Probing Metal Nanoparticles and Assemblies with Analytical Ultracentrifugation

by

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Abstract
Analytical Ultracentrifugation (AUC) is a powerful tool to obtain statistically relevant size and shape measurements for macromolecular systems. Metal nanoparticles coated by a ligand shell of thiolated molecules provide diverse functionality, from targeted cellular delivery to the formation of complex assemblies. Here I show that AUC can be used to determine particle size distribution, ligand shell density, shape, and hydrodynamic radius. It can also be used to probe complex mixtures of nanoparticle assemblies, from 2D dimers and chains, to 3D trimers, tetramers, and higher order assemblies, from a consideration of their hydrodynamic shape factor and its relation to the sedimentation coefficient. With AUC, the ease of sample preparation, ligand shell information, and dramatic increase in sample size are improvements compared with electron microscopy, and the ability to probe multiple, discrete absorbing wavelengths and globally analyze with interference information offers a measured improvement compared with dynamic light scattering (DLS). This work describes multiple calibrations and considerations as well as theoretical contributions concerning the application of AUC to nanoparticle systems.
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Chapter 1 Background

As nanoparticles become a more viable option for applications in energy, biology, and other complex fields\(^1\)\(^2\)\(^3\)\(^4\), the importance of complete characterization increases. Typical features remain elusive in the field, such as sample purity, molar mass, sedimentation coefficient, size and shape, stoichiometry, and binding affinity or kinetics, especially for complex assemblies of nanoparticles. Bulk sizing techniques such as electron microscopy (EM) and dynamic light scattering (DLS), and surface probing techniques such as atomic force microscopy (AFM) and scanning transmission microscopy (STM), contain many constraints\(^5\), including technical limitations, steep complexity, and low statistical significance. Most of these techniques don’t allow the observation of the nanoparticles in their native, biologically relevant state (e.g. suspended or in solution), with samples that instead must be immobilized on a surface and prepared with labels or tags. Still, these techniques are mostly unfit to directly measure many quantities associated with complex macromolecular assemblies, let alone in a single measurement. For example, DLS might be used to measure size, but is limited to ranges above our group’s nanoparticle diameter, or STM may give surface information, but is too low in resolution to provide size.

In this thesis, I will use analytical ultracentrifugation, sometimes combined with bulk fractionation, to elucidate many quantities that are normally independently measured for nanoparticles (such as size and shape) from first principle and to higher resolution than currently available without AUC. The objective of this thesis is to assist nanoparticle scientists to gain a greater understanding of AUC and to practically explain how modern sedimentation velocity experiments can be used to characterize nanoparticles and nanoparticle assemblies. Following this general treatment, I plan to examine how the surface structure influences the cell penetrating ability for a specific type of mixed-ligand nanoparticles.\(^2\)

1.1 Analytical Ultracentrifugation

1.1.1 Theory

Analytical Ultracentrifugation is a characterization technique popular in the fields of molecular biology, biochemistry, and polymer science. A sample being spun experiences centrifugal force, and the subsequent movement can be observed in real time with a set of onboard optics, utilizing the sample’s light absorption or optical refractive index differences between the sample and solvent. Many sample features can be elucidated, including size, shape, and sample-sample interactions. Because this technique also fractionates the sample, we measure not only average values, but the compete distributions of these values, such as molecular weight distributions (MWD), particle size distributions (PSD), and the density distribution (DD, chemical heterogeneity). Also, the polyelectrolyte character of dissolved macromolecules can be measured by multiple experiments using salt variation.

As the sample is pelleted by centrifugation, the evolution of the sample concentration is monitored and can be analyzed mathematically. The scope of the instrument involves two primary branches of experiments: sedimentation velocity (SV) and sedimentation equilibrium (SE) runs.\(^8\)\(^9\)

SV is a hydrodynamic method used to obtain size and shape information as determined by mass transport from both sedimentation and diffusion, while SE is a thermodynamic
method that reports only on mass, not shape, which relies on allowing the diffusion flux and sedimentation flux to equilibrate (no net flux). In this work, only sedimentation velocity is considered, as the density of metal nanoparticles eliminates the possibility of balancing diffusion and sedimentation, with sedimentation dominating. In general, SE and SV experiments are used to characterize reversible protein interactions, such as self-association, heterogeneous association, multi-protein complexes, binding stoichiometry, and the determination of association constants. Specifically, SV experiments are utilized to determine sedimentation coefficients by analyzing the moving boundaries of sedimenting species. The information contained in the sedimentation coefficient is related to many experimental details by the Svedberg equation. Since the AUC measurement takes place with molecules free in solution, the resulting size distribution is an accurate representation of the ensemble of particles in solution.

1.1.2 Instrumentation

A centrifuge is an apparatus used to create centrifugal fields by fast rotation of a rotor; ultra-centrifuges defined as a centrifuge capable of achieving more than ~5000 times the acceleration due to the Earth’s gravitation field (g). An analytical ultracentrifuge in an ultracentrifuge fitted with one or several optical detection systems, allowing the observation of the fractionation process of a species dissolved in solution. Beckman-Coulter Instruments developed two analytical ultracentrifuges, the XL-A and the XL-I, the former using UV and visible absorption optics and the latter integrating absorbance and interference optics. Absorbance optics are capable of detecting single or multi-wavelengths and interference optics detect small changes in refractive index between sample and solvent, which is useful for species which do not absorb, or absorb too poorly or abundantly at the concentration of interest. By simultaneous multi-optical detection, complex colloidal systems can be disentangled, even utilizing additional optical systems such as Schlieren, light scattering, and fluorescence optics. From an experimental standpoint, interference optics present disadvantages because refractive index variations are very sensitive to small impurities (high sample purity required), the optics must be independently adjusted before each experiment, and greater care must be taken to match the volume and chemical composition of the sample and reference cells (compared to absorbance). The choice of an appropriate optical detection system will be described in greater detail later. Because the rotor speed can be varied over a large range (600-60,000 rpm) detectable molecular weight and diameter ranges are large (300<M<10^{14} g/mol, 0.5<D<3000 nm), and SV runs typically require only 0.05 to 0.5 mg of material. Experimentally, small volumes (<450uL) of a dilute solution containing the macromolecule is loaded into a special sector-shaped cell, beside a reference cell containing only the naked solvent. The cells are loaded into one of two titanium rotors (An-50/60Ti which hold 1-7 cells and a counterbalance used for delay calibration of the optical systems) and the rotor is placed in a vacuum chamber at a controlled temperature during centrifugation.
1.1.3 Principles of Sedimentation Velocity

Dissolved or dispersed samples are exposed to high gravitational fields induced by the spinning of the centrifuge rotor, causing the solute macromolecules to drift from the meniscus to the bottom of centrifuge cell as a function of time. Each species is detected by optical detection systems which measure the concentration change with time and radius, \( c(r,t) \). Consider a single spherical particle of mass \( m_p \) and density \( \rho_p \) dissolved in a solvent \((\zeta)\) with density \( \rho_s \) and viscosity \( \eta_s \), in a gravitational field \( \omega^2 r \) at a radial distance \( r \) from the center of rotation as in Figure 1 (adapted from [15]).

*Figure 1: Forces on a centrifuging species*

Three forces are immediately apparent.

The first is the centrifugal force \( F_c \), induced by the acceleration \( \omega^2 r \) of the AUC rotor:

\[
F_{\text{centrifugal}} = m_p \cdot \omega^2 r = \frac{M}{N} \cdot \omega^2 r \quad (\text{Eq. 1.1})
\]

where \( M \) is the solute molar mass and \( N \) is Avogadro's number. This is the only force acting away from the center of rotation. Equation 1.1 illustrates that the centrifugal force increases with increasing mass of the particle, and thus larger particles sediment faster than smaller ones (so long as the mass of the particle is greater than the mass of the solvent).

The second is the buoyant force \( F_b \), governed by Archimedes principle and proportional to the mass of the displaced solvent \( m_s \) and \( \omega^2 r \), but opposite in direction to \( F_c \).

\[
F_{\text{buoyant}} = -m_s \cdot \omega^2 r = -\frac{M}{N} \cdot \bar{v}_p \cdot \rho_s \cdot \rho_p \cdot \omega^2 r \quad (\text{Eq. 1.2})
\]

with \( \bar{v}=(\rho_p)^{-1} \) as the solute partial specific volume or the reciprocal of particle density.

The final is the frictional force \( F_f \), produced by the movement of the particle through the solvent, according to the hydrodynamic treatment of drag around an object:

\[
F_{\text{frictional}} = -f \cdot u \quad (\text{Eq. 1.3})
\]
with frictional coefficient \( f \) and the sedimentation velocity \( u \) of the solute.

The three forces come into balance very quickly (~10^{-13} \text{ secs}) after the application of a centrifugal force and the particle achieves its terminal or sedimentation velocity \( u \) (the observed velocity of the particle):

\[
F_e + F_s + F_f = 0 = \frac{M}{N} \cdot \omega^2 r - \frac{M}{N} \cdot \bar{v}_p \cdot \rho_s \cdot \omega^2 r - f \cdot u \quad \text{(Eq. 1.4)}
\]

By rearranging (Eq. 1.4):

\[
\frac{M(1-\bar{v}_p \cdot \rho_s)}{N \cdot f} = \frac{u}{\omega^2 r} = s \quad \text{(Eq. 1.5)}
\]

This contains the meaning of the sedimentation coefficient, \( s \), now defined as the terminal velocity per centrifugal field. The frictional coefficient, \( f \), is well known from hydrodynamic theory, and depends on the shape and size of the particle. For a smooth, compact sphere under the Reynolds limit (small size), the Stokes-Einstein (Eq. 1.6) and Stokes’ equation (Eq 1.7) give \( f \):

\[
f = \frac{kT}{D} = \frac{RT}{ND} \quad \text{(Eq. 1.6)}
\]

\[
f = 3\pi \cdot \eta_s \cdot d_p \quad \text{(Eq. 1.7)}
\]

with Boltzmann constant \( k \), gas constant \( R \), and particle diameter \( d_p \) of a spherical particle.

Merging Eq. 1.5 and Eq. 1.6 leads to the Svedberg equation:

\[
M = \frac{s \cdot RT}{D(1-\bar{v}_p \cdot \rho_s)} \quad \text{(Eq 1.8)}
\]

now independent of \( f \) (size and shape). Combining Eq. 1.7 and 1.8 gives the basis for measuring Stokes-equivalent spherical diameters:

\[
d_p = \sqrt[18]{\frac{18 \cdot \eta_s \cdot s}{(\rho_p - \rho_s)}} \quad \text{(Eq 1.9)}
\]

The determination of \( s \) and its concentration in the sample are the primary outputs of the AUC.

1.1.4 Lamm Equation Modeling

A more accurate thermodynamic approach to modeling particle sedimentation with AUC utilizes the general Lamm equation, which describes the change in sample concentration with radius and time during an ultracentrifugation experiment. The partial differential Lamm equation describes the spatial and temporal behavior of concentration, considering mass transport of both sedimentation and diffusion. Diffusion describes the cooperative solute spreading effect produced by individual molecule Brownian motion. It
can be mathematically stated as a constant, the diffusion coefficient $D$, which is the spread of the molecule over time (units: cm$^2$/sec). The diffusion and sedimentation can be separated, but contribute additively to the boundary shape, which is described fully by the general Lamm equation15:

$$\frac{dc}{dt} = D\left(\frac{\partial^2 c}{\partial r^2} + \frac{1}{r} \frac{\partial c}{\partial r}\right) - \omega^2 s\left(r \frac{\partial c}{\partial r} + 2c\right) \quad (Eq. 1.10)$$

\[\text{diffusion term} \quad \text{sedimentation term}\]

A solution is a spatially and temporally resolved concentration function, $c(r,t)$. The AUC records the concentration (absorbance) profile by scanning (at up to three wavelengths of interest) at stepped intervals down in the radial direction down the cell. The mathematical analysis of the boundary movement of a species (with radius and time) allows the experimental measurement of the diffusion and sedimentation coefficients. Figure 2 illustrates the separation of the two terms for a single ideal species at a given time:

![Figure 2: Centrifugal Mass Transport: Sedimentation and Diffusion](image)

Bottom: A step function solution accounting for the sedimentation (black), with the actual solution (red) considering diffusion, which causes significant boundary broadening. Top: a possible configuration of a single ideal species in an AUC sector-shaped cell, which can be mathematically approximated by the red solution to the Lamm equation below.

A single species (wholly defined by a sedimentation coefficient $s$) sediments as a single boundary.11 The red concentration profile $c(r)$ pictured in the lower panel of Figure 2 can be treated as a graphical solution to the Lamm equation for a given time. Computer software (such as Sedfit, available from www.analyticalultracentrifugation.com) can fit an array of boundary profiles at different times to a set of experimental data points. The resulting $c^*(s)$ is a function that predicts an $s$-distribution and their relative concentrations (frequency). The asterisk indicates that the distribution is of the apparent $s$-value, at a given temperature and solvent condition. Generally, this is converted to the standard $s_{20,w}$ value, by the following the equation15.
The theoretical c*(s) fits the experimental data to user inputs of assumed values (solvent viscosity, density, solute partial specific volume, s range and resolution, regularization confidence, and frictional ratio). This is important, because AUC boundary analysis requires initial guesses that are close to the true values, therefore more independent measurements of these solution parameters will offer the best interpretation of the experimental data. The software performs a least-squares regression to minimize the error between the simulated solutions (generated in silico from the predicted c*(s) solution) and the experimentally measured data points. The measure of fit, or 'rmsd,' can be used as a measure to predict the validity of modeled solutions compared to the experimental data.

1.1.5 Microheterogeneity in Sedimentation Coefficient

At this point, the thermodynamic Lamm treatment has only been applied to a single, ideal species. Consider two sedimenting species with separated s values from Figure 3:

![Figure 3: Bimodal species](image)

(Left): The top and middle panels illustrate the two species measured separately, and the lower panel shows the bi-stepped Lamm equation solution after superposition. (Right): Illustrating the resulting superposed boundary when considering a bimodal species close in s.

When the two species have separated sedimentation coefficients, two clear steps can be resolved in the combined boundary plot. They are normalized (height) by their relative concentrations in solution. The right panel illustrates the difficulty in resolving steps for discrete species that are close in s-value. Because diffusion also serves as a boundary broadening effect, it becomes increasingly difficult to disentangle the sedimentation coefficient, s, from the diffusion coefficient, D, as species are added to the solution. However, diffusion and sedimentation are two mass transport phenomena that occur on different time scales, and at the limit of infinite time, sedimentation dominates. Experimentally one can increase the speed to simulate this effect, and in fact running the same sample over a range of speeds can lead to a very accurate measurement of each sedimentation coefficient, but at the expense of the diffusion coefficient. The general idea is
to deconvolute the effects of diffusion, so that it doesn’t obscure the existence of multiple species, and then take diffusion into account to accurately predict other thermodynamic quantities such as hydrodynamic radius ($R_H$), molar mass ($M$). The deviation of the hydrodynamic radius (measured from the experimentally fitted $D$)

While the analysis of sedimentation velocity experiments is straightforward and very accurate for s-monodisperse species, heterogeneity in $s$ results in difficult complications that should be considered, namely that the $s$-heterogeneity is tough to distinguish from diffusion. Normally, the general $c(s)$ is computed by the inversion of a Fredholm integral equation that has Lamm equation solutions as kernel$^{16-18}$, a method that implements a diffusion coefficient that scales with the sedimentation coefficient, according to a hydrodynamic scaling law $D(s)$ (derived from the Stokes-Einstein relationship and Svedberg equation)$^{19}$ based on the estimation of a single, weight-average frictional ratio ($f/f_0$):

$$D(s) = \frac{\sqrt{2}}{18\pi} kT s^{-1/2} \left( \eta \left( \frac{f}{f_0} \right)_s \right)^{-3/2} \left( \frac{\bar{v}}{1 - \bar{v}\rho} \right)^{-1/2}$$  \hspace{1cm} (Eq. 1.12)

with Boltzmann constant $k$, absolute temperature $T$.$^{16}$

While this treatment is acceptable for chemically homogeneous macromolecules,$^{20}$ it isn’t sufficient for ensembles of macromolecules exhibiting microheterogeneity in the sedimentation coefficient, especially in the case of metal nanoparticles, which exhibit a strong, non-linear increase in density ($\bar{\chi}^{-1}$) with increasing sedimentation coefficient (Sec. 2.2.1). An accurate description of the sedimentation must then consider heterogeneity of the solute properties, rather than interpreting the time-dependent spread of the sedimentation boundary as arising from a single, apparent diffusion coefficient. Luckily, the model can be expanded into a more general, two-dimensional diffusion distribution, $c(s,f)$ free of any assumptions of scaling laws,$^{12}$ which can be transformed to hydrodynamic radii, molar mass, and (assuming a theoretical particle density) $f/f_0$. The $c(s,f)$ approach provides a diffusion-deconvoluted, sedimentation coefficient distribution without any thermodynamic modeling assumption for $D(s)$.

A differential distribution of sedimentation coefficients and frictional ratios $c(s,f)$ can be defined as

$$a(r,t) = \iiint c(s,f) \chi(s,D(s,f),r,t) dsdf,$$  \hspace{1cm} (Eq. 1.13)

with $a(r,t)$ representing the total signal as a function of distance from the center of rotation, $r$, and time, $t$, $x(s,D,r,t)$ representing the solution of the Lamm equation, and $f$, abbreviated for $f/f_0$. $D(s,f)$ characterizes the diffusion coefficient dependence on the sedimentation coefficient and frictional ratio ($Eq. 1.12$). The $c(s,f)$ distribution can be transformed easily also to related distributions of hydrodynamic parameters, because any pair of two of the hydrodynamic and thermodynamic quantities $s$, $D$, $M$, $R_H$, and $f$, (except for $D$ and $R_H$, because the latter is solely determined from the former) determine all others.$^{15}$

Lamm equation solutions ($x(s,D,r,t)$ are calculated using finite element solutions, combined with Tikhonov-Philips (TP) regularization,$^{21}$ which penalizes the fit with the integral over the square of the second derivate of $c(s,f)$ along both dimensions. In the work of this thesis, a one standard deviation confidence level was used ($p=0.683$). The Fredholm equation ($Eq. 1.13$) is ill-posed, in that it might not have a solution in the strict sense, or solutions might not be unique or depend continuously on the data. Regularization is
commonly used to overcome this problem, and is a very important aspect in forming concentration distributions, so that systematic noise isn’t amplified to become the dominating feature of the distribution.

Although the s-range is typically discretized into a square grid, custom s-grids can be programmed. In this work, after an initial square grid run, a custom grid was created to improve the resolution in more ‘crowded’ areas of the distribution, and decrease the resolution in empty areas. This drastically improved the overall rmsd fit and improved computational time. Adding or removing s-ranges and checking the increase/decrease of the rmsd of the fit determine the acceptable limits of the s-grid.

While the $c(s_f) 2$-D distribution drastically improves the rmsd of the fit and also the fit of the simulated lines to the experimental data by visual inspection, the $s$ is more determined than the sedimentation coefficient. In fact, the $f_r$ dimension is hardly of use for large nanoparticles or at fast speeds, because diffusion is not appreciable or doesn’t perturb the boundaries enough to distinguish from noise. Typically, the rmsd shows a systematic deviation from the experimental data with increasing size, probably describing this problem.

1.1.5 Lamm Equation assumptions

There are multiple criteria that must be considered when applying the Lamm equation, none more important than the assumption of no heat convection (the passive transport of heat by a fluid motion). In a straight-walled tube, solute molecules displace and disturb the surrounding solvent while sedimenting, producing waves which fan heat down the tube and create a temperature gradient. The solvent viscosity and density, which both characteristically decrease with increased temperature, would also form a gradient, and so the buoyant mass of the solute would decrease non-linearly. The first consideration to minimize convection is to always allow the AUC rotor, cells, and samples to equilibrate at the desired run temperature (usually 20°C) for at least one hour before each run. Unfortunately, this limitation cannot be relaxed, and more than a 0.5°C variation in temperature during a run can cause abnormal sedimentation error, leading to increased $s$-value error. The sector shape of the AUC cell is the other important characteristic used to minimize convection (top Figure 2), so that the solute experiences a radial dilution while sedimenting. The extra spreading room decreases the solute-solute collisions, but increasingly dilutes the sample as it sediments. This effect can be ignored if sample $s$-values show low concentration and pressure dependence, which would both contribute non-ideally with dilution. The concentration dependence of $s$ and $D$ must be ensured experimentally by running the same sample over a range of concentrations (section 2.1.1).

As a general treatment of error in AUC measurement, examine the following experimental sedimenting boundary (a Lamm equation solution for a given time):
Figure 4: Error in the Lamm Equation

Due to the nature of optical systems, systematic time-invariant and radial-invariant noise components exist, and can be calculated algebraically and removed. Following this analysis, the $s$-values are determined. Because the boundary plateaus (B3 and D1) contain many points, the concentration (frequency after scaling with absorption) of $s$-values is well determined. The boundary midpoint is better calculated for C2 than in A4, so the $s$-value of the former is better determined. This type of analysis (determining how many points actually describe a species) can be used to find which $s$-values are more accurate than others. Heterogeneity spreads a single boundary, and so the C2 boundary may actually represent several steps of polydisperse solute species, each with diffusion. At higher speeds, the elucidation of these species becomes possible as the boundary separates into discernable steps, and so $s$-resolution increases dramatically.

Sedphat, a version of Sedfit for global modeling of multiple experiments, can be applied to a system run at varying speeds, concentrations, and temperatures, to account for non-ideality and achieve more accurate sedimentation coefficients. The main limitation of Sedphat is the inability to fit a two-dimensional $c(s,*_d)$ profile to the data.
Metal Nanoparticles

1.2.1 Background

Nanoparticles (NPs) have been intensely investigated due to their electronic\textsuperscript{5} and optical\textsuperscript{3} properties and their potential for use in systems such as chemical or biological sensing\textsuperscript{1} and catalysis.\textsuperscript{3} A common type of NP consists of a gold core coated with a self-assembled monolayer (SAM) of thiolated molecules. The SAM, or ligand shell, imparts many properties to the NP, such as solubility\textsuperscript{25} and assembly into complex structures.\textsuperscript{26} A thorough understanding of the composition and morphology of the ligand shell is essential for controlling the properties and behavior of NPs. Figure 5 illustrates AFM images of monolayer SAMs on flat gold surfaces (a) and spherical surfaces (b,c).

![AFM images](image)

\textbf{Figure 5: AFM of Mixed Ligand Monolayers on Flat Au Substrates and Spherical Au NPs.}\textsuperscript{45}

(a-c) AFM imaging of mixed and homogenous SAMs in ultrapure water. The topographic image (left) and the corresponding phase image (right) are presented. The phase image is an indication of the energy dissipated by the vibrating tip in the surface solvation layers and consequently the work of adhesion. For flat SAMs (a), the phase image can distinguish between regions coated with the different ligands (1-octanethiol is darker and Mhol is lighter, borderline highlighted). On the nanoparticles, mixed SAMs spontaneously self-assemble into striped domains (arrows in (b)), also visible in the corresponding phase image where maxima in the local energy dissipation are visible (lighter regions). When imaging nanoparticles coated with one ligand, no clear ordering is visible on the nanoparticles (c), as confirmed in the phase image.

In a SAM composed of two immiscible molecules on a flat surface, phase separation occurs and the molecules segregate into randomly shaped patterns.\textsuperscript{27} My group has previously demonstrated\textsuperscript{25,28,29} that when a NP is coated with such a mixture, the ligands phase separate into ordered, striped domains, or “ripples.” These stripes form because of a
competition between enthalpic losses and entropic gains (when mixing molecules of different length) at phase boundaries. The alignment of stripes is driven by topological constraints inherent in assembling what should be a 2D crystal (the SAM) onto a 3D surface. Ripples impart unique properties. For example, a series of NPs with an increasing ratio of hydrophobic to hydrophilic ligands shows anomalous non-monotonic solubility in various solvents.

Figure 6: 3D rendering of STM height images of gold nanoparticles (a) Decanethiol/MPA (2:1 molar ratio) showing ripples and (b) OT/MPA (10:1 molar ratio) showing packed, phase-separated domains but not ripples. (c),(d) Schematic drawings of (a) and (b), respectively.

Rippled NPs also are highly resistant to protein nonspecific adsorption. We recently predicted the existence of a size range for ordered striped domains in simulations. On small NPs, a binary mixture of surfactants separates into two “bulk” phases. On increasing the NP radius, phase separation into ordered ripples occurs. When the radius is further increased, disordered stripes and patchy domains form. The experimental size range where ordered ripples (and consequently structure-determined properties) are present was not determined.

1.2.2 Synthesis

Synthesis of nanoparticles: 2 mmol of gold salt (HAuCl4) was dissolved in 100 mL ethanol. 1 mmol of the desired thiol ligand mixture (2:1, MUS:OT or all MUS) was dissolved in 10mL methanol, and then added into the stirring reaction solution. 1 g of sodium borohydride (NaBH4) was dissolved in 75 mL ethanol, and added dropwise to the gold/thiol mixture, accompanied by a distinct color change (yellow to dark purple). Once there was no further color change, the remaining volume was added at once. The solution was stirred for 90s min and placed in a refrigerator to precipitate for 2 hrs. The precipitated particles were collected via vacuum filtration with qualitative filter paper, washed with ethanol, methanol, and acetone and dried under vacuum.

1.2.3 Assemblies

The potential for the introduction of divalency is a major consequence of ripples in NPs; that is, rippled NPs possess two highly reactive polar point defects. Molecules in a SAM have a tilt angle relative to the surface normal to maximize interactions with their
neighbors.\textsuperscript{27,33} For NPs, this tilt angle remains consistent relative to an axis of the NP rather than changing on each facet of the crystalline core.\textsuperscript{34} Arranging such a vectorial order (projections of the SAM molecules) onto a topological sphere (a NP core) is only possible if two defect points form in diametrically opposed positions on the sphere (Hairy Ball Theorem).\textsuperscript{35,36} This implies that two defect sites exist in the ligand shell at which the molecules are not optimally stabilized by intermolecular interactions.

\begin{center}
\textbf{Figure 7: Chaining of Striped Nanoparticles}\textsuperscript{37}
\end{center}

(a) Two steps of chain formation: pole functionalization of rippled NPs with MUA then interfacial polymerization. (b) NP size distributions measured from TEM images. Red is that of the starting NPs, green is that of chained NPs, and blue is that of unchained NPs. Bars represent the actual distribution; lines are Gaussian fits. The graphs are normalized for better visualization; the average NPs size after chaining is 2.7nm in this case. Below left is a cartoon of a rippled NP; below right is a representative TEM image of NP chains, scale bar 25 nm.

Previously, our group proved that these sites, or “poles”, are highly reactive and can be selectively functionalized with a place-exchange reaction.\textsuperscript{31} Specifically, we placed carboxylic acid-terminated molecules at the poles to generate divalent NPs (\textit{Figure 7}); subsequent reaction of these NPs with diamine molecules generated chains of NPs. Notably, this chemical divalency cannot be introduced in homoligand NPs. Previously, we’ve shown that divalent NPs (and consequently rippled NPs) exist only in a certain size range; smaller and larger NPs cannot form chains (\textit{Figure 8}).\textsuperscript{37} With AUC, the true existence and quantification of these assemblies can be accomplished, and characterization of their kinetics can be accomplished by systematic study.
Figure 8: Size distributions before and after chaining for three different sets of NPs. Chains form only for NPs in a certain size range. Bars represent actual data; lines are Gaussian fits. Each type of line (solid, dashed, or dashed-dotted) represents one complete set of NPs (starting, unchained, and chained distributions). (a-c) Simulation snapshots of phase separation in mixtures of ligands of unequal length on spheres of increasing diameter.
Chapter 2 AUC Results

Any adaptation of a technique to a new macromolecular system should be carefully calibrated. In the case of AUC, many factors contribute to sedimentation, including concentration, temperature, polydispersity, diffusion, shape, rotor speed, path length and shape, etc. In order to standardize the AUC of gold nanoparticles, I began with control experiments to account for any non-ideality and to find acceptable ranges (or at least reasonable initial parameters) for many of these variables. After finding typical conditions for SV runs, a simple mathematical treatment was derived in order to transform the sedimentation coefficients to a particle size distribution. AUC was then applied to characterize a systematic study of NP synthesis parameters that have remained elusive with other techniques, and finally to complex assemblies of nanoparticles.

2.1 Design of Experiments
For these controls, the c(s) model was used. The 2D c(s,f) model is computationally intensive, and because the following experiments were designed to probe an experimental parameter and measure s variation, the 2D model was not necessary.

2.1.1 Concentration
Several pseudo-monodisperse (pseudo meaning monodisperse in size, >10%, but not in s) samples at varying sizes of homoligand (MUS), water-soluble gold nanoparticles were prepared by fractionation (to be discussed) for the completion of several control experiments (referred to as control samples). The physical origin of hydrodynamic non-ideality is considered.

Both s and D are concentration-dependent quantities, because the flow field around the particles decreases with 1/R (inverse distance between neighboring solute molecules). If R decreases, each other’s flow fields influence pairs of particle and s and D are affected. To ensure that our experiments occur in the range of ideal concentration (independent for each system), the control samples were tested at multiple concentrations:

![Figure 9: Average Sed. Coeff vs. concentration (All MUS Au NPs)](image)

No variation of sedimentation coefficient with concentration in the local, workable concentration ranges.

(no error bars because the test was ran once per concentration)
Actually, because our system uses gold nanoparticles which uniquely absorb light at \( \lambda = 520 \text{nm} \), our minimum and maximum concentrations are in the limit of acceptable arbitrary absorbance units, above and below which the noise in absorbance becomes intolerable anyway. In this range, the average sedimentation coefficient remains statistically unchanged (Fig. 3). Experimentally, this means the concentration should be between 1 and 2 AU, measured first with UV/Vis spectroscopy. A consideration of error and statistics explains that the concentration should be maximized in this range, to increase the spatial boundary resolution for subsequent Sedfit analysis. The experiments in this thesis are all performed in this concentration range.

In addition to experimental consideration of maximum concentration, a mathematical treatment approximates an acceptable ‘volume fraction’. Hydrodynamic theory predicts that the statistics of particle pairs close in vicinity due simply to random motion can lead to reduced drag on the pair (a pseudo-dimer), and increasing the volume fraction dramatically enhances this effect. Therefore, the true \( s \) is determined empirically as:

\[
s = s^0 (1 - 6.55\Phi) \quad (\text{Eq. 2.1})
\]

This equation is valid up to volume fraction \(< 0.05\) and for non-interacting systems. In the range of concentration determined above (e.g. 0.01mg to 0.1mg), our volume fraction ranges from 0.0005 to 0.0008, thus our error in \( s \) is less than \( > 0.01\% \), much less than the limiting error due simply to absorbance noise (\( \sim 1-2\% \)) and is thus acceptable.

2.1.2 Temperature

During the duration of a single SV experiment, the temperature should fluctuate no more than \( 0.5^\circ \text{C} \). The advanced temperature detection system in the XL-I (a radiometer that accurately senses the actual rotor temperature within \( 0.05^\circ \text{C} \) advertised precision) records the temperature at each radial scan. Fluctuations in temperature greater than \( 0.5^\circ \text{C} \) during a single experiment leads to a convective instability that is best avoided. Experimentally, when the sample is loaded into the AUC and allowed to come to desired temperature, 1 hour should be allowed for sample equilibration and thus convective stability. For most applications and for every experiment in this thesis, 20°C was used for the absolute temperature. The range of allowed temperatures in the AUC is from 4°C to 22°C, but these weren’t tested with our system.

2.1.3 Polydispersity, diffusion, and speed

As acknowledged in the introduction, the Lamm equation modeling of complex boundaries from polydisperse samples can be problematic, due to the uncertainty in detangling multiple steps in the concentration profile from noise. Because Lamm equation solutions can be superpositions of single particle solutions, the shape of the boundary is fitted to approximate multiple particles. Two species with close values of sedimentation coefficient run at sufficiently fast speed will separate into two steps in a single concentration profile (Fig. 3). Sedimentation mass transport occurs linearly with time, while diffusion goes with the square root of time, so at the limit of infinite time, sedimentation dominates. Experimentally, this means that high-speed spins can extensively decrease the influence of diffusion (particularly useful for quick c(s) modeling to get a general size distribution), and the elucidation of accurate \( s \) values can be ascertained, with the confidence that the boundary spreading is due to heterogeneity in \( s \), not from diffusion. I have found that the best way to accomplish this is to find a speed in which the visible resolution of steps (not always possible
for very heterogenous species) can be obtained, for example, in Figure 10:

![Graph showing absorbance vs. distance from rotor center](image)

**Figure 10: Experimental boundary profile showing resolvable steps for separate species.**

At long times (yellow to red) the nanoparticles visibly separate into discrete sedimenting bands. The set of functions that fit these experimental curves is a solution to the general Lamm equation, yielding \( s \) and \( D \). The red arrows indicate the clear stepped boundary midpoints formed by discrete, sedimenting species.

As a loose experimental rule for metal nanoparticles, 20 krpm serves a good starting point for an initial run to capture your species. Eventually speeds upwards of 40 krpm can be used for smaller species. Monodisperse species, however, can be run at a much slower speed and analysis of the boundary shape (which would be a theoretical step function without the diffusion term in the Lamm equation) can give information on diffusion and a comparison of diameter from diffusion and diameter from sedimentation can be achieved. Prior fractionation of polydisperse samples followed by AUC can be used in the same way to gain shape (and thus surface structure) information and is treated in Section 3.3.1. In general high rotor speed (above 30,000 rpm) leads to higher resolution in \( s \) and lower influence of \( D \). Using complete data sets from the beginning to the end of sedimentation also increases resolution (without sufficient sedimentation time, \( c(s) \) is ill-defined).

**2.1.4 Radial Step Size**

While scanning across the cell, an adjustable parameter is the rate of scanning by selecting a scanning step size. The default step size (for a 14 mm cell) is 0.03 mm, however this can be adjusted depending on how many cells are ran concurrently, as the instrument scans only one cell at one step at a time. For three cells, the first cell is scanned from 0 to 14 mm at 0.03 mm steps (~450 points), followed then with the second and third cell, before coming back around to the first. At high speeds, significant sedimentation occurs in each cell in between scans, and much information can be missed. In this case, it is advantageous to decrease the radial resolution by increasing step size to 0.1 mm. Generally, samples are themselves run ‘quick-and-dirty’ (20°C, 20 krpm, 0.1 mm) in an effort to elucidate the preferred step size, and whether or not other samples can be run concurrently without reducing the available sedimentation information. This information isn’t wasted, because it can be combined in a global analysis with the eventual high quality information at the optimal parameters. In the end, each sample has its own optimization process, and this should be perfected to achieve the most accurate size-distribution information.
2.2 Particle Size Distribution

2.2.1 c(s) model

Considering that the NP is made of two concentric spheres, each with constant density ($\rho_{\text{core}}$ and $\rho_{\text{ligand}}$), a weighted density equation can be derived (full derivation in Appendix A):

$$\rho_p = \rho_{\text{lig}} + \frac{r_{\text{core}}^3}{r_p^3} (\rho_{\text{core}} - \rho_{\text{lig}}) \quad (Eq. 2.3)$$

and combining with Eq. 1.9, a functional dependence between NP diameter ($d_p$) and sedimentation coefficient ($s$), (given $\rho_{\text{core}}$, and $\rho_{\text{ligand}}$) goes as:

$$s = \frac{d_p^3}{18 \cdot \eta_s} \left( \rho_{\text{lig}} - \rho_s \right) + \left( \frac{d_p - l_{\text{lig}}}{d_p} \right)^3 \left( \rho_{\text{core}} - \rho_{\text{lig}} \right) \quad (Eq. 2.4)$$

Plotting particle diameter ($d_p$) as a function of $s$ for multiple values of ligand radius and taking constant the core density and shape (spherical gold core: $\rho_{\text{core}}$=19.3 g/cm$^3$), ligand density ($\rho_{\text{ligand}}$≈1.2 g/cm$^3$) solvent viscosity ($\eta_s$=0.01002 N sec/m$^2$), and solvent density ($\rho_w$=0.997 g/cm$^3$), yields the following curves:

![Figure 11: NP Diameter as a function of Sedimentation Coefficient for Varying Ligand Systems.](image)
A species of MUS:OT NPs was run at varying speeds and globally analyzed to obtain a best-fit \( c(s) \) function:

\[
\begin{align*}
0.02 & \\
0.018 & \\
0.016 & \\
0.014 & \\
0.012 & \\
0.01 & \\
0.008 & \\
0.006 & \\
0.004 & \\
0.002 & \\
0 & \\
100 & \\
200 & \\
300 & \\
400 & \\
500 & \\
600 & \\
\end{align*}
\]

\( \text{Sedimentation Coefficient (S)} \)

**Figure 12: \( c(s) \) for MUS:OT (2:1) AuNPs.**

Top: \( c(s) \) distribution function; Bottom: visual representation of residual error under a bitmap with \( (x,y,\text{contrast}(-0.5\text{black},0.5\text{white})=(\text{radius from rotor center, time, local residuals}) \).

To convert to core diameter, Eq. 2.4 can be written as a polynomial (substituting \( x \) for total diameter, and defining a few constants):

\[
s = a \cdot x^2 \left( d + b \frac{(x - c)^3}{x^3} \right) \tag{Eq. 2.5a-c}
\]

\[
a = \frac{1}{18} \cdot \eta_c, \quad b = (\rho_{\text{core}} - \rho_{\text{ag}}), \quad c = l_{\text{ag}}, \quad d = (\rho_{\text{ag}} - \rho_c)
\]

\[
s = (ad + ab)x^2 - 3abcx + 3abc^2 - \frac{abc^3}{x}
\]

This polynomial can be solved for each value of \( s \) iteratively using mathematical analysis software (such as Matlab) to yield the following distribution:
This conversion from s to diameter takes into account assumptions such as the approximation of all sedimenting species as ideal, non-interacting hard spheres. The ligand length was taken as the approximate length of the outer ligand, MUS = 1.8nm (calculated in ChemDraw®).

The c(s) distribution is highly smoothed, but well determined, and normalized from total loading concentration, so the frequency is quantitative when multiplied by the loading value. A more accurate approach would take into account a variable frictional coefficient, which in reality actually frees the model from any thermodynamic assumption of a D(s) scaling law (Eq. 1.12).

2.2.2 c(s, f/f0) 2D model

The c(s,f/f0) model outputs a two dimensional value distribution plot following fitting, including s, R_H, D, f/f0, and c(s,f/f0). The s and D are experimentally determined from fitting to the data by a least-squares regression, free of any scaling laws for D(s) or anything else.¹² The D is converted to R_H, and f/f0 is fitted for each value of s and D and the constant input partial specific volume (v). In our case, the partial specific volume is in fact not constant, so rather a value was chosen (<0.1cm³/g) as to avoid f/f0 values close to zero, which compresses the 2D contour curves. This makes the f/f0 value ‘fake’ for our system, instead compensating for a variable v.

The two values s and D can independently be converted to diameter, the s from iteritively solving Eq. 2.5c and D from:

\[
d_r(D) = \frac{RT}{ND \cdot 3\pi \cdot \eta_s} \quad (Eq. 2.6)
\]

These values should be in good agreement for spherical particles in which our input values for densities and viscosites are valid. A Matlab script was written to easily convert from the 2D distribution to a diameter_r-diameter_D plot (Appendix B).
The simplest, near-ideal case that could be experimentally measured and modeled with $c(s,f/f_0)$ is a population of average-sized, monodisperse, homoligand gold nanoparticles. Dodecanethiol (DDT=1.6nm) gold nanoparticles were synthesized by the Stucky method. Figure 14 shows the particle distribution:

**Figure 14: Particle Size Distribution for DDT homoligand monodisperse NPs**

The above image represents the $c(s,f/f_0)$ for the DDT monodisperse NPs. The lower image represents the diameter-diameter plot, each from the sedimentation coefficient and diffusion coefficient, respectively. (Svedberg and Stokes-Einstein equations).

The DDT NPs exhibit a nearly symmetric diameter-diameter plot. The greater spreading in diameter_D compared to diameter_S is due to the lower input resolution in Sedfit. Recall from the discussion of error that $s$ is much better defined than the diffusivity, due to the shape of the boundary curves. The regularization is high in the D direction, to achieve a more parsimonious agreement to the data, which translates to uncertainty in the shape of the projected f/f_0 axis $c(s,f/f_0)$. In other words, the single peak pictured in Fig. 14 may actually be thinner or contain multiple thin peaks, which are unresolvable under the current experimental conditions.
2.3 Assemblies

2.3.1 Dimers

Due to the ability to functionalize the surface of the NPs, assemblies can be formed through chemical linkage. The following distribution of NPs were synthesized, coated with a 2:1 molar mixture of hexadecanethiol and 11-mercaptopoundecanamine:

![Figure 15: c(s,f/f0) for HDT:MUAM (2:1) AuNPs.]

Because the synthesis was tailored in order to produce a small size distribution of NPs, in which the ligands theoretically phase separate into two domains due to the high surface curvature. These two-faced ‘Janus’ particles would only be able to form dimers, trimers, or tetramers by this reaction scheme. The NPs were reacted with activated dicarboxylic acid groups in order to link the NPs into dimers.
There is a noticeable peak which appears at ~80 S, which corresponds to a dimer 30 S particle. The existence of the dimer peak is obvious. The sedimentation coefficient of the first peak in the c(s,*) distribution is 30 S.

Consider the following treatment for the prediction of a dimer and compact trimer and tetramer for a 30 S nanoparticle (the s-value for the peak in Fig. 15). The density of an assembly is unchanged from that of the monomer, and can be calculated using Eq. 2.3. A 29 S nanoparticle with ligands HDT:MUAM (2:1) in chloroform corresponds to a total NP diameter of 5.95 nm (by Eq. 2.4). For the dimer of a 5.95 nm NP, the density is unchanged ($\rho=2.32$ g/cm$^3$) but the axial radius is doubled:

\[ f_{sphere} = 6\pi\eta_s \cdot r_M \]  

\textit{(Eq. 1.7)}
with $r_M$ the radius of a single NP or monomer. For a non-spherical dimer, the frictional coefficient is:

$$f_{\text{dimer}} = f_{\text{sphere}} \cdot f_P$$  \hspace{1cm} (Eq. 2.6)$$

where $f_P$ is the Perrin frictional factor and $f_{\text{sphere}}$ is the frictional factor for a hard sphere with the same volume as the ellipsoid of interest:\textsuperscript{41}

$$f_{\text{dimer}} = 6\pi \eta_s \cdot r_H = 6\pi \eta_s \left( \frac{3V_D}{4\pi} \right)^{1/3} = 6\pi \eta_s \cdot (2)^{1/3} \cdot r_M$$

$$f_P = \frac{2p^{2/3}}{S}$$

$$p = \frac{a}{b}$$  \hspace{1cm} (Eqs. 2.7 a-e)

$$S = \frac{2\cdot a \tanh \xi}{\xi}$$

$$\xi = \sqrt{\frac{p^2 - 1}{p}}$$

where $V_D$ is the volume of a dimer, which is twice that of its monomer, $V_D = 2V_M$. Plugging this into the equation adds a factor of $2^{2/3}$ to the frictional ratio. Finally, the frictional factor for a non-spherical ellipsoidal ‘dimer’ is:

$$f_{\text{dimer}} = 6\pi \eta_s \cdot r_M \cdot \left( \frac{5\sqrt{3}}{7} \right) = f_{\text{monomer}} \cdot 1.24$$  \hspace{1cm} (Eq. 2.8)$$

This form of the Perrin translational frictional factor simply multiplies a small correcting factor by the frictional factor for the monomer (given by the Stokes Eq. 1.7). A modified Svedberg equation is then:

$$d_{\text{dimer}} = \sqrt{\frac{18 \cdot \eta_s \cdot s}{(\rho_P - \rho_s) \cdot 1.24}}$$  \hspace{1cm} (Eq. 2.9)$$

Even though the Svedberg equation is derived by assuming a spherical volume, the approximation of the dimer as an equal volume sphere enables the use of the equation for dimers and assemblies.

Applying this treatment to the 30 S peak in the pre-dimer reaction NP distribution yields an approximate value of 80 S, the location of a new peak in the post-dimer NP distribution.

2.3.2 Chains

To synthesize chains, gold NPs coated with a 2:1 molar mixture of hexadecanethiol and 11-mercaptoundecaneamine typically undergo a two-step reaction.\textsuperscript{37} The first step is pole functionalization, a place-exchange reaction at the polar defect sites, in which the NPs are
stirred with a small excess (typically ~20-fold) of 11-mercaptoundecanoic acid (MUA), and then filtered to remove unreacted MUA. The second step is an interfacial polymerization in which the NPs in toluene react with a water solution of 1,6-diaminohexane. A precipitate appears at the interface, indicating the formation of insoluble chains. Transmission electron microscopy (TEM) is normally used to characterize the precipitate and the supernatant and to determine the NP size distribution. However, in this work, because the terminal functional group of the outer ligand is an amine, the chaining reaction is simplified to a single combined step of a one-phase pole functionalization with the dicarboxylic acid linker molecule. The following relatively complex c(s,*') distribution of NPs were synthesized, yet unlike the NPs prepared for dimerization, these NPs are larger in size, in the expected size range for ordering, or 'striping'.

The NPs were then reacted with linker molecules to selectively functionalize the poles of the 'striped' population, in order to form linear assemblies, from dimers to trimers and n-mer chains. The resulting monomer/assembled solution was examined with AUC and overlaid on the initial raw data:
Figure 19: c(s,f,f0) for HDT:MUAM (2:1) AuNPs and its Assemblies.

There are a series of peaks that appear at higher $s$ values, corresponding to discrete chains. This is the first direct measurement of chains in solution.

Future work will quantify and categorize each peak by analyzing the more general form of Eq. 2.7a, which can be applied for any $n$-mer linear chain:

$$f_{n\text{-mer chain}} = 6\pi\eta_s \cdot (n)^{1/3} \cdot r_M \cdot f_p$$  \hspace{1cm} (Eq. 2.7b)

This reinforces the idea that only NPs in a certain size range are divalent. In conclusion, this work demonstrates that chaining occurs only with NPs in a certain size range; this, by extension, implies that the aligned rippled organization of the ligand shell only exists in that specific size range as predicted by simulation. We believe that these size limitations will have implications for other properties of rippled NPs. Moreover, here we have presented a simple approach to determine whether NPs have “rippled” ligand shells, by starting with a distribution monodisperse in $s$ (by preparative fractionation) followed by chaining and analysis.
Chapter 3 Fractionation

Fractionation of nanoparticles describes the separation by sedimentation constant. Given the unique surface properties for nanoparticles, specifically as a function of increasing size or change in shape, the need for selective bulk separation is clear. Fortunately AUC instrumentation allows the measurement of the s-distribution. The treatment above can be used to determine diameter and shape, and small changes in surface structure can be inferred. This approach enables the testing of nanoparticle function over a range of sizes. The 'resolution' of fractionation methods available currently is basically unusable, and no preparative method exists to fractionate tightly distributed bands of nanoparticles.

3.1 Experimental Procedures

A sucrose gradient was implemented for the bulk preparative fractionation of water-soluble nanoparticles. The density range of the gradient varies linearly from the tube top to the bottom, such that the density of the particles is greater than the medium at all points. The basic principle is that larger particles sediment faster through the gradient than small ones, just like in a uniform solvent (this is also the fundamental principle of AUC).16

A small aliquot of nanoparticles in solution ($M = 5$ mg/mL) was carefully layered on top of a sucrose gradient (20%-50%) in a 13 mL preparative centrifuge tube (for the SW41 Ti Rotor). The sample was accelerated to 41000 rpm (rotor max speed) and removed after some time (on the order of one hour).

The following was observed for centrifugation of MUS:OT (2:1) NPs. The existence of bands can be seen overlayed on a diffuse continuous distribution of nanoparticles. The position can be measured and selectively fractionated with a Biocomp® fractionator, capable of sub millimeter fraction resolution.

Figure 20: Density Gradient Fractionation Band Measurement
3.2 General Gradient Model

The ability to run NPs through a density gradient and fractionate the bands by is useful, but the inability to target a specific monodisperse population of an originally polydisperse sample of NPs is problematic. After blind band fractionation, each unknown sample must be cleaned from sucrose, re-dissolved, and characterized individually by AUC to find the respective s-values (and thus particle size) of the collected bands. The ideal workflow would be to synthesize a batch of polydisperse NPs, perform an AUC run to find the c(s) distribution and desired s-values of the targeted population(s), and then selectively fractionate those NPs with the knowledge of how far in the radial direction of the preparative tube the desired s-value travels for a given time and rotor speed. This would eliminate the need for further characterization. In order to find this information, a mathematical treatment was developed in order to predict distance travelled as a function of s, time, and rotor speed (ω), then tested for accuracy by attempting to fractionate the following peaks from a general s-distribution obtained by AUC.

\[ r_{\text{band}}(s, \omega, t) \]

The treatment begins from the general definition of the sedimentation coefficient, terminal velocity per centrifugal field:

\[ s_{r,\zeta} = \frac{u}{\omega^2 r} = \frac{dr/dt}{\omega^2 r} \quad (Eq. 3.1) \]

where \( \zeta \) represents the local solvent conditions and \( r \) is the radial distance from the rotor center. To transform the sedimentation constant into standard conditions (to match AUC data taken in water at 20°C), Eq. 3.2 is applied:
Combining Eq. 3.1 and Eq. 3.2 leads to:

\[ s_{20,w} \omega^2 dt = \left[ \frac{1}{\eta_{20,w}} \right] \frac{\eta_{T_c} (\rho_p - \rho_{20,w})}{(\rho_p - \rho_{T_c})} \frac{1}{r} dr \]  

(Eq. 3.3a)

Eq. 3.3 is general for any centrifugation process in a uniform media, but in a sucrose gradient both the viscosity (\(\eta_{T_c}\)) and the density (\(\rho_{T_c}\)) are functions of the sucrose concentrations and hence of the distance of the medium from the rotor center, r.

\[ s_{20,w} \omega^2 dt = \left[ \frac{1}{\eta_{20,w}} \right] \eta_{T_c} (r) \left[ \rho_p (r) - \rho_{20,w} \right] \frac{1}{r} \]  

(Eq. 3.3b)

with \(\eta_{20,w}\) and \(\rho_{20,w}\) as constants. A major disadvantage of sucrose gradient centrifugation is the necessity of knowing the partial specific volume (\(\rho_p^{-1}\)) in order to determine the true \(s_{20,w}\). For this treatment, the particle density is determined recursively by initializing an estimated value for each r (from a calibration experiment), which gives an initial s-value that is transformed to \(\rho_p\) by rearranging Eq. 1.9:

\[ \rho_p = \frac{18 \cdot \eta_c (r) \cdot s}{(d_p(s))^2} + \rho_s (r) \]  

(Eq. 2.4)

taking the local solvent viscosity, \(\eta_c\), and local solvent density, \(\rho_s\), which are both functions of radius, r, and \(d_p(s)\) solved by a Matlab code using Eq. 2.5.

The left hand side of Eq. 3.3 can be integrated over the time of centrifugation, \(t\):

\[ \int_0^t s_{20,w} \omega^2 dt = s_{20,w} \omega^2 t \]  

(Eq. 3.4)

The right hand side of Eq. 3.3b can be numerically integrated using the trapezoidal rule, which approximates definite integrals:

\[ \int_0^{r_{i+1}} F(r) dr = \sum_{i=0}^{i+1} F(r_i)(r_{i+1} - r_i) + \frac{1}{2} \sum_{i=0}^{i+1} \left[ F(r_{i+1}) - F(r_i) \right] (r_{i+1} - r_i) \]  

(Eq. 3.5)

The expected s-value for each position is solved by calculating equal-spaced arbitrary distances (\(r_i\)) from the rotor center (for the SW41 Ti starting at the sucrose density meniscus, 71.4 mm) to the last point at which the centrifuge tube is perfect cylindrical (146.1 mm, for 89 mm standard tubes). Using standard values of sucrose molarity versus density and viscosity at given temperatures, F(r) can be calculated for each distance, \(r_i\).

For this treatment the time of centrifugation (\(t\)) and rotor speed (\(\omega\)) should be corrected to account for acceleration and deceleration of the rotor, which is unique for each centrifuge and should be found empirically. For the centrifuge used in these experiments, the
acceleration is included in the run time, while the deceleration occurs after, and so the total time of centrifugation was taken from the beginning of acceleration to the end of deceleration. The following linear rotor acceleration was found (and the converse was found for deceleration):

<table>
<thead>
<tr>
<th>Recorded Acceleration/Deceleration</th>
<th>Recorded Acceleration/Deceleration for Optima LE-80K UC</th>
</tr>
</thead>
<tbody>
<tr>
<td>45000</td>
<td>-</td>
</tr>
<tr>
<td>40000</td>
<td>-</td>
</tr>
<tr>
<td>35000</td>
<td>-</td>
</tr>
<tr>
<td>30000</td>
<td>-</td>
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<td>25000</td>
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<tr>
<td>20000</td>
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<tr>
<td>15000</td>
<td>-</td>
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<tr>
<td>10000</td>
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</tr>
<tr>
<td>5000</td>
<td>-</td>
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<tr>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

**Figure 22: Slow acceleration/deceleration for the Optima LE-80K Ultracentrifuge**

Using this treatment to blindly test for the expected s-values from Fig. 21 resulted in huge errors, especially further away from the sucrose meniscus. The particles acted much lighter (pelleted faster) than anticipated, and it was apparent that this treatment was flawed and that other non-idealities must be considered.

### 3.2.2 Hydration Correction

Solvent and cosolvent effects should be considered due to their quantitative effect on solute/solute interactions. A comprehensive analysis should include a Gibbsian thermodynamic treatment. The classical Svedberg equation (rearranged to solve for $s$):

$$ s = \frac{D(1 - \rho_s \bar{v}_2)M_2}{RT} \quad (Eq. 1.8b) $$

where $M_2$ is the molar mass of the macromolecular component 2, is in the limit of vanishing particle concentration and is derived only for two component systems.

The quantity $(1 - \rho_s \bar{v}_2)M_2$ is the Archimedes buoyant mass of the component 2. For charged systems, additional components such as sugars have to be considered, and the buoyant mass should be adjusted to include solvent and cosolvent interactions. The hydration of macromolecules has been shown to affect their sedimentation velocity in density.
gradients, and the hydration can be added to the treatment above.

A density gradient of a mixed solvent system can be approximated with 4 components: 1; a light solvent (water), 2- the macromolecule of interest (NPs) 3-a salt, and 4-a heavy neutral component (sucrose). For each position, the sedimentation coefficient at infinite dilution (subscript 0) is given by:

\[ s^0 = \frac{D(\frac{\partial \rho}{\partial c})_\mu \cdot M_r}{RT} \]  

(Eq. 3.6)

where the buoyant molar mass is replaced by the Gibbsian quantity of a density increment at constant chemical potential, \( \mu \), defined as:

\[ \left( \frac{\partial \rho}{\partial c} \right)_\mu = (1 - \nu \rho) + \xi(1 - \nu \rho) + ... \]  

(Eq. 3.7)

where \( \xi \) is a fitting parameter for the particular non-ideality considered. For hydration, \( B \), the hydration constant was used from a tabulated value of 0.3 g/g for sucrose.

The conversion to standard conditions with the hydration correction is:

\[ s_{20,\omega} = s_{T,\omega} \frac{\eta_{T,\omega}}{\eta_{20,\omega}} \left( \frac{1 - \bar{\nu}_p \rho_{20,\omega} + B_{T,\omega}(1 - \bar{\nu}_{T,\omega}\rho_{20,\omega})}{1 - \bar{\nu}_p \rho_{T,\omega} + B_{T,\omega}(1 - \bar{\nu}_{T,\omega}\rho_{T,\omega})} \right) \]  

(Eq. 3.8)

and the final integral is simplified to:

\[ s_{20,\omega} = \left[ \frac{1}{\eta_{20,\omega}} \int_0^x \left( \frac{\rho_{T,\omega} \cdot (\rho_p - \rho_{20,\omega}) + \rho_{T,\omega}B \cdot (\rho_{T,\omega} - \rho_{20,\omega})}{\rho_{T,\omega} \cdot (\rho_p - \rho_{T,\omega})} \right) \cdot \eta_{T,\omega} \ dx \right] \]  

(Eq. 3.9)

which can be evaluated by trapezoidal approximation or better, Matlab function analysis. This treatment resulted in a much closer fit to the actual fractionated bands as shown in Fig. 23:
Figure 23: Average NP Diameter Down the Tube Following Band Fractionation
Average Particle Diameter (core) as a function of position from the gradient meniscus as measured by TEM. 1-5 represents increasing diameter corresponding to the data points. The dispersity of each diameter was less than 15%. (Note dark aggregates in TEM images)

The fractionated bands were analyzed again using AUC to determine the accuracy of selection. Fig. 24 represents the c(s,i/f0) and calculated c(d,d_D):

Figure 24: Fract. ‘1’: Bimodal Distribution of NP Monomer/Aggregate.
From the c(s,f/f0) distribution, the experimentally measured s and D coefficients can be transformed separately into particle diameter. The diameter from D can be considered as a function of size-and-shape, the hydrodynamic size. The diameter from D depends on size and shape, but also strongly with density. The sharp peak in Fig. 24 measures ~7.3 nm in each dimension (from both s and D), indicating that population of NPs to be spherical and ideal, fulfilling the assumption of core and ligand density (19.3 g/cm³, 1.2 g/cm³) and ligand length (outer ligand MUS: 1.8 nm). Small adjustments and deviations are expected due to assumptions (constant ligand density and length). The spread in diameter of the main peak is from 7.21 to 7.42, a dispersity of 3%. The second peak has a hydrodynamic diameter about ~7 times that of the diameter from sedimentation coefficient. This peak is likely due to aggregation of a few nanoparticles around sucrose molecule impurities left over from the gradient centrifugation. By the integrated Eq. 3.9, fract. ‘1’ was predicted to be in the s-range of ~135 which gives an error of about 0.5 nm from the theoretical value.
Conclusion

AUC is a powerful tool for investigating nanoparticle size distributions and for detecting nanoparticle assemblies in solution in their natural hydrated state. A straightforward theoretical treatment enables the elucidation of nanoparticles, assemblies, and their properties. The program Sedfit serves as a tool to mathematically interpret the raw AUC sedimentation boundary data. For the future, many other types of measurements are possible with AUC, such as multi-wavelength measurements that could measure the stoichiometry of fluorescently tagged ligands to NPs, in order to study the kinetics of functionalization. Also, more detailed surface structure information or variation of size distribution with change in synthetic parameters can be determined by systematic study. Finally, the sedimentation distributions provide a map for bulk fractionation of the nanoparticles, which can be separated for function or study.

Future Work

For future work, I will attempt to sediment nanoparticle samples in a series of mixed solvents, in order to elucidate the ligand density distribution, measuring ligand density as a function of size. Also, multi-wavelength experiments are possible, in which the stoichiometry of ligands to nanoparticle can be determined. This could lead to kinetic studies of surface functionalization and coverage. Also, interacting systems (such as nanoparticle synthesis) will be monitored and characterized. AUC can also be used to systematically study nanoparticle synthesis by separately analyzing multiple NP batches that only had one synthetic parameter changed (e.g. ligand-gold molar ratio: 2:1, 3:1, 4:1). This will enable better synthetic control for targeted size distributions.
Appendix A: Derivations

Start with the definition of density:

$$\rho = \frac{m}{V} \quad (Eq. A.1)$$

where m is the mass and V is the volume.

$$\rho_P = \frac{m}{V} = \frac{m_{\text{lig}} + m_{\text{core}}}{V_T} = \frac{V_{\text{lig}} \cdot \rho_{\text{lig}} + V_{\text{core}} \cdot \rho_{\text{core}}}{V_T} = \rho_{\text{lig}} \cdot \frac{V_{\text{lig}}}{V_T} + \rho_{\text{core}} \cdot \frac{V_{\text{core}}}{V_T}$$

$$\rho_P = \rho_{\text{lig}} \cdot \frac{(4\pi / 3) \cdot (r_T^3 - r_{\text{core}}^3)}{(4\pi / 3) \cdot r_T^3} + \rho_{\text{core}} \cdot \left( \frac{(4\pi / 3) \cdot r_{\text{core}}}{(4\pi / 3) \cdot r_T} \right)^3 = \rho_{\text{lig}} \cdot \frac{(r_T^3 - r_{\text{core}}^3)}{r_T^3} + \rho_{\text{core}} \cdot \left( \frac{r_{\text{core}}}{r_T} \right)^3$$

$$\rho_P = \rho_{\text{lig}} \cdot \frac{(r_T^3 - r_{\text{core}}^3)}{r_T^3} + \rho_{\text{core}} \cdot r_{\text{core}}^3 = \rho_{\text{lig}} \cdot \frac{r_T^3 + r_{\text{core}}^3 (\rho_{\text{core}} - \rho_{\text{lig}})}{r_T^3}$$

$$\rho_P = \rho_{\text{lig}} + \frac{r_{\text{core}}^3}{r_T^3} (\rho_{\text{core}} - \rho_{\text{lig}})$$
Appendix B: MATLAB code for \( c(s, f/f_0) \) to \( c(d_s, d_D) \)

```matlab
% Clears all values
clear all

% prompt to get file
[fileName, pathName] = uigetfile(’*.txt’, ’Select AUC Data File’); % prompt to get file
raw_data = fileName; % to define the basename of your AUC file

% change command directory to the specified pathName
cd(pathName);

% Load AUC data txt file into the matrix 'data'
data = load(fileName);

ff0vec = data(:,3);
ff0min = min(ff0vec(2:length(ff0vec)));
ff0max = max(ff0vec(2:length(ff0vec)));
ff0inc = max(diff(ff0vec(2:length(ff0vec))));
nff0 = 1 + round((ff0max - ff0min) / ff0inc);

nD = length(data);
ns = length(data) / nff0;

NS = num2str(ns);

% Prompt for the solvent parameters
prompt = {'s-Resolution:', 'Solvent Viscosity (kg/s\times m):', 'Solvent Density (g/cm\^3):', 'Core Density (g/cm\^3):', 'Ligand Density (g/cm\^3):', 'Ligand Length (nm):', 'Temperature (K):'};
dlg_title = 'Input';
num_lines = 1;
def = {NS, '0.0005553', '1.490', '19.32', '1.2', '1.17', '293.15'};
solvent = inputdlg(prompt, dlg_title, num_lines, def);

svec = data(:,1);
Mvec = data(:,2); Mvec = Mvec / 1000;
Dvec = data(:,4); Dvec = Dvec * 1e7;
Rvec = data(:,5);
cvec = data(:,6);
smin = min(svec);
smax = max(svec);

% sinc = max(diff(svec));
Mmin = min(Mvec); Mmax = max(Mvec);
Dmin = min(Dvec); Dmax = max(Dvec);
Rmin = min(Rvec); Rmax = max(Rvec);
cmin = min(cvec); cmax = max(cvec);
svals = linspace(smin, smax, ns);
ff0vals = linspace(ff0min, ff0max, nff0);
sf0mat = zeros(ns, nff0);
sfmat = zeros(nD, ns);

for s = 1:ns,
    for f = 1:nff0,
        sf0mat(s,f) = cvec(s+(f-1)*ns);
    end;
end;

for s = 1:ns,
    for d = 1:nD,
        sfmat(d,s) = cvec(s+(f-1)*ns);
    end;
end;
```
[S,F] = meshgrid(svals,ffvals);
figure(3); clf;
h = contour(S,F,sff0mat');
ylabel('f/f0');
xlabel('s-value (S)');
colorbar;

aa=cell2mat(solvent(2,1));
aa=str2double(aa);  % solvent viscosity numerical value
bb=cell2mat(solvent(3,1));
bb=str2double(bb);  % solvent density numerical value
cc=cell2mat(solvent(4,1));
cc=str2double(cc);  % core density numerical value
dd=cell2mat(solvent(5,1));
dd=str2double(dd);  % ligand density numerical value
ee=cell2mat(solvent(6,1));
ee=str2double(ee);  % ligand length numerical value
ff=cell2mat(solvent(7,1));
ff=str2double(ff);  % temperature numerical value

a = (1/(18*aa));
b = 1000*(cc-dd);
c = (2*ee)*(10^-9);
d = -1000*(dd-bb);
R = 8.314472;
N = 6.022*10^23;
clear x
syms x
clear f
s = (10^13)*((a*b-a*d)*x^2-3*a*b*c*x+3*a*b*c^2-(a*b*c^3)/x);
t = 1;
u = cell2mat(solvent(1,1));
v = str2double(u);
for g=1:v;
f(g,1) = s-data(g,1);
end
p=1;
g = zeros(v, 2);
for i=1:numel(f);
h = solve(f(i));
h(2:3,:)=[];
g(i,1) = data(i,1);
g(i,2) = double(h)*(10^9);
end
real_g = real(g);
function_D(:,1) = data(:,4);
for z=1:numel(function_D);
frct(z,1) = (R*ff)/(N*(function_D(z)));
frct(z,2) = (10^13)*(frct(z,1))/(3*pi*aa);
end
data_FINAL = horzcat(data, frcet);
SD_FINAL = repmat(real_g, nff0,1);

% GRAPHICS

fvec = data_FINAL(:,7);
DDvec = data_FINAL(:,8);
SDvec = SD_FINAL(:,2);

fmin = min(fvec); fmax = max(fvec);
DDmin = min(DDvec); DDmax = max(DDvec);
SDmin = min(SDvec); SDmax = max(SDvec);
SDvals = linspace(SDmin, SDmax, ns);
DDvals = linspace(DDmin, DDmax, nD);

[SD, DD] = meshgrid(SDvals, DDvals);

Cmat = zeros(ns,nff0);
Smat = zeros(ns,nff0);
Mmat = zeros(ns,nff0);
Dmat = zeros(ns,nff0);
Rmat = zeros(ns,nff0);
Fmat = zeros(ns,nff0);
SDmat = zeros(ns,nff0);
DDmat = zeros(ns,nff0);

for s=1:ns,
    for f=1:nff0,
        Cmat(s,f)=cvec(s+(f-1)*ns);
        Smat(s,f) = svec(s+(f-1)*ns);
        Mmat(s,f) = Mvec(s+(f-1)*ns);
        Dmat(s,f) = Dvec(s+(f-1)*ns);
        Rmat(s,f) = Rvec(s+(f-1)*ns);
        Fmat(s,f) = ff0vec(s+(f-1)*ns);
        SDmat(s,f) = SDvec(s+(f-l)*ns);
        DDmat(s,f) = DDvec(s+(f-l)*ns);
    end;
end;

RDmat = 2*Rmat;

Yy=0;
YY=5+max(max(RDmat));
Xx=(min(min(SDmat)))-1;
XX=1+max(max(SDmat));
figure(5); clf;
h = surf(SDmat,RDmat,Cmat);
hold on;
for i=2:length(h),
    newz = get(h(i),'Zdata') + zoffset;
    set(h(i),'Zdata',newz);
end;
hold off;
xlabel('diameter from s (nm)');
ylabel('diameter from Diff. Coeff. (nm)');
zlabel('c(s,M)');
zoffset =0;
xlim([Xx XX]); ylim([Yy YY]);
view(0,90);
%axis([xmin xmax ymin ymax])
rotate3d;

%%%
%plot s-f/f0 3D
figure(1); clf;
h = surfc(S,F,sff0mat');
%contour(S,F,sff0mat,100);
ylabel('f/f0');
xlabel('s-value (S)');
zlabel('c(s,f/f0)');
zoffset = -0.3*max(max(sff0mat));
for i=2:length(h),
    newz = get(h(i),'Zdata') + zoffset;
    set(h(i),'Zdata',newz);
end;
axis([ smin smax ffimin ff0max zoffset 1.1*max(cvec)]);
view(30,90);
% shading interp;
alpha(0.1);
rotate3d;
figure(2); clf;
h = meshc(S,F,sff0mat');
ylabel('f/f0');
xlabel('s-value (S)');
zlabel('c(s,f/f0)');
zoffset = -0.4*max(max(sff0mat));
for i=2:length(h),
    newz = get(h(i),'Zdata') + zoffset;
    set(h(i),'Zdata',newz);
end;
view(0,90);
% shading interp;
alpha(0.1);
rotate3d;
Bibliography