Proof of performance

Orbitrap Exploris 240 mass spectrometer

Faster time to results–intelligent data acquisition and processing with applications software

Summary

This document highlights why the Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer is the best-in-class, high-resolution, accurate-mass (HRAM) Orbitrap system in this category that rapidly delivers results on time, every time.

With technology innovations (see XX-65862 proof of performance note) and versatile data acquisition options easily accessed through intelligent and ready-to-run instrument method templates (see XX-65879 proof of performance note), the Orbitrap Exploris 240 MS delivers high quality data in a very short time. Moreover in combination with application specific data processing software, the system delivers a faster turnaround time from sample to results when compared to traditional proteomics workflows.

Quantitative performance and sampling throughput efficiency increase using sample multiplexing. Thermo Scientific™ TMTpro™ 16plex isobaric tags provide the highest number of channels compared to any other current commercially available isobaric technique. Utilizing the optimized method setup for data acquisition and fit-for-purpose data analysis with Thermo Scientific™ Proteome Discoverer™ software, we demonstrate the increased sample throughput using TMTpro 16plex reagents on an Orbitrap Exploris 240 MS, easy to setup method and optimized data processing leading to a faster time to results compared to other end-to-end proteomics solutions.

Best-in-class productivity: When high throughput and ease-of-use are required, the Orbitrap Exploris 240 MS delivers intelligent acquisition strategies built into optimized methods and data analysis software designed for rich Thermo Scientific™ Orbitrap™ analyzer data and proteomics applications. These factors combine to create a streamlined process, significantly reducing time from sample to results compared to other solutions.

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Experimental conditions

Experimental design

Five different cell growth conditions were analyzed, with each condition measured in triplicate, in a single LC-MS analysis (Figure 1). Additionally, one reporter ion channel was utilized as a pooled sample. This 16th sample then functions to normalize reporter ion intensities across multiple TMTpro 16plex experiments, enabling up to 30 samples to be quantified in two LC-MS analyses, as depicted in Figure 1.
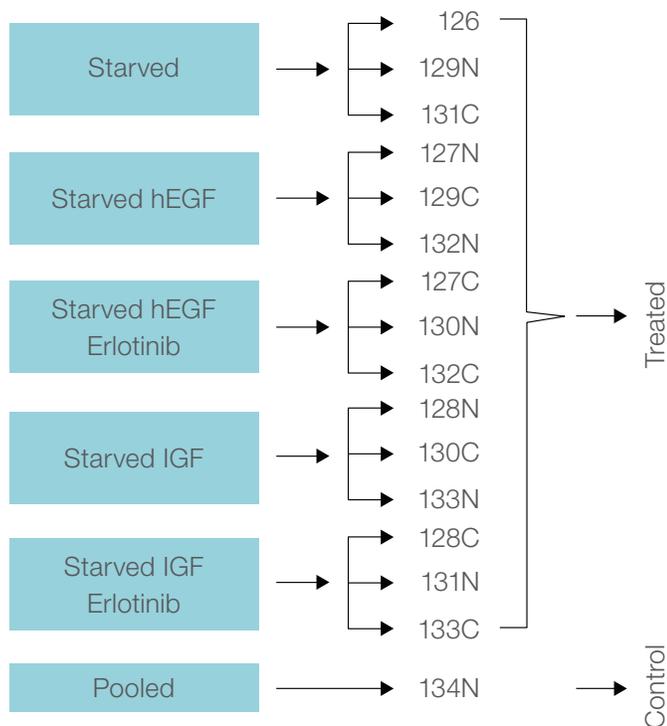
Figure 1. Tandem Mass Tags Multiplex Protein Quantitation: Experimental setup. Starved HeLa cell lines were treated under 4 different conditions with 3 technical replicates yielding a total of 16 samples, included a single pooled sample. Samples were labeled with TMTpro 16plex reagents, pooled together and 500 ng of the final pooled sample was analyzed in 3 technical replicates using a 2 hour LC-MS separation on a 25 cm Aurora column (IonOpticks).

Sample

- 500 ng Starved HeLa cells with 4 different conditions

- Thermo Scientific™ TMTpro™ 16plex Label Reagent Set, 1 × 5 mg (Cat # A44520)

LC method

- Thermo Scientific™ NanoSpray Flex™ ion source (Cat # ES071)

- Sonation Column Oven (PRSO-V2) operating at 40 °C

- IonOpticks Aurora column, 25 cm

- 120 min gradient

- Mobile phase A: 0.1% formic acid (FA),
  Mobile phase B: 80% acetonitrile (ACN) in 0.1% FA

Time (min)	B%
0	3
1	3
73	19
101	29
121	41
126	95
140	95

Instrumentation

- Thermo Scientific™ EASY-nLC™ 1200 system (Cat # LC140)

- Thermo Scientific™ EASY-Spray™ Source (Cat # ES081)

- Thermo Scientific™ FAIMS Pro™ interface (Cat # FMS02) (compensation voltage: -60 V/-80 V)

MS method

Figure 2. Optimized Method Templates to support TMTpro 16plex reagents. The Orbitrap Exploris 240 Instrument Control Software (ICSW) supports pre-built and optimized method templates that are customizable through drag-and-drop method creation. Shown here is the pre-built method template for experiments using TMTpro 16plex reagents analyzed with the FAIMS Pro interface on an Orbitrap Exploris 240 MS. Additional method templates for classic TMT and TMT11plex yeast digest standard are also included.

Data processing

Proteome Discoverer software, version 2.4 with 1% PSMs FDR

Data

Approximately 4,000 proteins across 16 samples were quantified with a single 120 min LC-MS experiment (Figure 3a), while monitoring differences in protein expression due to the growth conditions as exemplified by Ubiquitin-conjugating enzyme E2 C (UBE2C) protein reporter ion intensity (Figure 3b). These advances in instrumentation and new multiplex reagent development allow for maximum quantitative insights that are precise and accurate with high confidence for whole proteome profiling/quantitation while maximizing sample throughput.

Data processing with Proteome Discoverer software

Proteome Discoverer software was used to perform the database search, TMT quantification and statistical analysis. In the example shown in Figure 4, a pre-installed TMT workflow template with defined parameters was used, among those parameters precursor mass tolerance was set to 10 ppm with fixed TMT modification on lysines and peptide n-termini. For this experiment, a SwissProt™ saccharomyces cerevisae database was used.
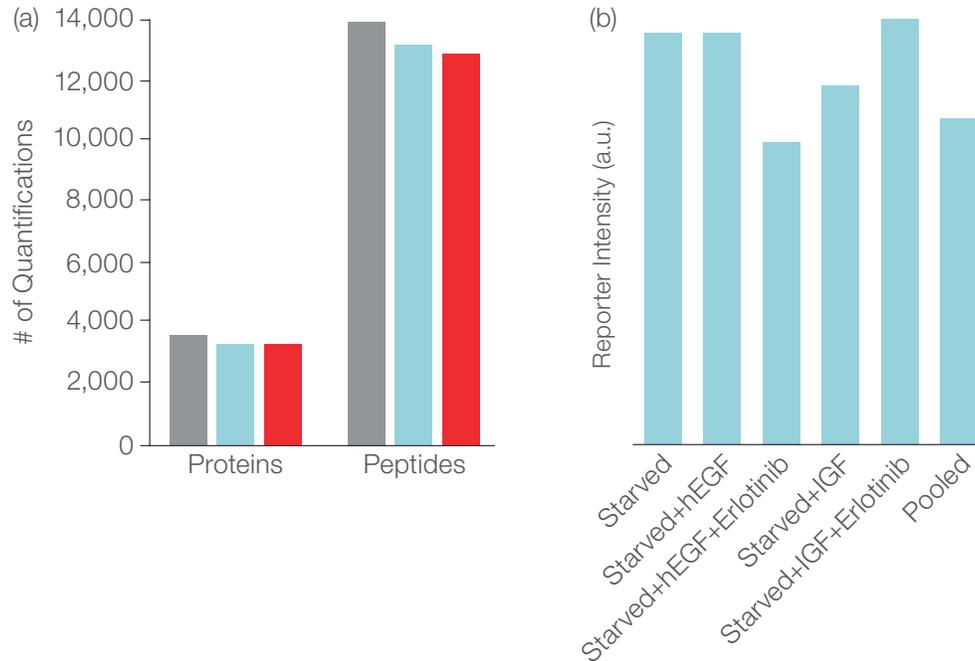
Figure 3. Bar plots highlighting the number of peptides and proteins quantified per run across the different samples. Bar plots shows quantitation value of protein; UBE2C in each cell line under those treatments.
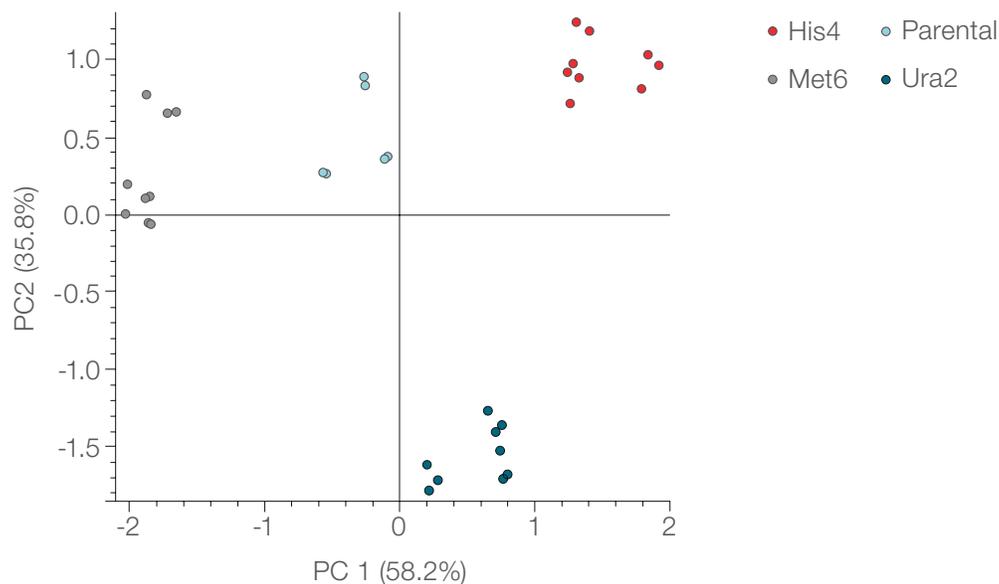
Figure 4. Principal component analysis (PCA) plot using the quantified proteins. The first two principal components (PCs) are plotted and colored according to the knockout protein. Percentage of variation accounted for by each PC is shown in brackets with the axis label.
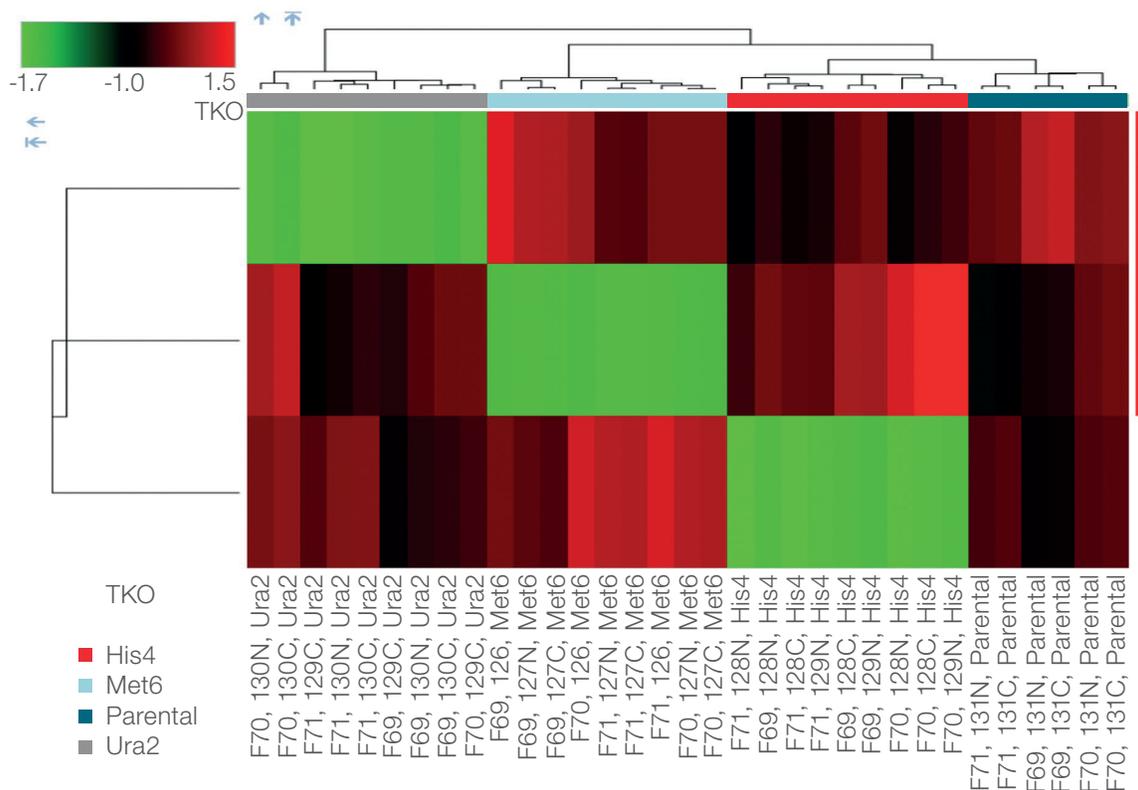
Figure 5. Unsupervised hierarchical clustering of the protein profiles for the knockout proteins. Samples were clustered. The dendrograms are generated using a hierarchical clustering algorithm based on the average-linkage method. In the matrix, each column represents a sample and each row represents a protein. The color scale bar shows the relative peptide expression changes normalized by the standard deviation (0 is the mean expression level of a given peptide).

Results

- The combination of TMTpro 16plex reagents and high-resolution Orbitrap Exploris 240 MS enable the quantitation of up to 16 samples in a single LC-MS analysis with no missing values.

- Optimized method setup enables greater ease-of-use and less time spent on instrument setup.

- Application specific software delivers results specific for TMT multiplexed quantitation, extracting rich HRAM data acquired by the Orbitrap mass analyzer and turning it into knowledge.

- Workflows with Orbitrap Exploris 240 MS deliver a fast turnaround of sample to results.

Outlook

The Orbitrap Exploris 240 MS system is designed for proteomics scientists in research and core laboratories looking to increase sample throughput, with ease-of-use in method setup and data processing that leads to a faster turnaround time of sample-to-results while meeting the goals of your research, and your clients.

Conclusion

The Orbitrap Exploris 240 MS enables TMTpro 16plex analysis with higher sample throughput in a single LC-MS run. Unlike QTOF technology, Orbitrap technology is capable of providing resolving power at low *m/z* sufficient to baseline resolve TMTpro reporter isotopologues without compromising speed and sensitivity. The Orbitrap Exploris 240 mass spectrometer accurately quantifies these reporter ions from a 16plex experiment.

Find out more at
thermofisher.com/OrbitrapExploris240Proof

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