

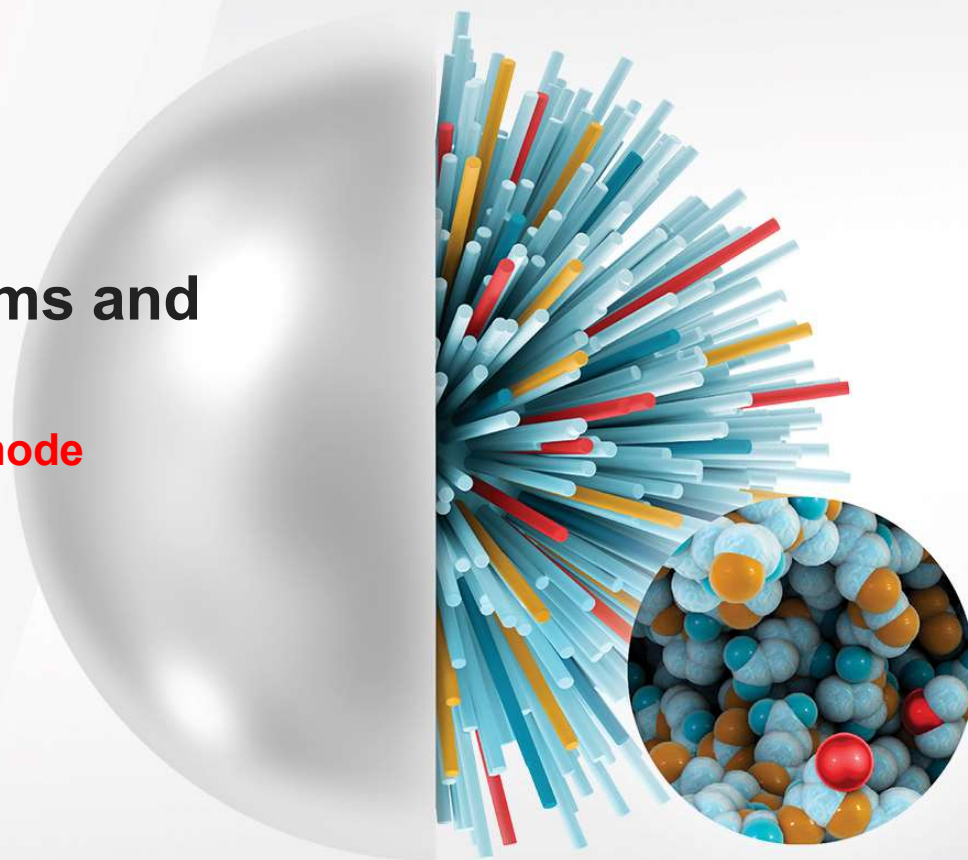
New Tools for Confident Proteoforms and Macromolecules Analysis

Thermo Scientific™ Direct Mass Technology™ mode

Weijing Liu

Omics Vertical Marketing

 The world leader in serving science



Outlines

- **How does it work?**

Mass spectrometry vs. Direct Mass Technology mode

- **End-to-end workflow**

1. Calibration: Charge state calibration
2. Data acquisition: Direct Mass Technology mode
3. Data processing: STORlboard software

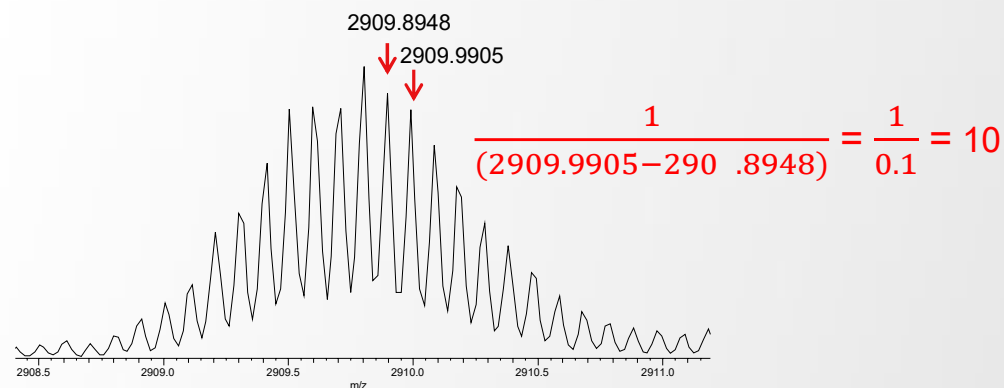
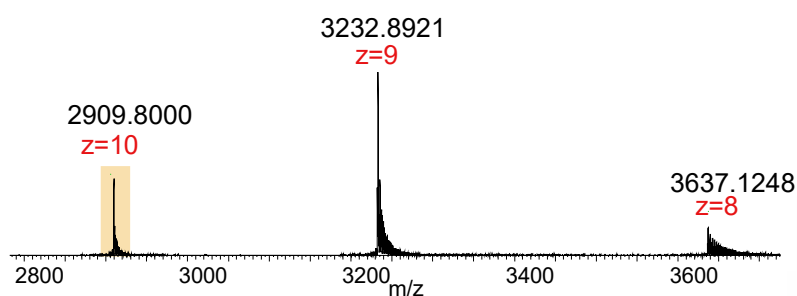
- **Applications**

Glycoproteins, membrane proteins, virus

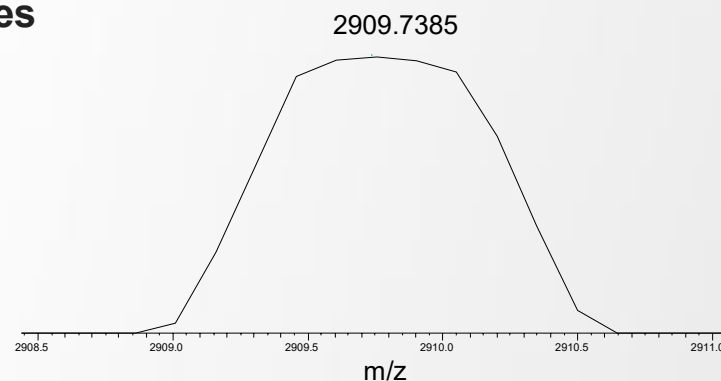
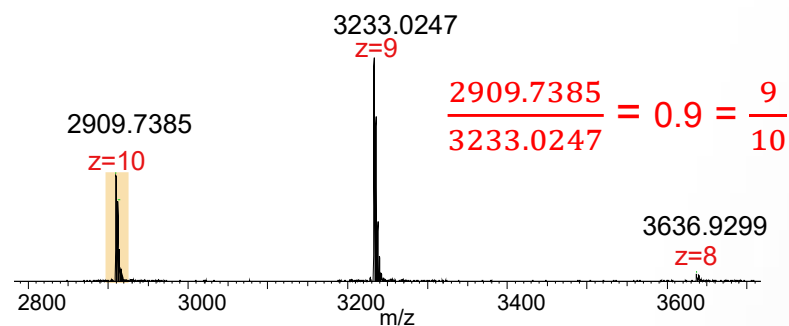
How does it work?

“Mass” Spectrometry: we only measure m/z

- z is determined from the resolved isotopes



- z is determined from the resolved charge envelopes

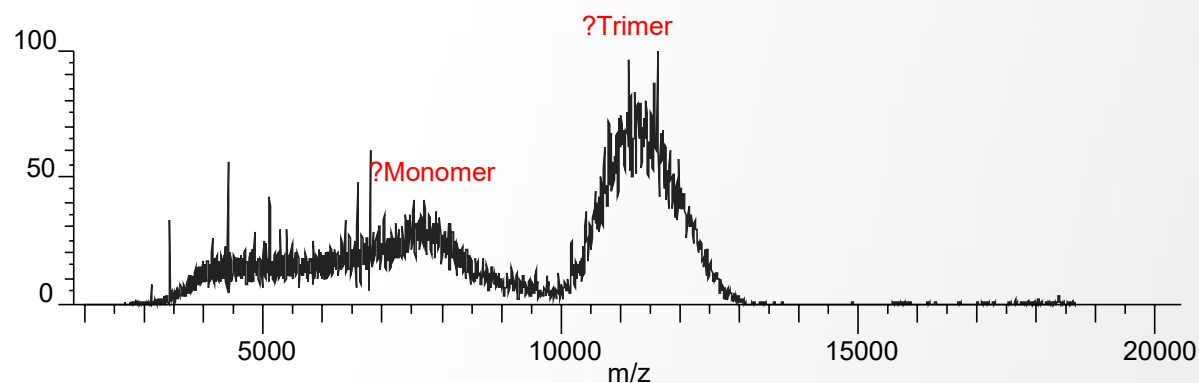


“Mass” spectrometry are measuring m/z , not measuring “mass”

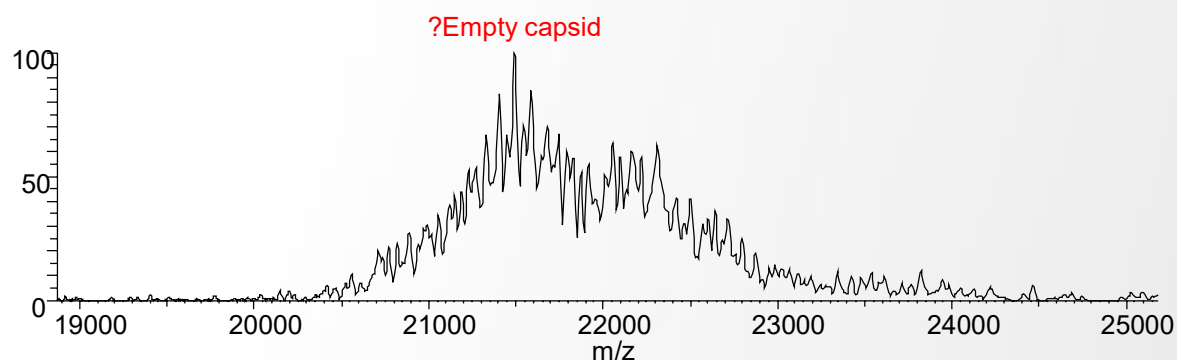
Mass calculation relies on the charge state deduced by m/z

Challenge: spectrum *without* resolved charge states and isotopes

- Spike protein



- Adeno-Associated Virus



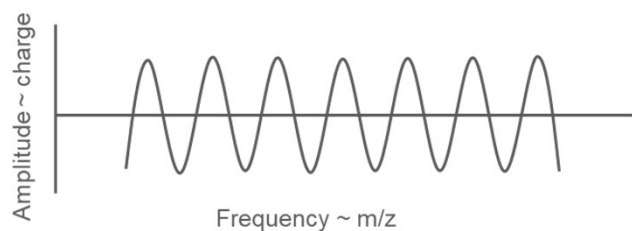
Charge states and/or isotopes are **NOT** resolved



• z cannot be determined
• mass cannot be calculated

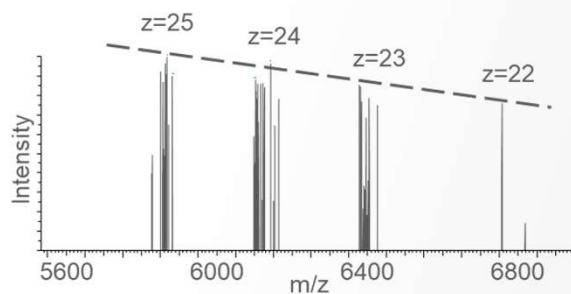
Solution: Charge detection mass spectrometry measures m/z and z

m/z from frequency
 z from amplitude



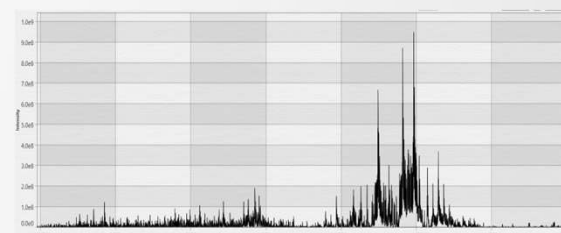
- m/z is measured from the frequency of signal
- z is measured from the amplitude of signal
- m/z and z are measured simultaneously

Many individual ion scans



- Hundreds of **individual ions** per spectrum in m/z space
- Collect hundreds of scans

Signals in mass domain



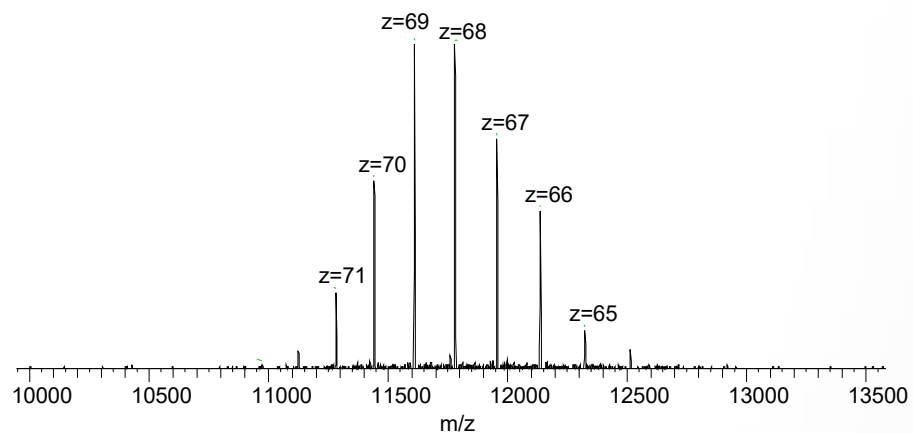
- Ion **Mass** is calculated from m/z and z

$$m/z \times z \rightarrow M$$

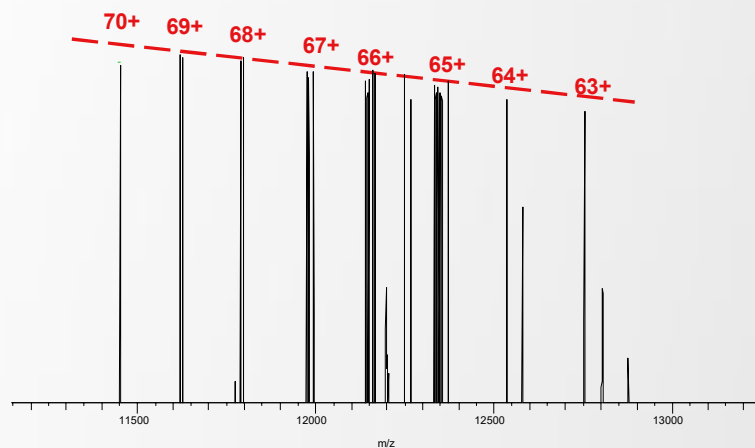
Direct Mass Technology mode

Comparison of ensemble vs. Direct Mass Technology

Ensemble native MS of GroEL (801 kDa)



Direct Mass Technology of GroEL (801 kDa)



Fit-for-purpose instrumentation for native MS

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- 1 Ultra-High Mass Range
✓ m/z 350 to 80,000
- 2 High mass quadrupole
✓ Isolation up to m/z 25,000
- 3 Desolvation and dissociation
✓ In-source trapping or CID, and HCD

NEW

- 4 Direct Mass Technology Mode
✓ Enabling charge detection MS

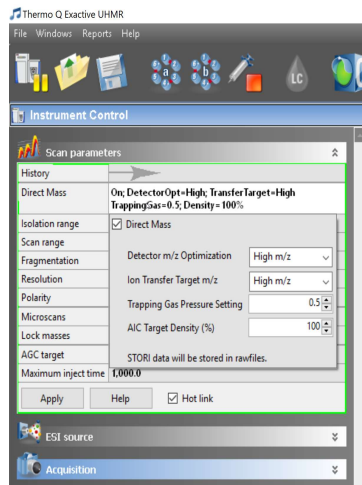


Thermo Scientific™ Q Exactive™ UHMR Hybrid
Quadrupole-Orbitrap™ Mass Spectrometer

End-to-end workflow

An end-to-end workflow

Data Acquisition



New Data Format

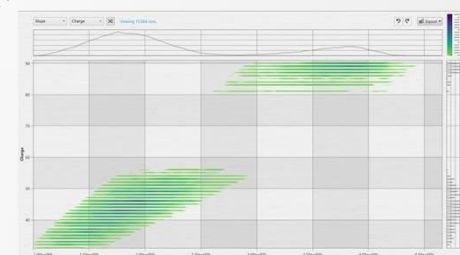


.raw files containing
STORI* data



Hundreds of **individual ions**
per spectrum in *m/z* space

New Data Processing



- Charge calibration
- Data analysis by STORIboard
- Create high resolution spectrum in **Mass domain**
- Export **centroid .raw Mass spectrum** for annotation

*Selective Temporal Overview of Resonant Ions (STORI)

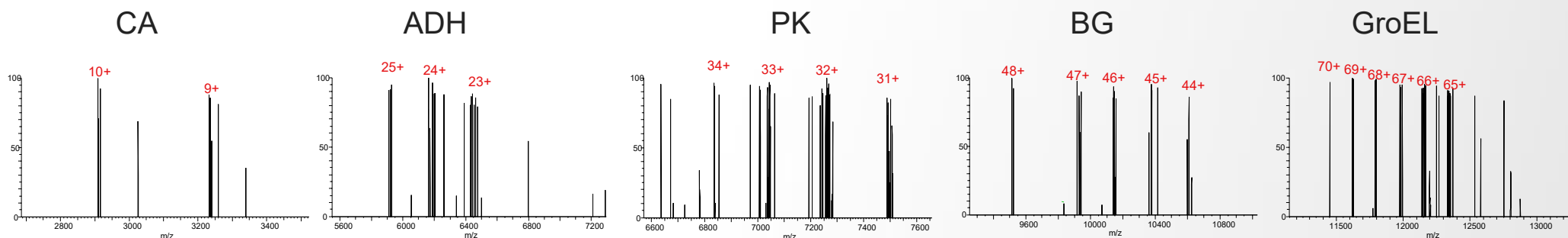
Direct Mass Technology mode workflow

1. Charge calibration produce
Build calibration curve with protein standards
2. Direct Mass Technology mode acquisition
Charge detection with STORI data embedded into .raw file
3. STORlboard software
Process and visualize data

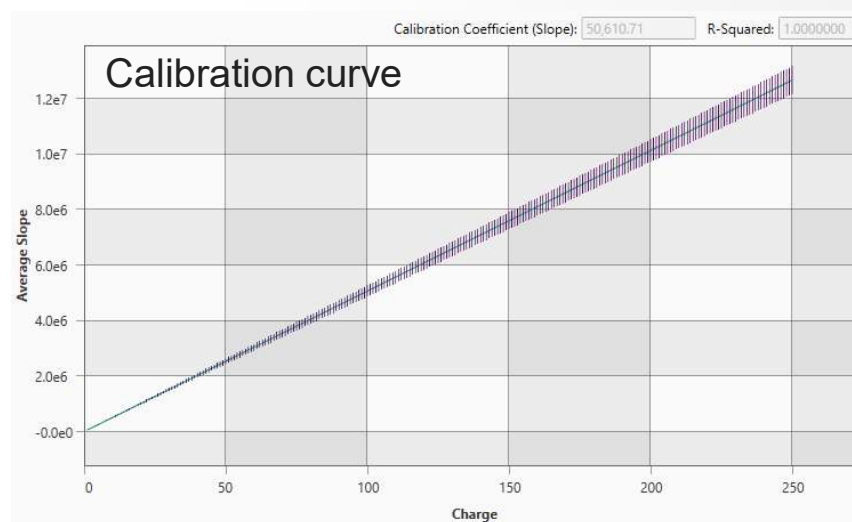


1. Charge calibration is necessary

Current recommended calibrants:



.raw files containing
STORI data



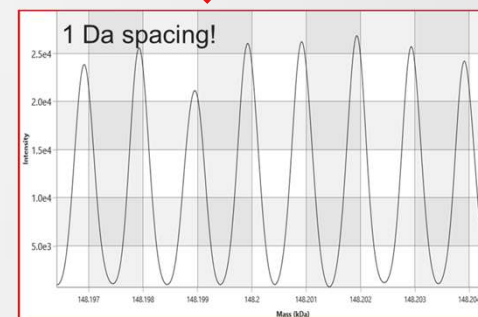
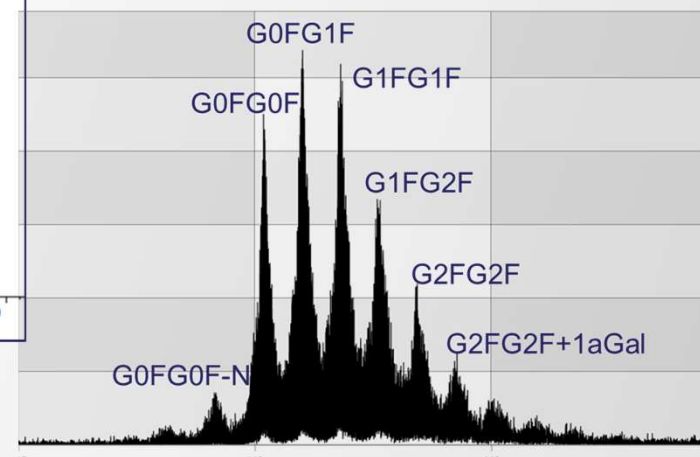
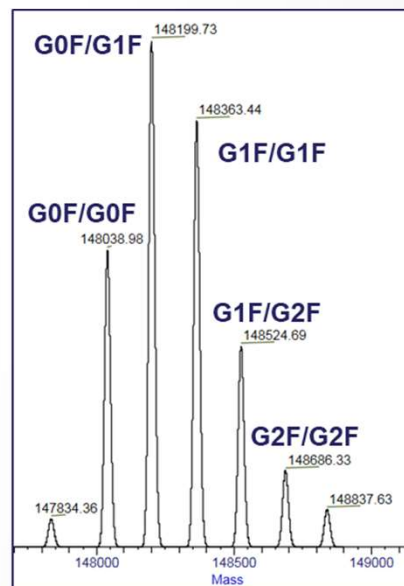
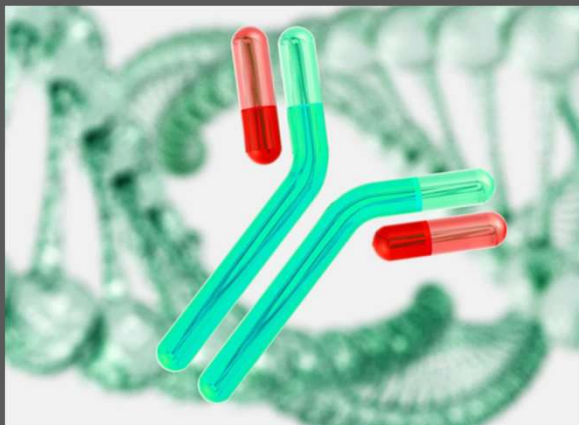
CA: Carbonic anhydrase
ADH: Alcohol dehydrogenase
PK: Pyruvate kinase
BG: Beta-galactosidase
GroEL: Chaperonin 60

Analysis of monoclonal antibody with exceptional resolution

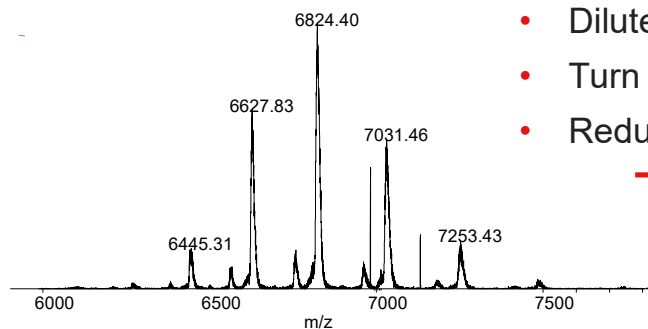
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NIST Antibody (RM 8671)

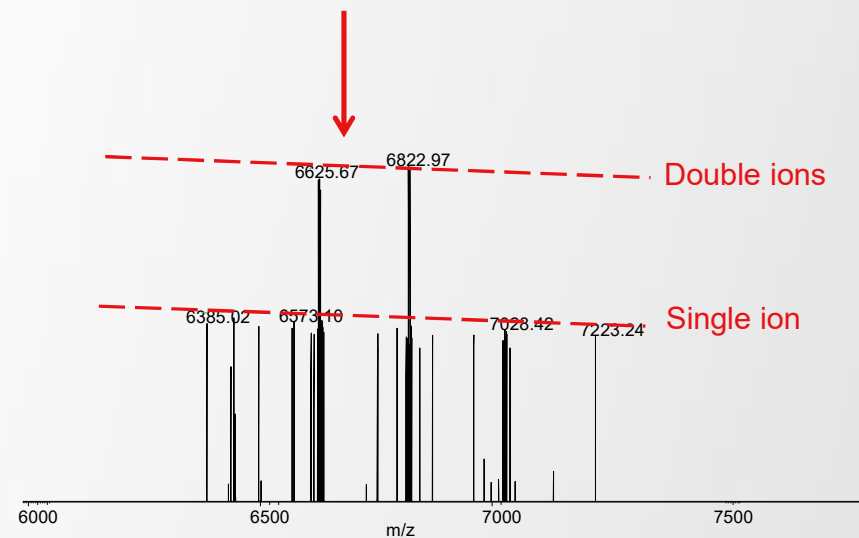
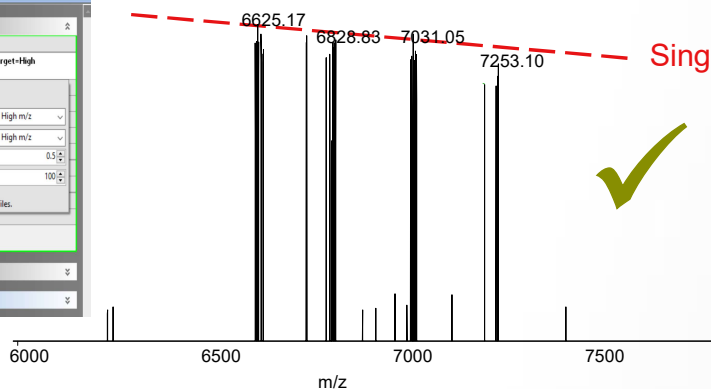
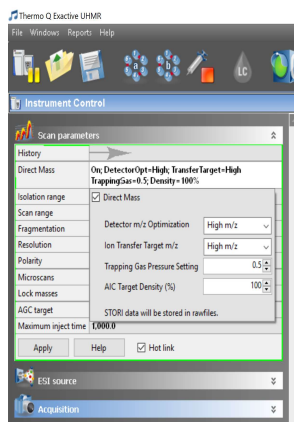
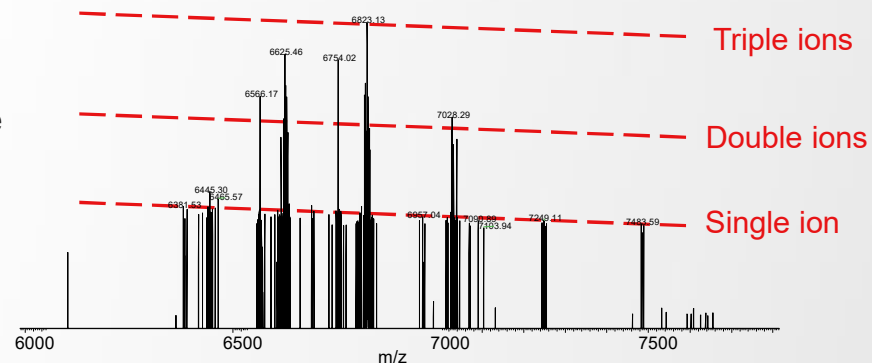
- Direct Mass Technology mode reveals more glycoforms/proteoforms than the native MS measurement
- Isotopically resolved peaks



2. Signals attenuation to reach single ion scan

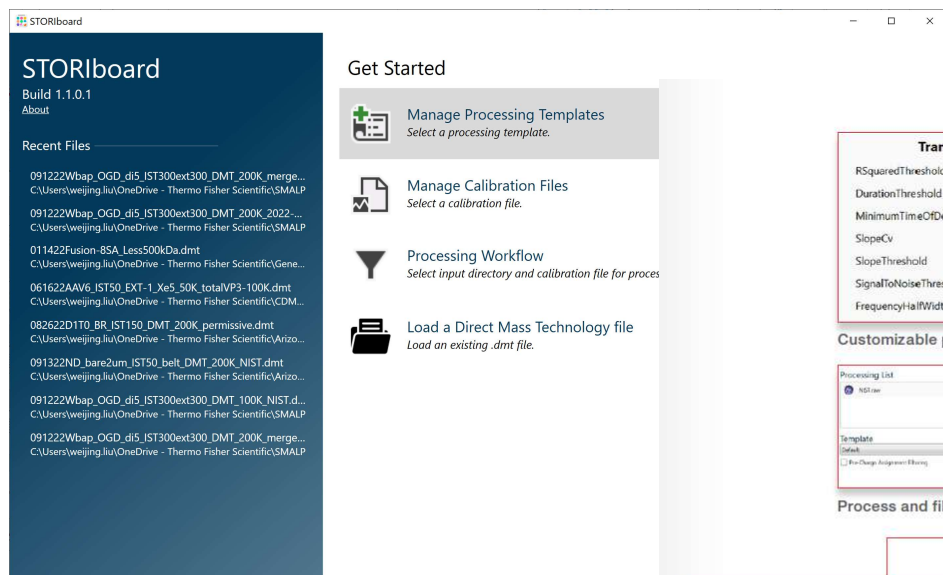


- Dilute sample
- Turn down trapping gas pressure
- Reduce injection time

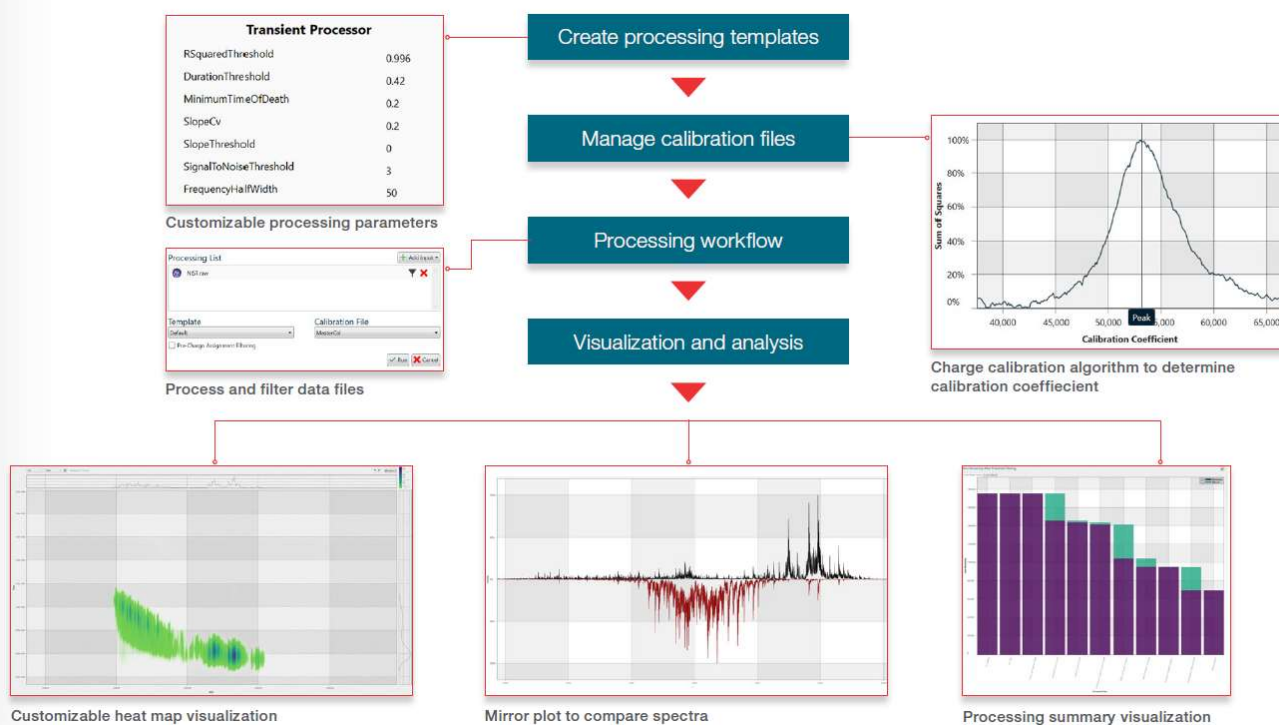


- Automated ion control (AIC) for dynamic ion injection

3. Data processing with STORlboard



STORlboard processing and visualization functionality



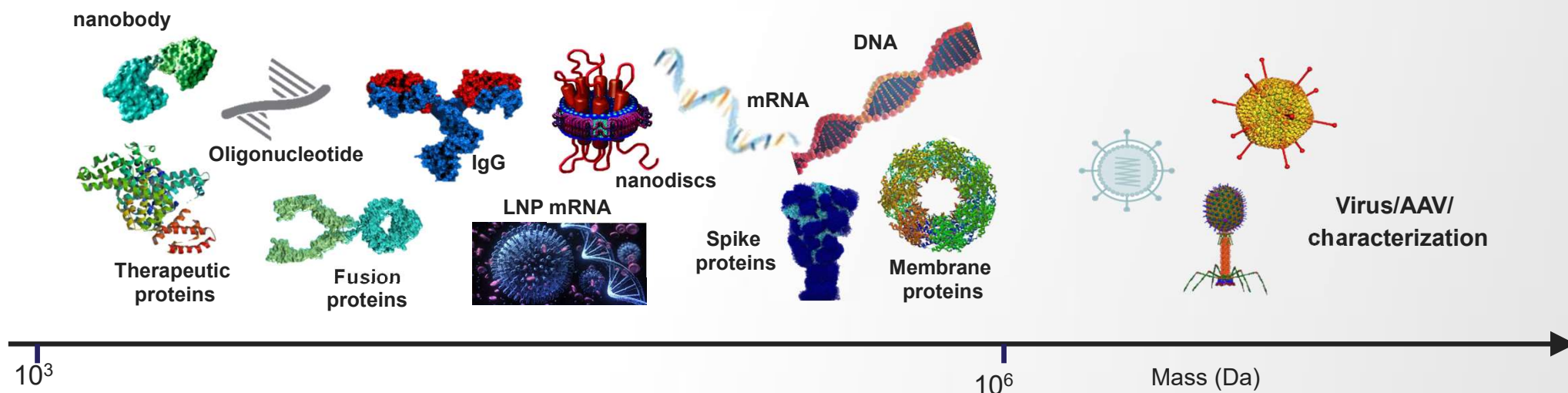
*STORlboard developed in collaboration with Proteinaceous

Applications

Direct Mass Technology mode: Fields of Use

Address astounding complexity and rapidly growing analytical need

Emerging larger and more diverse space with high mass and complexity

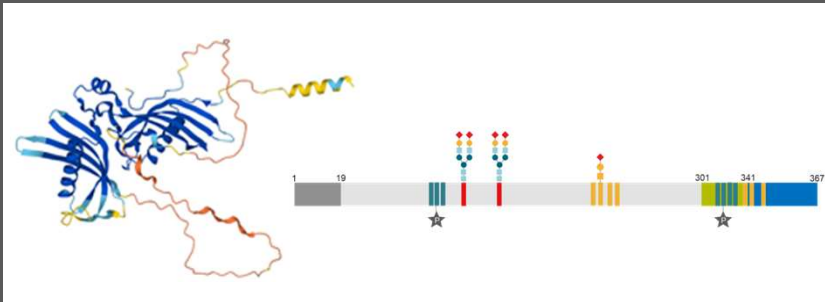


Determine molecular weight and heterogeneity with Direct Mass Technology mode

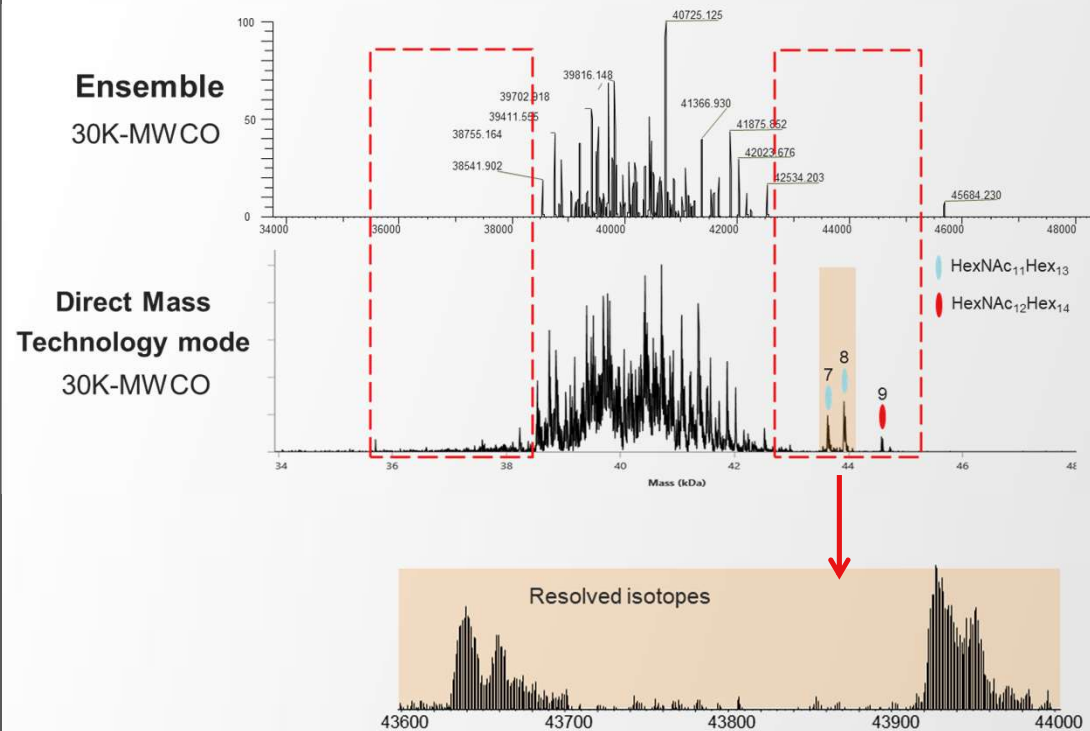
Reveal proteoforms in greater details

Human Fetuin A

- Predicted mass: ~37 kDa
 - 2 N-linked glycosylation sites
 - Suggested 5 O-linked glycosylation and 6 phosphorylation sites
- Increased levels of hFet have been linked to higher risk of cardiovascular disease (CVD) and incident type 2 diabetes (T2DM)*



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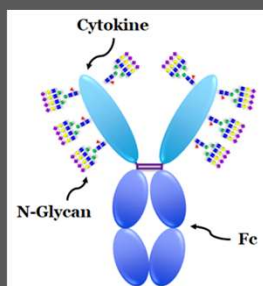
- Directed Mass Technology mode isotopically resolved a larger number of low abundant glycoforms

Heavily glycosylated Fc fusion biotherapeutics

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Fc fusion proteins

- Heavily glycosylated Fc-fusion protein
- Samples with 8, 12, and 15 glycosylation sites
- Over 10 different glycans per site

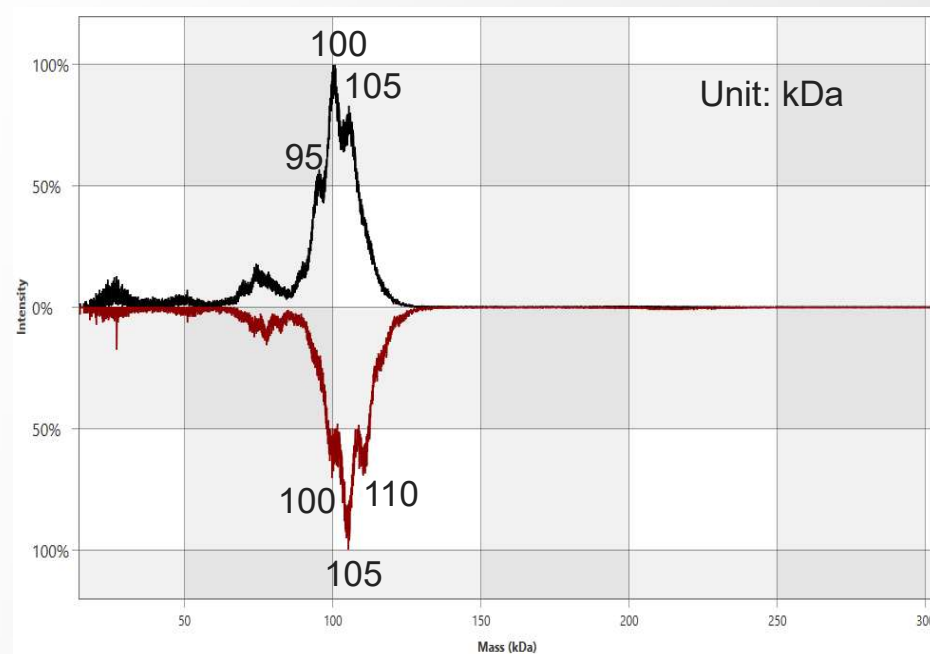


Samples courtesy of:
Wendy Sandoval

Genentech

SA8

SA15



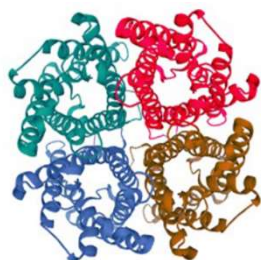
- Directed Mass Technology mode reveals the MW profile difference from sample to sample

Increased dynamic range for membrane protein characterization

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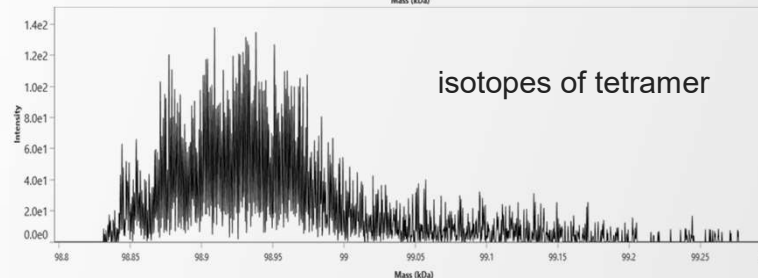
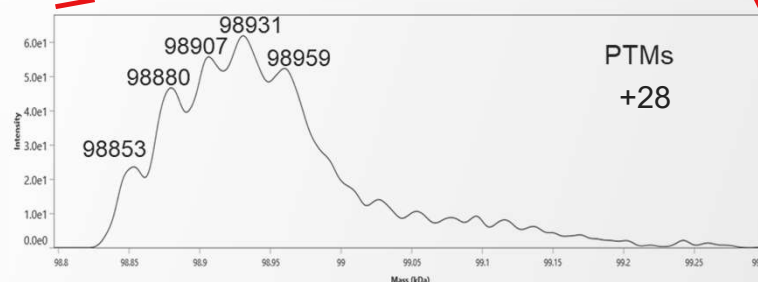
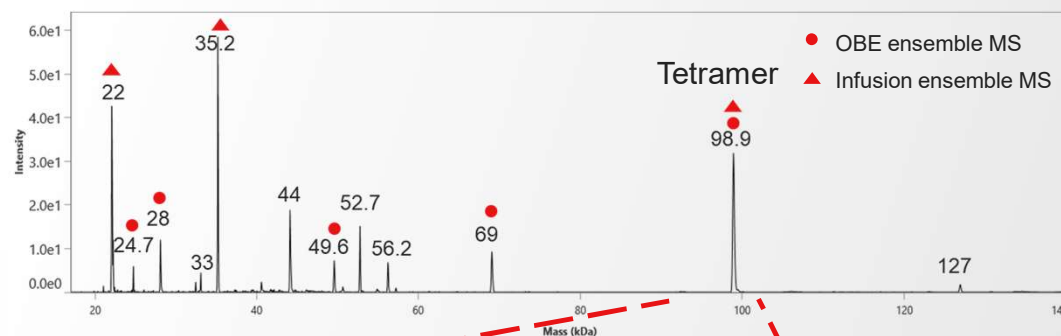
Aquaporin Z

- E. coli* Aquaporin Z (AqpZ) is an approximately 99 kDa membrane protein comprised of four subunits



99 kDa tetramer

Sample courtesy of
Prof. Michael Marty
University of Arizona



- Direct Mass Technology mode demonstrates much wider dynamic range than the ensemble MS measurement

Resolve membrane protein in complex matrix

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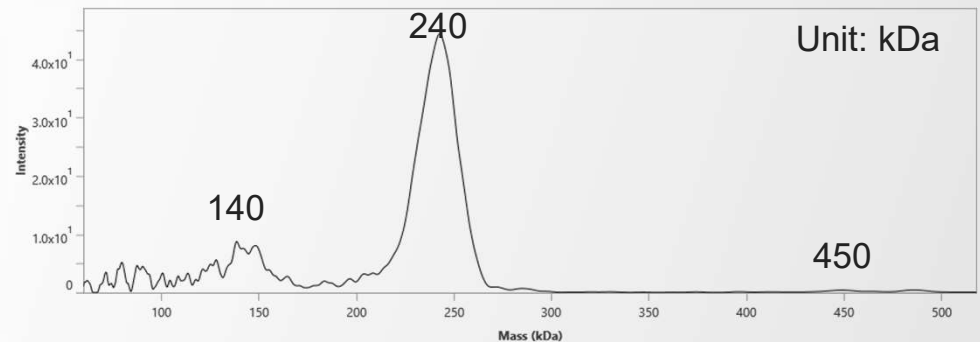
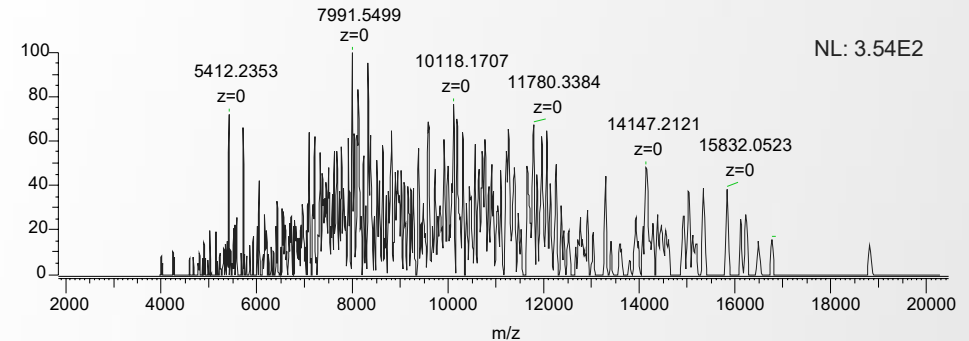
WbaP

- WbaP is a 60.85 kDa membrane protein prepared in SMALP (styrene maleic acid lipid particles)

Samples courtesy of:

Dr. Gregory Dodge

Prof. Barbara Imperiali



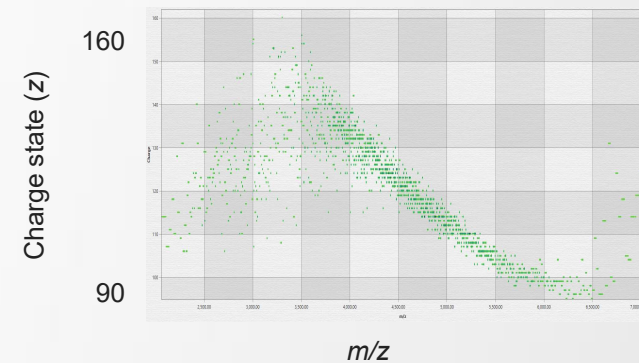
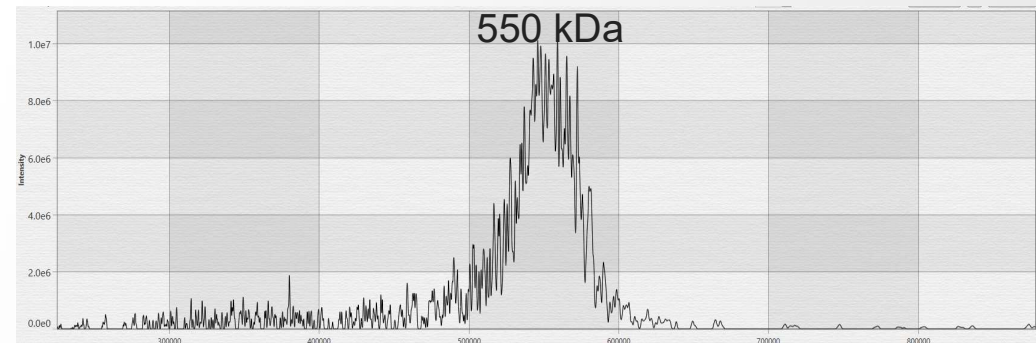
- Direct Mass Technology mode provides MW profile to confirm the oligomeric forms

Access intact mRNA mass measurements

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Intact mRNA

- CleanCap® Firefly Luciferase Mrna
- 1929 nucleotides
- Complementary technique to sequencing via digestion and oligonucleotide mapping



- Direct Mass Technology mode elucidates the MW matching well with theoretical MW

Characterization of gene therapy vectors

Intact Adeno Associated Virus (AAV)

EMPTY

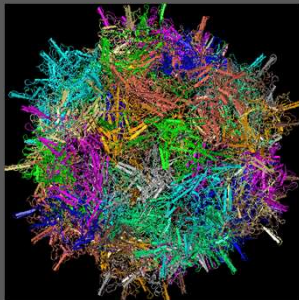


- 1 icosahedral capsid consisting of **60 subunits** of 3 distinct viral proteins

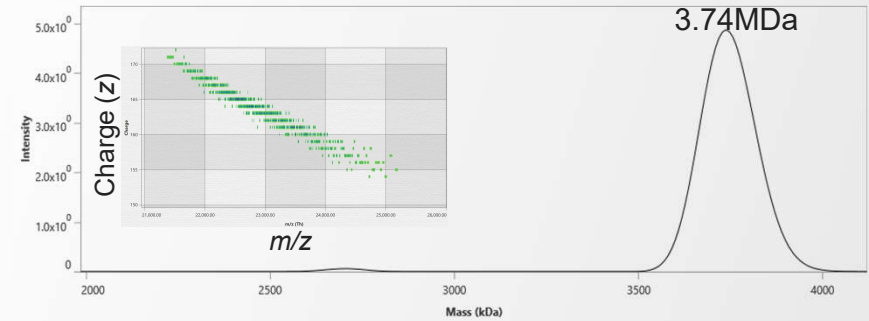
FULL



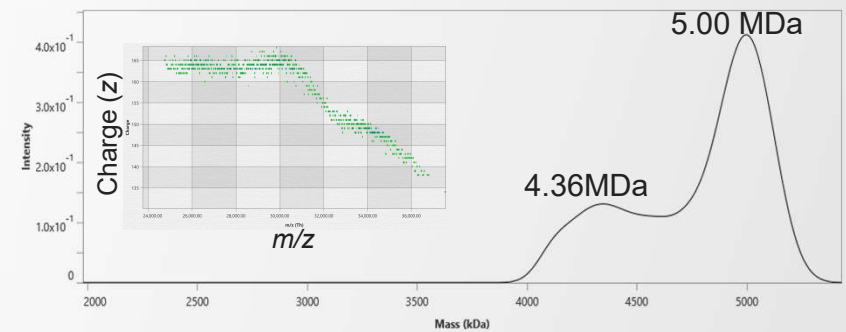
- **Genome material** packaged inside the capsid
- **3 - 5 MDa** size



Empty



Partial/Full



Key Points of Direct Mass Technology mode

WHAT?

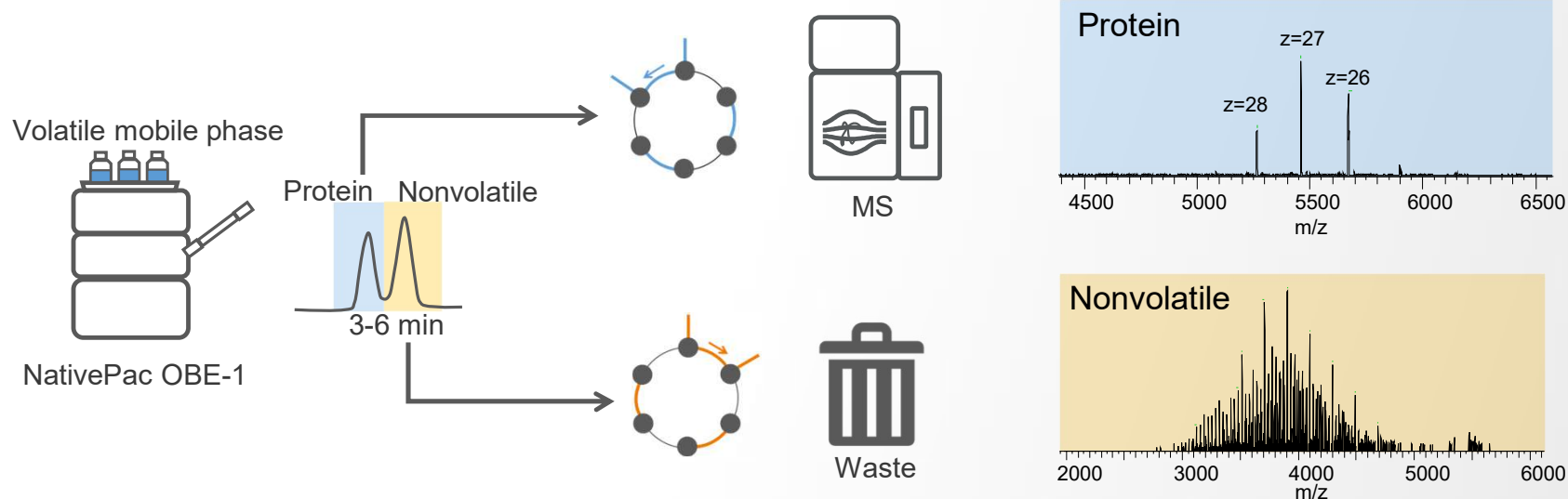
- Simultaneous determination of charge and mass to charge
- Individual ion detection
- Parallel measurement of hundreds of individual ions
- Improved performance at high mass

BENEFIT?

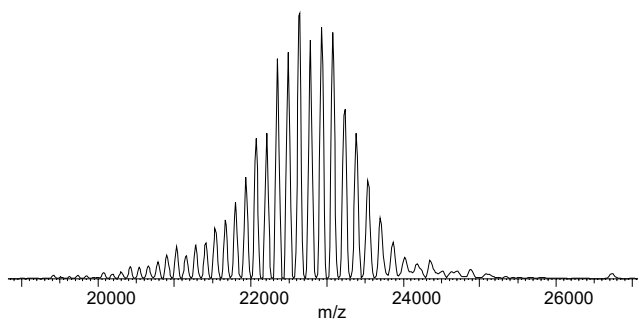
- Determine mass where it was impossible before
- Improved dynamic range
- Increased mass range, kDa-MDa
- Increased insight into complexity
- Improved sensitivity
- Ease-of-use

Rapid Protein Screening Using Online buffer exchange-native MS

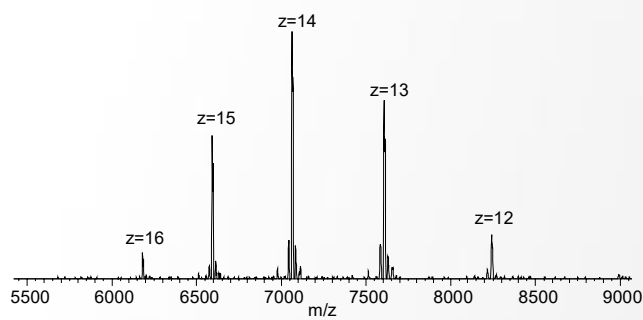
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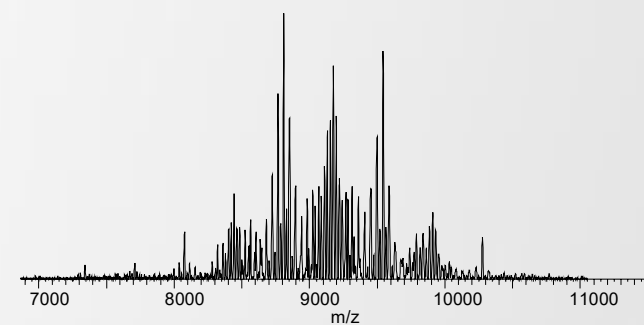
Adeno-associated virus



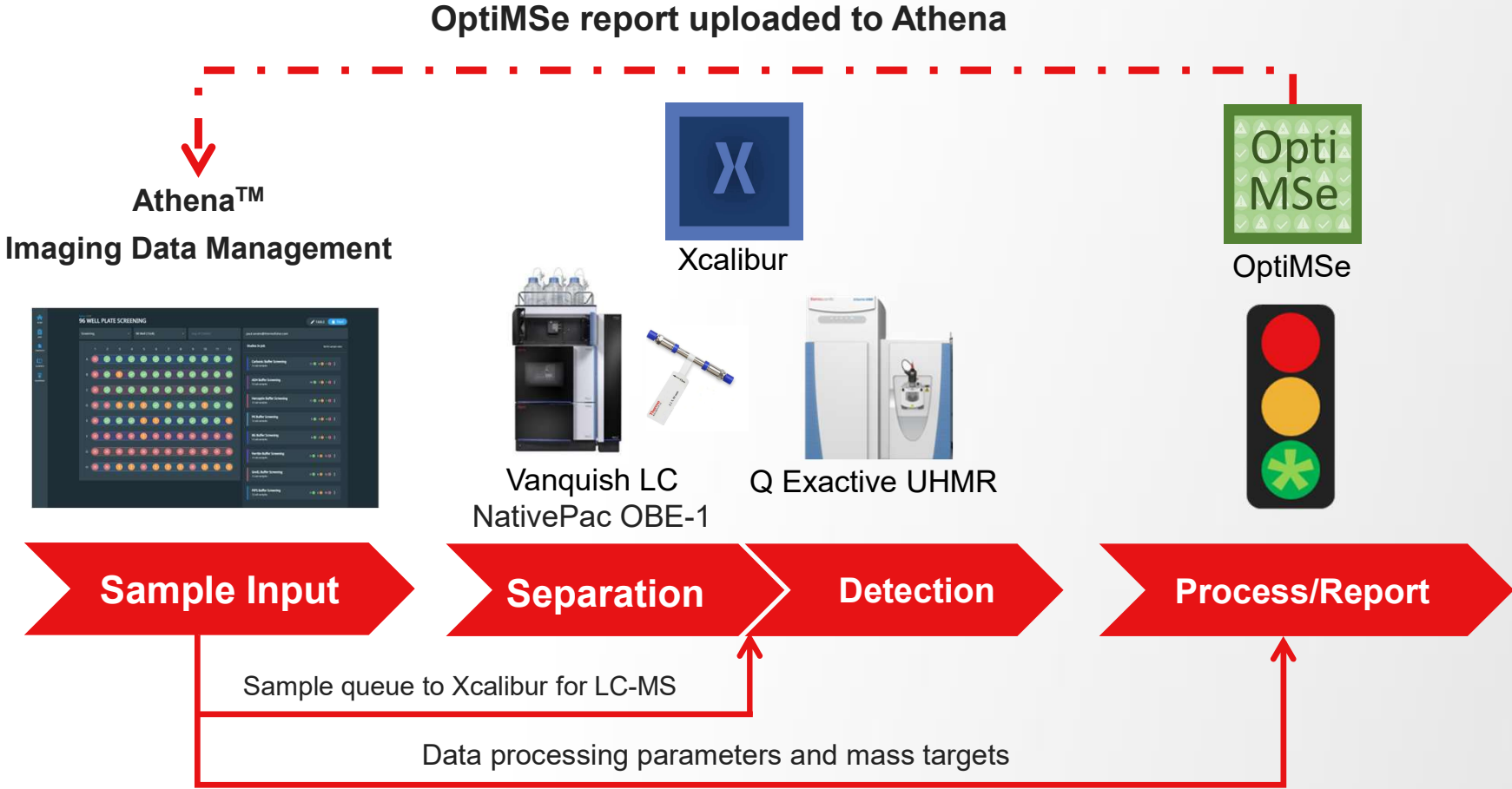
Membrane proteins in detergent



Membrane proteins in nanodiscs



OptiMSe: End-to-end Structural Biology Screening



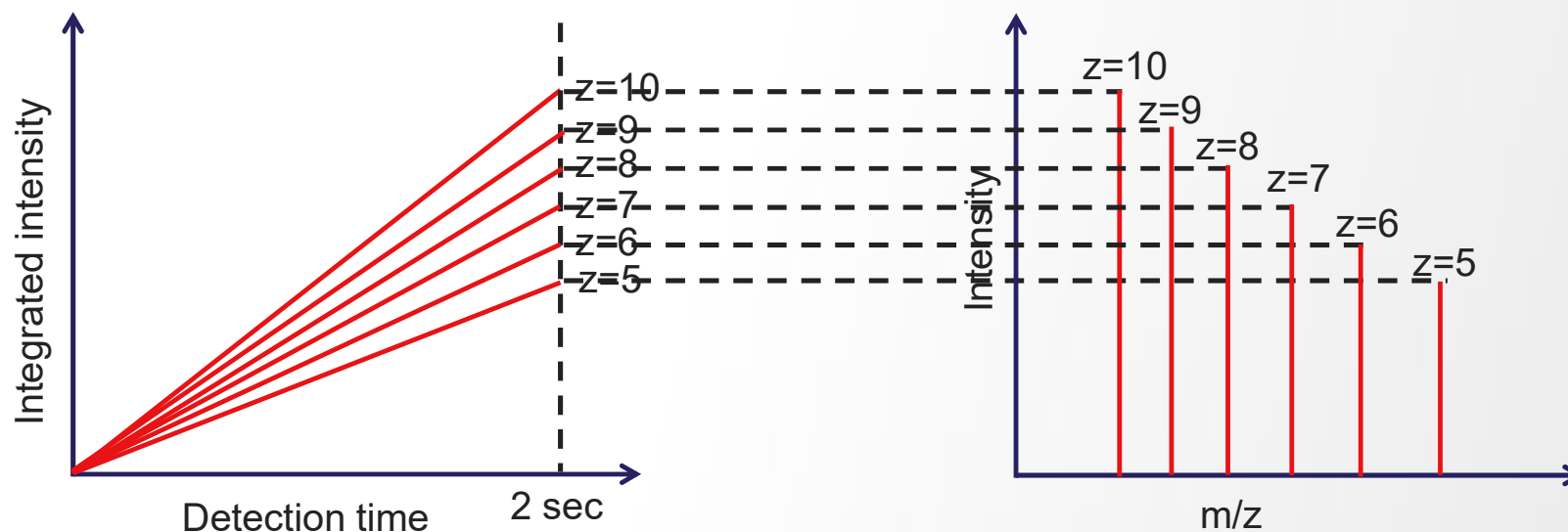
Thank you

The line has been unmuted for questions.



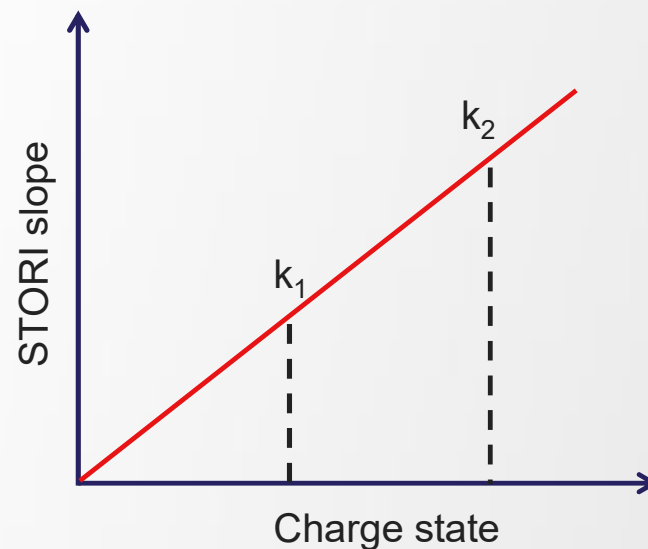
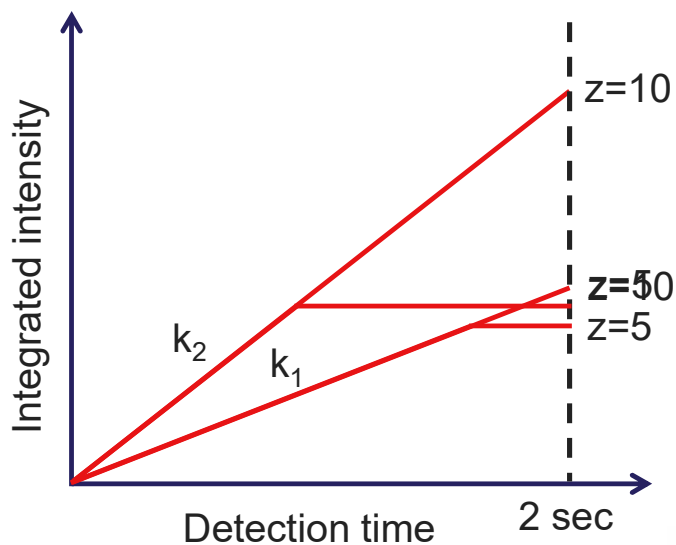
Data processing: STORI plots

Selective Temporal Overview of Resonant Ions (STORI)



Kafader, Jared O., et al. "STORI plots enable accurate tracking of individual ion signals." *Journal of The American Society for Mass Spectrometry* 30.11 (2019): 2200-2203.

Data processing: STORI plots



If intensity is possibly disproportional, what else is still valid?

STORI slope vs charge

- STORIboard uses STORI slope vs charge state to build calibration curve
- STORI approach helps more accurately determine charge

Kafader, Jared O., et al. "STORI plots enable accurate tracking of individual ion signals." *Journal of The American Society for Mass Spectrometry* 30.11 (2019): 2200-2203.