

Role of increased resolution and scan speed for top-down proteomics with ETD/PTR

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Introduction

- Increased resolving power with Maximum Resolution scan mode in a seamless scan range @ 4600 u/sec enabling the detection of multiply charged fragments ($z = 1-6$).
- Top-Down Proteomics: Sequence characterization on intact proteins with ETD/PTR. SNAPII data processing leads to mass list of singly charged fragments. Highly confident Protein ID by Mascot search.
- ID and sequence characterization of biomarker candidate with ETD/PTR.

Methods

ETD MS/MS were carried out on an amaZon ion trap. An improved control of the non-linear ejection process and the development of the trap environment support faster scan modes as well as a higher mass resolution. The purified proteins were introduced into the ion trap with offline nanospray (Triversa Nanomate). ETD/PTR of the isolated protein is performed with reagent anions dedicated for either ETD or PTR. For protein marker ID and sequence characterization tissue lysate was treated with ultrasonication in an ice bath. Extract was centrifuged with Vivaspin 5000 ultra-filtration unit. Supernatant was collected and separated with an Agilent mRP column into a 96-well plate. Fractions were measured with MALDI TOF MS for peak localization and combined protein fractions subsequently measured with amaZon ETD and TriVersa Nanomate.

Protein	MW _{mono}	m/z (prec.)	charge state	Mascot Score
Ubiquitin	8560	714	12	375
RNase A*	13682	978	14	194
Lysozym C*	14303	842	17	207
Myoglobin**	16941	653	26	393

Table 1: Top-down analysis of intact protein

ETD/PTR MS/MS applying the new maximum resolution scan mode, data processing with SNAP II™

*treatment with DTT for the reductively opening of the cystein SS-bridges

**data base search with Mascot TD, surpass Mascot's precursor mass limit > 16kDa

Results

Dedicated MS/MS-techniques for top-down proteomics are electron-induced fragmentation processes like electron capture or electron transfer dissociation (ETD). The interpretation of ETD MS/MS data of highly charged proteins can be rather complicated, because a plethora of multiply charged and overlaid fragment ions can be expected.

The complexity of the ETD MS/MS-data is significantly reduced when the initial ETD-step is followed by a subsequent proton transfer reaction (PTR). For those ion traps having lower resolving power than the present amaZon the charge reduction step is controlled leading to singly (and partially) doubly charged ETD-fragments. However, the charge reduction down to singly charged ions lowers the resulting data quality with respect to sequence coverage as well as sensitivity.

Increased resolving power in maximum resolution scan mode leads to the detection of isotopically resolved ETD-fragments ($z > 1-6$). Charge deconvolution with SNAP II™ generates a list of singly charged monoisotopic masses, as e.g. given for the ETD/PTR MS/MS of myoglobin, ([M+26H]²⁶⁺) in Fig. 1c). Mascot data base searches of ETD/PTR MS/MS of standard proteins (MW up to 16 kDa) result in the identification of the intact protein (Table 1).

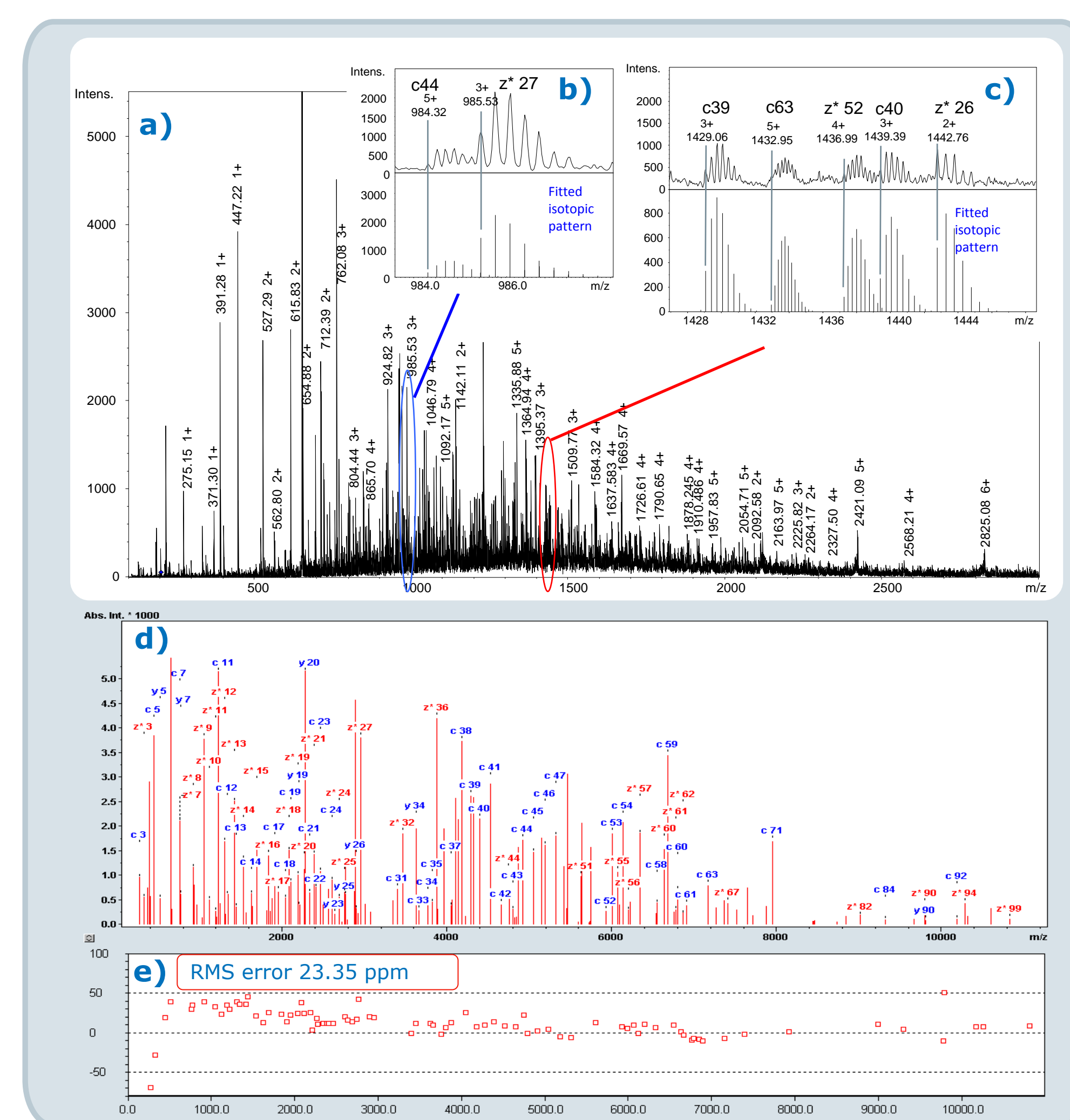


Fig 1. ETD/PTR of intact Myoglobin (200 fmol/μl); m/z = 653 [M+26H]²⁶⁺

- ETD MS/MS -> PTR; data processing with SNAP II™
- c) inset into Fig. 1a including the assignment of the c and z*-fragment
- BioTools annotation of TD Mascot search result (score = 393; Table 1) of the processed spectrum
- RMS mass error 23 ppm from the Mascot data base search (Fig. 1d)

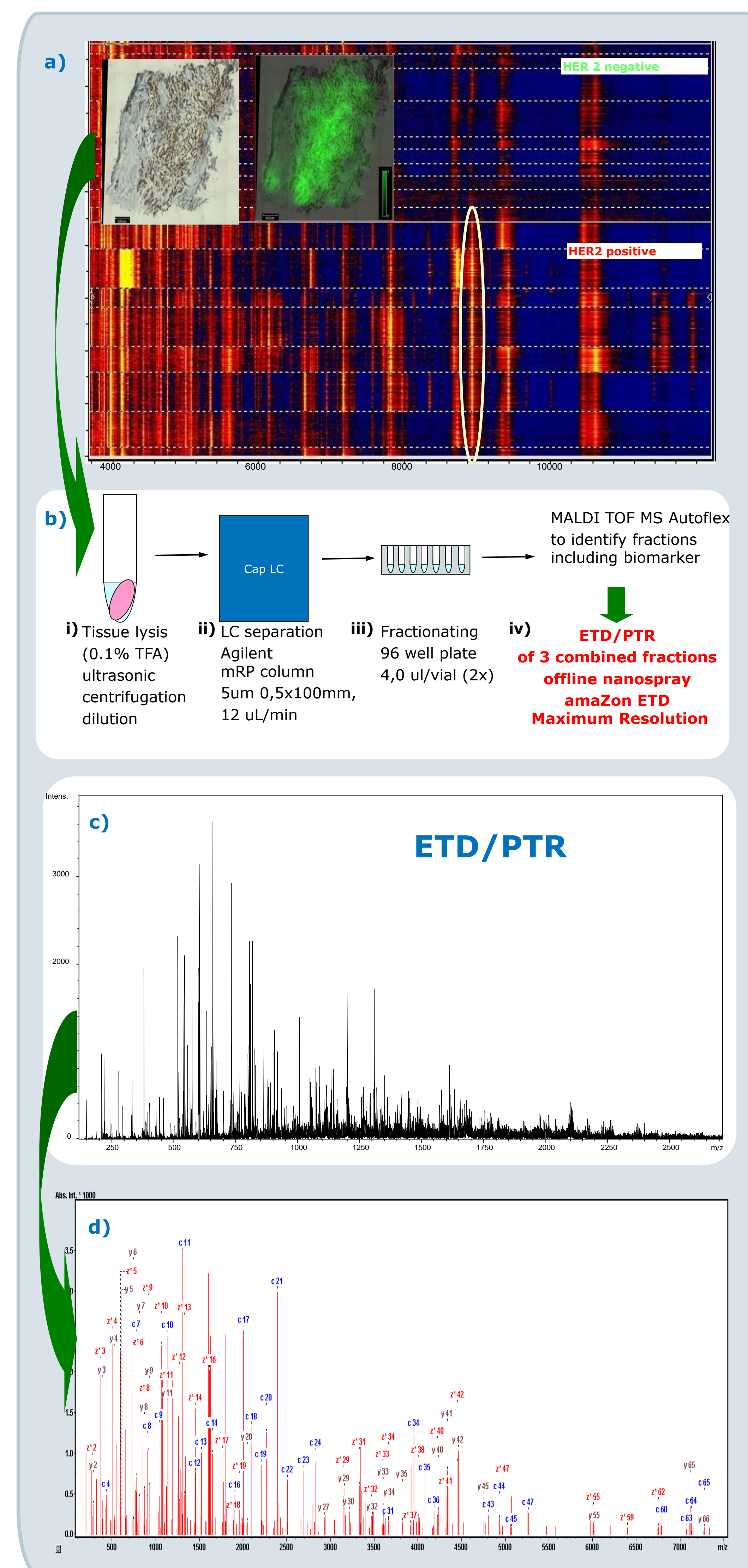


Fig 3: Top-Down ID and Sequence characterization of an intact biomarker protein

- MALDI Imaging results of a breast cancer tissue; HER2 positive/negative cells
- workflow for sample processing
 - Lysate of entire tissue cells, ii) LC-separation and fractionation, iii) biomarker identification, iv) sequence characterization with ETD MS/MS
- ETD/PTR spectrum of an intact protein from a cell lysate (top); maximum resolution scan mode (4800 u/sec, resolution up to 5+ ions) data processing with SNAP II™
- BioTools annotation of Mascot search result of the processed spectrum

ID protein marker for breast cancer

Protein biomarker discovered by a MALDI-TOF MS Imaging experiment of a breast cancer tissue (Fig 3a) is success-fully extracted and purified (Fig 3b).

ETD/PTR experiment (Fig. 3 c) of the isolated intact multiply charged protein leads to multiply charged ETD fragments ion ($z = 1-5$) detected with the new Maximum Resolution Scan mode.

Data processing with SNAPII results in singly charged monoisotopic masses. Mascot Data leads to biomarker identification with Mascot Score 126.

Acknowledgement

Breast cancer protein biomarker was kindly provided by Axel Walch und Sandra Rauser (Helmholtz-Zentrum, Munich, Germany).

Summary

- amaZon's maximum resolution scan mode (4600 u/sec) allows the seamless and fast detection of a plethora of multiply charged ETD-fragments ($z = 1 - 6$)
- Data processing with SNAP II generates a list of monoisotopic ETD-fragment masses, enabling the ID of the intact protein by a Mascot data base search
- In depth top-down sequence characterization of proteins up to MW of 20 kDa

Conclusions

⇒ ETD/PTR with the amaZon ion trap reaches new levels for Top-Down proteomics and is ready to become a powerful tool for characterization of intact protein biomarker.