filter the table.
Level	Indicates that the row is displaying raw data file information (lower level).
No.	Displays a sequential number for each raw data file. This list of numbers is the same for every component and should match the total number of raw data files used for the experiment.
Raw File Name	Displays the name of the raw data file.
Condition	Displays the condition assigned to the raw data file; otherwise, this cell is empty.
MS Area	Displays the area for the component, specific to each individual raw data file.
Delta (ppm)	Displays the mass difference between the theoretical mass of the peptide and the experimental measured mass. These masses are from the raw data file and might be different from the masses at the component level. Delta (ppm) = 1 000 000 × ([Mono Mass Exp. – Mono Mass Theo.] ÷ Mono Mass Theo.) If the component is not identified, this cell is empty. If the application cannot confidently determine the Mono Mass Exp. value, it uses the Avg Mass Exp. value instead.
Confidence Score	Displays the quality of the match between the predicted spectra and the experimental spectra. This column displays a value between 0 and 100%. See the Confidence Score column at the component level for more details. If the component is not identified, this cell is empty.
Average Structural Resolution	Displays the same average structural resolution value as the value on the fragment coverage map in the Peptide Sequence Coverage pane.

Column	Description
ID Type	Displays the type of peptide identification, specific to each individual raw data file. • MS2: The Average Structural Resolution value is > 0.00. • Full: The Average Structural Resolution value is = 0.00. The MS2 scan provides more confidence in identifying the component than a Full MS scan. If the component is not identified, this cell is empty.
RT (min)	Displays the retention time for the component that is specific to each individual raw data file.
RT Start (min)	Displays the start of the retention time range for the component that is specific to each individual raw data file.
RT Stop (min)	Displays the end of the retention time range for the component that is specific to each individual raw data file.
M/Z	Displays the mass-to-charge ratio of the calculated monoisotopic mass for the component that is specific to each individual raw data file.
Charge State	Displays the charge state for the component that is specific to each individual raw data file.
Mono Mass Exp.	Displays the experimental monoisotopic mass for the component that is specific to each individual raw data file. If the application cannot confidently determine the Mono Mass Exp. value, this cell displays a zero value. **Note:** For a targeted peptide mapping experiment with multiple raw data files, the values displayed in this cell might be the same for all of the loaded raw data files. In this case, during the identification process, the application compares the experimental monoisotopic mass in each raw data file against the mass in the file with the highest MS area (the best file). If all of the compared masses are outside of the tolerance limit, the application assigns the mass from the best file to this cell, for all of the raw data files.
Avg Mass Exp.	Displays the experimental average mass for the component that is specific to each individual raw data file. If the application cannot confidently determine the Avg Mass Exp. value, this cell displays a zero value.
Mono Mass Theo.	Displays the theoretical monoisotopic mass for the component that is specific to each individual raw data file. If the component is not identified, this cell is empty.

Modification parameters

The following table describes the format of the modification information for Peptide Mapping Analysis available in the Results table on the Process and Review page.

Table 30 Modification format

Modification type	Description
Variable modification	For example, 7:T95–R119 = 2544.30062m(C98+Carbamidomethylation) shows: • C98 = the modification on the cysteine at position 98 in the protein sequence • Carbamidomethlytation = the modification type
Unspecified modification	For example, 1:D1–R24 = 2587.29857m(~V2+57.0261) shows: • ~V2 = the modification is approximately on the V amino acid at position 2 in the protein sequence • +57.0261 = the addition of the mass of 57.0261 You enable the mass search for unspecified modifications to determine the unspecified mass modification.
Multiple modifications	For example, 1:S25–K50 = 3023.47773m(~N33+57.0083)(~H31–58.0273) shows two modifications. The modification at approximately N33 has an addition of mass, whereas the modification at approximately H31 has a subtraction of mass. See the format for Unspecified modification.
Dimer	For example, 1:D1–R24 = 2587.29857m[2x] shows the application identified the peptide as a dimer (2x).
Nonspecific protease	For example, 1:S10–R24 = 1588.78791m[nonspecific]
Adducts	For example, 1:V83–K108 = 2844.34288m(Na+) shows Na+ is the adduct.
Gas phase oxidation	For example, 1:S179–K188 = 1067.55326m(GasPhaseOxidation)
Disulfide bonds	For example, 1:D1–R24/1:V83–K108 = 5315.583m[1ss] shows the modification is 1ss.
Added or subtracted unspecified mass	For example, 57.0083 or –58.0273
Glycan	For example, A4S2G0 or A2S2FAc. For glycans appended with "Ac", this format stands for acetylation. Sialic acid residues are often acetylated, so whenever sialic acid is present, the application also searches its acetylated forms. Each sialic acid residue can have a maximum of 2 acetylation groups. When you see "Ac2", this format means two acetylations.

Results table commands

Right-clicking the Results table for Peptide Mapping Analysis on the Process and Review page opens a shortcut menu with the commands listed in the following table.

Table 31 Results table shortcut menu

Command	Description
Filters	Manages the filters in the Results table.
Apply	Applies all of the filters from a saved file to the table.
Save As	Saves all of the filters to a file with the .cfg extension.
Clear All	Clears all filters from the table.
Export All Components	Exports all data in the Results table to an Excel, a CSV, or a BPF file.
Export Checked Components	Exports data only for the selected components in the Results table to an Excel, a CSV, or a BPF file.
Create .mgf File	Creates an MGF file that the Mascot™ search engine can read.
Run De Novo Processing/Cancel De Novo Processing	Starts or cancels the de novo sequencing for a particular component.
Show Component Information	Displays information for an identified component, including other possible identifications from de novo sequencing.
Save As Peptide Workbook	Saves all or selected component results to a workbook that is Chromeleon-compatible and used for targeted peptide processing.

Viewing the chromatograms for peptide mapping analysis

The Chromatogram pane on the Process and Review page displays the base peak chromatogram (BPC) plot at the top and the selected ion chromatogram (SIC) plot at the bottom.

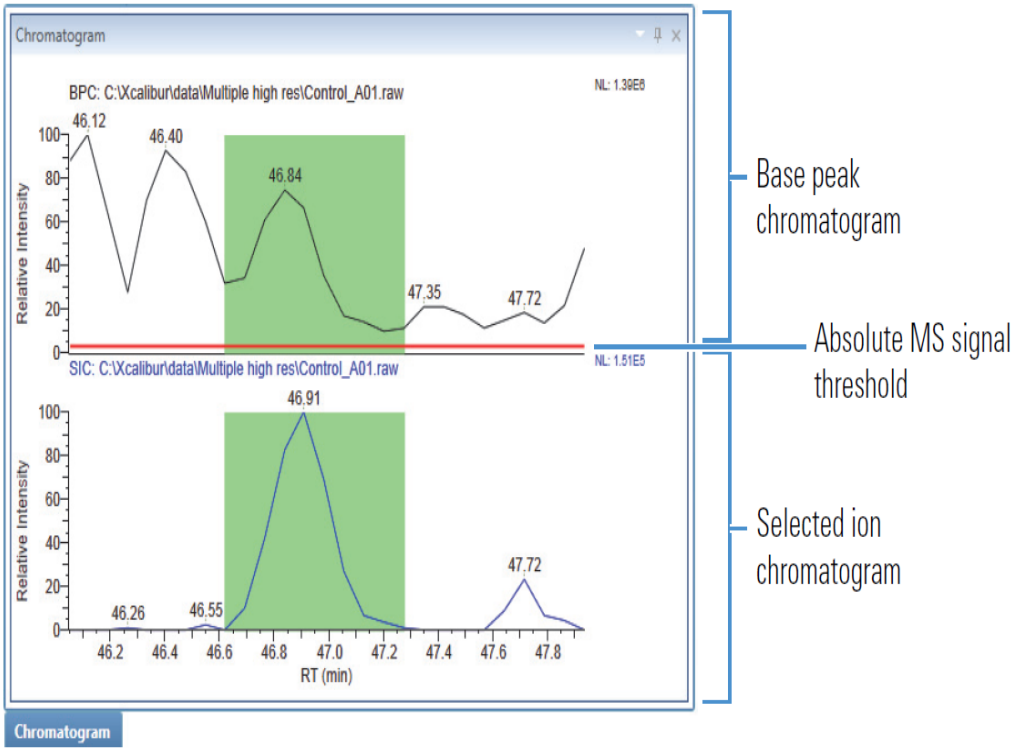


Figure 118 Chromatogram pane showing a BPC and an SIC

For more details, see the following topics:

- Viewing the chromatograms (page 260)
- Chromatogram plot types (page 260)
- Displaying multiple chromatogram plot types for one file (page 260)
- Displaying same chromatogram plot type for multiple files (page 263)
- Chromatogram pane commands (page 265)

Viewing the chromatograms

To view the chromatograms in the Chromatogram pane

1. Click the **Process and Review** tab if necessary.
2. Do either of the following:
   - Click the row of a component in the Results table.
     The plots in the Chromatogram pane show the peak information stored in the first raw data file in the list.

   –or–

   - Click the plus (+) sign to the left of a component row, and then click the row of one of the raw data files in the Results table.
     The plots show the peak information stored in the selected raw data file.

Chromatogram plot types

The base peak chromatogram (BPC) shows only the most intense peak in each MS spectrum at every point in the analysis. The BPC also displays a red horizontal line that represents the absolute MS signal threshold that you set as a component detection parameter in the method assigned to the experiment.

Note: BPCs for each raw data file often have a cleaner look and are therefore more informative than total ion current (TIC) chromatograms, which include noise and background signals. For intact protein spectra, the TIC often looks better because it adds together multiple charge states. The BPC is usually best for smaller molecules where all of the signal exists in a single charge state.

If you click a scan on the BPC, the available spectral plots for the deconvoluted, full-scan, and experimental MS spectra show the information from the selected scan.

The SIC (also known as an extracted ion chromatogram [XIC]) plots the intensity of the signal observed at a chosen *m/z* as a function of retention time.

Note: The green shaded area on these chromatograms (if visible) indicates the identified component peaks.

In the chromatogram plots, the *x* axis represents the retention time range and the *y* axis indicates the relative intensity. The plots label the peaks with their individual retention times (by default), and display the plot type and raw data file name at the top and the Normalized Largest (NL) intensity on the upper right side. They do not display peak labels, such as the scan number, or the header information.

Displaying multiple chromatogram plot types for one file

To display multiple chromatogram plot types for the same raw data file in the Chromatogram pane

1. Click the **Process and Review** tab if necessary.
2. Select a component in the Results table.

3. Right-click the Chromatogram pane and choose **Select Chromatogram**.

   The Select Chromatogram dialog box opens.

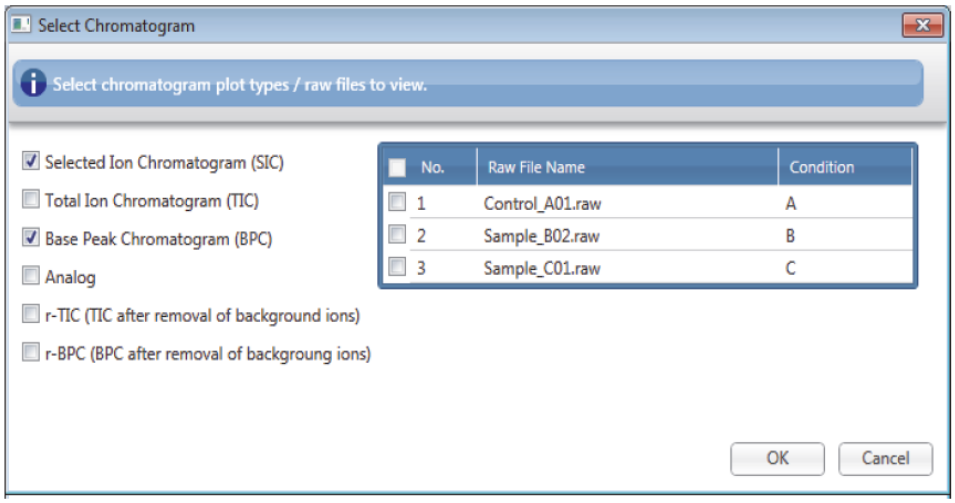


Figure 119 Select Chromatogram dialog box

4. Select the appropriate check boxes from the list on the left side of the dialog box.

   The available chromatogram plot types include the following:

   - Selected Ion Chromatogram (SIC)
   - Total Ion Chromatogram (TIC)
   - Base Peak Chromatogram (BPC)
   - Analog
   - r-TIC (TIC after the removal of background ions)
   - r-BPC (BPC after the removal of background ions)

   IMPORTANT! If you select only one plot type from the list on the left, you can select multiple raw data files from the list on the right. Conversely, to select multiple plot types, you must select only one raw data file.

   Your raw data file selections in the Select Chromatogram dialog box control the chromatogram plots. To return the control of the chromatogram plots to your selections in the Results table, you must clear all raw data file selections in this dialog box and then click **OK**.

5. Select the raw data file from the list on the right side of the dialog box.

   The following figure gives an example of one raw data file and multiple plots selected.

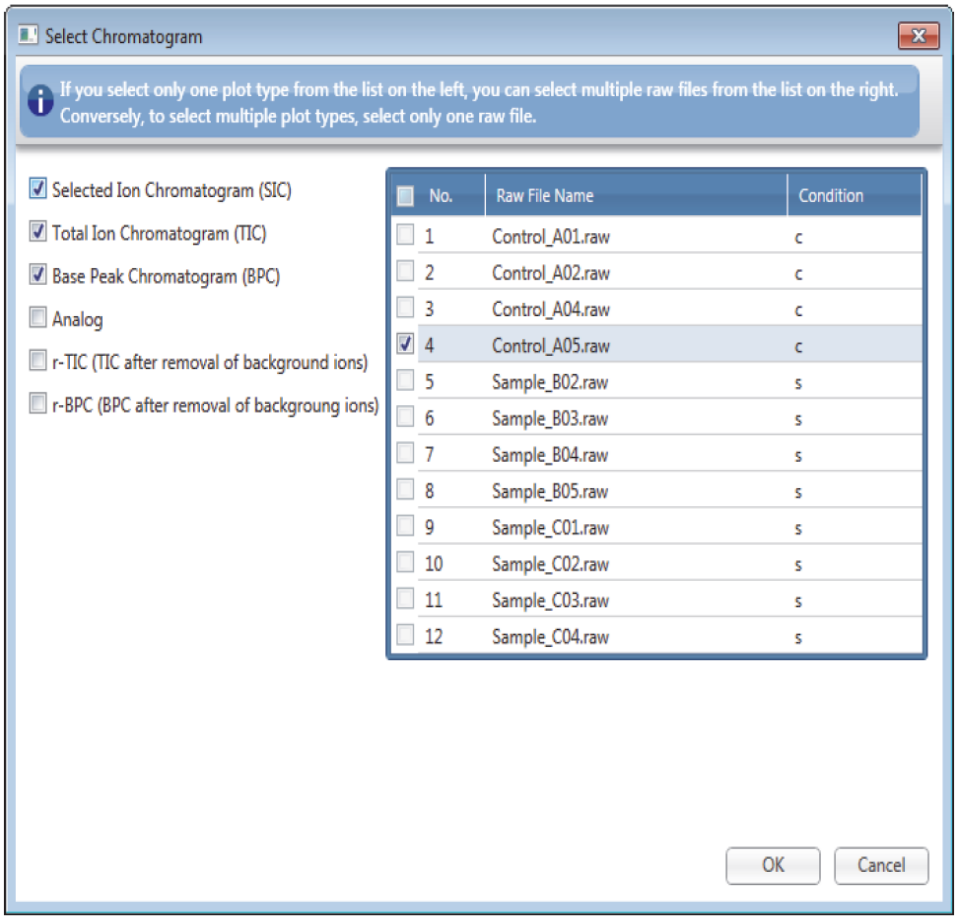


Figure 120 Selecting multiple plots for a raw data file

6. Click **OK** to update the chromatogram plots.

   The Chromatogram pane displays the selected plot types stacked on top of each other for the selected raw data file.

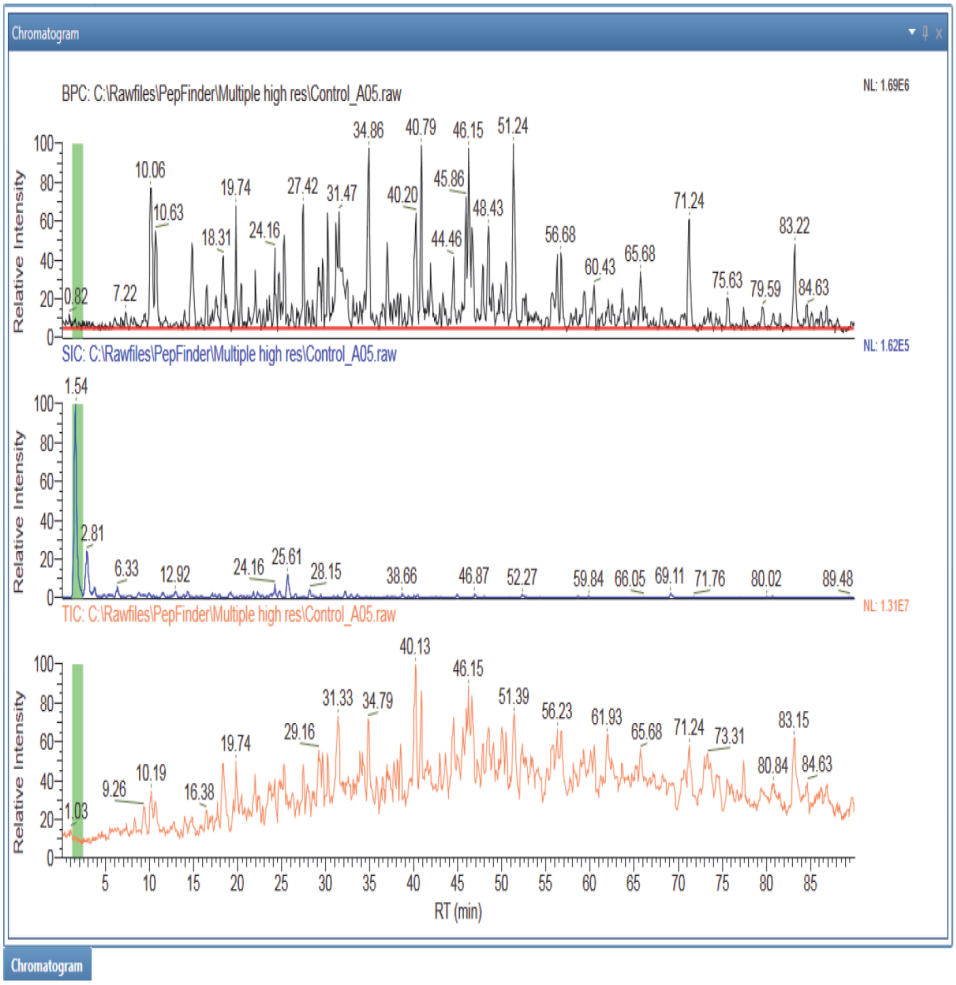


Figure 121 Different types of chromatograms displayed for a raw data file

Displaying same chromatogram plot type for multiple files

To display the same chromatogram plot type for multiple raw data files in the Chromatogram pane

1. Click the **Process and Review** tab if necessary.
2. Select a component in the Results table.
3. Right-click the Chromatogram pane and choose **Select Chromatogram**.
4. In the Select Chromatogram dialog box, select the type of chromatogram to display from the list on the left side.
5. On the right side of the dialog box, select the raw data files whose chromatograms you want to display. To select all raw data files, select the **No.** check box in the column header.

   The following figure gives an example of one type of plot and selections of multiple raw data files.

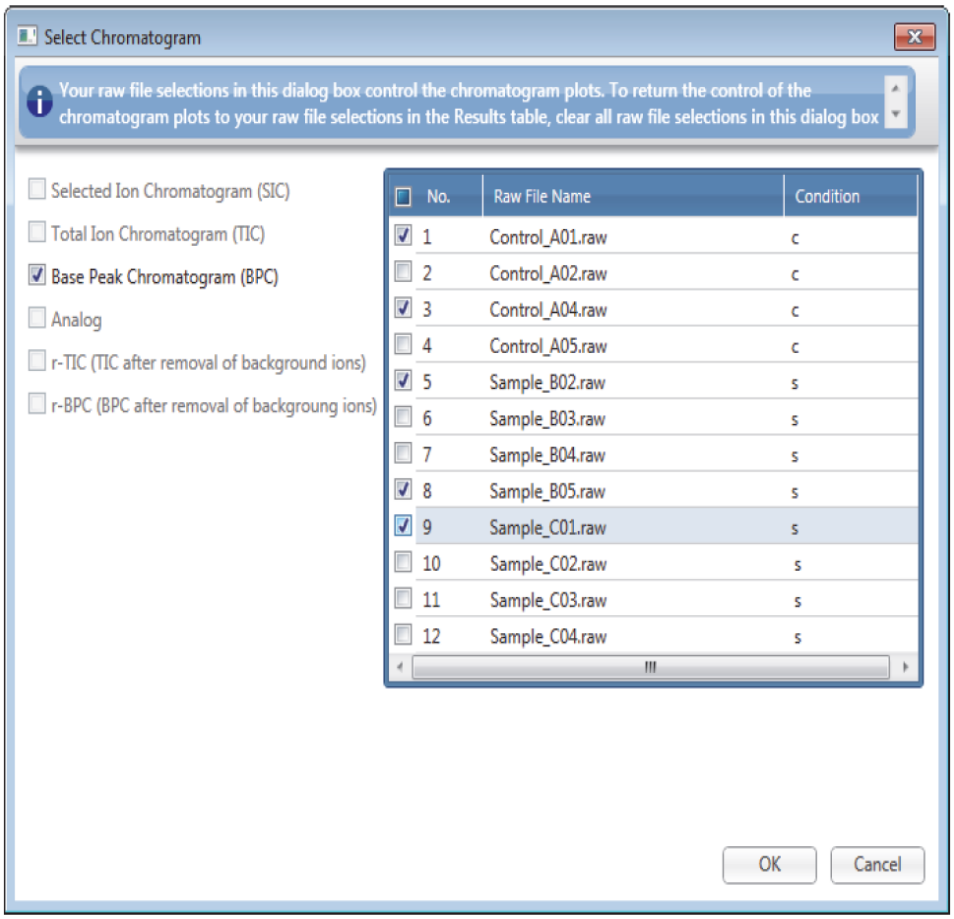


Figure 122 Selecting a plot for multiple raw data files

6. Click **OK** to update the chromatogram plots.

   The following figure shows a BPC chromatogram displayed for many different raw data files.

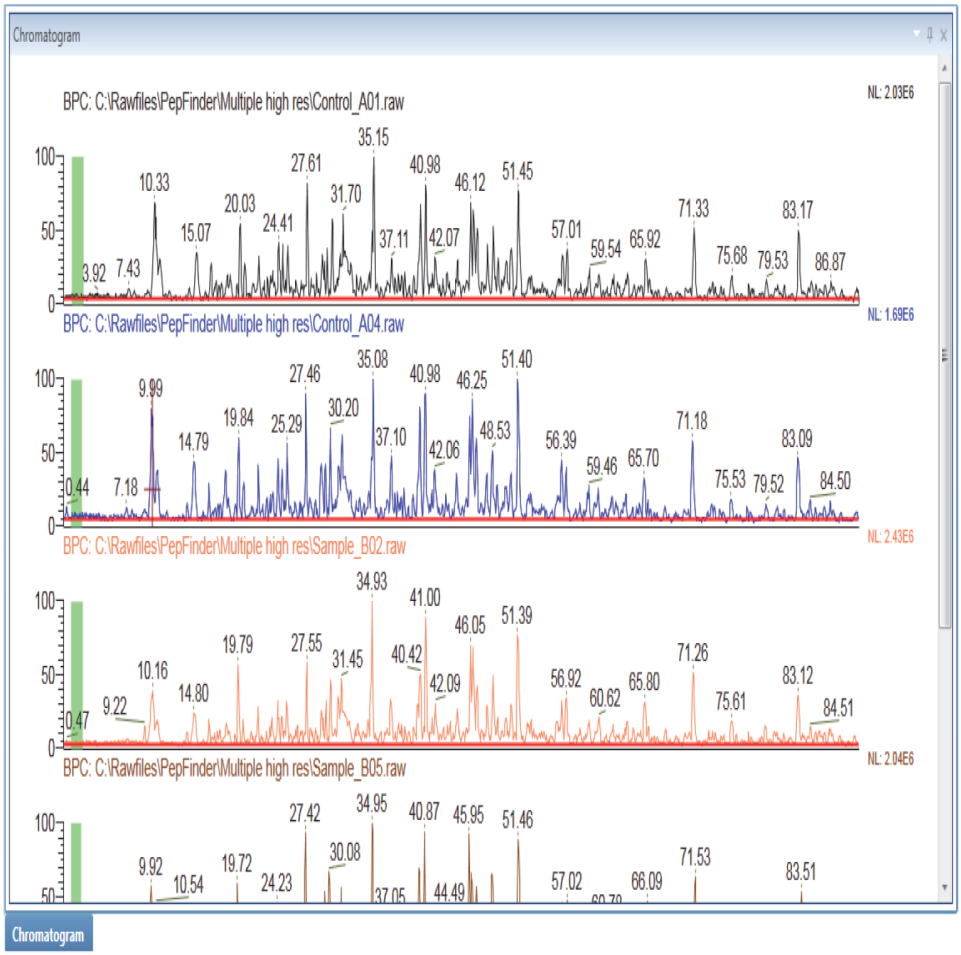


Figure 123 BPC chromatograms displayed for many different raw data files

Chromatogram pane commands

Right-clicking the Chromatogram pane on the Process and Review page opens a shortcut menu with the commands listed in the following table.

Table 32 Chromatogram pane shortcut menu

Command	Description
Select Chromatogram	Opens a dialog box to select which chromatogram or chromatograms to view.
Reset Scale	Restores the original scale that first appeared in the pane.
Copy	Copies the image in the pane to the Clipboard including all visible labeling and shading.
Label	Labels the peaks in the chromatograms with retention times or peptide information. **Note:** If the peptide is modified, an asterisk symbol, "*", appears at the end of the peptide label.

Viewing the trend ratio plot for peptide mapping analysis

The Trend Ratio pane on the Process and Review page displays the bar plots for the ratios between various conditions and the reference condition for the component that you select in the Results table.

Note: The Trend Ratio pane is visible only for experiments with multiple raw data files.

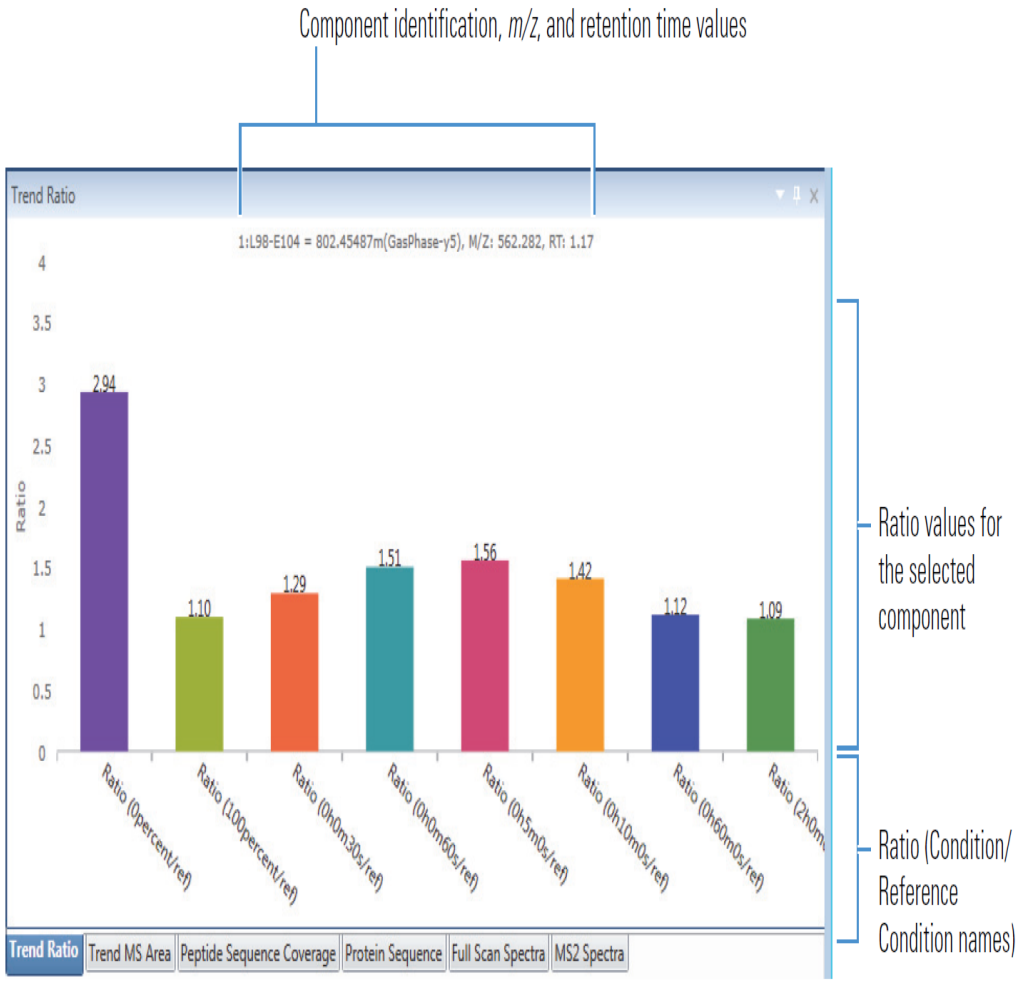


Figure 124 Trend Ratio pane

To view the trend ratio plot

1. Click the **Process and Review** tab and then click the **Trend Ratio** subtab.
2. Select the row for one component (or a raw data file under a particular component) in the Results table.

   The plot shows the Avg MS Area value for a particular condition ÷ Avg MS Area value for the reference condition, for the selected component. The ratio values in the plot are from the Ratio (Condition/Reference Condition) columns in the Results table. The plot displays each ratio in a different color. The order of the conditions underneath the bars in the plot is the same order as the entered conditions on the Peptide Mapping Analysis page.

Viewing the trend MS area plot for peptide mapping analysis

The Trend MS Area pane on the Process and Review page displays the bar plots for the MS Area values for each condition-raw data file pairing used in the experiment, for the component that you select in the Results table.

Note: The Trend MS Area pane is visible only for experiments with multiple raw data files.

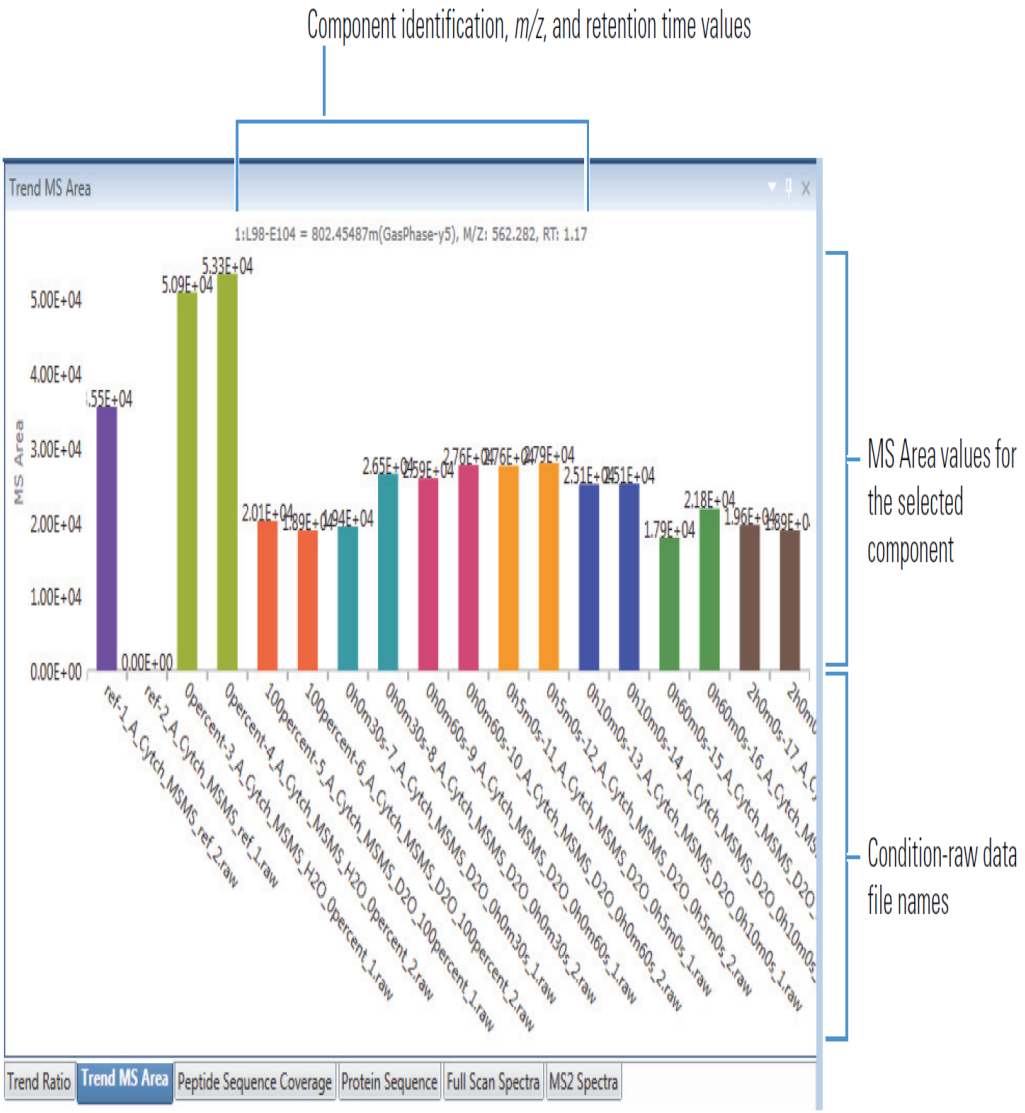


Figure 125 Trend MS Area pane

To view the trend MS area plot

1. Click the **Process and Review** tab and then click the **Trend MS Area** subtab.
2. Select the row for one component (or a raw data file under a particular component) in the Results table.

   The plot shows the MS Area value for the selected component and for each condition-raw data file pairing used in the experiment. The MS Area values in the plot are from the MS Area columns in the Results table. The plot displays each condition associated with a group of raw data files in a different color. The order of the conditions underneath the bars in the plot is the same order as the entered conditions on the Peptide Mapping Analysis page.

Viewing the fragment coverage map for peptide mapping analysis

The Peptide Sequence Coverage pane on the Process and Review page displays the fragment coverage map with color-coded peptide information.

Note: The fragment coverage map only displays MS2 spectra if the first raw data file of the selected component is of type MS2 and the component has a peptide identification.

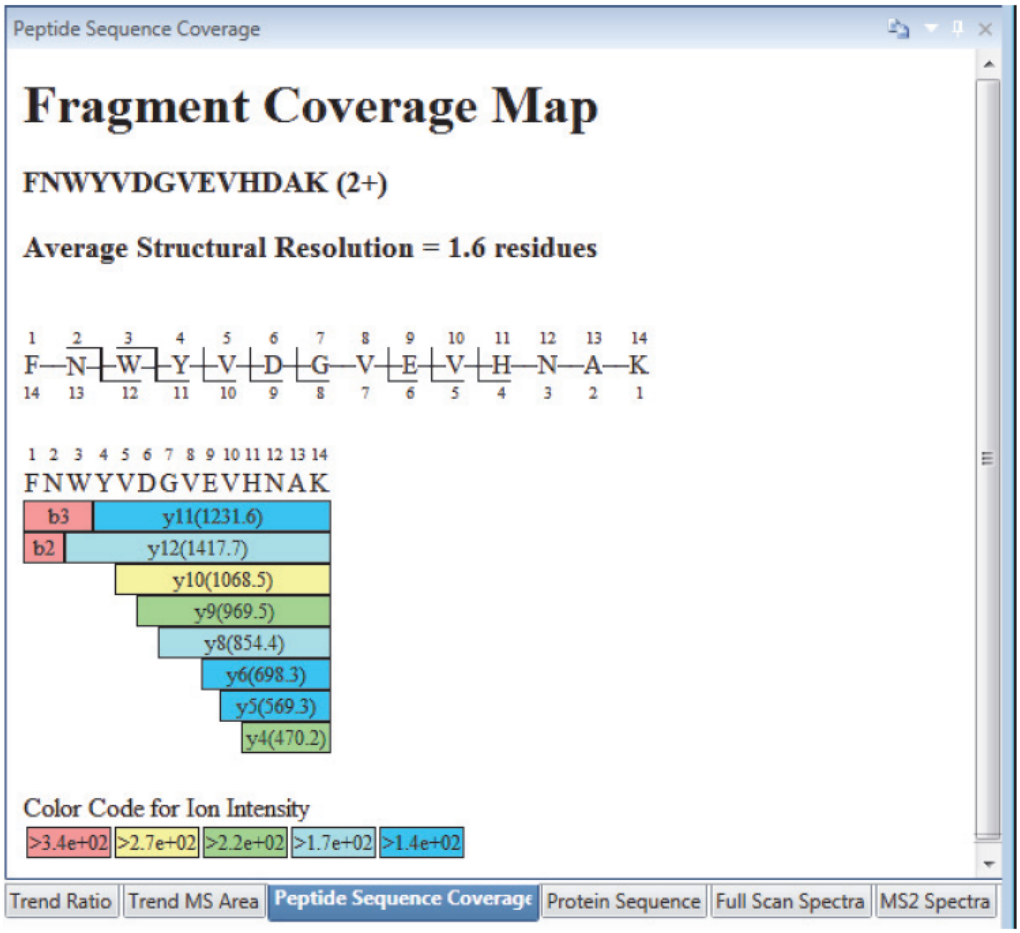


Figure 126 Fragment coverage map

Note: When the peptide is large, the Copy and Paste functions do not capture the fragment coverage map appropriately. Instead, use a screen capture application to capture the fragment coverage map as a screenshot to paste into a Microsoft™ application.

For more details, see the following topics:

- Viewing the fragment coverage map (page 271)
- Fragment coverage map display (page 271)

Viewing the fragment coverage map

To view the fragment coverage map in the Peptide Sequence Coverage pane

1. Click the **Process and Review** tab if necessary.
2. Click the **Peptide Sequence Coverage** subtab.
3. Do one of the following:
   - Click the row of an identified component in the Results table.
     The map in the Peptide Sequence pane shows the fragment coverage information stored in the reference raw data file.

     Note: By default, the BioPharma Finder application considers the first raw data file in an experiment as the reference raw data file.

   –or–

   - Click the plus (+) sign to the left of an identified component row, and then click the row of one of the raw data files in the Results table.
     The map shows the fragment coverage information predicted from MS2 spectra in the selected raw data file.

Fragment coverage map display

The fragment coverage map in the Peptide Sequence Coverage pane displays the following:

- Peptide sequence with its corresponding modification and charge state

  Note: Sometimes an amino acid letter in the peptide sequence changes to a different letter, based on a specific modification at that site. See Table 26 for examples of these modifications and the changed letters.
- Average structural resolution score (in number of residues) with a value of 1 indicating the best fit

  The application calculates the average structural resolution as follows:

  $$\frac{\textit{Total number of amino acids}}{\textit{Number of peptide fragments}}$$

- Peptide sequence with the numbered amino acid sequence and the identified fragment lines
  - The map labels the fragment ions on the peptides (b and c ions are on top, and y and z ions are on the bottom).
  - The first position is the peptide's N-terminus and the last position is the C-terminus of the peptide.

- Graphic showing the identified fragment ions using a color code for ion intensity (red, yellow, green, cyan, and blue), with red as most intense and blue as the least intense
  - The graphic shows the peptide sequence again and lists the identified ions.
  - Each ion includes the assignment and in some cases the mass-to-charge ratio.

    For example: y5-2H2O(626.9)
  - The map color codes the ions according to ion intensity and provides the color key below the graphic.

    Note: For components without significant b/y or c/z fragment ions, the color key for each color displays "ND" (not detectable).

When you select a different type of MS2 scan in the MS2 Spectra pane—that is, a different combination of fragmentation type and resolution type—the fragmentation coverage map automatically updates to display the results for that selection.

When you select a component identified as a disulfide bond, the application updates the Fragment Coverage Map and the MS2 Spectra panes to show information for the selected peptide.

Viewing the protein sequence for peptide mapping analysis

The Protein Sequence pane on the Process and Review page displays the protein sequence assigned to the current experiment with the identified peptide sequence highlighted in yellow.

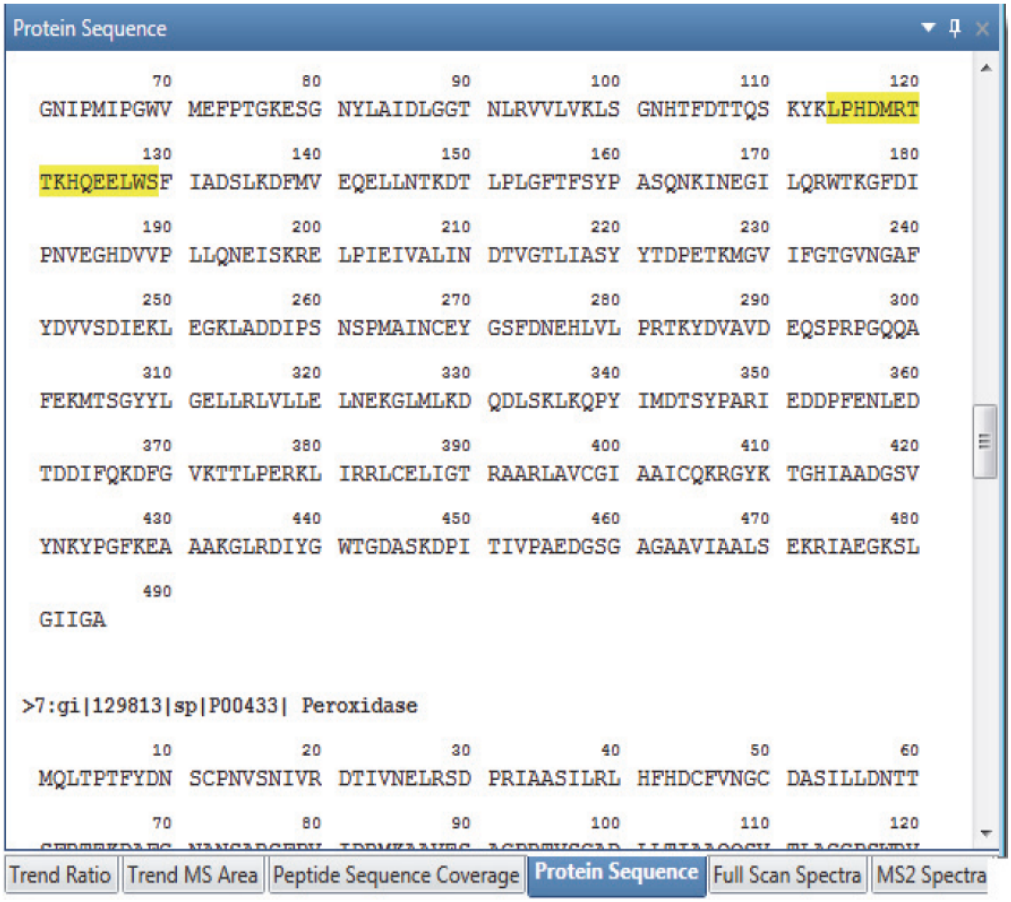


Figure 127 Protein sequence with the selected identified peptide sequence highlighted in yellow

For more details, see the following topics:

- Viewing the protein sequence (page 273)
- Protein sequence display (page 274)

Viewing the protein sequence

To view the protein sequence

1. Click the **Process and Review** tab if necessary.
2. Click the **Protein Sequence** subtab.
3. Click the row of an identified component in the Results table.

   –or–

Click the plus (+) sign to the left of an identified component row, and then click the row of one of the raw data files in the Results table.

Protein sequence display

The Protein Sequence pane displays the following:

- All of the chains in the protein sequence, including the comment lines from the FASTA file that begin with the greater-than sign (>) to distinguish each chain
- The amino acids in the sequence divided into groups of ten, separated by spaces
- The position labels, in groups of ten, displayed above the last amino acid in each group
- If an identification row is selected, the identified peptide sequence for the component selected in the Results table, highlighted in yellow

  Note: If the component (or raw data file) selected in the Results table contains a disulfide bond identification, the Protein Sequence pane highlights all of the different peptide sequences involved in the disulfide bond.

If necessary, expand the Protein Sequence pane.

Viewing the deconvoluted and Full-Scan MS Spectra for peptide mapping analysis

The Full Scan Spectra pane on the Process and Review page displays the noise-reduced deconvoluted spectrum stacked above the full-scan spectrum.

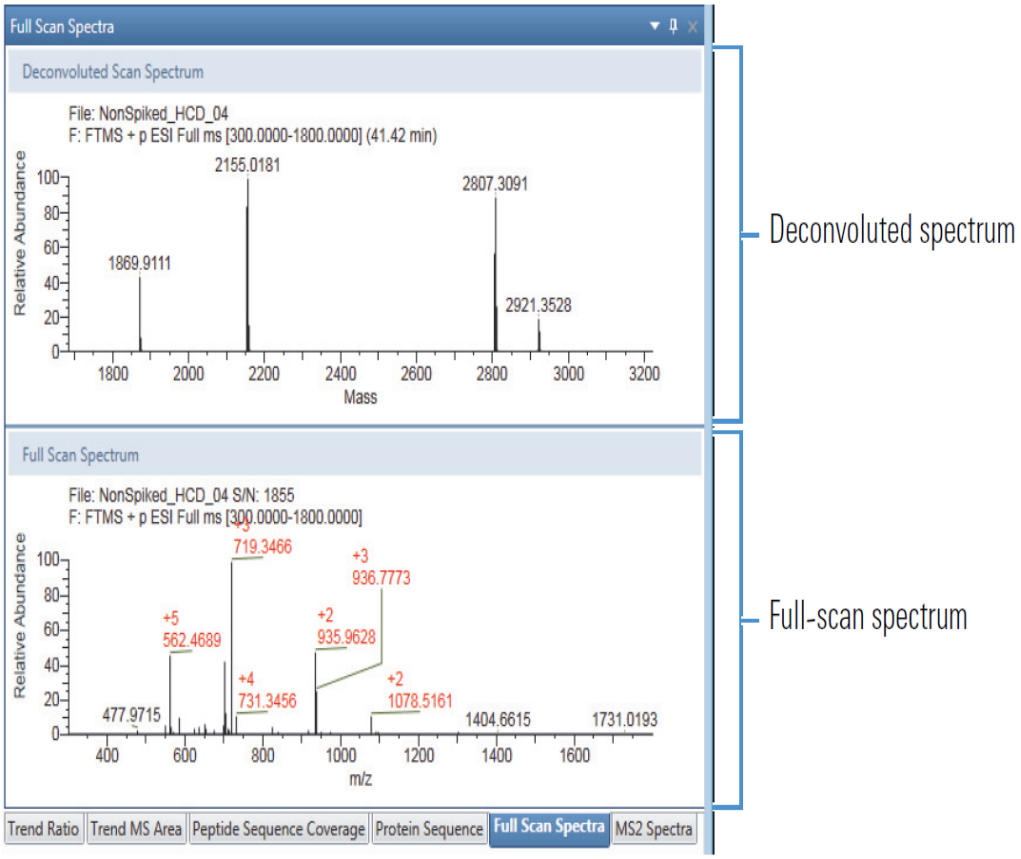


Figure 128 Deconvoluted and full-scan spectra

For more details, see the following topics:

- Viewing the deconvoluted and Full-Scan MS Spectra (page 276)
- Deconvoluted and Full-Scan MS Spectra display (page 276)
- Full scan Spectra pane commands (page 277)

Viewing the deconvoluted and Full-Scan MS Spectra

To view the deconvoluted and full-scan spectra

1. Click the **Process and Review** tab if necessary.
2. Click the **Full Scan Spectra** subtab.
3. Do one of the following:
   - Click the row of a component in the Results table.
     The available spectral plots in the Full Scan Spectra pane show the information stored in the reference raw data file.
     Note: By default, the application considers the first raw data file in an experiment as the reference raw data file.
   - Click the plus (+) sign to the left of a component, and then click the row of one of the raw data files in the Results table.
     The available spectral plots show the information stored in the selected raw data file.

   –or–

   - Click a scan on the BPC in the Chromatogram pane.

   The available spectral plots show the information from the selected scan.

Deconvoluted and Full-Scan MS Spectra display

In the deconvoluted spectral plot of the Full Scan Spectra pane, the *x* axis represents the mass. In the full-scan spectral plot, the *x* axis represents the mass-to-charge ratio (*m/z*). In both plots, the *y* axis indicates the relative abundance. The plots label the centroid spectra with their individual mass or *m/z* values, with four decimal digits for high-resolution data and two decimal digits for low-resolution data.

The area above the deconvoluted spectrum plot displays the following:

- Raw data file name
- Scan header information
- Retention time associated with the selected scan in the chromatogram

In the same area, the full-scan spectrum plot displays the same information as the deconvoluted spectrum plot, but instead of the retention time, it displays the signal-to-noise value. In addition to the *m/z* values, the labels in this plot also show the charge states. The labels appear in red for the identified peaks so that you can quickly spot the identified ions.

Full scan Spectra pane commands

Right-clicking the Full Scan Spectra pane on the Process and Review page opens a shortcut menu with the commands listed in the following table.

Table 33 Full Scan Spectra pane shortcut menu

Command	Description
Reset Scale	Restores the original scale that first appeared in the pane.
Copy	Copies the image in the pane to the Clipboard.

Viewing the predicted and experimental MS2 Spectra for peptide mapping analysis

The experimental data from a Peptide Mapping Analysis experiment might contain any of the following fragmentation and resolution types or multiple DD bonds:

- Fragmentation types: CID, HCD, and either ETD or ECD
- Resolution types: High or Low
- DD bonds: Peptide fragments joined by disulfide bonds

The BioPharma Finder application determines the specific types present in the data from the scan headers in the raw data files that you load for the experiment. If the data contains multiple types, the application displays a separate spectral plot for every combination of fragmentation type and resolution type.

At the top of the MS2 Spectra pane on the Process and Review page, the Activation list provides the available fragmentation types (CID, HCD, ETD, or ECD), and the Res. list provides the available resolution types (High or Low). When a sample processed by MS/MS includes peptide fragments that are joined by disulfide bonds (as denoted by the slash (/) in the sequence name), the DD Bond list also appears so that you can select different disulfide peptide fragments. When you select these disulfide peptide fragments, the Fragment Coverage Map and the labels in the MS2 Spectra change to reflect your selected peptide fragment.

Select which combination of fragmentation and resolution types (and optional disulfide peptide fragment) that you want to view in a spectral plot from these lists. Each plot displays the predicted spectrum stacked above the experimental spectrum for a particular combination if it is available.

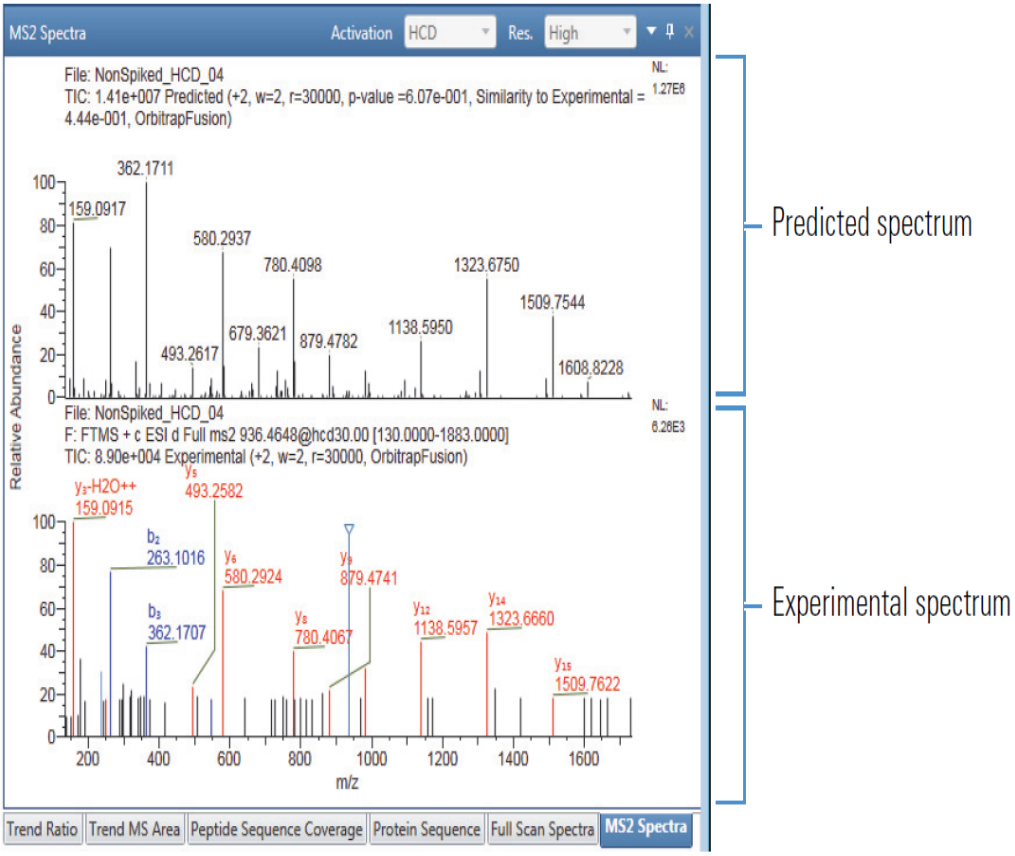


Figure 129 Predicted and experimental spectra on the MS2 Spectra pane

For more details, see the following topics:

- Viewing the predicted and experimental MS2 Spectra (page 278)
- Predicted and experimental MS2 Spectra display (page 279)
- Predicted and experimental MS2 Spectra fragment ions (page 280)
- MS2 Spectra pane commands (page 282)
- Comparing MS2 Spectra on the process and review page (page 282)
- Switching the header on the process and review page (page 284)
- Manual integration of change start and stop time of a component (page 286)

Viewing the predicted and experimental MS2 Spectra

To view the predicted and experimental spectra for a particular combination

1. Click the **Process and Review** tab if necessary.
2. Click the **MS2 Spectra** subtab.

3. Do one of the following:
   - Click the row of a component in the Results table.
     The spectral plots in the pane show the information stored in the reference raw data file if it is available.

     Note: By default, the application considers the first raw data file in an experiment as the reference raw data file.
   - Click the plus (+) sign to the left of a component, and then click the row of one of the raw data files in the Results table.
     The spectral plots show the information stored in the selected raw data file if it is available.

   –or–
   - Click a scan on the BPC in the Chromatogram pane.
     The experimental spectrum shows the information from the selected scan if it is available.

4. In the Activation and Res. lists, select the fragmentation type and resolution type, respectively, to view the MS2 spectra for this particular combination.

5. (Optional, when disulfide bonds exist) In the DD Bond list, select a particular disulfide peptide fragment, to view the MS2 spectra for this fragment.

Predicted and experimental MS2 Spectra display

In the spectral plots of the MS2 Spectra pane, the *x* axis represents the *m/z* and the *y* axis indicates the relative abundance. The plots label the centroid spectra with their individual *m/z* values, with four decimal digits for high-resolution data and two decimal digits for low-resolution data.

The area above the predicted spectrum plot displays the raw data file name, the Normalized Largest (NL) intensity, the TIC information, and a description line containing the following parts:

- Predicted/experimental spectrum indicator
- Charge state of the fragmented ion, for example, "+2"
- Isolation window used for fragmentation, for example, "w=2"
  If no window was found, this part shows "w=0".
- Resolution at *m/z* 400, for example, "r=17500"
  If no resolution at 400 was found, this part shows "r=0."
- (Predicted spectrum plot only) P-value (probability value)
  The lower this value is, the better the match is between the primary and secondary spectra.
- (Predicted spectrum plot only) Similarity to experimental value
  The higher this value is, the better the match is between the predicted and experimental spectra.
- Instrument model, for example, "QExactive"
  If no instrument was used, this part shows "LTQ".

You can regenerate the predicted spectrum by performing the Kinetic MS/MS model prediction.

The area above the experimental spectrum plot displays the same information as the predicted spectrum plot, along with the scan header information. This spectrum also displays an inverted triangle marker at the top of the spectral line for the theoretical precursor ion.

The labels appear in color for the identified peaks (so that you can quickly spot the identified ions) and also show their fragment ion assignments and charge states, for example, "y_9", "b_3", or "M_{2+}" (doubly charged precursor ion).

The color for the lines and labels for the identified ions in the experimental spectrum vary based on the ion type:

- Light blue for a ions with a charge on the N-terminal side
- Dark blue for b ions with a charge on the N-terminal side
- Dark green for c ions with a charge on the N-terminal side
- Light green for c–1 ions with a charge on the N-terminal side and one fewer proton
- Lighter green for c+1 ions with a charge on the N-terminal side and one more proton
- Orange for x ions with a charge on the C-terminal side
- Red for y ions with a charge on the C-terminal side
- Purple for z ions with a charge on the C-terminal side
- Light purple for z+1 ions with a charge on the C-terminal side and one more proton
- Lighter purple for z+2 ions with a charge on the C-terminal side and two more protons

Predicted and experimental MS2 Spectra fragment ions

The following table summarizes the types of fragment ions that appear in the spectra in the MS2 Spectra pane.

Table 34 Fragment ions

Fragment	Description
Ion series	
a	A ion with a charge on the N-terminal side
b	B ion with a charge on the N-terminal side
c	C ion with a charge on the N-terminal side
c·	C ion with a charge on the N-terminal side, one less proton (c–1.0078)
x	X ion with a charge on the C-terminal side
y	Y ion with a charge on the C-terminal side

Fragment	Description
z	Z ion with a charge on the C-terminal side
z·	Z ion with a charge on the C-terminal side, one more proton (z +1.0078)
z·'	Z ion with a charge on the C-terminal side, two more protons (z +2(1.0078))
Neutral losses	
$-H_2O$	Fragment that has lost water (–18 Da)
$-NH_3$	Fragment that has lost ammonia (–17 Da)
PTMs	
H_3PO_4	Phosphorylation loss (designated –P)
Other	
Immonium	Immonium ion
M	Precursor ion, M Neutral loss, for example: • Water loss, M–H20 • Loss from phosphorylated residues, M–98
R	Neutral loss from arginine with a minus mass value, for example, R–44
Glycans	There are several types of glycan labels: • Glycan fragments, which appear in parentheses, for example, (Gn) • Glycan fragment losses, for example, –A2S1G1F • Glycan core cleavages labeled with the B or Y convention, for example, Bn, Bn–1, Y0, Y1 • Common glycan structure, for example, GlcNAc Each type can have a charge associated with it. Glycan labels include capital letters to distinguish them from ion series labels. See Chapter 37, “Glycans”, for a list of the most common glycans and the monoisotopic mass that the sequence-matching algorithm adds to them.

MS2 Spectra pane commands

Right-clicking the MS2 Spectra pane on the Process and Review page opens a shortcut menu with the commands listed in the following table.

Table 35 MS2 Spectra panes shortcut menu

Command	Description
Reset Scale	Restores the original scale that first appeared in the pane.
Copy	Copies the image in the pane to the Clipboard.
Predict Peptide MS/MS (Kinetic Model)	Uses the Kinetic model to generate the predicted spectrum in the MS2 Spectra pane.
Delete Predicted MS/MS Spectrum	Deletes the predicted spectrum in the MS2 Spectra pane. To redisplay the predicted spectrum, you can either reselect the result row or right-click in the MS2 Spectra pane, and then select the Predicted Peptide MS/MS (Kinetic Model) command from the shortcut menu.

Comparing MS2 Spectra on the process and review page

By default, the predicted MS/MS spectrum is displayed on the top of the experimental MS/MS spectrum. You can compare the experimental spectra with different activation types.

To use the modified MS2 spectrum instead of the predicted MS2 spectrum

1. Click a component in the results table that has an MS2 spectrum.

   The MS2 Spectra view displays the predicted spectrum on the top and the experimental spectrum on the bottom.

2. Click **MS2 Spectra**, and then right-click the MS2 Spectra view.

   By default, the Predicted view check box is selected.

3. Choose **Set bottom spectrum as a reference (move to top)**.

   The Predicted view check box is cleared. The spectrum on the bottom moves to the top view and replaces the predicted spectrum. This spectrum is now known as the reference spectrum.

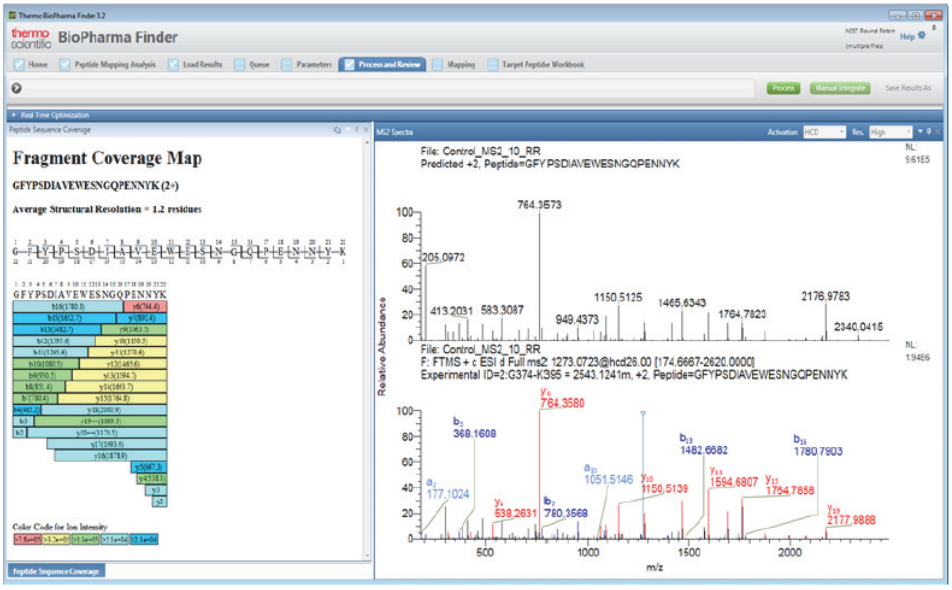


Figure 130 Comparing MS2 Spectra

4. To compare the spectrum, select another component.

   The spectra are stacked with the reference spectrum displayed on the top.

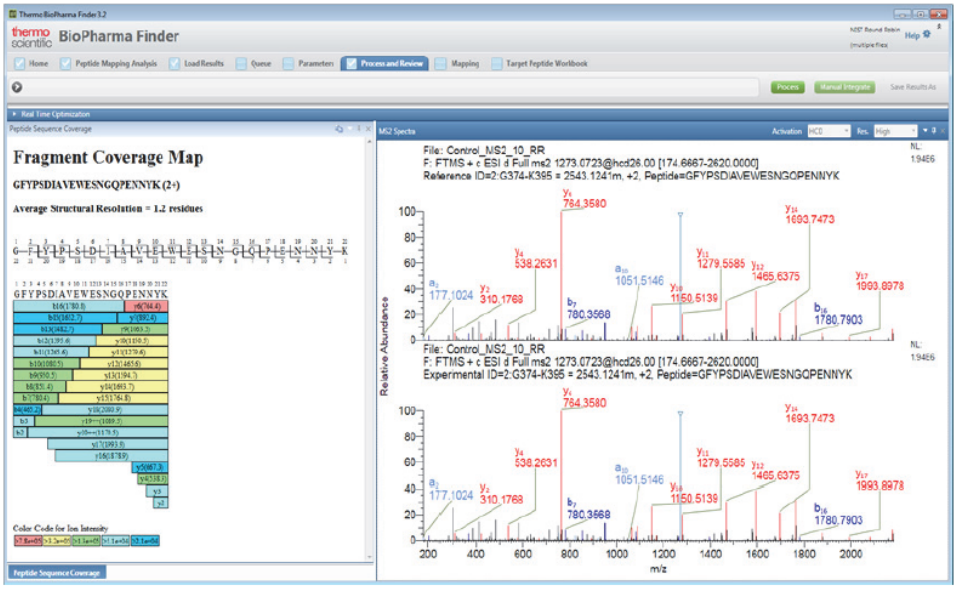


Figure 131 Reference spectra displayed on top

Note: The reference spectrum is not linked between pages—that is when you set a peptide as the reference on the Process and Review, this information is not passed to the Mapping page.

The Predicted peptide MS/MS (Kinetic Model) is deactivated.

The reference spectrum remains in this location until you right-click and select the Predicted view check box. Then the reference spectrum is replaced with the predicted spectrum of the experimental spectrum in the bottom.

Switching the header on the process and review page

This feature displays the Identification and the Peptide Sequence information in place of the TIC on both the top and bottom spectrum.

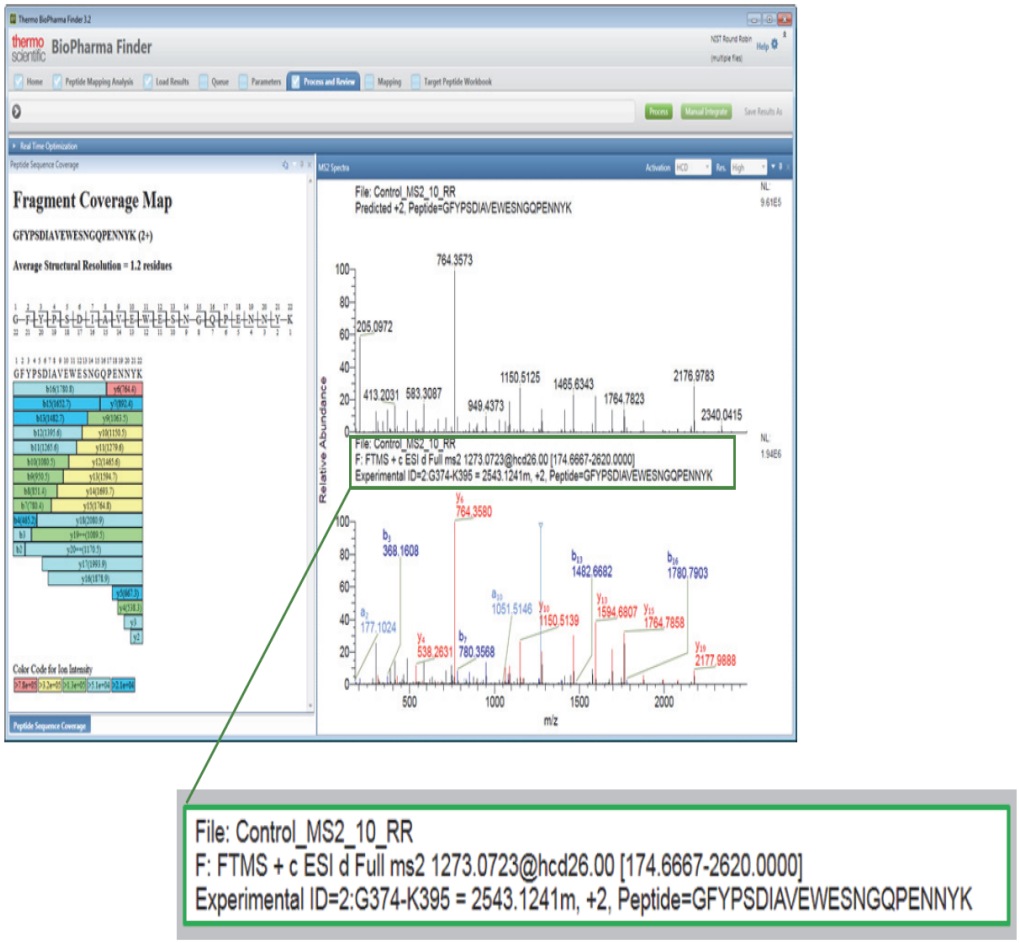


Figure 132 Switching the Header

To switch the header

1. Right-click the MS2 Spectra view.
2. Choose **Header**, and then select the **Show TIC Information** check box.

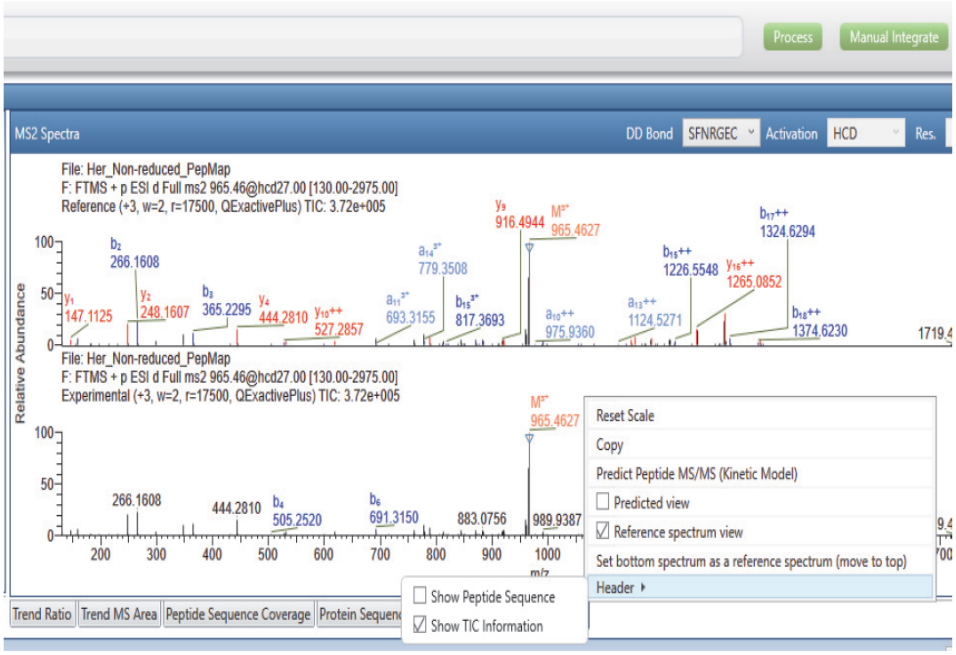


Figure 133 Shows the TIC Information

3. To switch back to Peptide information, right-click the MS2 Spectra view, choose **Header**, and then select the **Show Peptide Sequence** check box.

Manual integration of change start and stop time of a component

When you manually re-integrate the peak using the SIC trace, the column automatically changes to manual, the MS areas update with the new value, and the SIC trace green shading changes to the new start and stop time.

Figure 134 Default state displays in green

The manually integrated peak is displayed in blue.

Figure 135 Manually integrated peak

To manually integrate the peak

1. On the Load Results page, select the experiment name, and then click **Load Results**.

   The Process and Review page displays the results.

2. Click **Chromatogram**, and then select the **Select Chromatogram** check box.

3. Make sure the SIC check box is selected and the BPC check box is cleared. Then, select all of their raw data file names.

4. Click **OK**.

5. In the Results pane, select the component of interest.

6. Click **Enable Manual Integration**, and then drag the pointer to adjust the start and end points for the chromatographic peak.

7. Click **Manual Integrate**.

   The chromatographic peak where the RT time is adjusted is displayed in blue and the integration type changes to Manual for that raw file.

   Note: The BPC for each raw data file often have a cleaner look and are therefore more informative than total ion current (TIC) chromatograms, which include noise and background signals. For intact protein spectra, the TIC often

looks better because it adds together multiple charge states. The BPC is usually best for smaller molecules where all of the signal exists in a single charge state.

14 Viewing the coverage page

The Coverage page displays the results of the peptide mapping analysis.

Viewing the coverage page

After the BioPharma Finder application completes the analysis of a peptide mapping experiment, you can open the results of that analysis on the Coverage page and view the color-coded chromatogram, the sequence coverage map, and the Results table. The Results table groups together by the specific type of protein the components that were identified using de novo sequencing. The table also groups together the unidentified components and proteins.

To view the results on the Coverage page

1. Open the results from the Queue page or from the Load Results page.

   The application transfers you to the Process and Review page. The current experiment name appears in the upper right corner of the page. If you load only one raw data file for the experiment, its name also appears in this area; otherwise "(multiple files)" appears.

2. Click the **Mapping** tab.

   By default, the application opens the Coverage page under the Mapping tab, which displays the color-coded chromatogram in the Chromatogram pane, the sequence coverage map in the Protein Coverage Map pane, and the Results table.

Figure 136 Coverage page

Viewing the coverage results table

The Results table on the Coverage page displays the color-coded results of the analysis, organized by the types of proteins at the top level. It then displays protein coverage information specific to each raw data file at the next level. If the analysis identified a protein at the top level, then the table displays the component-specific information grouped by a particular raw data file at the lowest level.

When you select a protein, raw data file, or one of its components in the Results table, you can view related information in the Chromatogram and Protein Coverage Map panes of the Coverage page.

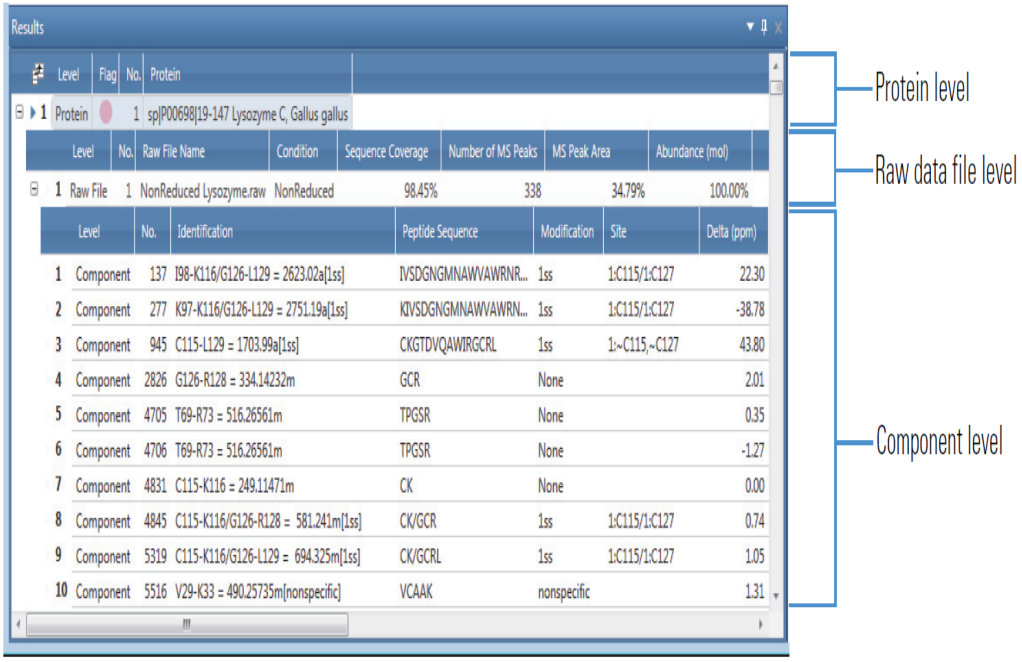


Figure 137 Results table on the Coverage page

For more details, see the following topics:

- Viewing the results table for protein coverage (page 293)
- Exporting the results table data (page 293)
- Results table parameters (page 294)
- Results table commands (page 295)

Viewing the results table for protein coverage

To view the Results table on the Coverage page

1. Click the **Mapping** tab and then click the **Coverage** subtab if necessary.

   The Coverage page displays the color-coded proteins in the Results table. For a description of the columns in this table.

2. Click the plus icon, ⊞, at the left side of a protein row to view protein-related information, specific to each raw data file that was loaded for the experiment.

3. Click the plus icon, ⊞, at the left side of a raw data file row (if a protein is identified at the top level) to display information related to that raw data file that is specific to a component.

   Note: The numbers in the No. column of this table correspond to the order of detection. These numbers are different from the numbers in the No. column for the components of the Results table on the Process and Review page.

Exporting the results table data

To export the data in the Results table

1. Click the **Mapping** tab and then click the **Coverage** subtab if necessary.

2. Right-click anywhere in the Results table on the Coverage page and choose **Export**.

   The Save As dialog box opens.

3. Locate or type the name of the Excel file to store the exported results in.

   By default, the file name is the same name as the experiment.

4. Click **Save**.

   The application stores the data at the protein and raw data files levels for all proteins in the table in the specified file. If you do not locate a different folder, the application places the exported file by default in the folder containing the raw data files for the experiment.

Results table parameters

The following table describes the types of information in the Results table of the Coverage page.

Table 36 Results table parameters

Parameter	Description
Protein level	
+	Shows or hides the lower level of raw data file information related to the current protein row.
Row number	Displays the row number for each protein or chain in a sequence.
Field Chooser 	Displays the Field Chooser dialog box so that you can select the columns to display in the Results table.
Level	Indicates that the row is displaying protein information (top level).
Flag	Displays the color code for each type of identified or unidentified protein.
No.	Displays a sequential number for each protein.
Protein	Displays the name identifier of the protein or chain from the sequence. If the protein/component is not identified, this cell displays "Unidentified."
Raw data file level	See “Results table parameters” on page 103 for descriptions of additional columns.
Sequence Coverage	Displays the identified amino acids as a percentage of the total number of amino acids in the sequence for a specific raw data file.
Number of MS Peaks	Displays the number of identified peaks for the protein or chain for a specific raw data file.
MS Peak Area	Displays the peak area percentage for a specific raw data file.
Abundance (mol)	Displays the abundance percentage for a specific raw data file.
Component level	Displays the third level, nested in the Results table. First being the Protein level, followed by the Rawfile level and the Component Level.

Results table commands

Right-clicking the Results table on the Coverage page opens a shortcut menu with the command listed in the following table.

Table 37 Results table shortcut menu

Command	Description
Show Protein Coverage Map Parameters	Opens a dialog box in which you can modify the sequence coverage map parameters.
Export	Exports to an Excel file the data at the protein and raw data files levels for all proteins in the table.

Viewing the coverage chromatogram

The Chromatogram pane on the Coverage page displays the base peak chromatogram (BPC) with color-coded protein coverage. The various shading colors correspond to the types of identified proteins shown at the top level in the Results table.

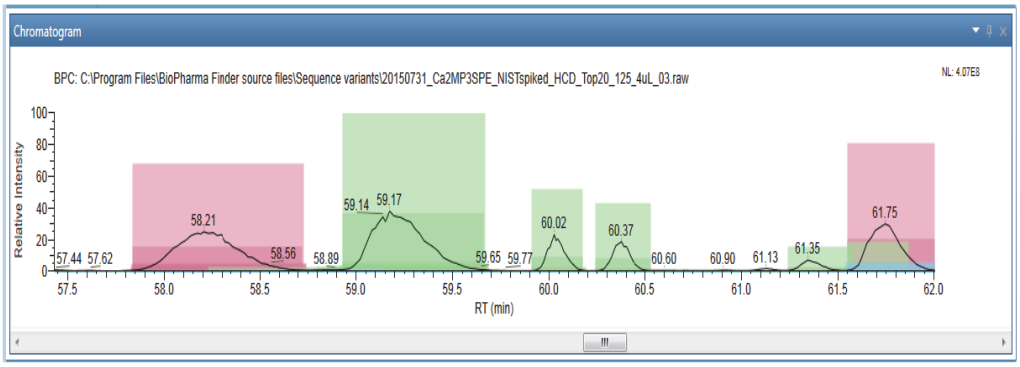


Figure 138 Chromatogram pane zoomed in showing color-coded protein coverage

The shading is semi-transparent so that you can view coeluting peaks on top of each other. The height of the shaded block indicates the intensity (peak height) of a component. You can view this height value in the MS Height column at the component level in the Results table.

The chromatogram shows you at a glance which peptides were identified for each chain and which remained unidentified. For example, the following figure displays the peptides identified for a light chain in red and a heavy chain in green. It shows the unidentified peptides in blue.

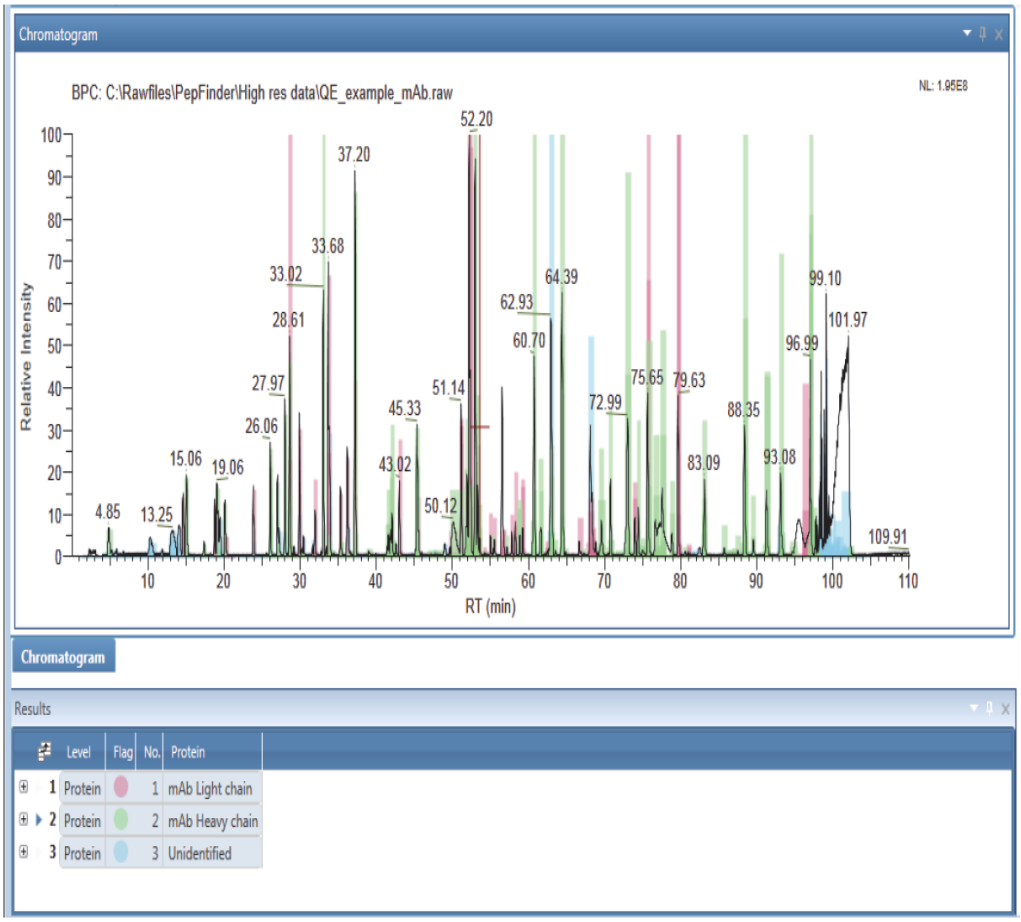


Figure 139 Identified and unidentified peptides in the Chromatogram pane

For more details, see the following topics:

- Viewing the Color-Coded chromatogram (page 297)
- Modifying the shading settings (page 297)
- Chromatogram pane commands (page 299)

Viewing the Color-Coded chromatogram

To view the results in the Chromatogram pane

1. Click the **Mapping** tab and then click the **Coverage** subtab if necessary.
2. Do one of the following:
   - Click the row of a protein in the Results table.

     If you click the protein level, the application uses the first raw data file to shade a protein on identified peaks.

     If you click the raw data file level, the application uses a specific raw data file.

     The BPC shows the shaded peak height for all of the components grouped under a particular protein type if one is identified. If some component peaks are not identified, the application groups them in the "Unidentified" protein type.

     Each selected protein row corresponds to only one shading color on the chromatogram. Use the CTRL or SHIFT key to select multiple rows to see the various colors for the multiple protein types.
   - Click the plus icon,  , at the left side of a protein row, and then click the row of one of the related raw data files in the Results table.

     The BPC shows the shaded peak height for each of the components in a particular raw data file, grouped under a particular protein type and shaded by the color assigned to that protein type.

   –or–

   - Click the plus icon,  , at the left side of a raw data file row (if a protein is identified at the top level), and then click the row of one of its components in the Results table.

     The BPC shows the same information as when you select the row for the related raw data file. In addition, the application also displays the SIC of the selected component below the BRC, in the Chromatogram pane.

In the chromatogram plots, the *x* axis represents the retention time range and the *y* axis indicates the relative intensity. The plots label the peaks with their individual retention times (by default) and display the file name at the top and the Normalized Largest (NL) intensity at the top right. They do not display peak labels, such as the scan number, or the header information.

Modifying the shading settings

To modify the shading parameter settings

1. Click the **Mapping** tab, and then click the **Coverage** subtab.
2. Right-click the Chromatogram pane and choose **Shading Parameters**.

   The Shading Parameters dialog box opens.

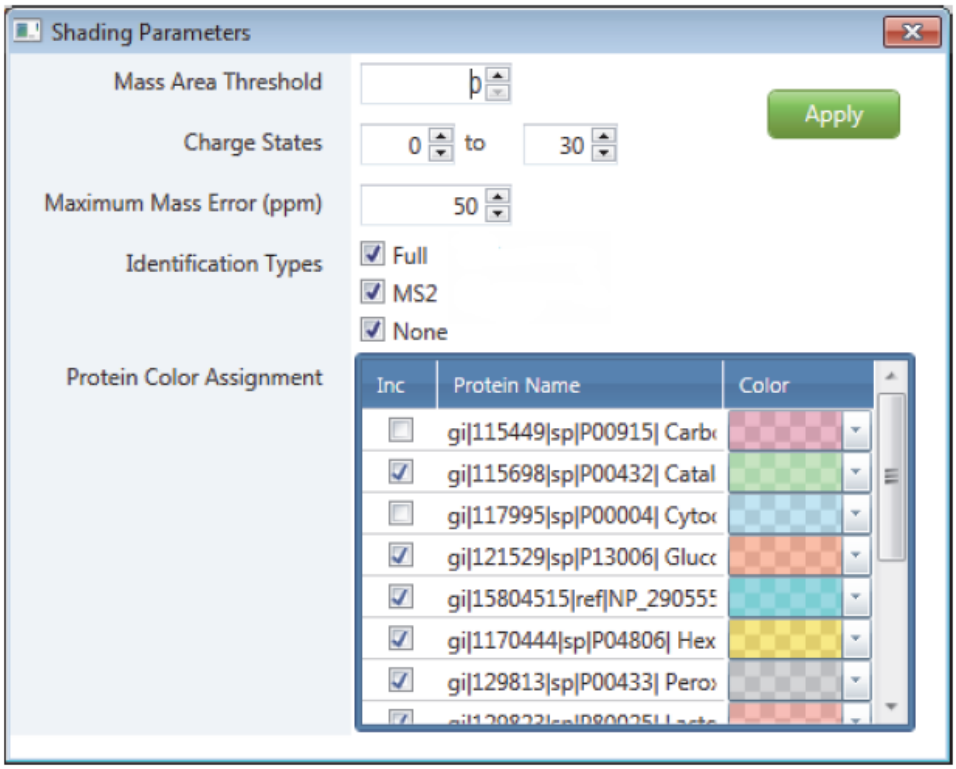


Figure 140 Shading Parameters dialog box

3. Enter the following settings:
   - Mass Area Threshold: Type a number for the minimum area threshold.
     The application does not display in the chromatogram any signals with areas below this threshold.
   - Charge States: Type the minimum and maximum values for the range of charge states to display in the chromatogram.
   - Maximum Mass Error (ppm): Type a value in ppm for the maximum mass threshold.
     The application does not display in the chromatogram any signals with a delta mass above this threshold.
   - Identification Types: Select the check boxes to view (in the chromatogram and in the component level of the Results table) only the components identified by the selected identification types. Select only the None check box to view only unidentified components.

- Protein Color Assignment table: The table contains all of the protein types from the Results table.
  - Inc: Select or clear this check box for a particular protein row if you want to show or hide both the same protein row in the Results table and its corresponding shading in the Chromatogram pane.

    You can select or clear multiple check boxes as needed.
  - (Not editable) Protein Name: The type of protein, as listed in the Results table.
  - Color: Click to select a shading color for a particular protein type.

Note: Your protein row selections in the Results table do not affect the Inc selections in the Protein Color Assignment table.

If you define specific components in the Results table on the Process and Review page to update the sequence coverage map, those selections override the previous settings in the Protein Color Assignment table when you go back to the Coverage page. Once you are back on the Coverage page, future filters in the Protein Color Assignment table apply until you redefine the components again.

4. Click **Apply**.

   The chromatogram updates based on your settings. The shading parameters affect the shading in the chromatogram and the records in the protein Results table. They do not affect the protein coverage map.

Chromatogram pane commands

Right-clicking the Chromatogram pane on the Coverage page opens a shortcut menu with the commands listed in the following table.

Table 38 Chromatogram pane shortcut menu

Command	Description
Shading Parameters	Opens a dialog box in which you can modify the chromatogram parameters, such as threshold, identification types, and shading colors.
Reset Scale	Restores the original full-scale chromatogram.
Copy	Copies the image in the pane to the Clipboard.
Label	Labels the peaks in the chromatograms with retention times or peptide identification information.

Viewing the coverage map

The upper portion of the Protein Coverage Map pane on the Coverage page displays a table of proteins with coverage and abundance values. This table displays some of the same columns as the Results table of the Coverage page at the raw data file level. Below this table are several values from the Coverage Map Options dialog box.

The lower portion of the pane displays the sequence coverage map with color-coded peptide information for a selected protein type, raw data file, or component in the Results table. Scroll down as necessary to see the rest of the color-coded peptide information. The color coding indicates the MS signal intensity of the predicted fragments. The sequence coverage map changes according to the raw data file that you select.

The application labels each peptide in the map with its retention time and color-codes the peptides by intensity with red, orange, yellow, green, cyan, blue, shades of gray, and white colors, showing red as most intense and white as least intense.

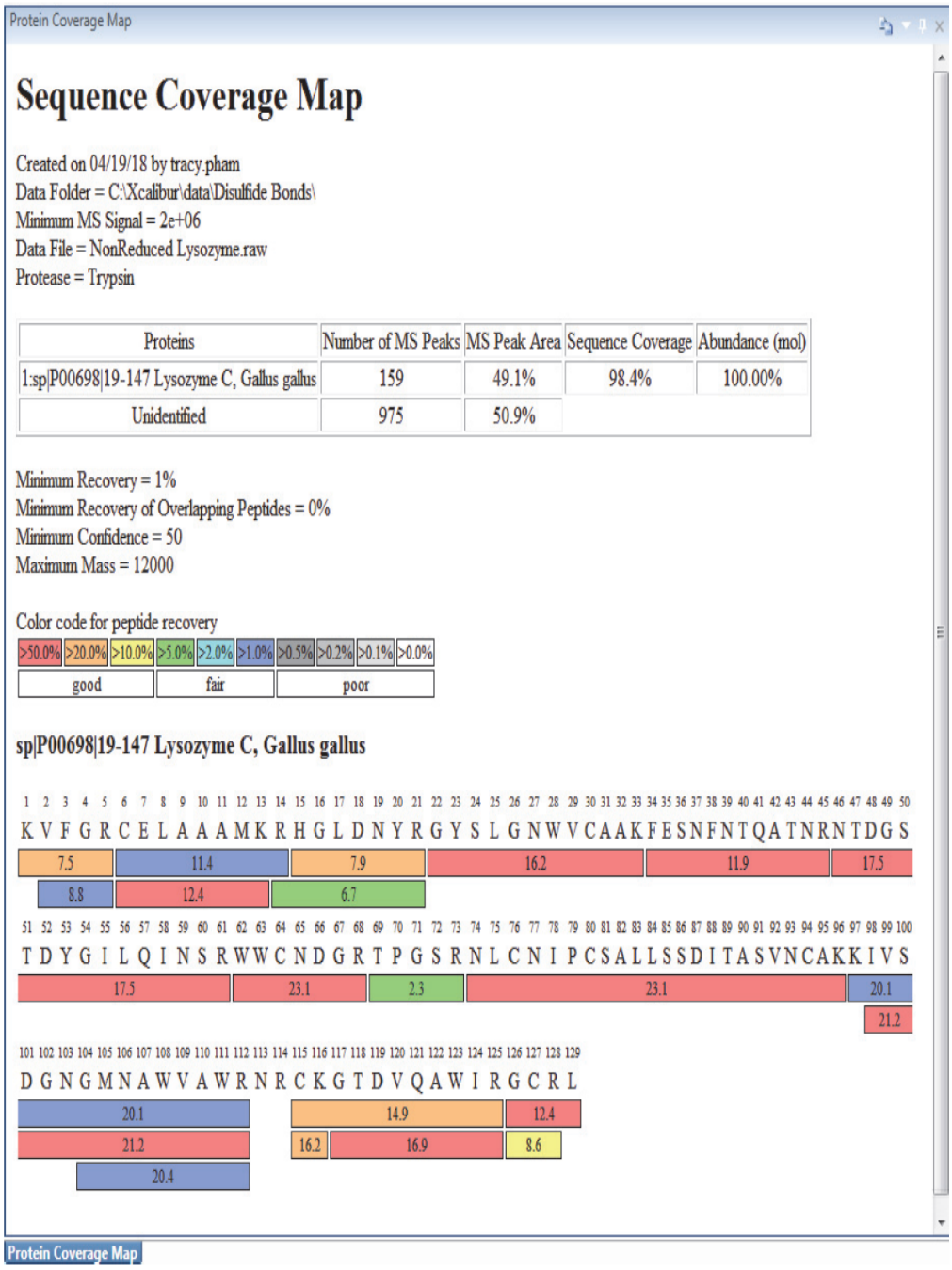


Figure 141 Sequence coverage map with a color-coded protein sequence

For more details, see the following topics:

- Viewing the sequence coverage map (page 302)
- Selecting the sequence coverage map components (page 302)
- Changing the sequence coverage map parameters (page 303)

Viewing the sequence coverage map

1. Click the **Mapping** tab, and then click the **Coverage** subtab.
2. Do one of the following:
   - Click the row of a protein in the Results table.
     The Protein Coverage Map pane displays the sequence coverage map for the reference (first) raw data file.
   - Click the plus icon,  , at the left side of a protein row, and then click the row of one of the raw data files in the Results table.
     The Protein Coverage Map pane displays the sequence coverage map for the selected raw data file.

   –or–

   - Click the plus icon,  , at the left side of a raw data file row, and then click the row of one of its components in the Results table.
     The Protein Coverage Map pane displays the sequence coverage map for the raw data file that the selected component belongs to.

Selecting the sequence coverage map components

To select the components to be included in the coverage map

1. Click the **Process and Review** tab in the navigation bar.
2. In the Results table, select or clear the check boxes for the components that you want to include in or exclude from the sequence coverage map on the Coverage page.
3. Click the **Mapping** tab and then click the **Coverage** subtab if necessary.

   Note: By default all Protein Levels are selected in Results table and Chromatogram are stacked for all raw files in the Chromatogram tab. Use the keys **CTRL** or **SHIFT** key to select multiple desired Proteins.

   The sequence coverage map title indicates that the map is now user defined. The map shows only the selected components. If you select multiple components from the same peptide sequence, the map displays the most abundant component in the table at the top but does not change the color-coded peptide information at the bottom.

   Tip: To return to the default sequence coverage map, in the Results table on the Process and Review page, select the check box in the table header row to clear all of the check boxes and deselect all components.

Changing the sequence coverage map parameters

To change the coverage map parameters

1. Click the **Mapping** tab and then click the **Coverage** subtab if necessary.
2. Right-click anywhere in the Results table on the Coverage page or in the title bar of the Protein Coverage Map pane, and choose **Show Protein Coverage Map Parameters**.

   The Coverage Map Options dialog box opens.

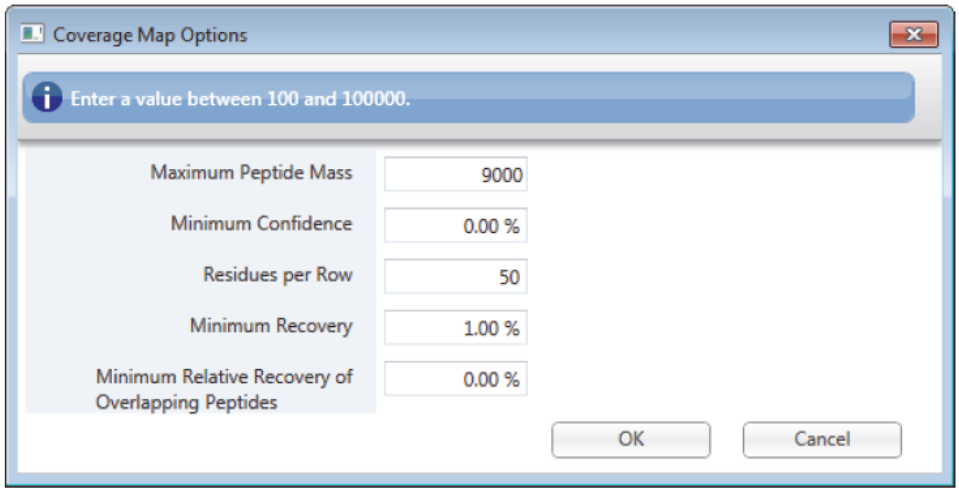


Figure 142 Coverage Map Options dialog box

Note: If you define specific components for the sequence coverage map, you can update only the Residues per Row parameter. The other parameters are read-only.

The parameter settings in the Coverage Map Options dialog box do not affect the data in the protein coverage Results table and the shading control for the color-coded chromatogram.

3. Enter the following settings:
   - Maximum Peptide Mass: Type a value to specify the maximum peptide mass to be included in the coverage map.
   - Minimum Confidence: Set the minimum confidence level to be included in the coverage map for a peptide assignment, on a 0 to 100% scale, with 100% being the highest confidence.

     IMPORTANT! If you set the Search by Full MS Only option to Yes in the method, set this value to 0.00%. Otherwise, you cannot see any coverage.
   - Residues per Row: Type the maximum number of amino acids (residues) to display in each row of the proteins in the coverage map.
   - Minimum Recovery™: Type a value to specify the minimum recovery threshold on a 0 to 100% scale, with 100% being the highest recovery. A peptide must have a recovery value higher than this threshold to be included in the coverage map for a peptide assignment.

     For more details on recovery values, see the Recovery™ parameter.
   - Minimum Relative Recovery™ of Overlapping Peptides: Type a value to specify the minimum relative abundance threshold on a 0 to 100% scale, with 100% being the highest threshold. A peptide must have a relative abundance value higher than this threshold to be included in the coverage map for a peptide assignment.

   Note: In the Minimum Confidence, Minimum Recovery™, and Minimum Relative Recovery™ of Overlapping Peptides fields, enter a value between 0.00 and 1.00 and the application automatically converts this value to a percentage value between 0.00% and 100.00%.

4. Click **OK** to update the coverage map based on your settings.

Coverage page parameters

The following table describes the types of information on the Coverage page.

Table 39 Coverage page parameters

Parameter	Description
Results table for protein coverage	At the top level, displays the identified and unidentified proteins, along with their color-coded flags. At the next level, the table displays the raw data files. At the lowest level, it displays the component information.
Chromatogram pane	Displays the chromatogram related to the row that you select in the Results table.
Relative Intensity (*y* axis)	Displays the ratio of each peak intensity relative to the maximum intensity in the spectrum.
RT (min) (*x* axis)	Displays the retention time—that is, the time after injection at which a compound elutes.
Protein Coverage Map pane	Displays the sequence coverage map including the proteins, sequence coverage information, and the color-coded peptides.

15 Viewing the modification summary page

After the BioPharma Finder application completes the analysis of a peptide mapping experiment, you can open the results of that analysis on the Modification Summary page and view the modification summary report that shows the recovery status and abundance of all detected modifications.

To view the results on the Modification Summary page

1. Open the results from the Queue page or from the Load Results page.

   The application transfers you to the Process and Review page. The current experiment name appears in the upper right corner of the page. If you load only one raw data file for the experiment, its name also appears in this area; otherwise, "(multiple files)" appears.

2. Click the **Mapping** tab and then click the **Modification Summary** subtab.

   The Modification Summary page opens and displays information in the following panes:

   - Modification Results pane—the modification summary
   - Components pane—the components table
   - Modification Plot pane—the plot of abundance percentages for the selected modifications in the Modification Results pane, grouped by conditions and the names of the loaded raw data files

     Note: By default, the Modification Plot pane is visible and all of the following panes are automatically hidden. To view any of the following panes, click its tab at the right side of the page. You can keep these panes from auto-hiding by clicking the pin icon, , to change it back to its open position,  .
   - Chromatogram pane—the chromatograms

- Visible only for experiments with multiple raw data files:
  - Trend Ratio pane—bar plot of ratio values
  - Trend MS Area pane—bar plot of MS Area values
- Peptide Sequence Coverage pane—the fragment coverage map
- Protein Sequence pane—the protein sequence
- Full Scan Spectra pane—the deconvoluted and full-scan spectra
- MS2 Spectra pane—the predicted and experimental spectra

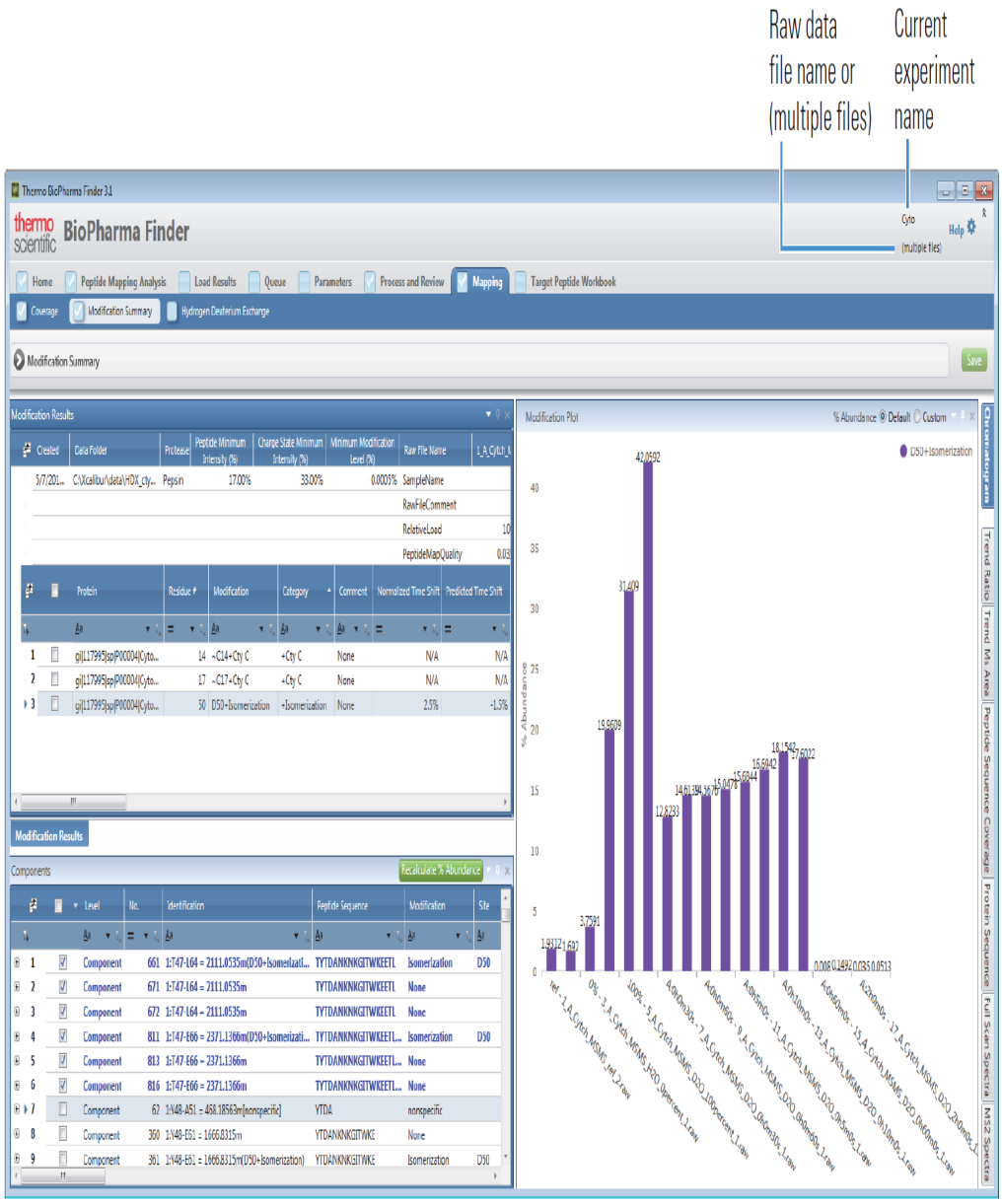


Figure 143 Modification Summary page

Modification summary page parameters

The following table describes the types of information available on the Modification Summary page. To display the content of a pane that is not currently visible, click its subtab.

Table 40 Modification Summary page parameters

Parameter	Description
Modification Results pane	Displays the modification summary results.
Components table	Displays component-related information.
Modification Plot pane	Displays the plot of the modification abundance percentages for the selected modifications in the Modification Results pane, grouped by conditions and raw data file names.
% Abundance (*y* axis)	Displays the percentages of abundance for the selected modifications in the Modification Results table.
Condition-Raw data file (*x* axis)	Displays the names of all conditions and their corresponding raw data files loaded for the experiment.
Chromatogram pane	Displays the chromatograms for the component that you select in the Components table. This pane is similar to the same pane on the Process and Review page.
Relative Intensity (*y* axis)	Displays the ratio of each peak intensity relative to the maximum intensity in the spectrum.
RT (min) (*x* axis)	Displays the retention time of the scan—that is, the time after injection at which a compound elutes.
Trend Ratio pane	Displays the bar plots for the ratios between various conditions and the reference condition for the component that you select in the Results table.
Ratio value (*y* axis)	Displays the Avg MS Area value for a particular condition ÷ Avg MS Area value for the reference condition.
Ratio conditions (*x* axis)	Displays the names of the various conditions and the name of the reference condition.
Trend MS Area pane	Displays the bar plots for the MS Area values for the component that you select in the Results table, for each raw data file used in the experiment.
MS Area value (*y* axis)	Displays the MS Area values from each of the individual raw data files assigned to a particular condition.
Condition-Raw data file (*x* axis)	Displays the names of the various conditions and their assigned raw data files used in the experiment.
Peptide Sequence Coverage pane	Displays the fragment coverage map, which includes the peptide sequence information and the color-coded fragment ions. This pane is similar to the same pane on the Process and Review page.

Parameter	Description
Protein Sequence pane	Displays the protein sequence assigned to the experiment. This shows the highlighted identified peptide sequence that you select from the Components table. This pane is similar to the same pane on the Process and Review page.
Full Scan Spectra pane	Displays the deconvoluted and full-scan spectra with mass and *m/z* information. This pane is similar to the same pane on the Process and Review page.
Relative Abundance (*y* axis)	Displays the ratio of the abundance of a specific peak to the abundance of the peak with the highest abundance.
Mass or *m/z* (*x* axis)	Displays the mass or mass-to-charge ratio of the ions formed from molecules. This ratio is the quantity formed by dividing the mass of an ion, in daltons, by the number of charges that the ion carries.
MS2 Spectra pane	Displays the predicted spectrum stacked on top of the experimental spectrum. This pane is similar to the same pane on the Process and Review page.
Activation	(Enabled only when multiple fragmentation types are used to generate the data) Displays a list of the available fragmentation types (CID, HCD, ETD, or ECD) that you can select from to view the MS2 spectra for this particular combination.
Res.	(Enabled only when multiple fragmentation types are used to generate the data) Displays the available resolution types (High or Low) that you can select to view the MS2 spectra for this particular combination.
DD Bond .	(Visible only when multiple peptides are identified for a disulfide bond [peptides are separated by a slash "/"]) Displays the fragments joined by a disulfide bond
Relative Abundance (*y* axis)	Displays the ratio of the abundance of a specific peak to the abundance of the peak with the highest abundance.
m/z (*x* axis)	Displays the mass-to-charge ratio of ions formed from molecules. This ratio is the quantity formed by dividing the mass of an ion, in daltons, by the number of charges that the ion carries.

Viewing the modification summary results

The Modification Results pane on the Modification Summary page displays the modification summary results as tabular data. In the upper table, the results include the date that the application created the summary, the data folder that holds the raw data files, the protease selected for the experiment, the summary filter options, and other information for each raw data file. The lower table lists the protein, residue, modification, abundance for each raw data file, and other data.

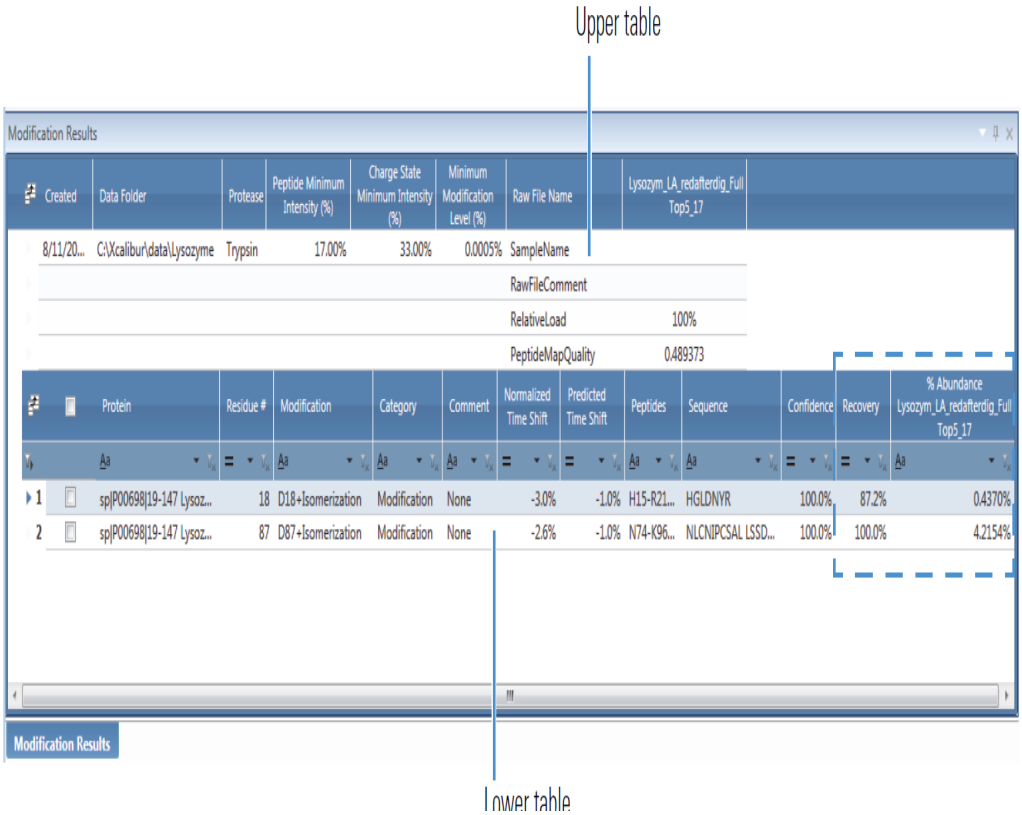


Figure 144 Modification Results pane

The % Abundance columns (one for each raw data file used in the experiment) in the lower table display the abundance of the modification in the sample as a percentage.

For a non-targeted peptide mapping experiment, the application uses the following equation when calculating the values in this column for a particular raw data file:

$$\%\ \text{Abundance} = \frac{\text{Sum of the MS area for all modified components}}{\text{Sum of the MS area for all selected components}} \times 100$$

The selected components appear in blue in the Components table.

For a targeted peptide mapping experiment, the application uses the following equation when calculating the values in this column:

$$\% \text{ Abundance} = \frac{\textit{Numerator value}}{\textit{Denominator value}} \times 100$$

where

Numerator value = Sum of the MS area for all components from relative quantitation group *x* with modification *y*

Denominator value = Sum of the MS area for all components from relative quantitation group *x*

x is each Relative Quantitation Group Number value in the peptide workbook used as the protein sequence for a targeted experiment

y is each modification associated with the components in the same relative quantitation group

Note: Move the scroll bar to the right to see more % Abundance columns if needed. You can select components to recalculate custom % Abundance values. You can also view the modification plot of the abundance percentages, grouped by conditions and raw data file names.

The Recovery™ column in the lower table displays the general abundance of the modified peptide, which is the total peak area (including the modified and unmodified forms of the peptide) as a percentage of the self-weighted average of all peptides. This means that higher-intensity peptides weigh more than lower-intensity peptides. The recovery value can be over 100% because of the way the application calculates the reference intensity. Typically, a good recovery is considered to be greater than 10%; a fair recovery, greater than 1%; and a poor recovery, less than 1%.

For more details, see the following topics:

- Viewing the modification results pane (page 312)
- Changing the modification summary options (page 313)
- Exporting the modification summary (page 314)
- Upper table of modification results pane parameters (page 314)
- Lower table of modification results pane parameters (page 315)
- Modification results pane commands (page 317)

Viewing the modification results pane

To view the modification summary in the Modification Results pane

1. Click the **Mapping** tab and then click the **Modification Summary** subtab if necessary.

2. In the Modification Results pane, select the row for the peptide modification that you are interested in, as shown in the following figure.

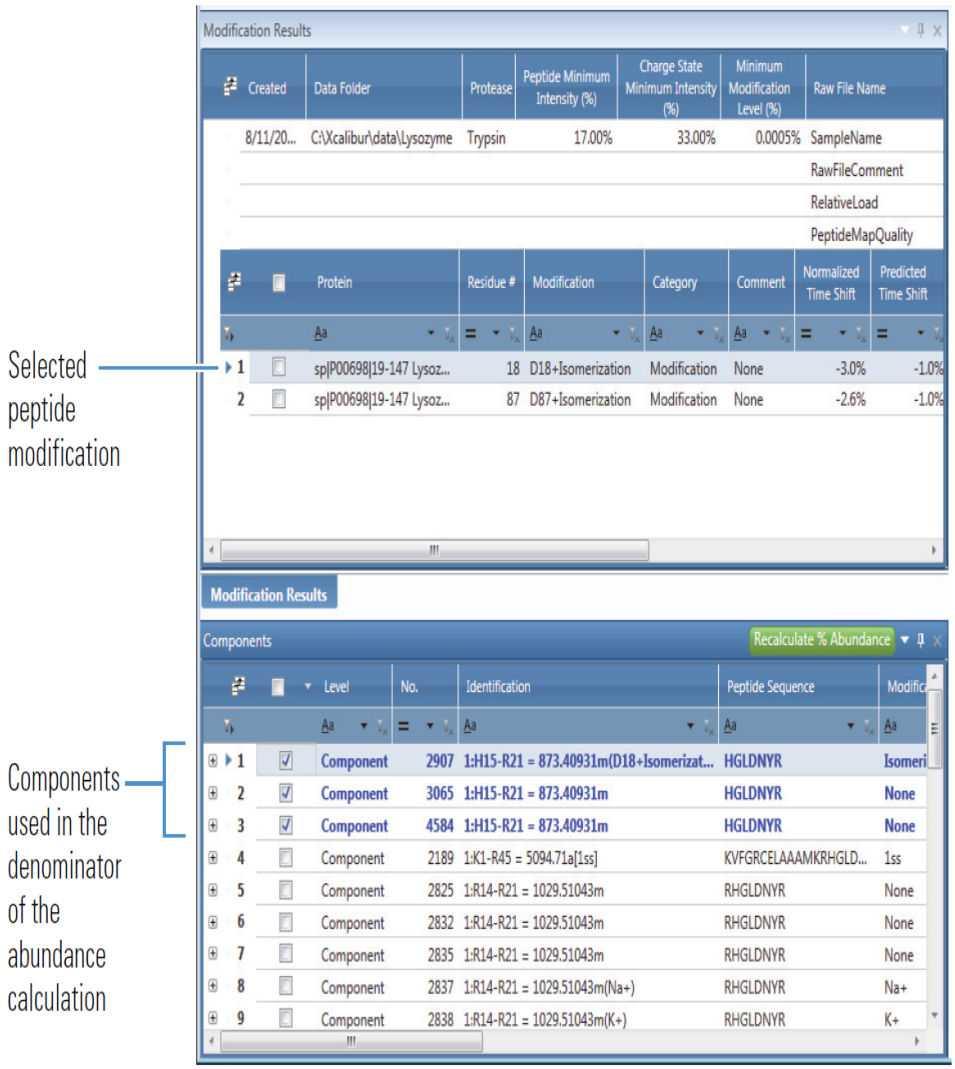


Figure 145 Components of a peptide modification selected in the Modification Results lower table

The selected row highlights in blue.

The Components pane lists all the components with the same residue as the selected peptide modification.

Changing the modification summary options

Note: You cannot change the options for the modification summary of a targeted peptide mapping experiment.

To change the options for the modification summary of a non-targeted peptide mapping experiment

1. On the Modification Summary page, right-click the Modification Results pane and choose **Set Summary Options**.

   The Summary Options dialog box opens.

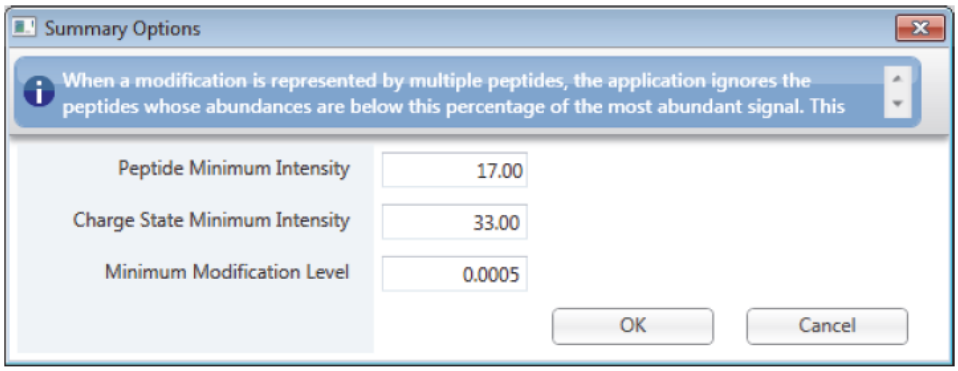


Figure 146 Summary Options dialog box

2. In the Peptide Minimum Intensity box, type a value to define the peptide area threshold as a minimum percentage of the most abundant signal.

   Missed cleavages or nonspecific digestion can cause more than one peptide to represent a modification. When the application uses the information from all of these peptides, unnecessary interferences might result in an improper abundance calculation. To avoid this problem, the modification summary does not include the peptides whose total peak area is below the percentage value set in the Peptide Minimum Intensity box—that is, below the minimum percentage of the most abundant signal. This filter results in the removal of less abundant peptides from the calculation.

3. In the Charge State Minimum Intensity box, type a value to define the charge state threshold as a minimum percentage of the most abundant signal.

   When there is a large variation in intensities, the abundance calculation might underestimate the low abundant peptides. To avoid this problem, the modification summary does not include the charge states whose abundances are below the percentage value set in the Charge State Minimum Intensity box—that is, below the minimum percentage of the most abundant charge-state signal. This filter results in the removal of less abundant charge states from the calculation.

4. In the Minimum Modification Level box, type a value to specify the minimum modification level to report in the summary.

5. Click **OK** to update the modification summary.

   Note: If you change the list of components used in the % Abundance calculation, your modified settings in the Summary Options dialog box apply only to the default % Abundance value for each raw data file. They do not apply to the recalculated Custom % Abundance values.

Exporting the modification summary

To export the modification summary

1. On the Modification Summary page, right-click anywhere in the Modification Results pane and choose one of the following:
   - **Export All Modifications to Excel** to export all of the summary results to an Excel file.

   –or–

   - **Export Checked Modifications to Excel** to export only the selected summary results to an Excel file.

     To select a modification row to export, select the check box in that row in the lower table.

     To select or deselect all of the rows, select or clear the check box in the table header row.

   The Save As dialog box opens.
2. Browse to or type the name of the file to store the exported results in.
3. Click **Save**.

Upper table of modification results pane parameters

The following table describes the types of information in the upper table of the Modification Results pane on the Modification Summary page.

Table 41 Modification Results pane, upper table parameters

Column	Description
Field Chooser 	Displays the Field Chooser dialog box so that you can select the columns to display in the upper table of the Modification Results pane.
Created	Displays the date on which the application created the Modification Summary and the name of the person who created the Summary.
Data Folder	Displays the folder containing the raw data files used in the experiment.
Protease	Displays the name of the protease selected for the method on the Identification page.
Peptide Minimum Intensity (%)	(Applies only to non-targeted peptide mapping experiment) Displays the filter value for peptide intensity as a minimum percentage of the most abundant intensity.

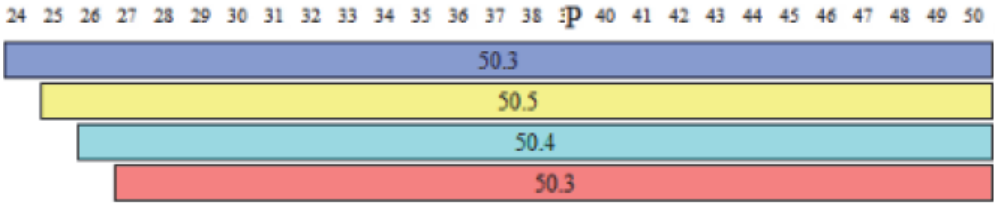
Column	Description
Charge State Minimum Intensity (%)	(Applies only to non-targeted peptide mapping experiment) Displays the value of the charge state intensity threshold as a minimum percentage of the most abundant intensity.
Minimum Modification Level (%)	(Applies only to non-targeted peptide mapping experiment) Displays the minimum modification level to report in the summary.
Raw File Name	Displays information imported from the raw data file. • SampleName: Displays the information saved in the Sample Name field in the raw data file. • RawFileComment: Displays the information saved as a comment in the raw data file. • RelativeLoad: Displays a measure of the protein quantification, using the top three peptides normalized to 100% for the first file. • PeptideMapQuality: Displays a measure of the quality of the digestion. A value of 1 indicates that the peptides in the sample are neither under-digested nor over-digested. The names of the raw data files used in the experiment appear in the columns to the right of the Raw File Name column, for example, Control_A01, Sample_B02, and Sample C_01.

Lower table of modification results pane parameters

The following table describes the types of information in the lower table of the Modification Results pane on the Modification Summary page.

Table 42 Modification Results pane, lower table parameters

Parameter	Description
Field Chooser 	Displays the Field Chooser dialog box so that you can select the columns to display in the lower table of the Modification Results pane.
	Select or clear the check box in the table header row to select/deselect all rows in the lower table. You can also select the check box in individual rows for export.
Protein	Displays the name of the protein imported from the FASTA file.
Residue #	Displays the position of the amino acid in the protein sequence that is modified.
Modification	Displays the amino acid on which the modification occurs and the type of modification.

Parameter	Description
Category	Displays the type of modification by category. • Unknown Modification: Modifications usually resulting from the unspecified modification search when the application cannot identify the exact location of the modification. Unknown modifications contain the tilde (~) mark. • Modification: Common modifications that the application identified. • Artifact: Something observed in a scientific investigation or experiment that is not naturally present but occurs as a result of the preparative or investigative procedure. • Sequence Variant: Modifications from sequence variants. • N-Glycan or O-Glycan: Modifications from N-linked or O-linked glycans. • Glycoform: Modifications from glycopeptides. • Clipped: For targeted peptide mapping experiments, this category designates that the modification belongs to a peptide clipped either at the N-terminus or the C-terminus. For example, this portion of the sequence coverage map shows several peptides each clipped by one amino acid at the N-terminus. 
Comment	Displays any comments about the modification to support the identification, the abundance percentage, or both. Examples of comments are None, Possible artifact, or Poor recovery.
Normalized Time Shift	Displays the experimental shift of the retention time after modification as a percentage of the abundance-weighted average retention time of all identified peptides. A negative indicates that the modified peptide elutes earlier than the unmodified peptide. A positive value indicates that the modified peptide elutes later than the unmodified peptide.
Predicted Time Shift	Displays the predicted retention time shift, which is based on the empirically determined value of the normalized time shift of common modifications.
Peptides	Displays the names of the peptides used for quantification.
Sequence	Displays the amino acid sequence.
Confidence	Displays the confidence score of the modified peptide.
Recovery™	Displays the general abundance of the modified peptide, which is the total peak area (including modified and unmodified forms of the peptide) as a percentage of the self-weighted average of all peptides. This means that higher-intensity peptides weigh more than lower-intensity peptides. The recovery value can be over 100% because of the way the application calculates the reference intensity. Good: Recovery™ ≥ 10% Fair: 1% < Recovery™ < 10% Poor: Recovery™ ≤ 1%

Parameter	Description
% Abundance *raw_data_file_name*	Displays the abundance of the modification in a particular raw data file as a percentage.
Custom % Abundance *raw_data_file_name*	(Visible only when you change the list of components used for the % Abundance calculation) Displays the custom abundance of the modification in a particular raw data file as a percentage.

Modification results pane commands

Right-clicking the Modification Results pane on the Modification Summary page opens a shortcut menu with the commands listed in the following table.

Table 43 Modification Results pane shortcut menu

Command	Description
Set Summary Options	(Active only for non-targeted peptide mapping experiments) Opens the Summary Options dialog box so that you can set new values for the Peptide Minimum Intensity (%), Charge State Minimum Intensity (%), and Minimum Modification Level (%) columns in the upper table of the Modification Results pane.
Export All Modifications to Excel	Exports data for all modifications in the Modification Results pane to an Excel file.
Export Checked Modifications to Excel	Exports data for only the selected modifications in the lower table in the Modification Results pane to an Excel file.

Viewing the modification summary components

The Components table on the Modification Summary page displays the components of a completed experiment related to a particular modification site that you select in the lower table of the Modification Results pane.

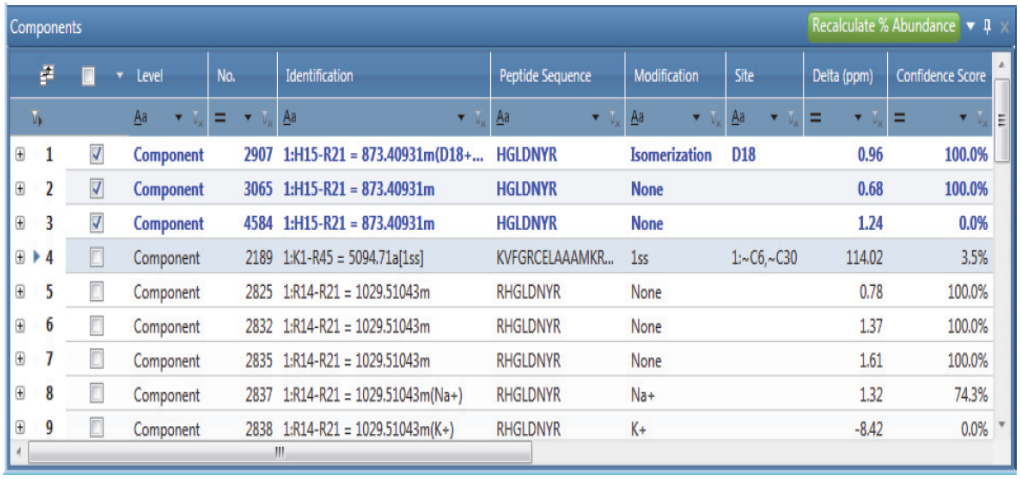


Figure 147 Components table

The information in this table is similar to the information in the Results table on the Process and Review page but is filtered to show only the peptides with the same site as the selected peptide modification.

The BioPharma Finder application uses blue text for the components whose modifications it used to calculate the abundance and recovery data and selects their corresponding check boxes, ☑. Below these components, the application uses black text for the components whose modifications it did not use in these calculations and clears their check boxes, ☐.

For more details, see the following topics:

- Viewing the components table (page 319)
- Changing the abundance calculation (page 319)
- Exporting the component results (page 320)
- Saving a peptide workbook from the modification summary page (page 321)
- Components table commands (page 323)

Viewing the components table

To view the Components table on the Modification Summary page

1. Click the **Mapping** tab and then click the **Modification Summary** subtab.
2. Click the row of a modification in the lower table of the Modification Results pane.

   The Components table displays the components related to the selected modification site.
3. Select a row in the Components table to view information related to that component in these other panes on this page:
   - Chromatogram
   - Visible only for experiments with multiple raw data files:
     - Trend ratio plot
     - Trend MS area plot
   - Peptide sequence coverage
   - Protein sequence
   - Full scan spectra
   - MS2 spectra

   Note: The previous linked topics describe the features on the Process and Review page. Their functionality is very similar to those on the Modification Summary page.

Changing the abundance calculation

To change the components used in the Abundance calculation

1. In the Components table on the Modification Summary page, select or clear the check boxes in the component rows to select or deselect the components to be used in the Abundance calculation of the modification summary.

   Note: To select or deselect all of the rows at once, select or clear the check box in the header row.

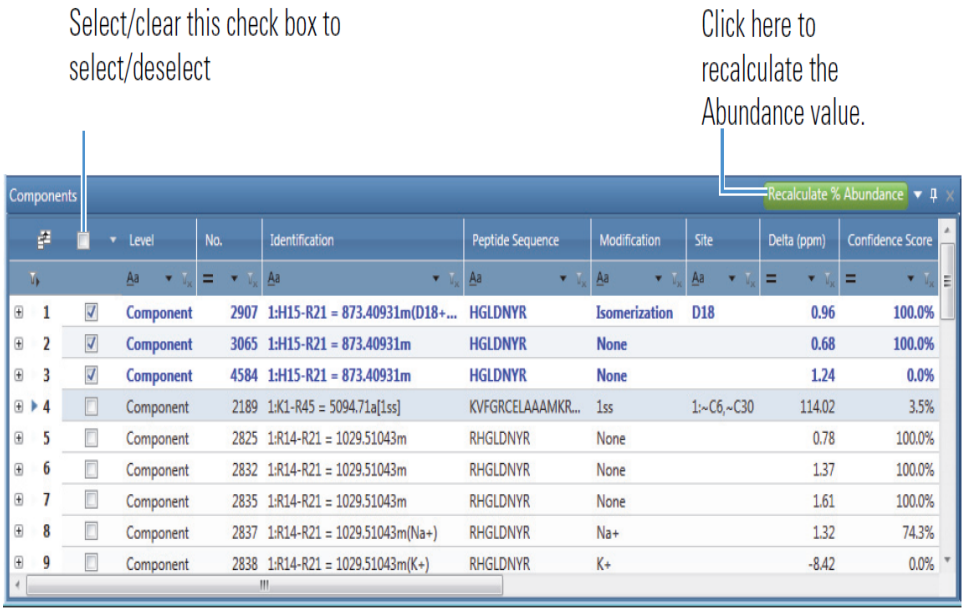


Figure 148 Components shown in the Components pane

2. Click **Recalculate % Abundance**.

   The application recalculates the % Abundance value using the selected components. It updates this value in the Custom % Abundance *raw_data_file_name* column for each raw file, in the lower table of the Modification Results pane. The default % Abundance value remains in the % Abundance *raw_data_file_name* column for each raw data file.

3. If you are satisfied with the recalculated value, click **Save** in the upper right corner of the Modification Summary page.

Exporting the component results

To export the component results

1. Click the row of a peptide modification in the lower table of the Modification Results pane on the Modification Summary page.

2. Right-click anywhere in the Components table on the Modification Summary page and choose one of the following:

   - **Export All Components**
     - **As Displayed** to export data at all levels for all components to an Excel file, exactly as currently displayed in the table
     - **Excel Workbook** to export data only at the component level for all components to an Excel file in the default format

   –or–

- Export Checked Components
  - **As Displayed** to export data at all levels for only the selected components to an Excel file, exactly as currently displayed in the table
  - **Excel Workbook** to export data only at the component level for only the selected components to an Excel file in the default format

  To select a component row to export, select the check box in that row.

  To select or deselect all of the rows, select or clear the check box in the table header row.

The Save As dialog box opens.

3. Browse to or type the name of the file to store the exported results in.
4. Click **Save**.

The exported data reflects the filtering, sorting, and reordering of columns of the Components table only for the As Displayed option. The application exports the table rows based on the sequential order of the numbers in the No. column.

Saving a peptide workbook from the modification summary page

A peptide workbook is a saved set of peptides that is compatible with the Chromeleon data system, containing some selected results from a Peptide Mapping Analysis experiment (or from the Theoretical Protein/Peptide Manager page, but not the protein sequences and the method parameters used to process those results.

To save the components on the Modification Summary page to a workbook

1. Click the row of a peptide modification in the lower table of the Modification Results pane on the Modification Summary page.
2. (Optional) In the Components table on the Modification Summary page, select the check box in the row of each component that you want to save to the workbook.

   To select/deselect all of the rows, select/clear the check box in the column header.
3. Right-click anywhere in the Components table and choose one of the following:
   - **Save As Peptide Workbook ▸ All** to save all peptides in the Components table to a workbook.
   - **Save As Peptide Workbook ▸ Checked** to save the peptides of the selected rows in the table to a workbook.

IMPORTANT! The application does not support saving components with unspecified modifications, dimers, adducts, disulfide bonds, or any type of gas phase modifications to the workbook. You must clear the check boxes for these components to save the workbook.

---

4. In the Save Peptide Workbook As dialog box, do the following:
   a. Select one of these options: **Create a New Peptide Workbook** or **Select an Existing Workbook**.
   b. (For the Create a New Peptide Workbook option) In the Workbook Name box, type a new name to create a new workbook or keep the same name to overwrite the workbook if it already exists.

      The default workbook name is the same as the experiment name.

      Note: Use only alphanumeric, space, underscore "_", and period "." characters in the workbook name.

      –or–

      (For the Select an Existing Workbook option) Select the check box for an existing workbook in the table.

      The application adds data from your selection of components to that workbook. A peptide workbook can contain added data from multiple different experiments.
   c. (Optional) In the Description box, type a description for the workbook.
   d. In the Number of Isotopes per Peptide box, specify the number of isotopes for the selected components.

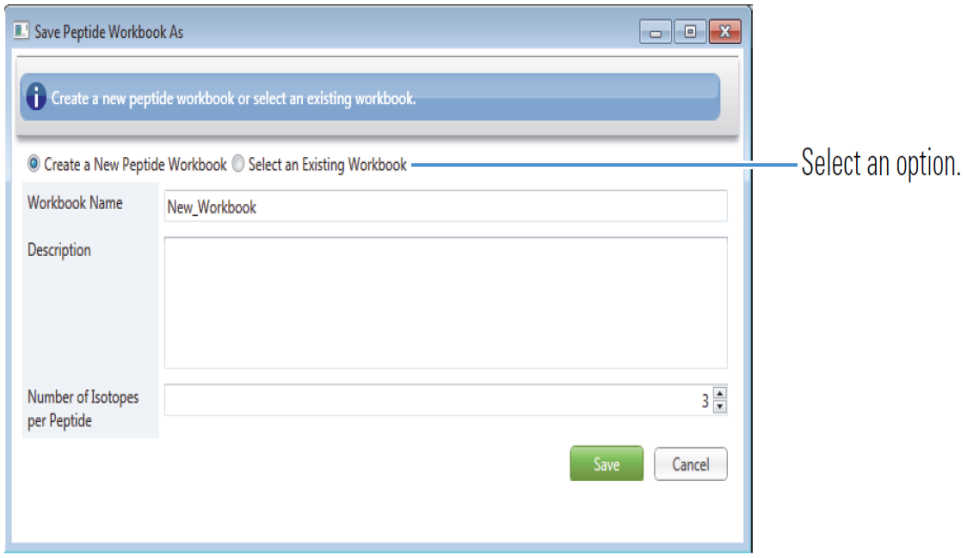


Figure 149 Save Peptide Workbook As dialog box

5. Click **Save**.

   Note: If the workbook is currently open for editing on the Workbook Editor page, indicate whether you want the application to automatically save and close the open workbook before proceeding.

If you are creating a new workbook, the following occur:

- Multiple peptide components with the same set of data—identification, sequence, modification, site, theoretical monoisotopic mass (within a tolerance that is ±10 ppm of each other), protein name, and RT (within a tolerance that is ± half of the typical chromatographic peak width for the experiment)—merge into one entry in the workbook. However, the merged entry saves the charge state distribution from the individual components.
- The application automatically assigns a relative quantitation group number to all workbook entries with the same protein and sequence, starting with group number "1" and incrementing by one for each new group.

If you are adding to an existing workbook, the following occur:

- The application does not merge the added components that have the same data as the components already in the workbook. In this case, application inserts the added components to the workbook as duplicates.
- The application automatically adjusts the relative quantitation group number. The added entries with the same protein and sequence as some existing entries in the workbook receive the same relative quantitation group number as the existing entries. All other added entries receive incremented group numbers.

After saving, you can manage the workbook using the Target Peptide Workbook page.

Components table commands

Right-clicking the Components table on the Modification Summary page opens a shortcut menu with the commands listed in the following table.

Table 44 Components pane shortcut menu

Command	Description
Export All Components	Exports data for all components in the Components pane to an Excel file.
Export Checked Components	Exports data for only the selected components in the Components pane to an Excel file.
Save As Peptide Workbook	Saves all or selected peptides to a workbook that is Chromeleon-compatible and used for targeted peptide processing.

Viewing the modification plot

The Modification Plot pane on the Modification Summary page displays a plot of the abundance percentages for the selected modifications in the Modification Results table, grouped by the conditions and corresponding raw data files loaded for the experiment.

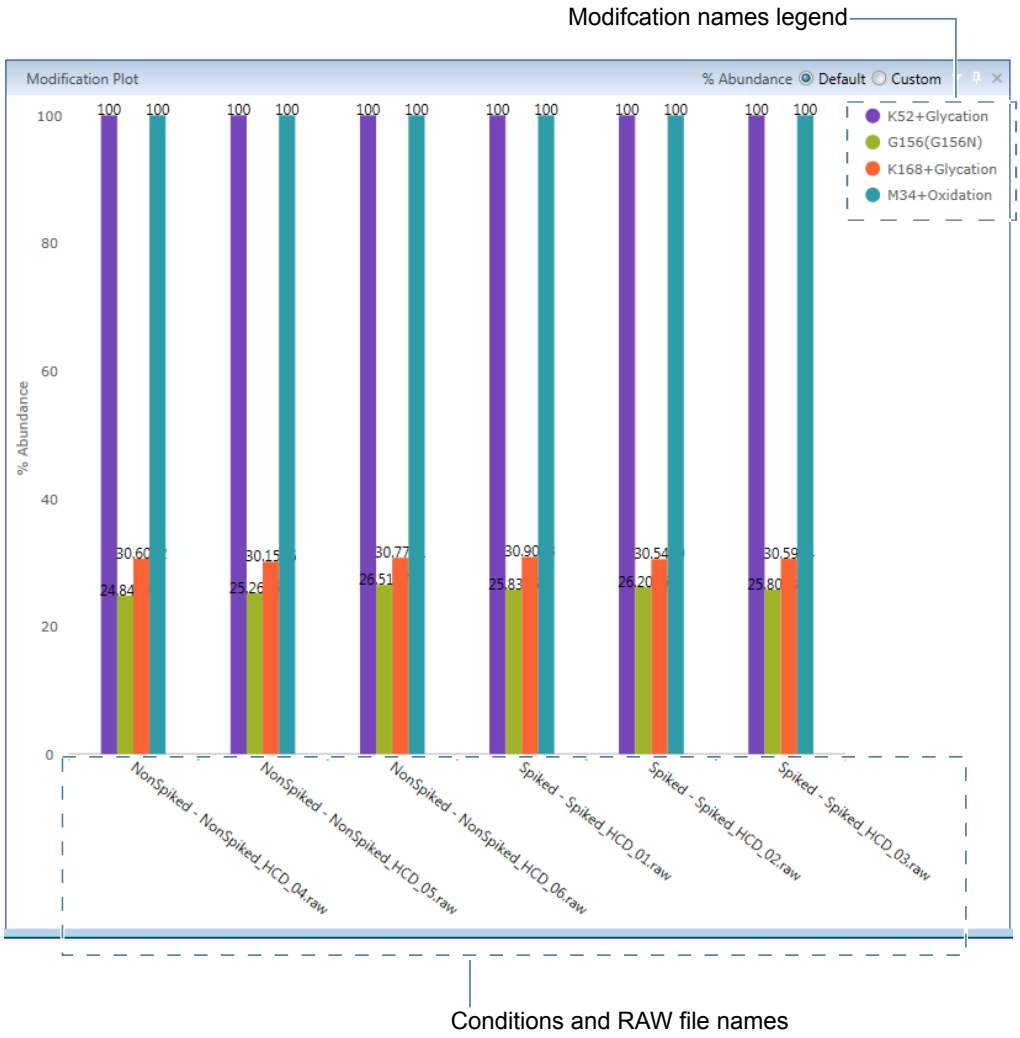


Figure 150 Modification Plot pane

To view the modification plot

1. Click the **Mapping** tab, and then click the **Modification Summary** subtab.
2. Select the check box for one or more rows of proteins and modifications in the lower table of the Modification Results pane.

   The Modification Plot pane shows the *default* abundance percentages for the selected rows, grouped by the conditions and corresponding raw data file names. The percentage values in the plot are from the % Abundance *raw_data_file_name* columns in the table. The plot displays each modification in a different color according to the legend in the upper right of the pane.
3. (Optional) If you recalculated *custom* abundance percentages, view them in the plot by selecting the **Custom** option in the title bar of the Modification Plot pane.

   The plot now uses the values from the Custom % Abundance *raw_data_file_name* columns in the table. If a selected row does not have a value in this column, the Custom option is inactive.

   To view the default percentages again for the selected rows, select the **Default** option.

For more information, see these topics:

- Comparing MS2 Spectra on the modification summary page (page 325)
- Switching the header on the modification summary page (page 327)

Comparing MS2 Spectra on the modification summary page

By default, the predicted MS/MS spectrum is displayed on the top of the experimental MS/MS spectrum. You can compare the experimental spectra with different activation types.

To use the modified MS2 spectrum instead of the predicted MS2 spectrum

1. Click a component in the results table that has an MS2 spectrum.

   The MS2 Spectra view displays the predicted spectrum on the top and the experimental spectrum on the bottom.
2. Click **MS2 Spectra**, and then right-click the MS2 Spectra view.

   By default, the Predicted view check box is selected.
3. Choose **Set bottom spectrum as a reference (move to top)**.

   The Predicted view check box is cleared. The spectrum on the bottom moves to the top view and replaces the predicted spectrum. This spectrum is now known as the reference spectrum.

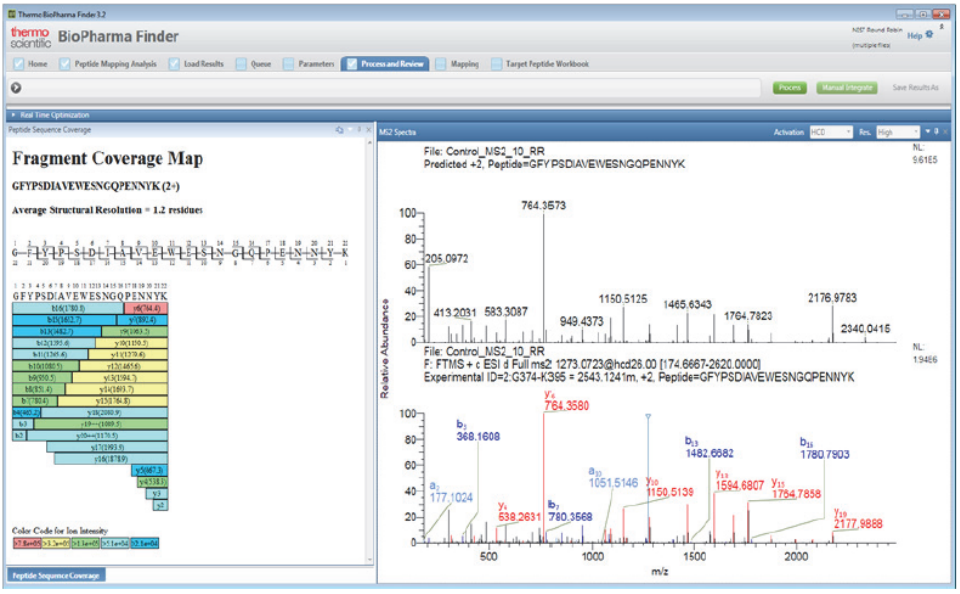


Figure 151 Comparing MS2 Spectra

4. To compare the spectrum, select another component.

   The spectra are stacked with the reference spectrum displayed on the top.

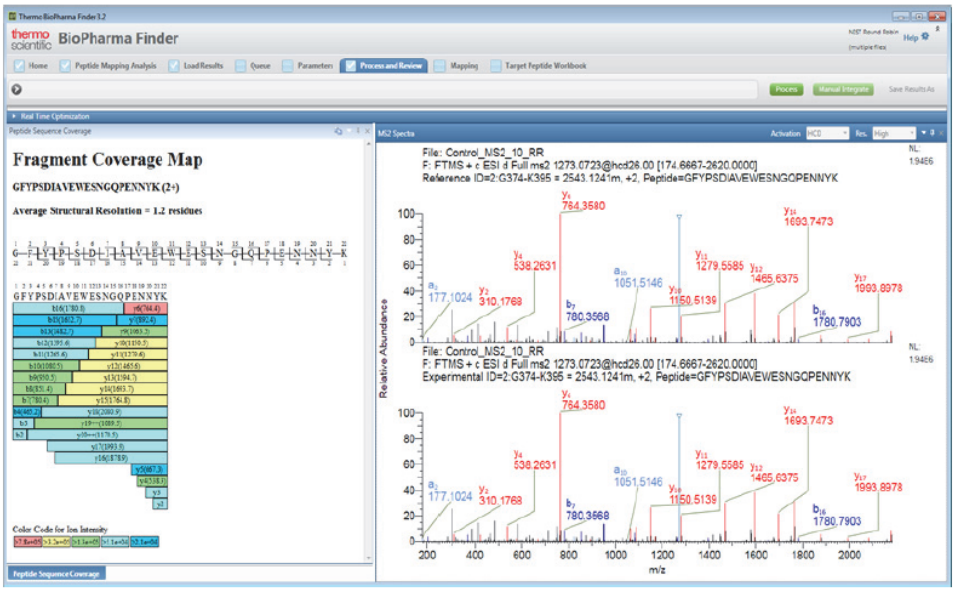


Figure 152 Reference spectra displayed on top

Note: The reference spectrum is not linked between pages—that is when you set a peptide as the reference on the Modification Summary page, this information is not passed to the Mapping page.

The Predicted peptide MS/MS (Kinetic Model) is deactivated.

The reference spectrum remains in this location until you right-click and select the Predicted view check box. Then the reference spectrum is replaced with the predicted spectrum of the experimental spectrum in the bottom.

Switching the header on the modification summary page

This feature displays the Identification and the Peptide Sequence information in place of the TIC in both spectrum plots.

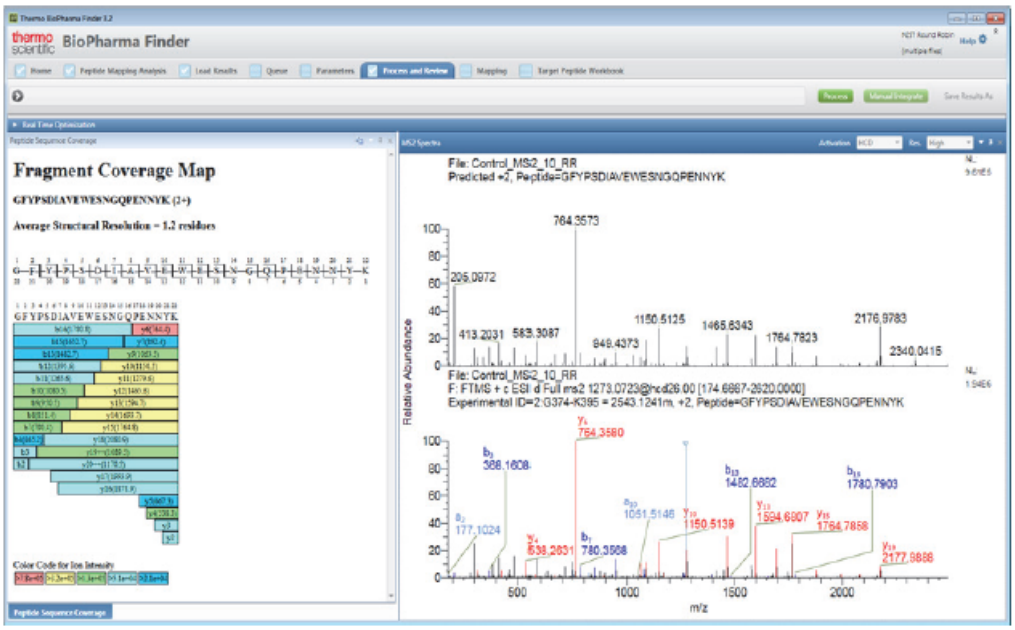


Figure 153 Switching the Header

To switch the header

1. Right-click the view.
2. Choose **Header**, and then select the **Show TIC information** check box.

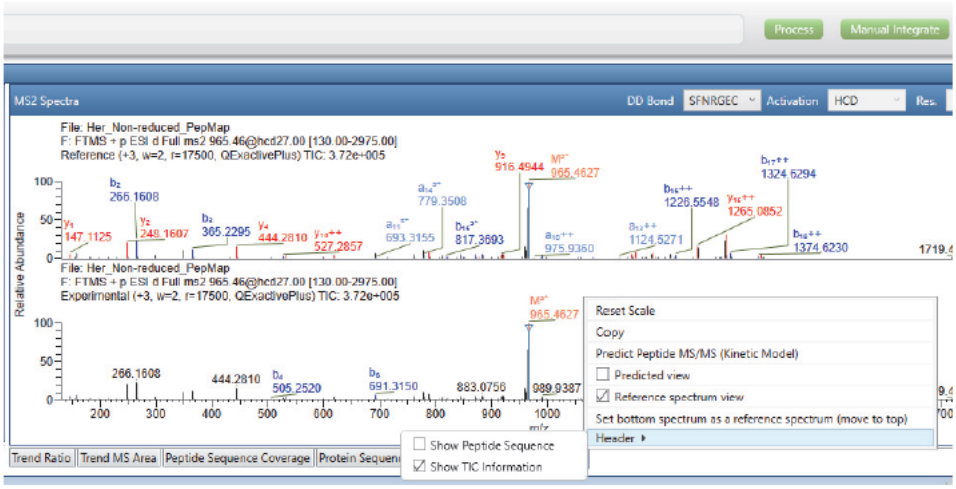


Figure 154 MS2 Spectra view with its shortcut menu displayed

3. To switch back to Peptide information, right-click the view, choose **Header**, and select the **Show Peptide Information** check box.

Part V

Intact Mass Analysis

16 Intact mass analysis features

Electrospray ionization (ESI) of intact peptides and proteins produces mass spectra that contain series of multiply charged ions with associated mass-to-charge ratio (*m/z*) values. The resulting spectrum is complex and difficult to interpret, requiring mathematical algorithms for the analysis of the data. Through a process called deconvolution, Intact Mass Analysis in the BioPharma Finder application uses such algorithms to transform a charge state series into a molecular mass.

The application identifies multiple peaks in the mass spectrum associated with different charge states of the same component and displays information about the masses and abundance of that component. For example, peaks at *m/z* 1000, 1111, and 1250 might be the charge states 10, 9, and 8 for a protein with a mass of 10 000 Da.

In addition, you can use the features of Intact Mass Analysis to do the following:

- Produce more than one deconvoluted spectrum for any given mass spectrum.
- Perform a manual or automated deconvolution.
- Compare spectra.
- Generate a report containing the deconvoluted spectrum and the mass spectrometry evidence for it or other customized reports.

Deconvolution algorithms

Intact Mass Analysis includes two independent deconvolution algorithms for mass spectral data:

- Xtract, which deconvolves isotopically resolved mass spectra—that is, spectra in which it is possible to distinguish separate peaks for different isotopic compositions of the same component
- ReSpect, which deconvolves isotopically unresolved (or unseparated) mass spectra—that is, spectra in which it is not possible to distinguish the separate peaks for different isotopic compositions of the same component

Whether mass spectra are isotopically resolved or unresolved depends not on the specific instrument but on the resolution of the instrument, the mass of the compounds involved, and the details of the experiment run.

The Xtract algorithm is designed for use on isotopically resolved spectra. ReSpect is designed for use on isotopically unresolved spectra. Attempting to use either algorithm on the wrong type of spectra can lead to unreliable results. In most cases, the Xtract algorithm fails to identify any components if you apply it to isotopically unresolved spectra, since these do not have any isotopic profiles. If you apply the Respect algorithm to isotopically resolved spectra, it might attempt to identify each isotopic peak as a separate component.

Xtract algorithm

The Xtract algorithm uses a fitting scheme similar to the THRASH algorithm to deconvolve and deisotope isotopically resolved mass spectra of peptides and proteins. First, it examines a cluster of isotopically resolved peaks and uses the peak spacing of a cluster to determine an initial estimate of the mass of the relevant component. Then it fits an averagine distribution to the observed peak profile in that cluster to determine the monoisotopic mass that best reproduces that profile. Finally, it combines results for all observed charge states for each mass component to produce a single mass value for that component. The resulting spectrum shows only the monoisotopic masses for the components that the algorithm identified.

Note: Senko, M.W.; Beu, S.C.; McLafferty, F.W. Determination of monoisotopic masses and ion populations for large biomolecules from resolved isotopic distributions. *J. Am. Soc. Mass Spectrometry*. **1995,** *6*, 226–233.

When used properly, the Xtract algorithm reduces spectral noise and provides a high-intensity mass spectrum of monoisotopic peaks. You can use the Results table, called the monoisotopic mass list, of the deconvolved mass-spectral peaks or the extracted spectra (not the original MS2 spectra) as the input to various search engines.

The following figure shows an isotopically resolved mass spectrum.

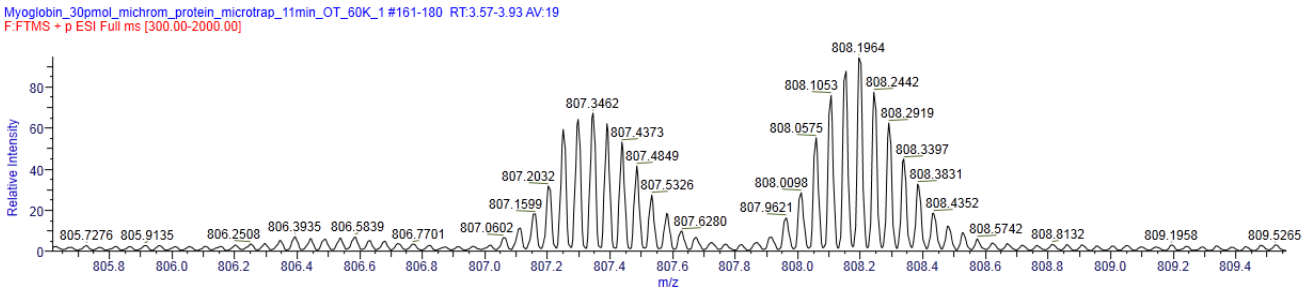


Figure 155 Isotopically resolved mass spectrum

ReSpect algorithm

The ReSpect algorithm from Positive Probability, Ltd. (PPL) is a robust and efficient data-fitting method that deconvolves isotopically unresolved complex mass spectra from biomolecules, such as small and large proteins, to the neutral average mass of each molecule. It determines the *m/z* of every peak in an ESI mass spectrum and evaluates all possible charge states for any particular peak as determined by the mass ranges.

For analyzing spectra, the ReSpect algorithm includes an optional spectrum preconditioning method, including automated baseline subtraction, and a number of automated and semiautomated peak-modeling facilities.

The following figure shows an isotopically unresolved mass spectrum.

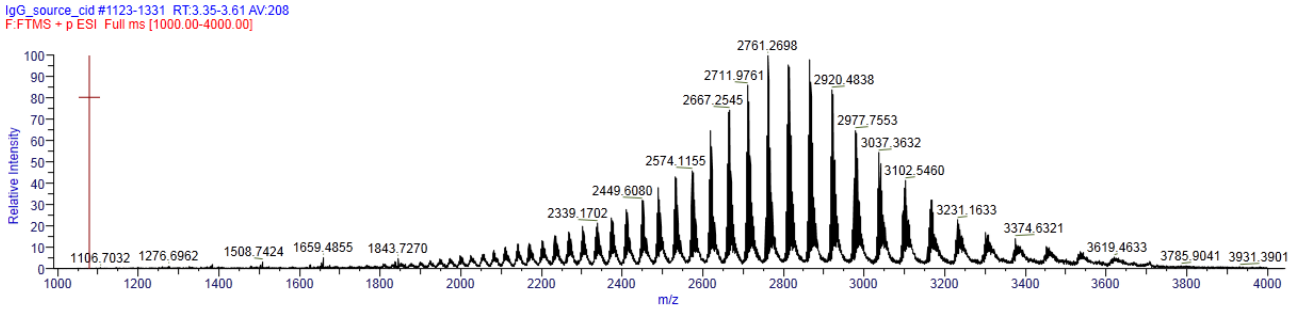


Figure 156 Isotopically unresolved mass spectrum

The ReSpect algorithm first performs a baseline subtraction. Next it performs a peak deconvolution to produce a list of peaks, and then it filters these peaks. Lastly, it performs a charge deconvolution to convert the remaining peaks from a mass-to-charge spectrum to a mass spectrum. The ReSpect algorithm uses peak spacing patterns, which are indicative of mass, to determine what the average mass should be.

The ReSpect algorithm can accommodate both low-charge-state spectra and data with a low signal-to-noise ratio, so it does not require high-quality data to produce meaningful results. You can use it to confirm molecular masses of proteins.

For more details, see the following topics:

- Spectra deconvolution (page 332)
- Important parameters (page 332)
- Default native method (page 333)
- Default ion trap method (page 333)
- Protein quality score (page 333)

Spectra deconvolution

The ReSpect algorithm deconvolves spectra by following these general steps:

1. It generates a peak model, using parameters provided by the method. These parameters include a right- and left-side width and a shape that describe the width of a peak and its deviation from a Gaussian. The BioPharma Finder application calculates a shape for a target protein using the target mass in the ReSpect method and the instrument resolution, along with the right- and left-peak shape parameters from the method. The ReSpect algorithm then processes this shape.

2. It deconvolves the spectrum using this peak model to detect a list of peaks and their associated parameters, such as *m/z* value, intensity, and an uncertainty in *m/z*. It filters this peak list by confidence level and *m/z* range.

3. It deconvolves charge states in the list of filtered peaks to identify patterns of peaks that could represent individual components with series of associated charge states.

The ReSpect charge state deconvolution is somewhat liberal on purpose. In particular, it accepts a significant false positive rate to be sure that there are no false negatives. For this reason, the ReSpect algorithm leaves the scoring to a separate scoring algorithm that can distinguish which components are most reliable.

Important parameters

The ReSpect algorithm accepts several parameters that control the peak detection process and the charge state deconvolution, but the following are the most important:

- m/z Range
- Model Mass Range
- Target Mass
- Number of Peak Models
- Deconvolution Mass Tolerance
- Peak Detection Quality Measure
- Resolution at *m/z* 400

Default native method

For the ReSpect algorithm, you can use the Default Native method, which is suitable for the structural analysis of proteins—that is, for measuring and studying intact proteins and protein complexes in their active form under native or non-denaturing conditions. Use this method to analyze very large, non-covalent complexes and antibody-drug conjugates that are held together by non-covalent bonding.

Default ion trap method

For the ReSpect algorithm, you can also use the Default Ion Trap method, which accounts for differences in resolution and characteristics that ion trap instruments receive from other instruments. In particular, the resolution specified in this method provides the optimum peak model. Use this method as a starting point for ion trap data.

Protein quality score

The ReSpect algorithm calculates a protein quality score for each component and displays it in the Results table so that you can determine whether each component is valid or spurious because of noise, harmonics, or other factors.

Manual and automatic modes

You can run Intact Mass Analysis in manual or automatic mode, as follows:

- In manual mode, you set up the chromatogram, select the chromatographic peaks, deconvolve the spectra with the ReSpect or Xtract algorithm, and report the results separately, one step at a time.

  You might want to run the BioPharma Finder application in manual mode when something about your workflow changes and you want to determine the optimal settings for selecting the chromatographic peaks, deconvolving the spectrum, or both. For example, you might introduce a new protein, a new chromatographic setup, or different mass spectrometry settings. Once you determine the optimal settings, you can save them to a processing method and use the automatic processing from then on for subsequent analyses for that type of sample.

  For manual mode, you can deconvolve spectra in two ways with the Xtract or ReSpect algorithm:

  - Use a chromatographic peak-picking method called *average over RT* deconvolution. From the chromatogram, you select the *single-scan* source spectrum by picking a particular retention time/scan or the *averaged* source spectrum by dragging across a range of retention times/scans. For this method, select the Average Over Selected Retention Time option for source spectra in the processing method.
  - Use *sliding windows* deconvolution. For this method, select the Sliding Windows option for source spectra in the processing method.

IMPORTANT! In manual mode, you cannot run experiments using the *Batch Processing* result format or the Auto Peak Detection option for source spectra. You can run these experiments only in automatic mode.

- In automatic mode, the application detects chromatographic peaks, extracts averaged mass spectra, deconvolves isotopically unresolved or resolved peptides or proteins, and generates a component list—all in a single step without any intervention. You can place up to 1000 samples in the run queue for automatic processing.

  In addition to the single and sliding windows deconvolution methods, you can also process experiments in *automatic* mode with the Xtract or ReSpect algorithm using an *auto-peak detection* method called Parameterless Peak Detection (PPD). For this method, select the Auto Peak Detection option for source spectra in the processing method. The application performs deconvolution for each detected peak and groups the peak results together.

Sliding Windows deconvolution

Sliding windows deconvolution is a new approach to identifying components in LC/MS data. It completely skips the chromatographic peak identification step in identifying chromatographic peaks and then averaging them over the associated retention time ranges, deconvolving the average spectra, and compiling the resulting components produced by the deconvolution into a list. Instead, it averages spectra over a succession of sliding windows in retention time, deconvolves each of these averaged spectra, and then merges similar masses to identify components.

You can use sliding windows deconvolution with both the Xtract and ReSpect algorithms by setting up the appropriate method parameters.

For more details, see the following topics:

- Sliding Windows advantages (page 334)
- Sliding Windows steps (page 335)

Sliding Windows advantages

The sliding windows deconvolution approach has several advantages over the conventional deconvolution approach:

- It avoids all of the problems involved in trying to identify the complicated and poorly defined chromatographic peaks associated with large molecules.
- It identifies and characterizes components that coelute at overlapping retention time ranges.
- It produces a meaningful elution profile for each component that it identifies.
- It greatly reduces the rate of false positives.

Sliding Windows steps

Using sliding windows deconvolution involves two steps:

1. Sliding window step: Applies a conventional sliding window along a retention time axis to generate a succession of time-averaged spectra, as shown in the following figure.

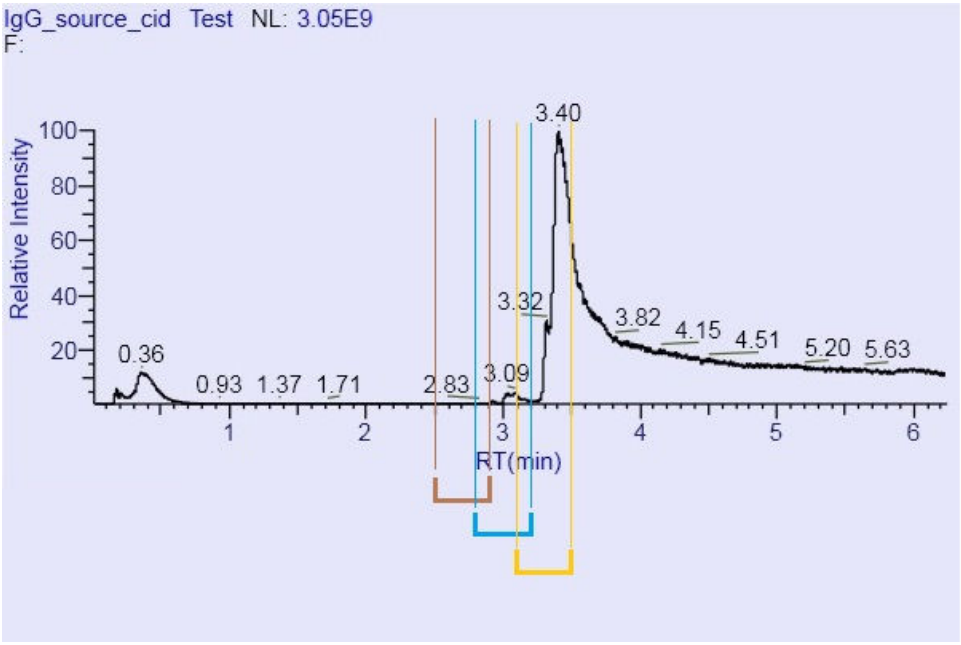


Figure 157 Three successive sliding windows in retention time

The application deconvolves the average spectrum from each sliding window and compiles the resulting components into a list of member components. Each of these member components has five parameters: mass, start retention time, stop retention time, intensity, and for results from the ReSpect algorithm, a fitness score.

2. Mass merge step: Takes the list of the member components produced by the first step and merges them to produce a list of merged components. The application incrementally examines the results from successive windows along a retention time axis and applies a sliding window along a mass axis to identify member components with similar masses. You specify the mass tolerance for this window. The application then applies additional tests and discards components with an implausibly small number of time steps and other false positives.

   The application follows these substeps during a mass merge:

   a. For ReSpect results, it discards the components with a score below the threshold.

   b. It sorts the remaining components by mass.

   c. It applies the sliding windows along the mass axis to merge the component peaks associated with the same component.

d. It discards the merged components with a number of time steps less than the number you specified.

e. For each of the remaining merged components, it examines the distance in retention time between the endpoints (for example, the stop and start retention times) of successive component peaks. If this value exceeds a user-specified distance, it splits the merged component in two.

Chromatographic peak detection and spectral peak modeling

For average over RT deconvolution experiments, you manually select the spectra to deconvolve. For auto peak detection experiments in automatic mode, Intact Mass Analysis uses the Parameterless Peak Detection (PPD) algorithm to analyze the raw data and to separate peaks from noise in chromatograms. This algorithm does the following to locate peaks in a chromatogram:

- Constructs a chromatogram after applying parameters that you set on the Parameters > Component Detection page or the Process and Review page.
- Assigns peak numbers.
- Generates a peak list.
- Determines the peak start and peak end points.

To locate peaks in spectra, the ReSpect algorithm fits a peak model to the spectrum to locate potential peaks. This peak model resembles a Gaussian distribution. You can control the characteristics of this peak model by modifying the relevant method parameters on the Parameters > Component Detection page or the Process and Review page using real-time optimization.

Batch and multiconsensus result formats

For Intact Mass Analysis experiments with multiple loaded raw data files, you can run the experiment using either of these result formats:

- The **Batch Processing** format maintains separate results from each of the multiple raw data files.

  In this format, the BioPharma Finder application processes one experiment individually for each loaded raw data file, so you can process multiple experiments at one time but retain individual results. For the name for each batch experiment, the application concatenates the specified experiment name on the Intact Mass Analysis page with a unique date-and-time stamp. The results for each batch experiment are the same as if you ran an experiment with just a single raw data file.

  IMPORTANT! You cannot run experiments using the *Batch Processing* result format in manual mode. You can run these experiments only in automatic mode.

–or–

- The **Multiconsensus** format merges the results from the multiple raw data files together.

  In this format, the application processes one experiment and merges the deconvolution results from all of the loaded raw data files together. By default, the name of this multiconsensus experiment uses the same name as the specified experiment name on the Intact Mass Analysis page.

  Tip: You can set the merging parameters for this format on the Parameters > Identification page when you edit a processing method.

Target sequence matching

For Intact Mass Analysis, the application can match the measured masses of the components that it detects to the masses of user-specified target sequences, aiding in the identification of the components. These target sequences can include site-specific and global fixed modifications, variable modifications, glycosylations, and disulfide links. If the measured mass of some components lies within a user-specified tolerance of the associated target sequence mass, the application displays the matched target sequence in additional columns in the Results table.

The application applies these modifications in the following order:

1. Disulfide links, which provide the bonds between heavy chains and light chains or bonds within the same chain.

2. Site-specific fixed modifications, which are side chain or terminal modifications to a user-specified site.

3. Global fixed modifications, which are side chain or terminal modifications applied universally to every instance of a user-specified amino acid or terminus.

4. Glycosylation, which is a process where chains of saccharides are linked to produce glycans that can be attached to glycosylation sites in the target sequence. You can have zero or one glycosylation per consensus site.

   The appendix lists all of the N-linked glycans included in the default modifications list, the N-linked glycans included in the N-glycan-specific search and sorted by host cell-line type, and the O-linked glycans that are supported by Peptide Mapping Analysis.

   Note: The algorithm that detects possible glycosylation sites was designed for use on intact proteins. If it is applied to peptides, it might fail to identify motifs that have been truncated by a cleavage. When you want to use a peptide as a target sequence, you can address this issue by appending an amino acid to the sequence to complete the motif, and then defining and applying a custom modification that subtracts the mass of that amino acid.

5. Variable modifications, which are possible side-chain or terminal modifications whose specific sites and number of occurrences might not be known. The application applies all possible combinations between zero and a maximum number of user-specified variable modifications to the target sequence.

When you select glycosylations and variable modifications, the application also generates additional masses in cases where glycosylations and variable modifications occur together.

You can use target sequence matching with both the Xtract and ReSpect algorithms for average over RT deconvolution, sliding windows deconvolution, or auto peak detection experiments, using either the Batch Processing or Multiconsensus result format.

Extracted ion chromatogram calculation for deconvoluted Spectra

Intact Mass Analysis can calculate an extracted ion chromatogram (XIC) from a selected component. It displays this chromatogram under a red curve in the Chromatogram pane of the Process and Review page. In addition, the Process and Review page features the Start Time and Stop Time columns in the Results table, which display the retention time range of the averaged source spectrum for the given component. The chromatogram plot also shows this retention range as a red box for the given component.

Component XICs and abundance traces

An XIC for a component shows the total signal associated with all the different charge states, the isotopes of a particular component, or both. It is qualitatively different from an individual mass XIC, which only shows the part of the signal for a single *m/z* value. A component XIC is a sum of all the conventional XICs that can be associated with a component. The component XIC can include portions of the original signal that are not already used in the component itself. For this reason, do not use component XICs for quantification.

The abundance trace generated by the sliding windows algorithm is the fraction of the signal that the deconvolution algorithm associated with a given component. This calculation accounts for the discarded parts of the signal belonging to dimers, multimers, half-antibodies, and other components that might share some *m/z* values with the primary component. In contrast, the component XIC calculation sums over all parts of the signal in the relevant *m/z* ranges. You can use the abundance trace for quantitative purposes to examine how the relative abundances of different components vary with retention time, although the actual numerical values returned by the deconvolution itself offer more accuracy.

Drug-to-Antibody ratio (DAR) values

A drug-to-antibody ratio (DAR) value is the average number of drugs conjugated to the antibodies, which is an important attribute of the antibody-drug conjugates (ADCs). ADCs are complex molecules composed of an antibody linked to a biologically active cytotoxic (anticancer) drug.

The DAR value affects the efficacy of the drug, as low drug loading reduces the potency, while high drug loading can negatively affect pharmacokinetics and toxicity. With the current conjugation chemistry—that is, lysine side-chain amidation or cysteine interchain disulfide bond reduction—a drug load of 0 to 8 drugs (D0 to D8) per antibody is commonly observed.

The BioPharma Finder application automatically calculates a drug load for each component. It also calculates the average DAR values for each raw data file loaded for an experiment, as well as for the entire experiment, and displays these values when you view the results.

Spectra comparison

To measure consistency among batches of proteins, you can compare the deconvoluted spectra from two raw data files or even two different portions of the same deconvoluted spectrum. You add all of the spectra that you want to compare to a library, and then select two spectra at a time from this library for a comparison plot.

Intact Mass Analysis displays a mirror plot of the two spectra, which you can enlarge to see whether the structures and the relative abundance of masses in the two spectra are divergent or the same. Major differences in these areas between the spectra can indicate that target protein sequences have been modified by post-translational modifications such as phosphorylation or glycosylation. You can then use top-down proteomics techniques or peptide mapping to determine the exact cause of these changes.

This ability to compare spectra is particularly important in determining how closely a biosimilar recombinant monoclonal antibody imitates an innovator recombinant monoclonal antibody. When used with peptide mapping and glycosylation profiling, spectral comparison can help you identify and quantify an intact protein mass, a primary sequence, and post-translational modifications. It also helps you visualize very small differences between the two spectra.

Note: The mirror plot does not display modification information.

Intact mass analysis inputs

The type of input file used in Intact Mass Analysis is the raw data file from a mass spectrometry experiment. The raw data file can contain LC/MS data or an individual mass spectrum.

You can use Intact Mass Analysis with data from various mass spectrometry systems: ion trap, Thermo Scientific™ Exactive Series and Orbitrap™ Series, and Fourier transform mass spectrometry (FTMS) series.

IMPORTANT! For the ReSpect algorithm, the application cannot process ion trap data acquired in centroid mode and requires profile data only. For the Xtract algorithm, the application cannot process ion trap data because Xtract does not support this type of data.

Intact mass analysis outputs

As output, Intact Mass Analysis produces deconvoluted spectra and component/peak information. It generates the following:

- A report on the results of the deconvolution that appears on the Reporting page of the BioPharma Finder application window. You can save this report to a PDF file.
- Database records that contain both the method parameter settings that you applied to the raw data files and the results of the deconvolution. This information appears on the Process and Review page.

  When you want to view the results in another session, you can load the previously saved results from the Load Results page to review the deconvolution data.

Performing an intact mass analysis experiment

The following steps show how to perform an Intact Mass Analysis experiment in automatic mode:

1. (Optional) Create a new processing method or edit the parameters in an existing method.
2. Create a new experiment by naming it, load the raw data file or files, add the conditions for the loaded files (optional), choose the result format (if you load multiple files), and select one or more protein sequences (optional) and a processing method.
3. Submit the experiment for processing and monitor the status of the experiment by using the queue.
4. View the results of the analysis.

5. (Optional) Compare the samples.
6. (Optional) Use real-time optimization to change the parameters and reprocess the results.
7. (Optional) Generate and view the reports.

The following figure shows how to perform an Intact Mass Analysis experiment in manual mode. The workflow is nearly the same for the Xtract algorithm as it is for the ReSpect algorithm.

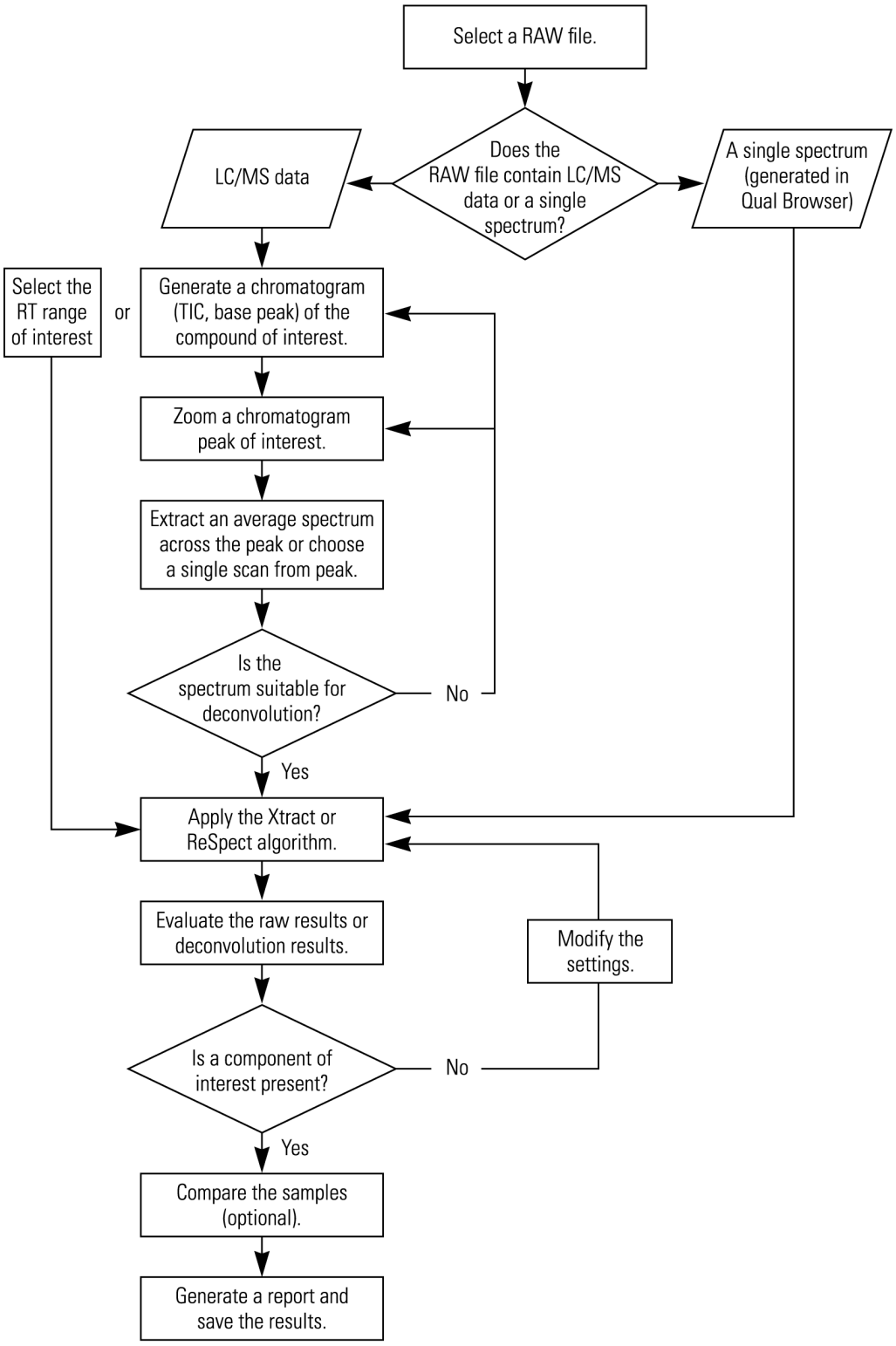


Figure 158 Intact Mass Analysis manual workflow

Running an intact mass analysis

After you add a sequence for intact mass analysis to the Sequence Manager, you can start an intact mass analysis experiment by clicking Intact Mass Analysis on the Home page.

Spectral deconvolution for intact mass analysis

Through a process called deconvolution, Intact Mass Analysis in the BioPharma Finder application uses algorithms to transform a charge state series into a molecular mass. The application identifies multiple peaks in the mass spectrum associated with different charge states of the same component and displays information about the masses and abundance of that component.

When you generate a deconvoluted spectrum from an isotopically resolved or unresolved protein mass spectrum, the source MS spectrum can be a single spectrum from an LC/MS data file, an averaged spectrum from an LC/MS data file, or a single spectrum from a raw data file containing only that spectrum. For Intact Mass Analysis, the Xtract or ReSpect algorithm transforms this source spectrum into a mass spectrum and displays it in a new pane labeled with mass units rather than with the mass-to-charge ratio on the *x* axis.

You can run spectral deconvolution in either manual or automatic mode, using average over RT deconvolution, sliding windows deconvolution, or auto peak detection.

You can save more than one deconvoluted spectrum for any given source spectrum. For example, if the sample contains more than one protein, you might want to perform two deconvolutions with two different mass ranges: 22 000 to 24 000 for immunoglobulin G (IgG) light chain and 50 000 to 52 000 for IgG heavy chain.

Starting a New Intact Mass Analysis Experiment

Use the Intact Mass Analysis page to create a new intact mass analysis experiment. Enter the experiment name, load the raw data file or files, choose a result format option (if you load multiple raw data files), select one or more sequences (optional), and select a processing method to start processing.

Use the global settings to specify the default folder from which you want to load your raw data files and also the precision for the intact mass analysis experiments.

To start a new experiment for Intact Mass Analysis

1. On the Home page, click **Intact Mass Analysis.**

   The Intact Mass Analysis page opens.

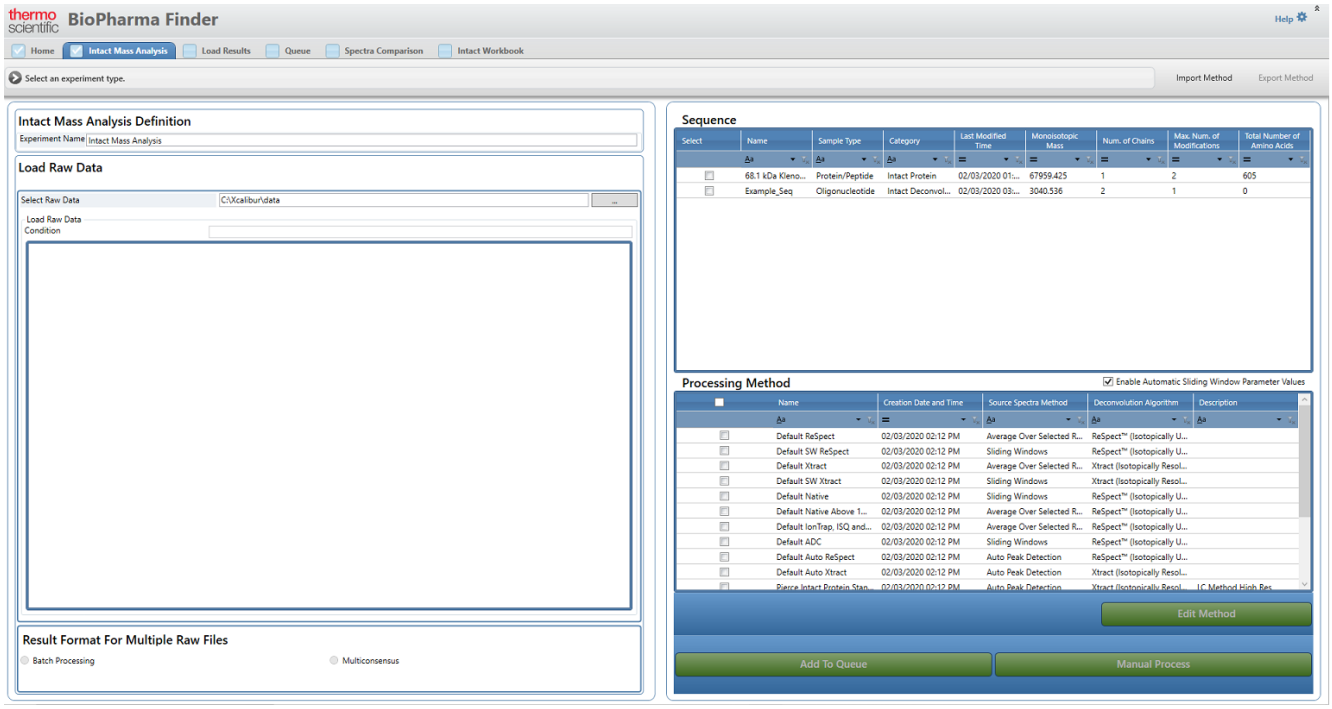


Figure 159 Intact Mass Analysis Page

2. In the Intact Mass Analysis Definition area, in the Experiment Name box, type the name of the experiment.

   Note: Use only alphanumeric, space, underscore "_", and period "." characters, up to 50 characters maximum, in the experiment name.

   If an existing experiment is already completed or canceled, you can overwrite it by entering the same experiment name. You cannot overwrite an experiment that is currently submitted to the run queue or is actively processing or canceling.

3. In the Load Raw Data area, load the raw data file or files for the experiment.

   (Optional) If you load multiple files, in the Condition box, type the conditions by which to group the raw data files, separated by spaces. Then, in the Condition column in the Load Raw Data table, select a condition for each raw data file. The application automatically groups together all files assigned to the same condition for processing the results related to conditions.

   IMPORTANT! For the ReSpect algorithm, the application cannot process ion trap data acquired in centroid mode and requires profile data. For the Xtract algorithm, the application cannot process ion trap data because Xtract does not support this type of data.

4. (For experiments with multiple loaded raw data files only) In the Result Format for Multiple Raw Files area, select either option:
   - **Batch Processing** to run each raw data file as a separate experiment.
   - **Multiconsensus** to merge the results from the multiple raw data files together.

     Note: To select the Multiconsensus result format, you can load a maximum of 10 raw data files.

5. (Optional) To run a target sequence matching experiment, in the table in the Sequence area, select the check box for one or more sequences for the experiment.

   IMPORTANT! For Intact Mass Analysis, the protein sequences with a category of Intact Protein and Oligonucleotide sequences with categories of Intact or Deconvolution appears in the table.

6. In the table in the Processing Method area, select the check box for a processing method for the experiment.

   You can select a custom method that you created or one of the provided default methods.

7. (For a sliding windows experiment only) To have the application automatically optimize parameters in a default sliding windows method, select the **Enable Automatic Sliding Window Parameters Values** check box above the table.

   With the check box selected, when you edit the default method, manually process the experiment, or automatically process by using the queue, the application automatically determines the best values for these parameters:
   - In the Sliding Windows Definition area
     - Target Avg Spectrum Width
     - Target Avg Spectrum Offset: Scan Offset and % Offset
   - In the Sliding Windows Merging Parameters area
     - Min. Number of Detected Intervals

The application reads the scan data from the raw data file (for an experiment using only one file) or from the first file (for an experiment using multiple files) to optimize these parameter values, instead of using the default values in the processing method.

IMPORTANT! The application does *not* optimize these parameter values in a custom method.

---

8. (Recommended) Click **Edit Method** to review the method parameter information for the selected method, make adjustments to the deconvolution and other method parameters, and then save the method before processing.

   You can create a new custom method by editing the parameters in an existing method and then saving it to a different name. You cannot overwrite a default method.

   If you are loading multiple raw data files, the application displays the chromatograms and source spectra for up to 10 individual raw data files.

   Note: Thermo Fisher Scientific recommends that you review and update the parameters before you begin processing the experiment.

   For example, to process an experiment using an m/z Range of 400 to 600, regardless of the raw data files used, create a custom processing method with the m/z Range set from 400 to 600, and save this method. After you save the method, the application returns to the Intact Mass Analysis page. When you create a new experiment, select the saved method to run the experiment with the specified range.

9. Do one of the following:

   - Click **Add to Queue.**

     The application uses the parameters in the selected method:

     - To automatically detect chromatographic peaks using auto peak detection.
     - To extract averaged mass spectra.
     - To deconvolve isotopically unresolved or resolved peptides or proteins.
     - To generate a results list.

     In this automatic protein deconvolution mode, you add jobs to a run queue to perform the processing.

     To start processing a new job, the application requires the experiment name, the raw data file or files, the result format selection when you load multiple raw data files, and a processing method. When all requirements are met for processing, the application saves all entered information for the new job and then opens the Queue page.

     Note: If you select a custom method, the chromatogram parameters might not be set correctly. In this case, a warning message informs you that the method might have incorrect chromatogram settings and suggests that you evaluate these settings in manual mode before running the automated mode.

If you select the Batch Processing option for the result format, the application automatically appends a date-and-time stamp to the experiment name for each loaded raw data file. For example, if you load 20 raw data files for the experiment, the application submits to the queue 20 separate jobs, each named *experiment name_date_time.* Each job generates individually processed results.

–or–

- Click **Manual Process.**

  The application automatically transfers you to the Process and Review page where you can perform the following one step at a time:

  – Manually set up the chromatogram.
  – Select the source spectra if needed.
  – Edit the algorithm parameters to deconvolve the spectra.
  – Review the results.

  Use this manual protein deconvolution mode when you want to make changes to determine the optimal settings before saving to a new method.

IMPORTANT! You might select a combination of the Result Format for Multiple Files option and the Source Spectra™ Method option in the processing method that the application does not support. In this case, the Add to Queue or Manual Process button is dimmed. To enable these buttons, select a different Result Format for Multiple Files option, processing method, or Source Spectra™ Method option in the method.

Working in manual mode

When you click Manual Process on the Intact Mass Analysis page, the Process and Review page opens in manual mode. The Chromatogram pane displays the chromatogram plot from each loaded raw data file, the Deconvoluted Spectrum pane is empty, and the Source Spectrum pane is either empty or displays the selected source spectra.

For average over RT deconvolutions, if the Source Spectrum pane is empty, you must select one or more source spectra before you can manually process the experiment. To select a spectrum, either click a single retention time or scan, or select a retention time range or a range of scans on the chromatogram in the Chromatogram pane.

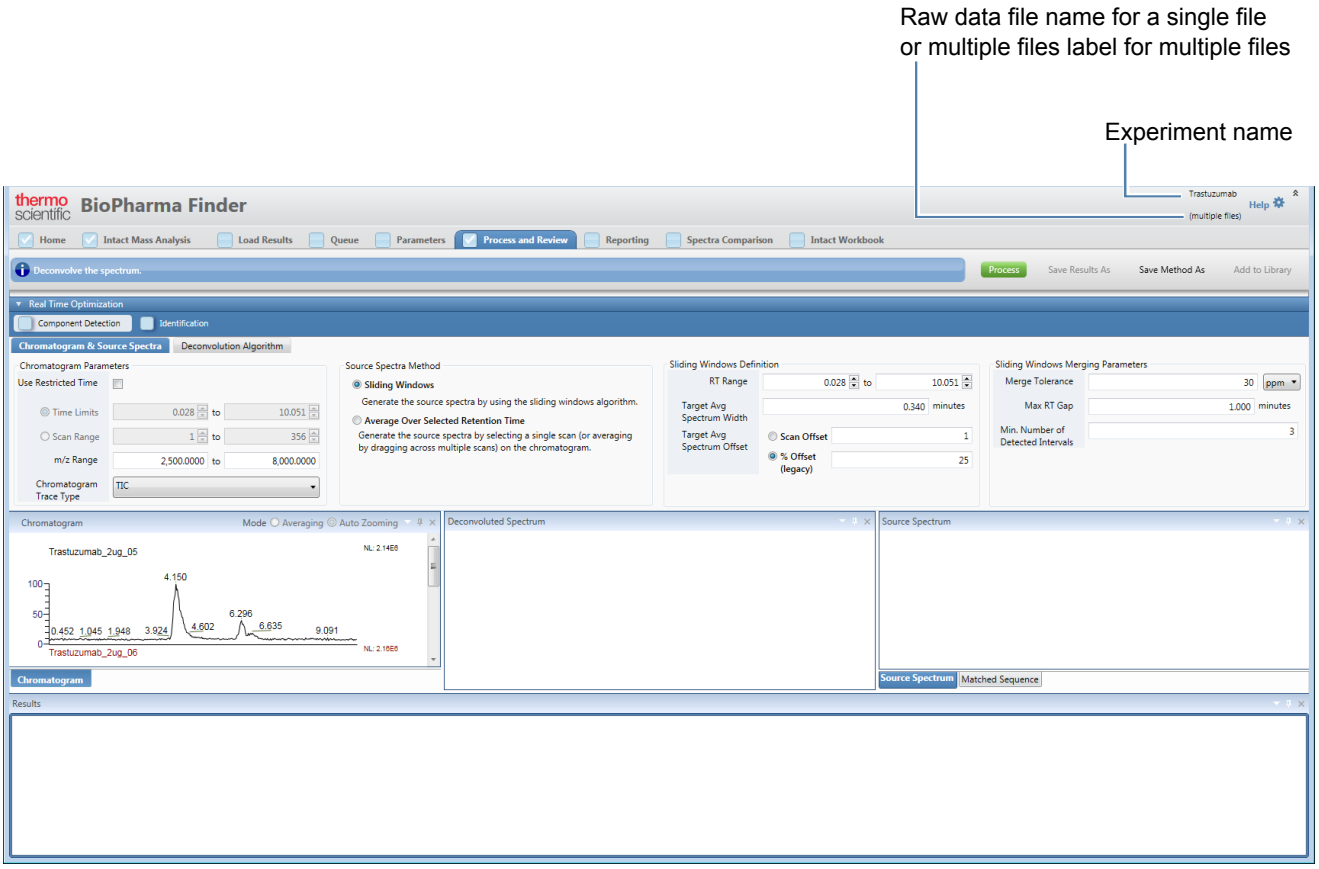


Figure 160 Process and Review Page in manual mode

For more details, see the following topics:

- Manual mode processing (page 348)
- Deconvolving in manual mode (page 349)

Manual mode processing

Use the Process and Review page to deconvolve the selected spectra and view the resulting data to ensure that the results make sense. You can also export the data into an Microsoft™ Excel™ spreadsheet file for use in other applications and copy the chromatograms and all spectra to the Clipboard.

Set up the method parameters for processing with real-time optimization and use the chromatogram to select one or more source spectra, as needed. You can zoom in and out of the chromatogram and spectra views.

IMPORTANT! If an experiment uses the Auto Peak Detection option in the processing method for source spectra generation, you cannot deconvolve the spectrum and process the results in manual mode. You can process this type of experiment only in automatic mode.

Deconvolving in manual mode

To deconvolve the spectra in manual mode

1. (Optional) Adjust the parameters in the Real Time Optimization pane, and then use the Chromatogram and Source Spectrum panes to set up one or more source spectra for deconvolution, as needed.

   You work with these parameters and panes the same way that you set them up in a processing method.

   IMPORTANT! When you are in manual mode, after processing at least once, you can go back to the pages under the Parameters tab to change the method parameters. These changes automatically update on the Process and Review page. However, the BioPharma Finder application does not display the chromatogram and source spectra on the Parameters > Component Detection page, you cannot select the Auto Peak Detection option, and you cannot save the parameter changes using the Save Method page. To save any changes to parameters, use the Save Method As command on the Process and Review page.

2. Click **Process** in the command bar.

   When you generate a deconvoluted spectrum from an isotopically resolved/unresolved intact protein or peptide, the Xtract/ReSpect algorithm transforms a source spectrum into a mass spectrum. The mass spectrum opens in the Deconvoluted Spectrum pane labeled with mass units rather than the mass-to-charge ratio on the *x* axis.

   During processing for a sliding windows deconvolution, a green status bar appears to indicate the real-time percentage of completion. The sliding windows appear and move across each chromatogram. For a multiconsensus sliding windows experiment, the status bar shows the status of each raw data file individually.

   Note: To stop the sliding windows processing, click the **Cancel** icon, . The application does not save the results. To activate the Process button after canceling, change one or more parameter settings on the Process and Review page.

   When you process a sliding windows deconvolution, a warning box similar to the one shown in the following figure might appear if the selected parameters are suboptimal for obtaining results. For multiconsensus experiments, the application reads data from the first loaded raw data file to determine the optimal settings.

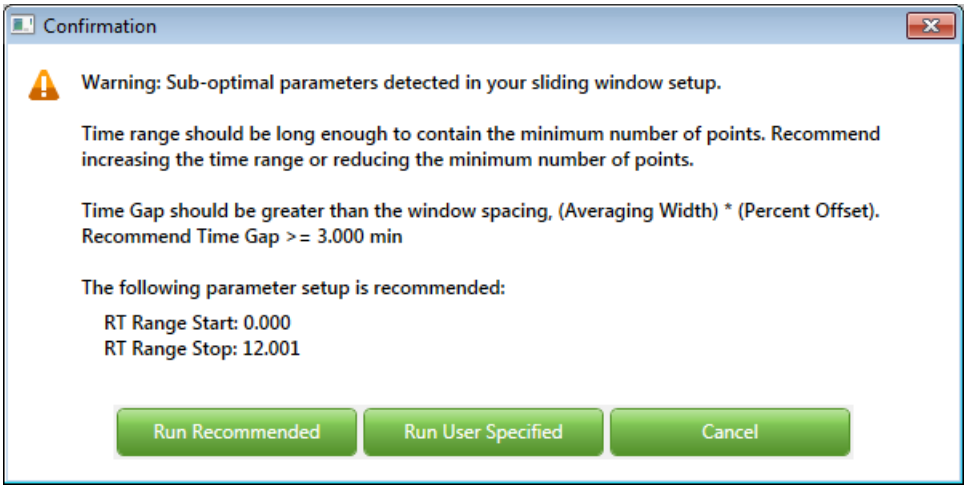


Figure 161 Parameter settings warning box example

If this warning box appears, take one of the following steps:

- Use the recommended parameter settings shown in the box by clicking **Run Recommended**. This setting guarantees that the application will run to completion.
- Use your original parameter settings by clicking **Run User Specified**. This setting cannot guarantee that the application will run to completion.
- Click **Cancel** and enter other parameter settings. Then, review the processed results.

3. View the results from the intact protein processing.

4. When you are satisfied with the parameter settings, the results, or both, save them as needed:
   - Click **Save Method As** in the command bar. In the dialog box, enter a new method name (or retain the current name to overwrite the current method parameters), a description (optional), and then click **OK**.

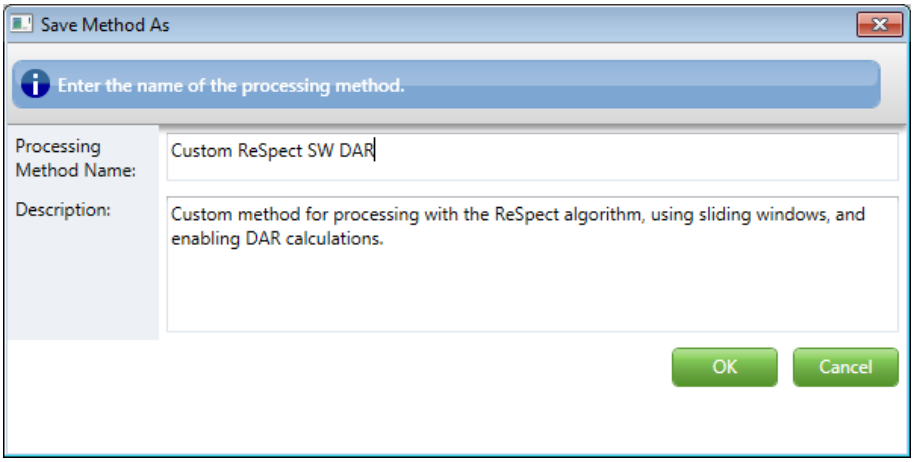


Figure 162 Save Method As dialog box

Note: You cannot overwrite a default method. If you change the parameters in a default method, save the method to a new name.

Use only alphanumeric, space, underscore "_", and period "." characters in the method name.

You have the option to use this saved method for future automatic deconvolution processing.

- Click **Save Results As** in the command bar. This saves the processed results to a database that contains both the method parameter settings that you applied to the loaded raw data files and the results of the deconvolution. In the dialog box, enter a new experiment name (or retain the current name to overwrite the current experiment), a description (optional), and then click **OK**.

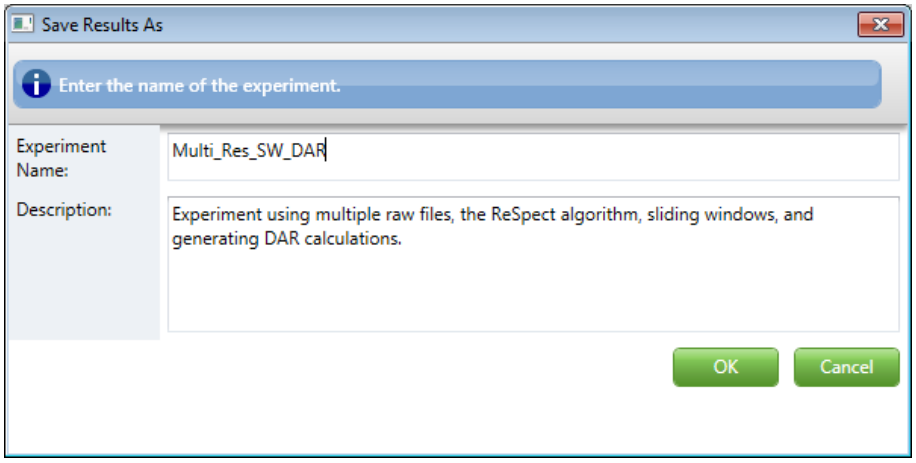


Figure 163 Save Results As dialog box

Note: If an existing experiment is already completed or is canceled, you can overwrite it by using the same name. You cannot overwrite an experiment that is currently submitted to the run queue or is actively processing or canceling. For details, see.

Use only alphanumeric, space, underscore "_", and period "." characters in the experiment name.

The entry of new names saves your changes to new areas of the database to ensure that you do not overwrite the previous results and method parameters.

You can open the saved results for future viewing from the Load Results page.

Otherwise, if you are not satisfied, return to step 1 on page 349.

18 Working with an Intact Mass processing method

The BioPharma Finder application provides several default processing methods for Intact Mass Analysis. If needed, you can use the editing wizard to edit the parameters in one of these methods and save them to a new file to create a custom method for your experiment.

Using a processing method for intact mass analysis

To create a new method or edit a current method

1. On the Home page, click **Intact Mass Analysis** in the left pane or below the splash graphic.

   The Intact Mass Analysis page opens.

2. (Optional) Enter the experiment name, load the raw data file or files and then enter the conditions if needed, choose a result format if you load multiple raw data files, and select one or more protein sequences for target sequence matching.

   Note: If you load one raw data file or run a batch experiment, the application derives the default narrow *m/z* range based on information retrieved from one file.

   For a multiconsensus experiment with multiple raw data files, by default, the application uses the *m/z* range from the first loaded file. The application determines the other component detection and identification parameters for the experiment and displays these parameters on the pages under the Parameters tab.

3. In the Processing Method area, select a processing method in the table to edit, and then click **Edit Method**.

   Note: To create a custom method, modify the parameters in a default method, and then save them to a new method using a different name.

The editing wizard opens the Component Detection page under the Parameters tab, showing the component detection parameters in the current method. The method name appears in the upper right corner of the page.

4. Use the editing wizard on the Component Detection and Identification pages to specify the parameters for the following:
   - For the Xtract algorithm: average over RT deconvolution, sliding windows deconvolution, auto peak detection, or target sequence matching.
   - For the ReSpect algorithm: average over RT deconvolution, sliding windows deconvolution, auto peak detection, target sequence matching, deconvolution with ion trap data, or the structural analysis of proteins.

When you are done editing the parameters on each of these pages, click **Next** in the command bar to go to the Report page. Set the reporting parameters and then click **Next** to go to the Save Method page to save all of the modified parameters to a method. You can then select that method to use for processing another experiment.

For more details, see the following topics:

- Batch and multiconsensus result formats (page 336)
- Left side of the component detection page (page 355)
- Right side of the component detection page (page 359)
- Saving a processing method (page 144)

Editing component detection parameters for intact mass analysis

When you want to create a new processing method or edit an existing method for Intact Mass Analysis, go to first page of the editing wizard, the Component Detection page. The parameters on this page vary depending on the chosen option for source spectra generation and the selected deconvolution algorithm: Xtract or ReSpect.

Before editing the parameters on the Component Detection page, see the following topics:

- Opening the component detection page (page 355)
- Left side of the component detection page (page 355)
- Right side of the component detection page (page 359)
- Editing the component detection page (page 363)

For more information regarding various parameters and commands, see the following topics:

- Chromatogram parameters area parameters (page 365)
- Source Spectra method area parameters (page 367)
- Xtract deconvolution parameters (page 370)
- ReSpect deconvolution parameters (page 374)
- Component detection page commands (page 380)

Opening the component detection page

To open the Component Detection page

1. (Optional) On the Intact Mass Analysis page, enter an experiment name, load the raw data files, choose a result format if you load multiple raw data files, and select one or more protein sequences

2. Select a method and then click **Edit Method**.

   The Component Detection page opens showing several areas on the left and two panes, Chromatogram and Source Spectrum, on the right. If you navigate away from this page and want to return to it, click the **Parameters** tab in the navigation bar, and then click the **Component Detection** subtab.

For more details, see the following topics:

- Left side of the component detection page (page 355)
- Right side of the component detection page (page 359)

Left side of the component detection page

An example of the left side of the Component Detection page for Intact Mass Analysis shows the parameters that are available for the Chromatogram pane, Average Over Selected Retention Time source spectra option, and Xtract algorithm.

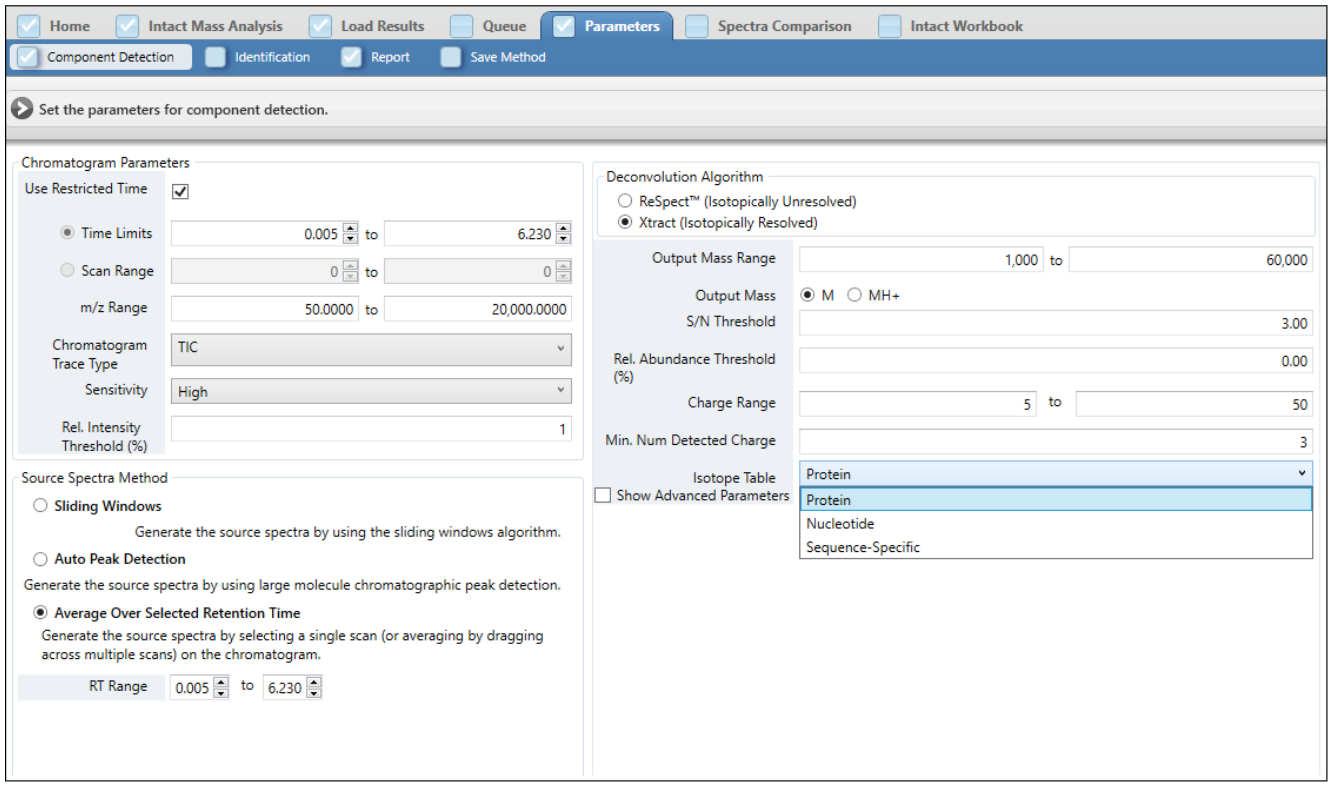


Figure 164 Component Detection page with parameters for the Chromatogram pane, Average Over Selected Retention Time option, and Xtract algorithm

Another example of the left side of the Component Detection page shows the parameters that are available for the Chromatogram pane, Sliding Windows Source Spectra Method option, and ReSpect algorithm.

Component Detection page with parameters for the Chromatogram pane, Average over Selected Retention Time option, and Xtract algorithm

- Output Mass Range
- Output Mass, that contains radio button options for M and MH+
- S/N Threshhold
- Relative Abundance Threshold (%)
- Charge Range
- Min Num Detected Charge
- Isotope Table, that contains the drop down with three options for, Protein, Nucleotide and Sequence Specific for Xtract Algorithm and Sequence Specific option is not available for ReSpect algorithm.
- **Show Advanced Parameters** checkbox

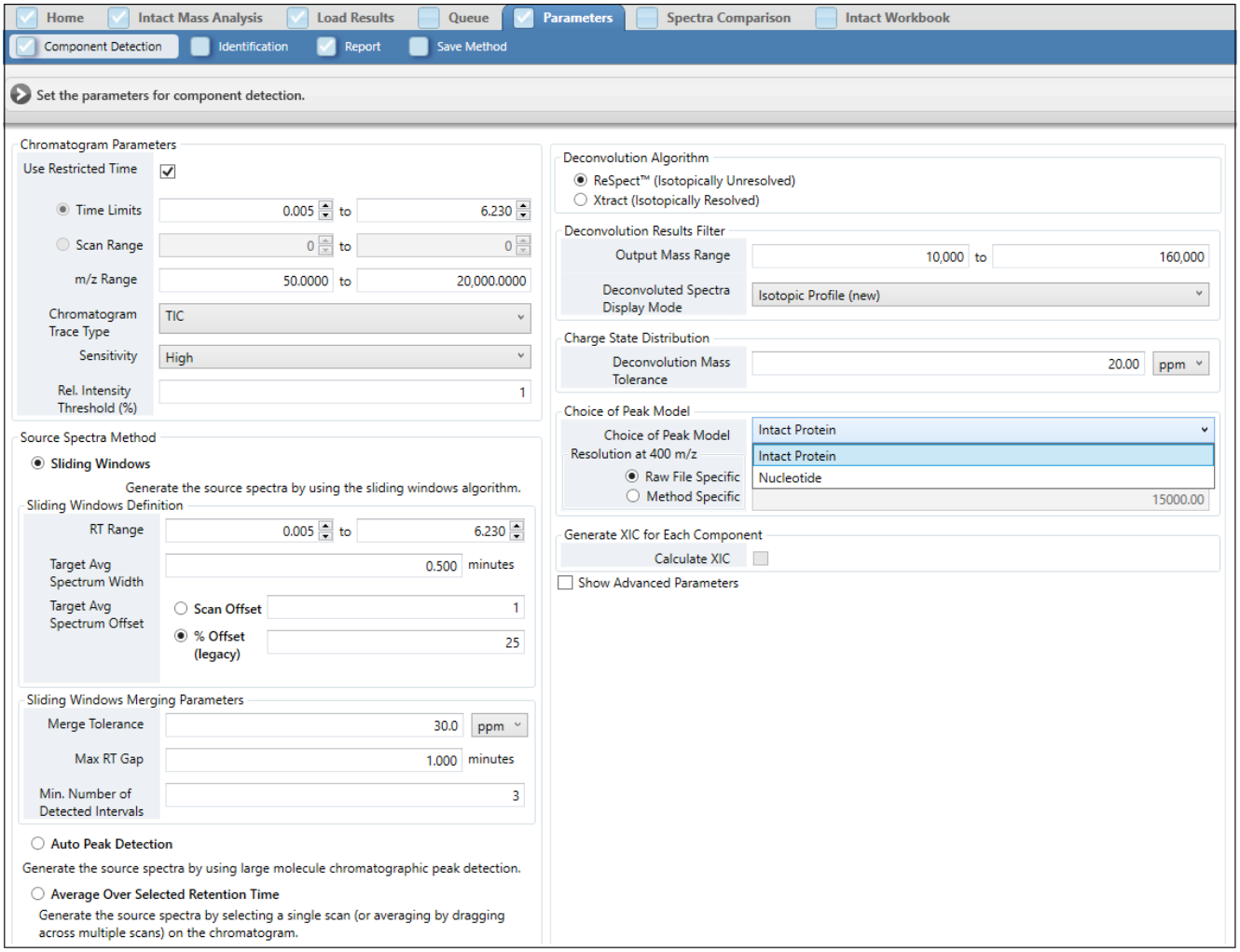


Figure 165 Component Detection page with parameters for the Chromatogram pane, Sliding Windows option, and ReSpect algorithm

Component Detection page with parameters for the Chromatogram pane, Sliding Windows option, and ReSpect algorithm

- **Chromatogram Parameters** area: Displays the parameters that determine the appearance of the chromatogram in the Chromatogram pane.
- **Source Spectra Method** area: Displays the methods for source spectra generation and the corresponding parameters, Sliding Windows, Auto Peak and Averge Over Selected Retention Time radio buttons

  The Sliding Windows area: Displays the Sliding Windows Definition and the Sliding Windows Merging Parameters.

  The Sliding Windows Definition area: Displays the RT Range, Target Average Spectrum Width

  The Sliding Windows Merging Parameters are: Displays the Merge Tolerance, the Max RT Gap and the Minumum Number of Detected Intervals.
- The **Auto Peak Detection** area: Displays the methods for source spectra generation by using large molecule chomatographic peak detection.

  This area contains the Sensitivity and Relative Intensity Threshold (%). fields.
- The Average Over Selected Retention Time are: Displays the mehtods for source spectra generation by selecting a single scan on the chromatogram.

  This area contains the RT Range selection option field
- **Deconvolution Algorithm** area: Displays the parameters for one of two deconvolution algorithms, Xtract and ReSpect.

  Note: You can edit advanced options by selecting the **Show Advanced Parameters** check box. However, these advanced parameters are hidden by default and typically need no modifications.
- **Deconvolution Results Filter** area: Displays the Output Mass Range and the Deconvoluted Spectra Display Mode. The latter has a drop down of two choices Mass Proability Distribution Profile (legacy) and Isotopic Profile (new)
- **Charge State Distribution** area: Displays the Deconvolution Mass Tolerance
- **Choice of Peak Model** area: Displays the Resolution at two options, Raw File Specific and Method Specific. Here you can select from two options, Intact Protein and Nucleotide.
- **Generic XIC for Each Component** area: Contains the check box for Calculate XIC.
- **Show Advanced Parameters** checkbox

  Checking the Show Advanced Parameters box, it displays the following fields:
  - Calculate XIC checkbox
  - Fit Factor (%)
  - Remainder Threshold
  - Consider Over laps checkbox
  - Resolution at 400 m/z, displays options for Raw File Specific and Method Specific radio buttons
  - Negative Charge check box
  - Charge Carrier, displays radio button options for N+, K+ Na+ and Custom.

- Maximum Intensity
- Unexpected Intensity Error

For more details, see the following topics:

- Chromatogram parameters area parameters (page 365)
- Source Spectra method area parameters (page 367)
- Xtract deconvolution parameters (page 370)
- ReSpect deconvolution parameters (page 374)

Right side of the component detection page

The following figure shows the right side of the Component Detection page for Intact Mass Analysis. In this example, the Chromatogram and Source Spectrum panes for each loaded raw data file reflect the Average Over Selected Retention Time option for source spectra generation. The application displays a red box to indicate the selected retention time range. A tab appears at the bottom of the panes for each raw data file loaded into the experiment. The application displays a maximum of ten raw data file tabs. Click a tab to see the chromatogram and source spectrum for a particular file. Scroll to the right to see more tabs as needed.

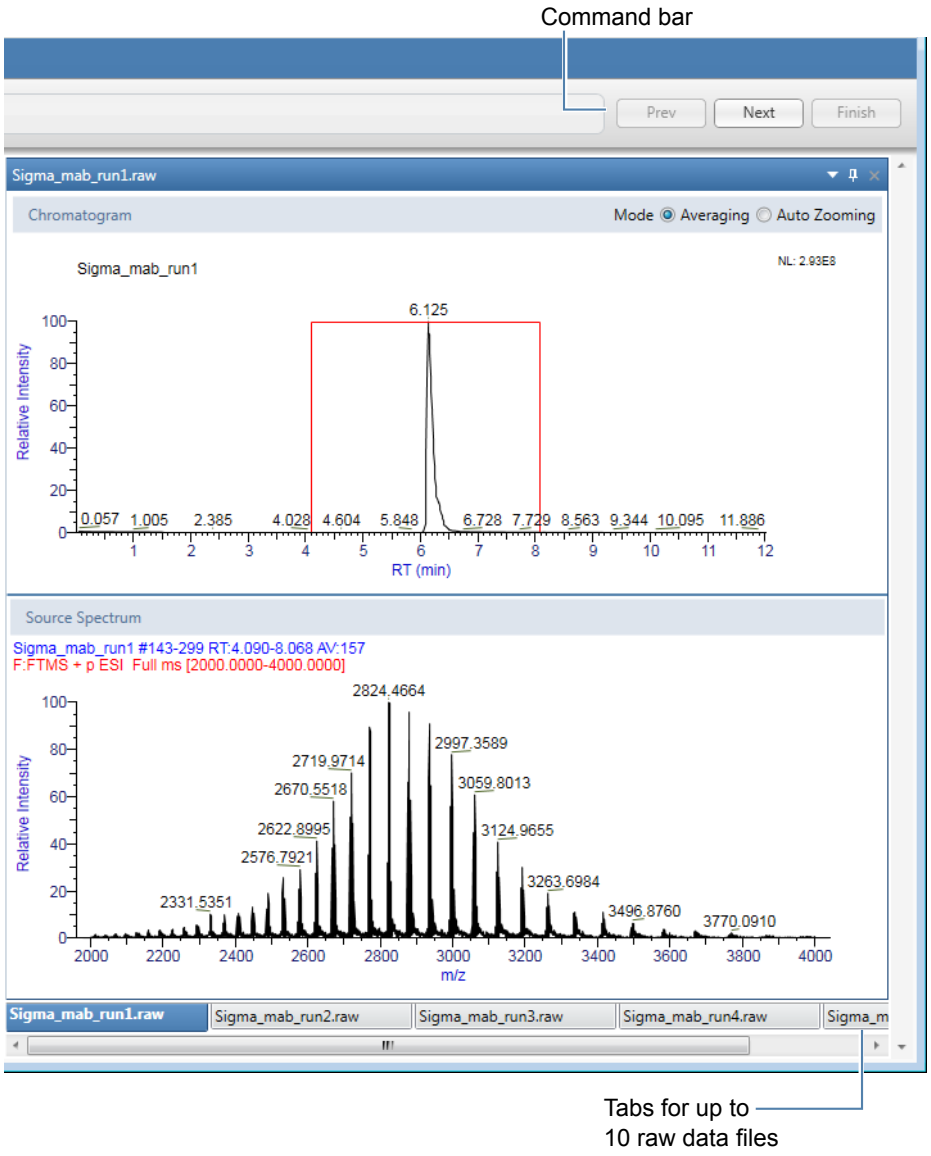


Figure 166 Component Detection page (right side) with two panes and multiple tabs

The following figure shows the Chromatogram pane when you use the Auto Peak Detection method for source spectra generation. The application displays the auto-detected peaks in blue.

Note: You can use this method for single-file or batch experiments only, not for multiconsensus experiments.

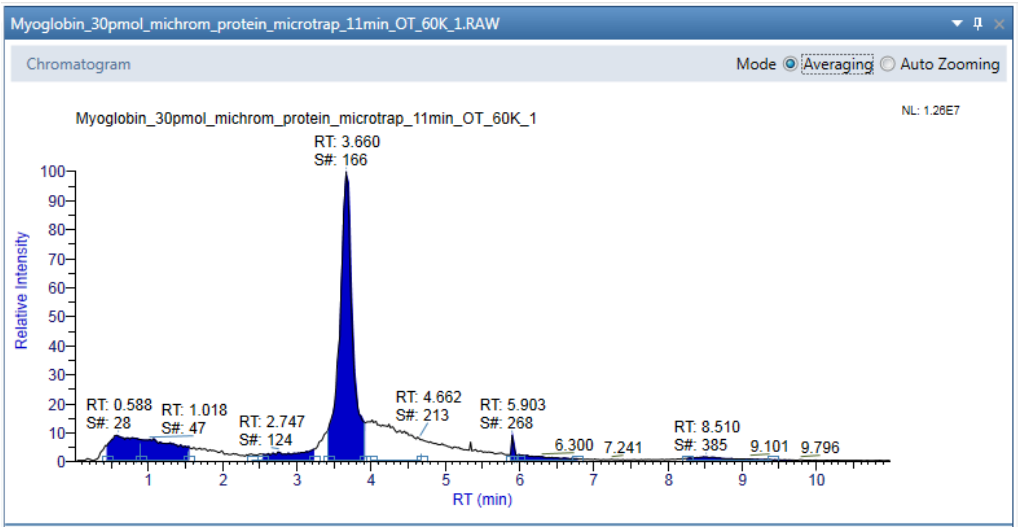


Figure 167 Chromatogram pane with the auto-detected peaks in blue

Descriptions of the two panes on the right side of the page follow:

- **Chromatogram** pane: Displays the chromatogram of the data in each loaded raw data file.

  Note: If you did not load a raw data file before you started editing the method, the Chromatogram pane is empty.

  Use the parameters in the Chromatogram Parameters area to adjust the chromatogram displayed in the Chromatogram pane.

  A chromatogram plot shows the intensities of one or more masses as a function of time. By default, the Chromatogram pane displays a total ion current (TIC) chromatogram, as shown in the previous figures. The chromatogram is fully magnified. You can use the zooming mode in this pane to enlarge a region of the spectrum or use the averaging mode to generate a new source spectrum by selecting a new retention time range.

  The pane displays the chromatogram based on the parameters in the Chromatogram Parameters area. Other features of the chromatogram depend on the Source Spectra Method option set in the processing method:

  - For the Auto Peak Detection option, the application uses the Parameterless Peak Detection (PPD) algorithm as the internal peak selection mechanism to select the chromatographic peaks and displays the detected peaks in blue.
  - For the Average Over Selected Retention Time option, the RT Range in the default methods is from 0.000 to 0.000. You specify the appropriate RT Range values and a red box appears on the chromatogram for that range.

  Use the Chromatogram pane to select the best possible spectrum for the target protein for deconvolution.

The Mode options in the upper right corner of the pane determine the function to apply when you drag the cursor over an area of the Chromatogram pane.

- Averaging: The application averages all the scans in the selected area to generate the source spectrum and displays it in the Source Spectrum pane.
- Auto Zooming: The application enlarges the selected area without changing the view displayed in the Source Spectrum pane.

The header in the Chromatogram pane displays the following information:

- The name of the raw data file, for example, Myoglobin_30pmol_microm_protein_microtrap_11min_OT_60K_1.
- NL: The intensity of the most abundant peak in the entire LC/MS run, for example, 8.51E7.

- **Source Spectrum** pane: Displays the spectrum to deconvolve a region of the chromatogram, either single-scan or averaged.

Note: If you did not load a raw data file before you started editing the method, the Source Spectrum pane is empty.

If the method uses the Average Over Selected Retention Time option for source spectra generation, select the best possible spectrum for the target protein for deconvolution from the Chromatogram pane.

The header in the Source Spectrum pane displays the following information:

- Name of the raw data file, for example, Myoglobin_30pmol_microm_protein_microtrap_11min_OT_60K_1.
- Scan number or range of scan numbers, for example, #149–187.
- RT: Retention time, which is the time in the mass chromatogram when any particular precursor ion is observed, for example, 3.30–4.08.
- NL (for single scans): The intensity of the most abundant peak in the entire LC/MS run, for example, 3.83E5.

  –or–

  AV (for multiple scans): The number of spectra that were averaged to create the source spectrum, for example, 39.
- F: The scan filter used during the LC/MS run, for example, FTMS + p ESI Full ms [300.00–2000.00]. The scan filter indicates the type of mass analyzer used to acquire the data in the raw data file and the ionization technique used. If this field is blank, no scan filter was used.

For more details, see the following topics:

- Source Spectra method area parameters (page 367)
- Chromatographic peak detection and spectral peak modeling (page 336)
- RT Range (page 367)
- To edit the parameters, chromatogram, and source spectra (page 363)

Editing the component detection page

Use the various areas and panes on the Component Detection page to edit your processing method.

To edit the parameters, chromatogram, and source spectra

1. Enter the appropriate parameter values in the areas on the left side of the Component Detection page.

2. Adjust or copy the view in the Chromatogram pane as necessary.

   Tip: Use the parameters in the Chromatogram Parameters area to adjust the chromatogram from a raw data file that is displayed in the Chromatogram pane.

   For the Auto Peak Detection source spectra option, if there is no obvious chromatographic peak, you can find it by changing the limits of the m/z Range parameter.

   The same chromatogram also appears on the Process and Review page for deconvolution in manual mode.

3. (For the Average Over Selected Retention Time option) Create a source spectrum by editing the RT Range parameter or by doing one of the following in the Chromatogram pane:

   - For a single scan: Use the red cross-shaped cursor to select a single scan on the chromatogram. The Source Spectrum pane displays the associated single-scan mass spectrum at that time point.

     You can use the left and right arrow keys to move to the previous or next time point in the chromatogram. The Source Spectrum pane automatically updates.
   - For multiple scans: Select a region of the chromatogram to display the averaged spectrum for all the scans within that region. To select this region, select the **Averaging** option in the Mode area of the Chromatogram pane. Drag the red cross-shaped cursor across the area of interest.

     The horizontal line of this cursor aids in assessing peak height. The application calculates an average spectrum for the selected interval and displays it in the Source Spectrum pane.

     The averaging method is better suited to complex data than the single-scan method. Averaging spectra produces higher signal-to-noise ratios, so higher-quality spectra are highly recommended for optimal deconvolution results.

   Tip: You can enter the chromatogram parameters, adjust the view of the chromatogram, and select the source spectrum in Qual Browser in the Xcalibur™ data system. Then, right-click and choose **Export ▸ Write to RAW File** to export the raw data file so that you can import it into the BioPharma Finder application.

   Note: For the Sliding Windows or Auto Peak Detection option, you can select either the Averaging or Auto Zooming option for the Mode to update the chromatogram and spectrum plots, but not to select the spectra to deconvolve.

For the Sliding Windows option, the application deconvolves the portion of the spectrum in the raw data file specified by the RT Range parameter and ignores the spectrum in the Source Spectrum pane.

For the Auto Peak Detection option, the application uses the Parameterless Peak Detection (PPD) algorithm to detect the peaks and ignores the spectrum in the Source Spectrum pane. You can use this option only for single-file or batch experiments in automatic mode.

4. Adjust or copy the view in the Source Spectrum pane as necessary.

   If you select the Average Over Selected Retention Time option for source spectra generation, the Source Spectrum pane shows the actual spectrum, either single-scan or averaged, to be deconvolved. It displays apex information for major peaks and *m/z* information for deconvolved components. It also shows peak apex information as a marker, along with an accompanying label that describes the *m/z* value for the most abundant peak from the spectrum at that retention time.

   In single-scan processing mode, the most abundant *m/z* for a component agrees with the *m/z* shown for the corresponding peak in the source spectrum. In averaged-scan processing mode, the two values might be different because of the way the application displays averaged spectra. This difference is small—approximately 0.001.

   The Xtract algorithm can deconvolve centroid spectra and profile spectra. The ReSpect algorithm can deconvolve only profile spectra. The application automatically chooses the appropriate type of spectrum. The Source Spectrum pane displays profile information if it is available and centroid information if the profile information is not. The two types of information differ as follows:

   - Centroid data represent mass spectral peaks using two parameters: the centroid (the weighted center of mass) and the intensity (the normalized area of the peak). The data are displayed in a bar graph of relative intensity versus *m/z*.
   - Profile data represent the entire spectrum as a succession of points, in *m/z*, and relative intensity. The data are displayed in a line graph of relative intensity versus *m/z*.

   The source spectrum also appears on the Process and Review page for deconvolution in manual mode.

   Note: Unlike adjustments in the Chromatogram pane, which you use to select a source spectrum for processing, adjustments in the Source Spectrum pane do not affect the spectrum that the application deconvolves. In particular, they do not change the *m/z* range that the deconvolution algorithm uses.

5. When you are done editing the parameters on the Component Detection page, click **Next** in the command bar to advance to the Identification page.

For more details, see the following topics:

- Chromatogram parameters area parameters (page 365)
- Source Spectra method area parameters (page 367)
- Xtract deconvolution parameters (page 370)
- ReSpect deconvolution parameters (page 374)
- Using basic chromatogram functions (page 661)
- Using copy and paste functions (page 666)
- Working in manual mode (page 347)
- Viewing the chromatograms for intact mass analysis (page 427)
- m/z Range (page 366) parameter
- RT Range (page 367) parameter
- Viewing the source Spectra for intact mass analysis (page 437)

Chromatogram parameters area parameters

The following table describes the parameters in the Chromatogram Parameters area on the Component Detection page.

Table 45 Chromatogram Parameters area on the Component Detection page

Parameter	Description
Use Restricted Time	Select to zoom the part of the chromatogram that you define with the Time Limits/Scan Range parameters. When cleared (default), the application displays the entire chromatogram.
Time Limits	(Enabled only when you select the Use Restricted Time check box and a chromatogram is visible in the Chromatogram pane) Specifies the beginning and end retention times of the range of the chromatogram that you want to view. The default values for both limits depend on the data in the raw data file. **Note:** The values that you enter link to the values that appear in the Scan Range boxes and update both sets of parameters.
Scan Range	(Enabled only when you select the Use Restricted Time check box and a chromatogram is visible in the Chromatogram pane) Specifies the beginning and end scans of the range of the chromatogram that you want to view. The default values for both limits depend on the data in the raw data file. **Note:** The values that you enter link to the values that appear in the Time Limits boxes and update both sets of parameters.

Parameter	Description
m/z Range	Specifies the range of *m/z* values used as input to the deconvolution. You can use this parameter to select a narrower range. The deconvolution algorithm ignores the portions of the spectrum outside this range. You might want to create a narrower range because the intact proteins are usually at a higher *m/z* value, and any small molecule contaminants and background are below 600 *m/z*. Instead of creating a TIC using the full *m/z* range, the deconvolution algorithm calculates a TIC by summing those protein peaks within the narrower *m/z* range. The resulting TIC is basically an XIC. The deconvolution algorithm redraws a BPC with the most intense peak within the selected *m/z* range rather than the whole spectrum. In both cases, when you select the *m/z* range around the protein signals of interest, any peaks for the background components generally disappear from the chromatogram, and the only peak left is for the target proteins. **Note:** Exercise caution in specifying the width of the *m/z* range. Using an *m/z* range that is too large might cause the deconvolution algorithm to incorporate weak, noisy, and poorly characterized peaks into some of its fittings, with a corresponding loss in the quality of the results. In general, try to restrict the *m/z* range to the more intense regions of the spectrum.
Chromatogram Trace Type	Determines the type of chromatogram displayed in the Chromatogram pane: • TIC: Displays a total ion current chromatogram, which shows the summed intensity across the entire range of masses being detected at every point in the analysis. The range is typically several hundred mass-to-charge units or more. In complex samples, the TIC chromatogram often provides limited information because multiple analytes elute simultaneously, obscuring individual species. A TIC in combination with a narrow *m/z* range is effectively an XIC. • BPC: Displays a base peak chromatogram, which shows only the most intense peak in each spectrum. The BPC represents the intensity of the most intense peak at every point in the analysis. BPCs for each spectrum often have a cleaner look and are therefore more informative than TICs because the background is reduced by focusing on a single analyte at every point. For intact protein spectra, the TIC often looks better. The BPC is usually better for smaller molecules where the entire signal exists in a single charge state.
Sensitivity	Specifies the sensitivity with which the chromatographic peak detector identifies peaks. • Low: Directs the chromatographic peak detector to perform one pass at the default sensitivity. • High: Directs the chromatographic peak detector to perform a second pass at higher sensitivity—that is, with a slightly narrower width threshold—to identify narrow shoulders or noise-like peaks that the peak detector might have missed in the first pass. This option increases sensitivity at the cost of a potential increase in the false positive rate.
Rel. Intensity Threshold (%)	Sets an intensity threshold for peaks in the chromatogram, as a percentage. The application ignores peaks with relative intensities below this threshold. This parameter is different from the Rel. Abundance Threshold (%) (Xtract) or Rel. Abundance Threshold (%) (ReSpect) parameter, which sets a lower intensity for signals in the spectrum, *not* in the chromatogram.

For more details, see the following topics:

- Left side of the component detection page (page 355)
- Rel. Abundance Threshold (%) (page 371) parameter (Xtract)
- Rel. Abundance Threshold (%) (page 377) parameter (ReSpect)

Source Spectra method area parameters

The following table describes the parameters in the Source Spectra Method area on the Component Detection page.

Table 46 Source Spectra Method area on the Component Detection page

Parameter	Description
Source Spectra Method	
	Displays the three methods for generating the source spectra: • Sliding Windows: The application averages spectra over a succession of sliding windows in the retention time range specified by the RT Range parameter. It deconvolves each of these averaged spectra and then merges similar masses to identify components. • Auto Peak Detection: The application generates the source spectra using the Parameterless Peak Detection (PPD) algorithm for auto-peak detection of large molecules. You can use this source spectra method only in automatic mode. • Average Over Selected Retention Time: You select the source spectrum for an average over RT deconvolution. From the chromatogram, you can select either the *single-scan* source spectrum by picking a particular retention time/scan or the *averaged* source spectrum by dragging across a range of retention times/scans. Instead of dragging in the chromatogram, you can also enter the RT Range values directly for this range. In the default methods, the RT Range is from 0.000 to 0.000. You must specify the appropriate range values before processing.
Parameters visible for the Sliding Windows option	
Sliding Windows Definition area	
RT Range	Displays the retention time range that the sliding windows deconvolution applies to, in minutes. By default, these values automatically correspond to the values in the Time Limits boxes in the Chromatogram Parameters area. You can change these values to limit the range for sliding windows deconvolution. Afterwards, if you change the Time Limits values, the RT Range values reset automatically to match the Time Limits values. **Note:** When you load multiple raw data files for the experiment, if the RT Range changes for any raw data file, the change applies to all of the loaded raw data files.

Parameter	Description
Target Avg Spectrum Width	Specifies the retention time, or the width, of the sliding window, in minutes. This value is also called the averaging width, determined by calculating the auto-correlation function of the chromatogram to determine the characteristic scale width of peaks. Using the averaging width has these advantages over attempting to identify and examine a subset of the chromatographic peaks: it is significantly more robust and objective, and is less sensitive to the parameter choices, the baseline, and the peculiarities of individual features in the chromatogram. Reducing this value improves time resolution but reduces execution speed and possibly sensitivity. Increasing this value increases execution speed but reduces time resolution and might increase sensitivity. You can achieve the best results when the window width is between one-quarter and twice the width of the characteristic peaks in the spectrum. For most usage, the optimum value might be half the width of the characteristic peaks. For example, if those peaks have a width of one minute, the optimum width would be 0.5 minutes. If you select the Enable Automatic Sliding Window Parameters Values check box above the Processing Method table before editing the method parameters or before processing, this parameter value is optimized based on the scan data from a loaded raw data file. You can edit the value as needed. However, each time you change the RT Range parameter or select the Target Avg Spectrum Offset—Scan Offset option, the application automatically resets this parameter to the optimized value.
Target Avg Spectrum Offset	Specifies the offset between successive sliding windows as a number of scans or as a percentage value. You can select from these two options: • Scan Offset This mode offsets each window from its predecessor by the user-specified number of scans. A zero offset means that successive windows all occur at the same time, but you cannot set this parameter to zero. An offset of *n* means that each window begins *n* scans after the beginning of its predecessor. If you select the Enable Automatic Sliding Window Parameters Values check box above the Processing Method table before editing the method parameters or before processing, this parameter value is set to 1 or the lowest value that ensures the number of windows remains less than 3000. You can edit the value as needed. • % Offset This mode offsets each window from its predecessor by the user-specified percentage of the window width. A 0% offset means that successive windows all occur at the same time, but you cannot set this parameter to 0%. An offset of 30% means that each window begins 30% after the beginning of its predecessor and overlaps the last 70%. An offset of 100% means that successive windows are adjacent with no overlap. If you select the Enable Automatic Sliding Window Parameters Values check box above the Processing Method table before editing the method parameters or before processing, this parameter value is optimized based on the scan data from a loaded raw data file. You can edit the value as needed. **Note:** In general, the product of the Target Avg Spectrum Width value and the % Offset value should be comparable to or less than the width of the features of interest in the chromatogram. Reducing the offset produces better resolution but a slower execution speed. However, values less than 25% can be wasteful; 25% is a good default.

Parameter	Description
Merging Parameters area	
Merge Tolerance	Determines how close two components in successive sliding windows must be in mass for the application to identify them as a single component. A value that is too high might result in merging components that should remain separate. A value that is too low might result in false positives when components remain separate that should have merged. Select the unit for this parameter: • ppm: Specifies the merge tolerance in parts per million. • Da: Specifies the merge tolerance in daltons. The best value for this parameter remains to be determined, but tests suggest the following: • For Xtract deconvolution, 10 ppm might be a good starting point. • For ReSpect deconvolution, 20 ppm might be a good starting point when you use the Default ReSpect method and 30 ppm when you use the Default Native method.
Max RT Gap	Specifies the maximum allowed separation in retention time between two successive individual members of a merged component identified by the sliding windows algorithm, in minutes. If the separation exceeds this value, the application divides the candidate component into two merged components separated by a gap in retention time. As with the Merge Tolerance parameter, a value that is too high for this parameter might result in merging components that should remain separate. A value that is too low might result in false positives when components remain separate that should have merged. This parameter should be comparable to or slightly less than the expected separation in retention time between distinct components with the same mass.
Min. Number of Detected Intervals	Specifies the minimum number of sliding window intervals that a component must appear in for the application to consider the component valid. A value that is too low might allow noise peaks to appear as false positives. A value that is too high might result in legitimate components being discarded. Set this parameter to a value large enough to exclude results that are implausibly narrow in retention time but small enough to include results of realistic duration. Values in the range of 3 through 8 generally give good results. A good approach is to use whichever is larger: 3 or the minimum number of windows that can fit into a peak. If you select the Enable Automatic Sliding Window Parameters Values check box above the Processing Method table before editing the method parameters or before processing, the application automatically sets this parameter to an optimized value by reading the scan data from a loaded raw file. You can edit the value as needed. However, each time you reselect the Target Avg Spectrum Offset—Scan Offset option, the application resets this parameter to the optimized value.

Parameter	Description
Parameters visible for the Auto Peak Detection option **The Sensitivity and Rel. Intensity Threshold (%) parameters in the Chromatogram Parameters area control the same values displayed for this option.**	
Parameter visible for the Average Over Selected Retention Time option	
RT Range	Displays the retention time range that the average over RT deconvolution applies to, in minutes. By default, these values are from 0.000 to 0.000. To select the source spectrum, you can enter the appropriate values or change the values by averaging an area on the chromatogram display. Afterwards, if you change the Time Limits range, the RT Range values do not change, unless any portion of the RT range is outside of the current Time Limits range. In this case, the RT Range values reset automatically to fit inside the Time Limits range. **Note:** When you load multiple raw data files for the experiment, if the RT Range changes for any raw data file, the change applies to all of the loaded raw data files.

For more details, see the following topics:

- Left side of the component detection page (page 355)
- Sliding Windows deconvolution (page 334)
- Manual and automatic modes (page 333)
- Chromatogram parameters area parameters (page 365)
- Recommended values for sliding Windows deconvolution parameters (page 396)
- To edit the parameters, chromatogram, and source spectra (page 363)

Xtract deconvolution parameters

The following table describes the parameters for the Xtract deconvolution algorithm on the Component Detection page.

Table 47 Xtract parameters on the Component Detection page

Parameter	Description
Deconvolution Algorithm	Select the Xtract option for the deconvolution algorithm used for processing.
Main Parameters (Xtract) **These parameters might change often. They also appear on the Process and Review page for real-time optimization.**	
Output Mass Range	Specifies the range for the displayed masses on the *x* axis of the plot in the Deconvoluted Spectrum pane.

Parameter	Description
Output Mass	Determines whether the Xtract algorithm returns a single peak at either the monoisotopic mass or the monoisotopic MH+ mass for each of the detected components. • M: Specifies that the results file contain a single peak for the monoisotopic mass for each of the detected components. This option generates masses without adducts. • MH+: Specifies that the results file contains a monoisotopic MH+ mass for each of the detected components. This option generates masses with adducts.
S/N Threshold	Specifies a signal-to-noise (S/N) threshold, *x*, above which the Xtract algorithm considers a measured peak to be a real (accepted) peak. The Xtract algorithm ignores peaks below this threshold. Any spectral peak must be *x* times the intensity of the calculated noise for that spectrum before the Xtract algorithm considers it.
Rel. Abundance Threshold (%)	Specifies a threshold below which the application filters out data for data reporting. This option sets a relative threshold as a percentage of the most abundant component in the spectrum. The most abundant peak in the deconvoluted spectrum has a relative abundance of 100 percent, and all other peaks are calculated relative to that one. In the Results table on the Process and Review page, the application shows only those components that are greater than or equal to this relative abundance threshold in the deconvoluted spectrum. For example, if the highest peak has an absolute abundance of 1000 and the relative abundance threshold is 1 percent, the application filters out all peaks below an absolute abundance of 10. For this value, 0% displays all results, and 100% displays only the most abundant component.
Charge Range	Specifies the charge state range to be deconvolved, from the lowest charge state to the highest. For example, if you set this parameter range from 2 through 5, the Xtract algorithm considers only charge states 2 through 5 for deconvolution. It ignores charge states 1 and higher than 6.
Min. Num Detected Charge	Specifies the minimum number of charge states required to produce a component. No components with less than this minimum number appear in the deconvoluted spectrum. This parameter must be an integer greater than or equal to 1.

Parameter	Description
Isotope Table	Specifies the type of isotope table to use. Isotope tables simulate the distribution of isotopic peaks, in *m/z*, for different choices of the monoisotopic mass. The Xtract algorithm chooses the monoisotopic mass with the best fit between the theoretical and the observed isotope distribution. To generate an isotope table, the BioPharma Finder application uses a chemical formula to describe the type of molecule. You can choose one of the following formulas: • Protein: Uses an averagine[1] formula to generate the isotope table. The Default Xtract method uses this formula. • Nucleotide: Uses an elemental formula typical for nucleotides to generate the isotope table. • Sequence Specific: Use the sequence specific isotope table for multiple sequences and correct mass.
Advanced Parameters (Xtract) **(Visible only when you select the Show Advanced Parameters check box) These parameters only infrequently need changing. Only experienced users should change these parameters. They also appear on the Process and Review page for real-time optimization.**	
Calculate XIC	When selected, the application calculates the extracted ion chromatogram for each detected component. This check box is not available for single spectra, because there is no chromatogram. Using this parameter can result in a much longer analysis time, so you might avoid using it with complex data or with data where the displayed XICs are unnecessary.
Fit Factor (%)	Measures the quality of the match between a measured isotope pattern and an averagine distribution of the same mass. Enter a value between 0 and 100%: • 0% requires a low fit only. • 100% means that the measured isotope profile is identical to the theoretical averagine isotope distribution.
Remainder Threshold (%)	Specifies the height of the smaller overlapping isotopic cluster, as a percentage, with respect to the height of the most abundant isotopic cluster when the Xtract algorithm attempts to resolve overlapping isotopic clusters. For example, if one isotopic cluster in a spectrum has an abundance of 100 and you set the Remainder Threshold parameter to 30%, the Xtract algorithm ignores any overlapping clusters with an abundance less than 30.

Parameter	Description
Consider Overlaps	When selected (default), indicates the Xtract algorithm is more tolerant of errors when the spectrum intensity is significantly higher than expected for the theoretical isotopic cluster. Because this option can lead to increased false positives, select it only in cases where you expect overlapping isotopic clusters in a data set.
Resolution at 400 m/z	Defines the resolution of the source spectrum at an *m/z* value of 400. For more details, see Resolution at 400 m/z for the ReSpect algorithm.
Negative Charge	When cleared, indicates the data was acquired in positive charge mode during the ESI process. When selected, indicates the data was acquired in negative charge mode. You might want to use this option when processing compounds that contain nucleotides like those found in DNA and RNA. When these compounds are acquired in negative mode, the resulting mass spectra are often clearer. Deprotonation of nucleotides, which are acidic, occurs when the compound is dissolved in a basic solution and negative voltage is applied to produce negatively charged ions. **IMPORTANT!** Do not select the Negative Charge check box if your data was acquired in positive mode. Results will not be usable.
Charge Carrier	Specifies the adduct ions used during ESI processing. Adduct ions bring the charge to the molecule, and this charge converts it to an ion. • H+ (1.00727663): Specifies that the adduct was hydrogen. • K+ (38.9631585): Specifies that the adduct was potassium. • Na+ (22.9892213): Specifies that the adduct was sodium. • Custom: Specifies that the adduct was a charge carrier other than hydrogen, potassium, or sodium. When you select this option, a box opens so that you can type the mass of the custom charge carrier. **Note:** In negative mode, these adduct ions correspond to deduct rather than adduct masses.
Minimum Intensity	Specifies a minimum intensity threshold to filter out possible background noise, even when you set the S/N Threshold parameter to zero.
Expected Intensity Error	Specifies the permissible percentage of error allowed in calculating the ratio of the most abundant isotope to the next isotope higher in mass in the isotope series.

[1] Senko, M.W.; Beu, S.C.; McLafferty, F.W. Determination of monoisotopic masses and ion populations for large biomolecules from resolved isotopic distributions. *J. Am. Soc. Mass Spectrometry*. **1995,** *6*, 226–233.

For more details, see the following topics:

- Left side of the component detection page (page 355)
- Viewing the deconvoluted Spectra for intact mass analysis (page 433)
- Viewing the results table for intact mass analysis (page 422)
- Resolution at 400 m/z (page 375)

ReSpect deconvolution parameters

The following table describes the parameters for the ReSpect deconvolution algorithm on the Component Detection page.

Table 48 ReSpect parameters on the Component Detection page

Parameter	Description
Deconvolution Algorithm	Select the ReSpect option for the deconvolution algorithm used for processing.
Main Parameters (ReSpect) **These parameters might change often. They also appear on the Process and Review page for real-time optimization.**	
Deconvoluted Result Filter	
Output Mass Range	Specifies the range for the displayed masses on the *x* axis of the deconvoluted spectral plot.
Deconvoluted Spectra Display Mode	Specifies the mode to display the deconvoluted spectra.
Charge State Distribution	
Deconvolution Mass Tolerance	Specifies the global allowable error for the *m/z* values of peaks in a charge state series as they appear in the input spectrum. Also compensates for calibration errors and the effects of local noise, peak overlaps, and other sources of mismatches between the model and the actual peak profiles. This parameter is important in the ReSpect algorithm, because it controls the tolerance for peaks, in *m/z*, when the algorithm uses these peaks to fit to a particular component. As you increase the value of this parameter, the ReSpect algorithm expands the plausible set of charge state peaks, but at the same time it correspondingly increases the false positive rate. The ReSpect algorithm can exclude these false positives to some degree. For most purposes, the ppm setting provides better results than the Da setting. A ppm setting between 20 and 50 is a good starting point. Select the unit for this parameter: • ppm: Specifies the mass tolerance in parts per million. • Da: Specifies the mass tolerance in daltons.

Parameter	Description
Choice of Peak Model	
Choice of Peak Model	Specifies the appropriate peak model for the data. The expected peak shapes for nucleotides are different from those for proteins and peptides because of their different isotopic composition. • Intact Protein: Specifies peak model widths that are appropriate for use with the intact protein data produced by Orbitrap™ MS instruments. • Nucleotide: Specifies peak model widths that are appropriate for use with the nucleotide data produced by Orbitrap™ MS instruments.
Resolution at 400 m/z	Defines the resolution of the source spectrum at an *m/z* value of 400. Select one of these options: • Raw File Specific: The application automatically uses the resolution from each raw data file loaded for the experiment to process the deconvolution for that particular file. If the acquisition used more than one resolution, the application takes the first resolution value from the raw data file. With this option, you cannot edit the resolution value; however, you can process multiple raw files acquired at different resolutions. • Method Specific: You specify the resolution in the processing method to process the deconvolution for all of the loaded raw data files in the experiment. By default, the application displays the resolution value from the first (or only) raw data file. If the acquisition used more than one resolution, the application takes the first resolution value from the file. With this option, you can change the resolution value for this method; however, the application processes all of the raw data files using the same resolution. If the files were acquired at different resolutions, this option might not be suitable. Only use this option when instrument method information is not available in an exported mass spectrum file (-qb.raw file format). For this type of file, the application uses a default resolution of 12374. If this resolution is not appropriate, you can modify the resolution value.
	In cases where the mass spectrometer measured the resolution in the raw data file at an *m/z* value other than 400, the ReSpect algorithm scales it as follows to account for the variation in instrument resolution versus *m/z*: $R_{converted} = R_{measured} \times \sqrt{\frac{M_{measured}}{400}}$ where: • $R_{converted}$ is the resolution to be converted. • $R_{measured}$ is the resolution at the measured mass-to-charge ratio. • $M_{measured}$ is the measured mass-to-charge ratio other than 400.

Parameter	Description
Generate XIC for Each Component	
Calculate XIC	When selected, calculates the extracted ion chromatogram for each detected component from a range of deconvoluted spectra. **Note:** Selecting the Calculate XIC check box can result in a much longer analysis time, so you might avoid selecting it with complex data or with data where the displayed XICs are unnecessary, for example, in an infusion sample run.
Advanced Parameters (ReSpect) **(Visible only when you select the Show Advanced Parameters check box) These parameters only infrequently need changing. Only experienced users should change these parameters. They also appear on the Process and Review page for real-time optimization.**	
Charge State Distribution	
Model Mass Range	Specifies the required mass range from the minimum (lowest) end to the maximum (highest) end of the range.
Charge State Range	Sets the allowable range for the number of charge states that must appear for a component to be recognized. The ReSpect algorithm rejects potential components with fewer than the minimum or greater than the maximum number of charge states.
Minimum Adjacent Charges (low & high model mass)	Specifies the minimum number of charge-state peaks that must appear in a row for components of the low model mass and of the high model mass. For example, if the specified values are 6 and 10, and the model mass range is 10 000 to 160 000, a component with a mass of 10 000 would require at least 6 adjacent charge states, and a component with a mass of 160 000 would require at least 10 adjacent charges states. **Note:** The performance of the ReSpect algorithm improves as the number of adjacent charge states increases. Tests suggest that this algorithm always yields reliable results if the minimum numbers of allowed adjacent charge states at the low and high end of the *m/z* range are 6 and 10, respectively. Therefore, these numbers are the default parameters. In cases where a sample does not provide this number of charge states, parameter values of 6 and 6 should still give high-quality results. If the number of adjacent charge states falls below 4 and 6, reliability might decline, so you should confirm results by other means, if possible.

Parameter	Description
Noise Parameters	
Rel. Abundance Threshold (%)	Specifies a threshold below which the application filters out data for data reporting. This option sets a relative threshold as a percentage of the most abundant component in the spectrum. The most abundant peak in the deconvoluted spectrum has a relative abundance of 100 percent, and all other peaks are calculated relative to that one. In the Results table on the Process and Review page, the application shows only those components that are greater than or equal to this relative abundance threshold in the deconvoluted spectrum. For example, if the highest peak has an absolute abundance of 1000, the relative abundance is 1 percent, and no peaks below an absolute abundance of 10 will appear in the deconvoluted spectrum. For this value, 0% displays all results, 100% displays only the most abundant component.
Deconvolution Quality	
Quality Score Threshold	Specifies a minimum protein quality score that components must have to be displayed in the Results table. The application discards components that have a score beneath this threshold.
Choice of Peak Model	
Target Mass	Specifies an expected target mass, in daltons, to use in calculating the peak model. This parameter is critical but does not have to be exact; a value within 5 and 10 percent of the actual target is sufficient for best performance. For samples where the range of masses is broad, choose a mass somewhere in the middle of the range. For example, if the IgG light (~20 kDa), heavy chains (~50 kDa), and intact antibody are found in the same sample, choose 75 kDa as the target mass. **Note:** When you modify the maximum value of the Output Mass Range on page 374, the application automatically updates the Target Mass on page 377 value to match the modified value. However, modifying the Target Mass on page 377 value does not affect the Output Mass Range on page 374 values. Previous versions of the BioPharma Finder and Protein Deconvolution applications used a default value of 150 000 for this parameter.
Peak Model Parameters **These parameters place restrictions on the width and shape that a peak must have to be associated with a compound.**	
Number of Peak Models	Controls the resolution of the peak modeling process by dividing the observed *m/z* range into a uniformly spaced set of regions equal to this number. The application generates a single peak model for each of these regions on the basis of the observed *m/z* value and instrument resolution at the midpoint of each region.
Left/Right Peak Shape	Defines the sharpness of a peak.

Parameter	Description
Peak Filter Parameters **These parameters control how potential peaks in the spectrum that might be associated with compounds are identified and which ones are excluded as being too small.**	
Peak Detection Minimum Significance Measure	Specifies a significance level, in standard deviations, that determines whether the ReSpect algorithm discards a peak as a noise feature (spectrum noise from the background) or retains it as a legitimate peak. The ReSpect algorithm retains peaks equal to or greater than this selected significance level. The higher the significance level, the more stringent this filtering is.
Peak Detection Quality Measure	Removes noise and irrelevant features from the list of peaks. Noise is calculated from the spectrum background. This parameter is important in the ReSpect algorithm because it controls how plausible a peak must be before the algorithm uses it in a deconvolution. Reducing the confidence level for this parameter increases the number of peaks but allows more noise. Tests suggest that a confidence level of 95 percent works well in most cases and that you can relax it to 68 percent if necessary. A confidence level of 99 percent is rarely required. A confidence level of 50 percent or less might produce an unacceptable number of false positives. • No Noise Rejection: Retains all peaks and features. • 50% Confidence: Rejects all features up to a significance corresponding to 0.7 standard deviations. • 68% Confidence: Rejects all features up to a significance corresponding to 1 standard deviation. • 95% Confidence: Rejects all features up to a significance corresponding to 2 standard deviations. • 99% Confidence: Rejects all features up to a significance corresponding to 3 standard deviations.
Specialized Parameters	
Peak Model Width Factor	Specifies the adjustment to the peak width in the deconvolution model. A larger value makes the peak width wider and vice versa.
Intensity Threshold Scale	Specifies how intense a possible charge state peak must be to be included in the wings of a charge state distribution. • 0.005 • 0.01 (legacy) Reducing this threshold value can increase sensitivity at the expense of a possible increase in the false-positive rate for weak low-scoring components. **Note:** Previous versions of the BioPharma Finder and Protein Deconvolution applications used a default value of 0.01 for this parameter.

Parameter	Description
Deconvolution Parameters	
Noise Compensation	When selected (default), the ReSpect algorithm improves signal detection where the noise level varies across the data.
Charge Carrier	Specifies the adduct ions used during ESI processing. Adduct ions bring the charge to the molecule that converts it to an ion. • H+ (1.00727663): Specifies that the adduct was hydrogen. • 2H+ (2.013553): Specifies that the adduct was deuterium. • Na+ (22.9892213): Specifies that the adduct was sodium. • Custom: Specifies that the adduct was a charge carrier other than hydrogen, deuterium, or sodium. When you select this option, a box opens so that you can type the mass of the custom charge carrier. **Note:** In negative mode, these adduct ions correspond to deduct rather than adduct masses.
Negative Charge	When cleared (default), indicates the data was acquired in positive charge mode during the ESI process. When selected, indicates the data was acquired in negative charge mode. You might want to use this option when processing compounds that contain nucleotides like those found in DNA and RNA. Deprotonation of nucleotides, which are acidic, occurs when the compound is dissolved in a basic solution and negative voltage is applied to produce negatively charged ions. **IMPORTANT!** Do not select the Negative Charge check box if your data was acquired in positive mode. Results will not be usable.

For more details, see the following topics:

- Left side of the component detection page (page 355)
- Viewing the deconvoluted Spectra for intact mass analysis (page 433)
- Deconvoluted Spectra display mode (page 390)
- Optimizing the protein quality score (page 392)
- Model mass range information (page 394)
- Viewing the results table for intact mass analysis (page 422)

Component detection page commands

Right-clicking the Chromatogram or Source Spectrum pane of the Component Detection page opens a shortcut menu that contains the commands listed in the following table.

Table 49 Chromatogram/Source Spectrum pane shortcut menu commands

Commands	Description
Reset Scale	Restores the original view that first appeared in the pane.
Copy	Copies the image in the pane to the Clipboard.
Zoom Out	Shrinks the view in the pane by a factor of 2.
Zoom In	Enlarges the view in the pane by a factor of 2.

For more details, see the following topics:

- Using copy and paste functions (page 666)

Editing identification parameters for intact mass analysis

To create a method for target sequence matching (matching the measured masses of the components that the application detects to the masses of target sequences that you specify), to define your sample as an ADC for the application to determine the average DAR values, or to set the merge options for the Multiconsensus result format, you must set the parameters on the Identification page.

For target sequence matching and to select a modification candidate for the DAR calculations, you can add predefined modifications or custom modifications that you create to a protein sequence for the experiment.

For more information about the Identification page, see the following topics:

- Opening the identification page (page 381)
- Identification page layout (page 381)
- Editing the identification parameters (page 382)
- Left side of the identification page parameters (page 384)
- Right side of the identification page tables (page 385)

Opening the identification page

To open the Identification page

- On the Component Detection page, click **Next** in the command bar.

–or–

- In the navigation bar, click the **Parameters** tab, and then click the **Identification** subtab.

For more details, see the following topics:

- Editing component detection parameters for intact mass analysis (page 354)
- Identification page layout (page 381)

Identification page layout

The items on the Identification page vary depending on whether you are editing the processing method with or without first entering the experiment name and raw data file or files on the Intact Mass Analysis page.

If you did *not* specify the experiment name and raw data file or files before editing the method, the Identification page shows only some areas at the left side of the page.

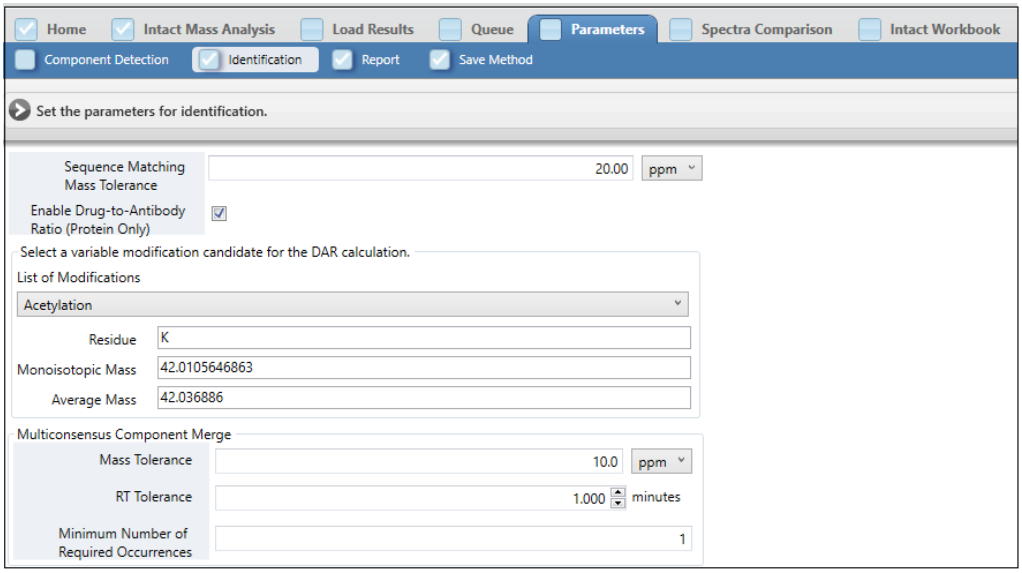


Figure 168 Identification page parameters (left side)

If you *already* specified the experiment name and raw data file or files before editing the method, in addition to the parameters on the left side of the Identification page, you also see the Sequences Added to Experiment and Global Sequence Reference tables on page 385 on the right side.

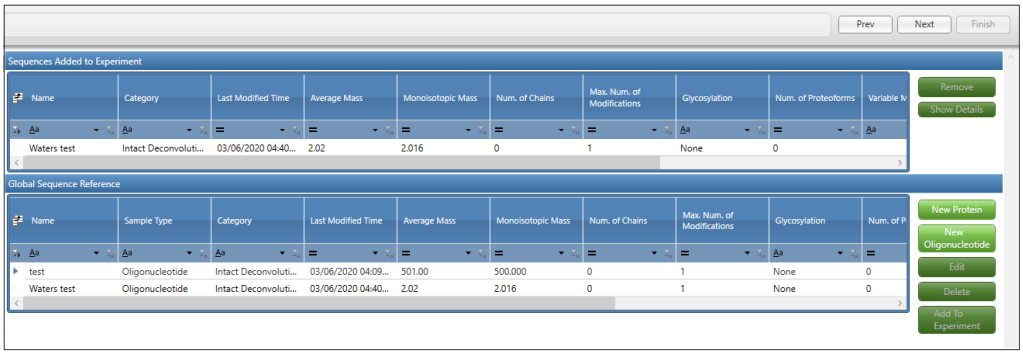


Figure 169 Identification page parameters (right side)

Editing the identification parameters

To edit the identification parameters

1. Enter the appropriate parameter values on the left side of the Identification page.
2. (Optional, for target sequence matching) Perform the following steps as needed on the right side of the page:
   a. Create a new protein sequence by clicking **New** next to the Global Sequence Reference table.

      The Protein Sequence Editor appears, where you can import a FASTA file containing a new sequence of interest or manually enter the sequence.

   b. Edit or delete an existing sequence by selecting it in the Global Sequence Reference table and then clicking **Edit** or **Delete**. You can also edit a sequence by double-clicking its row in the table.

      When you edit the sequence, the Protein Sequence Editor opens and displays the sequence information for you to make the necessary changes.

      When you delete the sequence, it disappears from the Global Sequence Reference table.

   c. Add a sequence to the experiment by selecting it in the Global Sequence Reference table and clicking **Add to Experiment**.

      If the sequence is already included in the experiment, a message appears to inform you.

      After you add the sequence to the experiment, the application populates a row of the Sequences Added to Experiment table.

d. Change a sequence after adding it to your experiment.

If the experiment already includes the sequence that you want to change or update, remove the sequence by selecting it in the Sequences Added to Experiment pane and clicking **Remove**. Then, create a new sequence (step a on page 382) or edit the existing sequence (step b on page 382), add the new modifications as needed, and then add the sequence to the experiment (step c on page 382).

To determine whether the experiment includes the latest version of the sequence, you can compare the data in the Last Modified Time, Average Mass, or Monoisotopic Mass columns of the Sequences Added to Experiment table and the Global Sequence Reference table.

e. Display the full sequence, including disulfide bonds, by selecting it in the Sequences Added to Experiment table, and then clicking **Show Details**.

The application displays the entire protein sequence.

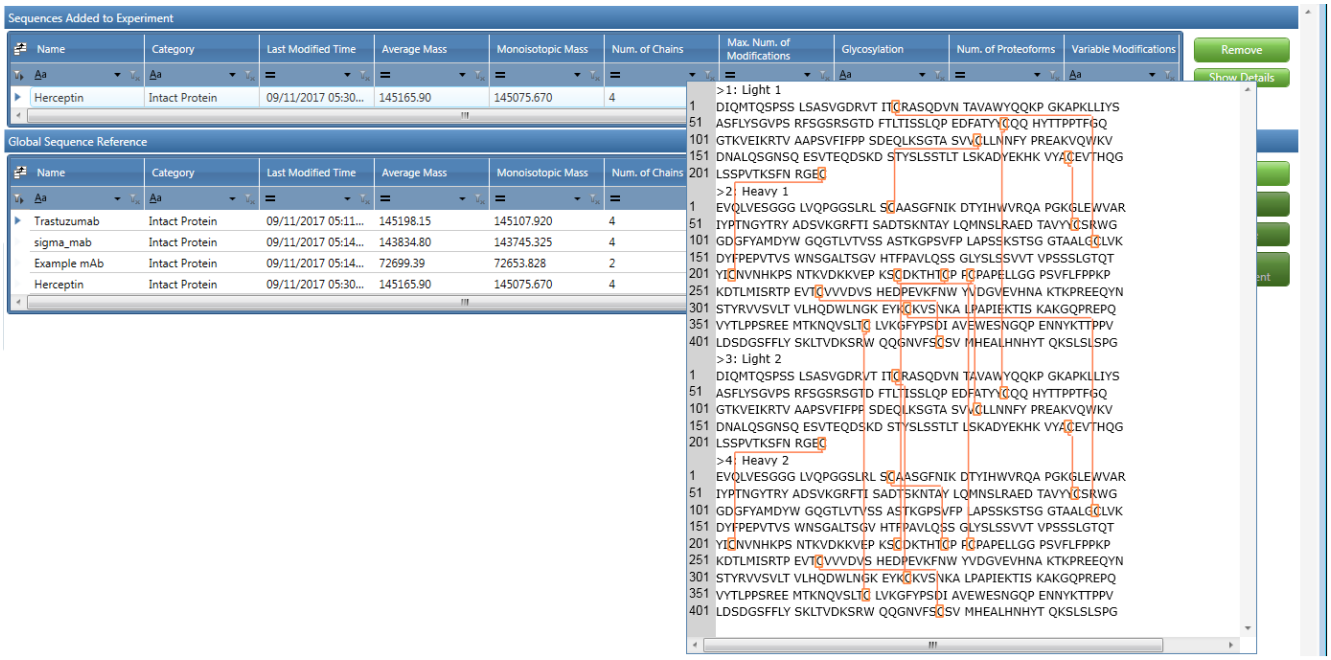


Figure 170 Details of protein sequence

3. When you are done editing the parameters on this page, click **Next** in the command bar to advance to the Report page.

For more details, see the following topics:

- Left side of the identification page parameters (page 384)
- Right side of the identification page tables (page 385)
- Identification page layout (page 381)

Left side of the identification page parameters

The following table describes the parameters on the left side of the Identification page.

Table 50 Parameters on the left side of the Identification page

Parameter	Description
Sequence Matching Mass Tolerance	Specifies the mass tolerance, in Da or ppm, within which the masses of the sequence and a component must fall to be considered a match.
Enable Drug-to-Antibody Ratio	Enables the application to determine the average DAR value based on the drug load assignments to the identifications. You must select a modification candidate.
Select a variable modification candidate for the DAR calculation **These parameters show the information for the selected variable modification candidate.**	
List of Modifications	(Enabled only when you select the Enable Drug-to-Antibody Ratio check box) Lists all of the side chain variable modifications available in the Protein Sequence Editor. An N-terminal or a C-terminal modification that is not already included as a side chain modification is not available in this list. If you want to select a missing modification, add it as a custom modification to the list of side chain modifications using the Protein Sequence Editor. Select one modification from this list to be the drug linker. The residue, monoisotopic mass, and average mass information for the selected modification appears. When you select a drug linker from this list, after the application finishes the matched sequence identification step, it automatically searches for all of the modifications that match the selected drug linker, and then reports the number of matched modifications as the Drug Load value on the deconvoluted spectra and in the Matched Sequence pane of the Process and Review page. **IMPORTANT!** If you select the Enable Drug-to-Antibody Ratio check box but do not select a modification from this list, the following occur: • If you manually process the experiment, the application displays an error message informing you that a modification selection is required. • If you automatically process the experiment, the application does not display an error message but the resulting drug load is 0.
Residue	(Not editable) Residue of the selected modification.
Monoisotopic Mass	(Not editable) Monoisotopic mass of the selected modification.
Average Mass	(Not editable) Average mass of the selected modification.

Parameter	Description
Multiconsensus Component Merge **These parameters control the merging of the multiple raw data files when you select Multiconsensus as the result format on the Intact Mass Analysis page.**	
Deconvolution Mass Tolerance	The mass tolerance in ppm or Da that components from individual files must satisfy to be merged into a multiconsensus component. The application excludes single file components whose masses differ by more than this tolerance from a multiconsensus component.
RT Tolerance	The maximum gap in retention time that components from individual files must satisfy to be merged into a multiconsensus component. The application discards single file components whose retention times are separated by more than this gap from a multiconsensus component.
Minimum Number of Required Occurrences	The minimum number of single raw data files that must be included in a multiconsensus component for it to be considered valid. The application discards multiconsensus components with fewer than this number of files.

For more details, see the following topics:

- Identification page layout (page 381)
- Viewing the deconvoluted Spectra for intact mass analysis (page 433)
- Viewing the matched sequence information for intact mass analysis (page 440)
- Drug Load (page 442) column
- Working in manual mode (page 347)
- Manual and automatic modes (page 333)

Right side of the identification page tables

The following table describes the tables on the right side of the Identification page.

IMPORTANT! These tables are visible only when you have specified the experiment name and raw data file or files on the Intact Mass Analysis page before you edit a method.

Table 51 Tables on the right side of the Identification page

Column	Description
Sequences Added to Experiment table **Displays the sequences added to the experiment after you click Add to Experiment.**	
Buttons	
Remove	Removes the sequence from the Sequences Added to Experiment table.
Show Details	Displays all of the chains in the selected sequence, including the disulfide bonds, if any.

Column	Description
Global Sequence Reference table **Lists all of the existing protein sequences. The columns in this table are the same as those on the Sequence Manager page.**	
Buttons	
New	Opens the Protein Sequence Editor so that you can import or create a new protein sequence.
Edit	Opens the Protein Sequence Editor so that you can edit the selected sequence.
Delete	Deletes the selected sequence from the Global Sequence Reference table.
Add to Experiment	Adds the selected sequence to the experiment. The added sequence appears in the Sequences Added to Experiment table.

For more details, see the following topics:

- Identification page layout (page 381)
- Sequence Manager page parameters (page 48)

Editing report parameters for intact mass analysis

Use the Report page to define the settings for generating reports containing deconvolution results and parameters.

Note: The default method settings provide a good balance between sensitivity and report size. If you adjust these parameters so that a report becomes filled with a large number of low-intensity noise peaks, a system without sufficient memory might hang. If your system hangs, restart the BioPharma Finder application and rerun it with a more restrictive set of parameters.
The BioPharma Finder application does not support the report feature for a multiconsensus experiment. If you set up this type of experiment before you edit the processing method, all of the report parameters are inactive.

For more information about the Report page, see the following topics:

- Editing the report page (page 387)
- Report page layout (page 387)
- Report page parameters (page 388)

Editing the report page

To edit the Report page

1. On the Identification page, click **Next** in the command bar.

   –or–

   In the navigation bar, click the **Parameters** tab, and then click the **Report** subtab.

2. Select the appropriate parameter options on the Report page.

When you are done editing the parameters on this page, click **Next** in the command bar to advance to the Save Method page.

For more details, see the following topics:

- Editing identification parameters for intact mass analysis (page 380)
- Saving a processing method (page 144)
- Report page parameters (page 388)

Report page layout

The Report page includes two different areas, Reporting Parameters at the top and Automation Parameters at the bottom. Enter the parameters to create a report that summarizes the results of the deconvolution.

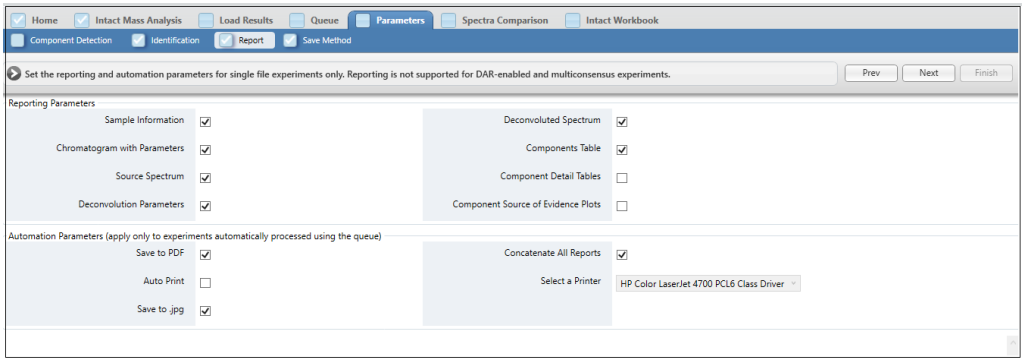


Figure 171 Report page areas

For more details, see the following topics:

- Report page parameters (page 388)

Report page parameters

The following table describes the parameters on the Report page.

Table 52 Parameters on the Report page

Parameter	Description
Reporting Parameters area **Determines, by selecting their corresponding check box, which sections of the report the application generates and displays.**	
Sample Information	Generates the Sample Information section of the report.
Chromatogram with Parameters	Generates the Chromatogram Parameters and Chromatogram sections of the report.
Source Spectrum	Generates the Source Spectrum section of the report.
Deconvolution Parameters	Generates the Main Parameters, Advanced Parameters, Source Spectra Parameters, and Sequences Information sections of the report.
Deconvoluted Spectrum	Generates the Deconvoluted Spectrum section of the report.
Components Table	Generates in the Masses Table section of the report showing the information that appears in the Results table on the Process and Review page.
Component Detail Tables	Generates the Monoisotopic Mass table (for Xtract) or Average Mass table (for ReSpect) for each component in the Component Detail Tables section of the report.
Component Source of Evidence Plots	Generates the Source Spectrum Evidence Plot section of the report for each component.
Automation Parameters area **Displays parameters that control the report for experiments that you run in automatic mode. The parameters in this pane apply only to experiments automatically processed using the run queue.**	
Save to PDF	When selected (default), saves the automatically generated report to a PDF file in the same folder as the raw data files, with the following file name: *RawFileName_ExperimentName.pdf*
Auto Print	When selected, automatically prints the generated deconvolution report contained in the PDF file. When you select this option, the Select a Printer box becomes available so that you can select a printer.

Parameter	Description
Save to .jpg	When selected (default), saves chromatographic peak data to both a JPG file and a CSV file. When cleared, saves chromatographic peak data only to a CSV file. For an automatic average over RT deconvolution/auto peak detection experiment, the application saves each peak to an individual file. For an automatic sliding windows deconvolution experiment, it saves all of the peaks to one file. The file names have the following format: *RawFileName_ExperimentName_ScanRange_DateStamp_TimeStamp_[A* M|PM]_[XT|RSP]_AUTO.jpg *RawFileName_ExperimentName_ScanRange_DateStamp_TimeStamp_[A* M|PM]_[XT|RSP]_AUTO.csv where XT is for Xtract and RSP is for ReSpect. Here are examples of each type of file: IgG_source_cid_ExperimentOne_2434_3410_8_15_2016_4_45_37_PM_RSP_AUTO.csv IgG_source_cid_ExperimentOne_2434_3410_8_15_2016_4_45_37_PM_RSP_AUTO.jpg
Concatenate All Reports	When selected (default), combines all reports for all deconvoluted spectra from the same raw data file into one report PDF file. When cleared, creates a report PDF file for each chromatographic peak in the raw data file.
Select a Printer	Displays a list of available printers that can print the deconvolution report (PDF) file. This option becomes available when you select the Auto Print option.

For more details, see the following topics:

- Report page layout (page 387)
- Sample information section (page 476)
- Chromatogram parameters section (page 477)
- Chromatogram section (page 478)
- Source spectrum section (page 483)
- Main parameters section (page 479)
- Advanced parameters section (page 480)
- Source Spectra parameters section (page 482)
- Sequences information section (page 483)
- Deconvoluted spectrum section (page 484)
- Masses table section (page 485)
- Component detail tables section (page 486)
- Source spectrum evidence plot section (page 487)
- Manual and automatic modes (page 333)
- Using the run queue (page 161)

19 ReSpect and Sliding Windows method information

The BioPharma Finder application provides several default processing methods that include the Respect algorithm and the sliding windows algorithm. You can edit the parameter settings for these methods to create custom methods for your experiments.

Deconvoluted Spectra display mode

One of the parameters for the ReSpect deconvolution algorithm is the Deconvoluted Spectra Display Mode.

You can select from two options for this mode:

- Mass Probability Distribution Profile (legacy)

  This mode is the same display mode in version 2.0 and earlier. It shows the probability (that a component has a particular average mass) multiplied by the component's abundance. If all of the masses can be determined precisely, this mode displays a centroid spectrum. In practice, the spectral plot shows a set of

Gaussian peaks, with the widths proportional to their uncertainties, typically measured in a few ppm.

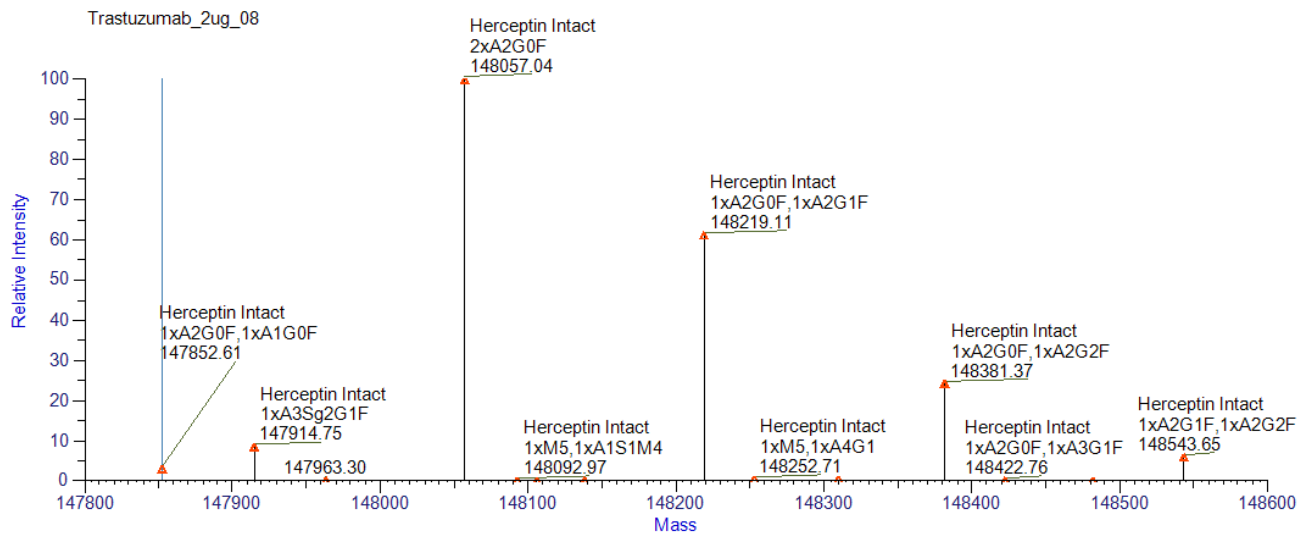


Figure 172 Mass Probability Distribution Profile

For sliding window experiments in Intact Mass Analysis, the application displays the deconvoluted spectrum as centroids of the average mass. For other types of experiments, the application displays the spectrum as probability distributions for the average mass. In most cases, these profiles are significantly narrower than the isotopic profiles from the Isotopic Profile mode.

- Isotopic Profile (new)

  This default mode, available in version 3.0 and later, displays the deconvoluted spectrum in profiles that show wider peaks for all types of experiments. It shows the isotopic distributions for all of the components identified by the deconvolution, plotted versus mass. The spectrum shows what the peaks in the original *m/z* spectrum would resemble if they are multiplied by their associated charge states and plotted versus mass. You can use the spectrum from this

mode as a visual comparison against the original *m/z* spectrum to examine how the deconvolution assigned the peaks.

Note: The spectrum from this mode does not include the background signal, which is identified and removed as part of the deconvolution process.

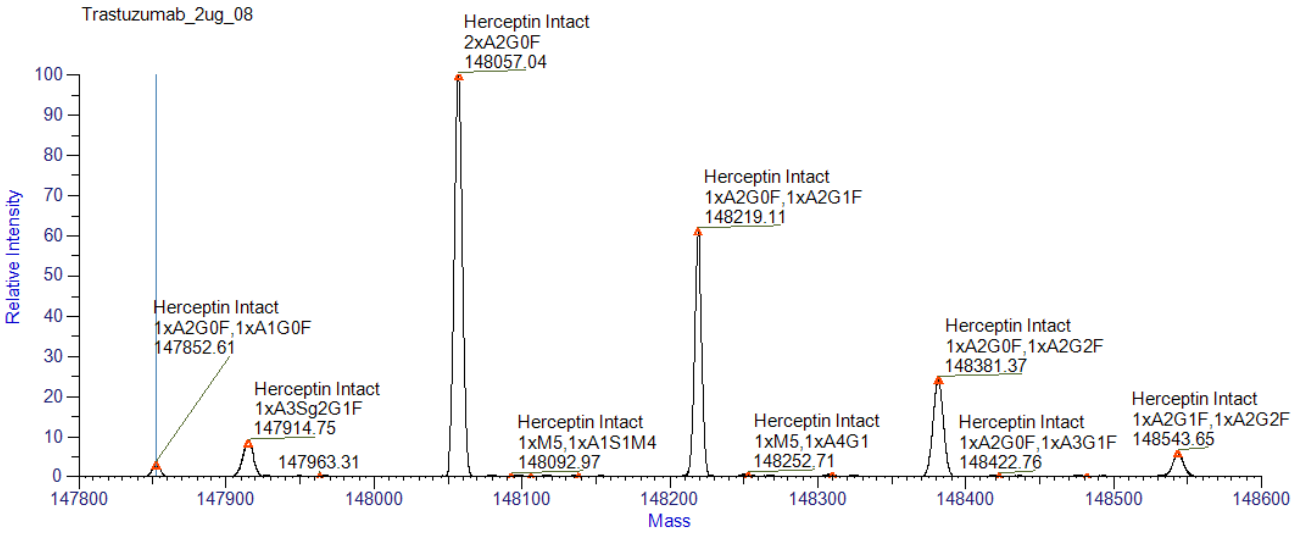


Figure 173 Isotopic Profile

If the original peaks are very close together, they might be merged into one. Often, the smaller of the original peaks appears as a shoulder of the larger peak in the spectral plot. In general, the merging process can shift the location of the larger peak very slightly towards the smaller one.

Optimizing the protein quality score

When the application runs the ReSpect algorithm, it calculates a protein quality score for each component shown in the Results table and displays it in the Score column so that you can easily assess the quality of the deconvolved components. These scores can help you determine whether each component is valid or spurious because of noise, harmonics, or other factors.

For more details, see the following topics:

- Scoring algorithm (page 393)
- Specifying a minimum score (page 394)
- Viewing and sorting the scores (page 394)
- Viewing the results table for intact mass analysis (page 422)
- Score (page 457)

Scoring algorithm

The ReSpect algorithm accepts a significant false positive rate to avoid the risk of false negatives. For this reason, the application applies a scoring algorithm to the ReSpect results to determine which components are the most reliable. This scoring algorithm reduces the false positive rate by calculating a Bayesian fitness measure that distinguishes reliable components from components that could represent harmonics, fortuitous fits to noise, or both. The algorithm compares the probability that a predicted component might produce peaks with an observed set of *m/z* positions and an intensity profile to the probability that this particular pattern could occur by chance. It then applies an additional set of penalties to exclude low-intensity components, harmonics, and superfluous fits to successive charge states of successive valid components.

The score calculation algorithm does not require you to set any control parameters. It copies any parameters that it might need from the corresponding control parameters used by the ReSpect algorithm or derives them from the ReSpect results themselves. However, you can use the Quality Score Threshold parameter to set a threshold value and exclude components with scores below the threshold value.

Deconvolution scores are absolute rather than relative so that you can compare scores between spectra, as well as compare scores within an individual spectrum. The higher the score, the better the component quality is. "Better" components in one spectrum have higher scores than "worse" ones in another spectrum. For example, a score of 100 in one spectrum represents a more reliable fit than a score of 50 in another spectrum. As another example, a score of 83 in spectrum A is better than a score of 54 in spectrum B, even if 83 is the lowest score in spectrum A and 54 is the highest score in spectrum B. In general, components with higher molecular weights, higher intensities, or both that might tend to have more charge states, receive higher scores.

You might not be able to specify ahead of time what the best threshold for a particular spectrum might be. For high-quality spectra, you might want to ignore components with scores below 40. For lower-quality spectra, this threshold might be 10 or lower. In general, scores indicate the following:

- In almost all cases, scores below 0 signify "bad" components. In particular, scores significantly below –100 are associated with harmonics and other unreliable fits that you should ignore.
- Scores between 0 and 20 indicate components that might be questionable. The precise value of this threshold depends on the quality of the spectra themselves. For clean spectra with well-defined and widely separated components, all results with a score greater than 0 might be good. As the noise increases and the spacing between components decreases, this threshold might rise. The precise threshold below which you might want to ignore components depends on the quality of the spectrum, the peak confidence level, and the mass tolerance that the ReSpect algorithm uses. Tests indicate that 20 might be a good starting point when you select a threshold.
- Scores between 20 and 40 generally indicate "good" components.
- Scores above 40 indicate components of very high quality.

The deconvolution score calculation algorithm is powerful, but it cannot overcome a poor choice of control parameters for the ReSpect algorithm. In particular, if you run the algorithm with an inappropriate mass or *m/z* range, too low a peak confidence level, or too high a mass tolerance, the results could include fits to noise that no scoring algorithm could identify.

For more details, see the following topics:

- Quality Score Threshold (page 377)

Specifying a minimum score

To specify a minimum component quality score

In the Quality Score Threshold box on the Component Detection page, specify the minimum quality score that a component must have to be displayed in the Results table.

You can enter any floating-point number. The default is 0.00.

The ReSpect algorithm discards components that do not meet this minimum score.

For more details, see the following topics:

- Quality Score Threshold (page 377) parameter

Viewing and sorting the scores

To view and sort the quality score

1. After the deconvolution processing of the spectrum, view the quality scores in the Score column of the Results table.
2. (Optional) Sort the scores from high to low by clicking the down arrow next to the Score column header, or sort the scores low to high by clicking the up arrow.

For more details, see the following topics:

- Viewing the results table for intact mass analysis (page 422)
- Score (page 457)

Model mass range information

For the ReSpect algorithm, the Model Mass Range parameter restricts the allowable mass range for the charge-state deconvolution. It produces the best fit possible to the observed set of peaks in a spectrum, using only components that fall within the specified mass range. But in ReSpect processing, results for a restricted model mass range are not a simple subset of runs for a larger model mass range. The results for two adjacent mass ranges, such as 35 000 to 40 000 and 40 000 to 45 000, need not always form proper subsets of the results for the combined mass range—in this case, 35 000 to 45 000.

Your choice of parameter values affects the components that the algorithm detects in subsets of the model mass range. If you set the Model Mass Range parameter to values that do not span the expected range of component masses, the ReSpect algorithm adds superfluous, low-amplitude components with physically implausible masses to its results list to fit the peaks that would have been associated with the missing masses. These components receive low deconvolution scores and disappear when you run the ReSpect algorithm with the full model mass range. If you run the algorithm with an inappropriate model mass range, these appearing and disappearing components can make the results unreliable.

For the initial processing, select a model mass range that includes all of the expected components and their immediate neighbors. You can narrow the mass range in subsequent runs.

For more details, see the following topics:

- Model Mass Range (page 376)

Best results with the ReSpect algorithm

Low outlying peaks in the source spectrum are less accurate than high peaks and fade into noise. Follow these suggestions to increase the stringency of the ReSpect deconvolution, decrease noise, and produce better results:

- Narrow the m/z Range as much as possible around the more abundant peaks in the distribution—perhaps those above 10 to 20 percent relative abundance.
- Adjust the values of the Model Mass Range parameter. Harmonics (overtones) are a byproduct of the ReSpect algorithm and other deconvolution algorithms. They are normal in a distribution, but you can avoid them by narrowing the range to the region around the target mass.
- Reduce the value of the Deconvolution Mass Tolerance parameter to make the results cleaner. As noted in its description, specifying this value using the ppm option might also improve results. When you decrease this value, the delta mass value for each charge state also drops.
- Raise the values of the Minimum Adjacent Charges (low & high model mass) parameter.

For more details, see the following topics:

- m/z Range (page 510)
- Model Mass Range (page 376)
- Deconvolution Mass Tolerance (page 374)
- Minimum Adjacent Charges (low & high model mass) (page 376)

Recommended values for sliding Windows deconvolution parameters

The sliding windows algorithm is significantly more powerful than conventional deconvolution and therefore more sensitive to parameter settings. The BioPharma Finder application checks the sliding windows parameters in the Source Spectra Method area on the Component Detection page before deconvolving a spectrum and issues a warning if these are markedly suboptimal. The optimal choice of parameters can depend on features such as the width of chromatographic peaks.

The sliding windows algorithm is comparatively robust, but it might produce less than optimal results if the parameter settings are inappropriate. If possible, avoid the following:

- Requesting too many sliding window cycles
- Setting the Min. Number of Detected Intervals parameter to a value that is too low
- Specifying a value that is too low for the Max RT Gap parameter

The following discussion describes these potential pitfalls in more detail.

- Too many sliding window cycles

  Execution time increases with the number of sliding window cycles.

  You can estimate the expected number of sliding window cycles by using this equation for the Target Avg Spectrum Offset—Scan Offset option:

  $N_{cycles} = (T_{stop} - T_{start}) \times (N_{scan\ offset} \div T_{scan})$

  where:

  - T_{stop} is the stop time.
  - T_{start} is the start time.
  - $N_{scan\ offset}$ is the value of the Scan Offset parameter.
  - T_{scan} is the width of a single scan.

  Or, use this equation for the Target Avg Spectrum Offset—% Offset option:

  $$N_{cycles} = \frac{T_{stop} - T_{start}}{\left(T_{width} \times \left(\frac{Offset}{100}\right)\right)}$$

  where:

  - T_{stop} is the stop time.
  - T_{start} is the start time.
  - T_{width} is the value of the Target Avg Spectrum Width parameter.
  - *Offset* is the value of the % Offset parameter.

  Execution time can increase to unacceptable levels as the number of window cycles climbs above 500. Too many sliding window cycles can also make the selection of an appropriate value for the Min. Number of Detected Intervals parameter more difficult.

- A value that is too low for the Min. Number of Detected Intervals parameter

  The number of sliding windows that a merged component must appear in if it is to be considered meaningful does not affect the mass merge operation itself. If you set the value of this parameter too low, the application could treat every individual component peak, however isolated it might be from other component peaks, as a valid merged component. The result is a number of false positives that could be associated with noise. If you set this value too high, the application discards components that it should accept as real.

  Make this value large enough to exclude results that are implausibly narrow in retention time but small enough to include results of realistic duration. A good approach is to use whichever is larger: 3 or the minimum number of windows that can fit into a peak. The following equation expresses this idea.

$$N_{points} > \max\left(3, \frac{T_{expected}}{\left(T_{width} \times \left(\frac{Offset}{100}\right)\right)}\right)$$

  where:

  - $T_{expected}$ is the expected width of a peak in retention time.
  - T_{width} is the setting of the Target Avg Spectrum Width parameter.
  - *Offset* is the setting of the Target Avg Spectrum Offset—% Offset parameter.

- A value that is too low for the Max RT Gap parameter

  If you set the Max RT Gap parameter to a value that is too low, the mass merge step in the sliding windows algorithm breaks up each merged component into an assortment of meaningless pieces. This value should be comparable to or slightly less than the expected gap in retention time between distinct components.

For more details, see the following topics:

- Source Spectra method area parameters (page 367)
- Min. Number of Detected Intervals (page 369)
- Max RT Gap (page 369)
- Target Avg Spectrum Offset (page 368)
- Target Avg Spectrum Width (page 368)

20 Viewing the intact mass analysis results

You can view the Intact Mass Analysis results from multiple pages in the BioPharma Finder application.

Opening the results from the queue page

When you run an Intact Mass Analysis experiment in automatic mode, the Queue page displays the run queue containing all of the completed, running, submitted, or canceled jobs. After a job is completed, you can open its results or report from the Queue page and view the processed data.

Note: A completed job displays "Completed" in the Status column.
You cannot open the results if the application has not yet analyzed the experiment or is in the process of analyzing it. In this case, the Open Results and Open Report buttons are inactive until processing of the selected job is completed.
Opening the results or report does not stop the application from analyzing subsequent jobs in the queue.

To view the results of an experiment from the Queue page

1. On the Home page, click **Intact Mass Analysis** in the left pane or below the BioPharma Finder splash graphic.

   The Intact Mass Analysis page opens.

2. Click the **Queue** tab.

   The Queue page opens showing the queued jobs in a table.

3. In the table, do one of the following:
   - Double-click a row to select the completed job and view its results, or click a row to select the completed job and then click **Open Results**.

The application transfers you to the Process and Review page, which displays the following:

- Parameters used for processing in the Real Time Optimization pane
- Chromatograms in the Chromatogram pane
- Deconvoluted spectra in the Deconvoluted Spectrum pane
- Source spectra in the Source Spectrum pane
- Components in the Results table

You can adjust the size or location of the panes on this page.

–or–

- Click a row to select the completed job and then click **Open Report**.

  The application transfers you to the Reporting page, which displays the report generated when the application processed the experiment.

For more details, see the following topics:

- Manual and automatic modes (page 333)
- Using the run queue (page 161)
- Queue page parameters (page 172)
- Viewing the process and review page for intact mass analysis (page 416)
- Rearranging the panes (page 655)
- Viewing an intact mass analysis report (page 472)

Opening the results from the load results page

Because you can delete jobs in the run queue on the Queue page, after deletion, you can no longer view the related results. However, the BioPharma Finder application saves all of the completed jobs on the Load Results page so that you can view any previously saved experimental results.

For Intact Mass Analysis, you can manually process experiments and save their results after modifying parameters or rerunning a sample. The application saves the manually processed results to the Load Results page only. You can also import or export the results of an experiment.

To view, delete, or convert the results of an experiment from the Load Results page

1. On the Home page, click **Intact Mass Analysis** in the left pane or below the splash graphic.

2. Click the **Load Results** tab.

   The table on the Load Results page displays all of the previously saved Intact Mass Analysis results, in order of completion time.

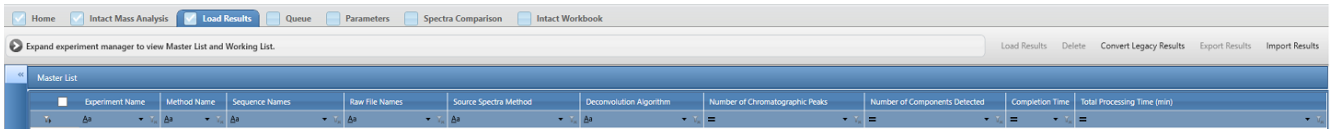


Figure 174 Load Results page

The table lists the name of each experiment, the raw data file names, the processing method and protein sequence or sequences (optional) assigned to that analysis, along with other information.

You can sort the columns or filter the data in the table.

Note: If you process an experiment in manual mode or use real-time optimization to reprocess an experiment, the Total Processing Time cell displays 0.00. This cell displays the actual total processing time only when you process an experiment in automatic mode.

For experiments that use the Auto Peak Detection option for source spectra in the processing method, a sub-level of peak information appears below the experiment row as follows:

- Peak #: Number of each peak in increasing order.
- Scan Range: The range of scans for a peak.

3. In the table on the Load Results page, do any of the following:
   - Double-click a top-level row to select an experiment and view its results, or select a top-level row and then click **Load Results** in the command bar.

     The application transfers you to the Process and Review page.
   - Select one or more top-level rows and then click **Delete** in the command bar.

     Select multiple rows by using the SHIFT key for contiguous rows or the CTRL key for noncontiguous rows.

     The application deletes this set of experiments from the database. If the selected experiments still exist in the run queue on the Queue page, the application removes them from the queue as well.

     The numbering in the Record Number cell for an experiment does not change when you make a deletion. For example, if you have six experiments numbered 1–6, and you delete experiments 3 and 4, experiments numbered 5 and 6 do not change to become experiments 3 and 4.
   - Click **Convert Legacy Results** in the command bar. Then, browse to legacy results files (SQLite files) from any version of the Protein Deconvolution application or from version 1.0 of the BioPharma Finder application on a different computer, and then click **Open**.

     The BioPharma Finder application converts all legacy results. Each experiment and its related information appear in a row in the Load Results table. Double-click that row, or select the row and then click **Load Results**, to view the corresponding results.

     If the legacy results file has the same name as an existing experiment, the application automatically appends "-legacy *version*" to its experiment name in the row.

To import the results of an experiment from the Load Results page

1. On the Home page, click **Intact Mass Analysis** in the left pane or below the splash graphic.
2. Click the **Load Results** tab.

   The table on the Load Results page displays all of the previously saved Intact Mass Analysis results in the order of completion time.
3. Click **Import Results**.
4. In the dialog box, locate and select one or more Intact Mass Analysis result file (*Filename*_intact.resultsbpf) that you want to import. The files must be in the same folder when importing multiple files simultaneously.
5. Click **Open**.

   A message appears indicating the confirmation of the import.

   The application adds the imported result in the experiment result table of the Master List and Working List folders.

   Note: If an existing method has the same name as the imported method, the application appends _imported to the file name of the new method.

To export the results of an experiment from the Load Results page

1. On the Home page, click **Intact Mass Analysis** in the left pane or below the splash graphic.
2. Click the **Load Results** tab.

   The table on the Load Results page displays all of the previously saved Intact Mass Analysis results in order of completion time.
3. Select one or more check boxes corresponding to the results of the experiments that you want to export, and do one of the following:
   - Choose **Export Results ▸ Export Experiment with Raw Files** in the command bar.

     –or–
   - Choose **Export Results ▸ Export Experiment without Raw Files** in the command bar.

   Note: You can only view the result when you include the raw files in the export of the experiment result file. To display the spectra when you import the exported experiment result file, you must store the raw file in the same directory as processed.

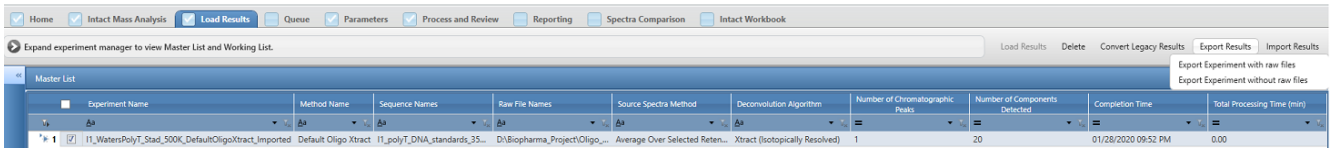


Figure 175 Export Results options in the Load Results page

4. In the dialog box, browse to the appropriate folder, and click **OK**.

   For export of a single experiment result, the Browse dialog box opens to the *drive*:\Xcalibur™\data\ folder by default.

   A message appears indicating the confirmation of the export. The application saves the exported experiment result (*Filename*_intact.resultsbpf) on the specified folder.

For more details, see the following topics:

- Using the run queue (page 161)
- Using basic table functions (page 670)
- Filtering data in a table (page 673)
- Queue page parameters (page 172)
- Manual and automatic modes (page 333)
- Using Real-Time optimization for intact mass analysis (page 402)
- Data conversion from legacy applications (page 43)

Using Real-Time optimization for intact mass analysis

On the Process and Review page, you can adjust the parameters in the processing method, the parameters in the protein sequence for target sequence matching, or both sets of parameters, and then process or reprocess the experiment without leaving this page, for real-time optimization and manual processing.

To process or reprocess the experiment with the modified method or sequence parameters

1. As necessary, click the **Process and Review** tab and then click the title bar of the **Real Time Optimization** pane to see the Component Detection and Identification subtabs.

   Tip: You can also click this title bar again to collapse the Real Time Optimization pane and provide more space for the other panes on the Process and Review page.

   The Component Detection pane expands automatically and displays the chromatogram parameters and source spectra method used for processing the current experiment.

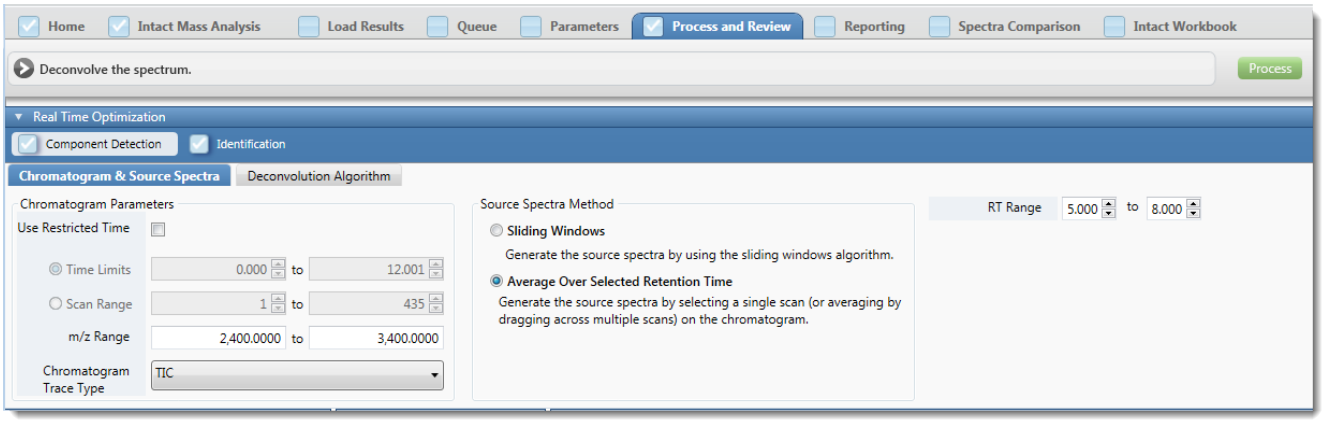


Figure 176 Component Detection pane for real-time optimization

2. Click the **Chromatogram & Source Spectra** or **Deconvolution Algorithm** subtab to update the corresponding parameters as needed.

   Under the Deconvolution Algorithm subtab, click the **Basic** subtab to update the basic parameters or the **Advanced** subtab to edit the advanced parameters.

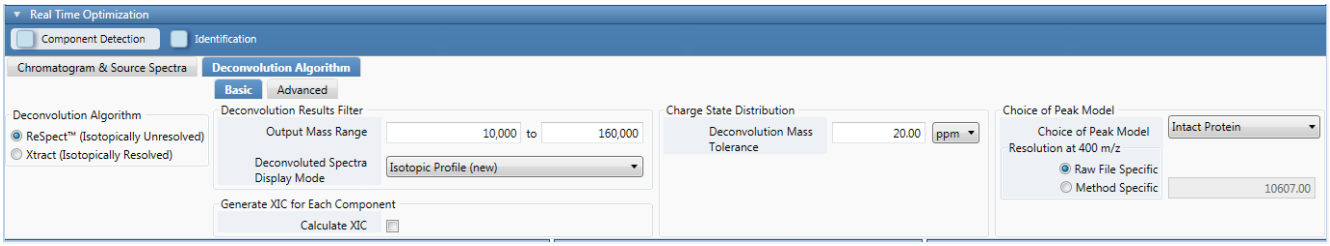


Figure 177 Component Detection pane showing the parameters under the Deconvolution Algorithm subtab

3. Click the **Identification** tab and update the identification parameters as needed.

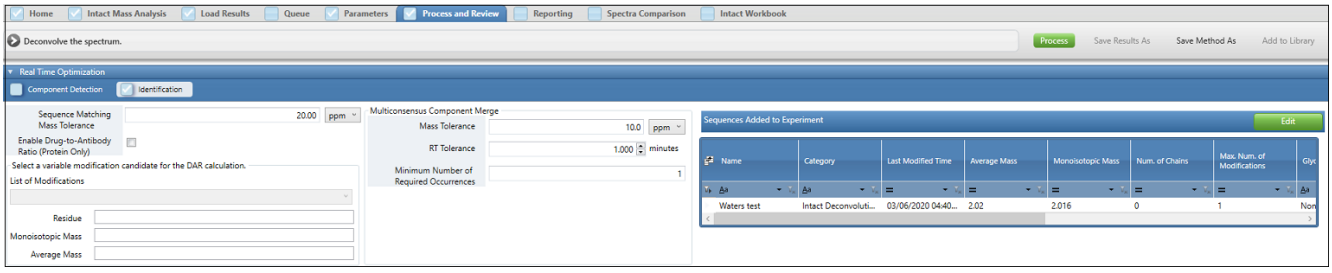


Figure 178 Identification pane for real-time optimization

Note: To change the protein sequence or sequences for target sequence matching, click **Edit** at the upper right area of the Sequences Added to Experiment table. The application opens the Parameters > Identification page where you can add one or more different sequences to the experiment or make other sequence-related changes as needed.

You can only change the modifications, glycosylation, or disulfide bonds information for a protein sequence. You cannot edit the chains.

When you are finished, click the Process and Review tab to return to the Process and Review page for processing. If you add different sequences to the experiment, they appear in the Sequences Added to Experiment table.

4. Click **Process** in the command bar.

   If the application finds invalid or suboptimal parameter entries, it displays an error dialog box to inform you. To continue, enter the required or recommended parameter values within the appropriate ranges.

   Otherwise, the application processes the experiment with your updated parameter settings and displays the new results.

5. When you are satisfied with the parameter settings and the results, do any of the following:

   - Click **Save Method As** in the command bar to save all updated parameter changes to a processing method. In the dialog box, enter a new method name or retain the current name to overwrite the current method parameters, (optional) describe the method, and then click **OK**.

     Note: You cannot overwrite a default method. If you change the parameters in a default method, save the method to a new name.

     Use only alphanumeric, space, and underscore "_", and period "." characters in the method name.

     Thermo Fisher Scientific recommends that you save the method after reprocessing and before saving the results to keep your method and results consistent. You now have the option to use this saved method for future automatic deconvolution processing.

   - Click **Save Results As** in the command bar to save the processed results of the deconvolution to a database. In the dialog box, enter a new experiment name or retain the current name to overwrite the current experiment, (optional) describe the experiment, and then click **OK**.

     In manual mode, the application does not save your results automatically when you process or reprocess an experiment. You must manually save your latest results.

     Note: If an existing experiment is already completed or is canceled, you can overwrite it by using the same name. You cannot overwrite an experiment that is currently submitted to the run queue or is actively processing or canceling.

     Use only alphanumeric, space, underscore "_", and period "." characters, up to 50 maximum, in the experiment name.

     The entry of new names saves your changes and ensures that you do not overwrite the previous results and method parameters.

     You can open the saved experiment results file to view later.

   Otherwise, if you are not satisfied, return to step 2 on page 403.

For more details, see the following topics:

- Working in manual mode (page 347)
- Manual and automatic modes (page 333)
- Editing component detection parameters for intact mass analysis (page 354)
- Editing identification parameters for intact mass analysis (page 380)
- Using the run queue (page 161)
- Opening the results from the load results page (page 399)

Comparing intact mass analysis Spectra

For spectra comparison, you can compare the deconvoluted spectra from two different samples, or you can compare two different averaged spectra from the same LC/MS run.

When you compare any two deconvoluted spectra, the BioPharma Finder application displays a mirror plot. One spectrum in the plot is in the positive direction and the other spectrum in the plot is in the negative direction. You can use the mirror plot to see whether the structures and the relative abundance of masses in the two spectra are divergent or the same. Divergence can indicate that the target protein sequences have been modified by post-translational modifications such as phosphorylation or glycosylation. You can then use top-down proteomics techniques or peptide mapping to determine the exact cause of these changes.

You can compare two spectra generated by the same or different deconvolution algorithms. You can compare a spectrum generated in automatic mode to a spectrum generated in manual mode. You can also view the results generated in automated mode and compare against samples from previously saved results.

The application saves the following information in the database for each spectrum, so that, if necessary, you can reconstruct how you created it:

- The absolute path and name of the original raw data file used to produce each spectrum
- The source spectra method and deconvolution algorithm used to process each spectrum
- The scan range and retention time range that the spectrum was derived from
- The mass of the most abundant component and the total number of components
- The spectrum's creation time (the time when you add it to the library) and description

You can view this information in the Deconvoluted Spectra Library table on the Spectra Comparison page, as shown in the following figure.

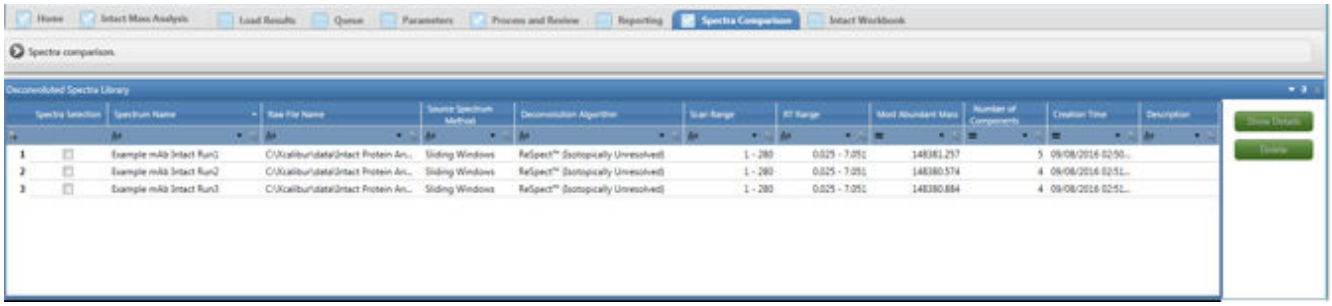


Figure 179 Spectra Comparison page showing the library table

For more details, see the following topics:

- Saving a spectrum to the library (page 406)
- Comparing two deconvoluted Spectra (page 407)
- Displaying spectrum parameters (page 409)
- Deleting Spectra from the library (page 409)
- Spectra comparison page parameters (page 409)
- Spectra comparison page commands (page 410)
- Opening the results from the load results page (page 399)
- Manual and automatic modes (page 333)
- Opening the results from the load results page (page 399)

Saving a spectrum to the library

To save a deconvoluted spectrum to the spectra library for comparison

1. Be sure that the deconvoluted spectrum that you want to save as a comparison spectrum is currently visible in the Deconvoluted Spectrum pane on the Process and Review page.

2. Click **Add to Library** in the upper right corner of the page.

   Note: The Add to Library command is inactive in the following instances:
   - A deconvoluted spectrum is not visible in the Deconvoluted Spectrum pane. In this case, process the experiment to deconvolve the spectrum and enable this command.
   - When you select a row at the component level in the Results table for multiconsensus or DAR-enabled experiments. Instead, select a row at the raw data file level or charge state level to enable this command.
   - When you edit one or more parameters in the Real Time Optimization pane. After editing, you must click Process to reprocess the experiment to enable this command.

3. In the Add to Library dialog box, type the name of the spectrum and optionally a description, and click **OK**.

   Note: Use only alphanumeric, space, underscore "_", and period "." characters in the spectrum name.

If a spectrum of the same name already exists, you can overwrite it. However, you cannot overwrite a spectrum in an experiment that is currently submitted to the run queue, processing, or canceling.

The added spectrum appears in the Deconvoluted Spectra Library table on the Spectra Comparison page. By default, the table sorts the added spectra by creation time (the time when you add it to the library), with the most recently added spectra at the top.

For more details, see the following topics:

- Viewing the deconvoluted Spectra for intact mass analysis (page 433)
- Using the run queue (page 161)

Comparing two deconvoluted Spectra

To compare two deconvoluted spectra

1. Start the experiments to deconvolve the source spectra that you want to compare.
2. After processing, on the Process and Review page, select each of the comparison spectra to add to the library.
3. Click the **Spectra Comparison** tab.
4. (Optional) In the Deconvoluted Spectra Library table, sort the data in each column by clicking in the column header and then clicking either the up arrow, , to sort the data from low to high or the down arrow, , to sort the data from high to low.
5. In this table, select the check boxes for the two comparison spectra.

   Note: You can only select two spectra at a time.

   The Mirror Plot pane now displays a mirror plot with one spectrum in the negative direction and another spectrum in the positive direction. Text at the top and at the bottom of the pane identifies the spectra and raw data file names.

   The mirror plot is scaled to 100 percent in both directions, but the actual intensities of the spectra can be completely different. You can tell the different intensities of the peaks in each spectrum by looking at the NL values for each spectrum.

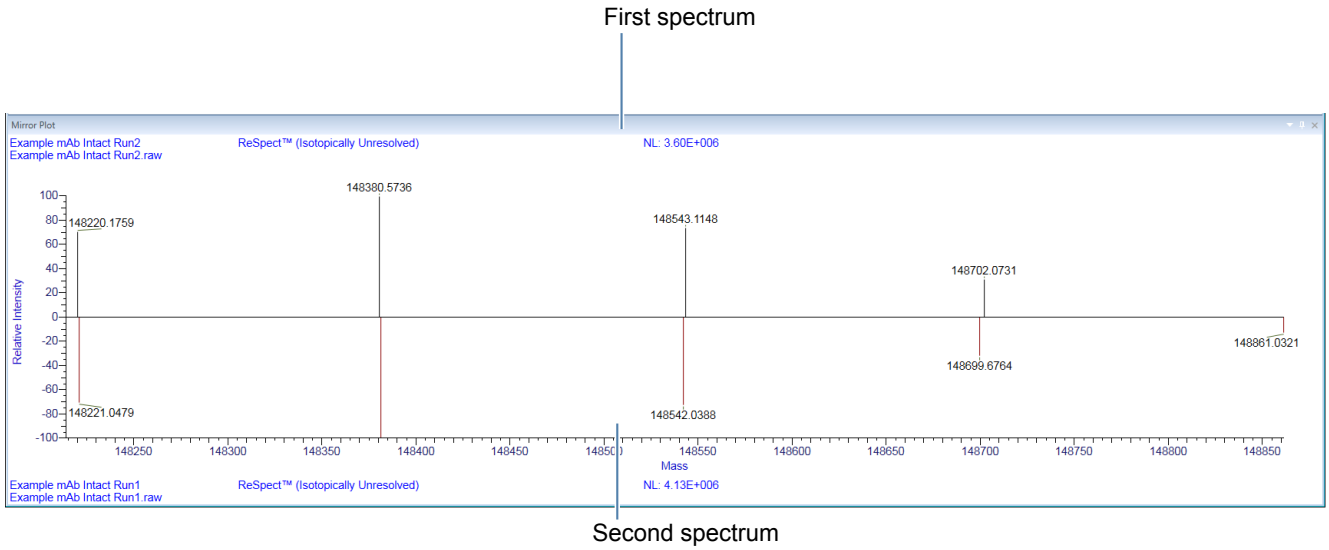


Figure 180 Mirror plot showing the two comparison spectra

If the two compared spectra do not cover the same mass range, the application automatically expands the range in the mirror plot to include the lowest limit and the highest limit of both spectra. For example, if one spectrum has a range of *m/z* 15 000 to 100 000, and the other spectrum has a range of *m/z* 25 000 to 160 000, the application expands the range to *m/z* 15 000 to 160 000 in the mirror plot.

6. (Optional) Right-click in the Mirror Plot pane and choose **Zoom In** to enlarge the plot, or drag the cursor beneath the part of the spectrum that you are interested in.

   The zoom setting in the mirror plot remains the same until you change the deconvolution mass range or load another result. This behavior is the same as that of the deconvoluted spectrum in the Deconvoluted Spectrum pane of the Process and Review page.

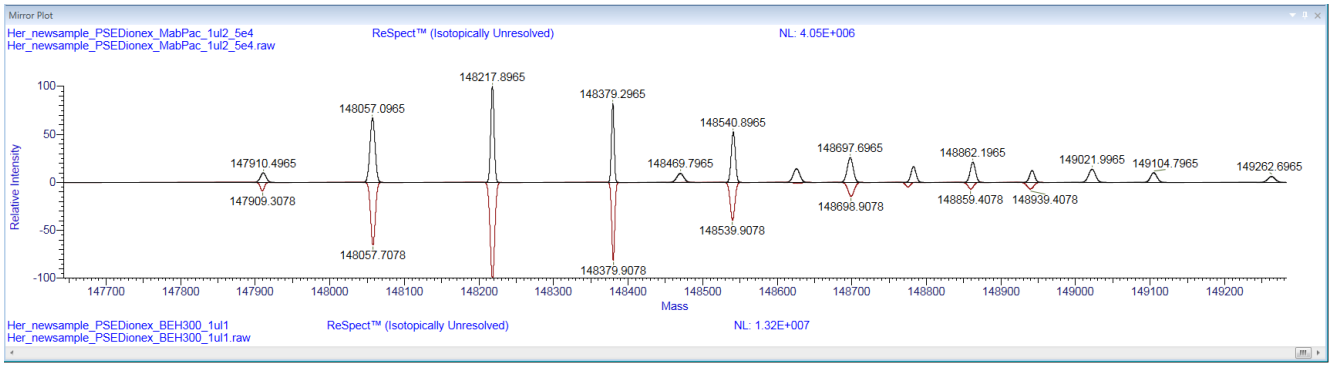


Figure 181 Enlarged mirror plot example

For more details, see the following topics:

- Saving a spectrum to the library (page 406)
- Viewing the deconvoluted Spectra for intact mass analysis (page 433)

Displaying spectrum parameters

To display the settings of the parameters used to generate a comparison spectrum

1. Click the **Spectra Comparison** tab if necessary.
2. Select the row for one spectrum in the Deconvoluted Spectra Library table.
3. Click **Show Details** to display all the deconvolution parameters used to generate the selected spectrum (*not* the currently loaded parameters).

   You can determine whether the two spectra are comparable by comparing these parameters to the parameters that you set to generate the spectrum for the current experiment.

Deleting Spectra from the library

To delete spectra from the library

1. Click the **Spectra Comparison** tab if necessary.
2. Select the rows for the appropriate spectra in the Deconvoluted Spectra Library table and click **Delete**.
3. In the confirmation box, click **Yes**.

Spectra comparison page parameters

The following table lists the parameters that are available on the Spectra Comparison page.

Table 53 Spectra Comparison page parameters

Parameter	Description
Deconvoluted Spectra Library table	Displays the spectra that you can select to show in the mirror plot for comparison.
Row number	The number assigned to each spectrum in the table. This sequential numbering does not change when you sort or filter the table.
Spectra Selection ☐	Select the check box for a spectrum that you want to compare using the Mirror Plot. You can select only up to two spectra at a time.
Spectrum Name	Displays the name of each spectrum available in the library.
Raw File Name	Displays the name of the original raw data file that produced each spectrum.
Source Spectrum Method	Displays the source spectrum method used to process each spectrum.
Deconvolution Algorithm	Displays the deconvolution algorithm used to process each spectrum.

Parameter	Description
Scan Range	Displays the scan range that the each spectrum was derived from.
RT Range	Displays the retention time range that each spectrum was derived from.
Most Abundant Mass	Displays the mass of the most abundant component in the each spectrum.
Number of Components	Displays the number of components in each spectrum.
Creation Time	Displays the date and time of when you add each spectrum to the library.
Description	Displays the description of each spectrum as you specified it in the Add to Library dialog box.
Buttons	
Show Details	Opens a Spectrum Information box displaying the parameters used to produce the spectrum that you selected in the library.
Delete	Deletes the selected spectra in the library.
Mirror Plot Pane	Displays the mirror plot of the selected spectra for comparison.
Spectrum Name	Displays the name of each spectrum that you specify when you add it to the library.
Raw Data File	Displays the name of the raw data file used to produce each spectrum.
Deconvolution Algorithm	Displays the deconvolution algorithm used to produce each spectrum.
NL	Displays the intensity of the most abundant peak in each spectrum.
Relative Intensity (*y* axis)	Displays the ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity.
Mass (*x* axis)	Displays the mass of the ions formed from molecules.

Spectra comparison page commands

Right-clicking the Mirror Plot pane on the Spectra Comparison page opens a shortcut menu that contains the commands listed in the following table.

Table 54 Mirror Plot pane shortcut menu commands

Command	Description
Reverse Order	Reverses the order of the two spectra in the Mirror Plot pane.
Reset Scale	Restores to the scale of the original plot that first appeared in the Mirror Plot pane.
Copy as Displayed	Copies the image in the Mirror Plot pane to the Clipboard. You cannot save the mirror plot, but you can copy its contents to the Clipboard to use in third-party applications.

Command	Description
Copy per Global Settings	Copies the image in the Mirror Plot pane to the Clipboard based on the global image dimensions set in the Intact Mass Analysis Settings dialog box. You cannot save the mirror plot, but you can copy its contents to the Clipboard to use in third-party applications.
Zoom Out	Shrinks the view in the pane by a factor of 2.
Zoom In	Enlarges the view in the pane by a factor of 2.

For more details, see the following topics:

- Using copy and paste functions (page 666)
- Specifying the image dimensions (page 37)

Organizing the experimental results

Use the Experiment Management pane to organize the experimental results in different folders. The Master List contains all of the previously saved Intact Mass Analysis experimental results. In the Working List, you can create folders or subfolders and drag and drop results from the Working List to any folder that you created. The Master List folder shows all the experimental results regardless of the folder in which they reside.

To access the Experiment Management pane

1. On the Home page, click **Intact Mass Analysis** in the left pane or below the BioPharma Finder splash graphic.

   The Intact Mass Analysis page opens.

2. Click the **Load Results** tab.

   The Load Results page opens to the Master List by default. The BioPharma Finder application displays all of the previously saved Intact Mass Analysis experimental results in the Master List.

3. Do one of the following to access the Experiment Management pane.

   - Click « to expand the Experiment Management pane.

     The Experiment Management pane opens to display the Master List and the Working List.

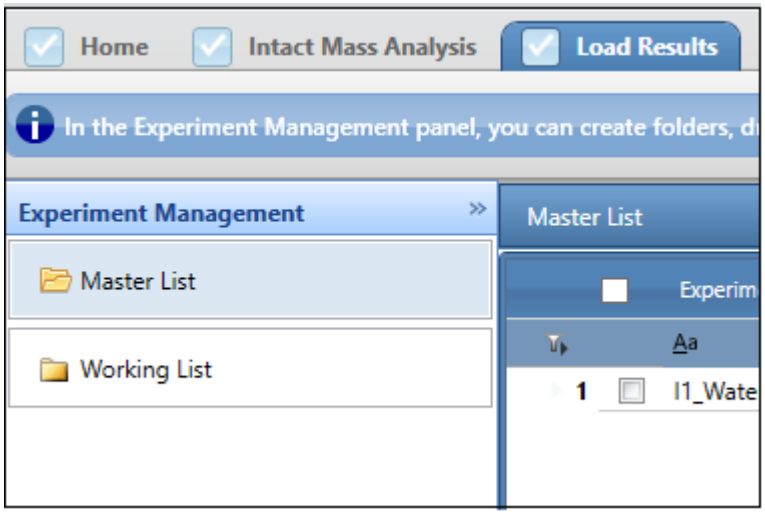


Figure 182 Experiment Management pane

–or–

- Click the **Experiment Management** pane on the left.
  A window appears showing the Master List and the Working List.

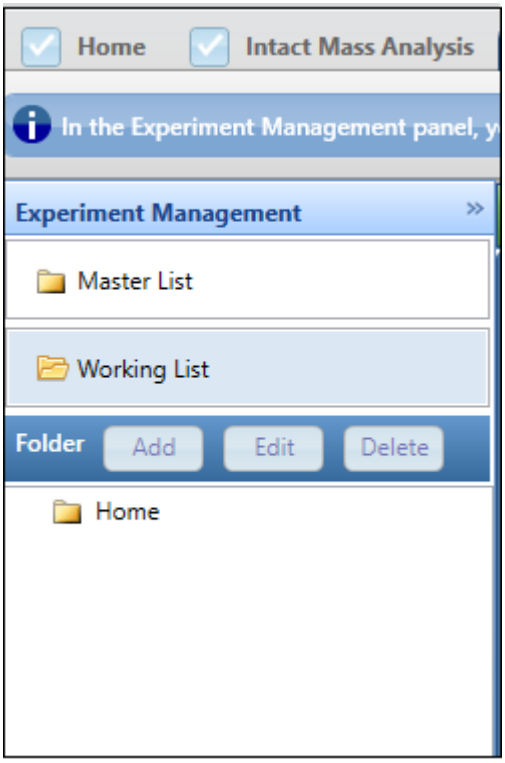


Figure 183 Experiment Management window

To create a folder in the Working List

1. In the Experiment Management pane of the Load Results page, select the **Working List**.
   By default, the Working List contains the Home folder.
2. Click the **Home** folder, and then click **Add**.

   Note: You can only create a new folder under the Home folder of the Working List. You cannot create a folder in the Master List.
3. In the Add New Folder dialog box, type the Folder Name, and then click **Add**.
   A new folder appears under the Home folder in the Working List.
4. To create a subfolder, select the folder to which you want to add a subfolder, and then click **Add**.
   A new subfolder appears under the folder.
   Each folder can contain subfolders, and each subfolder, in turn, can contain more subfolders, and so on.

To rename a folder

1. In the Experiment Management pane of the Load Results page, select the **Working List** to view its folder structure.
   When the Working List opens, only the Home folder appears in the pane.
2. To navigate through the folder structure, click the arrow next to a folder.
3. To rename a folder, select the folder of interest, and then click **Edit**.
4. In the dialog box, type the new name.

   Note: You can only rename a user-created folder.

To move an experiment result to a folder

1. In the Experiment Management pane of the Load Results page, select the **Working List** to view its folder structure.
   The right pane shows the contents of the Working List.
2. From the Working List, select an experiment result or use the CTRL key to select multiple results, and then drag and drop to the destination folder of interest.
   The moved experimental result or results appear in the new destination folder.

To delete an experiment result from a folder

1. In the Experiment Management pane of the Load Results page, select the **Working List** to view its folder structure.
   When the Working List opens, only the Home folder appears in the pane.
2. To navigate through the folder structure, click the arrow next to a folder.
3. Select the folder that contains the result to delete.
   The right pane shows the contents of the selected folder.
4. To delete an experiment result, select the check box corresponding to the result.
   You can also select multiple check boxes to delete more than one result.
   In the command bar, click **Delete**, and do one of the following:
   - In the Delete Selected Results from Folder dialog box, select **Delete Results from Folder**.
     The application deletes the experiment result from the folder and moves it to the Working List.
     –or–
   - In the Delete Selected Results from Folder dialog box, select **Delete Results from Folder and Master List**.
     The application deletes the experiment result from both the folder and the Master List.

To delete a folder

1. In the Experiment Management pane of the Load Results page, select the **Working List** to view its folder structure.
   When the Working List opens, only the Home folder appears in the pane.
2. To navigate through the folder structure, click the arrow next to a folder.
3. To delete a folder, select the folder.
4. In the Experiment Management pane, click **Delete**.

5. In the Delete Selected Folder dialog box, do one of the following:
   - Select **Delete Folder**.
     The application deletes the folder and moves its experiment result content (if any) to the Working List.
     –or–
   - Select **Delete Folder and Results from Master List**.
     The application deletes both the folder and its experiment result content from the Master List.

21 Viewing the process and review page for intact mass analysis

You can see the results of an intact protein experiment on the Process and Review page when the application has completed processing. At completion, the areas on this page display the chromatograms, spectra, and Results table.

In manual mode, after setting the appropriate parameters on the Process and Review page, click **Process** to see the deconvolution results. You can then save the results to view from the Load Results page.

In automatic mode, after processing is completed, use the Queue page or the Load Results page to open the results. For an auto peak detection experiment in automatic mode, the application displays a result for each peak shown in the Number of Chromatographic Peaks column on the Queue page. For example, if the Number of Chromatographic Peaks column displays 4, the Results table on the Process and Review page displays four result rows.

The current experiment name appears in the upper right corner of the page. If you load only one raw data file for the experiment, its name also appears below the experiment name; otherwise, "(multiple files)" appears. The values in the columns of the Results table represent the outputs of the deconvolution.

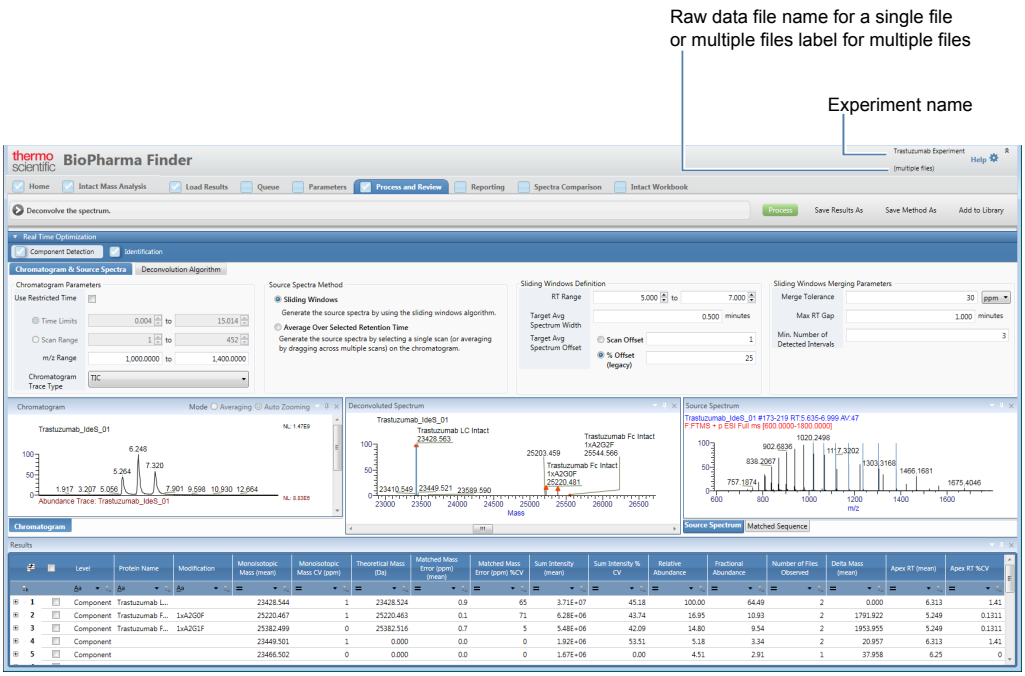


Figure 184 Process and Review page

Note: When you do any of the following, the layout dimensions of the Process and Review page remain fixed:

- Navigate away from the Process and Review page to another page.
- Open results from the run queue.
- Load previous results.
- Switch algorithms from Xtract to ReSpect or from ReSpect to Xtract.

However, after you close and reopen the application, the layout dimensions revert to their defaults.

For more details, see the following topics:

- Manual and automatic modes (page 333)
- Working in manual mode (page 347)
- Opening the results from the load results page (page 399)
- Opening the results from the queue page (page 398)
- Queue page parameters (page 172)

Process and review page parameters for intact mass analysis

The following table describes the types of information on the Process and Review page. To display the content of a pane that is not currently visible, click the corresponding subtab. You can also adjust the size or location of the panes on this page.

Table 55 Process and Review page parameters

Parameter	Description
Results table	At the upper level, displays the components detected during the deconvolution and their masses, intensities, along with their quality scores. At the lower levels, the table displays the raw data file (for Multiconsensus result format only) and charge state information.
Chromatogram pane	Displays the chromatogram from each raw data file loaded for the experiment.
Relative Intensity (*y* axis)	Displays the ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity.
RT (min) (*x* axis)	Displays the retention time of the spectrum, which is the time after injection at which a compound elutes. Retention time can also refer to the total time that the compound is retained on the chromatograph column.
Deconvoluted Spectrum pane	Displays each deconvoluted spectrum that results from applying the Xtract/ReSpect algorithm.
Relative Intensity (*y* axis)	Displays the ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity.
Mass (*x* axis)	Displays the mass of the ions formed from molecules.
Source Spectrum pane	Displays each source spectrum before deconvolution. For as average over RT deconvolution, you can use the Chromatogram pane to select the source spectra.
Relative Intensity (*y* axis)	Displays the ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity.
m/z (*x* axis)	Displays the mass-to-charge ratio of ions formed from molecules. This ratio is the quantity formed by dividing the mass of an ion, in daltons, by the number of charges carried by the ion.
Matched Sequence pane	Displays the matched component, its drug load value, and the matched sequence information. You can specify a different drug load or select a different identification in this pane to update the calculated drug-to-antibody ratio (DAR) values.

Parameter	Description
Average DAR pane	(Visible only if Enable Drug-to-Antibody Ratio is selected in the processing method) Displays the calculated average drug-to-antibody ratio (DAR) values. You can specify a different drug load in the Matched Sequence pane or select a different subset of components in the Results table to update the calculated values.
Real Time Optimization pane	Displays the same parameters as those on the Parameters > Component Detection and Identification pages, so that you can adjust these parameters in manual mode and perform real-time optimization. When you make a change to the parameters on the Component Detection and Identification pages, the application automatically updates the parameters on the Process and Review page, and vice versa.

Note: If the *x*- or *y*-axis label in a pane is not visible, enlarge the pane or change the pane to a larger floating window.

For more details, see the following topics:

- Rearranging the panes (page 655)
- Viewing the results table for intact mass analysis (page 422)
- Viewing the chromatograms for intact mass analysis (page 427)
- Viewing the deconvoluted Spectra for intact mass analysis (page 433)
- Viewing the source Spectra for intact mass analysis (page 437)
- Viewing the matched sequence information for intact mass analysis (page 440)
- Enable Drug-to-Antibody Ratio (page 384) parameter
- Viewing the average DAR values for intact mass analysis (page 443)
- Manual and automatic modes (page 333)
- Using Real-Time optimization for intact mass analysis (page 402)

Process and review page commands for intact mass analysis

The following table describes the commands in the command bar of the Process and Review page.

Table 56 Commands on the Process and Review page

Command	Description
Process	Processes the intact protein experiment and deconvolves the spectra with any or all of these parameters: modified protein sequence, component detection, and identification. Also, displays the new results. **IMPORTANT!** To activate the Process button, you must modify the experiment parameters. If the experiment uses the Average Over Selected Retention Time option for the source spectra method, all of the loaded raw data files need an available source spectrum before you can process the experiment. If the experiment uses the Auto Peak Detection option for the source spectra method, the Process button is always dimmed. You can only view the parameters and results; you cannot process the experiment.
Save Results As	Saves the latest results in a database after you process a deconvolution analysis. Click this button to open a dialog box where you can enter a new experiment name or retain the same name to overwrite previously saved results in the current experiment with the new results. You can also enter a description for the experiment. This button is inactive if you modified any processing method parameter for real-time optimization. In this case, click **Process** to reprocess the experiment and reactivate this button. **Note:** Use only alphanumeric, space, underscore "_", and period "." characters, up to 50 maximum, in the experiment name. You cannot overwrite an experiment that is currently submitted to the run queue or is actively processing or canceling. If you want to analyze another averaged spectrum from the same LC/MS data file, edit the component detection parameters to select a different source spectrum from the Chromatogram pane. You can delete the previously saved results from the Load Results page.

Command	Description
Save Method As	Saves the updated parameters in the Real Time Optimization pane, the chromatograms in the Chromatogram pane, and the source spectra in the Source Spectrum pane, to a processing method in a database. Click this button to open a dialog box where you can enter a new method name or retain the same name to overwrite all saved parameters in the method with the new parameter settings. You can also enter a description for the method. **Note:** Use only alphanumeric, space, underscore "_", and period "." characters in the method name. You cannot overwrite a default method.
Add to Library	Saves the current deconvoluted spectrum to the spectra library, along with the associated data. You can then compare it to any other saved spectrum in this library. This button is inactive if you modified any processing method parameter for real-time optimization. In this case, click **Process** to reprocess the experiment and reactivate this button.

For more details, see the following topics:

- Using Real-Time optimization for intact mass analysis (page 402)
- Using the run queue (page 161)
- Editing component detection parameters for intact mass analysis (page 354)
- To view, delete, or convert the results of an experiment from the Load Results page (page 399)
- To save a deconvoluted spectrum to the spectra library for comparison (page 406)

Canceling Sliding Windows processing

On the Process and Review page during processing for an Intact Mass Analysis sliding windows experiment, a green status bar appears to indicate the real-time percentage of completion. The sliding windows appear and move across each chromatogram. For a multiconsensus sliding windows experiment, the status bar shows the status of each raw data file.

To stop the sliding windows processing

Click the **Cancel** icon,  , in the command bar of the Process and Review page.

Note: When you cancel, the application does not save the results. To enable the Process button after canceling, change one or more parameter settings on the Process and Review page.
For other types of experiments, during processing, the cursor becomes a progress circle. You cannot stop the processing for these experiments.

Note: If you are viewing results of a sliding windows experiment while another experiment is running in the background, some of the data might be affected. If you suspect that the source spectra, abundance trace, or XIC is missing, close and reopen the sliding windows experiment to refresh the results display.

Viewing the results table for intact mass analysis

The Results table on the Process and Review page displays the results of a completed Intact Mass Analysis experiment, organized by the components at the upper level and by either of these possibilities:

- The charge states at the lower level for single-file or batch experiments

–or–

- The raw data files and charge states at the lower levels for multiconsensus experiments

Note: For a single-file or batch experiment with the drug-to-antibody ratio (DAR) feature enabled (Enable Drug-to-Antibody Ratio is selected in the processing method), the Results table shows the raw data file level, which is similar to the Results table for a multiconsensus experiment.

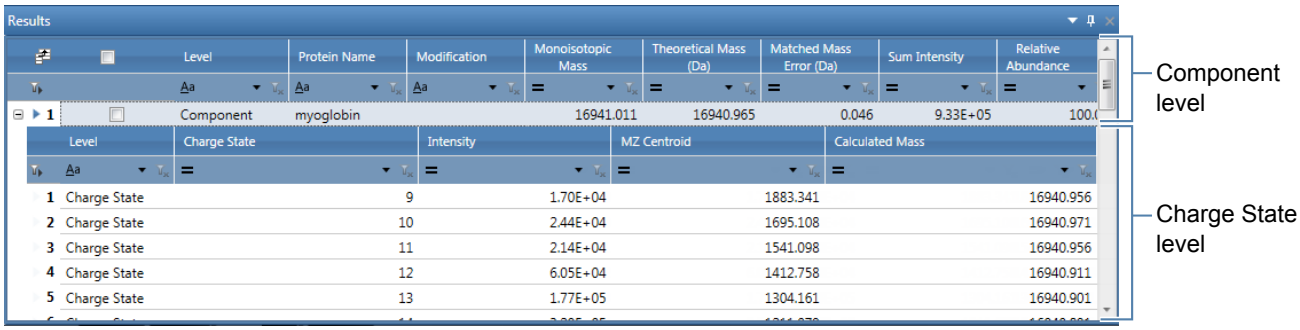


Figure 185 Results table on the Process and Review page with two levels

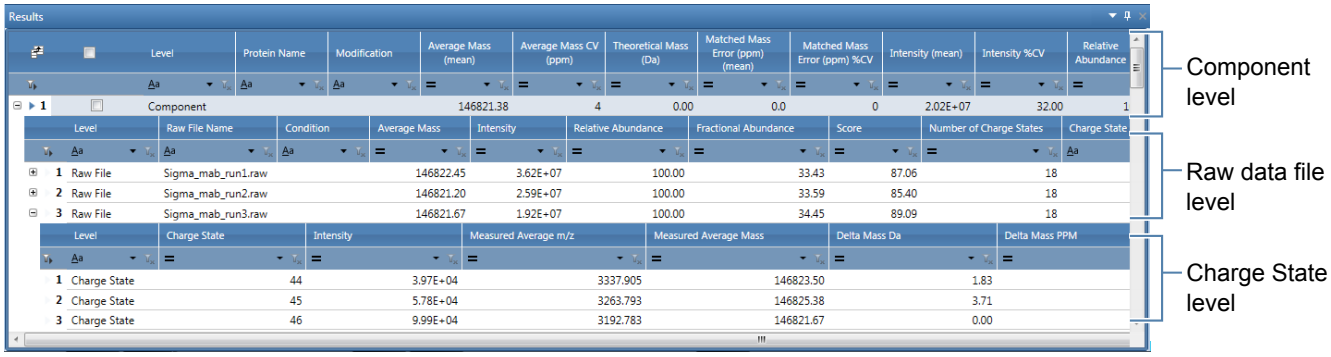


Figure 186 Results table on the Process and Review page with three levels

The columns and levels in the Results table vary depending on the selection of the Result Format for Multiple Files option for the experiment, the Source Spectra Method option, the Deconvolution Algorithm option, whether the DAR feature is enabled, and other settings in the processing method.

IMPORTANT! Because the BioPharma Finder application uses an updated algorithm, the calculated mass values in the Results table from this application might be slightly different from the calculated masses from the Protein Deconvolution application.

For more details, see the following topics:

- Viewing the intact results table (page 423)
- Exporting the results table (page 424)
- Saving an intact workbook (page 425)
- Results table commands (page 426)
- Selecting a reference mass to calculate mass differences (page 426)

Viewing the intact results table

To view data in the Results table

1. Click the **Process and Review** tab.

   The Process and Review page displays the component results for Intact Mass Analysis in the Results table.

2. Click a component row in the table to view information that is related to that component in the other panes on this page.

3. (For multiconsensus or DAR-enabled experiments only) Click the plus icon,  , at the left side of a component row to view raw data file information that is related to the top-level component.

4. Click the plus icon,  , at the left side of a component row (or a raw data file row) to view charge state information that is related to the top-level component.

   The values in the bottom level columns represent the isotopic clusters with different charge states from the source spectrum that was used to produce the peak in the deconvoluted spectrum.

For more details, see the following topics:

- Various results tables for intact mass analysis (page 448)
- Viewing the results table for intact mass analysis (page 422)

Exporting the results table

To export the data in the Results table

1. On the Process and Review page, right-click anywhere in the Results table for Intact Mass Analysis and choose from these options:
   - **Export All** to export all results of a certain type to an Excel file:
     - **Component Level Only**—To export only the results at the component level in the current Results table.

       The exported results do not include the data in the rows that appear when you click the plus icon,  , at the left side of each component row.
     - **All Levels**—To export the results at all levels in the current Results table.

   –or–
   - **Export Checked** to export only the *selected* results of a certain type to an Excel file:
     - **Component Level Only**—To export only the *selected* results at the component level in the current Results table.

       The exported results do not include the data in the rows that appear when you click the plus icon,  , at the left side of each component row.
     - **All Levels**—To export only the *selected* results at all levels in the current Results table.

     To select/deselect a row of results to export, select/clear the check box in that row.

     To select/deselect all of the rows, select/clear the check box in the column header.

2. In the Save As dialog box, browse to or type the name of the file to store the results in.

3. Click **Save**.

   The application stores the exported data in the specified file. If you do not browse to a different folder, the application places the exported file by default in the folder with the raw data files for the experiment.

   Note: For an experiment using the Multiconsensus result format (or with the DAR feature enabled), the export of the component level results includes the following columns from the raw data file level for each raw data file:
   - Intensity
   - Monoisotopic Mass (for Xtract)

     –or–

     Average Mass (for ReSpect)

For more details, see the following topics:

- Intensity (page 455) column
- Monoisotopic Mass (page 466) column
- Average Mass (page 468) column

Saving an intact workbook

An intact workbook contains the intact deconvolution results saved in an Intact Mass Analysis experiment and the method parameters used to process those results, but not the protein sequences. You can export data from a workbook to a file compatible with the Chromeleon data system.

IMPORTANT! You can save data to a workbook only for average over RT or sliding windows experiments, not for auto peak detection experiments.

To save the results and parameters for Intact Mass Analysis to a workbook

1. Load an experiment to open the Process and Review page and view the results.
2. (Optional) In the Results table, select the check box in the row of each component that you want to save to the workbook.

   To select/deselect a row of results to save, select/clear the check box in that row.

   To select/deselect all of the rows, select/clear the check box in the column header.
3. To save the results and processing parameters to a workbook, right-click anywhere in the Results table, choose **Save As Intact Workbook** and then choose one of these submenu options:
   - **All** to save all of the results in the table to the workbook
   - **Checked** to save only the selected results in the table to the workbook
4. In the Save Intact Workbook As dialog box, type a name for the workbook and, optionally, a description.

   The default workbook name is the same as the experiment name, and you can enter a new workbook name as needed.

   Note: Use only alphanumeric, space, underscore "_", and period "." characters in the workbook name.
5. Click **OK**.

   If a workbook of the same name already exists, indicate whether you want to overwrite it.

   Note: If the workbook is currently open on the Workbook Editor page for editing, indicate whether you want the application to automatically save and close the open workbook before overwriting.

   The application saves your selection of components to the workbook. After saving, you can manage the workbook using the Intact Workbook page.

For more details, see the following topics:

- Viewing the intact mass analysis results (page 398)
- Editing a workbook (page 154)
- Managing a workbook (page 150)

Results table commands

Right-clicking the Results table for Intact Mass Analysis on the Process and Review page opens a shortcut menu that contains the commands listed in the following table.

Table 57 Results table shortcut menu

Command	Description
Set As Reference Component	Sets the mass of the chosen component as the reference mass. Resets the value in the Delta Mass column of the Results table to 0 for the chosen component. Also, recalculates the delta mass value for all other components in the Results table relative to the chosen reference mass.
Export All	Exports both the selected and deselected results in the Results table to a file.
Export Checked	Exports only the *selected* results in the Results table to a file.
Save As Intact Workbook	(For average over RT or sliding windows experiments) Saves the component results and processing parameters to a workbook that is Chromeleon-compatible.

For more details, see the following topics:

- Delta Mass (page 451) column
- Selecting a reference mass to calculate mass differences (page 426)
- Exporting the results table (page 424)
- Saving an intact workbook (page 425)

Selecting a reference mass to calculate mass differences

A reference mass is usually the mass of the most abundant peak in the results of an Intact Mass Analysis experiment. The application compares the mass of all other peaks in the data set to the reference mass and places these differences in the Delta Mass column of the Results table on the Process and Review page.

The delta mass is useful in revealing the possible modifications based on the mass losses or gains compared to reference mass. However, you can select the mass of another component in the table to use as the reference mass for a given deconvoluted spectrum. The default Delta Mass of this new reference component is 0. The application then recalculates the mass of other peaks in the data set compared to this new reference mass and updates the Delta Mass values for all of these components in the Results table.

To select a new reference mass and reset the Delta Mass values

1. In the Results table on the Process and Review page, right-click the row of the component whose mass you want to use as the reference mass.
2. Choose **Set as Reference Component**.

The results of an auto peak detection experiment include results for multiple chromatographic peaks. Each peak's deconvolution results have a maximum intensity mass that the application uses to calculate the delta mass values for that peak.

If you save the results, the application saves the new reference mass and updated Delta Mass values. If you export the results to the spreadsheet application, the spreadsheet file reflects the updated state of the reference mass.

When you load results that you previously saved, you can change the reference component, but the application does not update the reports to reflect the change to the Results table because it cannot change reports that it already generated.

Viewing the chromatograms for intact mass analysis

The Chromatogram pane on the Process and Review page displays the chromatogram plot at the retention time range specified by the Time Limits/Scan Range parameters on the Parameters > Component Detection page, or in the Real Time Optimization > Component Detection pane of the Process and Review page.

The chromatogram plot can be any of these types, depending on the parameter settings for the processing method:

- Total ion current (TIC) if you select TIC for the Chromatogram Trace Type parameter
- Base peak chromatogram (BPC) if you select BPC for the Chromatogram Trace Type parameter

  The BPC shows only the most intense peak in each MS spectrum at every point in the analysis.
- Extracted ion chromatogram (XIC) if you select the check box for the Calculate XIC parameter
- Abundance trace if you select Sliding Windows for the Source Spectra Method parameter
- Chromatogram with detected peaks in the blue shaded areas if you select Auto Peak Detection for the Source Spectra Method parameter

For example, the following figure shows the chromatogram plots when you select the TIC option for the Chromatogram Trace Type parameter in the method, with the RT Range indicated by the red box. If you load multiple raw data files for the experiment, the Chromatogram pane displays an individual chromatogram plot for each of the raw data files, stacked on top of each other. The raw data file name appears at the top of each plot.

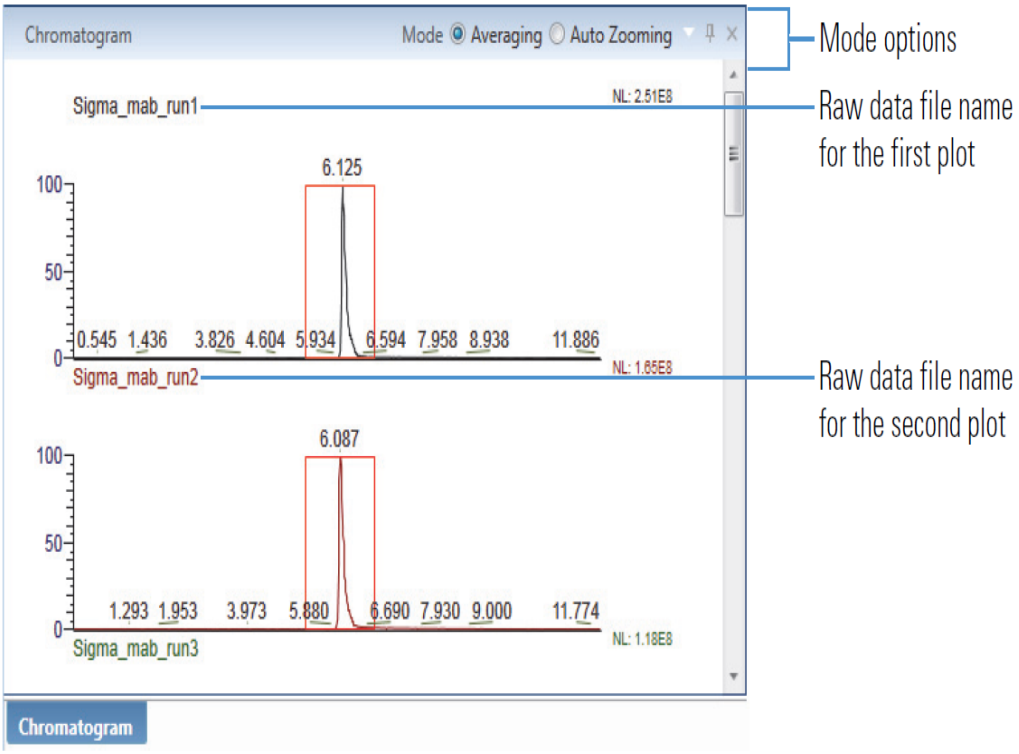


Figure 187 Chromatogram pane showing multiple plots with TIC as the trace type

Note: If the pane is too small for you to see the Mode options at the upper right corner or the *y* axis label, adjust the width or height of the pane.

For more details, see the following topics:

- Viewing the chromatograms (page 429)
- Chromatogram pane options (page 429)
- Chromatogram pane commands (page 430)
- Average over RT deconvolution and auto peak detection chromatograms (page 430)
- Sliding windows deconvolution chromatograms (page 431)

Viewing the chromatograms

To view the chromatograms in the Chromatogram pane

1. Click the **Process and Review** tab if necessary.
2. Do one of the following:
   - Click the row of a component in the Results table.

     The Chromatogram pane shows one chromatogram plot (for a single loaded raw data file in the experiment) or multiple stacked plots (for multiple loaded raw data files).

   –or–

   - (If available) Click the plus (+) sign to the left of a component row, and then click the row of one of the raw data files in the Results table.

     The Chromatogram pane shows the chromatogram plot from the selected raw data file.

In the chromatogram plot, the *x* axis represents the retention time range and the *y* axis indicates the relative intensity. The plot displays the plot type and raw data file name at the top and the Normalized Largest (NL) intensity on the upper right side. It does not display peak labels, such as the scan number, or the header information.

Chromatogram pane options

The following table lists the options in the upper right corner of the Chromatogram pane on the Process and Review page.

Table 58 Chromatogram pane options

Command	Description
Mode	Determines the available options: • **Averaging:** Averages the spectra for all the scans in the retention time range that you drag the cursor over (left to right) and displays them in the Source Spectrum pane. • **Auto Zooming:** Enlarges the area (intensity and time) that you drag the cursor over without changing the view displayed in the Source Spectrum pane.

Chromatogram pane commands

Right-clicking the Chromatogram pane on the Process and Review page opens a shortcut menu with the commands listed in the following table.

Table 59 Chromatogram pane shortcut menu

Command	Description
Reset Scale	Restores the original chromatogram that first appeared in the pane.
Copy as Displayed	Copies the image in the pane to the Clipboard.
Copy per Global Settings	Copies the image in the pane to the Clipboard based on the global image dimensions set in the Intact Mass Analysis Settings dialog box.
Zoom Out	Shrinks the view in the pane by a factor of 2.
Zoom In	Enlarges the view in the pane by a factor of 2.

For more details, see the following topics:

- Using copy and paste functions (page 666)
- Specifying the image dimensions (page 37)

Average over RT deconvolution and auto peak detection chromatograms

When you select the Calculate XIC check box on the Parameters > Component Detection page or in the Process and Review > Real Time Optimization pane for the processing method, after processing, the Chromatogram pane also displays the isotopic cluster's XIC for each component in the Results table. (If you did not select the Calculate XIC check box, the application displays a chromatogram in the Chromatogram pane but does not display the XIC for a given component.)

The XIC, which is the red peak in the following figure, indicates where the compound eluted. It is unique to the Chromatogram pane on the Process and Review page.

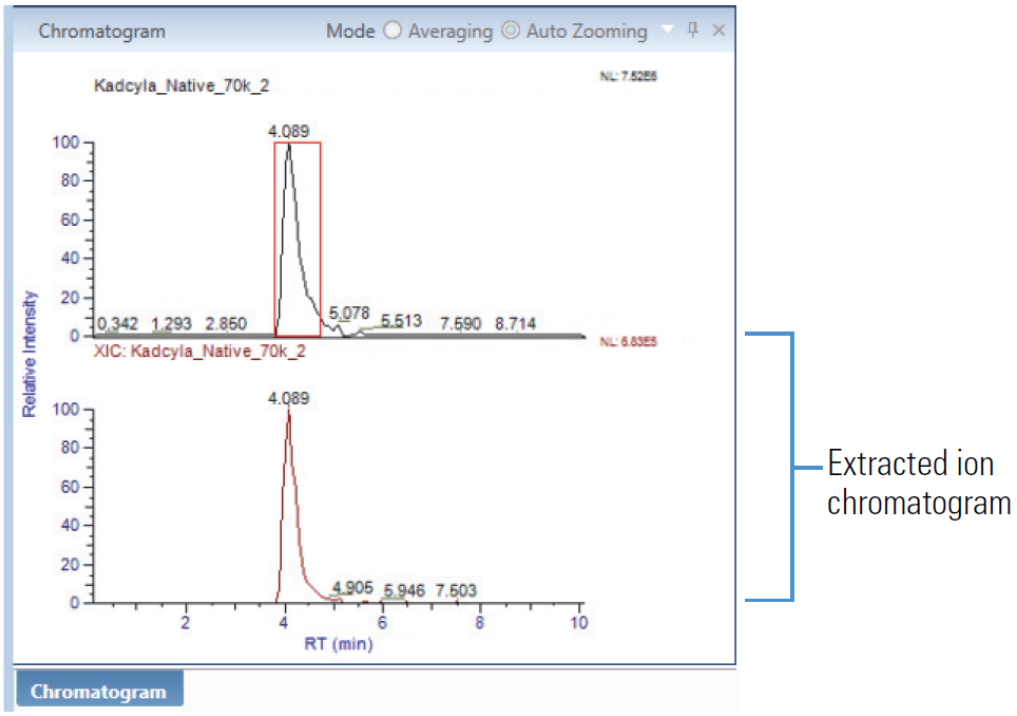


Figure 188 Chromatogram pane showing an extracted ion chromatogram

The Chromatogram pane also highlights in a red box the range used to produce the source spectrum for the deconvolved result.

When you click an individual charge state of a selected isotopic cluster, the XIC in the Chromatogram pane does not change.

For more details, see the following topics:

- Calculate XIC (page 376) check box

Sliding windows deconvolution chromatograms

By default, the Chromatogram pane on the Process and Review page displays the retention time range used in the raw data file or files.

When you set the RT Range parameter on the Parameters > Component Detection page, the Process and Review > Real Time Optimization pane of the Process and Review page displays that same retention time range, and vice versa.

When you select the Use Restricted Time check box and set the values of the Time Limits/Scan Range parameters on the Parameters > Component Detection page or in the Process and Review > Real Time Optimization pane, the Chromatogram pane displays the range set by these parameters, but it starts processing the spectrum at the lower value set by the RT Range parameter.

The following occurs during or after processing:

- The application displays a green bar indicating the progress of the deconvolution. Sliding window deconvolutions take longer than average over RT deconvolutions.
- The Chromatogram pane of the Process and Review page displays the following:
  - During processing, a red box that progressively moves across each group of spectra that the application deconvolves
  - When processing is completed, the abundance trace below the chromatogram plot

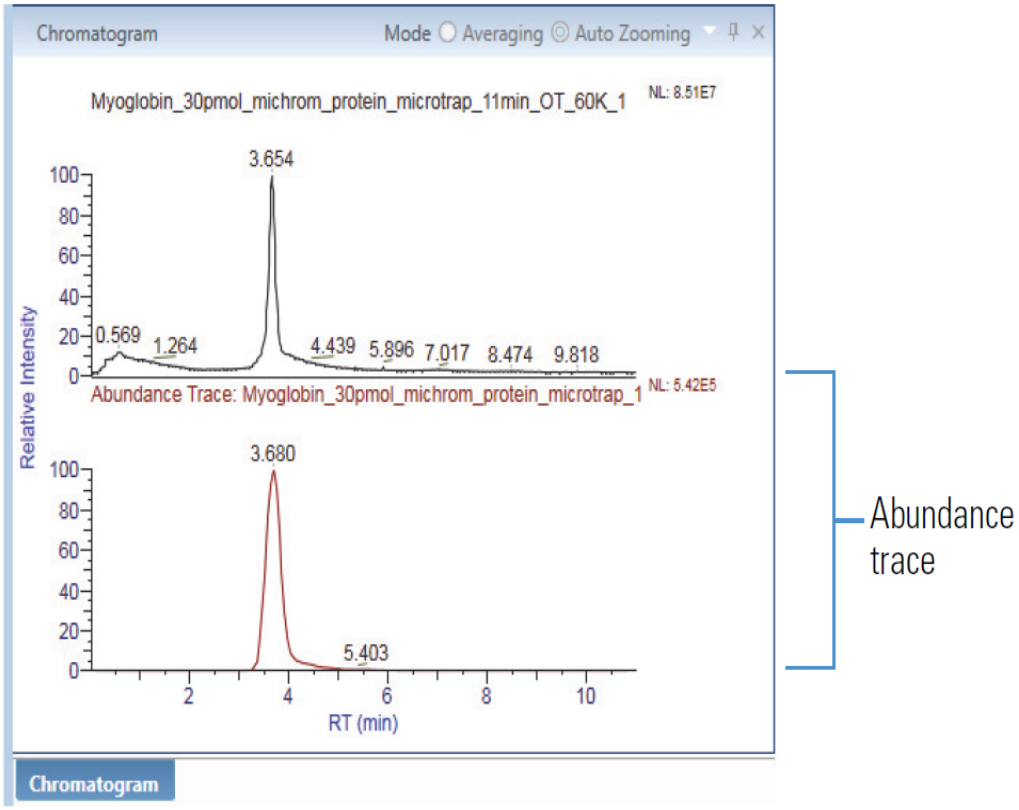


Figure 189 Chromatogram pane showing an abundance trace

For more details, see the following topics:

- RT Range (page 370) parameter
- Use Restricted Time (page 365) check box
- Time Limits (page 365) parameter
- Scan Range (page 365) parameter

Viewing the deconvoluted Spectra for intact mass analysis

The Deconvoluted Spectrum pane on the Process and Review page displays the deconvoluted spectra with the identified masses after the BioPharma Finder application applies the Xtract/ReSpect algorithm. For the Xtract algorithm, this pane displays each deconvoluted spectrum as a set of centroid peaks in mass and relative intensity. For the ReSpect algorithm, this pane displays each deconvoluted spectrum as a profile in mass and intensity with a set of peak labels.

In addition, for ReSpect, you can select from two different modes to display the deconvoluted spectra: Mass Probability Distribution Profile (legacy) and Isotopic Profile (new).

When you load multiple raw data files for the experiment, this pane displays a deconvoluted spectrum per raw data file, stacked one on top of the other. The raw data file name appears at the top of each spectral plot.

When you select a component row in the Results table, each spectral plot also displays a blue line, that represents the following:

- In an Xtract deconvolution, the monoisotopic mass of the selected component (in the Monoisotopic Mass column at the component or raw data file level)
- In a ReSpect deconvolution, the average mass of the selected component (in the Average Mass column at the component or raw data file level)

  In a spectrum deconvolved with the ReSpect algorithm, the area of a peak is proportional to the intensity of the associated component (charge-peak state), so lower-quality results are associated with wider peaks. If two components have equal intensities, the component with the lower-quality results has a wider peak.

  The range processed for the Deconvoluted Spectrum plot is limited by the setting of the Model Mass Range values on the Parameters > Component Detection page or in the Process and Review > Real Time Optimization pane. Using the same settings of the Model Mass Range parameter, you can deconvolve several spectra with the exact same range and compare them.

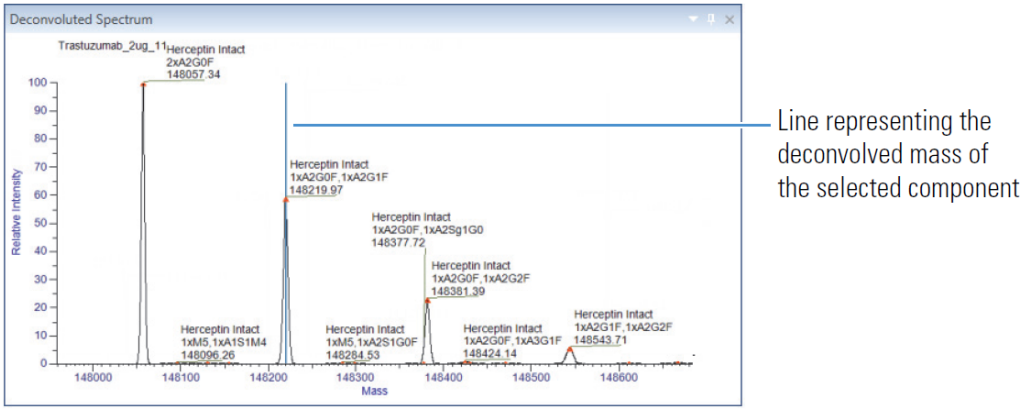


Figure 190 Deconvoluted Spectrum pane

The displayed mass range for the *x* axis of each plot is based on the Output Mass Range (for Xtract) or Output Mass Range (for ReSpect) values that you set on the Parameters > Component Detection page for the method or in the Process and Review > Real Time Optimization pane for real-time optimization.

For target sequence matching experiments, by default, an orange marker appears on each identified peak. You can turn this marker on and off, as well as change its color.

For more details, see the following topics:

- Deconvoluted Spectra display mode (page 390)
- Viewing the results table for intact mass analysis (page 422)
- Monoisotopic Mass (page 466)
- Average Mass (page 468)
- Model Mass Range (page 376)
- Model mass range information (page 394)
- Output Mass Range (page 370)(for Xtract)
- Output Mass Range (page 374)(for ReSpect)

Viewing the deconvoluted Spectra

To view the spectra in the Deconvoluted Spectrum pane

1. Click the **Process and Review** tab if necessary.
2. Do one of the following:
   - Click the row of a component in the Results table.

     The Deconvoluted Spectrum pane shows one deconvoluted spectrum plot (for a single loaded raw data file) or multiple stacked plots (for multiple loaded raw data files) with a blue line representing the deconvolved mass of the selected component.
   - (If available) Click the plus (+) sign to the left of a component row, and then click the row of one of the raw data files in the Results table.

     The Deconvoluted Spectrum pane shows one deconvoluted spectrum plot with a blue line for the top-level component and the results from the selected raw data file.

   –or–

   - (If available) Click the plus (+) sign to the left of a component (or raw data file) row, and then click the row of one of the charge states in the Results table.

     The Deconvoluted Spectrum pane shows one deconvoluted spectrum plot with a blue line for the top-level component and the results from the upper-level raw data file.

For more details, see the following topics:

- Viewing the results table for intact mass analysis (page 422)

Deconvoluted Spectra display for DAR

If the Enable Drug-to-Antibody Ratio check box is selected in the processing method for an Intact Mass Analysis experiment to enable DAR calculations, each spectrum plot displays the Average DAR value under the raw data file name, and also shows the Drug Load value on top of the peaks.

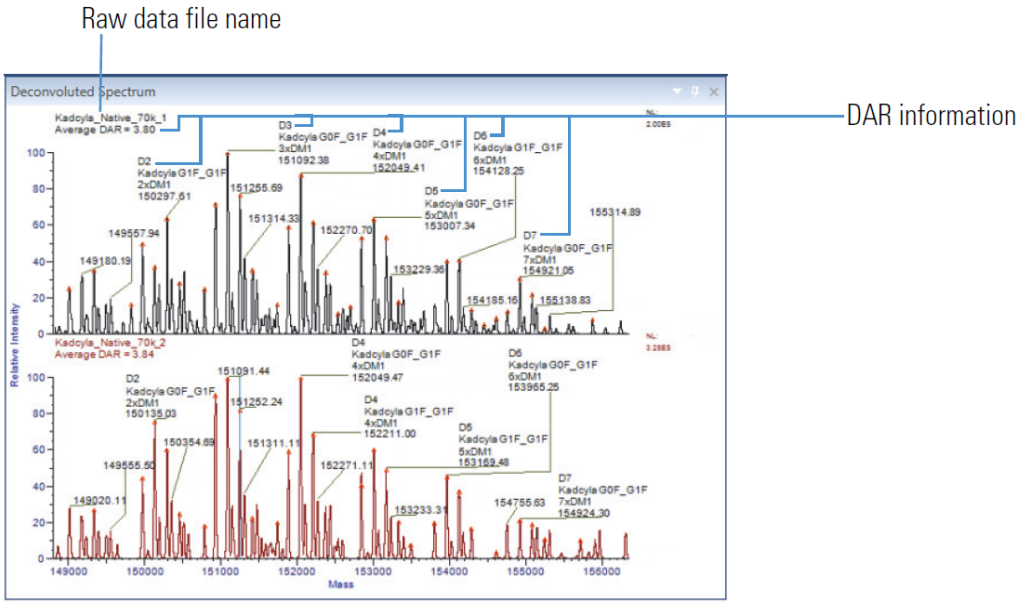


Figure 191 Deconvoluted Spectrum pane showing multiple plots with DAR information

For more details, see the following topics:

- Enable Drug-to-Antibody Ratio (page 384) check box
- Average DAR (page 446) value
- Drug Load (page 442) value

Deconvoluted spectrum pane commands

Right-clicking the Deconvoluted Spectrum pane on the Process and Review page opens a shortcut menu that contains the commands listed in the following table.

Table 60 Deconvoluted Spectrum pane shortcut menu

Command	Description
Reset Scale	Restores the original spectrum that first appeared in the pane.
Copy as Displayed	Copies the image in the pane to the Clipboard.
Copy per Global Settings	Copies the image in the pane to the Clipboard based on the global image dimensions set in the Intact Mass Analysis Settings dialog box.

Command	Description
Copy Data	Copies mass data (*x* axis) and intensity data (*y* axis) from the Deconvoluted Spectrum pane to the Clipboard so that you can paste it into an Excel™ spreadsheet or another application. For an Xtract deconvolution, the saved data consists of a centroid spectrum. For a ReSpect deconvolution, the saved data consists of a profile spectrum.
Zoom Out	Shrinks the view in the pane by a factor of 2.
Zoom In	Enlarges the view in the pane by a factor of 2.
Show Identification Markers	Turns on and off to show or hide the markers for identified peaks. This command is active only for target sequence matching experiments. When you open a new experiment, this command is on by default.
Identification Markers Color	Changes the color of the identification markers. This command is active only when the Show Identification Markers command is turned on. Select a different color from the palette or click **Advanced** to enter specific RGB, HSL, or CMYK values for the new color. The new color does not persist after you close the application. The next time you open the application, the color reverts back to default orange.

For more details, see the following topics:

- Using copy and paste functions (page 666)
- Specifying the image dimensions (page 37)

Viewing the source Spectra for intact mass analysis

The Source Spectrum pane on the Process and Review page displays the source spectra overlaid with blue lines. These lines represent the *m/z* values of the component's individual charge states.

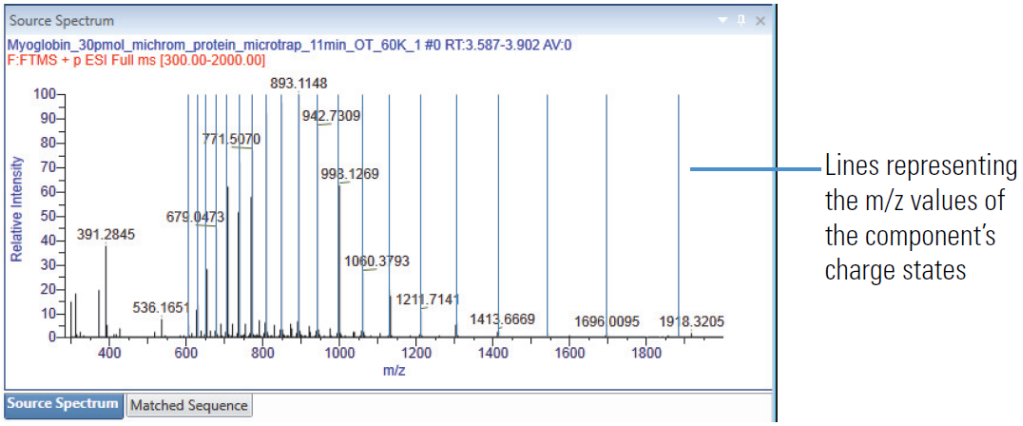


Figure 192 Source Spectrum pane

If you selected a source spectrum on the Parameters > Component Detection page, this pane displays the same spectrum. If you have not selected a source spectrum, this pane is empty.

If you load multiple raw data files for the experiment and selected source spectra, this pane displays an individual source spectrum plot for each of the raw data files, stacked on top of each other. The raw data file name and filter information (if available) appear on top of each plot.

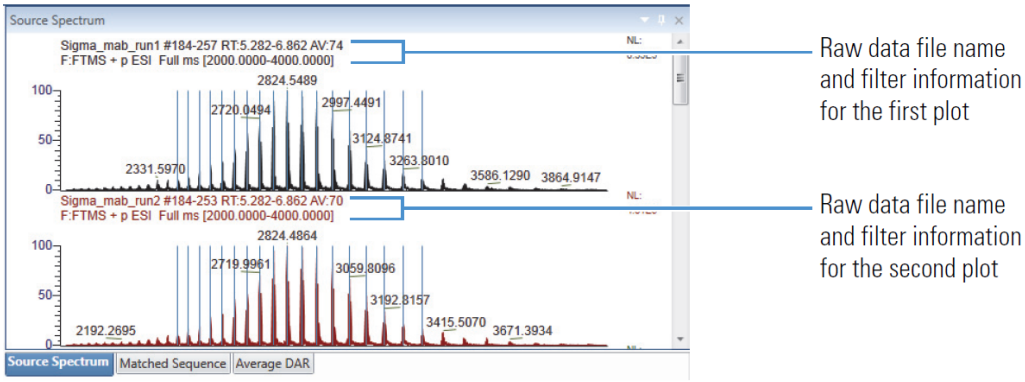


Figure 193 Source Spectrum pane showing multiple plots

For more details, see the following topics:

- Viewing the source Spectra (page 438)
- Source spectrum pane commands (page 439)
- Editing component detection parameters for intact mass analysis (page 354)

Viewing the source Spectra

To view the spectra in the Source Spectrum pane

1. As necessary, click the **Process and Review** tab and then click the **Source Spectrum** tab.

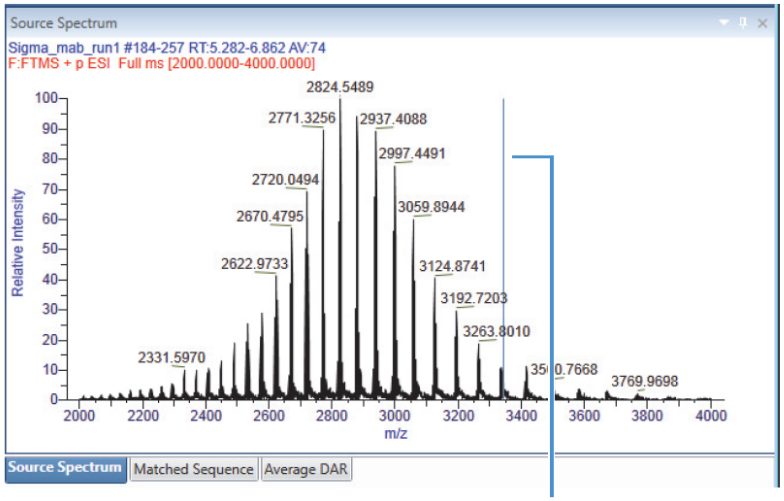
2. Do any of the following:
   - Click the row of a component in the Results table.

     The Source Spectrum pane shows the source spectrum plot or plots of the selected component.
   - (If available) Click the plus (+) sign to the left of a component row, and then click the row of one of the raw data files in the Results table.

     The Source Spectrum pane shows the source spectrum plot with a single blue line representing the top-level component and the masses and intensities from the selected raw data file.

   –or–
   - (If available) Click the plus (+) sign to the left of a component (or raw data file) row, and then click the row of one of the charge states in the Intact Deconvolution Results table.

     The Source Spectrum pane shows the source spectrum plot with a single blue line for the selected charge state, as shown in the following figure.



Line representing the calculated *m/z* value or m/z centroid value of an individual charge state

Figure 194 Line in Source Spectrum pane

For an average over RT deconvolution or auto peak detection, this line represents the following:

- For an Xtract deconvolution, the calculated monoisotopic *m/z* value of that charge state (shown in the Calculated Monoisotopic m/z column of the Intact Deconvolution Results table)
- For a ReSpect deconvolution, the measured average *m/z* value of that charge state (shown in the Measured Average m/z column of the Intact Deconvolution Results table)

For a sliding windows deconvolution, this line represents the *m/z* centroid value of that individual charge state (shown the in the MZ Centroid column of the Intact Deconvolution Results table).

For more details, see the following topics:

- Viewing the results table for intact mass analysis (page 422)
- Calculated Monoisotopic m/z (page 562) column
- Measured Average m/z (page 457) column
- MZ Centroid (page 455) column

Source spectrum pane commands

Right-clicking the Source Spectrum pane on the Process and Review page opens a shortcut menu that contains the commands listed in the following table.

Table 61 Source Spectrum pane shortcut menu

Command	Description
Reset Scale	Restores the original spectrum that first appeared in the pane.
Copy as Displayed	Copies the image in the pane to the Clipboard.
Copy per Global Settings	Copies the image in the pane to the Clipboard based on the global image dimensions set in the Intact Mass Analysis Settings dialog box.
Zoom Out	Shrinks the view in the pane by a factor of 2.
Zoom In	Enlarges the view in the pane by a factor of 2.

Note: To minimize storage space, the BioPharma Finder application does not save the source spectra for sliding windows experiments to the database. When you open the results for a sliding windows experiment, the application retrieves the source spectra from the processing method settings (see “Editing the component detection page” on page 363).

For more details, see the following topics:

- Using copy and paste functions (page 666)
- Specifying the image dimensions (page 37)

Viewing the matched sequence information for intact mass analysis

The Matched Sequence pane on the Process and Review page displays the selected component's mass and intensity, its drug load value when the Enable Drug-to-Antibody Ratio check box is selected in the method, and the matched sequence information from target sequence matching.

You can specify a different drug load value in this pane to update the calculated average drug-to-antibody ratio (DAR) values. You can also select a different identification in this pane and that change automatically updates the identification values in the Results table. However, you cannot modify the drug load value for a non-identified mass component.

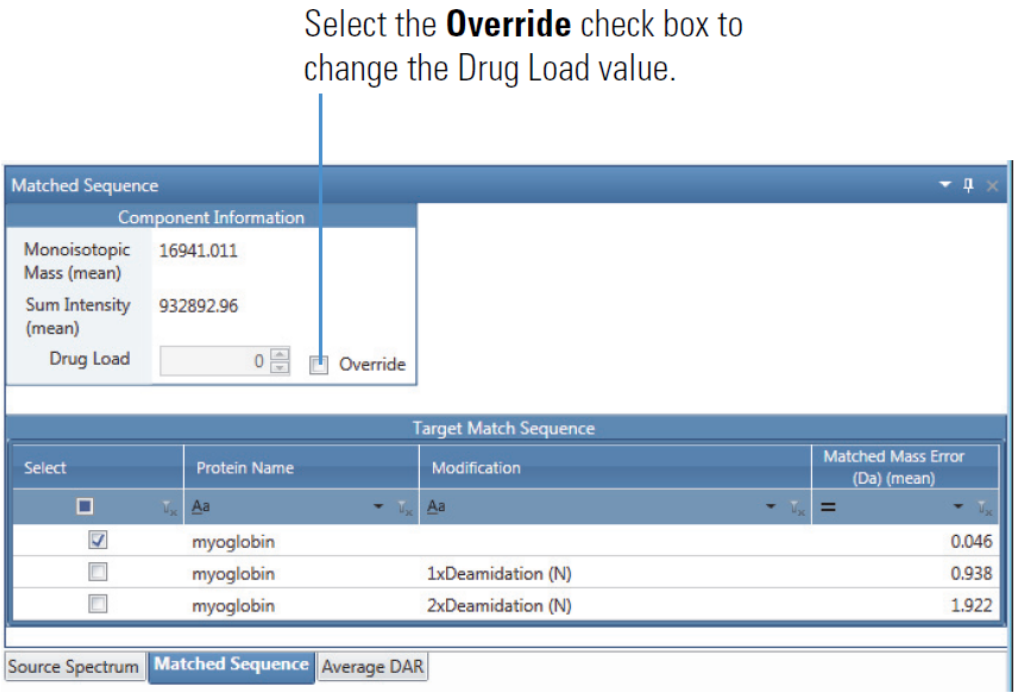


Figure 195 Matched Sequence pane

For more details, see the following topics:

- Viewing the matched sequence information (page 441)
- Target match sequence table parameters (page 443)
- Enable Drug-to-Antibody Ratio (page 384)
- Editing identification parameters for intact mass analysis (page 380)
- Viewing the average DAR values for intact mass analysis (page 443)
- Viewing the results table for intact mass analysis (page 422)

Viewing the matched sequence information

To view and modify the information in the Matched Sequence pane

1. As necessary, click the **Process and Review** tab and then click the **Matched Sequence** tab (next to the Source Spectrum tab).

2. Click the row of a matched component in the Results table.

   In the Matched Sequence pane, the Component Information table shows the mass and intensity information for the component that you select in the Results table, as well as the drug load value if the Enable Drug-to-Antibody Ratio check box is selected in the method. The Target Match Sequence table shows the matched identifications for the selected component.

3. (Optional) Next to the Drug Load value, select the **Override** check box, modify the Drug Load value, and then press ENTER.

   The application recalculates the DAR values based on your modified drug load and updates the tables in the Average DAR pane. The Results table and the drug load label on the spectra in the Deconvoluted Spectrum pane also automatically update with the modified drug load value.

4. (Optional) In the Target Match Sequence table, select a check box for a specific identification row.

   This selection overwrites the default best match identification. The application recalculates the DAR values based on your selection and updates the Average Drug to Antibody Ratio table in the Average DAR pane. The selected component row in the Results table and the labels on the spectra in the Deconvoluted Spectrum pane also automatically update with the identification data from the selected row in the Target Match Sequence table.

For more details, see the following topics:

- Viewing the results table for intact mass analysis (page 422)
- Enable Drug-to-Antibody Ratio (page 384) check box
- Component information table parameters (page 442)
- Target match sequence table parameters (page 443)
- Viewing the average DAR values for intact mass analysis (page 443)
- Viewing the deconvoluted Spectra for intact mass analysis (page 433)

Component information table parameters

The following table describes the information in the Component Information table at the top of the Matched Sequence pane on the Process and Review page.

Table 62 Component Information table rows

Row	Description
Monoisotopic Mass or Monoisotopic Mass (mean), Average Mass or Average Mass (mean), Sum Intensity or Sum Intensity (mean), Intensity or Intensity (mean)	See the corresponding descriptions in "Viewing the results table for intact mass analysis" on page 422. **Note:** These rows vary depending on the number of loaded raw data file or files, type of deconvolution algorithm, and result format used for the experiment: batch or multiconsensus.
Drug Load	Displays the drug-to-antibody ratio (DAR) value for a specific component when the Enable Drug-to-Antibody Ratio check box is selected in the processing method. The application calculates the drug load value based on the matched target sequence identification, for only the identified components in the Results table. This value is different from the Experimental Average DAR value, which is the average DAR for a complete set of measurements for an experiment, or the Average DAR value, which is the average DAR for a particular raw data file. **Note:** This value is not editable by default. To change the Drug Load value for an identified component, select the Override check box, edit the value, and then press ENTER. Any change here automatically updates the Drug Load value in the Results table, the Average DAR and Drug Load information on the deconvoluted spectra, and all calculated DAR values in the Average DAR pane.
Check Box	
Override	Enables the Drug Load box to be editable.

For more details, see the following topics:

- Enable Drug-to-Antibody Ratio (page 384) check box
- Experimental Average DAR (page 446) value
- Average DAR (page 446) value
- Results for a DAR-Enabled experiment (page 470)
- Viewing the deconvoluted Spectra for intact mass analysis (page 433)
- Viewing the average DAR values for intact mass analysis (page 443)

Target match sequence table parameters

The following table describes the information in the Target Match Sequence table at the bottom of the Matched Sequence pane on the Process and Review page.

Table 63 Target Match Sequence table columns

Column	Description
Select ☐	Select this check box to select the identification listed in a particular table row. **Note:** You can select only 1 identification row in the table. If you select a different identification, the following data automatically update based on your selection: • The Protein Name and Modification columns in the Results table • The average DAR labels on the deconvoluted spectra
Protein Name, Modification, Matched Mass Error (ppm/Da) or Matched Mass Error (ppm/Da) (mean)	See the corresponding descriptions in Table 71. **Note:** These columns vary depending on the number of loaded raw data files or the result format used for the experiment: batch or multiconsensus.

For more details, see the following topics:

- Protein Name (page 462) column
- Modification (page 315) column
- Results for a target sequence matching experiment (page 461)
- Viewing the deconvoluted Spectra for intact mass analysis (page 433)

Viewing the average DAR values for intact mass analysis

If the Enable Drug-to-Antibody Ratio check box is selected in the processing method for an Intact Mass Analysis experiment, the Average DAR pane on the Process and Review page is visible and displays by default the calculated average drug-to-antibody ratio (DAR) values using all of the components that have a drug load identification.

To update these calculated values, you can enter a different drug load value or select a different identification in the Matched Sequence pane, or choose a different set of components in the Results table. The Component Specific Summary table at the bottom of the Average DAR pane lists only the components used in the DAR calculations.

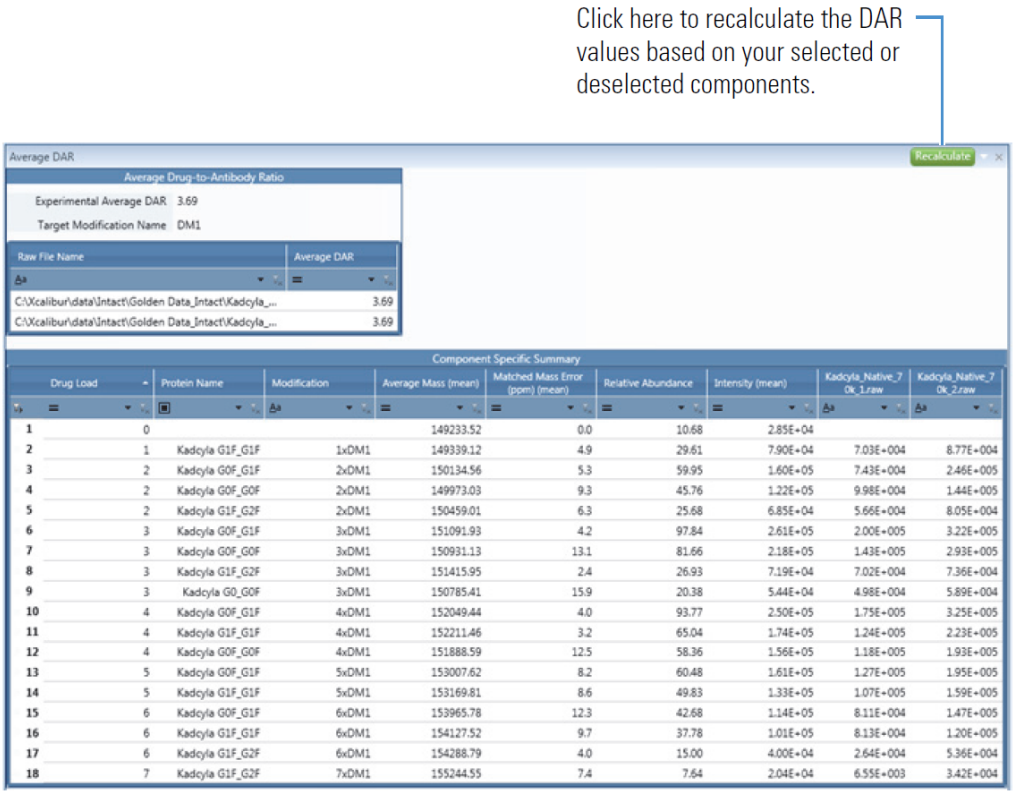


Figure 196 Average DAR pane displaying the DAR values and components used in the calculations

For more details, see the following topics:

- Viewing the average DAR values (page 444)
- Average DAR table parameters (page 446)
- Component specific summary table parameters (page 447)
- Average DAR pane command (page 447)
- Enable Drug-to-Antibody Ratio (page 384)
- Viewing the matched sequence information for intact mass analysis (page 440)
- Viewing the results table for intact mass analysis (page 422)

Viewing the average DAR values

To view and modify the information in the Average DAR pane

1. As necessary, click the **Process and Review** tab and then click the **Average DAR** tab (next to the Matched Sequence tab).

2. (Optional) In the Matched Sequence pane, select the **Override** check box and then modify the Drug Load value.

   The BioPharma Finder application recalculates the average DAR values based on your modified drug load and updates the tables in the Average DAR pane as well as the labels on the deconvoluted spectra. The Results table also automatically updates with the modified drug load value.

3. (Optional) In the Target Match Sequence table in the Matched Sequence pane, select a check box for a different identification row.

   The application recalculates the average DAR values based on your selection and updates these values in the Average Drug-to-Antibody Ratio table, as well as the labels on the deconvoluted spectra. The Results table also automatically updates with the identification information from the selected row.

   Note: Each *Raw File Name* column in the Average Drug-to-Antibody Ratio table displays the intensity value or values (when there are multiple peaks) for a particular raw data file. These values are actually strings, not numbers, so the filtering for this column does not work the same as for a numerical value.

4. (Optional) Select or clear the check boxes for the component rows in the Results table and then click **Recalculate**.

   If you select one or more component rows, the application recalculates the average DAR values using only the selected components and updates the values in the Average Drug-to-Antibody Ratio table.

   If you clear all of the components in the Results table, the application automatically recalculates the average DAR values in the Average Drug-to-Antibody Ratio table using only the default components that have a drug load identification.

   The Component Specific Summary table at the bottom of the Average DAR pane also updates to show only the list of components used in the calculations. Scroll to the right or down to see more columns and rows in this table as needed.

After you make modifications to the identifications and DAR calculations, you can click Save Results As in the command bar of the Process and Review page to save to a new experiment or overwrite the current experiment results.

Average DAR table parameters

The following table describes the information in the Average Drug-to-Antibody Ratio table at the top of the Average DAR pane on the Process and Review page. This table is not editable.

Table 64 Average Drug-to-Antibody Ratio table parameters

Parameter/Column	Description
Experimental Average DAR	Displays the average drug-to-antibody ratio (DAR) value for the experiment, calculated as the average of all the Average DAR values from all of the raw data files loaded for the experiment. **Note:** Any change to the Drug Load value automatically updates this value.
Target Modification Name	Displays the target modification found for the matched target sequence. This is the drug linker that you selected in the List of Modifications in the method.
Raw File Name	Displays the name or names of the raw data file or files loaded for the experiment.
Average DAR	Displays the calculated average DAR value for each raw data file. **Note:** Any change to the Drug Load value automatically updates this value here and in the deconvoluted spectra labels.

Component specific summary table parameters

The following table describes the information in the Component Specific Summary table at the bottom of the Average DAR pane on the Process and Review page. This table is not editable.

Table 65 Component Specific Summary table parameters

Column	Description
Row number	The number assigned to each component row in the Component Specific Summary table. This sequential numbering does not change when you sort or filter the table.
Drug Load	Displays the drug load values for only the components used in the DAR calculations. **Note:** Any change to the Drug Load value in the Matched Sequence pane automatically updates this column.
Protein Name, Modification, Monoisotopic Mass or Monoisotopic Mass (mean) or Average Mass or Average Mass (mean), Matched Mass Error (ppm/Da) or Matched Mass Error (ppm/Da) (mean), Relative Abundance, Sum Intensity or Sum Intensity (mean) or Intensity or Intensity (mean), *Raw File Name*	See the descriptions in "Viewing the results table for intact mass analysis" on page 422. **Note:** These columns vary depending on the number of loaded raw data file or files, the type of deconvolution algorithm, and the result format used for the experiment. A column with *Raw File Name* displays the intensity value for that particular raw data file.

Average DAR pane command

Right-clicking in the Average DAR pane on the Process and Review page opens a shortcut menu that contains the command listed in the following table.

Table 66 Average DAR pane shortcut menu

Command	Description
Export Component Specific Summary	Exports the values in the Component Specific Summary table to an Excel™ file. The default name of the file is *Experiment Name*_DAR_ComponentSummary.xls. If you do not browse to a different folder, the application places the exported file in the folder with the raw data files for the experiment.

22 Various results tables for intact mass analysis

When you process an Intact Mass Analysis experiment, the displayed Results table varies depending on the deconvolution algorithm, source spectra method, and other parameters used to generate the experiment.

Results for Single-File/Batch experiment using xtract and average over RT deconvolution

For this type of Intact Mass Analysis experiment, the Process and Review page displays the component list in the Results pane as a table of masses, intensities, and charge state information.

Displayed results table

The following figure shows the Results table for a single-file/batch Intact Mass Analysis experiment using Xtract and average over RT deconvolution. This type of experiment results in the same deconvoluted spectrum and RT range (values in the Start Time to Stop Time columns) for all of the components.

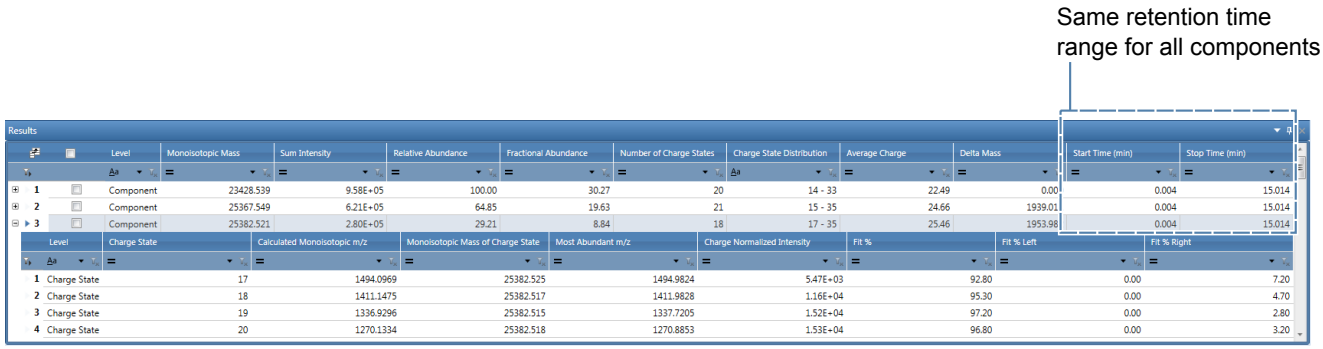


Figure 197 Results table for an average over RT deconvolution using the Xtract algorithm

Results table columns

The following table describes the table columns for a single-file/batch Intact Mass Analysis experiment using Xtract and average over RT deconvolution.

Table 67 Results table columns for an Xtract average over RT deconvolution experiment

Column	Description
Component level **Displays the following data related to each specific component.**	
+/–	Click to show or hide the lower level of information related to the current component row.
Row number	The number assigned to each visible component row in the table. This sequential numbering does not change when you sort or filter the table.

Column	Description
☐	Select this check box if you want to export the results for the components in the selected rows to a spreadsheet file, using the shortcut menu. You can also select this check box if you want to use only the components in the selected rows for the average drug-to-antibody ratio (DAR) calculations. **Note:** To select or clear all of the check boxes at once, select or clear the check box in the column header. If you filter the table, the following occurs: • Clearing all check boxes affects all of the original rows in the table, before any filtering. • Selecting all check boxes affects only the filtered and currently visible rows.
Level	Indicates that the row is displaying component information (top level).
Monoisotopic Mass	Displays a weighted average of the monoisotopic masses of each charge state: $$Monoisotopic\ Mass = \frac{\sum_{1}^{i}(Monoisotopic\ Mass\ of\ This\ Charge \times Charge\ Normalized\ Intensity)}{Sum\ Intensity}$$ where *i* is the sequential order of the charge in the Charge State column.
Sum Intensity	Displays the sum of the intensities of the isotopic clusters in a charge state. **Note:** If you enter conditions to group the raw data files when you create the experiment, the application calculates this value individually for each condition.
Relative Abundance	Displays the relative abundance of the peaks in the sample that are above the threshold set by the Rel. Abundance Threshold (%) (for Xtract) or Rel. Abundance Threshold (%) (for ReSpect) parameter. The application assigns the most abundant peak in a deconvoluted spectrum a relative abundance of 100 percent. The number in the Relative Abundance column represents the Sum Intensity (for Xtract) or Intensity (for ReSpect) value for a particular peak divided by the largest value in the Sum Intensity or Intensity column, and then multiplied by 100. For example, if the largest peak in a deconvoluted spectrum has an intensity of 1000, the application assigns it a relative abundance of 100 percent. If the next most abundant peak has an intensity of 500, the application assigns it an abundance of 50 percent: $$\frac{500}{1000} \times 100\% = 50\%$$ This value is averaged from all of the loaded raw data files. For an auto peak detection experiment, this value is based on each peak.

Column	Description
Fractional Abundance	Displays the fractional abundance of a component, which is the abundance for that peak relative to the total abundance of all peaks in the spectrum, expressed as a percentage. The sum of all fractional abundances of all peaks in a deconvoluted spectrum is 100 percent. This value is averaged from all of the loaded raw data files. For an auto peak detection experiment, this value is based on each peak.
Number of Charge States	Displays the number of detected isotopic clusters for a given deconvolved mass. This is the same number as the number of rows at the charge state level below the component level.
Charge State Distribution	Displays the range of charge states detected for the component, from the lowest to the highest charge state. See the Charge State column at the lower charge state level.
Average Charge	Displays the average of the charge numbers in the Charge State column.
Delta Mass	Displays the difference between the mass of a specific component and the mass of the highest-intensity component.
Start Time (min)	Displays the start of the retention time range of the averaged source spectrum for the given component.
Stop Time (min)	Displays the end of the retention time range of the averaged source spectrum for the given component.
Apex RT	(Visible only when the Calculate XIC check box is selected in the method) Displays the retention time for the chromatographic peak when a component has a calculated XIC.
Charge state level **Displays the following data related to each specific charge state.**	
Row number	The number assigned to each visible charge state row in the table. This sequential numbering does not change when you sort or filter the table.
Level	Indicates that the row is displaying charge state information (lower level).
Charge State	Displays the imbalance between the number of protons (in the nuclei of the atoms) and the number of electrons that a molecular species (or adduct ion) possesses. If the species possesses more protons than electrons, its charge state is positive. If it possesses more electrons than protons, its charge state is negative.
Calculated Monoisotopic m/z	Displays the mass-to-charge ratio of the calculated monoisotopic mass for a specific charge state.
Monoisotopic Mass of Charge State	Displays the detected monoisotopic mass for a specific charge state.
Most Abundant m/z	Displays the mass-to-charge ratio of the most abundant isotope, or the height of the tallest peak in the isotopic distribution.

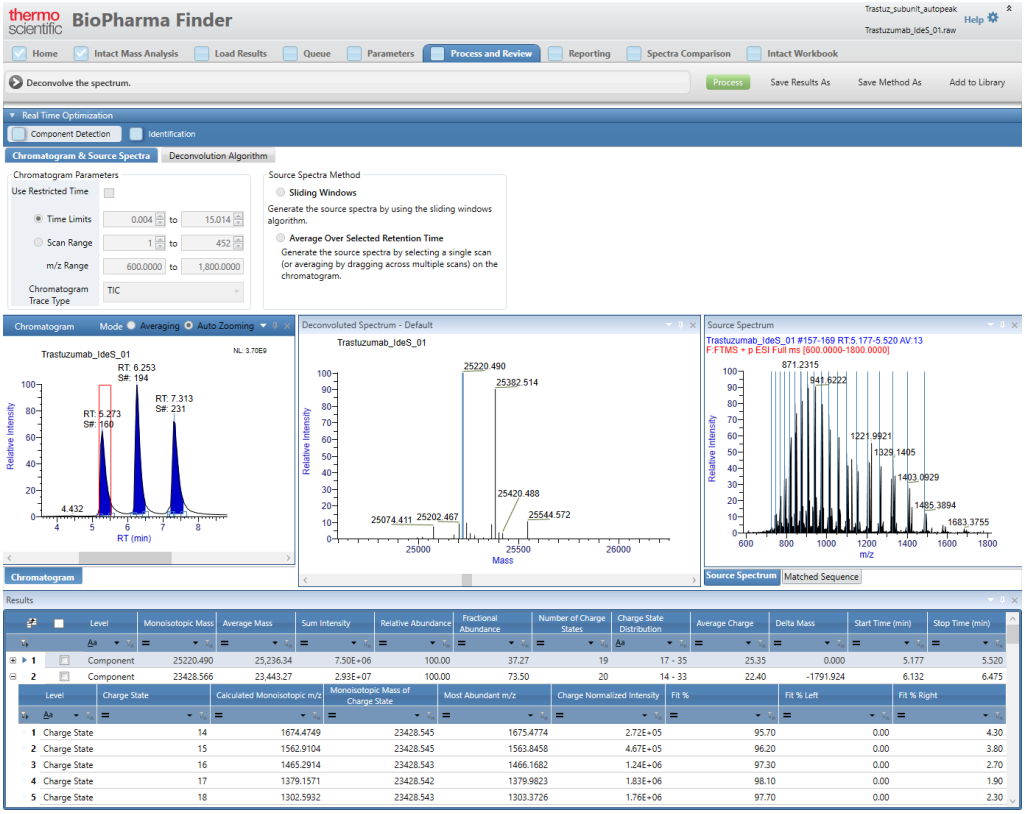
Column	Description
Charge Normalized Intensity	Displays the quotient of the intensity divided for this charge by the relevant charge.
Fit %	Displays the quality of the match between a measured isotope pattern and an averagine distribution of the same mass. This column displays a value between 0 and 100 percent. • 0% requires only a poor fit between the measured pattern and the averagine pattern. • 100% requires a very good (even though not exact) fit between the measured pattern and the averagine pattern. A fit factor of 100% means that the observed peaks in the measured isotope profile are absolutely identical to those in a theoretical averagine distribution and that any missing peaks fall below a restrictive threshold.
Fit % Left	Displays the quality of the match between a measured isotope pattern and an averagine distribution that is one dalton smaller than the calculated monoisotopic mass.
Fit % Right	Displays the quality of the match between a measured isotope pattern and an averagine distribution that is one dalton larger than the calculated monoisotopic mass.

For more details, see the following topics:

- Results table commands (page 426)
- Viewing the average DAR values for intact mass analysis (page 443)
- Filtering data in a table (page 673)
- Raw data files and sequences (page 134)
- Rel. Abundance Threshold (%) (page 371)(for Xtract)
- Rel. Abundance Threshold (%) (page 377)(for ReSpect)
- Intensity (page 455)
- Calculate XIC (page 376)

Results for a single File/Batch experiment using xtract and auto peak detection

The following figure shows the results for a single file/batch Intact Mass Analysis experiment using Xtract and auto peak detection.



	Level	Monoisotopic Mass	Average Mass	Sum Intensity	Relative Abundance	Fractional Abundance	Number of Charge States	Charge State Distribution	Average Charge	Delta Mass	Start Time (min)	Stop Time (min)
1	Component	25220.490	25,236.34	7.50E+06	100.00	37.27	19	17 - 35	25.35	0.000	5.177	5.520
2	Component	23428.566	23,443.27	2.93E+07	100.00	73.50	20	14 - 33	22.40	-1791.924	6.132	6.475

	Level	Charge State	Calculated Monoisotopic m/z	Monoisotopic Mass of Charge State	Most Abundant m/z	Charge Normalized Intensity	Fit %	Fit % Left	Fit % Right
1	Charge State	14	1674.4749	23428.545	1675.4774	2.72E+05	95.70	0.00	4.30
2	Charge State	15	1562.9104	23428.545	1563.8458	4.67E+05	96.20	0.00	3.80
3	Charge State	16	1465.2914	23428.543	1466.1682	1.24E+06	97.30	0.00	2.70
4	Charge State	17	1379.1571	23428.542	1379.9823	1.83E+06	98.10	0.00	1.90
5	Charge State	18	1302.5932	23428.543	1303.3726	1.76E+06	97.70	0.00	2.30

Figure 198 Results for an auto peak detection using the Xtract algorithm

The panes on the Process and Review page and the data in the Results table are the same as those for an average over RT deconvolution, except for the following:

- The chromatogram plot shows the detected peaks in blue.
- You cannot use real-time optimization or manually reprocess the experiment.
- Instead of the same RT range (values in the Start Time to Stop Time columns) and deconvoluted spectrum for all of the found peaks, each peak might have its own RT range and deconvoluted spectrum.

For more details, see the following topics:

- Chromatographic peak detection and spectral peak modeling (page 336)
- Results for Single-File/Batch experiment using xtract and average over RT deconvolution (page 449)
- Using Real-Time optimization for intact mass analysis (page 402)
- Working in manual mode (page 347)

Results for a single File/Batch experiment using xtract and sliding windows deconvolution

This type of Intact Mass Analysis experiment results in the same merged deconvoluted spectrum for all of the components, with each mass identified in multiple sliding windows within the given tolerances and from a specific RT range (values in the Start Time to Stop Time columns).

Displayed results table

The following figure shows the Results table for a single-file/batch Intact Mass Analysis experiment using Xtract and sliding windows deconvolution.

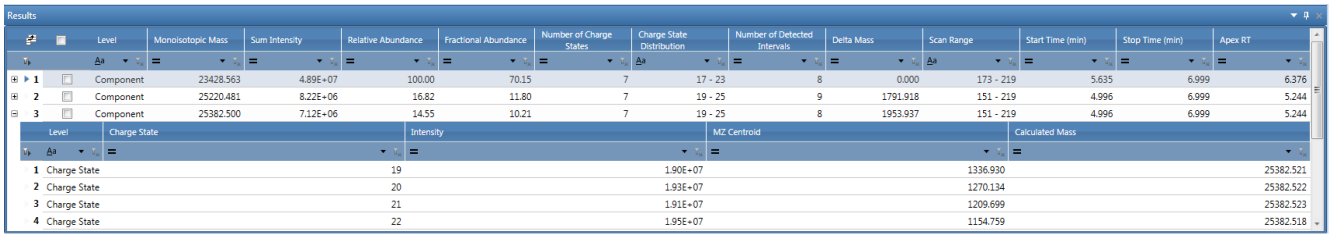


Figure 199 Results table for a sliding windows deconvolution using the Xtract algorithm

The Results table is the same as for an average over RT deconvolution, except for these column differences:

- At the component level, the table excludes the Average Charge column. Instead, it includes the Number of Detected Intervals, Start Time (min), Stop Time (min)and Apex RT columns.
- At the charge state level, the table excludes all of the columns after the Charge State column. Instead, it includes the Intensity, MZ Centroid, and Calculated Mass columns.

Results table columns

The following table describes the table columns that are different for a single-file/batch Intact Mass Analysis experiment using Xtract and sliding windows deconvolution.

Table 68 Results table columns for an Xtract sliding windows deconvolution experiment

Column	Description
Component level **Displays the following data related to each specific component.**	
Number of Detected Intervals	Displays the number of sliding windows within the specified tolerances where the application found the component of interest.

Column	Description
Start Time (min)	Displays the start of the retention time range that the sliding windows deconvolution covered. For mixed MS and MS2 data, the start time of the range might not necessarily be the first full MS scan that was used to generate that component.
Stop Time (min)	Displays the end of the retention time range that the sliding windows deconvolution covered. For mixed MS and MS2 data, the stop time of the range might not necessarily be the last full MS scan that was used to generate that component.
Apex RT	Displays the retention time for the chromatographic peak from the abundance trace.
Charge state level **Displays the following data related to each specific charge state.**	
Intensity	Displays the intensity of the charge state.
MZ Centroid	Displays the centroid position of the charge state.
Calculated Mass	Displays the mass associated with the MZ Centroid position.

For more details, see the following topics:

- Sliding Windows deconvolution (page 334)

Results for a single File/Batch experiment using ReSpect and average over RT deconvolution

For this type of Intact Mass Analysis experiment, the Process and Review page displays the component list in the Results pane as a table of masses, intensities, charge state information, and mass shifts. Each component in the Results table is composed of isotopic clusters. Each isotopic cluster in the original spectrum provides evidence for the peak in the deconvoluted spectrum.

Displayed results table

The following figure shows the Results table for a single-file/batch Intact Mass Analysis experiment using ReSpect and average over RT deconvolution. This type of experiment results in the same deconvoluted spectrum and RT range (values in the Start Time to Stop Time columns) for all of the components.

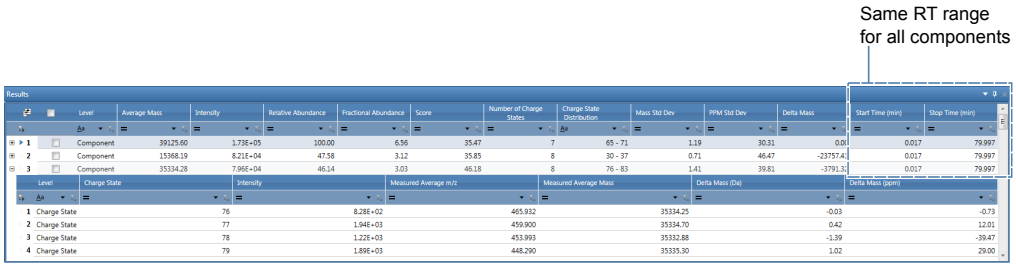


Figure 200 Results table for an average over RT deconvolution using the ReSpect algorithm

The Results table is the same as for an Xtract average over RT deconvolution experiment, except for these column differences:

- At the component level, the table excludes the Monoisotopic Mass, Sum Intensity, and Average Charge columns. Instead, it includes the Average Mass, Intensity, Score, Mass Std Dev, and PPM Std Dev columns.
- At the charge state level, the table excludes all of the columns after the Charge State column. Instead, it includes the Intensity, Measured Average m/z, Measured Average Mass, Delta Mass (Da), and Delta Mass (ppm) columns.

For more details, see the following topics:

- Monoisotopic Mass (page 450)
- Sum Intensity (page 450)
- Average Charge (page 451)
- Average Mass (page 468)
- Intensity (page 455)
- Score (page 457)

- Mass Std Dev (page 457)
- PPM Std Dev (page 457)
- Charge State (page 562)
- Intensity (page 455)
- Measured Average m/z (page 457)
- Measured Average Mass (page 458)
- Delta Mass (Da) (page 458)
- Delta Mass (ppm) (page 458)

Results table columns

The following table describes the table columns that are different for a single-file/batch Intact Mass Analysis experiment using ReSpect and average over RT deconvolution.

Table 69 Results table columns for a ReSpect average over RT deconvolution experiment

Column	Description
Component level **Displays the following data related to each specific component.**	
Average Mass	Displays the calculated mass of a molecule based on the average atomic weight of each element.
Intensity	Displays the sum of the intensities of the peaks for a charge state. **Note:** If you enter conditions to group the raw data files when you create the experiment, the application calculates this value individually for each condition.
Score	Displays the quality score of the deconvolved component.
Mass Std Dev	Displays the standard deviation, in daltons, of the delta masses for all the charge states of a component (for example, the standard deviation of the Delta Mass (Da) value).
PPM Std Dev	Displays the standard deviation, in parts per million, of the delta masses for all the charge states of a component (for example, the standard deviation of the Delta Mass (ppm) value).
Charge state level **Displays the following data related to each specific charge state.**	
Intensity	Displays the intensity of the charge state.
Measured Average m/z	Displays the mass-to-charge ratio of the peak in the source spectrum.

Column	Description
Measured Average Mass	Displays the mass calculated from the measured mass-to-charge ratio and the charge state. It represents the deconvolved mass for a particular charge state.
Delta Mass (Da)	Displays the difference between the average mass for a component and the calculated mass for that charge state, in daltons.
Delta Mass (ppm)	Displays the difference between the average mass for a component and the calculated mass for that charge state, in parts per million.

For more details, see the following topics:

- Raw data files and sequences (page 134)
- Optimizing the protein quality score (page 392)

Results for a single File/Batch experiment using ReSpect and auto peak detection

The following figure shows the results for a single file/batch Intact Mass Analysis experiment using ReSpect and auto peak detection.

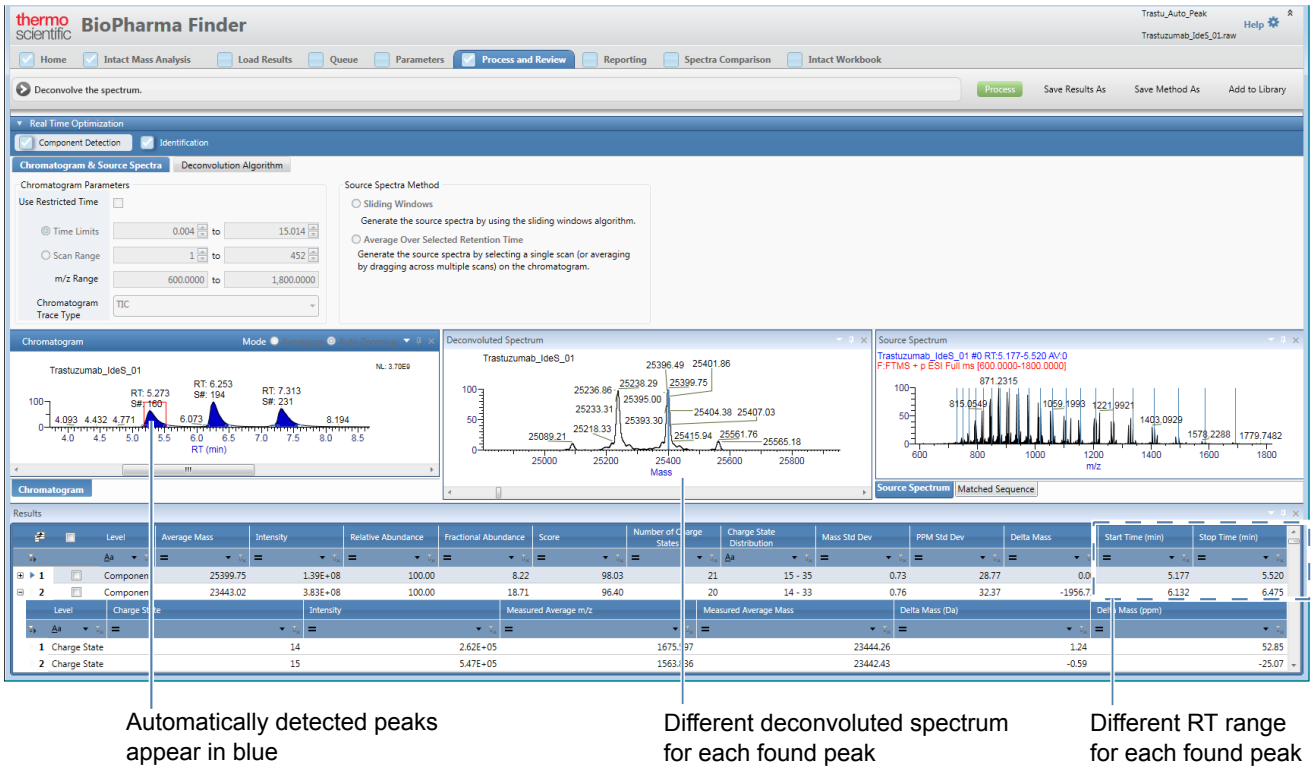


Figure 201 Results for auto peak detection using the ReSpect algorithm

The panes on the Process and Review page and the data in the Results table are the same as those for a ReSpect average over RT deconvolution experiment, except for the following:

- The chromatogram plot shows the detected peaks in blue.
- You cannot use real-time optimization or manually reprocess the experiment.
- Instead of the same RT range (values in the Start Time to Stop Time columns) and deconvoluted spectrum for all of the peaks, each peak might have its own RT range and deconvoluted spectrum.

For more details, see the following topics:

- Chromatographic peak detection and spectral peak modeling (page 336)
- Results for a single File/Batch experiment using ReSpect and average over RT deconvolution (page 456)
- Using Real-Time optimization for intact mass analysis (page 402)
- Working in manual mode (page 347)

Results for a single File/Batch experiment using ReSpect and sliding windows deconvolution

This type of Intact Mass Analysis experiment results in the same merged deconvoluted spectrum for all of the components, with each mass identified in multiple sliding windows within the given tolerances and from a specific RT range (values in the Start Time to Stop Time columns).

Displayed results table

The following figure shows the Results table for a single-file/batch Intact Mass Analysis experiment using ReSpect and sliding windows deconvolution.

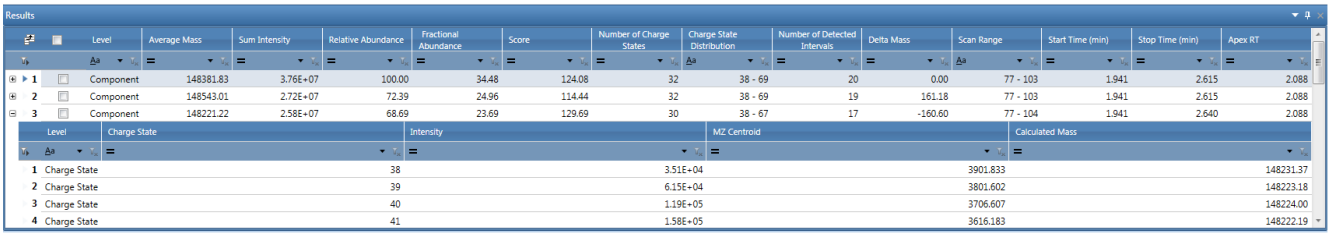


Figure 202 Results for a sliding windows deconvolution using the ReSpect algorithm

The Results table is the same as for an Xtract sliding windows deconvolution experiment, except for at the component level, the table excludes the Monoisotopic Mass column. Instead, it includes the Average Mass and Score columns. In addition, the Sum Intensity column contains different data.

Results table columns

The following table describes the table columns that are different for a single-file/batch Intact Mass Analysis experiment using ReSpect and sliding windows deconvolution.

Table 70 Results table columns for a ReSpect sliding windows deconvolution experiment

Column	Description
Component level **Displays the following data related to each specific component.**	
Average Mass	Displays the calculated mass of a molecule based on the average atomic weight of each element.
Sum Intensity	Displays the sum of all the successive component peaks identified by successive sliding windows. The component intensities are measurements of the relative abundance of components that Xtract/ReSpect identified. The charge state intensities are the actual intensities of peaks in the *m/z* spectra.
Score	Displays the quality score of the deconvolved component.

For more details, see the following topics:

- Optimizing the protein quality score (page 392)

Results for a target sequence matching experiment

For this type of Intact Mass Analysis experiment, the application displays in the Results table the best variable modification or glycosylation that matched the masses of the components. The target sequence mass includes any static modification or disulfide bonds. If the application finds more than one matching identification, it places each match in the Target Match Sequence table in the Matched Sequence pane.

Displayed results table

The following figure shows the results for a single-file/batch Intact Mass Analysis experiment with target sequence matching.

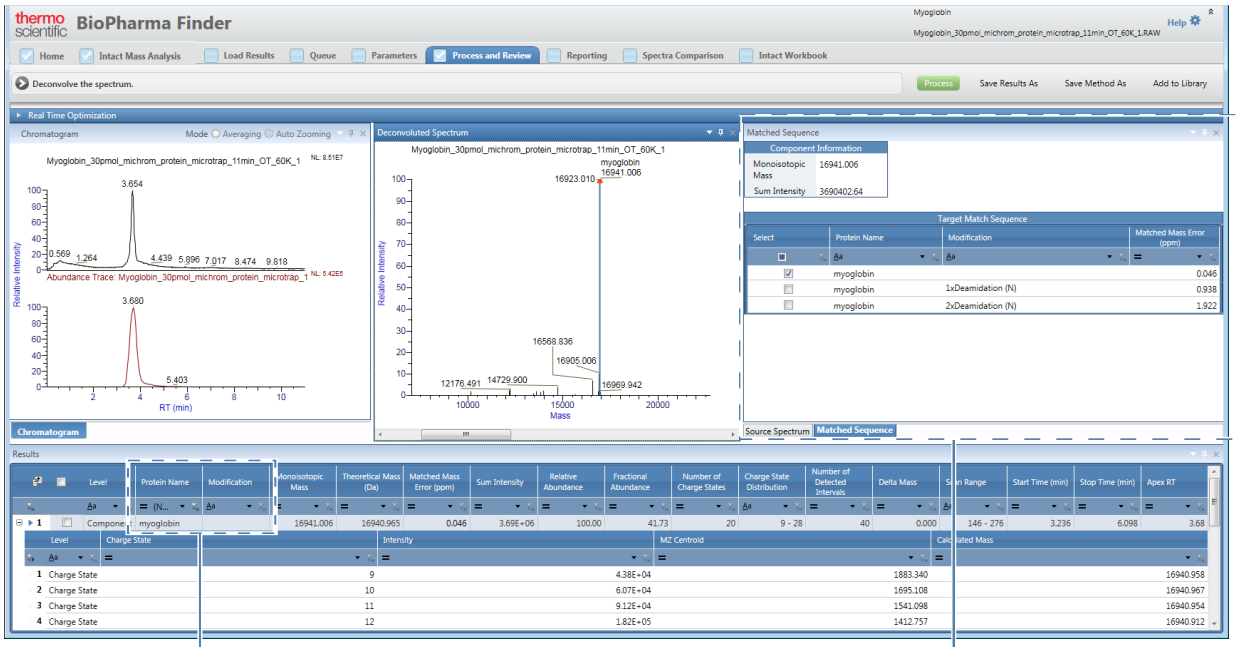


Figure 203 Results for a single-file/batch experiment with target sequence matching

An experiment with target sequence matching produces the same Results table as one without, except at the component level, the table includes the Protein Name, Modification, Theoretical Mass (Da), and Matched Mass Error (ppm/Da) columns.

For more details, see the following topics:

- Protein Name (page 462)
- Modification (page 315)
- Theoretical Mass (Da) (page 462)
- Matched Mass Error (ppm/Da) (page 462)

Results table columns

The following table describes the additional table columns for a single-file/batch Intact Mass Analysis experiment with target sequence matching.

Table 71 Results table columns for a target sequence matching experiment

Column	Description
Component level **Displays the following data related to each specific component.**	
Protein Name	Displays the name of the target sequence that matches this component within a given tolerance. If there is no matching sequence, this cell is empty. **Note:** If you change the matched sequence to another identification in the Matched Sequence pane, the changed protein name automatically appears in this cell.
Modification	Displays the modification of the target sequence that best matches this component within a given tolerance. The Matched Sequence pane displays all of the other modification matches. If there is no matching modification, this cell is empty. **Note:** If you select another identification in the Matched Sequence pane, the changed modification automatically appears in this cell.
Theoretical Mass (Da)	Displays the theoretical mass of the target sequence that matches this component within a given tolerance. If there is no matching sequence, this cell displays 0.00. **Note:** If you change the matched sequence to another identification in the Matched Sequence pane, the changed theoretical mass automatically appears in this cell.
Matched Mass Error (ppm/Da)	Displays the error associated with the best target sequence that matches this component.

For more details, see the following topics:

- Viewing the matched sequence information for intact mass analysis (page 440)

Results for a multiconsensus experiment

When you load multiple raw data files for an Intact Mass Analysis experiment, you can choose the Multiconsensus result format to process the experiment and merge the deconvolution results for all of the raw data files together.

The Results table includes a lower raw data file level that displays the data individually for each file. In addition, if you enter conditions to group the raw data files when you create the experiment, the application calculates the intensity and the number of files containing the detected component per condition. For a ReSpect experiment, the application does not provide the score per condition, but the score for each raw data file is available.

Displayed results table

The following figure shows the Results table for a multiconsensus Intact Mass Analysis experiment *without* conditions and uses the ReSpect algorithm and average over RT deconvolution.

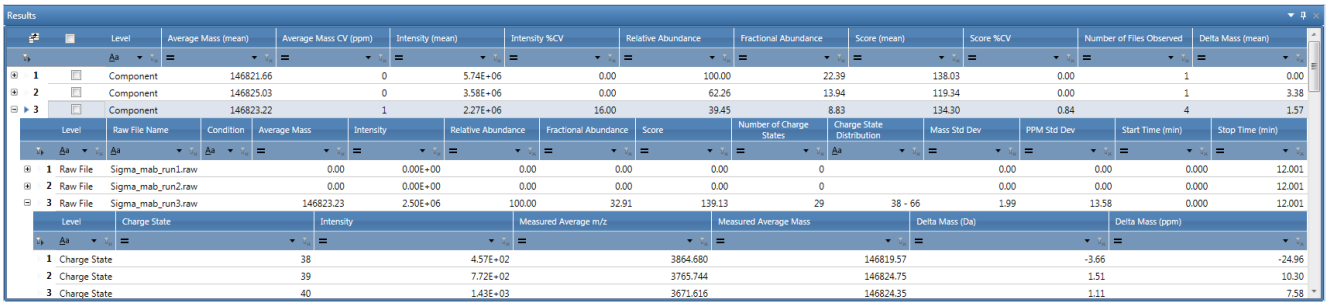
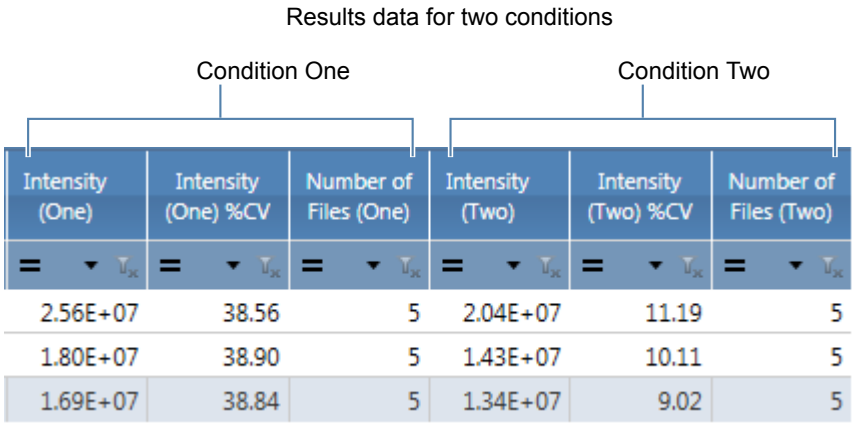


Figure 204 Results table for a multiconsensus ReSpect experiment without conditions

The following figure shows the extra top-level columns on the right side of the Results table for the same multiconsensus experiment *with* conditions. In this example, the conditions are "One" and "Two".



Intensity (One)	Intensity (One) %CV	Number of Files (One)	Intensity (Two)	Intensity (Two) %CV	Number of Files (Two)
2.56E+07	38.56	5	2.04E+07	11.19	5
1.80E+07	38.90	5	1.43E+07	10.11	5
1.69E+07	38.84	5	1.34E+07	9.02	5

Figure 205 Extra columns in Results table for a multiconsensus ReSpect experiment with two conditions

The Results table for a multiconsensus experiment is the same as for a single-file/batch experiment of the same type (see the previous topics), except for the following:

- At the component level, the table excludes the Monoisotopic Mass, Sum Intensity, Number of Charge States, Charge State Distribution columns (for Xtract) and Average Mass, Intensity, Score, Mass Std Dev, and PPM Std Dev columns (for ReSpect).

  Instead, it moves these columns down to the raw data file level and includes the "mean" and "CV (ppm)" or "%CV" versions of some of these columns at the component level.
- The table includes a raw data file level below the component level and above the charge state level.

For more details, see the following topics:

- Monoisotopic Mass (page 466)
- Sum Intensity (page 450)
- Number of Charge States (page 451)
- Charge State Distribution (page 376)
- Average Mass (page 468)
- Intensity (page 455)
- Score (page 457)
- Mass Std Dev (page 457)
- PPM Std Dev (page 457)

Results table columns

For descriptions of the columns that are different in the Results table for a multiconsensus Intact Mass Analysis experiment, see the following tables for Xtract and ReSpect deconvolution.

Table 72 Results table columns for a multiconsensus Xtract experiment

Column	Description
Component level **Displays the following data related to each specific component.**	
Monoisotopic Mass (mean)	Displays the average of the Monoisotopic Mass values from all of the raw data files used for the experiment.
Monoisotopic Mass CV (ppm)	Displays the coefficient of variation (CV) of the Monoisotopic Mass values from all of the raw data files used for the experiment. CV = standard deviation of the Monoisotopic Mass values from all of the raw data files ÷ the average of these values
Matched Mass Error (ppm/Da) (mean)	(Visible only for target matching experiments) Displays the average of the Matched Mass Error (ppm/Da) values from all of the raw data files used for the experiment.
Matched Mass Error (ppm/Da) %CV	(Visible only for target matching experiments) Displays the CV percentage of the Matched Mass Error (ppm/Da) values from all of the raw data files used for the experiment.
Sum Intensity (mean)	(Visible only for experiments *without* conditions) Displays the average of the Sum Intensity values from all of the raw data files used for the experiment.
Sum Intensity %CV	(Visible only for experiments *without* conditions) Displays the CV percentage of the Sum Intensity values from all of the raw data files used for the experiment.
Number of Files Observed	(Visible only for experiments *without* conditions) Displays the number of raw data files within which the component was detected.
Delta Mass (mean)	Displays the average of the Delta Mass values from all of the raw data files used for the experiment.
Apex RT (mean)	(Visible only for sliding windows experiments or when the Calculate XIC check box is selected) Displays the average of the Apex RT (sliding windows) or Apex RT (XIC) values from all of the raw data files used for the experiment.
Apex RT %CV	(Visible only for sliding windows experiments or when Calculate XIC is selected) Displays the CV percentage of the Apex RT (sliding windows) or Apex RT (XIC) values from all of the raw data files used for the experiment.
Sum Intensity (*Condition*) (mean)	(Visible only for experiments *with* conditions) Displays the average of the Sum Intensity values from all of the raw data files grouped under the *Condition* for the experiment.
Sum Intensity (*Condition*) %CV	(Visible only for experiments *with* conditions) Displays the CV percentage of the Sum Intensity values from all of the raw data files grouped under the *Condition* for the experiment.

Column	Description
Number of Files (*Condition*)	(Visible only for experiments *with* conditions) Displays the number of raw data files, grouped under the *Condition*, within which the component was detected.
Raw data file level **Displays the following data related to each specific raw data file.**	
Row number	The number assigned to each visible raw data file row in the table. This sequential numbering does not change when you sort or filter the table.
Level	Indicates that the row is displaying raw data file information (lower level).
Raw File Name	Displays the name of the raw data file.
Condition	Displays the condition assigned to the raw data file; otherwise, this cell is empty.
Monoisotopic Mass	Displays the Monoisotopic Mass for a specific raw data file.
Sum Intensity	Displays the Sum Intensity for a specific raw data file.
Relative Abundance	Displays the Relative Abundance for a specific raw data file.
Fractional Abundance	Displays the Fractional Abundance for a specific raw data file.
Number of Charge States	Displays the number of detected isotopic clusters for a given deconvolved mass.
Charge State Distribution	Displays the range of charge states detected for the component, from the lowest to the highest charge state. See Charge State at the lower charge state level.
Average Charge	(Visible only for average over RT deconvolution experiments) Displays the average of the charge numbers in the Charge State column.
Number of Detected Intervals	(Visible only for sliding windows deconvolution experiments) For a specific raw data file, displays the number of sliding windows within the specified tolerances where the application found the component of interest.
Scan Range	(Visible only for sliding windows deconvolution experiments) Displays the scan range for a specific raw data file.
Start Time (min)	Displays the start of the retention time range for a specific raw data file.
Stop Time (min)	Displays the end of the retention time range for a specific raw data file.
Apex RT	(Visible only for sliding windows experiments or when Calculate XIC is selected) Displays the Apex RT (sliding windows) or Apex RT (XIC) value for a specific raw data file.

Table 73 Results table columns for a multiconsensus ReSpect experiment

Column	Description
Component level **Displays the following data related to each specific component.**	
Average Mass (mean)	Displays the average of the Average Mass values from all of the raw data files used for the experiment.
Average Mass CV (ppm)	Displays the coefficient of variation (CV) of the Average Mass values from all of the raw data files used for the experiment.
Matched Mass Error (ppm/Da) (mean)	(Visible only for target matching experiments) Displays the average of the Matched Mass Error (ppm/Da) values from all of the raw data files used for the experiment.
Matched Mass Error (ppm/Da) %CV	(Visible only for target matching experiments) Displays the CV percentage of the Matched Mass Error (ppm/Da) values from all of the raw data files used for the experiment.
Intensity (mean)	(Visible only for average over RT deconvolution experiments *without* conditions) Displays the average of the Intensity values from all of the raw data files used for the experiment.
Intensity %CV	(Visible only for average over RT deconvolution experiments *without* conditions) Displays the CV percentage of the Intensity values from all of the raw data files used for the experiment.
Sum Intensity (mean)	(Visible only for sliding windows experiments *without* conditions) Displays the average of the Sum Intensity values from all of the raw data files used for the experiment.
Sum Intensity %CV	(Visible only for sliding windows experiments *without* conditions) Displays the CV percentage of the Sum Intensity values from all of the raw data files used for the experiment.
Score (mean)	Displays the average of the Score values from all of the raw data files used for the experiment.
Score %CV	Displays the CV percentage of the Score values from all of the raw data files used for the experiment.
Number of Files Observed	(Visible only for experiments *without* conditions) Displays the number of raw data files within which the component was detected.
Delta Mass (mean)	Displays the average of the Delta Mass values from all of the raw data files used for the experiment.
Apex RT (mean)	(Visible only when Calculate XIC is selected) Displays the average of the Apex RT values from all of the raw data files used for the experiment.
Apex RT %CV	(Visible only when Calculate XIC is selected) Displays the CV percentage of the Apex RT values from all of the raw data files used for the experiment.
Intensity (*Condition*) (mean)	(Visible only for average over RT deconvolution experiments *with* conditions) Displays the average of the Intensity values from all of the raw data files grouped under the *Condition* for the experiment.

Column	Description
Intensity (*Condition*) %CV	(Visible only for average over RT deconvolution experiments *with* conditions) Displays the CV percentage of the Intensity values from all of the raw data files grouped under the *Condition* for the experiment.
Sum Intensity (*Condition*) (mean)	(Visible only for sliding windows experiments *with* conditions) Displays the average of the Sum Intensity values from all of the raw data files grouped under the *Condition* for the experiment.
Sum Intensity (*Condition*) %CV	(Visible only for sliding windows experiments *with* conditions) Displays the CV percentage of the Sum Intensity values from all of the raw data files grouped under the *Condition* for the experiment.
Number of Files (*Condition*)	(Visible only for experiments *with* conditions) Displays the number of raw data files, grouped under the *Condition*, within which the component was detected.
Raw data file level **Displays the following data related to each specific raw data file.**	
Row number	The number assigned to each visible raw data file row in the table. This sequential numbering does not change when you sort or filter the table.
Level	Indicates that the row is displaying raw data file information (lower level).
Raw File Name	Displays the name of the raw data file.
Condition	Displays the condition assigned to the raw data file; otherwise, this cell is empty.
Average Mass	Displays the Average Mass for a specific raw data file.
Intensity	(Visible only for average over RT deconvolution experiments) Displays the Intensity for a specific raw data file.
Sum Intensity	(Visible only for sliding windows deconvolution experiments) Displays the Sum Intensity for a specific raw data file.
Relative Abundance	Displays the Relative Abundance for a specific raw data file.
Fractional Abundance	Displays the Fractional Abundance for a specific raw data file.
Score	Displays the Score for a specific raw data file.
Number of Charge States	Displays the number of detected isotopic clusters for a given deconvolved mass.
Charge State Distribution	Displays the range of charge states detected for the component, from the lowest to the highest charge state. See Charge State at the lower charge state level.
Mass Std Dev	(Visible only for average over RT deconvolution experiments) Displays the Mass Std Dev for a specific raw data file.
PPM Std Dev	(Visible only for average over RT deconvolution experiments) Displays the PPM Std Dev for a specific raw data file.

Column	Description
Number of Detected Intervals	(Visible only for sliding windows deconvolution experiments) For a specific raw data file, displays the number of sliding windows within the specified tolerances where the application found the component of interest.
Scan Range	(Visible only for sliding windows deconvolution experiments) Displays the scan range for a specific raw data file.
Start Time (min)	Displays the start of the retention time range for a specific raw data file.
Stop Time (min)	Displays the end of the retention time range for a specific raw data file.
Apex RT	(Visible only when Calculate XIC is selected) Displays the Apex RT for a specific raw data file.

For more details, see the following topics:

- Monoisotopic Mass (page 450)
- Matched Mass Error (ppm/Da) (page 462)
- Sum Intensity (page 450) value (Xtract)
- Delta Mass (page 451) value (Xtract)
- Calculate XIC (page 376)
- Apex RT (page 455)
- Apex RT (page 451) value (XIC)
- Relative Abundance (page 450)
- Fractional Abundance (page 451)
- Charge State (page 562)
- Start Time (min) (page 455)
- Average Mass (page 460)
- Intensity (page 460)
- Sum Intensity (page 460) value (ReSpect)
- Score (page 457)
- Mass Std Dev (page 457)
- PPM Std Dev (page 457)

Results for a DAR-Enabled experiment

For this type of Intact Mass Analysis experiment, you must define the appropriate parameters in the processing method for the application to determine the average DAR values for your ADC sample. You select the Enable Drug-to-Antibody Ratio check box and a drug linker from the List of Modifications. This enables the DAR calculations for the experiment.

Note: When you run a single-file/batch DAR-enabled experiment, the application displays the results the same as for a multiconsensus experiment.

For more details, see the following topics:

- Enable Drug-to-Antibody Ratio (page 384)
- List of Modifications (page 384) parameter
- Drug-to-Antibody ratio (DAR) values (page 339)
- Editing identification parameters for intact mass analysis (page 380)

Displayed results table

The following figure shows the results in the Results table for a DAR-enabled Intact Mass Analysis experiment.

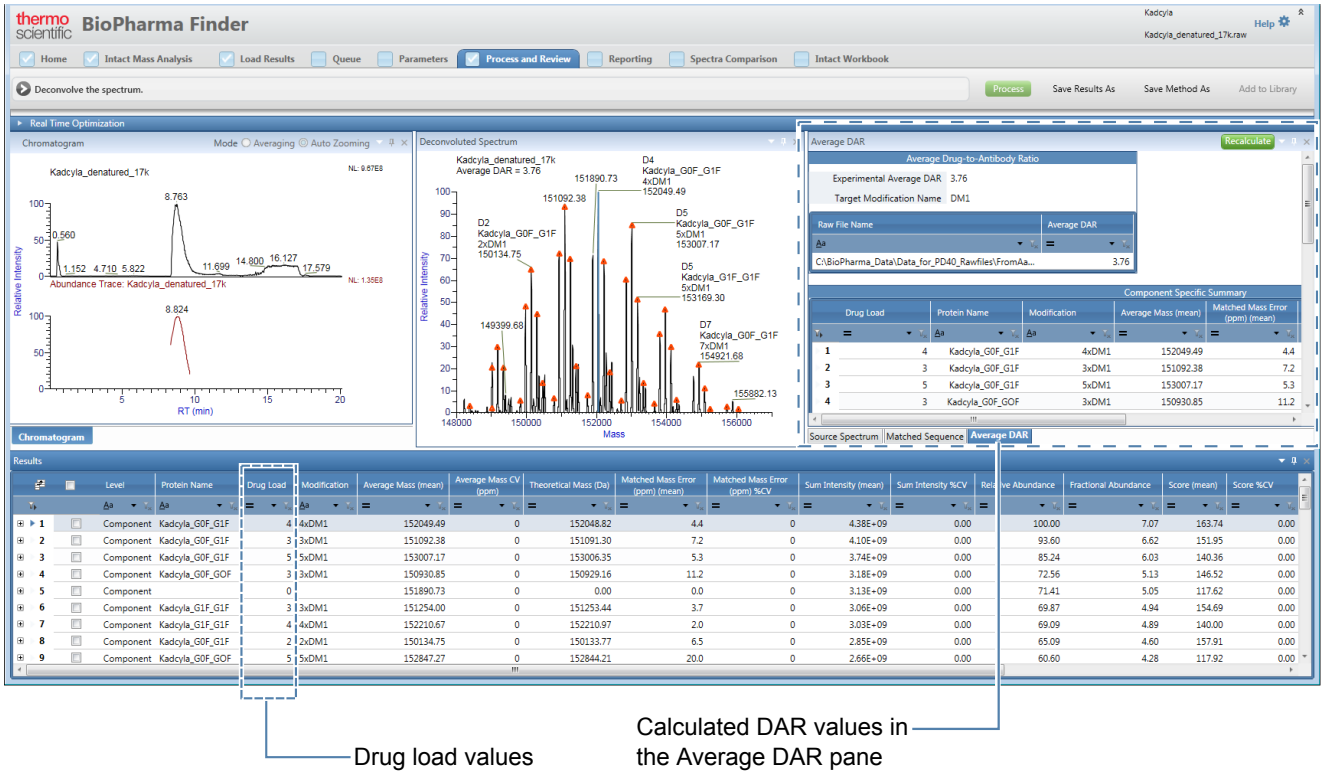


Figure 206 Results of a DAR-enabled experiment

The Results table is the same as for a multiconsensus experiment of the same type, except it includes the Drug Load column.

For more details, see the following topics:

- Results for a multiconsensus experiment (page 463)
- Drug Load (page 442) column

Results table additional column

The following table describes the additional column in the Results table for a DAR-enabled Intact Mass Analysis experiment. For details on the other columns in the Results table.

Table 74 Results table column for a DAR-enabled experiment

Column	Description
Component level **Displays the following data related to each specific component.**	
Drug Load	Displays the drug load value for a component. **Note:** When you change the drug load value in the Matched Sequence pane, the changed value automatically appears in this cell.

For more details, see the following topics:

- Results for a multiconsensus experiment (page 463)
- Viewing the matched sequence information for intact mass analysis (page 440)
- Displayed results table (page 470)

23 Viewing an intact mass analysis report

After you process an experiment, the BioPharma Finder application generates a report displaying various aspects of the deconvolution so that you can track the progression of the data. You can view this report on the Reporting page and save it as a PDF file. The report can only contain results from either Xtract or ReSpect, but not from both algorithms.

IMPORTANT! The application does not support the reporting feature for DAR-enabled (see the Enable Drug-to-Antibody Ratio on page 384 parameter in the processing method) and multiconsensus experiments.

Displaying a report

To display a report for an Intact Mass Analysis experiment

Do either of the following:

- In automatic mode, on the Queue page, select the completed job with the report that you want displayed, and click **Open Report**.

–or–

- From any other page, click the **Reporting** tab when the application has finished processing the data.

If you have the Acrobat Pro DC or Acrobat Reader DC application installed on your system, the report opens on the Reporting page. Otherwise, the report opens in a separate Acrobat window. It displays a summary of results and parameters for your experiment.

In automatic mode, if you select the Concatenate All Reports option in the Automation Parameters pane of the Parameters > Report page, the application automatically generates a single report concatenating all of the chromatographic

peaks. Otherwise, the application does not generate the report automatically. You generate the report in real-time when you click the Reporting tab to view it.

Note: The application does not support copying chromatograms and source spectra from the Reporting page.

For more details, see the following topics:

- Manual and automatic modes (page 333)
- Concatenate All Reports (page 389) option

Viewing specific sections in the report

To view the Component Detail Tables and Source Spectrum Evidence Plot sections

1. Select a processing method to edit.
2. Click the **Report** subtab and go to the Reporting Parameters pane of the Report page.
3. To generate the Monoisotopic Mass (for Xtract) or Average Mass (for ReSpect) table for each component in the report, select the **Component Detail Tables** check box.
4. To generate the spectrum of each component in the report, select the **Component Source of Evidence Plots** check box.
5. Save the method and use it for processing.
6. After you process the experiment, click the **Reporting** tab to view these sections.

For more details, see the following topics:

- Working with an Intact Mass processing method (page 353)
- Component detail tables section (page 486)
- Source spectrum evidence plot section (page 487)
- Saving a processing method (page 144)

Saving a report to PDF

To save a report as a PDF file

1. Click the **Reporting** tab and point to the top of the Reporting page.
   The Reporting page toolbar appears.
2. Click the **Save a Copy** icon, .
3. In the Save As dialog box, specify the path and name of a PDF file to store the report in, and click **Save**.
   The application saves the report in a file called *RawFileName_ExperimentName.pdf*. If you do not specify a folder, it places the file in the raw data file folder shown on the Intact Mass Analysis page.

Printing a report

To print a report

1. Click the **Reporting** tab and point to the top of the Reporting page.
   The Reporting page toolbar appears.
2. Click the **Print File** icon, .
3. In the Print dialog box, set the appropriate printing parameters, and click **Print**.

For more details, see the following topics:

- Reporting page toolbar (page 474)

Reporting page toolbar

You can activate the Reporting page toolbar, by pointing to the top of the Reporting page.

Figure 207 Reporting page toolbar

This toolbar contains the following icons.

Table 75 Icons on the Reporting page toolbar

Icon	Description
	Opens the Save a Copy dialog box so that you can save the report in a PDF file.
	Opens the Print dialog box so that you can print the reports.
	Shrinks the view.
	Enlarges the view.
	Activates an Adobe™ Acrobat application toolbar so that you can perform the functions available in an Acrobat file.

Report sections

For more details on the various sections in a report resulting from a processed Intact Mass Analysis experiment, see the following topics:

- Sample information section (page 476)
- Chromatogram parameters section (page 477)
- Chromatogram section (page 478)
- Main parameters section (page 479)
- Advanced parameters section (page 480)
- Source Spectra parameters section (page 482)
- Sequences information section (page 483)
- Source spectrum section (page 483)
- Deconvoluted spectrum section (page 484)
- Masses table section (page 485)
- Component detail tables section (page 486)
- Source spectrum evidence plot section (page 487)

Sample information section

The Sample Information section of a report, shown in the following figure, displays information about the sample from which the spectrum was taken.

Sample Information	
Raw File Name	C:\XCALIBUR\Intact\IgG_source_cid.raw
Instrument Method	C:\Xcalibur\Intact\mab_SS_1.meth
Vial	CStk1-01:23
Injection Volume (µL)	40
Sample Weight	0
Sample Volume (µL)	0
ISTD Amount	0
Dil Factor	1

Figure 208 Sample Information section

The following table lists the parameters in the Sample Information section. All the parameters in this section are read-only.

Table 76 Sample Information section parameters

Parameter	Description
Raw File Name	Displays the name of the original raw data file. If you rename the raw data file, the original name of the raw data file still appears on the report.
Instrument Method	Displays the name of the instrument method file.
Vial	Displays the position number of the sample in the autosampler.
Injection Volume (µL)	Displays the injection volume of the sample to be injected, in microliters.
Sample Weight	Displays the amount of a component in the sample.
Sample Volume (µL)	Displays the volume of a component in the sample.

Parameter	Description
ISTD Amount	Specifies the correction for the internal standard amount. If the value in this box is not 0.000, the value is used in an algorithm to correct for a case when any internal standard amounts specified in the active instrument method are correct, but when the amount of internal standard actually in one or more samples is different than the amount specified in the instrument method. This correction eliminates the necessity of remaking any samples to the internal standard concentrations or amounts specified in the instrument method and rerunning the samples.
Dil Factor	Specifies the dilution factor that was used to prepare the sample.

Chromatogram parameters section

The Chromatogram Parameters section of a report, shown in the following figure, displays the settings that you chose on the Chromatogram Parameters area of the Parameters > Component Detection or Process and Review page.

Chromatogram Parameters	
Use Restricted Time	False
Time Limits	0.005 - 6.230 minutes
Scan Range	1 - 3410
m/z Range	1000 - 4000
Chromatogram Trace Type	TIC
Sensitivity	High
Rel. Intensity Threshold (%)	1

Figure 209 Chromatogram Parameters section

Chromatogram section

The Chromatogram section of a report, shown in the following figure, displays the chromatogram contained in the raw data file. It is the same chromatogram that appears on the Chromatogram pane of the Parameters > Component Detection or Process and Review page.

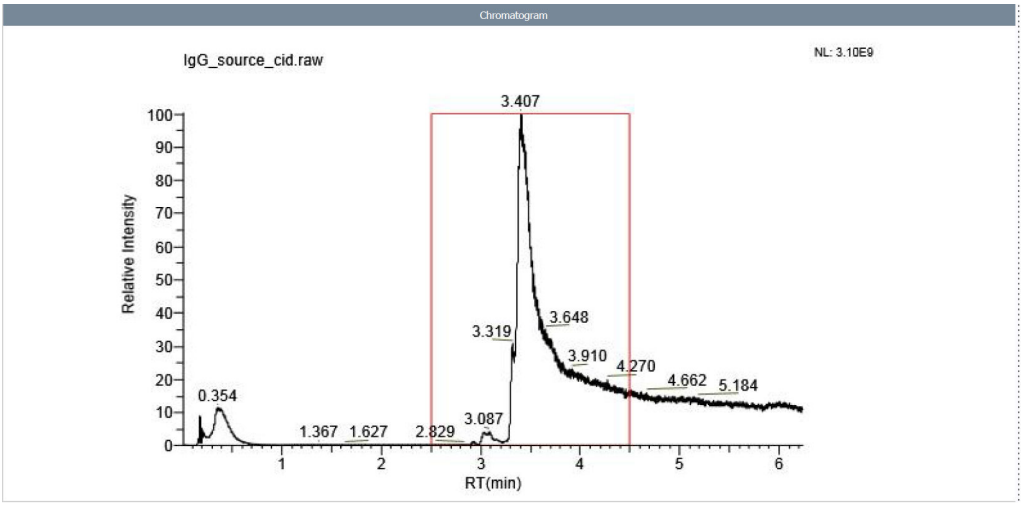


Figure 210 Chromatogram section

The following table lists the parameters in the Chromatogram section.

Table 77 Chromatogram section parameters for Xtract deconvolution

Parameter	Description
Relative Intensity (*y* axis)	Displays the ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity.
RT (min) (*x* axis)	Displays the retention time of the spectrum, which is the time after injection at which a compound elutes. Retention time can also refer to the total time that the compound is retained on the chromatograph column.

Main parameters section

The Main Parameters (Xtract) section of a report, shown in the following figure, displays the main parameter settings that you selected on the Parameters > Component Detection or Process and Review page for an Xtract deconvolution.

Main Parameters (Xtract)	
Output Mass Range	1000 - 60000
Output Mass	M
S/N Threshold	3
Rel. Abundance Threshold (%)	0
Charge Range	5 - 50
Min. Num Detected Charge	3
Isotope Table	Protein

Figure 211 Main Parameters (Xtract) section for Xtract deconvolution

The Main Parameters (ReSpect) section, shown in the following figure, displays the main parameter settings that you selected on the Parameters > Component Detection or Process and Review page for a ReSpect deconvolution.

Main Parameters (ReSpect™)	
Deconvolution Results Filter	
Output Mass Range	10000 - 160000
Deconvoluted Spectra Display Mode	Isotopic Profile (new)
Charge State Distribution	
Deconvolution Mass Tolerance	20 ppm
Choice of Peak Model	
Choice of Peak Model	Intact Protein
Resolution at 400 m/z	
Raw File Specific	12374
Generate XIC for Each Component	
Calculate XIC	False

Figure 212 Main Parameters (ReSpect) section for ReSpect deconvolution

Advanced parameters section

The Advanced Parameters (Xtract) section of a report, shown in the following figure, displays the advanced parameter settings that you selected on the Parameters > Component Detection or Process and Review page for an Xtract deconvolution.

	Advanced Parameters (Xtract)
Calculate XIC	False
Fit Factor (%)	80
Remainder Threshold (%)	25
Consider Overlaps	True
Resolution at 400 m/z	
Method Specific	60000
Negative Charge	False
Charge Carrier	H
Minimum Intensity	1
Expected Intensity Error	3

Figure 213 Advanced Parameters (Xtract) section for Xtract deconvolution

The Advanced Parameters (ReSpect) section, shown in the following figure, displays the advanced parameter settings that you selected on the Parameters > Component Detection or Process and Review page for a ReSpect deconvolution.

	Advanced Parameters (ReSpect™)
Charge State Distribution	
Model Mass Range	10000 - 160000
Charge State Range	10 - 100
Minimum Adjacent Charges (low & high model mass)	6 - 10
Noise Parameters	
Rel. Abundance Threshold (%)	0
Deconvolution Quality	
Quality Score Threshold	0
Choice of Peak Model	
Target Mass	160000 Da
Peak Model Parameters	
Number of Peak Models	1
Left/Right Peak Shape	2:2
Peak Filter Parameters	
Peak Detection Minimum Significance Measure	1 Standard Deviations
Peak Detection Quality Measure	95%
Specialized Parameters	
Peak Model Width Factor	1
Intensity Threshold Scale	0.01
Deconvolution Parameters	
Noise Compensation	True
Charge Carrier	H
Negative Charge	False

Figure 214 Advanced Parameters (ReSpect) section for ReSpect deconvolution

Source Spectra parameters section

The Source Spectra Parameters section of a report, shown in the following three figures, displays the parameter settings that you selected on the Parameters > Component Detection or Process and Review page for a particular source spectra method.

	Source Spectra Parameters
Source Spectra Method	Sliding Windows
Sliding Windows Definition	
RT Range	5.000 - 7.000 minutes
Target Avg Spectrum Width	0.5 minutes
Target Avg Spectrum Offset (%)	25
Sliding Windows Merging Parameters	
Merge Tolerance	30 ppm
Max RT Gap	1 minutes
Min. Number of Detected Intervals	3

Figure 215 Source Spectra Parameters section for the Sliding Windows method

	Source Spectra Parameters
Source Spectra Type	Auto Peak Detection
Sensitivity	High
Rel. Intensity Threshold (%)	1

Figure 216 Source Spectra Parameters section for the Auto Peak Detection method

	Source Spectra Parameters
Source Spectra Method	Average Over Selected Retention Time
RT Range	2.500 - 4.500 minutes

Figure 217 Source Spectra Parameters section for the Average Over Selected Retention Time method

Sequences information section

The Sequences Information section of a report, shown in the following figure, displays the protein sequence settings that you chose using the Protein Sequence Editor, including modification and identification parameters, for each protein sequence used in the experiment.

	Sequences Information
Protein Sequence Matching Mass Tolerance	20 ppm
Total Number of Protein Sequences	1
Protein Sequence	Disulfide_Bonds
Static Modifications	
Max. Number of Modifications	2
Variable Modifications	
Glycosylation	N-linked CHO Glycosylation

Figure 218 Sequences Information section

Source spectrum section

The Source Spectrum section of a report, shown in the following figure, displays the spectrum in the Source Spectrum pane of the Parameters > Component Detection or Process and Review page.

IMPORTANT! The report does not include this section for sliding windows deconvolutions because there is no single source spectrum for the results.

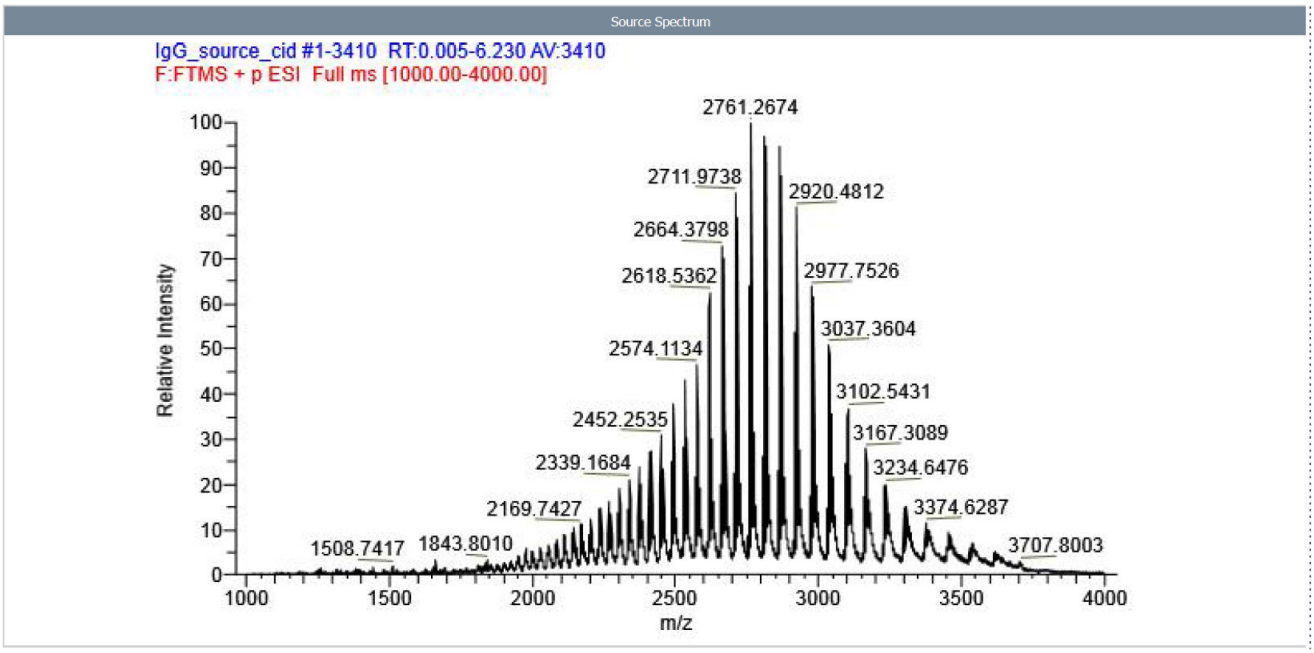


Figure 219 Source Spectrum section

The following table lists the parameters in the Source Spectrum section.

Table 78 Source Spectrum section parameters

Parameter	Description
Relative Intensity (*y* axis)	Displays the ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity.
m/z (*x* axis)	Displays the mass-to-charge ratio of ions formed from molecules. This ratio is the quantity formed by dividing the mass of an ion, in daltons, by the number of charges carried by the ion.

Deconvoluted spectrum section

The Deconvoluted Spectrum section of a report, shown in the following figure, displays the same spectrum that appears in the Deconvoluted Spectrum pane of the Process and Review page.

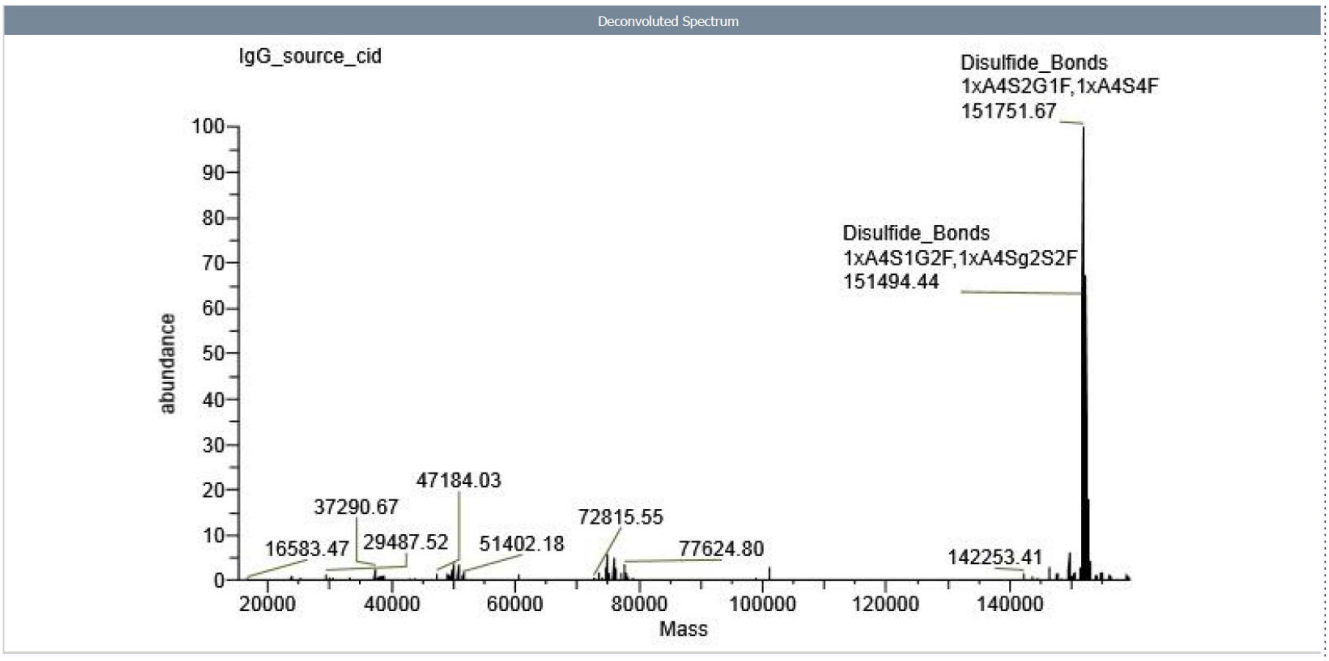


Figure 220 Deconvoluted Spectrum section

The following table lists the parameters in the Deconvoluted Spectrum section.

Table 79 Deconvoluted Spectrum section parameters

Parameter	Description
Abundance (*y* axis)	Displays the relative peak abundance.
Mass (*x* axis)	Displays the actual mass of an ion in atomic mass units.

Masses table section

The Xtract Masses Table section of a report displays the results of an Xtract deconvolution. For an example of a single or auto peak detection deconvolution, see the first following figure, and for an example of a sliding windows deconvolution, see the second following figure. This section contains the same columns as those in the Results table on the Process and Review page. The columns vary depending on the settings for the experiment.

Xtract Masses Table										
Row Number	Monoisotopic Mass	Sum Intensity	Relative Abundance	Fractional Abundance	Number of Charge States	Charge State Distribution	Average Charge	Delta Mass	Start Time (min)	Stop Time (min)
1	25220.490	7497468.06	100.00	37.27	19	17 - 35	25.35	0.000	5.177	5.520
2	25382.514	6777840.33	90.40	33.69	19	17 - 35	25.39	162.024	5.177	5.520
3	25544.572	800423.76	10.68	3.98	11	23 - 33	27.54	324.082	5.177	5.520
4	25238.464	732030.90	9.76	3.64	11	21 - 33	27.59	17.974	5.177	5.520
5	25202.467	699762.96	9.33	3.48	12	24 - 35	28.88	-18.023	5.177	5.520
6	25364.508	678201.27	9.05	3.37	11	24 - 34	28.86	144.018	5.177	5.520
7	25074.411	581217.93	7.75	2.89	12	23 - 34	27.82	-146.079	5.177	5.520
8	25402.501	311169.83	4.15	1.55	6	25 - 32	27.85	182.011	5.177	5.520

Figure 221 Xtract Masses Table section for a single/auto peak detection Xtract deconvolution

Sliding Windows Xtract Masses Table											
Row Number	Monoisotopic Mass	Sum Intensity	Relative Abundance	Fractional Abundance	Number of Charge States	Charge State Distribution	Number of Detected Intervals	Delta Mass	Scan Range	Start Time (min)	Stop Time (min)	Apex RT
1	23428.563	48902544.73	100.00	70.15	7	17 - 23	8	0.000	173 - 219	5.635	6.999	6.376
2	25220.481	8223064.99	16.82	11.80	7	19 - 25	9	1791.918	151 - 219	4.996	6.999	5.244
3	25382.500	7115985.52	14.55	10.21	7	19 - 25	8	1953.937	151 - 219	4.996	6.999	5.244
4	23449.521	2647658.67	5.41	3.80	7	17 - 23	4	20.958	181 - 210	5.868	6.735	6.376
5	23410.549	1172523.23	2.40	1.68	4	20 - 23	6	-18.014	177 - 219	5.751	6.999	6.376
6	14987.296	499475.08	1.02	0.72	3	13 - 15	4	-8441.267	181 - 210	5.868	6.735	6.376
7	23589.590	498743.88	1.02	0.72	4	20 - 23	3	161.027	181 - 206	5.868	6.619	6.244
8	25544.566	466196.36	0.95	0.67	3	23 - 25	5	2116.003	151 - 184	4.996	5.985	5.244

Figure 222 Xtract Masses Table for a sliding windows Xtract deconvolution

The ReSpect Masses Table section displays the results of a ReSpect deconvolution. For an example of a single or auto peak detection deconvolution, see the first following figure, and for an example of a sliding windows deconvolution, see the second following figure. This section contains the same columns as those in the Results table on the Process and Review page. The columns vary depending on the settings for the experiment.

ReSpect Masses Table											
Row Number	Average Mass	Intensity	Relative Abundance	Fractional Abundance	Score	Number of Charge States	Charge State Distribution	Mass Std Dev	PPM Std Dev	Delta Mass	Start Time (min)	Stop Time (min)
1	39125.60	172598.25	100.00	6.56	35.47	7	65 - 71	1.19	30.31	0.00	0.017	79.997
2	15368.19	82120.14	47.58	3.12	35.85	8	30 - 37	0.71	46.47	-23757.41	0.017	79.997
3	35334.28	79644.98	46.14	3.03	46.18	8	76 - 83	1.41	39.81	-3791.32	0.017	79.997
4	25102.57	77039.71	44.64	2.93	31.03	6	72 - 77	1.14	45.40	-14023.04	0.017	79.997
5	12967.21	70933.80	41.10	2.69	28.83	7	26 - 32	0.60	46.08	-26158.39	0.017	79.997
6	45663.50	66740.81	38.67	2.54	29.48	7	79 - 85	1.83	39.20	7537.90	0.017	79.997
7	19681.77	63414.99	36.74	2.41	28.97	6	43 - 48	0.83	42.09	-19443.83	0.017	79.997
8	28167.58	62350.82	36.12	2.37	30.84	6	44 - 49	1.40	49.67	-10958.03	0.017	79.997

Figure 223 ReSpect Masses Table section for a single/auto peak detection ReSpect deconvolution

Sliding Windows ReSpect Masses Table															
Row Number	Average Mass	Sum Intensity	Relative Abundance	Fractional Abundance	Score	Number of Charge States	Charge State Distribution	Number of Detected Intervals	Mass Std Dev	PPM Std Dev	Delta Mass	Scan Range	Start Time (min)	Stop Time (min)	Apex RT
1	148381.83	37567445.66	100.00	34.48	124.08	32	38 - 69	20	2.52	16.97	0.00	77 - 103	1.941	2.615	2.088
2	148543.01	27193773.35	72.39	24.96	114.44	32	38 - 69	19	3.40	22.90	161.18	77 - 103	1.941	2.615	2.088
3	148221.22	25805648.53	68.69	23.69	129.69	30	38 - 67	17	2.96	19.97	-160.60	77 - 104	1.941	2.640	2.088
4	148701.61	11475537.09	30.55	10.53	99.10	33	38 - 70	15	4.01	27.00	319.79	77 - 97	1.941	2.464	2.088
5	148014.27	3441095.52	9.16	3.16	92.70	18	42 - 59	7	5.40	36.48	-367.55	78 - 89	1.966	2.262	2.088
6	148860.68	2254640.88	6.00	2.07	69.16	15	40 - 54	4	7.28	48.88	478.85	78 - 86	1.966	2.186	2.088
7	149019.57	1057990.05	2.82	0.97	77.97	18	40 - 57	5	7.35	49.33	637.74	80 - 90	2.017	2.287	2.088
8	148378.75	147344.73	0.39	0.14	60.28	12	41 - 52	4	7.21	48.57	-3.07	76 - 110	1.916	2.791	2.665

Figure 224 ReSpect Masses Table section for a sliding windows ReSpect deconvolution

Component detail tables section

The Component Detail Tables section of a report displays a table for each component in the sample. For a single or auto peak detection deconvolution, see the first following figure (for Xtract) and the third following figure (for ReSpect), and for a sliding windows deconvolution, see the second following figure (for Xtract) and the fourth following figure (for ReSpect).

This section appears only if you select the Component Detail Tables option on the Parameters > Report page for the processing method. The table shows all the charge states that the BioPharma Finder application detected for that component. It displays the same parameters as those displayed in the Results table on the Process and Review page.

The following tables show only a partial list of values.

Monoisotopic Mass:16941.011								
Row Number	Charge State	Calculated Monoisotopic m/z	Monoisotopic Mass of Charge State	Most Abundanct m/z	Charge Normalized Intensity	Fit %	Fit % Left	Fit % Right
1	10	1695.1075	16940.973	1696.1090	247.5300	82.50	0.00	17.50
2	11	1541.0984	16940.971	1542.0022	342.3900	94.50	0.00	5.50
3	12	1412.7575	16940.938	1413.5888	583.3767	95.50	0.00	4.50
4	13	1304.1613	16940.907	1304.9242	1352.1785	97.20	0.00	2.80
5	14	1211.0789	16940.896	1211.7867	2269.3079	96.90	0.00	3.10
6	15	1130.4074	16940.885	1131.0669	3473.5833	97.60	0.00	2.40
7	16	1059.8199	16940.898	1060.4405	4910.5650	98.00	0.00	2.00
8	17	997.5368	16940.966	998.1264	7611.2888	91.20	0.00	8.80

Figure 225 Component Detail Tables section for a single/auto peak detection Xtract deconvolution

Monoisotopic Mass:16922.992				
Row Number	Charge State	Intensity	MZ Centroid	Calculated Mass
0	15	9372.63	1129.203	16922.987
1	16	20924.40	1058.692	16923.004
2	17	24272.79	996.477	16923.005
3	18	36773.10	941.172	16923.000
4	19	43455.75	891.690	16922.998
5	20	33690.26	847.156	16922.986
6	21	31022.74	806.862	16922.976
7	22	22870.25	770.232	16922.995
8	23	26740.44	736.788	16922.988

Figure 226 Component Detail Tables section for a sliding windows Xtract deconvolution

Average Mass:151815.27						
Row Number	Charge State	Intensity	Measured Average m/z	Measured Average Mass	Delta Mass (Da)	Delta Mass (ppm)
1	41	78878.01	3703.636	151807.77	-7.50	-49.39
2	42	90466.21	3615.732	151818.46	3.19	21.02
3	43	160037.56	3531.646	151817.49	2.22	14.63
4	44	197441.31	3451.451	151819.51	4.24	27.96
5	45	303060.51	3374.677	151815.12	-0.15	-0.99
6	46	352221.72	3301.369	151816.63	1.37	9.02
7	47	509951.75	3231.136	151816.05	0.78	5.16
8	48	624306.47	3163.862	151817.04	1.78	11.70

Figure 227 Component Detail Tables section for a single/auto peak detection ReSpect deconvolution

Monoisotopic Mass:151976.40				
Row Number	Charge State	Intensity	MZ Centroid	Calculated Mass
0	41	715205.63	3707.852	151980.65
1	42	2169397.71	3619.404	151972.65
2	43	2918569.78	3535.489	151982.71
3	44	2386771.83	3454.927	151972.48
4	45	3838859.07	3378.259	151976.34
5	46	6747962.65	3304.842	151976.40
6	47	11097249.54	3234.580	151977.90
7	48	11784068.16	3167.204	151977.43
8	49	21053891.56	3102.608	151978.44

Figure 228 Component Detail Tables section for a sliding windows ReSpect deconvolution

Source spectrum evidence plot section

The Source Spectrum Evidence Plot section of a report displays a source spectrum plot for each component in the sample. For an Xtract deconvolution, see the first following figure, and for a ReSpect deconvolution, see the second following figure.

This section appears only if you select the Component Source of Evidence Plots option on the Parameters > Report page for the processing method. The graph shows the peaks in the scan or the isotopic clusters that are associated with a particular component.

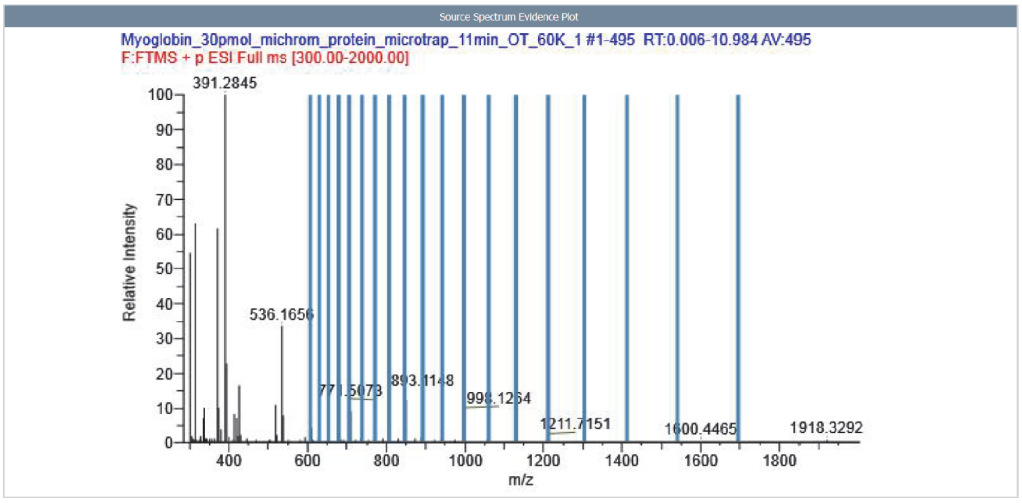


Figure 229 Source Spectrum Evidence Plot section for an Xtract deconvolution

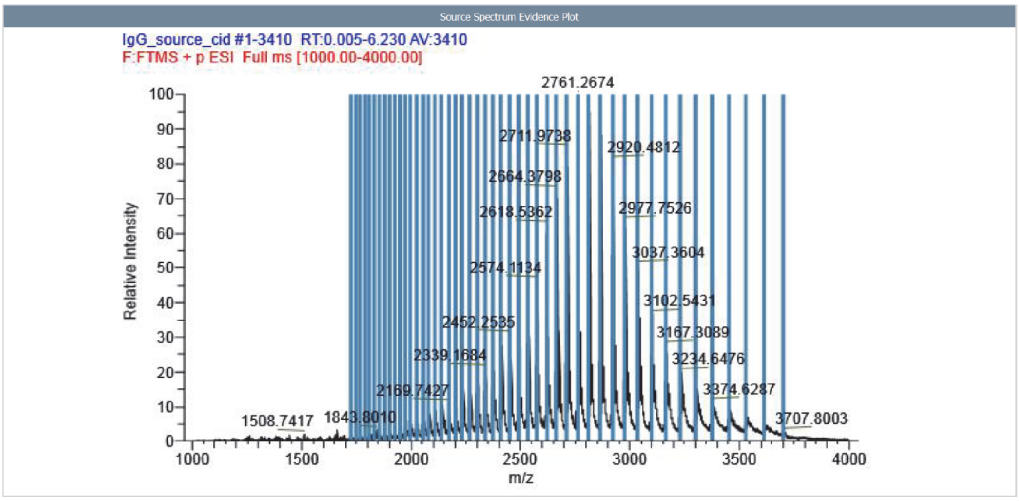


Figure 230 Source Spectrum Evidence Plot section for a ReSpect deconvolution

The following table lists the parameters for the source spectrum shown in the Source Spectrum Evidence Plot section.

Table 80 Source Spectrum Evidence Plot section parameters

Parameter	Description
Relative Intensity (*y* axis)	Displays the ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity.
m/z (*x* axis)	Displays the mass-to-charge ratio of ions formed from molecules.

Part VI

Top Down Analysis

24 Top down analysis features

Top Down Analysis provides precise identification and full characterization of molecular composition. The BioPharma Finder application processes this type of experiment by adding jobs to a run queue to search the protein sequences containing all the proteoforms for a specific organism based on its sequenced genome.

This type of experiment involves intact precursor protein molecules ionized and analyzed by mass spectrometry, which then isolates single peaks and subjects them to fragmentation. Each peak usually represents one charge state of the unknown protein but sometimes represents a small number of isobaric proteins.

The application then compares the MS mass measurements and MS2 fragment ions data to the proteoforms (with the known or predicted PTMs) saved in protein sequences to identify and characterize the unknown proteins. The matches resulting from these comparisons are not exact but are within a fragmentation tolerance. To determine the best match, the application then scores the matches by using various fragment-based scoring functions.

Features similar to intact mass analysis

Top Down Analysis provides several features that are similar to Intact Mass Analysis:

- It extracts the averaged mass spectra, deconvolves the isotopically unresolved or resolved mass for the proteins, and then generates the results.
- It uses the same two deconvolution algorithms for mass spectral data: Xtract and ReSpect.

  Note: Top Down Analysis currently supports the processing of MS2 spectra using only Xtract deconvolution. For processing Full MS spectra, it supports both the Xtract and ReSpect algorithms.
- When you run an experiment with multiple raw data files, you can choose either the Batch or Multiconsensus format for your results.
- You can set up one or more protein sequences and select them for target sequence matching when processing Full MS spectra.

Top down analysis inputs

The type of input file used in Top Down Analysis is the raw data file from a mass spectrometry experiment. The raw data file can contain LC/MS data or an individual mass spectrum.

You can use Top Down Analysis with data from various mass spectrometry systems: Thermo Scientific™ Exactive Series, Orbitrap™ Series, and Fourier transform mass spectrometry (FTMS) series.

Top down analysis outputs

As output, Top Down Analysis produces deconvoluted spectra and component/peak information, including fragment maps and modification data. It saves to database records both the method parameter settings that you applied to the raw data files and the results. This information appears on the Process and Review page.

When you want to view the results in another session, you can load the previously saved results from the Load Results page to review the deconvolution data.

Performing a top down analysis experiment

The workflows between Top Down Analysis and Intact Mass Analysis are similar. In both types, you can select the chromatogram to deconvolve in the BioPharma Finder application.

The following steps show how to perform a Top Down Analysis experiment:

1. Create a new experiment by naming it, load the raw data file or files, add the conditions and choose the result format (if you load multiple files), and then select the protein sequences and a processing method.
2. (Optional) Edit the parameters in an existing method and create a custom method. You must specify an RT range (this might be optional for a one scan mass spectrum).
3. Submit the experiment for processing and monitor the status of the experiment by using the queue.
4. View the results of the analysis.
5. (Optional) Use real-time optimization to change the parameters and reprocess the results.

25 Running a top down analysis

After you add a sequence for top down analysis to the Sequence Manager, you can start a top down analysis experiment by clicking Top Down Analysis on the Home page.

Spectral deconvolution for top down analysis

For Full MS scans, Top Down Analysis uses deconvolution algorithms to transform a charge state series into a molecular mass, similar to Intact Mass Analysis. The application identifies multiple peaks in the mass spectrum associated with different charge states of the same component and displays information about the masses and abundance of that component.

For MS2 scans, Top Down Analysis uses features of the ProSight Lite application to process the deconvolved MS2 spectral results. The application produces fragment maps and fragment mass labels for found matched components and modification masses.

Starting a new top down experiment

Use the Top Down Analysis page to create a new top-down experiment. Enter the experiment name, load the raw data file or files, choose a result format option (if you load multiple raw data files), select one or more protein sequences, and select a processing method to start processing.

To specify the following default items—the folder from which to load your raw data files, the global dimensions for copied images, and also the display precision for the top-down experiments.

To start a new experiment for Top Down Analysis

1. On the Home page, click **Top Down Analysis**.

   The Top Down Analysis page opens, as shown in the following figure.

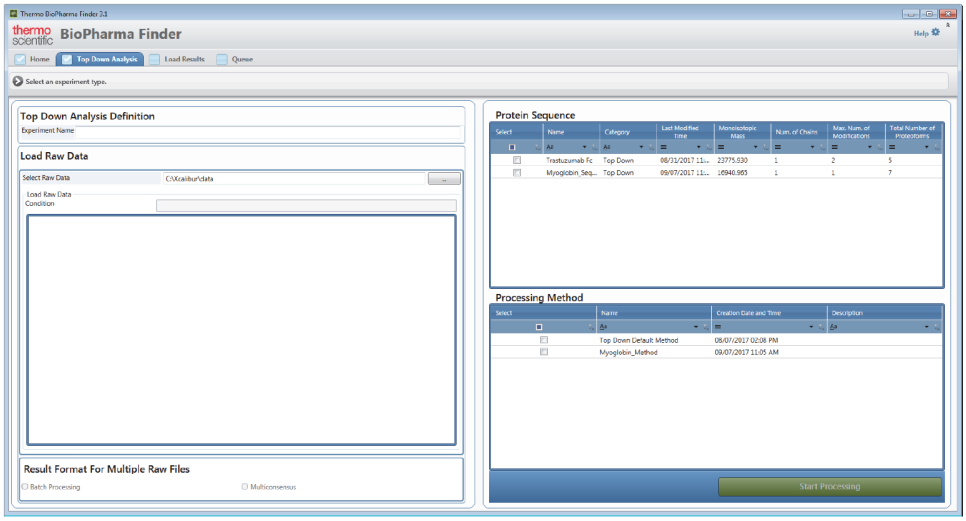


Figure 231 Top Down Analysis page

2. In the Top Down Analysis Definition area, type the name of the experiment.

   Note: Use only alphanumeric, space, underscore "_", and period "." characters, up to 50 maximum, in the experiment name. All of the names in the BioPharma Finder application are case-insensitive.

   If an existing experiment is already completed or canceled, you can overwrite it by entering the same experiment name. You cannot overwrite an experiment that is currently submitted to the run queue or is actively processing or canceling.

3. In the Load Raw Data area, load the raw data file or files for the experiment.

   (Optional) If you load multiple files, in the Condition box, type the conditions by which to group the raw data files, separated by spaces. Then, in the Condition column in the Load Raw Data table, select a condition for each raw data file. The application automatically groups together all files assigned to the same condition for processing the results related to conditions.

4. (For experiments with multiple loaded raw data files only) In the Result Format for Multiple Raw Files area, select either option:

   - **Batch Processing** to run each raw data file as a separate experiment.
   - **Multiconsensus** to merge the results from the multiple raw data files together.

     Note: To select the Multiconsensus result format, you can load a maximum of 10 raw data files.

IMPORTANT! For an experiment with MS2 scans and the Multiconsensus result format, unlike Full Scan processing, the application does not merge the results by using the merging parameters in the method. Instead, it concatenates the results from each raw data file together into one set of results for the experiment.

---

5. In the table in the Protein Sequence area, select the check box for one or more protein sequences for the experiment.

   IMPORTANT! For Top Down Analysis, only the protein sequences with a Category of Top Down appear in the table. If you want to use a sequence for Top Down Analysis and you do not see it in the table, change its Category value to Top Down.

6. In the table in the Processing Method area, select the check box for a processing method for the experiment.

   You can select a custom method that you created or the provided default method: **Top Down Default Method**. You can also import a method file from your computer.

7. To edit the method parameters and then start processing, click **Start Processing**.

   You can make adjustments to the peak, deconvolution, and other method parameters and then save the method before processing. You can also create a new custom method by editing the parameters in an existing method and then saving it to a different name.

   If you are loading multiple raw data files, the application displays the chromatograms and source spectra for up to 10 individual raw data files.

   Note: Thermo Fisher Scientific recommends that you review and update the parameters before you begin processing the experiment.

   For example, to process an experiment using an m/z Range of 400 to 600, regardless of the raw data files used, create a custom processing method with this range and save the method.

   When you create another experiment, select the saved method to run the experiment with the specified range.

For more details, see the following topics:

- Specifying global settings for Intact Mass Analysis or Top Down Analysis (page 35)
- Using the run queue (page 161)
- Loading the raw data files (page 135)
- Batch and multiconsensus result formats (page 336)
- Selecting one or more sequences (page 139)
- Working with a top down processing method (page 496)
- m/z Range (page 510) parameter

Top down experiment processing on the queue page

To start processing a new job, the application requires the experiment name, the raw data files, the result format selection when you load multiple raw data files, one or more protein sequences, and a processing method. When all requirements are met for processing, the application saves all entered information for the new job and then opens the Queue page.

If you select the Batch Processing option for the result format, the application automatically appends a date-and-time stamp to the experiment name for each loaded raw data file. For example, if you load 20 raw data files for the experiment, the application submits to the queue 20 separate jobs, each named *experiment name_date_time*. Each job generates individually processed results.

Note: You must manually select a protein sequence for each peak in a Top Down Analysis method.
If you select a custom method, the chromatogram parameters might not be set correctly. In this case, a warning message informs you that the method might have incorrect chromatogram settings and suggests that you evaluate these settings before running the experiment.

For more details, see the following topics:

- Using the run queue (page 161)
- Starting a new top down experiment (page 492)

26 Working with a top down processing method

The BioPharma Finder application provides a default processing method for Top Down Analysis. If needed, you can use the editing wizard to edit the parameters in this method and save it to a new file to create a custom method for your experiment. For this type of analysis, you cannot just edit a method without running an experiment.

Using a processing method for top down analysis

To create a new method or edit a current method

1. On the Home page, click **Top Down Analysis**.

   The Top Down Analysis page opens.

2. Enter the experiment name, load one or more raw data files and enter the conditions if needed, choose a result format if you load multiple raw data files, and select one or more protein sequences.

   Note: If you load one raw data file or run a batch experiment, the application derives the default scan filters, fragmentation type, *m/z* range, and resolution at 400 *m/z* based on information retrieved from each file.

   For a multiconsensus experiment with multiple raw data files, by default, the application uses the above information from the first loaded file.

   The application determines the other component detection and identification parameters for the experiment and displays these parameters on the pages under the Parameters tab.

3. In the Processing Method area, select a processing method in the table to edit, and then click **Start Processing**.

   Note: To create a custom method, modify the parameters in a default method, and then save them to a new method using a different name. You cannot overwrite a default method.

The editing wizard opens the Component Detection page under the Parameters tab, showing the component detection parameters in the current method. The experiment name appears in the upper right corner of the screen. Use the editing wizard on the Component Detection and Identification pages to specify the appropriate method parameters.

When you are done editing the parameters on each of these pages, click **Next** to go to the Save Experiment page to save all of the modified parameters to a method. You can then select that method to use for processing another experiment.

For more details, see the following topics:

- Starting a new top down experiment (page 492)
- Batch and multiconsensus result formats (page 336)
- Peak-specific parameters on the Component Detection page (page 498)
- Plots on the Component Detection page (page 502)
- Saving a processing method (page 144)

Editing component detection parameters for top down analysis

When you want to create a new processing method or edit an existing method for Top Down Analysis, go to first page of the editing wizard, the Component Detection page. The parameters on this page vary depending on the loaded raw data file or files and the selected deconvolution algorithm: Xtract or ReSpect.

Note: Top Down Analysis currently supports the processing of MS2 spectra using only Xtract deconvolution. For processing Full MS spectra, it supports both the Xtract and ReSpect algorithms.

Before editing the parameters on the Component Detection page, see the following topics:

- Opening the Component Detection page (page 498)
- Peak-specific parameters on the Component Detection page (page 498)
- Plots on the Component Detection page (page 502)
- Editing the Component Detection page (page 504)

For more information regarding various parameters and commands, see these topics:

- Xtract algorithm (page 330)
- ReSpect algorithm (page 331)
- Peak selection area parameters (page 507)
- Fragmentation types (page 509)
- Xtract deconvolution parameters (page 510)
- ReSpect deconvolution parameters (page 511)
- Component detection page commands (page 512)

For more information regarding various ReSpect parameters similar to those used for Intact Mass Analysis, see these topics:

- Optimizing the protein quality score (page 392)
- Model mass range information (page 394)
- Best results with the ReSpect algorithm (page 395)

Opening the Component Detection page

To open the Component Detection page

1. (Optional) On the Top Down Analysis page, enter an experiment name, load one or more raw data files, choose a result format if you load multiple files, and select one or more protein sequences.

2. Select a method and then click **Start Processing**.

   The Component Detection page opens showing several areas on the left and three panes on the right: Chromatogram, Peak # - Intact Fragmentation Source Spectrum, and Peak # - Intact Deconvolution Source Spectrum. If you navigate away from this page and want to get back to it, click the **Parameters** tab, and then click the **Component Detection** subtab.

For more details, see the following topics:

- Starting a new top down experiment (page 492)
- Raw data files and sequences (page 134)
- Selecting one or more sequences (page 139)
- Peak-specific parameters on the Component Detection page (page 498)
- Plots on the Component Detection page (page 502)

Peak-specific parameters on the Component Detection page

The left side of the Component Detection page for Top Down Analysis contains all of the parameters specific to each peak. The Peak Selection area displays the RT range, scan filter, activation type, protein sequence, and fragmentation mass tolerance parameters. The Peak # -Deconvolution Parameters area displays the Intact Fragmentation and Intact Deconvolution pages. Each page contains the parameters specific to a deconvolution algorithm for each type of spectrum (MS2 for the Intact Fragmentation page or Full MS for the Intact Deconvolution page).

Note: Top Down Analysis currently supports the processing of MS2 spectra using only Xtract deconvolution. For processing Full MS spectra, it supports both the Xtract and ReSpect algorithms.

An example of the left side of the Component Detection page shows various data in the upper area and the Intact Fragmentation Xtract parameters in the lower area—all specific to the selected peak.

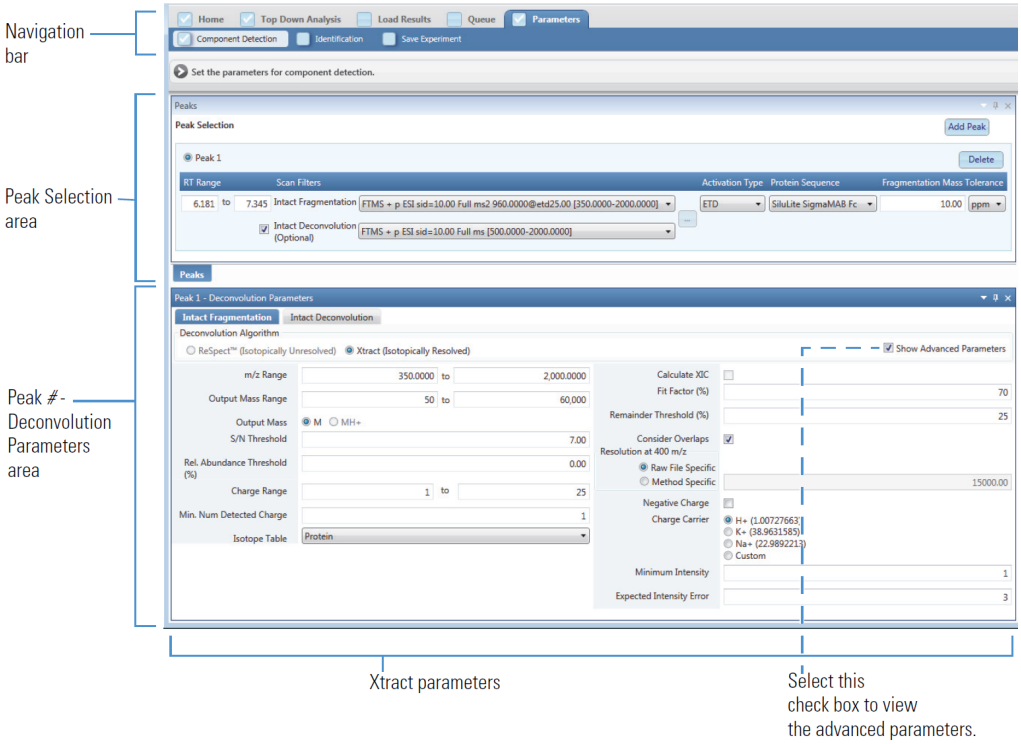


Figure 232 Component Detection page (left side) with peak-specific Intact Fragmentation Xtract parameters

Another example of the left side of the Component Detection page shows various data in the upper area and the Intact Deconvolution ReSpect parameters in the lower area—all specific to the selected peak.

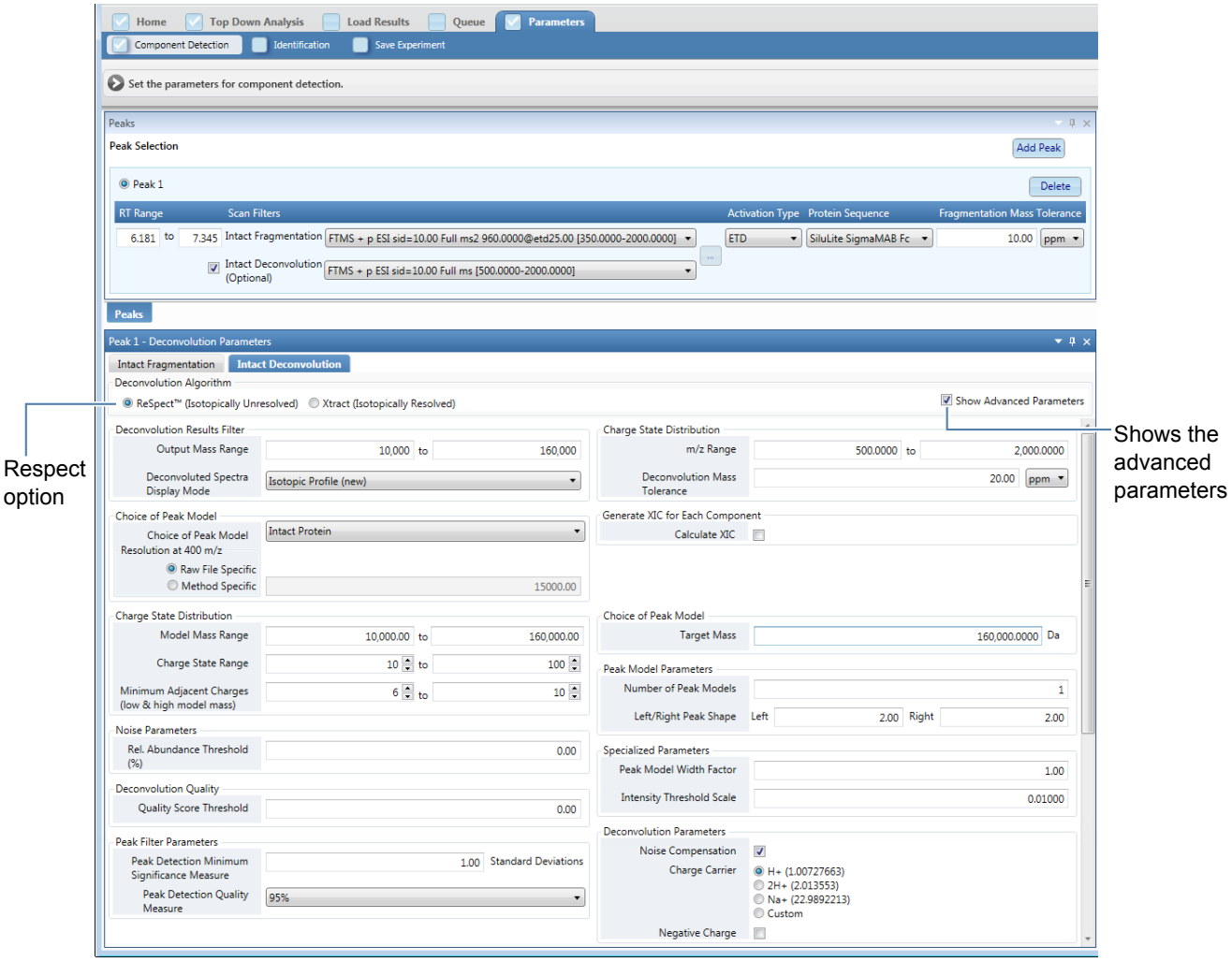


Figure 233 Component Detection page (left side) with peak-specific Intact Deconvolution ReSpect parameters

The left side of the page contains these areas where you define the peak-specific parameters:

- **Peak Selection**: Displays the parameters specific to each peak, including the retention time (RT) range of the selected peak in the Chromatogram pane, scan filters from the loaded raw data files, activation type, protein sequence, and fragmentation mass tolerance.

  Note: In the Chromatogram pane, the shaded box indicates the currently selected peak.

  IMPORTANT! The parameters in this area for processing the Full MS spectrum are optional. To set the parameters to process the deconvolution of the Full MS spectrum, select the **Intact Deconvolution** check box.

  For an experiment with multiple loaded raw data files, click the **Multiple File Parameters** button, ..., so that you can select the scan filters and activation type for each file.

- **Peak # - Deconvolution Parameters:** Displays the parameters specific to each peak on either the Intact Fragmentation page for MS2 spectra or the Intact Deconvolution page for Full MS spectra. For MS2 spectra, the application supports only Xtract deconvolution. For Full MS spectra, the application supports either the Xtract or ReSpect algorithm.

  Note: You can edit advanced options by selecting the Show Advanced Parameters check box. However, these advanced parameters are hidden by default and typically need no modifications.

  IMPORTANT! You must select the Intact Deconvolution check box in the Peak Selection area to make the Intact Deconvolution page active.

For more details, see the following topics:

- Editing the Component Detection page (page 504)
- Peak selection area parameters (page 507)
- Xtract deconvolution parameters (page 510)
- ReSpect deconvolution parameters (page 511)

Plots on the Component Detection page

The following figure shows the three panes on the right side of the Component Detection page for Top Down Analysis. In this example, the Chromatogram plot displays colored boxes to indicate the selected RT ranges for several peaks. The shaded box indicates the currently selected peak. For that peak, the Peak # - Intact Fragmentation Source Spectrum plot displays the MS2 source spectrum and the Peak # - Intact Deconvolution Source Spectrum plot displays the Full MS source spectrum.

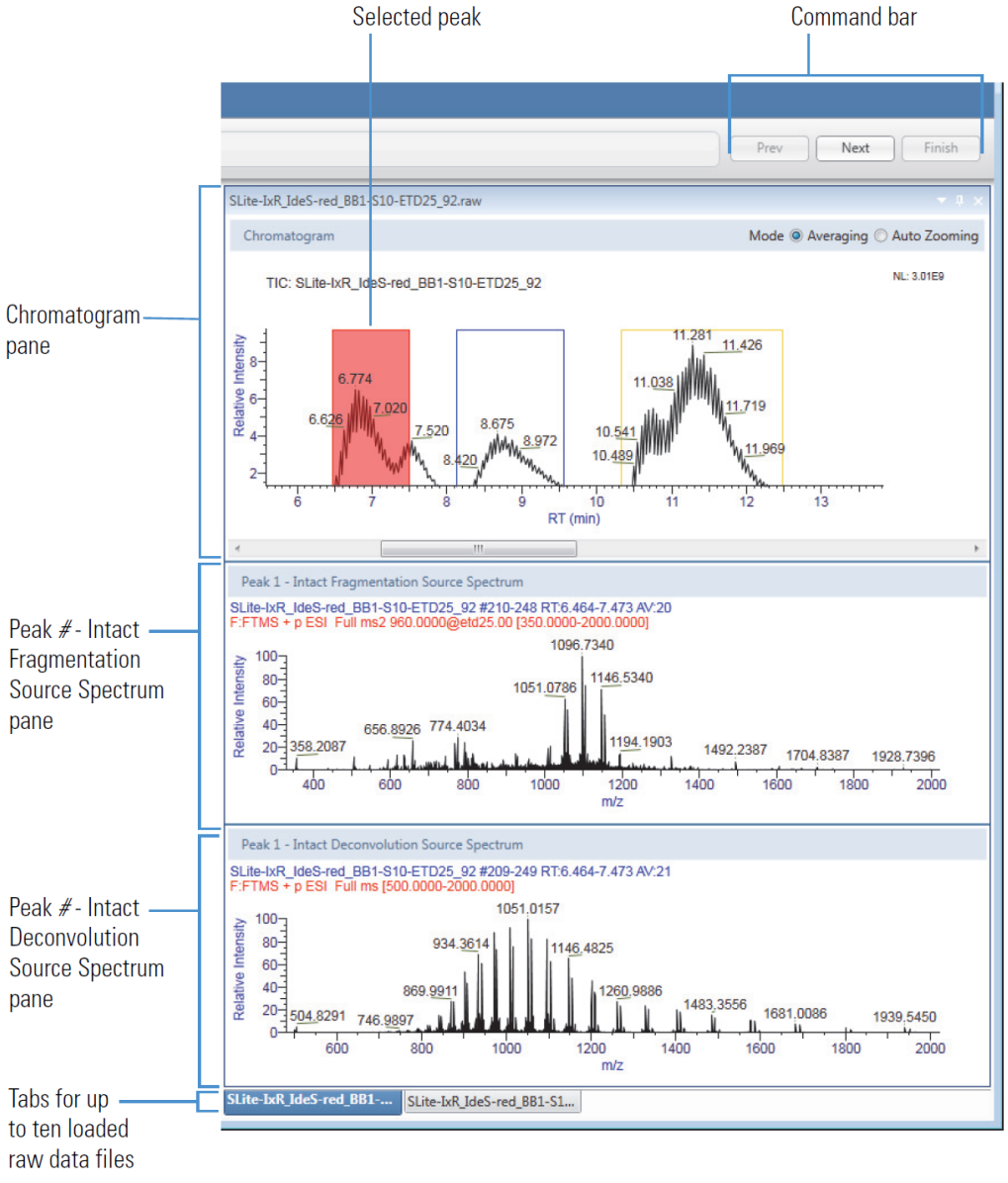


Figure 234 Component Detection page (right side) with the three panes and tabs

IMPORTANT! You must select the Intact Deconvolution check box in the Peak Selection area to make the Peak # - Intact Deconvolution Source Spectrum plot active.

A tab appears at the bottom of these panes for each raw data file loaded into the experiment. The application displays a maximum of ten raw data file tabs. Click a tab to see the chromatogram and source spectra for a particular file. To see more tabs, scroll to the right as needed.

See the following descriptions:

- **Chromatogram** pane: Displays the chromatogram of the data in each loaded raw data file.

  A chromatogram plot shows the intensities of one or more masses as a function of time. By default, the Chromatogram pane displays a TIC. You can view the BPC by right-clicking the pane and choosing **Chromatogram Trace Type ▸ BPC**. The chromatogram is fully magnified. Use the zooming mode in this pane to enlarge a region of the spectrum, or use the averaging mode to generate a new source spectrum by selecting a new retention time range.

  This pane displays a colored box for each selected peak in the chromatogram. To select a peak, enter the appropriate RT Range values in the Peak Selection area. Or, select a single scan or a range of averaged scans directly on the chromatogram, and a colored line or box appears on the chromatogram for that range. The default RT Range is from 0.000 to 0.000.

  Use the Chromatogram pane to select the peaks to generate the best possible spectra for deconvolution of the target protein.

  The Mode options in the upper right corner of the pane determine the function to apply when you drag the cursor over a retention time area of the Chromatogram pane.

  - Averaging: Averages all the scans in the selected area to generate the source spectrum and displays it in a source spectrum pane.
  - Auto Zooming: Enlarges the selected area without changing the view displayed in a source spectrum pane.

  The header in the Chromatogram pane displays the following information:

  - TIC (total ion chromatogram) or BPC (base peak chromatogram)
  - The name of the raw data file, for example, SLite-IxR_IdeS-red_BB1-S10-ETD25_92
  - The intensity of the most abundant peak in the entire LC/MS run, for example, NL: 3.01E9

- **Peak # - Intact Fragmentation Source Spectrum** and **Peak # - Intact Deconvolution Source Spectrum** panes: Display the source spectra to deconvolve a region of the chromatogram, either single-scan or averaged, for the currently selected (active) peak.

  The MS2 source spectrum appears in the top pane and the Full MS source spectrum appears in the bottom pane.

  From the Chromatogram pane, select the best possible spectra for the deconvolution of the target protein.

The header for the source spectra displays the following information:

- The name of the raw data file, for example, SLite-IxR_IdeS-red_BB1-S10-ETD25_92
- The scan range, for example, #210-248
- The retention time range, for example, RT:6.464-7.473
- The number of spectra that were averaged to create the source spectrum, for example, AV:20
- The scan filter used during the LC/MS run, for example, F:FTMS + p ESI Full ms2 960.0000@etd25.00[350.0000–2000.0000]

  The scan filter indicates the type of mass analyzer that acquires the data in the raw data file and the ionization technique. If this field is blank, no scan filter was used.

  This filter matches the selected Intact Fragmentation or Intact Deconvolution scan filter selected for the active peak for each loaded raw data file (to the left of the Component Detection page).

For more details, see the following topics:

- RT Range (page 507) parameter
- Editing the Component Detection page (page 504)
- Peak selection area parameters (page 507)

Editing the Component Detection page

Use the various areas and panes on the Component Detection page to modify your top down processing method.

To edit the parameters, chromatogram, and source spectra

1. In the Peak Selection area at the upper left side of the Component Detection page, specify the appropriate parameter values for each individual peak, and add or delete peaks as necessary.

   Select each peak before you define its parameters. Only the parameters for the active peak are editable.

   When you click **Add Peak**, the parameter settings of the new peak are the same as the parameter settings from the first peak by default. The new peak becomes the active peak where you can update the parameters as needed. You can add up to 10 peaks. At least one parameter value must be different for each peak.

   When you click **Delete Peak**, the application deletes the selected peak and all of its corresponding parameters.

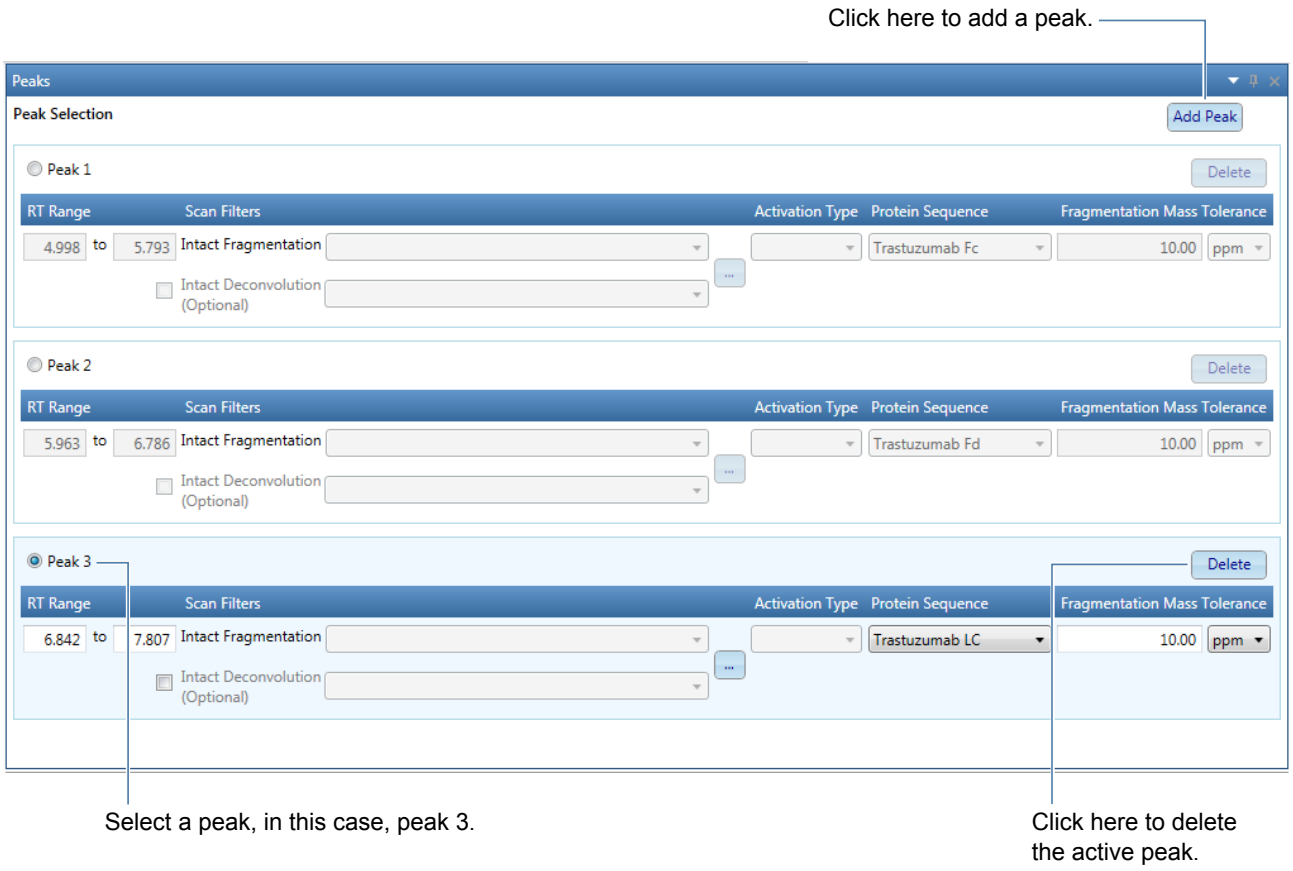


Figure 235 Selecting, adding, or deleting peaks

2. In the Peak # - Deconvolution Parameters area at the lower left side of the Component Detection page, do the following:

   a. Click the **Intact Fragmentation** tab to specify the appropriate parameter values for processing the MS2 spectra.

   –or–

   Click the **Intact Deconvolution** tab to specify the values for processing the Full MS spectra.

   b. Select a particular deconvolution algorithm, **Xtract** or **ReSpect**, and enter the corresponding parameters.

   Note: Top Down Analysis currently supports the processing of MS2 spectra using only Xtract deconvolution, so the ReSpect option is inactive. For processing Full MS spectra, it supports both the Xtract and ReSpect algorithms.

   The values you set apply to the currently selected peak at the upper left area of the page and all of the raw data files loaded for the experiment.

3. Adjust or copy the view in the Chromatogram pane on the right side of the Component Detection page as necessary.

   The same chromatogram also appears on the Process and Review page.

4. For each peak selected on the left side of the Component Detection page, change the source spectra by editing the RT Range parameter in the Peak Selection area at the left side of the page or by doing one of the following in the Chromatogram pane:
   - (For a single scan) Use the red cross-shaped cursor to select a single scan on the chromatogram. The Peak # - Intact Fragmentation Source Spectrum and Peak # - Intact Deconvolution Source Spectrum panes display the associated single-scan mass spectra at that time point.

     You can use the left- and right-arrow keys to move to the previous or next time point in the chromatogram. The source spectra panes automatically update.
   - (For multiple scans) Select a region of the chromatogram to display the averaged spectrum for all the scans within that region. To select this region, select the **Averaging** option in the Mode area. Drag the red cross-shaped cursor across the area of interest.

     The horizontal line of this cursor aids in assessing peak height. The application calculates the average spectra for the selected interval and displays them in the Peak # - Intact Fragmentation Source Spectrum and Peak # - Intact Deconvolution Source Spectrum panes.

     The averaging method is better suited to complex data than the single-scan method. Averaging spectra produces higher signal-to-noise ratios, so higher-quality spectra are highly recommended for optimal deconvolution results.

Tip: You can enter the chromatogram parameters, adjust the view of the chromatogram, and select the source spectrum in Qual Browser in the Xcalibur™ data system. Then, right-click and choose **Export ▸ Write to RAW File** to export the raw data file so that you can import it into the BioPharma Finder application.

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5. Adjust or copy the view in the Peak # - Intact Fragmentation Source Spectrum and Peak # - Intact Deconvolution Source Spectrum panes as necessary.

   These panes show the actual MS2 and Full MS source spectra, either single-scan or averaged, to be deconvolved. They display apex information for major peaks and *m/z* information for deconvolved components. They also show peak apex information as a marker, along with an accompanying label that describes the *m/z* value for the most abundant peak from the spectrum at that retention time.

   In single-scan processing mode, the most abundant *m/z* for a component agrees with the *m/z* shown for the corresponding peak in the source spectrum. In averaged-scan processing mode, the two values might be different because of the way the application displays averaged spectra. This difference is small—approximately 0.001.

   The Xtract algorithm can deconvolve centroid spectra and profile spectra. The ReSpect algorithm can deconvolve only profile spectra. The application automatically chooses the appropriate type of spectrum (centroid or profile) based on your selected algorithm. The source spectra panes display profile

information if it is available; otherwise, they display centroid information. The two types of information differ as follows:

- Centroid data represent mass spectral peaks using two parameters: the centroid (the weighted center of mass) and the intensity (the normalized area of the peak). The data are displayed in a bar graph of relative intensity versus *m/z*.
- Profile data represent the entire spectrum as a succession of points, in *m/z* and relative intensity. The data are displayed in a continuous line graph of relative intensity versus *m/z*.

The source spectra also appear on the Process and Review page.

Note: Zooming or scaling in the source spectra panes does not change the *m/z* range that the deconvolution algorithm uses.

6. Click **Next** in the command bar to advance to the Identification page.

For more details, see the following topics:

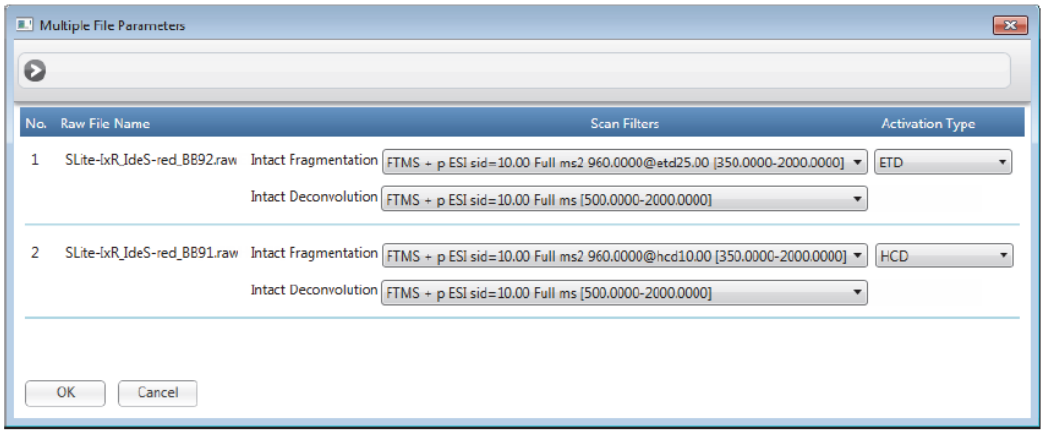
- "Editing the Component Detection page" on page 504
- Peak selection area parameters (page 507)
- Peak-specific parameters on the Component Detection page (page 498)
- Xtract deconvolution parameters (page 510)
- ReSpect deconvolution parameters (page 511)
- Using basic chromatogram functions (page 661)
- Using basic spectrum functions (page 664)
- Using copy and paste functions (page 666)
- RT Range (page 507) parameter
- Viewing the chromatograms for top down analysis (page 541)
- Viewing the source Spectra for top down analysis (page 549)

Peak selection area parameters

The following table describes the parameters in the Peak Selection area of the Component Detection page.

Table 81 Peak Selection area parameters

Parameter	Description
Peak #	Select an individual peak before defining its corresponding parameters.
RT Range	Specifies the beginning and end retention times of the range of the each peak. You can either type the range values, or select the range of each peak from the chromatogram in the Chromatogram pane. For multiple raw data files, select the range from the first file and this value applies to all loaded files.

Parameter	Description
Scan Filters	For an experiment with a single loaded raw data file, this parameter displays the two lists of scan filters within the entered RT Range, Intact Fragmentation (required), and Intact Deconvolution (optional), which the application automatically reads from the file. For Intact Fragmentation, the filter is per peak basis. For Intact Deconvolution, the filter is per raw data file. Select one filter from the Intact Fragmentation list. Optionally, to perform deconvolution of the Full MS spectrum, select the Intact Deconvolution check box and then select one filter from this list. The application uses the selected Intact Fragmentation filter and the features of the ProSight Lite application to process the MS2 scans. It uses the selected Intact Deconvolution filter to process the Full MS scans, similar to Intact Mass Analysis. For a multiconsensus experiment with multiple loaded raw data files, click the Multiple File Parameters button, ..., to open a pop-up box that displays a table with the data from each loaded raw data file. This data includes the file's name, scan filters, and activation types. From the table, select the appropriate scan filters and Activation Type for each file, and then click OK.  **Figure 236 Multiple File Parameters pop-up box** If no scan is present for a particular Scan Filter list, that list is empty. **Note:** In the pop-up box for multiple files, you might not be able to see the entire name of a raw data file if the name is long. In this case, point to the file name in the table to view the entire file name in a tooltip.
Activation Type	Displays the list of available fragmentation types for your selection. For a single loaded raw data file, the default fragmentation type appears automatically from the selected Intact Fragmentation scan filter, if available. For multiple loaded files, the application automatically applies a set of rules to derive the default fragmentation type. You can retain the default value or select a different fragmentation type as needed. Fragmentation types include CID, HCD, SID™, ETD, ECD, EThcD, IRMPD, and UVPD.

Parameter	Description
Protein Sequence	Displays the protein sequences selected in the Protein Sequence area of the Top Down Analysis page. From this list, select one sequence for each peak. The application automatically searches all proteoforms saved with the selected sequences. **IMPORTANT!** If you selected only one protein sequence on the Top Down Analysis page to use for the experiment, the application displays this sequence as selected for the current peak, by default. Otherwise, if you selected multiple protein sequences on the Top Down Analysis page and you select an Intact Fragmentation scan filter, you must also explicitly select a protein sequence for each peak before processing the experiment.
Fragmentation Mass Tolerance	Specifies the fragmentation tolerance for the MS2 scan, in Da or ppm, within which the masses of the sequence and a component must fall to be considered a match. Example: If you set your tolerance to 0.005 Da and your theoretical fragment ion is at 1154.1126 Da, observed fragment ions of 1154.1090 Da (–0.0034 Da from theoretical) and 1154.1167 (+0.0041 Da from theoretical) fall within the tolerance, but 1154.2312 (+0.1222) does not, because the mass difference is greater than your set tolerance.

For more details, see the following topics:

- Peak-specific parameters on the Component Detection page (page 498)
- Fragmentation types (page 509)
- Starting a new top down experiment (page 492)

Fragmentation types

The application supports the following fragmentation types for Top Down Analysis:

- CID: With the collision-induced dissociation method of fragmentation, molecular ions are accelerated to high kinetic energy in the vacuum of a mass spectrometer and then allowed to collide with neutral gas molecules such as helium, nitrogen, or argon. The collision breaks the bonds and fragments the molecular ions into smaller pieces.
- HCD: With the higher-energy collision-induced dissociation method of fragmentation, the ion optics accelerate the precursor ions into a high-pressure cell, where they collide with nitrogen gas. The projectile ion has laboratory-frame translation energy higher than 1 keV.
- SID™: With the surface-induced dissociation method of fragmentation, the molecular ions collide with a target surface composed of a relatively rigid material to maximize fragmentation.
- ETD: With the electron transfer dissociation method of fragmentation, singly charged reagent anions transfer an electron to multiply protonated peptides within an ion trap mass analyzer to induce fragmentation. ETD cleaves randomly along the peptide backbone while side chains and modifications, such as phosphorylation, are left intact. This method is used to fragment peptides and proteins.

- ECD: With the electron capture dissociation method of fragmentation, multiply protonated molecules are introduced to low-energy free electrons. Capture of the electrons releases electric potential energy and reduces the charge state of the ions by producing odd-electron ions, which easily fragment.
- EThcD: With the electron transfer higher-energy collision dissociation method, fragmentation is similar to HCD but involves an initial electron-transfer dissociation step and produces additional b and y ions.
- IRMPD: With the infrared multiphoton dissociation method of fragmentation, an infrared laser is directed at the ions in the vacuum of the mass spectrometer. The target ions absorb multiple infrared photons until they reach more energetic states and begin to break bonds, resulting in fragmentation.
- UVPD: With the ultraviolet photodissociation method of fragmentation, ultraviolet photons activate the proteins for fragmentation, providing ultra-high resolution for improved structural elucidation and quantitation of isobaric compounds.

Xtract deconvolution parameters

The following table describes the parameters for the Xtract deconvolution algorithm at the lower left area of the Component Detection page. These parameters are similar to the parameters used for Intact Mass Analysis; however, they are specific to a selected peak and are enabled on either the Intact Fragmentation page for processing MS2 spectra or the Intact Deconvolution page for processing Full MS spectra.

Table 82 Xtract parameters on the Component Detection page

Parameter	Description
Deconvolution Algorithm	Select the Xtract (Isotopically Resolved) option.
Main Parameters (Xtract) These are the same main parameters used for Intact Mass Analysis. However, for Top Down Analysis, the Output Mass parameter only provides one option, "M", and there is one additional m/z Range parameter as described next.	
m/z Range	Specifies the range of *m/z* values used as input for the deconvolution. You can use this parameter to select a narrower range. The deconvolution algorithm ignores the portions of the spectrum outside this range. The range value comes from the only (or first) raw data file loaded for the experiment, for each Scan Filters type. You can edit the range as needed. For more information, see the m/z Range on page 511 parameter.
Advanced Parameters (Xtract) (Visible only when you select the Show Advanced Parameters check box) These parameters need changing infrequently, and by experienced users only. These are the same advanced parameters used for Intact Mass Analysis, except the Calculate XIC check box is not enabled on the Intact Fragmentation page for processing MS2 spectra.	

For more details, see the following topics:

- Peak-specific parameters on the Component Detection page (page 498)
- Xtract deconvolution parameters (page 370)
- Output Mass (page 371) parameter
- Scan Filters (page 508) type
- m/z Range (page 366) parameter

ReSpect deconvolution parameters

The following table describes the parameters for the ReSpect deconvolution algorithm at the lower left area of the Component Detection page. These parameters are similar to the parameters used for Intact Mass Analysis; however, they are specific to a selected peak and are enabled on only the Intact Deconvolution page for processing the Full MS spectra.

Table 83 ReSpect parameters on the Component Detection page

Parameter	Description
Deconvolution Algorithm	Select the ReSpect (Isotopically Unresolved) option.
Main Parameters (ReSpect) These are the same main parameters used for Intact Mass Analysis. However, for Top Down Analysis, there is one additional m/z Range parameter as described next.	
m/z Range	Specifies the range of *m/z* values used as input for the deconvolution. You can use this parameter to select a narrower range. The deconvolution algorithm ignores the portions of the spectrum outside this range. The range value comes from the only (or first) raw data file loaded for the experiment, for each Scan Filters type. You can edit the range as needed. For more information, see the m/z Range on page 511 parameter.
Advanced Parameters (ReSpect) (Visible only when you select the Show Advanced Parameters check box) These parameters need changing infrequently, and by experienced users only. These are the same advanced parameters used for Intact Mass Analysis.	

For more details, see the following topics:

- Peak-specific parameters on the Component Detection page (page 498)
- ReSpect deconvolution parameters (page 374)
- Scan Filters (page 508) type
- m/z Range (page 366) parameter

Component detection page commands

Right-clicking the Chromatogram, Peak # - Intact Fragmentation Source Spectrum, or Peak # - Intact Deconvolution Source Spectrum pane of the Component Detection page opens a shortcut menu with the commands listed in the following table.

Table 84 Chromatogram/source spectra shortcut menu commands

Commands	Description
Reset Scale	Restores the chromatogram to full retention time range.
Copy as Displayed	Copies the image in the pane to the Clipboard as currently displayed.
Copy per Global Settings	Copies the image in the pane to the Clipboard based on the global image dimensions set in the Top Down Analysis Settings dialog box.
Zoom Out	Shrinks the view in the pane by a factor of 2.
Zoom In	Enlarges the view in the pane by a factor of 2.
Chromatogram Trace Type	(For the Chromatogram pane only) Selects which type of chromatogram to display in the Chromatogram pane: TIC or BPC.

For more details, see the following topics:

- Using copy and paste functions (page 666)
- Specifying the image dimensions (page 37)
- Chromatogram Trace Type (page 366)

Editing identification parameters for top down analysis

For MS2 scans, use the Identification page to add or edit protein sequences and then specify which ones to add to the experiment for processing.

For Full MS scans, if you select the Intact Deconvolution check box on the Parameters > Component Detection page, use the Identification page to do the following:

- Define the mass tolerance for target sequence matching (matching the measured masses of the components that the application detects to the masses of target protein sequences that you add to the experiment). For this function, assign various modifications to the protein sequences for the experiment.
- Set the merge options for the Multiconsensus result format.

For more information about the Identification page, see the following topics:

- Opening the identification page (page 513)
- Identification page layout (page 513)
- Editing the identification parameters (page 514)
- Left side of the identification page parameters (page 515)
- Right side of the identification page tables (page 516)

Opening the identification page

To open the Identification page

- On the Component Detection page, click **Next**.

–or–

- In the navigation bar, click the **Parameters** tab, and then click the **Identification** subtab.

Identification page layout

The following figure shows the left side of the Identification page.

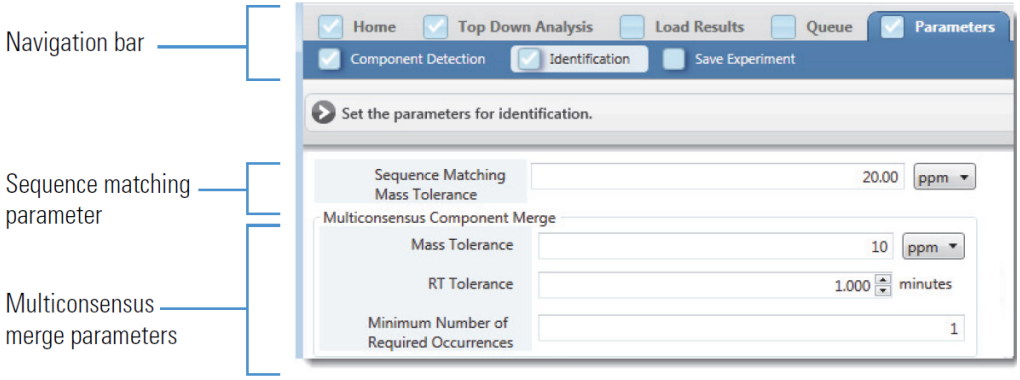


Figure 237 Identification page parameters (left side)

Note: These areas are inactive if you clear the Intact Deconvolution check box on the Parameters > Component Detection page for all of the peaks.

The following figure shows the Sequences Added to Experiment and Global Sequence Reference tables on the right side.

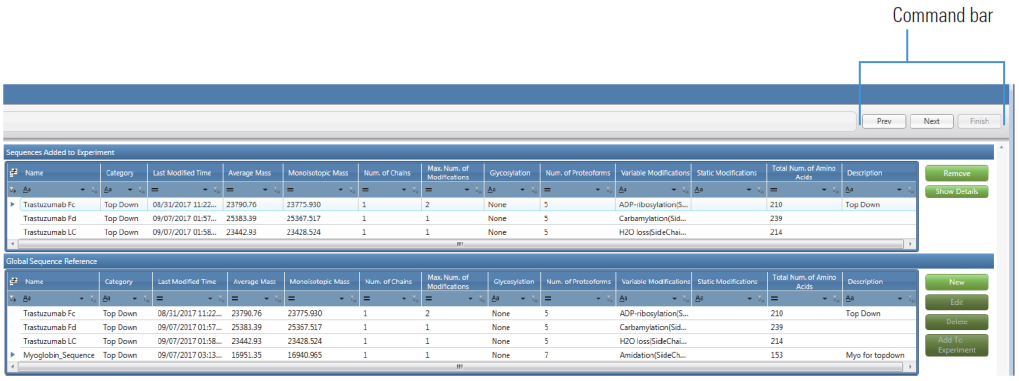


Figure 238 Identification page parameters (right side)

Editing the identification parameters

To edit the identification parameters

1. Enter the appropriate parameter values on the left side of the Identification page if those parameter boxes are active.

2. (Optional) Perform the following steps as needed:

   a. Create a new protein sequence by clicking New **next** to the Global Sequence Reference table.

   The Protein Sequence Editor appears, where you can import a FASTA file containing a new sequence of interest or manually enter the sequence.

   b. Edit or delete an existing sequence by selecting it in the Global Sequence Reference table and then clicking **Edit** or **Delete**.

   When you edit the sequence, the Protein Sequence Editor opens and displays the sequence information for you to make the necessary changes.

   When you delete the sequence, it disappears from the Global Sequence Reference table.

   c. Add a sequence to the experiment by selecting it in the Global Sequence Reference table and clicking **Add to Experiment**.

   If the sequence is already included in the experiment, a message appears to inform you.

   After you add the sequence to the experiment, the application populates a row of the Sequences Added to Experiment table.

   IMPORTANT! If you change any protein sequence in the Sequences Added to Experiment table, you must return to the Parameters > Component Detection page and reselect the protein sequence for each peak.

   d. Change a sequence after adding it to your experiment.

   If the experiment already includes the target sequence, select it in the Sequences Added to Experiment pane and click **Remove**. Then, create a new sequence (step a on page 514) or edit the existing sequence (step b on page 514), add the new modifications as needed, and then add the sequence to the experiment (step c on page 514).

   To determine whether the experiment includes the latest version of the sequence, you can compare the data in the Last Modified Time, Average Mass, or Monoisotopic Mass columns of the Sequences Added to Experiment table and the Global Sequence Reference table.

e. Display the full sequence by selecting it in the Sequences Added to Experiment table, and then clicking **Show Details**.

The application displays the entire protein sequence.

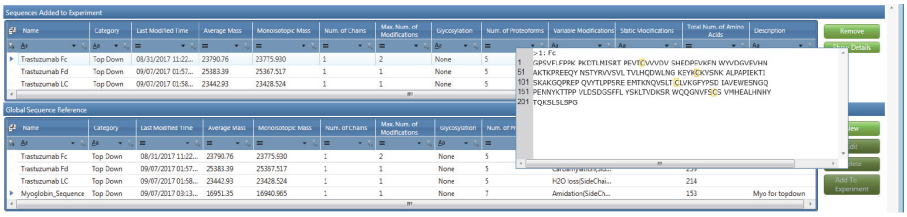


Figure 239 Details of protein sequence

3. Click **Next** in the command bar to advance to the Save Experiment page and save the method.

For more details, see the following topics:

- Left side of the identification page parameters (page 515)
- Identification page layout (page 513)
- Importing a FASTA file with a protein sequence (page 50)
- Editing component detection parameters for top down analysis (page 497)
- Saving a processing method (page 144)

Left side of the identification page parameters

The following table describes the parameters on the left side of the Identification page.

Note: To activate these parameters for processing Full MS scans, you must select the Intact Deconvolution check box on the Parameters > Component Detection page for at least one peak.

Table 85 Parameters on the left side of the Identification page

Parameter	Description
Sequence Matching Mass Tolerance	Specifies the Full MS scan mass tolerance, in Da or ppm, within which the masses of the sequence and a component must fall to be considered a match.
Multiconsensus Component Merge These parameters control the merging of the Full MS scan deconvoluted results for the multiple raw data files when you select Multiconsensus as the result format on the Top Down Analysis page. These are the same merge parameters used for Intact Mass Analysis.	

For more details, see the following topics:

- Identification page layout (page 513)
- Starting a new top down experiment (page 492)
- Left side of the identification page parameters (page 384)

Right side of the identification page tables

The tables on the right side of the Identification page are similar to the tables used for Intact Mass Analysis.

For more details, see the following topics:

- Right side of the identification page tables (page 385)

27 Viewing the top down analysis results

You can view the Top Down Analysis results from multiple pages in the BioPharma Finder application.

Opening the results from the queue page

When you run a Top Down Analysis experiment, the Queue page displays the run queue containing all of the completed, running, submitted, or canceled jobs. After a job is completed, you can open its results from the Queue page and view the processed data.

Note: A completed job displays "Completed" in the Status column.
You cannot open the results if the application has not yet analyzed the experiment or is in the process of analyzing it. In this case, the Open Results button is inactive until processing of the selected job is completed.
Opening the results does not stop the application from analyzing subsequent jobs in the queue.

To view the results of an experiment from the Queue page

1. On the Home page, click **Top Down Analysis** in the left pane or below the BioPharma Finder splash graphic.
2. Click the **Queue** tab.

   The Queue page opens showing the queued jobs in a table.
3. In the table, double-click a row to select the completed job and view its results, or click a row to select the completed job and then click **Open Results** in the command bar.

   The application transfers you to the Process and Review page, which displays the following:

   - Parameters used for processing in the Real Time Optimization pane
   - Chromatograms in the Chromatogram pane
   - Deconvoluted spectra in the Intact Fragmentation Deconvoluted Spectrum or Intact Deconvolution Deconvoluted Spectrum pane
   - Source spectra in the Intact Fragmentation Source Spectrum or Intact Deconvolution Source Spectrum pane
   - Results in the Intact Fragmentation Results or Intact Deconvolution Results tables

   You can adjust the size or location of the panes on this page as necessary.

For more details, see the following topics:

- Using the run queue (page 161)
- Queue page parameters (page 172)
- Viewing the process and review page for top down analysis (page 528)
- Rearranging the panes (page 655)

Opening the results from the load results page

Because you can delete jobs in the run queue on the Queue page, after deletion, you can no longer view the related results. However, the BioPharma Finder application saves all of the completed jobs on the Load Results page so that you can view any previously saved experimental results. You can also import or export the results of an experiment.

To view or delete the results of an experiment from the Load Results page

1. On the Home page, click **Top Down Analysis** in the left pane or below the splash graphic.
2. Click the **Load Results** tab.

   The table on the Load Results page displays all of the previously saved Top Down Analysis results in order of completion time.

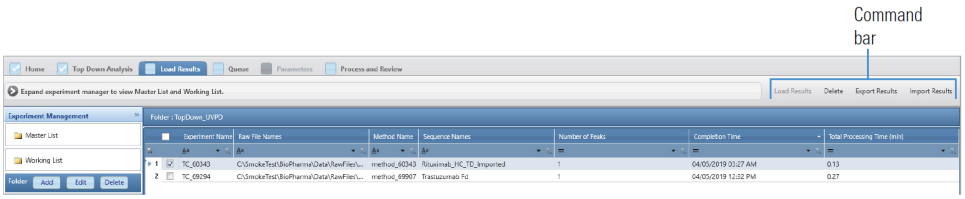


Figure 240 Load Results page

The table lists the name of each experiment, the raw data file names, the processing method and protein sequence or sequences assigned to that analysis, along with other information.

You can sort the columns or filter the data in the table.

Note: If you use real-time optimization to reprocess an experiment, the Total Processing Time cell displays 0.00. This cell displays the actual total processing time only when you process an experiment using the run queue.

3. In the table on the Load Results page, do any of the following:
   - Double-click a row to select an experiment and view its results, or select a row and then click **Load Results** in the command bar.

     The application transfers you to the Process and Review page.
   - Select one or more rows and then click **Delete** in the command bar.

     Select multiple rows by using the SHIFT key for contiguous rows or the CTRL key for noncontiguous rows.

     The application deletes this set of experiments from the database. If the selected experiments still exist in the run queue on the Queue page, the application removes them from the queue as well.

To import the results of an experiment from the Load Results page

1. On the Home page, click **Top Down Analysis** in the left pane or below the splash graphic.
2. Click the **Load Results** tab.

   The table on the Load Results page displays all of the previously saved Top Down Analysis results in the order of completion time.
3. Click **Import Results**.
4. In the dialog box, locate and select one or more Top Down Analysis result file(*Filename*_topdown.resultsbpf) that you want to import.

   The files must be in the same folder when importing multiple files simultaneously.
5. Click **Open**.

   A message appears indicating the confirmation of the import.

   The application adds the imported result in the experiment result table of the Master List and Working List folders.

   Note: If an existing method has the same name as the imported method, the application appends _imported to the file name of the new method.

To export the results of an experiment from the Load Results page

1. On the Home page, click **Top Down Analysis** in the left pane or below the splash graphic.
2. Click the **Load Results** tab.

   The table on the Load Results page displays all of the previously saved Top Down Analysis results in order of completion time.
3. Select one or more check boxes corresponding to the experiment results that you want to export, and do one of the following:
   - Choose **Export Results ▸ Export Experiment with Raw Files** in the command bar.

     –or–
   - Choose **Export Results ▸ Export Experiment without Raw Files** in the command bar.

     Note: You can only view the result when you include the raw files in the export of the experiment result file. To display the spectra when you import the exported experiment result file, you must store the raw file in the same directory as processed.

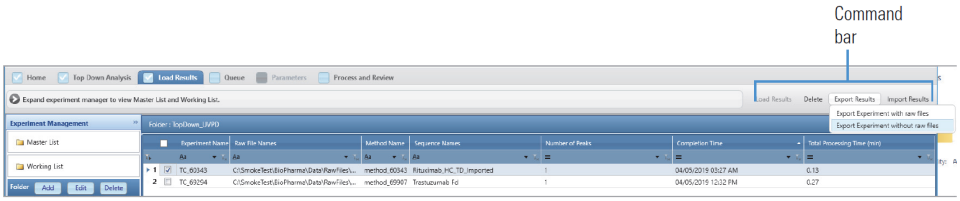


Figure 241 Export Results options in the Load Results page

4. In the dialog box, browse to the appropriate folder, and click **OK**.

   For export of a single experiment result, the Browse dialog box opens to the *drive*:\Xcalibur™\data\ folder by default.

   A message appears indicating the confirmation of the export. The application saves the exported experiment result (*Filename*_topdown.resultsbpf) on the specified folder.

For more details, see the following topics:

- Using the run queue (page 161)
- Using basic table functions (page 670)
- Filtering data in a table (page 673)
- Queue page parameters (page 172)
- Using Real-Time optimization for top down analysis (page 521)
- Viewing the process and review page for top down analysis (page 528)

Using Real-Time optimization for top down analysis

For real-time optimization, use the Process and Review page to adjust the parameters in the processing method, in the protein sequences, or in both sets of parameters. Then, reprocess the experiment without leaving this page.

To reprocess the experiment with the modified parameters

1. As necessary, click the **Process and Review** tab, and then click the title bar of the **Real Time Optimization** pane to see the Component Detection and Identification subtabs.

   Tip: You can also click this title bar to collapse the Real Time Optimization pane and provide more space for the other panes on the page.

   The Component Detection pane expands and displays the peak-specific parameters used for processing the current experiment.

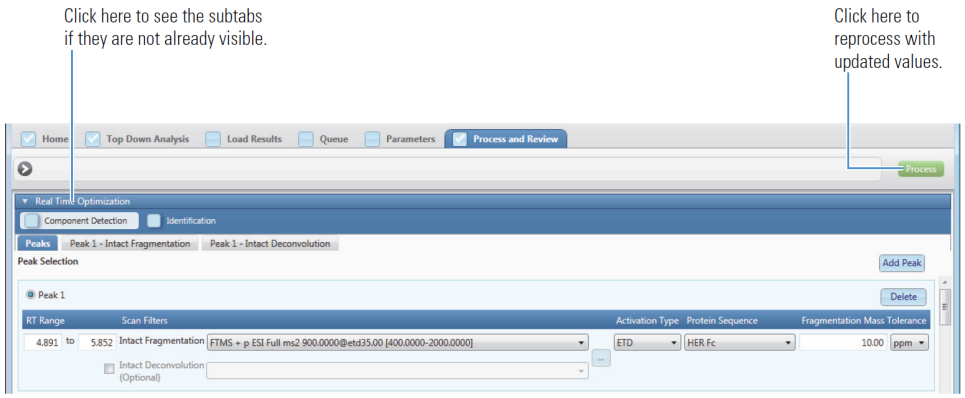


Figure 242 Component Detection pane for real-time optimization

2. Click the **Peaks, Peak #-Intact Fragmentation**, or **Peak #-Intact Deconvolution** subtab to update the corresponding parameters as needed.

   Instead of interacting with the chromatogram and updating the source spectrum on the Parameters > Component Detection page, you interact with the Process and Review > Chromatogram pane and update the Process and Review > Intact Fragmentation Source Spectrum/Intact Deconvolution Source Spectrum panes.

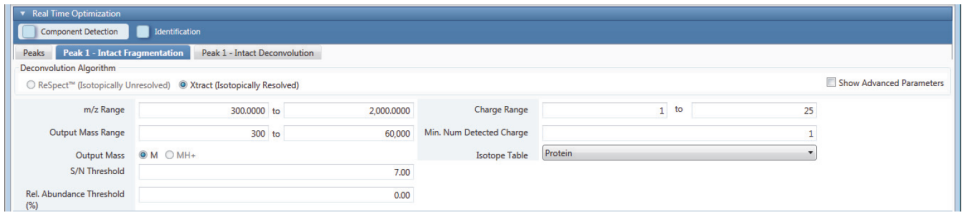


Figure 243 Component Detection pane showing the parameters under the Peak #-Intact Fragmentation subtab

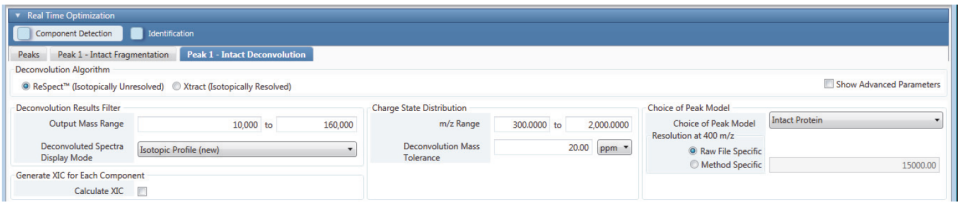


Figure 244 Component Detection pane showing the parameters under the Peak #-Intact Deconvolution subtab

3. Click the **Identification** tab and update the identification parameters as needed.

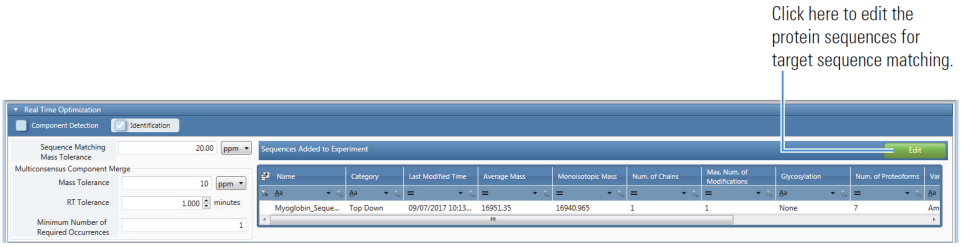


Figure 245 Identification pane for real-time optimization

Note: The parameters on the left side of the pane under the Identification subtab are active only if you select the Intact Deconvolution check box for at least one of the peaks under the Component Detection > Peaks subtab, because the application uses these parameters only for processing Full MS scans.

Tip: To change the protein sequences for target sequence matching, click **Edit** at the upper right area of the Sequences Added to Experiment table. The Parameters > Identification page opens where you can add different sequences to the experiment or make other sequence-related changes as needed. When finished, return to the Process and Review page for processing. If you add different sequences to the experiment, they appear in the Sequences Added to Experiment table.

If you change the sequences for the experiment, you must return to the Process and Review > Real Time Optimization > Component Detection pane and select each peak-specific sequence before reprocessing.

You can only change the modifications, glycosylation, or disulfide bonds information for a protein sequence. You cannot edit the chains.

---

4. Click **Process** in the command bar.

   If the application finds invalid or suboptimal parameter entries, it displays an error dialog box to inform you. To continue, enter the required or recommended range values.

5. If all of the entered parameters are valid, type the experiment and method names in the Reprocess Experiment dialog box, and then click **Reprocess**.

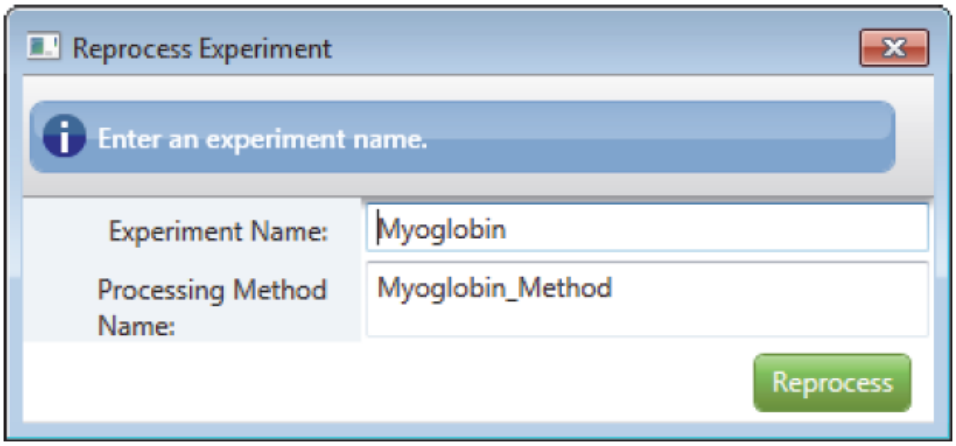


Figure 246 Reprocess Experiment dialog box

Note: If an existing experiment is already completed or is canceled, you can overwrite it by using the same experiment name. You cannot overwrite an experiment that is currently submitted to the run queue or is actively processing or canceling.

You cannot overwrite a default method. If you change the parameters in a default method, you can create a custom method by saving the method to a new name.

Use only alphanumeric, space, underscore "_", and period "." characters in the experiment and method names.

Entering new experiment and method names to save your changes to new files ensures that you do not overwrite the previous experiment results and method parameters.

The application clears all of the previous results on the Process and Review page and submits a new processing job with your changes to the run queue. The job moves to the bottom of the queue, below the currently running job. If no job is currently running, the application reprocesses the new job immediately. During this time, you remain on the Process and Review page and can navigate to other areas or open other results. When the reprocessing is completed, the Process and Review page automatically displays the new results, unless you open another results file while the application is reprocessing.

You can open the saved experiment results to view later from the Load Results page.

For more details, see the following topics:

- Editing component detection parameters for top down analysis (page 497)
- Editing identification parameters for top down analysis (page 512)
- Using the run queue (page 161)
- Opening the results from the load results page (page 518)

Organizing the experimental results

Use the Experiment Management pane to organize the experimental results in different folders. The Master List contains all of the previously saved Top Down Analysis experimental results. In the Working List, you can create folders or subfolders and drag and drop results from the Working List to any folder that you created. The Master List folder shows all the experimental results regardless of the folder in which they reside.

To access the Experiment Management pane

1. On the Home page, click **Top Down Analysis** in the left pane or below the BioPharma Finder splash graphic.

   The Top Down Analysis page opens.

2. Click the **Load Results** tab.

   The Load Results page opens to the Master List by default. The BioPharma Finder application displays all of the previously saved Intact Mass Analysis experimental results in the Master List.

3. Do one of the following to access the Experiment Management pane.

   - Click « to expand the Experiment Management pane.

     The Experiment Management pane opens to display the Master List and the Working List.

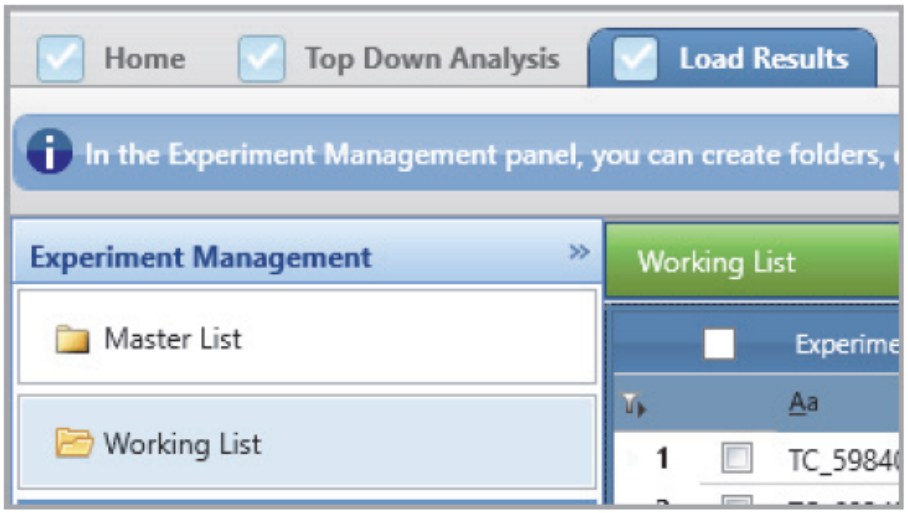


Figure 247 Experiment Management pane

–or–

- Click the **Experiment Management** pane on the left.
  A window appears showing the Master List and the Working List.

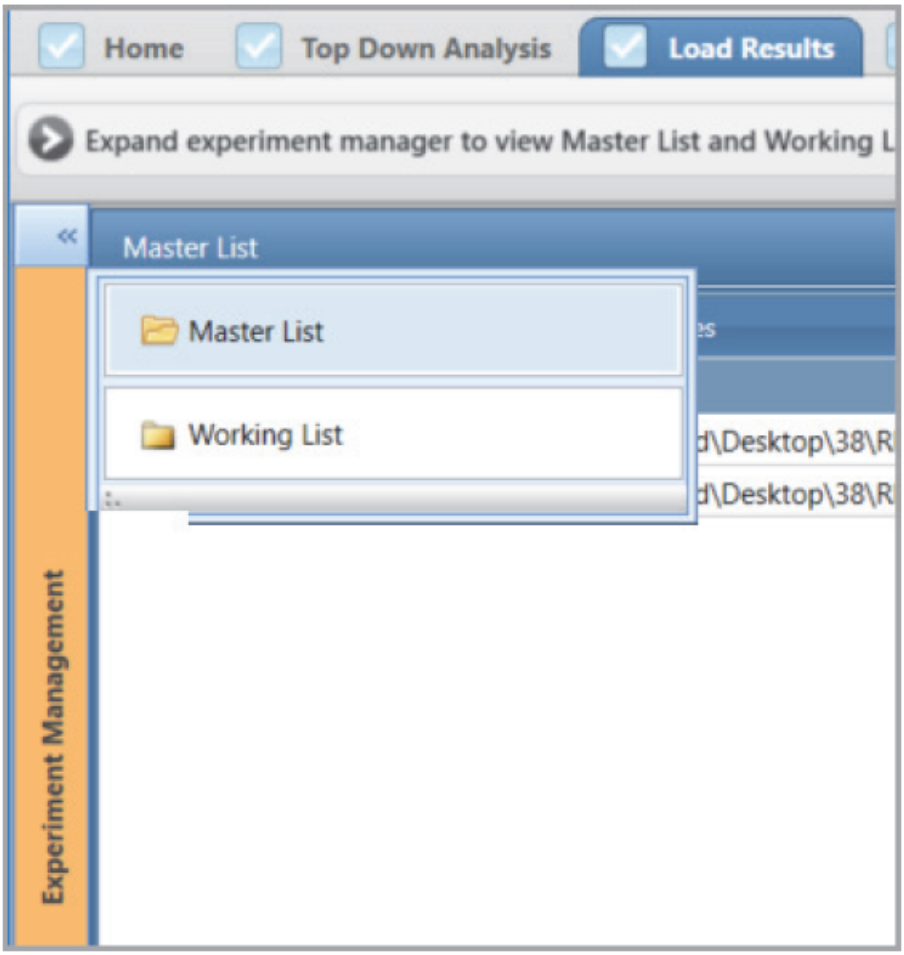


Figure 248 Experiment Management window

To create a folder in the Working List

1. In the Experiment Management pane of the Load Results page, select the **Working List**.
   By default, the Working List contains the Home folder.
2. Click the **Home** folder, and then click **Add**.

   Note: You can only create a new folder under the Home folder of the Working List. You cannot create a folder in the Master List.
3. In the Add New Folder dialog box, type the Folder Name, and then click **Add**.
   A new folder appears under the Home folder in the Working List.
4. To create a subfolder, select the folder to which you want to add a subfolder, and then click **Add**.
   A new subfolder appears under the folder.
   Each folder can contain subfolders, and each subfolder, in turn, can contain more subfolders, and so on.

To rename a folder

1. In the Experiment Management pane of the Load Results page, select the **Working List** to view its folder structure.
   When the Working List opens, only the Home folder appears in the pane.
2. To navigate through the folder structure, click the arrow next to a folder.
3. To rename a folder, select the folder of interest, and then click **Edit**.
4. In the dialog box, type the new name.

   Note: You can only rename a user-created folder.

To move an experiment result to a folder

1. In the Experiment Management pane of the Load Results page, select the **Working List** to view its folder structure.
   The right pane shows the contents of the Working List.
2. From the Working List, select an experiment result or use the CTRL key to select multiple results, and then drag and drop to the destination folder of interest.
   The moved experimental result or results appear in the new destination folder.

To delete an experiment result from a folder

1. In the Experiment Management pane of the Load Results page, select the **Working List** to view its folder structure.
   When the Working List opens, only the Home folder appears in the pane.
2. To navigate through the folder structure, click the arrow next to a folder.
3. Select the folder that contains the result to delete.
   The right pane shows the contents of the selected folder.
4. To delete an experiment result, select the check box corresponding to the result.
   You can also select multiple check boxes to delete more than one result.
   In the command bar, click **Delete**, and do one of the following:
   - In the Delete Selected Results from Folder dialog box, select **Delete Results from Folder**.
     The application deletes the experiment result from the folder and moves it to the Working List.
     –or–
   - In the Delete Selected Results from Folder dialog box, select **Delete Results from Folder and Master List**.
     The application deletes the experiment result from both the folder and the Master List.

To delete a folder

1. In the Experiment Management pane of the Load Results page, select the **Working List** to view its folder structure.
   When the Working List opens, only the Home folder appears in the pane.
2. To navigate through the folder structure, click the arrow next to a folder.
3. To delete a folder, select the folder.
4. In the Experiment Management pane, click **Delete**.

5. In the Delete Selected Folder dialog box, do one of the following:
   - Select **Delete Folder**.
     The application deletes the folder and moves its experiment result content (if any) to the Working List.
     –or–
   - Select **Delete Folder and Results from Master List**.
     The application deletes both the folder and its experiment result content from the Master List.

28 Viewing the process and review page for top down analysis

For real-time optimization, after setting the appropriate parameters on the Process and Review page, click **Process** to see the deconvolution results. You can then save the results to view from the Load Results page.

After processing is completed, use the Queue page or the Load Results page to open the results.

You can see the results of a top-down experiment on the Process and Review page when the application has completed processing. At completion, the areas on this page display the chromatograms, spectra, fragment maps, and results tables.

The experiment name and processing method appear in the upper right corner of the page. If you load only one raw data file for the experiment, its name also appears below the method name; otherwise, "(multiple files)" appears. The values in the columns of the Intact Fragmentation Results/Intact Deconvolution Results tables represent the outputs of the deconvolution.

Note: When you do any of the following, the layout dimensions of the Process and Review page remain fixed:

- Navigate away from the Process and Review page to another page.
- Open results from the run queue.
- Load previous results.
- Switch algorithms from Xtract to ReSpect or from ReSpect to Xtract.

However, after you close and reopen the application, the layout dimensions revert to their defaults.

Figure 249 Process and Review page

For more details, see the following topics:

- Using Real-Time optimization for top down analysis (page 521)
- Opening the results from the load results page (page 518)
- Opening the results from the queue page (page 517)

Process and review page parameters for top down analysis

The following table describes the types of information on the Process and Review page. To display the content of a pane that is not currently visible, click the corresponding subtab. You can adjust the size or location of the panes on this page as needed.

Table 86 Process and Review page parameters

Parameter	Description
Intact Fragmentation Results table	Displays at the upper level the peaks set in the method. At the lower levels, displays the proteoform and raw data file information.
Intact Deconvolution Results table	Displays the peaks set in the method and also the detected component data similar to the data in the Results table for Intact Mass Analysis.
Chromatogram pane	Displays the chromatogram from each raw data file loaded for the experiment.
Relative Intensity (*y* axis)	Displays the ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity.

Parameter	Description
RT (min) (*x* axis)	Displays the retention time of the spectrum, which is the time after injection at which a compound elutes. Can also refer to the total time that the compound is retained on the chromatograph column.
Intact Fragmentation Deconvoluted Spectrum or Intact Deconvolution Deconvoluted Spectrum pane	Displays the deconvoluted spectra that result from applying the Xtract/ReSpect algorithm.
Relative Intensity (*y* axis)	Displays the ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity.
Mass (*x* axis)	Displays the mass of the ions formed from molecules.
Intact Fragmentation Source Spectrum or Intact Deconvolution Source Spectrum pane	Displays the source spectra before deconvolution.
Relative Intensity (*y* axis)	Displays the ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity.
m/z (*x* axis)	Displays the mass-to-charge ratio of ions formed from molecules. This ratio is the quantity formed by dividing the mass of an ion, in daltons, by the number of charges carried by the ion.
ProSightBP Fragment Map pane	For MS2 scan processing, displays the fragment map and identified ion data generated by the ProSight Lite application.
ProSightBP Output pane	For MS2 scan processing, displays the monoisotopic mass, delta mass, charge state, and other ion information generated by the ProSight Lite application and the Xtract deconvolution algorithm for all of the searched fragment ions.
Intact Deconvolution Matched Sequence pane	For Full MS scan processing, displays the matched component and the matched target sequence information. You can select a different identification in this pane to update its value in the Intact Deconvolution Results table.
Real Time Optimization pane	Displays the same parameters as those on the Parameters > Component Detection and Identification pages so that you can adjust these parameters and perform real-time optimization.

Note: If the *x*- or *y*-axis label in a pane is not visible, enlarge the pane or change the pane to a larger floating window.

For more details, see the following topics:

- Rearranging the panes (page 655)
- Viewing the intact fragmentation results table (page 532)
- Viewing the intact deconvolution results table (page 537)
- Viewing the chromatograms for top down analysis (page 541)
- Viewing the deconvoluted Spectra for top down analysis (page 545)
- Viewing the source Spectra for top down analysis (page 549)
- Viewing the ProSightBP fragment map for top down analysis (page 553)
- Viewing the ProSightBP output results for top down analysis (page 557)
- Viewing the matched sequence information for top down analysis (page 564)
- Using Real-Time optimization for top down analysis (page 521)

Process and review page command for top down analysis

The following table describes the Process command on the Process and Review page.

Table 87 Command on the Process and Review page

Command	Description
Process	Processes the top-down experiment and deconvolves the source spectra with any or all of these parameters: modified protein sequence, component detection, and identification. Also, displays the new results. Saves the latest results in a database after you process an analysis. Click this button to open a dialog box where you can enter a new experiment/method name or retain the same experiment name to overwrite previously saved results/parameters in the current experiment with the new data. **Note:** To activate the Process button, you must modify the experiment parameters. Use only alphanumeric, space, underscore "_", and period "." characters, up to 50 maximum, in the experiment and method names. You cannot overwrite an experiment that is currently submitted to the run queue or is actively processing or canceling. You also cannot overwrite a default method but you can delete the previously saved results.

For more details, see the following topics:

- Using the run queue (page 161)
- To view or delete the results of an experiment from the Load Results page (page 218)

Viewing the results tables for top down analysis

The Intact Fragmentation Results and Intact Deconvolution Results tables on the Process and Review page contain the processed results for the deconvolution of MS2 spectra and Full MS spectra, respectively. Each table displays the results of a completed analysis, organized by multiple levels.

The columns and levels in the Intact Fragmentation Results or Intact Deconvolution Results table vary depending on the selection of the Result Format for Multiple Files option for the experiment, the Deconvolution Algorithm option, and other settings in the processing method.

For more details, see the following topics:

- Viewing the intact fragmentation results table (page 532)
- Exporting the intact fragmentation results table (page 533)
- Intact fragmentation results table parameters (page 534)
- Intact fragmentation results table commands (page 537)
- Viewing the intact deconvolution results table (page 537)
- Exporting the intact deconvolution results table (page 538)
- Intact deconvolution results table parameters (page 539)
- Intact deconvolution results table commands (page 540)
- Starting a new top down experiment (page 492)
- Working with a top down processing method (page 496)

Viewing the intact fragmentation results table

The Intact Fragmentation Results table on the Process and Review page displays the completed Top Down Analysis MS2 spectra experiment with the results from the ProSight Lite application. These results are organized by the peaks at the upper level, followed by the proteoforms at the next level, and then by the raw data files at the lowest level.

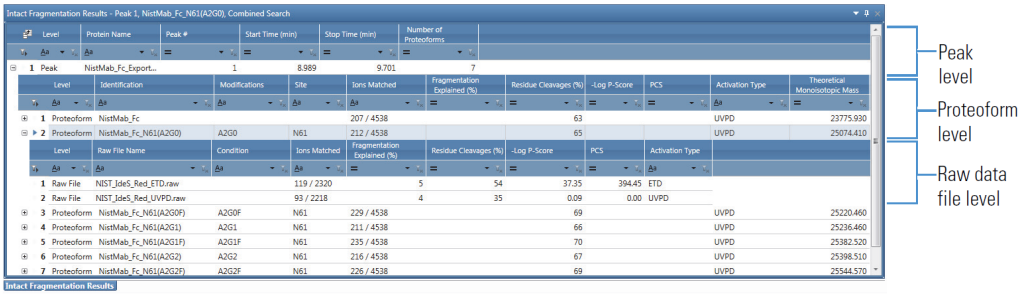


Figure 250 Intact Fragmentation Results table on the Process and Review page

To view the Intact Fragmentation Results table

1. Click the **Process and Review** tab if necessary.
2. At the bottom left of the page, click the **Intact Fragmentation Results** tab.

   By default, the Process and Review page displays the peak and first proteoform results in the Intact Fragmentation Results table.
3. Click a peak row in the table to view information related to that peak in the other panes on this page.
4. Click the plus icon,  , at the left side of a peak row to view information that is related to that peak and specific to each proteoform processed for the experiment.
5. Click the plus icon,  , at the left side of a proteoform row to view information that is related to a proteoform and specific to each raw data file loaded for the experiment.

For more details, see the following topics:

- Intact fragmentation results table parameters (page 534)

Exporting the intact fragmentation results table

To export the results in the Intact Fragmentation Results table

1. On the Process and Review page, right-click anywhere in the table and choose from these options:
   - **Export All ▸ All Levels**—To export the results at all levels in the table to a spreadsheet file.
   - **Export All ▸ Proteoform Level Only**—To export only the results at the proteoform level in the table to a spreadsheet file.
2. In the Save As dialog box, browse to or type the name of the file to store the results in.
3. Click **Save**.

   The application stores the exported data in the specified file. If you do not browse to a different folder, the application places the exported file by default in the folder containing the raw data files for the experiment.

Intact fragmentation results table parameters

The following table describes the types of information in the Intact Fragmentation Results table on the Process and Review page.

Table 88 Intact Fragmentation Results table parameters

Column	Description
Peak level	
+/–	Click to show or hide the lower level of proteoform information related to the current peak row.
Row number	The number assigned to each visible peak row in the table. This sequential numbering does not change when you sort or filter the table.
Level	Indicates that the row is displaying peak information (top level).
Protein Name	Displays the selected protein sequence in the processing method for each peak.
Peak #	Displays a number for each peak. This number matches each peak's number in the processing method.
Start Time (min)	Displays the start time in minutes that is set in the processing method for each peak.
Stop Time (min)	Displays the stop time in minutes that is set in the processing method for each peak. See RT Range on page 507.
Number of Proteoforms	Displays the total number of processed proteoforms for the experiment.
Proteoform level **For a multiconsensus experiment with multiple loaded raw data files, the data listed for this level are the results from processing the combined results. Otherwise, for a single-file or batch experiment, the data are the results from processing one raw data file.**	
+/–	Click to show or hide the lower level of raw data file information related to the current proteoform row.
Row number	The number assigned to each visible proteoform row in the table. This sequential numbering does not change when you sort or filter the table.
Level	Indicates that the row is displaying proteoform information (lower level).

Column	Description
Identification	Displays the name of the proteoform, consisting of the selected protein sequence for a peak, followed by the modification site and modification type (in parentheses) that were identified for an individual proteoform, if available. Commas separate multiple sites and modifications in the column. For example, "Rituximab_C4(Oxidation), N35(Deamidation)" indicates that • Rituximab is the protein sequence name, • C4 is the first modification site in the sequence, • Oxidation is the first modification type, • N35 is the second modification site in the sequence, and • Deamidation is the second modification type. If there is no identified modification, the site and modification parts are empty.
Modifications	Displays the same modification types as in the Identification column; otherwise, this cell is empty.
Site	Displays the same modification sites as in the Identification column; otherwise, this cell is empty.
Ions Matched	Displays for a proteoform the total number of identified ions/the total number of ions submitted for searching. For example, "80/400" indicates that • 80 is the total number of ions with fragment labels returned from the ProSight Lite application with an identification. This number matches the number of rows with identified ions in the ProSightBP Output table. • 400 is the total number of ions sent to the ProSight Lite application for searching.
Fragmentation Explained (%)	Displays a percentage representing the number of identified ions divided by the total number of ions submitted for searching. For example, if the Ions Matched column displays "80/400", then the value in this cell is 100 × (80 ÷ 400) = 20. For the combined results from multiple loaded raw data files, this value appears at the raw data file level, not at the proteoform level.
Residue Cleavages (%)	Displays a percentage representing the number of identified residue cleavage sites divided by the total number of residue cleavage sites.

Column	Description
-Log P-Score	Displays the –log base 10 value of the P-score, which is the probability of obtaining at least as good a match between the observed fragment list and a sequence as by chance. The P-score is a measure of confidence in the validity of a match. A low P-score means that the probability of obtaining at least this many fragments that match a sequence is low, so it is unlikely that random chance caused the association. For the combined results from multiple loaded raw data files, this value appears at the raw data file level, not at the proteoform level. **Note:** When the deconvolution algorithm returns no results and no fragment ion is identified, the Ions Matched column displays "0/0". In this case, the value in this column is "Infinity" since the P-score is 0.
PCS	Displays the Protein Characterization Score that uses shuffled protein sequences to create a decoy distribution to compare against the actual results. The more the actual results differ from the decoy distribution, the higher the PCS value. For the combined results from multiple loaded raw data files, this value appears at the raw data file level, not at the proteoform level.
Activation Type	Displays the fragmentation type selected in the processing method for a particular peak. For the combined results from multiple loaded raw data files, this value appears at the raw data file level, not at the proteoform level.
Theoretical Monoisotopic Mass	Displays the theoretical monoisotopic mass for a proteoform from the protein sequence. If the proteoform is not identified, this cell is empty.
Raw data file level **The data listed for this level are the results from processing each raw data file loaded for the experiment. Many columns in this level display the same information as in similar columns at the proteoform level, except the information is from one raw data file instead of from the combined results in the case of a multiconsensus experiment. For details on these columns, see the columns at the Proteoform level on page 534.**	
Row number	The number assigned to each visible raw data file row in the table. This sequential numbering does not change when you sort or filter the table.
Level	Indicates that the row is displaying raw data file information (lowest level).
Raw File Name	Displays the name of the raw data file.
Condition	Displays the condition assigned to the raw data file; otherwise, this cell is empty.

For more details, see the following topics:

- Protein Sequence (page 509) parameter
- RT Range (page 507) parameter
- Viewing the ProSightBP output results for top down analysis (page 557)
- Activation Type (page 508) parameter

Intact fragmentation results table commands

Right-clicking the Intact Fragmentation Results table on the Process and Review page opens a shortcut menu that contains the commands listed in the following table.

Table 89 Intact Fragmentation Results table shortcut menu

Command	Description
Export All	Exports the results in the table to an Excel™ file.
All Levels	Exports the results at all levels in the table to a file.
Proteoform Level Only	Exports only the results at the proteoform level in the table to a file.

For more details, see the following topics:

- Exporting the intact fragmentation results table (page 533)

Viewing the intact deconvolution results table

The Intact Deconvolution Results table on the Process and Review page displays the completed Top Down Analysis experiment with the Full MS spectra results from the deconvolution algorithm for each peak, organized by the peaks at the title bar level, components at the top level, raw data files at the next level, and then charge states at the lowest level.

The columns and levels in the Intact Deconvolution Results table vary depending on the selection of the Result Format for Multiple Files option for the experiment, the Deconvolution Algorithm option, and other settings in the processing method.

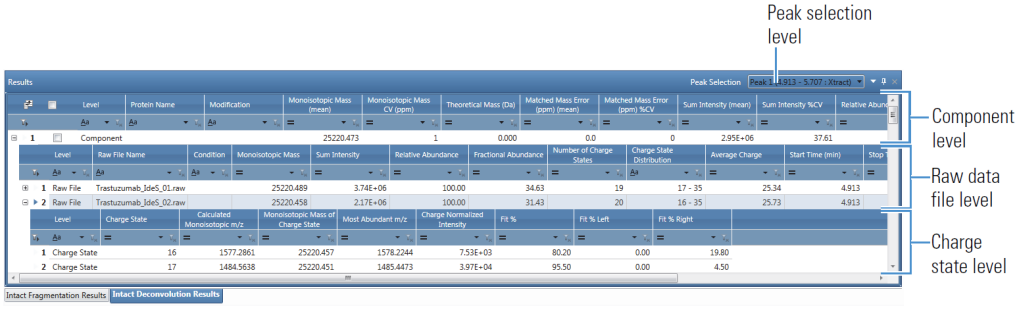


Figure 251 Intact Deconvolution Results table on the Process and Review page with four levels

IMPORTANT! The Intact Deconvolution Results table is accessible only if you select the Intact Deconvolution check box for at least one peak in one or both of these places: the Peak Selection area of the Parameters > Component Detection page for the processing method and the Process and Review > Real Time Optimization pane for real-time optimization.

To view the Intact Deconvolution Results table

1. Click the **Process and Review** tab if necessary.
2. At the bottom left of the page, click the **Intact Deconvolution Results** tab.

   The Process and Review page displays the component results in the Intact Deconvolution Results table.
3. In the title bar, select a peak from the Peak Selection list to show each peak number, along with its corresponding retention time range and deconvolution algorithm.

   The table displays the results specific to the selected peak.
4. Click a component row in the table to view information that is related to that component in the other panes on this page.
5. (For experiments with Multiconsensus result format) Click the plus icon,  , at the left side of a component row to view raw data file information that is related to the top-level component.
6. Click the plus icon,  , at the left side of a component row (or a raw data file row) to view charge state information that is related to the top-level component.

   The values in the bottom level columns represent the isotopic clusters with different charge states from the source spectrum that produced the peak in the deconvoluted spectrum.

For more details, see the following topics:

- Starting a new top down experiment (page 492)
- Working with a top down processing method (page 496)

Exporting the intact deconvolution results table

To export the results in the Intact Deconvolution Results table

1. On the Process and Review page, right-click anywhere in the Results table and choose from these options:
   - **Export All** to export all results of a certain type to an Excel™ file:
     - **Peak and Component Levels Only**—To export only the peak information and the results at the component level in the table.

       The exported data does not include the results at the raw data file and charge state levels.
     - **All Levels**—To export the results at all levels in the table.

   –or–

- **Export Checked** to export only the *selected* results of a certain type to an Excel™ file:
  - **Peak and Component Levels Only**—To export only the peak information and the *selected* results at the component level in the table.

    The exported data does not include the results at the raw data file and charge state levels.
  - **All Levels**—To export only the *selected* results at all levels in the table.

  To select/deselect a row of results to export, select/clear the check box in that row.

  To select/deselect all of the rows, select/clear the check box in the column header.

2. In the Save As dialog box, browse to or type the name of the file to store the results in.

3. Click **Save**.

   The application stores the exported data in the specified file. If you do not browse to a different folder, the application places the exported file by default in the folder with the raw data files for the experiment.

   Note: For an experiment using the Multiconsensus result format, the export of the component level results includes the following columns from the raw data file level for each raw data file:
   - Activation Type
   - Monoisotopic Mass (for Xtract)

     –or–

     Average Mass (for ReSpect)

For more details, see the following topics:

- Activation Type (page 508) column
- Monoisotopic Mass (page 450) column
- Average Mass (page 457) column

Intact deconvolution results table parameters

The columns in the Intact Deconvolution Results table for Top Down Analysis are similar to the columns in the Results table for Intact Mass Analysis, except the RT Range column does not appear in the table. Instead, the RT Range value appears for each peak in the Peak Selection list in the title bar of the table.

IMPORTANT! The calculated mass values in the Intact Deconvolution Results table from the BioPharma Finder application might be slightly different from the calculated masses from the Protein Deconvolution application. The BioPharma Finder application uses an updated algorithm.

The following topics describe the parameters in the Results table for Intact Mass Analysis. These parameters are very similar to the parameters in the Intact Deconvolution Results table for the various types of Full Scan data experiments with the various combinations of settings. The last topic in this list describes how to select a new reference mass to update the Delta Mass column.

- Results for Single-File/Batch experiment using xtract and average over RT deconvolution (page 449)
- Results for a single File/Batch experiment using ReSpect and average over RT deconvolution (page 456)
- Results for a target sequence matching experiment (page 461)
- Results for a multiconsensus experiment (page 463)
- Selecting a reference mass to calculate mass differences (page 426)

Intact deconvolution results table commands

Right-clicking the Intact Deconvolution Results table on the Process and Review page opens a shortcut menu that contains the commands listed in the following table.

Table 90 Intact Deconvolution Results table shortcut menu

Command	Description
Set As Reference Component	Sets the mass of the chosen component as the reference mass. Resets the value in the Delta Mass column of the Intact Deconvolution Results table to 0 for the chosen component. Also, recalculates the delta mass value for all other components in the table relative to the chosen reference mass.
Export All	Exports both the selected and deselected results in the table to a file.
Peak and Component Levels Only	Exports only the peak information and the component-level results in the Intact Deconvolution Results table to a file. The exported data does not include the results at the raw data file and charge state levels.
All Levels	Exports the results at all levels in the Intact Deconvolution Results table to a file.
Export Checked	Exports only the *selected* results in the table to a file.
Peak and Component Levels Only	Exports only the peak information and the *selected* component-level results in the Intact Deconvolution Results table to a file. The exported data does not include the results at the raw data file and charge state levels.
All Levels	Exports only the *selected* results at all levels in the Intact Deconvolution Results table to a file.

For more details, see the following topics:

- Delta Mass (page 451) column
- Selecting a reference mass to calculate mass differences (page 426)
- Exporting the intact deconvolution results table (page 538)

Viewing the chromatograms for top down analysis

The Chromatogram pane on the Process and Review page displays the chromatogram plot from the raw data file or files loaded for a Top Down Analysis experiment, at full range.

The chromatogram plot can be any of these types, depending on the selected settings from the shortcut menu and the processing method:

- Total ion current if you select TIC for the Chromatogram Trace Type parameter from the shortcut menu
- Base peak chromatogram if you select BPC for the Chromatogram Trace Type parameter from the shortcut menu

  The BPC shows only the most intense peak in each MS spectrum at every point in the analysis.
- Extracted ion chromatogram if you select the check box for the Calculate XIC parameter in the processing method for Xtract deconvolution of Full MS spectra

For example, the following figure shows the chromatogram plots when you select the TIC option for the Chromatogram Trace Type. If you load multiple raw data files for the experiment, the Chromatogram pane displays an individual chromatogram plot for each of the raw data files, stacked one on top of the other. The raw data file name appears at the top of each plot.

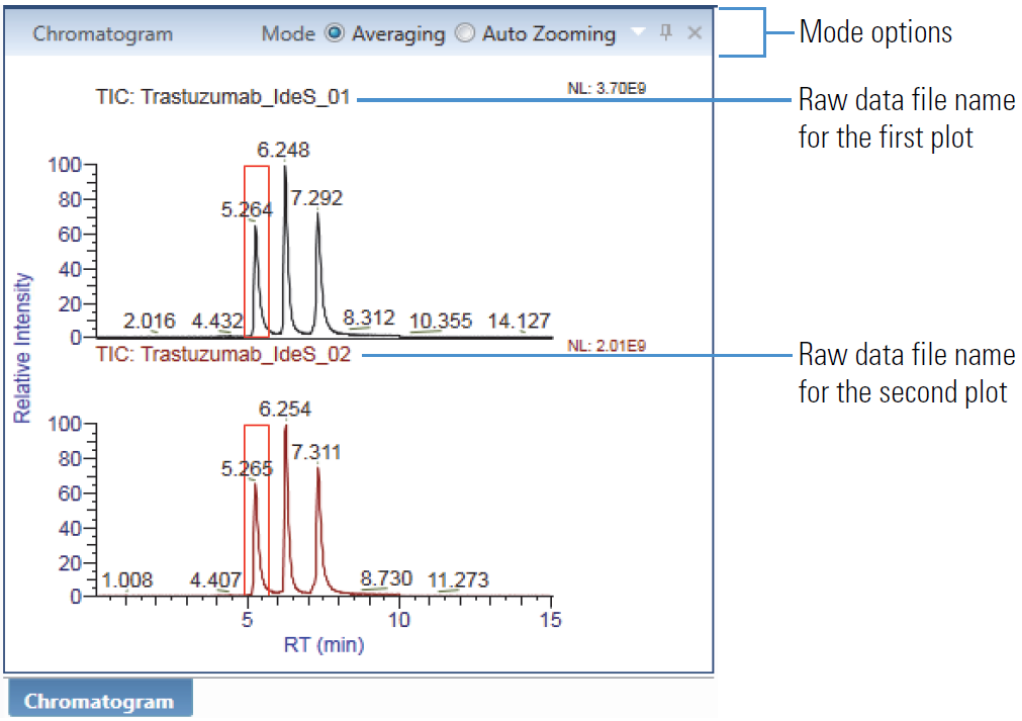


Figure 252 Chromatogram pane showing multiple plots with TIC as the trace type

Note: If the pane is too small for you to see the Mode options at the top right or the *y* axis label, adjust the width or height of the pane.

For more details, see the following topics:

- Viewing the chromatograms (page 542)
- Chromatogram pane options (page 543)
- Chromatogram pane commands (page 544)
- Chromatogram Trace Type (page 366) parameter
- Calculate XIC (page 372) parameter
- Collapsing the panes (page 660)

Viewing the chromatograms

To view the chromatograms in the Chromatogram pane

1. Click the **Process and Review** tab if necessary.
2. Do one of the following:
   - Click the row of a peak (or lower-level proteoform) in the Intact Fragmentation Results table or a peak in the Peak Selection list in the title bar of the Intact Deconvolution Results table.

     The Chromatogram pane shows one chromatogram plot (for a single loaded raw data file in the experiment) or multiple stacked plots (for multiple loaded raw data files). The plot displays a red box for the set RT Range of the selected (or upper level) peak.

–or–

- (If available) Click the plus (+) sign to the left of a proteoform row in the Intact Fragmentation Results table or a component row in the Intact Deconvolution Results table, and then click the row of a raw data file in the table.

  The Chromatogram pane shows the chromatogram plot from the selected raw data file.

In the chromatogram plot, the *x* axis represents the retention time range and the *y* axis indicates the relative intensity. The plot displays the plot type and raw data file name at the top and the Normalized Largest (NL) intensity on the upper right side. It does not display peak labels, such as the scan number, or the header information.

For more details, see the following topics:

- Viewing the intact fragmentation results table (page 532)
- Viewing the intact deconvolution results table (page 537)

Chromatogram pane options

The following table lists the options at the top right of the Chromatogram pane on the Process and Review page.

Table 91 Chromatogram pane options

Command	Description
Mode	Determines the options available in the upper right corner of the Chromatogram pane. • **Averaging:** Averages the spectra for all the scans in the retention time range that you drag the cursor over (left to right) and displays the averaged spectrum in a source spectrum pane. • **Auto Zooming:** Enlarges the area (intensity and time) that you drag the cursor over without changing the view displayed in a source spectrum pane.

Chromatogram pane commands

Right-clicking the Chromatogram pane on the Process and Review page opens a shortcut menu that contains the commands listed in the following table.

Table 92 Chromatogram pane shortcut menu

Command	Description
Reset Scale	Restores the original chromatogram that first appeared in the pane.
Copy as Displayed	Copies the image in the pane to the Clipboard as currently displayed.
Copy per Global Settings	Copies the image in the pane to the Clipboard based on the global image dimensions set in the Top Down Analysis Settings dialog box.
Zoom Out	Shrinks the view in the pane by a factor of 2.
Zoom In	Enlarges the view in the pane by a factor of 2.
Chromatogram Trace Type	Selects which type of chromatogram to display in the Chromatogram pane: TIC or BPC.

For more details, see the following topics:

- Using copy and paste functions (page 666)
- Specifying the image dimensions (page 37)
- Using copy and paste functions (page 666)

Viewing the deconvoluted Spectra for top down analysis

On the Process and Review page, the Intact Fragmentation Deconvoluted Spectrum pane displays the MS2 spectra. The Intact Deconvolution Deconvoluted Spectrum pane displays the Full MS deconvoluted spectra. Both panes show the identified masses after the application applies the Xtract/ReSpect algorithm.

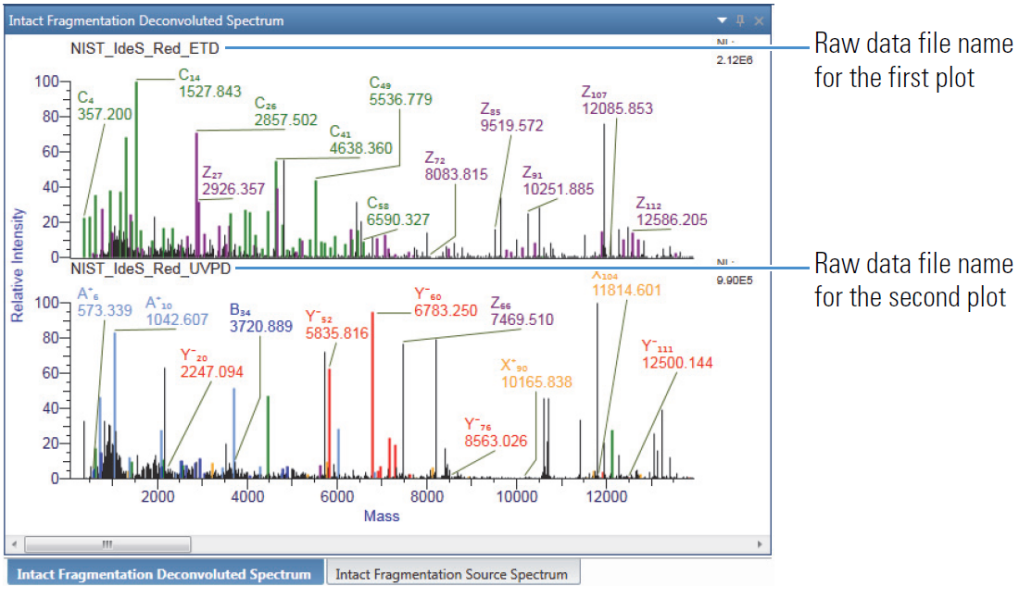


Figure 253 Intact Fragmentation Deconvoluted Spectrum pane showing multiple plots

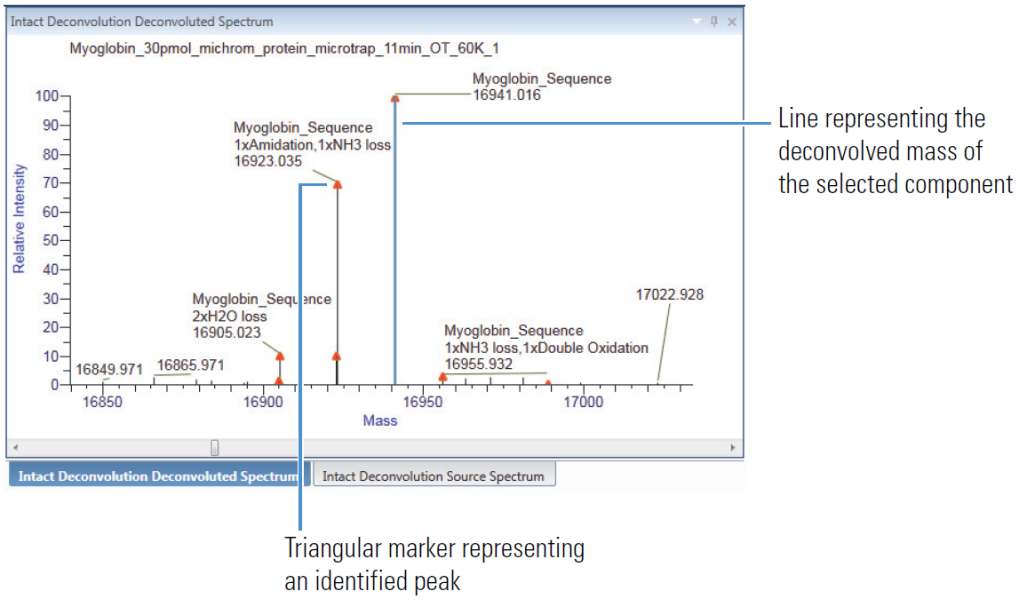


Figure 254 Intact Deconvolution Deconvoluted Spectrum pane showing a single plot

For the Xtract algorithm, these panes display each deconvoluted spectrum as a set of centroid peaks in mass and relative intensity. For the ReSpect algorithm, the panes display each deconvoluted spectrum as a profile in mass and intensity with a set of

peak labels. The upper right side of the spectrum plot displays the Normalized Largest (NL) intensity value.

In addition, for the ReSpect algorithm, you can select from two different modes to display the deconvoluted spectra: Mass Probability Distribution Profile (legacy) and Isotopic Profile (new).

Note: The BioPharma Finder application currently supports the deconvolution of MS2 spectra using only Xtract deconvolution. For processing Full MS spectra, the application supports both the Xtract and ReSpect algorithms.

When you load multiple raw data files for a multiconsensus experiment, these panes display a deconvoluted spectrum per raw data file, stacked one on top of the other. The raw data file name appears at the top of each spectrum.

The default mass range for the *x* axis of the plot is based on values that you set for the Output Mass Range (for Xtract deconvolution) or Output Mass Range (for ReSpect deconvolution) parameter. Set this range on the Parameters > Component Detection page for the method or in the Process and Review > Real Time Optimization pane for real-time optimization.

In the Intact Deconvolution Deconvoluted Spectrum pane, for target sequence matching experiments, an orange triangular marker appears by default for each identified peak. You can turn this marker on and off, as well as change its color.

For more details, see the following topics:

- Viewing the deconvoluted Spectra (page 546)
- Deconvoluted Spectra display mode (page 390)
- Output Mass Range (page 370) parameter (for Xtract deconvolution)
- Output Mass Range (page 374) parameter (for ReSpect deconvolution)

Viewing the deconvoluted Spectra

To view the deconvoluted spectra

1. Click the **Process and Review** tab if necessary.
2. Click the **Intact Fragmentation Results** tab or **Intact Deconvolution Results** tab at the lower left side of the screen if necessary.

   IMPORTANT! The Intact Deconvolution Results tab is available only if you select the Intact Deconvolution check box in the Peak Selection area of the Parameters > Component Detection page or the Process and Review > Real Time Optimization pane.

   If you click the **Intact Deconvolution Results** tab, select a peak from the Peak Selection list in the title bar of the Intact Deconvolution Results table.

   Note: When you click the Intact Fragmentation Results tab, you see the Intact Fragmentation Deconvoluted Spectrum subtab, not the Intact Deconvolution Deconvoluted Spectrum subtab.

Conversely, when you click the Intact Deconvolution Results tab, you see the Intact Deconvolution Deconvoluted Spectrum subtab, not the Intact Fragmentation Deconvoluted Spectrum subtab.

Click the visible subtab to view the corresponding pane.

The Intact Fragmentation Deconvoluted Spectrum pane or Intact Deconvolution Deconvoluted Spectrum pane shows one deconvoluted spectrum plot (for batch experiments or experiments with a single loaded raw data file) or multiple stacked plots (for multiconsensus experiments with multiple loaded raw data files).

3. Do any of the following:
   - Click the row of a peak in the Intact Fragmentation Results table or a component in the Intact Deconvolution Results table.

     For batch experiments or experiments with a single loaded raw data file, the plot in the Intact Fragmentation Deconvoluted Spectrum pane displays the deconvoluted spectrum for that one file. For a multiconsensus experiment, each plot displays the results for one loaded raw data file. The application sends the MS2 deconvolution mass results for the peaks from each raw data file to the ProSight Lite application for searching and then displays the individual returned search results.

     In each plot in the Intact Deconvolution Deconvoluted Spectrum pane, a blue line represents the deconvolved mass of the selected component.
   - (If available) Click the plus (+) sign to the left of a peak row in the Intact Fragmentation Results table, and then click the row of a proteoform.

     The Intact Fragmentation Deconvoluted Spectrum pane shows the same deconvoluted spectrum plots as for a peak row selection; however, these plots now display colored peaks and fragment ion labels. These labels are similar to the fragment ion labels in the MS2 experimental spectra for Peptide Mapping Analysis.
   - (If available) Click the plus (+) sign to the left of a proteoform row in the Intact Fragmentation Results table or a component row in the Intact Deconvolution Results table, and then click the row of a raw data file.

     The Intact Fragmentation Deconvoluted Spectrum pane shows a deconvoluted spectrum plot with the masses, intensities, colored peaks, and fragment ion labels from the selected raw data file.

     The Intact Deconvolution Deconvoluted Spectrum pane shows a deconvoluted spectrum plot with a blue line for the top-level component and the masses and intensities from the selected raw data file.

   –or–

   - (If available) Click the plus (+) sign to the left of a component (or raw data file) row, and then click the row of one of the charge states in the Intact Deconvolution Results table.

     The Intact Deconvolution Deconvoluted Spectrum pane shows a deconvoluted spectrum plot with a blue line for the top-level component and the masses and intensities from the upper-level raw data file.

For more details, see the following topics:

- Viewing the intact fragmentation results table (page 532)
- Viewing the intact deconvolution results table (page 537)
- Viewing the deconvoluted Spectra for top down analysis (page 545)
- Viewing the deconvoluted Spectra for intact mass analysis (page 433)
- Viewing the predicted and experimental MS2 Spectra for peptide mapping analysis (page 277)

Deconvoluted Spectra panes commands

Right-clicking the Intact Fragmentation Deconvoluted Spectrum or Intact Deconvolution Deconvoluted Spectrum pane on the Process and Review page opens a shortcut menu that contains the commands listed in the following table.

Table 93 Intact Fragmentation Deconvoluted Spectrum or Intact Deconvolution Deconvoluted Spectrum pane shortcut menu

Command	Description
Reset Scale	Restores the original spectrum that first appeared in the pane.
Copy as Displayed	Copies the image in the pane to the Clipboard as currently displayed.
Copy per Global Settings	Copies the image in the pane to the Clipboard based on the global image dimensions set in the Top Down Analysis Settings dialog box.
Copy Data	Copies mass data (*x* axis) and intensity data (*y* axis) from the pane to the Clipboard so that you can paste it into an Excel™ spreadsheet or another application. For Xtract deconvolution, the saved data consists of a centroid spectrum. For ReSpect deconvolution, the saved data consists of a profile spectrum.
Zoom Out	Shrinks the view in the pane by a factor of 2.
Zoom In	Enlarges the view in the pane by a factor of 2.

Command	Description
Show Identification Markers	(Intact Deconvolution Deconvoluted Spectrum pane only) Turns on and off to show or hide the markers for identified peaks. Active only for target sequence matching experiments. Default: On, when you open a new experiment
Identification Markers Color	(Intact Deconvolution Deconvoluted Spectrum pane only) Changes the color of the identification markers. Active only when the Show Identification Markers command is turned on. Select a different color from the palette, or click **Advanced** to enter specific RGB, HSL, or CMYK values for the new color. Default: Orange (When you close and reopen the application, the color you previously selected reverts back to orange.)

For more details, see the following topics:

- Using copy and paste functions (page 666)
- Specifying the image dimensions (page 37)

Viewing the source Spectra for top down analysis

On the Process and Review page, the Intact Fragmentation Source Spectrum pane displays the MS2 spectra. The Intact Deconvolution Source Spectrum pane displays the Full MS source spectra. Both panes show the masses before the application applies the deconvolution.

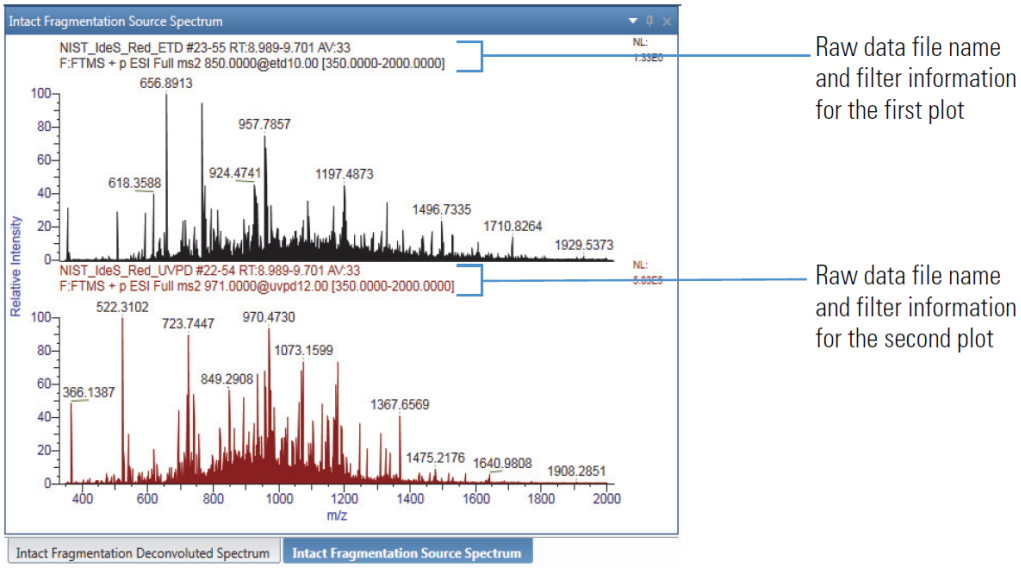


Figure 255 Intact Fragmentation Source Spectrum pane showing multiple plots

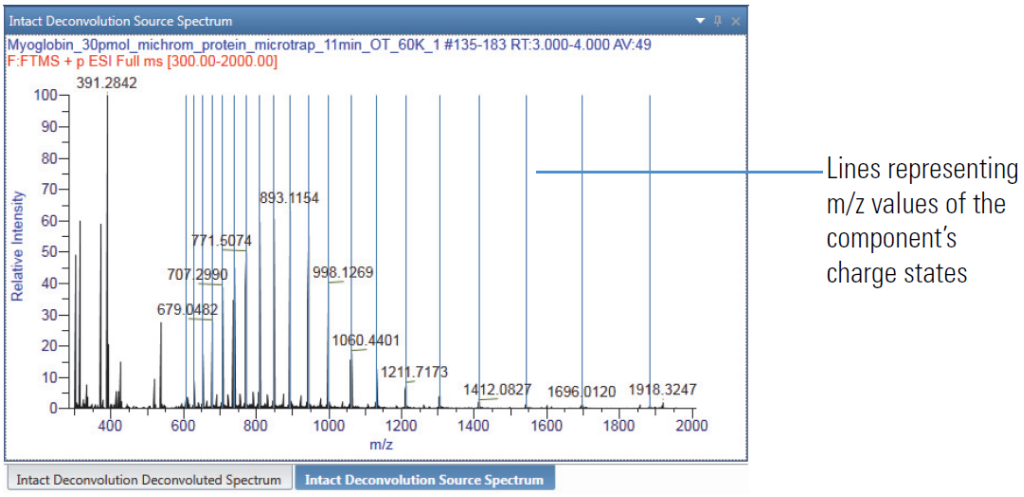


Figure 256 Intact Deconvolution Source Spectrum pane showing a single plot

These panes display the same source spectra as the ones you selected on the Parameters > Component Detection page.

When you load multiple raw data files for a multiconsensus experiment, these panes display a source spectrum per raw data file, stacked one on top of the other. The raw data file name and MS filter information appear at the top of each spectrum.

For more details, see the following topics:

- Viewing the source Spectra (page 550)
- Source Spectra panes commands (page 552)
- Editing component detection parameters for top down analysis (page 497)

Viewing the source Spectra

To view the source spectra

1. Click the **Process and Review** tab if necessary.
2. Click the **Intact Fragmentation Results** tab or **Intact Deconvolution Results** tab at the lower left side of the screen if necessary.

   IMPORTANT! The Intact Deconvolution Results tab is available only if you select the Intact Deconvolution check box in the Peak Selection area of the Parameters > Component Detection page or the Process and Review > Real Time Optimization pane.

   If you click the **Intact Deconvolution Results** tab, select a peak from the Peak Selection list in the title bar of the Intact Deconvolution Results table.

   Note: When you click the Intact Fragmentation Results tab, you see the Intact Fragmentation Source Spectrum subtab, not the Intact Deconvolution Source Spectrum subtab.
   Conversely, when you click the Intact Deconvolution Results tab, you see the Intact Deconvolution Source Spectrum subtab, not the Intact Fragmentation Source Spectrum subtab.

Click the visible subtab to view the corresponding pane.

The Intact Fragmentation Source Spectrum pane or Intact Deconvolution Source Spectrum pane shows one source spectrum plot (for batch experiments or experiments with a single loaded raw data file) or multiple stacked plots (for multiconsensus experiments with multiple loaded raw data files).

3. Do any of the following:
   - Click the row of a peak or lower-level proteoform in the Intact Fragmentation Results table or a component in the Intact Deconvolution Results table.

     The Intact Fragmentation Source Spectrum pane shows the source spectrum plot or plots of the selected or higher-level peak.

     The Intact Deconvolution Source Spectrum pane shows the source spectrum plot or plots of the selected component. These plots overlay the source spectra with blue lines. These lines represent the *m/z* values of the component's individual charge states.
   - (If available) Click the plus (+) sign to the left of a proteoform row in the Intact Fragmentation Results table or a component row in the Intact Deconvolution Results table, and then click the row of a raw data file.

     The Intact Fragmentation Source Spectrum pane shows a source spectrum plot with the masses and intensities from the selected raw data file.

     The Intact Deconvolution Source Spectrum pane shows a source spectrum plot with a single blue line representing the top-level component and the masses and intensities from the selected raw data file.

   –or–
   - (If available) Click the plus (+) sign to the left of a component (or raw data file) row in the Intact Deconvolution Results table, and then click the row of one of the charge states.

     The Intact Deconvolution Source Spectrum pane shows a source spectrum plot with a single blue line for the selected charge state, as shown in the following figure.

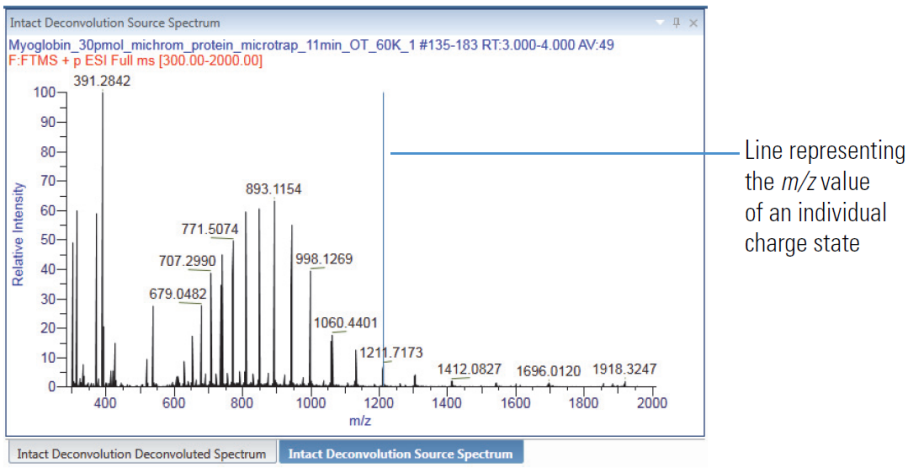


Figure 257 Line in the Intact Deconvolution Source Spectrum pane

This line represents the following:

- For Xtract deconvolution, the calculated monoisotopic *m/z* value of that charge state (shown in the Calculated Monoisotopic m/z column of the Intact Deconvolution Results table)
- For ReSpect deconvolution, the measured average *m/z* value of that charge state (shown in the Measured Average m/z column of the Intact Deconvolution Results table)

For more details, see the following topics:

- Viewing the intact fragmentation results table (page 532)
- Viewing the intact deconvolution results table (page 537)
- Viewing the source Spectra for top down analysis (page 549)
- Calculated Monoisotopic m/z (page 451) column
- Measured Average m/z (page 457) column

Source Spectra panes commands

Right-clicking the Intact Fragmentation Source Spectrum or Intact Deconvolution Source Spectrum pane on the Process and Review page opens a shortcut menu that contains the commands listed in the following table.

Table 94 Intact Fragmentation Source Spectrum or Intact Deconvolution Source Spectrum pane shortcut menu

Command	Description
Reset Scale	Restores the original spectrum that first appeared in the pane.
Copy as Displayed	Copies the image in the pane to the Clipboard.
Copy per Global Settings	Copies the image in the pane to the Clipboard based on the global image dimensions set in the Top Down Analysis Settings dialog box.
Zoom Out	Shrinks the view in the pane by a factor of 2.
Zoom In	Enlarges the view in the pane by a factor of 2.

For more details, see the following topics:

- Using copy and paste functions (page 666)
- Specifying the image dimensions (page 37)

Viewing the ProSightBP fragment map for top down analysis

The ProSightBP Fragment Map pane on the Process and Review page displays the proteoform-specific fragment map generated by the ProSight Lite application after processing the MS2 spectra. A fragment map shows the location of the PTMs and matching fragment ions.

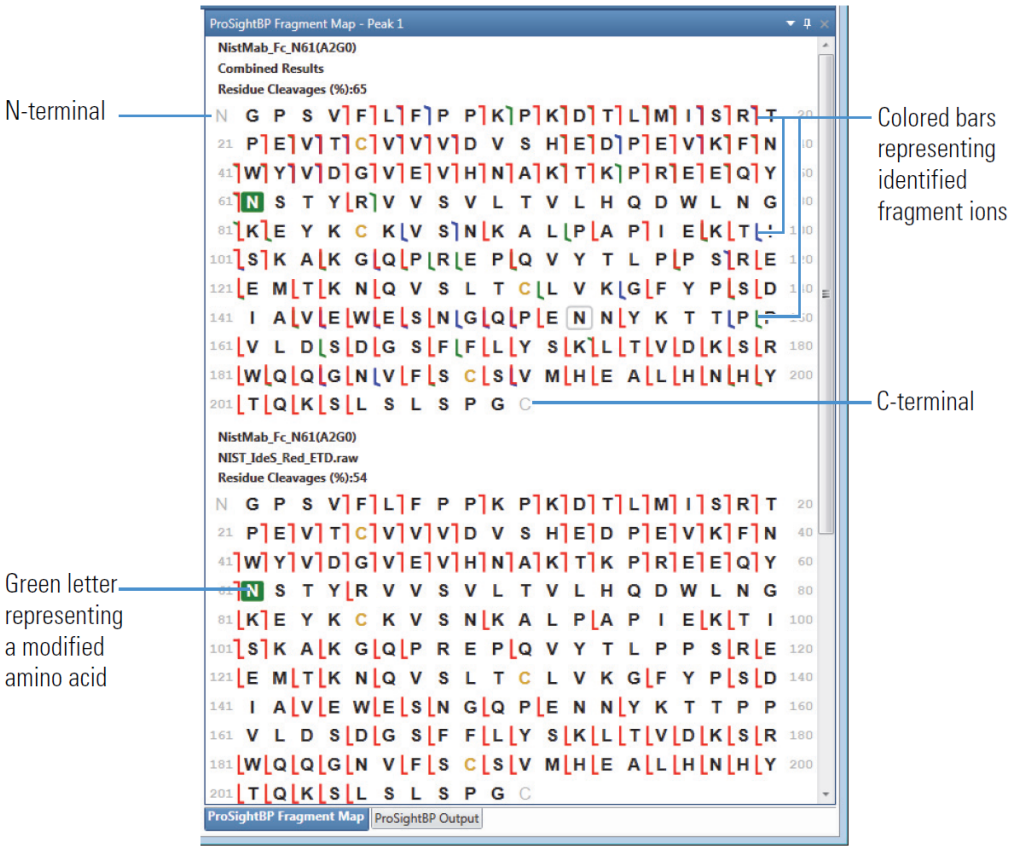


Figure 258 ProSightBP Fragment Map pane

This pane displays various fragment maps based on your interaction with the Intact Fragmentation Results table, the number of proteoforms saved in the protein sequences that were added to the experiment, and the number of loaded raw data files for the experiment.

The map lists the amino acid letters in the protein sequences from left to right and from top to bottom. All cysteines appear in yellow. All modified amino acids have green backgrounds. The gray "N" at the top left corner of the map represents the N-terminal and the gray "C" at the bottom of the map represents the C-terminal.

The map also contains different vertical bars, depending on the identified ion type. A red bar with a serif at the top pointing left represents the termination of a "c" ion and a serif at the bottom pointing right represents the start of a "z" ion. A blue bar with a serif at the top pointing left represents the termination of a "b" ion and a serif at the bottom pointing right represents the start of a "y" ion. A green bar with a serif at the top pointing left represents the termination of an "a" ion and a serif at the bottom

pointing right represents the start of an "x" ion. These bars can overlap if combinations of identified ions exist.

When you point to an individual amino acid letter, a tooltip appears showing the corresponding residue number. When you point to a colored bar, a tooltip appears showing the identified fragment ion's type, mass (measured in daltons), and number of identifications.

For more details, see the following topics:

- Viewing the ProSightBP fragment map (page 554)
- Matching fragment detail table parameters (page 556)
- ProSightBP fragment map pane commands (page 557)

For more details, see the following topics:

- Viewing the intact fragmentation results table (page 532)

Viewing the ProSightBP fragment map

To view the information in the ProSightBP Fragment Map pane

1. As necessary, click the **Process and Review** tab, and then click the **Intact Fragmentation Results** tab at the bottom left.
2. Click the **ProSightBP Fragment Map** subtab (next to the ProSightBP Output subtab) at the bottom right.
3. Do any of the following:
   - Click the row of a peak in the Intact Fragmentation Results table.

     The ProSightBP Fragment Map pane displays the fragment maps that show the identified fragment ion results for all of the proteoforms processed under the selected peak. The name of each proteoform appears above each map.

     If there is only one proteoform generated for the experiment, only one map appears that shows the identified fragment ions for that proteoform.

     If there are multiple proteoforms generated for the experiment, multiple maps appear: the first map with the results from the unmodified sequence if you select to include it as a searched proteoform, followed by one map for

each proteoform with results for that proteoform, stacked one on top of the other.

If you load only one raw data file for the experiment or you are running a batch experiment, the results for each map are from one file. If you load multiple files for a multiconsensus experiment, the results for each map are from the combined results.

To show the combined results, the application sends the MS2 deconvolution mass results from each individual raw data file to the ProSight Lite application for searching. After receiving the individual search results, the application then combines them for display.

Below the proteoform name at the top of each map, the name of the loaded raw data file (if this is a batch experiment or there is only one loaded file) or "Combined Results" (if this is a multiconsensus experiment with multiple loaded files) appears. Finally, the Residue Cleavages (%) value for each map appears above the map.

- Click the row of a proteoform (at the level under a peak row) in the Intact Fragmentation Results table.

  The ProSightBP Fragment Map pane displays the fragment maps that show the identified fragment ion results for the selected proteoform, related to the top-level peak. The name of the proteoform appears above each map.

  If you load only one raw data file for the experiment or you are running a batch experiment, only one map appears and its results are from one file. The name of that file appears below the proteoform name at the top of the map.

  If you load multiple files for a multiconsensus experiment, multiple maps appear: the first map with results from the combined results, followed by a map for each of the loaded raw data files used in the combined results. These subsequent maps contain individual results from each of the files, stacked one on top of the other. Below the proteoform name at the top of each map reads "Combined Results" for the first map and the names of individual raw data files for the other maps.

  Finally, the Residue Cleavages (%) value for each map appears above it.

–or–

- Click the row of a raw data file (at the level under a proteoform row) in the Intact Fragmentation Results table.

  The ProSightBP Fragment Map pane displays the fragment map that shows the identified fragment ion results for the selected raw data file, related to the higher-level proteoform and peak.

  The name of the proteoform, the name of the selected raw file, and then the Residue Cleavages (%) value appear above the map.

4. Double-click a colored bar or a set of overlapping colored bars on the fragment map.

   The Matching Fragment Detail table appears in a pop-up box displaying the masses and mass differences for the selected bars. Each row in the table represents one identified fragment ion for the selected location in the sequence.

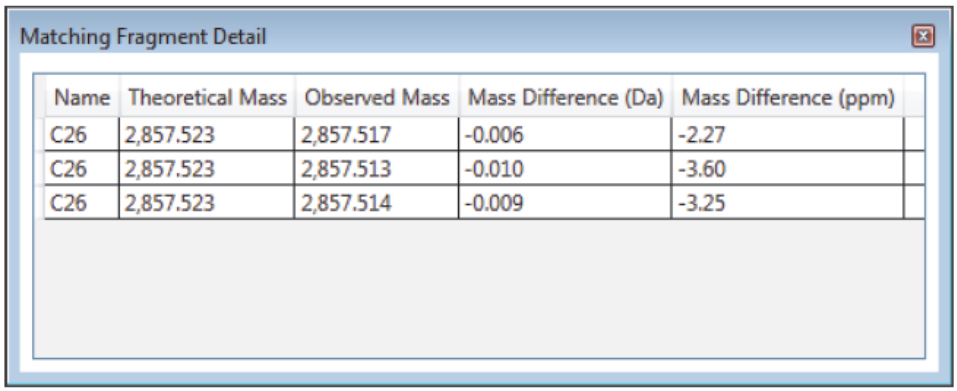


Figure 259 Matching Fragment Detail dialog box

Note: In this graphic example, there are multiple matches at the same theoretical mass.

For more details, see the following topics:

- Viewing the intact fragmentation results table (page 532)
- Residue Cleavages (%) (page 535) value
- Matching fragment detail table parameters (page 556)

Matching fragment detail table parameters

The following table describes the information in the Matching Fragment Detail table. This table appears when you double-click the colored bar or bars on the map in the ProSightBP Fragment Map pane, indicating one or more identified fragment ions.

Table 95 Matching Fragment Detail table

Column	Description
Name	Displays the name of the identified fragment ion.
Theoretical Mass	Displays the theoretical mass of the fragment ion, measured in daltons.
Observed Mass	Displays the observed mass of the fragment ion, measured in daltons.
Mass Difference (Da)	Displays the difference between the observed and theoretical fragment masses, measured in daltons.
Mass Difference (ppm)	Displays the difference between the observed and theoretical fragment masses, measured in parts per million.

ProSightBP fragment map pane commands

Right-clicking the ProSightBP Fragment Map pane on the Process and Review page opens a shortcut menu that contains the commands listed in the following table.

Table 96 ProSightBP Fragment Map pane shortcut menu

Command	Description
Save As (.png)	Saves the fragment map as an image file with a .png extension.
Copy	Copies the image in the pane to the Clipboard.

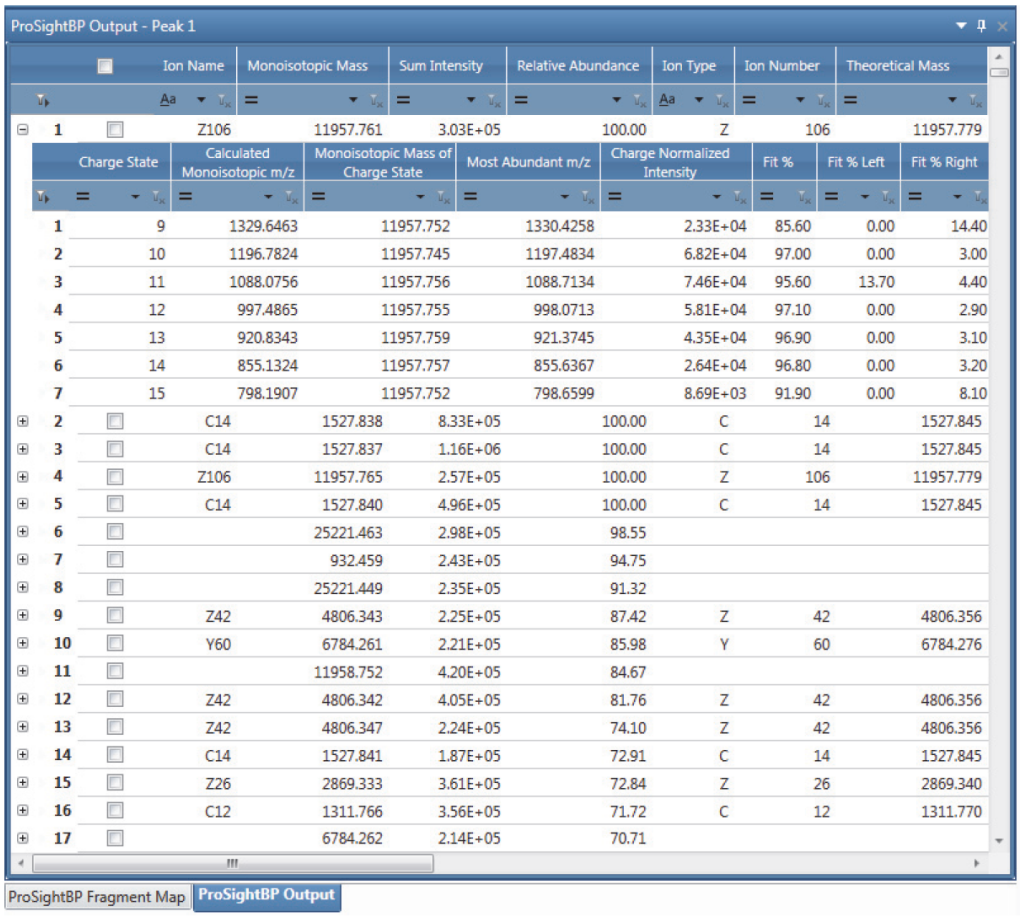
For more details, see the following topics:

- Using copy and paste functions (page 666)

Viewing the ProSightBP output results for top down analysis

The ProSightBP Output pane on the Process and Review page displays in tabular format the peak-specific results from processing the MS2 spectra. The search results of all fragment ions include the observed and theoretical monoisotopic masses, mass delta, relative abundance, charge state, and other ion information returned by the ProSight Lite application and the Xtract deconvolution algorithm.

Note: The BioPharma Finder application currently does *not* support the processing of MS2 spectra using the ReSpect deconvolution algorithm.



		Ion Name	Monoisotopic Mass	Sum Intensity	Relative Abundance	Ion Type	Ion Number	Theoretical Mass
1		Z106	11957.761	3.03E+05	100.00	Z	106	11957.779

	Charge State	Calculated Monoisotopic m/z	Monoisotopic Mass of Charge State	Most Abundant m/z	Charge Normalized Intensity	Fit %	Fit % Left	Fit % Right
1	9	1329.6463	11957.752	1330.4258	2.33E+04	85.60	0.00	14.40
2	10	1196.7824	11957.745	1197.4834	6.82E+04	97.00	0.00	3.00
3	11	1088.0756	11957.756	1088.7134	7.46E+04	95.60	13.70	4.40
4	12	997.4865	11957.755	998.0713	5.81E+04	97.10	0.00	2.90
5	13	920.8343	11957.759	921.3745	4.35E+04	96.90	0.00	3.10
6	14	855.1324	11957.757	855.6367	2.64E+04	96.80	0.00	3.20
7	15	798.1907	11957.752	798.6599	8.69E+03	91.90	0.00	8.10

2		C14	1527.838	8.33E+05	100.00	C	14	1527.845
3		C14	1527.837	1.16E+06	100.00	C	14	1527.845
4		Z106	11957.765	2.57E+05	100.00	Z	106	11957.779
5		C14	1527.840	4.96E+05	100.00	C	14	1527.845
6			25221.463	2.98E+05	98.55			
7			932.459	2.43E+05	94.75			
8			25221.449	2.35E+05	91.32			
9		Z42	4806.343	2.25E+05	87.42	Z	42	4806.356
10		Y60	6784.261	2.21E+05	85.98	Y	60	6784.276
11			11958.752	4.20E+05	84.67			
12		Z42	4806.342	4.05E+05	81.76	Z	42	4806.356
13		Z42	4806.347	2.24E+05	74.10	Z	42	4806.356
14		C14	1527.841	1.87E+05	72.91	C	14	1527.845
15		Z26	2869.333	3.61E+05	72.84	Z	26	2869.340
16		C12	1311.766	3.56E+05	71.72	C	12	1311.770
17			6784.262	2.14E+05	70.71			

Figure 260 ProSightBP Output pane

For more details, see the following topics:

- Viewing the ProSightBP output results (page 559)
- Exporting the ProSightBP output results (page 560)
- ProSightBP output pane parameters (page 560)
- ProSightBP output pane commands (page 563)

Viewing the ProSightBP output results

To view the results in the ProSightBP Output pane

1. As necessary, click the **Process and Review** tab, and then click the **Intact Fragmentation Results** tab at the bottom left.

2. Click the **ProSightBP Output** subtab (next to the ProSightBP Fragment Map subtab) at the bottom right.

3. Do any of the following:

   - Click the row of a proteoform (at the level under a peak row) in the Intact Fragmentation Results table.

     The table in the ProSightBP Output pane displays the fragment ion and charge state results for the selected proteoform that is related to the top-level peak in the Intact Fragmentation Results table, sorted in descending order of relative abundance. To view the charge state information, click the plus (+) sign to the left of a fragment ion row. Scroll to view more rows and columns in the table.

     If you load only one raw data file for the experiment or you are running a batch experiment, the results are from one file. If you load multiple files for a multiconsensus experiment, the results are from the combined results.

     To show the combined results, the application sends the MS2 deconvolution mass results from each raw data file to the ProSight Lite application for searching. After receiving the individual search results back, the application then combines them for display and calculates the combined sequence coverage.

   –or–

   - Click the row of a raw data file (at the level below a proteoform row) in the Intact Fragmentation Results table.

     The table displays the fragment ion and charge state results for the selected raw data file that is related to the higher-level proteoform and peak in the Intact Fragmentation Results table, sorted in descending order of relative abundance.

Exporting the ProSightBP output results

To export the results in the table to a spreadsheet file

In the ProSightBP Output pane, right-click anywhere in the table and choose one of the following:

- Export All
  - **Component Level Only**—To export all of the results at the top level in the table.

    The exported results do not include the data in the rows that appear when you click the plus icon, ,) at the left side of each fragment ion row.
  - **Component and Charge State Levels**—To export all of the results at all levels in the table.
- **Export Checked**
  - **Component Level Only**—To export only the selected results at the top level in the table.

    The exported results do not include the data in the rows that appear when you click the plus icon, , at the left side of each fragment ion row.
  - **Component and Charge State Levels**—To export only the selected results at all levels in the table.

To select a row of results to export, select the check box in that row.

To select all of the rows, select the check box in the column header.

The exported data reflects the filtering or sorting of the table.

ProSightBP output pane parameters

The following table describes the data in the table in the ProSightBP Output pane.

Table 97 ProSightBP Output table columns

Column	Description
+/–	Click to show or hide the lower level of charge state information related to the current fragment ion row.
Row number	The number assigned to each visible fragment ion row in the table. This sequential numbering does not change when you sort or filter the table.
	Select this check box if you want to export the results for the ions only in the selected rows to a spreadsheet file, using the shortcut menu. **Note:** To select or clear all of the check boxes at once, select or clear the check box in the column header. If you filter the table, the following occurs: • Clearing all check boxes affects all of the original rows in the table, before any filtering. • Selecting all check boxes affects only the filtered and currently visible rows.

Column	Description
Ion Name	Displayed as a letter followed by a number, for example "C24". The letter "C" represents the ion type and the number "24" represents the ion number. If the fragment ion is not identified, this cell is empty.
Monoisotopic Mass	Displays the observed monoisotopic mass for an individual fragment ion that results from the Xtract deconvolution algorithm.
Sum Intensity	Displays the sum of the intensities of the isotopic clusters for an individual fragment ion that results from the Xtract deconvolution algorithm.
Relative Abundance	Displays the relative abundance for a fragment ion. The application assigns the most abundant fragment ion in a deconvoluted spectrum a relative abundance of 100 percent. The number in the Relative Abundance column represents the Sum Intensity value for a particular fragment ion divided by the largest value in the Sum Intensity column, and then multiplied by 100. For example, if the largest fragment in a deconvoluted spectrum has an intensity of 1000, the application assigns it a relative abundance of 100 percent. If the next most abundant fragment has an intensity of 500, the application assigns it an abundance of 50 percent: $\frac{500}{1000} \times 100\% = 50\%$
Ion Type	Displays the ion type as a letter, as shown in the Ion Name column. If the fragment ion is not identified, this cell is empty.
Ion Number	Displayed as a number, as shown in the Ion Name column. If the fragment ion is not identified, this cell is empty.
Theoretical Mass	Displays the theoretical mass returned from the ProSight Lite application for an individual fragment ion If the fragment ion is not identified, this cell is empty.
Corrected Delta Mass (Da)	Displays the difference between the observed and theoretical masses of a fragment ion, measured in daltons. If the fragment ion is not identified, this cell is empty.
Corrected Delta Mass (ppm)	Displays the difference between the observed and theoretical masses of a fragment ion, measured in parts per million. If the fragment ion is not identified, this cell is empty.
Number of Charge States	Displays this number for an individual fragment ion that results from the Xtract algorithm.
Charge State Distribution	Displays the range of charge states for an individual fragment ion that the Xtract deconvolution algorithm detected, from the lowest to the highest charge state.
Start Time (min)	Displays the start of the retention time range for a particular peak as entered in the processing method.

Column	Description
Stop Time (min)	Displays the end of the retention time range for a particular peak as entered in the processing method.
Raw File Name	Displays the name of the loaded raw data file related to a particular fragment ion mass.
Activation Type	Displays the fragmentation type set in the processing method for a particular peak.
Charge state level **Displays the following data related to each specific charge state.**	
Row number	The number assigned to each visible charge state row in the table. This sequential numbering does not change when you sort or filter the table.
Charge State	Displays the imbalance between the number of protons (in the nuclei of the atoms) and the number of electrons that a molecular species (or adduct ion) possesses. If the species possesses more protons than electrons, its charge state is positive. If it possesses more electrons than protons, its charge state is negative.
Calculated Monoisotopic m/z	Displays the mass-to-charge ratio of the calculated monoisotopic mass for a specific charge state.
Monoisotopic Mass of Charge State	Displays the detected monoisotopic mass for a specific charge state.
Most Abundant m/z	Displays the mass-to-charge ratio of the most abundant isotope or the height of the tallest peak in the isotopic distribution.
Charge Normalized Intensity	Displays the quotient of the intensity divided by the relevant charge.
Fit %	Displays the quality of the match between a measured isotope pattern and an averagine distribution of the same mass. This column displays a value between 0 and 100 percent. • 0% requires only a poor fit between the measured pattern and the averagine pattern. • 100% requires a very good (even though not exact) fit between the measured pattern and the averagine pattern. A fit factor of 100% means that the observed peaks in the measured isotope profile are absolutely identical to those in a theoretical averagine distribution and that any missing peaks fall below a restrictive threshold.
Fit % Left	Displays the quality of the match between a measured isotope pattern and an averagine distribution that is one dalton smaller than the calculated monoisotopic mass.
Fit % Right	Displays the quality of the match between a measured isotope pattern and an averagine distribution that is one dalton larger than the calculated monoisotopic mass.

For more details, see the following topics:

- Intact fragmentation results table commands (page 537)
- Filtering data in a table (page 673)
- Sum Intensity (page 450) value
- Predicted and experimental MS2 Spectra fragment ions (page 280)
- RT Range (page 507) parameter
- Activation Type (page 508) parameter

ProSightBP output pane commands

Right-clicking the table in the ProSightBP Output pane on the Process and Review page opens a shortcut menu that contains the commands listed in the following table.

Table 98 ProSightBP Output pane shortcut menu

Command	Description
Export All	Exports all of the results in the table to a spreadsheet file.
Component Level Only	Exports all of the results at the top level in the table to a file.
Component and Charge State Levels	Exports all of the results at all levels in the table to a file.
Export Checked	Exports only the selected results in the table to a spreadsheet file.
Component Level Only	Exports only the selected results at the top level in the table to a file.
Component and Charge State Levels	Exports only the selected results at all levels in the table to a file.

For more details, see the following topics:

- Exporting the ProSightBP output results (page 560)

Viewing the matched sequence information for top down analysis

The Intact Deconvolution Matched Sequence pane on the Process and Review page displays the selected component's mass and intensity, as well as the matched sequence information from target sequence matching.

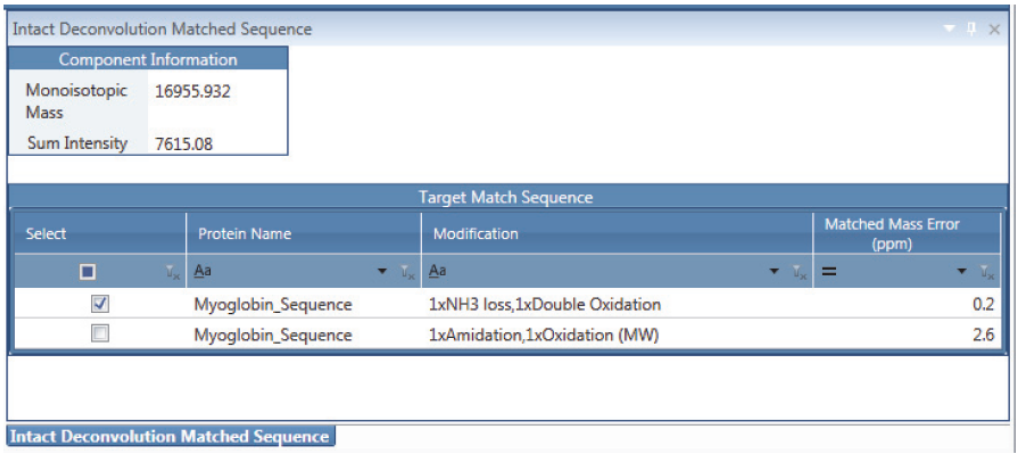


Figure 261 Intact Deconvolution Matched Sequence pane

You can select a different identification in this pane, which automatically updates the identification values in the Intact Deconvolution Results table.

For more details, see the following topics:

- Modifying the matched sequence information (page 565)
- Component information table parameters (page 565)
- Target match sequence table parameters (page 566)
- Viewing the intact deconvolution results table (page 537)
- Editing identification parameters for top down analysis (page 512)

Modifying the matched sequence information

To modify the information in the Intact Deconvolution Matched Sequence pane

1. As necessary, click the **Process and Review tab**, and then click the **Intact Deconvolution Results** tab at the bottom left.

   The Intact Deconvolution Matched Sequence pane opens to the right.

2. Click the row of a matched component in the Intact Deconvolution Results table.

   In the Intact Deconvolution Matched Sequence pane, the Component Information table shows the mass and intensity information for the component that you select in the Intact Deconvolution Results table. The Target Match Sequence table shows the matched identifications for the selected component.

3. (Optional) In the Target Match Sequence table, select a check box for a specific identification row.

   This selection overwrites the default best match identification. The selected component row in the Intact Deconvolution Results table and the labels on the spectra in the Intact Deconvolution Deconvoluted Spectrum pane also automatically update with the identification data from the selected row in the Target Match Sequence table.

For more details, see the following topics:

- Component information table parameters (page 565)
- Target match sequence table parameters (page 566)

Component information table parameters

The following table describes the information in the Component Information table at the top of the Intact Deconvolution Matched Sequence pane.

Table 99 Component Information table rows

Row	Description
Monoisotopic Mass or Monoisotopic Mass (mean), Average Mass or Average Mass (mean), Sum Intensity or Sum Intensity (mean), Intensity or Intensity (mean)	See the corresponding descriptions in “Viewing the intact deconvolution results table” on page 537. **Note:** These rows vary depending on the number of loaded raw data files, the type of deconvolution algorithm, and the result format used for the experiment: batch or multiconsensus.

Target match sequence table parameters

The following table describes the information in the Target Match Sequence table at the bottom of the Intact Deconvolution Matched Sequence pane.

Table 100 Target Match Sequence table columns

Column	Description
select	Select this check box to select the identification listed in a particular table row. **Note:** You can select only one identification row in the table. If you select a different identification, the Protein Name and Modification columns in the Intact Deconvolution Results table automatically update based on your selection.
Protein Name, Modification, Matched Mass Error (ppm/Da) or Matched Mass Error (ppm/Da) (mean)	See the corresponding descriptions in Table 71 and Table 72. **Note:** These columns vary depending on the number of loaded raw data files and the result format used for the experiment: batch or multiconsensus.

For more details, see the following topics:

- Protein Name (page 462) column
- Modification (page 462) column
- Viewing the intact deconvolution results table (page 537)

Part VII

Oligonucleotide Analysis

29 Oligonucleotide Analysis features

Use the Oligonucleotide Analysis features in the BioPharma Finder application to perform a complete characterization of nucleotide-based pharmaceuticals with superior Oligonucleotide Analysis. This type of analysis provides a high-confidence platform for fully automated characterization of these nucleotides. It performs component detection, nucleotide identification, and identification and quantification of post-translational modifications (PTMs) using Thermo Scientific™ instruments.

Experiment results display

Oligonucleotide Analysis displays the results of a completed experiment in a Results table that is organized by components at the upper level and by raw data files at the lower level. You can export the data in the Results table to a Microsoft™Excel™ or a CSV.

The analysis output also includes the Modification Summary page, which shows the recovery status and abundance of all detected modifications, and the Components table displaying the components of a completed experiment related to a particular modification.

Oligonucleotide Analysis provides the results in chromatograms, trend ratio and trend MS area plots, oligo sequence coverage, full-scan spectra, and MS2 spectra, including predicted kinetic MS2 spectra. You can view MS2 spectra for each fragmentation type and resolution combination.

The BioPharma Finder™ application generates both a BPC plot, which shows only the most intense peak in each spectrum, and the selected ion chromatogram (SIC) plot, which plots the intensity of the signal observed at a chosen mass-to-charge ratio (*m/z*).

Quantification of modifications

You can use the Modification Summary page to see the amount of a specific modification in the sample compared to the unmodified material. The BioPharma Finder™ application determines this level automatically.

Fragmentation

When you select a different type of MS2 scan in the MS2 Spectra pane—that is, a different combination of fragmentation type and resolution type—the fragmentation overage map automatically updates to display the results for that selection. See “Viewing the Fragment Coverage Map for Oligonucleotide Analysis” on page 620.

When you select a component identified as a disulfide bond, the application displays an MS2 Spectra pane for the selected in the bond.

For more details on how to interact with the spectra, see ““Using basic spectrum functions” on page 664.

Oligonucleotide analysis input

The type of input file used in Oligonucleotide Analysis is the raw data file from a mass spectrometry experiment. The raw data file can contain LC/MS data or an individual mass spectrum.

You can use Olignucleotide Analysis with data from various mass spectrometry systems: ion trap, Thermo Scientific™ Series and Orbitrap™ Series, and Fourier transform mass spectrometry (FTMS) series.

Oligonucleotide mapping analysis output

As output, Oligonucleotide Mapping Analysis produces peak and oligonucleotide information. It generates a Mapping File that contains both the parameter settings that you applied to the raw data file and the results of the analysis. These results appear in the Results table of the Process and Review page. See “Viewing the Results table for Oligonucleotide Analysis” on page 610.

When you want to view the results from another Oligonucleotide Mapping session, you can go to the Load Results page to load the results and display the results of that analysis. For more information on loading previous results, see “Opening the results from the Load Results page” on page 592.

30 Running an Oligonucleotide Analysis

After you add an oligonucleotide sequence to the Sequence Manager, you can start an oligonucleotide analysis experiment by clicking Oligonucleotide Analysis on the Home page.

Data acquisition and nucleotide identification

The BioPharma Finder™ application can be used for characterization of oligonucleotides by interpretation of high resolution accurate mass (HRAM) LC-MS and LC-MS/MS data. The monoisotopic mass of the molecule is determined using the HR full MS scan by the component detection algorithms, then the sequence of the oligonucleotide is confirmed by interpretation of the MS2 spectrum. The ideal data acquisition for oligonucleotide characterization is described below:

- HR full MS scan set to a resolution value that provides isotopically resolved data for the full length product is required. This data is used for component detection resulting in deconvolution monoisotopic or average mass of the molecule.
- Data-dependent MS2 method using HCD or CID or a combination of both fragmentation types are used to confirm the sequence of the molecule. Multiplexed targeted MS2 experiments can also be used to provide more complete sequence coverage as long as the full MS scan is part of the data acquisition method.

The BioPharma Finder component detection algorithms let you to detect impurities or metabolites never seen before and perform comparative analysis and new peak detection. The BioPharma Finder application automatically uses the associated MS2 spectrum to confirm the oligonucleotide sequence, determine the site specific location of the modification, and provide confirmation of expected and unexpected modifications.

Starting a new oligonucleotide experiment

Use the Oligonucleotide Analysis page to create a new oligonucleotide experiment. Before you can start processing, you must enter the experiment name, load the raw data files, select an oligonucleotide sequence and select a processing method.

Note: You can specify the default folder where you store the raw data files for your oligonucleotide experiments.

1. On the Home page, click **Oligonucleotide Analysis.**

   The Oligonucleotide Analysis page opens.

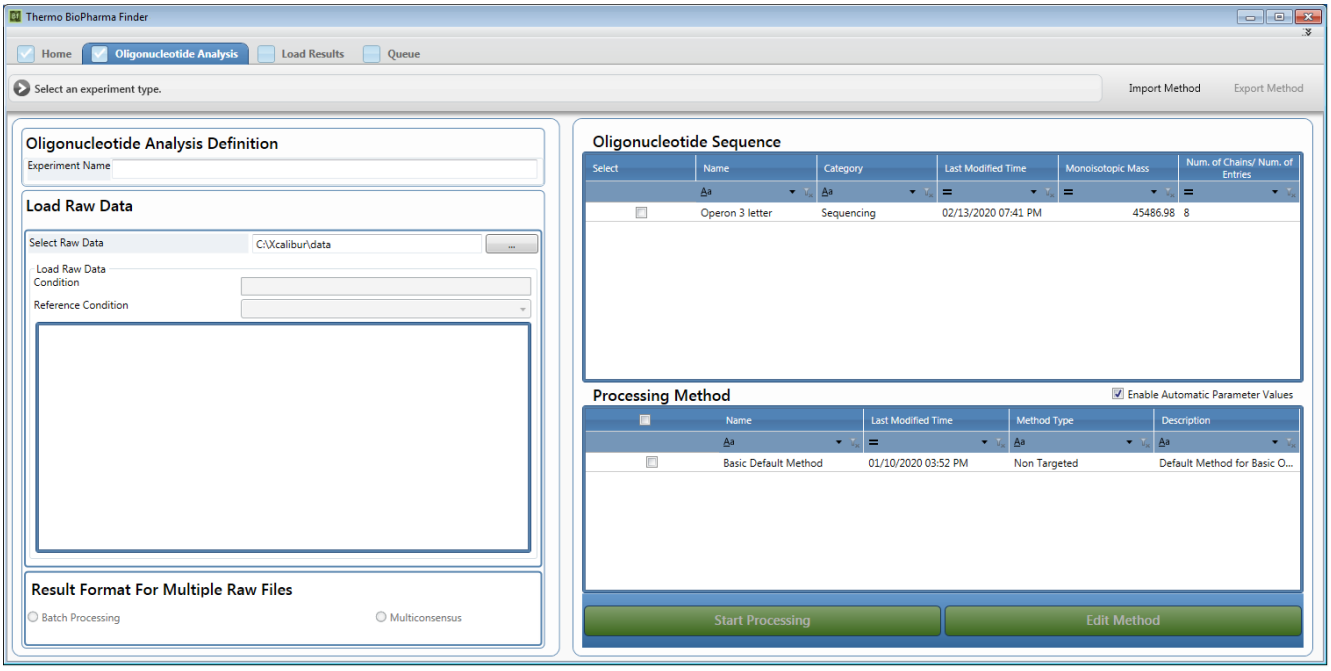


Figure 262 Oligonucleotide Analysis page

2. In the Oligonucleotide Analysis Definition area, in the Experiment Name box, type the name of the experiment.

   Use only alphanumeric, space, underscore "_", and period "." characters, up to 50 characters maximum, in the experiment name.

   IMPORTANT! If an existing experiment is already completed or canceled, you can overwrite it by entering the same experiment name. You cannot overwrite an experiment that is currently submitted to the run queue or is actively processing or canceling.

3. In the **Load Raw Data** area, load the raw data files for the experiment.

   See “Raw data files and sequences” on page 134.

4. If you load multiple files, enter the required condition information.

   The application automatically groups together all files assigned to the same condition and uses those groups for processing the results related to conditions.

5. In the table in the Oligonucleotide Sequence area, select the check box for the sequence for the experiment.

6. Do one of the following:

   - If you want the application to use certain peak detection or identification parameters that are stored in the loaded reference file for processing, Select the **Enable Automatic Parameters Values** check box above the Processing Method area.
   - To only use the parameter settings in the processing method, uncheck the **Enable Automatic Parameters Values** check box.

   When you select the Enable Automatic Parameters Values check box, the application uses the values from the raw data files for the following parameters on the Component Detection page:

   - Absolute MS signal threshold (10^3 counts, default S/N = 20)
   - Typical chromatographic peak width (min)
   - Maximum MS peak width (Da)
   - Restricted retention time range (start and stop times)
   - Mass tolerance (ppm for high-resolution or Da for low-resolution)
   - Maximum retention time shift (min)

   In addition, on the Identification page, the application uses the mass accuracy in ppm units.

7. In the Processing Method area, select a single method.

   The Edit Method button becomes available. Also, if you have entered all the required experiment information, the Start Processing button becomes available.

   Tip: If you select multiple methods, the application prompts you to select only one method. You can only select multiple methods only to export the methods.

8. Do one of the following:

   - To process the experiment without editing the method parameters, click **Start Processing**.

     The Queue page opens or the following prompt appears: The component detection interface now includes an improved visualization feature for viewing the Absolute MS Signal Threshold value. We recommend that you optimize this threshold value in the method before processing the experimient. Do you want to review and optimize the method settings?

     To edit the method, at the prompt, click **Yes**, and then edit the parameter settings as appropriate.

     Otherwise, at the prompt, click **No**. The Queue page opens.
   - To review and edit the experiment parameters before submitting the experiment to the run queue, click **Edit Method**. Edit the parameter settings as appropriate, and then click **Finish**.

     If the experiment meets all the requirements, the Queue page opens. Otherwise, the application returns to the Oligonucleotide Analysis page.

Batch and multiconsensus result formats for oligonucleotide analysis

For oligonucleotide analysis experiments with multiple loaded raw data files, you can run the experiment using either of these result formats:

- The **Batch Processing** format maintains separate results from each of the multiple raw data files.

  In this format, the BioPharma Finder application processes one experiment individually for each loaded raw data file, so you can process multiple experiments at one time but retain individual results. For the name for each batch experiment, the application concatenates the specified experiment name on the oligonucleotide analysis page with a unique date-and-time stamp. The results for each batch experiment are the same as if you ran an experiment with just a single raw data file.

–or–

- The **Multiconsensus** format merges the results from the multiple raw data files together.

  In this format, the application processes one experiment and merges the deconvolution results from all of the loaded raw data files together. By default, the name of this multiconsensus experiment uses the same name as the specified experiment name on the oligonucleotide analysis page.

Oligonucleotide experiment processing on the queue page

When all requirements are met for processing, the application saves all entered information for the new job and then opens the Queue page. If another job is already processing in the run queue, this experiment waits in line on the Queue page with a status of **Submitted**. Otherwise, the application immediately starts to process it with a status of **Running**. When the experiment is completed, its status shows **Completed**.

To view the processing results for an experiment, do one of the following:

- Select the completed job, and then click **Open Results**.
- Double-click the completed job.

The Process and Review page opens with the processing results for the experiment.

The following table describes the columns on the Queue page.

Table 101

Column Name	Comment
Record Number	Record number of the current experiment
Experiment Name	Name of the current experiment
Status	Status of the job: Submitted, Running,Cancelled, Completed, Abort, or Error
Experiment Type	Experiment type selected **Note:** In oligonucleotide analysis, this will always be Oligonucleotide
Method Name	Name of the processing method that you selelcted
Method Type	Method type selected **Note:** Currently only 'Non Targeted' is supported for oligonucleotide analysis
Sequence Name	Name of the sequence that you selected
Raw File Names	Names of raw files that you uploaded
Submit Time	Time when you submitted the experiment for processing
Start Time	Time when the application started processing the experiment
Completion Time	Time when the application completed processing the experiment
Total Processing Time (mins)	Time taken to process the experiment from Start Time to Completion Time

31 Working with an Oligonucleotide Processing Method

The BioPharma Finder application provides several default processing methods for Oligonucleotide Analysis. If needed, you can use the editing wizard to edit the parameters in one of these methods and save them to a new file to create a custom method for your experiment.

Using a processing method for Oligonucleotide analysis

To create a new method or edit a current method

1. On the Home page, click **Oligonucleotide Analysis** in the left pane or below the splash graphic.

   The Oligonucleotide Analysis page opens.

2. Enter the Experiment Name, under the Oligonucleotide Analysis Definition area.

   Size of the experiment name is limited to 50 characters. No symbols other than "_" are permitted.

3. In the Load Raw Data area, in the Select Raw Data field browse to the raw file, default location is C:\Xcalibur\data.

   - The Condition field in the Load Raw Data area, is active only when more then 1 raw file is loaded. The first condition is automatically assigned as the reference condition as well. The different conditions are separated using a space and required for multiple files.
   - The Reference Condition field in the Load Raw Data area, is automatically populated based on the information entered in the condition field.

     Note: Conditions are automatically assigned if the condition string entered matches part of the raw file name.

4. In the Processing Method area, select a processing method in the table to edit, and then click **Edit Method.**

   Note: If multiple methods are selected, the following message will appear, "Multiple methods selected. Please select only one method and try again."

   Tip: To create a custom method, modify the parameters in the Basic Default method, and then save it to a custom method using a different name.

   The editing wizard opens the **Component Detection** page under the Parameters tab, showing the Component Detection parameters in the current method.

   Note: If you create a new experiment and load multiple raw data files, the application determines the parameters for the whole group and displays them on the Component Detection and Identification pages under the Parameters tab.

   - If Enabled Automatic Parameters Values is selected on the Oligonucleotide Home page, the application reads the reference raw files and automatically populates the critical detection parameter. If this parameter is unchecked, then the values saved in the processing method are used.

5. Use the editing wizard on the Component Detection, Identification, and Save Method pages to specify the parameters for the following:

   - Peak detection
   - Ion alignment
   - Maximum Nucleotide mass
   - Nucleotide identification

   When you load raw data files for an experiment with Enable Automatic Parameter Values selected, the application uses them to automatically determine some of the parameters, such as the Absolute Signal Threshold and Maximum Retention Time Shift. You must manually set other parameters that are appropriate for the experiment, such as Task to be Performed, in Component Detection area and Use MS/MS in the Identification area in the Nucleotide Identification section.

When you finish editing the parameters on each of these pages, click **Next** in the command bar. When you get to the **Save Method** page, save all of the modified parameters to a method. You can then select that method to use for processing another experiment.

Editing Component Detection parameters for Oligonucleotide analysis

When you want to create a new processing method or edit an existing method for Oligonucleotide Analysis, go to the first page of the editing wizard, the Component Detection page.

Editing the component detection page

To edit the Component Detection page

1. On the Oligonucleotide Analysis page, enter an experiment name, load the raw data files, and select an oligonucleotide sequence.

2. Select a method and then click **Edit Method.**

   The Component Detection page opens. If you navigate away from this page and want to get back to it, in the navigation bar, click the Parameters tab, and then click the Component Detection subtab.

   In the Oligonucleotide homepage, if the Enable Automatic Parameters Values check box is selected, the algorithm automatically determines the best settings for peak detection such as MS Noise level and S/N Threshold that is very specific to the raw files.

3. Enter the appropriate values on the Component Detection page.

4. When you are done editing the parameters on this page, click **Next** in the command bar to advance to the Identification page.

   - **Note:** The **Previous** and **Next** buttons move you from one page to another under the parameters tab. The two buttons are also active if you make a change in any of the parameters. The application remembers the changes

when you go from one page to another using the buttons or if you click on the checkboxes for the different pages.

If an error is present in one of these parameters, the Next button becomes disabled.

The **Finish** button is not active until you get to the Save Method page

- Right below the **Set the Parameters for Component Detection,** you will find the **Select Task To Be Performed.**
  Here you can find a dropdown list for the tasks that can be performed, such as:
  Find All Ions in the Run - Default
  Find All Masses in the Run
  Find Peaks in the Total Ion Chromatogram
  Find Peaks in the Base Peak Chromatogram
  Find Peaks in the Analog Chromatogram
  Find All Ions with MS/MS
- Below the Select Task to be Performed field, is the Peak Detection area. It has the following fields:
  Absolute MS Signal Threshold (MS Noise Level * S/N Threshold), display only.
  Default (no raw): 1000
  Range: 0 to 1E+12
  Displayed as significant notation
  MS Noise Level
  Default (no raw): 1,000.00
  Range: 10 to 100,000
  Two decimal places
  Raw file specific
  S/N Threshold
  Default (no raw): 20.00
  Range: 0.1 to 1,000,000
  Two decimal places
  Raw file specific
  Beginning Peak Width (min)
  Default (no raw): 0.20
  Range: 0.02 to 20
  Two decimal places
  Raw file specific
  Typical Chromatographic Peak Width (min)
  Default (no raw): 0.20
  Range: 0.02 to 20
  Two decimal places
  Raw file specific
  Ending Peak Width (min)

Default (no raw): 0.20
Range: 0.02 to 20
Two decimal places
Raw file specific
Maximum Chromatographic Peak Width (min)
Default (no raw): 1.0
Range: 0.05 to 50
Two decimal places
Raw file specific
Use Restricted Time
Checkbox
Default not enabled
Time Limits
Only enabled when "user restricted time" is enabled.
First time and second time.
Two decimals places
The first time must be less than the second time.
Range: 0.01 to 999,999,999.99
Relative MS Signal Threshold (% of base) - advanced
Default (no raw): 1.0
Range: 0.00 to 100
Two decimal places
Relative Analog Threshold (% of highest peak) - advanced
Default (no raw): 1.0
Range: 0.00 to 99.9
Two decimal places
Width of Gaussian Filter (represented as 1/n of chromatographic peak width) - advanced
Default (no raw): 3
Range: 1 to 10
Three decimal places
Minimum Valley to be Considered as Two Chromatographic Peak (%) - advanced
Default (no raw): 80.00
Range: 5 to 99.9
Two decimal places
Minimum MS Peak Width (Da) - advanced
Default (no raw): 1.20
Range: 0.5 to 100
Two decimal places
Maximum MS Peak Width (Da) - advanced
Default (no raw): 4.20
Range: 2 to 200

Two decimal places

Mass Tolerance (ppm for high-res or Da for low-res) - advanced

Default (no raw): 0.30, this value is based on the raw files and will change depending on the files. Input should be provided from the backend, so please test to see if the value changes and matches what Mass Analyzer changes the value too.

Range: 0.01 to 50

Two decimal places

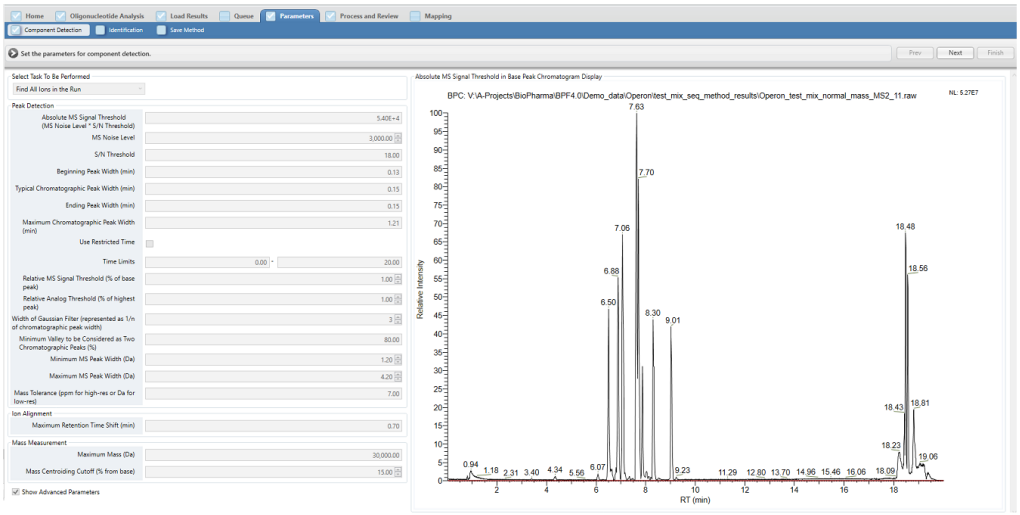
- **Ion Alignment**
  Maximum Retention Time Shift (min)
  Default (no raw): 0.50
  Range: 0.00 to 120
  Two decimal places
  Raw file specific
- **Mass Measurement**
  Maximum Mass (Da) - advanced
  Default (no raw): 30,000.00
  Range: 1,000 to 500,000
  Two decimal places
  Mass Centroding Cutoff (% from base) - advanced
  Default (no raw): 15.00
  Range: 0.00 to 99
  Two decimal places
- **Show Advance Parameters**
  The Show Advanced Parameters checkbox shows the advanced parameters, when selected.
- **Absolute MS Signal Treshold in Base Peak Chromatogram Display**
  Here, the BPC trace is displayed for the first raw file with a red line that displays the **Absolute MS Signal Threshold.** When you change the **Absolute MS Signal Threshold** by changing either the "MS Noise Level or the S/N Threshold" the red line will move accordingly to the changes. You can zoom in x and y-axis and double click to automatically zoom out. You can **Right Click** to either **Reset Scale** or **Copy.**

Component detection page layout

The Component Detection page includes five different areas: **Select Task to Be Performed**, **Peak Detection**, **Ion Alignment**, and **Mass Measurement** on the left side, and **Absolute MS Signal Threshold in Base Peak Chromatogram Display** on the right side.

Note: Select the **Show Parameters** check box to edit advanced options that are hidden by default. Normally, these advanced parameters do not need to be edited.

Figure 263 Component detection page


Home
Oligonucleotide Analysis
Load Results
Queue
Parameters
Process and Review
Mapping
Component Detection
Identification
Save Method
Set the parameters for component detection.
Prev
Next
Finish
Select Task To Be Performed
Find All Ions in the Run
Peak Detection
Absolute MS Signal Threshold (MS Noise Level * S/N Threshold)
5.40E+4
MS Noise Level
3,000.00
S/N Threshold
18.00
Beginning Peak Width (min)
0.13
Typical Chromatographic Peak Width (min)
0.15
Ending Peak Width (min)
0.15
Maximum Chromatographic Peak Width (min)
1.21
Use Restricted Time
Time Limits
0.00
20.00
Relative MS Signal Threshold (% of base peak)
1.00
Relative Analog Threshold (% of highest peak)
1.00
Width of Gaussian Filter (represented as 1/n of chromatographic peak width)
3
Minimum Valley to be Considered as Two Chromatographic Peaks (%)
80.00
Minimum MS Peak Width (Da)
1.20
Maximum MS Peak Width (Da)
4.20
Mass Tolerance (ppm for high-res or Da for low-res)
7.00
Ion Alignment
Maximum Retention Time Shift (min)
0.70
Mass Measurement
Maximum Mass (Da)
30,000.00
Mass Centroiding Cutoff (% from base)
15.00
Show Advanced Parameters
Absolute MS Signal Threshold in Base Peak Chromatogram Display
BPC: V:\A-Projects\BioPharma\BPF4.0\Demo_data\Operon\test_mix_seq_method_results\Operon_test_mix_normal_mass_MS2_11.raw
NL: 5.27E7
Relative Intensity
RT (min)
7.63
7.70
7.06
6.88
6.50
8.30
9.01
18.48
18.56
18.43
18.81
18.23
19.06
0.94
1.18
2.31
3.40
4.34
5.56
6.07
9.23
11.29
12.80
13.70
14.96
15.46
16.06
18.09

Component detection page parameters

The following table describes the parameters available on the Component Detection page under the Parameters tab.

Table 102 Component detection page parameters

Parameter	Description
Select Task to Be Performed	
(Task List)	Specifies the type of processing to perform. • Find All Ions in the Run: Displays information about each ion in the Results table, including the peptide sequence, retention time, *m/z,* MS peak area, charge state, and so forth. • Find All Masses in the Run: Combines multiple charge states into a single entry. • Find Peaks in the Total Ion Chromatogram: Detects peaks in the TIC, deconvolves the spectra, and identifies MS2 spectra associated with the major ions under these peaks. • Find Peaks in the Base Peak Chromatogram: Detects peaks in the BPC, deconvolves the spectra, and identifies MS2 spectra associated with the major ions under these peaks. • Find Peaks in the Analog Chromatogram: Detects peaks in the analog chromatogram. • Find All Ions with MS/MS: Detects ions that have an associated MS/MS.
Peak Detection	

Parameter	Description
Absolute™ MS Signal Threshold	(Not editable) Specifies the absolute MS signal threshold. Absolute™ MS Signal Threshold = MS Noise Level × S/N Threshold Adjust the value of this parameter by changing the MS Noise Level parameter or the S/N Threshold parameter, or both. The application provides a visual aid for this signal threshold by displaying a thin red horizontal line on the BPC displayed on the right side of the Component Detection page. In general, processing takes much more time if this red line is well below the background noise level in the BPC or total ion chromatogram (TIC).
MS Noise Level	Specifies the noise level in the raw data files.
S/N Threshold	Specifies the signal-to-noise threshold in the raw data files.
Beginning Peak Width (min)	Specifies the peak width in the beginning
Typical Chromatographic Peak Width (min)	Specifies the typical chromatographic peak width in the LC/MS run, in minutes. The application automatically determines the typical chromatographic peak width of the experimental LC/MS data file in minutes and sets the value to the width of the highest peak in the chromatogram.
Ending Peak Width	Specifies the peak width in the end
Maximum Chromatographic Peak Width (min)	Specifies the maximum chromatographic peak width in the LC/MS run, in minutes. The application automatically sets the maximum chromatographic peak width in the LC/MS run. It sets this initial value to the geometric mean of the width of the highest peak and the range of the chromatogram. The application considers any peak wider than this value to be part of the background and does not include it in the results.
Use Restricted Time	Activates the Time Limits boxes so that you can enter a restricted time range.

Parameter	Description
Time Limits	Specifies the retention time range used to truncate the chromatogram and reduce the results to an appropriate area.
Absolute™ MS Signal Threshold in Base Peak Chromatogram Display	Displays the red line representing the value shown in the Absolute™ MS Signal Threshold box. The value of the read-only Absolute™ MS Signal Threshold parameter changes when you adjust the MS Noise Level value, the S/N Threshold value, or both in the Peak Detection area. As the value of Absolute™ MS Signal Threshold changes, the red line on the BPC on the right side of the page moves to reflect this change. **Note:** To see the BPC with the red line, you must first load one or more raw data files on the Oligonucleotide Analysis page. If you load multiple raw data files, the application displays the BPC for the first raw data file.

Viewing the signal threshold

Use the Peak Detection area of the Component Detection page to define the MS Signal Threshold value.

As you change the MS Noise Level parameter, the S/N Threshold parameter, or both, the red line on the BPC on the right side of the page moves to reflect this change. You can zoom in to see the red line movement more clearly.

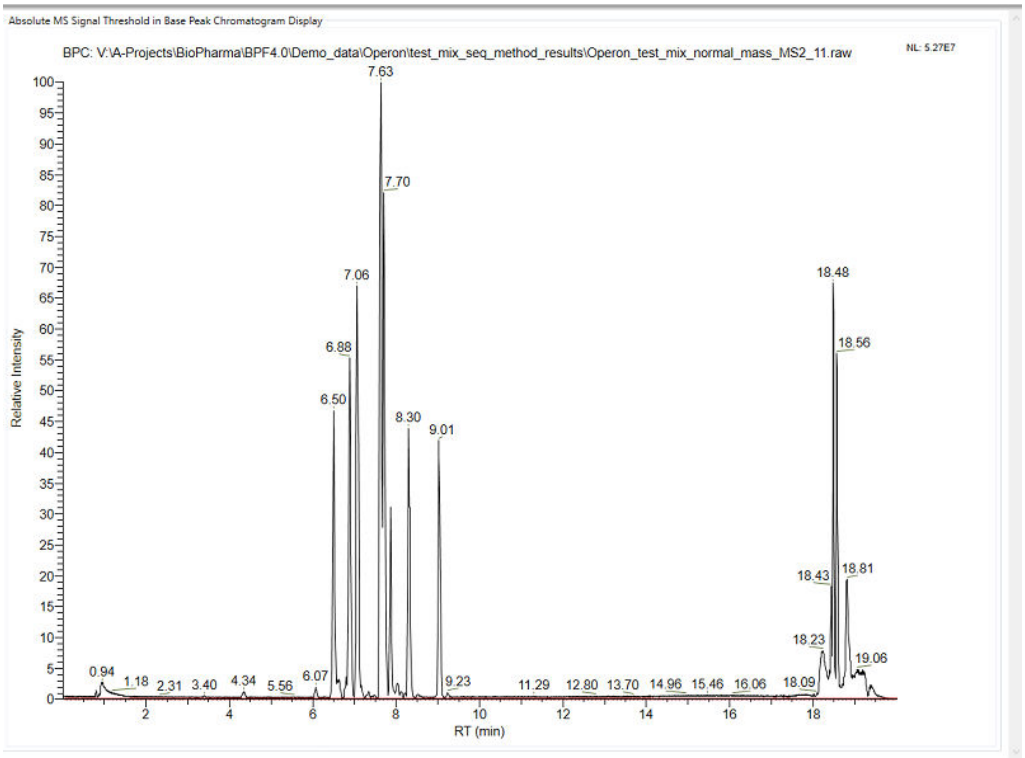


Figure 264 MS signal threshold line

To see detailed information about the retention time along the *x* axis in a certain area —for example, between the retention times of 10 and 15—drag the mouse horizontally along the bottom of the chromatogram from the lowest retention time to the highest. The following figure shows the magnified area between retention times 10 and 15 of the BPC.

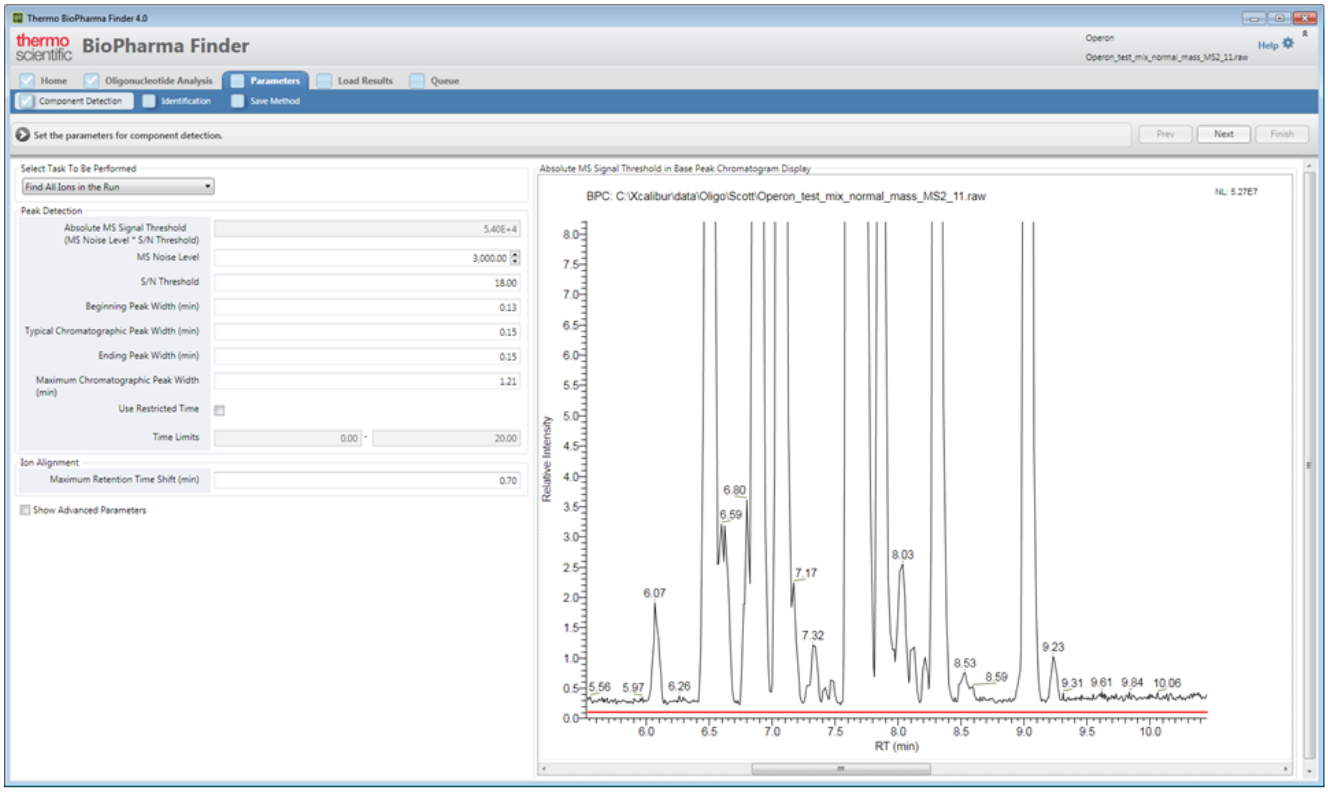


Figure 265 Magnified area of retention time

Figure 266 Magnified area of retention time

To see detailed information about the relative intensity along the *y* axis in a certain area—for example, between the relative intensities of 0 and 15—drag the mouse vertically along the left side of the chromatogram from the lowest intensity to the highest. The following figure shows the magnified area between the relative intensities of 0 and 15 of the BPC.

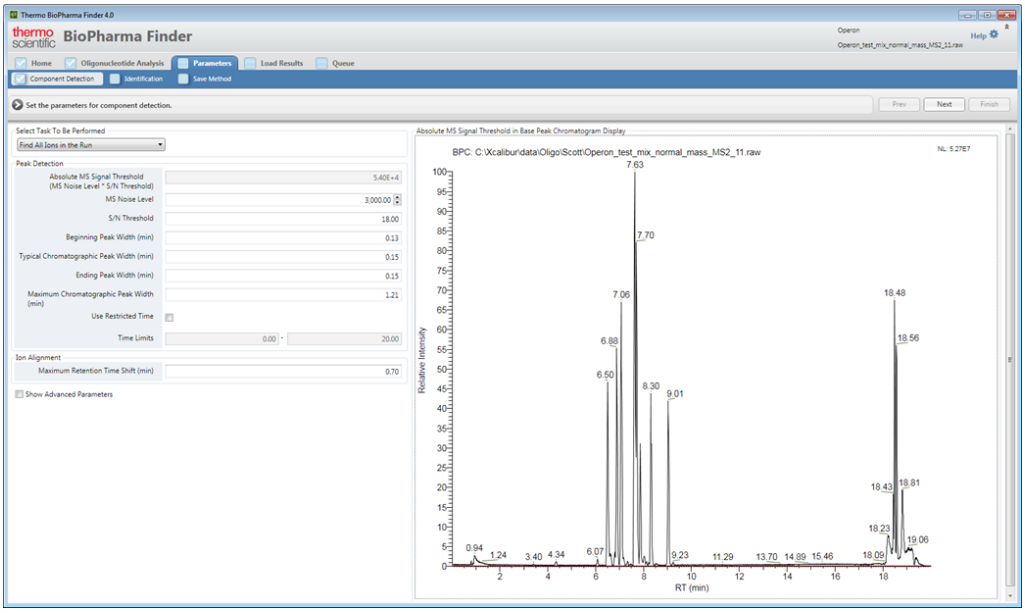


Figure 267 Magnified area of relative intensity

To return the BPC to its original scale, right-click and choose **Reset Scale.**

Editing identification parameters for Oligonucleotide analysis

You can advance to the second page of the editing wizard, the **Identification page** from the component detection page.

For more details, see the following topics:

- “Editing the identification page” on page 587
- “Identification page layout” on page 588
- “Identification page parameters” on page 588

Editing the identification page

1. On the Component Detection page, click **Next** in the command bar.

   –or–

   In the navigation bar, click the **Parameters** tab, and then click the **Identification** subtab.

   If you selected the **Enable Automatic Parameters Values** check box above the Processing Method table on the Oligonuceotide Analysis page,the application uses mass accuracy determined by the algorithm automatically based on the loaded raw data.

2. Enter the appropriate parameter values on the Identification page, in the Oligonucleotide Identification section.

3. You can select Enable Mass Search for Unspecified Modifications or make changes to Mass Changes for Unspecified Modificaiton in the Advanced Search section of this page.

4. When you are done editing the parameters on this page, click **Next** in the command bar to advance to the Save Method page.

Identification page layout

The Identification page includes three different areas: Navigation pane, Oligo Identification, and Search.

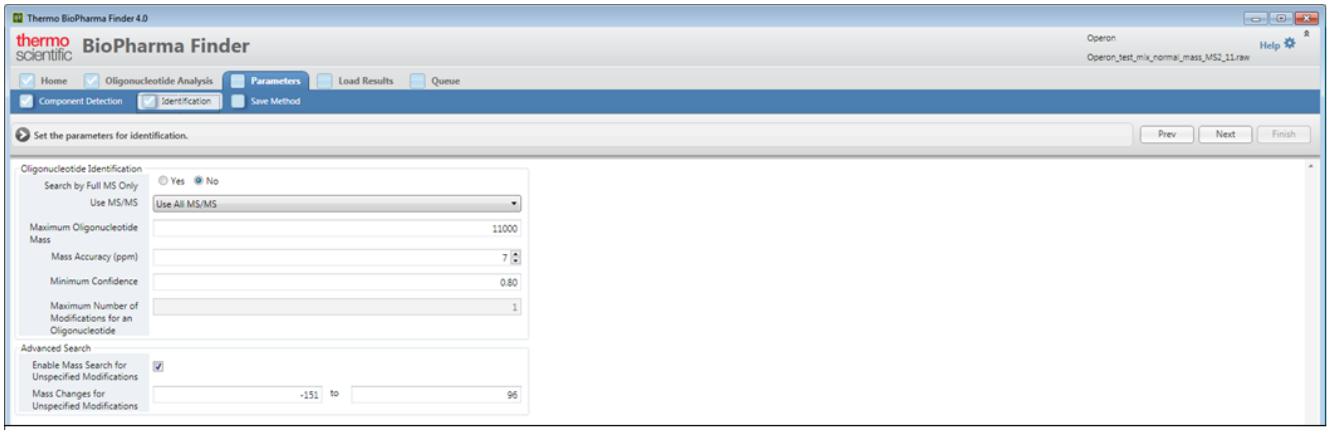


Figure 268 Identification page areas (highlighted)

Identification page parameters

The following table describes the parameters on the Identification page under the Parameters tab.

Table 103 Identification page parameters

Parameter	Description
Oligonucleotide Identification	
Search by Full MS Only	Indicates the type of data that the raw data file contains. • Yes: Indicates that the raw data file contains only full-scan data. • No: Indicates that the raw data file contains MS2 data.

Parameter	Description
Use MS/MS	Specifies the type of data to process. • Use All MS/MS: Uses all the MS2 spectra in the raw data file to identify the peptides. • Ignore MS/MS: Uses only the full-scan spectra in the raw data file to identify the peptides. • Use CID/HCD Only: Uses only the CID/HCD spectra in the raw data file to identify the peptides. • Use ETD/ECD Only: Uses only the ETD/ECD spectra in the raw data file to identify the peptides.
Maximum Oligonucleotide Mass	Specifies the maximum oligonucleotide mass to be identified.
Mass Accuracy (ppm)	Specifies the maximum mass deviation (ppm) when comparing the theoretical peptide mass to the calculated mass of a particular ion to determine identification.
Minimum Confidence	Specifies the minimum confidence level to be reported for a peptide assignment on a 0-to-1 scale, with 1 having the highest confidence.
Maximum Number of Modifications for an Oligonucleotide	(Read-only) Specifies the maximum number of modifications for each sequence. This value comes from the Oligonucleotide parameter in the Max™ # Modifications set in the Oligonucleotide sequence that you assign to the experiment.
Advanced™ Search	
Enable Mass Search for Unspecified Modifications	Determines whether the application performs a mass search for unspecified modifications. • Selected: Performs a mass search for unspecified modifications. • Cleared: Does not perform a mass search for unspecified modifications.

Parameter	Description
Mass Changes for Unspecified Modifications	• Enabled based on the "enabled mass search for unspecified modification" • First and second whole numbers • Default Values First: -151 Second: 96 • Whole numbers, no decimal places

Editing the Save Method page for Oligonucleotide Analysis

When you want to create a new method or edit an existing method, first go to the Component Detection and the Identification pages. Next, advance to the Save Method page, the third and last page of the editing wizard. The Method Name is populated with the selected method on the Oligonucleotide Analysis Home page, under the processing method section .

Here, choose a new name in the Method Name field, and then click **Finish**.

The BioPharma Finder takes you to the Oligonucleotide Analysis page. The new method name is added to the list in the Processing Method pane.

Note: When the Basic Default Method is selected, trying to save this method will prompt the message, "You cannot overwrite a Default Method". Enter a different Method Name". When a new name is entered, the method is saved and the applicaiton opens the Oligonucleotide Analysis Home page.
When an existing method name other than the Basic Default Method is entered, clicking on Save prompts the message, "Do you want to overwrite the existing method?".
Clicking **Yes** lets you click **Finish** and advance to the Oligonucleotide Analysis Home page.

You can now see the new method under the Processing Method area in the Name column.

32 Viewing the Oligonucleotide Analysis Results

You can view the oligonucleotide analysis results in the BioPharma Finder™ application

Opening the results from the queue page

When you run an Oligonucleotide Analysis experiment, the Queue page displays the run queue containing all of the completed, running, submitted, or canceled jobs. You can open any completed job by double clicking on the selected job. Jobs that are in submitted state or running state cannot be opened.

To view the results of an experiment from the Queue page

1. On the Home page, click **Oligonucleotide Analysis** in the left pane or below the splash graphic.

2. Click the **Queue** tab.

   The Queue page opens showing the queued jobs in a table that shows the columns:

   - Record Number
   - Experiment Name
   - Status
   - Experiment Type
   - Method Name
   - Method Type
   - Sequence Name
   - Raw File Names
   - Submit Time
   - Start Time

- Completion Time
- Total Processing Time (min)

You can sort the columns or filter the data in the table as needed.

3. Double click any completed job to open the result or select any completed job and click the "Open Results" Button.

4. In the command bar, click **Open Results.**

   The application opens the Process and Review page, which displays the following:

   - Chromatograms in the Chromatogram pane
   - Visible only for experiments with multiple raw data files:
     Trend ratios plot in the Trend Ratio pane
     Trend MS areas plot in the Trend MS Area pane
   - Oligo Sequence coverage map in the Sequence Coverage pane
   - Oligon sequence in the Oligo Sequence pane
   - Output spectra in the Full Scan Spectra™ and MS2 Spectra™ panes
   - Components in the Results table

   Note: You can adjust the size or location of the panes on this page as needed. In addition to the Process and Review page, you can also click the Mapping tab to view the Coverage page and the Modification Summary page.

Opening the results from the Load Results page

You can delete jobs in the run queue on the Queue page. However, the BioPharma Finder application saves all of the completed jobs on the Load Results page so that you can view any previously saved experimental results.

To view or delete the results of an experiment from the Load Results page

1. On the Home page, click **Oligonucleotide Analysis** in the left pane or below the splash graphic.

2. Click the **Load Results** tab.

   Note: To view the results of an oligonucleotide session, go to the Load Results page
   .

   The table on the Load Results page displays all of the previously saved oligonucleotide analysis results, in order of completion time.

The table provides information including the following:

- Experiment Name
- Raw File Name
- Method Name
- Method Type
- Method Description
- Sequence Name
- Completion Time
- Total Processing Time (min)

You can sort the columns or filter the data in the table as needed.

3. In the table on the Load Results page, do any of the following:
   - Double-click a row to select an experiment and view its results
     or
   - click a row and then click **Load Results** in the command bar.In addition to the Process and Review page,

   Note: You can also click the Mapping tab to view the Coverage page and the Modification Summary page.

4. Select one or more rows and then click **Delete** in the command bar.

5. Select multiple rows by using the SHIFT key for contiguous rows or the CTRL key for noncontiguous rows.

   The application deletes the selected set of results from the database. If the selected experiments still exist in the run queue on the Queue page, the application removes them from the queue as well.

   From the Load Results page, you can also import and export experimental results by using the Export Results or the Import Results option on the top right corner of the page.

   Note: See Export Results and Import Results behavior in table below

   The Exported file includes the following information:

   Sequence Information

   Sequence

   All types of modifications including site-specific, variable, and custom modification

   Targeted search -> workbook instead of the sequence

   Raw file option

   - Export Experiment with raw files
   - Export Experiment without raw files

   All parameters

   Peak detection

   Identification

Supporting files

Process Management Files (pmf)

Note: Multiple experiment export is supported.

Table 104 Export Results and Import Results behavior

Export Results	Import Results
Active only after you check at least one result using the check boxes.	Always active
You can select one or multiple results using the check boxes.	You can select one or multiple files and click import.
You can click Export Results and select from the two options: Export Experiment with raw files Export Experiment without raw files	You can click Import Results and the results will be created and saved in the Load Results tab in both the master list and the working list.
Opens the Windows C drive, where you can browse to save the file.	Opens the Windows C drive, or last folderthat was accessed, where you can browse to save the file.
The default name will be the same name as the saved results in the application.	Results will be created and saved in the Load Results tab in both the master list and the working list.
The filename exension will be: Oligonucleotide = *_olgioucleotide.resultsbpf Peptide Mapping = *_peptide.resultsbpf Intact = *_intact.resultbpf Top-down = *_topdown.resultbpf	The name of the imported results are the same as the saved result.
During export, the file will overwrite (replace) an existing result with the same name.	The import will always add and never overwrite a result because the check to make sure the results are exactly the same.
The application will check to make sure the drive has enough space to save the results.	If an existing result has the same name as the newly imported result, the application will tag the new result with _imported
During the export of multiple experiments with raw data files, a general message window will appear stating that one of the experimental results exceeds the maximum allowed space and the experiment will be exported without raw data files. This check is during the saving of the experiments.	When importing a single result or multiple results at one time, a dialog box appears and you can browse for exported results. This window only looks for the .resultbpf file(s) to simplify the searching. The individual workflow method will not be filtered. All results will be shown but you can use the *_oligonucleotide.resultsbpf to help distinguish that this is an oligo results file.

Note: While saving a file, there are no character limitations other than normal Windows file limitations and no case sensitivity.

IMPORTANT! When multiple experiments are exported with the raw files, if the experiment size is more than 5 GB, then a message will be displayed that the file size is bigger than 5 GB and it will not be exported. If you select 'ok', the experiment will not be exported with raw data files. The rest of the experiments will be exported without the raw data files and the message will not be displayed for other selected experiments.

Importing experiments with raw data files

The application checks the location in the experiment for the raw files. If they are not present, the application adds them. If they are present, the application will not overwrite the raw files.

If the raw data file location in the experiment is a networked drive or a drive not on the computer, an informative message is displayed that the raw data files will be saved to the C:\Xcalibur\BPF folder and you can remap the file location while opening the result.

The original folder path is retained inside of the C:\Xcalibur\BPF.

Using Real-Time optimization for Oligonucleotide Analysis

After viewing the results on the Process and Review page, you can change the parameters in the oligonucleotide sequence, the parameters in the processing method, or both sets of parameters, for real time optimization and then you can reprocess the experiment without leaving this page.

To reprocess the experiment with modified sequence or method parameters

1. Click the **Process and Review** tab if necessary.

   The Process and Review tab allows you to make changes to any of the processing parameters.

2. Click the **Real Time Optimization** title bar to see the Sequence, Component Detection, and Identification subtabs.

   The **Sequence grid** contains the following rows:

   - **Sequence:** lists the name of the sequence
   - **Sample Type:** this will always be Oligonucleotides
   - **Category:** Oligonucleotide Analysis
   - **Terminal Modifications:** lists the 5' and 3' modifications
   - **Variable Modifications:** lists any variable modifications

   Clicking **Edit** on this page opens the Oligonucleotide Sequence Editor for the specific sequence.

Note:

- The **Manual Input Sequence, Import Fasta File, Define Modification List** and **Save** buttons are unavailable because you cannot change these features for the selected sequence.
- The **Sequence Information, Assign Variable Modifications** and **Building Block and Variable Modification Editor** are all active. You can make changes to the modifications and create new modifications.
- You must save the sequence as new when saving changes. Therefore the **Save As New** button is active. This new sequence should appear in the sequence manager list after the Oligonucleotide Sequence Editor window closes.

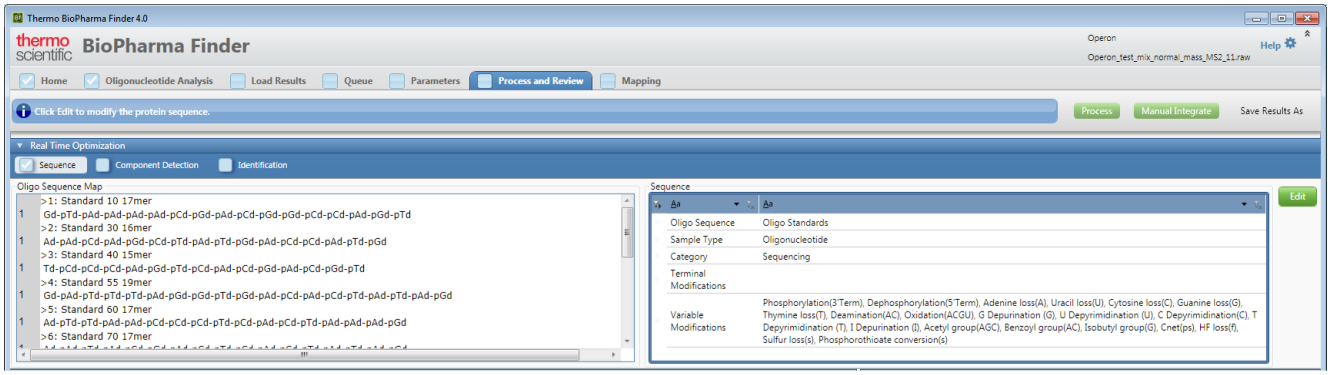


Figure 269 Sequence pane for real-time optimization experiment

When you click the other subtabs, the Component Detection and Identification panes display the parameter values in the method that are used for processing the currently open experiment.

3. Click the **Component Detection** tab. Change the parameters, and then click the **Next** subtab.

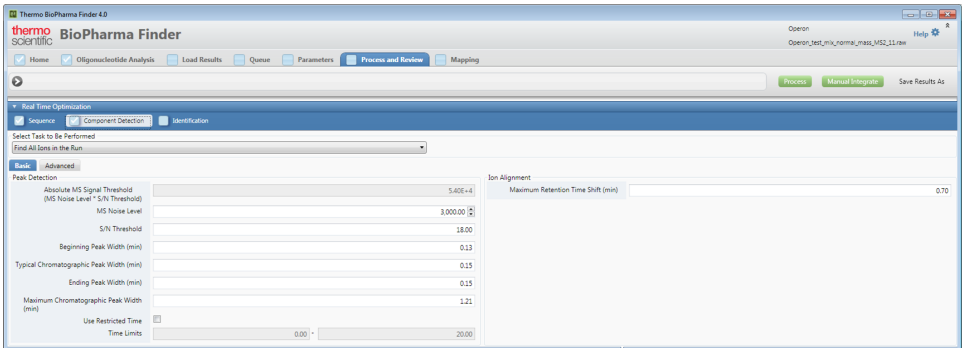


Figure 270 Component detection pane for real-time optimization

4. Click the **Identification** tab, and then in the Oligonucleotide Identification or Advanced Search areas, update the corresponding parameters as needed.

5. Click **Process** in the command bar of the Process and Review page.

   If the application finds invalid parameter entries, it displays an error dialog box to inform you. To continue, enter all required parameter values within the appropriate ranges.

6. If all of the entered parameters are valid, enter the experiment and method names in the Reprocess Experiment dialog box, and then click **Reprocess.**

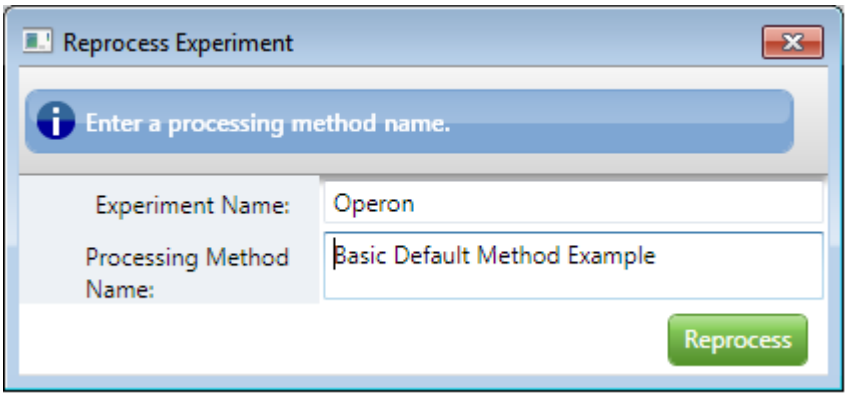


Figure 271 Reprocess experiment dialog box

Note: If an existing experiment is already completed or is canceled, you can overwrite it by using the same experiment name. You cannot overwrite an experiment that is currently submitted to the run queue or is actively processing or canceling.

Note: The default method name, Basic Default Method, cannot be overwritten. You select a new name.
If the method you are using is not a default method, you can overwrite it by using the same method name. Otherwise, if you change the parameters in a default method, save the method to a new name.
Use only alphanumeric, space, underscore "_", and period "." characters in the experiment and method names.
The entry of new experiment and method names saves your changes to new files to ensure that you do not overwrite the previous experiment results and method parameters.

When the processing is completed, the following occur:

- The application clears all of the previous results on the Process and Review page and submits a new processing job with your changes to the run queue.
- The job moves to the top of the queue, behind the currently running job.
- If no job is currently running, the application reprocesses the new job immediately.

  During this time, you remain on the Process and Review page and can navigate to other areas or open other results if you want.
- the Process and Review page automatically displays the new results, except when you open another results file while the application is reprocessing

  In this case, it does not update the display of the reprocessing results when they are completed.

If no job is currently running, the application reprocesses the new job immediately. During this time, you remain on the Process and Review page and can navigate to other areas or open other results if you want.

You can open the saved experiment results file later.

Performing the kinetic MS/MS model prediction

The BioPharma Finder application uses the kinetic model to predict oligonucleotide MS2 spectra. In the MS2 Spectra™ pane, you can change the parameters to regenerate the predicted spectrum using this kinetic model.

To perform spectral prediction for a nucleotide dataset using the kinetic model

1. On the Process and Review page, click the **MS2 Spectra** subtab.
2. Click a row in the Results table that displays "MS2" in the ID Type column.
3. Right-click the MS2 Spectra™ pane and choose **Predict Oligo MS/MS (Kinetic Model).**

   The application opens the Predict Oligo MS/MS (Kinetic Model) dialog box.

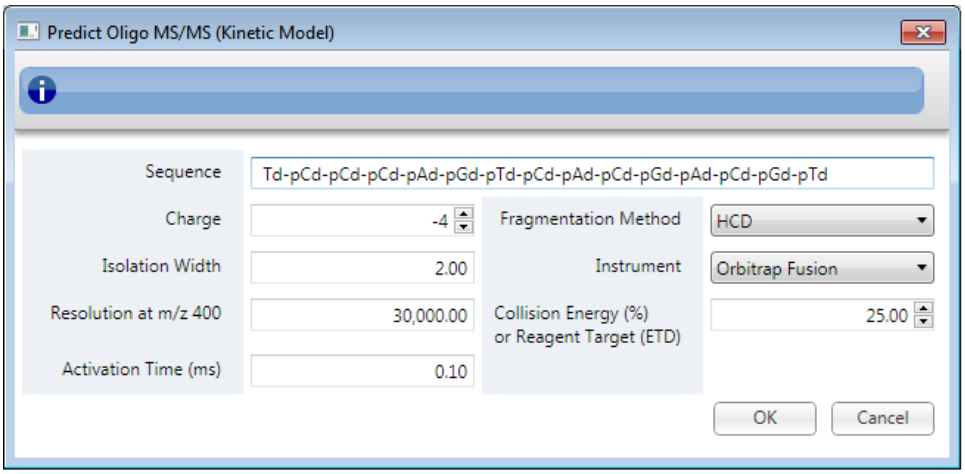


Figure 272 Predict Oligo MS/MS (Kinetic Model) dialog box

4. In the Sequence box, type the three-letter codes for a nucleotide sequence.

   Note: Use uppercase for all three-letter codes.

5. Enter values for the following parameters:
   a. To set the charge state, the isolation width used for fragmentation, the resolution at *m/z* 400, and the activation time (ms), enter values in their respective boxes.

b. To define a fragmentation method, select from the available options in the list.

   Valid values: CID, CID with WB activation, ETD, ETD with supplemental activation, ECD, and HCD

c. To choose an instrument, select an instrument name from the list.

   Valid values: LCQ, LTQ, Orbitrap™, LTQ FT, LTQ Velos, Velos Orbitrap™, Orbitrap™ Fusion™, Fusion™ Ion Trap , Q Exactive™, Q Exactive Plus™, and Q Exactive HF™

d. To define the collision energy or reagent target, enter a value in the box.

   Specify normalized collision energy as a percentage value (%), or specify the reagent ion target value for the ETD fragmentation method.

6. Click **OK** to see the MS2 predicted spectrum.

The application displays the changes in the predicted spectrum in the MS2 Spectra pane..

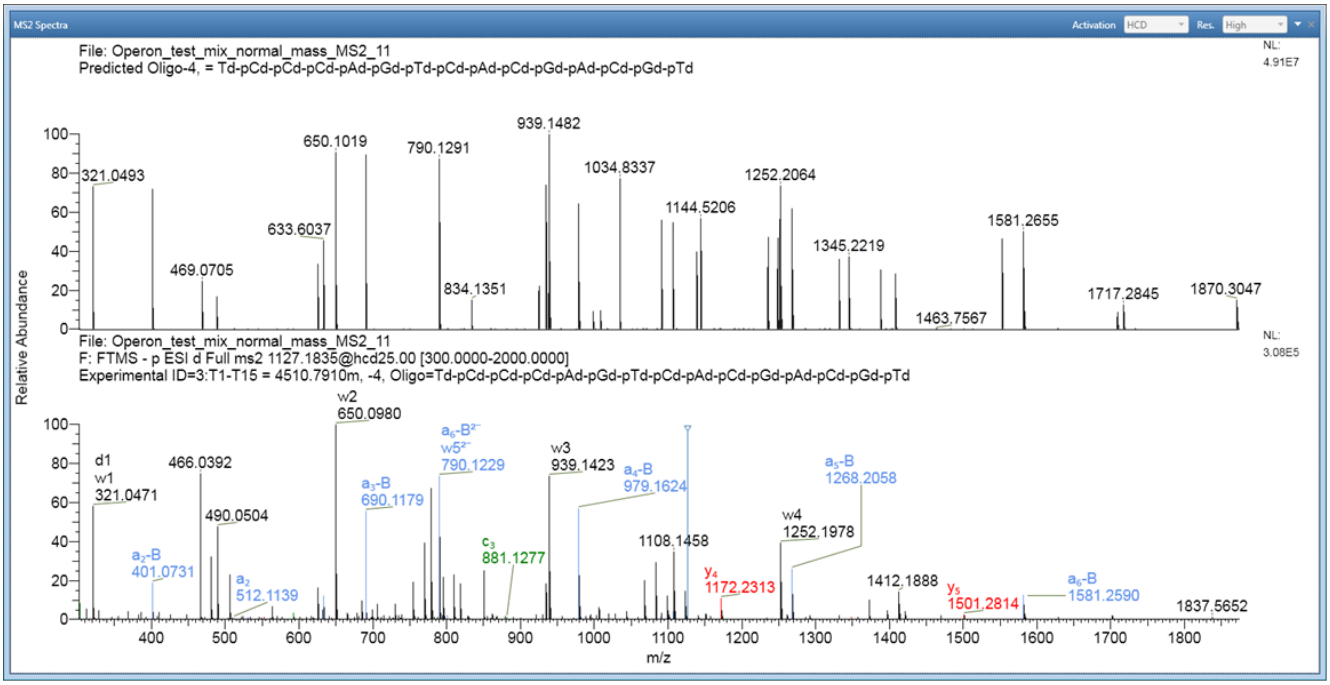


Figure 273 Predicted spectrum after applying the kinetic model

Organizing the Experimental Results

Use the Experiment Management pane to organize the saved experimental results in different folders. The experiment management pane consists of two lists: The Master list and the Working list. In the Working List, you can create folders or subfolders and drag and drop results from the Working List to any folder that you created. After an experiment is moved, it will be removed from the working list. The Master List contains all of the previously saved experimental results. The Master List folder shows all the experimental results regardless of the folder in which they reside.

To access the Experiment Management pane

1. On the Home page, click **Oligonucleotide Analysis** in the left pane or below the BioPharma Finder™ splash graphic. The Oligonucleotide Analysis page opens.

2. Click the **Load Results** tab.

   The Load Results page opens to the Master List by default. The BioPharma Finder application displays all of the previously saved experimental results in the Master List.

3. Do one of the following to access the Experiment Management pane:

   - Click the double arrows to expand the Experiment Management pane. The Experiment Management pane opens to display the Master List and the Working List.
   - Click the Experiment Management pane on the left. A window appears showing the Master List and the Working List.

Creating a folder in the Working List

1. In the Experiment Management pane of the Load Results page, select the **Working List**. By default, the Working List contains the Home folder.

2. Click the **Home** folder, and then click **Add**.

   Note: You can create a new folder only under the Home folder of the Working List. You cannot create a folder in the Master List.

3. In the **Add New Folder** dialog box, type the Folder Name, and then click **Add**.

   A new folder appears under the Home folder in the Working List.

4. To create a subfolder, select the folder to which you want to add a subfolder, and then click **Add**.

   A new subfolder appears under the folder.

   Tip: Each folder can contain a subfolder, and each subfolder, in turn, can contain more subfolders, and so on.

Deleting a folder

1. In the Experiment Management pane of the Load Results page, select the **Working List** to view its folder structure.
   When the Working List opens, only the Home folder appears in the pane.
2. To navigate through the folder structure, click the arrow next to a folder.
3. To delete a folder, select the folder.
4. In the Experiment Management pane, click **Delete**.
5. In the Delete Selected Folder dialog box, do one of the following:
   - Select **Delete Folder**.
     The application deletes the folder and moves its experiment result content (if any) to the Working List.
     –or–
   - Select **Delete Folder and Results from Master List**.
     The application deletes both the folder and its experiment result content from the Master List.

Deleting an experiment result from a folder

1. In the Experiment Management pane of the Load Results page, select the **Working List** to view its folder structure.
   When the Working List opens, only the Home folder appears in the pane.
2. To navigate through the folder structure, click the arrow next to a folder.
3. Select the folder that contains the result to delete.
   The right pane shows the contents of the selected folder.
4. To delete an experiment result, select the check box corresponding to the result.

   You can also select multiple check boxes to delete more than one result.
   In the command bar, click **Delete**, and do one of the following:
   - In the Delete Selected Results from Folder dialog box, select **Delete Results from Folder**.
     The application deletes the experiment result from the folder and moves it to the Working List.
   - In the Delete Selected Results from Folder dialog box, select **Delete Results from Folder and Master List**.
     The application deletes the experiment result from both the folder and the Master List.

Moving an experiment result to a folder

1. In the Experiment Management pane of the Load Results page, select the **Working List** to view its folder structure.
2. In the command bar, click **Delete**, and do one of the following:
   - From the Working List, select an experiment result
     - or -
   - Use the **CTRL** key to select multiple results, and then drag and drop to the destination folder of interest.

   The moved experimental result appears in the new destination folder.

Renaming a folder

1. In the Experiment Management pane of the Load Results page, select the **Working List** to view its folder structure.

   When the Working List opens, only the Home folder appears in the pane.
2. To navigate through the folder structure, click the arrow next to a folder.
3. To rename a folder, select the folder of interest, and then click **Edit**.

   Note: You can rename only a user-created folder.
4. In the dialog box, type the new name for the folder, then click **Edit** again to save the renamed folder.

33 Viewing the Process and Review page for Oligonucleotide Analysis

After the application completes the analysis of an experiment, you can open the results of that analysis and view the chromatograms, trend plots, sequence and fragment coverage maps, MS spectra, and Results table on the Process and Review page.

You can open the results from the Queue page or from the Load Results page.

The current experiment name appears in the upper right. If you load only one raw data file for the experiment, its name also appears below the experiment name; otherwise, "(multiple files)" appears.

1. On the Process and Review page, right-click the Chromatogram to select specific components from these four options:
   - Select Chromatogram
   - Reset Scale
   - Copy
   - Label
2. Select the Select Chromatogram to open the Select Chromatogram window.

Choose from the following display options:

- Selected Ion Chromatogram (SIC)
- Total Ion Chromatogram (TIC)
- Base Peak Chromatogram (BPC)
- Analog
- r-TIC (TIC after removal of background ions)
- r-BPC (BPC after removal of background ions)

3. Select the Reset Scale option to return to the original chromatogram results.
4. Select the Copy option to select and copy the chromatogram.
5. Select the Label option to open a window with the **Retention Time** and **Oligonucleotide.**

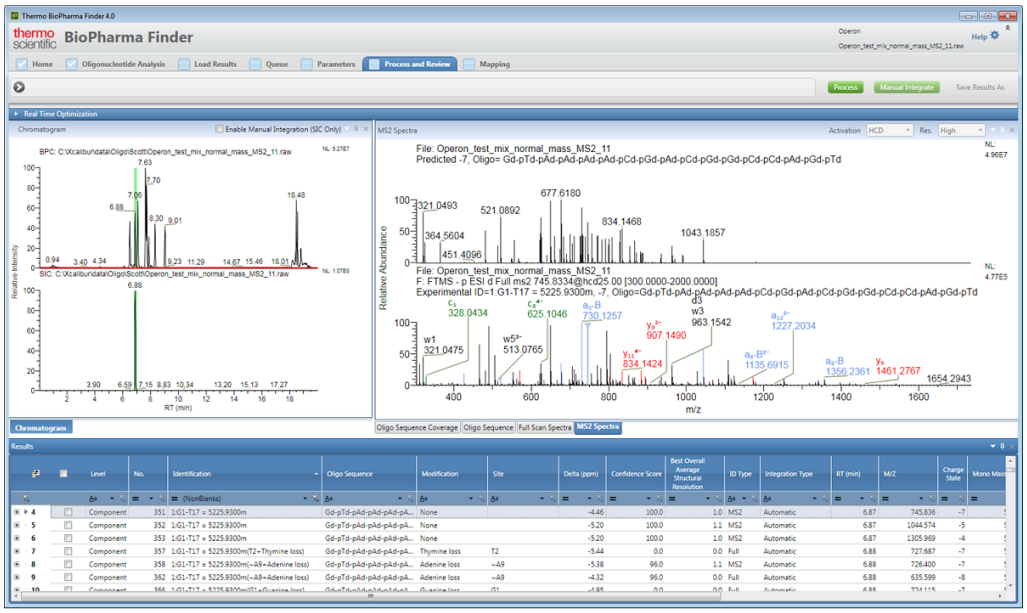


Figure 274 Process and Review page for Oligonucleotide Analysis

Process and Review page parameters for Oligonucleotide Analysis

The following table describes the types of information on the Process and Review page. To display the content of a pane that is not currently visible, click its subtab. You can adjust the size or location of the panes on this page as needed.

Table 105 Process and Review page parameters

Parameter	Description
Results table	Displays at the upper level the components and their peptide sequences, retention times, modifications, and detected masses, along with their confidence scores. At the lower level, the table displays all of the raw data files that are loaded for the experiment and their information.
Chromatogram pane	Displays the chromatograms for the component or raw data file that you select in the Results table.
Relative Intensity (*y* axis)	Displays the ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity.
RT (min) (*x* axis)	Displays the retention time of the spectrum, which is the time after injection at which a compound elutes. Retention time can also refer to the total time that the compound is retained on the chromatograph column.
Ratio value (*y* axis)	Displays the Avg MS Area value for a particular condition ÷ Avg MS Area value for the reference condition.
Ratio conditions (*x* axis)	Displays the names of the various conditions and the name of the reference condition.
Condition-Raw data file (*x* axis)	Displays the names of the various conditions and their assigned raw data files used in the experiment.
Oligonucleotide.Sequence Coverage pane	Displays the fragment coverage map, including the oligonucleotide sequence information and the color-coded fragment ions.
Oligo Sequence pane	Displays the oligonucleotide sequence assigned to the experiment, which shows the highlighted oligonucleotide sequence that you select from the Results table.
Full Scan Spectra™ pane	Displays the deconvoluted and full-scan spectra with mass and *m/z* information.
MS2 Spectra™ pane	Displays the predicted spectrum stacked on top of the experimental spectrum.
Relative Abundance (*y* axis)	Displays the ratio of the abundance of a specific peak to the abundance of the peak with the highest abundance.

Parameter	Description
m/z (*x* axis)	Displays the mass-to-charge ratio of ions formed from molecules. This ratio is the quantity formed by dividing the mass of an ion, in daltons, by the number of charges that the ion carries.
Real Time Optimization pane	Displays the same parameters as those on the Parameters > Component Detection and Identification pages, so that you can adjust these parameters and perform real-time optimization.

Note: If the *x*- or *y*-axis label in a pane is not visible, enlarge the pane or change the pane to a larger floating window.

Performing manual integration for Oligonucleotide Analysis

You can perform the manual integration on any raw file data using the **Manual Integrate** button in the Process and Review page for Oligonucleotide Analysis.

1. Open any oligonucleotide single raw data file experiment on the Process and Review page.
2. In the Chromatogram pane, select the check-box to enable the manual integration.
3. Re-define the RT start and stop for any SIC of the selected component. The **Manual Integrate** button becomes active now.
4. Click the **Manual Integrate** button to update the following information on the Process and Review page:
   - The new RT start and stop on the SIC in blue color.
   - The MS area for all raw data files at the raw data file level and the average MS area at the component level.
   - Ratio for the multiple file experiments.
   - All the plots that use the MS area, which includes Trend Ratio and Trend MS Area.
   - The Integration Type column in the results table is updated to Manual from the default value Automatic.
   - Coverage map and modification summary are also updated.

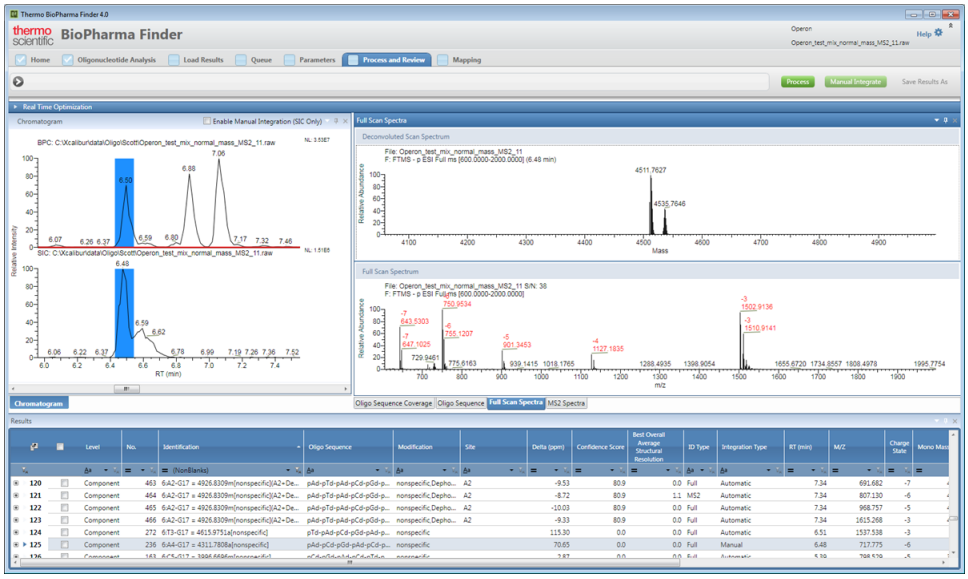


Figure 275 Manual Integration on Process and Review page

Process and Review page commands for Oligonucleotide Analysis

The following table describes the commands on the Process and Review page.

Table 106 Commands on the Process and Review page

Command	Description
Process	Processes the oligonucleotide experiment and deconvolves the source spectra with any or all of these parameters: modified oligonucleotide sequence, component detection, or identification. Also, displays the new results. Saves the latest results in a database after you process an experiment. Click this button to open a dialog box where you can enter a new experiment/method name or retain the same name to overwrite previously saved results/parameters in the current experiment with the new data. **Note:** To activate the Process button, you must modify the experiment parameters. Use only alphanumeric, space, underscore "_", and period "." characters, up to 50 maximum, in the experiment and method names. You cannot overwrite an experiment that is currently submitted to the run queue or is actively processing or canceling. You also cannot overwrite a default method.

Command	Description
Manual Integrate	Updates the following: • The new Real Time Optimization (RT) start and stop on the Selected Ion Chromatogram(SIC) in blue color • The MS Area for all raw data files at the raw file level and the average MS area at the component level • Ratio for multiple file experiments • All plots that use the MS area, including Trend Ratio and Trend MS Area • The Integration Type column in the results table is updated to Manual from the default Automatic. **Note:** The updates will be included in all exports, the coverage map, and the modification summary.
Save Results As	Saves the latest results in a database. Click this button to open a dialog box where you can enter a new experiment name or retain the same name to overwrite previously saved results in the current experiment with the new results. You can also enter a description for the experiment. This button is inactive if you modified any processing method parameter for real-time optimization. In this case, click **Process** to reprocess the experiment and reactivate this button. **Note:** Use only alphanumeric, space, underscore "_", and period "." characters, up to 50 maximum, in the experiment name. You cannot overwrite an experiment that is currently submitted to the run queue or is actively processing or canceling.

Viewing the Results table for Oligonucleotide Analysis

The Results table on the Process and Review page displays the results of a completed Oligonucleotide Analysis experiment, organized by the components at the upper level and by the raw data files at the lower level.

When you select the row for a component or one of the raw data files in the Results table, you can view related information in the various other panes of the Process and Review page.

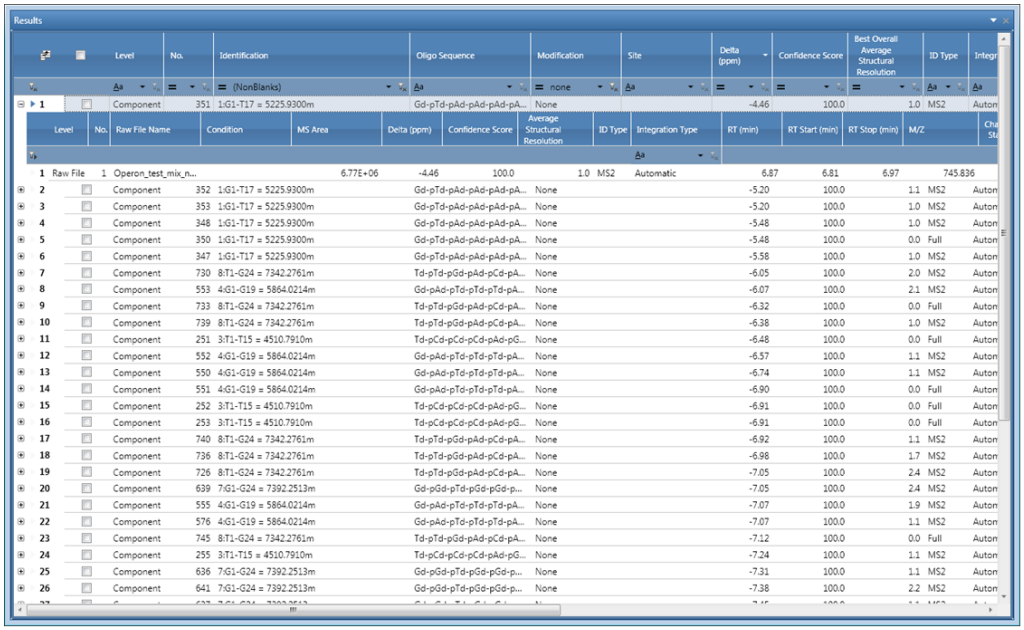


Figure 276 Results table on the Process and Review page

For more details, see the following topics:

- “Changing the reference condition” on page 246
- “Modification Summary page parameters” on page 637

Viewing the chromatograms for Oligonucleotide Analysis

The Chromatogram pane on the Process and Review page displays the Base Peak Chromatogram (BPC) plot at the top and the Selected Ion Chromatogram (SIC) plot at the bottom.

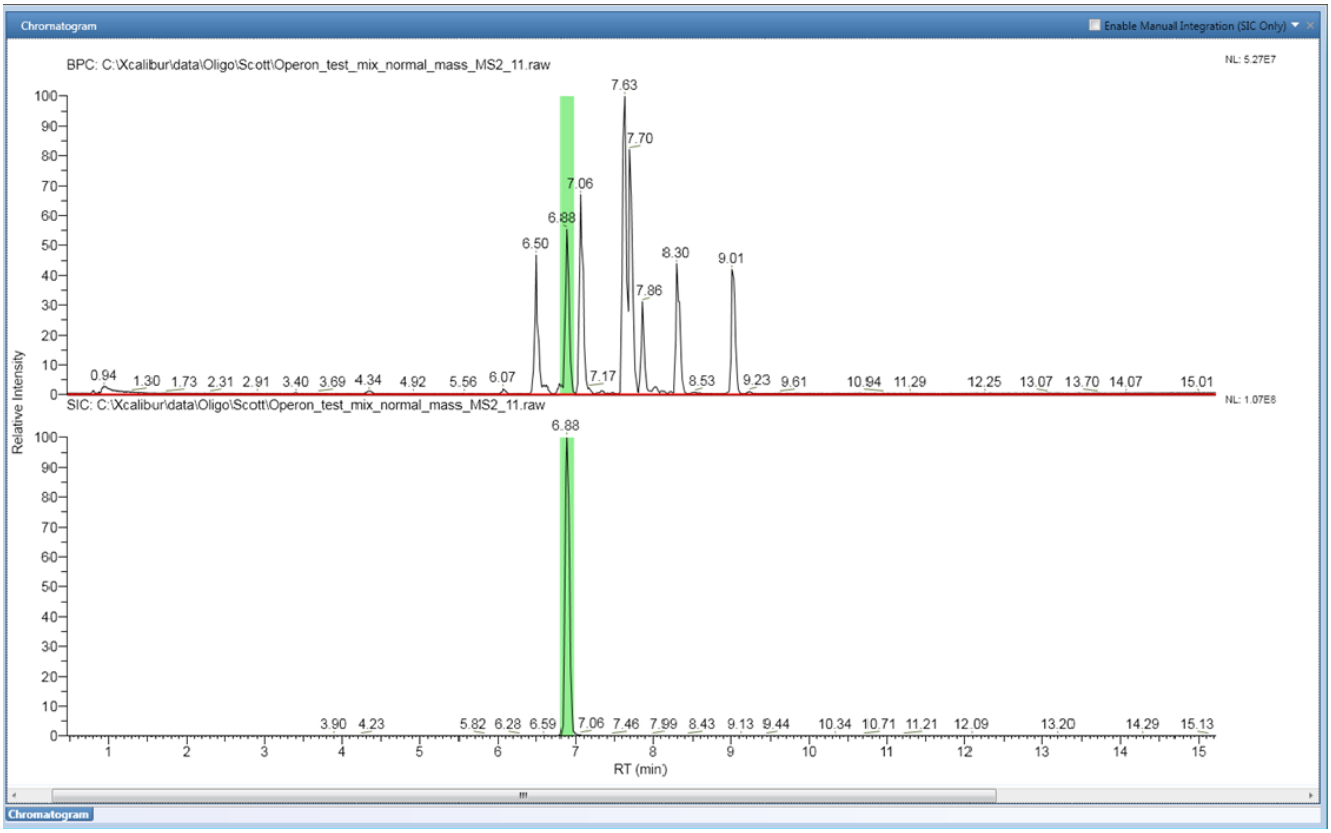


Figure 277 Chromatogram pane showing a BPC and an SIC

For more details, see the following topics:

- “Viewing the chromatograms for Oligonucleotide Analysis” on page 611
- “Chromatogram plot types” on page 612
- “Displaying multiple chromatogram plot types for one file” on page 613
- “Displaying same chromatogram plot type for multiple files” on page 615
- “Chromatogram pane commands” on page 632

Viewing the chromatograms

To view the chromatograms in the Chromatogram pane

1. Click the **Process and Review** tab.
2. Do one of the following:
   - Click the row of a component in the Results table.
     The plots in the Chromatogram pane show the peak information stored in the first raw data file in the list.

   –or–

   - Click the plus (+) sign to the left of a component row, and then click the row of one of the raw data files in the Results table.
     The plots show the peak information stored in the selected raw data file.

Chromatogram plot types

The base peak chromatogram (BPC) shows only the most intense peak in each MS spectrum at every point in the analysis. The BPC also displays a red horizontal line that represents the absolute MS signal threshold that you set as a component detection parameter in the method assigned to the experiment.

Note: BPCs for each raw data file often have a cleaner look and are therefore more informative than total ion current (TIC) chromatograms, which include noise and background signals. The BPC is usually best for smaller molecules where all of the signal exists in a single charge state.

When you click a scan on the BPC, the available spectral plots for the deconvoluted, full-scan, and experimental MS spectra show the information from the selected scan.

The SIC (also known as an extracted ion chromatogram [XIC]) plots the intensity of the signal observed at a chosen *m/z* as a function of retention time.

In the chromatogram plots, the *x* axis represents the retention time range and the *y* axis indicates the relative intensity. The plots label the peaks with their individual retention times (by default), and display the plot type and raw data file name at the top and the Normalized Largest (NL) intensity on the upper right side. They do not display peak labels, such as the scan number, or the header information.

For more details, see the following topics:

- “Editing Component Detection parameters for Oligonucleotide analysis” on page 577
- “Viewing the deconvoluted and full-scan MS spectra for Oligonucleotide Analysis” on page 622
- “Viewing the predicted and experimental MS2 spectra for Oligonucleotide Analysis” on page 622

Displaying multiple chromatogram plot types for one file

To display multiple chromatogram plot types for the same raw data file in the Chromatogram pane

1. Click the **Process and Review** tab.
2. Select a component in the Results table.
3. Right-click the Chromatogram pane and choose **Select Chromatogram.**
   The Select Chromatogram dialog box opens.

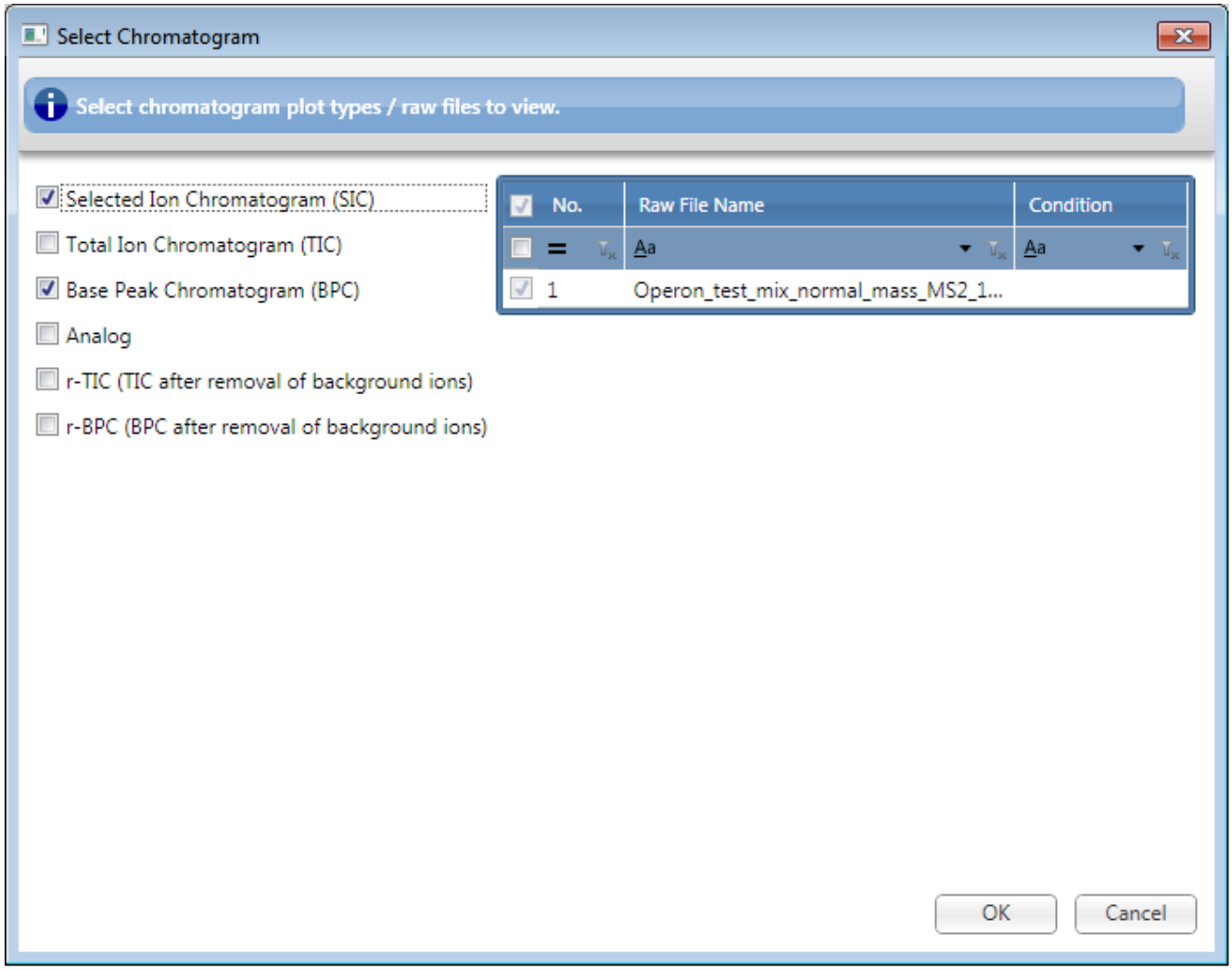


Figure 278 Select Chromatogram dialog box

4. Select the appropriate check boxes from the list on the left side of the dialog box.

   The available chromatogram plot types include the following:

   - Selected Ion Chromatogram (SIC)
   - Total Ion Chromatogram (TIC)
   - Base Peak Chromatogram (BPC)
   - Analog
   - r-TIC (TIC after the removal of background ions)
   - r-BPC (BPC after the removal of background ions)

   IMPORTANT! If you select only one plot type from the list on the left, you can select multiple raw data files from the list on the right. Conversely, to select multiple plot types, you must select only one raw data file.

   Your raw data file selections in the Select Chromatogram dialog box control the chromatogram plots. To return the control of the chromatogram plots to your selections in the Results table, you must clear all raw data file selections in this dialog box and then click **OK.**

5. Select the raw data file from the list on the right side of the dialog box.

   The following figure gives an example of one raw data file and multiple plots selected.

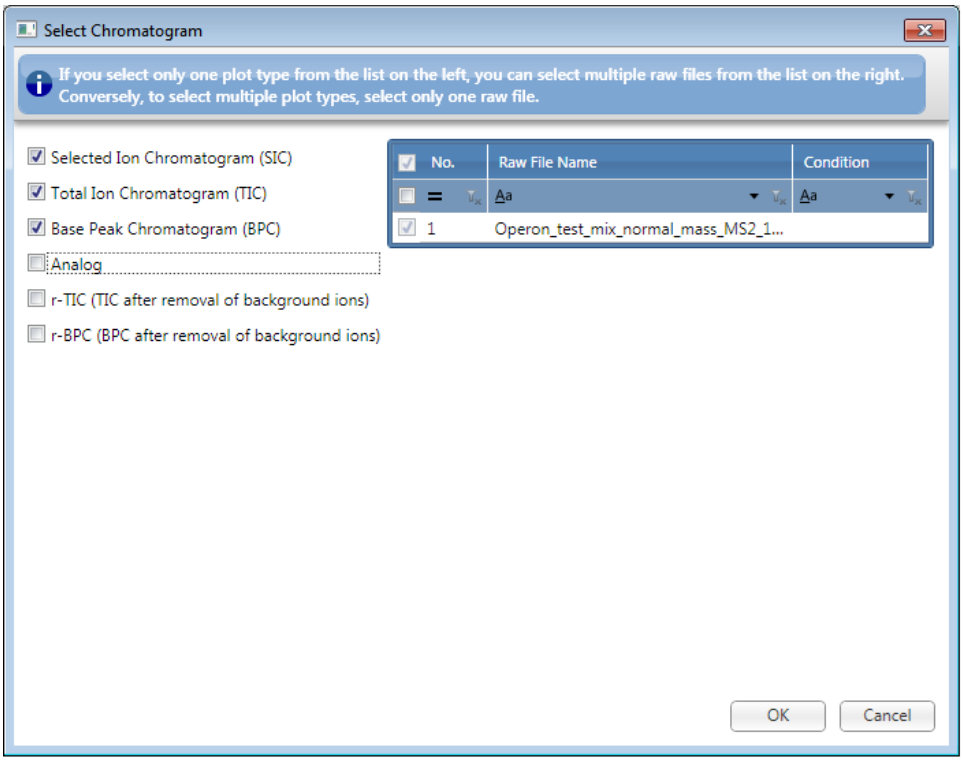


Figure 279 Selecting multiple plots for a raw data file

6. Click **OK** to update the chromatogram plots.

   The Chromatogram pane displays the selected plot types stacked on top of each other for the selected raw data file.

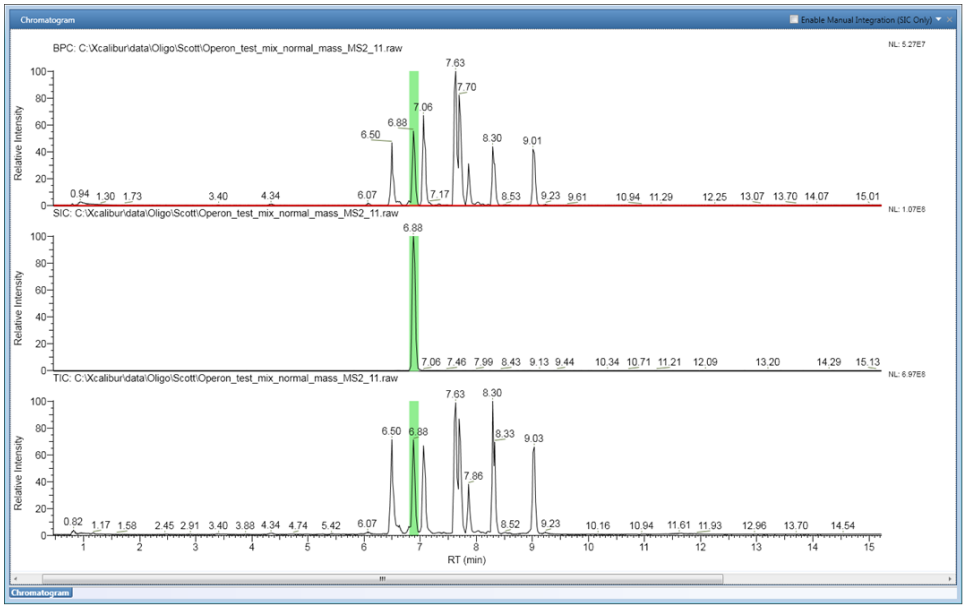


Figure 280 Different types of chromatograms displayed for a raw data file

Displaying same chromatogram plot type for multiple files

To display the same chromatogram plot type for multiple raw data files in the Chromatogram pane

1. Click the **Process and Review**
2. Select a component in the Results table.
3. Right-click the Chromatogram pane and choose **Select Chromatogram.**
4. In the Select Chromatogram dialog box, select the type of chromatogram to display from the list on the left side.
5. On the right side of the dialog box, select the raw data files whose chromatograms you want to display. To select all raw data files, select the **No.** check box in the column header.

   The following figure gives an example of one type of plot and selections of multiple raw data files.

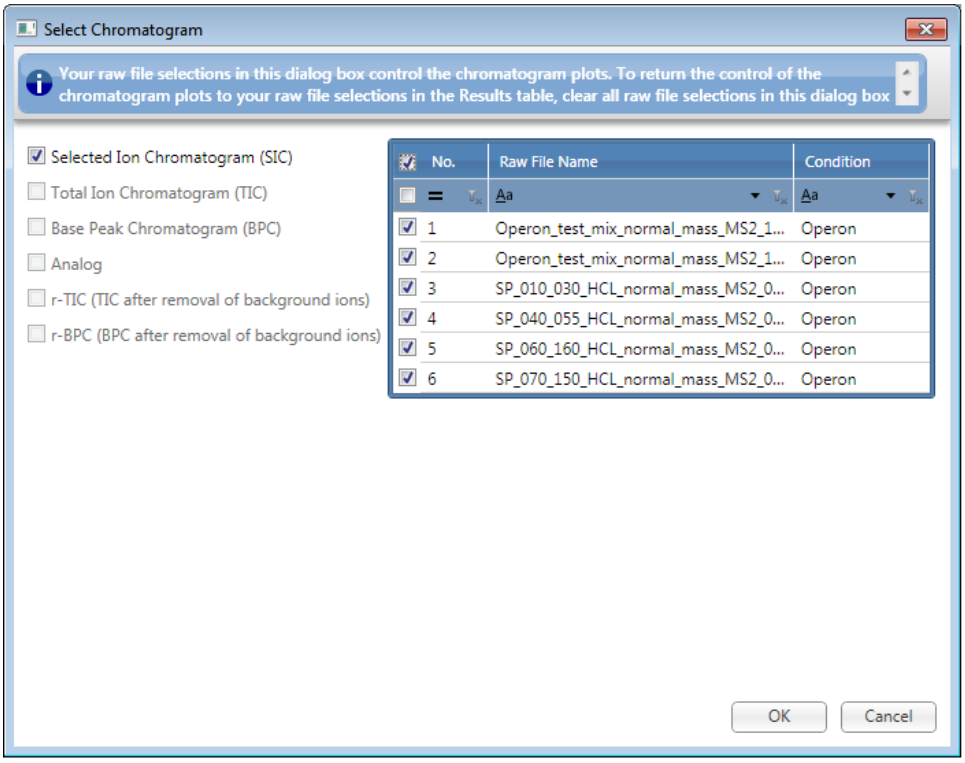


Figure 281 Selecting a plot for multiple raw data files

6. Click **OK** to update the chromatogram plots.

   The following figure shows a BPC chromatogram displayed for many different raw data files.

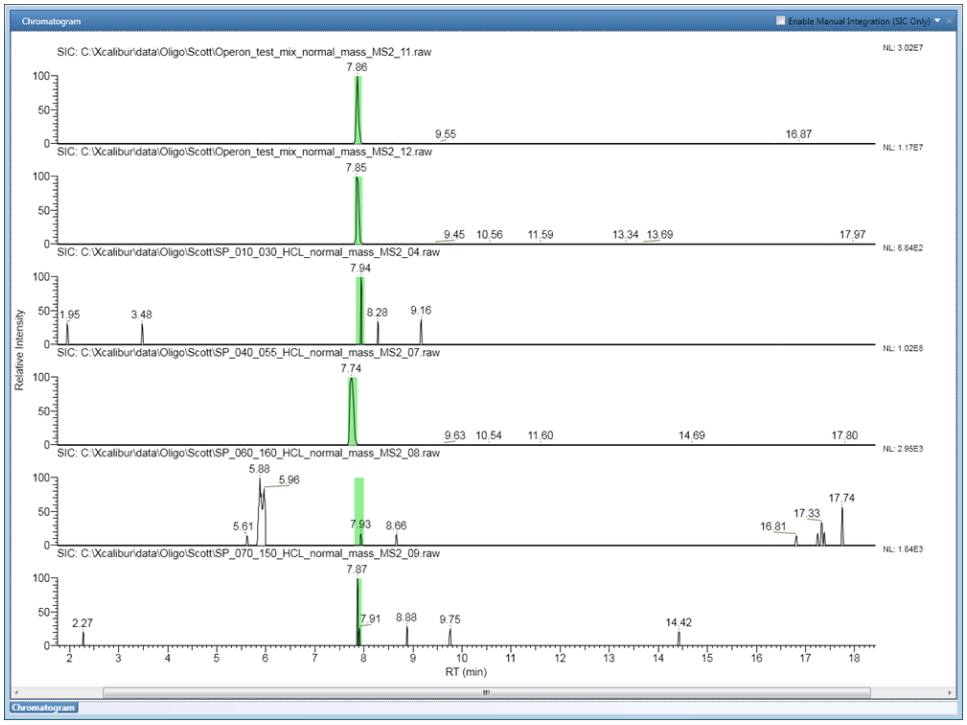


Figure 282 BPC chromatograms displayed for many different raw data files

Chromatogram pane commands

Right-clicking the Chromatogram pane on the Process and Review page opens a shortcut menu with the commands listed in the following table.

Table 107 Chromatogram pane shortcut menu

Command	Description
Select Chromatogram	Opens a dialog box to select which chromatogram or chromatograms to view.
Reset Scale	Restores the original scale that first appeared in the pane.
Copy	Copies the image in the pane to the Clipboard including all visible labeling and shading.
Label	Labels the peaks in the chromatograms with retention times or peptide information. **Note:** If the peptide is modified, an asterisk symbol, "*", appears at the end of the peptide label.

Viewing the Trend Ratio plot for Oligonucleotide Analysis

The Trend Ratio pane on the Process and Review page displays the bar plots for the ratios between various conditions and the reference condition for the component that you select in the Results table.

Note: The Trend Ratio pane is visible only for experiments with multiple raw data files.

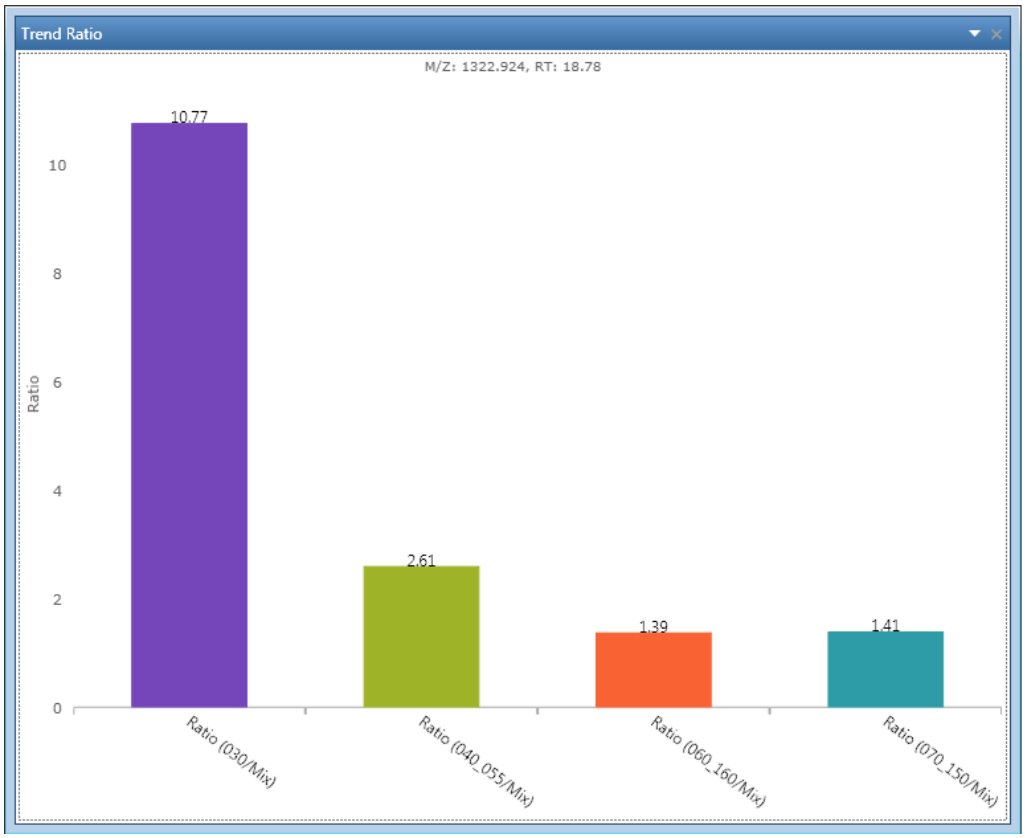


Figure 283 Trend Ratio pane

1. Click the **Process and Review** tab and then click the **Trend Ratio** subtab.
2. Select the row for one component (or a raw data file under a particular component) in the Results table.

   For the selected component, the plot shows the Avg MS Area value for a particular condition ÷ Avg MS Area value for the reference condition. The ratio values in the plot are from the Ratio (Condition/Reference Condition) columns in the Results table. The plot displays each ratio in a different color. The order of the conditions underneath the bars in the plot is the same order as the entered conditions on the Oligonucleotide Analysis page.

Viewing the trend MS area plot for Oligonucleotide Analysis

For the component that you select in the Results table, the Trend MS Area pane on the Process and Review page displays the bar plots for the MS Area values for each condition-raw data file pairing used in the experiment.

Note: The Trend MS Area pane is visible only for experiments with multiple raw data files.

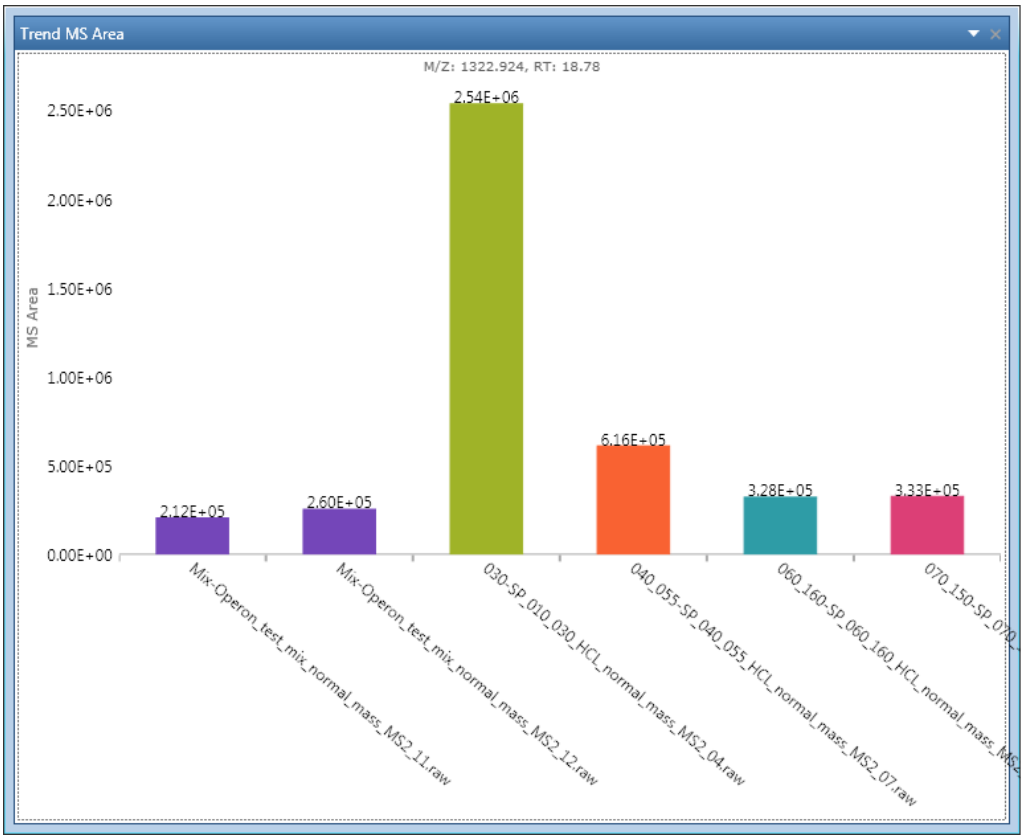


Figure 284 Trend MS Area pane

To view the trend MS area plot

1. Click the **Process and Review** tab and then click the **Trend MS Area** subtab.
2. Select the row for one component (or a raw data file under a particular component) in the Results table.

   The plot shows the MS Area value for the selected component and for each condition-raw data file pairing used in the experiment. The MS Area values in the plot are from the MS Area columns in the Results table. The plot displays each condition associated with a group of raw data files in a different color. The order of the conditions underneath the bars in the plot is the same order as the entered conditions on the Oligonucleotide Analysis page.

For more details, see the following topics:

- “Viewing the Results table for Oligonucleotide Analysis” on page 610
- “Starting a new oligonucleotide experiment” on page 571

Viewing the Fragment Coverage Map for Oligonucleotide Analysis

The Oligo Sequence Coverage pane on the Process and Review page displays the Fragment Coverage Map with color-coded oligonucleotide information.

Note: The Fragment Coverage Map displays MS2 spectra only if the first raw data file of the selected component is of type MS2 and the component has an oligonucleotide identification.

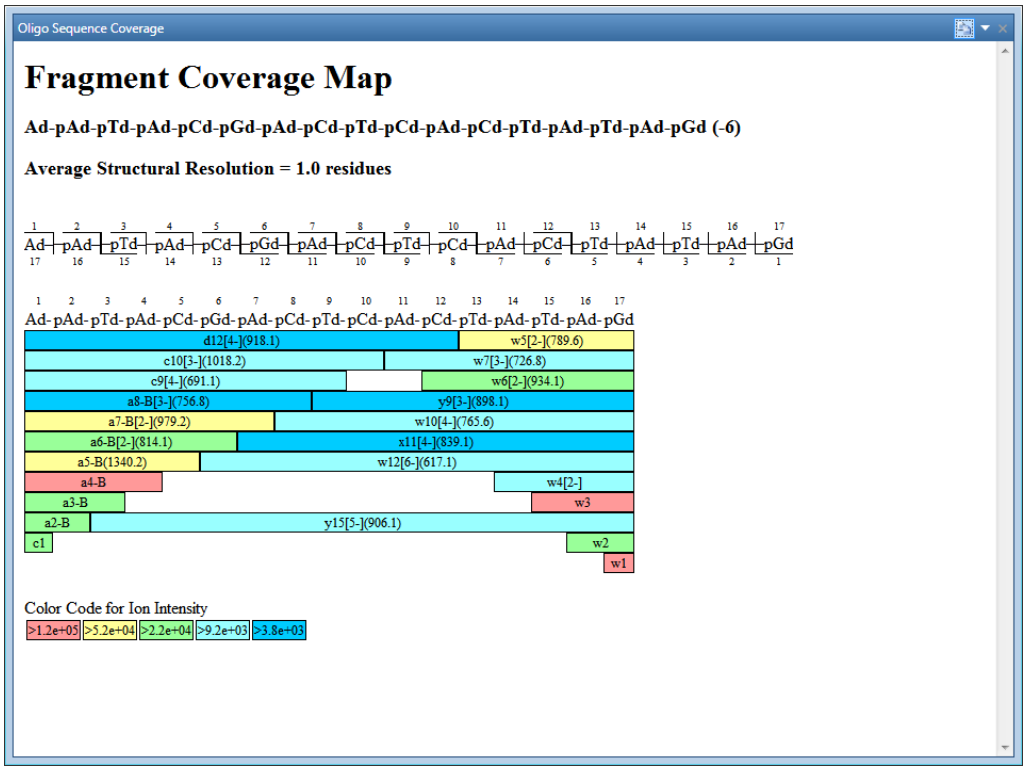


Figure 285 Fragment Coverage Map

Note: When the Oligonucleotide is large, the Copy and Paste functions do not capture the Fragment Coverage Map. Instead, use a screen capture application to capture the Fragment Coverage Map as a screenshot to paste into a Microsoft™ application.

For more details, see the following topics:

- “Viewing the Fragment Coverage Map for Oligonucleotide Analysis” on page 620
- “Using copy and paste functions” on page 666

Viewing the Oligonucleotide sequence for Oligonucleotide analysis

The Oligo Sequence pane on the Process and Review page displays the Oligonucleotide sequence assigned to the current experiment with the identified nucleotide sequence highlighted in yellow.

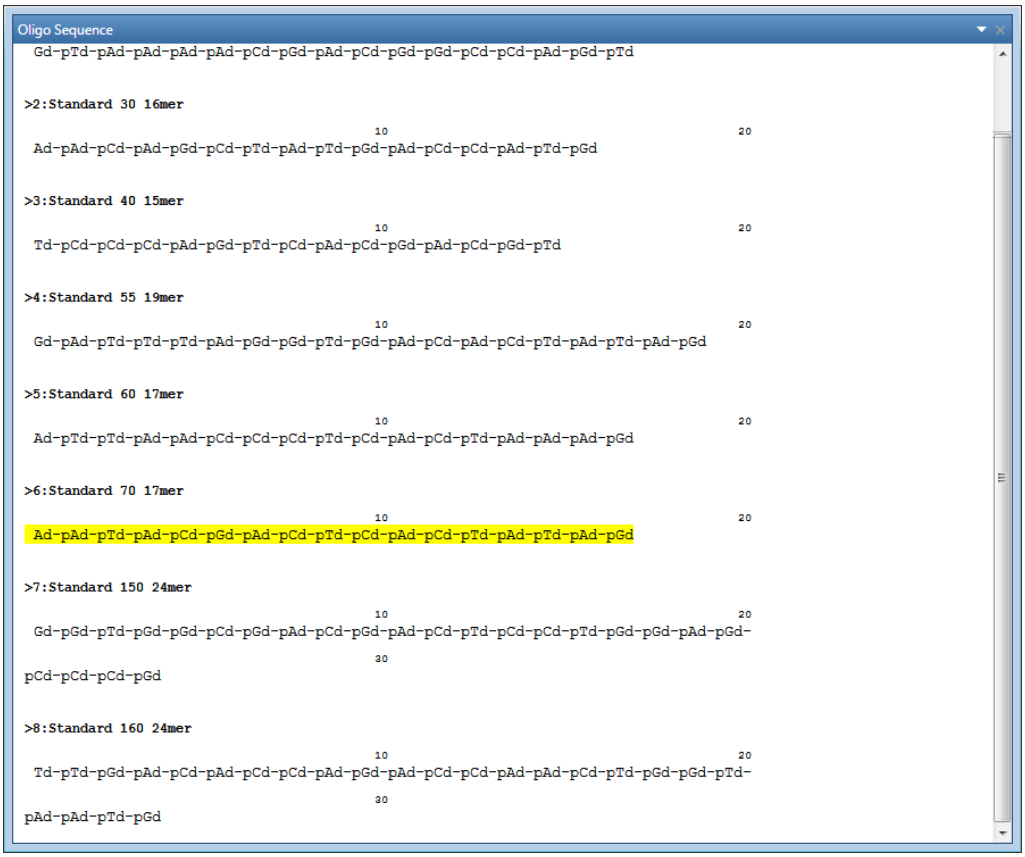


Figure 286 Oligonucleotide sequence with the selected identified oligonucleotide sequence highlighted in yellow

Viewing the deconvoluted and full-scan MS spectra for Oligonucleotide Analysis

The Full Scan Spectra pane on the Process and Review page displays the noise-reduced deconvoluted spectrum stacked above the full-scan spectrum.

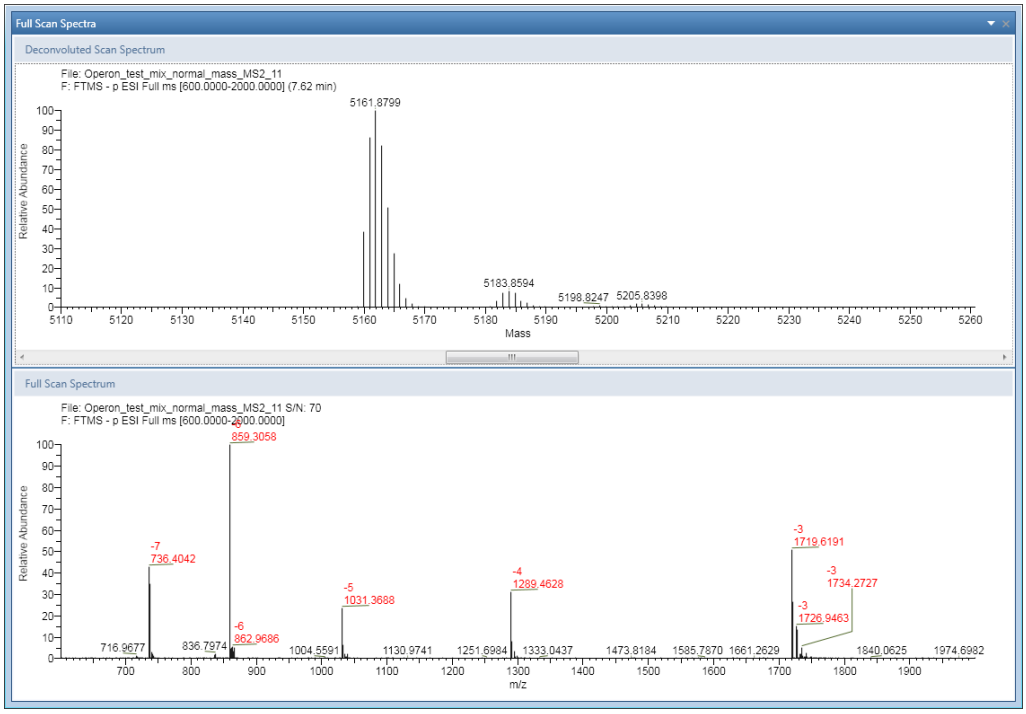


Figure 287 Deconvoluted and full-scan spectra

For more details, see the following topics:

- “Viewing the deconvoluted and full-scan MS spectra for Oligonucleotide Analysis” on page 622
- “Deconvoluted and Full-Scan MS Spectra display” on page 276
- “Full scan Spectra pane commands” on page 277

Viewing the predicted and experimental MS2 spectra for Oligonucleotide Analysis

The experimental data from an Oligonucleotide Analysis experiment might contain any of the following fragmentation and resolution types:

- Fragmentation types: CID or HCD.
- Resolution types: High or Low

The BioPharma Finder™ application determines the specific types present in the data from the scan headers in the raw data files that you load for the experiment. If the data contains multiple types, the application displays a separate spectral plot for every combination of fragmentation type and resolution type.

At the top of the MS2 Spectra pane on the Process and Review page, the Activation list provides the available fragmentation types (CID, HCD, ETD, or ECD), and the Res. list provides the available resolution types (High or Low).

From these lists, select which combination of fragmentation and resolution types you want to view in a spectral plot. Each plot displays the predicted spectrum stacked above the experimental spectrum for a particular combination if it is available.

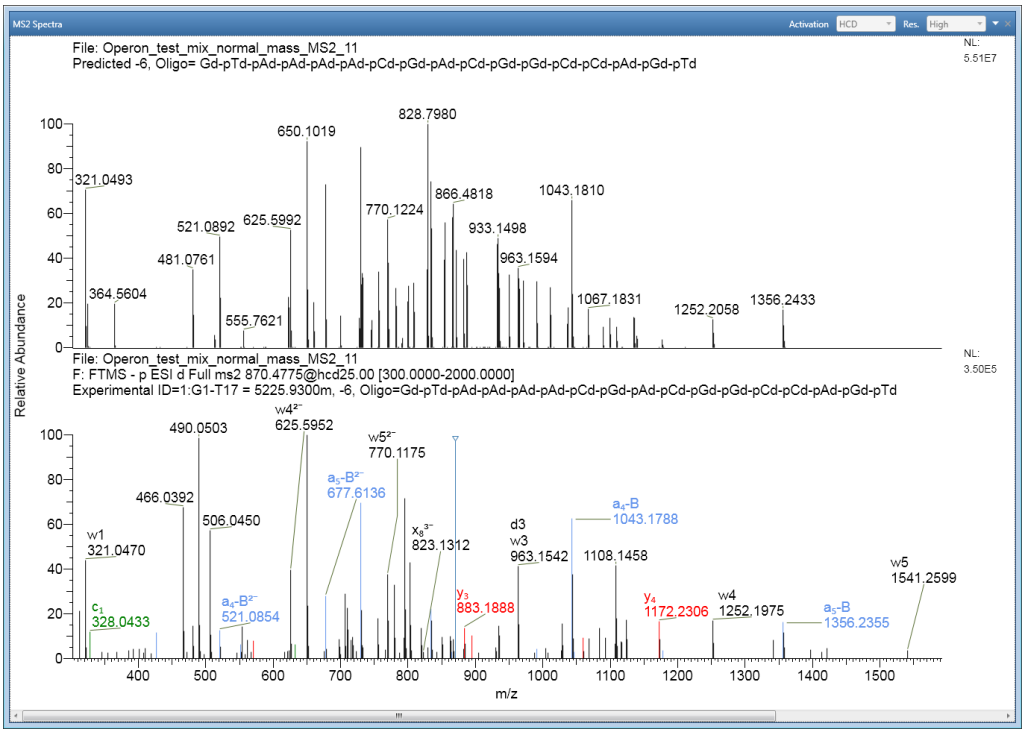


Figure 288 Predicted and experimental spectra on the MS2 Spectra pane

For more details, see the following topics:

- “Viewing the predicted and experimental MS2 spectra for Oligonucleotide Analysis” on page 622
- “Predicted and experimental MS2 Spectra display” on page 279
- “Predicted and experimental MS2 Spectra fragment ions” on page 280
- “MS2 Spectra pane commands” on page 282

34 Viewing the coverage page for oligonucleotide analysis

The Coverage page displays the results of the oligonucleotide analysis.

Viewing the Coverage page

After the BioPharma Finder™ application completes the analysis of an oligonucleotide experiment, you can open the results of that analysis on the Coverage page and view the color-coded chromatogram, the sequence coverage map, and the Results table. The Results table groups together the specific type of nucleotides and the unidentified components.

To view the results on the Coverage page

1. Open the results from the Queue page or from the Load Results page.

   The application opens to the Process and Review page. The current experiment name appears in the upper right corner of the page. When you load only one raw data file for the experiment, its name also appears in this area; otherwise“(multiple files)” appears.

2. Click the **Mapping** tab.

   By default, the application opens the Coverage page under the Mapping tab, which displays the color-coded chromatogram in the Chromatogram pane, the Oligonucleotide Sequence Coverage Map in the Oligonucleotide Coverage Map pane, and the Results table.

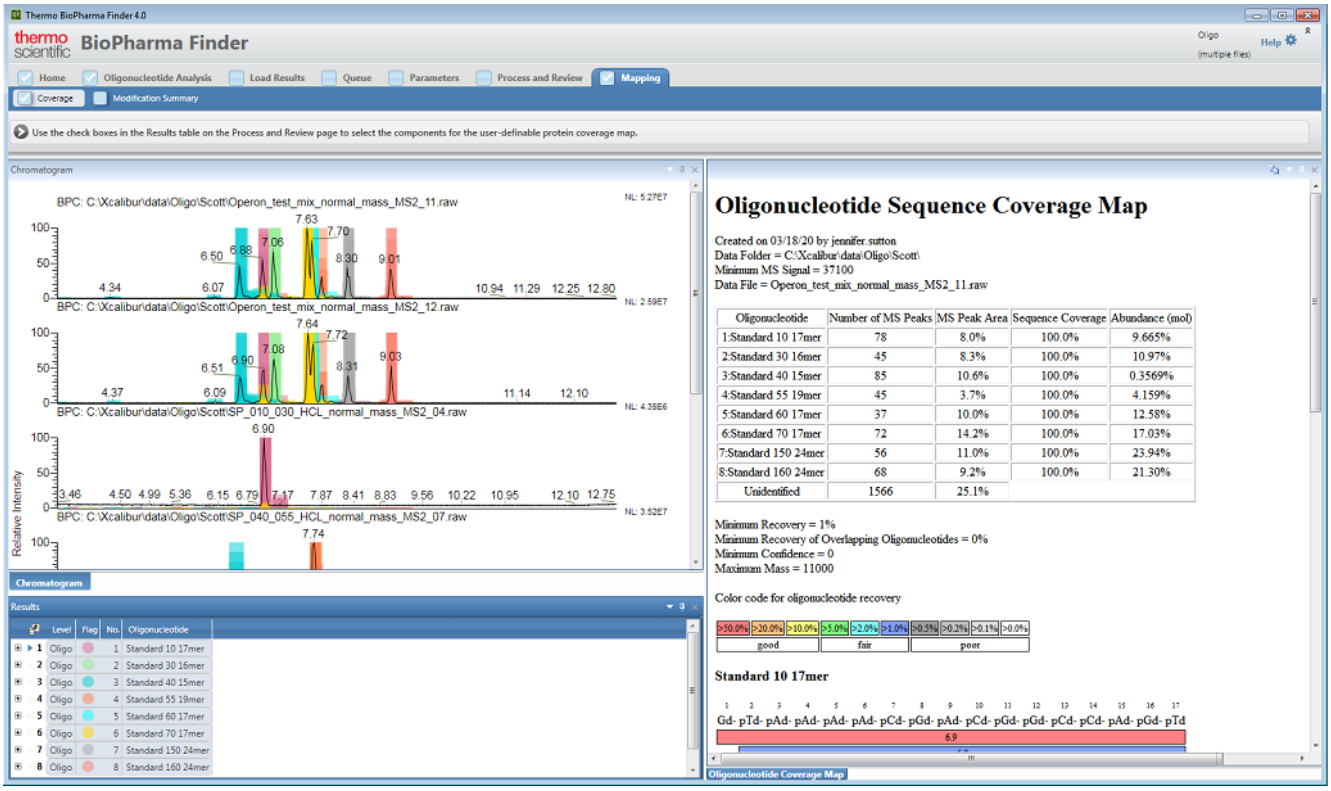


Figure 289 Coverage page

For more details, see the following topics:

- “Opening the results from the queue page” on page 591
- “Opening the results from the Load Results page” on page 592
- “Viewing the Coverage results table” on page 626

Viewing the Coverage results table

The Results table on the Coverage page displays the color-coded results of the analysis, organized by the types of proteins at the top level. It then displays oligonucleotide coverage information specific to each raw data file at the next level. If the analysis identified an oligonucleotide at the top level, then the table displays the component-specific information grouped by a particular raw data file at the lowest level.

When you select a protein, raw data file, or one of its components in the Results table, you can view related information in the Chromatogram and Oligonucleotide Coverage Map panes of the Coverage page.

Level 1 in the Results table includes the following columns:

Level- oligo

Flag

No.

Oligonucleotide

Level 2 in the Results table is displayed when clicking in the first column, which is Level (Oligo). It includes the following columns:

Level- raw file

No.

Raw File Name

Condition

Sequence Coverage

Number of MS Peaks

MS Peak Area

Abundance (mol)

Level 3 in the Results table is displayed when clicking the plus sign next to the Raw File in column 1 of Level 2. It includes the following columns:

Level (Component)

No.

Identification

Sequence

Modification

Site

Delta (ppm)

Confidence Score

ID Type

RT (min)

M/Z

Charge State

Mono Mass Exp.

Avg Mass Exp.

Theoretical Mass

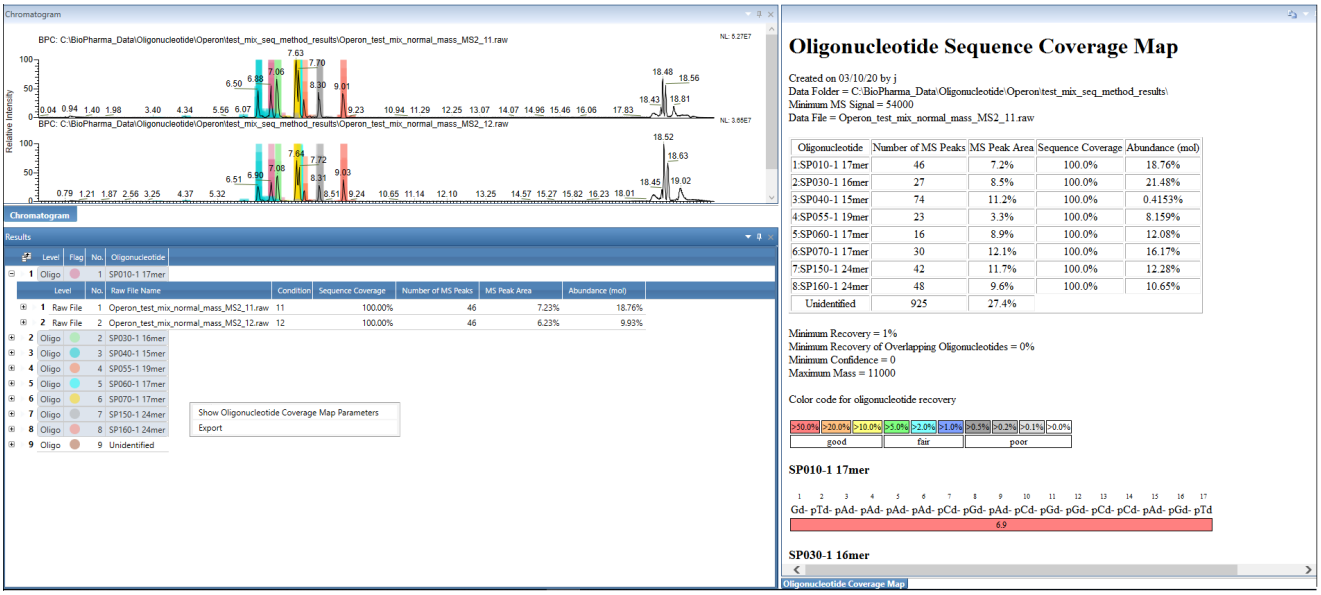
Oligonucleotide

MS Area

MS Height

You can right-click on any row to see the option to either **Show Oligonucloetide Coverage Map Parameters** or **Export.**

Note: Click **Export** to display the Windows browse option.
You can now browse to the desired folder location to save file extension as .xls by default.



Oligonucleotide	Number of MS Peaks	MS Peak Area	Sequence Coverage	Abundance (mol)
1:SP010-1 17mer	46	7.2%	100.0%	18.76%
2:SP030-1 16mer	27	8.5%	100.0%	21.48%
3:SP040-1 15mer	74	11.2%	100.0%	0.4153%
4:SP055-1 19mer	23	3.3%	100.0%	8.159%
5:SP060-1 17mer	16	8.9%	100.0%	12.08%
6:SP070-1 17mer	30	12.1%	100.0%	16.17%
7:SP150-1 24mer	42	11.7%	100.0%	12.28%
8:SP160-1 24mer	48	9.6%	100.0%	10.65%
Unidentified	925	27.4%		

Figure 290 Results table on the Coverage page

Note: Values displayed in the Results table under the Oligonucleotide level in the Raw File Name level match the values displayed in the Oligonucleotide Sequence Coverage Map table on the right.

For more details, see the following topic:

- Chapter 33, “Viewing the Process and Review page for Oligonucleotide Analysis”

Viewing the Chromatogram on the Coverage page

The Chromatogram pane on the Coverage page displays the base peak chromatogram (BPC) with color-coded oligonucleotide coverage. The various shading colors correspond to the types of identified nucleotides shown at the top level in the Results table.

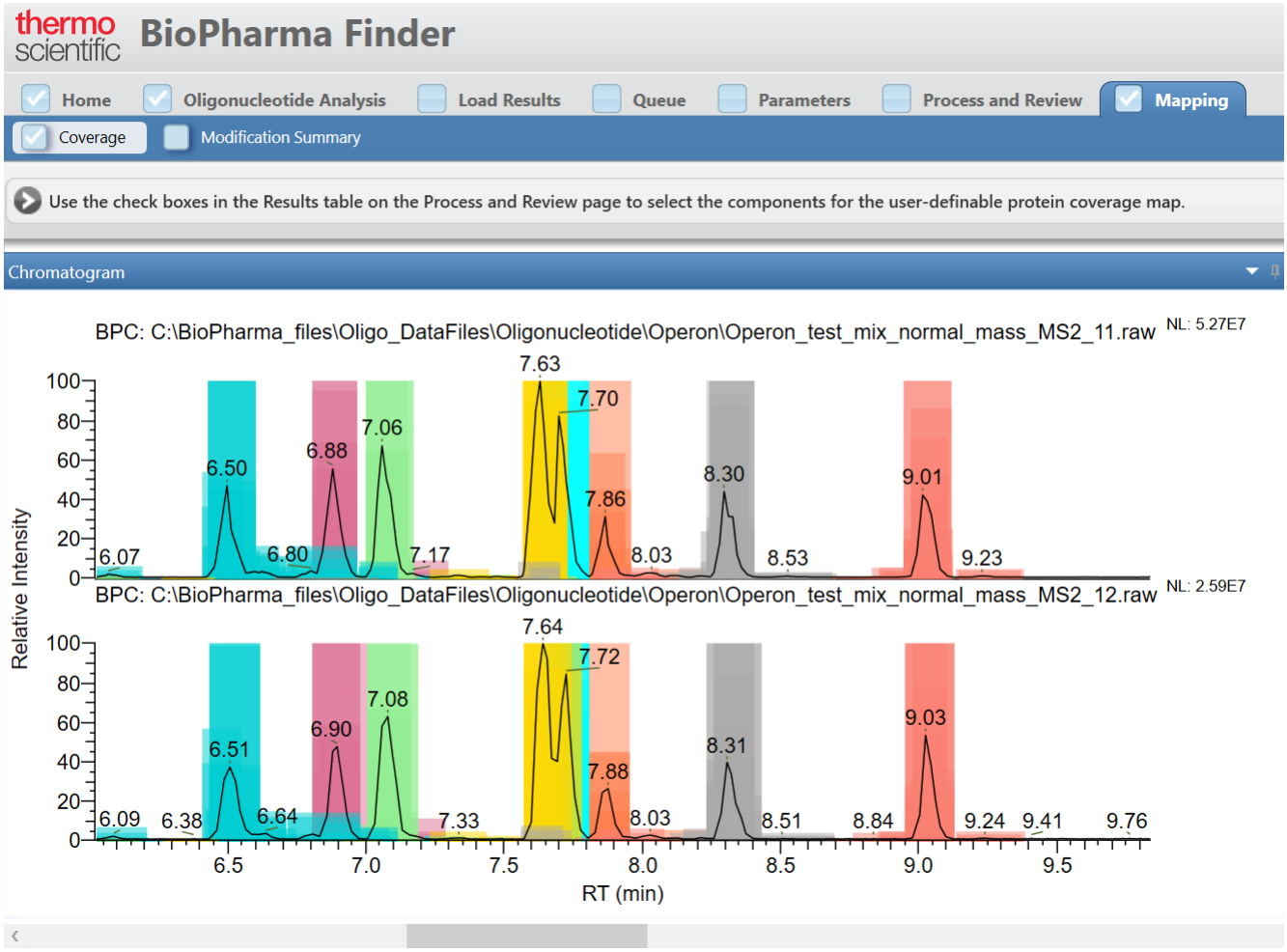


Figure 291 Chromatogram pane zoomed in showing color-coded sequence coverage

The shading is semi-transparent so that you can view coeluting peaks on top of each other. The height of the shaded block indicates the intensity (peak height) of a component. You can view this height value in the MS Height column at the component level in the Results table.

The chromatogram shows you at a glance which sequences were identified for each chain and which remained unidentified.

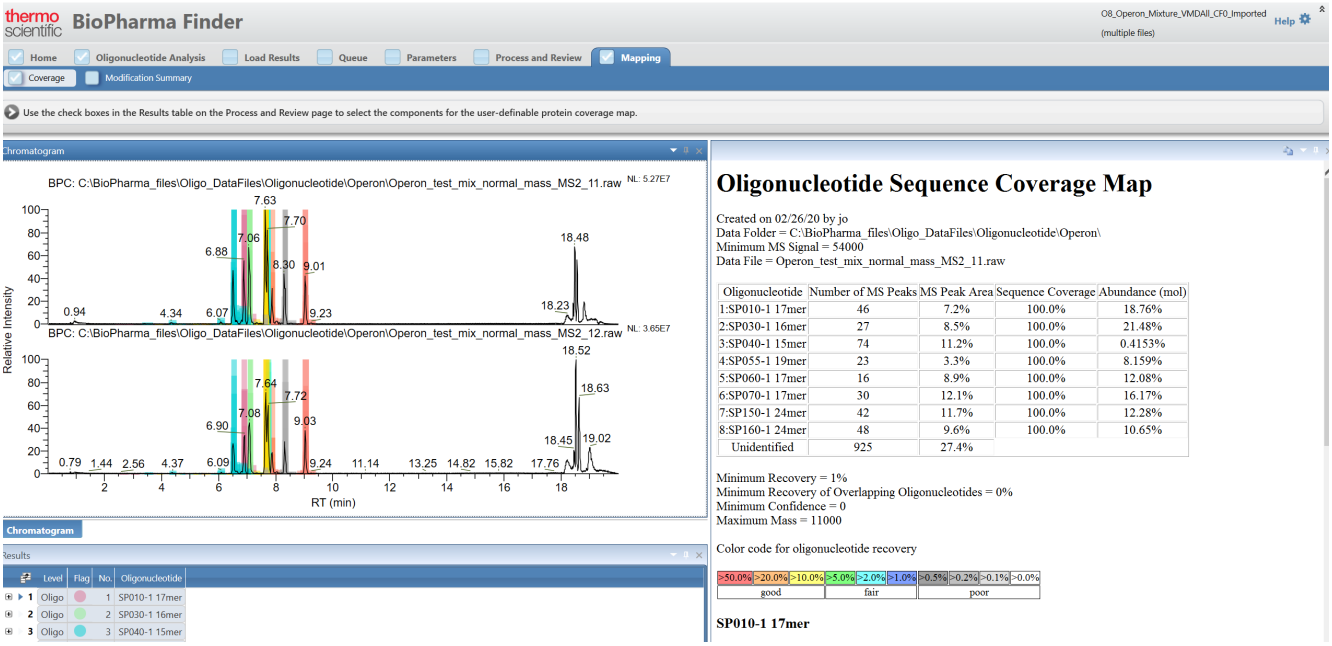


Figure 292 The Chromatogram pane

Viewing the color-coded chromatogram

To view the results in the Chromatogram pane

1. Click the **Mapping** tab and then click the **Coverage** subtab.
2. Do one of the following:
   - Click the row of a nucleotide in the Results table.

     If you click the nucleotide level, the application uses the first raw data file to shade a nucleotide on identified peaks.

     If you click the raw data file level, the application uses a specific raw data file.

     The BPC shows the shaded peak height for all of the components grouped under a particular nucleotide type if one is identified. If some component peaks are not identified, the application groups them in the "Unidentified" nucleotide type.

     Each selected nucleotide row corresponds to only one shading color on the chromatogram. Use the CTRL or SHIFT key to select multiple rows to see the various colors for the multiple nucleotide types.
   - Click the plus icon, ⊞, at the left side of a nucleotide row, and then click the row of one of the related raw data files in the Results table.

     The BPC shows the shaded peak height for each of the components in a particular raw data file, grouped under a particular oligonucleotide type and shaded by the color assigned to that oligonucleotide type.

–or–

- Click the plus icon, ⊞, at the left side of a raw data file row (if a nucleotide is identified at the top level), and then click the row of one of its components in the Results table.

  The BPC shows the same information as when you select the row for the related raw data file. In addition, the application also displays the SIC of the selected component below the BPC in the Chromatogram pane.

In the chromatogram plots, the *x* axis represents the retention time range and the *y* axis indicates the relative intensity. The plots label the peaks with their individual retention times (by default) and display the file name at the top and the Normalized Largest (NL) intensity at the top right. They do not display peak labels, such as the scan number, or the header information.

Modifying the Shading Parameters settings

To modify the shading parameter settings

1. Click the **Mapping** tab and then click the **Coverage** subtab.
2. Right-click the Chromatogram pane and choose **Shading Parameters.**

   The Shading Parameters dialog box opens.

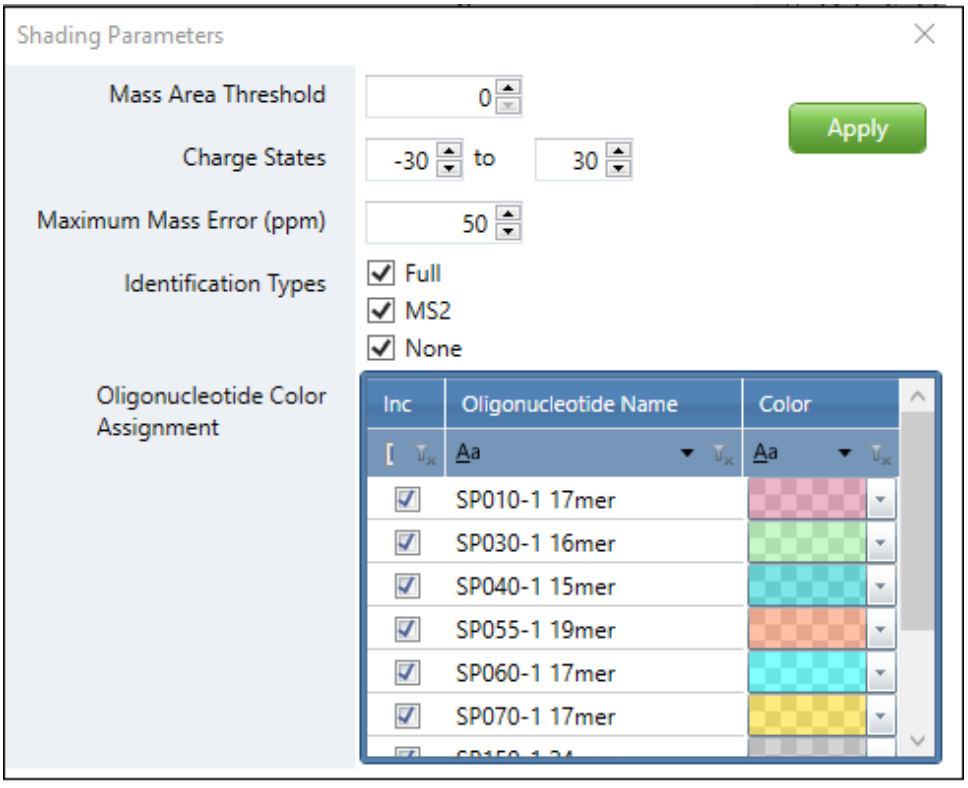


Figure 293 Shading Parameters dialog box

3. Enter the following settings:
   - Mass Area Threshold: Type a number for the minimum area threshold.

     The application does not display in the chromatogram any signals with areas below this threshold.
   - Charge States: Type the minimum and maximum values for the range of charge states to display in the chromatogram.
   - Maximum Mass Error (ppm): Type a value in ppm for the maximum mass threshold.

     The application does not display in the chromatogram any signals with a delta mass above this threshold.
   - Identification Types: Select the check boxes to view (in the chromatogram and in the component level of the Results table) only the components identified by the selected identification types. Select only the None check box to view only unidentified components.
   - Oligonucleotide Color Assignment table: The table contains all of the oligonnucleotide types from the Results table.
     - Select or clear this check box for a particular oligonucleotide row if you want to show or hide both the same oligonucleotide row in the Results table and its corresponding shading in the Chromatogram pane.

       You can select or clear multiple check boxes as needed.
     - (Not editable) Oligonucleotide Name: The type of oligonucleotide, as listed in the Results table.
     - Color: Click to select a shading color for a particular oligonucleotide type.

   Note: Your oligonucleotide row selections in the Results table do not affect the selections in the Oligonucleotide Color Assignment table.

   If you define specific components in the Results table on the Process and Review page to update the sequence coverage map, those selections override the previous settings in the Oligonucleotide Color Assignment table when you go back to the Coverage page. On the Coverage page, future filters in the Oligonucleotide Color Assignment table apply until you redefine the components again.

4. Click **Apply.**

   The chromatogram updates based on your settings. The shading parameters affect the shading in the chromatogram and the records in the Oligonucleotide Results table. They do not affect the sequence coverage map.

Chromatogram pane commands

Right-clicking the Chromatogram pane on the Coverage page opens a shortcut menu with the commands listed in the following table.

Table 108 Chromatogram pane shortcut menu

Command	Description
Shading Parameters	Opens a dialog box in which you can modify the chromatogram parameters, such as Mass area threshold, Charge States, Maximum Error (ppm), Identification types (default all checked), Oligonucleotide Color Assignment, and Shading Colors.
Reset Scale	Restores the original full-scale chromatogram.
Copy	Copies the image in the pane to the Clipboard.
Label	Labels the peaks in the chromatograms with retention times or oligonucleotide identification information.

For more details, see the following topics:

- “Modifying the Shading Parameters settings” on page 630

Viewing the Coverage Map

The upper portion of the Oligonucleotide Sequence Coverage Map pane on the Coverage page displays a table of oligonucleotides with coverage and abundance values. This table displays some of the same columns as the Results table of the Coverage page at the raw data file level. Below this table are several values from the Coverage Map Options dialog box.

The lower portion of the pane displays the sequence coverage map with color-coded nucleotide information for a selected nucleotide type, raw data file, or component in the Results table. Scroll down as necessary to see the rest of the color-coded nucleotide information. The color coding indicates the MS signal intensity of the predicted fragments. The sequence coverage map changes according to the raw data file that you select.

In the top-right portion of the pane, right-click the Copy icon to display the following options:

- Floating
- Dockable
- Tabbed Document
- Auto Hide

- Show Oligonucleotide Coverage Parameters

  When the Show Oligonucleotide Coverage Parameters option is selected, the Coverage Map Options are displayed. You can select from the following:

  Maximum Oligonucleotide Mass

  Default: 11 000

  Range: 100 and 100 000

  No decimals

  Minimum Confidence

  Default: 0.8

  Range: 0 to 1

  Residue per Row

  Default: 50

  Range: 2 to 500

  Minimum Recovery

  Default: 0.01

  Range: 0 to 1

  Minimum Relative Recovery of Overlapping Oligos

  Default: 0.0

  Range: 0 to 1

The application labels each oligonucleotide in the map with its retention time and color-codes them by intensity with red, orange, yellow, green, cyan, blue, shades of gray, and white colors, showing red as most intense and white as least intense.

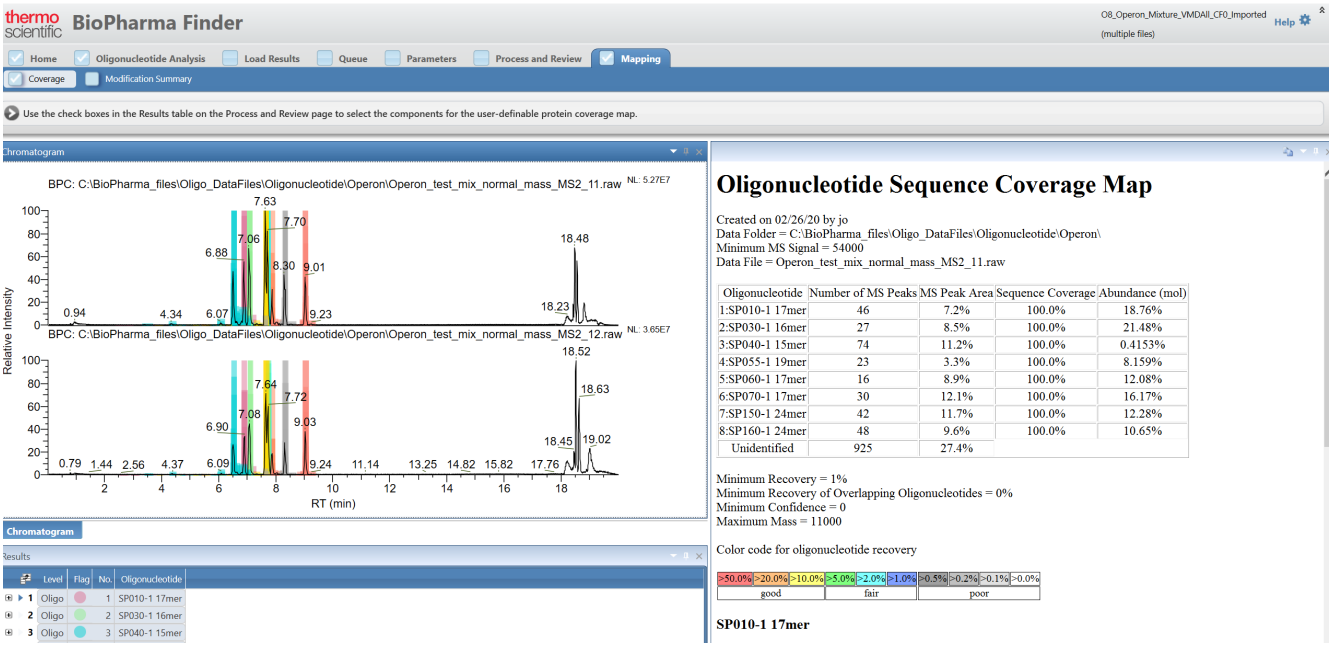


Figure 294 Oligonucleotide Sequence Coverage Map with a color-coded oligonucleotide sequence

For more details, see the following topic:

- “Viewing the Coverage results table” on page 626

Coverage page parameters

The following table describes the types of information on the Coverage page.

Table 109 Coverage page parameters

Parameter	Description
Oligonucleotide Coverage Map	At the top level, displays the identified and unidentified nucleotides, along with their color-coded flags. At the next level, the table displays the raw data files. At the lowest level, it displays the component information.
Chromatogram pane	Displays the chromatogram related to the row that you select in the Results table.
Relative Intensity (*y* axis)	Displays the ratio of each peak intensity relative to the maximum intensity in the spectrum.
RT (min) (*x* axis)	Displays the retention time—that is, the time after injection at which a compound elutes.
Sequence Coverage Map pane	Displays the sequence coverage map including the oligonucleotides, sequence coverage information, and the color-coded oligonucleotides.

For more details, see the following topics:

- “Viewing the Coverage results table” on page 626
- “Viewing the Chromatogram on the Coverage page” on page 628
- “Viewing the Coverage Map” on page 632

35 Viewing the Modification Summary page

After the BioPharma Finder™ application completes the analysis of an oligonucleotide experiment, open the results of that analysis on the Modification Summary page and view the modification summary report that shows the recovery status and abundance of all detected modifications.

To view the results on the Modification Summary page

1. Open the results from the Queue page or from the Load Results page.

   Process and Review page opens. The current experiment name appears in the upper right corner of the page. If you load only one raw data file for the experiment, its name also appears in this area; otherwise, multiple files appear.

2. Click the **Mapping** tab and then click the **Modification Summary** subtab.

   The Modification Summary page opens.

   On this page, the left pane contains the Modification results and the component table. The right pane contains component-specific information that is interactive with the component table. The right tab information reflects the currently selected component.

Table 110 Details of panes on the Modification Summary page

Left side of Modification Summary page	Details
Modification Results	The modification summary
Components	This is an interactive table. The right pane information updates based on the selection. By default the first component in the table is selected.
Right side of Modification Summary page	**Details**

Left side of Modification Summary page	Details
Modification Plot	The plot of abundance percentages for the selected modifications in the Modification Results pane, grouped by conditions, and the names of the loaded raw data files. **Note:** By default the first row in the modification summary is selected, and the selected row determines what is shown in the Modification Plot. By default the first row in the component table is selected, and this controls everything shown on the right tab. To view any of the following panes, click the tab on the right side of the page. Use the pin icons to turn off or turn on the auto-hiding feature.
Chromatogram	The chromatograms
Trend Ratio	Bar plot of ratio values
Visible only for experiments with multiple raw data files:	
Trend MS Area	Bar plot of MS area values per raw files
Oligo Sequence Coverage	The fragment coverage map
Oligo Sequence	The nucleotide sequence
Full Scan Spectra	The deconvoluted and full-scan spectra
MS2 Spectra	The predicted and experimental spectra **Note:** Everything described in the pane is for the selected component. When you select a different component, the information is refreshed.

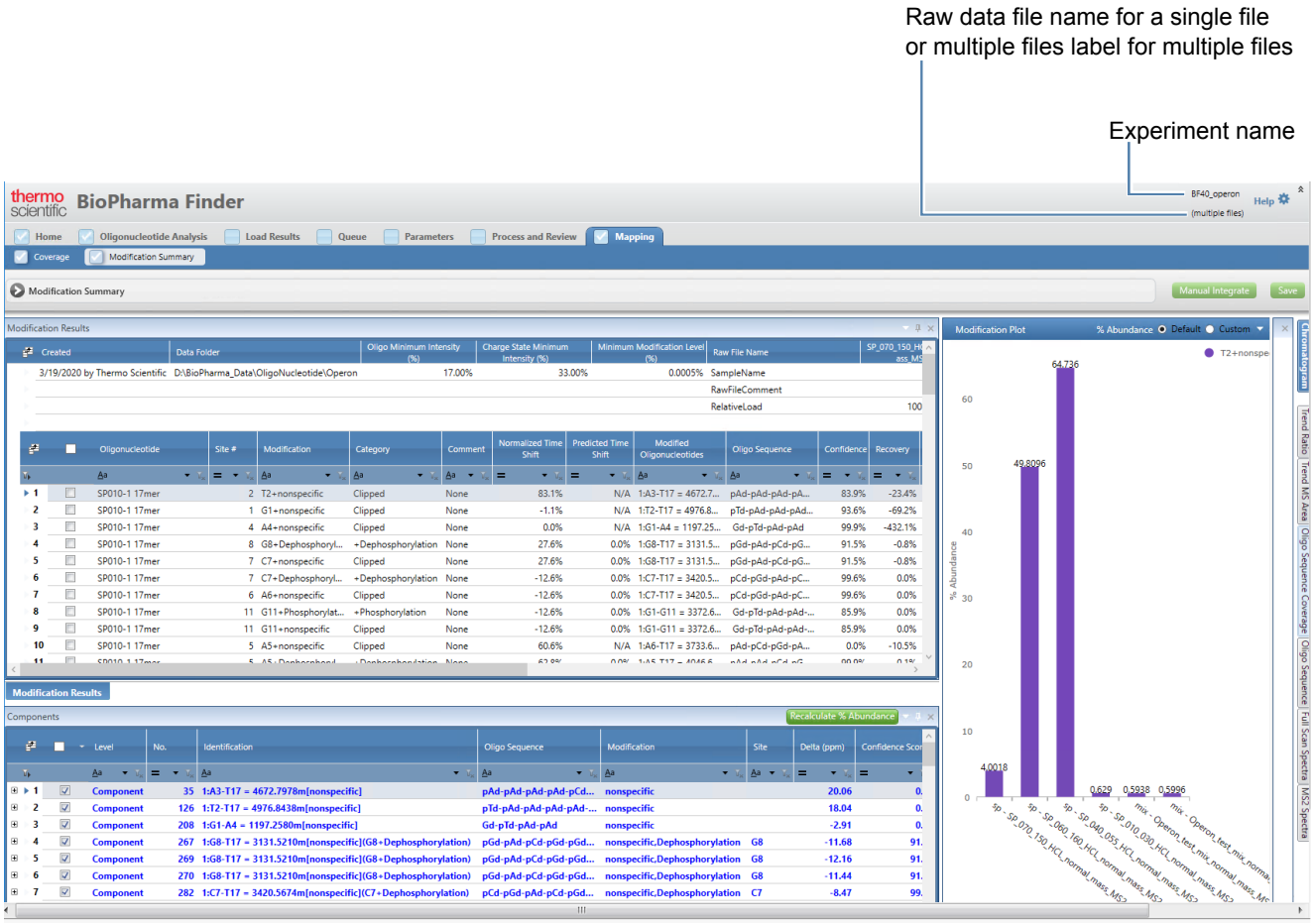


Figure 295 Modification Summary page

Note: You can pin the right tab control by selecting the pin icon, . This way, both modification plot and right tab control can be active at the same time.

Related Topics

Modification Summary page parameters

The following table describes the types of information available on the Modification Summary page. To display the content of a pane that is not currently visible, click its subtab.

Table 111 Modification Summary page parameters (Sheet 1 of 2)

Parameter	Description
Modification Results table	Displays the modification summary results in a table format
Components pane	Displays component-related information in a table format.

Parameter	Description
Modification Plot pane	Displays the plot of the modification abundance percentages for the selected modifications in the Modification Results pane, grouped by conditions and raw data file names.
% Abundance (*y* axis)	Displays the percentages of abundance for the selected modifications in the Modification Results pane.
Condition-Raw data file (*x* axis)	Displays the names of all conditions and their corresponding raw data files loaded for the experiment.
Chromatogram pane	Displays the chromatograms for the component that you select in the Components pane. This pane is similar to the same pane on the Process and Review page.
Relative Intensity (*y* axis)	Displays the ratio of each peak intensity relative to the maximum intensity in the spectrum.
RT (min) (*x* axis)	Displays the retention time of the scan—that is, the time after injection at which a compound elutes.
Trend Ratio pane	Displays the bar plots for the ratios between various conditions and the reference condition for the component that you select in the Modification Results pane.
Ratio value (*y* axis)	Displays the Avg MS Area value for a particular condition ÷ Avg MS Area value for the reference condition.
Ratio conditions (*x* axis)	Displays the names of the various conditions and the name of the reference condition.
Trend MS Area pane	Displays the bar plots for the MS Area values for the component that you select in the Modification Results table, for each raw data file used in the experiment.
MS Area value (*y* axis)	Displays the MS Area values from each of the individual raw data files assigned to a particular condition.

Parameter	Description
Condition-Raw data file (*x* axis)	Displays the names of the various conditions and their assigned raw data files used in the experiment.
Oligo Sequence Coverage pane	Displays the fragment coverage map, which includes the oligonucleotide sequence information and the color-coded fragment ions. This pane is similar to the same pane on the Process and Review page.
Oligo Sequence pane	Displays the nucleotide sequence assigned to the experiment. This shows the highlighted identified oligo sequence that you select from the Components table. This pane is similar to the same pane on the Process and Review page.
Full Scan Spectra pane	Displays the deconvoluted and full-scan spectra with mass and *m/z* information. This pane is similar to the same pane on the Process and Review page.
Relative Abundance (*y* axis)	Displays the ratio of the abundance of a specific peak to the abundance of the peak with the highest abundance.
Mass or *m/z* (*x* axis)	Displays the mass or mass-to-charge ratio of the ions formed from molecules. This ratio is the quantity formed by dividing the mass of an ion, in daltons, by the number of charges that the ion carries.
MS2 Spectra™ pane	Displays the predicted spectrum stacked on top of the experimental spectrum. This pane is similar to the same pane on the Process and Review page.
Activation	(Enabled only when multiple fragmentation types are used to generate the data.) Displays a list of the available fragmentation types (CID, HCD, ETD, or ECD) that you can select from to view the MS2 spectra for this particular combination.
Res.	(Enabled only when multiple fragmentation types are used to generate the data.) Displays the available resolution types (High or Low) that you can select to view the MS2 spectra for this particular combination.

Parameter	Description
Relative Abundance (*y* axis)	Displays the ratio of the abundance of a specific peak to the abundance of the peak with the highest abundance.
m/z (*x* axis)	Displays the mass-to-charge ratio of ions formed from molecules. This ratio is the quantity formed by dividing the mass of an ion, in daltons, by the number of charges that the ion carries.

For more details, see the following topics:

- “Viewing the modification summary results” on page 640
- “Upper table of Modification Results pane parameters” on page 648
- “Lower table of Modification Results pane parameters” on page 643
- “Viewing the modification summary components table” on page 649
- “Viewing the Modification Plot.” on page 652
- “Viewing the Trend Ratio plot for Oligonucleotide Analysis” on page 618
- “Viewing the trend MS area plot for Oligonucleotide Analysis” on page 619
- “Viewing the Fragment Coverage Map for Oligonucleotide Analysis” on page 620
- “Viewing the Oligonucleotide sequence for Oligonucleotide analysis” on page 621
- “Viewing the deconvoluted and full-scan MS spectra for Oligonucleotide Analysis” on page 622
- “Viewing the predicted and experimental MS2 spectra for Oligonucleotide Analysis” on page 622

Viewing the modification summary results

The Modification Results pane on the Modification Summary page displays the modification summary results as tabular data. In the upper table, the results include the date that the application created the summary, the data folder that holds the raw data files, the charge state, and other information for each raw data file. The lower table lists the oligonucleotide, modification, abundance for each raw data file, and other data.

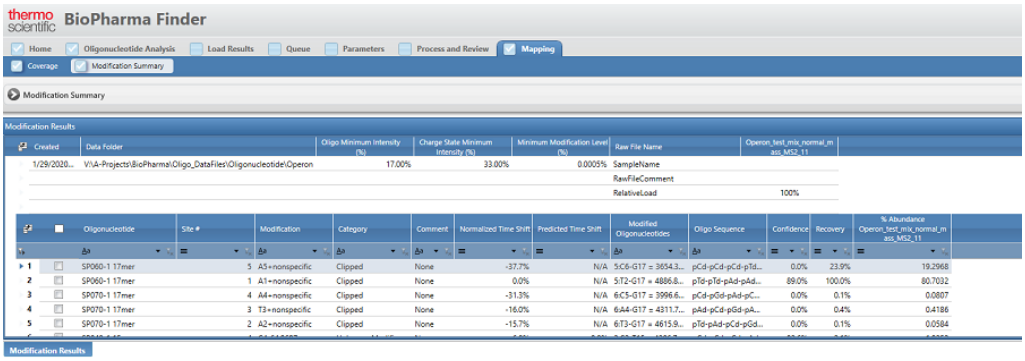


Figure 296 Modification Results pane displaying upper table and lower table

The % Abundance columns (one for each raw data file used in the experiment) in the lower table display the abundance of the modification in the sample as a percentage.

The selected components appear in blue in the Components table.

Note: Scroll to the right to see more % Abundance columns if needed.
You can select components to recalculate custom % Abundance values. You can also view the modification plot of the abundance percentages, grouped by conditions and raw data file names.

For more details, see the following topics:

- “Viewing the Modification Results pane” on page 646
- “Changing the modification summary options” on page 646
- “Exporting the modification summary” on page 641
- “Upper table of Modification Results pane parameters” on page 648
- “Modification Results pane commands” on page 642
- “Viewing the modification summary components table” on page 649
- “Changing the abundance calculation” on page 650
- “Viewing the Modification Plot.” on page 652

Exporting the modification summary

To export the modification summary

1. On the Modification Summary page, right-click anywhere in the Modification Results pane and choose one of the following:
   - **Export All Modifications to Excel** to export all of the summary results to an Excel file.

   –or–

- **Export Checked Modifications to Excel** to export only the selected summary results to an Excel file.

  To select a modification row to export, select the check box in that row in the lower table.

  To select or deselect all of the rows, select or clear the check box in the table header row.

The **Save As** dialog box opens.

2. Browse to or type the name of the file to store the exported results in.
3. Click **Save.**

Modification Results pane commands

Right-clicking the Modification Results pane on the Modification Summary page opens a shortcut menu with the commands listed in the following table.

Table 112 Modification Results pane shortcut menu

Command	Description
Set Summary Options	Opens the Summary Options dialog box so that you can set new values for the oligonucleotide Intensity (%), Charge State Minimum Intensity (%), and Minimum Modification Level (%) columns in the upper table of the Modification Results pane.
Export All Modifications to Excel	Exports data for all modifications in the Modification Results pane to an Excel file.
Export Checked Modifications to Excel	Exports data for only the selected modifications in the lower table in the Modification Results pane to an Excel file.

Lower table of Modification Results pane parameters

The following table describes the types of information in the lower table of the Modification Results pane on the Modification Summary page.

Table 113 Modification Results pane, lower table parameters

Parameter	Description
Field Chooser	Displays the Field Chooser dialog box so that you can select the columns to display in the lower table of the Modification Results pane.
	Select or clear the check box in the table header row to select/deselect all rows in the lower table. You can also select the check box in individual rows for export.
Oligonucleotide	Dsiplays the Oligonucleotide sequence.
Site #	Displays the site number.
Modification	Displays the sequence on which the modification occurs and the type of modification.

Parameter	Description
Category	Displays the type of modification by category. • Unknown Modification: Modifications usually resulting from the unspecified modification search when the application cannot identify the exact location of the modification. Unknown modifications contain the tilde (~) mark. • Modification: Common modifications that the application identified. • Artifact: Something observed in a scientific investigation or experiment that is not naturally present but occurs as a result of the preparative or investigative procedure. • Sequence Variant: Modifications from sequence variants. • N-Glycan or O-Glycan: Modifications from N-linked or O-linked glycans. **Note:** In the Modification column, the "nonspecific" tag is always assigned to the nucleotide on the left (5') side of the cleavage site, e.g. A4-C8 =1527.2596m[nonspecific] will show as G3+nonspecific because the cleavage happens between G3 and A4. G1- A4 =1388.2753m[nonspecific] will show as A4+nonspecific because the cleavage happens between A4 and C5.
Comment	Displays any comments about the modification to support the identification, the abundance percentage, or both. Examples of comments are None, Possible artifact, or Poor recovery.

Parameter	Description
Normalized Time Shift	Displays the experimental shift of the retention time after modification as a percentage of the abundance-weighted average retention time of all identified oligonucleotid. A negative indicates that the modified nucleotide elutes earlier than the unmodified oligonucleotide. A positive value indicates that the modified oligonucleotide elutes later than the unmodified oligonucleotide.
Predicted Time Shift	Displays the predicted retention time shift, which is based on the empirically determined value of the normalized time shift of common modifications.
Modified Oligonucleotides	Displays the names of the nucleotides used for quantification.
Oligo Sequence	Displays the oligonucleotide sequence.
Confidence	Displays the confidence score of the modified oligonucleotide.
Recovery™	Displays the general abundance of the modified oligonucleotide. which is the total peak area (including modified and unmodified forms of the oligonucleotide.) as a percentage of the self-weighted average of all oligonucleotide. This means that higher-intensity oligonucleotide weigh more than lower-intensity peptides. The recovery value can be over 100% because of the way the application calculates the reference intensity. Good: Recovery™ ≥ 10% Fair: 1% < Recovery™ < 10% Poor: Recovery™ ≤ 1%
% Abundance *raw_data_file_name*	Displays the abundance of the modification in a particular raw data file as a percentage.
Custom % Abundance *raw_data_file_name*	(Visible only when you change the list of components used for the % Abundance calculation.) Displays the custom abundance of the modification in a particular raw data file as a percentage.

Viewing the Modification Results pane

To view the modification summary in the Modification Results pane

1. Click the **Mapping** tab and then click the **Modification Summary** subtab if necessary.

2. In the Modification Results pane, select the row for the oligonucleotide modification that you are interested in, as shown in the following figure.

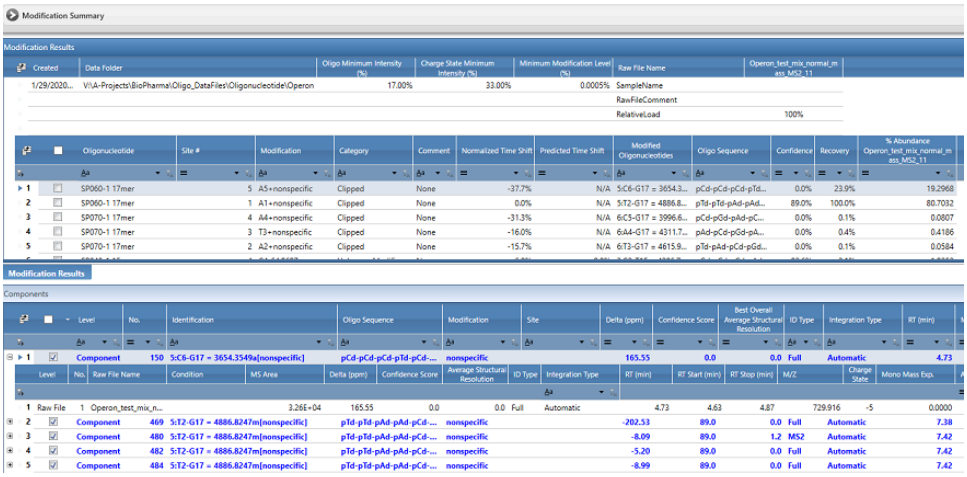


Figure 297 Components of an oligonucleotide modification selected in the modification results lower table

The selected row highlights in blue.

The Components pane lists all the components which were used to calculate the % Abundance of the selected oligonucleotide modification.

Changing the modification summary options

To change the options for the modification summary of an oligonucleotide experiment

1. On the Modification Summary page, right-click the Modification Results pane and choose **Set Summary Options.**

   The Summary Options dialog box opens.

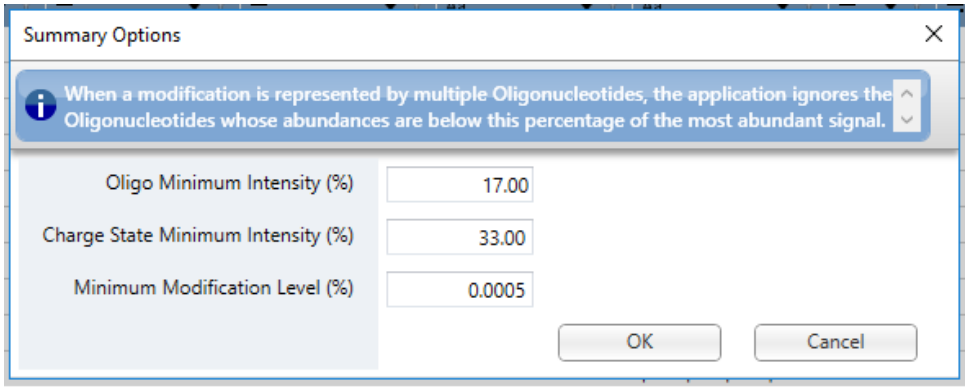


Figure 298 Summary Options dialog box

2. In the Oligo Minimum Intensity box, type a value to define the nucleotide area threshold as a minimum percentage of the most abundant signal.

   The oligonucleotide chain might break in some conditions, causing more than one sequence to represent a modification. When the application uses the information from all of these sequences, unnecessary interferences might result in an improper abundance calculation. To avoid this problem, the modification summary does not include the identifications whose total peak area is below the percentage value set in the Minimum Intensity box—that is, below the minimum percentage of the most abundant signal. This filter results in the removal of less abundant identifications from the calculation.

3. In the Charge State Minimum Intensity box, type a value to define the charge state threshold as a minimum percentage of the most abundant signal.

   When there is a large variation in intensities, the abundance calculation might underestimate the low abundant identifications. To avoid this problem, the modification summary does not include the charge states whose abundances are below the percentage value set in the Charge State Minimum Intensity box—that is, below the minimum percentage of the most abundant charge-state signal. This filter results in the removal of less abundant charge states from the calculation.

4. In the Minimum Modification Level box, type a value to specify the minimum modification level to report in the summary.

5. Click **OK** to update the modification summary.

   Note: If you change the list of components used in the % Abundance calculation, your modified settings in the Summary Options dialog box apply only to the default % Abundance value for each raw data file. They do not apply to the recalculated Custom % Abundance values.

For more details, see the following topics:

- “Viewing the modification summary components table” on page 649
- “Changing the abundance calculation” on page 650

Upper table of Modification Results pane parameters

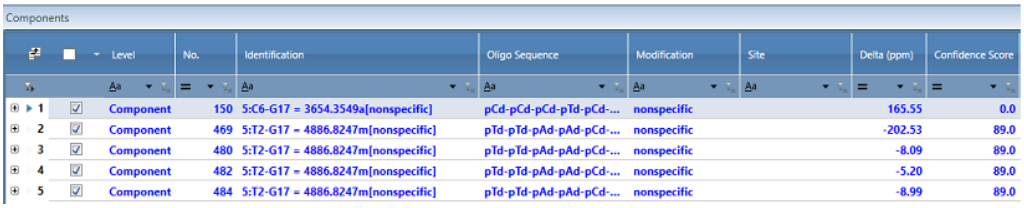
The following table describes the types of information in the upper table of the Modification Results pane on the Modification Summary page.

Table 114 Modification Results pane, upper table parameters

Column	Description
Created	Displays the date on which the application created the modification summary and the name of the person who created the summary.
Data Folder	Displays the folder containing the raw data files used in the experiment.
Oligo Minumum Intensity (%)	Displays minimum oligonucleotide intensity.
Charge State Minimum Intensity (%)	Displays the value of the charge state intensity threshold as a minimum percentage of the most abundant intensity.
Minimum Modification Level (%)	Displays the minimum modification level to report in the summary.
Raw File Name	Displays information imported from the raw data file. • SampleName: Displays the information saved in the Sample Name field in the raw data file. • RawFileComment: Displays the information saved as a comment in the raw data file. • RelativeLoad: Displays a measure of the nucleotide quantification, . The names of the raw data files used in the experiment appear in the columns to the right of the Raw File Name column, for example, Control_A01, Sample_B02, and Sample C_01.

Viewing the modification summary components table

The Components table on the Modification Summary page displays the components related to a particular modification site that you select in the Modification Results pane.


Components

		Level	No.	Identification	Oligo Sequence	Modification	Site	Delta (ppm)	Confidence Score
1	☑	Component	150	5:C6-G17 = 3654.3549a[nonspecific]	pCd-pCd-pCd-pTd-pCd-...	nonspecific		165.55	0.0
2	☑	Component	469	5:T2-G17 = 4886.8247m[nonspecific]	pTd-pTd-pAd-pAd-pCd-...	nonspecific		-202.53	89.0
3	☑	Component	480	5:T2-G17 = 4886.8247m[nonspecific]	pTd-pTd-pAd-pAd-pCd-...	nonspecific		-8.09	89.0
4	☑	Component	482	5:T2-G17 = 4886.8247m[nonspecific]	pTd-pTd-pAd-pAd-pCd-...	nonspecific		-5.20	89.0
5	☑	Component	484	5:T2-G17 = 4886.8247m[nonspecific]	pTd-pTd-pAd-pAd-pCd-...	nonspecific		-8.99	89.0

Figure 299 Components table

The information in this table is similar to the information in the Results table on the Process and Review page but is filtered to show only the nucleotides with the same site as the selected nucleotide modification.

The BioPharma Finder™ application uses blue text for the components whose modifications it used to calculate the abundance and recovery data and selects their corresponding check boxes, ☑. Below these components, the application uses black text for the components whose modifications it did not use in these calculations and clears their check boxes, ☐.

For more details, see the following topics:

- “Viewing the Components table” on page 649
- “Changing the abundance calculation” on page 650
- “Exporting the component results” on page 650
- “Components pane commands” on page 651

Viewing the Components table

To view the Components table on the Modification Summary page

1. Click the **Mapping** tab and then click the **Modification Summary** subtab.
2. Click the row of a modification in the lower table of the Modification Results pane.

   The Components table displays the components related to the selected modification site.
3. Select a row in the Components table to view information related to that component in these other panes on this page:
   - Chromatogram
   - Visible only for experiments with multiple raw data files:
     - Trend ratio plot
     - Trend MS area plot
   - Oligo Sequence Coverage
   - Oligo Sequence

- Full Scan Spectra
- MS2 Spectra

Changing the abundance calculation

To change the components used in the abundance calculation

1. In the Components pane on the Modification Summary page, select or clear the check boxes in the component rows to select or deselect the components to be used in the abundance calculation of the modification summary.

   Note: To select or deselect all of the rows at once, select or clear the check box in the header row.

Figure 300 Component table shown in the Components pane

2. Click **Recalculate % Abundance**.

   The application recalculates the % Abundance value using the selected components. It updates this value in the Custom % Abundance *raw_data_file_name* column for each raw file in the lower table of the Modification Results pane. The default % Abundance value remains in the % Abundance *raw_data_file_name* column for each raw data file.

3. When you are satisfied with the recalculated value, click **Save** in the upper right corner of the Modification Summary page.

Exporting the component results

To export the component results

1. Click the row of a oligonucleotide modification in the lower table of the Modification Results pane on the Modification Summary page.

2. Right-click anywhere in the Components table on the Modification Summary page and choose one of the following:

   - **Export All Components**
   - **Export Checked Components**

   When you hover your mouse over Export All Components and Export Checked Components you have two additional options:

   - **As Displayed**, to export data at all levels for all components to an Excel file
   - **Excel Workbook**, to export data to an Excel file, exactly as currently displayed in the table

   Note: To select a component row to export, select the check box in that row.

To select or deselect all of the rows, select or clear the check box in the table header row.

3. After you select an export option, the Save As dialog box opens. Browse or type the name of the file to save the exported results.

4. Click **Save.**

The exported data reflects the filtering, sorting, and reordering of columns of the components table only for the displayed option. The application exports the table rows based on the sequential order displayed in the Numbers column.

Components pane commands

You can right-click the Components pane on the Modification Summary page to open a shortcut menu with the commands listed in the following table:

Table 115 Components pane shortcut menu

Command	Description
Export All Components	Exports data for all components in the Components pane to an Excel file.
Export Checked Components	Exports data for only the selected components in the Components pane to an Excel file.

For more details, see the following topics:

- “Exporting the component results” on page 650

Viewing the Modification Plot.

The Modification Plot pane on the Modification Summary page displays a plot of the abundance percentages for the selected modifications in the Modification Results table, grouped by the conditions and corresponding raw data files loaded for the experiment.

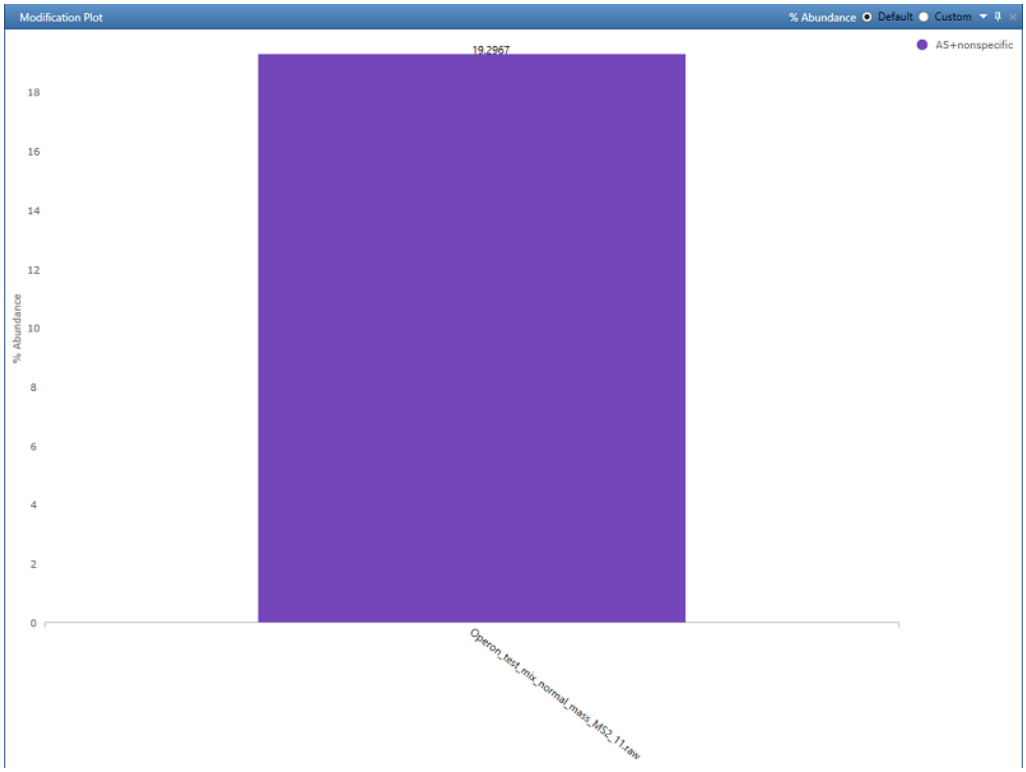


Figure 301 Modification Plot.

To view the Modification Plot

1. Click the **Mapping** tab and then click the **Modification Summary** subtab.

2. Select the check box for one or more rows of components in the lower table of the Modification Results pane.

   The Modification Plot pane shows the *default* abundance percentages for the selected rows, grouped by the conditions and corresponding raw data file names. The percentage values in the plot are from the % Abundance *raw_data_file_name* columns in the table. The plot displays each modification in a different color according to the legend in the upper right of the pane.

3. (Optional) If you recalculated *custom* abundance percentages, view them in the plot by selecting the **Custom** option in the title bar of the Modification Plot pane.

   The plot now uses the values from the Custom % Abundance *raw_data_file_name* columns in the table. If a selected row does not have a value in this column, the Custom option is inactive.

   To view the default percentages again for the selected rows, select the **Default** option.

Part VIII

Reference information

36 Interactive functions

To interact with the chromatograms, spectra, map and sequence panes, and results tables in the BioPharma Finder application, follow these procedures.

Rearranging the panes

You can rearrange various panes in the application by repositioning, collapsing, or resizing them.

For more details, see the following topics:

- Repositioning the panes (page 656)
- Rearranging the panes with the mouse (page 659)
- Collapsing the panes (page 660)
- Resizing the panes vertically (page 660)
- Resizing the panes horizontally (page 661)

Repositioning the panes

Use the following features, when available, to reposition the panes in the BioPharma Finder™ application.

To reposition a pane

1. Right-click the pane's title bar or click the down arrow, , on the right side of the title bar to open the shortcut menu.

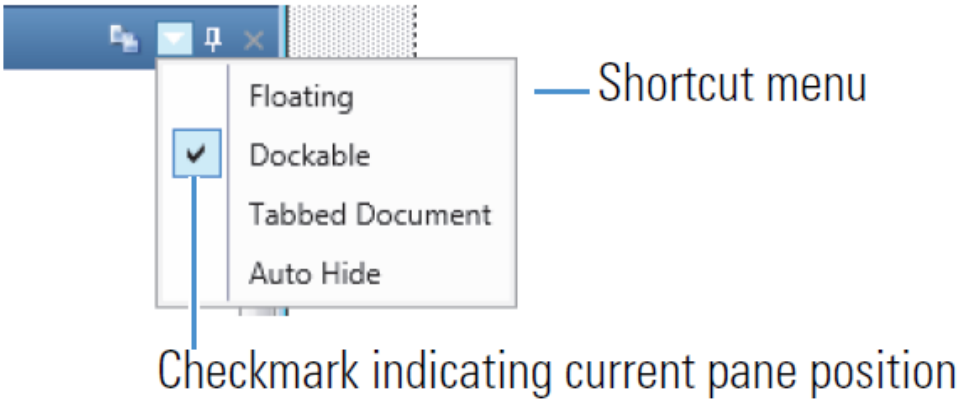


Figure 302 Pane shortcut menu

2. Choose the appropriate command:
   - To detach the pane into a floating window, choose **Floating**.
   - To dock the pane back to the main window, choose **Dockable**. Then, either double-click the pane's title bar or drag the pane on top of an up, down, left, or right icon in the View Arranger tool.
   - To change to a tabbed pane, choose **Tabbed Document**.

     You can have multiple tabbed panes overlaying each other. Click the tab to bring the contents of that pane to the front.

   –or–

   - To hide the pane temporarily, choose **Auto Hide**. You can also click the toggle-like pin icon, , on the right side of the title bar.

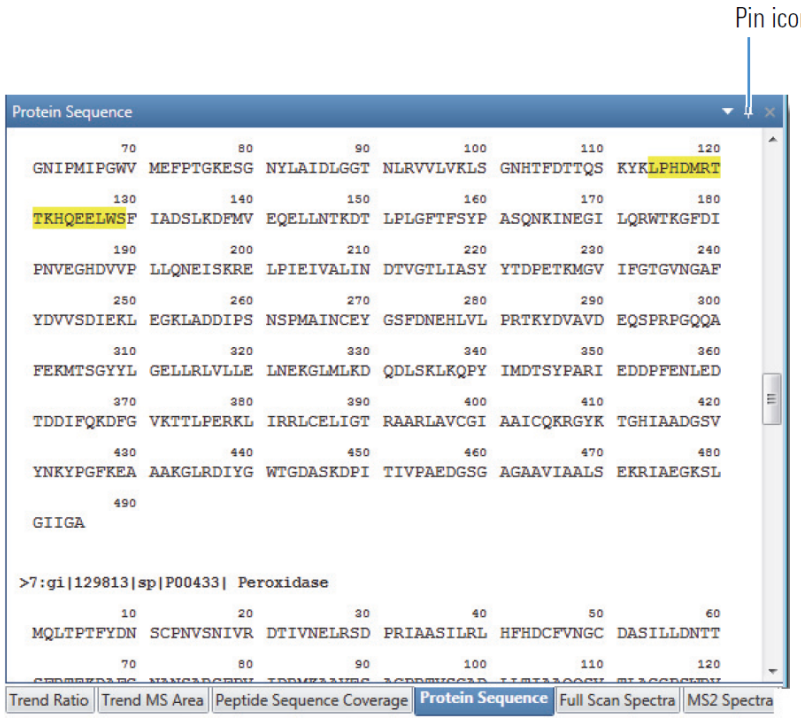


Figure 303 Pin icon

The application hides the pane but displays its tab next to the nearest window's edge—whether left, right, or bottom—whichever side is closest to the hidden pane.

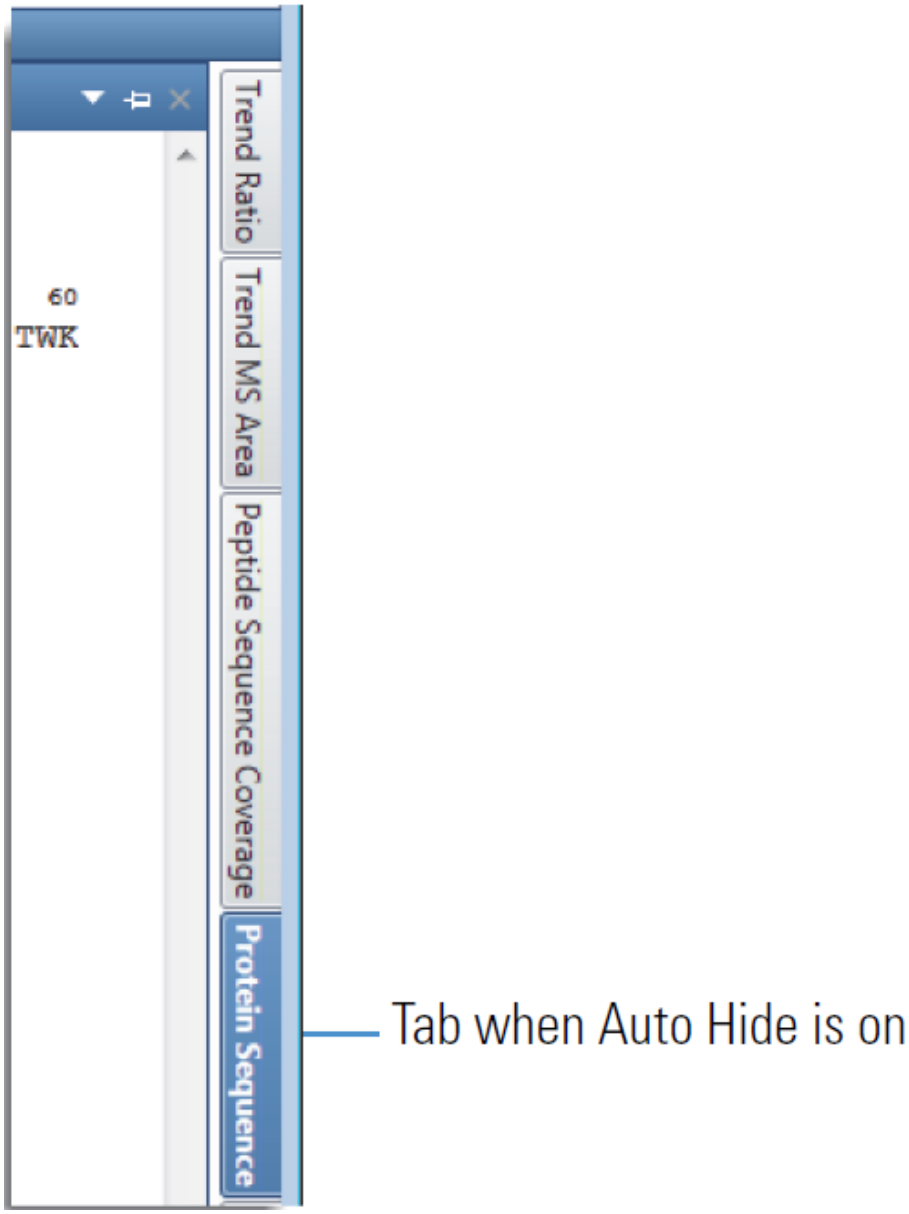


Figure 304 Tab for Auto Hide

To show the full pane, hold the cursor over the tab. Move your cursor away from the tab and the application auto-hides the pane.

When Auto Hide is on, the pin icon changes position, . To turn off the **Auto Hide** function, choose Auto Hide in the shortcut menu again to clear the check mark, or click the pin icon again to change it back to its open position, .

Note: To change a pane to a floating window, you can also drag the pane's title bar. However, when you dock this window, the application no longer displays the information in the pane. To display the data again, load the results from a different experiment, and then reload the results from the current experiment.

For more details, see the following topics:

- Rearranging the panes with the mouse (page 659)

Rearranging the panes with the mouse

To arrange panes with the mouse

1. Drag the title bar of the pane that you want to move to a second pane until the View Arranger tool appears, similar to the one in the following figure.

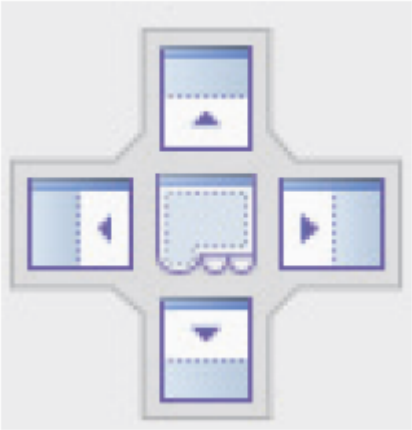


Figure 305 View Arranger tool

2. Do one of the tasks in the following table.

Table 116 View Arranger icons

Task	Procedure
Move the first pane above the second pane.	Drag the title bar to the up icon, .
Move the first pane below the second pane.	Drag the title bar to the down icon, .
Move the first pane to the left of the second pane.	Drag the title bar to the left icon, .
Move the first pane to the right of the second pane.	Drag the title bar to the right icon, .
Make both panes tabbed.	Drag the title bar to the tabs icon, . The application displays the first pane and creates a tab for the second pane.

Collapsing the panes

You can collapse a pane by clicking the blue title bar or the down arrow, , when available. To expand a pane, click title bar again or the side arrow, , when available.

Resizing the panes vertically

To adjust the pane size vertically

(Available for certain stacked panes) Drag the splitter bar up or down to adjust the height.

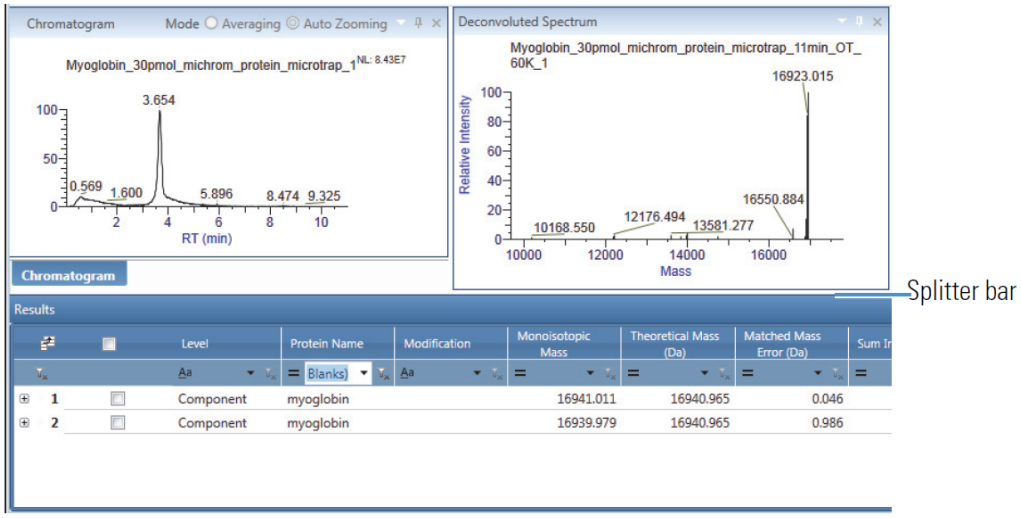


Figure 306 Splitter bars for sizing panes vertically

Resizing the panes horizontally

To adjust the pane size horizontally

(Available for certain panes that are side-by-side) Drag the splitter bar left or right to adjust the width.

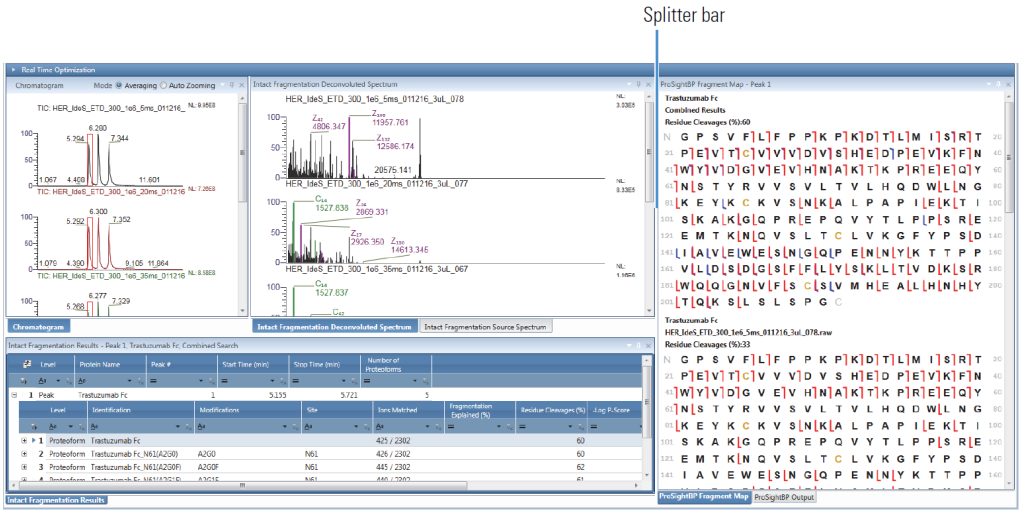


Figure 307 Splitter bar for sizing panes horizontally

Using basic chromatogram functions

Use the following features, when available, to interact with the displayed chromatograms in the Chromatogram pane:

- Zooming in (page 662)
- Zooming out (page 663)
- Resetting to original scale (page 663)
- Copying to the clipboard (page 663)
- Displaying labels (page 663)

Zooming in

To zoom in on the chromatogram

Do one of the following:

- Right-click the Chromatogram pane and choose **Zoom In** to zoom in on the entire chromatogram.
- (For Intact Mass Analysis or Top Down Analysis, select the **Auto Zooming** option from the pane title bar.) Drag the red cross-shaped cursor over the peak or peaks of interest to form a box.

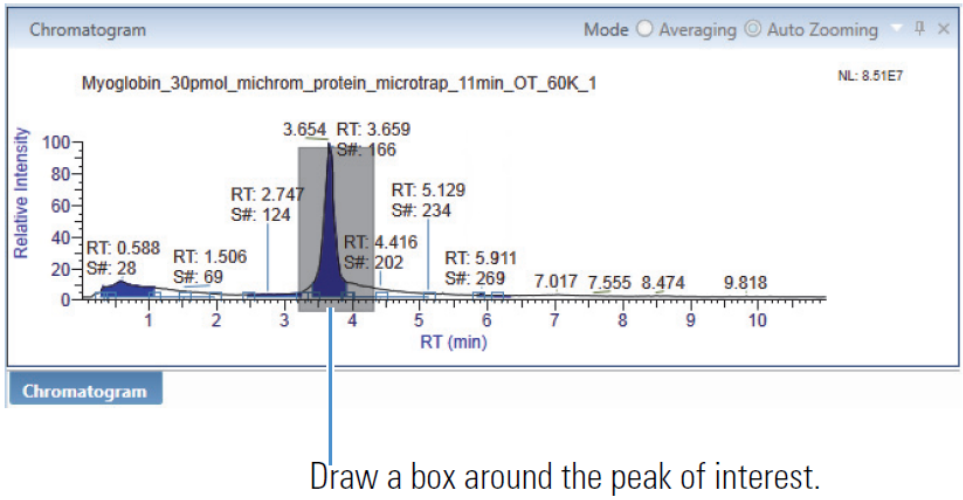


Figure 308 Enlarging a peak by drawing a box around it

- (For Intact Mass Analysis or Top Down Analysis, select the **Auto Zooming** option from the pane title bar.) Hold down the left mouse button and draw a line beneath the baseline of the peaks of interest.

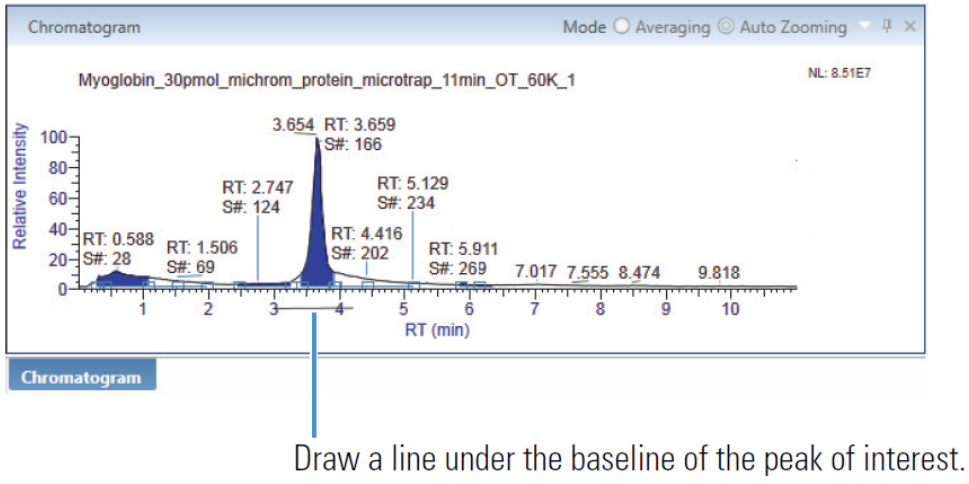


Figure 309 Enlarging a peak by drawing a line beneath its baseline

When you zoom in on a region of the chromatogram, the application recalculates the values on the *y* axis so that 100 percent represents the highest intensity in the displayed region. The intensities shown on the *y* axis remain at values relative to the most intense component in the plot.

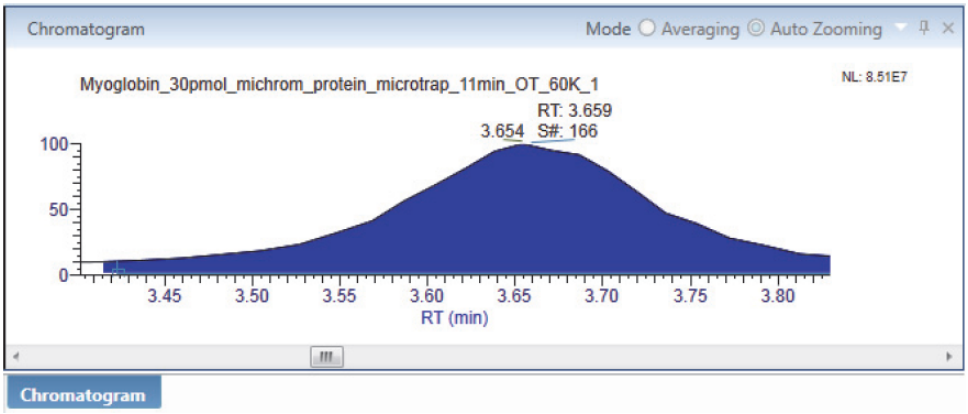


Figure 310 Chromatogram scaled to the maximum height of the peak

Zooming out

To zoom out of the chromatogram

To shrink the view of the entire chromatogram, right-click the Chromatogram pane and choose **Zoom Out** if available.

Resetting to original scale

To reset the view to the original scale

- Right-click the Chromatogram pane and choose **Reset Scale**.

–or–

- Double-click anywhere in the Chromatogram pane.

Copying to the clipboard

To copy the chromatogram to the Clipboard

Right-click the Chromatogram pane and choose **Copy**, **Copy as Displayed**, or **Copy per Global Settings**.

The application copies the entire view of the Chromatogram pane to the Clipboard including all visible labeling and shading. You can then paste the copied picture into a third-party application file.

Note: To paste a picture into a Microsoft™ application and create a PDF file of this content, you must install the Acrobat Pro software on your system.

Displaying labels

To display labels

- Right-click the Chromatogram pane and choose **Label ▸ Retention Time** if available.

  The application displays the retention time with one decimal digit on top of the peaks in all of the visible chromatograms.

–or–

- Right-click the Chromatogram pane and choose **Label** ▸ **Peptide** if available.
  The application displays the peptide information on top of the peaks in all of the visible chromatograms.

  Note: For Peptide Mapping Analysis, if the peptide is modified, an asterisk symbol, "*", appears at the end of the peptide label.

Using basic spectrum functions

Use the following functions to interact with the displayed spectra:

- Zooming in (page 664)
- Zooming out (page 665)
- Resetting to original scale (page 666)
- Copying spectrum to the clipboard (page 666)
- Copying data to the clipboard (page 666)

Zooming in

To zoom in on the spectrum

Do one of the following:

- Right-click the spectrum pane and choose **Zoom In** if available.
- Drag the cursor over the peak or peaks of interest to form a box and rescale a spectrum to the relative height of the box.

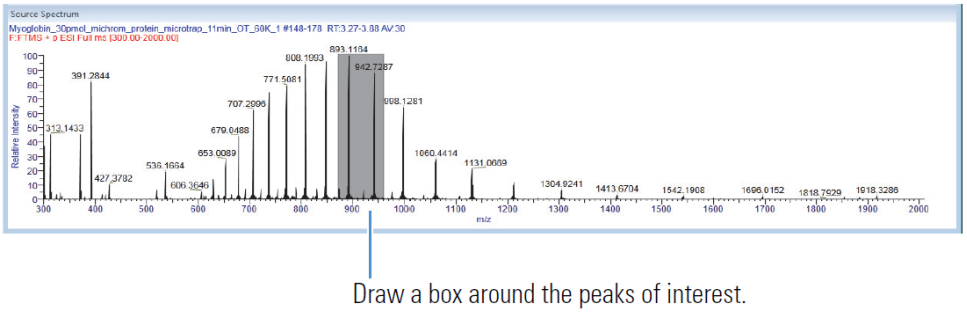


Figure 311 Enlarging an area by drawing a box around the peaks of interest

- Hold down the left mouse button and draw a line beneath the baseline of the peaks of interest.

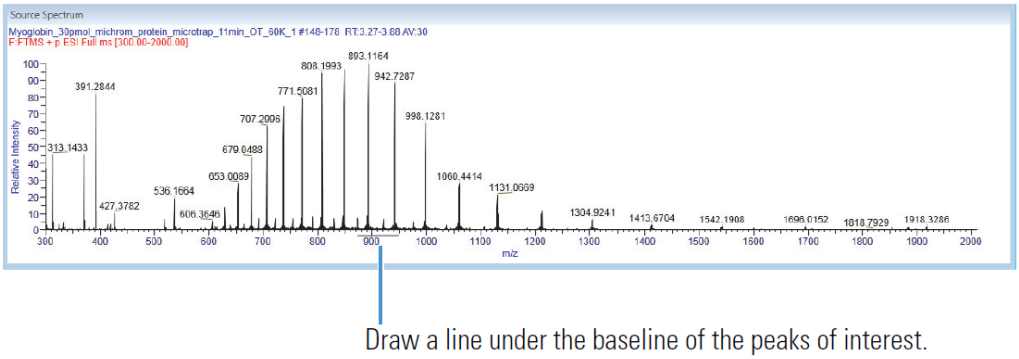


Figure 312 Enlarging an area by drawing a line beneath the baseline of the peaks of interest

When you zoom in on a region of the spectrum, the application recalculates the values on the *y* axis so that 100 percent represents the highest intensity in the displayed region. The intensities shown on the *y* axis remain at values relative to the most intense component in the plot.

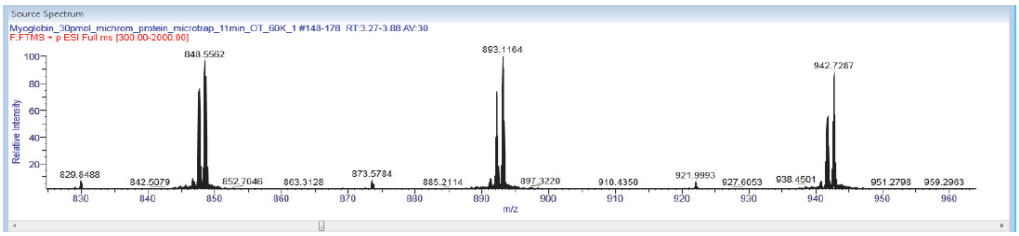


Figure 313 Enlarged peaks in the spectrum

Zooming out

To zoom out of the spectrum

To shrink the view of the entire spectrum, right-click the spectrum pane and choose **Zoom Out** if available.

Resetting to original scale

To reset the view to the original scale

- Right-click the spectrum pane and choose **Reset Scale**.

–or–

- Double-click anywhere in the spectrum pane.

Copying spectrum to the clipboard

To copy the spectrum to the Clipboard

Right-click the spectrum pane and choose **Copy**, **Copy as Displayed**, or **Copy per Global Settings**.

The application copies the entire image in the pane to the Clipboard including all visible labeling and shading. You can then paste the copied image into a third-party application file.

Note: To paste an image into a Microsoft™ application and create a PDF file of this content, you must install the Acrobat Pro software on your system.

For more details, see the following topics:

- Using copy and paste functions (page 666)

Copying data to the clipboard

To copy deconvoluted spectral data

Right-click the deconvoluted spectrum pane and choose **Copy Data**.

The application copies mass data (*x* axis) and intensity data (*y* axis) from the pane to the Clipboard so that you can paste it into a spreadsheet or another application.

Using copy and paste functions

Use the various copy commands to copy the content of the following panes to the Clipboard for the three types of BioPharma Finder analyses.

Table 117 Copy commands for data results by type of analysis and pane

Command	Page/Pane	Analysis		
		Peptide Mapping	Intact Protein	Top Down
Copy	Process and Review page • Chromatogram pane • Peptide Sequence Coverage pane • Full Scan Spectra pane • MS2 Spectra pane	✓		

Command	Page/Pane	Analysis		
		Peptide Mapping	Intact Protein	Top Down
Copy	Coverage page • Chromatogram pane • Protein Coverage Map pane	✓		
Copy	Modification Summary page • Chromatogram pane • Peptide Sequence Coverage pane • Full Scan Spectra pane • MS2 Spectra pane	✓		
Copy, Copy as Displayed, Copy per Global Settings	Process and Review page • Chromatogram pane		✓	✓
	• Deconvoluted Spectrum pane		✓	
	• Intact Fragmentation Deconvoluted Spectrum and Intact Deconvolution Deconvoluted Spectrum pane			✓
	• Source Spectrum pane		✓	
	• Intact Fragmentation Source Spectrum and Intact Deconvolution Source Spectrum pane			✓
Copy Data	Process and Review page • Deconvoluted Spectrum pane		✓	
	• Intact Fragmentation Deconvoluted Spectrum and Intact Deconvolution Deconvoluted Spectrum pane			✓
Copy as Displayed, Copy per Global Settings	Spectra Comparison page | Mirror Plot pane		✓	
Copy	Process and Review page | ProSightBP Fragment Map pane			✓

You can then paste the copied content into a third-party application

Note: If you cannot paste copied contents into a file, uninstall the Internet Explorer™ web browser and reinstall it.

Copying to a text editor or file

To copy the content of a pane to a text editor or file

1. In the title bar of the pane, click the **Copy** icon (if available), , to copy all of the pane's content.

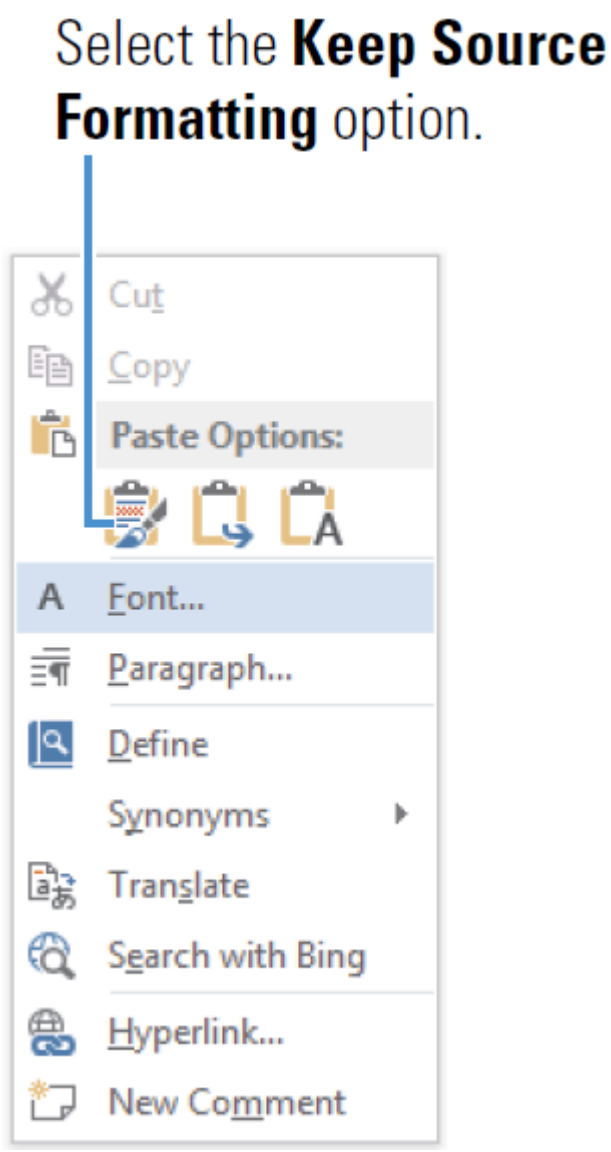
   –or–

   Right-click the pane and choose **Copy**, **Copy as Displayed**, **Copy per Global Settings**, or **Copy Data** if available.

   The Copy and Copy as Displayed commands copy the image in the pane as it is currently displayed. The Copy per Global Settings command copies the image at the set global dimensions.

2. To paste the content to a file, select the **Web Layout** option at the bottom right of the main window.

3. Right-click and choose the **Keep Source Formatting** option under Paste Options.



–or–

To paste the contents to a text file (edition 2010 and later), choose **Paste ▸ Special** in the Paste Special dialog box, select the **HTML** setting, and then click **OK**.

Note: The BioPharma Finder application does not support pasting some panes' content to the presentation application.

To paste the pane's content into the application and create a PDF file of this content, you must install the Acrobat Pro software on your system.

For more details, see the following topics:

- Specifying the image dimensions (page 37)

Copying a portion of the pane

To copy a portion of the pane

1. From the Sart menu of the text file application choose **All Programs ▸ Accessories ▸ Snipping Tool**.
2. Drag the cursor that appears around the area of the pane that you want to capture.
3. Right-click the screen capture in the Snipping Tool and choose **Copy**.
4. Paste the copied picture to a third-party application file.

Using basic table functions

Use the following functions, when available, to interact with various tables in the BioPharma Finder application:

- Sorting rows (page 670)
- Showing or hiding columns (page 671)

Sorting rows

To sort the rows based on the contents of a column

Click the column header to sort the rows in alphabetical or numerical order, based on the contents in that column. Click again to reverse the sort order.

For example, click the Delta (ppm) column header. The application displays the numbers in this column in order from lowest to highest values. Click the column header again to displays the numbers from highest to lowest values.

You can sort based on a maximum of two columns at a time. Select the first column header. The application sorts the table based on this header and this is the primary sorting order. Then, hold down the CTRL key before selecting the second column header. If there are some identical values in the first column, the application re-sorts their rows based on the order of the values in the second column.

Note:

- The sequential number column (first column) in the results table is not sortable.
- If you sort a column that contains check boxes, the application groups all cleared check boxes together at the top. Click again to display all selected check boxes at the top.

Note: The application does not sort the peptide sequences in the Identification column alphabetically by the order of the characters in the peptide string. It starts sorting based on the first protein before the colon character (for example, "1:" before "2:"). Next, the application orders the identified peptide sequences by the N-terminal

position (the first amino acid of the peptide sequence) and then by the C-terminal position (the last amino acid of the peptide sequence).
Before sorting based on the Identification column, first filter the table using the "Equals" operator and the "NonBlanks" operand for this column. This steps removes all of the unidentified components from the table.

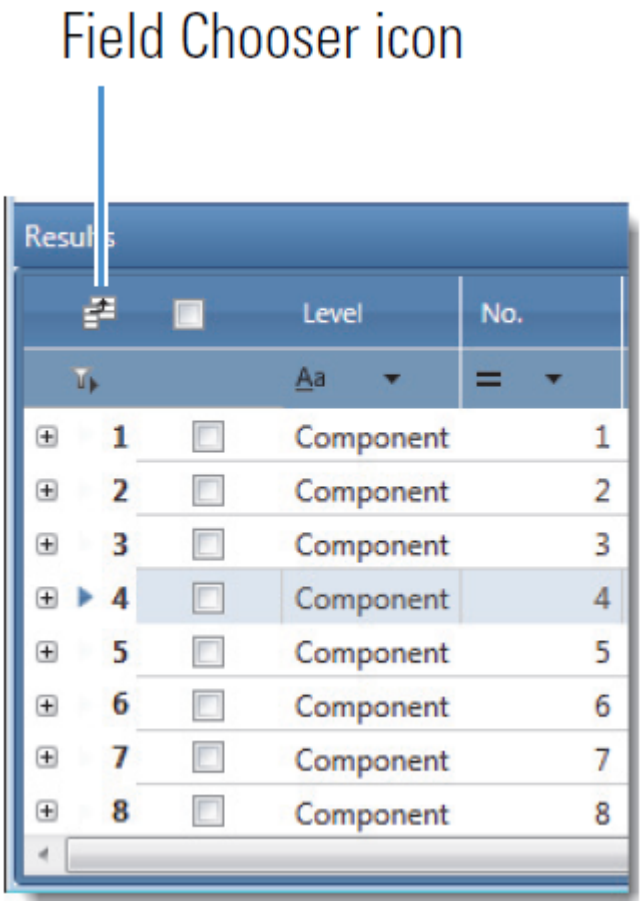
For more details, see the following topics:

- Filtering data in a table (page 673)

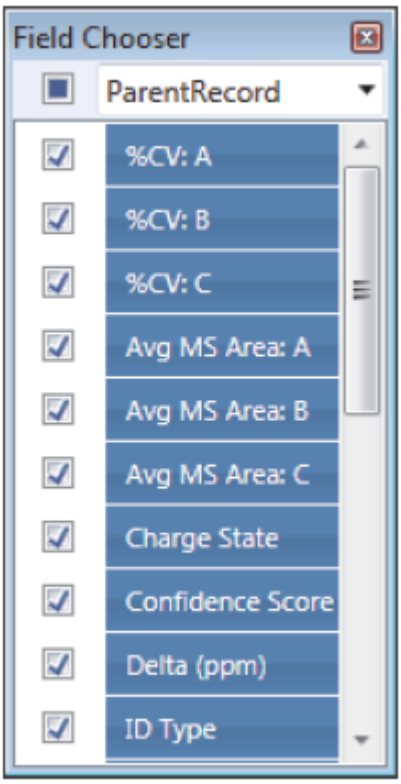
Showing or hiding columns

To show or hide selected columns

1. In the header row of the table, click the **Field Chooser** icon, , if available.



The Field Chooser dialog box opens listing all of the column headers for the current table.



2. Clear the check box for each column that you want to hide. To show those columns again, select their corresponding check boxes.

   The table updates and shows or hides your chosen columns immediately.

   Note:
   - For the results table on the Process and Review page, the Field Chooser affects only the top-level rows—for example, the list of components.
   - For the results table on the Mapping > Coverage page, the Field Chooser affects only the top-level rows—that is, the list of proteins.

   The application retains any modifications you make to the columns until you close it. Once you reopen the application, the results table columns return to their default states.

In addition to these table functions, you can also filter data based on selected conditions when those filtering functions are available.

For more details, see the following topics:

- Viewing the results table for peptide mapping analysis (page 245)
- Viewing the results table for intact mass analysis (page 422)
- Viewing the results tables for top down analysis (page 532)
- Viewing the coverage results table (page 292)
- Filtering data in a table (page 673)

Filtering data in a table

Use the following functions, when available, to filter a table to show only data that fulfill certain conditions:

- Filtering data rows (page 673)
- Selecting filter operators (page 674)
- Table filter operators (page 675)
- Selecting filter operands (page 677)
- Table filter operands (page 677)
- Setting up custom filters (page 678)
- Removing one filter (page 679)
- Removing all filters (page 679)
- Saving filters to a file (page 679)
- Applying saved filters (page 680)

Note: You cannot filter the sequential number column (first column) in the results table.

Filtering data rows

To filter the data rows in the table

1. In the filter row (below the column headers), select a filter operator.
2. Select a filter operand from the list of options for a particular filter column (if available), or type a value in the operand box for a condition.

   The table displays only the rows with values that fulfill the selected condition in the filter column.

   Note: Some of the operators do not apply to all of the available operands, or they can have other special operands.

   If you select operators and operands for multiple columns, the table shows only the rows with values that fulfill all of the conditions in the selected filter columns.

For more details, see the following topics:

- Selecting filter operators (page 674)
- Table filter operators (page 675)
- Selecting filter operands (page 677)
- Table filter operands (page 677)

Selecting filter operators

To filter the data in a table, select a filter operator from the list of options for a particular textual or numerical table column.

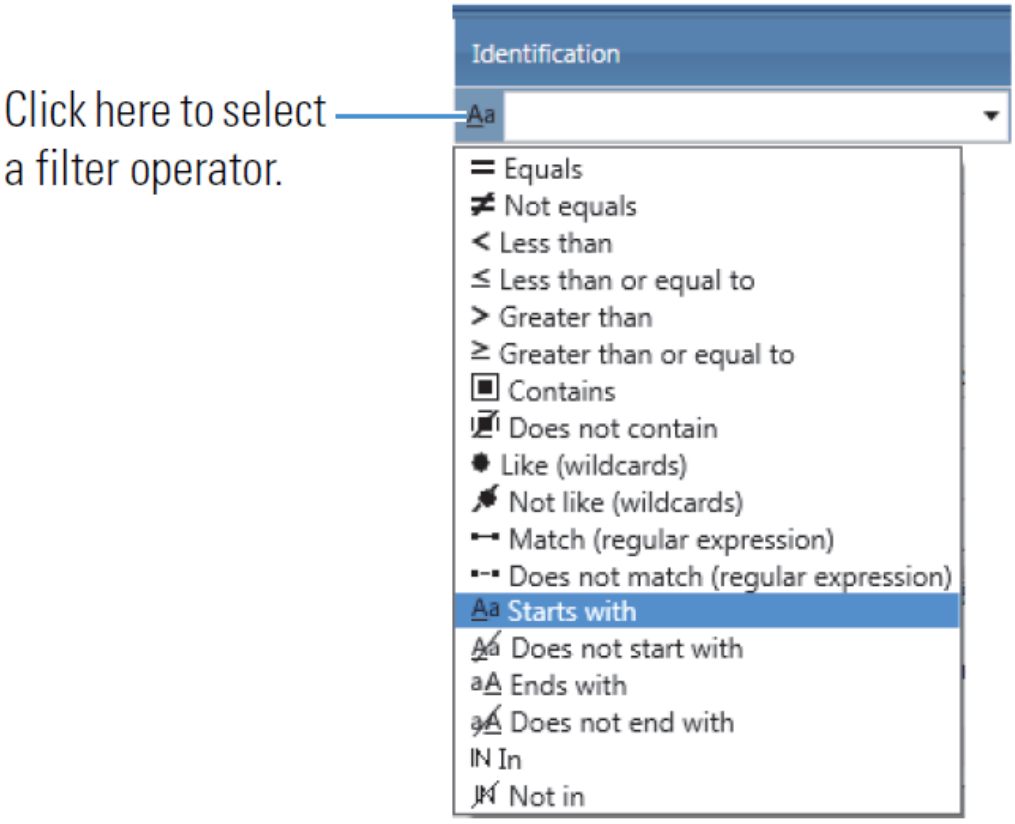


Figure 314 Selecting a filter operator for a textual column

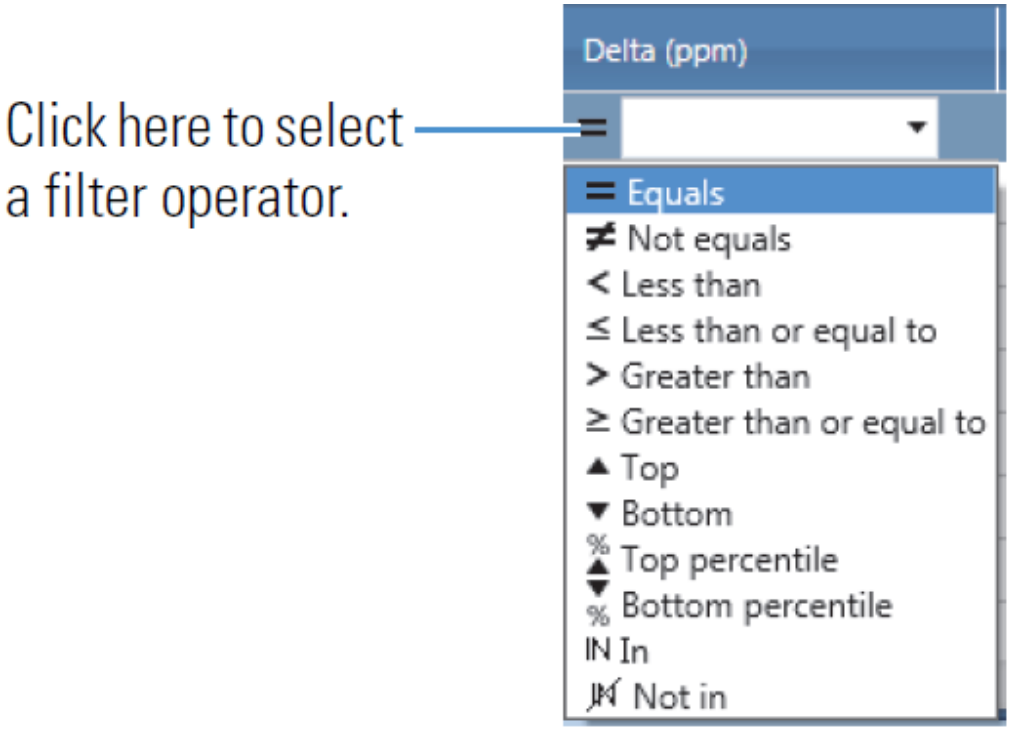


Figure 315 Selecting a filter operator for a numerical column

Table filter operators

The following table lists the various filter operators for the different types of data in the table columns.

Table 118 Filter operators for data in a column

Type of data	Filter operator	Condition description
Textual and Numerical	Equals	Data is equal to the selected filter operand[1] or a value that you enter in the operand box.
	Not equals	Data is not equal to the selected filter operand[1] or a value that you enter in the operand box.
	Less than	Data is less than the selected filter operand[1] or a value that you enter in the operand box.
	Less than or equal to	Data is less than or equal to the selected filter operand[1] or a value that you enter in the operand box.
	Greater than	Data is greater than the selected filter operand[1] or a value that you enter in the operand box.
	Greater than or equal to	Data is greater than the selected filter operand[1] or a value that you enter in the operand box.
	In	Data contains the selected filter operand[1] or a substring that you enter in the operand box.

Type of data	Filter operator	Condition description
Textual and Numerical	Not in	Data does not contain the selected filter operand[1] or a substring that you enter in the operand box.
Textual	Contains	Data contains the selected filter operand[1] or text that you enter in the operand box.
	Does not contain	Data does not contain the selected filter operand[1] or text that you enter in the operand box.
	Like (wildcards)	Data is like the selected filter operand[1], or text and a wildcard character that you enter in the operand box. Use these wildcard characters: • "*" to replace any characters • "?" to replace one character
	Not like (wildcards)	Data are not like the selected filter operand[1], or text and a wildcard character that you enter in the operand box. Use these wildcard characters: • "*" to replace any characters • "?" to replace one character
	Match (regular expression)	Data matches a regular expression that you enter in the operand box.
	Does not match (regular expression)	Data does not match a regular expression that you enter in the operand box.
	Starts with	Data starts with the selected filter operand[1] or text that you enter in the operand box.
	Does not start with	Data does not start with the selected filter operand[1] or text that you enter in the operand box.
	Ends with	Data ends with the selected filter operand[1] or text that you enter in the operand box.
	Does not end with	Data does not end with the selected filter operand[1] or text that you enter in the operand box.
Numerical	Top	Enter a number, *n*, in the operand box. The filter column displays only the highest *n* numbers out of all the numbers in that column.
	Bottom	Enter a number, *m*, in the operand box. The filter column displays only the lowest *m* numbers out of all the numbers in that column.

Type of data	Filter operator	Condition description
Numerical	Top Percentile	Enter a number, *x*, in the operand box. The filter column displays only the highest *x*% out of all the numbers in that column.
	Bottom Percentile	Enter a number, *y*, in the operand box. The filter column displays only the lowest *y*% out of all the numbers in that column.

[1] For details, see Table 119.

Selecting filter operands

To filter the data in a table, select a filter operand from the list of options for a particular textual or numerical table column (if available), or enter a value in the operand box.

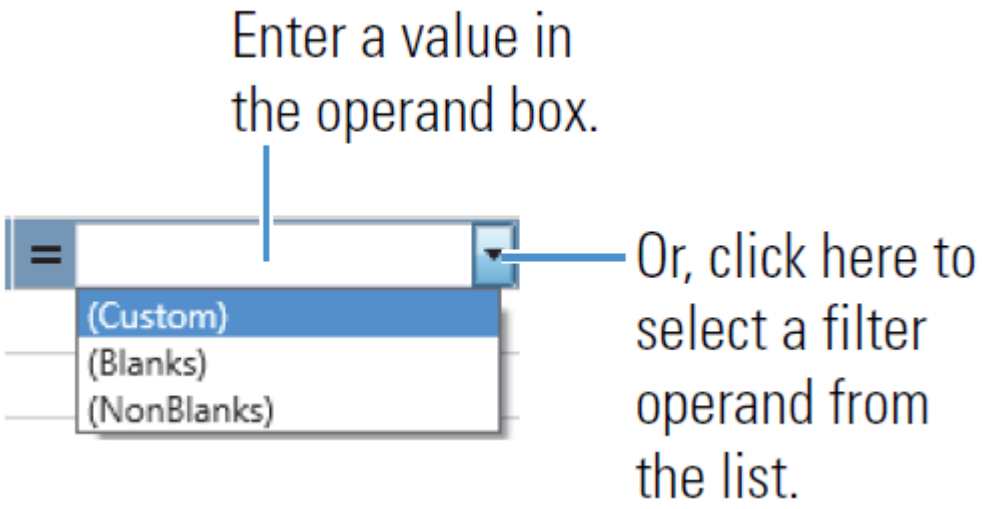


Figure 316 Entering or selecting a filter operand

Table filter operands

The following table lists the various filter operands for the different types of data in the table columns.

Table 119 Filter operands

Type of data	Filter operand	Operand description
Textual	Custom	Set up groups of multiple filter conditions.
	Blanks	Blank value
	NonBlanks	Not a blank value
	Text	A specific *text string*

Type of data	Filter operand	Operand description
Numerical	Custom	Set up groups of multiple filter conditions.
	Blanks	Blank value
	NonBlanks	Not a blank value
	Number	A specific *number*

Setting up custom filters

To set up a custom filter for a table by grouping multiple conditions

1. Select **(Custom)** from the filter operand list.
2. In the Custom Filter Selection dialog box, do the following for each condition that you want to add to the group:
   a. Click **Add Condition**.
   b. Select an operator from the Operator list, and then select an operand from the Operand list or type a specific text string or number.

   By default, the application applies the AND operator to all of the conditions in the group.

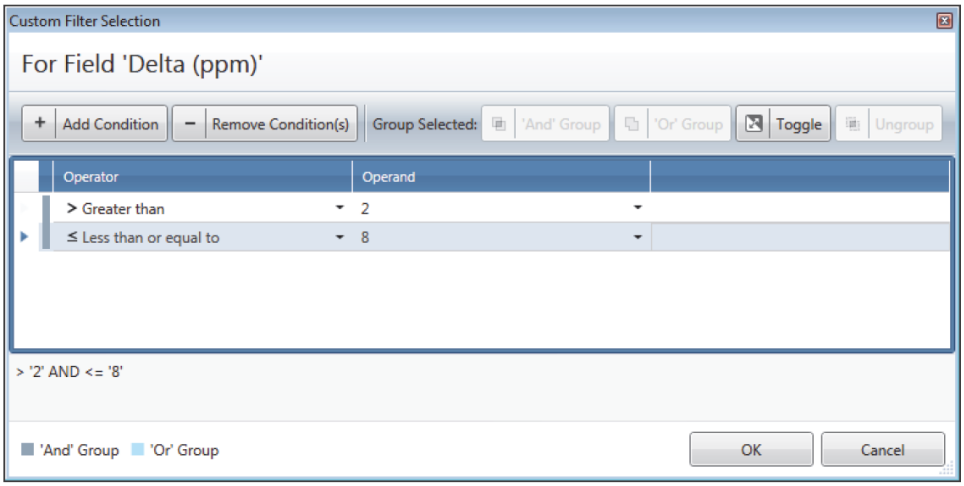


Figure 317 Custom Filter Selection dialog box

3. Click the space by the arrow icon, ▶, to the left of each condition row to select the row.

Tip: Select either contiguous rows by using the SHIFT key or noncontiguous rows using the CTRL key. Or, drag your pointer across the group of rows.

4. To change from applying the AND operator for the group to applying the OR operator, and vice versa, click **Toggle**.

   A gray bar to the left of the condition rows indicates an AND group. This custom filter passes only if all of the conditions in the group pass.

   A blue bar to the left of the condition rows indicates an OR group. This custom filter passes if any of the conditions in the group passes.

5. Click **OK**.

   The application applies the custom filter to the data in the selected filter column.

Removing one filter

To remove an individual filter in a table

1. Place your cursor in a particular filter operand box.
2. Select the filter value that you previously typed or selected in this box and then press the DELETE key.

Removing all filters

To remove all filters in a table

Click the funnel icon,  , to the left of the filter row.

–or–

(For Peptide Mapping Analysis only) Right-click the Results table on the Process and Review Page and choose **Filters ▸ Clear All**.

Saving filters to a file

To save the table filters to a file for Peptide Mapping Analysis

1. Right-click the Results table on the Process and Review Page and choose **Filters ▸ Save As**.

   The Save As dialog box opens.

2. Select a folder and a file with the .cfg extension to save all of the current filters at the top level (component level) in the table.

Applying saved filters

To apply the saved table filters from a file for Peptide Mapping Analysis

1. Right-click the Results table on the Process and Review Page and choose **Filters ▸ Apply**.

   The Open dialog box opens.

2. Select the file with the .cfg extension that contains the saved filters, and then click **Open**.

   The application applies all of the saved filters to the Results table.

   Note: All hidden columns become visible when you apply the saved filters, including the Sequence Variants column. This column is normally hidden until you set the Search for Amino Acid Substitutions option on the Identification page of the processing method to a value other than "None".
   When you save an experiment, the application does not save the filters automatically. You must manually save the filters to a file before saving the experiment, and then apply the saved filters when you reopen that experiment.

37 Glycans

See the following topics for information about the N-linked and O-linked glycans.

N-Linked glycans in the define modification list window

The following table lists the N-linked glycans that the BioPharma Finder application includes in the Define Modification List window.

Table 120 N-Linked glycans in the Define Modification List window

#	Glycan name	Formula	Average mass	Monoisotopic mass	Modification type
1	A1G0	C42H69N3O30	1095.40	1095.40	Side Chain
2	A1G0F	C48H79N3O34	1241.45	1241.45	Side Chain
3	A1G0M4	C48H79N3O35	1257.45	1257.45	Side Chain
4	A1G0M4F	C54H89N3O39	1403.51	1403.51	Side Chain
5	A1G0M5	C54H89N3O40	1419.50	1419.50	Side Chain
6	A1G0M5F	C60H99N3O44	1565.56	1565.56	Side Chain
7	A1G1	C48H79N3O35	1257.45	1257.45	Side Chain
8	A1G1F	C54H89N3O39	1403.51	1403.51	Side Chain
9	A1G1M4	C54H89N3O40	1419.50	1419.50	Side Chain
10	A1G1M4F	C60H99N3O44	1565.56	1565.56	Side Chain
11	A1G1M5	C60H99N3O45	1581.56	1581.56	Side Chain
12	A1G1M5F	C66H109N3O49	1727.61	1727.61	Side Chain
13	A1S1	C59H96N4O43	1548.54	1548.54	Side Chain
14	A1S1F	C65H106N4O47	1694.60	1694.60	Side Chain

#	Glycan name	Formula	Average mass	Monoisotopic mass	Modification type
15	A1S1M4	C65H106N4O48	1710.60	1710.60	Side Chain
16	A1S1M4F	C71H116N4O52	1856.66	1856.66	Side Chain
17	A1S1M5	C71H116N4O53	1872.65	1872.65	Side Chain
18	A1S1M5F	C77H126N4O57	2018.71	2018.71	Side Chain
19	A1Sg1	C59H96N4O44	1564.54	1564.54	Side Chain
20	A1Sg1F	C65H106N4O48	1710.60	1710.60	Side Chain
21	A2G0	C50H82N4O35	1298.48	1298.48	Side Chain
22	A2G0B	C58H95N5O40	1501.56	1501.56	Side Chain
23	A2G0F	C56H92N4O39	1444.53	1444.53	Side Chain
24	A2G0FB	C64H105N5O44	1647.61	1647.61	Side Chain
25	A2G0M4	C56H92N4O40	1460.53	1460.53	Side Chain
26	A2G0M5	C62H102N4O45	1622.58	1622.58	Side Chain
27	A2G0M5F	C68H112N4O49	1768.64	1768.64	Side Chain
28	A2G1	C56H92N4O40	1460.53	1460.53	Side Chain
29	A2G1B	C64H105N5O45	1663.61	1663.61	Side Chain
30	A2G1F	C62H102N4O44	1606.59	1606.59	Side Chain
31	A2G1FB	C70H115N5O49	1809.67	1809.67	Side Chain
32	A2G1M4	C62H102N4O45	1622.58	1622.58	Side Chain
33	A2G1M4F	C68H112N4O49	1768.64	1768.64	Side Chain
34	A2G1M5	C68H112N4O50	1784.63	1784.63	Side Chain
35	A2G1M5F	C74H122N4O54	1930.69	1930.69	Side Chain
36	A2G2	C62H102N4O45	1622.58	1622.58	Side Chain
37	A2G2B	C70H115N5O50	1825.66	1825.66	Side Chain
38	A2G2F	C68H112N4O49	1768.64	1768.64	Side Chain
39	A2G2FB	C76H125N5O54	1971.72	1971.72	Side Chain
40	A2G2M4	C68H112N4O50	1784.63	1784.63	Side Chain
41	A2G2M4F	C74H122N4O54	1930.69	1930.69	Side Chain
42	A2G2M5	C74H122N4O55	1946.69	1946.69	Side Chain

#	Glycan name	Formula	Average mass	Monoisotopic mass	Modification type
43	A2G2M5F	C80H132N4O59	2092.75	2092.75	Side Chain
44	A2S1G0	C67H109N5O48	1751.62	1751.62	Side Chain
45	A2S1G0B	C75H122N6O53	1954.70	1954.70	Side Chain
46	A2S1G0F	C73H119N5O52	1897.68	1897.68	Side Chain
47	A2S1G0FB	C81H132N6O57	2100.76	2100.76	Side Chain
48	A2S1G0M4	C73H119N5O53	1913.68	1913.68	Side Chain
49	A2S1G0M4F	C79H129N5O57	2059.73	2059.73	Side Chain
50	A2S1G0M5	C79H129N5O58	2075.73	2075.73	Side Chain
51	A2S1G0M5F	C85H139N5O62	2221.79	2221.79	Side Chain
52	A2S1G1	C73H119N5O53	1913.68	1913.68	Side Chain
53	A2S1G1B	C81H132N6O58	2116.76	2116.76	Side Chain
54	A2S1G1F	C79H129N5O57	2059.73	2059.73	Side Chain
55	A2S1G1FB	C87H142N6O62	2262.81	2262.81	Side Chain
56	A2S1G1M4	C79H129N5O58	2075.73	2075.73	Side Chain
57	A2S1G1M4F	C85H139N5O62	2221.79	2221.79	Side Chain
58	A2S1G1M5	C85H139N5O63	2237.78	2237.78	Side Chain
59	A2S1G1M5F	C91H149N5O67	2383.84	2383.84	Side Chain
60	A2S2	C84H136N6O61	2204.77	2204.77	Side Chain
61	A2S2B	C92H149N7O66	2407.85	2407.85	Side Chain
62	A2S2F	C90H146N6O65	2350.83	2350.83	Side Chain
63	A2S2FB	C98H159N7O70	2553.91	2553.91	Side Chain
64	A2S2M4	C90H146N6O66	2366.83	2366.83	Side Chain
65	A2S2M4F	C96H156N6O70	2512.88	2512.88	Side Chain
66	A2S2M5	C96H156N6O71	2528.88	2528.88	Side Chain
67	A2S2M5F	C102H166N6O75	2674.94	2674.94	Side Chain
68	A2Sg1G0	C67H109N5O49	1767.62	1767.62	Side Chain
69	A2Sg1G0F	C73H119N5O53	1913.68	1913.68	Side Chain
70	A2Sg1G1	C73H119N5O54	1929.67	1929.67	Side Chain

#	Glycan name	Formula	Average mass	Monoisotopic mass	Modification type
71	A2Sg1G1F	C79H129N5O58	2075.73	2075.73	Side Chain
72	A2Sg1S1	C84H136N6O62	2220.77	2220.77	Side Chain
73	A2Sg1S1F	C90H146N6O66	2366.83	2366.83	Side Chain
74	A2Sg2	C84H136N6O63	2236.76	2236.76	Side Chain
75	A2Sg2F	C90H146N6O67	2382.82	2382.82	Side Chain
76	A3G0	C58H95N5O40	1501.56	1501.56	Side Chain
77	A3G0F	C64H105N5O44	1647.61	1647.61	Side Chain
78	A3G1	C64H105N5O45	1663.61	1663.61	Side Chain
79	A3G1F	C70H115N5O49	1809.67	1809.67	Side Chain
80	A3G2	C70H115N5O50	1825.66	1825.66	Side Chain
81	A3G2F	C76H125N5O54	1971.72	1971.72	Side Chain
82	A3G3	C76H125N5O55	1987.71	1987.71	Side Chain
83	A3G3F	C82H135N5O59	2133.77	2133.77	Side Chain
84	A3S1G0	C75H122N6O53	1954.70	1954.70	Side Chain
85	A3S1G0F	C81H132N6O57	2100.76	2100.76	Side Chain
86	A3S1G1	C81H132N6O58	2116.76	2116.76	Side Chain
87	A3S1G1F	C87H142N6O62	2262.81	2262.81	Side Chain
88	A3S1G2	C87H142N6O63	2278.81	2278.81	Side Chain
89	A3S1G2F	C93H152N6O67	2424.87	2424.87	Side Chain
90	A3S2G0	C92H149N7O66	2407.85	2407.85	Side Chain
91	A3S2G0F	C98H159N7O70	2553.91	2553.91	Side Chain
92	A3S2G1	C98H159N7O71	2569.90	2569.90	Side Chain
93	A3S2G1F	C104H169N7O75	2715.96	2715.96	Side Chain
94	A3S3	C109H176N8O79	2861.00	2861.00	Side Chain
95	A3S3F	C115H186N8O83	3007.06	3007.06	Side Chain
96	A3Sg1G0	C75H122N6O54	1970.70	1970.70	Side Chain
97	A3Sg1G0F	C81H132N6O58	2116.76	2116.76	Side Chain
98	A3Sg1G1	C81H132N6O59	2132.75	2132.75	Side Chain

#	Glycan name	Formula	Average mass	Monoisotopic mass	Modification type
99	A3Sg1G1F	C87H142N6O63	2278.81	2278.81	Side Chain
100	A3Sg1G2	C87H142N6O64	2294.80	2294.80	Side Chain
101	A3Sg1G2F	C93H152N6O68	2440.86	2440.86	Side Chain
102	A3Sg1S1G0	C92H149N7O67	2423.85	2423.85	Side Chain
103	A3Sg1S1G0F	C98H159N7O71	2569.90	2569.90	Side Chain
104	A3Sg1S1G1	C98H159N7O72	2585.90	2585.90	Side Chain
105	A3Sg1S1G1F	C104H169N7O76	2731.96	2731.96	Side Chain
106	A3Sg1S2	C109H176N8O80	2876.99	2876.99	Side Chain
107	A3Sg1S2F	C115H186N8O84	3023.05	3023.05	Side Chain
108	A3Sg2G0	C92H149N7O68	2439.84	2439.84	Side Chain
109	A3Sg2G0F	C98H159N7O72	2585.90	2585.90	Side Chain
110	A3Sg2G1	C98H159N7O73	2601.89	2601.89	Side Chain
111	A3Sg2G1F	C104H169N7O77	2747.95	2747.95	Side Chain
112	A3Sg2S1	C109H176N8O81	2892.99	2892.99	Side Chain
113	A3Sg2S1F	C115H186N8O85	3039.05	3039.05	Side Chain
114	A3Sg3	C109H176N8O82	2908.98	2908.98	Side Chain
115	A3Sg3F	C115H186N8O86	3055.04	3055.04	Side Chain
116	A4G0	C66H108N6O45	1704.63	1704.63	Side Chain
117	A4G0F	C72H118N6O49	1850.69	1850.69	Side Chain
118	A4G1	C72H118N6O50	1866.69	1866.69	Side Chain
119	A4G1F	C78H128N6O54	2012.75	2012.75	Side Chain
120	A4G2	C78H128N6O55	2028.74	2028.74	Side Chain
121	A4G2F	C84H138N6O59	2174.80	2174.80	Side Chain
122	A4G3	C84H138N6O60	2190.79	2190.79	Side Chain
123	A4G3F	C90H148N6O64	2336.85	2336.85	Side Chain
124	A4G4	C90H148N6O65	2352.85	2352.85	Side Chain
125	A4G4F	C96H158N6O69	2498.90	2498.90	Side Chain
126	A4S1G0	C83H135N7O58	2157.78	2157.78	Side Chain

#	Glycan name	Formula	Average mass	Monoisotopic mass	Modification type
127	A4S1G0F	C89H145N7O62	2303.84	2303.84	Side Chain
128	A4S1G1	C89H145N7O63	2319.84	2319.84	Side Chain
129	A4S1G1F	C95H155N7O67	2465.89	2465.89	Side Chain
130	A4S1G2	C95H155N7O68	2481.89	2481.89	Side Chain
131	A4S1G2F	C101H165N7O72	2627.95	2627.95	Side Chain
132	A4S1G3	C101H165N7O73	2643.94	2643.94	Side Chain
133	A4S1G3F	C107H175N7O77	2790.00	2790.00	Side Chain
134	A4S2G0	C100H162N8O71	2610.93	2610.93	Side Chain
135	A4S2G0F	C106H172N8O75	2756.99	2756.99	Side Chain
136	A4S2G1	C106H172N8O76	2772.98	2772.98	Side Chain
137	A4S2G1F	C112H182N8O80	2919.04	2919.04	Side Chain
138	A4S2G2	C112H182N8O81	2935.04	2935.04	Side Chain
139	A4S2G2F	C118H192N8O85	3081.09	3081.09	Side Chain
140	A4S3G0	C117H189N9O84	3064.08	3064.08	Side Chain
141	A4S3G0F	C123H199N9O88	3210.14	3210.14	Side Chain
142	A4S3G1	C123H199N9O89	3226.13	3226.13	Side Chain
143	A4S3G1F	C129H209N9O93	3372.19	3372.19	Side Chain
144	A4S4	C134H216N10O97	3517.23	3517.23	Side Chain
145	A4S4F	C140H226N10O101	3663.29	3663.29	Side Chain
146	A4Sg1G0	C83H135N7O59	2173.78	2173.78	Side Chain
147	A4Sg1G0F	C89H145N7O63	2319.84	2319.84	Side Chain
148	A4Sg1G1	C89H145N7O64	2335.83	2335.83	Side Chain
149	A4Sg1G1F	C95H155N7O68	2481.89	2481.89	Side Chain
150	A4Sg1G2	C95H155N7O69	2497.88	2497.88	Side Chain
151	A4Sg1G2F	C101H165N7O73	2643.94	2643.94	Side Chain
152	A4Sg1G3	C101H165N7O74	2659.94	2659.94	Side Chain
153	A4Sg1G3F	C107H175N7O78	2805.99	2805.99	Side Chain
154	A4Sg1S1G0	C100H162N8O72	2626.93	2626.93	Side Chain

#	Glycan name	Formula	Average mass	Monoisotopic mass	Modification type
155	A4Sg1S1G0F	C106H172N8O76	2772.98	2772.98	Side Chain
156	A4Sg1S1G1	C106H172N8O77	2788.98	2788.98	Side Chain
157	A4Sg1S1G1F	C112H182N8O81	2935.04	2935.04	Side Chain
158	A4Sg1S1G2	C112H182N8O82	2951.03	2951.03	Side Chain
159	A4Sg1S1G2F	C118H192N8O86	3097.09	3097.09	Side Chain
160	A4Sg1S2G0	C117H189N9O85	3080.07	3080.07	Side Chain
161	A4Sg1S2G0F	C123H199N9O89	3226.13	3226.13	Side Chain
162	A4Sg1S2G1	C123H199N9O90	3242.13	3242.13	Side Chain
163	A4Sg1S2G1F	C129H209N9O94	3388.19	3388.19	Side Chain
164	A4Sg1S3	C134H216N10O98	3533.22	3533.22	Side Chain
165	A4Sg1S3F	C140H226N10O102	3679.28	3679.28	Side Chain
166	A4Sg2G0	C100H162N8O73	2642.92	2642.92	Side Chain
167	A4Sg2G0F	C106H172N8O77	2788.98	2788.98	Side Chain
168	A4Sg2G1	C106H172N8O78	2804.97	2804.97	Side Chain
169	A4Sg2G1F	C112H182N8O82	2951.03	2951.03	Side Chain
170	A4Sg2G2	C112H182N8O83	2967.03	2967.03	Side Chain
171	A4Sg2G2F	C118H192N8O87	3113.08	3113.08	Side Chain
172	A4Sg2S1G0	C117H189N9O86	3096.07	3096.07	Side Chain
173	A4Sg2S1G0F	C123H199N9O90	3242.13	3242.13	Side Chain
174	A4Sg2S1G1	C123H199N9O91	3258.12	3258.12	Side Chain
175	A4Sg2S1G1F	C129H209N9O95	3404.18	3404.18	Side Chain
176	A4Sg2S2	C134H216N10O99	3549.22	3549.22	Side Chain
177	A4Sg2S2F	C140H226N10O103	3695.28	3695.28	Side Chain
178	A4Sg3G0	C117H189N9O87	3112.06	3112.06	Side Chain
179	A4Sg3G0F	C123H199N9O91	3258.12	3258.12	Side Chain
180	A4Sg3G1	C123H199N9O92	3274.12	3274.12	Side Chain
181	A4Sg3G1F	C129H209N9O96	3420.18	3420.18	Side Chain
182	A4Sg3S1	C134H216N10O100	3565.21	3565.21	Side Chain

#	Glycan name	Formula	Average mass	Monoisotopic mass	Modification type
183	A4Sg3S1F	C140H226N10O104	3711.27	3711.27	Side Chain
184	A4Sg4	C134H216N10O101	3581.21	3581.21	Side Chain
185	A4Sg4F	C140H226N10O105	3727.27	3727.27	Side Chain
186	Gn	C8H13N1O5	203.08	203.079	Side Chain
187	GnF	C14H23N1O9	349.14	349.137	Side Chain
188	M3	C34H56N2O25	892.32	892.317	Side Chain
189	M4	C40H66N2O30	1054.37	1054.37	Side Chain
190	M5	C46H76N2O35	1216.42	1216.42	Side Chain
191	M6	C52H86N2O40	1378.48	1378.48	Side Chain
192	M7	C58H96N2O45	1540.53	1540.53	Side Chain
193	M8	C64H106N2O50	1702.58	1702.58	Side Chain
194	M9	C70H116N2O55	1864.63	1864.63	Side Chain
195	G0_G0F	C106H174N8O74S0	2744.54	2743.010	Side Chain
196	G0F_G0F	C112H184N8O78S0	2890.68	2889.068	Side Chain
197	G0F_G1F	C118H194N8O83S0	3052.82	3051.121	Side Chain
198	G1F_G1F	C124H204N8O88S0	3214.96	3213.173	Side Chain
199	G1F_G2F	C130H214N8O93S0	3377.10	3375.226	Side Chain
200	G2F_G2F	C136H224N8O98S0	3539.24	3537.279	Side Chain

N-Linked glycans with a CHO host Cell-Line type

The following table lists the N-linked glycans, sorted by CHO host cell-line type, that are included in the N-glycan-specific search.

Table 121 N-Linked glycans with a CHO host cell-line type

#	Glycan name	Antenna 1	Antenna 2	Antenna 3	Antenna 4	Formula	Monoisotopic mass	Man	Fuc	Bisecting Gn	Host cell line
1	M3	None	None	None	None	C34H56N2O25	892.317	3	0	0	CHO-N-glycan
2	M4	None	None	None	None	C40H66N2O30	1054.37	4	0	0	CHO-N-glycan
3	M5	None	None	None	None	C46H76N2O35	1216.42	5	0	0	CHO-N-glycan
4	M6	None	None	None	None	C52H86N2O40	1378.48	6	0	0	CHO-N-glycan
5	M7	None	None	None	None	C58H96N2O45	1540.53	7	0	0	CHO-N-glycan
6	M8	None	None	None	None	C64H106N2O50	1702.58	8	0	0	CHO-N-glycan
7	M9	None	None	None	None	C70H116N2O55	1864.63	9	0	0	CHO-N-glycan
8	A1G0	Gn-	None	None	None	C42H69N3O30	1095.4	3	0	0	CHO-N-glycan
9	A1G0F	Gn-	None	None	None	C48H79N3O34	1241.45	3	1	0	CHO-N-glycan
10	A1G0M4	Gn-	None	None	None	C48H79N3O35	1257.45	4	0	0	CHO-N-glycan
11	A1G0M4F	Gn-	None	None	None	C54H89N3O39	1403.51	4	1	0	CHO-N-glycan

#	Glycan name	Antenna 1	Antenna 2	Antenna 3	Antenna 4	Formula	Monoisotopic mass	Man	Fuc	Bisecting Gn	Host cell line
12	A1G0M5	Gn-	None	None	None	C54H89N3O40	1419.5	5	0	0	CHO-N-glycan
13	A1G0M5F	Gn-	None	None	None	C60H99N3O44	1565.56	5	1	0	CHO-N-glycan
14	A2G0	Gn-	None	Gn-	None	C50H82N4O35	1298.48	3	0	0	CHO-N-glycan
15	A2G0F	Gn-	None	Gn-	None	C56H92N4O39	1444.53	3	1	0	CHO-N-glycan
16	A2G0M4	Gn-	Gn-	None	None	C56H92N4O40	1460.53	4	0	0	CHO-N-glycan
17	A2G0M5	Gn-	Gn-	None	None	C62H102N4O45	1622.58	5	0	0	CHO-N-glycan
18	A2G0M5F	Gn-	Gn-	None	None	C68H112N4O49	1768.64	5	1	0	CHO-N-glycan
19	A3G0	Gn-	Gn-	Gn-	None	C58H95N5O40	1501.56	3	0	0	CHO-N-glycan
20	A3G0F	Gn-	Gn-	Gn-	None	C64H105N5O44	1647.61	3	1	0	CHO-N-glycan
21	A4G0	Gn-	Gn-	Gn-	Gn-	C66H108N6O45	1704.63	3	0	0	CHO-N-glycan
22	A4G0F	Gn-	Gn-	Gn-	Gn-	C72H118N6O49	1850.69	3	1	0	CHO-N-glycan
23	A1G1	G-Gn-	None	None	None	C48H79N3O35	1257.45	3	0	0	CHO-N-glycan
24	A1G1F	G-Gn-	None	None	None	C54H89N3O39	1403.51	3	1	0	CHO-N-glycan

#	Glycan name	Antenna 1	Antenna 2	Antenna 3	Antenna 4	Formula	Monoisotopic mass	Man	Fuc	Bisecting Gn	Host cell line
25	A1G1M4	G-Gn-	None	None	None	C54H89N3O40	1419.5	4	0	0	CHO-N-glycan
26	A1G1M4F	G-Gn-	None	None	None	C60H99N3O44	1565.56	4	1	0	CHO-N-glycan
27	A1G1M5	G-Gn-	None	None	None	C60H99N3O45	1581.56	5	0	0	CHO-N-glycan
28	A1G1M5F	G-Gn-	None	None	None	C66H109N3O49	1727.61	5	1	0	CHO-N-glycan
29	A2G1	G-Gn-	None	Gn-	None	C56H92N4O40	1460.53	3	0	0	CHO-N-glycan
30	A2G1F	G-Gn-	None	Gn-	None	C62H102N4O44	1606.59	3	1	0	CHO-N-glycan
31	A2G2	G-Gn-	None	G-Gn-	None	C62H102N4O45	1622.58	3	0	0	CHO-N-glycan
32	A2G2F	G-Gn-	None	G-Gn-	None	C68H112N4O49	1768.64	3	1	0	CHO-N-glycan
33	A2G1M4	G-Gn-	Gn-	None	None	C62H102N4O45	1622.58	4	0	0	CHO-N-glycan
34	A2G1M4F	G-Gn-	Gn-	None	None	C68H112N4O49	1768.64	4	1	0	CHO-N-glycan
35	A2G1M5	G-Gn-	Gn-	None	None	C68H112N4O50	1784.63	5	0	0	CHO-N-glycan
36	A2G1M5F	G-Gn-	Gn-	None	None	C74H122N4O54	1930.69	5	1	0	CHO-N-glycan
37	A3G1	G-Gn-	Gn-	Gn-	None	C64H105N5O45	1663.61	3	0	0	CHO-N-glycan

#	Glycan name	Antenna 1	Antenna 2	Antenna 3	Antenna 4	Formula	Monoisotopic mass	Man	Fuc	Bisecting Gn	Host cell line
38	A3G1F	G-Gn-	Gn-	Gn-	None	C70H115N5O49	1809.67	3	1	0	CHO-N-glycan
39	A4G1	G-Gn-	Gn-	Gn-	Gn-	C72H118N6O50	1866.69	3	0	0	CHO-N-glycan
40	A4G1F	G-Gn-	Gn-	Gn-	Gn-	C78H128N6O54	2012.75	3	1	0	CHO-N-glycan
41	A2G2M4	G-Gn-	G-Gn-	None	None	C68H112N4O50	1784.63	4	0	0	CHO-N-glycan
42	A2G2M4F	G-Gn-	G-Gn-	None	None	C74H122N4O54	1930.69	4	1	0	CHO-N-glycan
43	A2G2M5	G-Gn-	G-Gn-	None	None	C74H122N4O55	1946.69	5	0	0	CHO-N-glycan
44	A2G2M5F	G-Gn-	G-Gn-	None	None	C80H132N4O59	2092.75	5	1	0	CHO-N-glycan
45	A3G2	G-Gn-	G-Gn-	Gn-	None	C70H115N5O50	1825.66	3	0	0	CHO-N-glycan
46	A3G2F	G-Gn-	G-Gn-	Gn-	None	C76H125N5O54	1971.72	3	1	0	CHO-N-glycan
47	A4G2	G-Gn-	G-Gn-	Gn-	Gn-	C78H128N6O55	2028.74	3	0	0	CHO-N-glycan
48	A4G2F	G-Gn-	G-Gn-	Gn-	Gn-	C84H138N6O59	2174.8	3	1	0	CHO-N-glycan
49	A3G3	G-Gn-	G-Gn-	G-Gn-	None	C76H125N5O55	1987.71	3	0	0	CHO-N-glycan
50	A3G3F	G-Gn-	G-Gn-	G-Gn-	None	C82H135N5O59	2133.77	3	1	0	CHO-N-glycan

#	Glycan name	Antenna 1	Antenna 2	Antenna 3	Antenna 4	Formula	Monoisotopic mass	Man	Fuc	Bisecting Gn	Host cell line
51	A4G3	G-Gn-	G-Gn-	G-Gn-	Gn-	C84H138N6O60	2190.79	3	0	0	CHO-N-glycan
52	A4G3F	G-Gn-	G-Gn-	G-Gn-	Gn-	C90H148N6O64	2336.85	3	1	0	CHO-N-glycan
53	A4G4	G-Gn-	G-Gn-	G-Gn-	G-Gn-	C90H148N6O65	2352.85	3	0	0	CHO-N-glycan
54	A4G4F	G-Gn-	G-Gn-	G-Gn-	G-Gn-	C96H158N6O69	2498.9	3	1	0	CHO-N-glycan
55	A1S1	S-G-Gn-	None	None	None	C59H96N4O43	1548.54	3	0	0	CHO-N-glycan
56	A1S1F	S-G-Gn-	None	None	None	C65H106N4O47	1694.6	3	1	0	CHO-N-glycan
57	A1S1M4	S-G-Gn-	None	None	None	C65H106N4O48	1710.6	4	0	0	CHO-N-glycan
58	A1S1M4F	S-G-Gn-	None	None	None	C71H116N4O52	1856.66	4	1	0	CHO-N-glycan
59	A1S1M5	S-G-Gn-	None	None	None	C71H116N4O53	1872.65	5	0	0	CHO-N-glycan
60	A1S1M5F	S-G-Gn-	None	None	None	C77H126N4O57	2018.71	5	1	0	CHO-N-glycan
61	A2S1G0	S-G-Gn-	None	Gn-	None	C67H109N5O48	1751.62	3	0	0	CHO-N-glycan
62	A2S1G0F	S-G-Gn-	None	Gn-	None	C73H119N5O52	1897.68	3	1	0	CHO-N-glycan
63	A2S1G1	S-G-Gn-	None	G-Gn-	None	C73H119N5O53	1913.68	3	0	0	CHO-N-glycan

#	Glycan name	Antenna 1	Antenna 2	Antenna 3	Antenna 4	Formula	Monoisotopic mass	Man	Fuc	Bisecting Gn	Host cell line
64	A2S1G1F	S-G-Gn-	None	G-Gn-	None	C79H129N5O57	2059.73	3	1	0	CHO-N-glycan
65	A2S2	S-G-Gn-	None	S-G-Gn-	None	C84H136N6O61	2204.77	3	0	0	CHO-N-glycan
66	A2S2F	S-G-Gn-	None	S-G-Gn-	None	C90H146N6O65	2350.83	3	1	0	CHO-N-glycan
67	A2S1G0M4	S-G-Gn-	Gn-	None	None	C73H119N5O53	1913.68	4	0	0	CHO-N-glycan
68	A2S1G0M4F	S-G-Gn-	Gn-	None	None	C79H129N5O57	2059.73	4	1	0	CHO-N-glycan
69	A2S1G0M5	S-G-Gn-	Gn-	None	None	C79H129N5O58	2075.73	5	0	0	CHO-N-glycan
70	A2S1G0M5F	S-G-Gn-	Gn-	None	None	C85H139N5O62	2221.79	5	1	0	CHO-N-glycan
71	A3S1G0	S-G-Gn-	Gn-	Gn-	None	C75H122N6O53	1954.7	3	0	0	CHO-N-glycan
72	A3S1G0F	S-G-Gn-	Gn-	Gn-	None	C81H132N6O57	2100.76	3	1	0	CHO-N-glycan
73	A4S1G0	S-G-Gn-	Gn-	Gn-	Gn-	C83H135N7O58	2157.78	3	0	0	CHO-N-glycan
74	A4S1G0F	S-G-Gn-	Gn-	Gn-	Gn-	C89H145N7O62	2303.84	3	1	0	CHO-N-glycan
75	A2S1G1M4	S-G-Gn-	G-Gn-	None	None	C79H129N5O58	2075.73	4	0	0	CHO-N-glycan
76	A2S1G1M4F	S-G-Gn-	G-Gn-	None	None	C85H139N5O62	2221.79	4	1	0	CHO-N-glycan

#	Glycan name	Antenna 1	Antenna 2	Antenna 3	Antenna 4	Formula	Monoisotopic mass	Man	Fuc	Bisecting Gn	Host cell line
77	A2S1G1M5	S-G-Gn-	G-Gn-	None	None	C85H139N5O63	2237.78	5	0	0	CHO-N-glycan
78	A2S1G1M5F	S-G-Gn-	G-Gn-	None	None	C91H149N5O67	2383.84	5	1	0	CHO-N-glycan
79	A3S1G1	S-G-Gn-	G-Gn-	Gn-	None	C81H132N6O58	2116.76	3	0	0	CHO-N-glycan
80	A3S1G1F	S-G-Gn-	G-Gn-	Gn-	None	C87H142N6O62	2262.81	3	1	0	CHO-N-glycan
81	A4S1G1	S-G-Gn-	G-Gn-	Gn-	Gn-	C89H145N7O63	2319.84	3	0	0	CHO-N-glycan
82	A4S1G1F	S-G-Gn-	G-Gn-	Gn-	Gn-	C95H155N7O67	2465.89	3	1	0	CHO-N-glycan
83	A3S1G2	S-G-Gn-	G-Gn-	G-Gn-	None	C87H142N6O63	2278.81	3	0	0	CHO-N-glycan
84	A3S1G2F	S-G-Gn-	G-Gn-	G-Gn-	None	C93H152N6O67	2424.87	3	1	0	CHO-N-glycan
85	A4S1G2	S-G-Gn-	G-Gn-	G-Gn-	Gn-	C95H155N7O68	2481.89	3	0	0	CHO-N-glycan
86	A4S1G2F	S-G-Gn-	G-Gn-	G-Gn-	Gn-	C101H165N7O72	2627.95	3	1	0	CHO-N-glycan
87	A4S1G3	S-G-Gn-	G-Gn-	G-Gn-	G-Gn-	C101H165N7O73	2643.94	3	0	0	CHO-N-glycan
88	A4S1G3F	S-G-Gn-	G-Gn-	G-Gn-	G-Gn-	C107H175N7O77	2790	3	1	0	CHO-N-glycan
89	A2S2M4	S-G-Gn-	S-G-Gn-	None	None	C90H146N6O66	2366.83	4	0	0	CHO-N-glycan

#	Glycan name	Antenna 1	Antenna 2	Antenna 3	Antenna 4	Formula	Monoisotopic mass	Man	Fuc	Bisecting Gn	Host cell line
90	A2S2M4F	S-G-Gn-	S-G-Gn-	None	None	C96H156N6O70	2512.88	4	1	0	CHO-N-glycan
91	A2S2M5	S-G-Gn-	S-G-Gn-	None	None	C96H156N6O71	2528.88	5	0	0	CHO-N-glycan
92	A2S2M5F	S-G-Gn-	S-G-Gn-	None	None	C102H166N6O75	2674.94	5	1	0	CHO-N-glycan
93	A3S2G0	S-G-Gn-	S-G-Gn-	Gn-	None	C92H149N7O66	2407.85	3	0	0	CHO-N-glycan
94	A3S2G0F	S-G-Gn-	S-G-Gn-	Gn-	None	C98H159N7O70	2553.91	3	1	0	CHO-N-glycan
95	A4S2G0	S-G-Gn-	S-G-Gn-	Gn-	Gn-	C100H162N8O71	2610.93	3	0	0	CHO-N-glycan
96	A4S2G0F	S-G-Gn-	S-G-Gn-	Gn-	Gn-	C106H172N8O75	2756.99	3	1	0	CHO-N-glycan
97	A3S2G1	S-G-Gn-	S-G-Gn-	G-Gn-	None	C98H159N7O71	2569.9	3	0	0	CHO-N-glycan
98	A3S2G1F	S-G-Gn-	S-G-Gn-	G-Gn-	None	C104H169N7O75	2715.96	3	1	0	CHO-N-glycan
99	A4S2G1	S-G-Gn-	S-G-Gn-	G-Gn-	Gn-	C106H172N8O76	2772.98	3	0	0	CHO-N-glycan
100	A4S2G1F	S-G-Gn-	S-G-Gn-	G-Gn-	Gn-	C112H182N8O80	2919.04	3	1	0	CHO-N-glycan
101	A4S2G2	S-G-Gn-	S-G-Gn-	G-Gn-	G-Gn-	C112H182N8O81	2935.04	3	0	0	CHO-N-glycan
102	A4S2G2F	S-G-Gn-	S-G-Gn-	G-Gn-	G-Gn-	C118H192N8O85	3081.09	3	1	0	CHO-N-glycan

#	Glycan name	Antenna 1	Antenna 2	Antenna 3	Antenna 4	Formula	Monoisotopic mass	Man	Fuc	Bisecting Gn	Host cell line
103	A3S3	S-G-Gn-	S-G-Gn-	S-G-Gn-	None	C109H176N8O79	2861	3	0	0	CHO-N-glycan
104	A3S3F	S-G-Gn-	S-G-Gn-	S-G-Gn-	None	C115H186N8O83	3007.06	3	1	0	CHO-N-glycan
105	A4S3G0	S-G-Gn-	S-G-Gn-	S-G-Gn-	Gn-	C117H189N9O84	3064.08	3	0	0	CHO-N-glycan
106	A4S3G0F	S-G-Gn-	S-G-Gn-	S-G-Gn-	Gn-	C123H199N9O88	3210.14	3	1	0	CHO-N-glycan
107	A4S3G1	S-G-Gn-	S-G-Gn-	S-G-Gn-	G-Gn-	C123H199N9O89	3226.13	3	0	0	CHO-N-glycan
108	A4S3G1F	S-G-Gn-	S-G-Gn-	S-G-Gn-	G-Gn-	C129H209N9O93	3372.19	3	1	0	CHO-N-glycan
109	A4S4	S-G-Gn-	S-G-Gn-	S-G-Gn-	S-G-Gn-	C134H216N10O97	3517.23	3	0	0	CHO-N-glycan
110	A4S4F	S-G-Gn-	S-G-Gn-	S-G-Gn-	S-G-Gn-	C140H226N10O101	3663.29	3	1	0	CHO-N-glycan
111	A1Sg1	Sg-G-Gn-	None	None	None	C59H96N4O44	1564.54	3	0	0	CHO-N-glycan
112	A1Sg1F	Sg-G-Gn-	None	None	None	C65H106N4O48	1710.6	3	1	0	CHO-N-glycan
113	A2Sg1G0	Sg-G-Gn-	None	Gn-	None	C67H109N5O49	1767.62	3	0	0	CHO-N-glycan
114	A2Sg1G0F	Sg-G-Gn-	None	Gn-	None	C73H119N5O53	1913.68	3	1	0	CHO-N-glycan
115	A2Sg1G1	Sg-G-Gn-	None	G-Gn-	None	C73H119N5O54	1929.67	3	0	0	CHO-N-glycan

#	Glycan name	Antenna 1	Antenna 2	Antenna 3	Antenna 4	Formula	Monoisotopic mass	Man	Fuc	Bisecting Gn	Host cell line
116	A2Sg1G1F	Sg-G-Gn-	None	G-Gn-	None	C79H129N5O58	2075.73	3	1	0	CHO-N-glycan
117	A2Sg1S1	Sg-G-Gn-	None	S-G-Gn-	None	C84H136N6O62	2220.77	3	0	0	CHO-N-glycan
118	A2Sg1S1F	Sg-G-Gn-	None	S-G-Gn-	None	C90H146N6O66	2366.83	3	1	0	CHO-N-glycan
119	A2Sg2	Sg-G-Gn-	None	Sg-G-Gn-	None	C84H136N6O63	2236.76	3	0	0	CHO-N-glycan
120	A2Sg2F	Sg-G-Gn-	None	Sg-G-Gn-	None	C90H146N6O67	2382.82	3	1	0	CHO-N-glycan
121	A3Sg1G0	Sg-G-Gn-	Gn-	Gn-	None	C75H122N6O54	1970.7	3	0	0	CHO-N-glycan
122	A3Sg1G0F	Sg-G-Gn-	Gn-	Gn-	None	C81H132N6O58	2116.76	3	1	0	CHO-N-glycan
123	A4Sg1G0	Sg-G-Gn-	Gn-	Gn-	Gn-	C83H135N7O59	2173.78	3	0	0	CHO-N-glycan
124	A4Sg1G0F	Sg-G-Gn-	Gn-	Gn-	Gn-	C89H145N7O63	2319.84	3	1	0	CHO-N-glycan
125	A3Sg1G1	Sg-G-Gn-	G-Gn-	Gn-	None	C81H132N6O59	2132.75	3	0	0	CHO-N-glycan
126	A3Sg1G1F	Sg-G-Gn-	G-Gn-	Gn-	None	C87H142N6O63	2278.81	3	1	0	CHO-N-glycan
127	A4Sg1G1	Sg-G-Gn-	G-Gn-	Gn-	Gn-	C89H145N7O64	2335.83	3	0	0	CHO-N-glycan
128	A4Sg1G1F	Sg-G-Gn-	G-Gn-	Gn-	Gn-	C95H155N7O68	2481.89	3	1	0	CHO-N-glycan

#	Glycan name	Antenna 1	Antenna 2	Antenna 3	Antenna 4	Formula	Monoisotopic mass	Man	Fuc	Bisecting Gn	Host cell line
129	A3Sg1G2	Sg-G-Gn-	G-Gn-	G-Gn-	None	C87H142N6O64	2294.8	3	0	0	CHO-N-glycan
130	A3Sg1G2F	Sg-G-Gn-	G-Gn-	G-Gn-	None	C93H152N6O68	2440.86	3	1	0	CHO-N-glycan
131	A4Sg1G2	Sg-G-Gn-	G-Gn-	G-Gn-	Gn-	C95H155N7O69	2497.88	3	0	0	CHO-N-glycan
132	A4Sg1G2F	Sg-G-Gn-	G-Gn-	G-Gn-	Gn-	C101H165N7O73	2643.94	3	1	0	CHO-N-glycan
133	A4Sg1G3	Sg-G-Gn-	G-Gn-	G-Gn-	G-Gn-	C101H165N7O74	2659.94	3	0	0	CHO-N-glycan
134	A4Sg1G3F	Sg-G-Gn-	G-Gn-	G-Gn-	G-Gn-	C107H175N7O78	2805.99	3	1	0	CHO-N-glycan
135	A3Sg1S1G0	Sg-G-Gn-	S-G-Gn-	Gn-	None	C92H149N7O67	2423.85	3	0	0	CHO-N-glycan
136	A3Sg1S1G0F	Sg-G-Gn-	S-G-Gn-	Gn-	None	C98H159N7O71	2569.9	3	1	0	CHO-N-glycan
137	A4Sg1S1G0	Sg-G-Gn-	S-G-Gn-	Gn-	Gn-	C100H162N8O72	2626.93	3	0	0	CHO-N-glycan
138	A4Sg1S1G0F	Sg-G-Gn-	S-G-Gn-	Gn-	Gn-	C106H172N8O76	2772.98	3	1	0	CHO-N-glycan
139	A3Sg1S1G1	Sg-G-Gn-	S-G-Gn-	G-Gn-	None	C98H159N7O72	2585.9	3	0	0	CHO-N-glycan
140	A3Sg1S1G1F	Sg-G-Gn-	S-G-Gn-	G-Gn-	None	C104H169N7O76	2731.96	3	1	0	CHO-N-glycan
141	A4Sg1S1G1	Sg-G-Gn-	S-G-Gn-	G-Gn-	Gn-	C106H172N8O77	2788.98	3	0	0	CHO-N-glycan

#	Glycan name	Antenna 1	Antenna 2	Antenna 3	Antenna 4	Formula	Monoisotopic mass	Man	Fuc	Bisecting Gn	Host cell line
142	A4Sg1S1G1F	Sg-G-Gn-	S-G-Gn-	G-Gn-	Gn-	C112H182N8O81	2935.04	3	1	0	CHO-N-glycan
143	A4Sg1S1G2	Sg-G-Gn-	S-G-Gn-	G-Gn-	G-Gn-	C112H182N8O82	2951.03	3	0	0	CHO-N-glycan
144	A4Sg1S1G2F	Sg-G-Gn-	S-G-Gn-	G-Gn-	G-Gn-	C118H192N8O86	3097.09	3	1	0	CHO-N-glycan
145	A3Sg1S2	Sg-G-Gn-	S-G-Gn-	S-G-Gn-	None	C109H176N8O80	2877	3	0	0	CHO-N-glycan
146	A3Sg1S2F	Sg-G-Gn-	S-G-Gn-	S-G-Gn-	None	C115H186N8O84	3023.05	3	1	0	CHO-N-glycan
147	A4Sg1S2G0	Sg-G-Gn-	S-G-Gn-	S-G-Gn-	Gn-	C117H189N9O85	3080.07	3	0	0	CHO-N-glycan
148	A4Sg1S2G0F	Sg-G-Gn-	S-G-Gn-	S-G-Gn-	Gn-	C123H199N9O89	3226.13	3	1	0	CHO-N-glycan
149	A4Sg1S2G1	Sg-G-Gn-	S-G-Gn-	S-G-Gn-	G-Gn-	C123H199N9O90	3242.13	3	0	0	CHO-N-glycan
150	A4Sg1S2G1F	Sg-G-Gn-	S-G-Gn-	S-G-Gn-	G-Gn-	C129H209N9O94	3388.19	3	1	0	CHO-N-glycan
151	A4Sg1S3	Sg-G-Gn-	S-G-Gn-	S-G-Gn-	S-G-Gn-	C134H216N10O98	3533.22	3	0	0	CHO-N-glycan
152	A4Sg1S3F	Sg-G-Gn-	S-G-Gn-	S-G-Gn-	S-G-Gn-	C140H226N10O102	3679.28	3	1	0	CHO-N-glycan
153	A3Sg2G0	Sg-G-Gn-	Sg-G-Gn-	Gn-	None	C92H149N7O68	2439.84	3	0	0	CHO-N-glycan
154	A3Sg2G0F	Sg-G-Gn-	Sg-G-Gn-	Gn-	None	C98H159N7O72	2585.9	3	1	0	CHO-N-glycan

#	Glycan name	Antenna 1	Antenna 2	Antenna 3	Antenna 4	Formula	Monoisotopic mass	Man	Fuc	Bisecting Gn	Host cell line
155	A4Sg2G0	Sg-G-Gn-	Sg-G-Gn-	Gn-	Gn-	C100H162N8O73	2642.92	3	0	0	CHO-N-glycan
156	A4Sg2G0F	Sg-G-Gn-	Sg-G-Gn-	Gn-	Gn-	C106H172N8O77	2788.98	3	1	0	CHO-N-glycan
157	A3Sg2G1	Sg-G-Gn-	Sg-G-Gn-	G-Gn-	None	C98H159N7O73	2601.89	3	0	0	CHO-N-glycan
158	A3Sg2G1F	Sg-G-Gn-	Sg-G-Gn-	G-Gn-	None	C104H169N7O77	2747.95	3	1	0	CHO-N-glycan
159	A4Sg2G1	Sg-G-Gn-	Sg-G-Gn-	G-Gn-	Gn-	C106H172N8O78	2804.97	3	0	0	CHO-N-glycan
160	A4Sg2G1F	Sg-G-Gn-	Sg-G-Gn-	G-Gn-	Gn-	C112H182N8O82	2951.03	3	1	0	CHO-N-glycan
161	A4Sg2G2	Sg-G-Gn-	Sg-G-Gn-	G-Gn-	G-Gn-	C112H182N8O83	2967.03	3	0	0	CHO-N-glycan
162	A4Sg2G2F	Sg-G-Gn-	Sg-G-Gn-	G-Gn-	G-Gn-	C118H192N8O87	3113.08	3	1	0	CHO-N-glycan
163	A3Sg2S1	Sg-G-Gn-	Sg-G-Gn-	S-G-Gn-	None	C109H176N8O81	2892.99	3	0	0	CHO-N-glycan
164	A3Sg2S1F	Sg-G-Gn-	Sg-G-Gn-	S-G-Gn-	None	C115H186N8O85	3039.05	3	1	0	CHO-N-glycan
165	A4Sg2S1G0	Sg-G-Gn-	Sg-G-Gn-	S-G-Gn-	Gn-	C117H189N9O86	3096.07	3	0	0	CHO-N-glycan
166	A4Sg2S1G0F	Sg-G-Gn-	Sg-G-Gn-	S-G-Gn-	Gn-	C123H199N9O90	3242.13	3	1	0	CHO-N-glycan
167	A4Sg2S1G1	Sg-G-Gn-	Sg-G-Gn-	S-G-Gn-	G-Gn-	C123H199N9O91	3258.12	3	0	0	CHO-N-glycan

#	Glycan name	Antenna 1	Antenna 2	Antenna 3	Antenna 4	Formula	Monoisotopic mass	Man	Fuc	Bisecting Gn	Host cell line
168	A4Sg2S1G1F	Sg-G-Gn-	Sg-G-Gn-	S-G-Gn-	G-Gn-	C129H209N9O95	3404.18	3	1	0	CHO-N-glycan
169	A4Sg2S2	Sg-G-Gn-	Sg-G-Gn-	S-G-Gn-	S-G-Gn-	C134H216N10O99	3549.22	3	0	0	CHO-N-glycan
170	A4Sg2S2F	Sg-G-Gn-	Sg-G-Gn-	S-G-Gn-	S-G-Gn-	C140H226N10O103	3695.28	3	1	0	CHO-N-glycan
171	A3Sg3	Sg-G-Gn-	Sg-G-Gn-	Sg-G-Gn-	None	C109H176N8O82	2908.98	3	0	0	CHO-N-glycan
172	A3Sg3F	Sg-G-Gn-	Sg-G-Gn-	Sg-G-Gn-	None	C115H186N8O86	3055.04	3	1	0	CHO-N-glycan
173	A4Sg3G0	Sg-G-Gn-	Sg-G-Gn-	Sg-G-Gn-	Gn-	C117H189N9O87	3112.06	3	0	0	CHO-N-glycan
174	A4Sg3G0F	Sg-G-Gn-	Sg-G-Gn-	Sg-G-Gn-	Gn-	C123H199N9O91	3258.12	3	1	0	CHO-N-glycan
175	A4Sg3G1	Sg-G-Gn-	Sg-G-Gn-	Sg-G-Gn-	G-Gn-	C123H199N9O92	3274.12	3	0	0	CHO-N-glycan
176	A4Sg3G1F	Sg-G-Gn-	Sg-G-Gn-	Sg-G-Gn-	G-Gn-	C129H209N9O96	3420.18	3	1	0	CHO-N-glycan
177	A4Sg3S1	Sg-G-Gn-	Sg-G-Gn-	Sg-G-Gn-	S-G-Gn-	C134H216N10O100	3565.21	3	0	0	CHO-N-glycan
178	A4Sg3S1F	Sg-G-Gn-	Sg-G-Gn-	Sg-G-Gn-	S-G-Gn-	C140H226N10O104	3711.27	3	1	0	CHO-N-glycan
179	A4Sg4	Sg-G-Gn-	Sg-G-Gn-	Sg-G-Gn-	Sg-G-Gn-	C134H216N10O101	3581.21	3	0	0	CHO-N-glycan
180	A4Sg4F	Sg-G-Gn-	Sg-G-Gn-	Sg-G-Gn-	Sg-G-Gn-	C140H226N10O105	3727.27	3	1	0	CHO-N-glycan

#	Glycan name	Antenna 1	Antenna 2	Antenna 3	Antenna 4	Formula	Monoisotopic mass	Man	Fuc	Bisecting Gn	Host cell line
181	Gn	None	None	None	None	C8H13N1O5	203.079	0	0	0	CHO-N-glycan
182	GnF	None	None	None	None	C14H23N1O9	349.137	0	1	0	CHO-N-glycan

N-Linked glycans with a human host Cell-Line type

The following table lists the N-linked glycans, sorted by human host cell-line type, that are included in the N-glycan-specific search.

Table 122 N-Linked glycans with a human host cell-line type

#	Glycan name	Antenna 1	Antenna 2	Antenna 3	Antenna 4	Formula	Monoisotopic mass	Man	Fuc	Bisecting Gn	Host cell line
1	M3	None	None	None	None	C34H56N2O25	892.317	3	0	0	Human-N-glycan
2	M4	None	None	None	None	C40H66N2O30	1054.37	4	0	0	Human-N-glycan
3	M5	None	None	None	None	C46H76N2O35	1216.42	5	0	0	Human-N-glycan
4	M6	None	None	None	None	C52H86N2O40	1378.48	6	0	0	Human-N-glycan
5	M7	None	None	None	None	C58H96N2O45	1540.53	7	0	0	Human-N-glycan
6	M8	None	None	None	None	C64H106N2O50	1702.58	8	0	0	Human-N-glycan
7	M9	None	None	None	None	C70H116N2O55	1864.63	9	0	0	Human-N-glycan
8	A1G0	Gn-	None	None	None	C42H69N3O30	1095.4	3	0	0	Human-N-glycan

#	Glycan name	Antenna 1	Antenna 2	Antenna 3	Antenna 4	Formula	Monoisotopic mass	Man	Fuc	Bisecting Gn	Host cell line
9	A1G0F	Gn-	None	None	None	C48H79N3O34	1241.45	3	1	0	Human-N-glycan
10	A2G0	Gn-	None	Gn-	None	C50H82N4O35	1298.48	3	0	0	Human-N-glycan
11	A2G0B	Gn-	None	Gn-	None	C58H95N5O40	1501.56	3	0	1	Human-N-glycan
12	A2G0F	Gn-	None	Gn-	None	C56H92N4O39	1444.53	3	1	0	Human-N-glycan
13	A2G0FB	Gn-	None	Gn-	None	C64H105N5O44	1647.61	3	1	1	Human-N-glycan
14	A1G1	G-Gn-	None	None	None	C48H79N3O35	1257.45	3	0	0	Human-N-glycan
15	A1G1F	G-Gn-	None	None	None	C54H89N3O39	1403.51	3	1	0	Human-N-glycan
16	A2G1	G-Gn-	None	Gn-	None	C56H92N4O40	1460.53	3	0	0	Human-N-glycan
17	A2G1B	G-Gn-	None	Gn-	None	C64H105N5O45	1663.61	3	0	1	Human-N-glycan
18	A2G1F	G-Gn-	None	Gn-	None	C62H102N4O44	1606.59	3	1	0	Human-N-glycan
19	A2G1FB	G-Gn-	None	Gn-	None	C70H115N5O49	1809.67	3	1	1	Human-N-glycan
20	A2G2	G-Gn-	None	G-Gn-	None	C62H102N4O45	1622.58	3	0	0	Human-N-glycan
21	A2G2B	G-Gn-	None	G-Gn-	None	C70H115N5O50	1825.66	3	0	1	Human-N-glycan

#	Glycan name	Antenna 1	Antenna 2	Antenna 3	Antenna 4	Formula	Monoisotopic mass	Man	Fuc	Bisecting Gn	Host cell line
22	A2G2F	G-Gn-	None	G-Gn-	None	C68H112N4O49	1768.64	3	1	0	Human-N-glycan
23	A2G2FB	G-Gn-	None	G-Gn-	None	C76H125N5O54	1971.72	3	1	1	Human-N-glycan
24	A1S1	S-G-Gn-	None	None	None	C59H96N4O43	1548.54	3	0	0	Human-N-glycan
25	A1S1F	S-G-Gn-	None	None	None	C65H106N4O47	1694.6	3	1	0	Human-N-glycan
26	A2S1G0	S-G-Gn-	None	Gn-	None	C67H109N5O48	1751.62	3	0	0	Human-N-glycan
27	A2S1G0B	S-G-Gn-	None	Gn-	None	C75H122N6O53	1954.7	3	0	1	Human-N-glycan
28	A2S1G0F	S-G-Gn-	None	Gn-	None	C73H119N5O52	1897.68	3	1	0	Human-N-glycan
29	A2S1G0FB	S-G-Gn-	None	Gn-	None	C81H132N6O57	2100.76	3	1	1	Human-N-glycan
30	A2S1G1	S-G-Gn-	None	G-Gn-	None	C73H119N5O53	1913.68	3	0	0	Human-N-glycan
31	A2S1G1B	S-G-Gn-	None	G-Gn-	None	C81H132N6O58	2116.76	3	0	1	Human-N-glycan
32	A2S1G1F	S-G-Gn-	None	G-Gn-	None	C79H129N5O57	2059.73	3	1	0	Human-N-glycan
33	A2S1G1FB	S-G-Gn-	None	G-Gn-	None	C87H142N6O62	2262.81	3	1	1	Human-N-glycan
34	A2S2	S-G-Gn-	None	S-G-Gn-	None	C84H136N6O61	2204.77	3	0	0	Human-N-glycan

#	Glycan name	Antenna 1	Antenna 2	Antenna 3	Antenna 4	Formula	Monoisotopic mass	Man	Fuc	Bisecting Gn	Host cell line
35	A2S2B	S-G-Gn-	None	S-G-Gn-	None	C92H149N7O66	2407.85	3	0	1	Human-N-glycan
36	A2S2F	S-G-Gn-	None	S-G-Gn-	None	C90H146N6O65	2350.83	3	1	0	Human-N-glycan
37	A2S2FB	S-G-Gn-	None	S-G-Gn-	None	C98H159N7O70	2553.91	3	1	1	Human-N-glycan
38	Gn	None	None	None	None	C8H13N1O5	203.079	0	0	0	Human-N-glycan
39	GnF	None	None	None	None	C14H23N1O9	349.137	0	1	0	Human-N-glycan

O-Linked glycans

The following table lists the O-linked glycans that the application supports for the N-glycan-specific search in Peptide Mapping Analysis.

Table 123 O-Linked glycans

#	Glycan	Monoisotopic mass
1	GalNAc	203.079
2	GalNAc-3G	365.132
3	GalNAc-3GnG	568.212
4	GalNAc-3SG	656.228
5	GalNAc-6GGn	568.212
6	GalNAc-6GGn-3G	730.264
7	GalNAc-6GGn-3GnG	933.344
8	GalNAc-6GGn-3SG	1021.36
9	GalNAc-6Gn	406.159
10	GalNAc-6Gn-3G	568.212
11	GalNAc-6Gn-3GnG	771.291
12	GalNAc-6Gn-3SG	859.307

#	Glycan	Monoisotopic mass
13	GalNAc-6S	494.175
14	GalNAc-6S-3G	656.228
15	GalNAc-6S-3GnG	859.307
16	GalNAc-6S-3SG	947.323

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