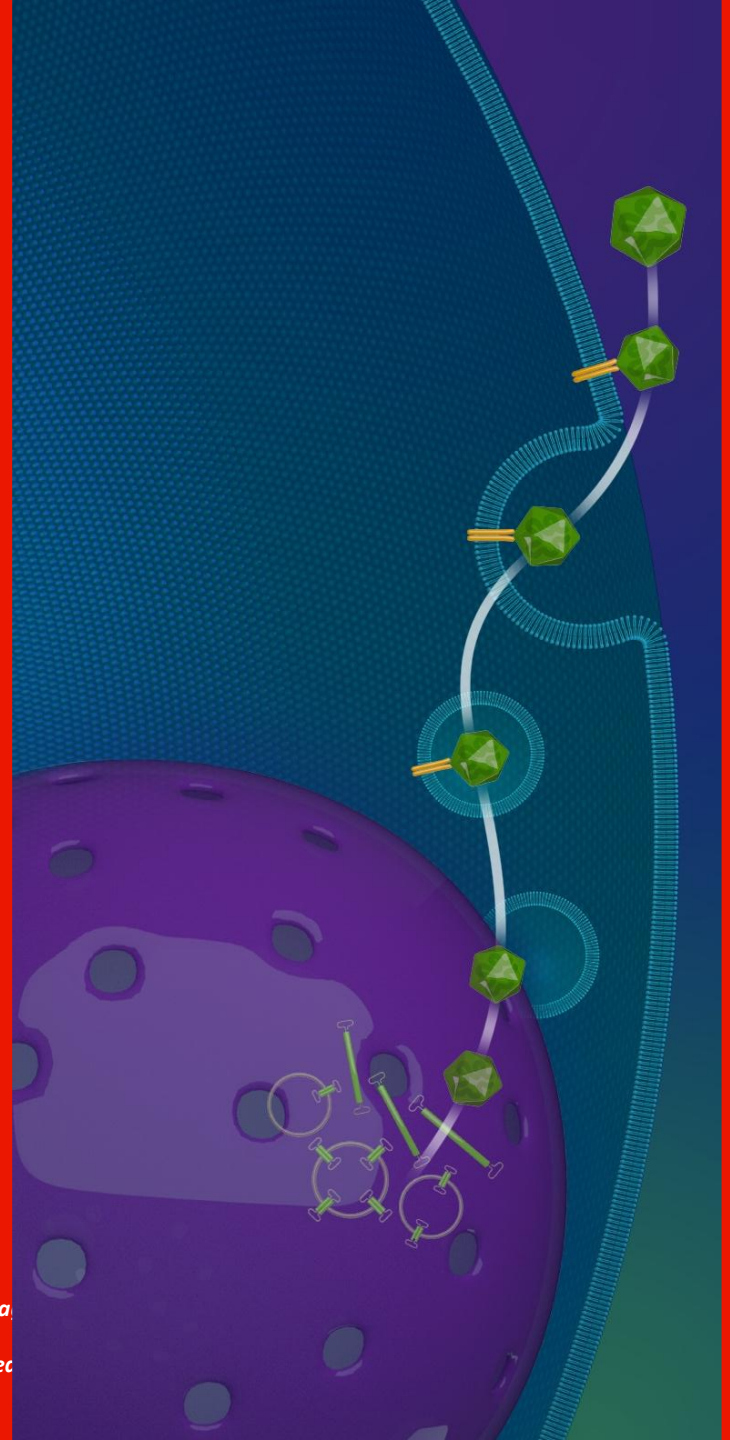
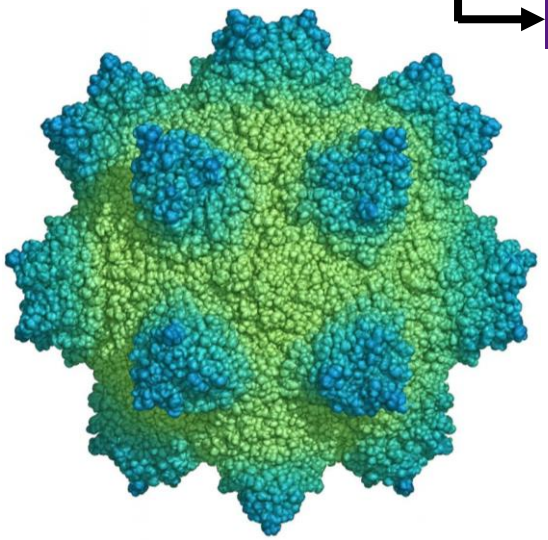
Biophysical Characterization and Control of AAV gene therapy products



Johnson&Johnson

Angus Hibbins (PhD, EMBA)

AAV Gene Therapy and generalized CQAs



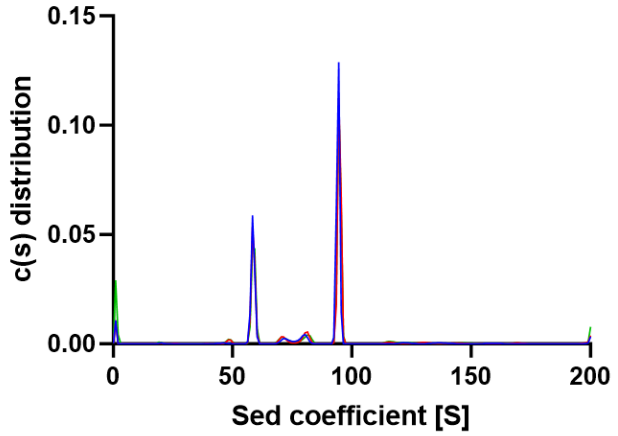
- AAV CQAs
  - Quantity and Identity
    - VG Titer
      - SEC-MALS
    - VP Titer
      - SEC-MALS
      - UPLC-AEX-FL
    - Capsid ID
      - UPLC-AEX-FL
      - UPLC-RP-FL
  - Purity
    - Capsid Purity
      - UPLC-RP-FL
    - Aggregation
      - SEC-MALS
      - SEC-UV
    - Viral Protein Ratio
      - UPLC-RP-FL
    - Empty/Full Ratio
      - SEC-MALS
      - UPLC-AEX-FL

CQA Determined at | DS & DP level | DS level | Process Support | QC Testing

What is analytical Strategy to Determine Empty/Full Ratio?

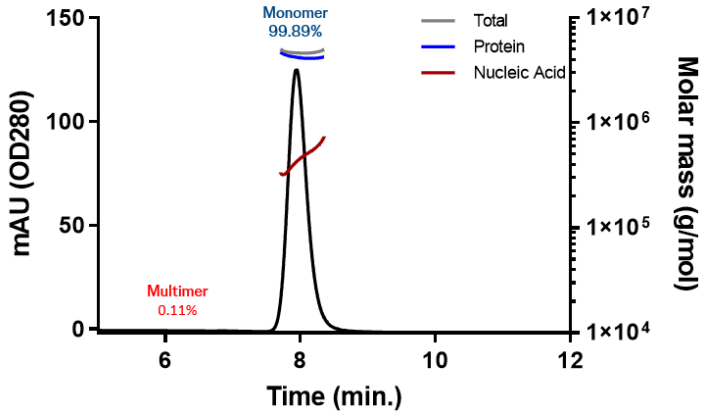
Historical Golden Standard

Analytical Ultracentrifugation

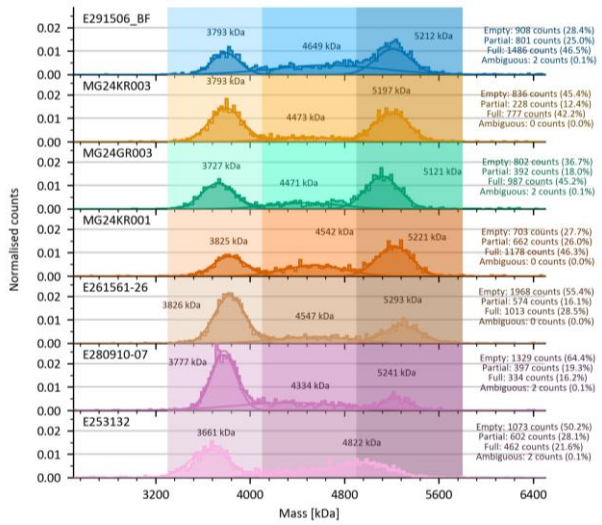


Process Analytical Support

SEC-MALS/DAD

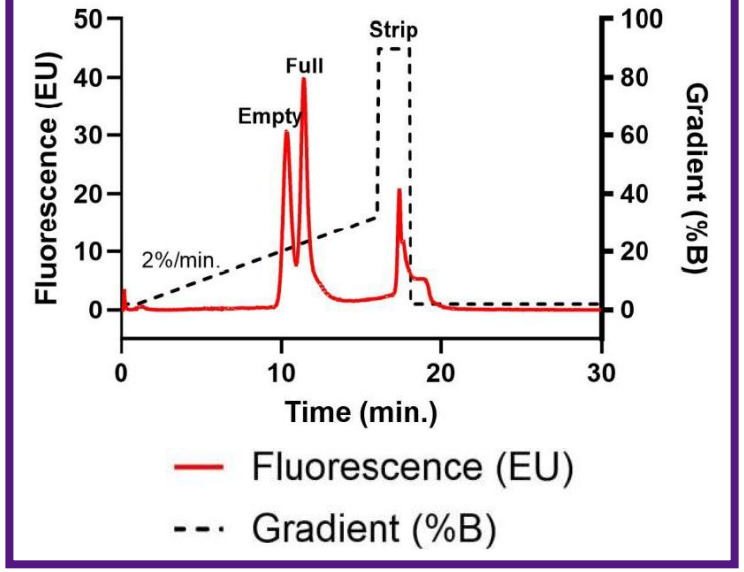


Mass Photometry



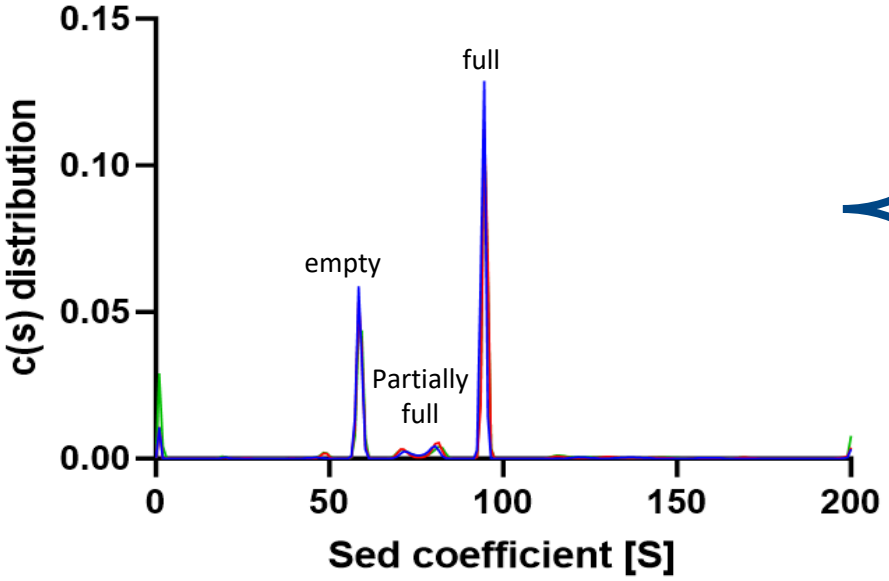
QC Release

UPLC-AEX-FL



How do we go from AUC to Orthogonal Methods?

Historical Golden Standard
Analytical Ultracentrifugation



Why is Analytical Ultracentrifugation (AUC) used?

- AUC separates AAV's based primarily on density
- Can separate empty, partially full, full AAV particles and aggregates
- Limited method development is required
- Established method with good reproducibility irrespective of the AAV serotype used
- Health Authorities currently expect to AUC data to be generated for filings

Why is AUC not recommended for process development?

- **Material Utilization**:
  Large quantities of material used volumetrically (1.2-1.3 mL)
- **Throughput**:
  Time consuming and low throughput (approximately 7 samples at once over 12 hours)
  Single CQA determination

Why is AUC not recommended for R&S in some QC labs?

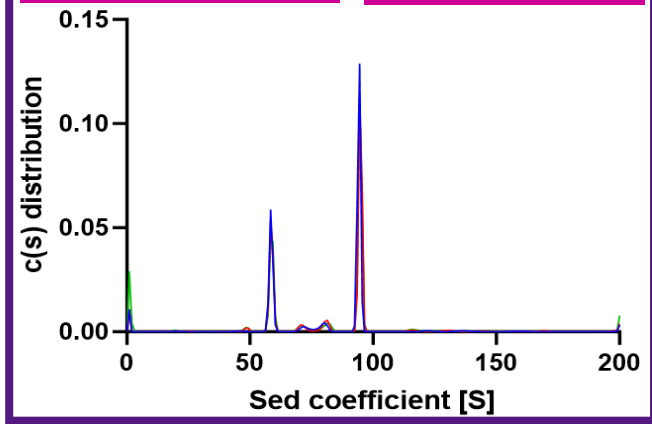
- **Material Utilization:**
  Large quantities of material used volumetrically (1.2-1.3 mL)
- **Throughput:**
  Time consuming and low throughput (approximately 7 samples at once)
  Single CQA determination
- **Compliance:**
  Currently not CFR21 part 11 compliant due to open database software

So how is the SEC-MALS approach leveraged for UPLC AEX?

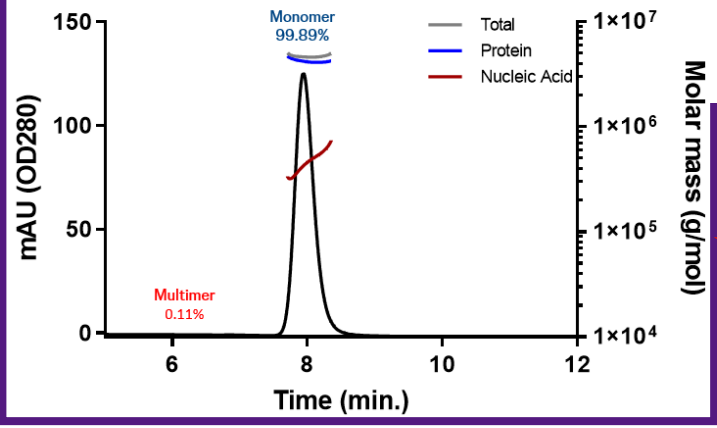
Analytical Ultracentrifugation

Partial's Determination

Empty/Full Ratio



SEC-MALS/DAD/LS



Empty/Full Ratio

Aggregation

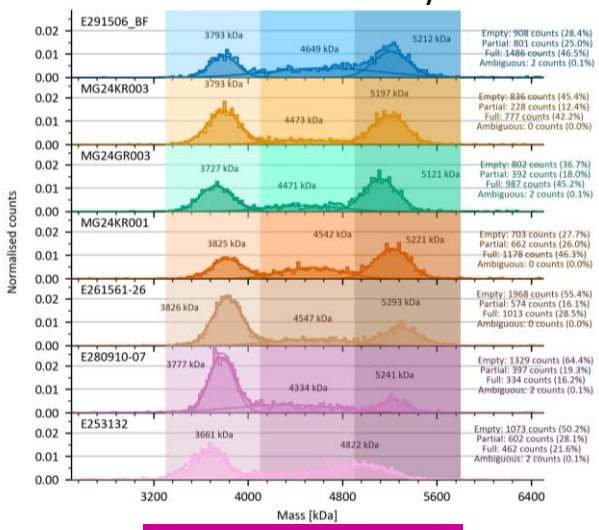
VP Titer

VG Titer

Capsid Size Distribution

AUC: 50.4% Full
SEC-MALS:
49±0.59% Full

Mass Photometry



Partial's Determination

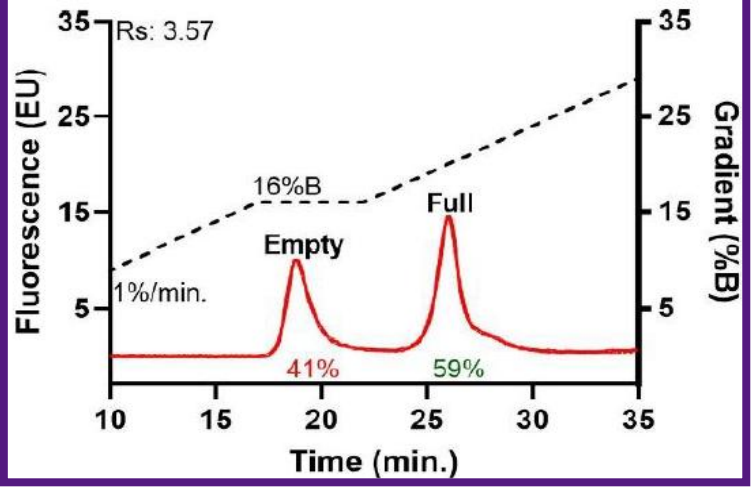
For Partially filled Capsids

VP Titer

Capsid ID

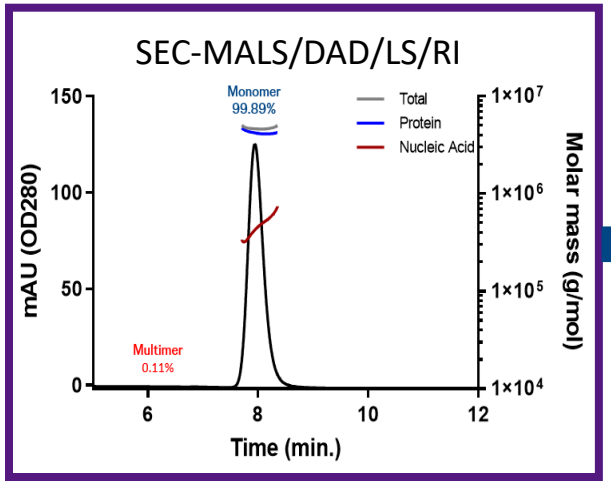
Empty/Full Ratio

AAV1.eGFP (Progen) Isocratic Hold 16%B



J&J

How do we maximize the method utility towards MAMs?



Multi-pCQA Determination for In-Process Support

- Empty/Full Ratio
- MW of Capsid and Encapsulated DNA
- Aggregation
- Capsid Size Distribution
- VP Titer
- VG Titer

Why is SEC-MALS/DAD/LS/RI used?

- SEC-MALS separates by hydrodynamic size and determines content from multi-angle light scattering
- Using theoretical calculations and WYATT AAV module, the Capsid mass in kDa. The DNA mass is also calculated in kDa. An OD260/OD280 is also calculated. From this, the Empty/Full, VP titer and VG titer are calculated.
- This has been compared to molecular bioassays (ELISA and dPCR)

Why is SEC-MALS recommended for process development?

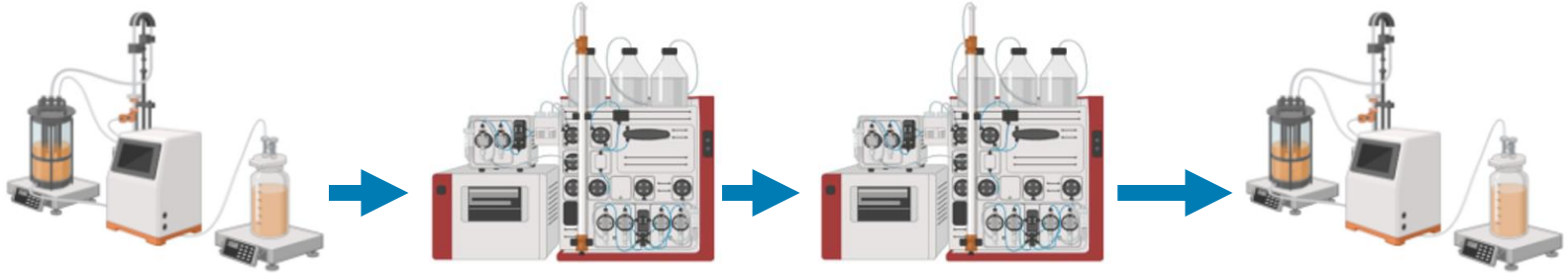
- **Material Utilization**:
  Limited quantities of material used volumetrically (30µL which is approximately 33 times less than AUC)
- **Throughput**:
  Approximately 12 minutes per samples with limited sample preparations
  Multiple CQA determinations per sample

Why is AUC not recommended for R&S in some QC labs?

- **Compliance:**
  Currently processing MALS within established QC preferred UPLC management software (Waters) is to be established

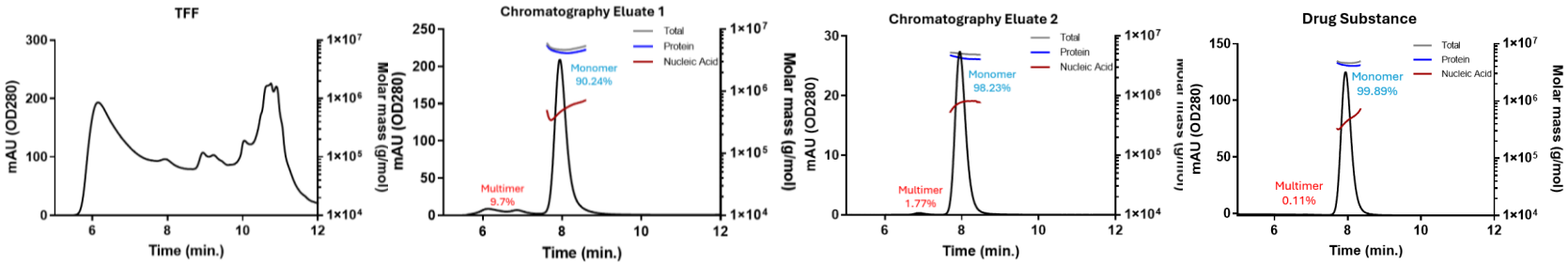
J&J

Quality Attribute: Monitoring over standard AAV process



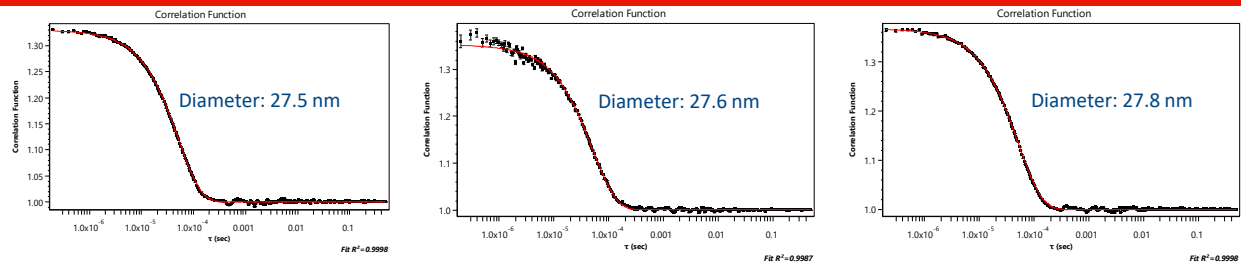
1. TFF1 2. Chromatography 3. Chromatography 4. TFF2

Aggregate determination by SEC-MALS



Size determination by SEC-MALS

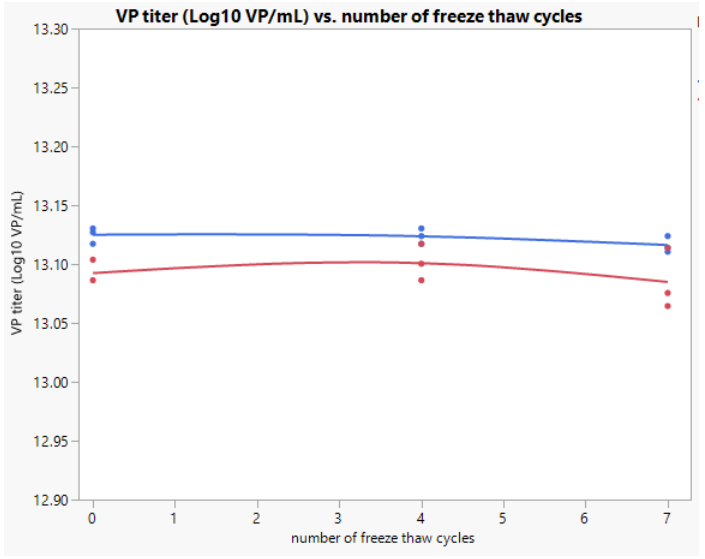
Not available

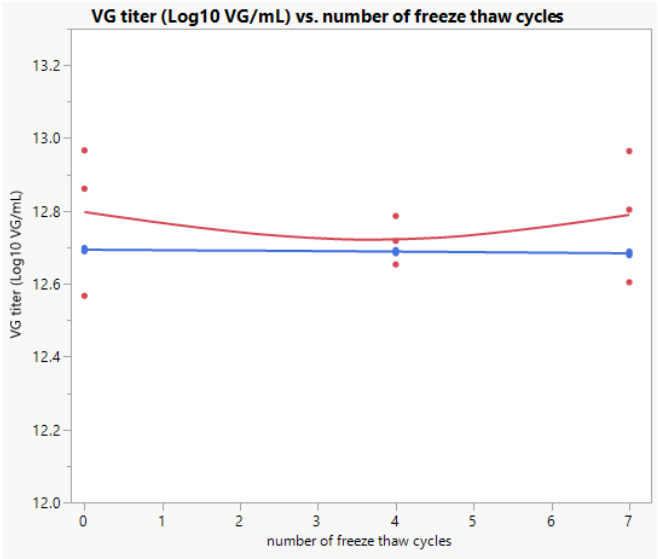


Multi-pCQA Determination for In-Process Support

- Empty/Full Ratio
- MW of Capsid and Encapsulated DNA
- Aggregation
- Capsid Size Distribution
- VP Titer
- VG Titer

SEC-MALS compared across process to ELISA and qPCR

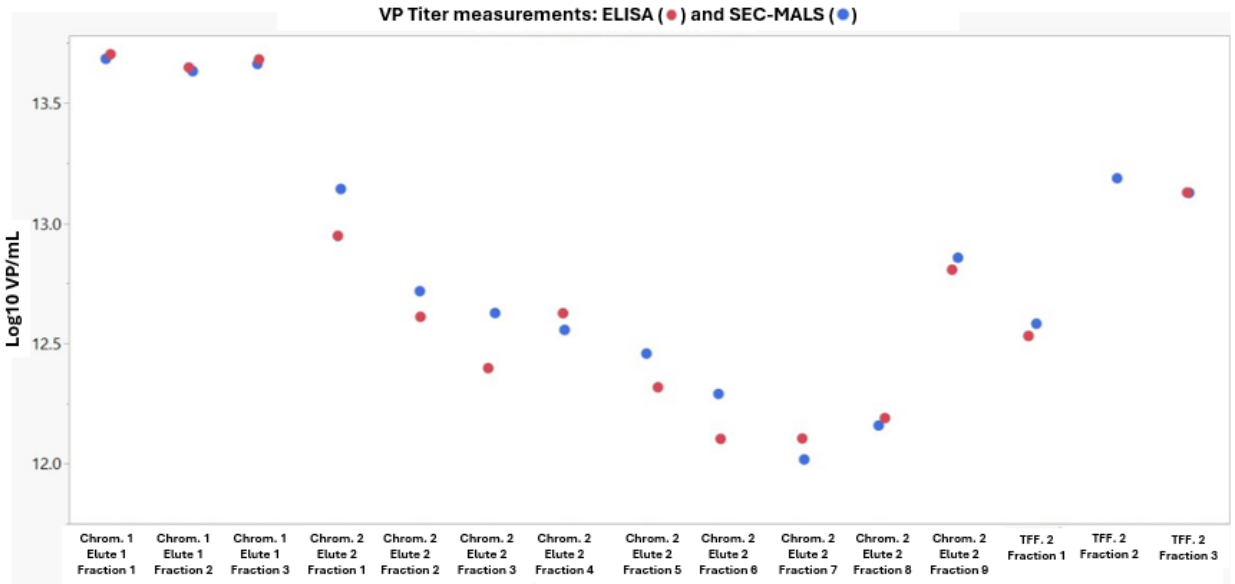




7 freeze thaw cycles were completed with AAV1 GFP and results between PCR, ELISA and SEC-MALS were quite comparable

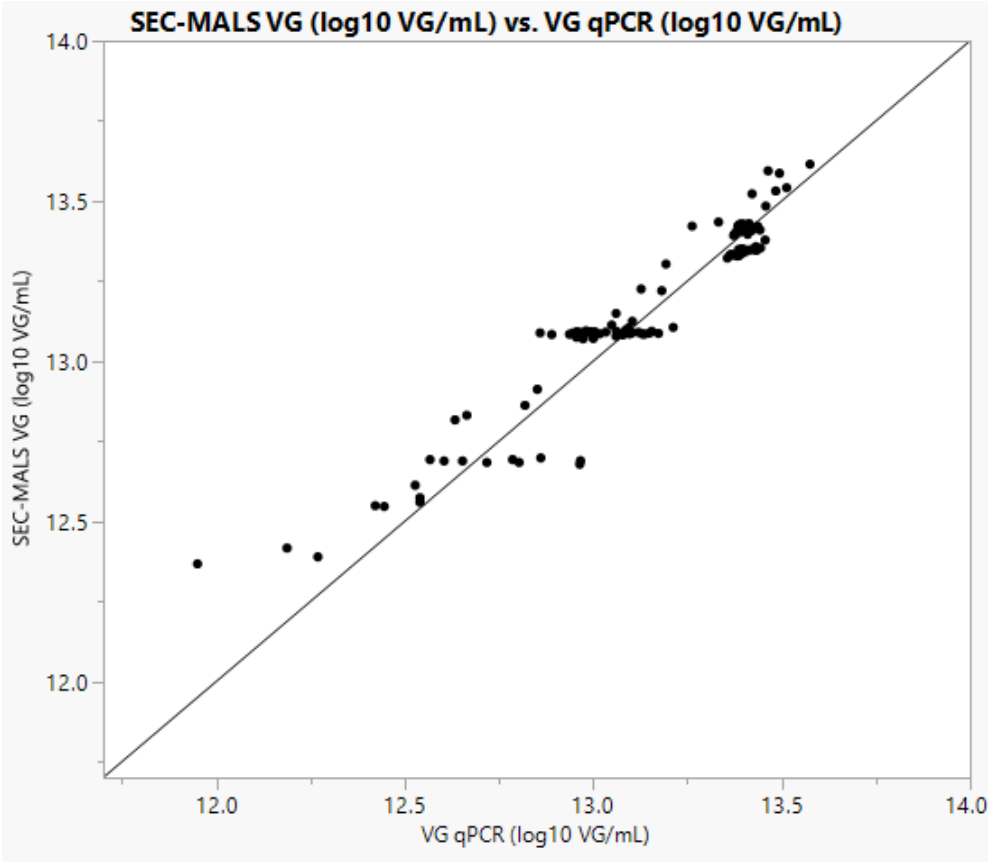
ELISA ( )

SEC-MALS ( )

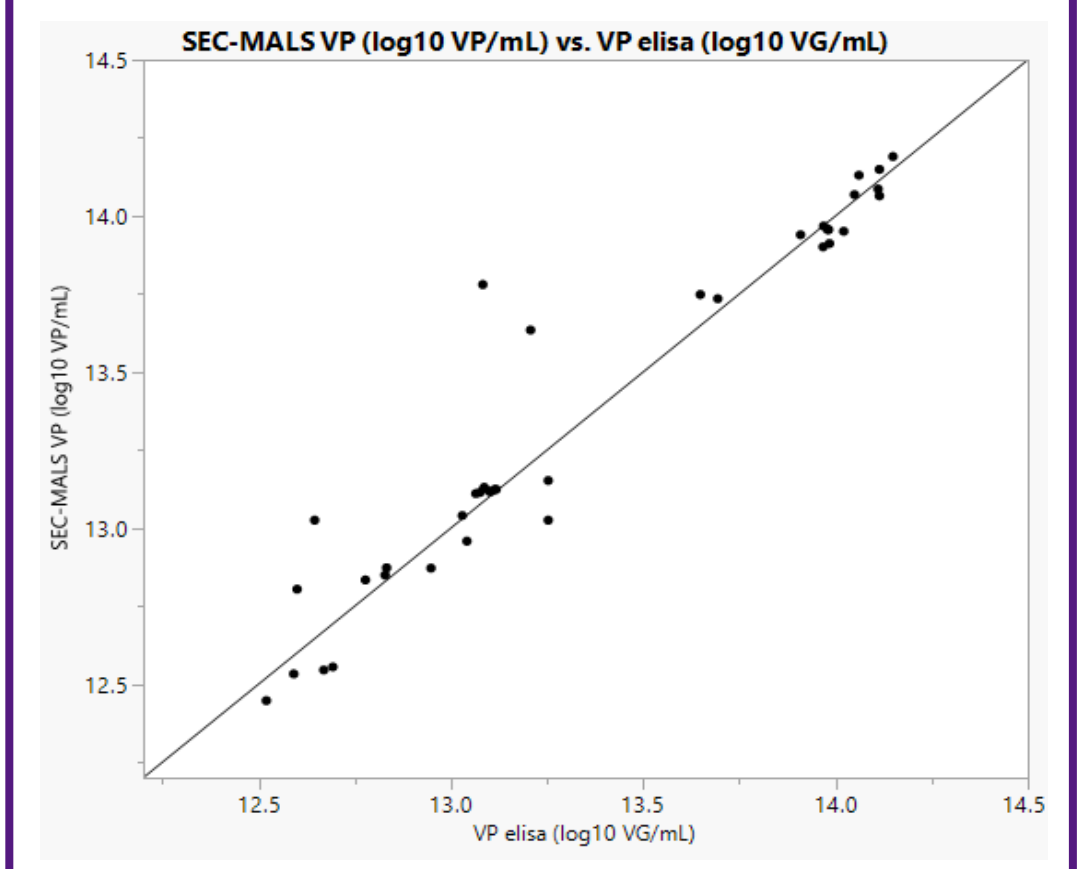


Analysis of process intermediates from AAVX elute to TFF product were analyzed with PCR, ELISA and SEC-MALS which was also quite comparable

SEC-MALS compared across process development samples



Average Difference between SEC-MALS and qPCR is 0.02 log10 VG/mL (approximately 5% error)



Average Difference between SEC-MALS and qPCR is 0.03 log10 VP/mL (approximately 5% error)

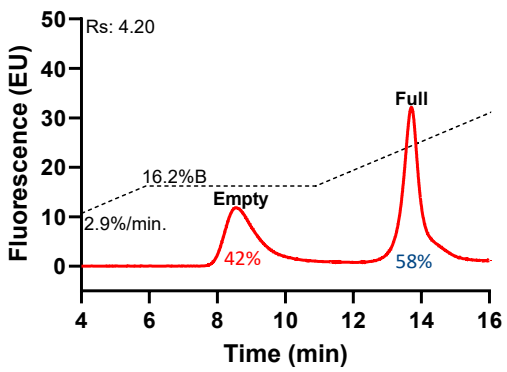
Note: VP ELISA and qPCR were determined in a 1x1 format and relative error is expected to decrease if format is increased

J&J

Quality Attribute: Impurities – Empty/Full determinations

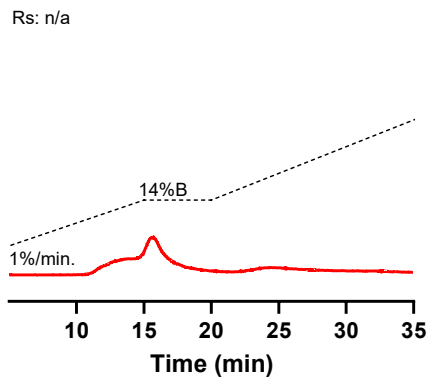
AAV1

AAV1. EGFP (Progen)
Isocratic Hold 16.2%B



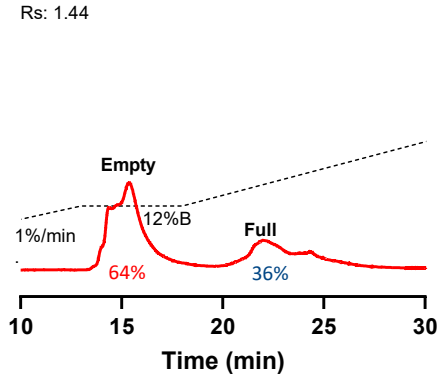
AAV2

AAV2.EGFP (Progen)
Isocratic Hold 14%B



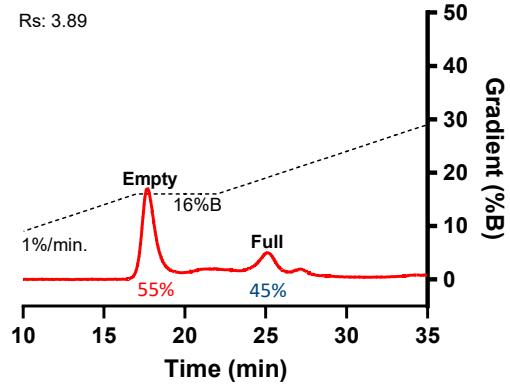
AAV5

AAV5.EGFP (Progen)
Isocratic Hold 12%B



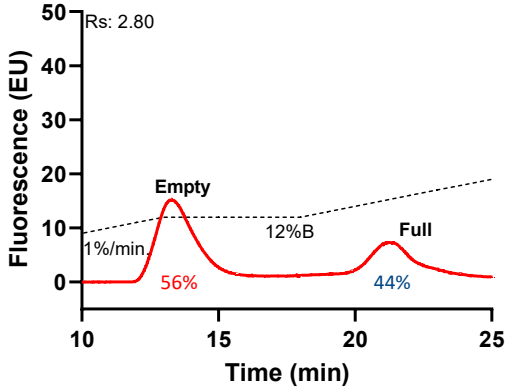
AAV6

AAV6.EGFP (Progen)
Isocratic Hold 16%B



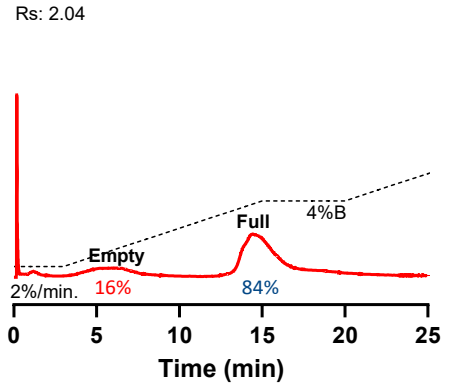
AAV8

AAV8.EGFP (Progen)
Isocratic Hold 12%B



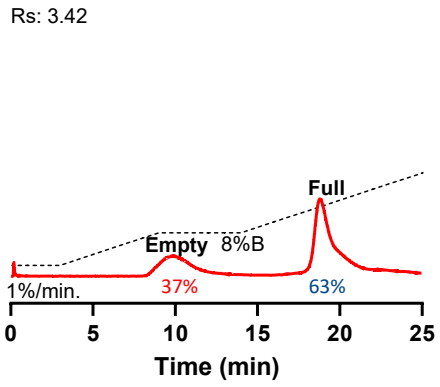
AAV9

AAV9.EGFP (Progen)
Isocratic Hold 4%B



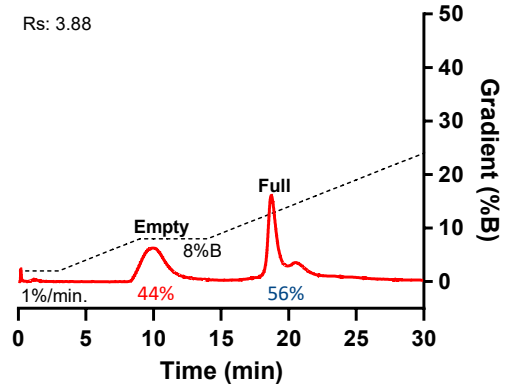
AAVrh.10

AAVrh10.EGFP (Progen)
Isocratic Hold 8%B



AAVrh74

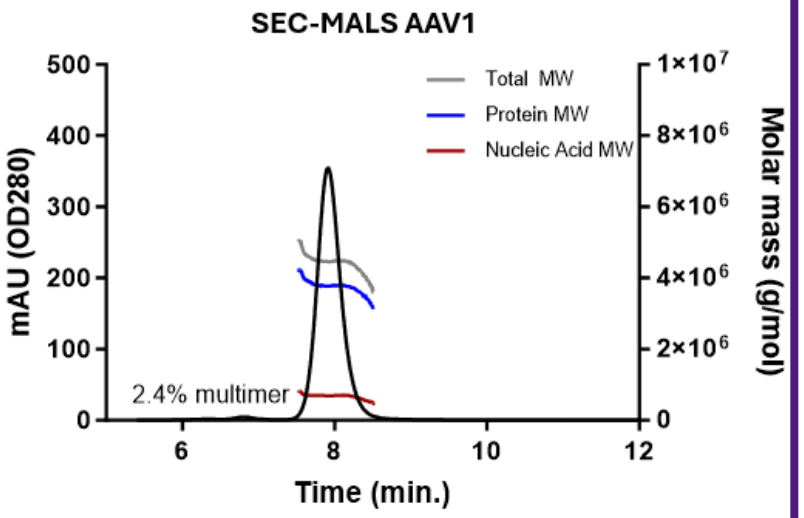
AAVrh74.EGFP (Progen)
Isocratic Hold 8%B



AAVs are sourced from Progen and contain the EGFP GOI construct

Setting up AEX for AAV1

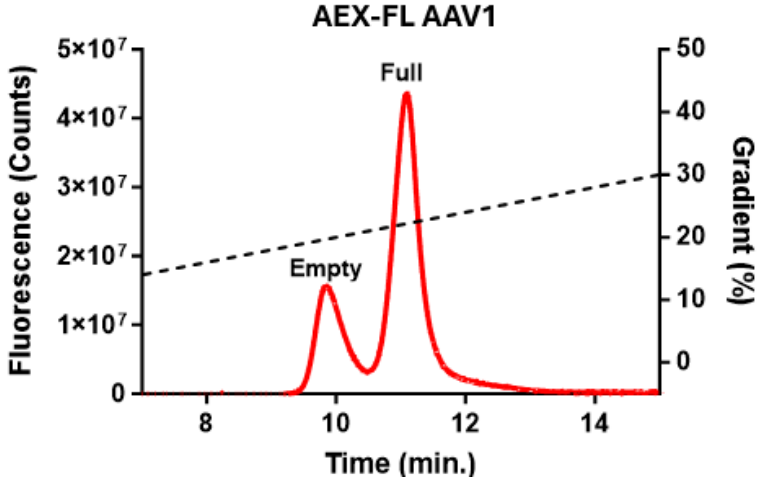
Isocratic gradient



- Column: Waters Premier GTx Resolve 450Å
- Mobile Phase: 20 mM NaPi, 350 mM NaCl, 0.001% (v/v) Pluronic F-68 pH7.4
- Detection: UV at 260 and 280 nm, µDawn

- VP titer: 5.39E+13
- VG titer: 2.78E+13
- Vg/Vp: 0.515 (= 51.5% Full)

Linear Gradient

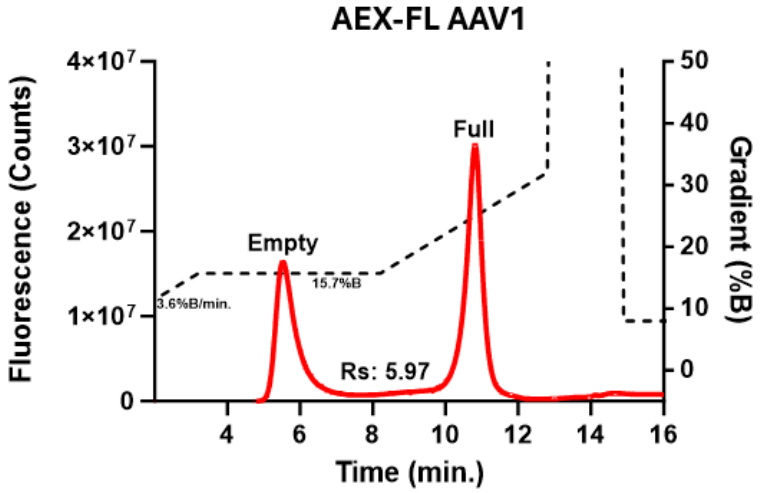


- Resolution factor: N/A

- No full baseline separation between empty and full particles

- Location of empty and full particles confirmed using OD260/280 ratios and reference materials

Step-gradient method

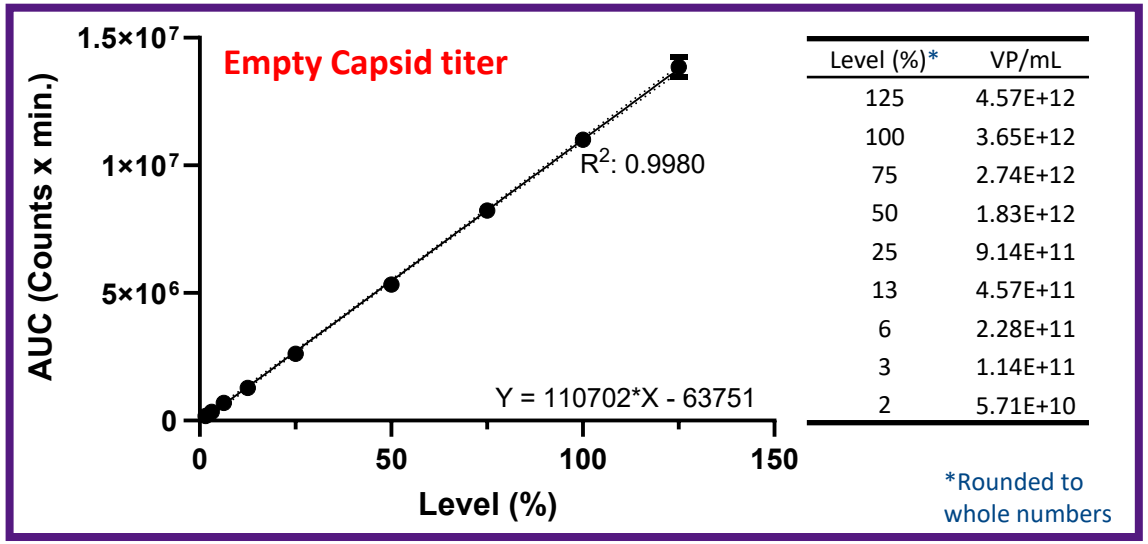


- Resolution factor: 5.97±1.48%

- Full baseline separation between empty and full particles

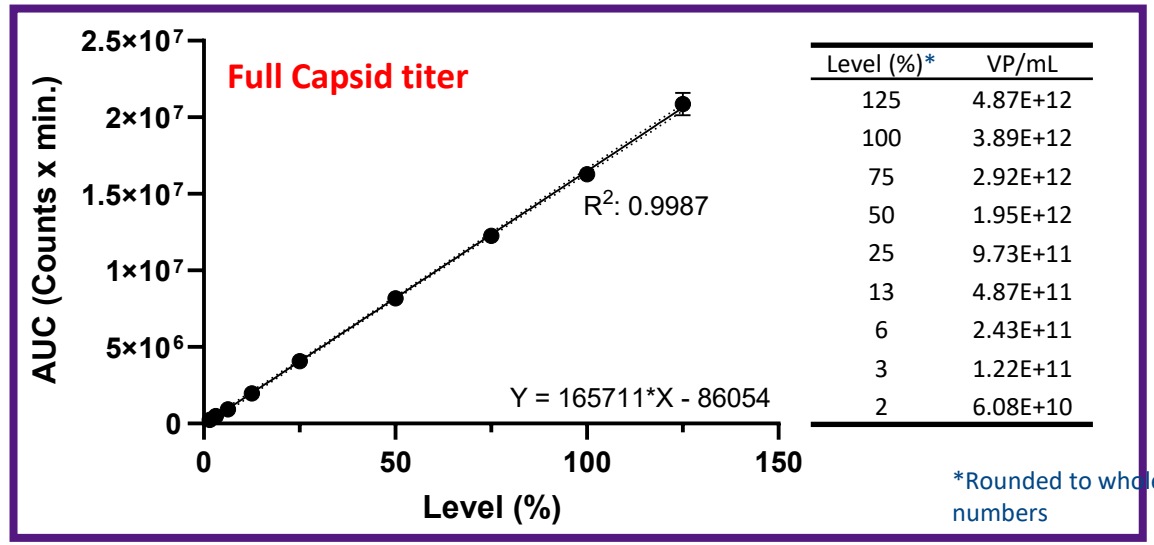
- Similar peak distribution (AUC) both species compared to base method

Linearity and range for titer determination for AAV1



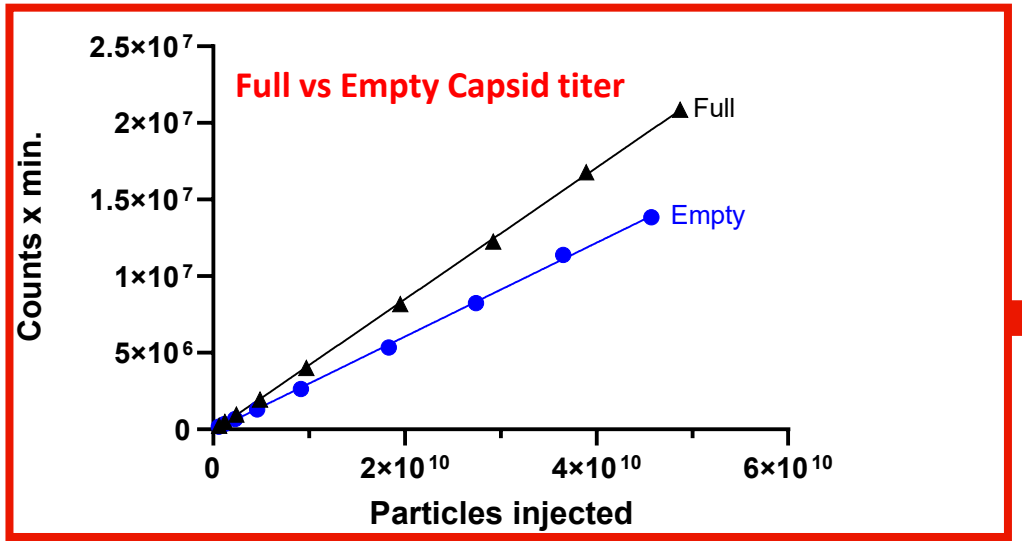
Level (%)*	VP/mL
125	4.57E+12
100	3.65E+12
75	2.74E+12
50	1.83E+12
25	9.14E+11
13	4.57E+11
6	2.28E+11
3	1.14E+11
2	5.71E+10

*Rounded to whole numbers

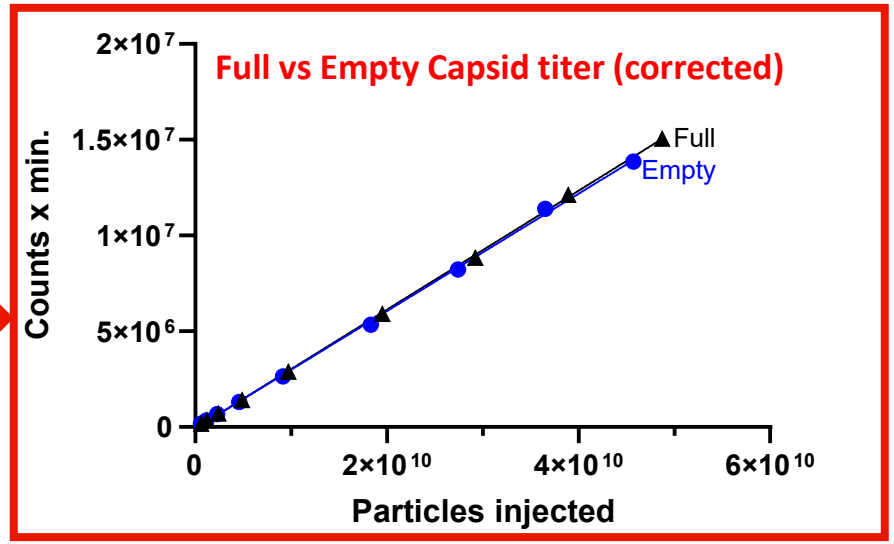


Level (%)*	VP/mL
125	4.87E+12
100	3.89E+12
75	2.92E+12
50	1.95E+12
25	9.73E+11
13	4.87E+11
6	2.43E+11
3	1.22E+11
2	6.08E+10

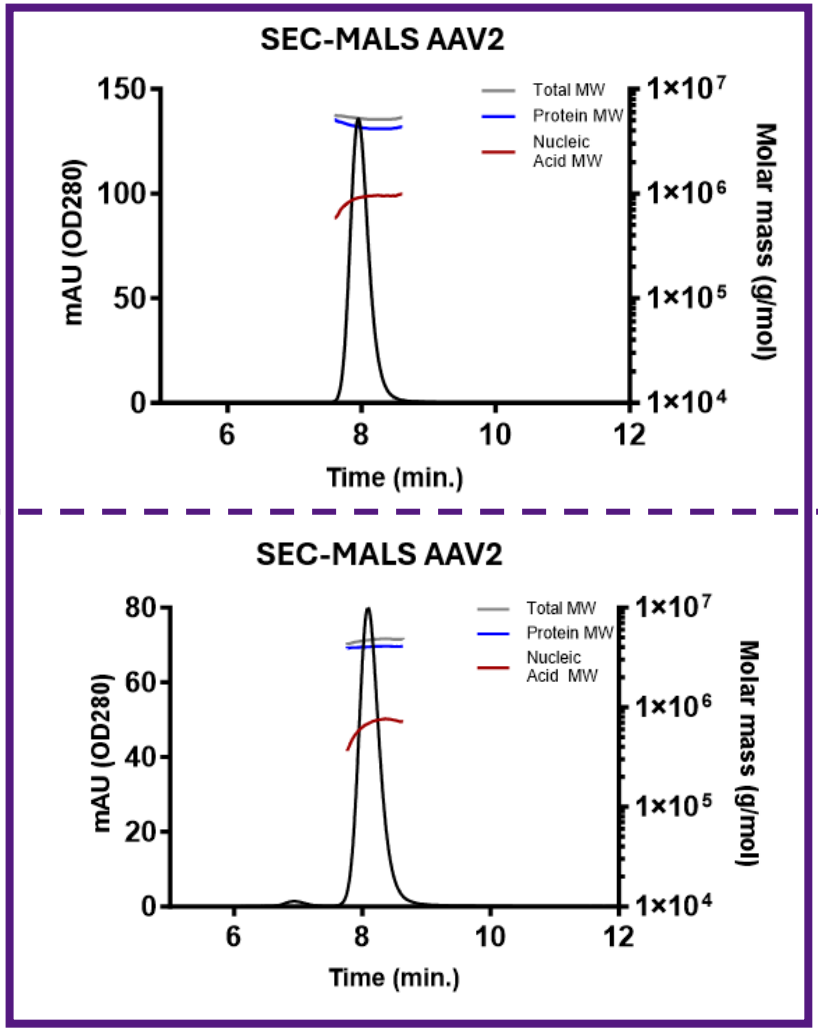
*Rounded to whole numbers



Correction factor
AUC_{Full}: 1.385

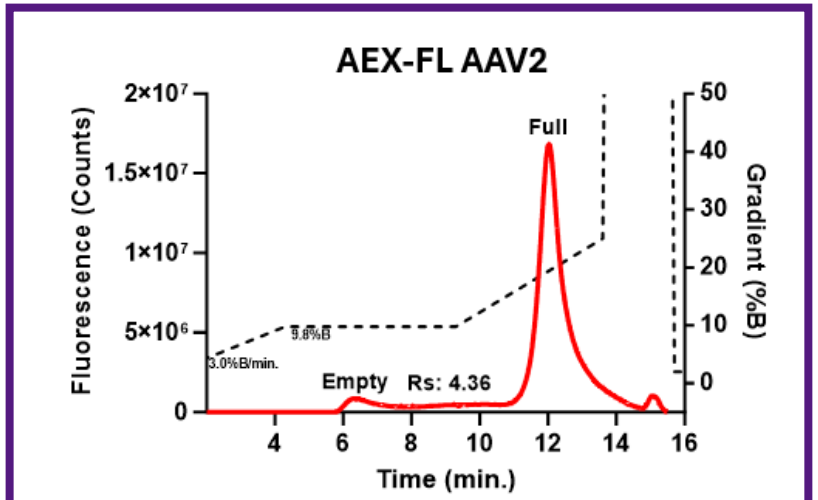


Setting up AEX for AAV2



Batch 1

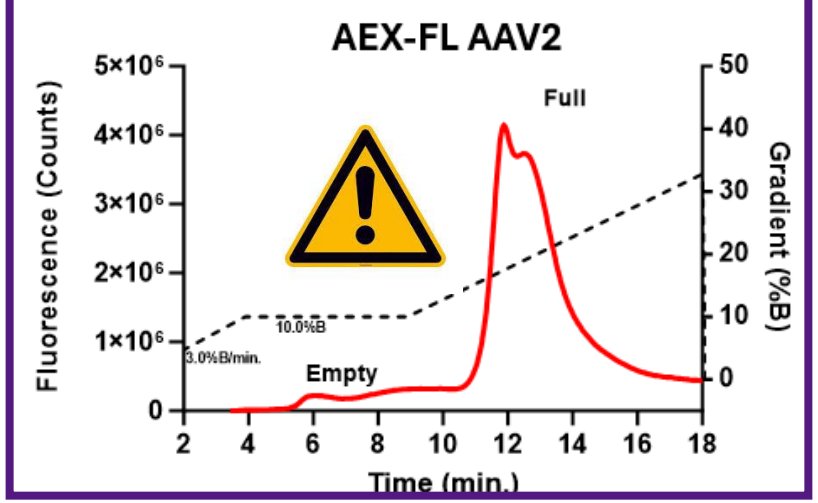
SEC-MALS and AEX results
88.9% Full
VP titer: 1.63E13 VP/mL



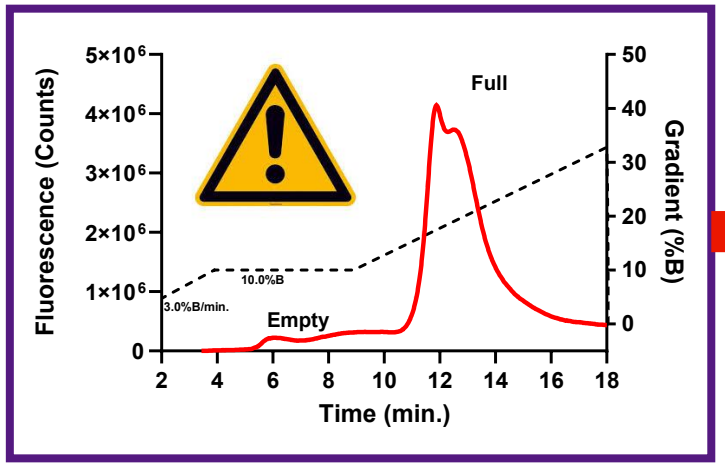
Batch 2

SEC-MALS
84.3% Full
VP titer: 1.23E13 VP/mL

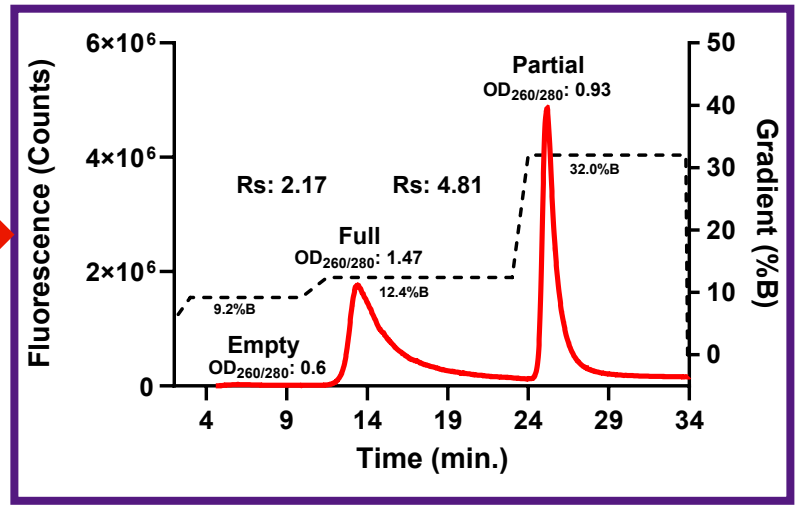
AEX
Full: undeterminable
VP titer: undeterminable



Separation through triple step elution



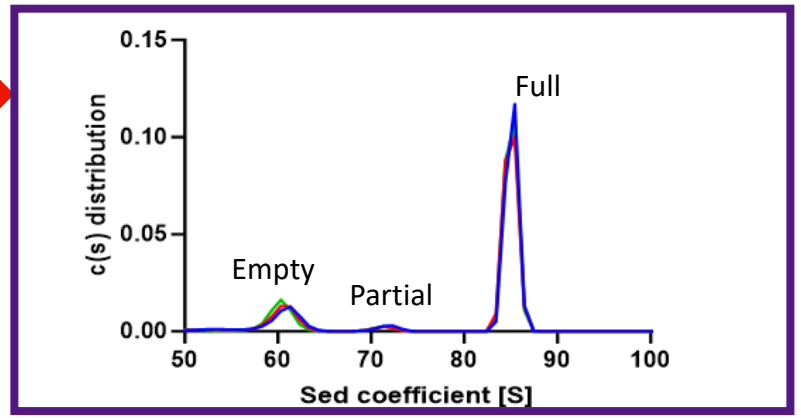
AEX: Triple step elution



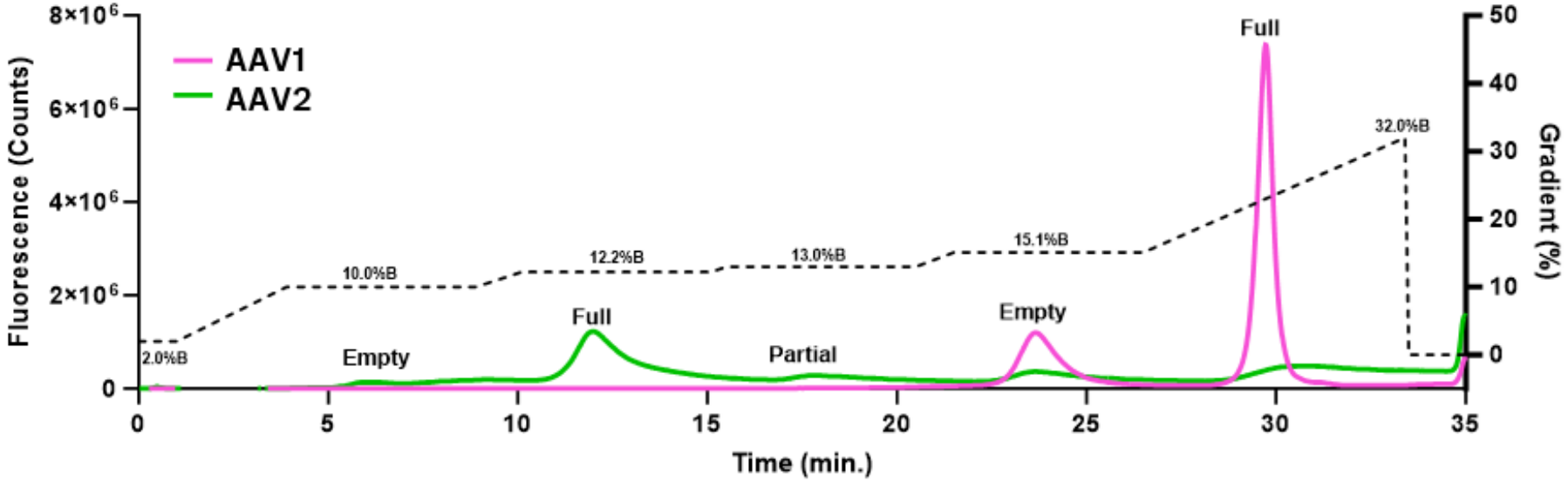
AUC determination was determined as follows:
%Full - 69.9%
%Empty 15.6%
Partial 2.65%
LMW+HMW species = 11.9%
AEX determination was determined as follows:
%Full – 70%;
%Empty 15.7%
Undetermined 14.3%

Only a ~0.1% difference between AUC and AEX-FL for E/F determination

AUC



Identity of AAV1 vs. AAV2



- Using AEX-FL resolving power to separate AAV serotypes allows for complementary Identity with Genome Identity
- This is particularly important for sites that have multiple gene therapy programs running in parallel from one site

In Summary

- **Mapping out** potential **CQAs** allows for the design of **Multi-Attribute Methods** (MAMs) for the purposes of Process Development, Characterization and QC purposes
- **Building correlations** between your MAMs and **orthogonal methods** are critical in understanding potential blind spots for analytical determinations
- **MAMs** are critical for **reducing material consumption** for the purposes of testing and enable richer datasets to be collected from a process which enables more rapid decision making throughout the developmental lifecycle