Application of Mass Spectrometry for AAV-based Gene Therapy Analysis

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Sep 17, 2020

Adeno-associated Virus (AAV) for Gene Therapy

Gene therapy is the therapeutic delivery of nucleic acid into a patient as a drug to treat disease.

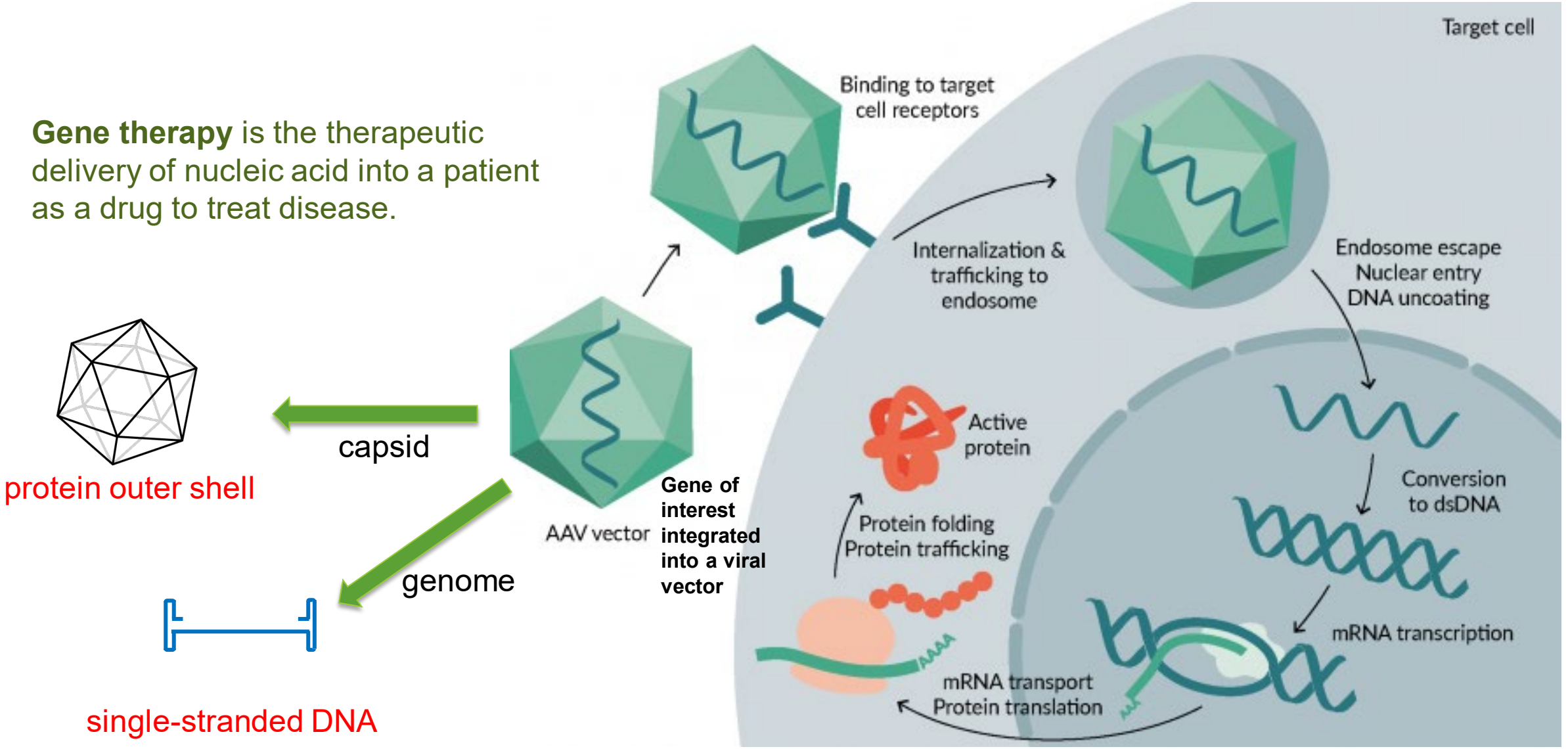
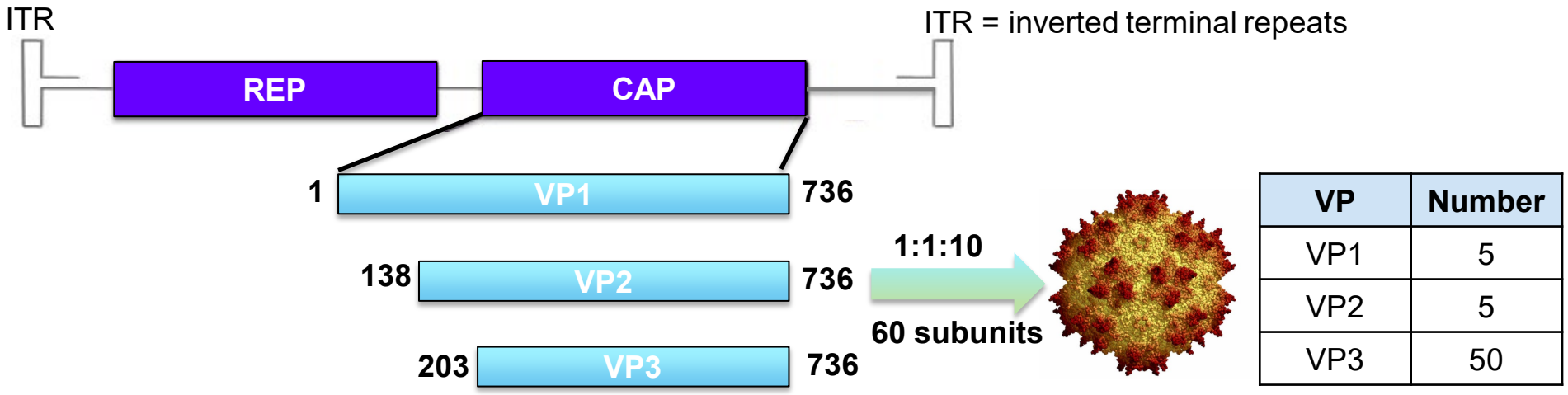


Figure adapted from: Li, *et al.* 2019, Cell & Gene Therapy Insights 2019; 5(4), 537–547

Wild Type AAV Structural Characteristics and Quality Attributes



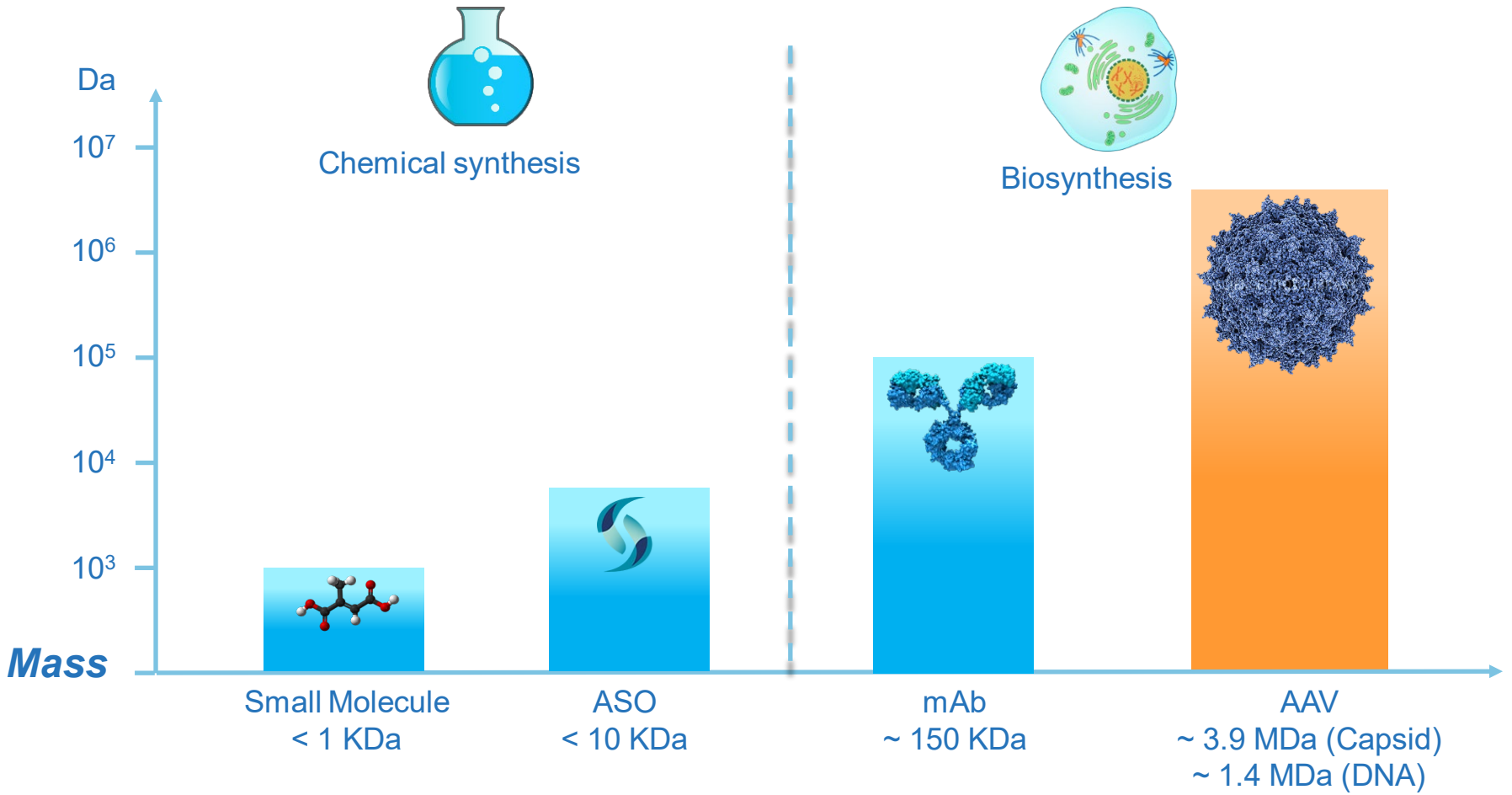
VP	Number
VP1	5
VP2	5
VP3	50

Capsid Proteins - Viral Proteins (VPs)

Adeno-Associated Virus
3.9 MegaDaltons (empty capsids)
Small icosahedral particles (20-25 nm in diameter)
Natively package ssDNA to ~ 4.7 kb
Replication-defective, nonenveloped virus
Non-pathogenic, mildly immunogenic; Low level integration, maintained episomally
Many distinct serotypes

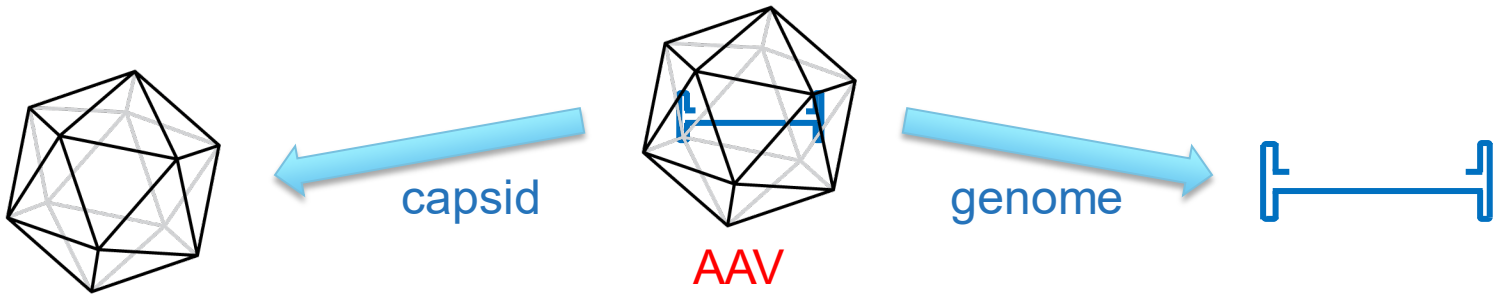
Examples of AAV attributes
Capsid purity
Capsid identity
Vector particle titer
Empty/full capsid

Comparing AAV Size with Other Drug Modalities



ASO: antisense oligonucleotide
mAb: monoclonal antibody

Key Structural Characteristics of AAV Products



Is it the right AAV capsid?

Capsid ID (serotype) and viral protein ratio

Are capsids filled with the transgene?

Empty/partial/full capsid

Does it contain the right transgene?

Gene sequencing and purity

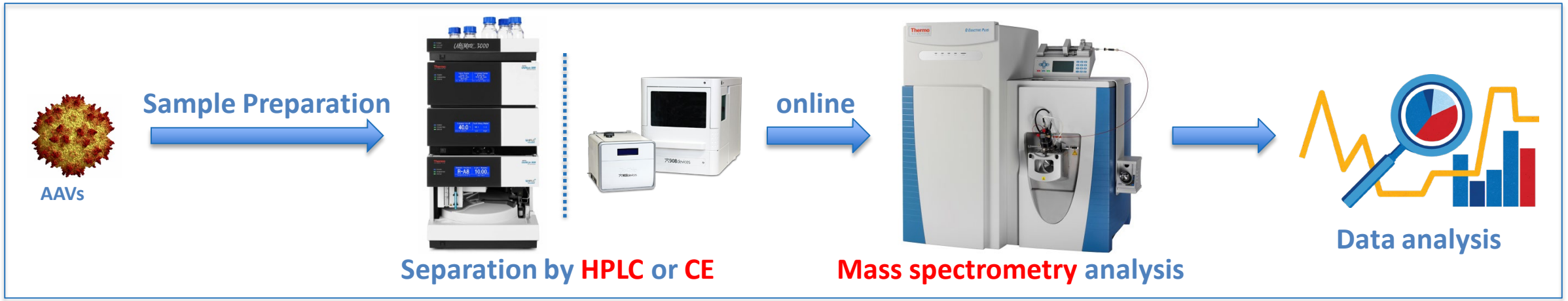
- What modifications could affect the potency/stability?
- Are there size/charge variants?
- How much residual impurities are there after purification?

Deep characterization and HCP/host cell DNA

Mass Spectrometry (MS) Applications for Gene Therapy Development

Approaches	Attributes	Methods
Intact AAV analysis	▪ ***AAV empty-to-full ratio, partially filled with truncated DNA;*** ▪ ***High molecular weight (HMW) species characterization***	• Native/charge detection MS
Intact viral protein analysis	▪ ***Serotype identification;*** ▪ ***Mutant identification***	• CE-MS • RPLC-MS
Peptide map	▪ ***Sequence coverage;*** ▪ ***Capsid PTM characterization;*** ▪ ***Mutant identification;*** ▪ ***Major HCPs identification/quantification***	• RPLC-MS
Process impurity analysis	▪ ***Residual impurity quantification***	• RPLC-MS
ssDNA characterization	▪ ***Sequence and size distribution (orthogonal to NGS)***	• Negative mode MS
Structure-function characterization	▪ ***Critical quality attributes (CQA)***	• Custom LC-MS workflow

Challenges in Gene Therapy Mass Spec Analysis



- **AAV is much larger in size and with complex heterogeneity**
  - ❖ Analyzing intact AAV in native state can provide rich information but requires advanced instruments with higher mass range and/or charge detection capability.
  - ❖ Heterogeneity could be introduced by capsid purity, genome integrity, and/or packaging behavior, etc.
- **Historical knowledge and literatures are limited**
- **Sample availability is limited, and sample concentration is low**

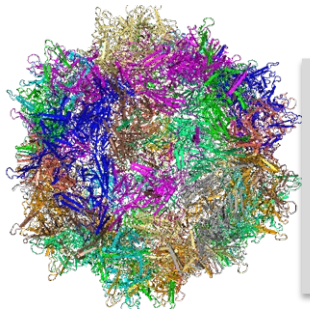
Case Study 1:
AAV Identification by Intact Viral Protein Analysis

Intact Protein Mass Analysis for AAV Identity

AAV Serotype	Viral Proteins (VPs)	Theoretical Mass (Da)
AAV1	Acetyl VP1 (2-736)	81286
	VP2 (139-736)	66093
	Acetyl VP3 (204-736)	59517
AAV2	Acetyl VP1 (2-735)	81856
	VP2 (139-735)	66488
	Acetyl VP3 (204-735)	59974
AAV9	Acetyl VP1 (2-736)	81291
	VP2 (139-736)	66210
	Acetyl VP3 (204-736)	59733
AAVRh10	Acetyl VP1 (2-738)	81455
	VP2 (139-738)	66253
	Acetyl VP3 (204-738)	59634

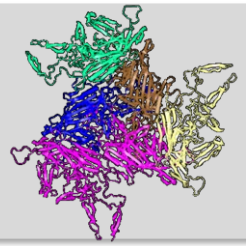
- The combination of mass measurement of intact VP1, VP2, and VP3 proteins is highly specific as an identity test.
- Potentially transferable to QC.
- The mass differences exist for wild type AAV serotypes from AAV1 to AAV12

ZipChip CE-MS Intact Mass Analysis



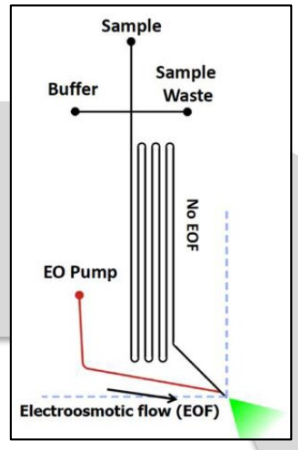
AAVs

Denaturation



Autosampler

Microfluidic CE-ESI



On-chip CE separation

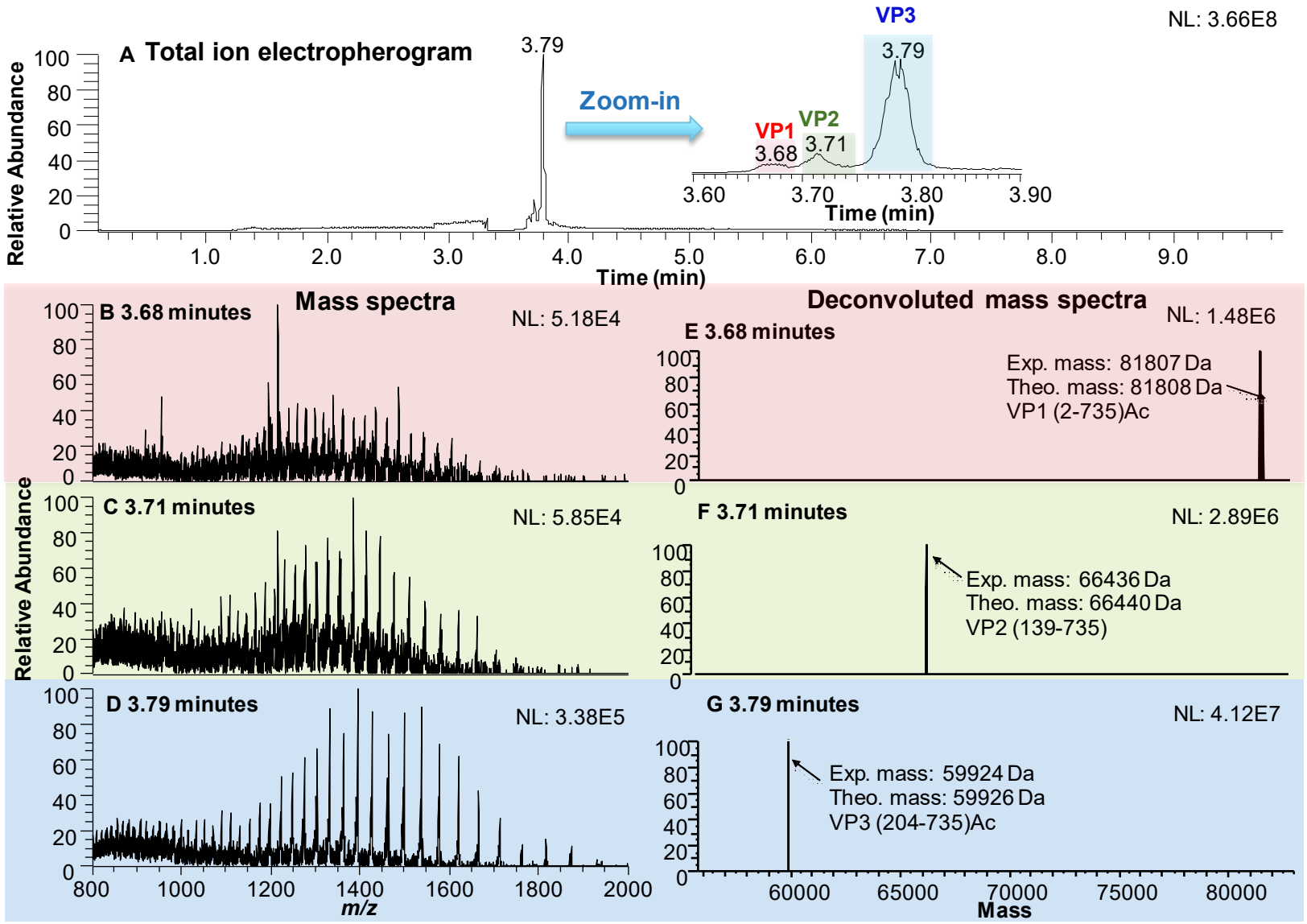
- 40 µL is loaded to the sample vial
- 5 nL for each analysis

Orbitrap MS

Mass spectrometry analysis

Data analysis: Protein Deconvolution

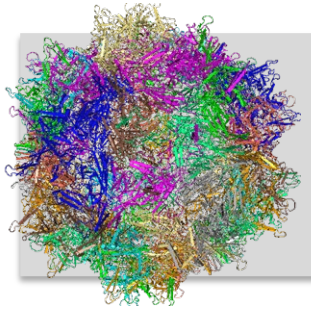
ZipChip CE-MS Intact Protein Analysis of AAV2tYF



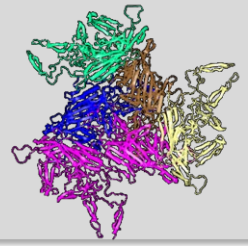
- The three capsid proteins of AAV2tYF were separated by CE and subsequently identified by MS
- The method only took 10 min with 5 nL of sample injected.

Figure adapted from: Zhang, Y. et al Analytical Biochemistry 555 (2018) 22–25

LC-MS Intact Protein Method



AAVs



Denaturation

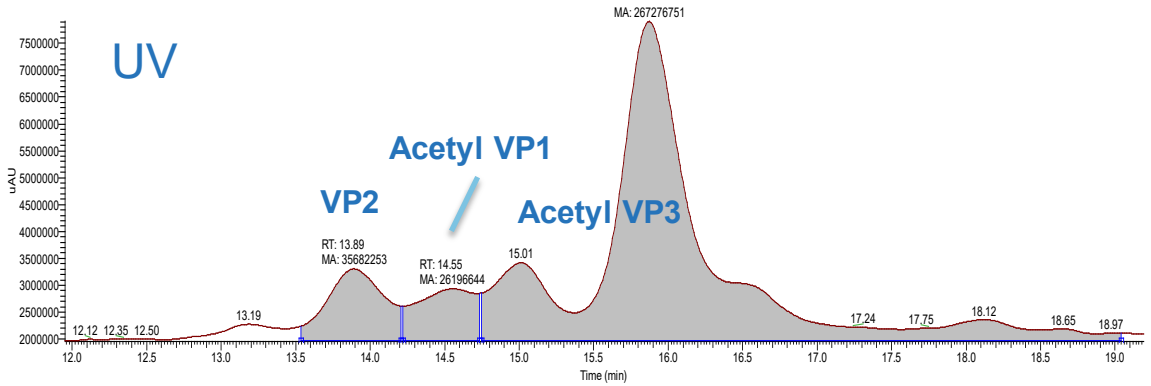
LC systems to separate viral proteins

Viral proteins analyzed by LC-MS on orbitrap MS

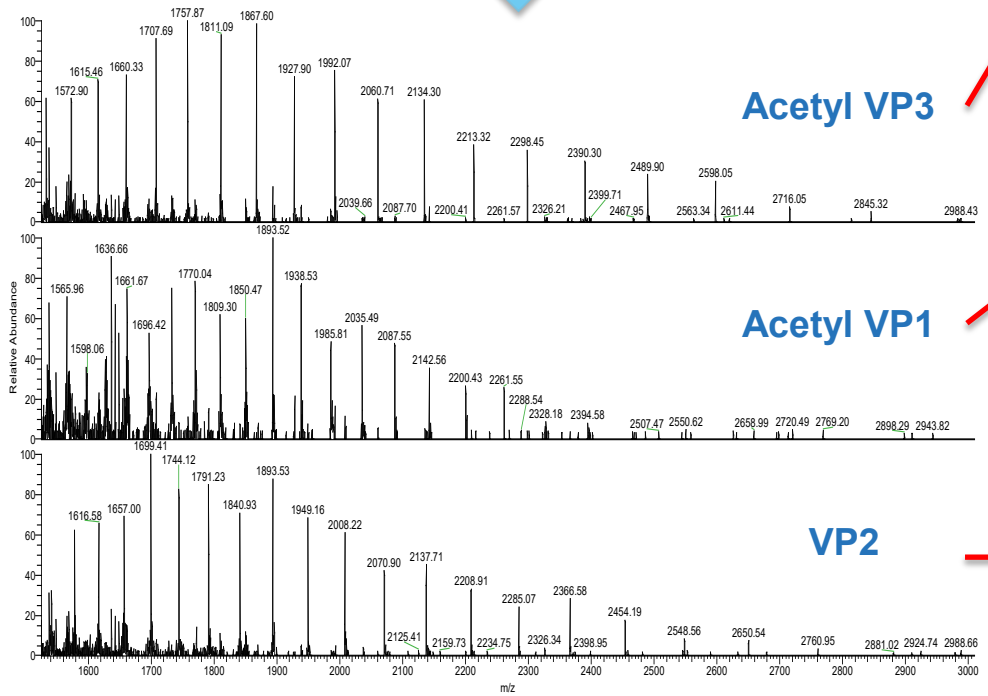
Data analysis:
Protein Deconvolution

Genedata
solutions in silico

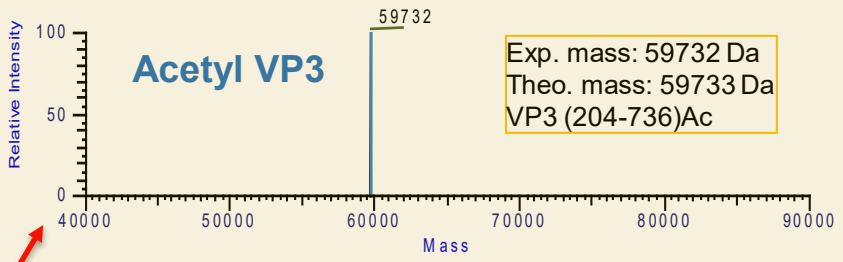
RP-C8-MS Intact Mass Analysis of AAV Serotype “A”

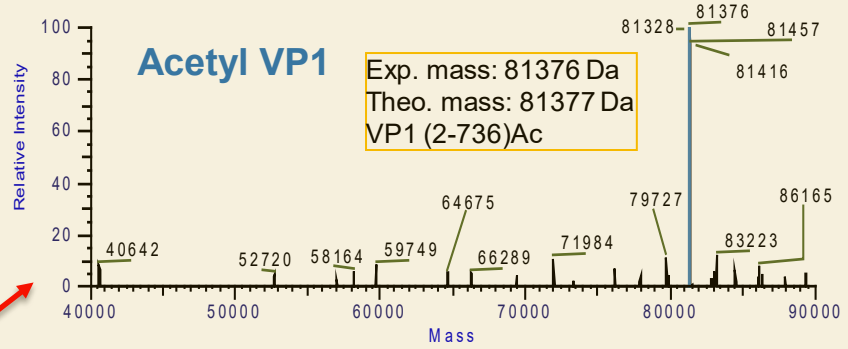


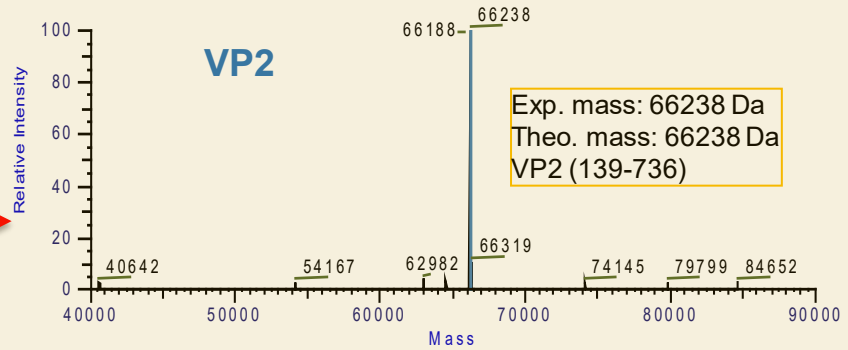
Mass spectrum



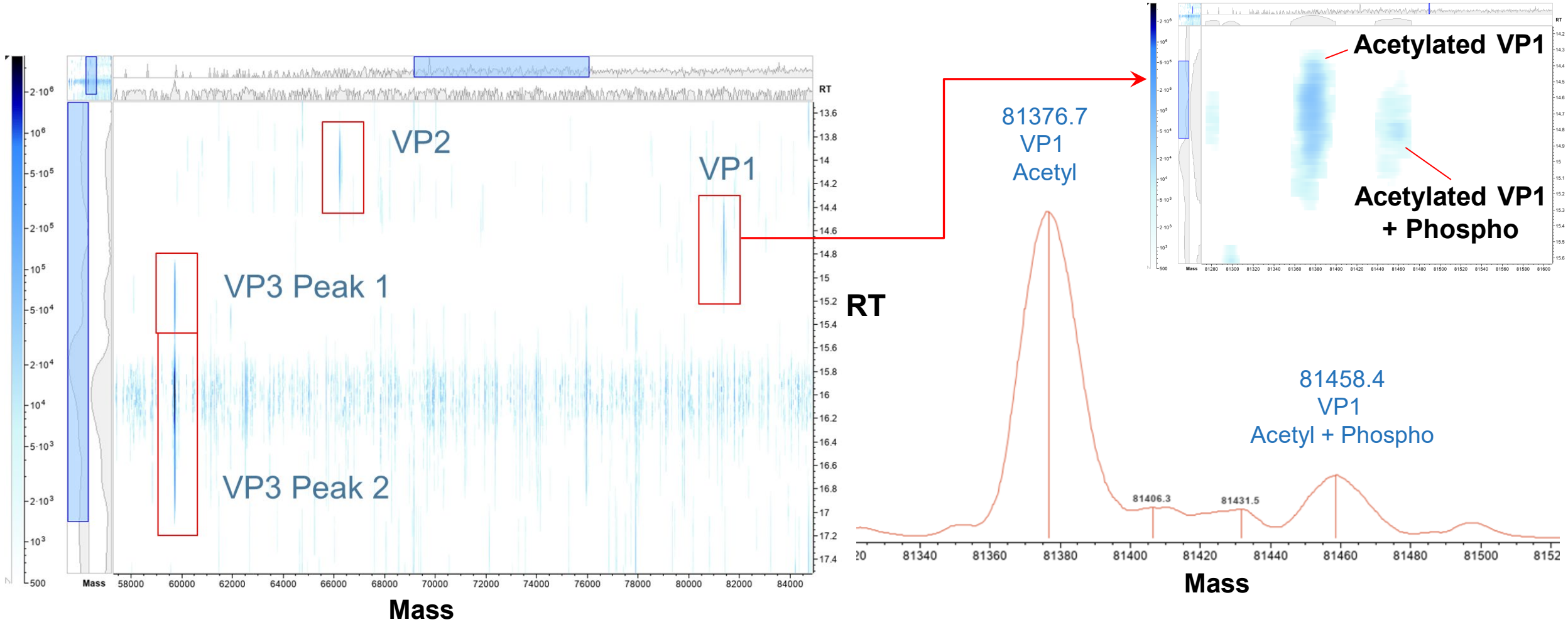
Deconvoluted MS







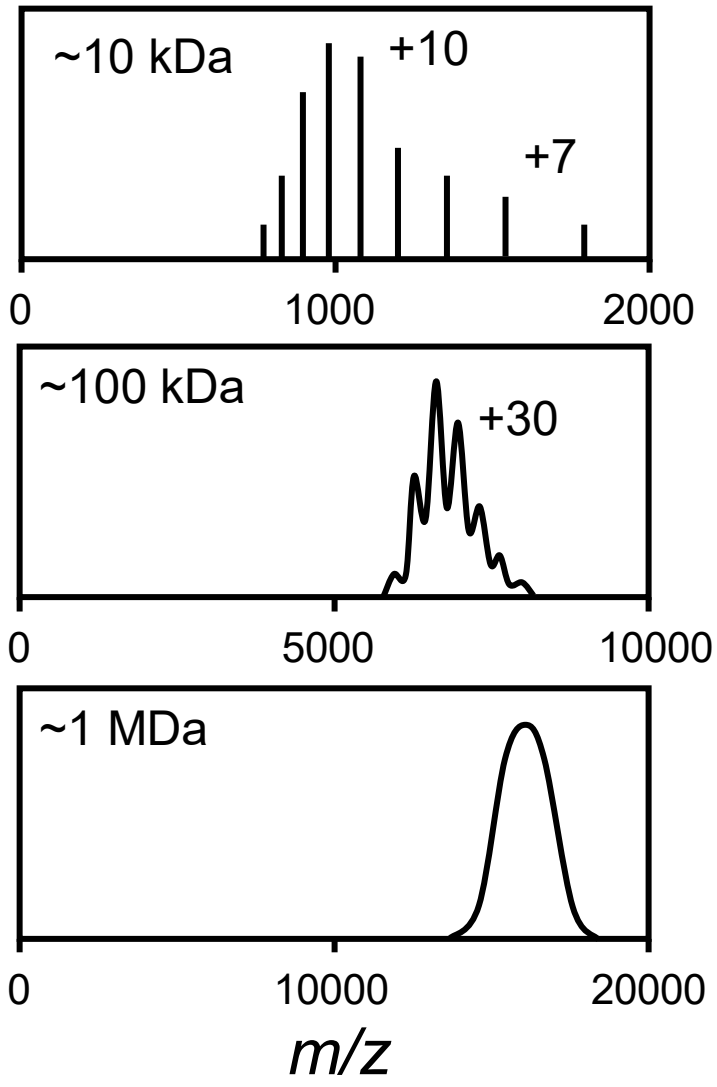
2D Deconvolution of Intact Protein Analysis (AAV Serotype "A")



- VP1 is partially overlapped with the pre-peak (peak 1) of VP3.
- VP1 is partially phosphorylated and the phosphorylated species co-elutes with unmodified one.
- VP3 contains two peaks with nearly identical mass, possibly due to presence of deamidated species.

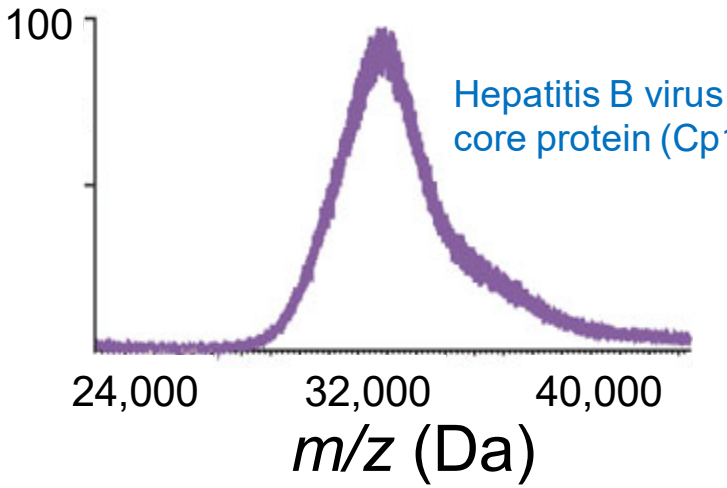
Case Study 2: Characterization of AAV Empty/Full Capsids by CDMS

Loss of Charge State Resolution of Large Molecules



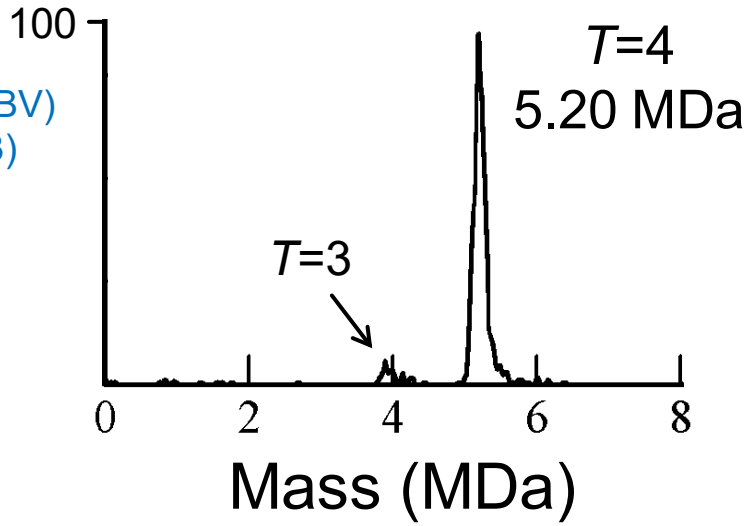
Conventional *m/z* spectrum

- Lack of charge state resolution of large molecules caused by heterogeneity
- *m* could not be determined



Charge detection MS (CDMS)

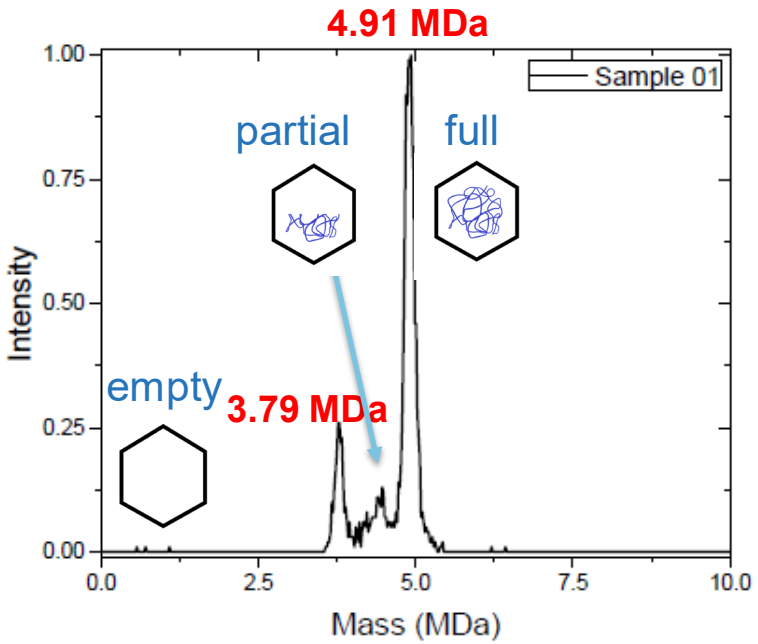
- Measure *m*/*z* and *z* for each ion
- *m*/*z* × *z* → *m* for each ion



T: triangulation numbers

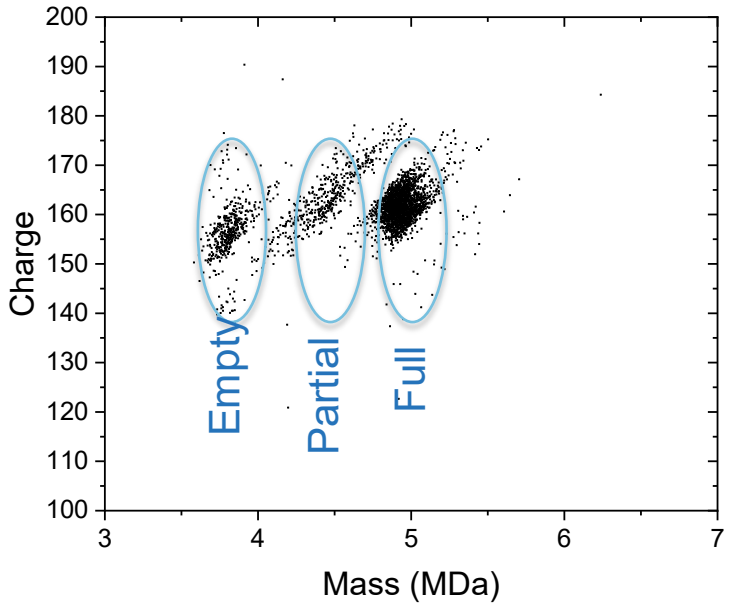
Figures adapted from: Benjamin E. Draper at Megadalton Solutions

CDMS of AAV

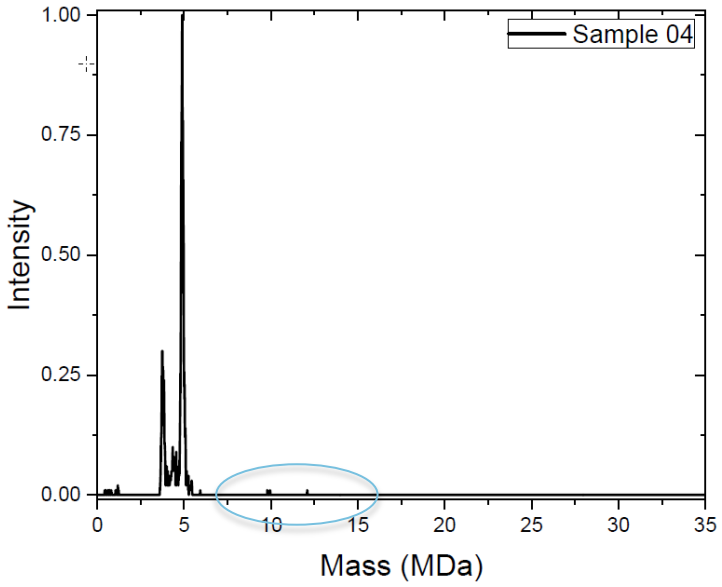


Theoretical Mass of Empty Capsid (1:1:8): 3.75 MDa

- Two primary populations of capsids detected corresponding to empty and full particles
- Some “intermediate” (partially filled) particles observed



- Empty, partial, and full capsids have similar charge characteristics.

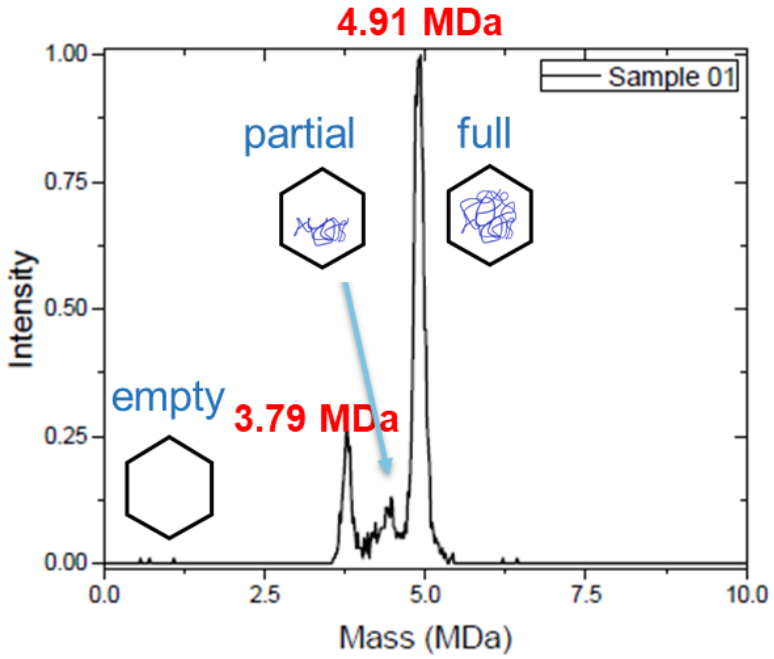


- High-molecular-weight (HMW) species could be characterized.

CDMS data provided by Megadalton Solutions

CDMS and Sedimentation Velocity Analytical Ultracentrifugation (SV-AUC)

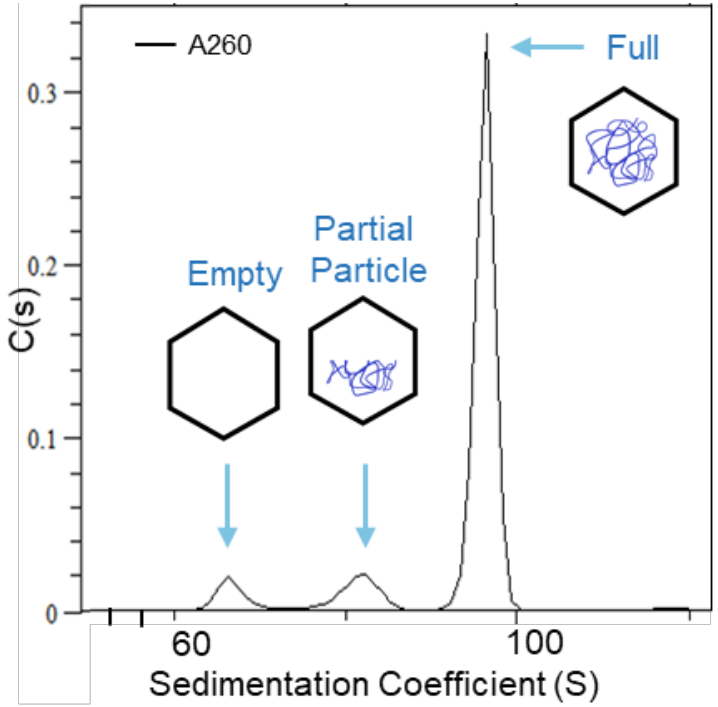
CDMS



Simultaneously measure m/z (mass to charge ratio) and z (charge)

- Resolves intermediate species
- Provide masses of particles
- Provide charge for each species
- Instrument not commercially available yet

SV-AUC

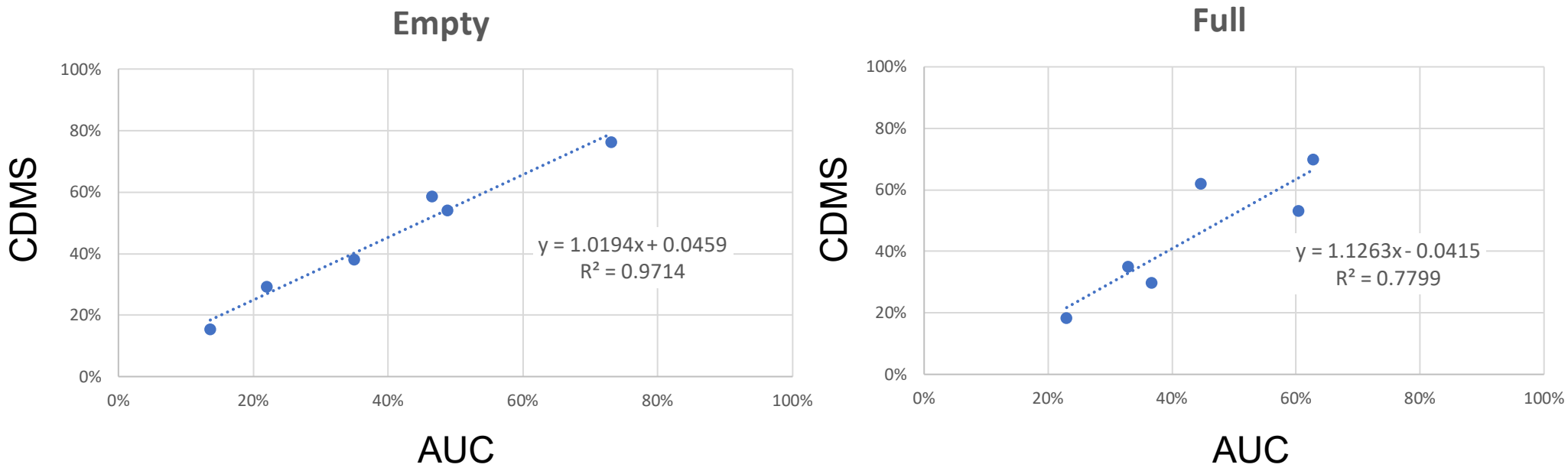


Separate and quantify based on size, shape and mass

- Resolves intermediate species
- Commercial instrument
- High sample amount required
- Low throughput
- Labor intensive

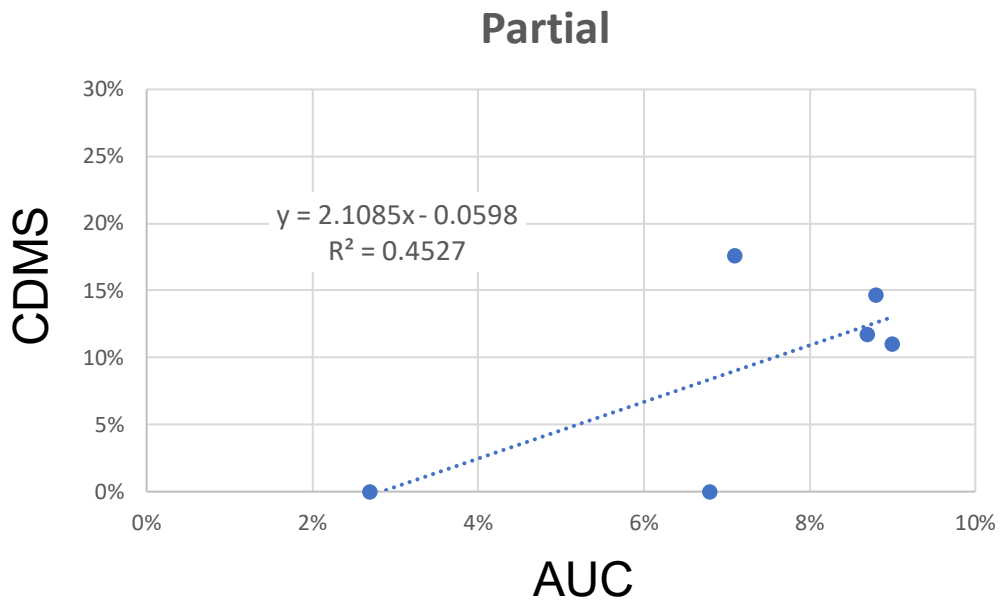
CDMS data provided by Megadalton Solutions

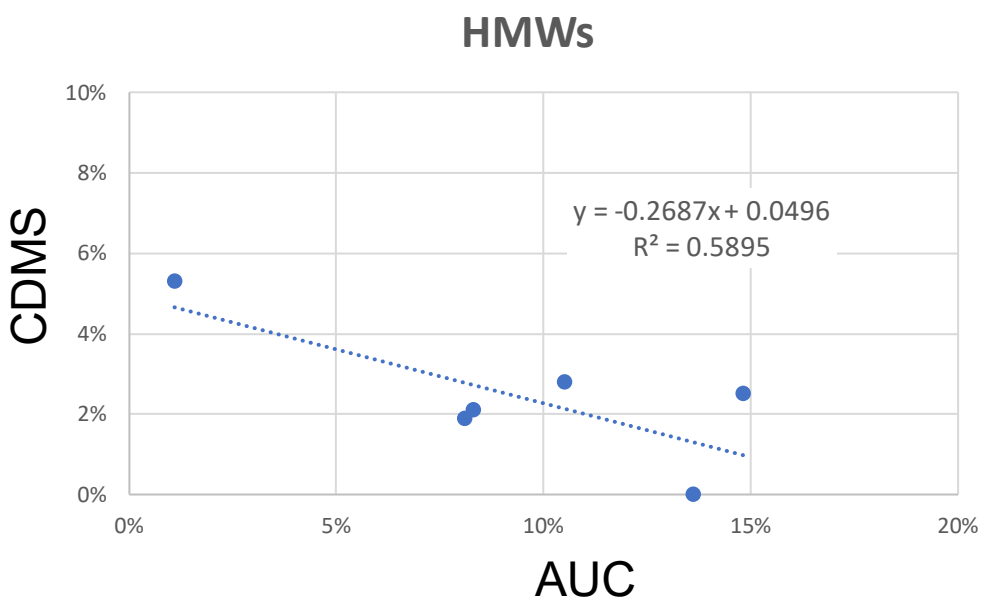
Good correlation between AUC and CDMS for Empty and Full



SV-AUC and CDMS are suitable for quantifying empty and full capsids

Poor correlation between AUC and CDMS for Partial and HMWs

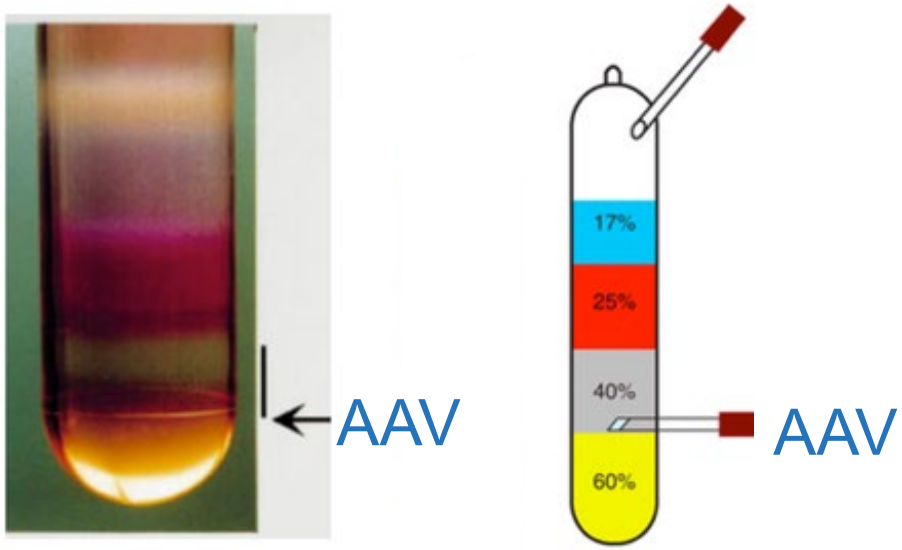




Case Study 3:
Residual Iodixanol Quantification to Support Process Development

Background

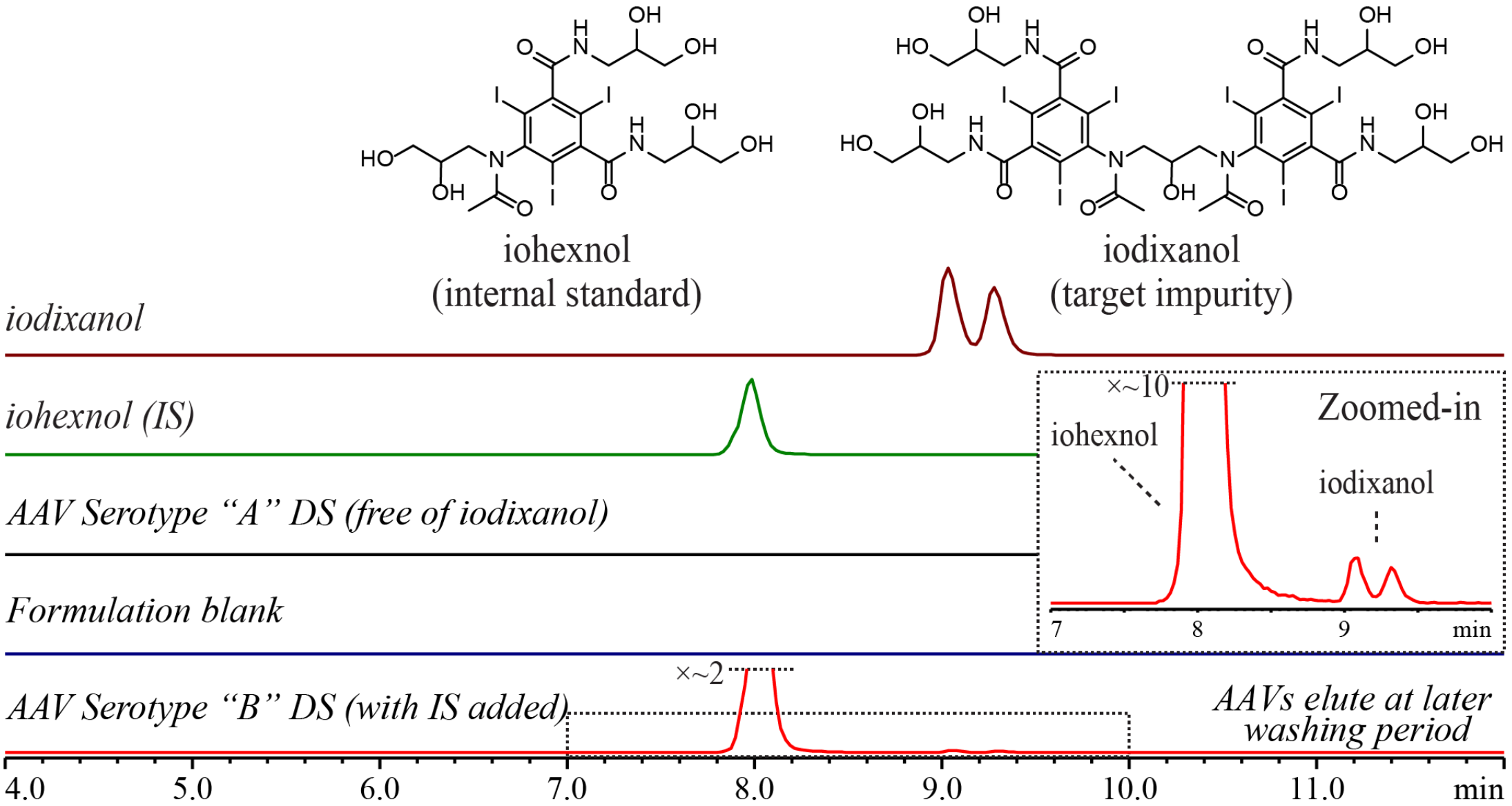
- Iodixanol-based density gradient is commonly used for AAV purification.



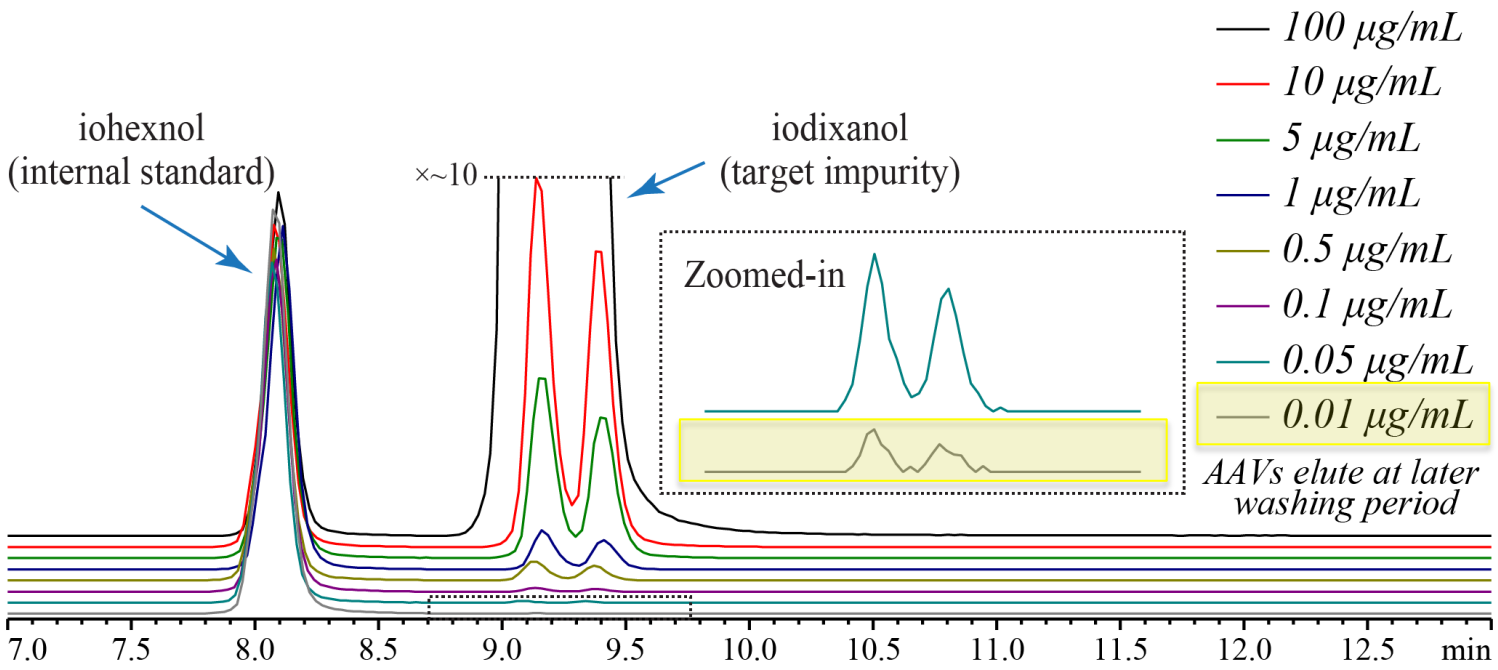
- However, residue iodixanol, as an in-process impurity, may present a safety concern.
- An analytical method with high sensitivity is essential to ensure sufficient clearance of iodixanol, and hence safety of AAV product.

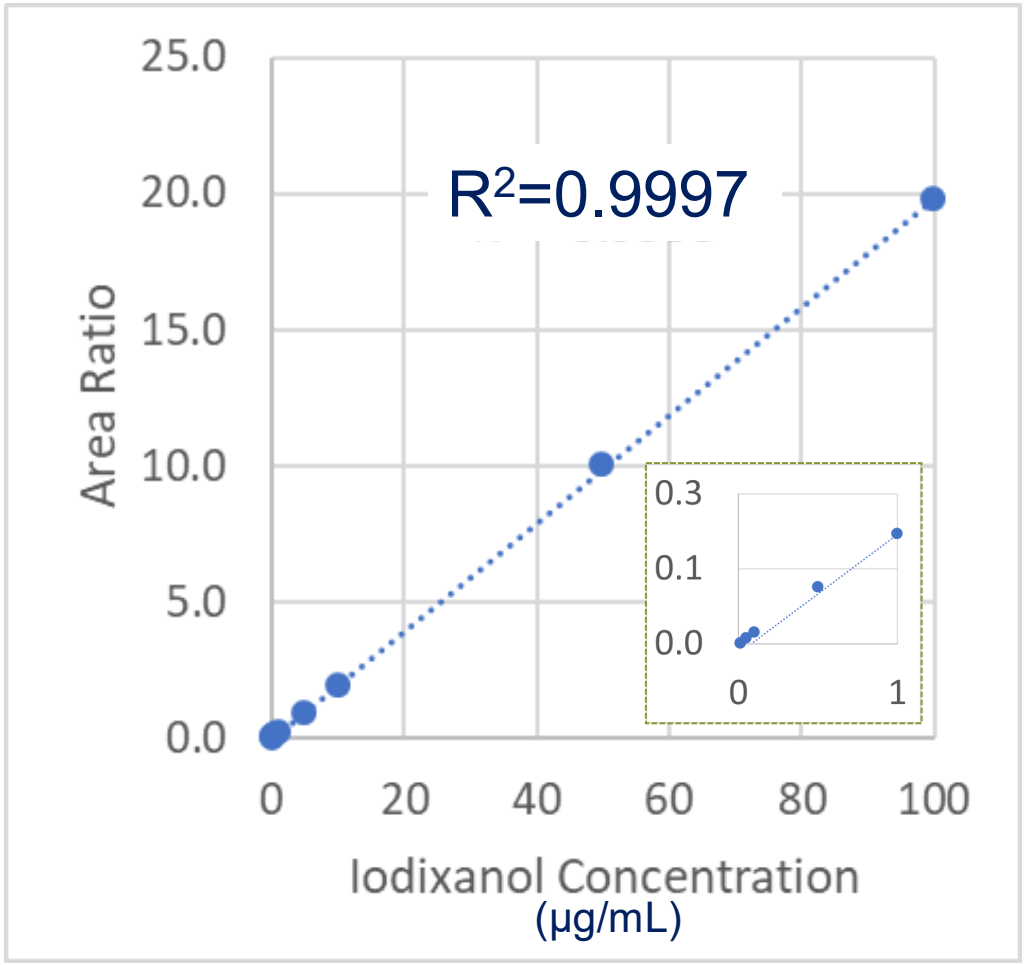
Figure adapted from: https://www.addgene.org/protocols/aav-purification-iodixanol-gradient-ultracentrifugation/

A RPLC-MS Method for Iodixanol Quantification



Sensitivity and Linearity

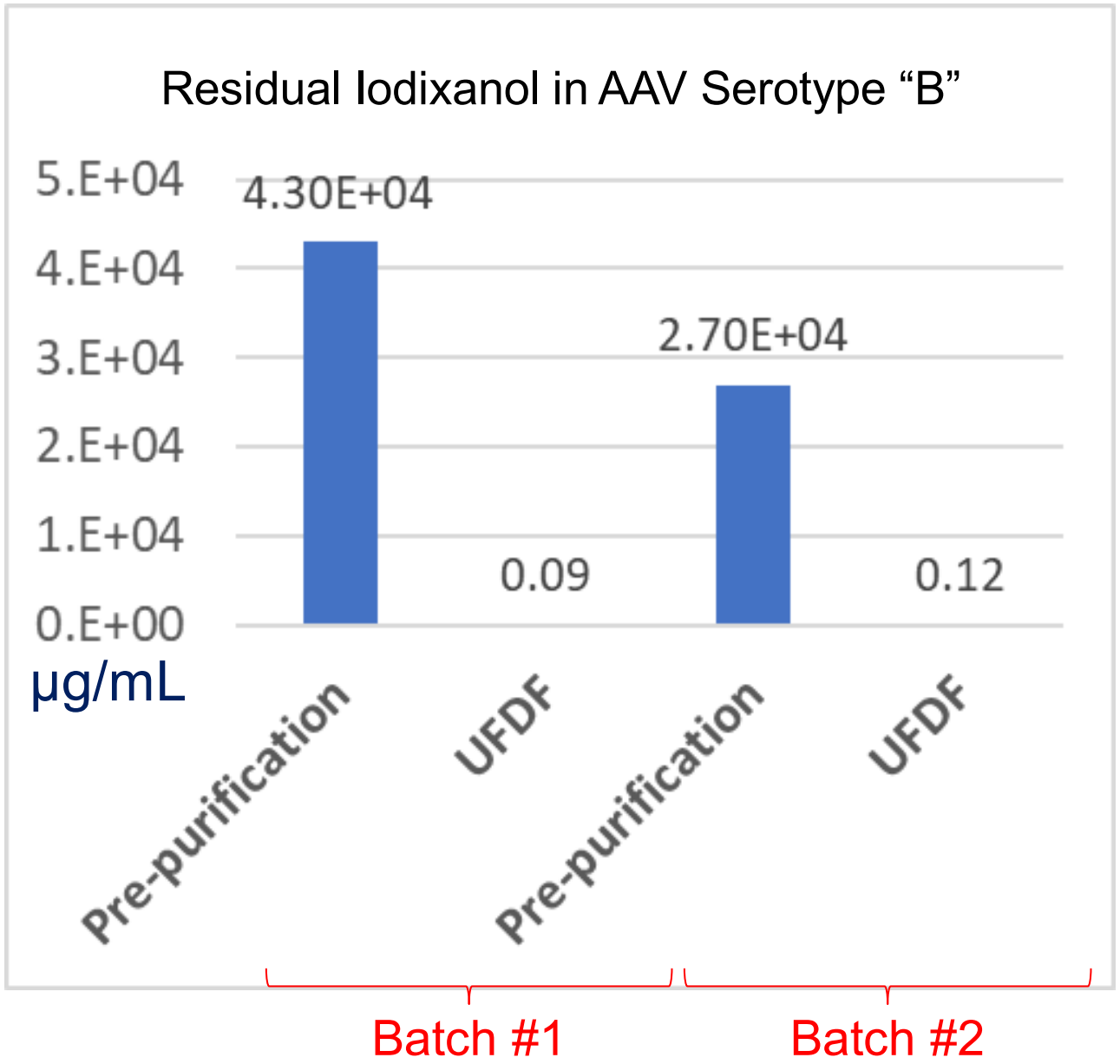




LOQ of 0.01 µg/mL can be achieved.

$$Area\ Ratio = \frac{Peak\ Area\ of\ iodixanol}{Peak\ Area\ of\ internal\ standard}$$

Application to Analysis of AAV In-process and DS Samples



- A highly efficient purification method was further explored for removal of the residual iodixanol.
- The two AAV batches after purification showed residual iodixanol levels well below the recommended safety threshold.

Conclusions

- Mass spectrometry (MS) is a powerful analytical tool that shows great promise in AAV-based gene therapy development.
- The combination of ***intact mass measurement*** of VP1, VP2, and VP3 proteins is highly specific as an identity test using CE-MS or LC-MS.
- SV-AUC and **CDMS** are suitable for characterizing empty and full capsids.
- A **MS-based method** for iodixanol quantification was successfully developed and applied in support of process development.

Acknowledgements

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