

Classification of HER2 Receptor Status in Breast Cancer Tissues by MALDI Imaging Mass Spectrometry

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Introduction

- Clinical laboratory testing for HER2 status in newly diagnosed, primary breast cancer tissues is critically important for therapeutic decision making.
- High Definition MALDI imaging mass spectrometry (HDMI) is a powerful tool for investigating proteins through the direct and morphology-driven analysis of tissue sections.
- Unlike immunohistochemistry (IHC), HDMI enables the acquisition of complex protein expression profiles without any labeling.
- We hypothesized that HDMI may determine HER2 status directly from breast cancer tissues.

Methods

Breast cancer tissues (n=48) predefined for HER2 status by IHC and fluorescence-in-situ-hybridization (FISH) were subjected to HDMI and protein profiles were obtained through direct analysis of tissue sections following standard procedures using an ultraflex III MALDI-TOF/TOF (Bruker). Lateral resolution was 200 μ m, 200 shots were accumulated per pixel in linear mode of operation with an observed mass range of 2.4-25 kDa.

Protein identification followed the scheme shown in Fig. 1 and is described in greater detail in Rauser et al.¹.

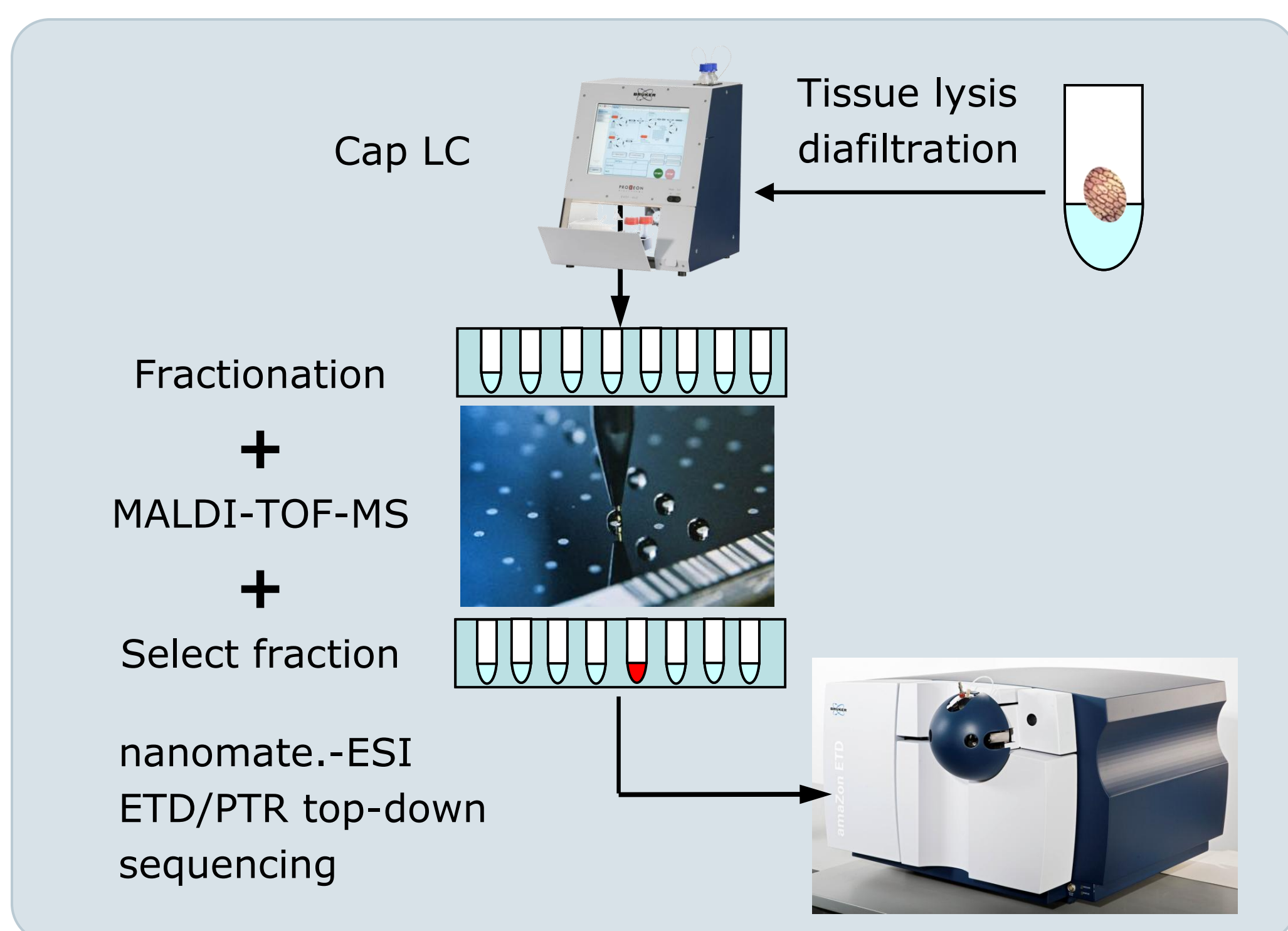


Figure 1. Protein identification workflow subsequent to MALDI Imaging: Tissue was lysed, followed by 5 kDa cutoff diafiltration and protein separation by Cap LC. Fractions were MALDI-MS analyzed to identify the fractions containing the m/z 8404 protein. These fractions were submitted to top-down protein ID on an ion trap using nanomate infusion-ESI-ETD/PTR and Mascot database searches

References

1. Rauser S. et al. (2010) Classification of HER2 Receptor Status in Breast Cancer Tissues by MALDI Imaging Mass Spectrometry. JPR, just accepted manuscript. DOI: 10.1021/pr901008d
2. Ma X.J. et al. (2003) Gene expression profiles of human breast cancer progression. Proc Natl Acad Sci U S A 100: 5974-9.

Results

Statistical analyses of the image mass spectra provided a panel of 7 peptides that classify HER+ and HER2- with 89 % overall accuracy (Fig. 2).

One of those, m/z 8404, was demonstrated to reliably stain HER2+ cancer tissue vs. stroma and HER2- cancer (Fig. 3).

The 8404 Da marker was isolated from HER2+ tissue (Fig. 1) and the molecular weight was confirmed by ESI-MS in the ion trap (Fig. 4).

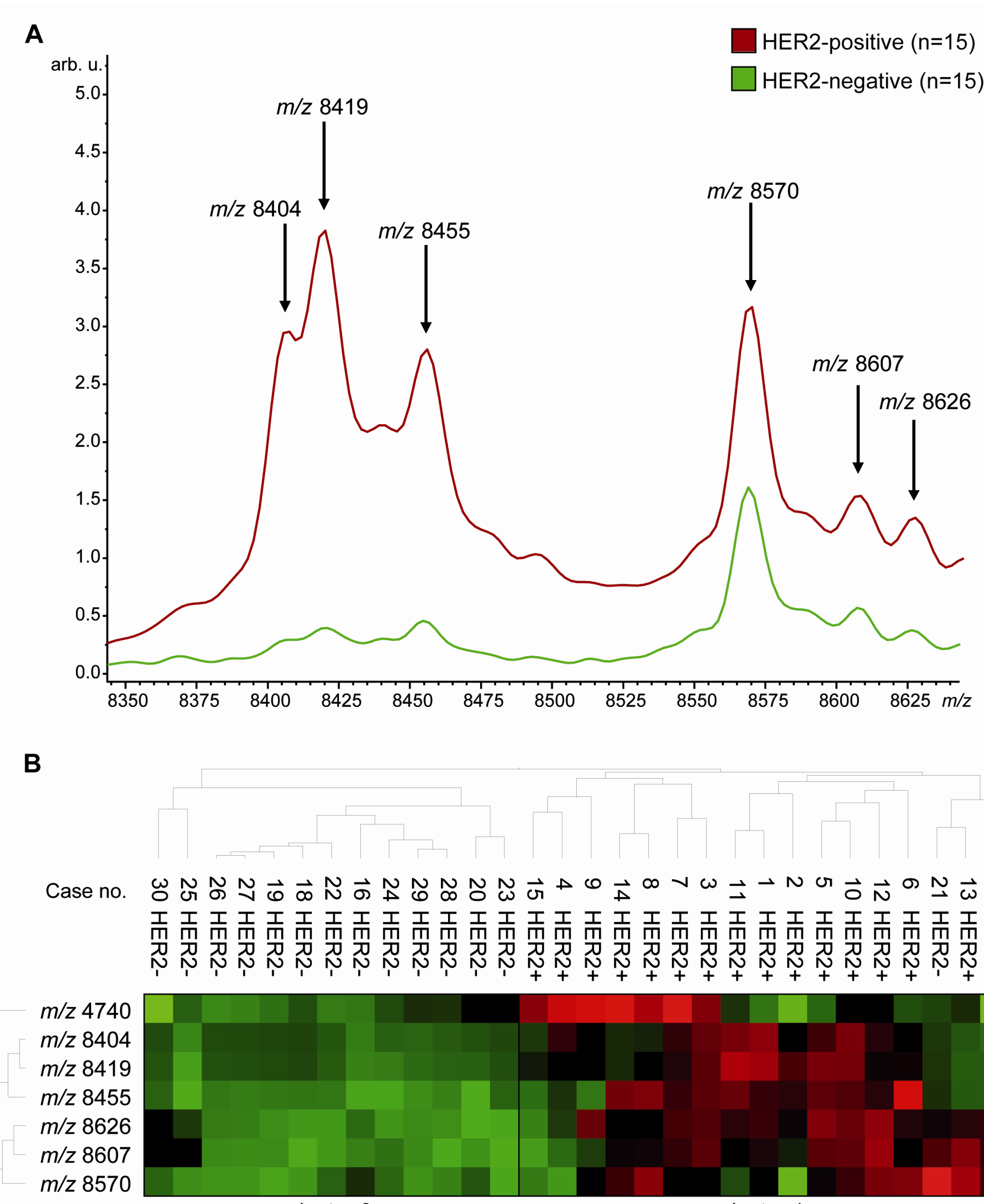


Figure 2. HER2 Marker Panel: A) Averaged MALDI-TOF MS spectra of HER2+ and HER2- breast cancer tissues (discovery set). The arrows mark 6 out of 7 peaks distinguishing the tissue groups. **B)** Hierarchical clustering analysis of the discovery set. All samples were clustered according to their expression pattern based on 7 discriminating peptide ions after intensity normalization. Over-expressed peptide signals are shown in red, under-expressed in green and unchanged ones in black. Two clusters (cluster 0 and cluster 1) were obtained. While cluster 0 harbors only HER2- samples, cluster 1 harbors the HER2+ samples. Only two of the HER2- cases were assigned as false positives to the HER2+ group.

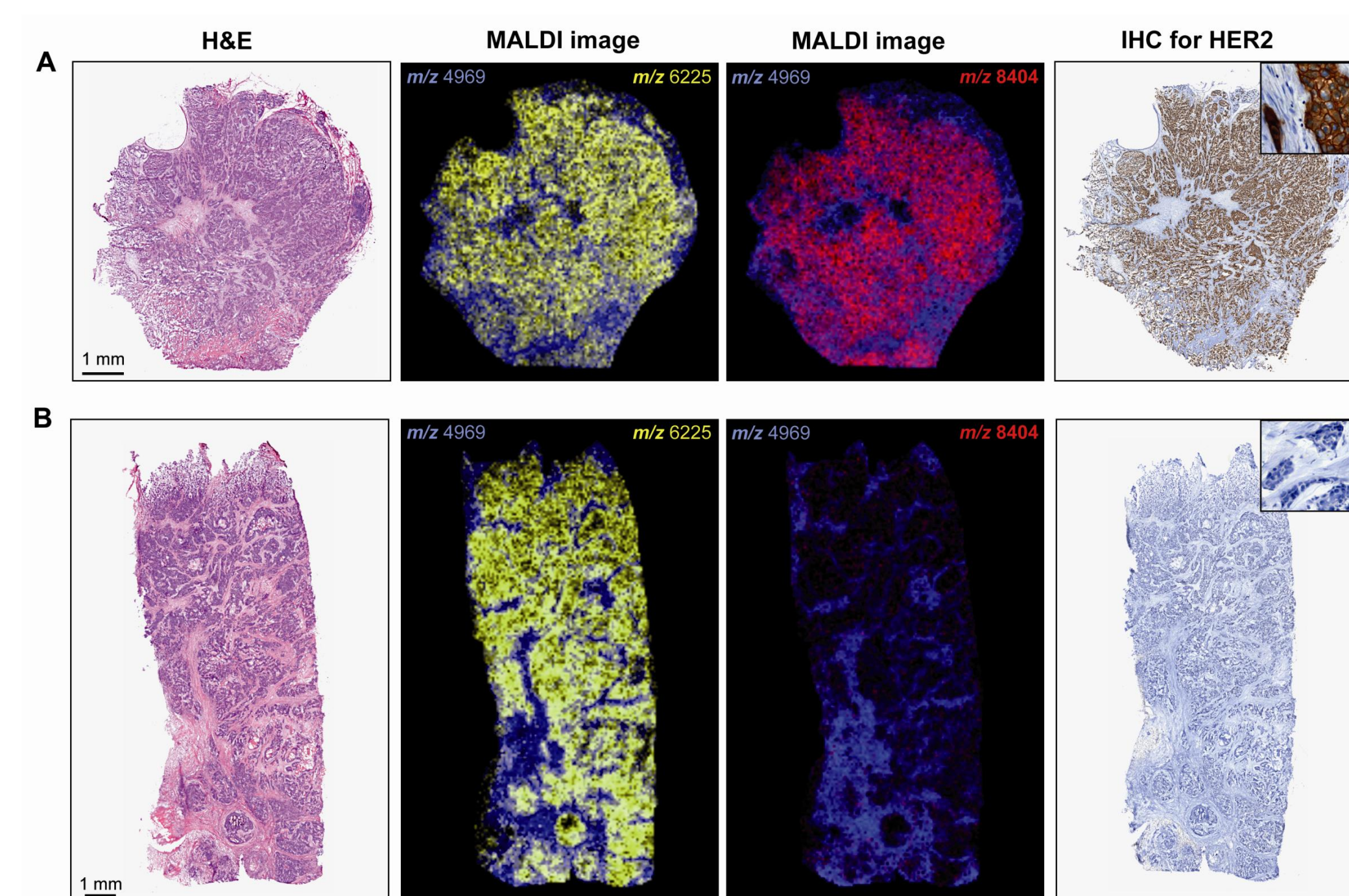


Figure 3. m/z 8404 as HER2 breast cancer biomarker: Analysis of two mamma carcinoma biopsies, one being HER2+ (top) and one HER2- (bottom). While H&E stained tissue (left) does not allow to distinguish the biopsies, immunohistochemical staining (anti-HER2, right) distinguishes HER2+ (brown) from HER2- (unstained = blue). The MALDI image (center panels), taken from the same section prior to H&E staining, allows to visualize tumor stroma (blue, m/z 4696), unspecified breast cancer (yellow, m/z 6225) and specifically HER2+ breast cancer (red, m/z 8404). Scale bars 1mm.

CRIP1

ETD-PTR analysis of the 8404 Da marker followed by a standard Mascot search allowed to identify Cysteine rich intestinal protein 1, CRIP1_HUMAN (Fig. 5).

CRIP1 is a cytosolic protein known from gene expression studies of various cancer types².

CRIP1 has not been detected as cancer related marker in proteomics studies before.

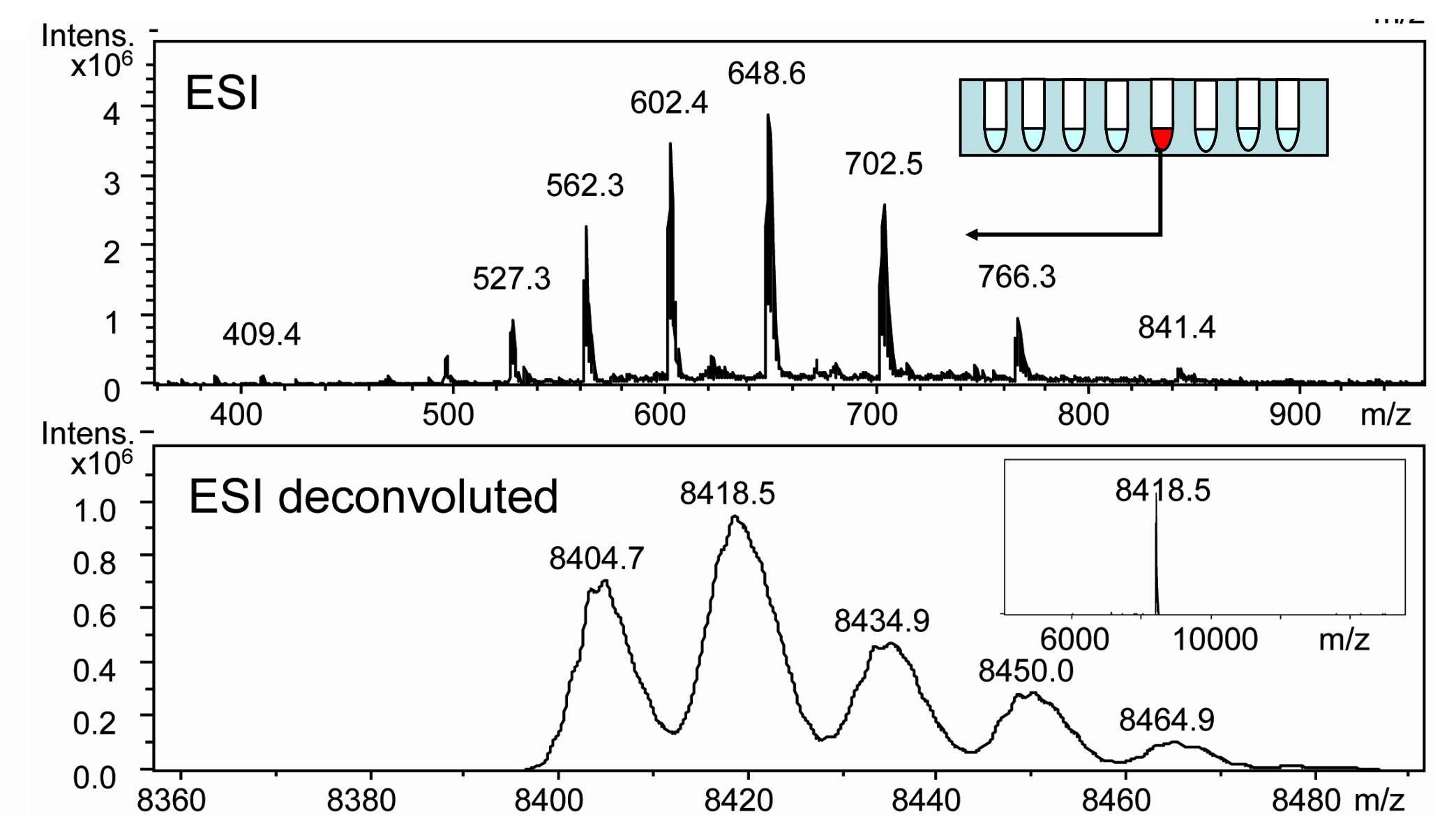


Fig. 4. MS analysis of the pooled fraction from the HER2+ classified tissue section: infusion ESI ion trap spectrum (top) and the charge deconvoluted spectrum (bottom) confirm the molecular weight of one HER2 marker candidate. The 14+ charge state (m/z 602.4) was selected and MS/MS analyzed by ETD/PTR (see Fig. 5).

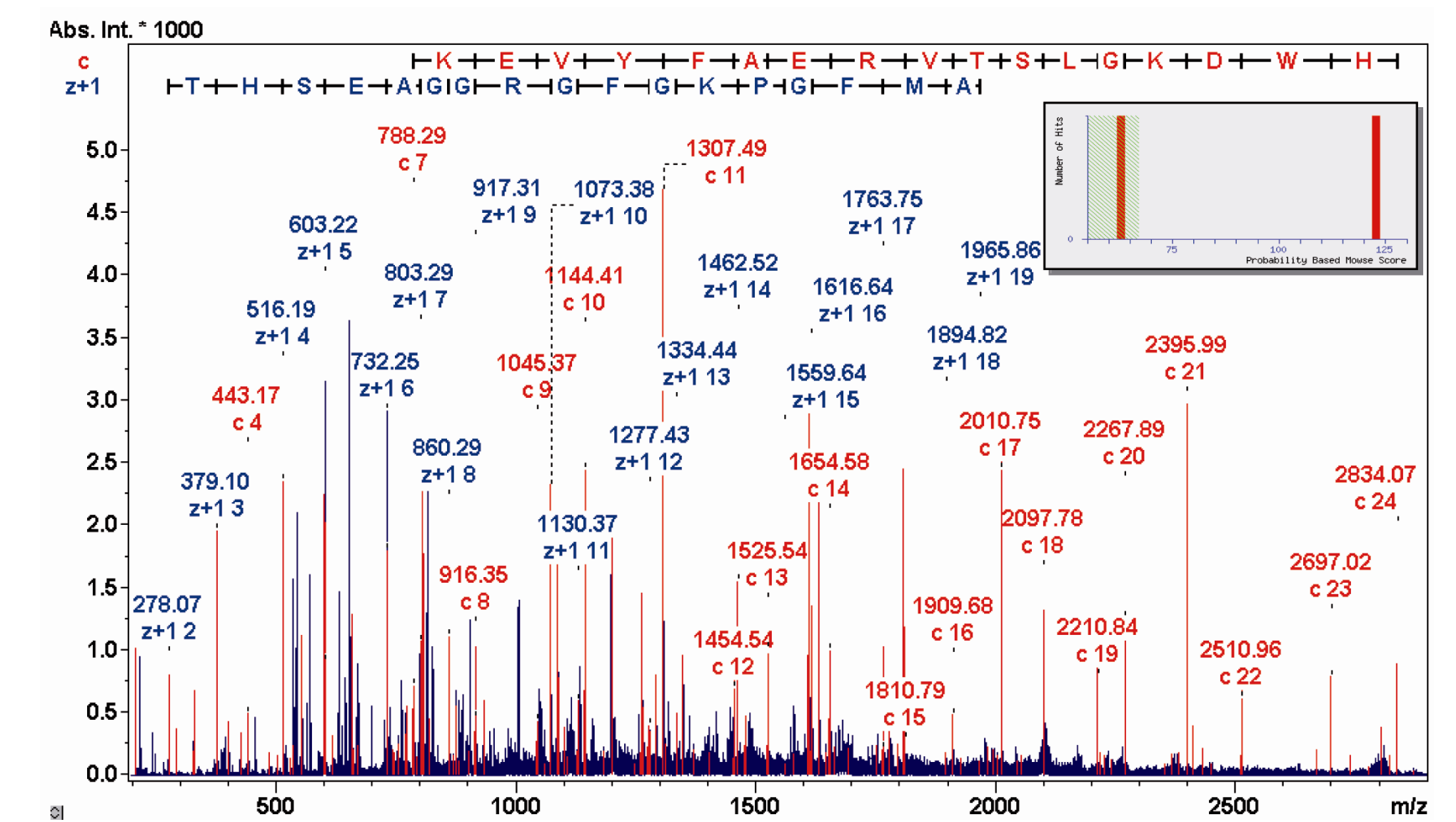


Figure 5. ETD/PTR top-down identification of CRIP1, the 8404 Da species. A Mascot protein sequence database search provided a high peptide identification score of 126 for CRIP1_HUMAN in UniProtKB/Swiss-Prot (insert).

Summary

- A peptide marker panel for HER2+ breast cancer was discovered using MALDI tissue imaging.
- A new workflow for the subsequent top-down protein identification was developed.
- CRIP1, a new cytosolic protein marker for HER2+ breast cancer tissue was identified.
- CRIP1 was detected as reduced, des-Met¹ protein.
- This study shows exemplarily how beneficially MALDI imaging and proteomics approaches can be combined.

Conclusions

A new 8.4 kDa biomarker candidate for HER2-positive breast cancer, the cytosolic protein CRIP1 was established by High Definition MALDI Imaging and Top-Down protein ID using ETD/PTR on an ion trap.

MALDI Imaging