

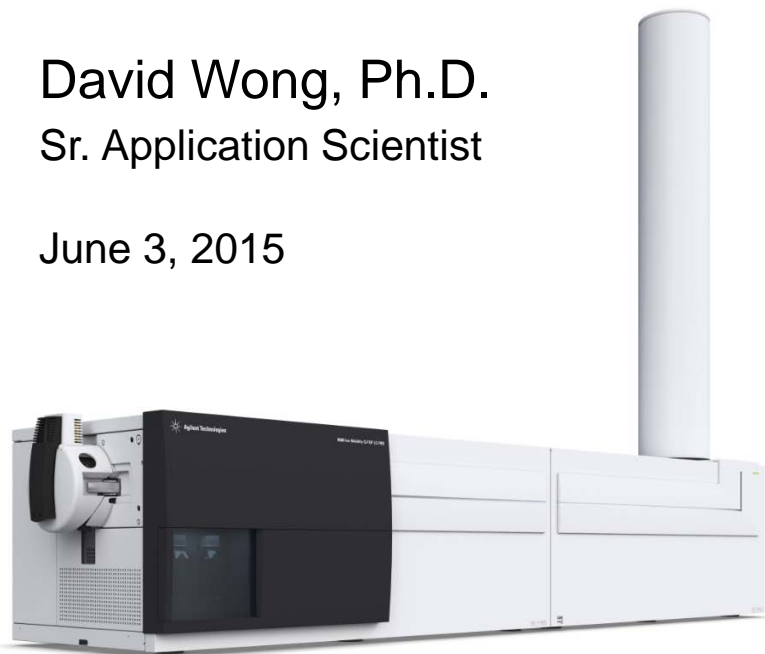


# Advantages of Ion Mobility QTOF for Characterization of BioPharma Molecules

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

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Sr. Application Scientist

June 3, 2015



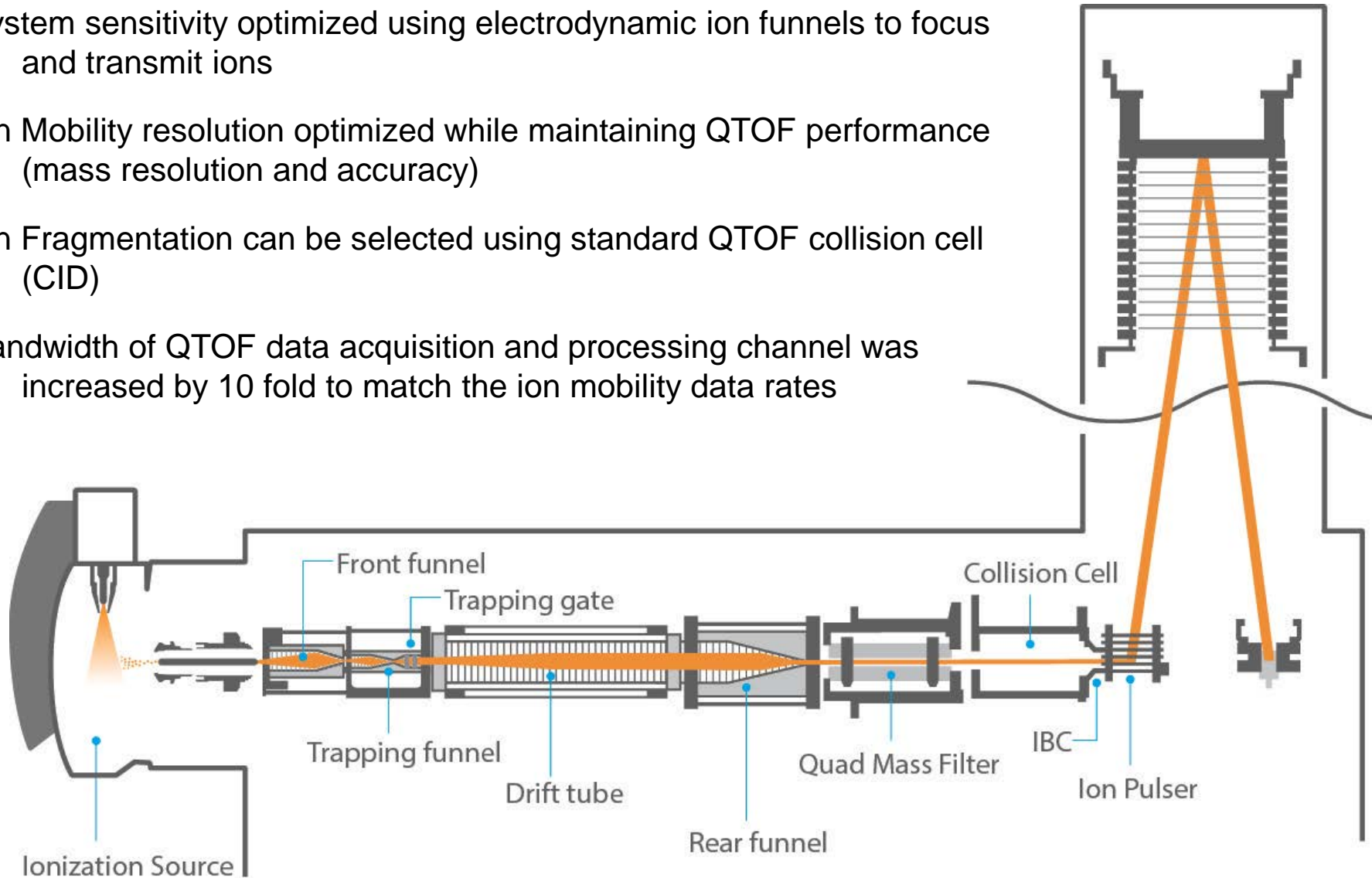
# IM-QTOF Instrument Overview

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

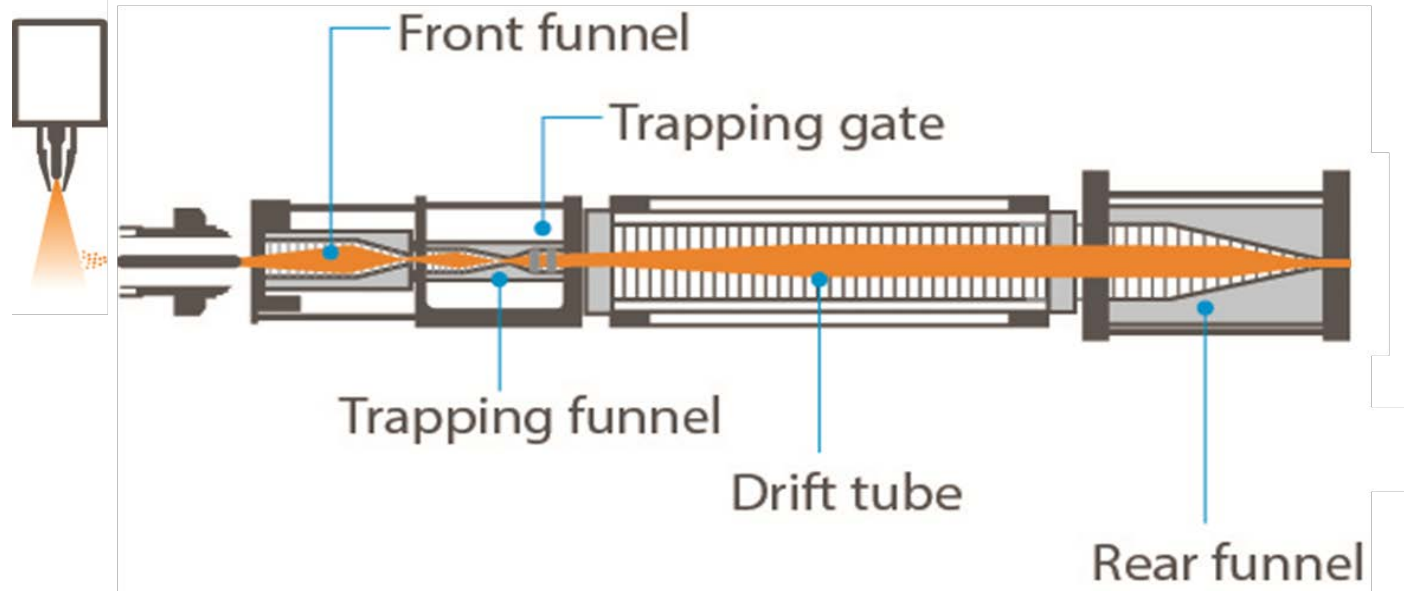
Ion Mobility resolution optimized while maintaining QTOF performance (mass resolution and accuracy)

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

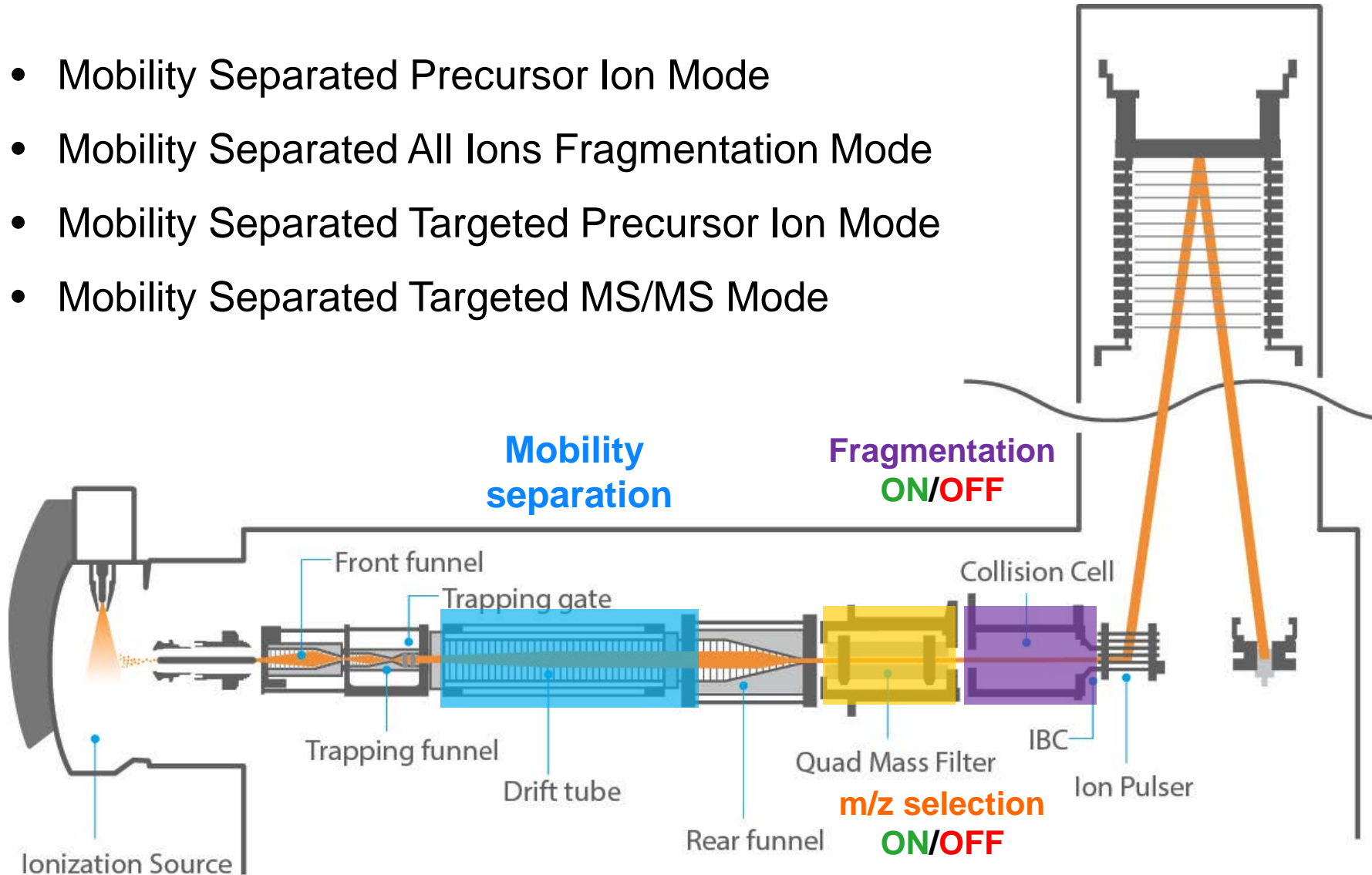
**Trap funnel:** Ion accumulation and introducing ion packets into drift cell

**Drift cell:** Uniform low field ion mobility allows direct determination of accurate CCS ( $\Omega$ )

**Rear funnel:** Efficient ion refocusing and introduction into mass analyzer

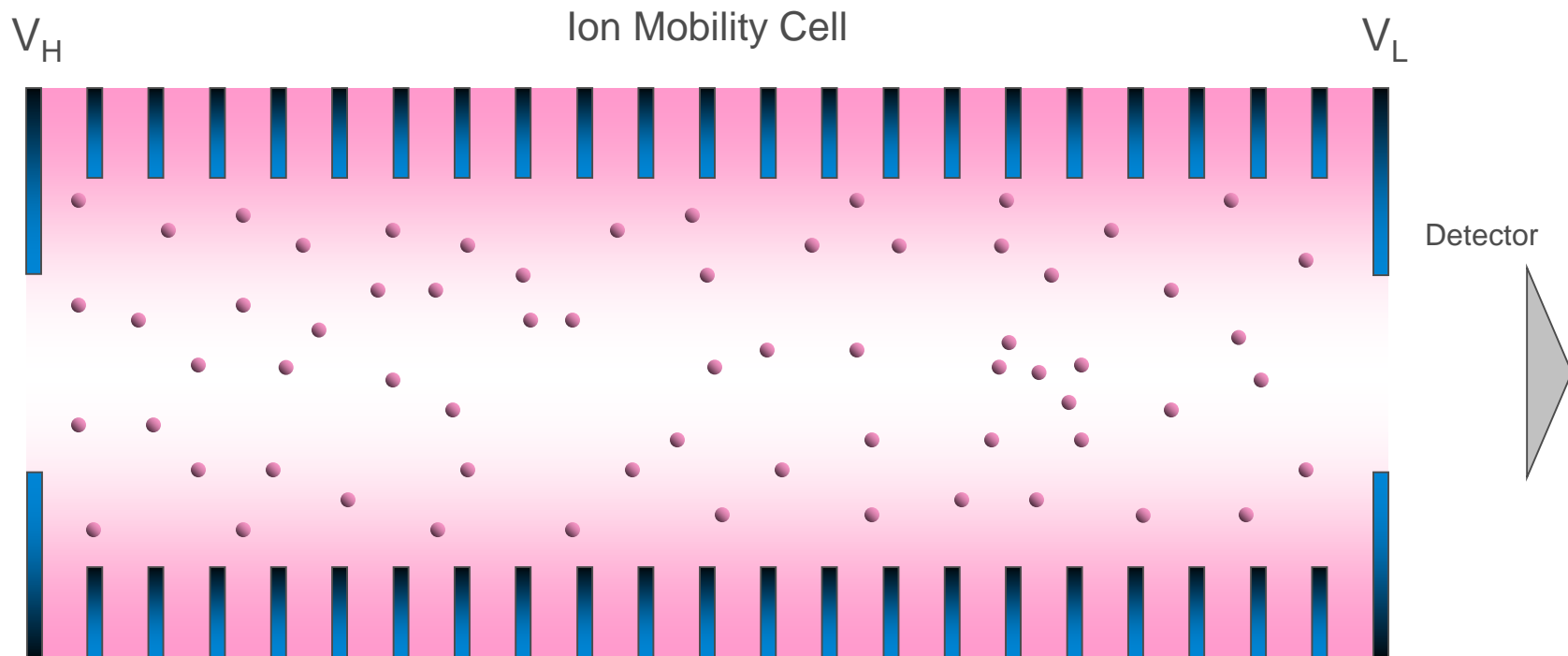
# IM Q-TOF/MS operational modes

- Mobility Separated Precursor Ion Mode
- Mobility Separated All Ions Fragmentation Mode
- Mobility Separated Targeted Precursor Ion Mode
- Mobility Separated Targeted MS/MS Mode



# Basic Operational Principle of Ion Mobility

## For Conventional DC Uniform Field IMS



Electric Field  
→  
*Stacked ring ion guide gives linear field*

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

# Benefits of Adding Ion Mobility to LC/Q-TOF/MS

## Adds Additional Separation Power

- A new dimension of separation for increased mass spectral purity especially for complex mixture analysis

## Improves Detection Limits

- Helps to eliminate interference from other analytes and background in the sample mixture
- Efficient ion focusing and transfer through the ion optics maximizes sensitivity for the overall system

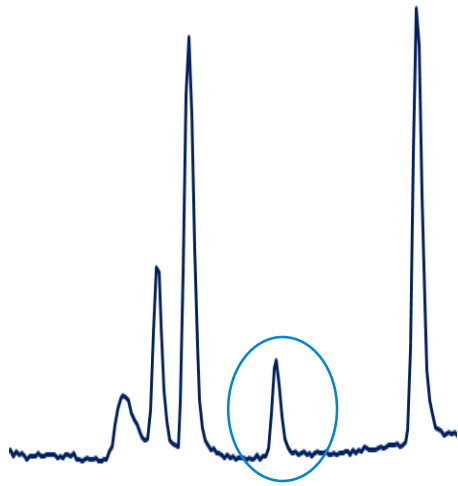
## Enhances Compound Identification

- Improves confidence in compound identification and ion structure correlation through accurate collision cross section measurements

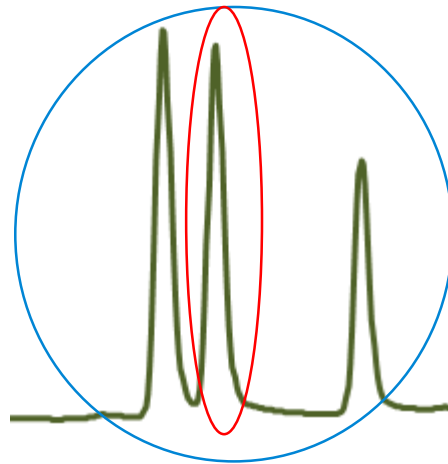
## Provides Native Molecule Structural Information

- Differentiates various protein conformers (native vs. S-S mis-matched)

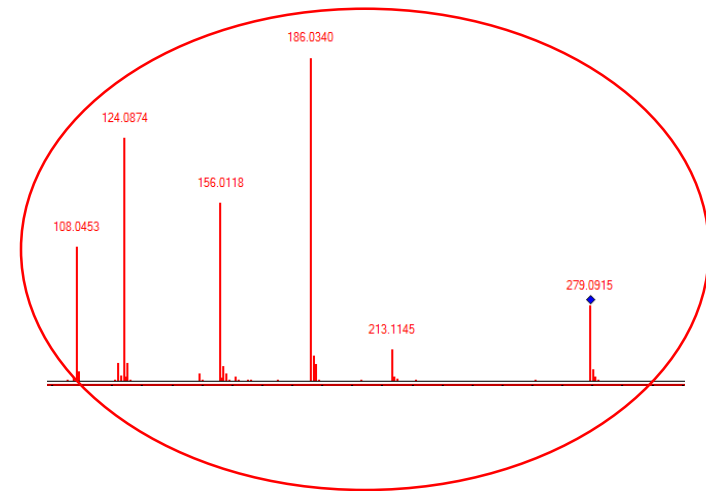
# It's All About Separation



~ minutes



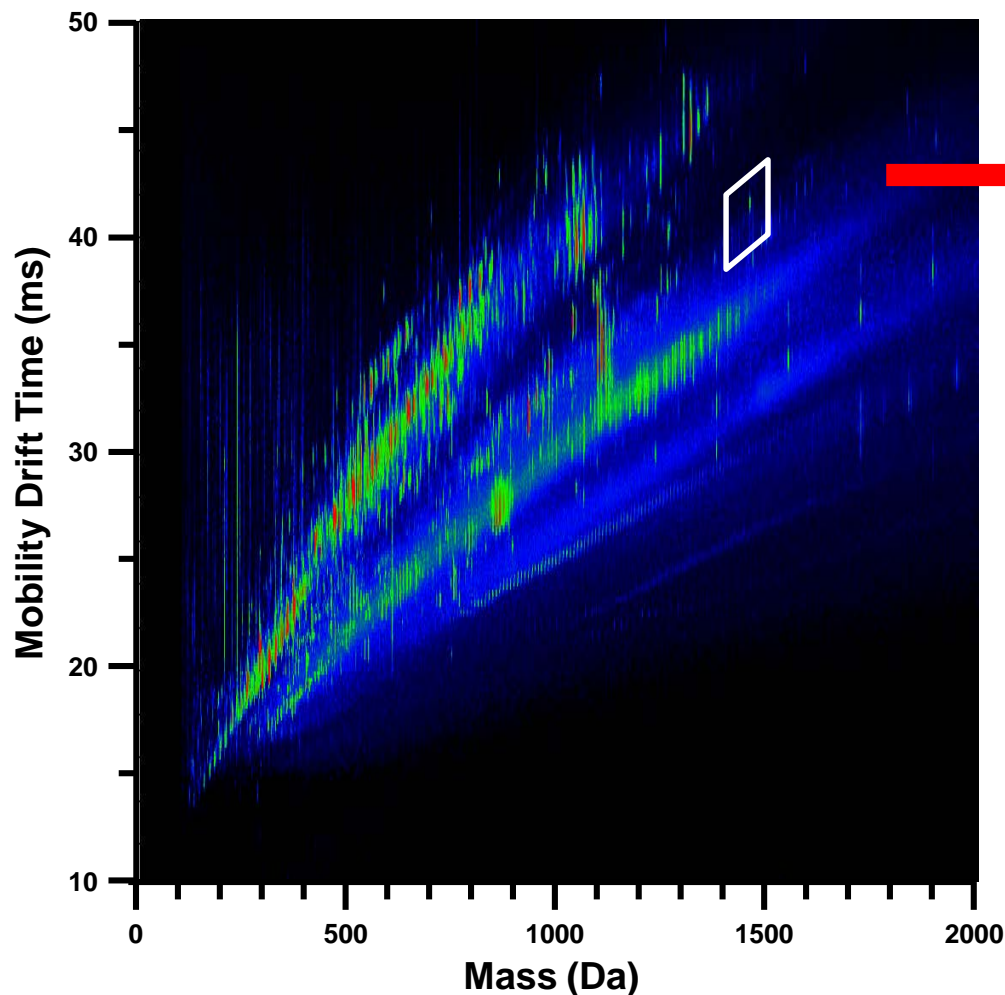
~ 60 milli-seconds



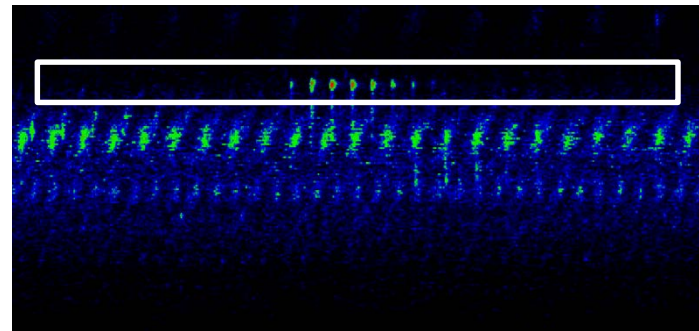
~ 100  $\mu$  seconds



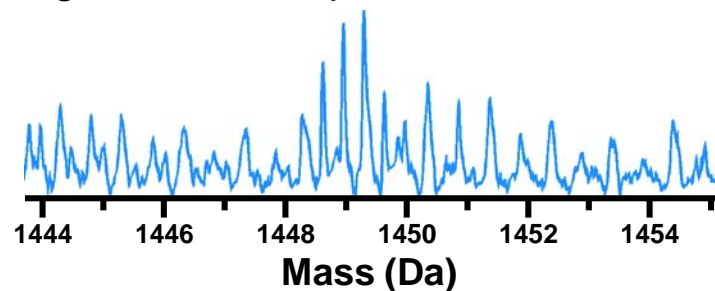
# Ion Mobility Provides Greater Specificity



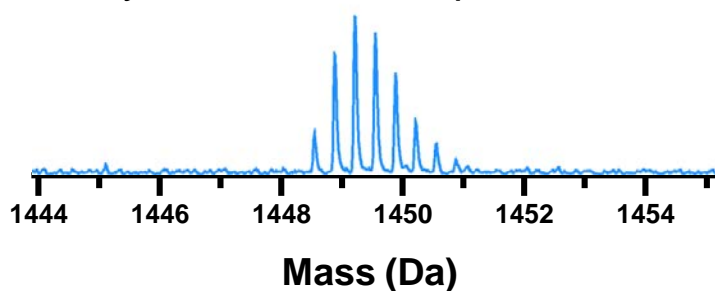
Crude bacterial extract  
(Prof. John McLean, Vanderbilt Univ.)



Integrated Mass Spectrum:



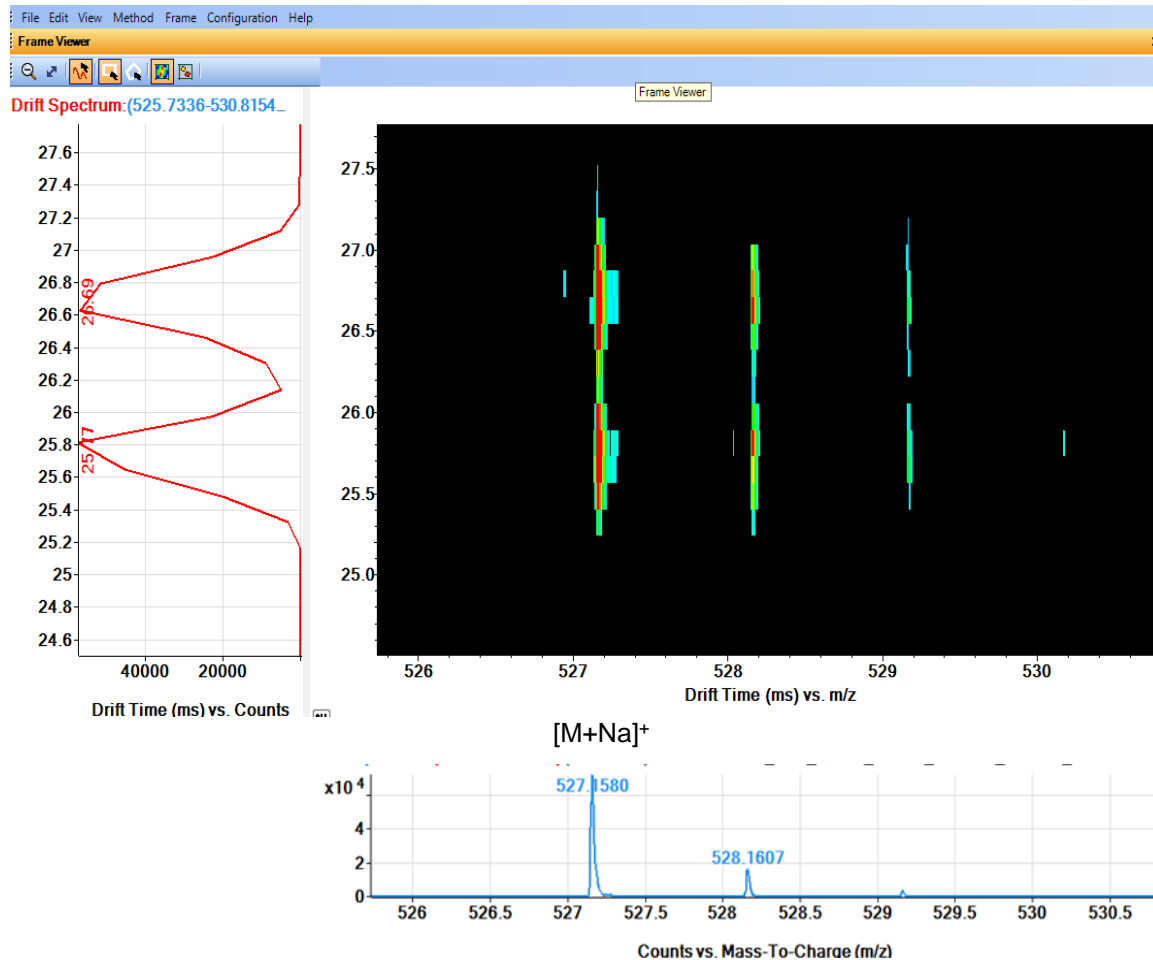
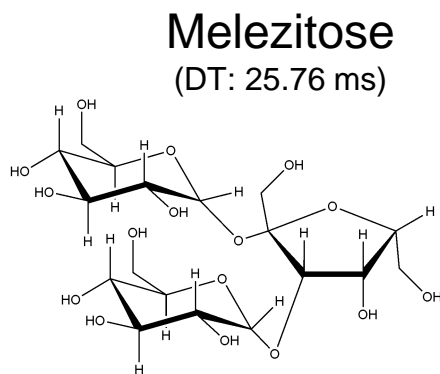
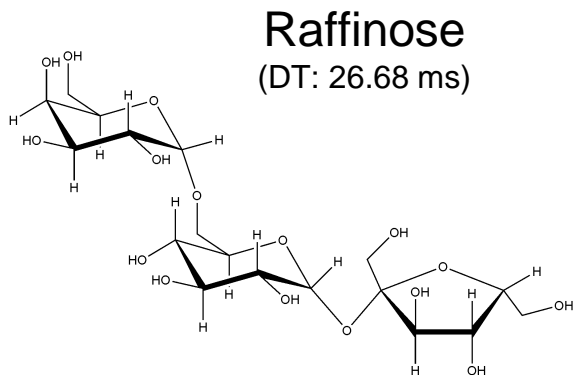
Mobility-Filtered Mass Spectrum:



**S/N increased significantly!**

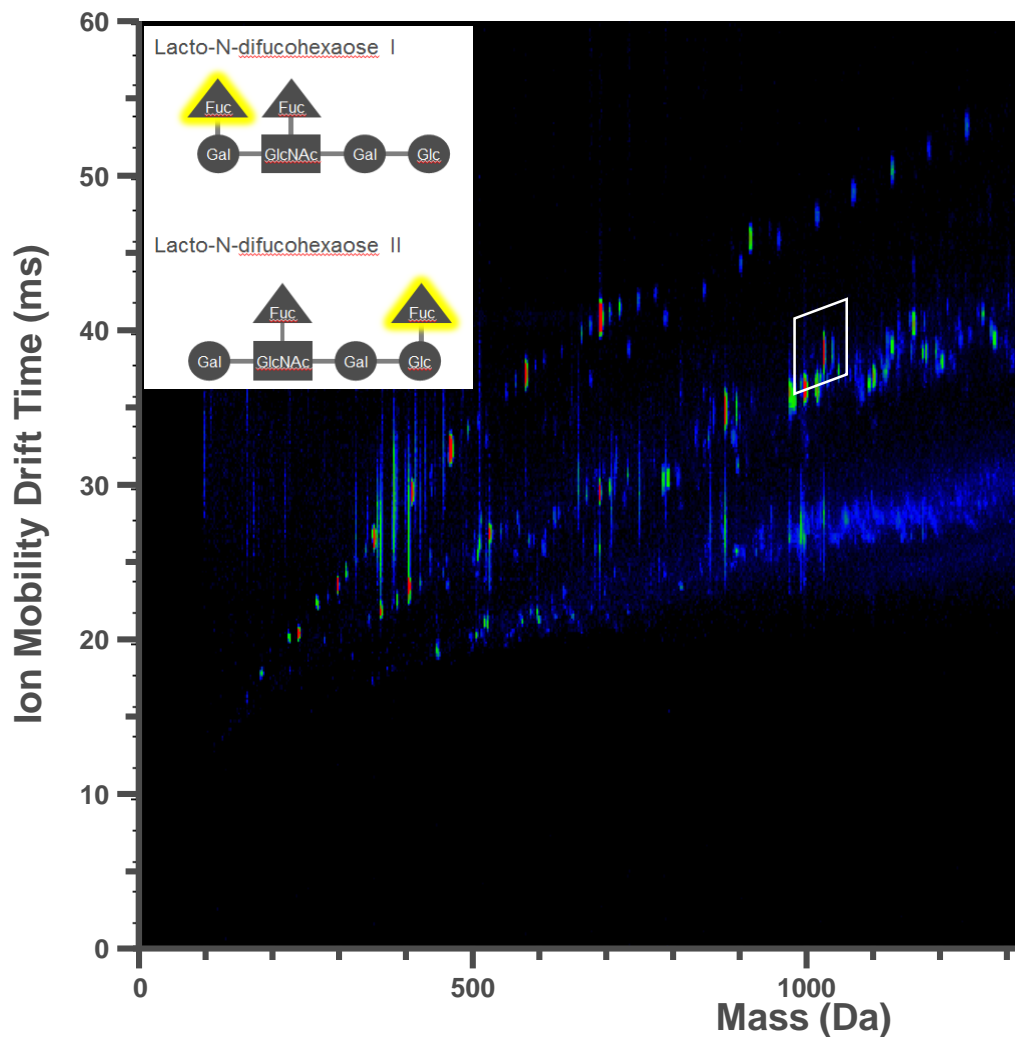


# Resolving Structural Sugar Isomers $C_{18}H_{32}O_{16}$



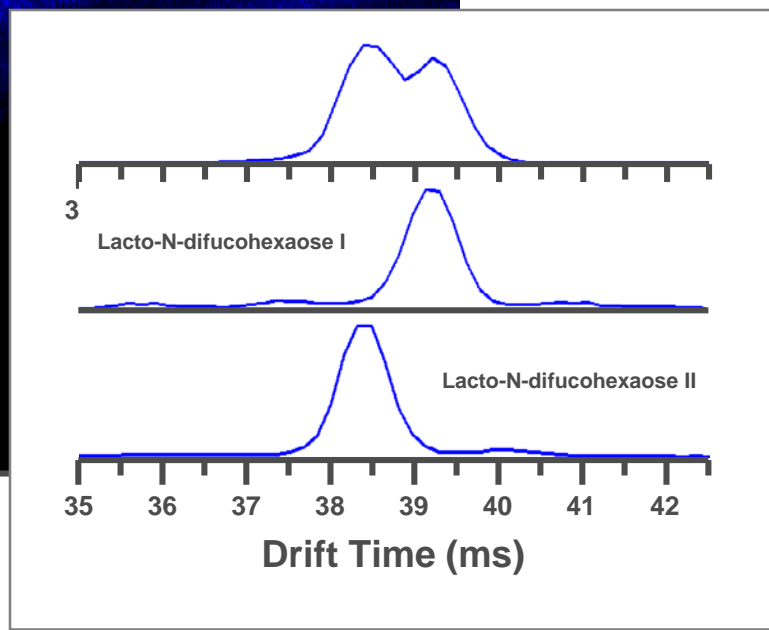
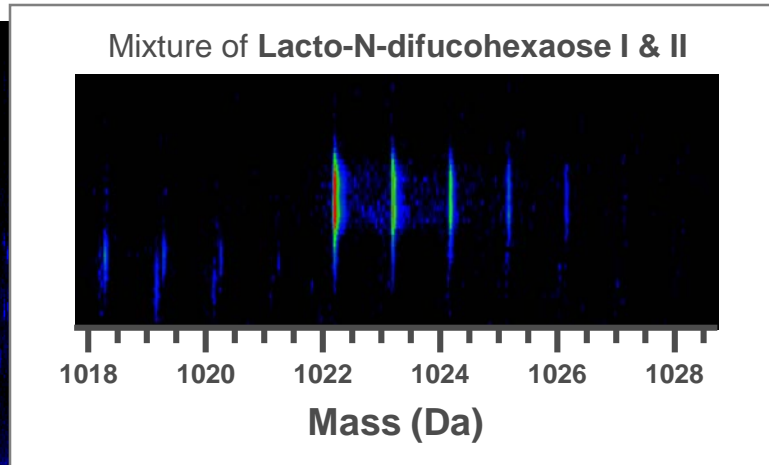
Resolving two isomeric tri-saccharides

# Carbohydrates Analysis by IM-MS

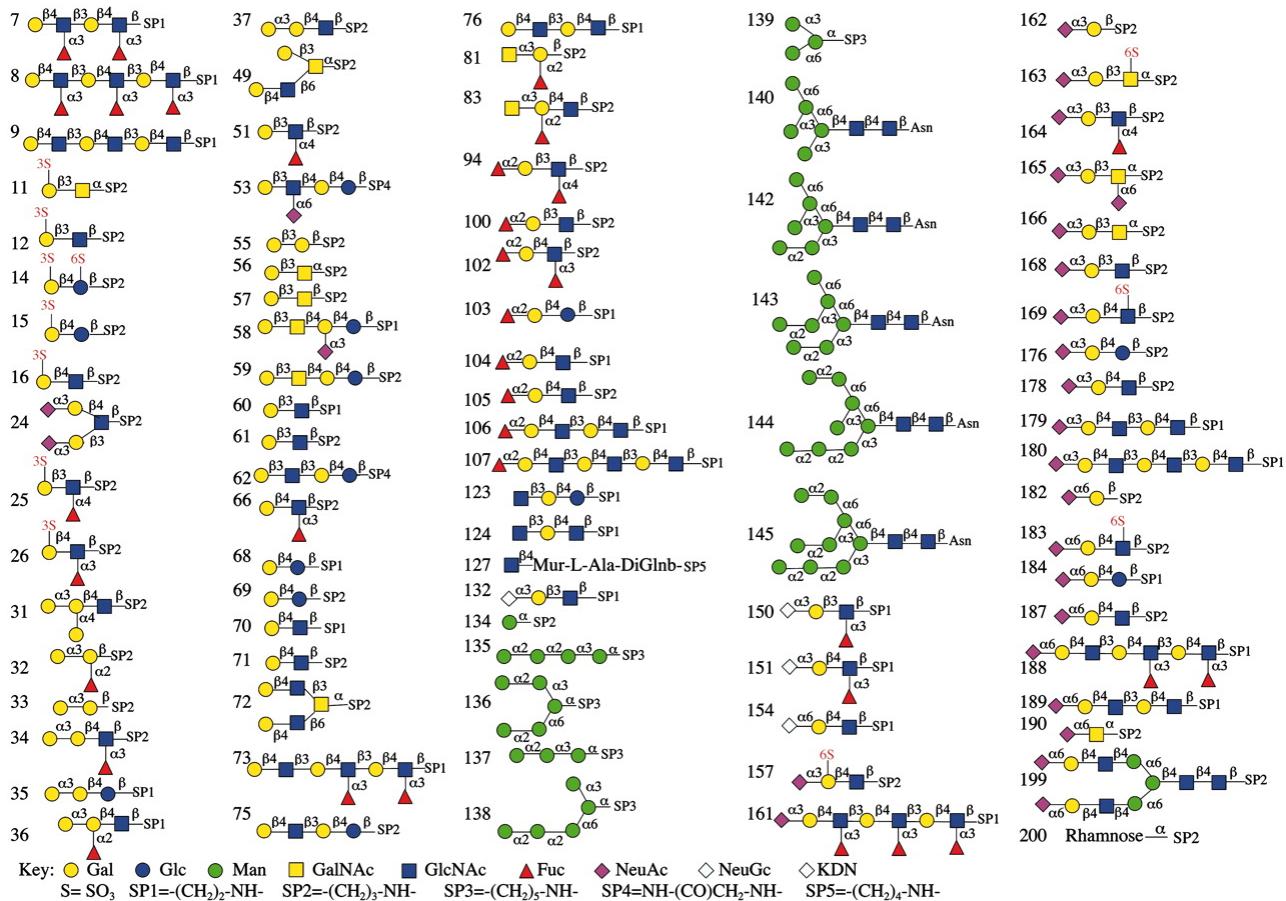


Oligosaccharide mixture

(Profs. John McLean and Jody May, Vanderbilt Univ.)



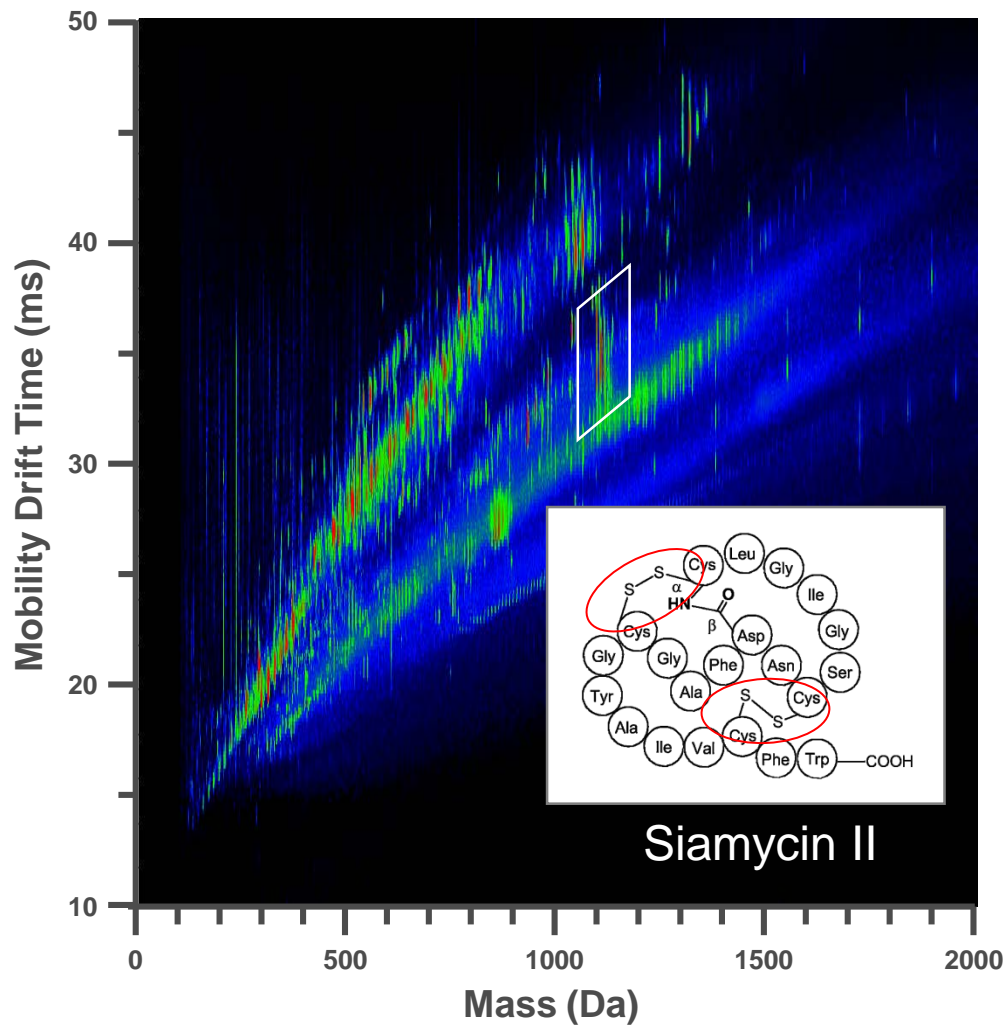
# Carbohydrates -- Great complexity by linkage



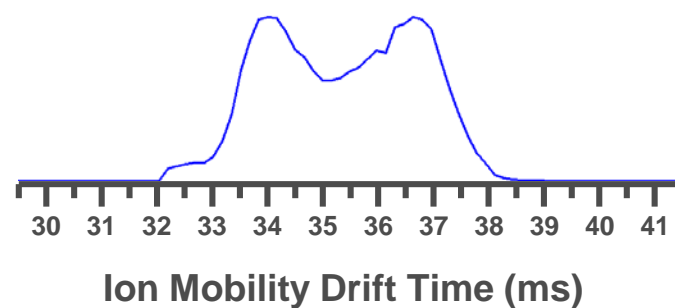
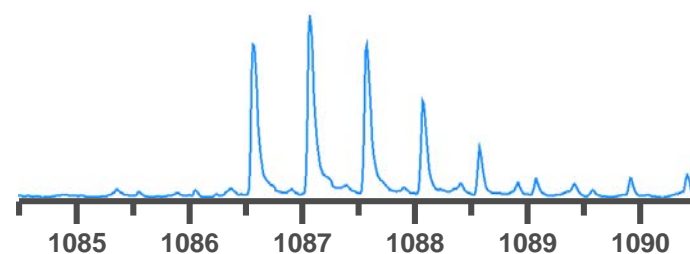
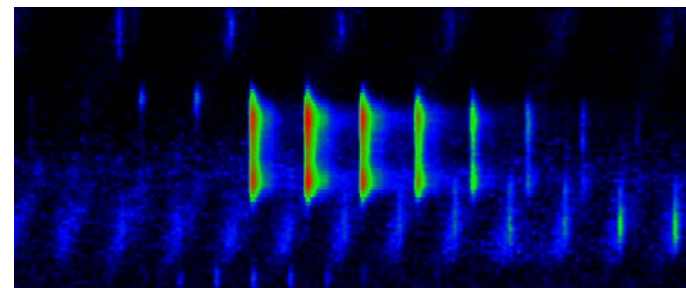
Source: Blixt et al., PNAS, 2004

## 4D (MS, DT, RT & TIC) Feature Finding or Library searches

# Detecting Miss-formed Disulfide Bonds: Siamycin II



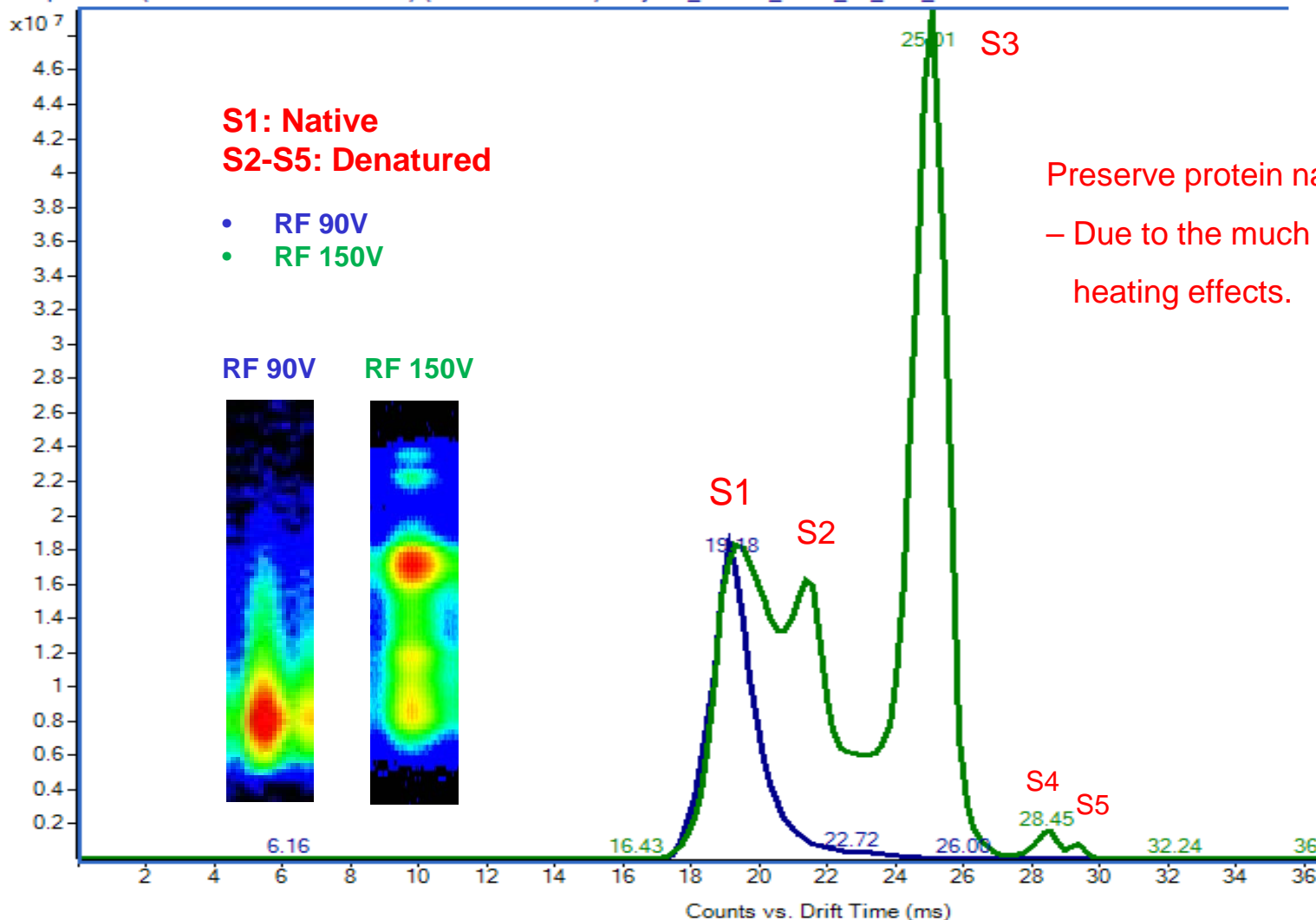
(Profs. John McLean and Jody May, Vanderbilt Univ.)



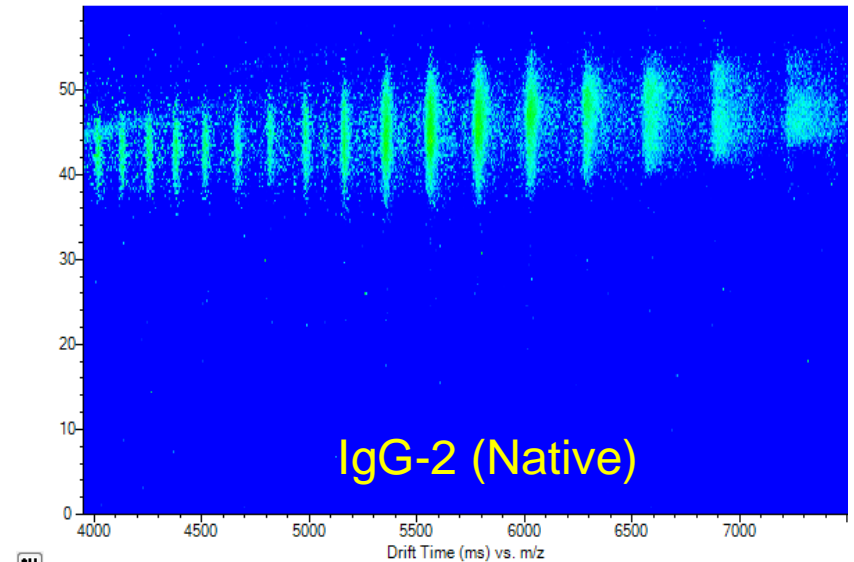
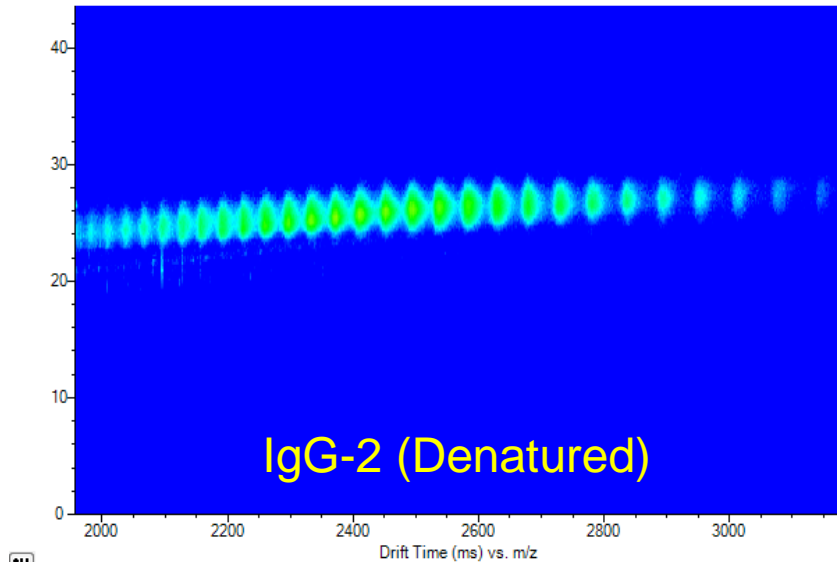
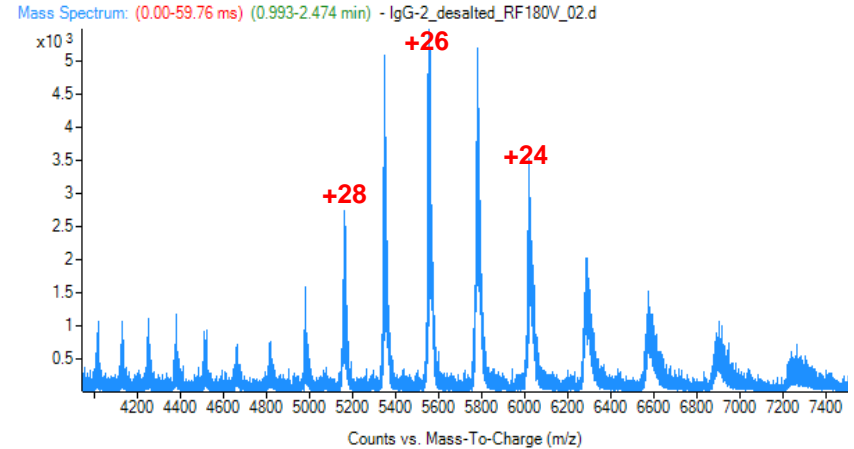
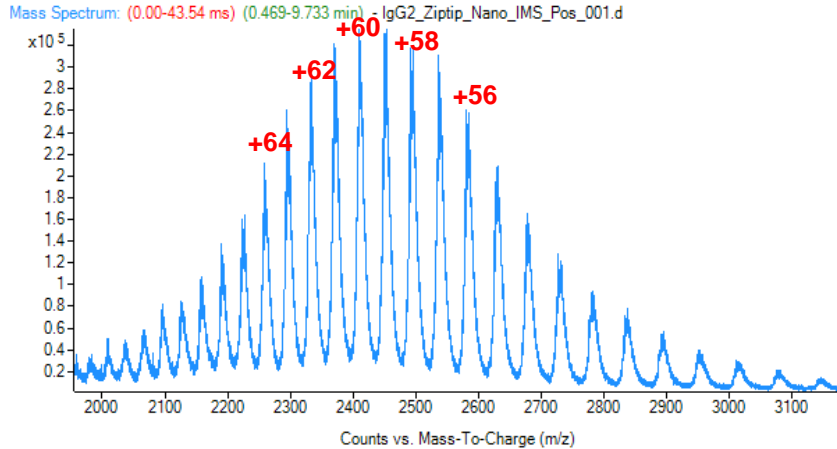
# IM Analysis of Cytochrom C (+8):

(Uniform Drift Tube)

Drift Spectrum: (1533.5688-1560.4894 m/z) (0.214-2.956 min) - CytoC\_RF90V\_250C\_IM\_Pos\_001.d



# IM Q-TOF/MS analysis of IgG-2 under the denatured and native conditions



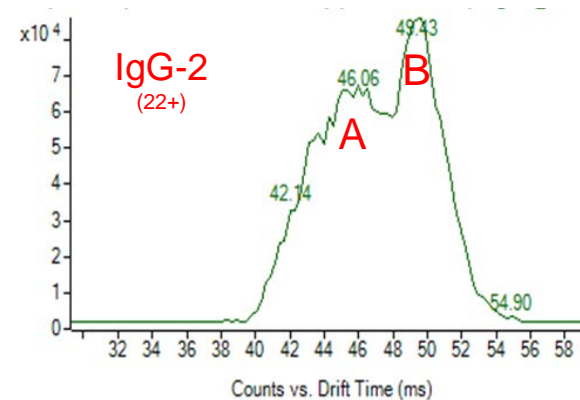
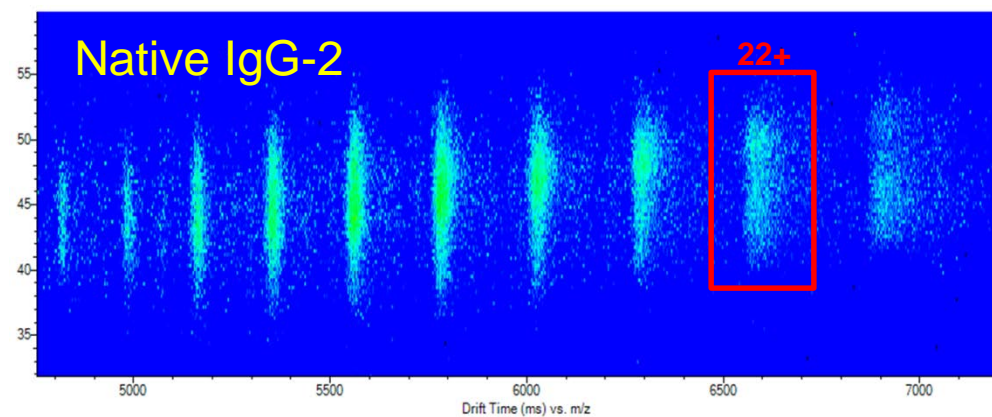
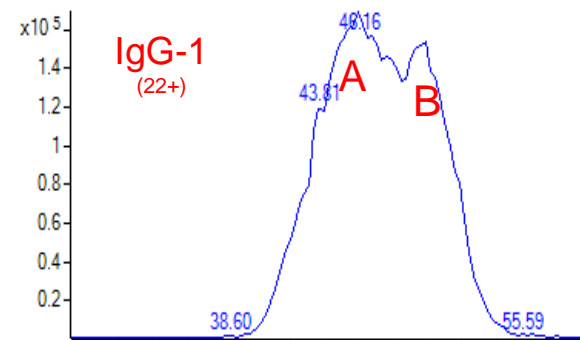
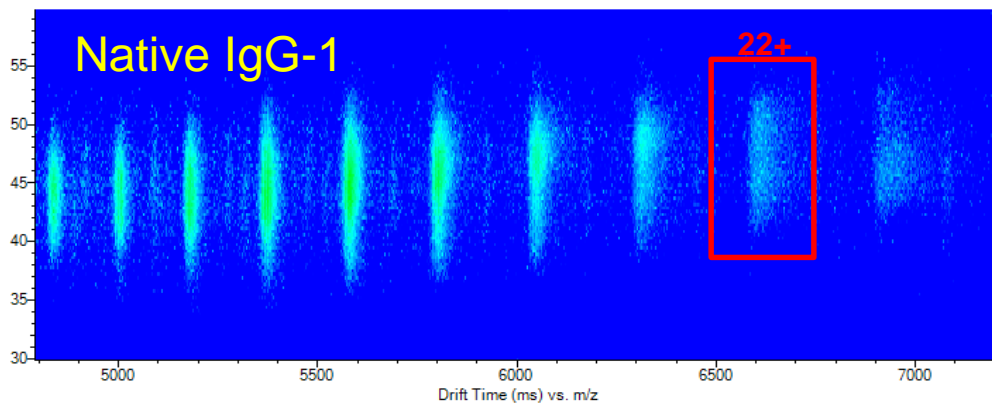
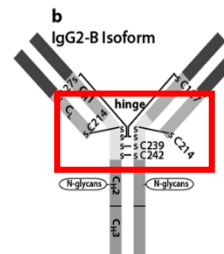
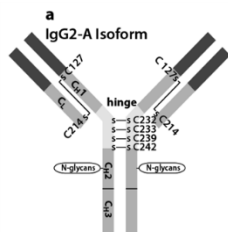
All charge ions of IgG-2 under denatured condition (+45 to +70) posed the much smaller drift times than the charge ions (+20 to +35) of native IgG-2.



# IM Q-TOF Comparison of IgG-1 and IgG-2 under native condition

mAb Structure:

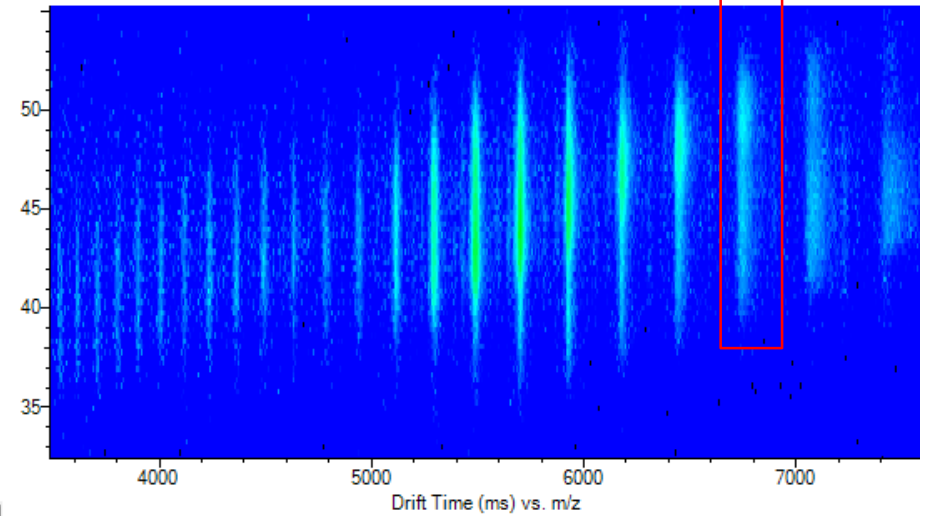
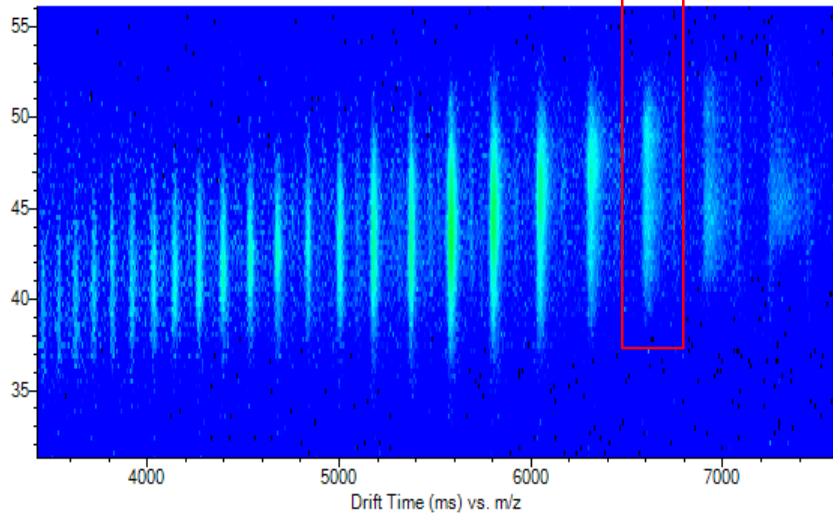
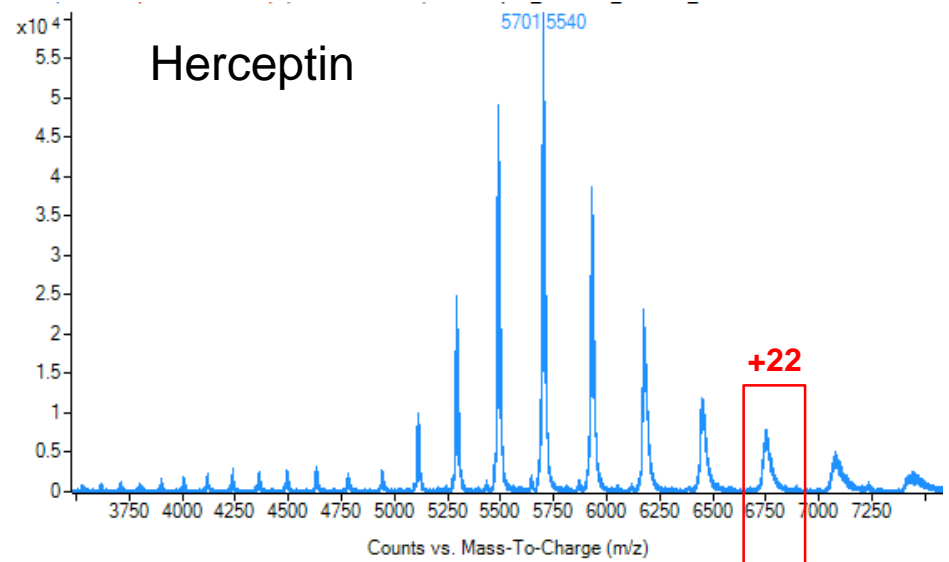
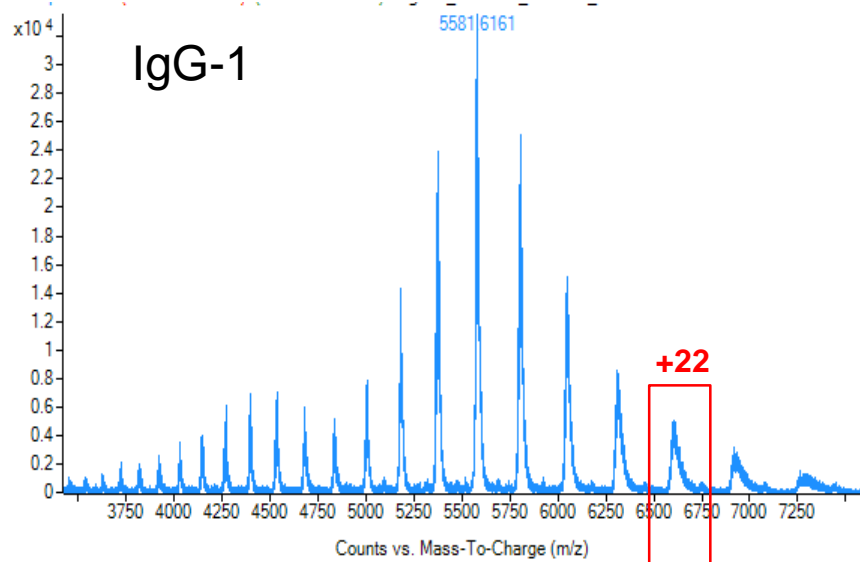
(Paul Schnier, Anal. Chem. 2010)



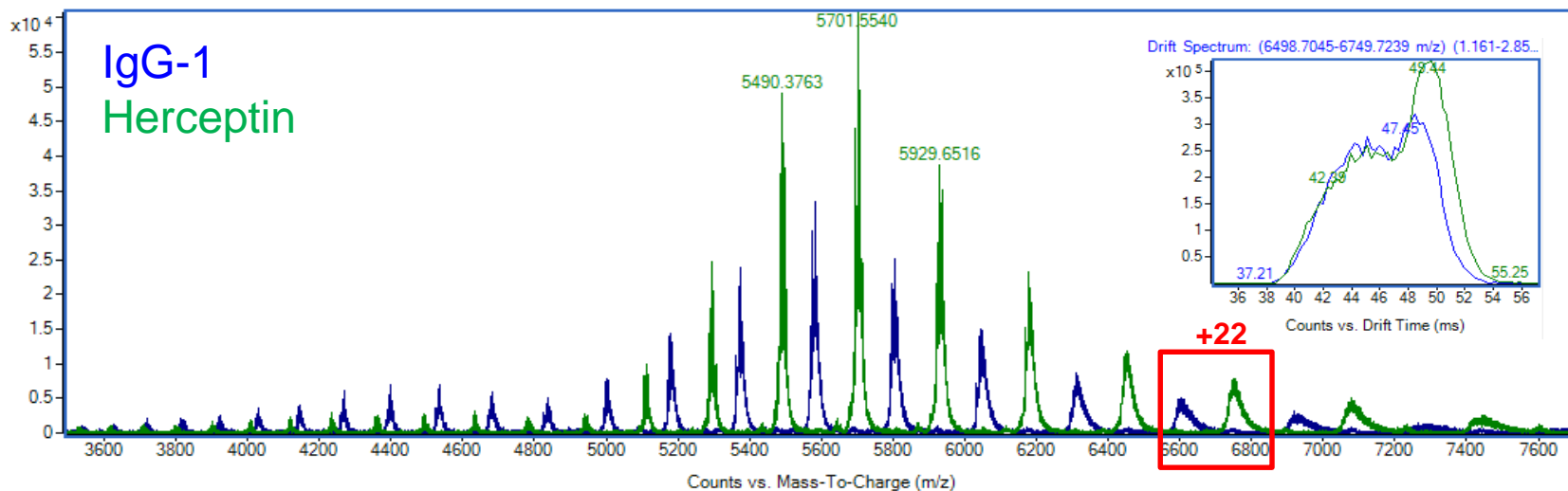
IgG-2 (22+ charge state) has more B isoform



# IM Q-TOF/MS analysis of IgG-1 and Herceptin under the native condition



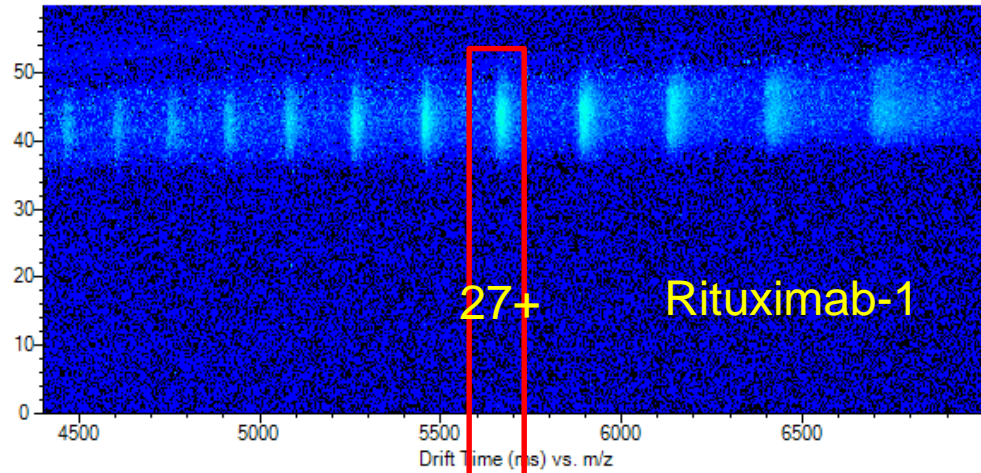
# Collision Cross Section (CCS) Comparison of IgG-1 and Herceptin



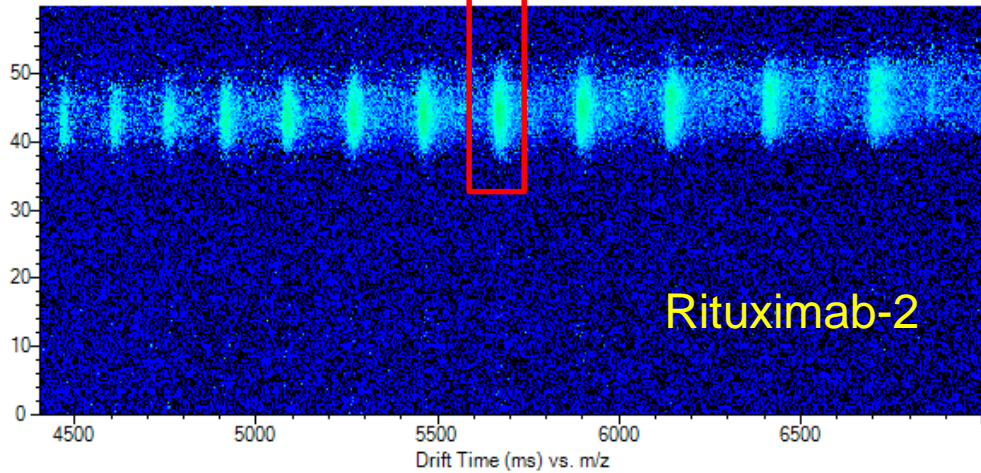
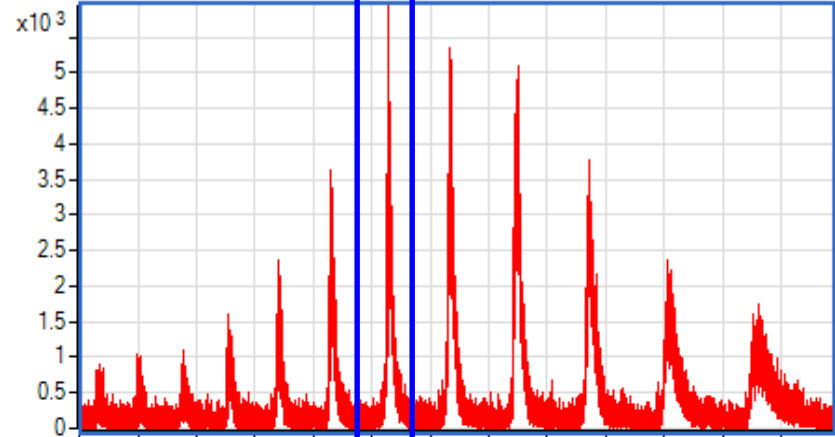
<u>IgG-1 (145,098 Da)</u>				<u>Herceptin (148,216 Da)</u>			
Charge State	Mass (m/z)	Drift Time (ms)	CCS (Å <sup>2</sup> )	Charge State	Mass (m/z)	Drift Time (ms)	CCS (Å <sup>2</sup> )
22	6598	48.45	8332.64	22	6737	49.43	8500.94
		44.95	7731.49			45.50	7825.94
23	6309	47.12	8472.57	23	6444	48.17	8661.10
24	6046	45.84	8601.11	24	6176	46.79	8779.10
25	5804	44.69	8735.03	25	5929	45.32	8857.98
26	5581	43.72	8887.54	26	5701	44.19	8982.92
27	5374	43.28	9136.62	27	5489	43.01	9079.69
28	5182	43.25	9468.45	28	5293	43.73	9573.36
29	5003	43.24	9804.35	29	5111	44.55	10100.92
30	4837	43.11	10111.98	30	4941	43.91	10299.33
31	4681	42.63	10332.88	31	4781	43.53	10550.68

IgG-1 posted slightly lower % of isoform B at its 22+ charge state. Overall, Herceptin has slightly larger CCS values than IgG-1 with the same charge states.

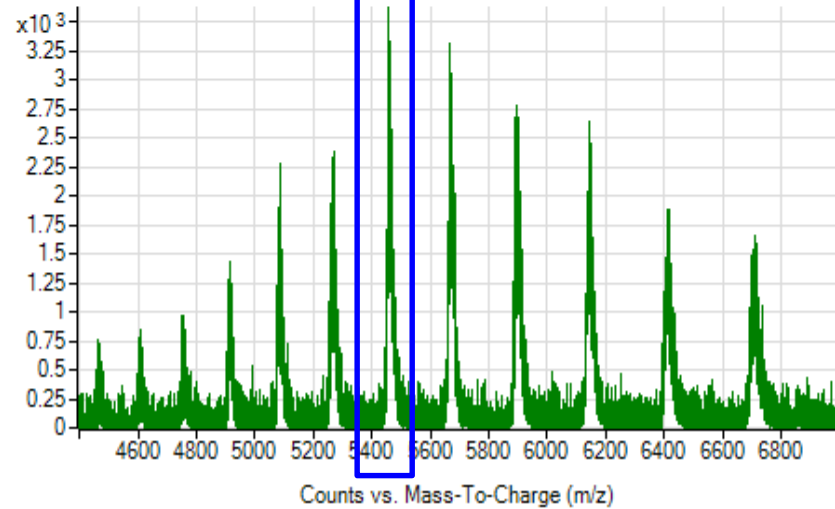
# IM Q-TOF Comparison of Rituximab-1 (Innovator) and Rituximab-2 (Biosimilar):



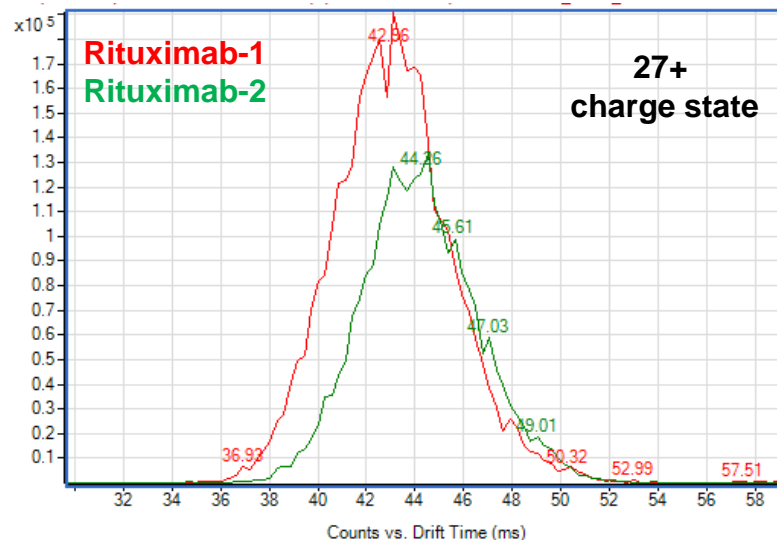
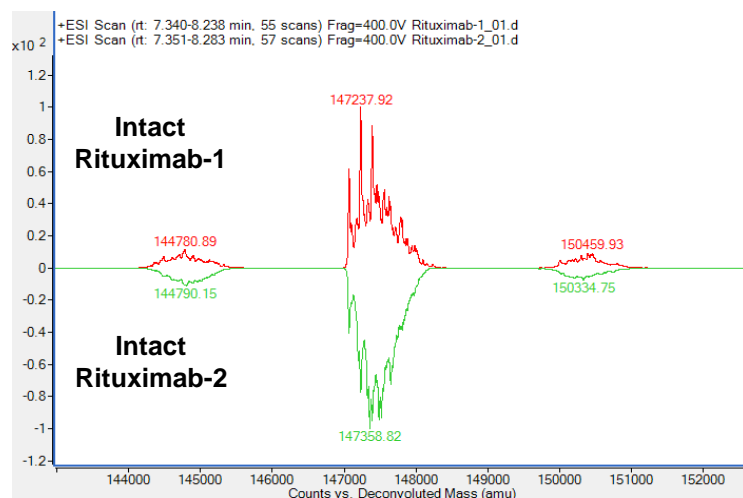
Mass Spectrum: (32.42-54.12 ms) (1.274-3.044 min) - Rituximab-1\_10XD\_04.d



Mass Spectrum: (0.00-59.76 ms) (1.308-2.888 min) - Rituximab-2\_10XD\_04.d



# Collision Cross Section (CCS) Comparison of Rituximab-1 (Innovator) and Rituximab-2 (Biosimilar):



## Rituximab-1 (Innovator)

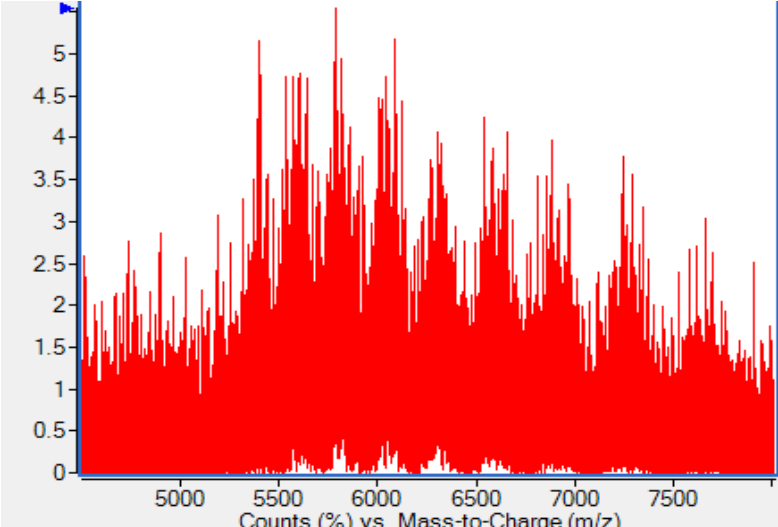
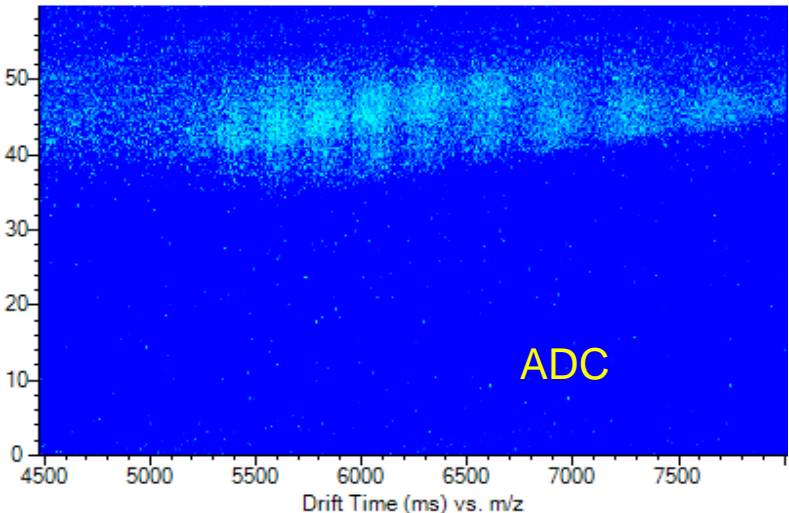
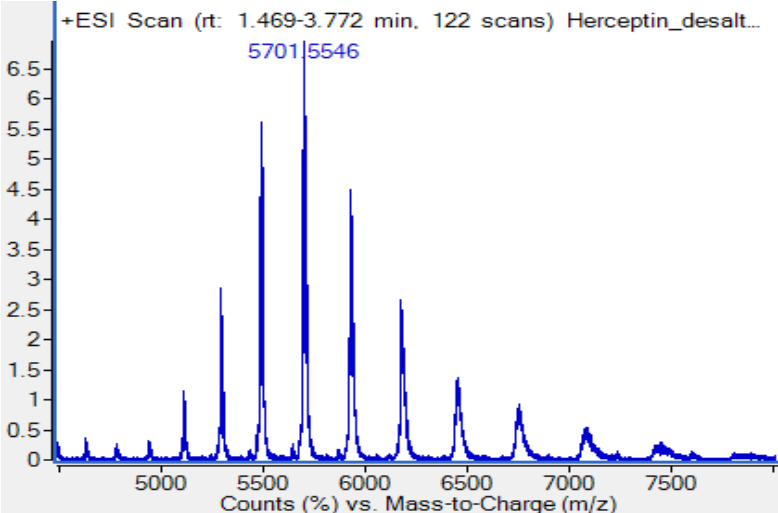
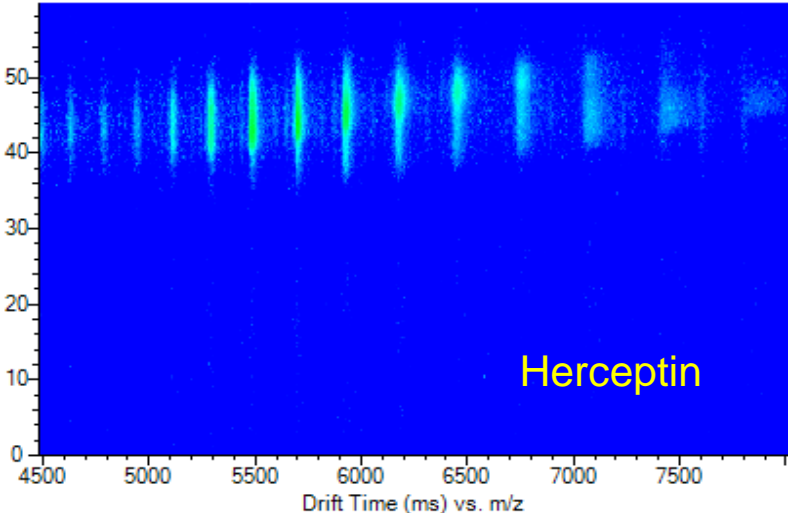
Charge State	Mass (m/z)	Drift Time (ms)	CCS (Å <sup>2</sup> )
22	6723	44.12	7583.31
23	6411	43.93	7893.76
24	6146	44.14	8276.46
25	5900	44.4	8672.25
26	5664	43.6	8856.15
27	5455	43.03	9076.18
28	5260	42.7	9339.93
29	5079	42.85	9707.59

## Rituximab-2 (Biosimilar)

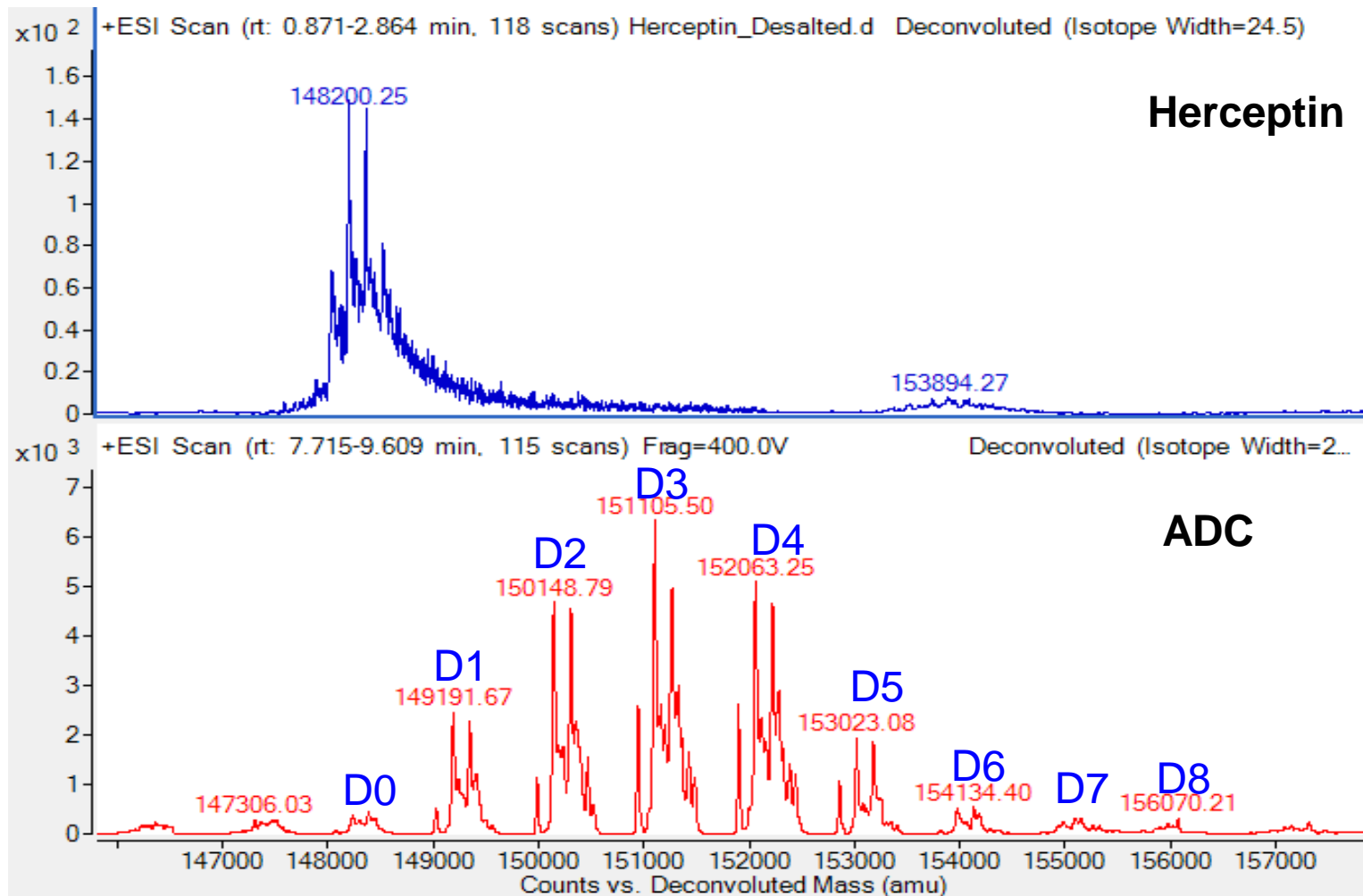
Charge State	Mass (m/z)	Drift Time (ms)	CCS (Å <sup>2</sup> )
22	6705	49.33	8481.46
23	6411	47.94	8616.47
24	6143	46.44	8709.00
25	5897	44.87	8764.32
26	5669	44.39	9017.10
27	5459	43.98	9277.17
28	5264	43.76	9572.50
29	5083	43.78	9918.92

The average size of glycans on the Rituximab-1 were slightly smaller than those on the Rituximab-2. The CCS of the 27+ molecule was larger for the Rituximab-2. Ion mobility can provide not only the size but also the molecule structural information in the Biosimilar study.

# IM Q-TOF Comparison of Herceptin and ADC



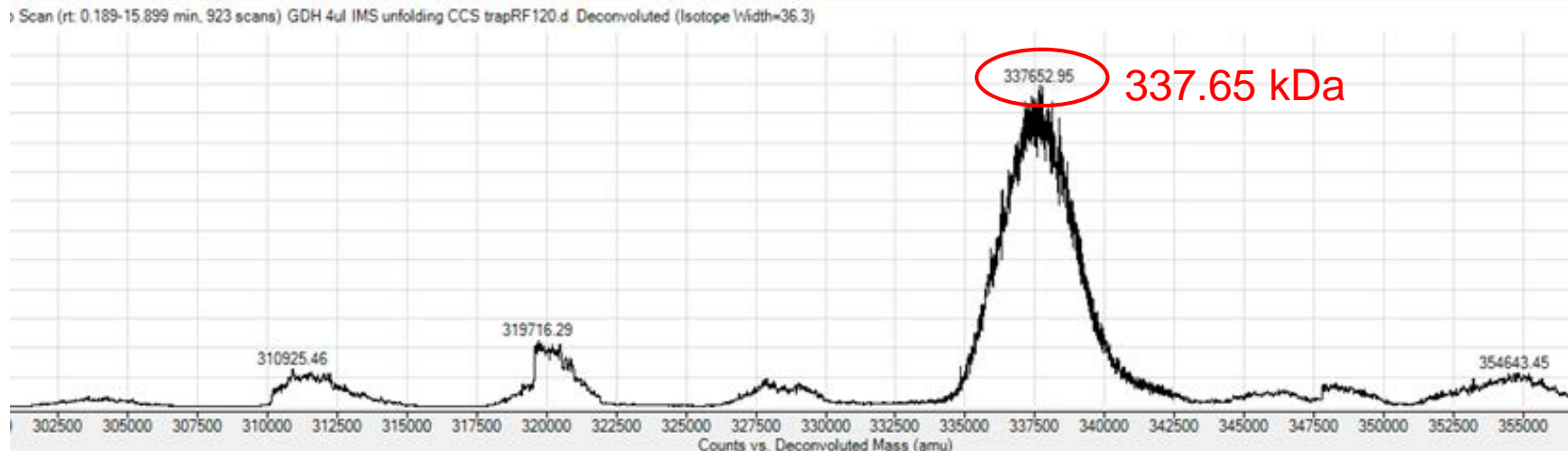
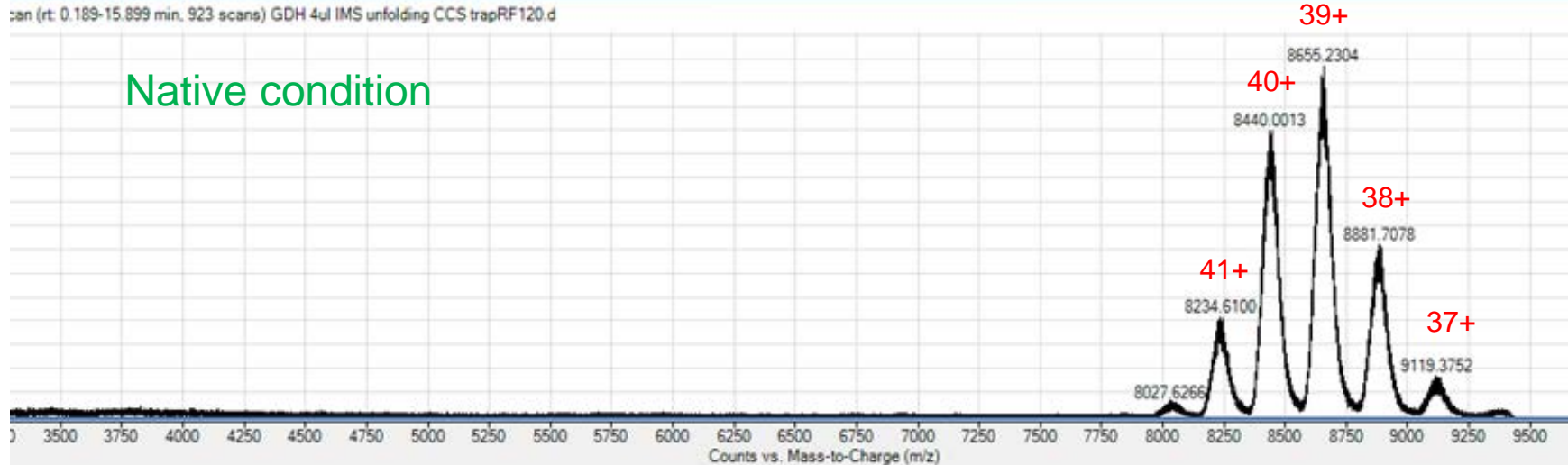
# Mass Spectrometric Comparison of Herceptin and ADC



The deconvoluted spectrum showed 8 major drug attachments and the calculated drug antibody ratio (DAR) was ~3.4



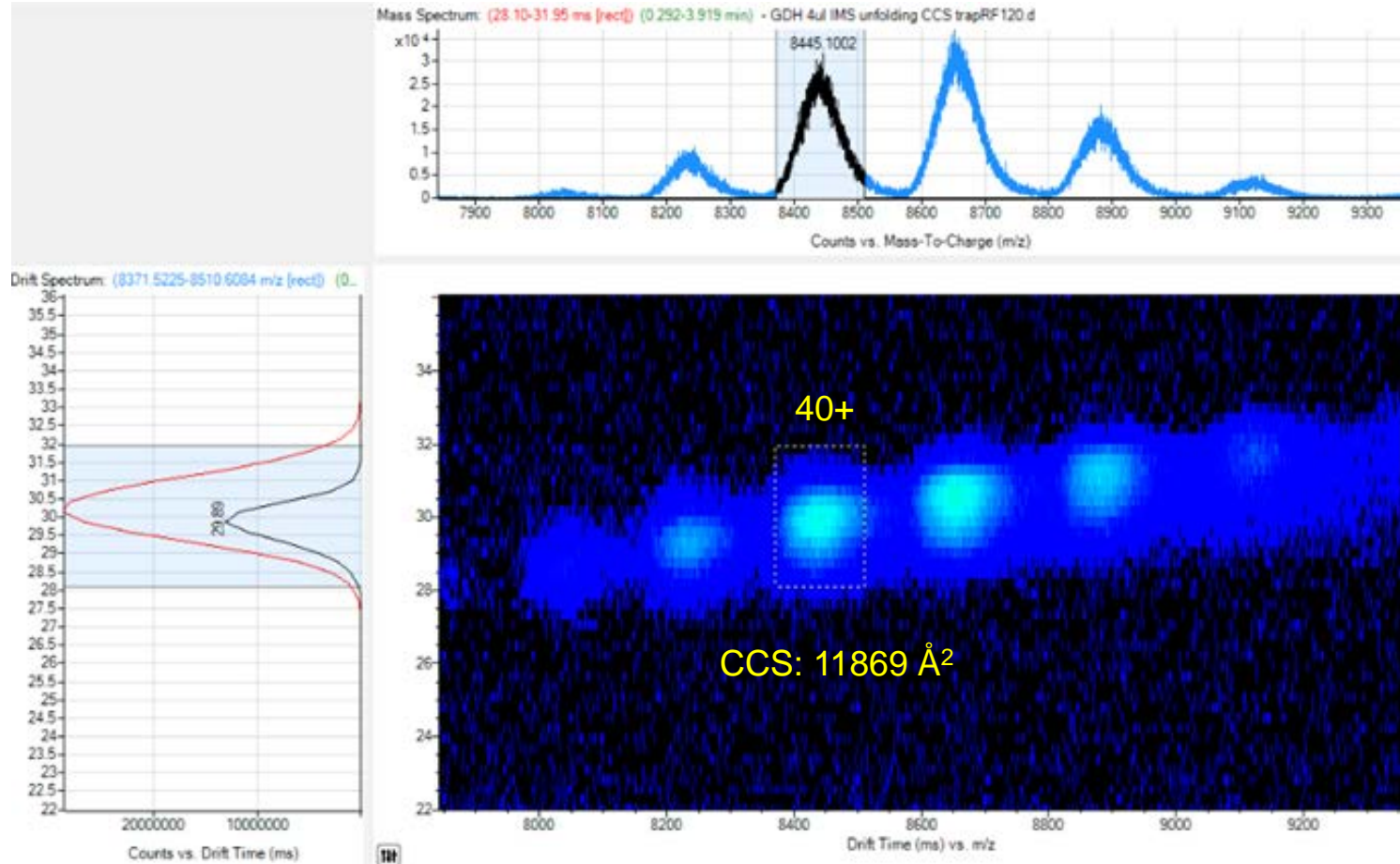
# Mass Spectrometric Analysis of Bovine Glutamate Dehydrogenase (GDH) Complex (Hexamer)



GDH is a hexamer of 500 residues with a molecular weight of ~56 kDa/each



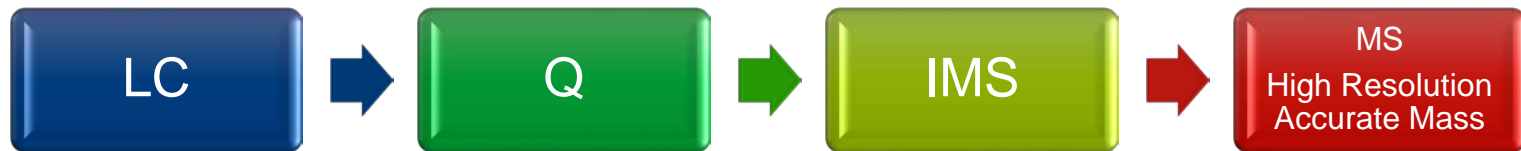
# IM Q-TOF/MS analysis of Bovine Glutamate Dehydrogenase (GDH) Complex (Hexamer)



# Ion Mobility Q-TOF Comparison



Feature	Drift Tube Ion Mobility (Agilent)	Travelling Wave Ion Mobility	Drift Mobility advantage
<b>Mobility Resolution</b>	Highest (can be > 80) 80cm drift tube (L) Higher voltage (E) No RF fields, Uniform low DC field	Generally around 30 10cm drift in TriWave, Multi-section device RF fields	Over 2X the IM resolution of T-wave
<b>Sensitivity</b>	High efficiency ion funnels - trapping and rear	Step wave lens Pressure barrier between Q and TriWave	10X to 50X better than T-wave
<b>Collision Cross Section (CCS) measurement (<math>\Omega</math>)</b>	Direct determination of $\Omega$ Low electric field and constant drift tube pressure	$\Omega$ cannot be directly determined from drift time. Need calibration tables.	1-2% precision  Much better than Synapt (5-10%)
<b>Molecular structures</b>	Lower RF fields, less ion heating.	Higher RF fields, tendency for higher fragmentation and ion heating	Lower RF allows preservation of molecular structures
<b>Duty cycle</b>	IM cycle time 10 to 100 ms is fully compatible with LC and MS duty cycles	Duty cycle 1 to 10 ms. No analytical benefit.	Drift IM is 10 to 50 more sensitive



# 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





# Dual AJS ESI Source Settings: 6560 IM Q-TOF MS

Parameter	Setting
Source	Dual Agilent Jet Stream
Acquisition Mode	Positive, Extended (10000 m/z) Mass Range (2 GHz)
Gas Temp	250 °C
Gas Flow	5 L/min
Nebulizer	20 psig
Sheath Gas Temp	275 °C
Sheath Gas Flow	12 L/min
VCap	4000 V
Nozzle Voltage	2000V
Fragmentor	400 V
Mass Range	300-10000 <i>m/z</i>
Scan Rate	0.9 frames/s
IM Trap Fill Time	50,000 us
IM Trap Release Time	300 us