Characterization of Viral Vector Particles with LC-MS

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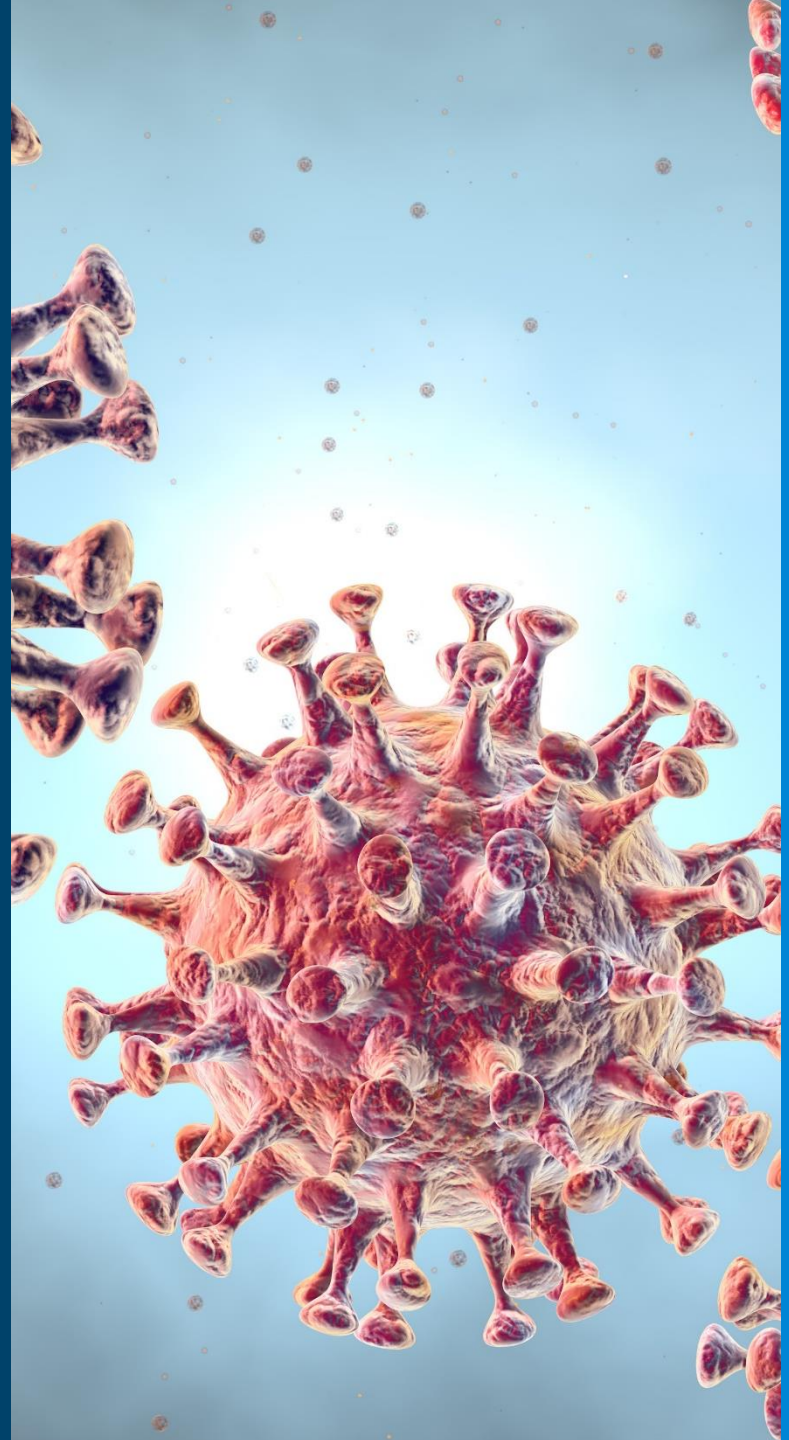
Agilent LC/MS Applications Scientist
January 13, 2021



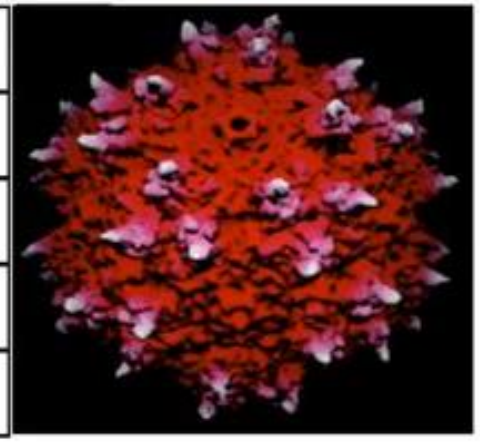
Agilent
Trusted Answers

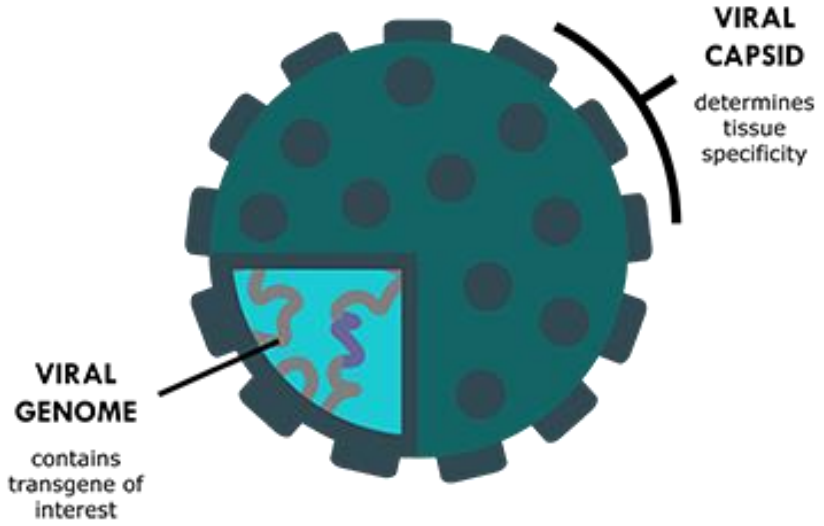
Agenda

- Introduction to Adeno-Associated Viruses.
- Introduction to LC/MS for Analysis of Viral Capsid Proteins.
- Reduced Analysis of Viral Capsid Proteins.
- Peptide Mapping of Viral Capsid Proteins.

Composition of AAV Particles

Particle radius	25nm
Molecular weight	
protein (74%)	M_r ~ 3750 kDa
DNA (26%)	M_r ~ 1350 kDa
total virus	M_r ~ 5100 kDa





- Capsid protects and delivers DNA into the cell.
- Protein capsid shell consists of 3 proteins: VP1, VP2, VP3.
- Protein ratio ~ 1:1:10
- Proteins share same C-terminus

Xie Q, Bu W, Bhatia S, et al. The atomic structure of adeno-associated virus (AAV-2), a vector for human gene therapy. Proc Natl Acad Sci U S A. 2002;99(16):10405-10410
https://www.vigenebio.com/aav-packaging/

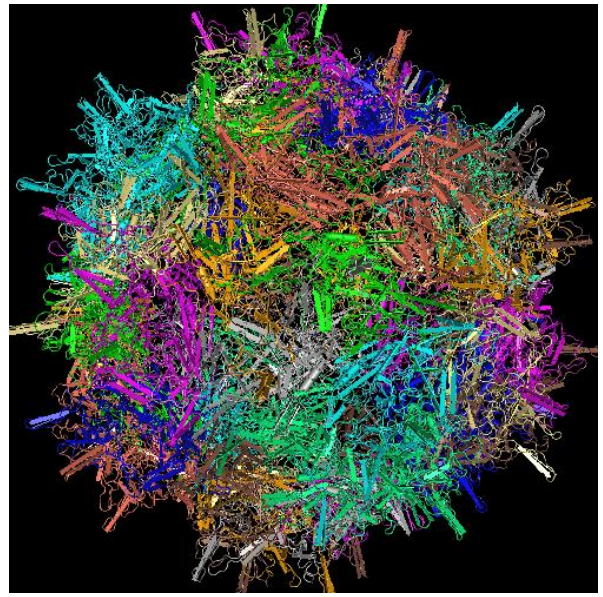
Recombinant AAV Serotypes

Lau CH and Suh Y. *In vivo* genome editing in animals using AAV-CRISPR system: applications to translational research of human disease. *F1000Research* 2017, **6**(F1000 Faculty Rev):2153.

Characterization and Comparability of AAV Capsid Proteins

Practical Challenges

- Attribute criticality –field still in early understanding
- Sample retain limitations due to small batch sizes
- Limitations of standard assays – poor sensitivity and high variability



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Current Analytical Approaches

- Vector Particle Titer assay – ELISA or SEC-HPLC
- Full / Partial / Empty Ratio: UV Spectroscopy, EM, AUC, Ion-exchange Chromatography
- Capsid Protein Analysis: SDS-Page, RP-HPLC, Intact LC-MS, Peptide Mapping
- Residual Host Cell DNA Characterization: Qualitative PCR

Agilent Biopharma Workflow Solutions for AAV Particles

- Aggregate/ Fragment Analysis
- Charge Variant Analysis
- Peptide Mapping
- Intact and Subunit Purity
- Titer Determination
- Glycan Analysis
- Amino Acid and Cell Culture Analysis
- Host Cell and Process Related Impurities
- Post Translational Analysis
- Extractables and Leachables
- Multi-Attribute Monitoring (MAM)
- Nucleic Acid Analysis

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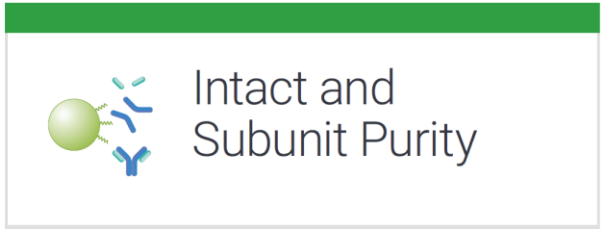
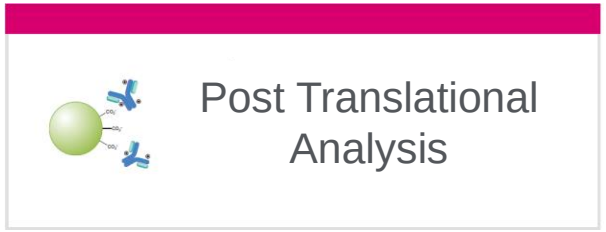
Mass Spectrometry of Capsid Particles

Intact Capsid Protein Analysis
– Denature capsid and separate by reversed phase chromatography prior to MS (RP-UHPLC/MS)

Peptide Mapping Protein Analysis
– Proteolytic Digest of Capsid Proteins
– Denature capsid and digest with proteolytic enzyme prior to RP-HPLC-MS/MS

Capabilities of LC-MS Methods
- Confirm amino acid sequence
- Monitor clips/truncations
- Determine cysteine oxidation state
- Identify capsid protein modifications
- Characterize unknowns/impurities





AAV Capsid Characterization Workflow

Separation → Detection → Data Processing & Report

1290 Infinity II LC

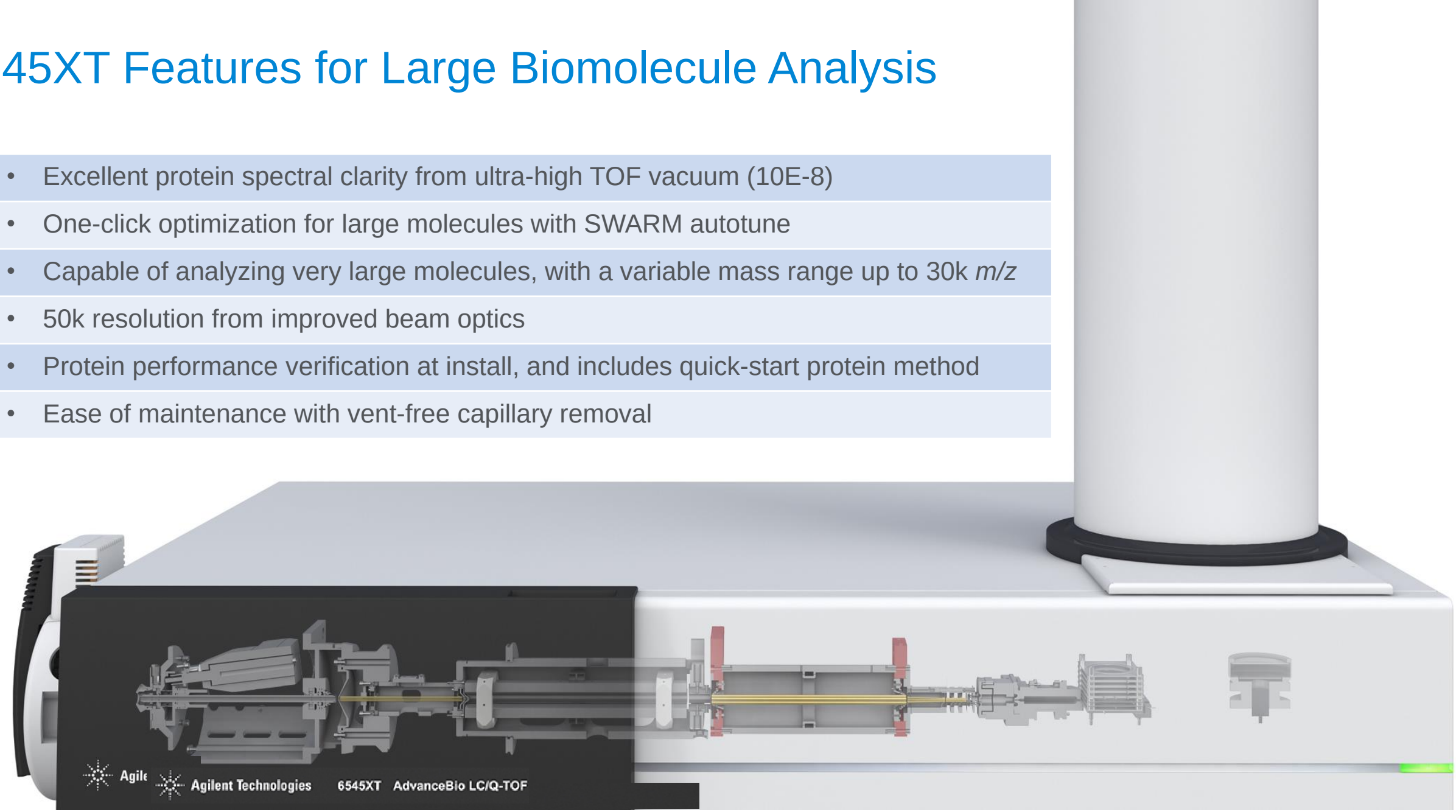
ZORBAX RRHD 300SB-Diphenyl
AdvanceBio Peptide Mapping

AdvanceBio 6545XT

BioConfirm 10.0

6545XT Features for Large Biomolecule Analysis

- Excellent protein spectral clarity from ultra-high TOF vacuum (10E-8)
- One-click optimization for large molecules with SWARM autotune
- Capable of analyzing very large molecules, with a variable mass range up to 30k *m/z*
- 50k resolution from improved beam optics
- Protein performance verification at install, and includes quick-start protein method
- Ease of maintenance with vent-free capillary removal

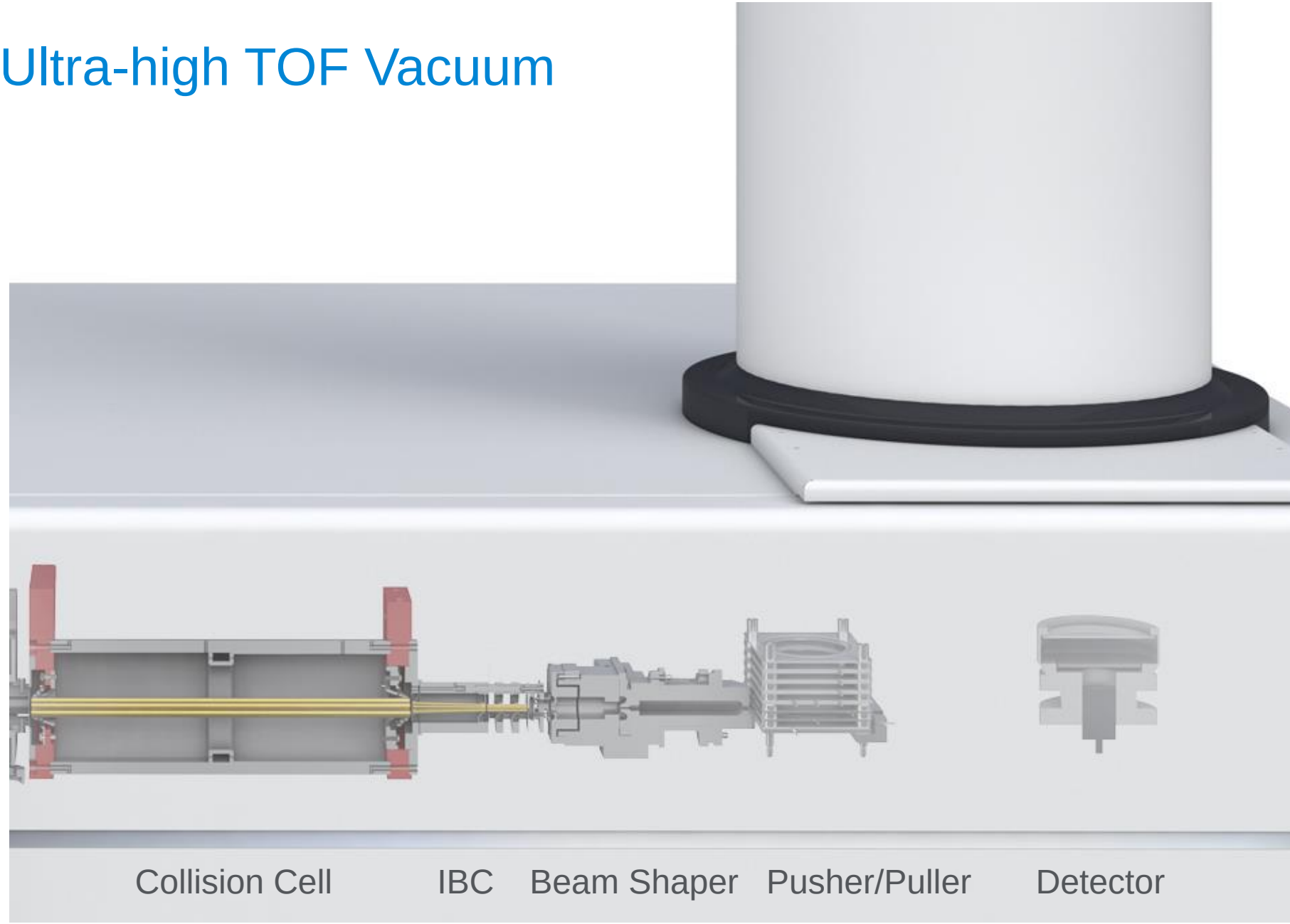


Design Innovation – Ultra-high TOF Vacuum

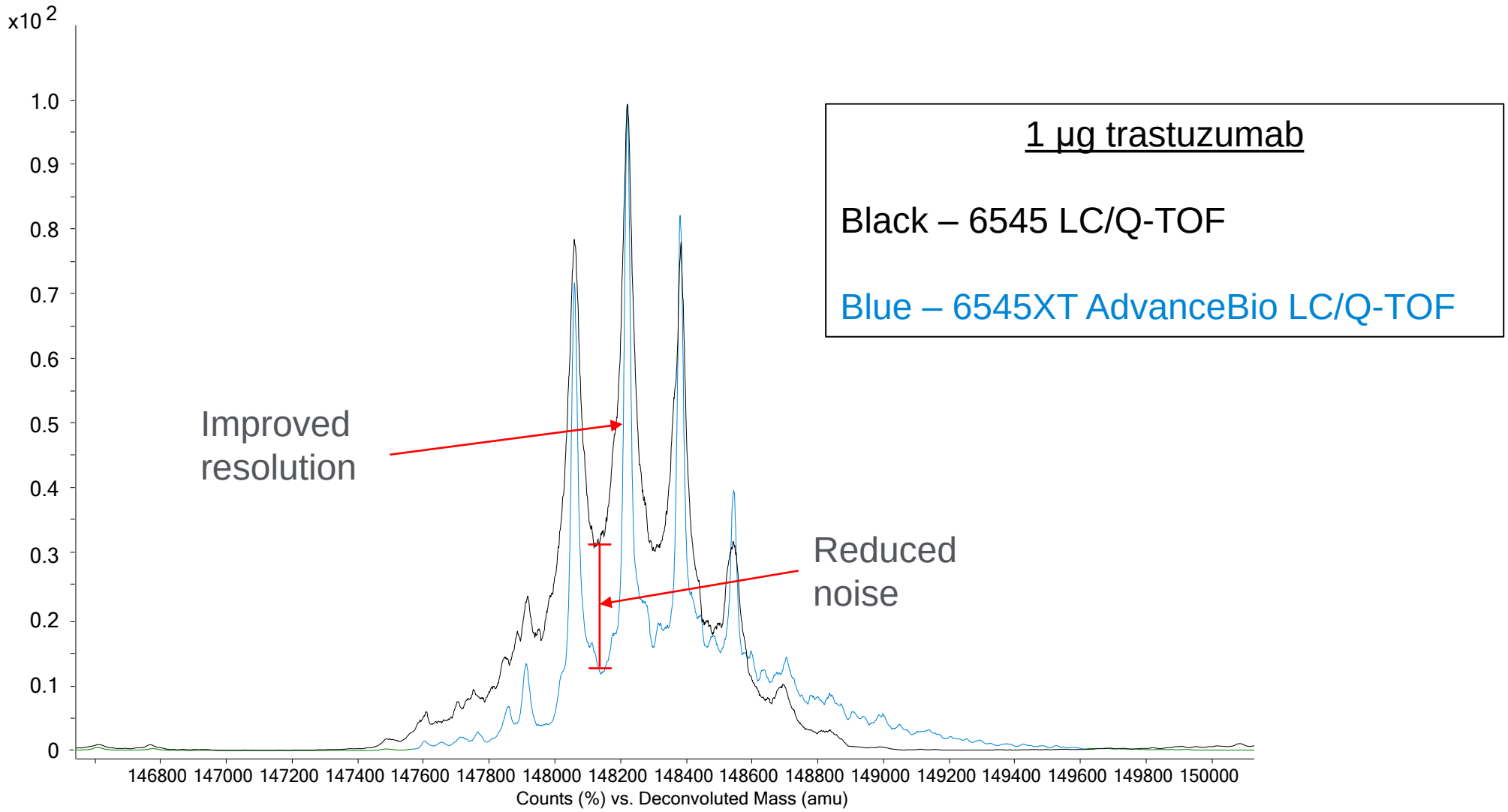
-Redesigned differential pumping

-Careful control of all materials within flight tube to minimize outgassing

-This higher vacuum leads to improved large molecule spectral quality by reducing noise



Improved Spectral Clarity from Improved TOF Vacuum



1 µg trastuzumab

Black – 6545 LC/Q-TOF

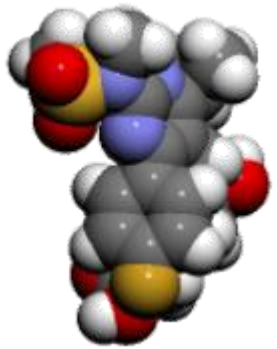
Blue – 6545XT AdvanceBio LC/Q-TOF

Design Innovation – Large Molecule SWARM Autotune

Crestor

$C_{22}H_{28}FN_3O_6S$

Molecular Mass 481.6 g/mol



Trastuzumab

$C_{6470}H_{10012}N_{1726}O_{2013}S_{42}$

Molecular Mass 145531.5 g/mol

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$C_{22}H_{28}FN_3O_6S$

Molecular Mass 481.6 g/mol

Trastuzumab

$C_{6470}H_{10012}N_{1726}O_{2013}S_{42}$

Molecular Mass 145531.5 g/mol

300x larger mass

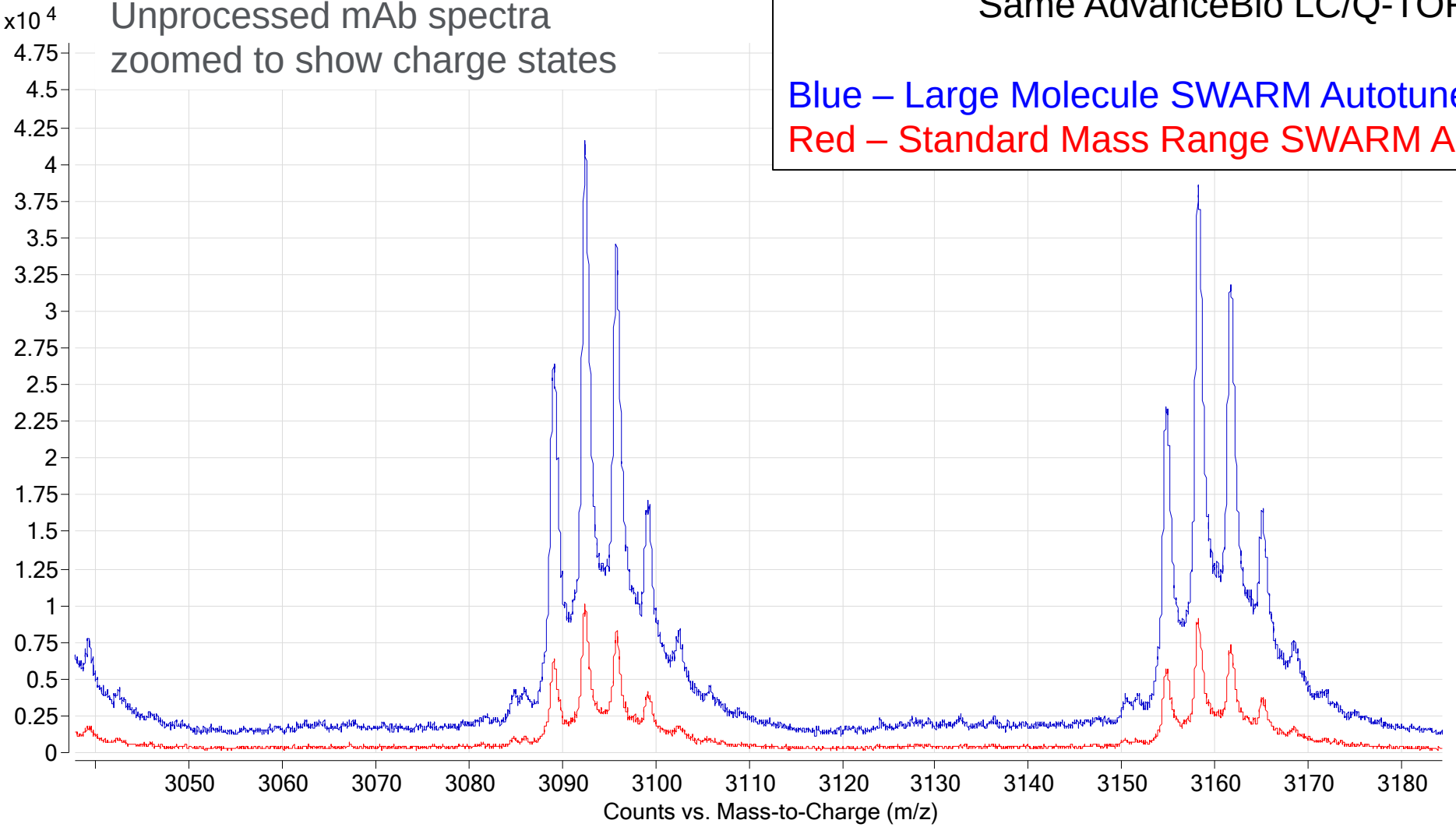
Small molecules and large molecules behave differently – A “one size” fits all approach doesn’t make sense for tuning the instrument.

Optimizing for Proteins with Large Molecule SWARM Autotune

Unprocessed mAb spectra
zoomed to show charge states

Same AdvanceBio LC/Q-TOF

Blue – Large Molecule SWARM Autotune
Red – Standard Mass Range SWARM Autotune

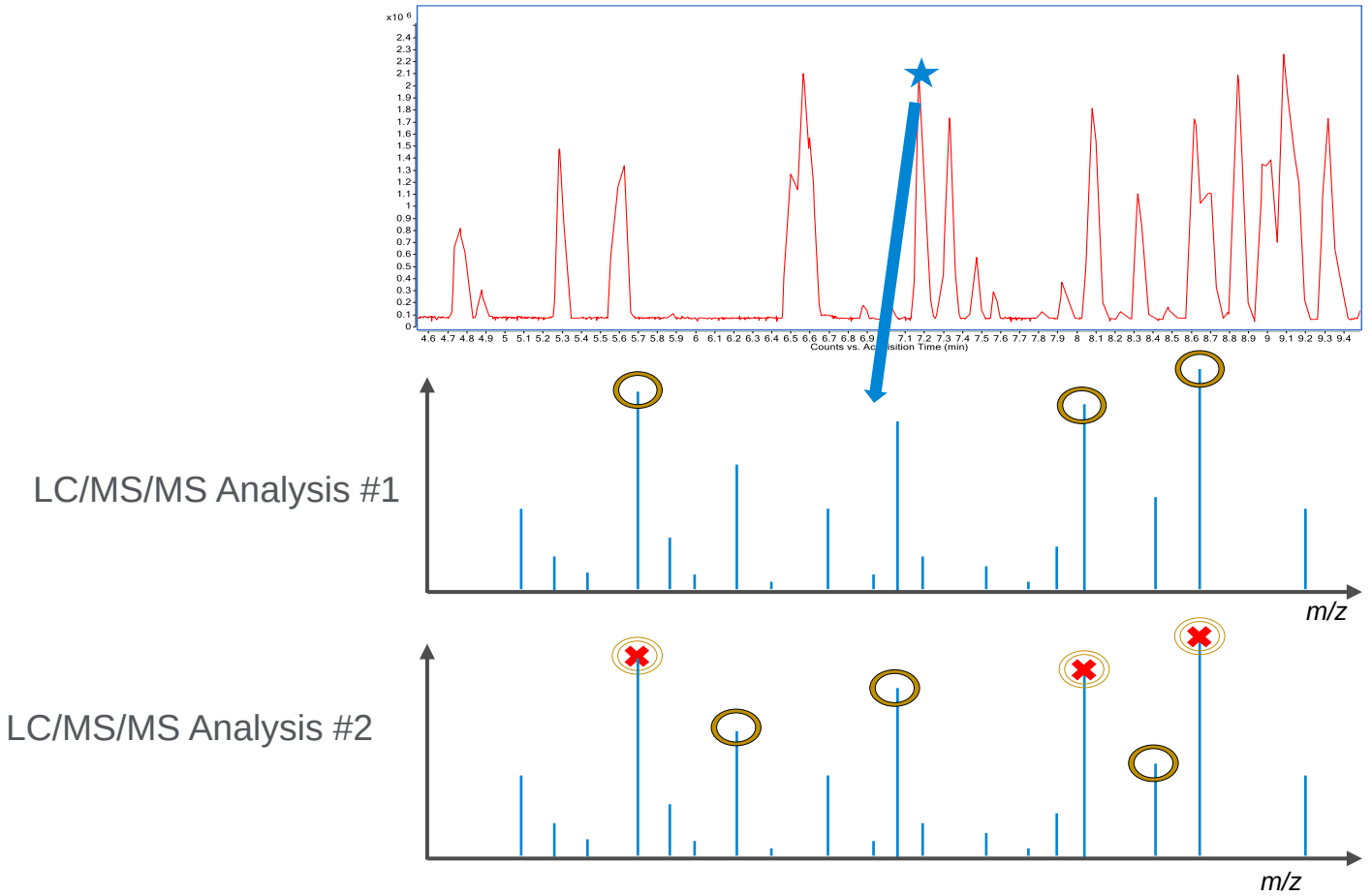


6545XT Features for Peptides

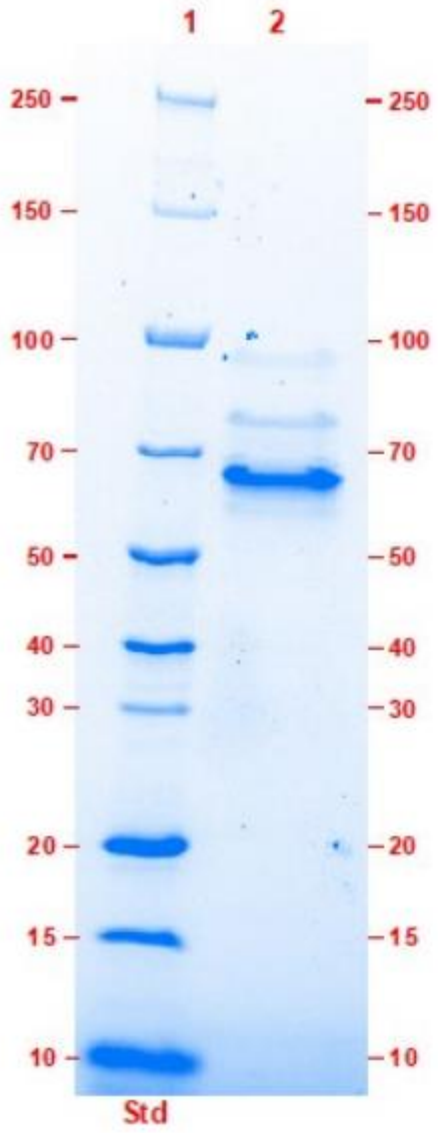
- Sensitive peptide detection featuring Agilent Jet Stream
- Quick-start peptide mapping method
- Access low intensity peptides/PTMs with the new Iterative MS/MS mode
- Sub-ppm mass accuracy with 50k resolution from improved beam optics
- Peptide fragmentation performance verification at install
- Ease of maintenance with vent-free capillary removal

For Research Use Only. Not for use in diagnostic procedures.

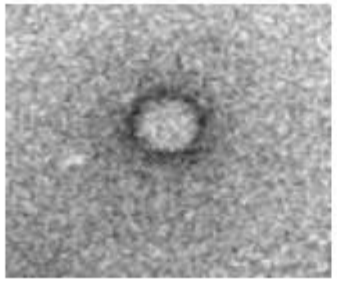
Iterative MS/MS



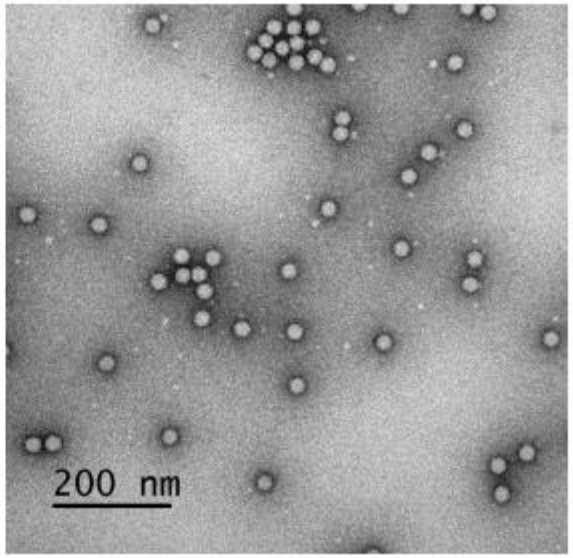
Production of Enriched AAV



Empty capsid



Full capsid



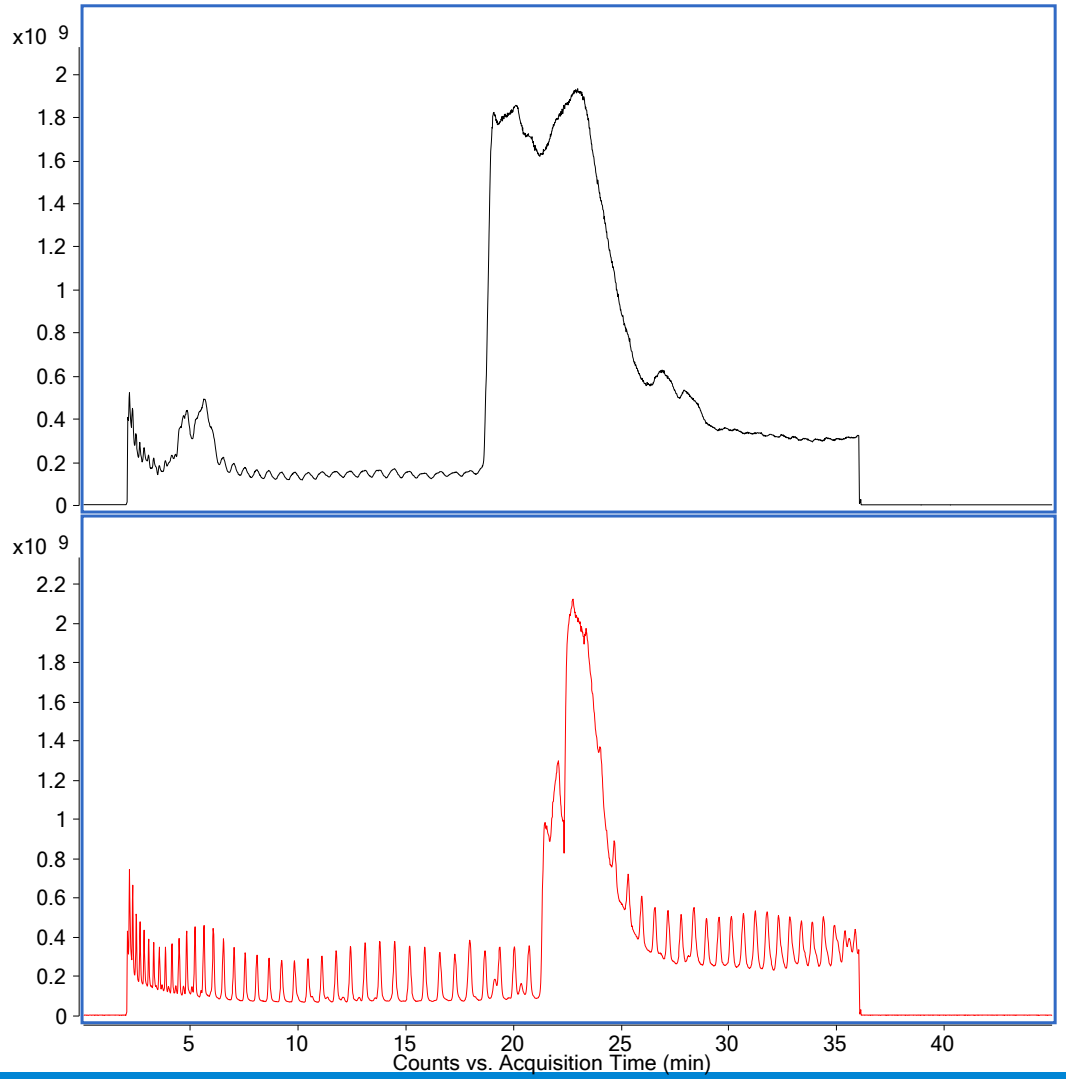
LakePharma
The Biologics Company

Agilent

Reduced Analysis

Importance of Mass Spec Friendly Buffers

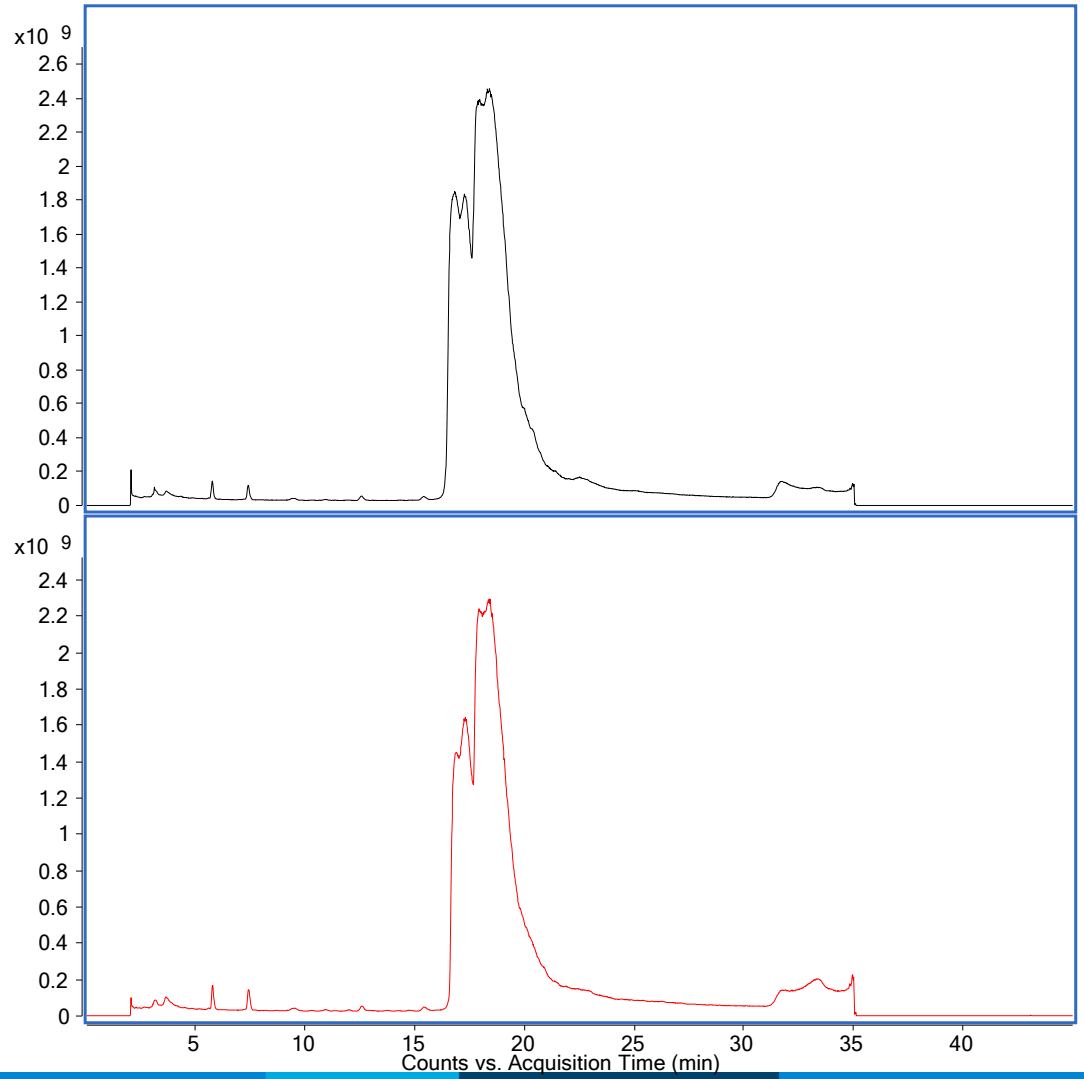
Before Buffer Exchange



Empty Capsids

Full Capsids

After Buffer Exchange

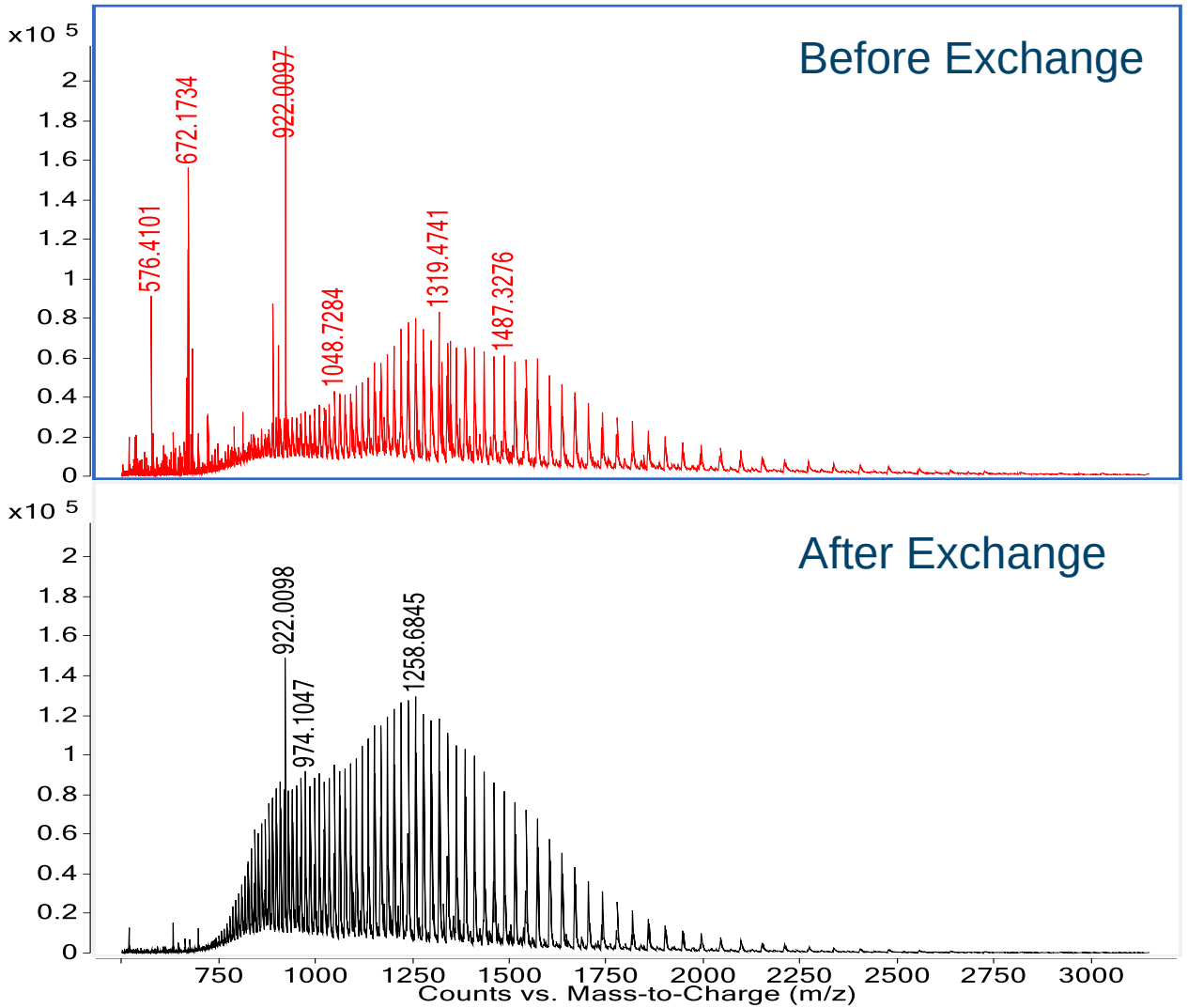


Sample Prep is Key!

10 kDa MWCO, 500 µL volume

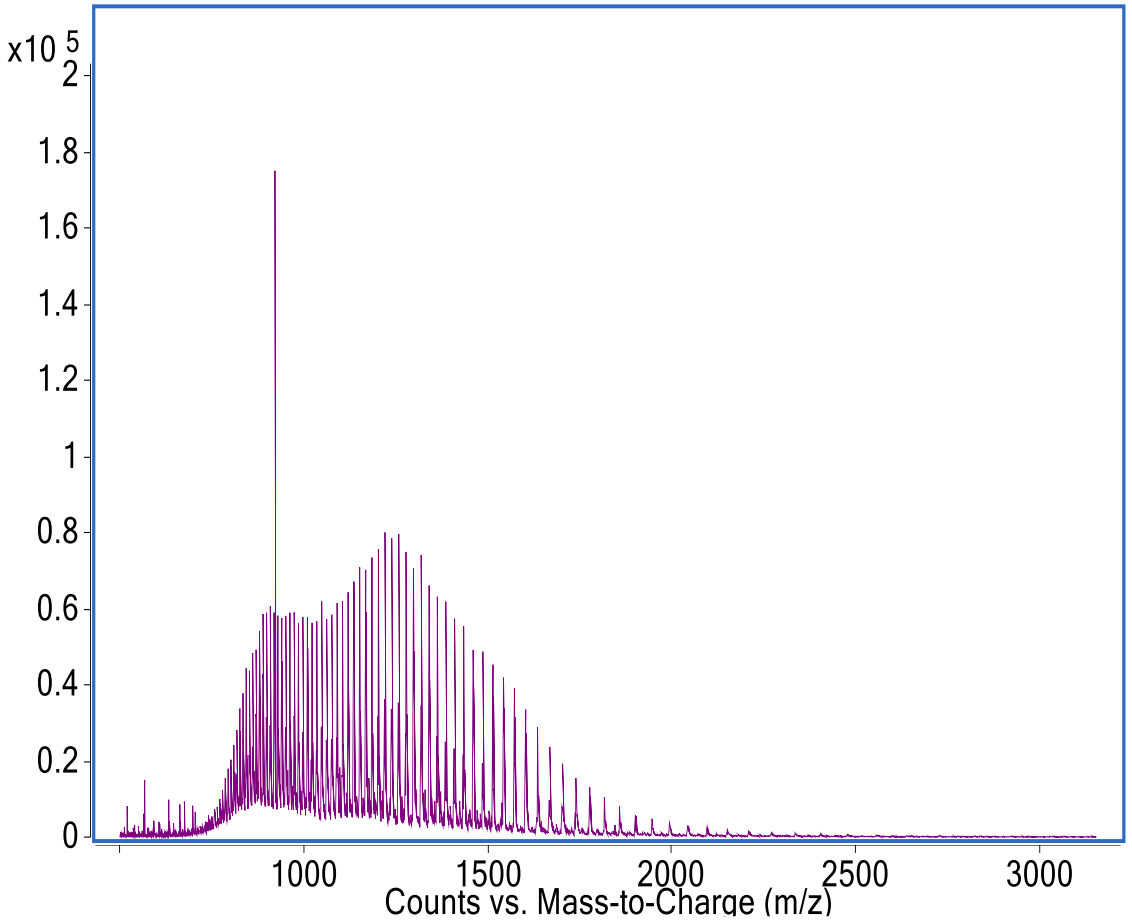
60 µL of AAV
440 µL of Exchange Buffer

Exchange Buffer: 80% 5 mM TCEP in H_2O, 20% ACN, 0.1% formic acid

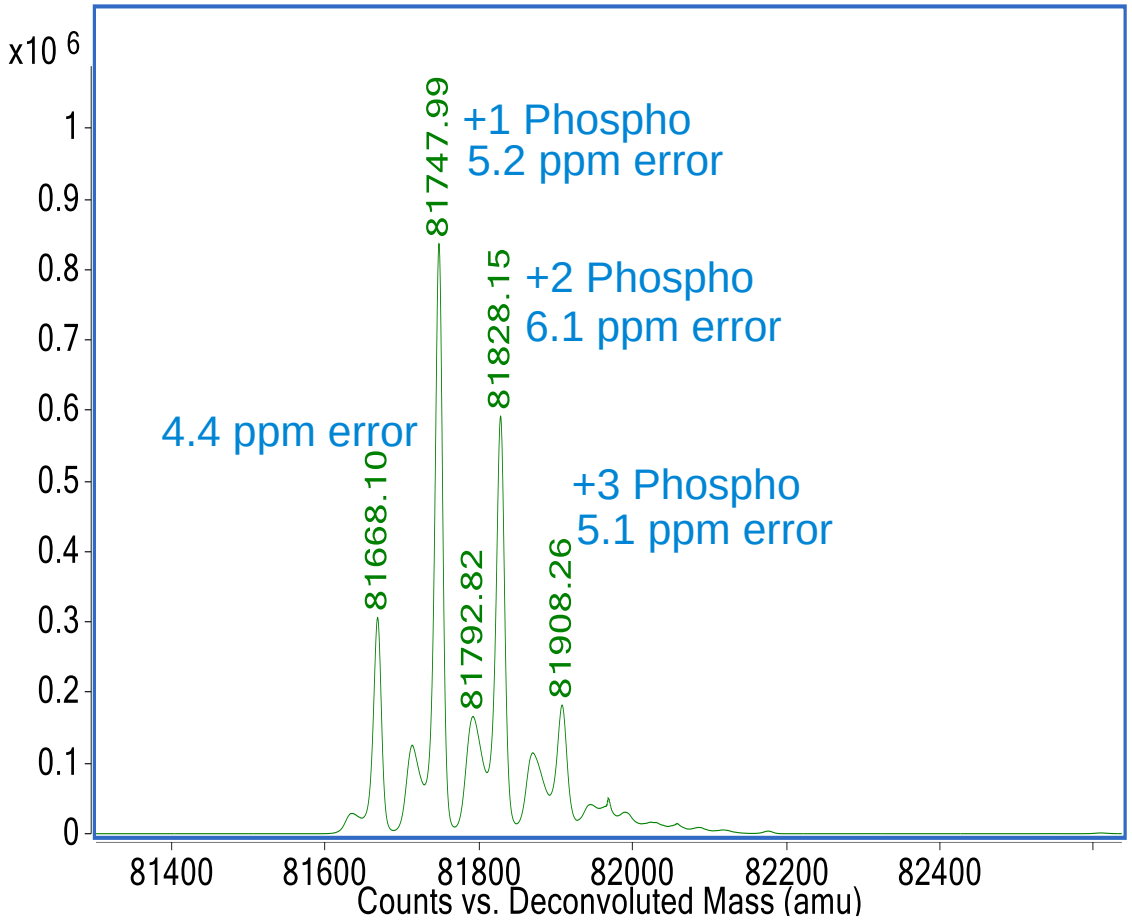


VP1 Raw and Deconvoluted Spectra

VP1 Raw Spectrum

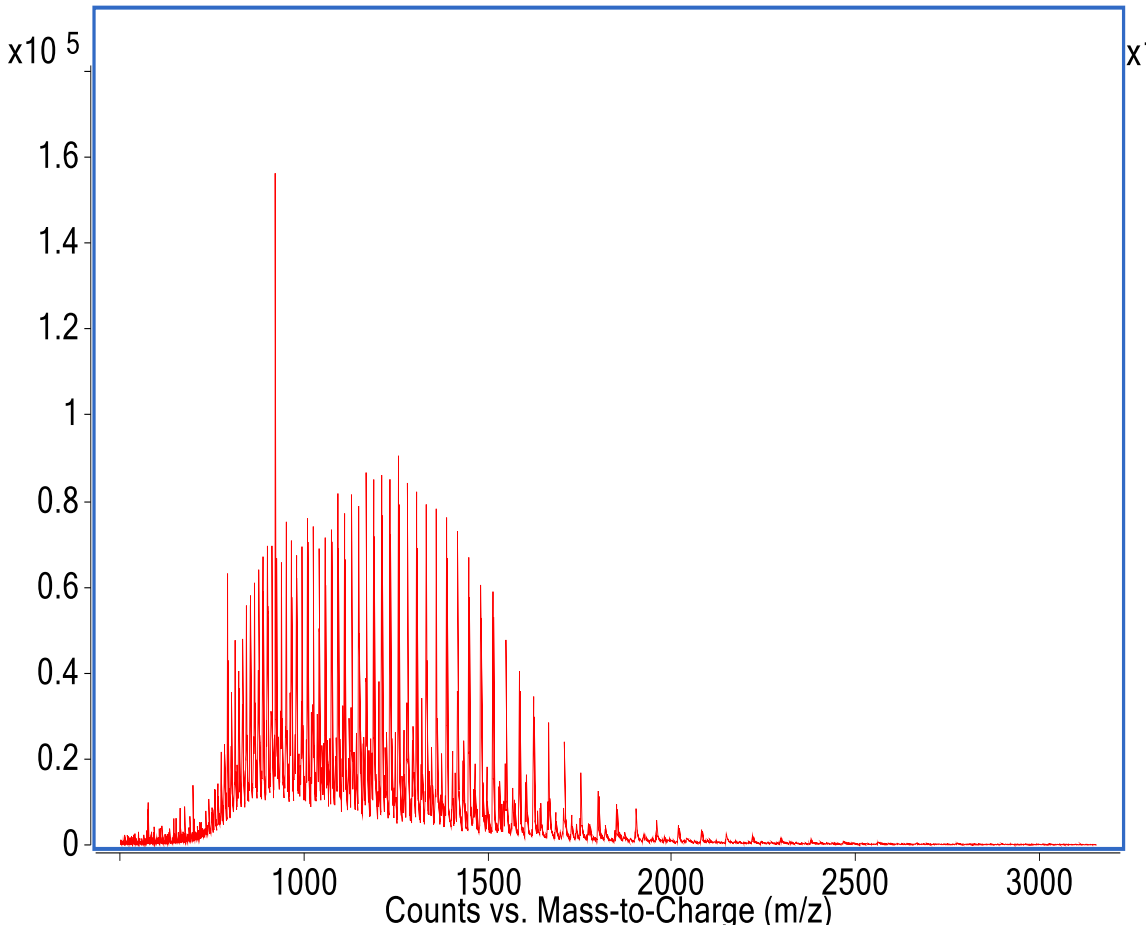


VP1 Deconvoluted Spectrum

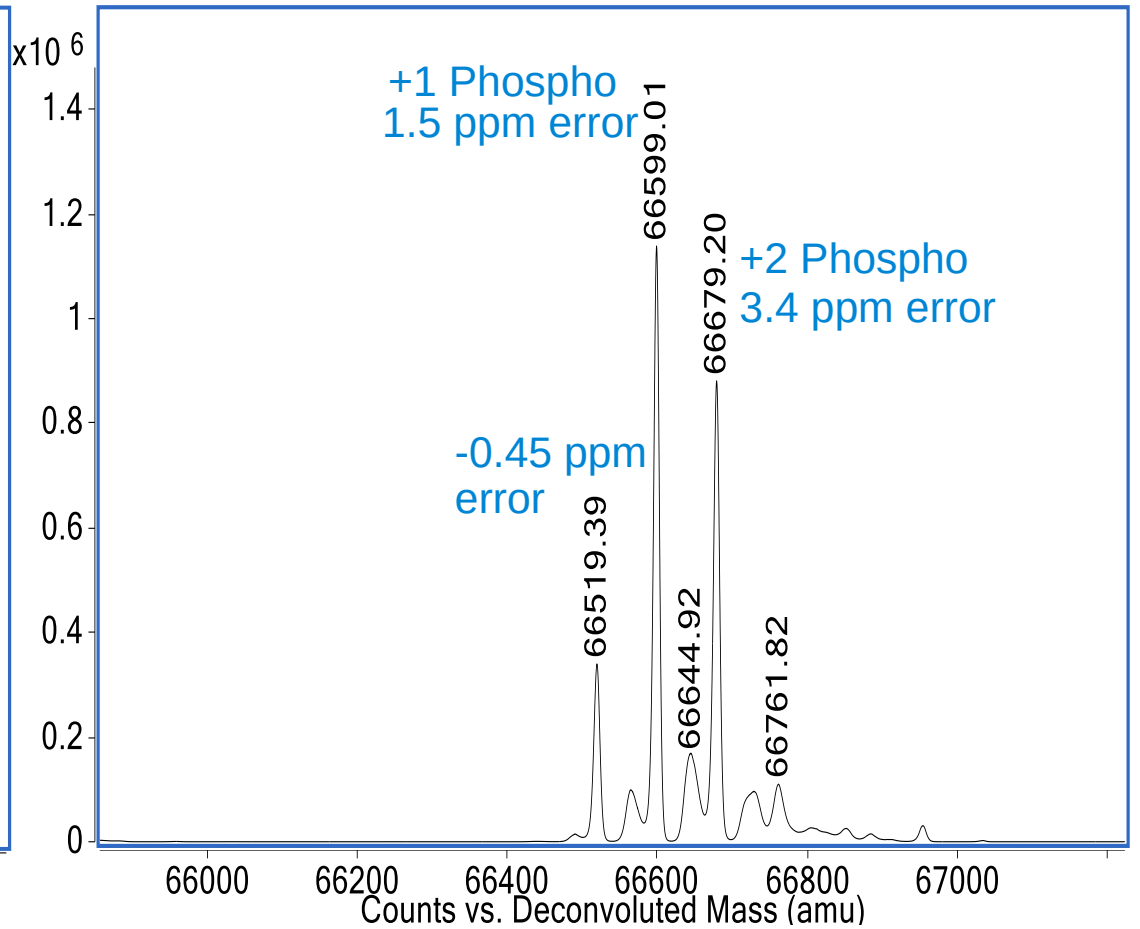


VP2 Raw and Deconvoluted Spectra

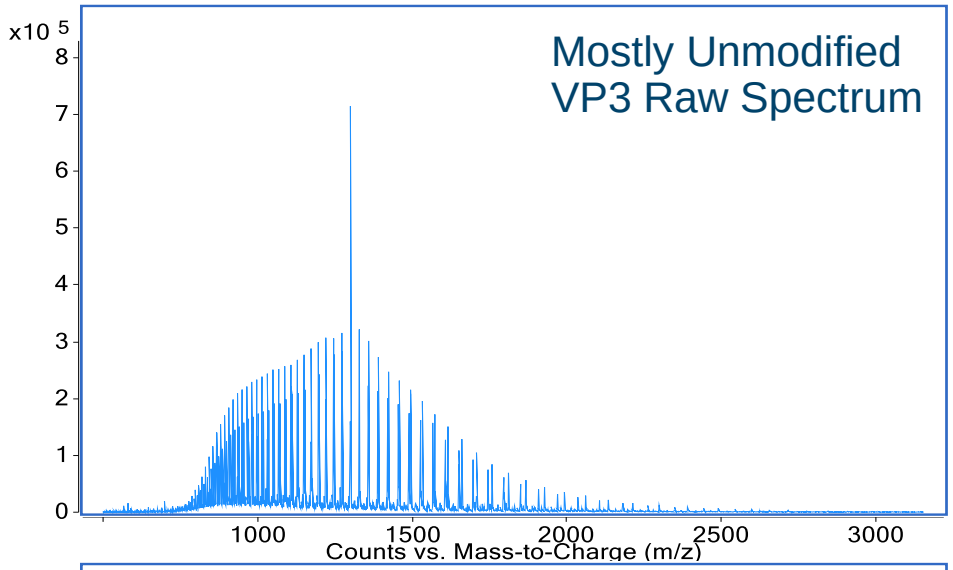
VP2 Raw Spectrum

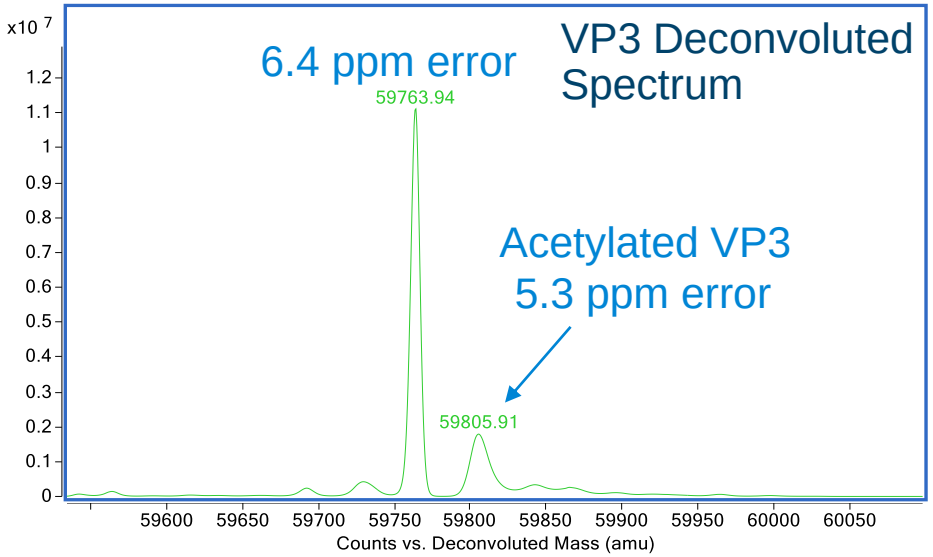


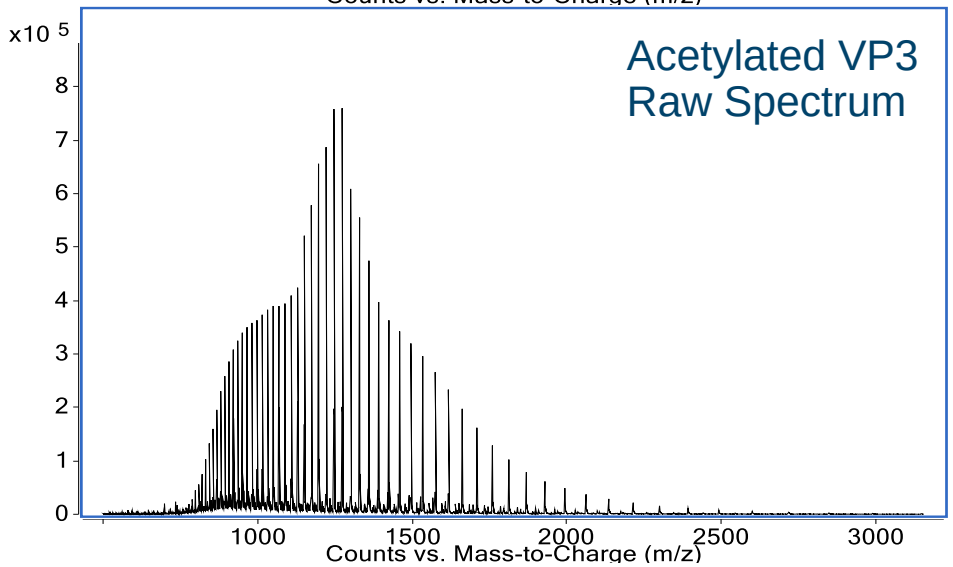
VP2 Deconvoluted Spectrum

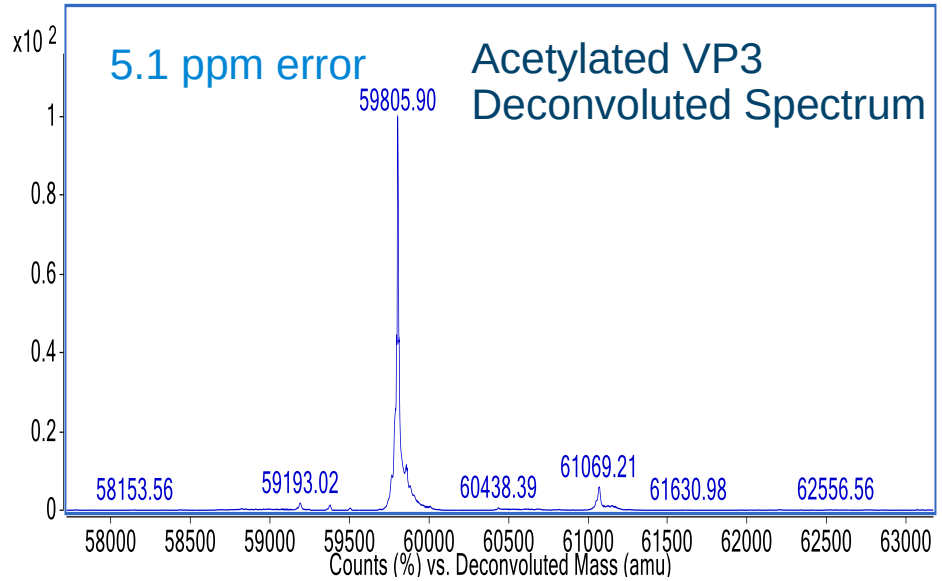


VP3 Raw and Deconvoluted Spectra



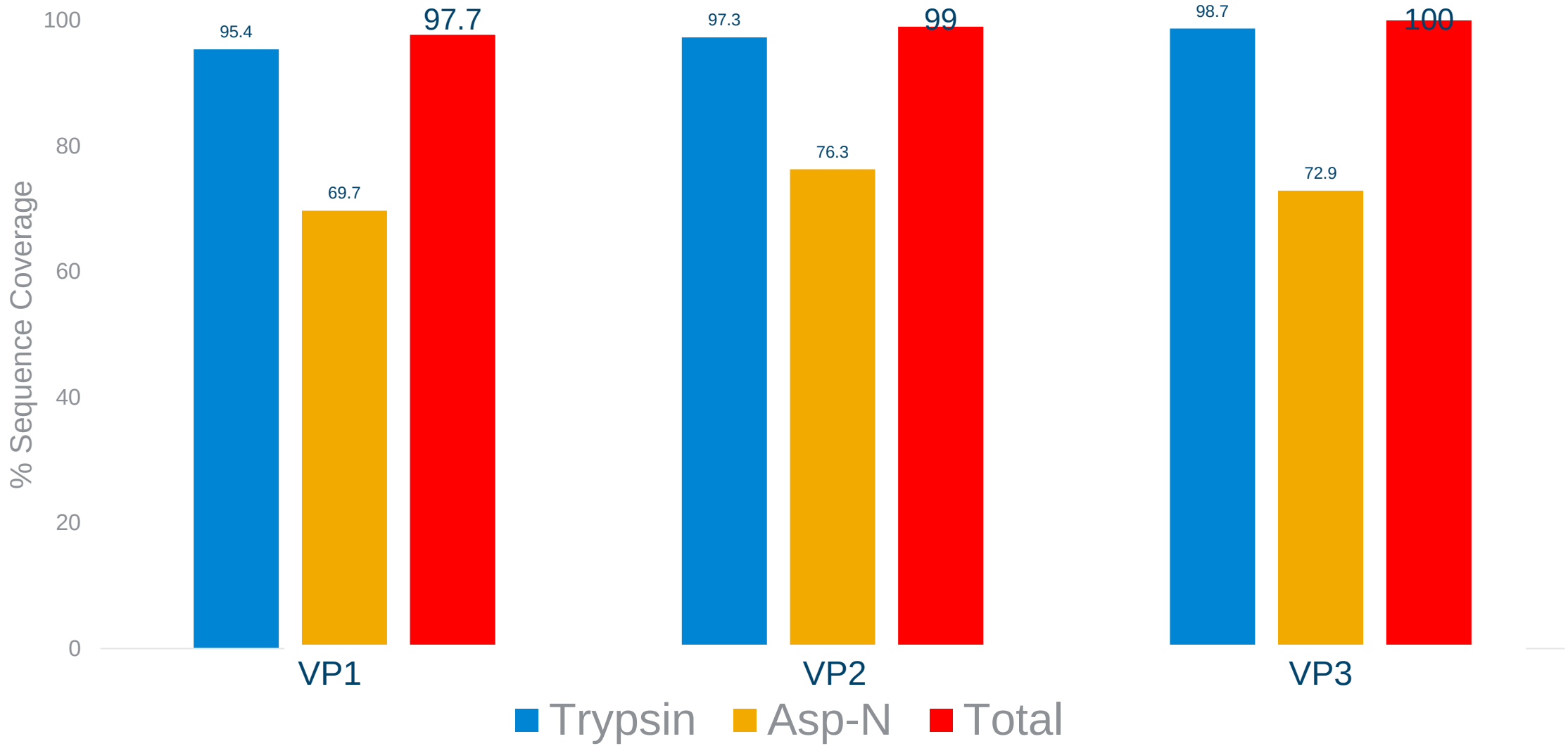




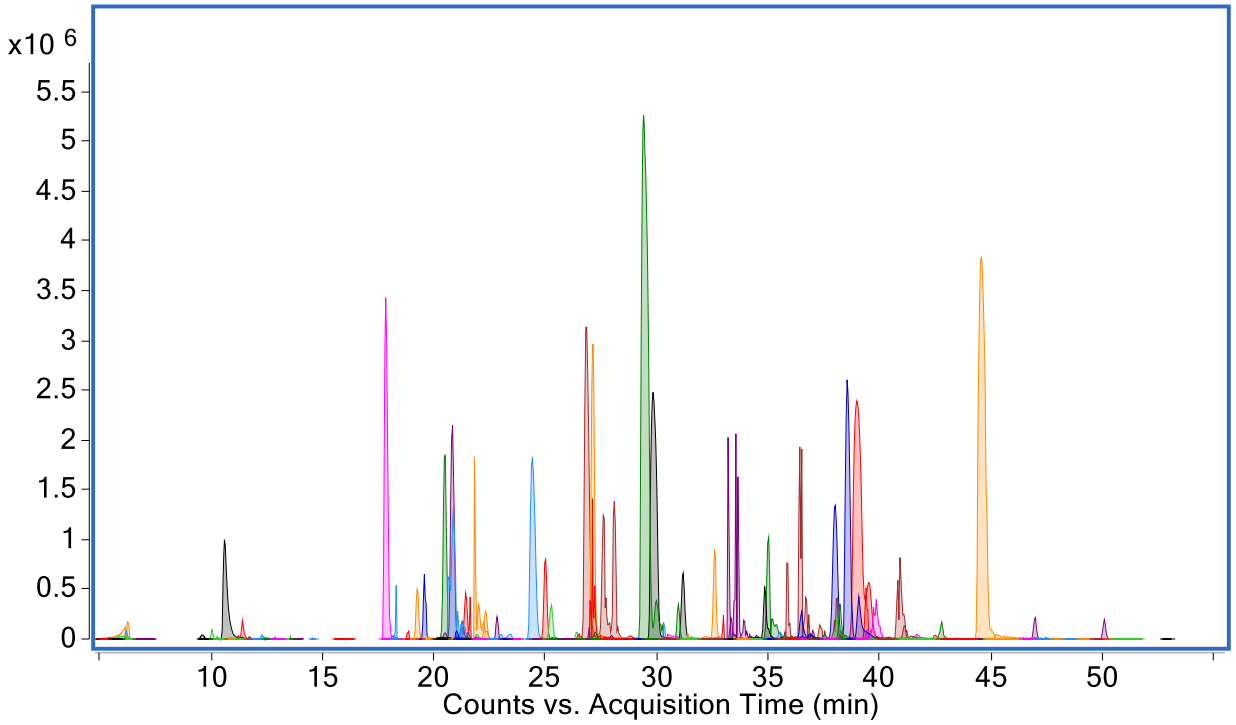


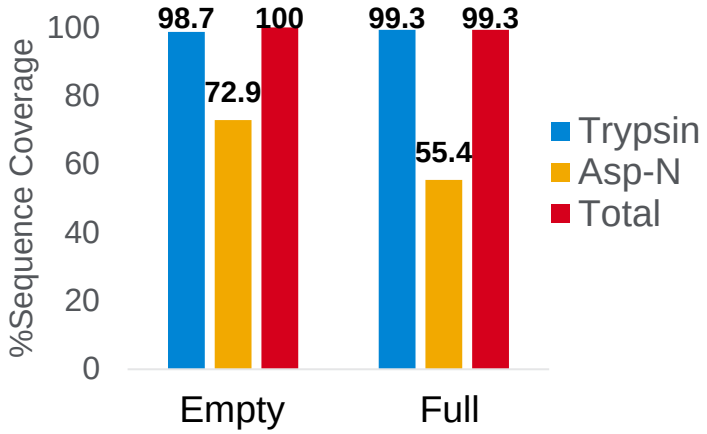
Peptide Mapping

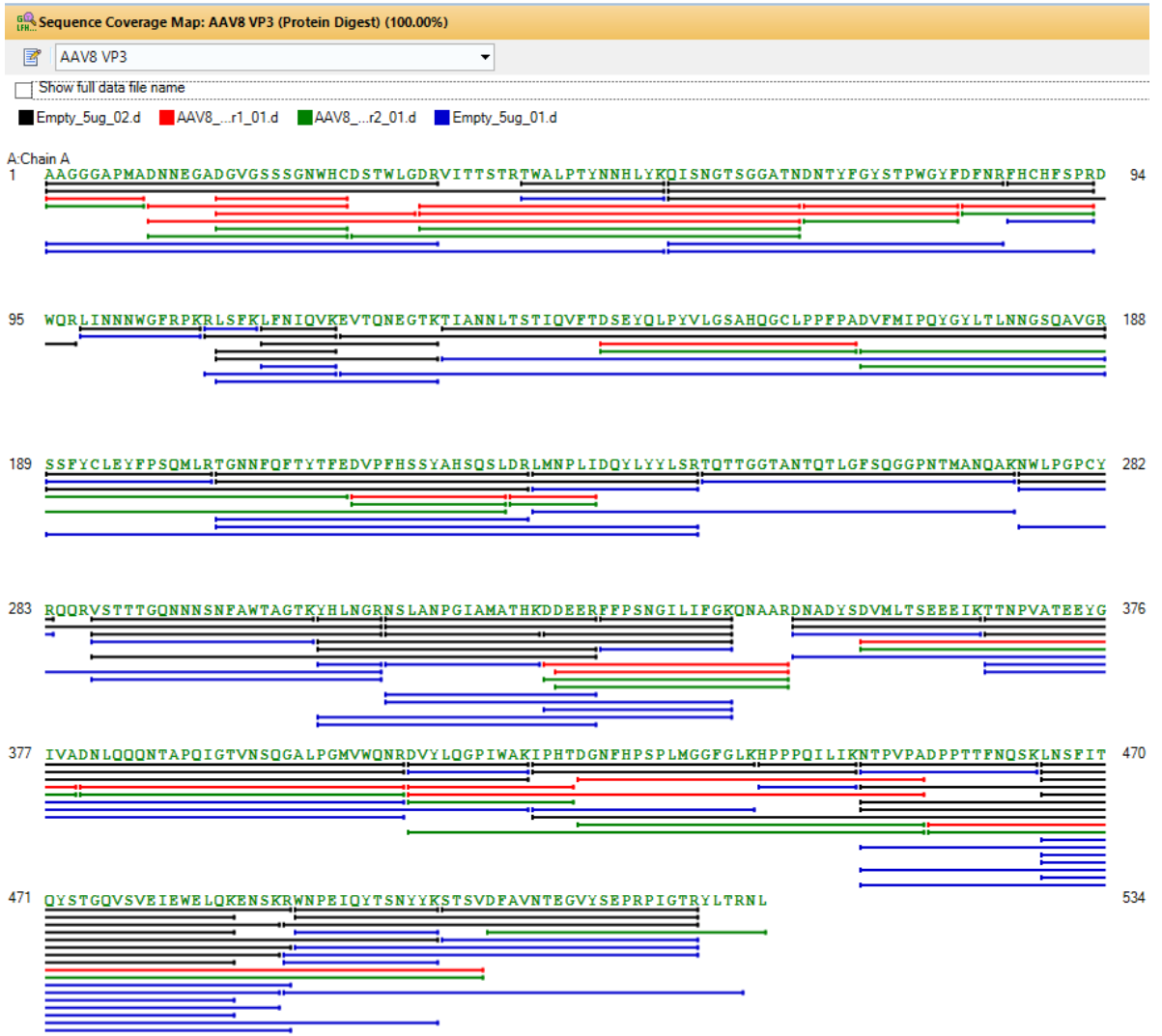
Peptide Mapping: Sequence Coverage of AAV8



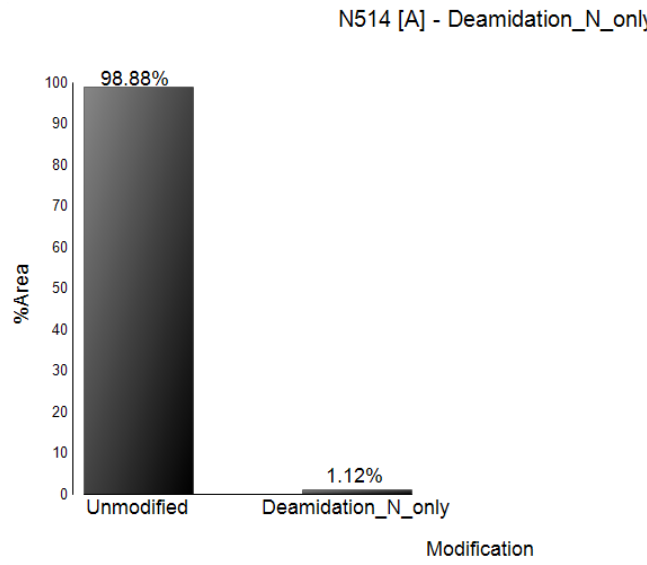
VP3 Peptide Mapping

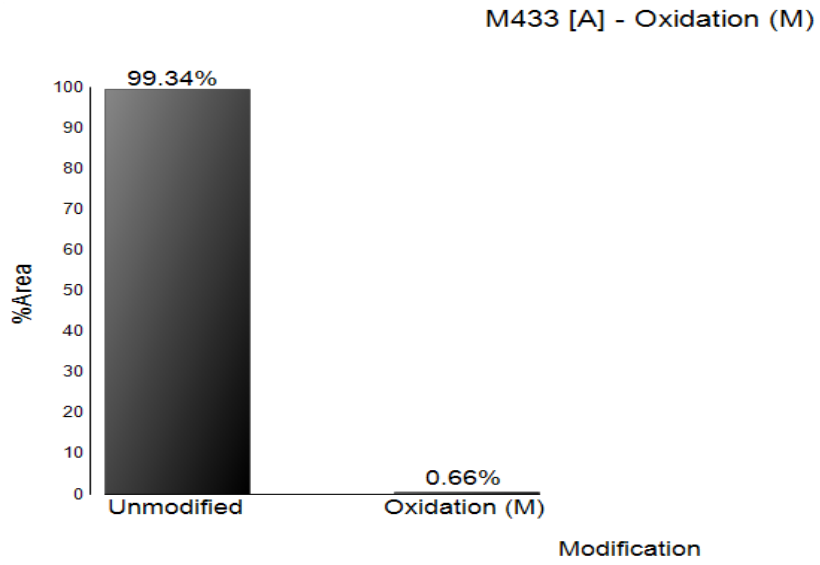


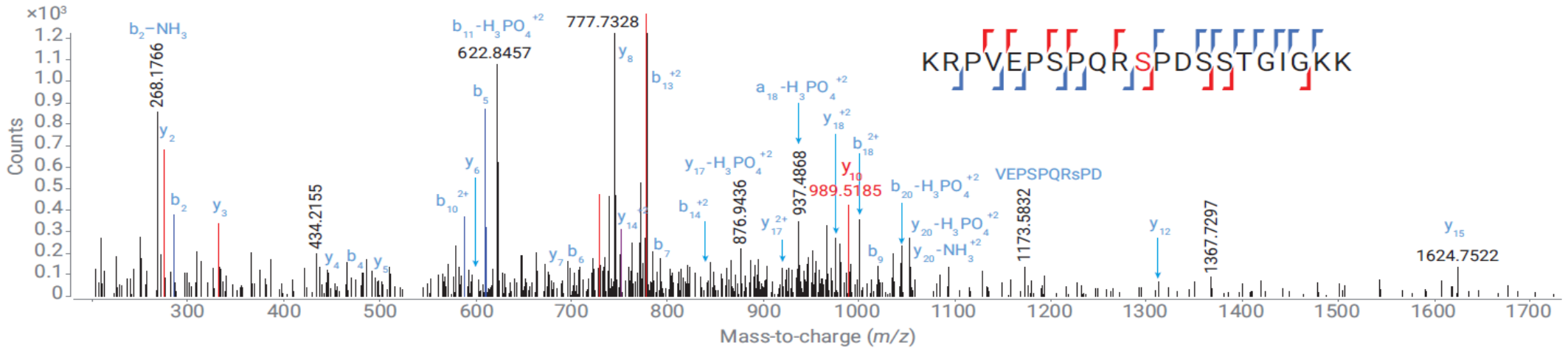




Post-Translational Modifications







Conclusions

- Adeno-Associated Viruses are an exciting and promising therapeutic for a variety of diseases and disorders.
- Ensuring critical quality attributes as AAVs progress is vital.
- Mass spectrometry at the intact level can confirm molecular weight with accurate mass information and provide some level of modification information (phosphorylation and acetylation).
- Mass spectrometry at the peptide level can determine site-specific post-translational modifications as well as confirm sequence information.

Acknowledgements

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