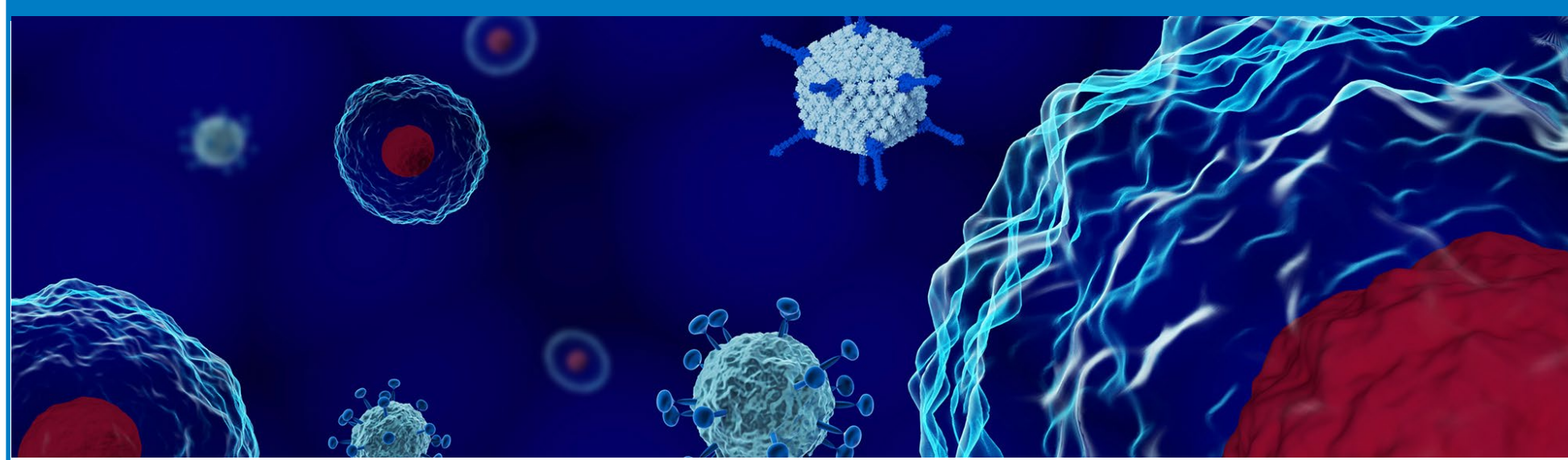


Next Steps in AAV Characterization via Light Scattering Measuring AAV Critical Quality Attributes

Anatolii Purchel, Ph.D., Michelle Chen, Ph.D., John Champagne, Ph.D., Wyatt Technology Corporation



Introduction



Adeno-associated virus (AAV). AAV has mild immune response, able to deliver its payload to a wide range of host cells, and is becoming a prominent delivery vehicle in gene therapy.

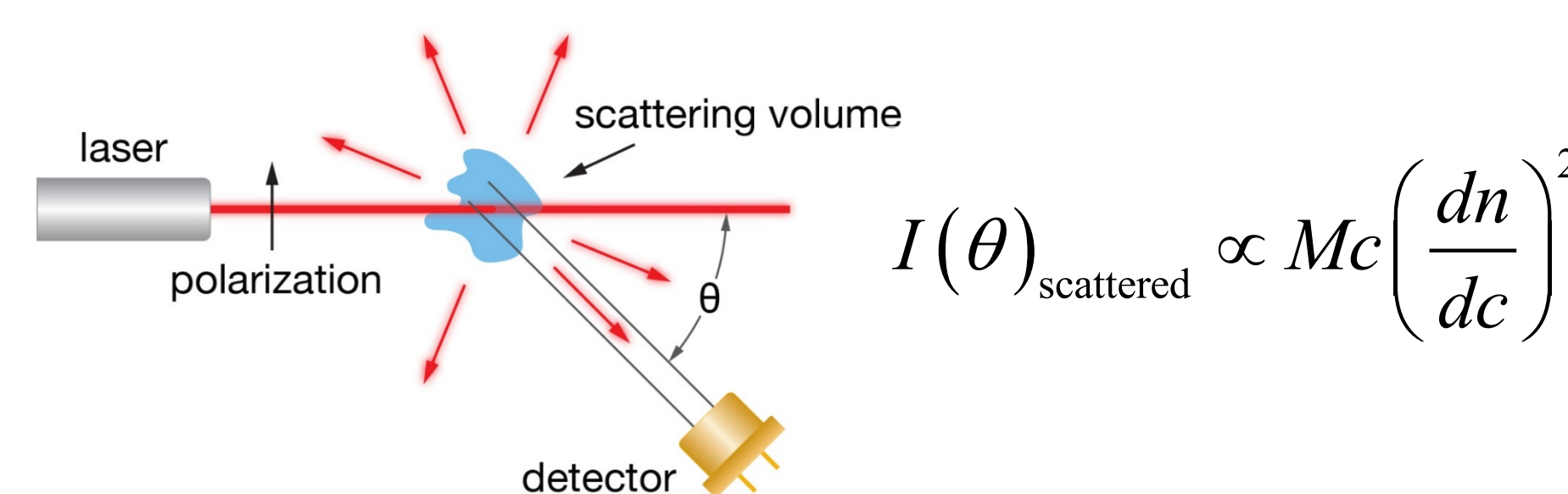
Multi-Angle Light Scattering (MALS) determines absolute molar mass and size directly, in solution, independent of elution time and conformation. This technique, combined with multiple concentration detectors allows independent protein and nucleic acid payload characterization.

AAV quality attributes. SEC-MALS method is presented for measuring total capsid particle concentration, relative capsid content, and percentage of monomer or aggregates.

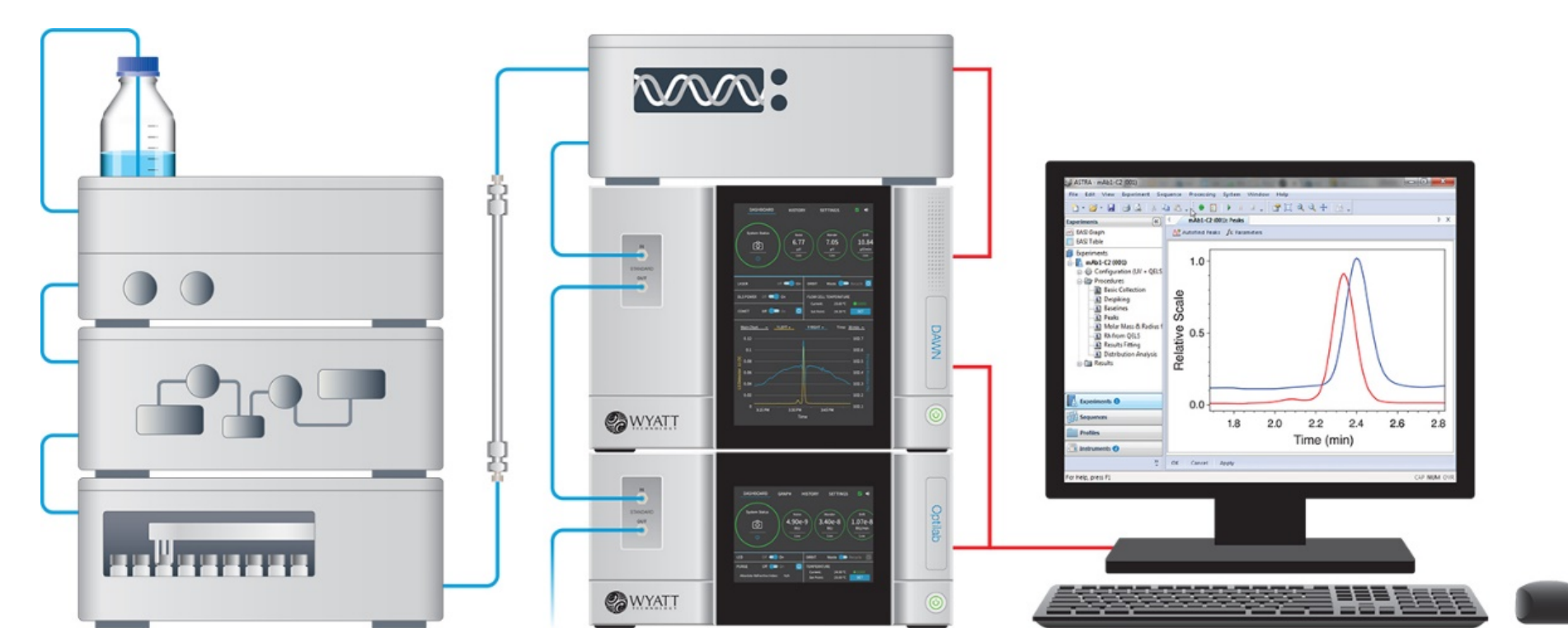
FFF-MALS and DLS. Field-flow fractionation and batch DLS were employed to further characterize amount of aggregates and particle concentration.

SEC-MALS Setup

SEC-MALS: Determines molar masses independently of elution time, and does not require calibration against reference molecules.

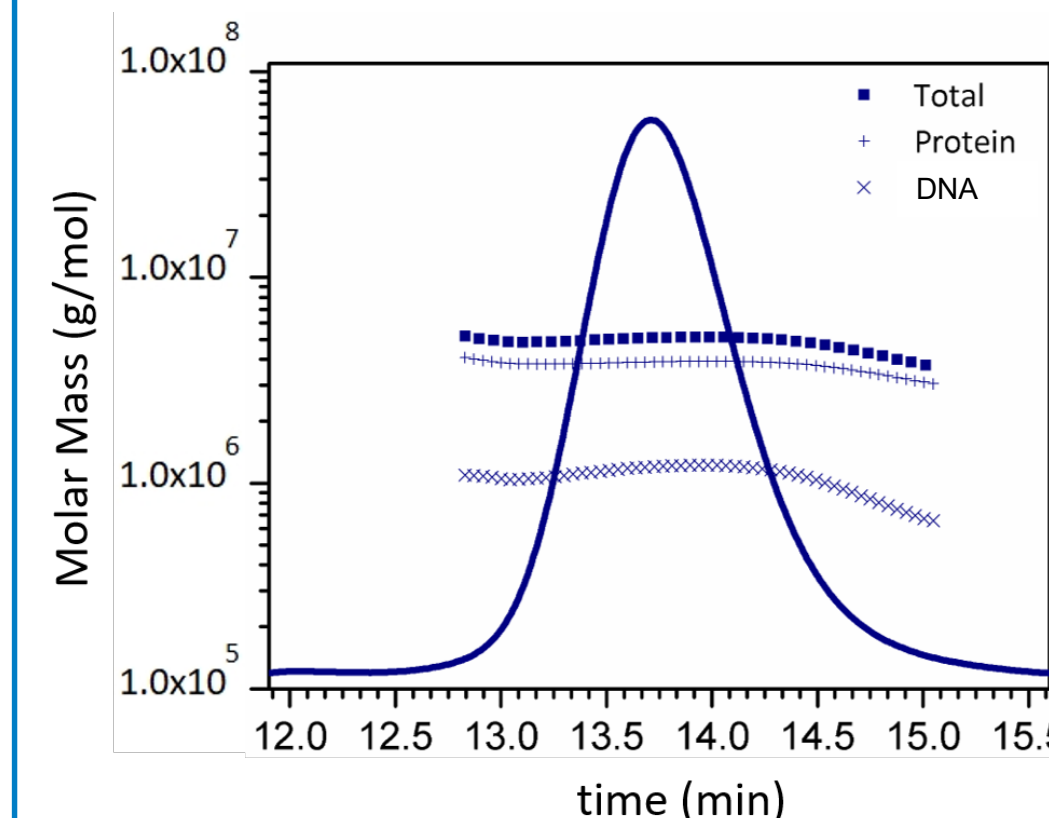


SEC-MALS typical setup consists of HPLC system, DAWN® and Optilab®. For AAV characterization, two distinct concentration detectors are necessary, e.g. RI and UV280



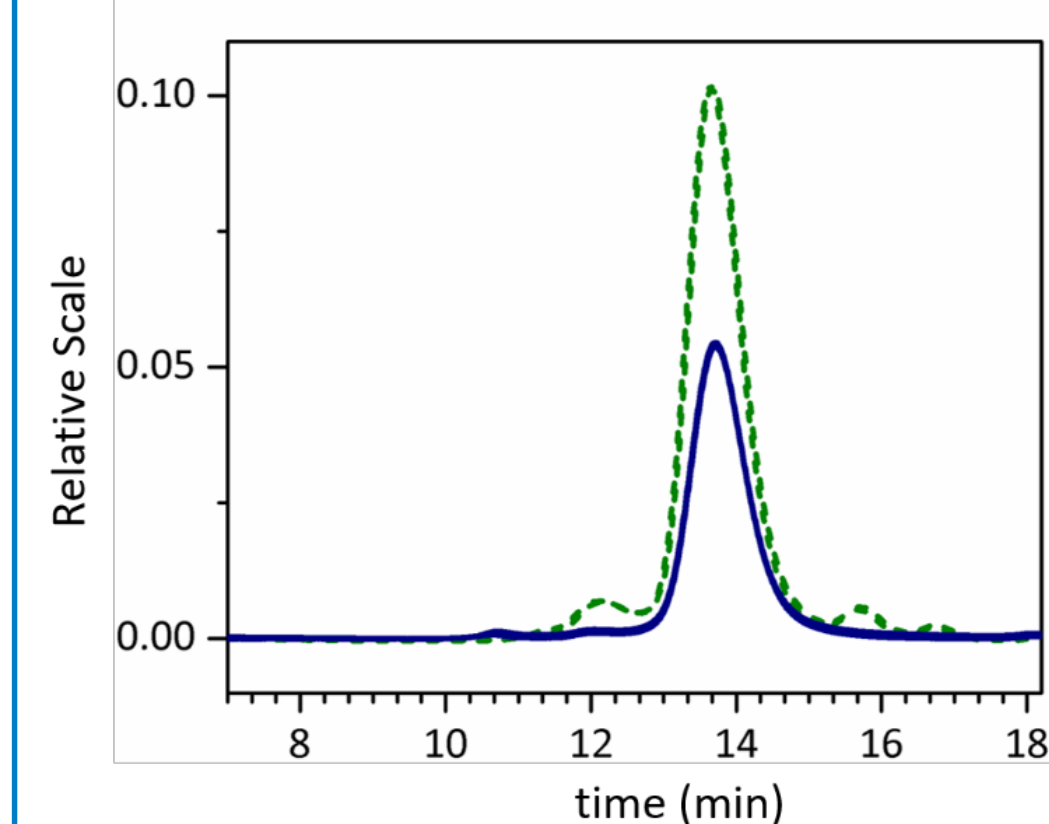
Protein Conjugate Analysis

Protein Conjugate Analysis: provides independent values for protein mass, DNA mass, and respective molar masses



Protein conjugate analysis determines molar mass of the protein and DNA components independently

Method development: appropriate column to assure 100 % mass recovery, peak symmetry, system validation with more than one standard



Chromatograms of Empty and Full AAVs
Two injections of each sample are overlaid

Calculating Number of Particles

Calculations: AAV protein and DNA mass (m_{capsid} and m_{DNA}) as well as AAV protein and DNA molar masses (M_{capsid} and M_{DNA}) are obtained from protein conjugate analysis.

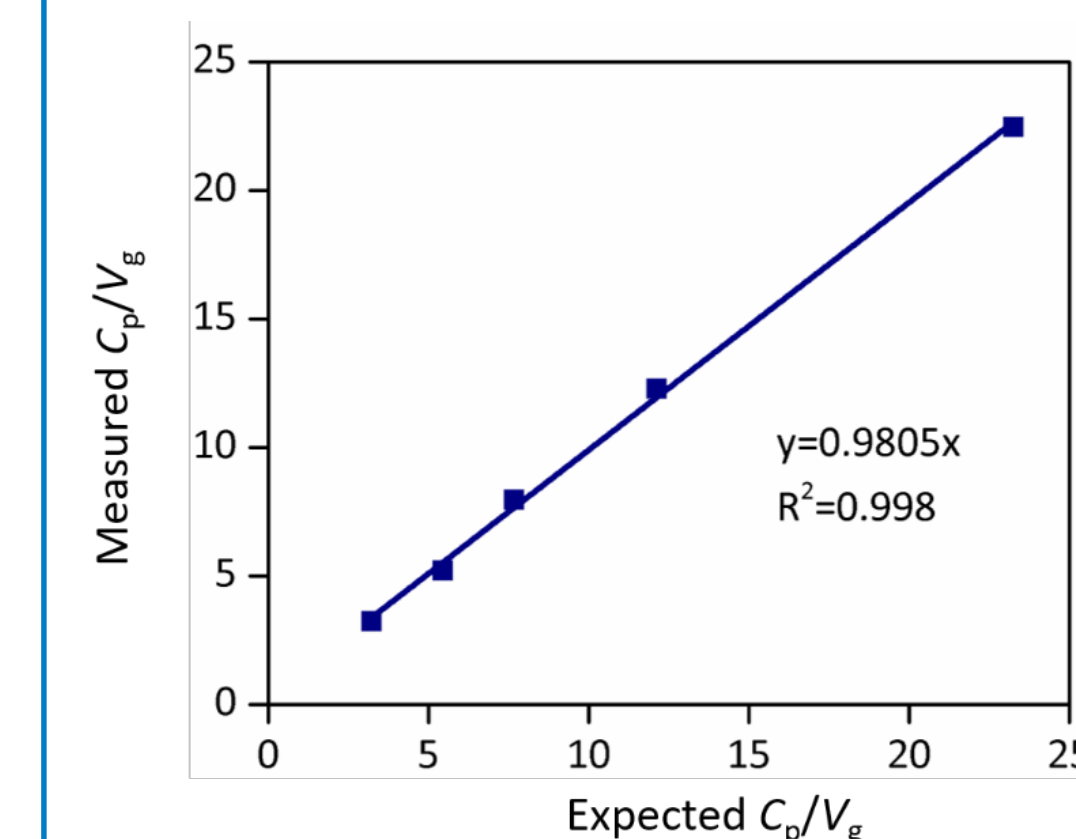
$C_{\text{Full}} = m_{\text{DNA}} \times N_A / (M_{\text{DNA}} \times v) - \text{full AAV particle concentration (v - injection volume)}$

$C_{\text{AAV}} = m_{\text{capsid}} \times N_A / (M_{\text{capsid}} \times v) - \text{total AAV particle concentration (v - injection volume)}$

Calculated CQA's: C_{Full} , C_{Empty} , V_g/C_p (V_g , C_p - genome and AAV particle titer)

Step by step guide:

1. Validate the system with two or more standards
2. Validate the system with empty and filled AAVs
3. Process the data using Protein Conjugate Analysis



C_g/V_p ratio measured using protein conjugate analysis and compared to the theoretical value based on mixing ratio

DLS

Batch DLS/SLS provides size and MW as well as concentration very quickly and easily. Use as high-throughput assays prior to preferred analytical (high resolution) approach afforded by SEC- or AF4-MALS.



Empty AAV:

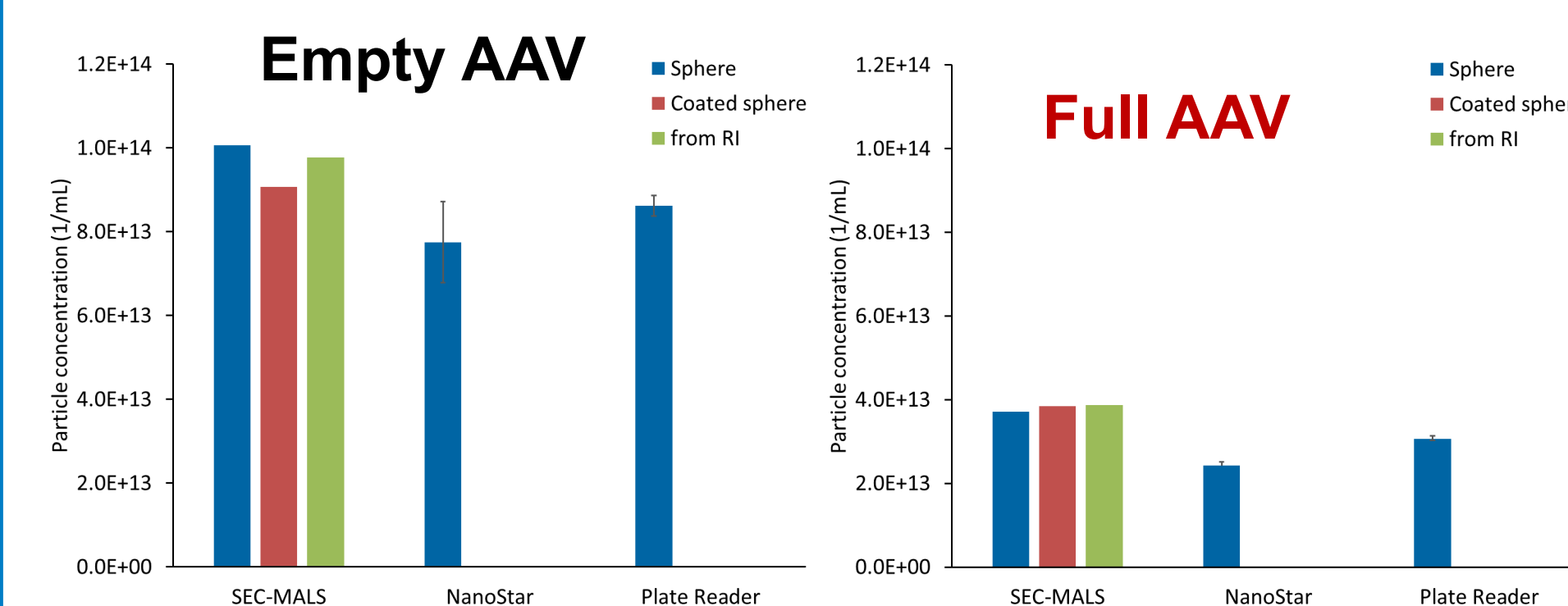
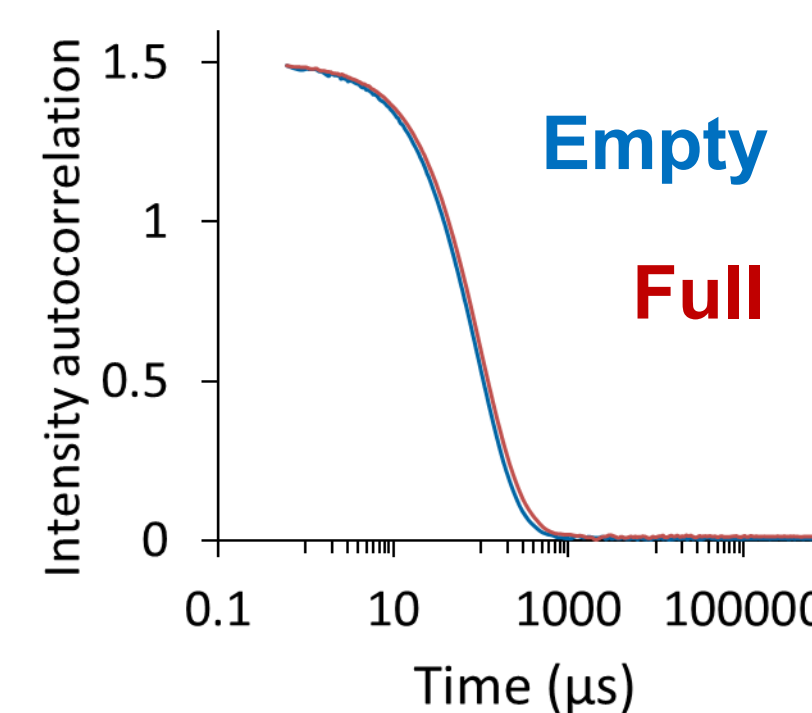
$$R_h = 14.7 \pm 0.7 \text{ nm}$$

$$M_w = 3.67 \pm 0.01 \text{ MDa}$$

Full AAV:

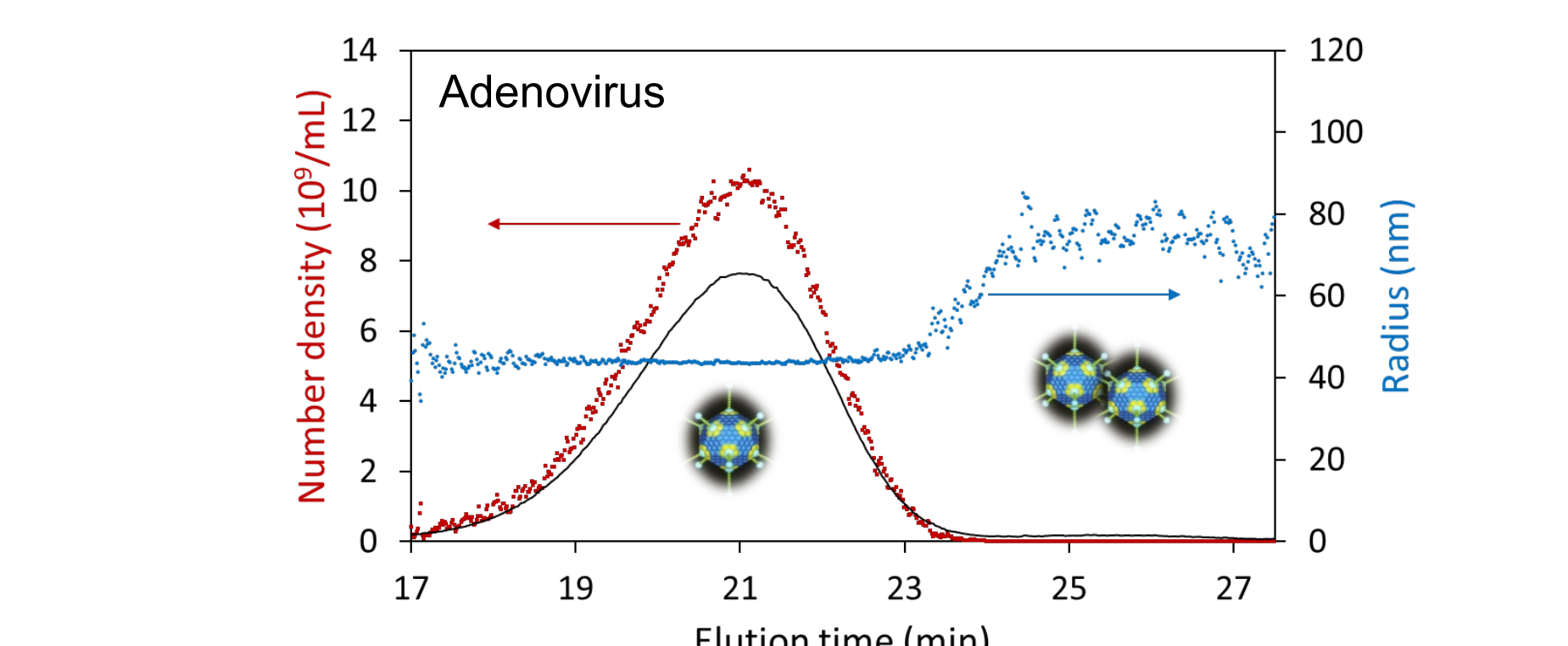
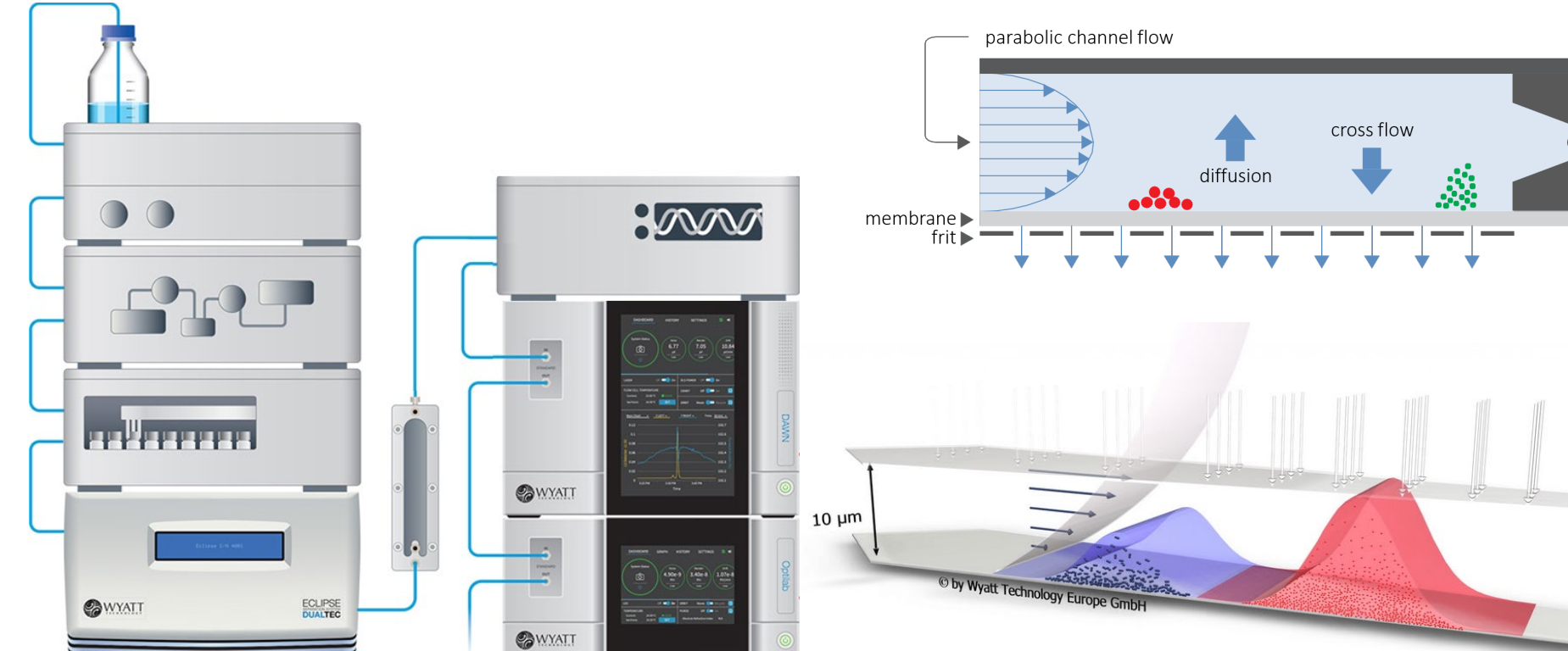
$$R_h = 15.6 \pm 0.1 \text{ nm}$$

$$M_w = 6.78 \pm 0.03 \text{ MDa}$$



FFF-MALS

FFF-MALS Typical setup consists of HPLC system, Eclipse™ AF4™, DAWN®, and Optilab®. Separation happens in a channel on a semi-permeable membrane

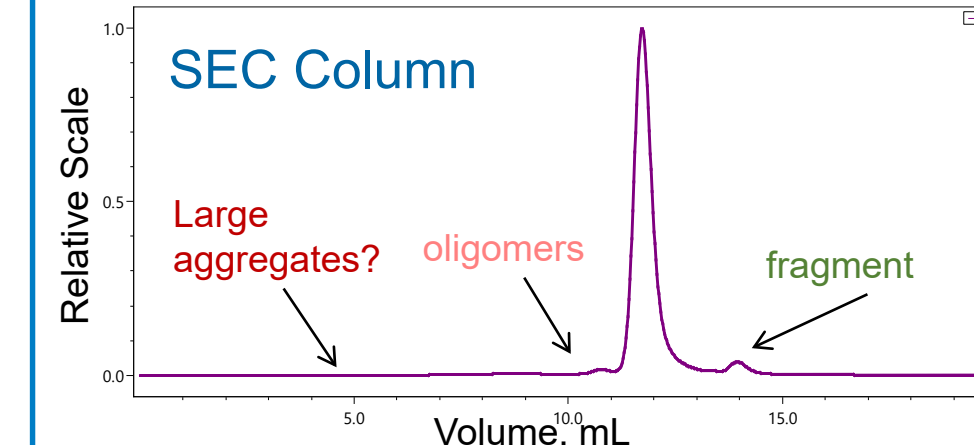
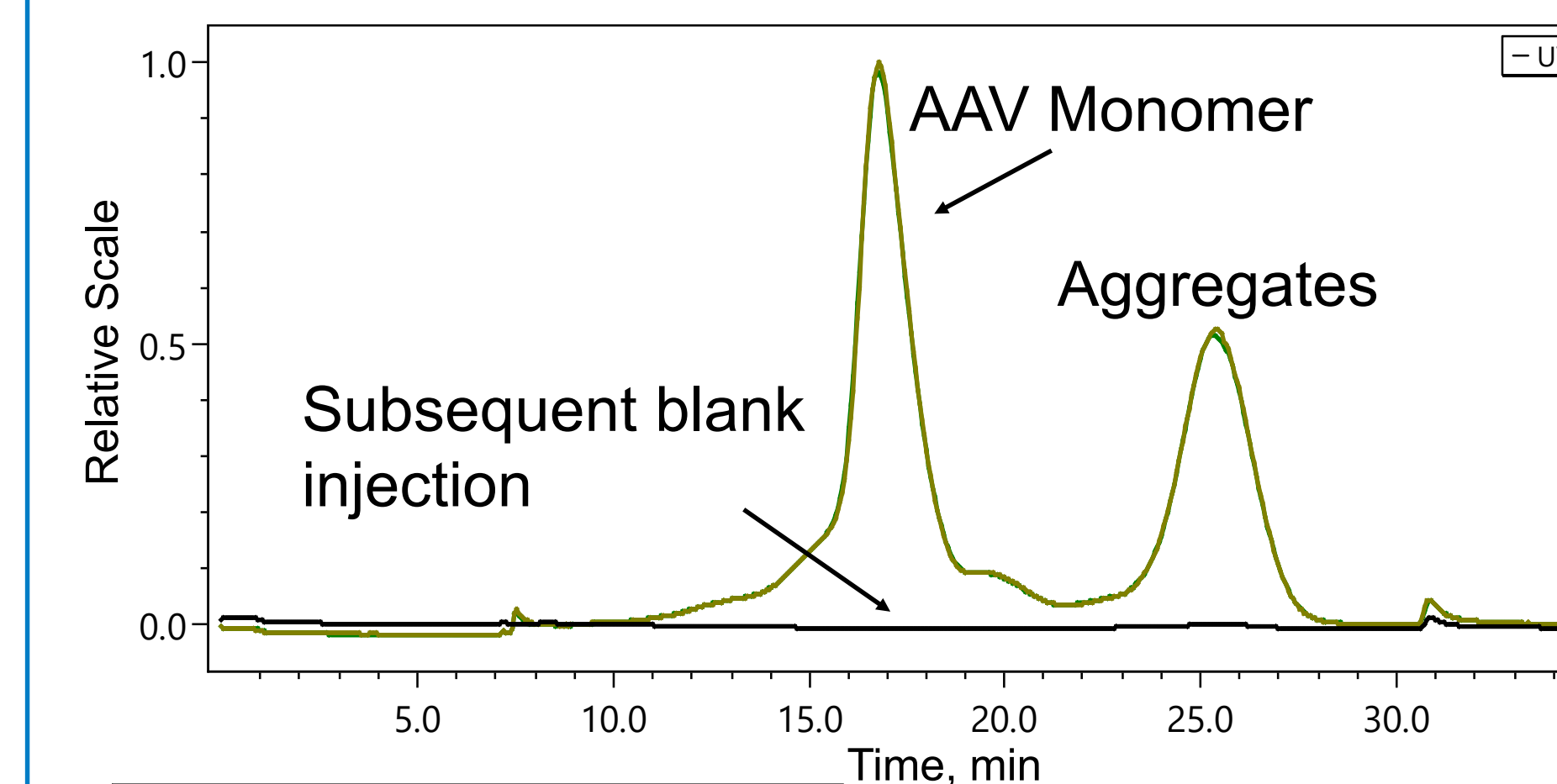


	AFM/TEM	FFF-MALS	% Difference
Total Particle Count	2.9×10^{10}	2.8×10^{10}	2
Average Radius	43.0	45.0	5

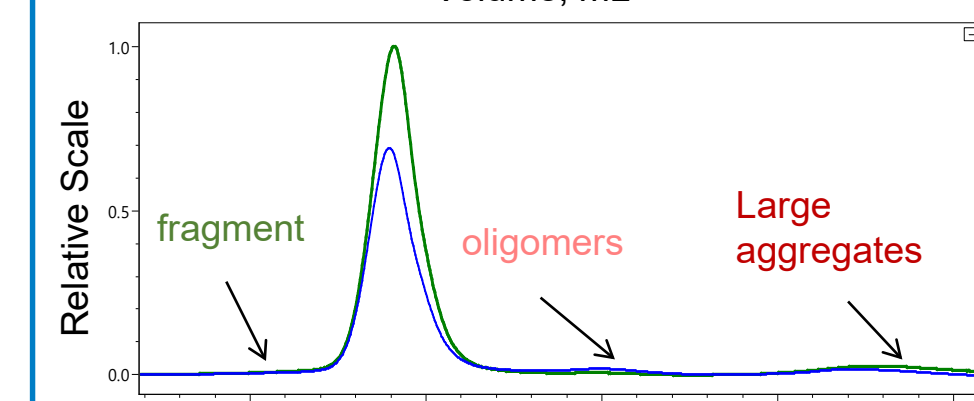
FFF-MALS

FFF is able to separate large AAV aggregates and monomers

A subsequent blank injection is useful for determination of sample retention on the membrane



AF4 allows large aggregates characterization



HMW aggregates visible by AF4-MALS may be removed by SEC column

Conclusions

The SEC-UV-MALS-dRI measures AAV particle concentration, relative capsid content, and aggregation all in a single run

- *Conformation-independent molar mass analysis* that does not rely on standards
- *Triple-detection system* allows independent quantification of AAV capsid protein and nucleic acid payload
- *No a priori knowledge* about the AAV structure or content is required
- *Batch DLS* can be used as high-throughput assays prior to preferred analytical (high resolution) approach afforded by SEC- or AF4-MALS
- *FFF-MALS* determines if aggregation occurred after AAV synthesis or processing

SEC-MALS App Note



DLS App Note



About FFF

