

Fast and Highly Accurate QTOF ETD MS/MS for Top-Down Protein Identification

Carsten Stoermer, Ralf Hartmer, Oliver Räther, Markus Lubeck and Dirk Wunderlich

Bruker Daltonik GmbH, Fahrenheitstraße 4, 28359 Bremen, Germany
dw@bdal.de

Introduction

Electron Transfer Dissociation (ETD) is an accepted dissociation technique for the sequence characterization of large peptide and protein molecules. Orthogonal TOF instruments offer high spectra rate (20 Hz MS and MS/MS), high resolving power (30,000 – 60,000) and high mass accuracy (low ppm).

Here we show a QTOF instrument enabled for ETD and present ETD MS/MS data of intact proteins and on LC time scale.

Methods

A quadrupole OTOF (Bruker maXis™) was equipped with a nCI-source and a hexapolar ETD reaction cell. ETD Reagent anion and analyte ions were mass selectively transmitted through a mass resolving quadrupole. ETD reactions are performed in the reaction cell following the quadrupole, where the ions of different polarity are mutually stored. As depicted in Figure 1, the ETD experiment (performed in trapping mode) consists of four steps. (C) cation accumulation, (A) anion accumulation (R) extension of the ETD reaction and finally the (D) detection of the product ions in the orthogonal TOF. While ions are extracted, the next ETD experiment is performed in the reaction cell thus maximizing duty cycle.

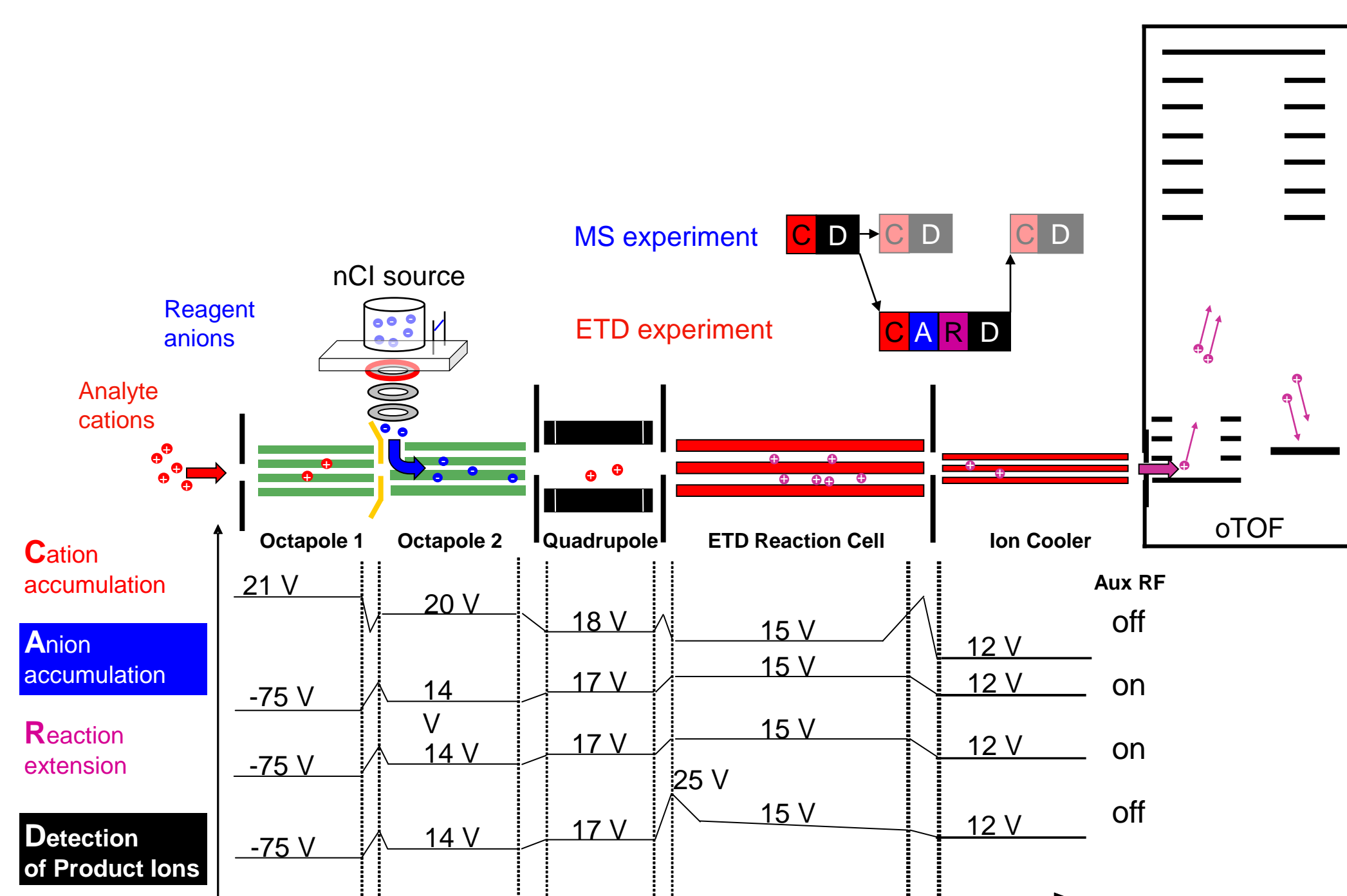


Fig. 1: Schematic depiction of the ETD maXis

a) The modification of the instrument include a nCI source, a segmented octapole ion guide, and a ETD reaction cell. The segmented octapoles are followed by a quadrupole, hexapolar ETD reaction cell, the ion cooler, and then the orthogonal accelerator and the TOF MS.
b) Plot of DC-potentials; applied to the ion transfer optics during the ETD experiments. Each ETD experiment consists of the steps of (c) cation accumulation, (a) anion accumulation, (r) reaction, and (d) detection of the product ions.

Results

Figure 2 shows the average of (a) 280, (b) 47 and (c) 5 ETD MS/MS spectra from the $[M+12H]^{12+}$ isolated ubiquitin ions ($m/z = 714.3087$). The TOF analyzer had a resolving power exceeding 40,000. In the case of Ubiquitin, with very complex ETD product ion spectra, this high resolution and mass accuracy was more than sufficient for protein identification by data base searching. As demonstrated in the enlarged views (b) and (c) of figure 2, averaging 47 experiments over a period of 10 s is sufficient to obtain ETD product spectra with a reasonable isotopic distribution, and 5 spectra (1s) is still enough to obtain a S/N-ratio resulting in valuable ion statistics to conclude the monoisotopic mass from the ETD MS/MS data.

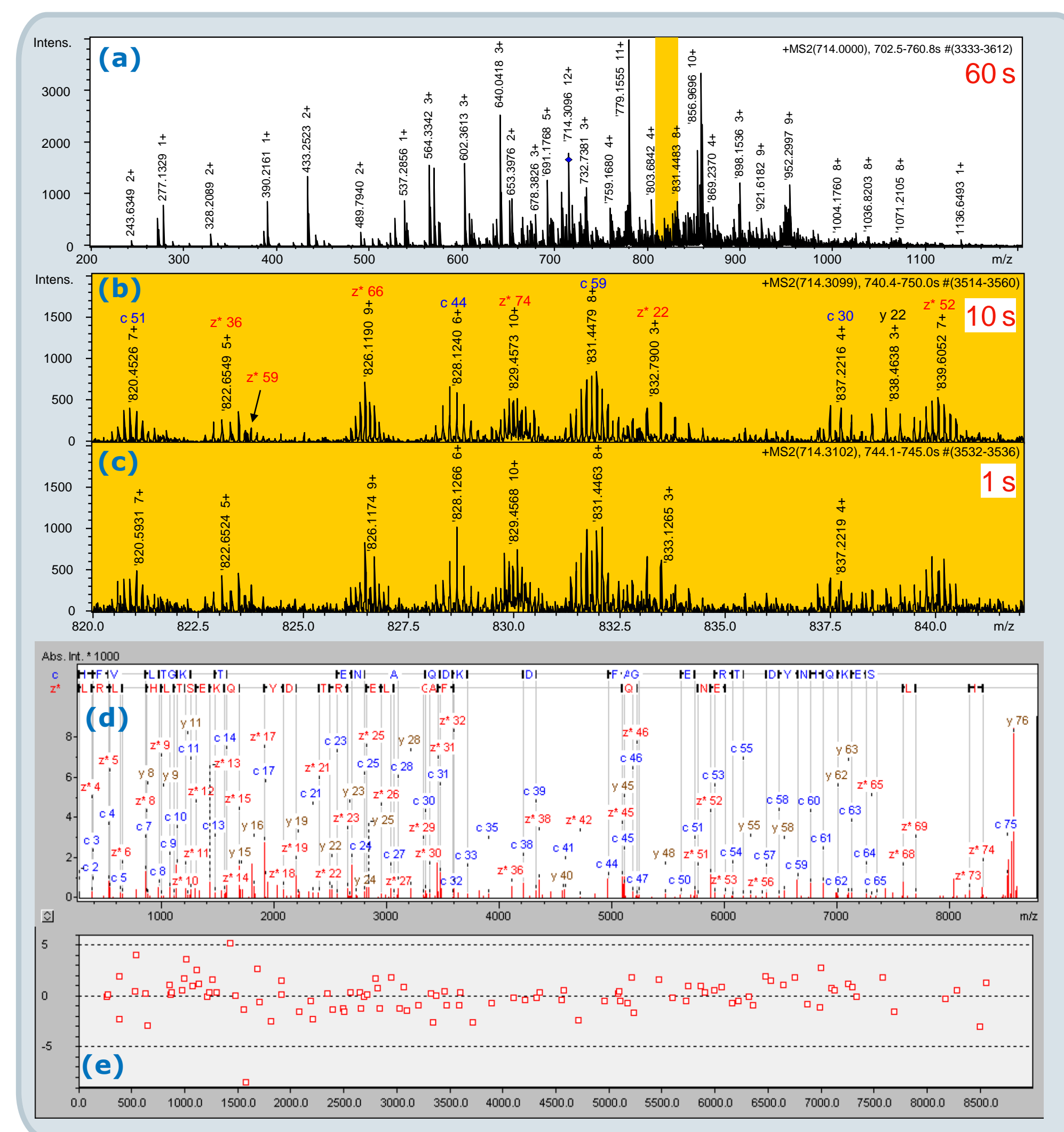


Fig 2. ETD MS/MS of intact ubiquitin, $m/z = 714.3087 [M+12H]^{12+}$
a) Average of 280 ETD MS/MS spectra over a period of 60 sec
b) enlarged view into the mass range indicated in (a), 47 ETD MS/MS spectra (10 s) averaged
c) enlarged view into the mass range indicated in (a), 5 ETD MS/MS spectra (1 s) averaged
d) BioTools annotation of Mascot search result (score = 416) of the processed spectrum. 47 of 75 c fragments and 45 of 75 z* fragments were identified.
e) RMS mass error 1.66 ppm from the Mascot data base search (Fig. 1d)

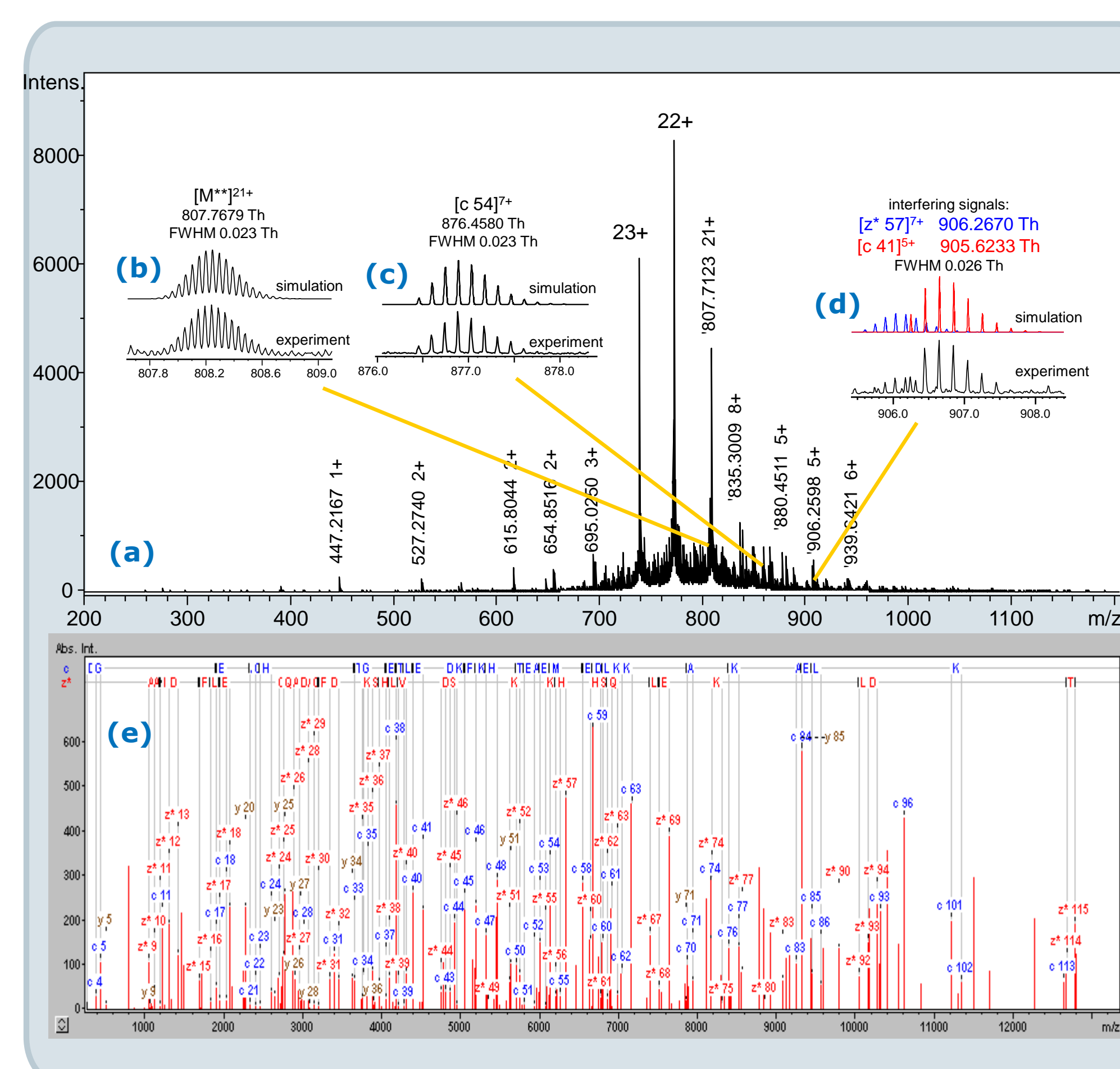


Fig 3. ETD MS/MS of intact myoglobin, $m/z = 737.5710 [M+23H]^{23+}$
a) Average of 840 ETD MS/MS spectra over a period of 240 sec
b), c), d) inset into ETD MS/MS spectra showing excellent match of the isotopic pattern and a resolving power of 35,000 to 38,000.
e) BioTools annotation of TD Mascot search result (score = 487) of the processed spectrum. 58 of 152 c fragments and 60 of 152 z* fragments were identified.

This is consistent with the Mascot data base searching from the SNAP II™ deconvoluted data resulting in probability scores of 416, 407, 208 for the averaging of 280, 47, 5 ETD MS/MS spectra of intact ubiquitin.

The average of 850 ETD experiments of intact myoglobin ($m/z = 738.5710$; $[M+23H]^{23+}$) over a period of 240 sec results in the Top-Down (TD) Mascot score of 487 (Figure 3). Insets (b), (c) and (d) show excellent consistency between experimental data and the simulated isotopic pattern of the ETD product ions. Within the m/z -range of 600 to 1000 at almost every nominal mass, an ETD product ion is obtained, so the interference of isotopic signals having different charge states becomes very likely (Figure 3 (d)). The data processing with SNAP II provides a charge deconvolution working extremely reliable and results in a list of singly charged monoisotopic masses which is particularly suitable for data base searching.

With the achieved rate ETD MS/MS experiments of 3-5 Hz the present instrument is ideally suitable for the coupling with online separation techniques. Figure 4 (a) shows an LC run from 100 fmol of a trypsin hydrolysis of BSA. 19 unique peptides were identified with a summed mascot score of 645 (standard scoring). ETD MS/MS data from the automatically selected precursor ion at $m/z 417.2119$ is shown in Figure 4 (b) identified with a Mascot score of 75.

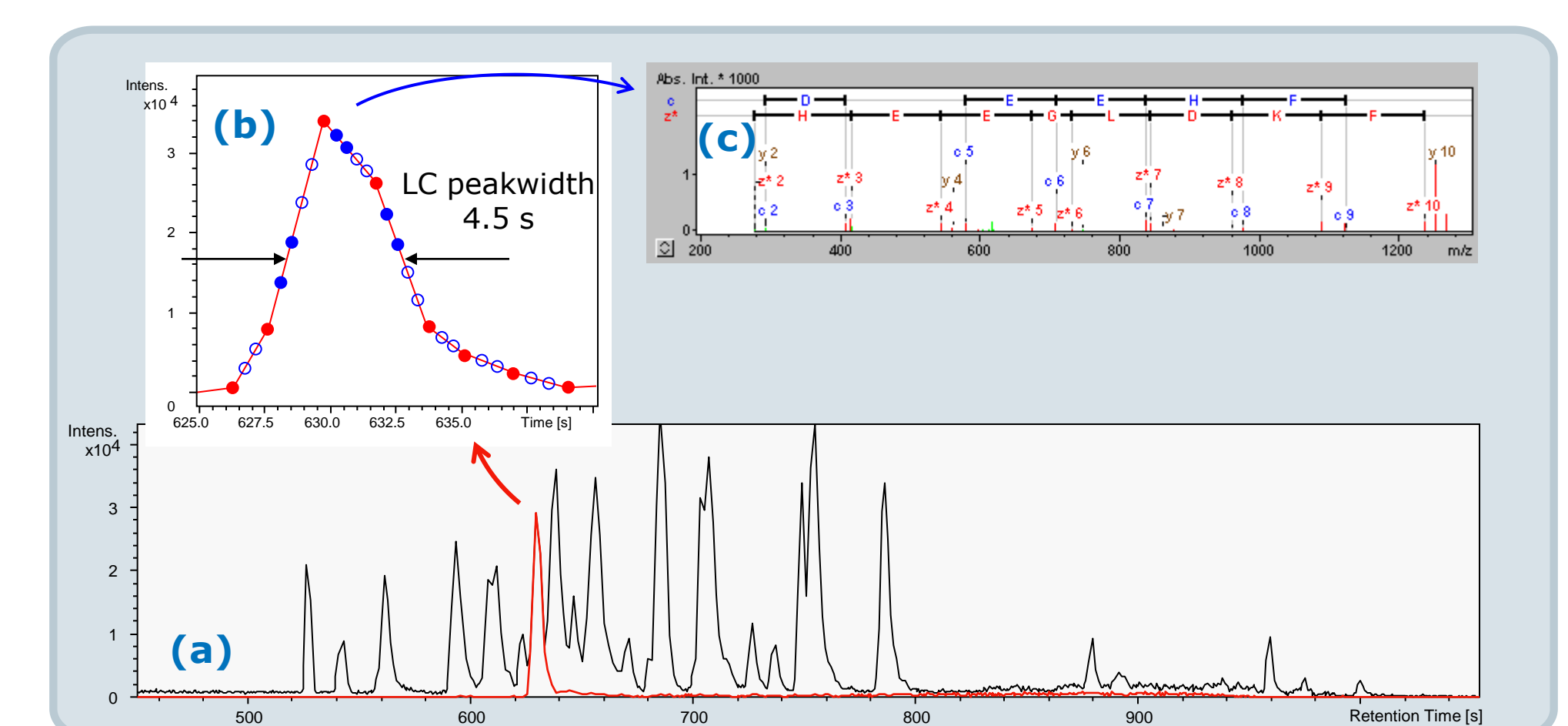


Fig 4. LC run of BSA (tryptic digest, 100 fmol)
a) Base peak chromatogram of the LC run and extracted ion chromatogram of the triply charged precursor ($m/z 417.2119$, sequence: FKDLGEEHFK) from the BSA-digest.
b) Inset showing a zoom into the extracted ion chromatogram indicating the sampling. Red circles: MS sampling points, blue circles: MSMS sampling points, filled blue circles: ETD-MSMS of FKDLGEEHFK.
c) BioTools annotation of Mascot search result (score = 75) of the processed spectrum.

Conclusions

- Ultra High Resolving OTOF is particularly suitable for sensitive and accurate detection of ETD fragments
- High Resolving Power of ETD-QTOF (~40,000) in combination with SNAP II provides a reliable tool for identification and discovery of intact proteins
- Fast ETD-QTOF spectra instrument (up to 5 ETD spectra/s) fits excellently to LC time scale of chromatographically separated peptides of protein digests

ETD quadrupole TOF enables highly accurate top-down proteomics and ETD MS/MS on LC-time scale

Top-Down Proteomics