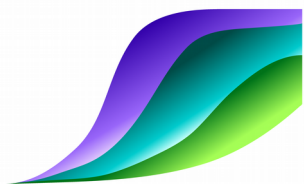


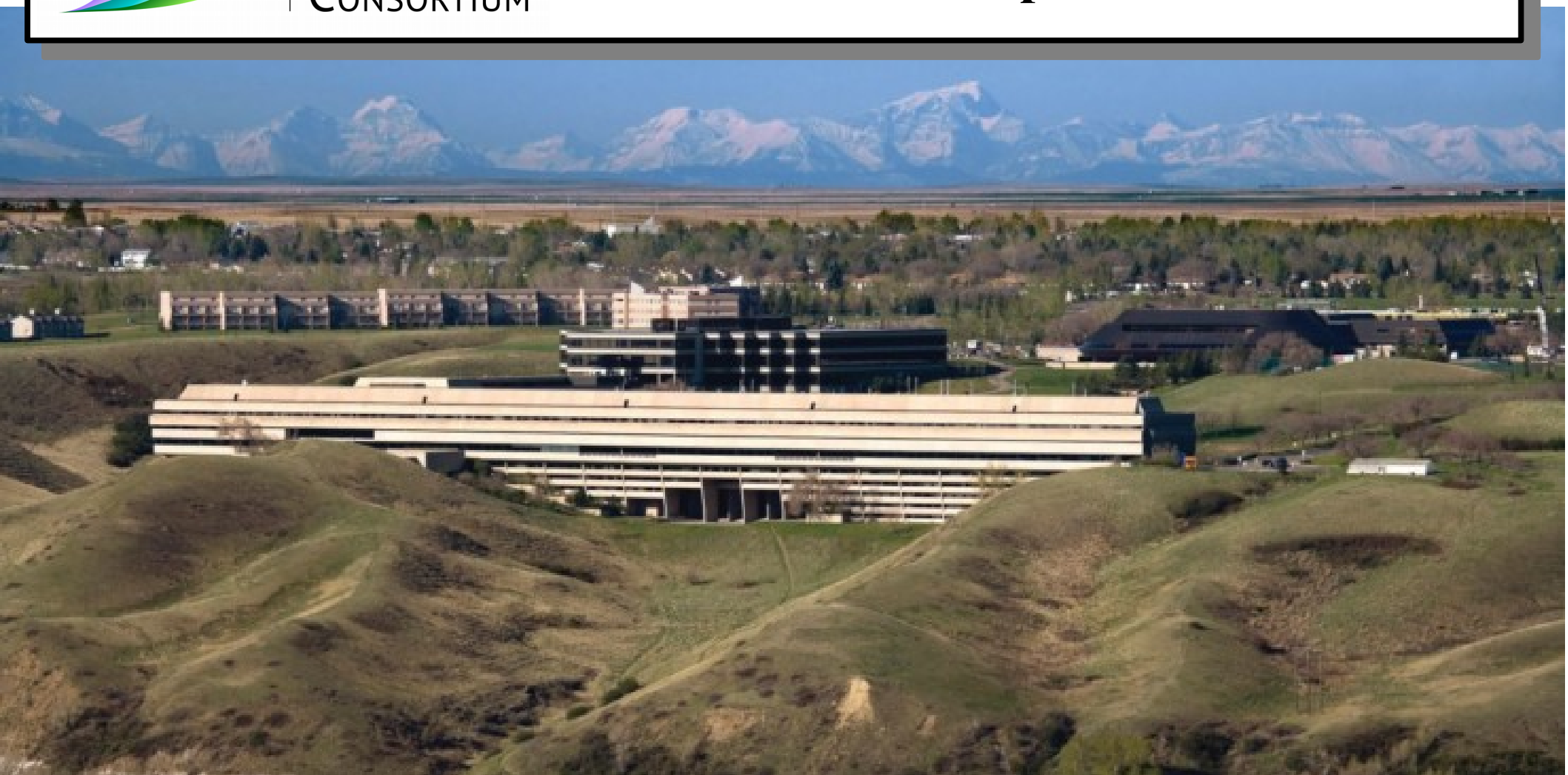


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NORTHWEST
BIOPHYSICS
CONSORTIUM

Custom Grid, Density and Partial Specific Volume



\bar{V} Determination

Standard velocity analysis provides s and D , but not the partial specific volume, \bar{V} . This parameter is needed to obtain an accurate absolute molecular weight.

\bar{V} is highly solvent dependent. There are multiple ways to get \bar{V} :

- Densitometer (sample must be homogeneous!)
- Density matching with H_2O_{18} or D_2O
- *Custom Grid* methods
 - Re-parameterize the sedimentation/diffusion space
 - Introduce prior knowledge to extend the solution to other parameters
- Analytical buoyant density centrifugation
 - The FDS is ideally suited for analytical buoyant density centrifugation:
 - High radial resolution
 - No refractive artifacts from gradient forming materials
 - High sensitivity – only very small amounts needed

\bar{V} Determination by Analytical Buoyant Density Gradient Centrifugation

Analytical Buoyant Density Gradient Centrifugation can provide \bar{V} by measuring the isopycnic position of a macromolecule in the density gradient.

This requires two components:

- a) The gradient forming material
 - Non-ionic: Nycodenz, Iodixanol, Metrizamide
 - Ionic: CsCl, CsSO₄
- b) The macromolecule to be measured

The sedimentation velocity experiment will then sediment the macromolecule in the co-sedimenting solute, subject to an ever changing density environment. The experiment is run until equilibrium is reached. The equilibrium radial position is observed, and the density is calculated from the known sedimentation properties of the gradient forming material. The calculation requires knowledge of:

- c) The meniscus position
- d) The bottom of the cell
- e) The v_{bar} and molecular weight of the gradient forming material
- f) The exact loading concentration of the gradient forming material

\bar{v} Determination by Analytical Buoyant Density Gradient Centrifugation

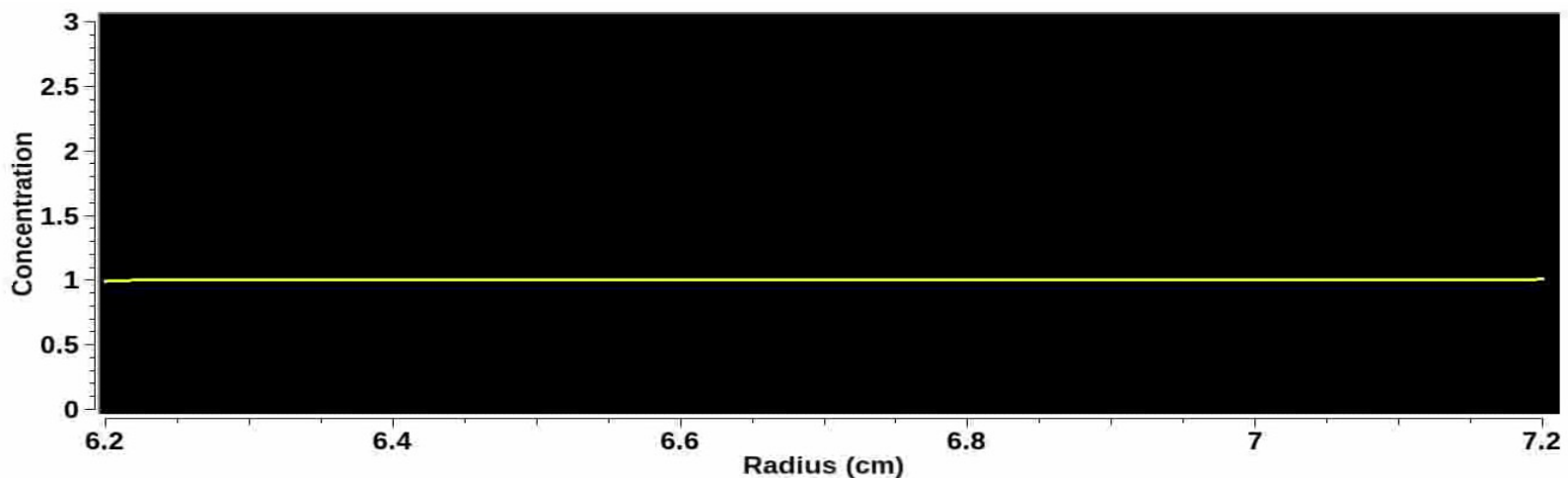
The equation of the concentration curve at equilibrium:

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

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

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

The macromolecule co-sediments with the gradient forming material:

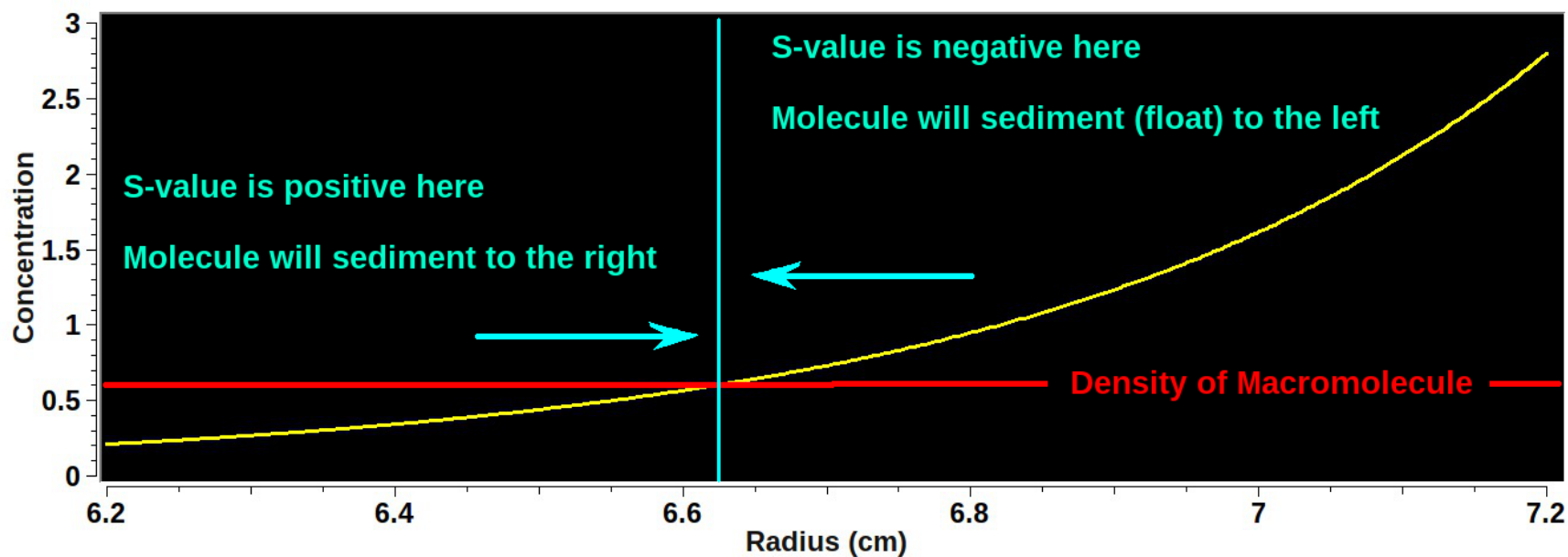


\bar{V} Determination by Analytical Buoyant Density Gradient Centrifugation

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

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

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

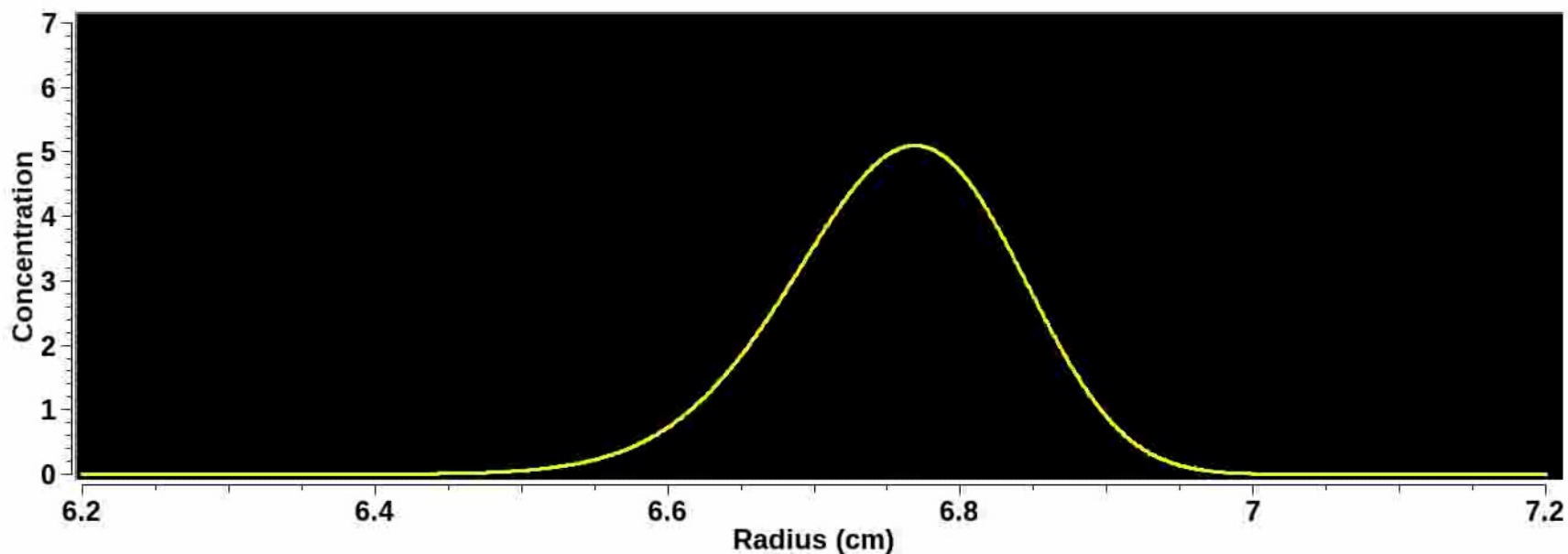


\bar{V} Determination by Analytical Buoyant Density Gradient Centrifugation

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

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

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



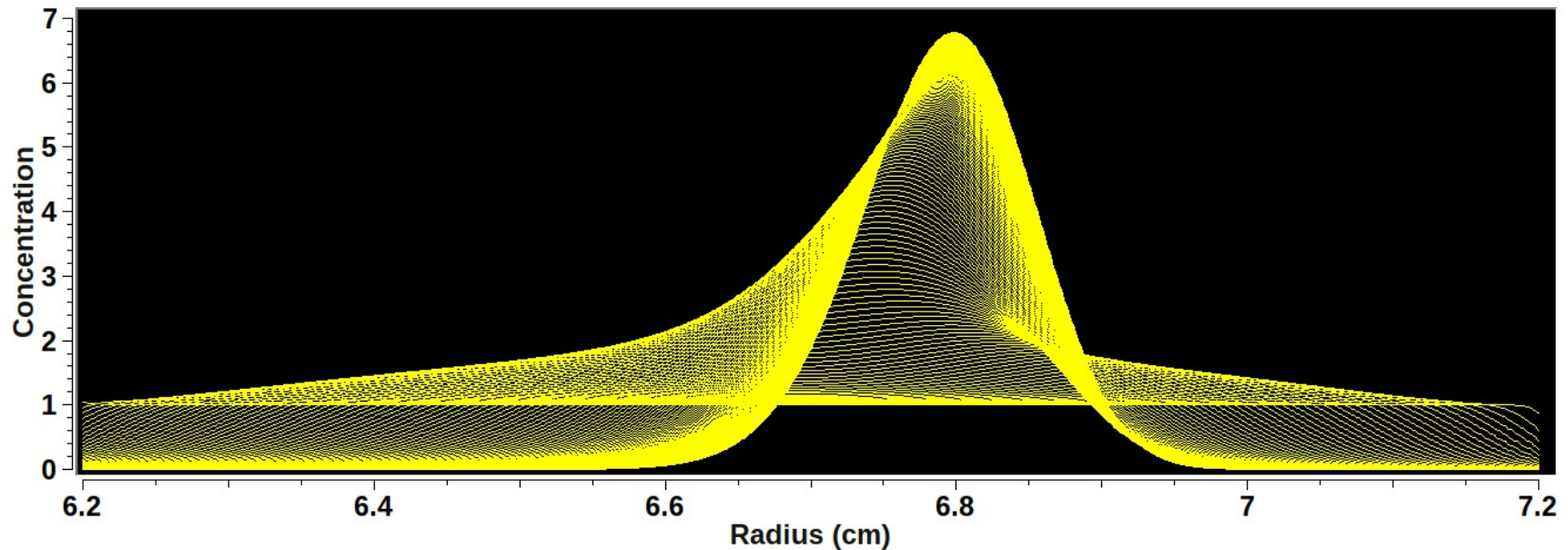
\bar{v} Determination by Analytical Buoyant Density Gradient Centrifugation

The equation of the concentration curve at equilibrium:

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

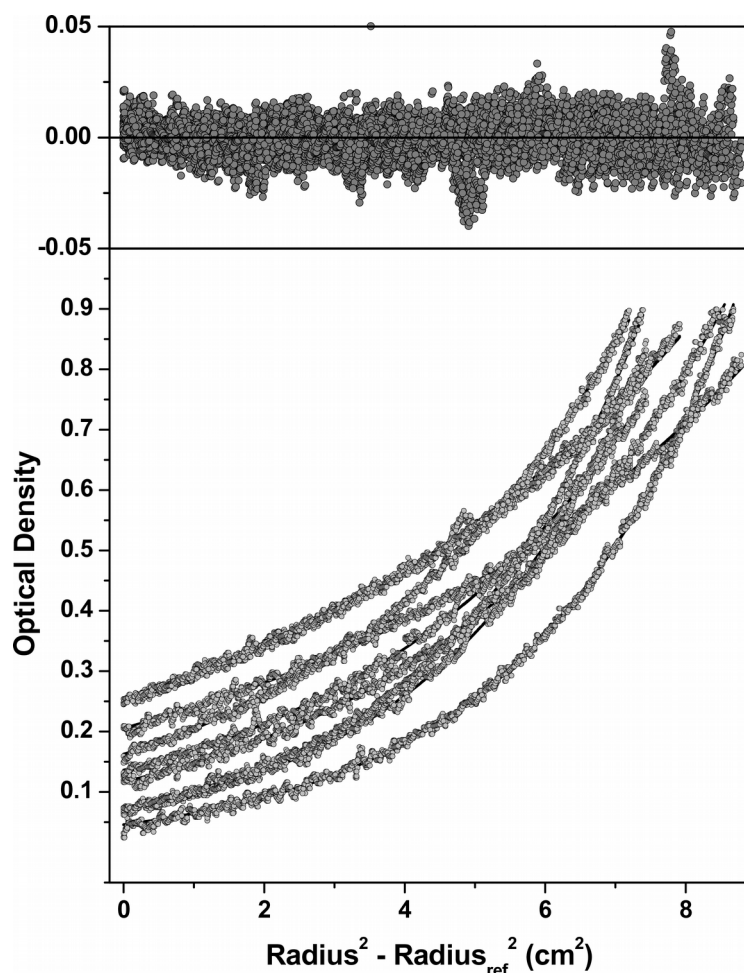
were: $\delta = \frac{M \omega^2}{2RT} (1 - \bar{v} \rho)$

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

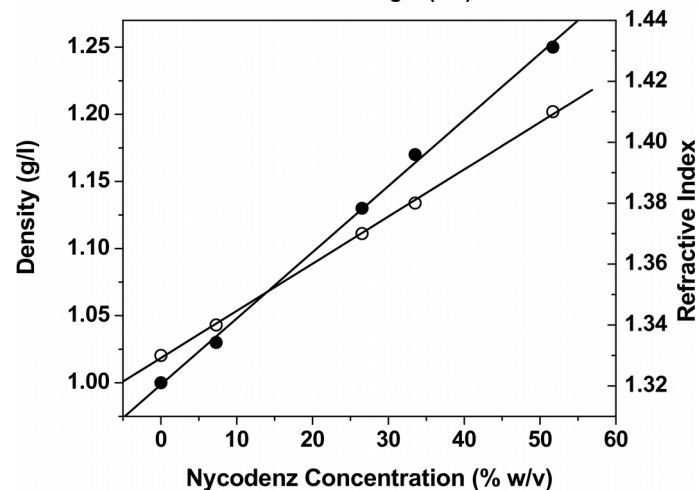
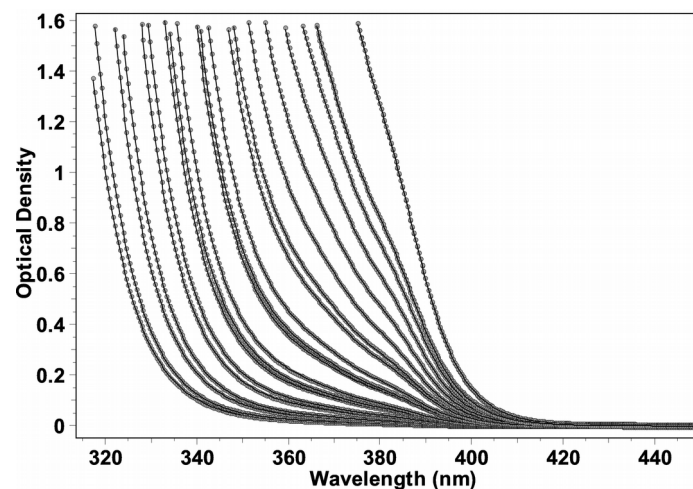


\bar{v} Determination by Analytical Buoyant Density Gradient Centrifugation

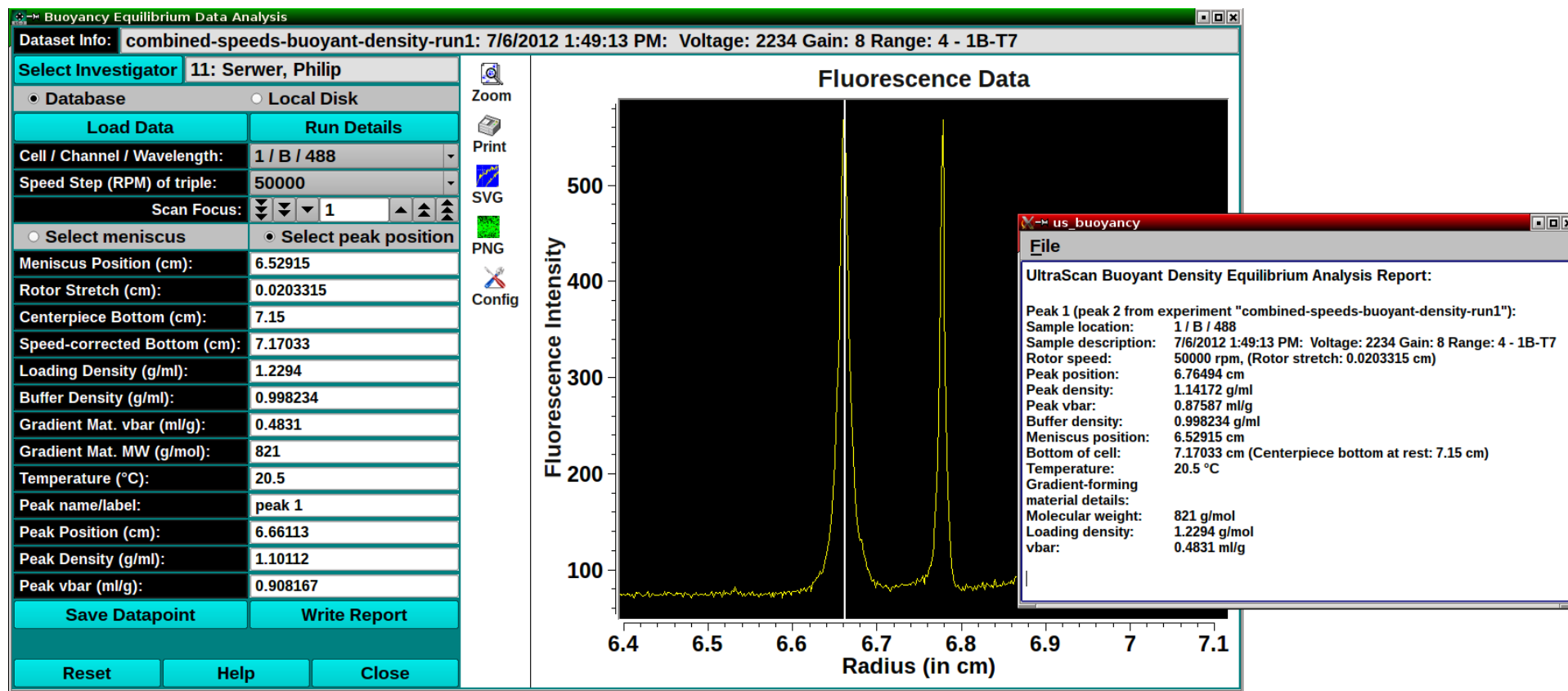
Determine the \bar{v} for the gradient forming materials with a sedimentation equilibrium experiment (MW is known), fix the MW, float the \bar{v} :



Determine the loading concentration for the gradient forming materials with a refractometer or by UV absorbance



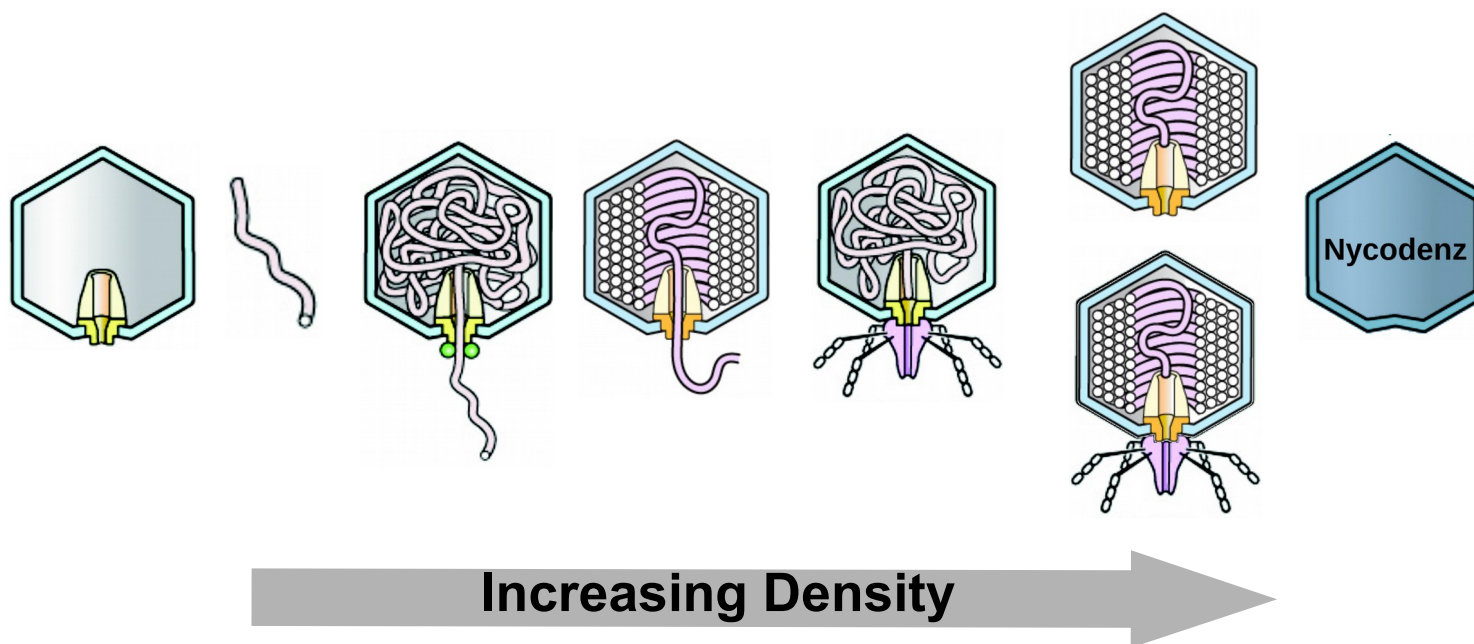
\bar{v} Determination by Analytical Buoyant Density Gradient Centrifugation



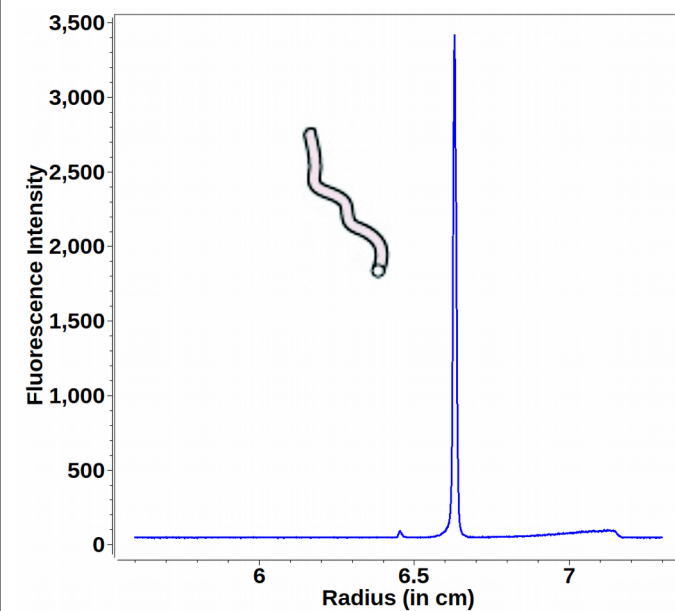
UltraScan-III Buoyancy Calculator

\bar{V} Determination by Analytical Buoyant Density Gradient Centrifugation

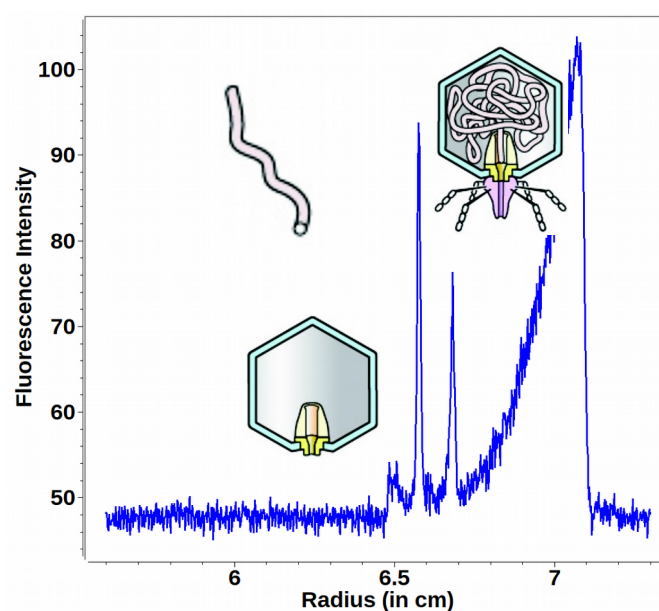
Characterization of phage DNA packaging intermediates by analytical Nycodenz buoyant density centrifugation.



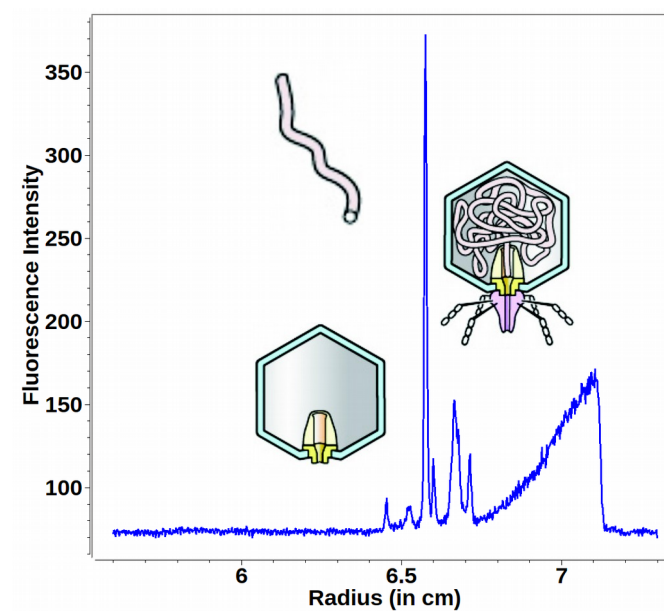
Nycodenz will separate based on hydration, which is very different for DNA and protein. In fact, DNA has a much lower density when suspended in Nycodenz due to hydration than protein.



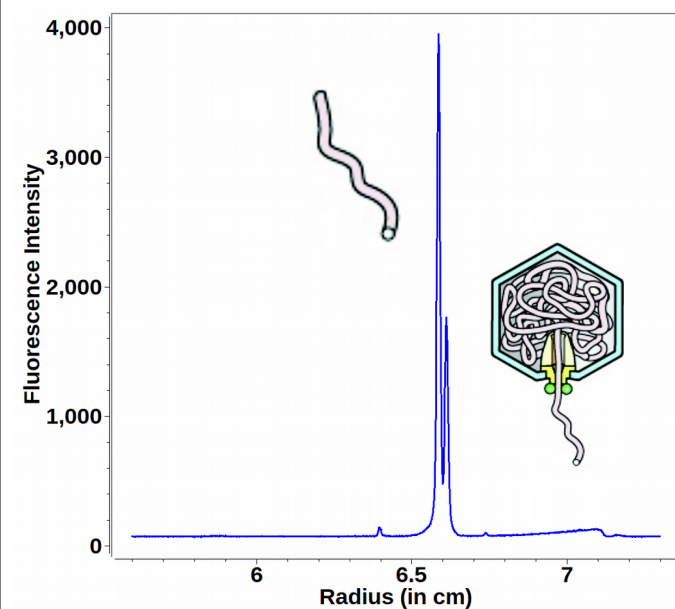
Purified T7Φ DNA



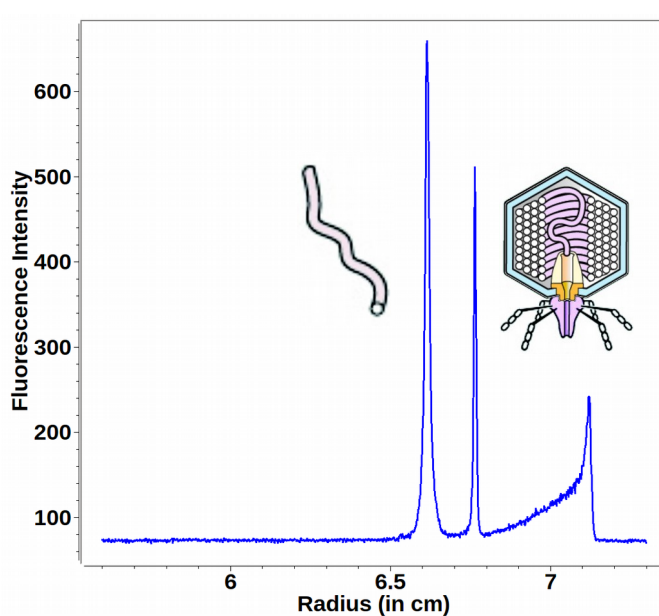
T3Φ



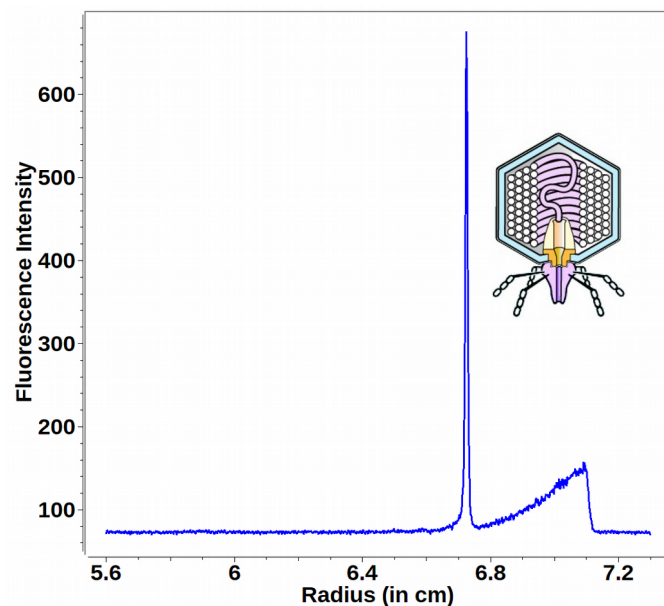
T3Φ



T7-heated

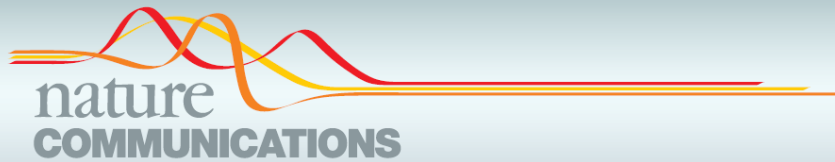


T7



T3

Differential Density Contrast Sedimentation



ARTICLE

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Solution-state conformation and stoichiometry of yeast Sir3 heterochromatin fibres

Sarah G. Swygert¹, Benjamin J. Manning¹, Subhadip Senapati², Parminder Kaur²,
Stuart Lindsay², Borries Demeler³ & Craig L. Peterson¹

This manuscript describes a how differential density contrast sedimentation is used to derive partial specific volumes of DNA-protein complexes by varying the density of the buffer and then derive accurate molecular weights and anisotropies. Using this approach, the stoichiometry of Sir3 binding to heterochromatin can be derived. Critical differences between histone H4 wildtype and mutant effects on binding Sir3, and on the conformational properties of heterochromatin fibers can be deduced.

Differential Density Contrast Sedimentation

Principle:

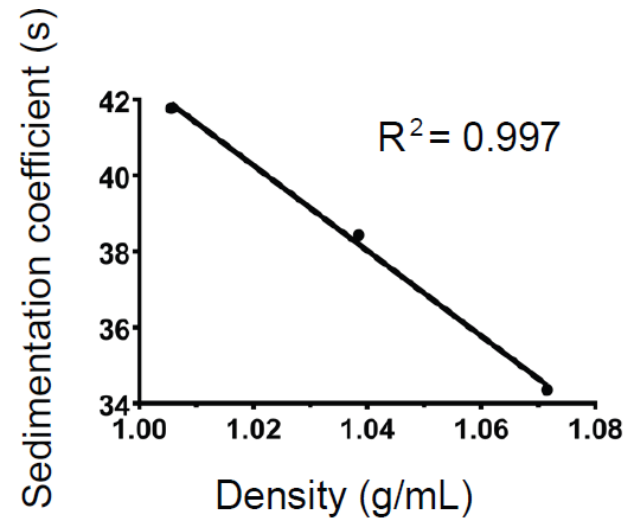
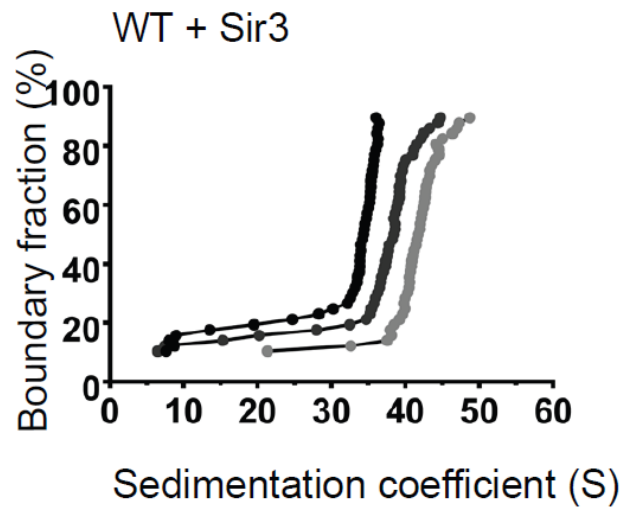
Measure the apparent sedimentation coefficient in 3 buffers with different densities. Use H₂¹⁸O instead of D₂O to avoid deuterium exchange since that would affect the \bar{v} . Use 0, 30% and 60% H₂¹⁸O and extrapolate the density to the point where sedimentation would cease – that's the isopycnic point where the density matches that of the analyte.

$$s = \frac{M (1 - \bar{v} \rho)}{Nf}$$

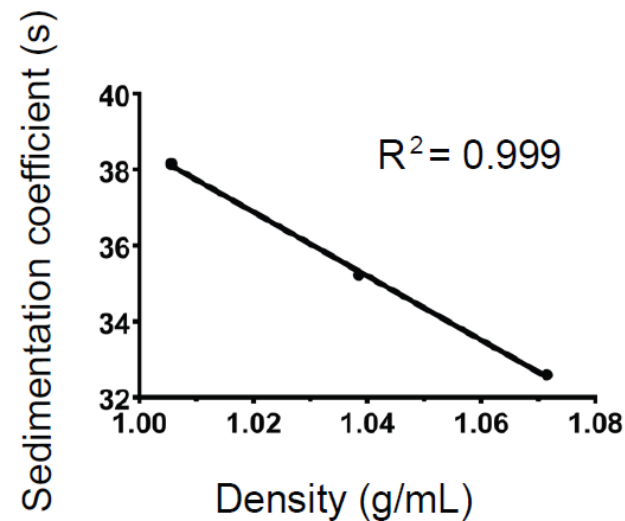
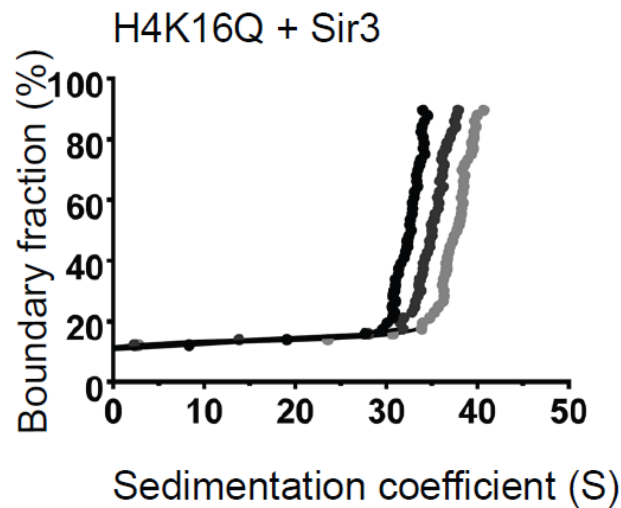
When the ratio of \bar{v}/ρ equals 1.0, the buoyancy term equals zero and sedimentation ceases.

Once the \bar{v} has been determined, it can be used to fit the data to a size/anisotropy grid with 2DSA or genetic algorithms to calculate accurate molecular weights and to determine conformational properties.

Differential Density Contrast Sedimentation



$$\bar{v} = 0.726 \text{ mL/g}$$



$$\bar{v} = 0.686 \text{ mL/g}$$

Characterization of Size, Anisotropy, and Density Heterogeneity of Nanoparticles by Sedimentation Velocity

Borries Demeler,^{*,†} Tich-Lam Nguyen,[‡] Gary E. Gorbet,[†] Virgil Schirf,[†] Emre H. Brookes,[†]
Paul Mulvaney,[‡] Ala'a O. El-Ballouli,[§] Jun Pan,[§] Osman M. Bakr,[§] Aysha K. Demeler,[†]
Blanca I. Hernandez Uribe,[†] Nabraj Bhattarai,[⊥] and Robert L. Whetten[⊥]

The sedimentation and diffusion coefficients can be re-parameterized when certain properties are known *a priori*. The *Custom Grid* approach allows the investigator to introduce prior knowledge, providing constant parameters known from external experiments to constrain the experiment's range and obtain the missing parameters.

Analysis is performed analogous to the two-dimensional spectrum analysis.

Custom Grid Method

The sedimentation and diffusion coefficients can be re-parameterized when certain properties are known *a priori*. The Lamm equation requires the sedimentation and diffusion coefficients for simulating transport.

$$D = \frac{RT}{Nf} \quad s = \frac{M(1 - \bar{v}\rho)}{Nf} \quad \bar{v} = ???$$

From densitometry, sequence, or density matching – fix \bar{v}

- Molecular weight and anisotropy can be obtained

From electron microscopy or SAXS or X-ray/NMR – fix anisotropy:

- \bar{v} and molecular weight can be obtained

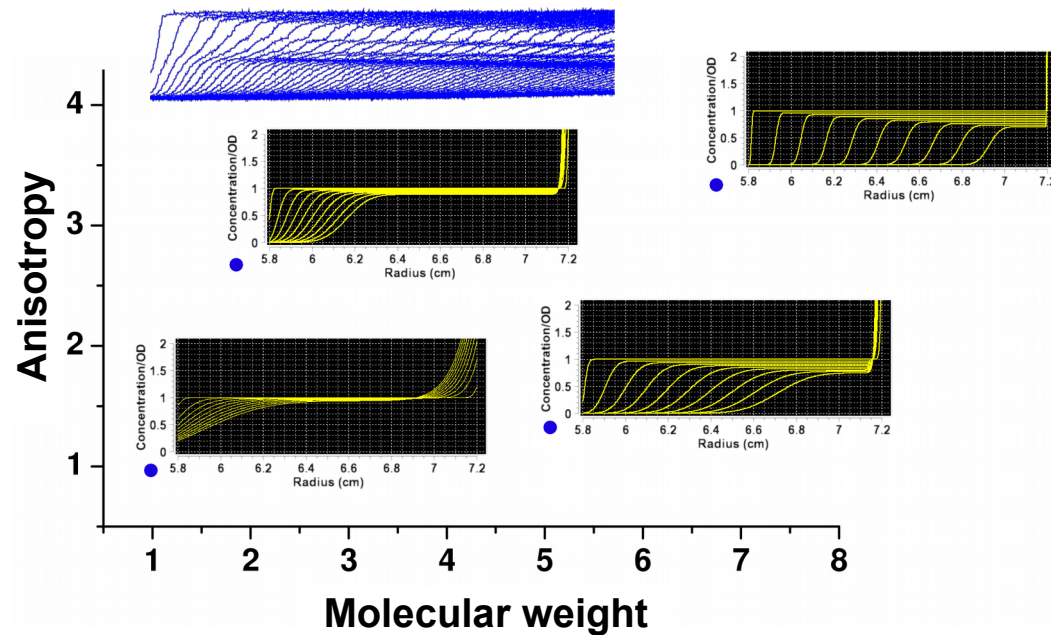
From mass spectrometry or polymer sequence – fix molecular weight:

- \bar{v} and anisotropy can be obtained

Custom Grid Method

From densitometry of polymer sequence – fix partial specific volume:

- Molecular weight and anisotropy can be obtained
- Create a grid over anisotropy (ϕ) and molecular weight (M)



$$V = \frac{M \bar{v}}{N}$$

$$r_0 = \left(\frac{3V}{4\pi} \right)^{1/3}$$

$$f_0 = 6\pi\eta r_0$$

$$f = \phi f_0$$

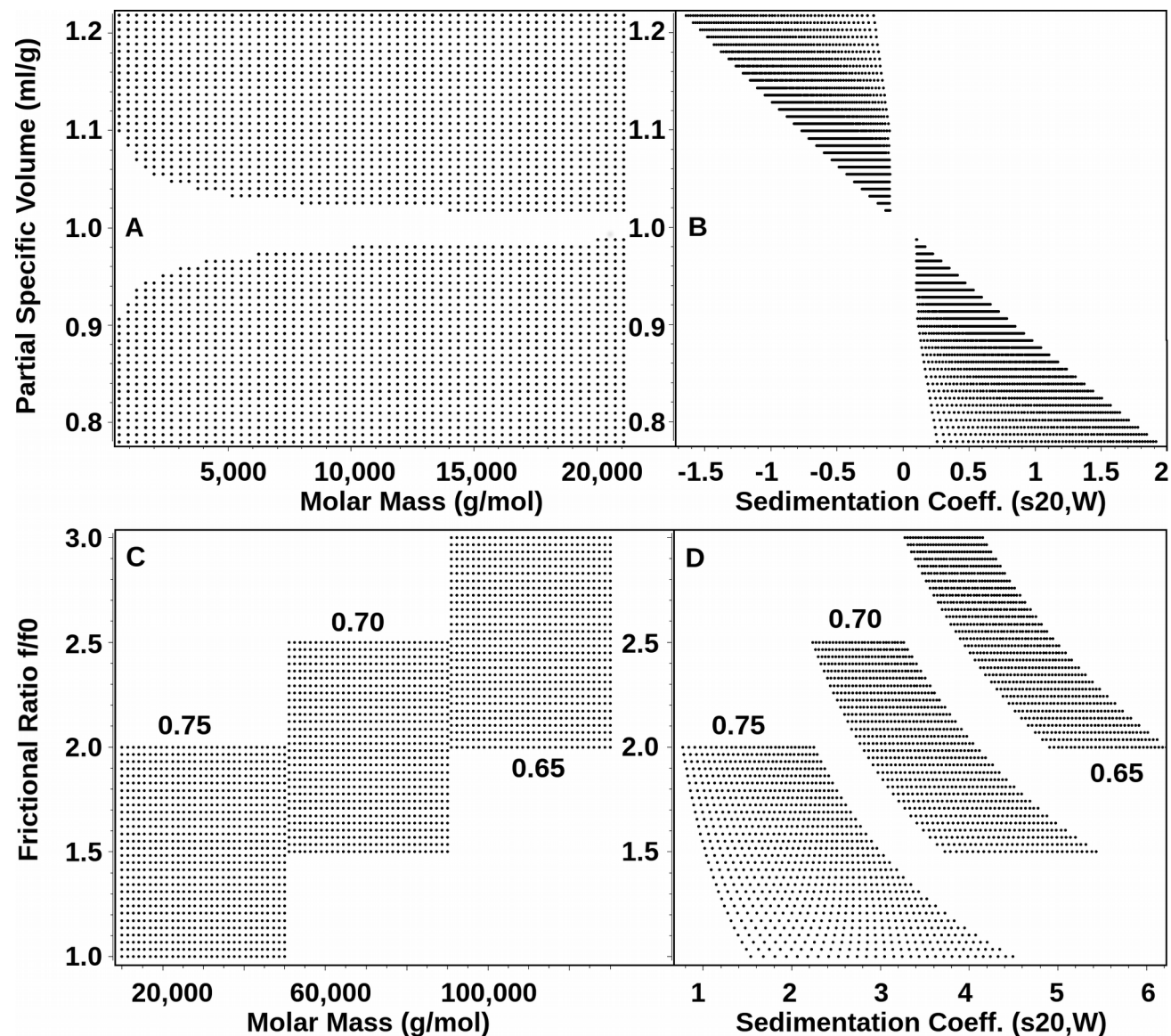
$$s = \frac{M(1 - \bar{v}\rho)}{N f}$$

$$D = \frac{RT}{N f}$$

$$\left(\frac{\partial C}{\partial t} \right)_r = \frac{-1}{r} \frac{\partial}{\partial r} \left[\underbrace{s \omega^2 r^2 C}_{\text{Sedimentation}} - \underbrace{D r \frac{\partial C}{\partial r}}_{\text{Diffusion}} \right]_t$$

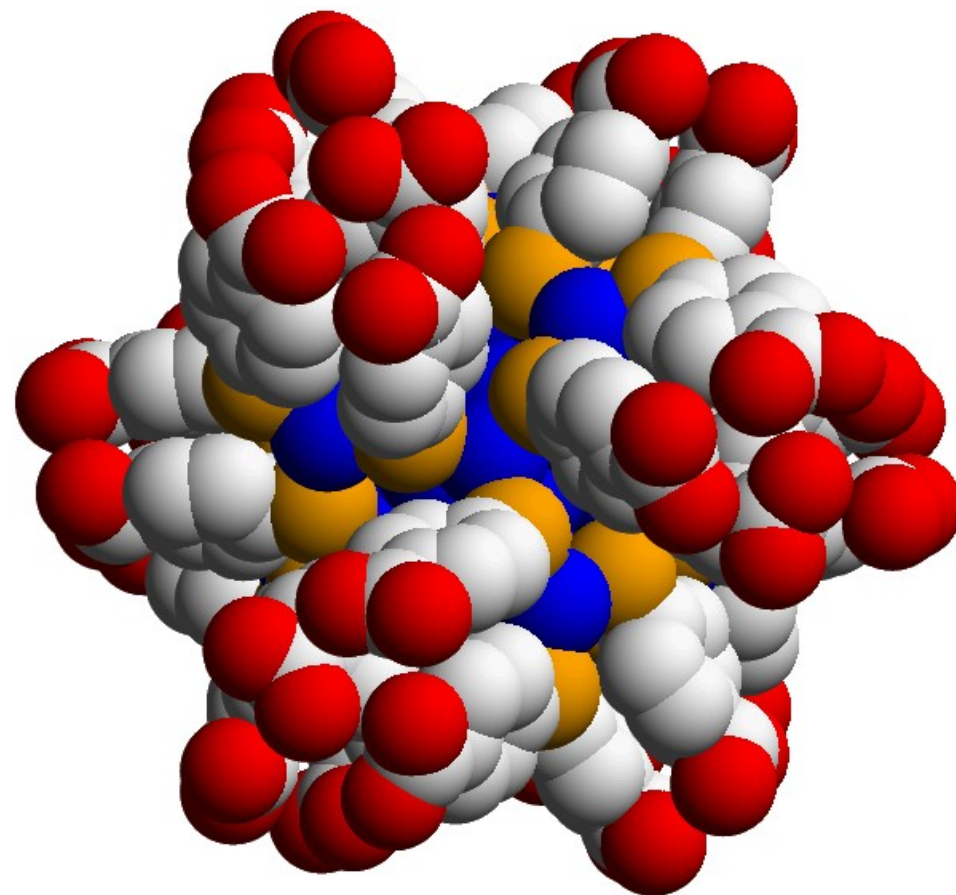
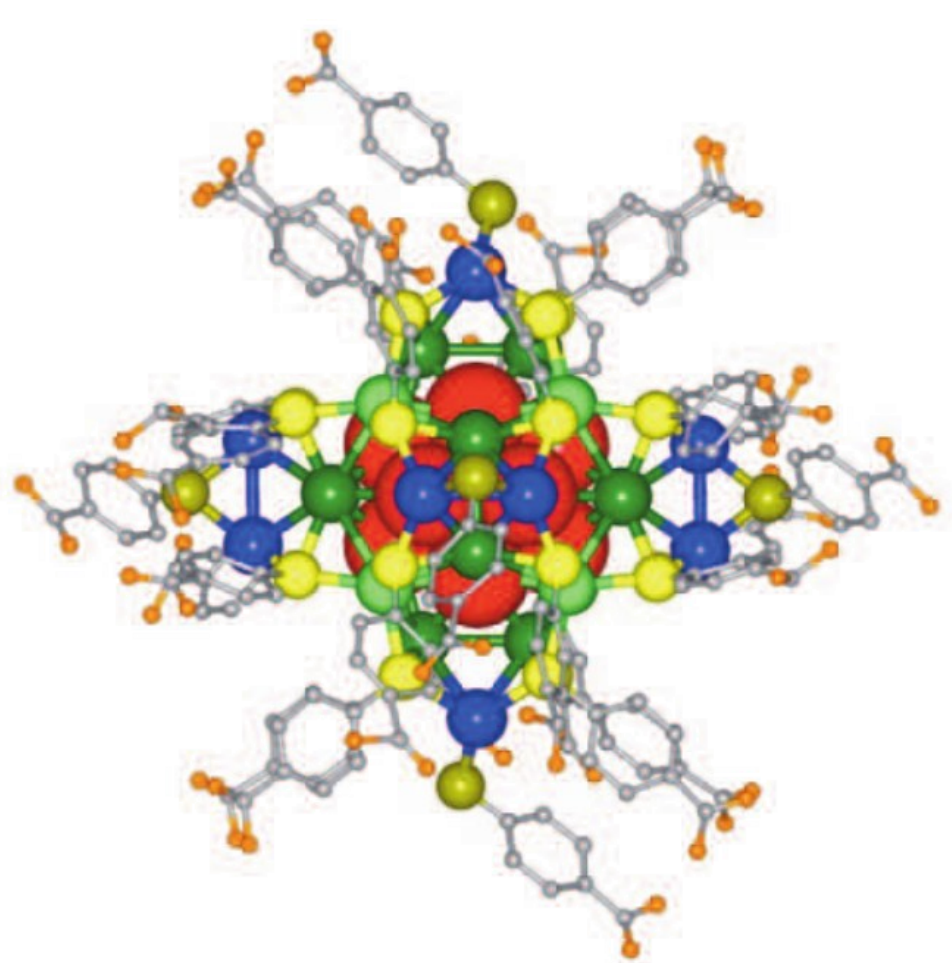
Concentration

Custom Grid Method



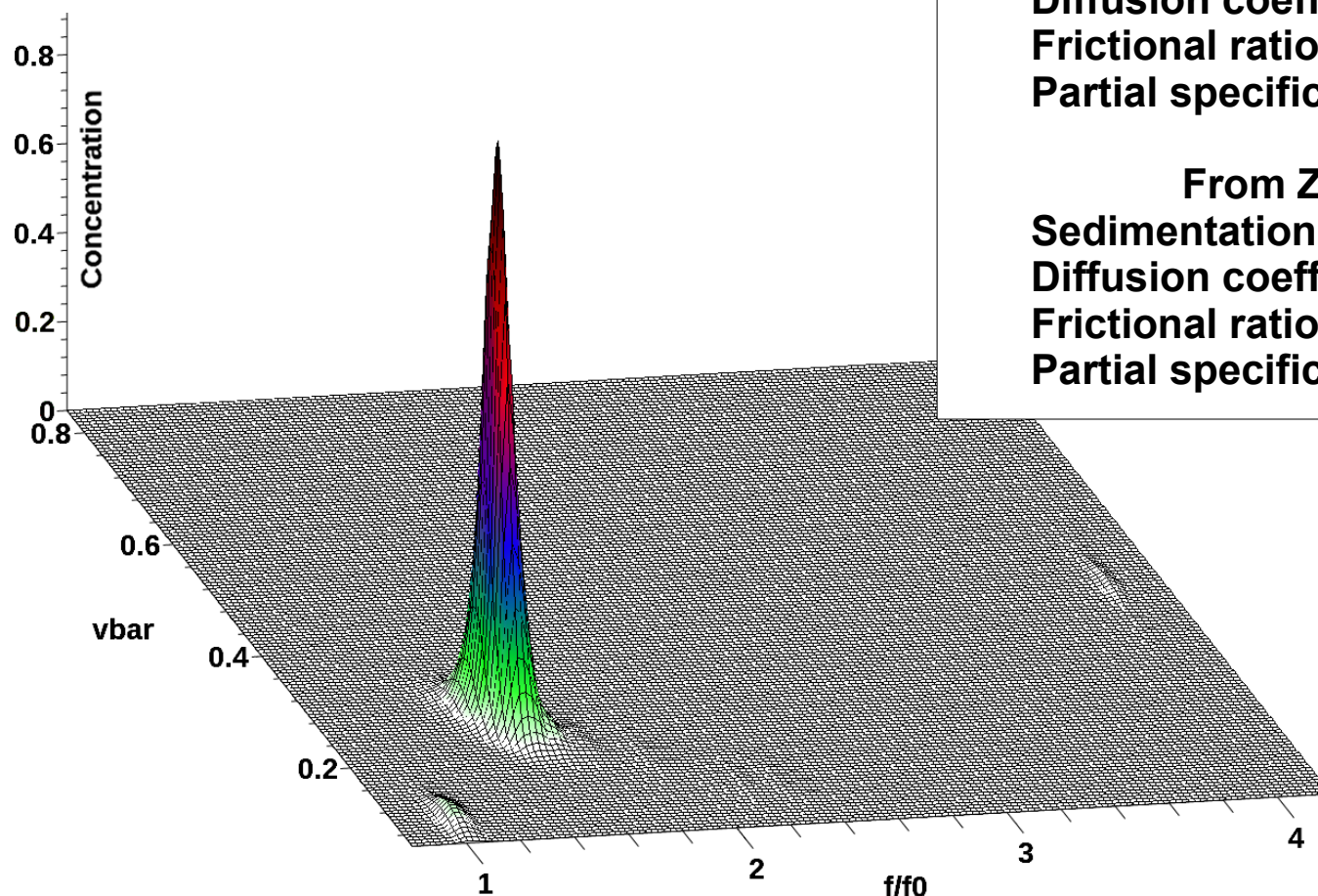
Ultrastable silver nanoparticles

Anil Desireddy¹, Brian E. Conn¹, Jingshu Guo¹, Bokwon Yoon², Robert N. Barnett², Bradley M. Monahan¹, Kristin Kirschbaum¹, Wendell P. Griffith¹, Robert L. Whetten^{3,4}, Uzi Landman² & Terry P. Bigioni^{1,5}



Density and Anisotropy – Molecular Weight fixed:

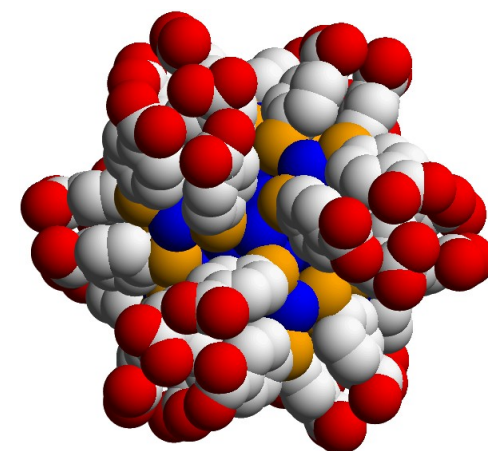
Re-parameterization of the sedimentation-diffusion space allows us to measure anisotropy and particle density:



From Structure:
Molecular weight: 9,621.8

From AUC:
Sedimentation coefficient: 4.19 (3.89, 4.49)
Diffusion coefficient: 14.5 (12.8, 16.2)
Frictional ratio: 1.47 (1.13, 1.80)
Partial specific volume: 0.27 (0.20, 0.34)

From Zeno Bead Modeling:
Sedimentation coefficient: 5.33
Diffusion coefficient: 18.5
Frictional ratio: 1.25
Partial specific volume: 0.24 (no H₂O)



Density and Molecular Weight – Anisotropy fixed:

Ligand-stabilized nano-particles and quantum dots:

CdSe 10.61 Å, CdSe 17.05 Å, CdSe 20.15 Å

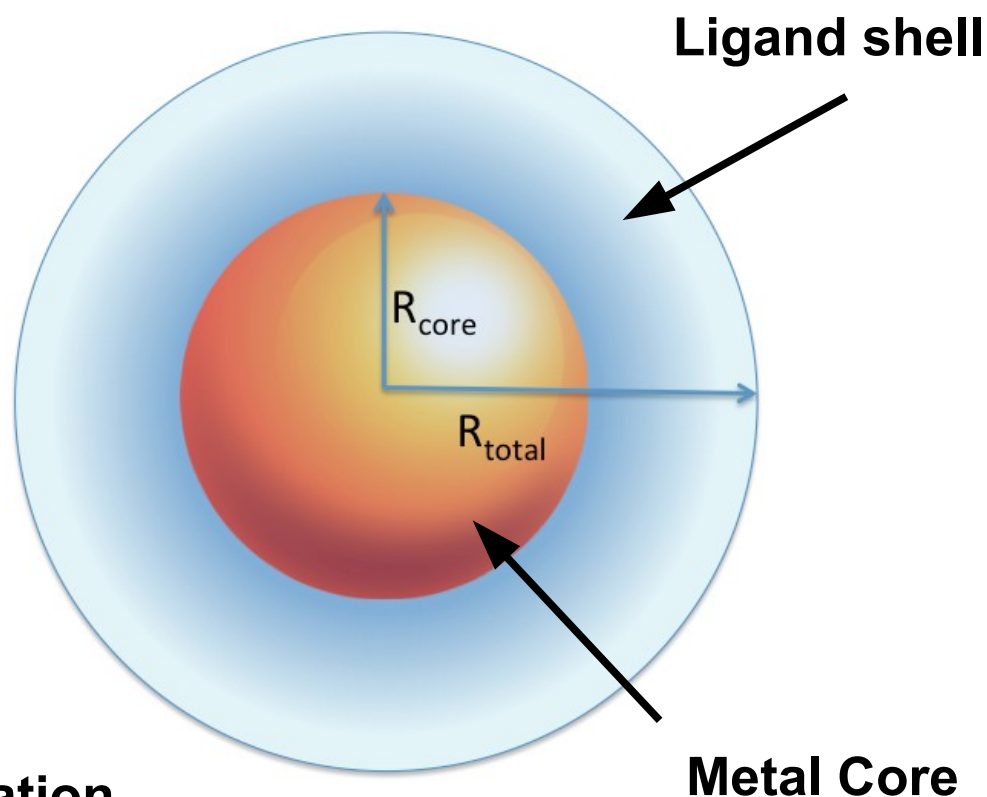
PbS, 12.6 Å, PbS, 15.0 Å

Question:

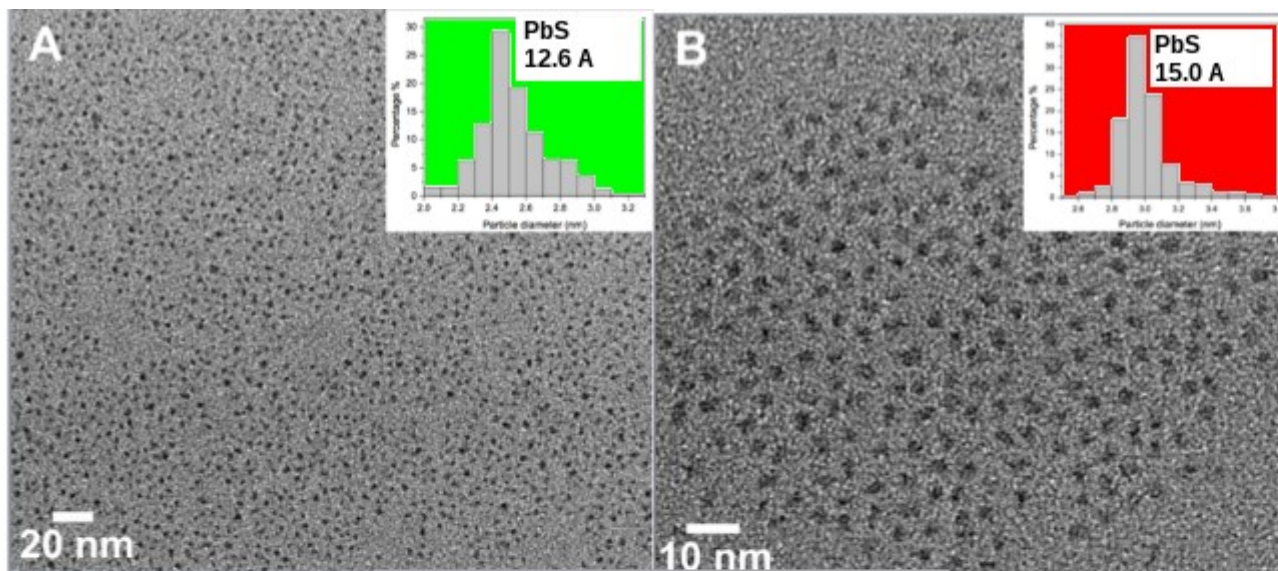
How much ligand is bound?

If we know the density of the ligand and the density of the core, and the core radius, we can measure the density of the entire particle and obtain the approximate amount of ligand Bound to the core.

Obtain: The level of functionalization



Density and Molecular Weight – Anisotropy fixed:



1. Verify anisotropy by TEM
2. obtain core radius from TEM (shell radius is invisible)

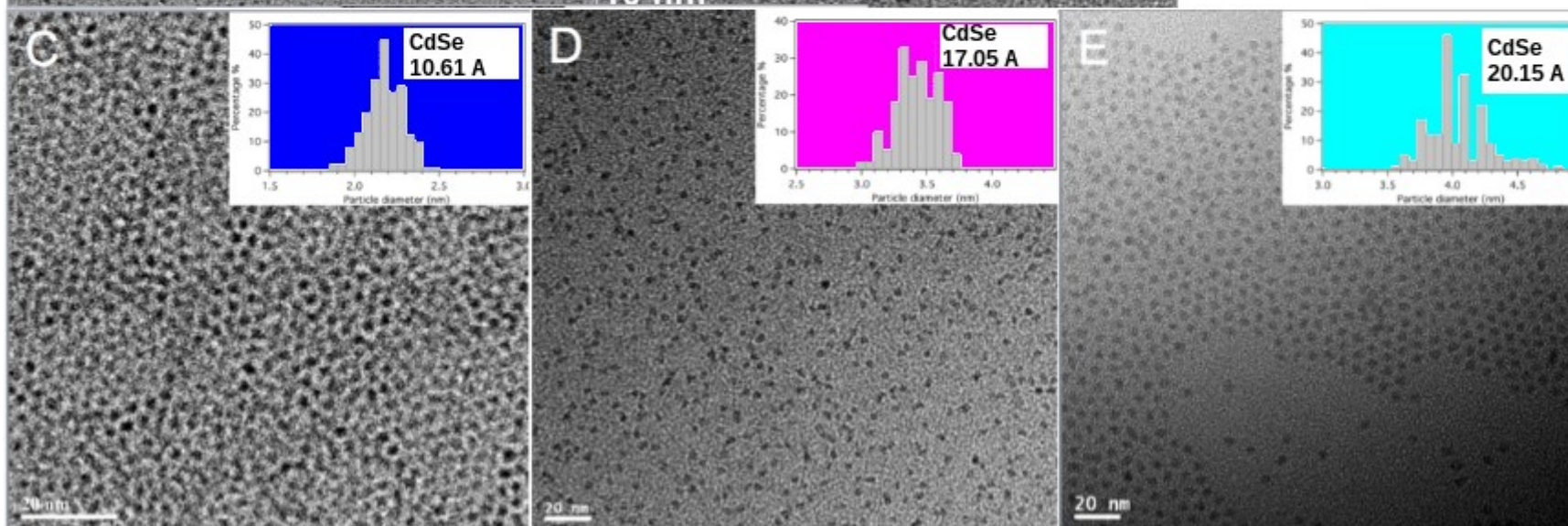
PbS, 12.6 Å

PbS, 15.0 Å

CdSe 10.61 Å

CdSe 17.05 Å

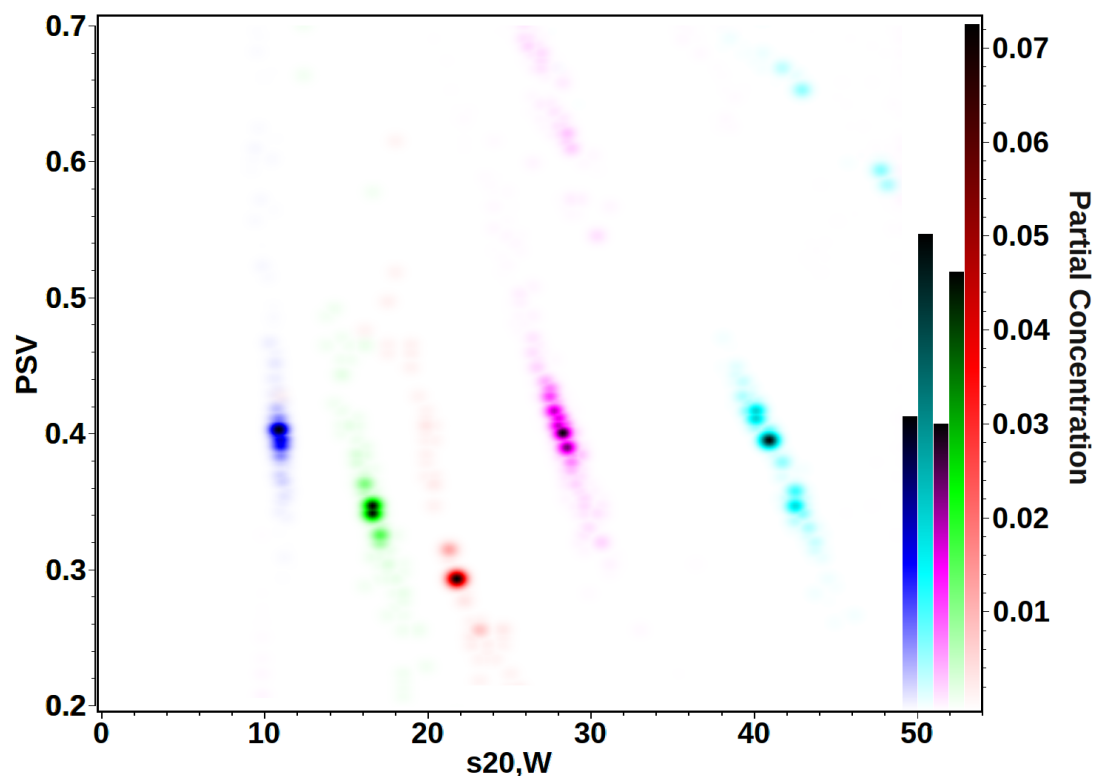
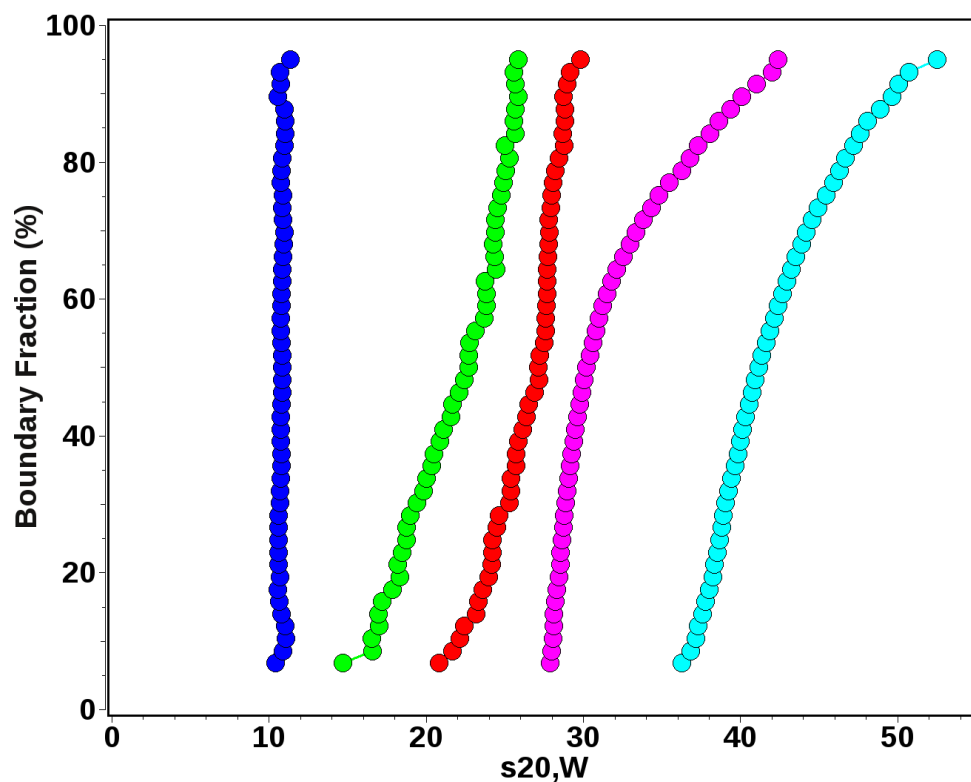
CdSe 20.15 Å



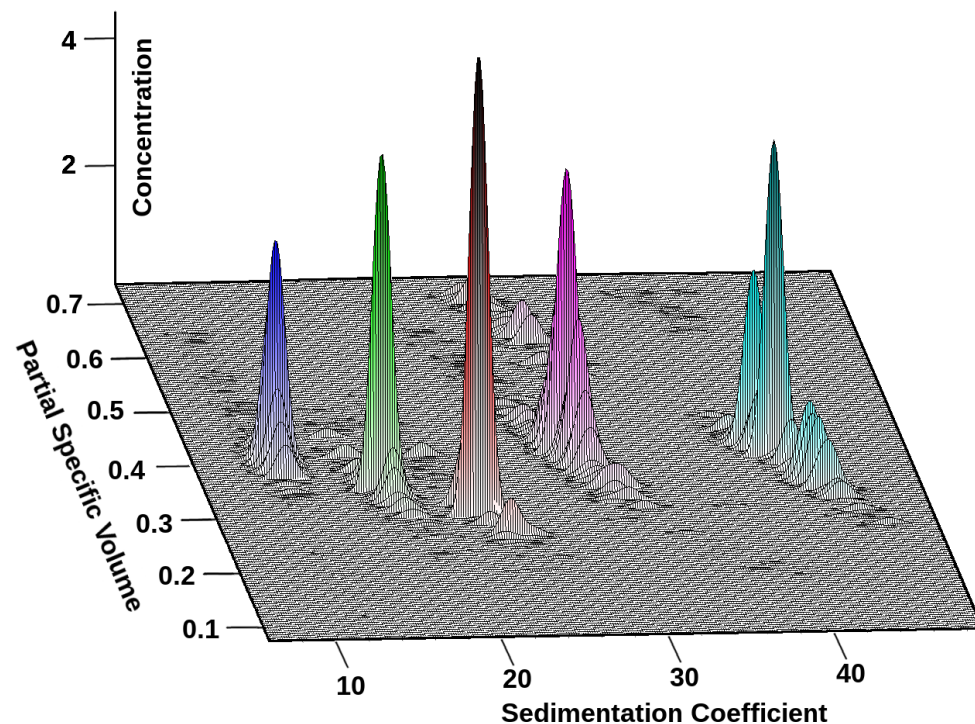
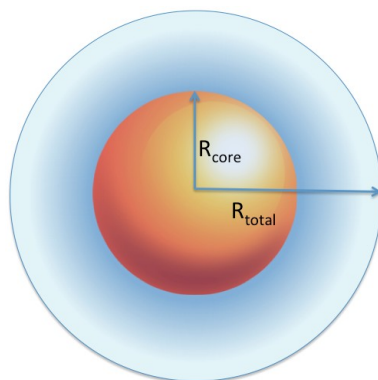
Density and Molecular Weight – Anisotropy fixed:

PbS, 12.6 Å
PbS, 15.0 Å
CdSe 10.61 Å
CdSe 17.05 Å
CdSe 20.15 Å

Re-parameterization of the sedimentation/diffusion space when the frictional ratio (anisotropy) is fixed provides density and molecular weight of the particle distributions



PbS, 12.6 Å
PbS, 15.0 Å
CdSe 10.61 Å
CdSe 17.05 Å
CdSe 20.15 Å



Sample	R_{core} (Å)	M_{total} (Da)	Sw (s)	D (cm^2/s)	\bar{v} (mL/g)	Density (g/mL)	R_{total} (Å)
CdSe 10.61	10.61	3.77E+04	1.09E-12	1.18E-06	0.4017	2.5131	18.20
PbS 12.60	12.60	5.68E+04	1.66E-12	1.08E-06	0.3433	2.9129	19.78
PbS 15.00	15.00	7.10E+04	2.17E-12	1.06E-06	0.2959	3.3795	20.27
CdSe 17.05	17.05	1.71E+05	3.07E-12	7.20E-07	0.3906	2.5605	29.80
CdSe 20.15	20.15	2.21E+05	4.15E-12	6.92E-07	0.3395	2.9459	31.00

Table 1: Results obtained from the custom grid analysis using sedimentation velocity data for CdSe and PbS QDs of radii between 10.61 Å and 20.15 Å, where R_{core} is the particle core radius obtained from TEM / absorption spectroscopy; M_{total} is the total molecular weight of ligand stabilized QDs; Sw is the standardized sedimentation coefficient, D is the diffusion coefficient, \bar{v} is the partial specific volume and R_{total} is the total particle radius including the organic ligand shell.