Beyond Aggregates:

Light Scattering Tools for Biophysical Characterization and Quantitation

Sophia Kenrick

Wyatt Technology Corporation






About Wyatt Technology Corporation

- ✓ Founded in 1982 by Dr. Philip J. Wyatt to commercialize multi-angle light scattering (MALS)
- ✓ Award-winning, robust, low maintenance, easy to use instruments that have been validated by thousands of peer-reviewed publications
- ✓ Leading provider of light scattering instruments for solution-based characterization of macromolecules and nanoparticles:

  molar mass, size, charge, & interactions
- ✓ Pioneer of SEC-MALS and FFF-MALS, now standard analytical tools in protein, biopharma, biopolymer, synthetic polymer labs and more
- ✓ Pioneer of plate-based dynamic light scattering (DLS), an essential technology for high-throughput protein and nanoparticle formulation

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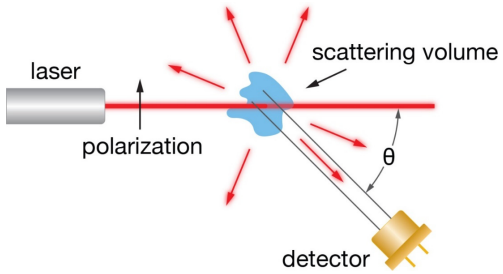
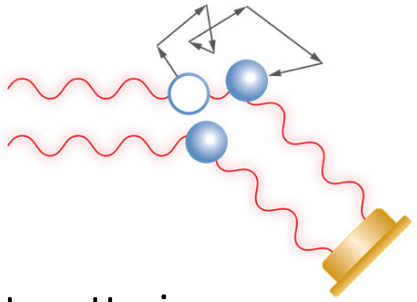
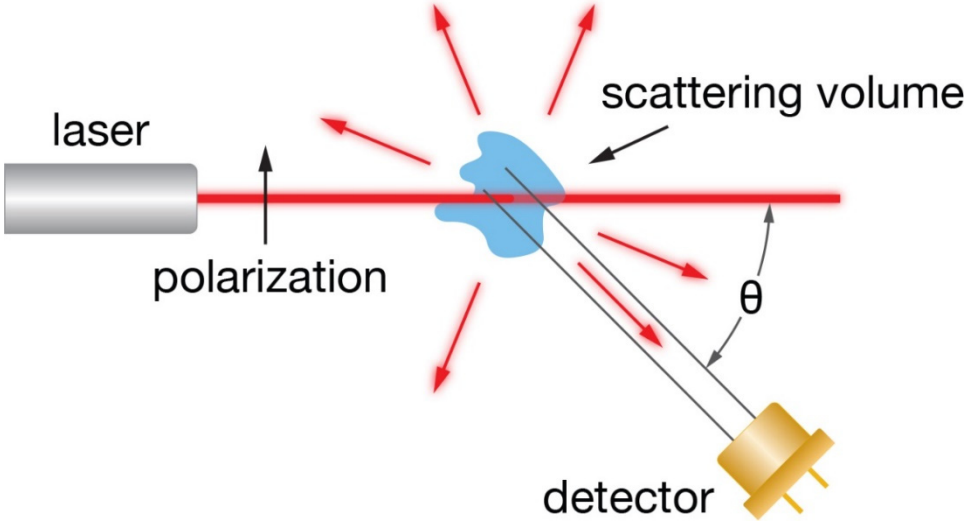
Light scattering provides critical attributes





	Static light scattering	Dynamic light scattering
Identity	✓ Molar mass ✓ Size (RMS radius) ✓ Conjugation/loading Extinction coefficient ✓ Concentration	✓ Hydrodynamic size Conformation
Quality	✓ Aggregate amount and size Fragment amount and size Heterogeneity	✓ Aggregate size Viscosity
Stability	Reversible associations ✓ Second virial coefficient	✓ Diffusion interaction parameter ✓ Transition temperatures

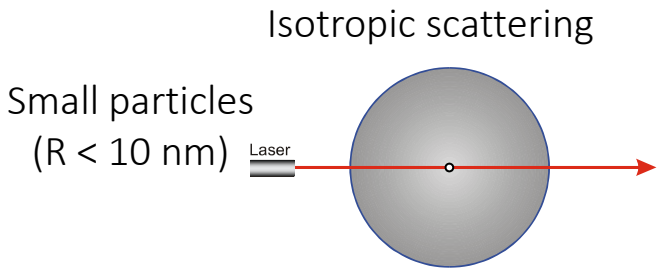
Multi-Angle Light Scattering

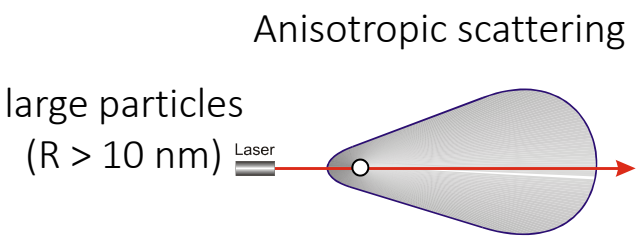


The amount of light scattered at 0° is directly proportional to the molar mass and mass concentration

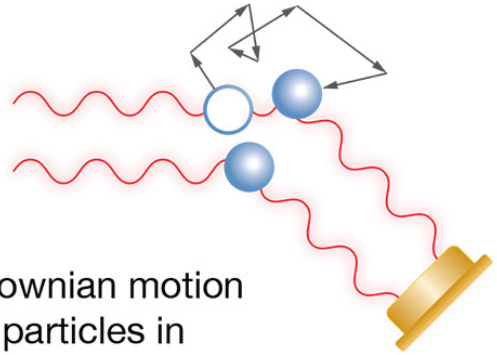
$$I_{scattered} \propto M \cdot c \cdot \left(\frac{dn}{dc}\right)^2$$

The variation of scattered light with scattering angle is proportional to the average size of the scattering molecules.



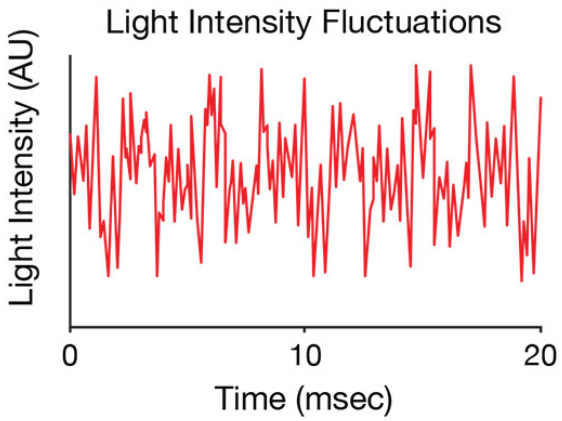


Dynamic light scattering (DLS)



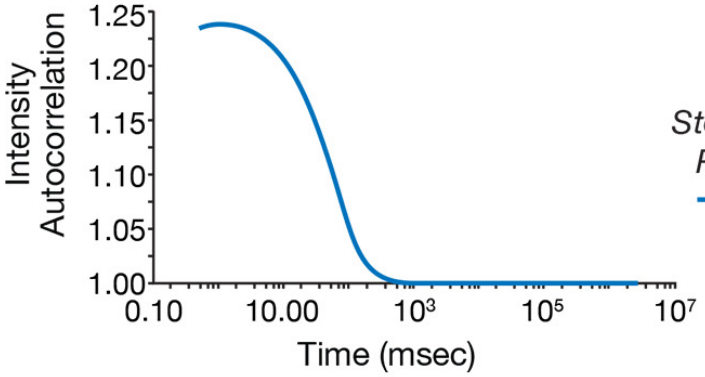
Brownian motion of particles in solution

Avalanche Photodiode



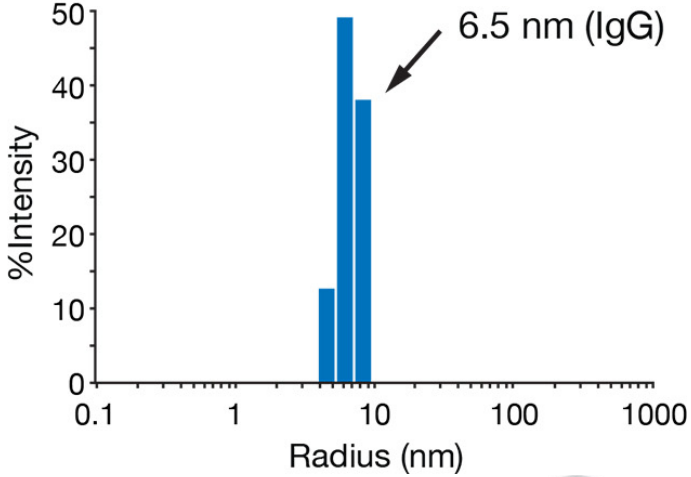
Autocorrelation Analysis

Decay rate ∝ Diffusion coefficient

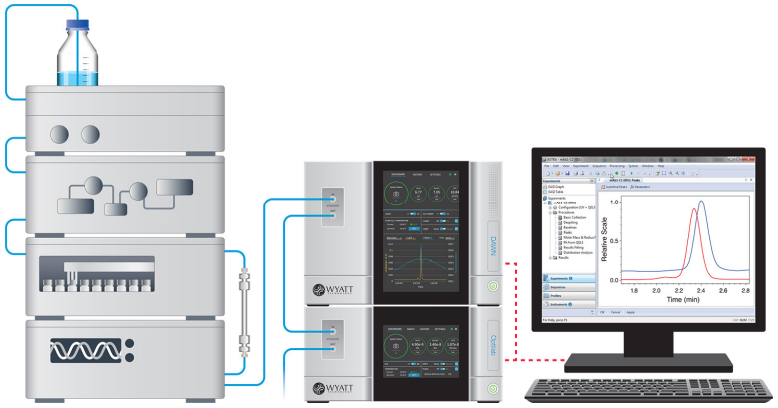


Stokes-Einstein Relationship

Hydrodynamic Radius

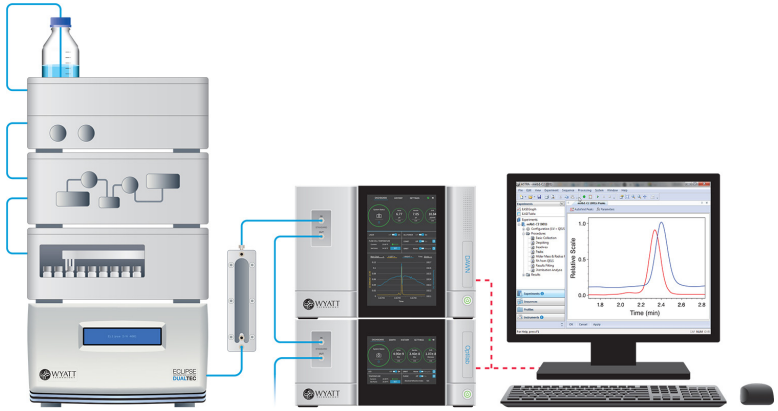


Typical MALS hardware setup and applications



SEC-MALS of biomolecules

- Proteins, polysaccharides, nucleic acids, conjugates
- Measure monomer and aggregate molar mass, molar mass distribution and polydispersity (M_w/M_n)
- Characterize branching and degree of conjugation

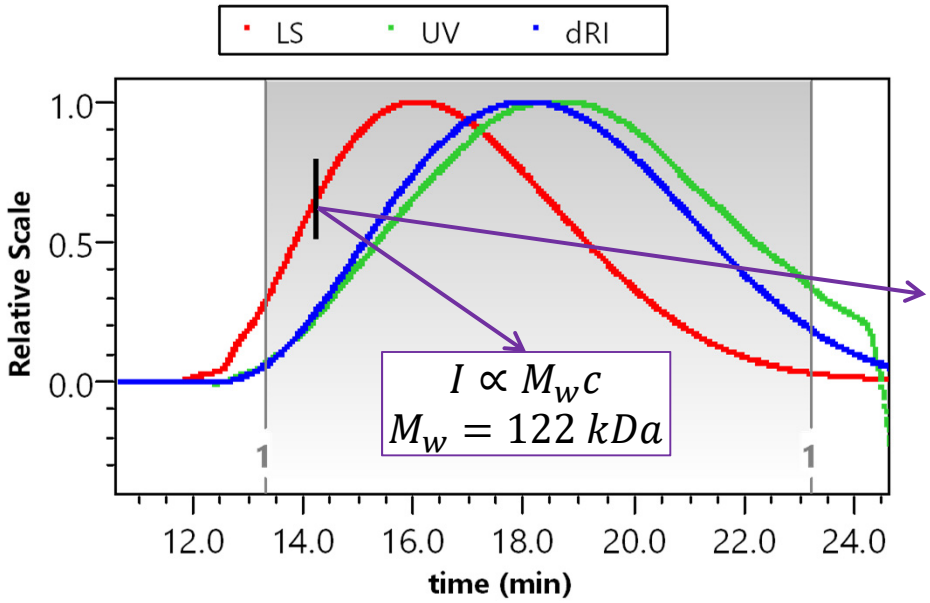


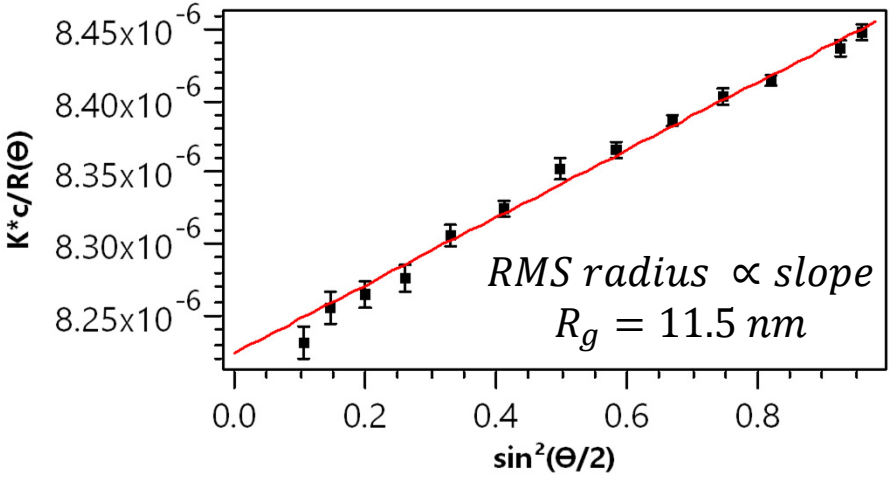
Eclipse AF4-MALS of BioNPs

- Viral vectors, EVs/exosomes, lipid or other NPs
- Isolate, identify, and quantify nanoparticle size, molar mass, and concentration
- Characterize shape/structure and payload/cargo content

Example:
Online Molar Mass and RMS Radius

Light scattering and refractive index data are measured for each eluting slice to yield *absolute* molecular weight, R_g, and (with DLS) R_h.





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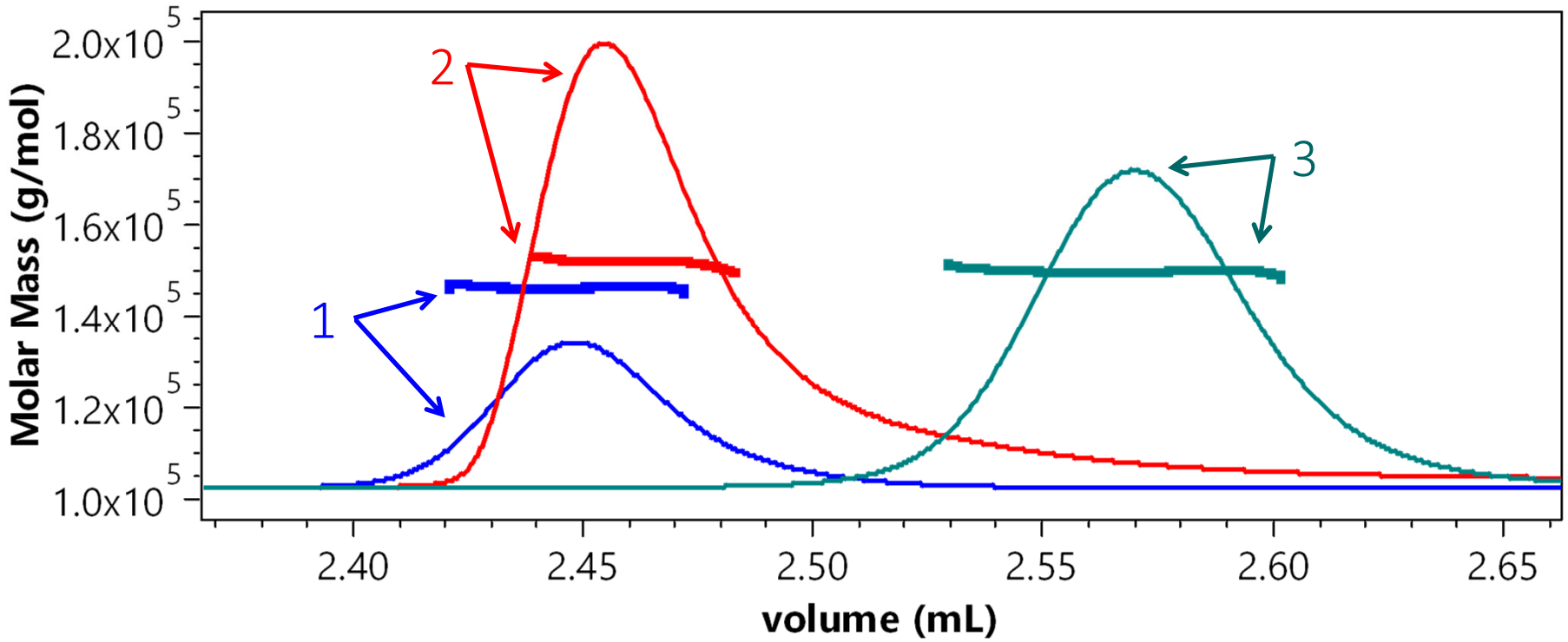
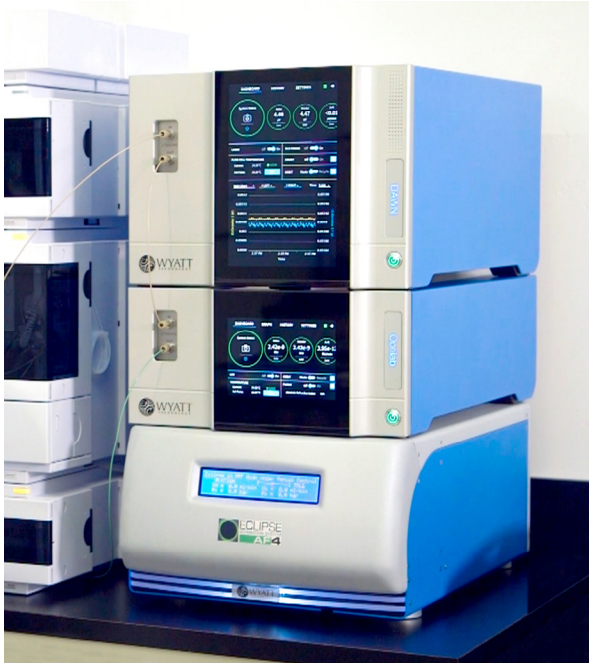


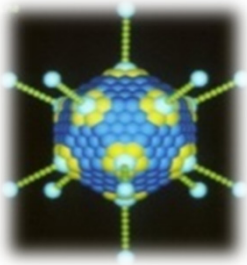


Case Study 1: Adenovirus

Measure size of monomer and aggregates

Quantify number of aggregates

Relate aggregation to stress conditions



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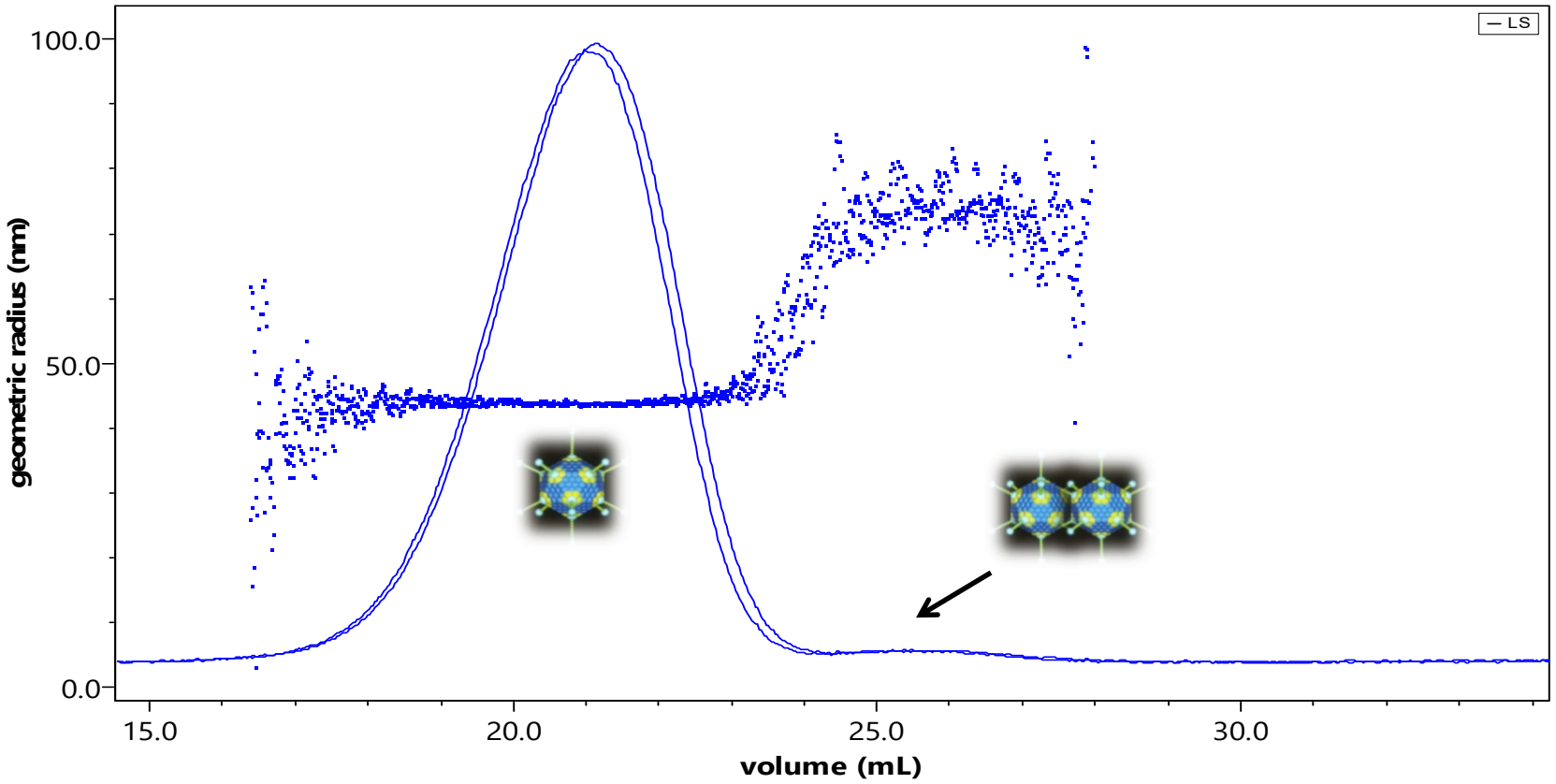
Adenovirus



- Small amount of aggregates will not be detected by batch DLS.
- R_g and R_h can be measured by MALS and online DLS, respectively.

Quantitation: size, particle counts, aggregate%

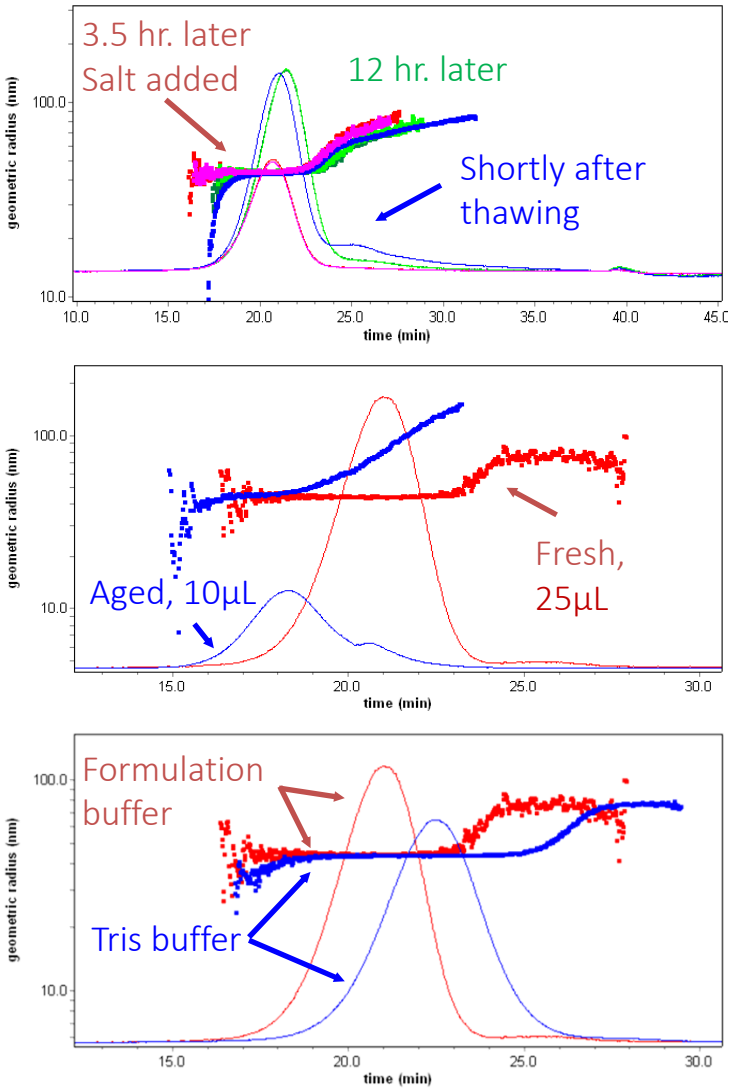
	Monomer		Aggregate				
	Radius (nm)	Number of particles	Radius (nm)	Number of particles	% by number (MALS)	% by mass (MALS)	% by UV peak area
Run 1	43.7	1.8×10^{10}	77	1.4×10^{7}	0.08%	2.76%	0.83%
Run 2	44	1.7×10^{10}	82	1.0×10^{7}	0.06%	2.09%	1.06%
Average	43.9	1.75×10^{10}	80	1.2×10^{7}	0.07%	2.4%	0.9%

Good reproducibility was obtained for both sizing and particle counting, despite very low amount of aggregate.

MALS provides more accurate quantitation of aggregate than traditional UV method.

- UV peak area may overestimate the percentage of large aggregates
- Scattering contribution in UV data is significant for particle radius >50 nm

How do stressors change the vector?



Freeze-thaw

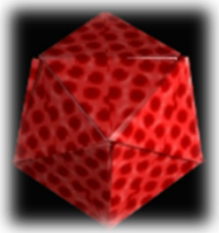
- Isolate and quantify virus size with Eclipse AF4-MALS
- Sensitive and robust aggregation assessment

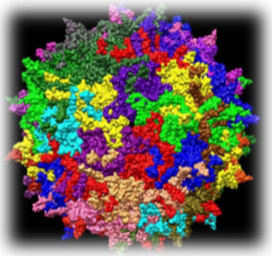
Fresh vs. aged

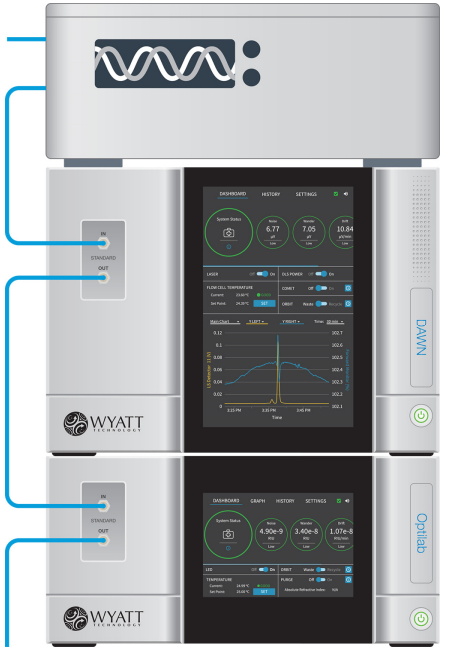
- Elution time and peak shape are not representative of size distribution
- Eclipse fractionation with DAWN MALS detector quantifies absolute size

Buffer effects

- Measure differences in size distribution between buffers
- Quantify number of particles: 1.7×10^{10} for each case







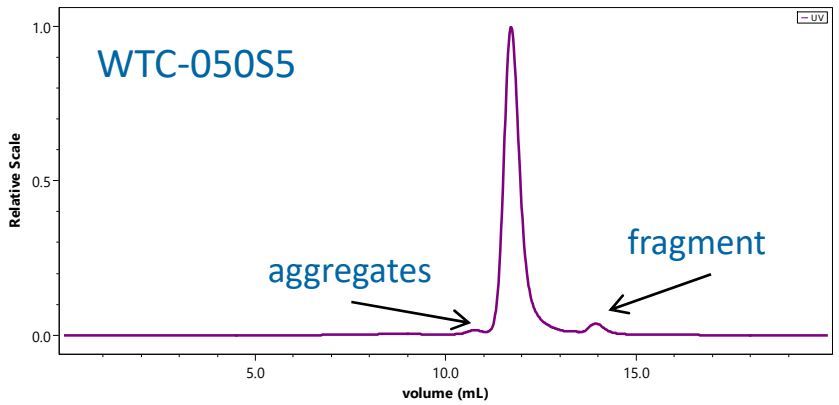
Case Study 2: Adeno-associated virus (AAV)

Comparison of separation techniques

Quantify genetic payload

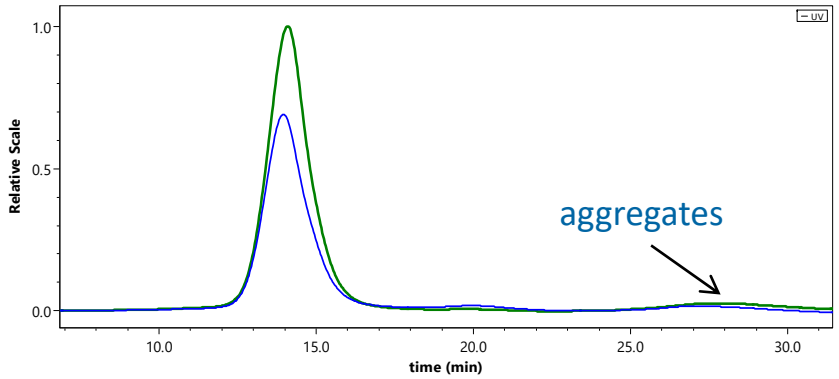
Determine structure

AAV by SEC-MALS and Eclipse AF4-MALS



SEC-MALS

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



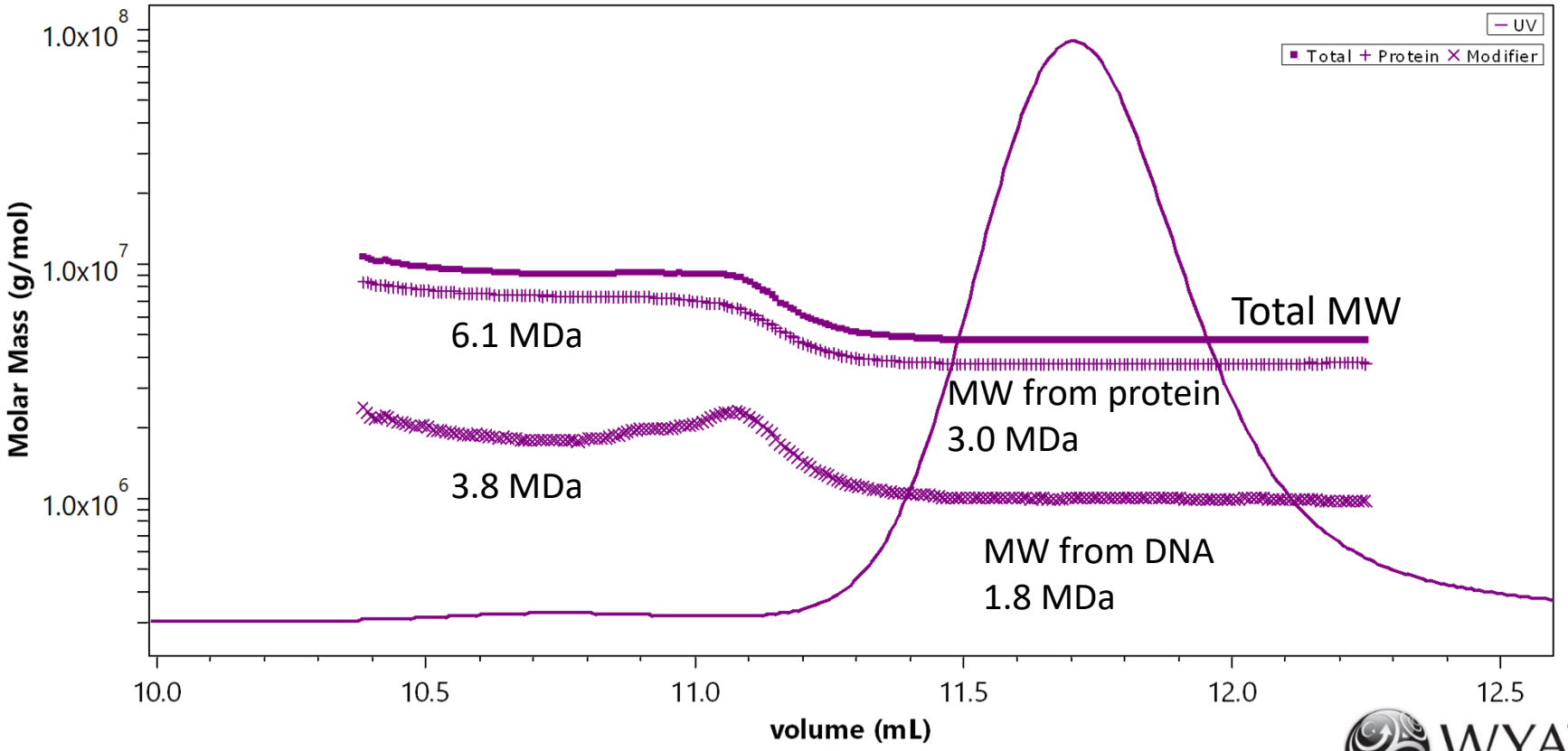
Eclipse AF4-MALS

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

AAV genetic payload by SEC with multi-detection

Quantify genetic payload

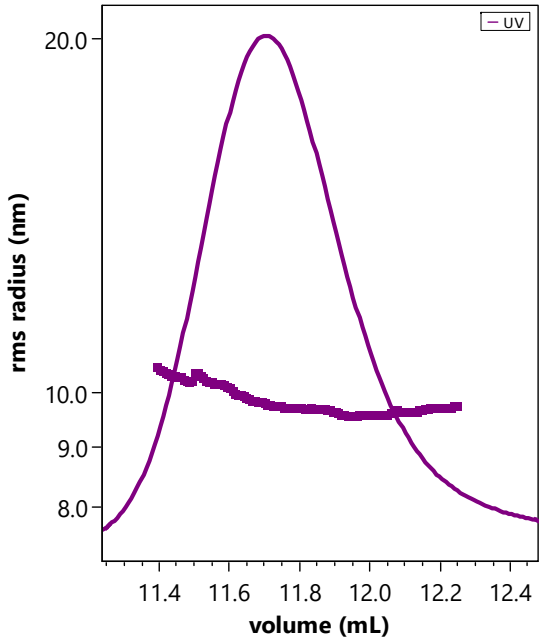
— SEC/FFF cannot resolve empty and filled AAVs, but the apparent MW data from MALS and dRI may correlate to the percentage of full AAV



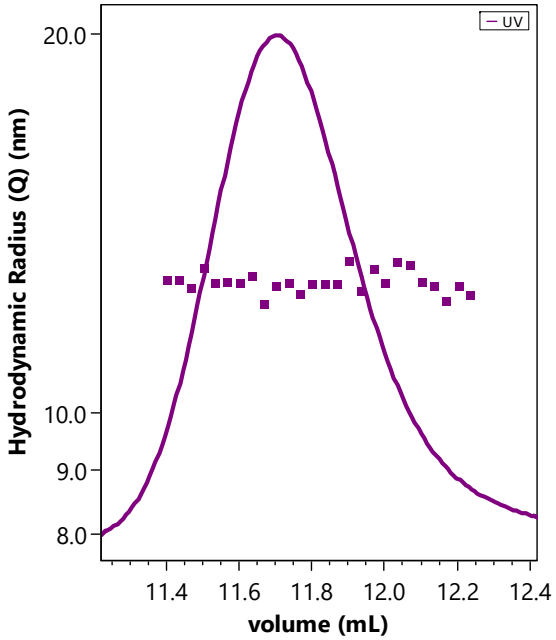
AAV structure by SEC with multi-detection

Confirm payload and measure shape

R_g from MALS

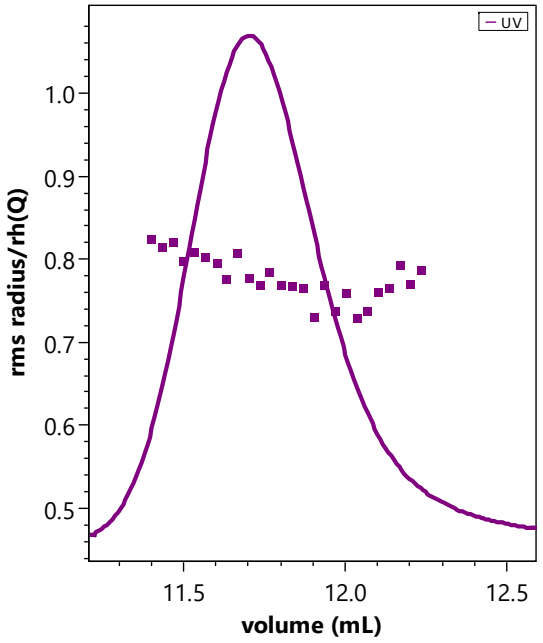


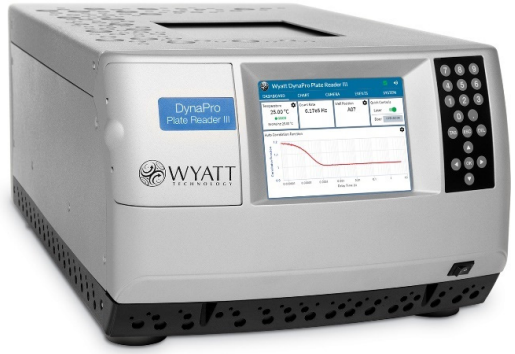
R_h from DLS

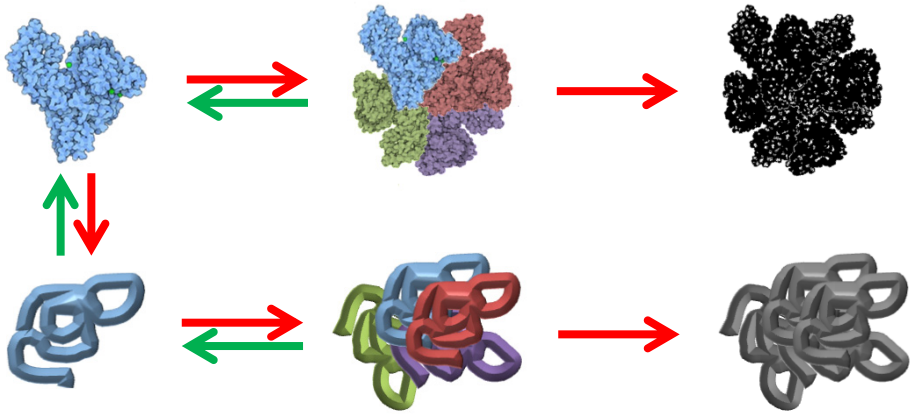


R_g/R_h ~ 0.8

filled sphere







Case Study 3: Formulation stability

High-throughput aggregate screening

Colloidal stability: k_D and A_2

Conformational stability: T_m and T_{agg}

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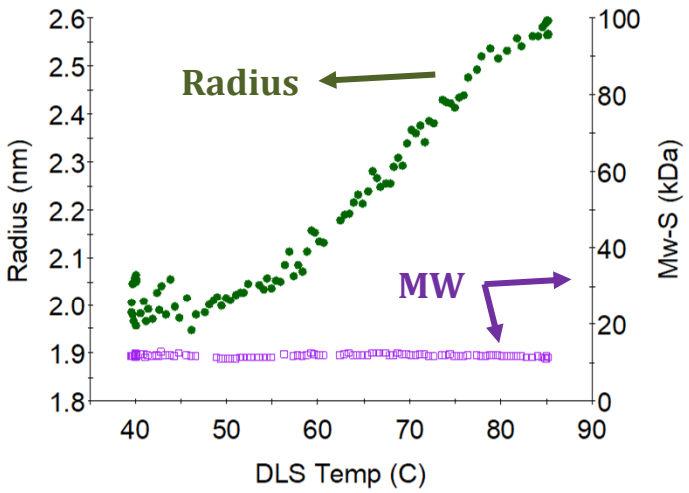
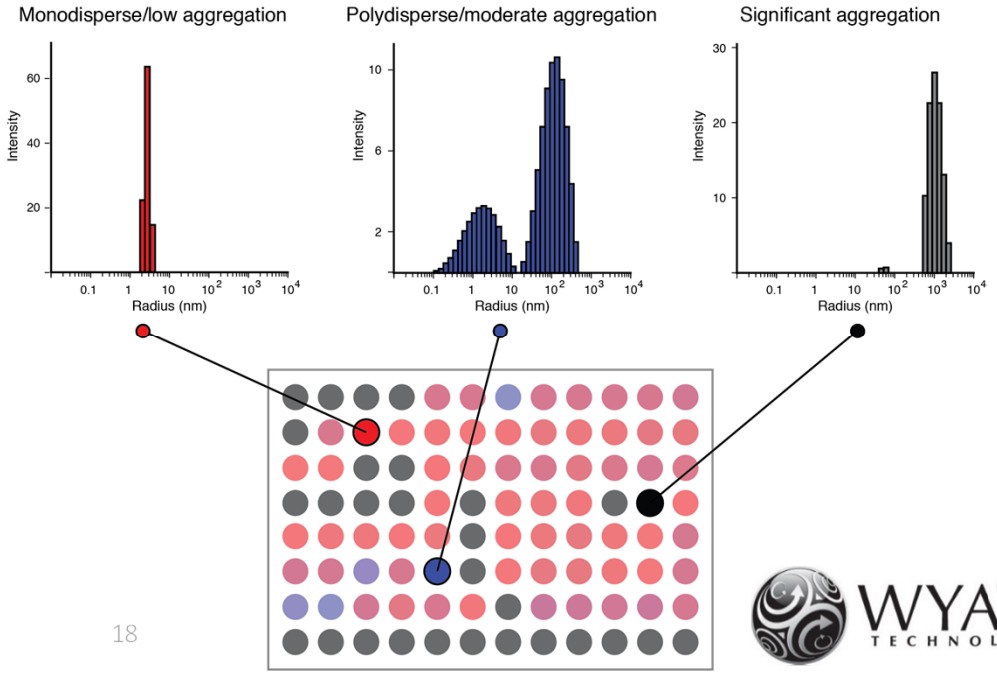
Benefits of batch DLS

Screening and characterization tool for formulation development

- High-throughput, low volume quality control for aggregates
- Perform studies as a function of time and temperature
- Screen small-molecule drugs: promiscuous inhibitors and binders
- Measure formulation viscosity





Measure interactions among molecules

Dynamic light scattering:

Diffusion Interaction Parameter, k_D

$$D_t = D_0(1 + k_D c)$$

$k_D < 0$ attraction

$k_D > 0$ repulsion

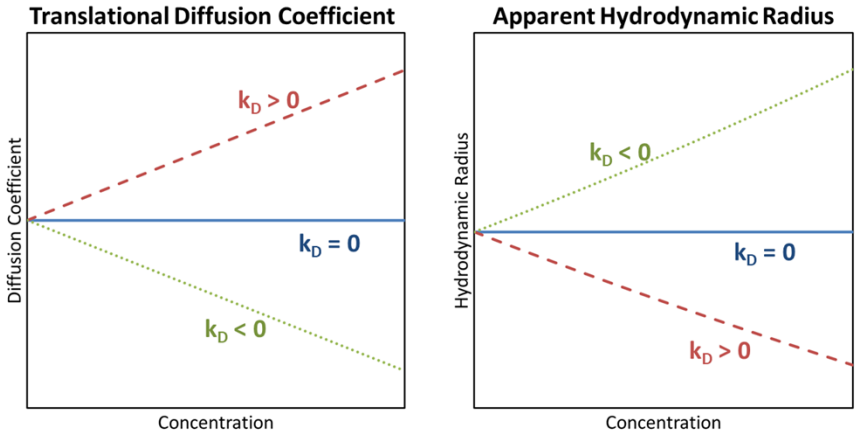
Static light scattering:

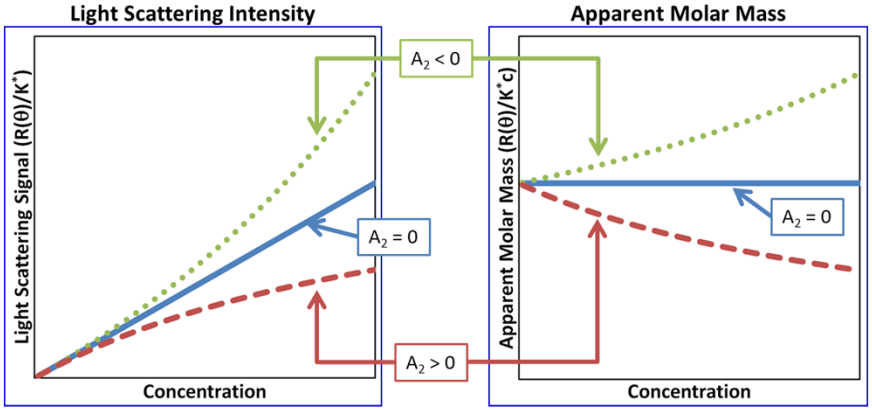
Second virial coefficient, A_2

$$R/K^* = Mc[1 - 2A_2Mc]$$

$A_2 < 0$ attraction

$A_2 > 0$ repulsion





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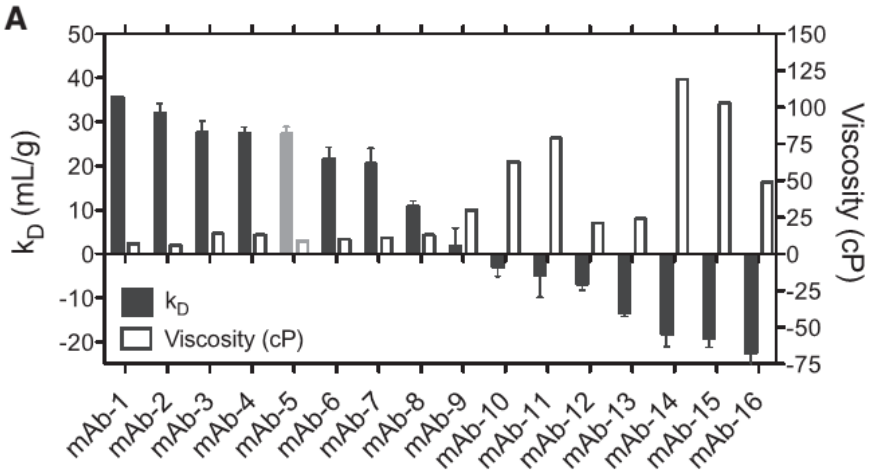
Why measure concentration dependence?

k_D correlates with solution properties

- $k_D > 0$ correlates to low viscosity
- $k_D \lesssim 0$ correlates to high viscosity
- $k_D \lesssim 0$ correlates with particles formation (e.g., after agitation)

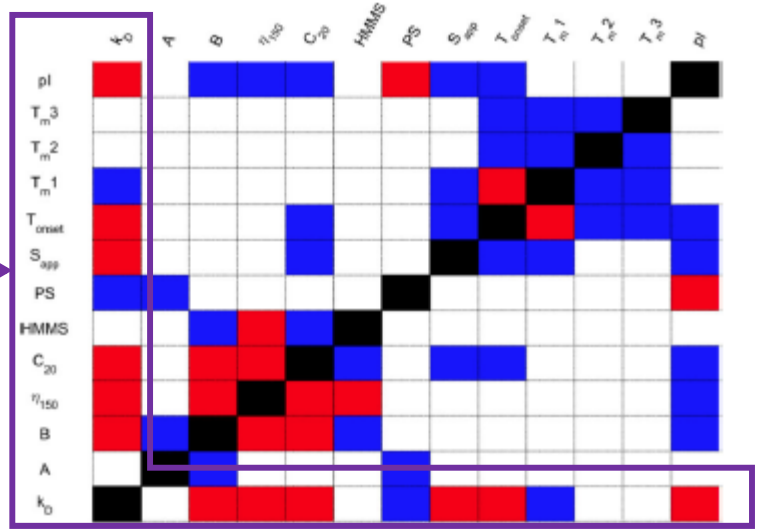
Sample formulation space and determining key stability attributes.

- Interactions as a function of pH, excipient, salts, etc.
- Observe correlations between k_D, A_2, and T_{agg}



Connolly, B.D. et al. *Biophys. J.* (2012) **103**(1):69-78.

Positive correlation between k_D and many other stability-indicating parameters!

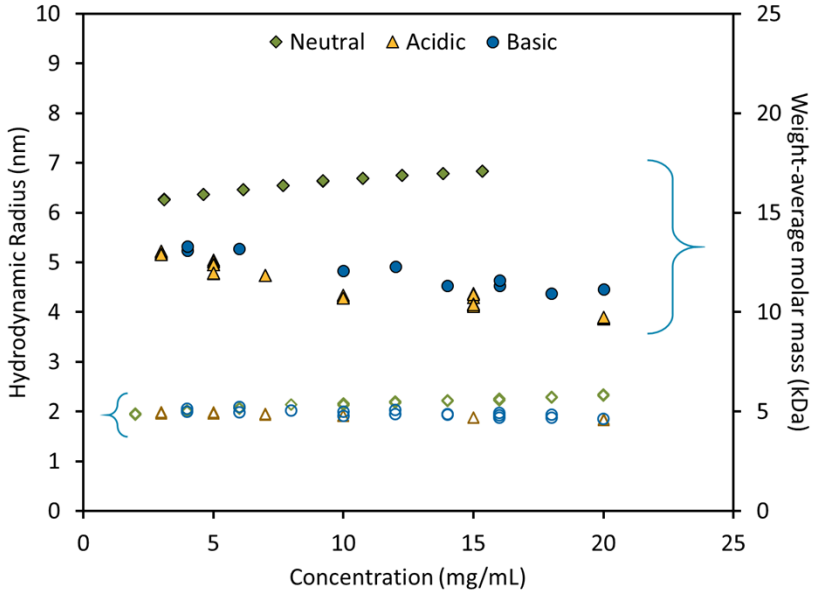


Tomar D.S., et al. (2018) *Pharm. Res*. **35**:193.

Quantify colloidal stability, k_D and A_2

Formulation pH influences intermolecular interactions.

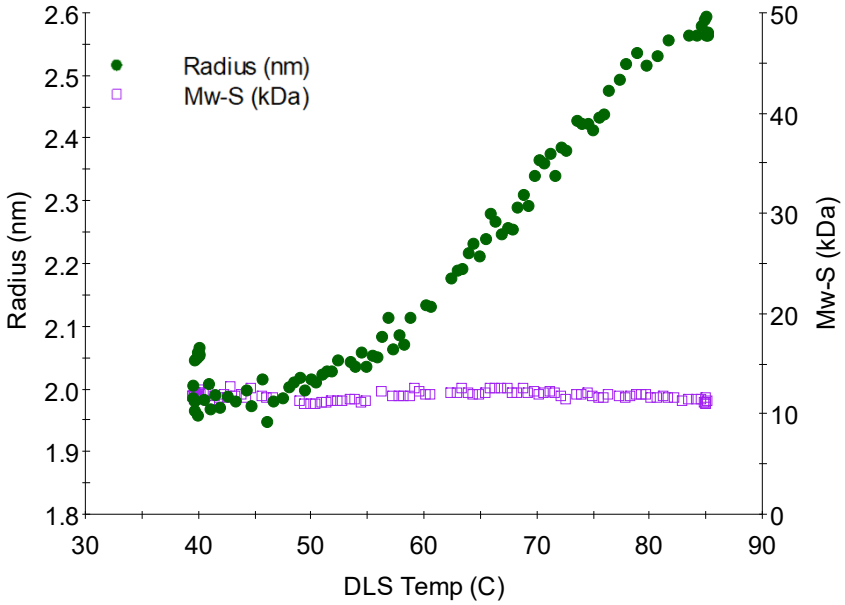
- Neutral pH causes undesirable attractive interactions
- Acidic and basic pH exhibit net repulsive interactions



	DLS		SLS	
	R_h (nm)	k_D (mL/g)	M_w (kDa)	A_2 (mol · mL/g^2)
Neutral	1.9	−8.5	15.0	-4.8×10^{-4}
Acidic	2.0	+4.6	13.5	$+7.3 \times 10^{-4}$
Basic	2.1	+3.7	14.7	$+5.0 \times 10^{-4}$

Conformational stability from temperature

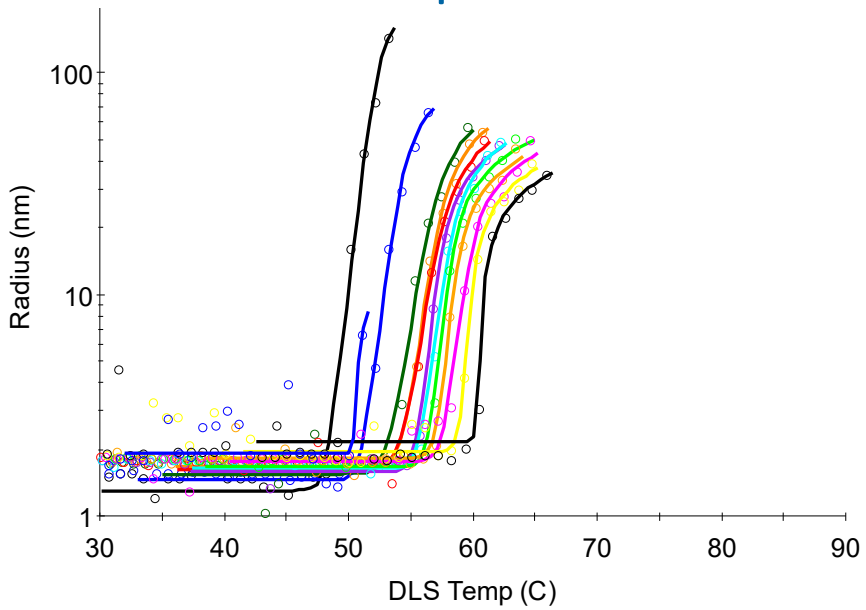
Acidic pH



Acidic pH provides conformational stability.

- Midpoint unfolding temperature $T_{\mathrm{m}} = 69\ ^{\circ}\mathrm{C}$
- Confirm unfolding (not aggregation) via constant measured M_{w}

Basic pH



Basic pH shows aggregation at elevated temperature

- Onset of aggregation/unfolding happens at lower T compared to acidic pH
- T_{agg} varies with concentration, ranging from 48 °C to 60 °C

Conclusion

Light scattering is not just for protein molecules and aggregates!

- Assess wide range of biotherapeutics, protein conjugates, and higher order structures.
- Extend characterization and quantitation to viruses, gene therapy and drug delivery vectors.

Static and dynamic light scattering provide a wide range of solutions for formulation stability.

- Measure nonspecific interactions and propensity to aggregate with DLS (k_D) or SLS (A_2).
- Characterize conformational stability (T_m, T_{agg}, time to aggregation, etc.)

Combine with complementary information for complete characterization.

For More Information

Sample application notes, webinars, & more at www.wyatt.com/Library.

Search over 14,000 peer-reviewed publications that feature Wyatt instruments at www.wyatt.com/Bibliography.

For information about a particular topic:

- Light Scattering Solutions: www.wyatt.com/Solutions
- SEC-MALS: www.wyatt.com/SEC-MALS
- DLS: www.wyatt.com/DLS
- CG-MALS: www.wyatt.com/CG-MALS