1000 Proteins Per Hour [pph] – Maximizing Protein ID From Complex Mixtures

Kai Scheffler[1], Eugen Damoc[2], and Thomas Moehring[2]
Thermo Fisher Scientific, [1] Dreieich, Germany; [2] Bremen, Germany

Overview

Purpose: Evaluate the performance of the newly developed hybrid linear ion trap-Orbitrap mass spectrometer for characterization of complex proteomes

Methods: NanoLC-MS/MS analyses of a complex protein proteolytic digest from *E. coli* whole cell lysate

Results: The increased sensitivity and improved duty cycle of the new instrument enables a dramatic increase in the number of protein IDs with increased sequence coverage and high confidence.

Introduction

Enormous improvements in mass spectrometry technology over the last few years have allowed for ever-deepening analysis of complex proteomes. However, many low-abundance proteins remain undetected in current large-scale proteomic analyses due to the limits on the rate at which MS/MS spectra can be acquired. To solve this undersampling problem, we developed a new hybrid linear ion trap-Orbitrap mass spectrometer (Thermo Scientific LTQ Orbitrap Velos hybrid mass spectrometer, Figure 1) that provides faster MS/MS scanning in the ion trap (up to 10 peptide sequences per second), thereby increasing the depth of analysis of complex protein mixtures. In the present work, we evaluated the ability of the newly developed hybrid instrument to identify more proteins, with increased sequence coverage and confidence.

Significant technology improvements have been implemented in the LTQ Orbitrap Velos™ mass spectrometer, including: (a) a progressive stacked-ring ion guide (S-Lens) with 5-10x increased ion transmission; (b) a dual-pressure differentially pumped ion trap with a higher-pressure cell for improved ion trapping, isolation, and CID efficiencies, and a lower-pressure cell for improved resolution and/or scan speed; (c) predictive AGC for increased scan speed; (d) an HCD-collision cell with axial field for improved performance; (e) improved vacuum in the Orbitrap chamber for better intact protein analysis.

Methods

Sample Preparation

E. coli protein digests were prepared from a whole cell lysate that was reduced with DTT and alkylated with iodoacetamide followed by digestion with a K/R-specific protease for 16 h at 37 °C.

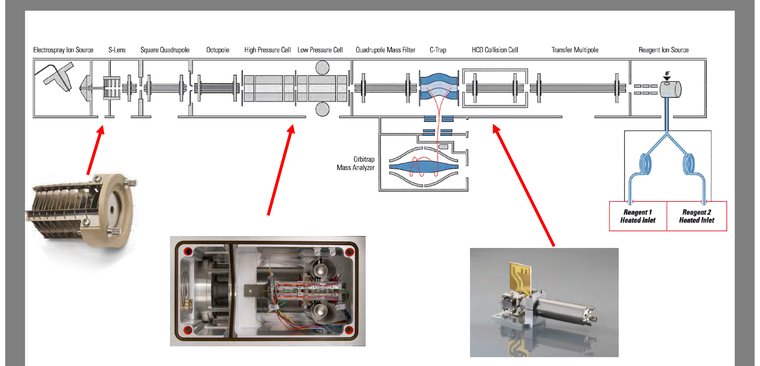
Liquid Chromatography

This complex peptide mixture was separated using a Thermo Scientific Surveyor LC and MicroAS autosampler with a reversed-phase peptide trap (100 µm inner diameter, 2 cm length) and a reversed-phase analytical column (75 µm inner diameter, 10 cm length, 3 µm particle size, both NanoSeparations, NL), at a flow rate of 300 nL/min.

Data Processing

Raw data files were searched with the Mascot™ algorithm (Matrix Sciences) using Thermo Scientific Proteome Discoverer software v. 1.2.

FIGURE 1. Schematic of the LTQ Orbitrap Velos hybrid mass spectrometer equipped with an ETD source.



Results

To evaluate the performance of the new linear ion trap-Orbitrap mass spectrometer for characterization of complex proteomes, nanoLC-MS/MS analysis of 1 µg, 100 ng, and 20 ng of a proteolytic digest of *E. coli* whole cell lysate was performed. Two different instrument methods were employed for this analysis:

(1) TOP20 ITMS^2 method (1 OT Full MS scan + 20 dd CID scans), and (2) TOP10 HCD method (1 OT Full MS scan + 10 dd HCD scans).

While the first method uses the ion trap to detect the CID fragment ions, the second method employs the Orbitrap to detect the HCD fragment ions, providing accurate masses for both MS and MS/MS scans. In addition to the different acquisition methods, two different gradient lengths were applied: 60 min and 90 min (2%-30% B). The methods and the results are summarized in Figures 2-6. NanoLC-MS/MS analyses of 1 µg *E. coli* digest with a 60 min gradient and a ddTOP20 CID method yields 1050 unique proteins based on 6582 unique peptides (results obtained with an FDR < 1%).

FIGURE 2. NanoLC-MS/MS analysis of 1 µg *E. coli* digest using TOP20 CID method (1x OT full scan + 20x ddCID scans). A) Base peak chromatogram displaying the scan speed for a full scan cycle. B) 10 consecutive CID MS/MS spectra and their assigned peptide sequences. C) Peptide ID from Protein Discover based on the CID spectra displayed in B.

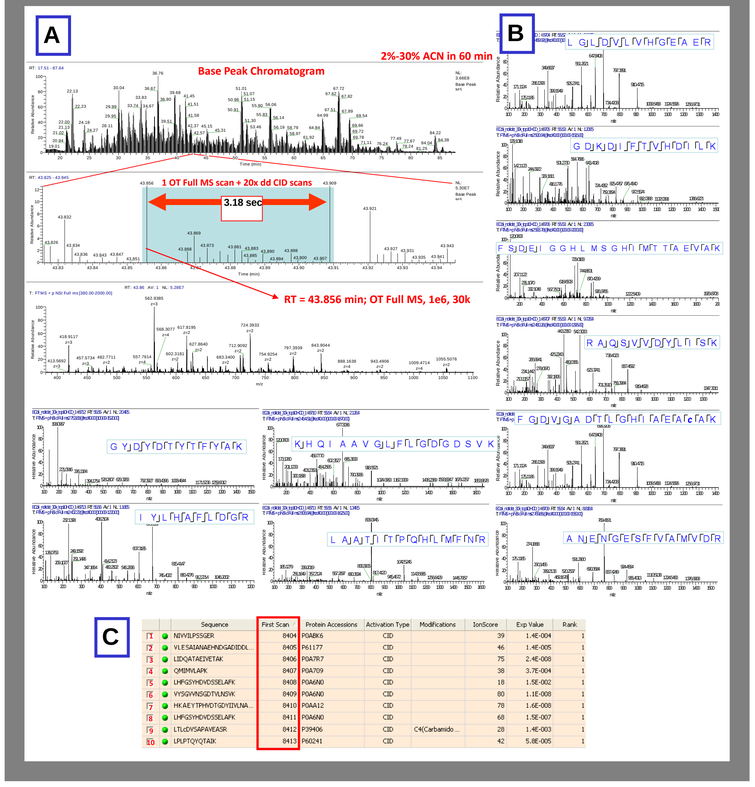


FIGURE 3. Unique proteins (A) and unique peptides (B) identified at <1% FDR from different sample amounts: a proteolytic digest of 1 µg, 100 ng, and 20 ng *E. coli* whole cell lysate, analyzed with a ddTOP20 CID method (2%-30% ACN in 60 min).

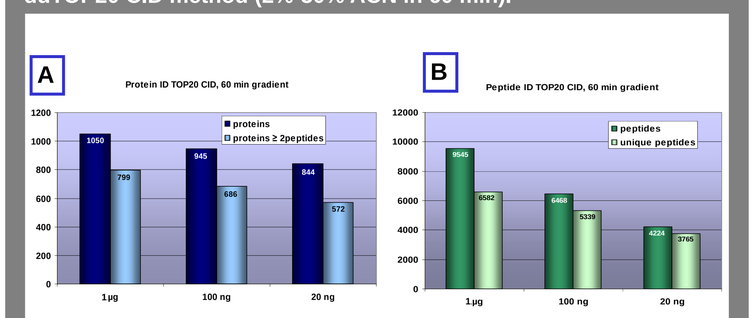
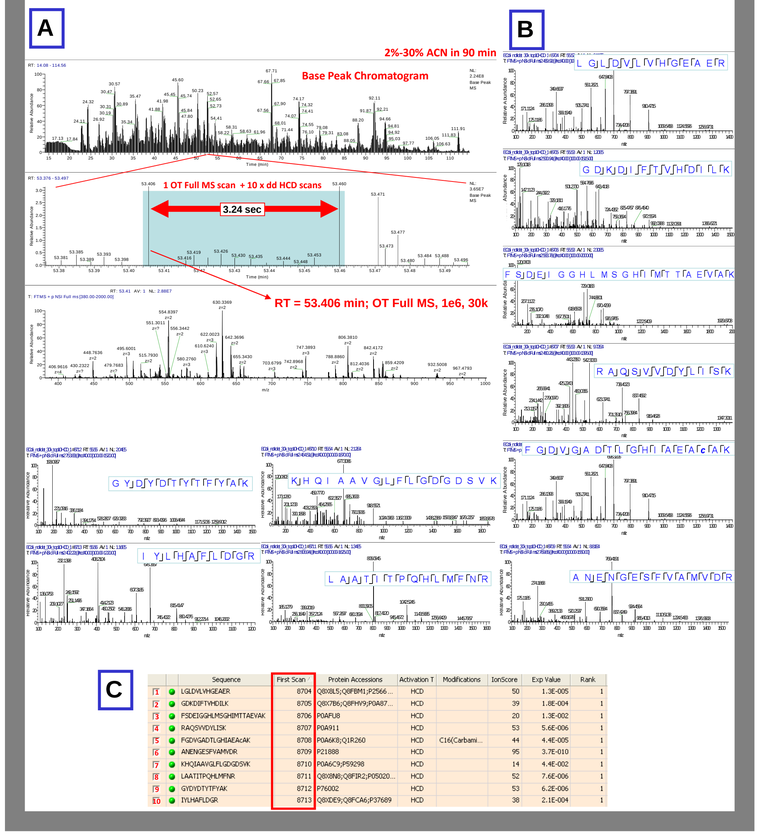


FIGURE 4. NanoLC-MS/MS analysis of 1 µg *E. coli* digest using TOP10 HCD method (1x OT full scan + 10x ddHCD scans). A) Base peak chromatogram displaying the scan speed for a full scan cycle. B) 10 consecutive HCD MS/MS spectra and their assigned peptide sequences. C) Peptide ID from Protein Discover based on the HCD spectra displayed in B).



1 µg of *E. coli* digest was separated over 90 min and analyzed with CID (ddTOP20 CID method) and HCD (ddTOP10 HCD method) in separate runs. The number of identified proteins was well above 1000 for each run, based on 7601 and 6344 peptides, respectively, (Figure 5) as an average from duplicate runs. The overlap of identifications from single runs using CID versus HCD was 81% on the protein level and 68% on the peptide level. The speed of CID and HCD MS/MS spectra acquisition is significantly increased on the LTQ Orbitrap Velos hybrid mass spectrometer, which is mainly due to the much reduced fill times.

FIGURE 5. Unique proteins (A) and unique peptides (B) identified from 1 µg and 100 ng of *E. coli* digest run with ddTOP20 and ddTOP10 HCD methods (<1% FDR, 2%-30% ACN in 90 min).

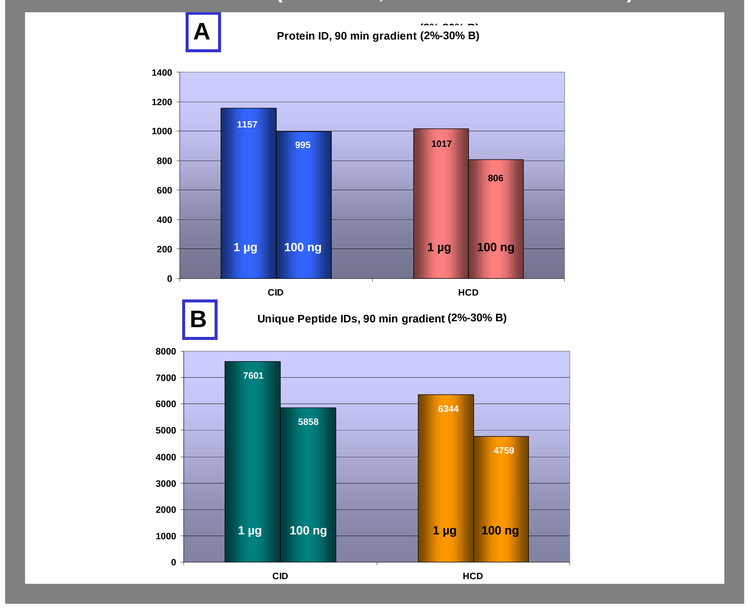
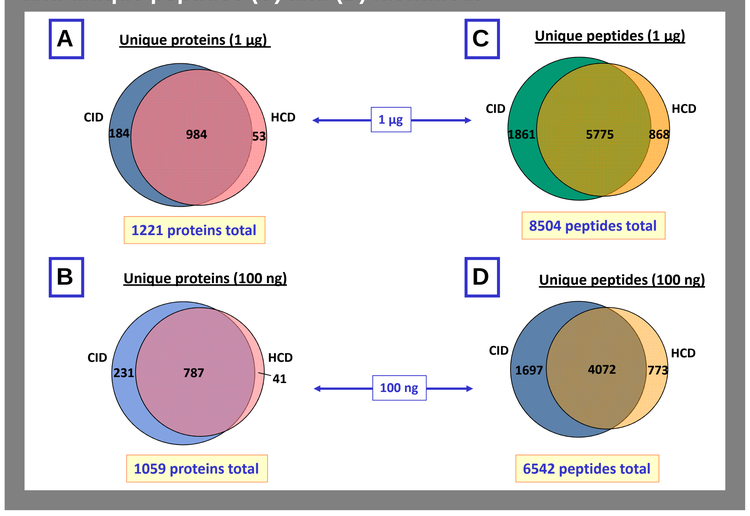


FIGURE 6. Venn diagram comparing single ddTOP20 CID and ddTOP10 HCD runs acquired from 1 µg and 100 ng *E. coli* digest with a 90 min gradient (2%-30% B) based on proteins (A) and (B) and unique peptides (C) and (D) identified.



Conclusions

The LTQ Orbitrap Velos hybrid mass spectrometer

- provides a significantly increased scan speed in MS and MS/MS mode due to its higher transmission ion source, dual pressure ion trap, and improved HCD cell.
- identifies more than 1,000 proteins per hour from the proteolytic digest of an *E. coli* whole cell lysate.
- provides a higher HCD scan speed and much improved spectral quality, opening up new perspectives for *de novo* sequencing, quantitation of chemically labeled samples, and analysis of modifications which require high mass accuracy.

References

1. Wouters, E.R., Splendore, M., Senko, M.W., Syka, J.E.P., Dunyach, J.-J., Design of a Progressively Stacked Ring Ion Guide for Improved Ion Transmission at High Pressure, ASMS 2008.
2. Pekar Second, T., Blethrow, J.D., Schwartz, J.C., Merrihew, J.E., MacCoss, M.J., Swaney, D.L., Russell, J.D., Coon, J.J., and Zabrouskov, V. Dual-Pressure Linear Ion Trap Mass Spectrometer Improving the Analysis of Complex Protein Mixtures, *Anal. Chem.* 2009**,** 81, 7757–7765.
3. Olsen J.V., Schwartz J.C., Griep-Raming J., Nielsen M.L., Damoc, E., Denisov, E., Lange, O., Remes, P., Taylor, D., Splendore, M., Wouters, E.R., Senko, M., Makarov, A., Mann, M., Horning, S. A Dual Pressure Linear Ion Trap - Orbitrap Instrument with Very High Sequencing Speed. *Mol Cell Proteomics.* 2009 Dec; 8(12):2759-69.