

The Role of Increased Resolution and Scan Speed of Ion Traps for Top-Down Proteomics with ETD/PTR

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Introduction

- Maximum Resolution Scan: enables the detection of multiply charged fragments (up to 6+) @ 4600 u/s in a seamless scan up to m/z 3000.
- ETD/PTR: ID and detailed sequence characterization of biomarker candidates.
- Top-Down Proteomics: SNAP II data processing of ETD/PTR data leads to a highly confident identification of intact proteins by BigMascot search.

Methods

- amaZon ion trap: improved control of the non-linear ejection and the further development on the spherical trap results in faster scan speed and higher resolution.
- The Triversa NanoMate was used to infuse purified proteins into the ion trap. ETD/PTR of the isolated intact protein is done with different reagent anions which are generated from a single neutral compound by altering the voltage settings of the nCI source.
- For the identification of a protein biomarker, the tissue lysate was treated with iced ultrasonication. The resulting extract was centrifuged (Vivaspin 5000), the supernatant was collected and separated on an Agilent mRP protein column into a 96-well plate. Fractions were measured with MALDI TOF MS for peak localization and then analyzed by the amaZon ETD / Nanomate combination.

Results

Dedicated MS/MS-techniques for top-down proteomics are electron-induced fragmentation processes like electron capture or electron transfer dissociation (ETD). ETD MS/MS of highly charged intact proteins results in rather complicated spectra because of multiply charged and overlaid fragment ions.

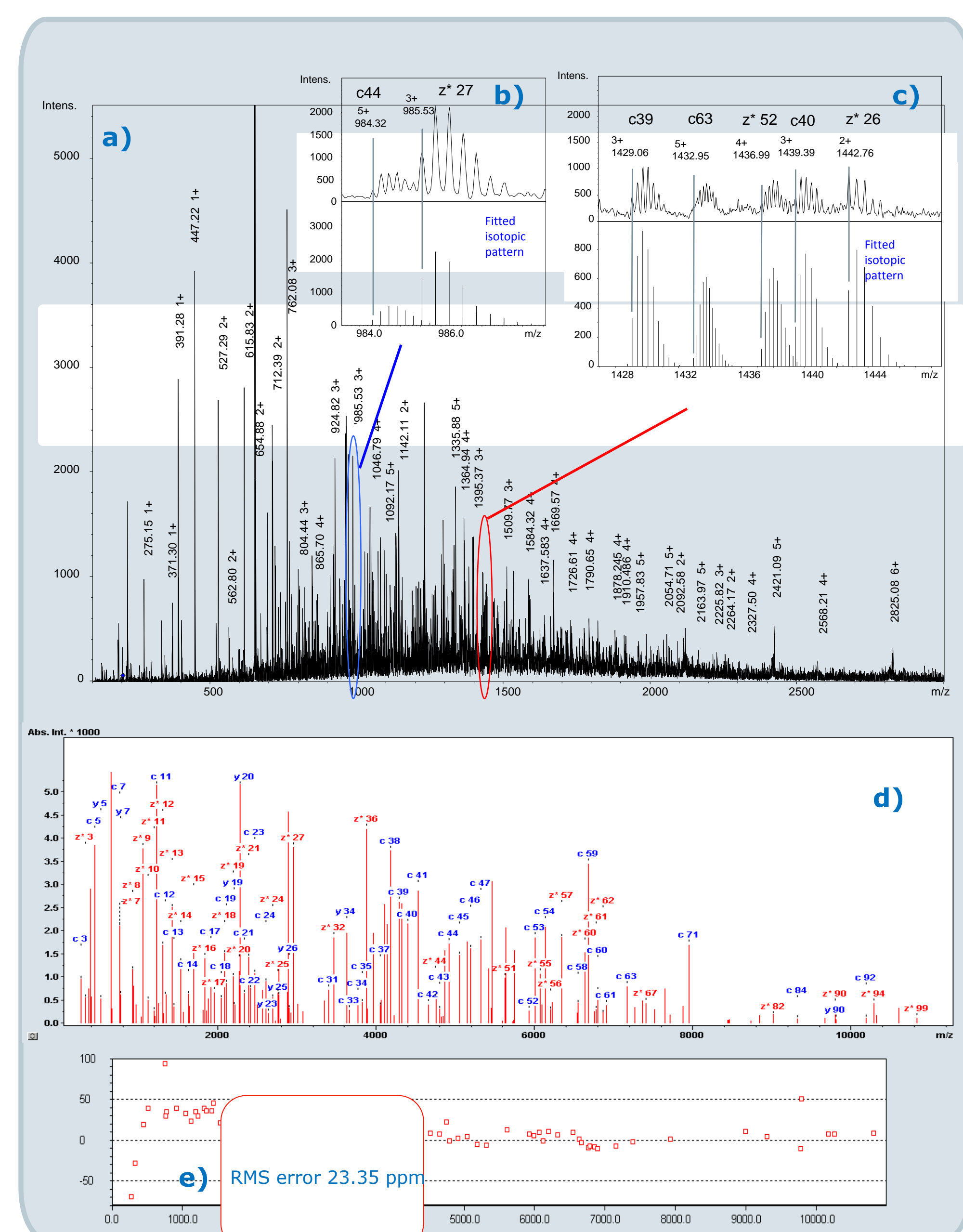


Fig 1. ETD/PTR of intact Myoglobin (200 fmol/μl); m/z= 653 [M+26H]²⁶⁺
a) ETD MS/MS → PTR; data processing with SNAP II™; b), c) inset into Fig. 1a including the assignment of the c and z* fragment; d) BioTools annotation of TD Mascot search result (score = 393; Table 1); e) RMS mass error 23 ppm from the Mascot data base search (Fig. 1d)

Subsequent proton transfer (PTR) significantly reduces the complexity by reducing the charge states to typically +1 to +6. However this still requires an excellent mass resolution and a sufficiently high m/z range as provided by the amaZon ETD. Charge deconvolution with SNAP II™ generates a list of singly charged monoisotopic masses, as e.g. for the ETD/PTR MS/MS of the [M+26H]²⁶⁺ ion of Myoglobin (Fig. 1c). From various proteins it can be shown that Mascot DB searches on ETD/PTR MSMS spectra result in the clear identification of the intact proteins (Table 1).

Protein	MW _{mono}	m/z (precursor)	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 proteins. ETD/PTR MS/MS with maximum resolution scan mode, data processing with SNAP II™

Sequence characterization of an intact Histone

Histones are highly basic K/R-rich DNA-binding proteins. Conventional bottom-up analysis by CID MS/MS is less useful for their PTM characterization. Fig. 2 shows the processed ETD/PTR data of a human histone ([M+22H]²²⁺; m/z 695) after charge deconvolution. Mascot DB search results in the identification of the Histone H3 protein (MW_{mono} = 15263 Da) with a Mascot score of 226.

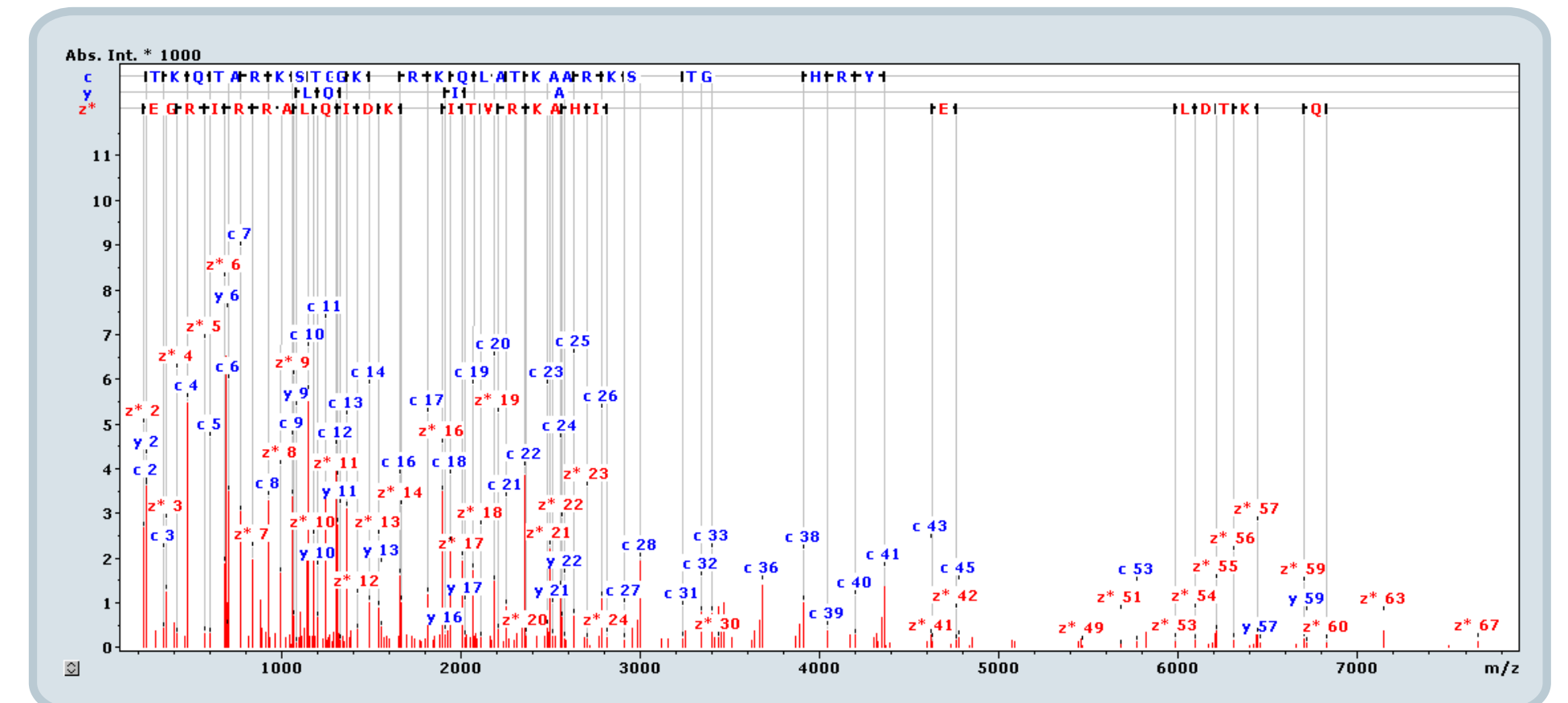


Fig 2. ETD/PTR of Histone H3, m/z =695 [M+22H]²²⁺. SNAP II data processing; BioTools annotation of Mascot result (score 226).

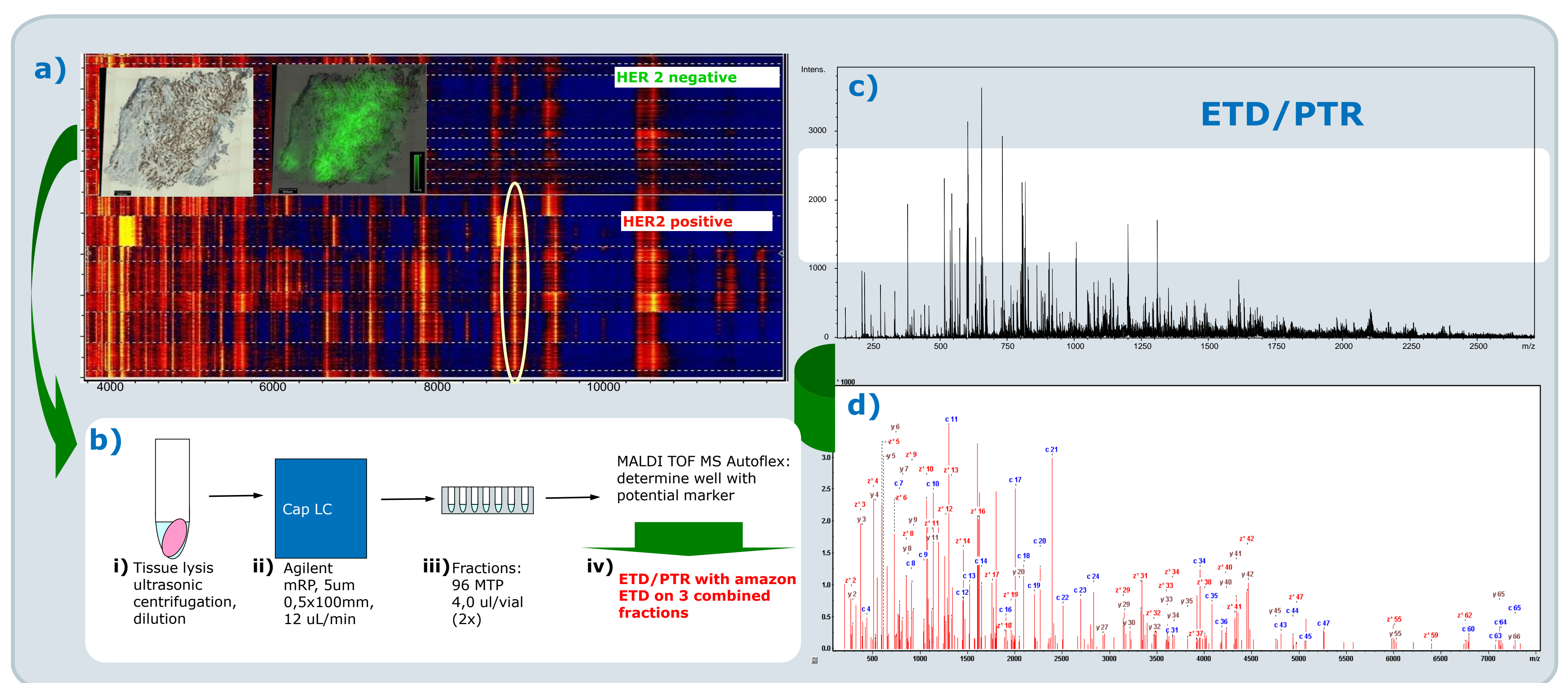


Fig 3: Top-Down discovery and identification of an intact biomarker protein
a) MALDI Imaging results of a breast cancer tissue; HER2 positive/negative cells
b) workflow for sample processing i) Lysate of entire tissue cells, ii) LC-separation and fractionation, iii) localize the fraction with the biomarker, iv) identification by ETD MS/MS
c) ETD/PTR spectrum of an intact protein from a cell lysate
d) BioTools annotation of Mascot search result of the processed spectrum

Identification of a protein biomarker for breast cancer

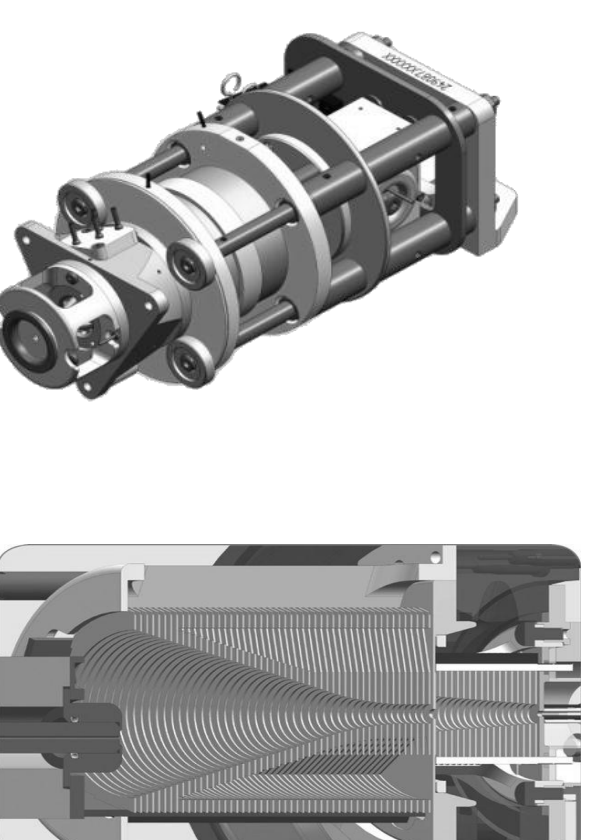
A potential protein biomarker was discovered in a MALDI Imaging experiment (autoflex MALDI-TOF) of a breast cancer tissue (Fig 3a). The protein was successfully extracted and purified (Fig 3b). The ETD/PTR analysis (Fig. 3 c) of the isolated intact multiply charged protein lead to an information-rich MS/MS spectrum with multiply charged fragments (+1 to +6), all resolved in MaximumResolution scan. After data processing with SNAP II, the BigMascot DB search resulted in a clear identification of the potential biomarker with a score of 126 (*Classification of HER2 Receptor Status in Breast Cancer Tissues by MALDI Imaging Mass Spectrometry*, S. Rauser, C. Marquardt, B. Balluff, S.-O. Deininger, C. Albers, E. Belau, R. Hartmer, D. Suckau, K. Specht, M. P Ebert, M. Schmitt, M. Aubele, H. Höfler and A. Walch, *J. Proteome Res.*, Just Accepted Manuscript DOI: 10.1021/pr901008d Publication Date (Web): February 19, 2010).

Acknowledgement

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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.

Top-Down Proteomics