

# Grant Application Resource

## Accelerating high-confidence insights for small- and large-molecule research using the Orbitrap Exploris 240 mass spectrometer

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### Goal

This document highlights how the Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer delivers leading performance with versatility to drive discovery for identification and quantitation, with high precision and accuracy to confidently scale up and achieve impact. Operational simplicity with intelligent data acquisition enables scientists to take the fast track to confident results across a range of small- to large-molecule applications. Proven Thermo Scientific™ Orbitrap™ mass analyzer technology provides the highest quality, intelligently collected high-resolution, accurate-mass (HRAM) data to address the challenges of undertaking impactful proteomics research, performing comprehensive characterization of complex biotherapeutics, and confidently identifying small-molecule unknowns. The information provided here describes the



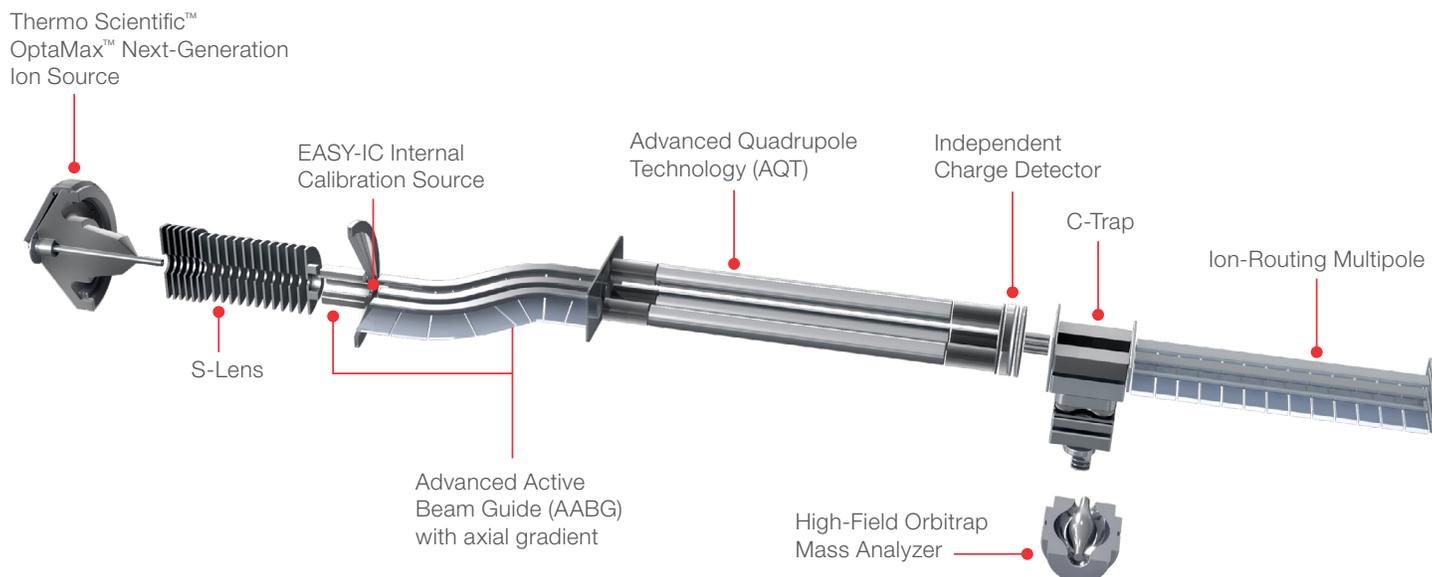
technologies that set the Orbitrap Exploris 240 mass spectrometer apart from Thermo Scientific™ Q Exactive™ hybrid quadrupole-Orbitrap™ mass spectrometers and quadrupole time-of-flight (Q-TOF) mass spectrometers.

### Overview

Discovery research laboratories and industry R&D departments solve complex challenges, ranging from understanding disease mechanisms and comprehensively characterizing complex biotherapeutics, to finding the next market-leading food ingredient. Likewise, core research facilities and Contract Research Organizations (CROs) must meet evolving client demands for analytical services while maintaining competitive pricing. Versatile technology that offers more insights or simplifies complex analytical workflows without sacrificing quantitation accuracy and precision is essential to these efforts. Numerous

hours spent on method development to address sample complexity can be avoided to allow shorter turnaround times and lower costs per sample. This white paper describes how the next-generation technology innovations in the Orbitrap Exploris 240 mass spectrometer hardware and software provide leading performance and versatility. Figure 1 and Table 1 summarize the feature improvements and new capabilities.

For all applications, the Orbitrap Exploris 240 mass spectrometer provides demonstrated gains in mass resolution, consistent mass accuracy, and speed to enhance performance and versatility. These enhancements are provided in a compact footprint and controlled in an easy-to-use interface that is consistent among Thermo Scientific™ next-generation mass spectrometers. In addition, improvements to robustness and serviceability maximize instrument uptime.



**Figure 1. Orbitrap Exploris 240 MS schematic with key technology innovations.** The improvements to this new compact mass spectrometer provide several compelling reasons to purchase.

**Table 1. Technologies associated with Orbitrap Exploris 240 MS benefits.**

Feature	Benefit	Technology
High resolution	High selectivity to resolve analytes down to a few mDa, with isotopic fidelity for enhanced confirmation and identification	240,000 (FWHM) at $m/z$ 200
Consistent sub-ppm mass accuracy	High selectivity and confidence in molecular formulae and compound identifications	Thermo Scientific™ EASY-IC™ internal calibrant source
Speed	Fast scan rates to improve compound identification and quantitation	Up to 22 Hz at resolution setting 15,000 (FWHM) at $m/z$ 200
Easy-to-use software	Pre-built method templates that are fully customizable using a drag-and-drop, flexible user interface	Thermo Scientific™ Orbitrap Exploris™ instrument control software
Orthogonal selectivity for proteomics	Improved signal-to-noise, reduced interferences, extended dynamic range, and increased number of proteins identified	Thermo Scientific™ FAIMS Pro™ interface (optional)
Multiplexed proteomics quantification	Increased quantitative performance compared to label-free quantitation (LFQ) limits missing values while increasing statistical confidence	Multiplexing with Thermo Scientific™ TMT 11plex reagents or Thermo Scientific™ TMTpro™ 16plex label reagent, and Thermo Scientific™ Tandem Mass Tag™ (TMT™) method templates
Scalable, standardized, reproducible workflows for translational research	Rigorous, relevant results to accelerate translation of discoveries into clinical applications	Thermo Scientific™ Ultra-High-Throughput Plasma Protein Profiling (uHTPPP) workflow
Native intact mass analysis	High-confidence characterization and quantitation of biotherapeutics	Mass range up to $m/z$ 8,000 with the Thermo Scientific™ BioPharma option, Thermo Scientific™ BioPharma Finder™ integrated software
Complete, in-depth small-molecule characterization	Comprehensive, automated sample profiling and experimental flexibility	Thermo Scientific™ AcquireX™ intelligent data acquisition workflow, Thermo Scientific™ Compound Discoverer™ software, and extended mass range down to $m/z$ 40

**Table 2. Application-specific comparison of the Orbitrap Exploris 240 mass spectrometer, Q Exactive mass spectrometers, and Q-TOF technology.**

Application	Orbitrap Exploris 240 instrument	Q Exactive instrument	Q-TOF technology
Sensitive and comprehensive protein identification and label-free quantitation (shotgun proteomics)	HRAM, FAIMS Pro interface, fully customizable method templates	HRAM	Lower resolution, less accurate mass
High-throughput, high-precision multiplexing with TMT 11plex and TMTpro reagents	FAIMS Pro interface (optional), customizable, flexible method templates	Method templates	Lower resolution limits to TMT 6plex or TMTpro 9plex analyses
High-performance targeted protein quantitation	Targeted MS <sup>2</sup> (t-MS <sup>2</sup> ) with pre-built method templates with product ion accumulation for enhanced sensitivity and spectral quality	PRM	PRM
Automated plasma profiling	uHTPPP workflow covering automated sample preparation, to HRAM MS analysis and QA/QC	HRAM	Lower resolution, less accurate mass
Biotherapeutic characterization and intact mass analysis	HRAM, mass range to <i>m/z</i> 8,000 with the BioPharma option	HRAM	Lower resolution, less accurate mass
Targeted, semi-targeted, and untargeted metabolomics, stable isotope labeling	AcquireX intelligent data acquisition workflow, extended low mass range to <i>m/z</i> 40, and high resolution for isotopic profile determination	HRAM	Lower resolution, less accurate mass, and limited polarity switching

For label-free quantitative (LFQ) proteome profiling or multiplexed protein quantitation using Thermo Scientific™ Tandem Mass Tag™ (TMT™) technology, the optional Thermo Scientific™ FAIMS Pro™ interface adds more performance and efficiency with differential ion mobility technology. When throughput and standardization for reliable translational and clinical research experiments are required, the Thermo Scientific™ Ultra-High-Throughput Plasma Protein Profiling (uHTPPP) workflow provides an automated solution for plasma profiling. This workflow includes sample preparation and QA/QC procedures that are reproducible and scalable, delivering rigorous, relevant results for large sample cohorts.

For biopharmaceutical applications, high resolving power, stable mass accuracy, and extended mass range to *m/z* 8,000 with the Thermo Scientific™ BioPharma option enable detailed structural characterization of large, complex species for top-down, subunit, and native analysis. The extended mass range is covered by a single mass calibration, providing simplified analysis of site modification, intact masses, and impurities. Intact protein mode with high-pressure settings permits analysis of intact proteins such as monoclonal antibodies (mAbs) and antibody drug conjugates (ADCs) under native conditions.

For small-molecule applications such as metabolomics and lipidomics studies, the Thermo Scientific™ AcquireX™ intelligent data acquisition workflow ensures the collection of meaningful high-quality MS<sup>2</sup> data, increasing small-molecule profiling efficiency with minimal manual method setup and streamlined data analysis. Thermo Scientific™ Compound Discoverer™ software makes it easier to transform the high-quality MS and MS<sup>2</sup> data obtained into known compounds, identify differences between sample groups, and make associations with biochemical pathways. With an extended low mass range down to *m/z* 40, high-confidence identification of isomers and small molecules is significantly improved.

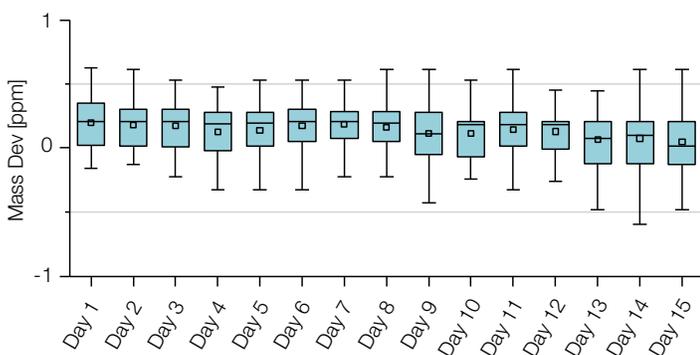
For research and core laboratories, the Orbitrap Exploris 240 mass spectrometer redefines versatility by expanding single-instrument capabilities to meet both proteomics and metabolomics needs while ensuring exceptional data quality and instrument robustness.

## New technology highlights

The highly optimized Orbitrap Exploris 240 mass spectrometer design allows for a smaller, bench-space-saving footprint while adding high-performance components and functionalities that substantially advance instrument performance. These new features are detailed below.

### EASY-IC ion source

High-confidence applications benefit from the highest possible mass measurement accuracy. The Thermo Scientific™ EASY-IC™ (internal calibration) ion source is an internal calibration system that achieves sub-ppm mass accuracies via automated introduction of an internal reference mass during sample analysis. The system provides confidence in every scan with <1-ppm mass measurement error for at least five consecutive days (Figure 2).



**Figure 2. The Easy-IC source internal calibration achieves consistently high mass accuracy.** This analysis of 15 veterinary drugs spiked into meat-muscle matrix using positive/negative polarity switching over 15 days following calibration demonstrates exceptional sub-1-ppm mass accuracy, providing the highest confidence in subsequent identification, characterization, and quantitation.

### Advanced quadrupole technology (AQT)

AQT features a segmented mass filter which enables precursor ion selection. The high-quality hyperbolic quadrupole rods, combined with novel RF-only segments at the device entrance and exit, provide very high ion transmission at narrow isolation widths, resulting in excellent selectivity with minimal sensitivity loss. The resolution of the mass filter can be set as high as 0.4 Da for ions <  $m/z$  400. AQT uniquely uses configuration switching in which the RF applied is switched between sets of quadrupole rods. Configuration switching significantly extends instrument performance uptime.

### Ion-routing multipole (IRM)

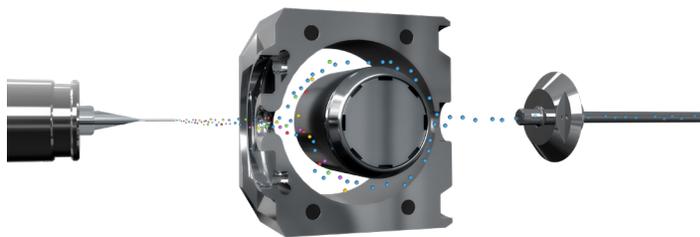
The ion-routing multipole provides high-performance ion routing and experimental versatility with higher-energy collisional dissociation (HCD) fragmentation. As with other Thermo Scientific next-generation mass spectrometers, HCD spectra can be generated using user-defined absolute collision energies (CEs) or normalized collision energies (NCEs), facilitating method development and cross-platform method transfer.

### High-field Orbitrap mass analyzer

The physical size of the Thermo Scientific™ Orbitrap™ central electrode, together with the ultra-high vacuum inside the Orbitrap analyzer, provides 240,000 (FWHM) mass-resolving power at  $m/z$  200.

### FAIMS Pro interface option

The optional FAIMS Pro interface (Figure 3) is a differential ion mobility device that performs online gas-phase fractionation prior to ion introduction to the mass spectrometer, enhancing precursor selectivity to improve qualitative and quantitative results for peptide and protein applications, and providing orthogonal separation capabilities.



**Figure 3. The FAIMS Pro interface cut-away image demonstrating the ion path around the cylindrical electrodes.**

The FAIMS Pro interface preferentially selects ion populations based on the application of a compensation voltage (CV) to the central cylindrical electrode, enabling new methods for data-dependent acquisition (DDA) or data-independent acquisition (DIA) workflows, including multiple-CV looping scans to increase proteome depth, while increasing selectivity and sensitivity. FAIMS Pro interface control is fully integrated into the instrument control software for easy integration into existing workflows.

### Extended mass range option

Adding the BioPharma option to the Orbitrap Exploris 240 mass spectrometer extends mass range from  $m/z$  6,000 to  $m/z$  8,000, enabling the analysis of even larger precursors.

## Robust and reliable design and manufacture

Designed for exceptional robustness, everyday reliability, and reproducibility, the Orbitrap Exploris 240 mass spectrometer maximizes uptime and productivity to meet large-scale study and sample-throughput requirements. Enhanced design architecture ensures reproducibility from one instrument to the other, facilitating larger scale research studies for translational and clinical research. Rigorous testing at every stage of manufacturing ensures the quality of each component and every instrument. The instrument can be operated continuously for over 125 days without performance decline. As described previously, the AQT with its novel configuration switching extends quadrupole maintenance intervals up to 200% without compromising performance.

## Serviceability

Going beyond previous generation hybrid-Orbitrap mass spectrometers, the Orbitrap Exploris 240 mass spectrometer adds features that enhance serviceability and uptime. The simplified ion source permits faster capillary replacement and ion source cleaning. In addition, the quadrupole assembly is easily removed and replaced for cleaning with no need to manually disassemble and reassemble electrical connections. Once the instrument is ready to pump down, automatic bake-out of the Orbitrap manifold minimizes downtime, while the single six-stage turbo pump maximizes the reliability of the system under vacuum.

## Instrument control software ecosystem

In contrast to Q-TOF technology, the Orbitrap Exploris mass spectrometer instrument control software delivers productivity, ease-of-use, and flexibility for users of all levels of expertise. Instrument calibration is simplified with the tune module, and method setup is intuitive and streamlined with ready-to-use, optimized data acquisition templates for common applications including metabolomics, peptide identification, peptide quantitation, post translational modification (PTM) characterization, and TMT multiplexing (Figure 4). 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 Thermo Scientific™ Orbitrap™ Tribrid™ mass spectrometers, the Orbitrap Exploris mass spectrometer platform, and Thermo Scientific™ TSQ™ triple-quadrupole mass spectrometers, streamlining training and method transfer.



**Figure 4. Method Editor window used to setup the mass spectrometer parameters.** Plug-and-play nodes enable users to easily setup and customize the analysis to maximize data quality and Instrument performance. In addition to the templates, intuitive tuning and simplified calibration across the mass range and polarity modes add to usability, data quality, and sample throughput.

## AcquireX intelligent data acquisition workflow

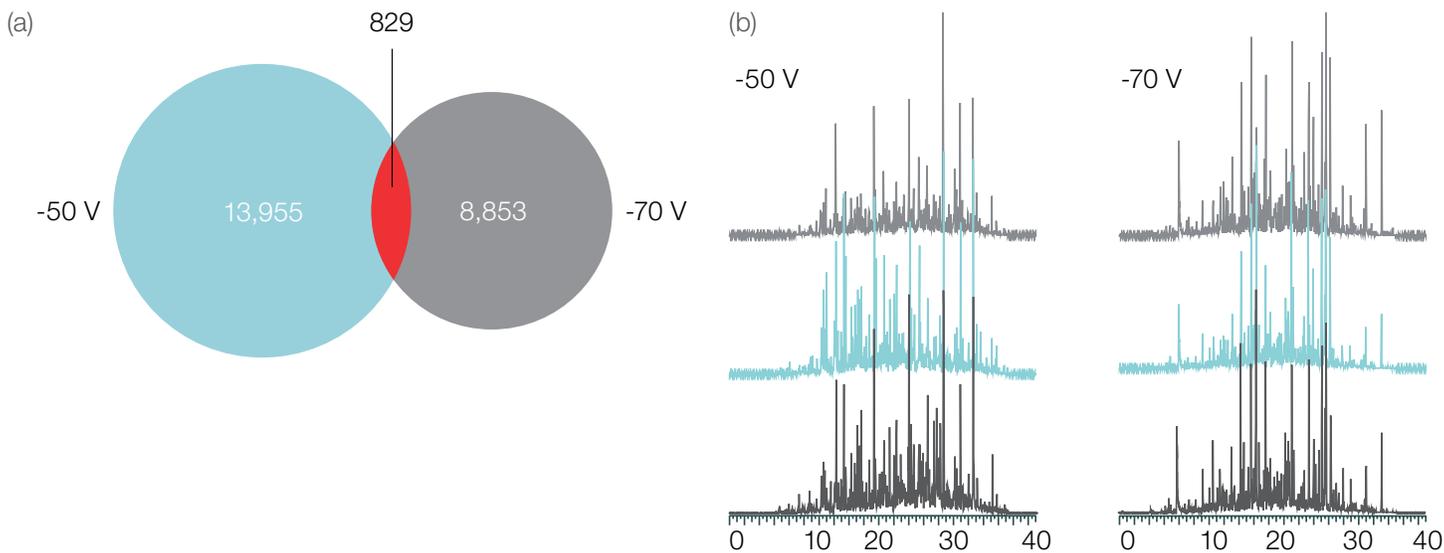
AcquireX intelligent data acquisition provides four unique experimental workflows that automate generation of meaningful MS<sup>2</sup> spectra for sample-relevant compounds: 1) background ion exclusion, 2) ion exclusion and component inclusion, 3) iterative precursor selection, and 4) deep scan for ultimate small-molecule sample interrogation. These workflows allow flexible experimental designs, while providing the highest data quality and increased coverage for small-molecule analysis.

## Improvements to application performance

### Comprehensive, versatile proteomics approaches

Proteome coverage is essential, not only for the identification of expressed proteins at a given time, but also for quantitative studies that monitor proteome expression across time and/or space to reveal more detailed insights about a biological system. Scientists must consider both the proteome depth achieved across a range of sample amounts and the time to complete the analysis.

The Orbitrap Exploris 240 mass spectrometer offers complete workflows for protein identification and quantitation, all of which increase sample throughput without compromising accuracy, coverage, or depth. In addition to accurate quantitation, spectral and fragment ion peak reproducibility across Orbitrap Exploris 240 mass spectrometers facilitates method transferability among other next-generation Thermo Scientific instruments. Together with the optional FAIMS Pro interface, the Orbitrap Exploris 240 mass spectrometer delivers the high spectral quality, improved selectivity, resolution, and mass accuracy that set it apart from Q-TOF analyzers.



**Figure 5. Three replicate injections of 200 ng of a Thermo Scientific™ Pierce™ HeLa Protein Digest Standard analyzed over a 30-min gradient at two CVs using the FAIMS Pro interface.** (a) Venn diagram of the identified peptides at CVs of -50 V and -70 V, showing minimal overlap in identifications. (b) Chromatographic traces measured at the same CV were highly reproducible, however, the traces for each CV were different.

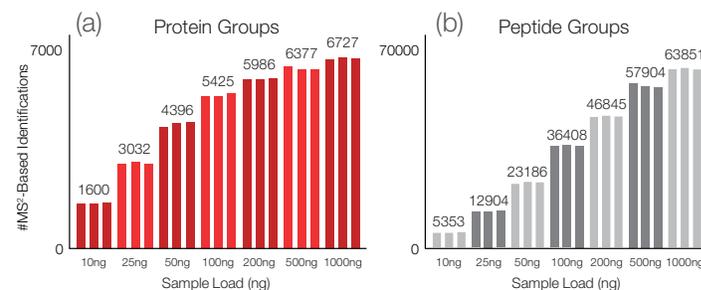
To meet diverse experimental needs, the flexibility of the Orbitrap Exploris 240 mass spectrometer enables laboratories to switch quickly between proteomics applications such as shotgun proteomics, gel-band protein identification, LFQ, and multiplexed proteome quantitation with isobaric tags.

### Deep protein and peptide identification

Protein and peptide identification is the foundation of qualitative or quantitative proteomics studies. The Orbitrap Exploris 240 mass spectrometer provides protein and peptide group identifications that are highly reproducible, even across instruments, with low coefficient of variations and false discovery rates (FDRs).

Protein identifications can be increased using the optional FAIMS Pro interface (Figure 5). Importantly, the FAIMS Pro interface can be seamlessly added to existing LFQ and TMT workflows to improve the signal-to-noise, and thus sensitivity, for low-abundance proteins while also reducing co-isolation interference to improve quantitation accuracy. Multiple CV settings can be repetitively sampled during analyses to increase the number of unique proteins identified and to minimize co-isolation of isobaric peptides, while reducing time-consuming offline fractionation. The orthogonal selectivity of gas-phase fractionation reduces the complexity of the precursor ions accumulated and analyzed per CV setting, increasing proteome coverage and decreasing interference. In addition to increasing coverage, the interface enhances data quality, quantitative confidence, workflow efficiency, and overall productivity.

When equipped with the optional FAIMS Pro interface, the Orbitrap Exploris 240 mass spectrometer delivers the best-in-class balance of throughput and sensitivity for proteome coverage, allowing analysis of limited sample amounts with unprecedented depth in less experimental time. Deep proteome coverage can be achieved over a wide range of sample loads. Very sensitive measurements of 10 ng of sample permit high-confidence identifications, averaging 1,600 protein groups and 5,300 peptide groups identified with at least one peptide spectrum match (Figure 6). Analysis of 1,000 ng of sample provides extensive proteome coverage with an average of 6,700 protein groups and 63,000 peptide groups identified (Figure 6). In addition, high numbers of protein and peptide identifications are possible across gradient conditions from 30 minutes up to 120 minutes, providing laboratories with the flexibility to balance proteome coverage with sample throughput.



**Figure 6. High-performance proteomics over a wide sample range.** 10–1,000 ng of Pierce HeLa Digest Standard was evaluated on the Orbitrap Exploris 240 MS equipped with the FAIMS Pro interface. Two compensation voltages (-50 and -70 V) were used during a 90-min analytical gradient with an Ionopticks 25 cm Aurora column. a) Protein Groups, and b) Peptide Groups, both at a 1% FDR.

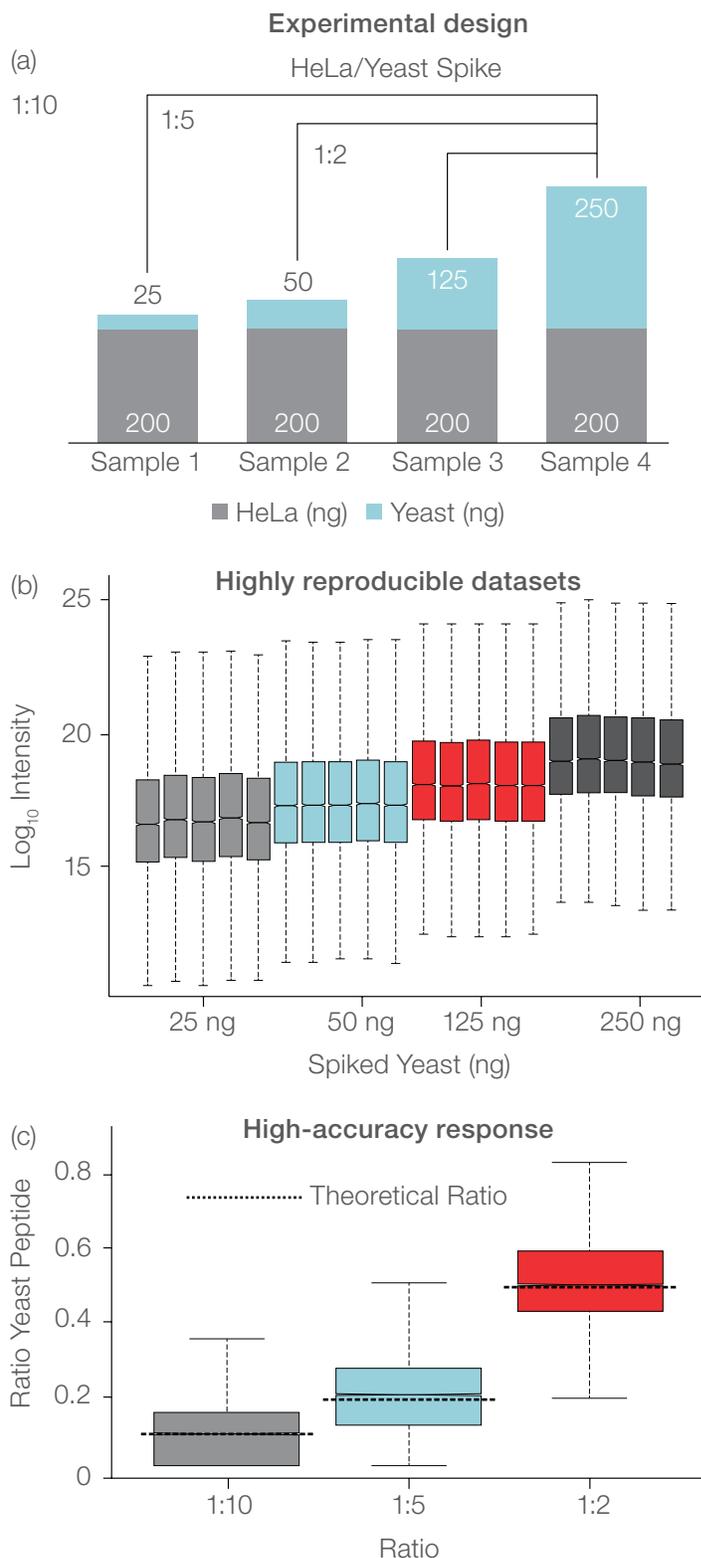
### Reproducible, accurate label-free quantitation

Quantitative proteomics is considerably complex due to the dynamic and interactive nature of proteins. The FAIMS Pro interface delivers highly reproducible data, enabling accurate quantitation with enhanced sensitivity and selectivity. LFQ is one approach to determine differential protein expression. Figure 7 illustrates the ability of the Orbitrap Exploris 240 mass spectrometer to perform reproducible and accurate LFQ on complex proteomes. A two-proteome mixture was created using a yeast protein digest spiked in different ratios in a background of HeLa protein digest. Protein abundance was highly reproducible across four technical replicates, and importantly, the abundance of the spiked yeast protein was accurately determined.

### High-throughput differential protein quantitation with TMT multiplexing

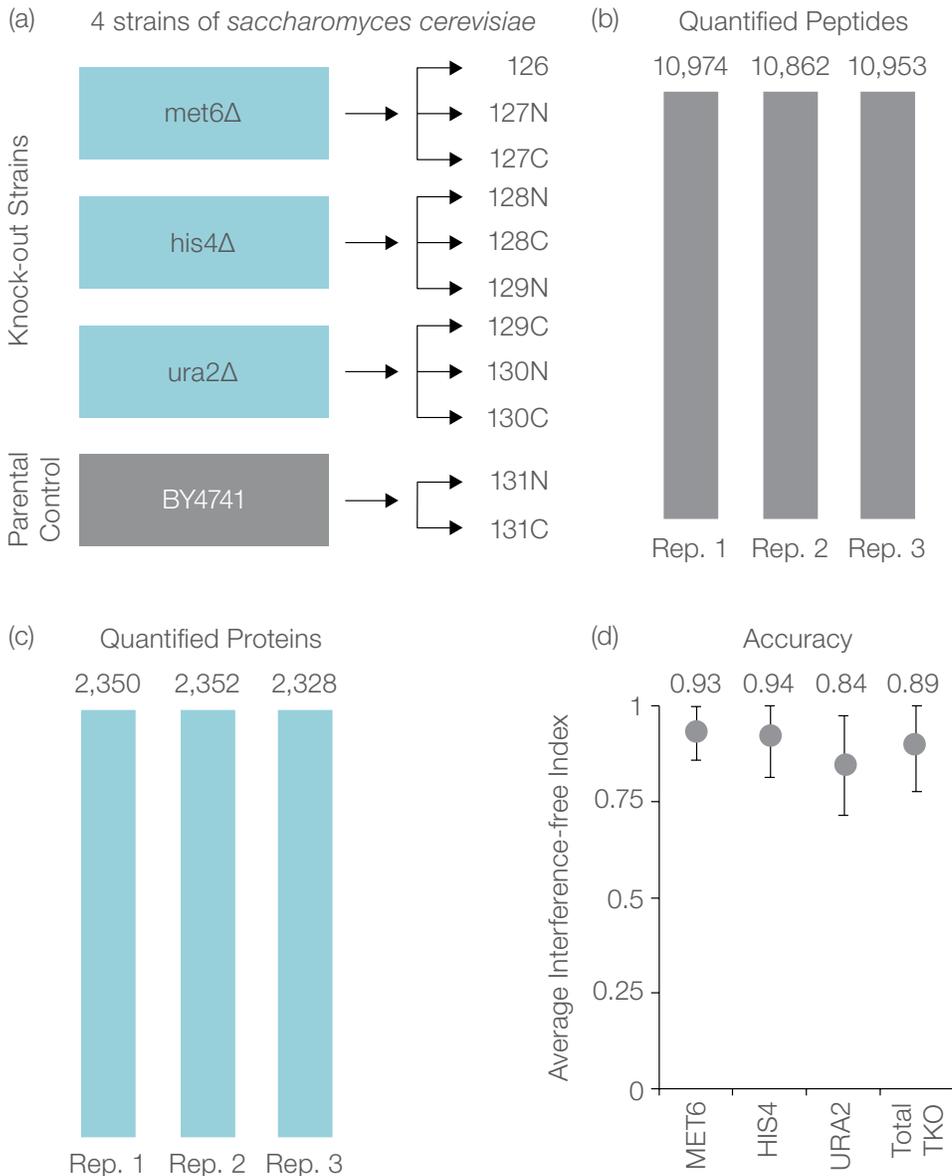
Quantitative performance can be increased beyond that of LFQ approaches using multiplexing with the FAIMS Pro interface and Thermo Scientific Pierce TMT 11plex or TMTpro isobaric reagents. Isobaric labeling methods using TMT reagents enable precise measurement of peptide or protein abundances in multiple samples in a single LC-MS run. This is useful for analysis of large sample sets for applications like thermal-shift assays and large-scale cohorts.

The high-resolution MS/MS scanning provided by Orbitrap technology is necessary to achieve accurate ratio determination in TMT multiplexing experiments. Orbitrap mass spectrometers also offer the highest number of channels for multiplexing with TMT or TMTpro reagents, allowing up to 11 or 16 samples, respectively, to be multiplexed in one LC-MS experiment. Shown in Figure 8, the FAIMS Pro interface further reduces the challenges associated with TMT quantitation, such as co-isolated interferences that can decrease the accuracy of ratio quantification, masking true differences in protein abundance across samples.



Theo. Ratio	0.10	0.20	0.50
Exp. Ratio	0.10	0.21	0.51

**Figure 7. Reproducible and accurate LFQ of a two-protein mixture using the Orbitrap Exploris 240 MS.** A yeast protein digest was spiked into a background of 200 ng Pierce HeLa Digest Standard at a ratio of 1:10, 1:5, 1:2. Data processing and LFQ were carried out in Thermo Scientific™ Proteome Discoverer™ software revision 2.4 using the Thermo Scientific™ Minora feature detector node.



**Figure 8. Reproducible and accurate TMT-based multiplexed quantitation using the Orbitrap Exploris 240 MS configured with the FAIMS Pro interface.** (a) Experimental setup for a mixed proteome analysis, evaluating quantitative precision and accuracy using three strains with specific protein knock outs and a parental control. (b) Bar plots highlighting the number of peptides (c) and proteins quantified per run across the different samples. (d) Plot highlighting the average interference-free index (IFI) for each of the knock-out proteins.

### Sensitive targeted protein quantification

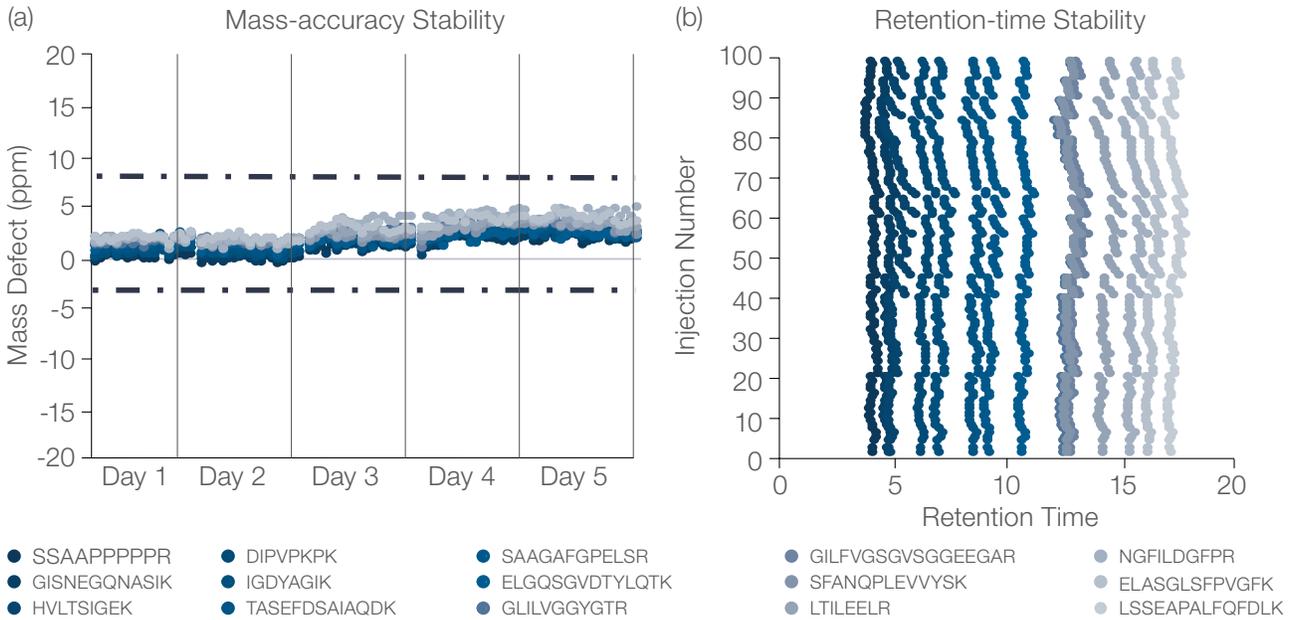
For the analysis of a few hundred analytes, targeted quantitation approaches similar to PRM are ideal methods that can be optimized to maintain high data quality, and that can be scalable, depending on how much instrument acquisition time is dedicated to the measurement of each analyte. The Orbitrap Exploris 240 mass spectrometer provides high-confidence HRAM measurements of proteins and peptides using targeted MS<sup>2</sup> (t-MS<sup>2</sup>) methods that improve upon the PRM strategy, achieving high precision even at detection limits.

### Scalable translational and clinical proteomics research

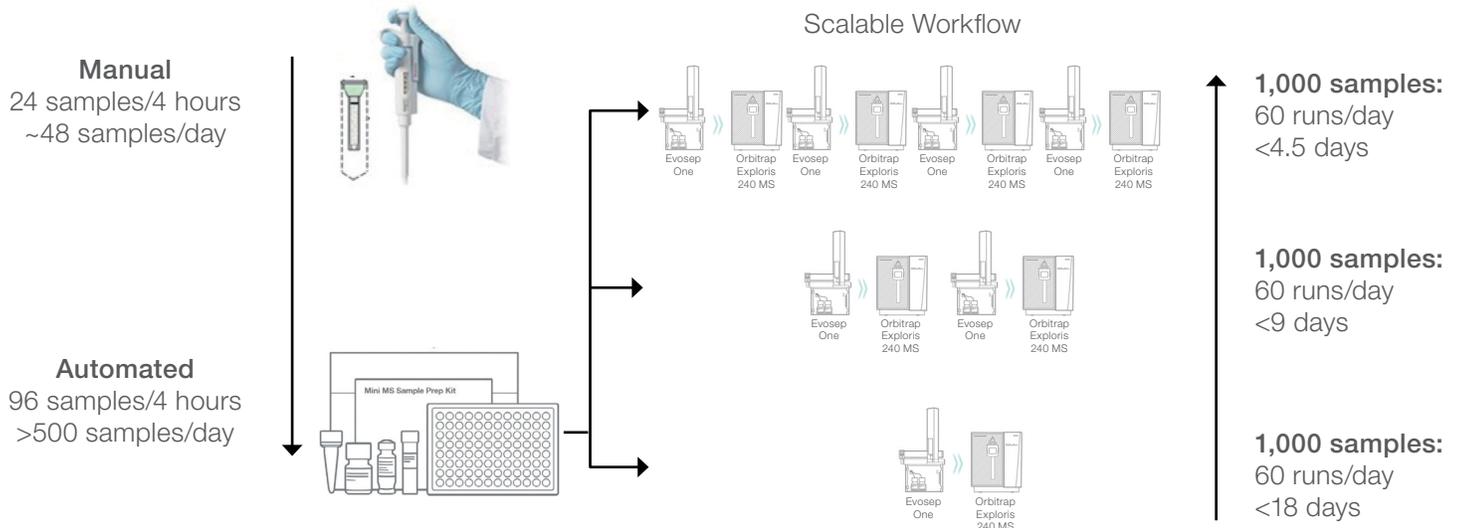
Scaling translational research discoveries to reliable clinical experiments demands standardized, reproducible workflows. As part of the uHTPPP workflow, the Orbitrap Exploris 240 mass spectrometer meets these demands. From integrated, automated sample preparation, sample cleanup, separations, and built-in QA/QC procedures, to industry-leading data processing, the workflow provides a complete solution for high-throughput, high-confidence quantitative proteome profiling of large-scale plasma and tissue sample cohorts that produces the rigorous and relevant results required to accelerate translation of discoveries into clinical applications.

The uHTPPP workflow delivers reliable and reproducible high-quality qualitative and quantitative data for plasma with stable mass accuracy over five days of continuous analysis, and stable retention times over 100 injections (Figure 9). Reproducible, high peptide recovery and protein

identification with a coefficient of variation of less than 5%, spanning a dynamic range of five orders of magnitude, can be obtained. The uHTPPP workflow also offers remarkable sample preparation, data acquisition, and processing time savings (Figure 10).



**Figure 9.** Over 100 depleted lung cancer serum samples were processed and analyzed over five days of continuous operation. Mass-accuracy and retention-time stability of Peptide Retention Time Calibration (PRTC) peptides spiked into each sample were monitored.



**Figure 10.** Comparison of a manual versus the scalable uHTPPP workflow. The uHTPPP workflow automates analysis from sample preparation to HRAM MS analysis, and uses QA/QC procedures that are included with the Orbitrap Exploris 240 mass spectrometer. Up to 96 samples can be processed in four hours using the Thermo Scientific™ EasyPep™ 96 MS Sample Prep Kit. The Evosep One LC system (available from Evosep Biosystems, Odense, Denmark) enables 30, 60, or 90 samples to be analyzed per day.

## Versatile, streamlined biotherapeutic characterization

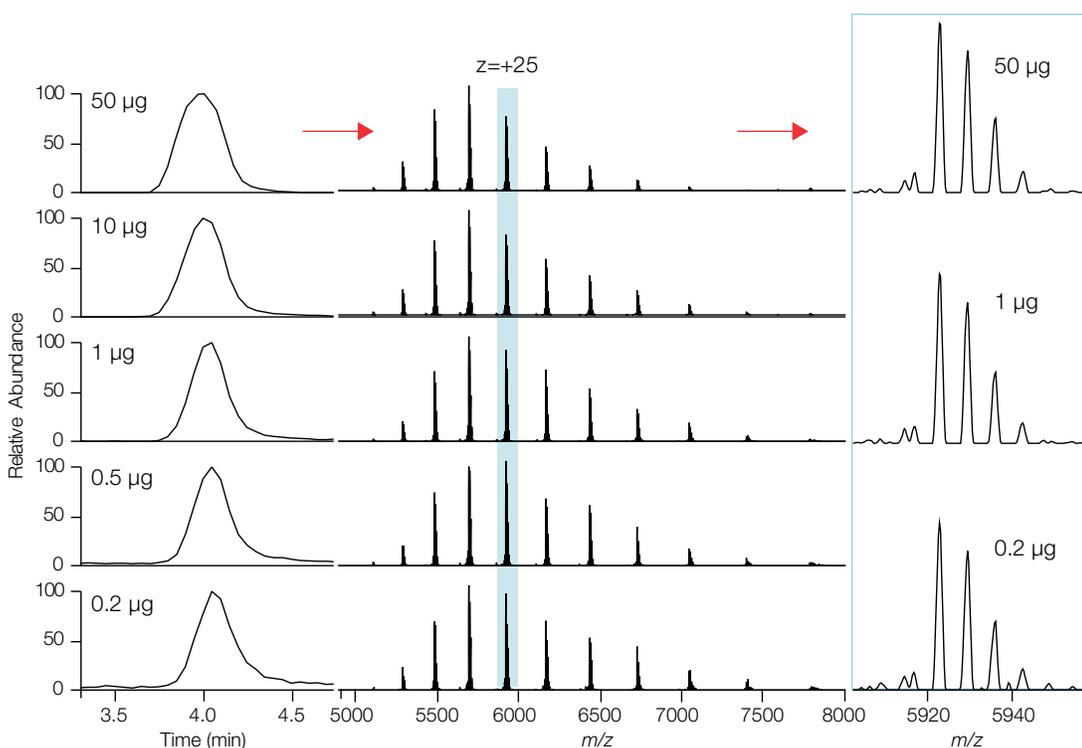
Biopharmaceutical development depends on protein analyses to confidently confirm sequences, verify structures, and reveal the precise locations of low-level modifications. Doing this work demands sophisticated analytical workflows that combine multiple analytical techniques and state-of-the-art technologies to produce sufficient information to obtain high-confidence structural insights. Using a single instrument to successfully acquire all the essential information for complete characterization—peptide mapping, subunit analysis, intact denatured and native investigation—is preferred for reasons of increased efficiency and productivity, and cost-effective analytical support.

## High-sensitivity intact and native protein analysis

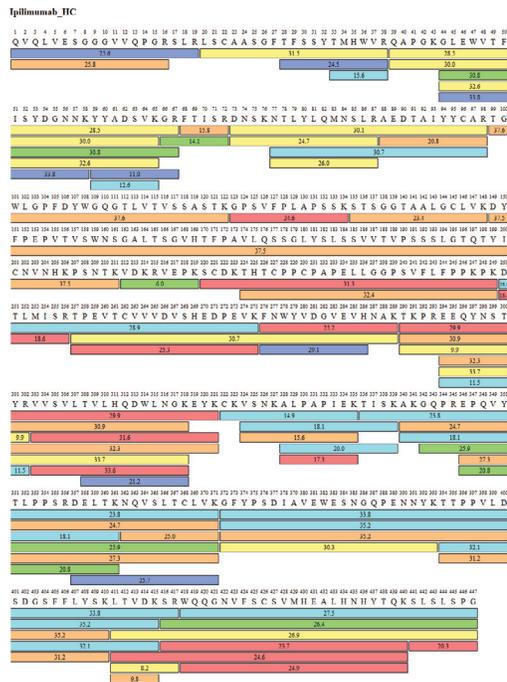
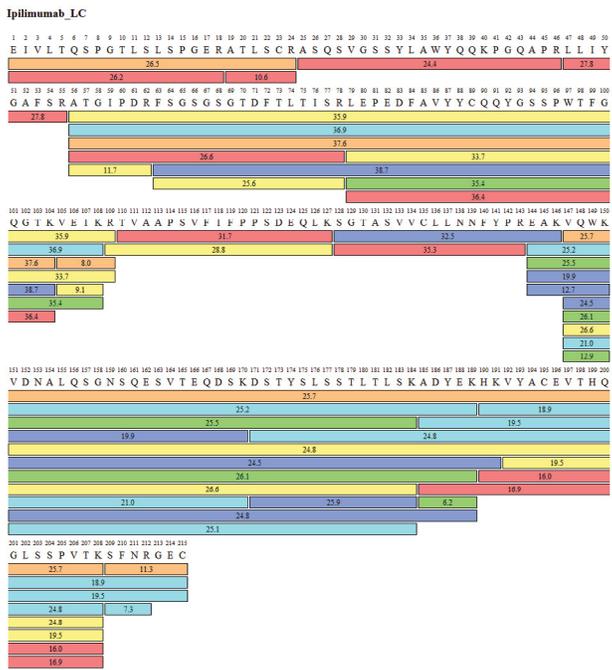
Intact native mass analysis of biotherapeutic molecules is a rapid and effective way to produce valuable data to enhance understanding of biological relevance, because it preserves non-covalent interactions, simplifies data analysis with reduced spectral complexity, and reduces the chance of experimentally induced artifacts. Additionally, charge variant analysis of native intact proteins can increase the amount of information obtained in a single experiment, reducing the need for sample preparation and multiple characterization steps.

The BioPharma option extends instrument mass range to  $m/z$  8,000, permitting intact native MS analysis of therapeutic proteins such as mAbs and ADCs. The extended mass range is covered by a single stable mass calibration across the entire mass range, simplifying instrument setup and providing reliable mass stability for monitoring applications. The application-specific pressure settings of the intact protein mode facilitate analysis of intact therapeutic proteins under native conditions. Superior ion transmission allows use of high-resolution settings to resolve protein modifications such as glycosylation and conjugations, even at low on-column protein loads. Enhanced ion-trapping efficiency reduces noise to remarkably low levels, resulting in superior mass spectrum quality. Structural insights are gained quickly when isoforms and glycoforms are easily and confidently differentiated using the powerful deconvolution capabilities of BioPharma Finder software (Figure 11).

Extended mass range and enhanced transmission also enable routine HRAM characterization of large, highly charged species such the individual light and heavy chains of antibodies up to 50 kDa. Low-level modifications or different conjugated species of an ADC can be routinely detected during intact denatured and protein subunit analyses.



**Figure 11. Size exclusion chromatographic (SEC) MS analysis of intact native trastuzumab from 50 to 0.2 µg on-column with the BioPharma option.** Simplified spectral data showed consistent glycoform distributions (right), even at low sample loadings, making routine native MS analysis possible.



**Figure 12. Complete coverage for the light and heavy chains of ipilimumab demonstrate the effectiveness of SMART Digest kit MS detection efficiency.** BioPharma Finder software with color-coding highlighted the confidence-level of the peptide assignments.

### High-confidence peptide mapping

With powerful sample preparation approaches such as the Thermo Scientific™ SMART Digest™ kits, consistently high coverage can be obtained across multiple digestion replicates, demonstrating the robustness and precision of the Orbitrap Exploris 240 mass spectrometer. High confidence in peptide mapping data is essential for complete characterization of therapeutic proteins. (Figure 12). Modifications with low mass differences, such as deamidation, require high-resolution mass spectral data down to the peptide level.

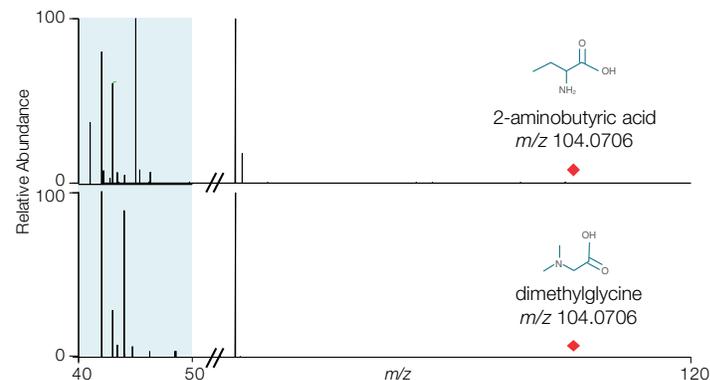
### Complete insights for small-molecule analysis

With HRAM performance, extended low mass range to  $m/z$  40, AcquireX intelligent data acquisition, and Compound Discoverer software for post-acquisition data analysis, the Orbitrap Exploris 240 mass spectrometer accelerates high-confidence insights for untargeted, semi-targeted, targeted, and stable isotope labeling small-molecule experiments from metabolomics to lipidomics, environmental, and food safety applications.

Robust performance with resolution up to 240,000 (FWHM) at  $m/z$  200 and unmatched mass accuracy make the Orbitrap Exploris 240 mass spectrometer ideal for 'omics studies. HRAM Orbitrap technology provides accurate mass assignments and resolves near-mass isobaric species from

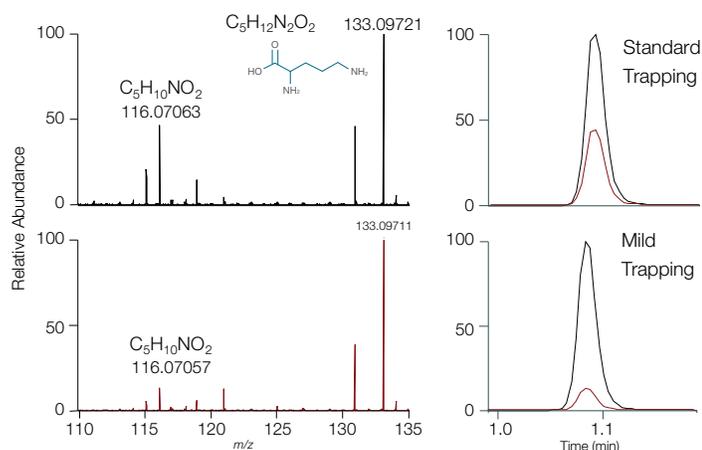
complex mixtures, increasing confidence in small-molecule identification and quantitation. High-confidence annotations require HRAM measurements to obtain high-quality fragmentation spectra for spectral matching against libraries, as well as to reveal isotope patterns and the presence of heteroatoms in unknowns, facilitating elemental formula prediction and elemental composition determination.

Low mass range extended down to  $m/z$  40 enables detection of additional fragment ions for high-confidence isomer differentiation and small-molecule identification (Figure 13).



**Figure 13. The additional five diagnostic fragment ions generated between  $m/z$  40 and 50 for the isomers 2-aminobutyric acid and dimethylglycine provided increased structural information that enabled the analyst to confidently differentiate between them.**

Labile compounds can fragment unintentionally, causing incorrect metabolite assignments. The Orbitrap Exploris 240 mass spectrometer allows use of mild trapping settings to reduce unintentional MS<sup>1</sup> precursor ion dissociation (Figure 14).

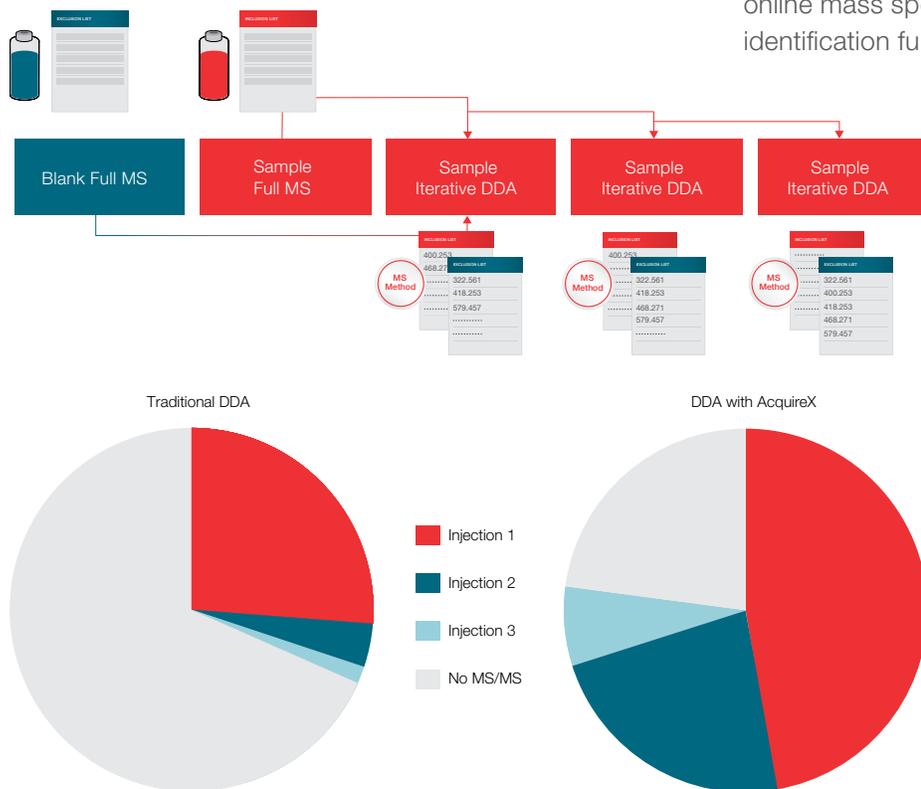


**Figure 14. The ornithine MS<sup>1</sup> product ion at m/z 116.0706 could be mistaken for proline, which shares an identical mass.** Shown in the red trace, compared to standard trapping, the amount of fragmentation is lower when mild trapping is used.

The unique AcquireX intelligent data acquisition workflow adds to the power of the Orbitrap Exploris 240 mass spectrometer by streamlining reliable acquisition of high-quality MS and MS<sup>2</sup> data to speed compound identification and characterization. Complex samples can limit the utility of DDA and DIA strategies due to the collection of irrelevant data from background matrices. The proven AcquireX intelligent data acquisition workflow collects more meaningful high-quality MS<sup>2</sup> data, increasing profiling efficiency with minimal manual setup and easier subsequent data interpretation.

In particular, the AcquireX intelligent acquisition workflow using deep-scan mode delivers improved MS<sup>2</sup> sampling by automatically excluding background ions and focusing acquisition on true sample components (Figure 15). This data-informed workflow minimizes irrelevant spectra and provides deeper metabolome coverage with exhaustive, in-depth analysis of complex samples, ensuring that nothing is missed. Low-intensity metabolites can be highly toxic, so ensuring that these compounds are detected is critically important. The AcquireX data acquisition workflow algorithm can be used in real time to determine the features corresponding to background contaminants and compound degeneracy, such as isotopes, adducts, and dimers.

Integration with Compound Discoverer software and cloud-based tools such as the Thermo Scientific™ mzCloud™ online mass spectral library streamlines unknown identification further, saving valuable analyst time.



**Figure 15. AcquireX intelligent data acquisition deep-scan analysis of pooled mouse plasma on a C18 column over 15-min LC gradient increased sample coverage compared to a traditional DDA approach.**

## Conclusion

The Orbitrap Exploris 240 mass spectrometer delivers leading performance with versatility for a wide range of small- to large-molecule applications and the quantitative precision and accuracy to easily scale up and confidently answer research questions. Proven Orbitrap mass analyzer technology provides the highest quality, intelligently collected HRAM data to address the challenges associated with undertaking impactful proteomics research, performing comprehensive characterization of complex biotherapeutics, and confidently identifying small-molecule unknowns.

As a next-generation mass spectrometer, the Orbitrap Exploris 240 instrument offers many advantages, and is technologically distinct from Q Exactive mass spectrometers and Q-TOF instruments. Exceptional accuracy and precision for quantitation improves performance for protein identification, quantitative

proteome profiling using LFQ with DDA or DIA, and multiplexed TMT quantitation. In addition, the instrument is capable of detailed structural characterization of proteins and biopharmaceuticals using peptide mapping, top-down, subunit, and native analysis methods to determine sequences, variants, sites of modifications, intact masses, and impurities. The AcquireX intelligent data acquisition workflow enables users to dig deeper into samples with on-the-fly background exclusion, component inclusion, iterative precursor exclusion, and deep scan.

Demonstrated gains in mass resolution, consistent mass accuracy, speed, and more, are all provided in a smaller system footprint. Robustness and serviceability maximize instrument uptime. An easy-to-use interface that is consistent among Thermo Scientific next-generation mass spectrometers provides operational simplicity and intelligent data acquisition, ensuring scientists can rapidly attain high-confidence insights.

### Technical and online support: peak performance for your instruments

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.

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### Protecting your investments: unparalleled laboratory services

Unity™ 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 ensure performance throughout your laboratory.

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### Thermo Scientific Tandem Mass Tag (TMT) quantitation

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### Thermo Scientific Proteome Discoverer software

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