ThermoFisher SCIENTIFIC

Scale up
your structural analysis

Orbitrap Ascend
Structural Biology Tribrid mass spectrometer

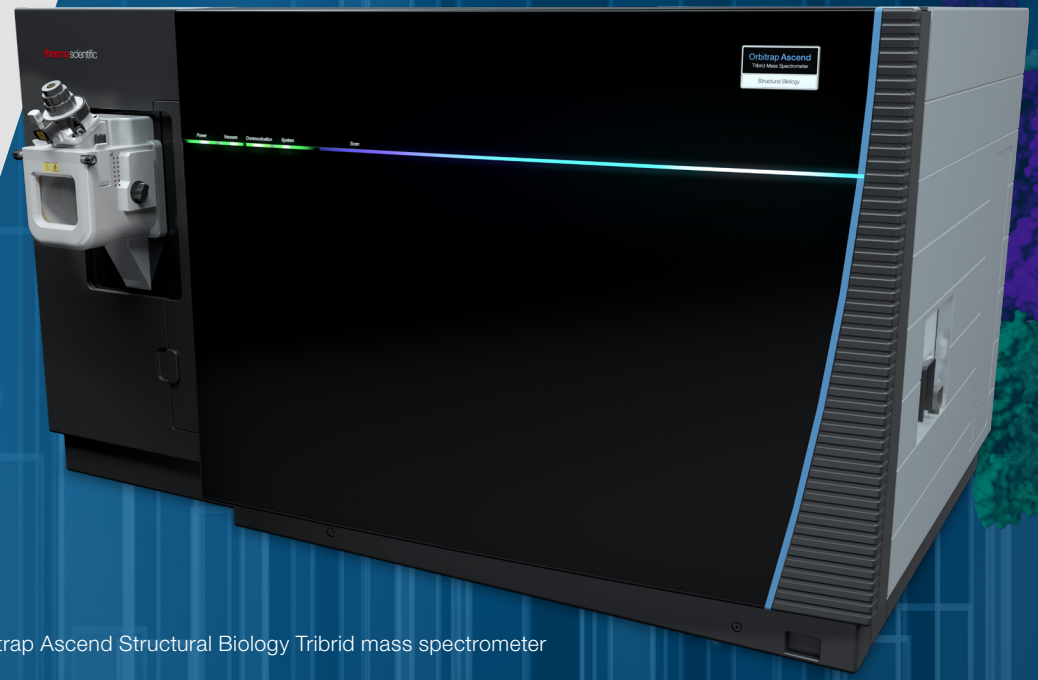
thermo scientific

Scale up insight from complex native structures

Unlock the intricacies of complex molecular structures

Built to meet the demands of structural biology, the Thermo Scientific™ Orbitrap™ Ascend Structural Biology Tribrid™ mass spectrometer adds innovations to scale up characterization of complex structures, including intact proteoforms, post-translational modifications (PTMs) and complex native structures, as well as peptide-level analyses such as hydrogen exchange MS and cross-linked peptides identification. Analyze challenging species up to *m/z* 16,000 with the Native MS option. With these capabilities and more, access unprecedented experimental throughput, versatility and usability to meet tomorrow's challenges while achieving more insight today.

Native proteomics	Analyze complex mixtures, cover higher mass range and perform orthogonal fragmentation techniques
Quantitation	Achieve accurate, proteome-wide quantitation for **structural characterization**
Gas-phase fractionation	Completely characterize protein complexes and proteoforms
Peptide-level structural analysis	Choose from the **widest selection** of methods for **identification and quantification of cross-linked peptides**, HDX-DIA
Ligand binding analysis	Characterize complex samples with wide dynamic range and analyze labile compounds



Orbitrap Ascend Structural Biology Tribrid mass spectrometer

Innovative technology addresses structural analysis needs

The Orbitrap Ascend Structural Biology Tribrid mass spectrometer features innovations to scale up your native structural characterization with throughput, versatility and ease-of-use.

Real-time database/ library search
Database search/spectral-library-directed MS^n acquisition

Back ion routing multipole
Enables parallel analysis, performs HCD at MS^{3+} stage

Optional UVPD
Unique fragmentation mode for analyte structure elucidation

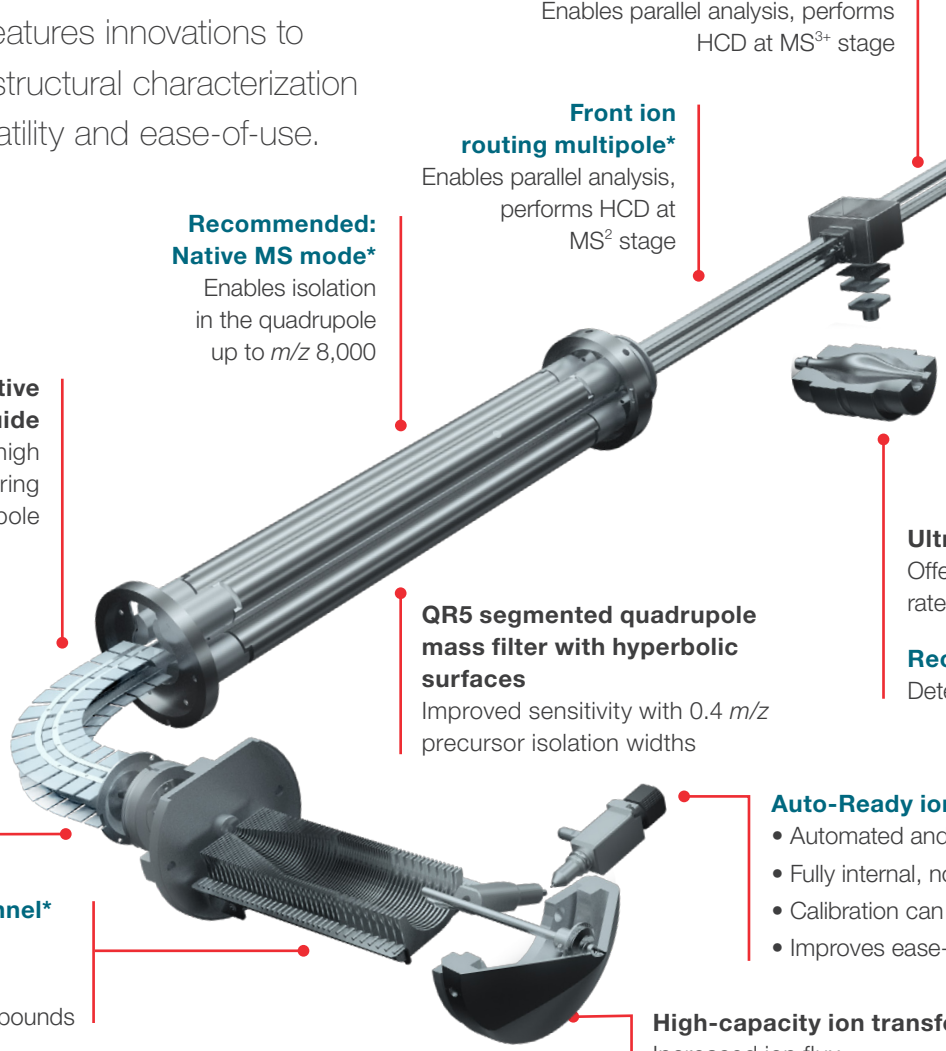
Front ion routing multipole*
Enables parallel analysis, performs HCD at MS^2 stage

Recommended: Native MS mode*
Enables isolation in the quadrupole up to *m/z* 8,000

Modified dual-pressure linear ion trap mass analyzer
- Up to 50 Hz MS^n and sensitive mass analysis
- Six fragmentation types: CID, HCD, ETD, EThcD, ETciD and UVPD

Recommended: Native MS mode*
Enables isolation up to *m/z* 8,000

Advanced active ion beam guide
Prevents neutrals and high velocity clusters from entering mass resolving quadrupole



Ultra-High-Field Orbitrap™ mass analyzer
Offers resolution >480 K FWHM and acquisition rates up to 45 Hz, TurboTMT

Recommended: Native MS mode*
Detection in the Orbitrap analyzer to *m/z* 16,000

QR5 segmented quadrupole mass filter with hyperbolic surfaces
Improved sensitivity with 0.4 *m/z* precursor isolation widths

Recommended: Thermo Scientific™ EASY-IC/ETD and PTCR ion source
Based on Townsend discharge, reliable and easy to use

Auto-Ready ion source*
- Automated and remote calibration
- Fully internal, no need to remove source (nESI, FAIMS)
- Calibration can be scheduled
- Improves ease-of-use and data consistency

Electrodynamic ion funnel*
- Efficient ion transfer
- Broad tuning curves
- Optimized for labile compounds

High-capacity ion transfer tube
Increased ion flux

OPTIONS IC I ETD I PTCR I Native MS* I UVPD I FAIMS Pro Duo interface

*New on this platform

Native complex

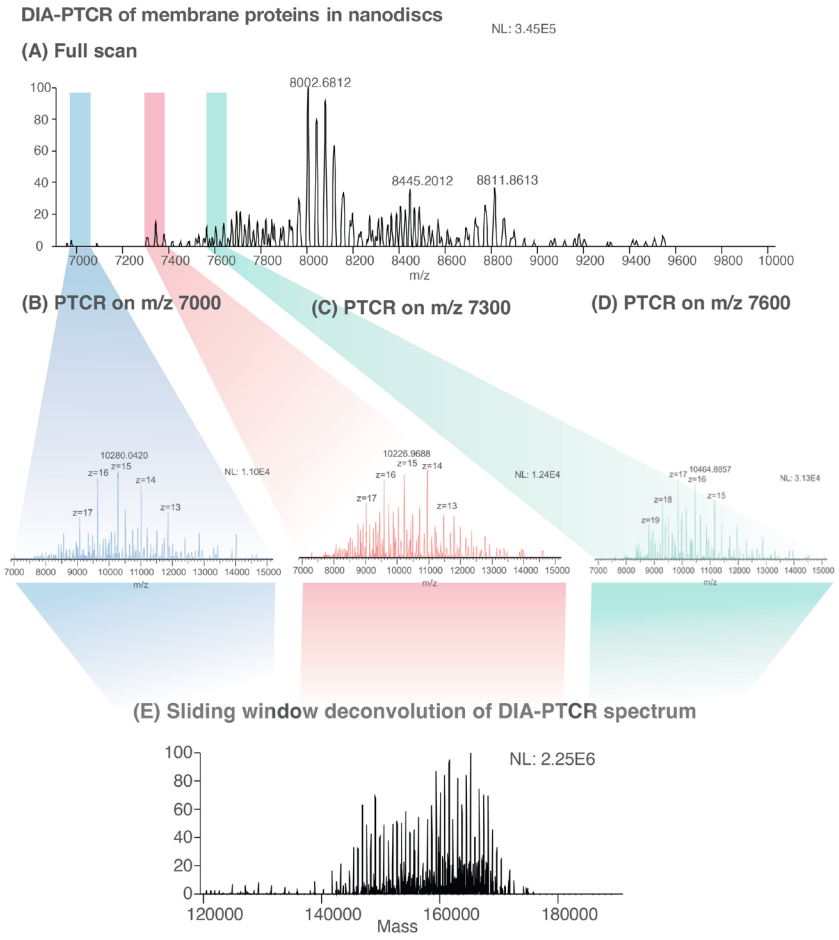
Identify more species across a broad *m/z* range in complex mixtures with DIA-PTCR

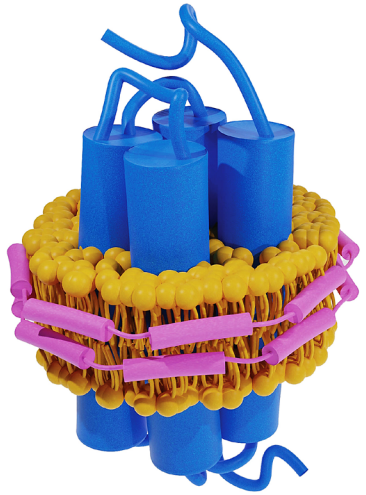
Scale-up complex sample characterization

The Native MS option extends the quadrupole isolation range to *m/z* 2,000–8,000 from *m/z* 50–2,000, and isolation width to *m/z* 5–3,000 from *m/z* 0.4–1,200. The proton transfer charge reduction (PTCR) ion source option generates perfluoroperhydrophenanthrene (PFPP) ions for subsequent gas-phase, ion-ion reactions. When narrower data independent acquisition (DIA) windows are used for PTCR analysis of complex native mixtures, the PTCR spectra generated are simplified and sensitivity is increased. In addition, extending the mass range detected enables elucidation of hidden peaks in the charge state envelope. For the analysis of membrane proteins in nanodisc-based experiments, researchers can use this capability to enhance structural insight, such as the **stoichiometry of different lipid classes** and bound proteins.

"I am very impressed by the high-mass quadrupole isolation, which enables exciting new DIA-PTCR experiments to characterize complex samples."

Michael T. Marty, PhD
Associate Professor of Chemistry & Biochemistry
University of Arizona





Orbitrap Ascend Structural Biology Tribrid mass spectrometer analysis of nanodiscs using extended quadrupole isolation and DIA-PTCR. Deconvolution of DIA-PTCR spectra produces a molecular weight profile of approximately 140–175 kDa. The spacing between adjacent deconvoluted peaks at 700–750 Da fall into the molecular weight range associated with nanodiscs lipids.

Data courtesy of Associate Professor Michael T. Marty, University of Arizona.

Identify native membrane proteins using PTCR and top-down techniques

Native top-down

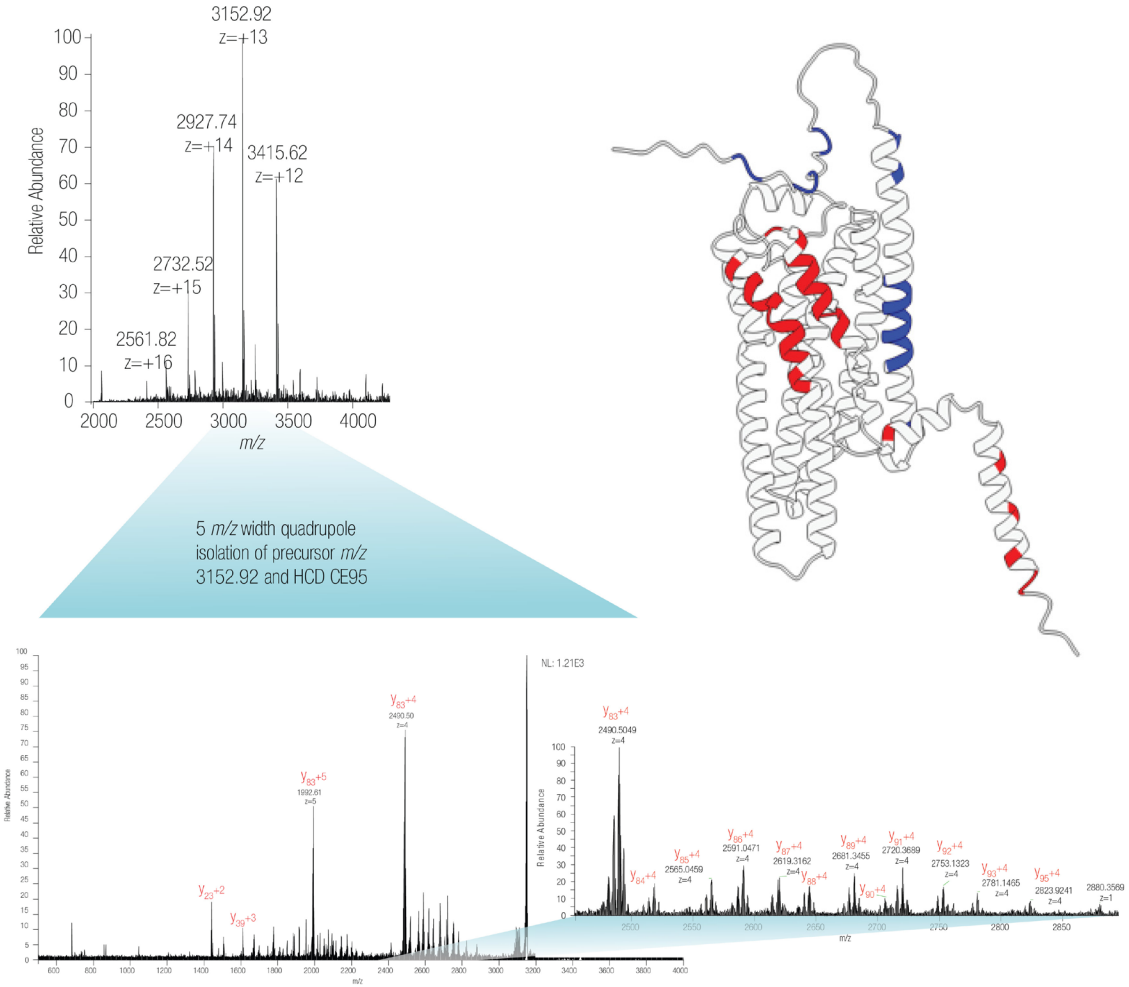
Scale up to realize the potential of native proteomics

With the Orbitrap Ascend Structural Biology Tribrid mass spectrometer, it's now possible to realize the potential of native proteomics, particularly for difficult-to-analyze membrane proteins. Using native protein sample preparation techniques, complexes can be directly injected into the mass spectrometer and the proteins elucidated using their precursor and fragmentation patterns. At the intact protein level, PTCR simplifies the spectra produced from complex samples. For top-down analysis, difficult-to-break proteins can be fragmented using multiple fragmentation options, generating enough sequence coverage for high-confidence identification of proteins within complex samples.

"I'm delighted that we now have an Orbitrap Ascend Structural Biology Tribrid mass spectrometer because it has transformed our ability to study complicated systems. It gives us the additional flexibility to break the intact molecule into its components and really identify what it is, and also what's there. Often, in these very large assemblies, there's a small molecule hidden. That's what we want to find. Is there a metabolite? Is there a drug that's changing the properties of this protein? That's very important for me."

Professor Dame Carol Robinson DBE FRS FMedSci FRSC
Director of the Kavli Institute for Nanoscience Discovery
University of Oxford

Fragments mapped on to structure of β1AR



Data courtesy of Corinne Lutomski, Jack Bennett and Tarick El-Baba, Professor Dame Carol Robinson's lab, University of Oxford, and Idlir Liko, OMass Therapeutics.

XL-MS

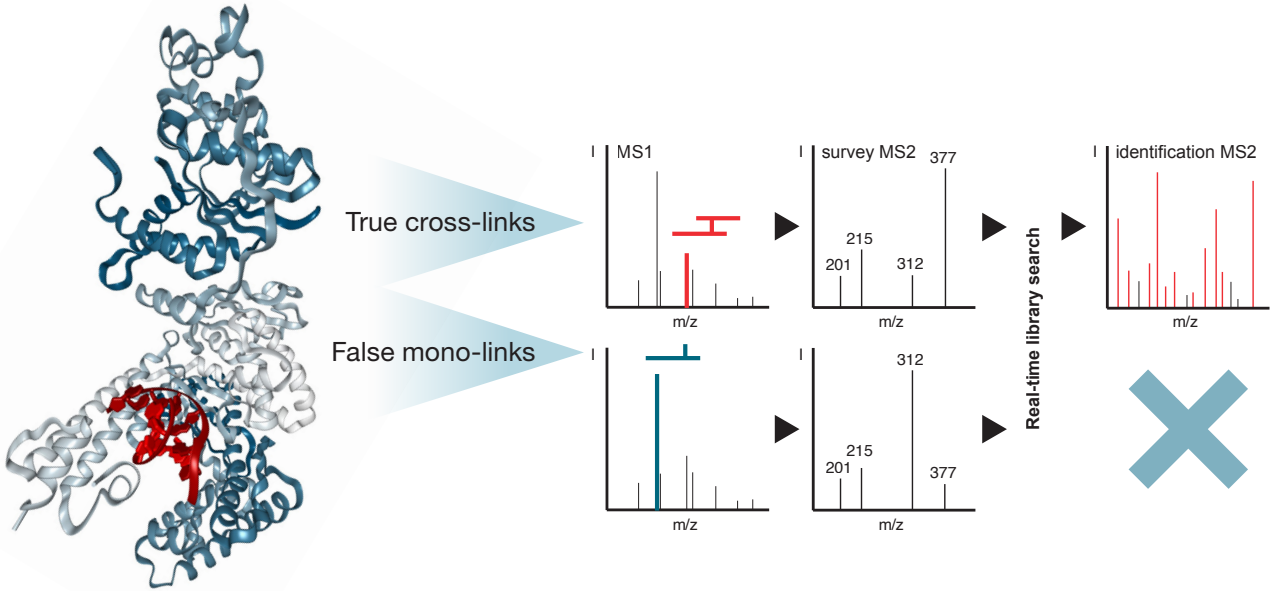
Enhance the productivity of cross-linking MS experiments with Real-Time Library Search

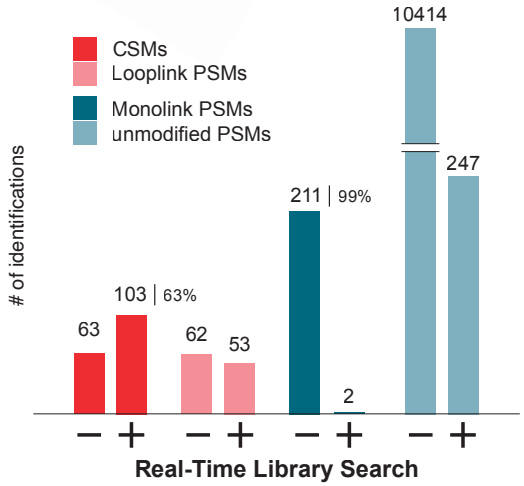
Scale up cross-link identification

The presence of mono-links complicates the process of cross-linked peptide identification by MS even after the enrichment. By analyzing intensity ratios of specific peaks with enrich-able cross-linkers, partial differentiation between the cross-links and mono-links in samples can be achieved. Real-Time Library Search triggering of high-resolution MS^2 scans based on the intensity ratios significantly enhances cross-linked precursors identification, predominantly for unenriched samples and short chromatography gradients. The approach particularly improves productivity for higher throughput and resource-constrained biological projects.

"The unique feature of Real-Time Library Search on the Orbitrap Ascend Structural Biology Tribrid mass spectrometer enables on-the-fly selection of cross-linked precursors for MS^2 sequencing. This is really exciting and has always been what we hoped to achieve over the last years—selective targeting and sequencing of cross-linked peptides."

Fan Liu, PhD
Professor of Biochemistry
Leibniz Research Institute for Molecular Pharmacology





Sixty-four human proteins were cross-linked with PhoX (p/n A52286). Cross-linked peptides were enriched and spiked into HeLa digest at a 1:20 ratio and analyzed on the Orbitrap Ascend Structural Biology Tribrid mass spectrometer with (+) and without (–) Real-Time Library Search. Cross-links were identified using Thermo Scientific™ Proteome Discoverer™ software revision 3.1 with XlinkX node.

Data courtesy of Max Ruwolt and Fan Liu, Leibniz-Research Institute for Molecular Pharmacology.

Perform disulfide mapping and quantitation with EThcD and the XlinkX node

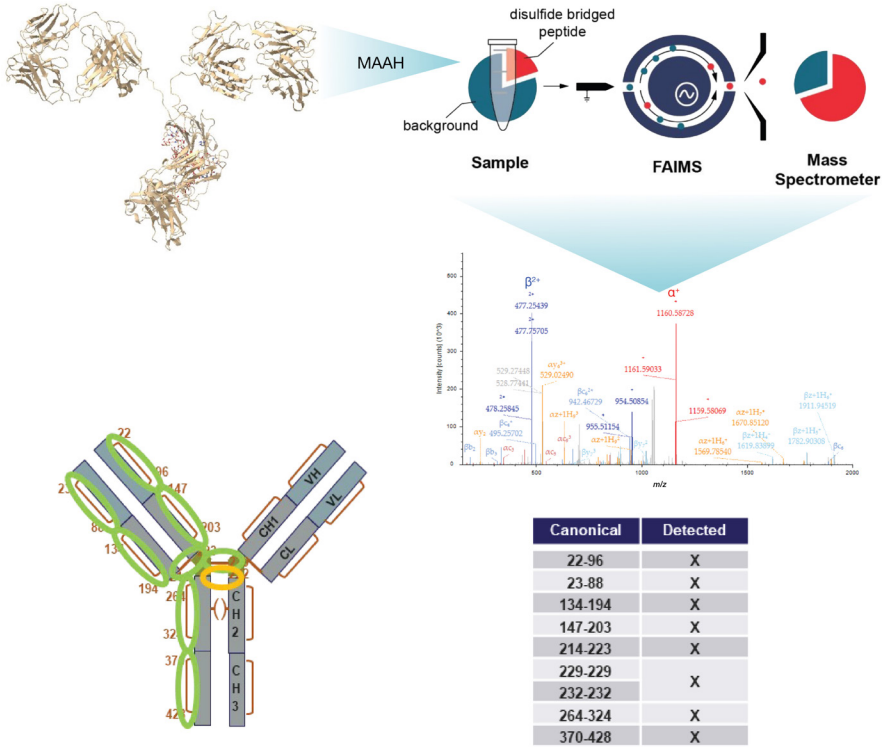
Scale up disulfide bridge assessment certainty

Quality control assessment of therapeutic recombinant proteins requires accurate measurement of critical attributes such as sequence fidelity, proper folding and PTMs. Errors can lead to diminished bioactivity and, in the context of therapeutic proteins, an elevated risk for immunogenicity. Though analytically challenging, assessment of disulfide bridges—the covalent bonds linking two Cysteine residues in a protein—is essential because they determine the correct folding and stability of proteins and thus have a major influence on their efficacy.

When equipped with the Thermo Scientific™ FAIMS Pro Duo interface and electron transfer dissociation (ETD) with supplemental activation (EThcD), the Orbitrap Ascend Structural Biology Tribrid mass spectrometer offers an efficient workflow for disulfide mapping. Microwave Assisted Acid Hydrolysis (MAAH) sample preparation effectively digests the protein of interest, generating massive peptide redundancy. Because MAAH is prone to background noise, the FAIMS Pro Duo interface uses differential ion mobility to remove interferences prior to MS analysis, bringing the desired signals into focus. EThcD generates highly informative spectra with ions diagnostic of disulfide-bridged peptides. XlinkX, a node in Proteome Discoverer software, enables rapid, high confidence identification of disulfide bridges at very low false discovery rates and quantifies the occupancy correctly.

“By combining novel sample preparation and advances in data analysis with the amazing FAIMS and EThcD capabilities of the Orbitrap Ascend Structural Biology Tribrid mass spectrometer, we are obtaining information on disulfide bridges at unprecedented detail in less than one hour. This transforms high performance QC of recombinant protein products.”

Richard Scheltema, PhD
Professor of Structural Proteomics
University of Liverpool



Canonical	Detected
22-96	X
23-88	X
134-194	X
147-203	X
214-223	X
229-229	X
232-232	
264-324	X
370-428	X

Trastuzumab is digested using MAAH. The subsequent background noise produced by MAAH is removed prior to MS analysis using the orthogonal selectivity provided by the FAIMS Pro Duo interface, enhancing signal-to-noise ratios for the ions of interest, including ions diagnostic of disulfide bridges.

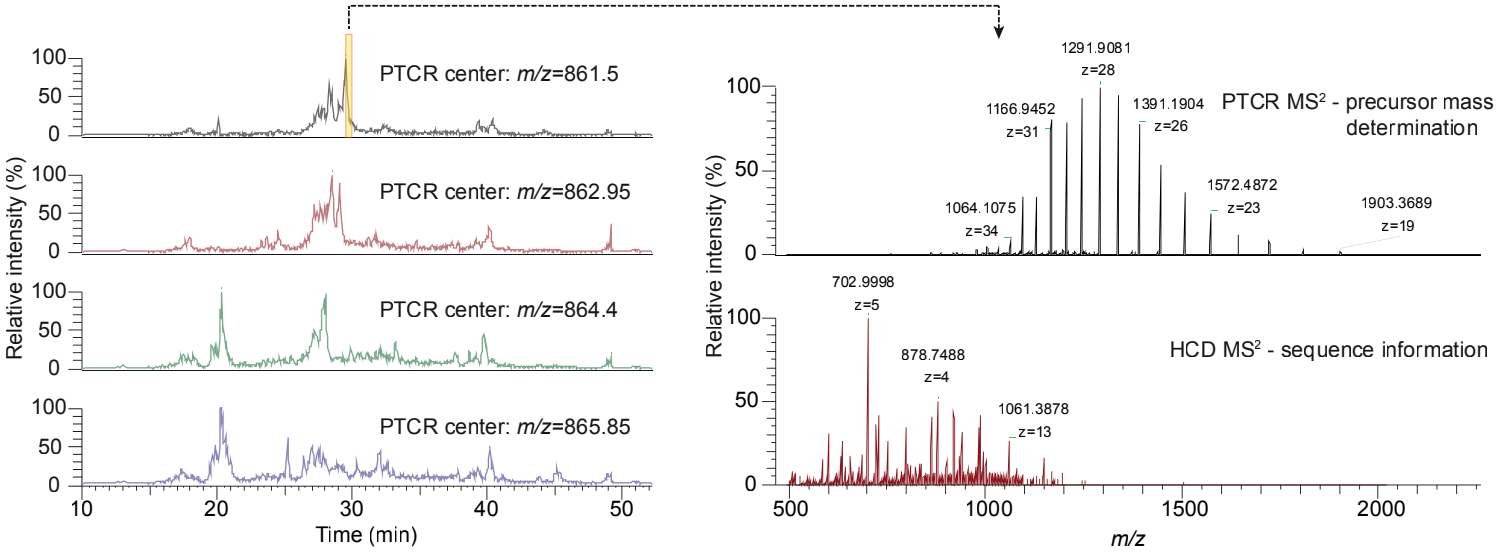
Data courtesy of Professor Richard Scheltema, University of Liverpool, and Søren Heissel, The Rockefeller University.

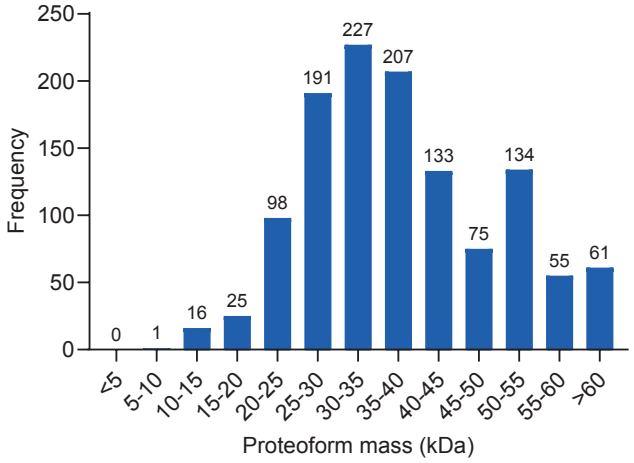
Proteoform detection

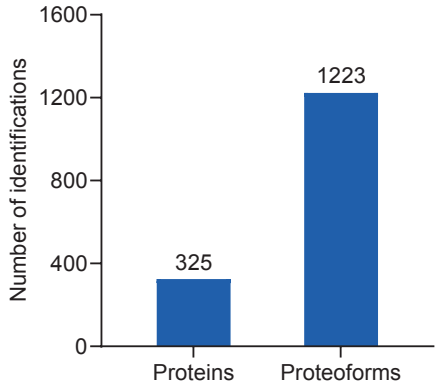
Access higher mass proteoforms with PTCR fractionation of the MS[1]

Scale up to measure larger proteoforms by top-down proteomics

Top-down proteomics enables scientists to identify and structurally characterize PTMs and protein isoforms—known collectively as proteoforms—as they occur in the cell. By combining PTCR and the Native MS option, which extends instrument mass range to *m/z* 16,000, proteoform characterization was expanded to a new level, exploring larger proteins above the 30 kDa range by simplifying complex MS[1] spectra using targeted isolation windows. The same *m/z* range is again isolated and fragmented for proteoform identification and primary structure characterization using Thermo Scientific™ ProSightPD™ software.







HEK293 cells were grown to 80–100% confluency before harvesting by trypsinization. Proteins were fractionated by molecular weight using 10% T gel. Fractions 2–14 were analyzed using tPRCT methodology.

Sample courtesy of Assistant Professor Luca Fornelli, University of Oklahoma.

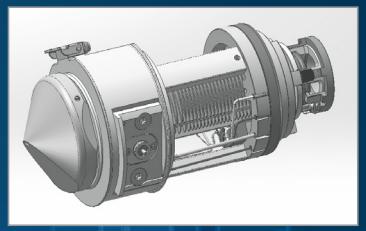
Experience more high-quality results with less hassle using automated, remote and schedulable system checks and calibrations

Scale up convenience and ease-of-use

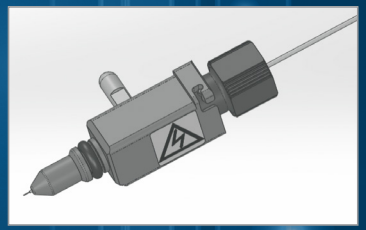
The Auto-Ready ion source is a fully integrated, standard, easy-to-use feature that increases laboratory productivity with automated, remote and schedulable system checks and internal calibrations. Because there is no need to remove the source (HESI, nESI or high-field asymmetric waveform ion mobility spectrometry [FAIMS]), there are no experimental setup interruptions required to perform internal calibrations. The user can automate the calibration to start at a scheduled time—for example, every week—when there are no experiments planned to run on the instrument. The calibration can run completely remotely, regardless of the nature of the last experiment. Because the calibration can be scheduled to occur regularly and automatically without interrupting vital work, users can expect to maintain mass spectrometer performance, improve data consistency and achieve more accurate and precise quantitation.

Auto-Ready ion source

Separate ion transfer tube

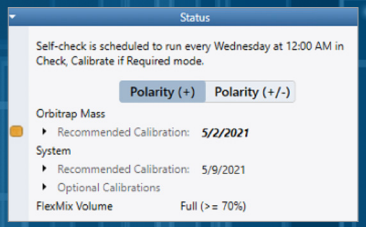


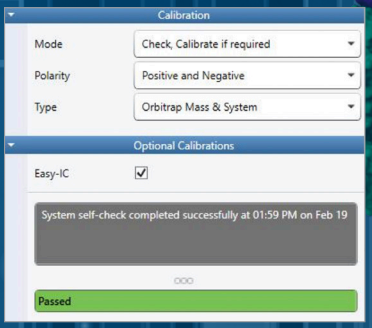
Dedicated emitter



Robust delivery system

Automated weekly calibrations





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