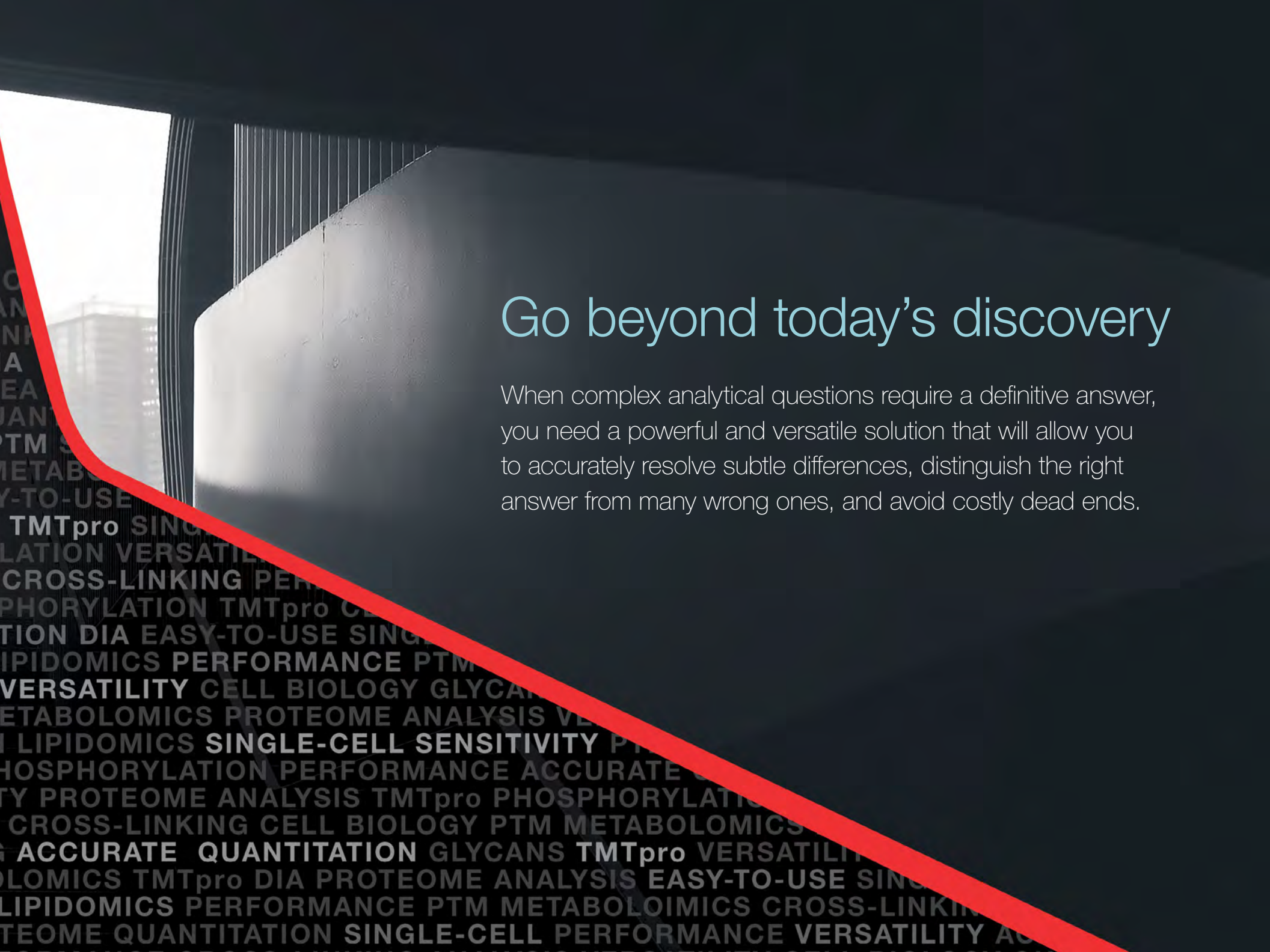
thermo scientific

Go beyond today's discovery

Orbitrap Eclipse Tribrid mass spectrometer

ThermoFisher
SCIENTIFIC



Go beyond today's discovery

When complex analytical questions require a definitive answer, you need a powerful and versatile solution that will allow you to accurately resolve subtle differences, distinguish the right answer from many wrong ones, and avoid costly dead ends.

Introducing the Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ mass spectrometer, our newest generation instrument designed with your most difficult analytical challenges in mind. With enhanced performance and usability, the Orbitrap Eclipse Tribrid mass spectrometer makes it easy to produce the high-quality data required to drive the right decisions, while expanding the breadth of your work and pushing your science beyond today's discovery.

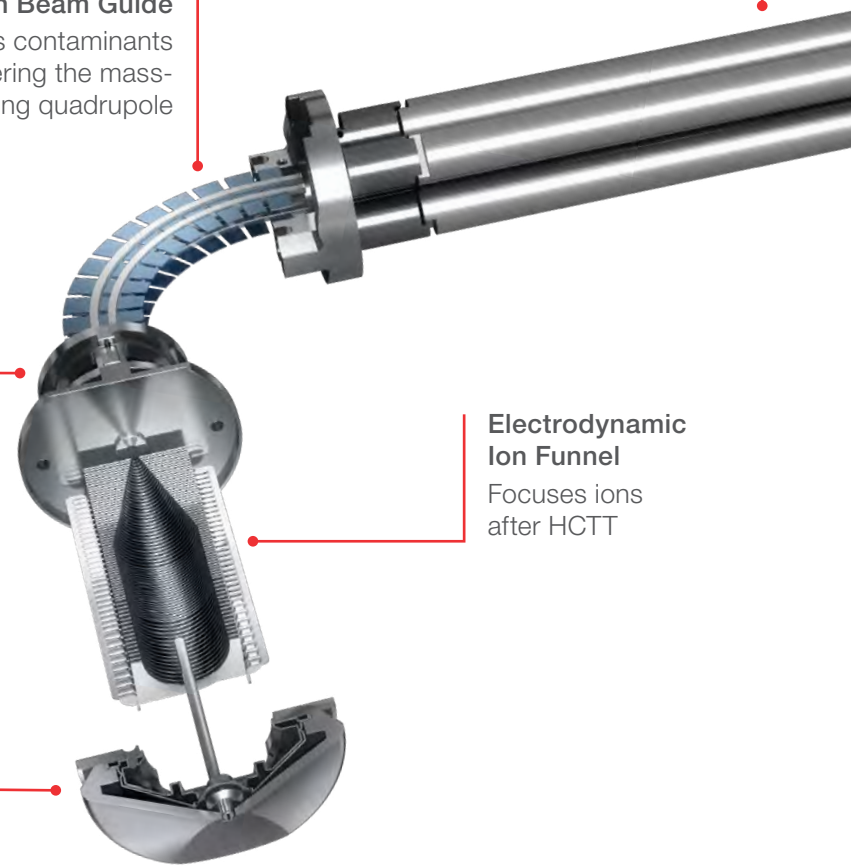
Building on revolutionary Tribrid architecture

The Orbitrap Eclipse Tribrid mass spectrometer amplifies the power and versatility of the innovative Thermo Scientific™ Tribrid™ design by incorporating the latest inventions in ion transmission and control, extended *m/z* range, and real-time decision making.

These new functionalities provide ground-breaking gains in sensitivity, selectivity and versatility, making the instrument ideally suited for obtaining comprehensive results from proteomics, structural biology, small-molecule, and biopharmaceutical characterization experiments.

QR5 Segmented Quadrupole Mass Filter with Hyperbolic Surfaces
Improves sensitivity with 0.4 *m/z* precursor isolation width

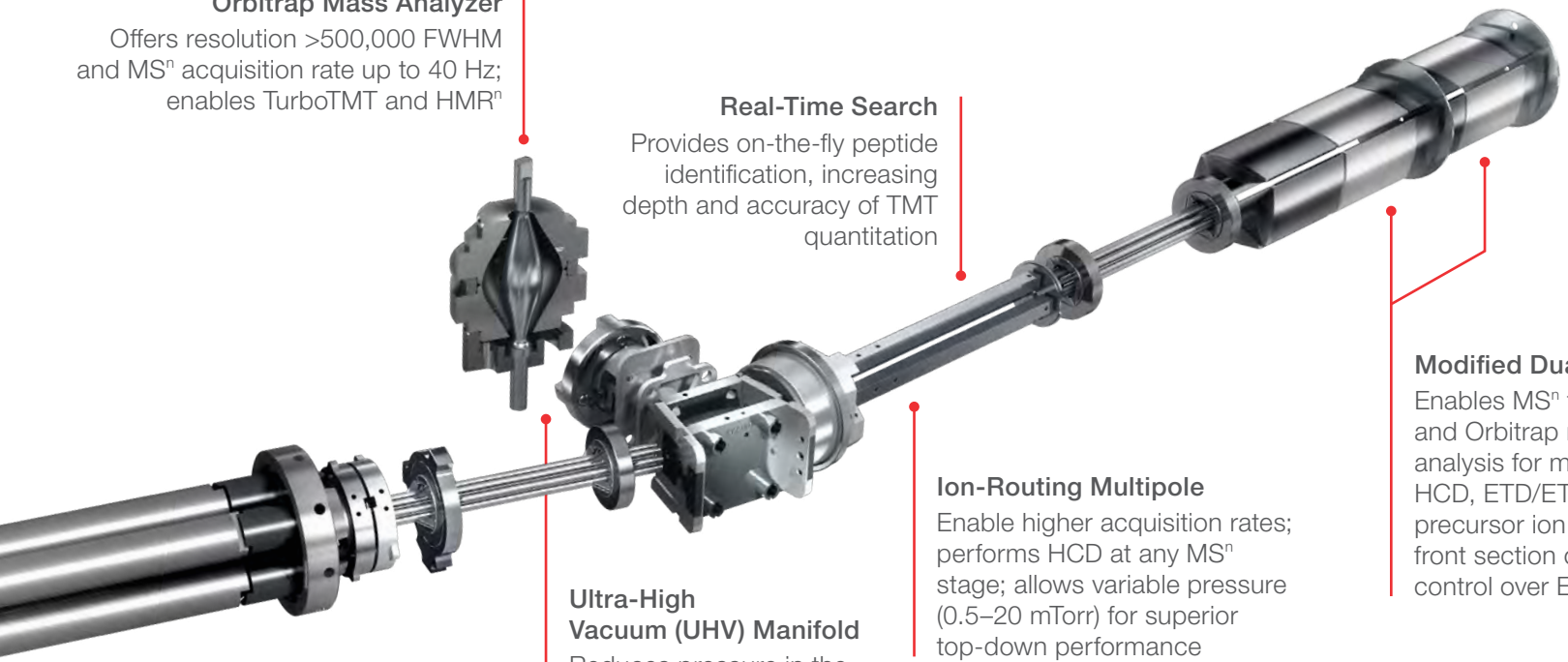
Advanced Active Ion Beam Guide
Prevents contaminants from entering the mass-resolving quadrupole



EASY-IC/ETD/PTCR Ion Source
Based on Townsend discharge; reliable and easy to use

Electrodynamic Ion Funnel
Focuses ions after HCTT

High-Capacity Transfer Tube (HCTT)
Increases ion flux into the mass spectrometer



Ultra-High-Field Orbitrap Mass Analyzer

Offers resolution >500,000 FWHM and MS^n acquisition rate up to 40 Hz; enables TurboTMT and HMR^n

Real-Time Search

Provides on-the-fly peptide identification, increasing depth and accuracy of TMT quantitation

Modified Dual-Pressure Linear Ion Trap

Enables MS^n for ion detection in both ion trap and Orbitrap mass analyzers; sensitive mass analysis for multiple fragmentation modes: CID, HCD, ETD/EThcD/ETciD and UVPD; and precursor ion isolation for HMR^n. The extended front section of the high-pressure cell improves control over ETD and PTCR reactions.

Ion-Routing Multipole

Enable higher acquisition rates; performs HCD at any MS^n stage; allows variable pressure (0.5–20 mTorr) for superior top-down performance

Ultra-High Vacuum (UHV) Manifold

Reduces pressure in the UHV region; improves ion detection in the Orbitrap mass analyzer

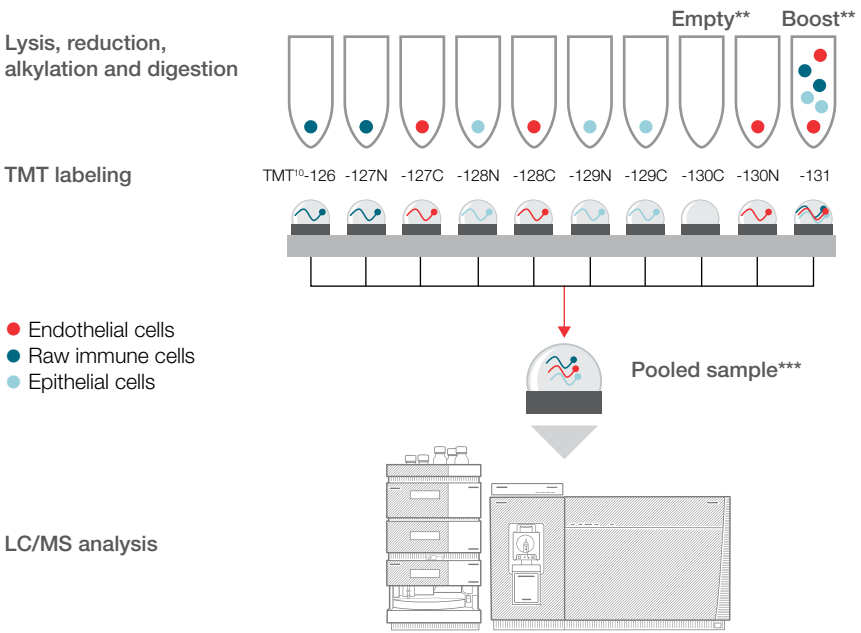
Options to expand experimental versatility

EASY-ETD	PTCR	HMR^n	FAIMS Pro	UVPD	EASY-IC	1M
Thermo Scientific™ EASY-ETD™ HD electron transfer dissociation ion source	Proton Transfer Charge Reduction	High Mass Range MS^n to *m/z* 8,000	Thermo Scientific™ FAIMS Pro™ interface	Thermo Scientific™ UV Photodissociation	Thermo Scientific™ Internal Calibration EASY-IC™ ion source	Thermo Scientific™ 1,000,000 FWHM

Unprecedented sensitivity for single-cell analysis

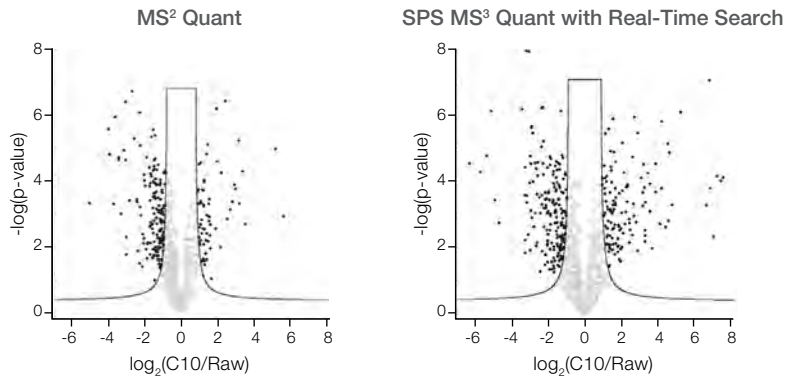
Cellular systems are comprised of many different cells, each with their own set of distinct molecular and functional properties. No two cells are identical. Comprehensive characterization of single-cell proteomes will provide a wealth of novel information about cellular development in the context of disease progression and response to treatment as a function of cell type. Yet, proteome analysis with single-cell resolution remains an enormous challenge due to the analytical sensitivity this experiment demands. The Orbitrap Eclipse Tribrid mass spectrometer was developed to meet the challenge of extracting unrivaled quantitative data from ultra-low-level samples, including from individual cells. Specifically, the MS^3-based Tandem Mass Tag (TMT) method, enhanced by Real-Time Search*, provides the throughput and sensitivity to achieve the proteome coverage and the quantitative accuracy needed to differentiate cell types and to capture their heterogeneity. Further, using novel Thermo Scientific™ TMTpro™ reagents, up to 16 single cells can be analyzed in one LC/MS run, providing quantitative comparison of thousands of proteins among individual cells.

Individual cells are isolated using FACS sorter, labeled, and analyzed using the Orbitrap Eclipse Tribrid mass spectrometer



Novel MS^3 workflow with Real-Time Search detected more than 300 differentially expressed proteins in each individual cell

	MS^2	SPS MS^3 with Real-Time Search
Proteins quantified	901	960
Upregulated in endothelial cells (C10)	67	113
Upregulated in raw immune cells	157	195

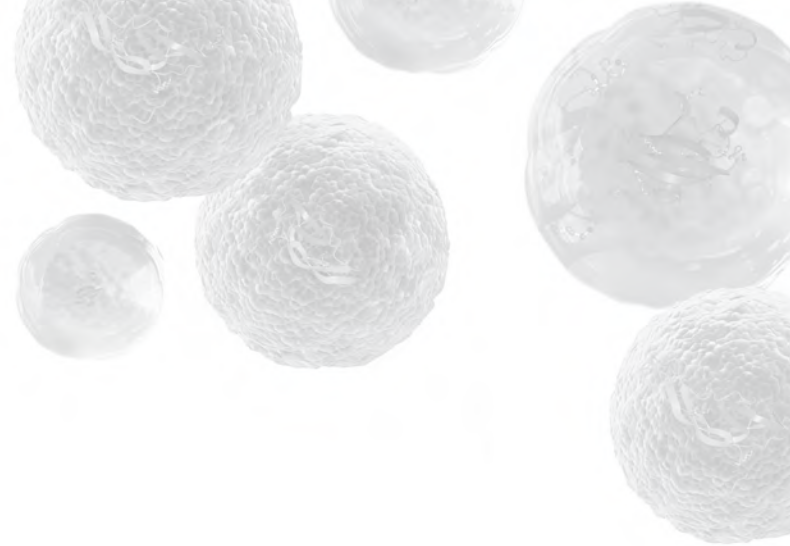


TMT analysis of two cell types revealed more differentially expressed proteins when using SPS MS^3 with Real-Time Search compared to the MS^2-based experiment. The MS^3 experiment allows for more accurate quantitation, enabling detection of subtle changes in more proteins for each individual cell.

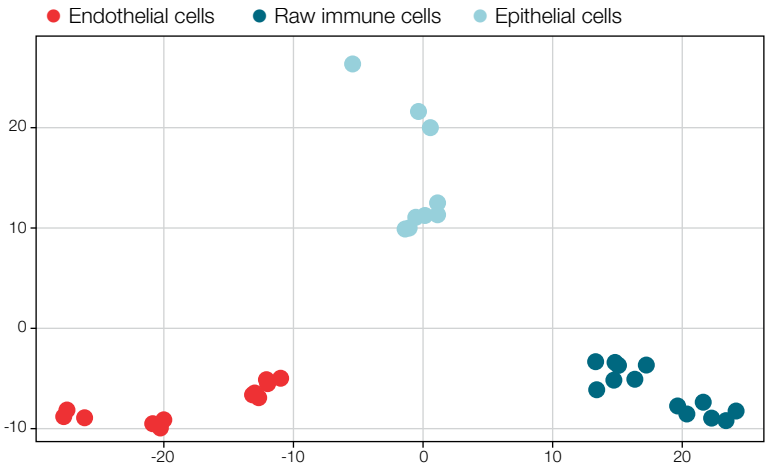
* For details on Real-Time Search see pp 8–9.
** Budnik *et al., Genome Biol.* 2018,*19*(1); Zhu *et al.,* 2018, *Nature Comm.,* 2018, *9*(882).
*** A total of 40 individual cells were analyzed in this study.

A revolution in single-cell proteomics is just beginning. The combination of nanoPOTS with the Orbitrap Eclipse Tribrid mass spectrometer, TMT reagents, and SPS MS³ with Real-Time Search provide the depth of coverage, quantitative accuracy, and throughput needed to propel this nascent field forward."

Ryan Kelly, Professor, Brigham Young University, UT

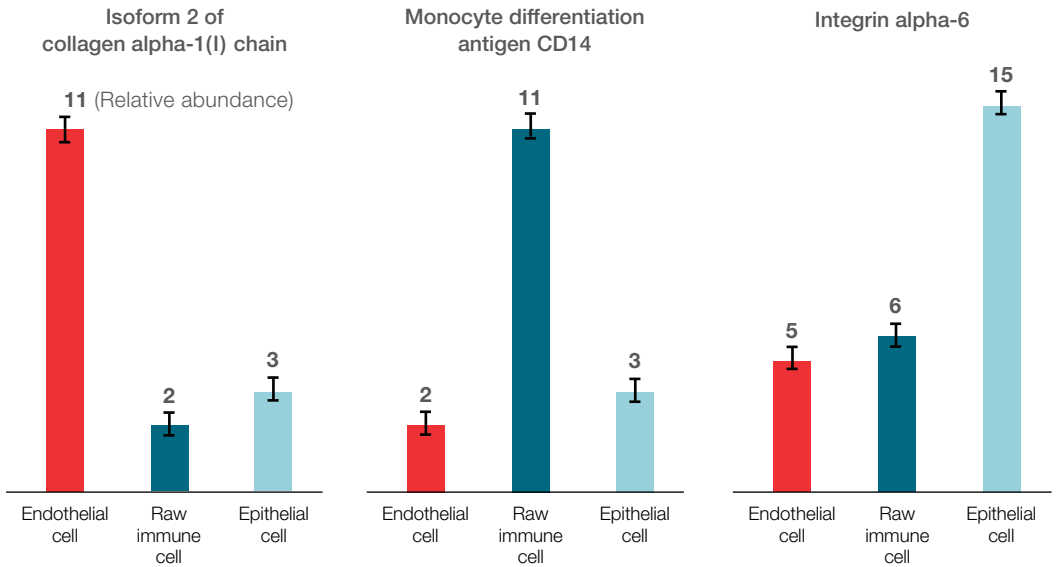


High-resolution classification of individual cells in an SPS MS³ experiment with Real-Time Search



PCA plot showing unsupervised classification of the three cell types using TMT data. Each point corresponds to the protein expression of a single cell. The plot shows clear classification of cell types and resolved heterogeneity within each type. This level of resolution is uniquely achievable using a combination of TMT-boost** and SPS MS³ with Real-Time Search data acquisition technologies.

Confirmation of overexpression of known protein biomarkers in individual cells of each type underscores the sensitivity of the workflow



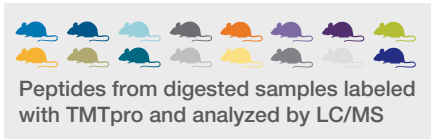
Hyper-accurate high-throughput protein quantitation using TMTpro 16plex and Real-Time Search

The standard for high-throughput quantitative comparisons of protein abundances is the TMT SPS MS^3 workflow, unique to Thermo Scientific™ Orbitrap™ Tribrid™ mass spectrometers. A significant advancement of the Orbitrap Eclipse Tribrid mass spectrometer is Real-Time Search, which can be used to identify peptide spectra on-the-fly to intelligently direct MS^3 data acquisition, resulting in accurate quantitation to depths often exceeding 8,000 proteins in up to 16 samples per LC/MS analysis.

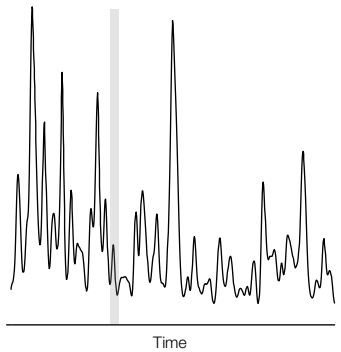
This new high-throughput workflow offers increased proteome coverage with improved accuracy and precision, boosting the number of quantifiable low-level peptides.

Multiplexing improves analytical throughput

Sample preparation



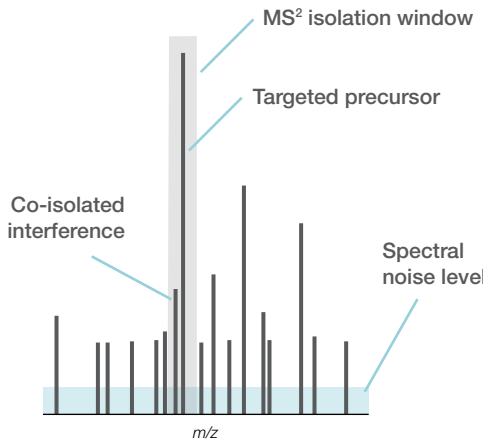
LC/MS analysis



Base peak extracted ion chromatogram of the multiplexed TMT sample. The shaded region highlights a selected MS spectrum.

Quantitation accuracy is dependent on isolation purity

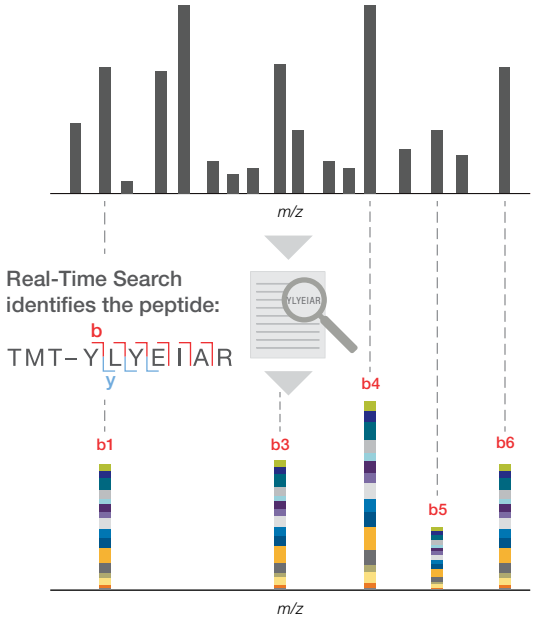
MS precursor spectrum



When analyzing complex peptide mixtures, co-isolated ion interferences can occur despite using narrow precursor isolation. Co-fragmentation of precursors of interest and interfering ions negatively impacts the quantitative accuracy of the TMT-based experiment.*

Real-Time Search improves accuracy by nearly eliminating isolation impurity

MS²



Every MS^2 spectrum is interrogated against a database of choice using Real-Time Search in parallel with the acquisition of the next MS^2 scan. If the search results in a peptide match, the instrument is directed to perform an SPS MS^3 scan using only the matched fragment ions that carry the TMT tags, while avoiding any fragments that may have originated from the interfering ions. For YLYEIAR, the TMT tag was found only on the N-terminus, so the SPS MS^3 is performed only on five *b*-ions.

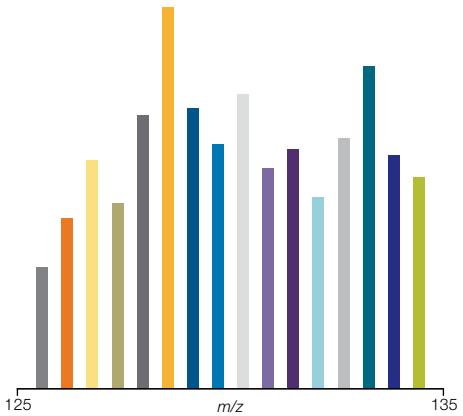
* Ting *et al., Nat. Methods*, **2011**, *8*(11).

The Orbitrap Eclipse Tribrid mass spectrometer provides several exciting advances which allow us to perform our analyses 100% faster with significantly improved quantification accuracy. Instead of 36 hours to perform a typical proteome-wide analysis, we can accomplish it now in 18 hours to reach between 8,000 and 10,000 quantified proteins in as many as 16 samples. This new mass spectrometer acts as if it were two instruments, collecting more accurate data in half the time, but at the same or even better depth."

Steven P. Gygi, Professor, Harvard University, MA

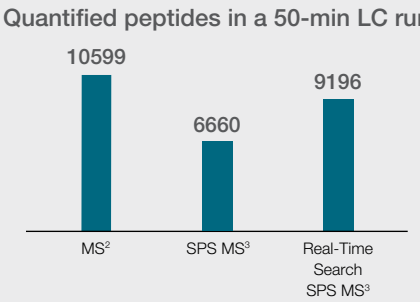
SPS MS^3 with Real-Time Search is the most accurate method for quantitative analysis of up to 16 samples simultaneously

TMTpro reporter ions



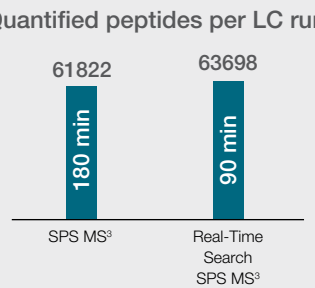
Selecting only identified fragments for the MS^3 event and excluding the ions that either do not carry the TMT tags (y-ions in this case) or that do not belong to the identified peptide increases the signal-to-noise ratio of the reporter ions and significantly improves the throughput and quantitative accuracy of the experiment (right).

Increased coverage



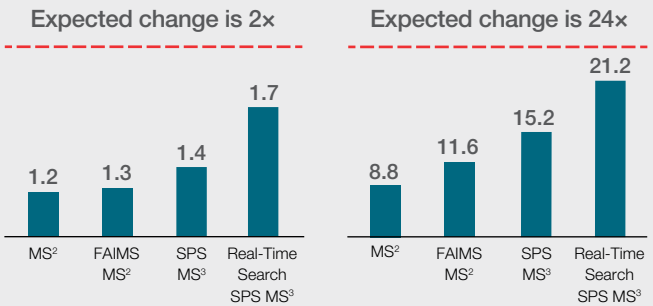
With Real-Time Search, 38% more peptides and 53% more proteins were quantified versus a classic SPS MS^3 experiment, approaching the results of the classic MS^2 experiment, but with much higher precision and accuracy (see bottom panel). Shown here are data for a TKO yeast standard (Thermo Fisher Scientific).

Increased throughput



Real-Time Search directs the mass spectrometer to perform MS^3 only on identified precursors. As a result, it doubles the throughput of SPS MS^3 experiments. Shown here are data for an HHM sample: three human cell lines labeled as biological replicates in 10plex (3-3-4). *(Data courtesy Devin Schweppe and Qing Yu, Harvard Medical School)*

Increased accuracy



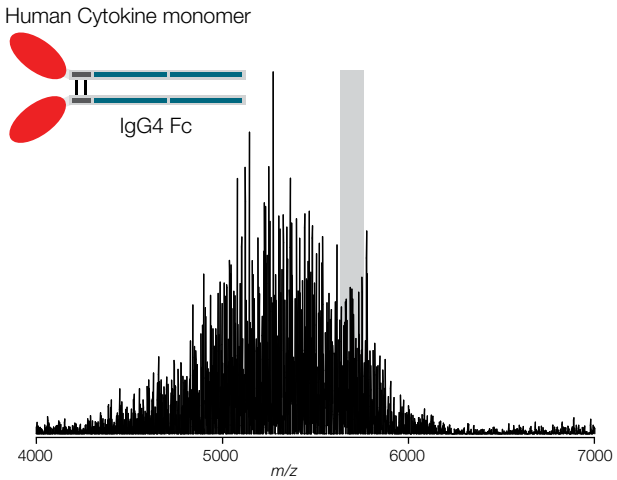
For each TMT channel, 4–100 pmol of a six-protein digest was added to 40 µg of HeLa digest, and then labeled, resulting in a spiked-in standard mixed in ratios of 2–24 (2, 4, 8, 12, 16 and 24). Only combined averages for the expected ratios of 2 and 24 are shown here. Higher accuracy afforded by Real-Time Search is indispensable for teasing out subtle differences in protein abundances, including when sub-classifying individual cells (pp 6–7).

Comprehensive native analysis of therapeutic proteins

Therapeutic proteins are successfully used to treat various cancers and a wide range of autoimmune diseases. However, their structural characterization presents a significant challenge because, unlike small molecule-based drugs, they exist as a heterogeneous mixture containing numerous modified proteoforms. Their highly complex ESI spectra can be simplified and made interpretable by increasing analyte *m/z*. This can be achieved by performing the ESI LC/MS experiment under native conditions where unfolded proteins accept fewer charges, or by performing precursor ion charge reduction in the mass spectrometer, or by both. For complete structural characterization, the mass spectrometer is required to not only detect ions within a higher *m/z* range, but to also fragment the selected precursor efficiently. The Orbitrap Eclipse Tribrid mass spectrometer is equipped with High Mass Range MS^n capability (HMR^n), Proton Transfer Charge Reduction (PTCR) and a multitude of dissociation techniques, including CID, HCD, ETD, EThcD and UVPD, making it the most powerful system available for comprehensive characterization of therapeutic proteins.

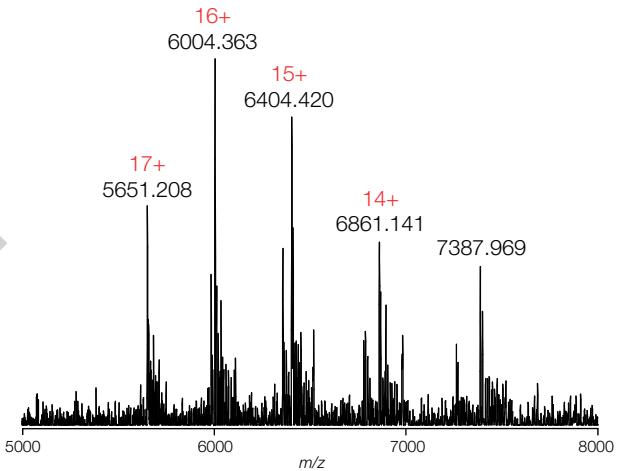
Simplifying highly complex spectra with PTCR

Proteoforms of native Cytokine Fc-fusion proteins are undecipherable



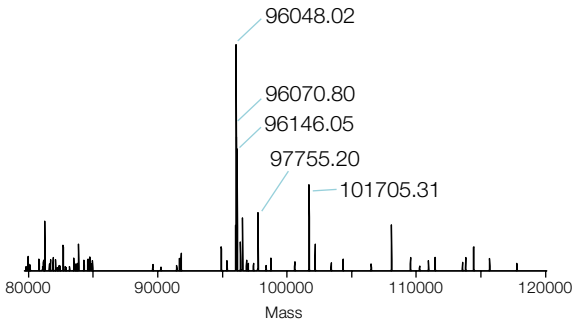
Native MS spectrum of intact desialyated Cytokine Fc-fusion protein after sialidase treatment with six N-linked glycosylation sites. The raw spectrum was highly complex due to the presence of multiple overlapping glycosylated proteoforms, making it challenging to interpret. The highlighted 80 *m/z* window represents the precursor ions that were isolated for subsequent PTCR.

PTCR elucidates the native proteoforms



PTCR dispersed the selected ion population to a higher *m/z* range, revealing individual molecular ion species at lower charge states. The interpretation of this spectrum provided accurate information about the mixture of different proteoforms, which were undecipherable in the original spectrum.

Multiple intact glycoforms are identified in deconvoluted PTCR spectrum

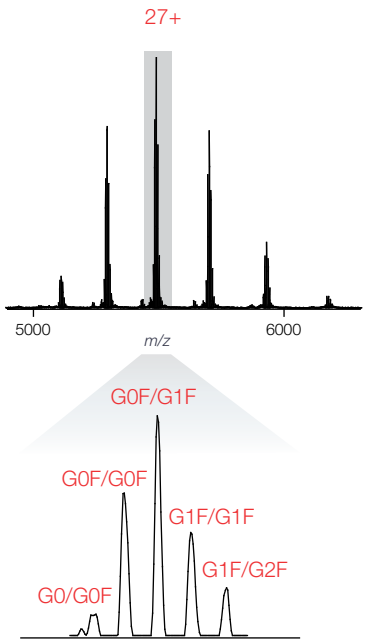


Average Mass	Relative Abundance	Charge State Distribution
96048.02	100.00	14–17
96070.80	58.35	14–17
96146.05	54.12	14–16
97755.20	25.85	13–16
101705.31	51.10	14–18
	• • •	

After deconvolution of the PTCR spectrum shown (ReSpect™ algorithm), several glycoforms of Cytokine Fc-fusion protein were identified. PTCR analysis of the entire native charge state envelope (*m/z* 4,000–7,000) resulted in the identification of >30 distinct glycoforms of this protein (data not shown).

Achieving high sequence coverage of CDR3 domain

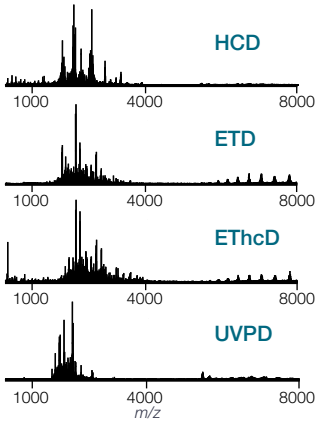
Native trastuzumab MS analysis



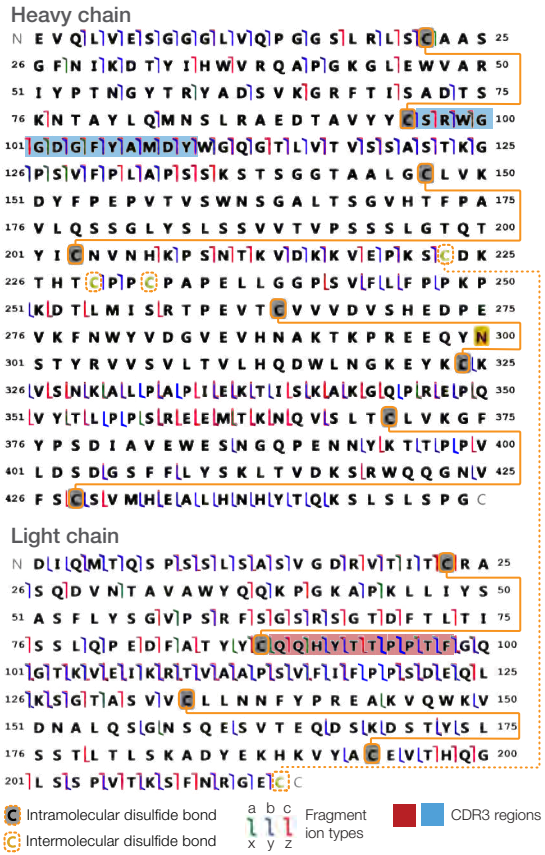
Glycoforms	Matched Mass Error (ppm)
G0/G0F	5.1
G0F/G0F	1.7
G0F/G1F	5.4
G1F/G1F	7.0
G1F/G2F	7.2

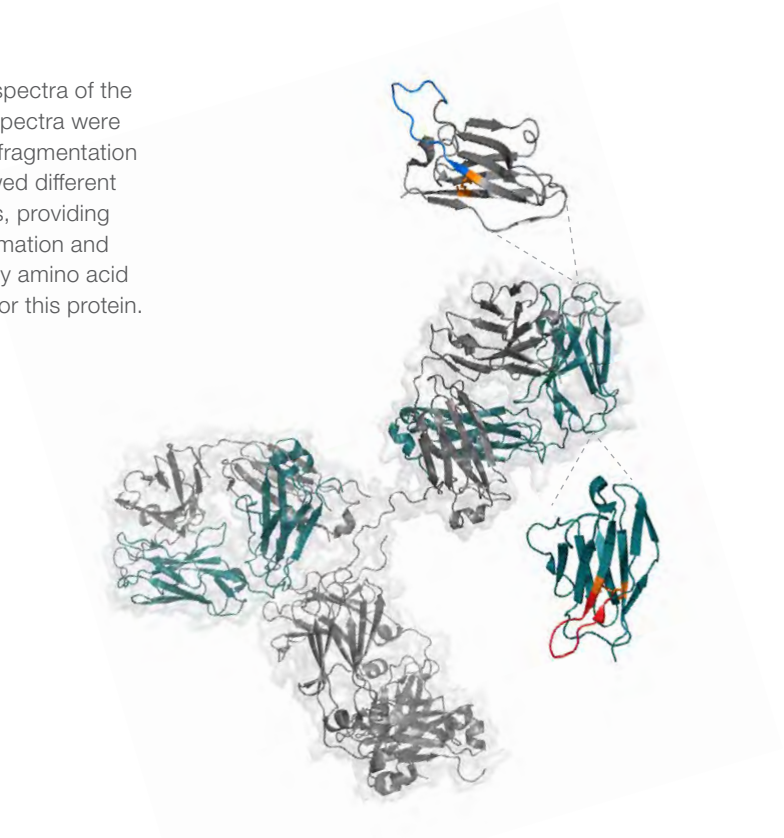
Native spectrum acquired using the HMR^n mode. The 27+ species showed baseline resolution of the major glycoforms.

Native trastuzumab MS^2 analysis for structural characterization of CDR3 regions



Representative MS^2 spectra of the 27+ parent ion. The spectra were obtained by multiple fragmentation techniques and showed different fragment ion patterns, providing complementary information and increasing the primary amino acid sequence coverage for this protein.



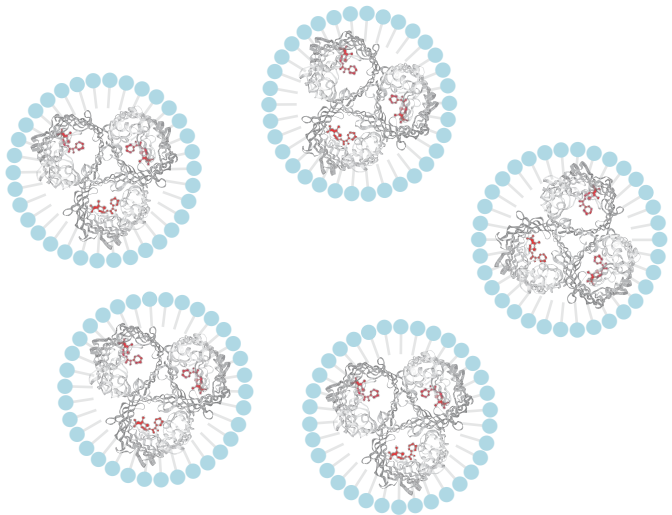


Combined fragmentation maps (left panel) of intact trastuzumab from the native HCD, ETD, EThcD and UVPD MS^2 data. Sequence coverage for light and heavy chains was 58% and 36% respectively, with combined coverage of 43% (fragment ion RMS error <3.7 ppm). Complementarity Determining Regions (CDR) 3 of the light and heavy chains are highlighted in red and blue respectively in the maps and in the crystal structure, showing 100% sequence coverage. Validation of CDR sequences is essential for studying binding affinity and efficacy of the antibody.

Characterization of native protein-ligand complexes using HMR^n

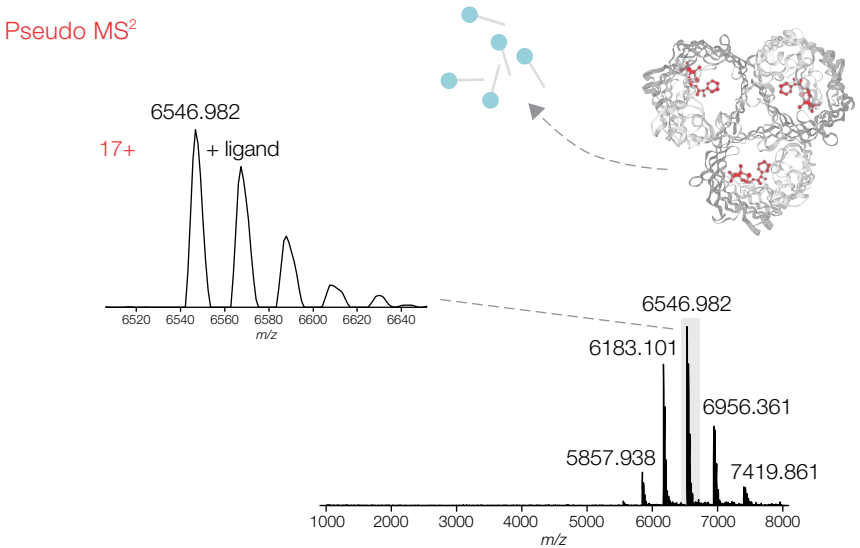
Ligands are inextricably linked to the regulation and function of the proteins to which they are bound. However, determining the identity of small-molecule ligands associated with membrane proteins is a significant challenge, underscored by the high prevalence of electron microscopy data with unassigned or poorly resolved ligand density. Direct MS identification of the ligands without losing ligand-protein complex associations is difficult because it requires a very wide mass range for retaining optimal ion transmission of intact protein assemblies of (hundreds of thousands of Daltons) and accurate detection of small-molecule ligands and their fragments (often smaller than 100 Daltons). The Orbitrap Eclipse Tribrid mass spectrometer offers a novel top-down MS^n approach to facilitate identification of ligands bound to membrane proteins, allowing elucidation of specific ligands interacting with specific proteins. This approach helps to elucidate a ligand's influence on the cascade of protein interactions that underlie cellular mechanisms, including mechanisms of cancer, diabetes, and Alzheimer's.

Intact protein-ligand assemblies in micelles are introduced directly into the mass spectrometer



Intact protein complexes are extracted from the membrane using detergent. Detergent molecules form a micelle layer around the protein complex that allows solubilization. The micelles are then ionized using nESI and introduced into the mass spectrometer.

Protein-ligand assemblies are released from the micelles



The intact membrane protein-ligand assembly is released from the detergent micelle using source-CID (pseudo MS^2) and detected in the Orbitrap mass analyzer. The 17+ parent ion with associated ligands is isolated (gray bar) for the next MS^n step.

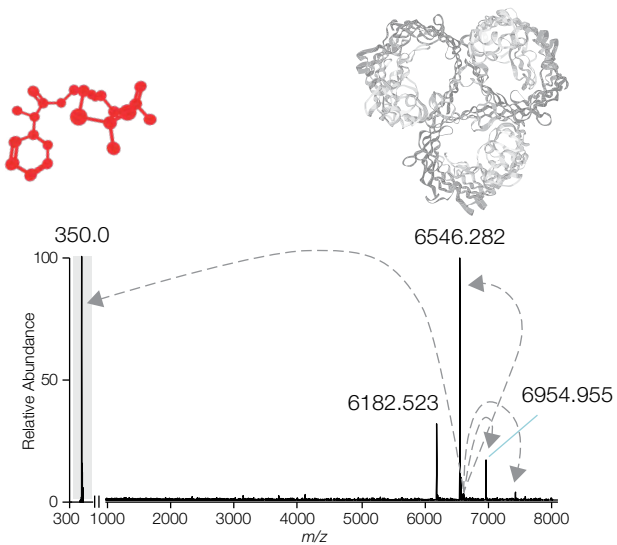
* Gault *et al.*, Nature Methods, in press

The Orbitrap Eclipse Tribrid mass spectrometer brings a new dimension to native MS, enabling us to discover and chemically define the ligands, lipids, and drugs that regulate the function of membrane protein assemblies within one single experiment."

Dr. Joseph Gault, University of Oxford, the UK

The ligand is released from the protein

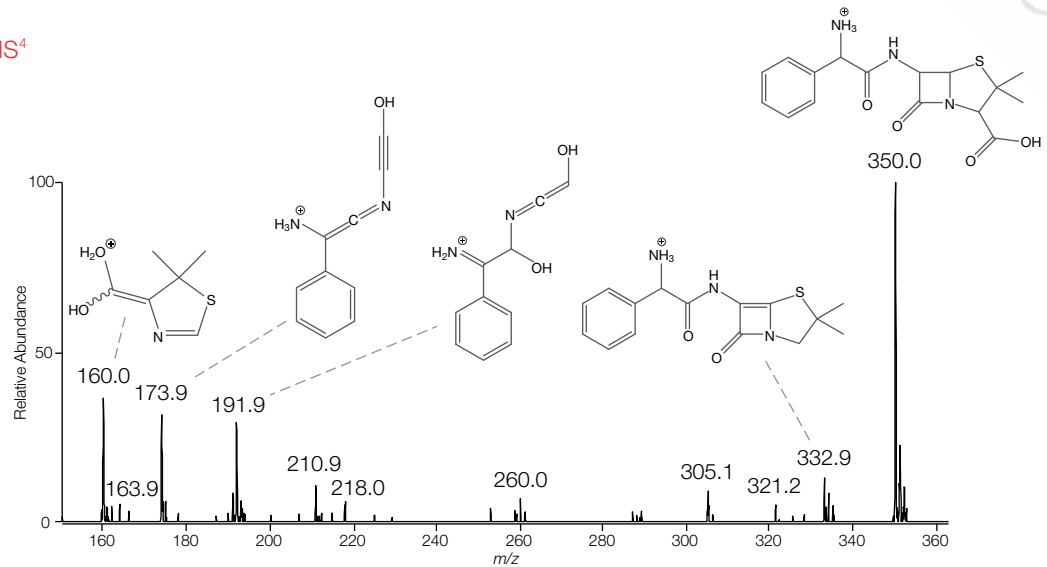
MS^3



MS^3-based isolation and activation of the intact membrane protein-ligand assembly promote the release of the ligand (*m/z* 350.0). This singly-charged ion is isolated (gray bar) for MS^4 step to allow full structural characterization. (Note: in this experiment the ligand at *m/z* 350.0 was detected in the ion trap. Both the Orbitrap and the linear ion trap mass analyzers can be used to detect ions across the entire *m/z* range).

The endogenous ligand is unambiguously identified

MS^4



The MS^4 spectrum contains ligand fragments detected in the ion trap. Unambiguous identification of the endogenous ligand bound to the protein is performed using established small-molecule characterization techniques. Here, ampicillin was identified bound to *E. coli* Outer Membrane Porin F (OmpF), forming a 111 kDa protein-ligand complex that mediates its intake. Further characterization of the protein's primary structure is also possible using this novel top-down MS^n approach.

Reducing protein mass spectral complexity with PTCR

Top-down mass spectrometry is used to directly characterize intact proteoforms. The ESI spectral complexity of proteoform mixtures, even after LC separation, is often very high due to the multitude of modified forms that overlap in the *m/z* domain. PTCR technology, unique to the Orbitrap Eclipse Tribrid mass spectrometer, reduces the average charge of the parent-ion distributions, shifting them to higher *m/z*. For overlapping indistinguishable proteoforms this reduces the signal overlap, and, as a result, easier-to-interpret spectra are obtained, enabling the proteoform mass calculation. For automated top-down experiments, this approach increases the number of distinguishable precursors available for data-dependent MS^n, ultimately increasing the number of characterized proteoforms.

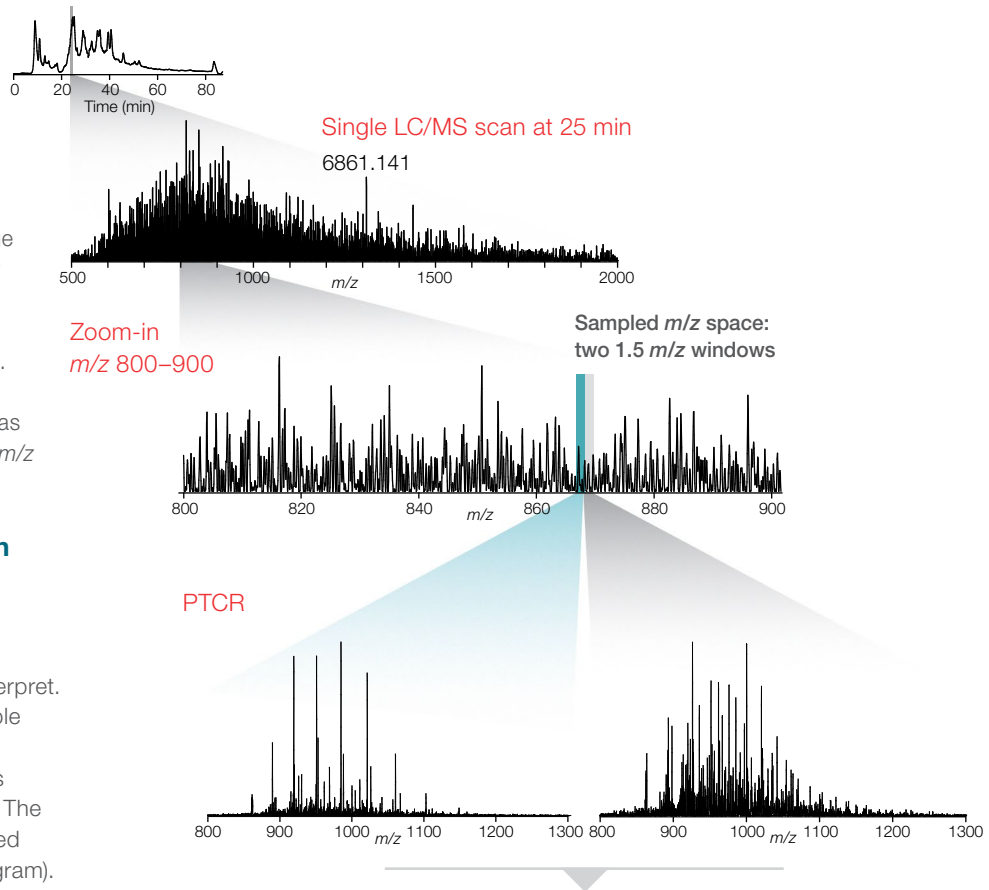
> *PTCR is required to study large proteoforms that previously would remain uncharacterized in LC/MS experiments. Unveiling the true complexity of the intact proteome beyond the 30 kDa barrier represents a substantial step forward for the proteomics community."*
>
> **Luca Fornelli**, Professor, University of Oklahoma, OK

MS spectra of intact proteins are often too complex

The co-elution of multiple proteoforms increases spectral complexity, particularly when analyzing proteins >25 kDa. These spectra are often impossible to interpret fully as few charge state distributions are distinguishable by deconvolution software (e.g., only two distinct masses of 16 and 25 kDa were identified in this MS spectrum at 25 min). The 800–900 *m/z* region shows the underlying spectral complexity. PTCR was performed on the precursors in two 1.5 *m/z* windows, highlighted in gray and teal.

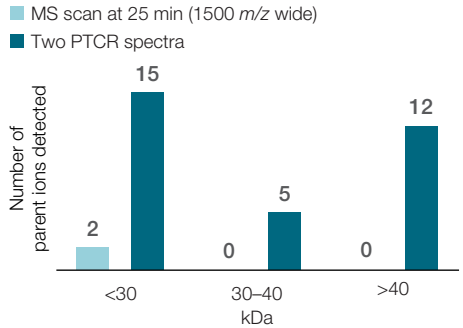
PTCR elucidates novel protein charge state distributions

PTCR formed lower charge state distributions of the parent ions that overlapped less and were easier to interpret. This increased the number of identifiable precursor ions from two species in the original full MS spectrum to 32 species identified after PTCR of a 3 *m/z* range. The newly identified masses were distributed over 10–70 kDa range (right, top histogram).

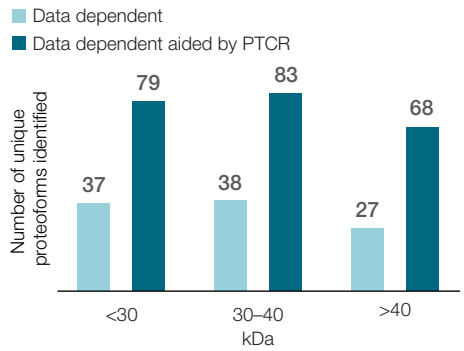


ReSpect deconvolution identified 32 species from these two spectra

PTCR enabled detection of more and larger proteoforms



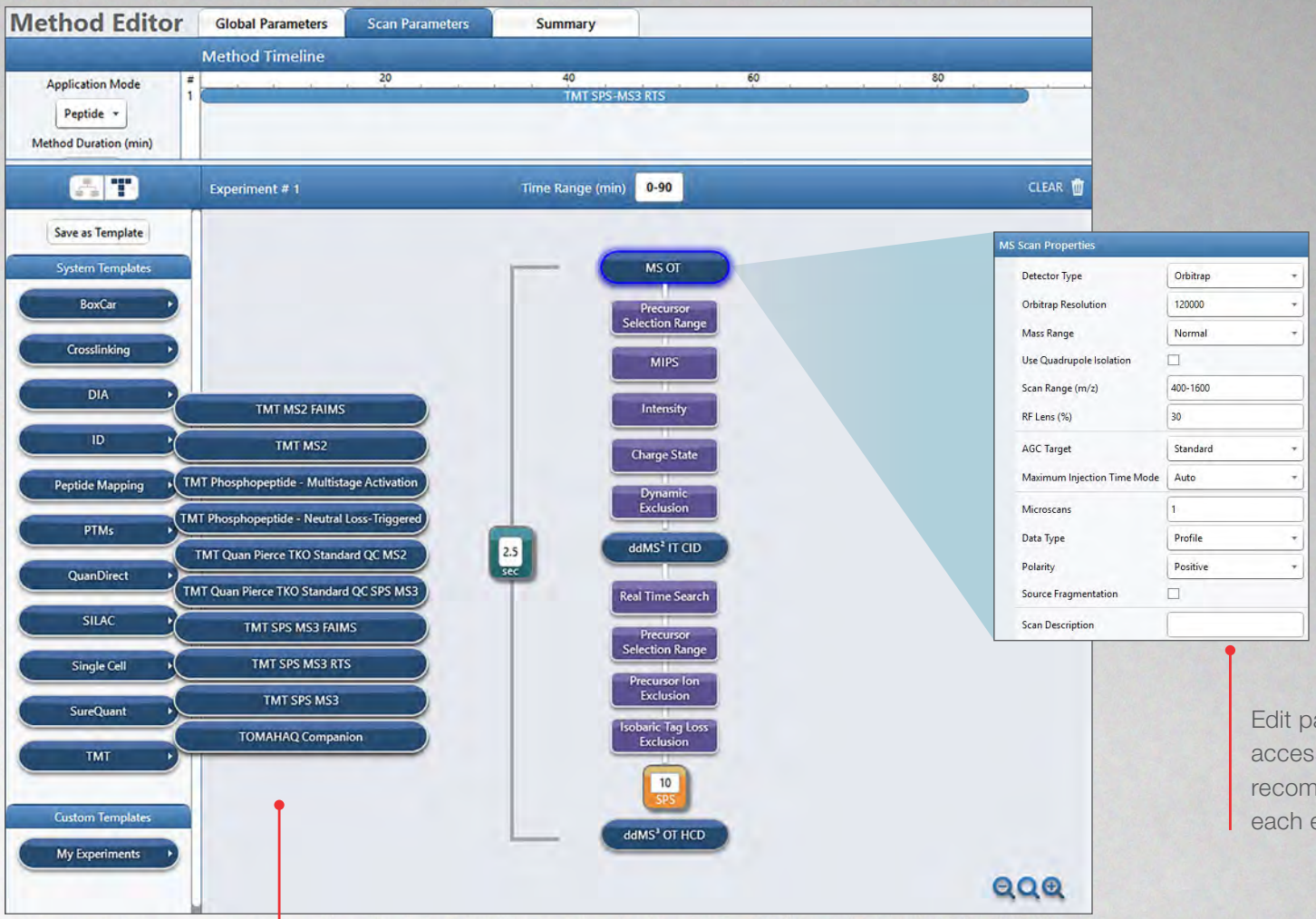
PTCR resulted in a significant increase in the number of identified proteoforms*



* Huguet, R. *et al. Anal. Chem.* **2019**, *91*(24).

Application-specific methods are one click away

The intuitive method editor features a drag-and-drop user-friendly interface with predefined, optimized method templates for a wide range of applications.



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