

Improving Protein Analysis in Orbitrap Mass Spectrometry

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Overview

Purpose: Demonstrate the ability of the new hybrid ion trap-Orbitrap™ mass spectrometer to characterize intact proteins on a chromatographic timescale.

Methods: Standard proteins were analyzed via LC-MS or direct infusion utilizing an Orbitrap Elite hybrid mass spectrometer.

Results: Significant improvement of top-down LC-MS/MS analysis by using the Orbitrap Elite mass spectrometer

Introduction

Major goals in every top-down proteomics experiment are protein identification and characterization. The strategy used to achieve these goals involves high-resolution mass measurement of the molecular weight of the intact protein and fragment ions. In spite of enormous improvements in terms of speed and sensitivity in the FT/MS instrumentation over the last few years, top-down LC-MS/MS in current large scale proteome analyses remains limited. Further improvement of higher-resolution analysis at faster detection speed is required to advance the development of proteome analysis. Recent innovations in the Orbitrap technology, such as high-field Orbitrap mass analyzer and advanced signal processing¹, implemented in the new Thermo Scientific Orbitrap Elite mass spectrometer, accelerated detection by around 3.6-fold.

Methods

Sample Preparation

Intact proteins (all from Sigma Aldrich, St. Louis, MO, USA, except the Fc fraction from EPFL) were dissolved in buffer A (98% water, 2% acetonitrile, 0.1% formic acid) prior to LC-MS analysis.

Liquid Chromatography

Proteins were separated on a nanobore analytical column (75 µm × 10 cm) with an integral fritted nanospray emitter (PicoFrit™, New Objective, Inc., Woburn, MA) containing 5 µm polymeric reversed-phase (RP) media (100 Å pore size). A trap column (150 µm i.d. × 2 cm) containing identical chromatographic media was used. The Thermo Scientific EASY-nLC system was operated at a flow rate of 300 nL/min.

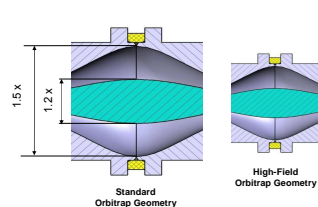
Mass Spectrometry

Experiments were carried out on a Thermo Scientific Orbitrap Elite™ hybrid mass spectrometer equipped with a compact high-field Orbitrap assembly (Figure 1).

Data Analysis

High-resolution mass spectra were deconvoluted using Thermo Scientific Xtract software and further processed using Thermo Scientific ProSigniPC software.

FIGURE 1. Comparison of a standard Orbitrap geometry with that of a high-field Orbitrap analyzer.



Results

Combining a new, compact Orbitrap mass analyzer geometry (Figure 1) with advanced signal processing, the resolution has been increased by nearly four-fold vs. the previous Orbitrap detector. For example, a 0.76 sec. transient provides nominal resolving power of about 240,000 which became the maximum resolving power setting on serial Orbitrap Elite instruments. This implementation enables high-resolution analysis at fast detection speed which makes the new instrument more suitable for top-down analysis on an LC time scale. With the Orbitrap Elite instrument we were able to achieve 3-4x shorter scan times vs. the previously highest-performance Orbitrap instrument, the Thermo Scientific LTO Orbitrap Velos mass spectrometer, at the same resolution. Top-down LC-MS/MS data shown in Figures 3-7 demonstrate that the Orbitrap Elite instrument is able to isotopically resolve proteins up to about 50 kDa. Furthermore, MS/MS averaging times can be reduced from 10 to 3 seconds without decreasing signal-to-noise performance, thus allowing us to identify proteins with even greater confidence (more fragment ions matched). The instrument method employed in the present work for top-down LC-MS/MS analysis is described in Figure 2.

FIGURE 2. Instrument method used for top-down LC-MS/MS analysis.

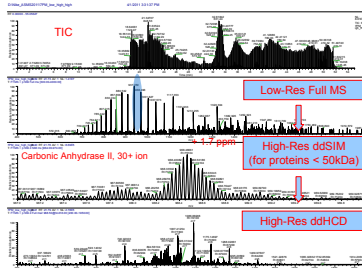


FIGURE 3. Top-down LC-MS/MS analysis of carbonic anhydrase II (29 kDa).

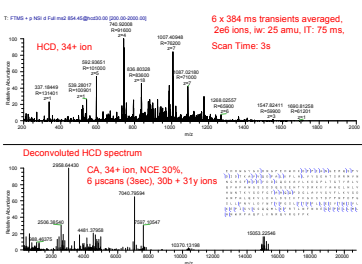


FIGURE 4. ProSigniPC graphical fragment mapper showing the identified b and y HCD fragment ions of carbonic anhydrase II

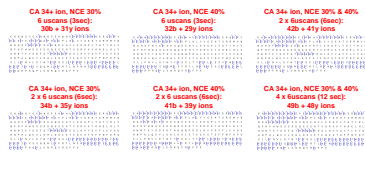


FIGURE 5. Top-down LC-MS/MS analysis of enolase (46.6 kDa).

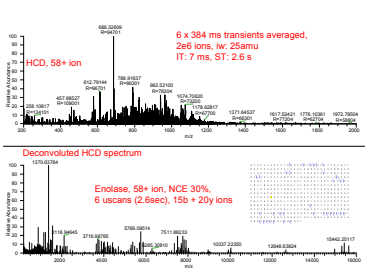


FIGURE 6. ProSigniPC™ graphical fragment mapper showing the identified b and y HCD fragment ions of enolase

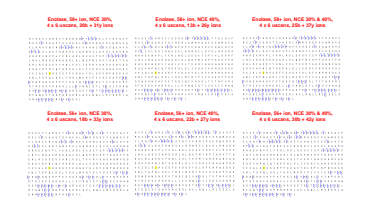
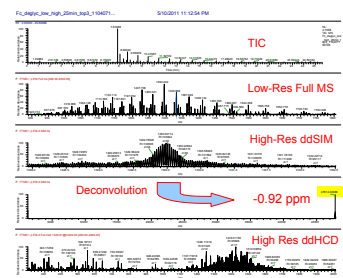


FIGURE 7. Top-down LC-MS/MS analysis of the Fc fragment (47.8 kDa) of an antibody.



In direct infusion experiments, proteins larger than 50 kDa (up to 78 kDa) could be isotopically resolved (see Figures 8-9).

FIGURE 8. Analysis of intact BSA (66.4 kDa) in 0.76s transients. Mass spectrum of +40 charge state was acquired in infusion mode at an average resolving power of 125,000.

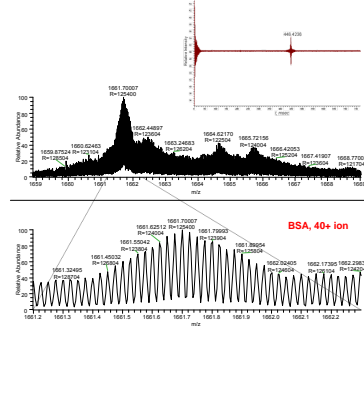
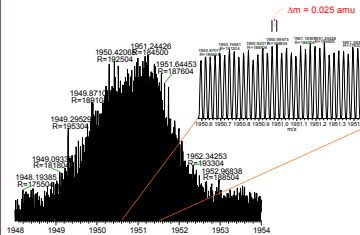


FIGURE 9. Analysis of intact apo-transferrin (78 kDa) in 1.5-second transients (developer's kit only). Baseline-resolved spectrum of +40 charge state was acquired in infusion mode (500 microseconds) at an average resolving power of 180,000.



Conclusion

- Top-down LC-MS/MS analysis is significantly improved using the compact, high-field Orbitrap analyzer and the advanced signal processing method implemented in the new Orbitrap Elite mass spectrometer.
- MS/MS averaging times can be reduced by more than three-fold without decreasing S/N and thus allowing to identify proteins with even more confidence (with more fragment ions matched).
- Intact proteins up to about 50 kDa can be isotopically resolved on a chromatographic timescale.
- Intact proteins up to 78 kDa can be isotopically resolved in direct infusion experiments.

References

- O. Lange, E. Damoc, A. Wiegand, A. Makarov, "Enhanced FT for Orbitrap Mass Spectrometry", Proc. 66th Conf. Amer. Soc. Mass Spectrom., Denver June 5-9, 2011, Poster MP093.

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