Determination of nanoparticle size distribution together with density or molecular weight by 2D analytical ultracentrifugation
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Nanoparticles are finding many research and industrial applications, yet their characterization remains a challenge. Their cores are often polydisperse and coated by a stabilizing shell that varies in size and composition. No single technique can characterize both the size distribution and the nature of the shell. Advances in analytical ultracentrifugation allow for the extraction of the sedimentation ($s$) and diffusion coefficients ($D$). Here we report an approach to transform the $s$ and $D$ distributions of nanoparticles in solution into precise molecular weight ($M$), density ($\rho_p$) and particle diameter ($d_p$) distributions. $M$ for mixtures of discrete nanocrystals is found within 4% of the known quantities. The accuracy and the density information we achieve on nanoparticles are unparalleled. A single experimental run is sufficient for full nanoparticle characterization, without the need for standards or other auxiliary measurements. We believe that our method is of general applicability and we discuss its limitations.
Hybrid inorganic–organic core–shell nanoparticles (NPs) are finding a wide range of applications in solar cells, optoelectronics, nanophotonics/plasmonics, catalysis, drug delivery and biomedical imaging agents. Their chemical, electronic, optical, magnetic and catalytic properties, and self-assembly inherently depend on their size and composition. Hence, as industrial and research needs grow more complex, it becomes imperative to find a versatile, reliable and scalable method for the full characterization of these particles. The total evaluation of a NP entails a global analysis that pieces together measurements taken from multiple techniques (Fig. 1). However, such an approach usually presents an incomplete picture of the sample in question since NPs are rarely perfectly monodisperse. Methods that characterize the organic shell give only macroscopic averages of the whole sample distribution, and size analysis techniques provide distribution data only of the total NP (see Fig. 1). Combined analyses are possible, but become challenging as the complexity (for example, polydispersity) of the sample increases. For example, the recent advances, where fractionation and size analysis are used in series, provide a wealth of information, but at the expense of numerous assumptions and laborious approaches. Arguably, NP characterization has become a rate-limiting step, hindering the development and prospective uses of these promising materials.

NP research lacks a single platform that quickly, easily and completely characterizes the size, density (an indicator of composition) and molecular weight of each unique particle species among a heterogeneous mixture, with a single experimental measurement. However, recent 2D mathematical and computational modelling advancements in sedimentation–velocity analytical ultracentrifugation (SV–AUC) allow for the mapping of sedimentation coefficient and diffusion coefficient distributions of species present in solution. AUC is performed using an ultracentrifuge fitted with one or more optical detection systems, allowing the observation of the fractionation process of a species dissolved in solution. The sedimentation process is monitored by a scanning UV/VIS optical detection system that records the concentration profile, $c(r, t)$, with respect to radial distance from the rotor ($r$) and time ($t$). The $c(r, t)$ is subsequently numerically modelled and transformed into a sedimentation coefficient ($S$) and diffusion coefficient ($D$) distribution, $c(s, D)$.

SV–AUC is a tool particularly suited for the study of NPs. It does not require any standard or calibration. A solution to the Lamm equation is a spatially and temporally resolved concentration function, $c(r, t)$, sigmoidal in shape, real-valued and differentiable. The analytical ultracentrifuge records in that we require no prior measurement nor make any assumptions regarding the density of the NPs and that we utilize both the diffusion data (ignored or unavailable in most studies) and the sedimentation data obtained from 2D SV–AUC to determine NP density. It should be further noted that we assume a 1:1 correspondence between the hydrodynamic Stokes’ diameter and the actual diameter of the particle, which we prove to be an accurate description for a wide range of NPs. As our methodology is simple, rapid, accurate and scalable, we expect the findings in this research article to be useful to anyone interested in the properties and applications of NPs. This method could become useful for those applications that are especially sensitive to NP size and overall variability.

**Results**

**Theory.** The Lamm equation describes the evolution of a solute concentration distribution under centrifugation:

$$\frac{\partial c}{\partial t} = D \left( \frac{\partial^2 c}{\partial r^2} + \frac{1}{r} \frac{\partial c}{\partial r} \right) - \omega^2 s \left( \frac{\partial c}{\partial r} + 2c \right)$$

A solution to the Lamm equation is a spatially and temporally resolved concentration function, $c(r, t)$, sigmoidal in shape, real-valued and differentiable. The analytical ultracentrifuge records...
In our treatment, we assume that the Stokes’ diameter and the particle diameter are approximately equal (that is, \(d_H = d_p\)), and combine Equations 3 and 4—and re-express Equation 2 in terms \(\rho_p\) to obtain formulas that allow us to determine the particle parameters \(\rho_p\), \(M\), and \(d_p\) in terms of the experimentally measured \(s\) and \(D\) value distributions:

\[
\rho_p = \rho_s + 18\eta_s s \left( \frac{1}{D} \frac{k_b T}{3\pi\eta_s} \right)^{-2}
\]

\[(5a)\]

\[
M = \frac{sRT}{D} \left( 1 - \frac{\rho_s}{\rho_p} \right)^{-1}
\]

\[(5b)\]

\[
d_p = \frac{18\eta_s s}{\sqrt{(\rho_p - \rho_s)}}
\]

\[(5c)\]

Equations 5a–c represent the theoretical basis underlying our 2D SV-AUC measurements. In contrast to most SV-AUC approaches where only Equation 5c is used by assuming a particle density \(\rho_p\), we find that the \(\rho_p\) can instead be calculated from \(s\) and \(D\) through Equation 5a. Our approach’s accuracy at predicting the density \(\rho_p\), molecular weight \(M\) and diameter of NPs \(d_p\) is demonstrated experimentally in the next section.

2D AUC of single monodisperse standard nanoclusters. As an illustrative test case, we first measured the ‘magic-sized’ NP \(\text{Au}_{116}(\text{SR})_{30}\) \((R = -\text{CH}_2\text{CH}_2\text{Ph})\) (synthesis33 and ESI-MS characterization detailed in Methods). 2D SV-AUC was performed on a sample of \(\text{Au}_{116}(\text{SR})_{30}\) nanoparticles (Experimental details provided in Methods). Figure 2 plots the analysis obtained from the data collected for the \(\text{Au}_{116}(\text{SR})_{30}\) cluster.

The particle density was first calculated by Equation 5a using the integrated, weight-averaged diffusion and sedimentation coefficients, \(\rho_p = 4.51\ \text{g cm}^{-3}\). With the particle density in hand, Equation 5b is utilized to calculate molecular weight, \(M = 35,260\pm 180\ \text{Da}\). Remarkably, the molecular weight predicted by theory for \(\text{Au}_{116}(\text{SR})_{30}\) is \(M = 36,597\ \text{Da}\), giving our method a 4% error. The molecular weight found by ESI-MS33 for the sample studied was \(M = 36,598\ \text{Da}\). Finally, the \(\rho_p\) and sedimentation coefficient are transformed into particle diameter \(d_p\) by Equation 5c. The distribution of the diameters is weight-averaged to a single value after normalizing by the concentration distribution, \(d_p = 2.83\pm 0.01\ \text{nm}\).

2D AUC of a mixture of monodisperse standard nanoclusters. As a next step, we tested the validity of our method on a mixture of three atomically discrete thioldated gold NPs with the same type of organic ligand, also of exactly known molecular formula35–38. Again we obtain an excellent match (~2–3% error) between the actual molecular weights measured by ESI-MS and the ones we obtained with our method. Three Au nanocluster standards were mixed together: \(\text{Au}_{116}(\text{SR})_{30}\) TOA, \(\text{Au}_{116}(\text{SR})_{24}\), and \(\text{Au}_{116}(\text{SR})_{26}\) \((R = -\text{CH}_2\text{CH}_2\text{Ph})\). The \(\text{Au}_{116}(\text{SR})_{26}\) TOA nanocluster was bound to a tetracylammonium ion (TOA⁺) due to its negative monovalency39. After AUC, sedimentation coefficient distributions were fit to the experimental data with SEDFIT \((\sigma = 0.683)\), and the \(s\)–\(D\)-distributions
(Fig. 3b) were transformed into diameters, densities and molecular weights, by the same procedure used for the single Au$_{133}$(SR)$_{50}$ nanoclusters. The 2D distributions can be integrated over all diffusion coefficients and reduced to a one-dimensional plot (Fig. 3a). Table 2 summarizes the 2D experimental data for the three NPs sedimented together in solution.

**Determination of particle composition.** In the very common case of a NP composed of two phases (for example, core–shell NPs and monolayer protected NPs) we can obtain a reasonable estimate of the composition of the particle by combining the measured $M$ and $p_p$ with the bulk densities (usually tabulated or easily measured) of the two phases. For example, the values for the core and ligand densities are: bulk gold: 19.32 g cm$^{-3}$; bulk ligand: 1.03 g cm$^{-3}$. Combining mass conservation

$$M_p = N_{core}M_{Au} + M_{shell}M_{ligand} \tag{6}$$

where $N_{core}$ and $N_{shell}$ represent the number of atoms in the core and the number of molecules in the shell, respectively, with an additive volume consideration:

$$p_p^{-1}M_p = p_{core}^{-1}N_{core}M_{Au} + p_{shell}^{-1}N_{shell}M_{ligand} \tag{7}$$

a set of equations that can be solved for $N_{shell}$ and $N_{core}$, and thus molecular formula:

$$N_{shell} = \frac{M_p p_{core}^{-1} - M_p p_{p}^{-1}}{M_{ligand} (p_{core}^{-1} - p_{shell}^{-1})} \tag{8a}$$

$$N_{core} = \frac{M_p p_{p}^{-1} - M_p p_{shell}^{-1}}{M_{Au} (p_{core}^{-1} - p_{shell}^{-1})} \tag{8b}$$

For the Au$_{133}$(SR)$_{50}$ nanoparticle, the estimated molecular formula by AUC was calculated with Equation 8 to be Au$_{133}$(SR)$_{50}$ a decent estimate of the actual molecular formula (Table 1). Given instead the actual molecular formula, it is possible to back calculate (by Equation 8) the density of the ligand shell. For the Au$_{133}$(SR)$_{50}$ nanocluster, the ligand shell density was found to be $p_{shell} = 1.2$ g cm$^{-3}$, slightly higher than the bulk ligand density, as expected for these types of curved systems.

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**Table 2 | Data from the 2D AUC distributions for both nanoclusters and PDT-capped nanoparticles.**

<table>
<thead>
<tr>
<th>Clusters</th>
<th>$s$ (S)</th>
<th>$D$ (cm$^2$ s$^{-1}$)</th>
<th>$d_p$ (nm)</th>
<th>$\rho_p$ (g cm$^{-3}$)</th>
<th>$M$ by AUC (Da)</th>
<th>$M$ by ESI-MS</th>
<th>MW error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au$<em>{13}$(SR)$</em>{26}$ TOA</td>
<td>6.8±0.1</td>
<td>2.8×10$^{-6}$</td>
<td>2.41±0.02</td>
<td>1.95</td>
<td>8,000±90</td>
<td>7,862</td>
<td>1.75</td>
</tr>
<tr>
<td>Au$<em>{13}$(SR)$</em>{24}$</td>
<td>9.6±0.1</td>
<td>2.7×10$^{-6}$</td>
<td>2.58±0.01</td>
<td>2.43</td>
<td>11,030±50</td>
<td>10,778</td>
<td>2.33</td>
</tr>
<tr>
<td>Au$<em>{13}$(SR)$</em>{50}$</td>
<td>27.1±0.4</td>
<td>2.5×10$^{-6}$</td>
<td>2.83±0.01</td>
<td>4.51</td>
<td>35,260±180</td>
<td>36,598</td>
<td>3.65</td>
</tr>
</tbody>
</table>

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**Figure 3 | 2D AUC distributions for both nanoclusters and PDT-capped nanoparticles.** The three ‘magic-sized’ clusters were mixed in toluene ($T = 20^\circ$C) and sedimented simultaneously, to illustrate the accuracy and ease of measuring sedimentation and diffusion coefficients for a distribution of species. (a) The integrated 1D sedimentation coefficient distribution over the respective values of diffusion coefficients. (b) 2D sedimentation and diffusion coefficient distributions for three thioltated gold nanoclusters. (c) The integrated one-dimensional distribution of sedimentation coefficients taken over all diffusion coefficients and the multi-peak Gaussian fit illustrating the resolution of two PDT-NP peaks. (d) The sedimentation and diffusion coefficient distributions for PDT-NPs.
2D AUC of PDT-coated nanoparticles. In order to prove the validity of our technique for large NPs, we applied the method to larger, pentadecanethiol gold nanoparticles (PDT-NPs) synthesized using the Stucky method (see Methods for details)\(^*\). These particles are illustrative of the often-encountered case of so-called ‘nearly’ monodisperse particles. The 2D sedimentation and diffusion plot for this sample is shown in Figure 3d. From this data we can extract the size, density and molecular weight of the sample, as outlined in previous paragraphs. Furthermore, we can use the bulk densities of the core \(\rho_{\text{core}} = 19.32 \text{ g cm}^{-3}\) and shell \(\rho_{\text{shell}} = 0.85 \text{ g cm}^{-3}\), to estimate the average molecular formula, the core diameter and the shell thickness. The data is summarized in Table 2. Unlike the previous NP standards (Fig. 3b), the PDT-NPs, although narrowly dispersed, are not discrete species. This signifies that the standard deviation in core diameter is indicative of sample properties rather than the intrinsic instrumental broadening of the peaks. By transmission electron microscopy (TEM) (Supplementary Fig. S2), the average core diameter \(d_{\text{core}}\) and standard deviation of the PDT-NPs was 6.7 ± 0.07 nm—essentially a perfect match with our AUC analysis. Yet our results reveal that AUC provides a more complete analysis as our results show that the core diameter is actually composed of a bi-modal distribution of nanoparticles, irresolvable with TEM, 6.68 ± 0.10 nm and 6.82 ± 0.06 nm.

<table>
<thead>
<tr>
<th>Actual formula</th>
<th>Estimated formula(^*)</th>
<th>Estimated ligand shell density (g cm(^{-3}))‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{Au}<em>{25}\text{(SR)}</em>{28}) TOA (^{†})</td>
<td>(\text{Au}<em>{22}\text{(SR)}</em>{22}), (\text{Au}<em>{27}\text{(SR)}</em>{18})</td>
<td>1.1</td>
</tr>
<tr>
<td>(\text{Au}<em>{16}\text{(SR)}</em>{24})</td>
<td>(\text{Au}<em>{16}\text{(SR)}</em>{24})</td>
<td>1.0</td>
</tr>
<tr>
<td>(\text{Au}<em>{14}\text{(SR)}</em>{30})</td>
<td>(\text{Au}<em>{14}\text{(SR)}</em>{30})</td>
<td>1.2</td>
</tr>
</tbody>
</table>

\(\) Actual number of core and ligand molecules compared with estimate numbers obtained from AUC and bulk density. The last column lists the estimated ligand shell density (g cm\(^{-3}\)).

\(\) Estimated from the data in Figure 3a, assuming bulk densities of thiol ligand and gold core.

\(\) Given first the molecular formula of each cluster and plugging into Equation 8.

\(\) For a sample with unknown components or counterions, it would be impossible to predict the composition based on the molecular weight.

**Table 1** | Estimated molecular formula and ligand shell density for standard nanoclusters.

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**Figure 4 | 2D AUC distribution of polydisperse gold nanoparticles.** (a) The integrated 1D distribution of sedimentation coefficients taken over all diffusion coefficients and the overlaid particle size distribution by TEM, to illustrate the increase in resolution by AUC. (b) The sedimentation and diffusion coefficient distributions for the polydisperse NP sample.

2D AUC of polydisperse gold nanoparticles. After proving the effectiveness of our technique for NPs of various sizes, we now demonstrate the power of our technique by characterizing truly polydisperse particles, the most general case encountered in undefined nanoparticle systems. To this end, we tested a polydisperse sample of sodium 11-mercaptoundecanesulfonate-capped AuNPs (MUS-NPs). The core diameter distribution of these particles ranges from ~4–9 nm, as suggested by TEM (Supplementary Fig. S2). The NPs were dissolved in 0.15 M NaCl and sedimented with AUC at 20 °C at a speed of 5,000 r.p.m. The 2D sedimentation and diffusion plot for this sample is shown in Figure 4.

**Discussion**

The molecular weights, densities and hydrodynamic diameters obtained by 2D SV-AUC are in extremely good agreement with the theoretical and experimental data in the literature for those clusters, and were all obtained in solution with a single experiment. Parameters are reported to three significant digits, because the radial positions and solvent density are known accurately to the same significance. A comparison between the cluster’s sedimentation behavior when run alone compared to when it was run together with the other clusters revealed no statistically significant difference (\(t\)-test, \(\sigma = 0.95\)) in the measured \(s\) and \(D\) values of the nanoclusters between both situations. We notice that the errors for the parameters determined for the three clusters vary between the three types. Even though they slightly increase with the cluster size at this point, we believe that the error is related to the non-ideality in the shape of the nanocrystals. Indeed, according to crystallographic data, \(\text{Au}_{16}\text{(SR)}_{24}\) TOA is quasi-spherical with an aspect ratio very close to 1, whereas \(\text{Au}_{25}\text{(SR)}_{18}\) is rod-like with an aspect ratio of ~1.3 (refs 35,39).

It is, therefore, not surprising that the error in molecular weight for \(\text{Au}_{16}\text{(SR)}_{24}\) is slightly higher compared with \(\text{Au}_{25}\text{(SR)}_{18}\) TOA, because...
the basis of our analysis is the validity of Stoke's Law for describing the diffusion of the particles.

The encouraging results obtained from the nanoparticle standards open the possibility of applying the 2D SV-AUC method outlined above to naturally polydisperse samples, which exhibit dispersity in size, density, and molecular weight. In this way, we are able to examine a particular population in a distribution, without requiring homogeneity in density (typically required in AUC experiments). These results also confirm that our methodology is robust enough to handle NPs that deviate from the spherical-shape requirement in Stokes Law (Equation 3); as the NPs in Figure 3b are known to be highly faceted or slightly prolate35,39. Yet, one should not push this concept too far. In samples with particles with widely varying aspect ratios, it would not be possible to apply our method without a priori knowledge on the aspect ratio of the specific (fractionated) populations. For example, the application of our method to analyse nanorods requires the knowledge of their aspect ratio.

Equation 8 was used to approximate the molecular formulas and ligand shell densities (Table 1), given the bulk core/ligand densities and theoretical molecular formulas. The estimated molecular formulas for the three NP standards confirm that the bulk ligand density could be used to reasonably approximate the actual ligand shell density. On the other hand, if prior knowledge of the molecular formula is available, one may be interested in using our methodology to measure the density of the ligand shell, as illustrated in Table 1.

It should be noted that nanoparticles in the size range of the PDT-NP sample (Fig. 3d), or larger, have small diffusion coefficients and typically sediment quickly, hence care must be taken in the choice of rotor speed. The general approach is to decrease the speed to increase the influence of diffusion during sedimentation while still maintaining sedimentation as the dominant transport mechanism. This yields a more accurate measurement of the diffusion coefficient, without compromising precision in the measurement of the sedimentation coefficient. In other words, a slower speed is necessary to accurately observe the diffusion of larger nanoparticles. The time scale of the experiment, however, remains only a few hours and still just a single run is required. Future work will be needed to determine a precise criterion to determine the optimal speed for a given sample.

For the sample of polydisperse MUS-coated NPs, the core diameter was calculated from the 2D sedimentation and diffusion plot and presented in Figure 4b. The core diameter distribution obtained by TEM was qualitatively overlaid onto the 1D AUC plot in Figure 4a to illustrate the level of resolution unobtainable by TEM, even when combined with other techniques. Even though the core size distributions show a good overall agreement in size range, 2D SV-AUC reveals the presence of distinct populations in the sample that are obscured by the comparatively tiny sampling capability of TEM. Previously, Colfen et al. have demonstrated the angstrom resolution of AUC analysis and its potential power in replacing other lower-resolution techniques41. SV-AUC distributions sample millions to billions of particles42, whereas TEM histograms can accommodate a few thousand particles at most. Given this level of detail, it should be immediately obvious to the reader that SV-AUC could be a powerful tool to understand nanoparticle growth43. Also, the core diameter distributions are the only parameters easily obtained by TEM. Even though insight into the ligand shell is in theory possible with advanced high-angle annular dark-field scanning transmission electron microscopy44, the technique is not practical for statistically assessing its properties, especially in an industrial setting. In a single experiment, our method provides the core size, overall particle diameter, and density for each population of NPs present in our sample without the need for a global analysis.

As well as the shape variations described in previous sections, charges on nanoparticles can also lead to non-ideality. The MUS-coated particles described above were prepared in salt solutions to screen the highly charged surface (zeta potential ($\zeta = -38 \pm 5.30 \text{mV}$)). The classical way to measure whether electrostatic gradients induced by centrifugation affect the sedimentation coefficient of a sample is to run the solute in a series of solutions differing in ionic strength35. Provided that one can accurately measure the density and viscosity of the ionic buffer solution, the application of our method can be applied to highly charged solutes.

We believe that our method has general applicability, even beyond nanoparticles; yet, we do acknowledge limitations for our approach. First, some non-ideal samples would require assumptions. For example, the analysis of rods with varying shape should be coupled with another sizing technique to predetermine the aspect ratio. For a solute which is relatively monodisperse in axial ratio, our analysis could be applied with little adaptation. Recent developments in multi-wavelength AUC by Colfen et al. could prove excellent for this type of sample by providing a third axis of information that could be used to eliminate the need for prior axial ratio determination35. Alternatively, preparative fractionation prior to AUC could significantly reduce the complexity of the solution. Future work by our group will explore this possibility. Other types of non-ideality could be samples with widely varying charge density, or with density varying with size, but in unpredictable ways. Moreover, the sample shown in Figure 4 is actually composed of nicely discrete population of particles; this helps to reduce the error in our analysis. Samples with continuously varying sedimentation coefficient distribution, such as polymers or polymer-coated nanoparticles, would require an artificial binning of the data that would introduce another source of error. For example, we tried to determine the iron content of ferritin type-I and found that we needed a set of additional assumptions to achieve a qualitative description of the complex45,46.

The 2D SV-AUC NP characterization approach described herein provides unprecedented access to the size, density and molecular weight distributions of NPs, from a single experiment and without the use of standards or auxiliary measurements—as is usually required for quantitative characterization with AUC. We proved the accuracy and the generality of our approach by testing NPs of various size distribution modes (for example, mixtures of atomically discrete particles, narrowly dispersed particles and polydisperse particles of a wide size distribution). We find that our approach delivers precise insight into the density, molecular weight and size distributions. Furthermore, no burdensome or speculative global analysis is needed to correlate these property distributions to one another—as is required when one studies different sample properties with multiple characterization methods.

Even though our methodology relies on Stokes' law to derive the various property distributions of NPs, we find that it is robust enough to handle NPs that slightly deviate (for example, oblate and faceted) from the spherical shape assumption of Stokes' law (formulated for a hard-sphere in a continuum liquid). Furthermore, we were able to accurately measure the properties of some of the smallest NPs that can be presently synthesized ($d_\text{s} \sim 2 \text{ nm}$) and thus exist in the regime of dominant intermolecular forces where Stokes' law is expected to break down. This discovery is of great importance to anyone interested in techniques that rely on Stokes' law for particle sizing (for example, dynamic light scattering (DLS), FFF and electrophoresis) and nanorheology in general.

NP research has seen an upsurge in size fractionation techniques in recognition of the need to minimize heterogeneity in NP samples that are intended for various applications. The methodology demonstrated in this work is ideally suited to work hand-in-hand with this trend, because it simultaneously maps a particle's density, size and molecular weight to its s and D values. Therefore, it provides not only the properties of the NPs present in a given sample, but also a solution allowing researchers to select (that is, fractionate) species in the sample based on pre-desired properties: a technique

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**Figure 3**

**Figure 4**

**Table 1**

**Equation 8**

**Supplementary Information**

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The Au clusters were precipitated and washed thoroughly with ethanol (or methanol) and then redispersed in the whole run (radial step size of 0.003–0.008 cm). SV runs typically required 0.5 to 0.5 mg of material in 400 µL solution. Each sample was prepared at varying concentrations to ensure that the sedimentation and diffusion coefficients were not concentration dependent. An inhomogeneous solvent model was applied to account for solvent compressibility caused by high pressure build-ups at the centrifugal field obtained at high rotor speeds. The sedimentation and diffusion coefficients are provided under normal conditions (20°C, water) and must be converted to the appropriate solvent.

Measurement of AUC experimental error. To obtain a measure of the experimental standard deviation of AUC, the Au(NP) NP was repeatedly measured a total of nine times at 80 °C. The results were found to be reproducible in both s and D, measured to be 27±0.1 and (2.5±0.1)×10−15 cm2 s−1, respectively. Hence, for both cases the standard deviation is limited by the line width measurement for a single experiment (~4% of the average). To compare the difference between AUC analysis of the standard nanoclusters sedimented separately and together, a t-test was used (G=0.95), assuming the data was normally distributed (visual test). For a grid of 10×10 pixels and values 30, the degrees of freedom n=300.

Synthesis of Au(NP)(SC2H3)2, TOA nanoclusters. HAuCl4·3H2O (0.4 mmol) was dissolved in 5 ml Nanopure water, and tetracetyltrimmonium bromide (TOAB, 0.47 mmol) was dissolved in 10 ml toluene; the two solutions were combined and vigorously stirred to facilitate phase transfer of Au(III) salt into the toluene phase. After phase transfer was completed, the aqueous phase was removed using a glass pipette. The toluene solution of Au(III) was cooled to 0 °C in an ice bath over a period of 30 min while constant magnetic stirring. PhC6H5CH2H (3 equivalents per mole of gold) was added and stirring was reduced to a very low speed. The deep red solution turned faint yellow over a period of ~5 min, and finally clear over ~1 h. The mixture was stirred at 55 °C for 1 h, and then cooled to room temperature. 100 ml of ethanol was then added to precipitate the NPs. The precipitated Au nanoclusters were dissolved in 1 ml toluene, and 0.5–0.8 ml neat PhC6H5CH2H was then added to the Au nanoclusters solution. The solution was heated to 80°C and maintained at 80 °C for about 24 h under constant magnetic stirring. After that, 20 ml ethanol was added to the solution to precipitate Au nanoclusters. Only Au34 nanoclusters and Au(II)-SCH2CH2Ph exist in the black precipitation. Au34 nanoclusters were exchanged with CH2Cl2, and Au(II)-SCH2CH2Ph residuals (poorly soluble) were discarded.

Molecular weight determination of nanoclusters. Electrospray ionization (ESI) mass spectra were used to determine the molecular weight of Au34(SC2H3)2, TOA, Au34(SC2H3)2, and Au34(SC2H3)2 nanoclusters (the latter two clusters are charge neutral) (see Supplementary Fig. S3). ESI mass spectra were recorded using a Waters Q-TOF mass spectrometer equipped with Z-spray source. The source temperature was kept at 70 °C. The sample was directly infused into the chromatography system with a flow rate of 1 μl/min. The spray voltage was kept at 2.20 kV and the cone voltage at 60 V. The ESI sample was dissolved in toluene (1 mg ml−1) and diluted (1:2 vol) by dry methanol (containing 50 mM CsOAc to enhance cluster ionization in ESI). The experimental error of formula weight determination is typically <0.3 Da (within the range of <10,000 Da).

Synthesis of pentadecanethiol gold nanoparticles. 0.25 mmol chlorotriphenylphosphine gold (AuPPh3Cl) was mixed with 0.75 mM PDDT in 20 ml of benzene to form a clear solution, to which 2.5 mmol of tert-butyl amine borane complex was then added. The mixture was stirred at 55°C for 1 h, and then cooled to room temperature. 100 ml of ethanol was then added to precipitate the NPs. The precipitated NPs were collected by centrifugation and washed three times with a mixture of benzene and ethanol. The highly reproducible monodisperse NPs were obtained without any further treatment.

References


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Author contributions
R.P.C. and O.M.B. conceived and designed the experiments. R.P.C. and H.M. performed the AUC experiments. H.M. and R.P.C. synthesized the nanoparticles, and J.Y.K., R.P.C. and O.M.B. conceived and designed the experiments. R.P.C. and H.M. performed the TEM characterization. R.P.C. analysed the data and co-wrote the paper with O.M.B. and F.S. All authors discussed the results and commented on the manuscript. Correspondence and requests for materials should be addressed to F.S. or O.M.B.

Additional information
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