

thermo scientific

# Extraordinary simplified

Orbitrap Exploris 480 mass spectrometer



**ThermoFisher**  
SCIENTIFIC

# Now, achieving the extraordinary is easier



The Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer coupled to the Thermo Scientific™ Vanquish™ Horizon UHPLC system.

Obtaining comprehensive, high-confidence research insights requires new levels of instrument performance, robustness, and ease of use. Welcome to the extraordinary accuracy, certainty, confidence, and simplicity of the Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer. Become empowered to translate each research step to a new level of insight with a next-generation ownership experience coupled with an intelligence-driven experimental approach. In addition to renowned qualitative performance, you'll obtain market-leading quantitative performance to solve your most complex challenges with ease. From exploratory to targeted quantitation, find the power and reliability to make large-scale studies possible, while reducing everyday hassles to increase productivity. And achieve it all, remarkably, within a compact footprint.

## Making genius simpler

- **Next-generation user experience**

Built on our next-generation unified architecture, the Orbitrap Exploris 480 mass spectrometer delivers ease-of-use without sacrificing high performance. Hardware and software harmonization across our LC-MS portfolio reduces procedures to learn and enables you to transfer accessories and methods, streamlining your ability to translate science to the next step.

- **High-performance, high-throughput insights**

Solve complex research issues with next-level quantitative and qualitative insights. Combine an intelligence-driven approach with market-leading sensitivity and spectral quality for game-changing productivity, proteome coverage, and maximum certainty in small and large-scale studies. Ready-to-use curated workflows based on intelligent method parameters leverage enhanced instrument performance to deliver high confidence and high throughput.

- **Robust and reliable everyday performance**

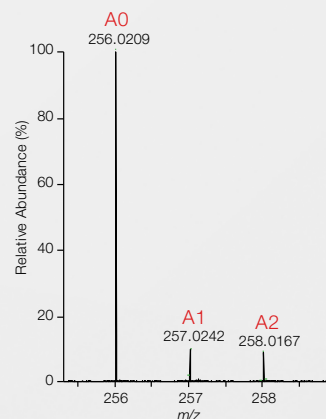
Exceptional instrument robustness and everyday reliability maximizes uptime and productivity to meet your large-scale study sample throughput requirements. The improved design architecture ensures reproducibility from one instrument to the next so you can focus your research.

- **Focus on your science, not on instrument setup**

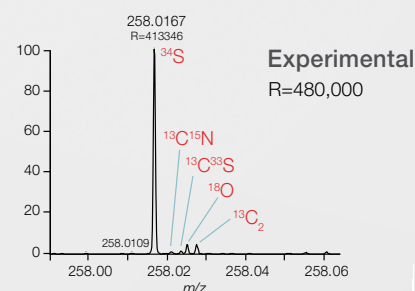
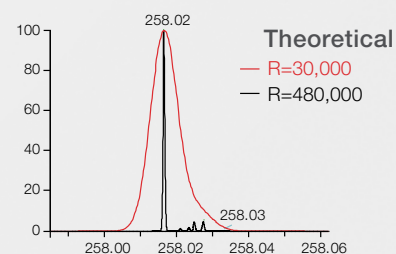
Integrated instrument control, data processing, and servicing software allow staff of all skill levels to focus on their science. Intuitive tuning and method setup, single calibration across the mass range, along with pre-defined methods, and common data acquisition schemes and components, add to usability, data quality, and throughput.

### Fine isotopic structure baseline resolution

**Sulfathiazole full scan**  
R=424,154



**Sulfathiazole A2**



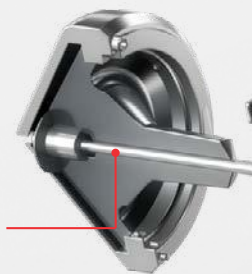
Full-scan mass spectrum of sulfathiazole, measured using the Orbitrap Exploris 480 mass spectrometer at a resolution (R) setting of 480,000 (FWHM) at *m/z* 200, demonstrates outstanding resolving power.

# Twenty years of know-how, designed into our fourth generation quadrupole-Orbitrap mass spectrometer

The Orbitrap Exploris 480 mass spectrometer combines technology refined over 20 years with advanced capabilities, intelligence-driven approaches, day-to-day reliability, and a compact footprint. Now scientists can more easily and extensively deploy MS for rigorous, high-throughput, high-sensitivity protein identification, quantitation, and structural characterization.

## OptaMax NG electrospray ion source

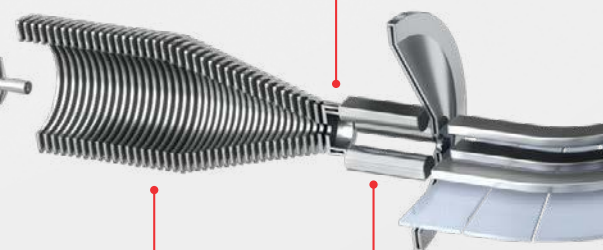
Positions reliably with easy-to-change probes and needles common across next-generation Thermo Scientific mass spectrometers



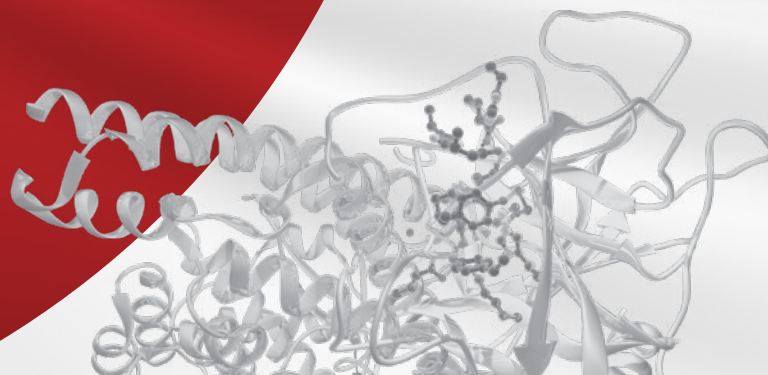
High-capacity transfer tube

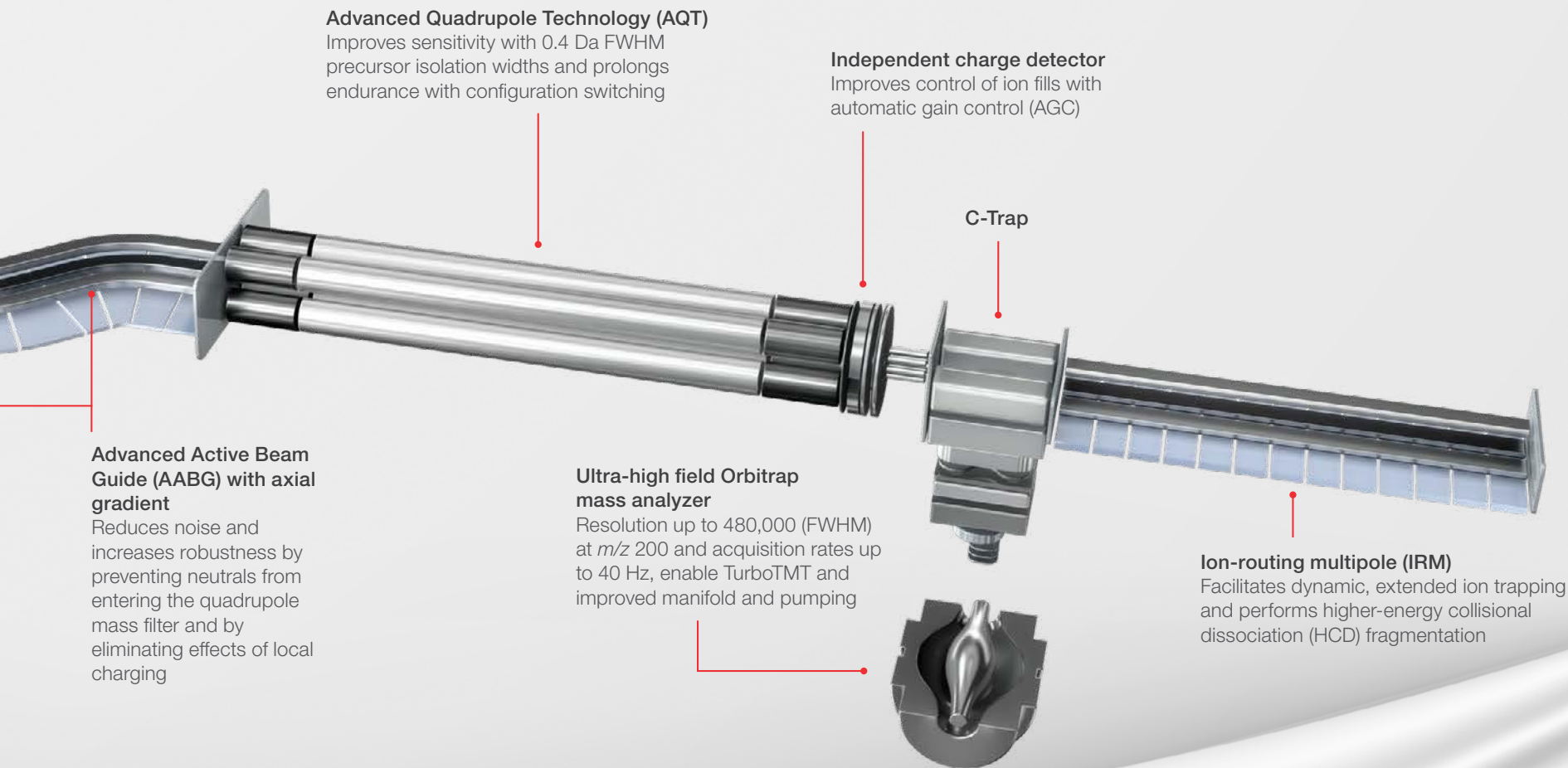
## EASY-IC internal calibration source

Ensures easy, reliable real-time mass calibration of spectra in positive and negative modes



Electrodynamic ion funnel





**Advanced Quadrupole Technology (AQT)**

Improves sensitivity with 0.4 Da FWHM precursor isolation widths and prolongs endurance with configuration switching

**Independent charge detector**

Improves control of ion fills with automatic gain control (AGC)

**C-Trap**

**Advanced Active Beam Guide (AABG) with axial gradient**

Reduces noise and increases robustness by preventing neutrals from entering the quadrupole mass filter and by eliminating effects of local charging

**Ultra-high field Orbitrap mass analyzer**

Resolution up to 480,000 (FWHM) at  $m/z$  200 and acquisition rates up to 40 Hz, enable TurboTMT and improved manifold and pumping

**Ion-routing multipole (IRM)**

Facilitates dynamic, extended ion trapping and performs higher-energy collisional dissociation (HCD) fragmentation

**Add performance and experimental flexibility**

- Thermo Scientific™ FAIMS Pro™ interface enhances precursor selectivity
- Thermo Scientific™ EASY-IC™ source provides real-time 1-ppm mass accuracy
- Thermo Scientific™ EASY-Spray™ NG ion source provides maximum nanoelectrospray performance
- Thermo Scientific™ APCI probe for the OptaMax NG ion source, upgradable to APPI
- Thermo Scientific™ BioPharma option extends mass transmission and detection up to  $m/z$  8000

# Reproducible, reliable, and robust everyday performance

Built for exceptional robustness and everyday reliability, the Orbitrap Exploris 480 mass spectrometer maximizes uptime and productivity. To meet the needs of your large-scale studies, we designed and verified individual components as well as the entire system to ensure reproducibility from one instrument to the next.

## **Sustained cleanliness, streamlined maintenance**

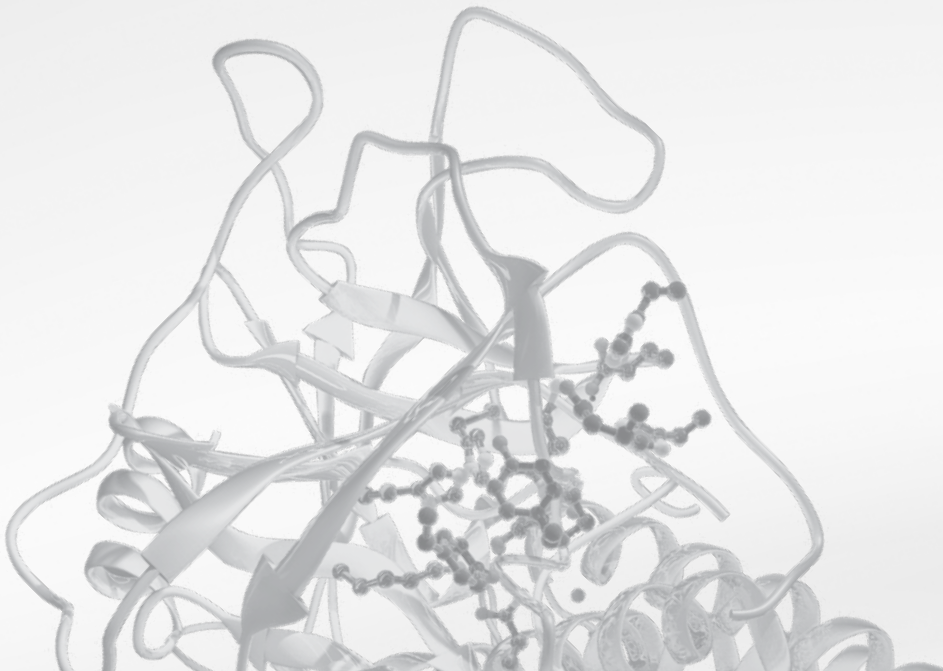
Easy-to-clean, ultra-robust ion source with probe capillary and ion transfer optics reduces quadrupole contamination to sustain sensitivity. Patented configuration switching extends quadrupole maintenance intervals up to 200%, while automatic bake-out following a power cycle saves time, getting you up and running samples faster. The six-stage pumping system controlled by a single turbo pump streamlines planned maintenance.

## **Calibrate once for all your analyses**

One robust calibration covers the entire mass range from  $m/z$  40 to 6,000 (or optionally to 8,000) with sub 3-ppm mass accuracy, making it remarkably easy to achieve consistently excellent mass accuracy for highest-quality data and confidence in results.

## **Real-time 1-ppm mass accuracy**

The standard EASY-IC source adds ease and confidence with automated internal calibrant delivery to achieve constant 1-ppm mass accuracy. The source delivers calibrant ion as a lock mass, and the instrument control software uses the known mass-to-charge ratio of the calibrant mass peak to provide real-time fine adjustment of the calibration.



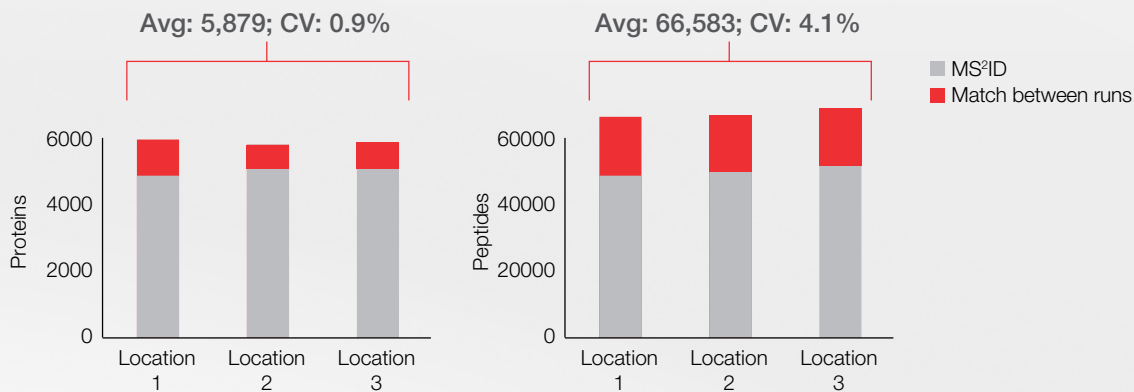


The Orbitrap Exploris 480 mass spectrometer has become our go-to system for large cohort studies. Over six months of constant operation, the instrument has delivered and maintained very high performance with minimal ongoing effort. The robustness of the system is excellent, both with and without the use of the FAIMS Pro interface, and for DIA studies which utilize high ion current.”

Jesper V. Olsen, Professor,  
The Novo Nordisk Foundation Center for Protein Research. Denmark

### Inter-system reproducibility for high-performance peptide and protein identification

The Orbitrap Exploris 480 mass spectrometer defines next-generation performance for protein and peptide identifications in its class with very high reliability across instruments—a mandatory requirement for large-scale studies.



To assess instrument reproducibility, three operators at three different locations analyzed 1 µg of Thermo Scientific™ Pierce™ HeLa Protein Digest Standard (n=5). Left figure indicates the number of proteins identified at 1% false discovery rate (FDR) and the right figure shows the number of identified peptides for each of the three locations.

# Focus on your science, not on instrument setup

The intuitive Method Editor features a drag-and-drop user-friendly interface with predefined optimized method templates for a wide range of applications and high-quality results, making method development routine and transferable.

The screenshot displays the 'Method Editor' software interface. At the top, there are tabs for 'Global Parameters', 'Scan Parameters', and 'Summary'. Below this is a 'Method Timeline' section with a horizontal axis from 0 to 120 minutes. A dropdown menu shows 'Peptide' and 'Method Duration (min)' is set to 42. The main workspace is titled 'Experiment 1' with a 'Time Range' of '0-120 min'. On the left, there is a 'Save as Template' button and a list of 'System Templates' including 'BoxCar', 'ID', 'Peptide Mapping', 'SureQuant', and 'TMT'. A red circle highlights the 'SureQuant' template. In the center, a list of templates is shown, with 'PQ500 Plasma - SureQuant' highlighted by a red circle. To the right, a flowchart diagram illustrates the scan sequence, starting with a 'Full Scan' followed by four parallel 'Targeted Mass' scans, each with 'Trigger' and 'MS1' sub-steps. A red circle highlights the 'Full Scan' step in the flowchart. A 'Properties' panel is visible on the far right.

Effortlessly run advanced experiments with less expertise across a variety of applications using verified method templates.

Ready-to-use templates combined with intelligent data acquisition and predefined instrument parameters enable generation of high-quality data regardless of analyte of interest.



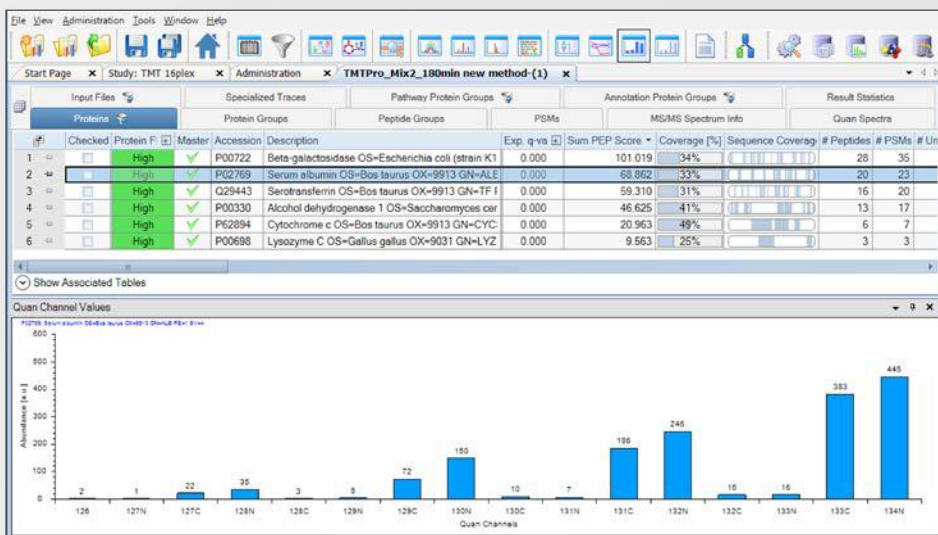
# Cutting through complexity to deliver real insights

Comprehensively characterizing potentially complex proteins requires advanced data processing technologies. Thermo Scientific™ Proteome Discoverer™ and Thermo Scientific™ BioPharma Finder™ software can interrogate data to deliver real insights and the complete picture for your protein analyses.

## Enable complete characterization of biotherapeutic proteins

Whether you are performing native or denatured intact protein mass analysis, top- and middle-down analysis, or peptide mapping workflows, BioPharma Finder software rapidly provides the insights your data contain.

Workflows facilitate comprehensive data interpretation and visualization, allowing you to confidently characterize biologics with speed and ease. From novel deconvolution algorithms that generate complete results, to easy-to-understand data visualization tools, the software helps you confidently characterize proteins.



Example of TMTpro 16-plex support in Proteome Discoverer software revision 2.4 and above.

## Identification and quantitation of proteins in complex samples

Proteomics research requires more than just identification. Proteome Discoverer software simplifies the identification and quantification of proteins in complex biological samples for a wide range of proteomic workflows, from protein and peptide identification, to PTM analysis, isobaric mass tagging, and both SILAC and label-free quantitation.

Mapping quantitation channels to biological information reveals the biological context with automated protein identification and annotation using gene ontology (GO) classifications, PTM sites, and literature references from public databases, while results are easily visualized and statistically validated.



Historically it took weeks to evaluate data, ruling out many false positives. BioPharma Finder software is a unique solution that has efficient workflows meaning that you don't need to spend hours fine-tuning parameters to get the right answer the first time. For comparative quantitative modification analyses, we have gone from two weeks of analysis down to one day for multi-batch samples."

Guilong Charles Cheng, Alexion Pharmaceutical Inc., USA

# Maximum certainty for deeper, faster qualitative proteomics

Obtaining high-quality protein and peptide identifications from complex biological samples over a wide dynamic range with maximum sensitivity is a challenging but necessary aspect of bottom-up proteomics, and the foundation of all discovery research whether for protein structure-function studies or protein quantitation. Superior mass resolution, accuracy, speed, and intelligent data acquisition approaches maximize the richness and certainty of identifications. With the Orbitrap Exploris 480 mass spectrometer, you make more unique peptide identifications per unit time, down to the single cell level, shortening your time-to-results so you can go beyond what you thought possible.



With FAIMS, a reduction in the precursor interference problems associated with the use of isobaric labeling in quantitative proteomics experiments can be achieved. Our research demonstrates FAIMS is now a reliable and straightforward technology to be incorporated as part of standard proteomics workflows.”

Pierre Thibault, Principal Investigator,  
Proteomics and Bioanalytical Mass Spectrometry Research Unit,  
IRIC Proteomics Core Facility Scientific Direction, Canada

## Application areas that can benefit from FAIMS

- Peptide identification
- Peptide quantitation
- TMT quantitation
- Crosslinked protein structure
- Data-independent acquisition (DIA)
- Single cell proteomics

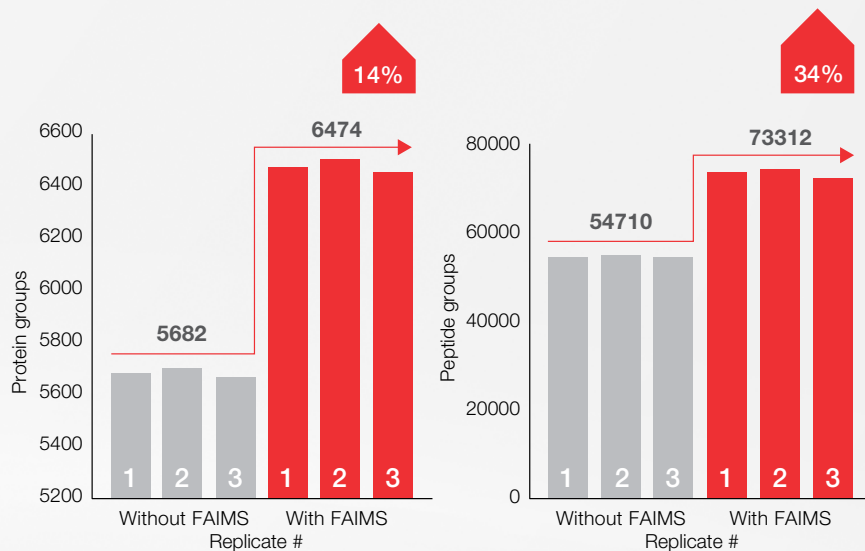


The effortless selectivity provided by the optional FAIMS Pro interface with the Orbitrap Exploris 480 mass spectrometer and the Thermo Scientific™ EASY-nLC™ 1200 system accelerates proteomics workflows with even broader and deeper analysis.

## FAIMS increases identifications for high-throughput studies

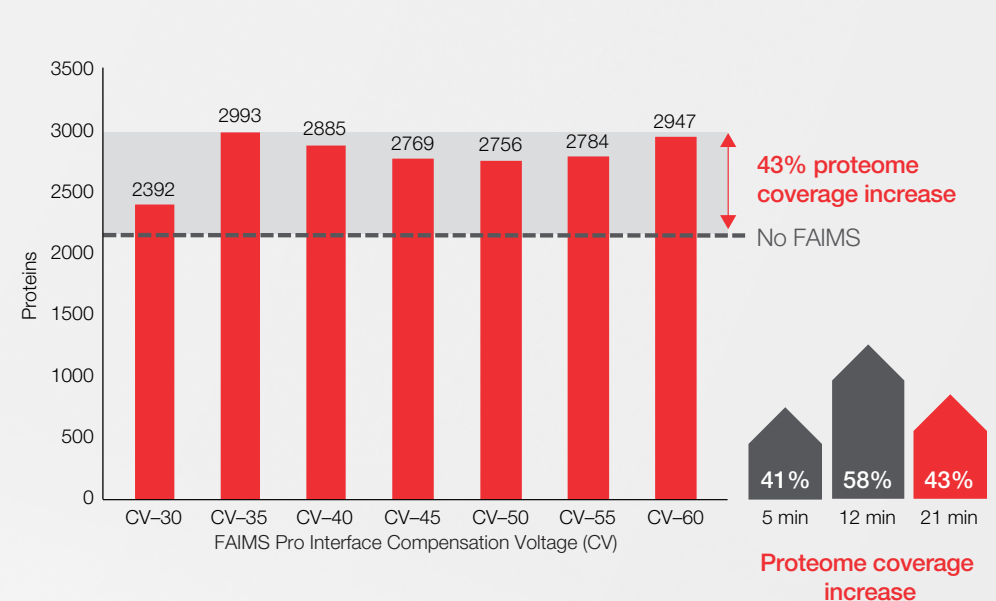
The Thermo Scientific™ FAIMS Pro™ interface minimizes co-isolation of isobaric peptides, increases the number of unique peptides identified, and improves signal-to-noise to sample low-abundance peptides, maximizing proteome coverage. Multiple CV (compensation voltage) settings may be repetitively sampled to augment protein and peptide identification rates using the same experimental parameters.

### Increase peptide and protein coverage



The Orbitrap Exploris 480 mass spectrometer together with the FAIMS Pro interface provides maximum coverage and outstanding reproducibility. In the analysis of a 200 ng HeLa digest over a 120-minute gradient with multiple intra-analysis CV steps between -50V and -70V, approximately 6,500 protein groups and 74,000 peptide groups were identified. Peptide group and protein group identifications were reproducible with <2% and <1% coefficient of variation respectively. The false discovery rate (FDR) was 1%.

### Increase proteome coverage up to 2x while decreasing run time



500 ng HeLa Digest using Evosep One LC system (Evosep Biosystems, Odense, Denmark), over 5, 12, and 21-minute gradient, analyzed using the FAIMS Pro interface. Proteome coverage was increased from 41% to 58% for gradients ranging from 5 to 21 minutes. The interface significantly increases protein coverage with short gradients enabling high-throughput applications. Increased proteome depth was observed for all single compensation voltages from CV-30 to CV-60, and across three different gradient lengths.

Data courtesy of Jesper V. Olsen, Professor, The Novo Nordisk Foundation Center for Protein Research.

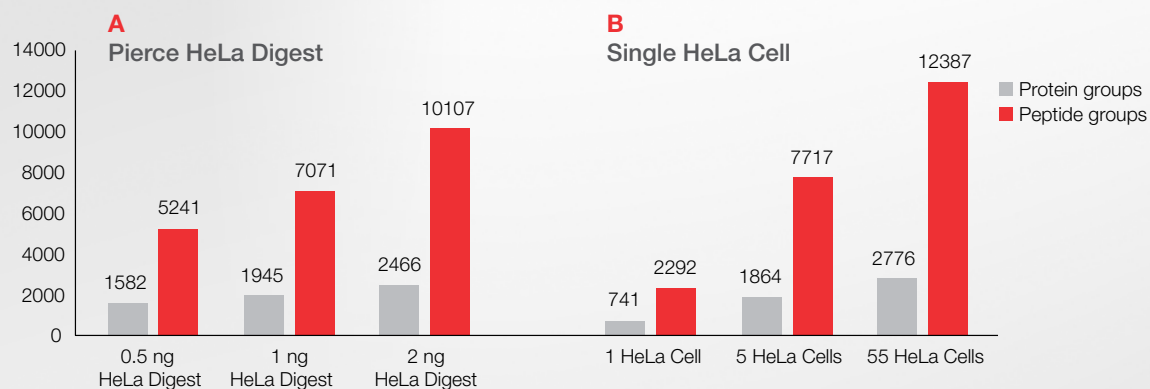
## FAIMS enhances sensitivity for limited samples and single-cell proteomics

Bottom-up proteomics workflows are now routine with the analysis of hundreds of nanogram to microgram sample amounts, representing the protein profile of a few hundred-thousand cells.

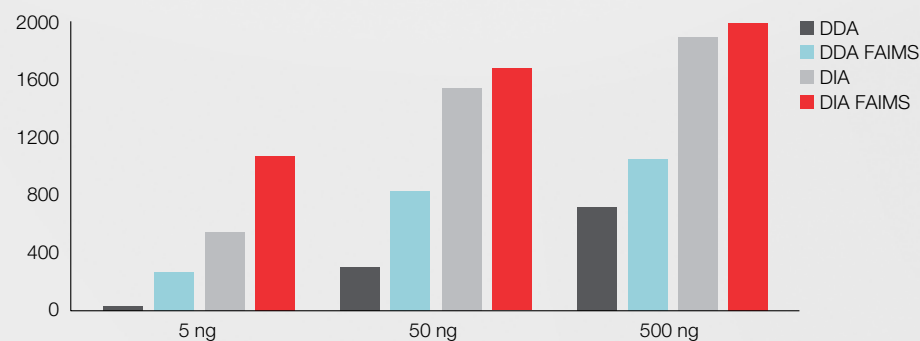
For large sample cohorts where throughput and sensitivity are priorities, the Orbitrap Exploris 480 mass spectrometer coupled with the FAIMS Pro interface and a data-independent acquisition strategy offers a boost in sensitivity for short gradients.

Most importantly, the DIA-FAIMS Pro interface workflow does not compromise the quantitative accuracy obtained for the additional proteins identified. Most are reproducibly quantified with a coefficient of variation below 10%\*.

However, many biologically relevant proteins are only present in trace amounts. With single-cell level sensitivity, the Orbitrap Exploris 480 mass spectrometer delivers more complete biological insights from low-level proteins to rare and individual cells, overcoming the challenges of identifying only the most abundant proteins.



The Orbitrap Exploris 480 mass spectrometer with the FAIMS Pro interface provides sensitivity from low-ng to single-cell levels. (A) Instrument performance at low nanogram levels (0.5-2 ng Pierce HeLa Digest Protein Standard), (B) Instrument performance at single-cell levels (1, 5, and 55 HeLa cell).



Combining the Orbitrap Exploris 480 mass spectrometer with the FAIMS Pro interface and a DIA strategy enables sensitive analysis for short gradients, high throughput (>200 samples per day), and identification of 2500 peptides per minute of gradient time. Overall, proteome coverage is increased by over 35% compared with data-dependent acquisition (DDA).

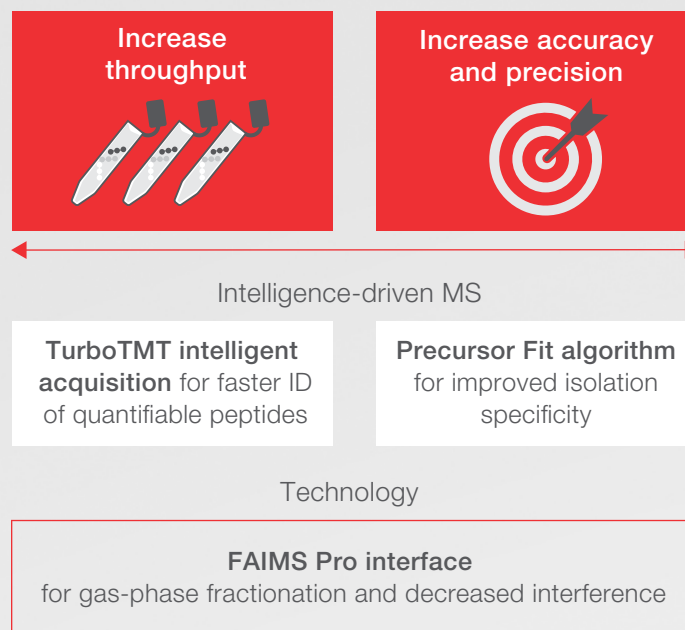
\* Jesper Olsen et al. <https://www.biorxiv.org/content/10.1101/860643v1>

# Best-in-class TMT multiplexed proteome quantification

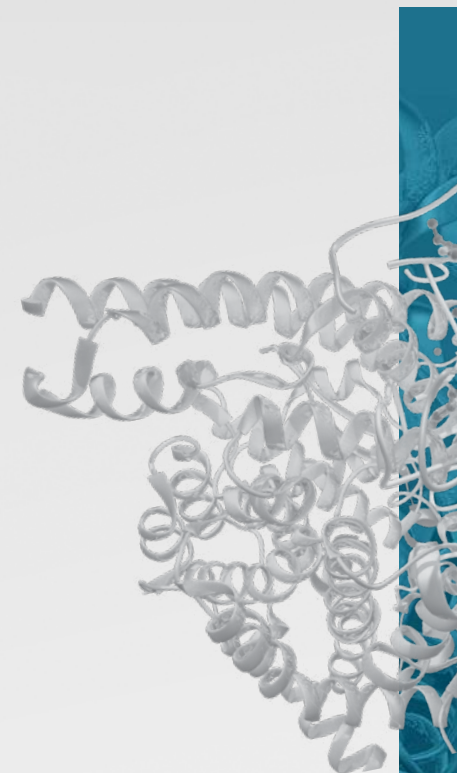
Isobaric tagging strategies using Tandem Mass Tags (TMT) are powerful tools for functional biology studies and analyzing large sample sets. The Thermo Scientific™ Tandem Mass Tag (TMT)™ MS<sup>2</sup> workflow delivers best-in-class quantitation for multiplexed proteome analysis. By leveraging intelligent data acquisition methods, you can confidently quantify proteins with minimal setup. In combination with TMT reagents and the FAIMS Pro interface, the Orbitrap Exploris 480 mass spectrometer has the resolving power and speed to perform Thermo Scientific™ TMT11plex™ and TMTpro™ 16plex experiments with unrivaled confidence and no compromise in coverage or depth.

## Next-level quantitative precision, accuracy, and throughput

Orbitrap mass spectrometer-based high-resolution accurate-mass (HRAM) MS with TMT-based quantitation is the most frequently published, leading isobaric tagging application available. We continue driving improvements to deliver the quantitative accuracy and precision needed to meet your research goals.



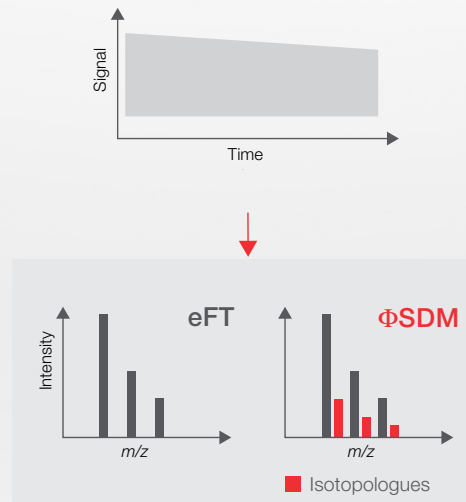
Intelligence-driven acquisition algorithms, and FAIMS Pro interface technology deliver highest-quality TMT MS/MS (MS<sup>2</sup>) quantitation.



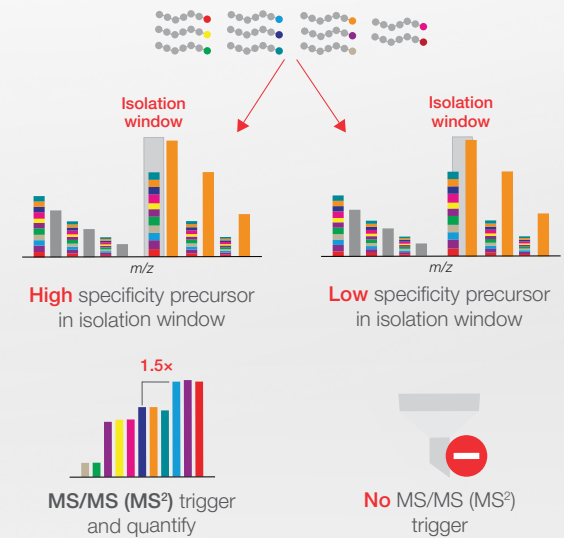
## Raw spectra to highest-quality data

TurboTMT intelligent acquisition based on novel  $\Phi$ SDM spectral processing further improves resolution to baseline resolve TMT reporter ion isotopologues and speed up spectral acquisition for TMT11plex experiments, increasing throughput for identification of quantifiable peptides.

The Precursor Fit algorithm reduces co-isolated ion interferences that mask true differences in protein abundance. Precursors are selected on highest specificity within the isolated window, resulting in highest MS/MS ( $MS^2$ ) quality for better quantitative accuracy and proteome depth.

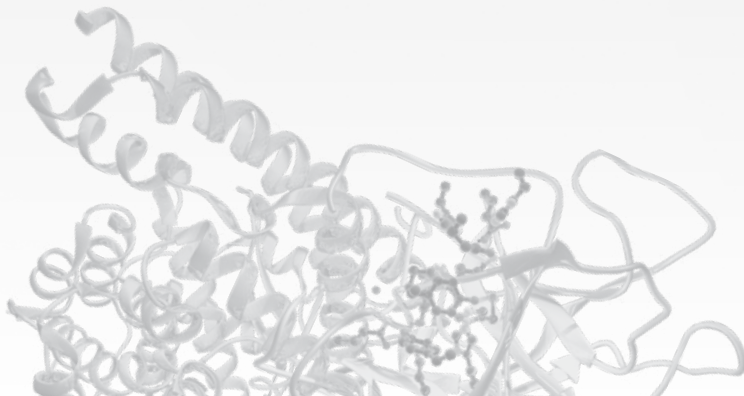


TurboTMT intelligent acquisition improves resolution to resolve reporter ion isotopologues



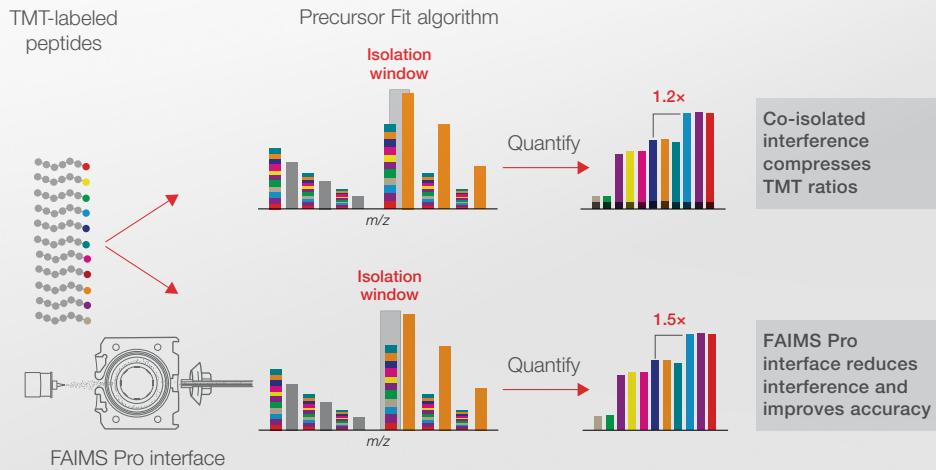
Precursor Fit algorithm improves isolation specificity

Intelligent data acquisition strategies and algorithms with enhanced mass spectrometer technology produce highest-quality data.

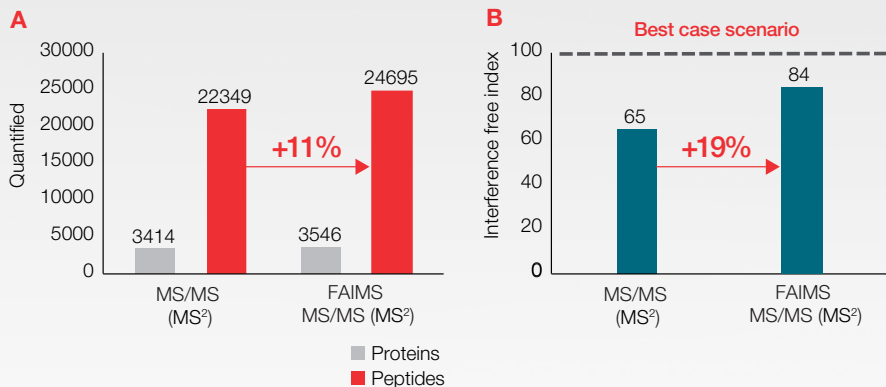


## FAIMS reduces interferences for more accurate quantitation

Incorporating the FAIMS Pro interface in the workflow increases precursor selectivity and reduces interference using gas-phase fractionation, resulting in greater accuracy for TMT-based quantitation.



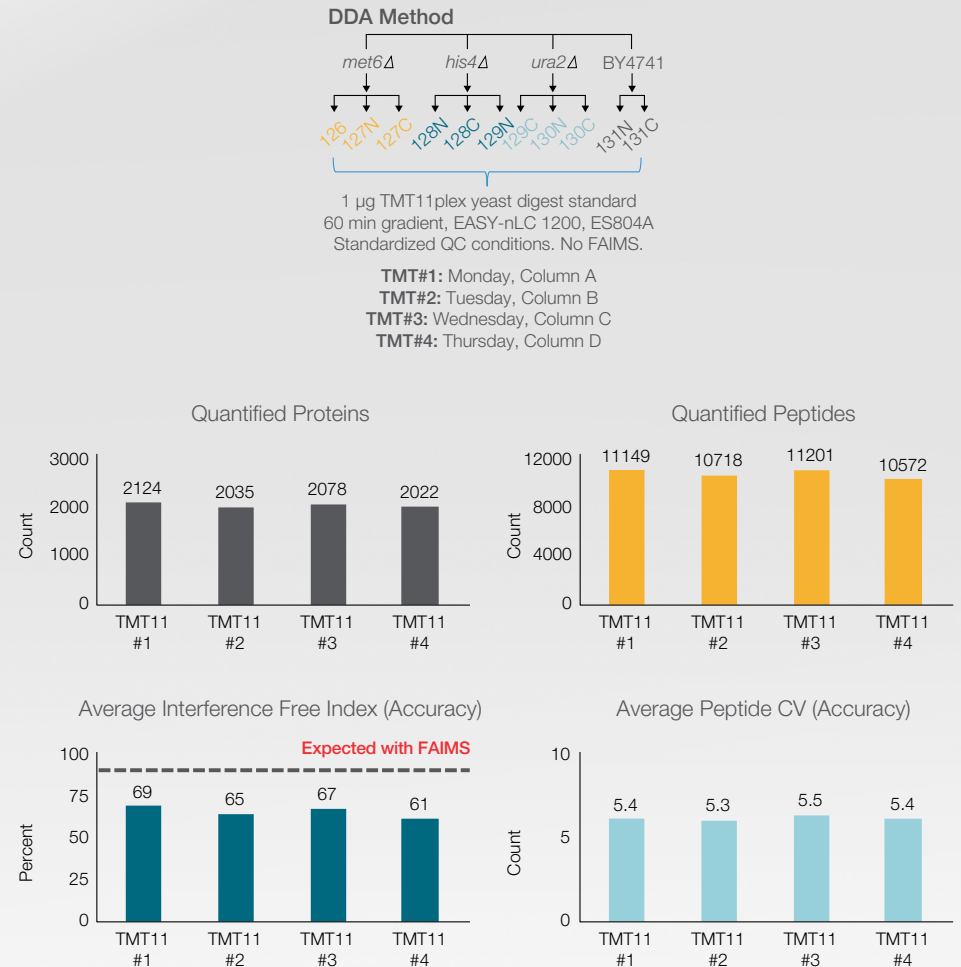
Thermo Scientific technology adds quantitative precision, accuracy, and throughput. The Precursor Fit algorithm increases precursor specificity in the isolation window, reducing co-isolated interferences.



The combination of the FAIMS Pro interface, TurboTMT mode, and Precursor Fit algorithm with the Orbitrap Exploris 480 mass spectrometer enables the TMT MS<sup>2</sup> workflow to deliver TMT11plex results faster with (A) increased proteome depth and quantifiable peptides and (B) improved quantification accuracy. 1 µg of TMT11plex yeast digest standard was analyzed using a four-hour gradient.

## Best-in-class reproducibility

The Orbitrap Exploris 480 mass spectrometer delivers everyday reproducibility and QC performance capabilities necessary for high-throughput multiplexing studies.



TMT11plex analysis of 1 µg of a yeast digest standard analyzed using a 60 minute gradient per injection. Interference Free Index (IFI): based on *met6Δ*, *his4Δ*, or *ura2Δ* yeast strains used to assess ratio distortion for TMT quantitation.

# New paradigm in targeted protein quantitation

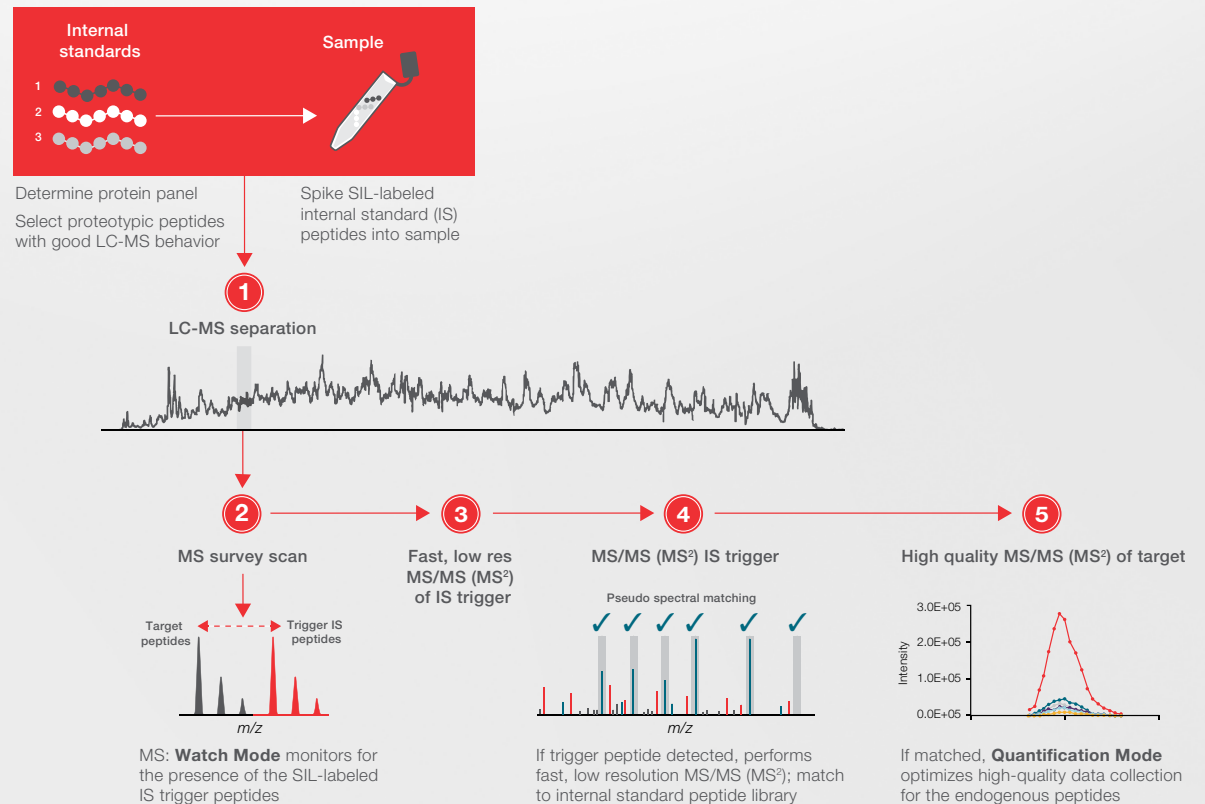
Verification of biomarkers and protein expression studies requires sensitive multiplexed quantitation of hundreds of protein targets, often in large sample studies. The Thermo Scientific™ SureQuant™ IS Targeted Protein Quantitation workflow enables life scientists to quantify proteins with the highest confidence with unique intelligent real-time monitoring of targets. By leveraging labeled internal standard peptides to guide the acquisition of endogenous peptides in real time, reliable absolute quantitation of hundreds of targets is possible with easy setup and no compromises. In combination with Thermo Scientific™ SureQuant™ targeted assay kits, we offer a complete assay, including sample preparation, for monitoring and quantitation of target peptides in a single sample.

## Precise quantitative results for targeted studies

To meet today's challenges, targeted quantitation methods had to evolve from standard selected reaction monitoring (SRM) to precise quantitation of a limited peptide using high-resolution MS. New strategies enable uncompromising quantification of more targets with high precision and accuracy, as well as easy setup to address biomarker development and biological questions in pathway monitoring studies.

## The new gold standard: Intelligence-driven acquisition for highest quantitative performance

By leveraging SureQuant targeted assay kits, the SureQuant method provides an easy-to-use assay that delivers quantitation of more targets without compromising sensitivity. The intelligence-driven SureQuant method uses IS to guide and dynamically manage quantitation of peptide targets, while maintaining the highest accuracy and precision.



Spiked stable-isotope-labeled (SIL) internal standards are monitored in “watch mode” as the instrument rapidly scans for the presence of the IS peptides. When an IS is detected, an MS/MS (MS<sup>2</sup>) scan is triggered and fragment ions are matched to an IS reference list. If matched, the method switches to “quantification mode” where spectra for the endogenous target peptides are acquired at high resolution for accurate quantitation.



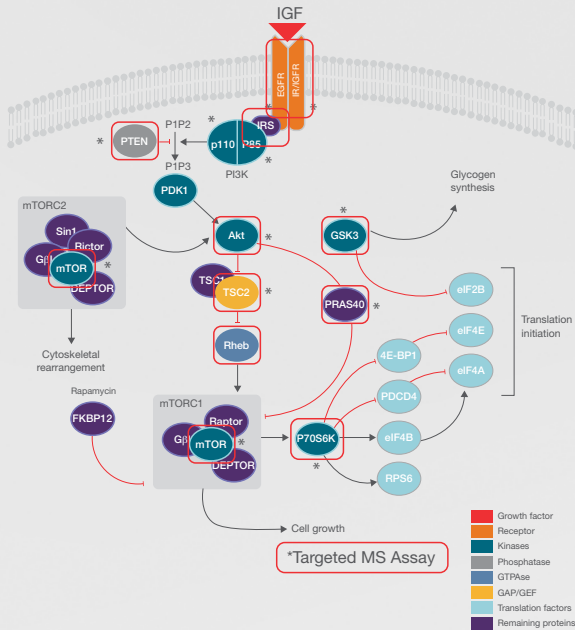
## Sensitive, accurate, precise, and more complete targeted protein analysis

Compared to traditional methods, the SureQuant method increases resolution and ion fill time, without compromising cycle time, resulting in higher sensitivity and more reliable detection of peptide targets.

## Flexible with higher throughput for targeted quantitation of pre-defined or custom peptides

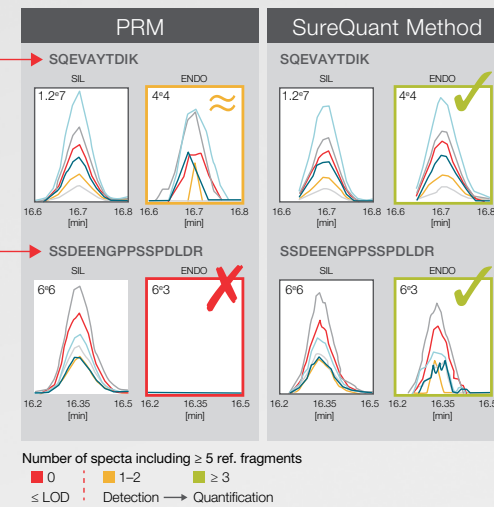
The SureQuant method is flexible and can be used to quantify custom peptide targets, and monitor large panels of proteins, such as in plasma with the PQ500 human plasma kit (Biognosys AG, Schlieren, Switzerland), with outstanding dynamic range and quantitative precision, making it possible to increase throughput without any loss in reproducibility.

### AKT/mTOR key cancer biology pathway



### From cancer pathway to reliable assay

Peptide	PRM Method	SureQuant Method
AENGPGVGLVLR	1	15
AQEALDFYGEVLR	10	11
DGFYPADDFR	0	1
ETSFNQAYGR	2	6
FEISETSVNLR	0	9
GALAEAAAR	5	13
GNNLQDTLR	0	6
GQPEGPLPSSSPR	0	8
GYTISDSAPSR	0	5
DIHSQ[Carbamidomethyl]NHEAEK	1	12
IYNLC[Carbamidomethyl]AER	0	15
LQ[Carbamidomethyl]DSGELVAIK	5	15
LFDAPAAPLSR	11	11
LLEYTPRAR	0	15
LPFYNQDHER	0	17
LVPPFKPQVTSQDTR	0	0
NDGTRFGYK	0	13
NNIDVVR	0	4
SDGSFQYK	0	13
SNPTDIYPSK	0	9
SQEVAYTDIK	1	9
SSDEENGPPSPDLDR	0	8
SVSAPQIINPIR	0	0
TFHVDTPPEER	0	0
TGIAAEVSLPR	0	0
TLDSQSPER	2	10
TPPEAIALQ[Carbamidomethyl]SR	1	20
TTINNEYNYR	0	7
VTTVATLGQGER	6	15
YSFQTHDR	0	10
# of endogenous peptides detected	11/30	26/30

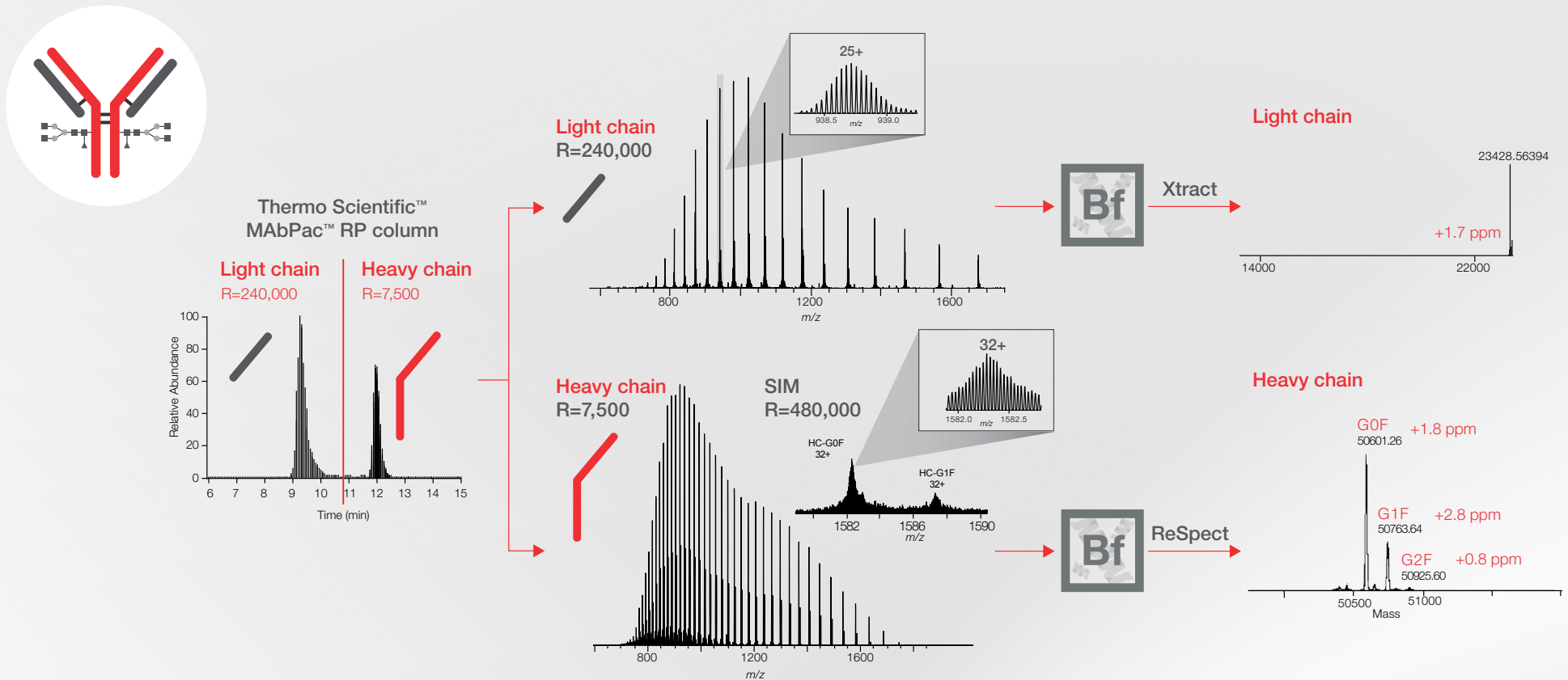


Number of spectra including  $\geq 5$  ref. fragments  
 ■ 0 ■ 1-2 ■  $\geq 3$   
 ≤ LOD ■ Detection → Quantification

Compared to PRM methods, the SureQuant method increases resolution by a factor of four, and fill time by a factor of six, without compromising cycle time. The result is higher sensitivity and more reliable detection of targets, as shown here for peptides from the protein targets GSK3 $\alpha$  and AKT1 from the AKT/mTOR pathway—a key cancer biology pathway.

# Spectral quality and sensitivity simplify Biopharma analyses

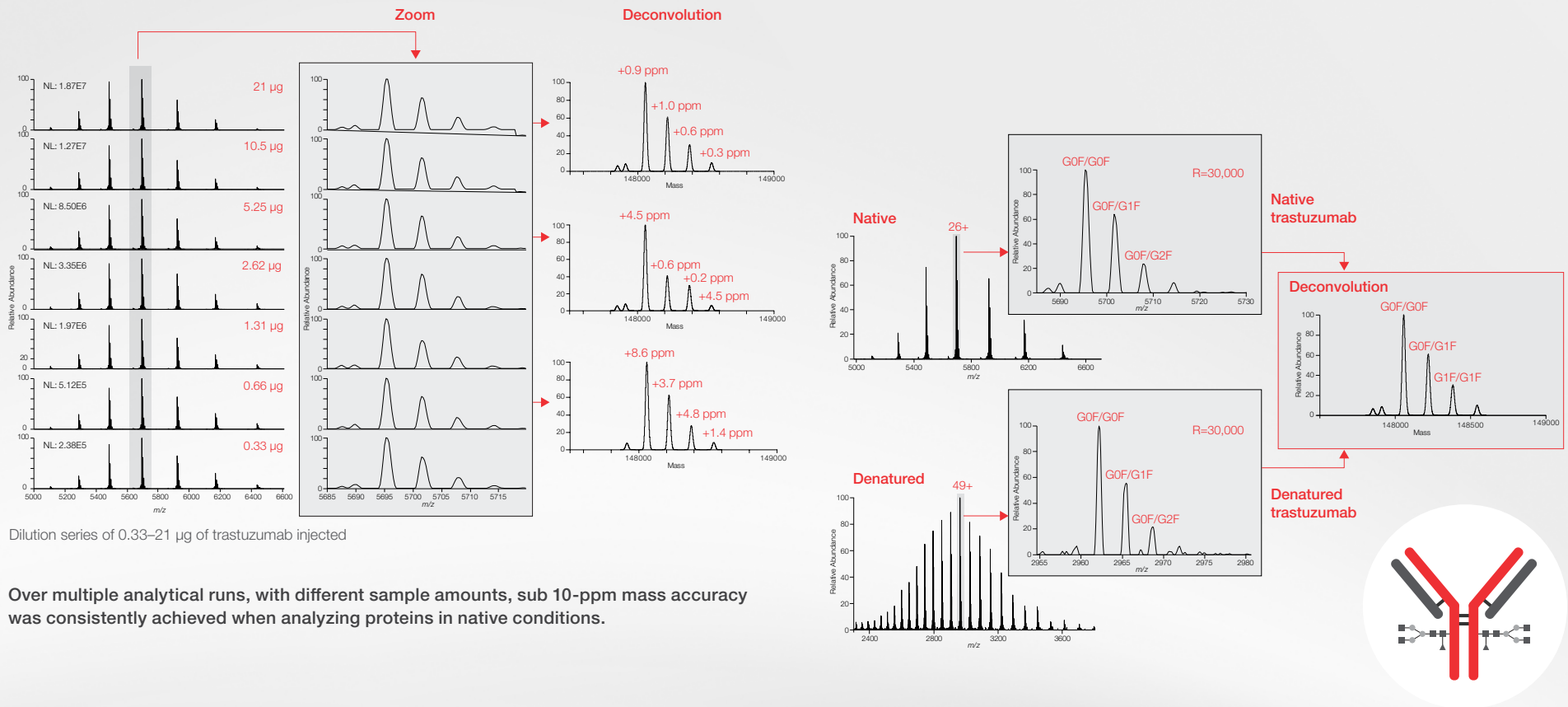
Achieve simple, robust, and high-spectral-quality intact-mass data acquisition for denatured protein analysis. The Orbitrap Exploris 480 mass spectrometer pushes the boundaries of routine analyses with added resolving power of up to 480,000 (FWHM) at  $m/z$  200. Obtaining baseline resolved peaks for a 50kDa heavy chain is now simple, providing deeper insights than previously were possible. HRAM analysis of the protein chains of monoclonal antibodies at the subunit level is easy, rapidly producing the answers you require with high mass accuracy and confidence.



Achieving structural insights at the subdomain level are effortless. Trastuzumab was reduced, and the light and heavy chains separated by reversed-phase liquid chromatography. The light-and heavy-chain spectra obtained were deconvoluted using the BioPharma Finder software Xtract and ReSpect algorithms, respectively. Information on the heavy chain with the glycoforms was revealed, while monoisotopic masses for the light chain were also provided.

## Routinely high-resolution for intact denatured and native protein analyses

The BioPharma option enables analysis of intact therapeutic proteins under native-like conditions. Superior ion transmission allows use of high-resolution settings to resolve protein modifications such as glycosylation and conjugations. Isoforms and glycoforms are easily and confidently differentiated and determined when analyzed in their intact native and denatured conditions for maximum structural insights.



Dilution series of 0.33–21 µg of trastuzumab injected

Over multiple analytical runs, with different sample amounts, sub 10-ppm mass accuracy was consistently achieved when analyzing proteins in native conditions.

Easily achieve accurate and robust native protein characterization with mass detection up to  $m/z$  8000. Confirm the glycoform profile under native and denatured conditions with exceptional data quality and spectral clarity to simplify interpretation.



## **Almanac web-based monitoring and management**

Stay connected to your science. See how the Thermo Scientific™ Almanac™ application can help you get the most out of your instruments.

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

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