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

Shannon Eliuk1, John F. Kellie2, David Horn1, Neil Kelleher2, Vlad Zabrouskov1

Thermo Fisher Scientific, San Jose, CA 95134, USA1; Northwestern University, Evanston, IL 60208, USA2

Introduction

The ability to characterize proteins in their intact forms allows thorough investigations of the composition of complex mixtures, essential information, in a non-equilibrated setting, which is critical for understanding the specific roles of proteins in cellular processes. Many of these findings are often not easily discernible using standard proteomics protocols involving protein digestion; however, the ability to deconvolute peptide sequence combinations of post-translational modifications, and determine in vivo protein structures, is highly relevant.

Here we introduce a newly developed Thermo Scientific Orbitrap Elite hybrid mass spectrometer, which combines unprecedented ion counting at extremely high mass resolution and accurate mass measurements with the ability to perform multiple reaction monitoring (MRM) analyses. The Orbitrap Elite is the next generation of Thermo Scientific hybrid mass spectrometers that combines the highest mass resolution with a mass accuracy of parts per million. The high resolution (R = 70,000) at m/z 200 and mass accuracy (δm/z < 1 ppm) allows for full MS analysis of intact proteins and identification of multiple fragmentations. The Orbitrap Elite is a top of the line mass spectrometer for the identification of intact proteins on an LC timescale. In this study we evaluated a complete top-down proteomics workflow from sample preparation to intact protein identification.

Purpose:

The protein identification capabilities of the Orbitrap Elite by LC/MS/MS were investigated with the use of a ProteoWizard peptide library. Identifications were based on accurate, high resolution mass measurements of both the intact protein and product ions. The large top-down characterization of proteins was evaluated using multiple fragmentation techniques including CID, HCD, and ETD. Data were analyzed using Progenesis QI 2.0.

Methods:

Figure 2: Typical Workflow for Top Down Proteomics

A robust two-dimensional separation for top-down tandem mass spectrometry.

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.

Figure 3: Identification of 60S Ribosomal Protein P2-8 and localization of phosphorylation sites

Multiple fragmentations improved fragment ion coverage for larger proteins.

Comparison of multiple fragmentation techniques including multiple reaction monitoring (MRM) for ETD, provided extensive characterization of intact proteins.

Summary and Conclusions:

The Orbitrap Elite is a new generation of the mass spectrometer for the identification of intact proteins on an LC timescale. The ability to perform multiple reaction monitoring (MRM) analyses for intact proteins is critical for understanding the specific roles of proteins in cellular processes. The new high resolution and accurate mass measurements of both the intact protein and product ions allows for full MS analysis of intact proteins and identification of multiple fragmentations.

References: