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

Supplemental Information

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Figure S1:

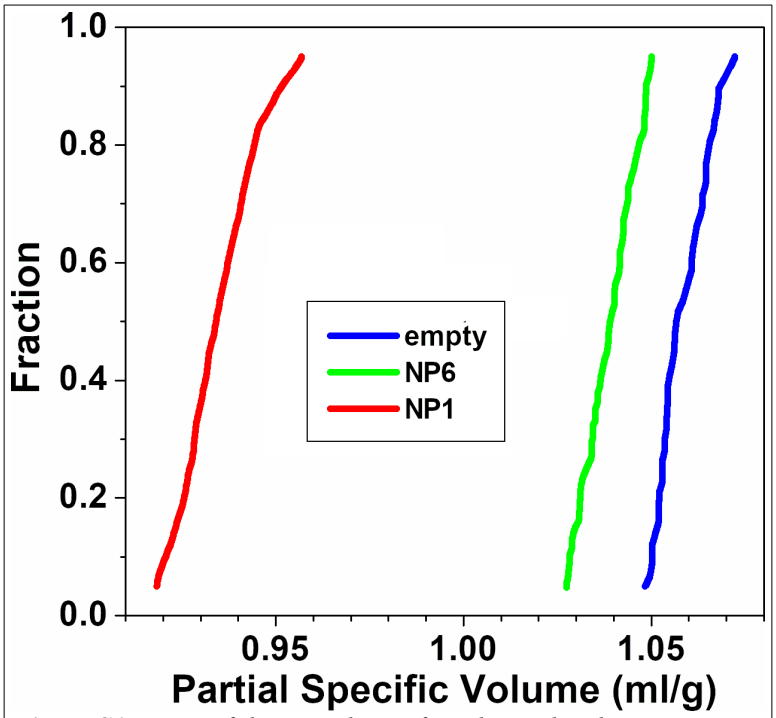

Figure S1: *Zoom of the partial specific volume plot shown in Figure 1 in the main text to emphasize the observed heterogeneity of PSV.*

Section S2: Multi-wavelength Analytical Ultracentrifugation (MW-AUC):

MW-AUC is a recent technique enabled by new instruments that allows the user to collect sedimentation velocity data in intensity mode at multiple wavelengths. This is useful when the sample includes multiple analytes, each with a different and distinct chromophore. In this study, the different chromophores derive from siRNA and from the Mie scattering of LNPs in the UV. When the spectra of each analyte are known *a priori*, it is possible to deconvolute the contribution of each analyte to each hydrodynamically distinct species in the mixture. In this case this allows us to determine if LNPs and siRNA sediment as free, individual species, or as a complex. If molar extinction coefficients are known for each measured wavelength it is possible to assign molar quantities of each analyte to each hydrodynamic species, otherwise, relative quantities can easily be determined from the spectral deconvolution. This approach has wide-reaching applications for studies of interacting or complex-forming systems as is present in this study. It provides a second, spectral dimension to aid in the characterization of the solutes present in a mixture, in addition to the hydrodynamic dimension, which separates individual species based on their hydrodynamic properties.

Approach:

Sedimentation velocity experiments (SVEs) for each wavelength are initially analyzed according to standard analysis protocols as described in [1]. In the last refinement step, an iterative two-dimensional spectrum analysis fit is generated [2], which is inspected for randomness in the residuals and to assure a sufficiently low RMSD for each triple. If both criteria are satisfactory, the solutes simulated by the fit represent the original dataset well, and can be used to simulate an equivalent SVE with arbitrary scan times, using the boundary conditions, speeds and hydrodynamic corrections from the original experiment. In order to perform a spectral decomposition, and treat each radial position as a complete wavelength scan, it is important that scans from different wavelengths are time-synchronized. In the Beckman Coulter Optima AUC instrument, each wavelength is acquired sequentially, i.e., at a different time point during the sedimentation process. In order to generate a time-synchronized dataset, each dataset is simulated with its best fit model, shifting all corresponding scans to identical time points. With those simulations in hand, a 3-dimensional surface (absorbance as a function of wavelength, radius and time) is generated for each scan time as shown in Figure S3, Simulations are accomplished using the ASTFEM simulation module of UltraScan [3, 4], and are based on the best fit model for each triple. At this point, each radial position from each simulated scan represents a wavelength scan that can be deconvoluted into its basis spectra, generating two or more separate two-dimensional datasets (absorbance as a function of radius and time), one for each spectral species [5]. The two-dimensional datasets can then be independently fitted for each species to derive sedimentation and diffusion distributions for each individual analyte, in this case siRNA and LNP.

Figure S3:

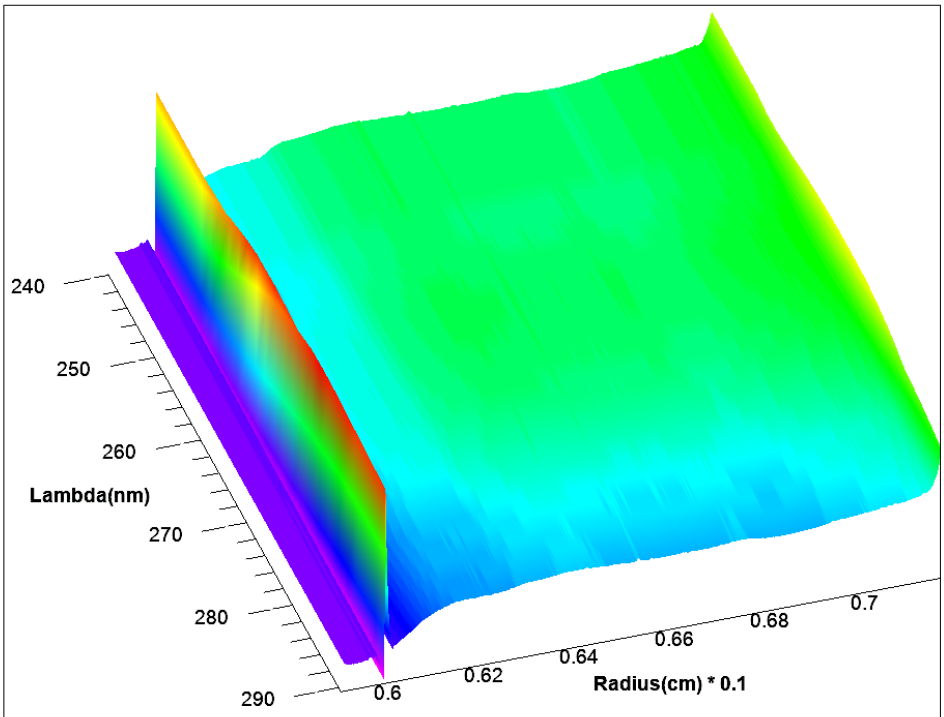

Figure S3: *A single multi-wavelength scan for a LNP experiment before simulation for time synchronization. The time distortion, while small, is resolved by simulating this surface with identical time stamps for each wavelength. The leading peak on the left represents the meniscus. For the time-synchronized surface, each radial position yields a complete wavelength scan that can be decomposed into its spectral basis vectors, generating a two-dimensional dataset for each basis spectrum [5].*

Figure S4:

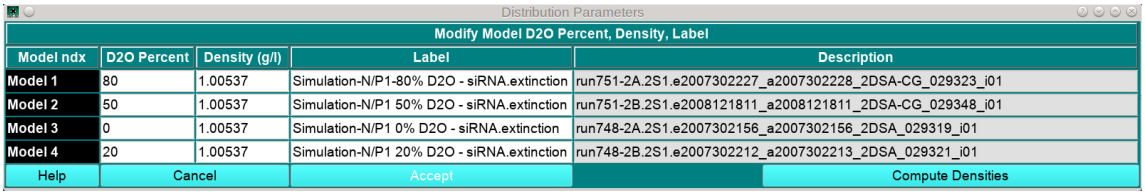

Figure S4: *UltraScan dialog for entering the D_2O percentage for each dataset used in the extrapolation for the PSV distribution.*

Figure S5:

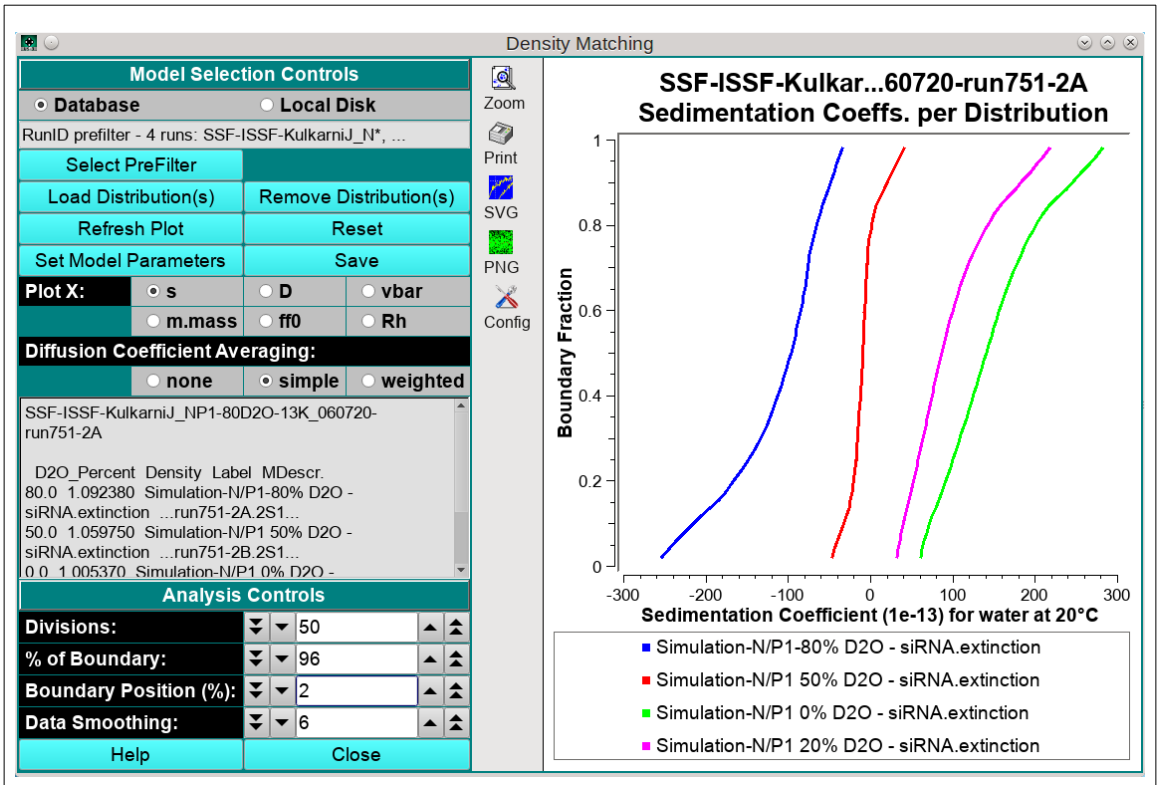

Figure S5: *Main screen of the UltraScan us_buoyancy module. Controls for loading distributions and selecting calculated parameters for the integral distributions are shown. Users can switch between displaying s, D, vbar/PSV, molar mass, frictional ratio and hydrodynamic radius. Diffusion coefficient averaging can be performed by using a simple average from all densities, or a weighted average when multiple measurements at the same density are included.*

Figure S6:

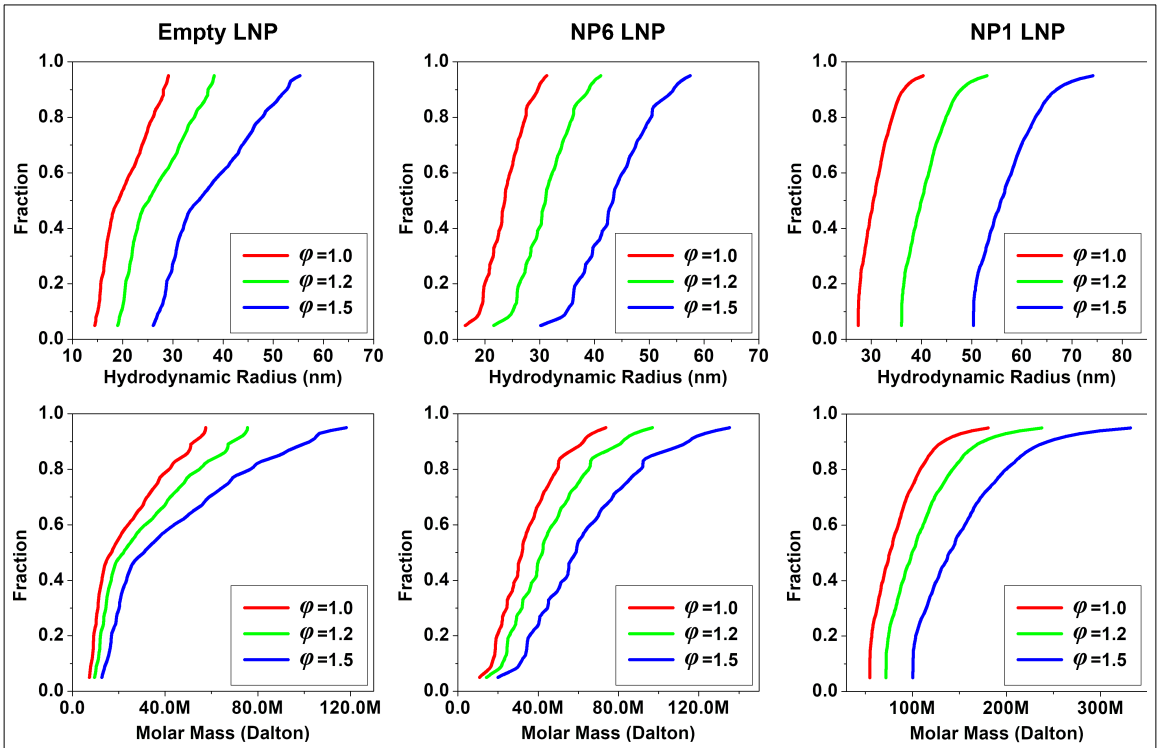

Figure S6: *Hydrodynamic radius (top row) and molar mass (bottom row) predictions for anisotropy constraints 1.0 (red), 1.2 (green) and 1.5 (blue). Comparisons of hydrodynamic radii predicted by DLS suggest that φ = ~1.0 is most consistent, which is also supported by the morphology observed in cryo-TEM images.*

Supplemental References:

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4 Zhang J, Pearson JZ, Gorbet GE, Cölfen H, Germann MW, Brinton MA, Demeler B. Spectral and Hydrodynamic Analysis of West Nile Virus RNA-Protein Interactions by Multiwavelength Sedimentation Velocity in the Analytical Ultracentrifuge. Anal Chem. 2017. 89(1):862-870.

5 Pearson JZ, Krause F, Haffke D, Demeler B, Schilling K, and H. Cölfen. Next-Generation AUC Adds a Spectral Dimension: Development of Multiwavelength Detectors for the Analytical Ultracentrifuge Methods in Enzymology. 2015. doi:10.1016/bs.mie.2015.06.033