

# Applications of Two-Dimensional Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

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### Overview

- 2D FT-ICR MS correlates precursor and fragment ions for all compounds in a complex sample without precursor ion isolation.
- We recorded the 2D mass spectrum of cholesterol using Atmospheric Pressure Photolonization (APPI) for the first time.
- We recorded the positive mode nanoESI 2D mass spectra of a tryptic digest of cytochrome C using both ECD and IRMPD as fragmentation modes.
- We discuss the advantages of 2D FT-ICR MS over MS/MS and LC-MS in terms of the information available in 2D mass spectra.

# Principle of 2D FT-ICR MS

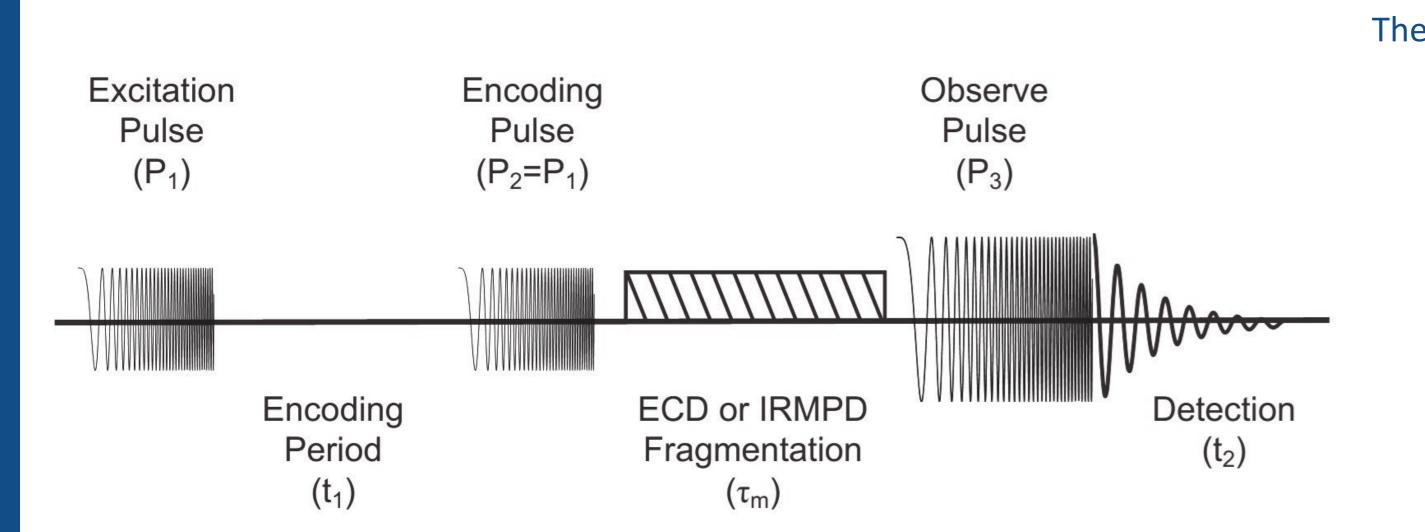


Figure 1: Pulse sequence for two-dimensional FT-ICR MS.

pulse sequence of this experiment is shown in Fig. 1 [1-9].

- Precursor ions are excited coherently from the center of the ICR cell by the excitation pulse  $P_1$ .
- During the encoding period  $t_1$ , precursor ions rotate at their own cyclotron frequency. At the end of t1, they have accumulated a phase  $\omega_{ICR} \times t_1$ .
- The encoding pulse P<sub>2</sub> changes the precursor ions' radius according to their phase: if ion motion is in phase with the closest excitation plate, ions are coherently excited, if ion motion is out of phase with the closest excitation plate, ions are coherently de-excited.

### At the end of $P_2$ , ion cyclotron radii are modulated according to cyclotron frequency and $t_1$ .

- A period of radius-dependent fragmentation (IRMPD, ECD, CID...) produces fragment ions with abundances that are dependent on the cyclotron radii of their precursors, i.e. their cyclotron frequency and t<sub>1</sub>.
- The observe pulse P<sub>3</sub> excites both precursor and fragment ions in order to measure the transient (detection date t<sub>2</sub>).

Transients are recorded with regularly incremented values of t<sub>1</sub>. A double Fourier transform according to t<sub>1</sub> and t<sub>2</sub> shows correlations between precursors and fragments in a two-

After mass calibration the 2D mass spectrum can be read with precursor m/z ratios vertically and fragment m/z ratios horizontally (fig. 2). 2D mass spectra show several characteristic

- The autocorrelation line (y = x) shows the correlation of the prescursor ion signal with their own cyclotron radius.
- Horizontal fragment ion spectra ( $y = m_{precursor}$ ) show the fragmentation patterns of each precursor ion.
- Vertical precursor ion spectra ( $x = m_{fragment}$ ) show the precursor ions of each fragment
- **Electron capture lines** (y = (n-p)\*x/n) show the capture of p electrons by n-charged precursor ions.
- **Neutral loss lines**  $(y = x + m_{neutral})$  show the loss of neutrals by precursor ions.

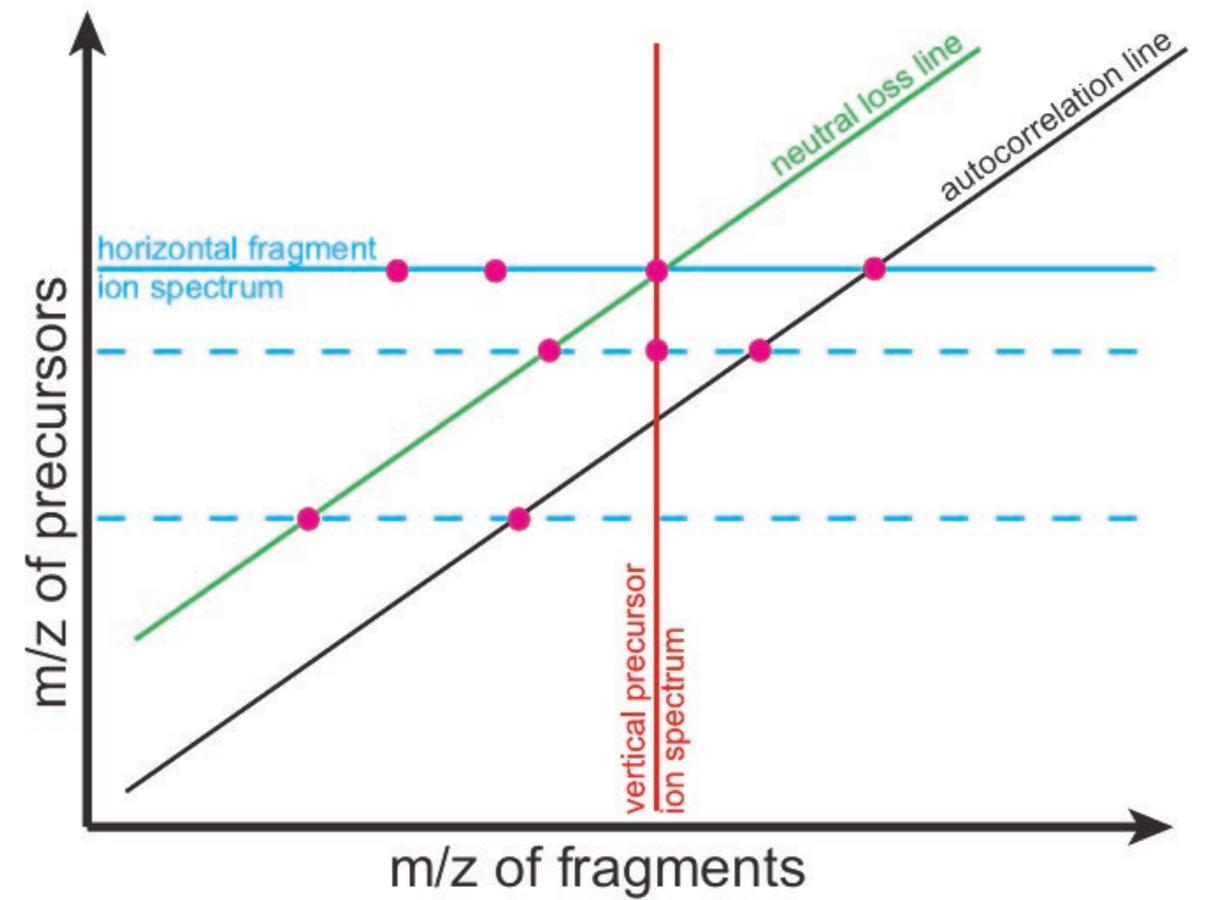
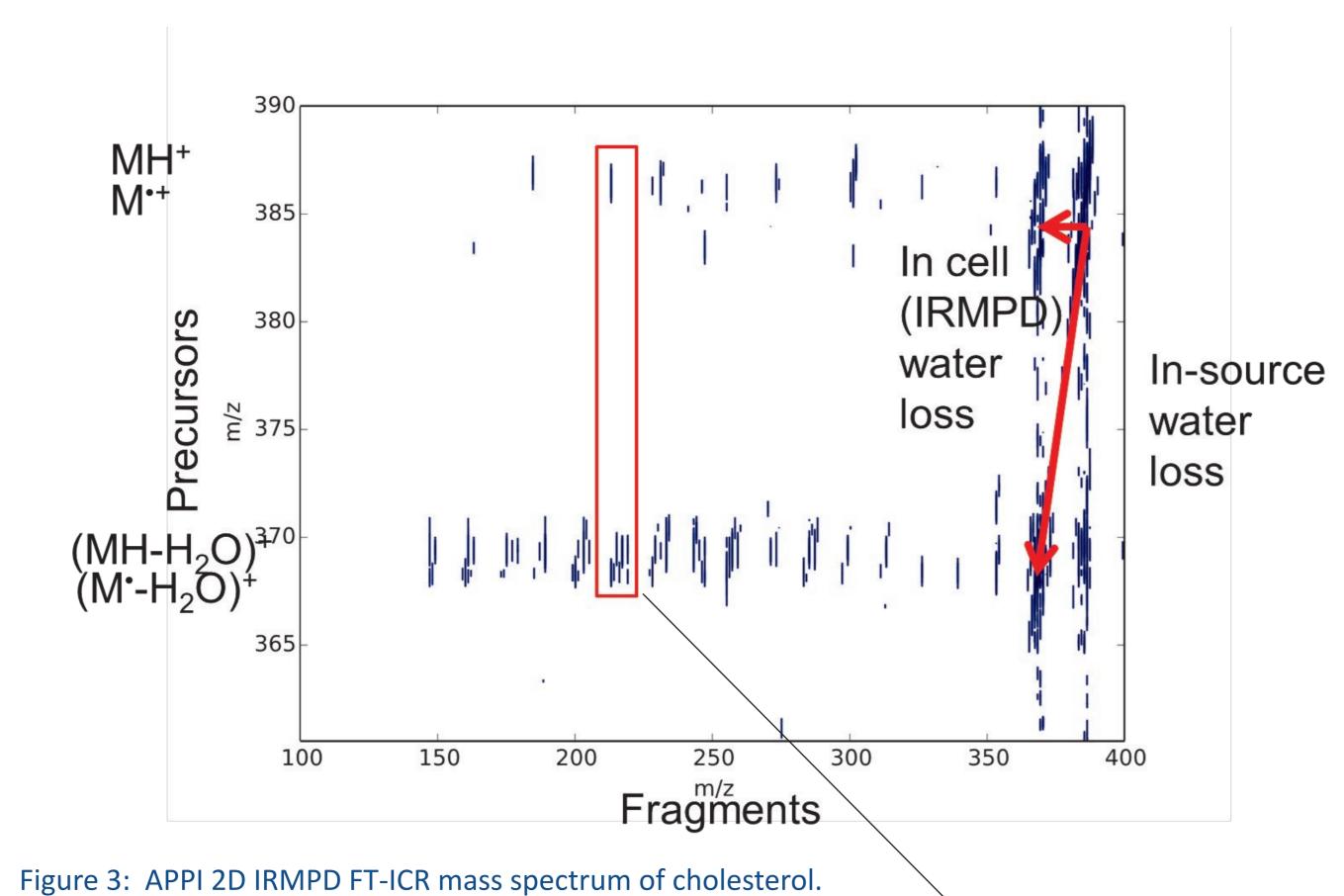


Figure 2: Interpretation of a 2D mass spectrum.

# APPI 2D IRMPD FT-ICR Mass Spectrum of Cholesterol



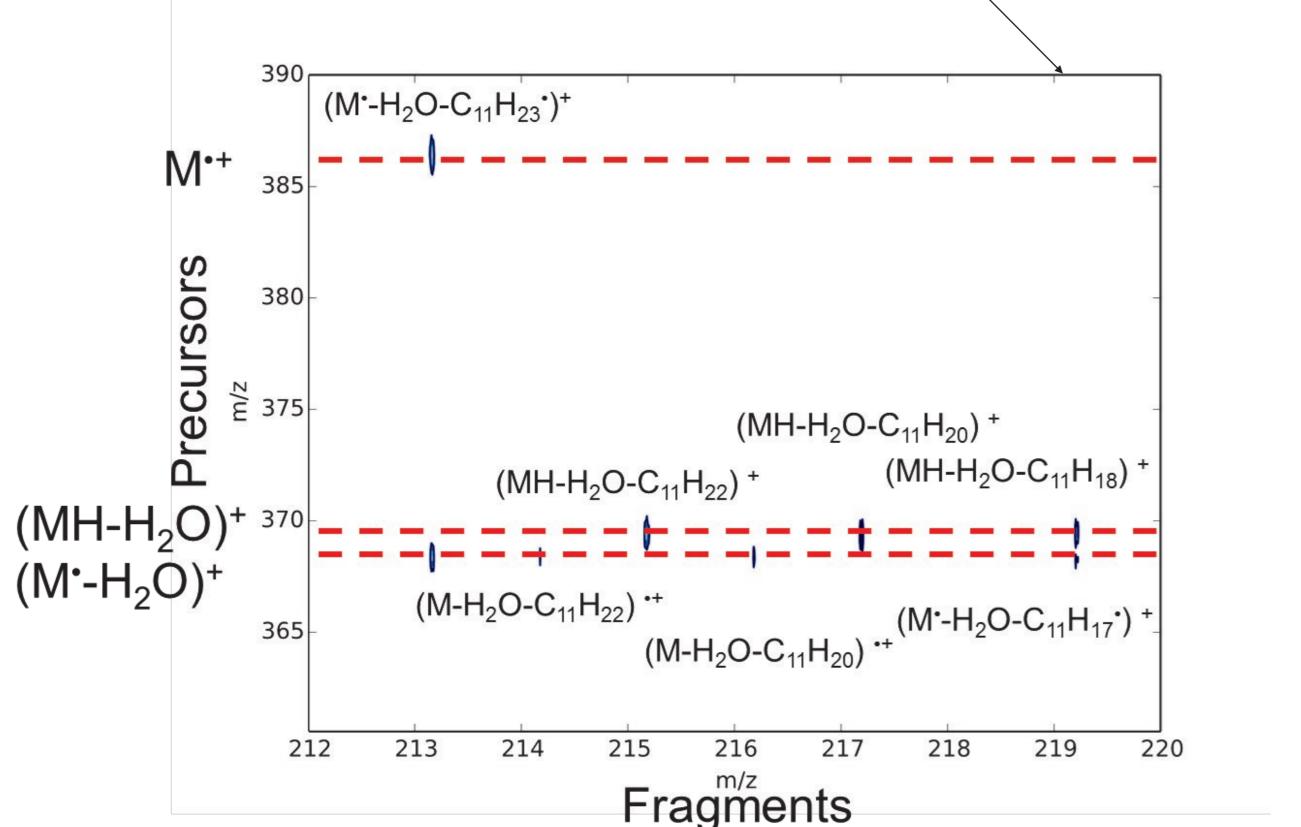


Figure 4: APPI 2D IRMPD FT-ICR mass spectrum of cholesterol (zoom).

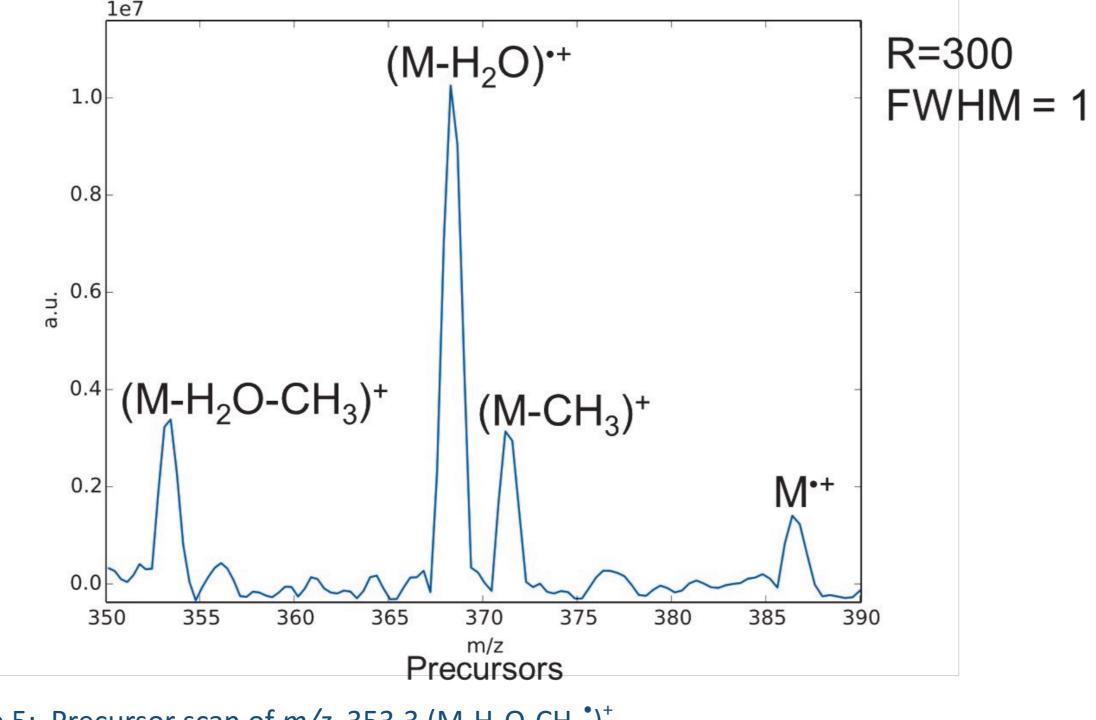


Figure 5: Precursor scan of m/z 353.3 (M-H<sub>2</sub>O-CH<sub>3</sub> $^{\bullet}$ ) $^{+}$ .

#### **Experimental Methods:**

- Cholesterol sample at 100 pmol/ $\mu$ L in acetonitrile/water (75:25).
- . 12 T SolariX FT-ICR mass spectrometer (Bruker) equipped with a Bruker II APPI source.
- 2048 scans of 128k datapoints were recorded over a m/z 36.9-500 horizontal and m/z184.27-500 vertical mass range.
- IRMPD: Synrad CO<sub>2</sub> laser (25 W), 10.6 μm wavelength, 0.1 s irradiation at 50% power.
- Data processing: NPK (NMR Processing Kernel), rewritten in 64-bit Python programming language. Processed datafiles in HDF5 file format.

### Results and Discussion:

- . APPI is a continuous ion source that generates both protonated and radical ion species. In the case of cholesterol, in-source ion loss of H<sub>2</sub>O and CH<sub>3</sub> are also observed.
- spectrometer are considered as precursors.

. In the context of 2D FT-ICR MS, all fragments generated in the front-end of the mass

- . The 2D mass spectrum shows the fragmentation patterns of all precursors (sample, contaminants and in-source fragments).
- The number of datapoints in the vertical (precursor ion) dimension lead to a 1 Da separation for precursor m/z ratios: we can see the difference between the fragmentation pattern of the protonated ion and the radical ion.
- The vertical precursor ion scans enable the identification of the precursors of each fragment ion generated during the fragmentation period of the 2D FT-ICR pulse
- Vertical precursor scans lead us to information on the fragmentation mechanism of cholesterol: loss of CH<sub>3</sub> can happen before of after loss of H<sub>2</sub>O. (figure 5)

# nanoESI 2D IRMPD and ECD Mass Spectra of a Tryptic Digest of Cytochrome C

### **Experimental Methods:**

- . Tryptic digest of cytochrome C purchased from Thermo Scientific at 800 fmol/μL in acetonitrile/water (25:75).
- Positive mode nanoESI on 12 T SolariX FT-ICR mass spectrometer (Bruker).
- . 2048 scans of 128k datapoints were recorded over a m/z 147.4-3000 horizontal and m/z147.4-3000 vertical mass range for both 2D mass spectra.
- IRMPD: 0.2 s irradiation at 50% power. ECD: 0.05 s irradiation.
- Data processing: NPK (NMR Processing Kernel), rewritten in 64-bit Python programming language. Processed datafiles in HDF5 file format.

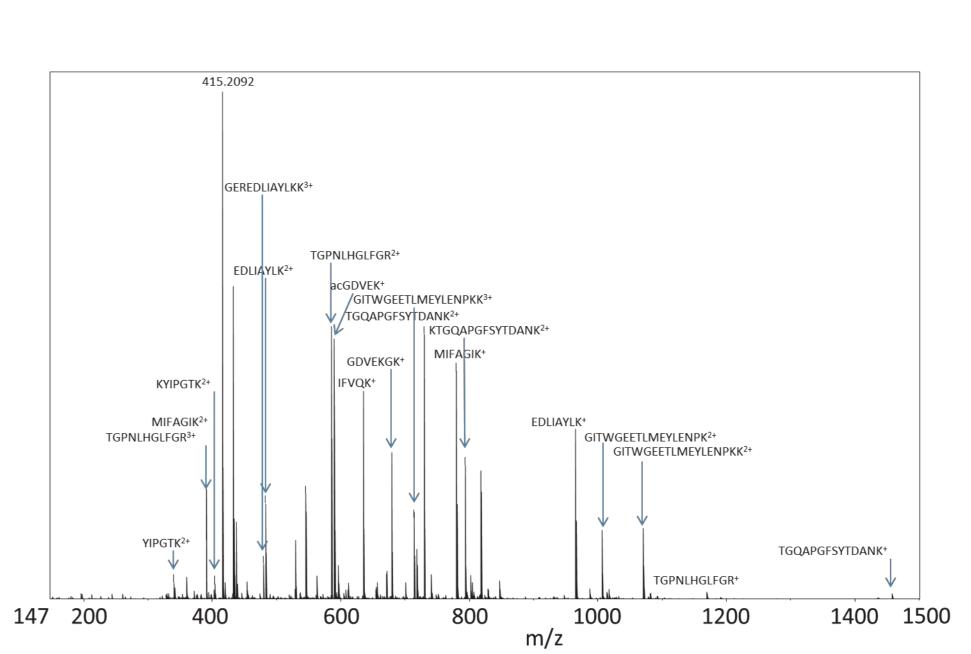


Figure 6: FT-ICR mass spectrum of a commercial tryptic digest of cytochrome C.

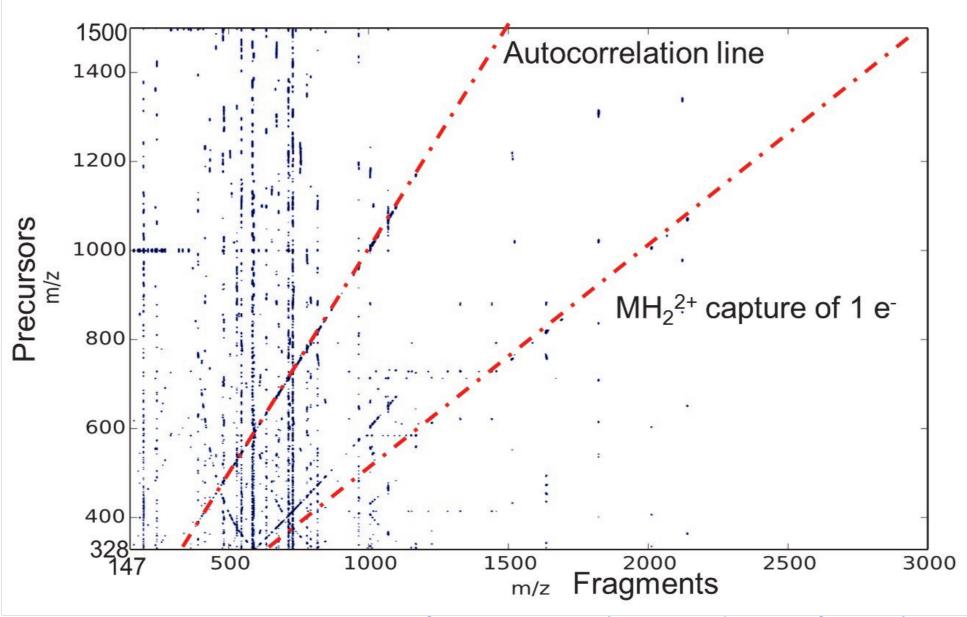


Figure 7: 2D ECD mass spectrum of a commercial tryptic digest of cytochrome C.

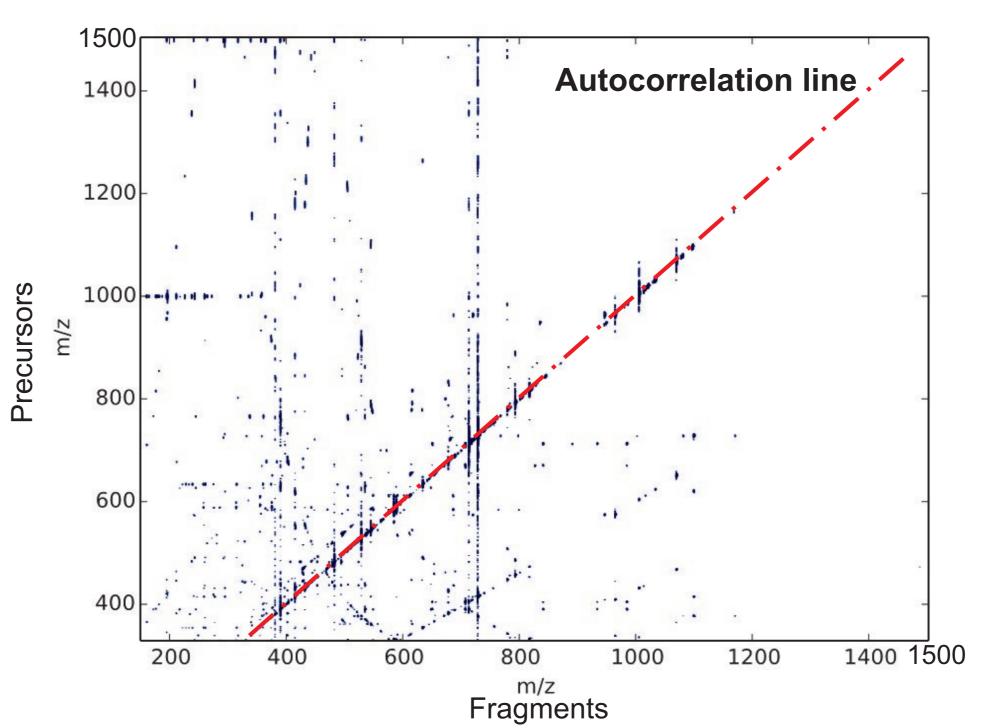


Figure 8: 2D IRMPD mass spectrum of a commercial tryptic digest of cytochrome C.

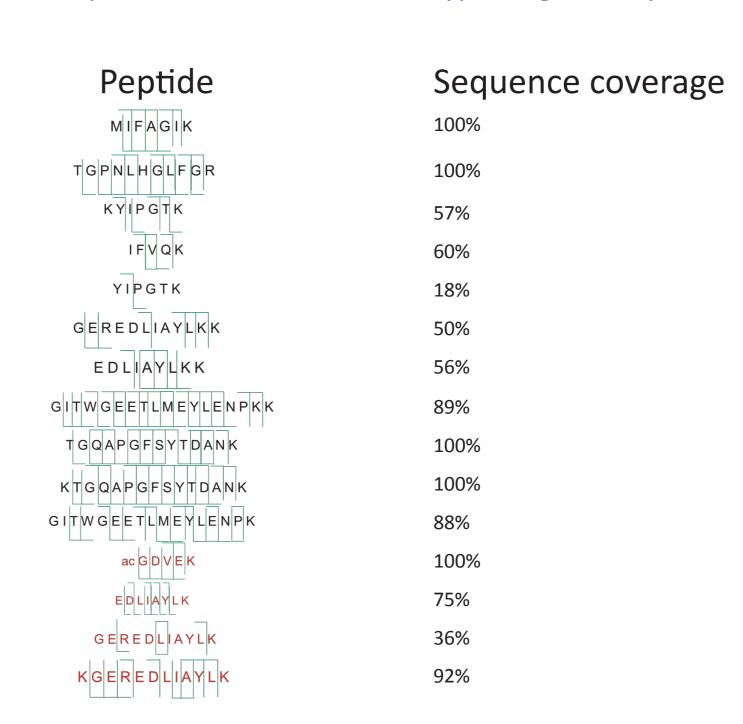
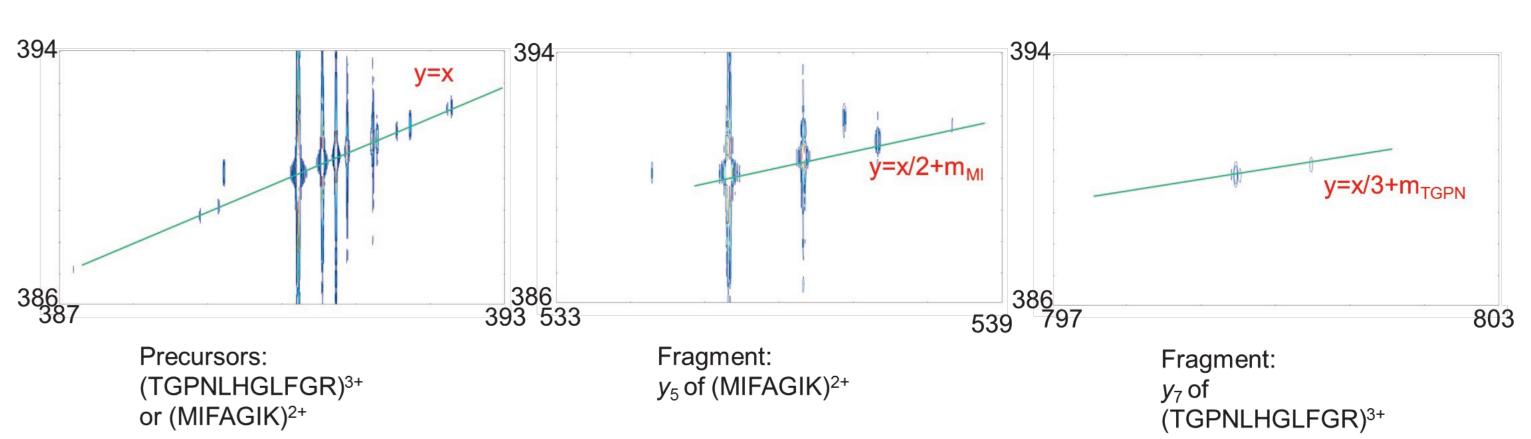


Table 1: Sequence coverage of the peptides from cytochrome C using both 2D mass spectra (black: peptides identified in the chromatogram, red: peptides identified in the high resolution FT-ICR mass spectrum).



### **Results and Discussion**

- . 2D FT-ICR mass spectrum of a tryptic digest of cytochrome C with ECD and with IRMPD (40 minutes of analysis time in total).
- . We obtain the fragmentation patterns of ion species in a range of abundances, for various charge states. Because 2D FT-ICR MS is data-independent, we observe the fragmentation patterns of ion species that are not listed in the chromatogram provided by the supplier.
- We observe charge reduction lines in the 2D ECD mass spectrum (Figure 7).
- We observe neutral loss lines that are parallel to the autocorrelation line in the 2D IRMPD mass spectrum (Figure 8).
- Sequence coverage of cytochrome C using both 2D ECD and IRMPD mass spectra after tryptic digest: 66%

Separation of Fragments of Ions of close m/z ratios but Different Charge States:

- TGPNLHGLGR<sup>3+</sup> at m/z 390.2122 and MIFAGIK<sup>2+</sup> at m/z 390.2278 require a separation power of m/ $\Delta$ m = 25000 in MS/MS correlate fragments and their precursors in MS/MS.
- Fragmentation: ion of mass m and charge z loses p charges and n mass:

$$y = \frac{z - p}{z} x + n$$

. The isotopic distribution of the fragment yields (z-p), and the slope along the peaks of the isotopic distribution of the fragments yields z: this enables the attribution of the precursor for each fragment.

## Conclusions

- We have expanded the capabilities of 2D FT-ICR MS to continuous ion sources other than El and nanoESI by using APPI as an ion source.
- 20 minute-long experiments lead to precursor ion separation of 1 Da, which enables the analysis of increasingly complex samples by 2D FT-ICR MS, as well as the study of the different fragmentation patterns of protonated and radical ions.
- 2D FT-ICR MS allows for the differentiation of fragments generated in the front-end of the instrument (on autocorrelation line) and fragments generated in the ICR cell (fragment peak), which gives us more accurate information on fragmentation mechanisms.
- The slope of fragment isotopic distributions allows for correct precursor identification when they are close in m/z ratio but with different charge states.

# Acknowledgments

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### References

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