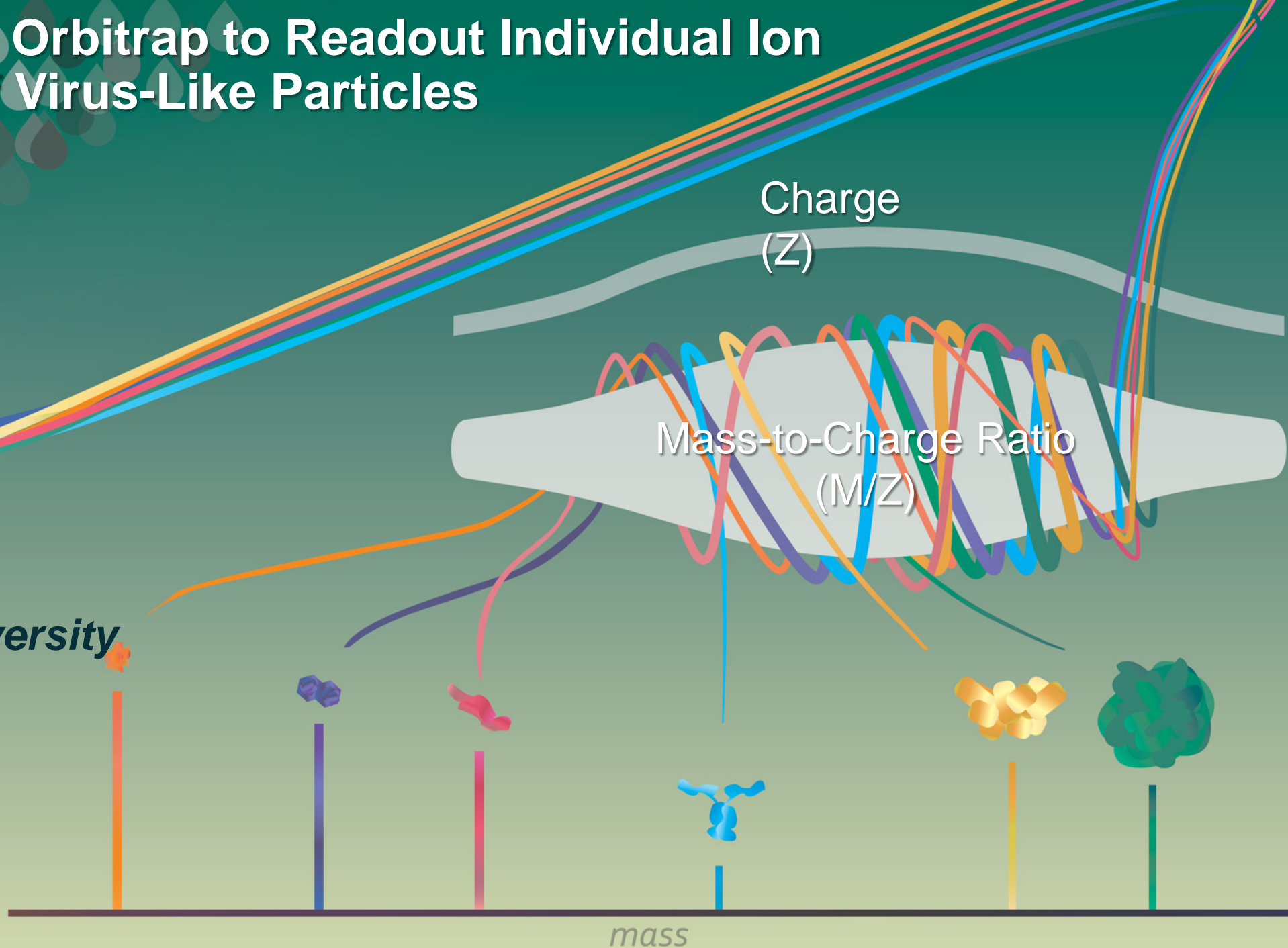


# Multiplexing the Orbitrap to Readout Individual Ion Mass Spectra of Virus-Like Particles

**Neil L. Kelleher**  
**Northwestern University**

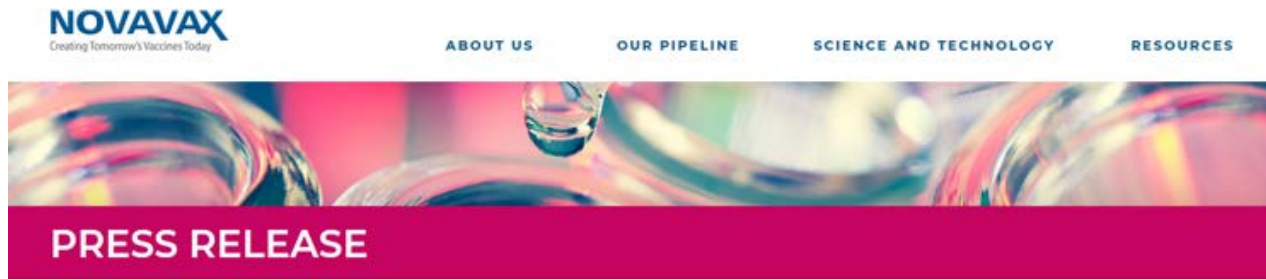
CASSS  
Mass Spec 2020  
September 17, 2020



# Executive Summary

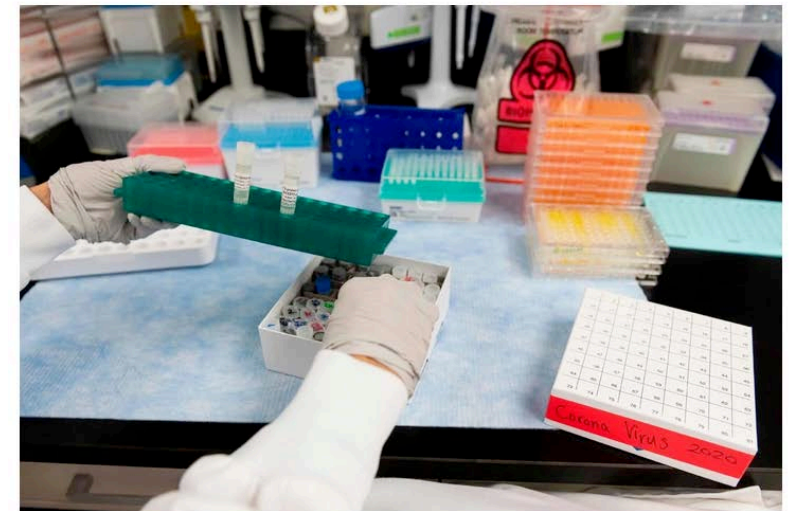
- Supporting COVID-19 Vaccine Development
- Individual Ion MS (I<sup>2</sup>MS or “i2MS”)
- Antibodies – Super Resolution
- Deconvoluting Protein Complexity
  - Protein Complexes
  - Virus-like Particles
- A New Sample Stream for Intacts and Complexes

# Novavax: Major Player in COVID-19 Vaccine Development



## *How a Struggling Company Won \$1.6 Billion to Make a Coronavirus Vaccine*

Novavax just received the Trump administration's largest vaccine contract. In the Maryland company's 33-year history, it has never brought a vaccine to market.



The coronavirus vaccine Novavax, a small biotech company, has developed is now in safety trials. Results are expected this month. Andrew Caballero-Reynolds/Agence France-Presse — Getty Images

By Katie Thomas and Megan Twohey

July 16, 2020 Updated 12:22 p.m. ET



[View printer-friendly version](#)

[<< Back](#)

### Novavax to Receive up to \$388 Million Funding from CEPI for COVID-19 Vaccine Development and Manufacturing

- Funds clinical development of NVX-CoV2373 through Phase 2
- Supports rapid scale-up of vaccine manufacturing
- Allows for increased production of Matrix-M adjuvant
- Reserves global large-scale manufacturing capacity

#### BIOTECH AND PHARMA

## Novavax soars after U.S. government awards firm \$1.6 billion for coronavirus vaccine development

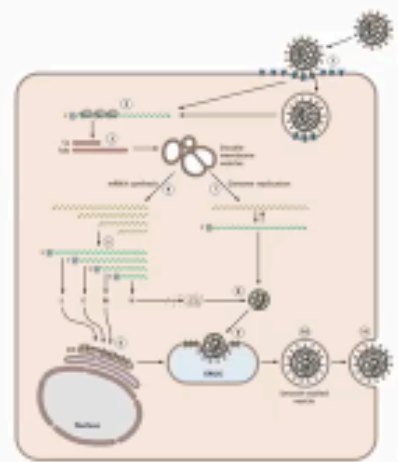
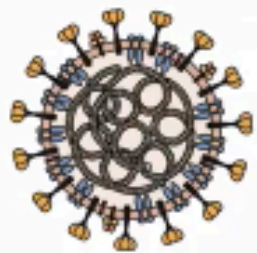
PUBLISHED TUE, JUL 7 2020 6:12 AM EDT | UPDATED TUE, JUL 7 2020 6:28 AM EDT

REUTERS

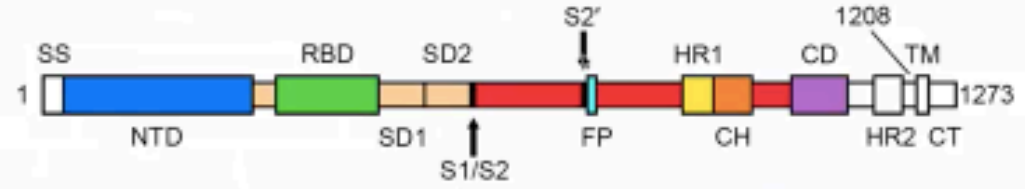
SHARE [f](#) [t](#) [in](#) [✉](#)

# Spike Protein

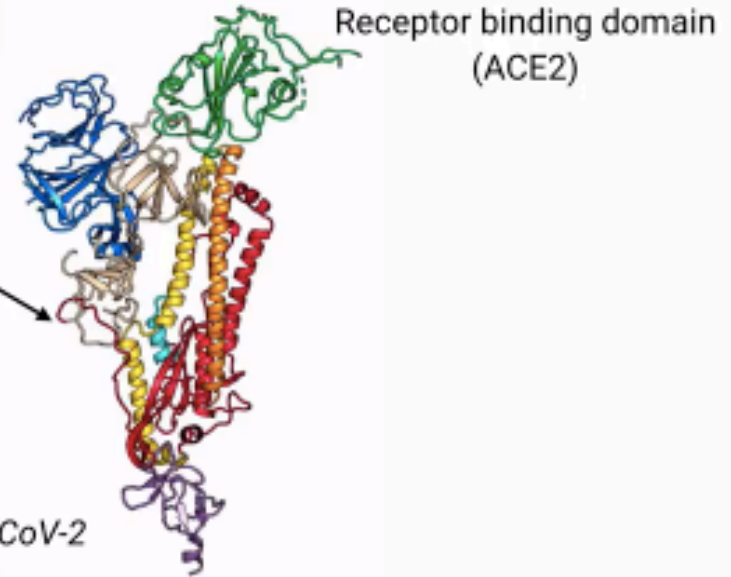
## S cleavage and zoonotic potential of SARS-CoV-2



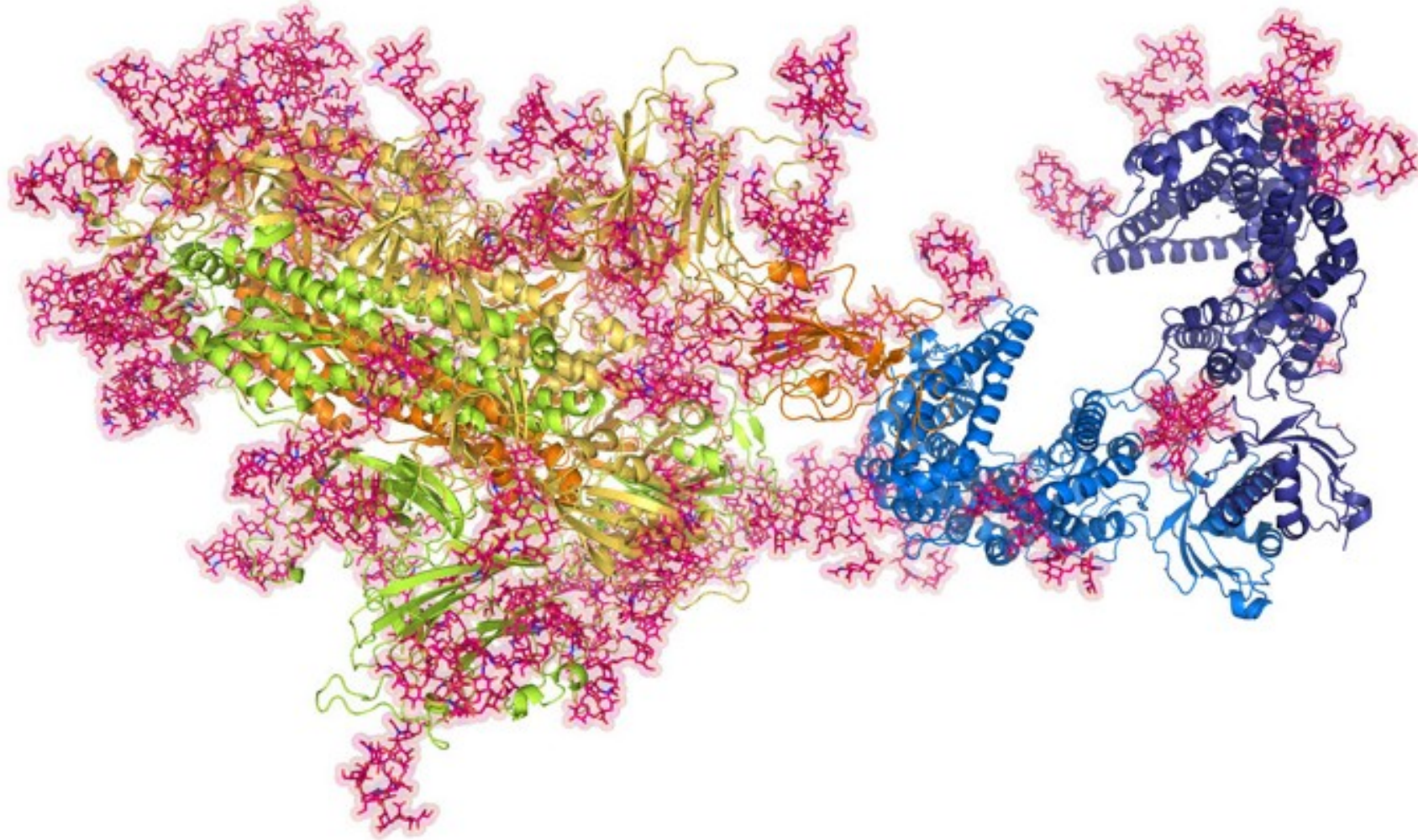
*S* cleaved by endosomal cathepsins, TMPRSS for SARS-CoV, SARS-CoV-2

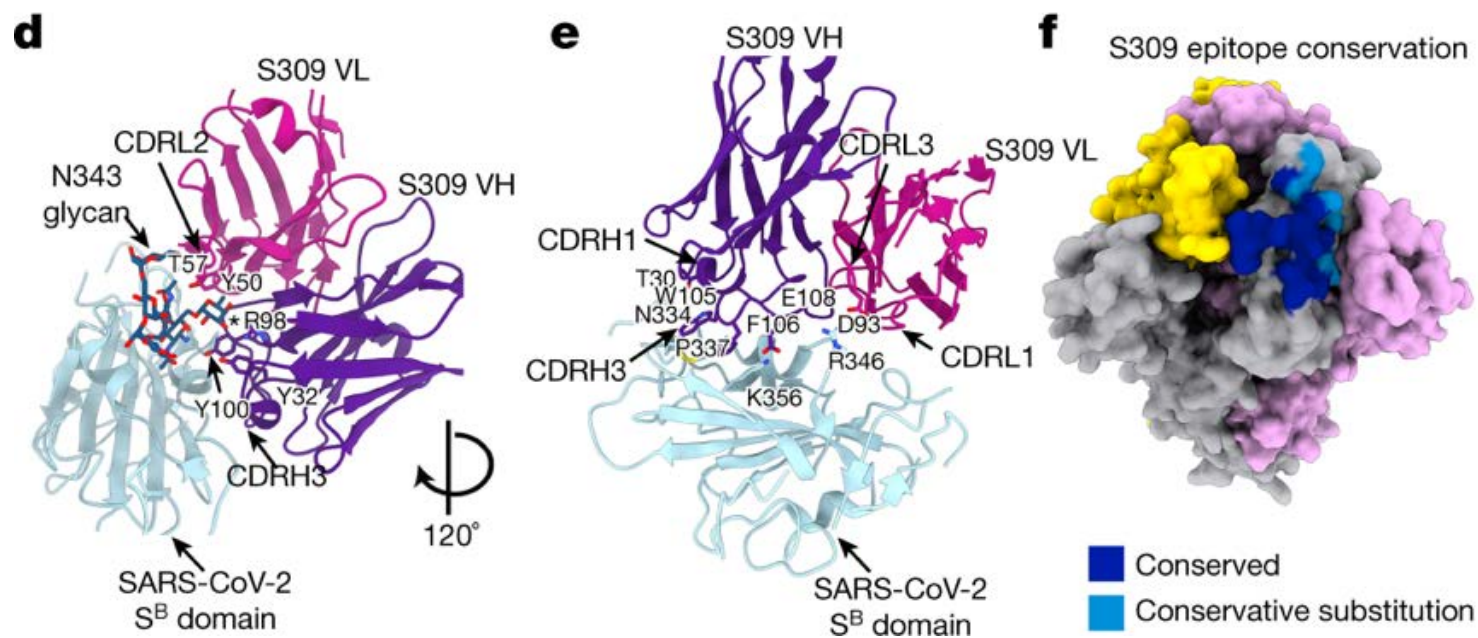
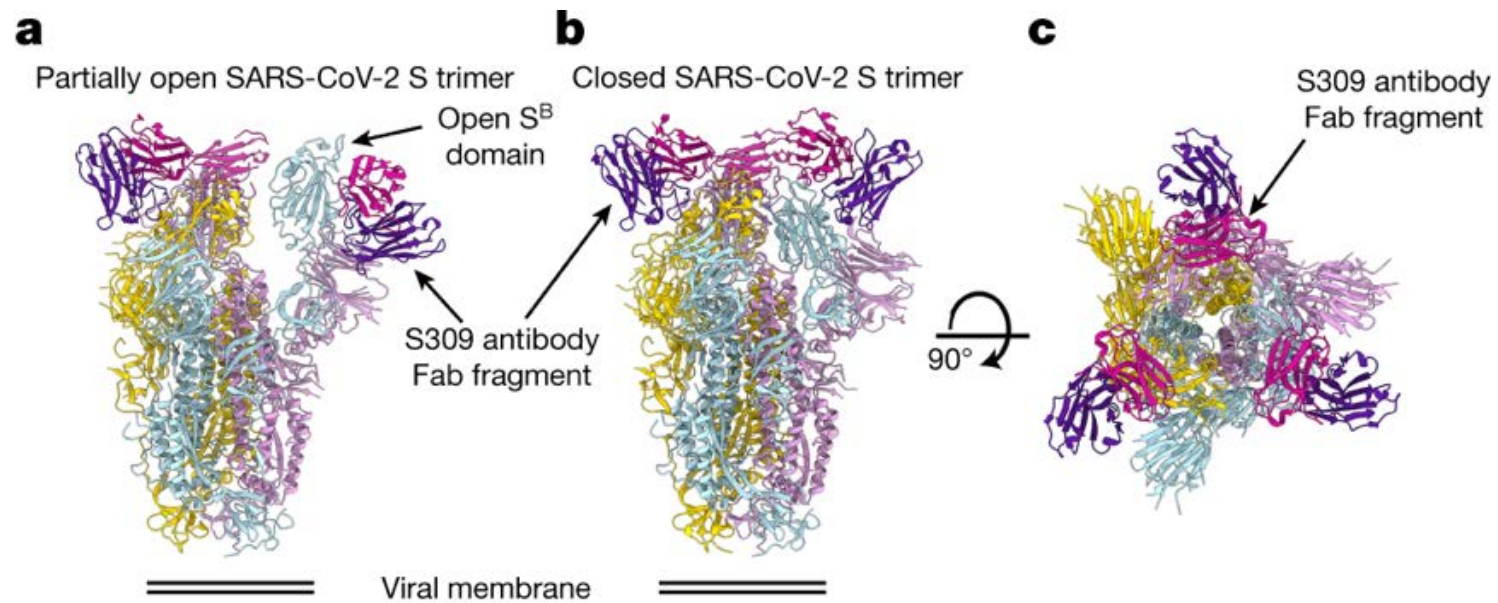


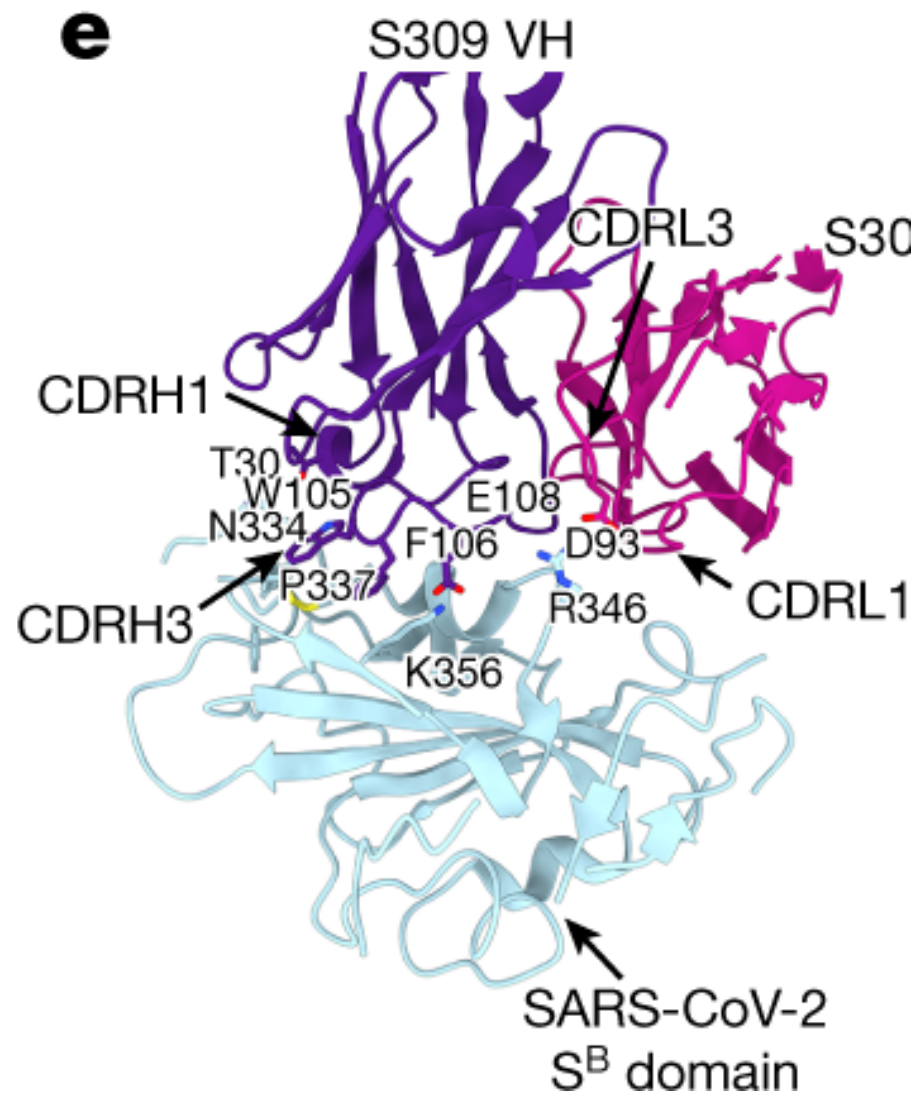
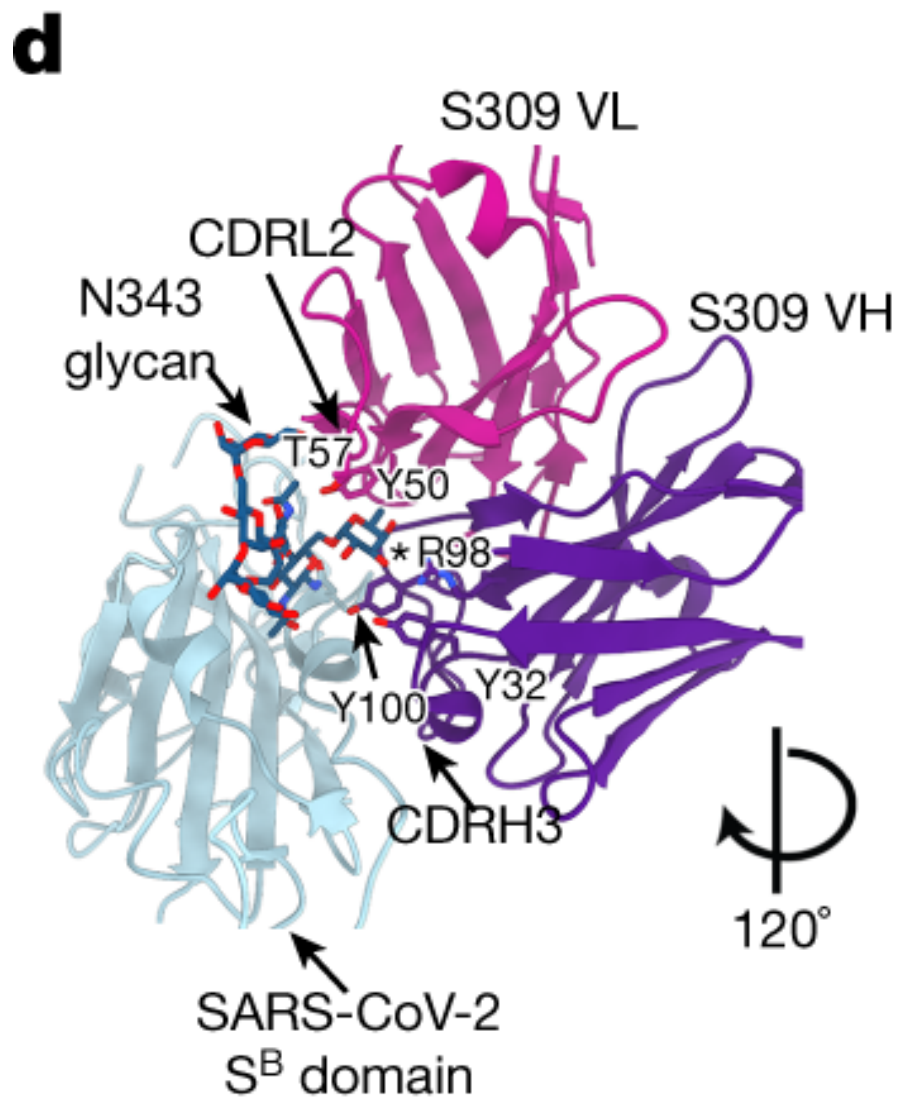
Furin cleavage site  
Not present in RaTG13,  
a bat SARS-like CoV  
isolated in 2013, the  
closest known isolate  
to SARS-CoV-2



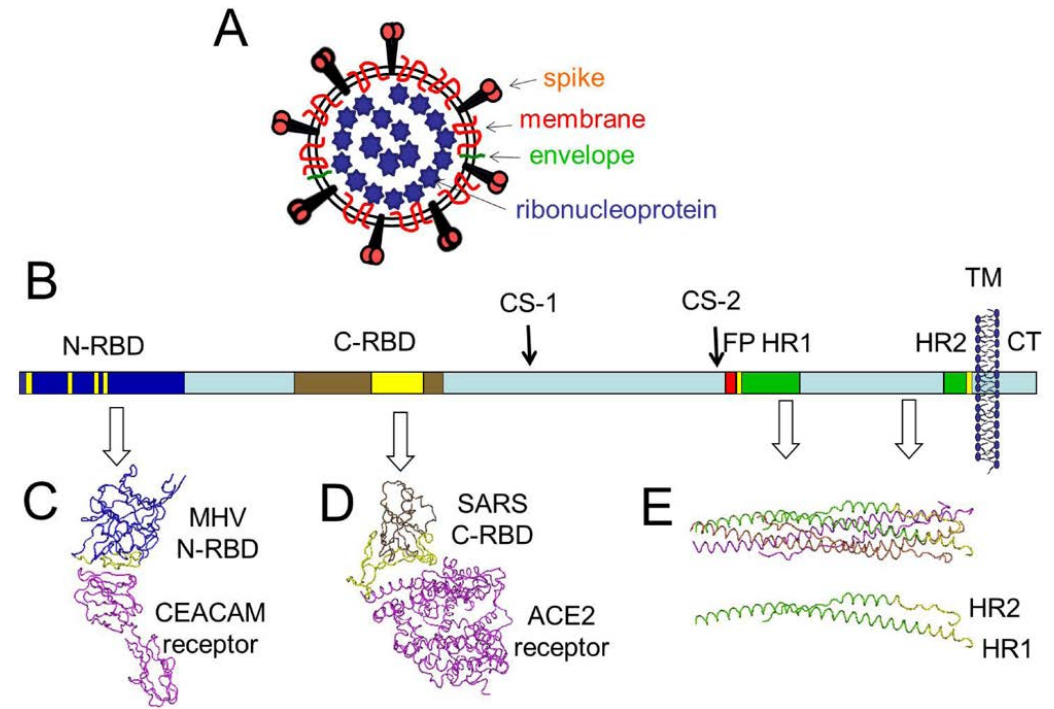
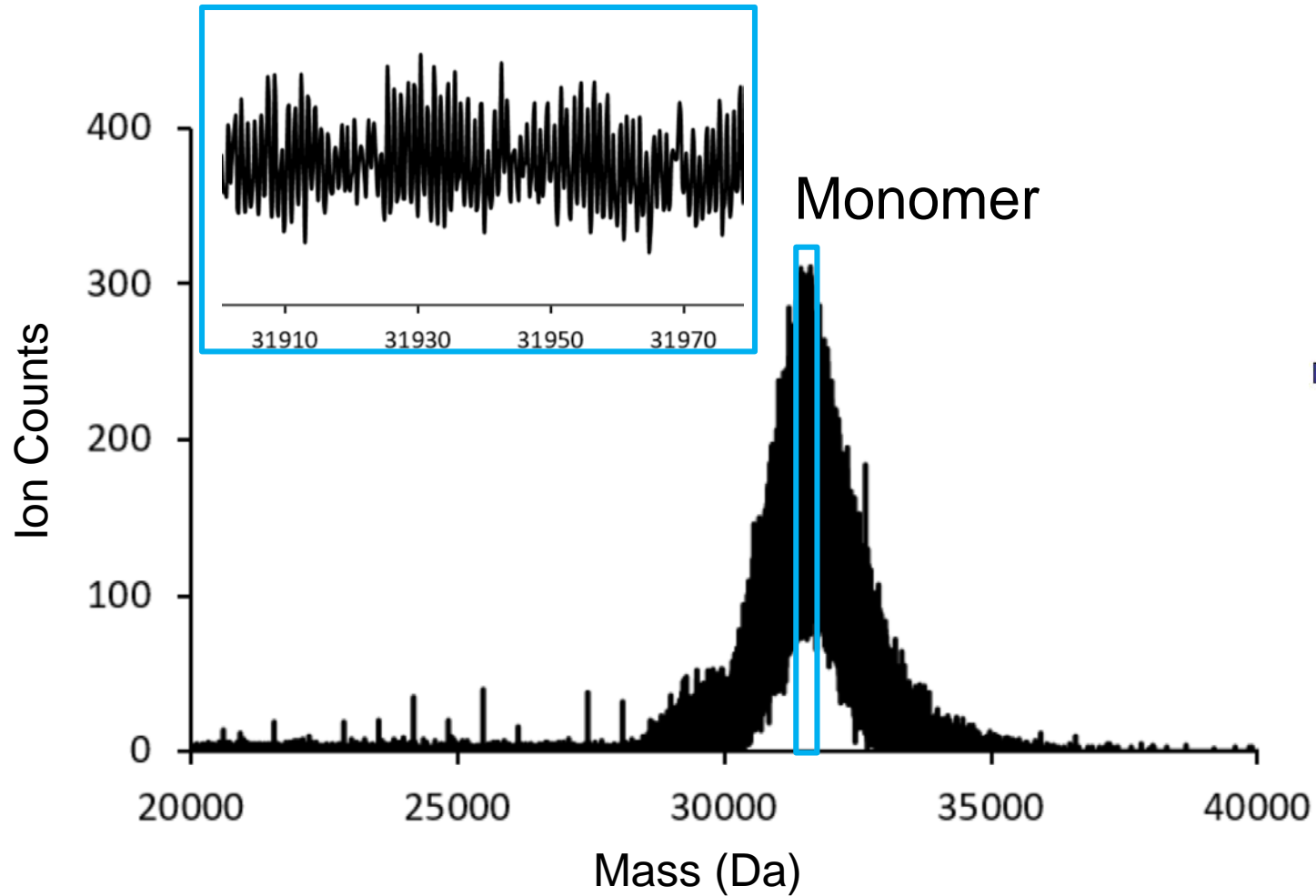
# Model of spike protein & the human ACE2 receptor (blue) and complex glycans (magenta)







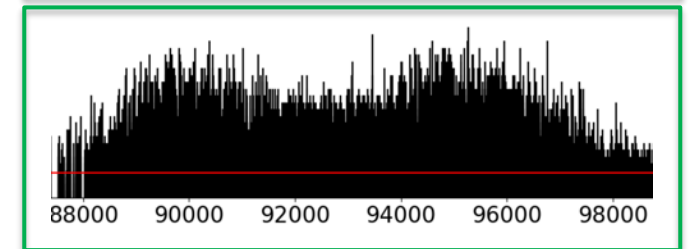
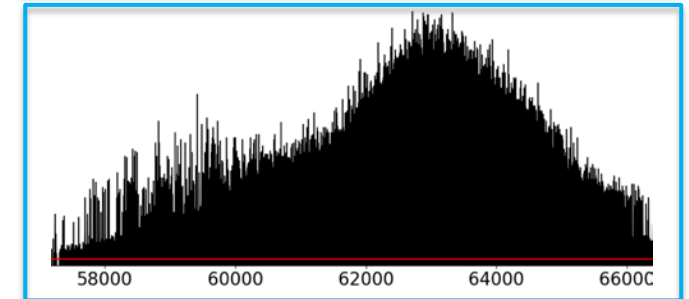
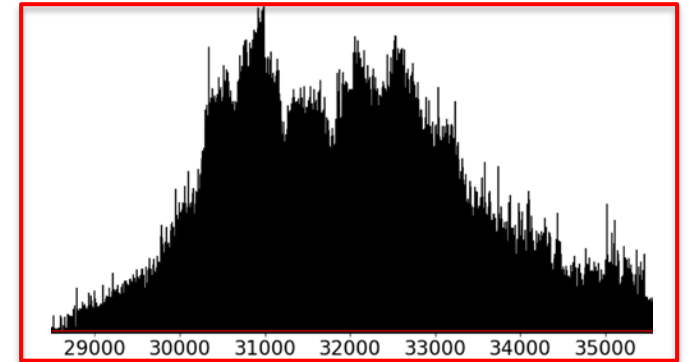
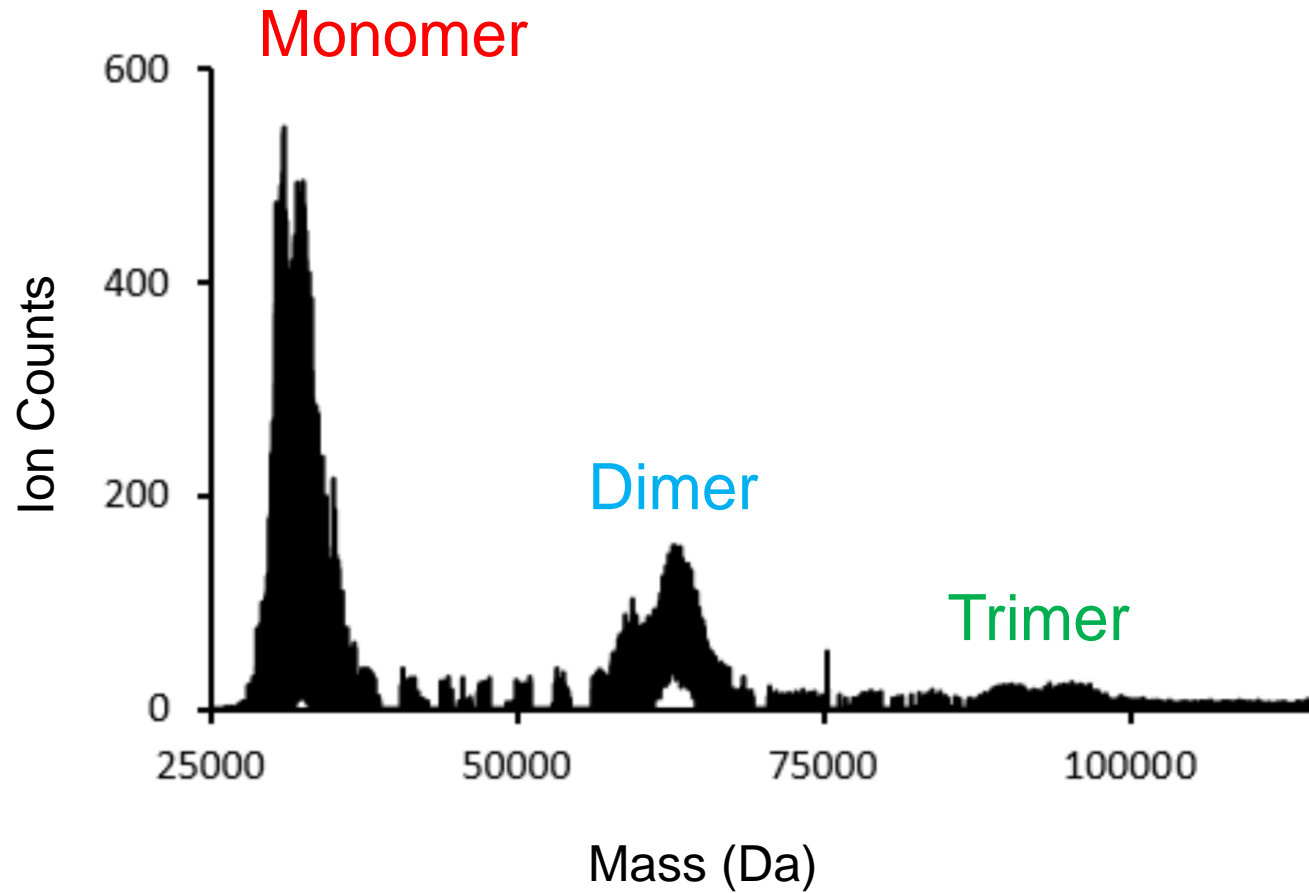
# Spike RBD Denatured



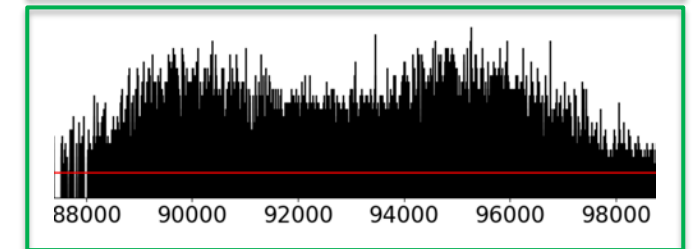
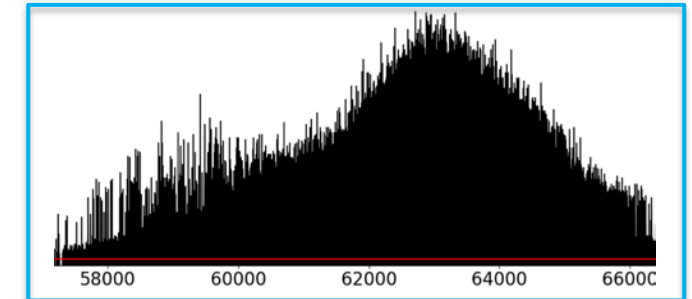
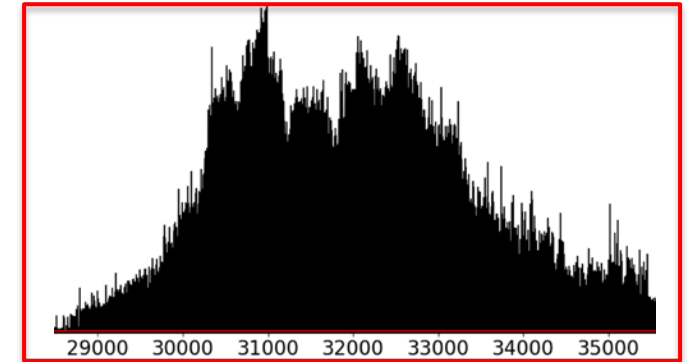
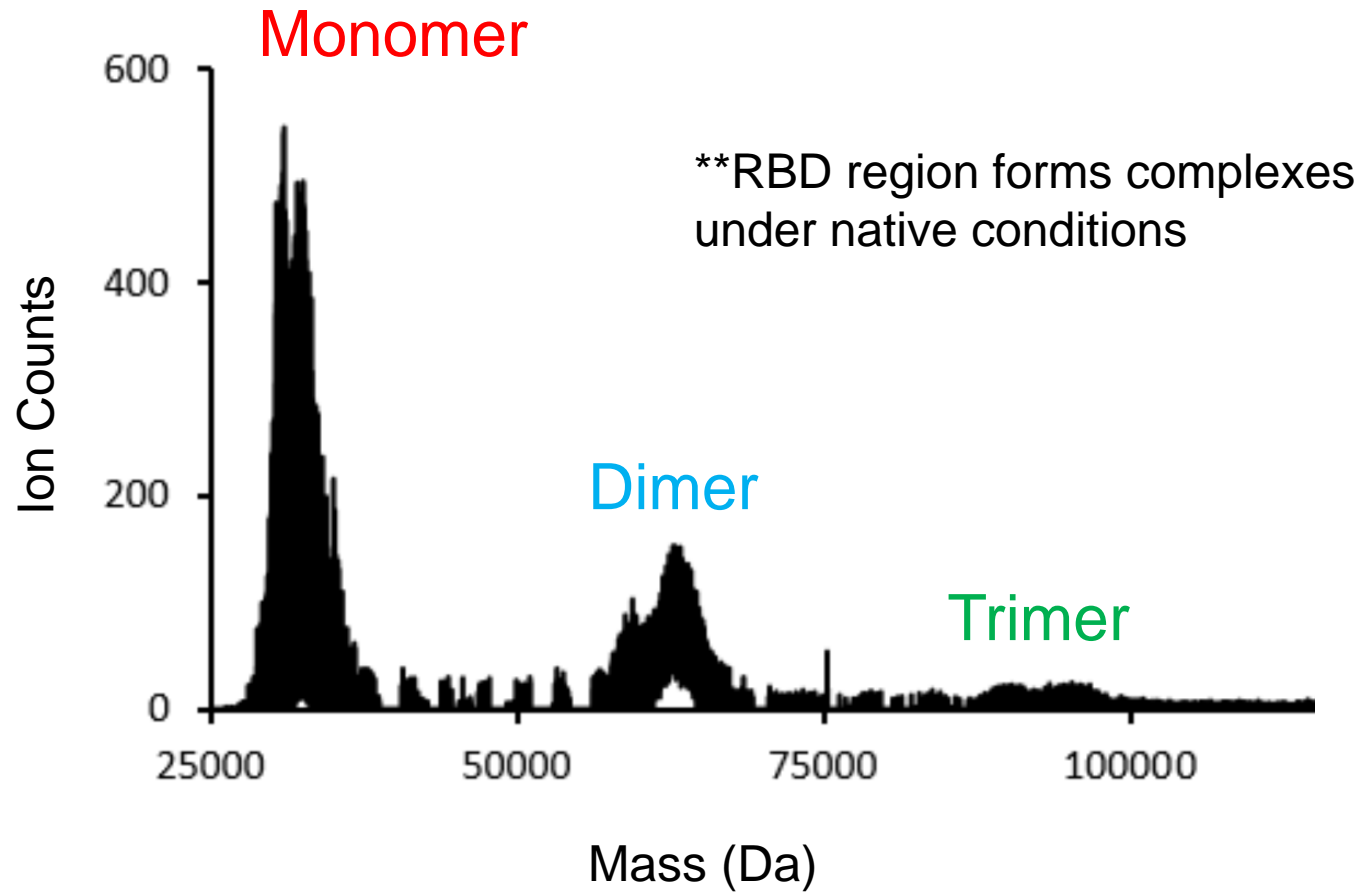
Viral complexity is astounding!!



# Spike RBD Native



# Spike RBD Native



Mass (Da)

# 4 Recent Publication on Individual Ion MS (I<sup>2</sup>MS)

**analytical chemistry** Article  
Cite This: *Anal. Chem.* 2019, 91, 2776–2783 pubs.acs.org/ac

## Measurement of Individual Ions Sharply Increases the Resolution of Orbitrap Mass Spectra of Proteins

Jared O. Kafader,<sup>†</sup> Rafael D. Melani,<sup>†</sup> Michael W. Senko,<sup>‡</sup> Alexander A. Makarov,<sup>§</sup> Neil L. Kelleher,<sup>†</sup> and Philip D. Compton<sup>\*,†</sup>

**nature methods** BRIEF COMMUNICATION  
<https://doi.org/10.1038/s41592-020-0764-5> Check for updates

## Multiplexed mass spectrometry of individual ions improves measurement of proteoforms and their complexes

Jared O. Kafader<sup>1</sup>, Rafael D. Melani<sup>1</sup>, Kenneth R. Durbin<sup>1</sup>, Bon Ikwuagwu<sup>2</sup>, Bryan P. Early<sup>1</sup>, Ryan T. Fellers<sup>1</sup>, Steven C. Beu<sup>3</sup>, Vlad Zabrouskov<sup>4</sup>, Alexander A. Makarov<sup>5</sup>, Joshua T. Maze<sup>6</sup>, Deven L. Shinholt<sup>6</sup>, Ping F. Yip<sup>4</sup>, Danielle Tullman-Ercek<sup>2</sup>, Michael W. Senko<sup>4</sup>, Philip D. Compton<sup>1</sup> and Neil L. Kelleher<sup>1</sup>

**ASMS** © American Society for Mass Spectrometry, 2019 Check for updates  
J. Am. Soc. Mass Spectrom. (2019) 30:2200–2203  
DOI: 10.1007/s13361-019-02309-0

SHORT COMMUNICATION

## STORI Plots Enable Accurate Tracking of Individual Ion Signals

Jared O. Kafader,<sup>1</sup> Steven C. Beu,<sup>2</sup> Bryan P. Early,<sup>1</sup> Rafael D. Melani,<sup>1</sup> Kenneth R. Durbin,<sup>1</sup> Vlad Zabrouskov,<sup>3</sup> Alexander A. Makarov,<sup>4</sup> Joshua T. Maze,<sup>5</sup> Deven L. Shinholt,<sup>5</sup> Ping F. Yip,<sup>3</sup> Neil L. Kelleher,<sup>1</sup> Philip D. Compton,<sup>1</sup> Michael W. Senko<sup>3</sup>

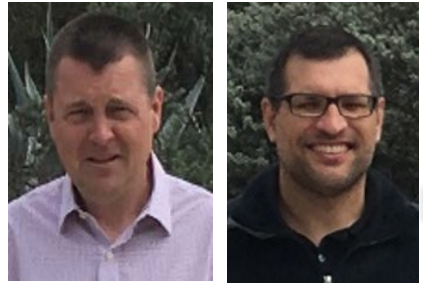
**Journal of proteome research** Letter  
pubs.acs.org/jpr

## Individual Ion Mass Spectrometry Enhances the Sensitivity and Sequence Coverage of Top-Down Mass Spectrometry

Jared O. Kafader, Kenneth R. Durbin, Rafael D. Melani, Benjamin J. Des Soye, Luis F. Schachner, Michael W. Senko, Philip D. Compton, and Neil L. Kelleher\*

Cite This: <https://dx.doi.org/10.1021/acs.jproteome.9b00797> Read Online

# Orbitrap-based Individual Ion Mass Spectrometry



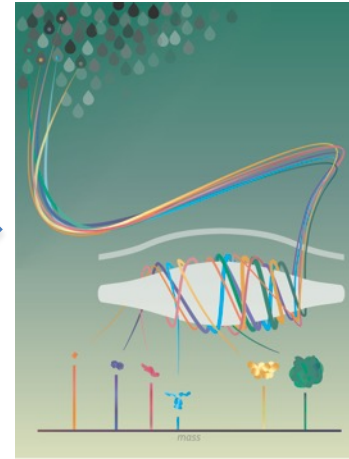
Initiation

Oct 2017



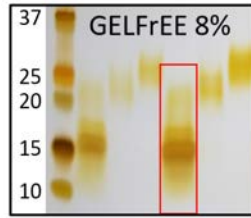
Development

2017-2018



Applications

2019



kDa



**ThermoFisher**  
SCIENTIFIC

**Northwestern**  
Proteomics



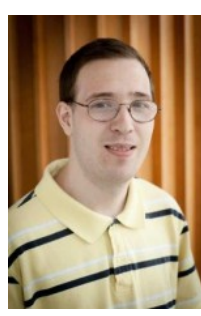
Jared Kafader



Rafael Melani



Ken Durbin



Bryan Early



Ryan Fellers



Ping Yip



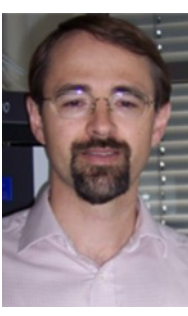
Steven Beu



Deven Shinholt



Joshua Maze

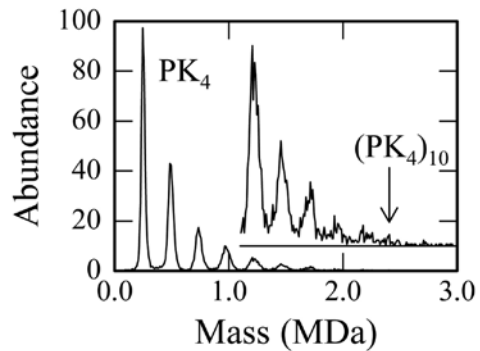
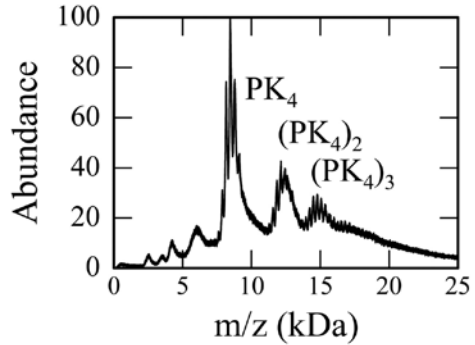


Alexander Makarov

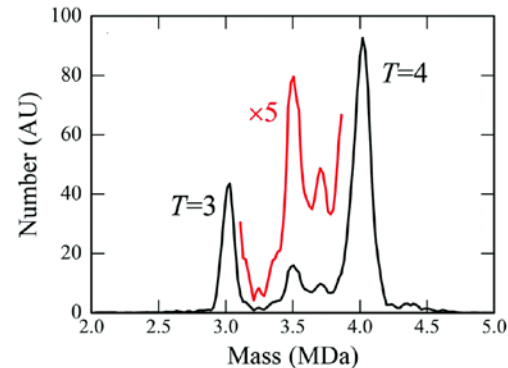
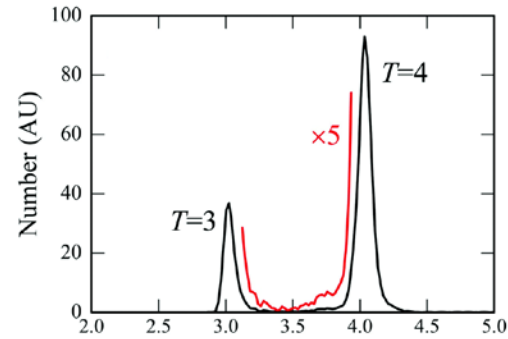
Ion Charge Assignment and True Mass Determination

# Charge Detection Mass Spectrometry

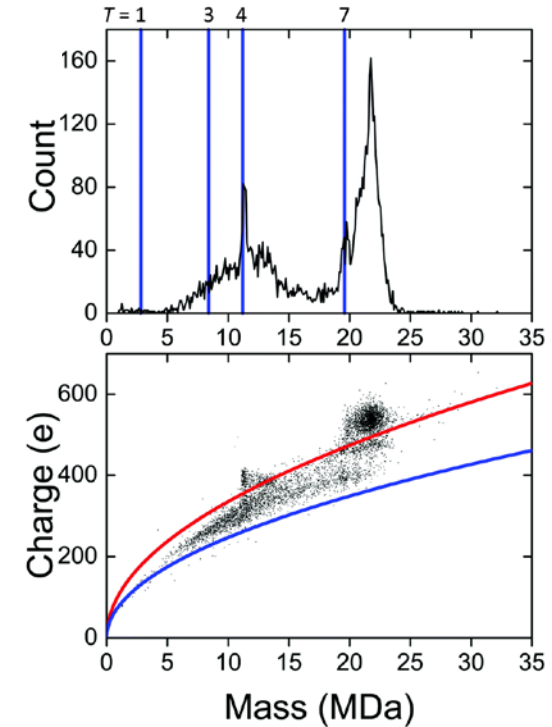
## Assemblies



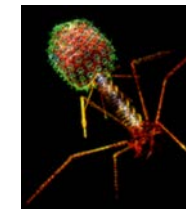
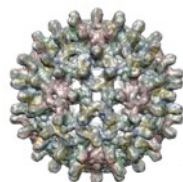
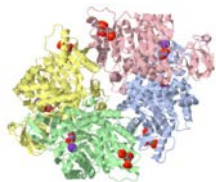
## Viruses



## Bacteriophages

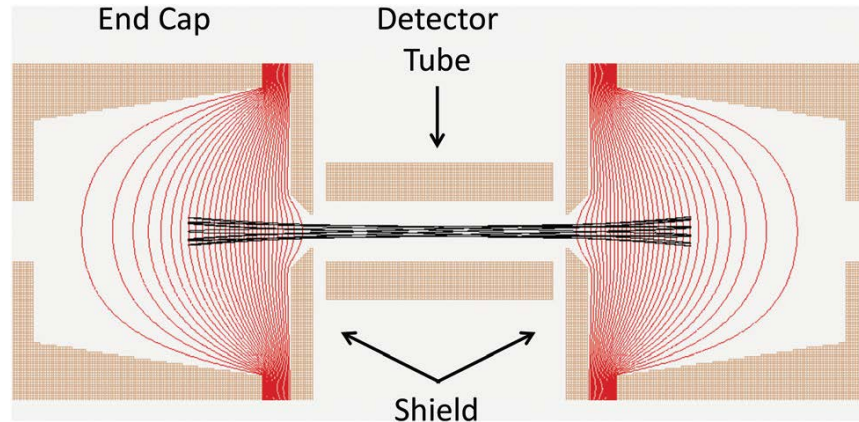


Increasing Molecular Weight



# Charge Detection Mass Spectrometry

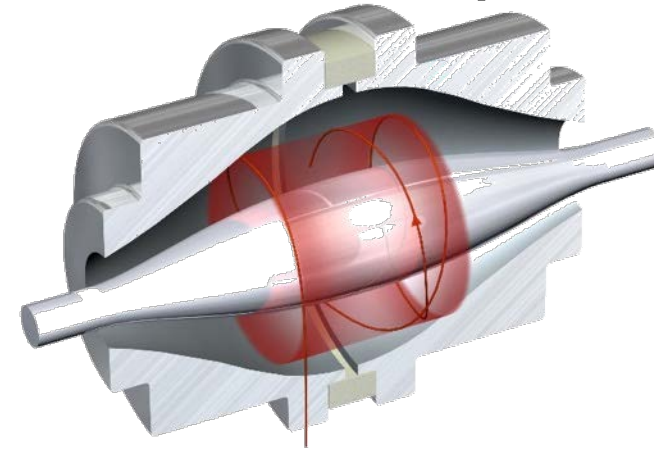
## Linear Ion Trap



### Pros

- High Resolution (z)**
- Low Capacitance
- Double Measurement Frequency
- Efficient Signal Pickup
- Ion trajectory Independent

## Orbitrap

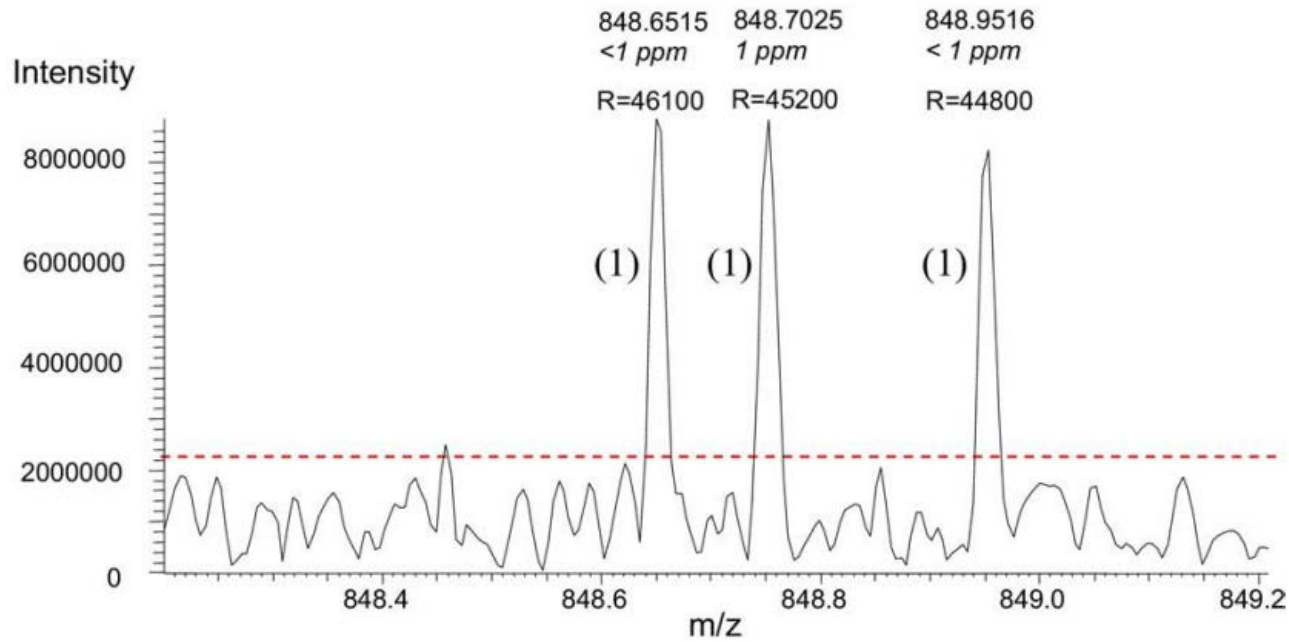


### Pros

- High Availability**
- High Resolution (m/z)**
- High Mass Accuracy**
- Harmonic Potential
- Precise Kinetic Energies
- 100x Ion Collection**
- Space Charge Tolerance
- High Vacuum
- Optimized Desolvation

# Orbitrap Single Ion Measurements

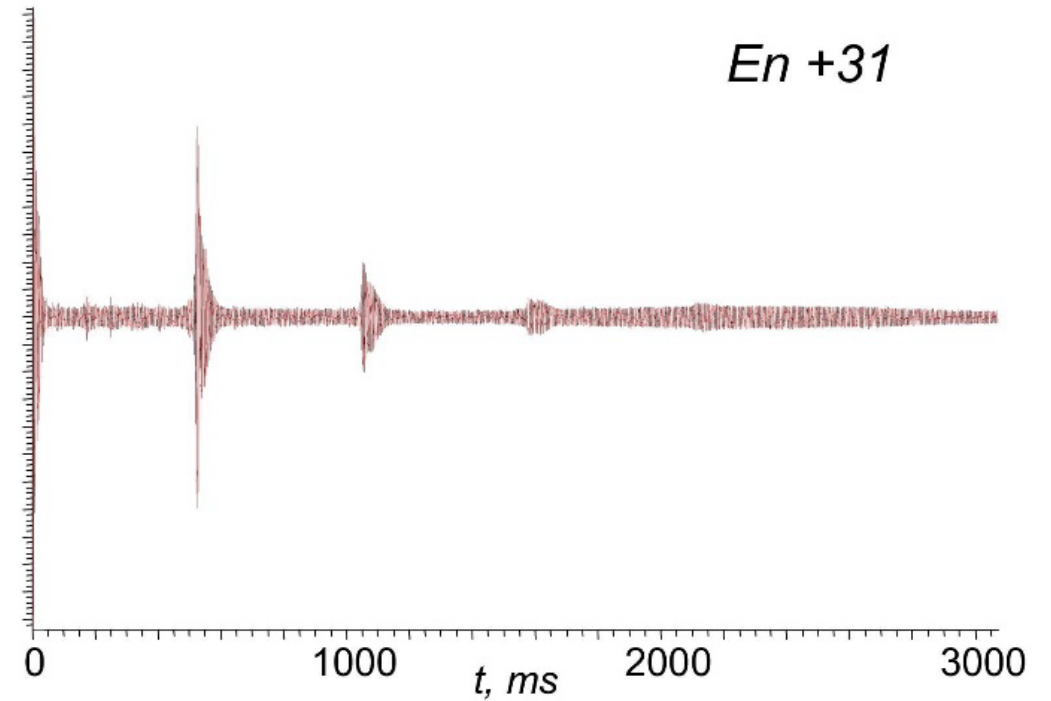
Orbitraps are sensitive enough to measure single ion events



Increase transient length

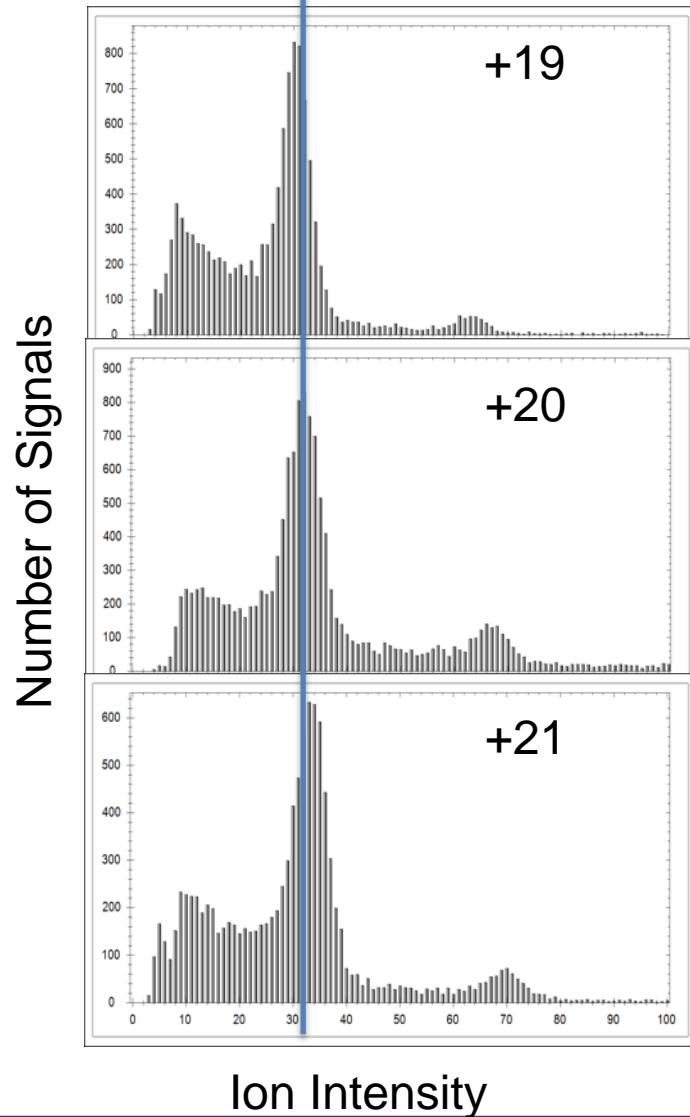


Increase Resolution



# Charge Detection

Using Only Intensity



## Problems

Decayed ions

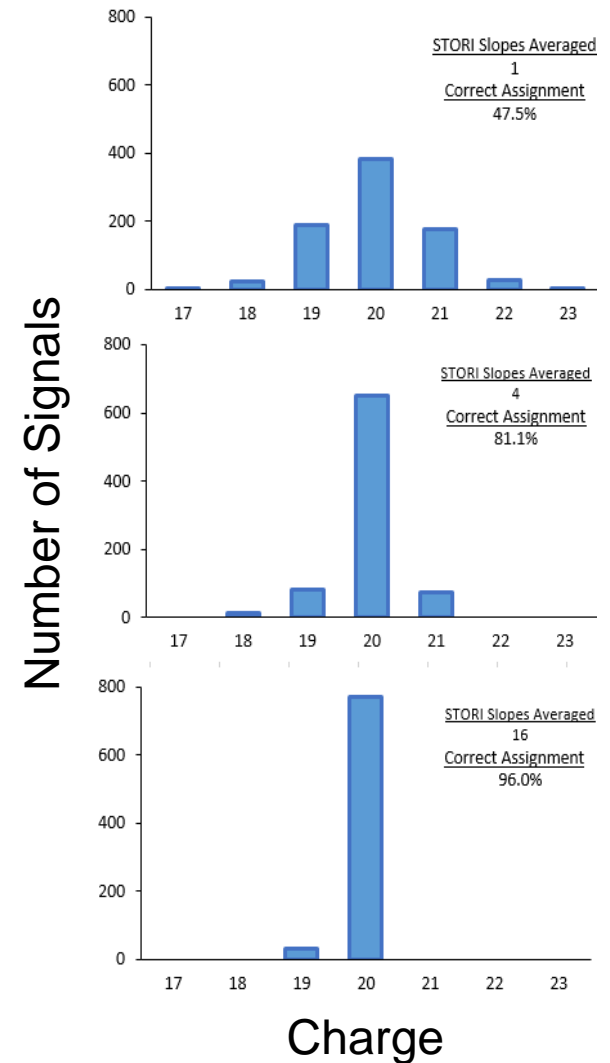
Large proteins

Not Filterable



**Solution:** Integration of ion signal over time (STORI plots)

Using Summation of Ion Signal Over Time





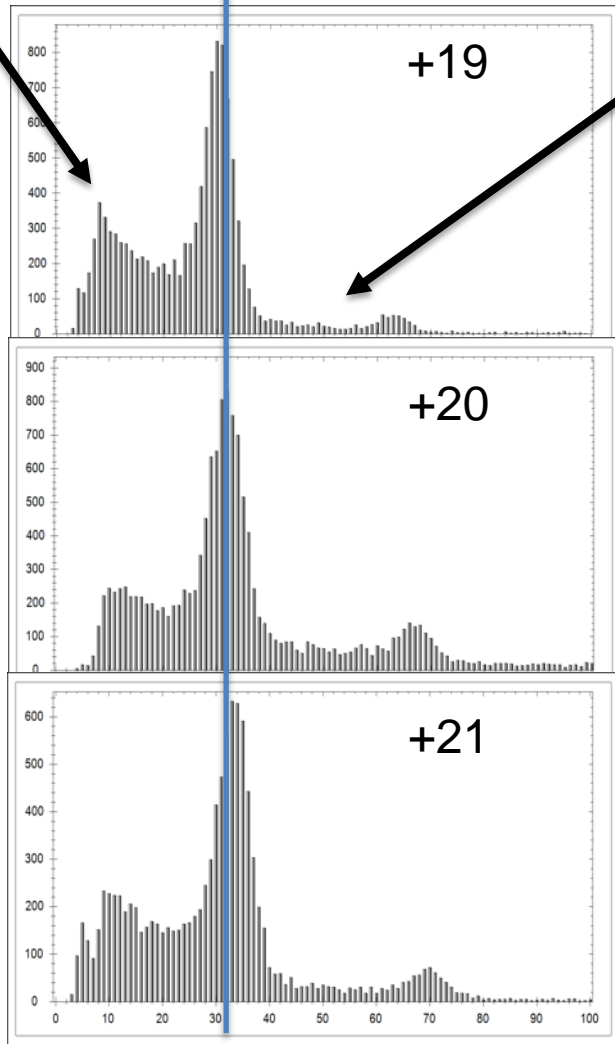
# Charge Detection

Ions Died During Detection

Using Only Intensity

Multiple Ions

Number of Signals



Ion Intensity

## Problems

Decayed ions

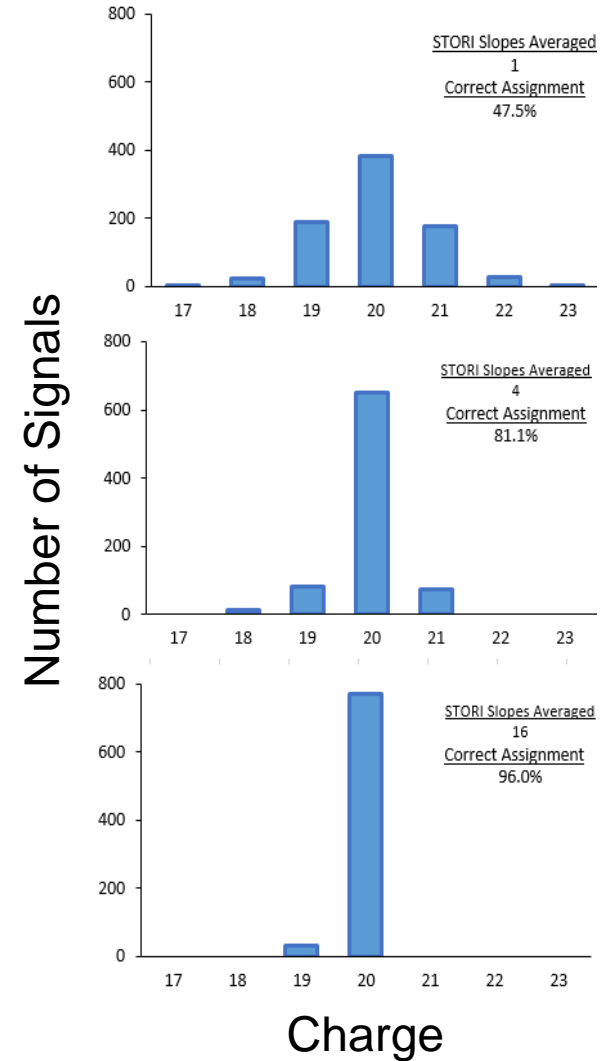
Large proteins

Not Filterable

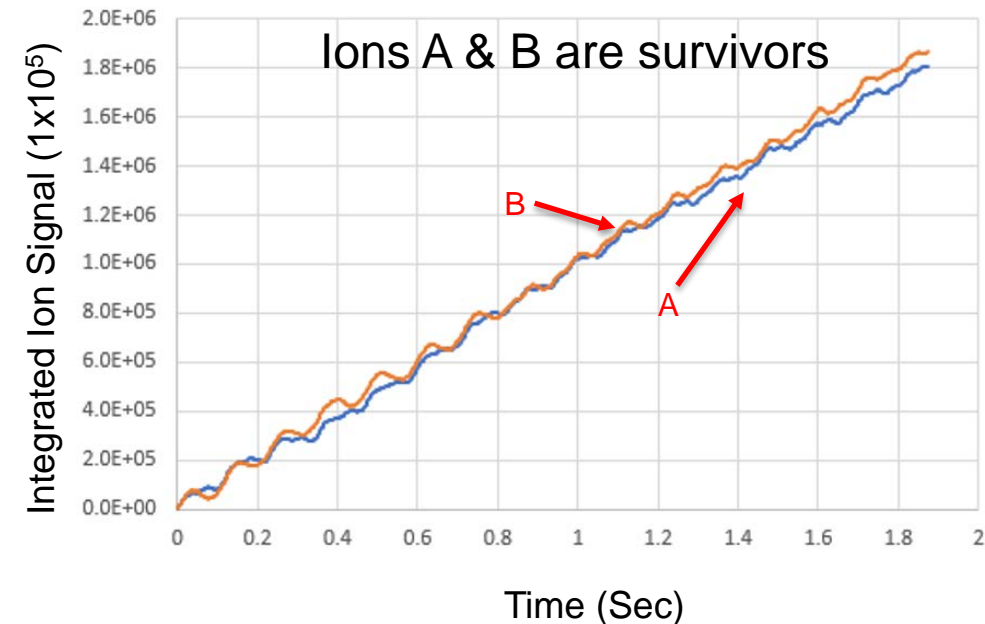
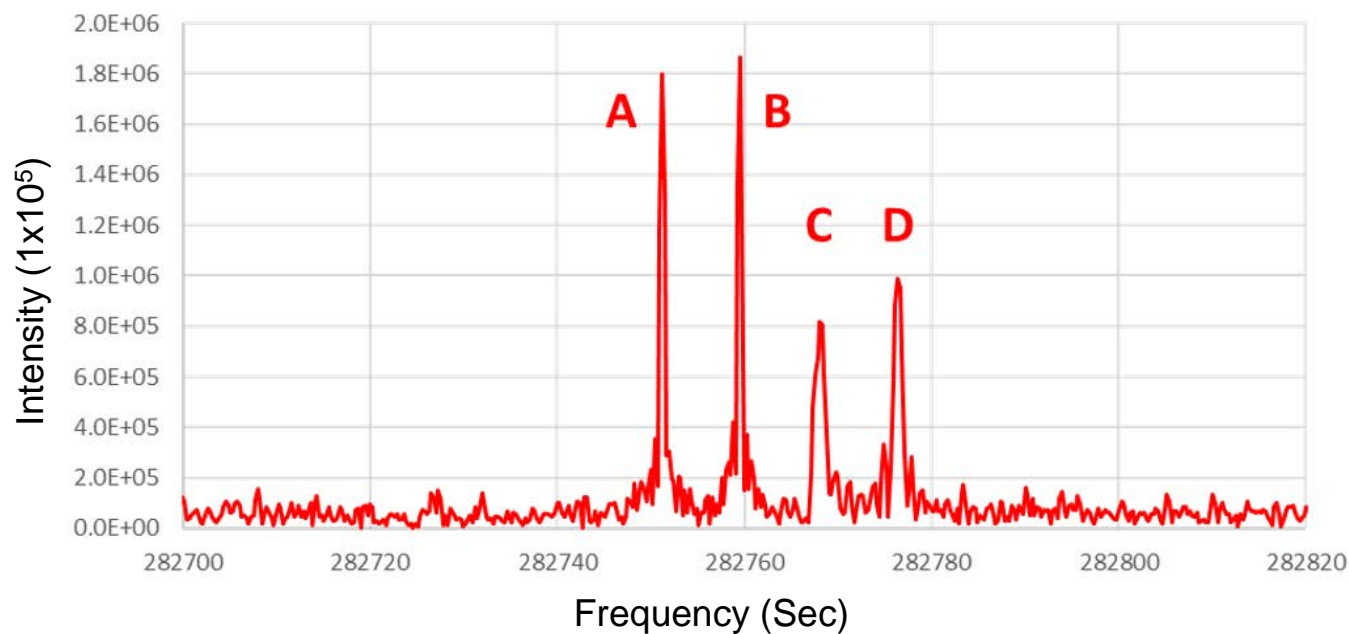


**Solution:** Integration of ion signal over time (STORI plots)

Using Summation of Ion Signal Over Time



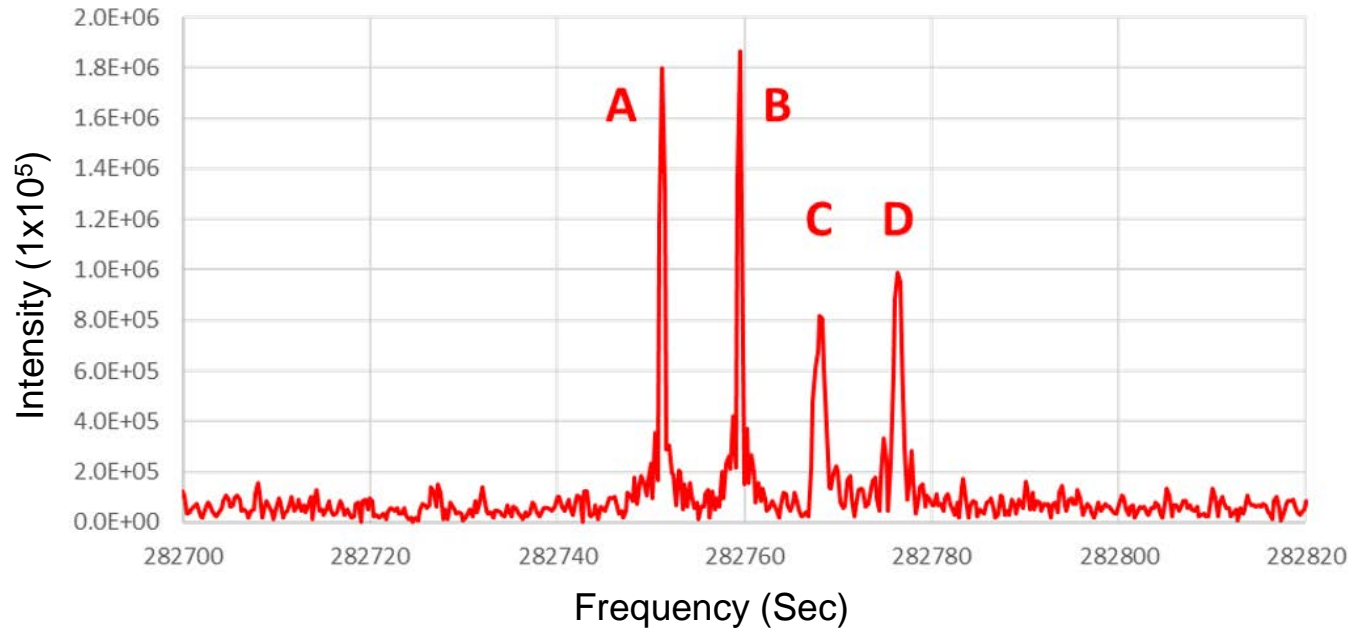
# Charge Detection



## **STORI analysis**

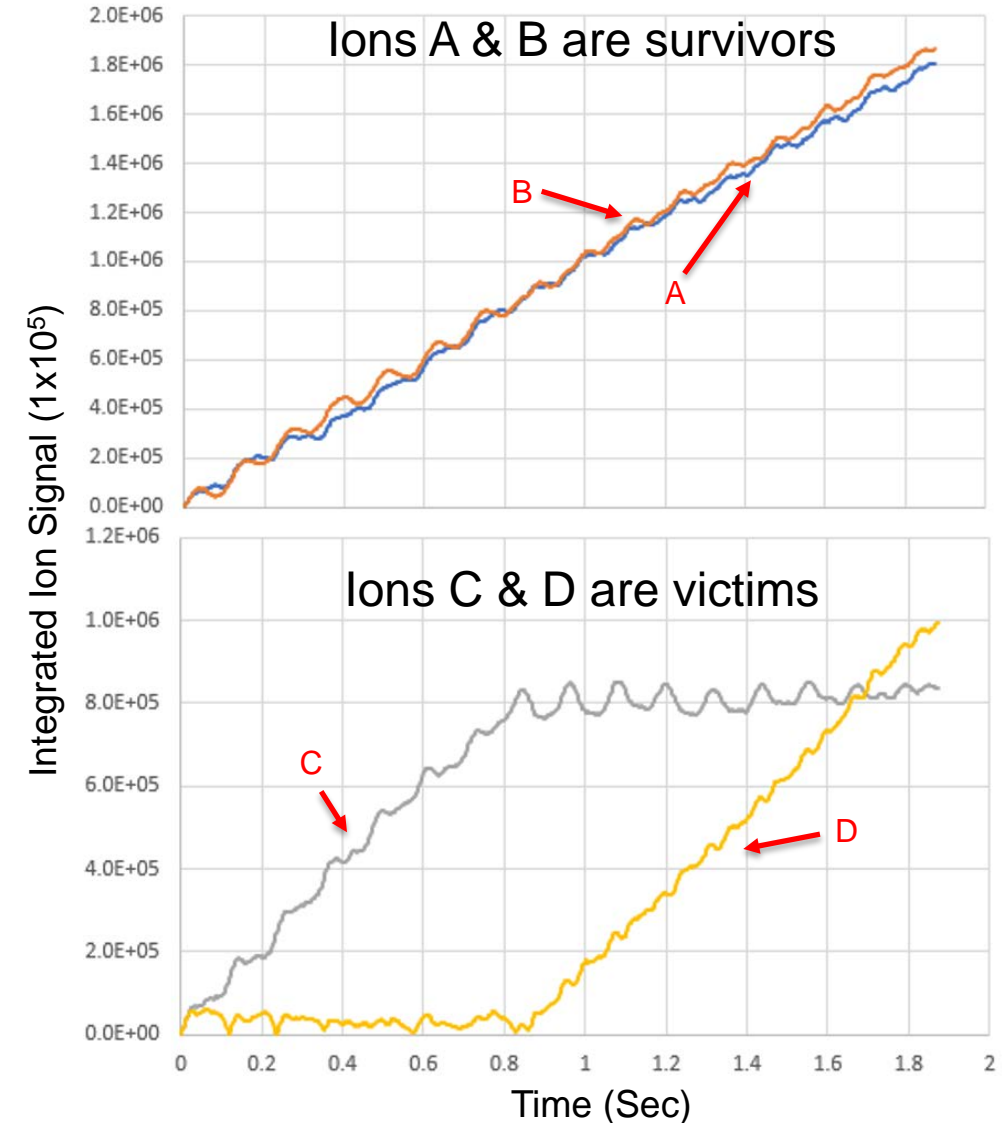
- 1) Correctly assigns slopes to +20 charge state
- 2) Higher precision
- 3) Filterable Results

# Charge Detection

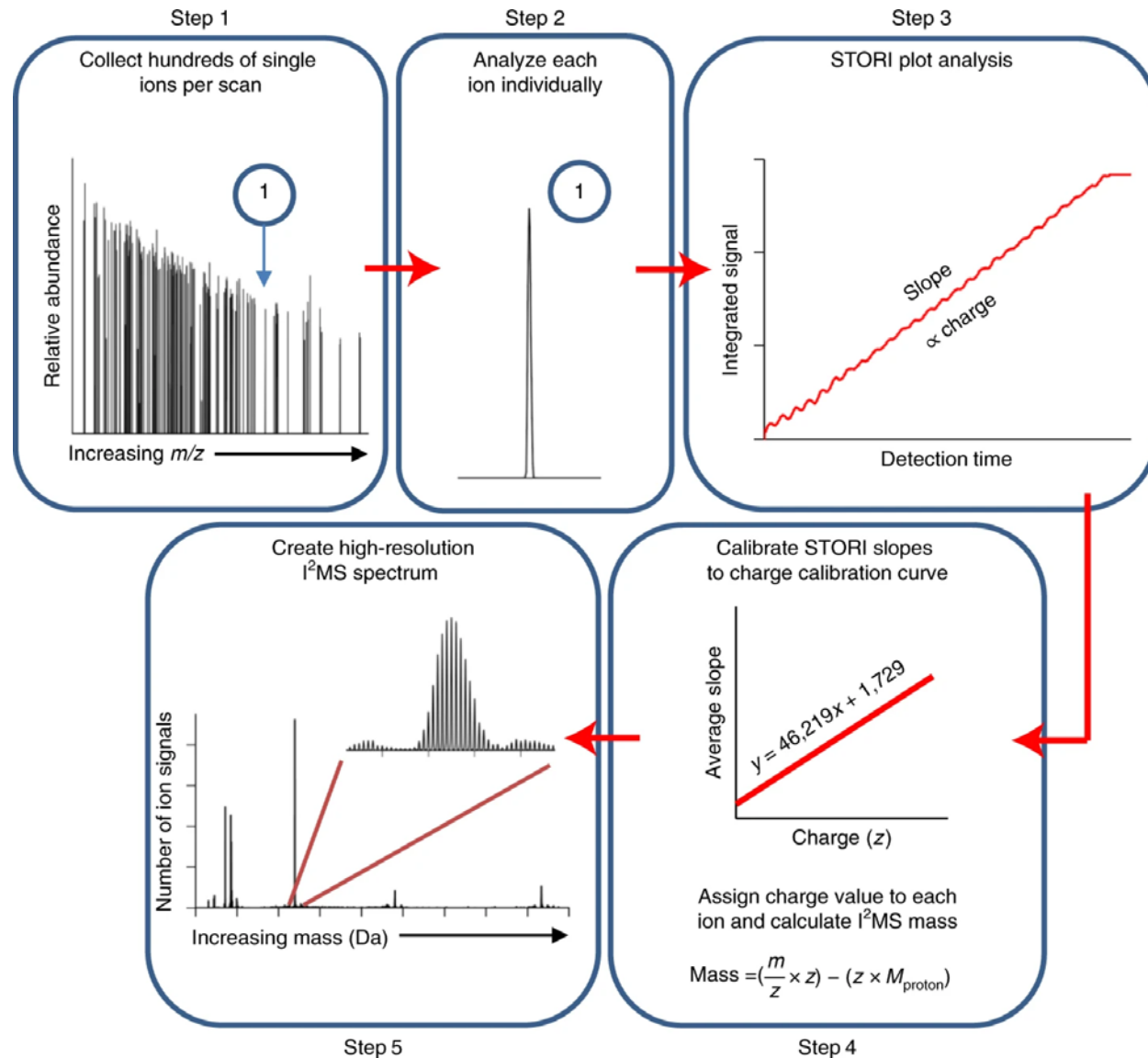


## STORI analysis

- 1) Correctly assigns slopes to +20 charge state
- 2) Higher precision
- 3) Filterable Results
- 4) Rescues decayed signals
- 5) Identifies events



# Individual Ion Workflow



Analyze individual ion signals to determine:

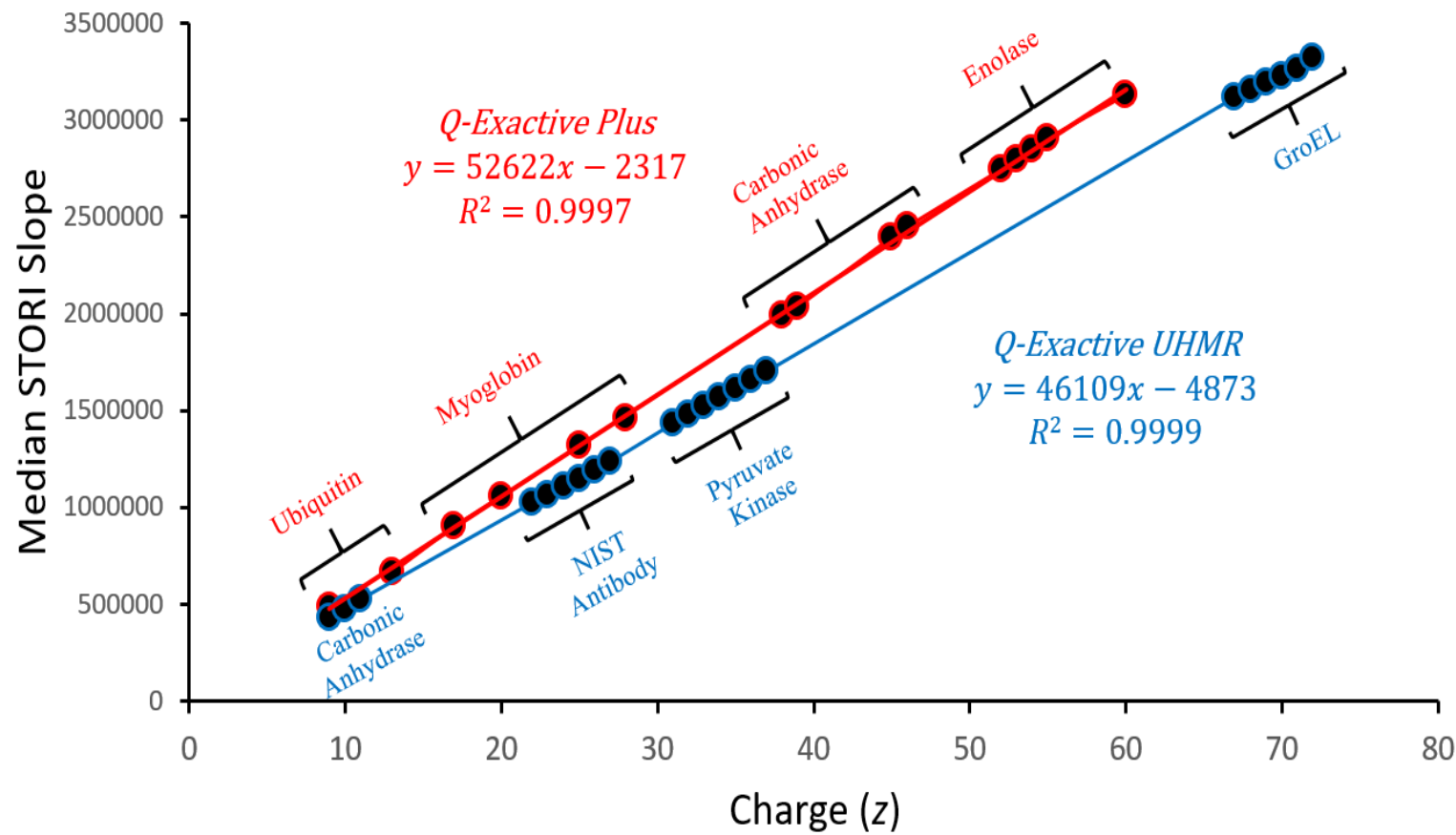
Mass-to-charge ratio (m/z)  
Normal FT-MS analysis

Charge value (z)  
Newly developed STORI analysis



Generate true MASS spectra  
1) Mass determination for large native complexes

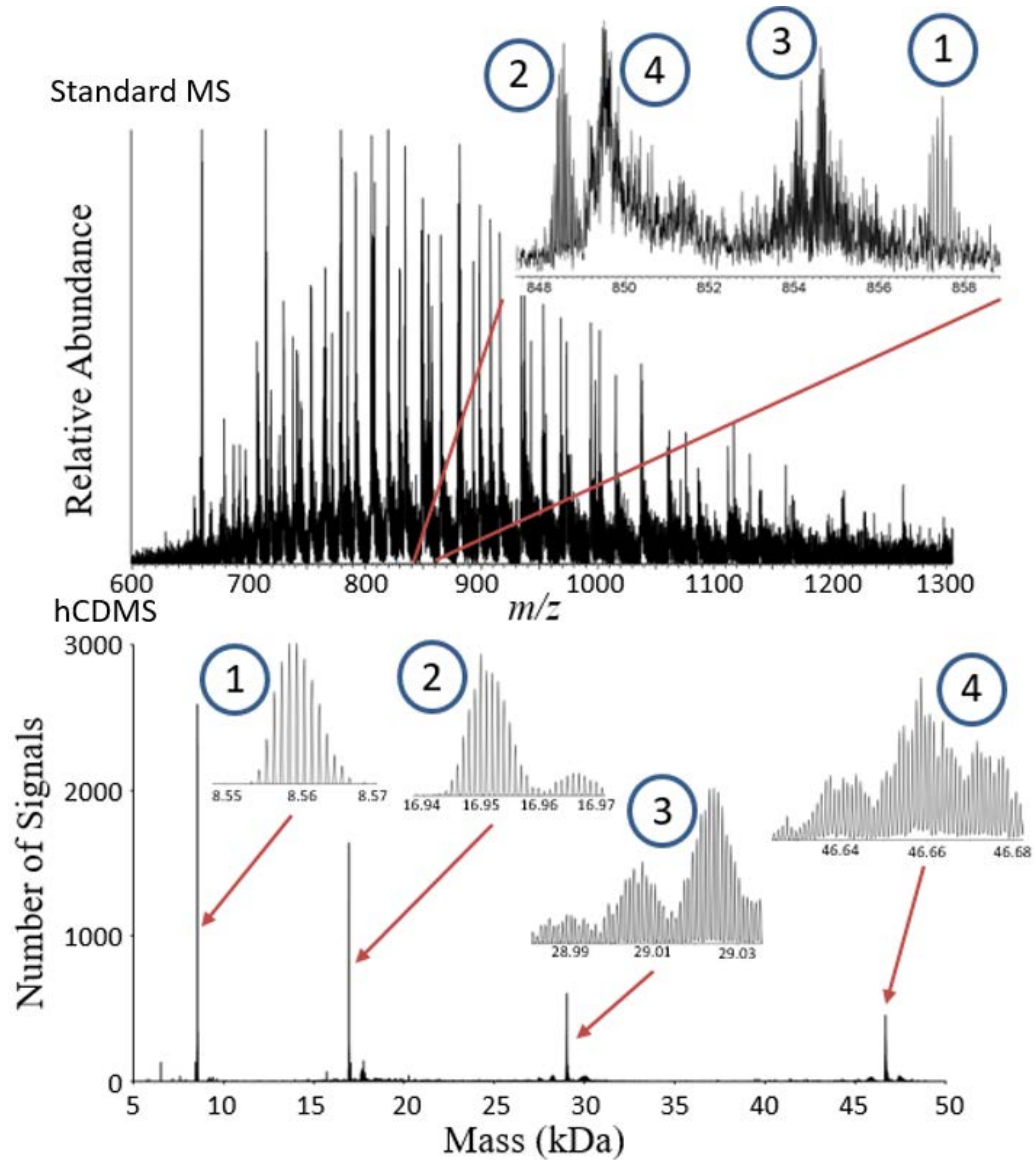
# STORI Slope to Charge Calibration



Denatured  
Proteins

Native  
Complexes

# Deconvolution: 4 Protein Mix

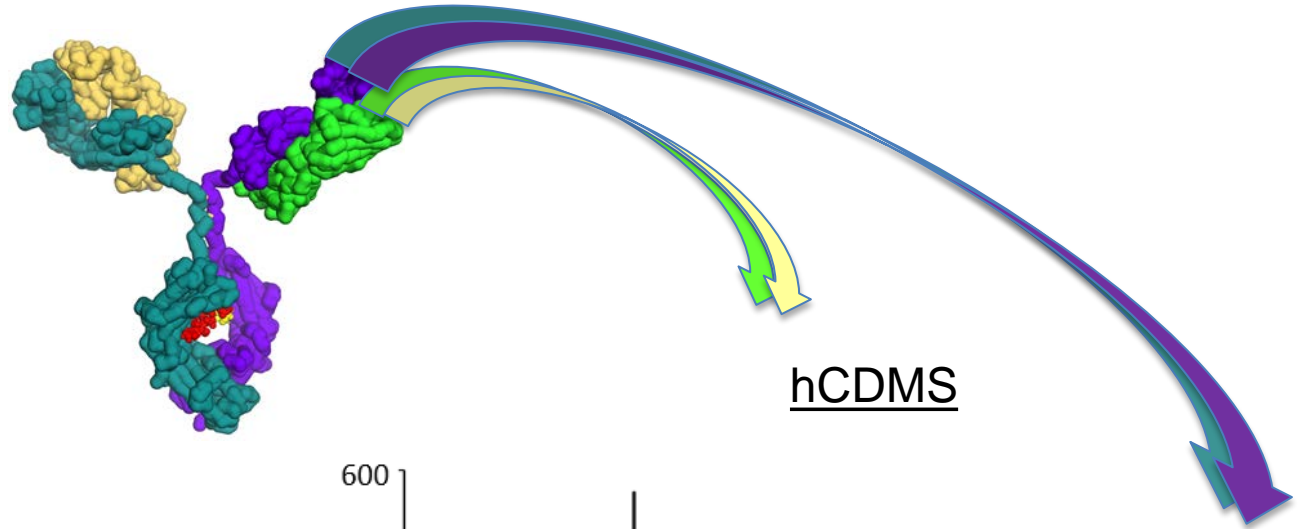


**Single Ion hCDMS Analysis Solves Charge State Congestion Problem**

#	Name	Monoisotopic Mass (Da)	Accuracy (ppm)	Resolution
1	Ubiquitin	8,559.6	3.5	43,000 @ 8.6 kDa
2	Myoglobin	16,941.0	11	77,000 @ 16.9 kDa
3	Carbonic Anhydrase	29,006.7	11.3	88,000 @ 29 kDa
4	Enolase	46,656.2	15	133,000 @ 46.7 kDa

# Deconvolution: Reduced Ab

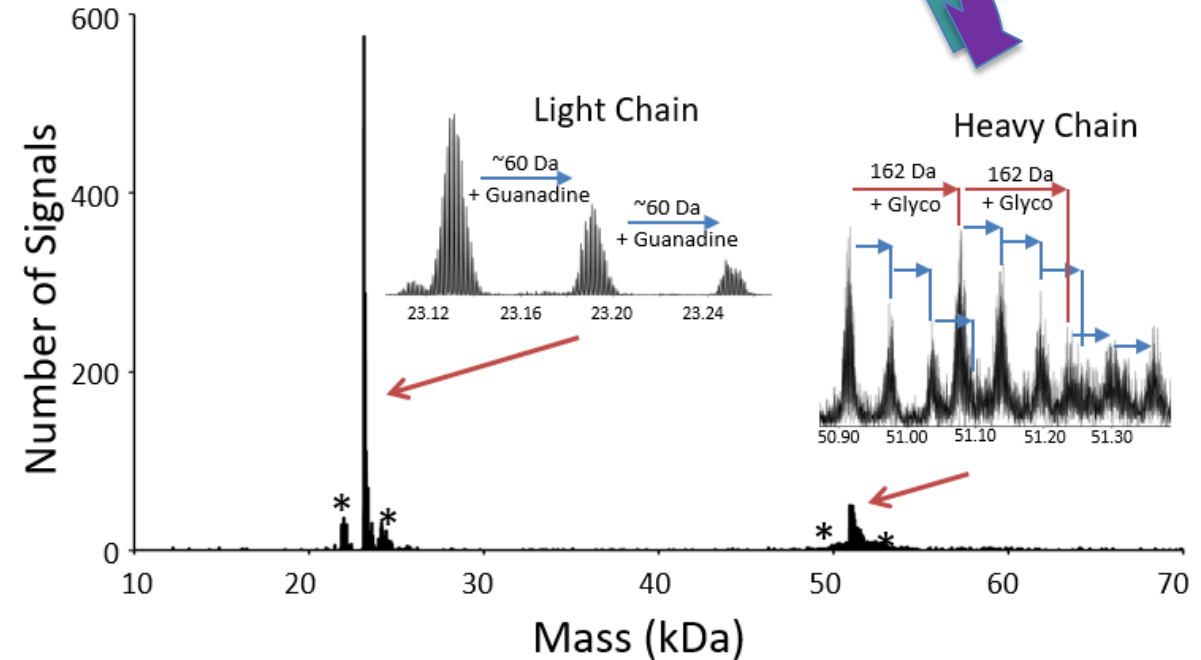
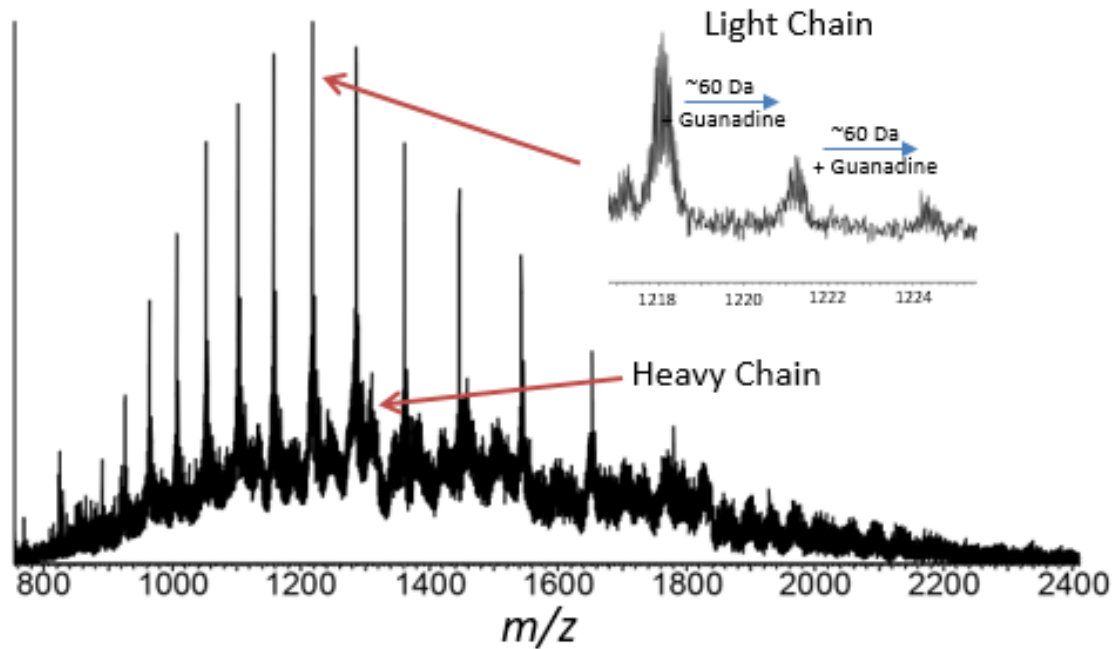
GdHCl  
TCEP - HCl



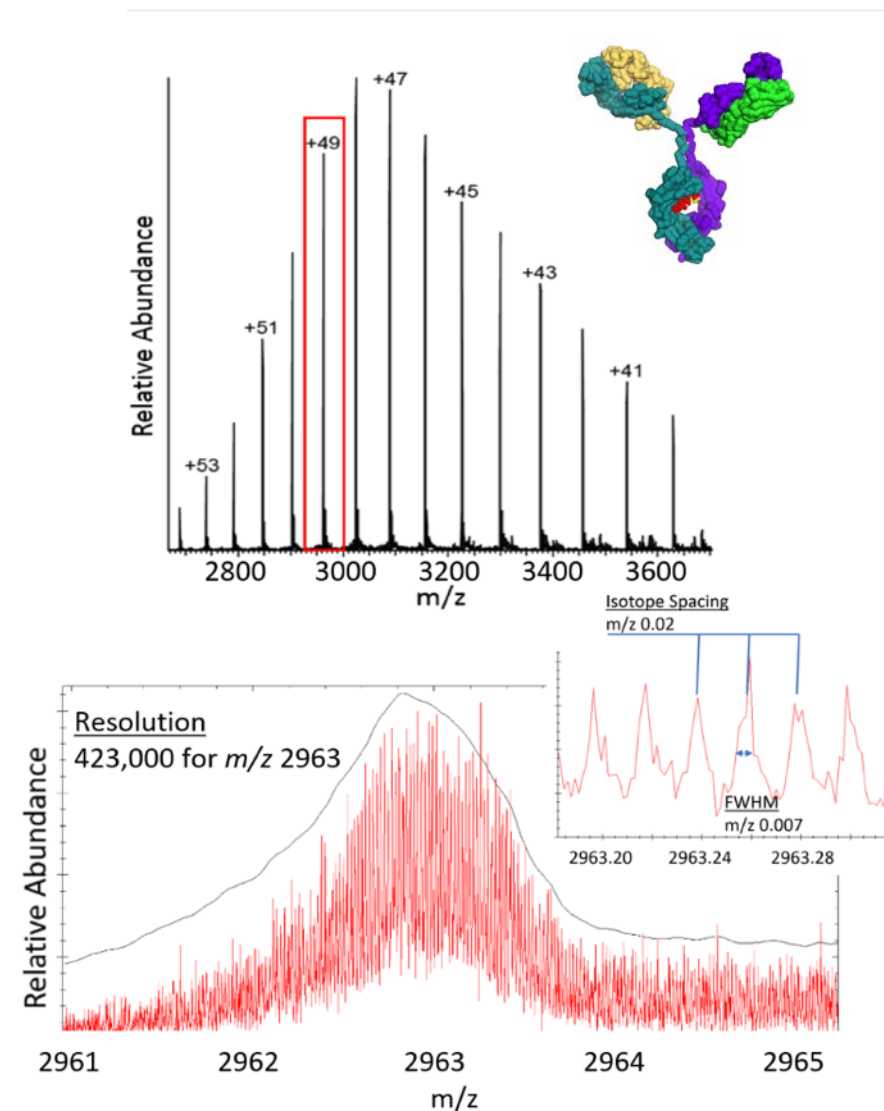
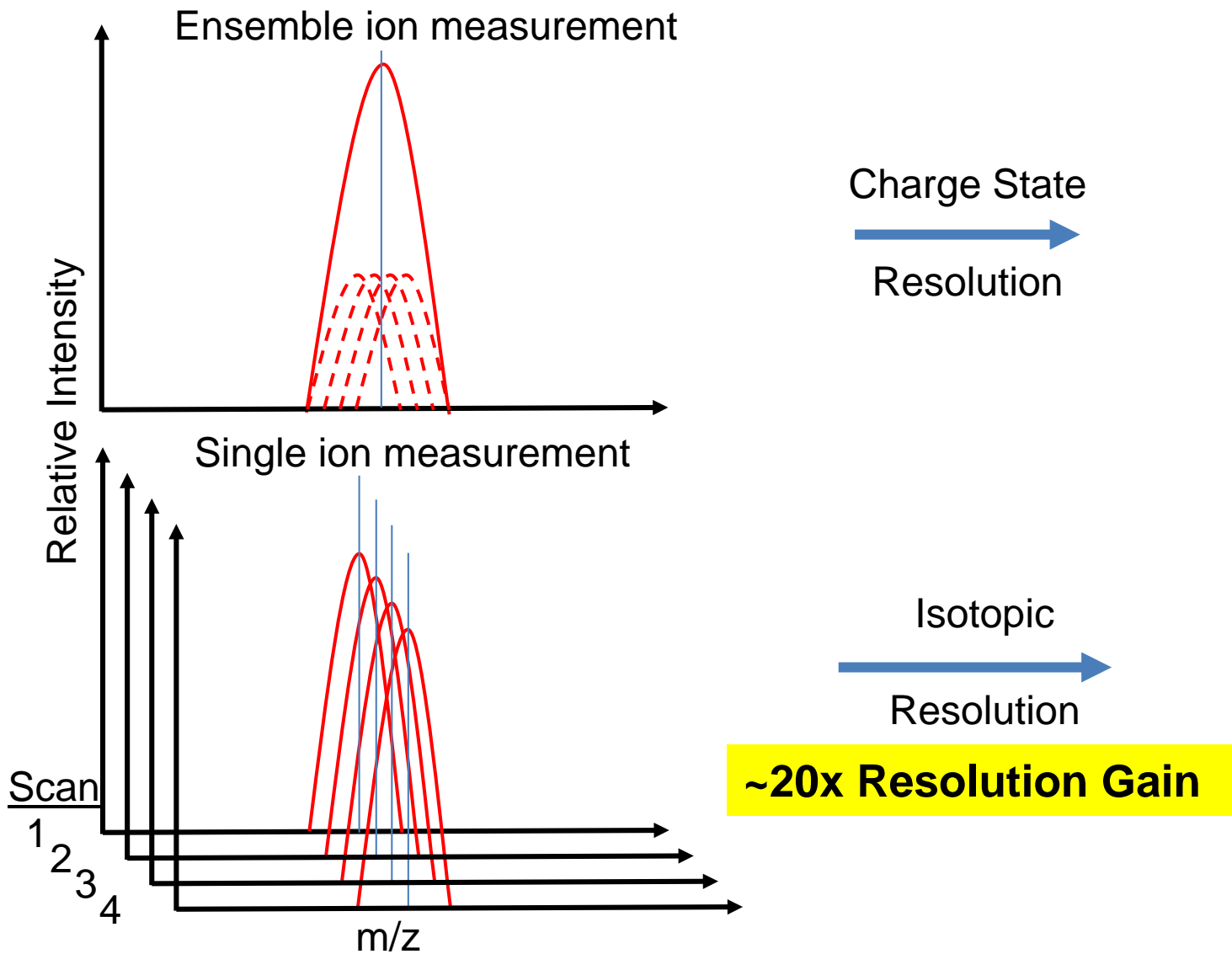
Standard MS

hCDMS

Relative Intensity (Arb.)

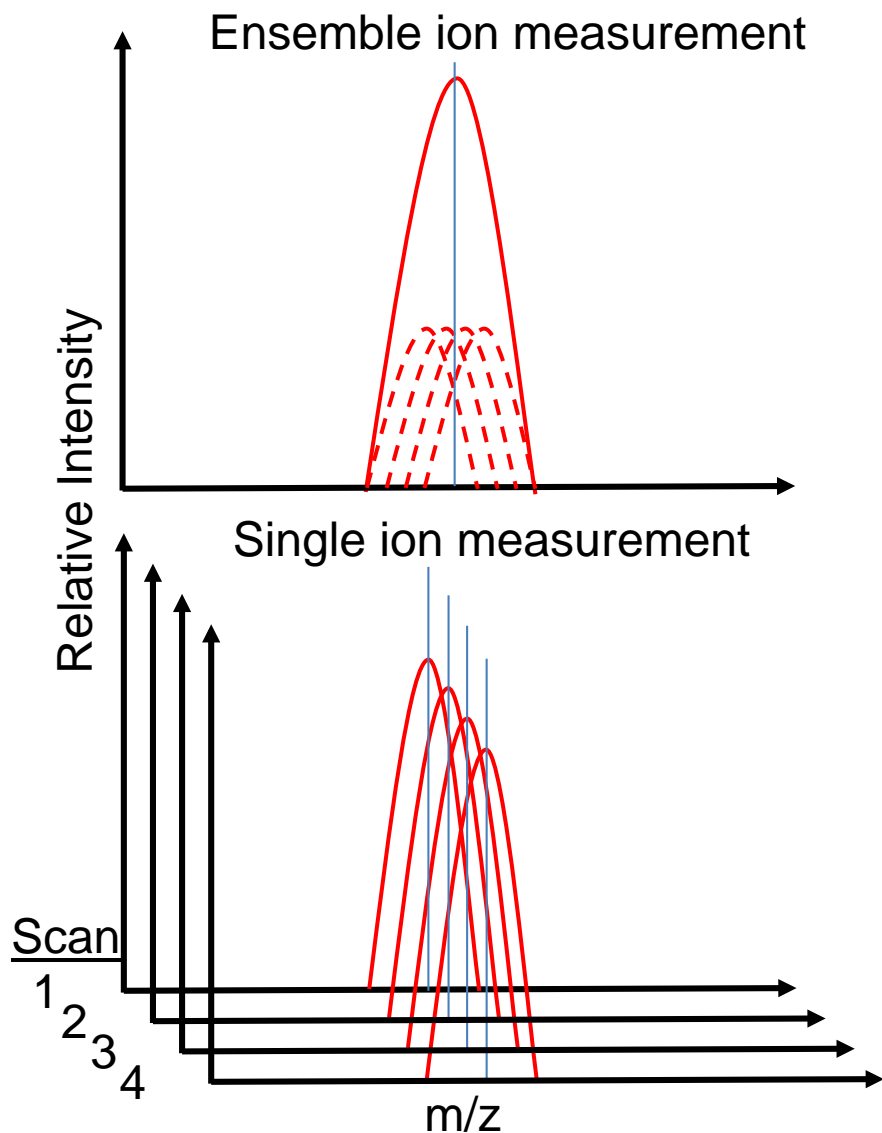


# Single Ion MS: Resolution Gain





# Individual Ion MS: Resolution Gain

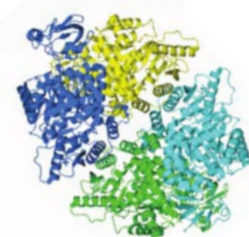
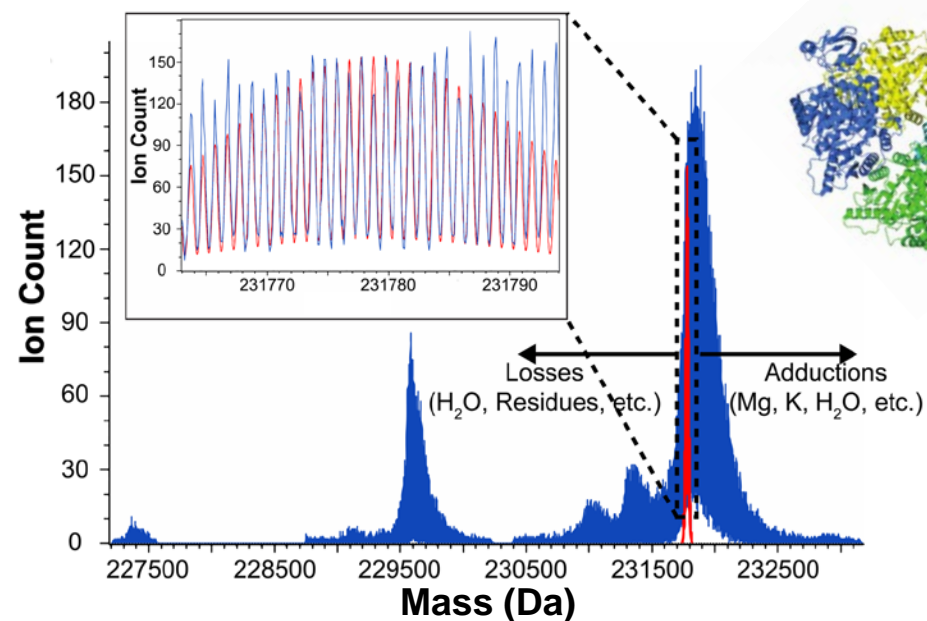
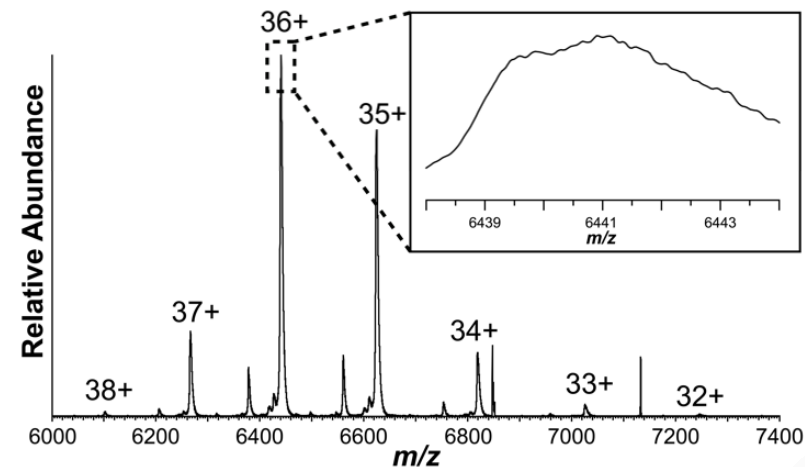


Charge State  
Resolution

Isotopic  
Resolution

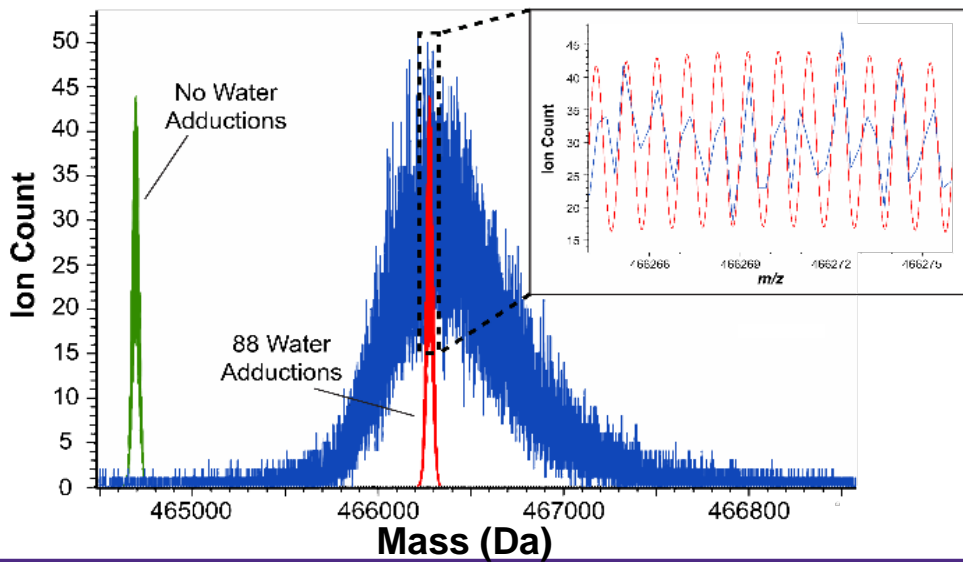
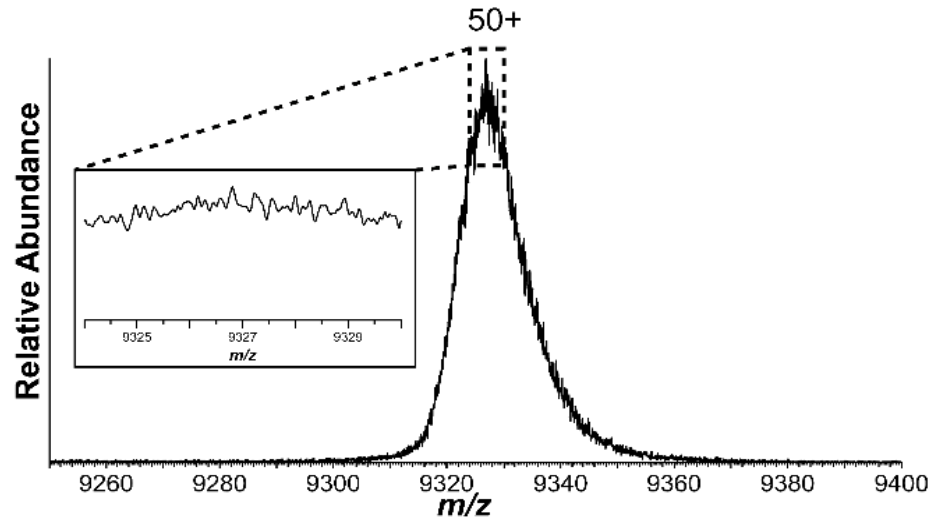
**~20x Resolution  
Gain**

## Pyruvate Kinase (Tetramer)

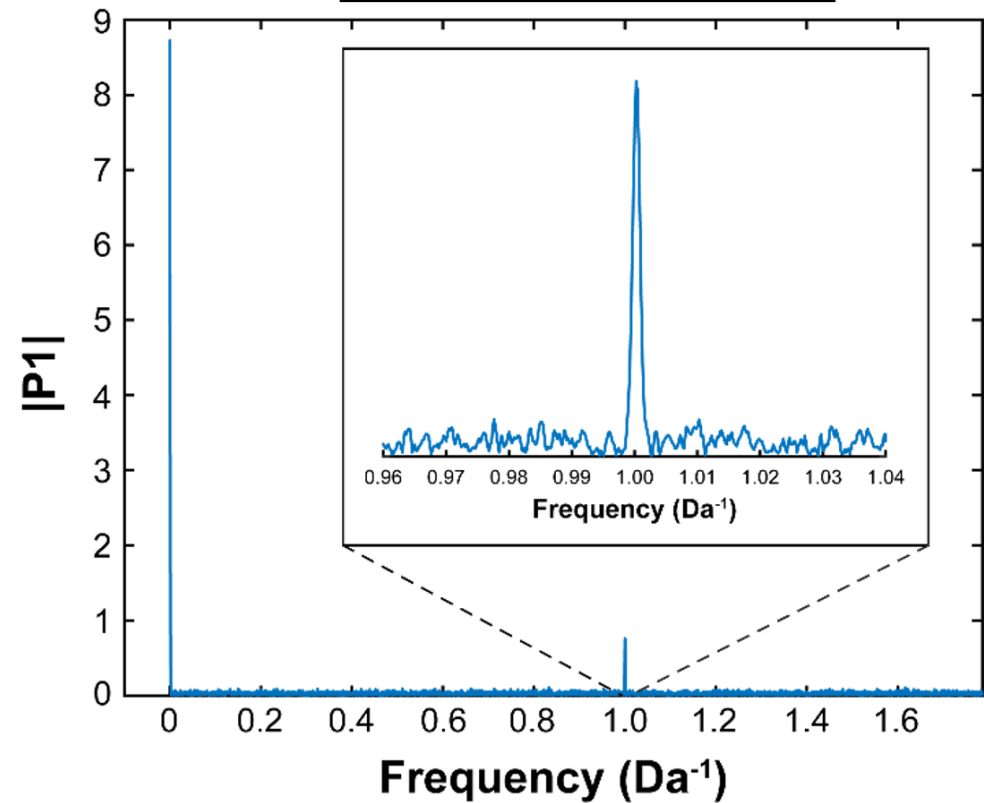


# Individual Ion MS: Resolution Gain

## $\beta$ -Galactosidase (Tetramer)



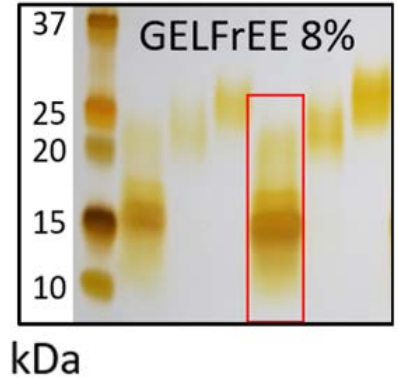
## Isotopic Confirmation



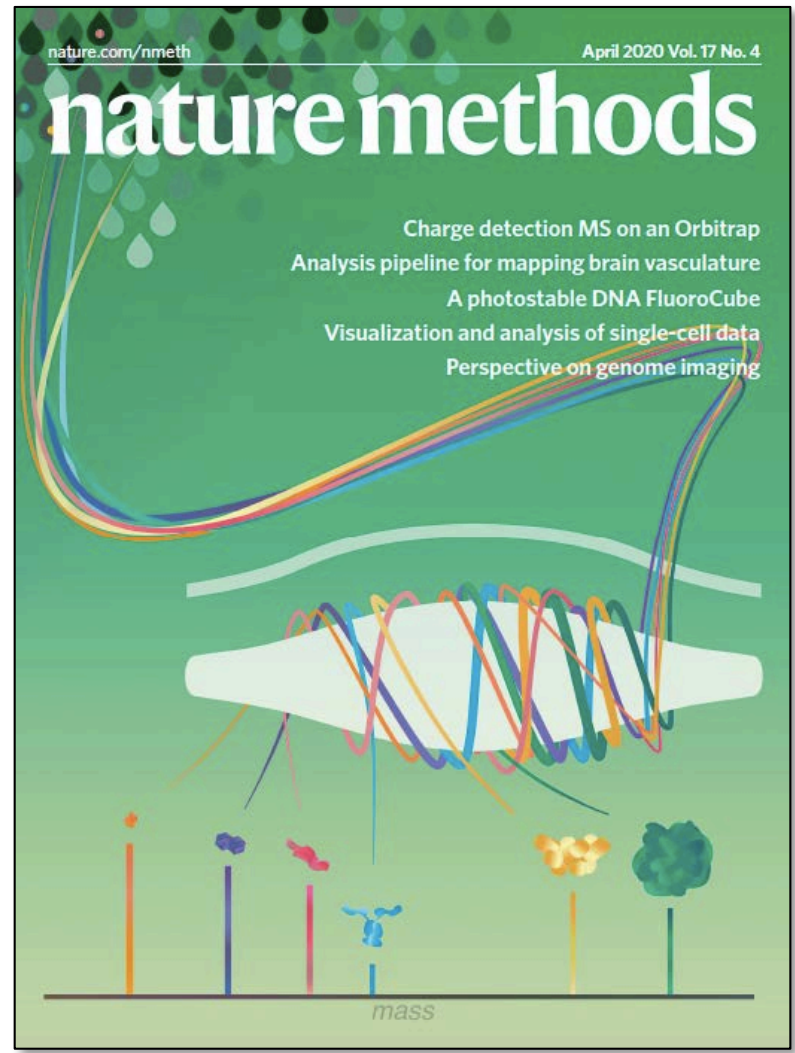
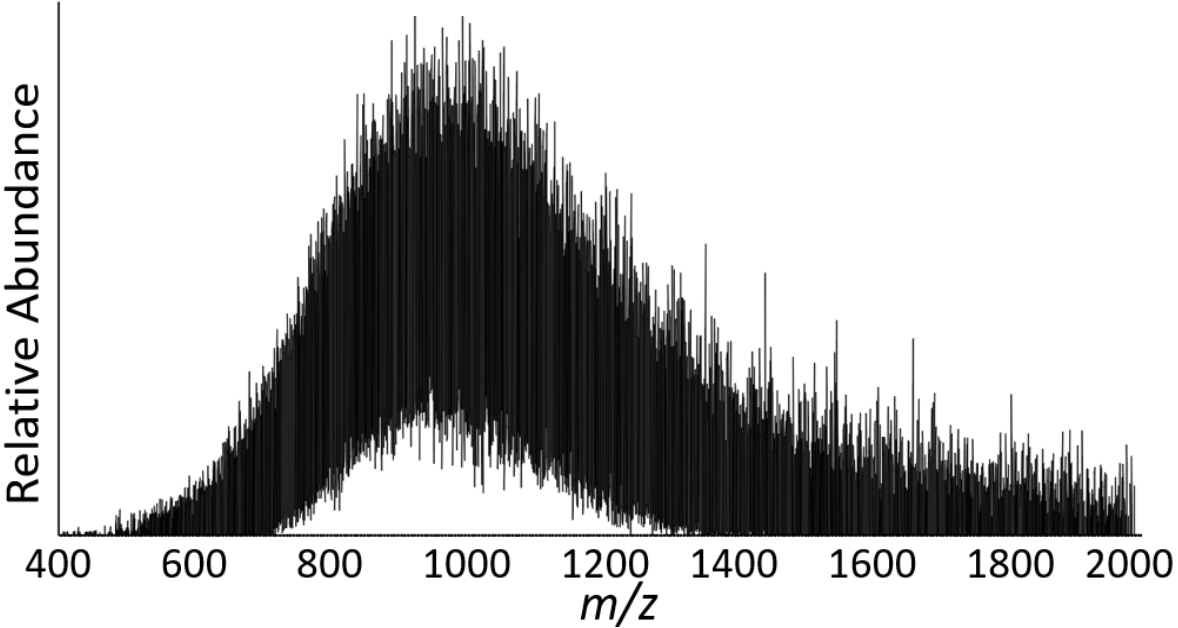
Jack McGee

# Deconvolution: Extreme Proteoform Complexity

5-30 kDa mix of human proteoforms



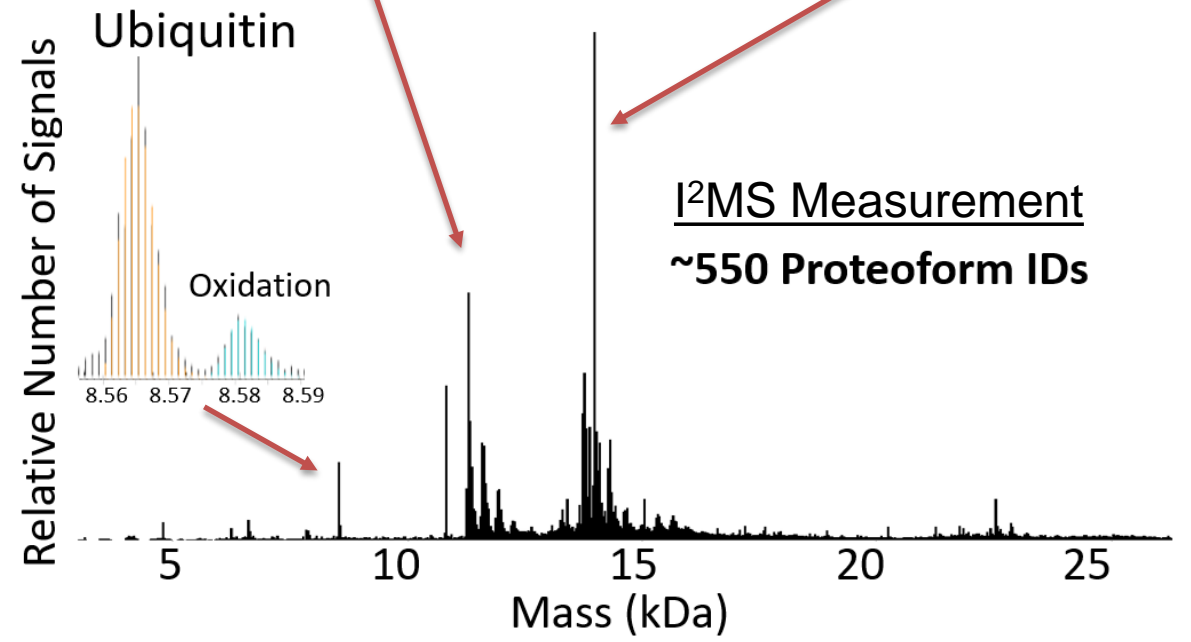
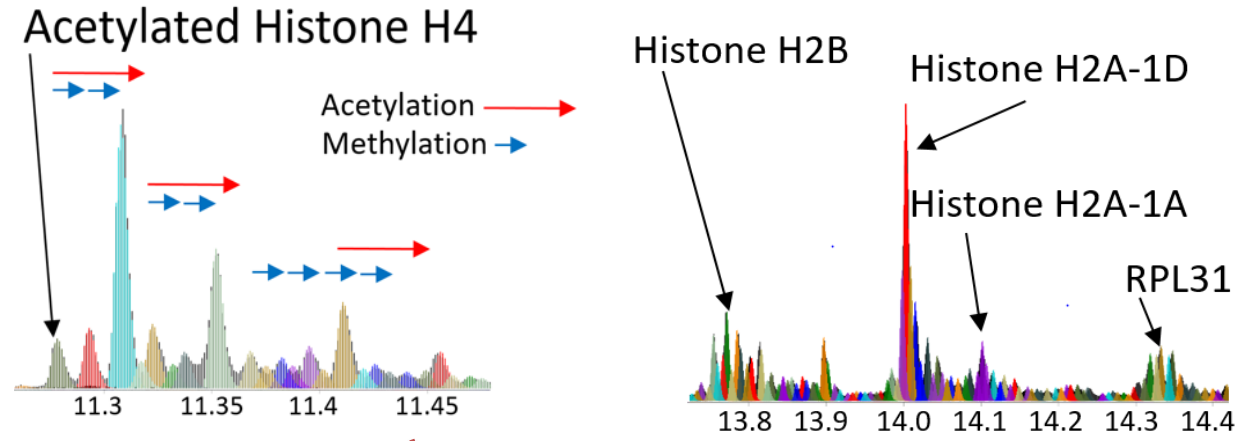
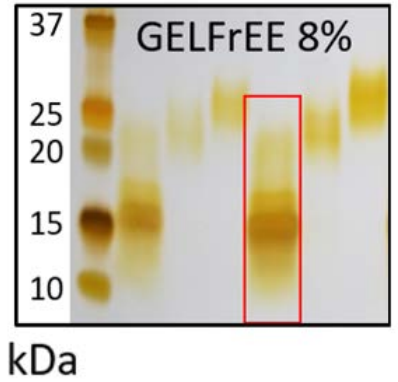
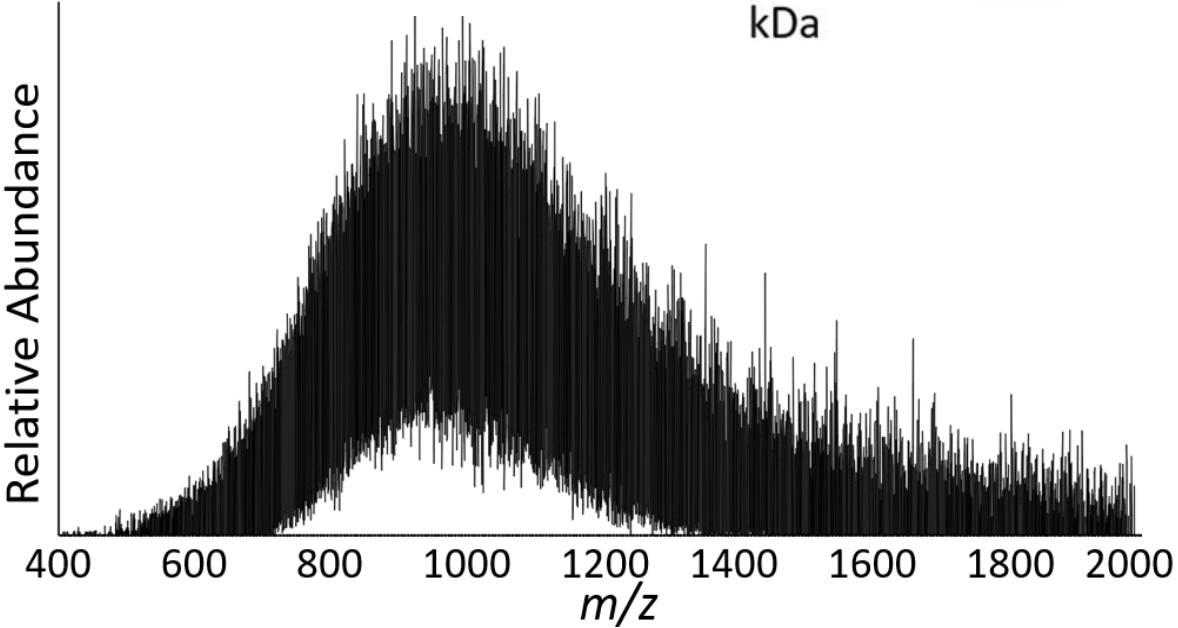
Ensemble Measurement  
**0 Proteoform IDs**



# Deconvolution: Extreme Proteoform Complexity

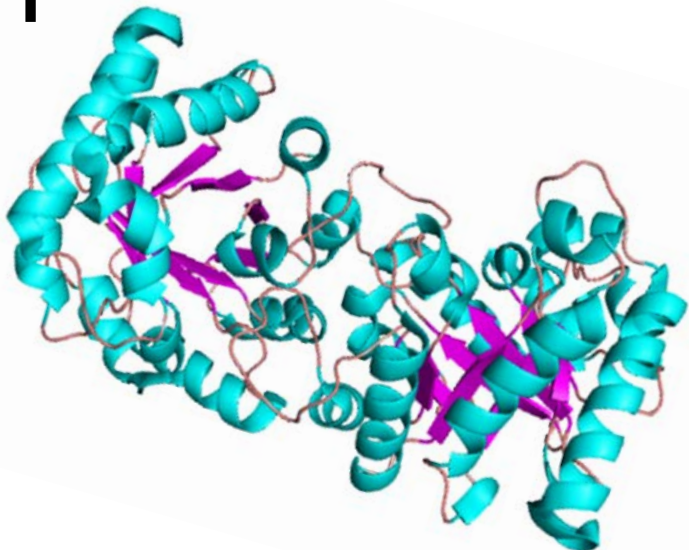
5-30 kDa mix of human proteoforms

Ensemble Measurement  
**0 Proteoform IDs**

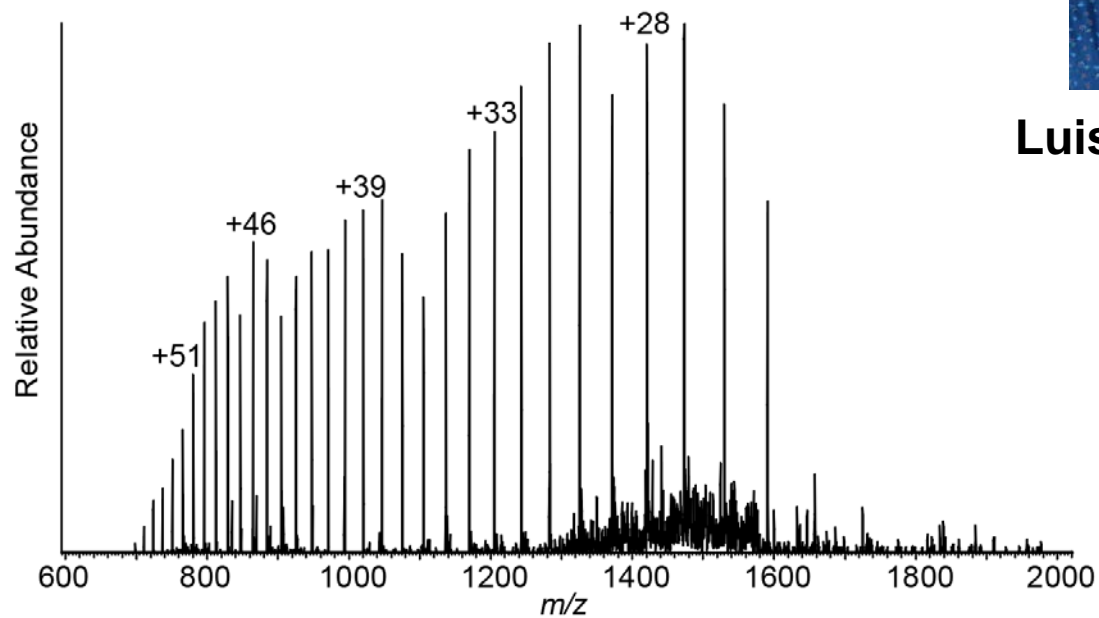


I<sup>2</sup>MS Measurement  
**~550 Proteoform IDs**

# Top Down MS: Triosephosphate Isomerase



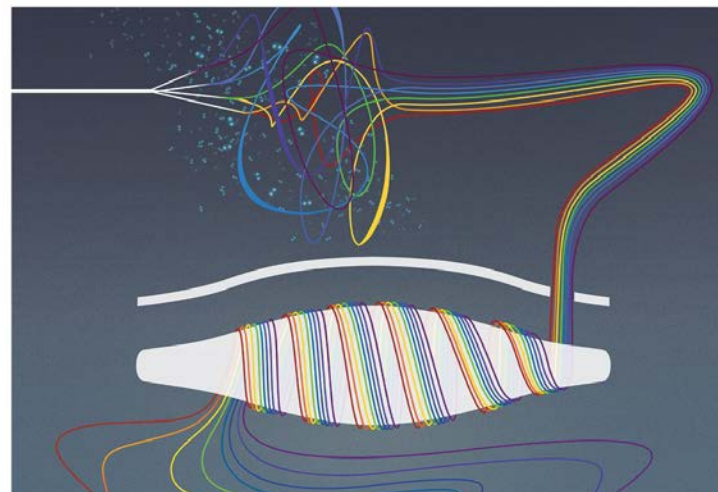
Luis Schachner



Journal of  
**proteome**  
research

March 2020

Volume 19 Number 3 pubs.acs.org/jpr

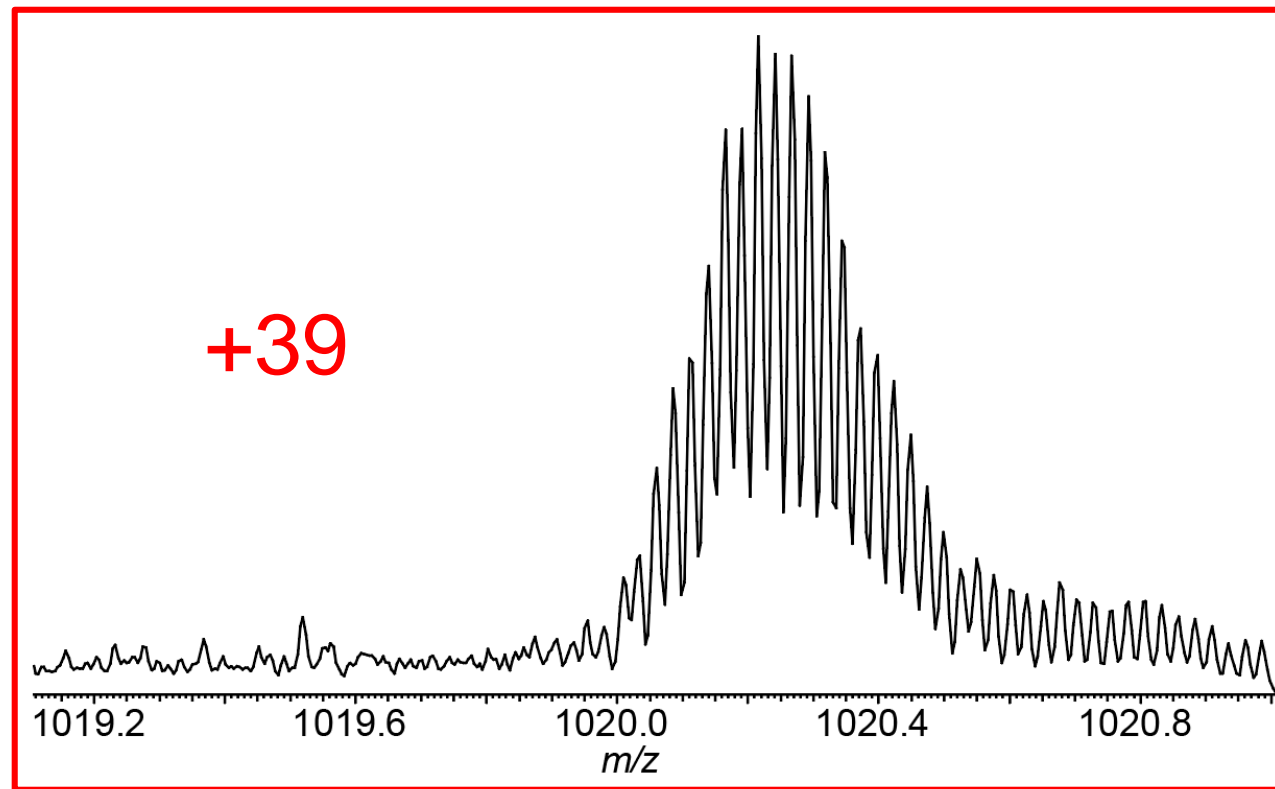
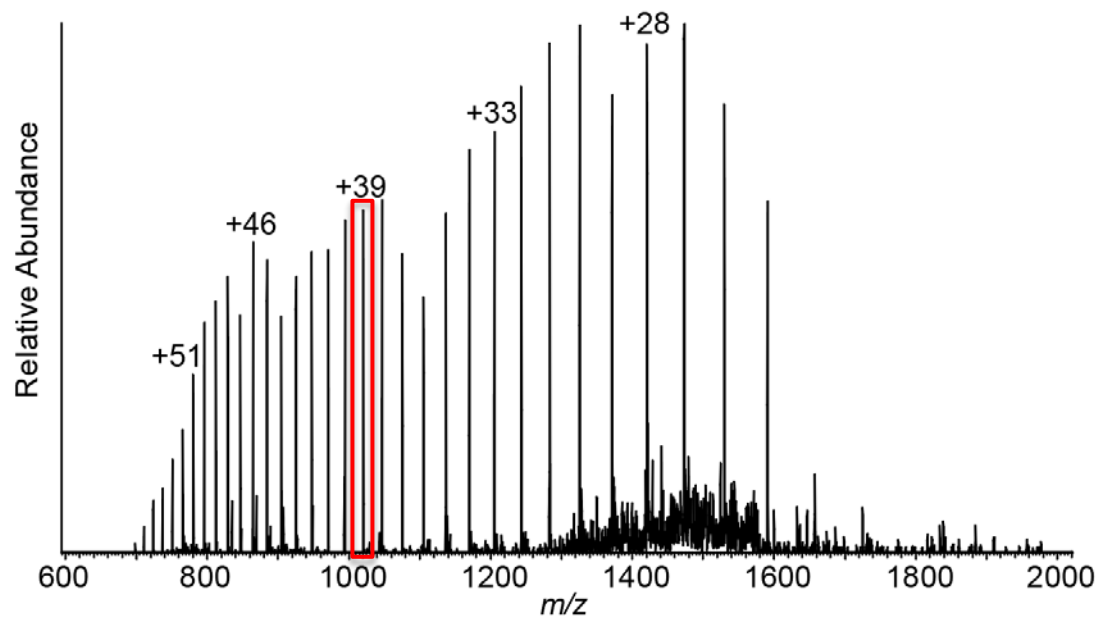
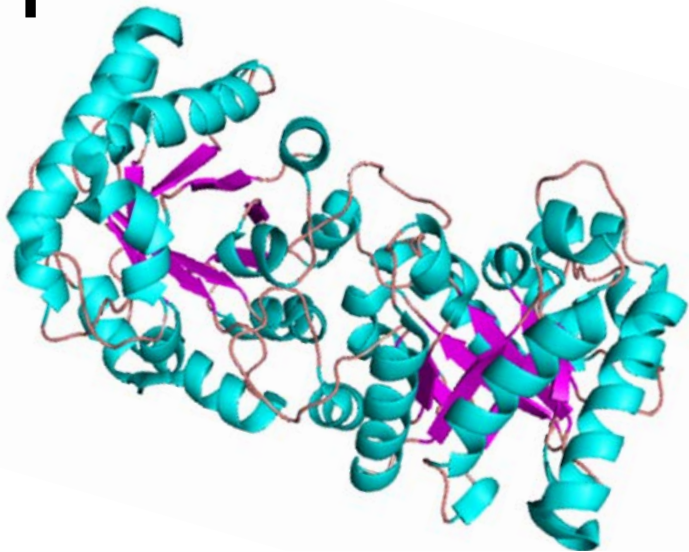


```
N  M E K K I R G S H H H H H M A S G S D S E I V N Q 25
26  E A K P E V K P E V K P E T H I N L K V I S D G S S 50
51  E I F F K I K K T T P L R R L M E A F A K R Q G K 75
76  E M D S L R F L Y D G I R I Q A D I Q T P E D I D M 100
101 E D N D I I I E A H R E Q I G G A P S R K F F V G G 125
126 N W K M N G R K Q S L G E L I I G I T L N A A K V P A 150
151 D T E I V I C A P P T A Y I D F A R Q K L D P K I A 175
176 V A A Q N C Y K V T N G A F T G E I S P G M I K E 200
201 C G A T W V V L G H S E R R H V F G E S D E L I G 225
226 L K P E F V D I I N A K Q C
```

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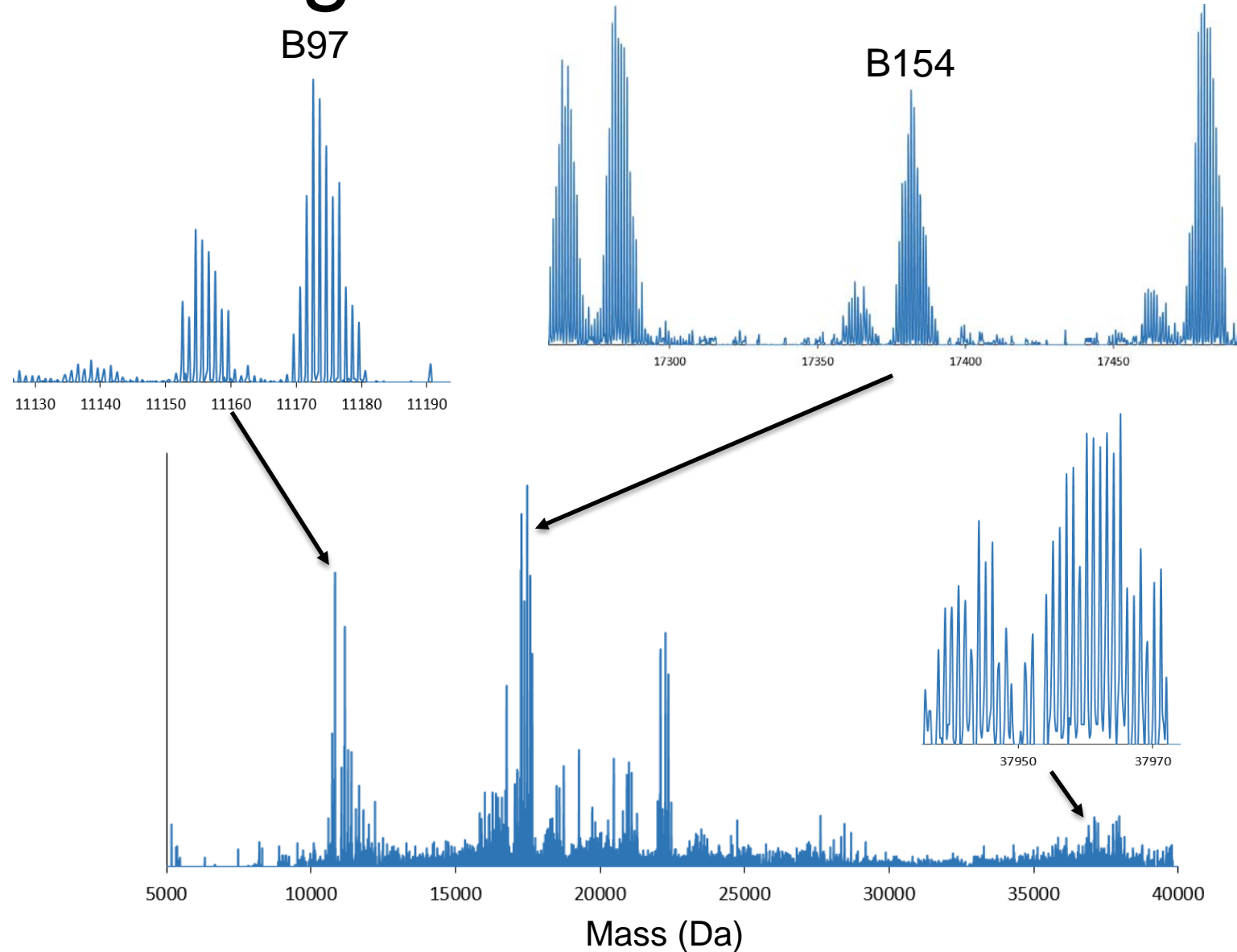
# Top Down MS: Triosephosphate Isomerase



# Top Down MS: Fragment Matches

hCDMS

N	M	E	K	K	I	R	G	S	H	H	H	H	H	M	A	S	G	S	D	S	E	L	V	N	Q	25
26	E	A	K	P	E	V	K	P	E	V	K	P	E	T	H	I	N	L	K	V	S	D	G	S	S	50
51	E	I	F	F	K	I	K	T	T	P	L	R	R	L	M	E	A	F	A	K	R	Q	G	K	75	
76	E	M	D	S	L	R	F	L	Y	D	G	I	R	I	Q	A	D	Q	T	P	E	D	L	D	M	100
101	E	D	N	D	I	I	E	A	H	R	E	Q	I	G	G	A	P	S	R	K	F	F	V	G	G	125
126	N	W	K	M	N	G	R	K	Q	S	L	G	E	L	I	G	T	L	N	A	A	K	V	P	A	150
151	D	T	E	V	V	C	A	P	T	A	Y	I	D	F	A	R	Q	K	L	D	P	K	I	A	175	
176	V	A	A	Q	N	C	Y	K	V	T	N	G	A	F	T	G	E	I	S	P	G	M	I	K	D	200
201	C	G	A	T	W	V	V	L	G	H	S	E	R	R	H	V	F	G	E	S	D	E	L	I	G	225
226	Q	K	V	A	H	A	L	A	E	G	L	G	V	I	A	C	I	G	E	K	L	D	E	R	E	250
251	A	G	I	T	E	K	V	V	F	E	Q	T	K	V	I	A	D	N	V	K	D	W	S	K	V	275
276	V	L	A	Y	E	P	V	W	A	I	G	T	G	K	T	A	T	P	Q	Q	A	Q	E	V	H	300
301	E	K	L	R	G	W	L	K	S	N	V	S	D	A	V	A	Q	S	T	R	I	I	Y	G	G	325
326	S	V	T	G	A	T	C	K	E	L	A	S	Q	P	D	V	D	G	F	L	V	G	G	A	S	350
351	L	K	P	E	F	V	D	I	I	N	A	K	Q	C												



# Top Down MS: Fragment Matches

## I<sup>2</sup>MS<sup>2</sup>

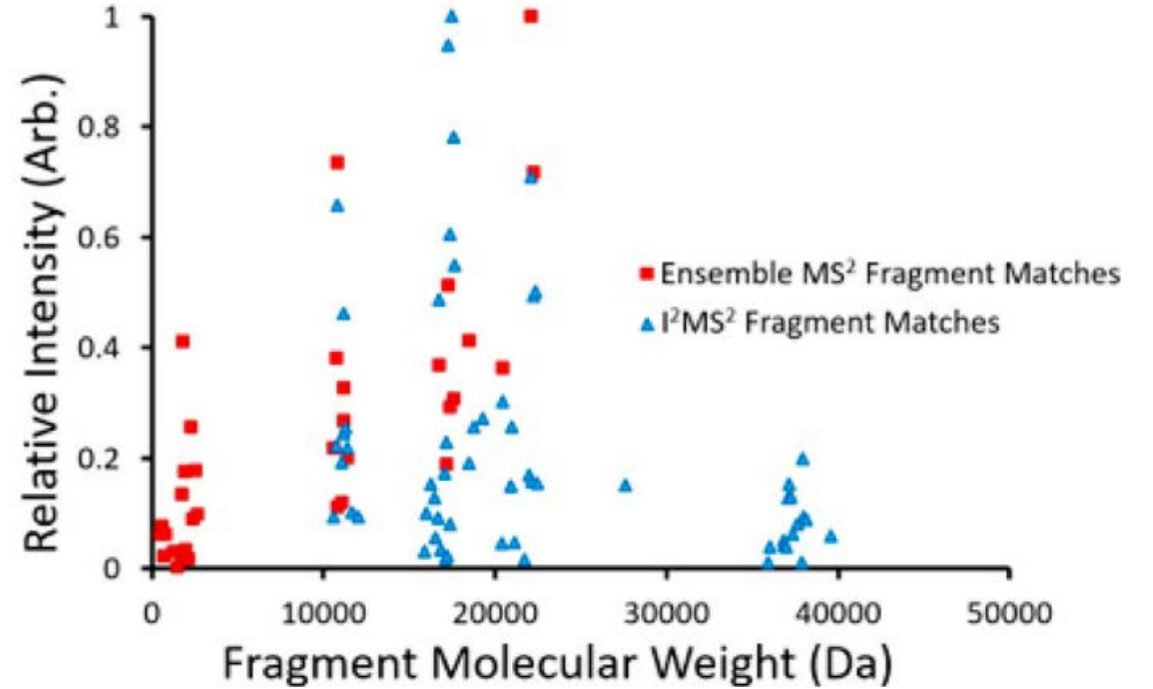
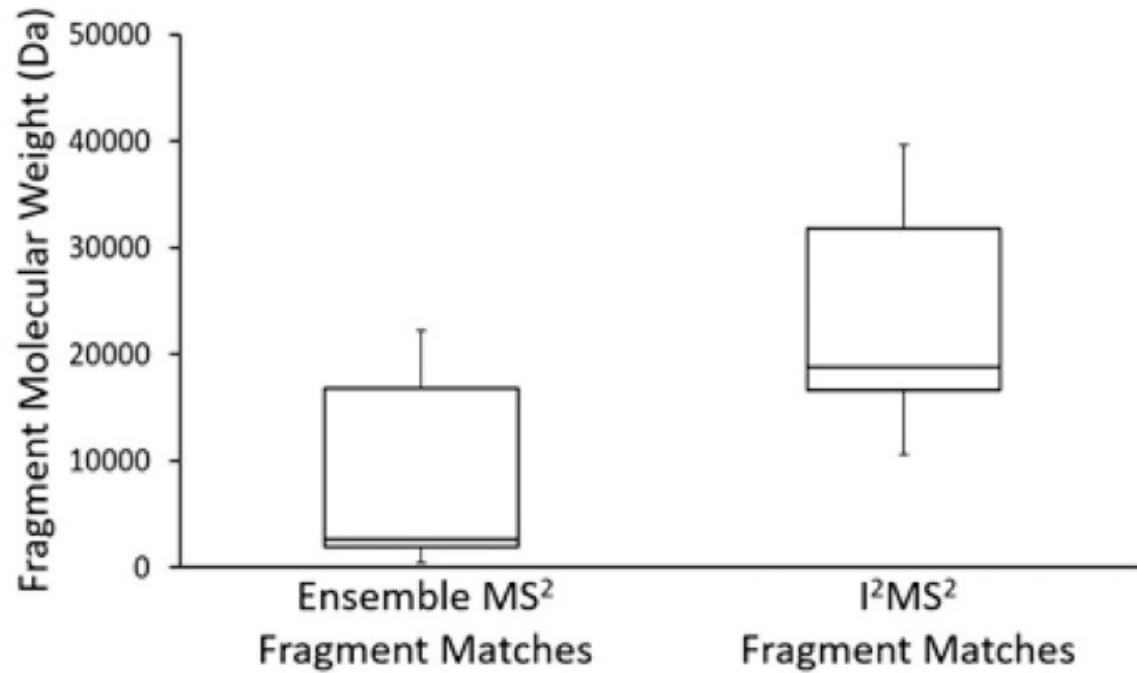
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N MEKKIRGSHHHHHM[A S G S D S]E[V]N Q 25
26 EAKPEVKPEVKPETHINLKVSDGSS 50
51 EIFFKIKKTTPLRRLMEAFKRQ GK 75
76 EMDSLRF LYDGI RI QAD]Q T]P E]D]L]D]M 100
101 EDNDI I EAHREQ I GGAPSRKFFVGG 125
126 NWKMNGRKQSLGEL I]G T]L]N A]A K]V]P A 150
151 D]T]E]V]V]C]A]P]P TAY I D F A R Q K L D]P]K I A 175
176 VAAQN C Y K V T N G]A]F]T]G E]I S P G M I K]D 200
201 C G A T]W V V L G H S E R R H V F G E S D E L I G 225
226 QKVAHALA E G L G V I A C I G E K L D E R E 250
251 A G I T E K V]V F]E Q T K V I A D N V K D W S K V 275
276 V L A Y E P V W A I G T G K T A T P Q Q A Q E V H 300
301 E K L R G W L K S N V S D A V A Q S T R I I Y G G 325
326 S V T G A T C K E L A]S Q]P D V D G]F]L]V]G G A]S 350
351 L K P E F V D I I N A K]Q C
```

## Standard MS<sup>2</sup>

```
N MEKKIRGSHHHH]HH]M]A]S G]S D]S]E]V]N Q 25
26 EAKPEVKPEVKPETHINLKVSDG]S S 50
51 EIFFKIKKTTPLRRLMEAFKRQ GK 75
76 EMDSLRF LYDGI RI QAD]Q T]P E]D]L]D]M 100
101 EDNDI I EAHREQ I GGAPSRKFFVGG 125
126 NWKMNGRKQSLGEL I G T L N A]A K]V]P A 150
151 D T]E]V]V]C]A]P P T A Y I D F A R Q K L D]P]K I A 175
176 VAAQN C Y K V T N G A F T]G E I S P G M I K]D 200
201 C G A T W V V L G H S E R R H V F G E S D E L I G 225
226 QKVAHALA E G L G V I A C I G E K L D E R E 250
251 A G I T E K V V F]E Q T]K V I A D N V K D W S K V 275
276 V L A Y E P V W A I G T G K T A T P Q Q A Q E V H 300
301 E K L R G W L K S N V S D A V A Q S T R I I Y G G 325
326 S V T G A T C K E L A S Q]P D V D G]F]L]V]G G A S 350
351 L K]P E F V]D]I]I]N A K Q C
```



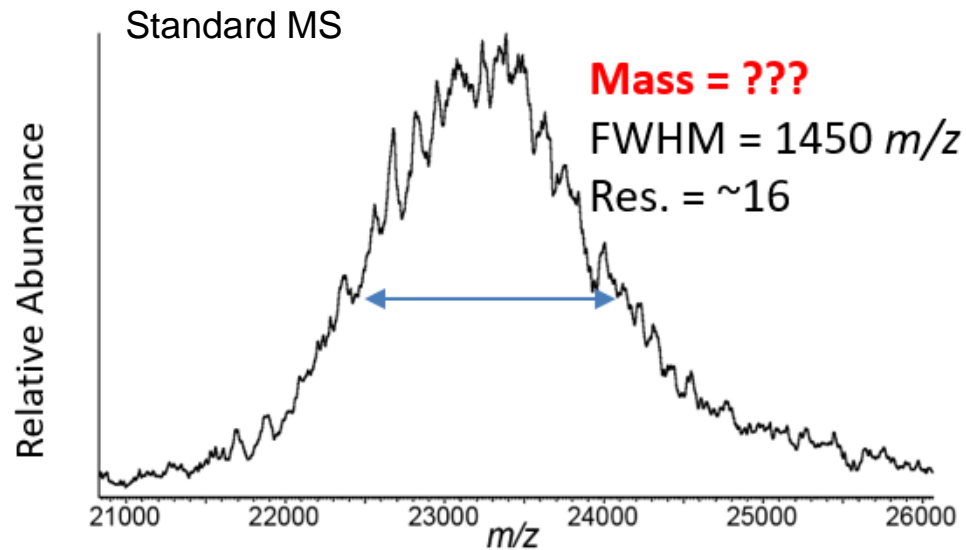
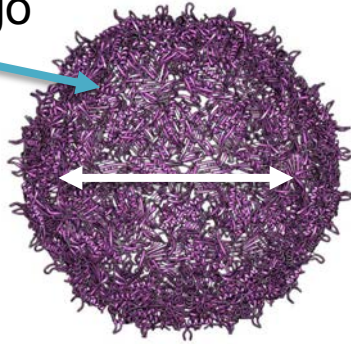
# Top Down MS: Fragment Matches



# Mass Determination: Virus-like Particles

Heterogeneous DNA  
and RNA cargo

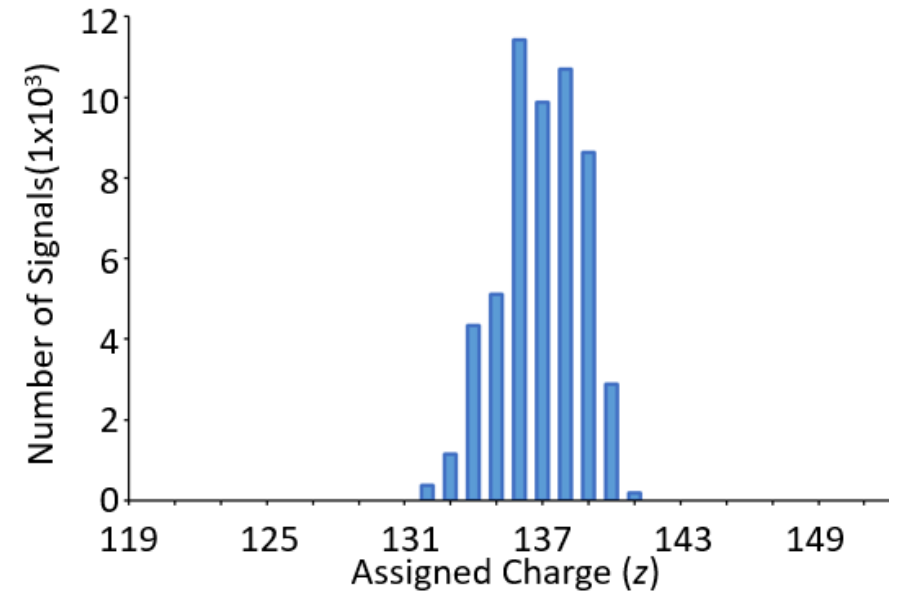
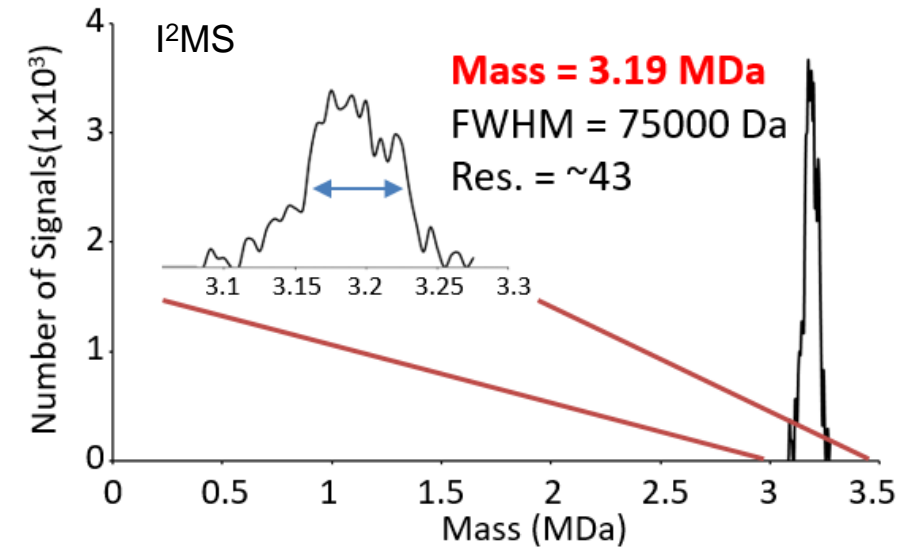
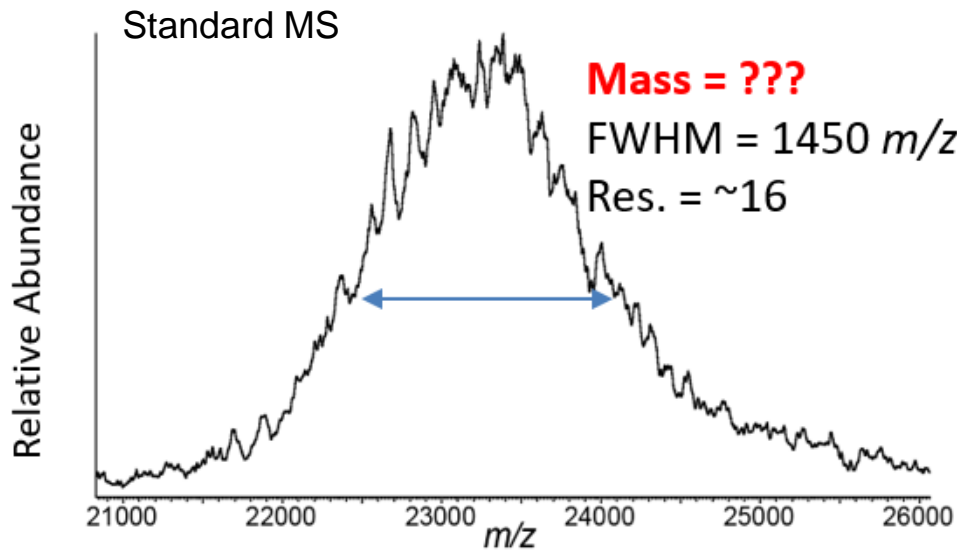
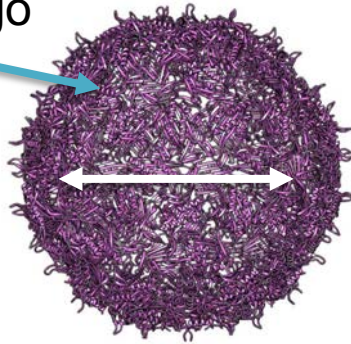
27 nm  
T= 3 Geometry



# Mass Determination: Virus-like Particles

Heterogeneous DNA  
and RNA cargo

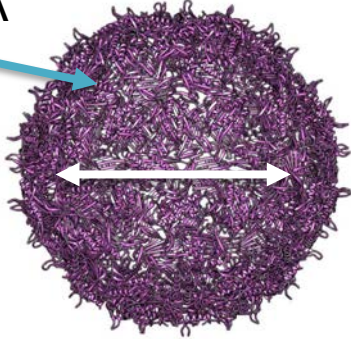
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T= 3 Geometry



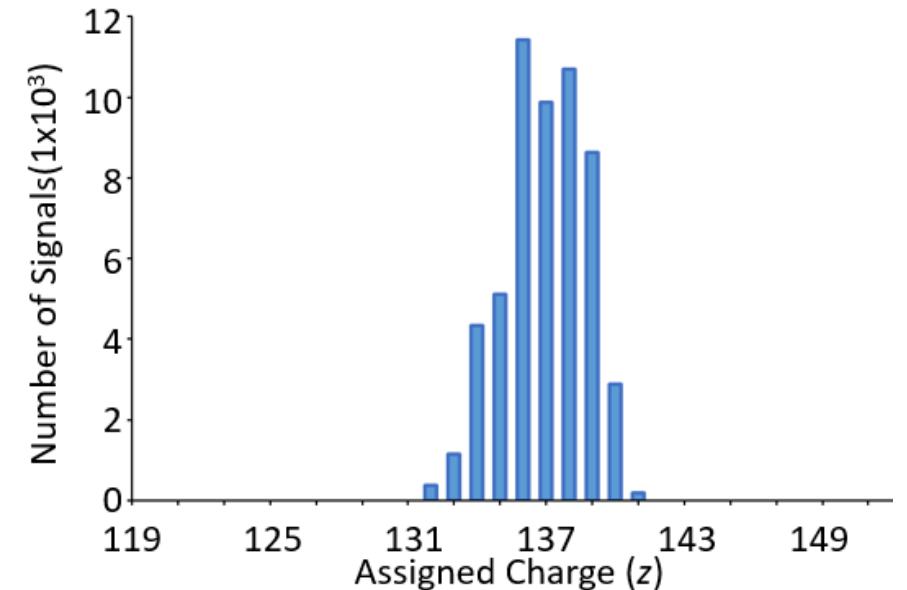
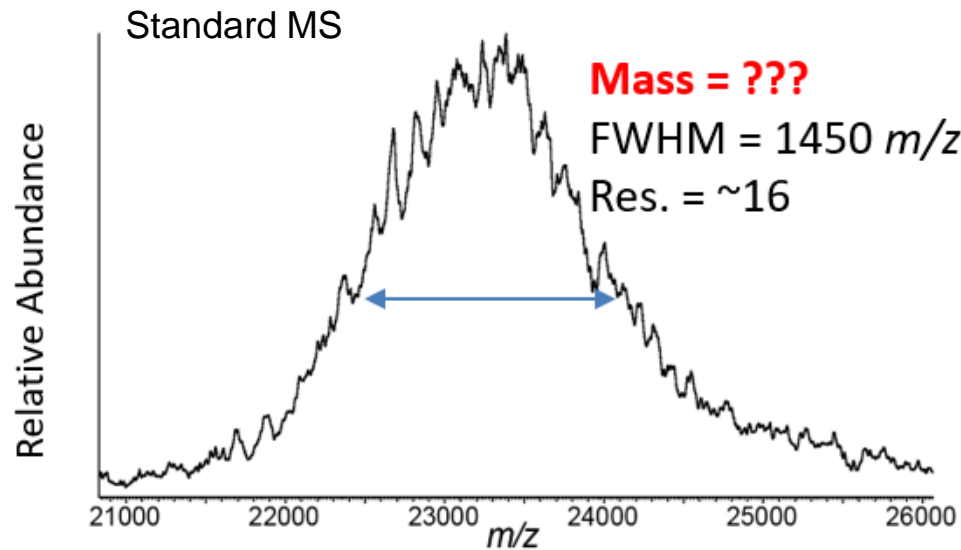
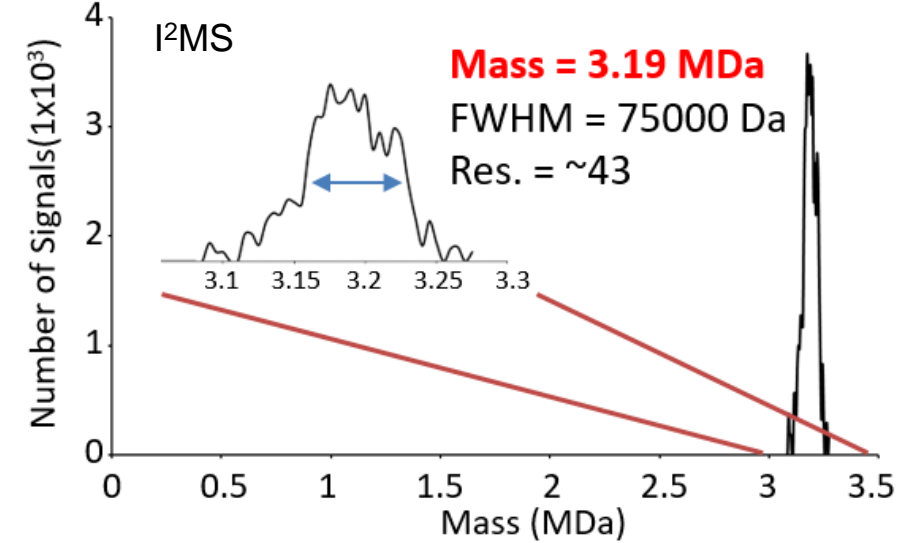
# Mass Determination: Virus-like Particles

Heterogeneous  
DNA **AND** RNA  
cargo

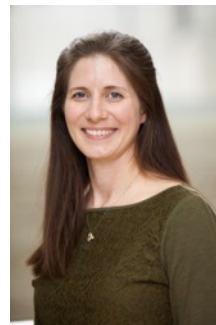
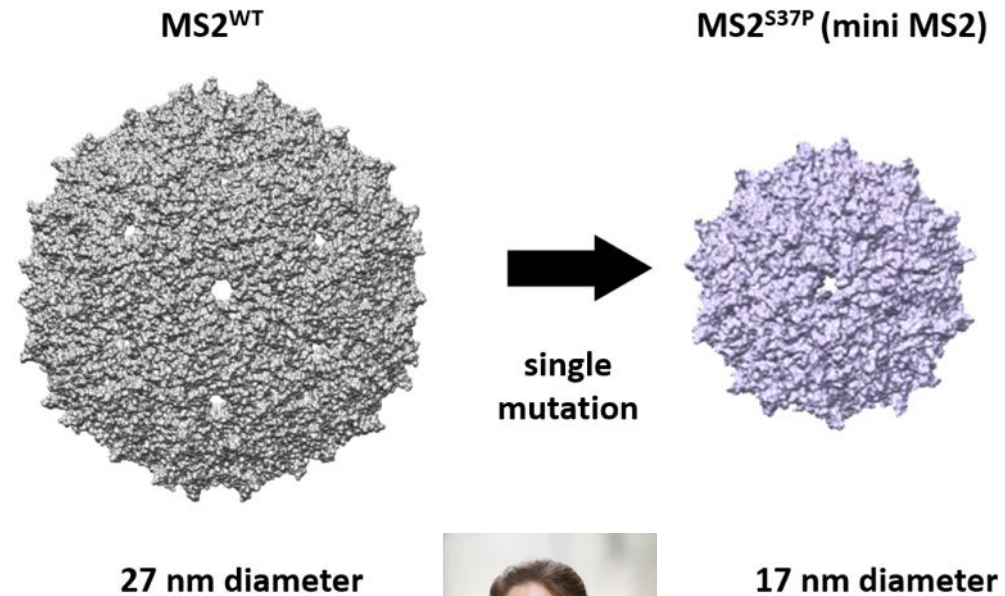
27 nm  
T= 3 Geometry



	MDa
Capsid	2.47
Cargo(DNA)	0.58
Cargo(RNA)	0.15
<b>TOTAL</b>	<b>~3.20</b>



# Virus-like Particles: WT vs MUTANT



**Dr. Danielle Tullman-Ercek**

# Virus-like Particles: WT vs MUTANT

180 Coat Proteins

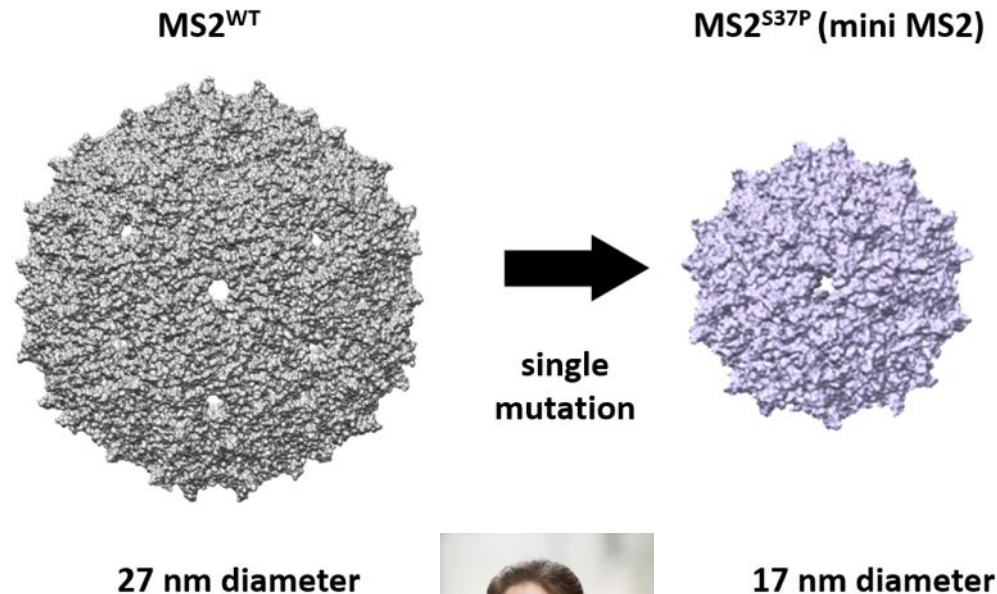
27 nm Diameter

10,300 nm<sup>3</sup> Volume

60 Coat Proteins

17 nm Diameter

2,600 nm<sup>3</sup> Volume



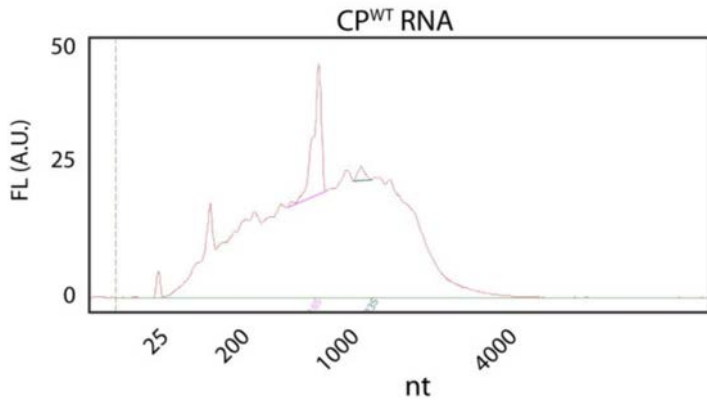
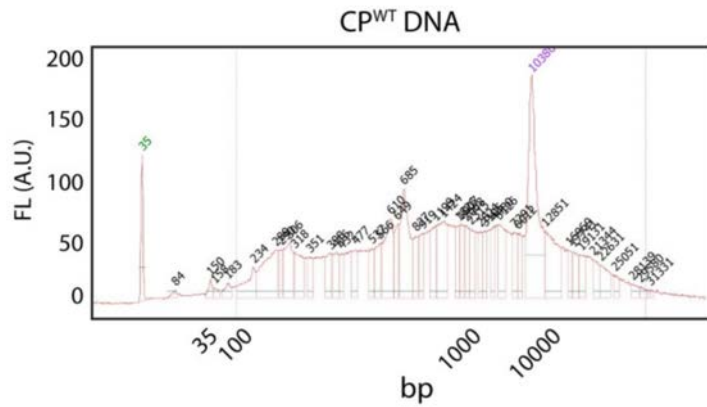
**Dr. Danielle Tullman-Ercek**

# Virus-like Particles: WT vs MUTANT

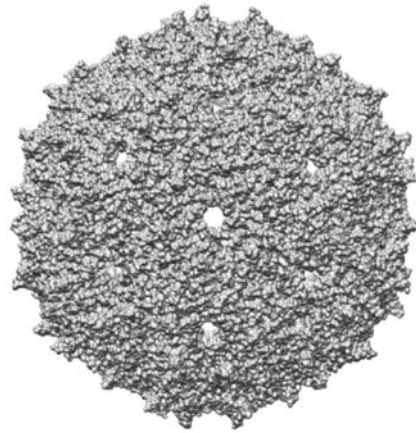
180 Coat Proteins

27 nm Diameter

10,300 nm<sup>3</sup> Volume

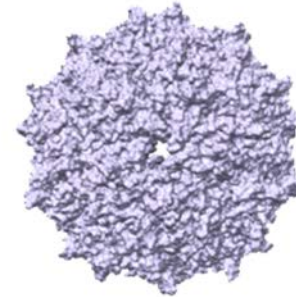


MS2<sup>WT</sup>



27 nm diameter

MS2<sup>S37P</sup> (mini MS2)



17 nm diameter

single  
mutation

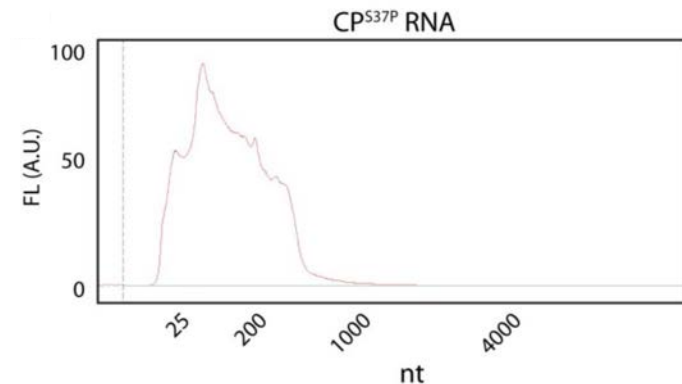
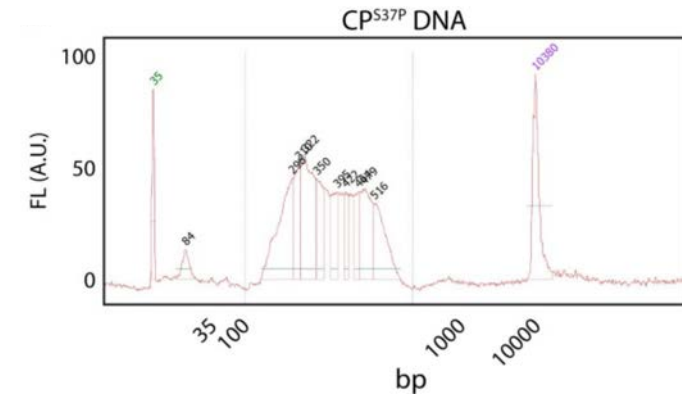


Dr. Danielle Tullman-Ercek

60 Coat Proteins

17 nm Diameter

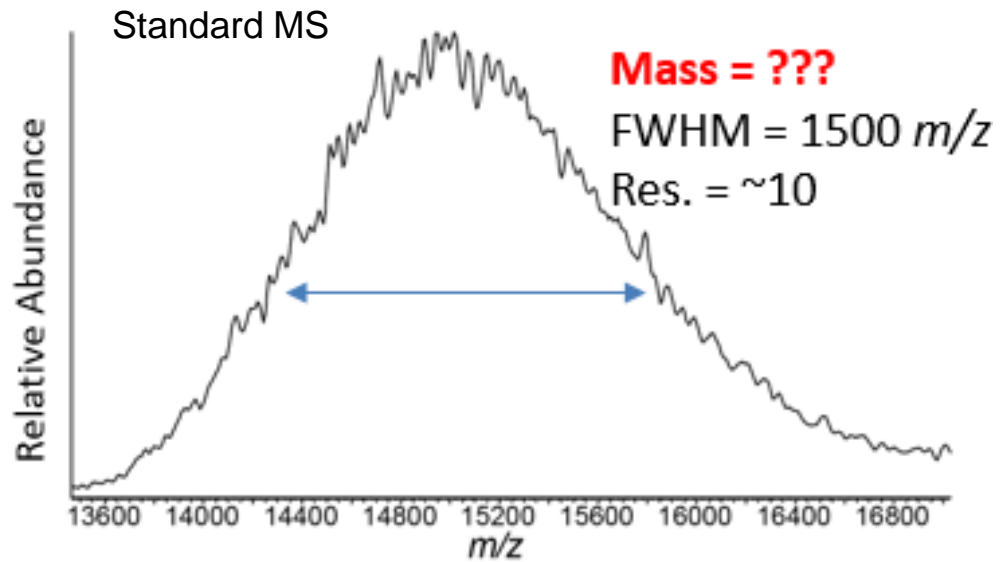
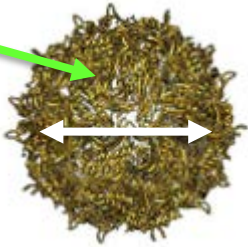
2,600 nm<sup>3</sup> Volume



# Mass Determination: Virus-like Particles

Heterogeneous DNA  
and RNA cargo

17 nm  
T= 1 Geometry

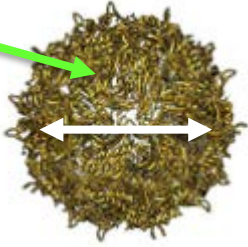




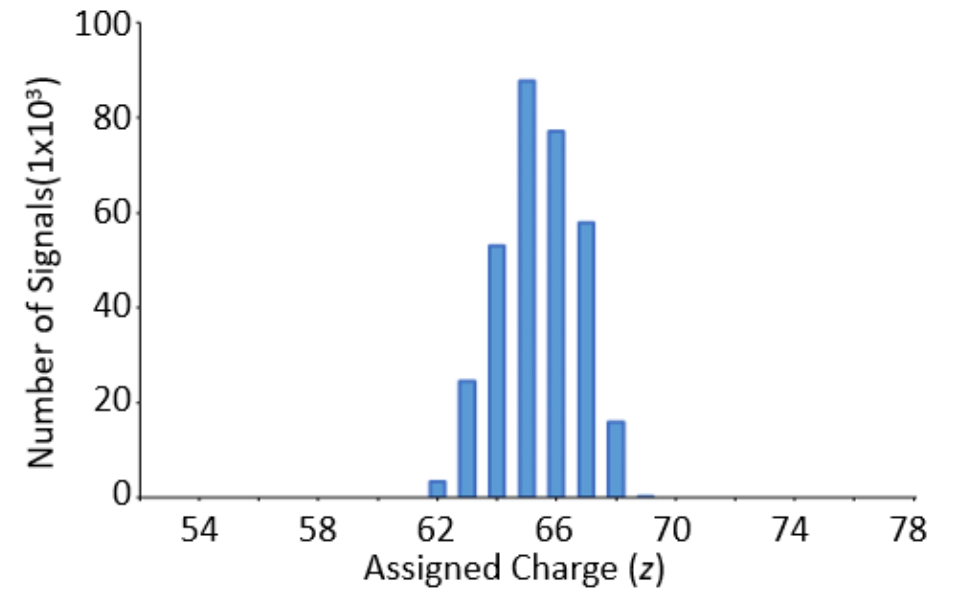
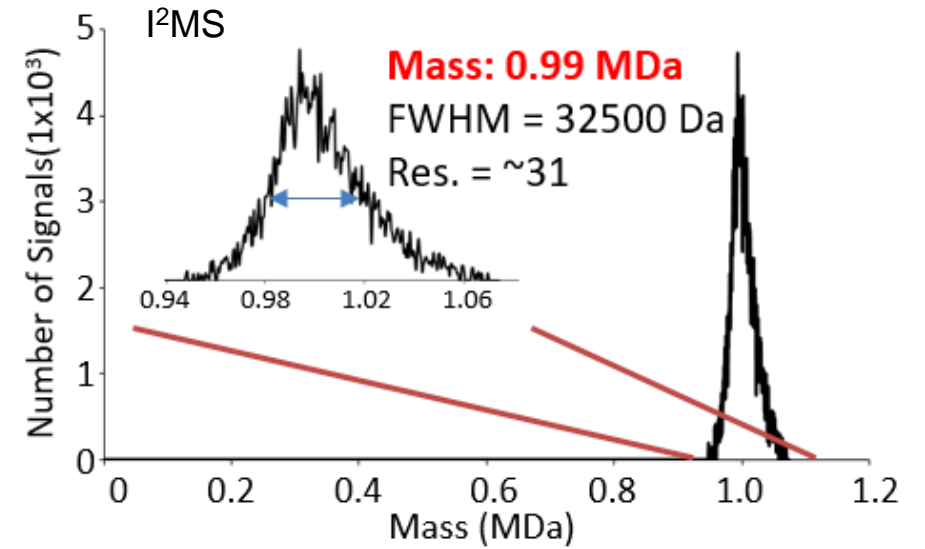
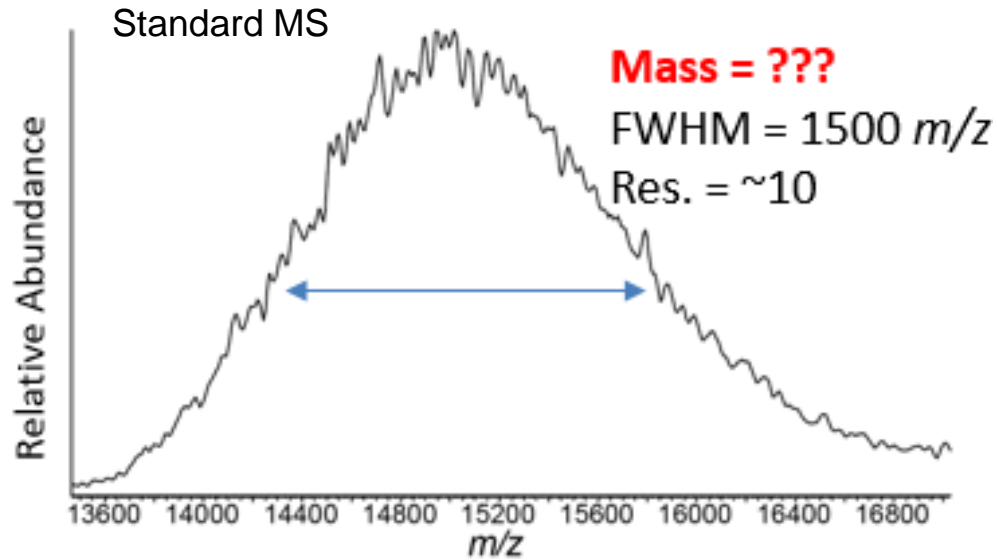
# Mass Determination: Virus-like Particles

Heterogeneous DNA  
and RNA cargo

17 nm  
T= 1 Geometry



	MDa
Capsid	0.82
Cargo(DNA)	0.24
Cargo(RNA)	0.03
<b>TOTAL</b>	<b>~1.09</b>

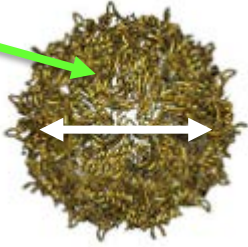


# Mass Determination: Virus-like Particles

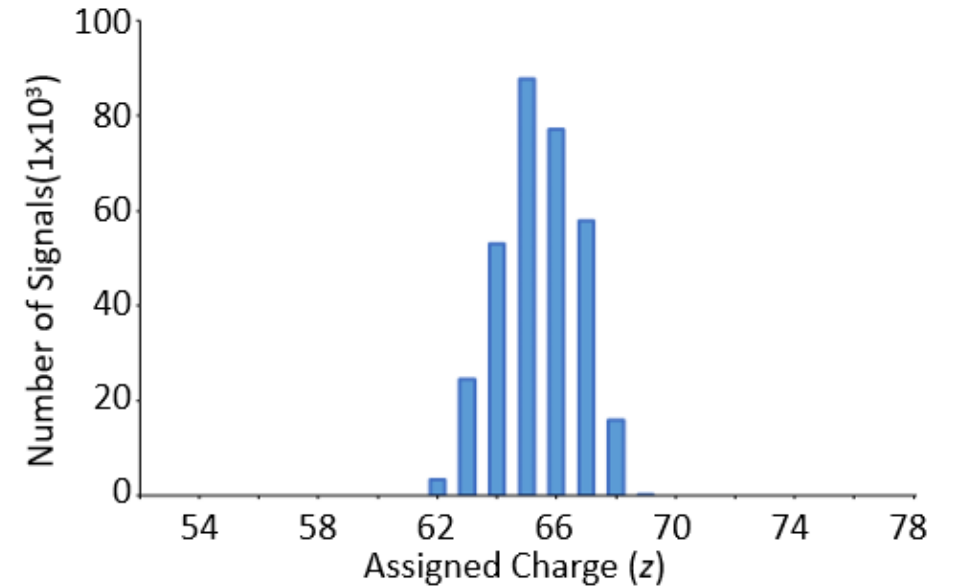
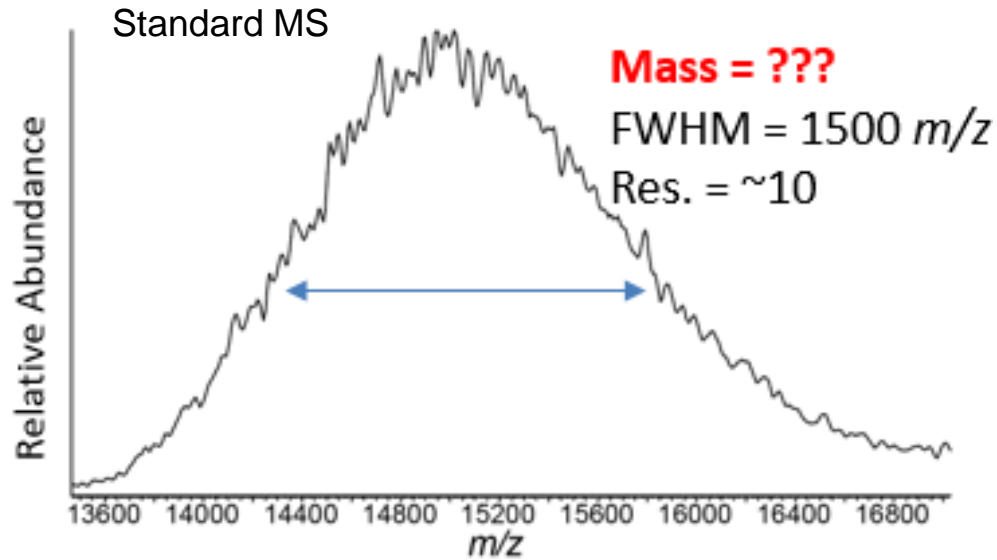
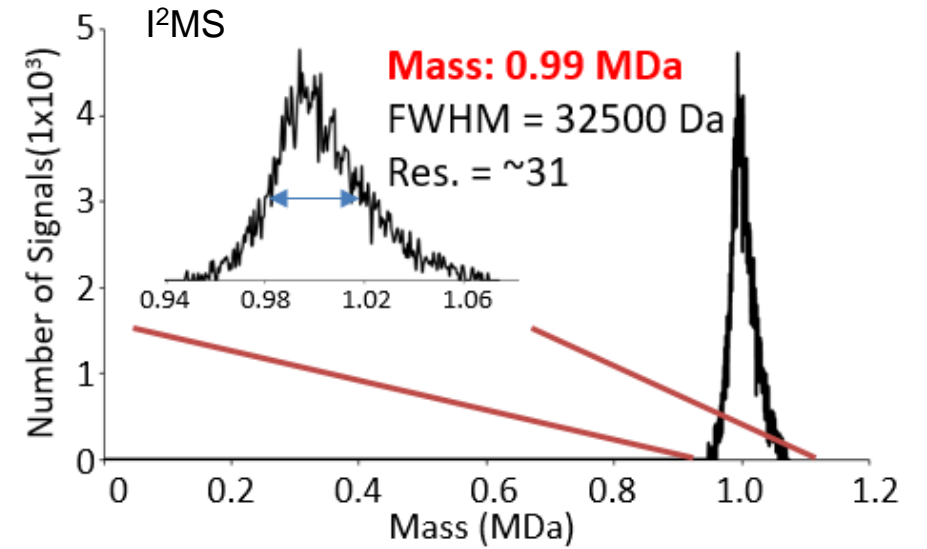
Heterogeneous DNA

**OR** RNA cargo

17 nm  
T= 1 Geometry



	MDa
Capsid	0.82
Cargo(DNA)	0.24
Cargo(RNA)	0.03
<hr/>	
<del>TOTAL</del>	<del>~1.09</del>



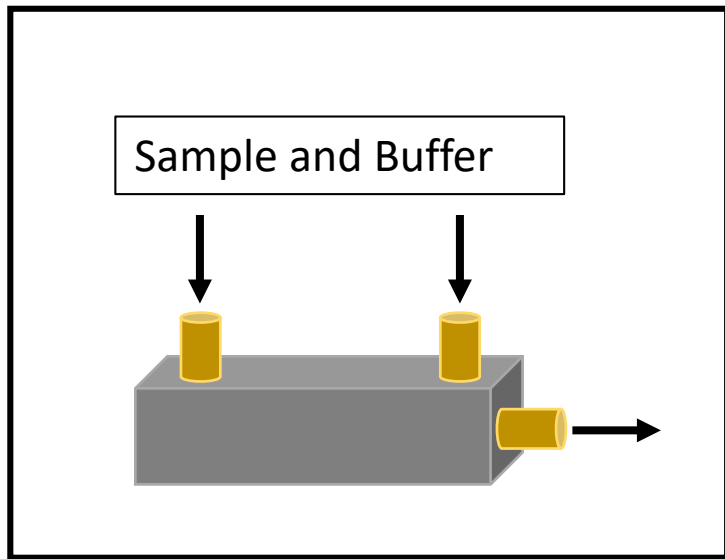
# Improved sample handling for native and denatured top-down MS

## New SampleStream tech:

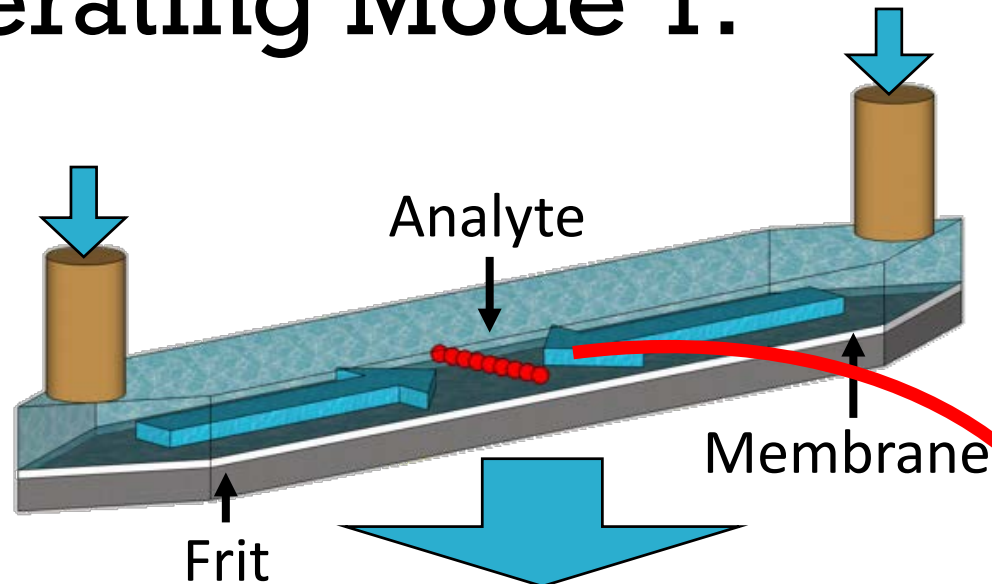
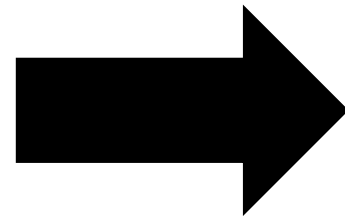
- Automated Buffer Exchange
- 20-100x Protein Concentration
- Detergent Removal
- nanograms to low micrograms



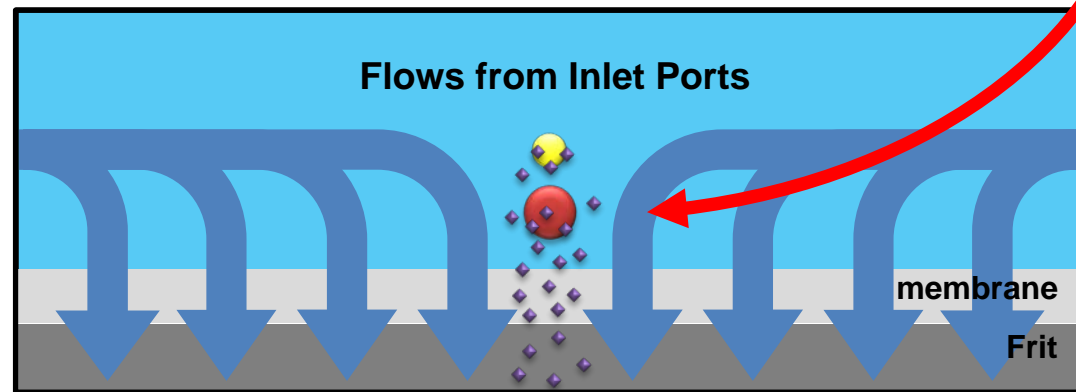
# SampleStream Operating Mode 1: Focusing



Sample introduced to one or both flows entering upper ports

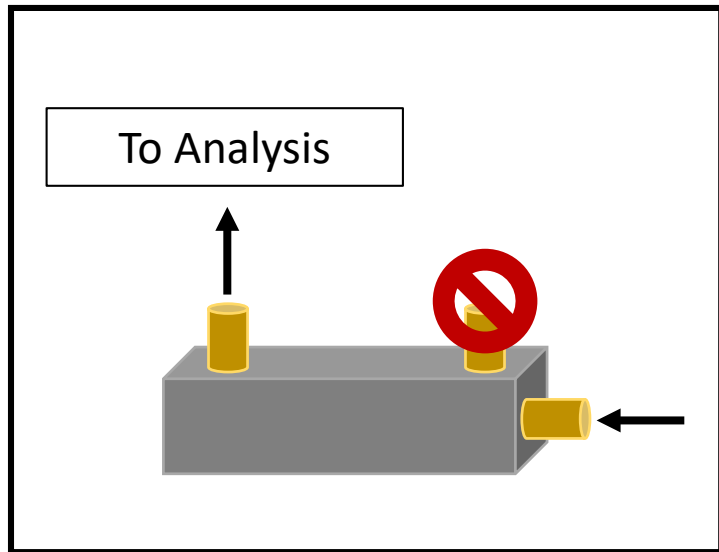


Analytes retained by MWCO membrane accumulate above membrane where flows cancel (concentration)

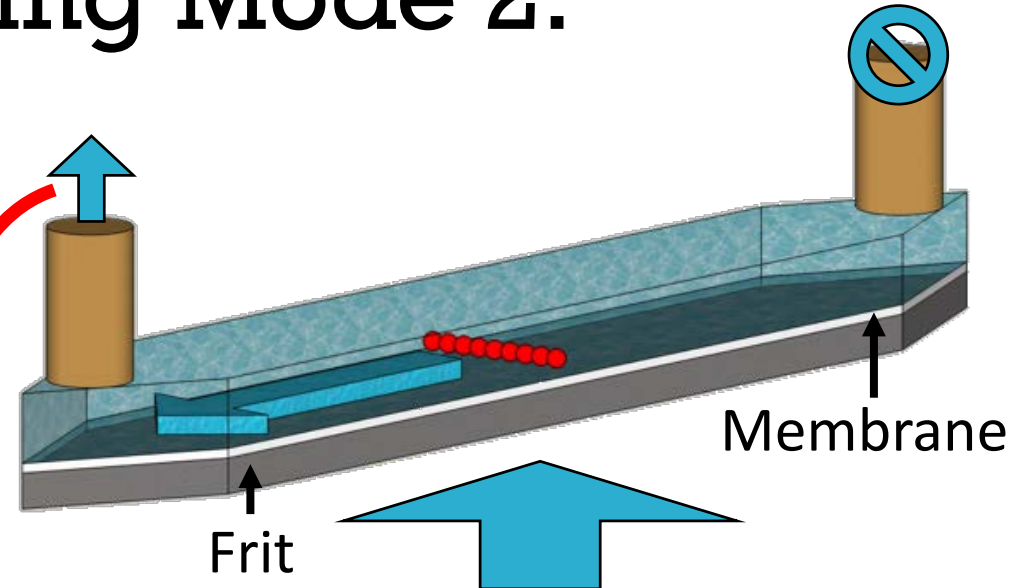
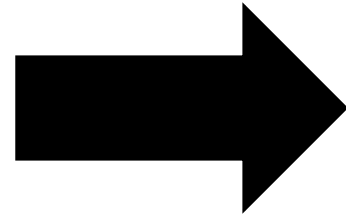


Buffer and small molecules pass through membrane to waste port (buffer exchange)

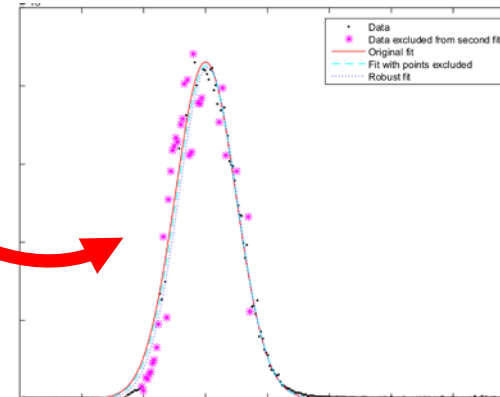
# SampleStream Operating Mode 2: Elution



Elution buffer delivered to waste port

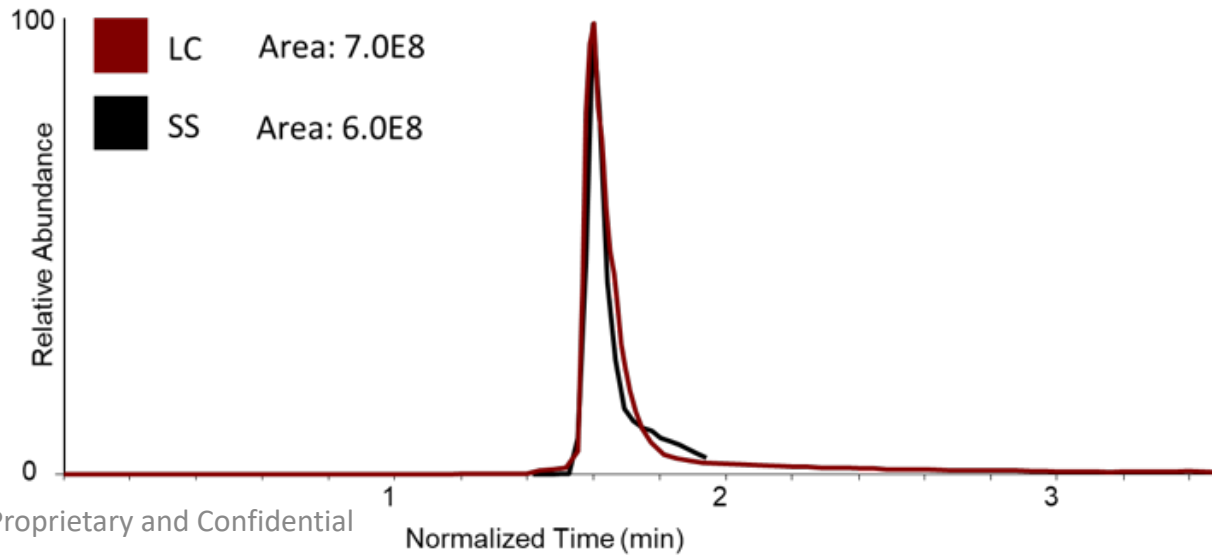
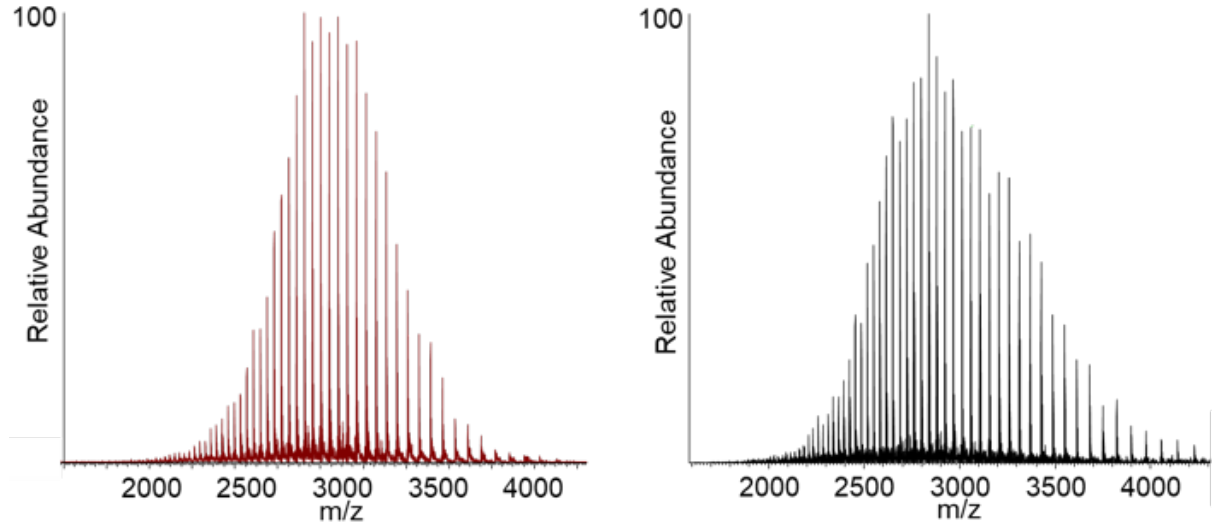


Retained sample band lifted off membrane and transported out top port to analysis



Generates elution profile reminiscent of liquid chromatography

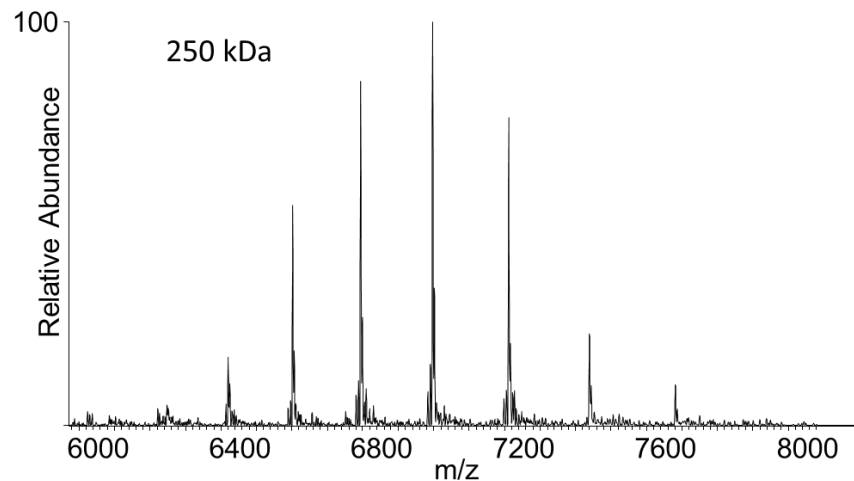
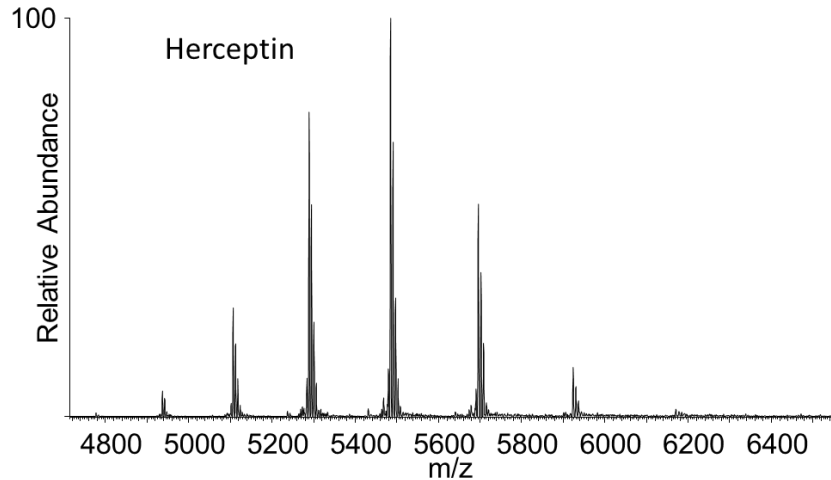
# Comparison of SS to LC



**Key Point:** Equivalent data from 20x more dilute samples with over 2x the throughput

- 500 ng Herceptin analyzed via optimized LC method provided by Genentech (red) and comparable SampleStream method (black)
- Identical mass spectrometer and tune file
- LC loading volume: 5  $\mu$ L
- SS Loading volume: 100  $\mu$ L
- LC run time: 6 min
- SS run time: 2.5 min

# SS & Native Mass Spectrometry



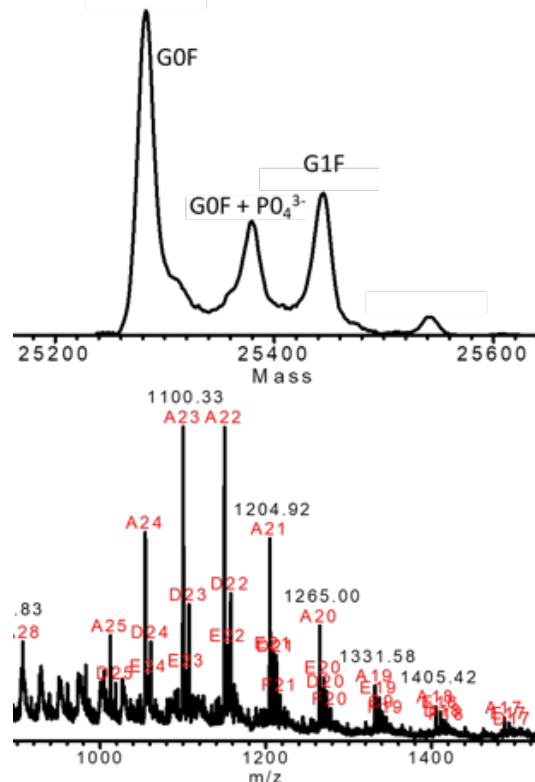
**Key Point:** Simple buffer change enables sensitive native analysis on the same platform → **can rapidly switch between native and denaturing modes**

- 3.3 pmol loaded for various molecules
- SampleStream module operated with native buffer
- Total method time: 80 sec

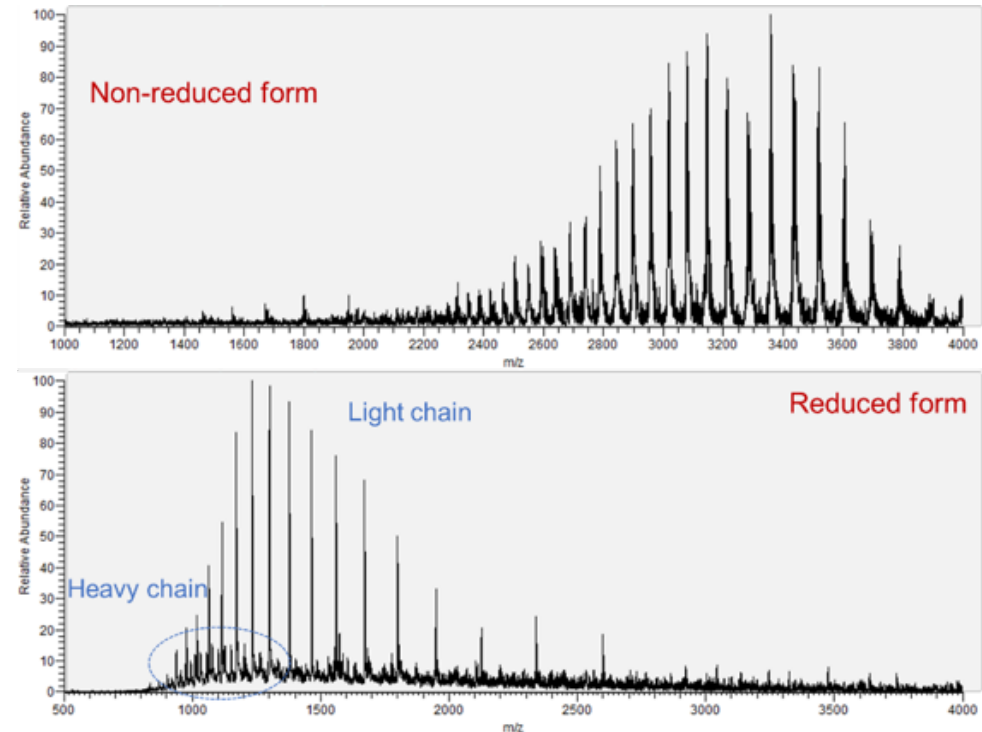
# Sample Preparation in the SS Channel

**Key Point:** Can perform enzymatic digestions and chemical transformation inside the channel

## Digestion



## Reduction







# The **SampleStream**<sup>TM</sup> Platform



Philip Compton, Ph.D. CEO

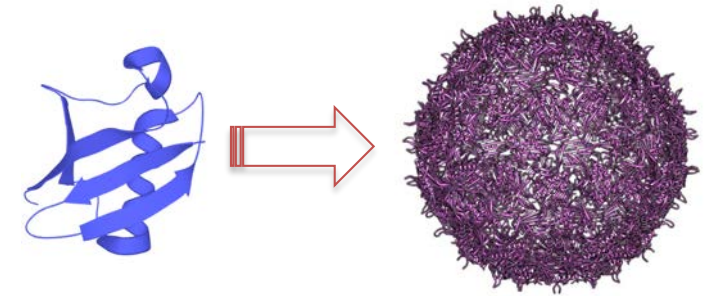
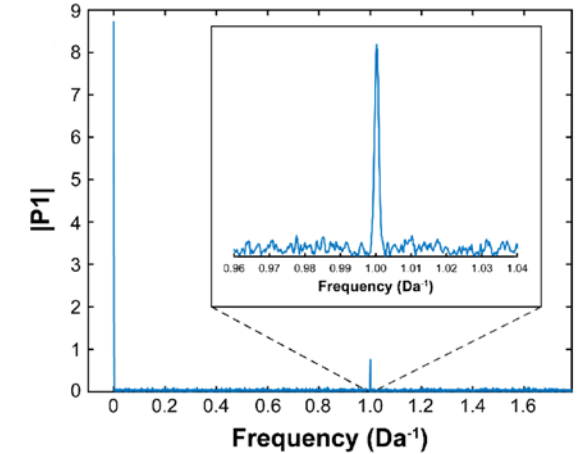
[Philip-Compton@IPTInc.com](mailto:Philip-Compton@IPTInc.com)

Jared Drader, Ph.D. CTO

[Jared-Drader@IPTInc.com](mailto:Jared-Drader@IPTInc.com)

# Concluding Remarks

- Orbitrap analyzers are capable of multiplexing CDMS analysis
  - ↳ New paradigm for MASS spectrometry
- I<sup>2</sup>MS can quickly produce native mass spectra (even isotopically resolved up to 466 kDa)
- Orbitrap-based I<sup>2</sup>MS has many applications for proteins in the kDa to MDa range, e.g., COVID-19 vaccine candidates with high glycosylation
- SampleStream for improved sample handling of intact proteins



# Acknowledgements



**Jared Kafader**  
**Philip Compton**  
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