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Ultra-High-Resolution Top-Down Proteomics Analysis

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Introduction

While peptide sequencing by LC-MS/MS has made great advances over the past years the analysis of intact proteins has been obstructed by the lack of efficient analytical tools. For the separation of proteins, polymeric monolithic columns have become an attractive alternative. Especially for the nanoLC-MS analyses of complex samples they offer high-efficiency separations due to the absence of inter-particle mass transfer. On the other hand ETD enabled ultra-high-resolution (UHR) TOF mass spectrometer appears to be a very good tool for the detection and identification of proteins.

Intact protein LC/MS Data with UHPLC and UHR-TOF

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Automated Processing
- Dissect: extract spectra for all proteins
- Max Entropy: deconvolution
- SNAP II algorithm: create mono-isotopic peak list for intact proteins

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Option for proteins of interest: ETD MS/MS in an offline experiment

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Biotools and MASCOT Top-Down Identification

Fig. 1: Workflow for ultra-high-resolution top-down proteomics analysis

Methods

LC/MS analysis
HPLC: UltiMate 3000 RSLCnano (Dionex)
MS: maXis UHR-TOF with ETD option (Bruker Daltonik GmbH)
Software: Compass 1.3 SR2, biotools 3.2 SR2, MASCOT 2.2 (Matrix Science)
Sample: 500 ng bacterial lysis supernatant (*E. coli*)
Columns: Dionex PepSwift monolithic PS-DVB, 100µm ID, 25 cm, Trapping column: 200µm ID, 5mm.
Gradient: 7% to 30% B in 27 min, 30% to 50% in 28 min, from 50 to 95% in 30, minutes, 700nl/min; Solvent B: 0.1% formic acid in 80% acetonitrile, 20% water; Sample loading/desalting for 5 minutes with 0.5% TFA in water at 20µl/min. Column compartment was kept at 60 °C.

The LC/MS analyses were automatically processed with the Compass software. The workflow is described in Figure 1.

ETD MS/MS
Sample: *E. coli* thioredoxin (Sigma Aldrich), 1pmol/µl was prepared in 50:50 acetonitrile/water with 0.1% formic acid and ionized by pneumatic electrospray at a rate of 3µl/min.

Fig. 2: Experimental setup
LC/MS analysis with UltiMate 3000 RSLCnano connected to a maXis UHR TOF with ETD option. LC separation with 25 cm monolithic columns.

Results

LC/MS analysis of intact E. coli lysate

The LC/MS raw data were processed with a special extraction algorithm (DISSECT) to generate compounds of similar chromatographic behavior; MaxEnt deconvolution and SNAP II applied on the resulting spectra gave the monoisotopic molecular weight of all proteins per compound. The workflow is described in Figure 1. In total we have detected 223 compounds or 1818 features, representing large peptides and intact proteins in the mass range from 5,000 to 40,000. The number of mono-isotopic masses per mass range is displayed in Table 1.

Chromatographic peak width are typically in the range of 7.5-20 s.

The resolving power for the intact proteins is in the range between 45,000 and 65,000. This allows isotopic resolution for most of the proteins in the observed mass range.

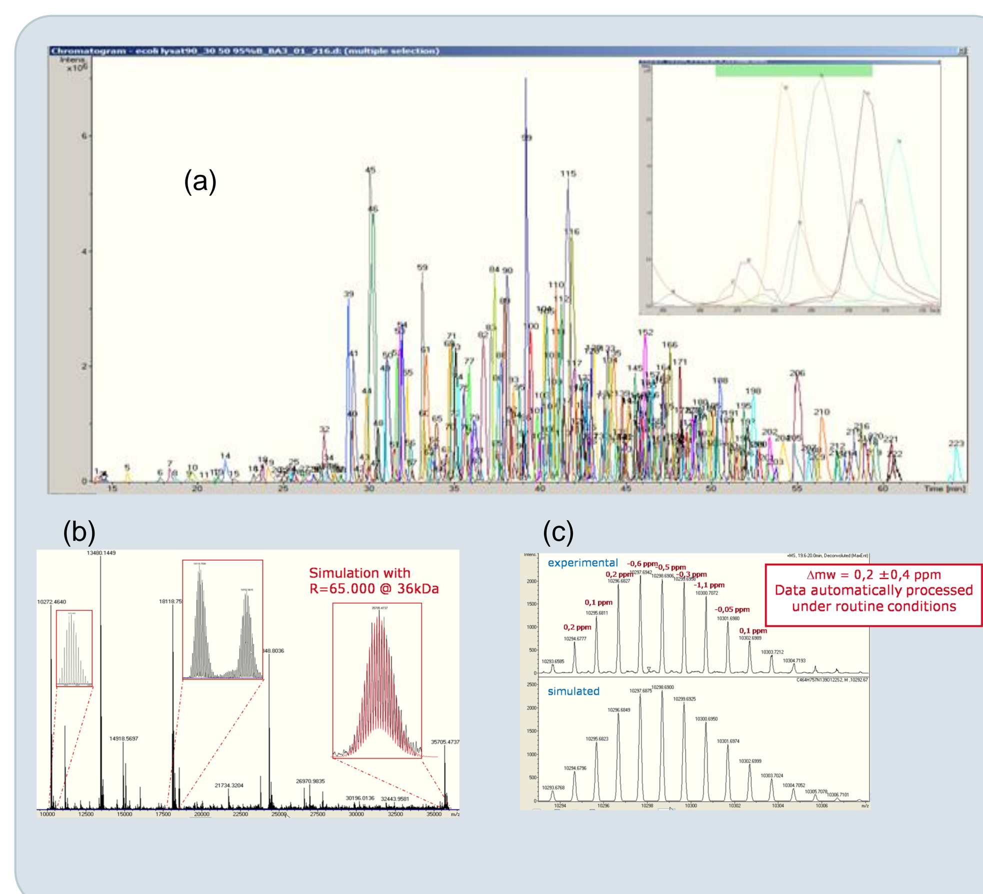

Fig. 3: Feature extraction using Dissect
(a) the complete chromatogram, 223 compounds have been extracted by Dissect; (b) a compound after MaxEnt deconvolution and SNAP II, every compounds contains multiple features, i.e. protein or peptide masses, mass resolution is typically > 50,000 allowing isotopic resolution in the observed mass range; (c) comparison of experimental data and calculated isotopic pattern, mass accuracy < 1 ppm.

MW_{mono} range	Number of features
5,000- 10,000	424
10,000-15,000	555
15,000-20,000	396
20,000-25,000	219
25,000-30,000	146
> 30,000	78

Table 1: Number of detected proteins and large peptides as function of the mass range.

ETD of intact Thioredoxin (11.7 kDa)

E. Coli thioredoxin could be identified by ETD fragmentation of the thioredoxin 15+ charge state as shown in Figure 3 (a). The high charge states of the fragments (up to 12+) require high resolution in MS/MS too. SNAP II gives in this case also the monoisotopic mass of the fragments that can be used for the MASCOT data base search.

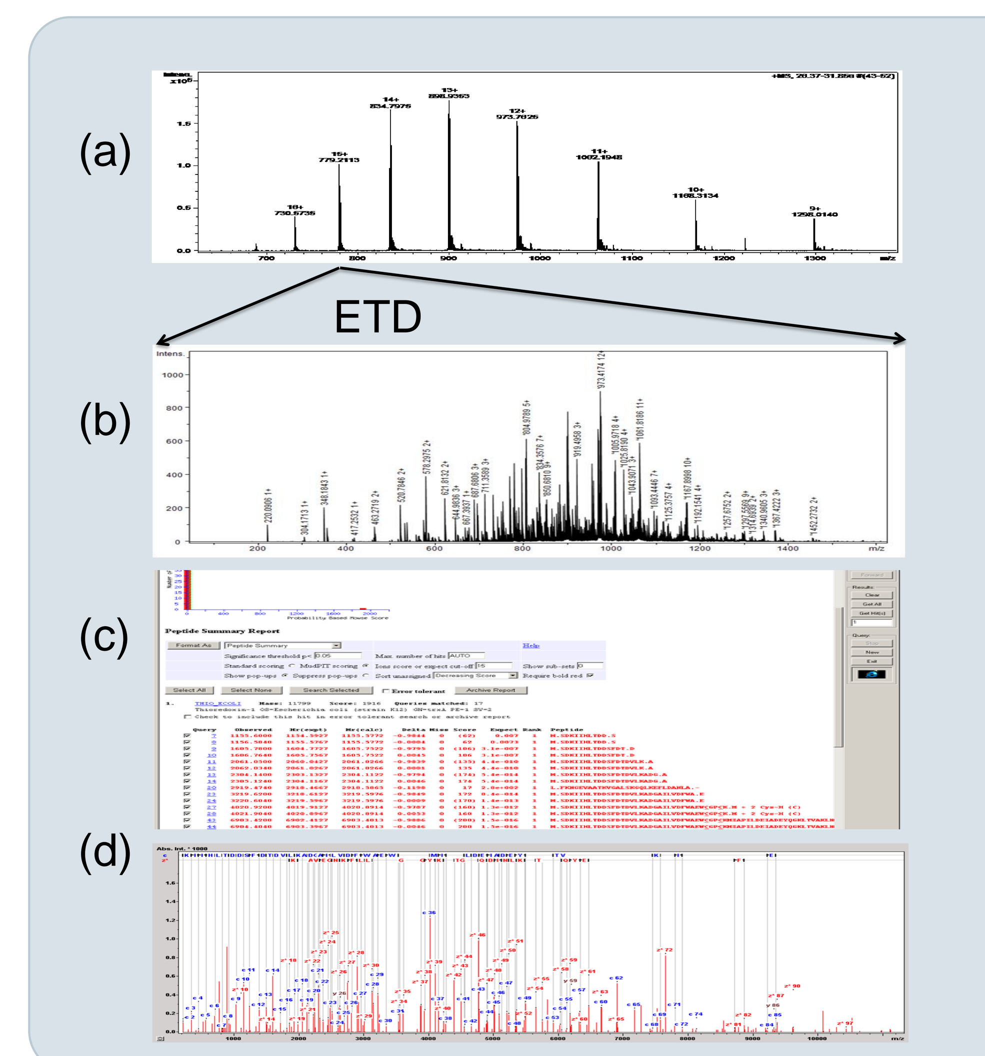

Fig. 4: ETD MS/MS of intact thioredoxin m/z 779.2 (z=15+)
(a) shows the different charge states; (b) multiply charged fragments; (c) MASCOT search results; (d) annotated sequence in Biotools

Summary

The combination of ultra-high-performance nanoLC and a fast UHR - TOF mass spectrometer with ETD capability enables a new approach for top-down proteome analysis, even for complex samples. Top-down proteome studies have some important advantages compared to bottom-up approaches: direct link between molecular weight and gene identity; full control over PTMs, truncation variance and isoforms.

Therefore top-down analysis could become an important complement to bottom-up proteome studies, possible on the same platform.

Conclusions

- Sensitive detection of intact proteins from complete cell lysates in a mass range up to 40 kDa.
- Excellent intact protein data with isotopic resolution on proteins < 40 kDa.
- Sensitive and information-rich ETD data from intact proteins

ETD MS/MS-UHR-TOF

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