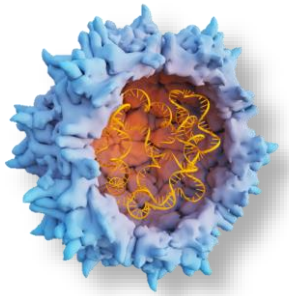
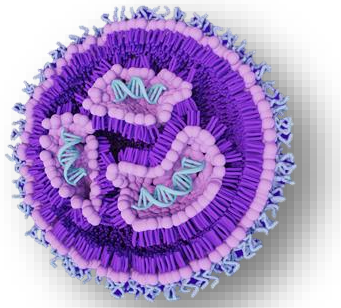


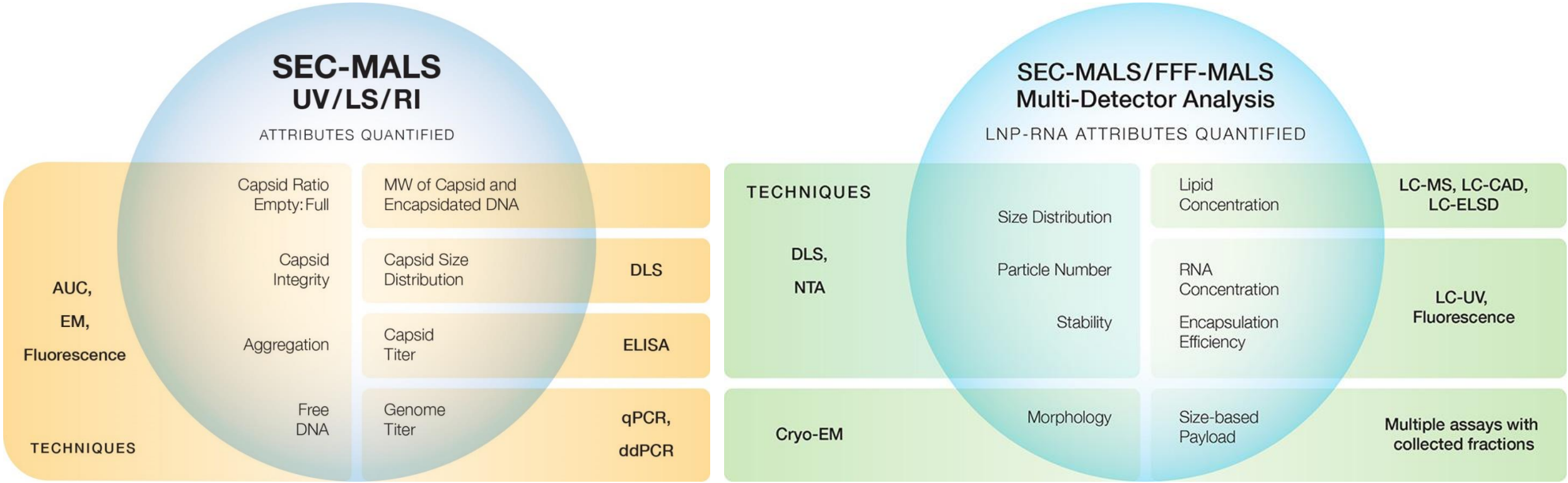
Light Scattering Tools for Quantifying Viral Vector Critical Quality Attributes

John Champagne, Ph.D.
Senior Applications Scientist
Wyatt Technology Corporation
Santa Barbara, CA



Outline

Characterization of gene therapy NPs is complex and hard. These are the solutions to make job of analytical biochemists easier and work more productive while providing accurate and precise quantification of essential attributes



Quantification of AAV properties

DLS Plate Reader

Screening tool for all viral vectors with built-in automation

SEC - MALS

Characterization tool for AAV production and QC
(3 CQAs in a single assay)

FFF - MALS

Characterization tool for large AAV aggregates (lenti, adeno VV, liposomes, LNP, extracellular vesicles)

Dynamic light scattering (DLS) solutions

DLS
NanoStar or Plate Reader

Screening tool for all viral vectors using small amount

- ✓ Size distribution, aggregation
- ✓ Particle concentration
- ✓ Stability screening
- ✓ <30 s per sample

Dynapro® NanoStar® II

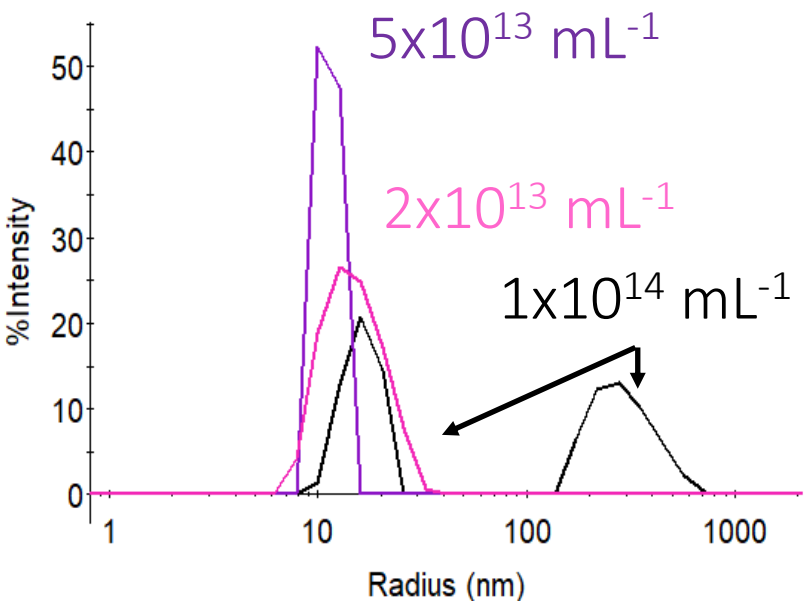
2 µL (quartz cuvette)
4 µL (disposable cuvette)

DynaPro® Plate Reader III

5 µL (1536 well plate)
25 µL (384 well plate)

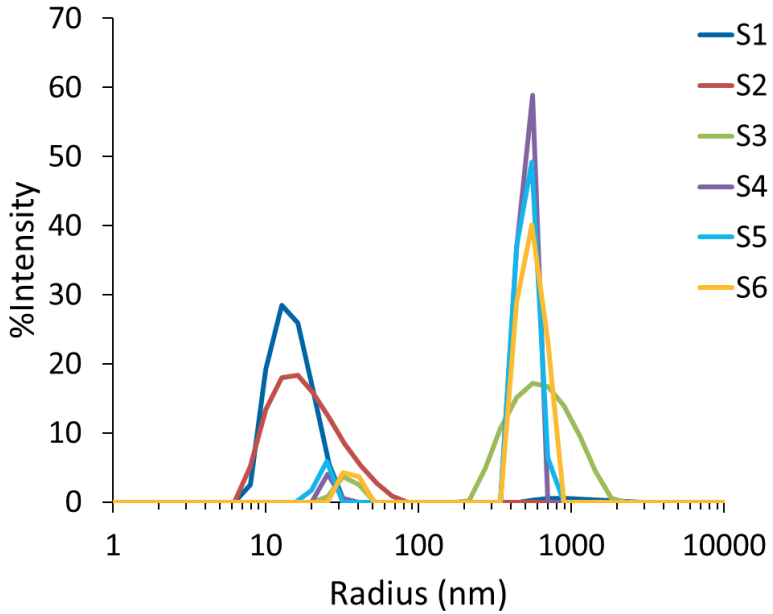
Stability screening AAVs with the Dynapro Plate Reader

Concentration



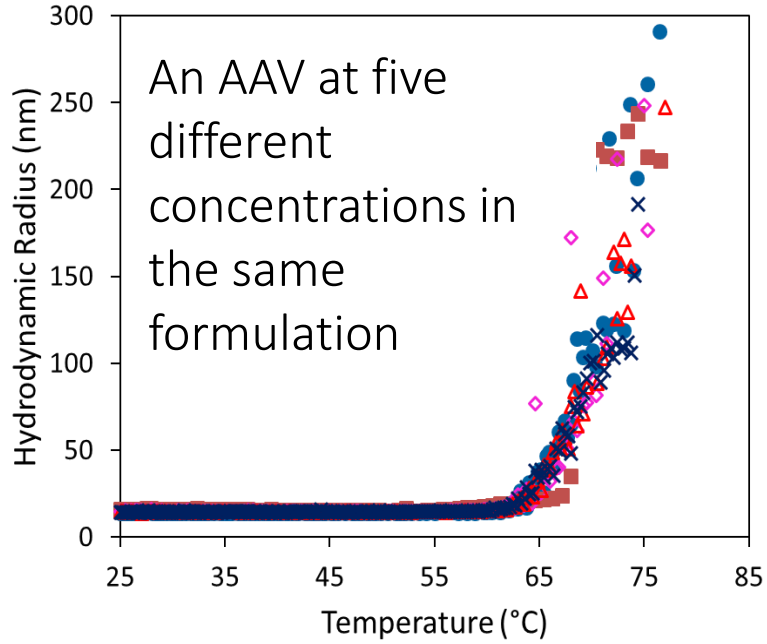
Aggregates appear to dissociate with decrease in concentration.

Buffer additive



Buffer additives affect aggregate size and content.

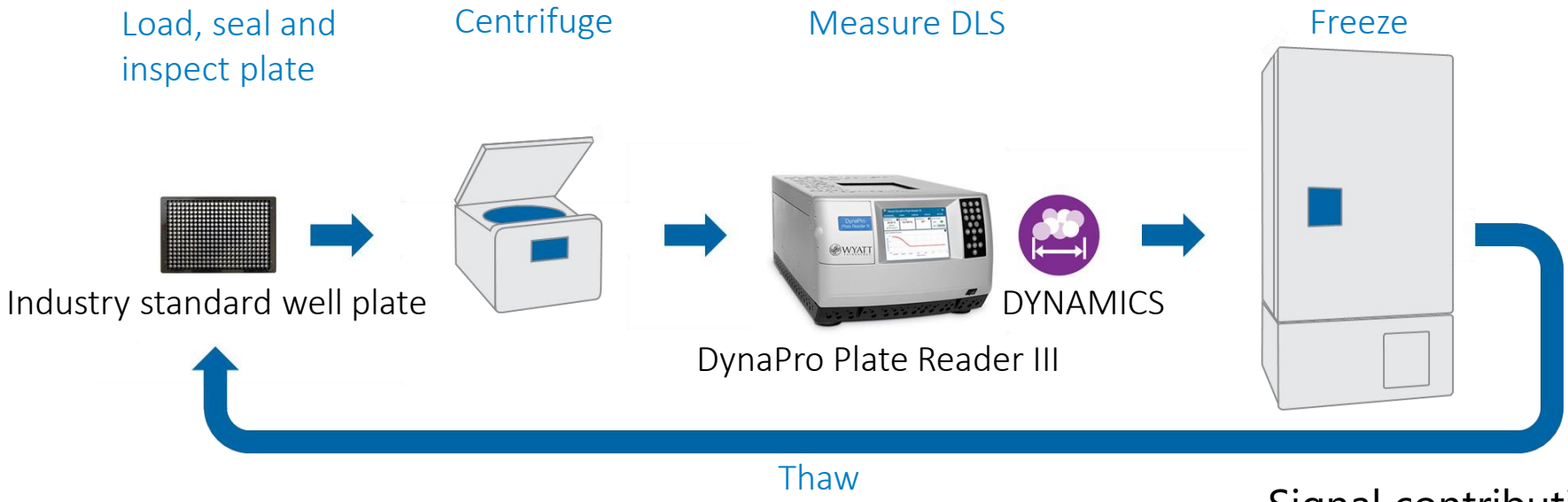
Temperature



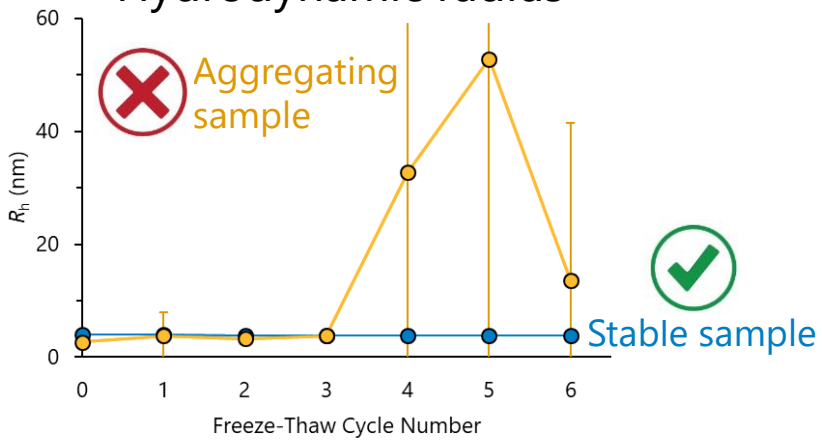
Similar thermal stability:

$T_{onset} = 62.5 \pm 0.5$ °C

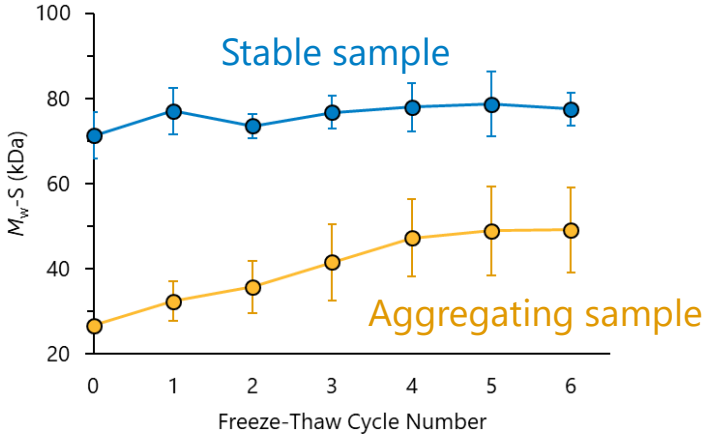
Convenient freeze-thaw stability studies with the DynaPro Plate Reader



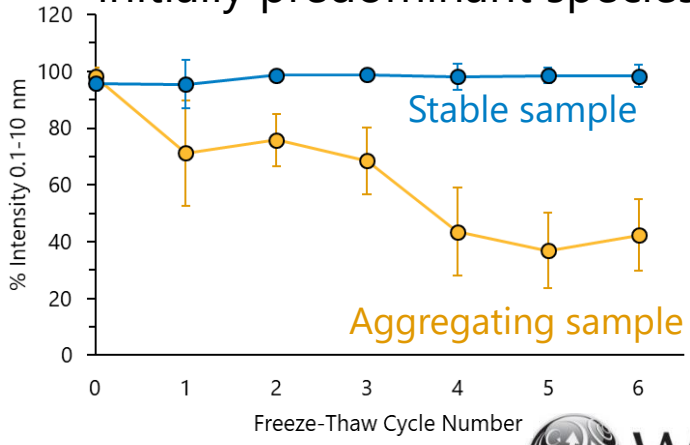
Hydrodynamic radius



Molar mass



Signal contribution from the initially predominant species



AAV Freeze Thaw

Three AAV samples prior to freeze-thaw

—No large aggregates

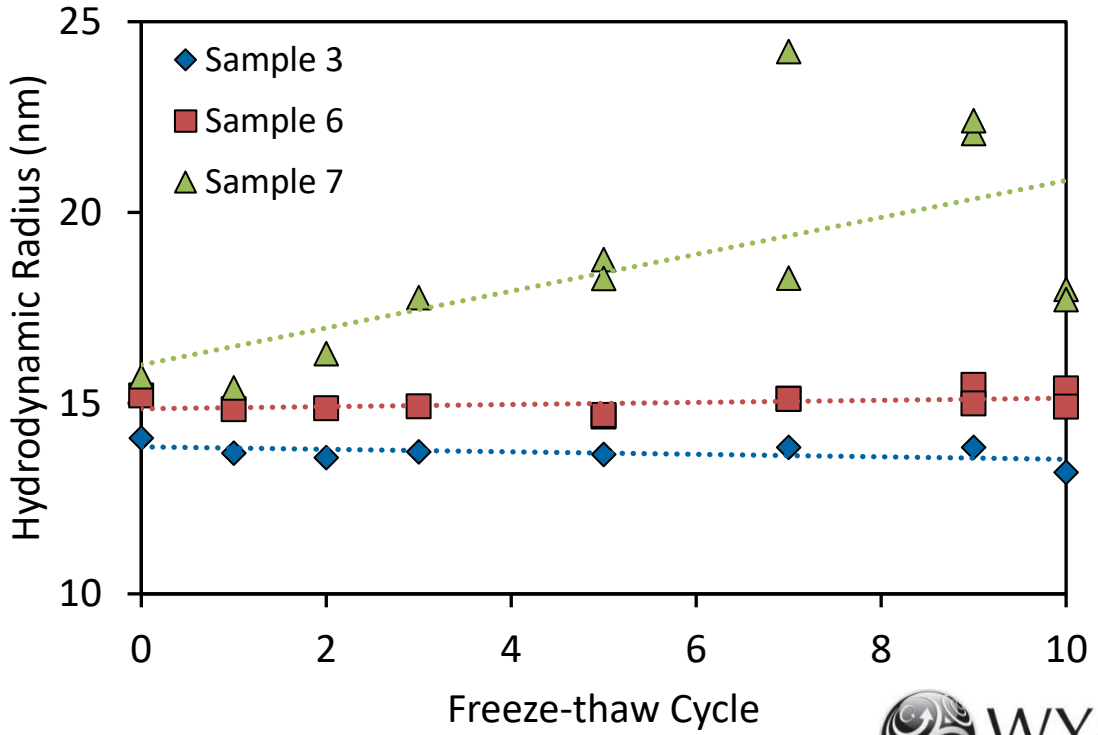
—Similar starting concentrations

After 10 freeze-thaw cycles

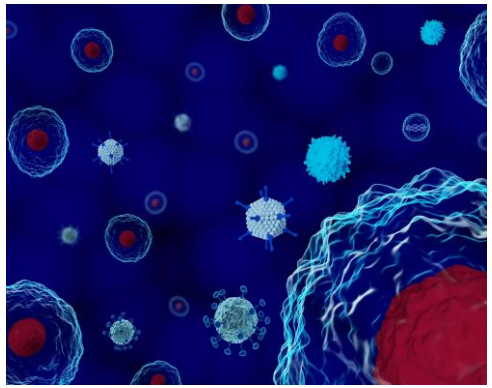
—Samples 3 and 6 appear stable

—Sample 7 shows significant aggregation

Sample	Radius (nm)	PDI	Part. Conc. (1/mL)
3	14.1	2 %	2.7×10^{10}
6	15.2	9 %	4.2×10^{10}
7	15.7	20 %	2.8×10^{10}



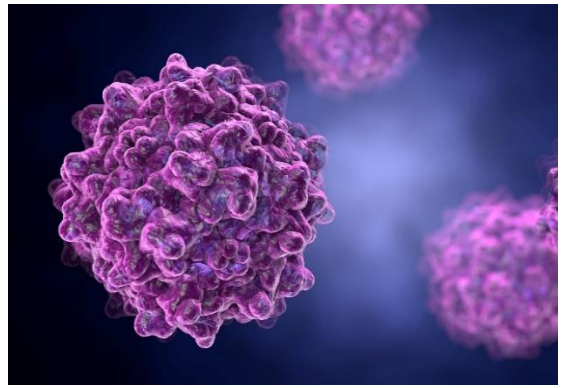
Multi-angle light scattering (MALS) solutions



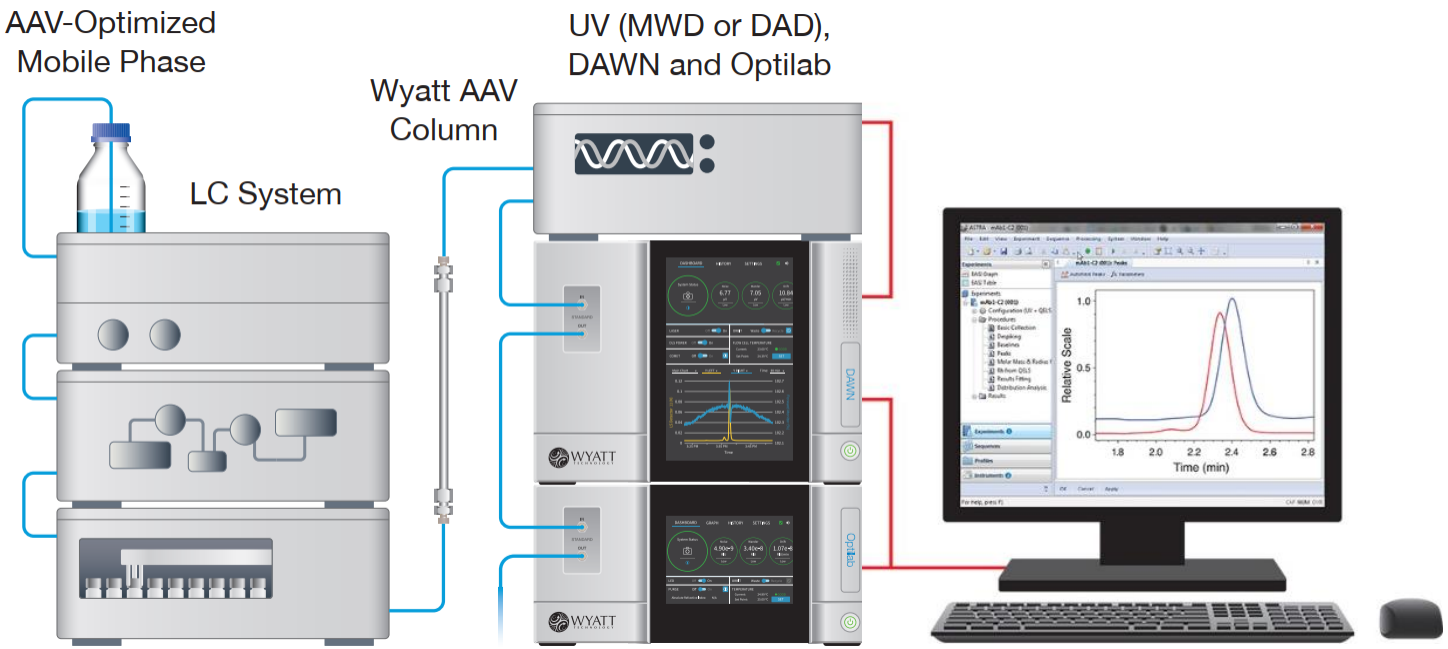
SEC-MALS

Quantify 3 AAV CQAs in one assay

- ✓ Particle concentration
- ✓ Capsid content
- ✓ Aggregation degree
- ✓ Easy implementation

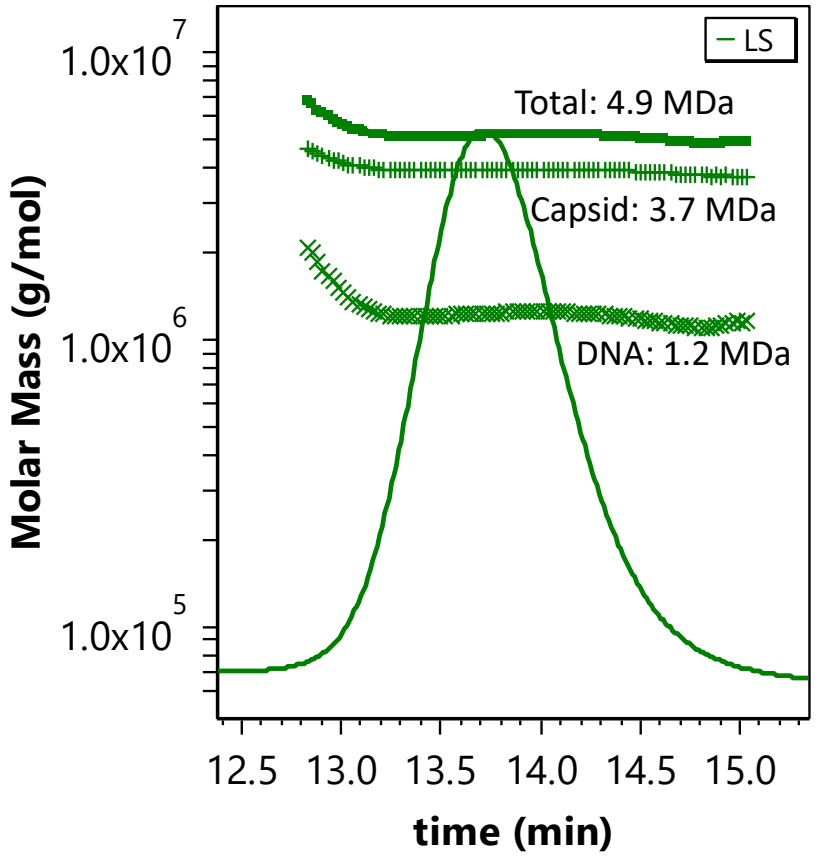


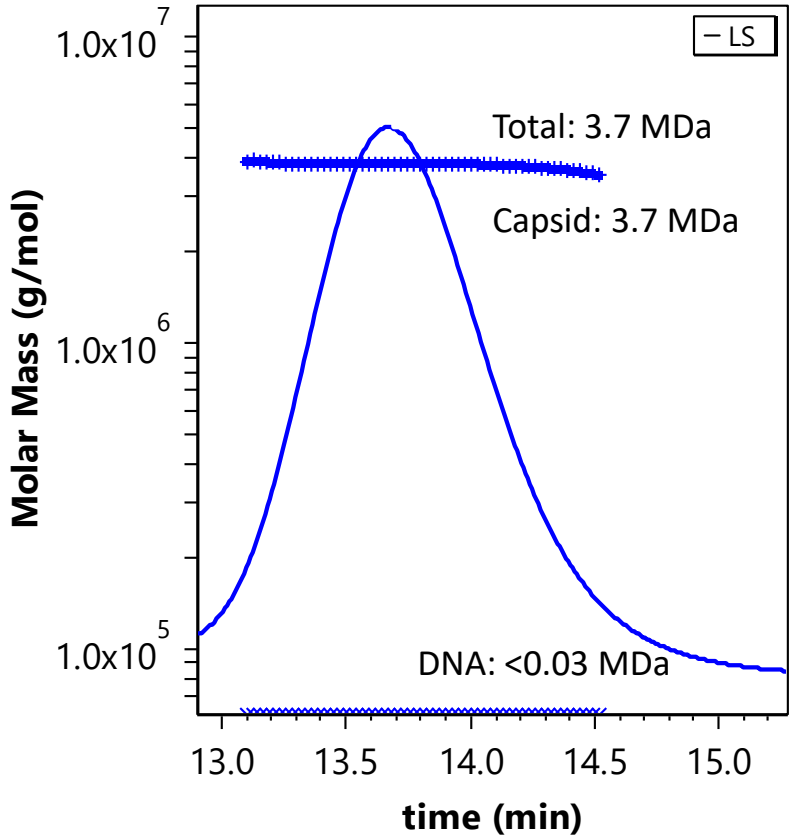
AAV analysis – experimental setup



- ✓ Well validated and used in QC for other biologics (PEGylated proteins, polysaccharides, protein-polysaccharide conjugates)
- ✓ 21CFR11 compliant software, IQ/OQ
- ✓ Robust instruments with outstanding technical support teams

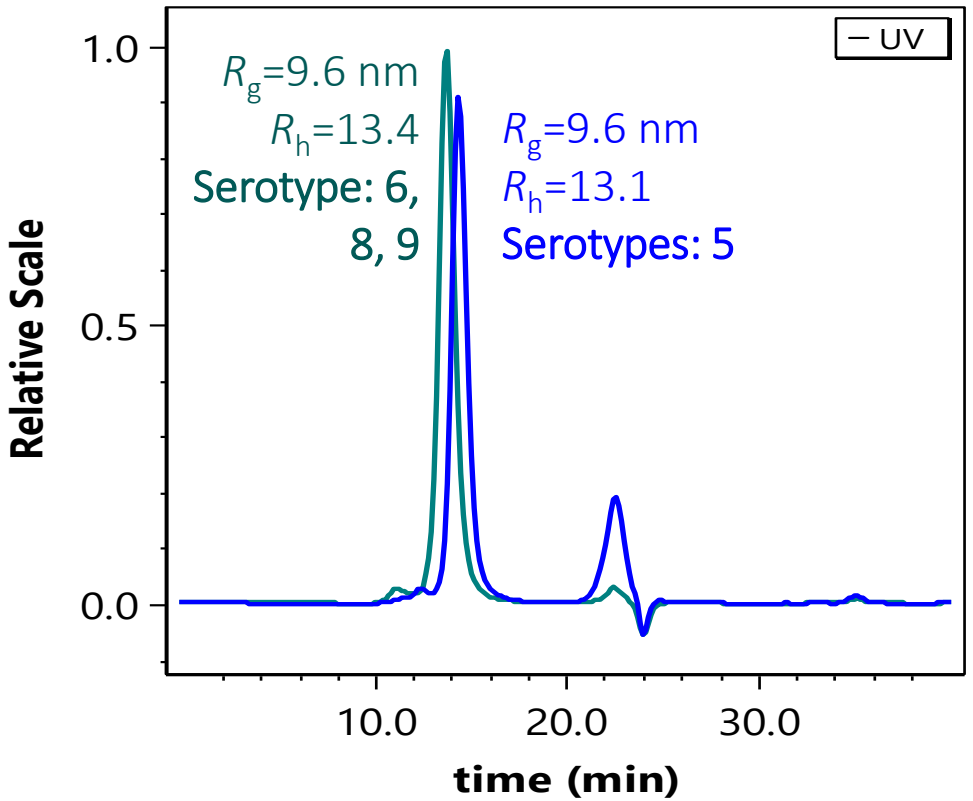
Basis - Protein Conjugate Analysis. AAV (Protein-NA Conjugate)

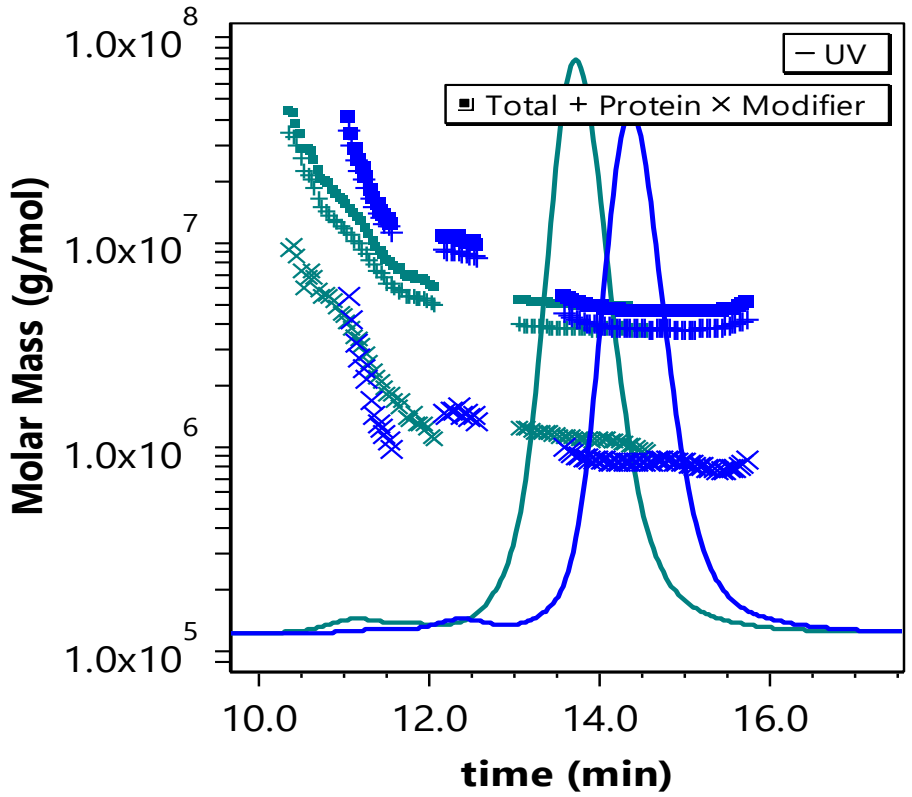




Determine MW for capsid, encapsulated DNA and the entire AAV particle.

Extended characterization: MW, size, and impurities





Measure R_g, R_h, R_g/R_h (shape factor).

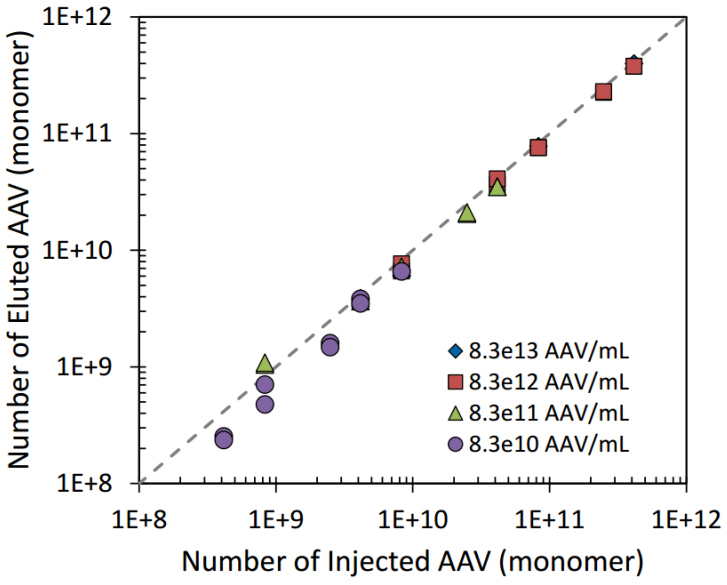
Different serotypes eluted at slightly different times.

Separation, detection, and characterization of other species present in the sample.

AAV quality attributes

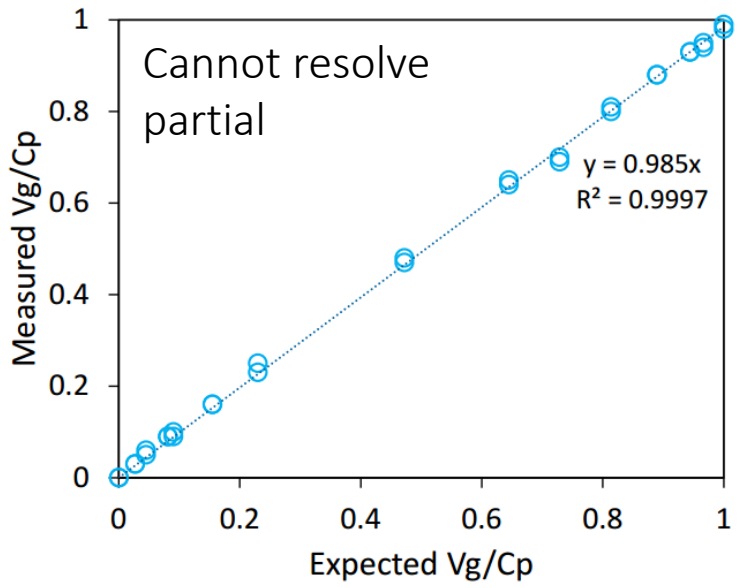
☑ Capsid concentration

Most accurate and precise method.



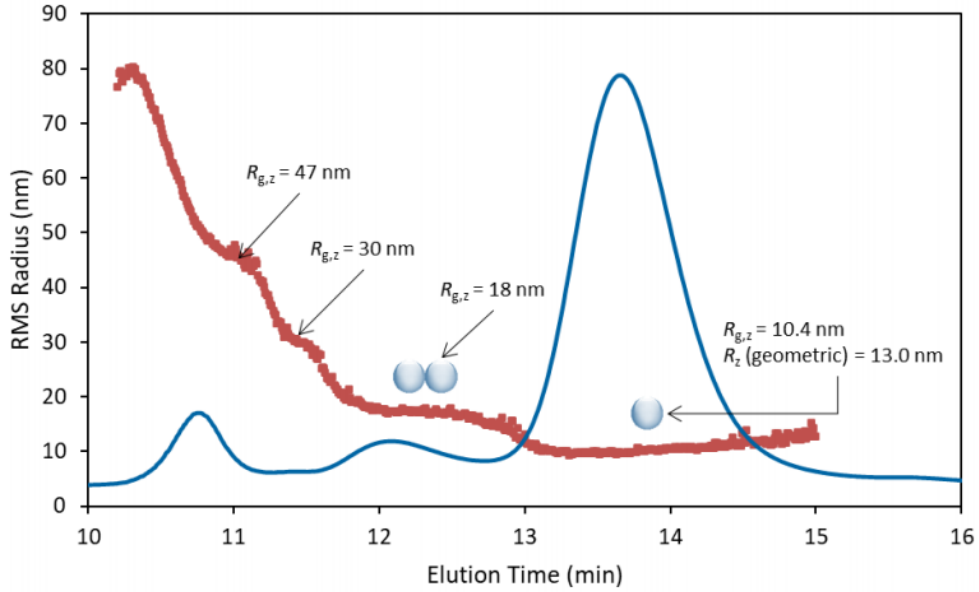
☑ Vg/Cp

Consistent and precise analysis for routine analysis.



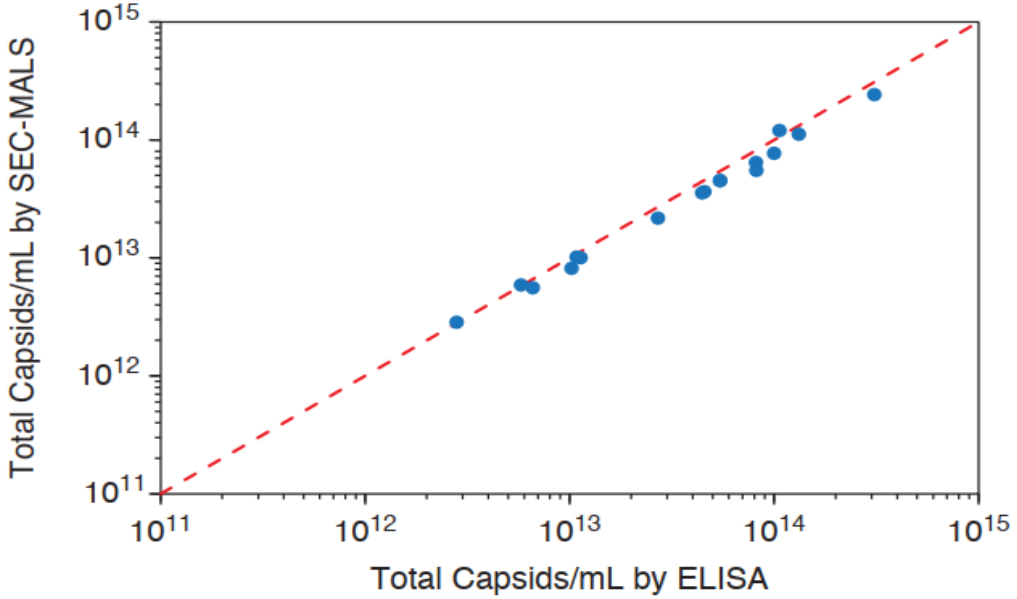
☑ Aggregates

High sensitivity and resolution. UHMW aggregates may be removed



AAV analysis: cross-verification

☑ Capsid concentration

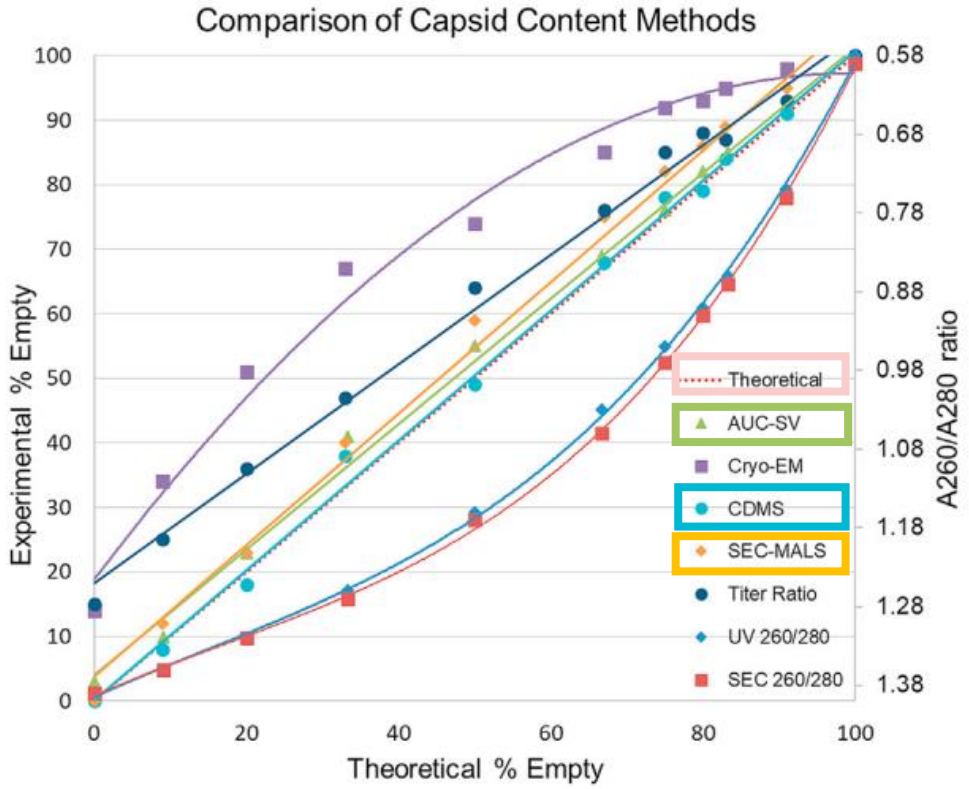


	AAV #1		AAV #2	
	C_px10^{14} [mL^{-1}]	RSD [%]	C_px10^{14} [mL^{-1}]	RSD [%]
SEC-MALS	1.04	0.3	1.13	0.1
microBCA	0.90	5	0.94	5

- Consistent correlation with other protein quantitation methods.
- The Wyatt SEC-MALS method works for AAV 1, 2, 3, 5, 6, 8, 9, 10 and more.

AAV analysis: cross-verification

☑ Vg/Cp



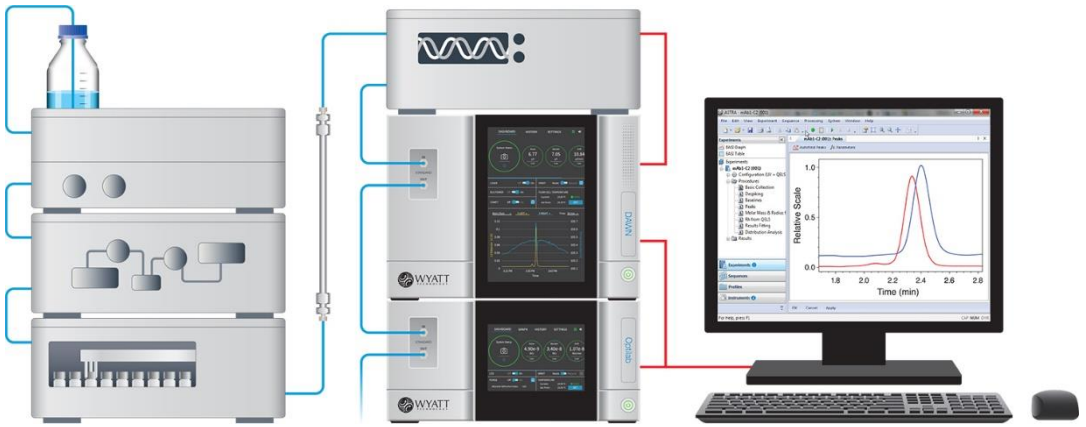
Werle, A. K. et al. Comparison of Analytical Techniques to Quantitate the Capsid Content of Adeno-Associated Viral Vectors. Molecular Therapy - Methods & Clinical Development (2021) https://doi.org/10.1016/j.omtm.2021.08.009.

Case Study 2

CryoTEM/AUC Result relative to SEC-MALS %Full:Empty					
Sample	Technique	In-Process samples			Drug Product
Batch 1	CryoTEM	98%	93%	93%	98%
	AUC	135%	108%	111%	105%
Batch 2	CryoTEM	98%	102%	105%	93%
	AUC	122%	106%	106%	102%

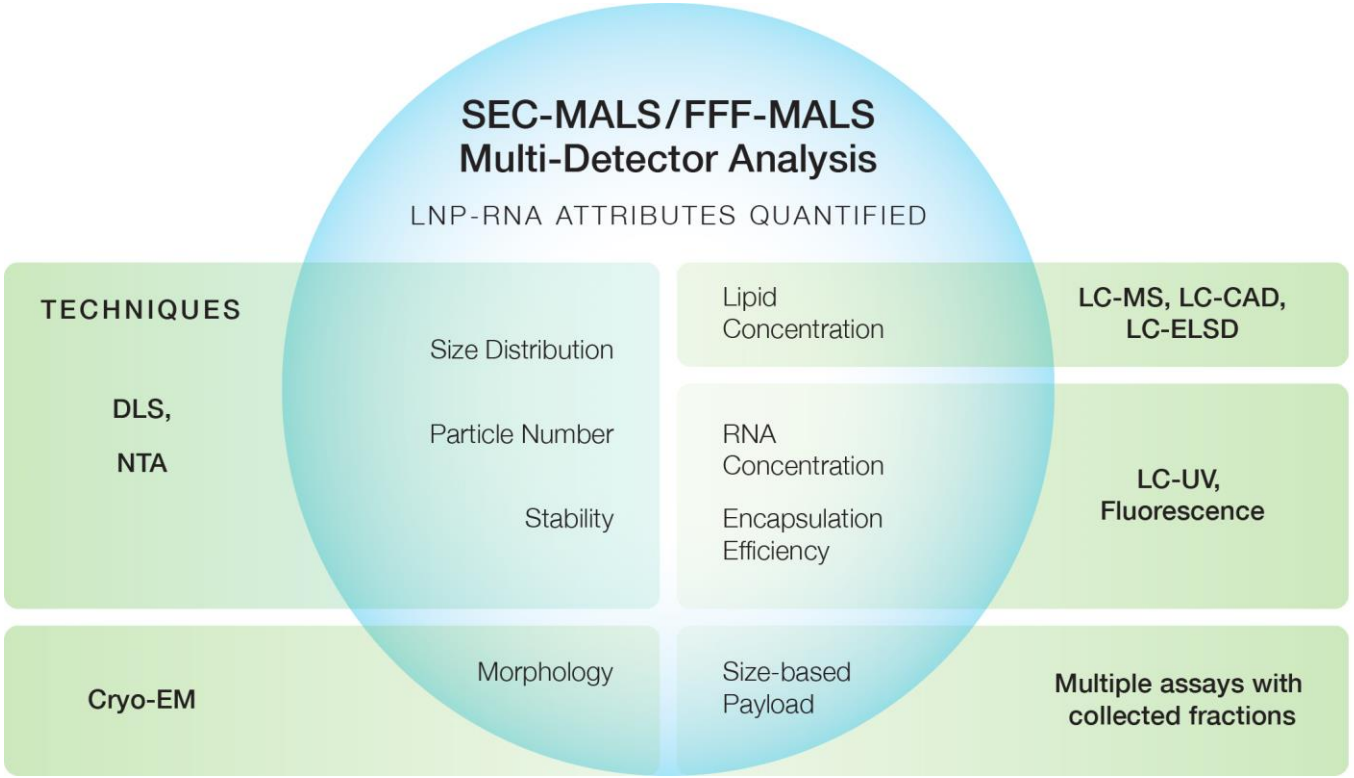
Excellent correlation AUC, cryoEM, and CDMS data, confirming SEC-MALS is a viable & orthogonal method

Complete solution from RD, AD, PD, to formulation, QC, and platform assay



- Well validated and used in QC (release assay) for other biologics (PEGylated proteins, polysaccharides, protein-polysaccharide conjugates)
- 21 CFR Part 11 compliant software, IQ/OQ, ready for CMC validation
- Viral Vector Analysis has excellent sensitivity ($5x10^{10}$ to $1x10^{15}$ AAV/mL), linearity, reproducibility, consistency, and robustness, which are required in QC
- SOP guidance manual is included to ensure proper adoption, routine analysis, and method transfer
- Robust instruments with outstanding technical support teams

SEC/FFF-MALS-UV-dRI: a comprehensive solution for LNP quantitation



- ✓ Ideal for LNPs, lentivirus, adenovirus, large AAV aggregates, EVs
- ✓ Well validated for other biologics (PEGylated proteins, polysaccharides, protein-polysaccharide conjugates)
- ✓ Software packages are 21 CFR Part 11 compliant

- ***Cross-validation of SEC-MALS for LNP-RNA size-based payload distribution:*** Jia, X. et al., *J Chromatogr B*. 1186, 123015 (2021). https://doi.org/10.1016/j.jchromb.2021.123015

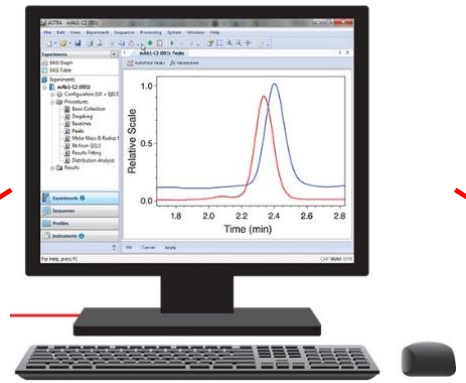
Selected peer-reviewed references using the Wyatt AAV and LNP methods

- ***SEC-MALS for comprehensive, quantitative characterization of AAV vectors:*** McIntosh, N.L., et al. *Sci Rep.* 11, 3012 (2021). https://doi.org/10.1038/s41598-021-82599-1
- ***SEC-MALS for AAV process development:*** Selvaraj N., et al. Hum Gene Ther. 32(15-16):850-861. https://doi.org/10.1089/hum.2020.054
- ***SEC-MALS compared to other analytical techniques for AAV capsid content quantitation:*** Werle, A.K. et al. *Mol Ther Methods Clin Dev.*23, 254-262 (2021). https://doi.org/10.1016/j.omtm.2021.08.009
- ***SEC-MALS for characterization of siRNA lipid nanoparticle polydispersity:*** Zhang, J. et al., *Anal Chem.* 84(14), 6088-6096 (2012). https://doi.org/10.1021/ac3007768
- ***FFF-MALS for LNP-RNA particle sizing and concentration measurements:*** Mildner, R., et al., *Euro J Pharm Biopharm.* 163 (2021): 252-265. https://doi.org/10.1016/j.ejpb.2021.03.004
- ***FFF-MALS for liposomal drug formulations:*** Parot, J. et al. *J Cont Rel.* 320, 495- 510 (2020). https://doi.org/10.1016/j.jconrel.2020.01.049
- ***SEC-MALS for LNP-RNA size-based payload distribution:*** Jia, X. et al., *J Chromatogr B*. 1186, 123015 (2021). https://doi.org/10.1016/j.jchromb.2021.123015
- ***IEX-MALS for AAV characterization:*** Wagner, C. et al., *Int. J. Mol. Sci.* 23(21), 12715 (2022). https://doi.org/10.3390/ijms232112715

Experimental setup for LNP characterization

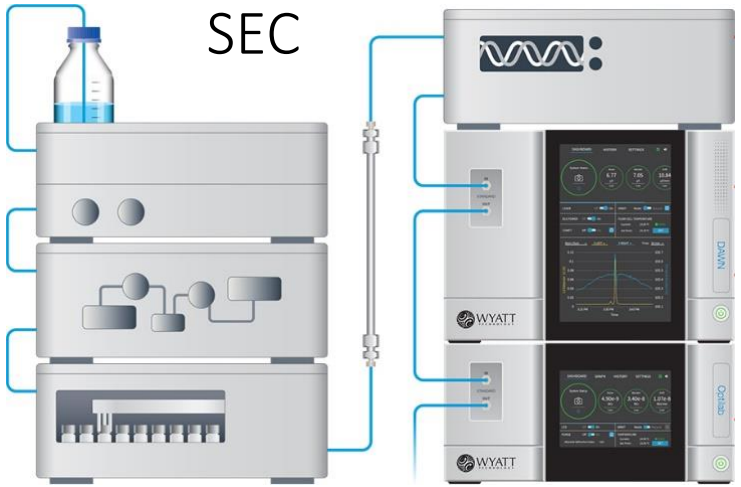
ASTRA

VISION

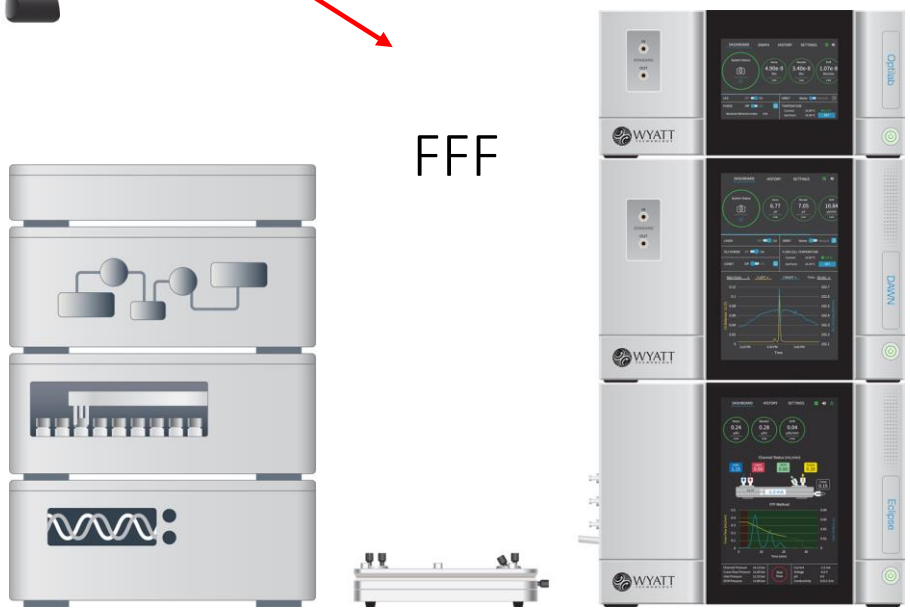


- *Online multiple-detector:* DAWN (MALS-DLS), UV (260 nm), Optilab (dRI)
- Software packages are 21 CFR Part 11 compliant

SEC



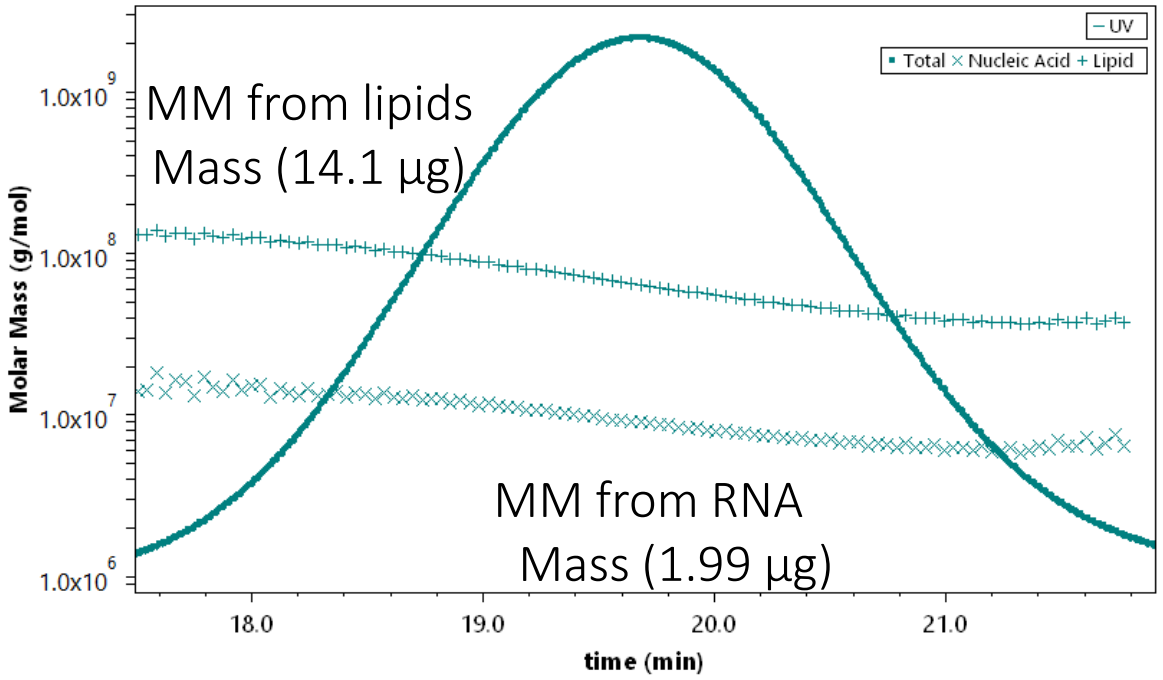
FFF



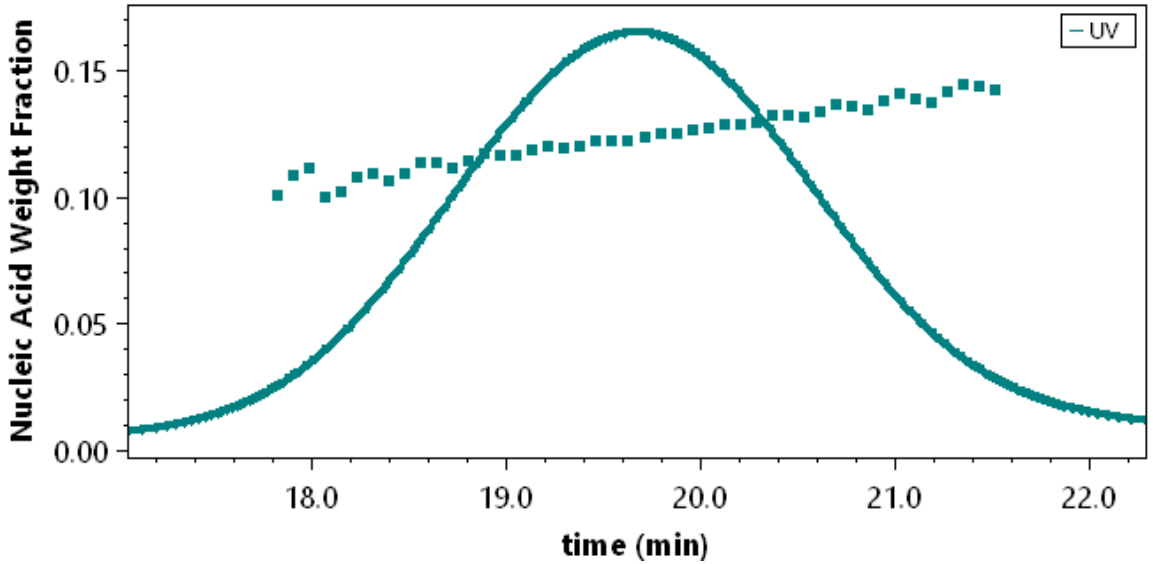
WYATT TECHNOLOGY

LNP and nucleic acid concentration (SEC-MALS)

The method removes the scattering contribution from the UV signal of the nanoparticle, and then applies a calculation similar to standard conjugate analysis (like AAV method).

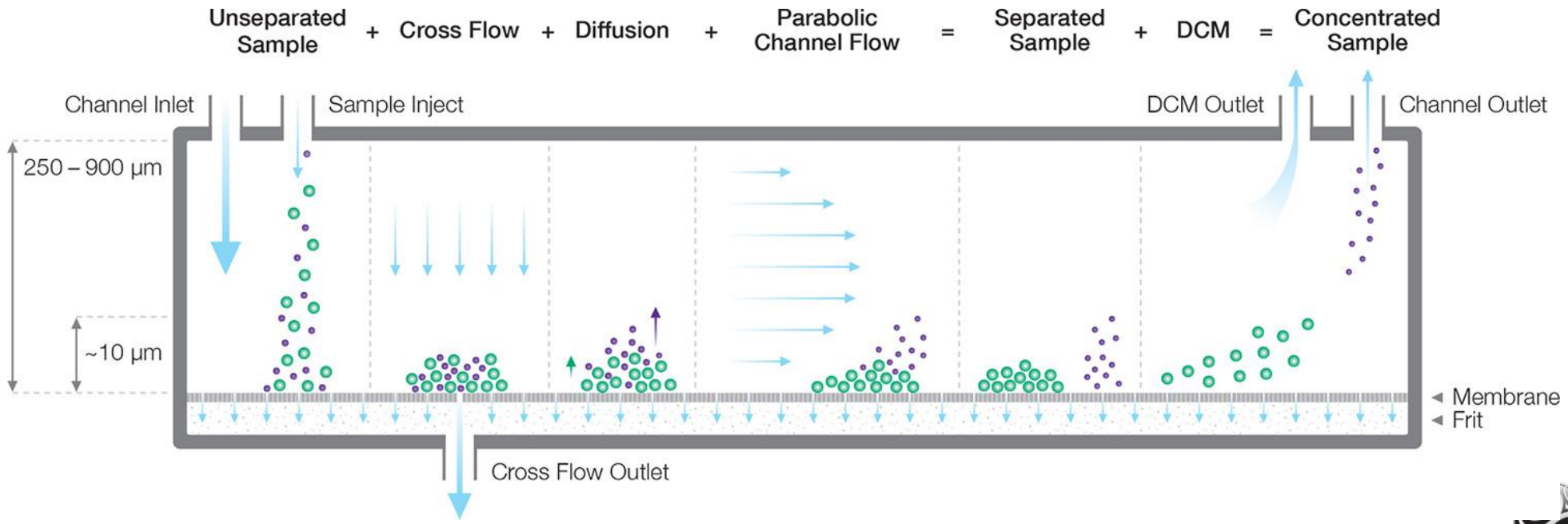


Comprehensive and accurate characterization in a single run

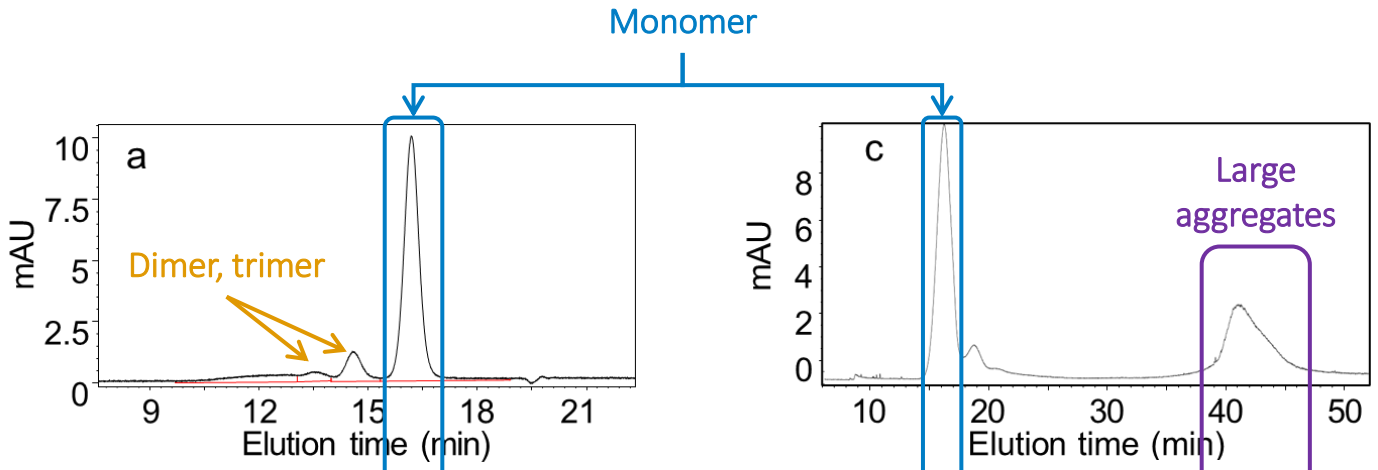
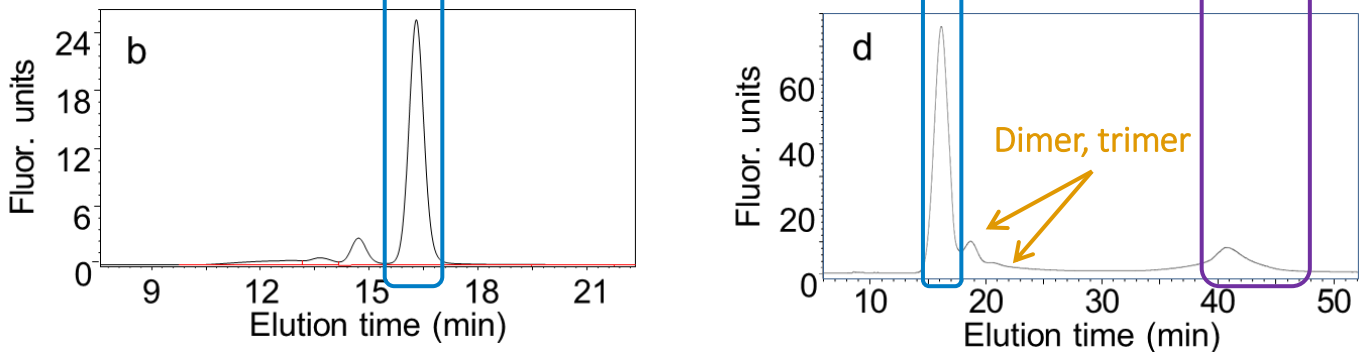


Alternative separation technique: FFF

- Bio-nanoparticles
- Proteins that adsorb to SEC columns
- Proteins with a wide MW range or large aggregates
- Orthogonal approach to SEC



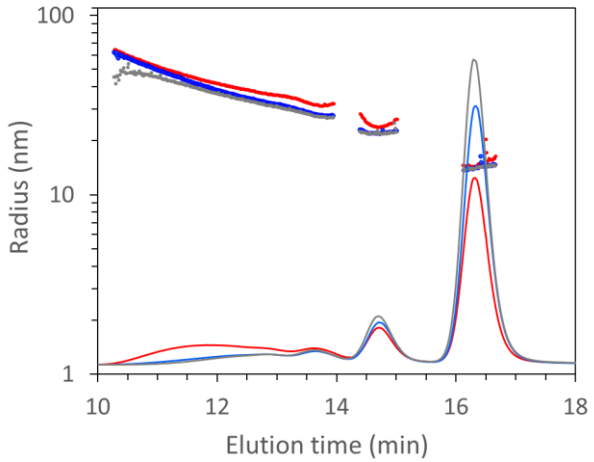
Case study: MALS required for large aggregate quantitation

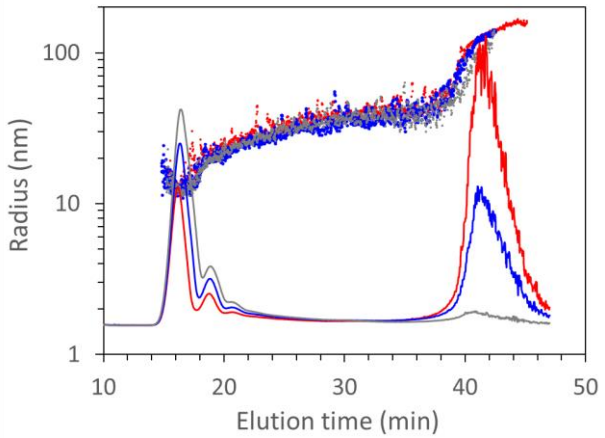
	Size-Exclusion Chromatography	Field-Flow Fractionation
UV absorbance (280 nm)		
Fluorescence 280 nm excitation 350 nm emission		

AN2004: Why and how to quantify AAV aggregates by FFF-MALS

Judit Bartalis, Novartis Gene Therapies, Michelle Chen and Daniel Some, Wyatt Technology Corporation

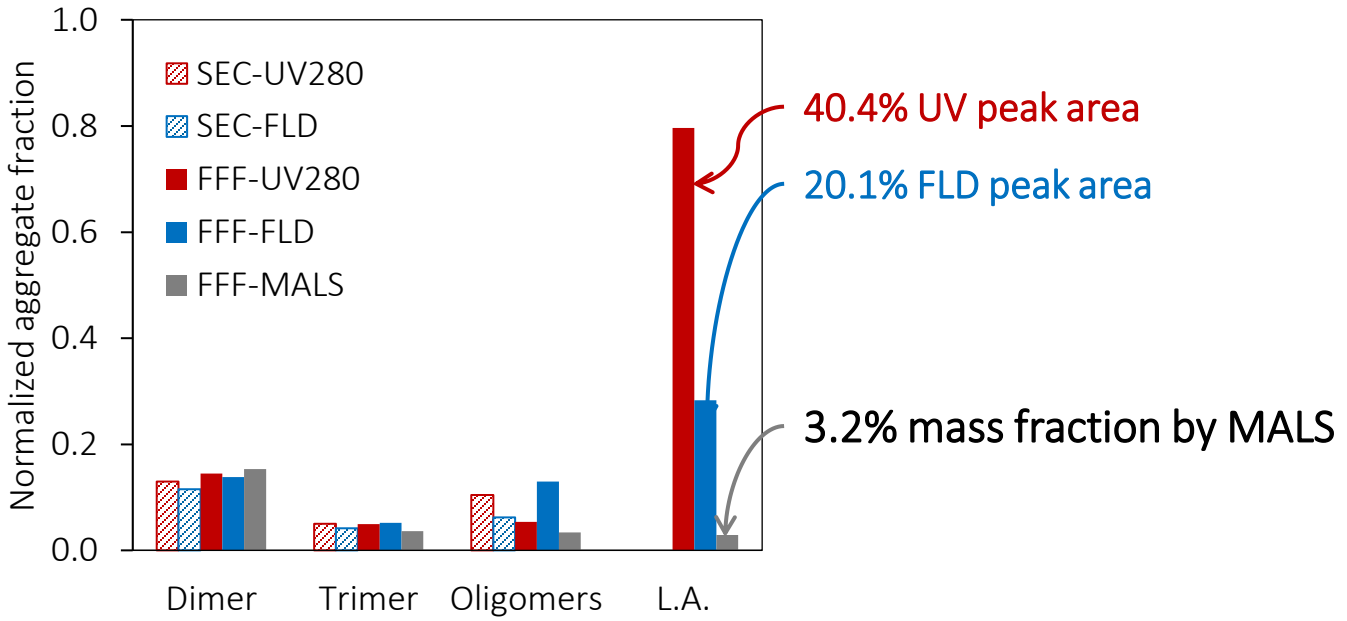
Only MALS correctly quantifies large aggregates





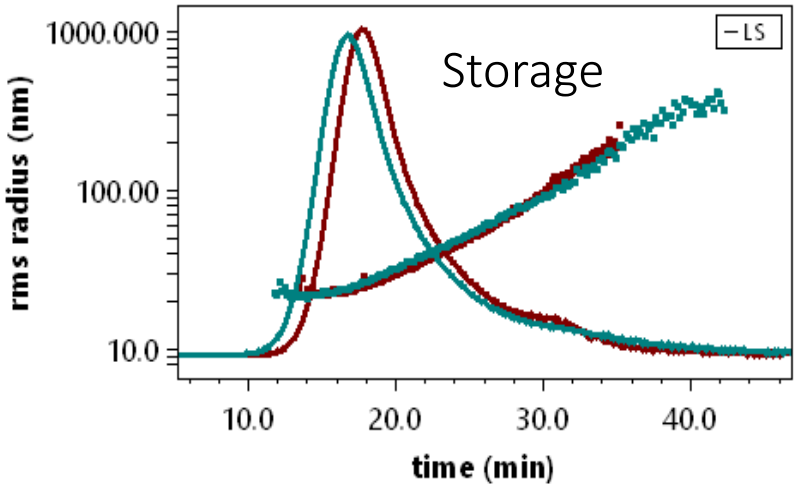
Large aggregates (LA) both absorb *and scatter* light, leading to overestimation of large aggregates.

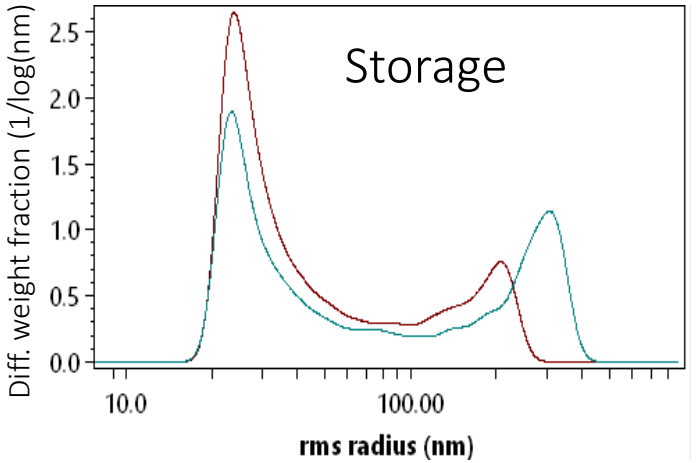
- Note that large aggregates are only detectable with FFF.
- Large aggregates filtered out by SEC are retained by FFF.

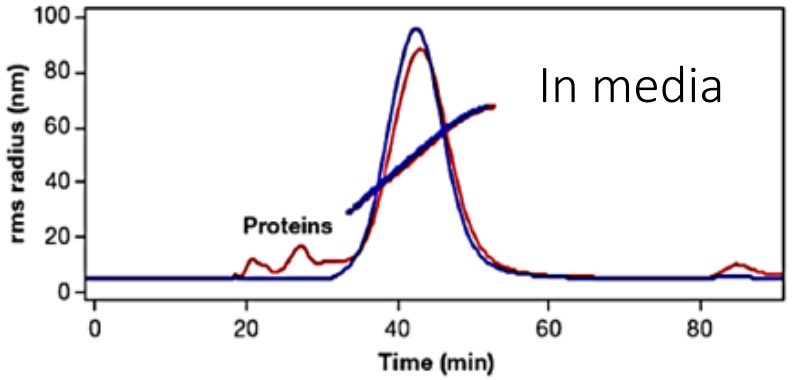


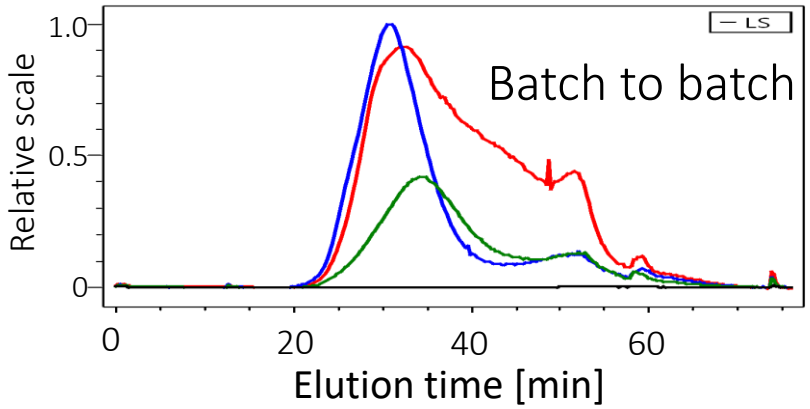
LNP stability ☑

Used as a next step after FFF-MALS characterization







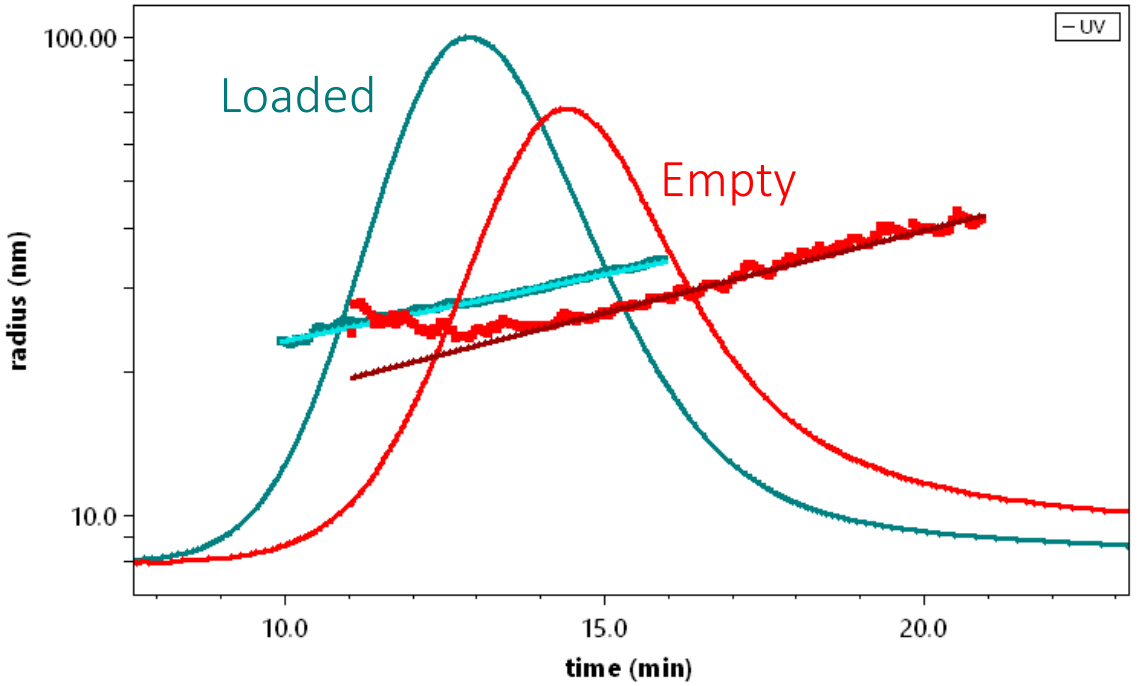


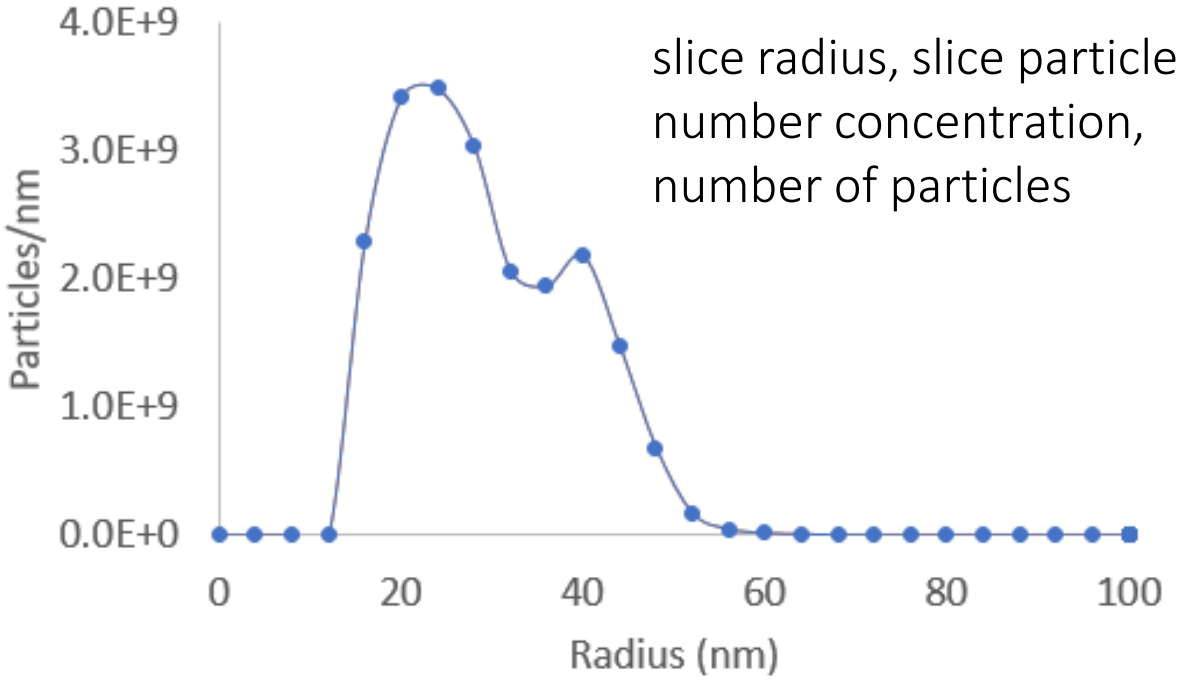
Graph courtesy of Dr. Fanny Caputo, SINTEF

LNP size and polydispersity ☑

Note strong UV absorption of Empty LNPs
→due to strong UV scattering

R_{min} [nm]	R_{max} [nm]	N	C_N [mL^{-1}]
0.0	38.0	6.49x10^{10}	3.25x10^{12}
38.0	100.0	1.84x10^{10}	9.22x10^{11}

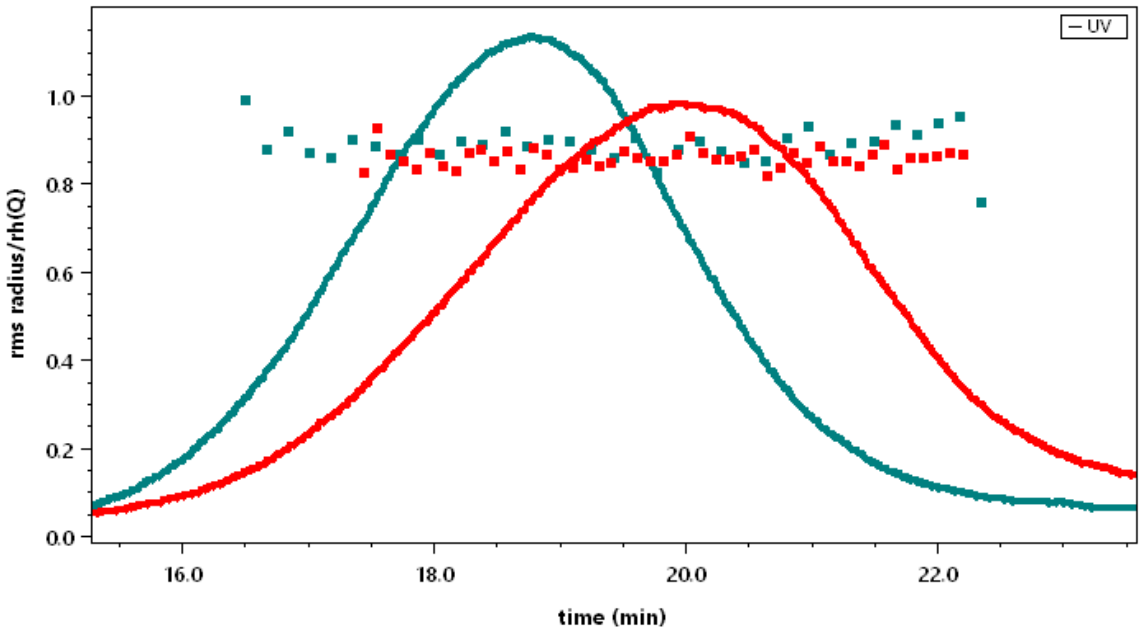




slice radius, slice particle number concentration, number of particles

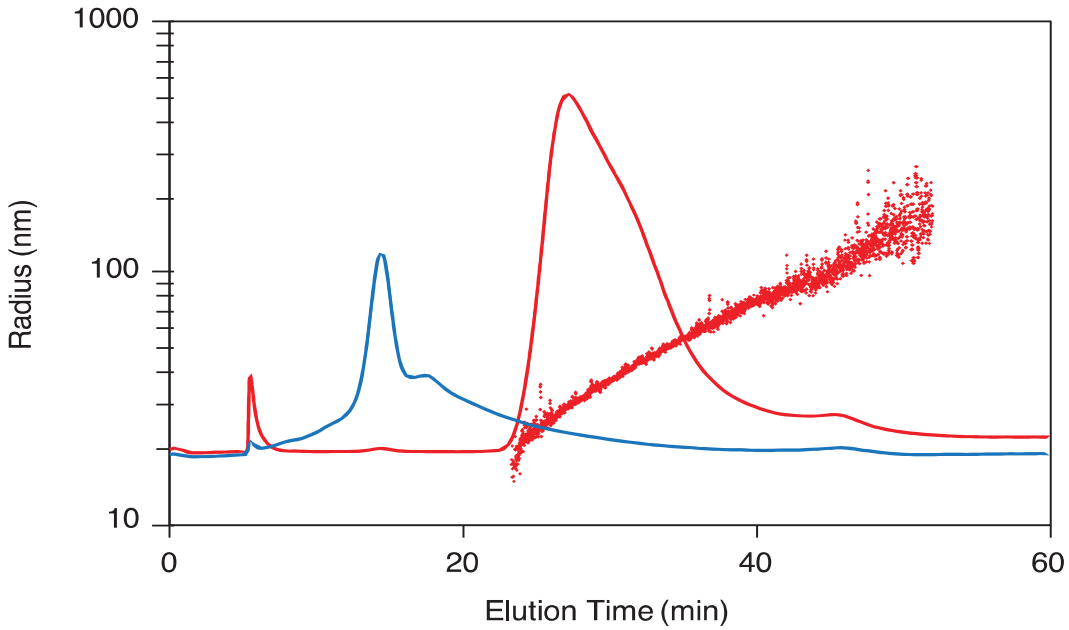
LNP particle morphology and encapsulation efficiency ☑

Comparison of R_g and R_h reveals particle density and morphology in the solution.

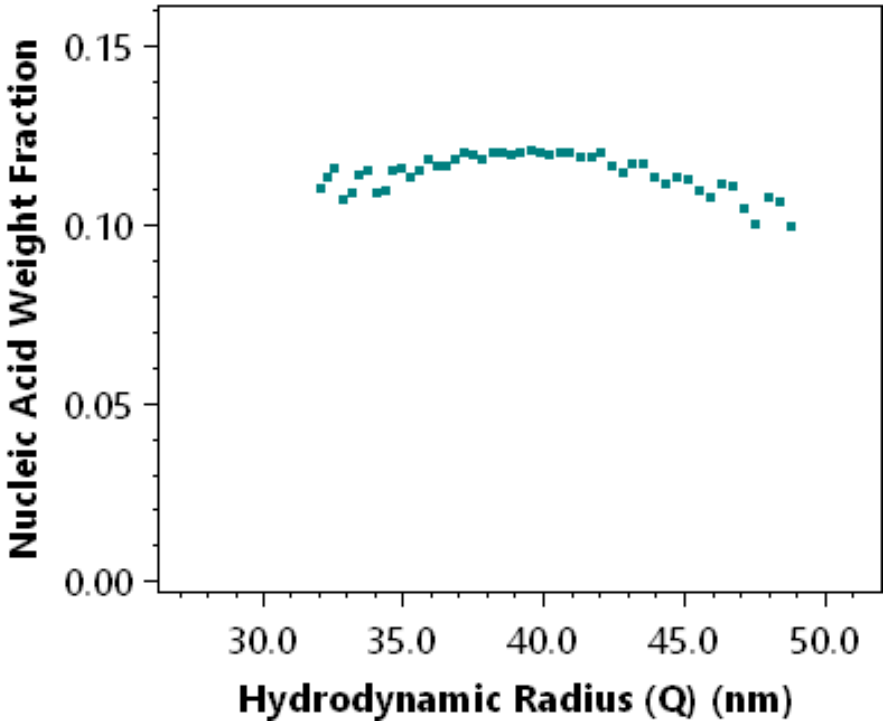


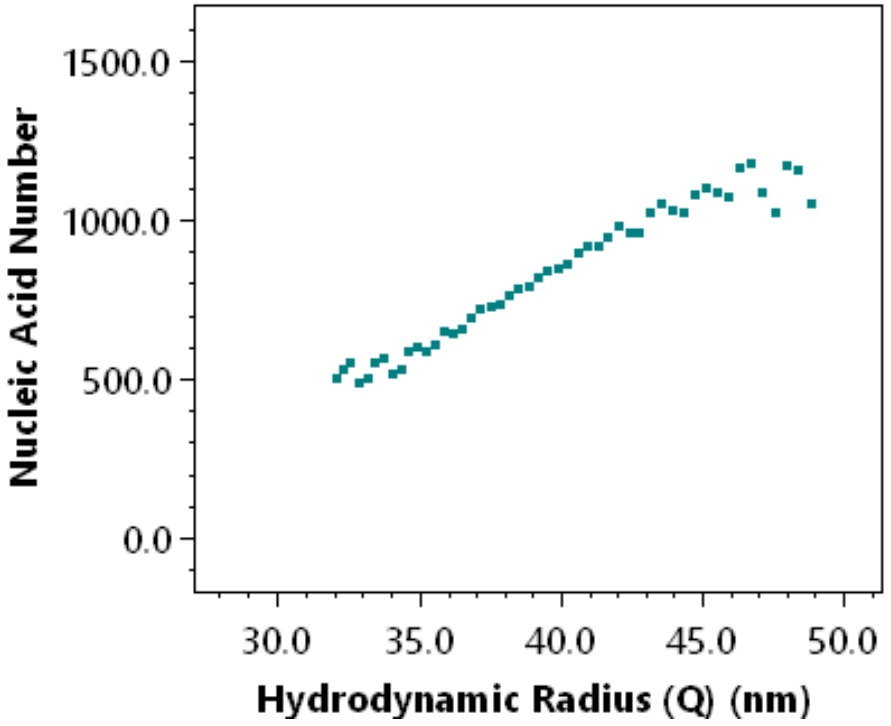
Encapsulation efficiency can be quantified thanks to separation

$EE=(C_{Total\ RNA}-C_{Free\ RNA})/C_{Total\ RNA}$



Additional LNP quantitation: Nucleic acid payload





	Mw (Nucleic Acid) (kDa)	Lipid Concentration (mg/ml)	Nucleic Acid Concentration (mg/ml)	Encapsulation Efficiency (%)	Nucleic Acid Number
F1a)	12778.2 (±0.5%)	10.67	1.42	97.8	819.1 (±0.5%)
F2a)	8805.0 (±1.3%)	7.36	0.97	96.8	564.4 (±1.3%)
F3a)	13180.6 (±0.9%)	7.95	1.07	97.5	844.9 (±0.9%)

Summary of the new LNP Analysis

- SEC or FFF separates LNP with high resolution.
- Online detectors (MALS, UV at 260 nm, and dRI) provide comprehensive characterization and multi-attribute quantitation.
- The new LNP Analysis method enables size-based nucleic acid payload
- MD-SEC and MD-FFF are essential tools for measuring LNP size, concentration, payload, and product quality
- Software packages are 21 CFR 11 compliant
- MD-SEC and MD-FFF are automated, robust, easy to adopt, minimum hands-on time, less prone to experimental errors

Key LS methods for proteins, AAVs, and LNPs

	DLS	SEC-MALS	FFF-MALS
Proteins	▪ Developability & Formulation study ▪ Aggregation screening	Platform method for: MW, composition, Aggregation, etc.	▪ Troubleshooting
AAVs (VVA Module)	▪ Size, polydispersity ▪ Titer ▪ Aggregation	**Platform method for MAQ:** ▪ Titer ▪ E/F ▪ Aggregation ▪ Extended characterization	▪ Quantify all AAV aggregates
LNPs (LNP Module)	▪ Size, polydispersity ▪ Particle concentration ▪ Aggregation	First sized-based separation and characterization tool for LNPs	**Platform method for MAQ:** ▪ High-res size distribution ▪ Size-based payload distribution ▪ Online lipid conc. ▪ RNA or DNA HOS ▪ Isolation of narrowly distributed fractions

Thank you!

- The new LNP Analysis method enables size-based nucleic acid payload.
- MD-SEC and MD-FFF are essential tools for measuring LNP size, concentration, payload, and product quality.
- MD-SEC and MD-FFF are automated, robust, easy to adopt, minimum hands-on time, less prone to experimental errors.
- Viral Vector Analysis has excellent sensitivity ($5x10^{10}$ to $1x10^{15}$ AAV/mL), linearity, reproducibility, consistency, and robustness, which are required in QC.
- SOP guidance manual is included to ensure proper adoption, routine analysis, and method transfer.
- Software packages are 21 CFR 11 compliant.

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