Current capabilities in 2DMS on FTICR mass spectrometers

Pui Yiu (Yuko) Lam, Chris Wootton, Johanna Paris, Tomos Morgan, Bryan Marzullo, Maria van Agthoven, Alina Theissen,

Peter B. O'Connor

University of Warwick, Coventry, United Kingdom

EU_FT-ICR_MS 2nd Advanced Users School
Prague, 26 - 30 September 2021

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Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

- Extremely high resolving power
- Many (new) types of MS^n
- New instrumentation

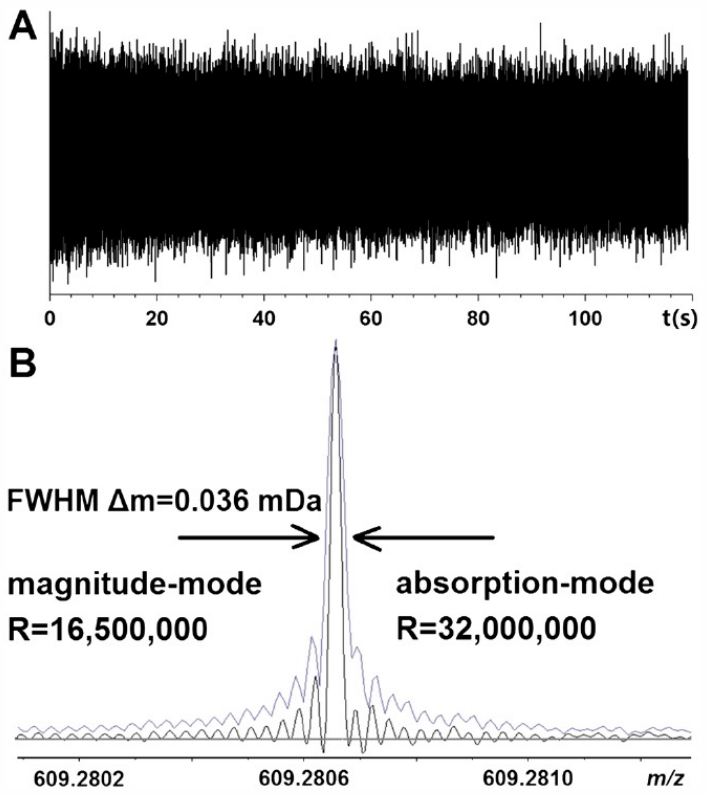


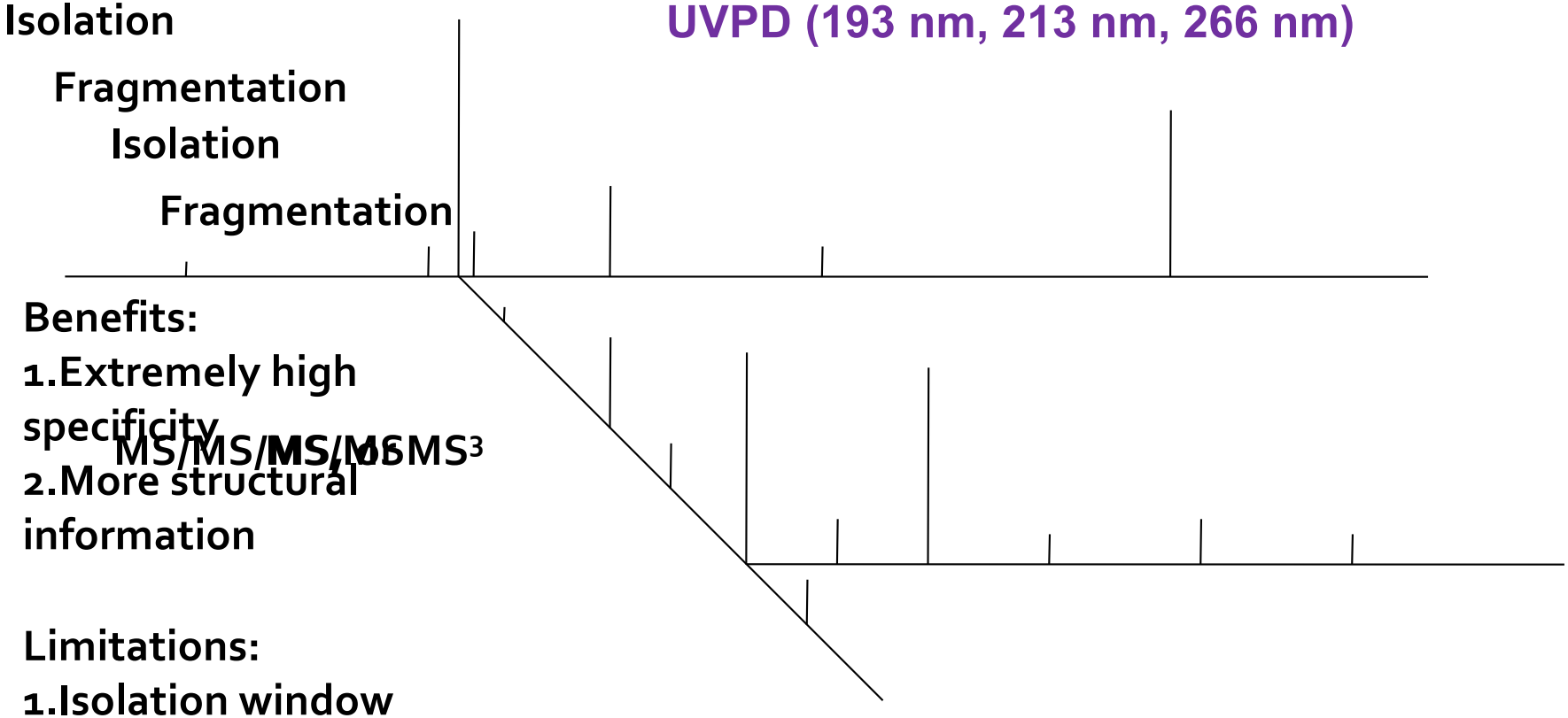
Figure 1. (A) Time-domain transient of the resperine $[M + H]^+$ ion in heterodyne mode, acquired for 2 min. (B) Spectrum in both magnitude- (grey) and absorption-mode (black), the peak width of the magnitude-mode is labelled.

THE UNIVERSITY OF WARWICK

Qi, Y.; Witt, M.; Jertz, R.; Baykut, G.; Barrow, M. P.; Nikolaev, E. N.; O'Connor, P. B., Absorption-mode spectra on the dynamically harmonized Fourier transform ion cyclotron resonance cell. *Rapid Commun. Mass Spectrom. 26 (17),(**2012**) 2021-2026.*

Tandem Mass Spectrometry or MS/MS

CID/CAD, IRMPD
ECD, ETC, EID, EDD, EIEIO
UVPD (193 nm, 213 nm, 266 nm)

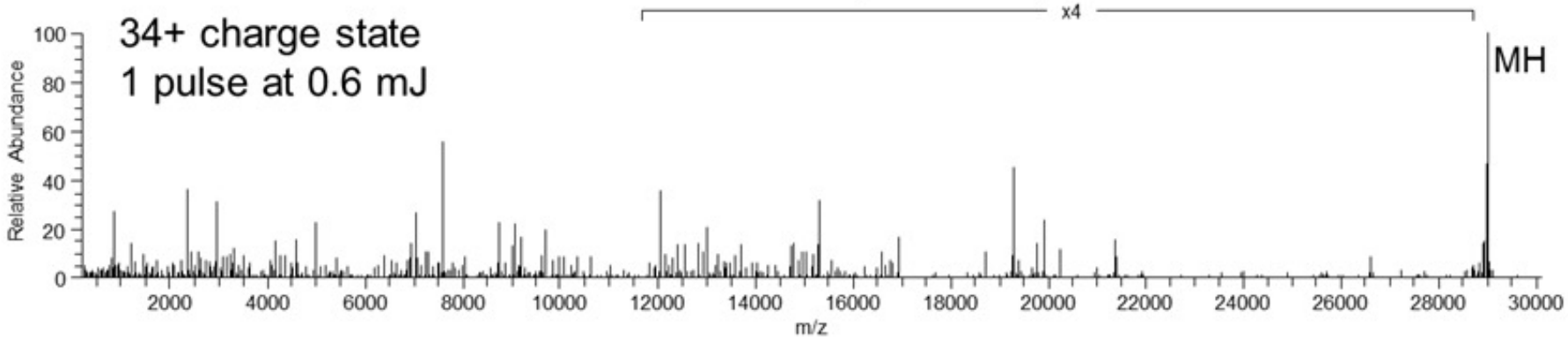


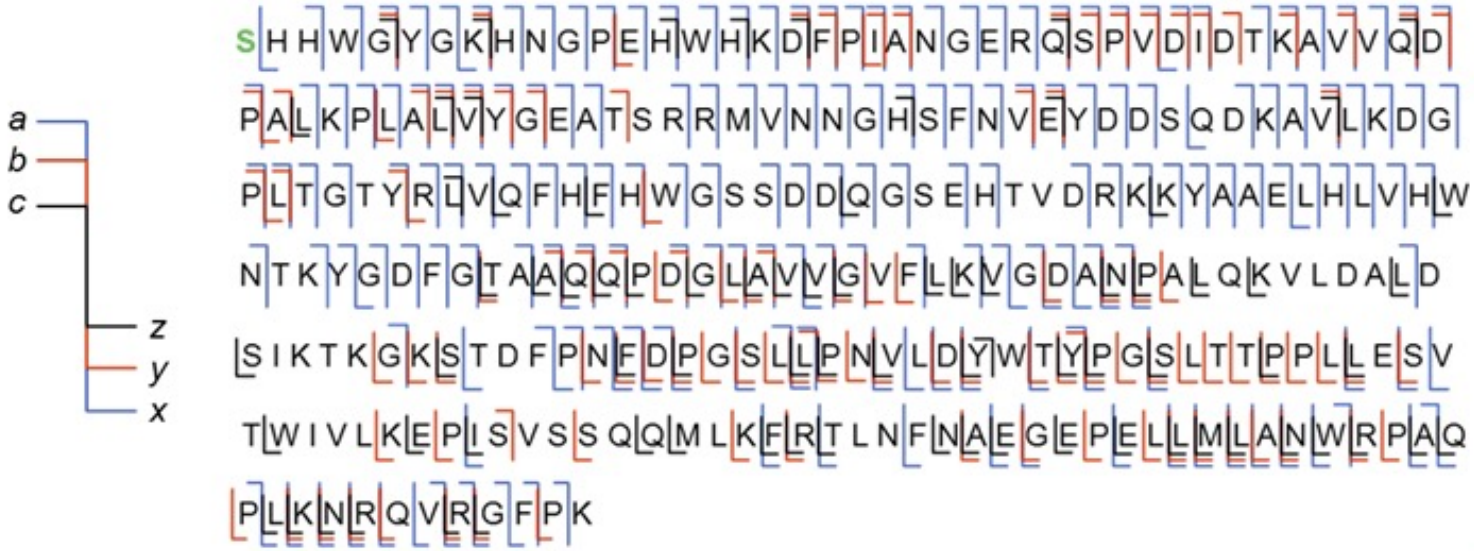
Benefits:
1. Extremely high specificity
2. More structural information

Limitations:
1. Isolation window
2. Fragmentation efficiency
3. Ion Losses

UVPD spectrum of carbonic anhydrase (34+) and its sequence map

259 residues (29 kDa)





Ions Series	Number Observed
a	150
b	38
c	14
x	54
y	71
z	64

Shaw, J. B.; ….; Brodbelt, J. S., Complete Protein Characterization Using Top-Down Mass Spectrometry and Ultraviolet Photodissociation. *J Am Chem Soc 2013, 135 (34), 12646-12651.*

But, what if we don't need to isolate prior to fragmentation?

J. Am. Chem. Soc. **1988**, *110*, 5625–5628

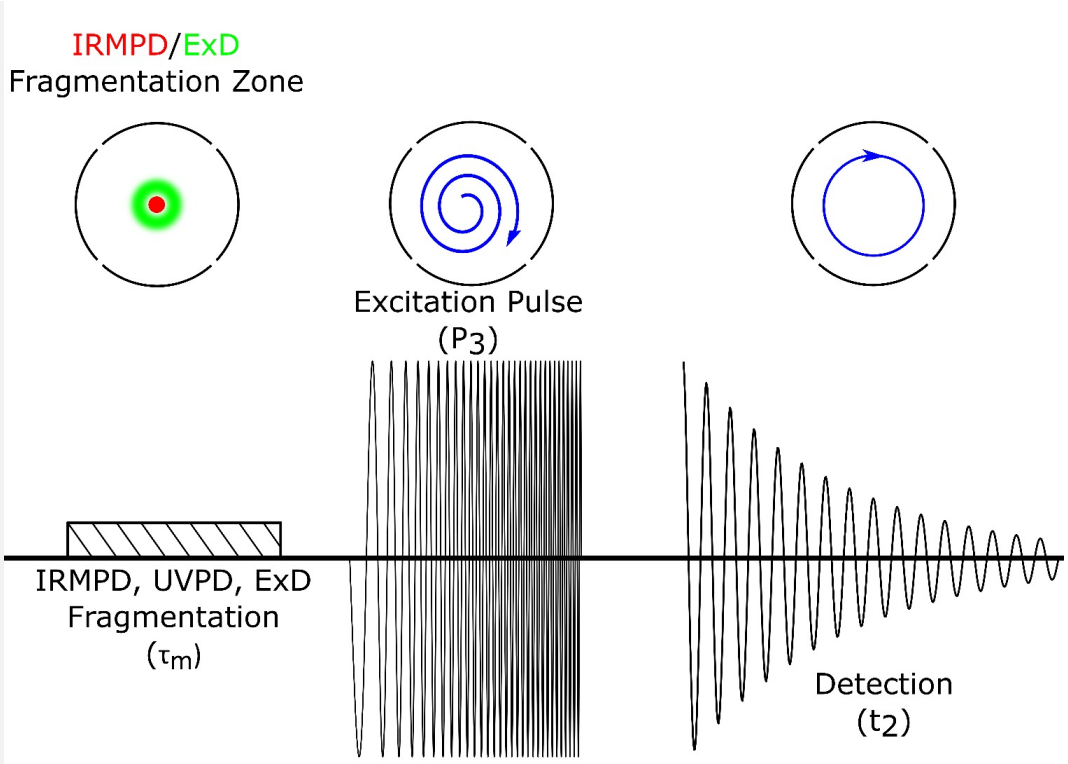
Broad-Band Two-Dimensional Fourier Transform Ion Cyclotron Resonance

Peter Pfändler,[†] Geoffrey Bodenhausen,*[†] Jacques Rapin,[‡] Marc-Etienne Walser,[‡] and Tino Gäumann*[‡]

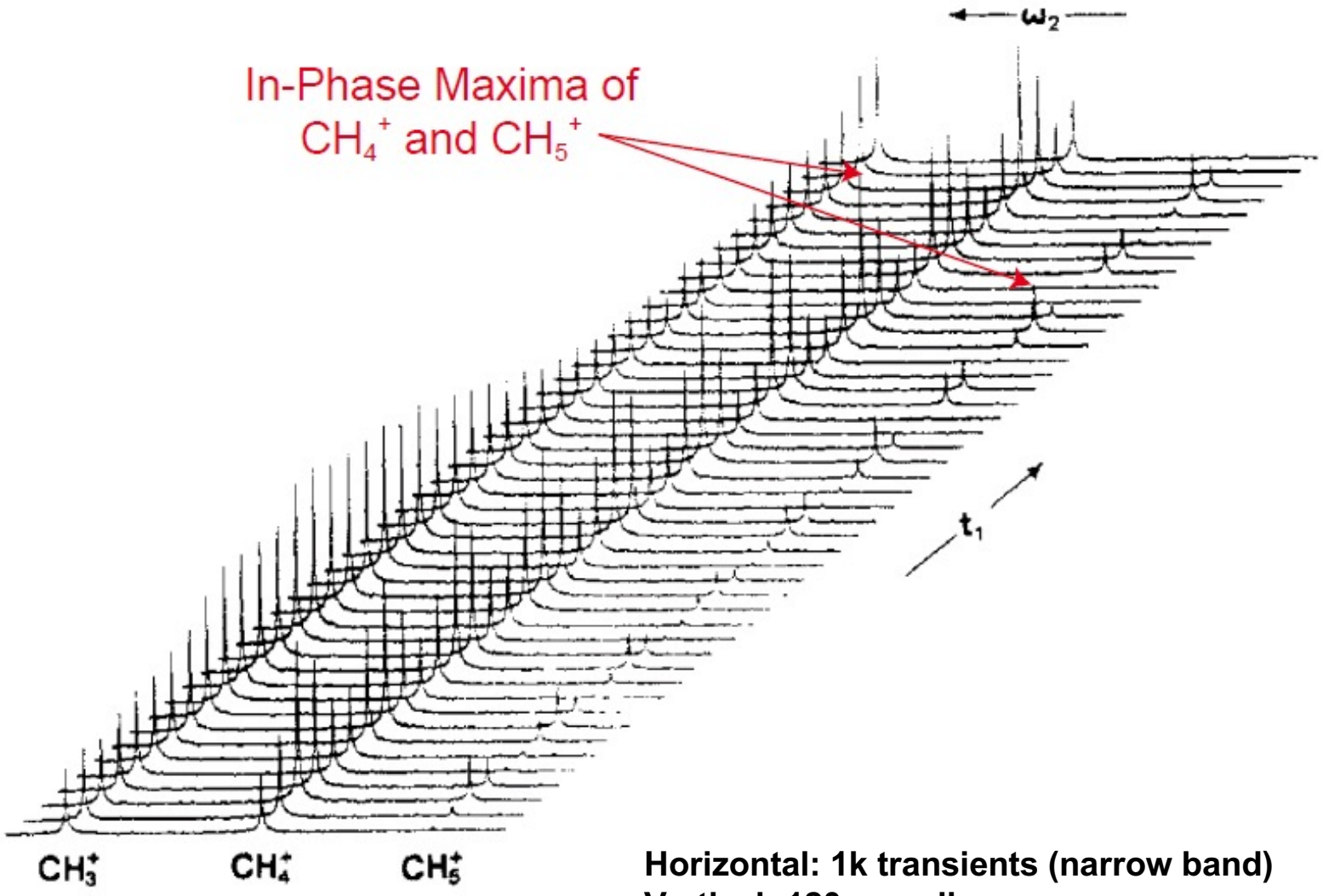
Contribution from the Institut de Chimie Organique, Université de Lausanne, Rue de la Barre 2, CH-1005 Lausanne, Switzerland, and Institut de Chimie Physique, Ecole Polytechnique Fédérale, CH-1015 Lausanne, Switzerland. *Received December 17, 1987*

Abstract: Two-dimensional Fourier transform ion cyclotron resonance (2D FT-ICR) allows one to obtain direct evidence for the occurrence of ion–molecule reactions. In 2D FT-ICR spectra, one observes resonances with two distinct frequency coordinates ω_1 and ω_2 that correspond to the mass-to-charge ratios of the ions that participate as reactants and products, respectively. It is possible to monitor a manifold of distinct reactions in one single 2D spectrum. Spectral ranges of the order of several MHz can be covered by using frequency-swept ("chirped") radio-frequency pulses.

Two-dimensional Mass Spectrometry



Principle of 2D FT-ICR MS: Modulation



Horizontal: 1k transients (narrow band)
Vertical: 120 scan lines.
Total: 120,000 data points

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

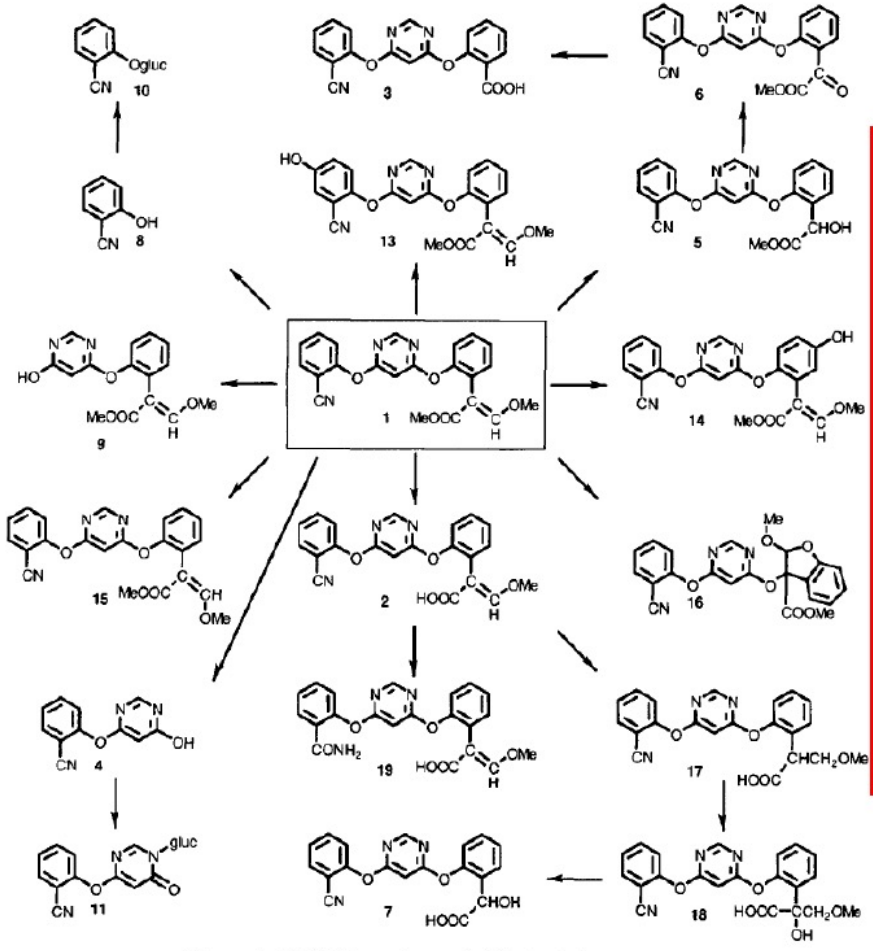
Lecture overview

- 2DMS for agrichemical analysis.
- 2DMS for polymer analysis.
- 2DMS for monoclonal antibodies.
- Distinguishing phosphopeptides using 2DMS
- 2DMS – UVPD
- 2DMS on a linear ion trap

Case study of 2DMS with Syngenta UK

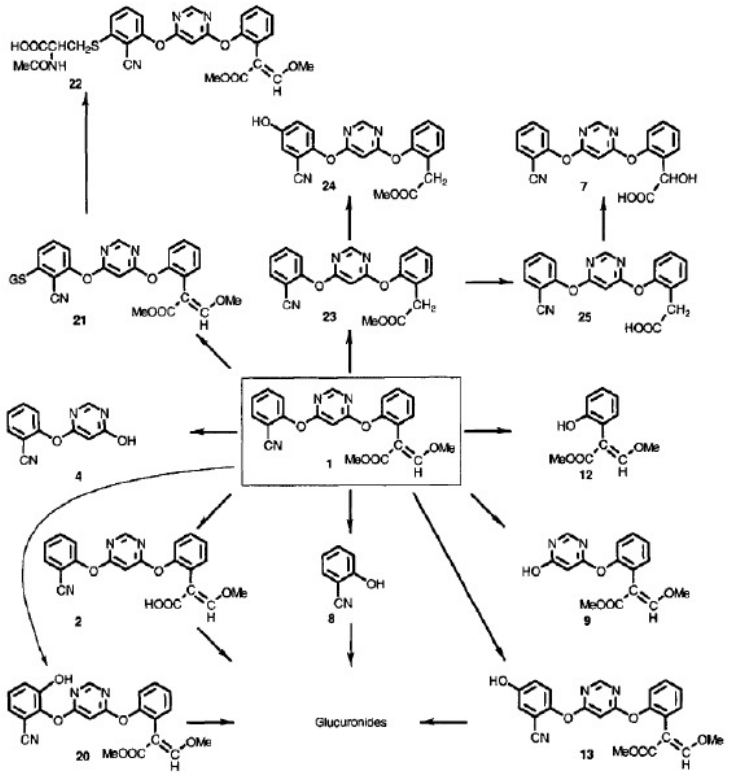
Agrochemical and pesticide analysis

Pesticides degrade and react with their local environment to produce many potentially dangerous compounds:



Scheme 2 Metabolism of azoxystrobin in plants.

We need methods to detect all of these species in real, complex samples to identify toxic compounds. Like 2DMS

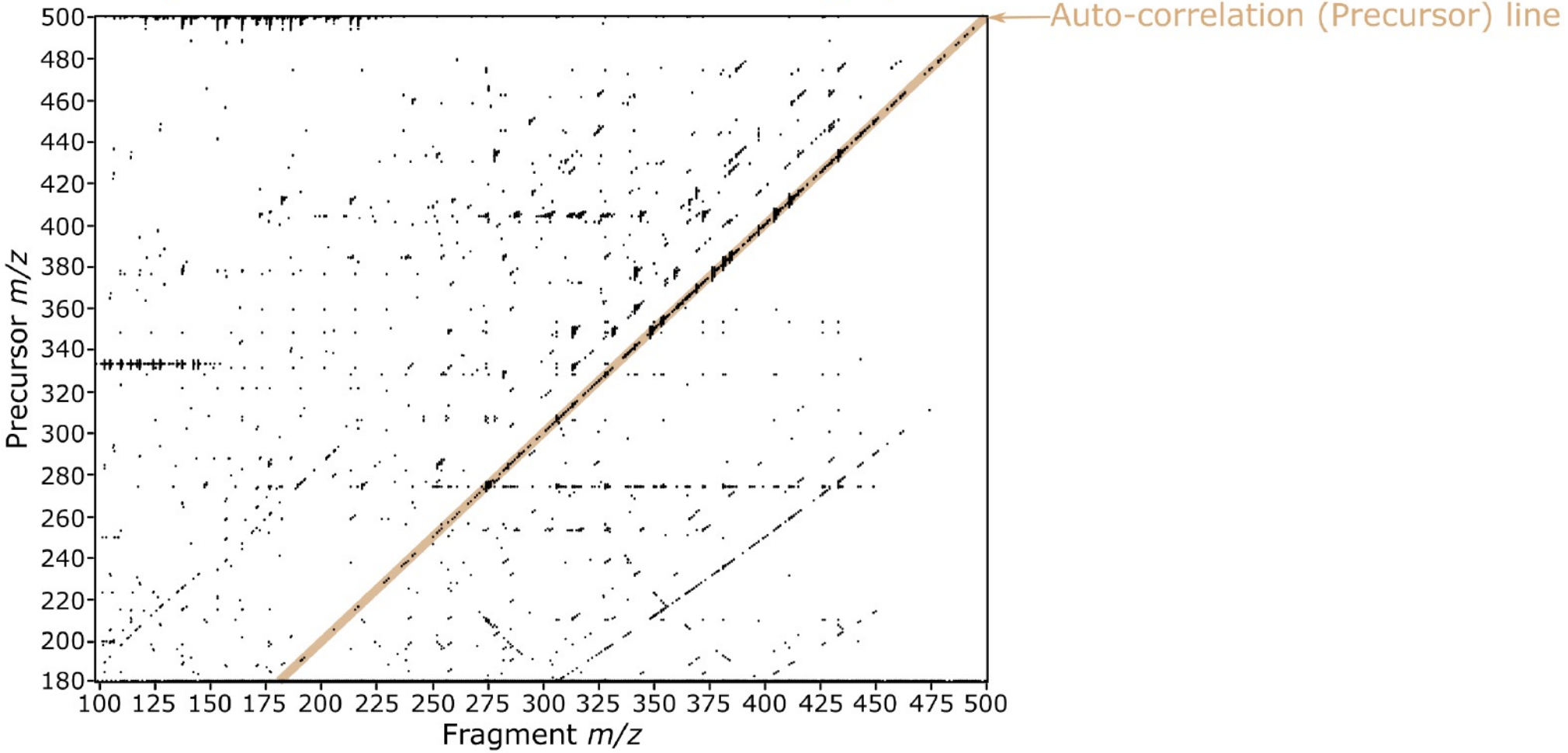


Scheme 3 Metabolism of azoxystrobin in the rat.

Copping, Leonard G. "Metabolic pathways of agrochemicals: part two–insecticides and fungicides, eds-in-chief T Roberts and D Hutson, Royal Society of Chemistry, Cambridge, 1999, 1475 pp, ISBN 085404 499 X." *Pest Management Science: formerly Pesticide Science* 56.1 (2000): 103-104.

2D IRMPD MS

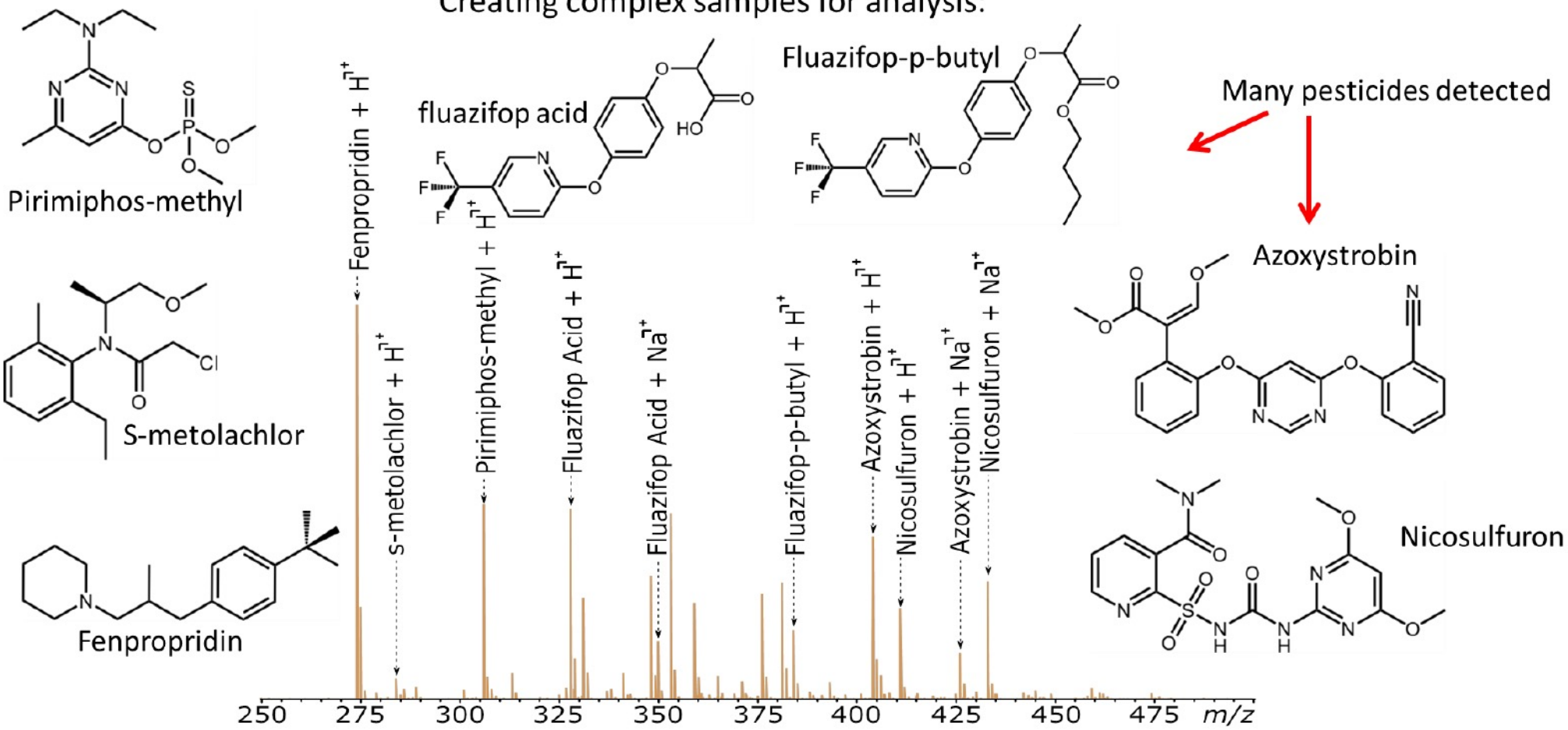
2DMS provides a wealth of information in a single plot:



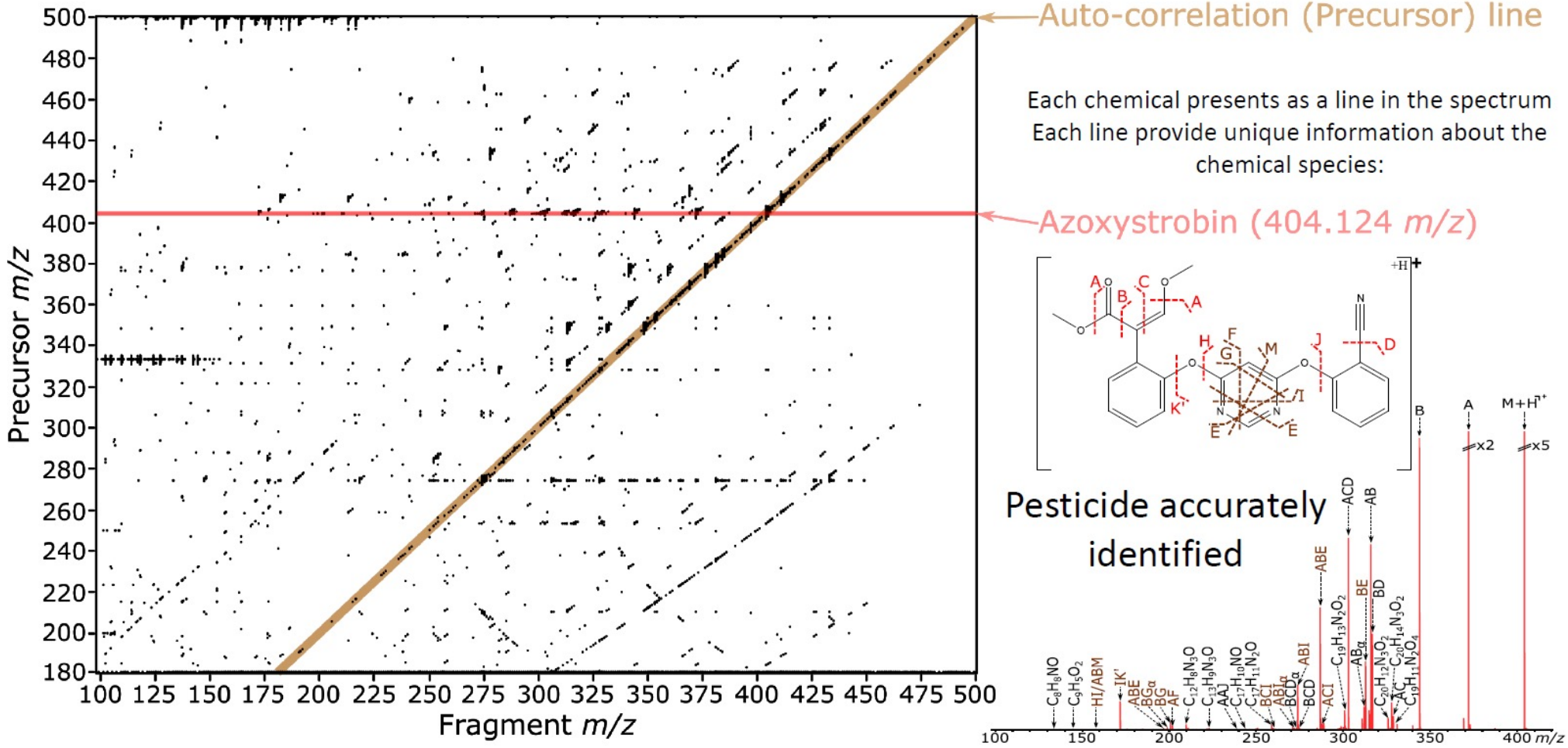
2D IRMPD MS – autocorrelation (precursor) line

Pesticides are often used in mixtures of active components

Creating complex samples for analysis:



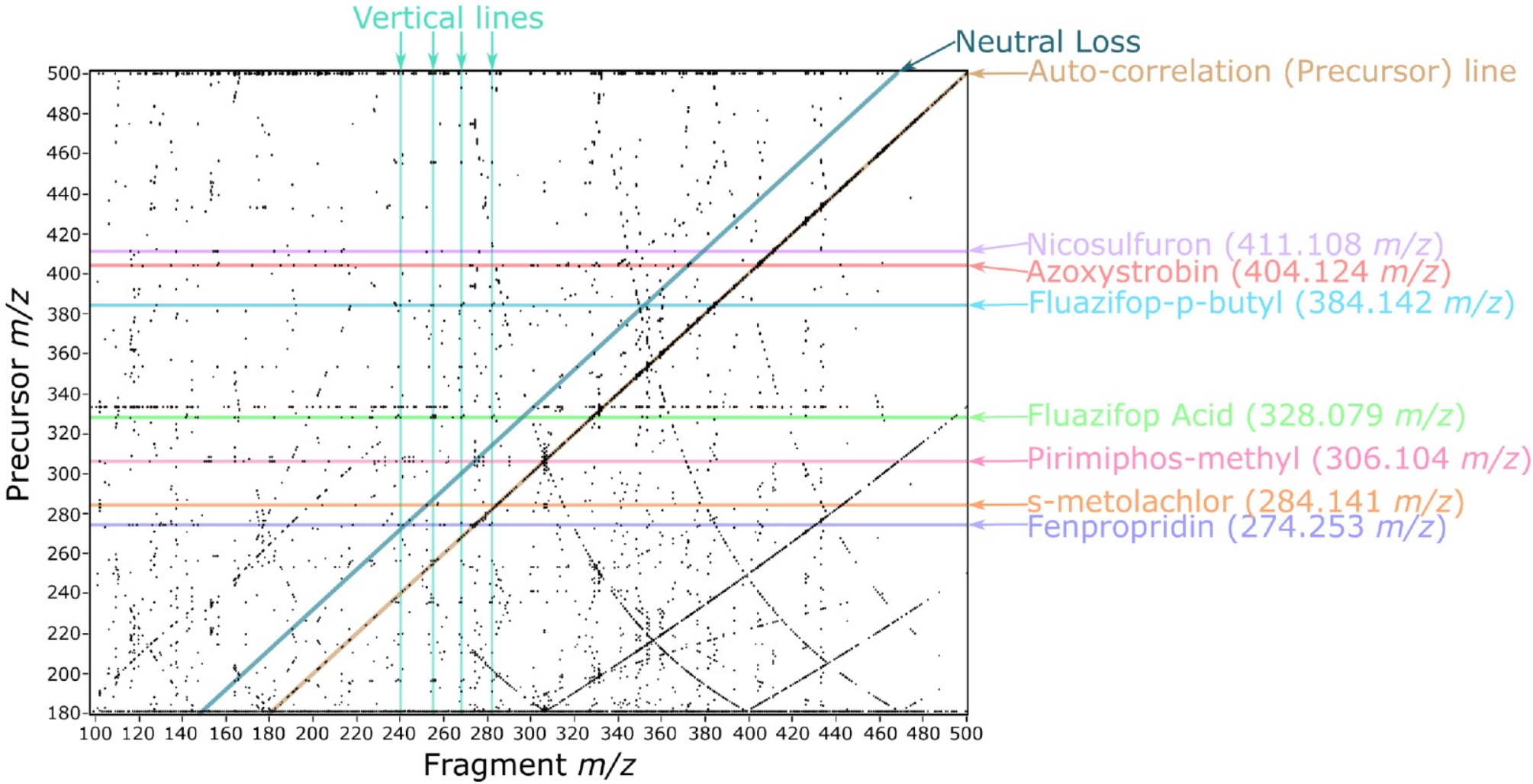
2D IRMPD MS



Each chemical presents as a line in the spectrum
Each line provide unique information about the chemical species:

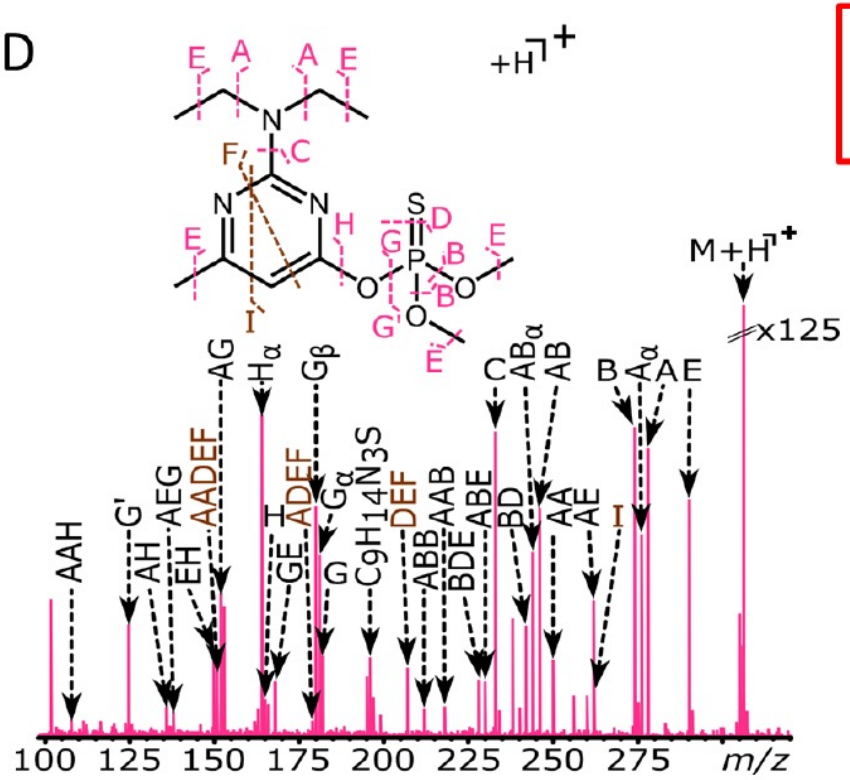
Pesticide accurately identified

2D EID MS

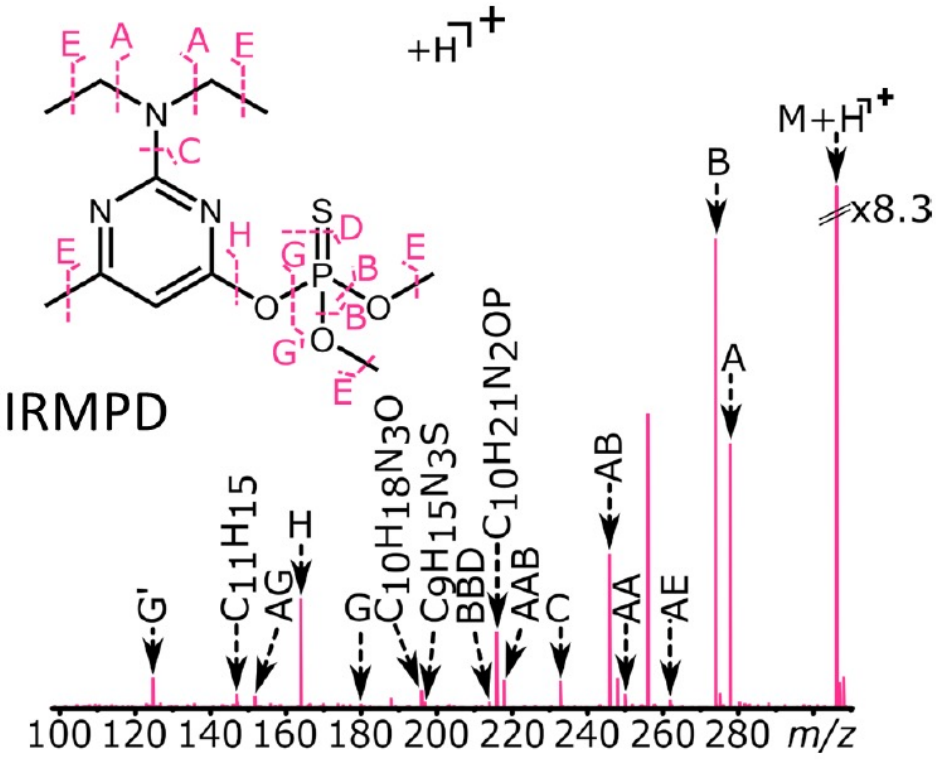

Vertical lines
Neutral Loss
Auto-correlation (Precursor) line
Nicosulfuron (411.108 m/z)
Azoxystrobin (404.124 m/z)
Fluazifop-p-butyl (384.142 m/z)
Fluazifop Acid (328.079 m/z)
Pirimiphos-methyl (306.104 m/z)
s-metolachlor (284.141 m/z)
Fenpropridin (274.253 m/z)
Precursor m/z
Fragment m/z
500
480
460
440
420
400
380
360
340
320
300
280
260
240
220
200
180
100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500

2D EID MS

EID

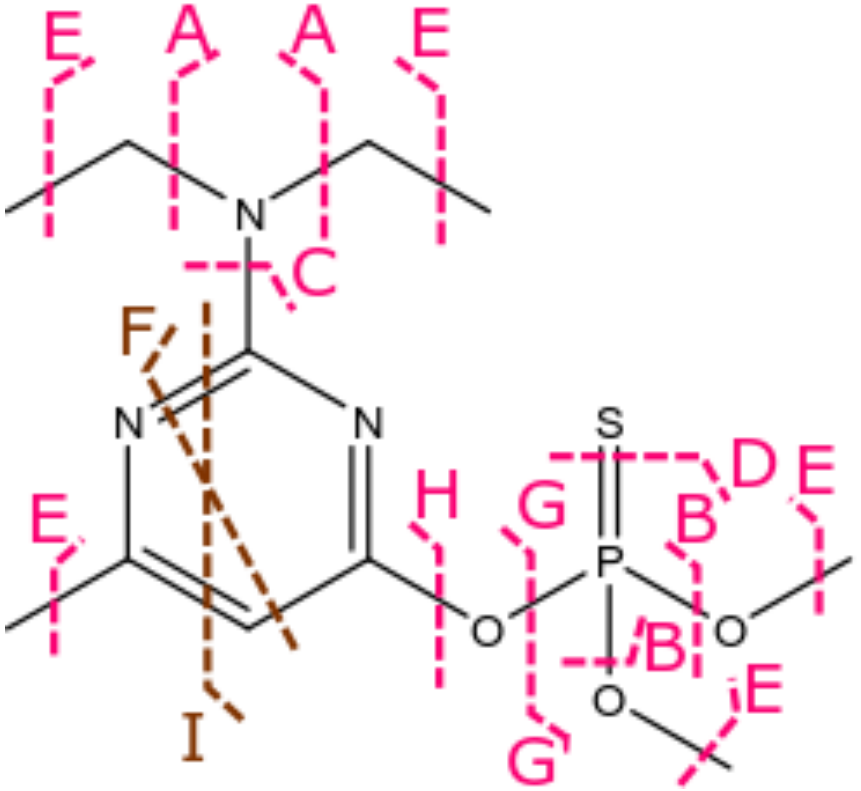


Extensive characterisation
Of every species
Allows upmost confidence of assignments



Allows both identification of known products / toxins
And characterisation of unknowns easily and accurately
Can use multiple types of analysis (EID or IRMPD shown)

Pirimiphos-methyl assignments

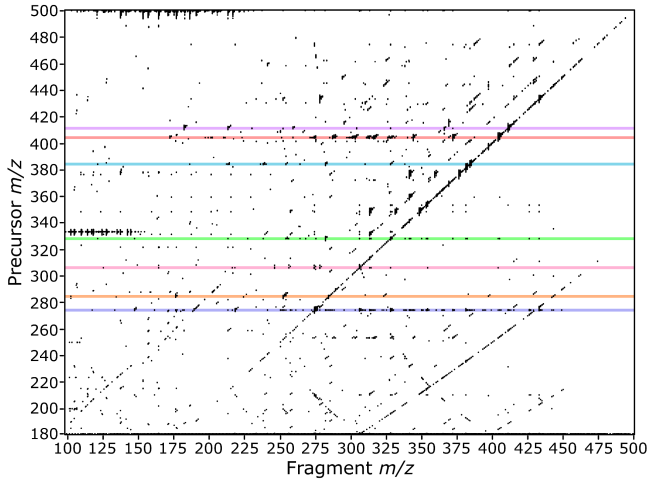


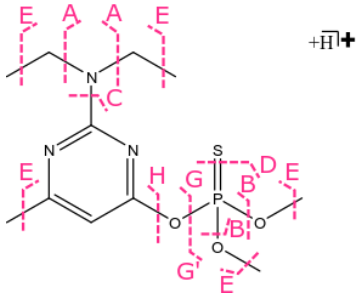
$+H^+$

m/z	Relative Intensity	Description	Theoretical Mass	Error
290.07239	0.47	C10H17N3O3PS	290.07228	0.411
278.07227	0.57	C9H16N3O3PSH	278.07228	-0.009
276.05665	0.39	C9H15N3O3PS	276.05663	0.075
274.13167	0.41	C11H21N3O3P	274.13150	0.596
274.07733	0.60	C10H16N3O2PSH	274.07736	-0.128
262.05342	0.09	C9H15N2O3PS	262.05355	-0.493
262.04083	0.27	C8H13N3O3PS	262.04098	-0.556
250.04110	0.15	C7H13N3O3PS	250.04098	0.489
246.04608	0.45	C8H13N3O2PS	246.04606	0.059
244.03039	0.37	C8H11N3O2PS	244.03041	-0.088
242.10537	0.22	C10H17N3O2P	242.10529	0.325
233.01443	0.59	C7H10N2O3PS	233.01443	0.012
230.01457	0.11	C7H9N3O2PS	230.01476	-0.825
228.08968	0.12	C9H15N3O2P	228.08964	0.165
218.01495	0.06	C6H9N3O2PS	218.01476	0.872
212.00441	0.06	C7H7N3OPS	212.00420	0.991
207.08940	0.14	C7H16N2O3P	207.08931	0.465
197.09803	0.08	C9H15N3S	197.09812	-0.459
196.14446	0.16	C10H18N3O	196.14444	0.124
196.09034	0.14	C9H14N3S	196.09029	0.226
195.13652	0.12	C10H17N3O	195.13661	-0.491
182.12885	0.16	C9H16N3O	182.12879	0.332
182.07458	0.08	C8H12N3S	182.07464	-0.349
181.12101	0.35	C9H15N3O	181.12096	0.255
180.11315	0.46	C9H14N3O	180.11314	0.070
179.05809	0.03	C5H12N2O3P	179.05801	0.488
168.11314	0.11	C8H14N3O	168.11314	-0.004
168.05902	0.11	C7H10N3S	168.05899	0.155
166.09747	0.07	C8H12N3O	166.09749	-0.098
166.04342	0.02	C7H8N3S	166.04334	0.480
165.12605	0.07	C9H15N3	165.12605	0.033
164.11825	0.62	C9H14N3	164.11822	0.172
163.11050	0.06	C9H13N3	163.11040	0.643
152.08186	0.28	C7H10N3O	152.08184	0.170
151.02672	0.13	C3H8N2O3P	151.02671	0.097
150.10263	0.18	C8H12N3	150.10257	0.368
138.06627	0.05	C6H8N3O	138.06619	0.617
136.08691	0.06	C7H10N3	136.08692	-0.090
124.98209	0.22	C2H6O2PS	124.98206	0.218
108.05562	0.04	C5H6N3	108.05562	0.000

Absolute Average:
0.312 ppm
Standard Deviation:
0.385 ppm

Assignments - 2D IRMPD

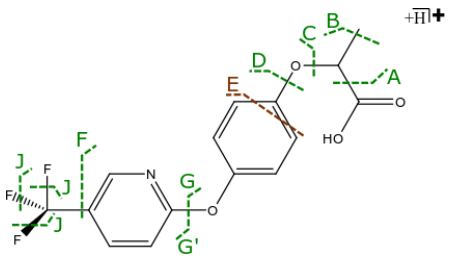




IRMPD

Abs. Ave. Std. Dev.

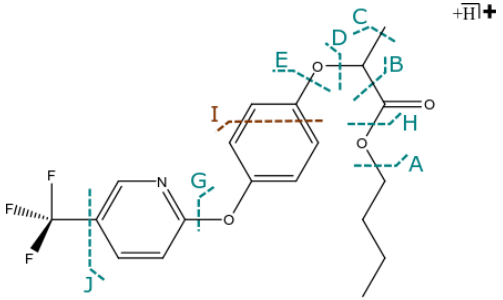
0.29 ppm 0.49 ppm



IRMPD

Abs. Ave. Std. Dev.

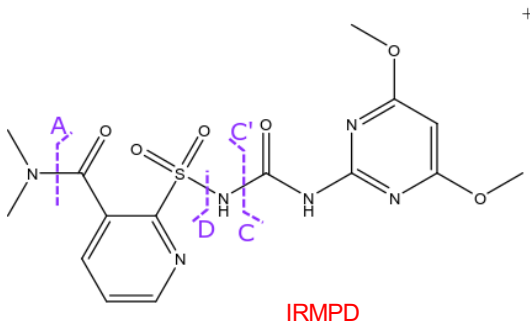
0.23 ppm 0.26 ppm



IRMPD

Abs. Ave. Std. Dev.

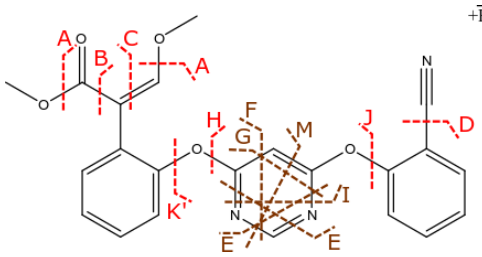
0.20 ppm 0.24 ppm



IRMPD

Abs. Ave. Std. Dev.

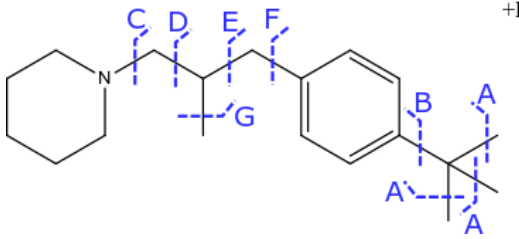
0.15 ppm 0.26 ppm



IRMPD

Abs. Ave. Std. Dev.

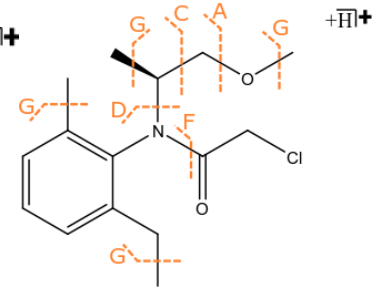
0.19 ppm 0.36 ppm



IRMPD

Abs. Ave. Std. Dev.

0.22 ppm 0.32 ppm

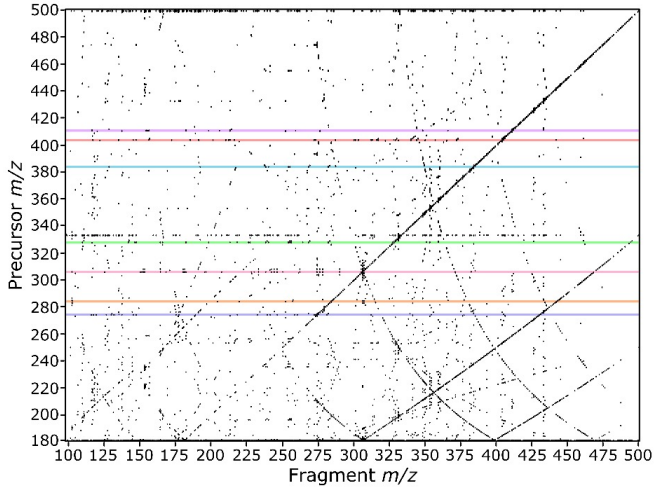


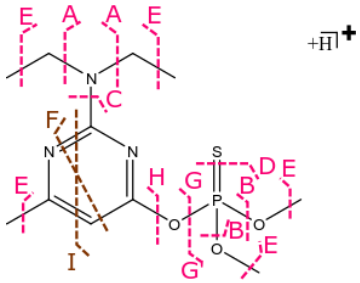
IRMPD

Abs. Ave. Std. Dev.

0.42 ppm 0.30 ppm

Assignments - 2D EID

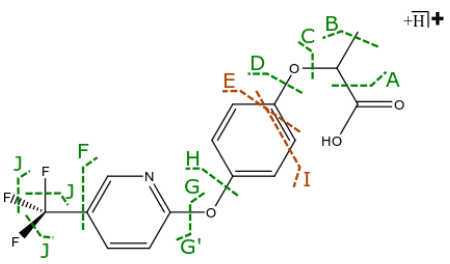




EID

Abs. Ave. 0.31 ppm

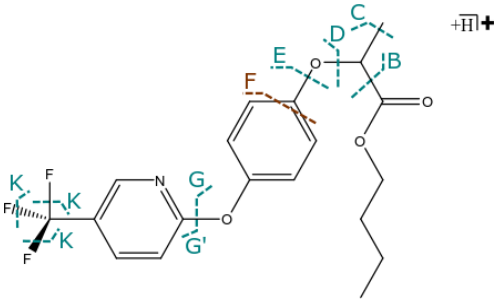
Std. Dev. 0.39 ppm



EID

Abs. Ave. 0.55 ppm

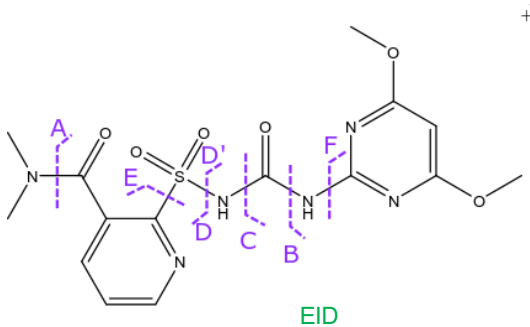
Std. Dev. 0.77 ppm



EID

Abs. Ave. 0.15 ppm

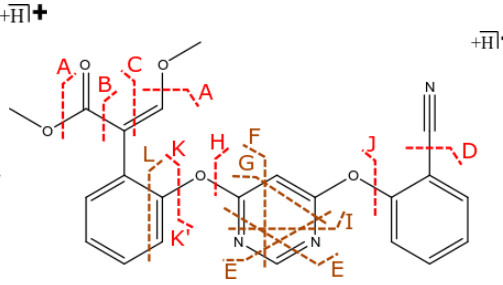
Std. Dev. 0.17 ppm



EID

Abs. Ave. 0.50 ppm

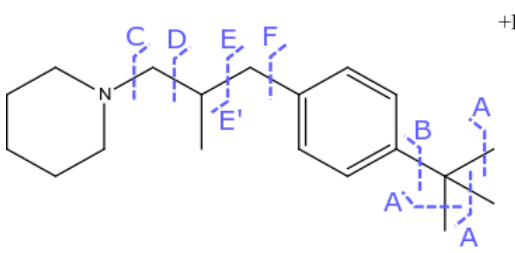
Std. Dev. 0.54 ppm



EID

Abs. Ave. 0.52 ppm

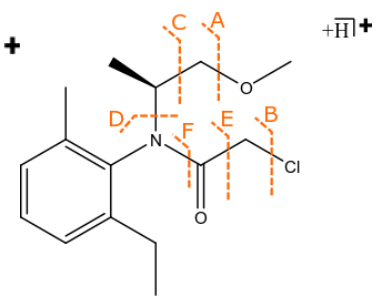
Std. Dev. 0.96 ppm



EID

Abs. Ave. 0.91 ppm

Std. Dev. 1.16 ppm



EID

Abs. Ave. 0.09 ppm

Std. Dev. 0.19 ppm

Case study of 2DMS with Syngenta UK

Agrochemical and pesticide analysis

Issues with current analysis:

- Unique method required for certain compounds – no one-size-fits-all
- LC-MS/MS based – fails to scale with complexity, induces bias in LC
- Restricted solvent systems makes certain compounds "off-limits" or require separate methods
- Large optimisations needed for each question, no future proofing for later questions – very long and expensive

What we achieved:

- Parallel analysis of all pesticide and degradation products in one run
- Extensive fragmentation patterns for each unique species – enables accurate identification
- Data independent analysis which is future-proof for unknown questions
- Less bias analysis, not reliant on solvent systems or LC compatibility

Lecture overview

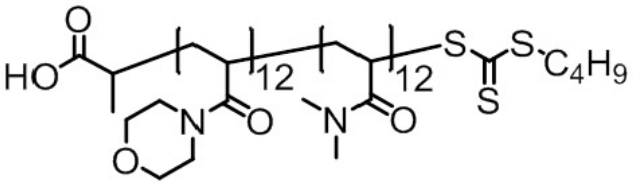
- 2DMS for agrichemical analysis.
- 2DMS for polymer analysis.
- 2DMS for monoclonal antibodies.
- Distinguishing phosphopeptides using 2DMS
- 2DMS – UVPD
- 2DMS on a linear ion trap

Case study of 2DMS with Astra Zeneca UK

Biologically relevant polymer analysis

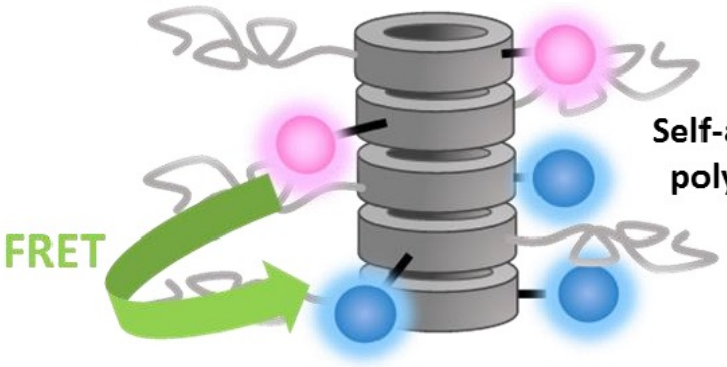
Polymer analysis by mass spectrometry

- Increased use of polymeric species as therapeutic agents
- Movement away from PEG due to chemical modification
- Focus on primary chemical structure and how this varies properties
- Presence of sample complexity as well as need to understand primary structure



Acrylamide copolymers

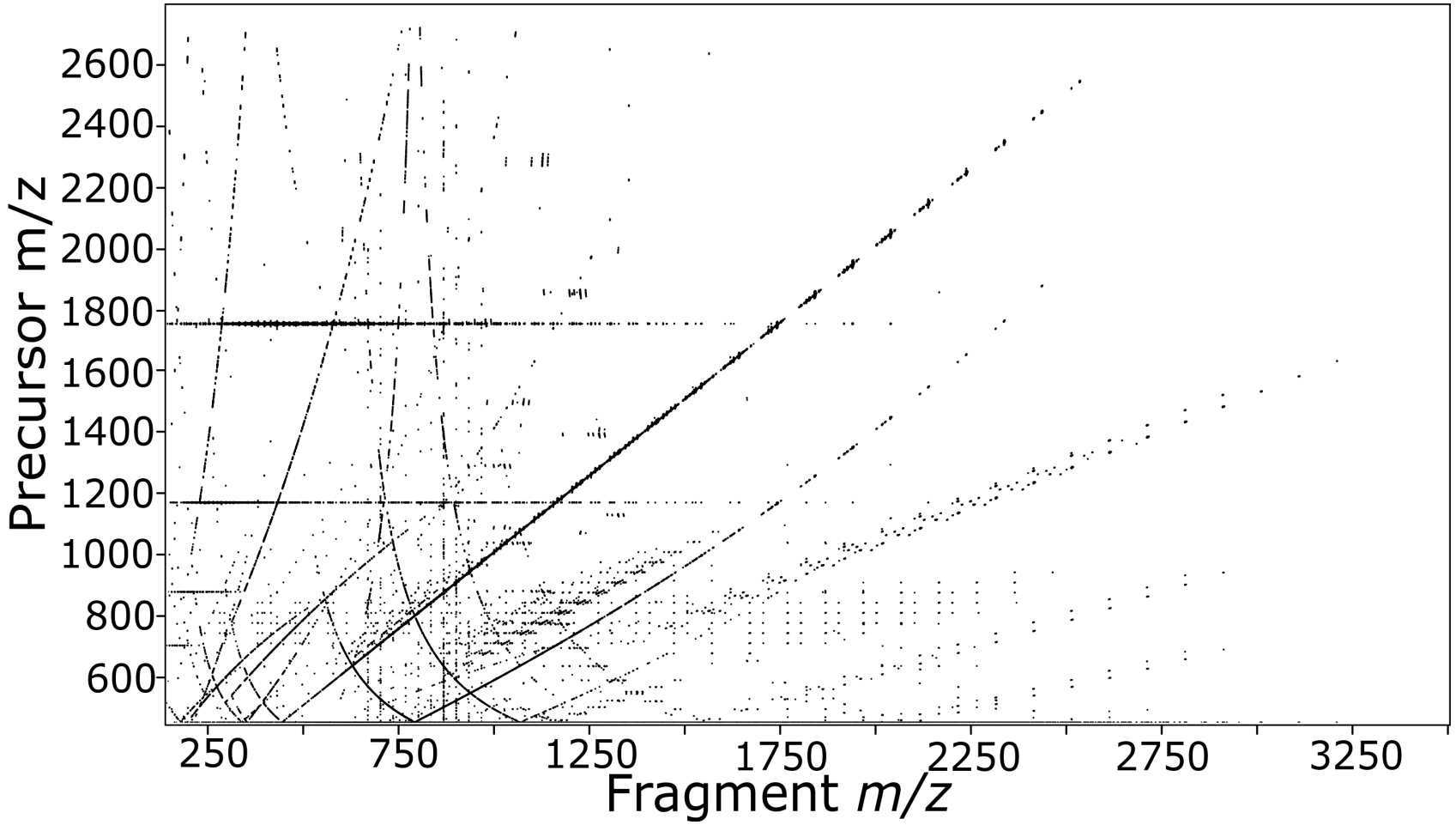
Polyoxazoline homopolymers



Self-assembly peptide polymer conjugates

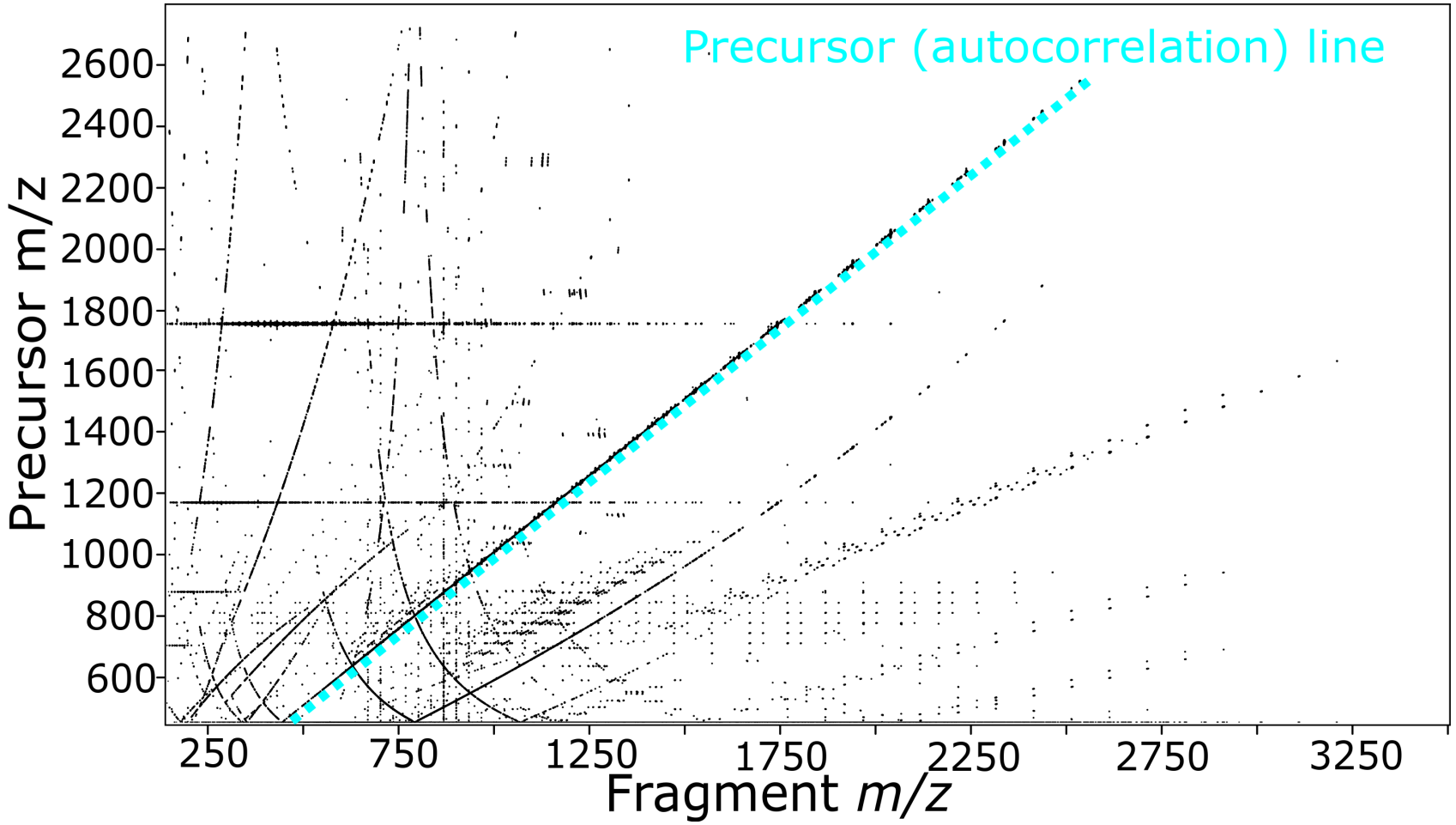
Cyclic-peptide polymer conjugates

Polymer analysis by 2D ECD



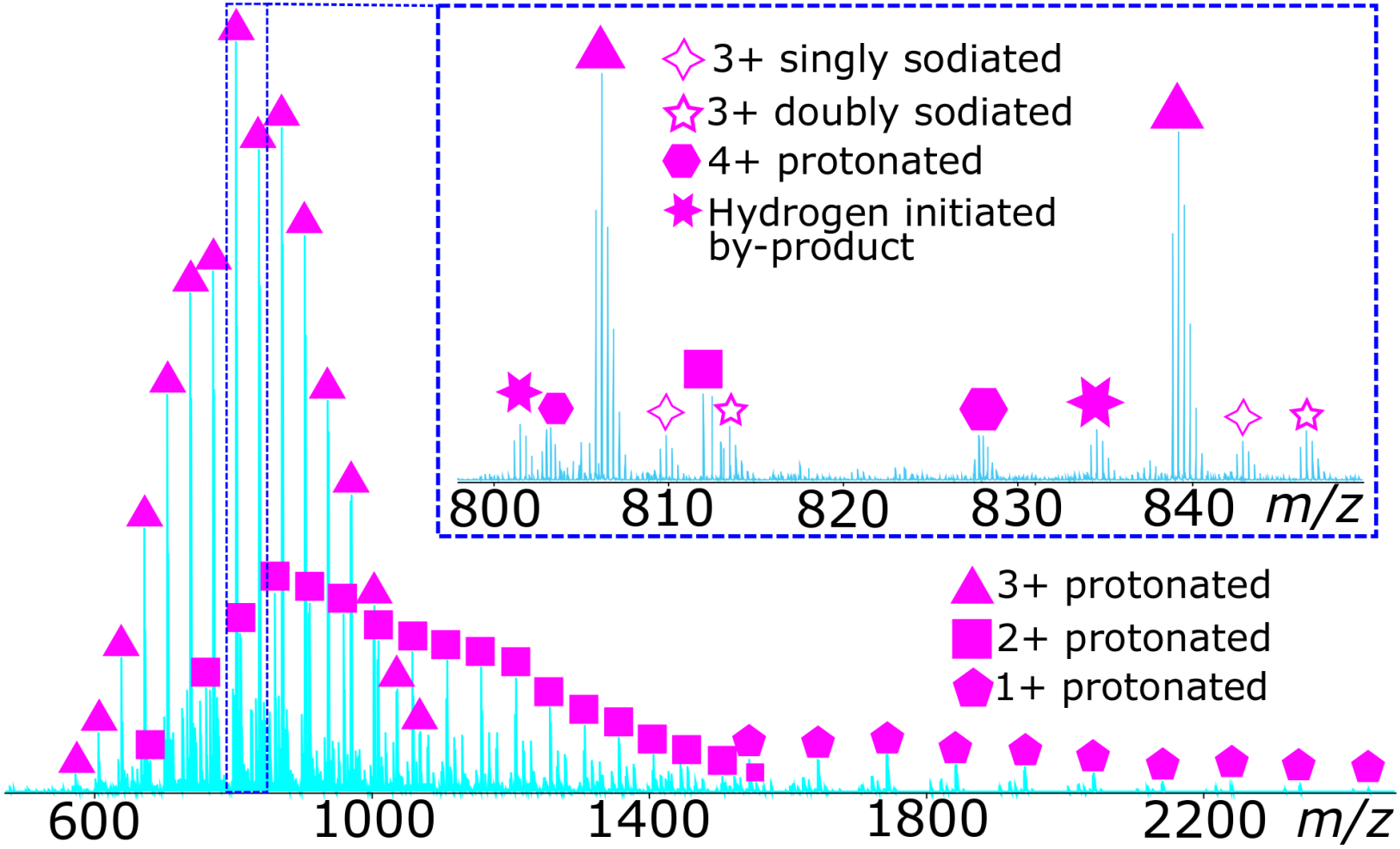
- Information dense 2D spectrum

Polymer analysis by 2D ECD

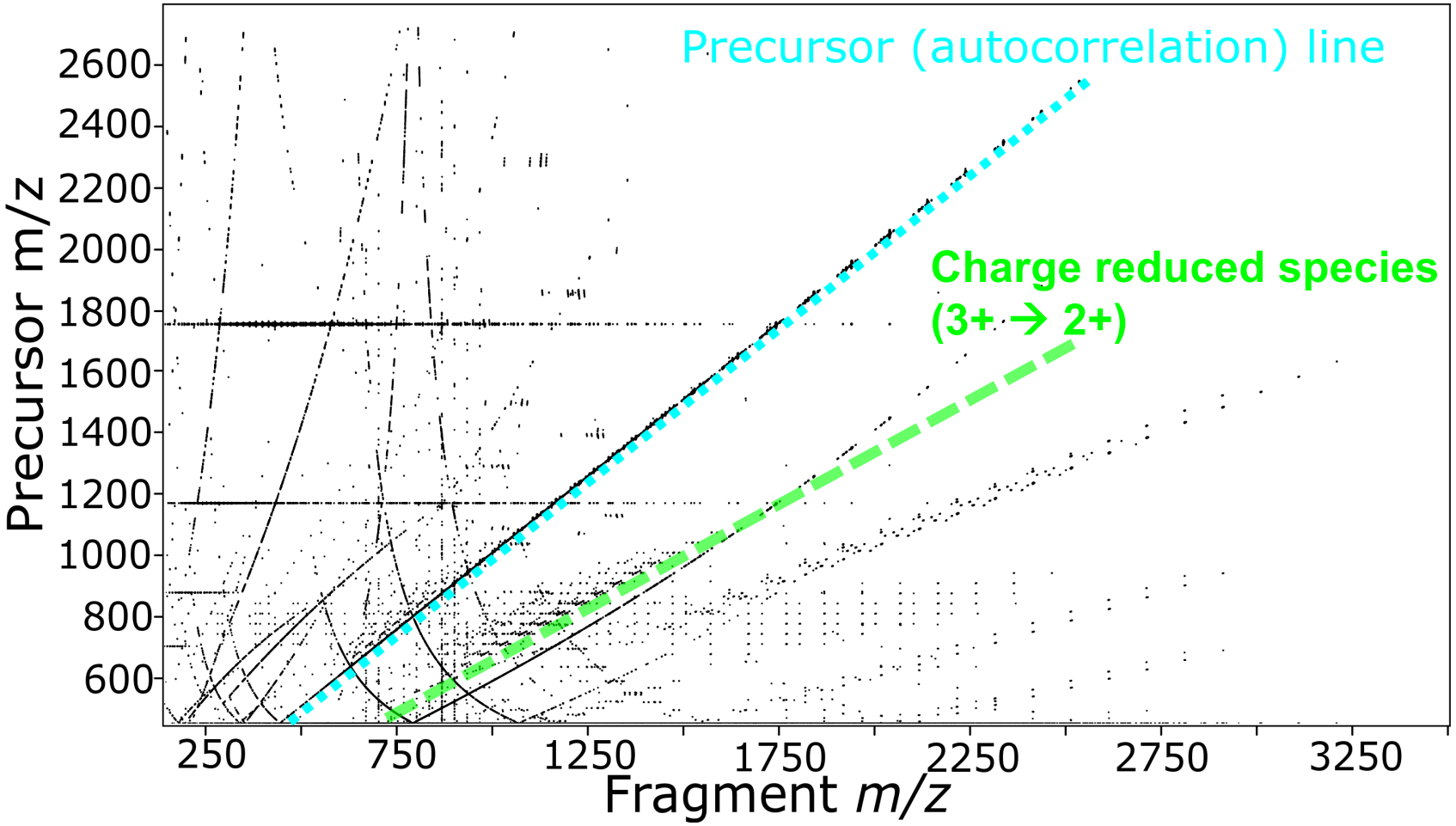


Polymer analysis by 2D ECD

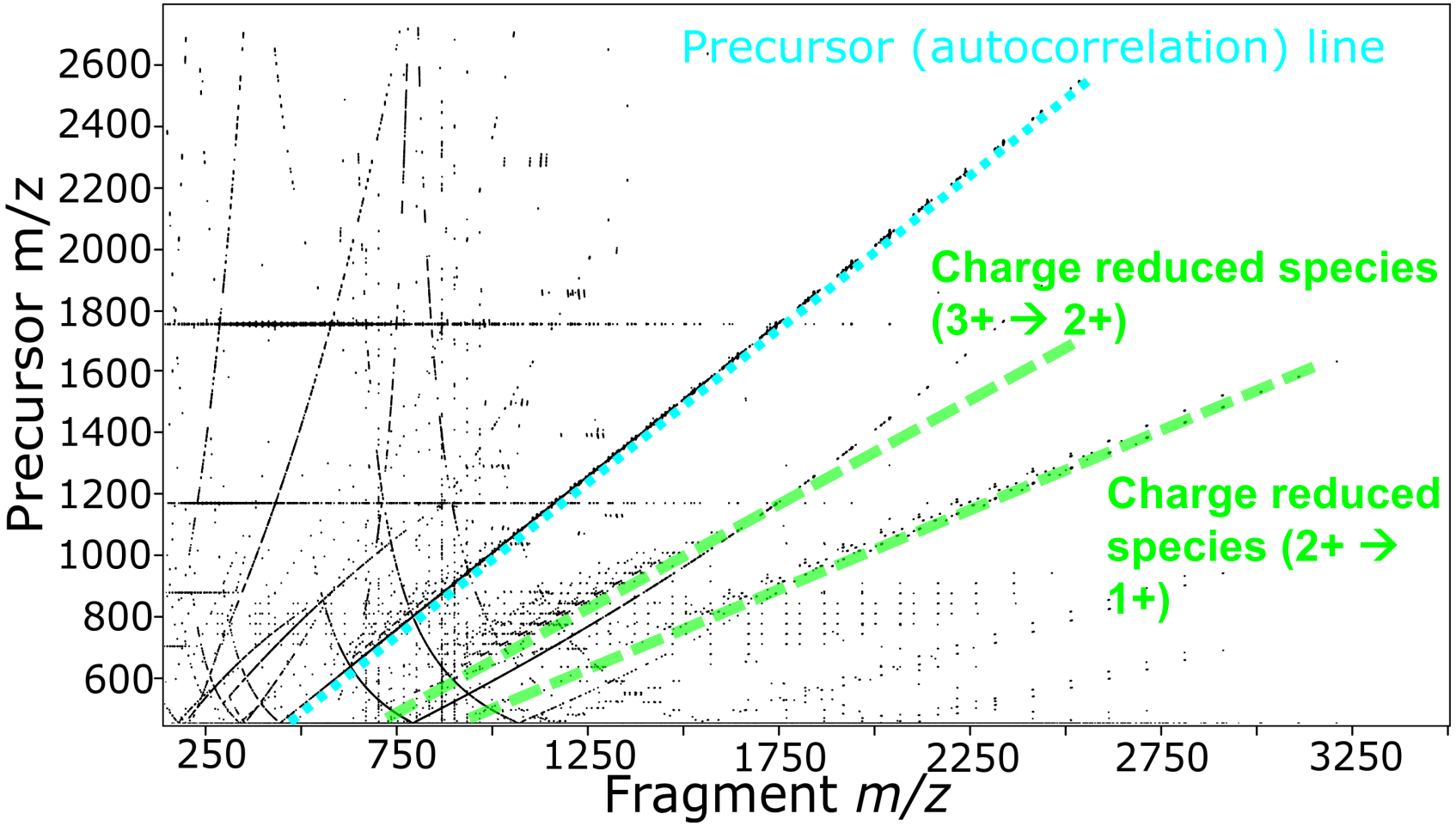
Autocorrelation line



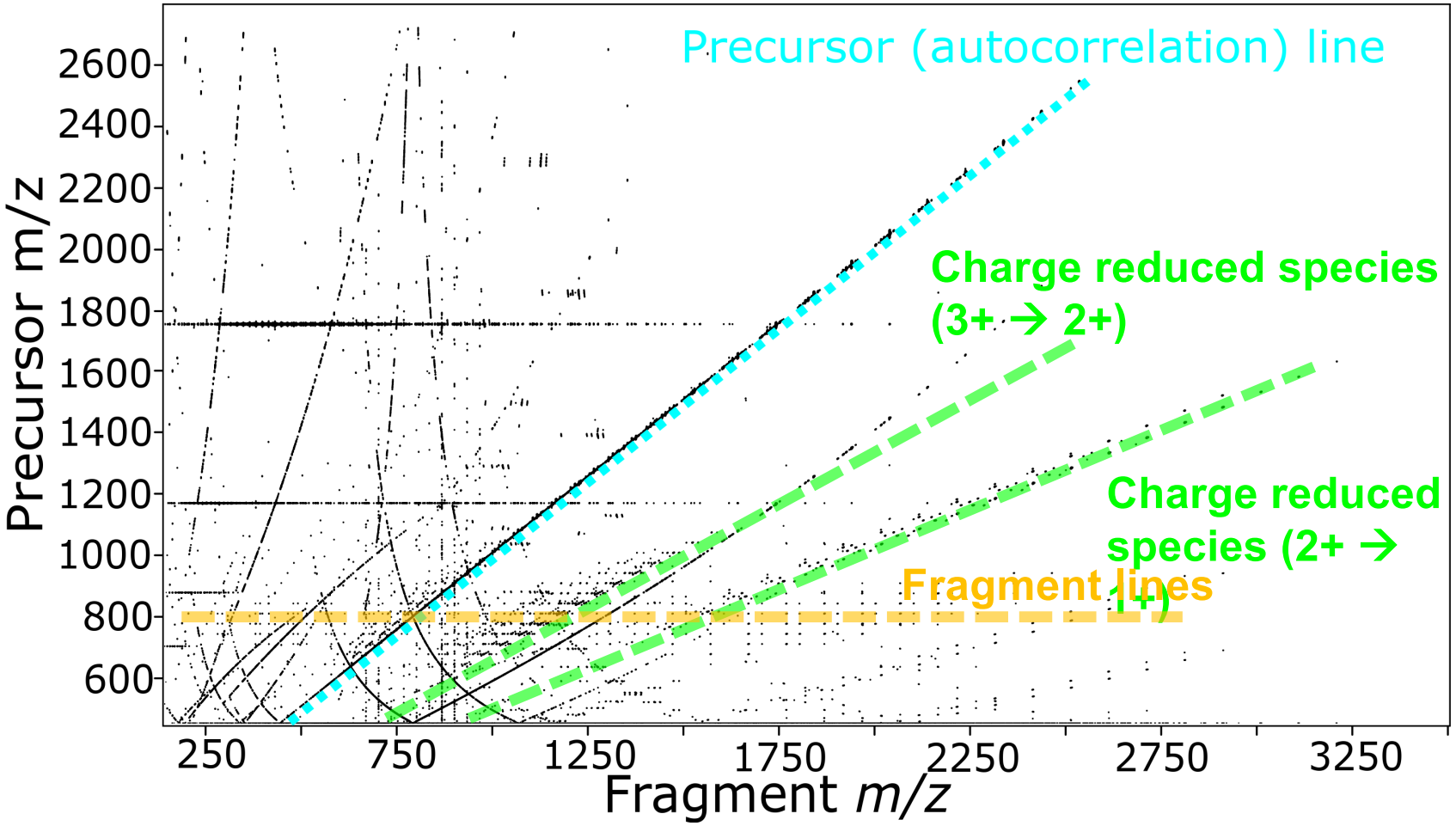
Polymer analysis by 2D ECD



Polymer analysis by 2D ECD

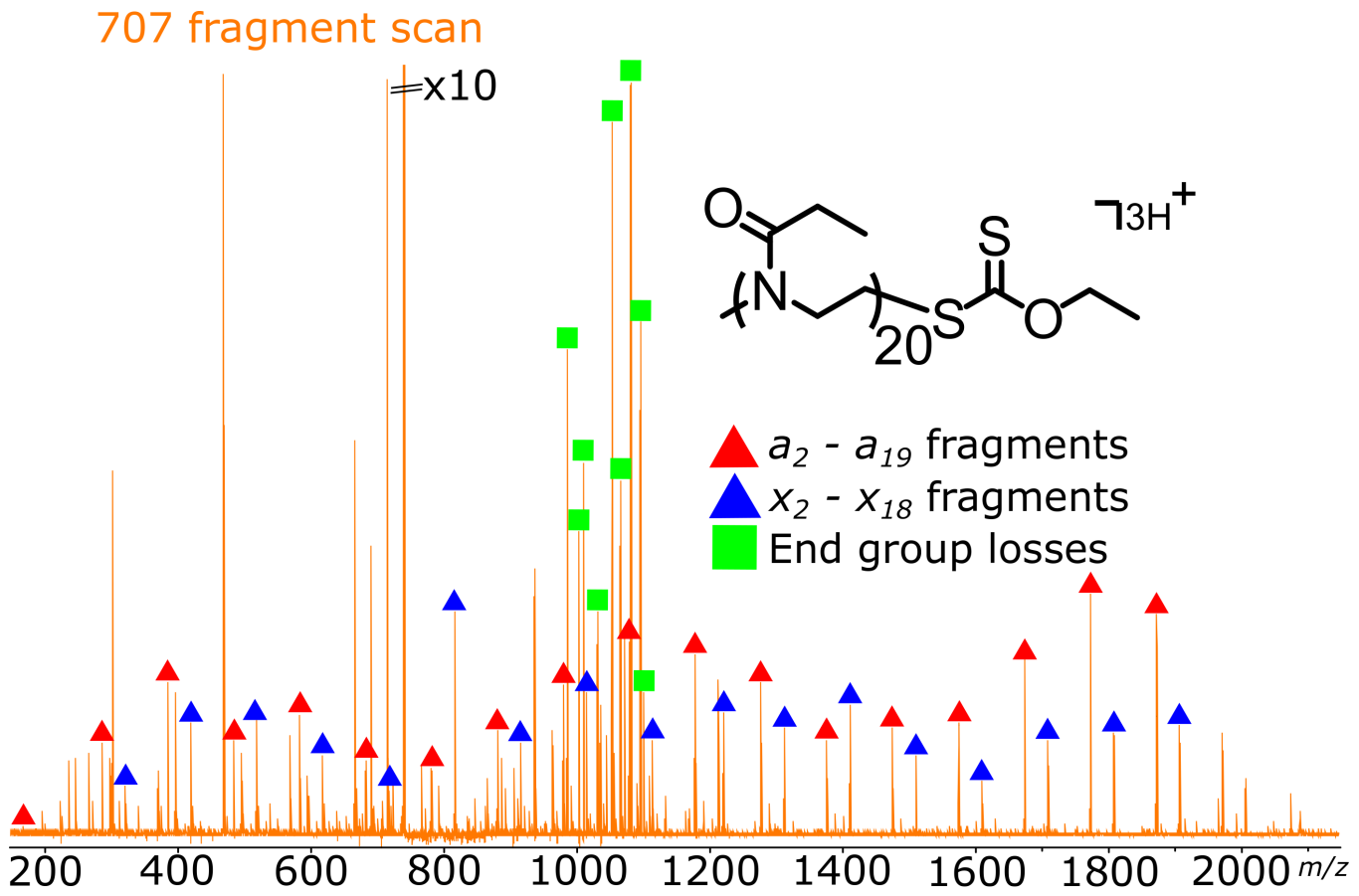


Polymer analysis by 2D ECD



Polymer analysis by 2D ECD

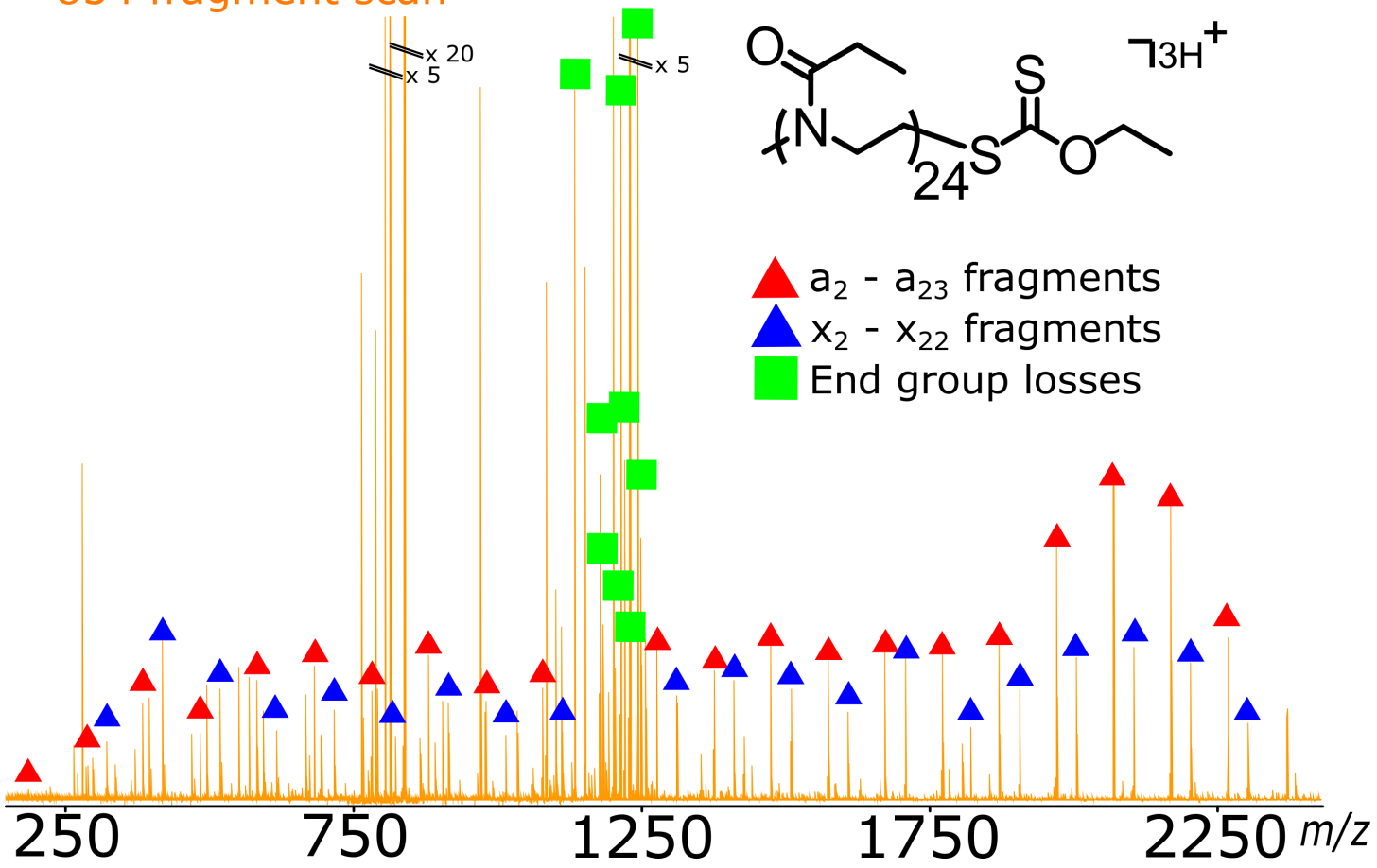
Fragment ion scans



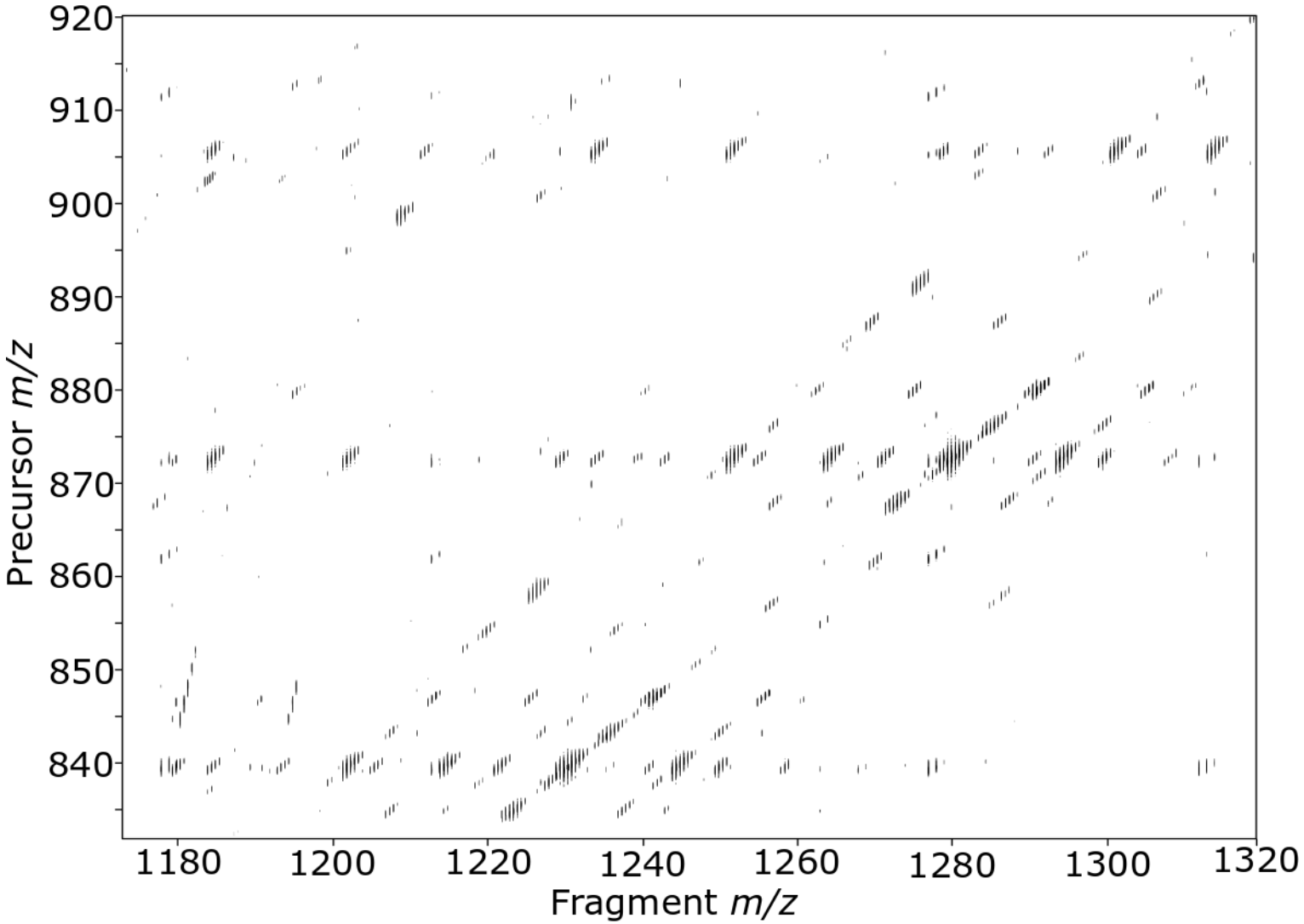
Polymer analysis by 2D ECD

Fragment ion scans

834 fragment scan



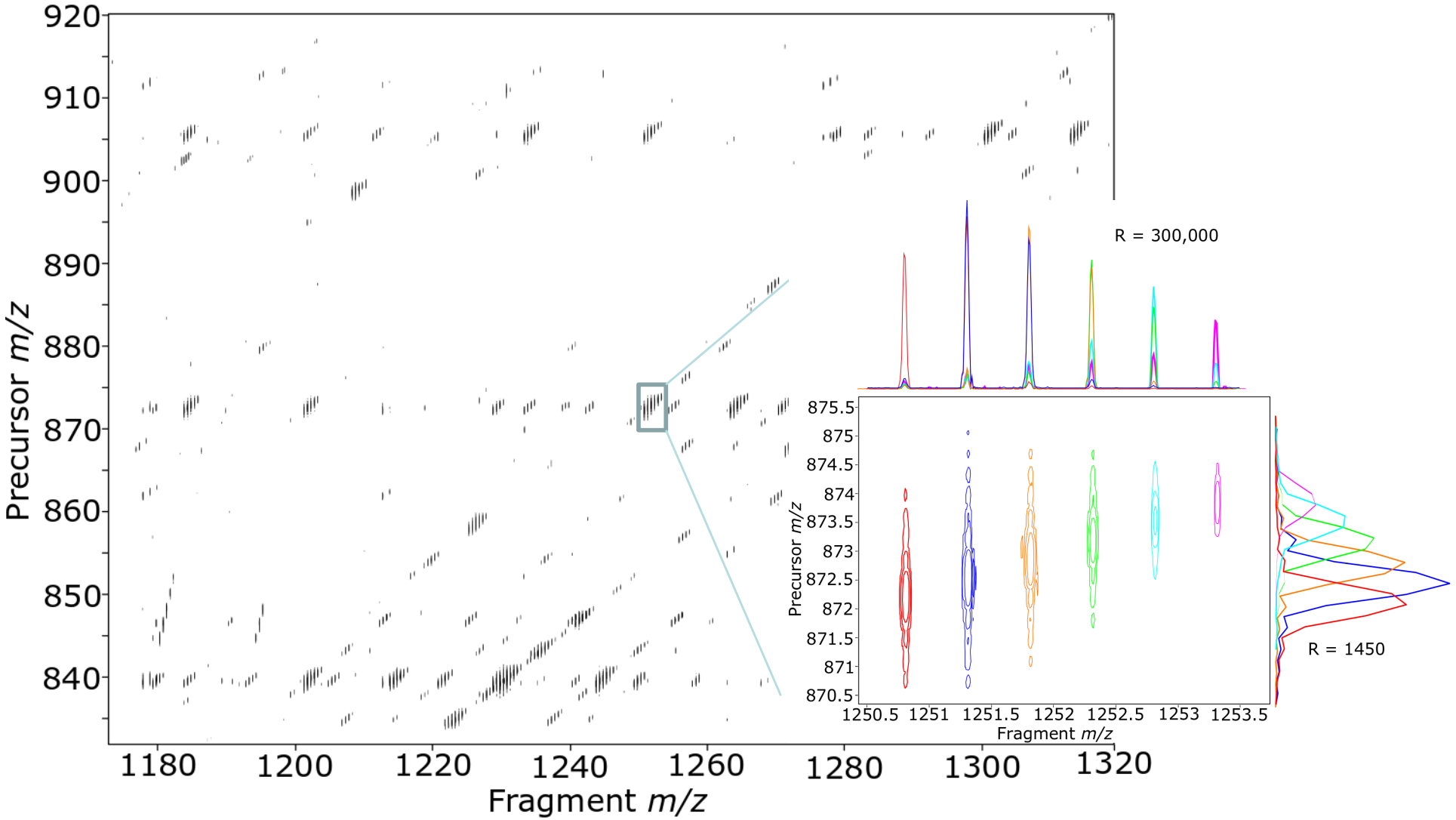
Polymer analysis by 2D ECD



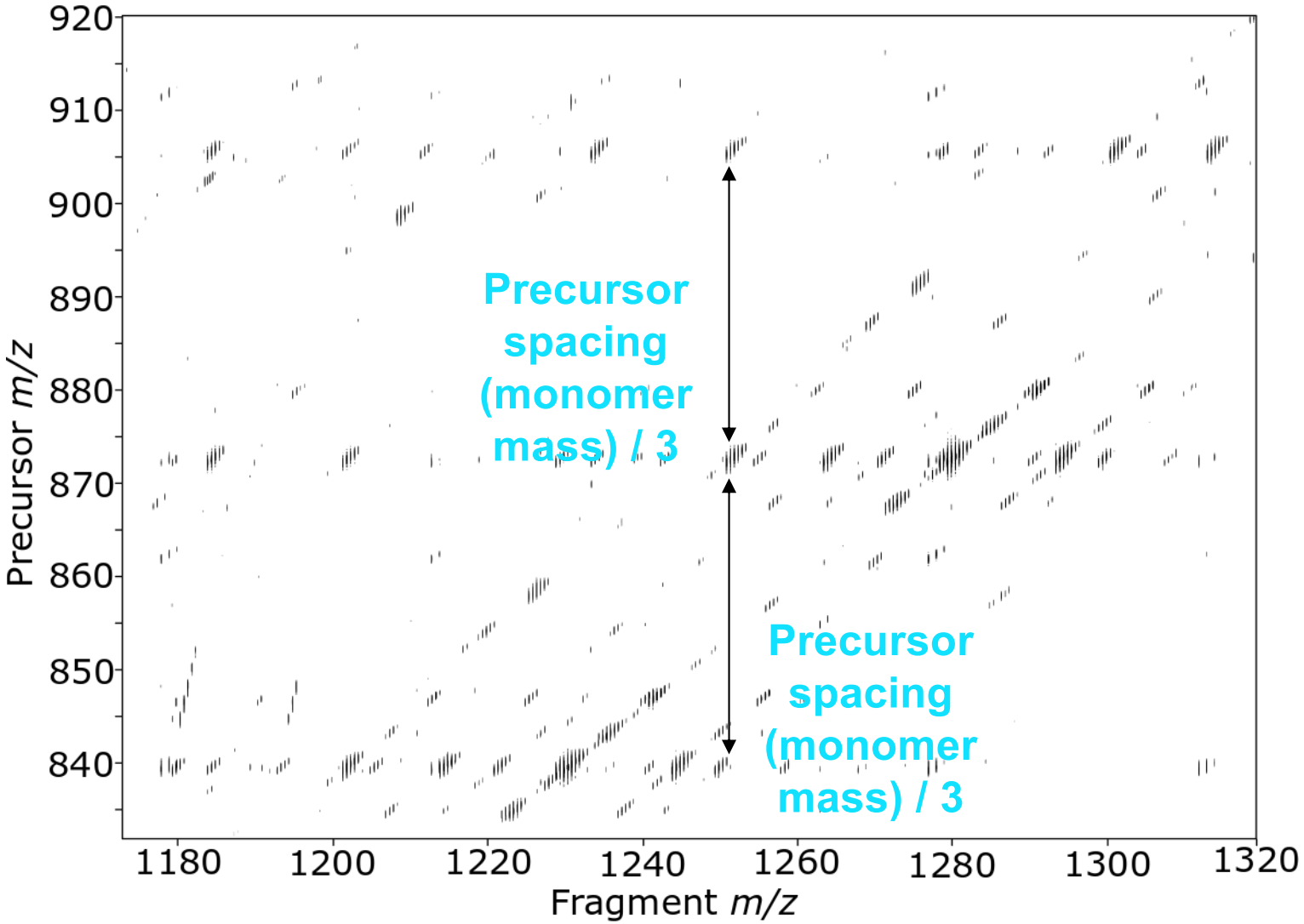
- Zoom in of region shows isotopic distribution

Polymer analysis by 2D ECD

Combined R = 435,000,000

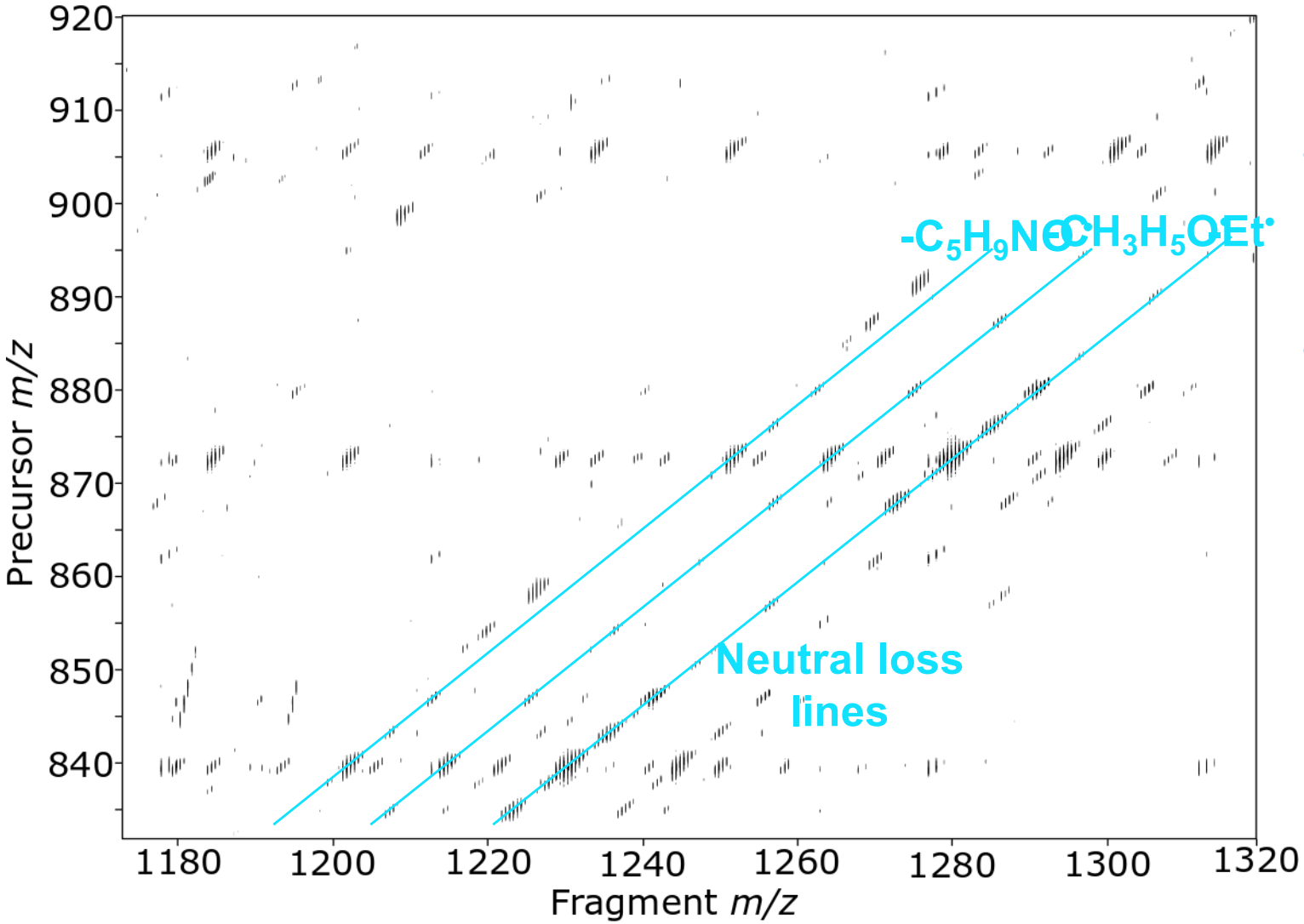


Polymer analysis by 2D ECD



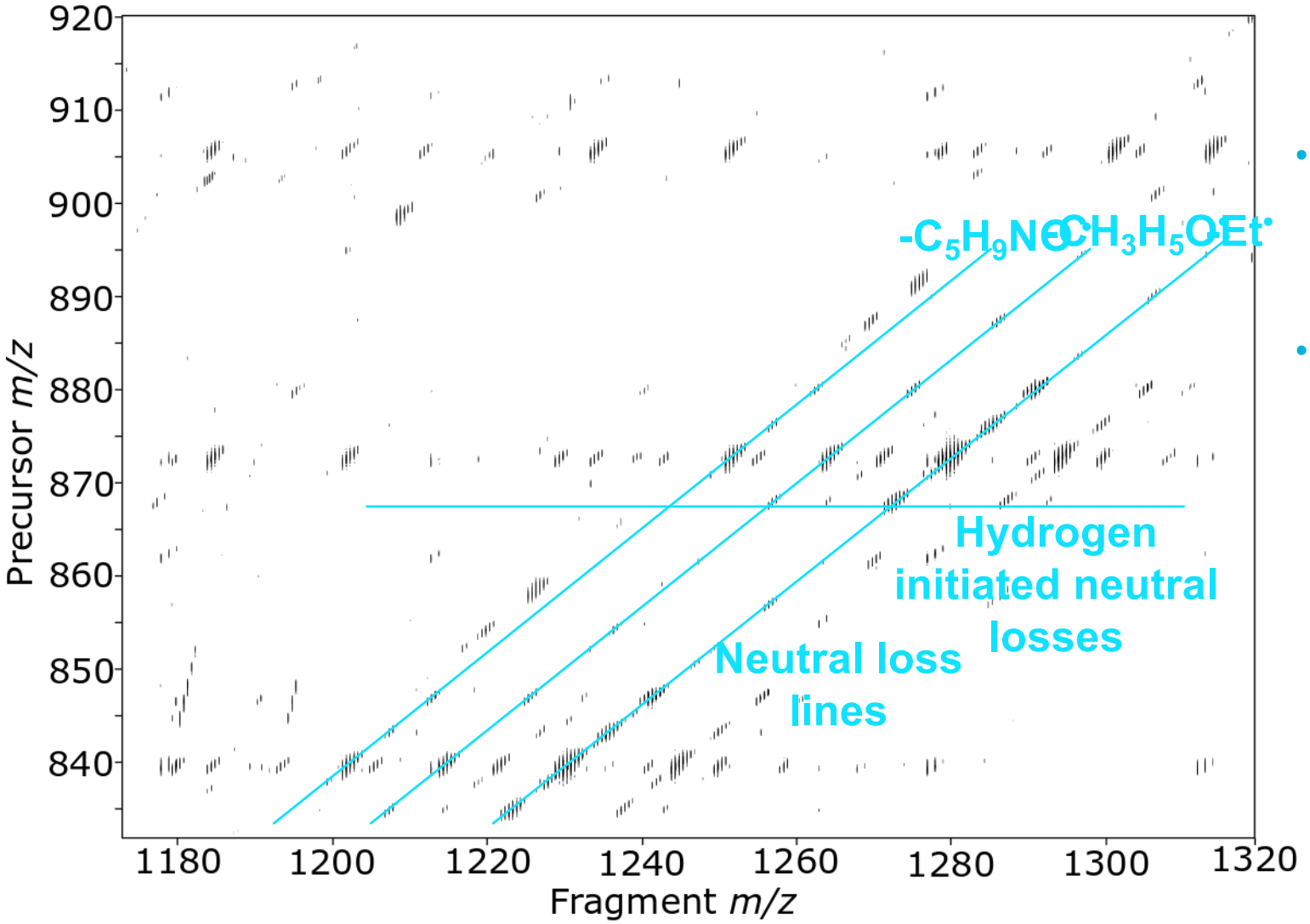
- Spacing of lines is equal to that of (monomer mass)/3 due to charge

Polymer analysis by 2D ECD



- Neutral losses from charged reduced species
- Fragments on the same intercept means the loss is equal from the charged reduced species

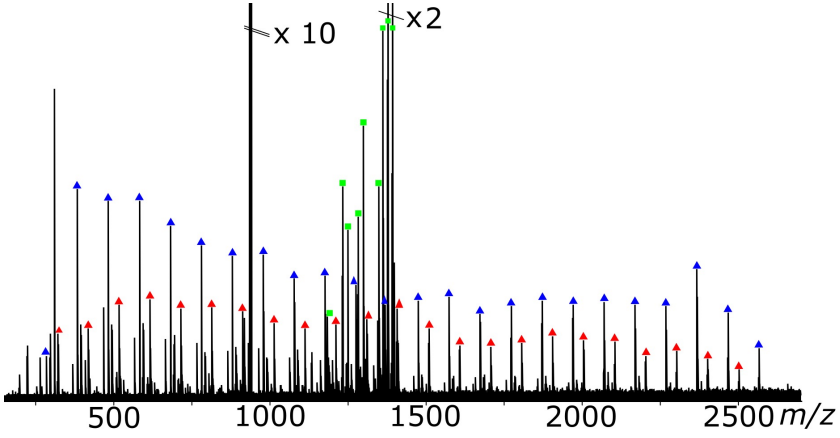
Polymer analysis by 2D ECD

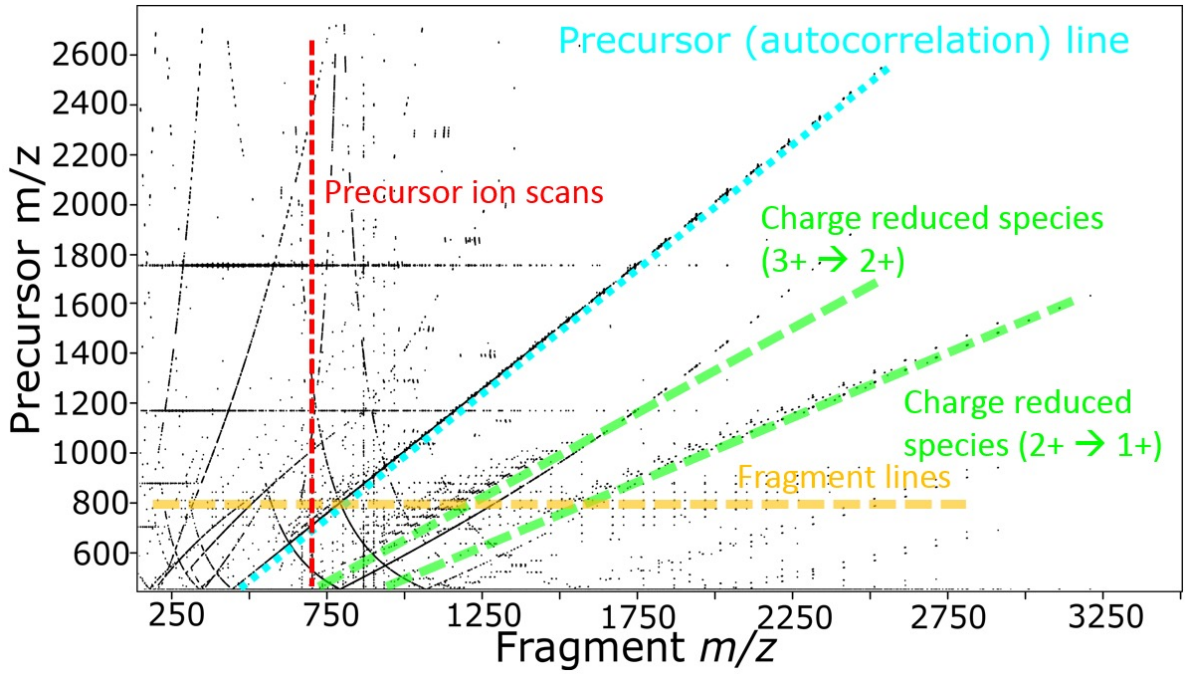


- Neutral losses from charged reduced species
- Fragments on the same intercept means the loss is equal from the charged reduced species

Conclusions

- ECD fragmentation can be used to analyze a polyoxazoline
- 2D mass analysis gives significant fragment information





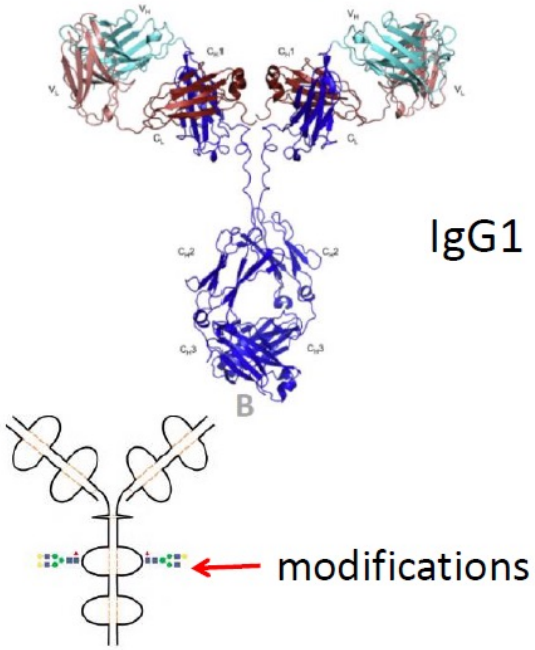
Lecture overview

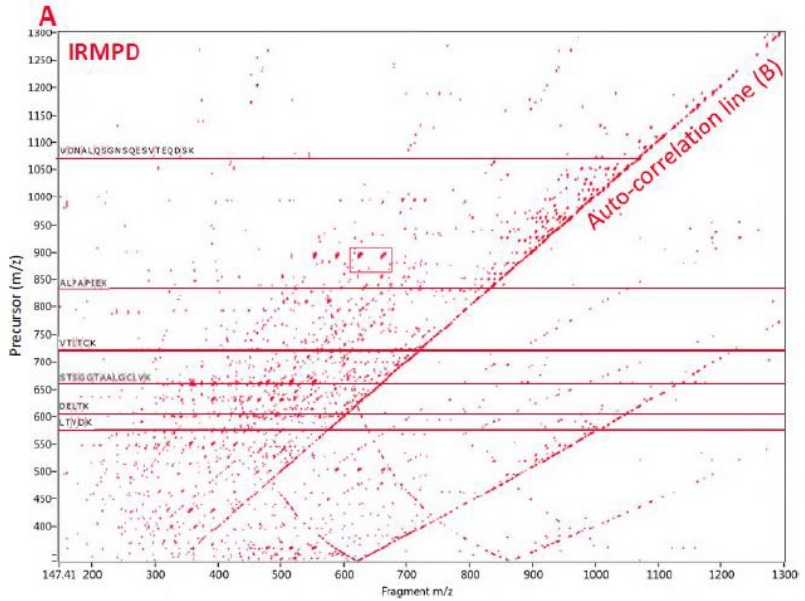
- 2DMS for agrichemical analysis.
- 2DMS for polymer analysis.
- 2DMS for monoclonal antibodies.
- Distinguishing phosphopeptides using 2DMS
- 2DMS – UVPD
- 2DMS on a linear ion trap

Case study of 2DMS with UCB

Antibody modification analysis

Antibody 2DMS





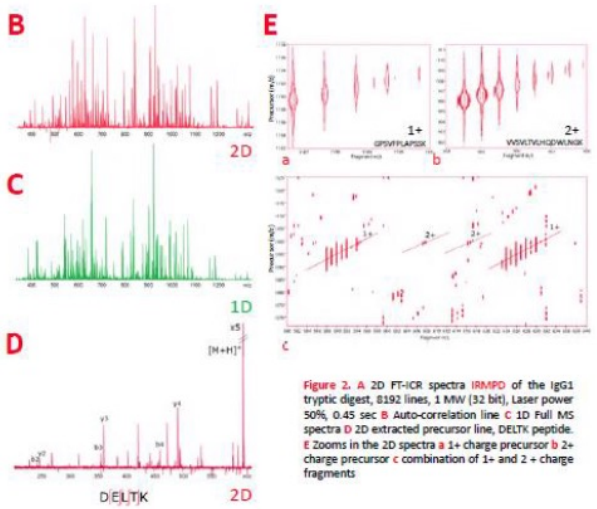


Figure 2. A 2D FT-ICR spectra IRMPD of the IgG1 tryptic digest, 8192 lines, 1 MW (32 bit), Laser power 50%, 0.45 sec B Auto-correlation line C 1D Full MS spectra D 2D extracted precursor line, DELTK peptide. E Zooms in the 2D spectra a 1+ charge precursor b 2+ charge precursor c combination of 1+ and 2 + charge fragments

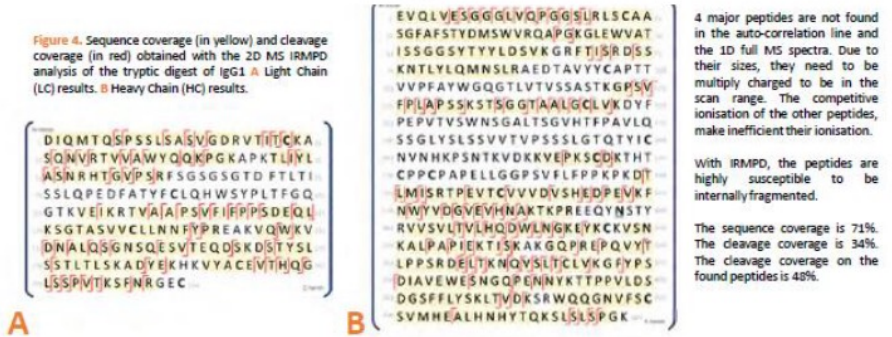


Figure 4. Sequence coverage (in yellow) and cleavage coverage (in red) obtained with the 2D MS IRMPD analysis of the tryptic digest of IgG1 A Light Chain (LC) results. B Heavy Chain (HC) results.

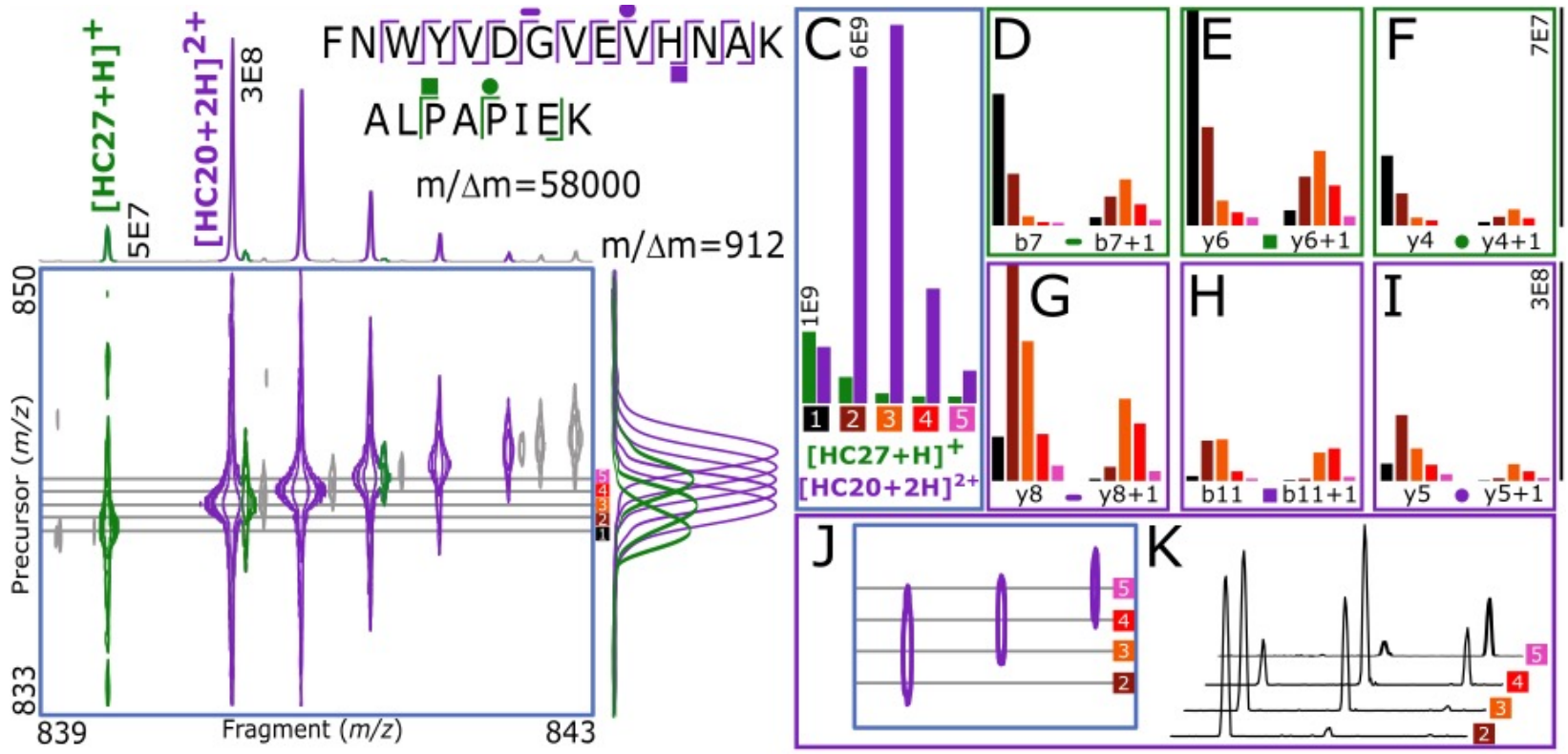
4 major peptides are not found in the auto-correlation line and the 1D full MS spectra. Due to their sizes, they need to be multiply charged to be in the scan range. The competitive ionisation of the other peptides, make inefficient their ionisation.

With IRMPD, the peptides are highly susceptible to be internally fragmented.

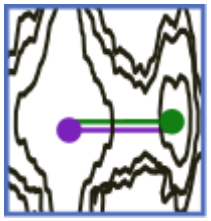
The sequence coverage is 71%. The cleavage coverage is 34%. The cleavage coverage on the found peptides is 48%.

Antibodies are large, complex biomolecules which are often biologically modified for function (good).
But also get modified/degrade during bio-production, leading to either little/no change (good)
Loss of function (bad)
Or different function (possibly very bad)
2DMS can handle complex samples where LC often fails

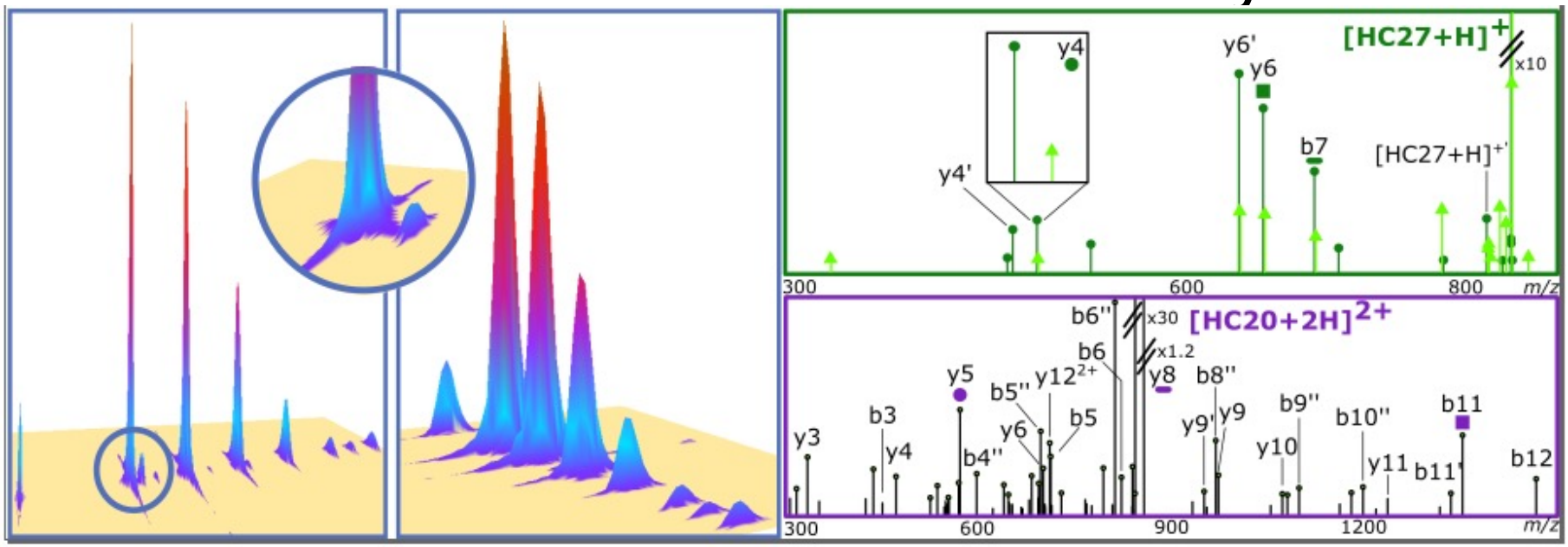
Extracted Lines Resolution



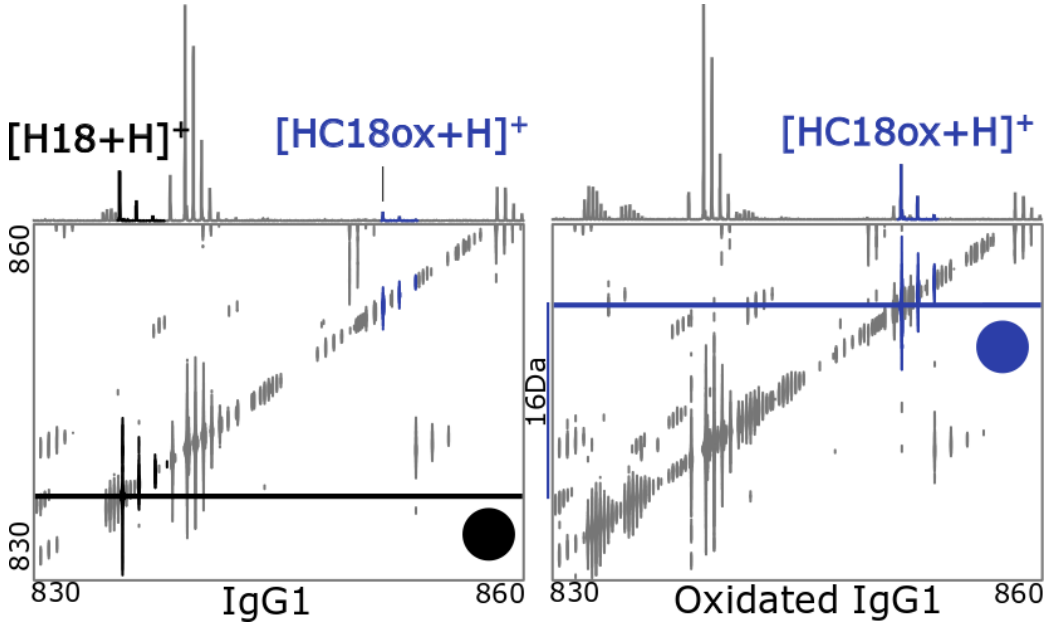
Peak Picking Resolution

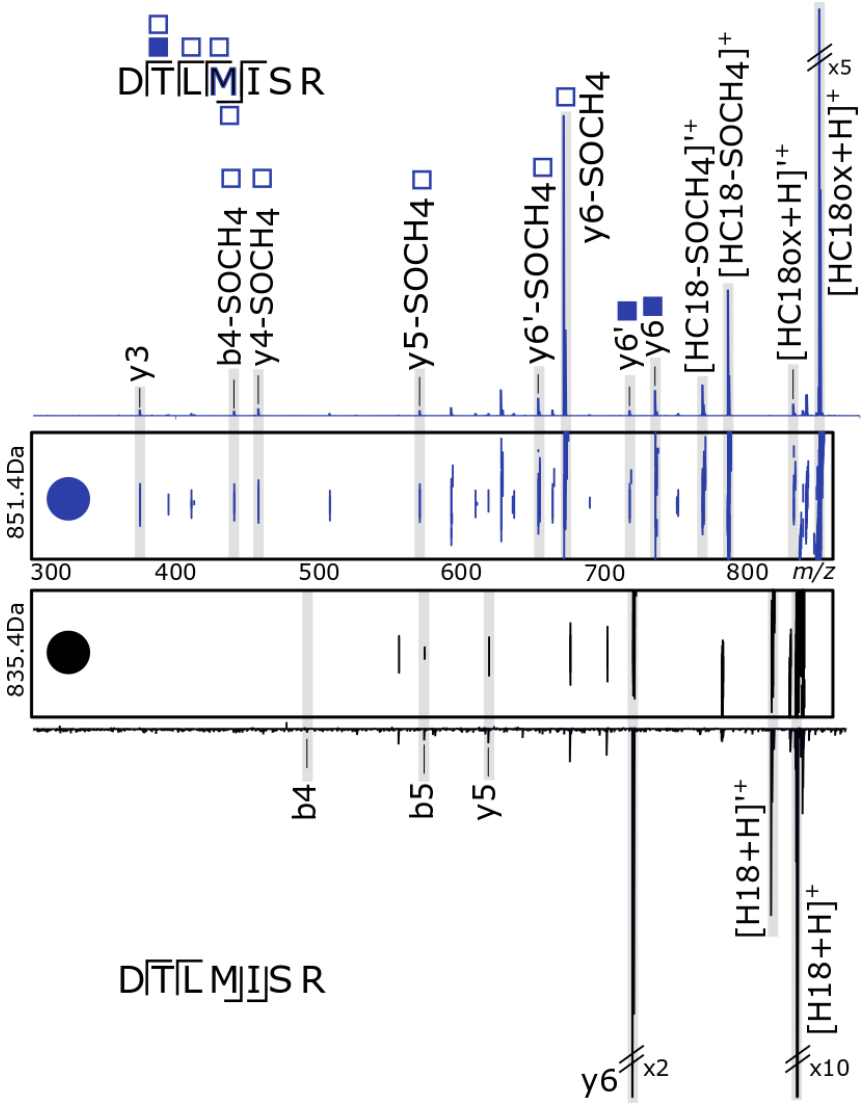


- Δx: 0.094 *m/z*
- Δy: 0.089 *m/z*



PTM Detection -Antibody





Lecture overview

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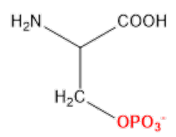
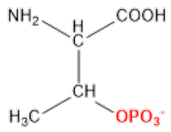
- 2DMS on a linear ion trap

Phosphorylation

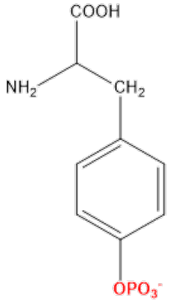
Phosphoserine



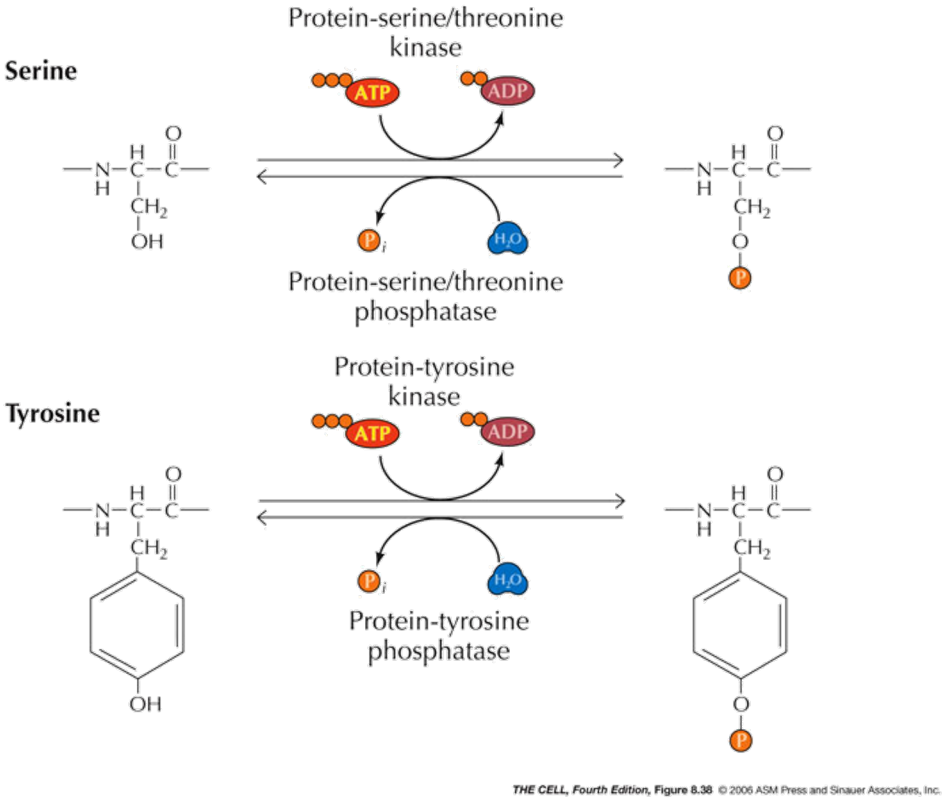
Phosphothreonine



Phosphotyrosine

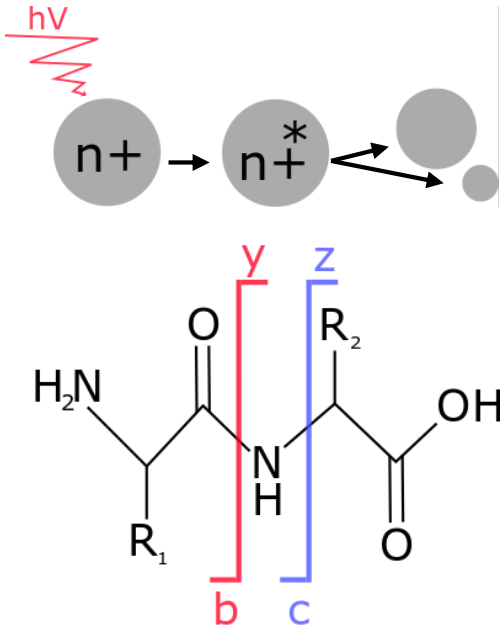


- Serine, Threonine or Tyrosine
- Phosphoester linkage.
- Regulation of intercellular processes
- Cell signalling pathway
- Change activity, subcellular location, degradation and interaction with other proteins
- Limit of RP-LC: the phosphopeptide loss during the sample preparation or the liquid chromatography



THE CELL, Fourth Edition, Figure 8.38 © 2006 ASM Press and Sinauer Associates, Inc.

InfraRed Multi-Photon Dissociation



- CO_2 laser (10.6 μm photons)
- Resonance with the P-O stretch of the phosphate (9.6-11 μm)

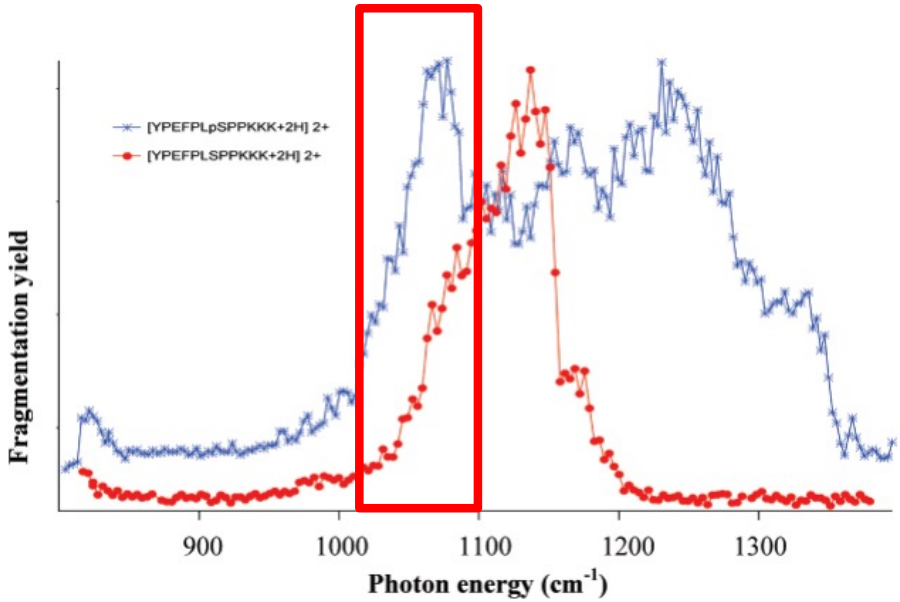


Figure. IRMPD spectra. Fragmentation efficiencies defined as -ln(P/F + P), with F being the sum of fragment abundances and P the abundance of the intact precursor.

Correia, C. F.; Balaj, P. O.; Scuderi, D.; Maitre, P.; Ohanessian, G. *J. Am. Chem. Soc.* 2008, *130*, 3359-3370.

2D MS IRMPD Analysis of Phosphopeptides

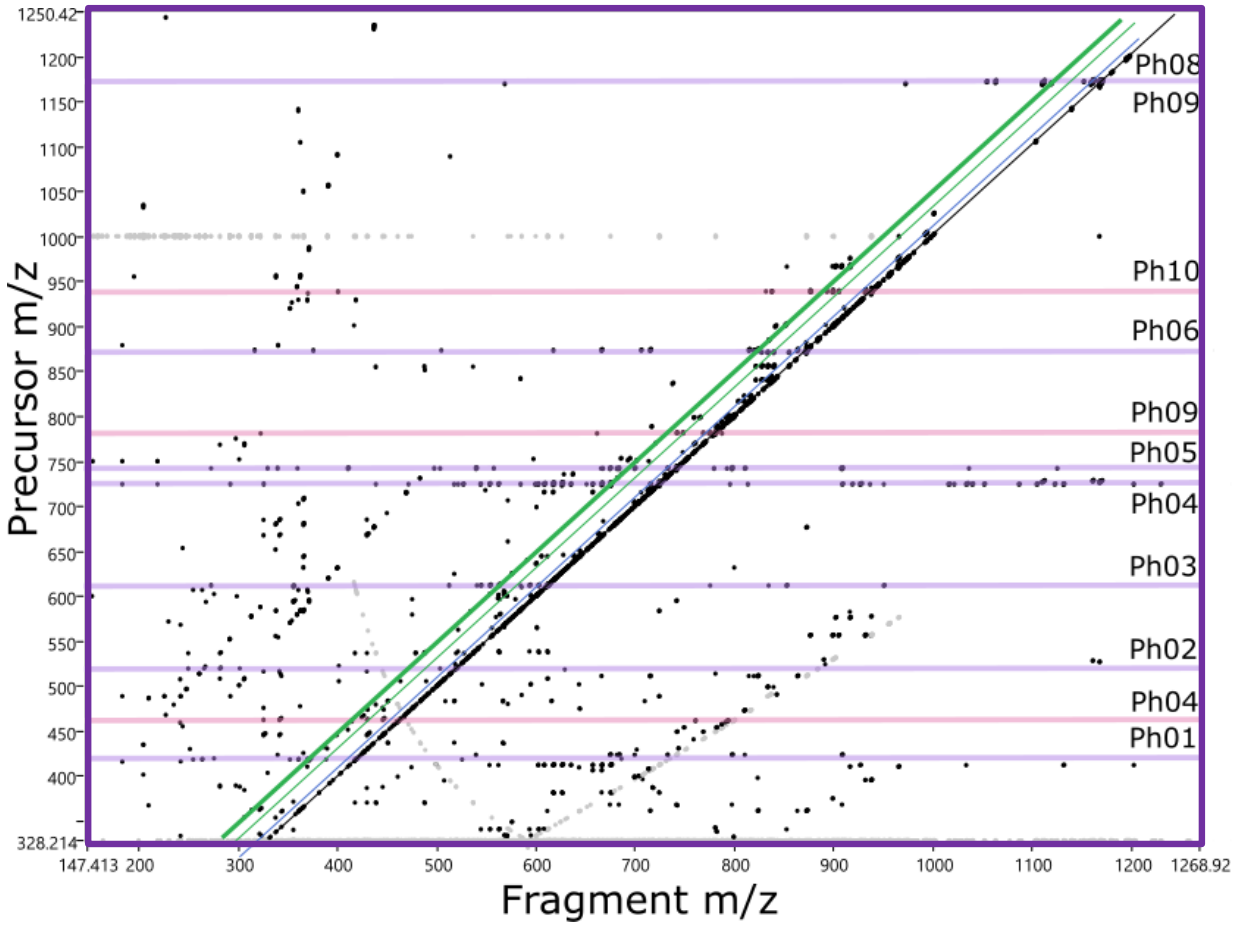


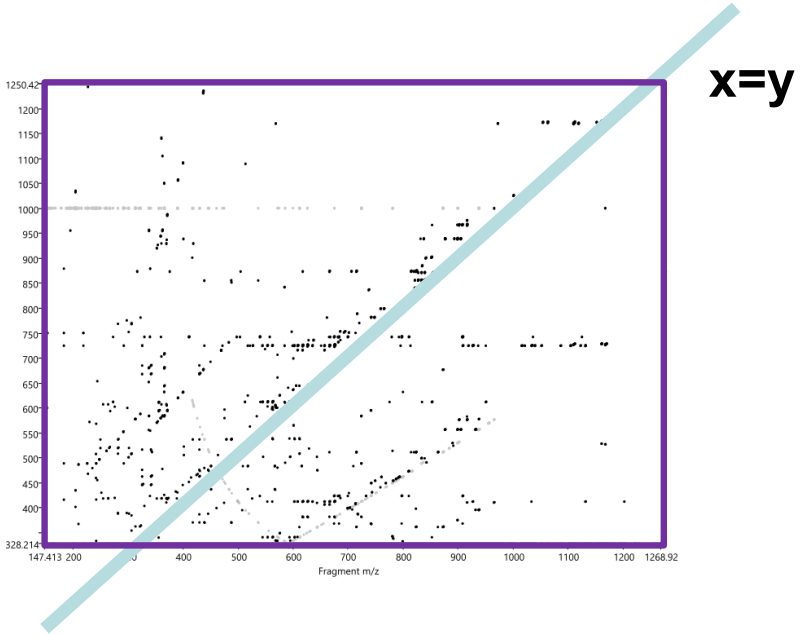
Figure. 2D FT-ICR spectra IRMPD of the Phosphomix, y: 8192 lines, x: 1 megapoints

Direct infusion.
10 Phosphopeptides.
No prior separation.

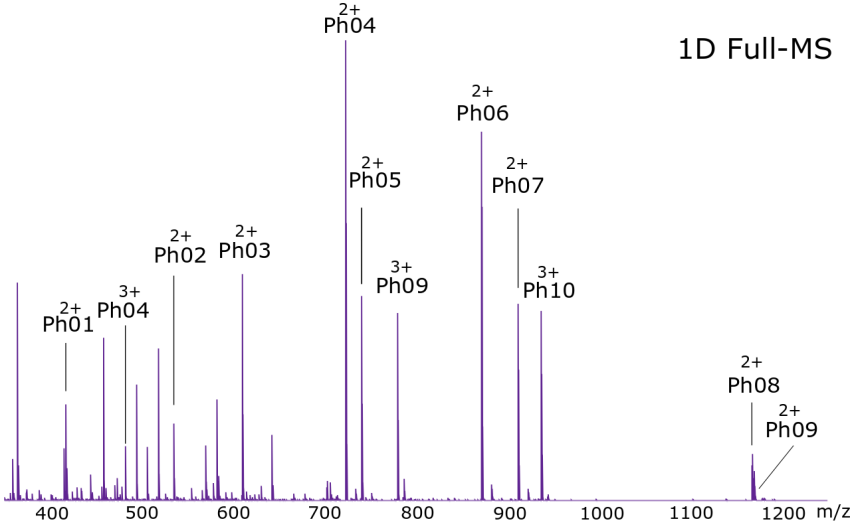
In pink and purple, precursor lines.
In black, auto-correlation line.
In blue and green, neutral loss lines.

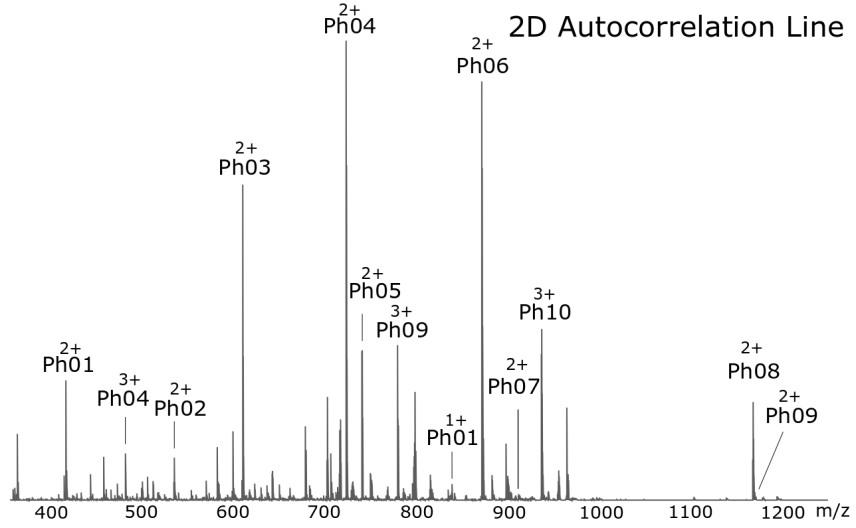
Horizontal: 1,048,576k transients (broadband)
Vertical: 8,192 scan lines.
Total: 8,589,934,592 data points

Comparison 1D/2D Full MS

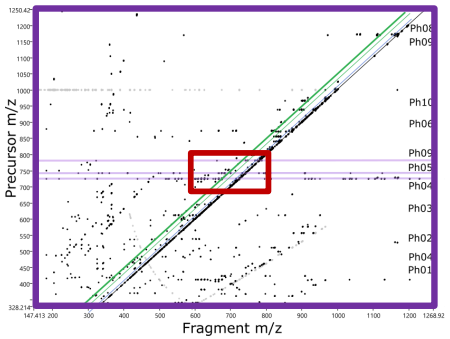


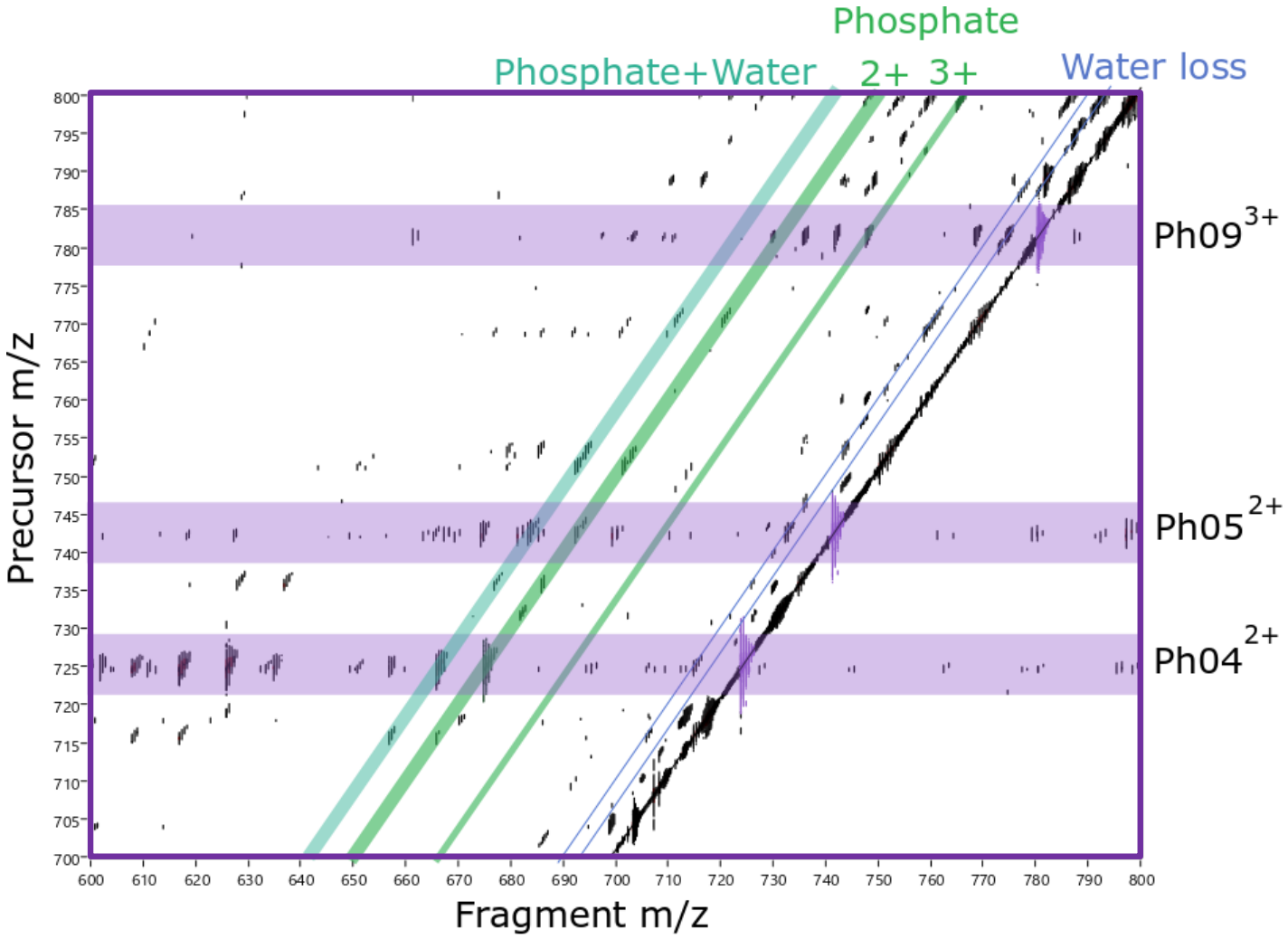
Loss of Ph07
Hydrophobic peptide



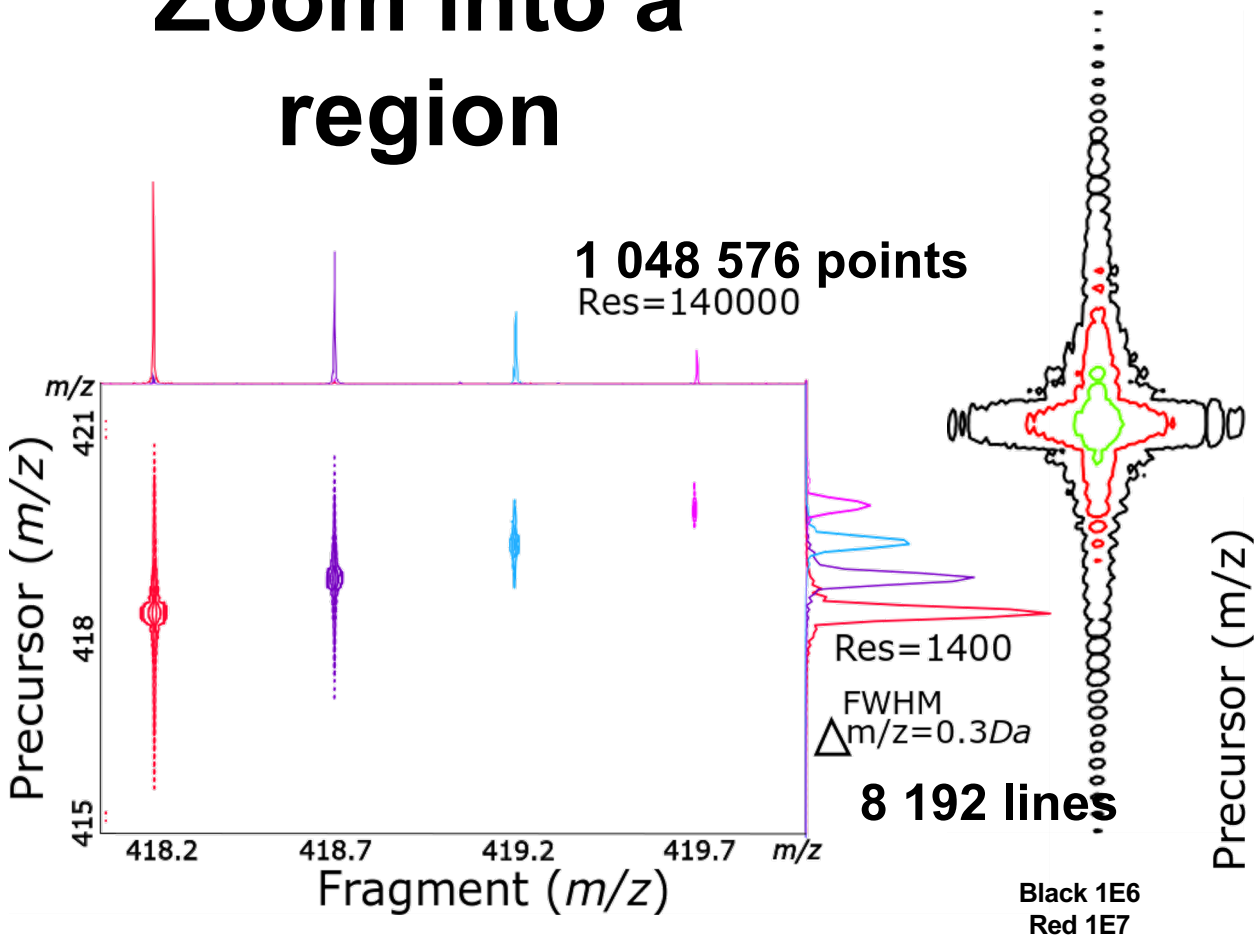


Zoom into a region

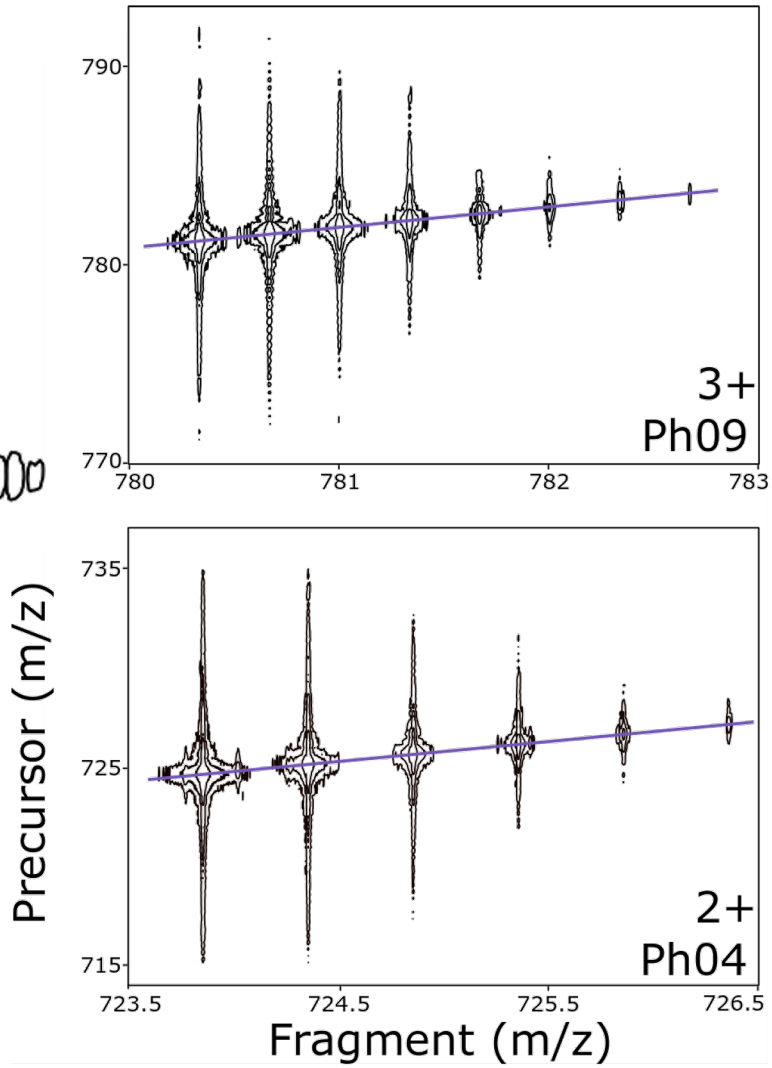




Zoom into a region

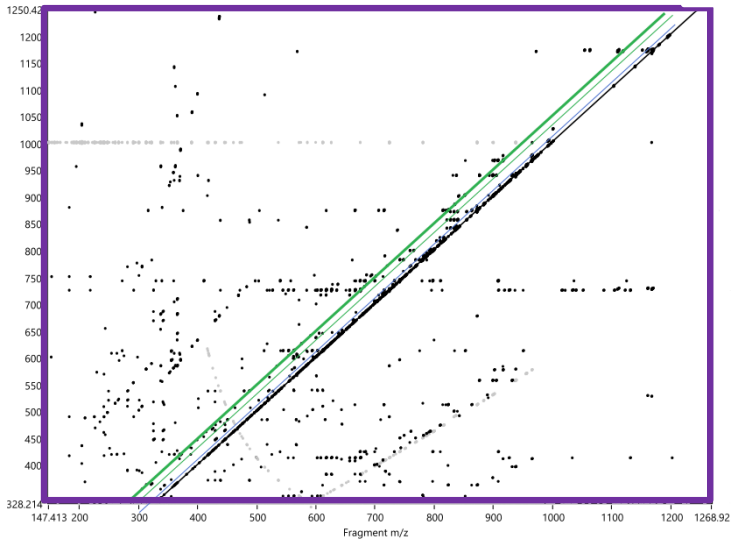


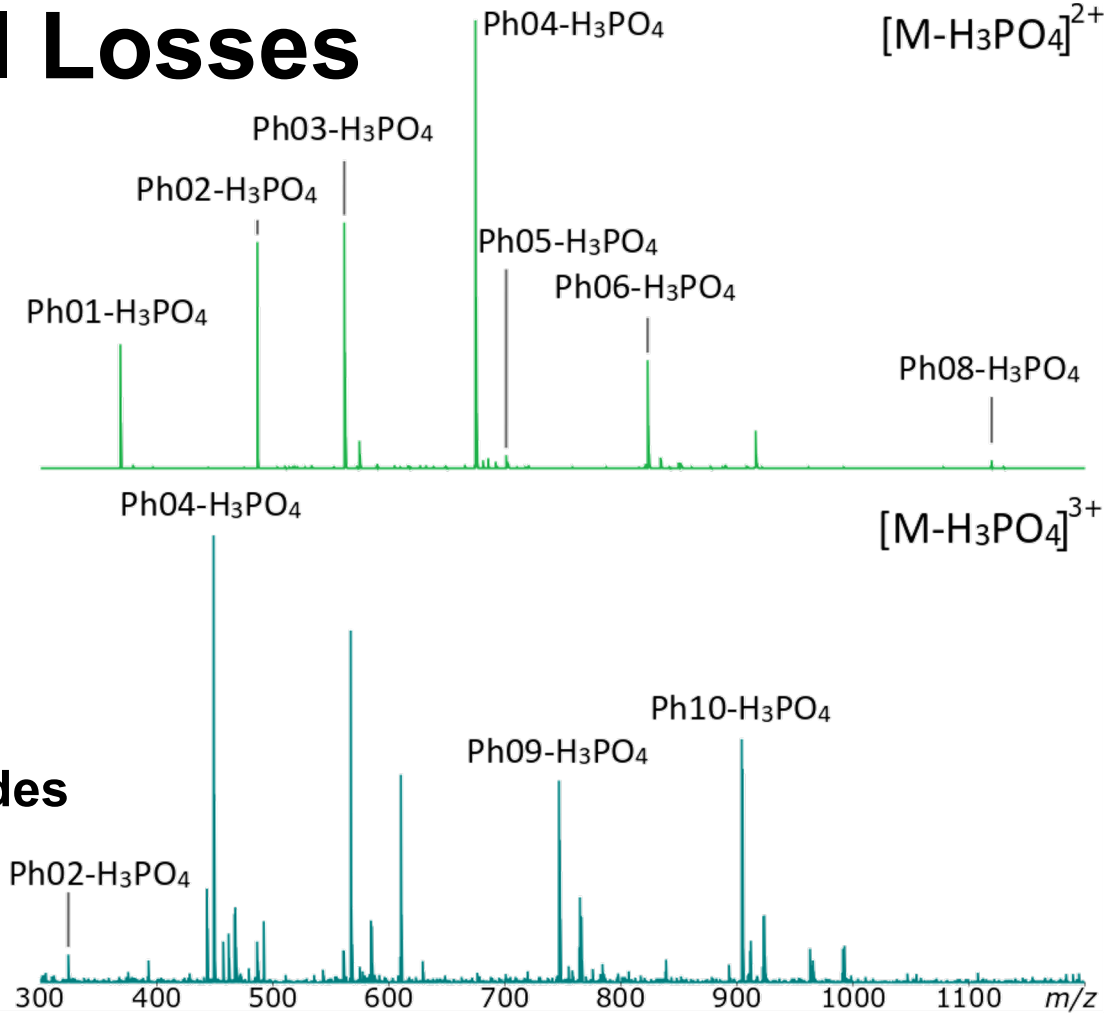
Black 1E6
Red 1E7
Green 1E8
Threshold



Horizontal: 1,048,576k transients (broadband)
Vertical: 8,192 scan lines.
Total: 8,589,934,592 data points

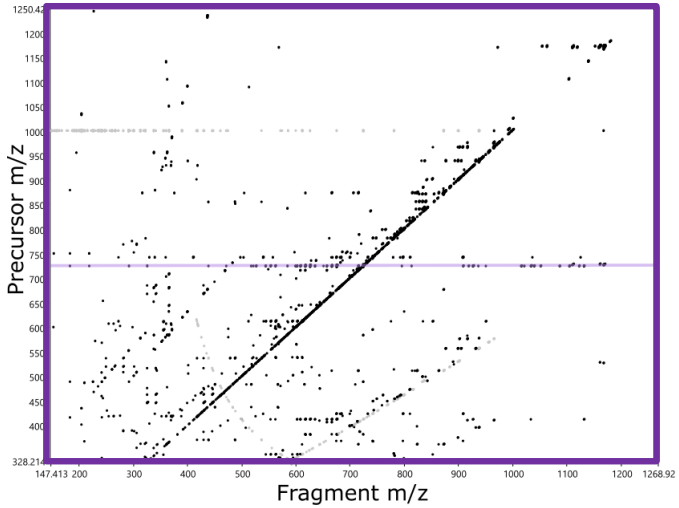
Phosphate Neutral Losses



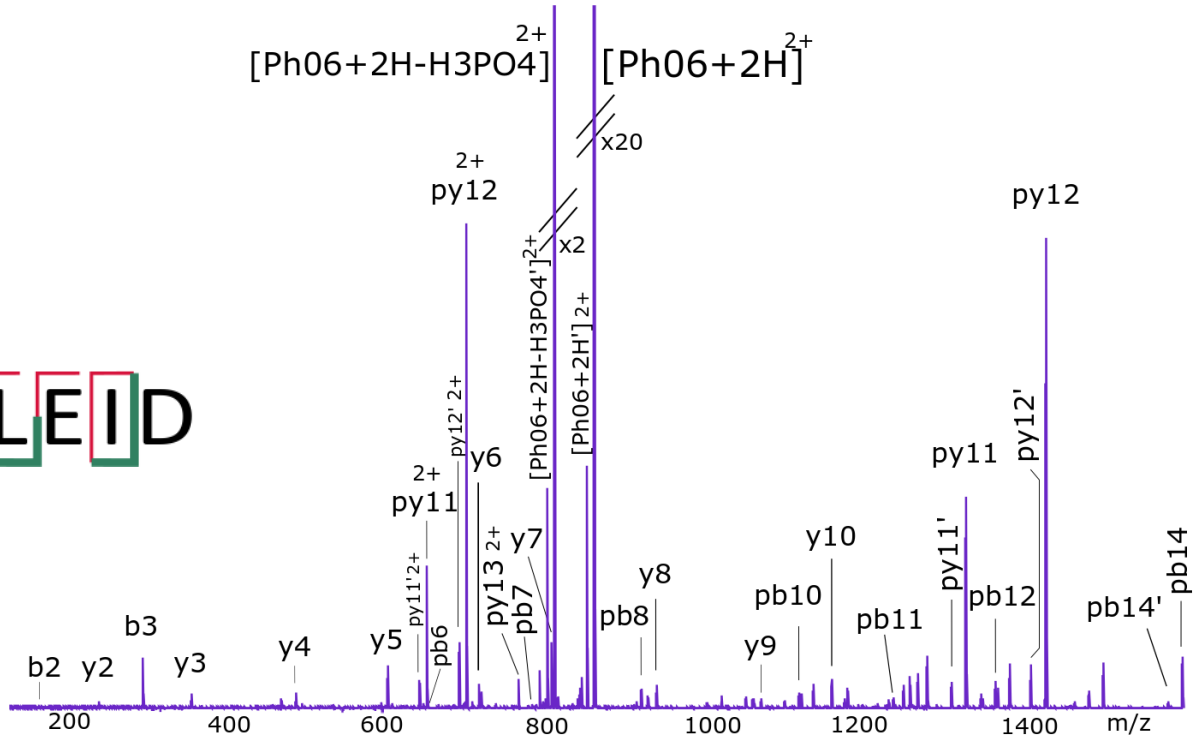


Detection of the Phosphopeptides

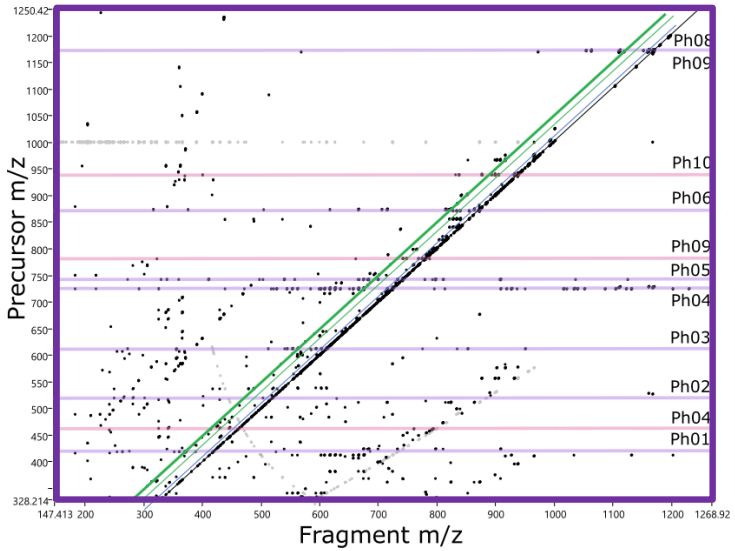
Extracted fragment Line of Ph06



In red: fragments without Phosphate
In green: fragments with Phosphate



All Results



Advantages of 2DMS strategy:

Direct infusion
Quick identification with neutral loss lines
IRMPD selectively fragment phosphopeptides

Fragmentation of the Phosphopeptides

In red fragment without Phosphate
In green fragment with Phosphate

Ph01 V L H S G pS R

Ph02 R S pY pS R S R

Ph03 R D S L G pT Y S S R

Ph04 pT K L I pT Q L R D A K

Ph05 E V Q A E Q P S S pS S P R

Ph06 A D E P pS S E E S D L E I D K

Ph08 F E D E G A G F E E S pS E T G D Y E E K

Ph09 E L S N pS P L R E N S F G pS P L E F R

Ph10 S P T E Y H E P V pY A N P F Y R P T pT P Q R

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- 2DMS for monoclonal antibodies.
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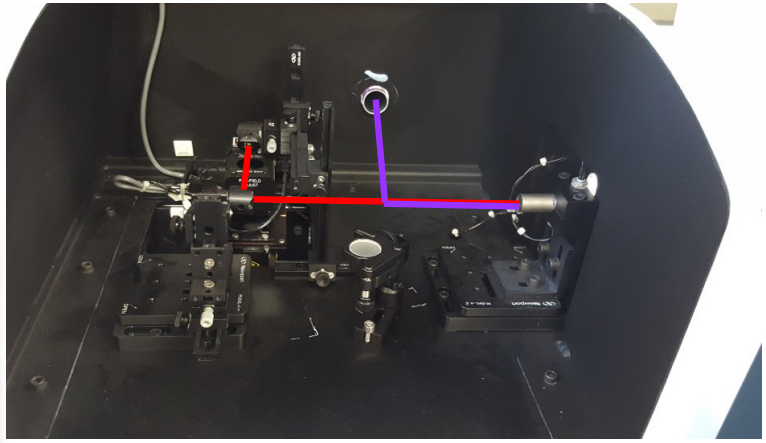
- 2DMS – UVPD
- 2DMS on a linear ion trap

solariX – Laser Setup

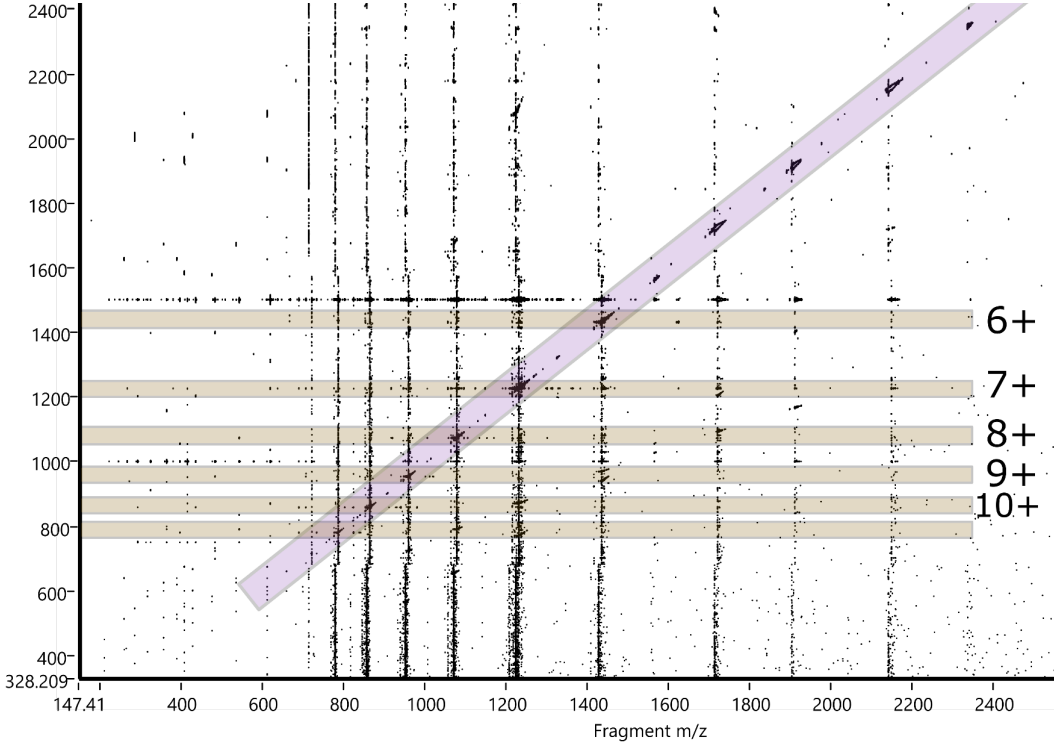


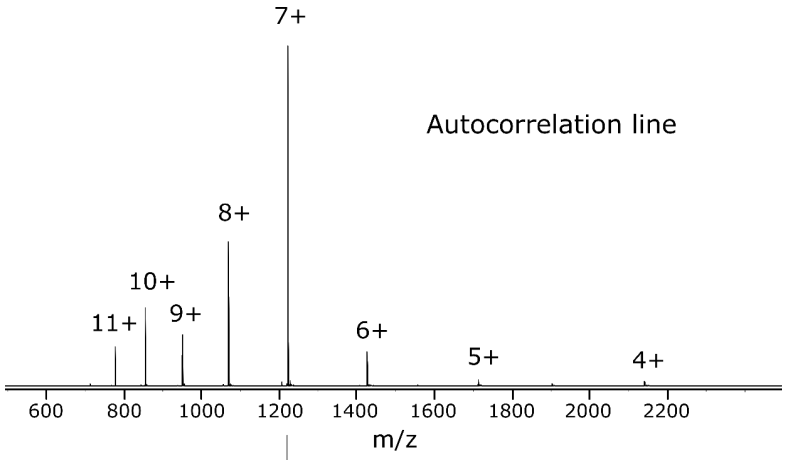
Dichroic mirror allows co-alignment of UV and IR beams

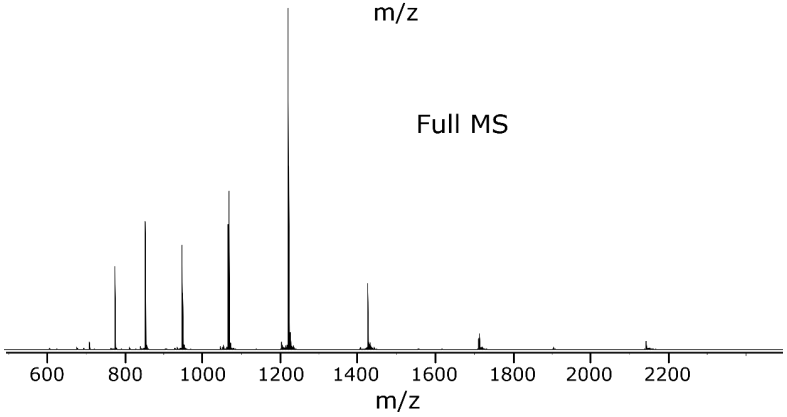
Coherent ExciStar XS, 193 nm
Synrad 48-2, 10.6 µm

UVPD-2DMS: Ubiquitin

8192 lines x 1 MW (just under 4 hours)







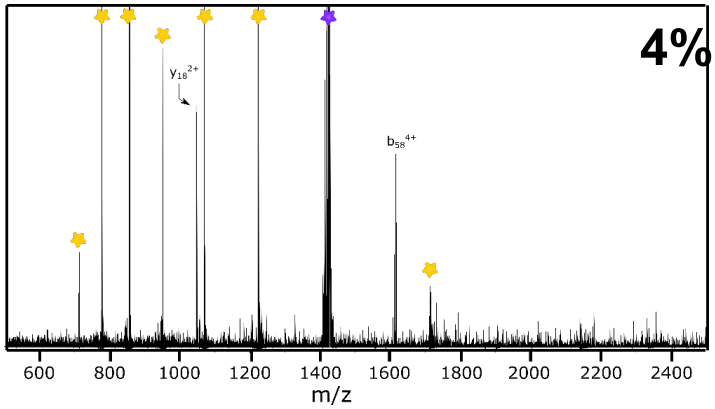
UVPD: 193 nm, 4 shots @ 6 mJ (~0.4 mJ in cell)

Horizontal lines

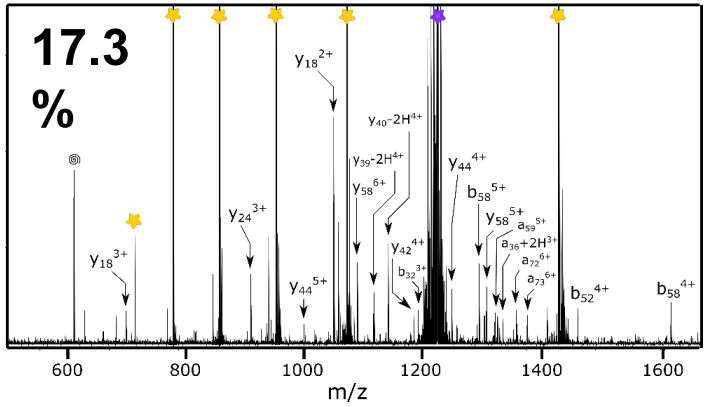
Scintillation noise

Precursor

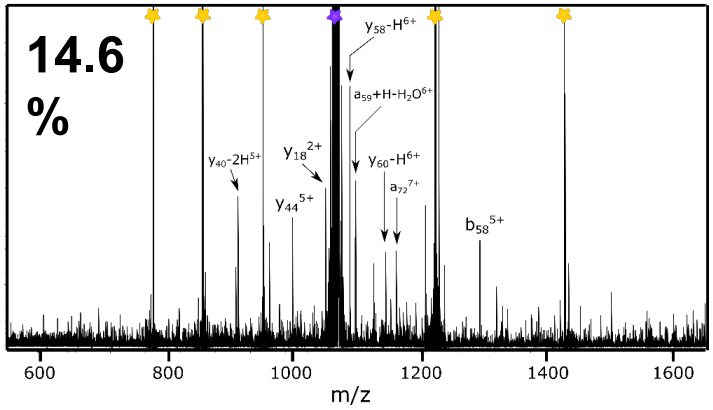
6+



7+



8+



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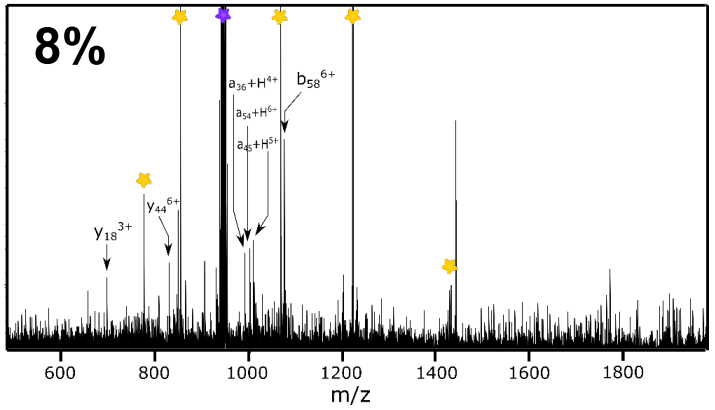


10+ : 9%

Lecture overview

- 2DMS for agrichemical analysis.
- 2DMS for polymer analysis.
- 2DMS for monoclonal antibodies.
- Distinguishing phosphopeptides using 2DMS
- 2DMS – UVPD
- 2DMS on a linear ion trap

Linear Ion Trap Geometry

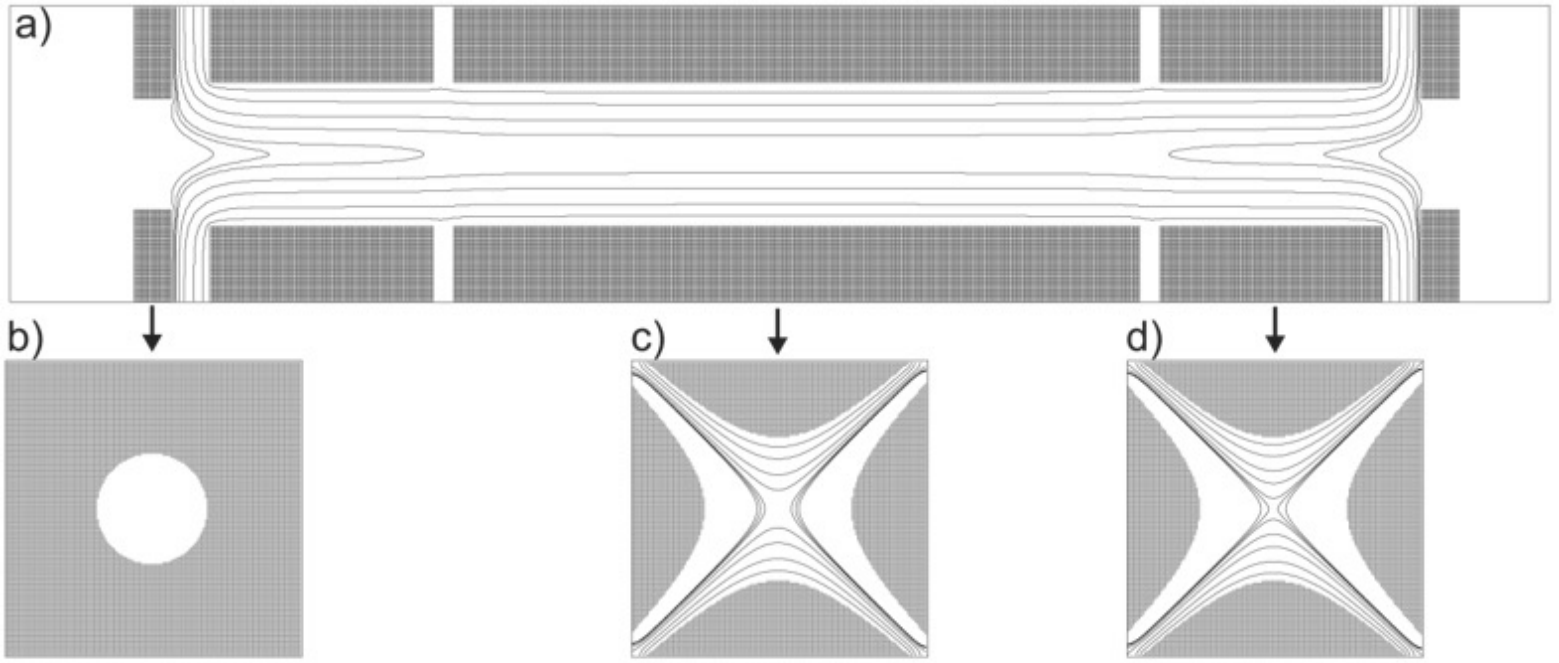
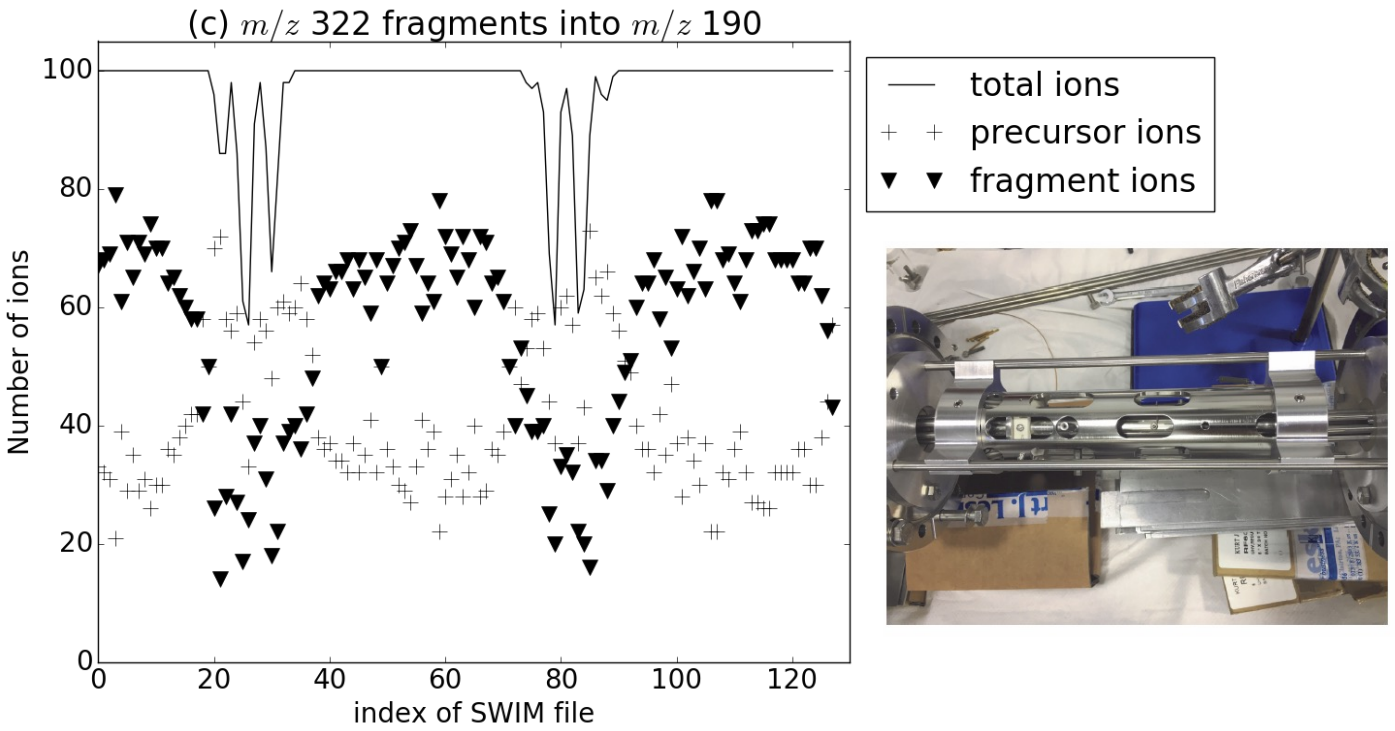


Figure 1. SIMION model of the linear ion trap with equipotential lines. a) Horizontal cut. b) Vertical cut of trapping electrode. c) Vertical cut of center quadrupole. d) Vertical cut of side quadrupole. The voltages applied on the LIT electrodes in this instance are +10.0 V_{DC} on the end-cap electrodes, +5.0 V_{DC} on the outer quadrupole rods, +/- 100.0 V_{RF} on the quadrupole rods. The equipotential lines are for: -100.0 V, -75.0 V, -50.0 V, -25.0 V, -10.0 V, 0.0 V, +2.5 V, 5.0 V, and 10.0 V.

1. Maria van Agthoven, Peter B. O'Connor, 2 Dimensional Mass Spectrometry in a Linear Ion Trap UK Patent GB1615469, 2016.
2. Maria van Agthoven and Peter B. O'Connor, Rapid Communications in Mass Spectrometry 2017, 31 (8), 674-684

2D MS in a Linear Ion Trap with SWIM

- Simion ion trajectory simulations show that 2DMS can be done on a linear ion trap.
- We have built a LIT-2DMS system and are in the process of testing the prototype.
- If coupled to a TOF mass analyzer, a LIT-TOF instrument could allow full 2DMS experiments in less than 10 seconds.



1. Maria van Agthoven, Peter B. O'Connor, 2 Dimensional Mass Spectrometry in a Linear Ion Trap UK Patent GB1615469, 2016.
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Tailored excitation for Fourier transform ion cyclotron mass spectrometry

Alan G. Marshall, Tao Chin Lin Wang, and Tom L. Ricca

Cite this: *J. Am. Chem. Soc.* 1985, 107, 26, 7893–7897
Publication Date: December 1, 1985
https://doi.org/10.1021/ja00312a015

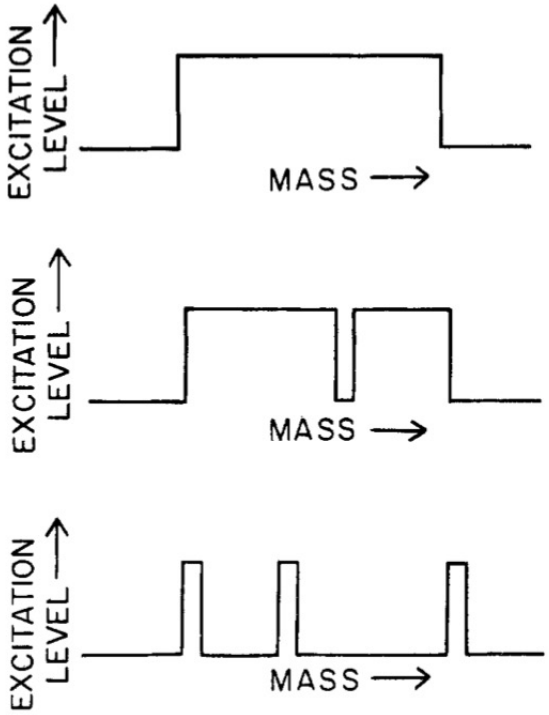


Figure 1. Desired excitation power spectra for Fourier transform ion cyclotron resonance mass spectrometry (FT/ICR). Top: Flat power over a specified mass range, for ordinary detection and/or isotope-ratio measurements. Middle: Flat excitation with a zero-power window, for use in the first stage of MS/MS experiments. Bottom: Multiple-frequency excitation for use in multiple-ion monitoring.

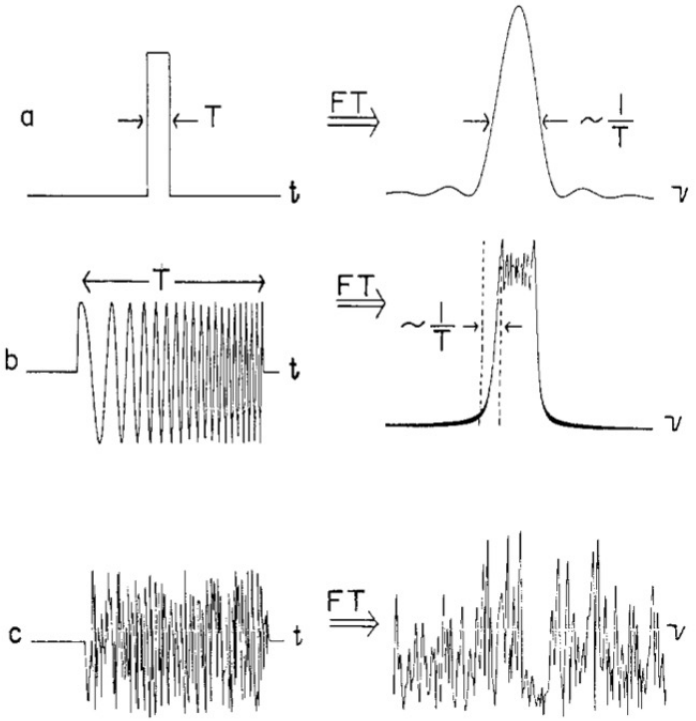


Figure 2. Previously employed time-domain excitation waveforms and their corresponding frequency-domain magnitude-mode spectra. Top: Single-pulse excitation (1). Middle: Frequency-sweep excitation (2, 12). Bottom: Pseudorandom noise excitation (13). Note the nonflat power and broad cutoff shoulders.

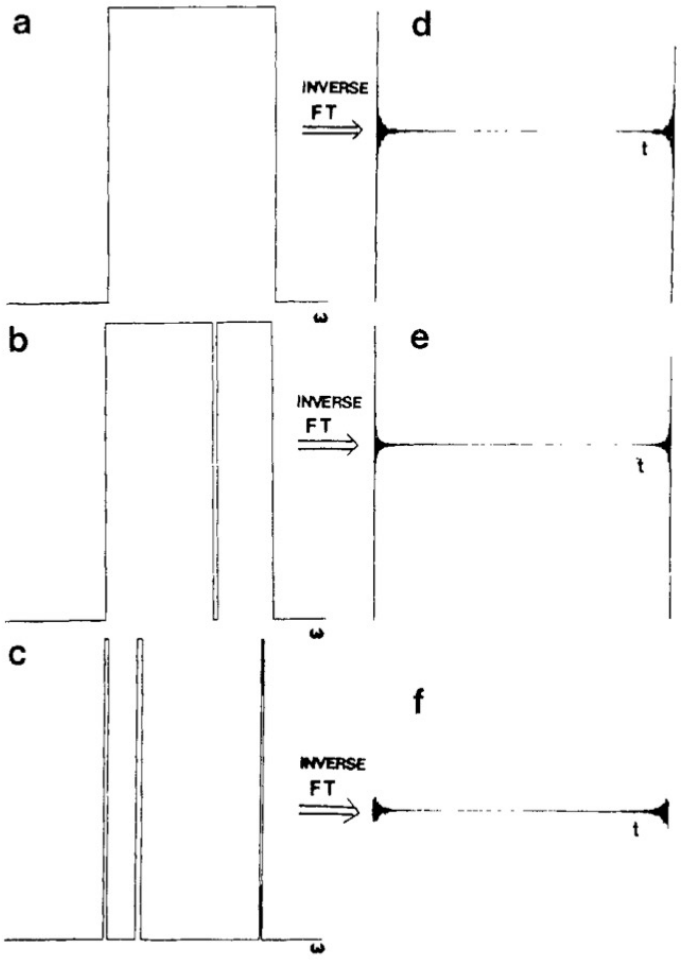


Figure 5. Time-domain excitation waveforms (right) generated by inverse Fourier transformation of the desired frequency-domain excitation spectra (left). Because mass and frequency are inversely related in ICR, the desired excitation mass spectra of Figure 1 are readily converted to those shown at the left in this figure.

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

Charles W. Ross, III, Shenheng Guan, Peter B. Grosshans,† Tom L. Ricca,‡ and Alan G. Marshall*,§

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.

The fundamental concept of SWIM:

Through each of the succeeding scans, calculate a SWIFT pulse with steadily incrementing cycles of a sine wave.

This will have the effect of modulating ion radius rapidly on the right end of the spectrum and slowly on the left end, and linearly varying modulation frequencies in between.

When combined with a radius-dependent fragmentation method, to create a 'fragmentation zone', this allows 2DMS.

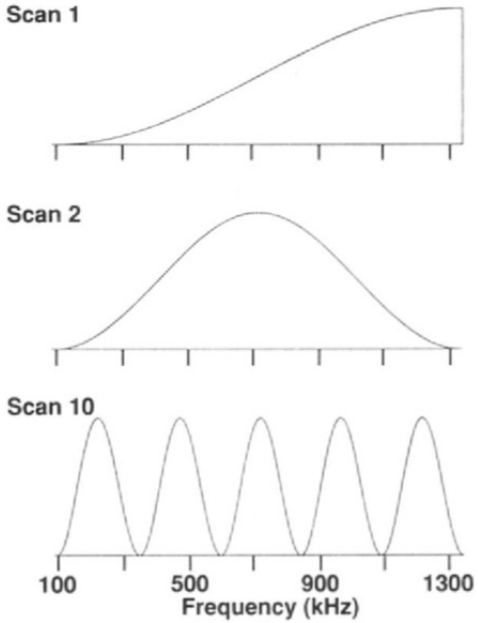


Figure 2. SWIFT magnitude-mode spectra (see eq. 3) of several parent ion modulation waveforms, for $j = 1, 2$, and 10. Note that the excitation amplitude for ions of the highest cyclotron frequency, 1333.0 kHz, is modulated the fastest in proceeding from one waveform to the next, whereas the excitation amplitude for ions of the lowest cyclotron frequency, 100 kHz, is not modulated at all from one waveform to the next. The mass-to-charge ratio range corresponding to these ion cyclotron frequencies is $34.8 < m/z < 463$ ($B \approx 3.02$ T).

Anal. Chem. **2002,** *74,* 4625−4633

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

Charles W. Ross, III,*,† William J. Simonsick, Jr.,‡ and David J. Aaserud‡

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

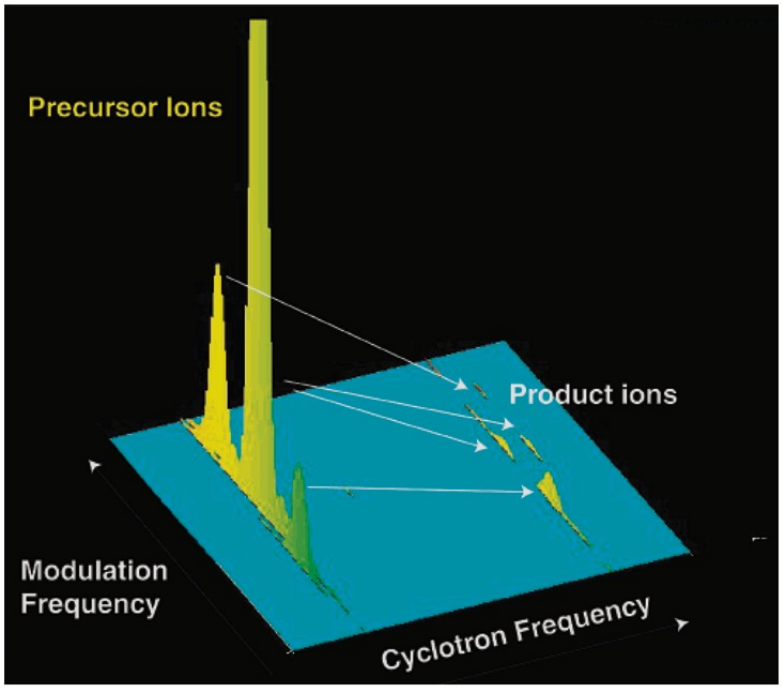
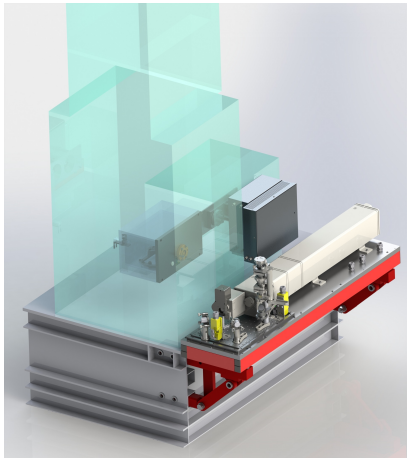


Figure 3. Power plot of the SWIM two-dimensional Fourier transform mass spectrum of the reactive oligomer intermediate.

Horizontal: 16k transients (narrow band)
Vertical: 128 scan lines.
Total: 2,097,152 data points

Verdel Instruments: 2DMS using UVPD on a Q-TOF

UV laser and beam optics



Quadrupole and lens control systems

MaXis ETD II Q-TOF

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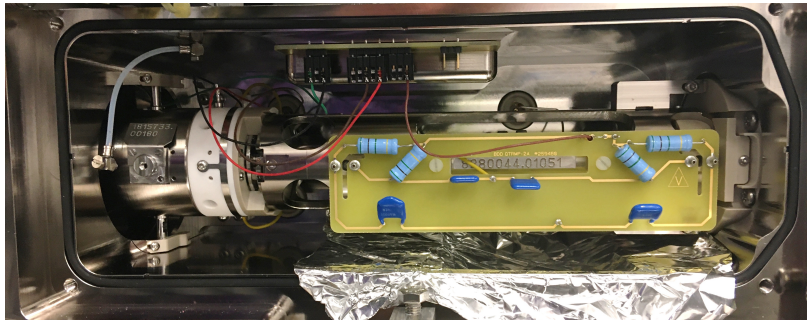
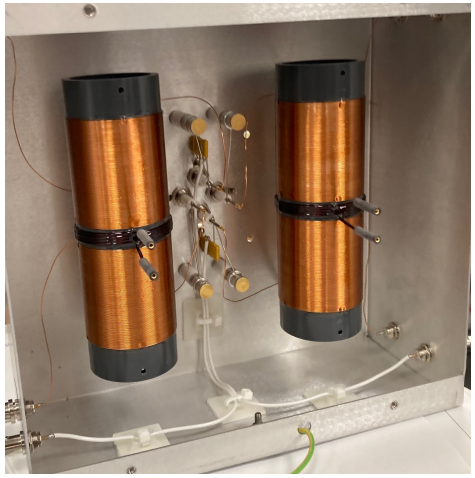
Instrument Modifications

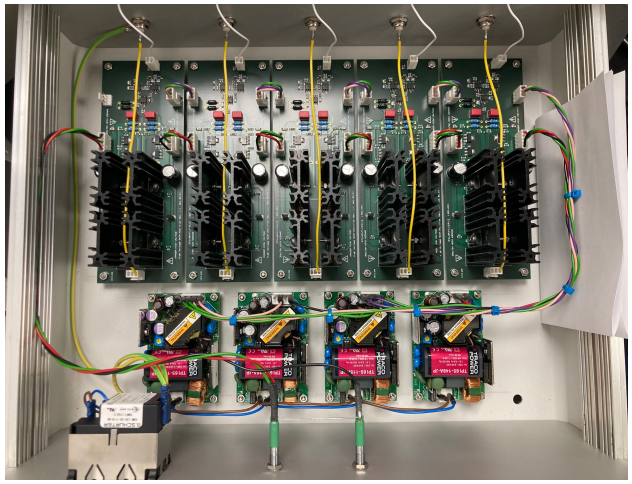
- Transformed quadrupole to linear ion trap (LIT).



- Drive the RF with our own tank circuit amplifier.



- Control of LIT lenses with DC trapping amp.



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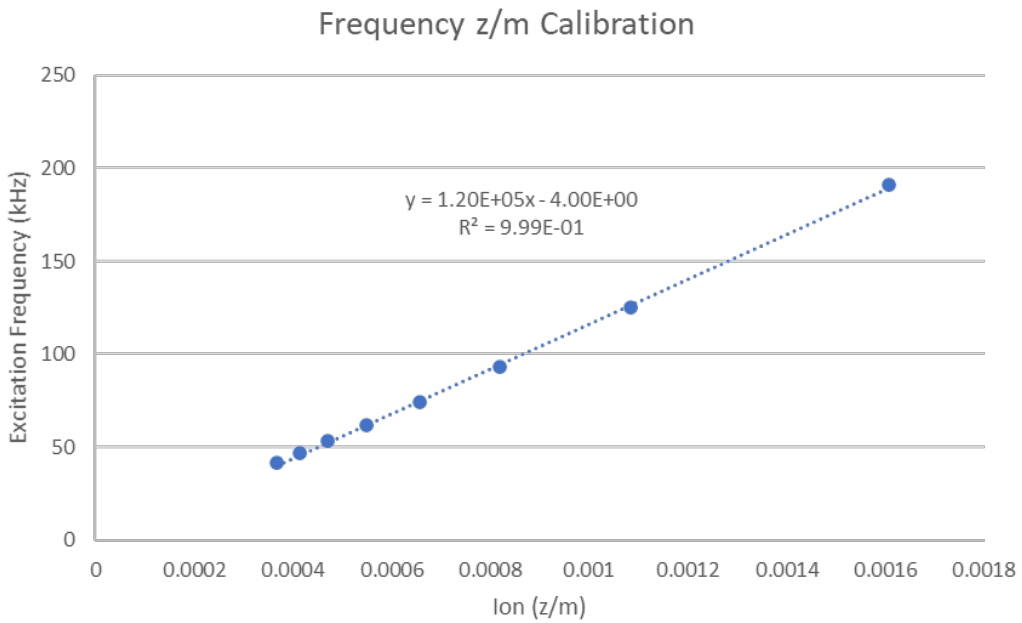
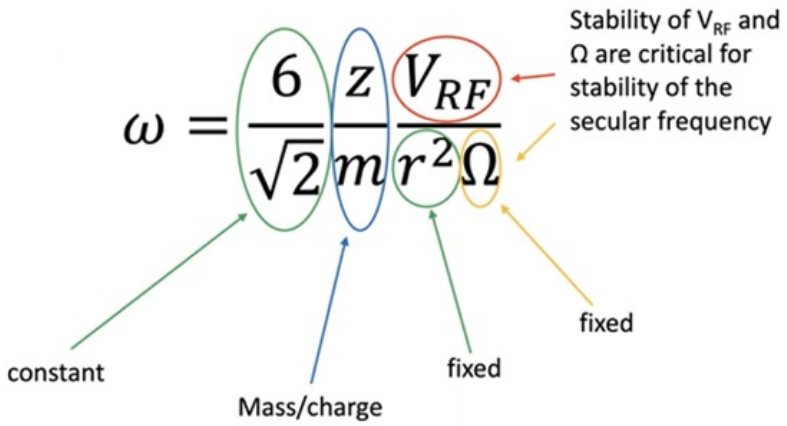
Excitation of Ions



- **Linear relation between excitation frequency and z/m of ejected ion**



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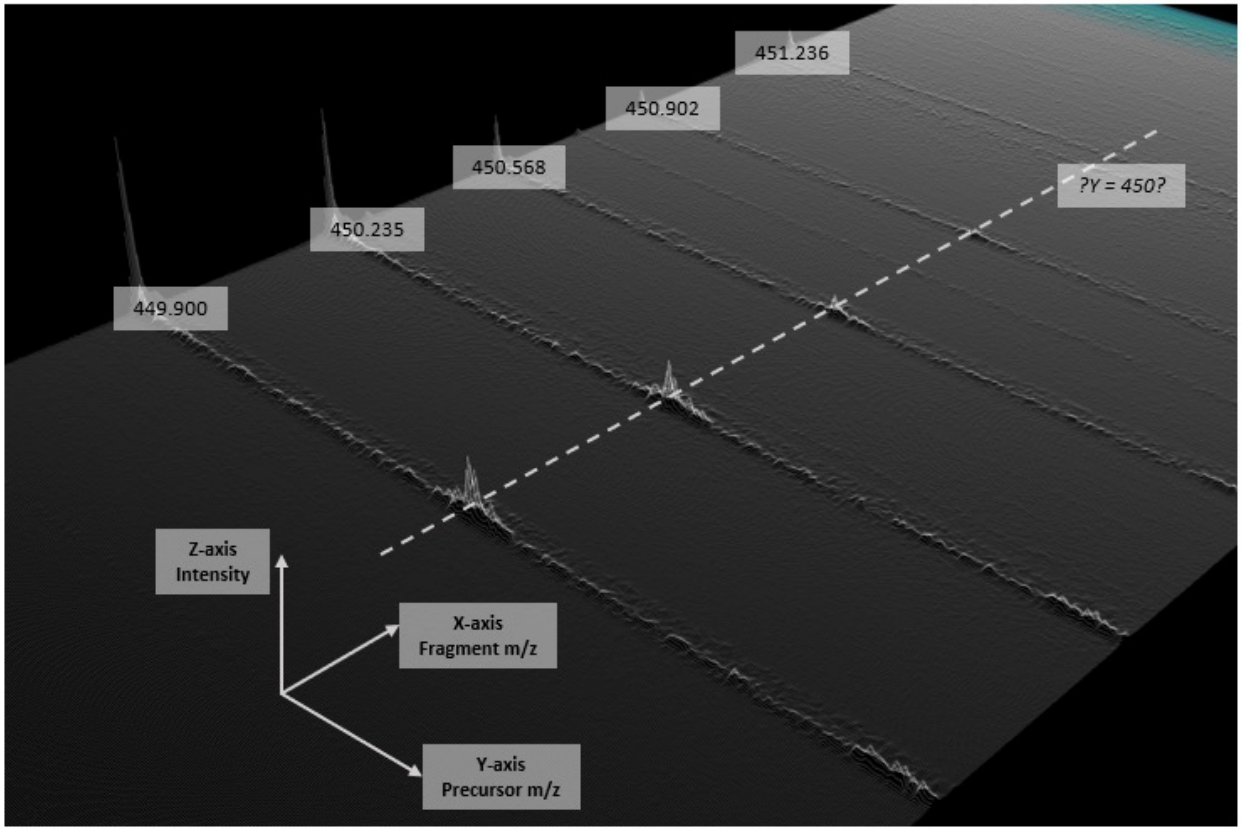
Substance P: 449.9 m/z isotope pattern



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LEADING INNOVATION IN TOC-MS™

TOTAL CORRELATION MASS SPECTROMETRY™

The Next Generation of Mass Spectrometry

Compatible with any mass spectrometer that uses linear ion trap technology.

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2-Dimensional mass spectrometry

- 2-dimensional mass spectrometry (2DMS) is now feasible, and bordering on the routine, for a wide range of samples in FTICR mass spectrometry.
- 2DMS can be achieved by modulating ions through a fragmentation zone (with frequencies dependent on m/z) using the Gaumann pulse sequence or the SWIM methods. Fragments will appear at the modulation frequencies thus correlating fragments with their precursors. The Fourier transform can extract those frequencies.
- 2DMS requires large data sets, currently up to 2B data points or more, which wasn't really feasible to process in the 1990's, but it now can be done.
- 2DMS can be done with a range of fragmentation methods, including IRMPD, ExD, and UVPD.
- 2DMS can be done on a linear ion trap, and we've spun out Verdel Instruments to achieve this commercially.

Acknowledgements

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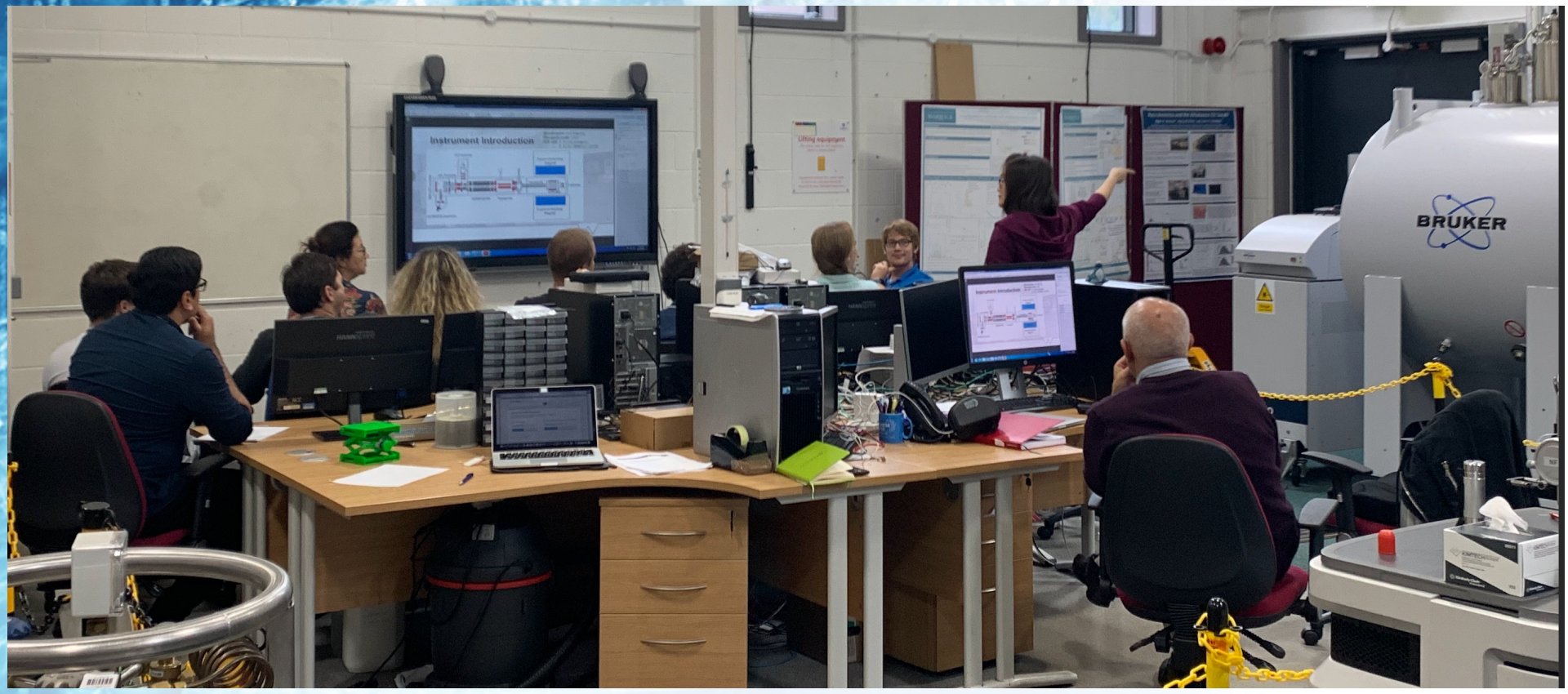
- EPSRC (J003022, N021630, N033191)
- BBSRC (P021875, R022399)
- Royal Society Translation Award
- Innovate UK iCure award
- H2020 EU-FT-ICR-MS Network
- Innovate UK / Verdel Instruments Ltd

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 731077.

EU FT-ICR MS



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CR MS

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