## Scale up your multiomics research

. **~~**\_\_\_\_

Orbitrap Ascend MultiOmics Tribrid mass spectrometer

thermo scientific

**Thermo Fisher** 

SCIENTLELC

## Scale up multiomics insight

## Be prepared for tomorrow's most challenging demands

Built to meet the demands of multiomics research, the Thermo Scientific<sup>™</sup> Orbitrap<sup>™</sup> Ascend MultiOmics Tribrid<sup>™</sup> mass spectrometer incorporates innovations to scale up multiplexed quantitative proteomics, targeted and untargeted metabolomics, lipidomics, glycoproteomics and glycomics experiments. Quantify more samples at lower concentrations with intelligent acquisition and Real-Time Search. Achieve greater coverage using a novel design featuring two ion routing multipoles that parallelize analyses. With these capabilities and more, you access unprecedented experimental throughput, versatility and usability to meet tomorrow's research challenges while making groundbreaking discoveries today.



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

## Innovative technology addresses your multiomics needs

**Optional Native** 

Enables isolation

in the quadrupole

up to *m/z* 8,000

MS mode\*

The Orbitrap Ascend MultiOmics Tribrid mass spectrometer features innovations to scale up your multiomics analyses with throughput, versatility and ease-of-use.

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

**Recommended:** Thermo Scientific<sup>™</sup> **EASY-IC/ETD** and optional 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

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

precursor isolation widths



**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

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

\*New on this platform

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

#### Scale up proteomics productivity

TMT

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 Orbitrap Ascend MultiOmics Tribrid 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.

#### 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 Thermo Scientific<sup>™</sup> Orbitrap<sup>™</sup> Ascend Tribrid<sup>™</sup> 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 Thermo Scientific<sup>™</sup> TMTpro<sup>™</sup> 32-plex reagents using the Orbitrap Ascend MultiOmics Tribrid mass spectrometer at 90,000 resolution.

# Experience unprecedented single-platform versatility for low and high sample loads of peptides using DIA methods

#### Scale up proteomics accuracy across sample loads

LFQ methods using data independent acquisition (DIA) significantly increase proteome coverage and reduce missing values by acquiring MS<sup>2</sup> data from all detected precursor ions. The approach also makes retrospective data analysis possible. For LFQ DIA experiments, the Orbitrap Ascend MultiOmics Tribrid mass spectrometer delivers coefficients of variance (CVs) of less than 10%, providing exceptional quantitative accuracy. On the Orbitrap Ascend MultiOmics Tribrid mass spectrometer, without any hardware changes, picograms to micrograms can be analyzed on a column. By selecting the appropriate method-specific template in the software, researchers can move from single-cell sensitivity to higher load identifications with ease.

#### Analyze a wide range of sample loads using the same instrument setup

LFQ DIA analysis of HeLa using the same Orbitrap Ascend MultiOmics Tribrid mass spectrometer setup with the FAIMS Pro Duo Interface Vanquish Neo UHPLC system with a Thermo Scientific<sup>™</sup> µPAC<sup>™</sup> Neo HPLC column. Protein groups were accurately identified and quantified from on-column sample loads ranging from 50 pg to 500 ng and beyond.





## PTMs

## 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



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. 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).

Tribrid MS

Data courtesy of Professor Nick Riley, University of Washington.

Tribrid MS

Improve phosphopeptide and glycopeptide analysis



## Double the number of lipid identifications

## Lipidomics

#### Scale up lipidomics experiments with AqcuireX software and alternative fragmentation capabilities

In lipidomics experiments, HCD and collision-induced dissociation (CID) of the Orbitrap Ascend MultiOmics Tribrid mass spectrometer can produce complementary fragments. The differences in fragmentations can be utilized to yield comprehensive lipid annotations. Diagnostic fragments of lipid fatty acid side chains using CID can be generated while lipid class-characteristic backbone fragments using HCD are produced, yielding comprehensive lipid molecular species characterization. At the same time, optional ultraviolet photodissociation (UVPD) can generate unique side-chain fragments to locate fatty acid double bonds.

In lipidomics experiments, traditional data-dependent acquisition (DDA) can miss low abundant precursors. Thermo Scientific<sup>™</sup> AcquireX<sup>™</sup> software solves this issue through fully automated iterative injections, where an initial survey scan will input all viable precursors on an inclusion list for automatic MS/MS acquisition. In subsequent injections, the mass spectrometer goes through the inclusion list until all precursors are selected at least once for MS<sup>2</sup>. Using this method, the number of lipid identifications can be doubled in the same amount of time.



Pooled lipid sample was injected on a Thermo Scientific<sup>™</sup> Accucore<sup>™</sup> C30 column (2.1 × 150 mm, 2.6 µm) using a Thermo Scientific<sup>™</sup> Vanquish<sup>™</sup> Horizon UHPLC system (flow rate of 260 µL/min). The AcquireX lipid characterization HCD-CID- MS<sup>3</sup> built-in template was used in the positive mode. Pooled samples were also analyzed by both HCD and CID using AcquireX software in negative mode. All data was searched using Thermo Scientific<sup>™</sup> LipidSearch<sup>™</sup> 5.1 software.

## **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



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





### Thermo Fisher

### **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.

Learn more at thermofisher.com/servicescentral



#### Technical and online support

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.

Learn more at thermofisher.com/technicalresources



#### Protect your investments with expert lab services

Unity<sup>™</sup> 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 help ensure performance throughout your laboratory.

Learn more at unitylabservices.com

### More resources

Thermo Scientific<sup>™</sup> Ardia<sup>™</sup> Platform thermofisher.com/ArdiaProteomics

Thermo Scientific<sup>™</sup> BioPharma Finder<sup>™</sup> software thermofisher.com/biopharmafinder

Thermo Scientific Compound Discoverer software thermofisher.com/CompoundDiscoverer

mzCloud mass spectral library thermofisher.com/mzCloud

Thermo Scientific Proteome Discoverer software thermofisher.com/ProteomeDiscoverer

#### Learn more at thermofisher.com/OrbitrapAscendMultiOmics

**General Laboratory Equipment – Not For Diagnostic Procedures.** © 2024 Thermo Fisher Scientific Inc. All rights reserved. TMT and TMTpro are trademarks of Proteome Sciences plc. All other trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details. 24-085-1055 | BR002864-EN 0524C

### thermo scientific