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28èmes Journées Scientifiques de ccCTA

Ultra-High-Resolution (UHR) MS: The Blessing, The Curse, The Data Overload

On the use, possibilities, & limitations of UHR MS (FT-ICR MS & Orbitraps)

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Villars-sur-Ollon, Near home
October 2, 2025; 15:10-15:40

Complex Mixture Analysis with UHR MS

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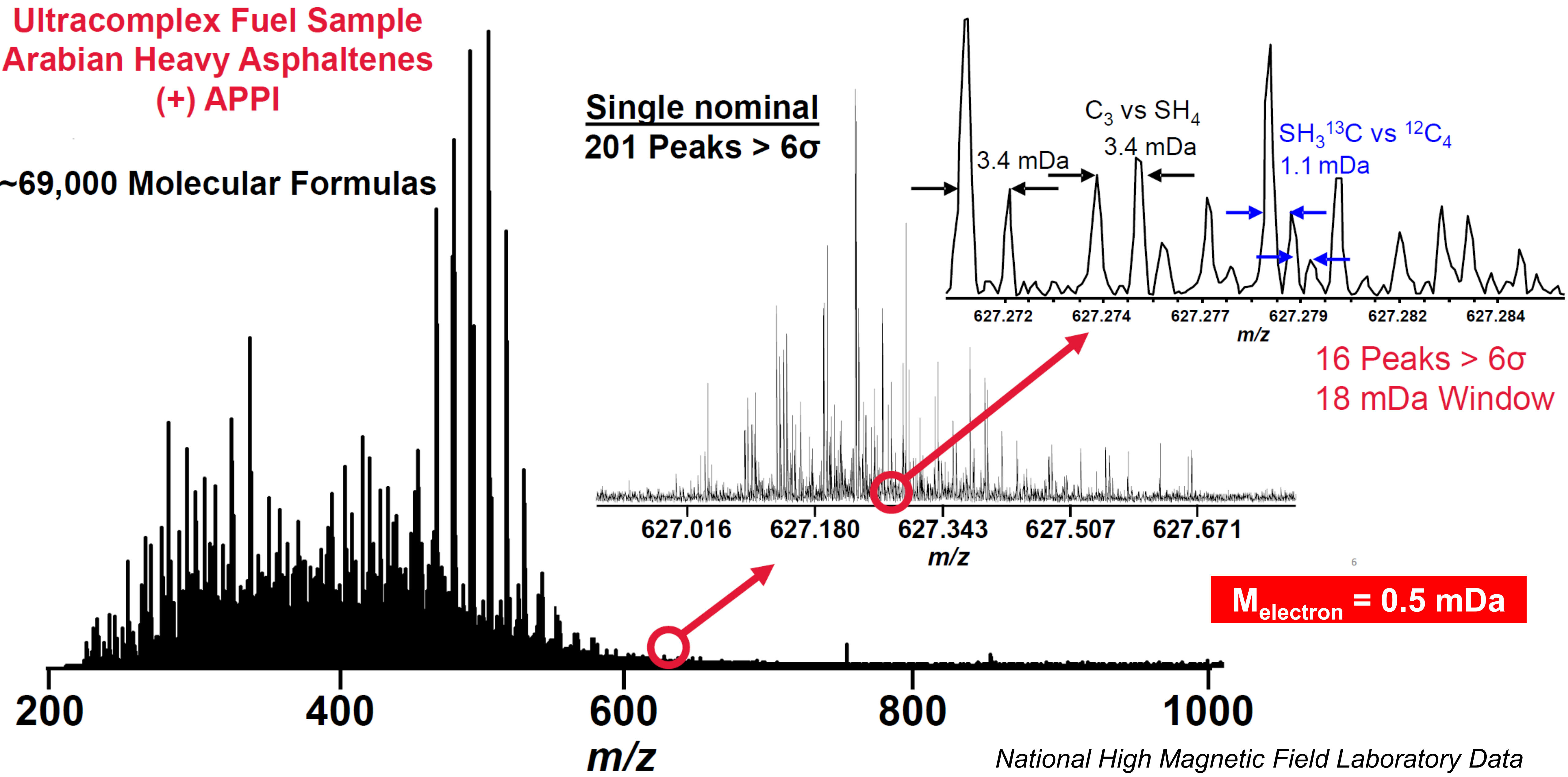

National High Magnetic Field Laboratory Data

Core Scientific Question UHR MS Answers:

Which exact elemental compositions are in this peak?

by separating what other (LRMS & HRMS) mass spectra blur together

- **Single vs. composite peak?** Distinguish a lone species from a superposition of near-isobars.
- **Unambiguous formula assignment.** Use exact mass + isotopic fine structure (e.g., ^{34}S vs $^{13}C2$; Cl/Br patterns) to pin down elemental composition, especially at higher *m/z*.
- **Heteroatom counting.** Determine the number of S/Cl/Br (and sometimes N/O) directly from isotopic fine structure.
- **Adduct/charge state clarity.** Separate overlapping adducts/charge states that would otherwise masquerade as one feature.
- **Deconvolution of congested mixtures.** In complex matrices (PFAS, environmental DOM, unknowns), map compositional space rather than a blur.

- **BUT…** UHR MS won't resolve constitutional/positional/stereo isomers - need MS/MS, chromatography, ion mobility, or all of the above.

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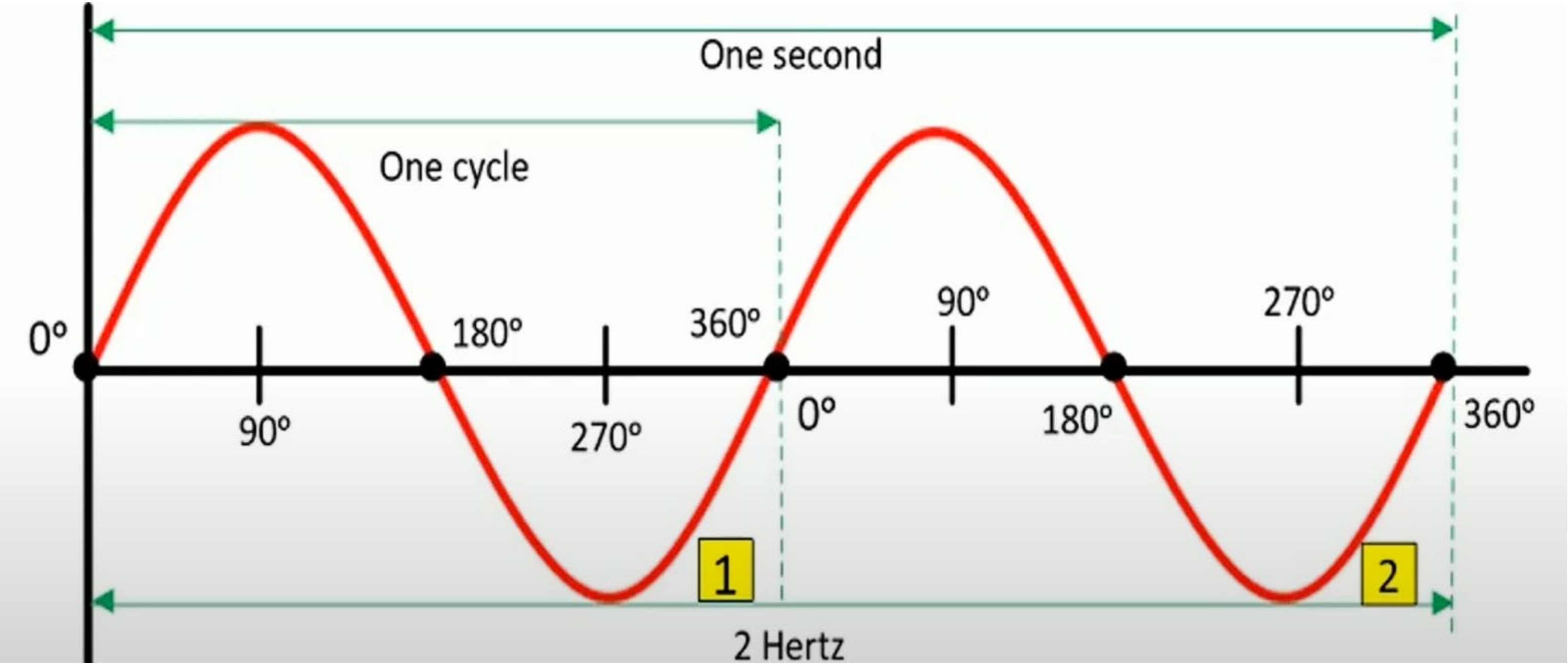
How to Achieve Ultra-High-Resolution (UHR) MS?

- Measure **frequency** of ion oscillations, not an arrival time on a detector (a single-shot measurement)
- Measure thousands to millions of oscillations over the detection time (can be seconds)
- Each oscillation contributes another “vote” for the true frequency, reducing **uncertainty**



- **frequency**

$$f = \frac{N}{T}\ (Hz)$$

- **uncertainty**

$$\Delta f \propto \frac{1}{T}$$

- **resolution**

$$R = f/\Delta f$$

How to establish an oscillation motion of ions and how to measure the frequency?

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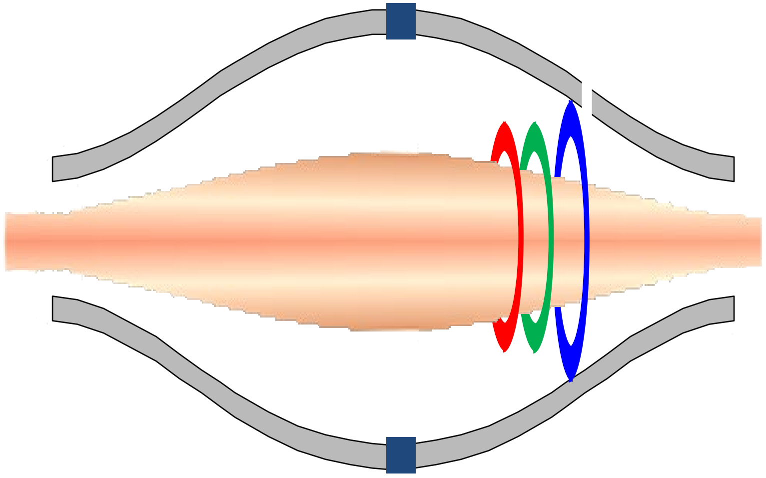
FTMS: Fourier Transform Mass Spectrometry

- Ion identity (*m/z*) is encoded as a frequency of ion oscillations in an ion trap
- Frequencies of ion oscillations are measured as time-domain signals (**transients**)
- Each transient comprises hundreds of thousands to millions of oscillations
- Fourier transform (FT) decodes transients to reveal frequencies (*m/z*) values

Electrostatic field-based

Orbitrap



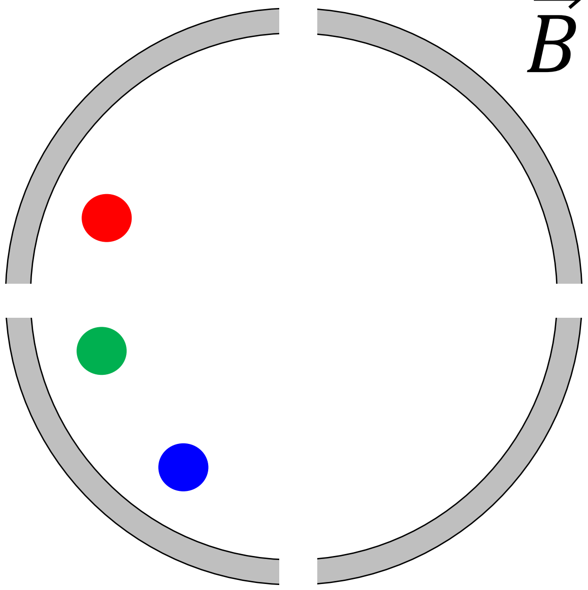
$$\omega \approx \sqrt{\frac{k}{m/z}}$$

Orbitrap™ families: LTQ Orbitrap; Exactive; Q Exactive; Exploris; Excedion; Fusion; Lumos; Eclipse, Ascend, Astral

Magnetic field-based

Ion Cyclotron Resonance (ICR)

$\vec{B} \odot$



$$\omega \approx \frac{1}{m/z}$$

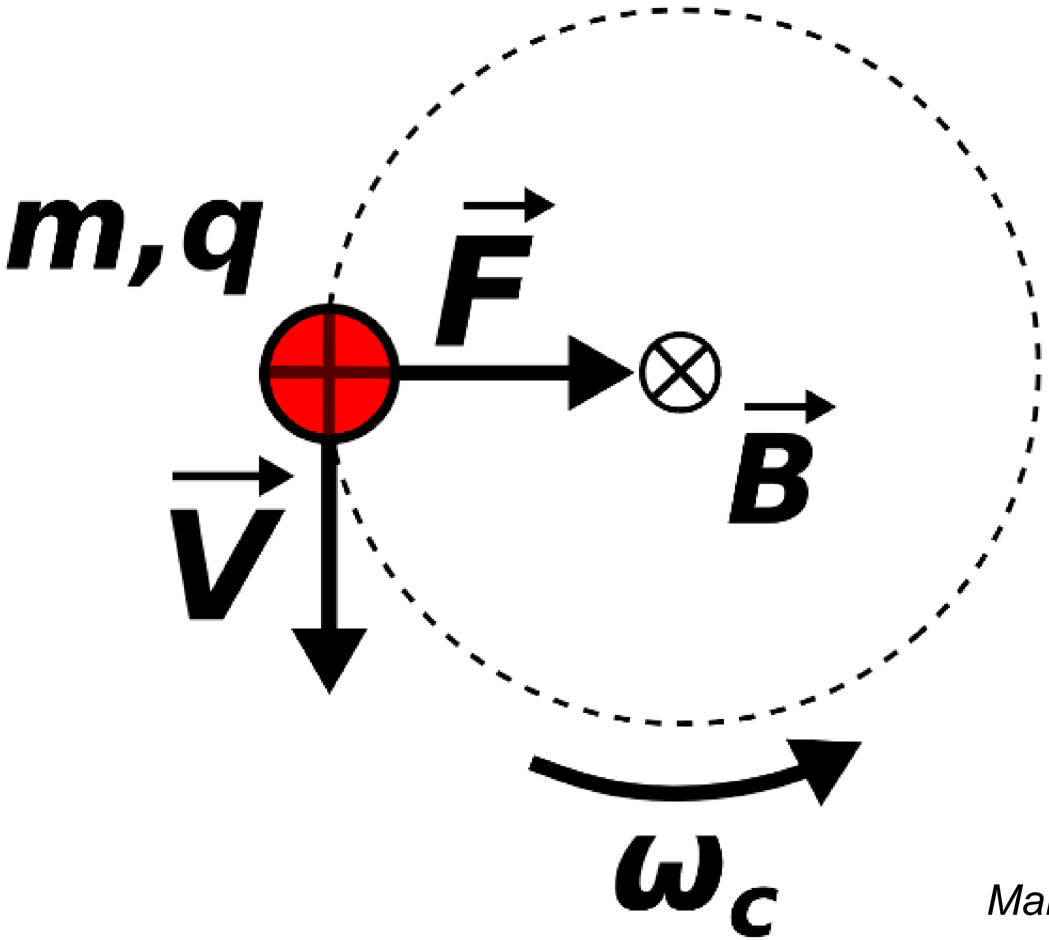
Apex
Solarix
Scimax
timsMRMS

FTMS is the highest resolution & mass accuracy MS technique

FT-ICR MS Concept: Ion Cyclotron Frequency

use of the magnetic field

- Cyclotron frequency is independent of the radius and velocity and therefore independent of the ion's kinetic energy
- All ions with the same charge-to-mass ratio rotate around magnetic field lines with the same cyclotron frequency



$$\omega_c = \frac{q}{m} B$$ **cyclotron frequency**

$$\boxed{f_c = \frac{qB}{2\pi m}}$$

ω_c angular velocity (rad/s)

f_c frequency (Hz)

Marshall et al., Mass Spectrometry Reviews (1998) 17:1-35

Ion Detec

Ion packet inject

⇩

Ion trapping (on-a

⇩

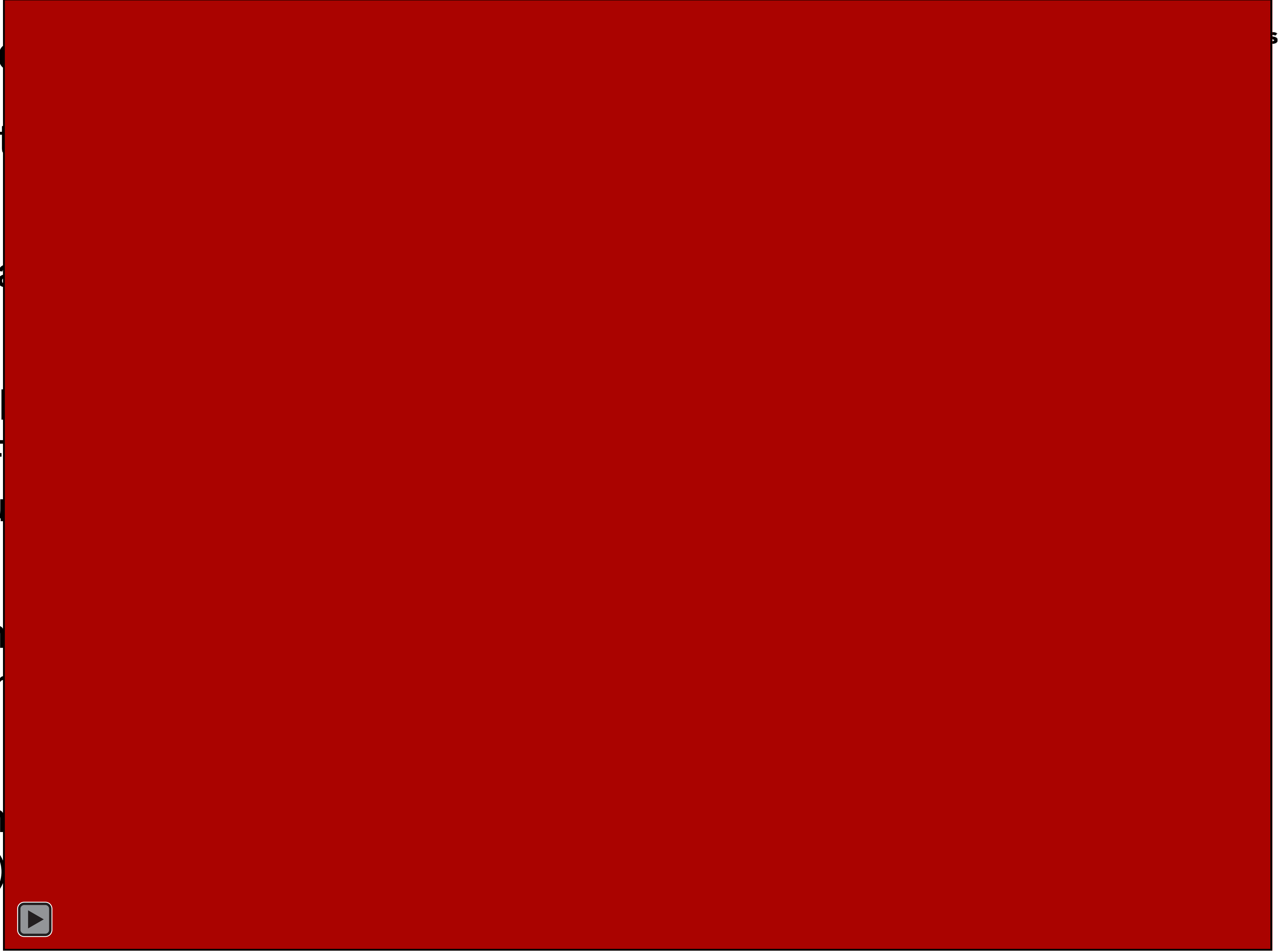
Ion excitation to a l
radius with an RF
(**compact clou**

⇩

Ion detection
(induced ion curr

⇩

Quench of ion
(ion ejection)



s

How Can We Convert Time-Domain Transients into Mass Spectra

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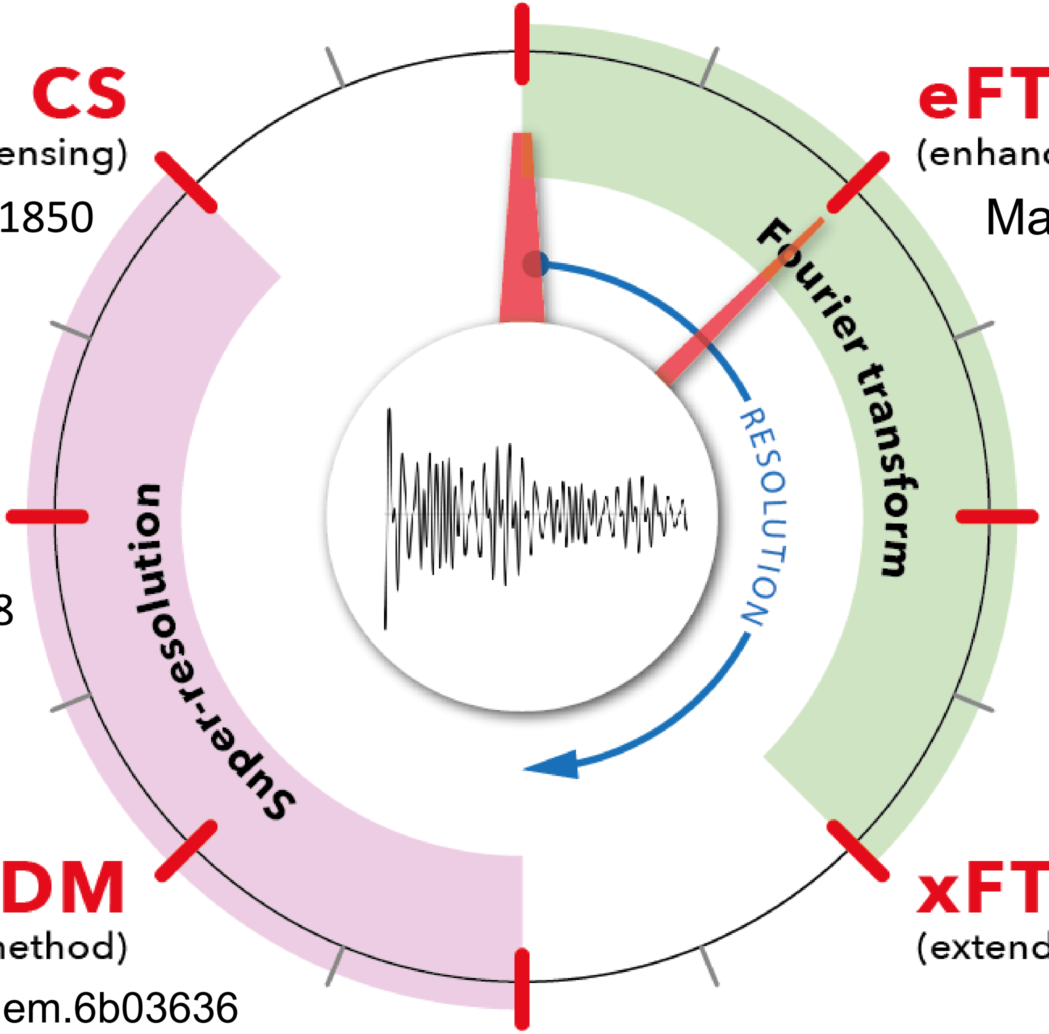
- **Different uncertainty principles** Δf

mFT
(magnitude mode FT)
O'Connor *et al.*, *MS Reviews* (2014) 33:333

CS
(compressed sensing)
DOI: 10.1021/acs.analchem.7b01850

eFT
(enhanced FT = mFT + aFT)
Makarov *et al.*, *IJMS* (2014) 369, 16-22



FDM
(filter diagonalization method)
DOI:10.1016/j.jasms.2006.02.018
DOI: 10.1021/ac203391z

aFT
(absorption mode FT)
O'Connor *et al.*, *MS Reviews* (2014) 33:333

ΦSDM
(phased spectrum deconvolution method)
DOI: 10.1021/acs.analchem.6b03636
turboTMT product from Thermo

xFT FT of non-sinusoidal signals (Tsybin)
(extended basis FT)

LSF
(least-squares fitting)
DOI: 10.1007/s13361-014-0888-x
DOI: 10.1002/mas.21681

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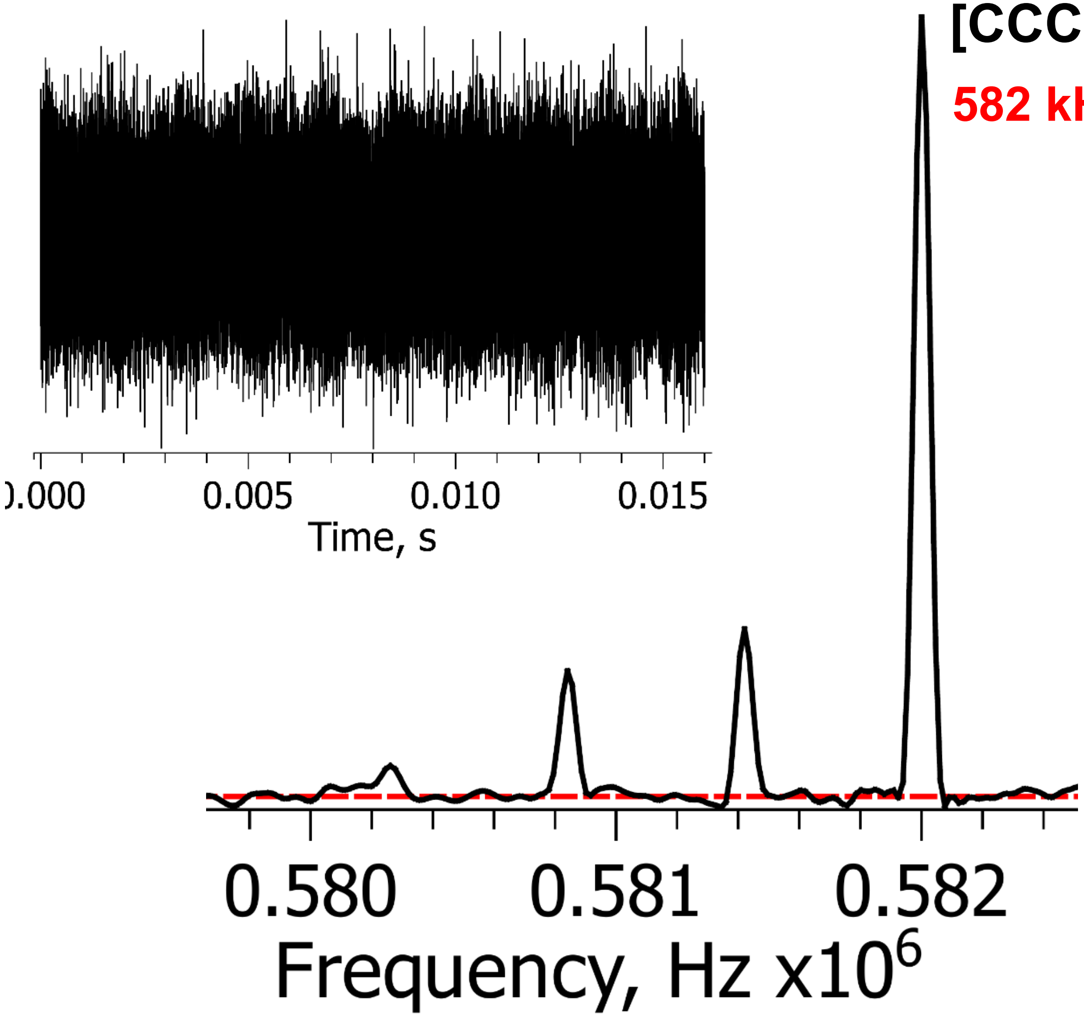
Data Processing in FTMS: from time to **frequency**, to ***m/z***

Time-domain transient → **FT** → **Frequency spectrum** → **Mass spectrum** → **Information output**

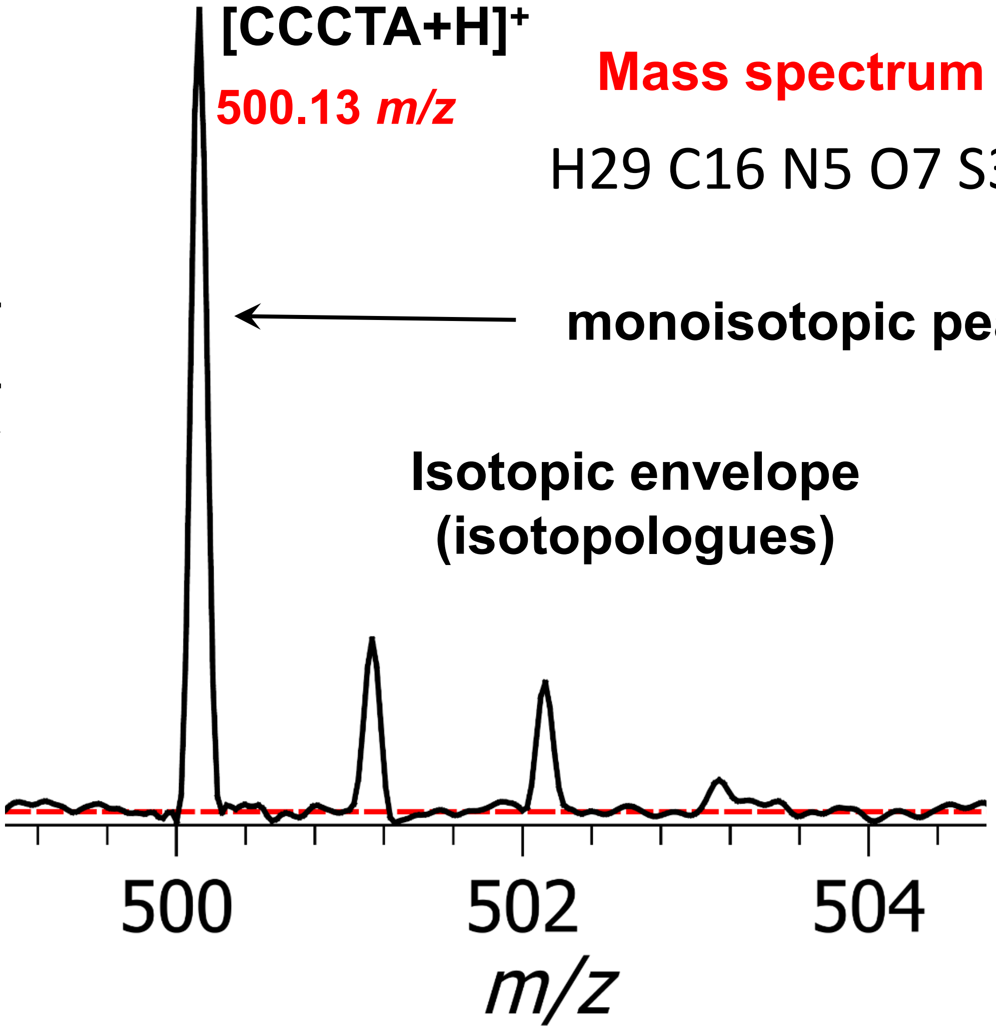


Orbitrap Exploris

$$\omega \approx \sqrt{\frac{k}{m/z}}$$

→

calibration



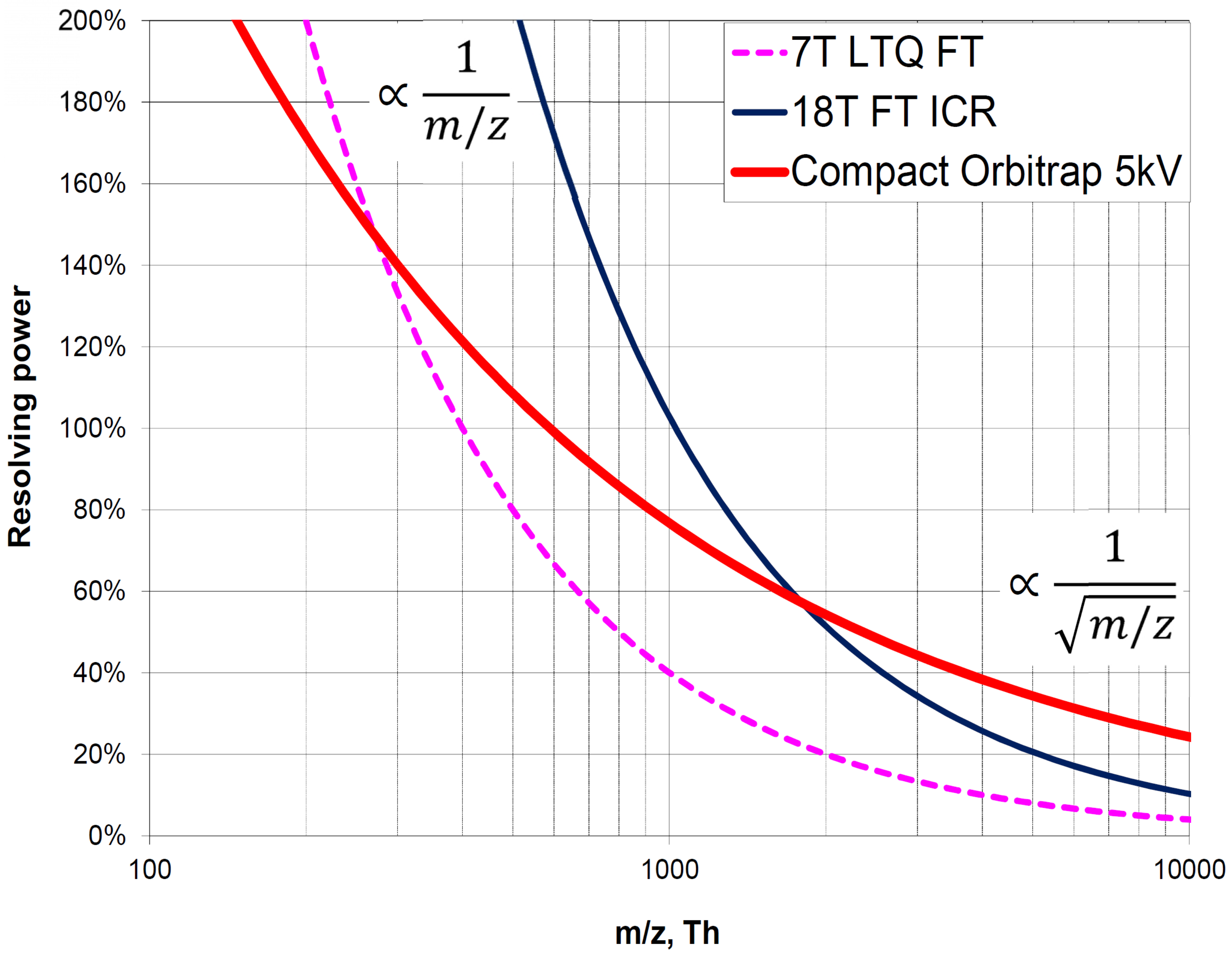
Mass spectrum

H29 C16 N5 O7 S3

monoisotopic peak

Isotopic envelope (isotopologues)

Resolution = function of frequency or *m/z*



$$R \propto f \cdot T$$

$$f_{ICR} \propto \frac{1}{m/z}$$

$$f_{Orbitrap} \propto \frac{1}{\sqrt{m/z}}$$

Complex Mixture Analysis with High-Field FT-ICR MS

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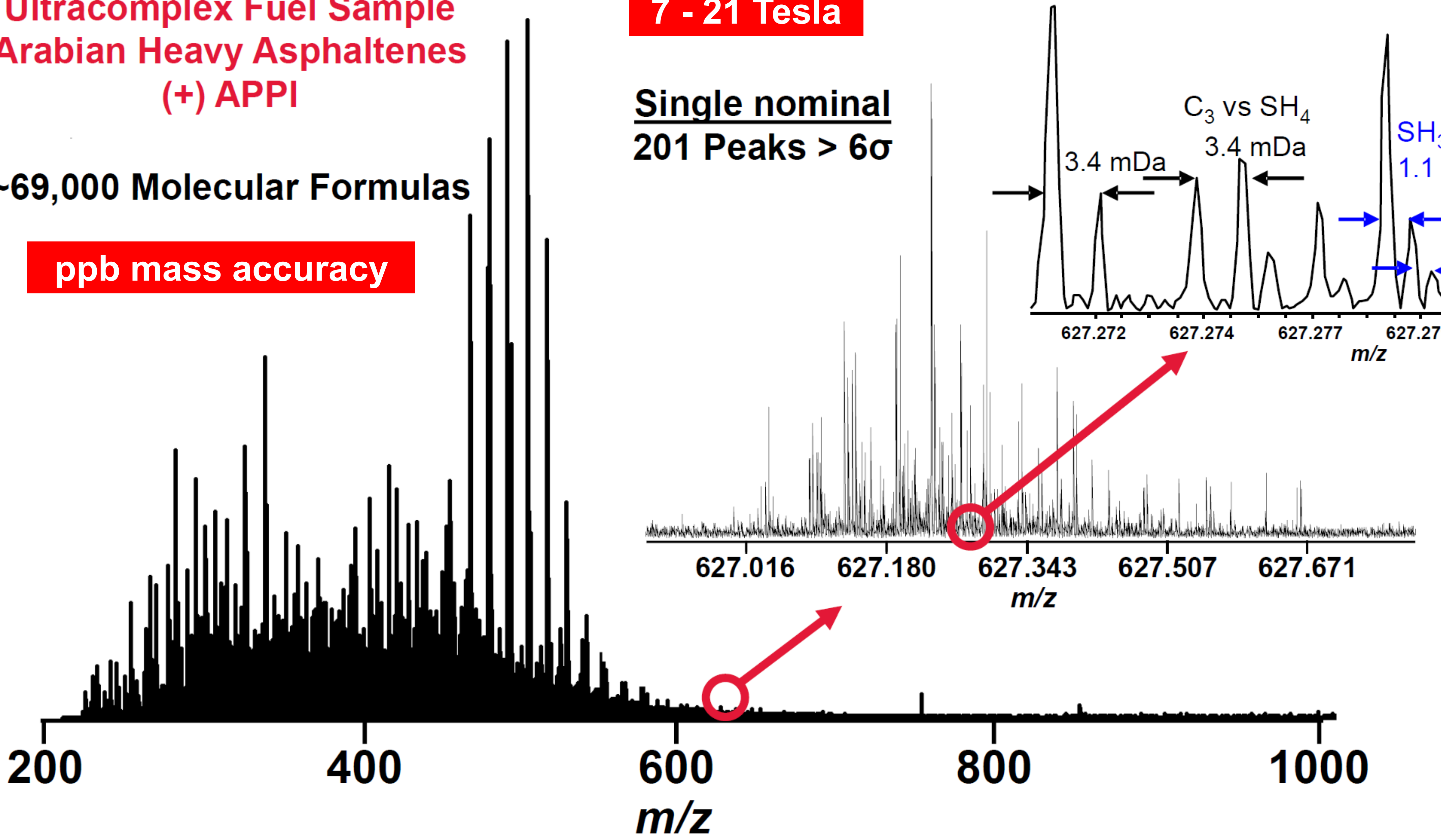
Ultracomplex Fuel Sample
Arabian Heavy Asphaltenes
(+) APPI

~69,000 Molecular Formulas

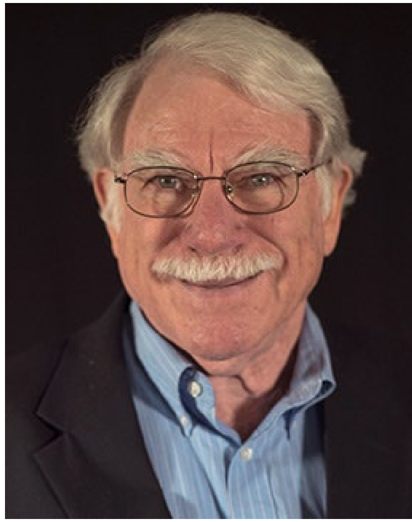
ppb mass accuracy

7 - 21 Tesla

Single nominal
201 Peaks > 6σ



Alan Marshall
1944-2025



FTMS Analytical Characteristics

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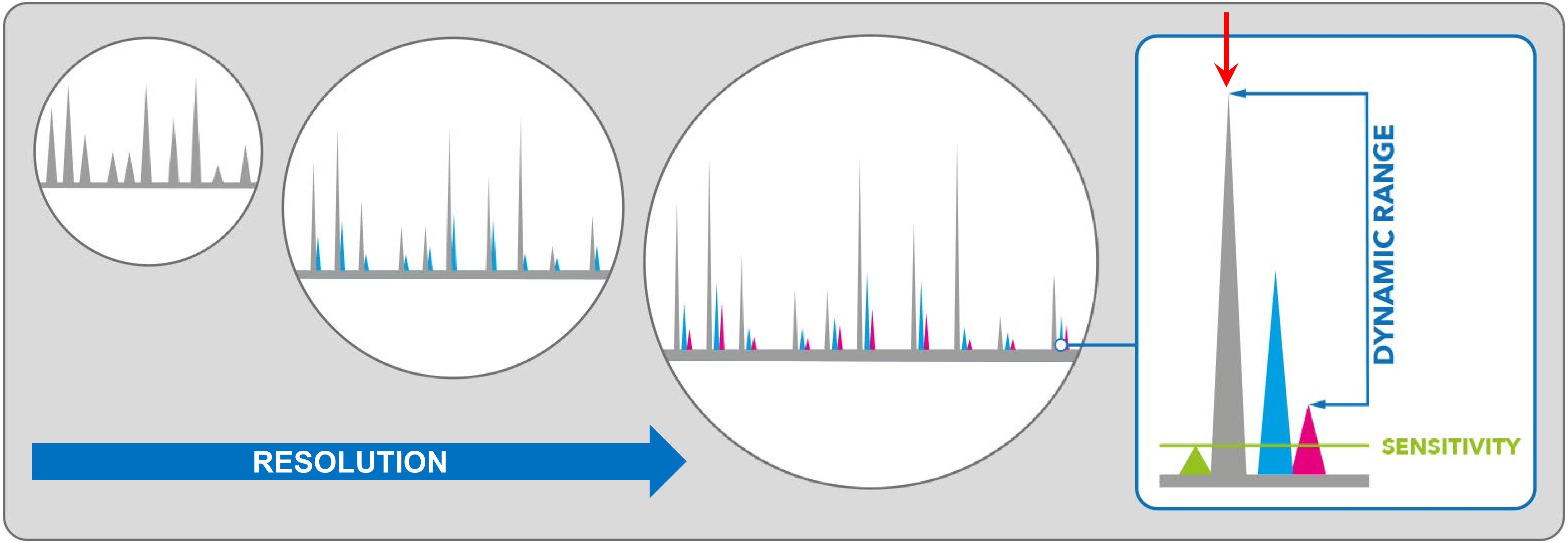
- **the highest resolution**
- **the highest mass accuracy**

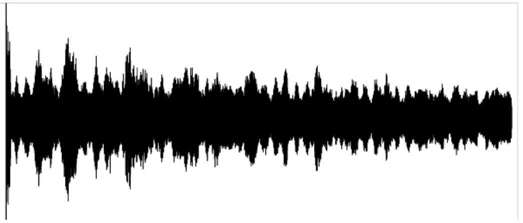
ABUNDANCE ACCURACY

MASS ACCURACY



DATA ACQUISITION SPEED (THROUGHPUT)

induced current ion detection (transients)



$$R \propto f \cdot T$$

What Instruments Offer UHR MS: ICRs & Orbitraps

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Kozhinov et al., Chimia 79 (2025) 77-83

+ DAQ

Orbitrap Astral

Exploris+TOF

+/- DAQ

Eclipse/Ascend

FT-ICR MS

+ DAQ

Q Exactive

+ DAQ

Exploris

+ DAQ

Excedion Pro

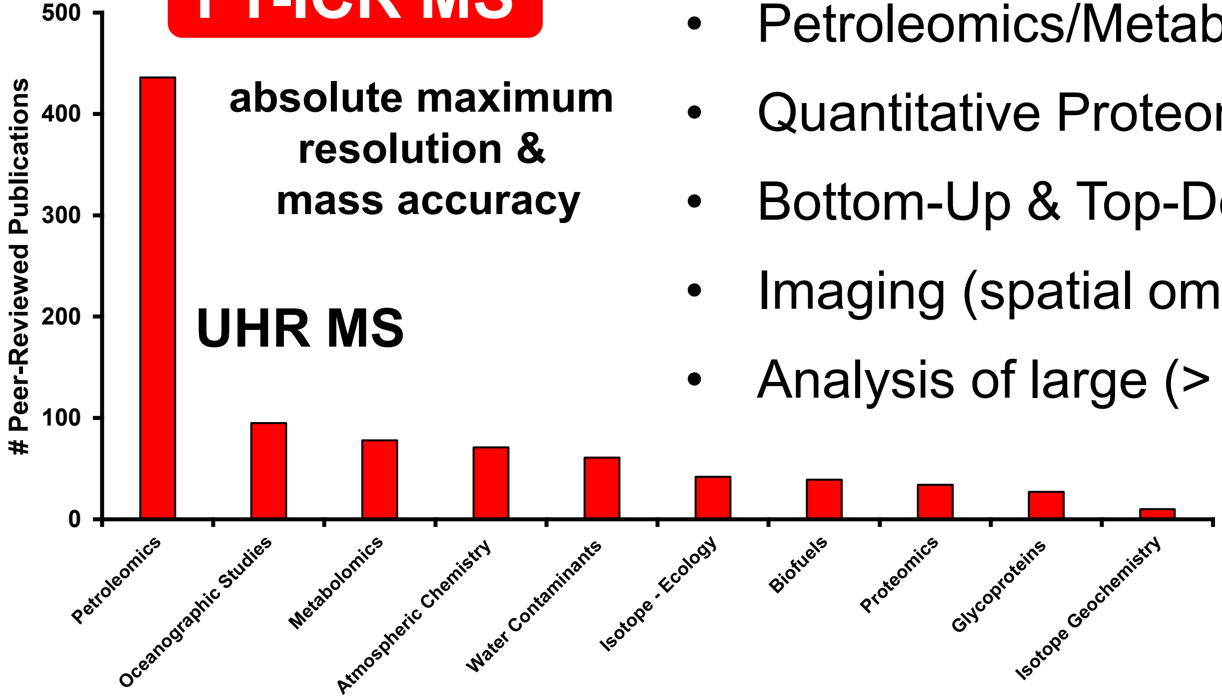
DAQ: high-performance Data Acquisition (DAQ) system

FTMS Applications: ICRs & Orbitraps

FT-ICR MS

absolute maximum resolution & mass accuracy

UHR MS

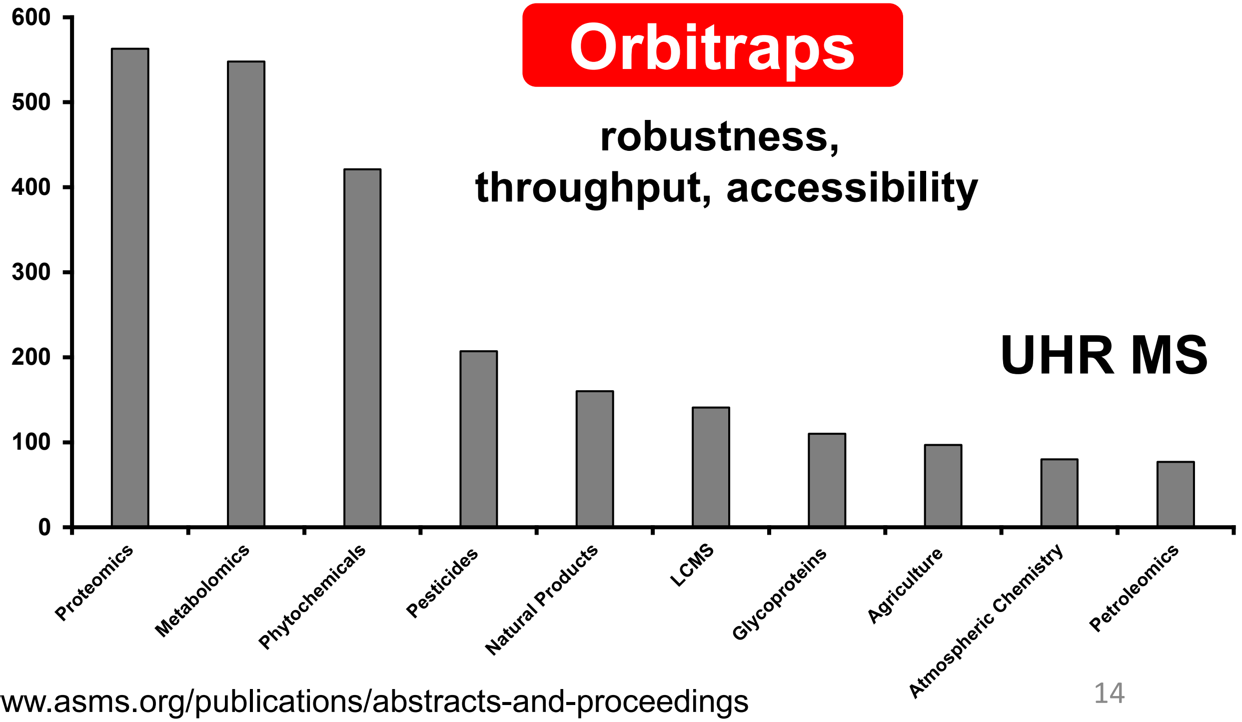


- Petroleomics/Metabolomics: isobars, isotopic fine structure (IFS)
- Quantitative Proteomics/Lipidomics: multiplexing with labeling
- Bottom-Up & Top-Down: higher sequence coverage, confidence
- Imaging (spatial omics): more IDs, higher confidence
- Analysis of large (> 1 MDa) biomolecules: individual (single) ion counting

Orbitraps

robustness, throughput, accessibility

UHR MS



FT-ICR MS:
Unmatched in resolution (>10,000,000), mass accuracy, & spectral complexity handling (better space charge control).

Orbitraps:
Sufficient for most HRMS & UHR MS needs (<1,000,000 resolution). Ideal for routine proteomics, biopharma, and clinical labs (easier LC-MS operation).

Top 10 Topics (Keyword in Title / Abstract) https://openalex.org/, https://www.asms.org/publications/abstracts-and-proceedings

In-Browser FTMS Simulator (Web-App): www.peakbypeak.com

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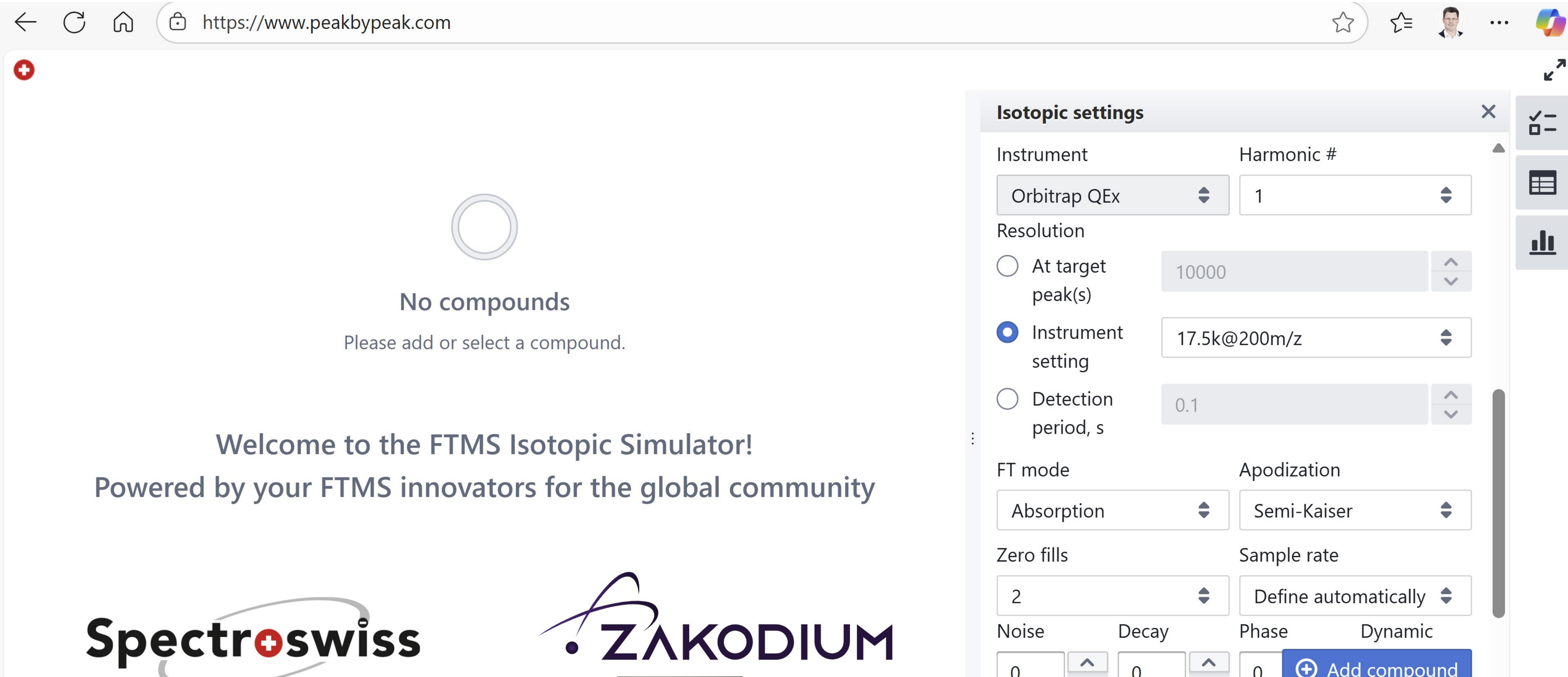

Luc Patiny

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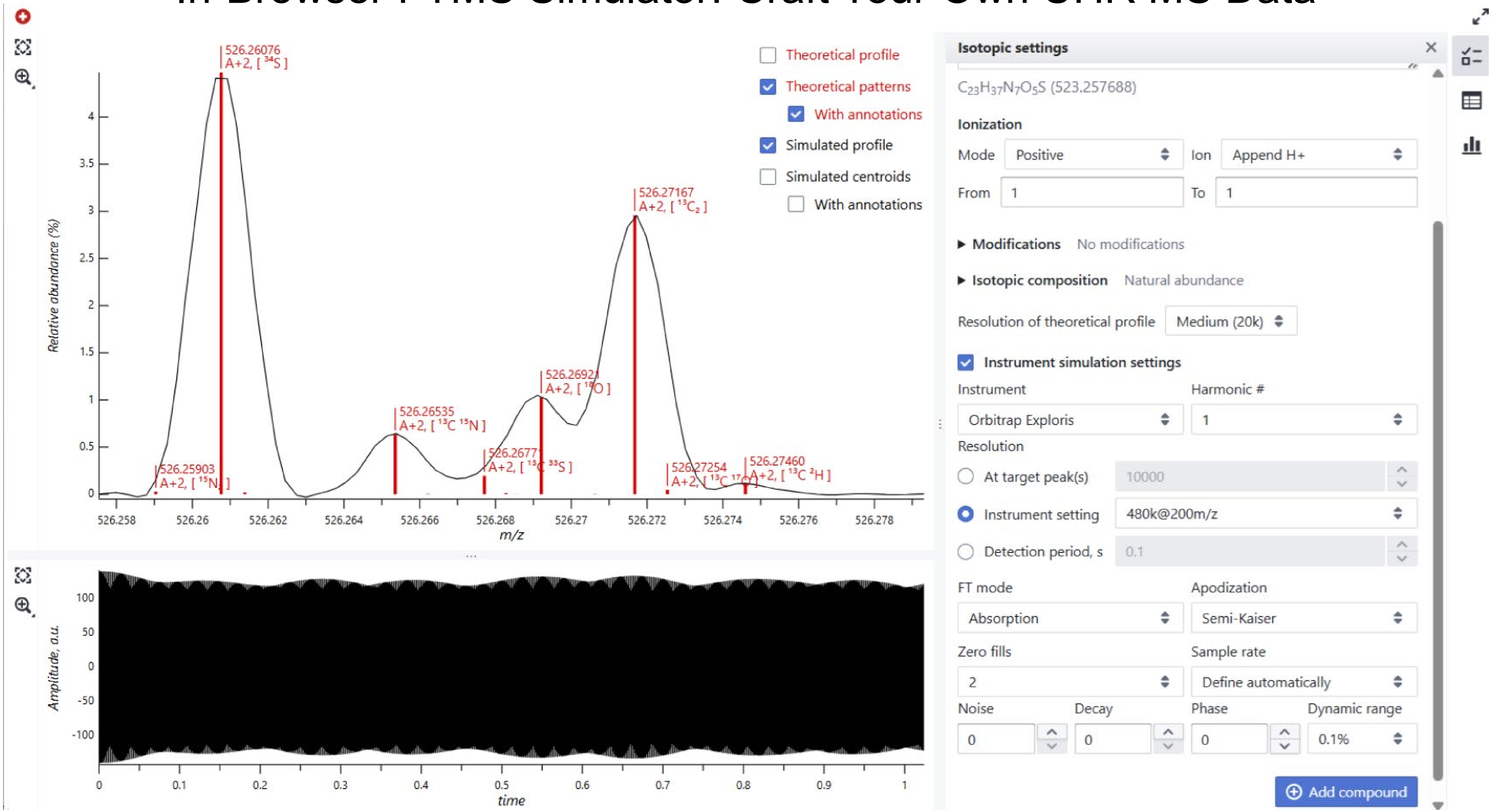
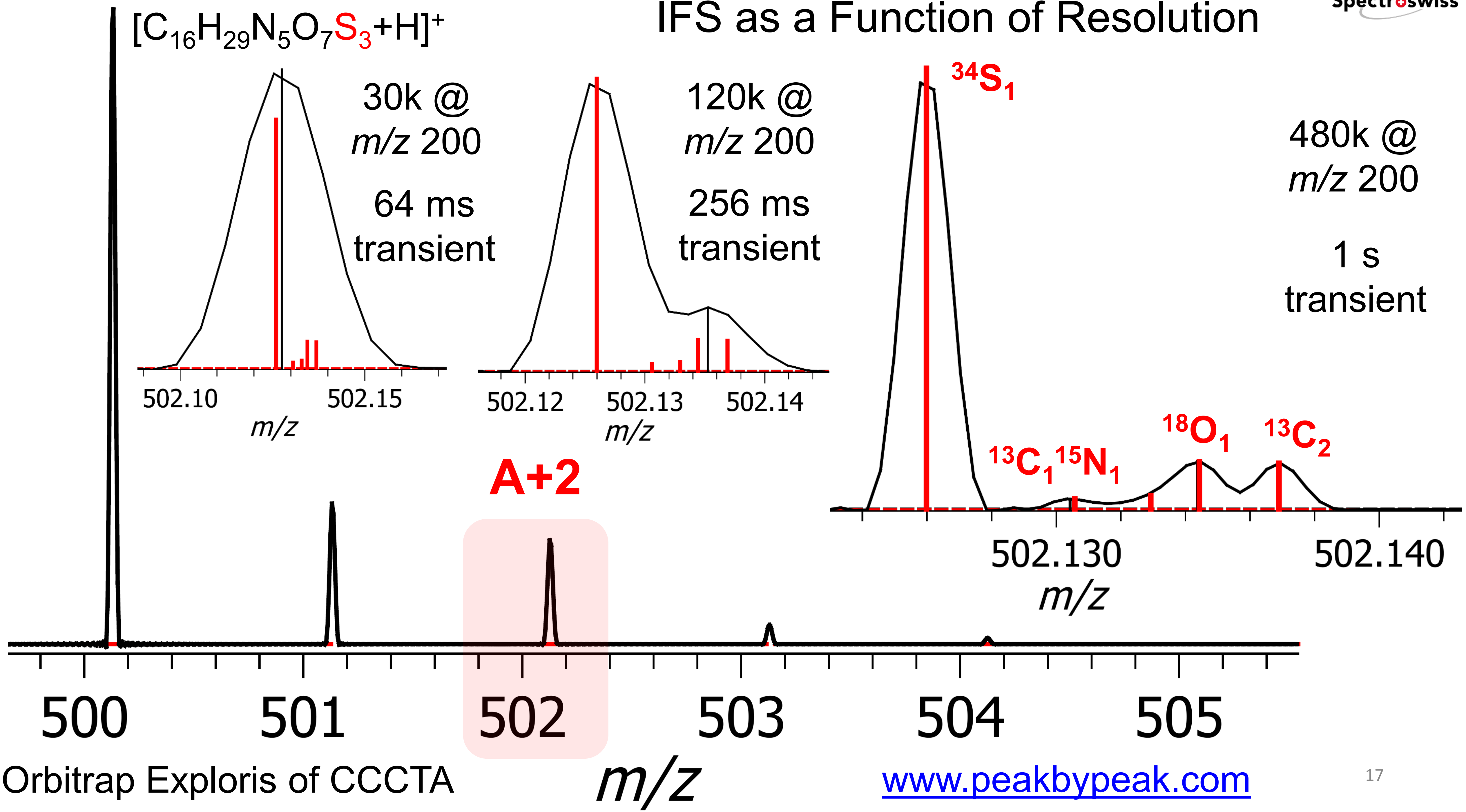
In-Browser FTMS Simulator: Craft Your Own UHR MS Data

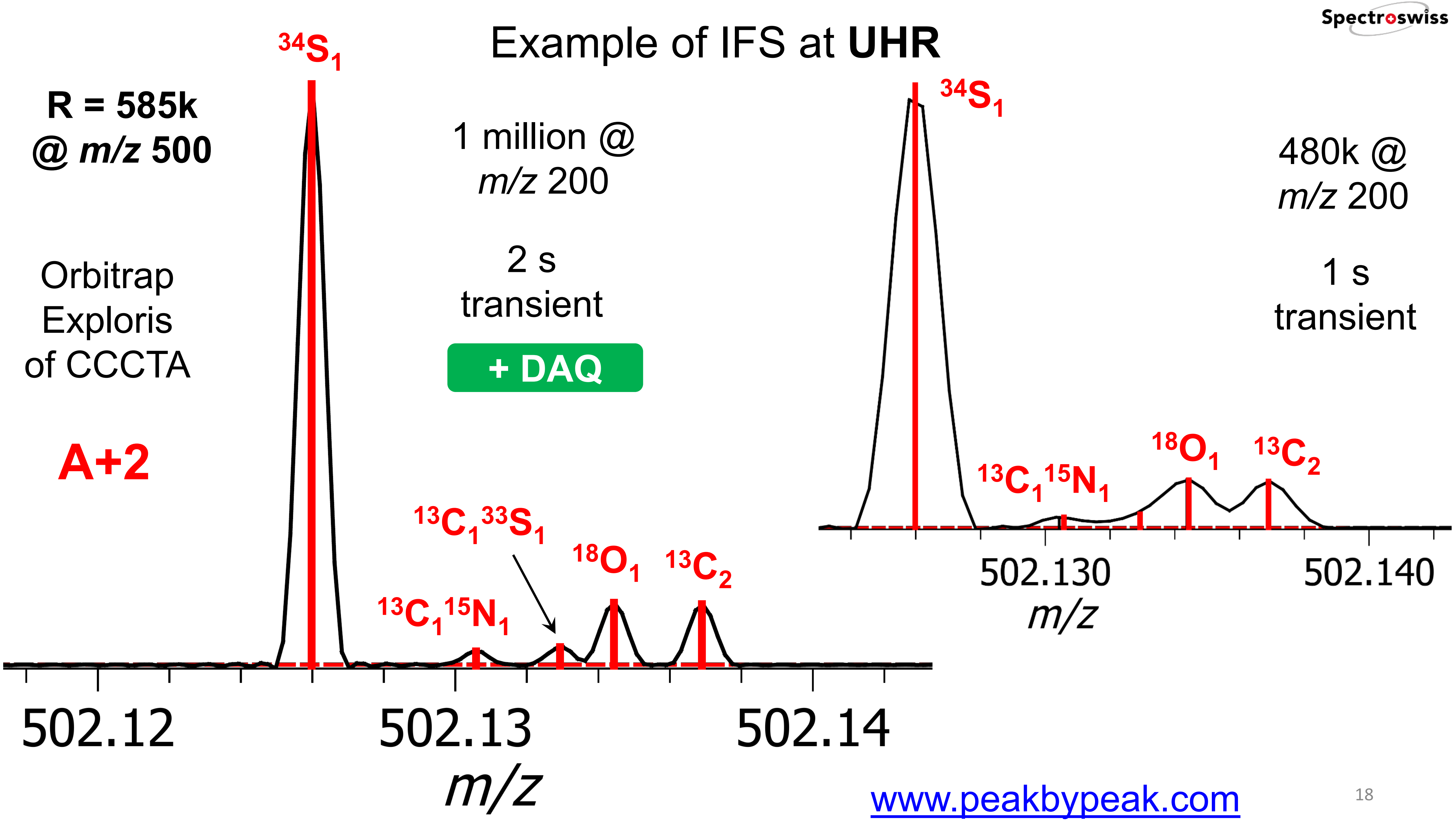


IFS as a Function of Resolution



Orbitrap Exploris of CCCTA

www.peakbypeak.com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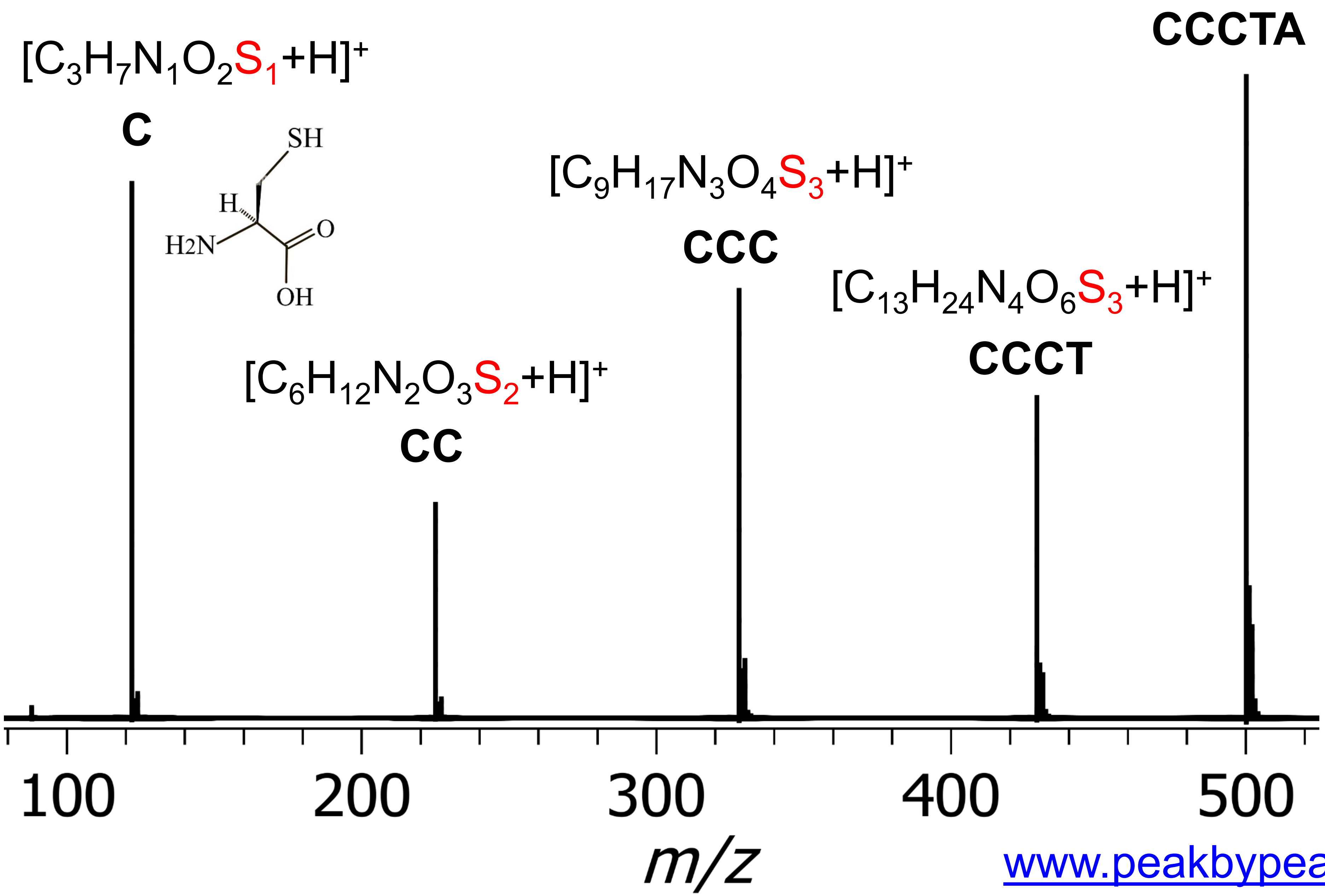
Example of IFS at **UHR**
R = 585k
@ *m/z* 500
Orbitrap
Exploris
of CCCTA
A+2
1 million @
m/z 200
2 s
transient
+ DAQ
$^{34}S_1$
$^{13}C_1{}^{33}S_1$
$^{13}C_1{}^{15}N_1$
$^{18}O_1$
$^{13}C_2$
502.12
502.13
502.14
m/z
480k @
m/z 200
1 s
transient
$^{34}S_1$
$^{13}C_1{}^{15}N_1$
$^{18}O_1$
$^{13}C_2$
502.130
502.140
m/z
www.peakbypeak.com

Resolution is a Function of *m/z*



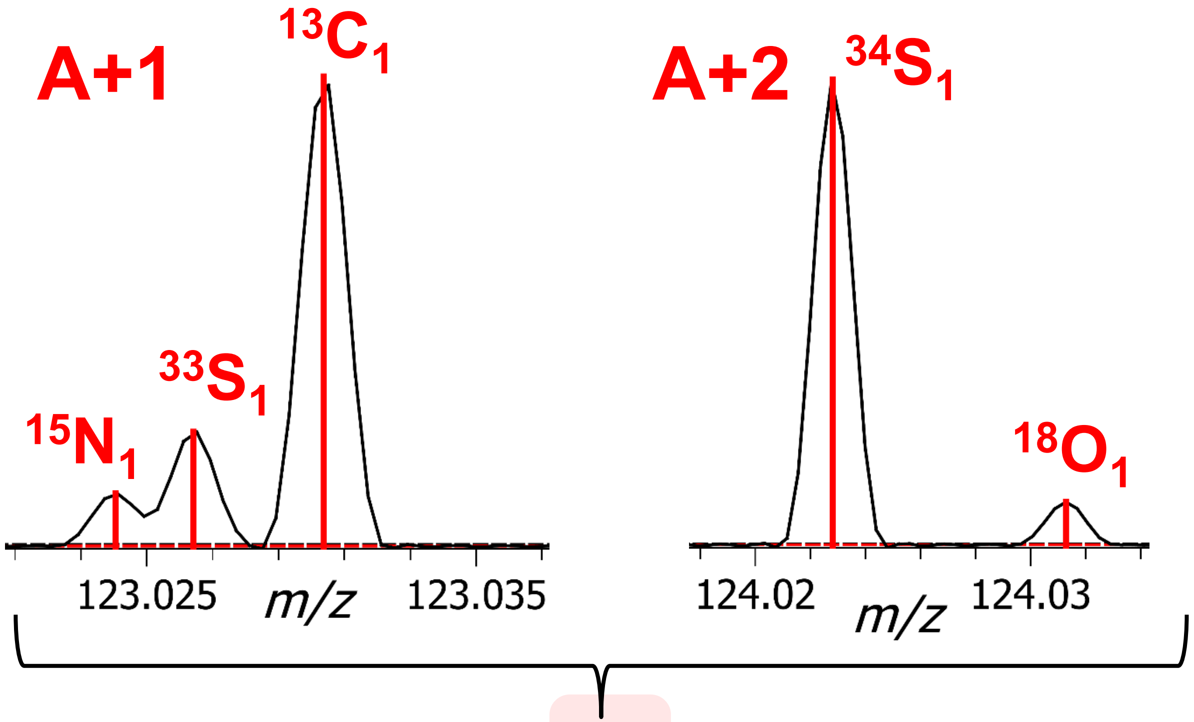
Orbitrap Exploris

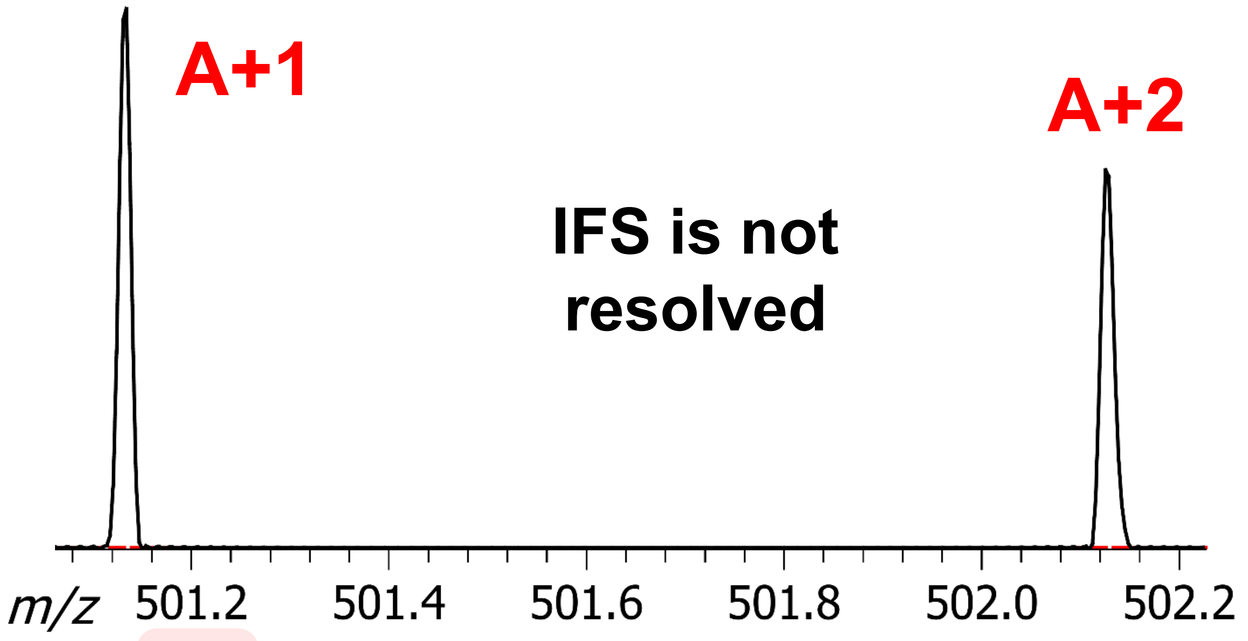
128 ms transient

60k @ *m/z* 200

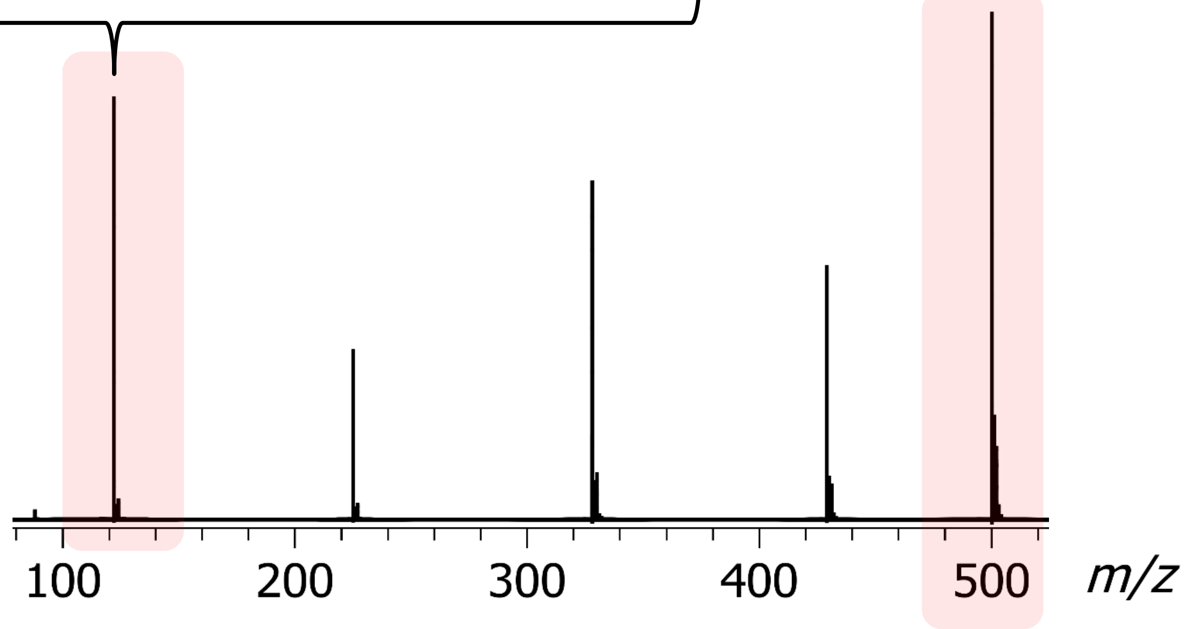
$$\boxed{\omega_z \approx \sqrt{\frac{k}{m/z}}}$$

UHR MS: Use with Caution!





$$\omega_z \approx \sqrt{\frac{k}{m/z}}$$



Orbitrap Exploris

128 ms transient

60k @ *m/z* 200

www.peakbypeak.com

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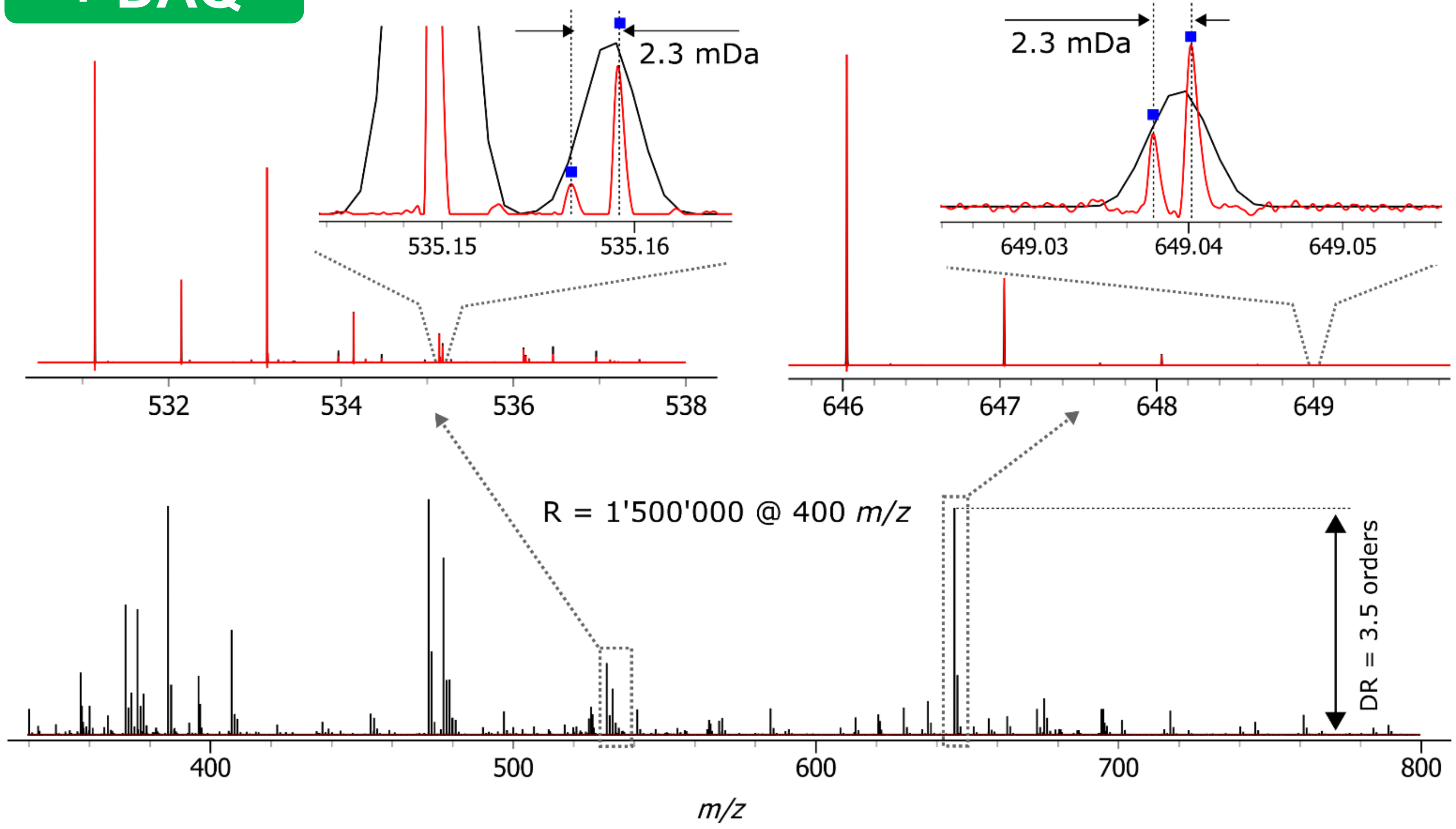
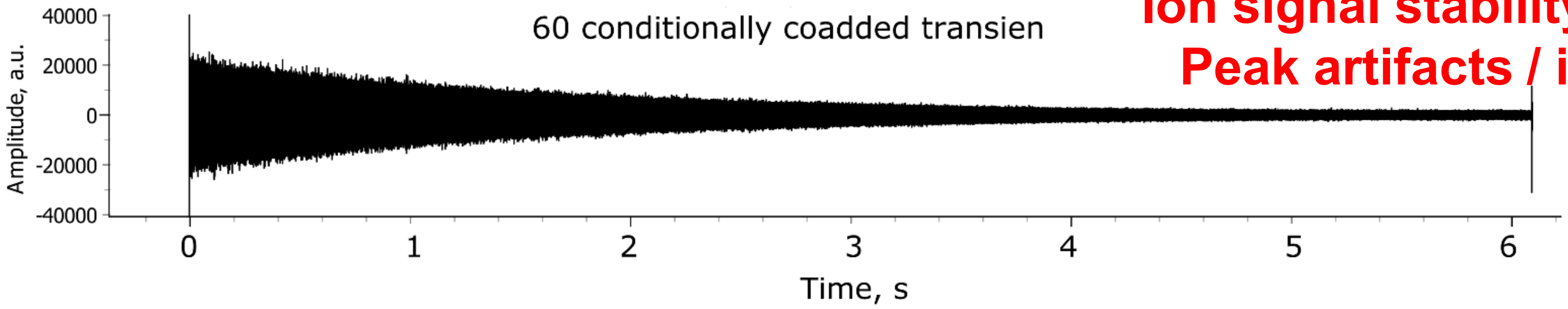
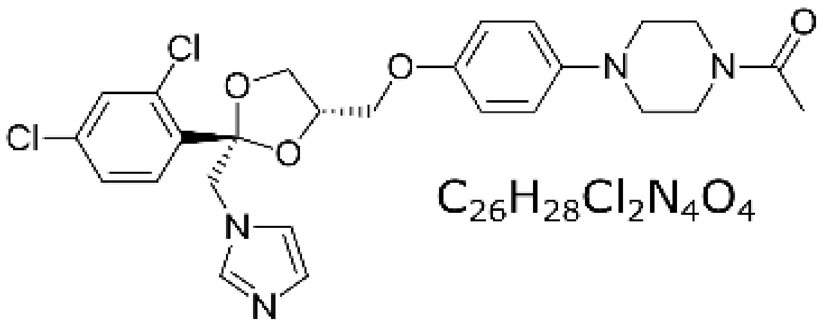
UHR MS of Metabolite Mixtures: **LC-MS**

+ DAQ



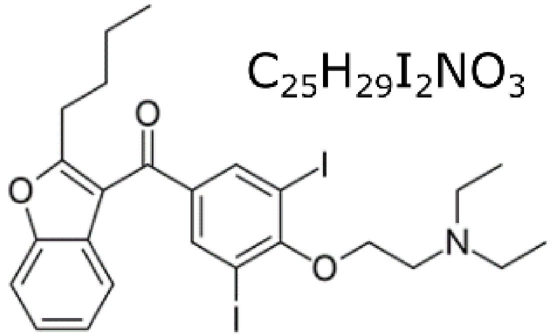


Ketoconazole



$C_{26}H_{28}Cl_2N_4O_4$

Amiodarone



$C_{25}H_{29}I_2NO_3$

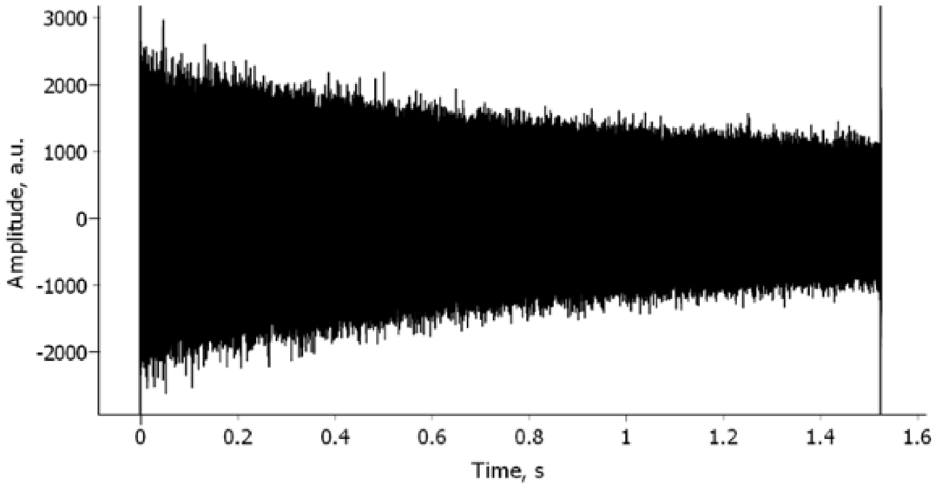
Ion signal stability / coherence?
Peak artifacts / interference?

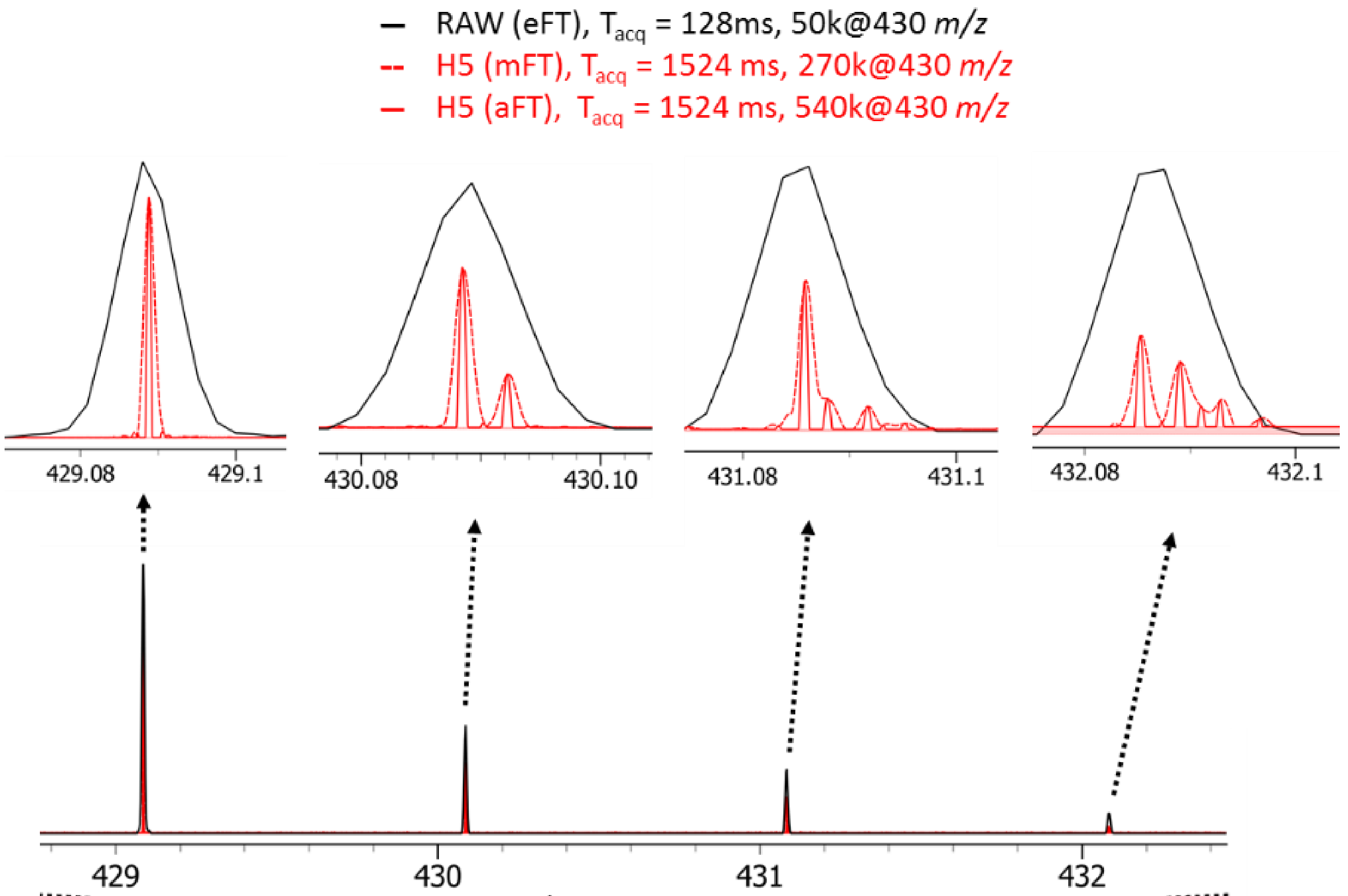
Laure
Menin

UHR MS of Volatile Compounds: **GC-MS**

+ DAQ

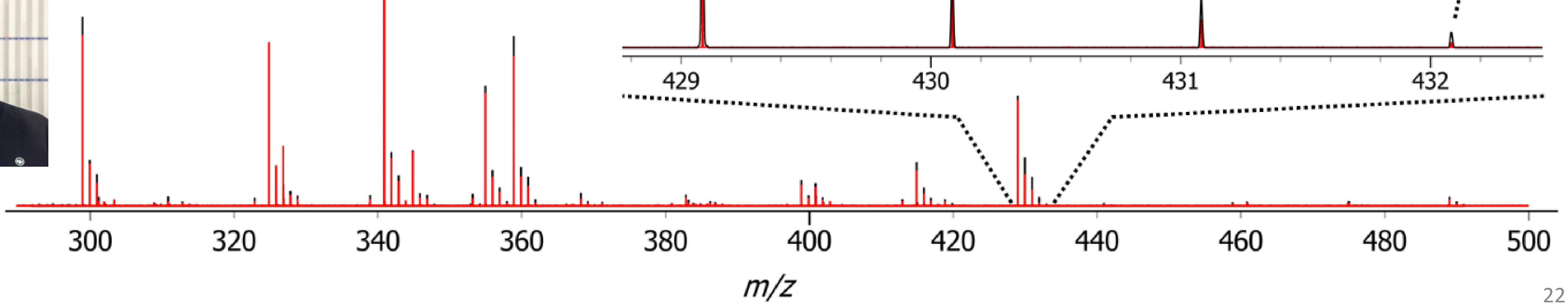
Dioxin analysis



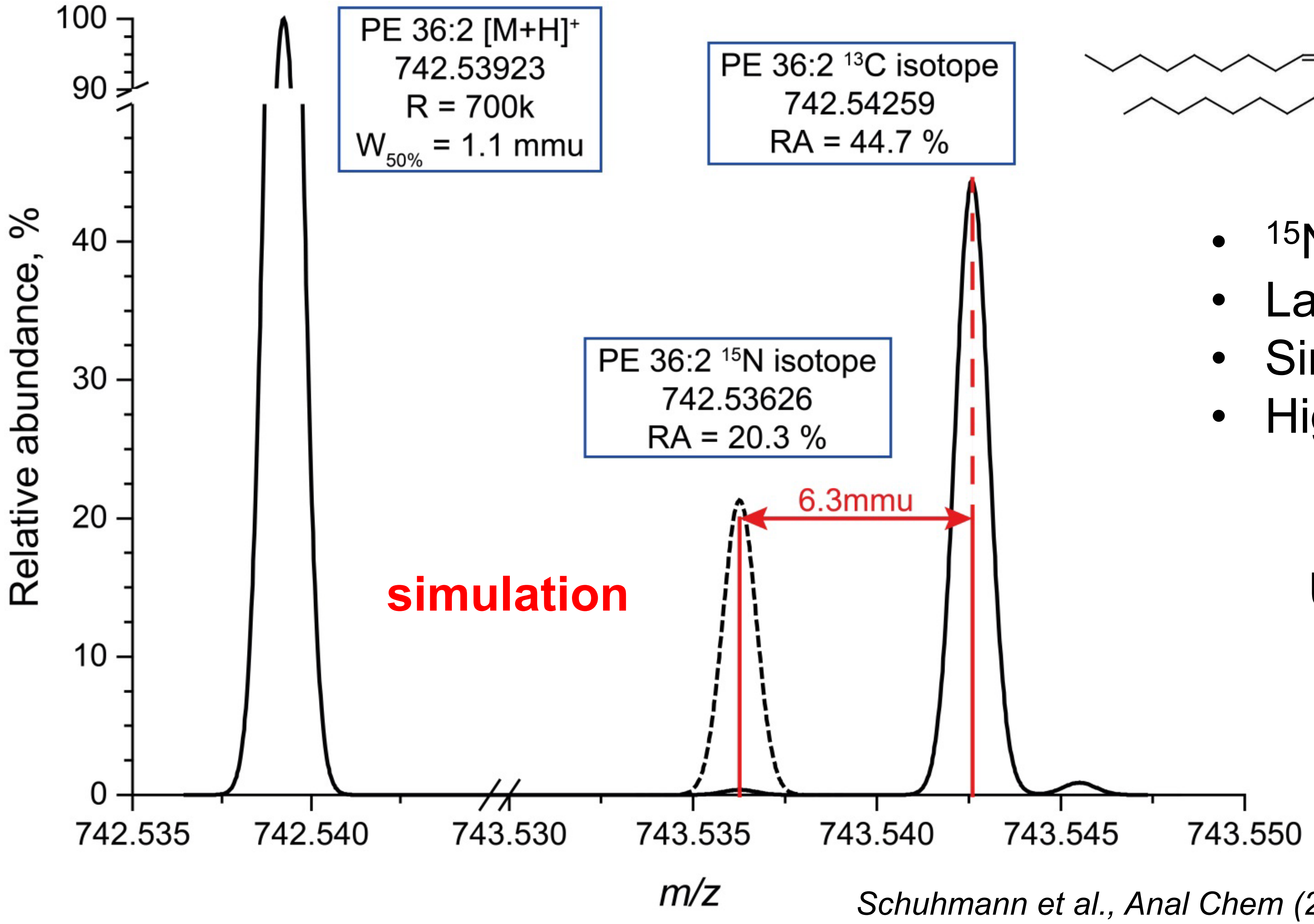


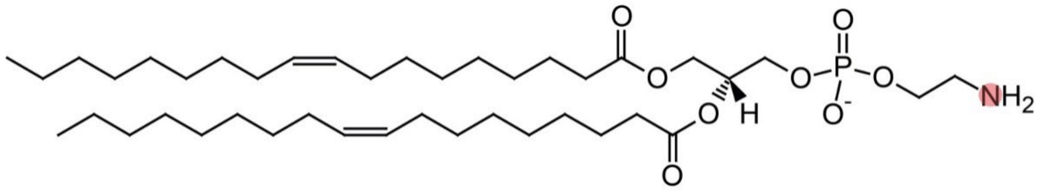
Bleiner et al., JASMS 2020, 31, 257-266

David Bleiner



UHR MS for **Shotgun Lipidomics**: Multiplexed Quantitation





- ^{15}N sources are affordable
- Labeling is specific
- Simple isotopic patterns
- High sensitivity

BUT
UHR MS is required

Andrej Shevchenko

CBG
Max Planck Institute of Molecular Cell Biology and Genetics

Schuhmann et al., Anal Chem (2017) 12857-12865

Metabolic ^{15}N labeling: Quantitative Shotgun Lipidomics

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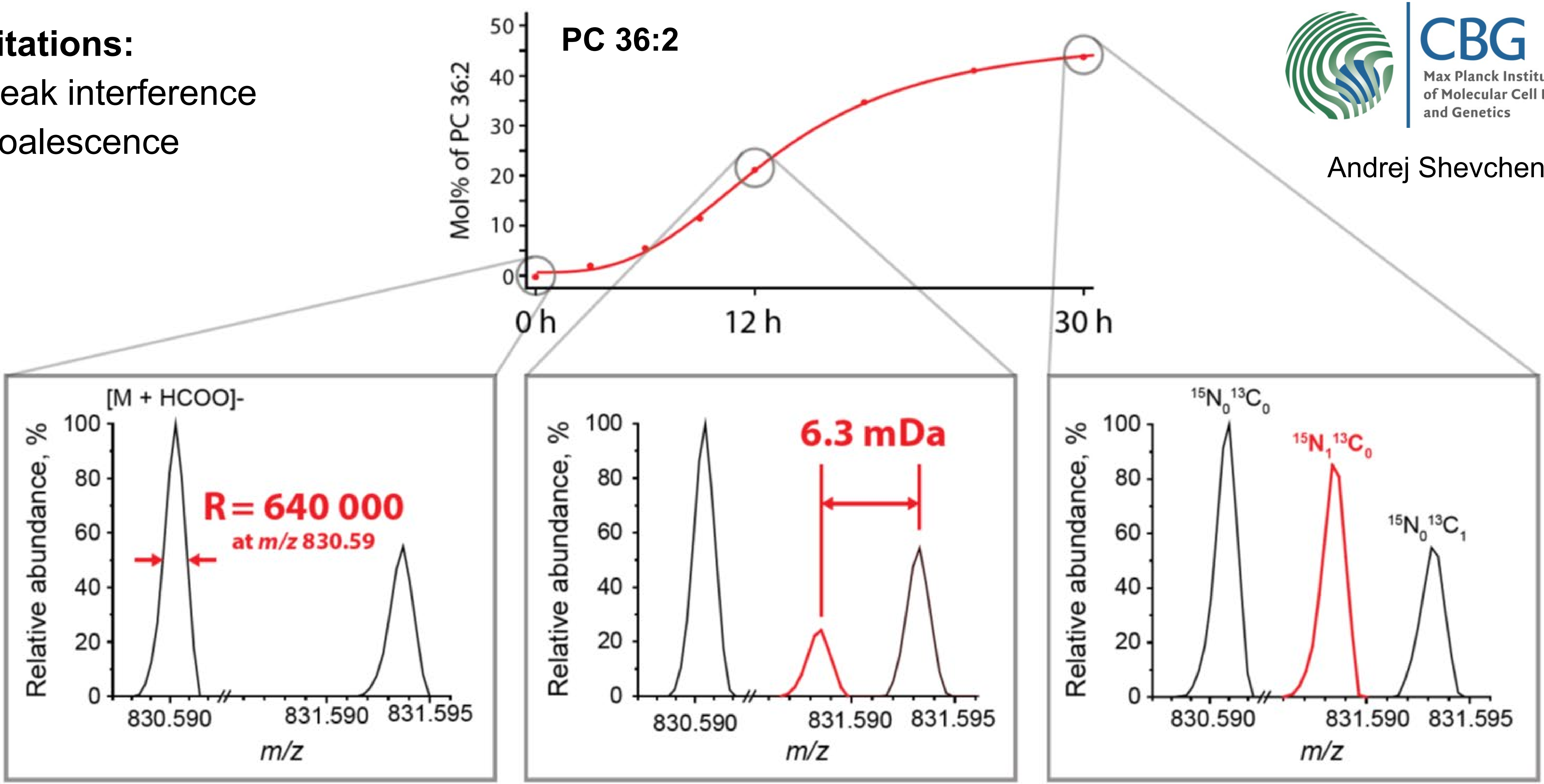
Limitations:
- peak interference
- coalescence



CBG
Max Planck Institute of Molecular Cell Biology and Genetics

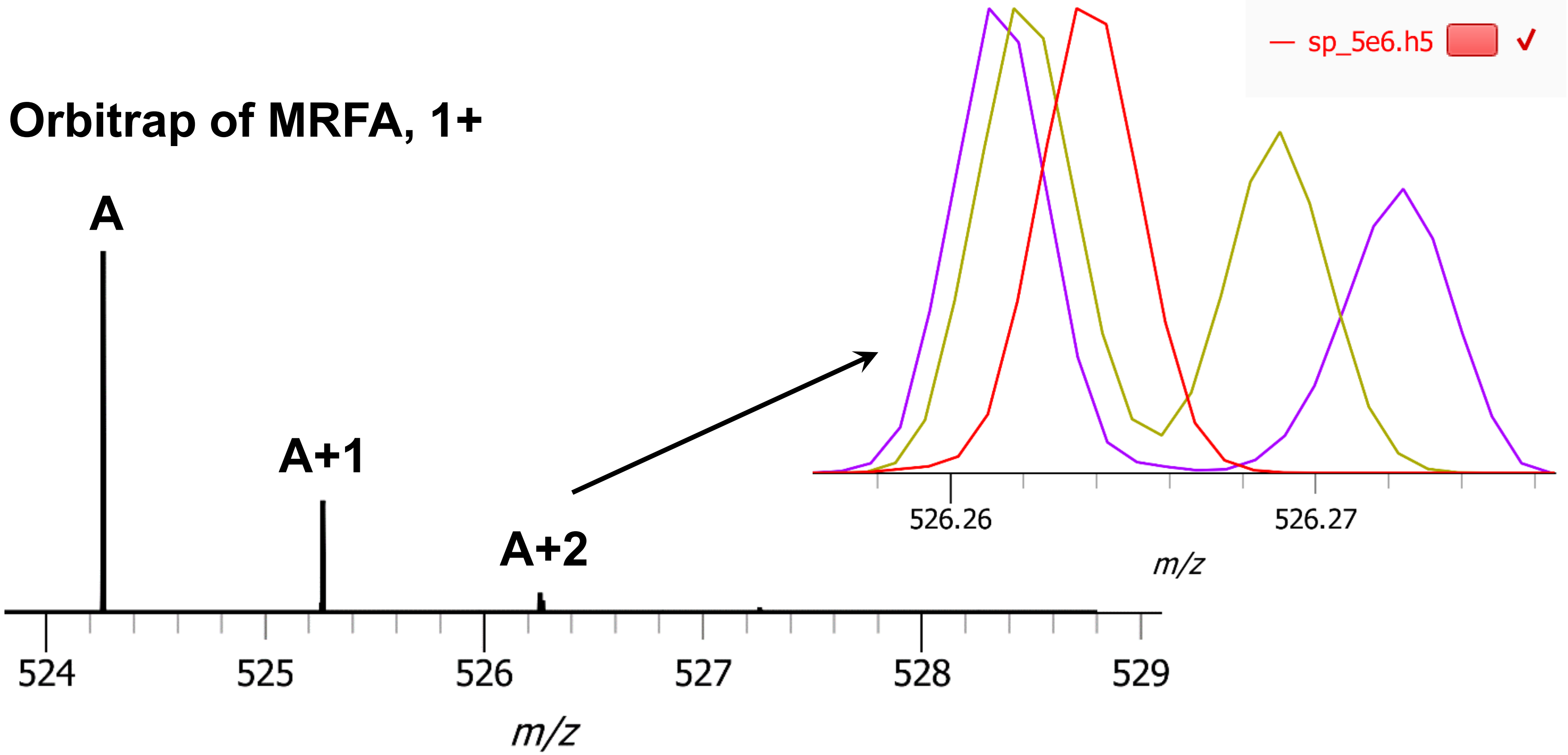
Andrej Shevchenko

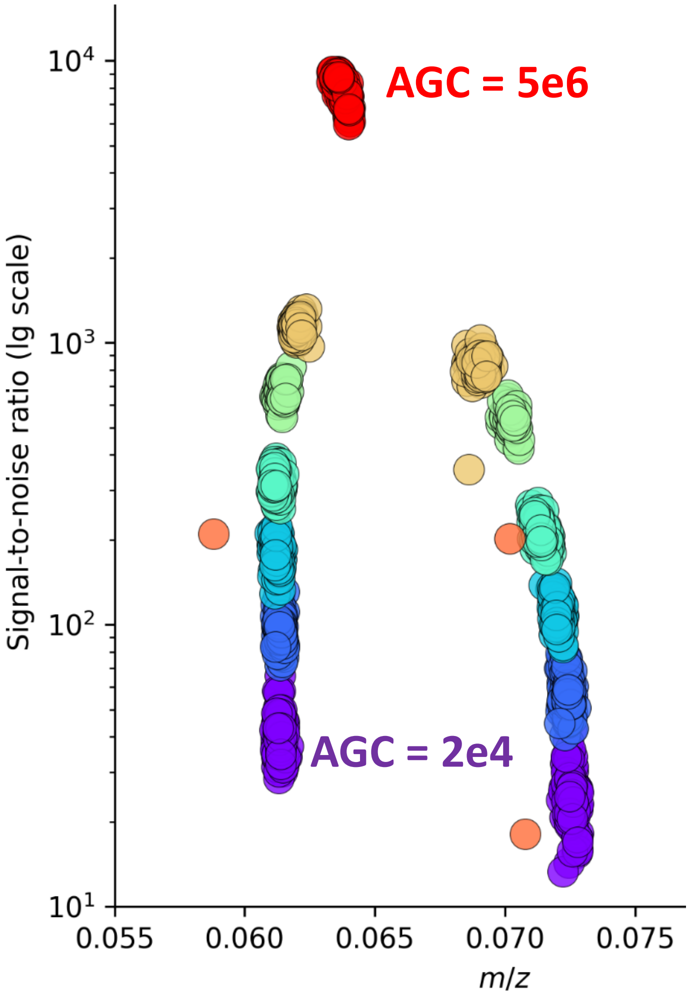
Schuhmann et al., Anal Chem (2017) 12857-12865

Space Charge Effects: Peak Artifacts

- Too many charges (ions): peak artifacts (*m/z*, abundance errors), coalescence
- Number of charges (ions) control is needed
- AGC: automatic gain control (Orbitraps)

Orbitrap of MRFA, 1+





DOI: abs/10.1002/rcm.6289; DOI: 10.1021/acs.analchem.7b03437

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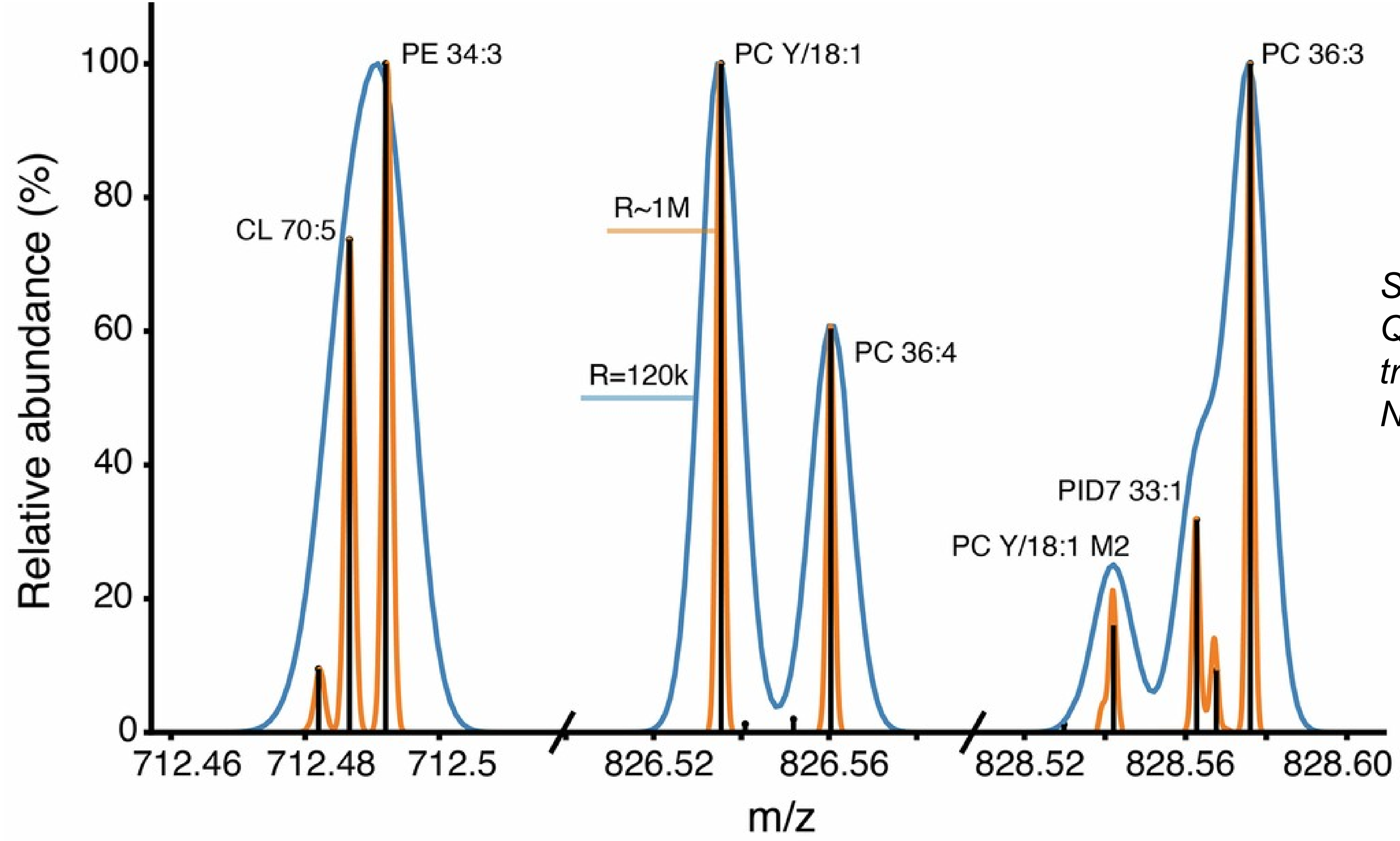
Shotgun UHR MS resolves lipid peaks spaced by a few mDa, matches bifunctional precursors & their metabolites in multiple lipid classes



Shevchenko et al.
Quantitative imaging of lipid transport in mammalian cells
Nature (2025), in print

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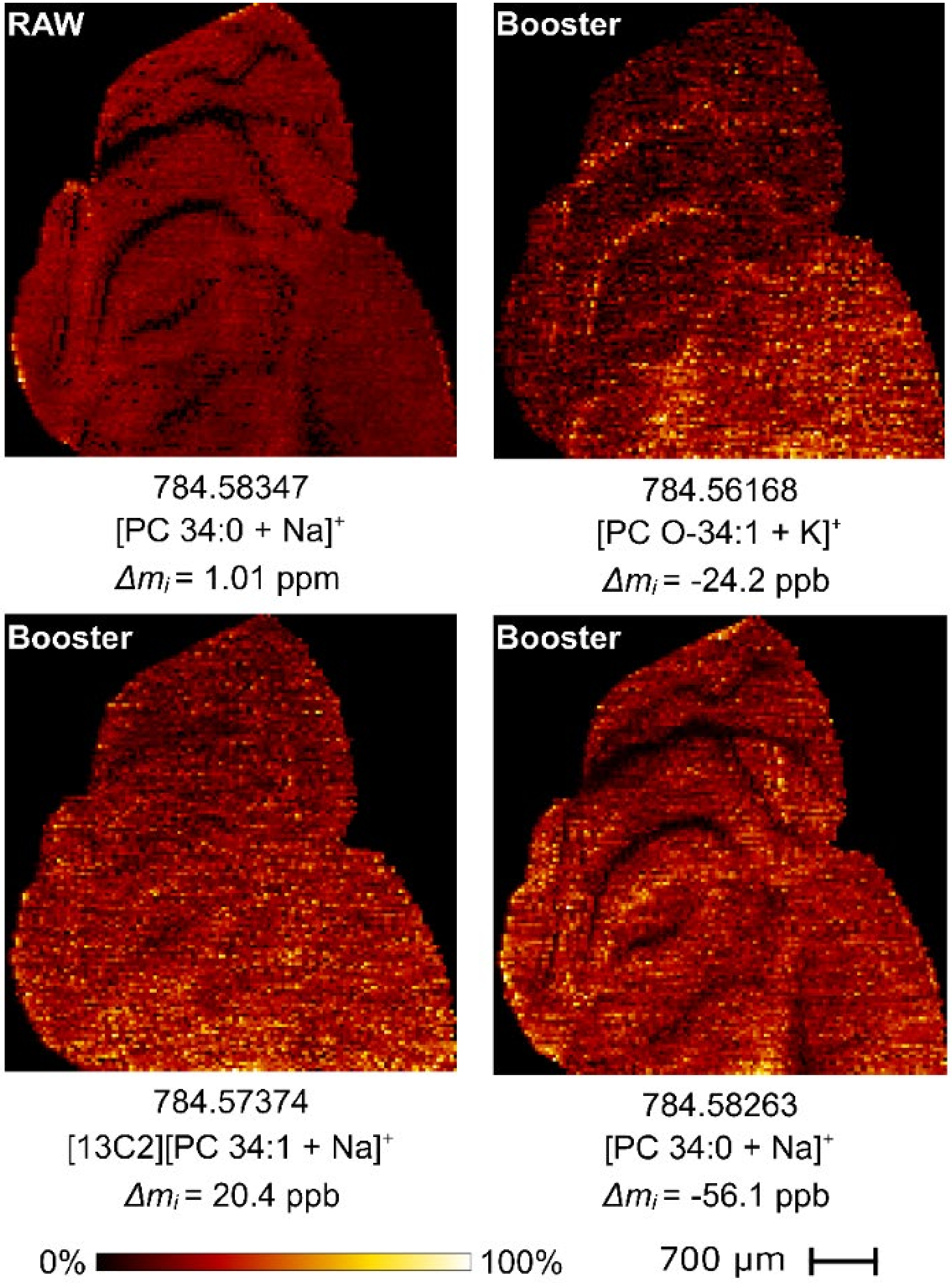
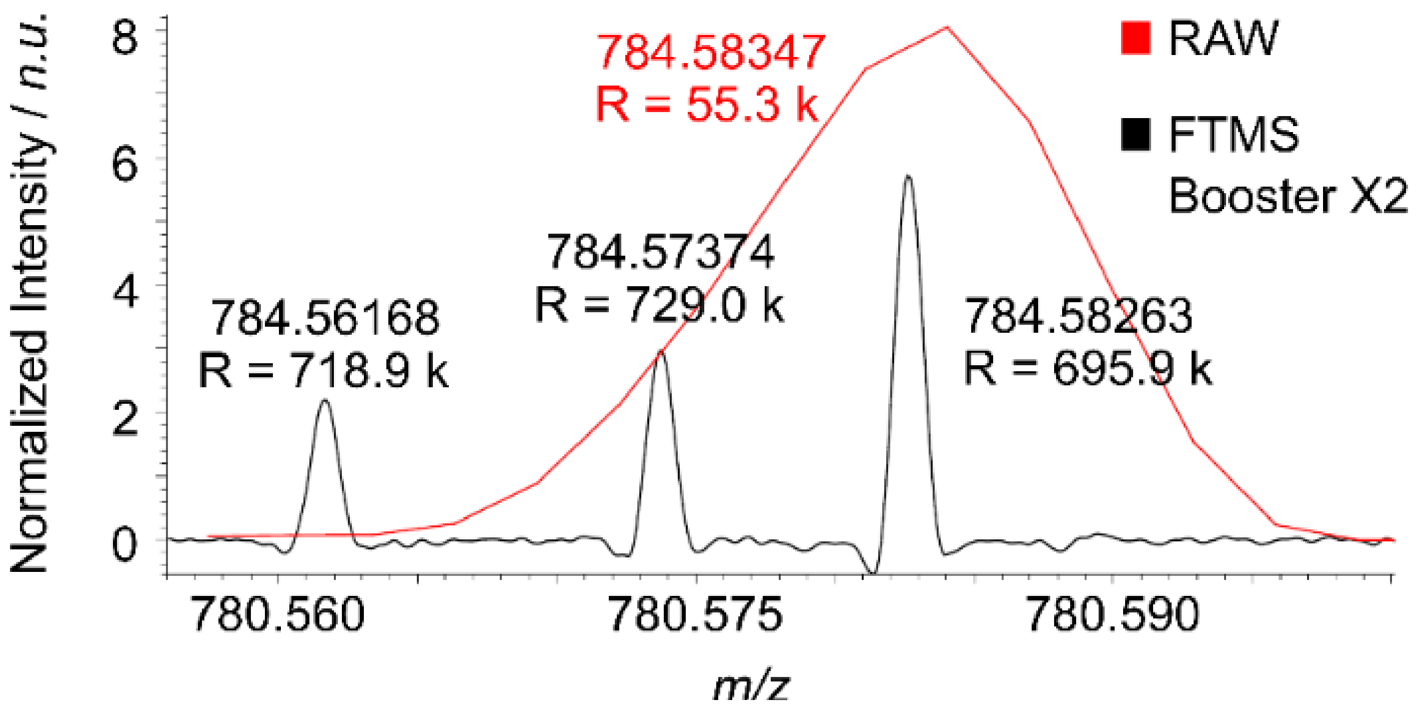
UHR MS **Imaging** of Lipids





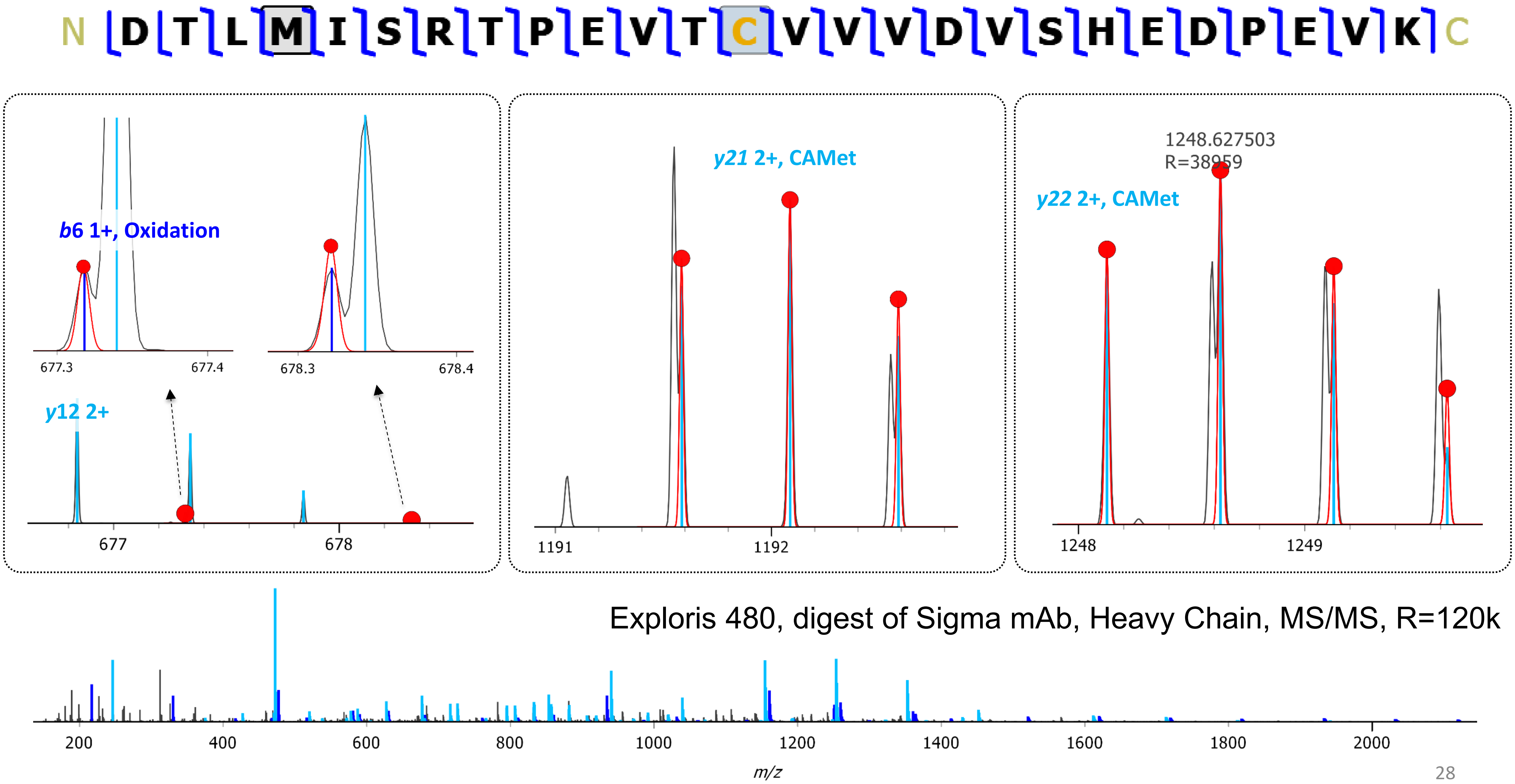
- A mouse brain tissue section analyzed at 70 μm^2 pixels in positive ion mode using a MALDI Orbitrap
- Resolution from **256 ms** & **7 s (Booster)** transients

Ron Heeren

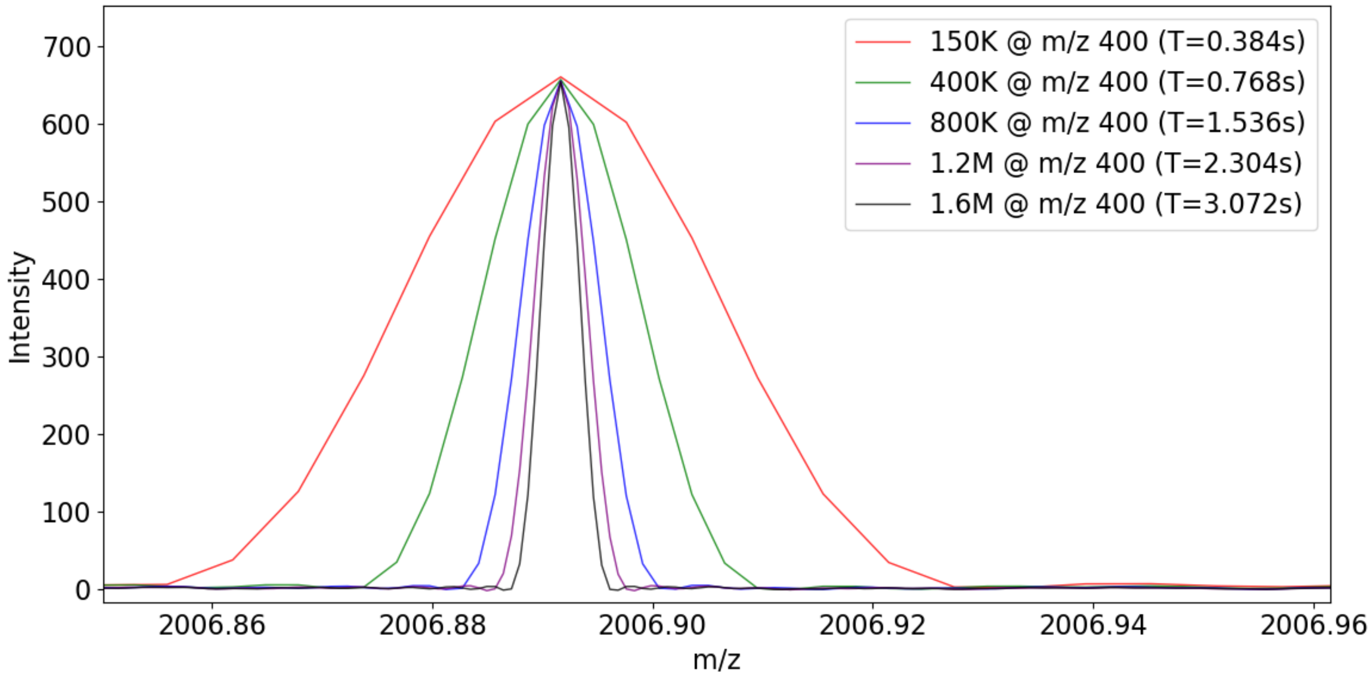
+ DAQ

Heeren et al., Anal. Chem. 2024, 96, 2, 794–801

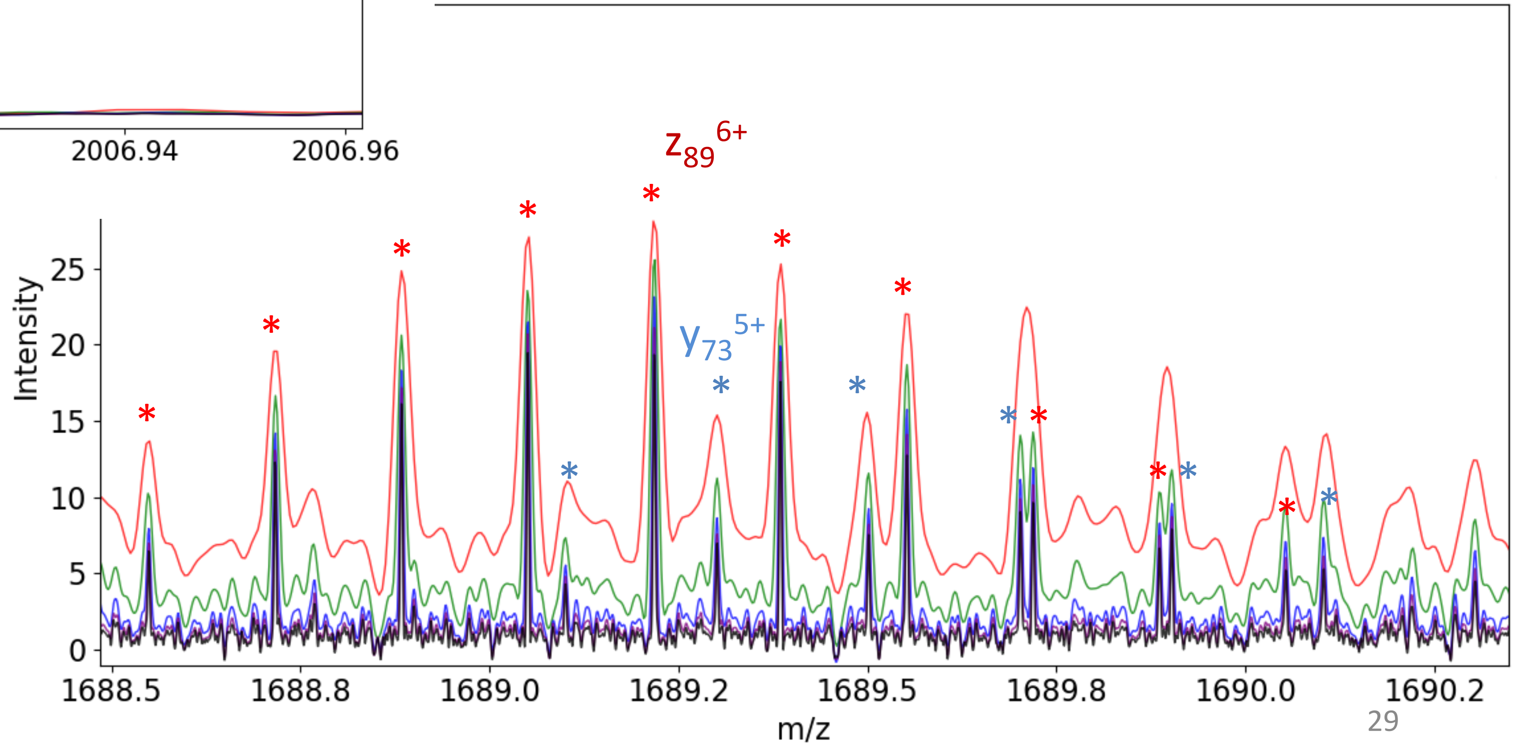
UHR Bottom-Up Proteomics (Peptide MS/MS)



UHR Top-Down Proteomics (Protein MS/MS): High Mass Ions



- better detection of the overlapping product ion distributions

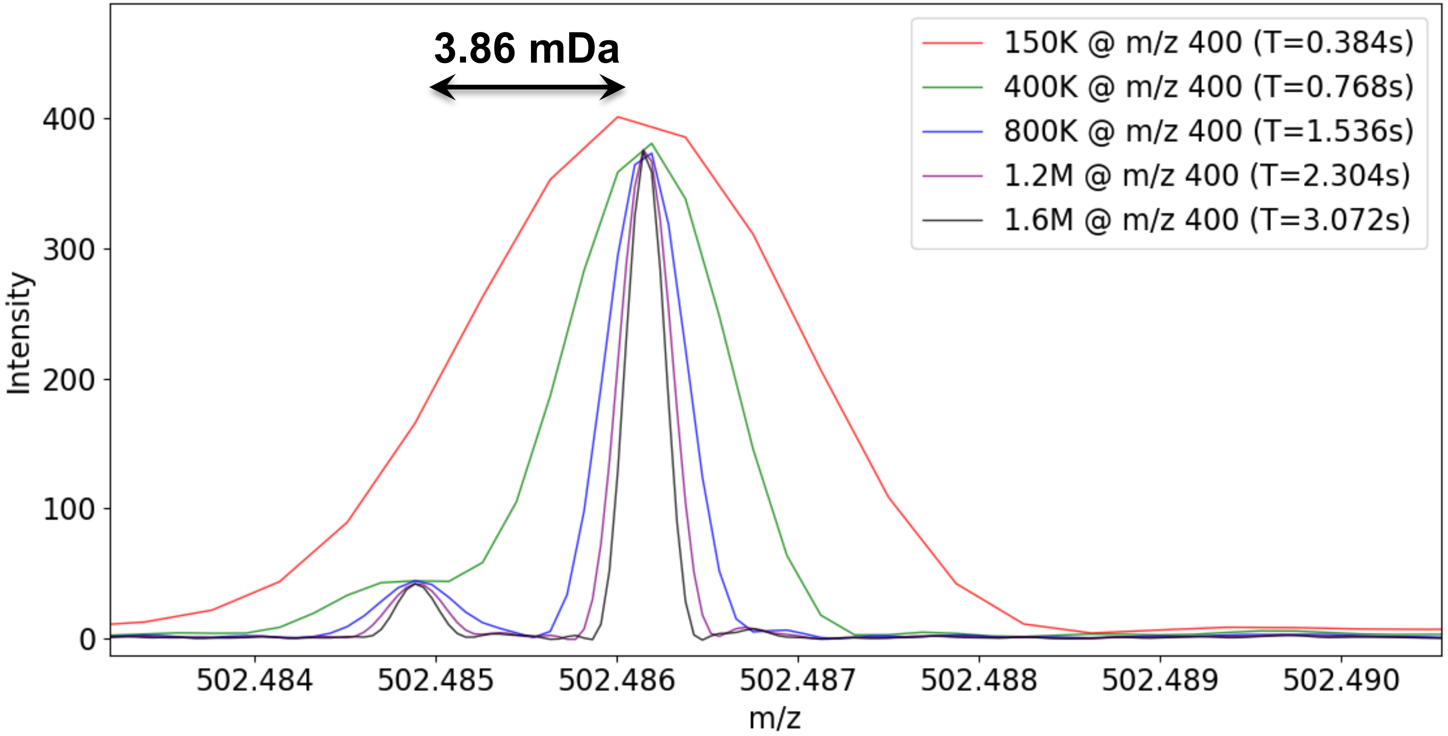


Analysis of
Carbonic Anhydrase II (29 kDa)
ETD of $[M+34H]^{34+}$
21 T FT-ICR MS

Nina Khristenko

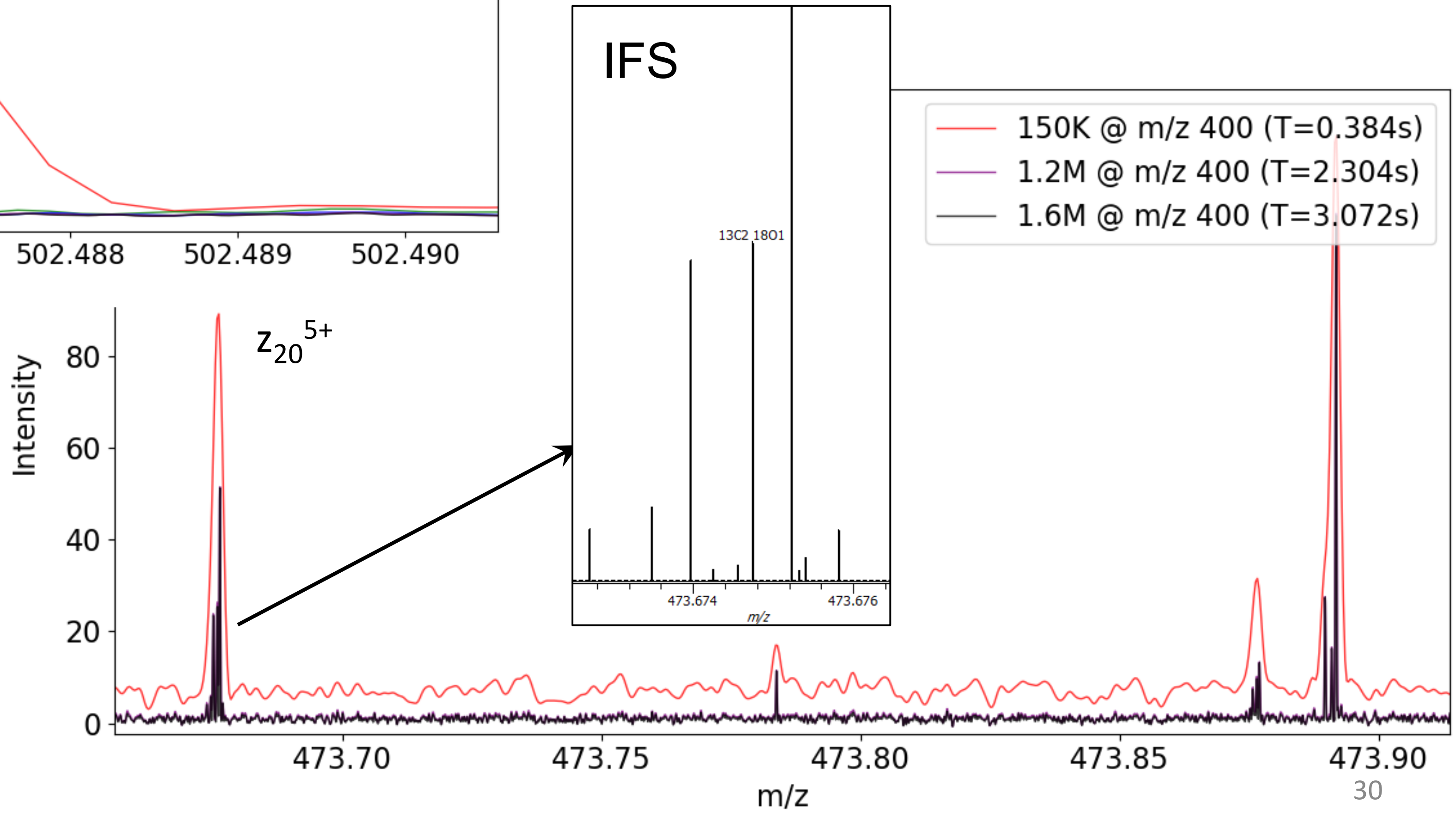
Spectroswiss

UHR Top-Down Proteomics (Protein MS/MS): Low Mass Ions



- Increased spectral complexity
- Harder charge state definition
- Longer MS annotation
- More false annotations

Analysis of
Carbonic Anhydrase II (29 kDa)
ETD of $[M+34H]^{34+}$
21 T FT-ICR MS



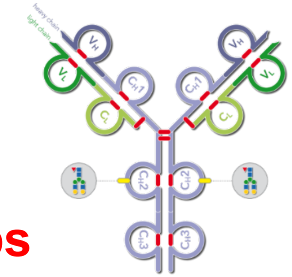
nature methods

Article https://doi.org/10.1038/s41592-024-02207-8

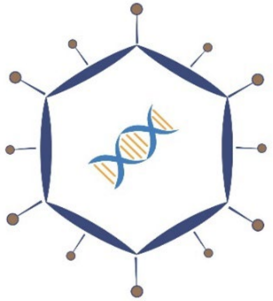
Ultralong transients enhance sensitivity and resolution in Orbitrap-based single-ion mass spectrometry

+ DAQ

150 kDa mAbs



1-10 MDa AAVs



Received: 20 June 2023

Accepted: 7 February 2024

Published online: 05 March 2024

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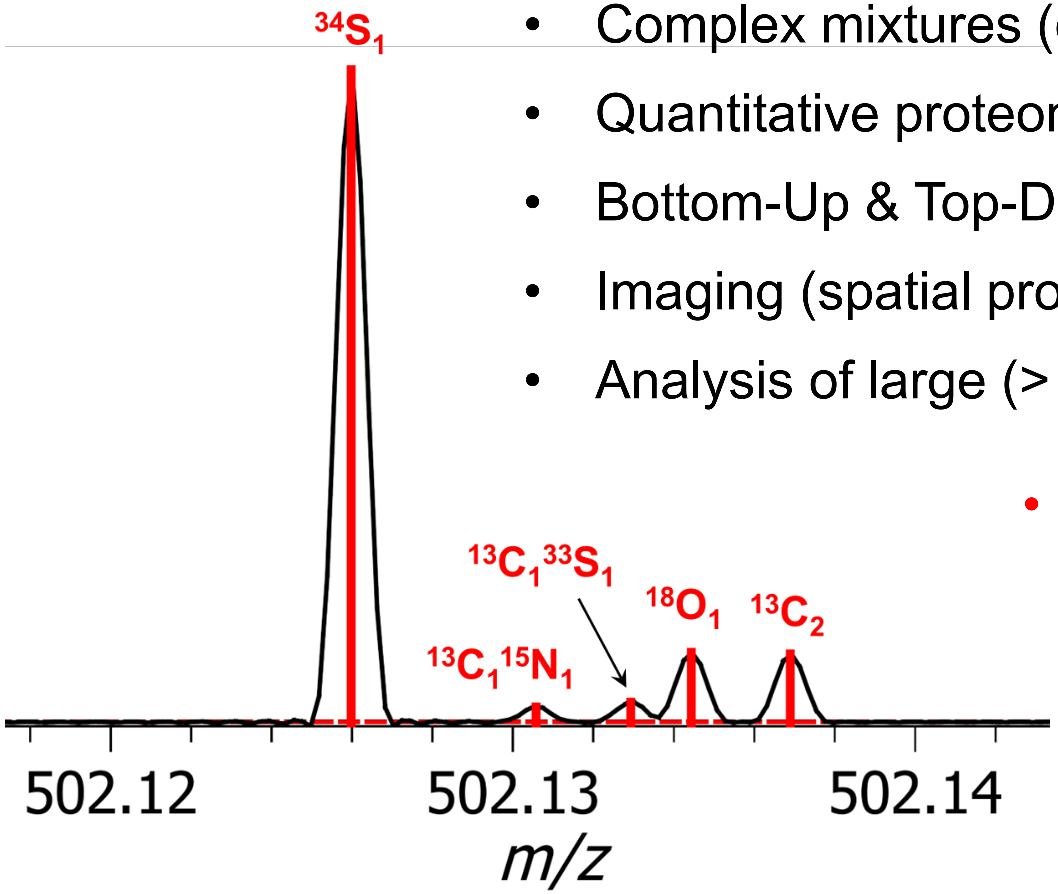
The UHR MS **Blessing**: Sharper Vision, Analytical Clarity

Spectroswiss

- Ultra-high-resolution is needed to separate isobaric compounds
- Improved confidence in annotation and identification of unknowns
- Exploring previously inaccessible chemical space (novel metabolites, toxins)



  - Complex mixtures (crude oils/biofuels/metabolites/drugs): isobars, IFS
  - Quantitative proteomics/lipidomics: multiplexing with isotopic labeling
  - Bottom-Up & Top-Down (proteins): higher sequence coverage, confidence
  - Imaging (spatial proteomics & metabolomics): more IDs, higher confidence
  - Analysis of large (> 1 MDa) biomolecules (viruses): high-performance CDMS

- **Direct infusion is great, but how about LC/GC?**
  - Small molecules are in the highest resolution range
  - Parallel ion accumulation / fragmentation / detection
  - Super-resolution methods instead of the FT methods

The UHR MS **Curse**: Bigger Burden

- **Longer experiment optimization:**
  - Space charge – control of ion (charge) number, fine tuning of all parameters.
- **Instrumental cost & complexity:**
  - Long transient acquisition times, maintenance, accessibility limited to few labs.
- **Sensitivity trade-offs:**
  - Sometimes resolution is gained at the expense of duty cycle or sensitivity.
- **Interpretation bottleneck:**
  - Higher resolution doesn't always mean better biological insight
- **Overfitting danger:**
  - Assigning meaning to noise/artifacts, spurious peak picking.

High level of expertise is required to overcome these limitations

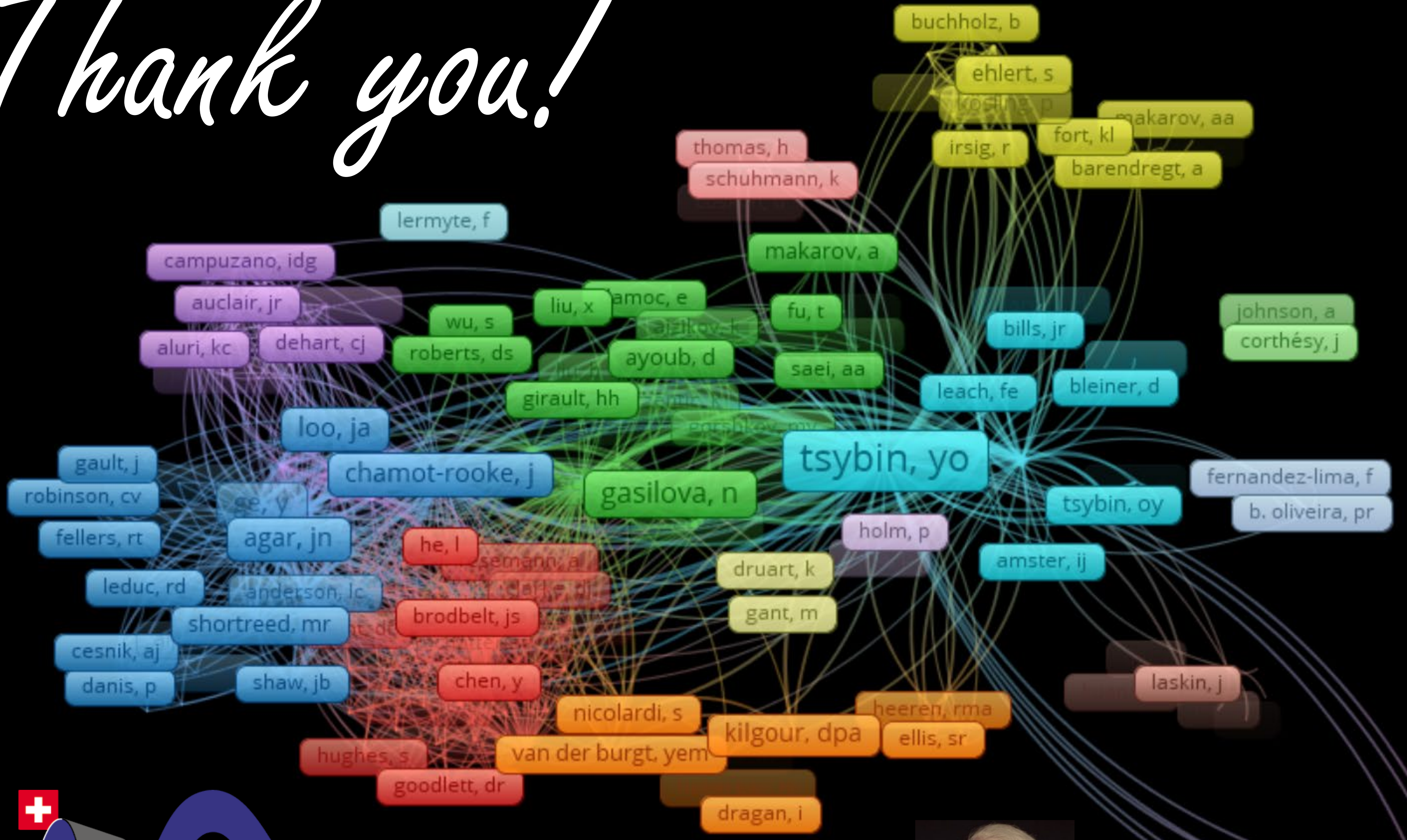
The UHR MS **Data Overload**: New Skills & Tools Needed

- **Explosion of peak lists:**
  - One LC-MS run generates millions and billions of datapoints.
- **Challenges in (toxicology) workflows:**
  - Database matching becomes slow and error-prone.
  - False positives increase if not carefully controlled.
- **Need for computational solutions:**
  - Peak filtering, data reduction, machine learning.
  - Examples: Kendrick mass defect analysis, network visualization, AI annotation.
- **Future perspective:**
  - Move toward “smart resolution” - adaptive acquisition, cloud computing, collaborative databases.

When does UHR MS change the scientific answer - and when doesn’t it?

Thank you!

Spectroswiss



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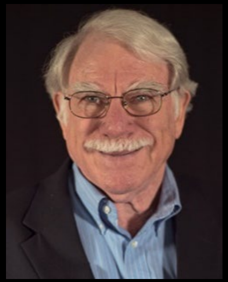

Alan Marshall
1944-2025