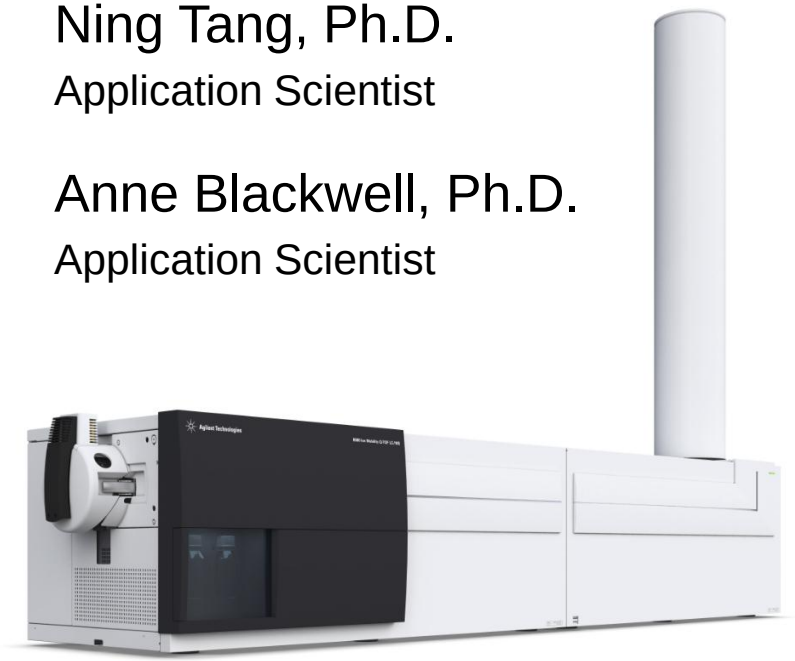
Advantages of Ion Mobility QTOF for Characterization of Large Molecules

Add a New Dimension to your Research Capability with Agilent's New Drift Ion Mobility QTOF System

Ning Tang, Ph.D.
Application Scientist

Anne Blackwell, Ph.D.
Application Scientist



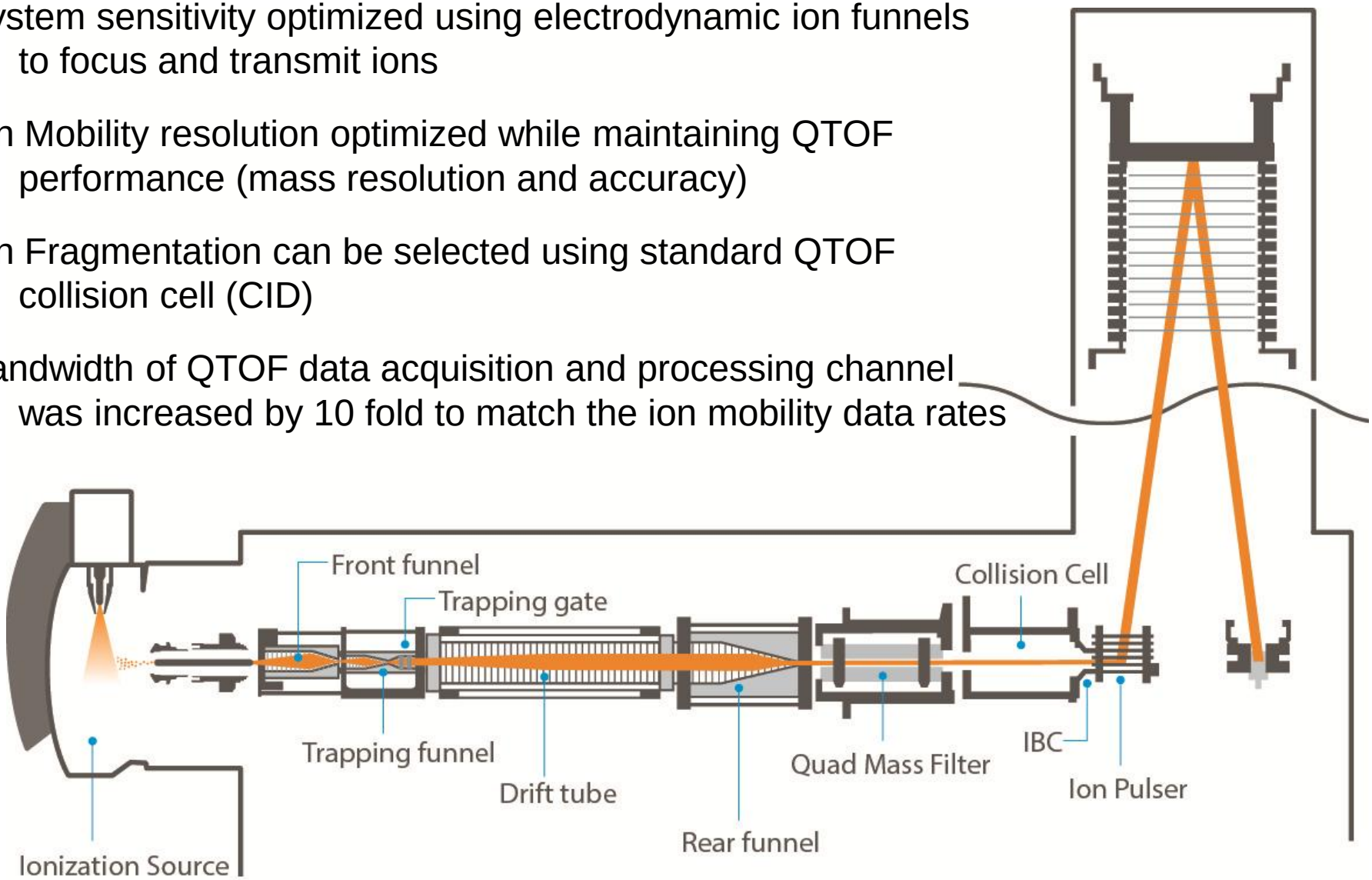
IM-QTOF Instrument Overview

System sensitivity optimized using electrodynamic ion funnels to focus and transmit ions

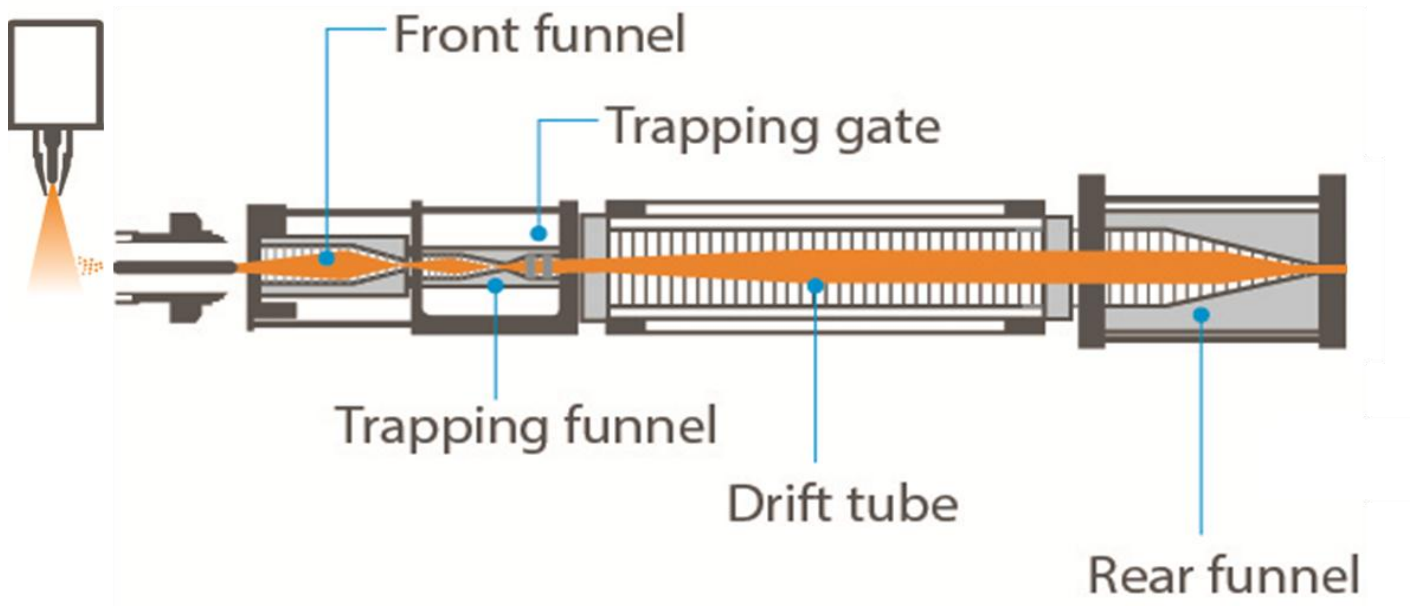
Ion Mobility resolution optimized while maintaining QTOF performance (mass resolution and accuracy)

Ion Fragmentation can be selected using standard QTOF collision cell (CID)

Bandwidth of QTOF data acquisition and processing channel was increased by 10 fold to match the ion mobility data rates



Ion Mobility System Design



Ionization source: Ion generation (ESI, AJS, Nano ESI, ChipCube, APCI etc.)

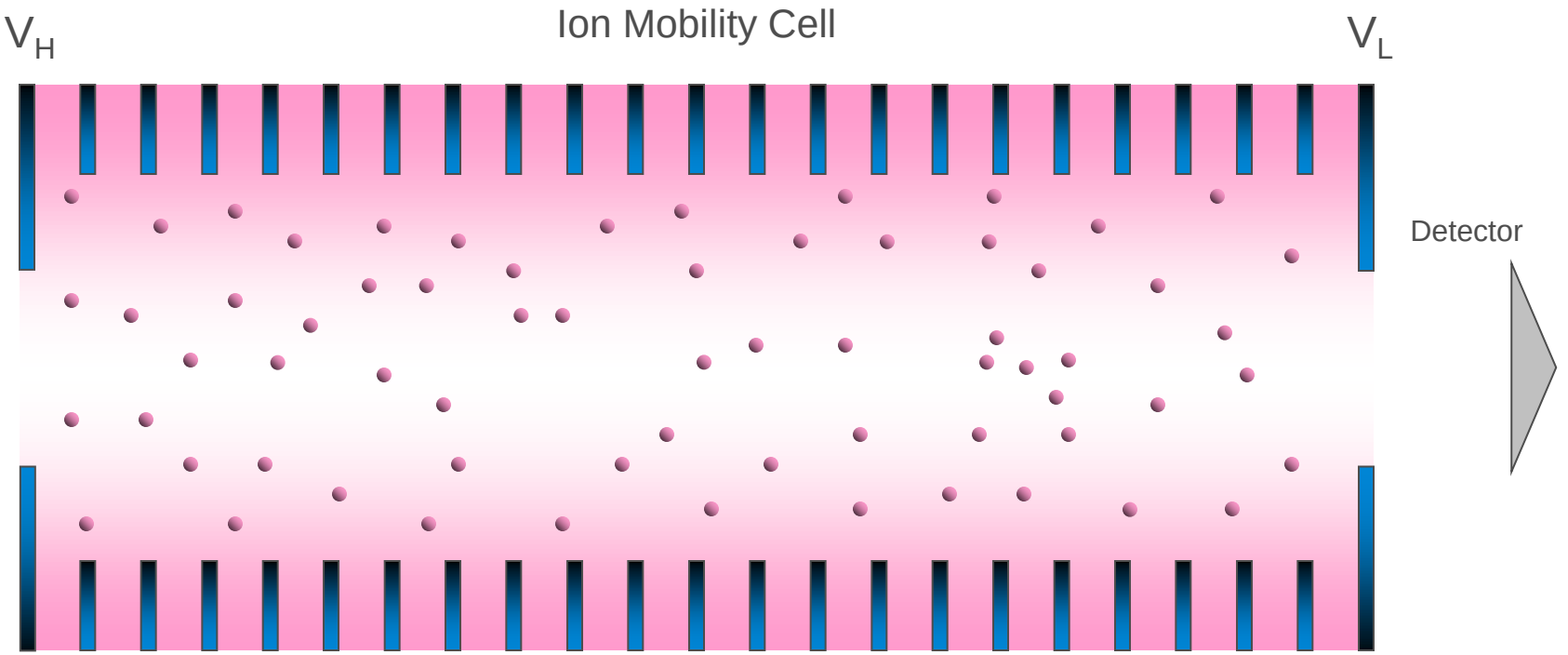
Front ion funnel: Efficient ion collection, desolvation and excess gas removal

Trap funnel: Ion accumulation and introducing ion packets into drift cell

Drift cell: Uniform low field ion mobility allows direct determination of accurate CCS (Ω)

Rear funnel: Efficient ion refocusing and introduction into mass analyzer

Basic Operational Principle of Ion Mobility
For Conventional DC Uniform Field IMS



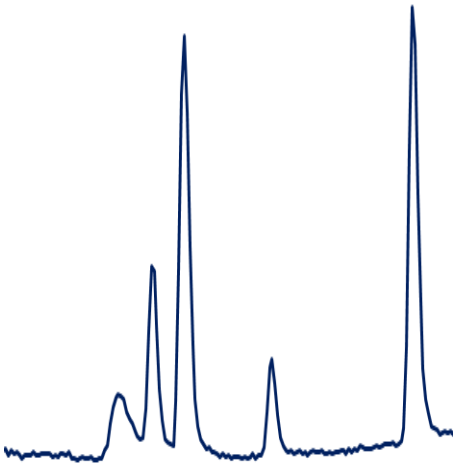
Electric Field

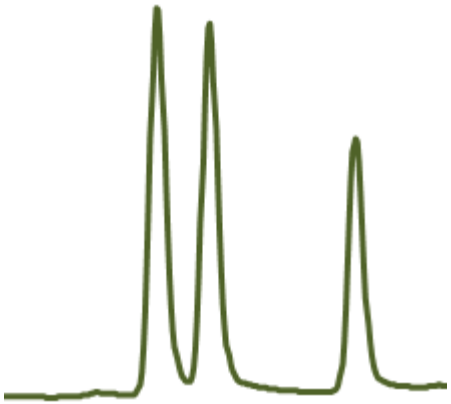
Stacked ring ion guide gives linear field

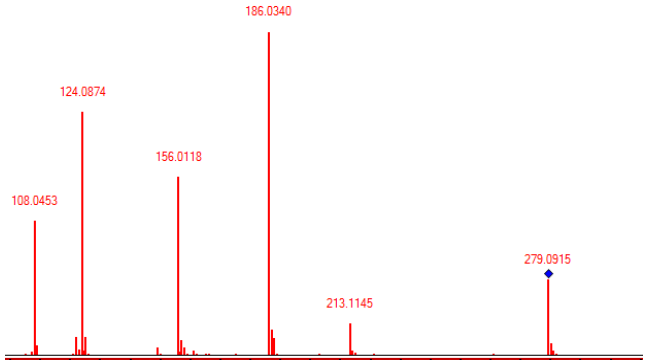
$$v = K\,E \propto \frac{e\,E}{P\sqrt{T}\,\Omega}$$

Resolution Is Important!

Chromatographic → Ion Mobility → Mass







~seconds

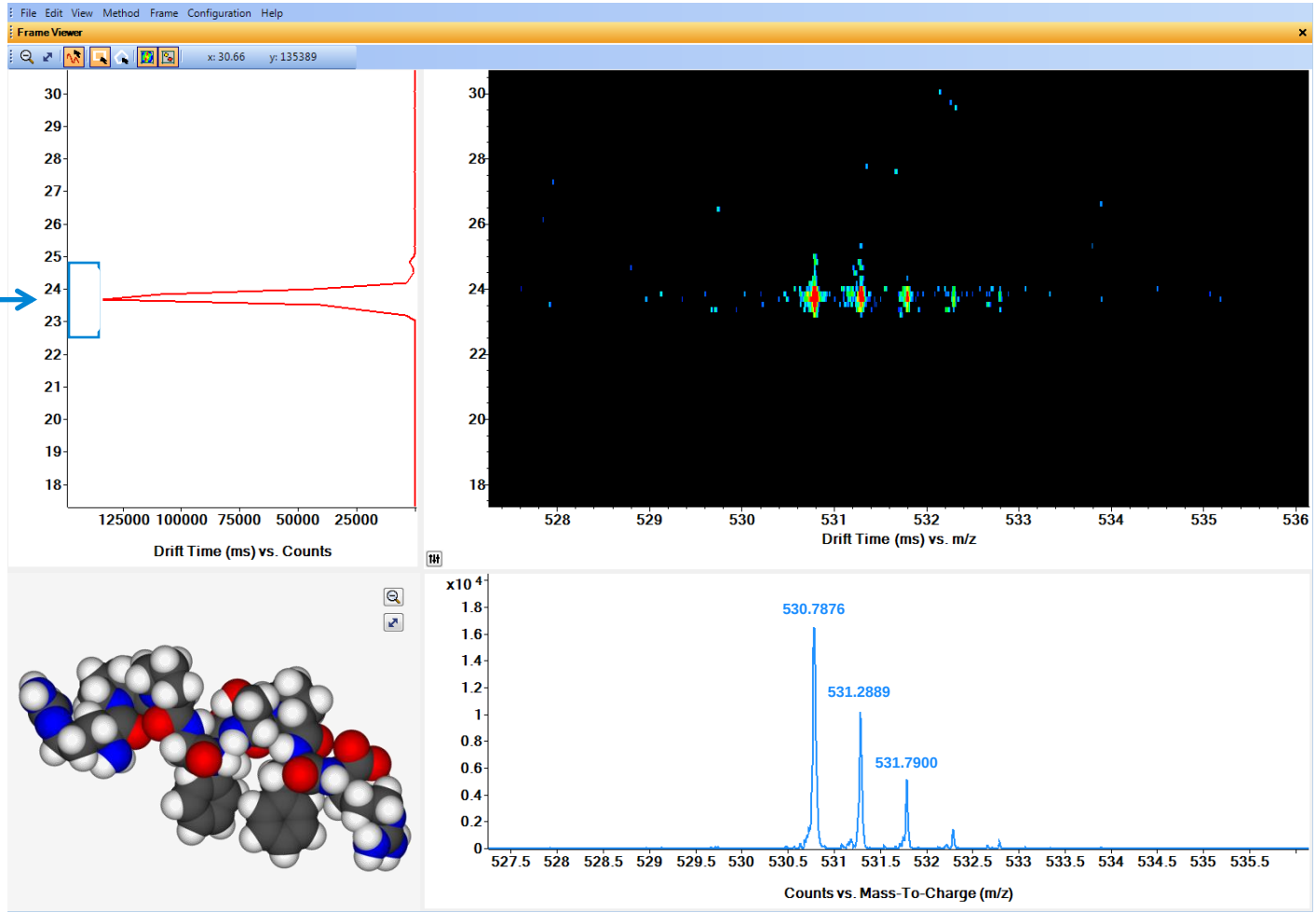
~60 milli-seconds

~ 100 μ seconds

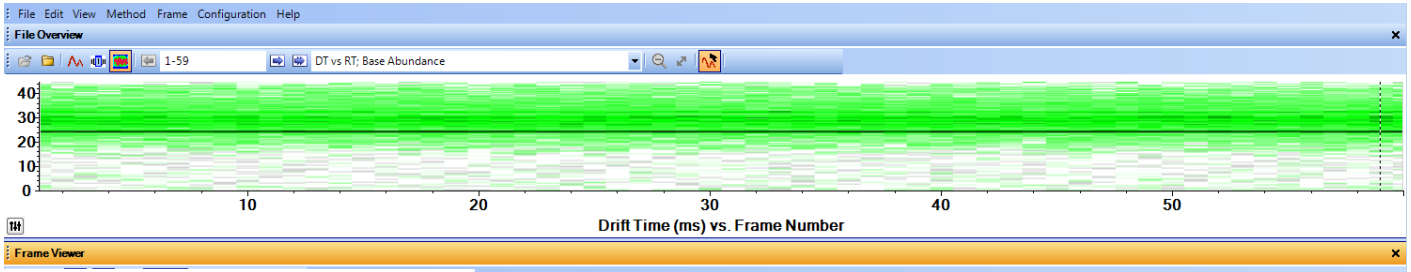
Ion Mobility Resolution

IMS Resolution

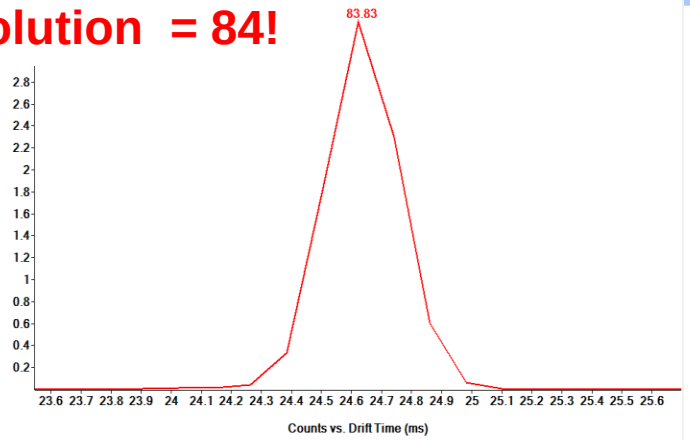
$$R = \frac{t_d}{\Delta t_d} = \sqrt{\frac{LEQ}{16kTln2}}$$

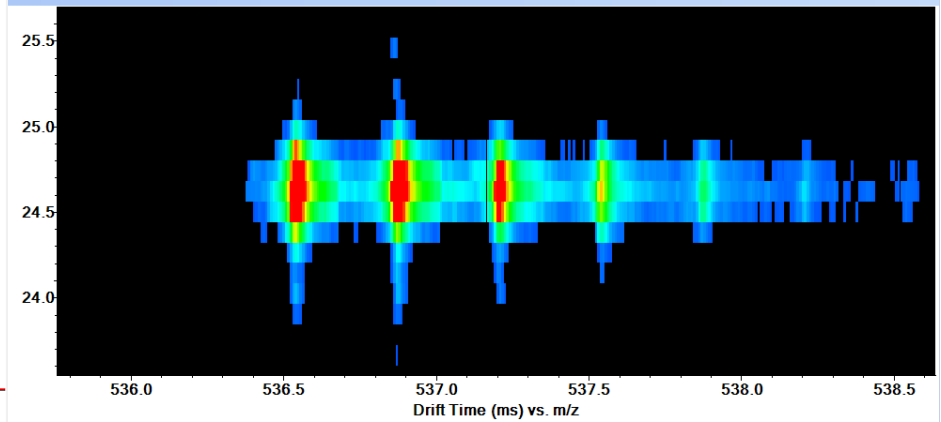


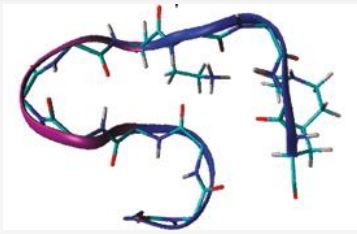
Ion Mobility Resolution - Continued

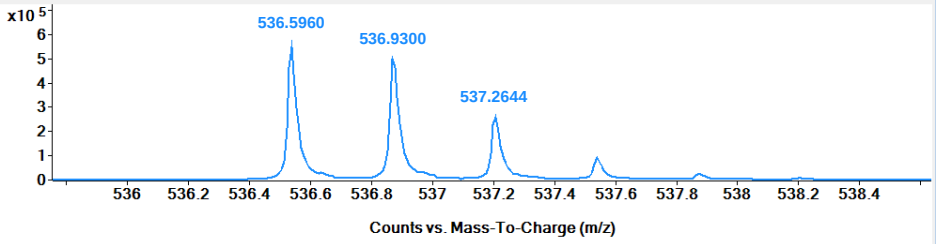


Resolution = 84!



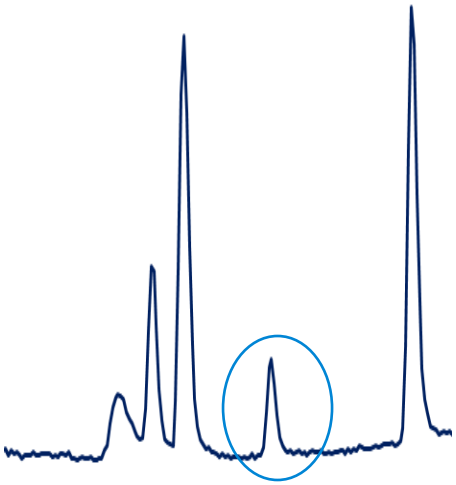




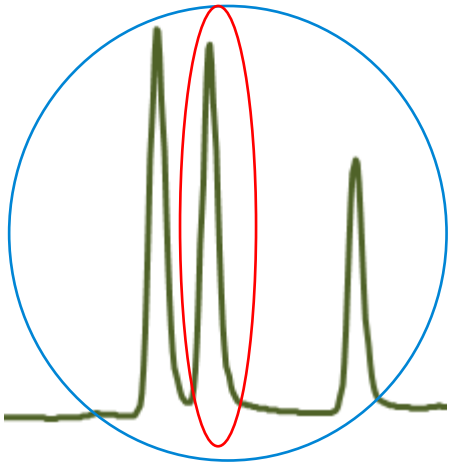


It's All About Separation

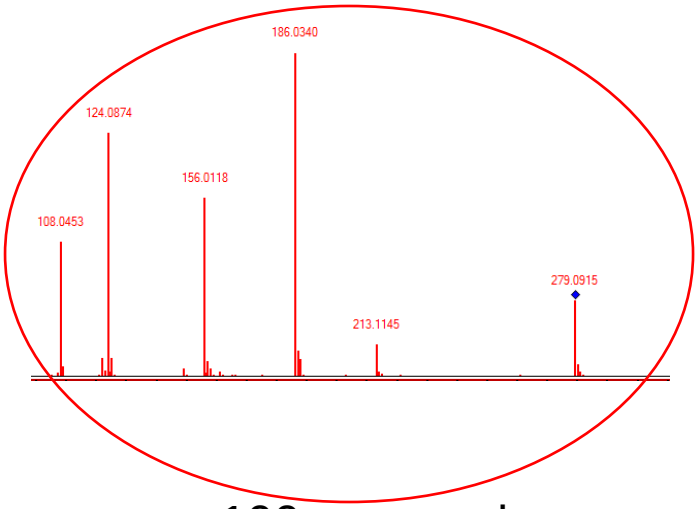
Chromatography → Ion Mobility → Mass



~seconds

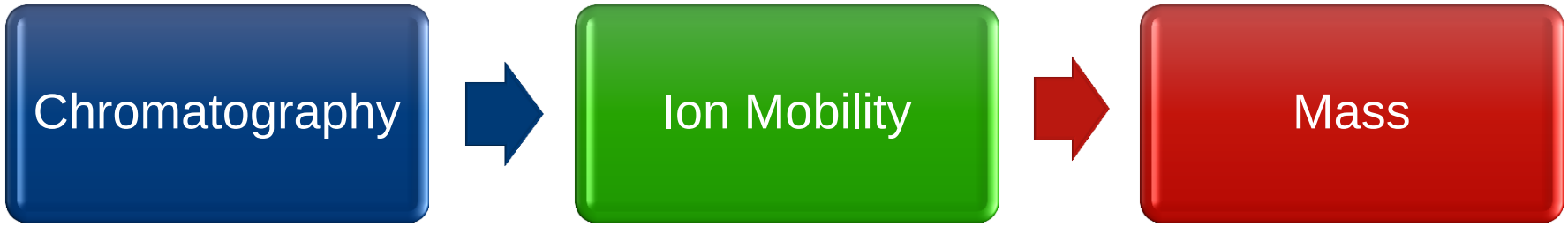


~60 milli-seconds



~ 100 μ seconds

It's All About Separation



Peak Capacity = $IM_{Resolution}$ x $Mass_{Resolution}$ x Orthogonality

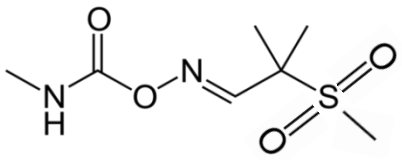
Peak Capacity = 60 x 40,000 x 14% = 336,600 ~ 8-fold increase

Dwivedi P, Schultz AJ, Hill HH,
Metabolic profiling of human blood by high-resolution ion mobility mass spectrometry (IM-MS).
Int J Mass Spectrom 298:78–90. 2010

Separation of Isobaric Pesticides

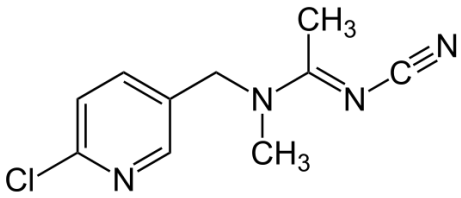
Aldicarb-sulfone ($C_7H_{14}N_2O_4S$)

$[M+Na]^+ = 245.056649$



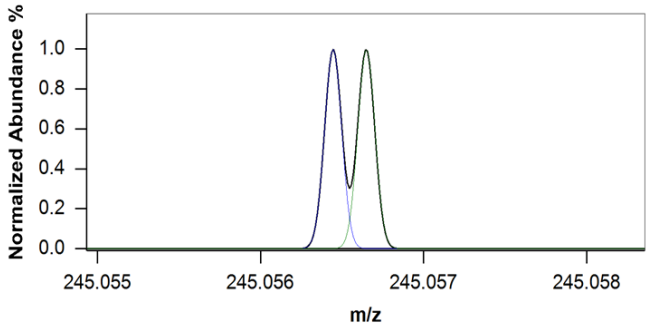
Acetamiprid ($C_{10}H_{11}ClN_4$)

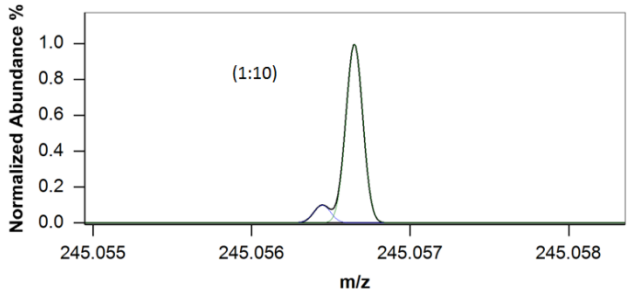
$[M+Na]^+ = 245.056445$



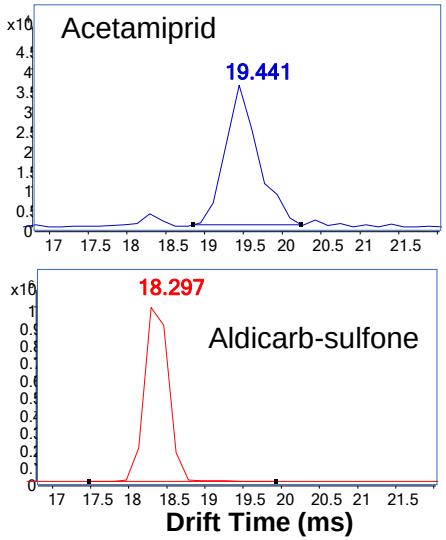
Δ mass is 0.2 mDa requires ~ 2,000,000 resolution !

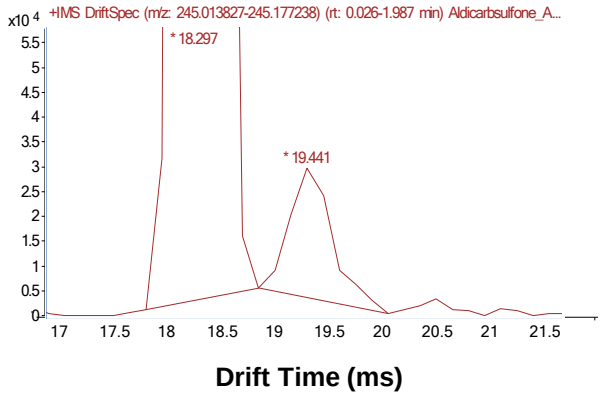
Theoretical Plot





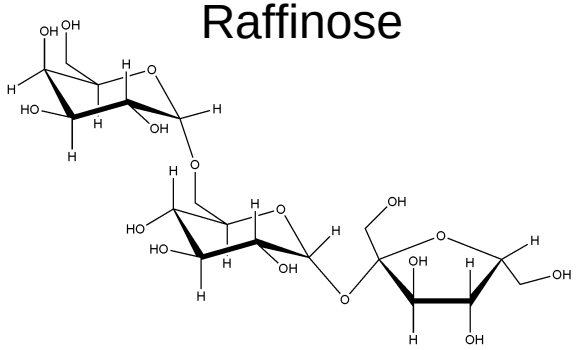
IMS Drift Separation



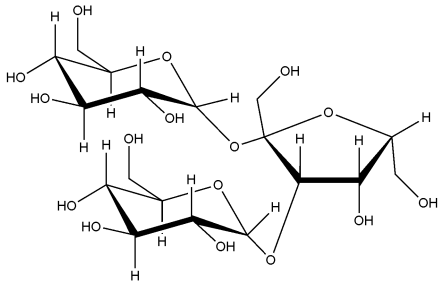


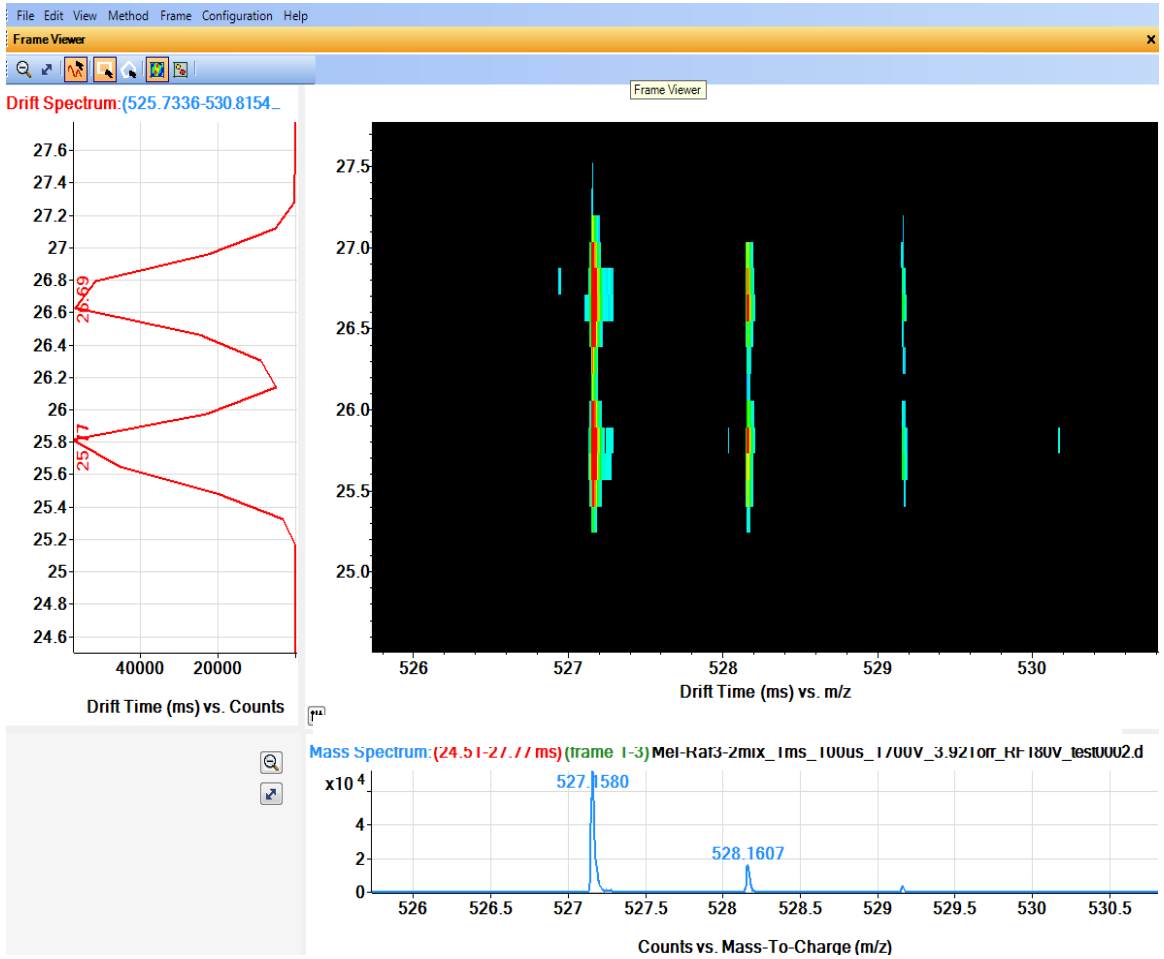
Resolving Structural Sugar Isomers $C_{18}H_{32}O_{16}$

Raffinose



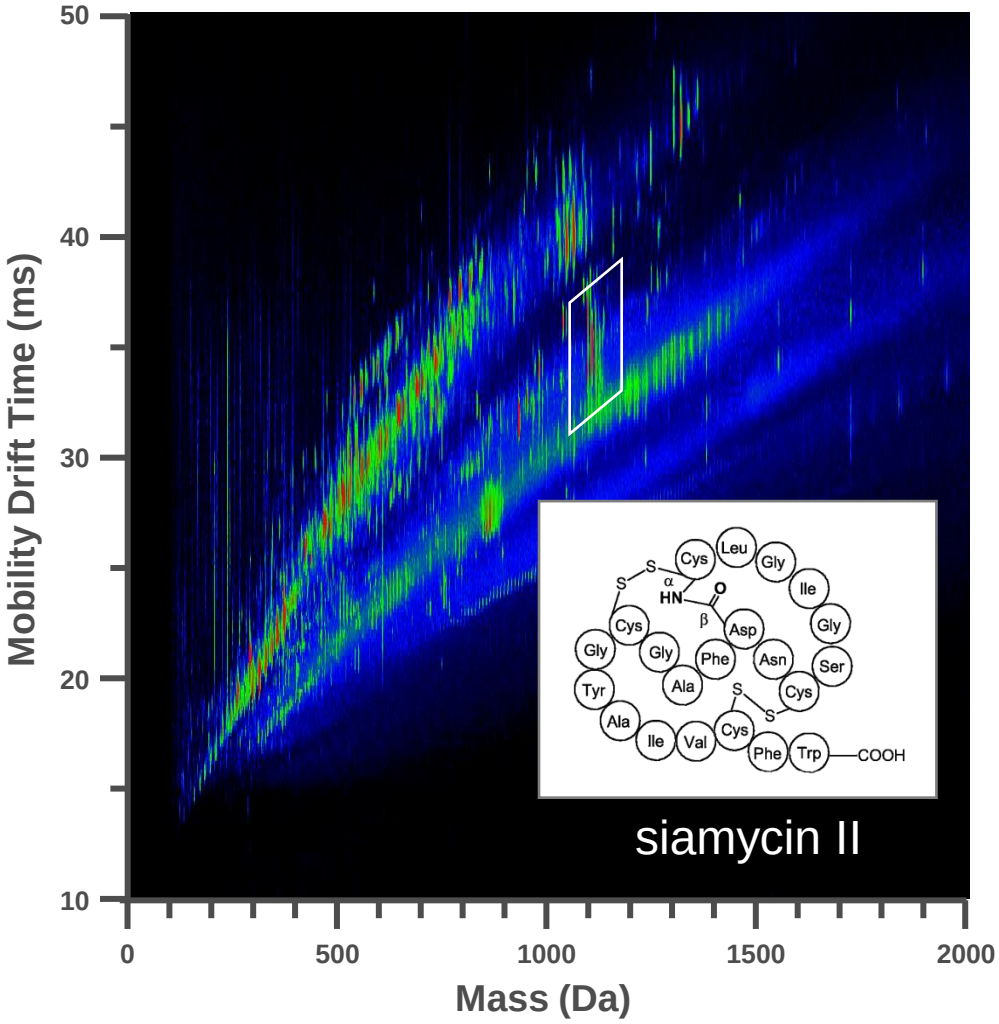
Melezitose

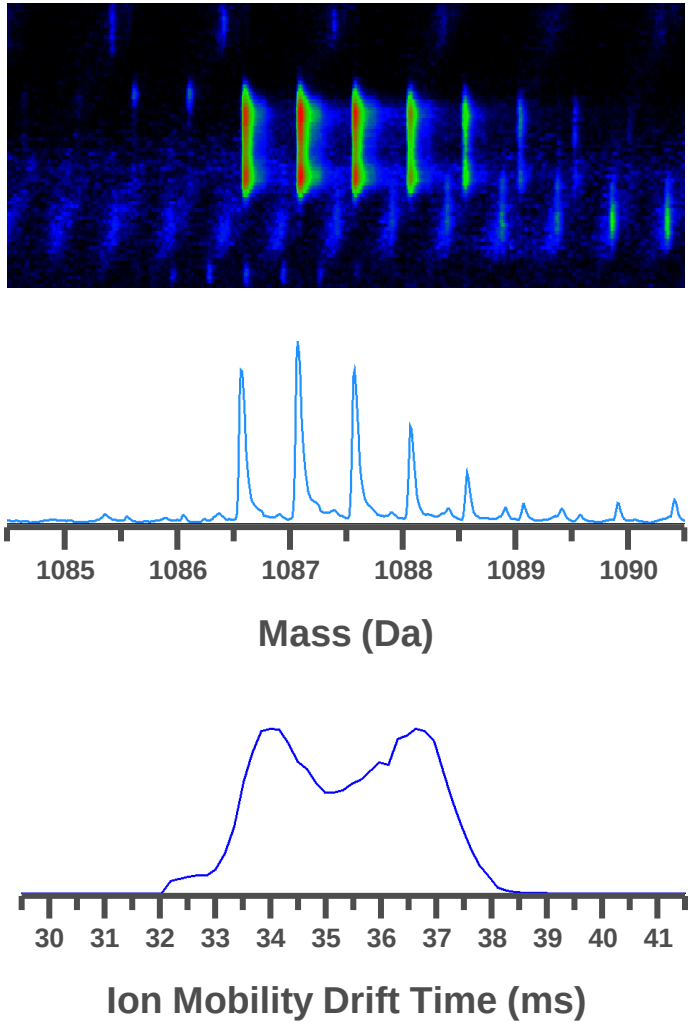




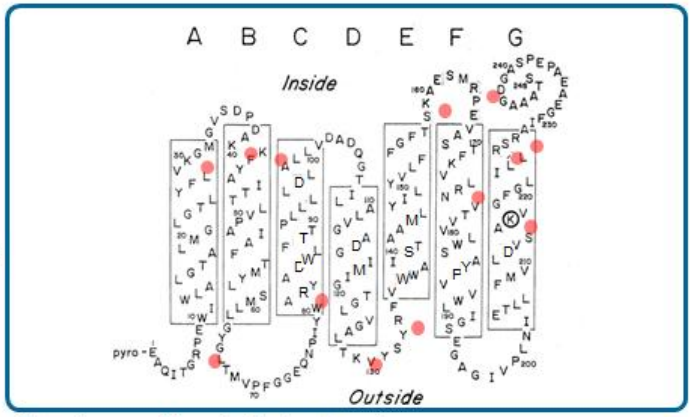
Resolving two isobaric tri-saccharides

Crude Bacterial Extract

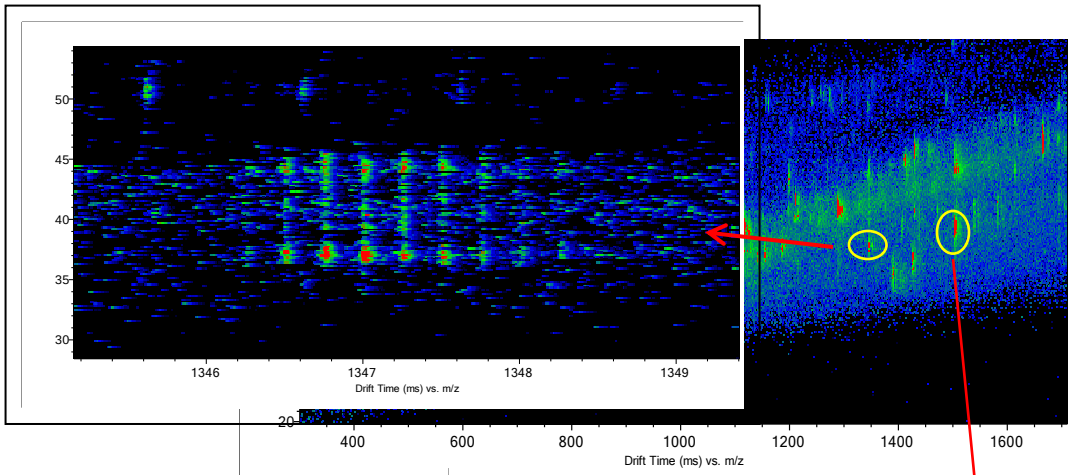


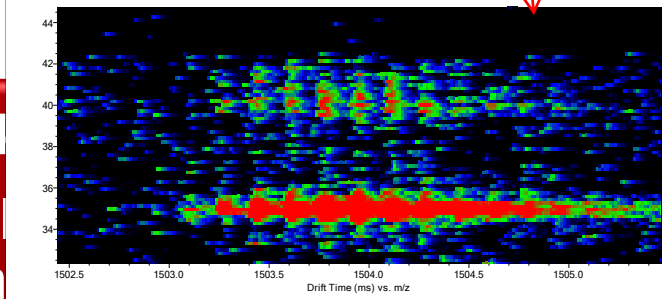


IMS-MS for Proteomics: Transmembrane Spanning Peptides



Structure of bacteriorhodopsin





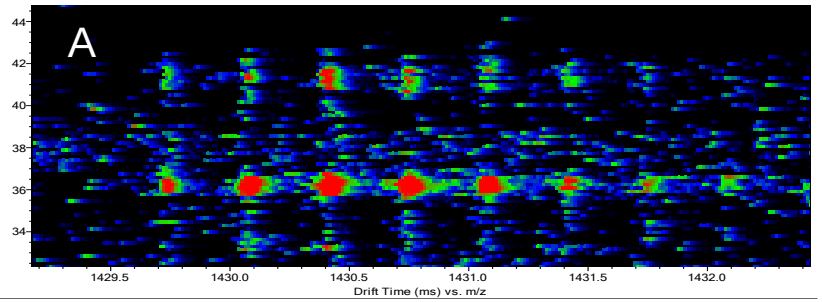
Characteristics:

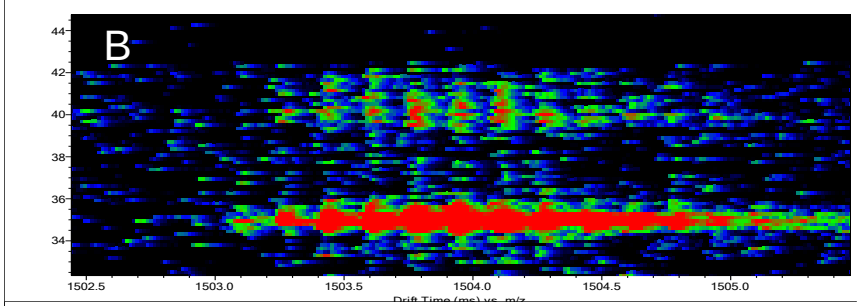
- Large hydrophobic peptides
- X-ray: 7-helical domains
- Confirmation impacts function of membrane
- Membrane proteins are drug targets

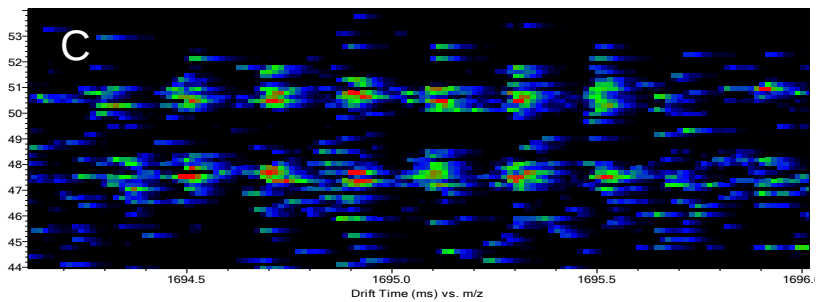
Worl

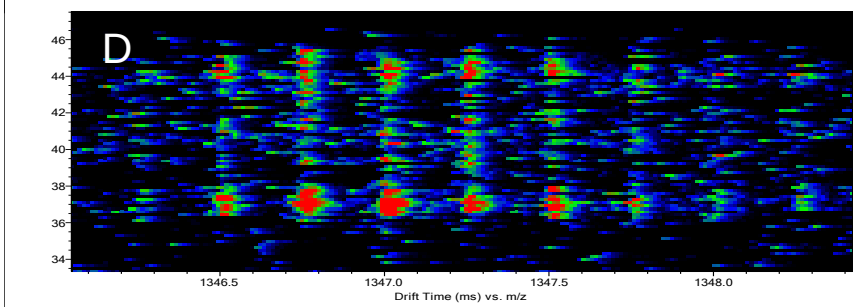
α-hel
span
intact, and differ in drift-time from denatured peptides

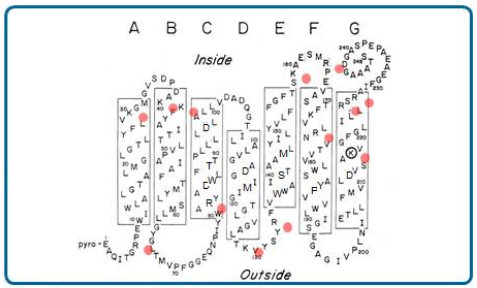
IMS-MS for Proteomics: Transmembrane Spanning Peptides







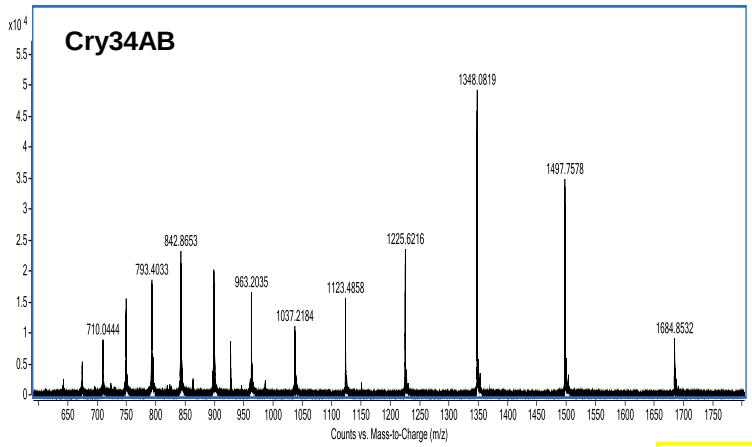


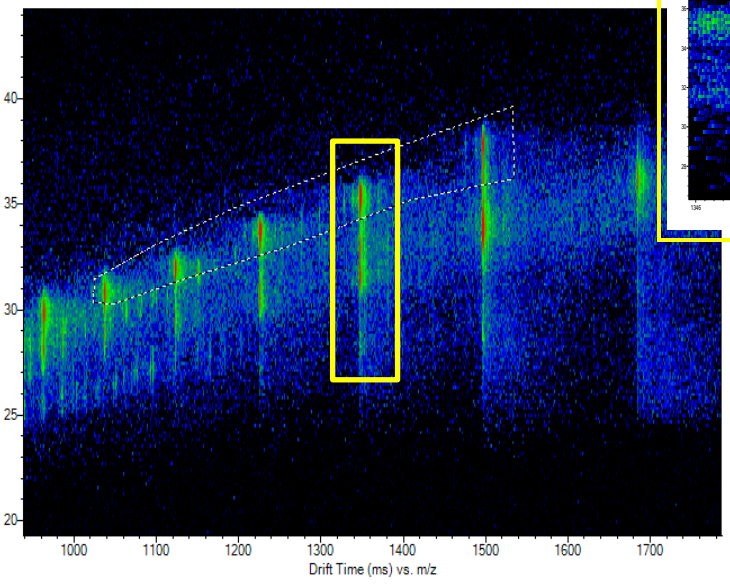


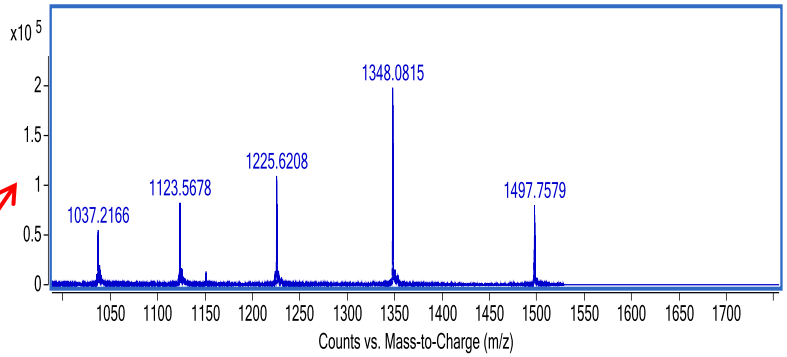
Structure of bacteriorhodopsin

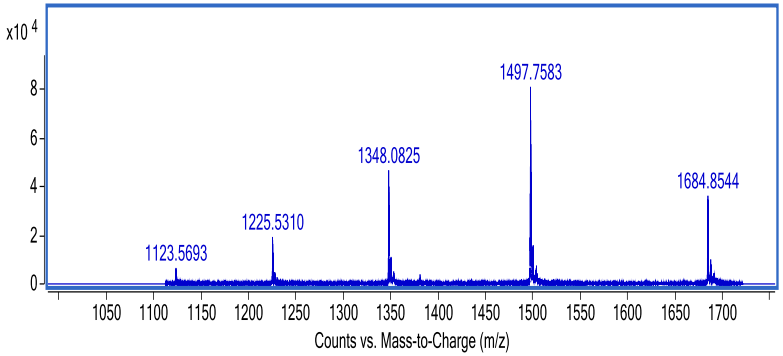
Figure	Seq. Loc.	Chg. State	Helix
A	1-40	$[M+3H]^{3+}$	A
B	1-82	$[M+6H]^{6+}$	A-B
C	83-159	$[M+5H]^{5+}$	C-D-E
D	176-225	$[M+4H]^{4+}$	F-G

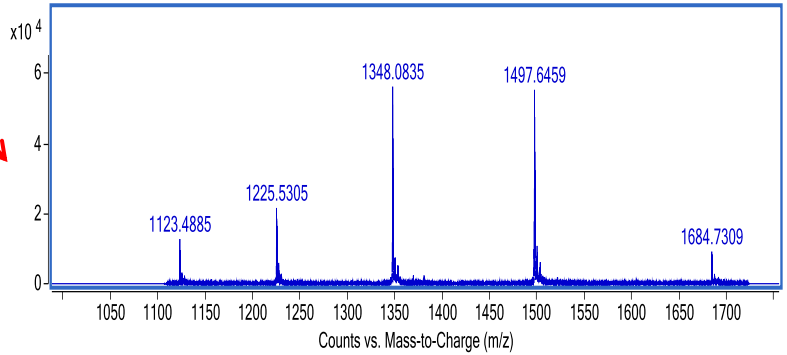
LC – Ion Mobility – QTOF Protein Isoform Analysis



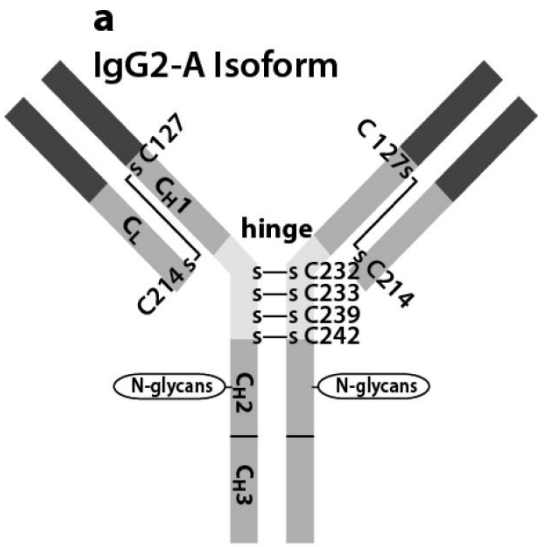


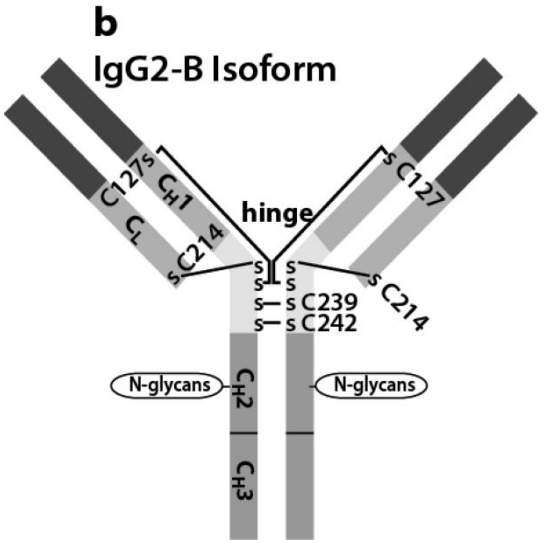


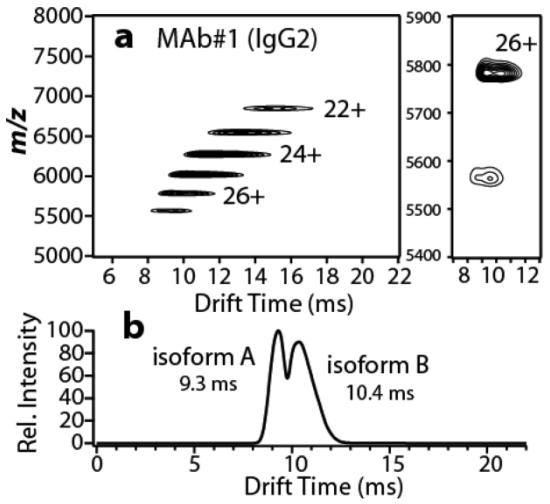


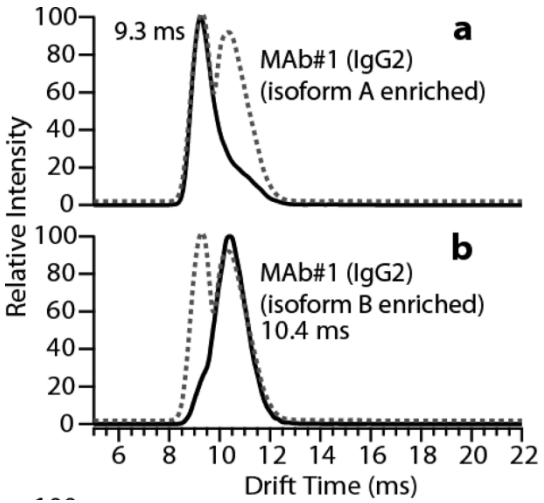


Resolving Isoforms of IgG2

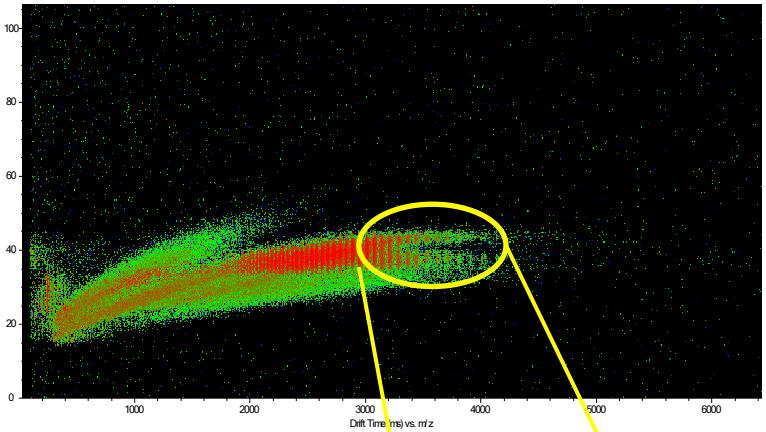


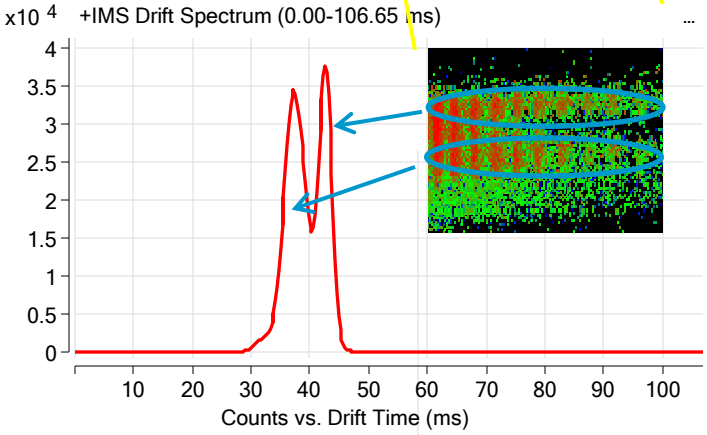


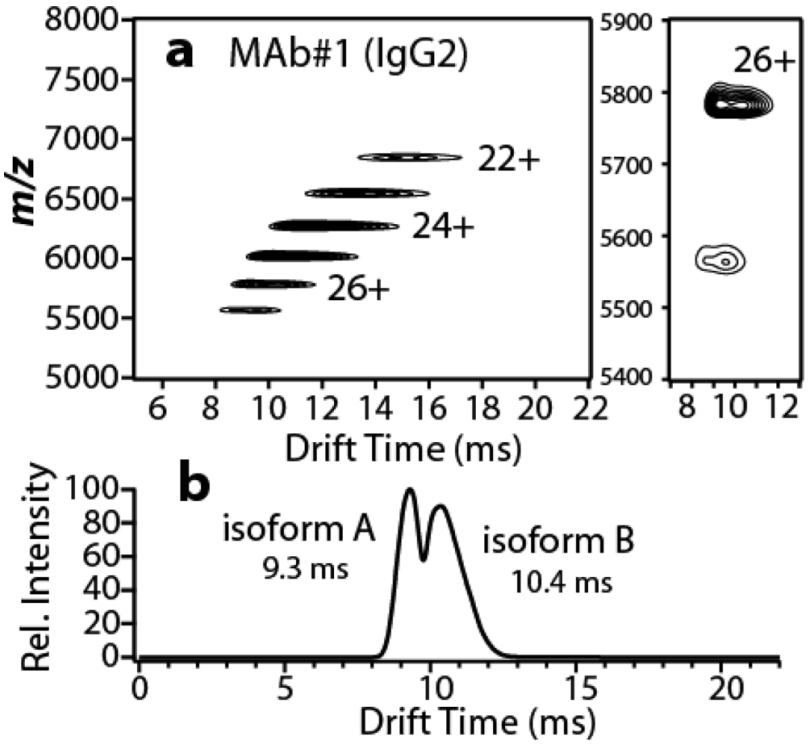




Resolving Isoforms of IgG2

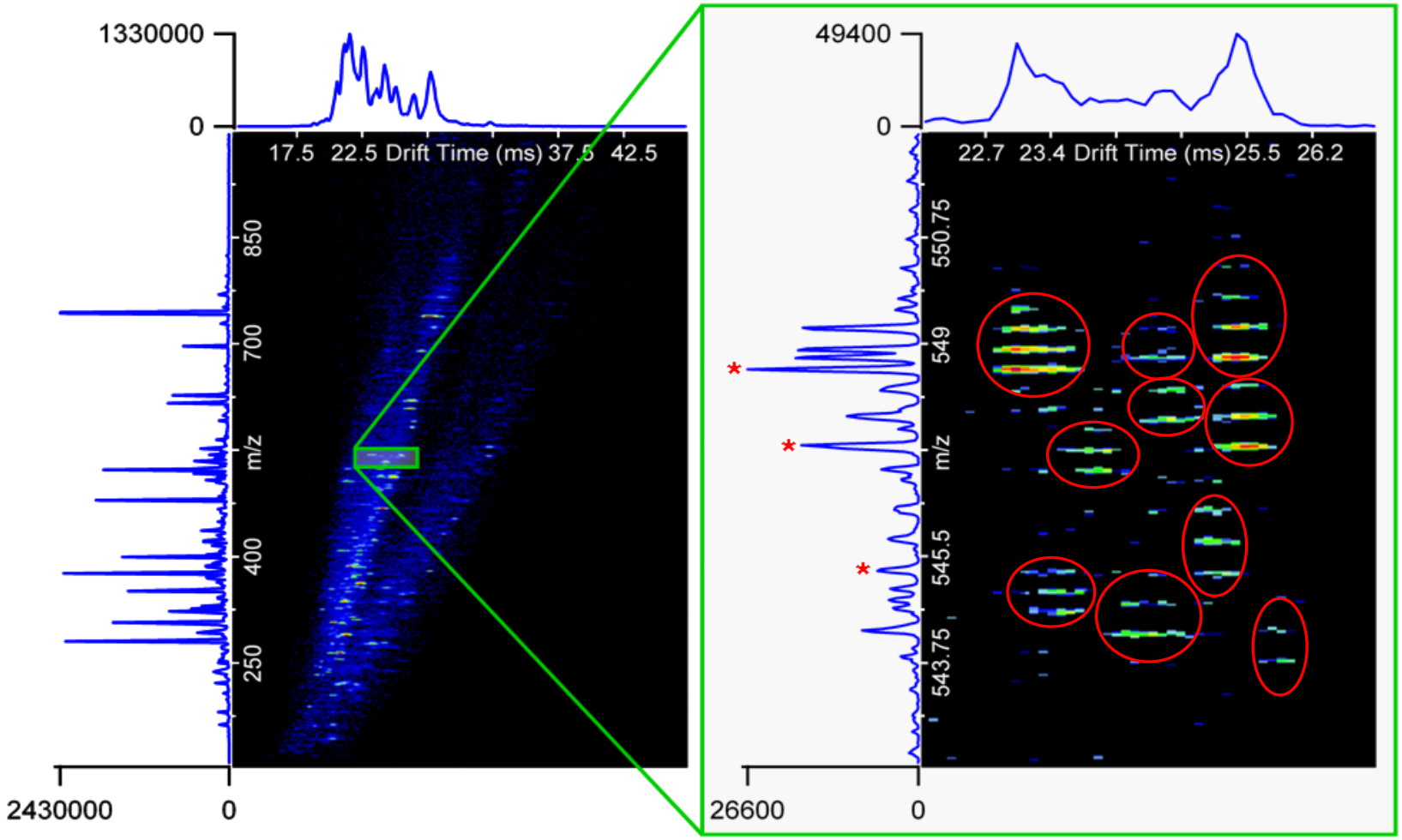






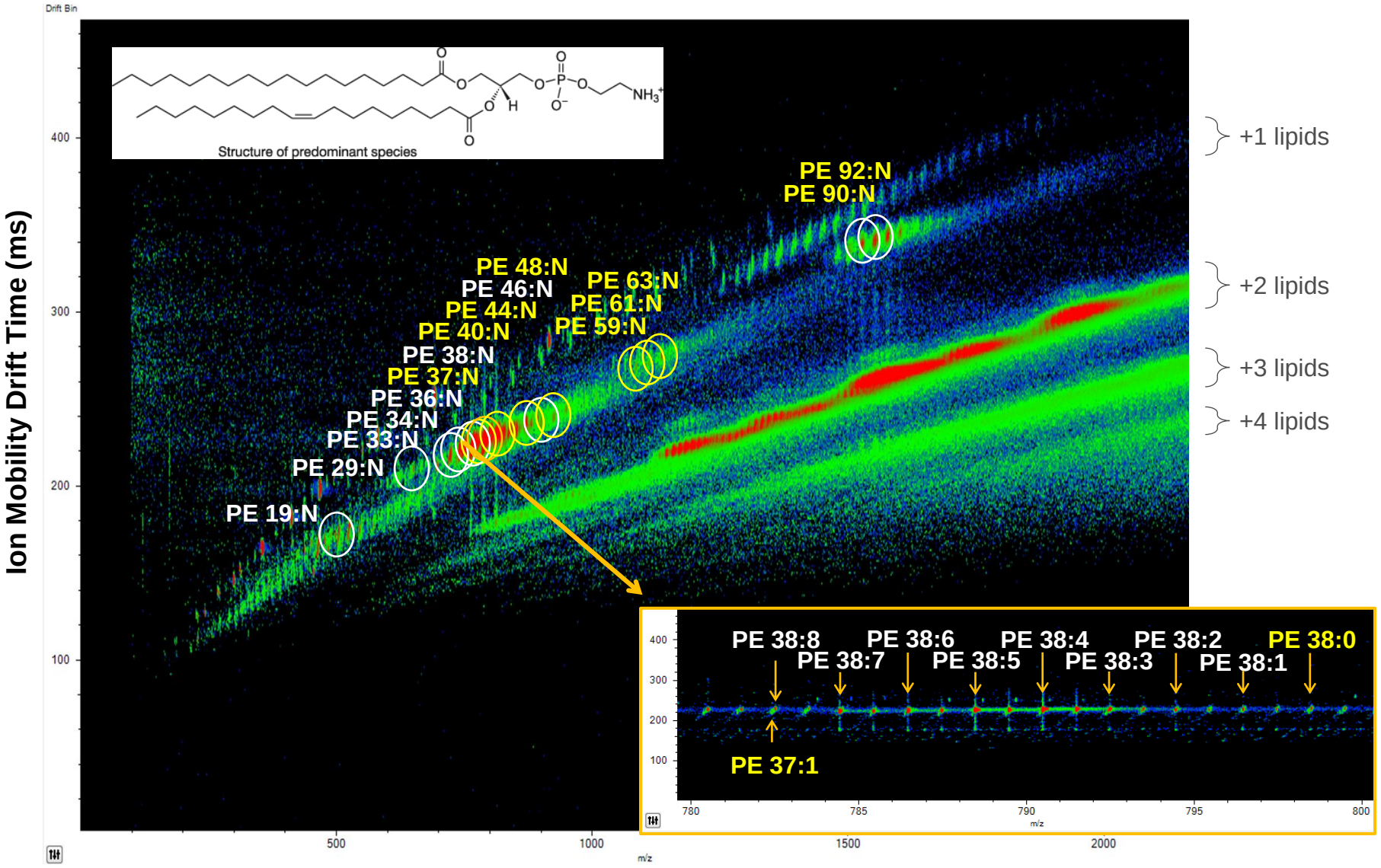
Publication Paul Schnier, Synapt:
3uM direct infusion in 160 mM NH_4Ac

Why Ion Mobility? Separation!

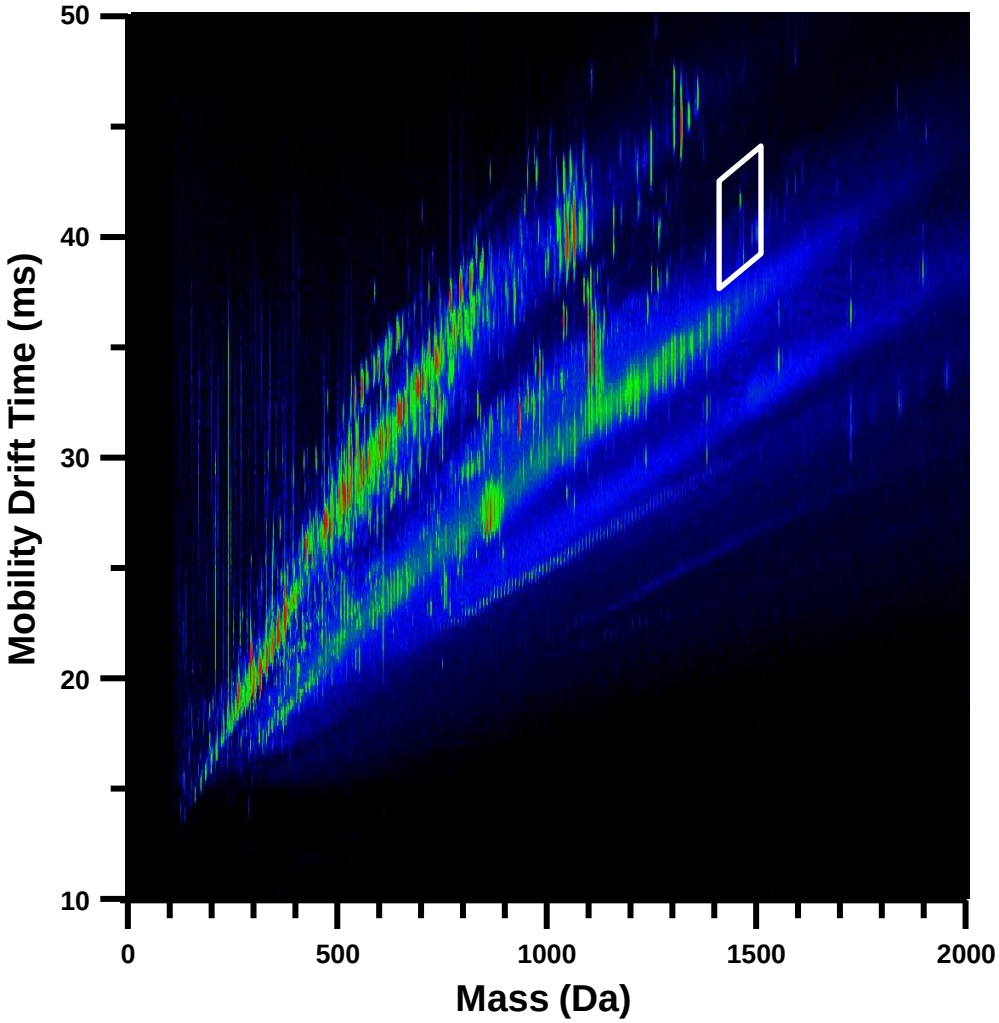


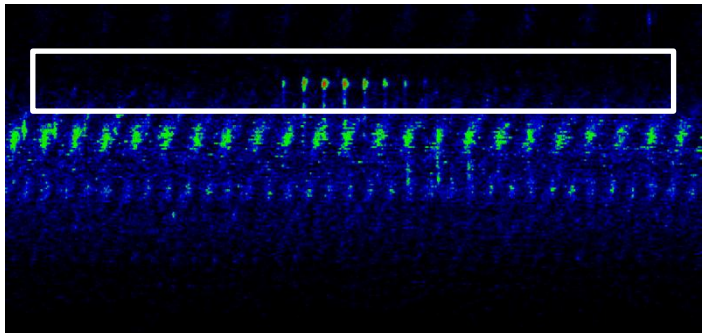
Protein Digest, Erin Baker, PNNL

Lipid Analysis: Mixture of L-α-phosphotidylethanolamine (PE) Lipids

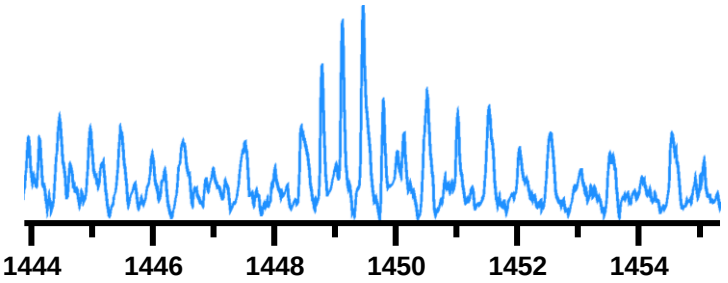


Why Ion Mobility? Specificity!

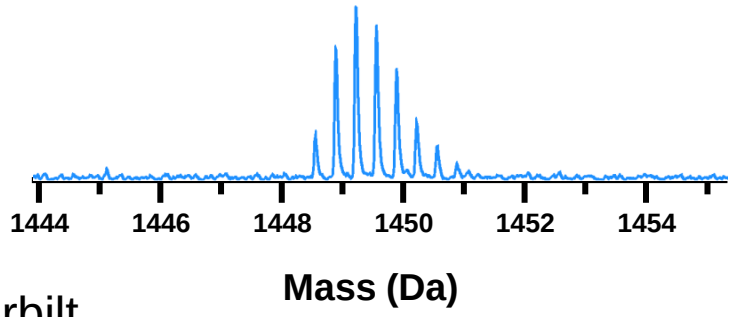




Integrated Mass Spectrum:

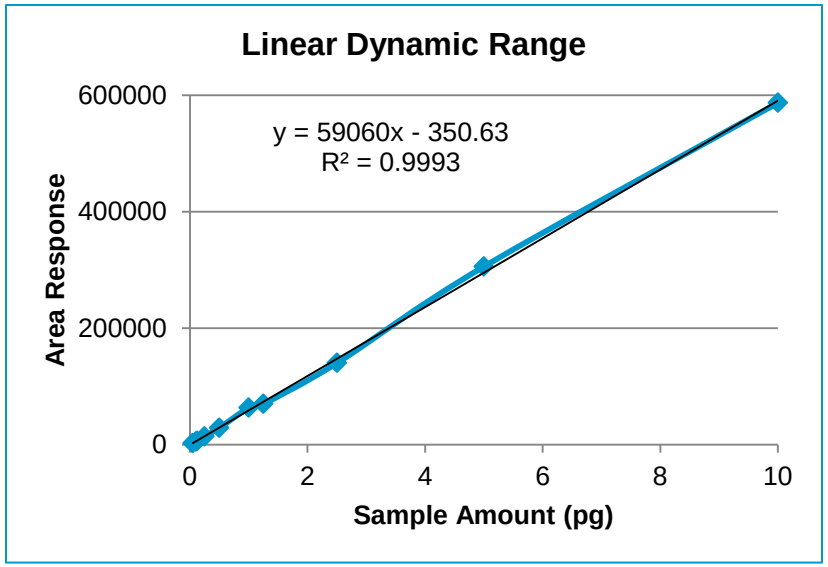


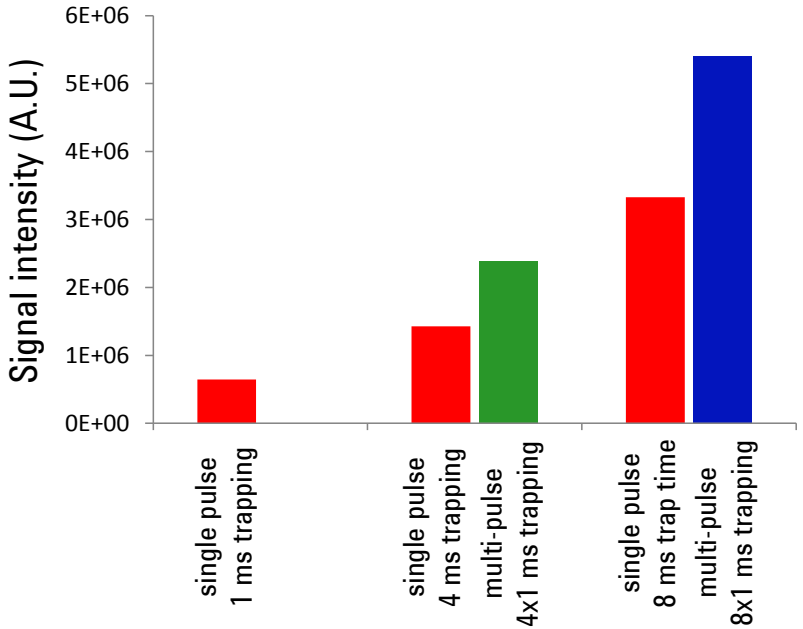
Mobility-Filtered Mass Spectrum:



Crude bacterial extract, John McLean, Vanderbilt

Why Ion Mobility? Sensitivity!



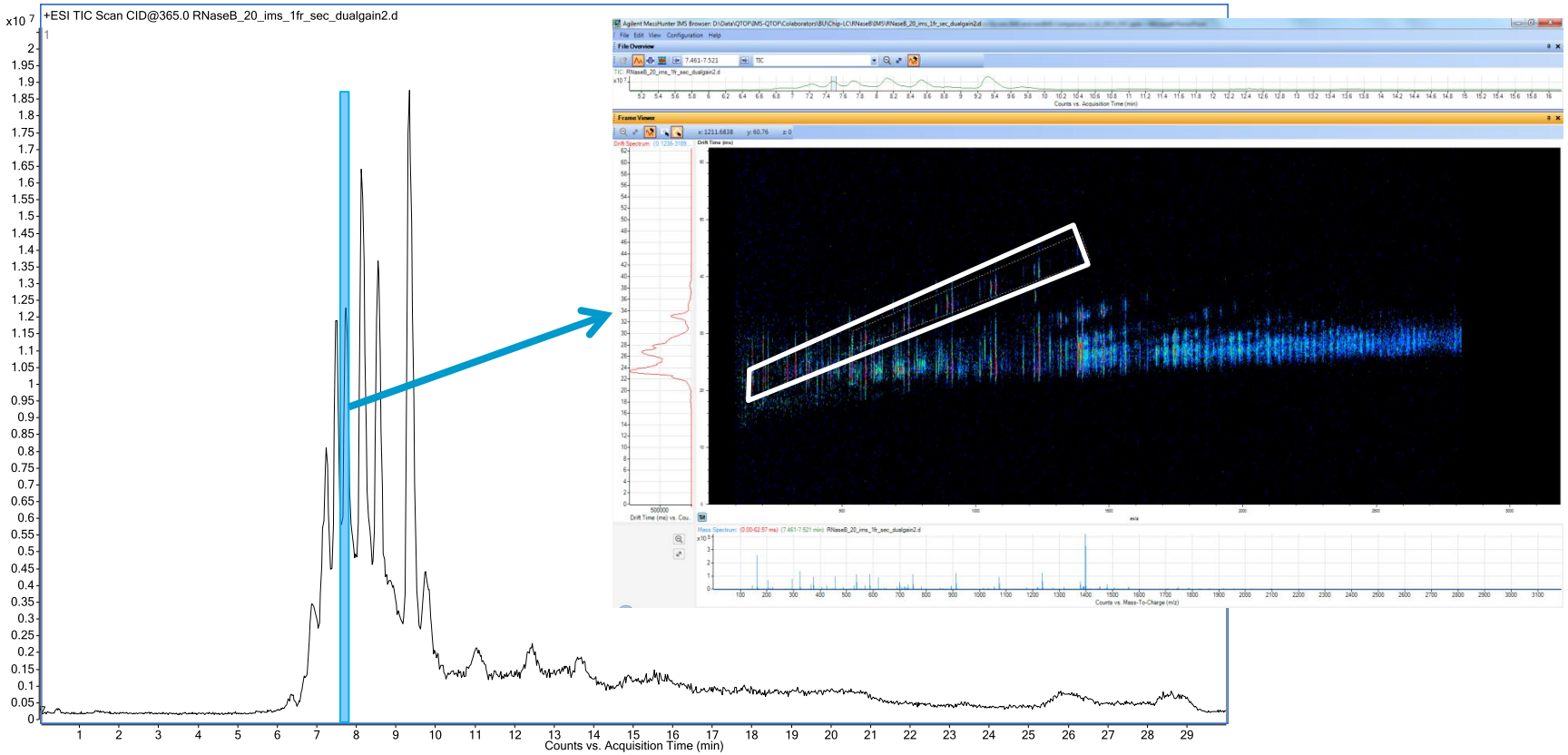


Sensitivity: ~ 50 fg of Reserpine

Dynamic range: ~ 3-4 orders

- Integrated signal intensity for tetrakis decyl ammonium bromide ion versus ion trapping time for single pulse and multi-pulse experiments.
- These data indicate that for the same amount of trapping time, multiplexing experiments result in at least 10X higher signal intensity possibly due to less space charge effects and detector saturation issues.

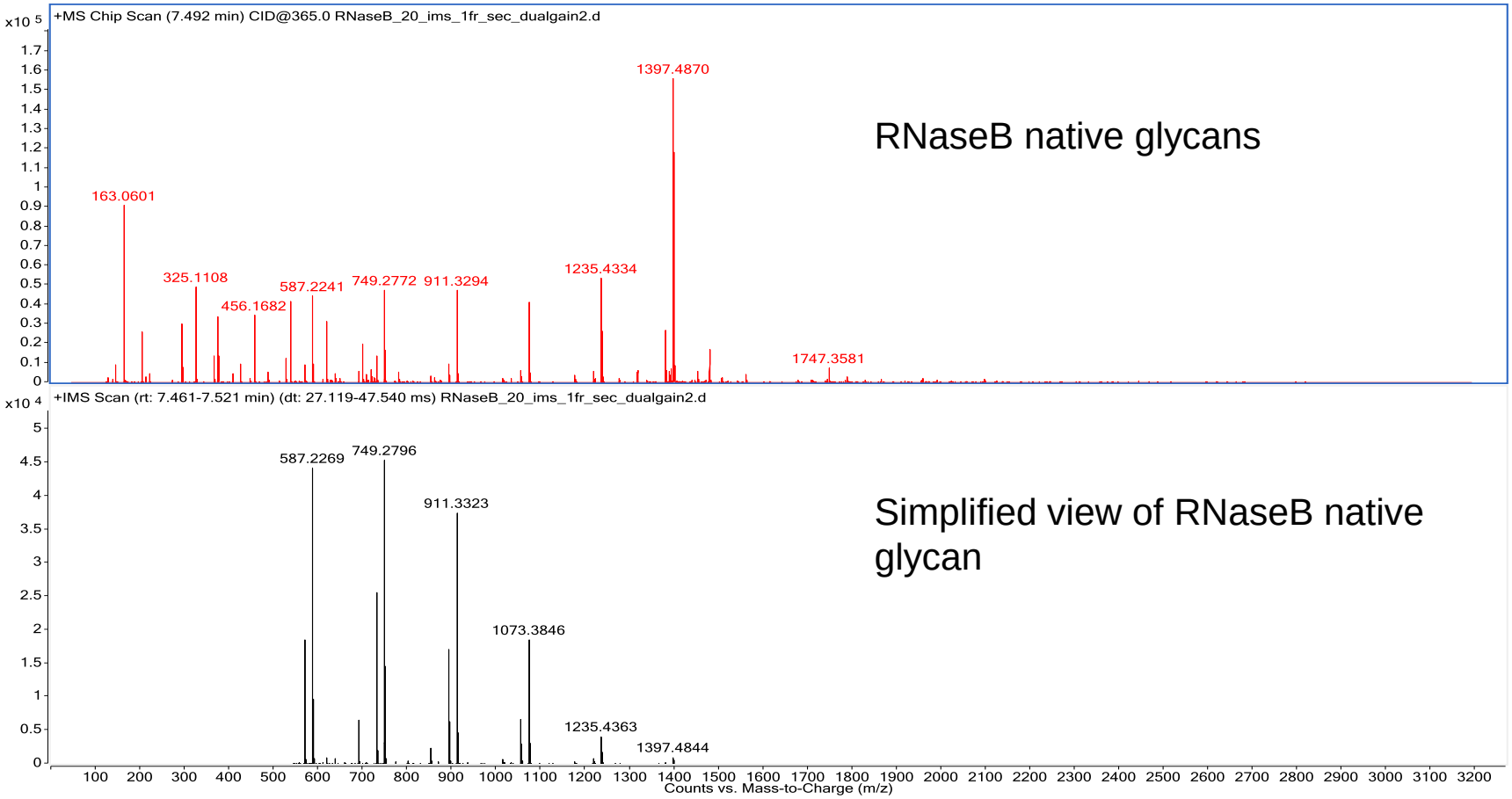
Why Ion Mobility? Selectivity!



Data browser can be used to "isolate" a group of glycans from matrix

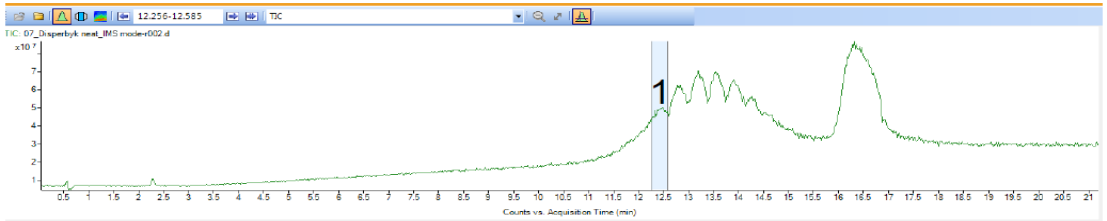
Isolation of RNAseB native Glycans, Cathy Costello, Boston University

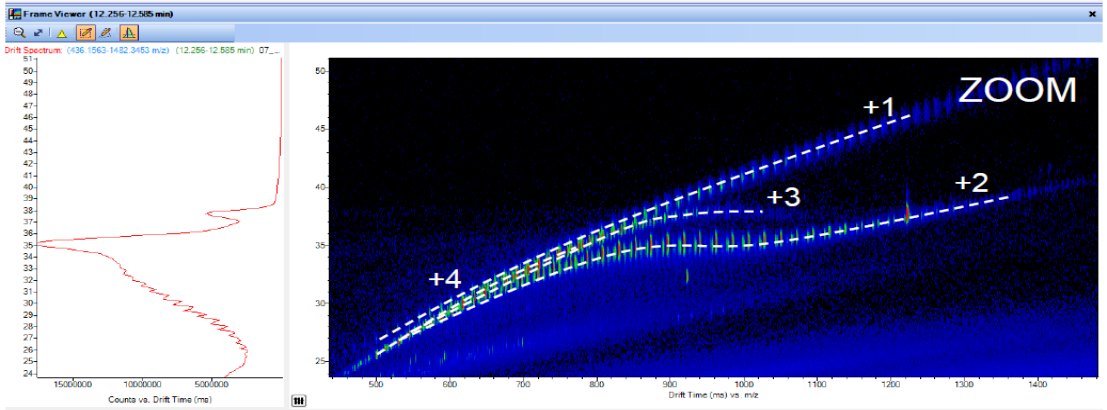
Why Ion Mobility? Selectivity!

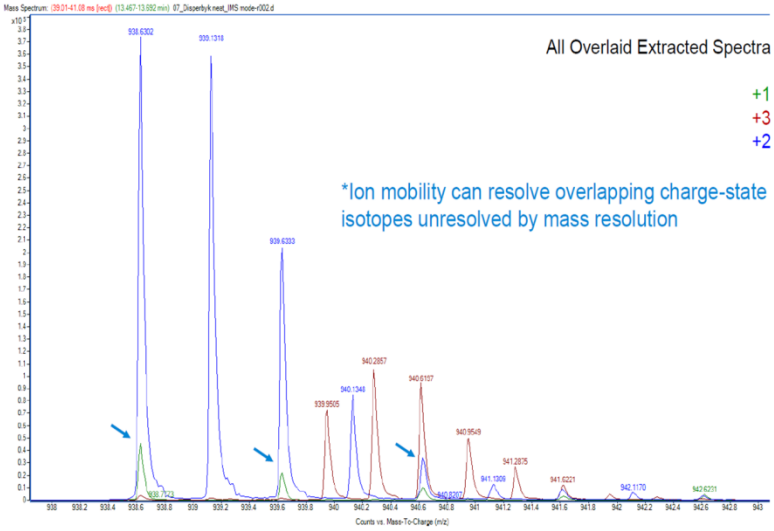


Glycan obscured by matrix is identified after cleaning up background chemical noise using ion mobility

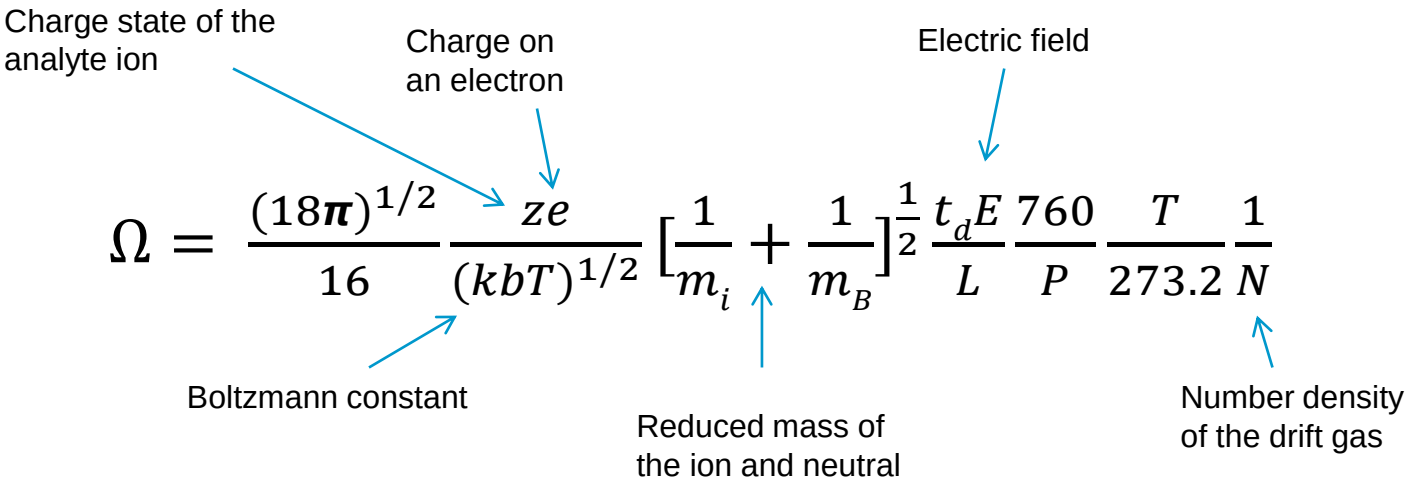
Ion Mobility of Polymeric Ink Dispersants







Determining Cross Sectional Areas



$$K_0 = \left(\frac{273\ K}{T}\right)\left(\frac{P}{760\ \text{Torr}}\right)\frac{L^2}{Vt_d}$$

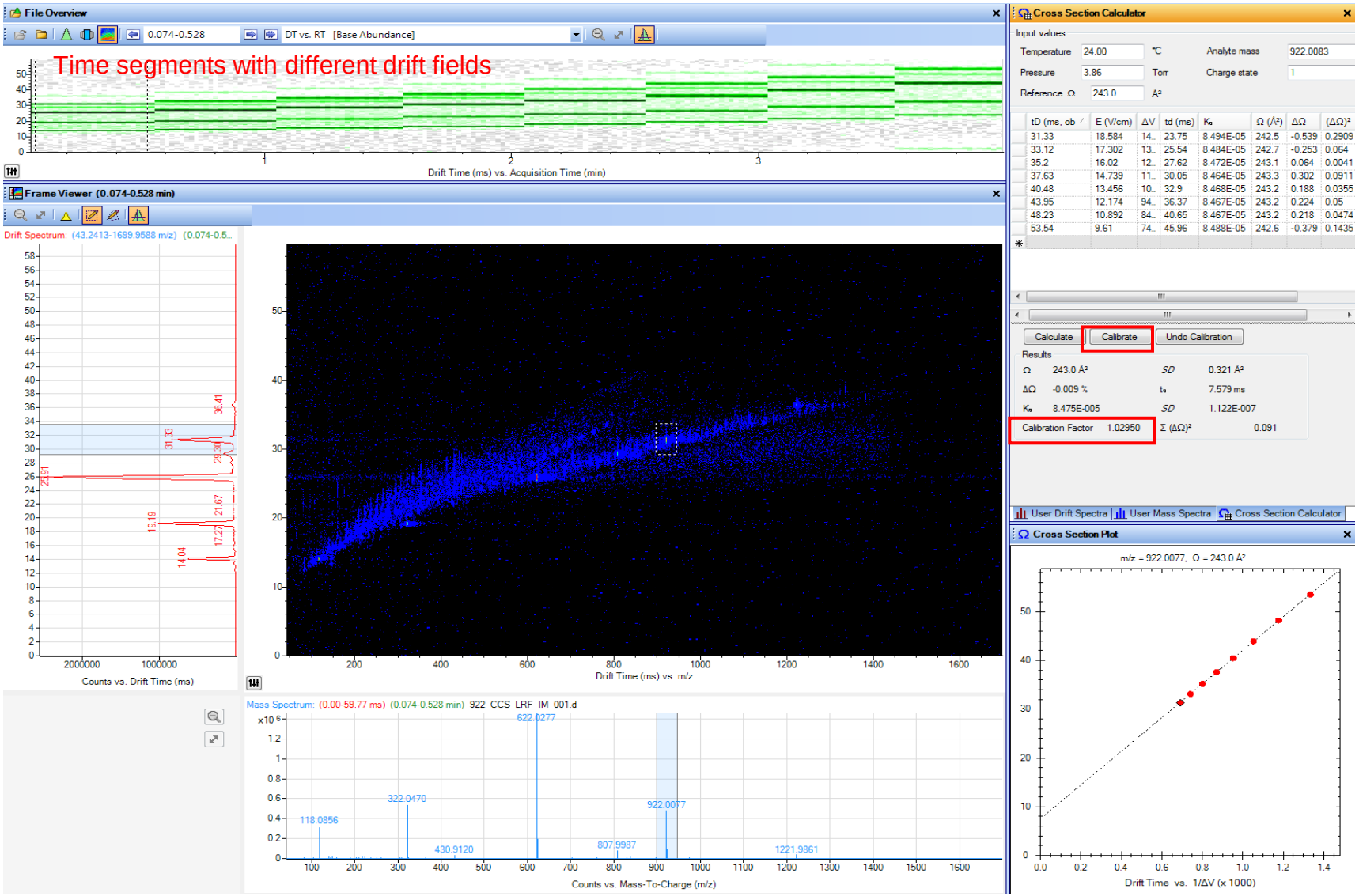
K_0 = Reduced Ion Mobility
T = Temperature
P = Pressure
L = Drift length
V = Voltage Drop across drift region
t_d = Drift time

Molecular size

- X-ray crystallography
- Ion mobility (using Helium)

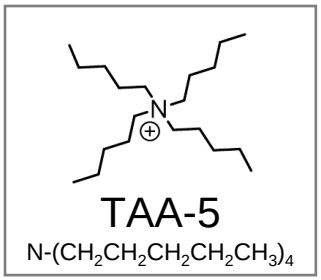
Standard Procedure for Calculating CCS

(Auto Charting, Curve Fitting and Calculating the CCS)

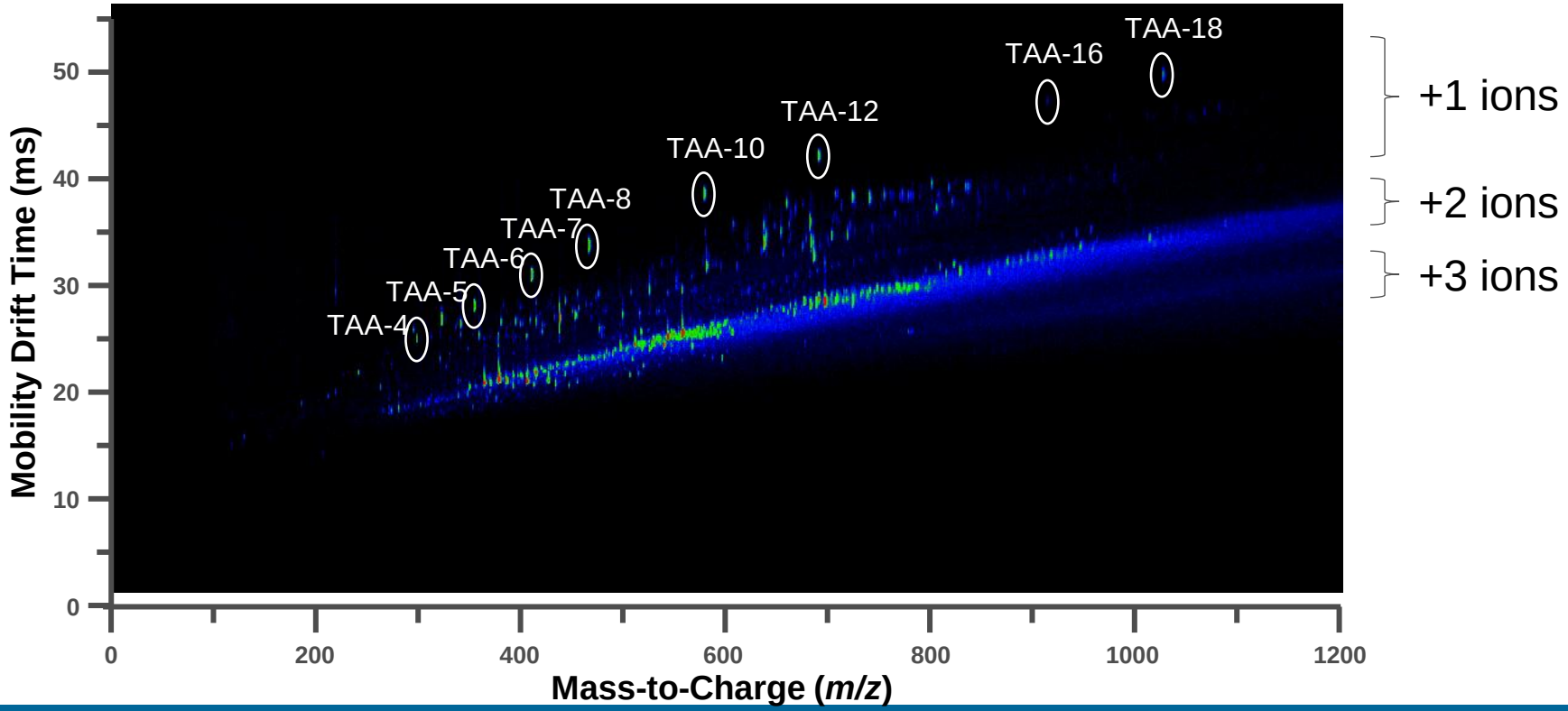


Collision Cross Section Benchmark

--- Vanderbilt University



- Tetraalkylammonium salts (TAA)
  - Proposed as an “ideal” ion mobility standard
  - Wide CCS range (TAA-4 to TAA-18; 100 to 400 Å^2)
  - TAA salts do not form clusters
  - Literature CCS values exist N_2 drift gas



Tetraalkylammonium Salts

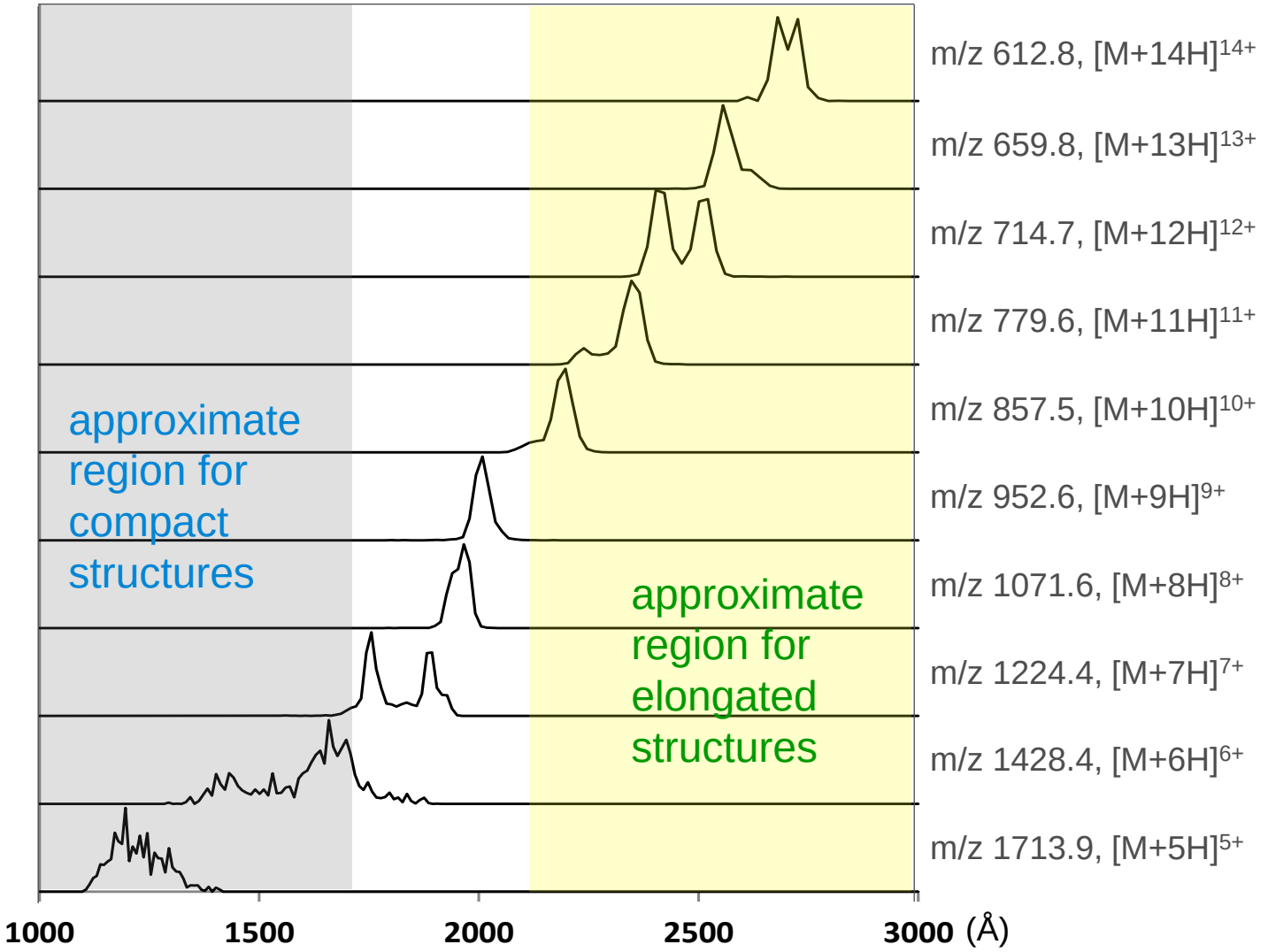
--- CCS Values Compared to Literature

Analyte	Measured Cross-Section [Å^2]	Literature Cross-Section [Å^2]	Relative Standard Deviation [%]
TAA-4	166.61 ± 0.5%	166.00 ± 0.3%	0.56
TAA-5	189.21 ± 0.6%	190.10 ± 0.1%	0.28
TAA-6	212.71 ± 0.3%	214.00 ± 0.3%	0.41
TAA-7	236.34 ± 0.2%	236.80 ± 0.2%	0.01
TAA-8	257.19 ± 0.1%	258.30 ± 0.4%	0.24
TAA-10	294.53 ± 0.1%		
TAA-12	323.62 ± 0.2%		
TAA-16	362.03 ± 0.2%		
TAA-18	381.58 ± 0.3%		

- High experimental precision (< 0.5% relative deviation)
- Agreement with literature (most < 0.5% deviation)

Cross Section Calculation of Ubiquitin Charge States

Automated collision cross section calculation without the use of calibration curves



Reference: Koeniger and Clemmer *J Am Soc Mass Spectrom* **2007**, *18*, 322-331

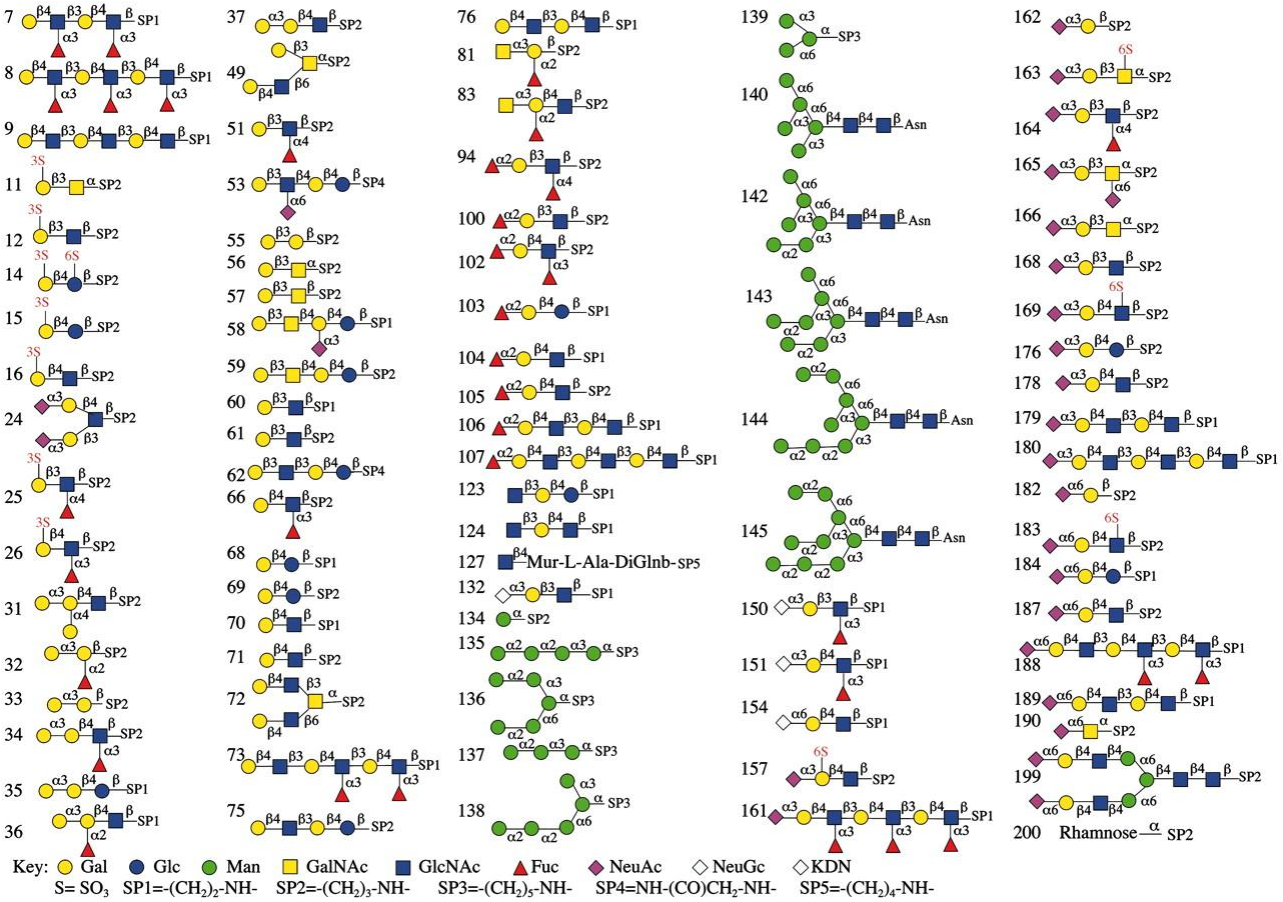
Cross Section Calculation of Ubiquitin Charge States

Ion	Charge State	CCS experimental (Å²)	CCS literature (Å²)
$[M+5H]^{5+}$	5	1196	
$[M+6H]^{6+}$	6	1431, 1658	
$[M+7H]^{7+}$	7	1755, 1886	1910
$[M+8H]^{8+}$	8	1966	1990
$[M+9H]^{9+}$	9	2008	2090
$[M+10H]^{10+}$	10	2114, 2197	2200
$[M+11H]^{11+}$	11	2239, 2348	2340
$[M+12H]^{12+}$	12	2412, 2511	2480
$[M+13H]^{13+}$	13	2556, 2620	2600
$[M+14H]^{14+}$	14	2680, 2726	

Automated collision cross section calculation without the use of calibration curves

Reference: Bush et al., *Anal Chem*. **2010**, *82*, 9557-9565.

Carbohydrates -- Great complexity by linkage

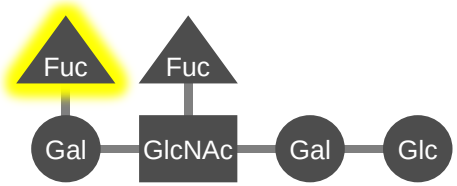


Source: Blixt et al., PNAS, 2004

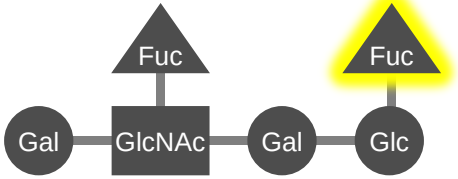
Current dominant strategies: MS^n or Library searches

Carbohydrates IM-MS

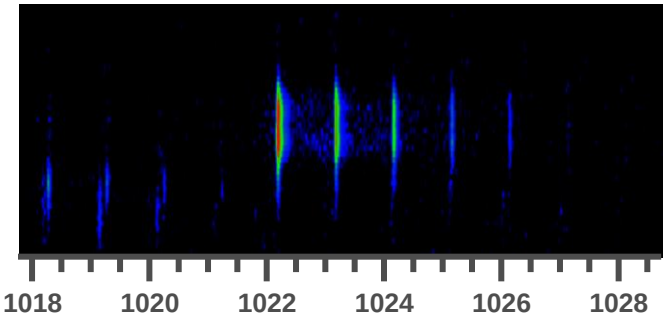
Lacto-N-difucohexaose I

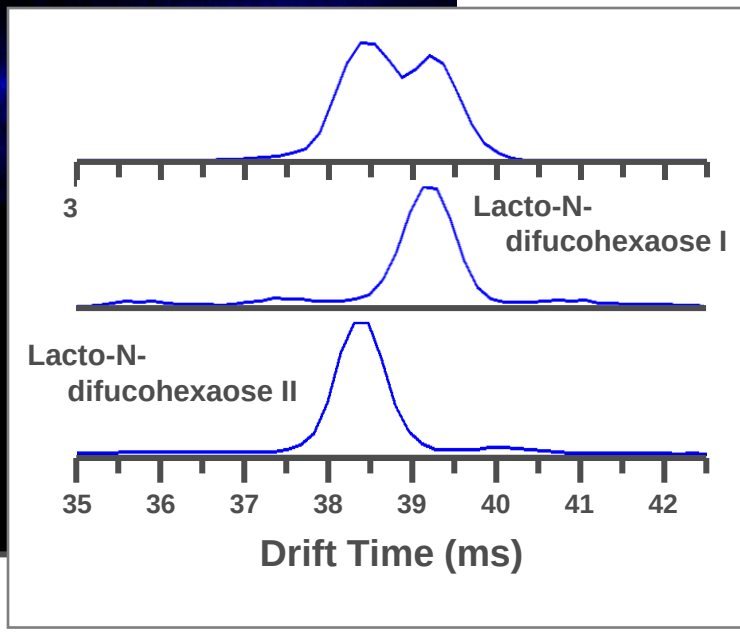


Lacto-N-difucohexaose II

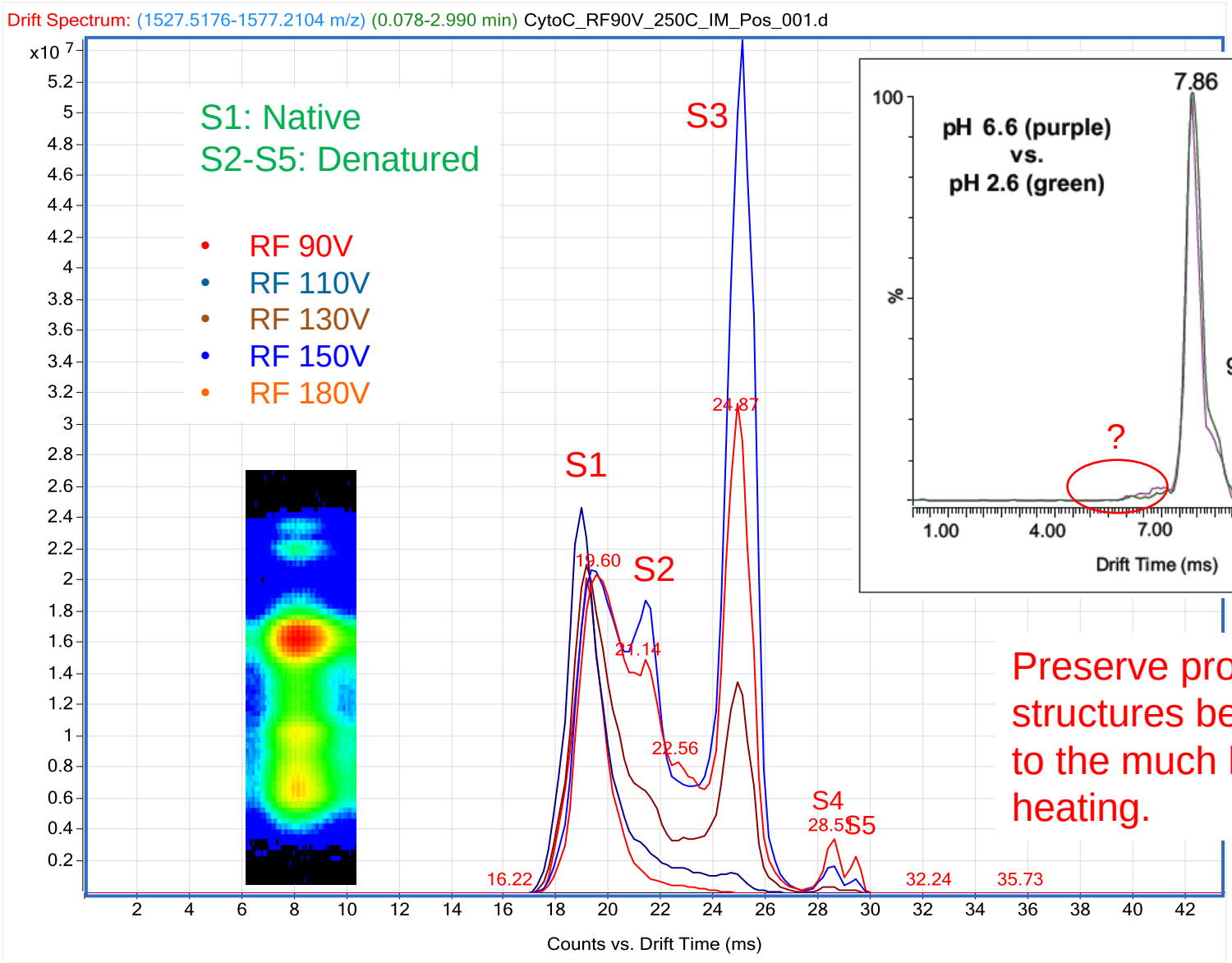


Mixture of **Lacto-N-difucohexaose I & II**





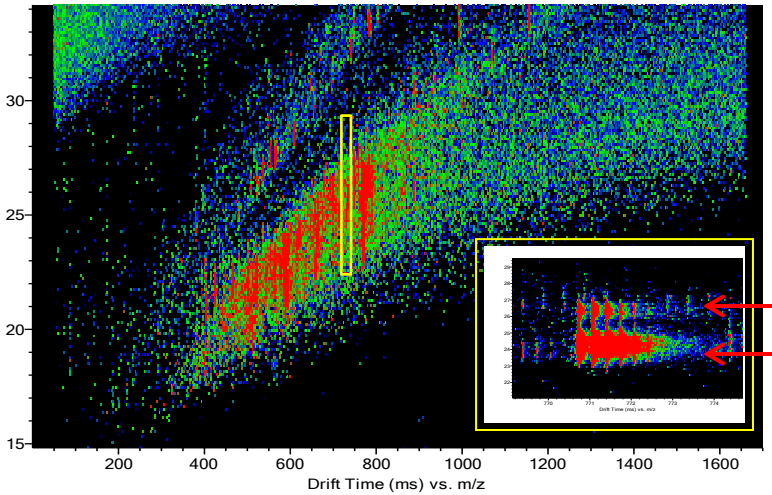
IM Drift Spectrum on Cytochrome C at various RFs:



Preserve protein native structures better! – Due to the much lower ions heating.

IMS-MS for Proteomics:

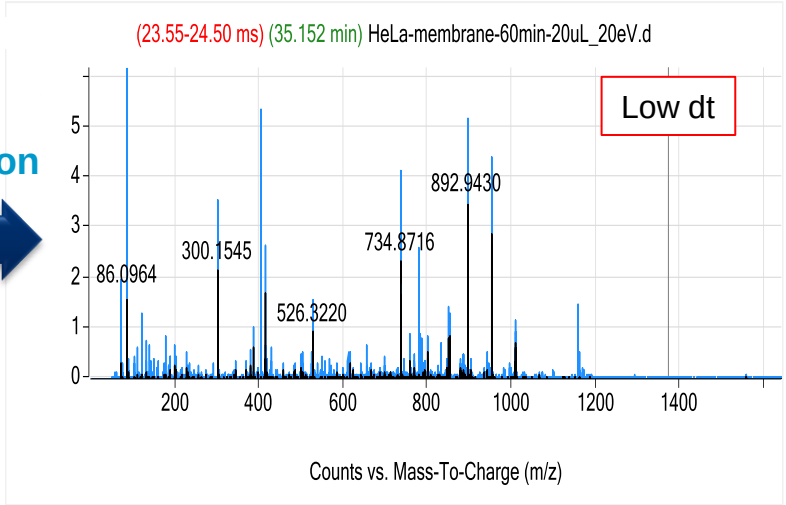
Transmembrane Spanning Peptides of HeLa digest

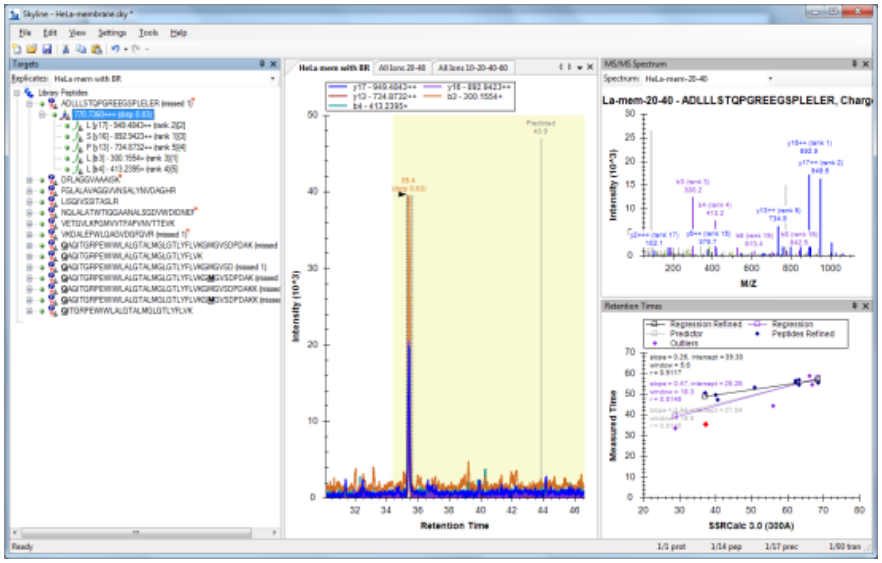


All Ions
Fragmentation

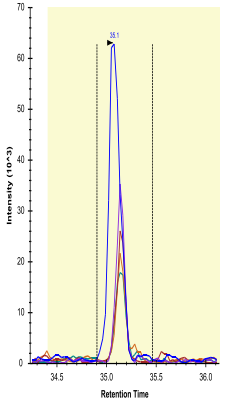
High dt

Low dt

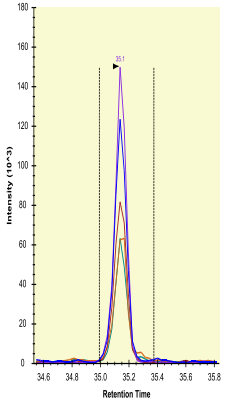




High dt



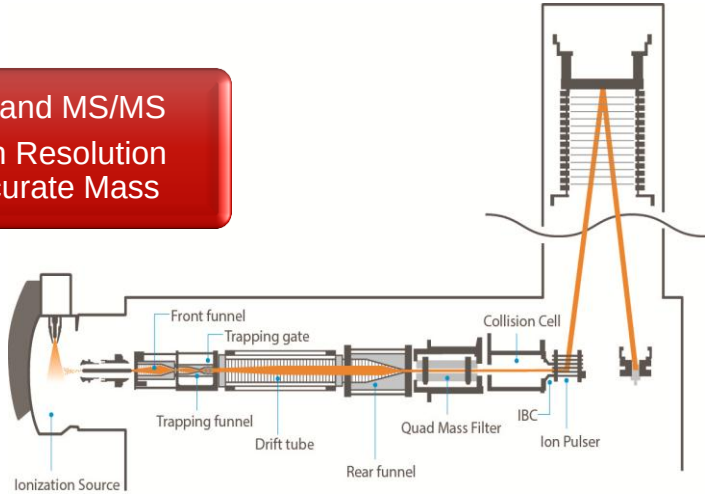
Low dt

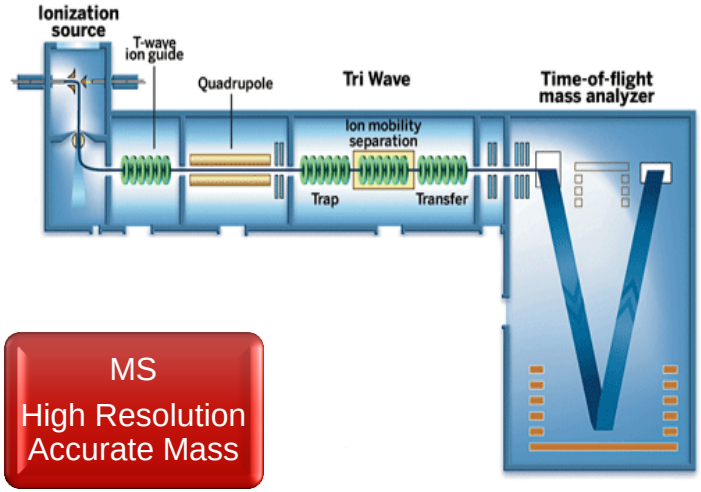


A Word About Instrument Design

LC → Drift IMS → MS and MS/MS High Resolution Accurate Mass

Feature	Agilent	Waters	Drift Mobility Advantage
Mobility Resolution	Highest (can be > 80) 80cm drift tube (L) higher voltage (E) No RF fields, Uniform low DC field	Generally around 30 25cm drift TriWave, Multi-section device RF fields	Over 2X the IM resolution of T-wave
Sensitivity	High efficiency ion funnels - trapping and rear	Step wave lens Pressure barrier between Q and TriWave	10X to 50X better than T-wave
Collision Cross Section (CCS) measurement (Ω)	Direct determination of Ω Low electric field and constant drift tube pressure	Ω cannot be directly determined from drift time. Need calibration tables.	1-2% precision Much better than Synapt (5-10%)
Molecular structures	Lower RF fields, less ion heating.	Higher RF fields, tendency for higher fragmentation and ion heating	Lower RF allows preservation of molecular structures





LC → Q → IMS → MS High Resolution Accurate Mass

Summary

- Next generation of IM Q-TOF Technology
- Added dimension of separation based on size, charge and molecular conformation
- Resolve and characterize the complex samples

  -- Increased peak capacity
- Direct determination collision cross sections
- Preservation of molecular structures

Acknowledgments

Ruwan Kurulugama

Alex Mordehai

Mark Werlich

Chris Klein

Tom Knotts

Ed Darland

Gregor Overney

Bill Barry

Nathan Sanders

Lane Howard

Mikhail Ugarov

Gene Wong

Yergeny Kaplun

George Stafford

Bill Frazier

Bruce Wang

Huy Bui

Crystal Cody

John Fjelsted

Ken Imatani

Robert Kincaid

Robin Scheiderer

Collaborators:

PNNL

NIH

Texas A&M

Vanderbilt University

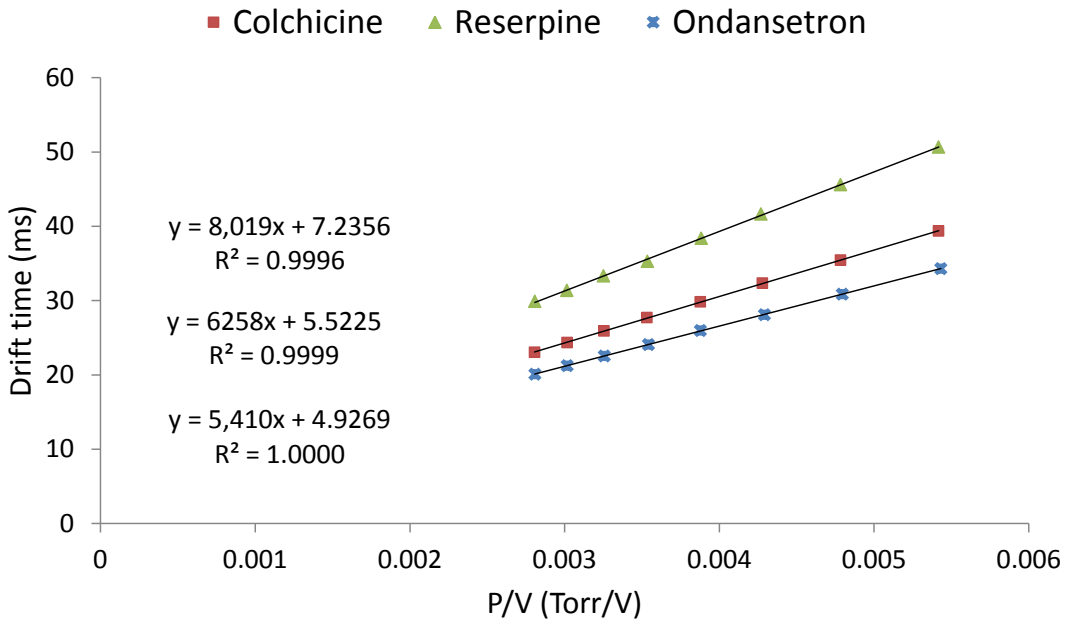
Boston University

Standard Procedure for Calculating CCS
(Drift Time versus P/V Curves)

$$t_d = \frac{L^2}{K_0}\frac{273.2}{760}\frac{1}{T}\frac{P}{V}$$

$$t_d = t_D - t_0$$

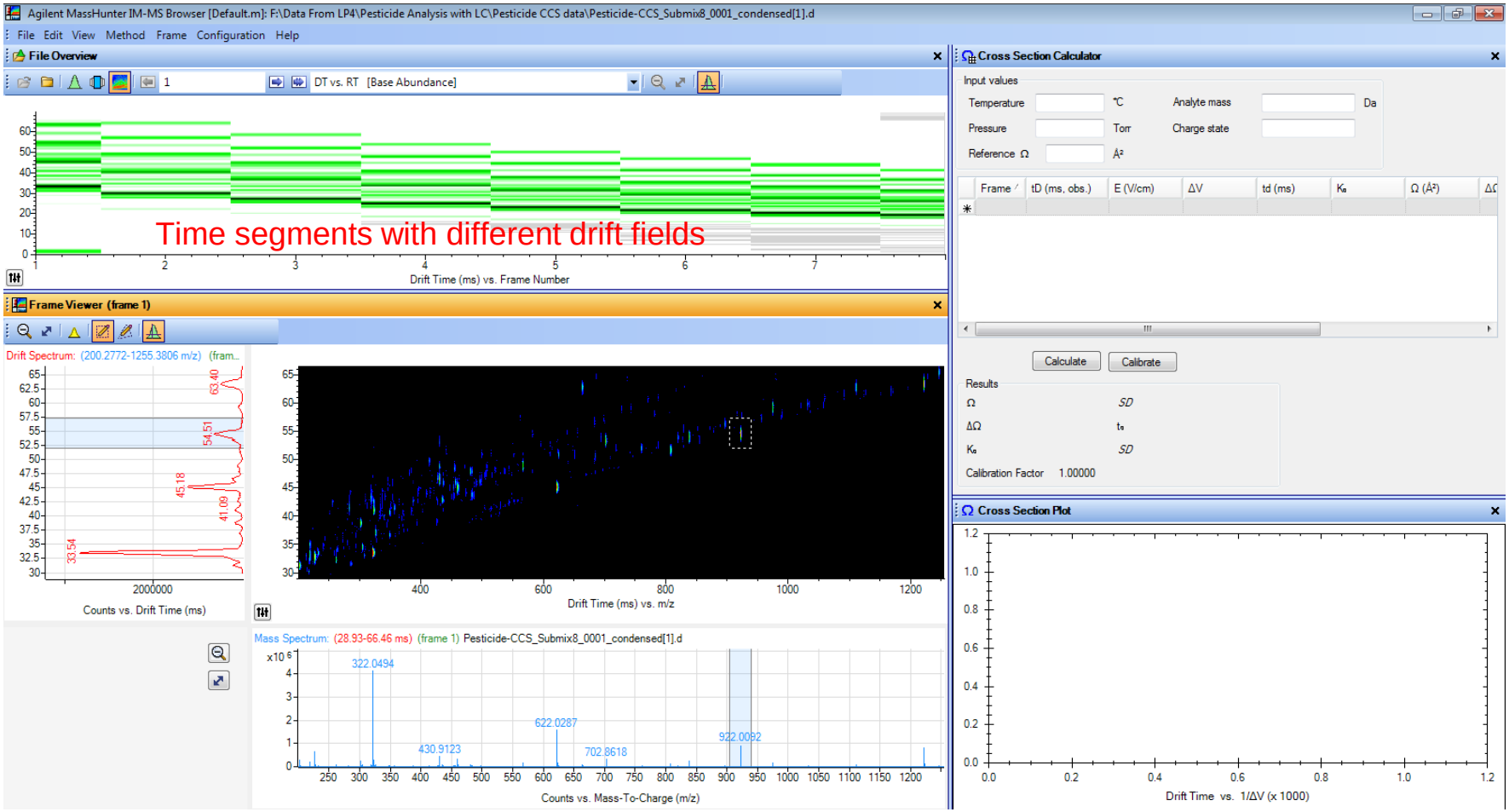
t_D = experimental drift time
t_0 = time ions spend out side the drift cell
t_d = corrected drift time



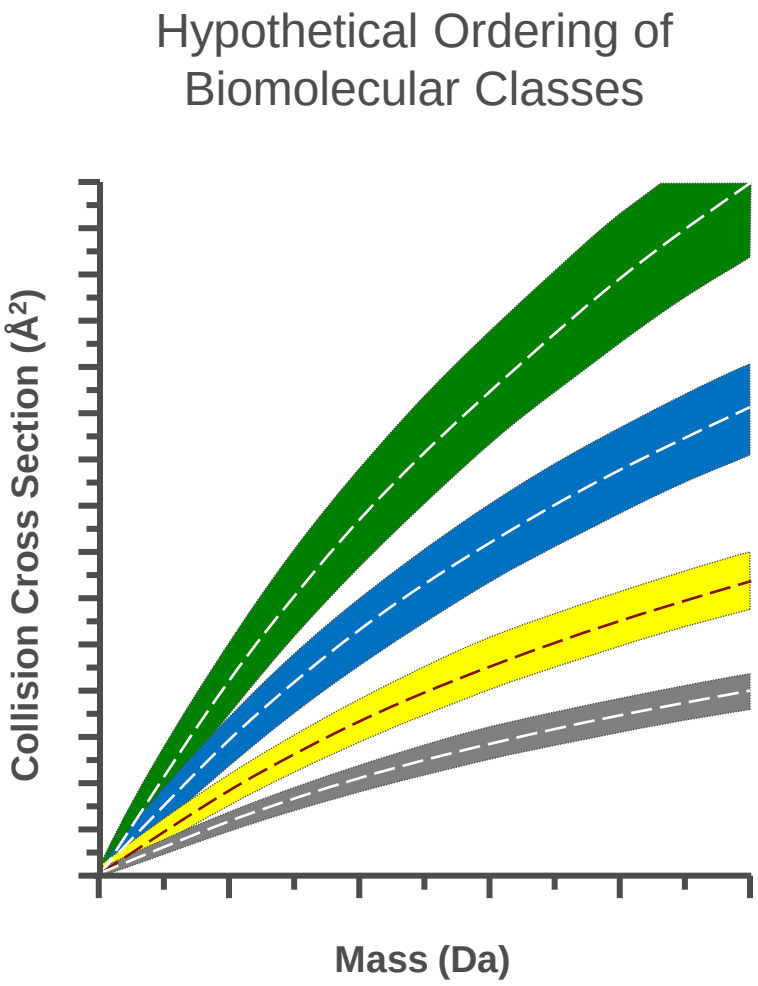
$$\Omega = \frac{(18\pi)^{1/2}}{16}\frac{ze}{(k_b T)^{1/2}}\left[\frac{1}{m_I}+\frac{1}{m_B}\right]^{1/2}\frac{t_d E}{L}\frac{760}{P}\frac{T}{273.2}\frac{1}{N}$$

Compound	Drift time (ms)	Slope	Intercept t_0(ms)
Ondansetron	20.09	5410	4.9269
Colchicine	23.03	6258	5.5225
Reserpine	29.89	8019	7.2356

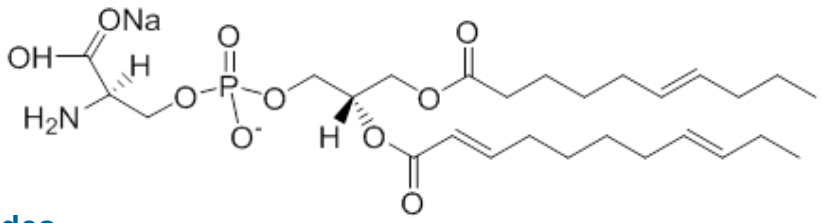
Standard Procedure for Calculating CCS - Auto Stepping the Low Field Gradient



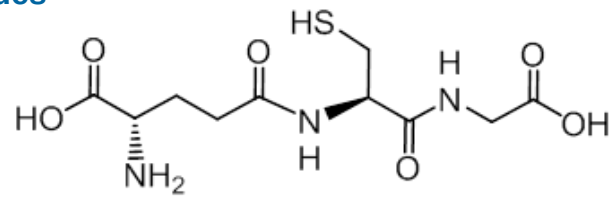
Conformational Space Occupancy of Biomolecules



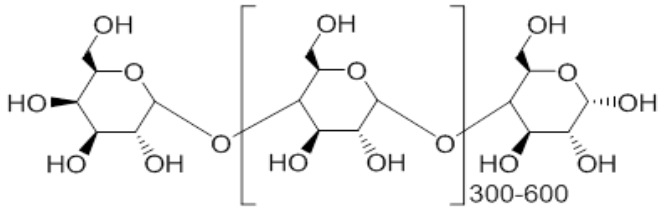
lipids



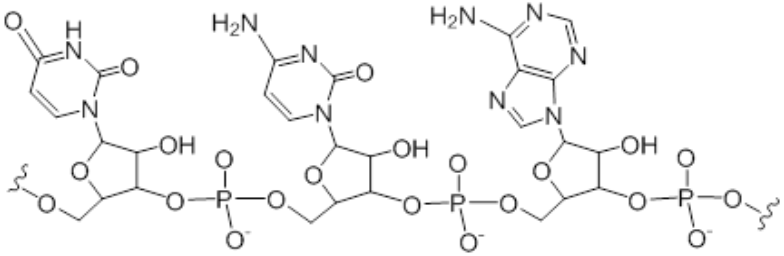
peptides



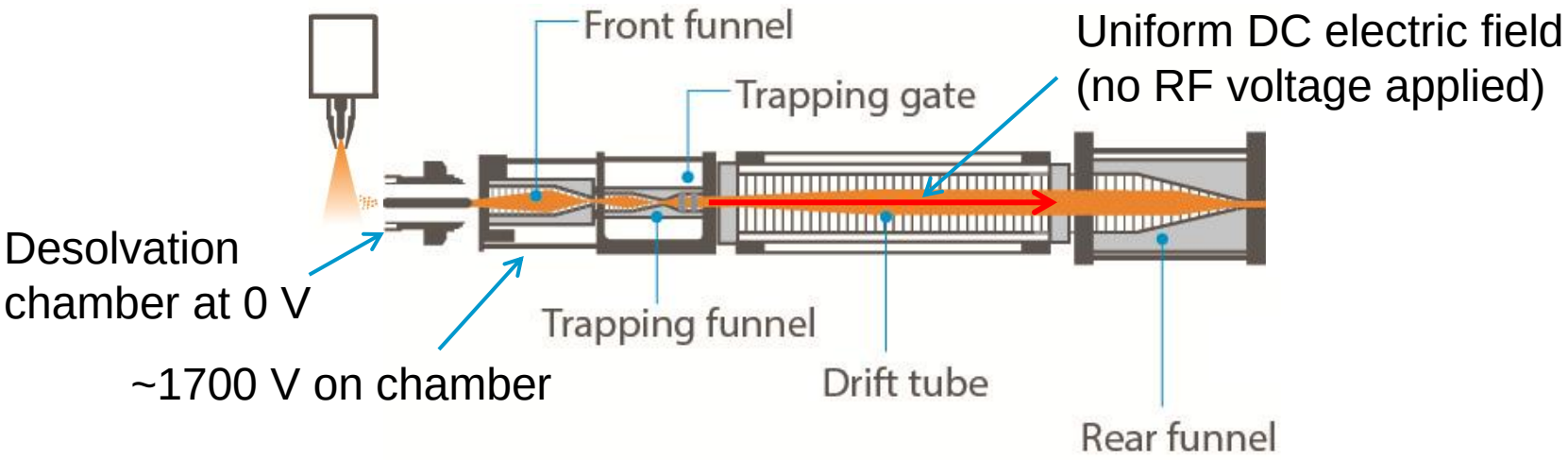
carbohydrates



oligonucleotides



Ion Mobility System Design



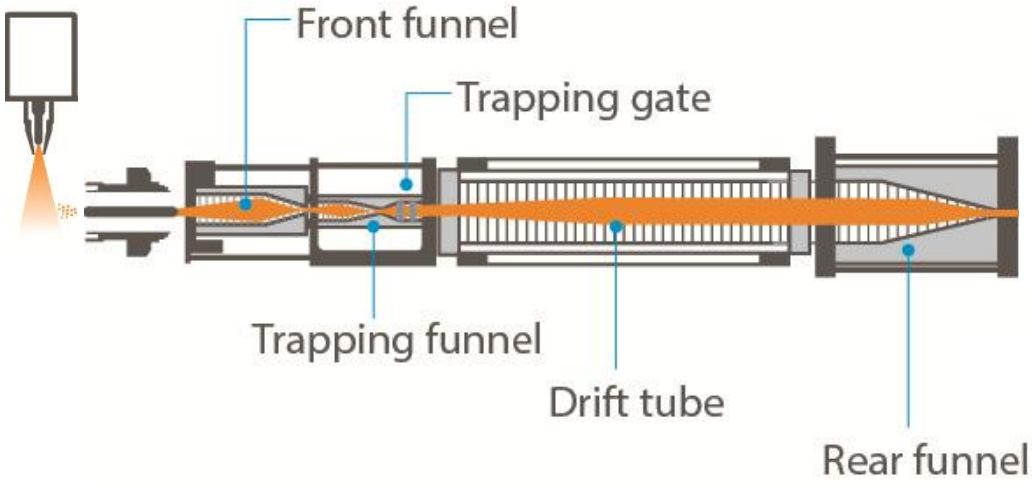
Ion source maintained at ground potential (no voltage offset)

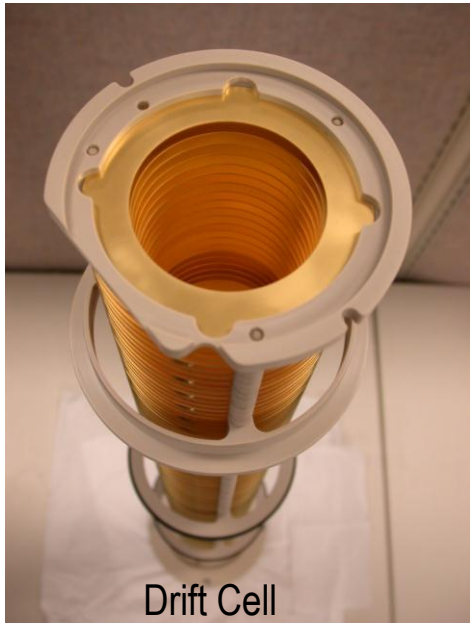
Uniform low static electric field across drift cell

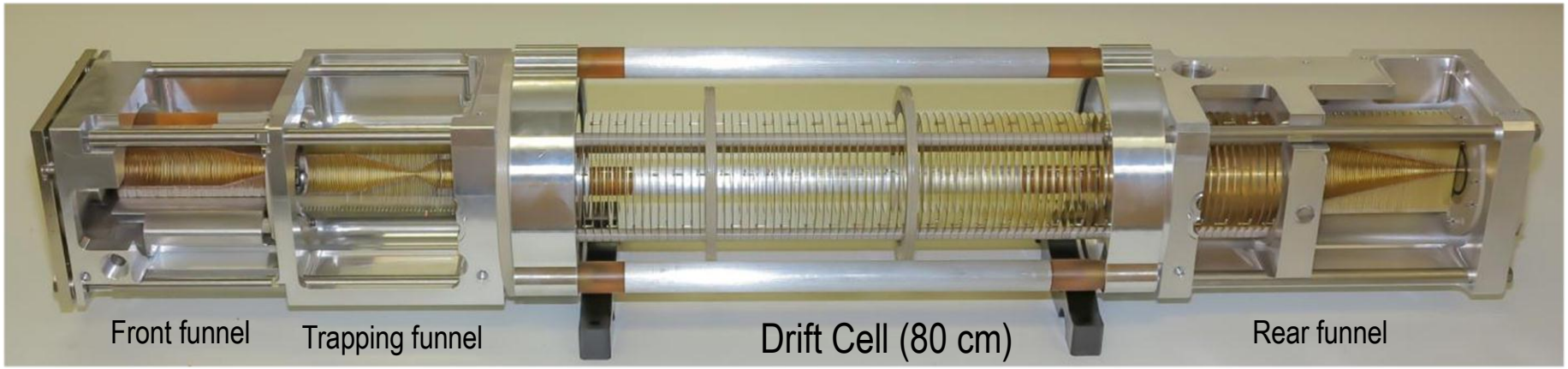
- 80 cm long, approximately 20 V/cm with 4 Torr Nitrogen buffer gas
- Mobility resolution approaches theoretical limit
- Minimizes ion excitation or heating (helps to maintain ion structures and conformations)

Uniform low field ion mobility allows direct determination of accurate CCS (Ω)

Ion Mobility Hardware Design







Ion funnel technology improves sensitivity