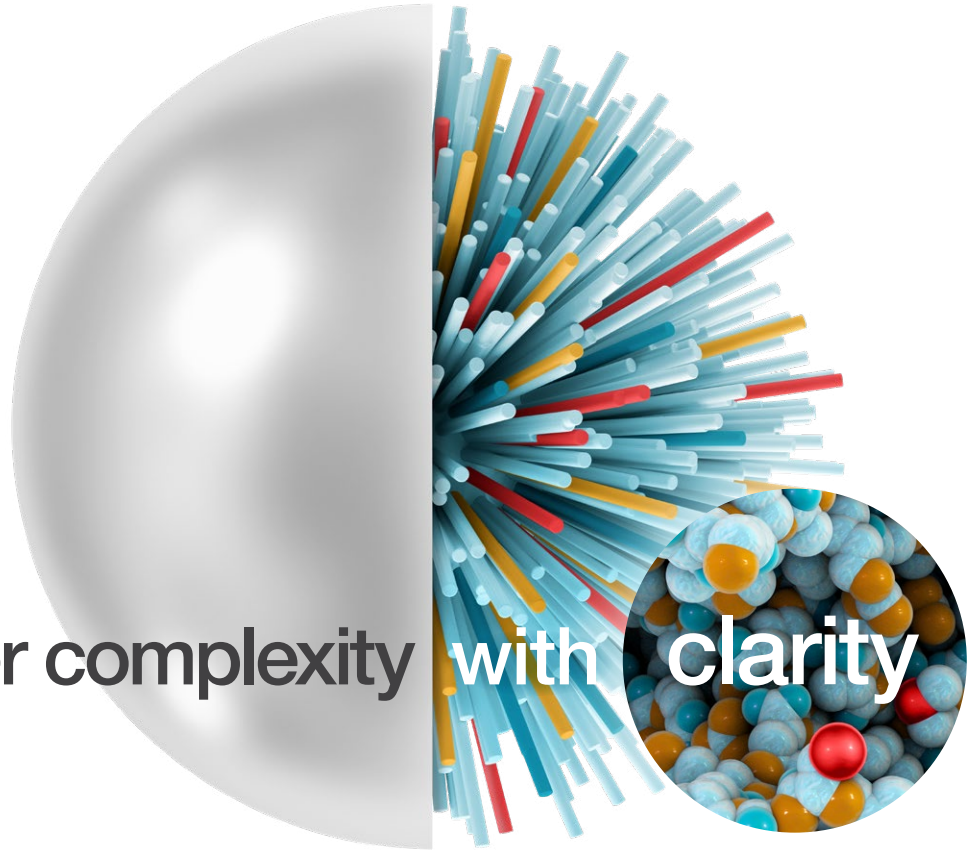
Mass spectrometry



Decipher complexity with clarity

Reveal biomolecular signatures with unprecedented detail

Thermo Scientific Direct Mass Technology mode

thermo scientific

Resolve complexity, achieve macromolecular clarity with the Direct Mass Technology mode

Thermo Scientific™ Direct Mass Technology™ mode redefines mass spectrometry by equipping Thermo Scientific™ Q Exactive™ UHMR Hybrid Quadrupole-Orbitrap™ mass spectrometers with charge detection capabilities. This revolutionary parallel individual ion measurement technique provides direct, accurate mass determination, enabling you to decipher protein complexes and biotherapeutics that are too complex to resolve using other methods. As a result, small changes to large molecules, for example post-translational modifications, are revealed in exquisite detail. A charge-detection-enabled Orbitrap mass analyzer unravels complexity and unlocks new insights into proteoforms, biotherapeutics, and next-generation drug modalities.

The Q Exactive UHMR mass spectrometer with the Direct Mass Technology mode enables an end-to-end workflow for data acquisition, processing, and interpretation of previously intractable targets.

- Maximize throughput and signal quality with parallel individual ion measurements
- Processing software streamlines charge calibration, error correction, and data visualization

Workflow for acquiring, processing, and interpreting Direct Mass Technology mode data

Data acquisition	Data format	Data processing
• Simultaneous charge determination • Toggle Direct Mass Technology mode on or off • Automated ion population control • Charge calibration procedure	• Selective Temporal Overview of Resonant Ions (STORI) data[1] • Parallel individual ion measurements of hundreds of single ions per spectrum in *m/z* space	• STORI plot analysis • Charge calibration algorithm • Create high resolution spectrum in mass domain • Export centroid .raw mass spectrum

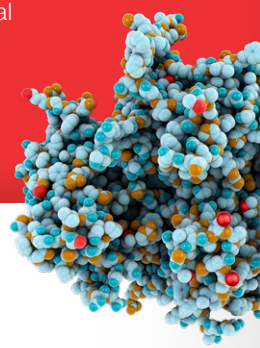
Direct Mass Technology mode | Resolve complexity | Achieve macromolecular clarity

Technology to match the scale of biology

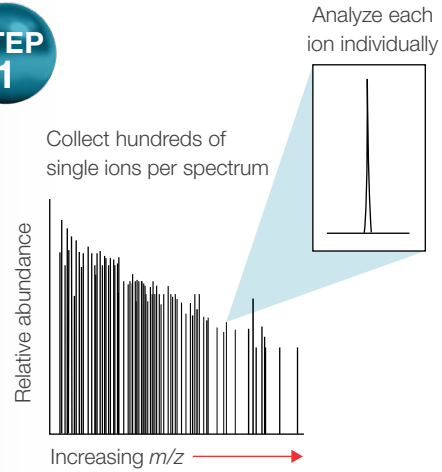
The Direct Mass Technology mode adds charge detection to the Orbitrap mass analyzer, enabling simultaneous measurement of both mass-to-charge (*m/z*) and charge (*z*) for hundreds of individual ions. This allows for direct calculation of analyte mass without the need for *m/z*-dependent deconvolution, which relies on resolved charge states and/or isotopically-resolved signals in ensemble measurements. The Direct Mass Technology mode increases resolution and dynamic range and increases the upper limit of accessible mass measurements, while allowing for the collection of more meaningful data from significantly less concentrated samples due to the sensitivity of individual ion measurements.

Benefits of Direct Mass Technology mode for structural biology and biopharmaceutical applications

- Accurate direct mass determination, eliminating need for *m/z*-based deconvolution
- As much as 20-fold increase in resolution relative to traditional ensemble ion measurements
- Requires less sample or less concentrated samples
- Access to measurements up to 80,000 *m/z* and mass measurements in the megadalton range



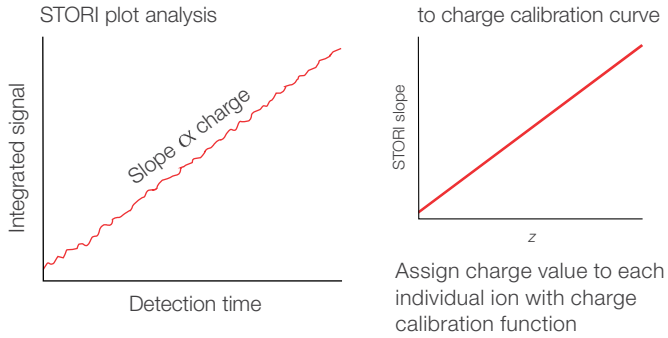
STEP 1



Collect hundreds of individual ions in parallel per spectrum

The Direct Mass Technology mode adds extra dimensionality to Orbitrap measurements by determining the frequencies and amplitudes of individual ions in each spectrum.

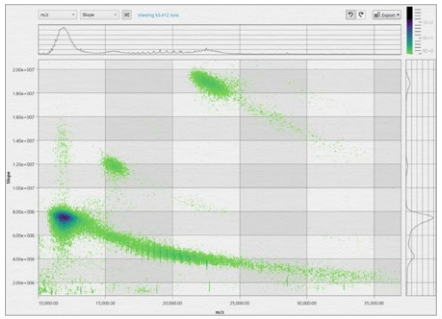
STEP 2



STORI plot analysis

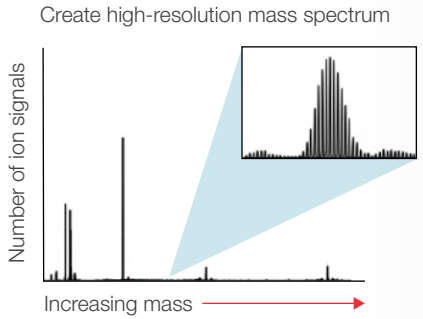
In an Orbitrap mass analyzer, the *m/z* is determined from the axial frequency of rotation along the central axis of the central electrode.

The Direct Mass Technology mode measures the induced charge on the outer electrode and calculates the integrated induced charge signal over time, which is known as STORI. The slope of the STORI plot of an individual ion is proportional to its charge, enabling determination of *z*.



Example visualization of STORI plot slopes (y-axis) and *m/z* (x-axis) in STORIboard demonstrating the differentiation of analytes with the same *m/z* but different charge states.

STEP 3



Calculate high-resolution mass domain spectrum

Multiply the *m/z* and *z* to calculate the mass of each individual ion.

Characterize complex heterogenous native proteins with unprecedented confidence

Native MS characterization of large heterogenous protein complexes is essential to understanding biological processes and the development of next generation biotherapeutics. When using traditional native MS techniques with ensemble measurements that measure *m/z* only, the overlap in charge-state distributions caused by residual solvation, ionic adducts, and post-translational modifications results in a degree of complexity that often yields spectra that are intractable to deconvolution.

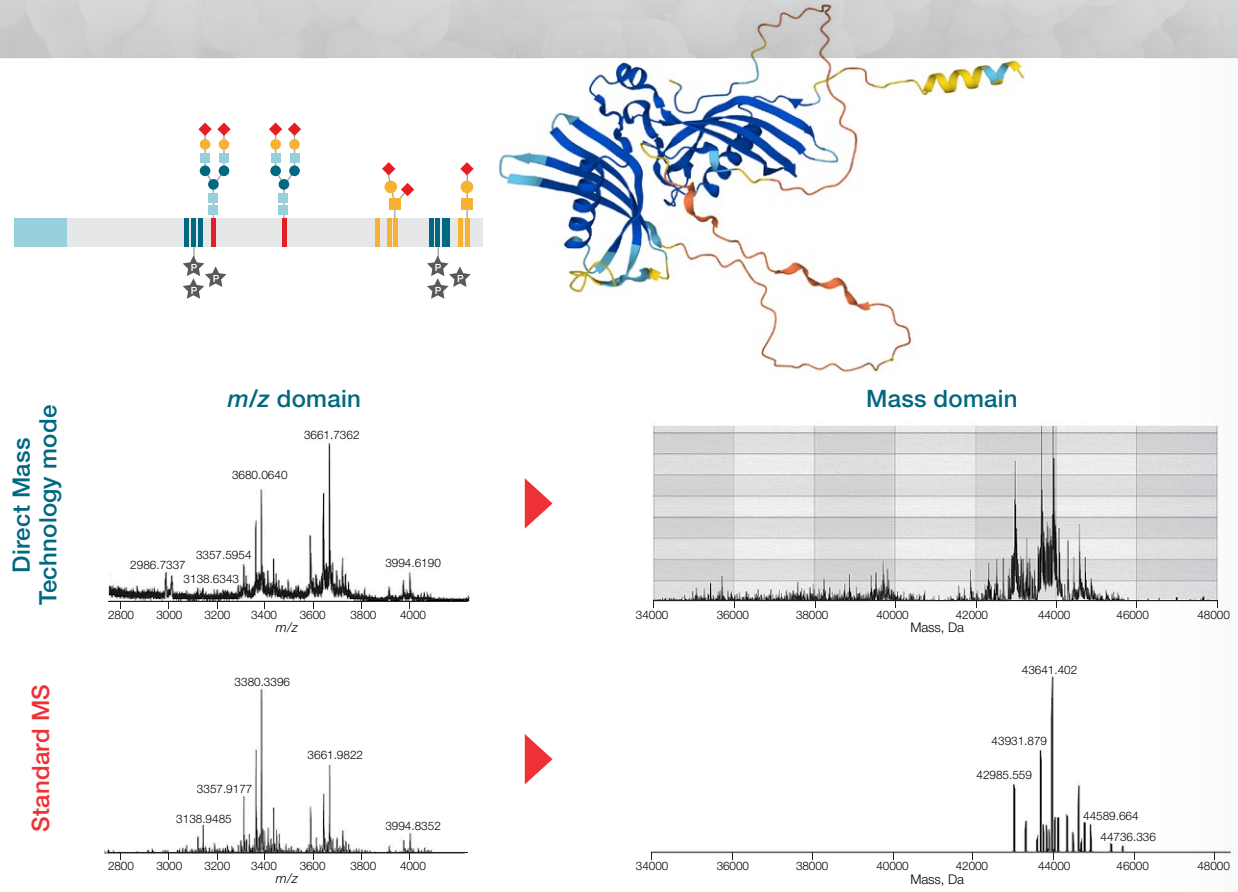
The Direct Mass Technology mode overcomes these challenges, enabling detailed characterization of membrane proteins, large noncovalent protein complexes, glycoproteins, and extremely heterogeneous protein- and nonprotein-based therapeutics, with enhanced resolution, sensitivity, and dynamic range.

Identify more glycoforms with exquisite detail

Glycoproteins are key in many physiological functions and in the progression of certain diseases. Due to heterogeneity, glycoprotein characterization using traditional intact and top-down MS methods has challenged researchers with extremely complex spectra. Additionally, glycoform detection by native MS has been limited by spatial resolution and dynamic range. The Direct Mass Technology mode unlocks new possibilities by adding resolution and dynamic range, enabling accurate, efficient, and detailed identification of glycoforms.

Comprehensive characterization of fetuin-A

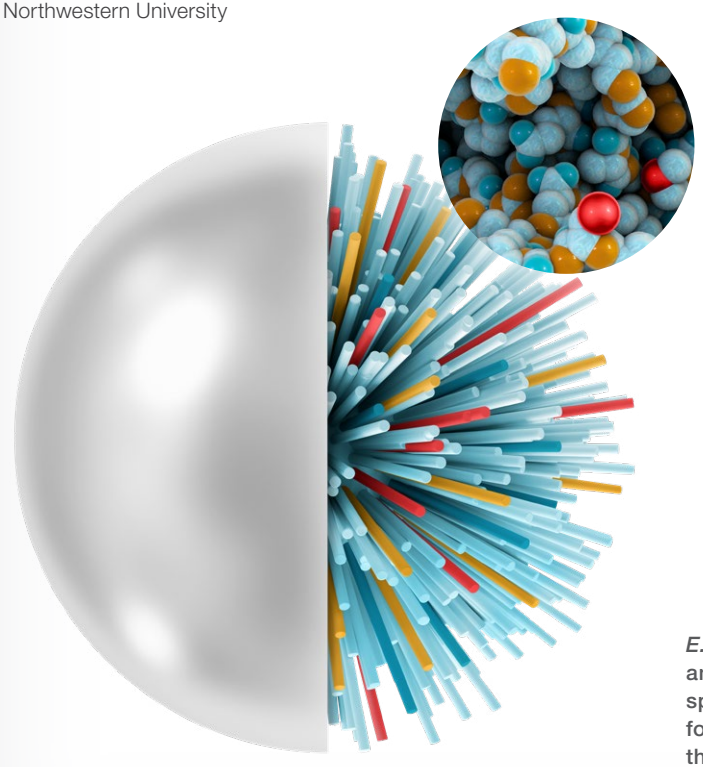
Human fetuin-A is a heterodimeric protein composed of A and B chains connected by a disulfide bond. Increased levels of this protein have been linked to higher risk of cardiovascular disease and Type 2 Diabetes.[2] The heterogeneity of native fetuin-A is due to its two N-glycosylation sites, up to seven O-glycosylation sites, and seven phosphorylation sites. Six disulfide bonds and sequence variants further complicate analysis. Using the Q Exactive UHMR mass spectrometer powered by the Direct Mass Technology mode, a more comprehensive glycoform profile for intact fetuin-A can now be obtained.



Analysis of native human fetuin-A (approximately 37 kDa without modifications, structure pictured from AlphaFold[3]) using traditional ensemble MS (bottom) and the Q Exactive UHMR mass spectrometer with the Direct Mass Technology mode (top) comparing both the raw *m/z* spectra (left panels) and deconvoluted mass domain spectra (right panels). Direct Mass Technology mode resolves more masses through simultaneous charge detection, more than doubling the number of uniquely assigned glycoforms.

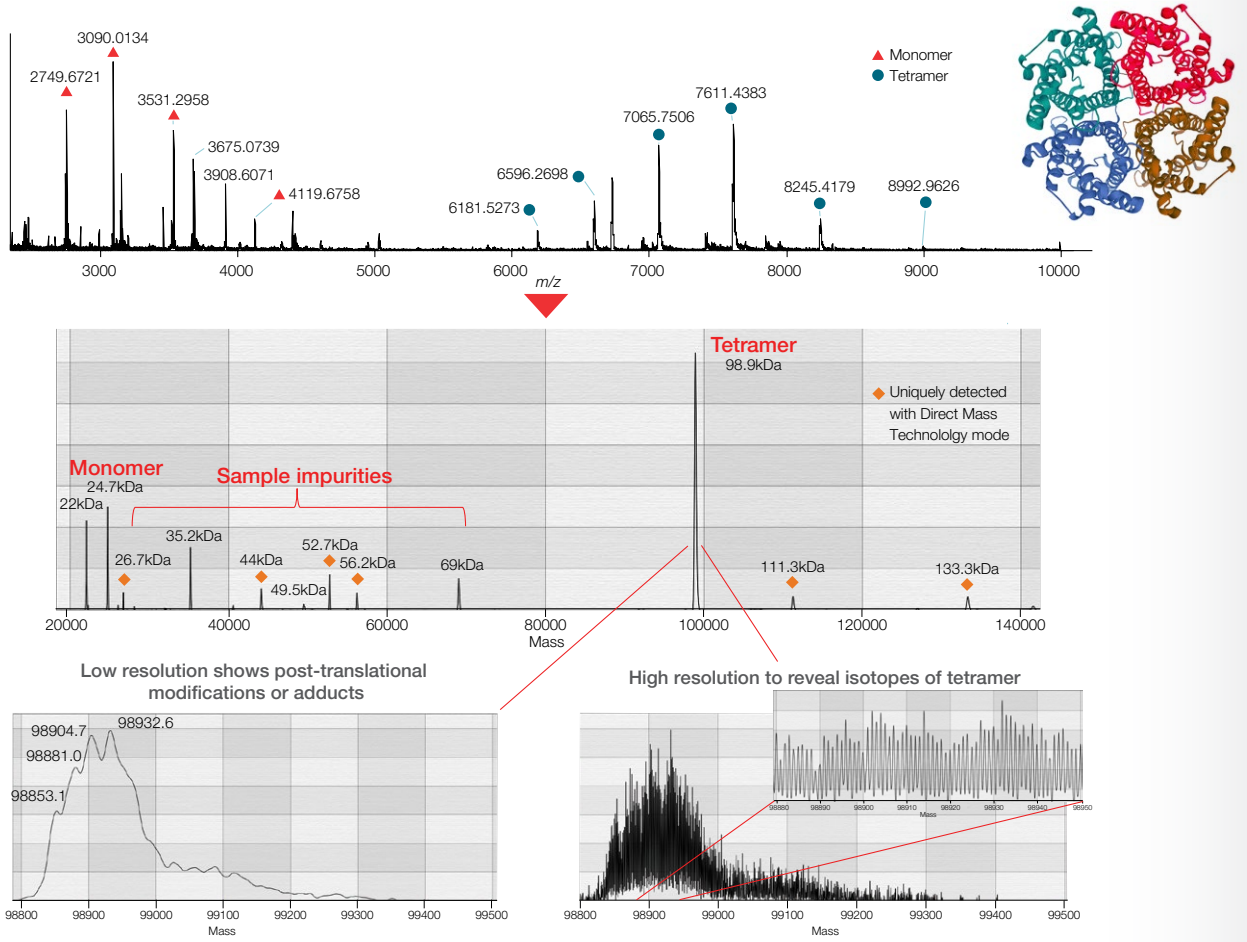
"One of the most exciting things about Direct Mass Technology mode is it can make the impossible seem very possible. And then, once possible, you get the belief that you can understand protein-level biology. And this is what proteomics needs. We need to understand that we're not overwhelmed by the scale of complexity in the human proteome, that we can actually build tools that match the scale of our biology with the scale of the solution.

Neil Kelleher, Ph.D., Director, Northwestern Proteomics and the Chemistry of Life Processes Institute, Northwestern University



Screen and characterize membrane proteins with remarkable sensitivity

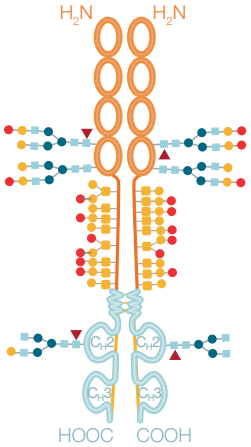
Membrane proteins and their complexes are responsible for diverse biological functions and are therefore popular drug targets, but their analysis can be challenging due to considerable sample preparation requirements. Via parallel individual ion measurements, the Direct Mass Technology mode delivers enhanced sensitivity and resolution, allowing hundreds-of-fold sample dilution to use minute amounts of precious samples and single-run sample characterization. It is now possible to perform screening, impurity identification, and isotopically-resolved characterization of native membrane proteins, including proteoforms and ligand binding, with unprecedented sensitivity.



E. coli **Aquaporin Z (AqpZ) is an approximately 99 kDa membrane protein comprised of four subunits. Native MS analysis of AqpZ with a high in source trapping setting to eject monomer using the Q Exactive UHMR mass spectrometer with Direct Mass Technology mode reveals two primary distributions of AqpZ in monomer and tetrameric forms (top). Converting this spectrum to the mass domain also reveals sample impurities (middle). Further analysis of the tetrameric peak with low resolution reveals post-translation modifications with 28 dalton shifts (bottom left) or high resolution allows isotopic resolution of the tetramer complex proteoforms in the mass domain (bottom right).**

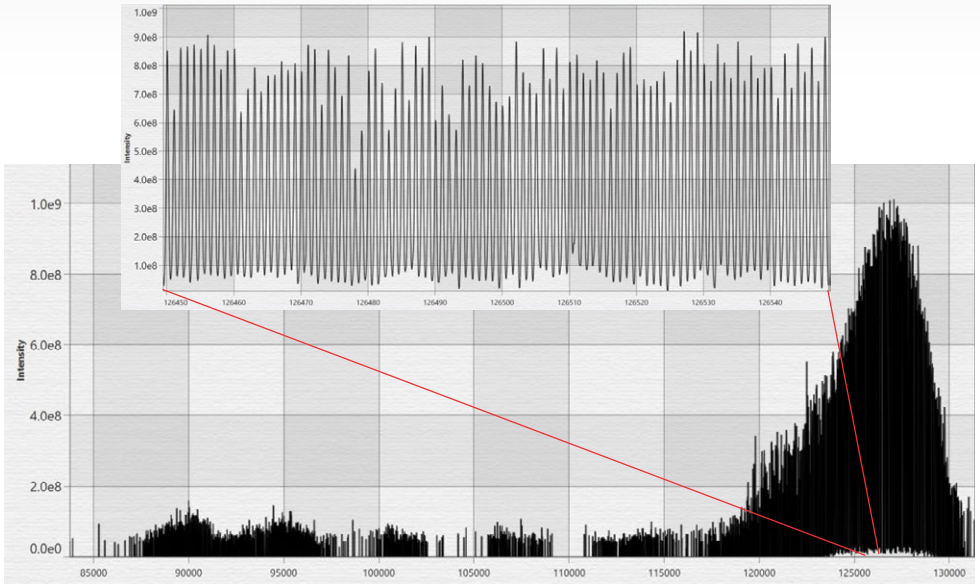
Confident characterization of Etanercept

Etanercept (Enbrel®), a FC-fusion protein, represents a new class of therapeutic biologics. Etanercept is a tumor necrosis factor (TNF) inhibitor that blocks the cytokine cascade that causes inflammation and joint destruction in rheumatoid arthritis and other autoimmune diseases. Characterization of complex biotherapeutics presents extensive analytical challenges that can require complex sample processing and multiple mass spectrometry experiments.[4] Compared to traditional ensemble MS methods, the Direct Mass Technology mode enables more confident characterization of complex biotherapeutics through the direct determination of charge states and mass to enable the visualization of a wider mass distribution and a more detailed picture of species present through increased resolution.



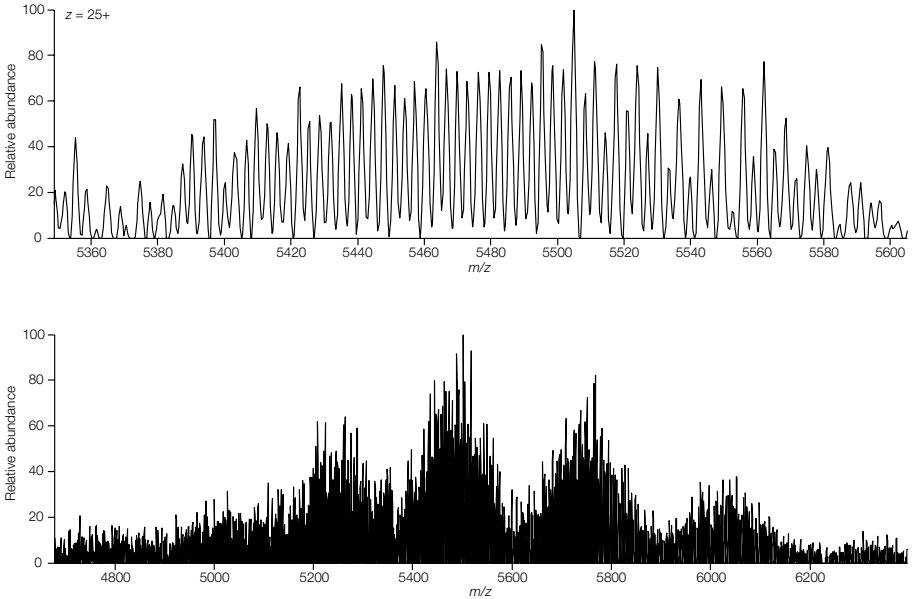
- **3 N-linked** glycosylation sites
- **13 O-linked** glycosylation sites

Direct Mass Technology mode



Molecular weight distribution of >45 kDa revealed

Standard MS



- Coexistence of over 100 glycoforms determined
- Requires multiple experiments
- Requires sample deglycosylation

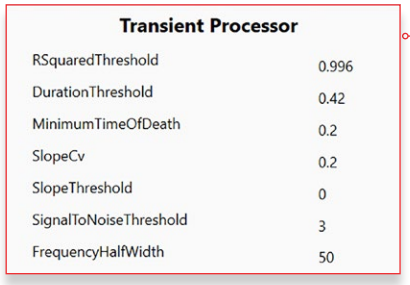
Analysis of the native Etanercept FC-fusion protein (approximately 125 kDa monomer) using traditional ensemble MS (bottom) and the Q Exactive UHMR mass spectrometer with the Direct Mass Technology mode (top). In contrast to traditional ensemble MS, the Direct Mass Technology mode allows for direct charge and mass determination to reveal a much wider proteoform distribution.

Data processing and visualization with STORIboard

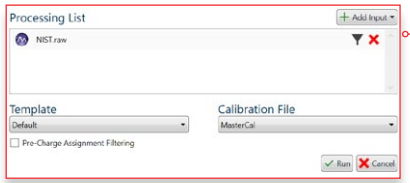
Direct Mass Technology mode .raw files containing STORI data are processed using STORIboard*, which provides an easy-to-use interface to process, visualize, and analyze results.

- Processing templates can be created, imported, customized, and stored
- Calibration data files are automatically processed with an algorithm to determine the optimal charge calibration coefficient
- Data processing of single or multiple data files with selection of processing template and calibration file, including the ability to filter data with multiple customizable parameters
- Data visualization and analysis to assist with data interpretation, data acquisition, and processing optimization

STORIboard processing and visualization functionality



Customizable processing parameters



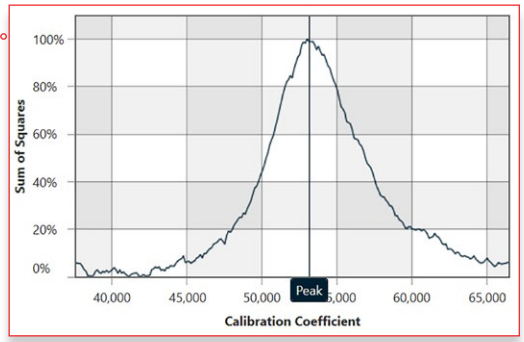
Process and filter data files

Create processing templates

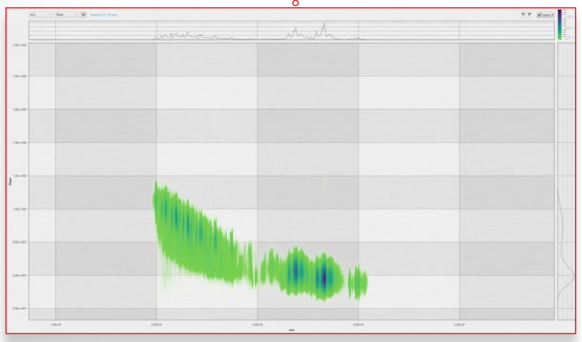
Manage calibration files

Processing workflow

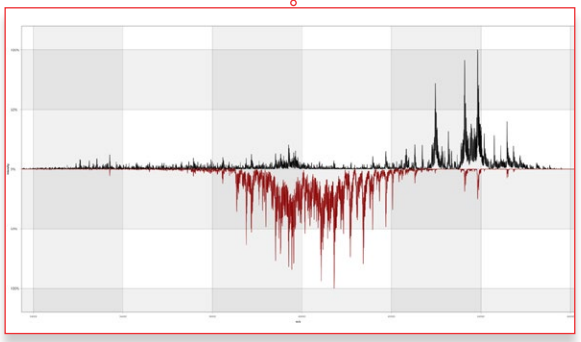
Visualization and analysis



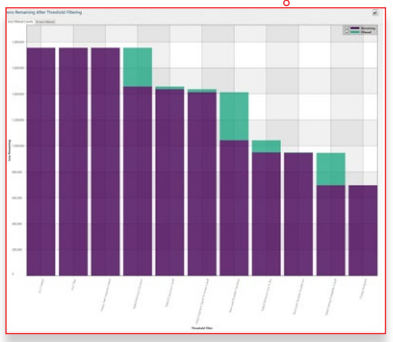
Charge calibration algorithm to determine calibration coeffiecient



Customizable heat map visualization



Mirror plot to compare spectra



Processing summary visualization

*STORIboard developed in collaboration with Proteinaceous, Inc.

ThermoFisher SCIENTIFIC

Almanac web-based monitoring and management

Stay connected to your science. See how the Thermo Scientific™ Almanac™ application can help you get the most out of your instruments.

thermofisher.com/almanac

Technical and online support: peak performance for your instruments

Helping you keep your instruments running at peak performance is our goal. Whether you're looking for an instrument manual or spare parts, want to submit a repair request, or check on the status of your warranty or service contract, we have every support option you're looking for.

thermofisher.com/technicalresources

Protecting your investments: unparalleled laboratory services

Unity™ Lab Services provides a single source for integrated lab service, support, and supply management. Our customized service offerings and world-class service experts have the flexibility and experience to address your laboratory's needs. We provide a complete portfolio of services and support solutions designed to help you improve productivity, reduce total cost of ownership, and enable performance throughout your laboratory.

unitylabservices.com

Thermo Scientific Q Exactive UHMR Hybrid Quadruople-Orbitrap mass spectrometer
thermofisher.com/QExactiveUHMR

Join our analyteguru.com community, where science meets social.
AnalyteGuru.com

[1] *Kafader et al., J. Am. Soc. Mass Spectrom. (2019) 30: 2200-2203.*
[2] *Lin et al. J. Proteome Res. (2018) 17: 2861-2869.*
[3] *Fetuin structure image from https://alphafold.ebi.ac.uk/entry/P02765.*
[4] *Wohlschlager et al., Nat. Commun. (2018) 9: 1713.*

Learn more at **thermofisher.com/DirectMassTechnology**

thermo scientific