Q Exactive – A True Qual-Quan HR/AM Mass Spectrometer for Routine Discovery and Target Quantification in Proteomics

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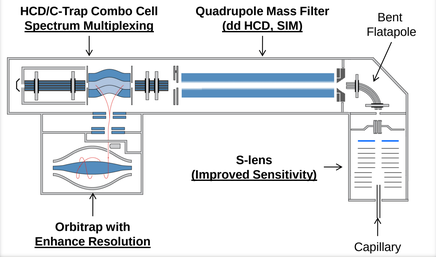
Overview

Purpose: The performance of a novel Q Exactive mass spectrometer was evaluated in both routine peptide identification and targeted peptide quantification.

Methods: The peptide identification capability was investigated through in-depth analysis of the yeast proteome. Discovery-based quantification accuracy and precision were evaluated using TMT labeled *E. coli* digests. The sensitivity and linear dynamic range for HR/AM targeted quantification were investigated with peptide standards spiked into a complex yeast digest background.

Results: The Q Exactive identified twice as many peptides/proteins as the AB Sciex TripleTOF 5600 in a well controlled head-to-head comparison. High precision (CV<13%) was routinely achieved for TMT-based quantification experiments on the Q Exactive. The quadrupole based SIM and ultra-high resolution of 140K resulted in accurate and sensitive HR/AM targeted quantification. A LOD of 10 amol was obtained on most peptide targets with low background.

FIGURE 1. Schematic of the Q Exactive

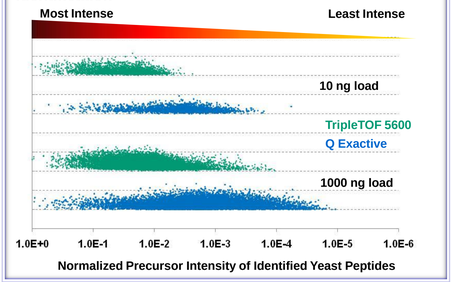


Discovery- Protein Identification

A Head-to-Head Comparison against TripleTOF 5600

Same sample, Same column, Same gradient

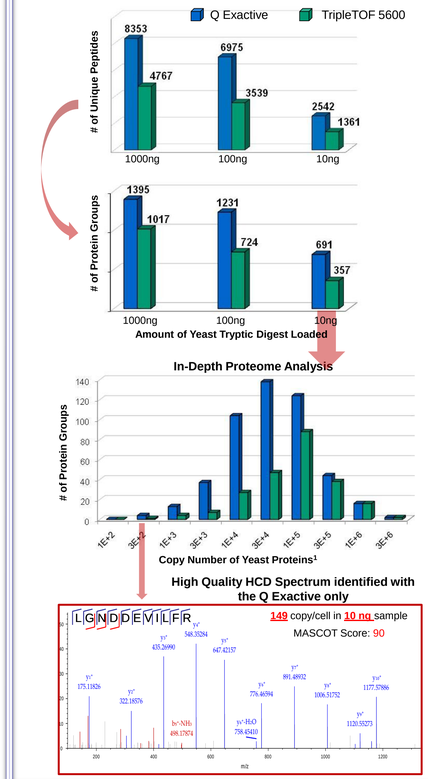
Figure 2: Q Exactive has 10x broader dynamic range of identification



Discovery- Protein Identification

High Dynamic Range, High Sensitivity, High Duty Cycle

Figure 3: Q Exactive identified 2x unique peptides and proteins and 4x lower abundant proteins than the TripleTOF due to its broader dynamic range, fast scan rate, and high quality HCD spectra.



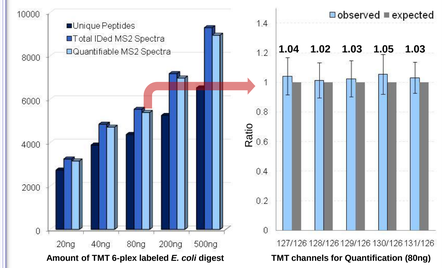
Discovery-based HR/AM Qual/Quan

High Precision, High Accuracy TMT Quantification

Figure 4: TMT 6-plex Quantitative Results on Q Exactive

A. Quantifiable MS2 spectra account for 97% of the total identified MS2.

B. Measured ratios are within 5% of expected values, with CV<13%.



Methods

Samples: heavy isotope labeled peptide retention standards (Thermo Fisher Scientific, Cat #: 88321), yeast tryptic digest, TMT 6-plex labeled *E. coli* tryptic digest.

LC: Split-free EASY-nLC from Proxeon, Solvent A: 0.1% formic acid in H2O, Solvent B: 0.1% formic acid in acetonitrile.

Head-to-Head Comparison of the Q Exactive against the AB SciexTripleTOF 5600 for Peptide Identification: Yeast tryptic digests at 10 ng, 100 ng and 1000 ng were analyzed with the same nano LC column (Dionex AcclaimPepMap100 C18, 75μm x 15cm, 3μm particle, 100Å pore size) and the same 60 min gradient on both the Q Exactive and TripleTOF 5600. The instrument parameters on the TripleTOF 5600 were set at values recommended by AB Sciex: 250 ms full scan with 30K resolution, top 20 MS/MS with 15K resolution and 50 ms beam time, MS^2 trigger threshold of 100 cps, dynamic exclusion of 30 s. On the Q Exactive, the resolution was 70K for full scan and 35K for MS^2, the AGC target was 1E6 for full scan and 1E5 for MS^2, the maximum IT was 100 ms for both full scan and MS^2 scan, top 10 HCD was selected with MS^2 trigger threshold of 5,000 and dynamic exclusion of 60s.

TMT Quantification Evaluation: *E. coli* tryptic digests were labeled with TMT 6-plex reagents at a ratio of 1:1:1:1:1:1. Labeled peptides (20, 40, 80, 200, 500 ng) were separated over a Michrom Magic C18 nano LC column (75μm x 20cm, 3μm particle) with a two-hour gradient, analyzed with a data dependent top 10 HCD method on the Q Exactive. The resolution was 70K for full scan and 17.5K for MS^2. The maximum IT was 250 ms for both full scan and MS^2 scan, top 10 HCD was selected with MS^2 trigger threshold of 1E5 and dynamic exclusion of 80s. The AGC targets were the same as above.

HR/AM Targeted Quantification: Heavy isotope labeled peptide retention standards at 0, 10 amol, 50 amol, 100 amol, 1fmol, 10 fmol, and 100 fmol were spiked into either 10 ng or 1000 ng of yeast tryptic digest. Each sample was analyzed three times with a 60 min LC gradient over a Michrom Magic C18 nano LC column (75μm x 15cm, 3μm particle) and a full-msx tSIM method on the Q Exactive. The resolution was set at 140k for both scan types. The AGC target was 1E6 for full scan and 5E5 for SIM scan. The maximum ion injection time was 100 ms for full scan and 500 ms for SIM scan. The isolation width for SIM scan was 4 amu. The peptide retention standards were monitored over a 4 min window. Multiplexing level was set at 4, which allowed isolation and accumulation of up to four peptide targets in the c-trap before they were transferred to Orbitrap for detection (Figure 7)

Data Analysis Peptide identification and TMT quantification data were processed and searched against their respective databases with Mascot using standard search parameter settings in Proteome Discoverer 1.3. Targeted quantification using LC peak areas of heavy peptide standards with ±5 ppm mass windows were automatically calculated for both full scan and SIM scan using Quan browser in Xcalibur 2.2. Linear regression fittings in both normal scale and log scale were performed to estimate LOD and LOQ.

HR/AM Target Verification/Quantification

High Selectivity, High Sensitivity, High Throughput

Figure 5: High resolution ensures accurate target selection

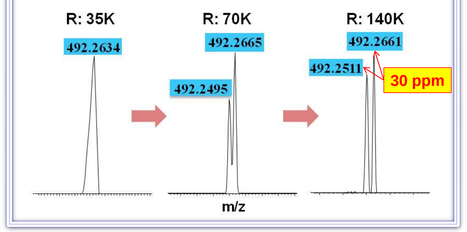


Figure 6: High sensitivity with quadrupole based SIM scan

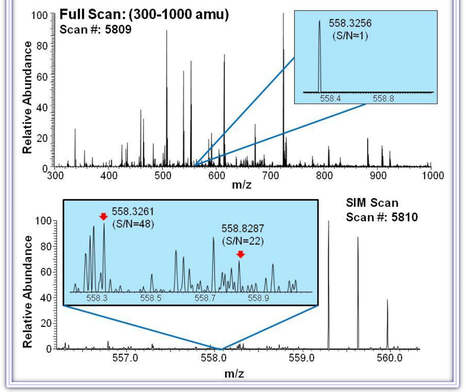


Figure 7: Spectrum multiplexing and concurrent injection/detection provides high throughput analyses

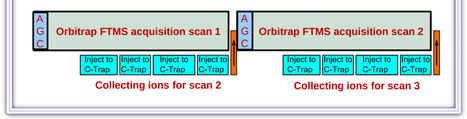
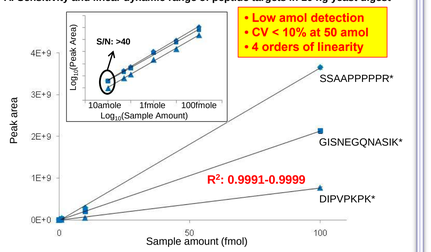
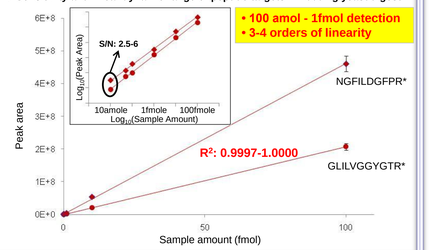


Figure 8: LOD of 10 amol or 100 amol is routinely achieved with HR/AM targeted quantification in medium or strong background, respectively

A. Sensitivity and linear dynamic range of peptide targets in 10 ng yeast digest



B. Sensitivity and linear dynamic range of peptide targets in 1000 ng yeast digest



Conclusion

- The Q Exactive is well suited for routine in-depth proteome analysis with its five orders of magnitude dynamic range, fast high-resolution MS/MS scan rate of 12Hz, and high quality HR/AM HCD spectrum.
- The Q Exactive generates accurate quantification data with high precision (CV<15%) in TMT based quantitative discovery analyses.
- The quadruple-based high-resolution SIM scans combined with the unique spectrum multiplexing functionality enable high throughput HR/AM quantification with high selectivity and sensitivity in target verification/quantification studies.
- The Q Exactive is a true Qual-Quan mass spectrometer, which allows seamless transition from discovery to target verification/quantification.

References

1. Ghaemmaghami S, Huh WK, Bower K, Howson RW, Belle A, Dephoure N, O'Shea EK, Weissman JS. *Nature* **2003**.16, 737–7341.

Acknowledgements

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