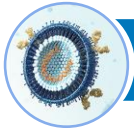
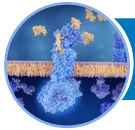
Determination of Critical Quality Attributes for ADC, NanoDDS and Gene Delivery tools from Research to Production

Dr. Abhigyan Sengupta and
Dr. David Golonka

Applications in Focus


Vaccines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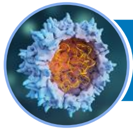
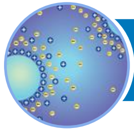
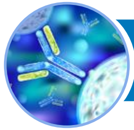
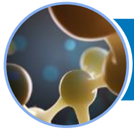
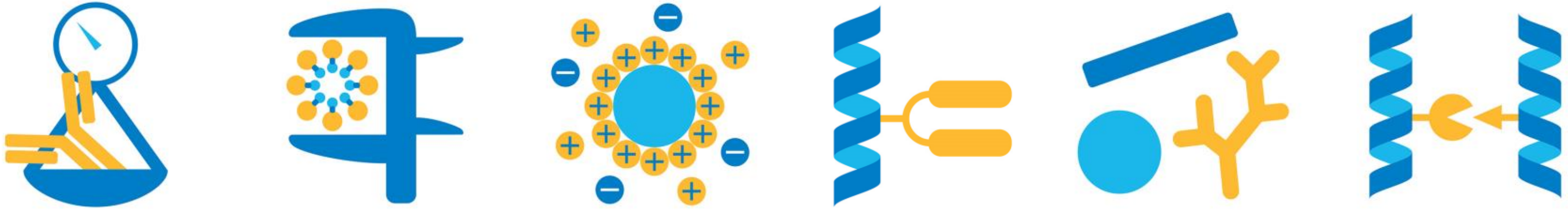

Gene Therapy


Nanoparticle


Biotherapeutics


Polymer

Biophysical Properties of Interest



Molar Mass | Size | Charge | Interaction | Conformation | Conjugation

Steps of Drug Development - Research to Production

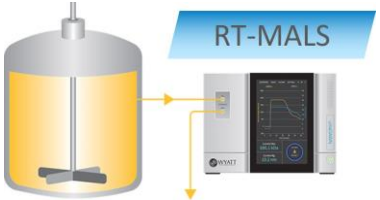
1. Research & Development
2. Scale up
3. Production
4. Quality Control

Wyatt Portfolio

Batch systems

Chromatography

Process



Dynamic Light Scattering (DLS)

Multi-Angle Light Scattering (MALS)

Wyatt Portfolio

Batch systems

Chromatography

Process

Dynamic Light Scattering (DLS)

Multi-Angle Light Scattering (MALS)

DLS

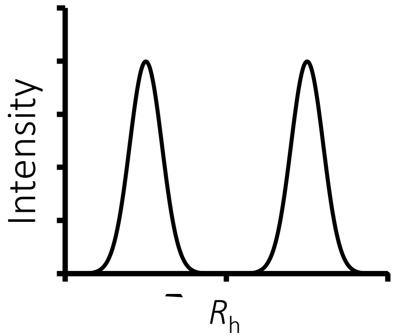
HTP-DLS

Dynamic Light Scattering

Theory

Measurement of hydrodynamic size

Determination of the diffusion coefficient from which the hydrodynamic radius is derived.



What can you measure?

- Size, size distribution and polydispersity
- Aggregate detection
- Protein quality
- Molar mass and particle concentration

NanoStar II

ZetaStar

- Disposable or quartz cuvettes
- Walk-up experiments
- Low sample volume
- Aggregate detection
- Protein quality

Plate Reader III

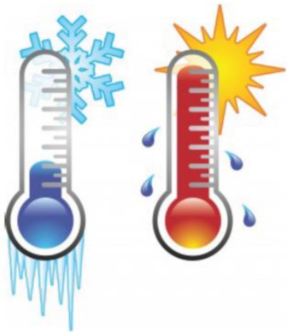
- Wellplates (96, 384, 1536)

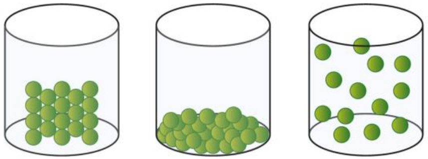
- Formulation Screening
- Colloidal stability (A_2/B_{22}, k_D)
- Thermal stability (T_{onset}, T_m, T_{agg})
- Viscosity
- Compatible with robotic systems

DLS Application Example
Formulation Screening

DLS Application Examples – Formulation Screening

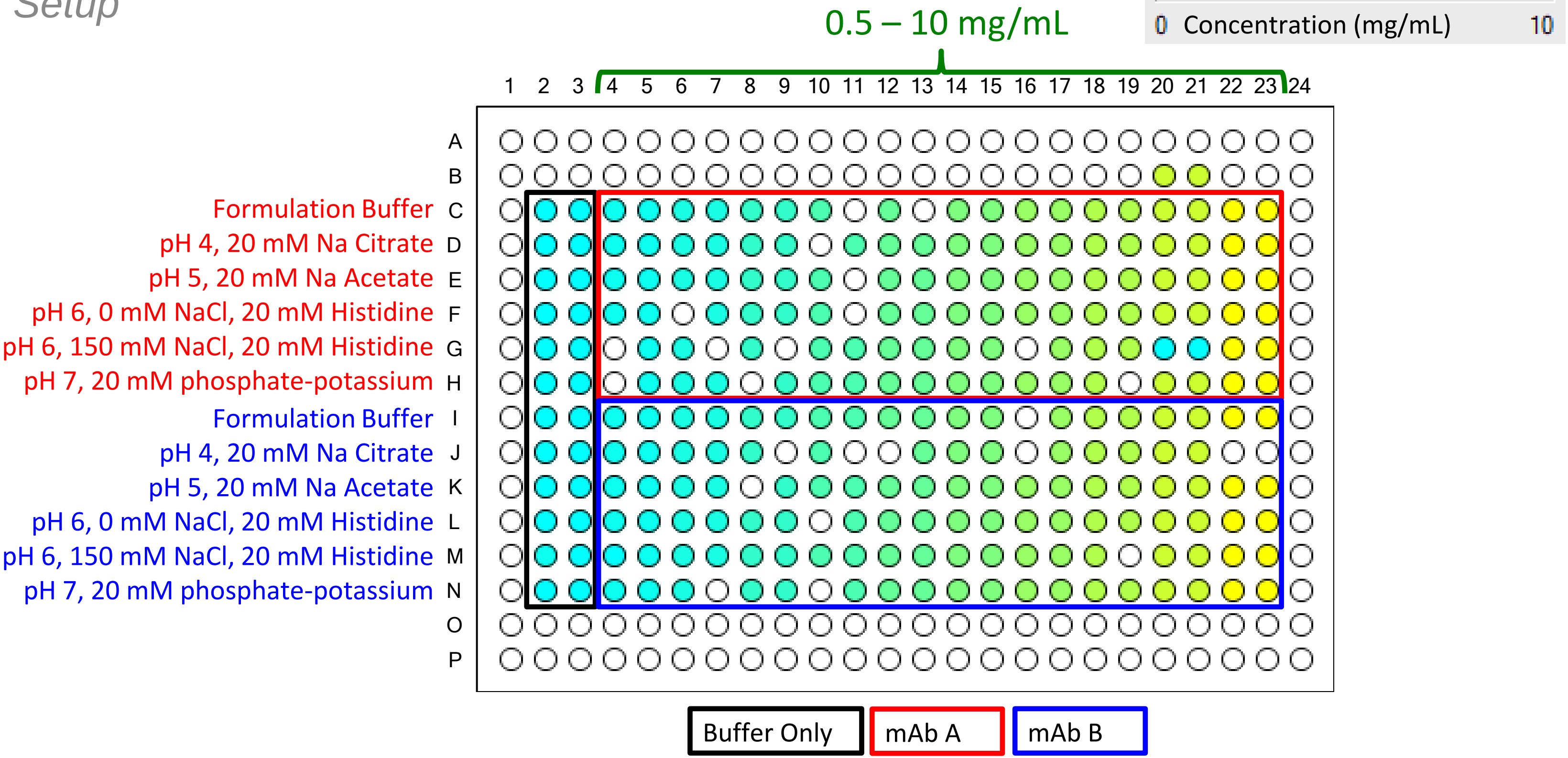
- Background: Antibody drug candidates can be screened by accelerated stability studies
- Experiment: Compare two antibody candidates in multiple formulations
  - Formulation Buffer
  - pH 4, 20 mM Na Citrate
  - pH 5, 20 mM Na Acetate
  - pH 6, 0 mM NaCl, 20 mM Histidine
  - pH 6, 150 mM NaCl, 20 mM Histidine
  - pH 7, 20 mM phosphate - potassium
- Challenges:
  - Limited sample amount
  - Time restrictions





DLS Application Examples – Formulation Screening

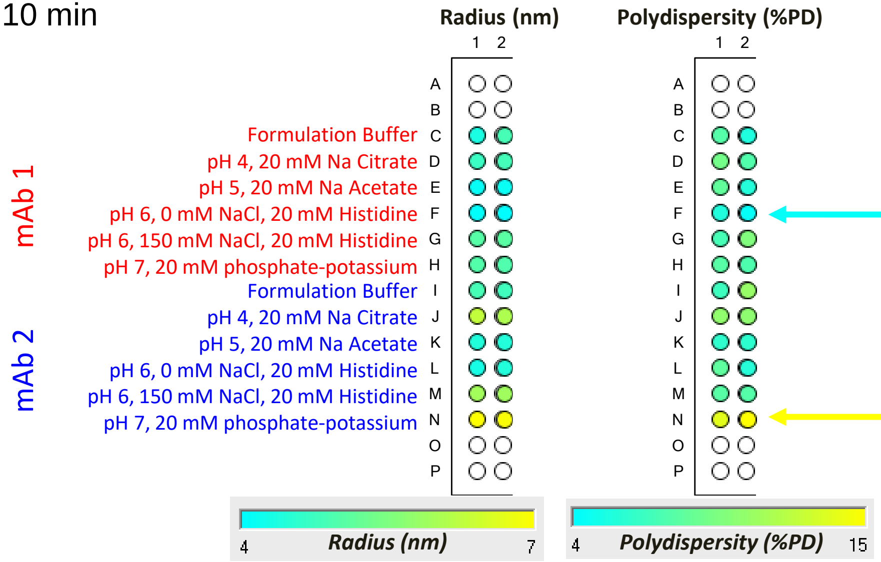
Experiment Setup

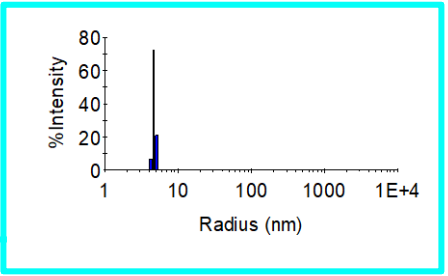


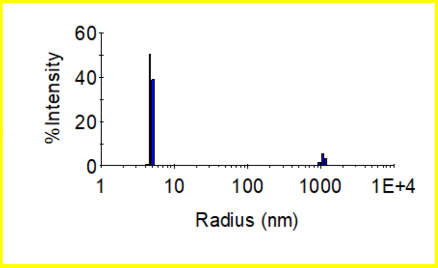
DLS Application Examples – Formulation Screening

Purity and Aggregation

Total Samples: 24
Total Time: 10 min





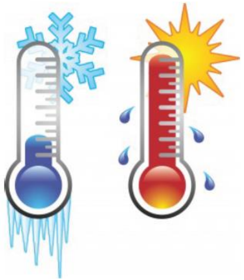


Quickly screen average size and polydispersity with spectral viewer

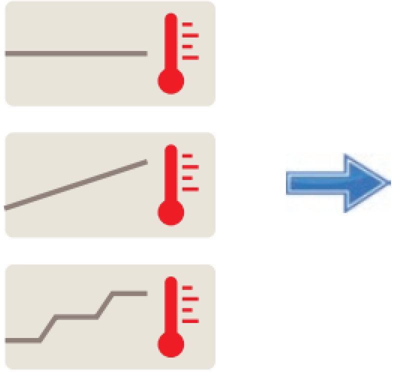
DLS sensitive to low concentration of large aggregates

DLS Application Examples – Formulation Screening

Thermal Stability Study

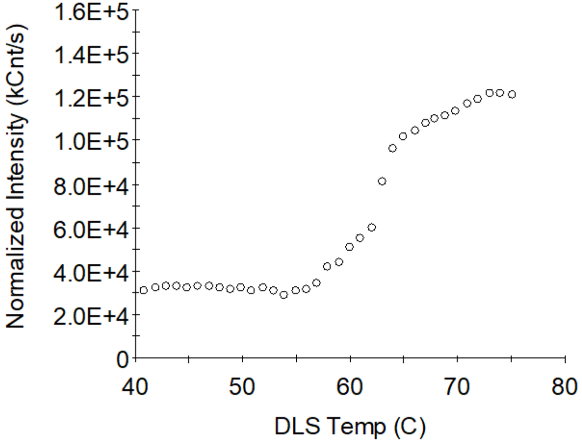


TN7001 Measuring Temperature Transitions Using the DynaPro Plate Reader



1. Select temperature profiles

Combine multiple profiles
for complex protocols.

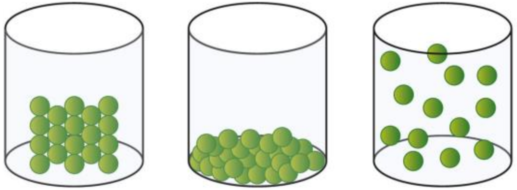


DLS Application Examples – Formulation Screening

Colloidal Stability, k_D and A_2

- Diffusion Interaction Parameter, k_D
  - measured by DLS
  - Measure of protein-protein interactions + some smaller contributions
  - Usually well correlated to A_2

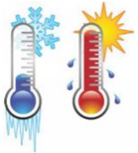
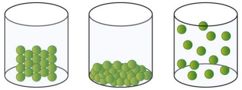
- Second Virial Coefficient A_2
  - Measured by SLS
  - Direct measure of protein-protein interactions



Relate to long-term stability

Traditionally challenging to measure

DLS Application Examples – Formulation Screening

Case Study: Conclusion		Sample Purity 	Thermal Stability 		Colloidal Stability 	**Sum**
		Rh, %PD, Histogram	Temp Ramp	Temp Cycle	kD & A2	
mAb 1	pH 4, 20 mM Na Citrate	☐	☒	☒	☐	**-2**
	pH 5, 20 mM Na Acetate	☑	☒	☒	☑	**0**
	pH 6, 0 mM NaCl, 20 mM Histidine	☑	☐	☐	☑	**2**
	pH 6, 150 mM NaCl, 20 mM Histidine	☐	☐	☐	☐	**0**
	pH 7, 20 mM phosphate - potassium	☐	☐	☐	☐	**0**
mAb 2	pH 4, 20 mM Na Citrate	☐	☐	☐	☐	**0**
	pH 5, 20 mM Na Acetate	☑	☐	☐	☑	**2**
	pH 6, 0 mM NaCl, 20 mM Histidine	☑	☐	☐	☑	**2**
	pH 6, 150 mM NaCl, 20 mM Histidine	☐	☐	☒	☐	**-1**
	pH 7, 20 mM phosphate - potassium	☒	☐	☒	☒	**-3**

ELS Application Example

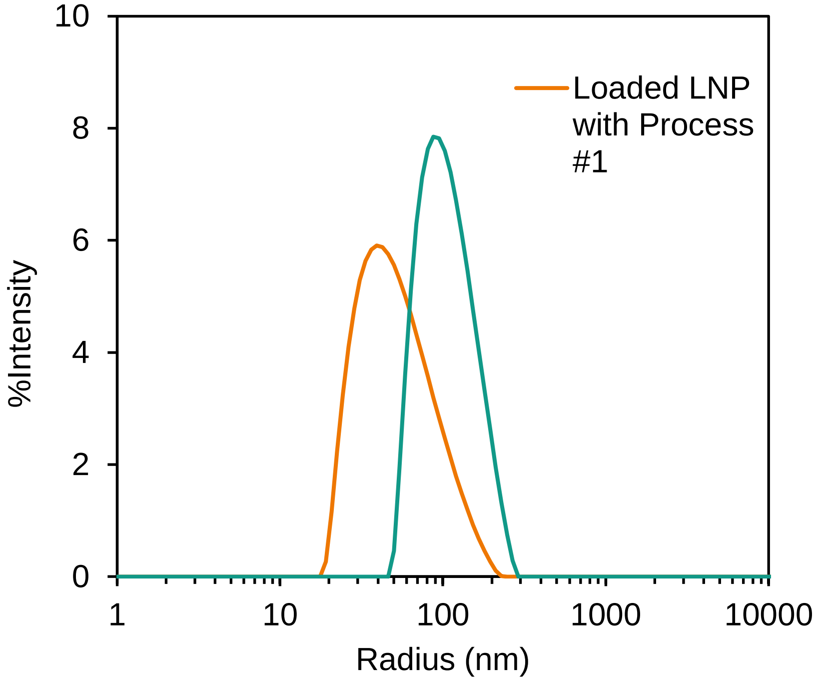
mRNA-LNP development and production

RNA-LNP development and production

- Helper lipid
  (DSPC, DOPE …)
- Ionizable lipid
  (MC3, SM102 …)
- Cholesterol
- Lipid-anchored PEG
  (DMP-PEG)
- mRNA

Mixing device

Assessing LNP preparation processes

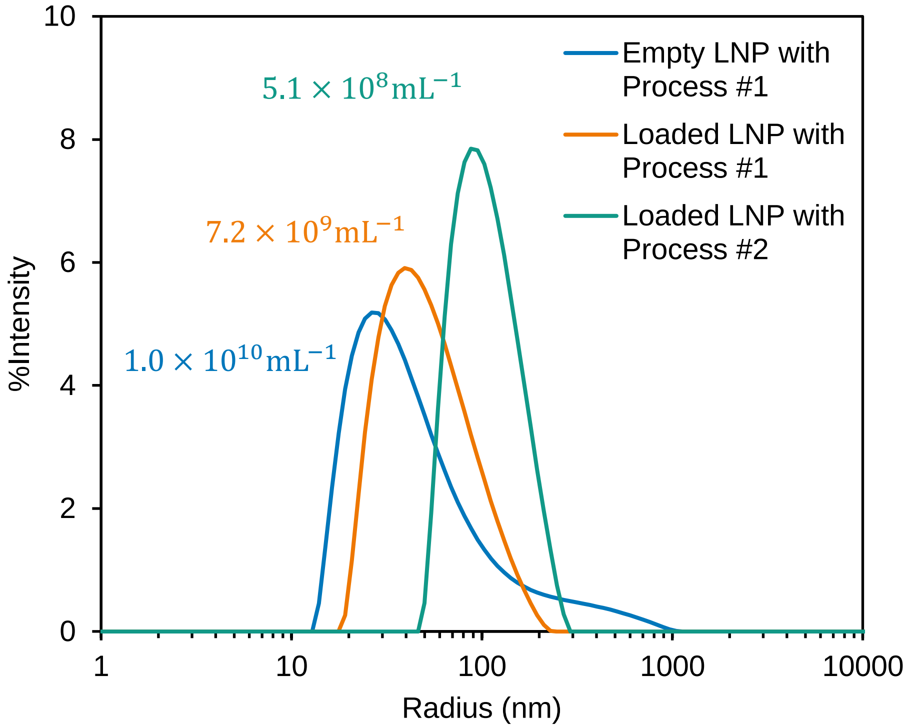


Rapid screening of product and process development

Assess various mixing process or devices

- Process #1: With Microfluidics-mixing
- Process #2: Without Microfluidics-mixing

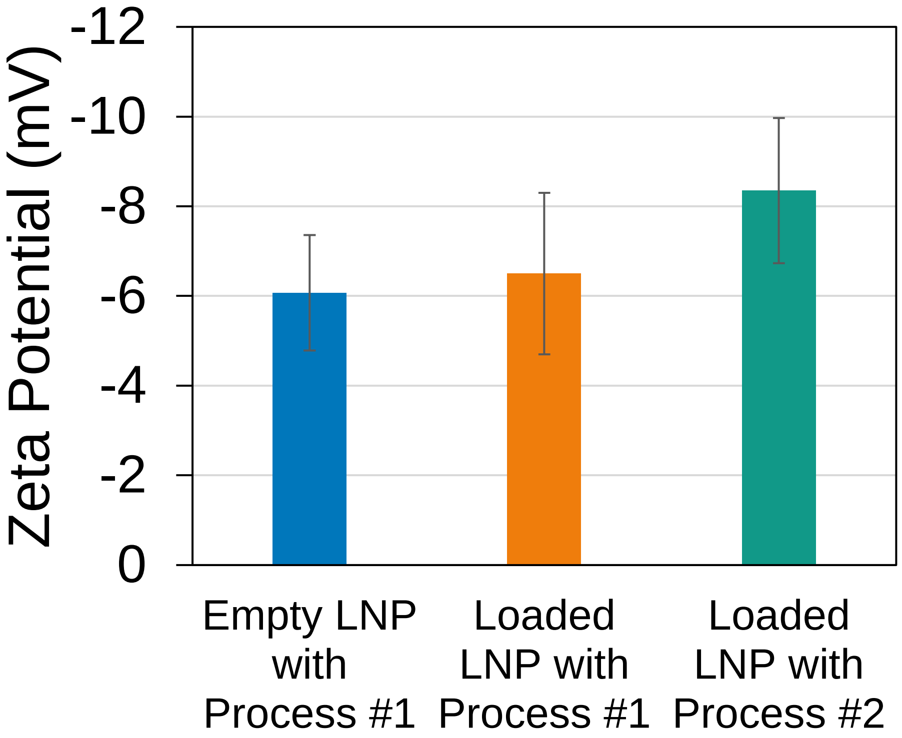
Characterizing size and particle concentration of LNPs



Determine size, polydispersity and size distribution of empty and loaded LNPs

- Empty LNP exhibit noticeable broad distribution
- Loaded LNP median is clearly larger (~ 45 nm)
- Particle concentration is quickly determined ~1×10^{10} particles/mL

Characterizing charge / zeta potential of LNPs



ELS determines charge / zeta potential of LNPs, which is intrinsically related to the ability to enter specific organs and cells and deliver its payload effectively and where needed.

- All samples are negatively charged under test conditions
- Process conditions seem to have no or little influence on LNP surface charge
- Measurements can be performed under physiological conditions

ZetaStar provides rapid answers for scientists to make faster and more informed decisions to improves LNP size consistency

Summary – DLS and ELS

- Fast and reliable measurements
- Identification of aggregates
- Automated measurements of large sample sets
- Powerful technique for proteins from research to production
  - Characterization of protein candidates and formulation screening in research
  - Assessing sample quality during scale up and production at-line
  - Quality control for protein products
- Zeta potential and structural insight for charged particles like LNPs

Wyatt Portfolio Multi-Angle Light Scattering

Waters™ | WYATT TECHNOLOGY

Batch systems

Chromatography

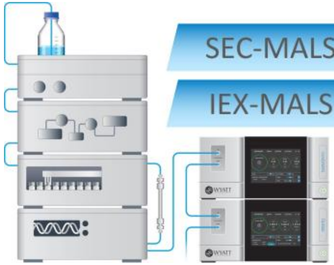
Process

DLS

HTP-DLS

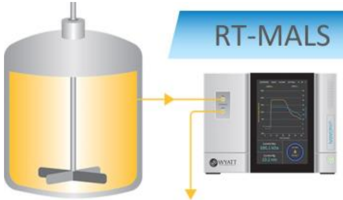
SEC-MALS

IEX-MALS



AF4-MALS

RT-MALS



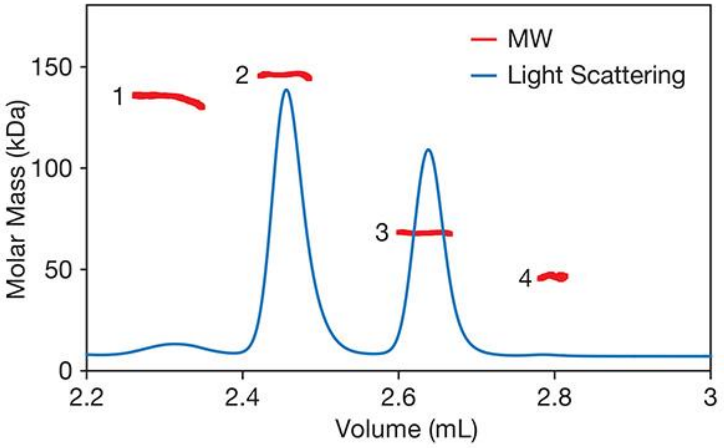
Dynamic Light Scattering (DLS)

Multi-Angle Light Scattering (MALS)

Multi-Angle Light Scattering

Theory

Absolute Molar Mass Distribution



What can you measure?

miniDAWN & DAWN

- Molar masses from 200 Da up to 10 MDa or 1 GDa
- No protein standards needed (no assumptions)

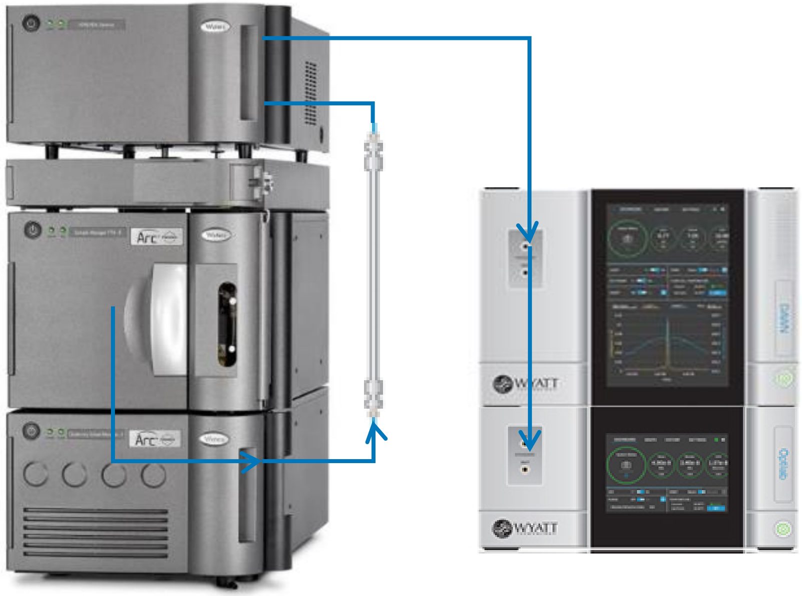
- Identification & quality
- Purification & aggregates
- Oligomerization state & complexes
- Conjugate analysis (glycol-proteins, PEGylated proteins, antibody drug conjugates, AAV)
- 21 CFR part 11

microDAWN

- for UHPLC and µSEC

SEC-MALS - Hardware

- ☑ Quantitative multi-wavelength UV
- ☑ Steady pressure during injection
- ☑ Pulseless pump



SEC-MALS interfaces to standard U/HPLC-SEC instrumentation

SEC-MALS Application Example
Protein Conjugate Analysis

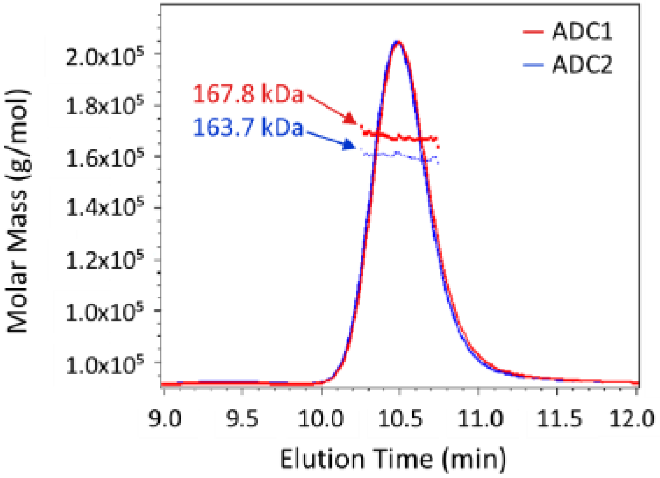
MALS Application Examples – Protein Conjugate Analysis

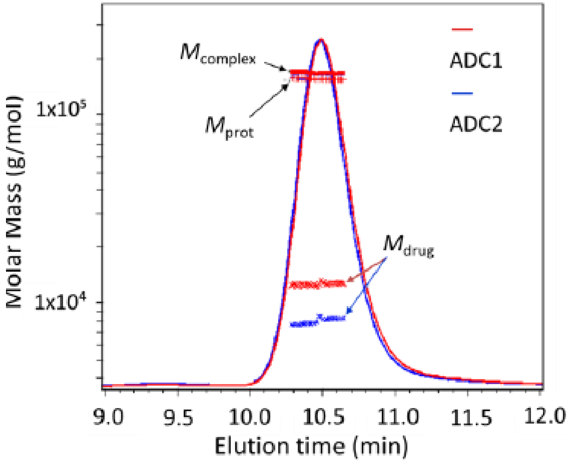
- Protein conjugate analysis uses MALS + UV + RI and measures:
  - Amount component A - Amount component B
  - Molar mass component A - Molar mass component B
  - Molar mass of the complex

- Protein conjugate analysis is used for:
  - Glycosylated proteins
  - PEGylated proteins
  - Membrane protein
  - Protein-protein-complex
  - Antibody-Drug-Ratios

Antibody-Drug-Ratio by Protein Conjugate

- Requirements
  - UV extinction coefficients and dn/dc values for antibody and drug need to be known
  - Antibody and drug must differ either in the UV extinction coefficient or in the dn/dc value
  - The modifier mass should be at least 5 % of the total molar mass
- Molar mass of a single drug molecule is 1.25 kDa





	M_w (kDa)			DAR
	Complex	Antibody	Drug	
ADC1	167.8	155.2	12.6	10.1
ADC2	163.7	155.6	8.1	6.5

SEC-MALS Application Example
Characterizing AAV

Multi-angle light scattering (MALS) solution for AAV

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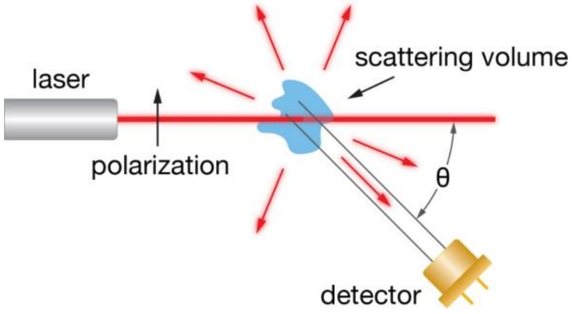
SEC-MALS

For AAV production and QC
(4 CQAs in a single assay)

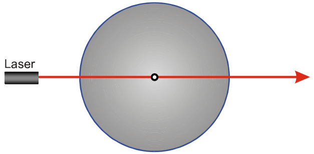
- ✓ Identity (molar mass)
- ✓ Particle concentration
- ✓ Capsid content
- ✓ Purity (aggregation)



$$I_{scattered} \propto M \cdot c \cdot \left(\frac{dn}{dc}\right)^2$$

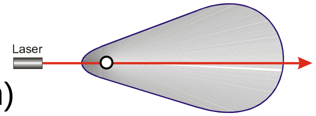
Isotropic scattering

Small particles (R < 10 nm)



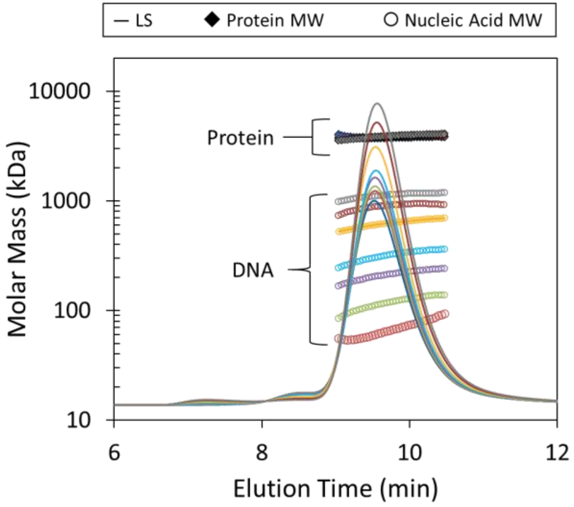
Anisotropic scattering

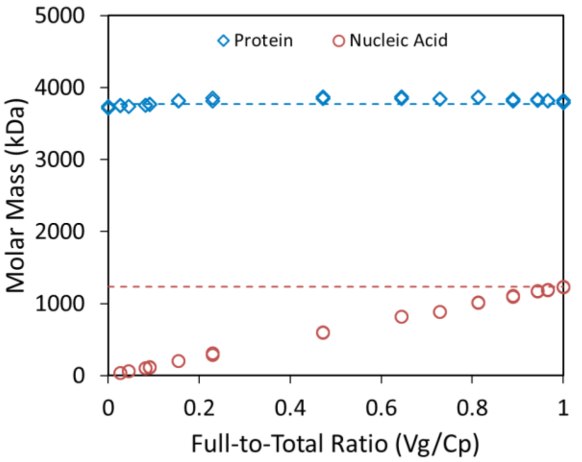
large particles (R > 10 nm)



CQA #1: Identity (Molar Mass)

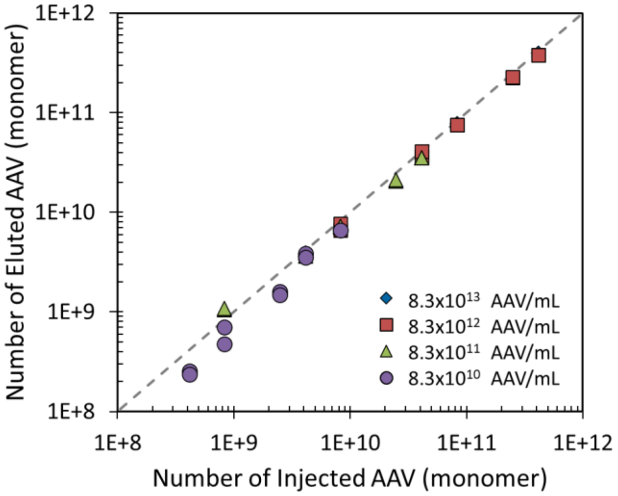
- MALS directly measures molar mass of each AAV sample to identify
  - Oligomeric state (monomer, dimer, higher order oligomer)
  - Capsid content (empty, full, or mixture)
  - Presence of self-complementary DNA or other nonideal DNA loading

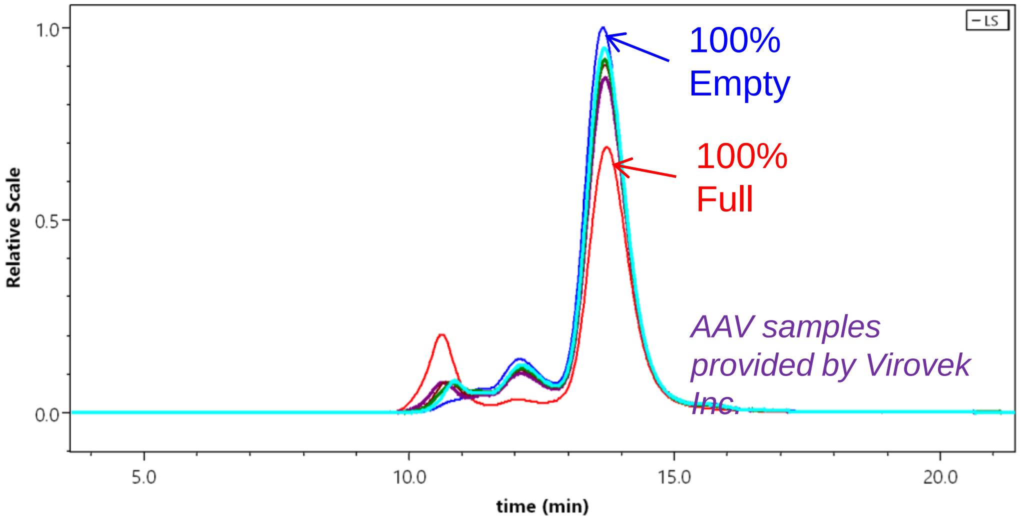




CQA #2: Particle concentration

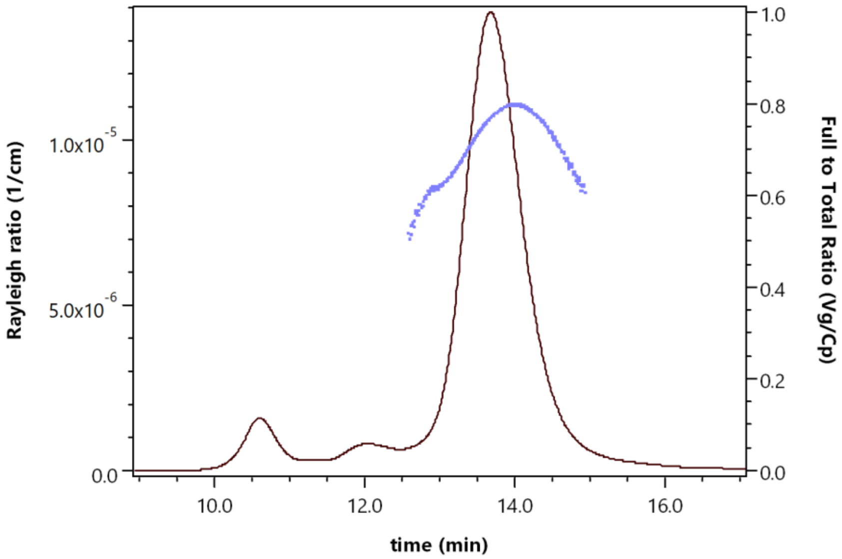
- SEC-MALS combined with UV and RI provides total, full, and empty AAV concentration.
  - Well-tested method that has been applied to serotypes 1, 2, 5, 6, 8, 9, 10
  - Complementary to AUC, ddPCR, ELISA, and other methods
  - Only assumption is 100% mass recovery

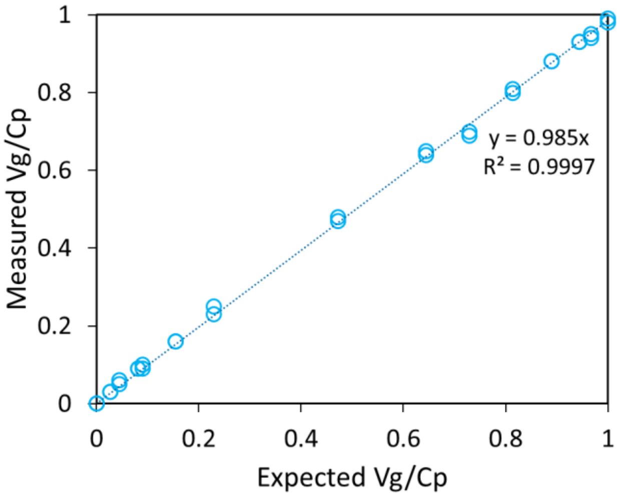




CQA #3: Capsid content (Vg/Cp or full/total)

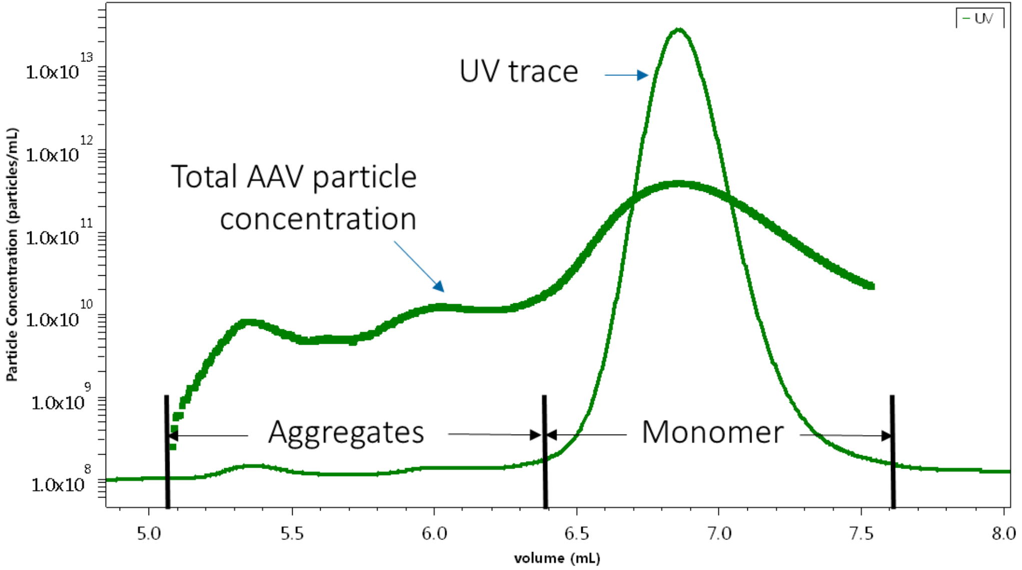
- Simultaneous with CQA 1, SEC-UV-MALS-RI provides capsid content (CQA 2).
  - Excellent correlation with AUC data to provide orthogonal, complementary information
  - Measured capsid content is linear and extends across a wide dynamic range





CQA #4: Purity (quantify aggregation)

- Particle concentrations are calculated for each data slice to quantify percent monomer and aggregates.
- SEC column may remove large aggregates and FFF is a better alternative. Please read Wyatt AN2004.



	Concentration [particles/mL]	Content [%]
Monomer	$4.22x10^{13}$	94.6
Aggregates	$0.24x10^{13}$	5.4
Total	$4.46x10^{13}$	100

Summary – SEC-MALS

- Measurements are absolute
  - No protein standards needed (no assumptions)
- 200 Da up to 1 GDa
- Automated measurements of large sample sets
- Powerful technique for proteins and AAV from research to production
  - Identify proteins, oligomeric states and purity of samples
  - Analyze modified proteins, protein-DNA-conjugates or protein-protein-complex
  - In-depth quality control for protein and AAV products

Wyatt Portfolio

Batch systems

Chromatography

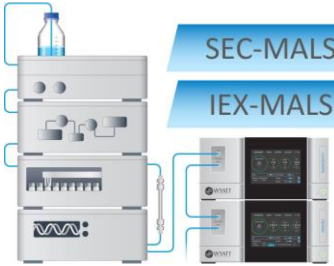
Process

DLS

HTP-DLS

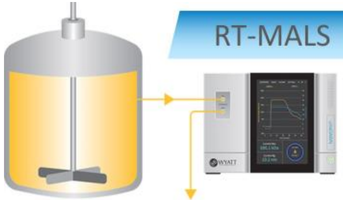
SEC-MALS

IEX-MALS



AF4-MALS

RT-MALS



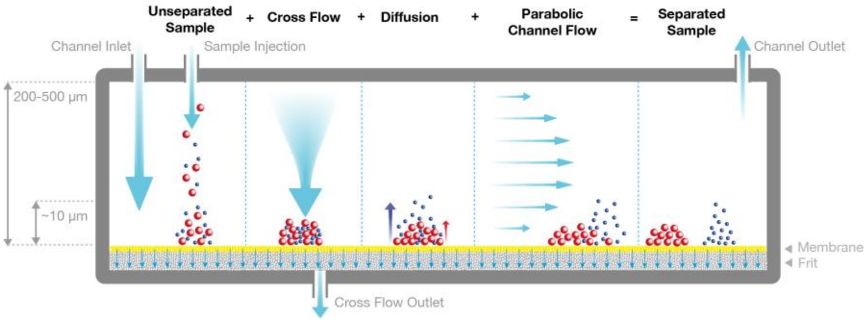
Dynamic Light Scattering (DLS)

Multi-Angle Light Scattering (MALS)

Field-Flow Fractionation

Theory

Orthogonal Separation Technique



- Laminar channel flow transports the sample
- Cross flow through membrane retains sample
- Separation based on cross flow / channel flow ratio
- Separation depends upon diffusion speed, hydrodynamic radius

What can you measure?

Eclipse FFF System

- Orthogonal technique to SEC
- Separation of sticky proteins, larger complexes, viruses, particles
- 21 CFR part 11

Eclipse FFF-MALS System

HPLC

Pump

Autosampler

UV detector

Fraction collector

DAWN MALS detector

Measures molar mass and radius R_g

R_h with integrated DLS detector

Optilab dRI detector

Measures concentration

FFF Channel

Separation in a flow field

No stationary phase

Eclipse FFF control module

Regulates the flows for the separation

Monitors all pressures and flows

Viral vector characterization by LS related techniques

	Challenges
	SEC-MALS
AAV	Good method for multiple CQAs May remove large aggregates
AD	High risk of low recovery and insufficient resolution, not a good fit
LV	

Viral vector characterization by LS related techniques

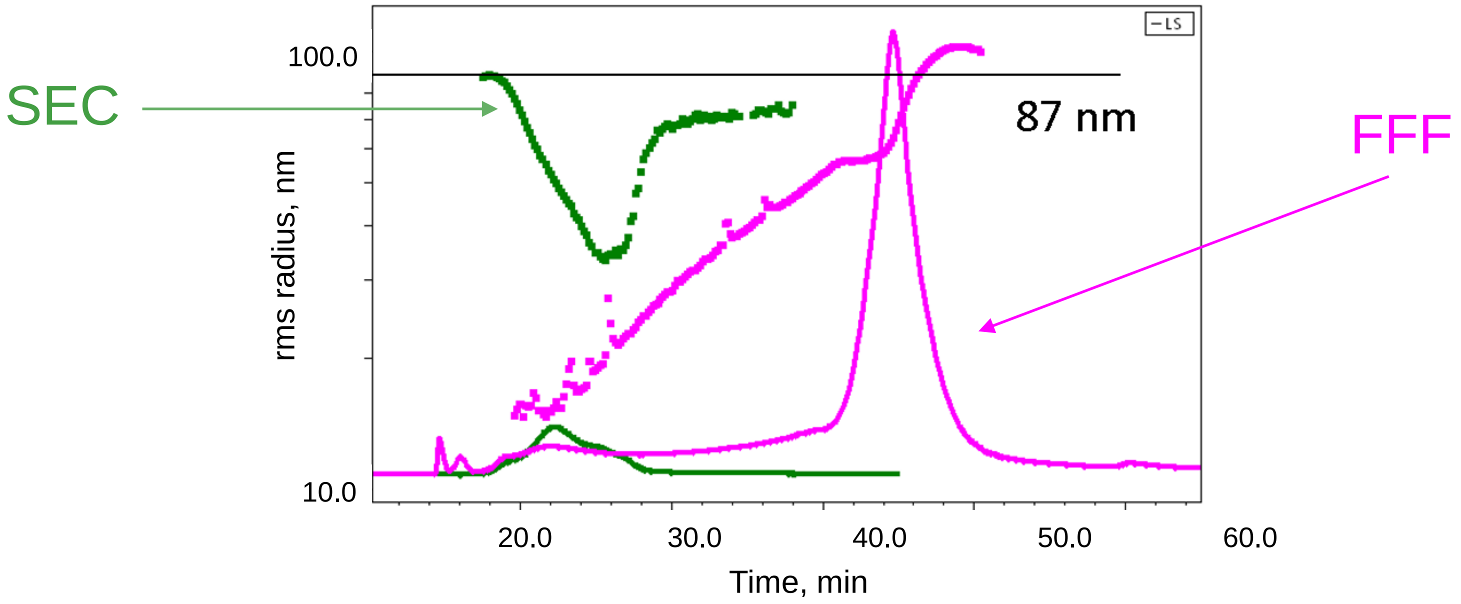
<table>
<tr><th></th><th colspan="2">Challenges</th></tr>
<tr><th></th><th>SEC-MALS</th><th>DLS</th></tr>
<tr><td>AAV</td><td>Good method for multiple CQAs
May remove large aggregates</td><td rowspan="3">Good screening method
Low resolution and low quantitation for in-depth characterization</td></tr>
<tr><td>AD</td><td rowspan="2">High risk of low recovery and insufficient resolution, not a good fit</td></tr>
<tr><td>LV</td></tr>
</table>

Viral vector characterization by LS related techniques

<table>
<tr><th></th><th colspan="2">Challenges</th><th>Solutions</th></tr>
<tr><th></th><th>SEC-MALS</th><th>DLS</th><th>FFF-MALS</th></tr>
<tr><td>AAV</td><td>Good method for multiple CQAs
May remove large aggregates</td><td rowspan="3">Good screening method
Low resolution and low quantitation for in-depth characterization</td><td>Orthogonal method for AAV aggregate quantification</td></tr>
<tr><td>AD</td><td rowspan="2">High risk of low recovery and insufficient resolution, not a good fit</td><td rowspan="2">Preferred method for high resolution separation, proper quantification of size/concentration/aggregation, and in-depth characterization (empty vs. full)</td></tr>
<tr><td>LV</td></tr>
</table>

Should we choose SEC or FFF

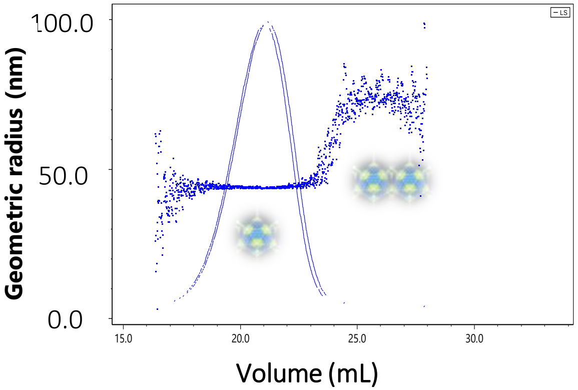
- SEC: poor mass recovery, compromised separation resolution, filters off larger components
- FFF: good mass recovery, high resolution, no sample degradation



FFF-MALS Application Example
Characterizing Viral Vectors

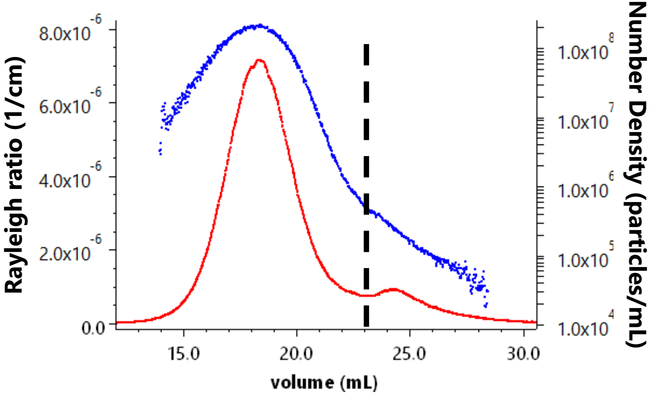
Adenovirus characterized by FFF-MALS

Detect aggregation with high sensitivity and resolution



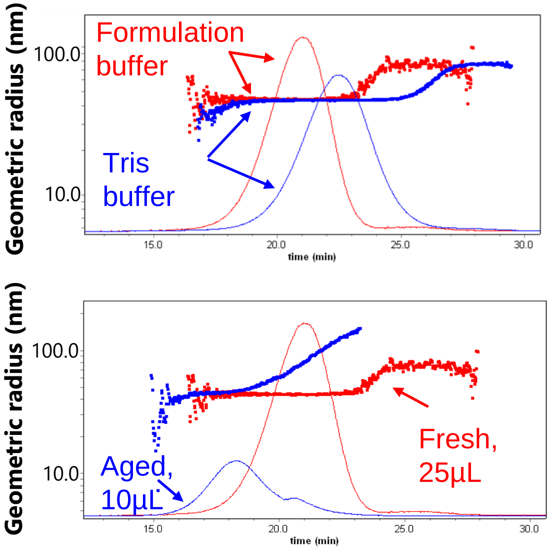
The minute amount of dimer cannot be detected by DLS.

Quantify physical titer and estimate empty to full ratio

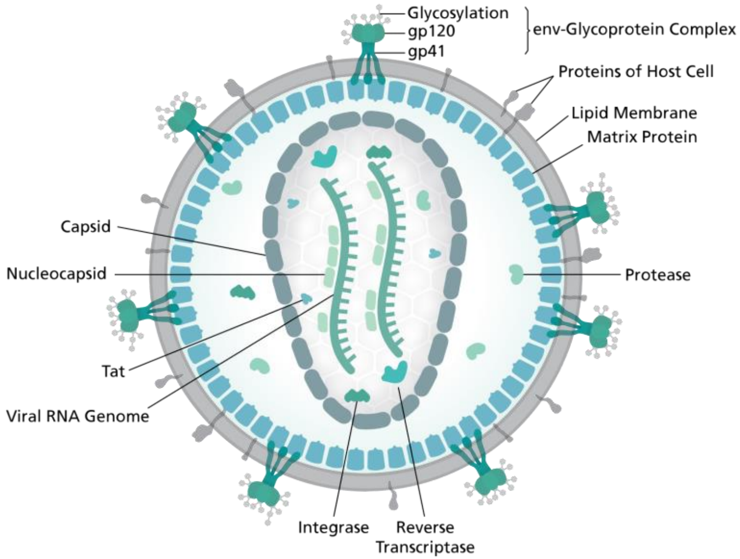


Monomer, mL^{-1}	6.5×10^{10}
Aggregates, mL^{-1}	8.8×10^{7}

Study formulation and stability



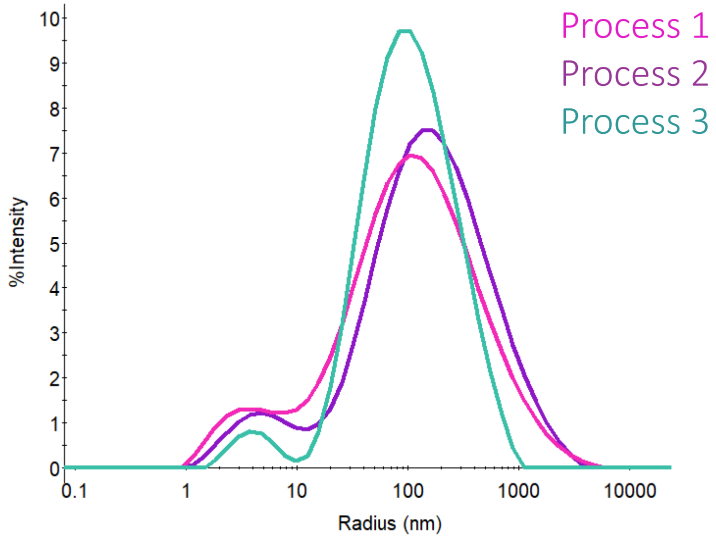
Lentivirus (LV)

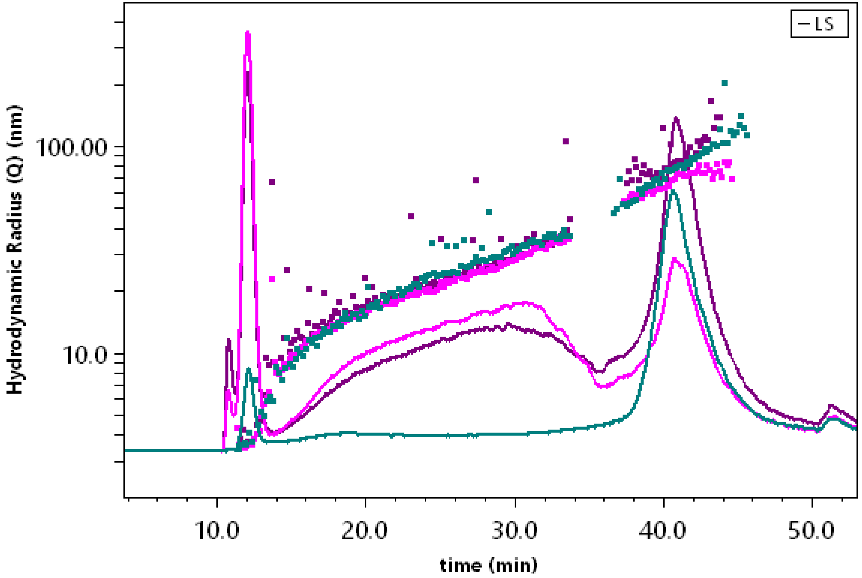


- A subtype of gamma retrovirus
- Used in both gene and cell therapies
- Genetic payload is low compared to the total capsid mass
- More complex than AAV and AD due to its large size and size heterogeneity
- Lack of characterization tools
- Low recovery and no separation by SEC

LV: understand the manufacturing parameters

- LVs from 3 slightly different process conditions. DLS to screen the samples first.
- FFF-MALS provides good resolution for LV and impurities, thus enables better understanding and control of manufacturing parameters and conditions.





FFF-MALS Application Example

Characterizing LNP

ASTRA LNP Analysis

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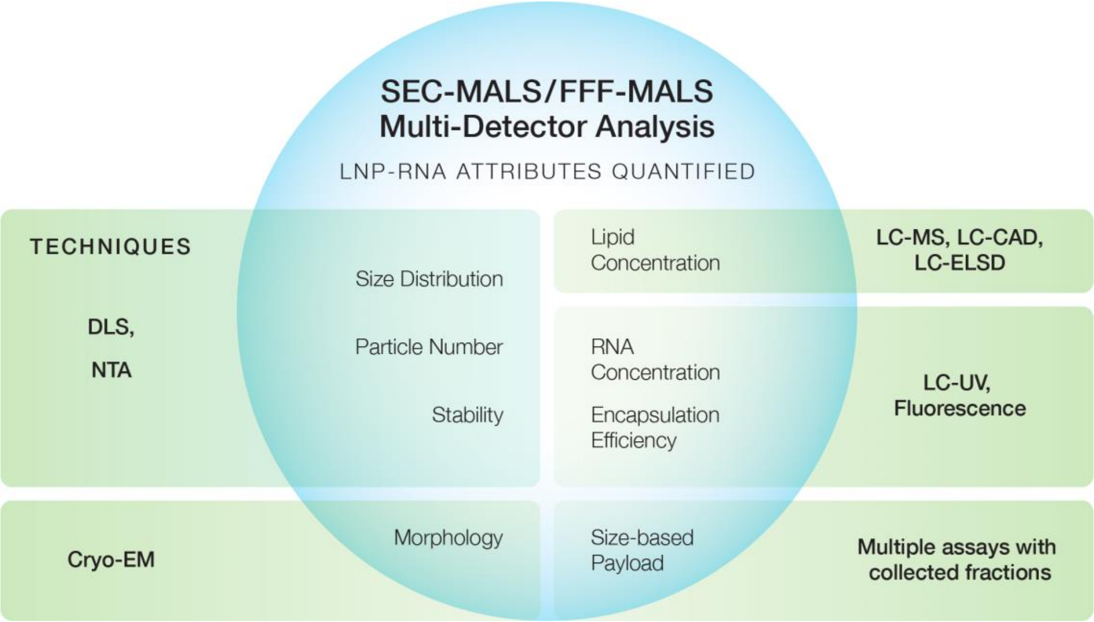
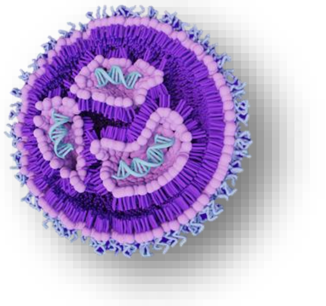
- SEC-MALS and FFF-MALS provide comprehensive LNP characterization and multi-attribute quantitation.





MALS-DLS-UV-dRI following SEC or FFF

SEC or FFF provides size-based separation

- Online detectors – MALS, DLS, UV, and dRI – provide data for biophysical analysis to quantify attributes

DAWN® (MALS-QELS)

- ✓ Molar mass
- ✓ Radius (R_g, R_h), shape
- ✓ Particle concentration

UV (260 nm)

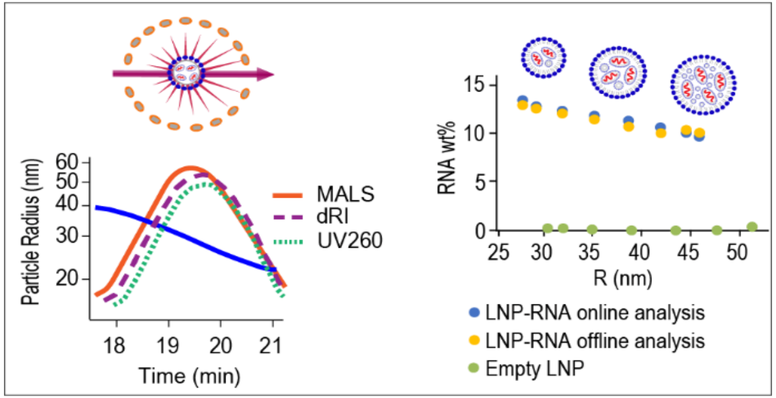
- ✓ Concentration
- ✓ Composition

Optilab ® (dRI)

- ✓ Concentration
- ✓ Composition

LNP-siRNA by SEC/FFF-MALS-UV-dRI

Size-based RNA payload



X. Jia, et al, "Enabling online determination of the size-dependent RNA content of lipid nanoparticle-based RNA formulations", submitted.

Offline analysis:

Total mass: use dRI signal from SEC

RNA mass:

1) Fractions collected;

2) LNP disrupted by 2% Triton X-100 and diluted;

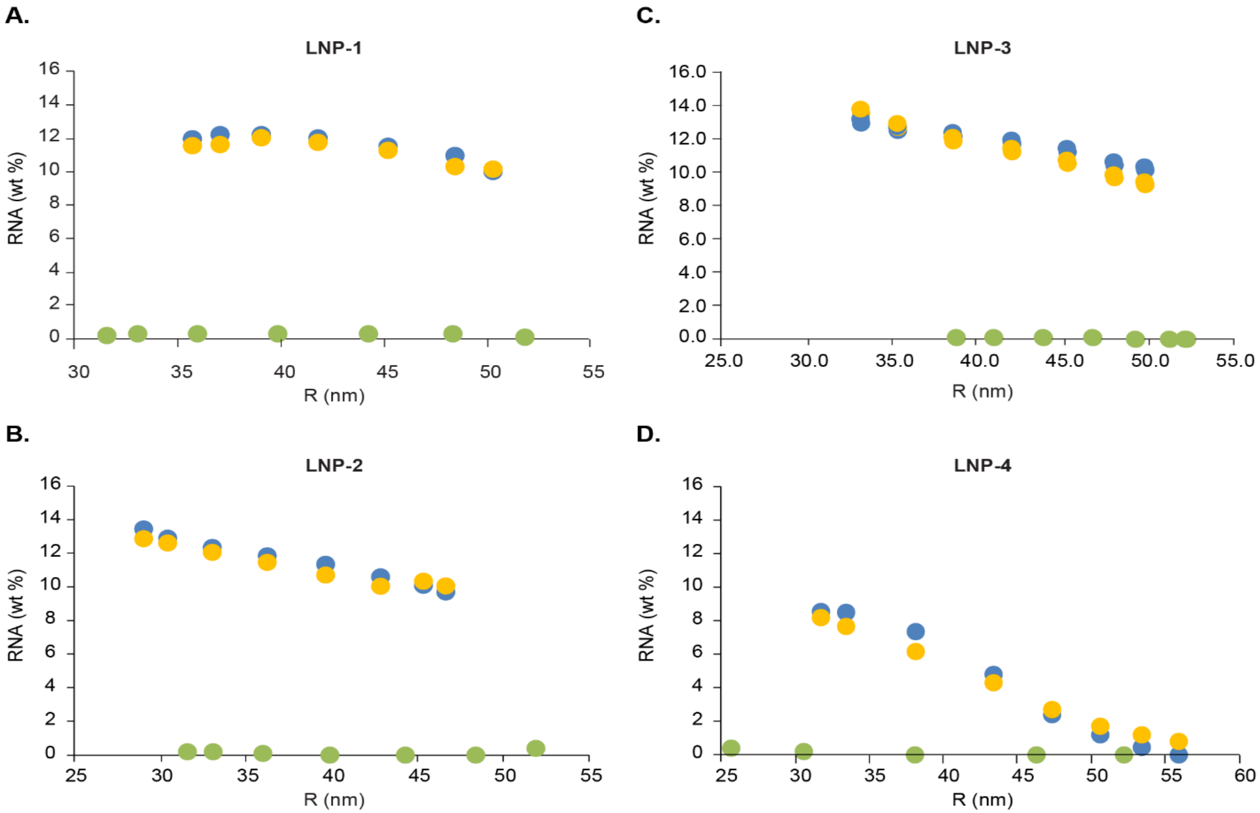
3) Analyzed by UHP-RPC-UV(260 nm) for RNA quantitation.

New

Online analysis (Wyatt LNP method):

Use data from ***online multiple-detector*** and ***Wyatt LNP Analysis*** to calculate lipid and RNA concentration of each eluting slice

LNP-siRNA by SEC/FFF-MALS-UV-dRI



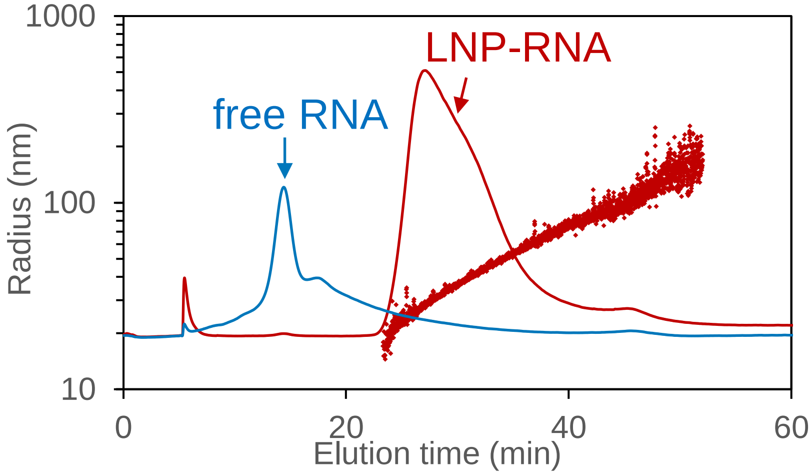
LNP-RNA, offline

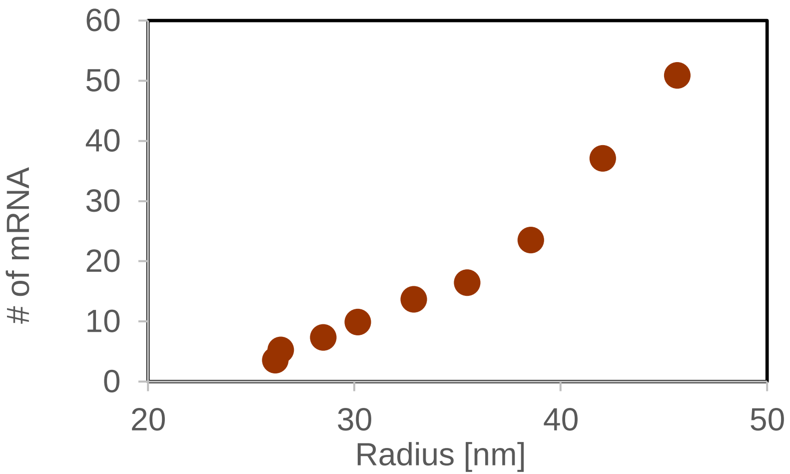
LNP-RNA, online

Empty LNP, online

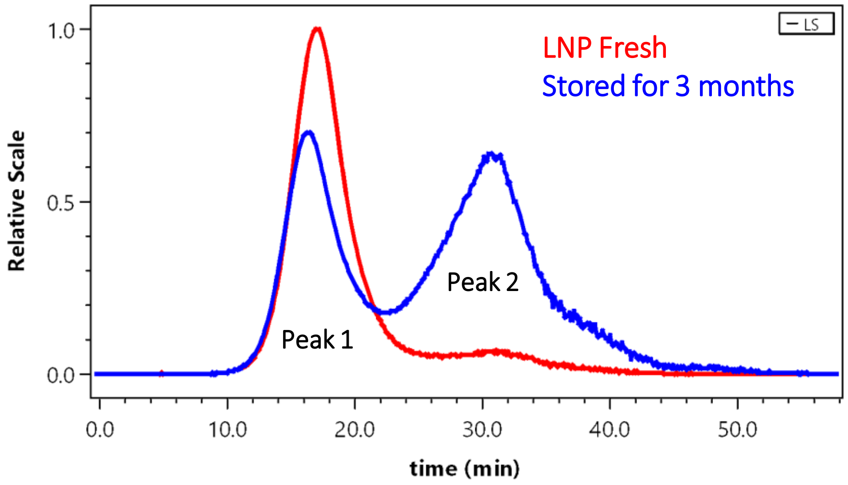
Lipid nanoparticle (LNP) encapsulated nucleic acids

- LNP is emerging as a platform to formulate various gene drug substance.
- Use FFF-MALS-UV-dRI to quantify MW and size distribution, RNA encapsulation efficiency, payload distribution, and structural information.

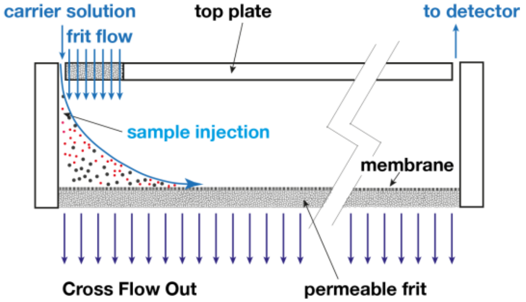




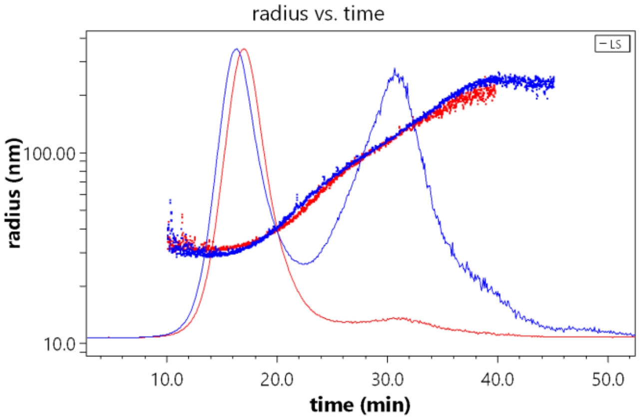
RNA-LNP Long-Term Storage Stability



Dispersion Inlet channel



	Peak 1 rms radius (nm)	Peak 1 particle concentration (mL^{-1})	Peak 2 particle concentration (mL^{-1})
Fresh sample	26.4	5.55 e12	2.24 e09
Stored sample	24.7	5.85 e12	2.01 e10



Summary – FFF-MALS

- Orthogonal technique to SEC
- Less shear effects
- Suitable for sticky proteins, larger complexes, viruses, particles
- Retains aggregates for analysis sometimes removed by SEC
- Higher flexibility compared to SEC
  - Adjust separation range to different products without change of separation channel
  - Vast range for buffer compatibility

Wyatt Protfolio Real-Time Multi-Angle Light Scattering

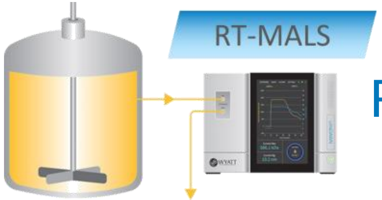
Batch systems

Chromatography

Process

Dynamic Light Scattering (DLS)

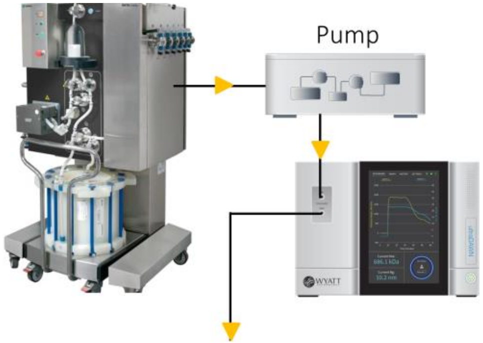
Multi-Angle Light Scattering (MALS)



Real-Time Multi-Angle Light Scattering

- Real-time measurements of molar mass, radius and particle concentration
- Triggers for fraction collection and process control

In-line	On-line
• Lab- and pilot scale FPLC, UF/DF • Short response time • Flow rates up to 200 mL/min (depends on allowed backpressure) • Soon: 800-1000 mL/min	• For high flow rates or static processes • Reactor vessel, homogenizer, fill-finish, chromatography, UF/DF



Multi-angle light scattering in process analytical technology

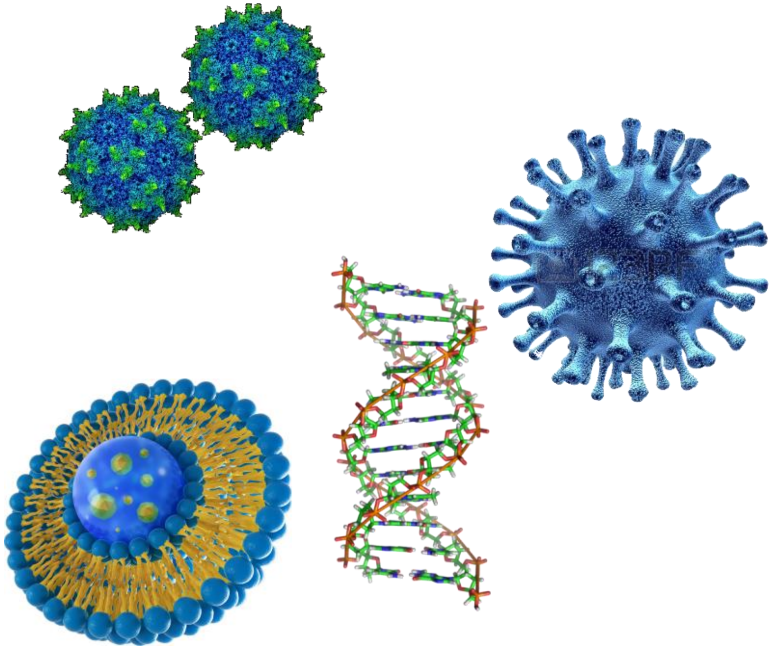
- **Traditional PAT measures *process or indirect parameters***
  - Temperature, pressure, flow rate, feedstock (e.g. via Raman spectroscopy)
  - UV signal (concentration of… something)
  - Extensive modeling required
- **Real-time multi-angle light scattering (RT-MALS) measures *direct product attributes***
  - Molar mass: 10^3 to 10^9 g/mol
  - Size: radius from 10 to 250 nm
  - Particle concentration (range depends on size)
  - Molar mass and size inform on aggregation
  - Payload: AAV full:total ratio (Vg/Cp)

PAT:

*"a system for …analyzing, and controlling manufacturing through **timely** measurements (i.e., during processing) of critical quality and performance attributes of …materials and processes, with the goal of ensuring final product quality." (FDA)*

Gene vector attributes monitored in DSP by RT-MALS

	M_w	R_g	R	Part. Conc.	other
Proteins, nucleic acids	✓	✓			Aggregate content
AAVs, small VLPs	✓		✓	✓	Vg/Cp, aggregation
Viruses, viral vectors			✓	✓	
Lipid nanoparticles			✓	✓	



RT-MALS Application Example

Characterizing AAV

AAV (and other small viral vectors): Vg/Cp, titer

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Attributes:

- Vg/Cp
- N_{capsid}
- N_{genome}
- MW_{capsid}
- MW_{genome}
- R_g

Waters™ | WYATT TECHNOLOGY APPLICATION NOTE

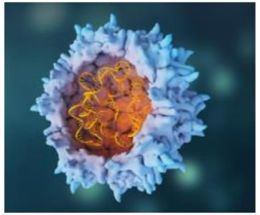
AN8008: Real-time monitoring and control of AAV chromatographic enrichment with RT-MALS

F. Michael Haller, Ph.D., Lonza Biologics and Dan Some, Ph.D. Waters | Wyatt Technology

Summary

Downstream purification and enrichment of full AAV capsids for gene therapy products is typically accomplished by ion-exchange chromatography (IEX). While the ratio of UV260 to UV280 absorption is often used during IEX as a proxy for the full:total capsid ratio Vg/Cp, this method does not afford process developers deep insight into accurate empty and full titers, or the presence of product-related impurities. Only when detailed offline analysis of fractions is complete does that information make its way back to process developers or manufacturing teams.

Real-time multi-angle light scattering (RT-MALS) operates in-line with bench-scale FPLC systems to monitor and quantify critical quality attributes (CQA) and identify impurities. RT-MALS provides immediate results for pool chloride or iodixanol density gradient, which has the benefit of being serotype-independent. However, ultra-centrifugation is not suitable for capsid enrichment at commercial/GMP scales where chromatographic separations are preferred.

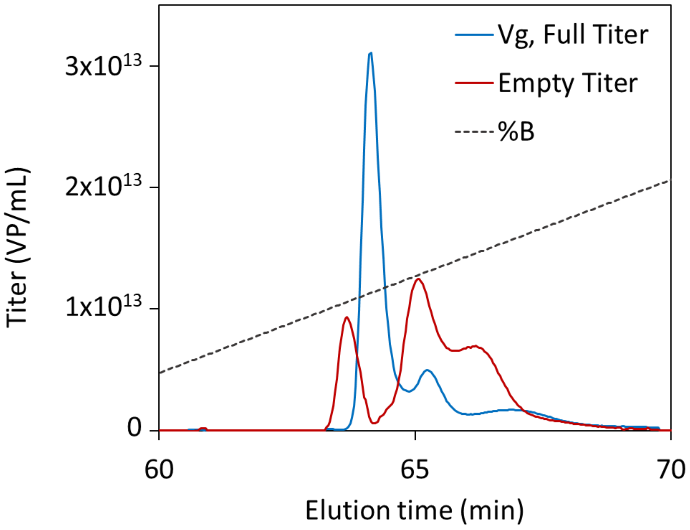


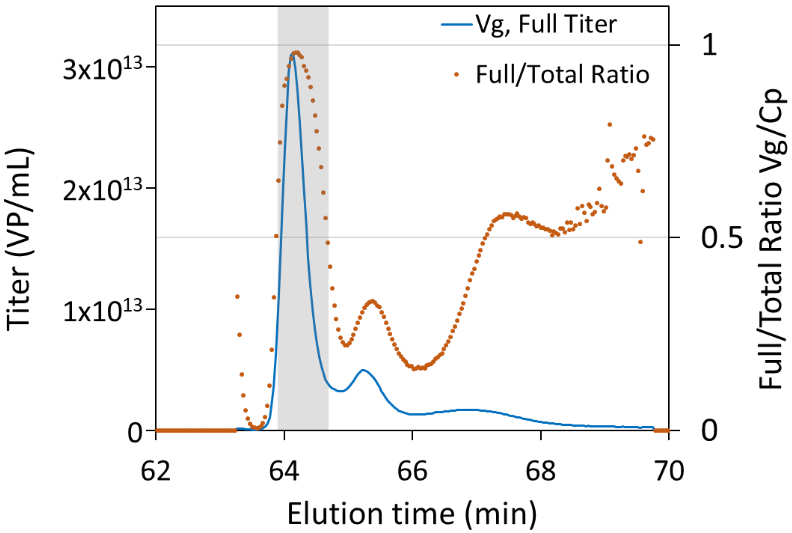
AAV enrichment by ion-exchange chromatography

AAV (and other small viral vectors): Vg/Cp, titer during linear salt gradient

Attributes:

- Vg/Cp
- N_{capsid}
- N_{genome}
- MW_{capsid}
- MW_{genome}
- R_g

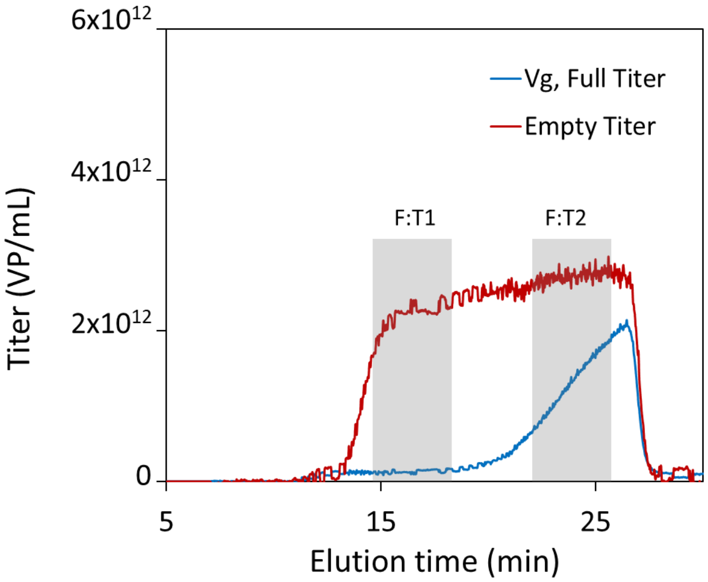




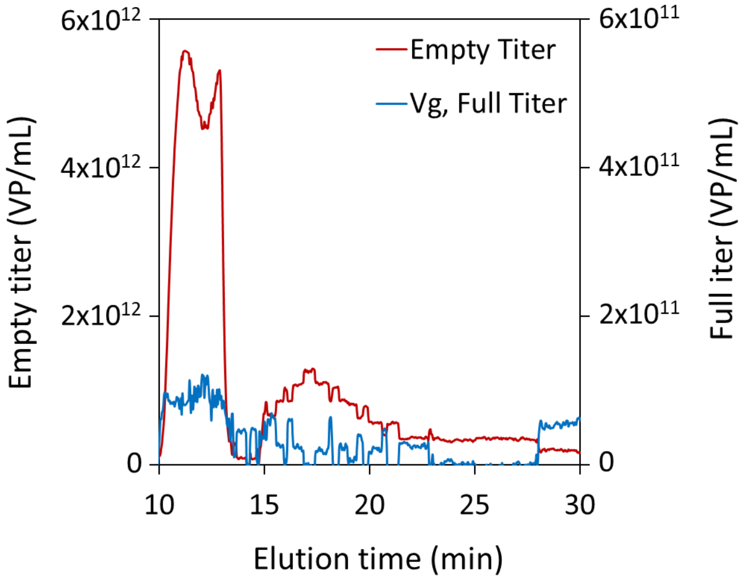
Slide courtesy of F. Michael Haller, Lonzac

AAV (and other small viral vectors): Vg/Cp, titer during column loading

Pre-optimal column loading

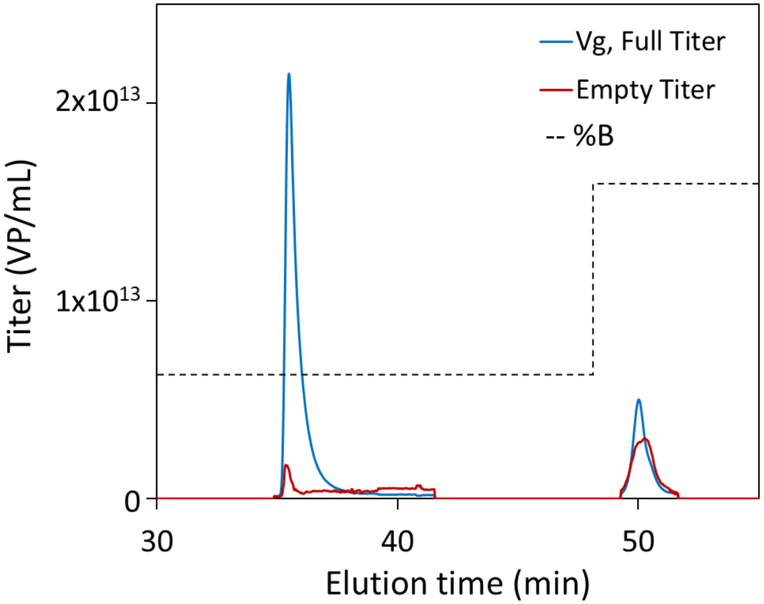


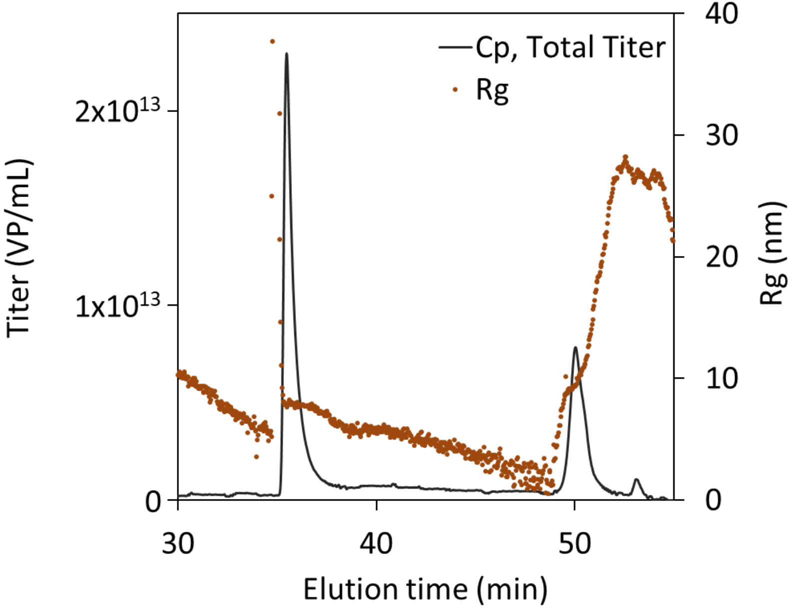
Optimized column loading



Slide courtesy of F. Michael Haller, Lonzac

AAV (and other small viral vectors): Vg/Cp, titer during step elution & strip





Slide courtesy of F. Michael Haller, Lonzac

Summary – RT-MALS

- Measurements of product parameters instead of secondary indicators
- No modelling involved
- Triggers for fraction collection and process control
- Applicable for FPLC, UF/DF, TFF and Fill-finish from scale-up to production
- Real-time molar mass, radius, particle concentration, and Vg/Cp (AAV)
  - Verify product quality in real time during production
  - Identify contaminations, HMW-species or fragments

Summary: DLS & MALS for biologics

Product quality attributes from research to production

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DLS & MALS

Identify formulation conditions for drug candidates in research by DLS

Perform in-depth characterization in development by SEC/FFF-MALS

(RT-)MALS

Increase efficiency in process development by SEC/FFF-MALS

Determine product attributes directly in process by RT-MALS

DLS & MALS

Perform in-line and at-line analytics in minutes by DLS & SEC/FFF-MALS

Real-time monitoring of product quality by RT-MALS

DLS & MALS

Detect aggregates in mere seconds with the help of DLS

Determine critical quality attributes for the product by SEC/FFF-MALS

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