Improving Protein Analysis in Orbitrap Mass Spectrometry

Eugen Damoc, Eduard Denisov, Oliver Lange, Thomas Moehring, and Alexander Makarov
Thermo Fisher Scientific, Bremen, Germany

Overview

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

Methods: Standards were analyzed via LC-MS/MS to directly utilize 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 gains in every top-down proteomics experiment are protein identification and characterization. The existing solution for these goals requires high-resolution and high-mass accuracy equipment, combined with substantial computational effort. This is in stark contrast to the number of intact protein applications, which include: intact proteins, post-translational modifications, crosslinking, and intracellular localization. The current generation of Orbitrap mass spectrometers, in either high-resolution and high-mass accuracy or field ionization modes, enables the analysis of large scale proteomic samples. Further gains in the development of top-down LC-MS/MS are possible with the combination of high-resolution and high-mass accuracy with advanced signal processing.

Methods

Sample Preparation

Tryptic peptide mix of Sigma Aldrich, BD, USA was used as the test mixture. All samples were filtered through 0.2 μm nylon syringe filters before LC-MS analysis.

Liquid Chromatography

Proteins were separated on a standard analytical column (35 μm x 50 mm) with an integrated fritted nanospray emitter (ProSciFit, New Objective, Inc., Woburn, MA) using a Thermo Scientific PROBO-500 chromatography system. A complete list of the MS conditions can be found in the Supporting Information.

Mass Spectrometry

Experiments were carried out in a Thermo Scientific Orbitrap Elite™ hybrid mass spectrometer equipped with a compact high-field Orbitrap analyzer. The field-free Orbitrap analyzer (with Orbitrap Elite™) was operated at a low field of 300 mTorr.

Data Analysis

High-resolution mass spectra were deconvoluted using Thermo Scientific ProFit software and further processed using Thermo Scientific ProFit/Proteo发现 toolset.

Results

Combining a new compact Orbitrap Elite analyzer geometry (9 μm) 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 excellent resolving power (R ~ 194 000) and the mass resolution curve is shown in Figure 7. As expected, the new instrument is more suitable for top-down analysis as the higher mass scale. The Orbitrap Elite instrument has been particularly useful for the advent of new Orbitrap technology (e.g., implemented in the new Orbitrap mass spectrometer). Accelerated detection by several fold.

Conclusions

- 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 newest 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.
- Top-down LC-MS/MS analysis can be conducted at lower S/N levels, thus allowing to identify proteins with more confidence.

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