Identification and characterization of intact proteins in complex mixtures using online fragmentation on the new Orbitrap Elite

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Overview

Purpose: To evaluate the performance of the new Orbitrap Elite hybrid mass spectrometer for the in-depth top down analysis of complex intact protein mixtures.

Methods: The protein identification capabilities of the Orbitrap Elite by LC-MS/MS were investigated with the use of a fractionated yeast lysate. Identifications were based on accurate, high resolution mass measurements of both the precursor and product ions. Improved top down characterization of proteins was evaluated using multiple fragmentation techniques including CID, HCD, and ETD. Data were analyzed using ProsightPC 2.0.

Results: The Orbitrap Elite hybrid mass spectrometer was very successful at identifying proteins in a complex mixture using online LC separation. The improved resolution and scan speed allowed the accurate identification of proteins with sensitive detection of fragment ions in a short period of time. The combination of multiple fragmentation types improved the sequence coverage and allowed the localization of post-translational modifications such as phosphorylation.

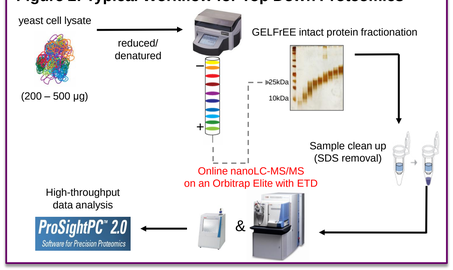
Introduction

The ability to fully characterize proteins in their intact forms allows thorough biological investigation of the functional importance of changes such as post-translational modifications, protein isoforms/sequence variations, and protease cleavages. Characterization of proteins at the intact level by nanoLC-MS/MS has long been of interest for mass spectrometrists as this technique can provide essential information, often in a non-targeted fashion, regarding modification chemistries localized to specific residues. Many of these findings are otherwise not easily discernable using standard proteomics practices involving protein digestion, this includes the ability to discern protein isoforms, localize combinations of post-translational modifications, and determine in vivo protease cleavage sites.

Here we introduce a newly developed Thermo Scientific Orbitrap Elite hybrid mass spectrometer which combines unprecedented scans speeds at extremely high resolution (>240,000) with high sensitivity and accurate mass characteristic of our hybrid Orbitrap platforms (Figure 1). The high-field Orbitrap and advanced signal processing has improved the analysis of level intact proteins in relatively complex mixtures on an LC timescale. In this study we evaluated a complete top down proteomics workflow from sample preparation to data analysis providing a robust and sensitive method for identification and characterization of proteins in a mixture using a variety of fragmentation techniques including CID, HCD, and ETD.

Methods

Figure 2: Typical Workflow for Top Down Proteomics



Sample: Yeast lysate (Sigma-Aldrich).

Lysate Desalting: The necessary desalting, required before GELFREE fractionation, was performed using the Thermo Fisher Scientific Zeba Spin Desalting columns with a 7 kDa MWCO (product # 89890).

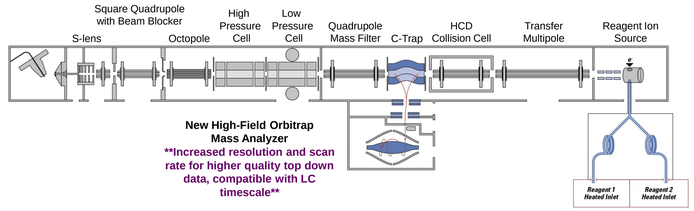
GELFREE™ Fractionation of Intact Protein Lysate: Fractionation of proteins in the mass range of 3.5-100 kDa was performed with a GELFREE 8100 10% Tris-Acetate Cartridge Kit on the GELFREE 8100 Fractionation System (Protein Discovery) according to the manufacturer's protocol.

Detergent Cleanup: Using the Thermo Scientific Pierce Detergent Removal Resin (product # 87777), we efficiently removed the detergents introduced for the GELFREE separation.

Online LC: Fractions 1 and 4 were analyzed by further LC-MS/MS as described here. Intact proteins were separated online using the split-free EASY-nLC (Thermo Scientific) with solvent compositions as follows: solvent A: 0.1% formic acid in water, solvent B: 0.1% formic acid in acetonitrile. The gradient consisted of 5-30% solvent B over 10 min, 30-65% solvent B over 45 min, and 65-90% solvent B over 5 min. The proteins were separated using a 5 µm, 1000 Å polymer (polystyrene divinylbenzene) reversed phase (PLRP-S) precolumn (3 cm x 150 µm ID) and analytical column (10 cm x 75 µm ID) purchased from New Objective.

MS Analyses: On the new Thermo Scientific Orbitrap Elite hybrid mass spectrometer, the resolution was set to 100K for full scan with 8 µscans and 1E6 AGC target. The resolution was set to 60K for MS^2 with 10 µscans and 2E6 AGC target. The maximum IT was 2s for both full scan and MS^2 scan. ETD, HCD, and CID fragmentation was performed on the most intense ion with the m/z as masses option selected to only perform MS/MS on the most intense charge state per protein. The protein identifications were performed with ProSightPC 2.0 software (Thermo Scientific).

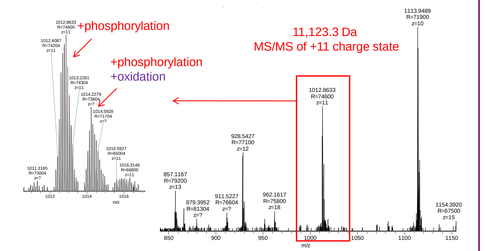
FIGURE 1. Schematic of the new Orbitrap Elite hybrid mass spectrometer

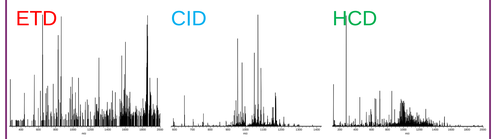


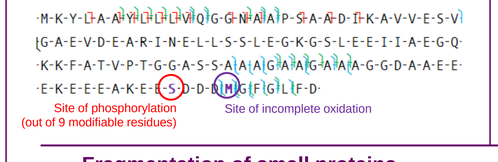
- New high-field Orbitrap with advanced signal processing providing more scans at a given resolution (>4 Hz at 60,000 resolution vs 1 Hz on LTQ Orbitrap Velos) and a higher maximum resolving power of 240,000.
- Robust and sensitive generation II ion optics with beam blocking.
- New pre-amplifier for improved S/N.

Localization of Modifications

Identification of 60S Acidic Ribosomal Protein P2-β and localization of phosphorylation site

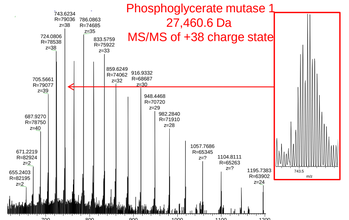




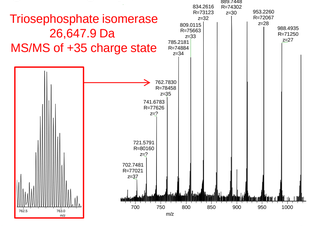


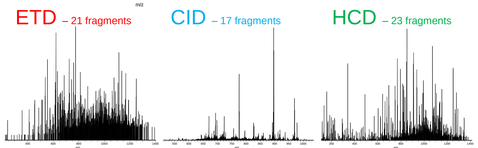
Identification of larger proteins

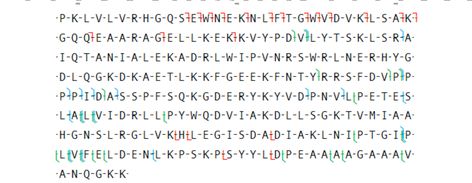
Multiple fragmentations improved fragment ion coverage for larger proteins

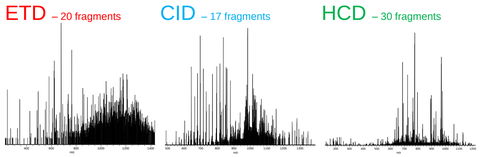


30 kDa proteins are baseline resolved with a single scan by LC-MS/MS. The online fragmentation enables accurate identification of the intact proteins.



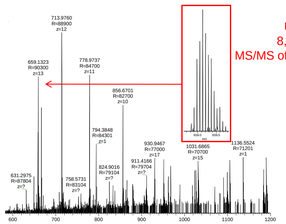




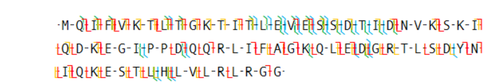


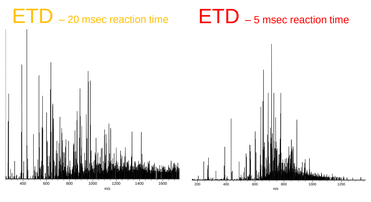
Fragmentation of small proteins

Full sequence characterization from both N and C termini



Combination of multiple fragmentation techniques, including multiple reaction times for ETD, provided extensive sequence coverage with single scan LC-MS/MS





Summary and Conclusions

- The Orbitrap Elite is a top of the line mass spectrometer for the identification of intact proteins on an LC timescale
  - High resolution MS allowed baseline resolution of isotopes for intact proteins up to 30 kDa with low ppm mass accuracy
    - 50 kDa proteins can be baseline resolved with resolution of 240,000
  - Multiple modes of fragmentation (CID, ETD, and HCD) improved fragment ion coverage of intact proteins and allowed localization of sites of modifications
  - Faster scan speeds at high resolution settings (60K resolution at ~4 Hz) allowed more microscans to be summed on the LC-timescale to improve both MS and MS/MS analyses
    - Improves signal to noise and ion statistics of intact proteins and fragment ions
    - Improves fragment ion coverage in less time
  - Ion trap accumulation prior to MS/MS allowed identification of low intensity proteins with high quality spectra

References

Lee, Kellie, et al. A robust two-dimensional separation for top-down tandem mass spectrometry of the low-mass proteome. *J. Am. Soc. Mass. Spectrom.* 2009 Dec;20(12):2183-91