# Scale up

Thermo Fisher SCIENTIFIC

Orbitrap Ascend Editions Tribrid mass spectrometer

thermo scientific

## Overcome the challenges in life sciences mass spectrometry

The Orbitrap Ascend Editions Tribrid mass spectrometers offer extraordinary sensitivity and versatility

With improved capabilities for multiplexed quantitative proteomics, post-translational modification (PTM) analysis, native protein characterization and metabolomics, the Thermo Scientific<sup>™</sup> Orbitrap<sup>™</sup> Ascend Editions Tribrid<sup>™</sup> mass spectrometers are among the most powerful, flexible platforms available. The MultiOmics, Structural Biology and BioPharma editions of the Orbitrap Ascend Tribrid mass spectrometer quantify more samples at lower concentrations, achieve greater coverage, analyze the toughest native complexes and characterize the largest biopharmaceuticals on a single platform—all with less instrument setup to optimize ease-of-use.



The Orbitrap Ascend Editions Tribrid mass spectrometer equipped with the Thermo Scientific<sup>™</sup> FAIMS Pro<sup>™</sup> Interface and the Thermo Scientific<sup>™</sup> Vanquish<sup>™</sup> Neo UHPLC system.

## Innovative technology addresses your research needs

The Orbitrap Ascend Editions Tribrid mass spectrometers feature innovations to scale up your analyses with throughput, versatility and ease-of-use.

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

Optional Thermo Scientific<sup>™</sup> EASY-IC/ETD and PTCR ion source Based on Townsend discharge, reliable and easy to use

#### Electrodynamic ion funnel\*

- Efficient ion transfer
- Broad tuning curves
- Optimized for labile compounds

Real-time database/ library search Database search/spectrallibrary-directed MS<sup>n</sup> acquisition

Back ion routing multipole Enables parallel analysis, performs HCD at MS<sup>3+</sup> stage

Front ion routing multipole\* Enables parallel analysis, performs HCD at MS<sup>2</sup> stage

Optional

Native MS mode\*

Enables isolation

in the quadrupole

up to *m/z* 8,000

MS<sup>2</sup> stage

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

precursor isolation widths

C L n s

**Optional UVPD** Unique fragmentation mode for analyte structure elucidation

### Modified dual-pressure linear ion trap mass analyzer

- Up to 50 Hz MS<sup>n</sup> and sensitive mass analysis
- Six fragmentation types: CID, HCD, ETD, EThcD, ETciD and UVPD

**Optional Native MS mode\*** 

Enables isolation up to m/z 8,000

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

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

#### 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

High-capacity ion transfer tube Increased ion flux

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

\*New on this platform

## Identify native membrane proteins using PTCR and top-down techniques

With the Thermo Scientific<sup>™</sup> Orbitrap<sup>™</sup> Ascend Structural Biology Tribrid<sup>™</sup> 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, Proton Transfer Charge Reduction (PTCR) simplifies the spectra produced from complex samples. For top-down analysis, very difficultto-break proteins can be fragmented using multiple fragmentation options, generating enough sequence coverage for high-confidence identification of proteins within complex samples.

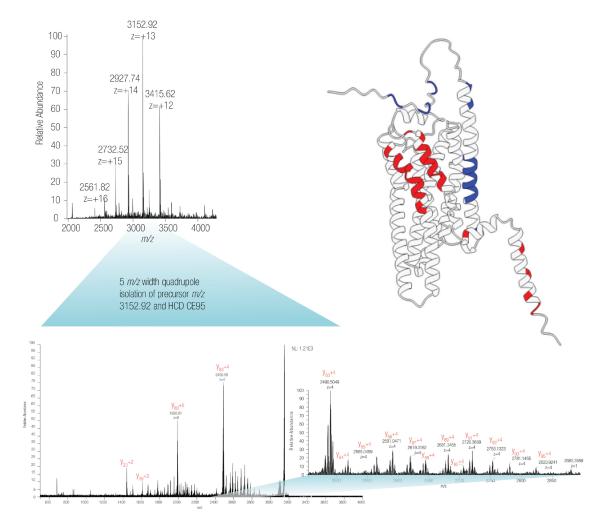


Native

"I'm delighted that we now have an Orbitrap Ascend Structural Biology edition, because this Tribrid mass spectrometer has transformed our ability to study complicated systems."

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

### Fragments mapped onto 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.

## Comprehensively analyze therapeutic proteins with Native MS and PTCR

### Biopharma

Protein therapeutics often generate undecipherable spectra due to their high levels of intrinsic heterogeneity. By using the Thermo Scientific<sup>™</sup> Orbitrap<sup>™</sup> Ascend BioPharma Tribrid<sup>™</sup> mass spectrometer's recommended Native MS and PTCR options, interpretable spectra can be generated from heterogeneous protein samples. The Native MS option extends the quadrupole isolation range to *m/z* 2,000–8,000, which can be used in combination with narrow data-independent acquisition (DIA) windows to simplify the ion population in each scan. The Native MS option also extends Orbitrap analyzer detection to *m/z* 16,000. These capabilities enable unambiguous identification of previously indiscernible or low-abundance proteoforms. PTCR generates perfluoroperhydrophenanthrene (PFPP) ions for subsequent gas-phase, ion-ion reactions that produce lower charge state distributions to enhance protein characterization.

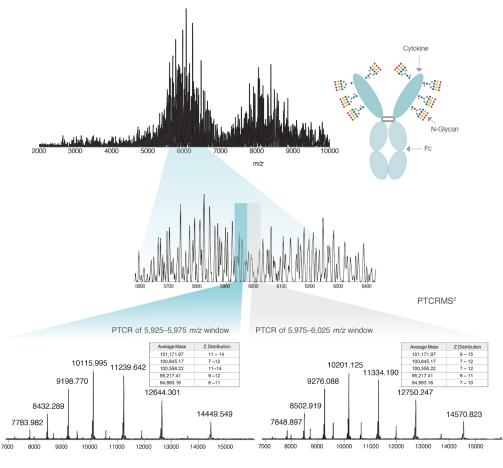
The flexibility of the Orbitrap Ascend BioPharma Tribrid mass spectrometer enables scientists to carry out unique experiments that combine the recommended Native MS option with PTCR, as well as other dissociation techniques, making it a powerful instrument for comprehensive characterization of therapeutic proteins in their native state.



"The m/z 16,000 range and large ion transmission capabilities of the Orbitrap Ascend Tribrid mass spectrometer enable full use of PTCR charge reduction for complete characterization of even the most complicated molecules."

Wendy Sandoval Distinguished Scientist





Data courtesy of Wendy Sandoval.

## TMT

# Quantify more low-level proteins with increased confidence and sample throughput using multiplexed quantitative proteomics

### Scale up proteomics productivity

Increasing quantitative coverage of low-abundance proteins is essential to detect biologically important molecules, understand biological systems and substantiate dataset claims. Improving experimental throughput is also desirable because it can proportionally reduce experimental costs. Compared to label-free quantitation (LFQ) methods, multiplexing is a powerful way to increase sample throughput.

With the Thermo Scientific<sup>™</sup> Orbitrap<sup>™</sup> Ascend MultiOmics Tribrid<sup>™</sup> mass spectrometer's fast scanning power and Thermo Scientific<sup>™</sup> Tandem Mass Tag (TMT<sup>™</sup>) multiplexing reagents, you obtain the high protein coverage you expect while analyzing about 30% fewer fractions and substantially increasing experimental productivity.

### Access the power of SPS mass spectrometry methods with Real-Time Search

The Synchronous Precursor Selection (SPS) MS<sup>3</sup> methods with Real-Time Search for TMT experiments provide unprecedented quantitative accuracy while maximizing the number of peptide identifications. The SPS MS<sup>3</sup> acquisition improves the quantitative ratio accuracy of TMT reporter ions, thereby increasing the number of peptides correctly quantified. The Real-Time Search adds speed and further improves quantification accuracy because the MS<sup>3</sup> scans are only triggered when a peptide-spectrum match (PSM) occurs from the preceding MS<sup>2</sup> scan.

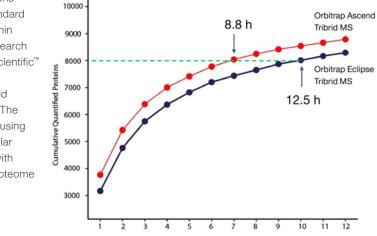


"We need to continue reducing the LC-MS acquisition time even further with a better depth of analysis of smaller numbers of fractionated biological samples. Orbitrap Ascend Tribrid mass spectrometer enables this solution."

Steven P. Gygi, PhD Professor of Cell Biology Harvard Medical School

### Identify more proteins per fraction and save time

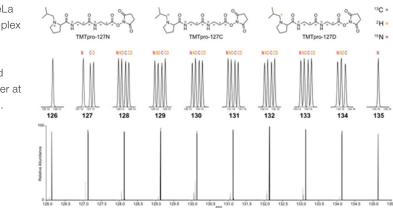
Twelve high-pH fractions of 1 µg of HYPER standard analyzed using a 65-min SPS MS<sup>3</sup> Real-Time Search method on Thermo Scientific<sup>™</sup> Orbitrap Eclipse<sup>™</sup> and Orbitrap Ascend Tribrid mass spectrometers. The data were processed using Harvard pipeline. Similar results are obtained with Thermo Scientific<sup>™</sup> Proteome Discoverer<sup>™</sup> software.



Sample courtesy of Professor Steven Gygi, Harvard Medical School.

### Multiplex analysis of more samples with next-generation TMT reagents

Analysis of 1:1 HeLa using TMTpro 32-plex reagents with the Orbitrap Ascend MultiOmics Tribrid mass spectrometer at 90,000 resolution.



Sample courtesy of Steven Shuken, Qing Yu and Professor Steven Gygi, Harvard Medical School.

## Increase phosphopeptide and glycopeptide IDs and site localization

#### Scale up PTM identification and localization

The Orbitrap Ascend MultiOmics Tribrid mass spectrometer increases confidence in results obtained from analysis of labile PTMs thanks to the availability of alternative fragmentation techniques such as electron transfer dissociation (ETD). ETD is better suited for labile PTMs such as glycopeptide because of their non-ergodic type of dissociation. ETD produces extensive fragmentation of the peptide backbone, enabling sequencing of the peptide while preserving glycans on the peptide backbone. This allows for unambiguous assignment of the glycosylation sites. ETD can be used in parallel with high-energy collisional dissociation (HCD) fragmentation, which provides information about glycan composition for thorough characterization of the glycopeptide structure. The availability of electron transfer/higher energy collision dissociation (EThcD) fragmentation can further increase identification and characterization confidence of labile PTMs. The electrodynamic ion funnel improves the capture and transmission of labile PTMs. The front ion routing multipole increases the speed of complex data acquisition—such as that encountered during O-linked glycopeptides EThcD analysis—increasing the number of MS<sup>2</sup> scans by almost 50%.



"Heterogeneity of protein glycosylation creates different analytical demands. Flexibility is key, making the Orbitrap Ascend MultiOmics Tribrid mass spectrometer particularly valuable for glycopeptide analysis. Its architecture allows manipulation of multiple ion populations simultaneously. This lets us accumulate more ions without slowing acquisition of MS/MS scans, or even speeds it up depending on the method design. This translates to more, and higher quality, MS/MS spectra that ultimately improve our glycopeptide characterization."

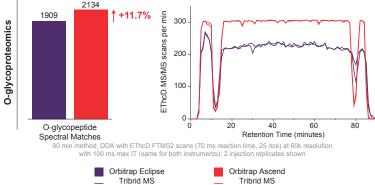
Nick Riley, PhD Assistant Professor of Chemistry University of Washington

10250 10250 10250 Phosphopeptides • Orbitrap Eclipse Tribrid MS • Orbitrap Ascend Tribrid MS

Phosphopeptide analysis of 0.5  $\mu$ g sample load in a 90-min gradient on an ES903 column using the Orbitrap Eclipse or Orbitrap Ascend MultiOmics Tribrid mass spectrometer at 15,000 resolution at *m/z* 200 and 27 ms maximum injection time.

Data courtesy of Yuchen He, Professor Joshua Coon's lab, University of Wisconsin–Madison.

#### Improve phosphopeptide and glycopeptide analysis



PTMs

Analysis of semi-complex mixtures of recombinant/purified glycoproteins in a 90-min gradient with Orbitrap Ascend MultiOmics Tribrid mass spectrometer using DDA, EThcD FTMS<sup>2</sup> scans (70 msec reaction time 25 nce, 60,000 resolution at *m/z* 200 and 100 ms maximum injection time).

Data courtesy of Professor Nick Riley, University of Washington.

### **Metabolomics**

## Get single-injection insight with the Simultaneous Quantitation and Discovery (SQUAD) workflow

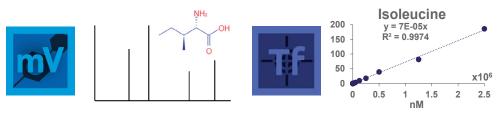
### Scale up metabolomics productivity

To increase productivity and accelerate time-to-insight, metabolomics researchers are looking to perform untargeted discovery profiling and targeted quantitation in a single analysis on one MS platform. This is also essential when dealing with limited sample amounts. SQUAD analysis combines the strengths of both methods. It quantifies a predefined set of metabolites, like targeted analysis, but also confidently annotates unknown metabolites like untargeted analysis. Thus, it enhances the understanding of molecular relationships within biological systems. The increased parallelization provided by the dual ion routing multipoles allows the Orbitrap Ascend MultiOmics Tribrid mass spectrometer to acquire more scans for both discovery and targeted quantitation when using the SQUAD workflow. The approach enables metabolomics researchers to save instrument time and samples without compromising the quality of their results. Compared to the previous Orbitrap Tribrid architecture, the new system measures 55% and 25% more MS and MS<sup>2</sup> ions, respectively.

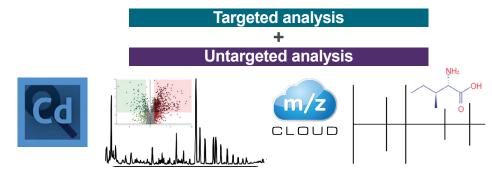


"The idea of combining true targeted MS/MS while screening for nontargeted compounds is fascinating. It not only extends the sensitivity and scope for metabolomics and exposome research, but it also opens the door for better clinical studies. The Orbitrap Ascend MultiOmics Tribrid mass spectrometer release is very timely and just what we need today."

Oliver Fiehn, PhD Director West Coast Metabolomics Center UC Davis



LOQ of 5 femtomole with 6 orders of magnitude linear dynamic range



Analysis of isoleucine using the SQUAD workflow on the Orbitrap Ascend MultiOmics Tribrid mass spectrometer. The single-injection workflow combines untargeted data analysis and interpretation using Thermo Scientific<sup>™</sup> Compound Discoverer<sup>™</sup> software with the mzCloud<sup>™</sup> advanced mass spectral database for feature extraction, differential analysis and annotation. Targeted quantification uses Thermo Scientific<sup>™</sup> TraceFinder<sup>™</sup> software. The mzVault<sup>™</sup> application can be used for offline searching of mzCloud MS<sup>2</sup>-level spectral data in either Compound Discoverer or TraceFinder software. Compared to similarly run sample and experiment on the Thermo Scientific<sup>™</sup> Orbitrap<sup>™</sup> IQ-X Tribrid<sup>™</sup> mass spectrometer.

### 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

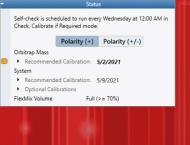
#### Robust delivery system



**Dedicated emitter** 



### Automated weekly calibrations





### **Resources and support**



### Services Central—All your service information at your fingertips

Spend less time searching for support and more time focusing on your important work. This online platform has what you need to easily manage your instruments and equipment.

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