WHITE PAPER 65659

Grant application resource: Using the Orbitrap Exploris 480 mass spectrometer to accelerate fundamental research

Keywords: Single Cell Sensitivity, TMT & TMTpro Multiplexing, SureQuant Method, Quantitative Proteomics, Orbitrap Exploris 480 MS, FAIMS Pro interface, Mass Spectrometry

Goal

This document highlights why the Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer is the premium quantitative high resolution, accurate mass (HRAM) Orbitrap system in category that delivers fastest time to results. With technology innovations and curated ready-to-use application specific method templates, this system delivers complete quantitative strategies for large scale studies, from untargeted proteome profiles to novel targeted experiments that enable quantification for proteins without compromising accuracy, coverage or depth. With industry-leading single cell level sensitivity, the Orbitrap Exploris 480 MS system is designed for proteomics scientists in research and core laboratories looking to expand into new biological research areas with the highest confidence and rigor that lead to impactful publications and results. The information provided here highlights technologies that are different from Q Exactive instrumentation as well as Quadrupole-Time of Flight (QTOF) analyzers.

Summary

Protein identification serves as a foundation for proteomics, with the need to identify more and at higher levels of sensitivity than ever before. Most proteomics studies rely on bulk samples with the analysis of thousands of proteins from nanograms to microgram sample amounts which represent the protein profile of a few hundred to thousands of cells in aggregate. Single cell analysis allows for insights that assess cell heterogeneity and requires more sensitive instrumentation than ever before that can deliver more proteins per cell from tens to hundreds of cells per day to analyze cell populations, cell types and states, on a cell-to-cell basis. This new level of sensitivity is essential for limited samples and for rare cells such as circulating tumor cells and requires the most advanced instrumentation. Because of the dynamic and interactive nature of proteins, quantitative

proteomics is considerably more complex than simply identifying proteins in a sample. Quantitative strategies such as label-free quantitation, multiplexing isobaric labeled proteomes or targeted quantitative proteomics — all with a new standard of sensitivity, accuracy and precision can provide further understanding of global protein kinetics and foster new biological discoveries.

Here, we highlight how next generation technologies in mass spectrometry hardware and software innovations detailed in Figure 1 and Table 1 achieve increased sensitivity and selectivity on the Orbitrap Exploris 480™. Combination of this intelligence-driven mass spectrometry with market-leading sensitivity and spectral quality, provides unprecedented proteome coverage, and maximum certainty in small and large-scale studies. Ready-to-use curated application specific method templates based on intelligent method parameters leverage enhanced instrument performance to deliver high confidence data and high throughput required to improve productivity of a proteomics core facility or laboratories focused on measuring and quantifying molecular components of cells.

We outline how quantitative performance and sampling throughput efficiency increase using differential ion mobility with the FAIMS Pro interface to enhance single cell proteomics, how sample multiplexing with Tandem Mass Tags™ (TMT™) increases throughput using Thermo Scientific™ TMT11 plex or TMTpro™ 16plex isobaric tags, as well as the ability to quantitate several hundreds of protein targets by the SureQuant™ method with an enhanced user experience (Table 2). Together, these advances allow for maximum quantitative insights that are precise and accurate with high confidence from whole proteome profiling/quantitation to targeted quantitation. With single cell level sensitivity, this enables a more complete biological picture with more proteins identified and quantified as well as the reality of single cell proteomics being accessible to the proteomics community.

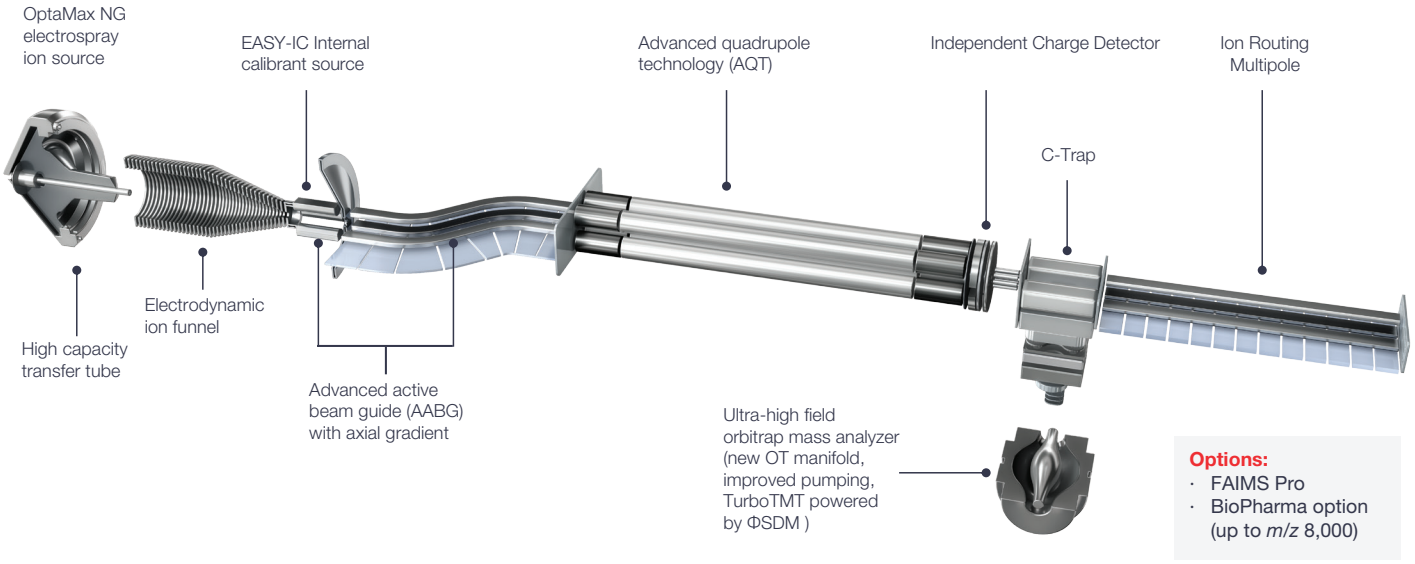

Figure 1: Schematic of fundamental features on Orbitrap Exploris 480 MS technology. Overall improvements provided on this new compact MS give the reason to upgrade.

Table 1. Technology specifications for fundamental benefits of Orbitrap Exploris 480 MS instrumentation.

Features	Benefits	Technology
Ultra-high resolution	High selectivity to resolve analytes down to a few mDa	480,000 (FWHM) at *m/z* 200
Sub-ppm mass accuracy	High selectivity and confidence in molecular formula	EASY-IC internal calibrant source
Speed	Fast scan rates for improving protein identification and quantitation	Up to 40 Hz at resolution setting 7,500 (FWHM) at *m/z* 200
Easy-to-use software	Pre-built method templates that are fully customizable using a drag-n-drop flexible user interface	Orbitrap Exploris instrument control software
Orthogonal selectivity for single cell proteomics	Improves signal-to-noise, reduces interferences, and increases the number of peptides and proteins identified.	FAIMS Pro interface (optional)
Acquisition modes including: DDA, DIA, PRM, SureQuant, TMT & TMTpro, BoxCar DDA & DIA	Multiple novel methods that improve protein and peptide quantitation for discovery or target verification	SureQuant IS Protein Quantitation TurboTMT power by ΦSDM BoxCar DDA and DIA templates

Table 2. Application specific technology comparisons with the Orbitrap Exploris 480 MS.

Applications area	Orbitrap Exploris 480	Q Exactive	QTOF
Single cell analysis/ limited sample	HRAM, FAIMS Pro interface, fully customizable method templates	HRAM	Low resolution, less accurate mass
DDA & DIA	Up to 40hz, fully customizable method templates	Up to 40hz, Pre-build method templates	Fast scanning with lower quality data
TMT11plex & TMTpro 16plex	FAIMS Pro interface, TurboTMT, precursor fit filter, fully customizable method templates	Pre-build method templates	Low resolution limits to TMT6plex or TMTpro 9plex
SureQuant Method	RT independent, fully customizable method templates , embedded pre-set method templates for commercialized kits.	Not available	Not available

Additional highlights

Application areas that can benefit from Orbitrap Exploris 480 MS with FAIMS Pro interface

- Peptide/protein identification
- Peptide/protein quantitation
- Sample multiplexing with TMT 11plex and TMTpro 16plex quantitation
- Targeted quantitation with SureQuant™ methods
- Data dependent acquisition (DDA) for label free quantitation (LFQ)
- Data independent acquisition (DIA)
- BoxCar acquisition for DDA or DIA methods
- Single cell proteomics

Benefits for proteomics

- Wider mass range 40-6000 *m/z* (optionally up to 8000)
- Higher resolutions up to 480,000 (FWHM) at *m/z* 200
- Sub-1ppm mass accuracy with Thermo Scientific™ EASY-IC™ source
- Higher uptime/improved robustness due to Advanced Quadrupole Technology (AQT) with novel, patented Configuration Switch Mode (CSM).

Single cell-level sensitivity

We have demonstrated label-free single cell proteomics performance on the Orbitrap Exploris 480 mass spectrometer coupled to the FAIMS Pro interface, enabling researchers to investigate cellular heterogeneity as well as rare cells or limited samples with this ultra-sensitive, high throughput LC-MS analysis. The FAIMS Pro interface together with the Orbitrap Exploris 480 mass spectrometer provides high selectivity and sensitivity required to analyze single cell proteomes. Figure 2 demonstrates unmatched sensitivity of the system yielding 741 proteins identified in a single HeLa cell, and 1864 proteins from 5 cells which were sorted by flow-cytometry. This platform also supports quantitative analysis of single cells (1), which can be accomplished in high throughput with the combination of TMT 11plex and TMTpro 16plex for increased sample throughput with precision quantitation (2). These application areas and how FAIMS Pro interface improves selectivity are discussed in the following sections.

FAIMS Pro interface increases sensitivity and selectivity

The FAIMS Pro interface is a differential ion mobility device that can be seamlessly added to existing workflows such as single cell proteomics or TMT multiplexing to improve the signal-to-noise of low-abundance peptides and thus maximize sensitivity. Multiple compensation voltages (CV) settings may be repetitively sampled to increase the number of unique peptides identified, and minimize co-isolation of isobaric peptides, while reducing time-consuming offline fractionation. As shown in Figure 3, utilization of FAIMS Pro interface boosts protein identification rates for single cell and low nanogram sample loads by greater than 50%.

The orthogonal selectivity of gas-phase fractionation adds efficiency to proteomics workflows, increasing productivity and data quality for every user from discovery of disease biomarkers to identification of new therapeutic targets. Differential ion mobility reduces the complexity of precursor ions accumulated and analyzed per CV setting, increasing proteome coverage, decreasing interference, and improving quantitative confidence.

Unmatched sensitivity from single cells to low nanogram sample injections.

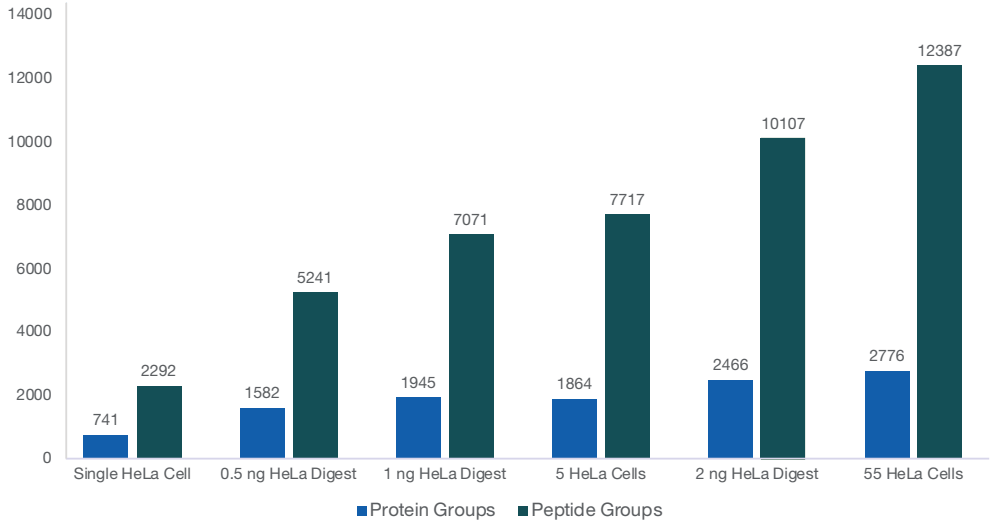

Figure 2. Protein digests from individual single cells or peptides from Pierce HeLa digest standard were analyzed using a 120min gradient on Orbitrap Exploris 480 MS. FAIMS Pro interface was used for online gas-phase peptide fractionation with intra-analysis CV stepping, CV -60 and -75. Protein Group identification with Thermo Scientific™ Proteome Discoverer™ 2.4 software was performed with SEQUEST HT at 1% False Discovery Rate (FDR) at MS^2 level.

Orthogonal selectivity with FAIMS Pro interface increases identifications by 50%.

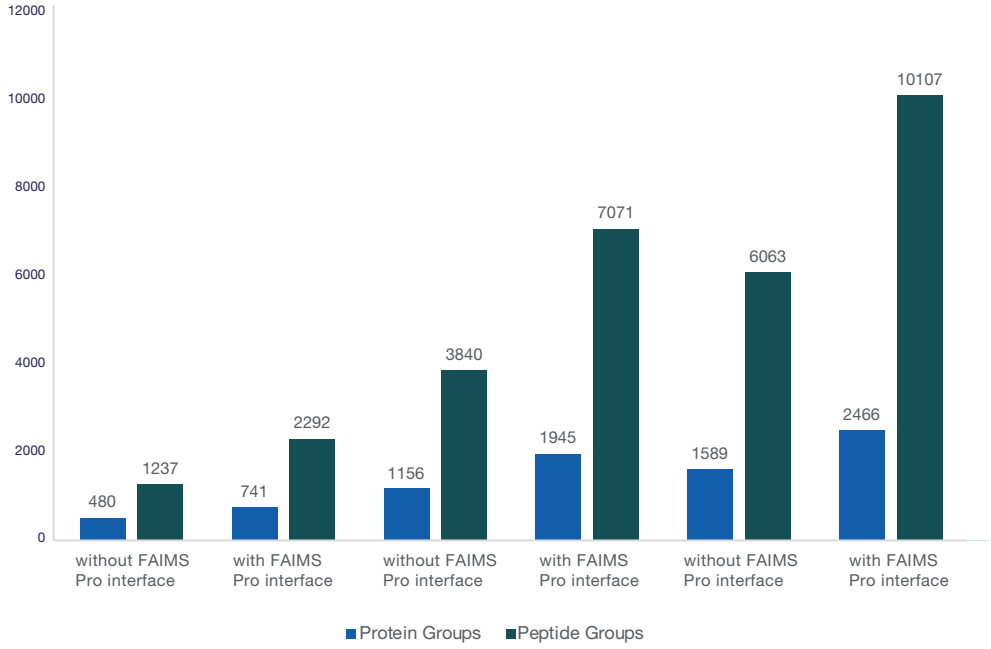

Figure 3. Protein digests from individual single cells or peptides from Pierce HeLa digest standard were analyzed using a 120min gradient on Orbitrap Exploris 480 MS with or without the FAIMS Pro interface using intra-analysis CV stepping, CV -60 and -75. Protein Group identification with Proteome Discoverer 2.4 software was done with SEQUEST HT at 1% False Discovery Rate (FDR) at MS^2 level.

High throughput TMT multiplexing with maximum quantitative precision and accuracy

Multiplexed quantitation strategies using Tandem Mass Tags (TMT) enable precise and accurate measurement of thousands peptides or proteins from multiple samples in a single LC-MS run, improving the analysis of large sample sets for applications such as thermal shift assays to single cells. Isobaric tagging strategies using TMT or TMTpro reagents allow up to 11 or 16 samples, respectively, to be multiplexed in a single high resolution LC/MS experiment. However, higher resolution MS/MS scanning is necessary for accurate ratio determination in greater than TMT6plex experiments which can reduce the frequency of acquisition. Additionally, co-isolated ion interference can suppress ratio quantification and thereby mask true differences in protein abundance across samples. The Orbitrap Exploris 480 MS delivers best-in-class TMT MS^2 level proteome quantitation and is designed to address the conventional challenges associated with TMT quantitation, including co-isolated interferences.

The Orbitrap Exploris 480 mass spectrometer has the resolving power and speed capabilities to perform TMT11plex and TMTpro 16plex experiments with unrivaled confidence and no compromise in coverage or depth.

TurboTMT intelligent acquisition, based on novel Phase-Constrained Spectrum Deconvolution Method “Φ-SDM” spectral processing, further improves resolution to baseline of TMT reporter ion isotopologues and speeds up spectral acquisition for TMT11plex experiments, increasing throughput for identification of quantifiable peptides. The “Precursor Fit” algorithm improves isolation specificity. Precursors are selected within the isolation window in a way to maximize the specificity, resulting in the highest MS^2 quality, and therefore the best quantitative accuracy and proteome coverage depth when combined with Advanced Peak Determination (APD). Additionally, the Orbitrap Exploris 480 MS uses a unified Instrument control software architecture and source housing as the Thermo Scientific™ Orbitrap™ Tribrid™ MS portfolio and Thermo Scientific™ TSQ Triple Quadrupole MS. The unified architecture makes parameter transfer between different classes of MS instruments easier, and enables the Orbitrap Exploris 480 MS to utilize the FAIMS Pro interface. High field asymmetric ion mobility spectrometry (FAIMS) reduces interferences for more accurate quantitation (3, 4). Incorporating the FAIMS Pro interface in the workflow increases precursor selectivity and reduces interference contamination using gas-phase fractionation, resulting in greater accuracy for TMT-based quantitation. As shown in Figure 4, combining the new

features on the Orbitrap Exploris 480 MS that benefit TMT workflow, more specifically TurboTMT, Precursor Fit filter, and FAIMS Pro interface, increases from the baseline quantitation accuracy by 19% and increases identification rates by 11%. Furthermore, we evaluated how gas-phase fractionation could be used to increase quantification accuracy when using TMTpro 16plex. FAIMS Pro interface decreased co-isolation interference, improving TMT MS^2 quantitation compared to traditional MS^2 methods (Figure 5). A Synchronous Precursor Selection (SPS) MS^3 approach using a Tribrid instrument such as Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ MS is required for applications requiring the highest quantitation accuracy. By leveraging intelligent data acquisition methods with pre-built method templates for TMT that are fully customizable, proteins can be confidently quantified with minimal setup preparation effort.

SureQuant™ method, a targeted strategy for the

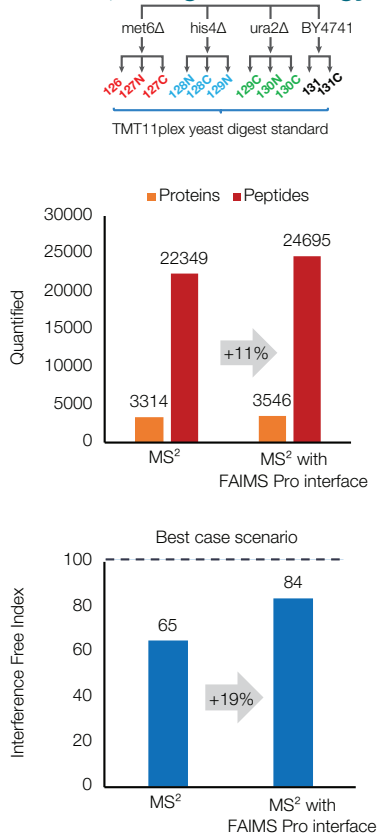

Figure 4. Larger numbers of peptides/proteins quantified with improved accuracy for TMT multiplexing on Orbitrap Exploris 480 MS. Combining TurboTMT at 30,000 resolution, Precursor Fit Filter at 70%, and FAIMS Pro interface with -45,-60,-75,-90 CVs increases quantified peptide and protein identifications by 11%, and improved the interference free index, a proxy for quantitation accuracy, by 19%. In order to assess the sensitivity of the instrumentation for TMT based quantitation, 500ng of the Thermo Scientific™ Pierce™ TMT11plex yeast digest standard was analyzed in 4 hour gradient. This standard sample provides users with a tool to measure the accuracy, precision, and proteome depth of TMT methods across different instrumentations (5).

FAIMS Pro™ interface and Precursor Fit filter decrease ratio compression.

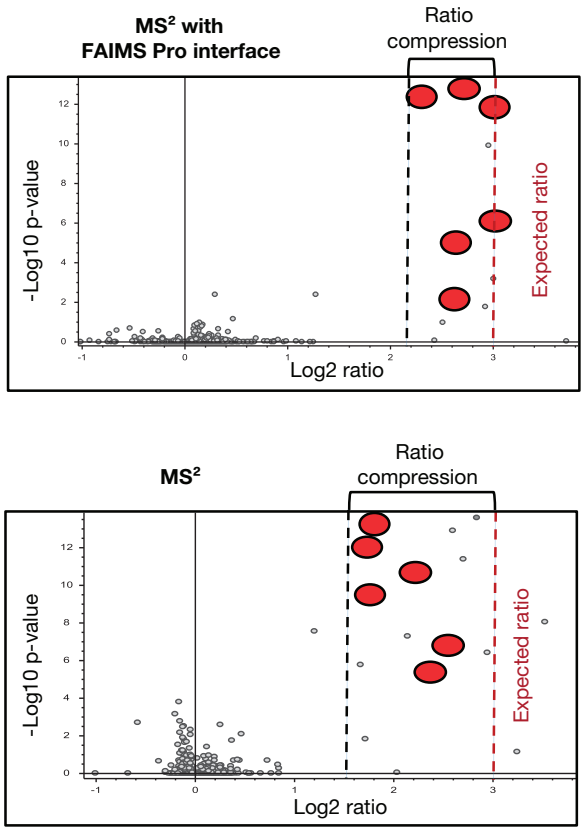

Figure 5. An equimolar mixture of Thermo Scientific™ Pierce™ 6 Protein Digest (red circles) was labeled with TMTpro 16plex, mixed in various ratios, and spiked into overwhelming background of Thermo Scientific™ Pierce™ HeLa Protein Digest Standard (gray circles). 1ug of peptides were measure on a 120min analytical gradient using a modified hybrid quadrupole-Orbitrap™ mass spectrometer, with or without the FAIMS Pro interface and the Precursor Fit Filter set at 70%. Data was analyzed and visualized in Proteome Discover 2.3 software.

confident identification of hundreds of protein targets

The Orbitrap Exploris 480 MS empowers the SureQuant™ acquisition workflow, which is a new paradigm for targeted protein quantification. This intelligent data acquisition scheme has been developed in order to overcome the limited acquisition efficiency of conventional targeted analyses (selected reaction monitoring or parallel reaction monitoring), which penalizes in general the overall outcome of experiments by requiring an inevitable trade-off between scale and analytical performance. The concept of SureQuant acquisition has been described previously under the name of internal standard-triggered parallel reaction monitoring (IS-PRM) (6). In its original implementation, the technique demonstrated significant improvement in acquisition efficiency in comparison with regular time-scheduled PRM analysis by minimizing the number of non-informative PRM scans and their associated

acquisition time. This approach leveraged spiked-in internal standards to dynamically drive in real-time the measurement of corresponding endogenous peptides of interest only while they were actually eluting. This allowed extra-time available to be used to increase the number of targeted peptides and/or the acquisition time devoted to their measurement. For instance, applied to the analysis of a large set of peptides (up to 600) in complex matrices, the IS-PRM method deliver reliable quantification without sacrificing data quality and measurement sensitivity. The SureQuant targeted quantification method is an evolution of the original IS-PRM approach, relying on the same rationale of using internal standard to drive the acquisition, but benefiting from refinements in terms of implementation, usability, and robustness, while still maintaining the highest level of quantitative performance.

More specifically, operated in SureQuant mode, the mass spectrometer alternates between two acquisition modes, *i.e.*, Watch mode and Quant mode, in which fill time and resolution settings of PRM scans are adjusted on-the-fly to maximize sensitivity and selectivity at precisely the time-point in the analysis when the targets of interest are eluting. In practice, the real-time process is supported by a native implementation of the method in the instrument control software of the Orbitrap Exploris 480 MS through a combination of various scan events and filters, with parameters optimized for the highest data quality and sensitivity of triggering, but also to retain sufficient triggering specificity (Figure 6).

Importantly, in the SureQuant implementation the

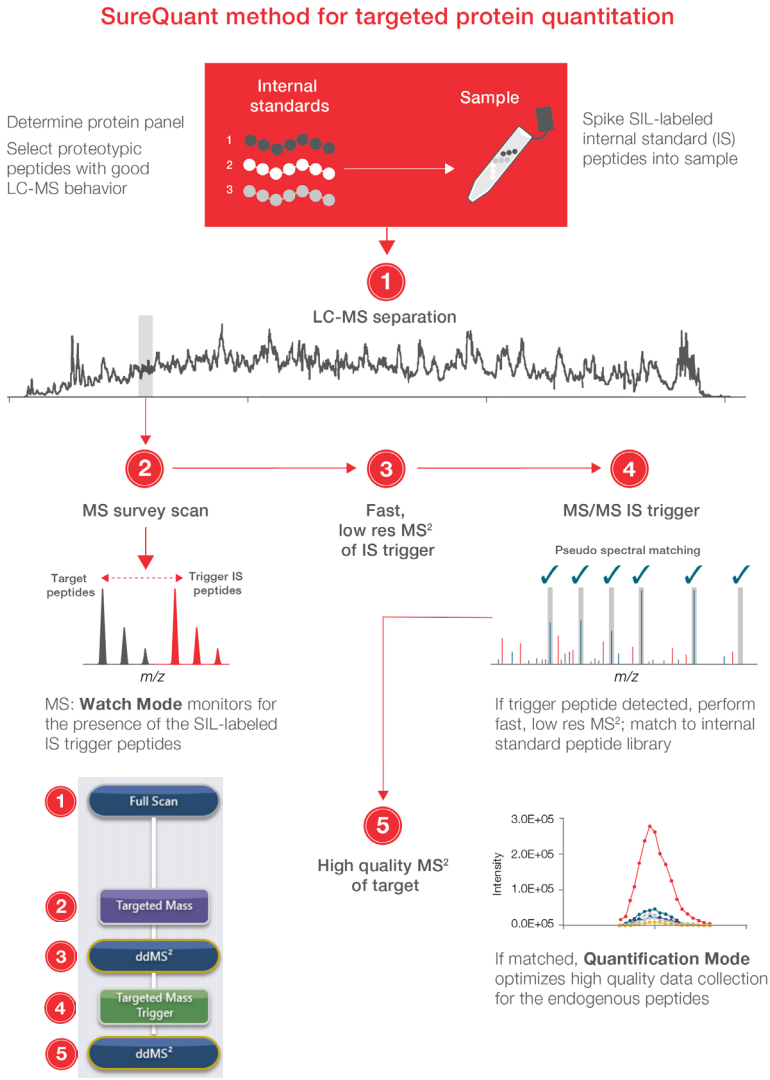

Figure 6: SureQuant acquisition scheme and method structure. First, synthetic internal standards corresponding to the targets of interest are spiked into the sample at easily detectable amounts, and then subjected to an LC-MS SureQuant analysis. During SureQuant analyses, a high resolution (120k) full scan MS 1] is acquired, using lock mass re-calibration, to monitor the predefined optimal precursor ions of the IS, based on the list of associated m/z values and intensity thresholds (typically defined at 1% of the expected MS1 intensity at the chromatographic peak apex) included in the 'Targeted Mass' filter 2]. In case of the detection of a candidate precursor ion satisfying the filtering criteria (MS peak with an m/z value within 3-10 ppm tolerance and intensity exceeding the threshold), it is subjected to fast MS/MS acquisition 3] performed at low resolution and short fill times (typically 7.5k/10 ms), which is used as a second layer of confirmation of the detection of the IS. Several fragment ions, predefined as associated with the IS (6 optimal frag.) are included in the 'Targeted Mass Trigger' filter 4], and the detection of a subset of them (at least 5 fragments with 10-20 ppm tolerance) confirms the actual elution of the IS. This two-step process interrogating MS1 and MS2 data to track IS elution is the 'watch' mode of the SureQuant method. The detection of an IS in watch mode triggers the 'quant' mode, and therefore MS/MS acquisition of the corresponding endogenous peptides 5] with parameters favoring data quality, i.e., high resolution and long fill times (typically 60k/116 ms). The process is repeated over the entire LC separation with a systematic cycle-to-cycle assessment of the elution of the IS.

acquisition no longer requires analyte chromatographic retention time scheduling of monitoring windows and therefore removes the associated inefficiencies inherent to conventional targeted methods. The superior acquisition efficiency offered by SureQuant acquisition is depicted in Figure 7 through a comparison with regular time-scheduled PRM for the measurement of a pair of internal standard and endogenous peptides. The monitoring of peptides in relatively long windows with respect to their actual elution time typically results in a low proportion of productive scans in retention time-scheduled PRM analysis, typically 10-15% of the total MS^2 acquisition time, collecting meaningful data on the target. By contrast, SureQuant acquisition systematically shows higher efficiency, typically delivering 80 to 90 % of productive MS^2 scans. This enhanced efficiency can be leveraged to gain several analytical advantages, including:

- **Enhanced data quality:** providing higher sensitivity and selectivity (higher resolution/fill time) to improve limits of detection, limits of quantitation.
- **Increased target scale:** A higher number of targets can be included in an experiment, and quantified in the same amount of total analysis time as PRM without sacrificing the duty cycle and data quality.
- **Increased throughput:** Higher productivity can be achieved by reliably quantifying targets in less total instrument time, while still achieving acceptable data quality.
- **Enhanced detection success rate without worrying about chromatographic variability:** More reliable and consistent target measurement can be achieved since the IS guides the measurement of the target of interest at precisely the right time, independently from retention time-scheduling. This leads to less intra-run and less inter-run missing values.

In addition, the method has other figures of merit, which should foster a broad adoption, such as its strong robustness or its kind of “load-and-play” execution, requiring minimal preliminary work, and allowing in fine a better user experience. This is especially true for the deployment of already developed SureQuant assays, for instance pre-set method templates embedded in the Orbitrap Exploris 480 instrument control software, which support the use of Thermo Scientific SureQuant Targeted MS Assay Kits and third party targeted panels such as the Biognosys PQ500 human plasma panel. For added flexibility, even custom peptide panels may also be used and is facilitated by generic SureQuant Method templates also available in the instrument control software. An example of SureQuant application based on embedded developed assay is presented in Figure 8. This SureQuant targeted plasma profiling application is intended to support translational proteomics. The assay has been developed from the Biognosys PQ500 kit, which includes 804 Stable Isotope Labeled (SIL) peptides corresponding to 582 plasma proteins, and applied to the triplicated analyses of 1 µg of a pooled non-depleted plasma sample. The 802 SIL peptides, on which the method was built, were systematically detected across the analyses, while the

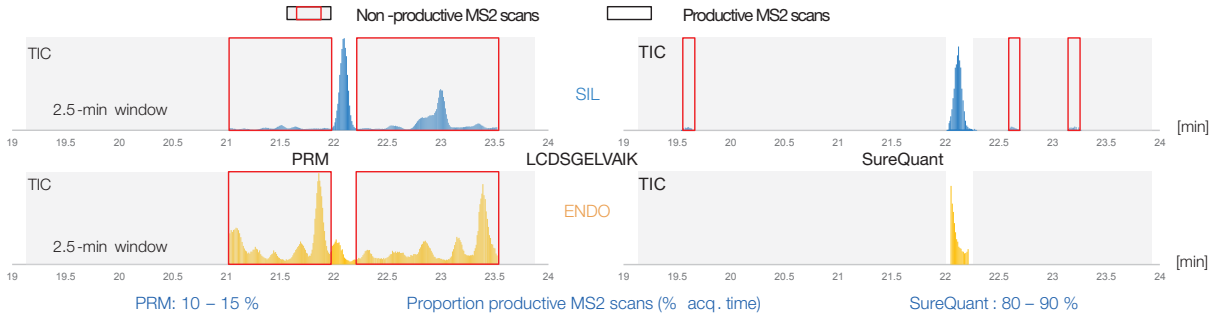

Figure 7: Comparison of MS data acquisition efficiency in conventional PRM and SureQuant analyses. A pair of internal standard and endogenous peptides was measured by time-scheduled PRM and SureQuant acquisitions. The PRM method used a 2.5-min monitoring window, resulting in low proportion of productive scans (10-15 % of the total MS^2 acquisition time) acquired while the peptides are actually eluting, so located outside from the red rectangle in these graphs (left panel). In SureQuant acquisition (right panel), using typical parameters previously mentioned (Figure 6), the peptide elution profiles were nicely captured and with very few non-productive MS^2 scans acquired for the IS peptide in the red rectangle, and none for the endogenous peptide. This is typical result obtained with SureQuant acquisition, which normally generates 80 to 90 % of productive MS^2 scans.

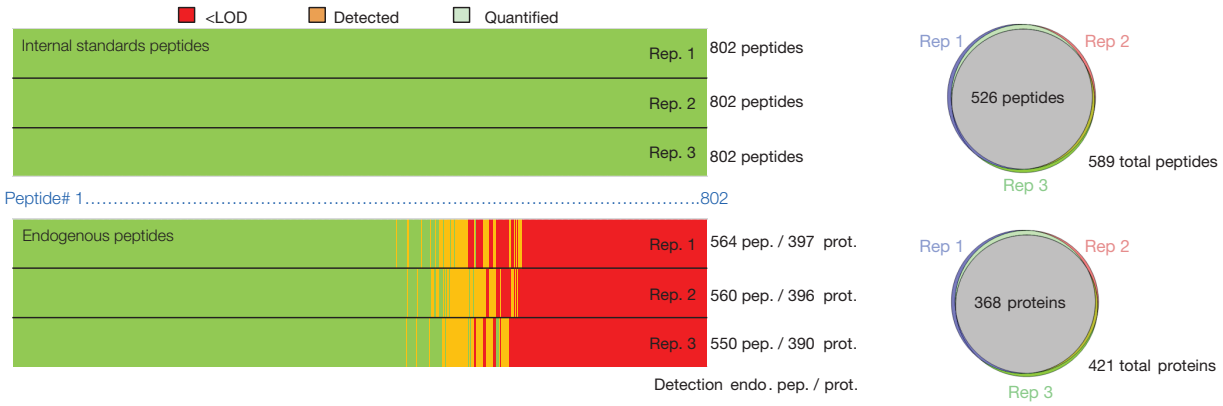

Figure 8: Peptide detection and quantification of the PQ500 protein panel of un-depleted plasma using SureQuant Acquisition. The SureQuant acquisition PQ500 workflow was applied to the analyses of 1 µg of un-depleted plasma sample supplemented with PQ500 kit (median spiked-in amount of SIL peptides of 80 fmol) using a 70-min LC gradient on an UltiMate 3000 RSLC system. The SureQuant survey run (directed DDA with inclusion list of pre-defined IS precursor ion under optimal charge state) revealed that 802 of 804 SIL peptides were compatible (intensity response and hydrophobicity) with the LC-MS setup used, and were retained for subsequent SureQuant analyses. The 802 retained internal standards were systematically detected in the triplicated SureQuant analyses of plasma samples, and triggered high quality measurement of the corresponding endogenous peptides, translating into a broad coverage of the un-depleted plasma proteome. Around 400 proteins were detected in each replicate (based on detection of 560 surrogate endogenous peptides), while 90% of the total sets were systematically quantified across triplicates.

plasma proteome was broadly covered, with around 400 proteins quantified in each replicate and 560 surrogate endogenous peptides. Therefore, the plasma proteome coverage obtained by the SureQuant workflow rivals that of a typical discovery workflow while still providing the quantification performance of targeted analyses, including an exquisite reproducibility (as reflected by 90 % of the endogenous peptides and proteins systematically quantified across replicates).

Native implementation of BoxCar acquisition expands the dynamic range of label-free quantification experiments.

The Orbitrap Exploris 480 MS features BoxCar acquisition methods, which increase the MS1 dynamic range of label-free quantification experiments. This method, which has been introduced previously for Q Exactive platforms and enabled by third-party software through application programming interface (7), benefited from a native implementation in the instrument control software of the new MS. Therefore, in this operation mode, the data acquisition schemes of conventional DDA or DIA are supplemented with BoxCar scans, allowing the full mass range to be split into multiple narrow m/z segments, for a sequential and balanced filling of the analyzer by intact precursor ions before their analysis in a single Orbitrap scan. The resulting approximate 10-fold increase in ion injection time, as compared with standard full scan, translates into more sensitive peptide measurement and in turn into more comprehensive proteome coverage. Figure 9 illustrates a 40-% increase in the number of proteins identified in the analysis of plasma sample by BoxCar DDA acquisition using a 45-min LC gradient.

Everyday usability assures performance

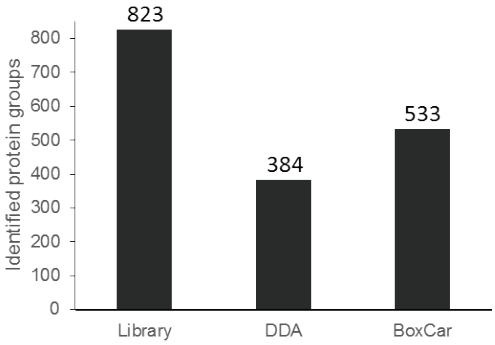

Figure 9. Increased dynamic range analysis of a human plasma sample using BoxCar DDA acquisition. A total of 500 ng of human plasma digest was analyzed by conventional DDA and BoxCar DDA analyses using a 45-min LC gradient on Orbitrap Exploris 480 MS. Preliminary DDA analyses of 24 fractions of plasma (prepared by high-pH reverse phase chromatography) allowed a peptide library to be built, covering 823 proteins. The data from regular DDA and BoxCar DDA analyses of the non-fractionated plasma sample were processed by MaxQuant software, using “match between runs” feature to transfer peptide identification from the library. While only 47% of the library was covered by DDA analysis, around 40% more proteins were identified with BoxCar DDA.

With automated tuning, data-acquisition method templates (Figure 10), and streamlined data processing setup, the use of the Orbitrap Exploris 480 MS in combination with FAIMS Pro interface is simplified, enhancing productivity regardless of user expertise. Seamless integration is designed to support maximum productivity for your most demanding science. Built on our next-generation unified software user interface architecture, the Orbitrap Exploris 480 mass spectrometer delivers ease-of-use without sacrificing high performance. Hardware and software harmonization across Thermo Scientific LC-MS portfolio simplifies learning process and enables straightforward transfer of accessories and method parameters, streamlining the ability to go from sample to data to translate into scientific insights. Pre-built method templates are organized by application area, such as Single Cell, LFQ, TMT, Boxcar, or SureQuant, and method templates are fully customizable to fit your needs focusing on your science, not on instrument and method setup. Integrated instrument control, data processing, and servicing software allow LC-MS users of all skill levels to execute methods with ease without compromising on data quality.

Designed and manufactured for exceptional robustness, everyday reliability, and reproducibility, the Orbitrap Exploris 480 mass spectrometer maximizes uptime and productivity to meet large-scale study and sample-throughput requirements, while reducing everyday hassles. Figure 11 shows that the Orbitrap Exploris 480 MS can be run with continuous operation for 125 days without decline in performance. Rigorous testing at every stage of the manufacturing process ensures the quality of every system that leaves the factory. Advanced Quadrupole Technology (AQT) with novel, patented Configuration Switch Mode (CSM) extends quadrupole maintenance intervals up to 200% without compromising on performance, while automatic bake-out after power cycle saves time to restore operational capabilities. The six-stage pumping system controlled by a single turbo pump streamlines planned maintenance. The improved design architecture ensures reproducibility from one instrument to the other, enabling stronger focus on research activities.

High-up time, High-performance, High-throughput

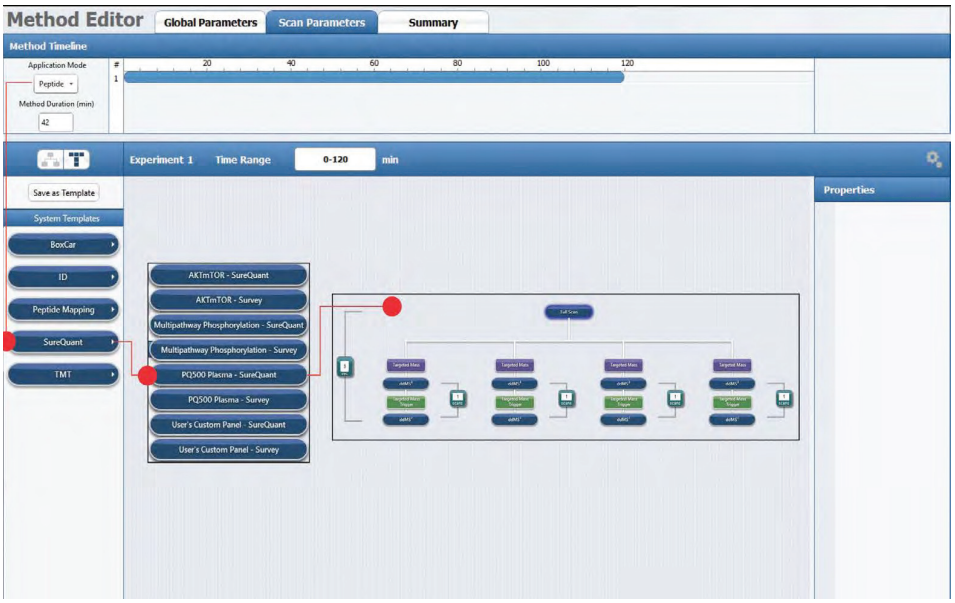

Figure 10. Pre-built method templates sorted by application area come ready to use in the Orbitrap Exploris 480 MS Instrument Control Software. Intuitive tuning and method setup, single calibration across the mass range and polarity modes, along the pre-defined methods, and common data acquisition schemes and components, add to usability, data quality, and throughput. Shown here is the pre-build, customizable method template for the SureQuant Targeted MS Assay using the Biognosys PQ500 human plasma panel.

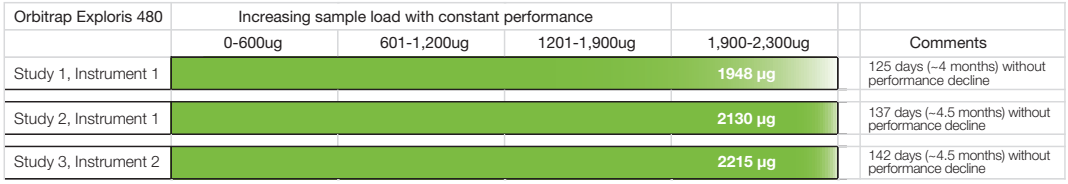

Figure 11. Longitudinal performance of the Orbitrap Exploris 480 MS. In each study, Instruments 1 and 2 ran without decline in performance for more than 125 days. The performance of Instrument 1 was fully restored after maintenance and lasted for another 137 days and analyzed more than a total of 2,000 µg injected from protein digests of plasma and whole cell lysates.

Conclusion

The Orbitrap Exploris 480 MS enables high resolution, accurate mass measurements of analytes with confidence. The increased sensitivity and selectivity of the instrumentation when combined with FAIMS Pro interface help investigate immerging application areas such as single cell proteomics, and TMTpro multiplexing strategies of up to 16 samples in a single injection, while the SureQuant method enables targeted protein quantitation with high usability and robustness. Collectively, these new technology developments and intelligent acquisition strategies improve identification rates, quantification accuracy and precision in analyses performed with the Orbitrap Exploris 480 mass spectrometers including for DIA and TMT strategies to profile changes in proteome expression (8). The new method editor and instrument control software increases ease of use, making the mass spectrometer easier to operate for all levels of expertise while achieving robust, certain, and confident results necessary to answer novel biological questions. In summary, the Orbitrap Exploris 480 MS is a novel, next-generation mass spectrometer that offers many advantages, and is technologically distinct, from Q Exactive and Quadrupole-Time of Flight (QTOF) analyzers.

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