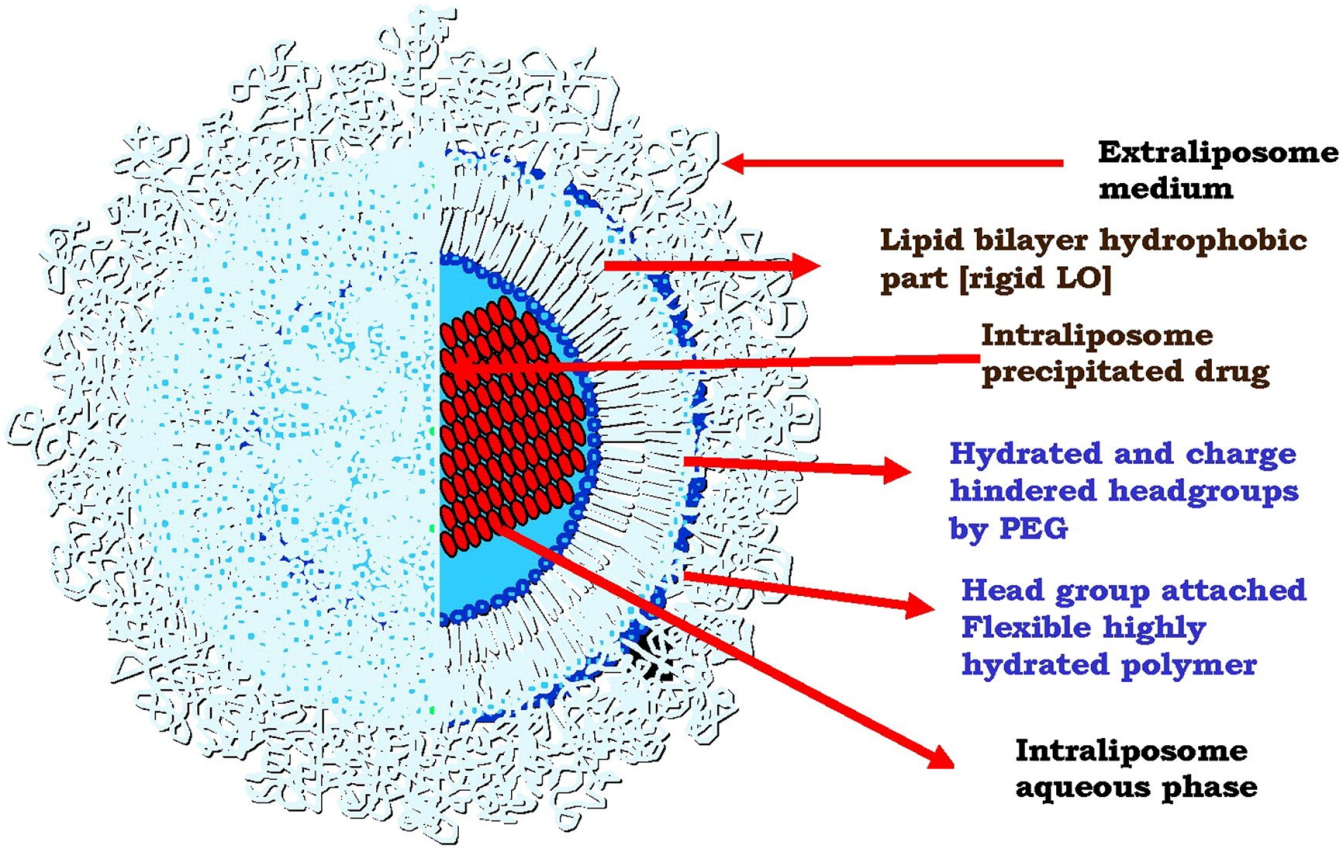
Lipid Nanoparticle (LNP) Characterization

LNPs are used to deliver cancer drugs and gene therapies:

- **Doxil (liposomal doxirubicin, treats solid tumors)**
- **Marqibo (liposomal vincristine, treats acute lymphoblastic leukemia)**
- **Onpattro (siRNA, treats polyneuropathy caused by hereditary transthyretin-mediated amyloidosis)**
- **SARS-CoV-2 vaccines (mRNA cargo, Covid-19 vaccines, Pfizer-Biontech, Moderna)**



Lipid Nanoparticle (LNP) Characterization by MW-AUC

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ARTICLE

Density Matching Multi-wavelength Analytical Ultracentrifugation to Measure Drug Loading of Lipid Nanoparticle Formulations

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Measuring Lipid Nanoparticle loading with RNA

Challenges:

- Heterogeneity in LNP size
- Heterogeneity in RNA loading
- Unknown amount of unincorporated RNA, if any
- Clinically relevant loaded LNPs and empty LNPs look identical in cryo-TEM
- DLS and SEC too low in resolution and don't differentiate by loading

Focus on what's different:

- Liposomes and RNA have *different absorbance spectra and densities*
- Liposomes with different RNA loading ratios have different *densities*
- Different sizes have different *hydrodynamic radii*
- Liposomes with different sizes and different RNA loading will vary in *molar mass*

Experimental Design:

- Measure Mie scattering and siRNA absorbance by MW-AUC
- SV-AUC differentiates based on *s* and *D*
- Perform D_2O density matching
  1) obtain partial specific volume distributions
  2) derive molar mass distribution
  3) derive hydrodynamic radii
- Integrate constraints from orthogonal methods (cryo-TEM)

$$M = \frac{s\,RT}{D\left(1-\bar{v}\,\rho\right)}$$

Step 1:

Perform D_2O density matching analysis
Approach: Measure RNA control in 4 D_2O Concentrations
Derive partial specific volume distribution and heterogeneity

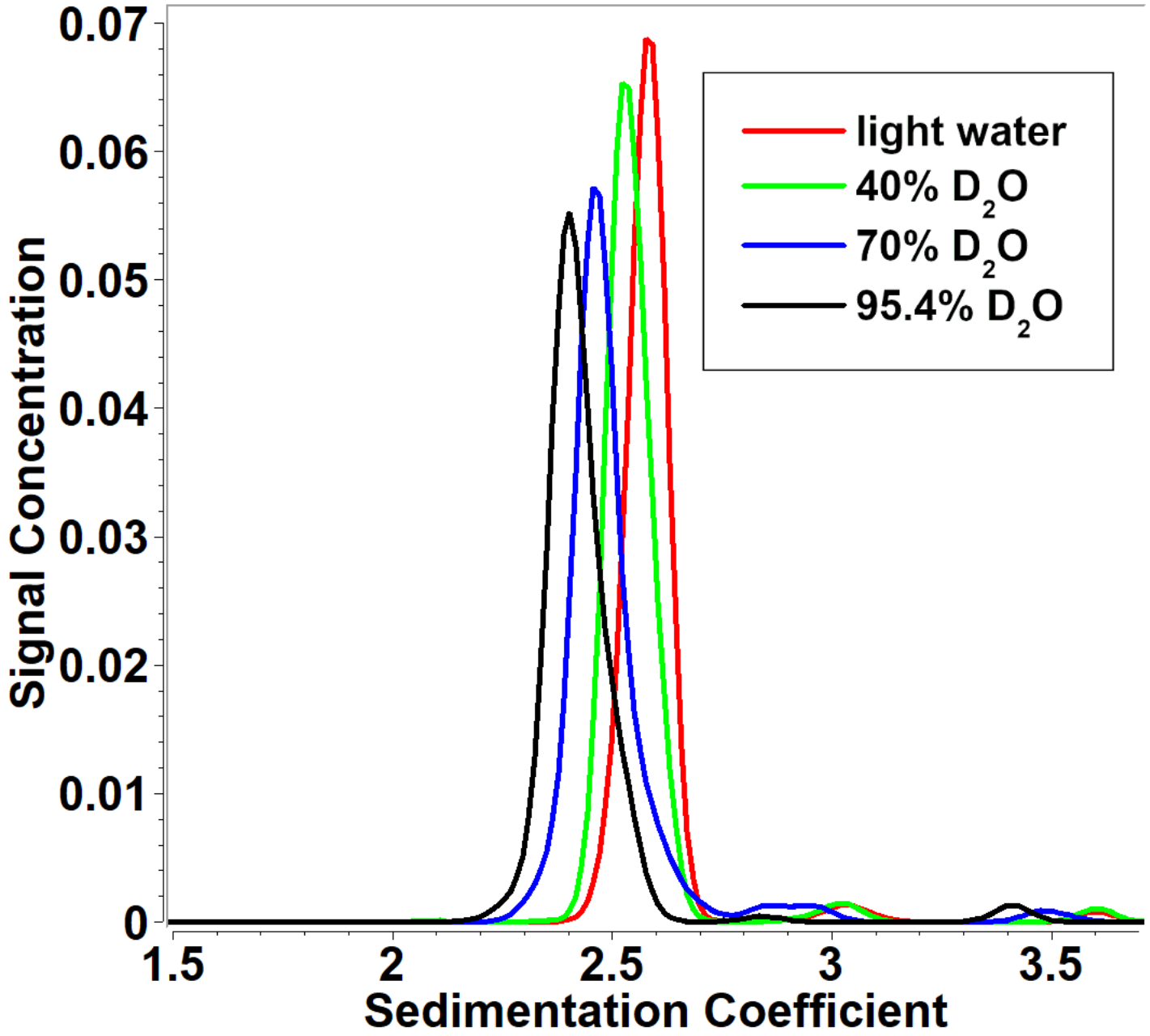
Step 2:

Repeat for Lipid Nanoparticle sample
Derive molar masses and hydrodynamic radii distributions

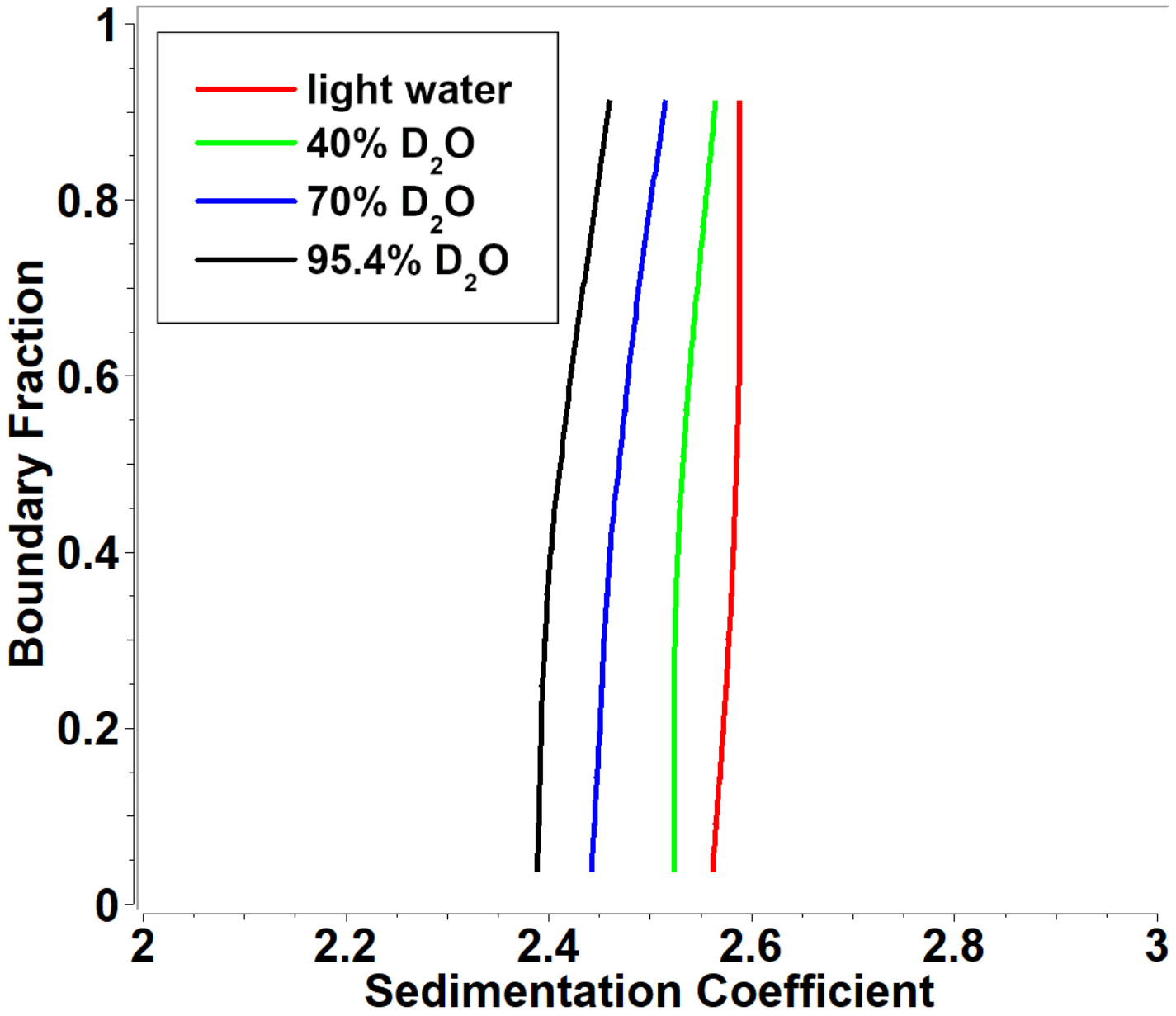
Step 3:

Perform MW-AUC experiment
Deconvolute nucleic acids and liposome
Validate partial specific volume distribution

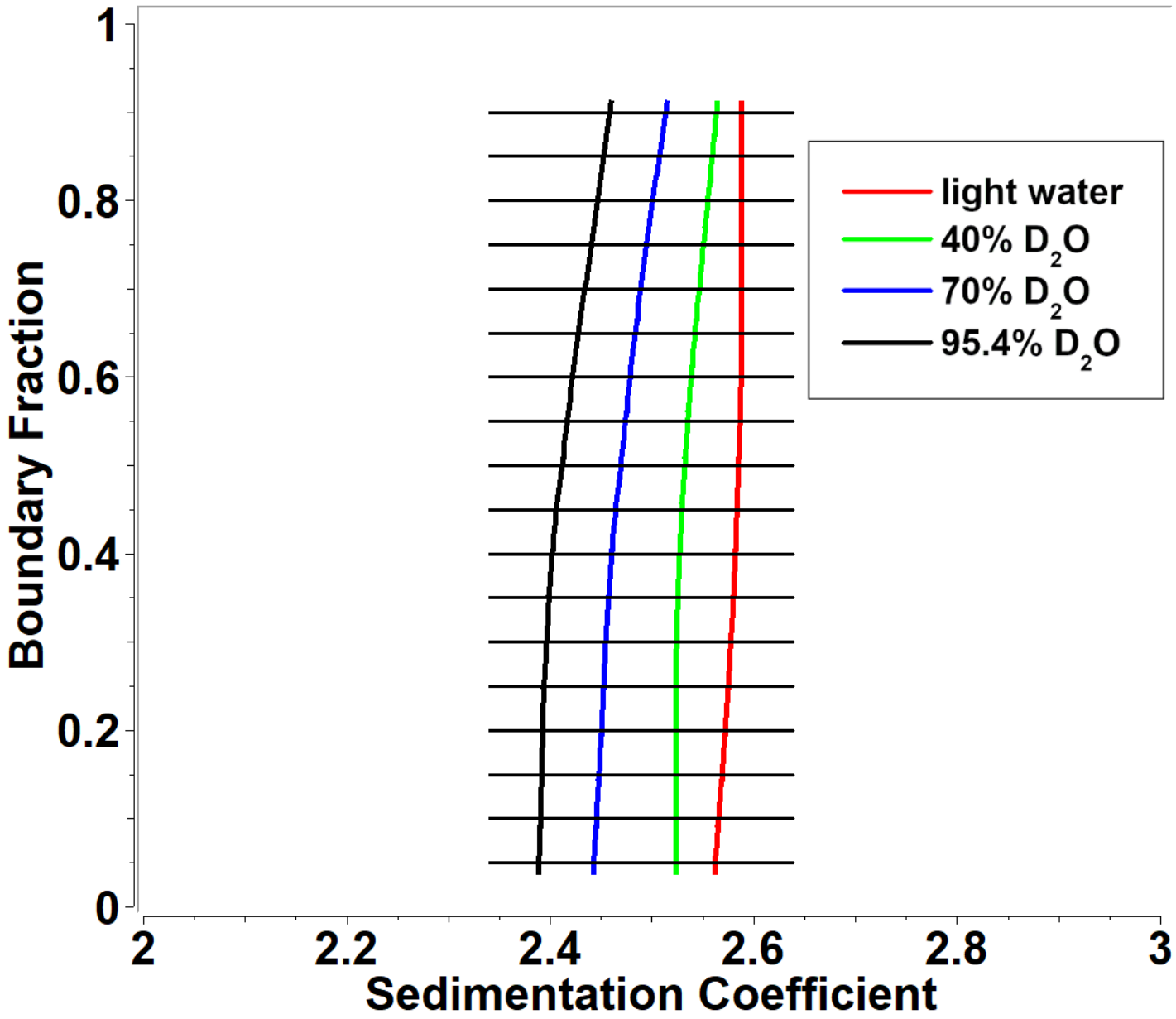
Step 1: Measure RNA control in 4 D_2O Concentrations



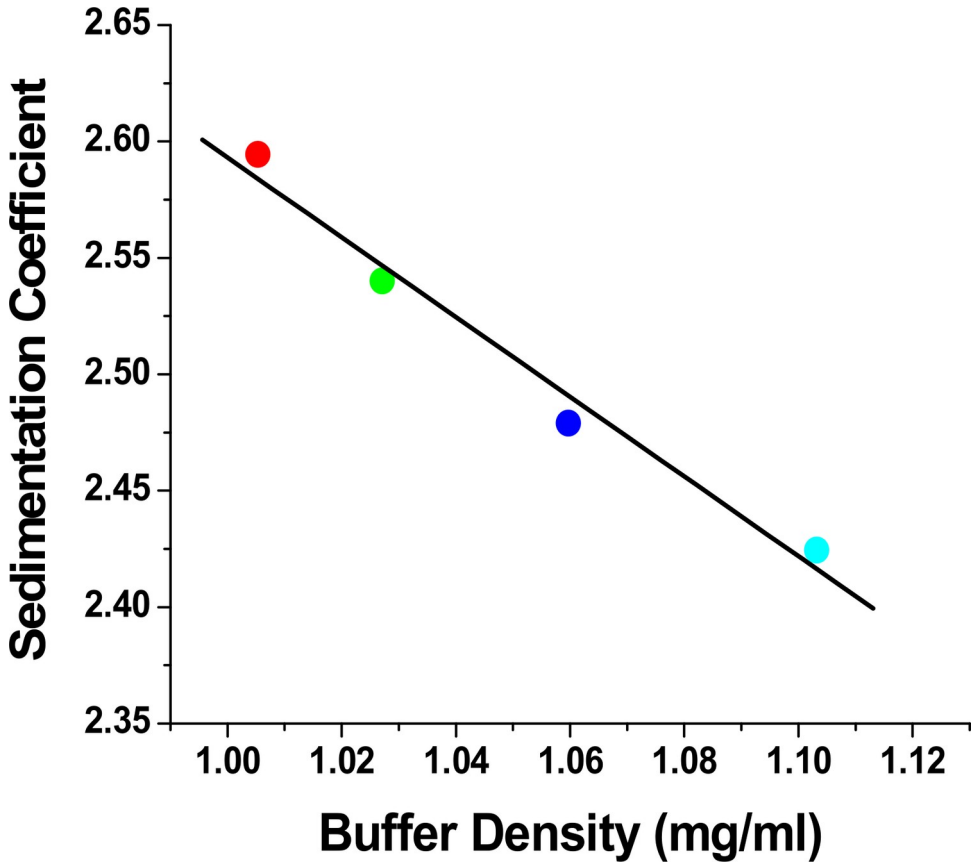
Step 2: Create Integral G(s) Distributions:

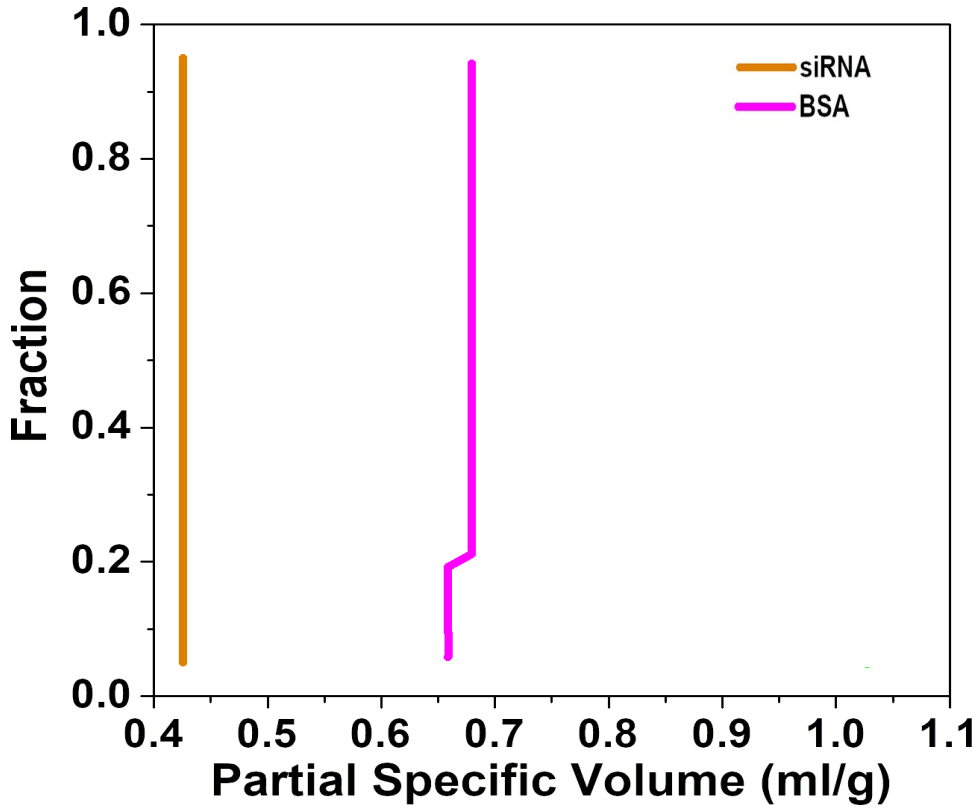


Step 3: Determine s-values at each boundary fraction



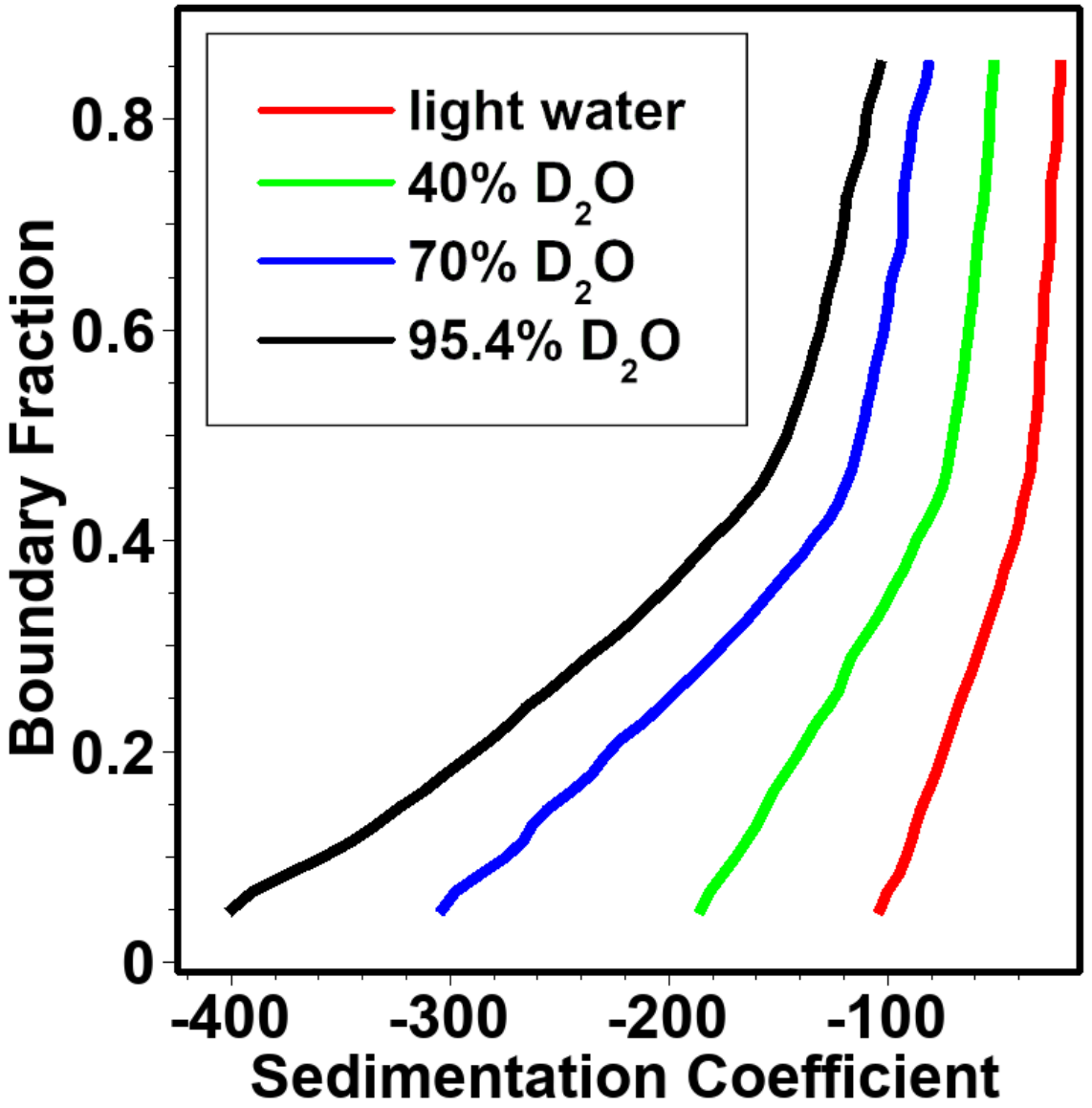
Step 3: Extrapolate equivalent boundary fractions to zero S





The inverse of the density at s=0 is equivalent to the partial specific volume at that boundary fraction. This can be repeated for each boundary fraction to derive a PSV distribution.

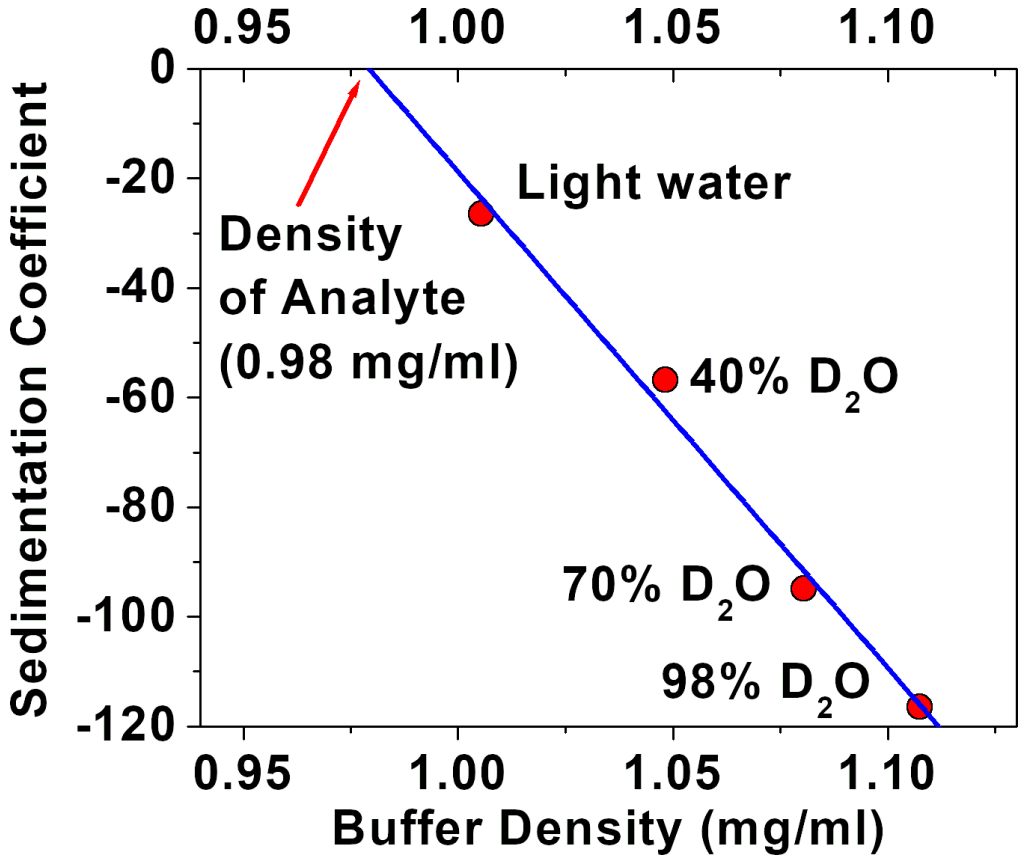
Repeat for each Lipid Nanoparticle Preparation:

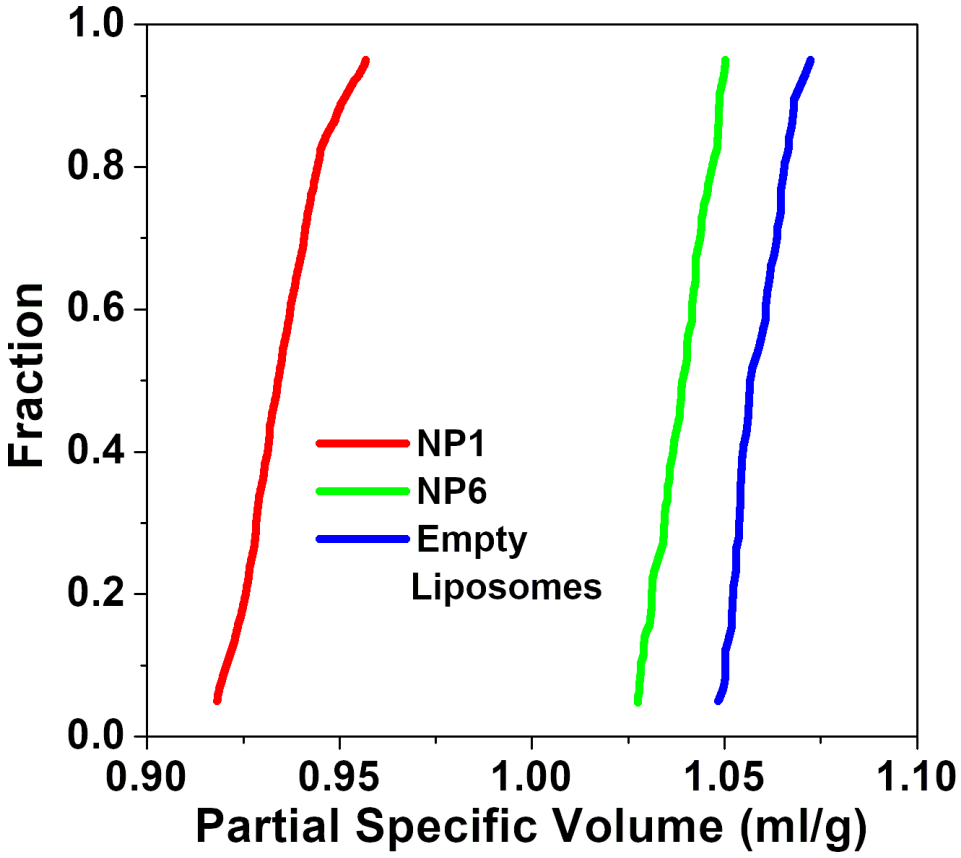


(Lipids are less dense than water or heavy water and float – negative s-values)

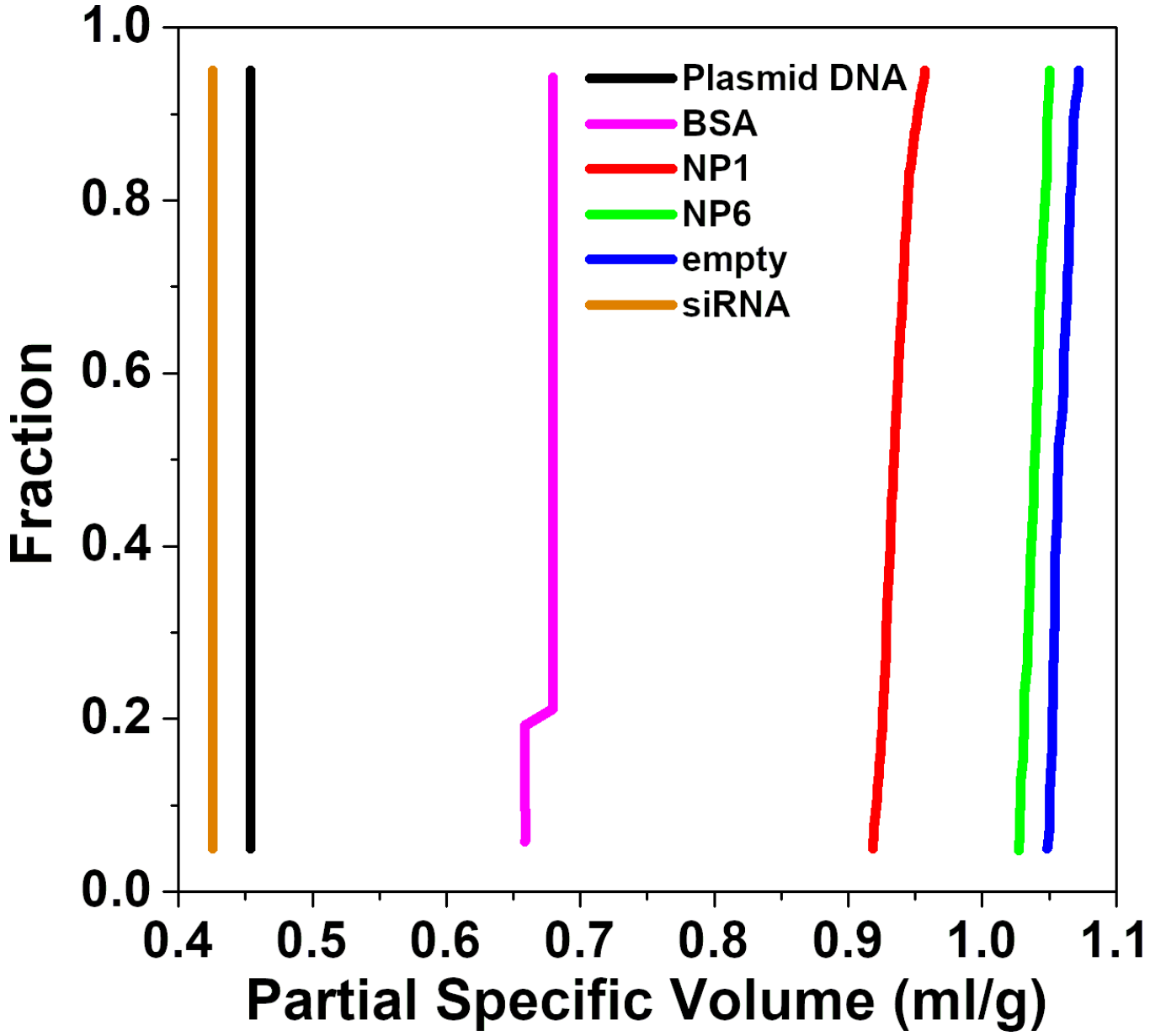
Repeat for each Lipid Nanoparticle Preparation:

Extrapolate each boundary fraction to zero sedimentation to derive the partial specific volume of this heterogeneous mixture:

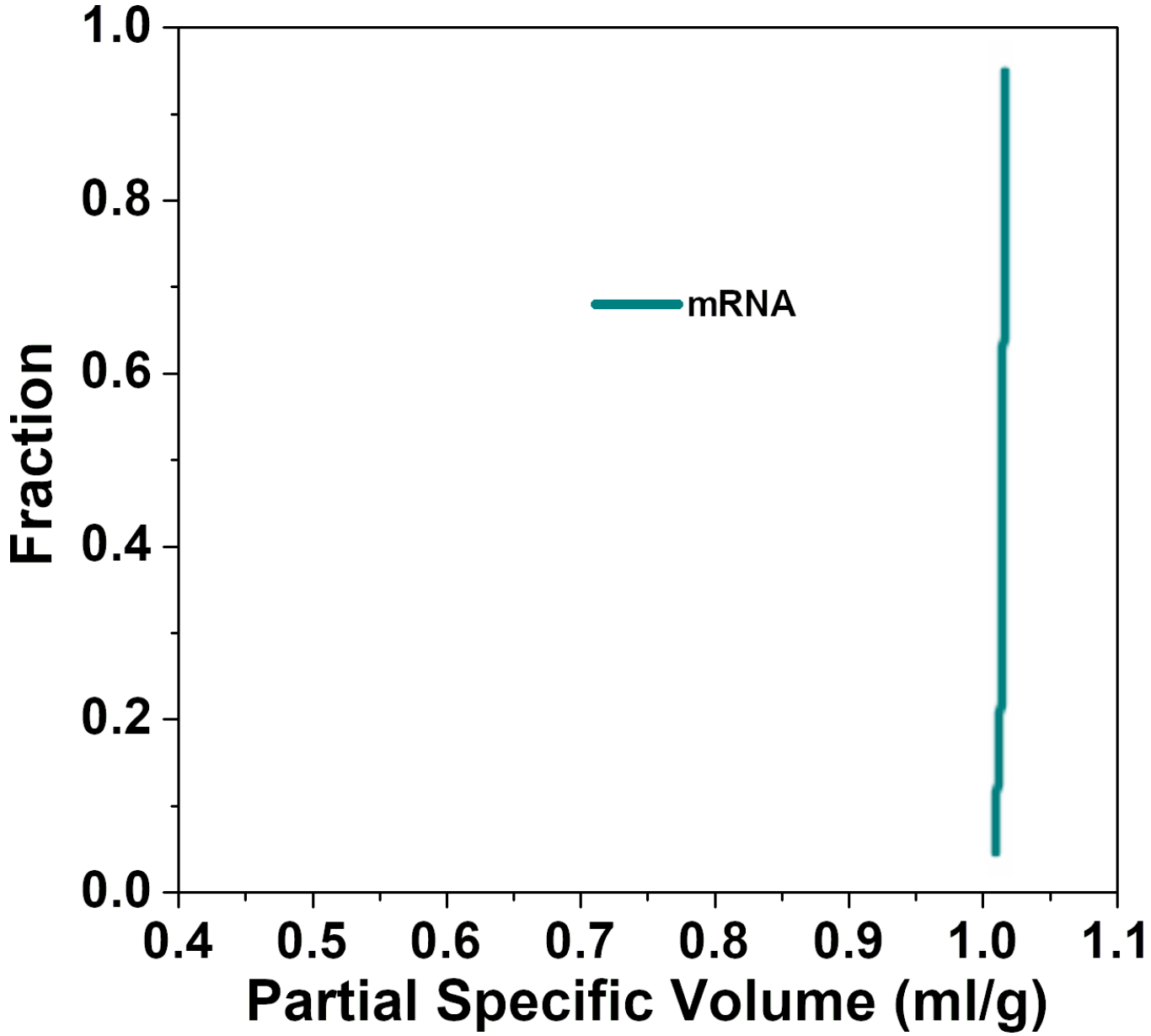




...or any other biopolymer:



...or any a different cargo (mRNA):



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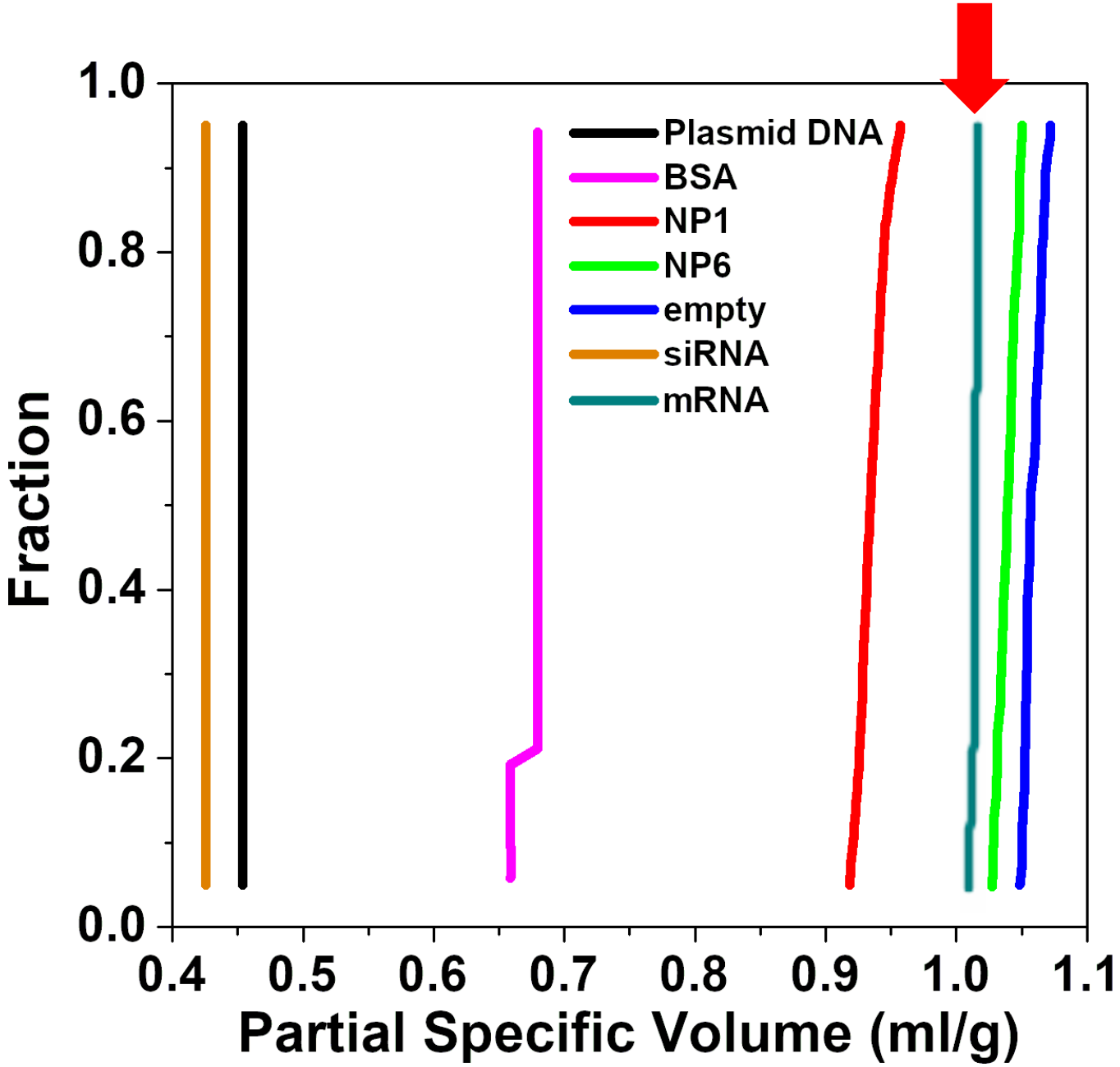
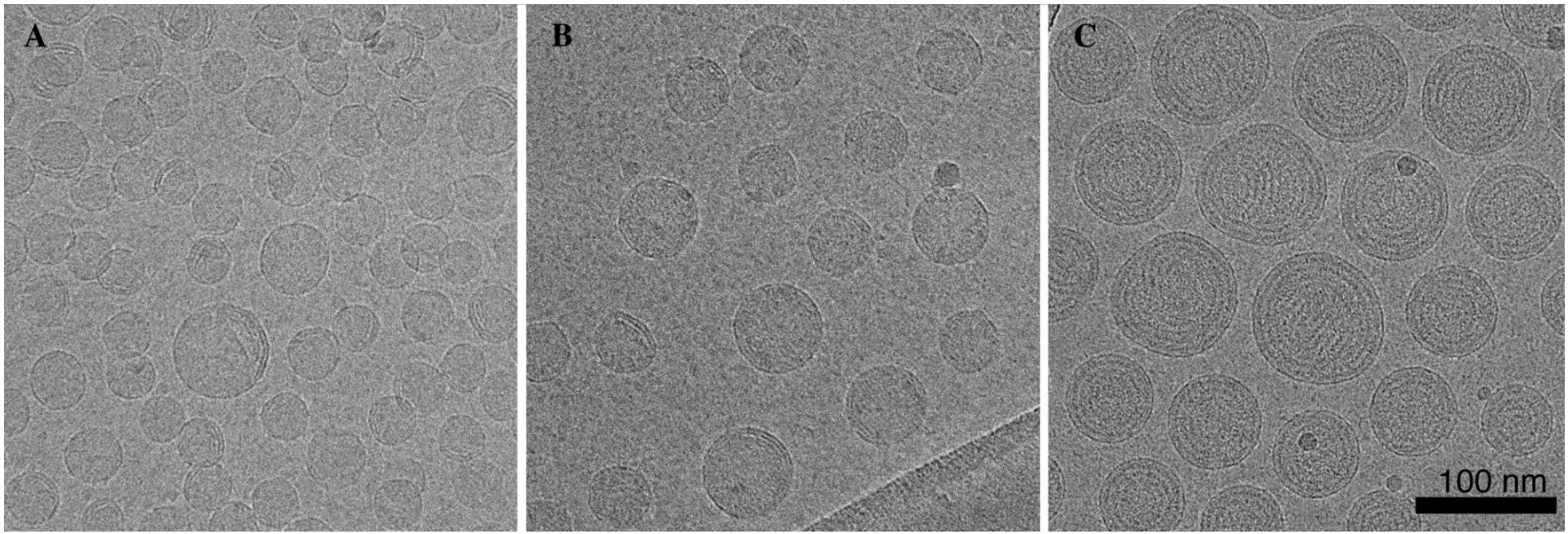


Report Particle Sizes based on known Anisotropy (φ):

Empty LNPs | NP6: clinically relevant | NP1: Over loaded



$$\phi = \frac{f}{f_0} = 1.0, \quad D = \frac{RT}{9\pi\eta\phi N}\left(\frac{2s\phi\,\bar{v}\,\eta}{1-\bar{v}\rho}\right)^{-0.5}$$

Report Particle Sizes and Molar Masses:

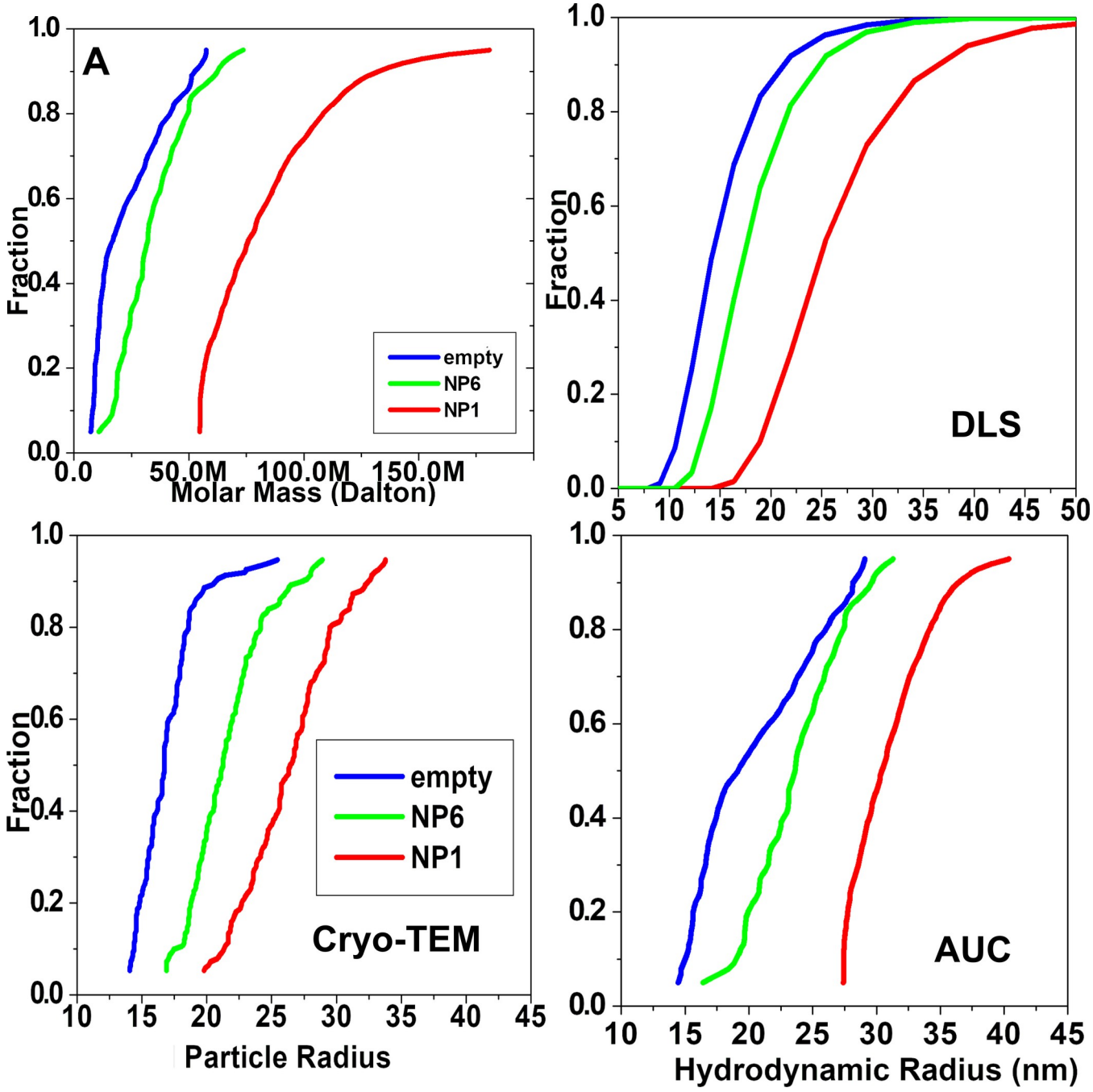
$$D = \frac{RT}{Nf}$$

$$f = 6\pi\eta r_h$$

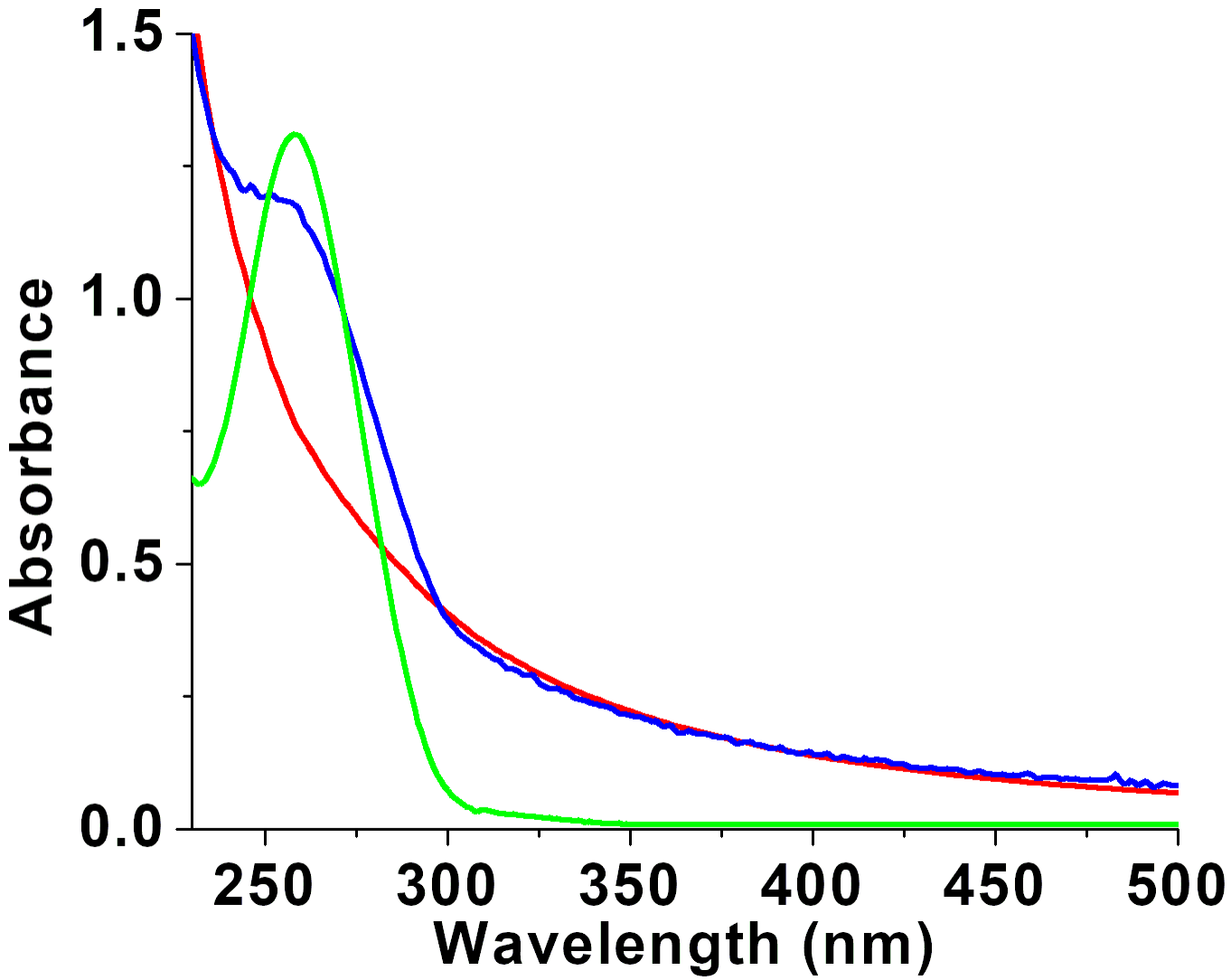
$$r_h = \frac{RT}{6\pi\eta N D}$$

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

$$M = \frac{sNf}{(1-\bar{\nu}\rho)}$$

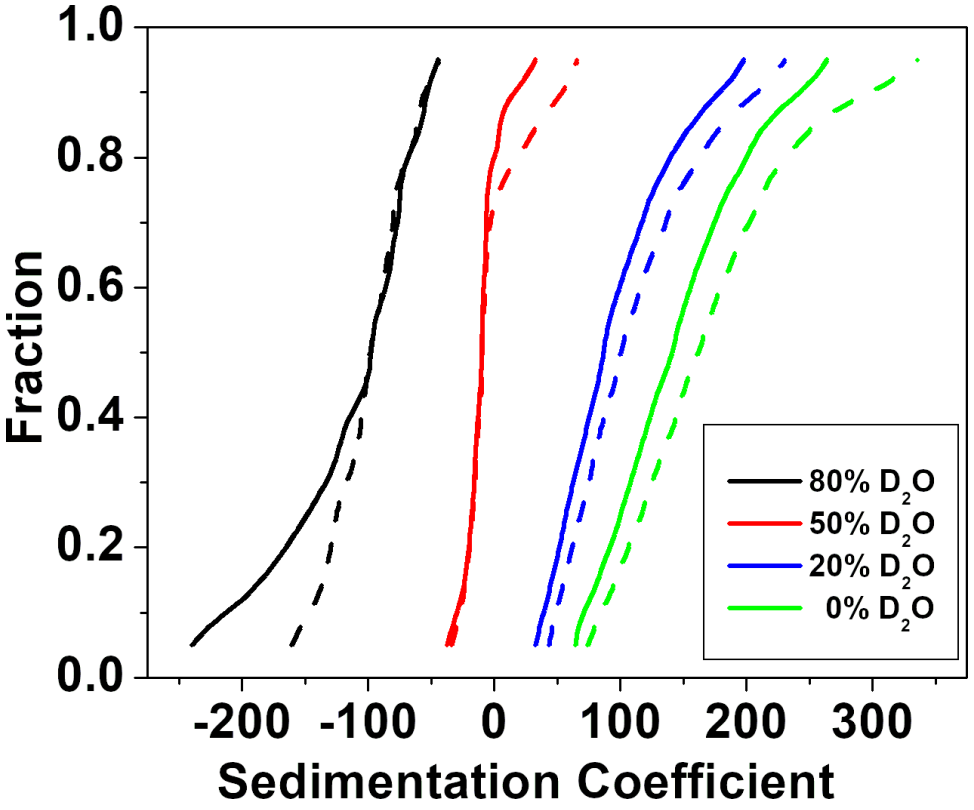


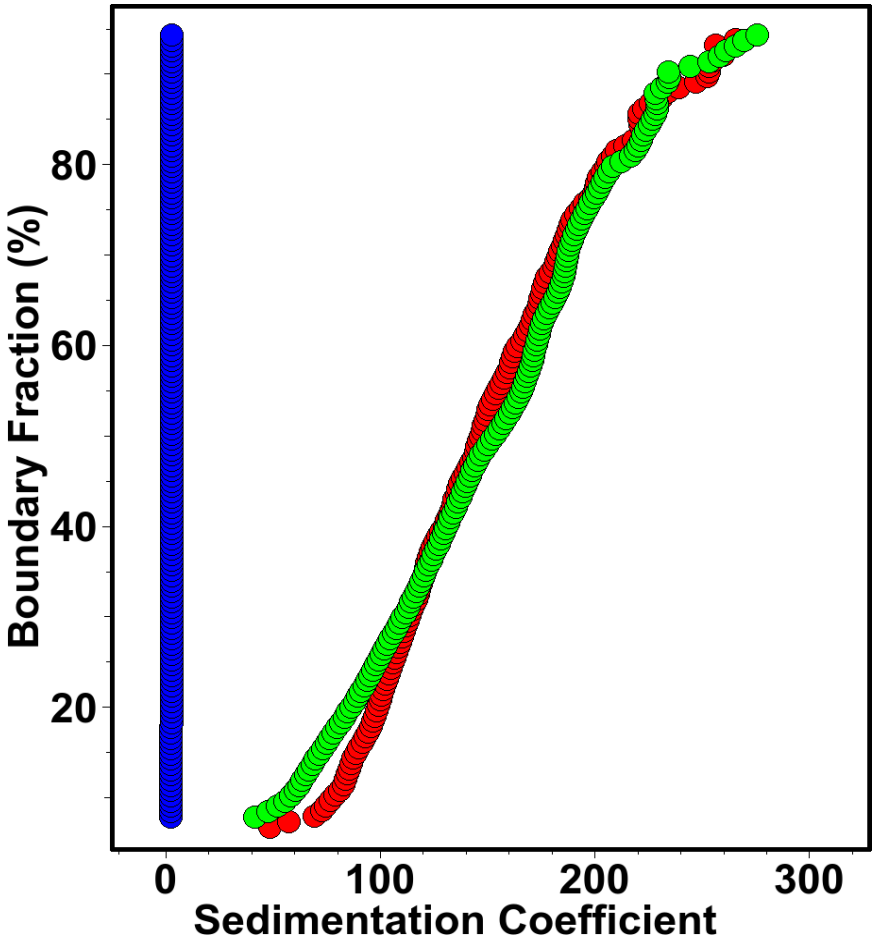
Deconvolute Liposome Absorbance from RNA Absorbance:



UV spectral properties of RNA (green), empty LNP (red), and RNA-loaded LNPs (blue).

Deconvolute Liposome Absorbance from RNA Absorbance:





Dashed Lines: Liposome scattering signal
Solid Lines: RNA absorbance signal

NP1 at 260 nm,
NP1 using fluorescence
siRNA at 260 nm (2.58s)

Software Demo – Custom Grid