Advances in Two-Dimensional ExD Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

Maria A. van Agthoven[1]; David Kilgour[1]; Andrew Soulby[1]; Juan Wei[1]; Lionel Chiron[2]; Marie-Aude Coutouly[3]; Marc-André Delsuc[2,3]; Mark Barrow[1] & Peter B. O'Connor[1]

1) University of Warwick, Coventry, UK; 2) IGBMC, Illkirch-Graffenstaden, France; 3) NMRTEC, Illkirch-Graffenstaden, France

Overview

- 2D FT-ICR MS correlates precursor and fragment ions for all compounds in a complex sample without precursor ion isolation.
- Pulse sequence optimization increases signal-to-noise ratios and minimizes harmonic peaks.
- First results in 2D FT-ICR MS using a 12 T Bruker SolariX instrument.
- Phase correction in transients of 2D mass spectra increases both signal-to-noise ratio and resolving power for fragment peaks.
- We present 2D mass spectra collected using fragmentation methods (EID and AI-ECD) which have not previously been used in 2D FT-ICR MS.

Principle of 2D FT-ICR MS

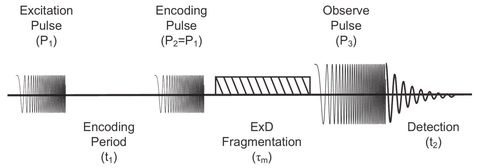

Figure 1: Pulse sequence for two-dimensional FT-ICR MS.

The pulse sequence of this experiment is shown in Fig. 1 [1-7].

- 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_2 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_1.
- The **observe pulse** P_3 excites both precursor and fragment ions in order to measure the transient (detection date t_2).

Transients are recorded with regularly incremented values of t_1. A double Fourier transform according to t_1 and t_2 shows correlations between precursors and fragments in a two-dimensional map.

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 lines :

- **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 ion.
- **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.

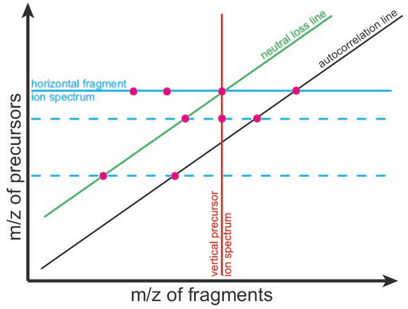

Figure 2: Interpretation of a 2D mass spectrum.

Optimisation of 2D ECD FT-ICR MS

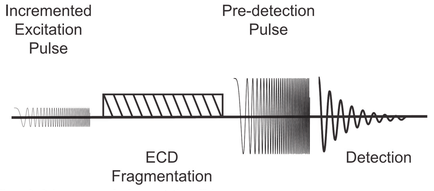

Figure 3: Pulse sequence for fragmentation efficiency measurements.

- Pulse sequence (fig. 3): Precursor ions are coherently excited before ECD fragmentation in order to measure fragmentation as a function of cyclotron radius.
- Quadrupole-isolated MH_2^{2+} of substance P was fragmented with the pulse sequence in fig. 3 using incrementally long pulses on a 12 T SolariX FT-ICR mass spectrometer using positive nanoESI ionization.
- Fig. 4 shows the percentage of fragmentation vs. pulse length (i.e. cyclotron radius of precursor) and gives an overview of the shape of the fragmentation zone for pulse sequence optimisation [10].

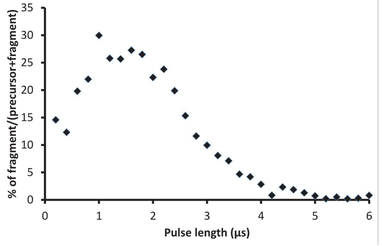

Figure 4: Percentage of fragmentation vs. pulse length for substance P using ECD as a fragmentation mode.

Phase Correction for Absorption-mode 2D FT-ICR MS

Phase Correction in the horizontal dimension (t_2, m/z of fragments)

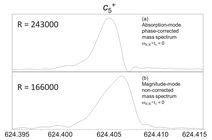

Figure 5: Fragment ion peak from a single ECD spectrum of substance P recorded with the pulse sequence in Fig. 1 with P_1 = 1.0 µs and t_1 = 1.0 µs. Each transient was recorded with 4 Mword datapoints. (a) Phase-corrected spectrum shown in absorption-mode. (b) Uncorrected spectrum shown in magnitude-mode.

- ECD spectra of substance P were recorded with the pulse sequence shown in Fig. 1 with various values of t_1. All spectra were phase-corrected using Autophaser 5.2.
- Fig. 5 shows that for fragments phase-correction increases the resolving power.
- Fig. 6 shows that fragments and precursors do not follow the same phase correction:

  Precursors are excited by P_1, P_2 and P_3, but fragments are only excited by P_3

  Excitation is t_1-dependent for precursors and t_1-independent for fragments
- Increasing the modulation amplitude leads to further phase shifts, both for precursor ions and fragment ions.
- Increasing t_1 induces frequency shifts for both precursor and fragment ions. Frequency shifts can lead to scintillation noise.
- Increasing the amplitude of modulation increases the frequency shift.

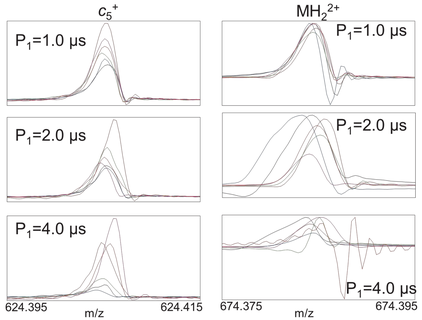

Figure 6: Phase-corrected absorption-mode peaks of c_5^+ (left) and MH_2^{2+} (right) for substance P recorded with the pulse sequence in Fig. 1 using several pulse lengths for modulation: P_1=1.0 µs (up), 2.0 µs (middle) and 4.0 µs (down), as well as several values of $\omega_{ICR} \times t_1$: 15° (red), 78° (pink), 157° (blue), 219° (green), 297° (brown) and 360° (black).

Phase Correction in the Vertical Dimension (t_1, m/z of precursors)

- The signal intensity of several fragment ions of substance P were recorded for 32 regularly incremented values of t_1 with P_1 = 4.0 µs in phase-corrected absorption mode for each transient.
- An FFT of the signal intensity after one zerofill was calculated in Excel. The frequency spectrum in magnitude mode and in absorption mode are compared in Fig. 7.
- The resulting vertical precursor frequency spectrum shows a peak at f ≈ 300 kHz corresponding to MH_2^{2+}.

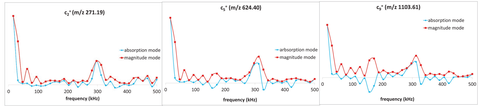

Figure 7: Non-corrected absorption-mode (blue) and magnitude mode (red) vertical precursor frequency spectra of c_2^+ (left), c_5^+ (middle) and c_9^+ (right) for substance P recorded with the pulse sequence in Fig. 1 with P_1=4.0 µs.

- The frequency spectrum in absorption mode shows a higher resolving power for all fragments, even without phase correction.
- Signal intensities in absorption mode and magnitude mode are comparable. However, the noise level may be lower in absorption mode.

2D Electron Induced Dissociation FT-ICR MS of a Sample Containing Bilirubin

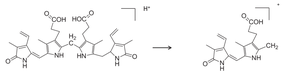

Figure 8: Bilirubin and its main fragment using EID as a fragmentation mode (adapted from [8]).

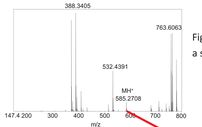

Figure 9: FT-ICR mass spectrum of a sample containing bilirubin.

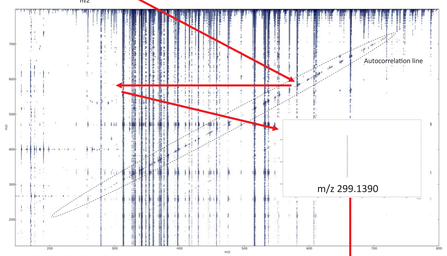

Figure 10: 2D EID FT-ICR mass spectrum of a sample containing bilirubin.

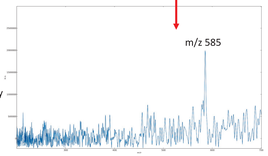

Figure 11: Vertical precursor ion spectrum extracted from the 2D mass spectrum in Fig. 10 at m/z 299.1390. This spectrum shows only one precursor peak at m/z 585.

- We show that EID can be used as a radius-dependent fragmentation mode in 2D FT-ICR MS, just like ECD has been shown to be usable [6].
- Although bilirubin is not the most abundant peak in the mass spectrum, its fragmentation pattern can nonetheless be identified despite the high level of scintillation noise in the 2D mass spectrum.

2D IRMPD-ECD FT-ICR MS of Calmodulin

- Calmodulin is a 17 kDa protein that is not fragmented easily using ECD alone.
- Although IRMPD and ECD have both been used separately in 2D FT-ICR MS before, they have not been used together in a single experiment.
- Being able to fragment intact proteins in the ICR cell using simultaneous IRMPD and ECD allows 2D FT-ICR MS to become a tool for top-down proteomics.
- Preliminary results show that 2D IRMPD/ECD FT-ICR MS can be used, although it needs to be optimized for a better visibility of fragment peaks.

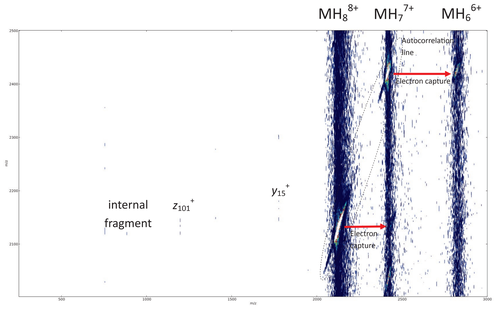

Figure 12: 2D mass spectrum of calmodulin using simultaneous IRMPD and ECD as a fragmentation mode.

Conclusions

- The 2D FT-ICR MS has been successfully adapted to a 12 T SolariX FT-ICR mass spectrometer.
- Phase correction in the horizontal dimension improves the resolving power of fragment ion peaks, although a different phase correction function needs to be used for precursor ion peaks.
- Preliminary results show that phase correction can also be used in the vertical dimension with a simple linear phase correction function: this can improve the resolving power of the vertical dimension without cost in terms of experimental time.
- Preliminary results show that EID and IRMPD/ECD can be used for 2D FT-ICR MS.

Acknowledgments

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