PRESENTATIONS

1. provide an overview of the problem and add any background necessary to understand the details

2. Explain the methods used in the paper

3. Show the results that were obtained

4. Discuss the importance, significance and impact of the paper, and explain what future work, if any, would be useful

Adeno-Associated Virus:

Characterization of Capsid Loading

Two Orthogonal Approaches using AUC

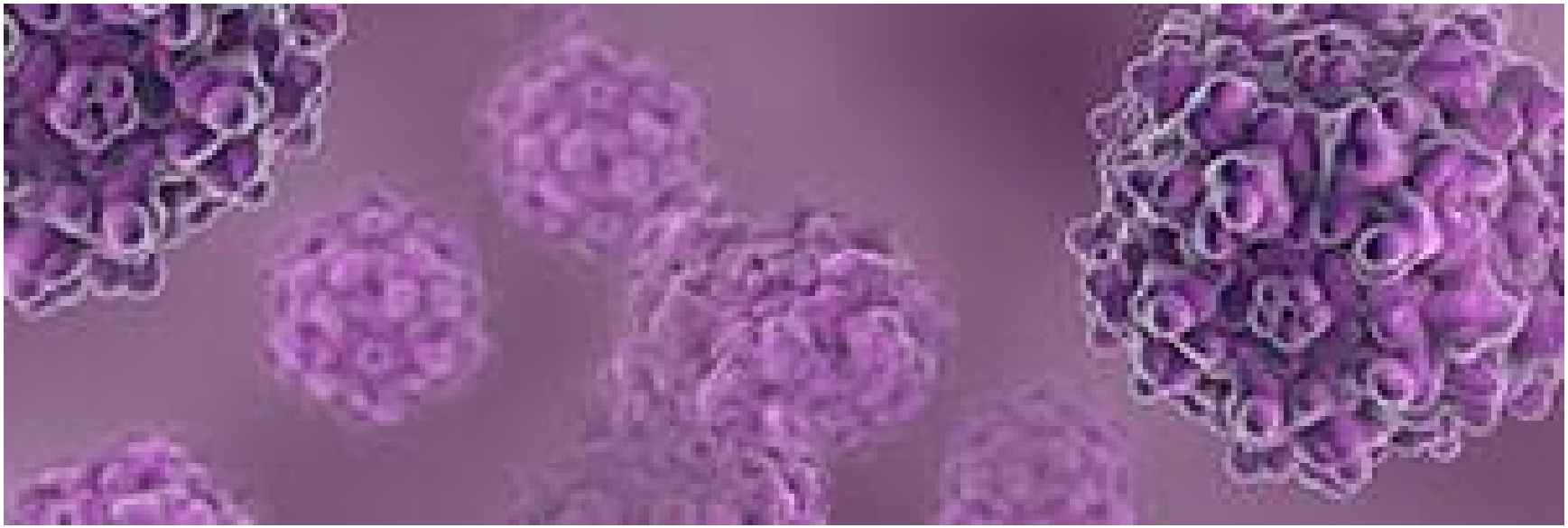
Adeno Associated Virus-based Gene Therapy/Editing Vectors

Essential Question:

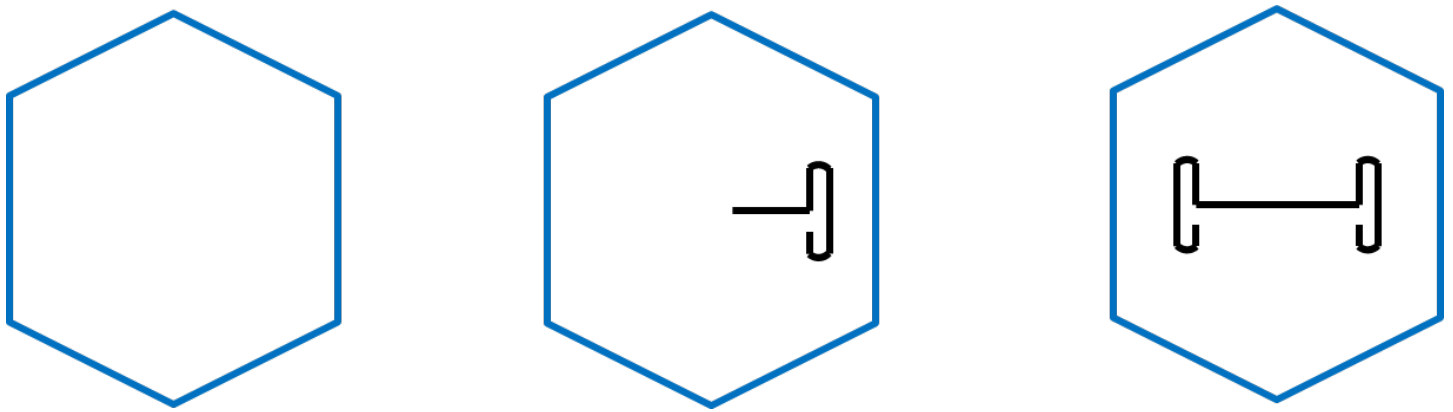
What is the degree of nucleic acid loading efficiency?

Objectives:

1) Minimize the amount of empty capsids
2) Minimize amount of partially filled capsids
3) Eliminate any free nucleic acids
4) Verify the loading state

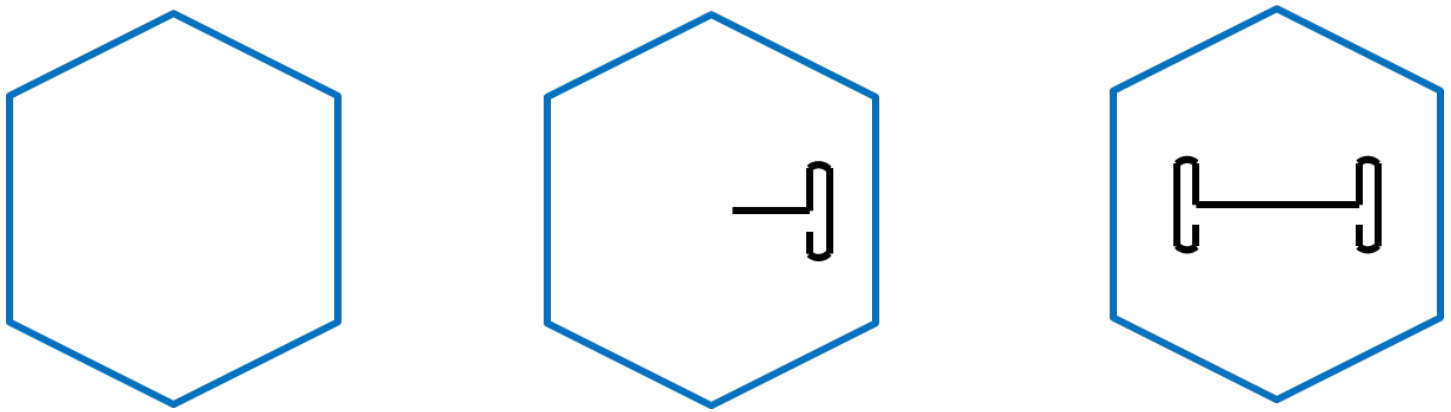


Adeno-Associated Viruses (AAV)



Empty Capsids	Partially Filled Capsids	Full Capsids
Lacks genome packaging	Contains a partial section of therapy or host cell DNA	Contains the therapeutic genome
Increases risk of immunogenicity and reduces transduction		Therapeutically Effective

Adeno-Associated Viruses (AAV)



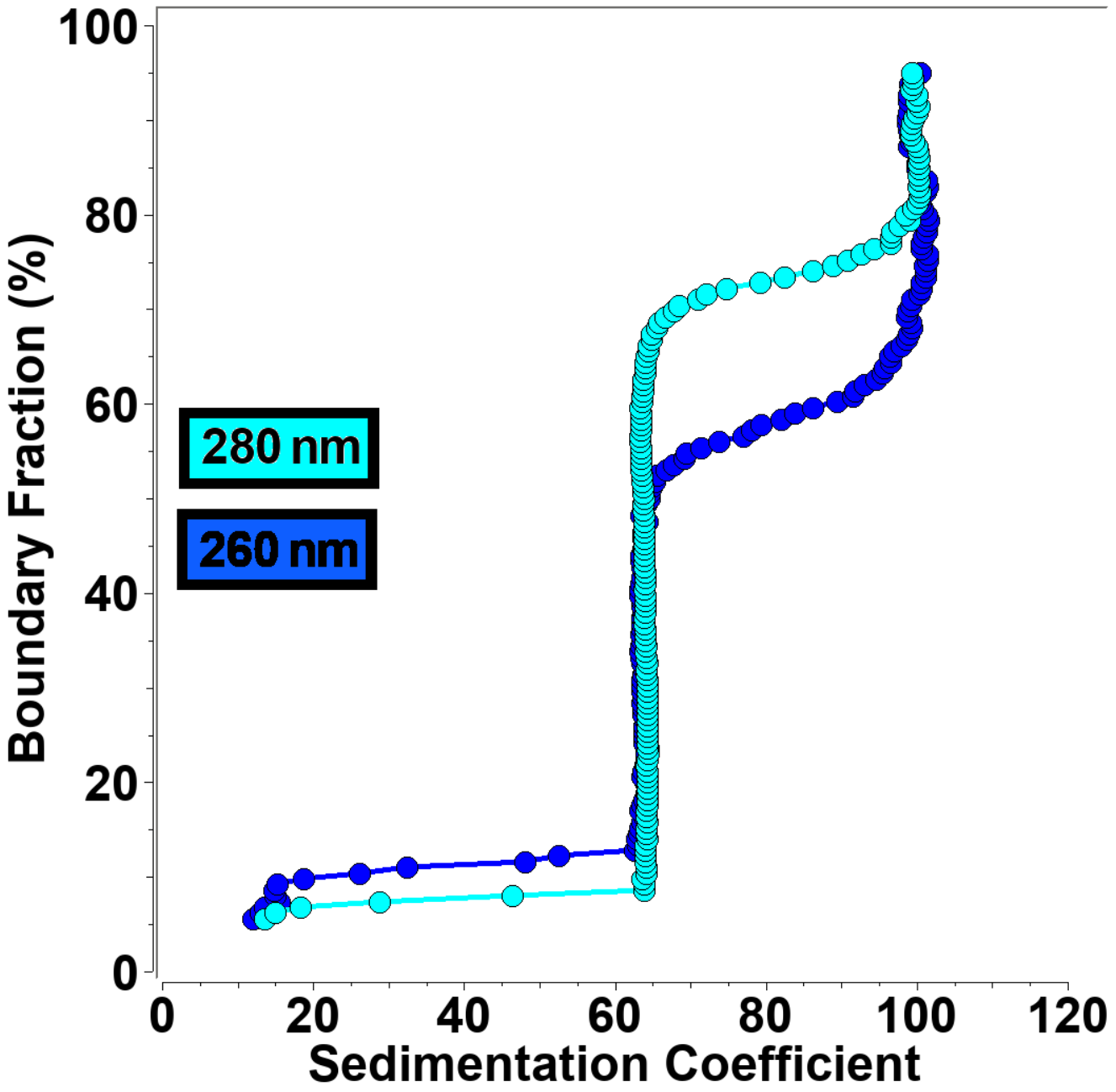
Empty Capsids	Partially Filled Capsids	Full Capsids
Lacks genome packaging	Contains a partial section of therapy or host cell DNA	Contains the therapeutic genome
Increases risk of immunogenicity and reduces transduction		Therapeutically Effective

AAV, whether loaded or empty, have the same size and shape. To distinguish these species by AUC, focus on what's different:

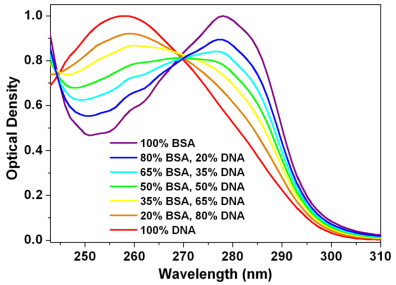
1. Density

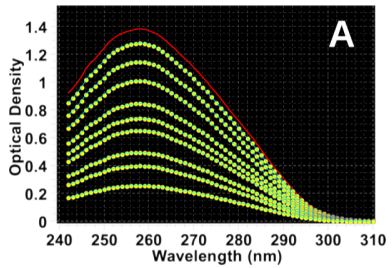
2. Molar mass

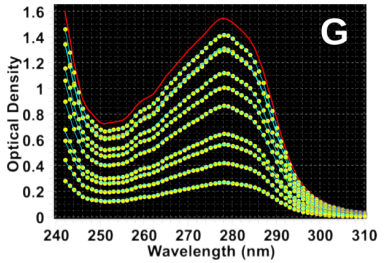
3. Absorbance


Boundary Fraction (%)
100
80
60
40
20
0
280 nm
260 nm
0
20
40
60
80
100
120
Sedimentation Coefficient

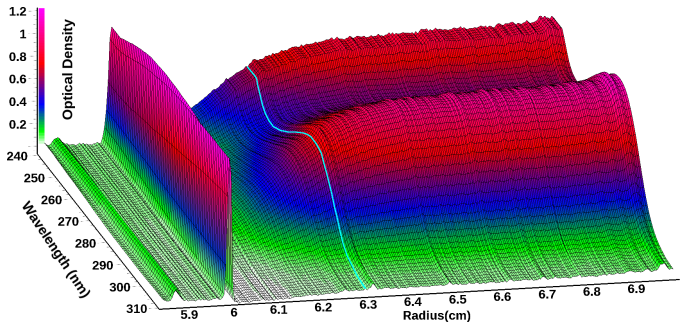
Use L_j Spectra for Spectral Decomposition







4D Multi-wavelength data:



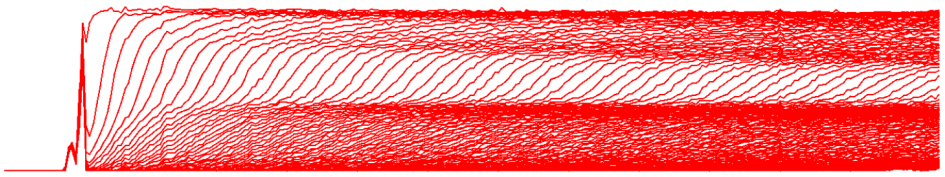
Scan1

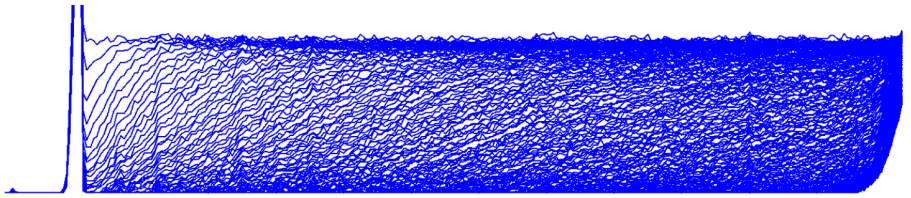
Scan n

NNLS

L_{DNA} $L_{Protein}$

$$\cdots \rightarrow C_{MWL} = x_a \begin{bmatrix} \epsilon_{a1} \\ \epsilon_{a2} \\ \cdots \\ \epsilon_{ai} \end{bmatrix}_{r,t} + x_b \begin{bmatrix} \epsilon_{b1} \\ \epsilon_{b2} \\ \cdots \\ \epsilon_{bi} \end{bmatrix}_{r,t}$$



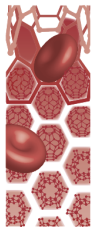


Adeno Associated Virus-based Gene Therapy/Editing Vectors

Multi-wavelength Sedimentation Velocity Experiment of AAV Prep:

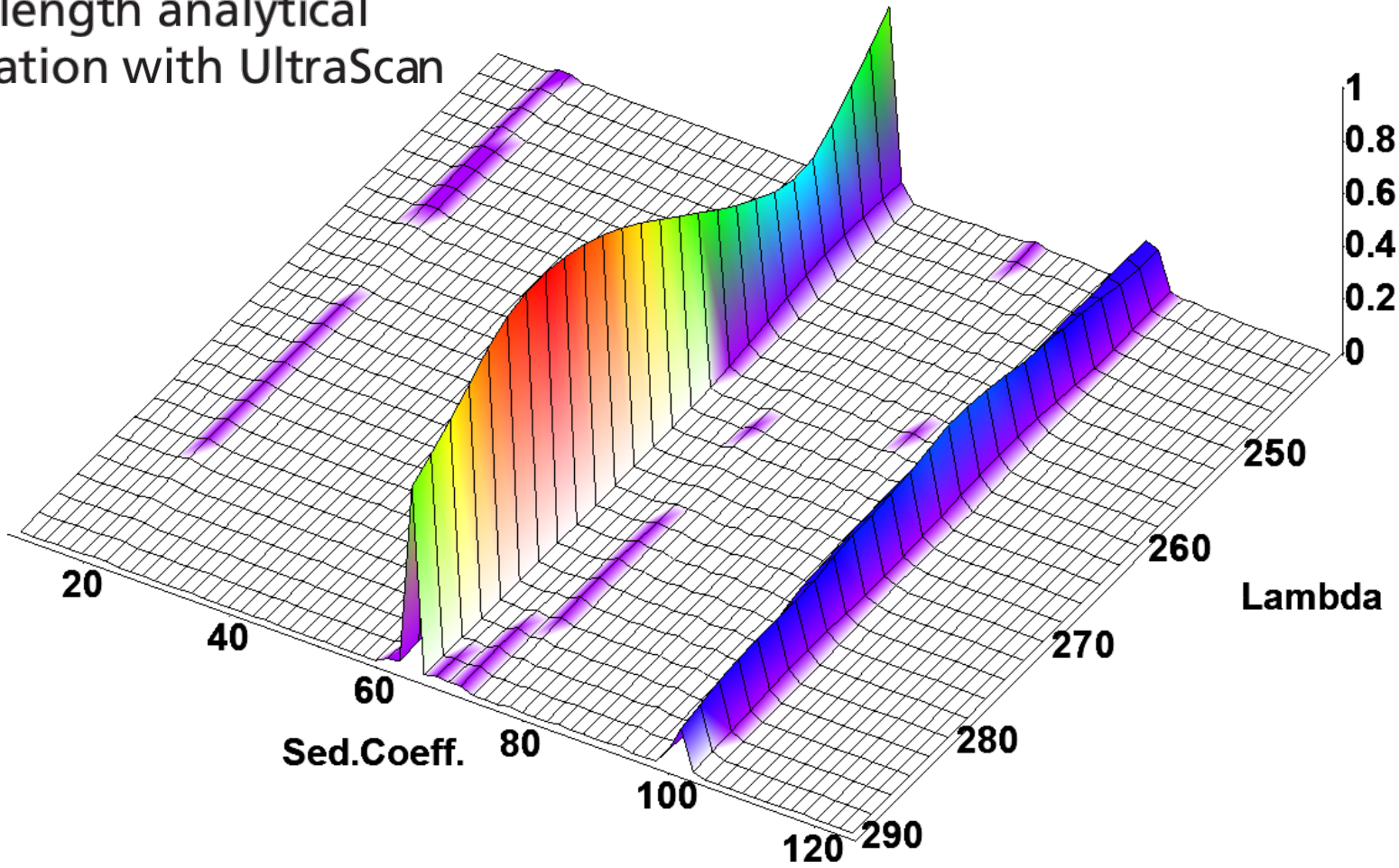
Nanomedicine

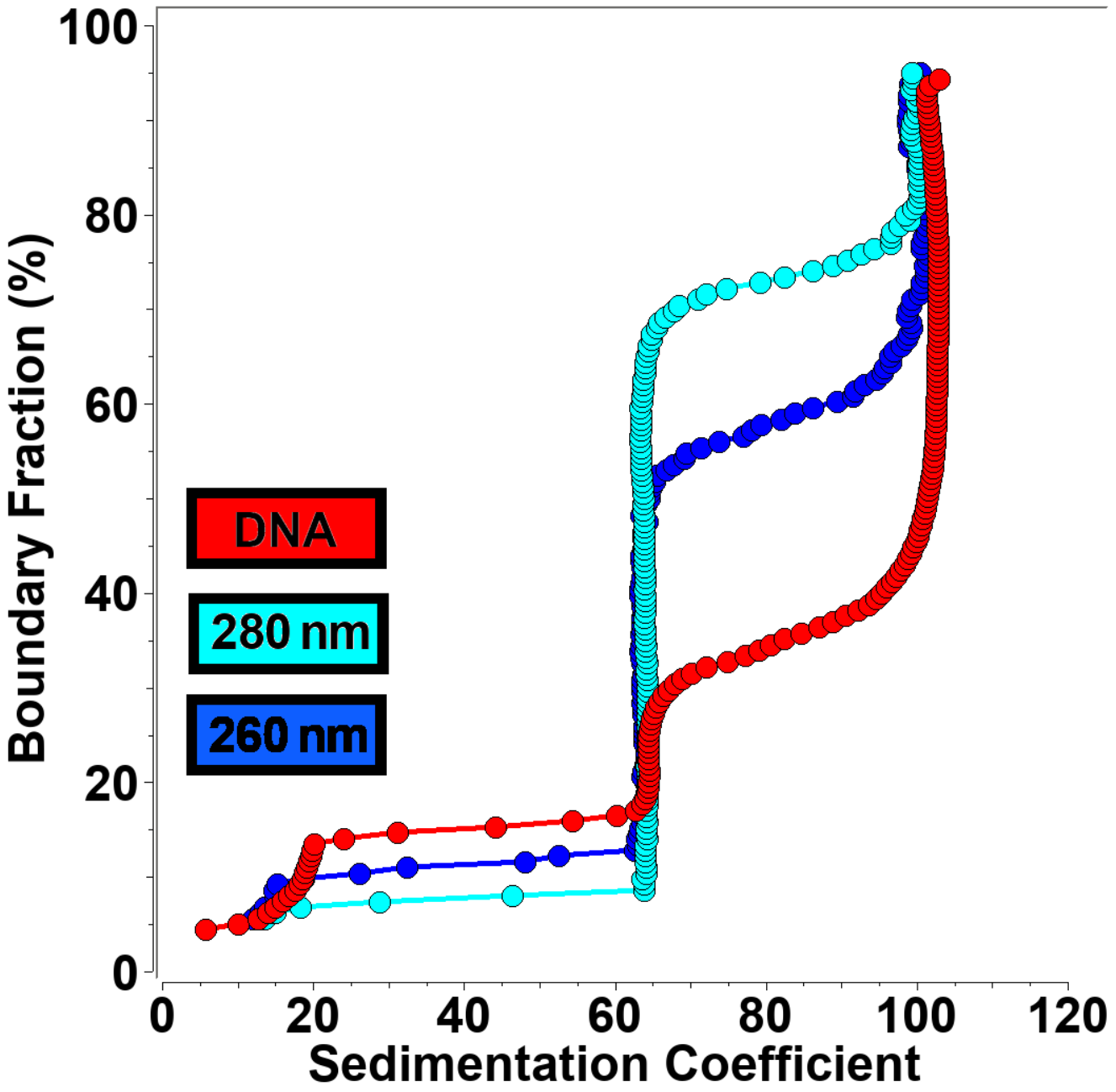
Amy Henrickson[1], Xiaozhe Ding[2], Austin G Seal[3], Zhe Qu[2], Lauren Tomlinson[4], John Forsey[4], Viviana Gradinaru[2], Kazuhiro Oka[3,5] & Borries Demeler*[,1,6]

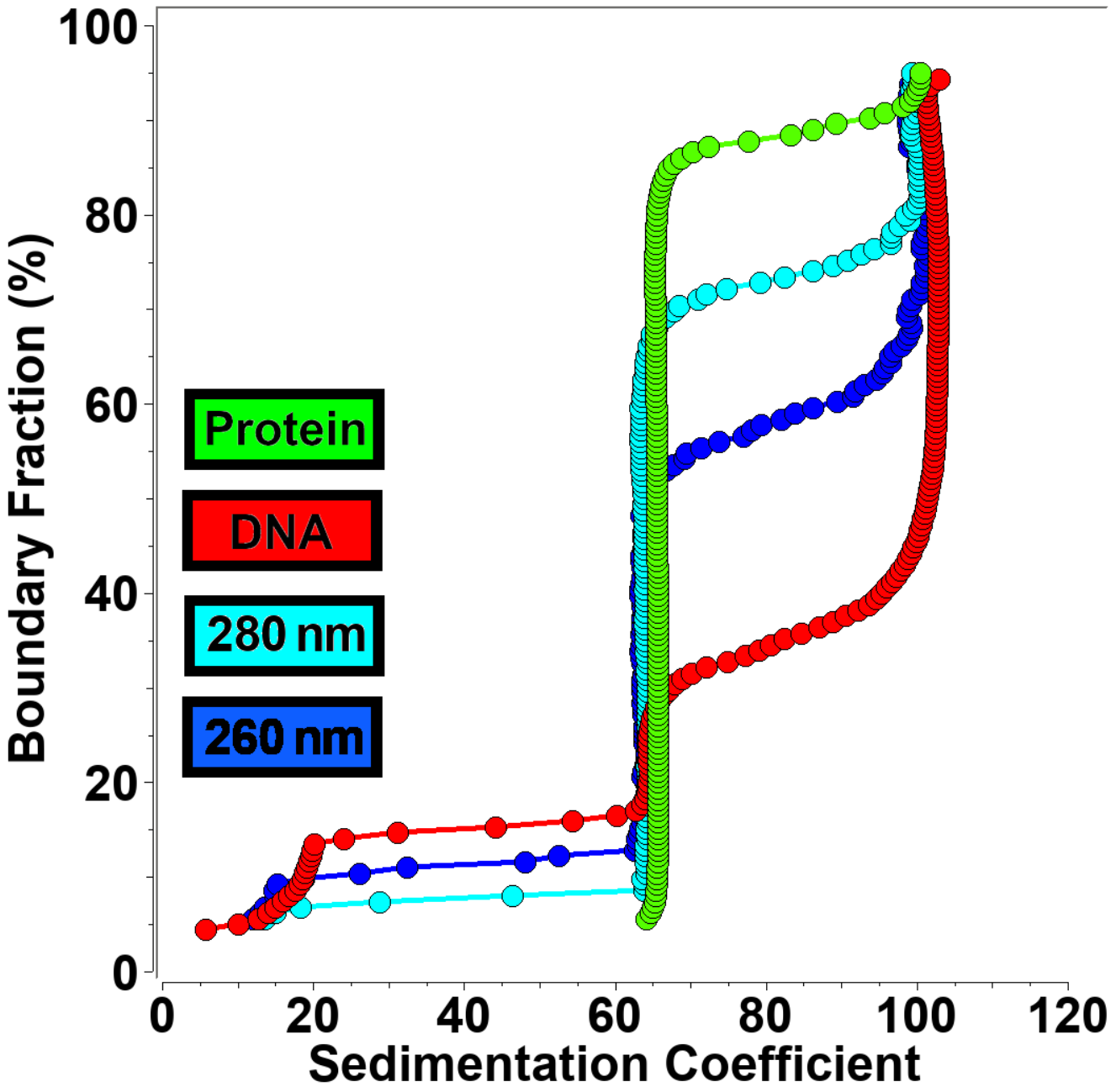


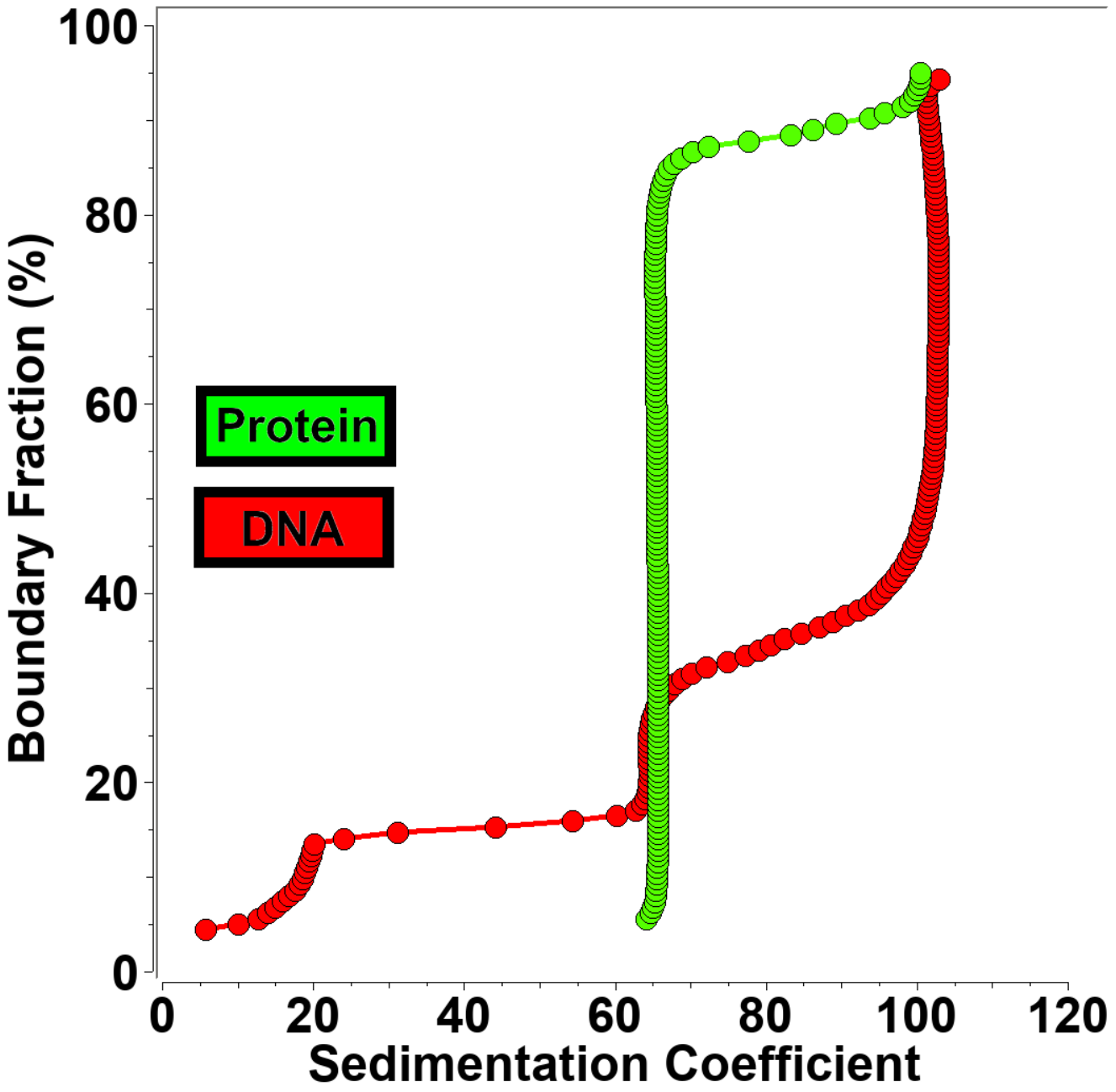
Characterization and quantification of adeno-associated virus capsid-loading states by multi-wavelength analytical ultracentrifugation with UltraScan

Amy Henrickson




Boundary Fraction (%)
100
80
60
40
20
0
0
20
40
60
80
100
120
Sedimentation Coefficient
DNA
280 nm
260 nm


Boundary Fraction (%)
100
80
60
40
20
0
0
20
40
60
80
100
120
Sedimentation Coefficient
Protein
DNA
280 nm
260 nm

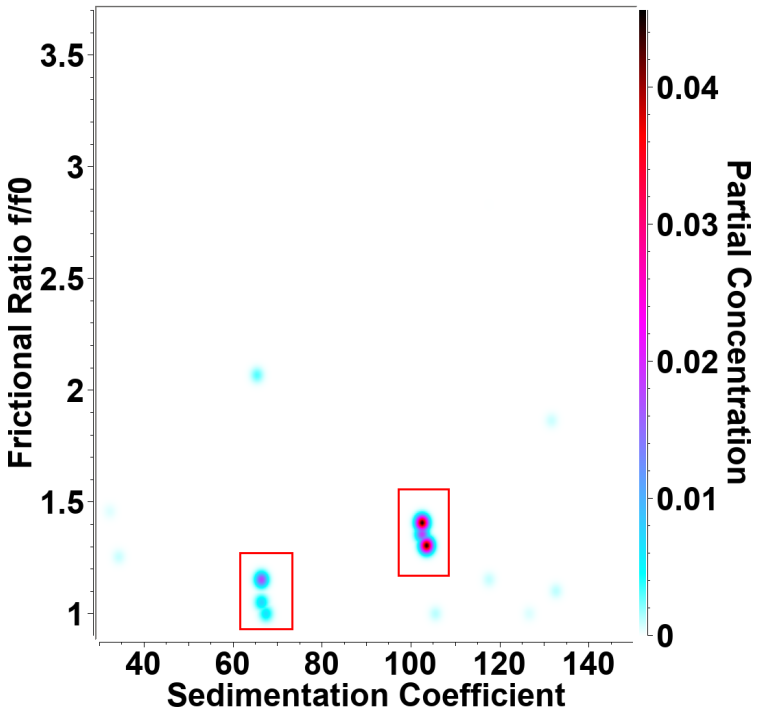

Boundary Fraction (%)
100
80
60
40
20
0
Protein
DNA
0
20
40
60
80
100
120
Sedimentation Coefficient

Integration results for major peaks of DNA:

Solute 1:
Sedimentation coefficient: 2.21e-12
Relative percentage: 14.0 %

Solute 2:
Sedimentation coefficient: 6.66e-12
Relative percentage: 11.1 %

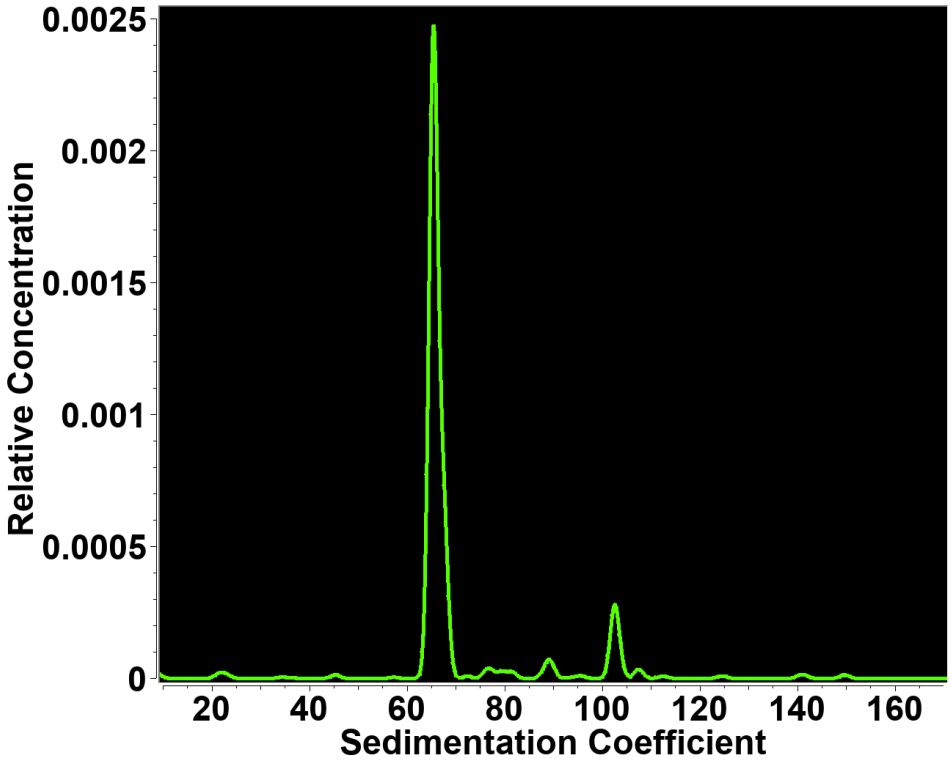
Solute 3:
Sedimentation coefficient: 1.03e-11
Relative percentage: 36.9 %



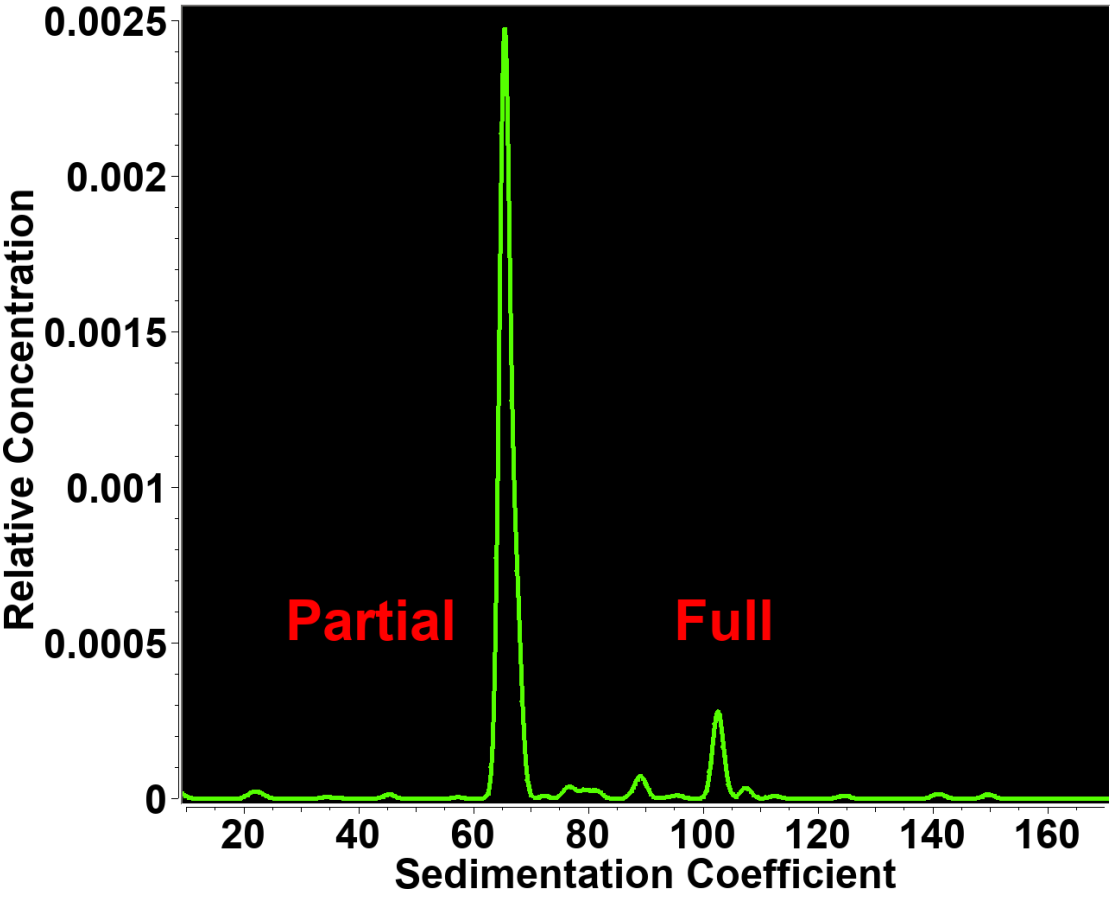
Integration results for major peaks of Protein:

Solute 1:
Sedimentation coefficient: 6.58e-12
Relative percentage: 75.6 %

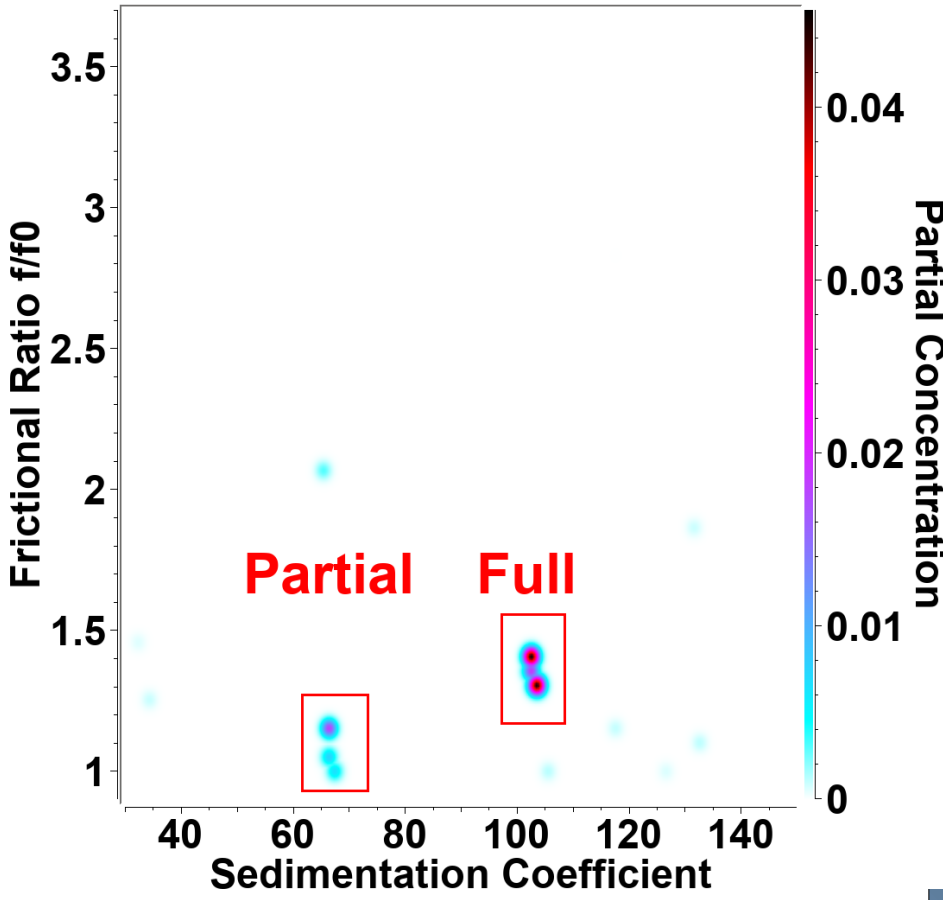
Solute 2:
Sedimentation coefficient: 1.03e-11
Relative percentage: 6.81 %



Capsid Protein Signal only



DNA Signal Only



Method 2:

Analytical Buoyant Density Equilibrium (ABDE)

$\bar{\nu}$ Determination by Analytical Buoyant Density Gradient Centrifugation

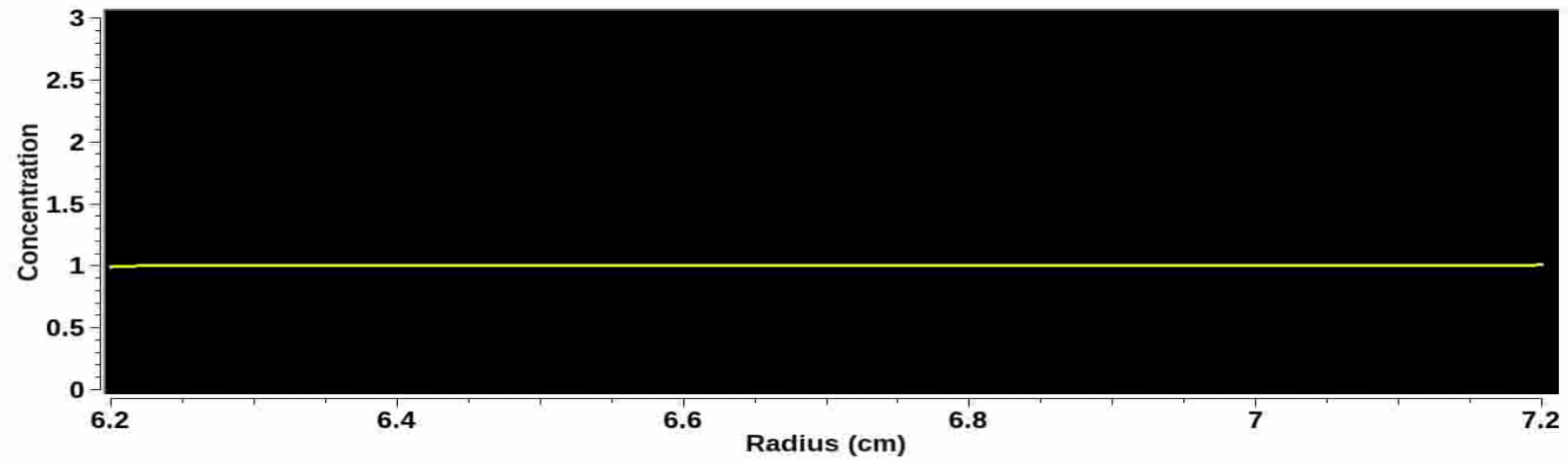
The equation of the concentration curve at equilibrium:

$$C(r) = C_0 e^{\delta(r^2 - r_m^2)}, \quad C(r) = C_{loading}\,\delta(r_b^2 - r_m^2)\frac{e^{\delta(r^2 - r_m^2)}}{e^{\delta(r_b^2 - r_m^2)} - 1}$$

were: $\delta = \dfrac{M\omega^2}{2RT}(1 - \bar{\nu}\rho)$

we need: $C_{loading}$, r_m, r_b, $\bar{\nu}$ (of gradient material)

The macromolecule co-sediments with the gradient forming material:

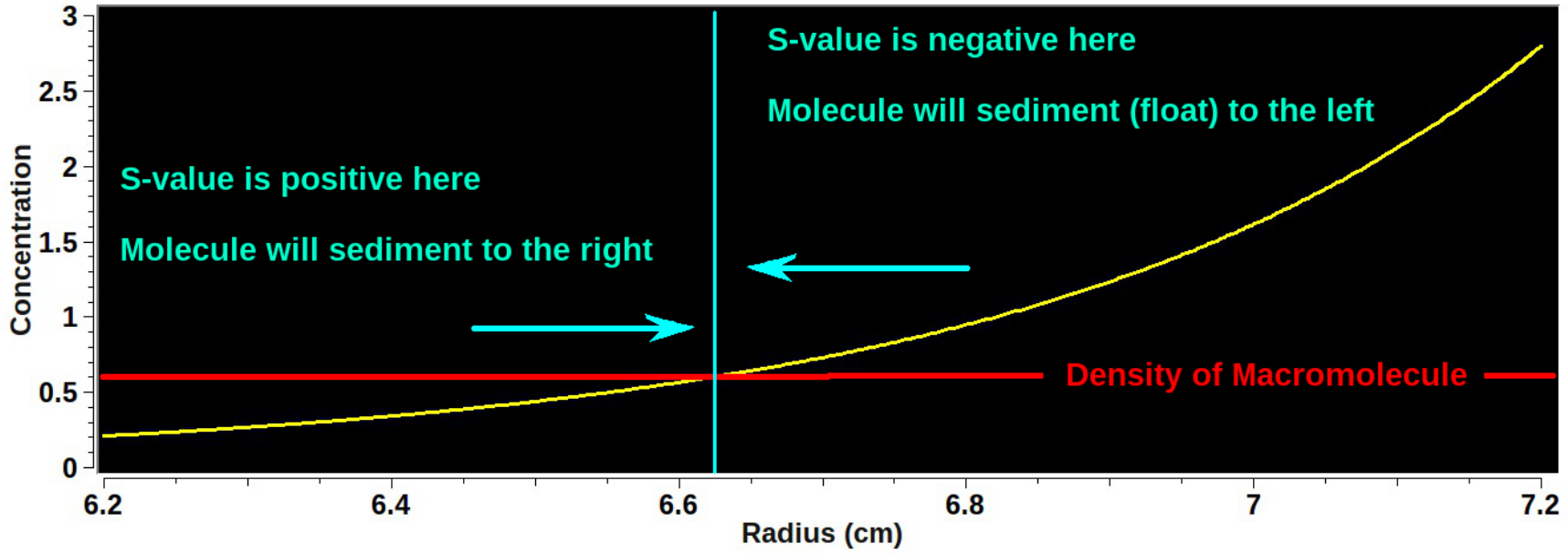


$\bar{\nu}$ Determination by Analytical Buoyant Density Gradient Centrifugation

The equation of the macromolecule's transport inside the gradient:

$$\left(\frac{\partial C}{\partial t}\right)_r = \frac{-1}{r}\frac{\partial}{\partial r}\left[s_{app}\,\omega^2 r^2 C - D_{app}\, r \frac{\partial C}{\partial r} \right]_t$$

$$\textit{with:} \quad s_{app} = s_0(\rho, \eta, r, t) \quad \textit{and} \quad D_{app} = D_0(\eta, r, t)$$



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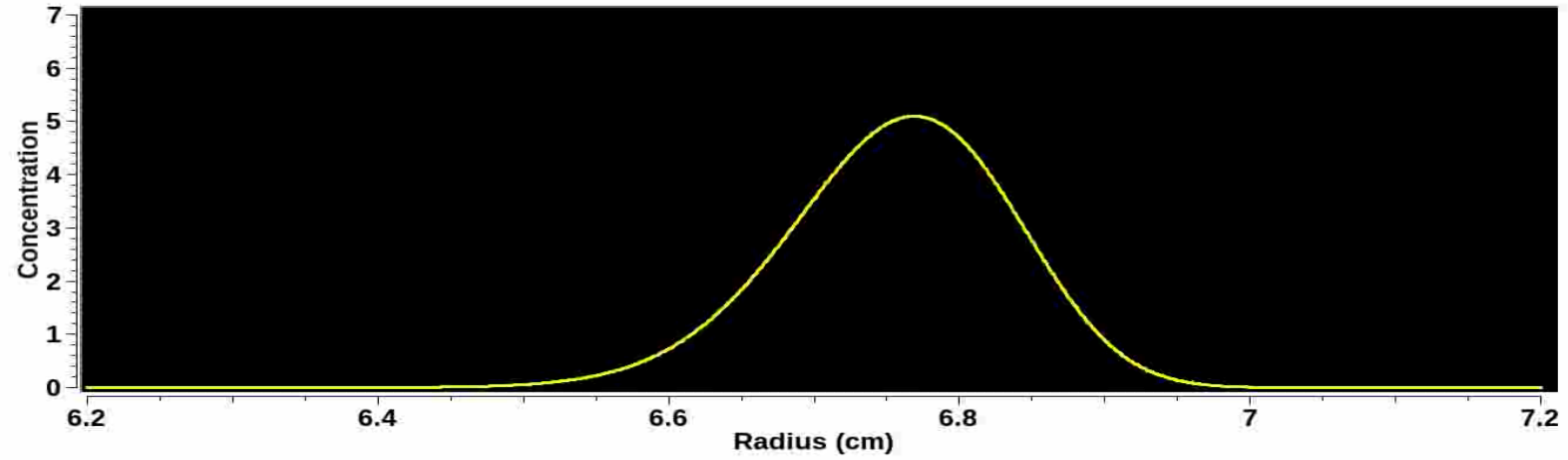

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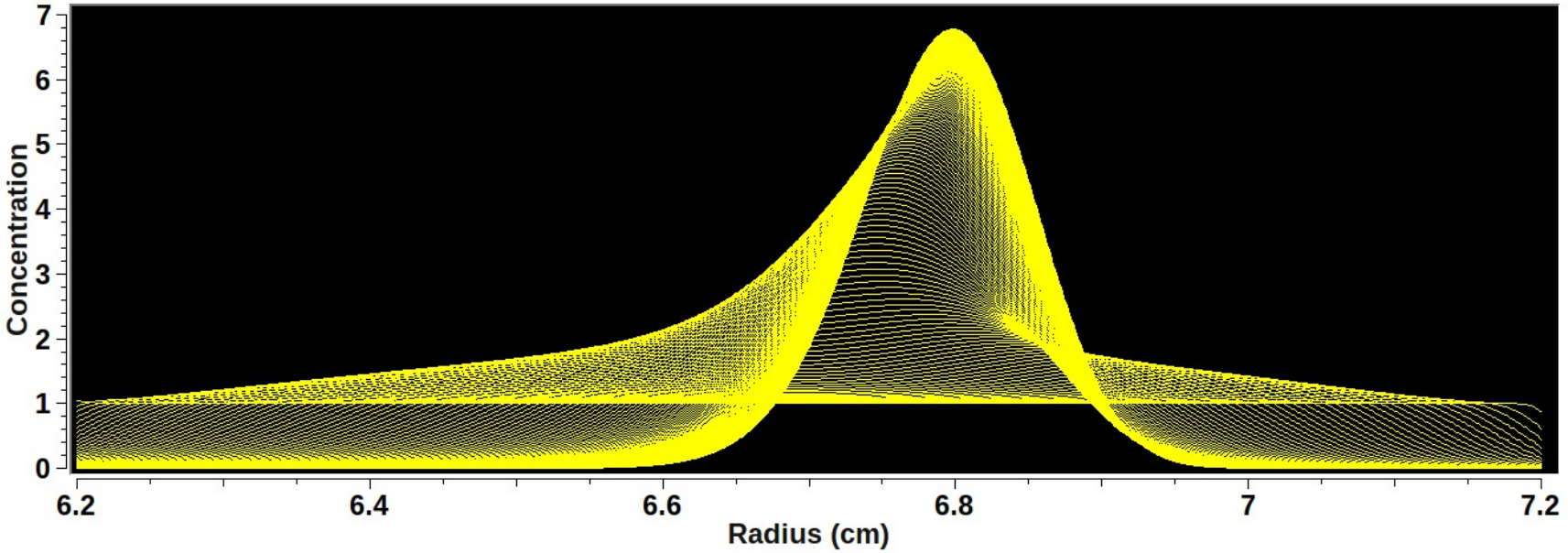
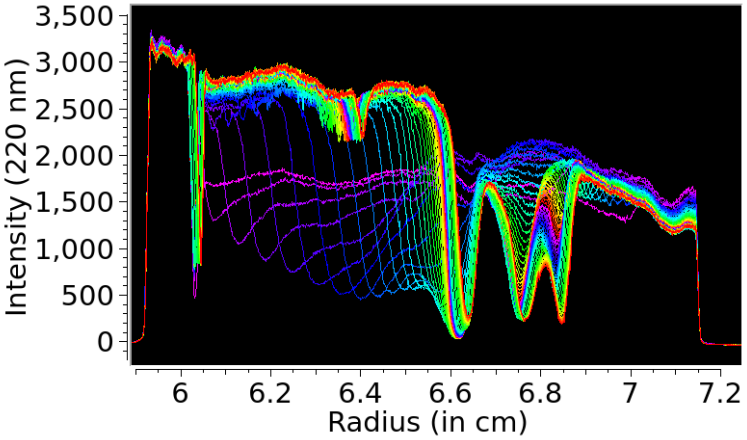
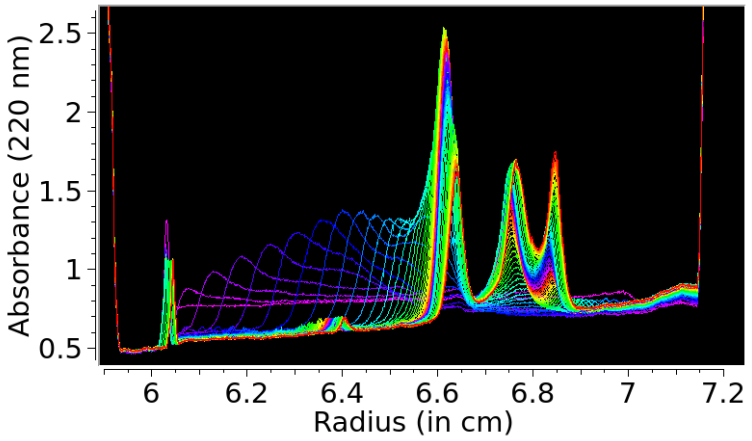
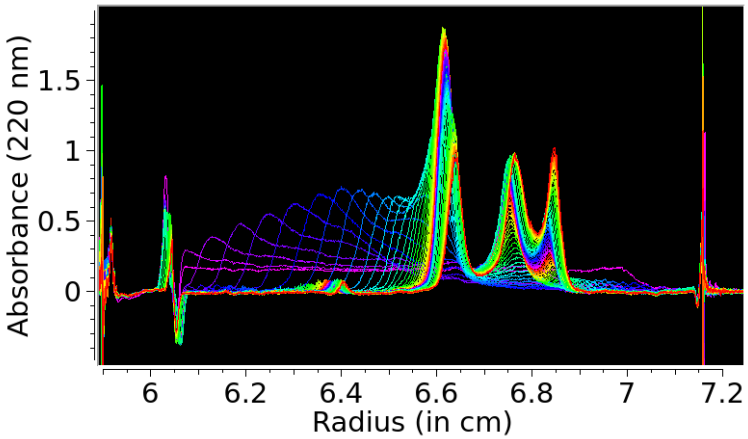


$\bar{\nu}$ Determination by Analytical Buoyant Density Gradient Centrifugation

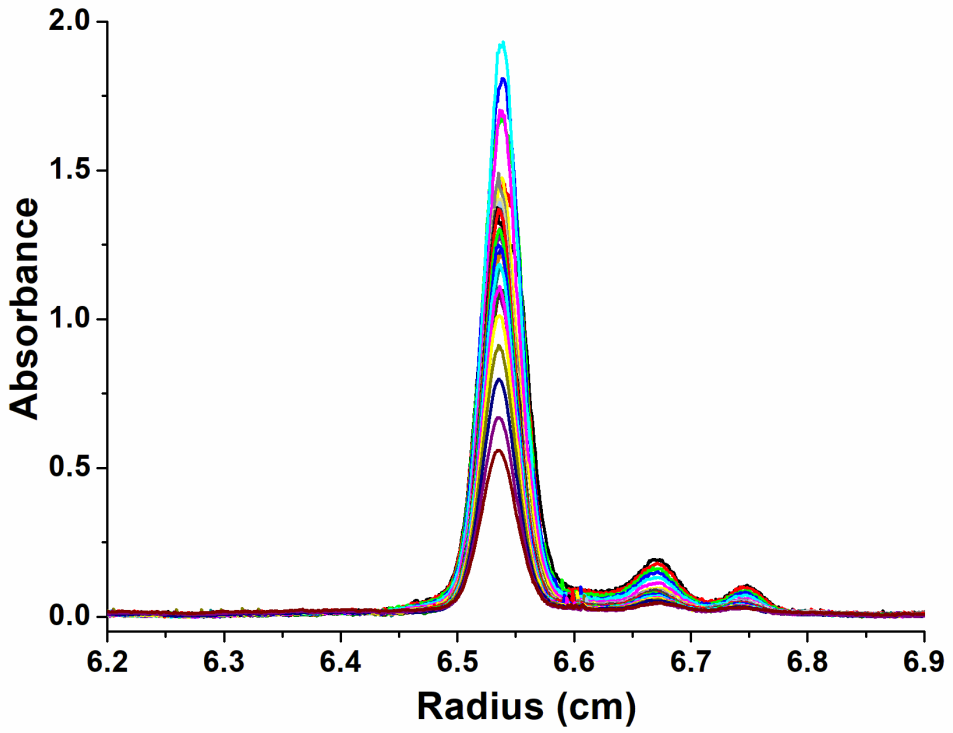






$\overline{\nu}$ *Determination by Analytical Buoyant Density Gradient Centrifugation*

Take advantage of the equilibrium condition of ABDE to collect up to 100 wavelengths:

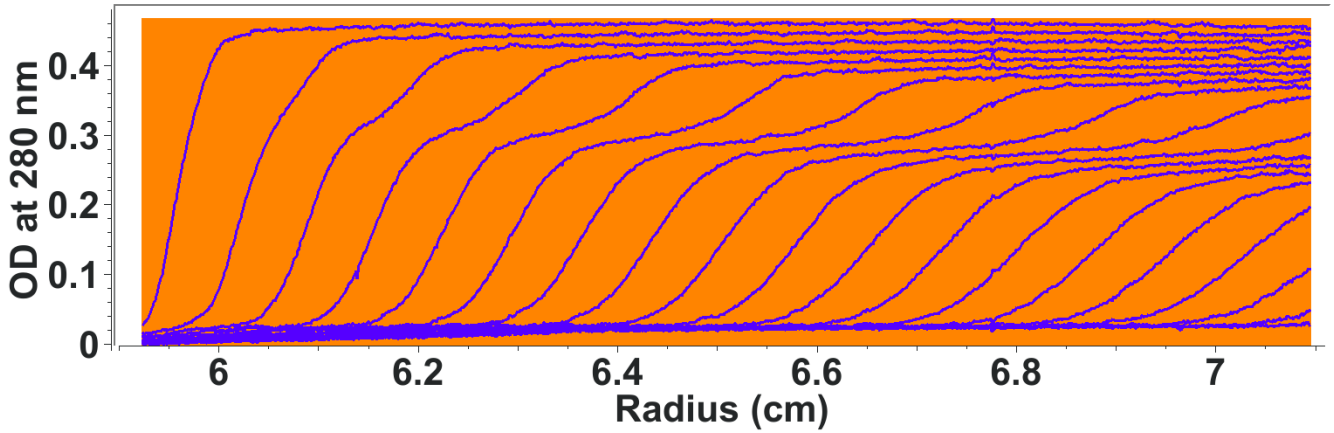


Obtain reliable spectral component separation and benefit from increased signal-to-noise due to inclusion of many wavelengths

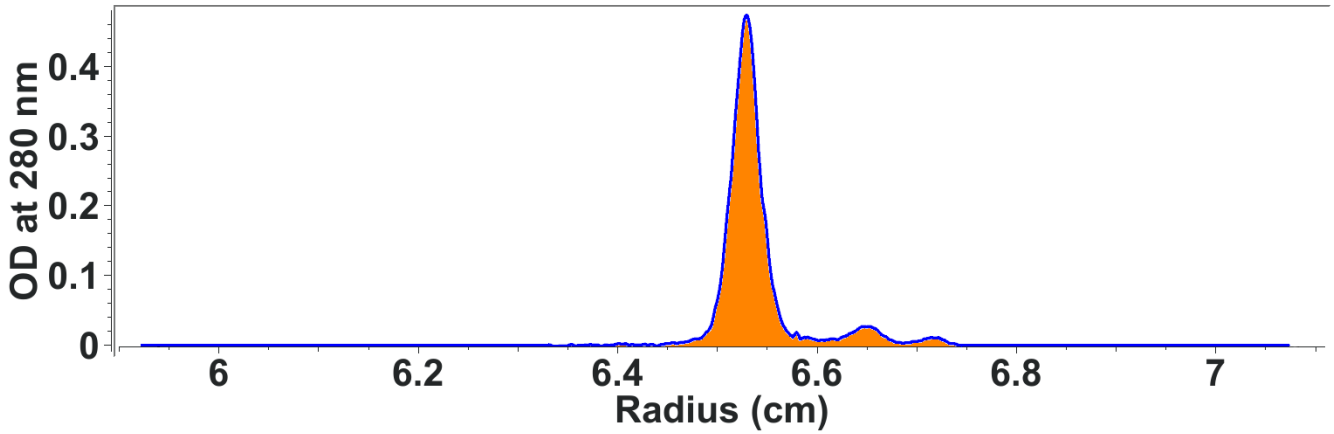
$\bar{\nu}$ *Determination by Analytical Buoyant Density Gradient Centrifugation*

Take advantage of the peak signal vs. boundary signal:

Sedimentation Velocity:

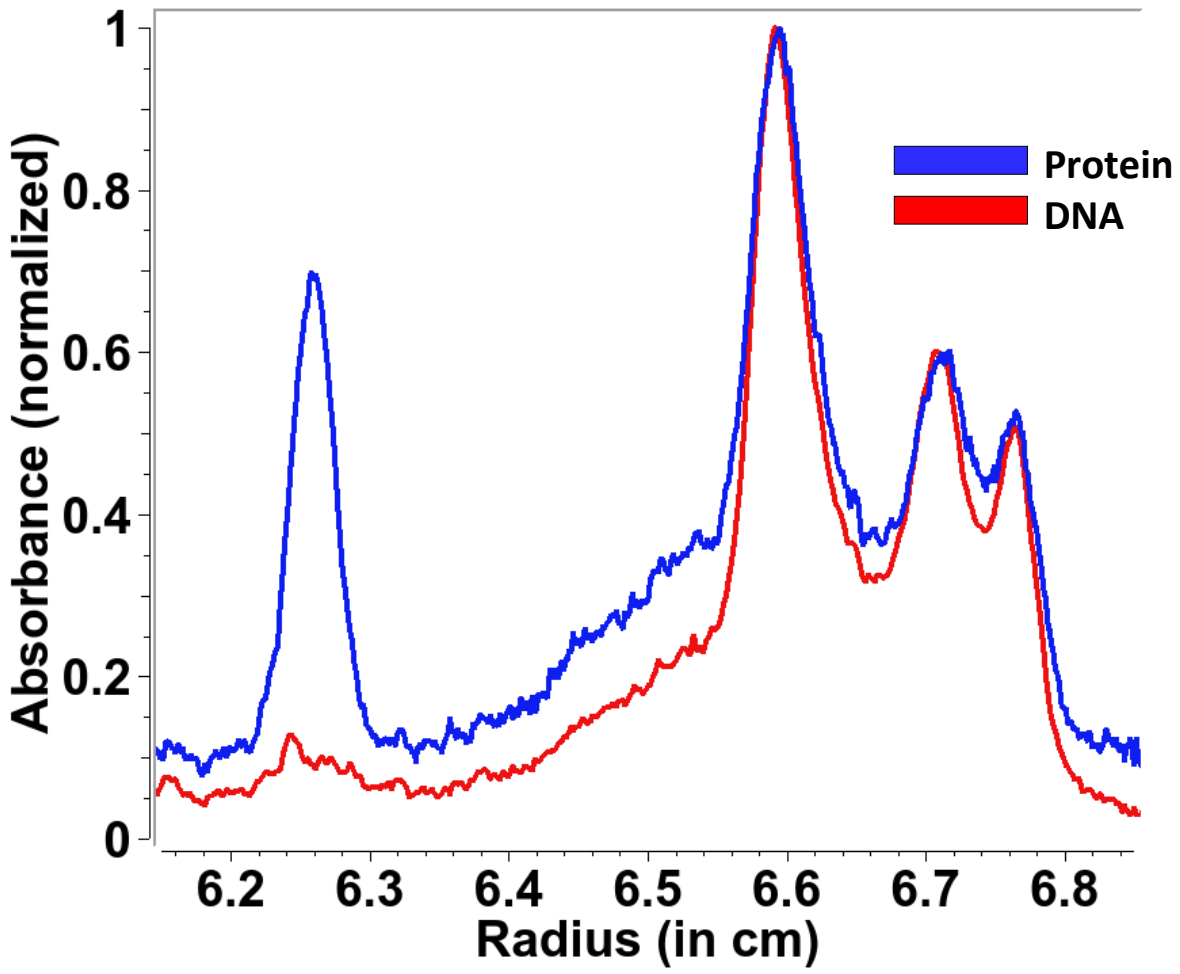


Analytical Buoyant Density Equilibrium (ABDE)



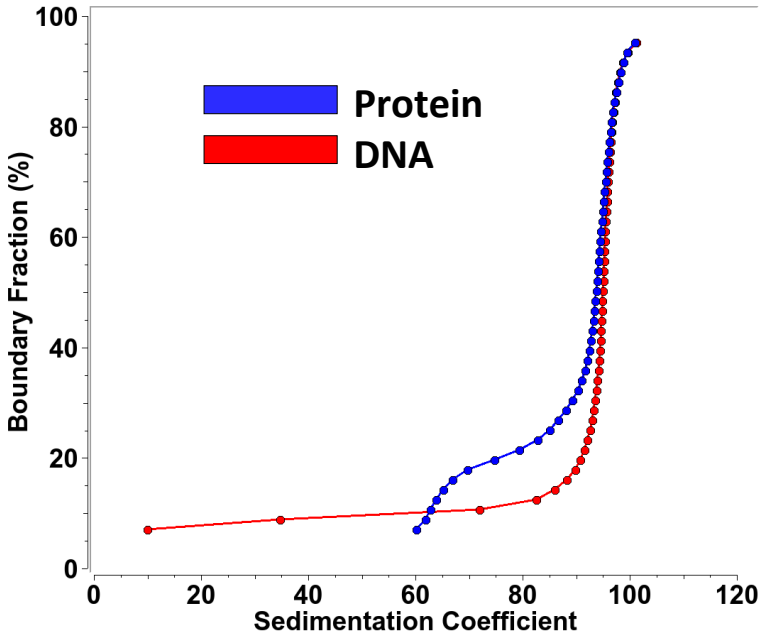
$\bar{\nu}$ *Determination by Analytical Buoyant Density Gradient Centrifugation*

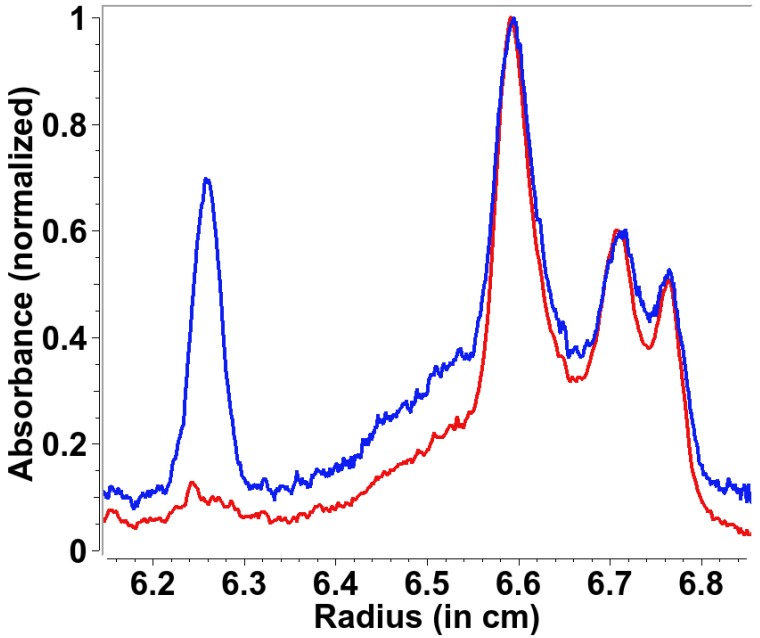
The equation of the concentration curve at equilibrium:



$\overline{\nu}$ *Determination by Analytical Buoyant Density Gradient Centrifugation*

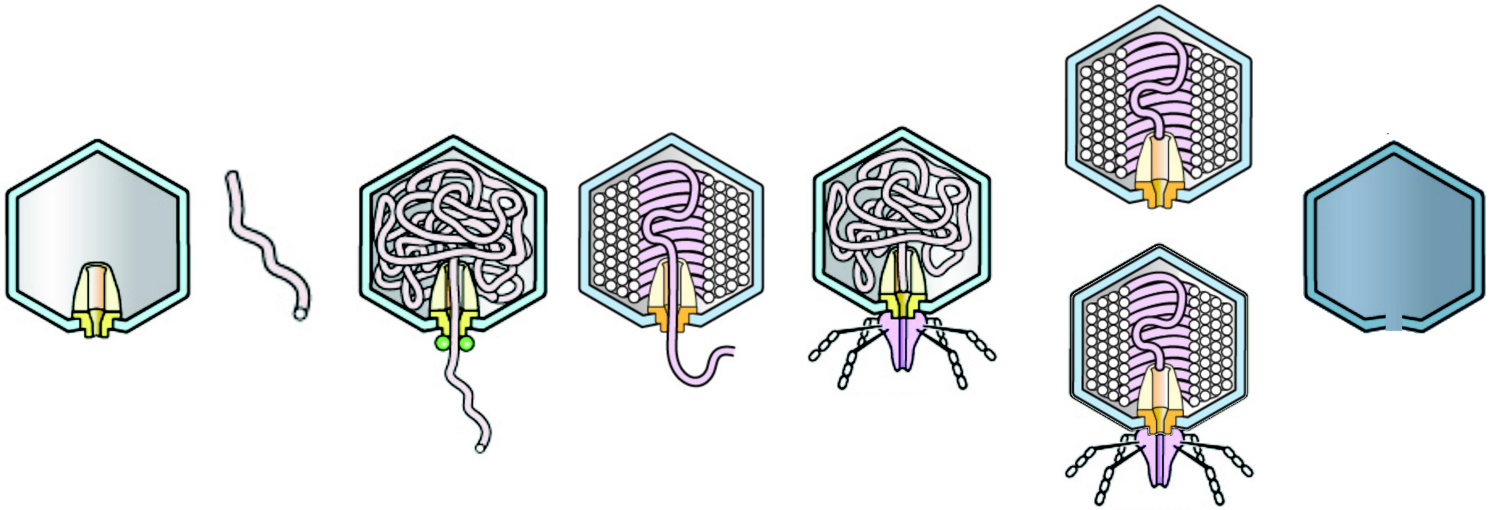
Sedimentation Velocity vs. ABDE





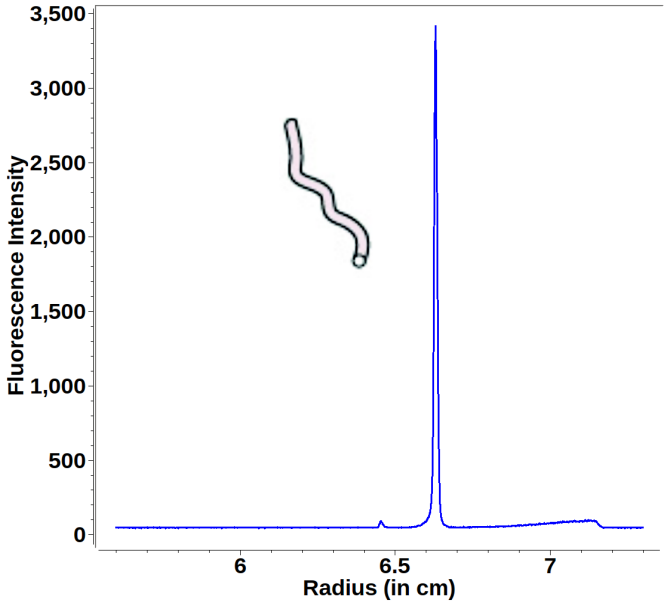
$\bar{\nu}$ *Determination by Analytical Buoyant Density Gradient Centrifugation*

Characterization of phage DNA packaging intermediates by analytical buoyant density centrifugation.

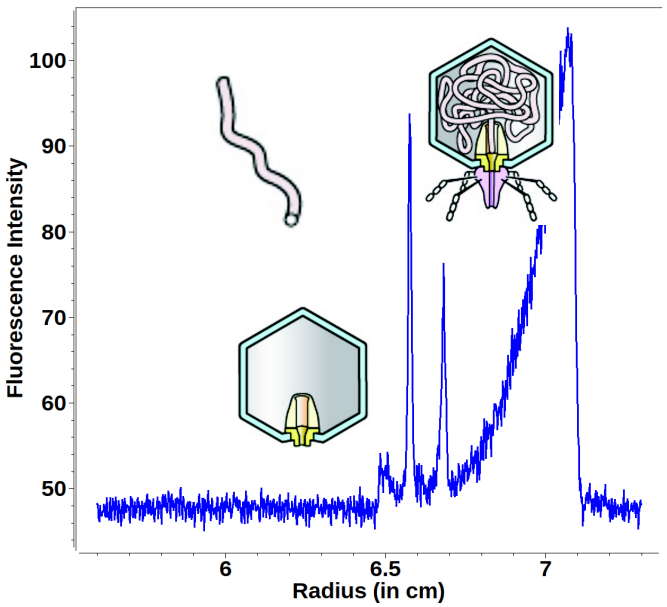


Increasing Density

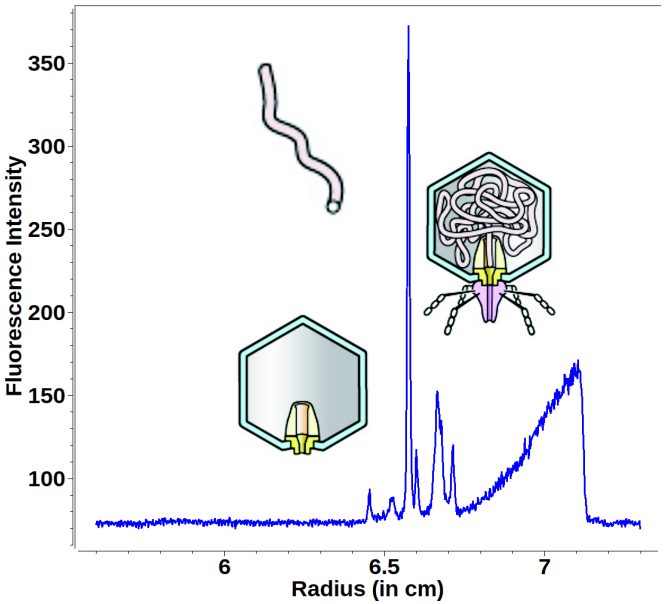
Observed density depends on the degree of hydration, which depends on the solvent!



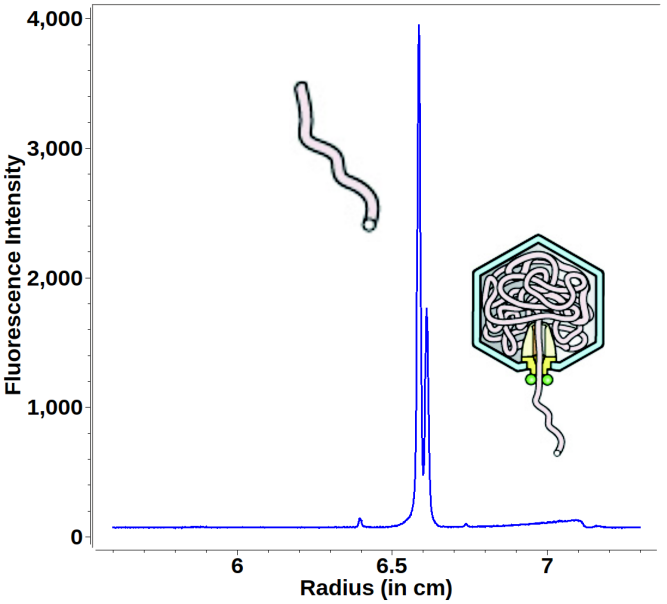
Purified T7Φ DNA



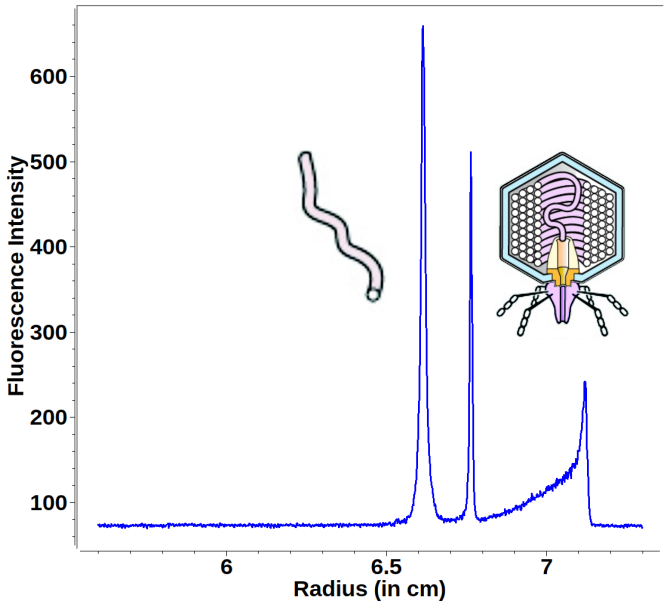
T3Φ



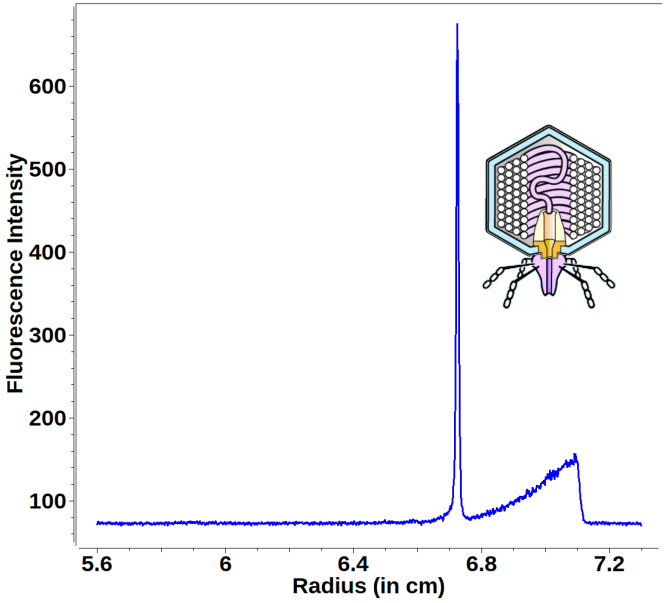
T3Φ



T7-heated



T7



T3