

Orbitrap Mass Spectrometry With Resolving Powers Above 500,000 and 1,000,000 on a Chromatographic Time Scale

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Overview

Purpose: Demonstrate ultrahigh resolving power on an Orbitrap™ mass analyzer.

Methods: 1.5-second and 3-second transients were acquired on a pre-selected compact high-field Orbitrap analyzer with advanced signal processing.

Results: Demonstrated resolving powers over 5×10^5 and 10^6 enable the determination of elemental composition of analytes and the identification of some important modifications.

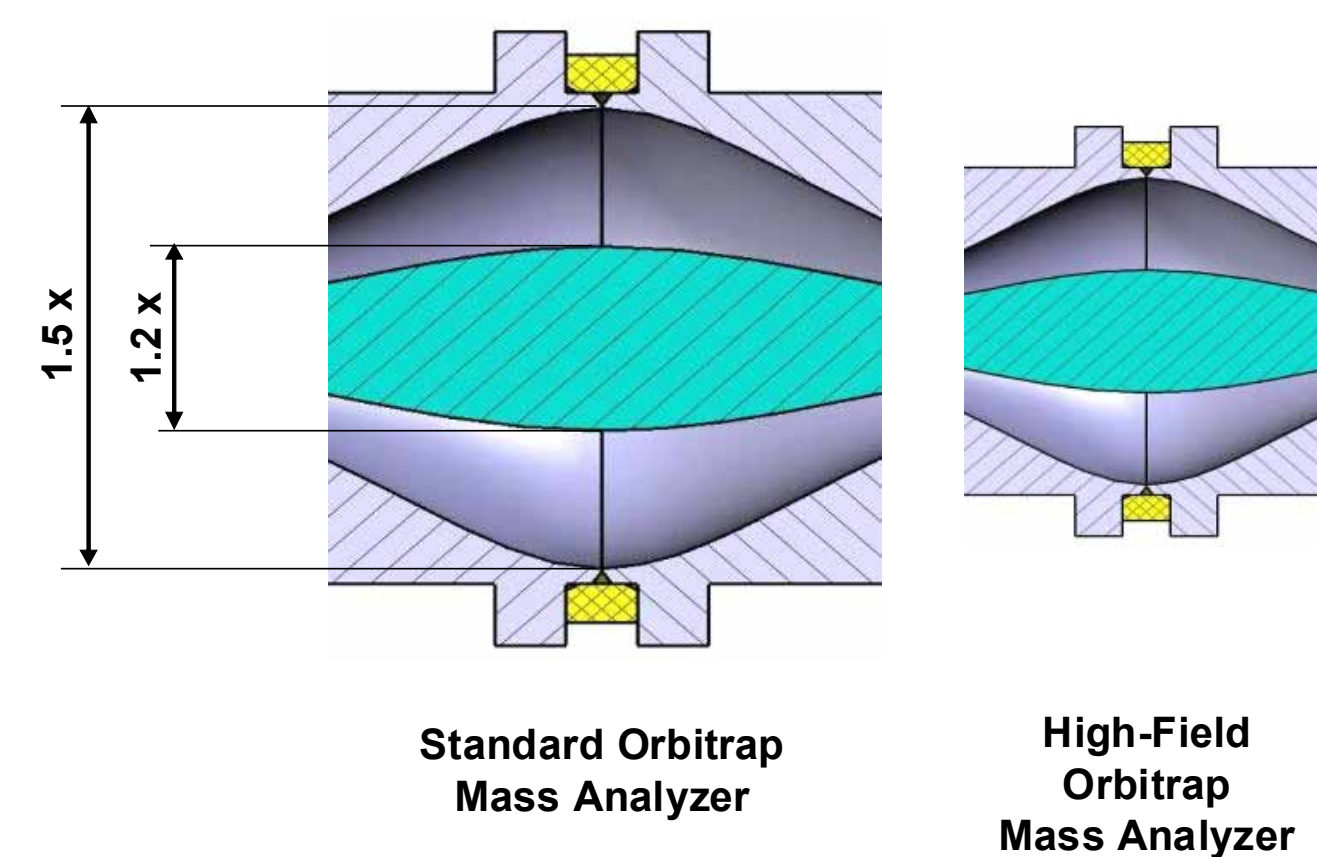
Introduction

Typically, an Orbitrap mass spectrometer is expected to provide resolving power of not higher than 100,000-200,000 which corresponds to detection time of 1-2 seconds. Recent innovations in Orbitrap technology, such as the high-field Orbitrap mass analyzer and advanced signal processing method, allowed us to accelerate detection rate by around 3.6-fold. This allows not only an accelerated spectral acquisition rate but makes it possible to do the opposite: to use longer transients to achieve higher resolving power while still remaining on time scale compatible with chromatographic separations.

Methods

Experiments were carried out on a Thermo Scientific Orbitrap Elite hybrid mass spectrometer equipped with a compact high-field Orbitrap mass analyzer (Figure 1) specifically selected from a batch of serial assemblies. Software was modified to allow transients up to 3 seconds long to be processed using advanced signal processing [1]. Peptides, small molecules, and intact proteins were analyzed in infusion mode. The instrument was manually tuned and mass-calibrated.

FIGURE 1. Comparison of a standard Orbitrap geometry with that of a compact, high-field Orbitrap mass analyzer.



Results

Instrument performance in ultrahigh resolution mode

Implementation of a compact Orbitrap mass analyzer and advanced signal processing with 0.76-second transients provides nominal resolving power of about 240,000 which became the maximum resolving power setting on Orbitrap Elite instruments. This represents a spectacular 3.5-fold improvement over the resolution of the Thermo Scientific LTQ Orbitrap Velos hybrid mass spectrometer. When compared for the same transient duration, the Orbitrap Elite instrument achieved higher resolving powers than Fourier transform ion cyclotron resonance (FT-ICR) instruments with strongest commercially available super-conducting magnets starting from m/z 330 (Figure 2).

However, FT-ICR allows for much longer transients in comparison with the Orbitrap analyzer. Though physics of ion motion and limitations of electronics make it impossible for Orbitrap analyzer to detect ions for tens of seconds as possible in FT-ICR, nevertheless it is possible to extend detection times several fold. A practical upper limit of detection duration is imposed by the chromatographic time scale and makes it impractical to detect longer than for a few seconds (e.g. 3 sec).

A 3-second transient corresponds to nominal resolving power of about 960,000 at m/z 400. At such long transients additional factors start to limit resolving power, such as collisions with residual gas and finite accuracy of Orbitrap electrode geometry. As the result, resolving power does not increase linearly with transient time any longer.

Resolving power in excess of 1,000,000 could easily be demonstrated for m/z below 300-350 (Figure. 3) and the thin structure of isotopes could be resolved and used for unequivocal determination of molecular composition, as shown for several model compounds.

FIGURE 2. Resolving power as a function of m/z for different FTMS instruments for 0.76 sec detection time:

- Standard Orbitrap analyzer with 3.5 kV central electrode voltage and single zero-filling and Kaiser-Bessel apodization;
- Compact high-field Orbitrap mass analyzer with 3.5 kV central electrode voltage and advanced signal processing;
- FT-ICR with a 15-Tesla superconducting magnet, single zero-filling and Kaiser-Bessel apodization.

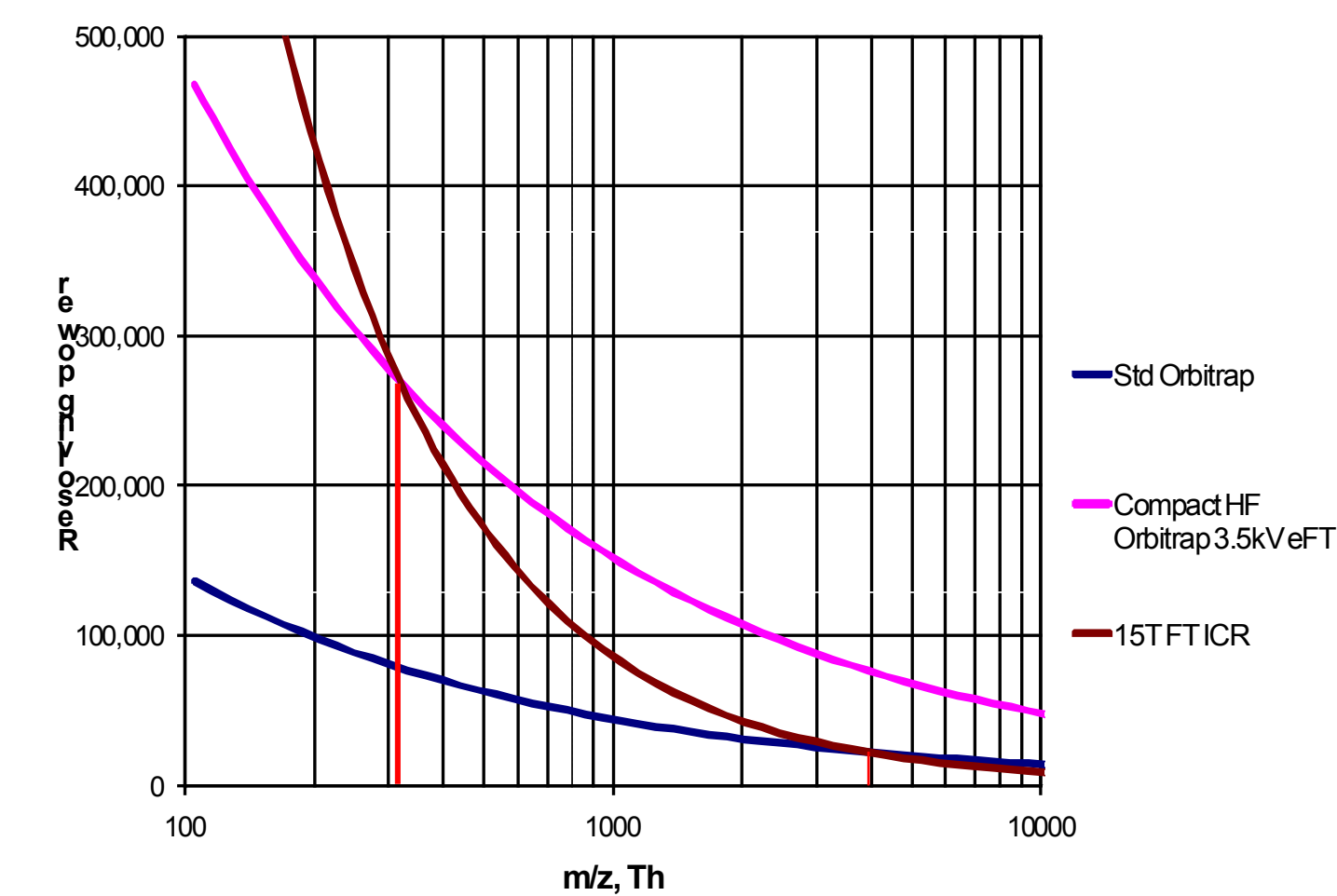
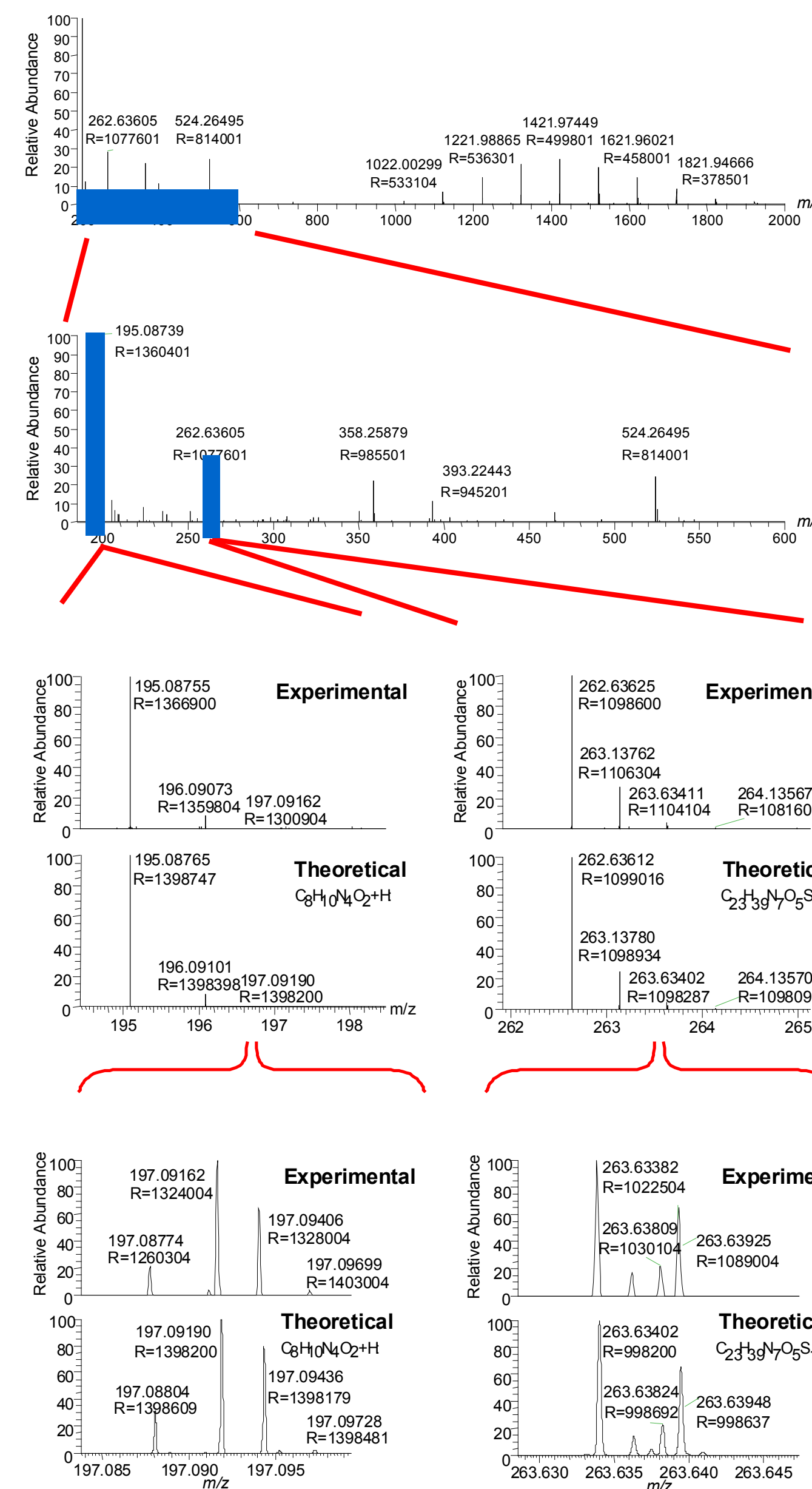


FIGURE 3. Ultimate resolving power obtained using a 3-second detection time (4 times longer than the detection time in Figure 2) and external calibration. Experimental values are presented on panels for corresponding m/z . Isotope clusters with thin structure are acquired in SIM mode and presented in insets along with corresponding theoretical patterns normalized to the largest peak.



Deamidation analysis using ultrahigh resolving power

Analytic utility of ultrahigh resolving power was also demonstrated on the example of substance P and melittin peptides. Both were analyzed at resolving power above 600,000 which allowed resolution of thin isotopic structure along with deamidation (Figures 4 and 5). For more intense isotopes, a single spectrum is sufficient for identification of this modification.

FIGURE 4. Identification of deamidation in panoramic mass spectra of substance P (isotopic envelope of +2 charge state is shown) using 3-second detection time and external calibration. Isotope clusters with thin structure (average of 17 spectra) are presented in insets along with corresponding theoretical patterns.

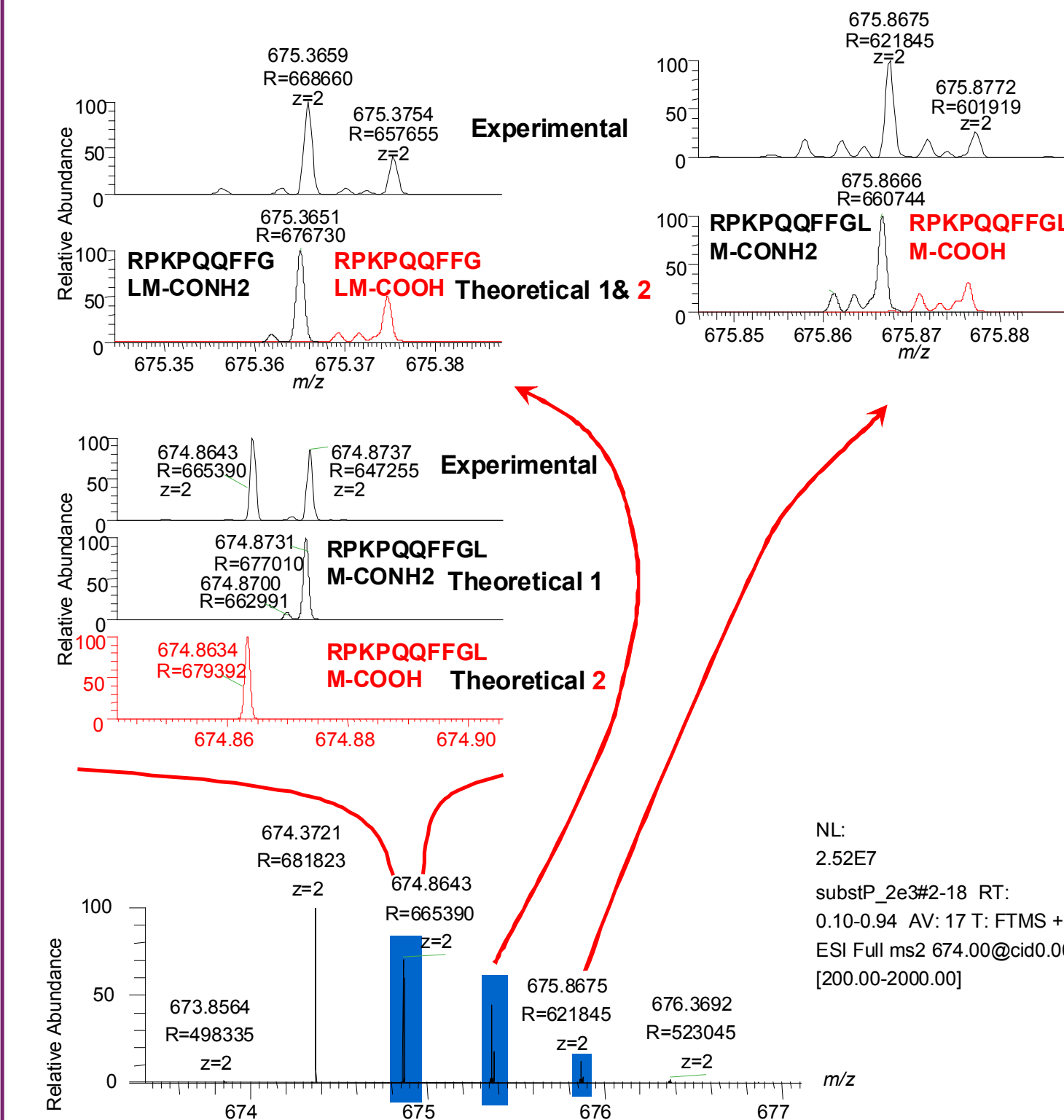
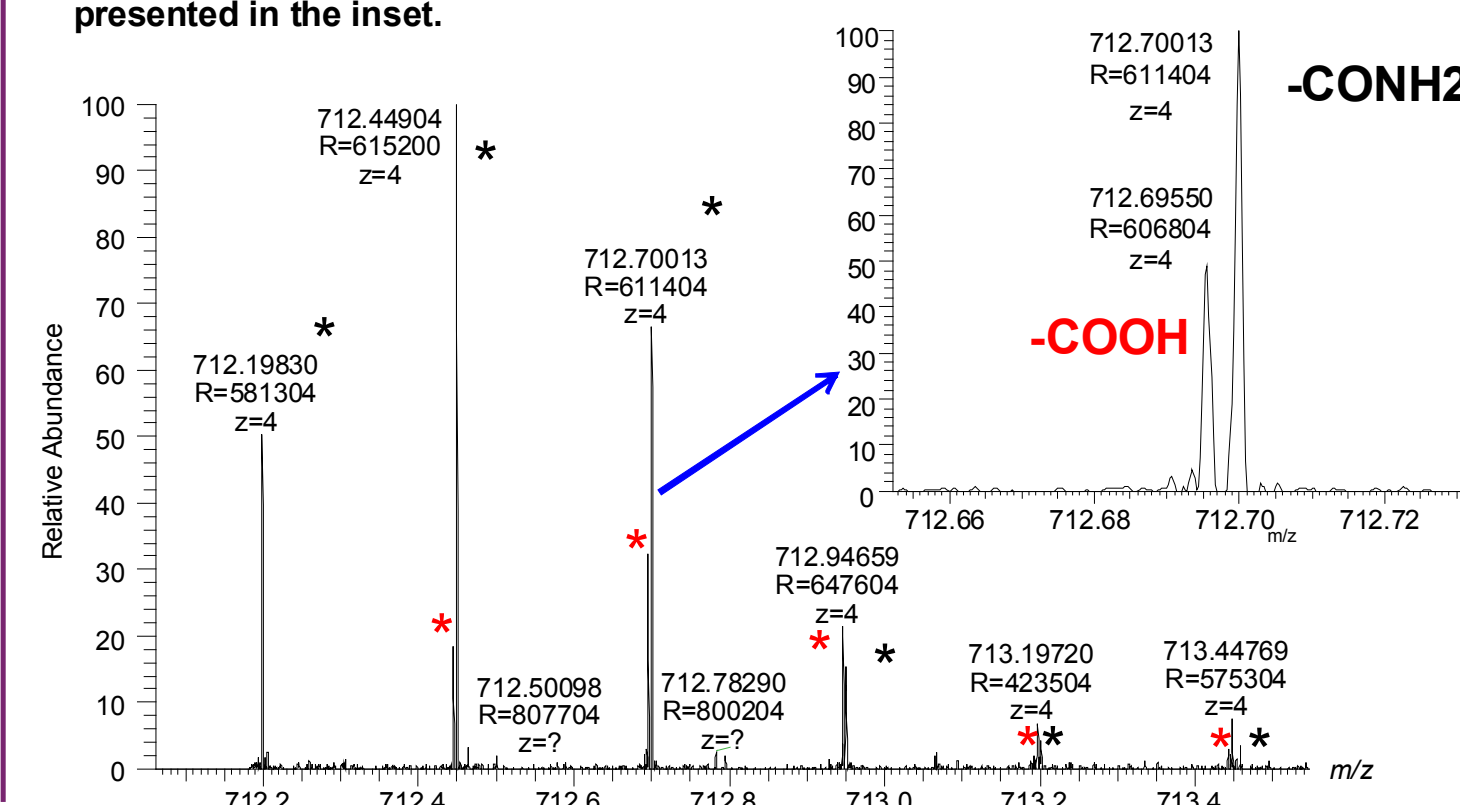


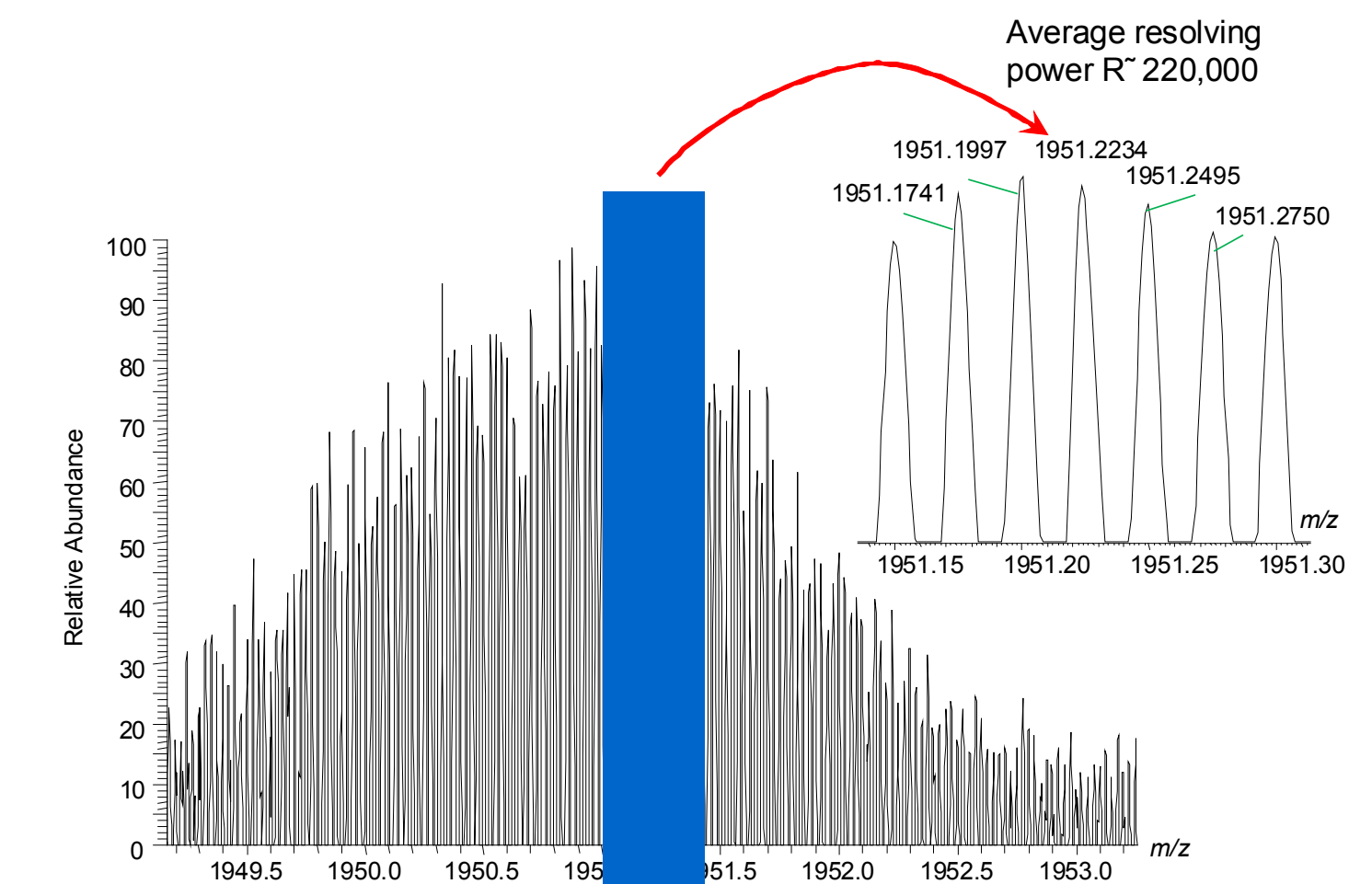
FIGURE 5. Identification of deamidation in a single panoramic mass spectrum of Melittin (isotopic envelope of +4 charge state is shown) using 3-second detection time and external calibration. One of isotope clusters with thin structure is presented in the inset.



Long transients for protein analysis

Long transients could be used also for analysis of heavier molecules such as intact proteins wherein baseline separation of isotopes of transferrin (78 kDa) has been demonstrated.

FIGURE 6. Analysis of proteins in 3-second transients on the example of transferrin (78 kDa). Baseline-resolved spectrum of +40 charge state was acquired in infusion mode (400 microscans) at an average resolving power of 220,000.



Conclusion

- Orbitrap technology is capable of ultrahigh resolving power in excess of 1,000,000 resolving power when appropriate tolerance and tuning requirements are met.
- ultrahigh resolving power could be achieved for 3-second detection time which makes it compatible with chromatographic separations.
- Resolving powers over 5×10^5 allow resolution of thin isotopic structure and identify some important modifications of peptides.

References

1. O. Lange; E. Damoc; A. Wiegand; A. Makarov. "Enhanced FT for Orbitrap Mass Spectrometry". *Proc. 59th Conf. Amer. Soc. Mass Spectrom.*, Denver June 5-9, 2011, Poster MP093.

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