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 FTMS 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 analyses. Recent innovations in the Orbitrap technology, such as high-field Orbitrap mass analyzer and advanced signal processing[1], 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 (PLRP) media (1000 Å pore size). A trap column (150µm 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.

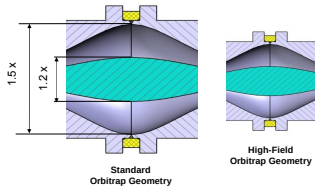
Mass Spectrometry

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 ProSightPC 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 LTQ 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 to it 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.

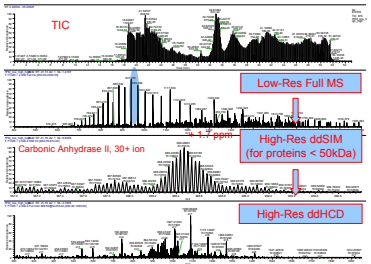


FIGURE 3. Top-down LC-MS/MS analysis of carbonic anhydrase II (29 kDa).

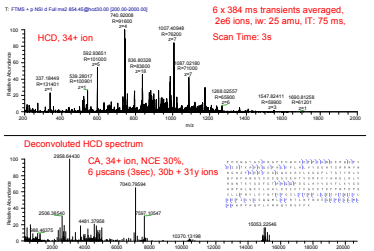


FIGURE 4. ProSightPC graphical fragment mapper showing the identified *b* and *y* HCD fragment ions of carbonic anhydrase II

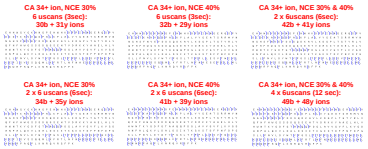


FIGURE 5. Top-Down LC-MS/MS analysis of enolase (46.6 kDa).

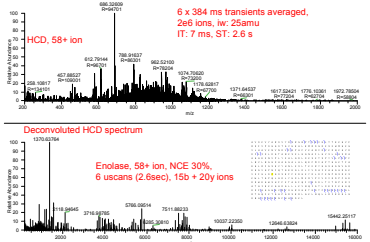


FIGURE 6. ProSightPC™ graphical fragment mapper showing the identified *b* and *y* HCD fragment ions of enolase

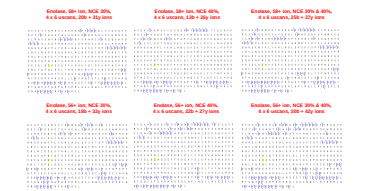
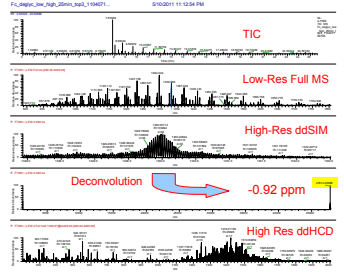


FIGURE 7. Top-down LC-MS/MS analysis of the Fc fragment (47.8 kDa) of an antibody.



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.

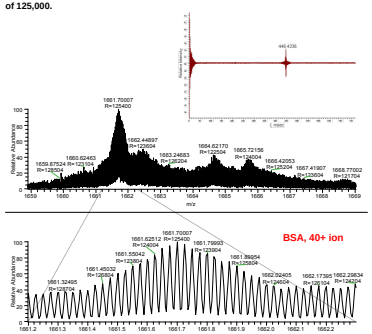
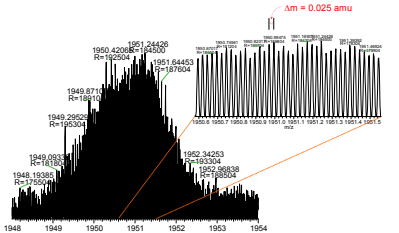


FIGURE 9. Analysis of intact apo-transferrin (78 kDa) in 1.5-second transients (developer's kit only). Baseline-resolved spectrum of +40 charge state was acquired in infusion mode (500 microscans) at an average resolving power of 180,000.



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

1. O. Lange; E. Damoc; A. Wieghaus; A. Makarov. "Enhanced FT for Orbitrap Mass Spectrometry". Proc. 59th Conf. Amer. Soc. Mass Spectrom., Denver June 5-9, 2011, Poster MP093.

Acknowledgements

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