The Benefits of 2D-Mass Spectrometry for Protein Structural Characterization

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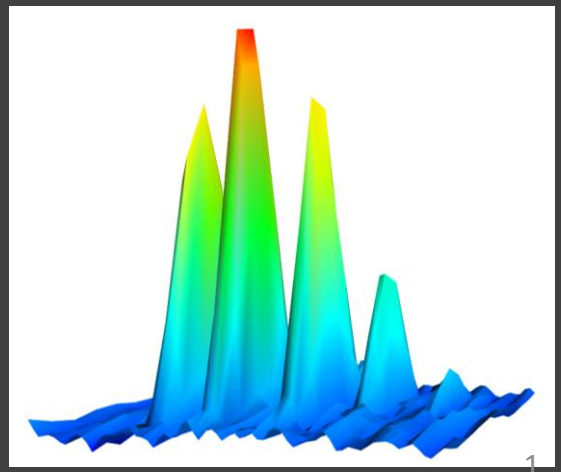


- Protein or RNA sequencing
- Modifications:
  - Identification
  - Location
  - Relative quantification

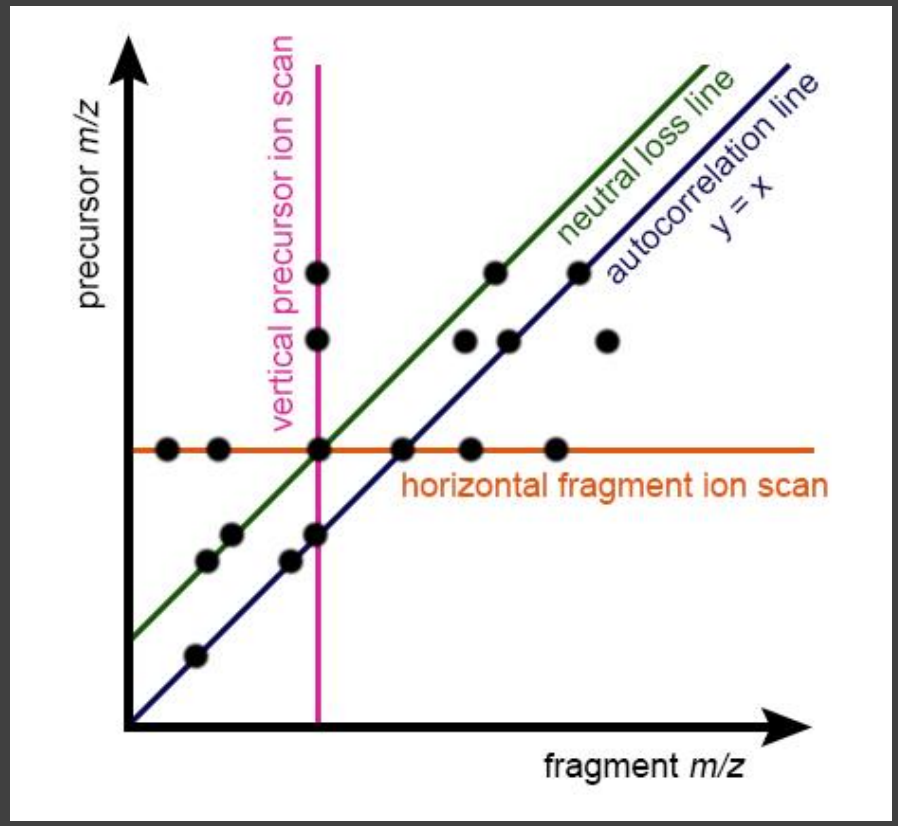
Two-dimensional FT-ICR MS

- All ions in a complex sample fragmented and visualised on one spectrum

- NO isolation
- YES fragmentation

- Horizontal axis: fragment *m/z*
- Vertical axis: precursor *m/z*

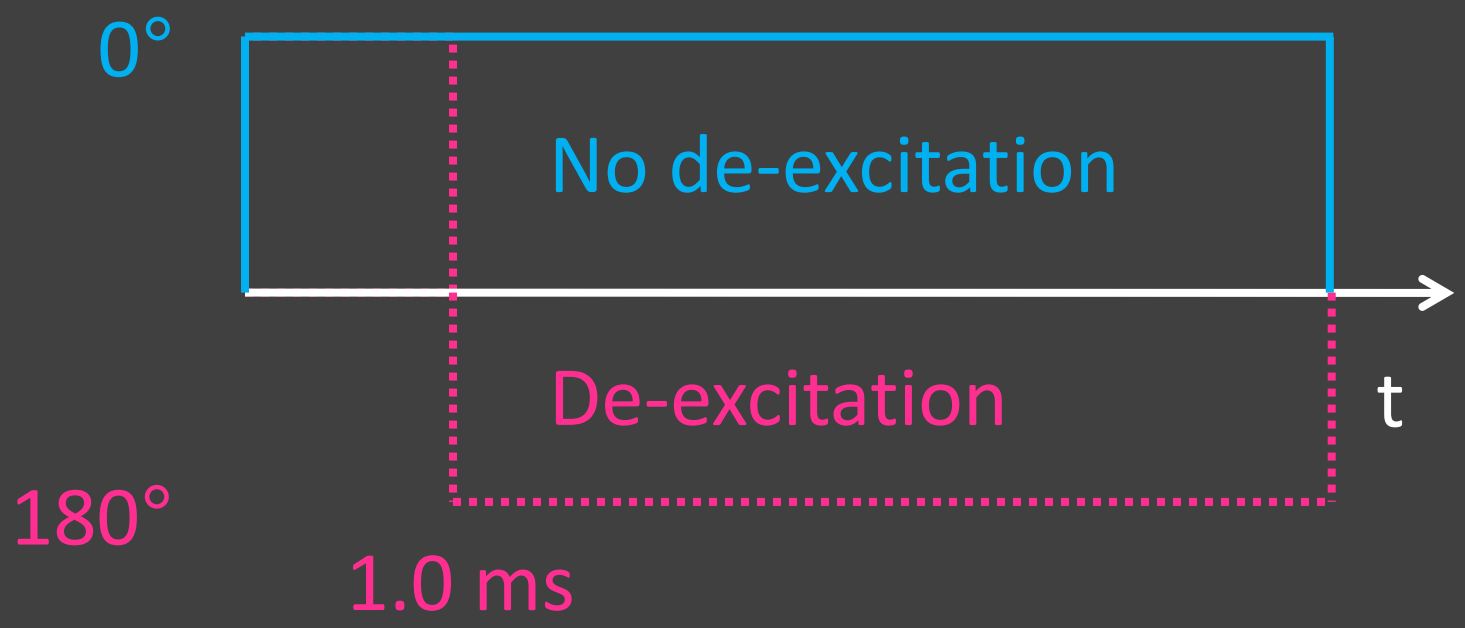
- Each peak corresponds to one fragmentation



M. van Agthoven et al., *Anal. Bioanal. Chem*. 405 (2013) 51.
M. van Agthoven et al., *Eur. Biophys. J.* 48 (2019) 213.

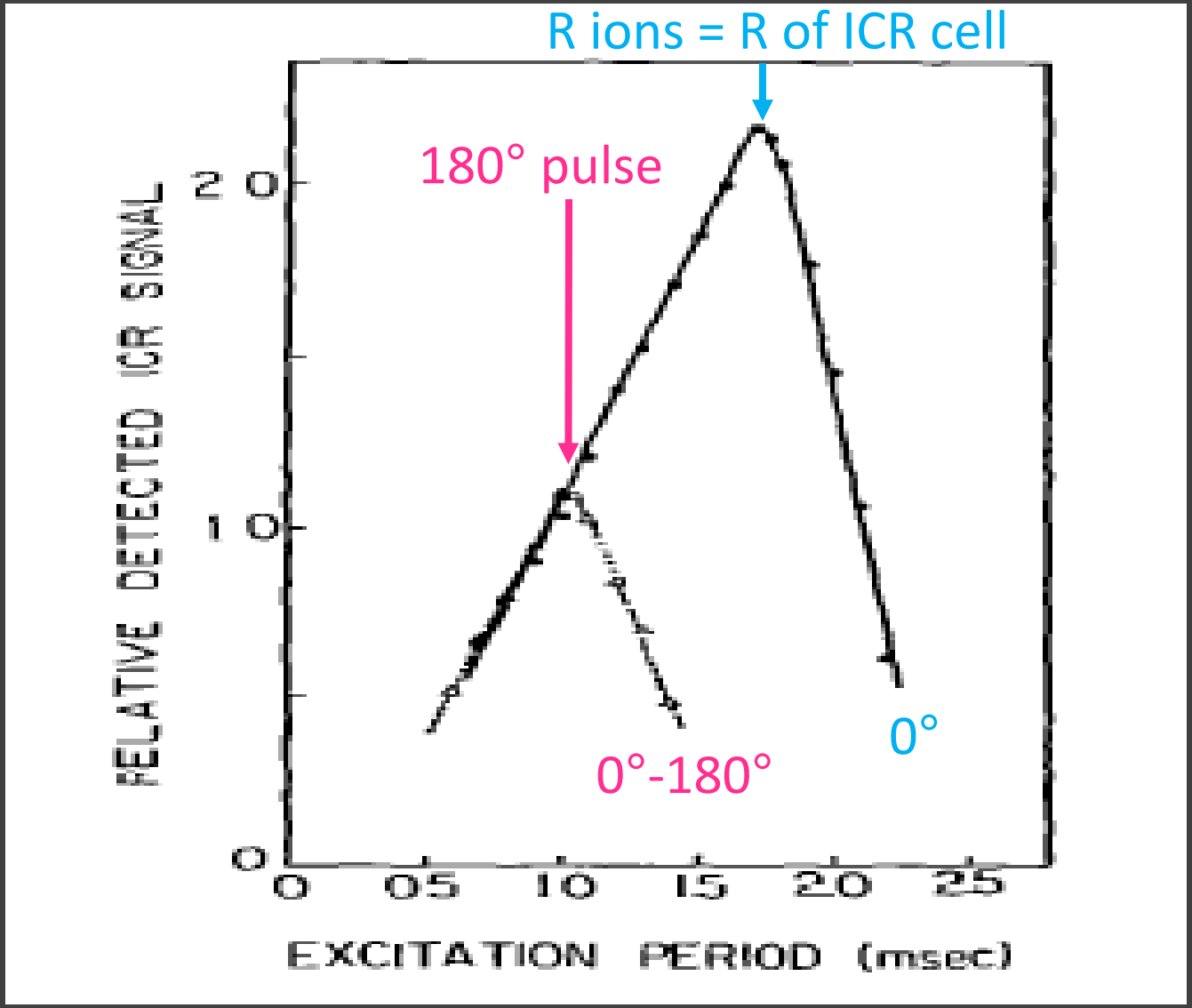
Back to basics: Original Ion De-excitation Experiment

Coherent excitation in the ICR cell



A.G. Marshall, T.C. Lin Wang, T. Lebatuan Ricca, *Chem. Phys. Letts.* 105 (1984) 233-236.

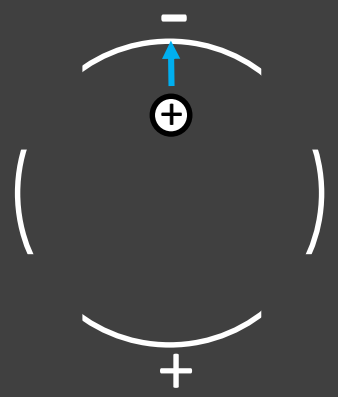
Original Ion De-excitation Experiment



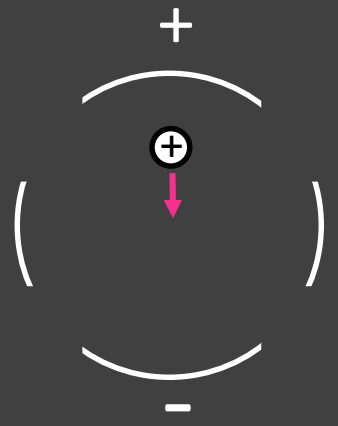
A.G. Marshall, T.C. Lin Wang, T. Lebatuan Ricca, *Chem. Phys. Letts.* 105 (1984) 233-236.

Original Ion De-excitation Experiment

Excitation voltage ***in phase with*** ion motion: ions excited to higher radius

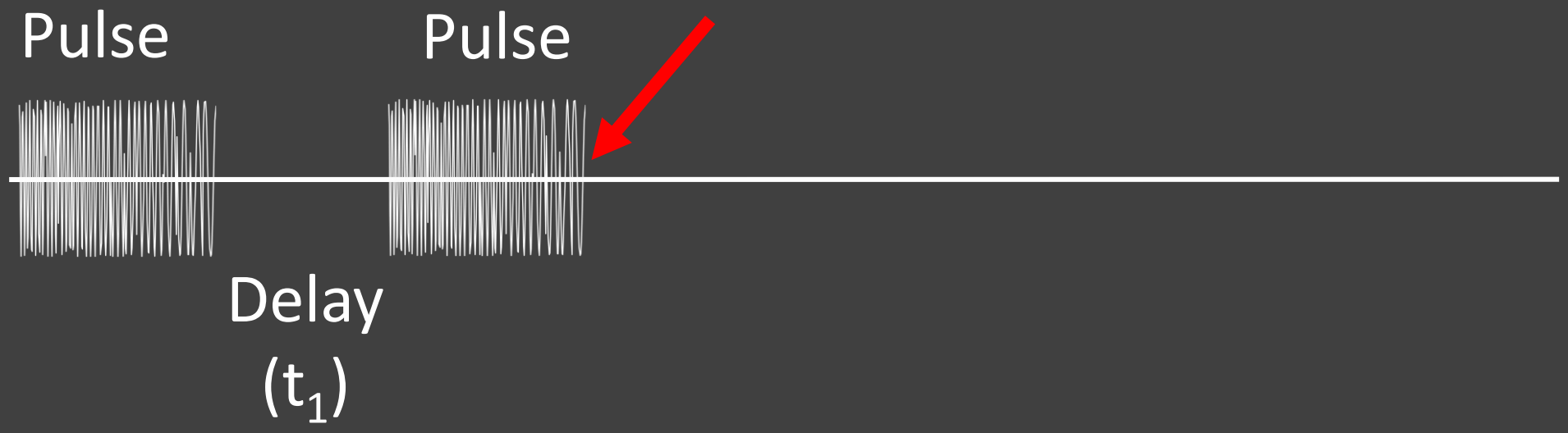


Excitation voltage ***in phase opposition with*** ion motion: ions de-excited to center of ICR cell.



A.G. Marshall, T.C. Lin Wang, T. Lebatuan Ricca, *Chem. Phys. Letts.* 105 (1984) 233-236.

2D MS Pulse Sequence



P. Pfändler, G. Bodenhausen, J. Rapin, M.-E. Walser, T. Gäumann, *J. Am. Chem. Soc.* 110 (1988) 5625-5628.
M. Bensimon, G. Zhao, T. Gäumann, *Chem. Phys. Letts.* 157 (1989) 97-100.
S. Guan, P.R. Jones, *J. Chem. Phys.* 91 (1989) 5291-5295.

Radius Modulation

$$\text{Phase} = \text{Frequency} \times \text{Delay}$$

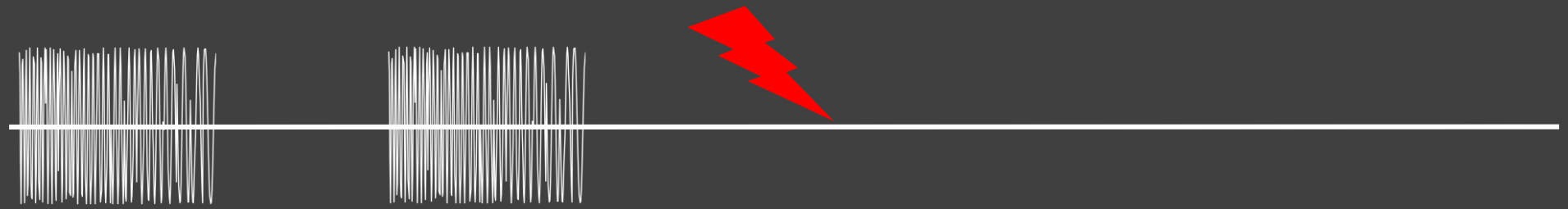
P. Pfändler, G. Bodenhausen, J. Rapin, M.-E. Walser, T. Gäumann, *J. Am. Chem. Soc.* 110 (1988) 5625-5628.
M. Bensimon, G. Zhao, T. Gäumann, *Chem. Phys. Letts.* 157 (1989) 97-100.
S. Guan, P.R. Jones, *J. Chem. Phys.* 91 (1989) 5291-5295.

Radius Modulation

$$radius \propto \sqrt{2\left(1 + cos(Frequency \times Delay)\right)}$$

P. Pfändler, G. Bodenhausen, J. Rapin, M.-E. Walser, T. Gäumann, *J. Am. Chem. Soc.* 110 (1988) 5625-5628.
M. Bensimon, G. Zhao, T. Gäumann, *Chem. Phys. Letts.* 157 (1989) 97-100.
S. Guan, P.R. Jones, *J. Chem. Phys.* 91 (1989) 5291-5295.

2D MS Pulse Sequence

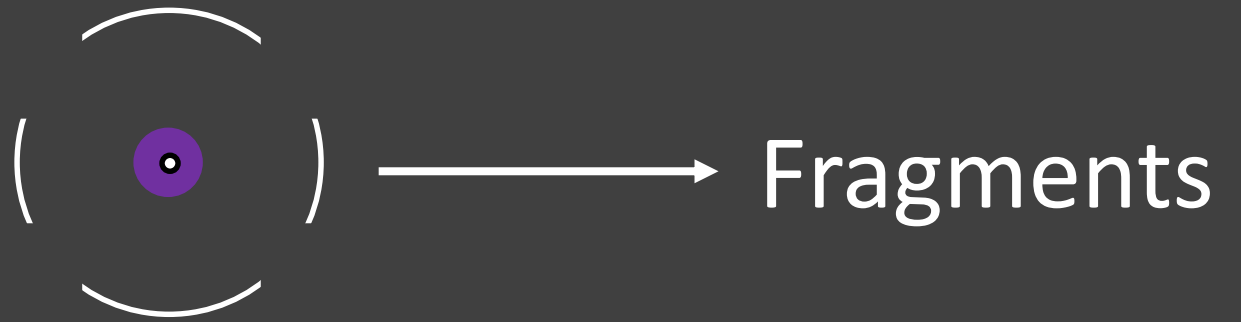


Fragmentation

P. Pfändler, G. Bodenhausen, J. Rapin, M.-E. Walser, T. Gäumann, *J. Am. Chem. Soc.* 110 (1988) 5625-5628.
M. Bensimon, G. Zhao, T. Gäumann, *Chem. Phys. Letts.* 157 (1989) 97-100.
S. Guan, P.R. Jones, *J. Chem. Phys.* 91 (1989) 5291-5295.

Radius Modulation

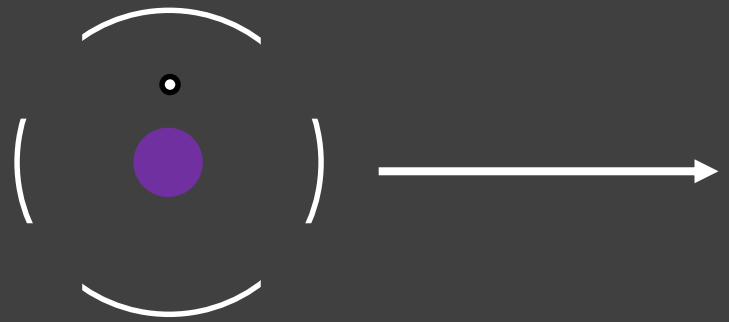
Phase = 180°



P. Pfändler, G. Bodenhausen, J. Rapin, M.-E. Walser, T. Gäumann, *J. Am. Chem. Soc.* 110 (1988) 5625-5628.
M. Bensimon, G. Zhao, T. Gäumann, *Chem. Phys. Letts.* 157 (1989) 97-100.
S. Guan, P.R. Jones, *J. Chem. Phys.* 91 (1989) 5291-5295.

Radius Modulation

Phase = 360°



No Fragments

P. Pfändler, G. Bodenhausen, J. Rapin, M.-E. Walser, T. Gäumann, *J. Am. Chem. Soc.* 110 (1988) 5625-5628.
M. Bensimon, G. Zhao, T. Gäumann, *Chem. Phys. Letts.* 157 (1989) 97-100.
S. Guan, P.R. Jones, *J. Chem. Phys.* 91 (1989) 5291-5295.

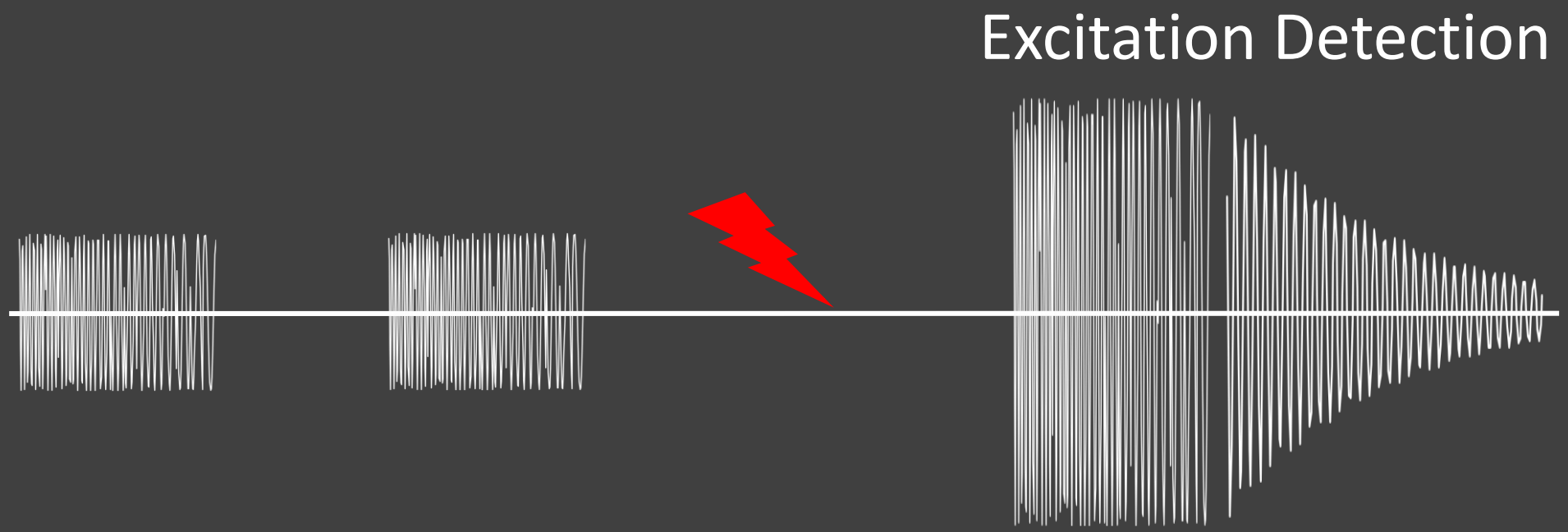
Precursor-Fragment correlation

Fragment abundance $\propto$ Precursor radius

Fragment abundance modulation =
Precursor radius modulation

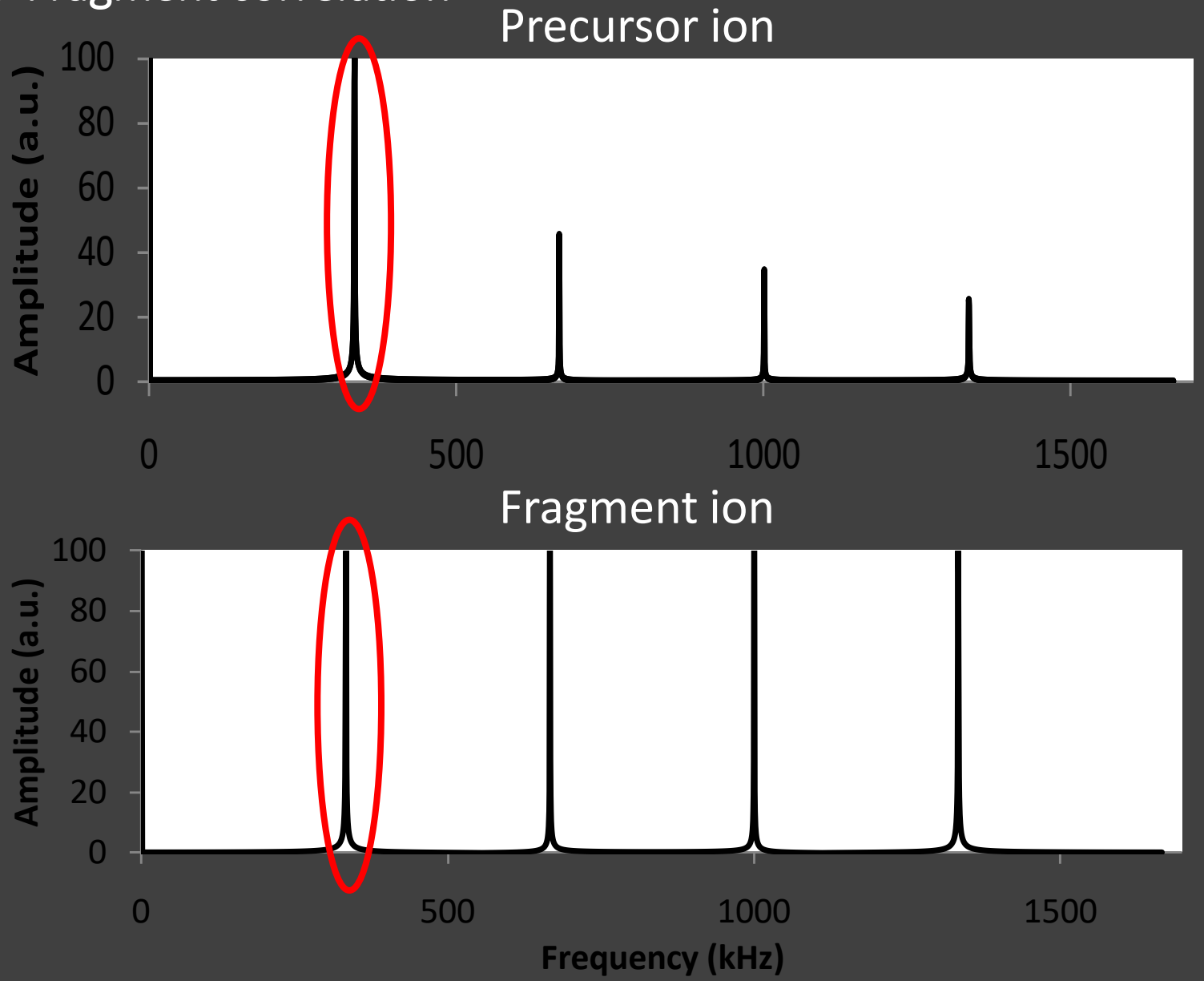
P. Pfändler, G. Bodenhausen, J. Rapin, M.-E. Walser, T. Gäumann, *J. Am. Chem. Soc.* 110 (1988) 5625-5628.
M. Bensimon, G. Zhao, T. Gäumann, *Chem. Phys. Letts.* 157 (1989) 97-100.
S. Guan, P.R. Jones, *J. Chem. Phys.* 91 (1989) 5291-5295.

2D MS Pulse Sequence



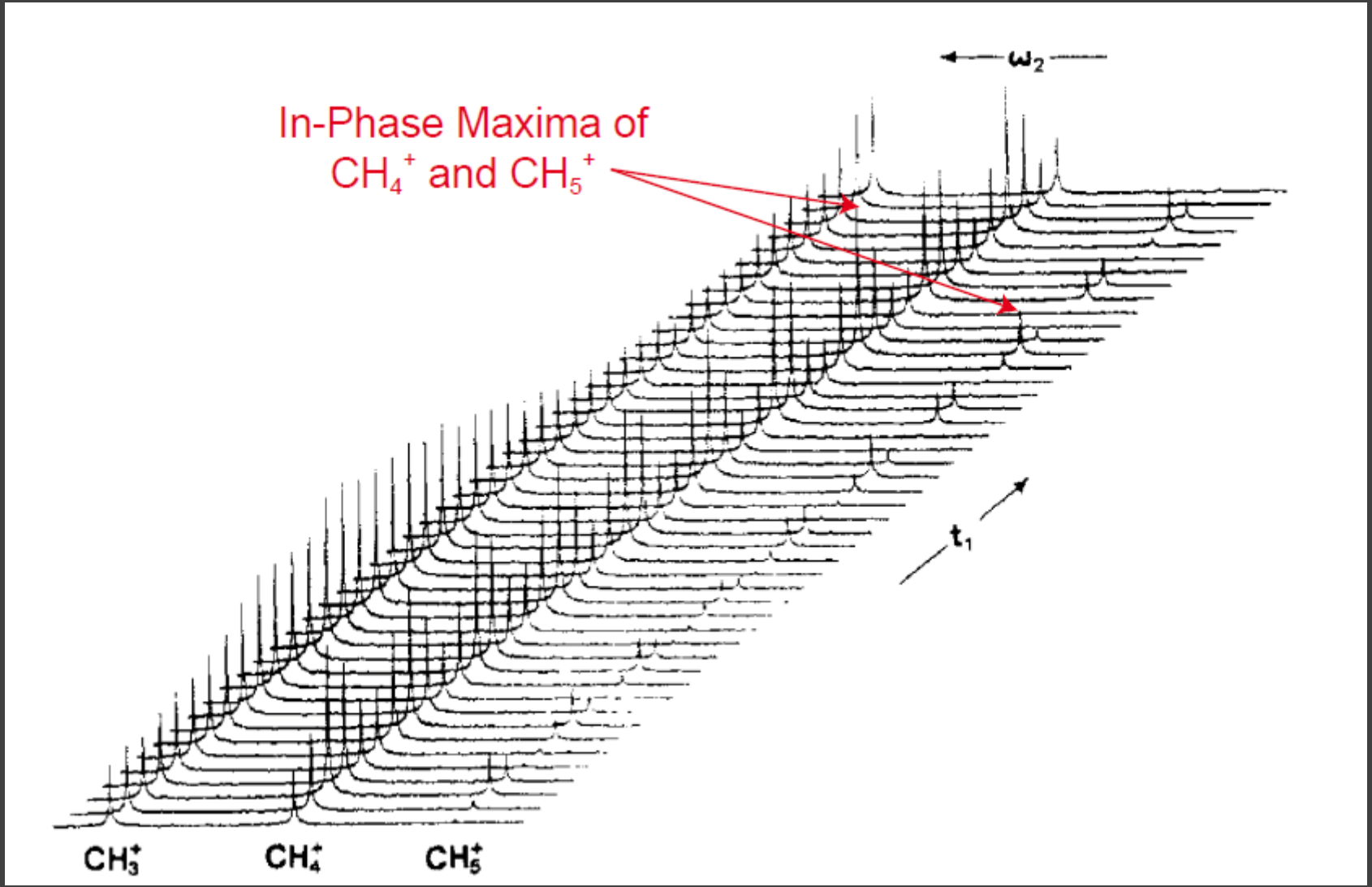
P. Pfändler, G. Bodenhausen, J. Rapin, M.-E. Walser, T. Gäumann, *J. Am. Chem. Soc.* 110 (1988) 5625-5628.
M. Bensimon, G. Zhao, T. Gäumann, *Chem. Phys. Letts.* 157 (1989) 97-100.
S. Guan, P.R. Jones, *J. Chem. Phys.* 91 (1989) 5291-5295.

Precursor-Fragment correlation



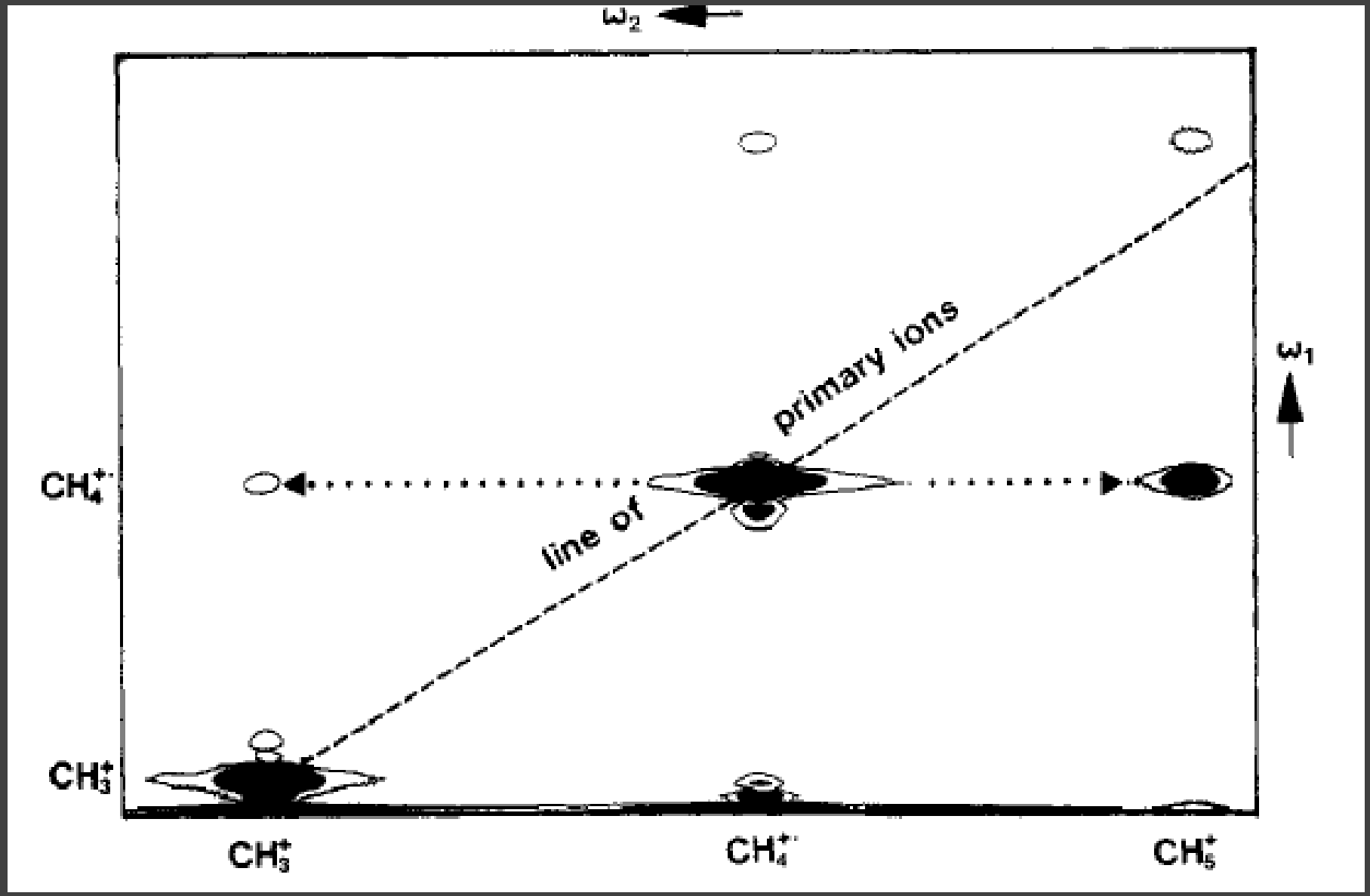
P. Pfändler, G. Bodenhausen, J. Rapin, M.-E. Walser, T. Gäumann, *J. Am. Chem. Soc.* 110 (1988) 5625-5628.
M. Bensimon, G. Zhao, T. Gäumann, *Chem. Phys. Letts.* 157 (1989) 97-100.
S. Guan, P.R. Jones, *J. Chem. Phys.* 91 (1989) 5291-5295.

First 2D FT-ICR Spectra
Ion-Molecule Reactions of CH_4^+



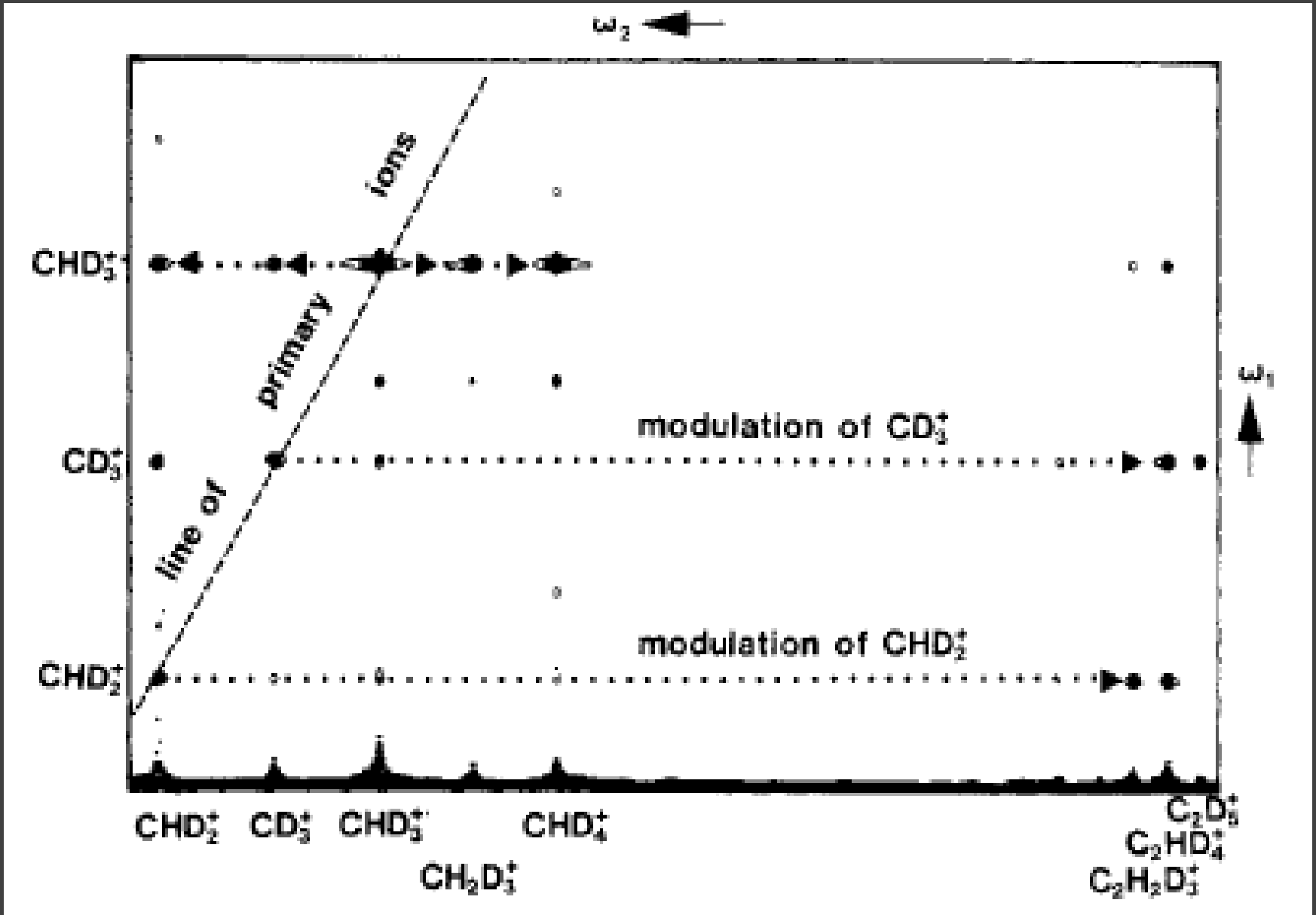
P. Pfändler, G. Bodenhausen, J. Rapin, M.-E. Walser, T. Gäumann, *J. Am. Chem. Soc.* 110 (1988) 5625-5628.

First 2D FT-ICR Spectra
Ion-molecule reactions of CH_4^+ (120×1k data points)



P. Pfändler et al., *J. Am. Chem. Soc.* 110 (1988) 5625-5628.

First 2D FT-ICR Spectra
Ion-molecule reactions of CHD_3 (256×1k data points)



P. Pfändler et al., *J. Am. Chem. Soc.* 110 (1988) 5625-5628.

Further 2D FT-ICR MS Studies

A theory for two-dimensional Fourier-transform ion cyclotron resonance mass spectrometry

Shenheng Guan and Patrick R. Jones
University of the Pacific, Chemistry Department, Stockton, California 95211

(Received 8 March 1989; accepted 27 July 1989)

A theoretical model, based on the Lorentz equations for ion motion and the mass action law, is developed for two-dimensional Fourier-transform mass spectrometry known as 2D FT–ICR or 2D FTMS. The theory illustrates that the modulation of 2D FT–ICR ion signals in the additional time dimension comes from the modulation of the primary ion speed by the 2D excitation pulses. The modulation of the primary ion speed is found not to be sinusoidal and the modulation of the ion signals in 2D FT–ICR spectra is found to be complicated even in the simplest chemical system. The complex modulation creates higher harmonic components in the spectra. Based on the model, a data processing algorithm is proposed. The results show that the Fourier transformation should be performed stepwise in order to obtain complete information, and that the phase portion of the frequency domain generated by the second Fourier transformation should not be discarded since it contains useful information.

Noise analysis for 2D tandem Fourier transform ion cyclotron resonance mass spectrometry

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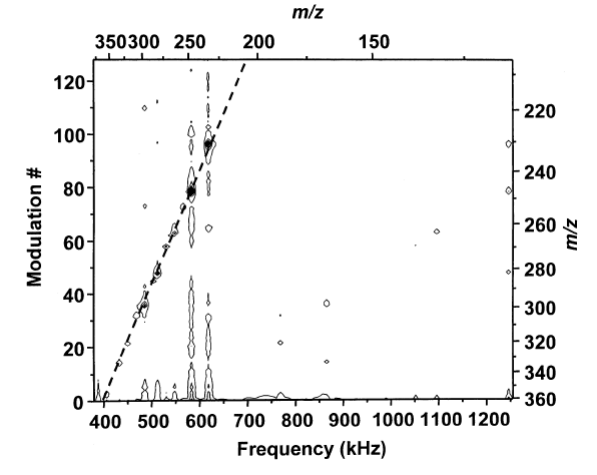
Center for Interdisciplinary Magnetic Resonance, National High Magnetic Field Laboratory, Florida State University, 1800 East Paul Dirac Drive, Tallahassee, FL 32310, USA

Received 31 December 2000; accepted 12 February 2001



7854 *J. Am. Chem. Soc.* **1993,** *115,* 7854–7861

Two-Dimensional Fourier Transform Ion Cyclotron Resonance Mass Spectrometry/Mass Spectrometry with Stored-Waveform Ion Radius Modulation

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Contribution from the Department of Chemistry, The Ohio State University, 120 West Eighteenth Avenue, Columbus, Ohio 43210

Received March 4, 1993

Abstract: A fundamentally new two-dimensional Fourier transform ion cyclotron resonance mass spectrometry experiment, SWIM ("stored-waveform ion modulation") 2D-FT/ICR MS/MS, is described. Prior encodement of the second dimension by use of two identical excitation waveforms separated by a variable delay period (analogous to 2D-NOESY NMR) is replaced by a new encodement in which each row of the two-dimensional data array is obtained by use of a single stored excitation waveform whose frequency-domain magnitude spectrum is a sinusoid whose frequency increases from one row to the next. In the two-dimensional mass spectrum, the conventional one-dimensional FT/ICR mass spectrum appears along the diagonal, and each off-diagonal peak corresponds to an ion-neutral reaction whose ionic components may be identified by horizontal and vertical projections to the diagonal spectrum. Fragmentation due to (e.g.) collision-induced dissociation results in peaks on only one side of the diagonal, whereas bidirectional ion–molecule reactions result in peaks on both sides of the diagonal. All ion–molecule reactions in a gaseous mixture may be identified from a single 2D-FT/ICR MS/MS experiment, without any prior knowledge of the system.

Anal. Chem. **2002,** *74,* 4625–4633

Application of Stored Waveform Ion Modulation 2D-FTICR MS/MS to the Analysis of Complex Mixtures

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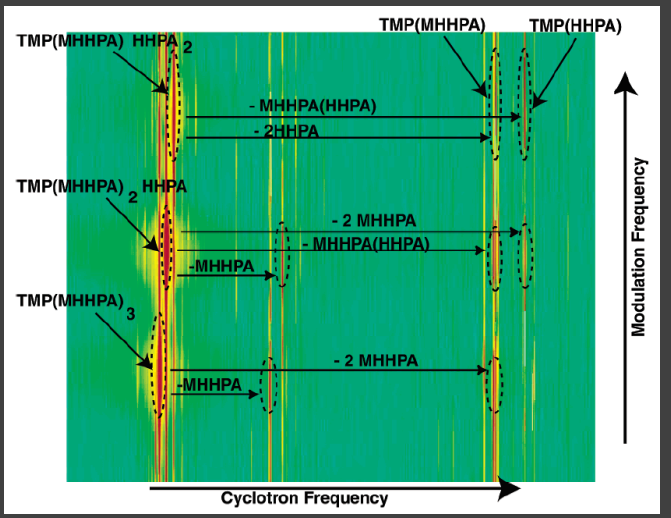
DuPont Marshall R & D Laboratory, Philadelphia, Pennsylvania 19146, and Merck Research Labs, Merck & Co. Inc., West Point, Pennsylvania 19486



Guan et al. J. Chem. Phys. 91 (1989) 5291-5295.
Ross et al. J. Am. Chem. Soc. 115 (1993) 7854-7861.
van der Rest et al. Int. J. Mass Spectrom. 210/211 (2001) 101-111.
Ross et al. Anal. Chem. 74 (2002) 4625-4633.

2D FT-ICR MS Renewal: Adaptation to IRMPD and ECD

Two-dimensional FT-ICR/MS with IRMPD as fragmentation mode

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ARTICLE INFO

Article history:
Received 27 August 2010
Received in revised form 22 October 2010
Accepted 27 October 2010
Available online xxx

Keywords:
FT-ICR
2-Dimensional
IRMPD
FTMS
Pulse sequence
Double-frequency

ABSTRACT

In 1988, Gäumann et al. introduced a pulse sequence for two-dimensional FT-ICR/MS correlating parent ions and fragment ions without the need for ion isolation. The improvement in computer technology makes this pulse sequence analytically useful in order to obtain structural information on complex samples. The pulse sequence can be applied to all cyclotron radius-dependent fragmentation modes, including gas-free fragmentation modes like IRMPD, which do not affect sensitivity and resolving power like the pulsing of a gas into the ICR cell does. This study shows the feasibility of 2D FT-ICR/MS and lays the groundwork to turn this method into a viable analytical tool.

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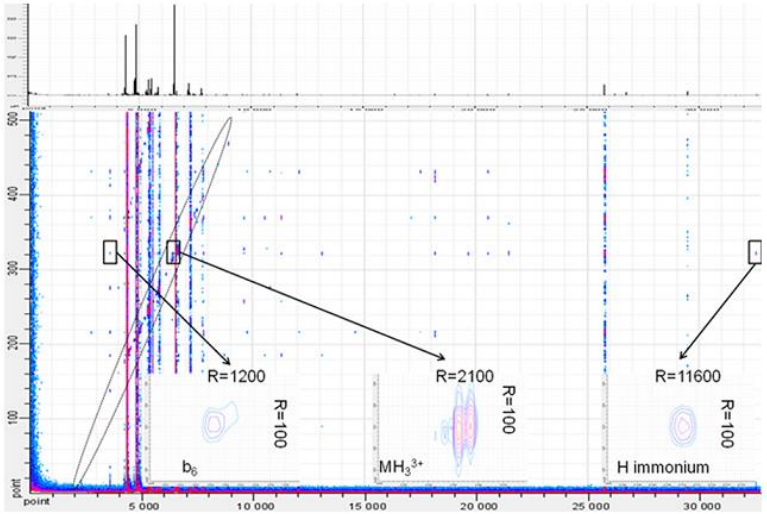

Fig. 2. 2D IRMPD mass spectrum of angiotensin I (1 pmol/μL in MeOH/water with 0.1% formic acid) using IRMPD (50% for 0.1 s). Cyclotron frequencies are represented horizontally ($f_{Nyquist}$ = 1667 kHz = 32,768 a.u., corresponding to a *m/z* 86–2000 mass range) and correlation frequencies are represented vertically ($f_{Nyquist}$ = 1667 kHz = 2048 a.u., corresponding to a *m/z* 86–2000 mass range). Although the size of the datafile is 32,768 × 2048, we chose to represent 32,768 × 512 for better visibility. Inserts: Self-correlation peak of MH_3^{3+} (*m/z* 433) with its ^{13}C isotopes and resolution of the peak in both directions. Correlation peaks of MH_3^{3+} and b_6 (*m/z* 784) and H immonium (*m/z* 110) and resolution of the peaks in both dimensions.

Two-Dimensional ECD FT-ICR Mass Spectrometry of Peptides and Glycopeptides

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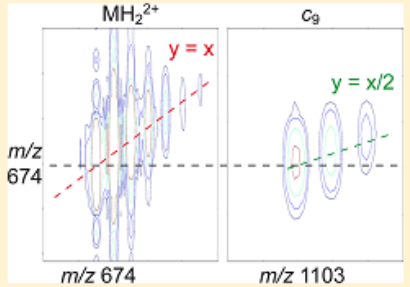
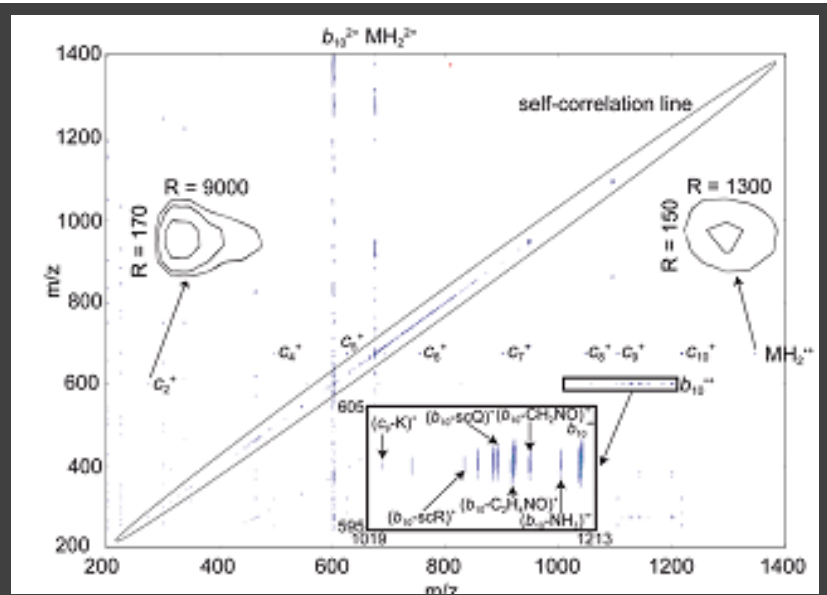
Supporting Information

ABSTRACT: 2D FT-ICR MS allows the correlation between precursor and fragment ions by modulating ion cyclotron radii for fragmentation modes with radius-dependent efficiency in the ICR cell without the need for prior ion isolation. This technique has been successfully applied to ion–molecule reactions, Collision-induced dissociation and infrared multiphoton dissociation. In this study, we used electron capture dissociation for 2D FT-ICR MS for the first time, and we recorded two-dimensional mass spectra of peptides and a mixture of glycopeptides that showed fragments that are characteristic of ECD for each of the precursor ions in the sample. We compare the sequence coverage obtained with 2D ECD FT-ICR MS with the sequence coverage obtained with ECD MS/MS and compare the sensitivities of both techniques. We demonstrate how 2D ECD FT-ICR MS can be implemented to identify peptides and glycopeptides for proteomics analysis.





van Agthoven et al. Int. J. Mass Spectrom. 306 (2011) 196-203.
van Agthoven et al. Anal. Chem. 84 (2012) 5589-5595.
SPIKE: http://www.bitbucket.org/delsuc/spike
Chiron et al. arXiv.org, e-Print Arch., Phys., 1-13 (2016).

2D FT-ICR MS Renewal: Adaptation to IR-ECD

analytical chemistry

Article

Cite This: *Anal. Chem.* 2018, 90, 3496–3504

pubs.acs.org/ac

Can Two-Dimensional IR-ECD Mass Spectrometry Improve Peptide de Novo Sequencing?

Maria A. van Agthoven,[§] Alice M. Lynch,[§] Tomos E. Morgan,[§] Christopher A. Wootton,[§] Yuko P.Y. Lam,[§] Lionel Chiron,[+] Mark P. Barrow,[§] Marc-André Delsuc,[#,+] and Peter B. O'Connor*[,§]

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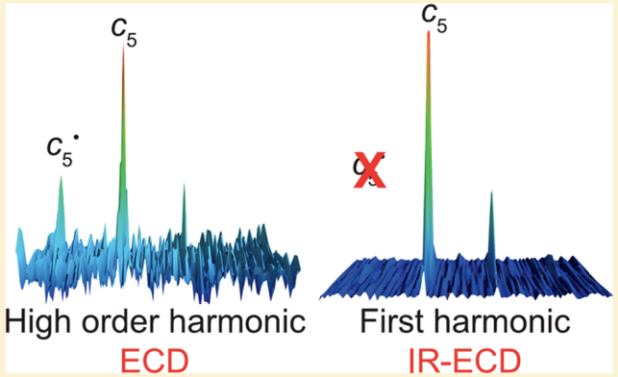
[#]Institut de Génétique et de Biologie Moléculaire et Cellulaire, INSERM, U596; CNRS, UMR7104, Université de Strasbourg, 1 rue Laurent Fries, 67404 Illkirch-Graffenstaden, France

Supporting Information

ABSTRACT: Two-dimensional mass spectrometry (2D MS) correlates precursor and fragment ions without ion isolation in a Fourier transform ion cyclotron resonance mass spectrometer (FTICR MS) for tandem mass spectrometry. Infrared activated electron capture dissociation (IR-ECD), using a hollow cathode configuration, generally yields more information for peptide sequencing in tandem mass spectrometry than ECD (electron capture dissociation) alone. The effects of the fragmentation zone on the 2D mass spectrum are investigated as well as the structural information that can be derived from it. The enhanced structural information gathered from the 2D mass spectrum is discussed in terms of how de novo peptide sequencing can be performed with increased confidence. 2D IR-ECD MS is shown to sequence peptides, to distinguish between leucine and isoleucine residues through the production of *w* ions as well as between C-terminal (*b*/*c*) and N-terminal (*y*/*z*) fragments through the use of higher harmonics, and to assign and locate peptide modifications.



van Agthoven et al. Anal. Chem. 90 (2018) 3496-3504.

2D FT-ICR MS Renewal: Adaptation to EID

analytical chemistry

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Advantages of Two-Dimensional Electron-Induced Dissociation and Infrared Multiphoton Dissociation Mass Spectrometry for the Analysis of Agrochemicals

Bryan P. Marzullo, Tomos E. Morgan, Christopher A. Wootton, Simon J. Perry, Mansoor Saeed, Mark P. Barrow, and Peter B. O'Connor*

Cite This: *Anal. Chem.* 2020, 92, 11687–11695 Read Online

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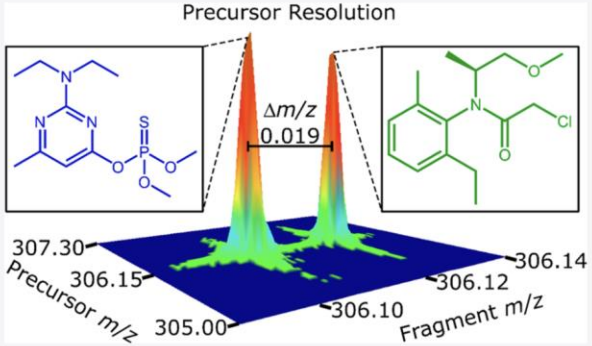
ABSTRACT: Analysis of agrochemicals in an environmental matrix is challenging because these samples contain multiple agrochemicals, their metabolites, degradation products, and endogenous compounds. The analysis of such complex samples is achieved using chromatographic separation techniques coupled to mass spectrometry. Herein, we demonstrate a two-dimensional mass spectrometry (2DMS) technique on a 12 T Fourier transform ion cyclotron resonance mass spectrometer that can analyze a mixture of agrochemicals without using chromatography or quadrupole isolation in a single experiment. The resulting 2DMS contour plot contains abundant tandem MS information for each component in the sample and correlates product ions to their corresponding precursor ions. Two different fragmentation methods are employed, infrared multiphoton dissociation (IRMPD) and electron-induced dissociation (EID), with 2DMS to analyze the mixture of singly charged agrochemicals. The product ions of one of the agrochemicals, pirimiphos-methyl, present in the sample was used to internally calibrate the entire 2DMS spectrum, achieving sub part per million (ppm) to part per billion (ppb) mass accuracies for all species analyzed. The work described in this study will show the advantages of the 2DMS approach, by grouping species with common fragments/core structure and mutual functional groups, using precursor lines and neutral loss lines. In addition, the rich spectral information obtained from IRMPD and EID 2DMS contour plots can accurately identify and characterize agrochemicals.



Marzullo et al. Anal. Chem. 92 (2020) 11687-11695.

2D FT-ICR MS Renewal: Pulse Sequence Optimisation and Data Processing

Optimization of the discrete pulse sequence for two-dimensional FT-ICR mass spectrometry using infrared multiphoton dissociation

Maria A. van Agthoven[a,1], Lionel Chiron[b], Marie-Aude Coutouly[c], Akansha Ashvani Sehgal[d], Philippe Pelupessy[d], Marc-André Delsuc[b,c], Christian Rolando[a,*]

[a] Miniaturisation pour la Synthèse, l'Analyse & la Protéomique (MSAP), USR CNRS 3290, and Protéomique, Modifications Post-traductionnelles et Glycobiologie, IFR 147 and Institut Eugène-Michel Chevreul, FR CNRS 2638, Université de Lille 1 Sciences et Technologies, 59655 Villeneuve d'Ascq Cedex, France
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[c] NMRTEC, Boulevard Sébastien Brandt, Bioparc, Batiment B, 67400 Illkirch-Graffenstaden, France
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ARTICLE INFO

Article history:
Received 15 March 2014
Received in revised form 9 June 2014
Accepted 23 June 2014
Available online xxx

Keywords:
FT-ICR
2-dimensional
IRMPD
FTMS
Pulse sequence
Optimization

ABSTRACT

2D FT-ICR MS, introduced by Pfändler et al. (*Chem. Phys. Lett.* 138 (1987) 195), allows one to correlate precursor and fragment ions in complex samples without requiring ion isolation. Recent advances in electronics, computer capacities, and gas-free in-cell fragmentation techniques open up new perspectives for 2D FT-ICR MS as an analytical technique. The pulse sequence consists of two encoding pulses separated by an incremental delay, followed by an observe pulse. In our previous 2D FT-ICR MS work we used three pulses of equal duration and amplitude. However, signal intensity was low because it was distributed over a series of intense harmonics. Using a simple theoretical model to analytically express ion fragmentation and 2D FT-ICR MS ion trajectories, we obtained a nearly pure signal when the maximum radius of the ions during the encoding pulses is within the laser beam. By adjusting the experimental parameters of the encoding pulses according to the calculation on the same cyclotron radius, we strongly decrease the intensity of harmonic peaks. We also discuss the effect of increasing the amplitude of the observe pulse, which affects precursor and fragment ion peaks differently in terms of signal-to-noise ratio. The 2D mass spectra obtained with the optimized pulse sequence show a much higher signal-to-noise ratio, even without using denoising algorithms.

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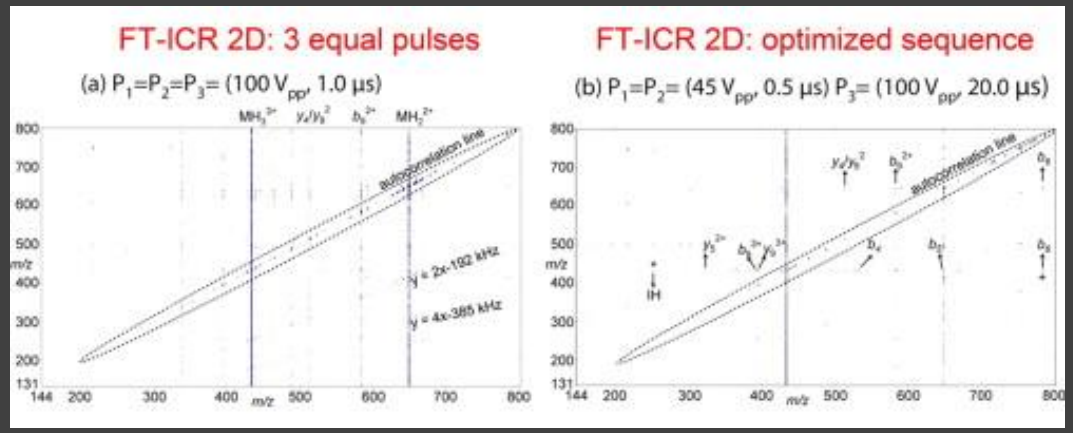

Two-dimensional Fourier transform ion cyclotron resonance mass spectrometry: reduction of scintillation noise using Cadzow data processing

Maria A. van Agthoven[1]*, Marie-Aude Coutouly[2], Christian Rolando[1] and Marc-André Delsuc[2,3]

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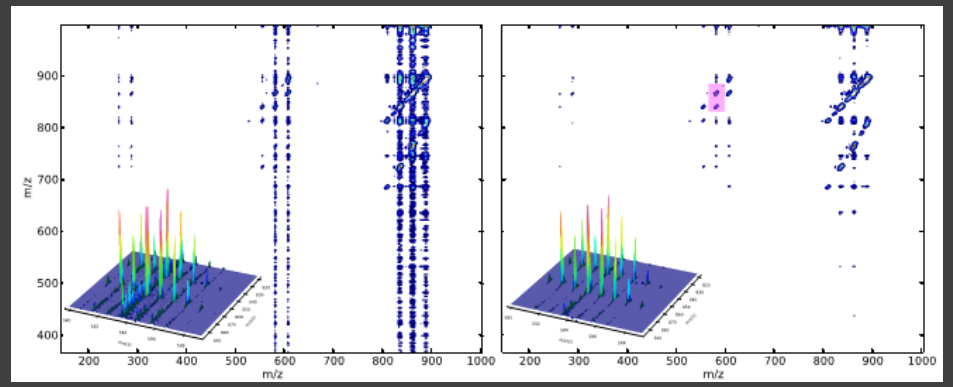
In two-dimensional Fourier transform ion cyclotron resonance mass spectrometry (2D FTICR-MS), scintillation noise, caused mostly by fluctuations in the number of ions in the ICR cell, is the leading cause for errors in spectrum interpretation. In this study, we adapted an algorithm based on singular value decomposition and first introduced by Cadzow *et al.* (*IEE Proceedings Pt. F* 1987, 134, 69) to 2D FTICR-MS and we measured its performance in terms of noise reduction without losing signal information in the 2D mass spectrum. **Copyright © 2011 John Wiley & Sons, Ltd.**

Efficient denoising algorithms for large experimental datasets and their applications in Fourier transform ion cyclotron resonance mass spectrometry

Lionel Chiron[a], Maria A. van Agthoven[b], Bruno Kieffer[a], Christian Rolando[b], and Marc-André Delsuc[a,1]



van Agthoven et al. Int. J. Mass Spectrom. 370 (2014) 114-124.
van Agthoven et al. Rapid Comm. Mass Spectrom. 25 (2011) 1609-1616.
Chiron et al. Proc. Nat. Acac. Sci. 111 (2014) 1385-1390.

2D FT-ICR MS Renewal: Non-Uniform Sampling

analytical chemistry

Letter

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Nonuniform Sampling Acquisition of Two-Dimensional Fourier Transform Ion Cyclotron Resonance Mass Spectrometry for Increased Mass Resolution of Tandem Mass Spectrometry Precursor Ions

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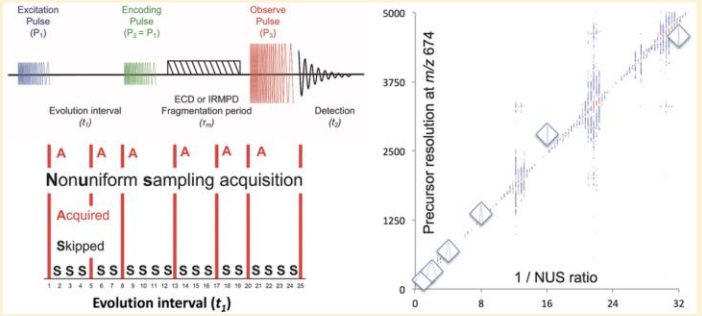
Supporting Information



ABSTRACT: Obtaining the full MS/MS map for fragments and precursors of complex mixtures without hyphenation with chromatographic separation by a data-independent acquisition is a challenge in mass spectrometry which is solved by two-dimensional (2D) Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS). Until now 2D FTICR MS afforded only a moderate resolution for precursor ion since this resolution is limited by the number of evolution interval steps to which the number of scans, the acquisition time, and the sample consumption are proportional. An overnight acquisition is already required to reach a quadrupole mass filter-like unit mass resolution. Here, we report that 2D FTICR MS using nonuniform sampling (NUS) obtained by randomly skipping points in the first dimension corresponding to the precursor selection gives access, after data processing, to the same structural information contained in a complex mixture. The resolution increases roughly as the inverse of the NUS ratio, up to 26 times at NUS 1/32, leading to an acquisition time reduced in the same ratio compared to a classical acquisition at the same resolution. As an example, the analysis of a natural oil is presented.

Bray et al. Ana. Chem. 89 (2017) 8589-8593.

2D FT-ICR MS Renewal: Narrowband Modulation

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analytical chemistry

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Narrowband Modulation Two-Dimensional Mass Spectrometry and Label-Free Relative Quantification of Histone Peptides

Matthias Halper, Marc-André Delsuc, Kathrin Breuker, and Maria A. van Agthoven*

Cite This: https://dx.doi.org/10.1021/acs.analchem.0c02843 Read Online

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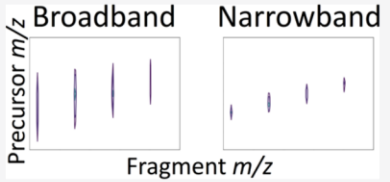
ABSTRACT: Two-dimensional mass spectrometry (2D MS) on a Fourier transform ion cyclotron resonance (FT-ICR) mass analyzer allows for tandem mass spectrometry without requiring ion isolation. In the ICR cell, the precursor ion radii are modulated before fragmentation, which results in modulation of the abundance of their fragments. The resulting 2D mass spectrum enables a correlation between the precursor and fragment ions. In a standard broadband 2D MS, the range of precursor ion cyclotron frequencies is determined by the lowest mass-to-charge (m/z) ratio to be fragmented in the 2D MS experiment, which leads to precursor ion m/z ranges that are much wider than necessary, thereby limiting the resolving power for precursor ions and the accuracy of the correlation between the precursor and fragment ions. We present narrowband modulation 2D MS, which increases the precursor ion resolving power by reducing the precursor ion m/z range, with the aim of resolving the fragment ion patterns of overlapping isotopic distributions. In this proof-of-concept study, we compare broadband and narrowband modulation 2D mass spectra of an equimolar mixture of histone peptide isoforms. In narrowband modulation 2D MS, we were able to separate the fragment ion patterns of all ^{13}C isotopes of the different histone peptide forms. We further demonstrate the potential of narrowband 2D MS for label-free quantification of peptides.



Halper et al. Ana. Chem. 92 (2020), 13945–13952

2D FT-ICR MS Renewal: Phase correction for Absorption Mode 2D MS

molecules

MDPI

Article

Phase Correction for Absorption Mode Two-Dimensional Mass Spectrometry

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2 CASC4DE, Pôle API, 300 Bd. Sébastien Grant, 67400 Illkirch-Graffenstaden, France
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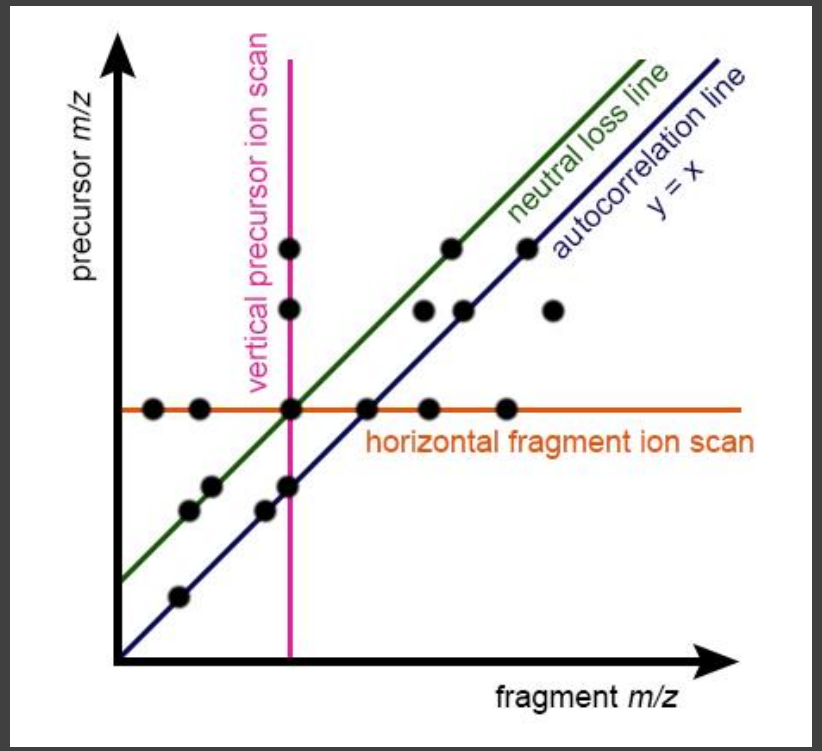
Abstract: Two-dimensional mass spectrometry (2D MS) is a tandem mass spectrometry method that relies on manipulating ion motions to correlate precursor and fragment ion signals. 2D mass spectra are obtained by performing a Fourier transform in both the precursor ion mass-to-charge ratio (*m/z*) dimension and the fragment ion *m/z* dimension. The phase of the ion signals evolves linearly in the precursor *m/z* dimension and quadratically in the fragment *m/z* dimension. This study demonstrates that phase-corrected absorption mode 2D mass spectrometry improves signal-to-noise ratios by a factor of 2 and resolving power by a factor of 2 in each dimension compared to magnitude mode. Furthermore, phase correction leads to an easier differentiation between ion signals and artefacts, and therefore easier data interpretation.

Halper et al. Molecules 26 (2021), 3388–3402

2D Mass Spectra

- Autocorrelation line = MS
- Fragment ion scan = MS/MS
- Precursor ion scan = all precursors that have the same fragment
  - ABBA → ABB
  - ABBC→ ABB
- Neutral loss line = all precursors that lose the same neutral
  - ABBA → BA
  - ABBC→ BC

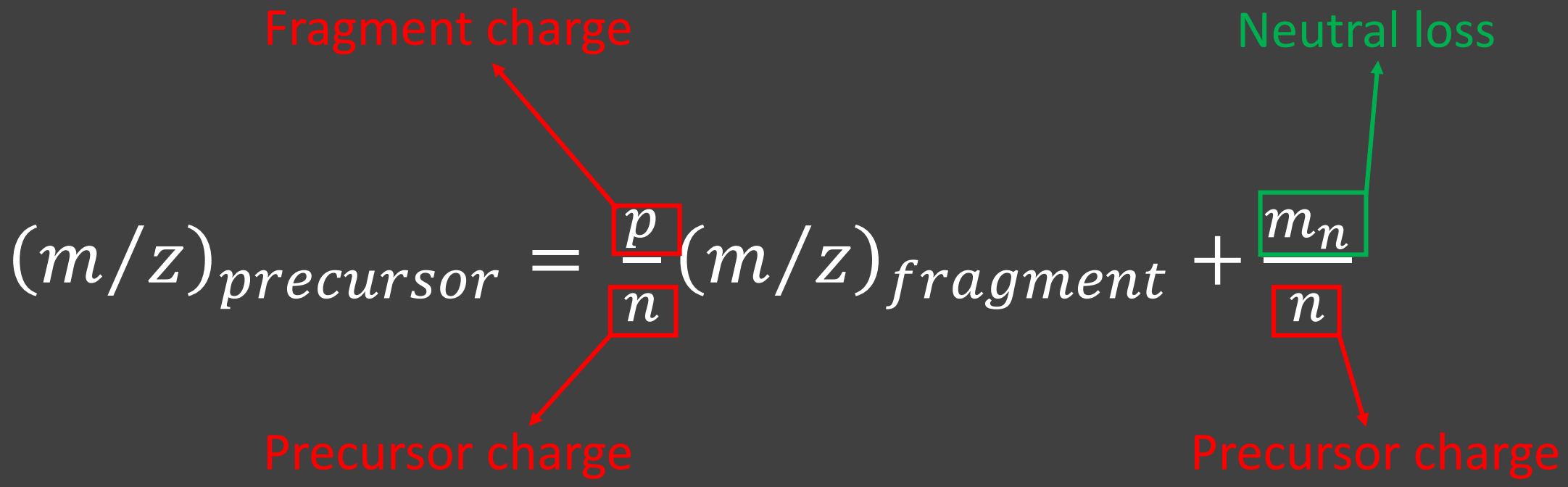


M. van Agthoven et al., *Anal. Bioanal. Chem*. 405 (2013) 51.
M. van Agthoven et al., *Eur. Biophys. J.* 48 (2019) 213.

Dissociation Lines in 2D Mass Spectra

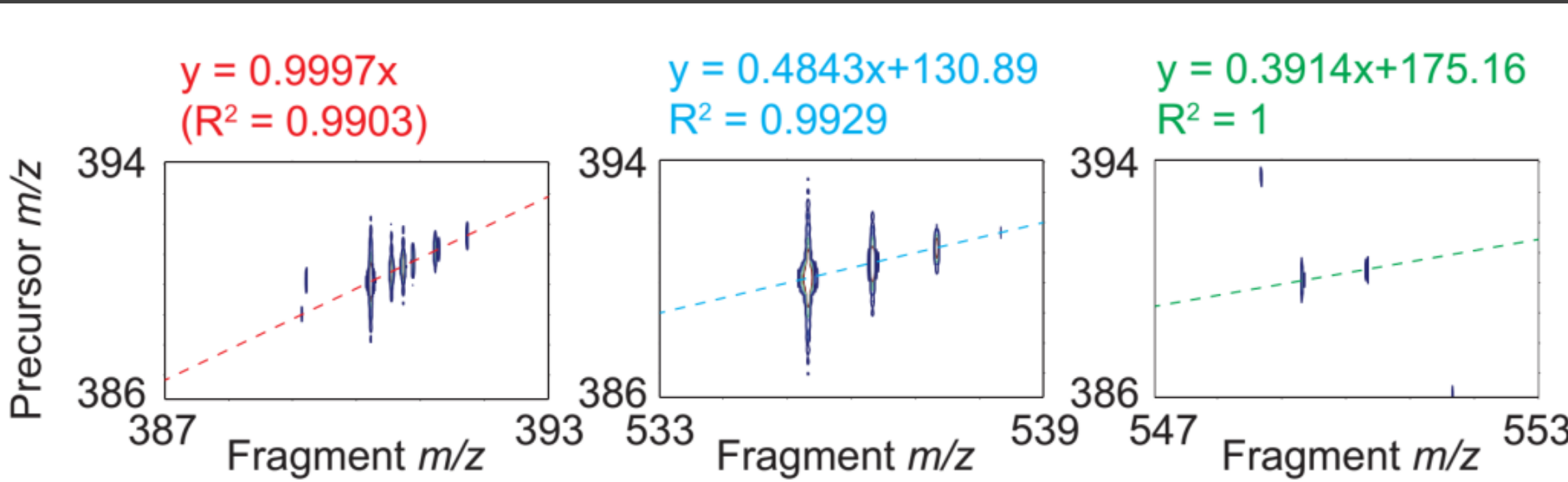
- Precursors of same charge state
- Lose the same charge
- Lose the same mass

M. van Agthoven et al., *Anal Chem.* 88 (2016) 4409-4417.



M. van Agthoven et al., *Anal Chem.* 88 (2016) 4409-4417.

Dissociation Lines in 2D Mass Spectra

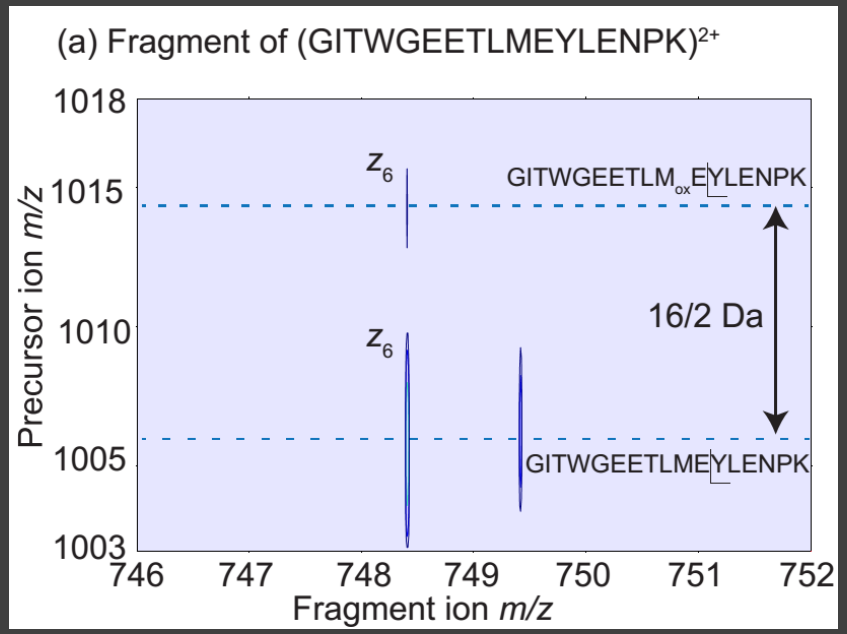


(a) Autocorrelation peaks for $(MIFAGIK)^{2+}$ and $(TGPNLHGLFGR)^{3+}$

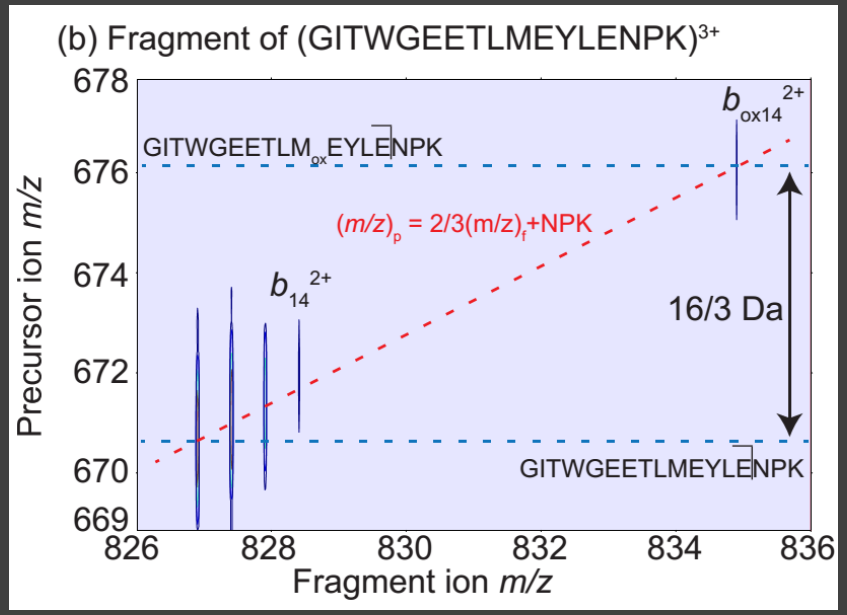
(b) Isotopic distribution for y_5 fragment of $(MIFAGIK)^{2+}$

(c) Isotopic distribution for y_5 fragment of $(TGPNLHGLFGR)^{3+}$

M. van Agthoven et al., *Anal Chem.* 88 (2016) 4409-4417.



Fragment without the modification: vertical

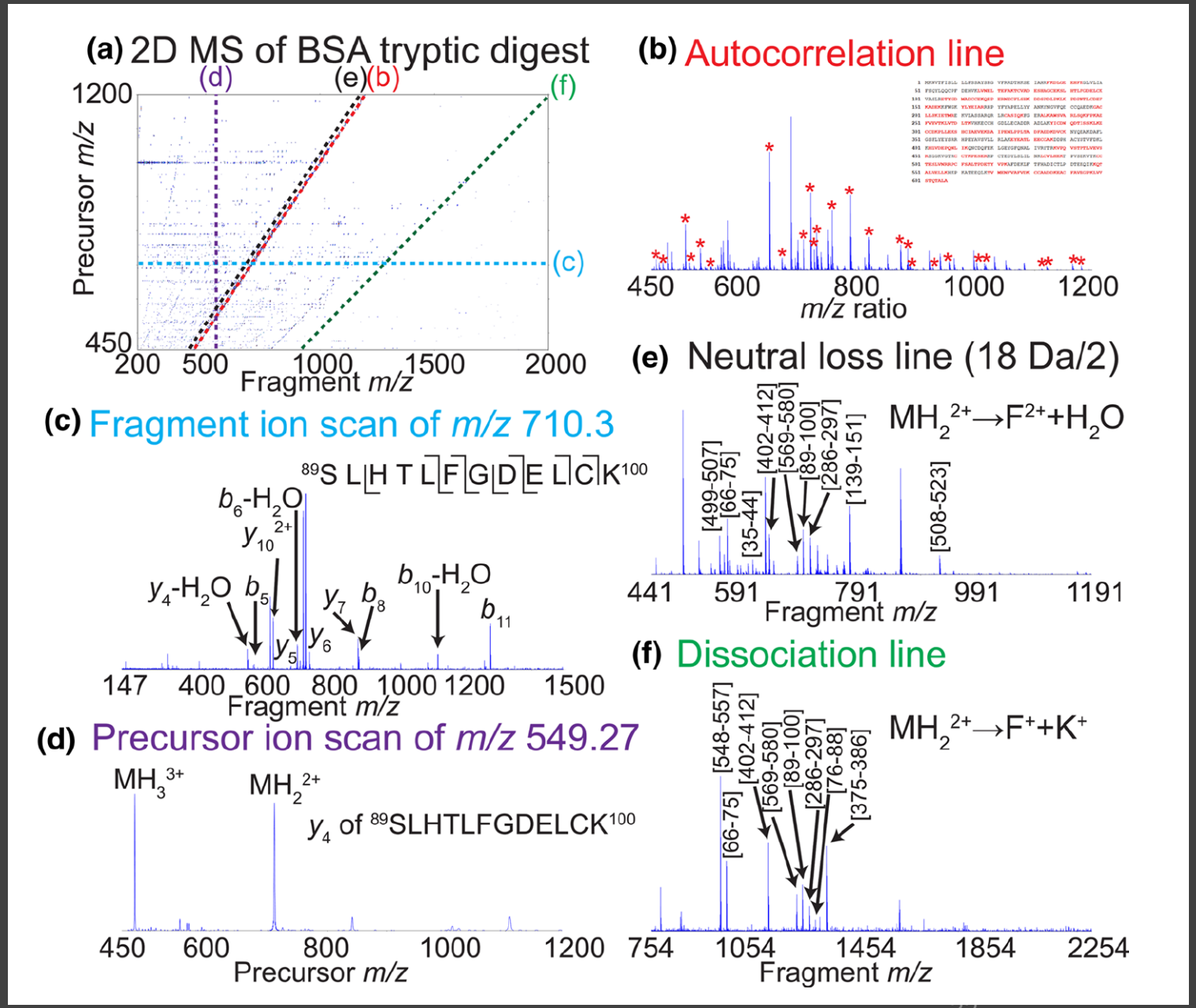


Fragment with the modification: on the same dissociation line

Easy method for assignment and location of peptide modifications!

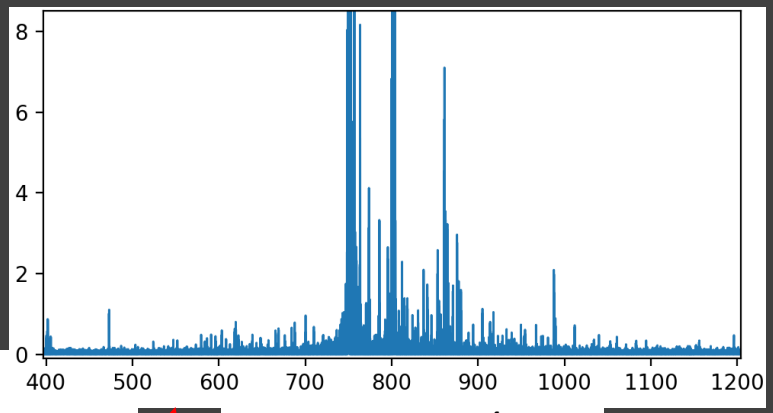
M. van Agthoven et al., *Anal Chem.* 90 (2018) 3496-3504.

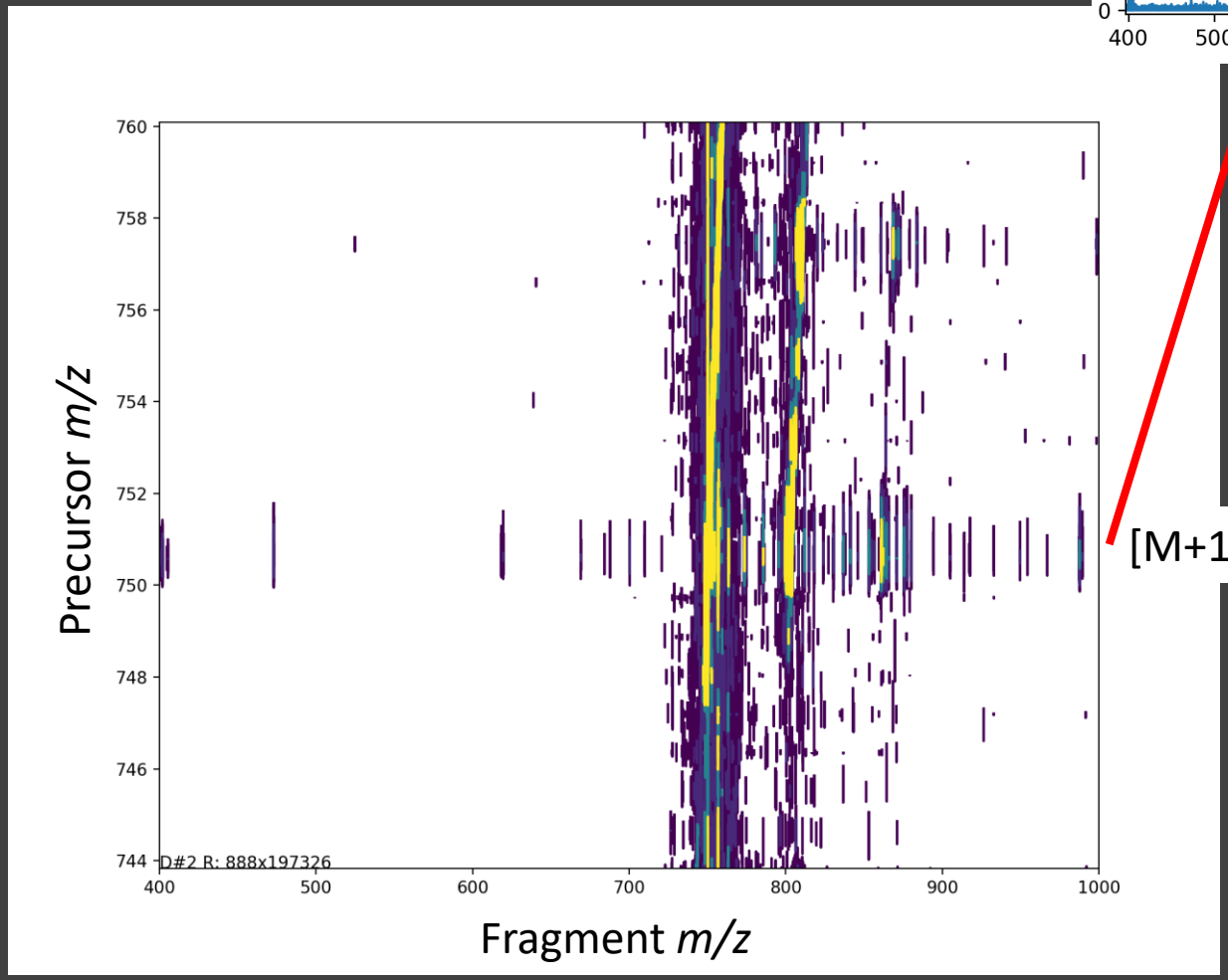
nanoESI 2D (IRMPD) FT-ICR Mass Spectrum of Bovine Serum Albumin Digest



M.A. van Agthoven, Y.P.Y. Lam, P.B. O'Connor, C. Rolando, M.-A. Delsuc, *Eur. Biophys. J.* 48 (2019) 213-229.

2D ECD MS of histone H4:





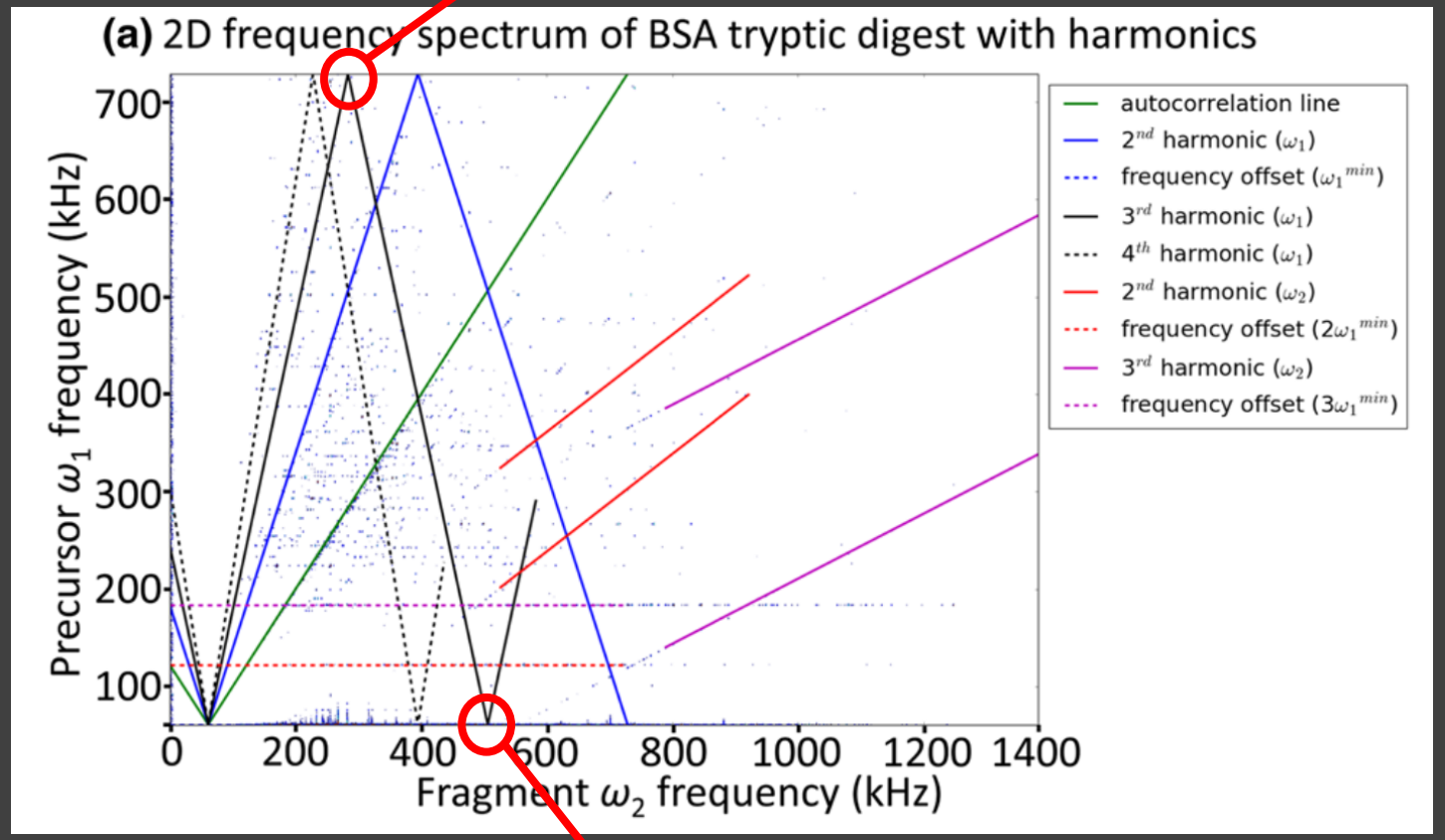
Vertical FWHM:
m/z 0.8 @ *m/z* 750

$[M+15H]^{15+}$

- Optimal signal-to-noise ratio
- Maximum precursor-fragment correlation

- Narrowband mode 2D MS
- Phase correction for absorption mode 2DMS

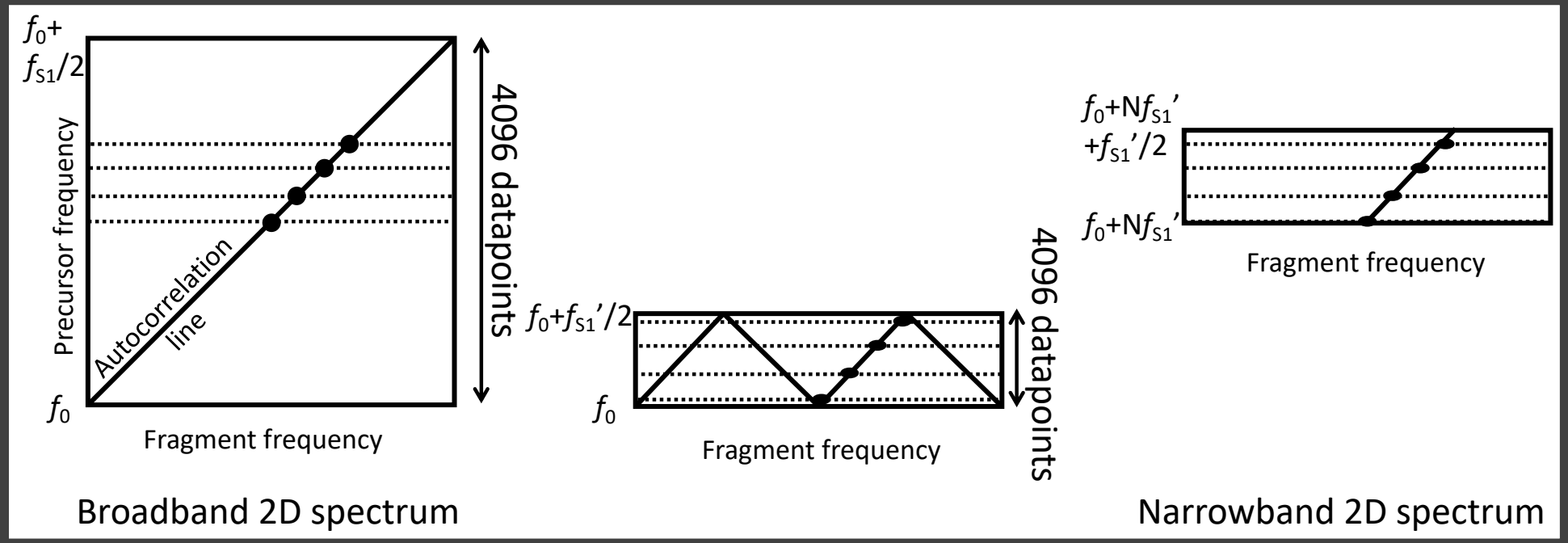
Narrowband 2D MS: Principle



Signals can be folded over at the borders of the spectrum!

M. van Agthoven et al., *Eur. Biophys. J.* 48 (2019) 213.

Narrowband 2D MS: Principle



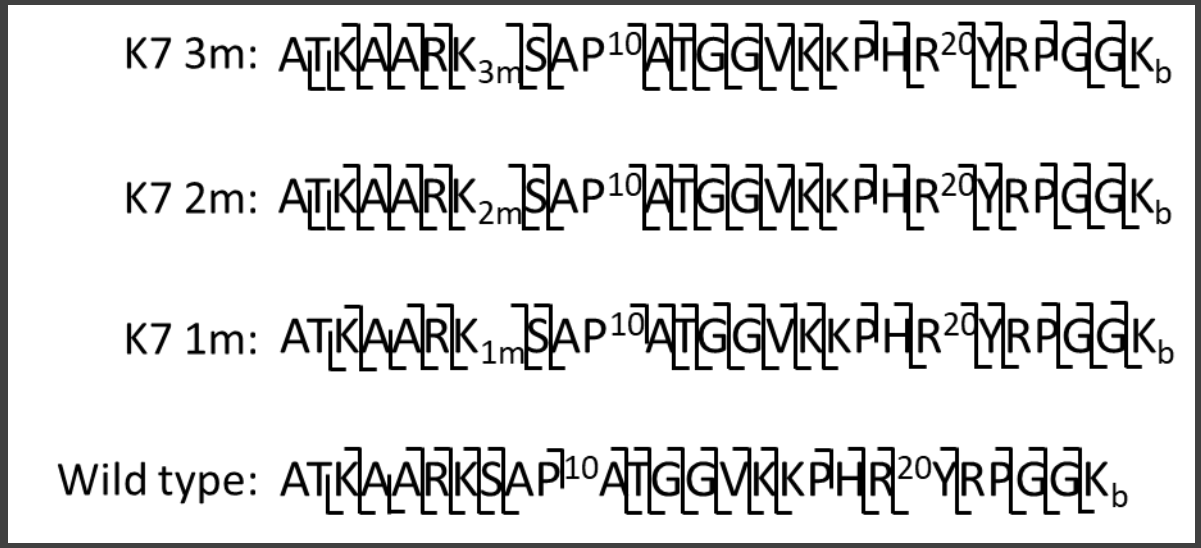
- Maximum frequency reduced to fold over autocorrelation line
- Same number of datapoints over smaller mass range
- Increase in resolving power/precursor-fragment correlation

M. Halper et al., *Anal Chem.* 92 (2020) 13945-13952.

Narrowband 2D MS: Experimental Conditions

- C-terminal GK-biotinylated histone H3 sequences (residues 21 to 44)
- Modified at K7 (1, 2, 3 methylations)
- Equimolar mixture

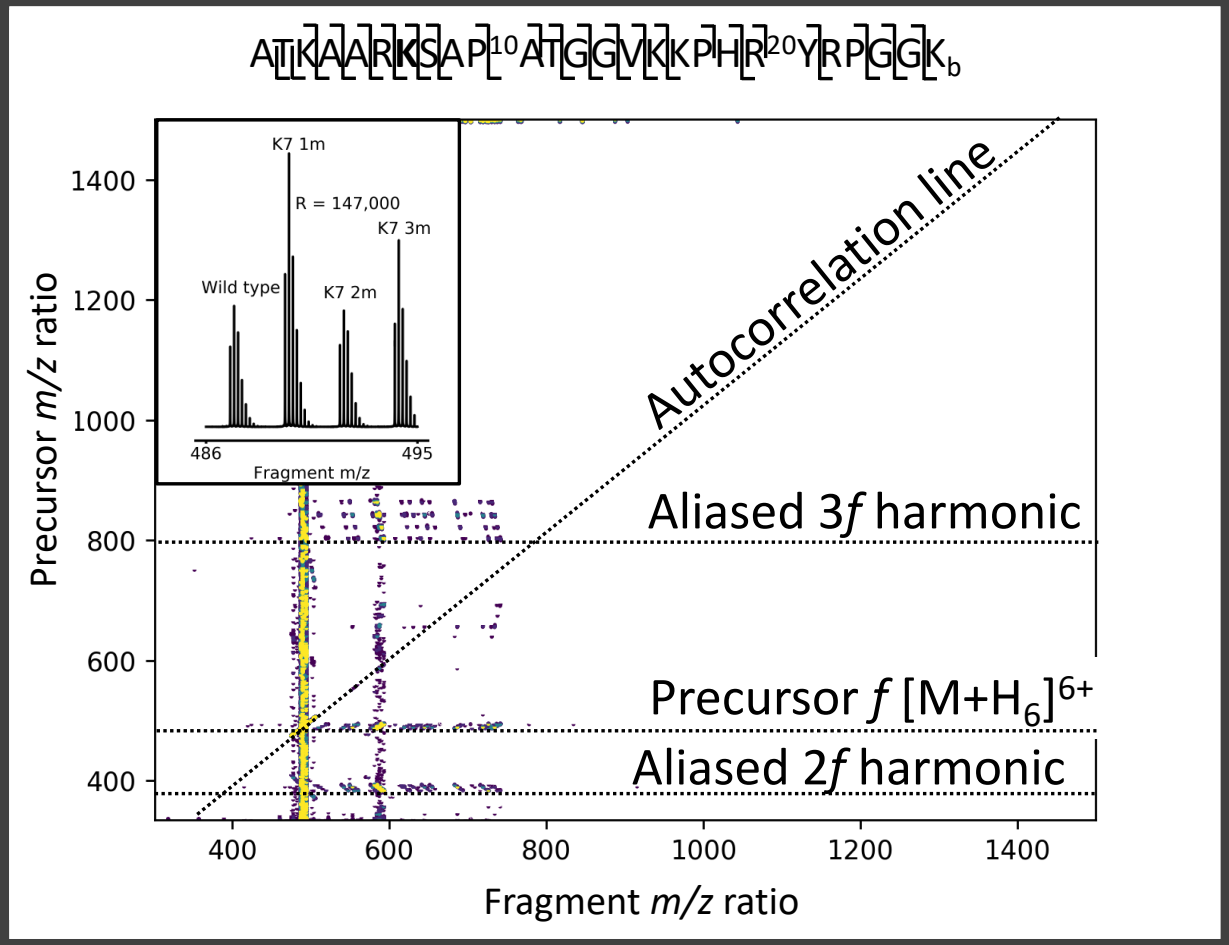
- 7 T ApexQE FT-ICR MS
- ECD fragmentation
- 2D MS maximum frequency: 250 kHz (broadband) and 62.5 kHz (narrowband)
- 2 x foldover in narrowband



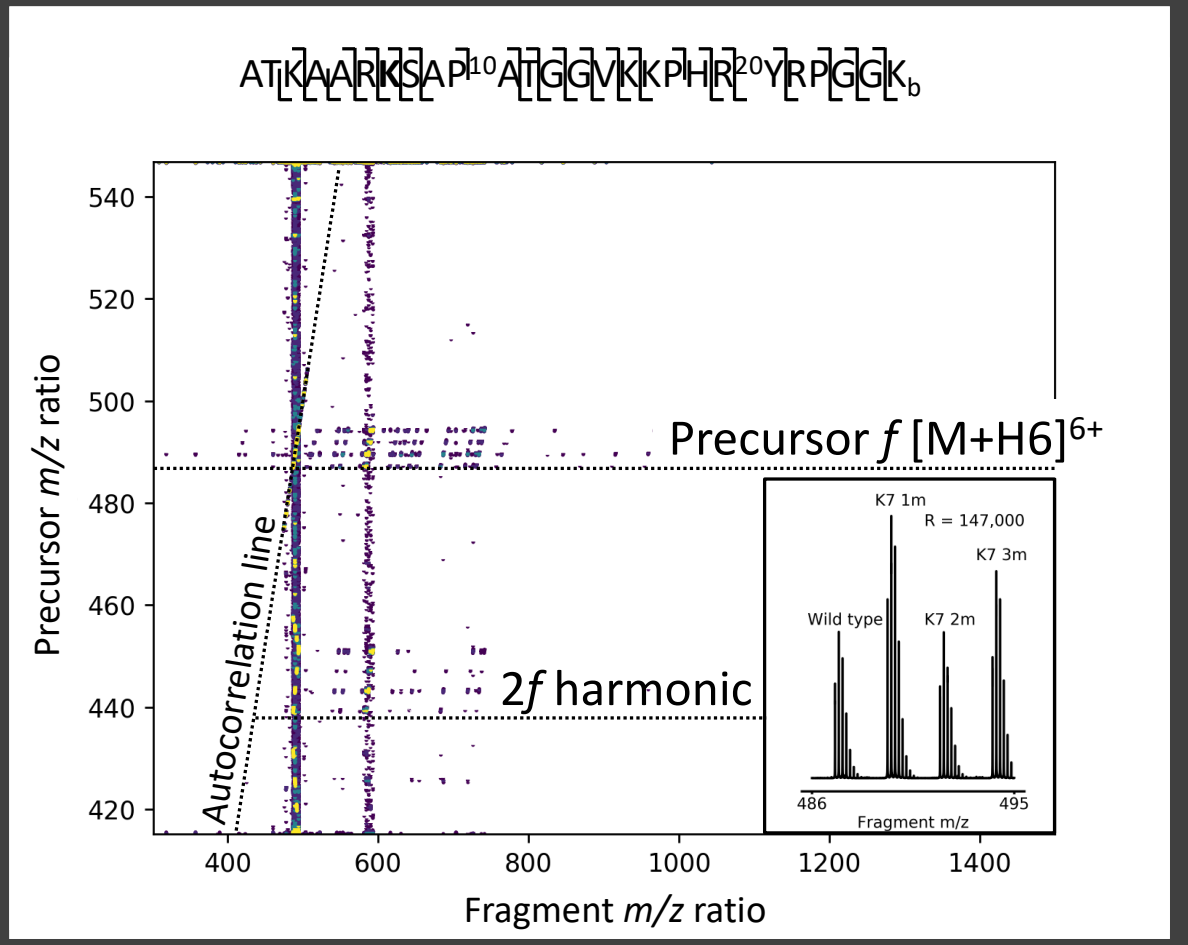
M. Halper et al., *Anal Chem.* 92 (2020) 13945-13952.

Broadband vs. Narrowband 2D MS

Broadband 2D mass spectrum

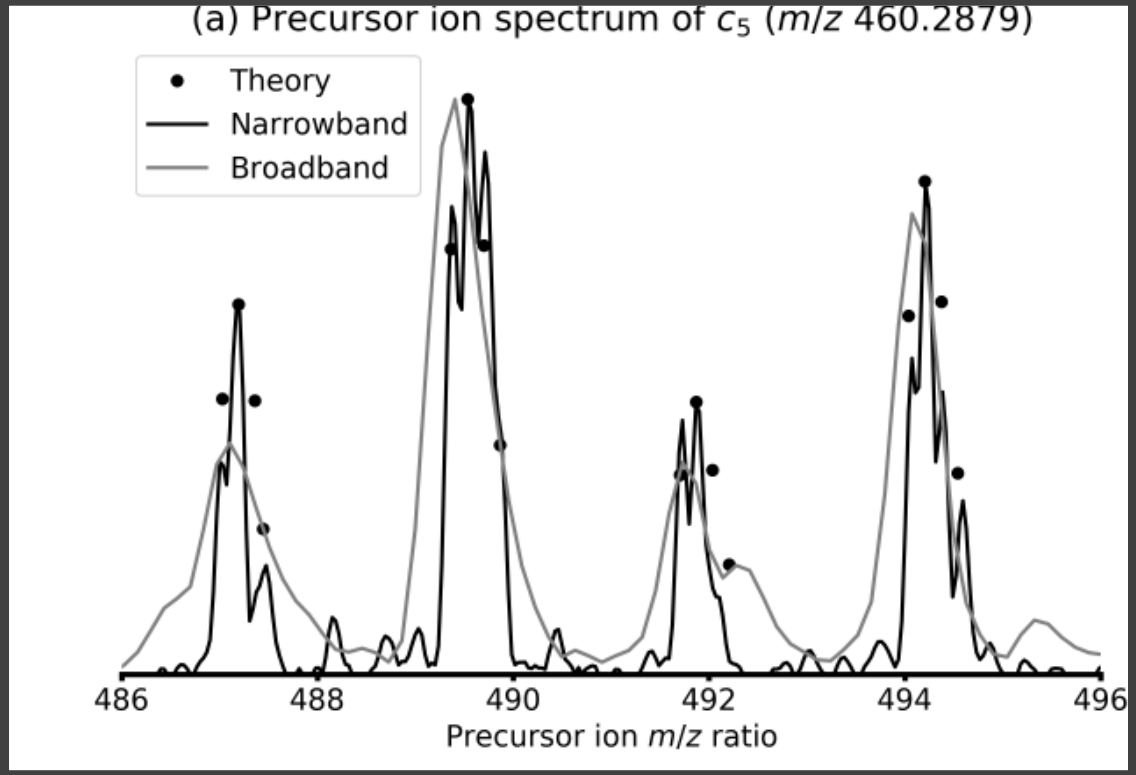


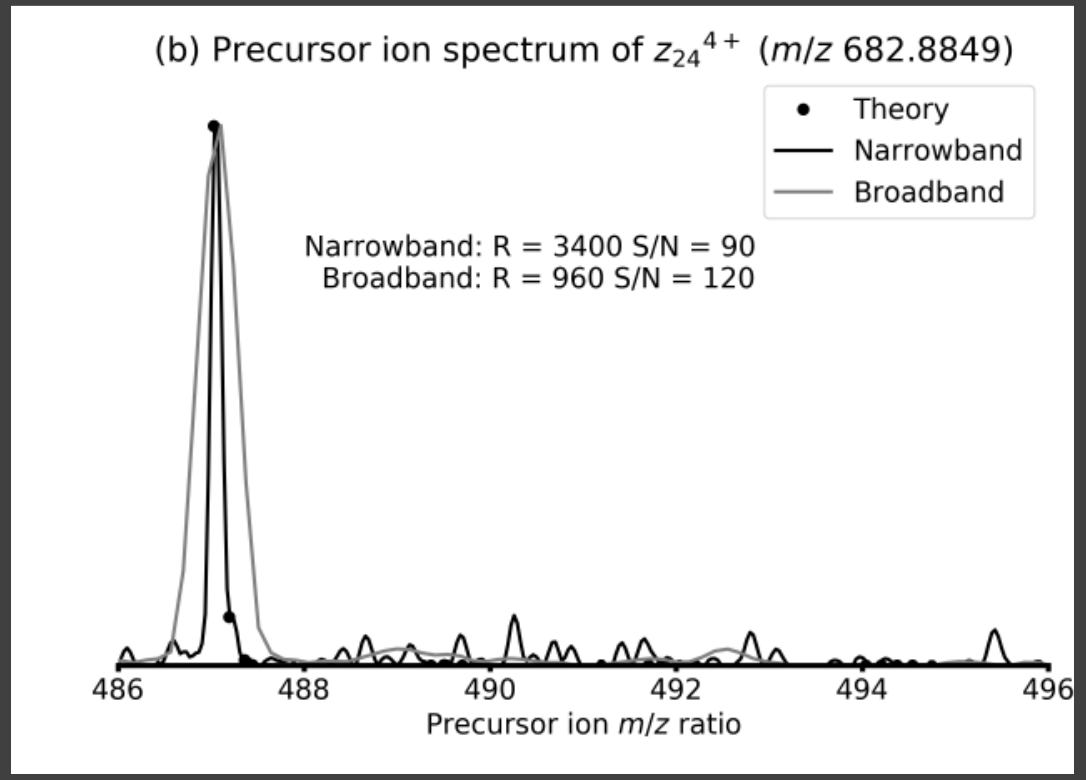
Narrowband 2D mass spectrum



M. Halper et al., *Anal Chem.* 92 (2020) 13945-13952.

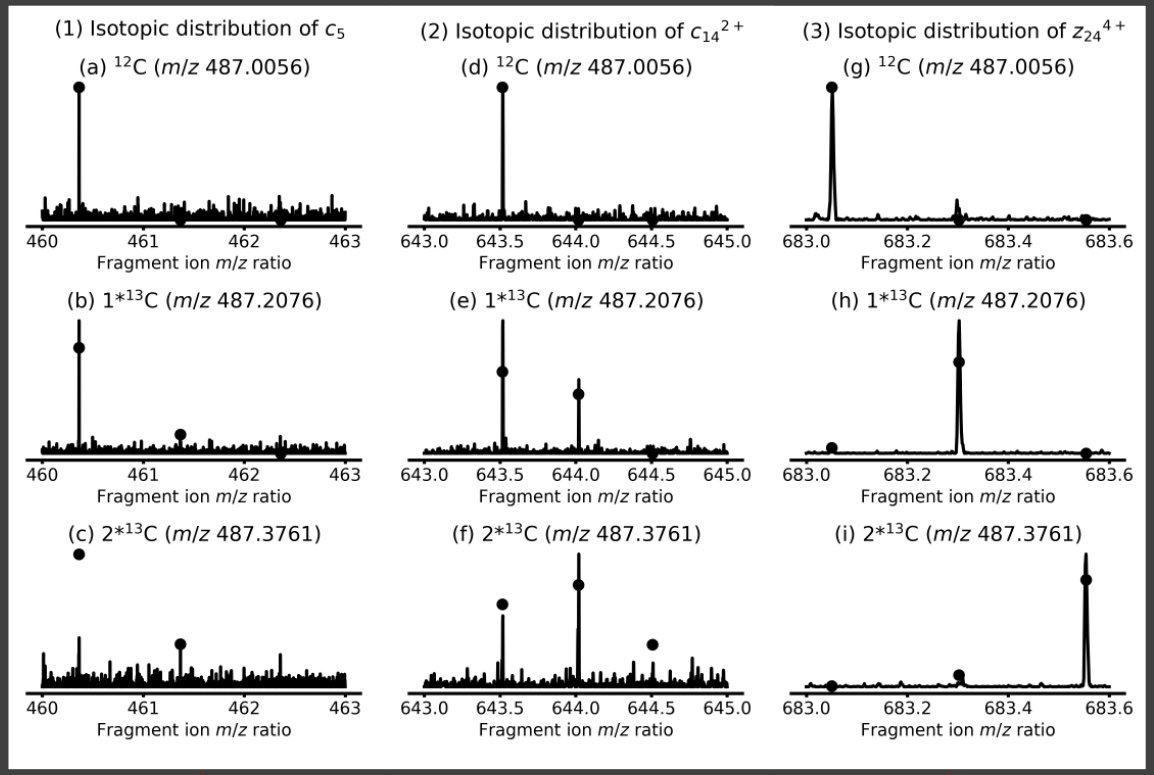
Broadband vs. Narrowband 2D MS





M. Halper et al., *Anal Chem.* 92 (2020) 13945-13952.

Isotopic Distributions in Narrowband 2D Mass Spectrum



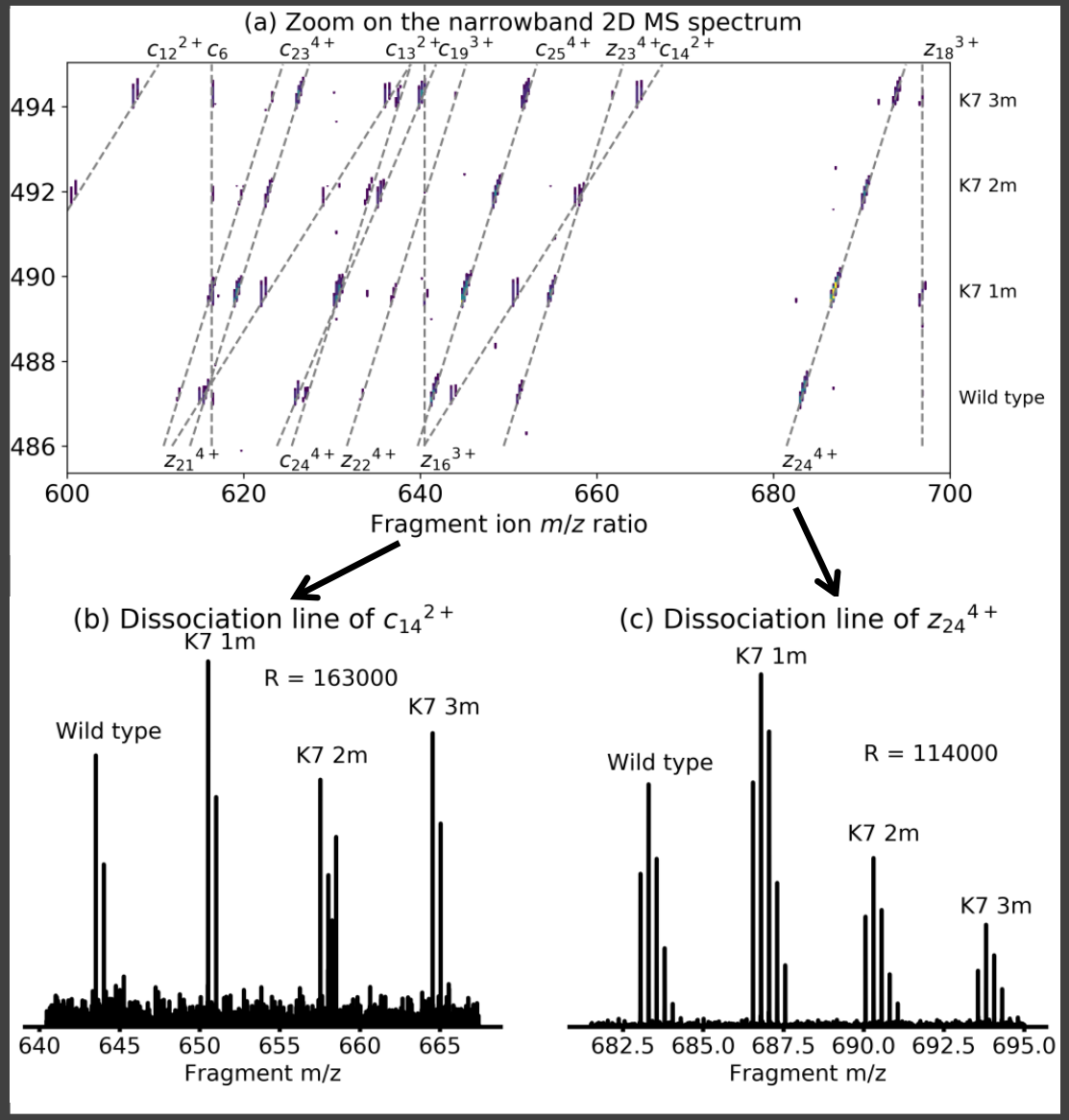
Small fragment: most ^{13}C isotopes in complement

Medium fragment: ^{13}C isotopes evenly distributed between fragment and complement

Big fragment: most ^{13}C isotopes in fragment

M. Halper et al., *Anal Chem.* 92 (2020) 13945-13952.

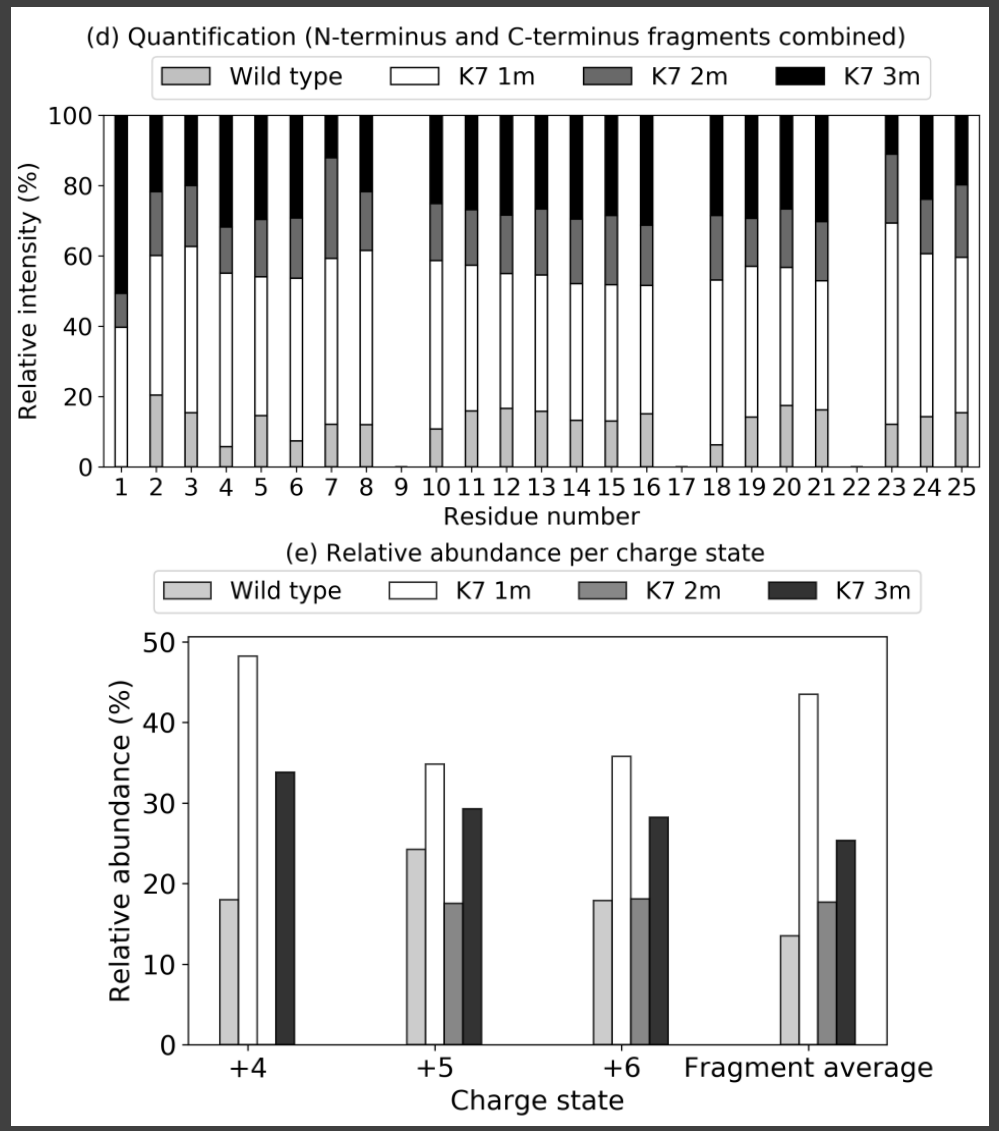
Narrowband 2D MS: Identification and Location of PTM



- Dissociation lines: slopes 0.33, 0.5, 0.67
  ⟹ confirm ID and location of PTM
- m/z difference: 14.0157 Da
  ⟹ methylations
- c_6 and z_{18}^{3+}: vertical precursor ion scans
  ⟹ PTMs on 7th residue

M. Halper et al., *Anal Chem.* 92 (2020) 13945-13952.

Narrowband 2D MS: Label-free relative quantification

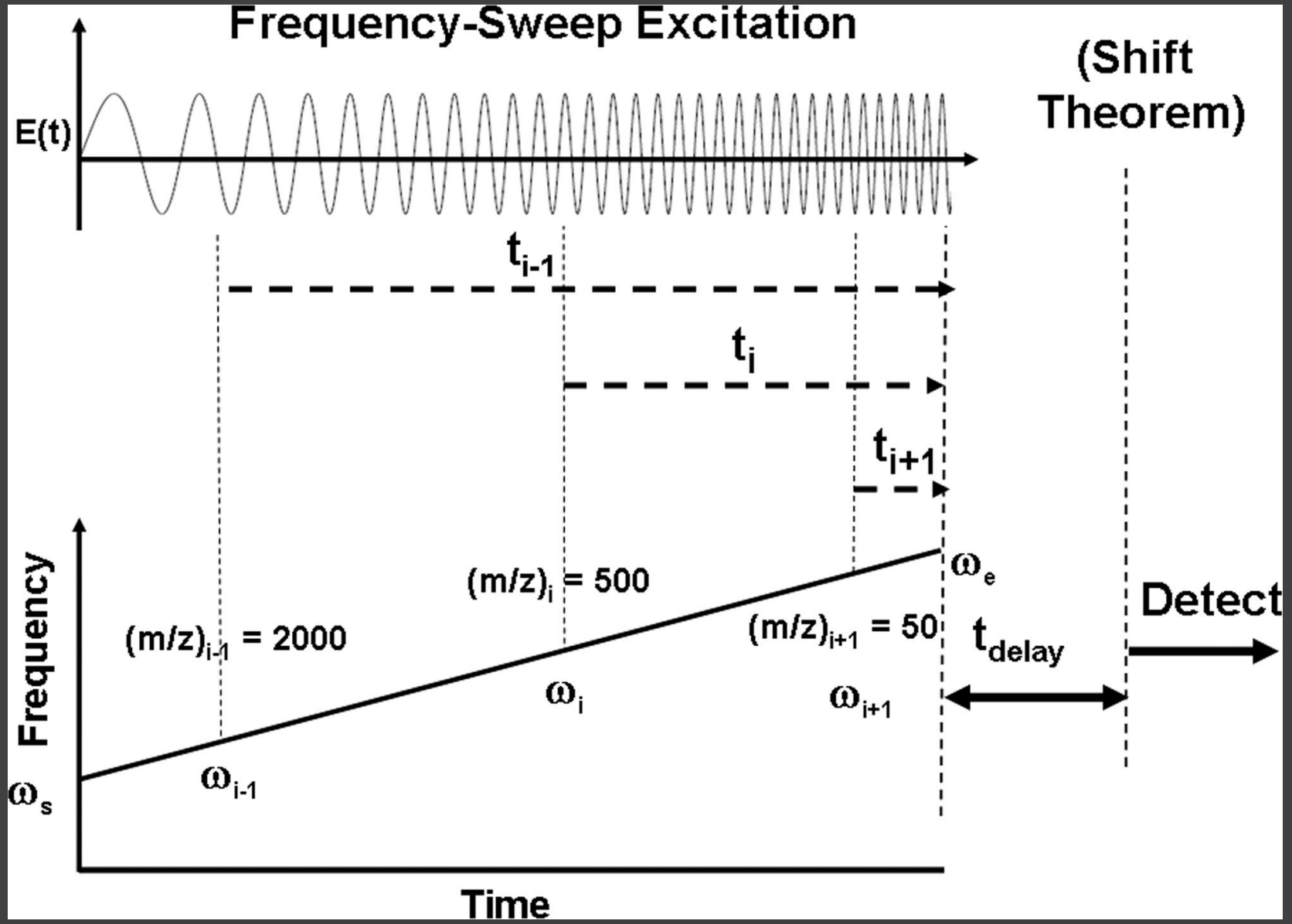


- PTM location achieved visually
  ⟹ relative intensities plotted without distinguishing fragment *m/z*

- Comparable results from intensities of precursor ions, charge-reduced species, and fragment ions
  ⟹ 2D MS can be used for label-free relative quantification

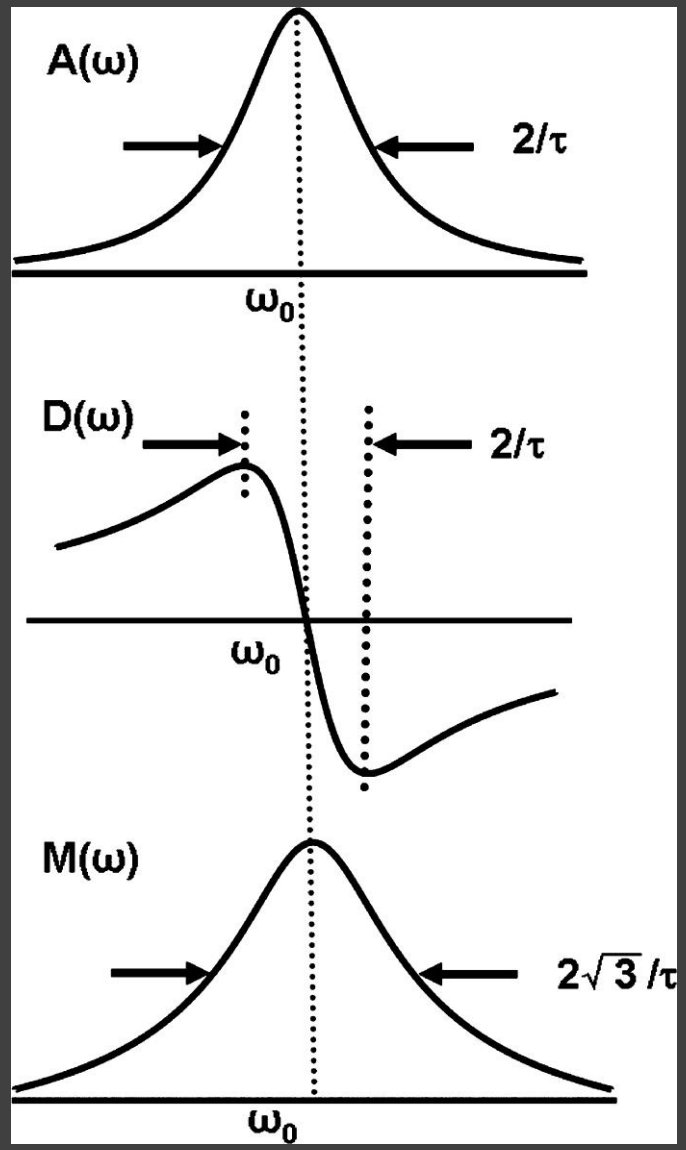
M. Halper et al., *Anal Chem.* 92 (2020) 13945-13952.

Phase correction in 1D FT-ICR MS



Feng Xian et al., *Anal Chem.* 82 (2010) 8807-8812.

Phase correction in 1D FT-ICR MS



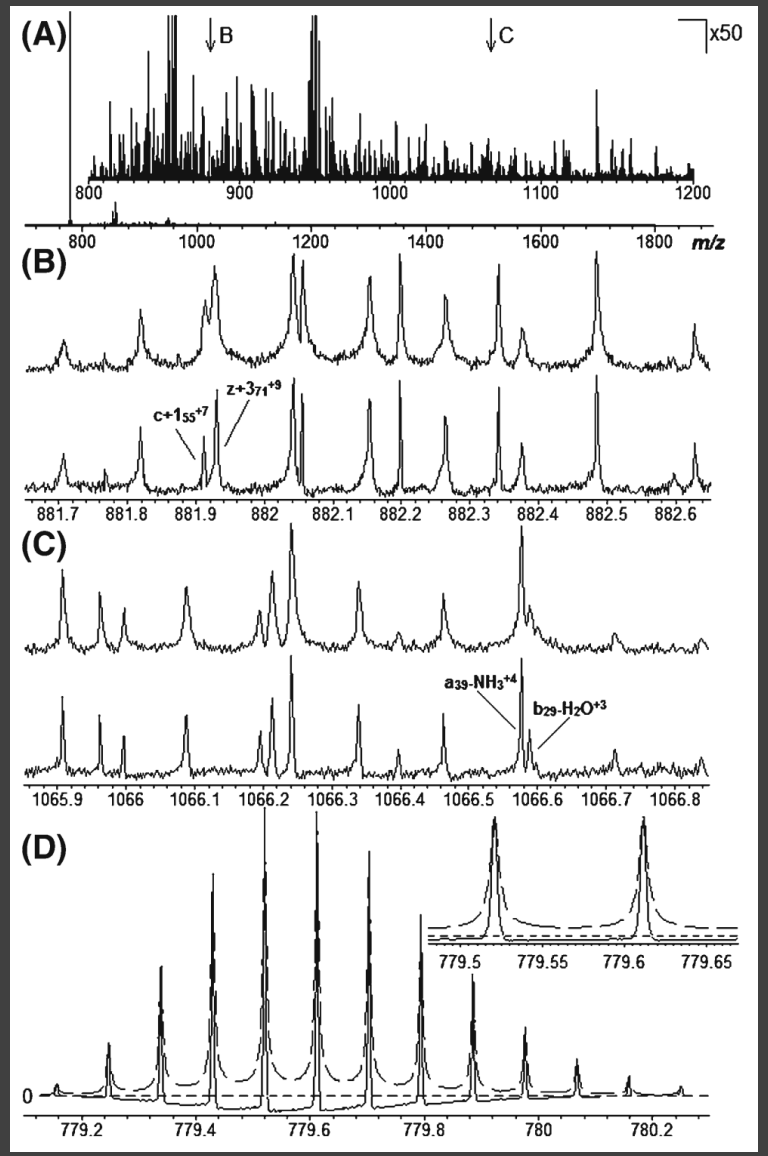
Absorption mode:
Gain in S/N and resolving power
Need to know phase of peak

Dispersion mode

Magnitude mode:
No need to know phase of peak

Feng Xian et al., *Anal Chem.* 82 (2010) 8807-8812.

Phase correction in 1D FT-ICR MS



ECD MS/MS of ubiquitin

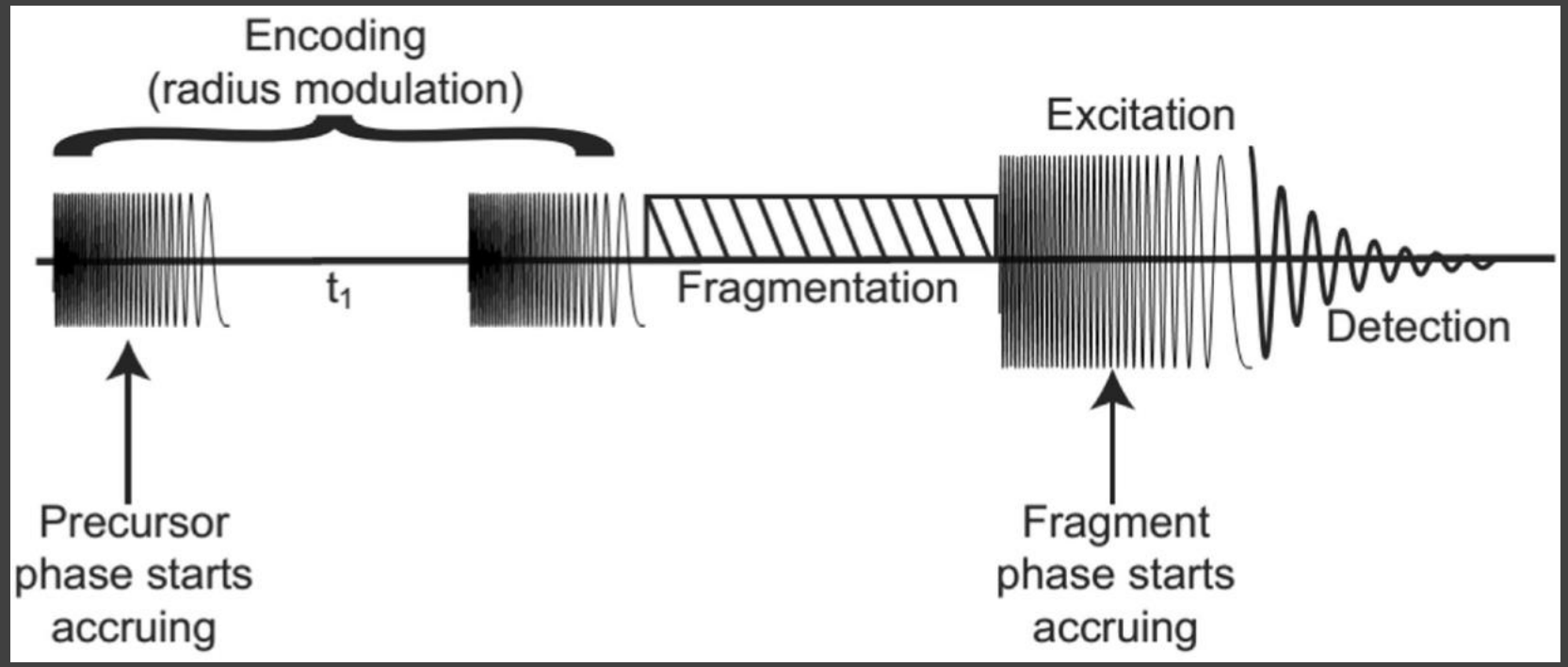
Zoom on *m/z* 882

Zoom on *m/z* 1066

Zoom on precursor ion

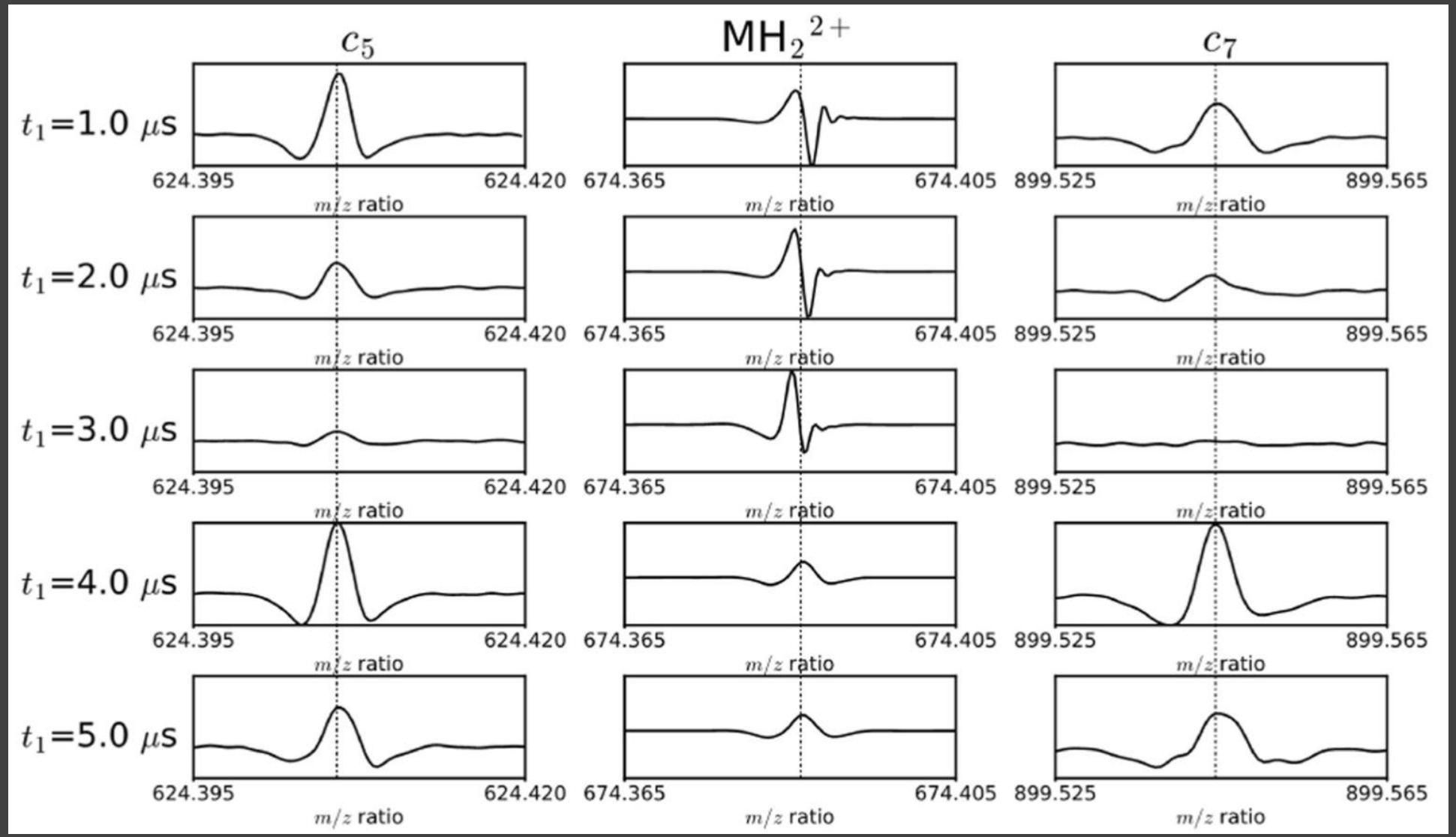
Yulin Qi et al., *J. Am. Soc. Mass Spectrom.* 22 (2011) 138-147.

Phase correction in 2D FT-ICR MS



M. van Agthoven et al., *J. Am. Soc. Mass Spectrom.* 30 (2019) 2594-2607.

Phase correction in 2D FT-ICR MS



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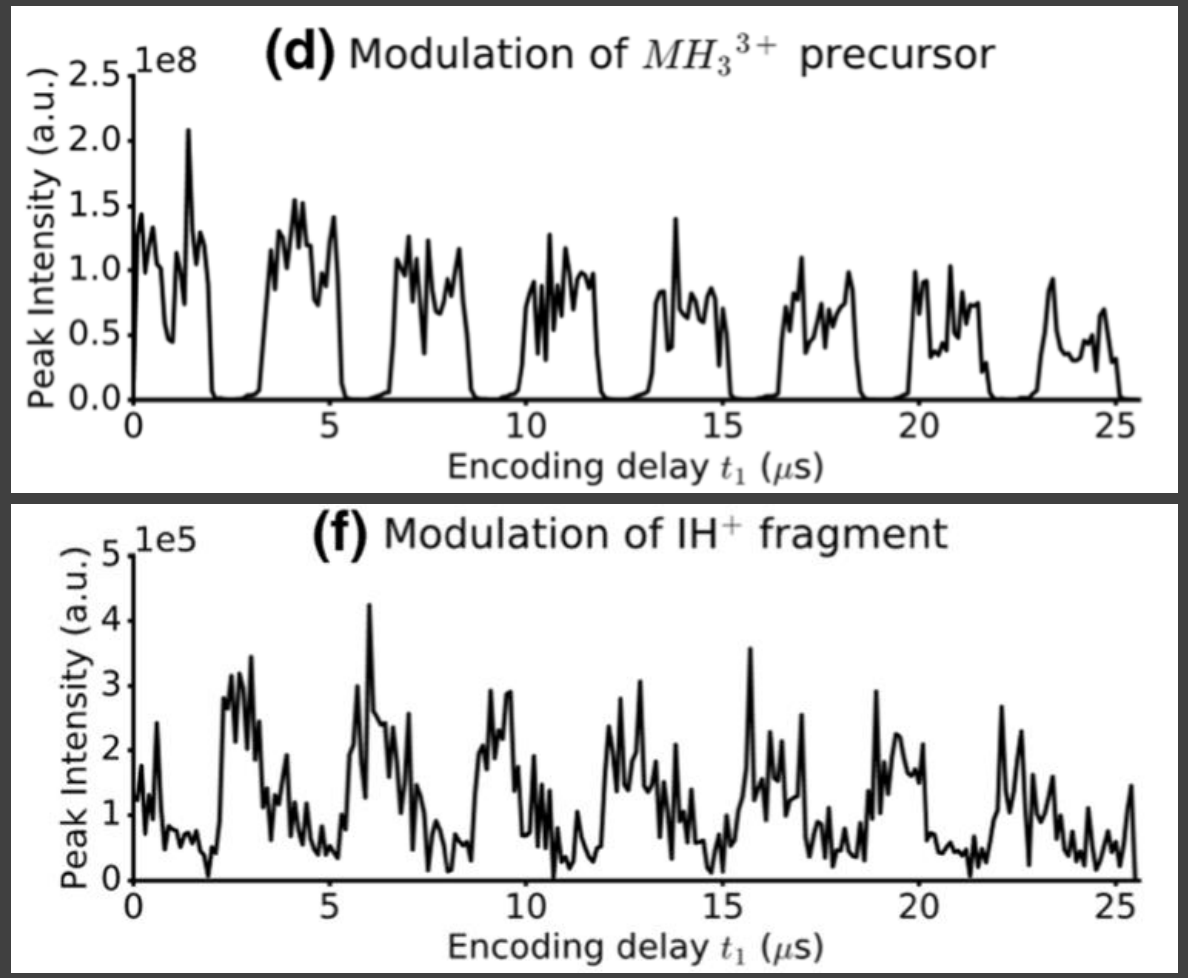


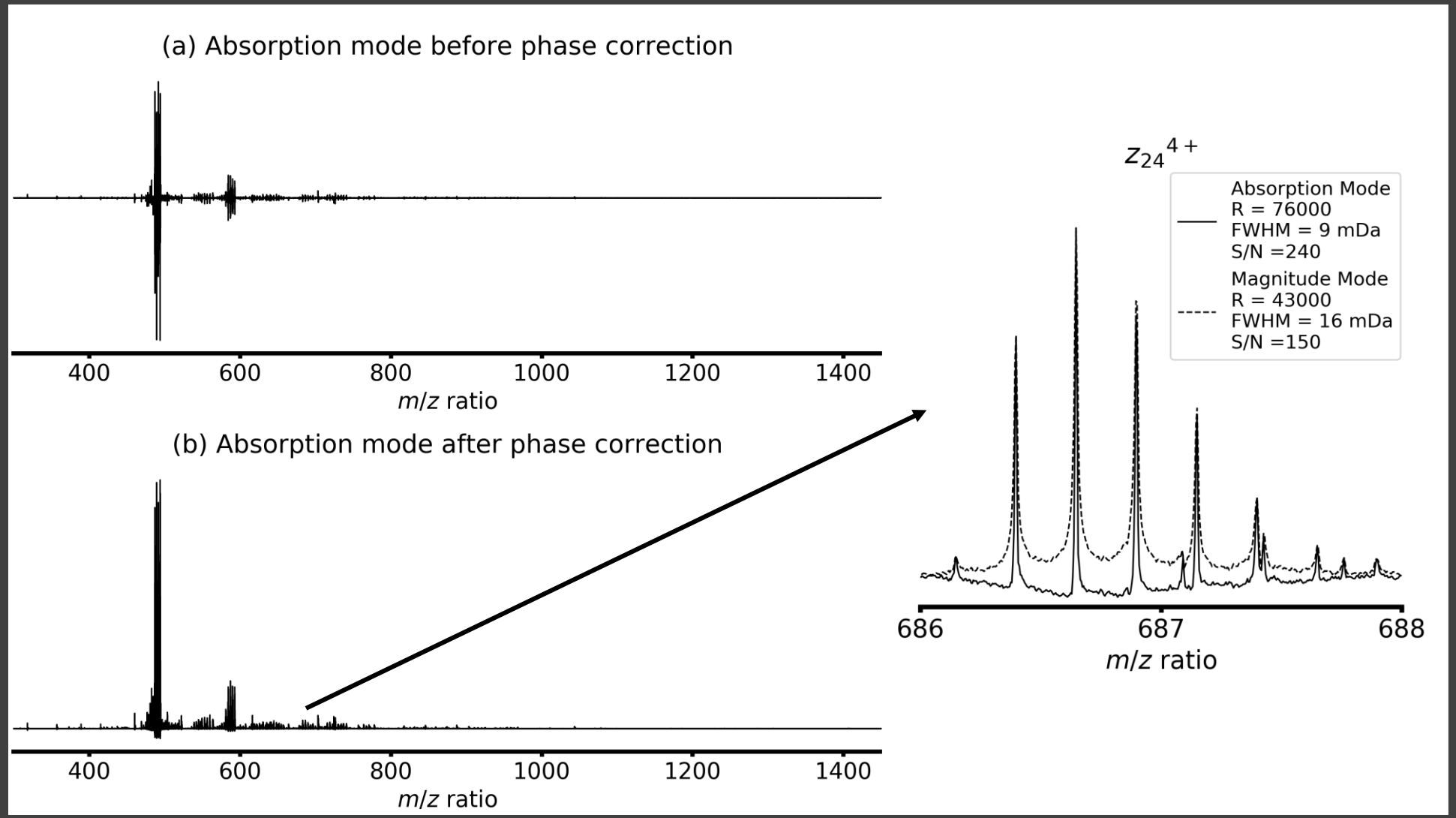
M. van Agthoven et al., *J. Am. Soc. Mass Spectrom.* 30 (2019) 2594-2607.

Phase correction in 2D FT-ICR MS

- Fragment ion (horizontal) dimension: quadratic phase correction
- Precursor ion (vertical) dimension: linear phase correction
- Resolving power expected x2
- Signal-to-noise expected x2

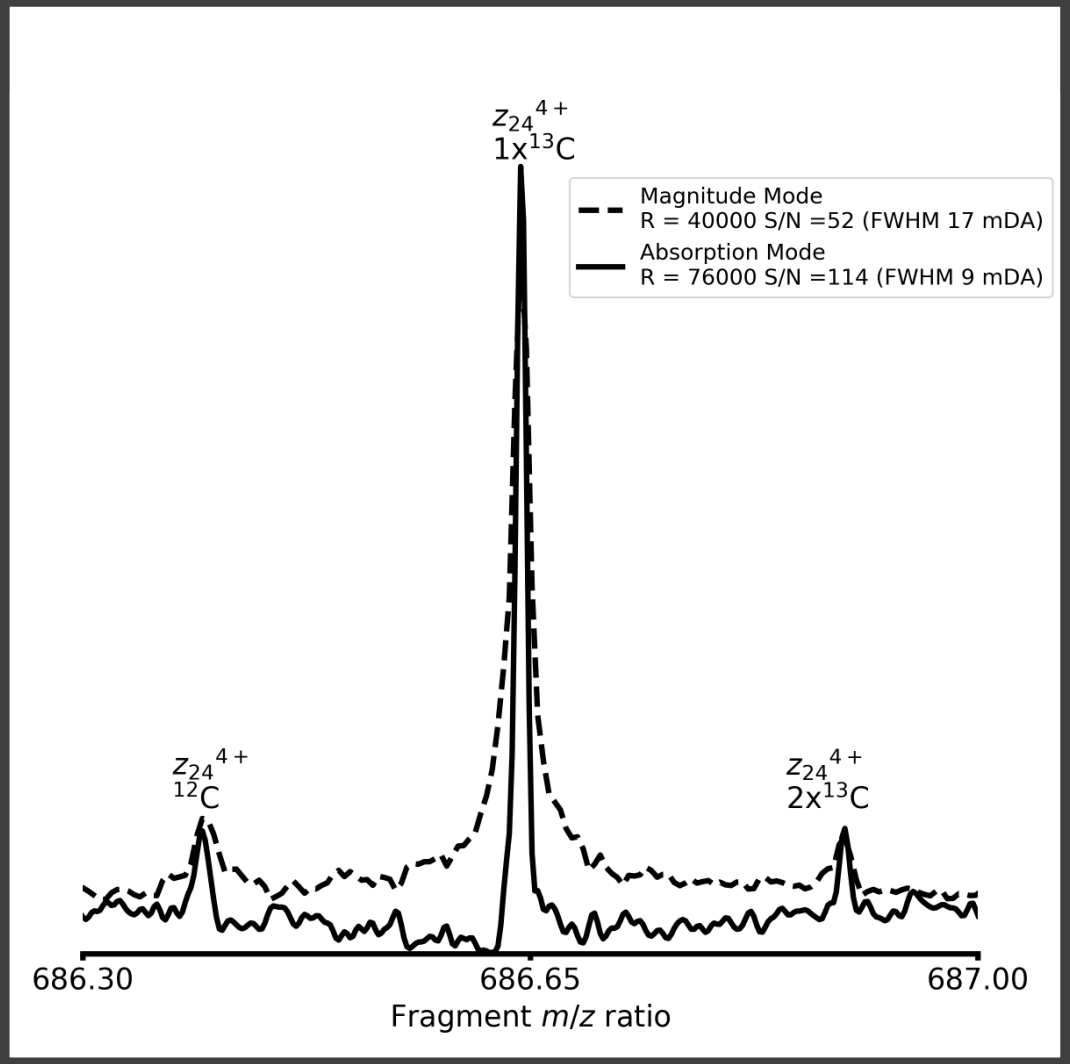
M. van Agthoven et al., *J. Am. Soc. Mass Spectrom.* 30 (2019) 2594-2607.

Phase correction in 2D FT-ICR MS:
Horizontal Phase correction Determined by Phasing MS/MS spectrum



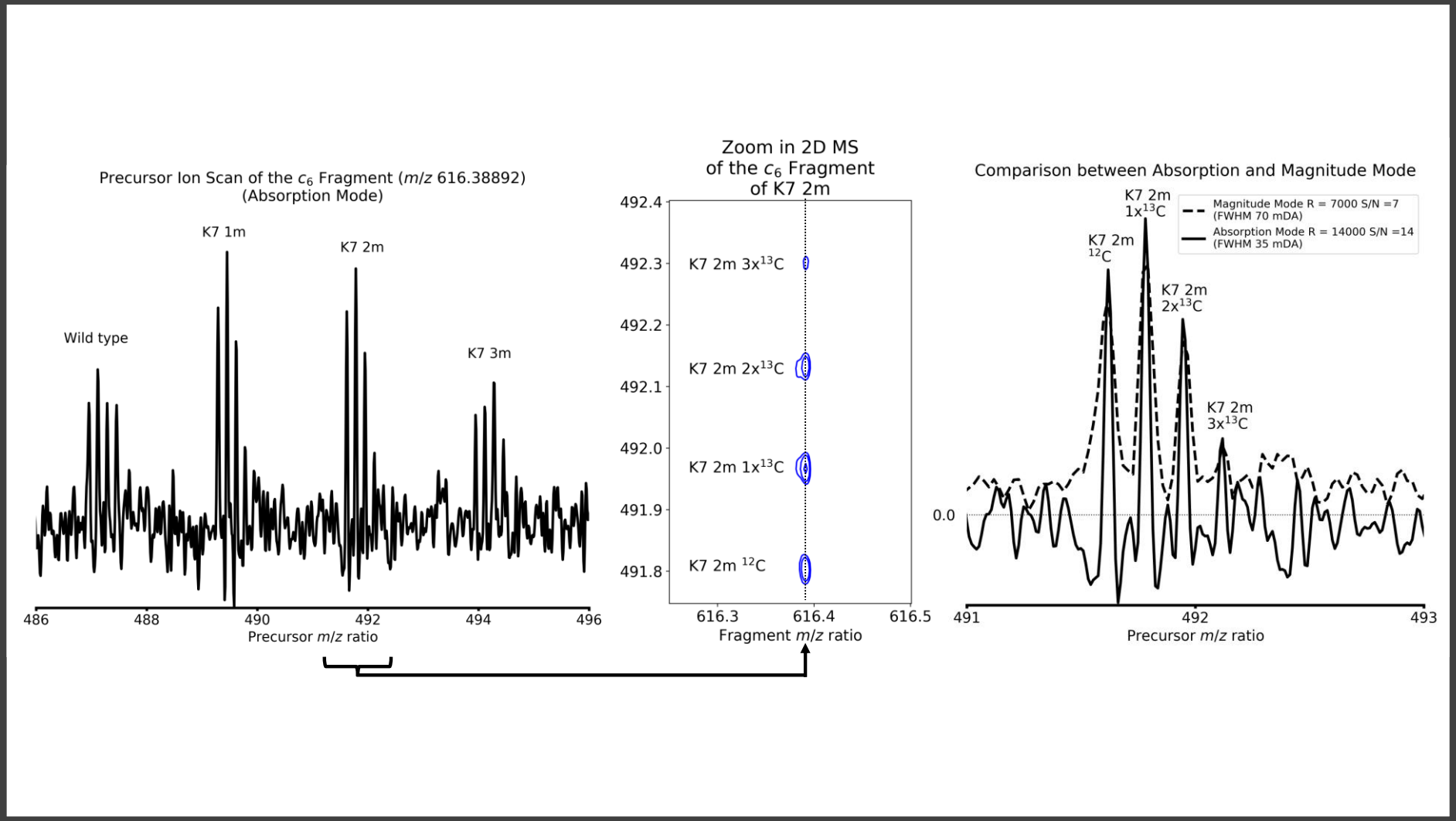
M.-A. Delsuc et al., *Molecules* 26 (2021) 3388.

Phase correction in 2D FT-ICR MS



M.-A. Delsuc et al., *Molecules* 26 (2021) 3388.

Phase correction in 2D FT-ICR MS: Vertical Linear Phase Correction



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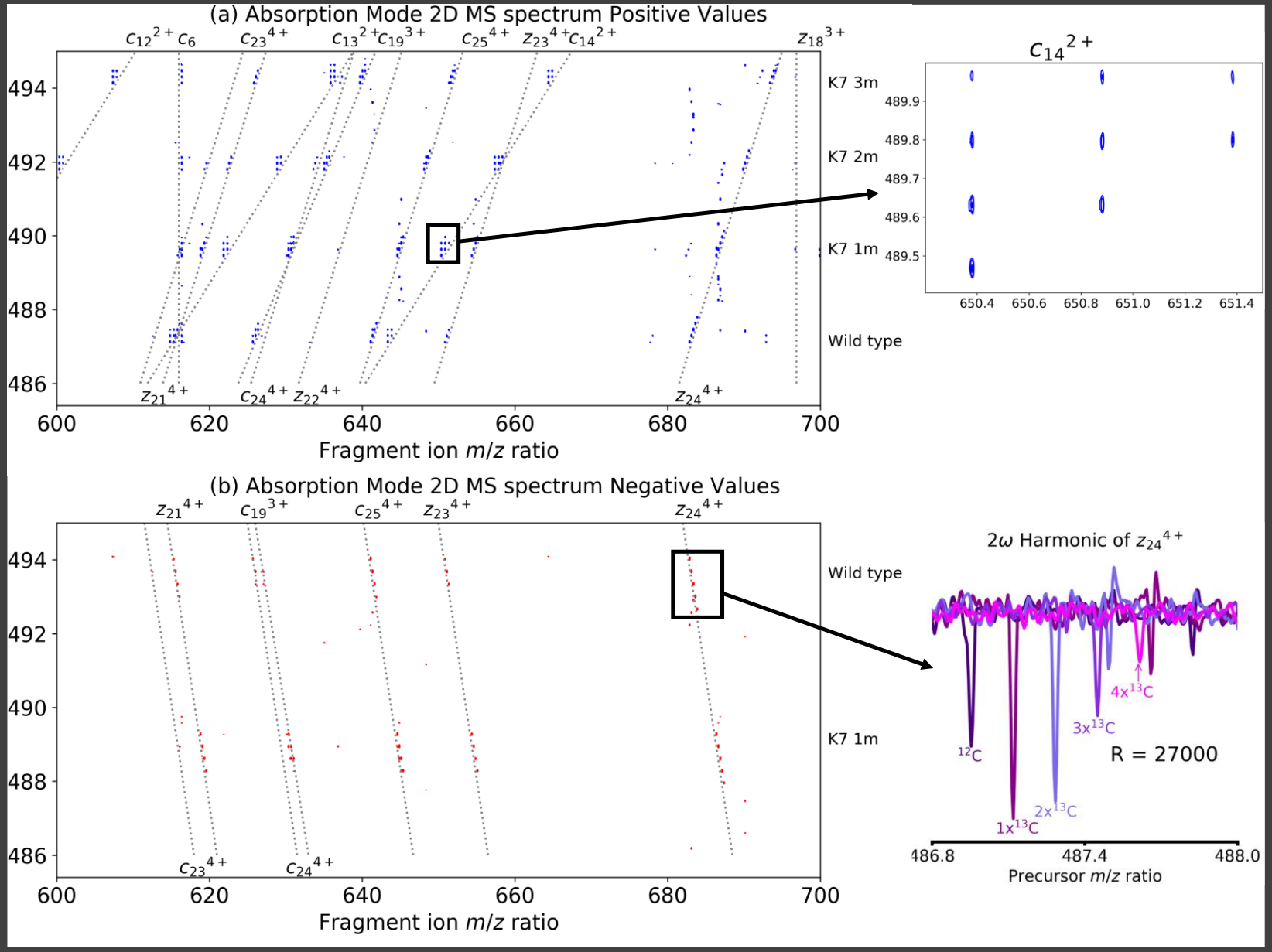
Phase correction in 2D FT-ICR MS



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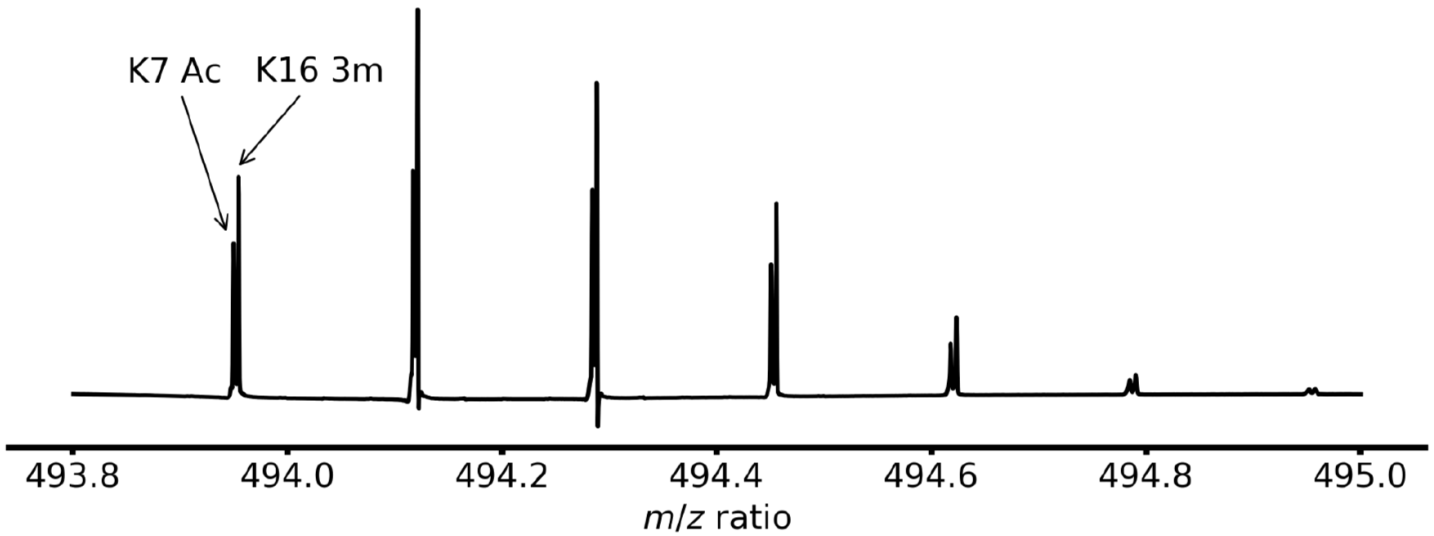
Can we separate a trimethylated and an acetylated histone peptide?



m/z(K7 Ac)	493.944715	f_{ICR} = 219173.83 Hz
m/z(K16 3m)	493.950779	f_{ICR} = 219171.14 Hz
$\Delta m/z$	0.006064	Δf_{ICR}= 2.69 Hz

ATKAARK_{Ac}SAP^{10} ATGGVKK PHR^{20} YRPGGK$_{biotin}$

ATKAARK SAP^{10} ATGGVKK_{3m}PHR^{20} YRPGGK$_{biotin}$

$c_{1\rightarrow6}$: Δm = 0	$c_{7\rightarrow15}$: Δm = 42.010564	$c_{16\rightarrow25}$: Δm = 0.0364
$z_{20\rightarrow25}$: Δm = -0.0364	$z_{11\rightarrow19}$: Δm = -42.046950	$z_{1\rightarrow10}$: Δm = 0

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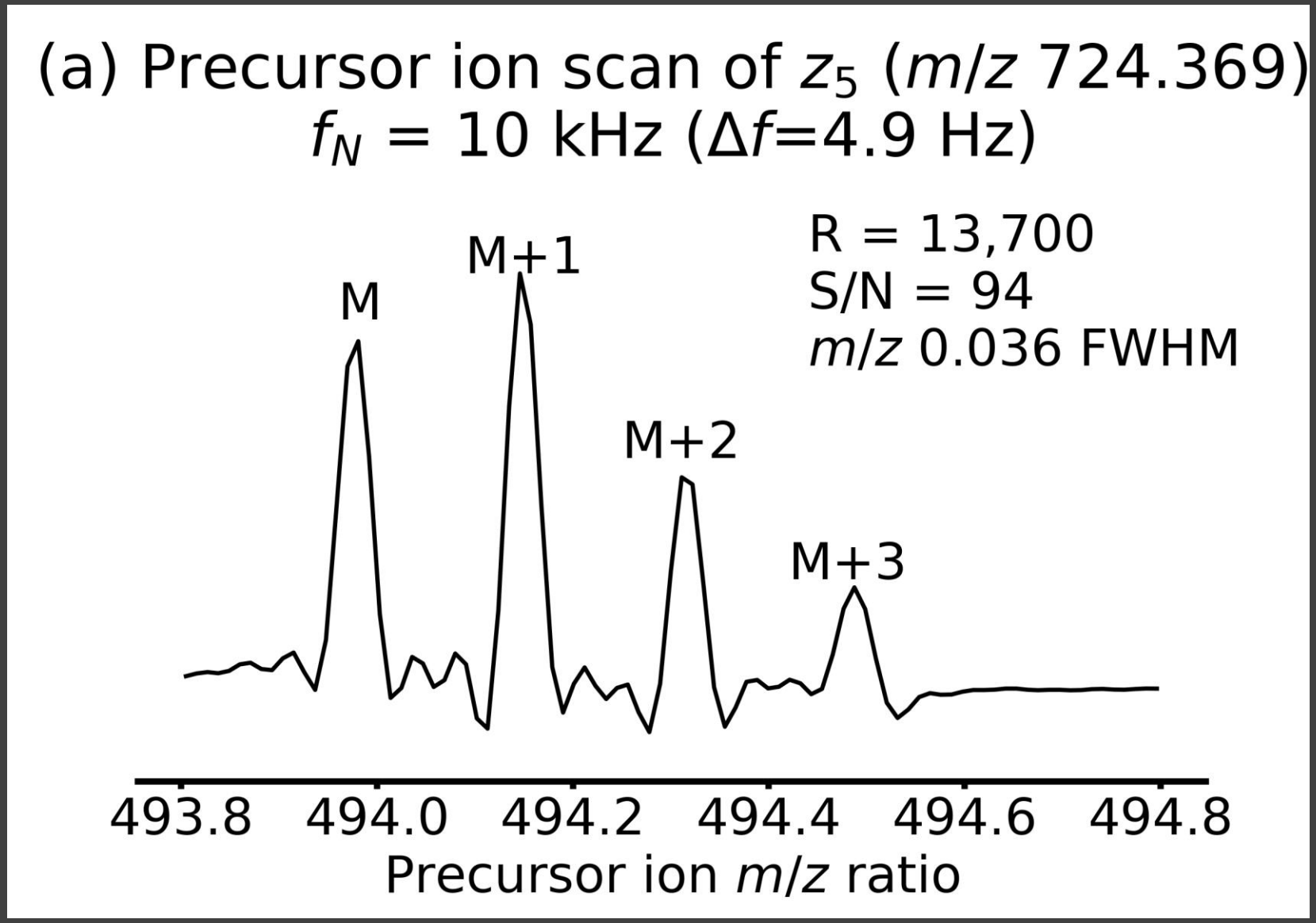

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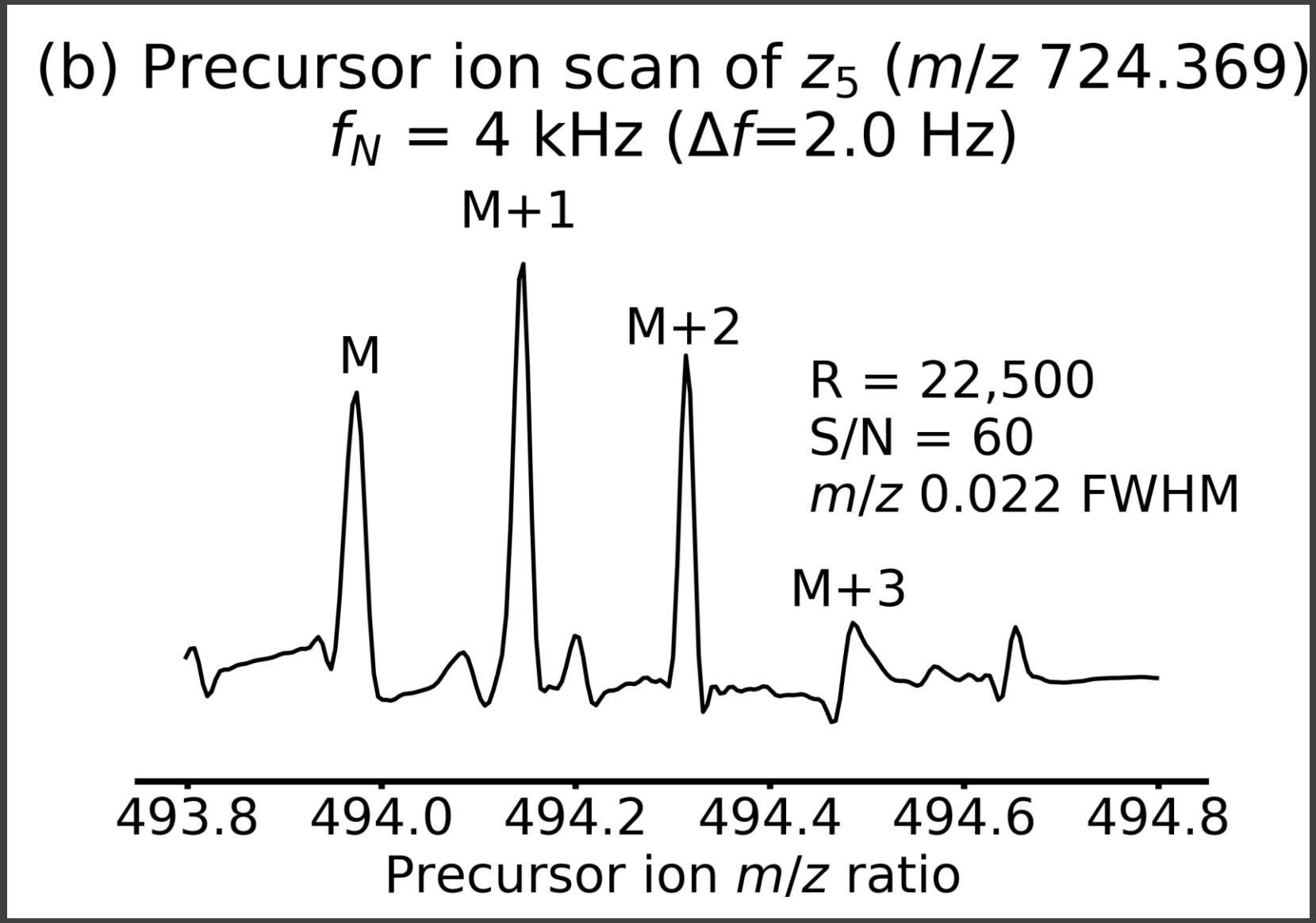

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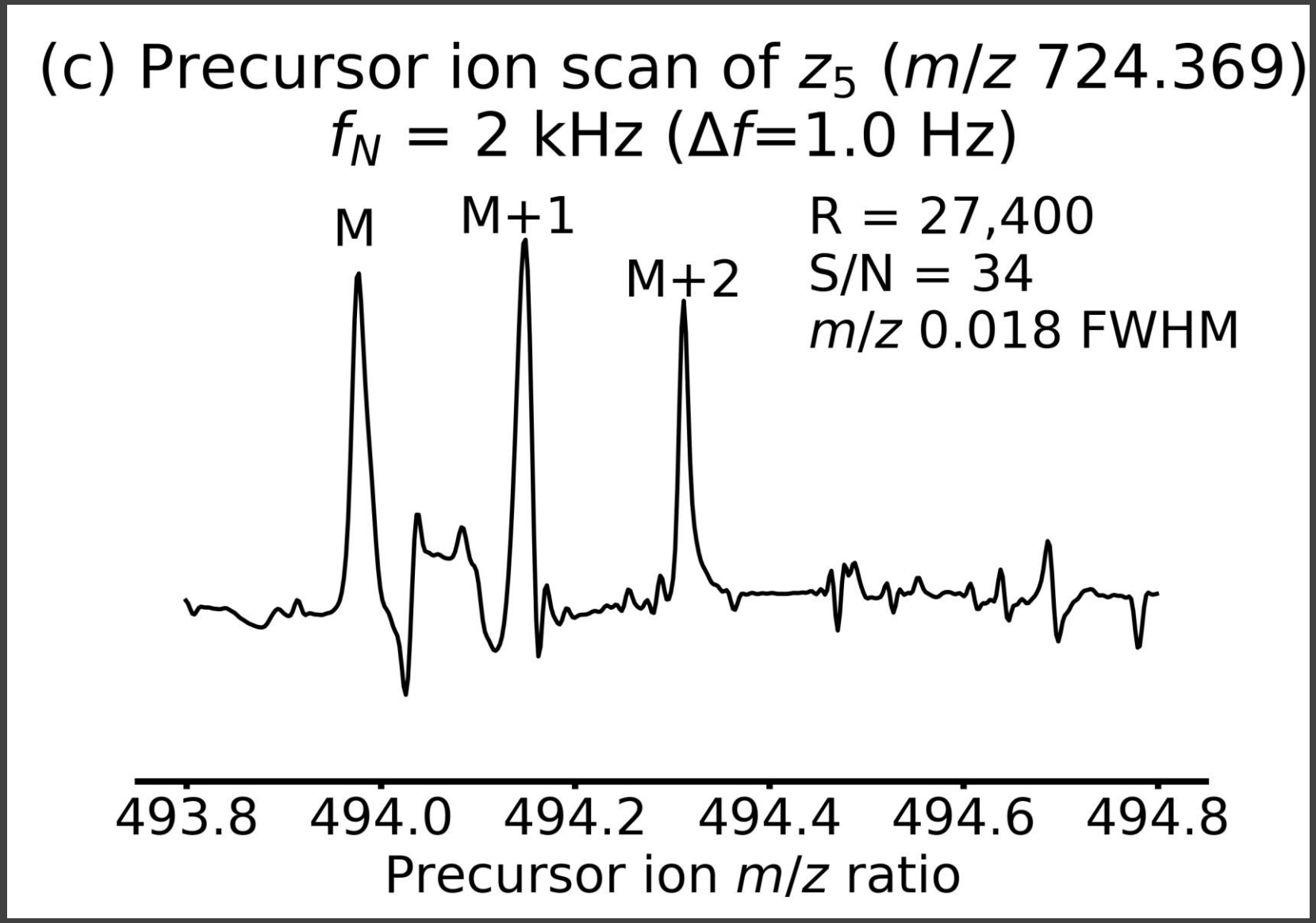

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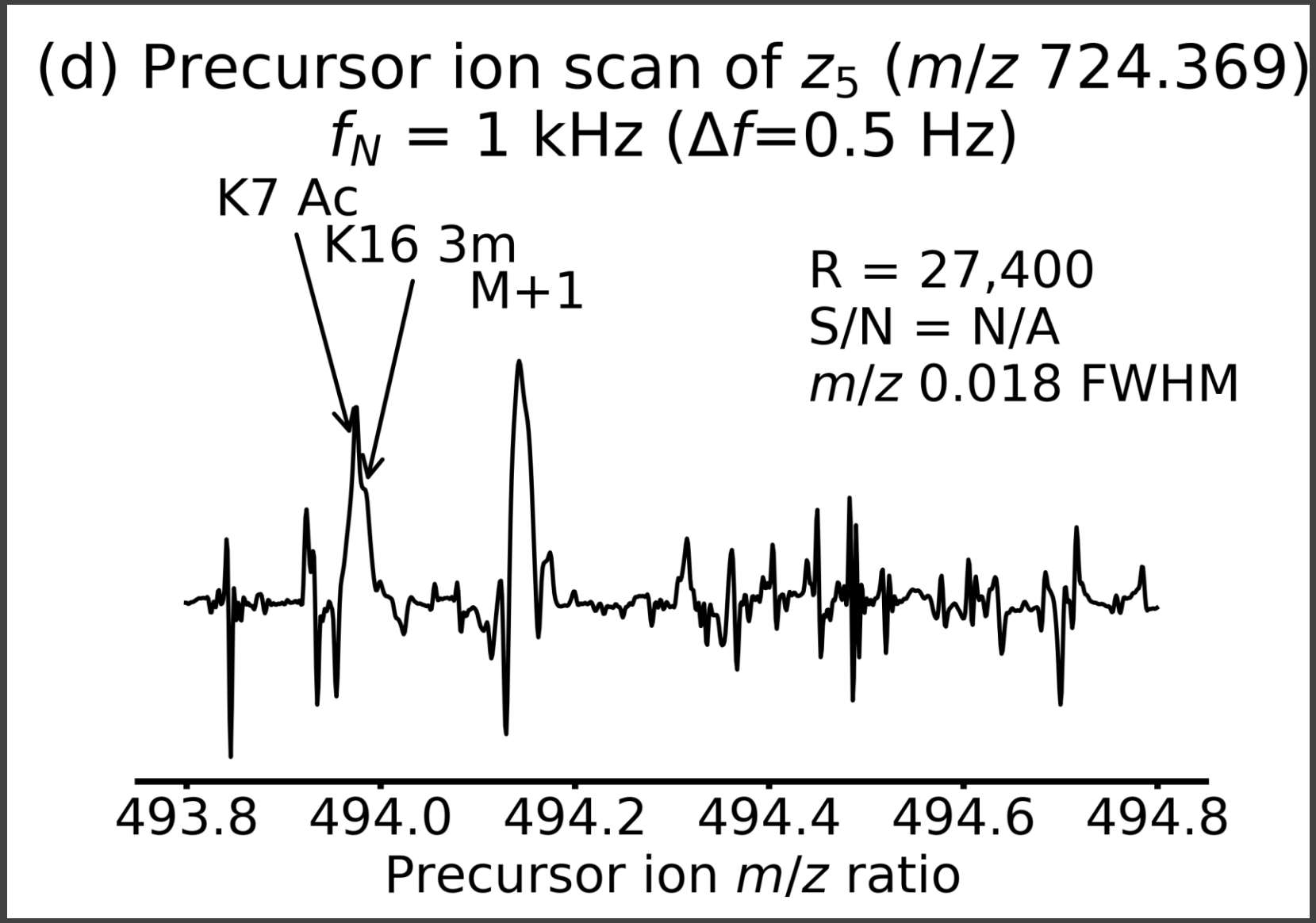

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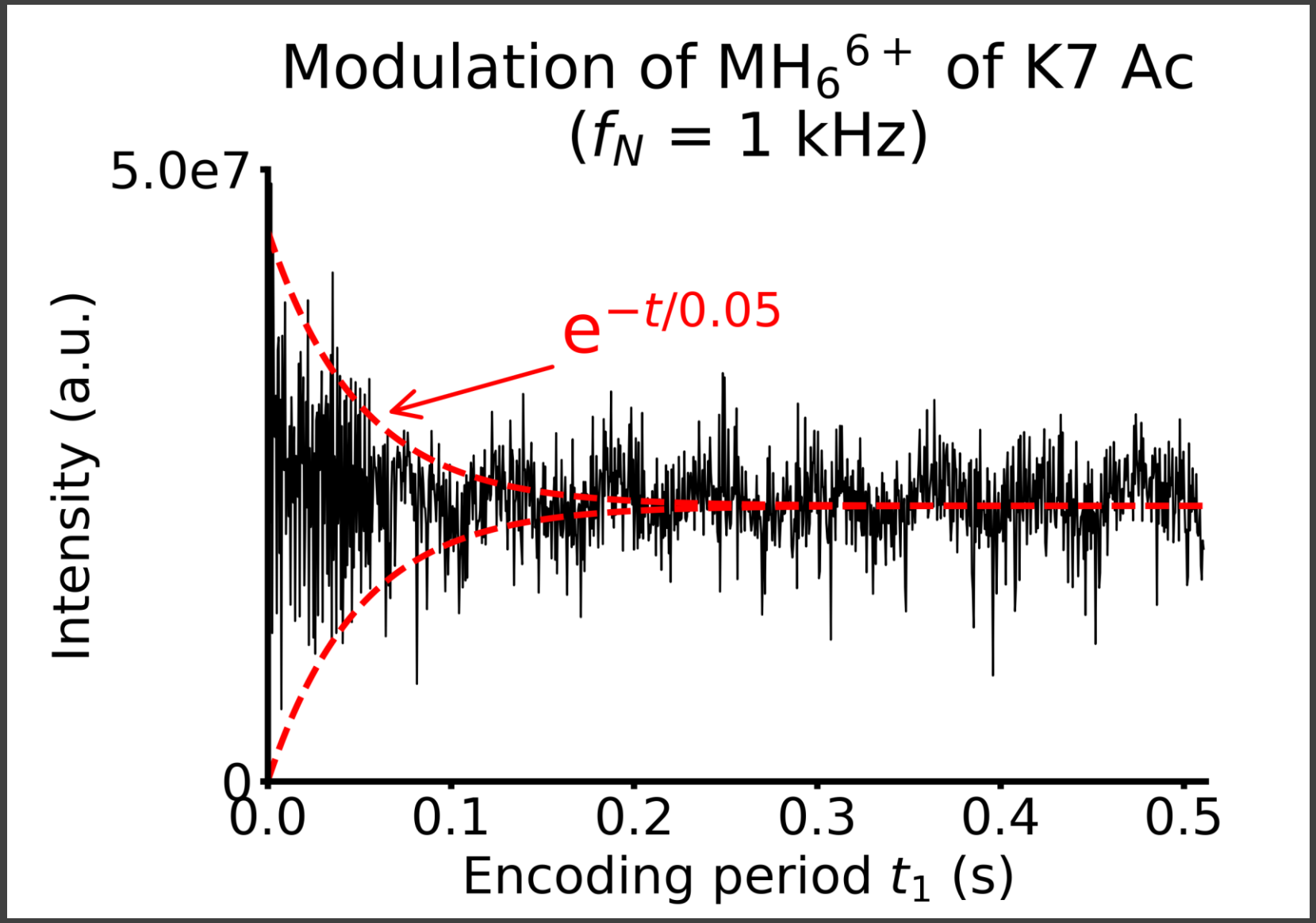

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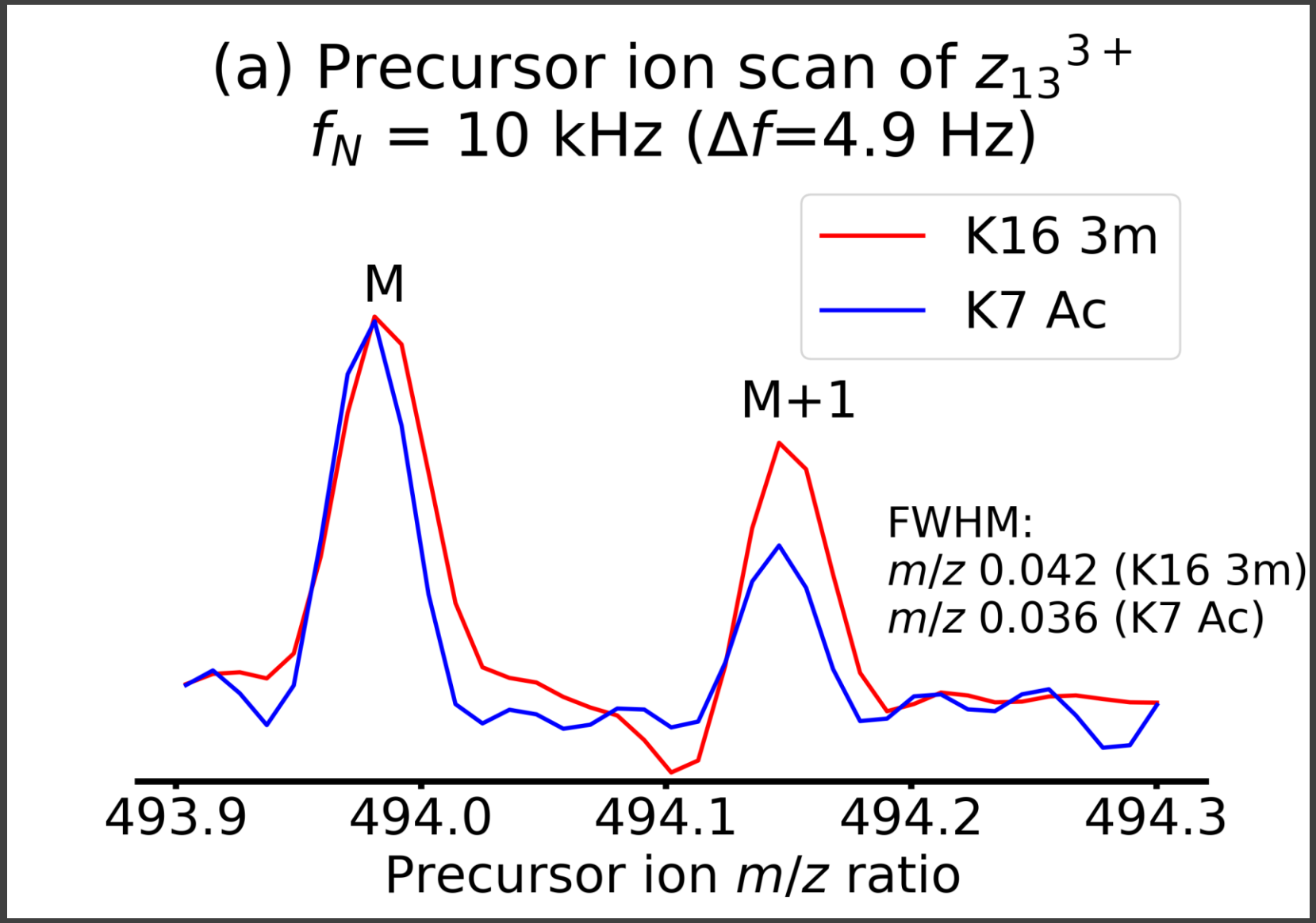

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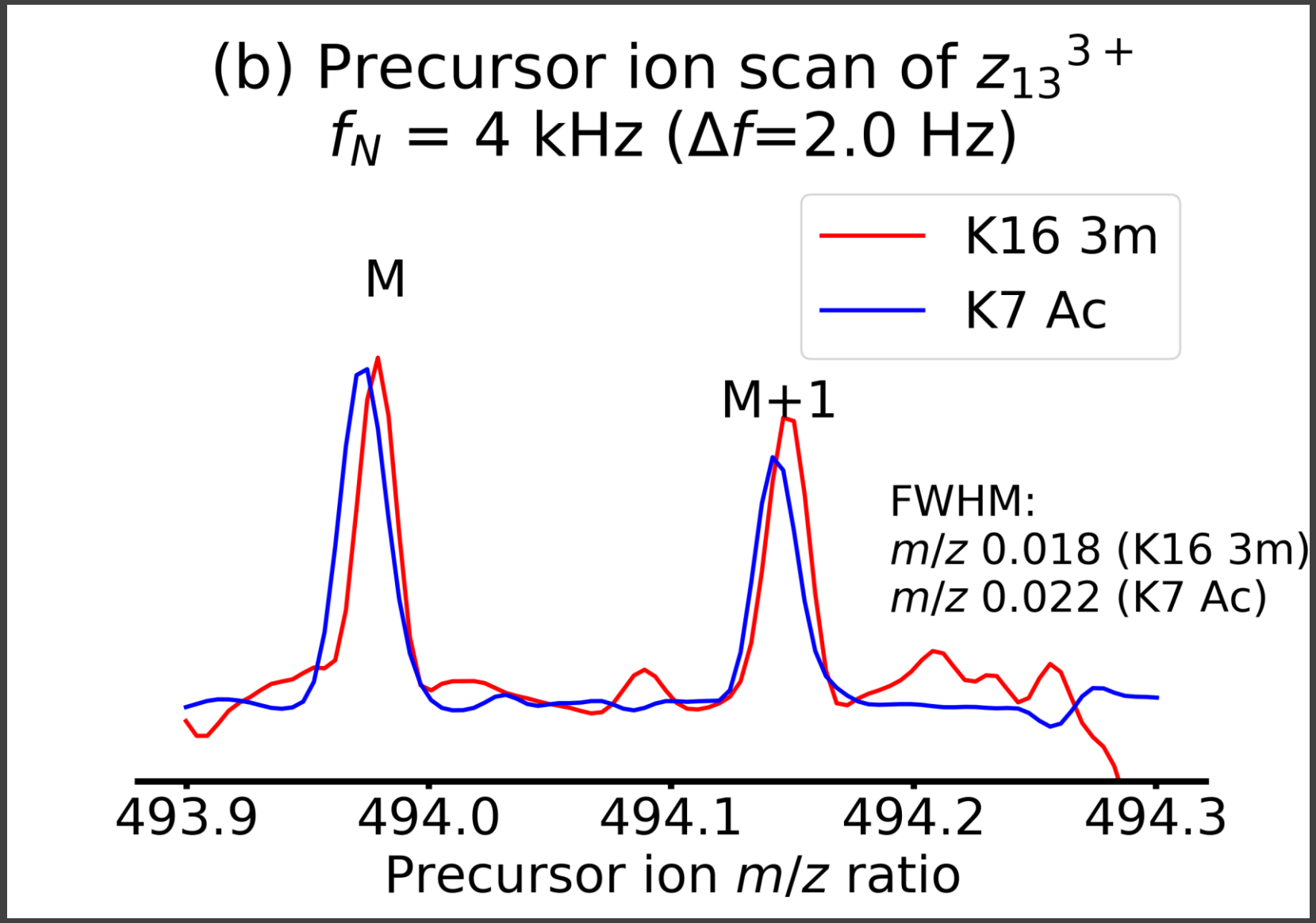

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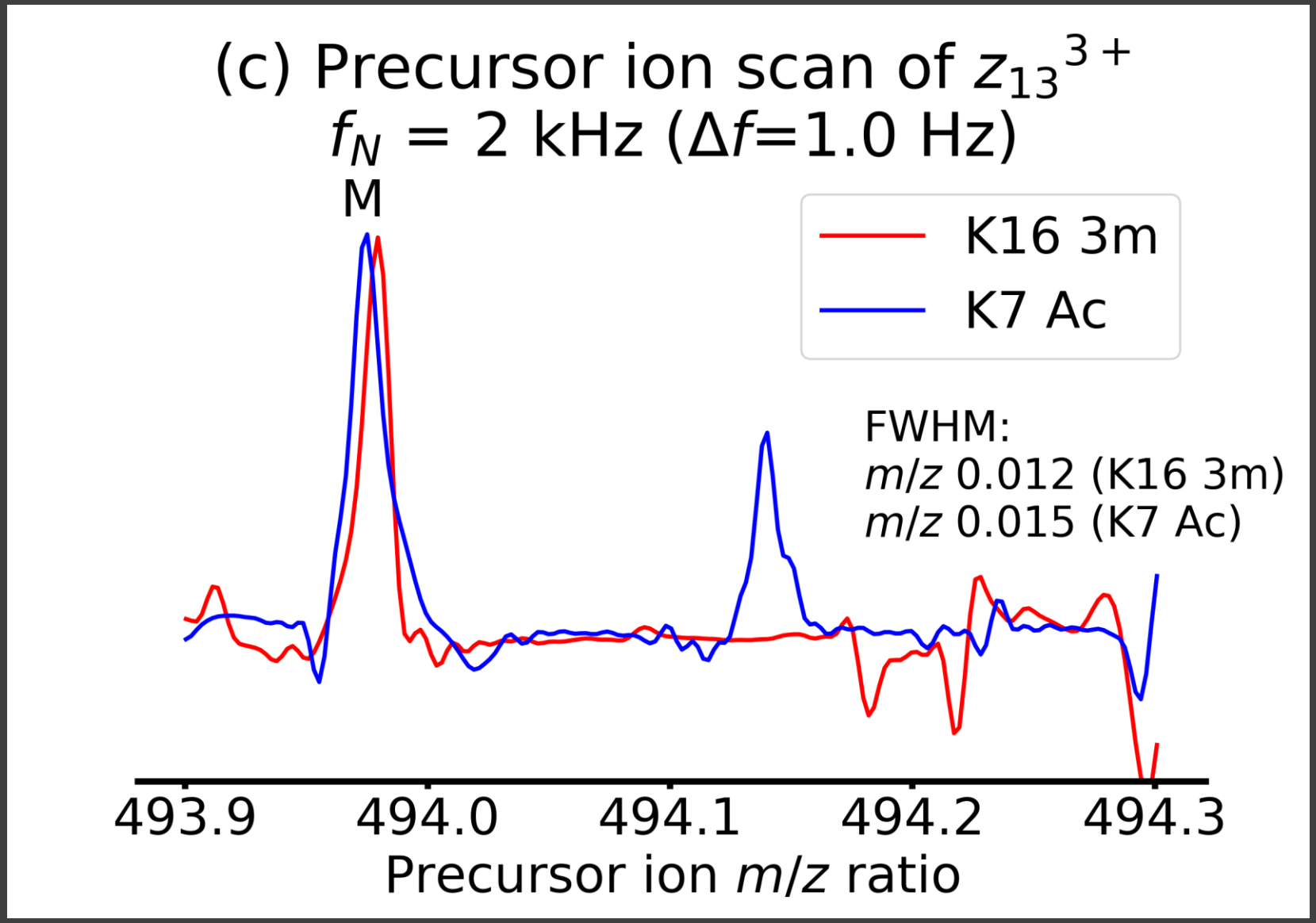

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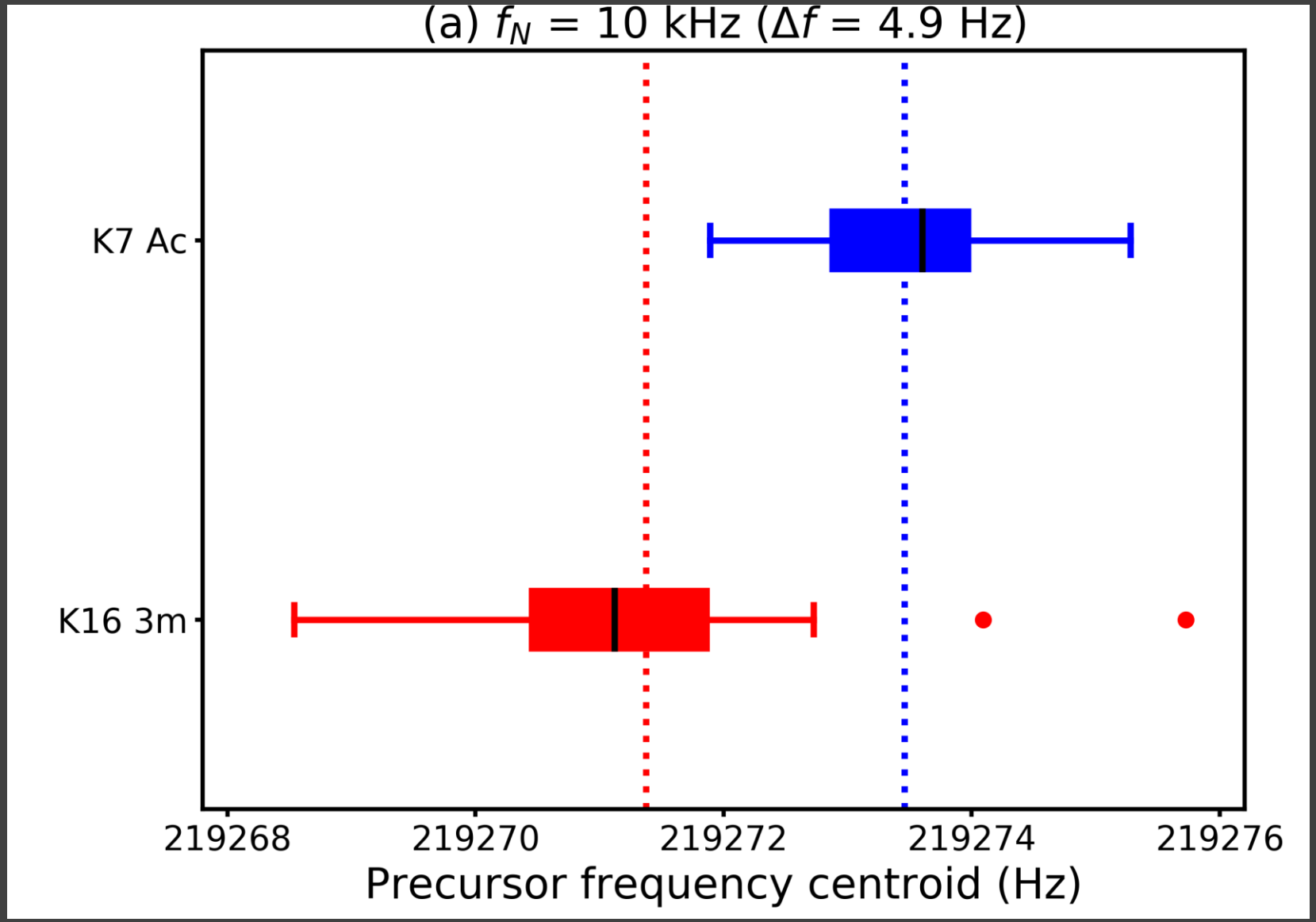

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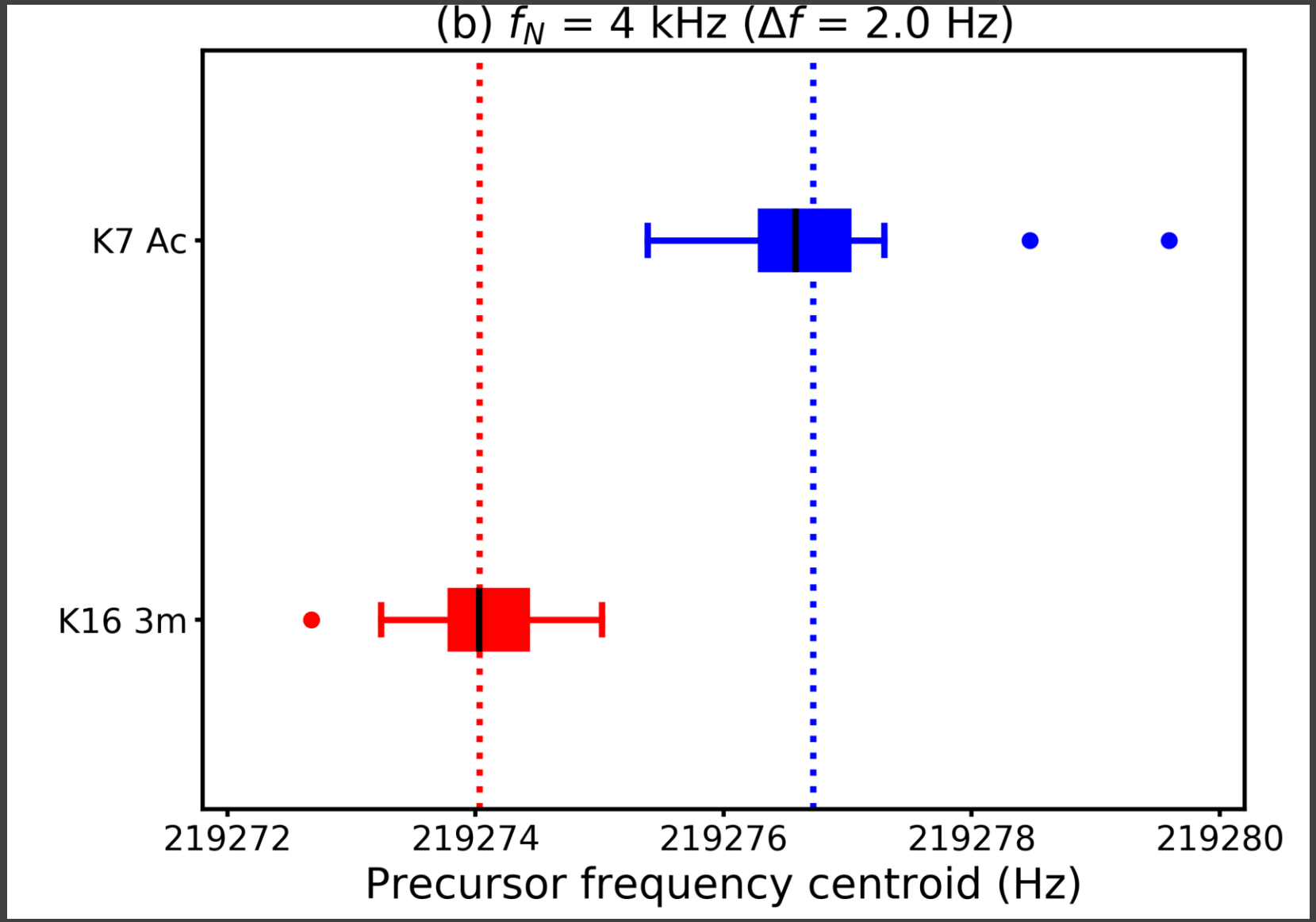

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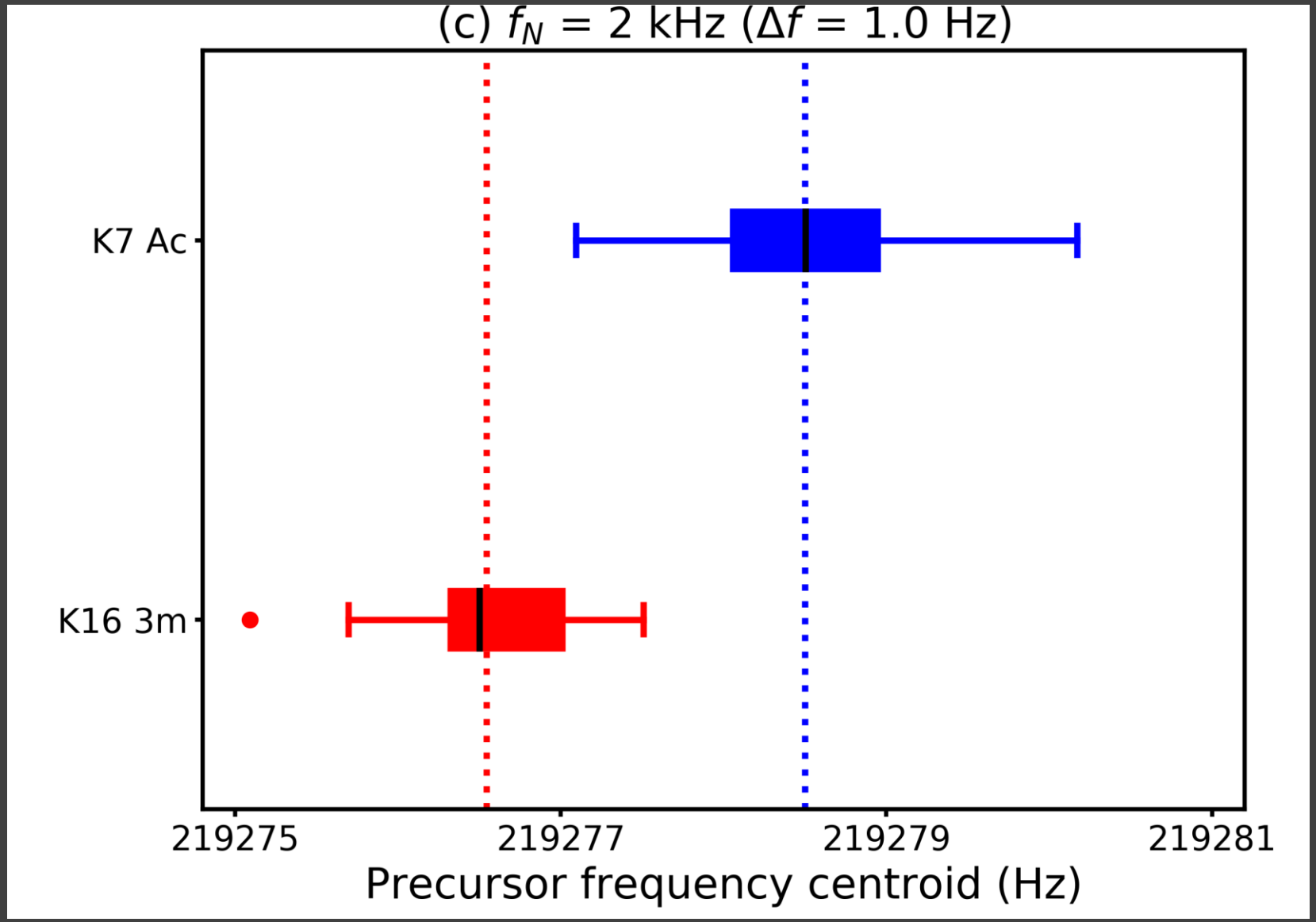


Conclusion

- Narrowband 2D MS: increased resolving power
- Absorption mode 2D MS: increased S/N and resolving power
- Application to top-down analysis of histones and RNA for modifications (identification, location, relative quantification)
- Separation in 2D MS goes beyond resolving power
- Further methods to increase resolving power: non-uniform sampling (NUS)

Acknowledgements

University of Warwick:
Prof. Peter O'Connor
Dr. Mark Barrow
Prof. Alex Jones
Dr. Federico Floris
Dr. Hayley Simon
Dr. Christopher Wootton
Dr. Yuko Lam
Alice Lynch
Dr. Tomos Morgan
Bryan Marzullo
Dr. Georgina Charlton

Université Lille 1:
Dr. Christian Rolando
Dr. Olga Plechakova
Dr. Fabrice Bray

IGBMC, CASC4DE, NMRTEC:
Dr. Marc-André Delsuc
Dr. Lionel Chiron
Dr. Marie-Aude Coutouly

University of Innsbruck:
Prof. Kathrin Breuker
Dr. Giovanni Calderisi
Matthias Halper
Michael Palasser
Sarah Heel

Funding:

- Région Nord Pas-de-Calais
- Agence Nationale de la Recherche
- TGE FT-ICR
- University of Warwick
- Engineering and Physical Sciences Research Council
- Biotechnology and Biological Sciences Research Council
- Warwick Ventures
- ICURe Programme
- Austrian Science Fund

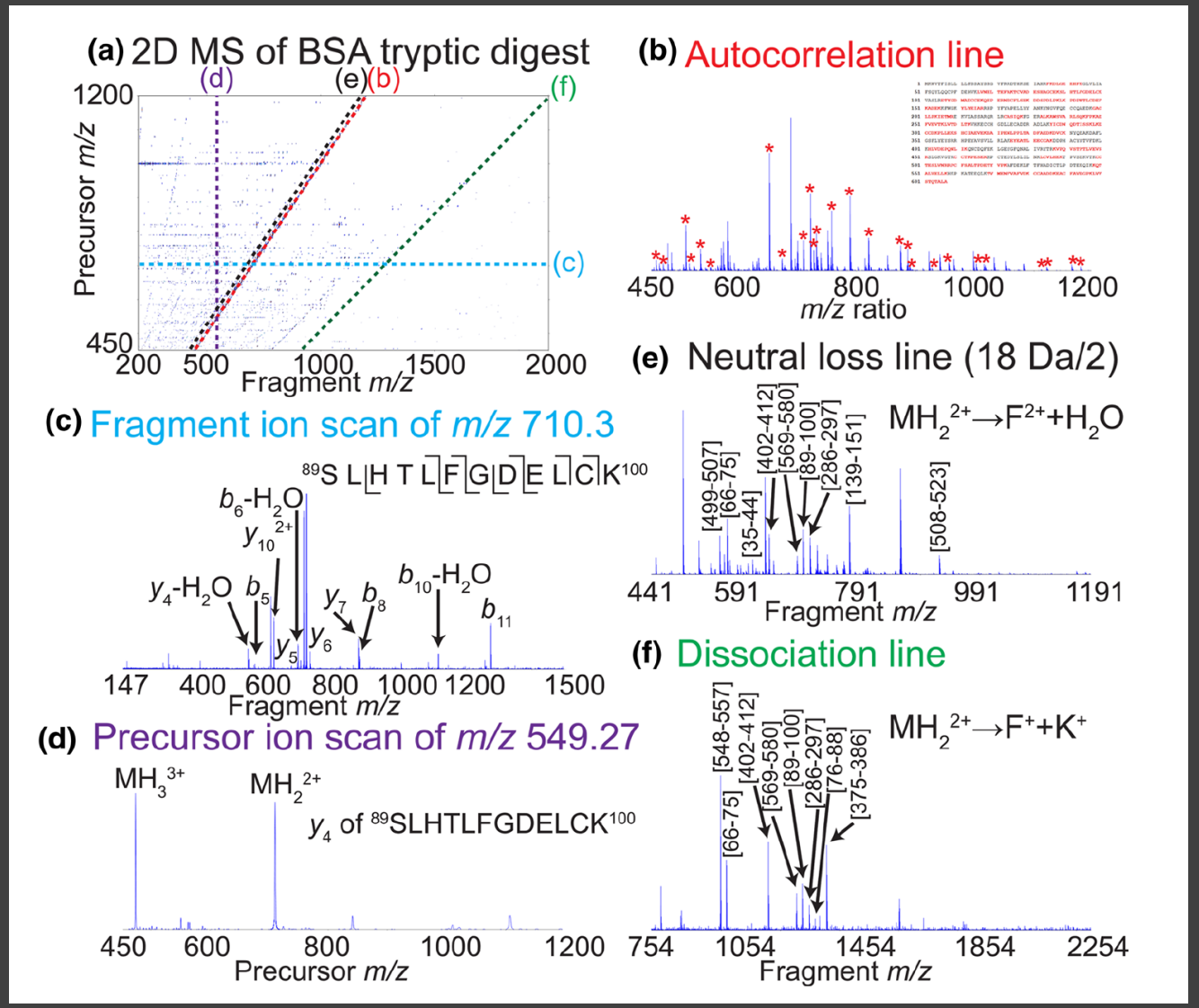
Lise Meitner Fellowship Project M-2757

- EU FT-ICR Network

FWF
Der Wissenschaftsfonds.

EU FT-ICR MS

Thank you for your attention!



Tutorial review:

M.A. van Agthoven, Y.P.Y. Lam, P.B. O'Connor, C. Rolando, M.-A. Delsuc, *Eur. Biophys. J.* 48 (2019) 213-229.

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