Increased Analytical Performance on a Hybrid Linear Ion Trap-FTMS Mass Spectrometer with a High-Field Orbitrap Mass Analyzer

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Overview

Purpose: Implementation of a next-generation high-field Orbitrap™ mass analyzer and advanced signal processing algorithm on a hybrid ion trap-Orbitrap mass spectrometer.

Methods: Various experiments (direct infusion, online LC-MS/MS) have been carried out to characterize the performance of the high-field Orbitrap mass analyzer on the Orbitrap Elite hybrid mass spectrometer.

Results: The new high-field Orbitrap mass analyzer, in combination with advanced data processing, provides increased performance for the characterization of intact proteins and complex mixtures.

Introduction

One of the biggest challenges in shot-gun proteomics of complex samples remains undersampling. Besides the ion accumulation time to reach the target value in ion trap hybrid mass spectrometer, the detect time in the Orbitrap detector has the biggest portion in the overall scan process. Improvements resulting in higher resolution or in higher scan speed at the same resolution significantly decrease the overall cycle time. In this work we present the advances in analytical performance using modifications to hardware and software on an ion trap-hybrid MS based instrument platform.

Methods

Sample Preparation

E. coli cell lysate (BioRad) was reduced, alkylated and enzymatically degraded. For the TMT experiments, the enzymatic digest was divided into six aliquots and labeled with *m/z* 126, 127, 128, 129, 130 and 131 TMT tags according to the manufacturer's protocol. Samples were subsequently mixed in various ratios, concentrated by a Thermo Scientific SpeedVac system and analyzed by LC-MS/MS. Intact proteins (all from Sigma Aldrich, St. Louis, MO, USA) were dissolved in 50% methanol containing 0.1% formic acid.

Liquid Chromatography

Peptides were separated on a Michrom Bioresources spray tip (75 µm inner diameter, 200 mm length) Magic™ C18 column or on a Thermo Scientific BioBasic peptide trap (100 µm inner diameter, 2 cm length) and a BioBasic™ C18 analytical column (75 µm inner diameter, 10 cm length) using a Thermo Scientific EASY-nLC with a flow rate of 300 nL/min. Mobile phase A was water containing 0.1% formic acid and phase B was acetonitrile containing 0.1% formic acid. Different LC gradients were used for the bottom-up proteomics experiments.

Mass Spectrometry

Data were acquired using Thermo Scientific Orbitrap Elite, LTQ Orbitrap Velos, and LTQ Orbitrap XL hybrid mass spectrometers, all equipped with electron transfer dissociation (ETD).

Data Analysis

Thermo Scientific Proteome Discoverer software version 1.2 with Mascot 2.3 search engine was used for protein identification. Carbamidomethyl modification on cysteine and TMTsixplex on lysine and N-terminal were set as fixed modifications for the TMT-labeled samples.

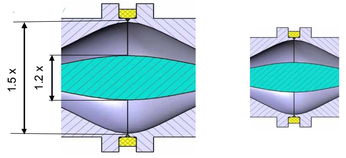
The SwissProt database with taxonomy *E.coli* and two missed-cleavages was searched with precursor mass accuracy of 10 ppm and fragment ions with 20 mmu. TMT reporter ions were quantified with 10 ppm tolerance. A 1% FDR was allowed.

Results

High-Field Orbitrap Mass Analyzer

One of the most important changes in the Orbitrap Elite™ mass spectrometer is the use of a new high-field Orbitrap mass analyzer. The new Orbitrap is 1.5 times smaller than the standard Orbitrap while the central electrode is relatively thicker. However, this down-scaling means that the entrance aperture is also reduced more than twice in cross-section. To avoid a loss of sensitivity, a miniature lens system was developed to focus ions into a much smaller spot. The increase in resolution on the high-field Orbitrap is about a factor of 1.8 compared to the standard Orbitrap. Figure 1 shows the standard Orbitrap and the new high-field Orbitrap mass analyzers true-to-scale.

FIGURE 1. Standard Orbitrap mass analyzer (left) and new high-field Orbitrap mass analyzer (right), true-to-scale



Standard Orbitrap High-Field Orbitrap

Advanced Signal Processing

The Orbitrap Elite instrument employs advanced signal processing based on enhanced Fourier transformation. This produces a high degree of synchronization of the ion motion in the Orbitrap mass analyzer. The resolution increase with this advanced signal processing is a factor of about 1.7.

The improvements in resolution permit a faster scan rate. The resolution settings and the resulting scan speed are summaried in Table 1.

Table 1. Summary of resolution and scan speed on the Orbitrap Elite hybrid mass spectrometer

Resolution settings Orbitrap Elite	Scan speed [Hz]	Resolution at *m/z* 400
15.000	7.7	15.700
30.000	6.9	31.700
60.000	4.0	60.000
120.000	2.3	125.000
240.000	1.2	248.000

Intact Protein Analysis and Top-Down Proteomics

Figure 2 shows on the example of the intact yeast enolase (47 kDa) the increase in resolving power using both the next generation Orbitrap mass analyzer and advanced data signal processing.

FIGURE 2. Intact yeast enolase infusion on the Orbitrap Elite (top trace) and Orbitrap Velos Pro™ (bottom trace) hybrid mass spectrometers showing the increased resolution of the Orbitrap Elite

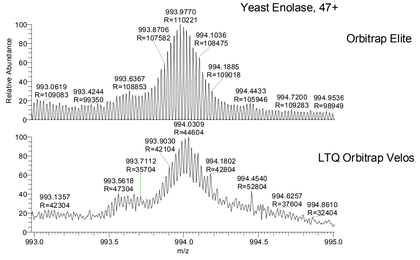


FIGURE 3. Histone H4 (12.3 kDa) HCD (top trace) and ETD (bottom trace) spectra and identification and annotation using Thermo Scientific ProSightPC software

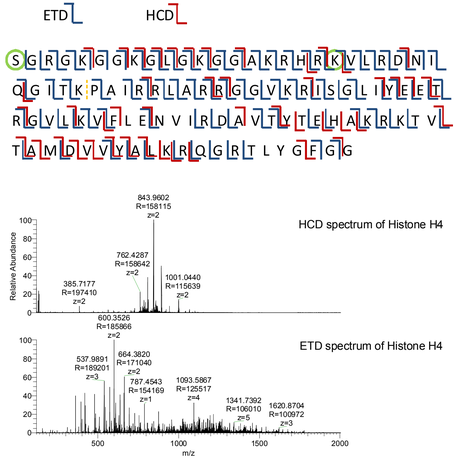
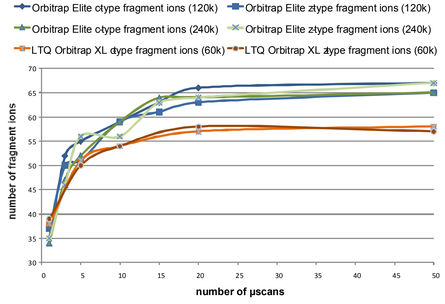


Figure 3 shows the HCD and ETD spectra of the Histone H4 protein, a 12.3 kDa protein. Top-down proteomics is done on-line on complex protein mixtures and usually employs serveral microscans to enhance the quality of the MS/MS spectrum. Figure 4 shows the benefit of higher resolution and higher sensitivity of the Orbitrap Elite hybrid mass spectrometer for top-down proteomics. More ETD fragment ions can be identified with higher resolution with fewer microscans, making the Orbitrap Elite suitable for on-line top-down proteomics.

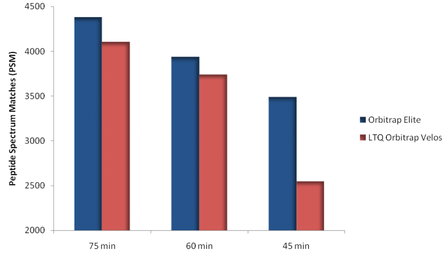
Figure 4. Influence of the number of microscans for an ETD top-down spectrum on the Orbitrap Elite and the LTQ Orbitrap XL™ hybrid mass spectrometers on the example of Ubiquitin



Bottom-Up Proteomics

Figure 5 shows the benefits of the higher scan speed and higher sensitivity for typical bottom-up proteomics experiments.

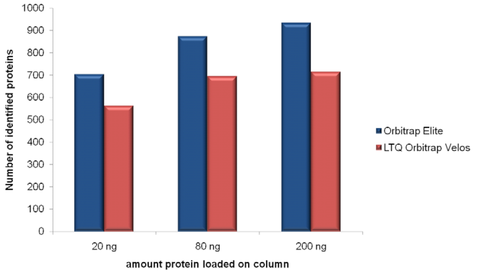
Figure 5. Number of peptide spectrum matches for different LC gradients on the Orbitrap Elite and the LTQ Orbitrap Velos hybrid mass spectrometers for a top 15 HCD method



The same *E.coli* sample was analyzed with the same LC setup on the Orbitrap Elite and the LTQ Orbitrap Velos hybrid mass spectrometers with a top 15 HCD method and with different gradient lengths. Due to the higher scan rate and sensitivity, substantially more peptide spectrum matches (PSM) were identified on the Orbitrap Elite. The performance increase also allows shorter LC gradients and increases analysis throughput.

The same performance increase can also be observed for TMT-labeled proteins with different protein amounts loaded on column. The Orbitrap Elite hybrid mass spectrometer identifies more proteins than the LTQ Orbitrap Velos mass spectrometer for both very low amounts (20 ng) and high amounts (200 ng). (Figure 6)

FIGURE 6. Number of identified TMT-labeled *E.coli* proteins with different protein amounts loaded on column on the Orbitrap Elite and the LTQ Orbitrap Velos hybrid mass spectrometers



Conclusion

- The new high-field Orbitrap mass analyzer and advanced signal processing enhance resolution, scan speed and sensitivity on the Orbitrap Elite hybrid mass spectrometer.
- Higher MW intact proteins can be isotopically resolved for accurate molecular mass determination.
- More fragment ions can be identified in top-down MS/MS spectra.
- More peptides and proteins can be identified in bottom-up proteomics experiments.

Acknowledgements

We would like to thank Rosa Viner (Thermo Fisher Scientific, San Jose, CA, USA) for the TMT analysis.