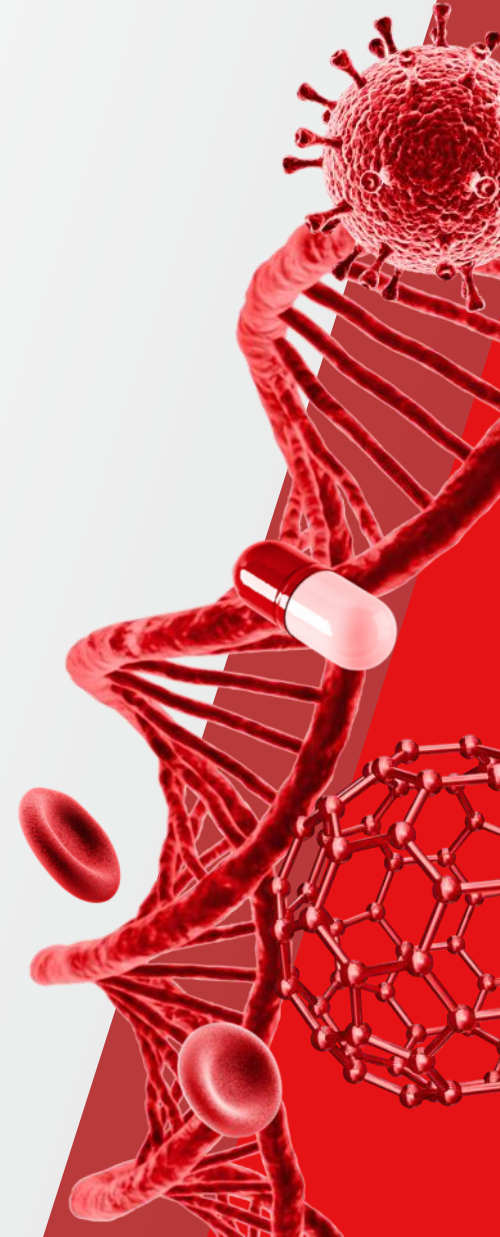


Getting Started on XlinkX 2.5 Node for Proteome Discoverer 2.5 Software

 The world leader in serving science



XlinkX 2.5 Node - New Features for Proteome Discoverer 2.5 Software

- New modifications dialog
 - Heterobifunctional crosslinkers support
 - Direct import of Unimod crosslinkers
- New FDR calculations including intra and inter validation
- New options for **results export** :
 - PyMol
 - xiView
 - Download: <https://thermo.flexnetoperations.com/control/thmo/download?element=12199007>

The image displays several key features of Proteome Discoverer 2.5:

- Add Chemical Modification Dialog:** A window for defining new modifications. It includes fields for Name (EDC_new) and Abbreviation (EDC_new), and tabs for General, Neutral Losses, Diagnostic Ions, and Crosslinking. The Crosslinking tab is active, showing a 'Non-cleavable Crosslink' dropdown and a 'Select the target amino acids and press "Add" button to enter the target pairs.' instruction. Target Pairs (Non-cleavable Crosslinkers) are listed as K <-> D and K <-> E.
- New Modification Dialog:** A callout box highlighting the 'Add Chemical Modification' dialog.
- New Options for Results Export:** A callout box highlighting the 'Export' menu in the Proteome Discoverer 2.5 interface, which includes options like 'To PyMOL...', 'To xiNET...', and various mass list formats.
- New FDR Validator:** A workflow diagram showing the integration of the 'New FDR Validator' (Node 6) into the search and validation process. It connects to 'XlinkX/PD Search' (Node 8) and 'XlinkX/PD Crosslink Grouping' (Node 6), which then feed into 'XlinkX/PD Validator' (Node 9) and 'XlinkX/PD Consensus Validator' (Node 7). A table below the diagram shows settings for the validator:

1. Input Data	
FDR threshold	0.01
2. Advanced	
Separate inter from intra	True
- 3D Protein Structure:** A 3D visualization of a protein structure (Serum albumin) with red spheres representing crosslinks and orange lines connecting them.

Outline

- New modifications dialog
 - Adding Crosslinkers
 - Adding Cleavable Crosslinkers
 - Adding Non-Cleavable Crosslinkers
 - Adding Monolinks Modification
- New FDR calculations
- Crosslinking Analysis Template
- Example of EDC Crosslinking Analysis
- New options for results export
 - Exporting Crosslinks to Pymol
 - Exporting Crosslinks to xiVIEW





New Modifications Dialog

Support Heterobifunctional Crosslinkers

1. Adding Crosslinkers

- Common crosslinkers is available in the list of modifications in Thermo Scientific™ Proteome Discoverer Software.
- A new crosslinker can be added manually:
 - In the Modification Manager, **Add a Modification** for the crosslinker.

1. Click

2. Click

3. Click

Thermo Proteome Discoverer 2.5.0.400

File View Administration Tools Window Help

Start Page Administration

Process Management

Job Queue

Content Management

FASTA Files

FASTA Indexes

FASTA Parsing Rules

Spectral Libraries

Chemical Modifications

Cleavage Reagents

+ Add Edit Remove Import Export

Is Active	Modification	Abbreviation	Delta Mass [Da]	Delta Average Mass [Da]
<input checked="" type="checkbox"/>	=	<input type="checkbox"/>	=	=
<input checked="" type="checkbox"/>	EDC	EDC	-18.01056	-18.01056

Add Chemical Modification

Name: Abbreviation:

General Neutral Losses Diagnostic Ions Crosslinking

Position: Any Unimod Accesion: 0

Delta Mass [Da]: 0 Delta Average Mass [Da]: 0

Substitution: Leaving Group:

2. Adding Cleavable Crosslinkers

Step 1. Define a crosslink by using **Name**, **Abbreviation**, **Substitution** and **Target Amino Acid Site(s)** in General Tab.

1. Input

2. Input

3. Click and Select

Add Chemical Modification

Name: Abbreviation:

General Neutral Losses Diagnostic Ions Crosslinking

Position: Unimod Accesion:

Delta Mass [Da]: Delta Average Mass [Da]:

Substitution: Leaving Group:

Amino Acid Site(s): Double click on a row to activate, press [ENTER] to add or [DEL]/Delete button to remove rows.

Target Amino Acid	Classification	
K	CID cleavable crosslink	<input type="checkbox"/>
N	CID cleavable crosslink	<input type="checkbox"/>
S	CID cleavable crosslink	<input type="checkbox"/>
W	CID cleavable crosslink	<input type="checkbox"/>
Y	CID cleavable crosslink	<input type="checkbox"/>
		<input type="checkbox"/>

Help OK Cancel

Adding Cleavable Crosslinkers

Step 2. Define a crosslink **type**, **fragments** in Crosslinking Tab.

- I. Click **Crosslink** to activate the crosslinking tab.
- II. Click and define a **Cleavable Crosslink** type.
- III. Click and Input crosslinking fragments **Name, Abbreviate, Substitution, Delta Mass and Target(s)** to the list.
- IV. Select Left Fragments 1 from the list and click Add Selected Fragments to the Left Fragments.
- V. Select Right Fragments 2 from the list and click Add Selected Fragments to the Right Fragments.
- VI. Repeat step IV and V to connect all fragments.
- VII. Click the **OK** button

Add Chemical Modification

Name: APDC4 Abbreviation: APDC4

General Neutral Losses Diagnostic Ions **Crosslinking**

Cleavable Crosslink Double click on a row to activate, press [ENTER] to add or [DEL]/Delete button to remove rows.

Crosslink Fragments:

Name	Abbreviate	Substitution	Delta Mass	Delta Average Mass	Target(s)	
Fragment1	F1	C4H4O	68.02621	68.07411	K	✖
Fragment2	F2	C10H18N2	166.147	166.26378	N,S,W,Y	✖
Fragment3	F3	C10H16N2C	180.12626	180.2473	K	✖
Fragment4	F4	C4H6	54.04695	54.09059	N,S,W,Y	✖
						✖

Connected Fragments: Add Selected Fragment

Left Fragment	Right Fragment	
Fragment1	Fragment2	✖
Fragment3	Fragment4	✖

Help OK Cancel

Adding Cleavable Crosslinkers

Step 3. Add Left Fragments in Connected Fragments.

- I. Click **Crosslink** to activate the crosslinking tab.
- II. Click and define a **Cleavable Crosslink** type.
- III. Click and Input crosslinking fragments **Name, Abbreviate, Substitution, Delta Mass and Target(s)** to the list.
- IV. Select **Left Fragments 1** from the list and click **Add Selected Fragments to the Left Fragments**.
- V. Select **Right Fragments 2** from the list and click **Add Selected Fragments to the Right Fragments**.
- VI. Repeat step IV and V to connect all fragments.
- VII. Click the **OK** button

Add Chemical Modification

Name: Abbreviation:

General Neutral Losses Diagnostic Ions **Crosslinking**

Cleavable Crosslink

Crosslink Fragments:

Name	Abbreviat	Substitutio	Delta Mass	Delta Average Mass	Target(s)	
Fragment1	F1	C4H4O	68.02621	68.07411	K	✖
Fragment2	F2	C10H18N2	166.147	166.26378	N,S,W,Y	✖
Fragment3	F3	C10H16N2C	180.12626	180.2473	K	✖
Fragment4	F4	C4H6	54.04695	54.09059	N,S,W,Y	✖
						✖

Connected Fragments:

Left Fragment	Right Fragment	
Fragment1	Fragment2	✖
Fragment3	Fragment4	✖

Add Selected Fragment

Help **OK** Cancel

Adding Cleavable Crosslinkers

Step 4. Add Right Fragments in Connected Fragments.

- I. Click **Crosslink** to activate the crosslinking tab.
- II. Click and define a **Cleavable Crosslink** type.
- III. Click and Input crosslinking fragments **Name, Abbreviate, Substitution, Delta Mass and Target(s)** to the list.
- IV. Select **Left Fragments 1** from the list and click **Add Selected Fragments to the Left Fragments**.
- V. Select **Right Fragments 2** from the list and click **Add Selected Fragments to the Right Fragments**.
- VI. Repeat step IV and V to connect all fragments.
- VII. Click the **OK** button

Add Chemical Modification

Name: Abbreviation:

General Neutral Losses Diagnostic Ions **Crosslinking**

Cleavable Crosslink Double click on a row to activate, press [ENTER] to add or [DEL]/Delete button to remove rows.

Crosslink Fragments:

Name	Abbreviat	Substitutio	Delta Mass	Delta Average Mass	Target(s)	
Fragment1	F1	C4H4O	68.02621	68.07411	K	✕
Fragment2	F2	C10H18N2	166.147	166.26378	N,S,W,Y	✕
Fragment3	F3	C10H16N2C	180.12626	180.2473	K	✕
Fragment4	F4	C4H6	54.04695	54.09059	N,S,W,Y	✕
						✕

Connected Fragments:

Left Fragment	Right Fragment	
Fragment1	Fragment2	✕
Fragment3	Fragment4	✕

Adding Cleavable Crosslinkers

Step 5. **Connect all fragments** in Crosslinking Tab.

- I. Click **Crosslink** to activate the crosslinking tab.
- II. Click and define a **Cleavable Crosslink** type.
- III. Click and Input crosslinking fragments **Name, Abbreviate, Substitution, Delta Mass and Target(s)** to the list.
- IV. Select **Left Fragments 1** from the list and click **Add Selected Fragments to the Left Fragments**.
- V. Select **Right Fragments 2** from the list and click **Add Selected Fragments to the Right Fragments**.
- VI. Repeat step **IV** and **V** to connect all fragments one by one.
- VII. Click the **OK** button

Add Chemical Modification

Name: APDC4 Abbreviation: APDC4

General Neutral Losses Diagnostic Ions **Crosslinking**

Cleavable Crosslink Double click on a row to activate, press [ENTER] to add or [DEL]/Delete button to remove rows.

Crosslink Fragments:

Name	Abbreviat	Substitutio	Delta Mass	Delta Average Mass	Target(s)	
Fragment1	F1	C4H4O	68.02621	68.07411	K	✖
Fragment2	F2	C10H18N2	166.147	166.26378	N,S,W,Y	✖
Fragment3	F3	C10H16N2C	180.12626	180.2473	K	✖
Fragment4	F4	C4H6	54.04695	54.09059	N,S,W,Y	✖
						✖

Connected Fragments:

Left Fragment	Right Fragment	
Fragment1	Fragment2	✖
Fragment3	Fragment4	✖

Help **OK** Cancel

3. Adding Non-Cleavable Crosslinkers

Step 1. Define a crosslink by using **Name**, **Abbreviation**, **Substitution** and **Target Amino Acid Site(s)** in General Tab.

1. Click and add

2. Click and add

3. Double click and select

Add Chemical Modification

Name: PhoX_Example Abbreviation: PhoX_Example

General Neutral Losses Diagnostic Ions Crosslinking

Position: Any Unimod Accesion: 0

Delta Mass [Da]: 209.97181 Delta Average Mass [Da]: 210.0805

Substitution: H(3)C(8)O(5)P Leaving Group:

Amino Acid Site(s): Double click on a row to activate, press [ENTER] to add or [DEL]/Delete button to remove rows.

Target Amino Acid	Classification
K	Crosslink
S	Crosslink
T	Crosslink

Help OK Cancel

Adding Non-Cleavable Crosslinkers

Step 2. Define **Diagnostic Ions** by using **Name** and **Formula**. **Monoisotopic Mass** and **Average Mass** will automatic calculate based on **Formula**.

1. Click

2. Double Click and add

Double click on a row to activate, press [ENTER] to add or [DEL]/Delete button to remove rows.

Name	Formula	Monoisotopic Mass	Average Mass	
PhoX	C18H22N2O5P	377.1266	377.3522	X
				X

Help OK Cancel

Adding Non-Cleavable Crosslinkers

Step 3. Define Target Pairs in Crosslinking Tab.

1. Click

2. Click, Select

3. Click, Select

4. Click

5. Add all Target Pairs one by one

6. Click

4. Adding Monolinks Modification

To set up the monolinks use the standard modification.

- Add a Chemical Modification of of PhoX dead ends by using **Name, Abbreviation, Substitution and Target Amino Acid Site(s)** in General Tab.

1. Click and add

Add Chemical Modification

Name: Abbreviation:

2. Click and add

General Neutral Losses Diagnostic Ions Crosslinking

Position: Unimod Accession:

Delta Mass [Da]: Delta Average Mass [Da]:

Substitution: Leaving Group:

Amino Acid Site(s): Double click on a row to activate, press [ENTER] to add or [DEL]/Delete button to remove rows.

Target Amino Acid	Classification	
K	Chemical derivative	✕
		✕

3. Double click and Select

5. Click

Help Cancel

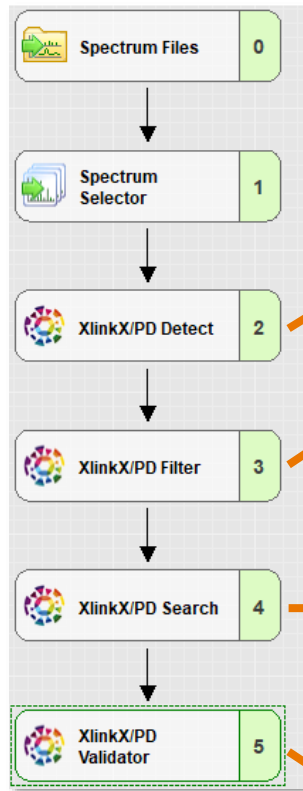


New FDR Calculations

Including intra and inter validation

XlinkX 2.5 Node – Data Analysis Driven by XlinkX/PD

Processing Workflow



1. Input Data	
Acquisition strategy	MS2_MS2
Crosslink Modification	DSSO / +158.004 Da (K)
Minimum S/N	1.5
Enable protein N-terminus linkage	False

Select Crosslinker Reagent and acquisition strategy

1. Output Data	
Select	Crosslinks

Filter for Spectra identified as being crosslinked

1. General Data	
Protein Database	pastrosepticum.fasta
Retain FASTA file indexes	True
Enzyme Name	Trypsin (Full)
Maximum Missed Cleavages	2
Minimum Peptide Length	5
2. Tolerances	
Precursor Mass Tolerance	10 ppm
FTMS Fragment Mass Tolerance	20 ppm
ITMS Fragment Mass Tolerance	0.5 Da
4. Static Modifications	
Static Modification	Carbamidomethyl / +57.021 Da (C)
Static Modification	None
Static Any N-term Modification	None
Static Any C-term Modification	None
Static Protein N-term Modification	None
Static Protein C-term Modification	None
5. Dynamic Modifications	
Dynamic Modification	Oxidation / +15.995 Da (M)
Dynamic Modification	None
Dynamic Modification	None
Dynamic Modification	None
Dynamic Modification	None
Dynamic Any N-term Modification	None
Dynamic Any N-term Modification	None
Dynamic Any N-term Modification	None
Dynamic Any N-term Modification	None
Dynamic Any C-term Modification	None
Dynamic Any C-term Modification	None
Dynamic Any C-term Modification	None
Dynamic Any C-term Modification	None
Dynamic Protein N-term Modification	Acetyl / +42.011 Da (N-Terminus)
Dynamic Protein C-term Modification	None

Select targeted modifications and protein database

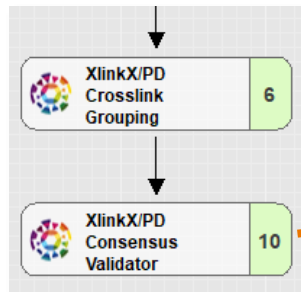
1. Input Data	
FDR threshold	0.01

Spectrum-Match-level (CSM) Validation (see slides below)

1. Input Data	
Cross-link FDR threshold	0.01
CSM FDR threshold	0.01

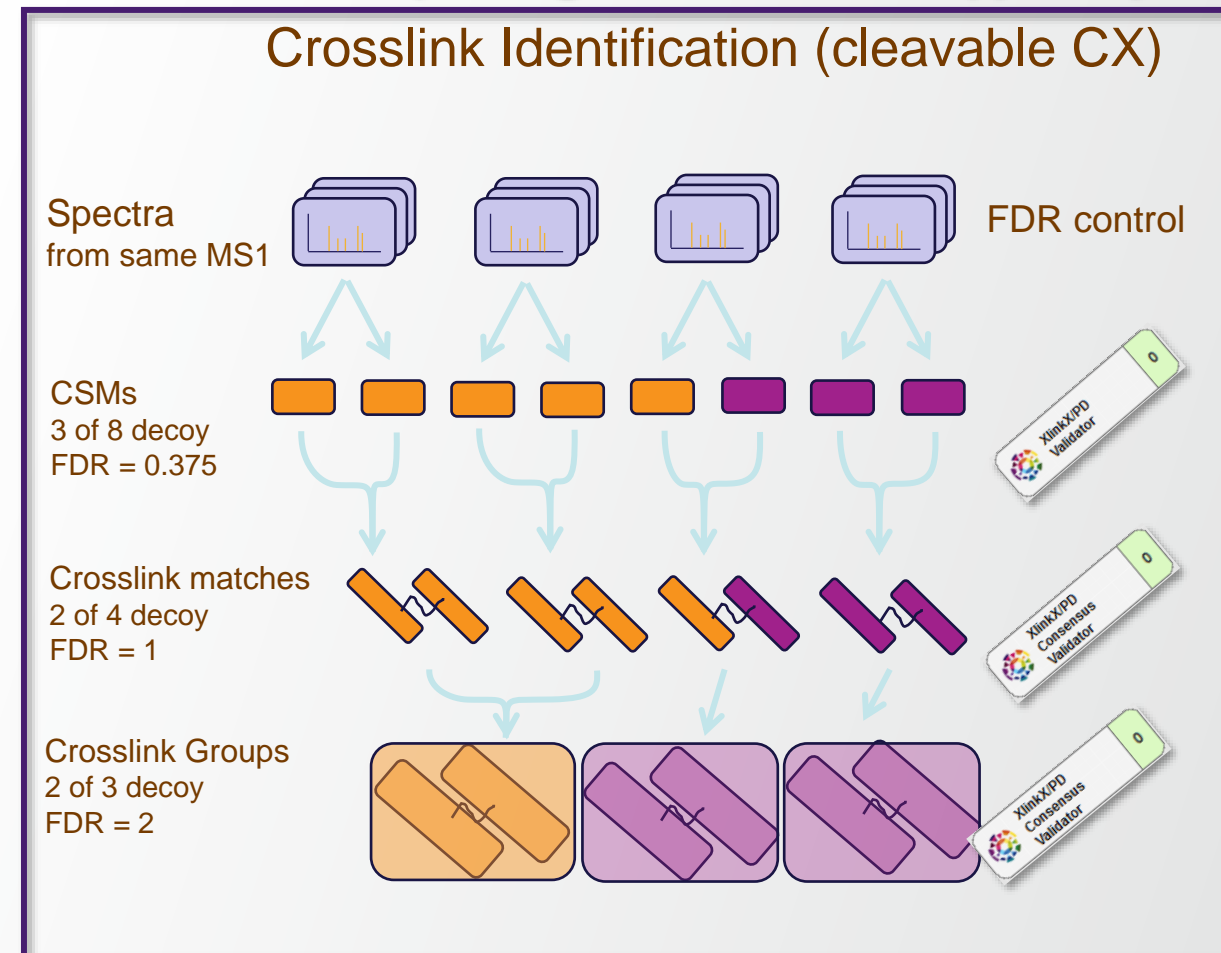
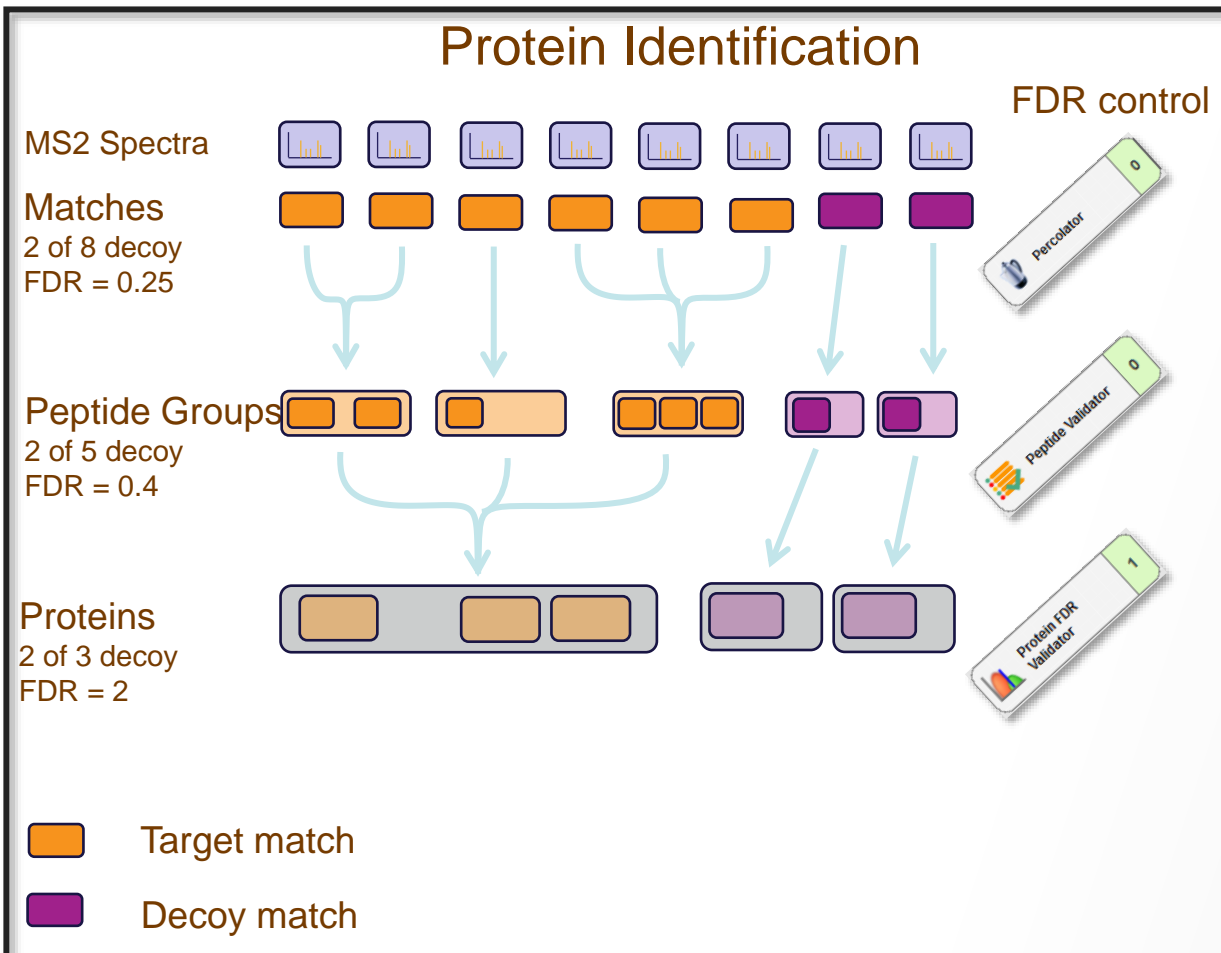
Crosslink-level Crosslink Validation (see slides below)

Consensus Workflow (cutout)



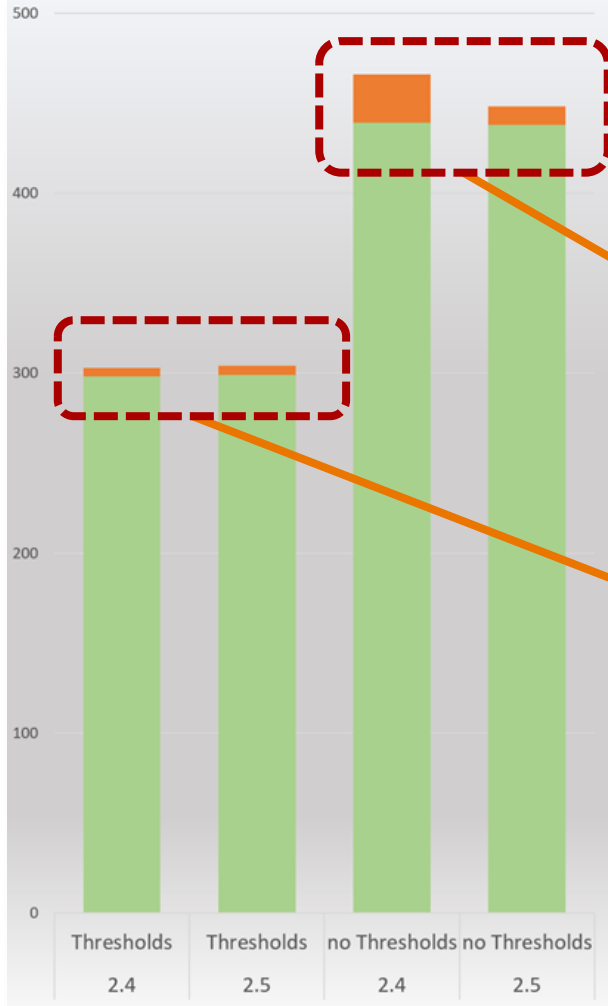
FDR Related Hierarchies Between Traditional and Crosslinked Analysis

- All levels of consolidation (Spectra, Matches, Peptide Groups, Proteins) need to be under FDR control
- Former versions of XlinkX node contained only FDR control on Matches – Level
- Higher level FDR was controlled by using Score Cutoffs
- In XlinkX 2.5 node FDR control on all Levels make Score Cutoffs obsolete (although these are still supported)



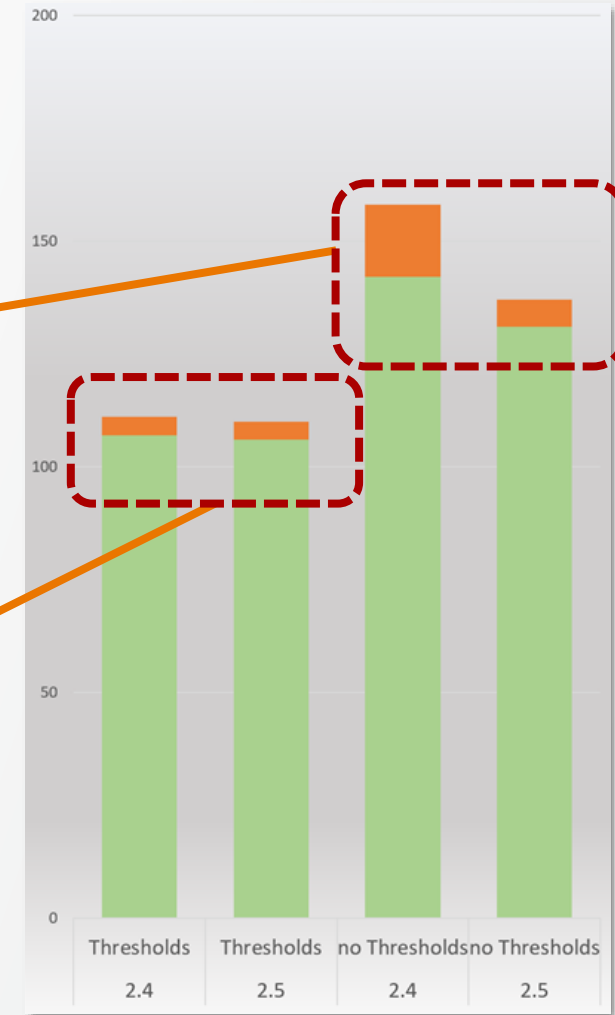
Case Studies – Improvements in Match Level

Synthetic Crosslinks from [2]



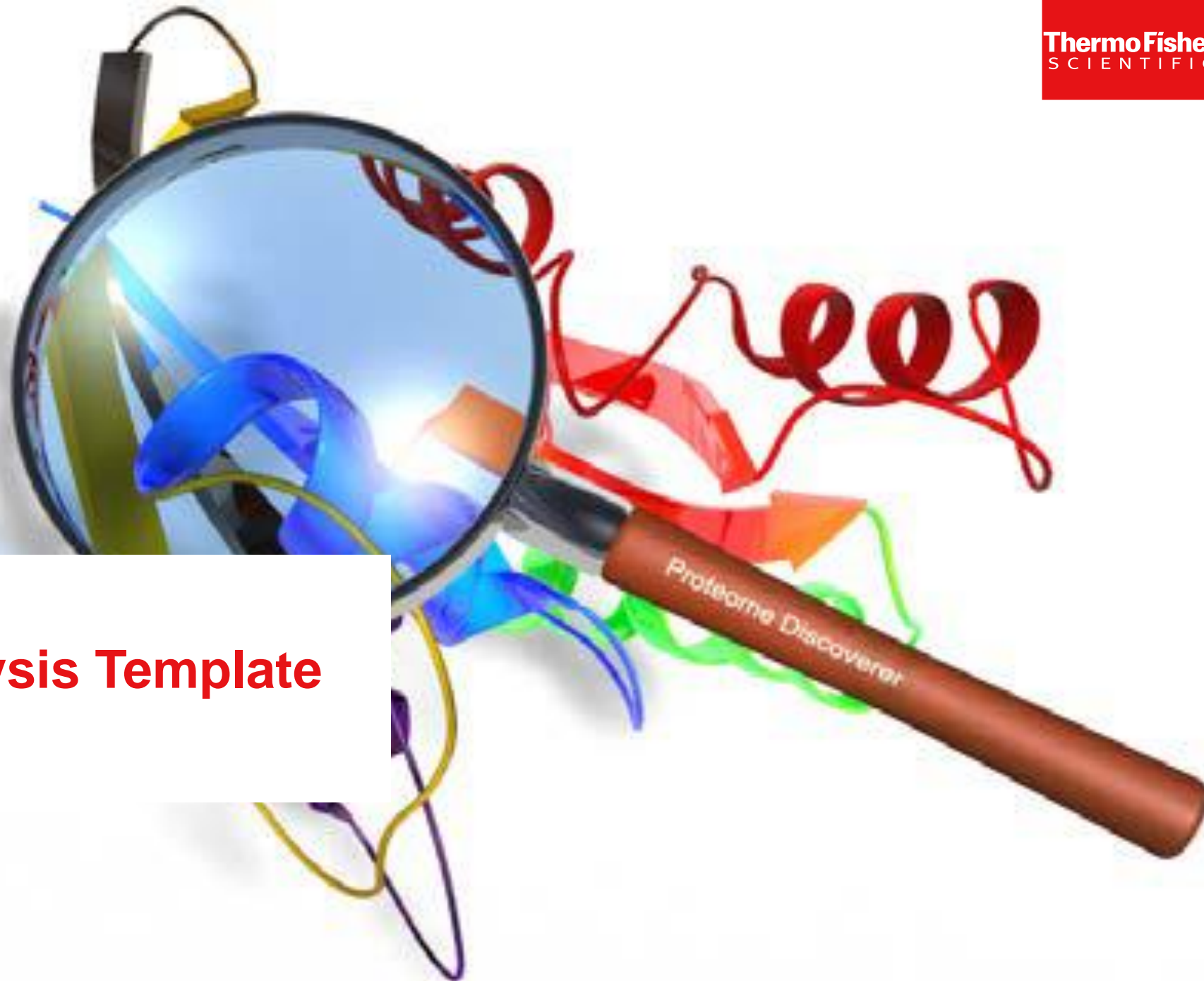
- Omitting Thresholds increases number of identified Crosslinks
- Omitting Thresholds drastically improves FDR in multi level approach
- Threshold based approach shows no difference between spectra-only FDR approach (PD 2.4) and hierarchical approach (PD 2.5)

CSM incorrect
CSM correct



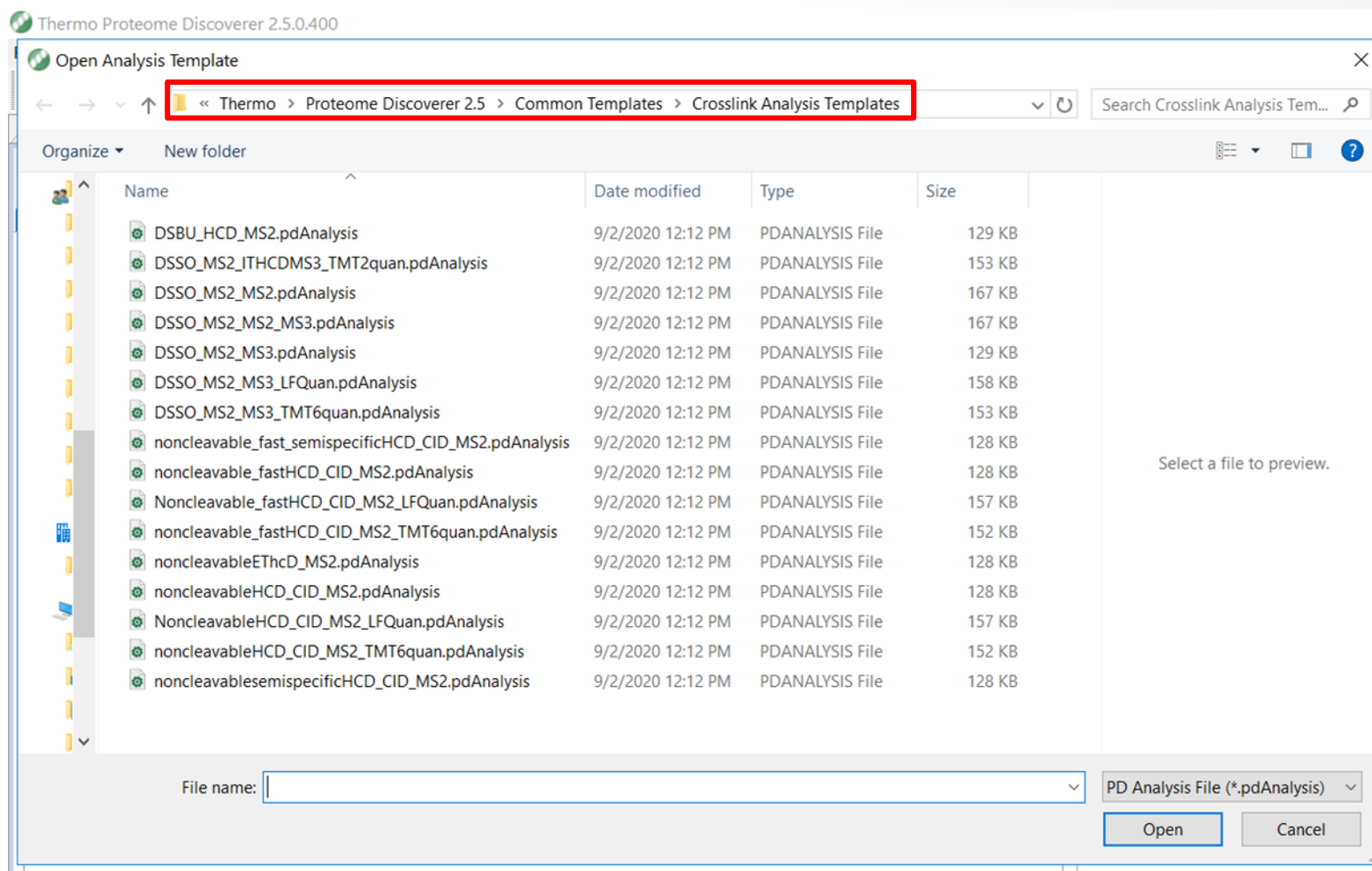
Crosslink incorrect
Crosslink correct

Crosslinking Analysis Template



Preinstalled Crosslinking Analysis Templates

Crosslinking Analysis Templates located in C:/Users/Public/Public Documents/ Thermo/ Proteome Discoverer 2.5/ Common Templates/ Crosslink Analysis Templates



Xinkx 2.5 Node with Proteome Discoverer 2.5 Software Support:

- Non-Cleavable (n^2 database search)
- Non-Cleavable-fast (search distinct signature crosslinked fragments and sequence tags spectrum to reduce the search time)
- Cleavable-MS2-MS2 (e.g. CID for reporter detection-ETD for sequence readout)
- Cleavable-MS2 only (e.g. HCD for reporter detection and sequence readout)
- Cleavable-MS2-MS3 (e.g. MS2 CID for reporter detection and MS3 HCD for individual peptides)
- Cleavable MS2-MS2-MS3 (e.g. MS2 CID for reporter detection, MS3 HCD and MS2 EThcD for individual peptides)
- Cleavable semi-specific crosslinking data
- LFQ and TMT quantification for crosslinking data

Analysis Template

Including Processing and Consensus workflow

The screenshot displays the Thermo Proteome Discoverer 2.5.0.400 software interface. The main window is titled "Study: New Study 1" and features a menu bar (File, View, Administration, Tools, Window, Help) and a toolbar with various icons. The interface is divided into several panels:

- Study Definition:** Contains a "Study Summary" section with fields for Study Name (New Study 1), Study Directory (D:\PD 2.5 Study\New Study 1), Study Type (General), Last Changed (10/15/2020 9:43:24 AM), and Creation Date (10/15/2020 9:43:24 AM). Below it is a "Study Description" field.
- Quantification Methods:** A panel with an "Add" button, currently empty.
- Study Factors:** A panel with "Paste", "Copy", and "Add" buttons, currently empty.
- Analysis:** A floating window containing a workflow configuration. It includes:
 - A "Consensus Step (Fully Processing)" highlighted in blue, with "Workflow: CWF_Basic_Xlinkx" and "Result File: Enter result file name." It has an "Edit" button and a warning icon.
 - A "Processing Step (Fully Processing)" highlighted in green, with "Workflow: WF_Fusion_Basic_SequestHT_XlinkxNoncleavable" and "Result File: Enter result file name." It has "Edit" and "Clone" buttons and a warning icon.
 - A "Child Steps: (1)" section with an "Add" button.
 - A "Files for Analysis: (0)" section with a "Clear All" button and a "Drag and drop from Input Files here" instruction.

Red annotations are present: "Consensus workflow" with an arrow pointing to the blue step, and "Processing workflow" with an arrow pointing to the green step.

Analysis Template for Non-cleavable CID/HCD Data

Select and Open Analysis template

File Explorer window showing the path: < Common Templates > Crosslink Analysis Templates. The search bar contains "Search Crosslink Analysis Tem...".

Name	Date modified	Type	Size
DSBU_HCD_MS2.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	129 K
DSSO_MS2_ITHCDMS3_TMT2quan.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	153 K
DSSO_MS2_MS2.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	167 K
DSSO_MS2_MS2_MS3.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	167 K
DSSO_MS2_MS3.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	129 K
DSSO_MS2_MS3_LFQuan.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	158 K
DSSO_MS2_MS3_TMT6quan.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	153 K
noncleavable_fast_semispecificHCD_CID_MS2.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	152 K
noncleavable_fastHCD_CID_MS2.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	128 K
Noncleavable_fastHCD_CID_MS2_LFQuan.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	152 K
noncleavable_fastHCD_CID_MS2_TMT6quan.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	152 K
noncleavableETHcd_MS2.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	128 K
noncleavableHCD_CID_MS2.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	128 K
NoncleavableHCD_CID_MS2_LFQuan.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	152 K
noncleavableHCD_CID_MS2_TMT6quan.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	152 K
noncleavablesemispecificHCD_CID_MS2.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	128 K

Callout boxes:

- Non-Cleavable-fast: search distinct signature crosslinked fragments and sequence tags spectrum
- Non-Cleavable: n^2 database search

Analysis Template for DSSO Cleavable MS2-MS2 Data

Select and open Analysis template

Support Cleavable-MS2-MS2 data : e.g. CID for reporter detection-ETD for sequence readout

Name	Date modified	Type	Size
DSBU_HCD_MS2.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	129 K
DSSO_MS2_ITHCDMS3_TMT2quan.pdAnalysis			
DSSO_MS2_MS2.pdAnalysis			
DSSO_MS2_MS2_MS3.pdAnalysis			
DSSO_MS2_MS3.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	129 K
DSSO_MS2_MS3_LFQuan.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	158 K
DSSO_MS2_MS3_TMT6quan.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	153 K
noncleavable_fast_semispecificHCD_CID_MS2.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	128 K
noncleavable_fastHCD_CID_MS2.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	128 K
Noncleavable_fastHCD_CID_MS2_LFQuan.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	157 K
noncleavable_fastHCD_CID_MS2_TMT6quan.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	152 K
noncleavableEThcD_MS2.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	128 K
noncleavableHCD_CID_MS2.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	128 K
NoncleavableHCD_CID_MS2_LFQuan.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	157 K
noncleavableHCD_CID_MS2_TMT6quan.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	152 K
noncleavablesemispecificHCD_CID_MS2.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	128 K

Select a file to preview.

Analysis Template for DSSO Cleavable MS2-MS2-MS3 Data

Select and Open Analysis template

Support Cleavable MS2-MS2-MS3 data: e.g. MS2 CID for reporter detection, MS3 HCD and MS2 EThcD for individual peptides

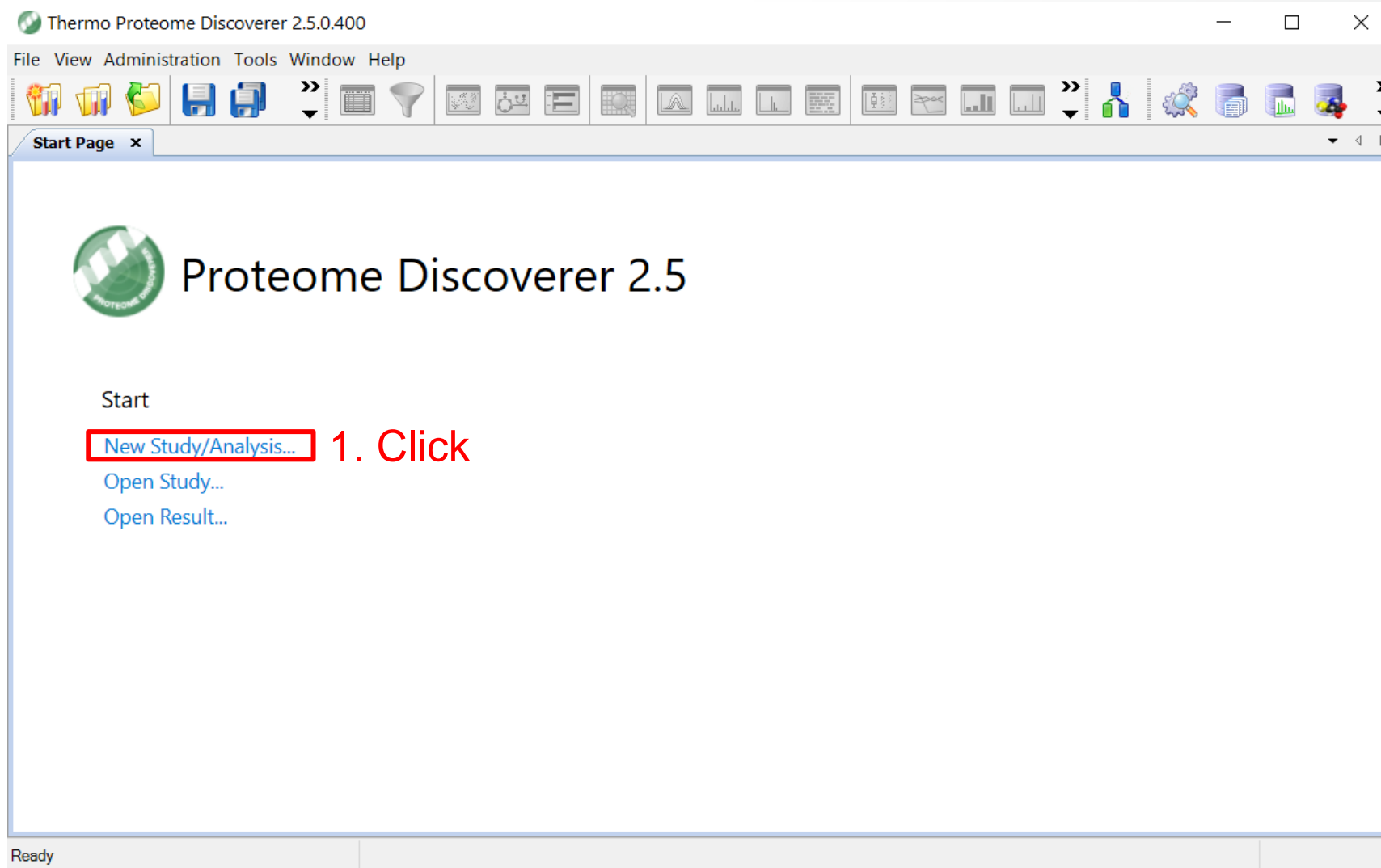
Name	Date modified	Type	Size
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DSSO_MS2_ITHCDMS3_TMT2quan.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	153 K
DSSO_MS2_MS2.pdAnalysis			
DSSO_MS2_MS2_MS3.pdAnalysis			
DSSO_MS2_MS3.pdAnalysis			
DSSO_MS2_MS3_LFQuan.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	158 K
DSSO_MS2_MS3_TMT6quan.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	153 K
noncleavable_fast_semispecificHCD_CID_MS2.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	128 K
noncleavable_fastHCD_CID_MS2.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	128 K
Noncleavable_fastHCD_CID_MS2_LFQuan.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	157 K
noncleavable_fastHCD_CID_MS2_TMT6quan.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	152 K
noncleavableEThcD_MS2.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	128 K
noncleavableHCD_CID_MS2.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	128 K
NoncleavableHCD_CID_MS2_LFQuan.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	157 K
noncleavableHCD_CID_MS2_TMT6quan.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	152 K
noncleavablesemispecificHCD_CID_MS2.pdAnalysis	9/2/2020 12:12 PM	PDANALYSIS File	128 K

Select a file to preview.



Example of EDC Crosslinking Analysis

Step 1. Create and Setup a New Study



Step 2. Define the New Study

The screenshot shows the Thermo Proteome Discoverer 2.5.0.400 software interface. The main window displays the Proteome Discoverer 2.5 logo and a 'Start' section with links for 'New Study/Analysis...', 'Open Study...', and 'Open Result...'. A 'New Study and Analysis' dialog box is open, containing the following fields:

- Study Name: New Study 1
- Study Root Directory: D:\PD 2.5 Study
- Import From File: (empty)
- Processing Workflow: (empty workflow)
- Consensus Workflow: (empty workflow)

At the bottom of the dialog box, there are 'OK' and 'Cancel' buttons. The 'OK' button is highlighted with a red box. Red annotations are present: '1. Define' is written above the 'Study Name' and 'Study Root Directory' fields, and '2. Click' is written above the 'OK' button.

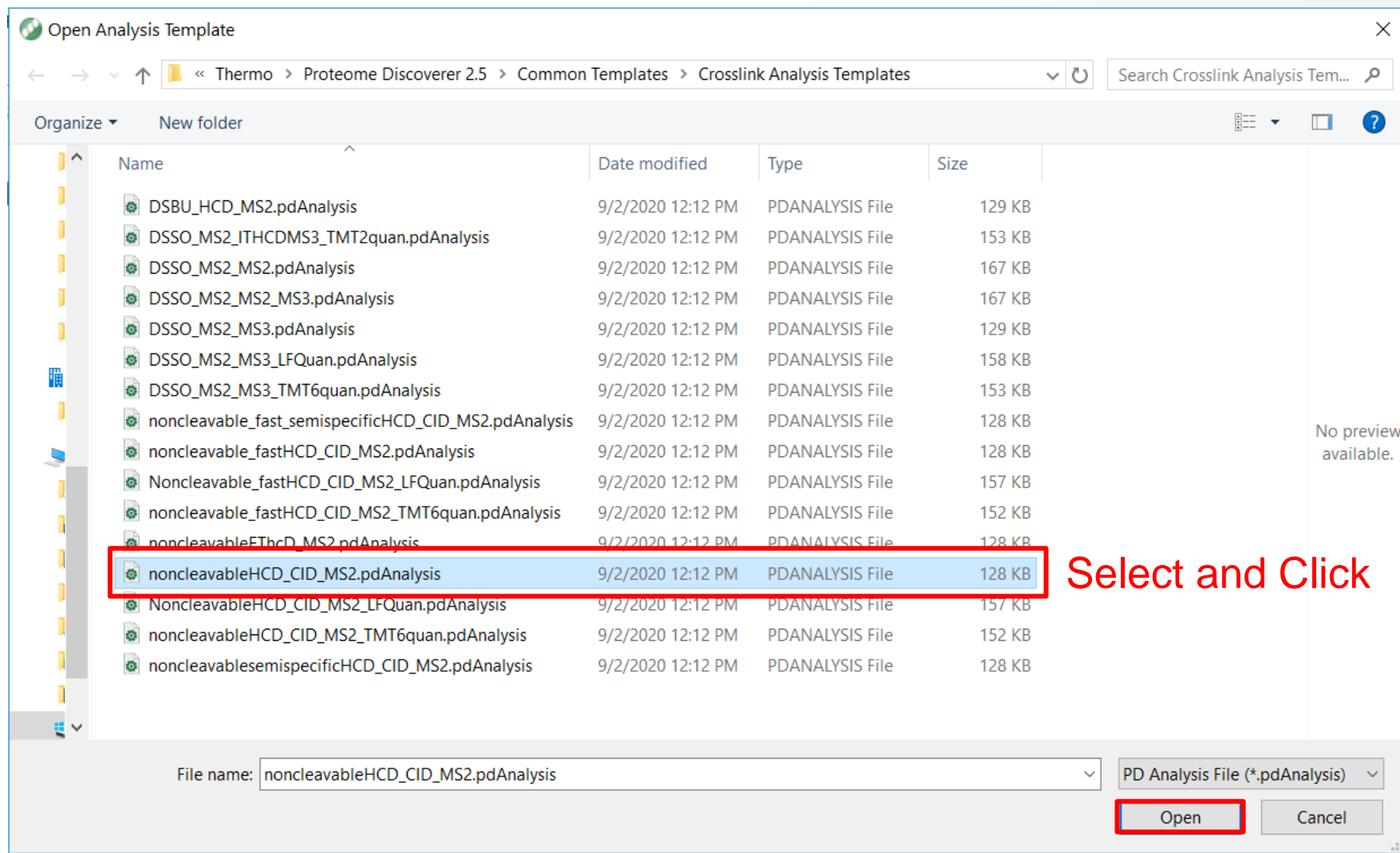
Study Page

The screenshot displays the Thermo Proteome Discoverer 2.5.0.400 software interface. The window title is "Thermo Proteome Discoverer 2.5.0.400". The menu bar includes "File", "View", "Administration", "Tools", "Window", and "Help". The toolbar contains various icons for file operations and analysis. The active window is "Study: New Study 1", which is highlighted with a red box and labeled "Study Page". Below the toolbar, there are buttons for "Add Files", "Add Fractions", "Remove Files", "Open Containing Folder", "New Analysis", and "Open Analysis Template". The main interface is divided into several sections: "Study Definition" (selected), "Input Files", "Samples", and "Analysis Results". The "Study Definition" section is further divided into "Study Summary" and "Study Description". The "Study Summary" section displays the following information: Study Name: New Study 1, Study Directory: D:\PD 2.5 Study\New Study 1, Study Type: General, Last Changed: 10/15/2020 9:43:24 AM, and Creation Date: 10/15/2020 9:43:24 AM. The "Study Description" section is currently empty. To the right of the "Study Summary" section is the "Quantification Methods" section, which is currently empty and has an "Add" button. Below the "Quantification Methods" section is the "Study Factors" section, which is also empty and has "Paste", "Copy", and "Add" buttons. The status bar at the bottom left shows "Ready".

Step 3. Select Analysis Template

The screenshot displays the Thermo Proteome Discoverer 2.5.0.400 software interface. The window title is "Thermo Proteome Discoverer 2.5.0.400". The menu bar includes "File", "View", "Administration", "Tools", "Window", and "Help". The toolbar contains various icons for file operations and analysis. The main window has a tab labeled "Study: New Study 1". Below the toolbar, there are buttons for "Add Files", "Add Fractions", "Remove Files", "Open Containing Folder", "New Analysis", and "Open Analysis Template". The "Open Analysis Template" button is highlighted with a red box, and the word "Click" is written in red above it. The interface is divided into several sections: "Study Definition" (with sub-tabs for "Input Files", "Samples", and "Analysis Results"), "Study Summary" (showing details like "Study Name: New Study 1", "Study Directory: D:\PD 2.5 Study\New Study 1", "Study Type: General", "Last Changed: 10/15/2020 9:43:24 AM", and "Creation Date: 10/15/2020 9:43:24 AM"), "Study Description", "Quantification Methods" (with an "Add" button), and "Study Factors" (with "Paste", "Copy", and "Add" buttons). The status bar at the bottom left shows "Ready".

Step 4. Example of EDC Data Analysis



Step 5. Define Processing Workflow

1) Active Processing workflow

Thermo Proteome Discoverer 2.5.0.400

File View Administration Tools Window Help

Start Page x Study: New Study 1 x

Add Files Add Fractions Remove Files Open Containing Folder New Analysis Open Analysis Template

Study Definition Input Files Samples Analysis Results Workflows Grouping & Quantification

Workflow: WF_Fusion_Basic_SequestHT_XlinkNoncleavable

Description: Crosslink processing workflow for noncleavable crosslinkers with target/decoy validation to be used for searches of low complexity samples or employing a small FASTA database. Specify the FASTA database and any additional modifications in

Workflow Tree

```
graph TD; A[Spectrum Files 0] --> B[Spectrum Selector 1]; B --> C[Sequest HT 2]; C --> D[Target Decoy PSM Validator 3];
```

Post-Processing Nodes

Current Workflow Issues

Node Name	Issue Description	Parameter Name	Value
Sequest HT	Missing value for parameter 'Protein Dat...	Protein Database	
XlinkX/PD Search	Missing value for parameter 'Protein Dat...	Protein Database	

Analysis

Consensus Step (Fully Processing) Edit ⚠ x

Workflow: CWF_Basic_Xlinkx

Result File: Enter result file name.

Child Steps: (1) Add

Processing Step (Fully Processing) Edit Clone ⚠

Workflow: WF_Fusion_Basic_SequestHT_XlinkNoncleavable

Result File: Enter result file name.

Files for Analysis: (0) Clear All

Drag and drop from Input Files here

Click

Processing workflow

Step 5. Define Processing Workflow

2). Define Sequest HT node

Open Open Common Save Save Common Auto Layout Clear

Workflow: WF_Fusion_Basic_SequestHT_XlinkNoncleavable
Description: Crosslink processing workflow for noncleavable crosslinkers with target/decoy validation to be used for searches of low complexity samples or employing a small FASTA database. Specify the FASTA

Workflow Tree

```

    graph TD
      A[Spectrum Files 0] --> B[Spectrum Selector 1]
      B --> C[Sequest HT 2]
      C --> D[Target Decoy PSM Validator 3]
      D --> E[Spectrum Confidence Filter 4]
      E --> F[XlinkX/PD Detect 5]
      F --> G[XlinkX/PD Filter 6]
      G --> H[XlinkX/PD Search 7]
      H --> I[XlinkX/PD Validator 8]
  
```

Click

Post-Processing Nodes

Node Name	Issue Description	Parameter Name	Value
Sequest HT	Missing value for parameter 'Prot...	Protein Database	
XlinkX/PD Search	Missing value for parameter 'Prot...	Protein Database	

Parameters of 'Sequest HT'

Show Advanced Parameters

1. Input Data

Protein Database US140923_13Proteins.fasta

Enzyme Name Trypsin (Full)

Max. Missed Cleavage Sites 2

Min. Peptide Length 6

Max. Peptide Length 150

2. Tolerances

Precursor Mass Tolerance 10 ppm

Fragment Mass Tolerance 0.02 Da

Use Average Precursor Mass False

Use Average Fragment Mass False

3. Spectrum Matching

Use Neutral Loss a ions True

Use Neutral Loss b ions True

Use Neutral Loss y ions True

Use Flanking Ions True

Weight of a ions 0

Weight of b ions 1

Weight of c ions 0

Weight of x ions 0

Weight of y ions 1

Weight of z ions 0

4. Dynamic Modifications

Max. Equal Modifications Per Peptide 3

1. Dynamic Modification None

2. Dynamic Modification Oxidation / +15.995 Da (M)

3. Dynamic Modification EDC / -18.011 Da (D, E, K)

4. Dynamic Modification None

5. Dynamic Modification None

6. Dynamic Modification None

5. Dynamic Modifications (peptide terminus)

1. N-Terminal Modification None

2. N-Terminal Modification None

3. N-Terminal Modification None

1. C-Terminal Modification None

2. C-Terminal Modification None

3. C-Terminal Modification None

6. Dynamic Modifications (protein terminus)

1. N-Terminal Modification Acetyl / +42.011 Da (N-Terminus)

2. N-Terminal Modification None

3. N-Terminal Modification None

1. C-Terminal Modification None

2. C-Terminal Modification None

3. C-Terminal Modification None

7. Static Modifications

Peptide N-Terminus None

Peptide C-Terminus None

1. Static Modification Carbamidomethyl / +57.021 Da (C)

2. Static Modification None

3. Static Modification None

4. Static Modification None

5. Static Modification None

6. Static Modification None

Step 5. Define Processing Workflow

3). Define XlinkX PD Detect node

Open Open Common Save Save Common Auto Layout Clear

Workflow: WF_Fusion_Basic_SequestHT_XlinkNoncleavable

Description: Crosslink processing workflow for noncleavable crosslinkers with target/decoy validation to be used for searches of low complexity samples or employing a small FASTA database. Specify the FASTA

Workflow Tree

Click

Post-Processing Nodes

Current Workflow Issues

Node Name	Issue Description	Parameter Name	Value
Sequest HT	Missing value for parameter 'Prot...	Protein Database	
XlinkX/PD Search	Missing value for parameter 'Prot...	Protein Database	

Parameters of 'XlinkX/PD Detect'

Show Advanced Parameters

1. Input Data

Acquisition strategy	NonCleavable
Crosslink Modification	EDC / -18.011 Da (D, E, K)
Minimum S/N	1.5
Enable protein N-terminus linkage	True

Acquisition strategy

The data acquisition strategy used. This will impact how the data is analyzed further down in the pipeline.

- 'MS2': Strategy for a gas-phase cleavable crosslinker, one MS2 scan containing diagnostic ions and fragments.
- 'MS2_MS2': Strategy for a gas-phase cleavable crosslinker, one MS2 spectrum to detect diagnostic peaks, and one MS2 to identify the fragments.
- 'MS2_MS2_MS3': Strategy for a gas-phase cleavable crosslinker, one MS2 spectrum to detect diagnostic peaks, one MS2 to identify the fragments and MS3 spectra from the diagnostic peaks.
- 'MS2_MS3': Strategy for a gas-phase cleavable crosslinker, one MS2 spectrum to detect diagnostic peaks and MS3 spectra from the diagnostic peaks.
- 'NonCleavable': Strategy for non cleavable crosslinkers, suitable for datasets with up to 100 proteins.
- 'NonCleavable_fast': Strategy for non cleavable crosslinkers, a faster algorithm allowing to analyze samples with up to 300 proteins.

Step 5. Define Processing Workflow

4). Define XlinkX/PD Detect node

Open Open Common Save Save Common Auto Layout Clear

Workflow: WF_Fusion_Basic_SequestHT_XlinkNoncleavable

Description: Crosslink processing workflow for noncleavable crosslinkers with target/decoy validation to be used for searches of low complexity samples or employing a small FASTA database. Specify the FASTA

Workflow Tree

Click

Post-Processing Nodes

Current Workflow Issues

Node Name	Issue Description	Parameter Name	Value
Sequest HT	Missing value for parameter 'Prot...	Protein Database	
XlinkX/PD Search	Missing value for parameter 'Prot...	Protein Database	

Parameters of 'XlinkX/PD Filter'

Show Advanced Parameters

1. Output Data

Select

Select

Determines which fragment scans will be forwarded to the search engine node coming after this node.

- 'Crosslinks': Spectra containing reporter peaks and connected spectra pass the filter.
- 'Peptides': Spectra without reporter peaks pass the filter.

Step 5. Define Processing Workflow

5). Define XlinkX/PD Search node

Open Open Common Save Save Common Auto Layout Clear

Workflow: WF_Fusion_Basic_SequestHT_XlinkNoncleavable
Description: Crosslink processing workflow for noncleavable crosslinkers with target/decoy validation to be used for searches of low complexity samples or employing a small FASTA database. Specify the FASTA

Workflow Tree

```

    graph TD
      A[Spectrum Files 0] --> B[Spectrum Selector 1]
      B --> C[Sequest HT 2]
      C --> D[Target Decoy PSM Validator 3]
      D --> E[Spectrum Confidence Filter 4]
      E --> F[XlinkX/PD Detect 5]
      F --> G[XlinkX/PD Filter 6]
      G --> H[XlinkX/PD Search 7]
      H --> I[XlinkX/PD Validator 8]
  
```

Click

Post-Processing Nodes

Current Workflow Issues

Node Name	Issue Description	Parameter Name	Value
Sequest HT	Missing value for parameter 'Prot...	Protein Database	
XlinkX/PD Search	Missing value for parameter 'Prot...	Protein Database	

Parameters of 'XlinkX/PD Search'

Show Advanced Parameters

1. General Data

Protein Database US140923_13Proteins.fasta

Retain FASTA file indexes True

Enzyme Name Trypsin (Full)

Maximum Missed Cleavages 2

Minimum Peptide Length 5

2. Tolerances

Precursor Mass Tolerance 10 ppm

FTMS Fragment Mass Tolerance 20 ppm

ITMS Fragment Mass Tolerance 0.5 Da

4. Static Modifications

Static Modification Carbamidomethyl / +57.021 Da (C)

Static Modification None

Static Any N-term Modification None

Static Any C-term Modification None

Static Protein N-term Modification None

Static Protein C-term Modification None

5. Dynamic Modifications

Dynamic Modification Oxidation / +15.995 Da (M)

Dynamic Modification None

Dynamic Modification None

Dynamic Modification None

Dynamic Modification None

Dynamic Any N-term Modification None

Dynamic Any N-term Modification None

Dynamic Any N-term Modification None

Dynamic Any C-term Modification None

Dynamic Any C-term Modification None

Dynamic Any C-term Modification None

Dynamic Any C-term Modification None

Dynamic Protein N-term Modification None

Dynamic Protein C-term Modification None

Protein Database
The sequence database to be searched.

Step 5. Define Processing Workflow

6). XlinkX/PD Detect node

Workflow: WF_Fusion_Basic_SequestHT_XlinkNoncleavable
 Description: Crosslink processing workflow for noncleavable crosslinkers with target/decoy validation to be used for searches of low complexity samples or employing a small FASTA database. Specify the FASTA

Workflow Tree

```

  graph TD
    A[Spectrum Files 0] --> B[Spectrum Selector 1]
    B --> C[Sequest HT 2]
    C --> D[Target Decoy PSM Validator 3]
    D --> E[Spectrum Confidence Filter 4]
    E --> F[XlinkX/PD Detect 5]
    F --> G[XlinkX/PD Filter 6]
    G --> H[XlinkX/PD Search 7]
    H --> I[XlinkX/PD Validator 8]
  
```

1. Click

Post-Processing Nodes

Current Workflow Issues

Node Name	Issue Description	Parameter Name	Value
Sequest HT	Missing value for parameter 'Prot...	Protein Database	
XlinkX/PD Search	Missing value for parameter 'Prot...	Protein Database	

Parameters of 'XlinkX/PD Validator'

Hide Advanced Parameters

- 1. Input Data
 - FDR threshold: 0.01
- 2. Advanced
 - Separate inter from intra: True

Parameters of 'XlinkX/PD Validator'

Show Advanced Parameters 2. Click

- 1. Input Data
 - FDR threshold: 0.01

FDR threshold
 Maximum FDR rate for a crosslinked peptide pair to pass

Minimum value = 0
 Maximum value = 1

Step 6. Active Consensus Workflow

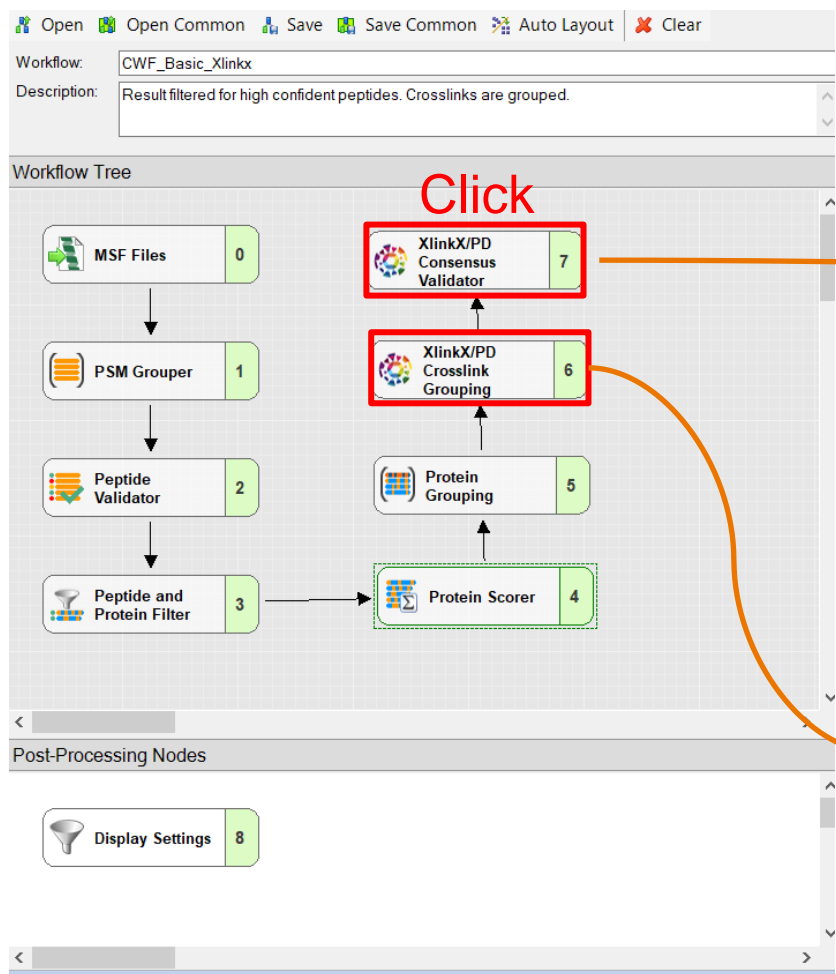
The screenshot displays the Thermo Proteome Discoverer 2.5.0.400 interface. The main window is titled "Workflow Editor" and shows a workflow tree with the following nodes:

- MSF Files (0)
- PSM Grouper (1)
- Peptide Validator (2)
- Peptide and Protein Filter (3)
- Display Settings (8)

The "Workflow" field is set to "CWF_Basic_Xlinkx" and the description is "Result filtered for high confident peptides. Crosslinks are grouped." The "Analysis" panel on the right shows a "Consensus Step (Fully Processing)" which is highlighted with a red box. An orange arrow points from the text "Consensus workflow" to this step. The "Analysis" panel also shows a "Processing Step (Fully Processing)" and a "Files for Analysis" section with a "Clear All" button. The "Consensus Step" panel includes an "Edit" button, a warning icon, and a close icon. The word "Click" is written in red next to the "Edit" button.

Step 7. Define Consensus Workflow

Define Xlinkx/PD Grouping and Validator node



Parameters of 'XlinkX/PD Consensus Validator'

Show Advanced Parameters

1. Input Data

Cross-link FDR threshold	0.01
CSM FDR threshold	0.01

Cross-link FDR threshold
Maximum FDR rate for a cross-link to pass

Minimum value = 0
Maximum value = 1

Parameters of 'XlinkX/PD Crosslink Grouping'

Show Advanced Parameters

1. General

Ignore reporter scan identification False

Ignore reporter scan identification
This option is active for the MS2_MS2 and MS2_MS3 workflows; when set to true, upon finalizing the peptide pair the software will not take any identification from the reporter scan into account.

Step 8. Add Files and Run Analysis

1. Click **Add Files**

2. Select **F2 t02630_150120_9mix_3hr_EDC .raw**

3. Drag and drop

4. Click **Run**

Error	ID	Name	File Type	Sample Information
	F2	t02630_150120_9mix_3hr_EDC	.raw	Sample Type: [Sample]

Analysis

Consensus Step (Fully Processing) Edit x

Workflow: CWF_Basic_Xlinkx
Result File: t02630_150120_9mix_3hr_EDC.pdResult

Child Steps: (1) Add

Processing Step (Fully Processing) Edit Clone

Workflow: WF_Fusion_Basic_SequestHT_XlinkxNoncleavable
Result File: t02630_150120_9mix_3hr_EDC.msf

Files for Analysis: (1) Clear All

- x F2 t02630_150120_9mix_3hr_EDC Sample Type: [Sample]



New Options for Results Export

Results Page

Thermo Proteome Discoverer 2.5.0.400

File View Administration Tools Window Help

Start Page x S140923_noncleavable_fast_semispecificHCD_MS2_EDC_XLScore30DeltaXLScore18_PD2.5.0.375 x

Proteins Protein Groups Peptide Groups PSMs MS/MS Spectrum Info Input Files Specialized Traces Study Information Result Statistics Crosslinks CSMS Crosslink MS2 Scans Crosslink Reporter Peaks Crosslink Summary

	Checked	Protein FDF	Master	Accession	Description	Exp. q-value	Coverage [%]	# Peptides	# Crosslinks	# CSMS	# PSMs	# Unique Peptides	# AAs	MW [kDa]	calc. pI	Score St	# Peptides	# Protein Groups
1	<input type="checkbox"/>	High	✓	B1J0T5	Beta-galactosidase OS=Escherichia coli (strain ATCC 8739 / D	0.000	69%	49	2	3	837	14	1024	116.4	5.58	3049.26	49	1
2	<input type="checkbox"/>	High	✓	P00918	Carbonic anhydrase 2 OS=Homo sapiens OX=9606 GN=CA2 F	0.000	81%	33	7	23	697	33	260	29.2	7.40	2158.96	33	1
3	<input type="checkbox"/>	High	✓	P02787	Serotransferrin OS=Homo sapiens OX=9606 GN=TF PE=1 SV	0.000	91%	95	14	22	611	95	698	77.0	7.12	1771.63	95	1
4	<input type="checkbox"/>	High	✓	P02768	Serum albumin OS=Homo sapiens OX=9606 GN=ALB PE=1 S	0.000	80%	52	21	38	417	52	609	69.3	6.28	1451.98	52	1
5	<input type="checkbox"/>	High	✓	A6T129	Beta-galactosidase 2 OS=Klebsiella pneumoniae subsp. pneum	0.000	53%	38	2	3	393	5	1024	116.2	5.53	1428.89	38	1
6	<input type="checkbox"/>	High	✓	P00432	Catalase OS=Bos taurus OX=9913 GN=CAT PE=1 SV=3	0.000	77%	42	6	8	374	42	527	59.9	7.28	1374.95	42	1
7	<input type="checkbox"/>	High	✓	P00489	Glycogen phosphorylase, muscle form OS=Oryctolagus cunicul	0.000	70%	66	9	10	379	57	843	97.2	7.21	1235.05	66	1
8	<input type="checkbox"/>	High	✓	P01012	Ovalbumin OS=Gallus gallus OX=9031 GN=SERPINB14 PE=1	0.000	88%	28	2	2	239	28	386	42.9	5.29	777.14	28	1
9	<input type="checkbox"/>	High	✓	A8AKB8	Beta-galactosidase OS=Citrobacter koseri (strain ATCC BAA-8	0.000	28%	15	0	0	192	9	1025	116.2	5.68	638.58	15	1
10	<input type="checkbox"/>	High	✓	P08515	Glutathione S-transferase class-mu 26 kDa isozyme OS=Schist	0.000	83%	23	10	17	149	23	218	25.5	6.54	430.11	23	1
11	<input type="checkbox"/>	High	✓	P13645	Keratin, type I cytoskeletal 10 OS=Homo sapiens OX=9606 GN	0.000	53%	27	1	1	84	27	584	58.8	5.21	241.89	27	1
12	<input type="checkbox"/>	High	✓	P04264	Keratin, type II cytoskeletal 1 OS=Homo sapiens OX=9606 GN	0.000	50%	31	1	10	42	30	644	66.0	8.12	142.58	31	1
13	<input type="checkbox"/>	High	✓	P35908	Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens OX	0.000	62%	30	0	0	41	29	639	65.4	8.00	140.31	30	1
14	<input type="checkbox"/>	High	✓	P35527	Keratin, type I cytoskeletal 9 OS=Homo sapiens OX=9606 GN	0.000	39%	18	0	0	34	18	623	62.0	5.24	136.27	18	1
15	<input type="checkbox"/>	High	✓	Q0VCM4	Glycogen phosphorylase, liver form OS=Bos taurus OX=9913 C	0.000	42%	25	2	2	37	21	851	97.4	7.12	107.37	25	1
16	<input type="checkbox"/>	High	✓	P00915	Carbonic anhydrase 1 OS=Homo sapiens OX=9606 GN=CA1 F	0.000	60%	12	0	0	25	12	261	28.9	7.12	99.01	12	1
17	<input type="checkbox"/>	High	✓	P11216	Glycogen phosphorylase, brain form OS=Homo sapiens OX=96	0.000	21%	12	4	5	40	2	843	96.6	6.86	96.72	12	1
18	<input type="checkbox"/>	High	✓	P48034	Aldehyde oxidase 1 OS=Bos taurus OX=9913 GN=AOX1 PE=1	0.000	29%	24	0	0	26	24	1339	147.5	7.28	87.13	24	1
19	<input type="checkbox"/>	High	✓	P02533	Keratin, type I cytoskeletal 14 OS=Homo sapiens OX=9606 GN	0.000	48%	19	0	0	25	17	472	51.5	5.16	82.08	19	1
20	<input type="checkbox"/>	High	✓	P08779	Keratin, type I cytoskeletal 16 OS=Homo sapiens OX=9606 GN	0.000	39%	16	0	0	21	14	473	51.2	5.05	70.47	16	1
21	<input type="checkbox"/>	High	✓	P15924	Desmoplakin OS=Homo sapiens OX=9606 GN=DSP PE=1 SV	0.000	15%	29	1	1	31	29	2871	331.6	6.81	66.84	29	1
22	<input type="checkbox"/>	High	✓	P62976	Polyubiquitin OS=Cricetulus griseus OX=10029 PE=2 SV=2	0.000	92%	14	1	2	21	14	658	73.9	8.66	62.40	14	1
23	<input type="checkbox"/>	High	✓	P00761	Trypsin OS=Sus scrofa OX=9823 PE=1 SV=1	0.000	42%	5	0	0	17	5	231	24.4	7.18	49.18	5	1

Show Associated Tables

Ready 23 Proteins; 23 Protein Groups; 742 Peptide Groups; 4139 PSMs; 49454 MS/MS Spectrum Info; 1/2 Input Files; 1 Study Information; 2 Specialized Traces; 224 Result Statistics; 72 Crosslinks; 134 CSMS; 134 Crosslink MS2 Scans; 45320 Crosslink Repor...

Decoy CSMs

1. Click

2. Click

3. Click

Ready 23 Proteins; 23 Protein Groups; 742

	Checked	Protein FDF	Master	Accession	Description	Exp. q-value	Coverage [%]	# Peptides	# Crosslinks	# CSMs	# PSMs	# Unique Peptides	# AAs	MW [kDa]	calc. pI	Score Sr	# Peptides	# Protein Groups
1	<input type="checkbox"/>	High	✓	B1J0T5	Beta-galactosidase OS=Escherichia coli (strain ATCC 8739 / D	0.000	69%	49	2	3	837	14	1024	116.4	5.58	3049.26	49	1
2	<input type="checkbox"/>	High	✓	P00918	Carbonic anhydrase 2 OS=Homo sapiens OX=9606 GN=CA2 F	0.000	81%	33	7	23	697	33	260	29.2	7.40	2158.96	33	1
3	<input type="checkbox"/>	High	✓	P02787	Serotransferrin OS=Homo sapiens OX=9606 GN=TF PE=1 SV-	0.000	91%	95	14	22	611	95	698	77.0	7.12	1771.63	95	1
4	<input type="checkbox"/>	High	✓	P02768	Serum albumin OS=Homo sapiens OX=9606 GN=AI PE=1 SV=1											1451.98	52	1
5	<input type="checkbox"/>	High	✓	A6T129	Beta-galactosidase 2 OS=Escherichia coli (strain ATCC 8739 / D											1428.89	38	1
6	<input type="checkbox"/>	High	✓	P00432	Catalase OS=Bos taurus OX=9606 GN=CA3 F											1374.95	42	1
7	<input type="checkbox"/>	High	✓	P00489	Glycogen phosphorylase OS=Homo sapiens OX=9606 GN=PYGL F											1235.05	66	1
8	<input type="checkbox"/>	High	✓	P01012	Ovalbumin OS=Gallus gallus OX=9606 GN=OVA F											777.14	28	1
9	<input type="checkbox"/>	High	✓	A8AKB8	Beta-galactosidase OS=Escherichia coli (strain ATCC 8739 / D											638.58	15	1
10	<input type="checkbox"/>	High	✓	P08515	Glutathione S-transferase OS=Homo sapiens OX=9606 GN=GSTA1 F											430.11	23	1
11	<input type="checkbox"/>	High	✓	P13645	Keratin, type I cytoskeletal class I OS=Homo sapiens OX=9606 GN=KRT1A F											241.89	27	1
12	<input type="checkbox"/>	High	✓	P04254	Keratin, type II cytoskeletal class I OS=Homo sapiens OX=9606 GN=KRT2A F											142.58	31	1
13	<input type="checkbox"/>	High	✓	P35908	Keratin, type II cytoskeletal class II OS=Homo sapiens OX=9606 GN=KRT2B F											140.31	30	1
14	<input type="checkbox"/>	High	✓	P35527	Keratin, type I cytoskeletal class II OS=Homo sapiens OX=9606 GN=KRT1B F											136.27	18	1
15	<input type="checkbox"/>	High	✓	Q0VCM4	Glycogen phosphorylase OS=Homo sapiens OX=9606 GN=PYGL F											107.37	25	1
16	<input type="checkbox"/>	High	✓	P00915	Carbonic anhydrase 1 OS=Homo sapiens OX=9606 GN=CA1 F											99.01	12	1
17	<input type="checkbox"/>	High	✓	P11216	Glycogen phosphorylase OS=Homo sapiens OX=9606 GN=PYGL F											96.72	12	1
18	<input type="checkbox"/>	High	✓	P48034	Aldehyde oxidase 1 OS=Homo sapiens OX=9606 GN=APOX1 F											87.13	24	1
19	<input type="checkbox"/>	High	✓	P02533	Keratin, type I cytoskeletal class I OS=Homo sapiens OX=9606 GN=KRT1A F											82.08	19	1
20	<input type="checkbox"/>	High	✓	P08779	Keratin, type I cytoskeletal class II OS=Homo sapiens OX=9606 GN=KRT1B F											70.47	16	1
21	<input type="checkbox"/>	High	✓	P15924	Desmoplakin OS=Homo sapiens OX=9606 GN=DSPL F											66.84	29	1
22	<input type="checkbox"/>	High	✓	P62976	Polyubiquitin OS=Cricetulus cricetus OX=9606 GN=UBI1 F											62.40	14	1
23	<input type="checkbox"/>	High	✓	P00761	Trypsin OS=Sus scrofa OX=9606 GN=TPST F											49.18	5	1

Decoy CSMs Page

Thermo Proteome Discoverer 2.5.0.400

File View Administration Tools Window Help

Start Page x S140923_noncleavable_fast_semispecificHCD_MS2_EDC_XLScore30DeltaXLScore18_PD2.5.0.375 x

Proteins Protein Groups Peptide Groups PSMs MS/MS Spectrum Info Input Files Specialized Traces Study Information

Result Statistics Crosslinks CSMs Crosslink MS2 Scans Crosslink Reporter Peaks Crosslink Summary

Decoy CSMs

Checked Sequence Crosslinker Crosslink Type Crosslink Strategy Identified By # Identified MS2 Scans XlinkX Score ΔXlinkX Score m/z [Da] Charge MH+ [Da] First Scan RT [min] ΔM [ppm] Reporter Ion Score Sequence A Modifications A Cross

Decoy CSMs
0 items shown (0 filtered out)

Show Associated Tables

Ready 23 Proteins; 23 Protein Groups; 742 Peptide Groups; 4139 PSMs; 49454 MS/MS Spectrum Info; 1/2 Input Files; 1 Study Information; 2 Specialized Traces; 224 Result Statistics; 72 Crosslinks; 134 CSMs; 134 Crosslink MS2 Scans; 45320 Crosslink Repor...

XlinkX Score Visualization

Thermo Proteome Discoverer 2.5.0.400

File View Administration Tools Window Help

Start Page x S140923_noncleavable_fact...MS2_EDC_XLScore30DeltaXLScore18_PD2.5.0.375 x

Report Item Distribution

Scatter Plot Histograms Bar Charts Pie Charts Venn Diagrams Volcano Plots PCA Plots Sample Abundances Heat Map

Options

Load Save Factor Defaults Data Source: CSMs - XlinkX Score Refresh

1. Click (on Histograms icon)

2. Click (on CSMs - XlinkX Score dropdown)

3. Select (on the chart)

1. Chart Options

- Chart Type: Column
- Show Cumulative: False
- Horizontal Grid Lines: None
- Vertical Grid Lines: None

2. Column Options

- Show Column Amount: False
- Show Percentages: False
- Column Display: Flat
- Column Width: 0.8
- Column Label Font: Arial, 8pt

3. Axis Options

- X-Axis Number Format: Decimal
- X-Axis Title: **CSMs - XlinkX Score**
- Y-Axis Type: Linear
- Y-Axis Title: **Count**
- Reduce Number of Axis Labels: True
- Axis Title Font: Arial, 12pt
- Axis Scale Font: Arial, 10pt

4. Binning Options

- Binning Method: Auto
- Number of Bins: 20
- Bin Width: 1
- Use Full Series Value Range: True
- Minimum Value: 30
- Maximum Value: 140

5. Legend Options

- Show Legend: None
- Legend Font: Arial, 8pt

6. Series Options

- Show Only Checked Items: False
- Show Decoy Items: **True**
- Show Excluded Items: False
- Target Series Color: Firebrick
- Decoy Series Color: Black
- Excluded Target Series Color: Salmon
- Excluded Decoy Series Color: Silver

Show Decoy Items
If set to TRUE, also the decoy items are shown as a separate series.

CSMs - XlinkX Score	Count
30	31
40	31
50	23
60	18
70	16
80	5
90	7
100	1
110	0
120	1
130	1
140	0

Crosslink Reporter Peaks Crosslink Summary Decoy CSMs

Proteins Protein Groups Peptide Groups

PSMs MS/MS Spectrum Info Input Files

Specialized Traces Study Information Result Statistics

Crosslinks **CSMs** Crosslink MS2 Scans

Checked	Sequence
<input type="checkbox"/>	DGAGDVAFVKHSTIFENLANKADR-DQYELLCLDNTR
<input type="checkbox"/>	SLHTLFGDKLCTVATLR-LVNEVTEFAK
<input type="checkbox"/>	FKDLGEENFK-DAHKSEVAHR
<input type="checkbox"/>	NPDWPWAKNLNEKYELLCLDGTR-KPVEEYANCHLAR
<input type="checkbox"/>	LVRPEVDVMCTAFHDNEETFLK-YKAAFTECCQAADK
<input type="checkbox"/>	KLNFNGEGEPEELMVDNWRPAQPLK-VVDVLDISK
<input type="checkbox"/>	KLNFNGEGEPEELMVDNWRPAQPLK-VVDVLDISK
<input type="checkbox"/>	KSASDLTWDNLK-DYELLCLDGTR
<input type="checkbox"/>	LYAEERYPILPEYLQCVKELYR-GGLEPINFQTAADQAR
<input type="checkbox"/>	KLNFNGEGEPEELMVDNWRPAQPLK-VVDVLDISK
<input type="checkbox"/>	DFETLKVDFLSK-IAYSKDFETLK
<input type="checkbox"/>	HQTVPQNTGGKNPDPWAKNLNEK-DYELLCLDGTR
<input type="checkbox"/>	KLNFNGEGEPEELMVDNWRPAQPLK-VVDVLDISK
<input type="checkbox"/>	KWLSLPGETRPLILCEYAHAMGNSLGGFAK-VDEDQPFPA
<input type="checkbox"/>	KLNFNGEGEPEELMVDNWRPAQPLK-VVDVLDISK
<input type="checkbox"/>	AAQKPDVLTGGGNPVGDKLNSLTVGPR-LVNANGEAVY
<input type="checkbox"/>	NLNEKYELLCLDGTR-KSASDLTWDNLK
<input type="checkbox"/>	KSASDLTWDNLK-DYELLCLDGTR
<input type="checkbox"/>	KLNFNGEGEPEELMVDNWRPAQPLK-GKSADFTNFDPR
<input type="checkbox"/>	RIEAIQIDKYLK-LLEYLEEK
<input type="checkbox"/>	SLHTLFGDKLCTVATLR-LVNEVTEFAK
<input type="checkbox"/>	NKYEDEINKR-NMQDMVEDYR
<input type="checkbox"/>	LDSELNMQDMVEDYR-NKYEDEINKR
<input type="checkbox"/>	NVLQPSSVDSQTAMVLVNAIVFKGLWEK-ISQAVHAAHAE
<input type="checkbox"/>	LVRPEVDVMCTAFHDNEETFLK-YKAAFTECCQAADK
<input type="checkbox"/>	SLHTLFGDKLCTVATLR-LVNEVTEFAK
<input type="checkbox"/>	LCMGSGNLNCEPNNKEGYGYTGAFR-IECVSAETTEDCL
<input type="checkbox"/>	NKYEDEINKR-NMQDMVEDYR

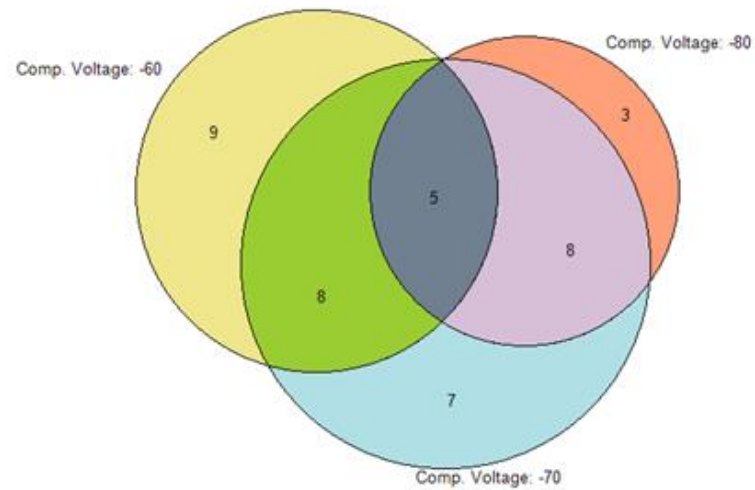
Show Associated Tables

Ready 23 Proteins; 23 Protein Groups; 742 Peptide Groups; 4139 PSMs; 49454 MS/MS Spectrum Info; 1/2 Input Files; 1 Study Information; 2 Specialized Traces; 224 Result Statistics; 72 Crosslinks; 134 CSMs; 134 Crosslink MS2 Scans; 45320 Crosslink Repor...

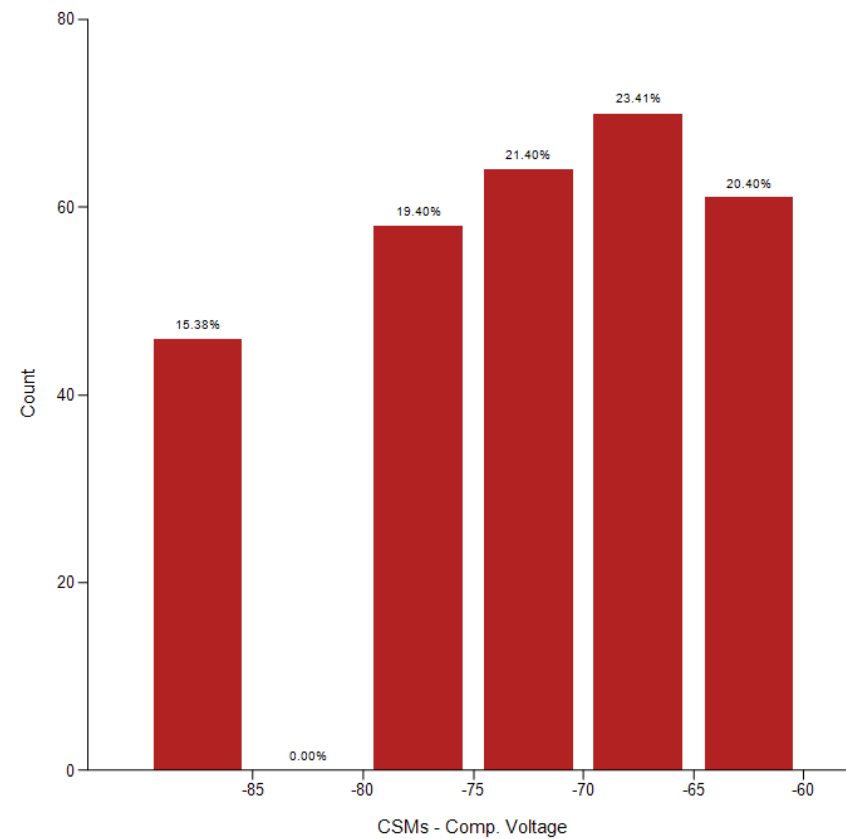
Compensation Voltage in CSM Table for FAIMS Data

Data Source: Crosslinks Refresh

1. Group: Comp. Voltage: -60 2. Group: Comp. Voltage: -80 3. Group: Comp. Voltage: -70



	Exclusive	Total	Label
A	9	22	Comp. Voltage: -60
B	3	16	Comp. Voltage: -80
C	7	28	Comp. Voltage: -70
B C	8	13	Comp. Voltage: -80 Comp. Voltage: -70
A C	8	13	Comp. Voltage: -60 Comp. Voltage: -70
A B C	5	5	Comp. Voltage: -60 Comp. Voltage: -80 Comp. Voltage: -70
Sum		40	

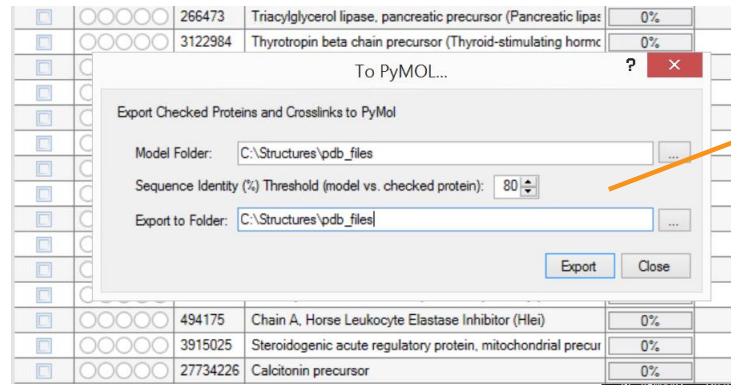




Exporting Crosslinks to Pymol

Automatic Export to Pymol (incl crosslink distances)

Export proteins containing crosslinkers in PD



Path to folder containing structure files for the exported protein, sequence similarities threshold between structure and PD sequence and destination folder

Color-coded display of protein sequence for easy highlighting of

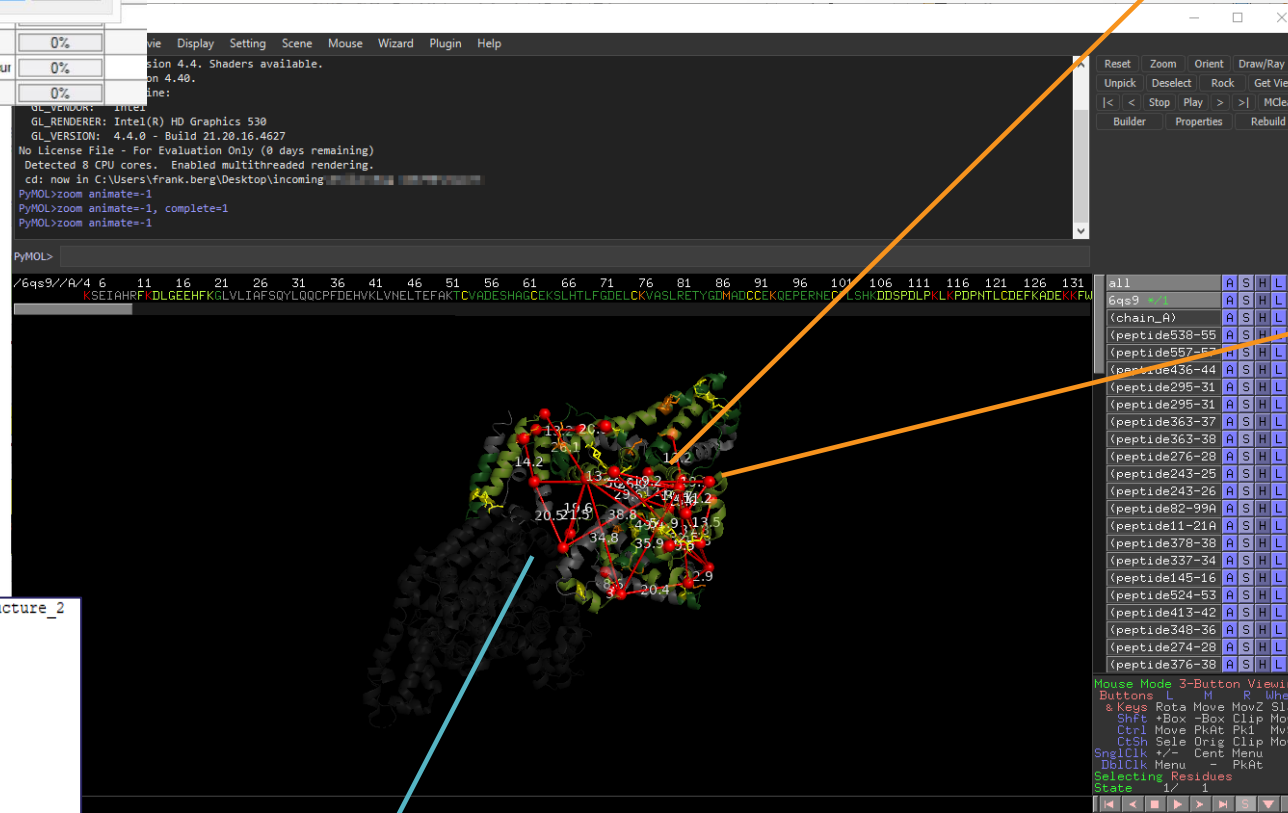
- Links (red)
- Found Modifications (orange, yellow)
- Identified peptide chains (green)

... for pymol
Protein_chain.py

... for Xlink Analyzer
Protein_chain.txt

... add distances file
Protein_chain.Distances

PDB_id	Chain_1	Position_in_structure_1	Chain_2	Position_in_structure_2	Position_in_PDresult_1	Position_in_PDresult_2	Type
Description Distance (Angstrom)							
6QS9	A	4	A	439	28	463	intra
6QS9	A	431	A	131	455	155	intra
6QS9	A	537	A	431	561	455	intra
6QS9	A	4	A	431	28	455	intra
6QS9	A	221	A	131	245	155	intra
6QS9	A	439	A	431	463	455	intra
6QS9	A	4	A	221	28	245	intra
6QS9	A	132	A	131	156	155	intra
6QS9	A	439	A	221	463	245	intra
6QS9	A	4	A	239	28	263	intra
6QS9	A	12	A	4	36	28	intra
6QS9	A	12	A	131	36	155	intra
6QS9	A	242	A	431	266	455	intra
6QS9	A	221	A	431	245	455	intra
6QS9	A	413	A	535	437	559	intra



Graphical display of links including calculated crosslink distances

All positions aligned to PDB structure (done automatically)

Download a Protein Structure File

Access protein structure database <https://www.rcsb.org/>

The screenshot shows the RCSB PDB homepage. At the top, there is a navigation bar with links for Deposit, Search, Visualize, Analyze, Download, Learn, and More, along with a MyPDB button. Below this is a search bar with the placeholder text "Enter search term(s)" and a search icon. The main content area features a "Welcome" message, a "Deposit" section, and a "Search" section. A sidebar on the left contains navigation links for Welcome, Deposit, Search, Visualize, Analyze, Download, and Learn. The main content area also includes a "A Structural View of Biology" section, a "COVID-19 CORONAVIRUS Resources" banner, and an "October Molecule of the Month" section featuring a 3D model of the Capsaicin Receptor TRPV1.

RCSB PDB Deposit Search Visualize Analyze Download Learn More MyPDB

RCSB PDB 169963 Biological Macromolecular Structures Enabling Breakthroughs in Research and Education

Enter search term(s)

Advanced Search | Browse Annotations

October Molecule of the Month

Capsaicin Receptor TRPV1

Download a Protein Structure File in .pdb or .cif Format

The screenshot shows the RCSB PDB website interface. At the top, a navigation bar includes 'RCSB PDB', 'Deposit', 'Search', 'Visualize', 'Analyze', 'Download', 'Learn', and 'More'. A search bar contains the ID '4F5S'. Below the search bar, a 'Download Files' dropdown menu is open, listing various file formats: FASTA Sequence, PDB Format, PDB Format (gz), PDBx/mmCIF Format, PDBx/mmCIF Format (gz), and PDBML/XML Format (gz). The main content area displays the protein structure '4F5S: Crystal Structure of Bovine Serum Albumin' with a 3D ribbon model. The 'Download Files' menu is highlighted with an orange box and labeled '2. Click'. The search bar is also highlighted with an orange box and labeled '1. Search'. The 'Download Files' menu is open, showing options for downloading the structure in various formats. The 'Download Files' menu is open, showing options for downloading the structure in various formats. The 'Download Files' menu is open, showing options for downloading the structure in various formats.

1. Search

2. Click

4F5S

Crystal Structure of Bovine Serum Albumin

DOI: 10.2210/pdb4F5S/pdb

Classification: TRANSPORT PROTEIN

Organism(s): Bos taurus

Mutation(s): Yes

Deposited: 2012-05-13 Released: 2012-10-03

Deposition Author(s): Bujacz, A., Bujacz, G.

Experimental Data Snapshot

Method: X-RAY DIFFRACTION

Resolution: 2.47 Å

R-Value Free: 0.259

R-Value Work: 0.197

R-Value Observed: 0.199

wwPDB Validation

Metric

Rfree

Clashscore

Ramachandran outliers

Sidechain outliers

RSRZ outliers

Worse

Perce

Perce

FASTA Sequence

PDB Format

PDB Format (gz)

PDBx/mmCIF Format

PDBx/mmCIF Format (gz)

PDBML/XML Format (gz)

Biological Assembly 1

Biological Assembly 2

Structure Factors (CIF)

Structure Factors (CIF - gz)

Validation Full PDF

Validation XML

3D View: Structure | Electron Density | Ligand Interaction

Global Symmetry: Asymmetric - C1

Global Stoichiometry: Monomer - A1

Export Crosslink Result to Pymol

Thermo Proteome Discoverer 2.5.0.400

File View Administration Tools Window Help

3 Administration x US111571_F1_Agl5_20150717_FL_BSA_DSSO_MS2_OTCID_MS3_OTCID_PD2.5.0.185-(1) x

Peptide Groups PSMS MS/MS Spectrum Info Input Files Specialized Traces Study Information Crosslinks CSMS Crosslink MS2 Scans Crosslink MS3 Scans Crosslink Reporter Peaks Crosslink Summary

	Max. XlinkX Score	Crosslinker	Crosslink T _y	# CSMS	# Proteins	Sequence A	Modifications A	Accession A	Position A	Sequence B	Modifi
3	141.48	DSSO	Intra	1	1	CCT[K]PESER	2×Carbamidomethyl [C1: C2]; 1×DSSO [K4]	BSA	439	SLG[K]VGTR	1×DS
4	180.36									KJAEFVEVTK	1×DS
5	196.27									KJAEFVEVTK	1×DS
6	112.84									KJAWSVAR	1×DS
7	65.04									CLLP[K]IETMR	1×Car
8	123.86									KJEYATLEECCAK	2×Car
9	88.68									JVLTSSAR	1×DS
10	464.17	DSSO	Intra	2	1	ECCHGDLLCADDRADLA[K]YICNDQDTISSK	4×Carbamidomethyl [C2: C3: C			SHCIAEVEK	3×Car
11	132.24	DSSO	Intra	2	1	DDSPDLP[K]LKPDPNTLCDEFKADEK	1×Carbamidomethyl [C17]; 1×D				1×DS
12	183.79	DSSO	Intra	1	1	LA[K]JEYATLEECCAK	2×Carbamidomethyl [C12: C13]				1×DS
13	186.83	DSSO	Intra	1	1	LFTFHADICTLPDTE[K]QIK	1×Carbamidomethyl [C9]; 1×DS				1×DS
14	40.92	DSSO	Intra	1	1	L[K]PDPNTLCDEFK	1×Carbamidomethyl [C9]; 1×DS				1×DS

1. Click

2. Click

3. Define

4. Click

5. Click

Export

Close










Export completed.

OK

Ready 1 Proteins; 1 Protein Groups; 39 Peptide Groups; 68 PSMS; 3375 MS/MS Spectrum Info; 1/2 Input Files; 1 Study Information; 2 Specialized Traces; 23 Crosslinks; 36 CSMS; 27 Crosslink MS2 Scans; 177 Crosslink MS3 Scans; 179 Crosslink Reporter Pea...

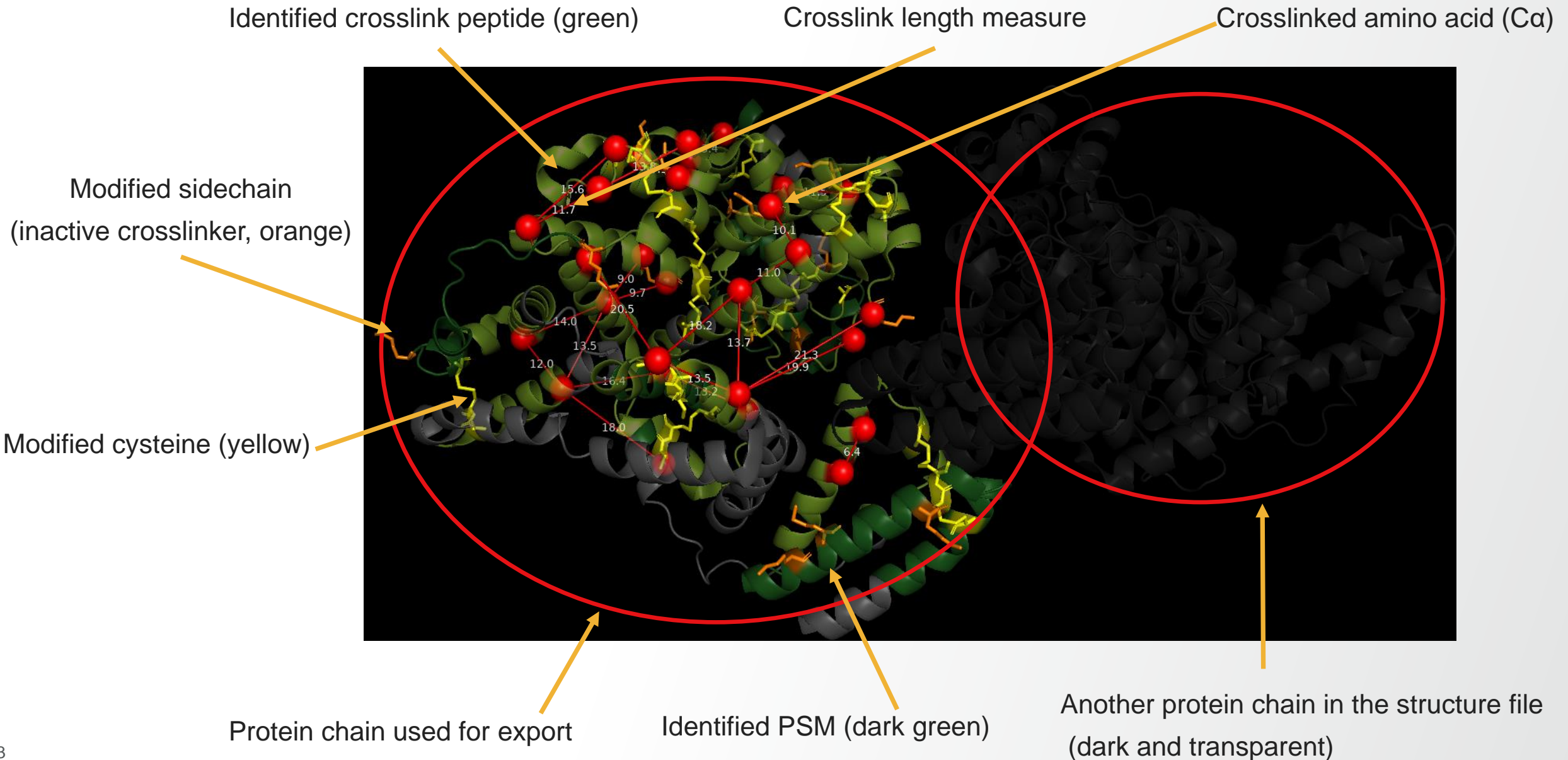
Pymol Folder

> DATA (D:) > BSA_DSSO

<input type="checkbox"/>	Name	Date modified	Type	Size
<input type="checkbox"/>	 4f5s.pdb	10/20/2020 6:14 PM	Protein Data Bank ...	1,589 KB
<input type="checkbox"/>	 BSA_4f5spdbchainA.log	10/20/2020 7:53 PM	Text Document	1 KB
<input checked="" type="checkbox"/>	 BSA_4f5spdbchainA.py	10/20/2020 7:53 PM	Python Script	5 KB
<input type="checkbox"/>	 BSA_4f5spdbchainA_cxList.txt	10/20/2020 7:53 PM	Text Document	2 KB
<input type="checkbox"/>	 BSA_4f5spdbchainA_distances.txt	10/20/2020 7:53 PM	Text Document	2 KB
<input type="checkbox"/>	 BSA_4f5spdbchainB.log	10/20/2020 7:53 PM	Text Document	1 KB
<input type="checkbox"/>	 BSA_4f5spdbchainB.py	10/20/2020 7:53 PM	Python Script	5 KB
<input type="checkbox"/>	 BSA_4f5spdbchainB_cxList.txt	10/20/2020 7:53 PM	Text Document	2 KB
<input type="checkbox"/>	 BSA_4f5spdbchainB_distances.txt	10/20/2020 7:53 PM	Text Document	2 KB

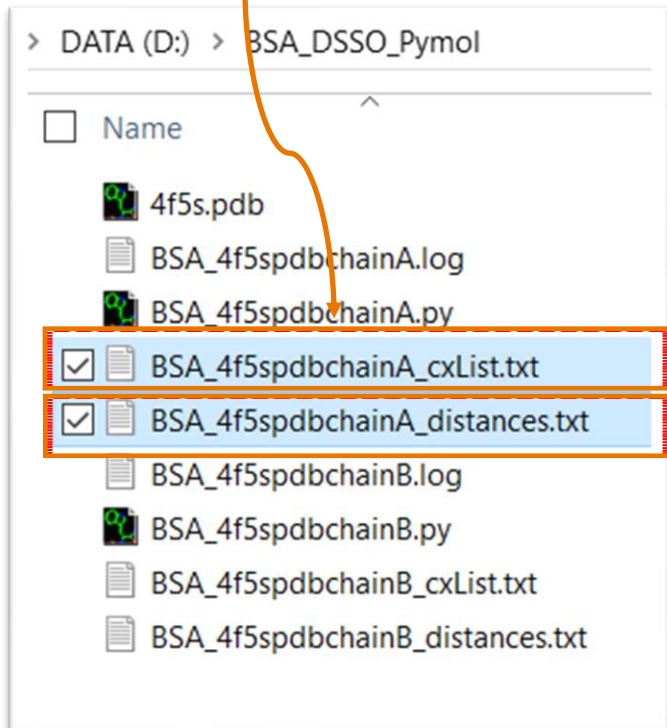
Double Click

3D Display of Protein



Extra Exporting

Select and double click



```
BSA_4f5spdbchainA_cxList.txt - Notepad
File Edit Format View Help
ID      Protein1      Protein2      AbsPos1 AbsPos2
CCTKPESER-SLGKVGTR-a4K-b4K      4F5S      4F5S      436      428
CCTKPESER-EKVLTSAR-a4K-b2K      4F5S      4F5S      436      184
CCTKPESER-LSQKFPK-a4K-b4K      4F5S      4F5S      436      218
EKVLTSAR-SLGKVGTR-a2K-b4K      4F5S      4F5S      184      428
FKDLGEEHFK-DTHKSEIAHR-a2K-b4K  4F5S      4F5S      12       4
LCVLHEKTPVSEK-CASIQKFGER-a7K-b6K  4F5S      4F5S      462      201
LAKEYEATLEECCAK-VTKCCTESLVNR-a3K-b3K  4F5S      4F5S      347      471
VHKECCHGDLLECCADDRADLAK-ALKAWSVAR-a3K-b3K  4F5S      4F5S      239      208
LAKEYEATLEECCAK-CASIQKFGER-a3K-b6K  4F5S      4F5S      347      201
LVTDLTKVHK-ALKAWSVAR-a7K-b3K      4F5S      4F5S      236      208
YICDNQDTISSKLECCDKPILLEK-a12K-b5K  4F5S      4F5S      270      277
LKPDPNTLCDEFK-SLGKVGTR-a2K-b4K      4F5S      4F5S      114      428
LAKEYEATLEECCAK-ALKAWSVAR-a3K-b3K  4F5S      4F5S      347      208
DSDPDLPKLKPDPNTLCDEFKKADEK-SLGKVGTR-a8K-b4K  4F5S      4F5S      112      428
ECCHGDLLECCADDRADLAKYICDNQDTISSK-LKECCDKPILLEKSHCTAEVEK-a19K-b12K  4F5S      4F5S      258      282
YNGVFECCQAEEDKGACLLPKIET-EKVLTSAR-a21K-b2K  4F5S      4F5S      178      184
VYQEAADAFGLSFLYEYSR-LAKEYEATLEECCAK-a6K-b3K  4F5S      4F5S      319      347
HPYFYAPELLYYANKYNGVFECCQAEEDK-GACLLPKIET-a15K-b7K  4F5S      4F5S      157      178
VYQEAADAFGLSFLYEYSR-ALKAWSVAR-a6K-b3K  4F5S      4F5S      319      208
LKECCDKPILLEK-FPKAEFVEVTK-a2K-b3K      4F5S      4F5S      272      221
YICDNQDTISSKLECCDKPILLEK-FPKAEFVEVTK-a12K-b3K  4F5S      4F5S      270      221
LFTFHADICTLPDTEKQIK-KQALVELLK-a16K-b1K  4F5S      4F5S      517      521
DLGEEHFKGLVLIAFSQQYLCQPFDEHVK-KFWGK-a8K-b1K  4F5S      4F5S      20       130
```

... for Xlink Analyzer
Protein_chain.txt

```
BSA_4f5spdbchainA_distances.txt - Notepad
File Edit Format View Help
'DB_id Chain_1 Position_in_structure_1 Chain_2 Position_in_structure_2 Position_in_PDresult_1 Position_in_PDresult_2 Type Description Distance(Angstrom)
IF5S A 439 A 431 439 431 intra intra_K439_K431 13.51
IF5S A 439 A 187 439 187 intra intra_K439_K187 18.22
IF5S A 439 A 221 439 221 intra intra_K439_K221 20.54
IF5S A 187 A 431 187 431 intra intra_K187_K431 13.69
IF5S A 12 A 4 12 4 intra intra_K12_K4 13.38
IF5S A 465 A 204 465 204 intra intra_K465_K204 13.24
IF5S A 350 A 474 350 474 intra intra_K350_K474 17.99
IF5S A 242 A 211 242 211 intra intra_K242_K211 9.73
IF5S A 350 A 204 350 204 intra intra_K350_K204 16.43
IF5S A 239 A 211 239 211 intra intra_K239_K211 8.96
IF5S A 273 A 280 273 280 intra intra_K273_K280 13.82
IF5S A 116 A 431 116 431 intra intra_K116_K431 21.27
IF5S A 350 A 211 350 211 intra intra_K350_K211 13.54
IF5S A 114 A 431 114 431 intra intra_K114_K431 19.9
IF5S A 261 A 285 261 285 intra intra_K261_K285 9.07
IF5S A 180 A 187 180 187 intra intra_K180_K187 10.97
IF5S A 322 A 350 322 350 intra intra_K322_K350 11.98
IF5S A 159 A 180 159 180 intra intra_K159_K180 10.07
IF5S A 322 A 211 322 211 intra intra_K322_K211 13.99
IF5S A 275 A 224 275 224 intra intra_K275_K224 15.59
IF5S A 273 A 224 273 224 intra intra_K273_K224 11.66
IF5S A 520 A 524 520 524 intra intra_K520_K524 6.42
IF5S A 20 A 132 20 132 intra intra_K20_K132 11.51
```

... add distances file
Protein_chain.Distance



Exporting Crosslinks to xiVIEW

Selecting the correct set of spectra for proteins when exporting mzIdentML and mzML for uploading to XiView

Step 1: Check Proteins to be exported (only the Proteins), then select checked Proteins and tag all associated Items (sub tables) of these Proteins with A (Blue Tag)

The screenshot shows the Thermo Proteome Discoverer 2.5.0.400 interface. The 'Proteins' table is visible with columns for 'Checked', 'Tags', 'Master', 'Accession', 'Description', and 'Coverage [%]'. A protein entry for 'BSA' is highlighted. A 'Field Chooser' dialog is open, showing various fields with checkboxes. The 'Tags' field is checked. A 'Setting Tags' dialog is also open, showing a 'Setting Tags' window with a blue tag selected and the 'In this and all sub-tables' option chosen. The 'Apply' button is highlighted.

1. Check Protein(s)

2. Click

3. Check

4. Right click

5. Click and select blue

6. Click

Step 2: Select all blue-tagged CSMs and tag all associated items with tag B (Red Tag)

1. Click

2. Check

The screenshot displays the Proteome Discoverer interface with a table of Crosslink MS2 Scans (CSMs). The table has columns for Checked, Tags, Sequence, Crosslinker, Crosslink Type, Crosslink Strategy, Identified By, # Proteins, # Identified MS2 Scans, # Identified MS3 Scans, XlinkX Score, Δ XlinkX Score, m/z [Da], Charge, MH+ [Da], First Scan, and RT [min]. A 'Setting Tags' dialog box is open over the table, showing a red tag selected for 'Selected items'. The dialog box also has radio buttons for 'In this table' and 'In this and all sub-tables', and 'Apply' and 'Cancel' buttons.

Checked	Tags	Sequence	Crosslinker	Crosslink Type	Crosslink Strategy	Identified By	# Proteins	# Identified MS2 Scans	# Identified MS3 Scans	XlinkX Score	Δ XlinkX Score	m/z [Da]	Charge	MH+ [Da]	First Scan	RT [min]
<input checked="" type="checkbox"/>	●○○○○	CCTKPESER-SLGKVGTR	DSSO	Intra	MS2_MS3	MS3	1	1	4	141.48	81.57	536.000061	4	2140.978414	2505	24.5336
<input checked="" type="checkbox"/>	●○○○○	CCTKPESER-EKVLTSAR	DSSO	Intra	MS2_MS3	MS3	1	1	4	110.14	110.14	579.267273	4	2314.047262	2527	24.6510
<input checked="" type="checkbox"/>	●○○○○	CCTKPESER-LSQKFPK	DSSO	Intra	MS2_MS3	MS3	1	1	4	138.54	138.54	543.503784	4	2170.993307	2866	26.5672
<input checked="" type="checkbox"/>	●○○○○	EKVLTSAR-SLGKVGTR	DSSO	Intra	MS2_MS3	MS3	1	1	4	151.44	91.04	492.016144	4	1965.042745	3064	27.6412
<input checked="" type="checkbox"/>	●○○○○	FKDLGEEHFK-DTHKSEIAHR	DSSO	Intra	MS2_MS3	MS3	1	1	2	82.42	82.42	520.849914	5	2600.220466	3088	27.7269
<input checked="" type="checkbox"/>	●○○○○	FKDLGEEHFK-DTHKSEIAHR	DSSO	Intra	MS2_MS3	MS3	1	1	4	205.83	205.83	520.850098	5	2600.221382	3251	28.5905
<input checked="" type="checkbox"/>	●○○○○	LCVLHEKTPVSEK-CASIQKFGFR	DSSO	Intra	MS2_MS3	MS3	1	1	4	226.40	226.40	723.856262	4	2892.403219	4446	33.0607
<input checked="" type="checkbox"/>	●○○○○	LCVLHEKTPVSEK-CASIQKFGFR	DSSO	Intra	MS2_MS3	MS3	1	1	4	140.31	140.31	579.286682	5	2892.404304	4461	33.0918
<input checked="" type="checkbox"/>	●○○○○	LAKEYEATLEECCA-KVTKCTESLVNR	DSSO	Intra	MS2_MS3	MS3	1	1	4	316.83	316.83	860.390991	4	3438.542135	4560	33.4142
<input checked="" type="checkbox"/>	●○○○○	VHKECCHGDLLECADDR-ALKAWSVAR	DSSO	Intra	MS2_MS3	MS3	1	1	4	358.71	358.71	818.873352	4	3272.471578	4792	34.1784
<input checked="" type="checkbox"/>	●○○○○	VHKECCHGDLLECADDR-ALKAWSVAR	DSSO	Intra	MS2_MS3	MS3	1	1	4	318.40	318.40	655.301575	5	3272.478767	4808	34.2337
<input checked="" type="checkbox"/>	●○○○○	VHKECCHGDLLECADDRADLAK-ALKAWSVAR	DSSO	Intra	MS2_MS3	MS3	1	1	4	124.12	113.66	754.956421	5	3770.752998	4882	34.4633
<input checked="" type="checkbox"/>	●○○○○	VHKECCHGDLLECADDRADLAK-ALKAWSVAR	DSSO	Intra	MS2_MS3	MS3	1	1	1	78.65	78.36	539.543884	7	3770.763530	4929	34.5923
<input checked="" type="checkbox"/>	●○○○○	LAKEYEATLEECCA-KASIQKFGFR	DSSO	Intra	MS2_MS3	MS3	1	1	4	293.82	293.82	792.609863	4	3167.417623	5078	35.0524
<input checked="" type="checkbox"/>	●○○○○	LVTDLTKVHK-ALKAWSVAR	DSSO	Intra	MS2_MS3	MS3	1	1	4	248.81	240.44	578.827453	4	2312.287985	5324	35.9005
<input checked="" type="checkbox"/>	●○○○○	LVTDLTKVHK-ALKAWSVAR	DSSO	Intra	MS2_MS3	MS3	1	1	3	258.00	253.09	463.263641	5	2312.289100	5338	35.9506
<input checked="" type="checkbox"/>	●○○○○	YICDNQDTISSK-LK-ECCDKPLLEK	DSSO	Intra	MS2_MS3	MS3	1	1	3	89.54	89.54	784.110779	4	3133.421286	5399	36.1577
<input checked="" type="checkbox"/>	●○○○○	LKPDPNTLCDEFK-SLGKVGTR	DSSO	Intra	MS2_MS3	MS3	1	1	0	40.92	40.92	638.570312	4	2551.259420	5544	36.6267
<input checked="" type="checkbox"/>	●○○○○	LAKEYEATLEECCA-ALKAWSVAR	DSSO	Intra	MS2_MS3	MS3	1	1	3	183.79	183.79	744.109985	4	2973.418112	5723	37.1886
<input checked="" type="checkbox"/>	●○○○○	DDSPDLPKLPDPNTLCDEFKADEK-SLGKVGTR	DSSO	Intra	MS2_MS3	MS3	1	1	3	132.24	132.24	773.178223	5	3861.862007	6120	38.4685
<input checked="" type="checkbox"/>	●○○○○	ECCHGDLLECADDRADLAKYICDNQDTISSK-LKECCDKPLLE	DSSO	Intra	MS2_MS3	MS3	1	1	4	464.17	463.71	1070.145386	6	6415.835931	6202	38.7410
<input checked="" type="checkbox"/>	●○○○○	ECCHGDLLECADDRADLAKYICDNQDTISSK-LKECCDKPLLE	DSSO	Intra	MS2_MS3	MS3	1	1	3	176.54	176.54	917.414062	7	6415.854778	6211	38.7711
<input checked="" type="checkbox"/>	●○○○○	YNGVFQECQAEDKGAQLLPKIETMR-EKVLTSAR	DSSO	Intra	MS2_MS3	MS2	1	1	0	45.63	45.63	853.803955	5	4264.990669	6839	40.9069
<input checked="" type="checkbox"/>	●○○○○	NYQEAKDAFLGSFLYEYSR-LAKEYEATLEECCA	DSSO	Intra	MS2_MS3	MS2	1	1	1	80.44	80.44	1068.985473	4	4272.920065	8811	50.1421
<input checked="" type="checkbox"/>	●○○○○	NYQEAKDAFLGSFLYEYSR-LAKEYEATLEECCA	DSSO	Intra	MS2_MS3	MS2	1	1	0	123.86	123.86	1068.986572	4	4272.924459	8884	50.4879
<input checked="" type="checkbox"/>	●○○○○	HPYFYAPPELLYANKYNGVQECQAEDK-GAQLLPKIETMR	DSSO	Intra	MS2_MS3	MS2	1	1	0	65.04	65.04	1033.479126	5	5163.366524	9180	51.7805
<input checked="" type="checkbox"/>	●○○○○	NYQEAKDAFLGSFLYEYSR-ALKAWSVAR	DSSO	Intra	MS2_MS3	MS3	1	1	2	112.84	101.94	865.673828	4	3459.673483	9220	51.9566
<input checked="" type="checkbox"/>	●○○○○	CCTKPESER-EKVLTSAR	DSSO	Intra	MS2_MS3	MS3	1	0	3	84.17	71.81	579.268066	4	2314.050436	2575	24.9869
<input checked="" type="checkbox"/>	●○○○○	CCTKPESER-LSQKFPK	DSSO	Intra	MS2_MS3	MS3	1	0	3	101.64	101.64	543.503357	4	2170.991598	2798	26.2274

3. Select Red

Step 3: Filter all MS/MS Spectrum Info items that carry tag A or B (Red or Blue)

Thermo Proteome Discoverer 2.5.0.400

File View Administration Tools Window Help

Start Page x Study: BSA_DSSO x Administration x F1_Agi5_20150717_FL_BSA_DSSO_MS2_OTCID_MS3_OTCID_ x

Display Filter

Load Save Clear Clear All Apply Cancel

2. Click

3. Add filters

1. Click

MS/MS Spectrum Info

OR

- Tags is true in tag A Remove
- Tags is true in tag B Remove

Add property

Study Information Crosslinks CSMs Crosslink MS2 Scans Crosslink Reporter Peaks Crosslink Summary

Proteins Protein Groups Peptide Groups PSMs MS/MS Spectrum Info Input Files Specialized Traces

Checked	Tags	File ID	RT [min]	First Scan	Mass Analyzer	Activation Type	MS Order	# PSMs	# CSMs	# Peptide Groups	Isolation Intolerance [%]	Ion Inject. time [ms]	# Precursors	# Identified Precursors	Precursor m/z [Da]	Precursor MH+ [Da]	Precursor Charge	Spectrum File
63	●○○○○	F1	55.1162	9770	FTMS	CID	MS2	1	0	1	6	50.000	1	1	623.82312	2492.27065	4	F1_Agi5_20150717
64	●○○○○	F1	52.6890	9355	FTMS	CID	MS2	1	0	1	22	52.000	1	1	936.40967	3742.61684	4	F1_Agi5_20150717
65	●○○○○	F1	52.7068	9359	FTMS	CID	MS2	1	0	1	10	50.000	1	1	1134.01440	4533.03579	4	F1_Agi5_20150717
66	●○○○○	F1	52.7742	9375	FTMS	CID	MS2	1	0	1	22	50.000	1	1	949.16229	3793.62734	4	F1_Agi5_20150717
67	●○○○○	F1	50.4181	8871	FTMS	CID	MS2	1	0	1	23	52.000	1	1	988.18884	3949.73354	4	F1_Agi5_20150717
68	●○○○○	F1	28.0926	3162	FTMS	CID	MS2	1	0	1	0	50.000	1	1	788.07336	3149.27163	4	F1_Agi5_20150717
69	●○○○○	F1	29.5838	3471	FTMS	CID	MS2	1	0	1	10	50.000	1	1	653.79663	2612.16469	4	F1_Agi5_20150717
70	●○○○○	F1	28.1414	3168	FTMS	CID	MS2	1	0	1	0	50.000	1	1	529.22662	2113.88466	4	F1_Agi5_20150717
71	●○○○○	F1	29.5435	3457	FTMS	CID	MS3	1	0	1	0	120.000	1	1	653.79651	2612.16421	4	F1_Agi5_20150717
72	●○○○○	F1	29.5993	3478	FTMS	CID	MS3	1	0	1	0	33.408	1	1	749.98480	2247.93985	3	F1_Agi5_20150717
73	●○○○○	F1	53.2836	9487	FTMS	CID	MS2	1	0	1	7	50.000	1	1	875.87891	3500.49380	4	F1_Agi5_20150717
74	●○○○○	F1	53.2625	9484	FTMS	CID	MS2	1	0	1	2	52.000	1	1	649.93732	3245.65748	5	F1_Agi5_20150717
75	●○○○○	F1	38.7799	6213	FTMS	CID	MS3	0	1	0	0	120.000	1	0	880.76526	2640.28122	3	F1_Agi5_20150717
76	●○○○○	F1	38.7410	6202	FTMS	CID	MS2	0	1	0	1	52.000	1	0	1070.14539	6415.83593	6	F1_Agi5_20150717
77	●○○○○	F1	38.7485	6204	FTMS	CID	MS3	0	1	0	0	120.000	1	0	880.76562	2640.28232	3	F1_Agi5_20150717
78	●○○○○	F1	38.7520	6205	FTMS	CID	MS3	0	1	0	0	120.000	1	0	1253.51978	3758.54477	3	F1_Agi5_20150717
79	●○○○○	F1	39.3504	6385	FTMS	CID	MS2	0	1	0	11	52.000	1	0	684.53931	3418.66743	5	F1_Agi5_20150717
80	●○○○○	F1	38.7554	6206	FTMS	CID	MS3	0	1	0	0	120.000	1	0	891.42334	2672.25547	3	F1_Agi5_20150717
81	●○○○○	F1	38.7589	6207	FTMS	CID	MS3	0	1	0	0	120.000	1	0	1242.86206	3726.57163	3	F1_Agi5_20150717

Show Associated Tables

Step 4: Check all MS/MS Spectrum Info items that carry tag A or B (Red or Blue)

Thermo Proteome Discoverer 2.5.0.400

File View Administration Tools Window Help

Start Page x Study: BSA_DSSO x US111571_F1_Agi5_20150717_FL_BSA_DSSO_MS2_OTCID_MS3_OTCID_PD2.5.0.185-(1) x

Display Filter

Load Save Clear Clear All Apply Cancel

ON Proteins
ON Protein Groups
ON Peptide Groups
ON PSMs
ON MS/MS Spectrum Info
ON Input Files

MS/MS Spectrum Info

OR Add group

Tags is true in tag A Remove
Tags is true in tag B Remove

Add property

Check	Tags	File ID	RT [min]	First Scan	Mass Analyzer	Activation Type	MS Order	# PSMs	# CSMs	# Peptide Groups	Isolation Interference [%]	Ion Inject Time [ms]	# Precursors	# Identified Precursors	Precursor m/z [Da]	Precursor MH+ [Da]	Precursor Charge	Spectrum File
<input checked="" type="checkbox"/>	●○○○○○	F1	30.0147	3608	FTMS	CID	MS2	1	0	1	4	50.000	1	1	636.04767	2541.16884	4	F1_Agi5_20150717_FL...
<input checked="" type="checkbox"/>	●○○○○○	F1	29.9870	3599	FTMS	CID	MS2	1	0	1	7	50.000	1	1	653.79730	2612.16738	4	F1_Agi5_20150717_FL...
<input checked="" type="checkbox"/>	●○○○○○	F1	29.6161	3484	FTMS	CID	MS2	1	0	1	6	52.000	1	1	509.03955	2541.16865	5	F1_Agi5_20150717_FL...
<input checked="" type="checkbox"/>	●○○○○○	F1	29.6789	3505	FTMS	CID	MS2	1	0	1	32	52.000	1	1	436.20032	2612.16552	6	F1_Agi5_20150717_FL...
<input checked="" type="checkbox"/>	●○○○○○	F1	29.8009	3543	FTMS	CID	MS2	1	0	1	19	50.000	1	1	573.23053	2289.90029	4	F1_Agi5_20150717_FL...
<input checked="" type="checkbox"/>	●○○○○○	F1	53.2836	9487	FTMS	CID	MS2	1	0	1	7	50.000	1	1	875.87891	3500.49380	4	F1_Agi5_20150717_FL...
<input checked="" type="checkbox"/>	●○○○○○	F1	30.3668	3688	FTMS	CID	MS2	1	0	1	34	52.000	1	1	636.04700	2541.16616	4	F1_Agi5_20150717_FL...
<input checked="" type="checkbox"/>	●○○○○○	F1	27.1634	2978	FTMS	CID	MS2	1	0	1	69	50.000	1	1	423.58292	2113.88547	5	F1_Agi5_20150717_FL...
<input checked="" type="checkbox"/>	●○○○○○	F1	29.5856	3472	FTMS	CID	MS2	1	0	1	19	52.000	1	1	523.23871	2612.16444	5	F1_Agi5_20150717_FL...
<input checked="" type="checkbox"/>	●○○○○○	F1	26.8303	2912	FTMS	CID	MS2	1	0	1	6	50.000	1	1	419.19788	1673.76967	4	F1_Agi5_20150717_FL...
<input checked="" type="checkbox"/>	●○○○○○	F1	26.6633	2881	FTMS	CID	MS2	1	0	1	35	50.000	1	1	575.51428	2299.03530	4	F1_Agi5_20150717_FL...
<input checked="" type="checkbox"/>	●○○○○○	F1	26.5461	2863	FTMS	CID	MS2	1	0	1	13	50.000	1	1	527.76849	2108.05214	4	F1_Agi5_20150717_FL...
<input checked="" type="checkbox"/>	●○○○○○	F1	27.0582	2951	FTMS	CID	MS2	1	0	1	14	50.000	1	1	529.22650	2113.88418	4	F1_Agi5_20150717_FL...
<input checked="" type="checkbox"/>	○●○○○○	F1	38.7410	6202	FTMS	CID	MS2	0	1	0	1	52.000	1	0	1070.14539	6415.83593	6	F1_Agi5_20150717_FL...
<input checked="" type="checkbox"/>	○●○○○○	F1	38.7485	6204	FTMS	CID	MS3	0	1	0	0	120.000	1	0	880.76562	2640.28232	3	F1_Agi5_20150717_FL...
<input checked="" type="checkbox"/>	○●○○○○	F1	38.7520	6205	FTMS	CID	MS3	0	1	0	0	120.000	1	0	1253.51978	3758.54477	3	F1_Agi5_20150717_FL...
<input checked="" type="checkbox"/>	○●○○○○	F1	38.7554	6206	FTMS	CID	MS3	0	1	0	0	120.000	1	0	891.42334	2672.25547	3	F1_Agi5_20150717_FL...
<input checked="" type="checkbox"/>	○●○○○○	F1	38.7589	6207	FTMS	CID	MS3	0	1	0	0	120.000	1	0	1242.86206	3726.57163	3	F1_Agi5_20150717_FL...

Show Associated Tables

Ready 1 Proteins; 1 Protein Groups; 39 Peptide Groups; 68 PSMs; 244/3375 MS/MS Spectrum Info; 1/2 Input Files; 1 Study Information; 2 Specialized Traces; 23 Crosslinks; 36 CSMs; 27 Crosslink MS2 Scans; 177 Crosslink MS3 Scans; 179 Crosslink Reporter...

1.Check

Step 5: Export checked Proteins to mzIdentML

1. Click

2. Click

3. Define

4. Click

5. Click

The screenshot shows the Thermo Proteome Discoverer 2.5.0.400 interface. The 'File' menu is open, and the 'Export' option is selected. The 'Export' submenu is also open, and 'To mzIdentML...' is highlighted. The 'Export to mzIdentML' dialog box is displayed, showing the destination folder as 'SSO_MS2_OTCID_MS3_OTCID_PD2.5.0.185-(1).mzid' and the 'Checked Proteins Only' option checked. The 'Export' button is highlighted. A final 'Export completed' message box is shown with the 'OK' button highlighted.

Coverage [%]	# Peptides	# Crosslinks	# CSMs	# PSMs	# Unique Peptides	# AAs	MW [kDa]	calc. pI	Score St	# Peptides	# Protein Groups
54%	29	28	48	75	29	583	66.4	5.86	102.63	29	1

Step 6: Export checked MS/MS Spectrum Info to mzML file.

1. Click

2. Click

3. Define

4. Click

The screenshot shows the Thermo Proteome Discoverer 2.5.0.400 interface. The 'File' menu is open, and the 'Export' option is selected. The 'Export' submenu is also open, showing various export options. The 'Spectra...' option is selected. The 'Export Spectra' dialog box is open, showing the 'Destination Folder' and 'File Type' fields. The 'Items to Be Exported' section is also visible, with 'From MS/MS Spectrum Info' selected. The 'Export Spectra' dialog box is also open, showing the 'Export completed.' message and the 'OK' button.

Protein	Peptide	PSM	Activation Type	MS2	MS3	# Identified Precursors	Precursor m/z [Da]	Precursor MH+ [Da]	Precursor Charge	Spectrum File
56	✓	●	CID			1	636.04767	2541.16884	4	F1_Agi5_20150717_F1...
57	✓	●	CID			1			4	F1_Agi5_20150717_F1...
58	✓	●	CID			1			5	F1_Agi5_20150717_F1...
59	✓	●	CID			1			4	F1_Agi5_20150717_F1...
60	✓	●	CID			1			4	F1_Agi5_20150717_F1...
61	✓	●	CID			1			4	F1_Agi5_20150717_F1...
62	✓	●	CID			1			4	F1_Agi5_20150717_F1...
63	✓	●	FTMS			1			5	F1_Agi5_20150717_F1...
64	✓	●	FTMS			1			4	F1_Agi5_20150717_F1...
65	✓	●	FTMS			1			4	F1_Agi5_20150717_F1...
66	✓	●	FTMS			1			4	F1_Agi5_20150717_F1...
67	✓	●	FTMS			1			4	F1_Agi5_20150717_F1...
68	✓	●	FTMS			1			4	F1_Agi5_20150717_F1...
69	✓	●	FTMS			1			6	F1_Agi5_20150717_F1...
70	✓	●	FTMS			1			3	F1_Agi5_20150717_F1...
71	✓	●	FTMS			1			3	F1_Agi5_20150717_F1...
72	✓	●	FTMS			1			3	F1_Agi5_20150717_F1...
73	✓	●	FTMS			1			3	F1_Agi5_20150717_F1...

Step 7: Export crosslinks to xiNET CSV format

1. Click

3. Define

4. Click

Activation Type	MS Order	# PSMs	# CSMS	Peptide Groups	Isolation Interference [%]	Ion Inject Time [ms]	# Precursors	# Identified Precursors	Precursor m/z [Da]	Precursor MH+ [Da]	Precursor Charge	Spectrum File
	CID	MS2							636.04767			20150717_F1_...
	CID	MS2							653.79730			20150717_F1_...
	CID	MS2							509.03958			20150717_F1_...
	CID	MS2							436.20033			20150717_F1_...
	CID	MS2							573.23055			20150717_F1_...
	CID	MS2							878.87633			20150717_F1_...
	CID	MS2							636.04700			20150717_F1_...
	CID	MS2							423.58292			20150717_F1_...
	CID	MS2							523.23871			20150717_F1_...
	CID	MS2							419.19788			20150717_F1_...
	CID	MS2							575.51428			20150717_F1_...
	CID	MS2							527.76849	2108.05214	4	F1_Agi5_20150717_F1_...
	CID	MS2							529.22650	2113.88418	4	F1_Agi5_20150717_F1_...
	CID	MS2							1070.14539	6415.83593	6	F1_Agi5_20150717_F1_...
	CID	MS3	0	1	0	0	1	0	880.76562	2640.28232	3	F1_Agi5_20150717_F1_...
	CID	MS3	0	1	0	0	0	0	1253.51978	3758.54477	3	F1_Agi5_20150717_F1_...
	CID	MS3	0	1	0	0	0	0	891.42334	2672.25547	3	F1_Agi5_20150717_F1_...
	CID	MS3	0	1	0	0	0	0	1242.86206	3726.57163	3	F1_Agi5_20150717_F1_...

Note the xiVIEW URL on the 'To xiNET...' dialog (for later upload to website)

Step 8: Export result to *.fasta file

1. Click

2. Click

3. Click

4. Click

Export To FASTA

Destination Folder

DSSO_MS2_OTCID_MS3_OTCID_PD2.5.0.185-(1).fasta

Options

Checked Proteins Only

Export

Close

Export To FASTA

Export completed.

OK

Peptide Groups	PSMs	MS/Mz	Layout	Max. Xlink	MS3 Scans	Crosslink
				388.87	QQAL	
				371.03	L[K]G	
				353.78	GISCI	
				335.99	ASFA	
				332.26	YT[K]	
				332.26	IM[K]NEIQDLQTK	1×DSSO [K3]
				327.06	NFGP[K]GFGFGGAGALVHSE	1×DSSO [K5]
				322.87	GLTSVINQ[K]LKDDEVAQLK	1×DSSO [K9]
				316.48	L[K]SELVANNVTLPAGEQRK	1×DSSO [K2]
				312.58	MQQNIQEELEEEESARQ[K]LQLEK	1×DSSO [K21]

Ready 1158/2823 Proteins; 212 Crosslinks; 292 CSMs; 380 Crosslink MS2 Scans; 1496 Crosslink MS3 Scans; 1959 Crosslink Reporter Peaks; 1 Crosslink Summary

Step 9. Exporting crosslinks to xiNET

Access <http://crosslinkviewer.org/> in Chrome

xiNET

CROSS-LINK VIEWER

HOME

EXAMPLES

UPLOAD

Click

CONTACT

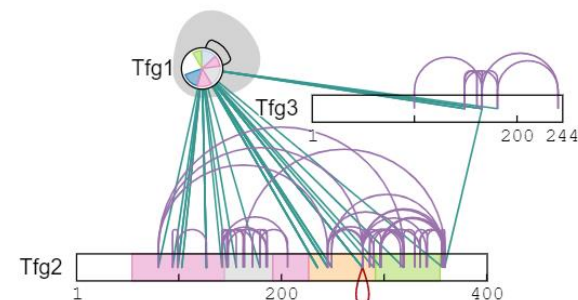
RAPPSILBER
LABORATORY

Supported by
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A tool for exploring and communicating cross-linking / mass spectrometry data.

xiNET displays:

- residue resolution positional information including linkage sites and linked peptides;
- all types of cross-linking reaction product;
- ambiguous results;
- additional sequence information such as domains.



Citation: [Combe, C. W., Fischer, L. & Rappsilber, J. xiNET: Cross-link Network Maps With Residue Resolution. *Mol Cell Proteomics* 14, 1137–1147 \(2015\).](#)

Click 'UPLOAD' then 'Chose File' to upload

xiNET
CROSS-LINK VIEWER

HOME
EXAMPLES
UPLOAD
CONTACT

NEW! Try xiNET's successor at xiVIEW.org

Upload Your Own Data

Cross-link CSV file: No file chosen

FASTA file: No file chosen

Annotation CSV file: No file chosen

UPLOAD

You will be redirected to a unique URL for your data which you can share with others.

You can view your results by uploading [cross-link data](#) in a Comma Separated Values (CSV) file.

Optionally, this can be accompanied by a [FASTA file](#) giving the protein sequences and/or a CSV file containing [annotations](#).

For further information on the file formats see:

- [Cross-link CSV format](#)
- [FASTA files / protein IDs](#)
- [Annotations CSV format](#)

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```
graph TD; A[Cross-link Data] --> B[Explore data in xiNET]; C[Protein Sequence Data] --> B; D[Annotations] --> B; B --> E[Share interactive web page]; B --> F[Export Figure SVG]; E -.-> B; Key[Key: Data input (solid arrow), Communication (dashed arrow)]; F --> G[Export Figure SVG icon];
```

Upload CSV and FASTA files

xiNET
CROSS-LINK VIEWER

HOME
EXAMPLES
UPLOAD
CONTACT

RAPPSILBER
LABORATORY

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NEW! Try xiNET's successor at xiVIEW.org

Upload Your Own Data

Cross-link CSV file: FASTA file: Annotation CSV file:

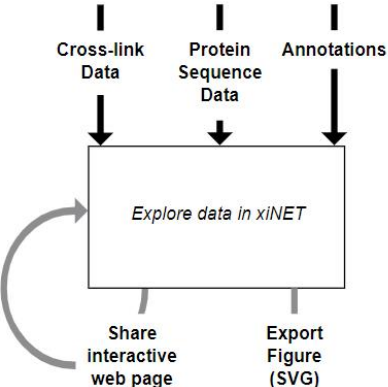
You will be redirected to a unique URL for your data which you can share with others.

You can view your results by uploading [cross-link data](#) in a Comma Separated Values (CSV) file.

Optionally, this can be accompanied by a [FASTA file](#) giving the protein sequences and/or a CSV file containing [annotations](#).

For further information on the file formats see:



- [Cross-link CSV format](#)
- [FASTA files / protein IDs](#)
- [Annotations CSV format](#)



```
graph TD; A[Cross-link Data] --> B[Explore data in xiNET]; C[Protein Sequence Data] --> B; D[Annotations] --> B; B --> E[Share interactive web page]; B --> F[Export Figure SVG];
```

Upload CSV and FASTA files

> PD 2.4 Study > DSSO-MS2-MS2-MS3

<input type="checkbox"/>	Name	Date modified	Type	Size
<input checked="" type="checkbox"/>	 fraction37_tm5_human-(1).fasta	12/13/2019 12:22 PM	FASTA File	964 KB
<input checked="" type="checkbox"/>	 fraction37_tm5_human-(1).csv	12/13/2019 11:56 AM	Microsoft Excel Co...	14 KB

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HOME

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UPLOAD

CONTACT

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NEW! Try xiNET's successor at xiVIEW.org

Upload Your Own Data

Cross-link CSV file:

fraction37_tm5_human-(1).csv

FASTA file:

fraction37_tm5_human-(1).fasta

Annotation CSV file:

No file chosen

UPLOAD

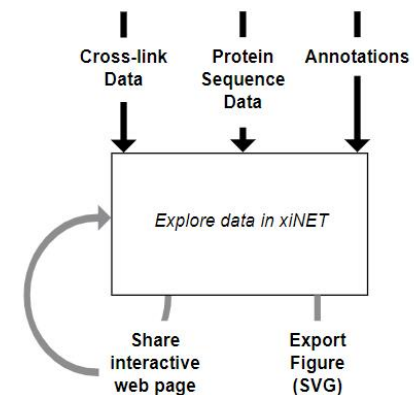
You will be redirected to a unique URL for your data which you can share with others.

You can view your results by uploading [cross-link data](#) in a Comma Separated Values (CSV) file.

Optionally, this can be accompanied by a [FASTA file](#) giving the protein sequences and/or a CSV file containing [annotations](#).

For further information on the file formats see:

- [Cross-link CSV format](#)
- [FASTA files / protein IDs](#)
- [Annotations CSV format](#)



Visualization in xiNET

Displaying the crosslinks in xiNET

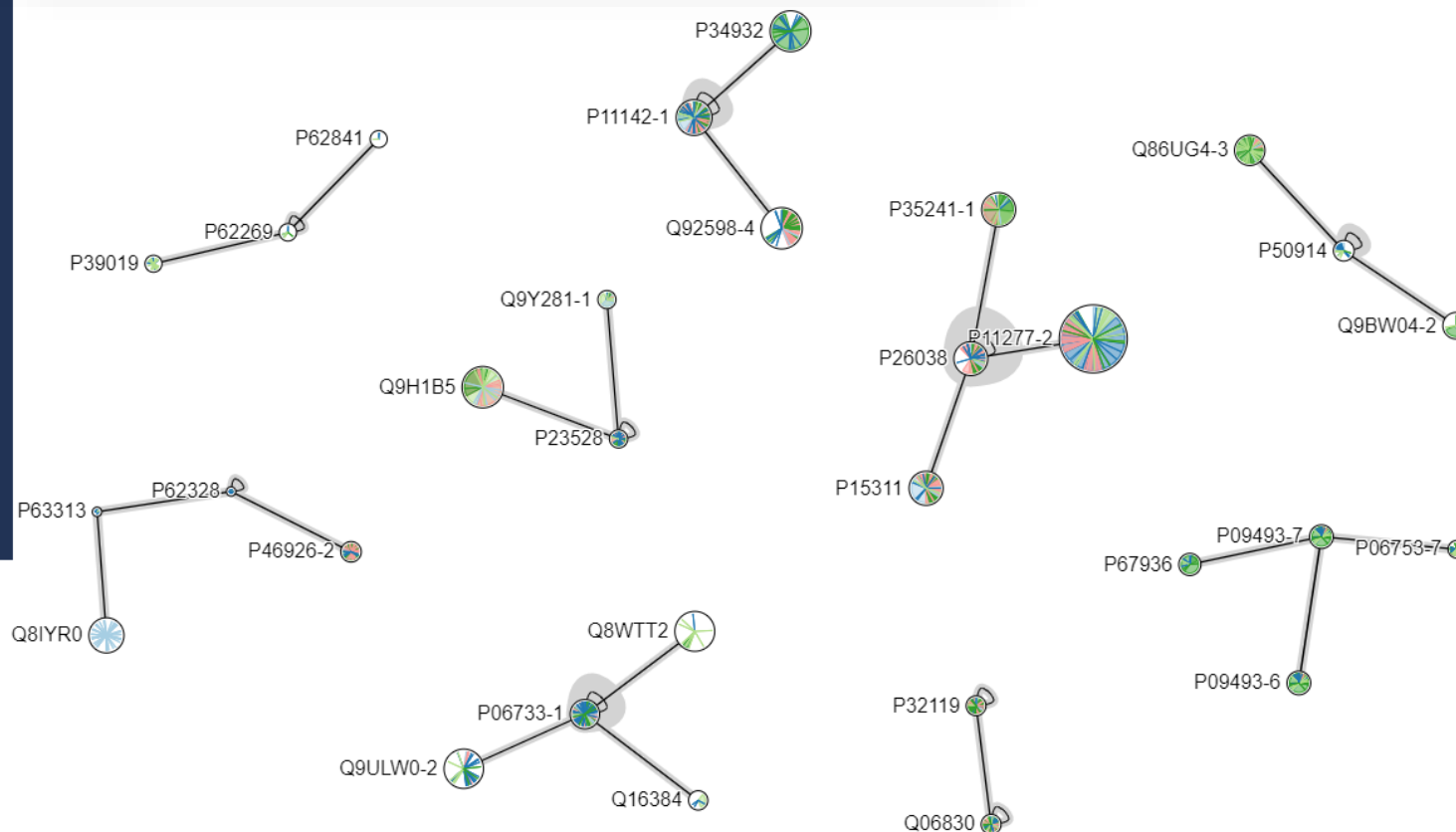
Protein-protein level

- Cross-link
- - - Ambiguous
- ▬ Multiple linkage sites
- ⤷ Self-link, possibly inter- or intra-molecular
- ⤶ Self-link, includes confirmed inter-molecular

Residue level

- Cross-link
- - - Ambiguous
- ⤷ Self-link (inter- or intra-molecular)
- ⤶ Inter-molecular self-link (homomultimeric link)
- ⤵ Intra-molecular self-link (e.g. from internally linked peptide)
- ⤴ Linker modified peptide (unfilled = ambiguous)
- ⤳ Highlighted linked peptide

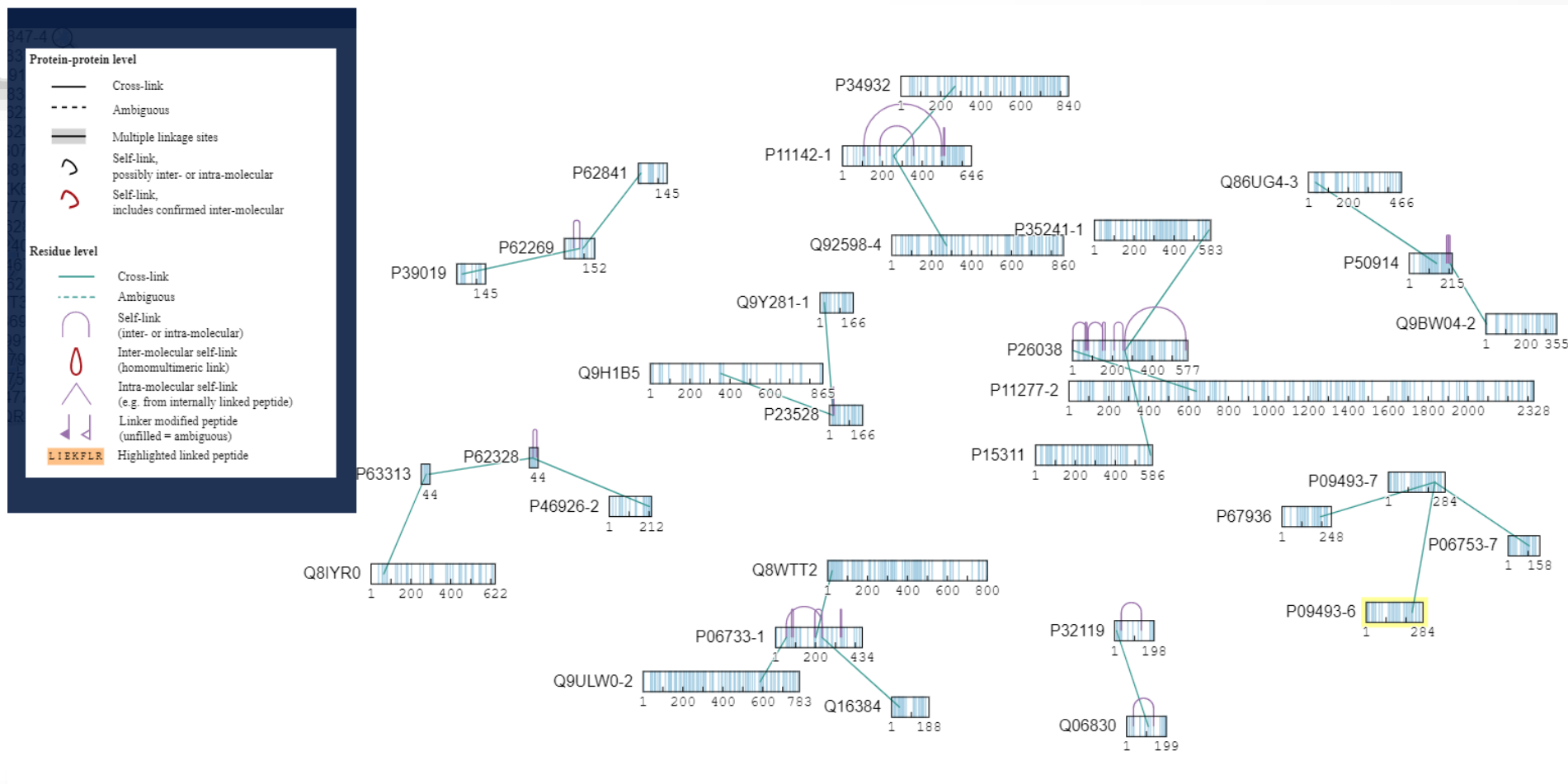
L I E K F L R



Self-Links Ambig. Decoys Score:40.0

388.9 (40.0) Annot. UniprotKB

Expanding the proteins to show the linked positions (lysines) in the sequence.



Self-Links Ambig. Decoys Score:40.0 — 388.9 (40.0) Annot. UniprotKB

Step 10. Exporting crosslinks to xiVIEW

Access https://xiview.org/xiNET_website/index.php in Chrome

Sign into your account.

xiVIEW | Home

xiview.org/xiNET_website/index.php

xiVIEW

HOME

CREATE ACCOUNT

SIGN IN

DEMO

MZIDENTML

CSV FORMATS

PRIVACY

CONTACT

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Home

xiView is a web-based visualisation tool for the analysis of cross-linking / mass spectrometry results, it is independent of the search software used. It provides multiple, linked views of the data, including:

- 2D network ([xiNET](#) or circular)
- the supporting annotated spectra using [xiSPEC](#).
- 3D structure view using [NGL](#).

The [video tutorials](#) give an overview of xiView's many features.

xiView is an open source project on [GitHub](#). Report issues and request features [here](#).

When using XiView please cite: [Graham, M., Combe, C. W., Kolbowski, L. & Rappsilber, J. xiView: A common platform for the downstream analysis of Crosslinking Mass Spectrometry data. doi: 10.1101/561822.](#)

New User?

CREATE NEW ACCOUNT

ANNOTATIONS VIEW LOAD DATA EXPORT PIN SELECT AUTO LAYOUT SAVE LAYOUT EXPORT GRAPHIC

Create

DOWNLOAD IMAGE AS SVG

SHOW RESIDUE LABELS IF FEW

Spectrum

Navigate to the Upload page

Choose files

xiVIEW | Upload

xiview.org/xiNET_website/upload.php

xiVIEW

- HOME
- UPLOAD -**
- MY DATA
- SIGN OUT
- DEMO
- MZIDENTML
- CSV FORMATS
- PRIVACY
- CONTACT

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Upload

Data upload video tutorial

CHOOSE FILE(S) SUBMIT DATA

Identification file: Select a mzIdentML or csv file to upload
Peak list file(s): No peak list file(s) selected - spectra will be unavailable
Sequence file: No FASTA file selected, protein identifiers must be UniprotKB accession numbers

xiView accepts three types of input data:

- Peptide Identifications (required)**
Supported file formats: [mzIdentML](#) (file extension must be '.mzid') and [Comma Separated Values](#) (file extension '.csv').
- Peak Lists (optional)**
Supported file formats: [mzML](#), [mgf](#), and [ms2](#) (& zip/gz archives of these). File extension must be '.mzML', '.mgf', '.ms2' or '.zip'.
If peak list data is uploaded then it must be complete, i.e. all spectra identified must be present, or the upload process will result in an error.
mzML tip: Filter out MS1 spectra to reduce file size and upload/parsing time. (e.g. 'MS level 2' in [MSconvert](#))
- Protein Sequences (optional)**
Supported file formats: [FASTA](#) (file extension must be '.fasta'), sequences can also be contained in mzIdentML files.
If you do not provide a FASTA file, then your protein IDs must be valid UniProtKB accession numbers.
If you do provide a FASTA file, then your protein IDs must all match identifiers in the FASTA file.

Only the peptide identifications file is required, but without uploading peak lists you won't be able to inspect the supporting spectra using [xiSPEC](#).

There is a 1GB size limit on uploaded files.

```
graph TD; A[Peptide Identifications] --> B[Explore data in xiView]; C[Peak Lists] --> B; D[Protein Sequences] --> B; B --> E[Share interactive web page]; B --> F[Export Figures SVG]; F --> G[The Journal of Proteomics];
```

After the upload progress reaches 100%, submit the data

The screenshot shows the xiVIEW Upload interface. On the left is a dark blue sidebar with navigation links: HOME, UPLOAD, MY DATA, SIGN OUT, DEMO, MZIDENTML, CSV FORMATS, PRIVACY, CONTACT RAPP SILBER LABORATORY, and Supported by wellcome trust. The main content area has a dark header with the word 'Upload'. Below it is a link for 'Data upload video tutorial'. A file upload section features a 'CHOOSE FILE(S)' button, a red progress bar at 100%, and a 'SUBMIT DATA' button highlighted with an orange dashed border. Below the progress bar, three files are listed with checkmarks: 'Identification file: F1_Agi5_20150717_FL_BSA_DSSO_MS2_OTCID_MS3_OTCID_mzid', 'Peak list file(s): F1_Agi5_20150717_FL_BSA_DSSO_MS2_OTCID_MS3_OTCID_mzML', and 'Sequence file: F1_Agi5_20150717_FL_BSA_DSSO_MS2_OTCID_MS3_OTCID_fasta'. A diagram on the right shows 'Peptide Identifications', 'Peak Lists', and 'Protein Sequences' as inputs to a box labeled 'Explore data in xiView'. From this box, arrows point to 'Share interactive web page' and 'Export Figures (SVG)', with a small thumbnail image of a figure below the latter.

xiVIEW

HOME
UPLOAD
MY DATA
SIGN OUT
DEMO
MZIDENTML
CSV FORMATS
PRIVACY
CONTACT RAPP SILBER LABORATORY
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Upload

[Data upload video tutorial](#)

CHOOSE FILE(S) 100% SUBMIT DATA

Identification file: F1_Agi5_20150717_FL_BSA_DSSO_MS2_OTCID_MS3_OTCID_mzid ✓
Peak list file(s): F1_Agi5_20150717_FL_BSA_DSSO_MS2_OTCID_MS3_OTCID_mzML ✓
Sequence file: F1_Agi5_20150717_FL_BSA_DSSO_MS2_OTCID_MS3_OTCID_fasta ✓

xiView accepts three types of input data:

- Peptide Identifications (required)**
Supported file formats: [mzIdentML](#) (file extension must be '.mzid') and [Comma Separated Values](#) (file extension '.csv').
- Peak Lists (optional)**
Supported file formats: [mzML](#), [mgf](#), and [ms2](#) (& zip/gz archives of these). File extension must be '.mzML', '.mgf', '.ms2' or '.zip'.
If peak list data is uploaded then it must be complete, i.e. all spectra identified must be present, or the upload process will result in an error.
mzML tip: Filter out MS1 spectra to reduce file size and upload/parsing time. (e.g. 'MS level 2-' in [MSconvert](#))
- Protein Sequences (optional)**
Supported file formats: [FASTA](#) (file extension must be '.fasta'), sequences can also be contained in

Peptide Identifications, Peak Lists, Protein Sequences

Explore data in xiView

Share interactive web page, Export Figures (SVG)

Depending upon file size, this process may take a little while...

The screenshot shows the xiVIEW Upload interface. At the top, a dark header contains the 'Upload' title. Below it, a navigation menu on the left lists options: HOME, UPLOAD, MY DATA, SIGN OUT, DEMO, MZIDENTML, CSV FORMATS, PRIVACY, and CONTACT. The main content area features a 'Data upload video tutorial' link, a 'CHOOSE FILE(S)' button, and a progress bar at 100%. A 'SUBMIT DATA' button is visible to the right. Below the progress bar, there are fields for 'Identification file', 'Peak list file(s)', and 'Sequence file'. A modal window is overlaid in the center, displaying a loading spinner and the text: 'Your data is being processed. Please wait... Depending on the size of your data this process may take up to several minutes.' To the right of the modal, a flowchart shows the data processing pipeline: 'Peptide Identifications', 'Peak Lists', and 'Protein Sequences' feed into 'Explore data in xiView', which then leads to 'Share interactive web page' and 'Export Figures (SVG)'. The bottom of the page includes the RAPPESILBER LABORATORY logo, the Wellcome Trust logo, and a list of notes: 'Only the peptide identifications file is required, but without uploading peak lists you won't be able to inspect the supporting spectra using xiSPEC.' and 'There is a 1GB size limit on uploaded files.'

If there are no errors, click 'CONTINUE'

The screenshot shows the xiVIEW Upload interface. A modal dialog box is displayed in the center, containing the following text:

2 warning(s) and 0 error(s) occurred parsing your data.
▼ [Show log for more information.](#) ▼

Your input file did not specify fragment ion types.
Select and update ion types below. Then click continue to view your data.

peptide, b, y

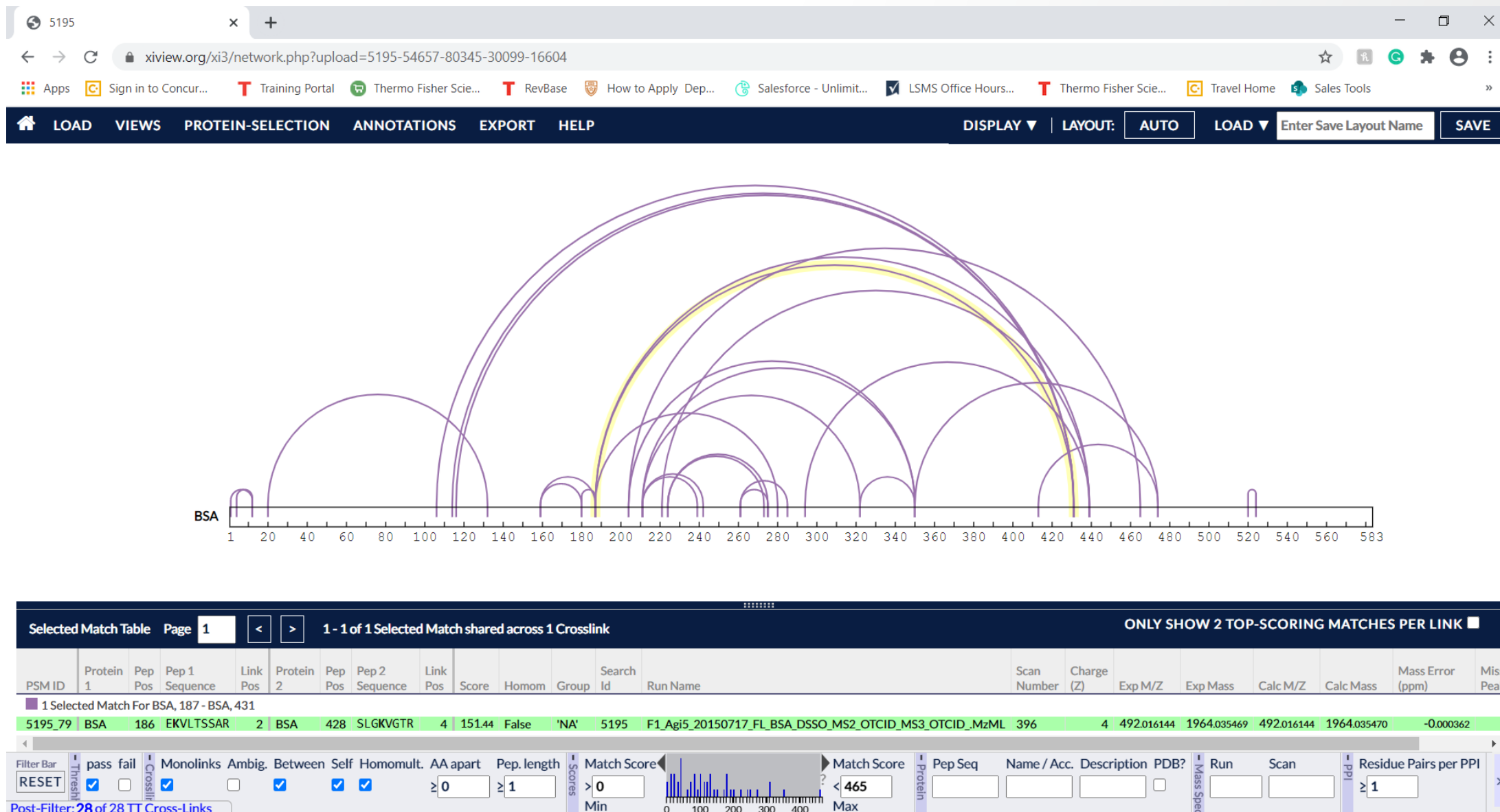
<input checked="" type="checkbox"/> Peptide ion	<input type="checkbox"/> A ion	<input checked="" type="checkbox"/> B ion
<input type="checkbox"/> C ion	<input type="checkbox"/> X ion	<input checked="" type="checkbox"/> Y ion
	<input type="checkbox"/> Z ion	

UPDATE IONS

CANCEL CREATE ISSUE **CONTINUE**

The background page shows the 'Upload' section with a 'SUBMIT DATA' button and a flowchart on the right. The flowchart illustrates the data processing pipeline: Peptide Identifications, Peak Lists, and Protein Sequences are input into 'Explore data in xiView', which then allows for 'Share interactive web page' and 'Export Figures (SVG)'. The 'CONTINUE' button in the dialog is highlighted with an orange border.

Viewing Your Data Online



Re-arrange Proteins, Display Annotations and Views

Spectrum view

Views Legend:

- LEGEND & COLOURS
- CIRCULAR
- 3D (NGL)
- MATRIX
- PROTEIN INFO
- SPECTRUM
- HISTOGRAM
- SCATTERPLOT
- ALIGNMENT
- SEARCH SUMMARIES

Spectrum View Panel:

MOVE LABELS MEASURE M/Z: 208 1592 RESET ZOOM

Precursor: exp m/z=746.88; calc m/z=746.88; z=4; error=1.17 ppm

Peptide Sequence: L K [E] C [C] D [K] P [L] L [E] K

Fragment Sequence: F P K | A E E F V E | V T K

Peaks: y2, y3, y5, y6, y7, y8, y9, y10

Quality control plots: error (ppm) vs Intensity, error (ppm) vs m/z

Selected Match Table: 1 - 2 of 2 Selected Matches shared across 1 Crosslink

PSM ID	Protein 1	Pep Pos	Pep 1 Sequence	Link Pos	Protein 2	Pep Pos	Pep 2 Sequence	Link Pos	Score
2 Selected Matches For BSA, 224 - BSA, 275									

Filter Bar: pass fail, Monolinks Ambig. Between Self Homomult. AA apart, Pep. length, Match Score, Pep Seq, Name / Acc. Description PDB?, Run, Scan, Residue Pairs per PPI

The End

