



Mass spectrometry

## Grant application resource

### Scale up your science with the Orbitrap Ascend Tribrid mass spectrometer

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#### Summary

Scale up your science. With new capabilities for multiplexed quantitative proteomics and native protein characterization, the Thermo Scientific™ Orbitrap™ Ascend Tribrid™ mass spectrometer delivers ultimate experimental throughput, versatility, and ease-of-use. This instrument enables end users to quantify more samples at lower concentrations using faster acquisition, while achieving greater coverage using a revolutionary new hardware design featuring dual ion routing multipoles.

The information provided in this document highlights the technologies where the Orbitrap Ascend Tribrid mass spectrometer surpasses other mass spectrometry instrumentation, specifically outlining:

1. How softer ionization and faster, smarter acquisition improves the analysis of post-translational modifications (PTMs) including phosphorylation.
2. How to quantify more samples at lower concentrations with better accuracy and precision.
3. How an optional mass range up to  $m/z$  16,000 enables the characterization of the largest biopharmaceuticals and new modalities.
4. All while producing more biological data with less instrument setup by utilizing the automated Auto-Ready ion source for calibration, and pre-built, optimized method templates to maximize convenience and ease-of-use.

## What's new

From single cell sensitivity to analyzing proteins up to 800 kDa in size, the Orbitrap Ascend Tribrid MS produces high quality data with ease, enabling you to tackle challenging analytes required to scale up your science. With its built-in versatility, leading performance, and MS<sup>n</sup> capabilities, collectively, these features make this instrument uniquely suited for high throughput, single cell proteomics, characterization of small molecule-based pharmaceuticals, and deciphering higher-order native protein structures, all on a single platform.

Here we highlight innovative hardware and software enhancements that enable unprecedented sensitivity, increased sample throughput, and improved efficiency of the Orbitrap Ascend Tribrid mass spectrometer. Adding the new features and options shown in Figures 1 and 2 enable the Orbitrap Ascend Tribrid mass spectrometers to surpass previous-generation Tribrid instrumentation and deliver a new level of experimental flexibility and performance for the wide range of applications.

Innovative hardware improvements that provide expanded experimental capabilities include:

- Ultra-high-field Orbitrap mass analyzer provides up to 45 Hz MS/MS acquisition rates with 7500 resolution at  $m/z$  200
- A next-generation dual pressure linear ion trap mass analyzer with up to 50 Hz MS/MS acquisition rates
- Enhanced ion optics and QR5 segmented quadrupole mass filter improve sensitivity at  $m/z$  0.4 precursor isolation and increase transmission at higher precursor isolation resolution

- Dual ion routing multipoles (IRMs) increases scan rates and sensitivity. Each IRM can be used to store, transfer, accumulate, or fragment ions obtained with higher energy collisional dissociation (HCD). Dual IRMs enable the simultaneous manipulation of up to three ion populations in parallel, increasing scan rate for methods like Tandem Mass Tags™ (TMT™) that require long ion injection times.
- New HMR<sup>n</sup>+ extended mass range up to  $m/z$  16000 for the analysis of macromolecules and new modalities with improved detection and sensitivity
- Auto-Ready ion source for calibration enables remote and automated system calibrations without distribution to the LC-MS set-up or changing ion sources, so end users can spend more time on results, less time on instrument set-up
- Extended high-pressure cell in the linear ion trap enables multiple fragmentation types and improved kinetic control of ETD and PTCR reactions
- Thermo Scientific™ EASY-IC™/ETD (internal calibration/electron transfer dissociation) ion source with Proton Transfer Charge Reduction (PTCR) capability
- Thermo Scientific™ FAIMS Pro Duo interface option for online gas-phase fractionation prior to MS analysis provides orthogonal selectivity
- Enhanced Orbitrap Tribrid Instrument Control software (ICSW) delivers additional productivity, ease-of-use, and flexibility with application modes and pre-build method templates



**Figure 1. Orbitrap Ascend Tribrid mass spectrometer with the Thermo Scientific™ Vanquish™ Neo UHPLC system, FAIMS Pro Duo interface, and Thermo Scientific™ AcceleOme™ automated sample preparation platform. An end-to-end solution for proteomics when combined with Thermo Scientific™ Proteome Discoverer™ software.**

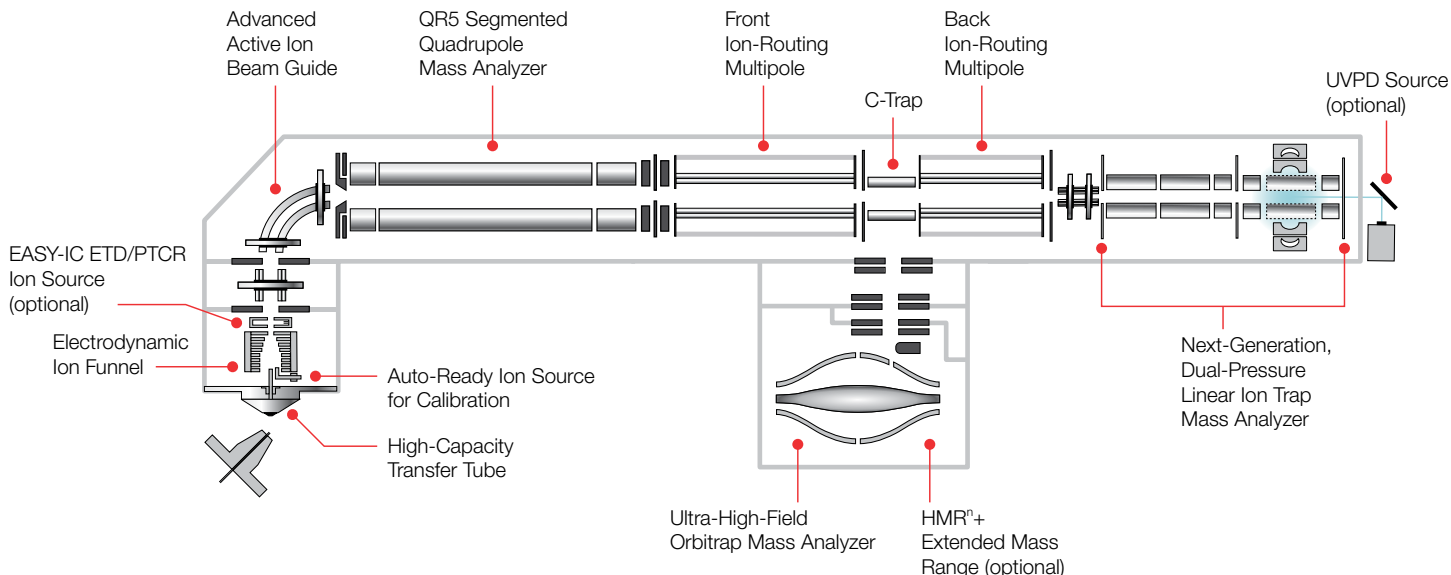


Figure 2. Orbitrap Ascend Tribrid mass spectrometer schematic showing key hardware innovations

### Intuitive user experience and Instrument Control software

The Orbitrap Ascend Tribrid Instrument Control Software (ICSW) provides ease-of-use, and flexibility for operators of all levels of expertise. Instrument and method setup are intuitive and streamlined with ready-to-use, optimized data acquisition templates for common applications including metabolomics, peptide identification and quantitation, post-translational modification (PTM) characterization, and TMT multiplexing (Figure 3). The Method Editor module provides templates that are fully customizable to meet specific application needs. The software environment is harmonized and consistent across next-generation Thermo Scientific mass spectrometers, including the Thermo Scientific™ Orbitrap Exploris™ mass spectrometer platform, and Thermo Scientific™ TSQ™ Triple Quadrupole mass spectrometers, streamlining training and operation.



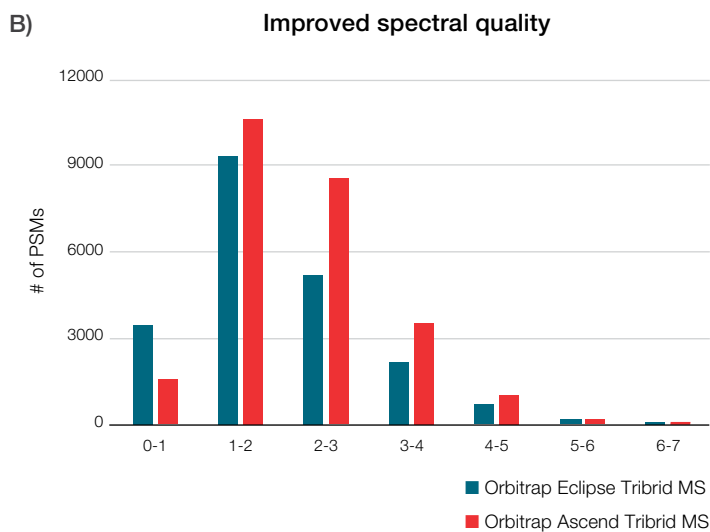
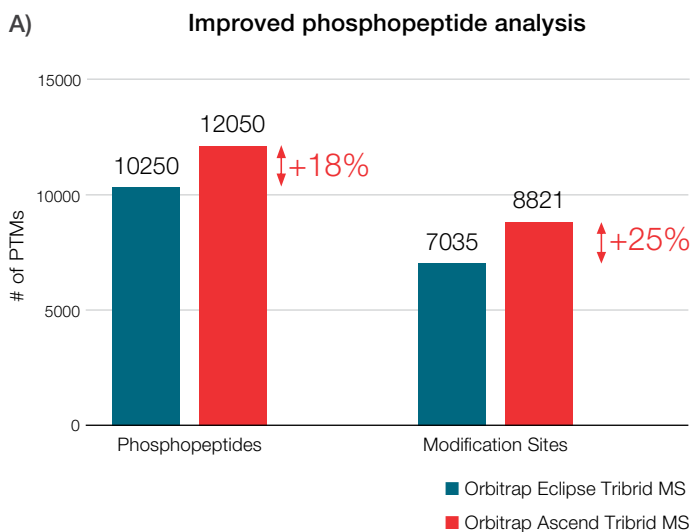
Figure 3. Orbitrap Ascend Tribrid MS intuitive method editor features a drag-and-drop user-friendly interface with predefined, optimized method templates for a wide range of applications. This allows scientists to generate high-quality data easily for applications including single cell proteomics, PTMs, TMT multiplexed quantitation, Data-independent Acquisition (DIA), Metabolomics, Lipidomics, Native protein characterization, and Thermo Scientific™ SureQuant™ methods and more.

Additional experimental options and capabilities further differentiate the Orbitrap Ascend Tribrid mass spectrometer from competitive high-end instruments. Table 1 provides a summary of

the key technologies and features of the Orbitrap Ascend Tribrid mass spectrometer.

**Table 1. Technology and features of the Orbitrap Ascend Tribrid mass spectrometer, compared to previous generation Tribrid instrumentation**

|  | Thermo Scientific™ Orbitrap™ IQ-X™ Tribrid™ mass spectrometer  | Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer   | Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ mass spectrometer  | Thermo Scientific Orbitrap Ascend Tribrid mass spectrometer   |
|--|--|---|--|---|
| <b>Ideal applications</b>  | Small molecule characterization, including metabolomics, lipidomics, Metabolite ID, extractables and leachables, industrial environmental health and more                | Multi-omics, TMT, Intact/top-down proteomics, Targeted Quantification, Protein-Protein Crosslinking   | Multi-omics, TMT, Intact/top-down proteomics, Targeted Quantification, Protein-Protein Crosslinking, Native Omics, Top-down mass spectrometry, biotherapeutics   | High throughput, single cell proteomics, multi-omics, TMT, Intact/top-down proteomics, Targeted Quantification, Protein-Protein Crosslinking, Native Omics, Top-down mass spectrometry, biotherapeutics, and new modalities   |
| <b>Analyses</b>  | MS and MS <sup>n</sup> , Qualitative and Quantitative, Profiling, Screening and Targeted workflows   |   |  |   |
| <b>Advanced data-dependent experiments</b>                               | Universal Method, Product Ion & Neutral Loss Triggered-MS <sup>n</sup> , Isolation offset, Intelligent MS <sup>n</sup> with Real-Time Library Search and Library builder | SPS MS <sup>3</sup> , Universal Method, Product Ion & Neutral Loss Triggered-MS <sup>n</sup> , Isolation offset, Quantitation/Confirmation Acquisition, SureQuant | Real-Time Search SPS MS <sup>3</sup> , Universal Method, Product Ion & Neutral Loss Triggered-MS <sup>n</sup> , Isolation offset, Quantitation/Confirmation Acquisition, SureQuant, Precursor Fit Filter | Real-Time Search and Real-Time Library Search for small molecules and peptides, SPS MS <sup>3</sup> , Universal Method, Product Ion & Neutral Loss Triggered-MS <sup>n</sup> , Isolation offset, Quantitation/Confirmation Acquisition, SureQuant, Precursor Fit Filter |
| <b>Thermo Scientific™ AcquireX intelligent data acquisition workflow</b> | Requires Thermo Scientific Orbitrap Tribrid Series 4.2 SP1QF1, 3.1 or greater  |   |  |   |
| <b>Thermo Scientific™ Almanac web-based monitoring and management</b>    | Requires Foundation 3.1 SP1 or later   |   |  |   |
| <b>Auto-Ready ion source for calibration</b>                             | No   |   |  | Yes   |
| <b>Optional upgrades</b>   |  |   |  |   |
| <b>EASY-ETD HD</b>   | No   | Yes   |  |   |
| <b>UVPD Laser</b>  | Yes  |   |  |   |
| <b>High mass range</b>   | No   |   | Up to <i>m/z</i> 8000  | Up to <i>m/z</i> 16000  |
| <b>Proton Transfer Charge Reduction (PTCR)</b>                           | No   |   | Yes  |   |
| <b>EASY-IC ion source</b>  | Yes  |   |  |   |
| <b>1 Million (1M) resolution</b>   | Yes  |   |  | No  |



**Figure 4. Increased phosphopeptide identifications and spectral quality.** Ti-IMAC beads were used to enrich phosphorylated peptides. 500 ng was injected and analyzed over a 90 min gradient using Orbitrap/Orbitrap data-dependent methods. Sample courtesy Yuchen He, Coon Laboratories, University of Wisconsin-Madison.

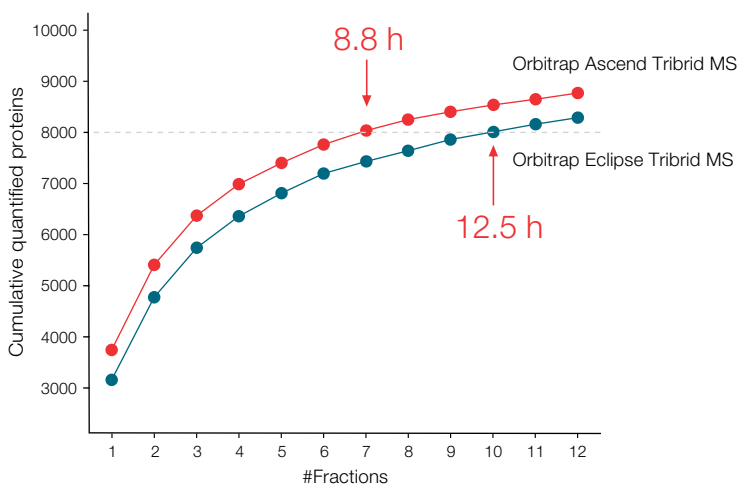
**Highlights: Increased application performance for proteomics and native protein analysis applications**

**Increase sample throughput: Unprecedented sensitivity for quantitative post-translational modification identification and site-localization**

Proteome analysis with PTMs remains challenging due to its unique analytical requirements of sample collection, sample preparation, and the mass spectrometer itself. While new methods and techniques have been developed to minimize sample loss during collection, preparation, and chromatographic delivery to the mass spectrometer, MS advancements are also needed to increase comprehensive characterization and relative quantitation for PTMs including phosphorylation experiments. The Orbitrap Ascend Tribid mass spectrometer was developed to meet the challenge of acquiring and extracting qualitative and quantitative data from challenging samples. Improvements in ion transmission combined with faster data acquisition rates of 45 Hz with 7500 resolution at  $m/z$  200 provide a 20% increase in phosphoproteome coverage compared to the Orbitrap Eclipse Tribid mass spectrometer (Figure 4A). Additionally, the Orbitrap Ascend Tribid MS provided higher quality spectra for phosphopeptides, as observed by an increased Xcorr score, enabling a 25% increase in the PTM site localization (Figure 4B). Important for fundamental biological research, these modification site improvements increase the ability to detect which specific amino acid of Ser or Thr is phosphorylated on a phosphopeptide.

**Quantify more of the proteome with better accuracy and precision**

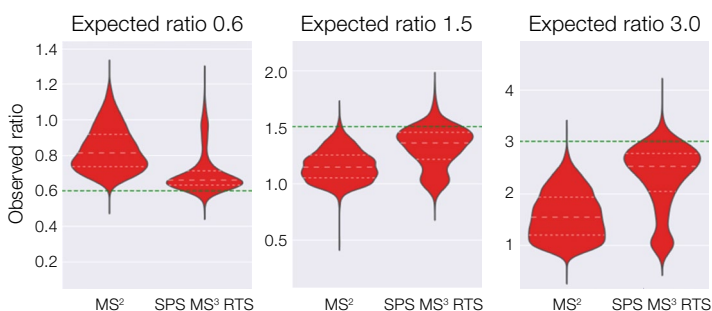
Sample multiplexing using tandem mass tags (TMT) is a powerful approach for mass spectrometry-driven drug discovery and characterization. However, sensitivity and throughput remain limiting factors toward achieving routine, full-proteome coverage. Figure 5 shows that the throughput can be improved by ~30% on the Orbitrap Ascend Tribid MS compared to previous instrumentation. This is due to a combination of improved ion transfer and increased scan rate leading to increased quantifiable peptides in TMTpro 18plex experiments. Improvement in experimental throughput is important, as it reduces the cost of the entire experiment proportionally.



**Figure 5. Quantify up to 18 samples with coverage to >8,000 proteins in <9 hours.** Human cells were labeled with TMTpro 18plex reagents, combined, fractionated, and subsequently 12 high pH fractions were analyzed using 75 min gradient with SPS MS<sup>3</sup> Real-Time Search methods. The Orbitrap Ascend Tribid MS was able to achieve the sample depth of proteome coverage in 30% less time than the Orbitrap Eclipse Tribid MS. Sample courtesy of Steven Shuken, Qing Yu, and Steven Gygi, Harvard Medical School.



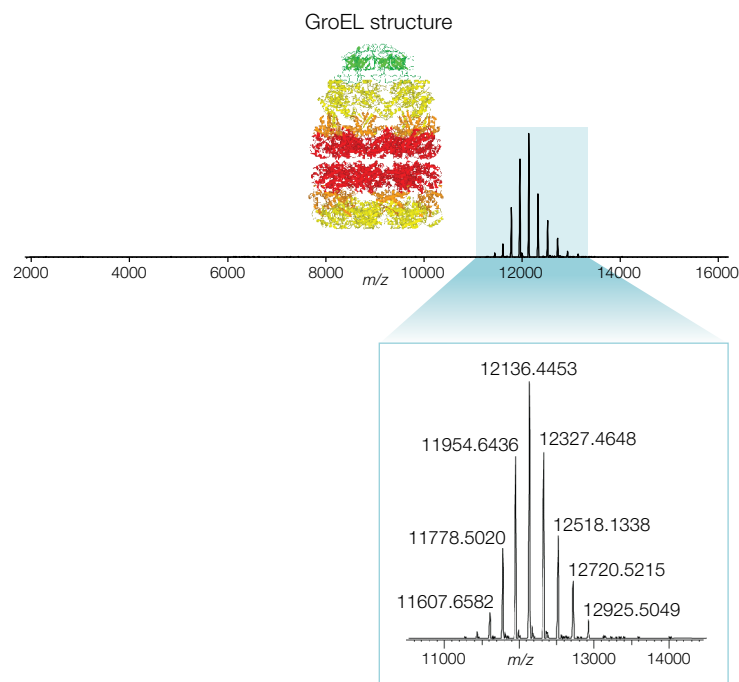
Additionally, accurate quantitation is critical for success of the biological experiment. Only MS3-based methods provide sufficient accuracy and precision in TMT-based quantitation throughout wide concentration ranges. In Figure 6, the Orbitrap Ascend Tribrid MS is able to better quantify the expected ratio utilizing the unique capabilities of a Tribrid instrument, including SPS MS<sup>3</sup> with Real-Time Search. The closer the median of protein abundance is to the green line (expected ratio), the more accurate the quantitative method. MS<sup>2</sup> results are significantly less accurate due to the interference in the reporter ion region. Improvements in quantitative accuracy of the proteome is fundamentally important, especially when the gain comes from lower abundant proteins.



**Figure 6. Excellent quantitative accuracy and precision using SPS MS<sup>3</sup> with Real-Time Search (RTS).** The HYPER standard, a quantitative mixture of TMT-labeled yeast peptides mixed into a high background of TMT-labeled human peptides was used to assess quantitation accuracy across a 65 min gradient using either SPS MS<sup>3</sup> with Real-Time Search or MS<sup>2</sup> on the Orbitrap Ascend Tribrid MS. Superior quantitative accuracy and precision was observed in the SPS MS<sup>3</sup> with Real-Time Search experiment.

## Go bigger: Comprehensive characterization of native proteoforms and intact proteins

Preserving the native state of large molecules during analysis has many benefits, most notably to reduce sample preparation related artifacts, preserve non-covalent interactions, and to obtain analytical information about the molecule in as close to its biological state as possible. With an efficient ion source, enhanced QR5 quadrupole mass filter with increased transmission efficiency, the ability to modulate the neutral gas pressure in the IRM, enhanced vacuum technology to maintain high vacuum in the C-trap and Orbitrap mass analyzer region and extended high mass range detection with precursor detection up to  $m/z$  16000, the Orbitrap Ascend Tribrid mass spectrometer provides the versatility and compatibility for in-depth native MS protein characterization. As shown in Figure 7, the new HMR<sup>n+</sup> extended mass range up to  $m/z$  16000 enables the detection of very large proteins up to 800 kDa in size. Additionally, the Orbitrap Ascend Tribrid mass spectrometer offers multiple fragmentation modes that can be performed at any MS<sup>n</sup> stage, including HCD/CID, ETD, ETciD, EThcD, and UVPD (Thermo Scientific™ UV Photodissociation.) The combination of these fragmentation methods generates complementary product ions that significantly enhance characterization of intact proteins and proteoforms.



**Figure 7. Demonstration of  $m/z$  16,000 mass range on purified GroEL protein (~800 kDa) on a single Orbitrap scan at 7500 resolution at  $m/z$  200.** Critical core functionalities include AGC controlled ion measurements and compatible with advanced ion manipulation techniques including: ETD, PTCR, UVPD, and CID/HCD.

### Auto-ready ion source details

Automated instrument calibration using the Auto-Ready ion source hardware ensures the instrument is ready to go when you are set (Figure 8). Trigger on-demand or scheduled system checks and calibrations from anywhere without disruption to your LC-MS system. Standardize calibration and maintenance with dedicated hardware including a separate ion transfer tube and emitter for calibration further supporting the consistent performance of the instrument over time. The Auto-Ready ion source for calibration means you can spend less time on instrument set-up, more time focused on biological questions.

### Conclusion

The Orbitrap Ascend Tribrid mass spectrometer is the newest generation Tribrid instrument designed to address today's most difficult analytical problems and be ready for tomorrow's most challenging demands. This next-generation Orbitrap Ascend Tribrid mass spectrometer offers many advantages and is technologically distinct from other mass spectrometry instrumentation. The latest inventions in ion transmission and control, extended  $m/z$  range, intelligent and faster acquisition, and multiple fragmentation modes provide significant gains in sensitivity, higher sample throughput, and increased versatility, making the instrument ideally suited to expanding the breadth of proteomics, structural biology, and protein characterization experiments made possible today.

Auto-Ready ion source

Orbitrap Ascend Tribrid mass spectrometer



**Figure 8. Spend less time on instrument set-up with the integrated Auto-Ready ion source.** Trigger on-demand or scheduled system calibrations remotely and without disruption to your LC-MS system.

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