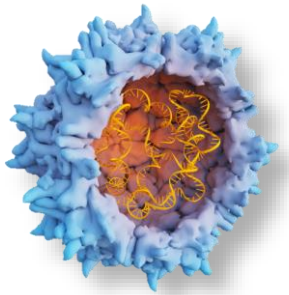

Biophysical characterization and quality control of LNP and viral vectors with light scattering

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Senior Field Application Scientist

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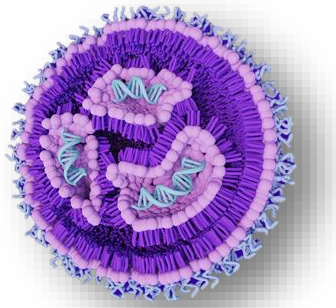

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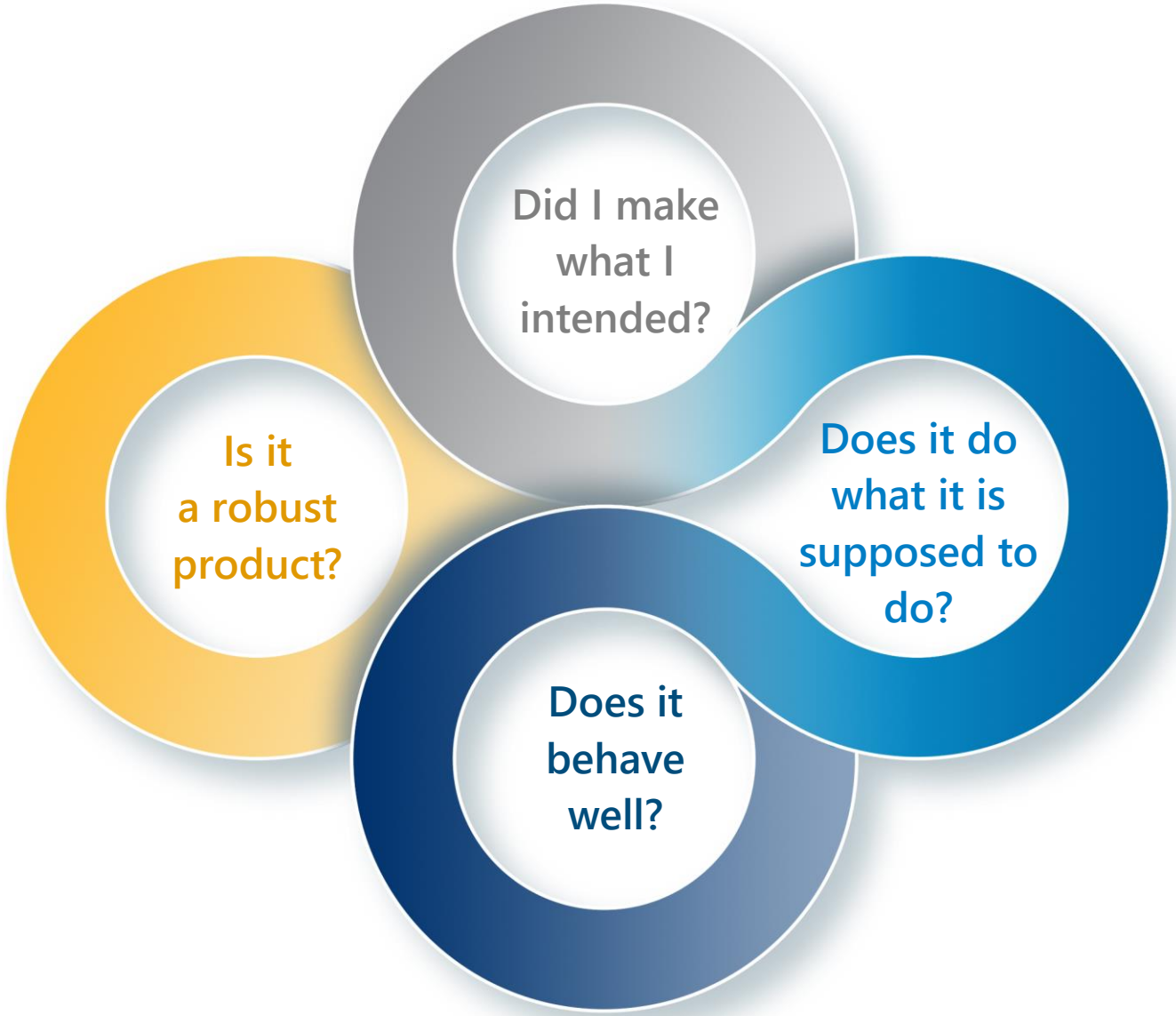

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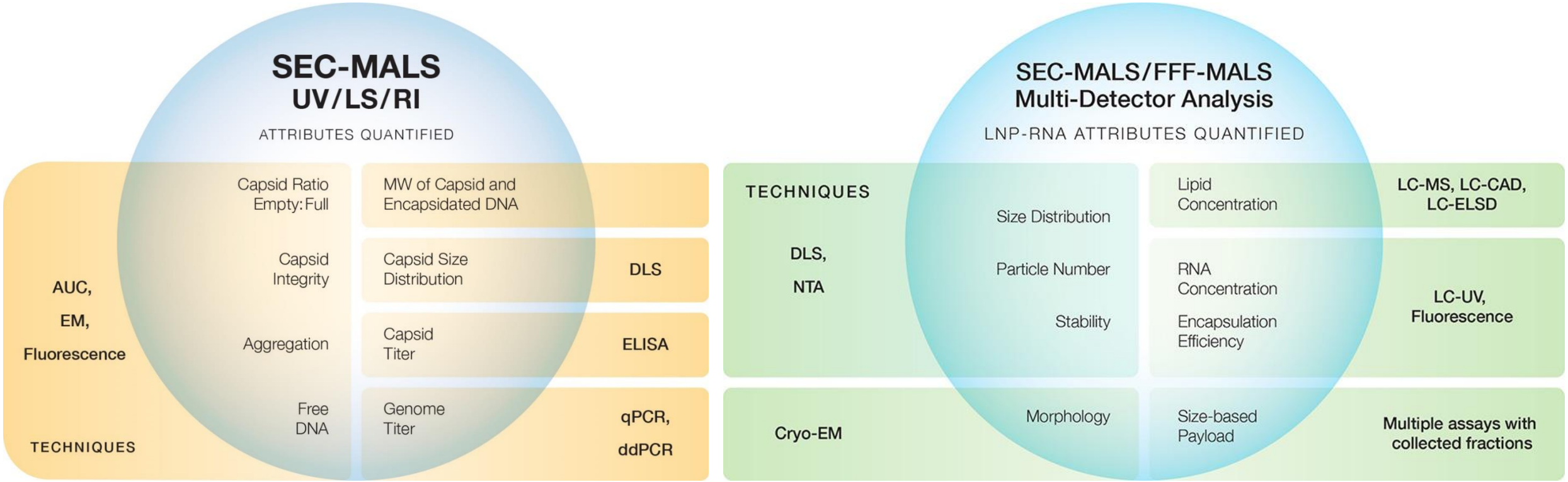
Outline

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



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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)

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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)

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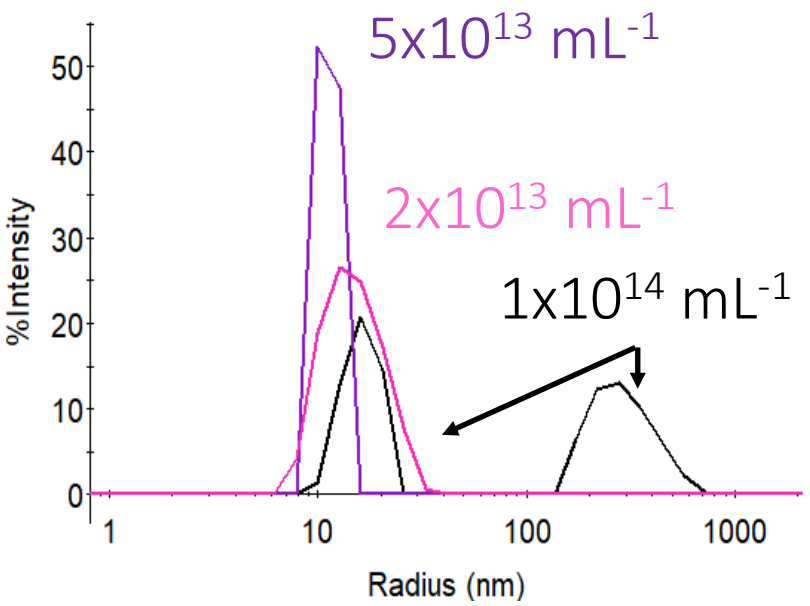
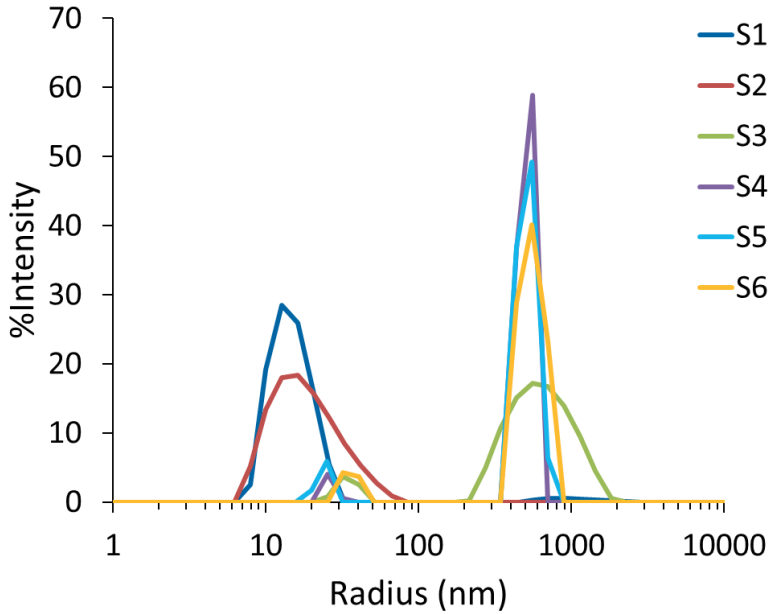
Stability Screening With the Dynapro Plate Reader

Concentration



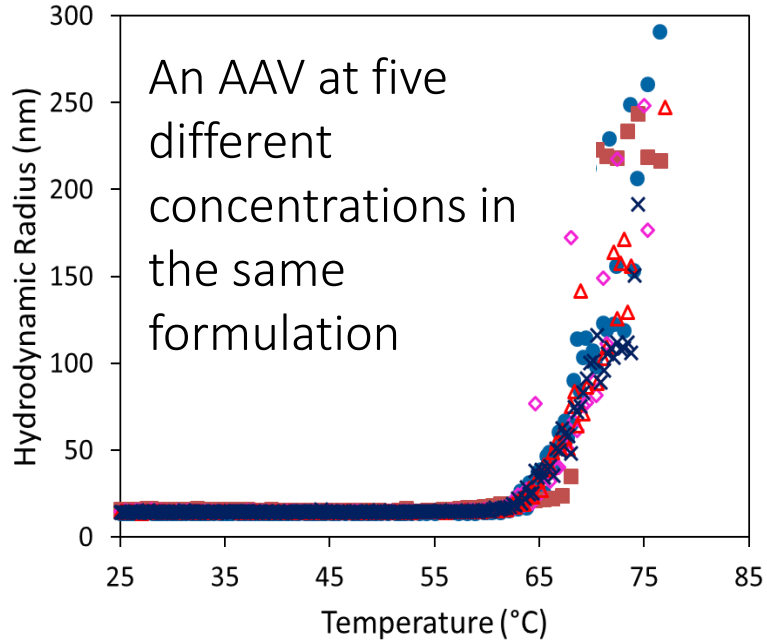
Aggregates appear to dissociate with decrease in concentration.

Buffer additive



Buffer additives affect aggregate size and content.

Temperature



Similar thermal stability:

$T_{onset} = 62.5 \pm 0.5$ °C

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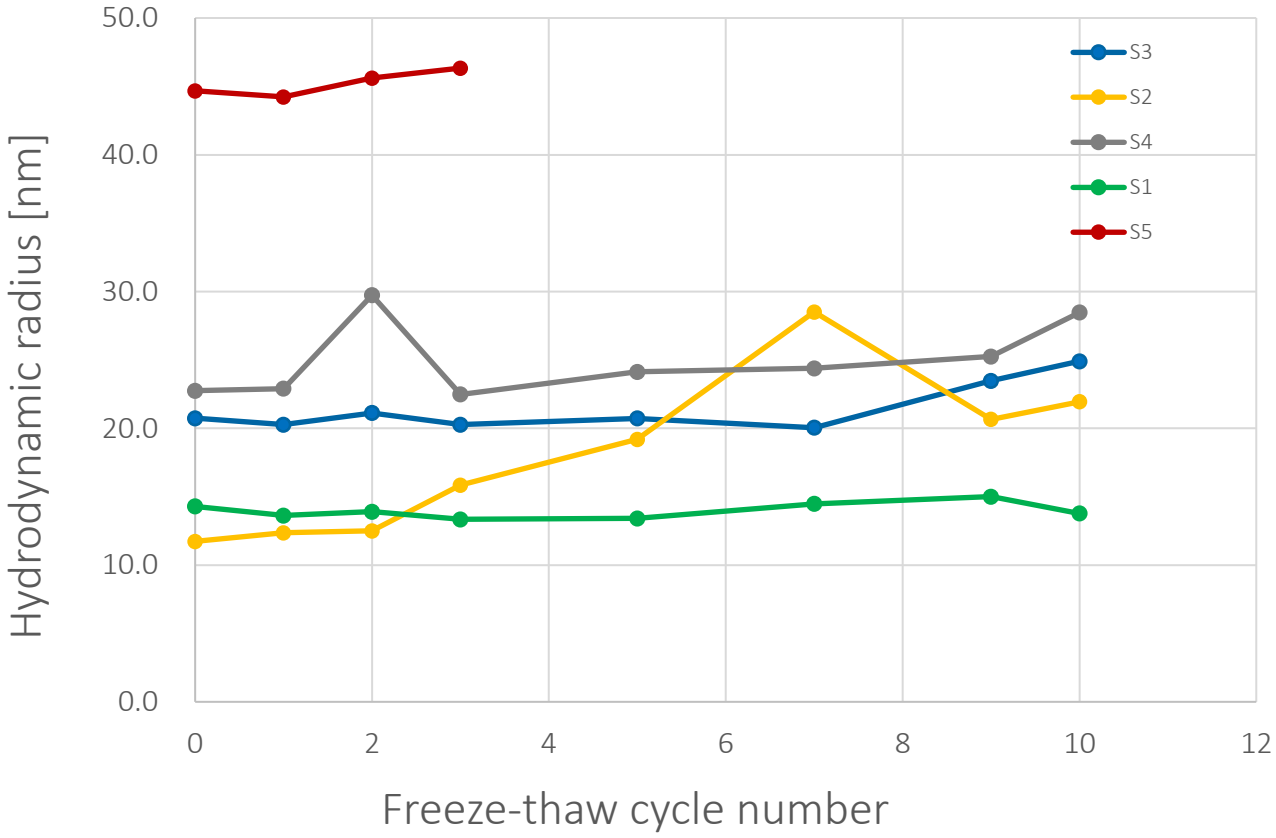
Freeze-thaw Study



Plates facilitate formulation studies, such as freeze-thaw cycles.

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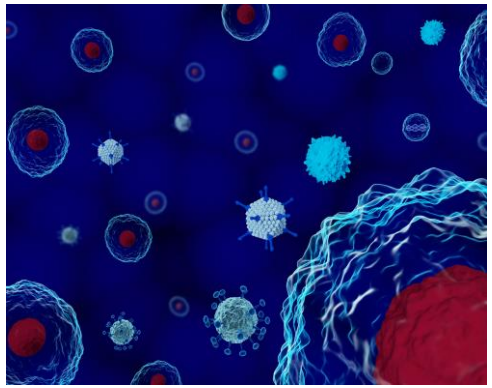
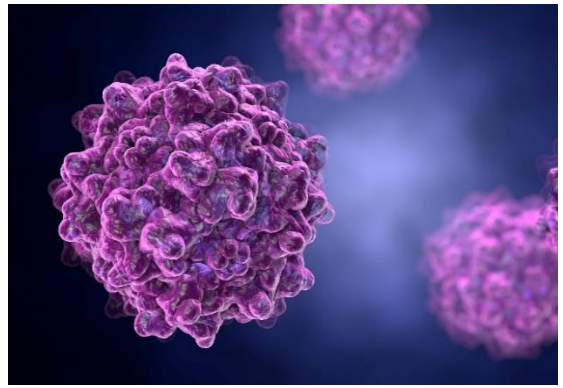
Multi-angle Light Scattering (MALS) Solutions



SEC-MALS

Quantify 3 AAV CQAs in one assay

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



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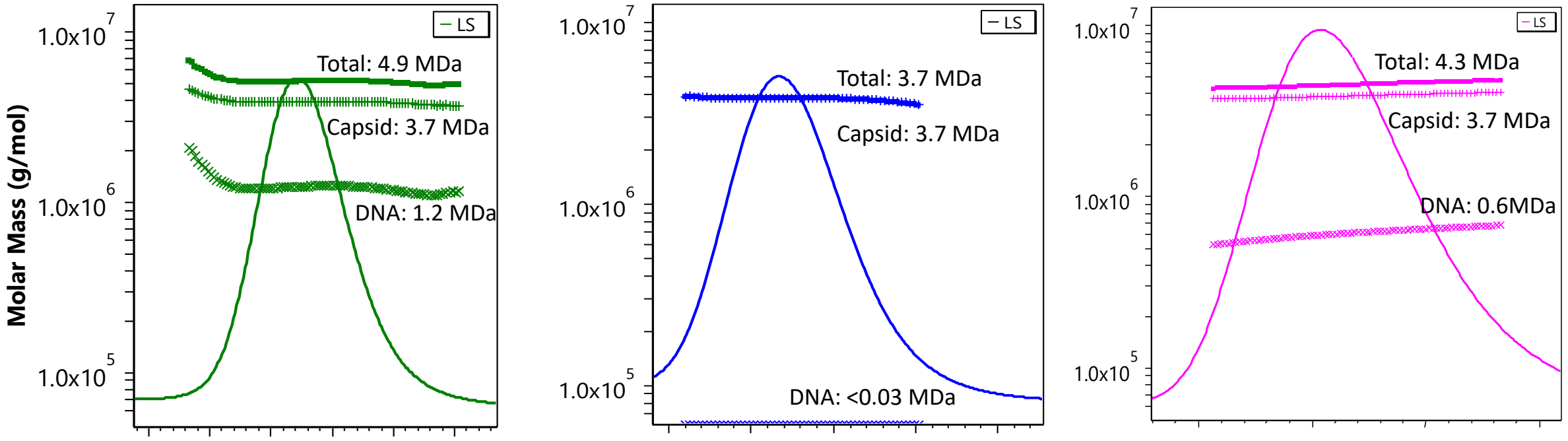
Protein Conjugate Analysis: AAV (Protein-DNA Conjugate)



- Determine MW and mass for capsid and encapsidated DNA.
- Equivalent to simultaneous ELISA/microBCA and ddPCR/qPCR analyses.
- Viral Vector Analysis module calculates the AAV specific attributes.
- To learn more details: AN 1617 on www.wyatt.com (U.S. patent pending)

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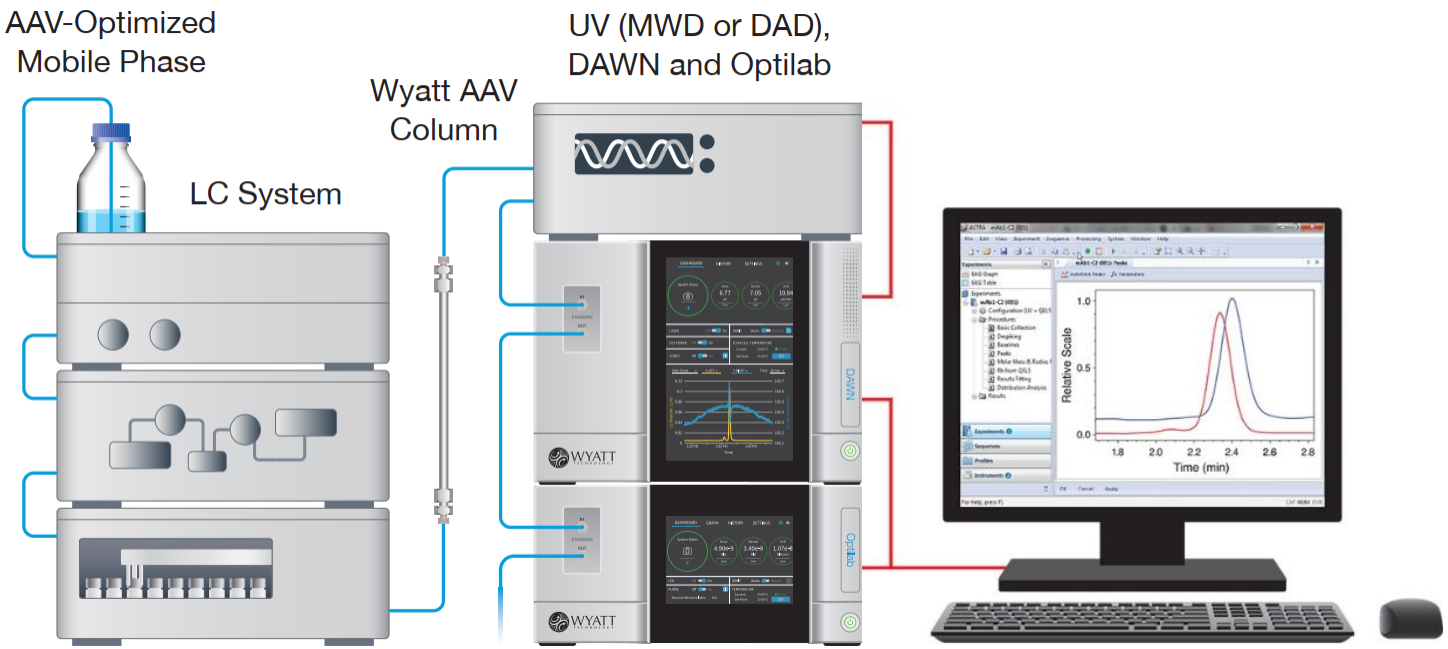
AAV Analysis – Experimental Setup



- ✓ 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

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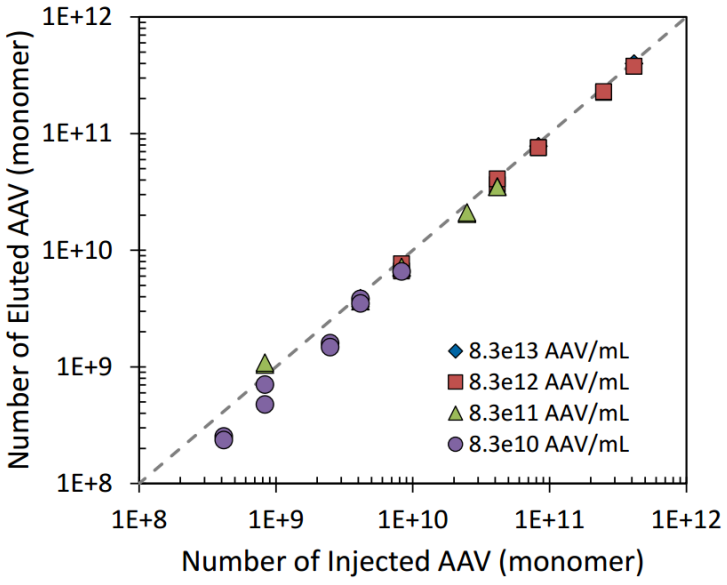
AAV Quality Attributes

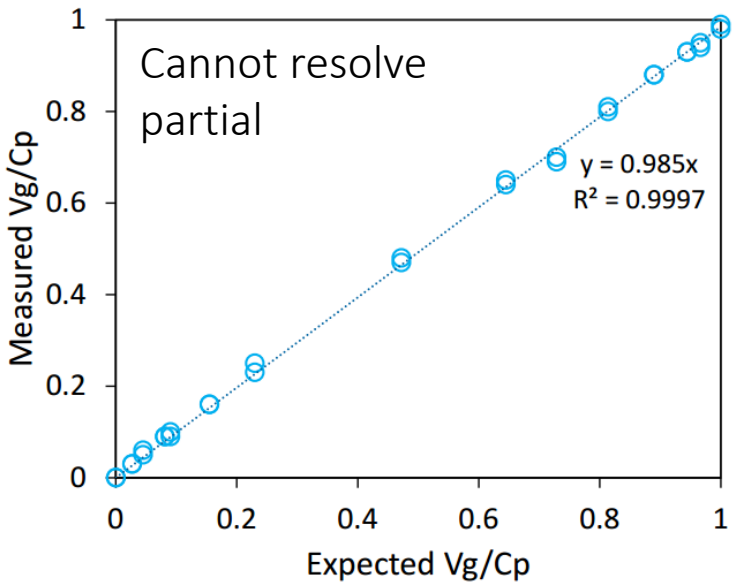
☑ Capsid concentration

Most accurate and precise method.



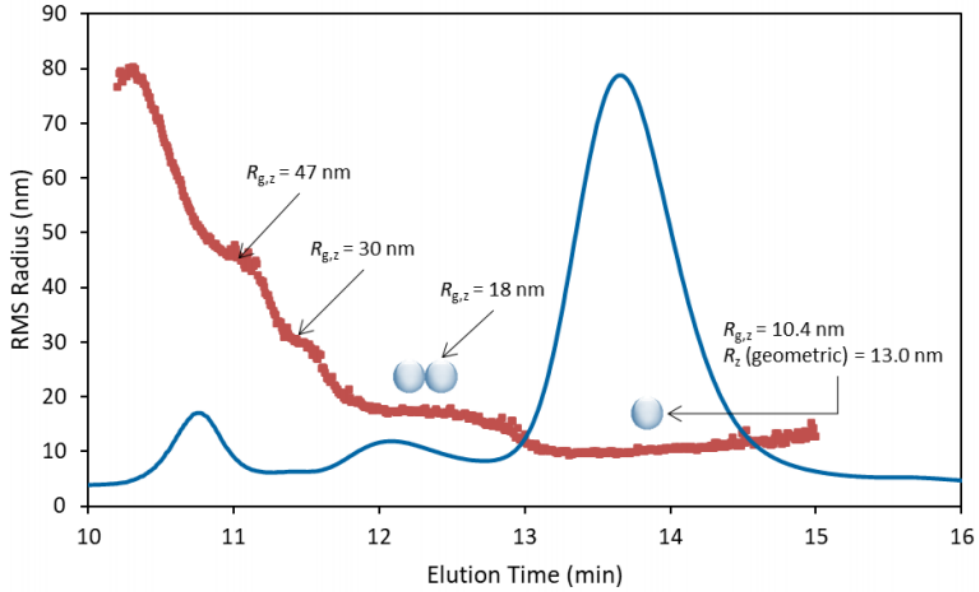
☑ Vg/Cp

Consistent and precise analysis for routine analysis.



☑ Aggregates

High sensitivity and resolution. UHMW aggregates may be removed



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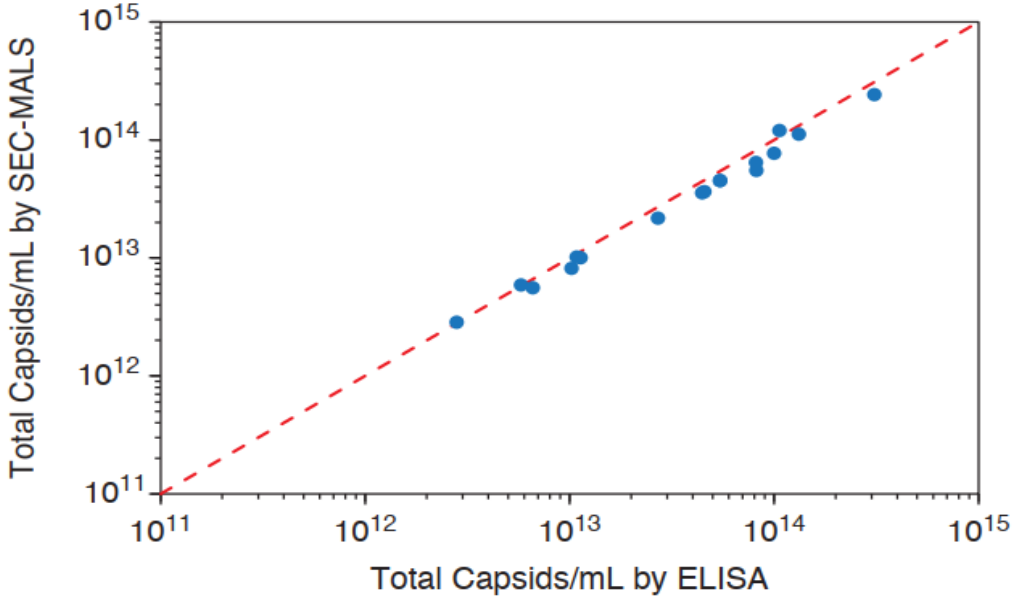
AAV Analysis: Cross-verification

☑ 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.

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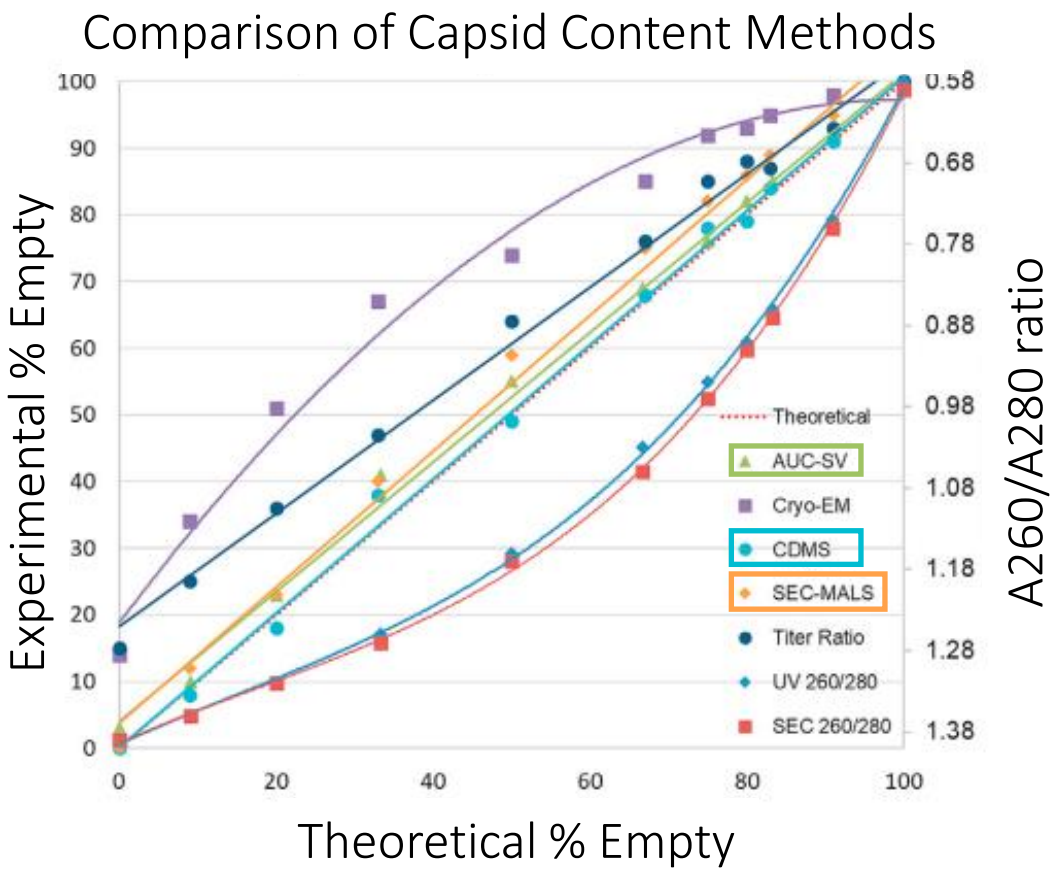
AAV Analysis: Cross-verification

☑ Vg/Cp



	AAV #1 V_g [mL^{-1}]	AAV #2 V_g [mL^{-1}]
SEC-MALS	(2.3±0.1)x10^{12}	(1.2±0.1)x10^{12}
dd PCR	2.3x10^{12}	1.2x10^{12}
qPCR	1.2x10^{13}	8.5x10^{12}

- Comparison graph is from a paper by Pfizer Inc. https://doi.org/10.1016/j.omtm.2021.08.009
- CRO qPCR and in-house ddPCR data as well as AAV samples were kindly provided by Ronald Yeh, Discovery Biologics Seattle, Novo Nordisk.

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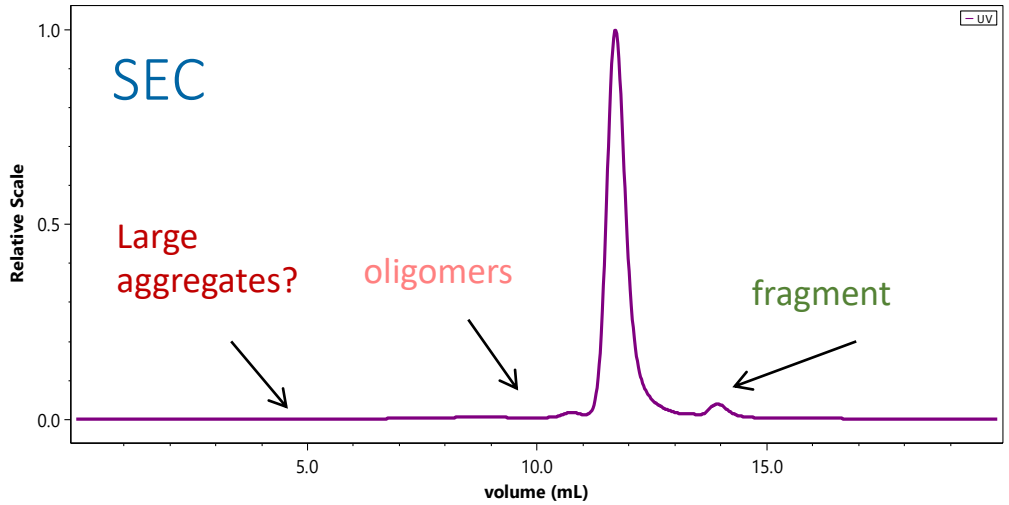
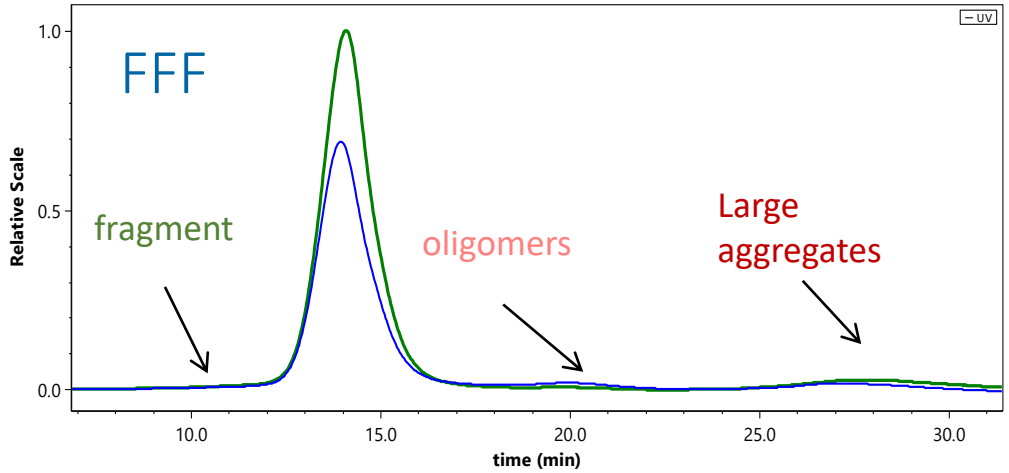
AAV Aggregates by SEC & FFF





SEC-MALS

- SEC is able to resolve monomer and oligomers
- SEC may not be the best tool to quantify large aggregates

FFF-MALS

- HMW aggregates visible by AF4-MALS may be removed by SEC column
- AF4 provides better separation of large aggregates and confirmation of aggregate %

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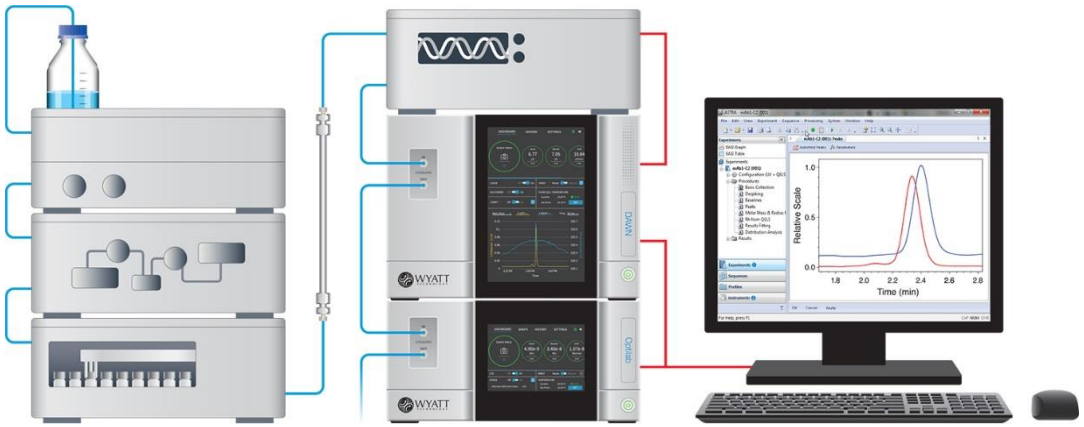
Complete Solution from RD, AD, PD, to Formulation, QC, and Platform Assay



- 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

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Physical Attributes and Assays for LNPs

Attribute	Assay	SEC/FFF-MALS-UV-dRI
mRNA integrity	Gel, qPCR	✓
LNP size	DLS analysis	✓
LNP distribution	DLS analysis	✓
Physical stability	DLS analysis	✓
LNP number	NTA analysis	✓
LNP morphology	TEM, cryo-EM	✓ (R_g/R_h)
LNP charge	Zeta potential	Possibly with EAF4
Encapsulation efficiency	Fluorescence	✓, new
mRNA concentration	Fluorescence	✓, new
Lipid concentration	LC-MS analysis	✓, new

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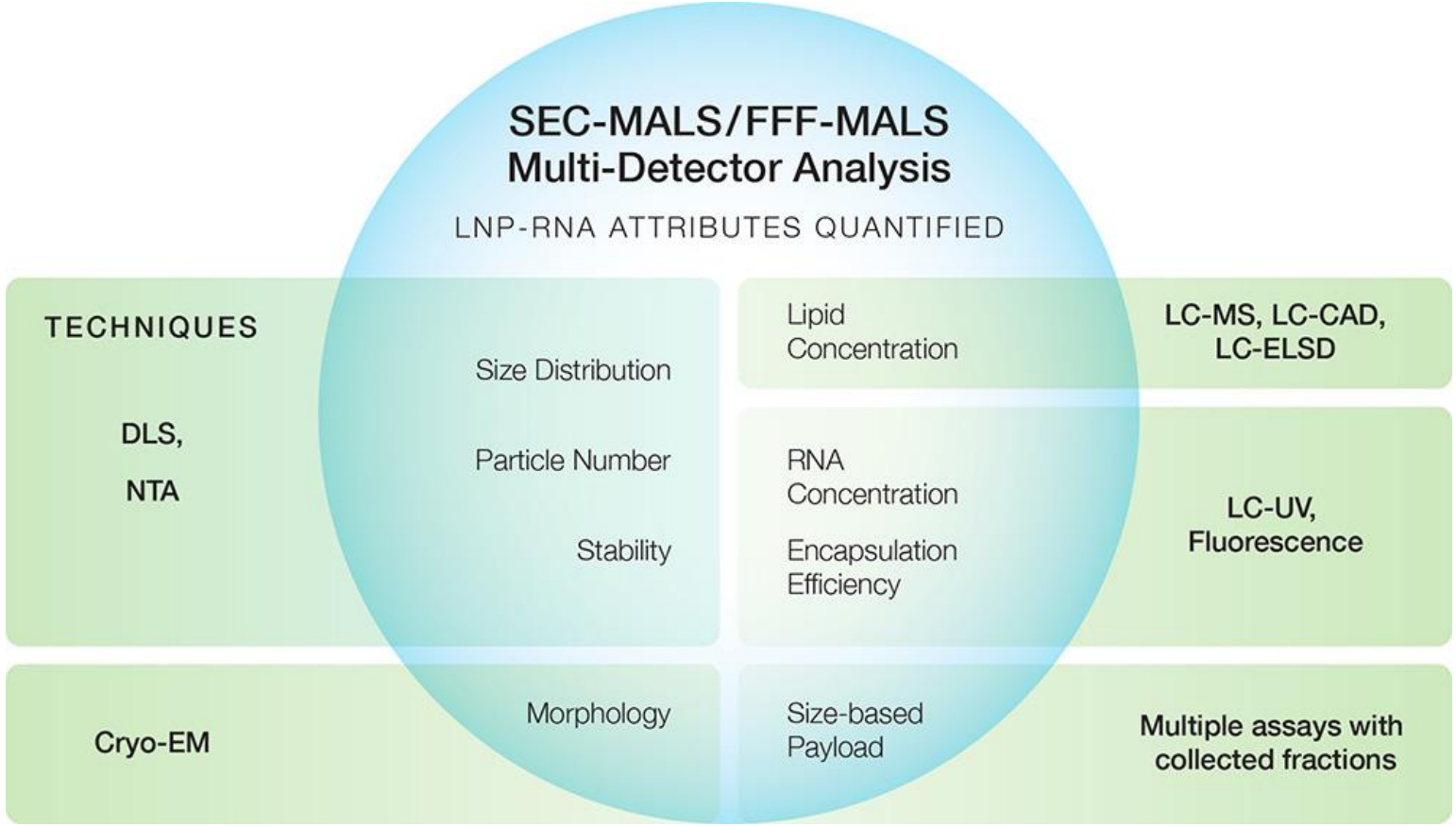
Wyatt Solutions for Quantifying LNP-RNA Attributes

ASTRA's new LNP-RNA Analysis quantifies payload, encapsulation efficiency and more



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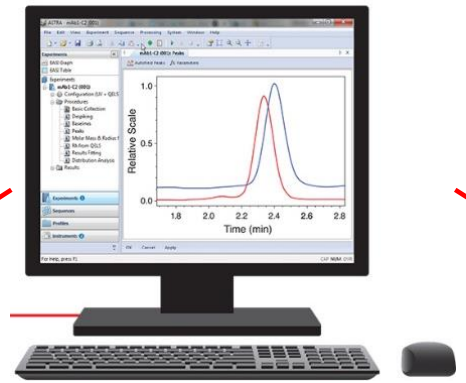
Experimental Setup for LNP Characterization

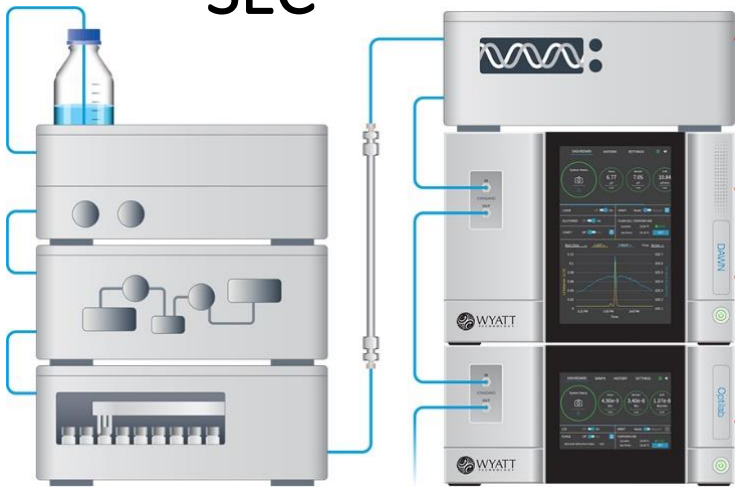
ASTRA

VISION

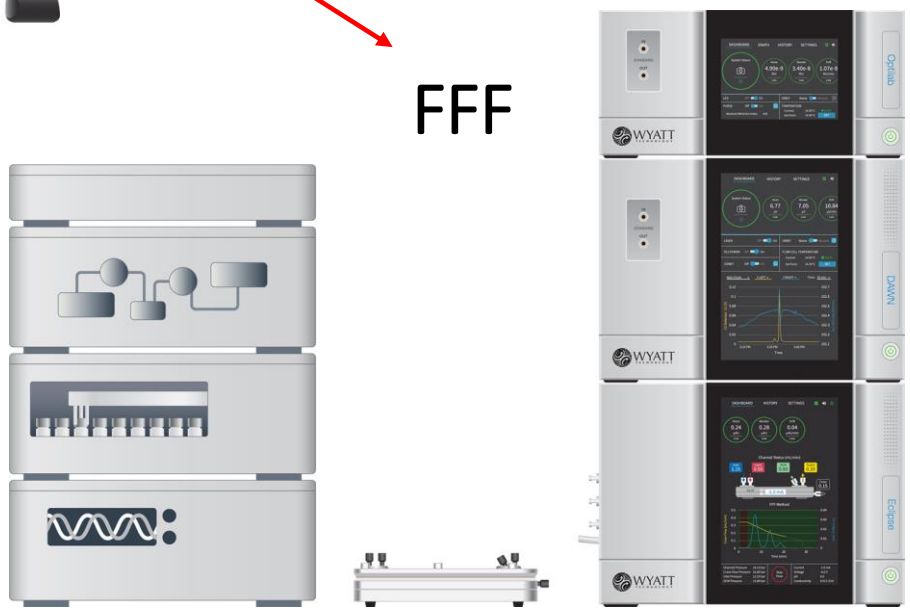


- *Online multiple-detector:* DAWN (MALS-DLS), UV (260 nm), Optilab (dRI)
- Software packages are 21 CFR Part 11 compliant

SEC



FFF



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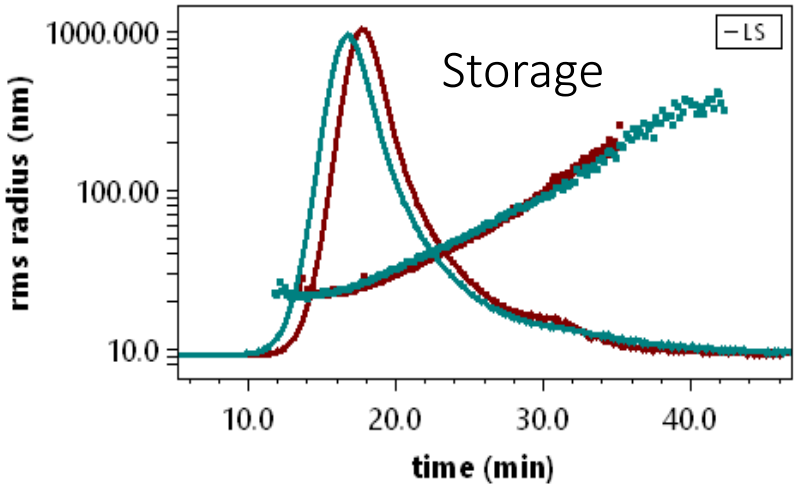
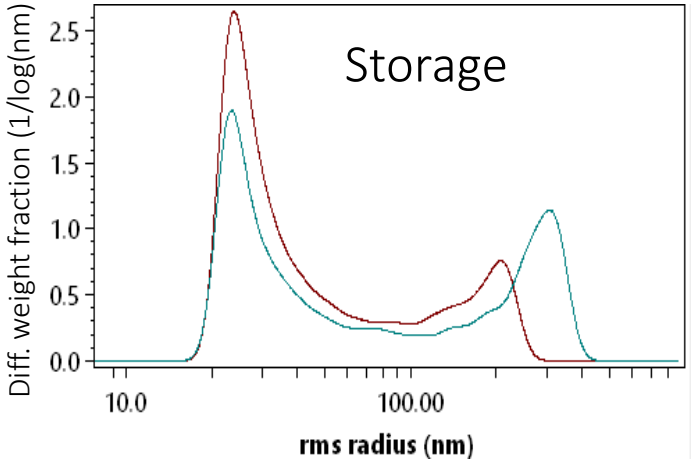
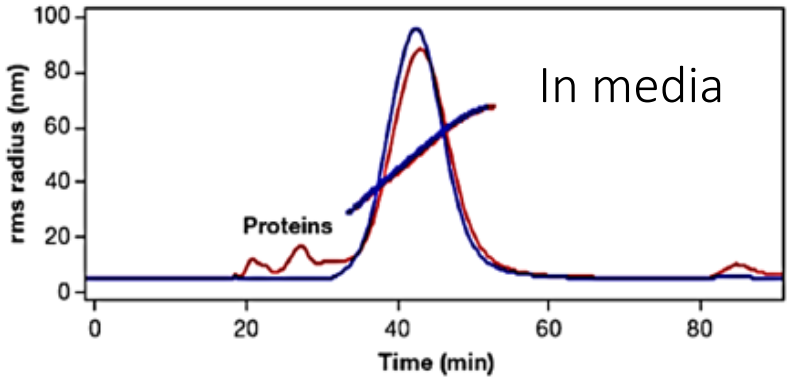
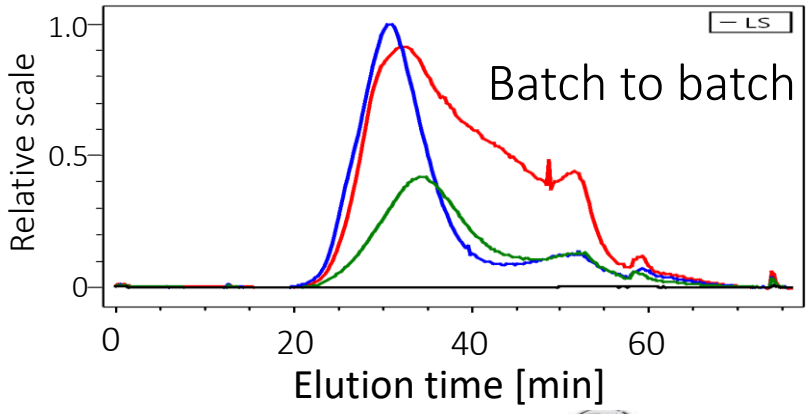
LNP Stability ☑

Used as a next step after HT-DLS characterization









Graph courtesy of Dr. Fanny Caputo, SINTEF

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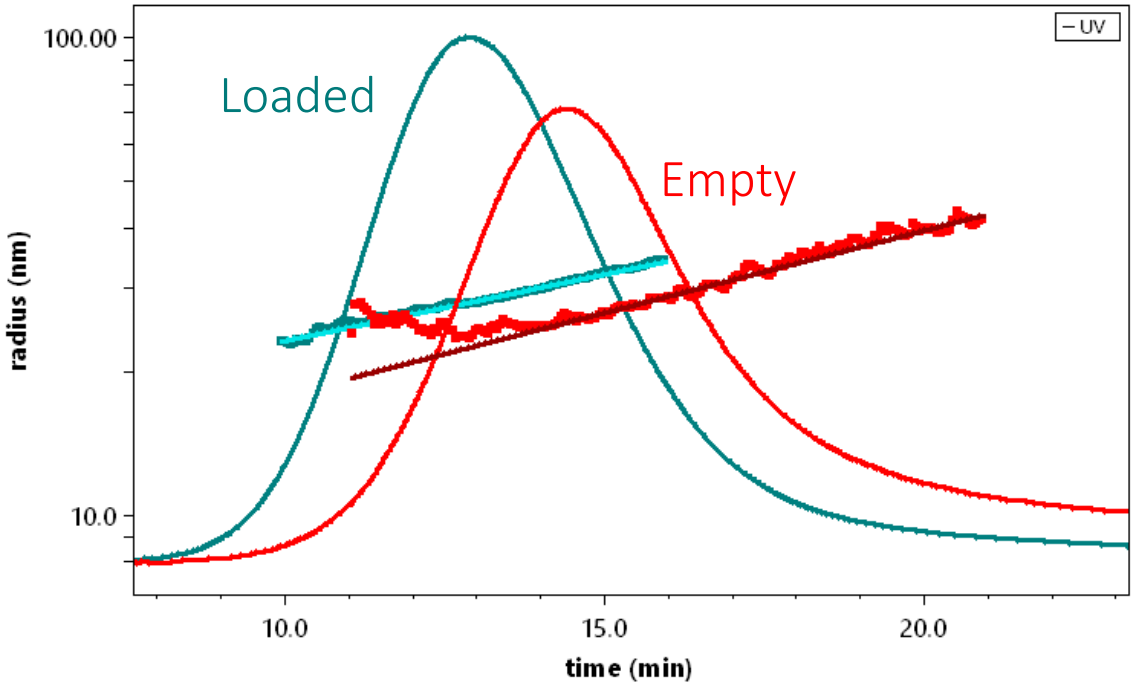
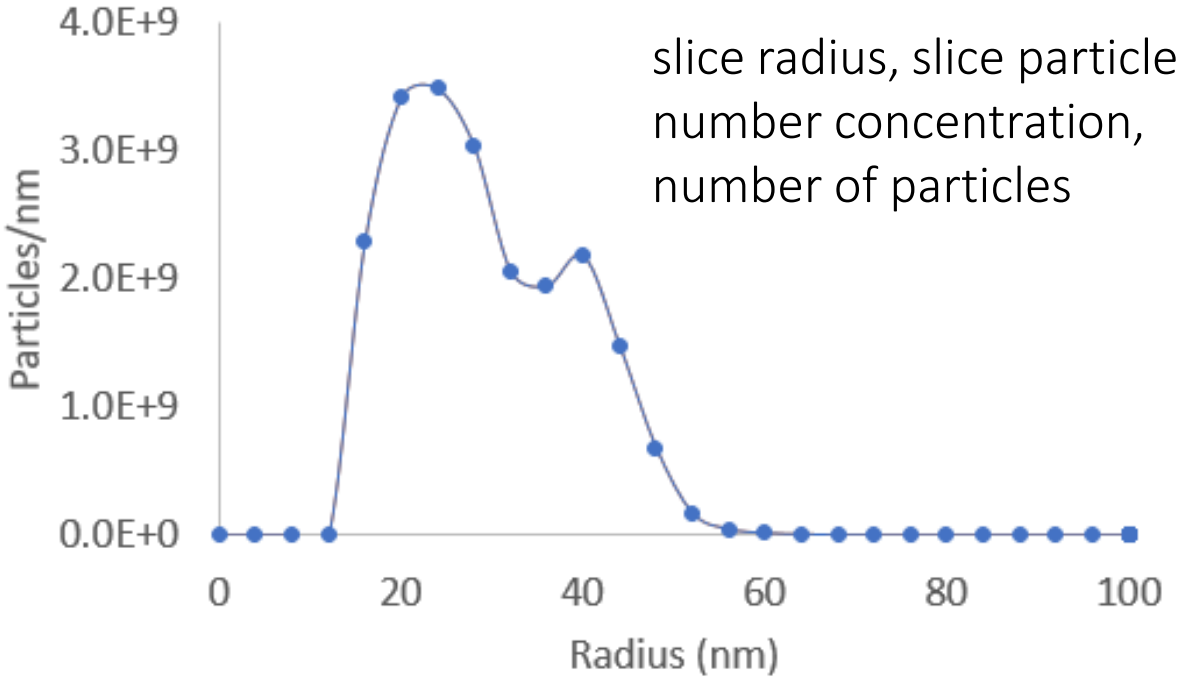
LNP Size and Polydispersity ☑

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	6.49×10^{10}	3.25×10^{12}
38.0	100.0	1.84×10^{10}	9.22×10^{11}





slice radius, slice particle number concentration, number of particles

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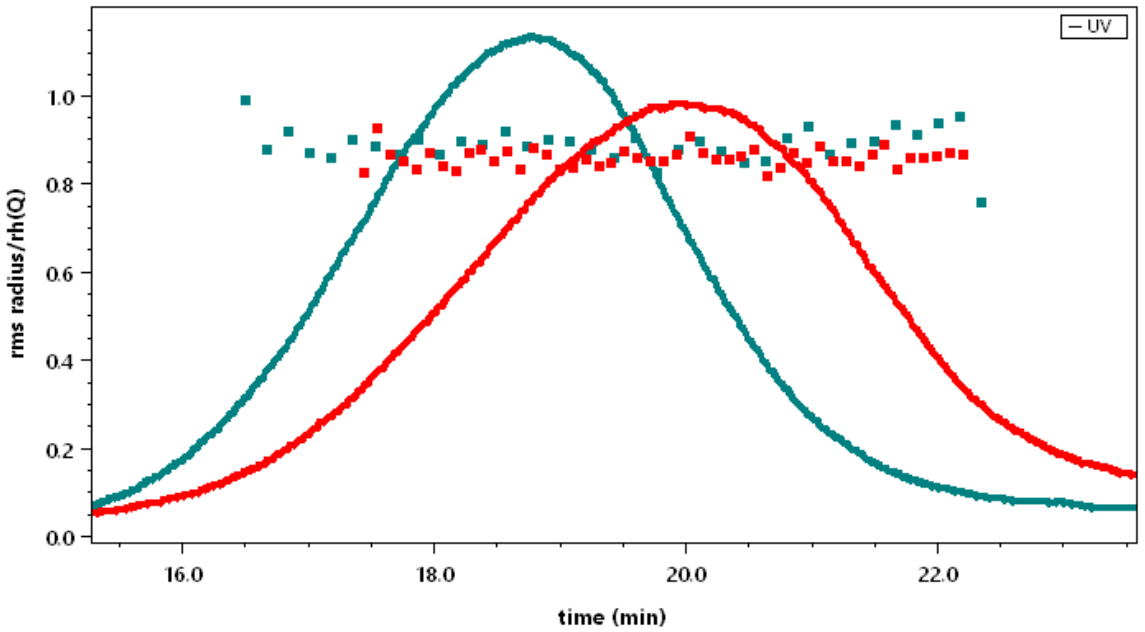
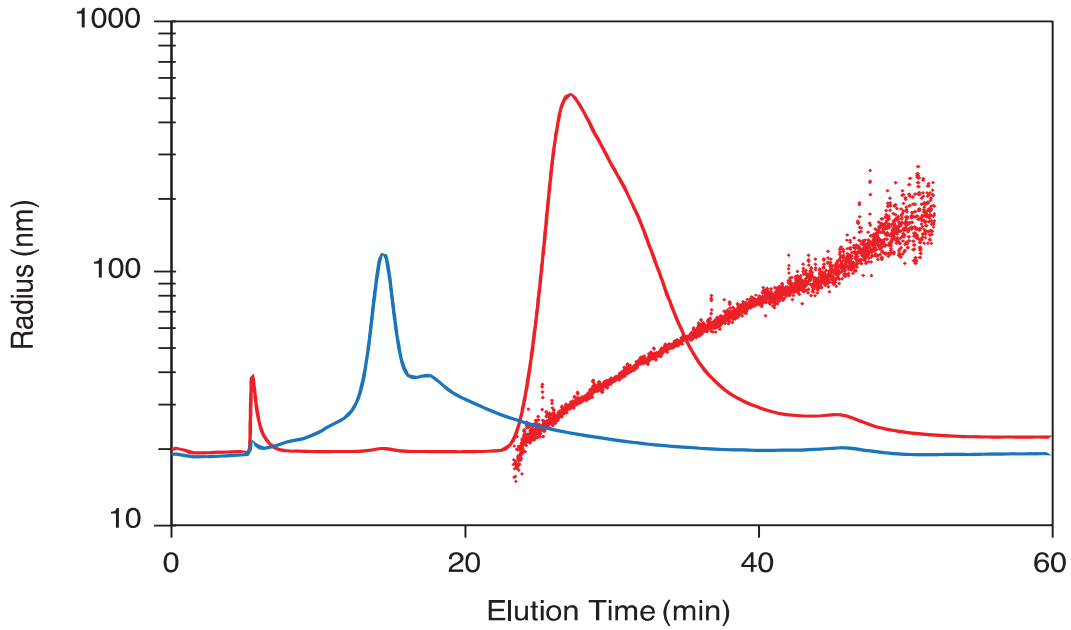
LNP Particle Morphology and Encapsulation Efficiency ☑

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



Encapsulation efficiency can be quantified thanks to separation

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



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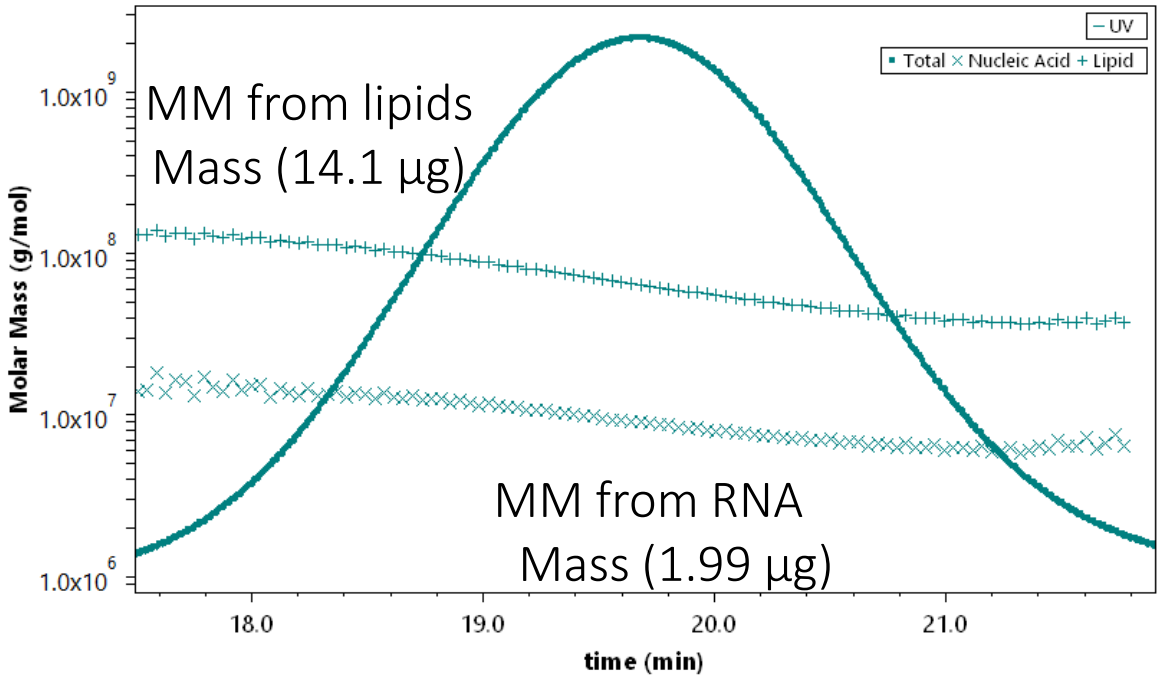
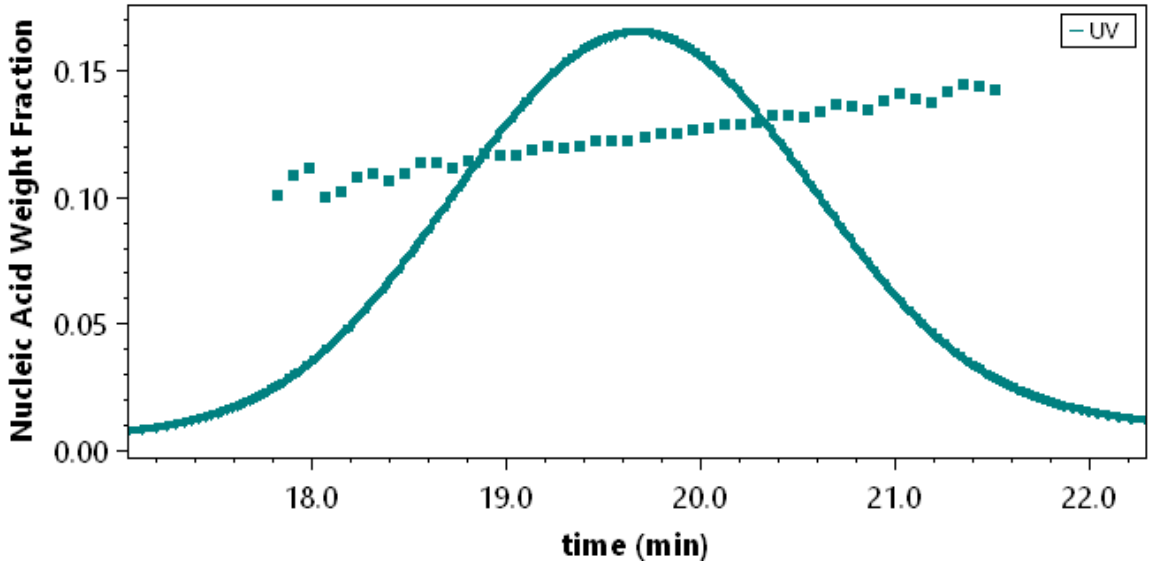
LNP and Nucleic Acid Concentration

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



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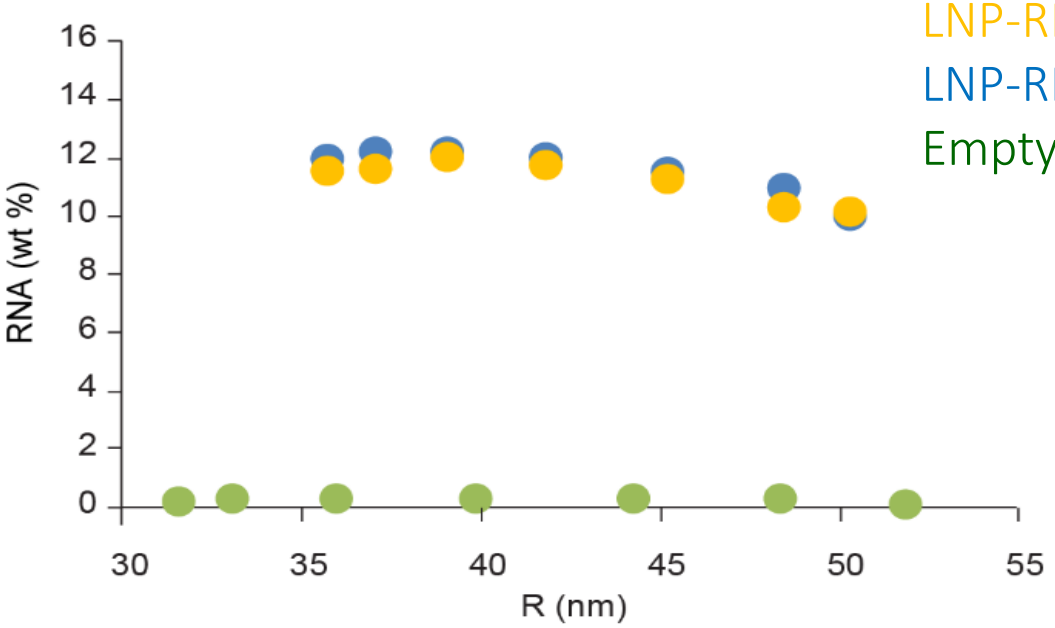
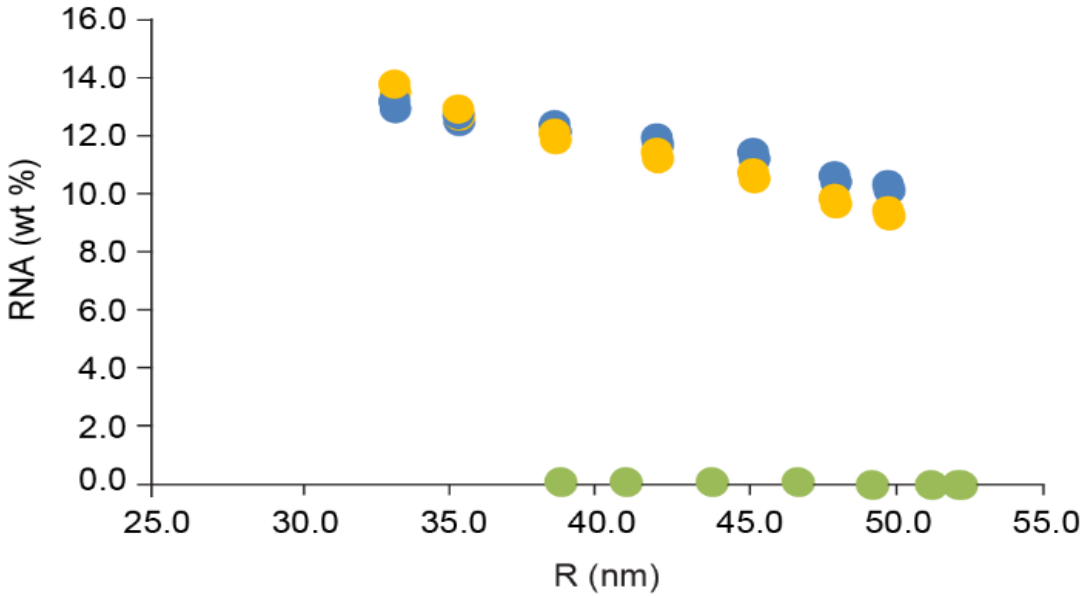
LNP Analysis: Cross-verification

Publication in collaboration with Merck

(https://doi.org/10.1016/j.jchromb.2021.123015)





Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

Enabling online determination of the size-dependent RNA content of lipid nanoparticle-based RNA formulations

Xiujuan Jia [a,*], Yong Liu [a], Angela M. Wagner [b], Michelle Chen [c], Yuejie Zhao [a], Katelyn J. Smith [d], Dan Some [c], Andreas M. Abend [a], Justin Pennington [a]

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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 New
- 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

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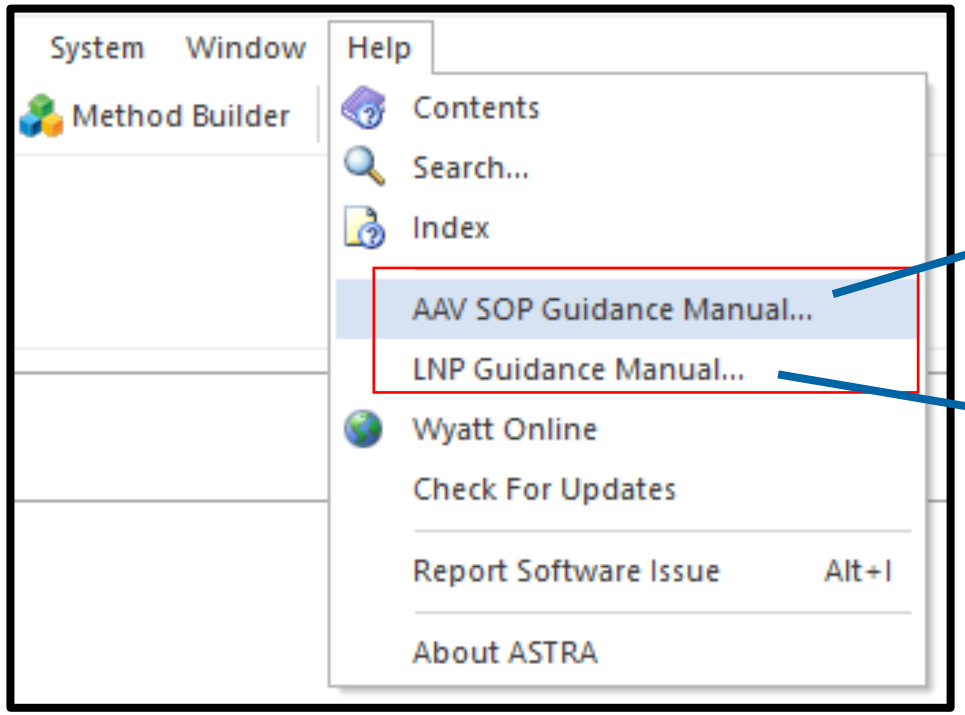
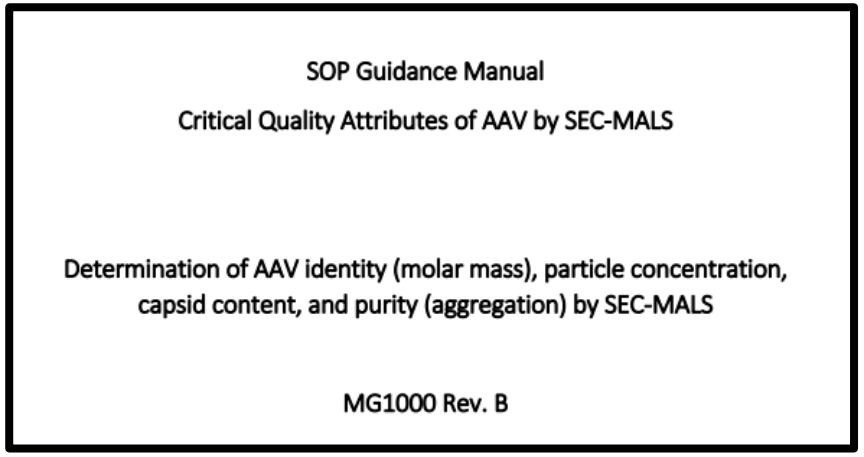
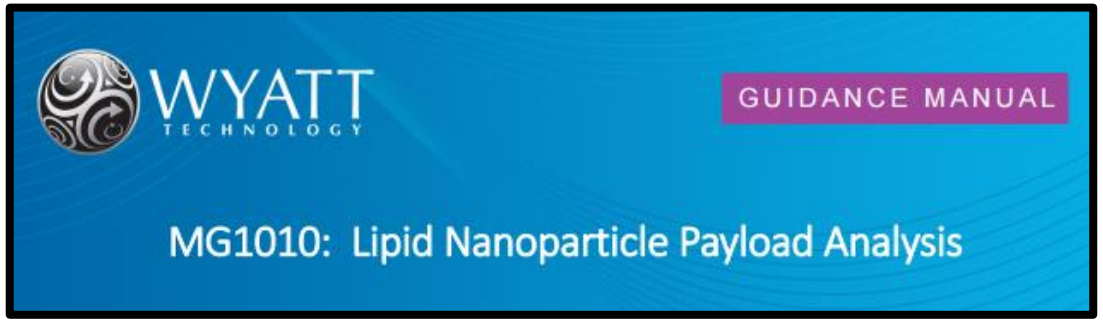
How Difficult is the Implementation?

Guidance manuals are available within the ASTRA software







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Implemented by Industry Leaders in Gene Therapy

- ***DLS Plate Reader, lipid nanoparticles for therapeutic mRNA delivery:*** Nogueira, S.S et al., *ACS Appl Nano Mat.* 3(11), 10634-10645 (2020). https://doi.org/10.1021/acsanm.0c01834
- ***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

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ultraDAWN: MALS for in-process decision-making

- LNP
- mRNA
- Large Viruses
- Therapeutic prot
- AAV
- Polysaccharides

Fundamental, molecular attributes to guide process development and (ultimately) manufacturing.

AKTA ™

TFF

Honomgenizer, Sonicator, etc

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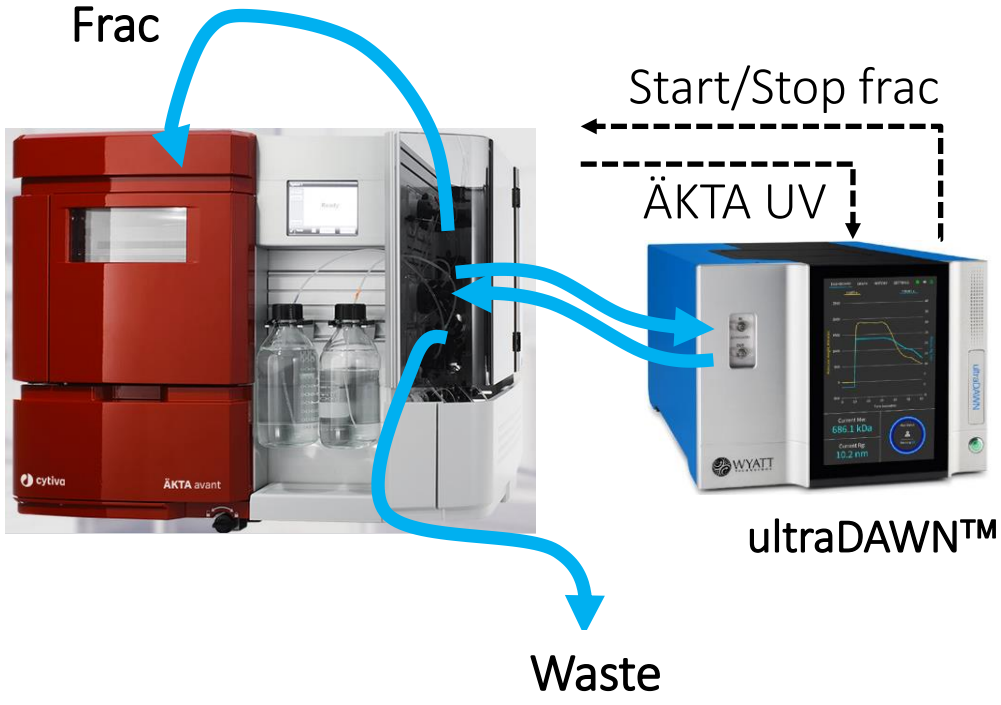
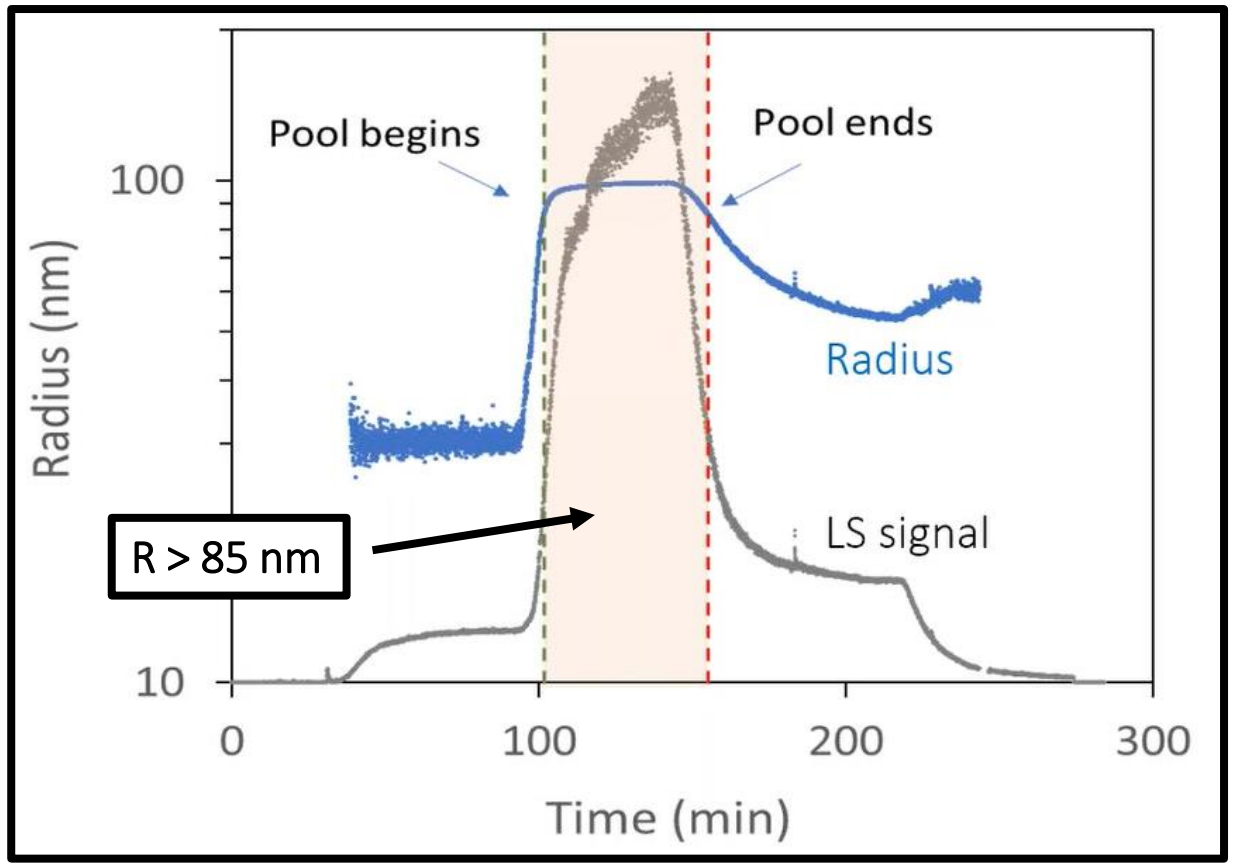
Virus Purification





Also calculates virus titer from light scattering (appropriate for TFF as well!)

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