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Stable Aqueous Nanoparticle Film Assemblies with Covalent and Charged Polymer Linking Networks

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Abstract

The construction of highly stable and efficiently assembled multilayer films of purely water-soluble gold nanoparticles is reported. Citrate stabilized-nanoparticles (CS-NPs) of average core diameter of 10 nm are used as templates for stabilization-based exchange reactions with thioctic acid to form more robust aqueous NPs that can be assembled into multi-layer films. The thioctic acid stabilized nanoparticles (TAS-NPs) are networked via covalent and electrostatic linking systems, employing dithiols and the cationic polymer poly-L-lysine, respectively. Multi-layer films of up to 150 nm in thickness are successfully grown at biological pH with no observable degradation of the NPs within the film. The characteristic surface plasmon band, an optical feature of certain NP film assemblies that can be used to report the local environment and core spacing within the film, is preserved. Growth dynamics and film stability in solution and in air are examined, with poly-L-lysine linked films showing no evidence of aggregation for at least fifty days. We believe these films represent a pivotal step toward exploring the potential of aqueous NP film assemblies as a sensing apparatus.

Keywords:

Nanoparticles, assembled films, citrate-stabilized, thioctic acid-stabilized, aqueous

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Introduction

Substantial interest exists in the broad field of nanometer-scale materials because of their importance to both fundamental and application-driven science. Specifically, the electronic and optical properties of materials such as nano-rods,[1,2] carbon nanotubes,[3-5] and nano-wires,[6,7] for example, have received much attention due to their potential use as sensors and in nanoelectronics.[8] Of particular interest in this area are metallic nanoparticles (NPs) which feature optical properties as a function of particle spacing, size, and composition.[9,10] Thus, in addition to the fundamental properties of NPs, the interconnection of these materials into ordered arrays and layered assemblies is also studied in order to take advantage of the sensing potential optics of NPs.

To avoid spontaneous aggregation to bulk metal, NPs are often synthesized with a peripheral layer of protective ligands.[11] One of the most versatile NPs of this nature is the alkanethiol-based monolayer protected cluster or MPC. Synthesized with the Brust reaction[12] and prominently investigated by Murray and coworkers[13] among others, MPCs have a peripheral layer of alkanethiols that stabilize the metallic nanoparticle cores. Known for their high degree of stability, the dry form of alkanethiolate-coated MPCs can be readily dissolved in and extracted from organic solvents without significant degradation. Variations in the core size (typically <6 nm in diameter) and functionality of the peripheral alkanethiol ligands can be easily achieved during the synthesis and via exchange reactions with ω-substituted alkanethiol ligands, respectively.[14,15] The combination of their superior stability and ease of functionalization has led to extensive research aimed at using MPCs as potential sensing materials.[16,17] Nanoparticles of this nature, however, are limited in this capacity in that their hydrophobic periphery renders them insoluble in water.

Much progress has been made in recent years with regard to water-soluble NPs that feature a variety of protective monolayers. Examples of water-soluble NPs that have been investigated include those with peripheral layers composed of tiopronin,[18-22] coenzyme A,[21] polyethylene glycol,[23-25] and glutathione.[20,26,27] There are also reports of polar NPs synthesized with stabilizing ligands of 4-hydroxythiolphenol,[28] mercaptobenzoic acid,[29] mercaptosuccinic acid,[30] mercaptoammonium,[31] and sulfonic acids[20,32] though the solubility of these materials in water is dependent on solution conditions. Another important example of a widely used water-soluble NP is the citrate stabilized NP (CS-NP).[33-35] In almost all of these cases, however, the NPs are predominantly studied in solution.

CS-NPs are protected by a peripheral layer of citrate ions that electrostatically stabilize the metallic core while maintaining water solubility of the material.[33-35] Reports investigating aspects of CS-NPs can be found throughout the literature. Similar to MPCs, the core size of CS-NPs (typically 10-20 nm in diameter) can be readily manipulated during their synthesis.[36] Likewise, there are reports of modifying CS-NPs in solution with functional ligands through exchange reactions.[17,37] Compared to their smaller alkanethiolate-protected counterparts or even the reasonably stable glutathione-stabilized NPs, however, CS-NPs are notoriously unstable and susceptible to aggregation. Unlike the alkanethiolate MPCs, CS-NPs cannot be reconstituted once extracted from an aqueous solution as they usually aggregate with extended exposure to air.[38]

A major aspect of NP research is the exploration of their controlled assembly into stable film geometries. As previously mentioned, most NPs of this size have optical properties that depend on core size, spacing, and local environment. Therefore, films of NPs can be useful for fundamental electron transfer studies[39] as well as for developing innovative chemical sensors.[40-48]

Films of alkanethiolate MPCs, ranging from simple dropcast materials[40-42] to highly networked assemblies[43-45] with a variety of linking mechanisms have been investigated as chemical vapor or metal ion sensing materials in several labs including our own.[46-48] In stark contrast, the number of reports focusing on water-soluble NP films of this nature is significantly less and void of sensing applications.

The most significant work with films of water-soluble NPs stems from efforts in the Murray and Natan laboratories. Specifically, Natan and Mallouk[49] embedded a monolayer film of CS-NPs within a layered structure of inorganic solid and polyelectrolyte to create novel metal-insulator systems. Murray et al. has explored sulfonic acid-protected NPs in the form of molten salts.[32] Film assemblies with intentional architectures are less common, however, with Murray's films of alternating ionic layers of carboxylic-acid modified MPCs (anionic) and mercaptoammonium protected clusters (cationic) serving as one example of such films.[31] While successfully grown, these electrostatic films were not tested for stability, required the synthesis of two different types of NPs, and showed asymmetric growth efficiency most likely related to the mixing of NPs with different solubility in water.[50]

In a series of seminal reports, Natan and coworkers[51-56] attached CS-NPs onto silanized glass surfaces to form stable monolayers of water-soluble NPs. In addition, the group attempted to construct multi-layer films of CS-NPs using a variety of linking ligands including dithiols, 2-mercaptoethanol, and 2-mercaptoethylamine linkages.[57] Careful inspection of the results, however, reveals that these multi-layer films undergo significant aggregation during growth, ultimately resulting in a bulk gold film rather than a stable multi-layer film of networked gold nanoparticles. The loss of NP integrity during film assembly is observable both visually and spectroscopically in this work.[57] While other water-soluble NP films have been employed for various applications in the literature,[58-62] the stability of these films is often left unaddressed and the integrity of the NPs within the film is unknown. Thus, largely because of their inherent instability, most research with water-soluble NPs is done in solution rather than with films.

In this paper, we present stable multi-layer films of purely water-soluble NPs with larger cores than previously assembled into networked films. Integrating NPs with larger cores into film geometries enables spectroscopic observation of a prominent surface plasmon band which, because of its sensitivity to the local NP environment, can be used as a diagnostic tool for certain sensing applications.[46,48,63] Starting with CS-NPs and using a novel stabilization technique developed by Chen and coworkers,[64] we employ an electrostatic-based, polymer linking system to construct highly stable water-soluble NP films. These NP films are of substantial thickness and show no signs of aggregation throughout the assembly process. NP integrity is maintained and the films are stable in both air and aqueous solutions for extended periods of time. In addition to film stability, our study also focuses on growth dynamics of films with different linking strategies. Ultimately, the ability to efficiently construct stable films of networked water-soluble NPs is an important step for exploring the use of such materials in water-based sensors and as novel biological interfaces for fundamental science.[65]

Experimental Details

Synthesis of Citrate-Stabilized Nanoparticles (CS-NPs)

All materials were available from Sigma/Aldrich unless otherwise stated. The procedure used by Natan and coworkers[53] was followed for the preparation of citrate-stabilized gold nanoparticles. In brief, all glassware was cleaned with aqua-regia (3:1 HCl/HNO_3) and rinsed thoroughly with 18 MΩ nanopure water (NP). Water-soluble gold colloids were synthesized from 1 mM $HAuCl_4$

by first bringing the gold salt to a boil with constant stirring in a round-bottom flask equipped with a reflux condenser. This was followed by the rapid addition of 38.8 mM sodium citrate solution. The color of the solution immediately turned from light yellow to colorless and finally to dark burgundy. The solution was boiled for an additional 10 minutes, allowed to cool to room temperature, and then vacuum filtered through a 0.8μm membrane filter (Gelman). The resulting filtrate was stored in the dark at room temperature prior to further functionalization. Transmission electron microscopy (TEM) imaging, operating at 80 kV, was used to verify the average core size and reproducibility of the procedure (see Supporting Information). TEM samples were prepared by dropcasting material from solution onto 400 mesh copper grids coated with Formvar and carbon (Electron Microscopy Sciences).

Thioctic Acid Stabilization of Nanoparticles

Stabilization of the CS-NPs with thioctic acid (TA) was carried out immediately following the preparation of gold colloids in order to avoid aggregation, as the CS-NPs do not display sufficient long-term stability. Following protocol developed by Chen et al.,[64] the pH of the CS-NP solution was adjusted to pH 11 with the dropwise addition of 0.5 M NaOH as a means of ensuring charge stabilization during the TA-functionalization process. To do this, an equal molar quantity of TA to the amount of $HAuCl_4$ used in the citrate-stabilization was added to the CS-NP solution and stirred overnight in the dark. The TA-exchanged solution was then centrifuged for 20-30 minutes at 15,900 *g* and 10°C (Sorvall Superspeed RC2-B). The supernatant was decanted, and the remaining solid dissolved in equal parts of NP water, resulting in a burgundy colored solution that was stored in the dark until further use. TEM imaging was used to verify the average core size and reproducibility of the procedure (see Supporting Information).

Nanoparticle Film Growth

All NP films were grown on pre-cut, Piranha cleaned glass slides. *Warning: Piranha solution (2:1, concentrated sulfuric acid to hydrogen peroxide) reacts violently with organic material and should be handled with extreme caution.* Clean glass slides were then silanized with either 3-(mercaptoproyl)trimethoxy silane (3-MPTMS) or 3-(aminopropyl)trimethoxy silane (3-APTMS), following previously described procedures.[48, 52, 66]

CS-NP and TAS-NP films were grown using the previously reported "dip-cycle" method[47, 48, 67, 68] where the silanized slide is immersed in alternating solutions of NPs and linker ligands. For dithiol-linked TAS-NP films, dip cycles consisted of a one-hour exposure to TAS-NP solution followed by a one-minute dip in 2.5mM dithiol (1,6-hexanedithiol or 1,9-nonanedithiol) solution in ethanol. After each exposure, the films were rinsed in excess with their respective solvent (water or ethanol) and, depending on the silanizing agent and specific plan of the film, either stored in that particular solvent or dried in a stream of nitrogen. In a similar fashion, TAS-NP films assembled with poly-L-lysine (PL) linkers were dipped in TAS-NP solution for one hour prior to a 30-minute exposure to refrigerated 0.03 mM PL in 8.8mM sodium phosphate buffer (pH 8.5). PL-linked films were rinsed thoroughly with NP water before and after each dip and kept wet at all times. Dip sequences for both types of growth were repeated (dip cycles) in order to achieve multi-layer films of NPs. Film growth was monitored using a UV-Vis diode array spectrometer (Agilent 8453) recording spectra after every two dip cycles and monitoring the absorbance at 528 nm. Spectroscopy of dithiol-linked CS-NP and TAS-NP films was performed either "wet," in a water filled cuvette or "dry," after films were thoroughly dried in a stream of nitrogen. Contact angle measurements of water droplets on the films were measured using a goniometer (Rame-Hart-300) affixed with a nitrogen-purged environmental chamber.

Results and Discussion

Aqueous Nanoparticle Stability

A substantial difference in stability persists between the nonaqueous soluble, alkanethiolate-protected nanoparticles and the aqueous soluble, citrate-stabilized nanoparticles (CS-NPs). In spite of their wide-spread use, solutions of CS-NPs are inherently unstable for long periods of time as the citrate stabilization is a much less effective layer of protection than their alkanethiolate counterparts. The instability of the CS-NPs is easily seen using transmission electron microscopy (TEM) imaging or UV-Vis spectroscopy. **Figure 1A** shows a typical TEM image of CS-NPs immediately after synthesis. The image is very common for this traditional synthesis, displaying relatively mono-disperse particles with an average particle size of 9.3 ± 1.9 nm (see Supporting Information for TEM histogram). Imaging of this same solution after storage for one week in the dark reveals particle coalescence into aggregated material as the result of a breakdown in the citrate protection (**Figure 1B).** The large aggregates of material observed in these later images are noticeably absent from images taken immediately after the synthesis. Likewise, large areas of monodispersed, unaggregated particles were relatively rare in images of the week-old material.

UV-Vis spectroscopy of these solutions (Supporting Materials) reveals corresponding evidence of aggregation over time. Just after synthesis, a single, sharp surface plasmon band at approximately 530 nm is easily observed. Upon aggregation, the surface plasmon band appears red-shifted, with a pronounced peak in the range of 600-650 nm growing at the expense of the original surface plasmon band. This red-shifted spectra is commonly seen with NP aggregation[17] and is the basis of flocculation parameters used to track such transformations.[69] More importantly, this type of aggregation is also observed during functionalization of the CS-NPs through exchange reactions where thiol groups of functional ligands displace the surface citrate molecules. The immediate aggregation of the NPs upon this exchange has been attributed to a sudden loss of electrostatic stabilization of the cores, initiated by the strong affinity of the thiol to covalently bond with the gold.[64] The ability to form stable (i.e., non-aggregated) NPs bearing functionalized ligands is a vital step for potential sensing applications of NPs,[70] including their assembly into films.

Stable aqueous NPs have been achieved by derivatizing the CS-NPs with thioctic acid (TA) as shown by Chen et al.[64] **Figures 1C and 1D** correspond to images of freshly prepared TA-stabilized NPs (TAS-NPs) immediately after derivatization and after one week of storage, respectively. Both images show relatively mono-dispersed NPs with a mean particle size of 10.3 ±3.6 nm that does not appreciably change after one week of storage. Considering that TAS-NPs are made from CS-NPs, the comparability in core size is expected. Likewise, UV-Vis spectra of the TAS-NP solutions revealed no aggregation over time (Supporting Materials). By modifying gold cores with thioctic acid, the NPs remain water-soluble but no longer depend on the electrostatic interaction between the gold and the citrate ion for stabilization. Instead, the NPs are stabilized by TA molecules that feature two thiol groups per molecule. It is believed that the two thiol-to-gold binding sites on each TA molecule significantly slow the kinetics of the exchange reaction, allowing for electrostatic charge compensation throughout the process.[64]

Stability of Aqueous Nanoparticle Films

A major area of interest for using NPs in sensing applications is their interconnection and assembly into a film geometry. Stability and reasonable assembly times are key aspects of NP film construction. Here, we address the stability of multi-layer films of aqueous NPs both in terms of overall film and individual NP integrity. With any NP film assembly, the stability of an initial, anchoring monolayer of NPs is vital and is usually accomplished using silanized glass. In this case, however, the specific silanizing agent proved to have a significant impact on the stability of this layer. Glass slides silanized with (3-mercaptopropyl)-trimethoxysilane (3-MPTMS) and exposed to NPs showed instantaneous destabilization of the NPs directly on the substrate. As seen with CS-NP solutions undergoing functionalization, the very potent penetration of any thiols to the gold surface of the NPs causes a catastrophic disruption of the electrostatics stabilizing the cores and spawns irreversible aggregation as indicated by a red-shifted surface plasmon band at ~650nm in the UV-Vis spectrum of 3-MPTMS supported CS-NP monolayers. CS-NPs on 3-aminopropyltrimethoxysilane (3-APTMS) silanized glass faired much better, resulting in an immobilized and stable monolayer affixed to the surface through electrostatic interaction between the cationic amino group and the anionic CS-NPs. UV-Vis spectra of these monolayers in water showed no substantial change in the position (λ_{max}) of the surface plasmon band compared to corresponding solutions of CS-NPs (**Figure 2A**) and were then used as substrates for multi-layer films (*vide infra*).

Natan and coworkers formed monolayers of CS-NPs to 3-APTMS modified glass as described above. All Natan's attempts to grow stable multi-layer films with these materials, however, resulted in films with substantial aggregation toward bulk gold. This was observed regardless of the type of linker ligand employed and reiterates the fragile nature of the CS-NPs.[57]

This phenomenon was observed in our lab as well when multi-layer growth of CS-NP films was attempted with dithiols, a successful bridging ligand used for alkanethiol-protected NP films.[48] In this case, aggregation of the CS-NPs films occurred immediately after the second layer of CS-NPs was added to the monolayer film of CS-NPs (**Figure 2A**), a result consistent with the established susceptibility of CS-NPs toward thiol destabilization that has been previously described. The spectra of CS-NPs in solution and in a monolayer show stable surface plasmon band peaks at 528 nm and 535 nm, respectively, and confirm the stability of CS-NPs in these two mediums. The slight red shift seen in the monolayer spectrum indicates that the nanoparticles in the film are more compact than in solution. In contrast to the monolayer's spectrum, the spectrum of the CS-NPs "film" with multiple layers shows a strongly red shifted surface plasmon band signal at ~620 nm, indicative of significantly aggregated NP material. Similar spectroscopic evidence of aggregation was observed for films formed from CS-NP solution, dried, and measured in air (Supporting Materials).

In order to successfully grow multilayer films of aqueous NPs that are resistant to aggregation, TAS-NPs were employed. In spite of their obvious improved stability in solution, attempts to anchor an initial layer of TAS-NPs on 3-MPTMS modified glass failed. Instead of aggregation, however, UV-Vis spectra taken after the initial monolayer showed absolutely no increase in absorbance when compared to the 3-MPTMS silanized glass slide baseline. This result suggests that, while stabilizing the NPs, the peripheral TA layer, featuring a five carbon chain, is now too thick to be penetrated by the thiolated propyl chain of the 3-MPTMS and cannot form thiol-gold anchors at the core surface. Since TA is terminated with a carboxylic acid group, however, electrostatic interactions can also be used to form a monolayer on 3-APTMS modified glass slides. UV-Vis spectra taken of monolayers of TAS-NPs on 3-APTMS silanized slides show sharp surface plasmon bands at 540 nm indicating that a stable monolayer was successfully formed. Likewise, there is virtually no change in the surface plasmon band's position (λ_{max}) as dithiol linking ligands are used to successfully grow multilayer films of TAS-NPs with no loss of NP integrity (i.e., aggregation). These results are illustrated in **Figure 2B** which shows the spectrum of a TAS-NPs solution compared to that of a monolayer and multi-layer (dithiol-linked) TAS-NP films, both measured in solution. The film growth from a monolayer to a multi-layer film depicted in Figure 2B was achieved with exposing the substrate to eight consecutive cycles of TAS-NPs and dithiol solutions. The slow growth rate is evident from Figure 2B where the overall addition of approximately eight layers of dithiol-linked TAS-NPs results in only a very modest increase in absorption compared to single monolayer of material (e.g., an increase of only 0.05 a.u. is observed). The rate of growth is typical for films utilizing dithiol-linking mechanisms,[47,48] as assembly is limited to immobilizing a monolayer or less of NPs with each exposure. It is noteworthy that the spectrum of the dithiol-linked film of TAS-NPs was taken fifty days after the film construction and is identical to those taken during film growth. Again, the slight red shift seen in the spectra for the films indicates that the nanoparticles in the film are simply more compact than in solution. Corresponding measurements of dry films measured in air revealed the same observed trends, showing almost no sign of aggregation (Supporting Materials).

Enhancement of Nanoparticle Film Growth

Despite the stability and simplicity of TAS-NP films using dithiol linkages, growth of the film is extremely inefficient and represents a major disadvantage to using these films in any application. It was speculated that the length of dithiol linking ligand may influence the growth rate of the TAS-NP films. However, there was no appreciable difference between the growth rate of films using hexanedithiol versus nonanedithiol linking ligands as after eight exposures to TAS-NPs the films showed respective absorbances at 528 nm of only 0.12 and 0.14 a.u. (**Figure 3A**). The modestly slower growth of hexanedithiol-linked films suggests that the linker may have slightly more trouble bridging NPs that are not completely interdigitated. As previously reported with dithiol-linked films of this nature, negligible growth was recorded when the dip cycles excluded exposure to dithiol solution.[48]

In an effort to enhance the growth of TAS-NP films, we looked at alternative linking mechanisms. Linking mechanisms of nonaqueous NP (MPC) films have been extensively studied by our laboratory and others[67,68] but are largely unexplored for purely aqueous systems. In addition to covalent linkages (e.g., dithiol[48] or ester-coupled[46]), MPCs bearing carboxylic acid functional groups have also been linked with metal cations acting as an electrostatic linker between carboxylates on neighboring clusters.[67,68] While these metal-linked films were able to be grown rapidly, it was noted that metal ions not participating in the film's connectivity could easily leech from the system – a potential contaminant in certain applications of nanoparticle films (e.g., metal sensing). A recent study by Murray featured an alternative electrostatic linking system where a cationic polymer, poly(allylamine) hydrochloride, was used to network carboxylic acid functionalized MPCs from ethanol solutions.[50,71]

In the current study, the negative charge stemming from the TAS-NPs was viewed as conducive to an electrostatic linkage system. Bowden and coworkers used a cationic bridge, poly-L-lysine (PL), to adsorb a monolayer of anionic cytochrome b5 protein to a self-assembled monolayer composed of carboxylic acid terminated alkanethiols.[72] The success of this work prompted us to try to use PL as a linking layer between the initial TAS-NP monolayer on the silanized glass and subsequent layers of TAS-NPs within a multi-layer assembly (**Scheme I**). Previous characterization of PL adsorbed at carboxylic acid self-assembled monolayers suggests that *most* of the polymer backbones are oriented approximately parallel to the substrate.[73] As shown in **Figure 3,** the use of PL as a linking bridge in the TAS-NP films was highly successful for improved film growth. UV-Vis spectra of assembled films beyond the initial monolayer show a consistently sharp surface plasmon band at 550 nm that increases in intensity very dramatically with each exposure to the TAS-NP solutions from which the films are grown. After eight successive exposures to the linking PL solution and the TAS-NP solution, the absorbance of the film at 528 nm approaches 1.4 a.u., an over 10-fold increase in film growth efficiency compared to the dithiol-linked films (**Figure 3**). Control experiments where slides were only exposed to TAS-NP solution without subsequent exposure to PL solution resulted in no film growth (not shown).

As in previous reports,[46-48, 63] the measured absorbance can be used to estimate the thickness of the film and track the growth. After eight dipping cycles, typical absorbance measurements at 528 nm were approximately 0.145 a.u. for dithiol-linked TAS-NP films and 1.37 a.u. for PL-linked TAS-NP films, translating into films with average thicknesses of 57 nm and 130 nm, respectively. The *average* absorbance increase per dip cycle, 0.08 a.u. for PL-linked films versus 0.0013 a.u. for dithiol-linked films, illustrates a dramatic enhancement of film growth with the use of PL. Based on our estimations, a single dip cycle involving PL deposits 2.3 pmol/cm^2 or 1-2 monolayers of material (i.e., multilayers) *on average* compared to only 0.036 pmol/cm^2 (i.e., significantly less than 1 monolayer) for a single dip cycle with dithiols.[74] Considering the polymeric nature of the PL-linker (Scheme I), multi-layer deposition per exposure is not surprising having also been observed with metal-linked alkanethiol MPC films.[67,68]

As with the dithiol-linked TAS-NP film assemblies, the PL-linked systems were extremely stable and exhibited no change in their spectra during extended storage in pure water (50 days) or 8.8 mM phosphate buffer at pH = 8 (14 days).[75] The general stability of the PL-linked TAS films was also assessed at a variety of different solution conditions including high and low temperatures, ionic strengths, and pH conditions (Supporting Materials).[76] The PL-linked films were stable at low temperature (9°C) as well as temperatures well above room temperature (40 °C). High temperature did not destabilize the film until the solution was boiled at 100 °C, resulting in a significant red shift. Likewise, solutions of high ionic strength (μ), including phosphate buffer (35 mM; μ = 75mM) and potassium chloride (1 M; μ = 1 M), able to desorb electrostatically adsorbed protein from self-assembled monolayers, had no effect on the electrostatics of the PL-linked films. PL-linked TAS-NP films were also stable when exposed to phosphate buffer at pH 5 for over a week but showed expected degradation at pH 10 after only one day of exposure because the pH was close to the pK_a (~10.5) of the PL's epsilon amino groups and likely eliminated the cationic charges within the film. The long term stability at biological pHs, however, remains the primary asset of these films as they were designed as biological interfaces. Along these lines, we note that the PL interface is sufficiently hydrophilic, with measured contact angles of water at the surface measured to be 37.4° (standard error = ±1.7), compared to 88.1° (±1.0) for films of alkanethiol-based MPCs which denature biomolecules upon attachment to such hydrophobic interfaces.[59]

In conclusion, we have successfully shown the efficient assembly of water-soluble NPs into a stable, multi-layer film that resists aggregation. To our knowledge, this is the first film of this nature to exhibit this level of stability and growth while maintaining the presence of a surface plasmon band from nonaggregated NPs. Hydrophilic NP film assemblies of significant thickness and stability have widespread application as interfacial materials in many facets of bioanalytical research including, for example, as a sensing material for physiological targets, as a component in biosensor designs requiring immobilized enzymes, or as an electrode modifier in protein electrochemistry. By achieving significant growth at biological pH levels (pH = 7-8) these films are especially suited for such applications. Our laboratory is now exploring the many possibilities these highly stable aqueous NP films offer.

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Supporting Information Available:

TEM histogram analysis of CS-NPs and TAS-NPs and UV-Vis spectra of these solutions immediately after synthesis and over time; UV-Vis spectra and TEM imaging of CS-NPs before and after ligand exchange reaction; UV-Vis spectra of dithiol-liked CS-NP and TAS-NP films in air and under various solution conditions including various temperature, ionic strengths, and pH. These materials are available free of charge via the Internet at http://pubs.acs.org.

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(74) Film thickness estimates were carried out as in previous reports[45-48, 63] using Abs = $1000\varepsilon\Gamma$, where ε is the previously determined molar absorptivity at 528 nm (3.6×10^7 $M^{-1}cm^{-1}$) for ~10 nm diameter aqueous nanoparticles[17] and Γ is the surface coverage (mols/cm^2). Surface concentrations were converted to approximate number of monolayers based on a model assuming a hexagonal close-packed system[22] with negligible interdigitation of the charged peripheral layers of the TAS-NPs. A single monolayer of TAS-NPs was determined to be 1.98 pmol/cm^2 and ~13 nm thick. Molecular modeling (Spartan) was used to obtain bond lengths of ligands and calculations were adjusted to compensate for different linking ligands and for film growth on both sides of the glass slide.

(75) PL-Linked TAS-NP films showed signs of degradation when exposed to air for prolonged periods of time (e.g., several days) and, for this reason, it is recommended that these films be stored in aqueous solution until use.

(76) A more systematic investigation of the growth dynamics and stability over a range of conditions is currently underway and includes examination of concentration and PL molecular weight effects on growth.

Captions

Scheme I. (*top*) Poly-L-lysine (PL) linking system for networked film of TAS-NPs. (*bottom*) Simple film schematic showing the potential of these films to have multi-layer deposition of NPs per growth cycle. Numbers represent the exposures of the substrate to NP solution during growth.

Figure 1. *(top)* Typical transmission electron microscopy (TEM) images of CS-NPs from solution immediately after synthesis **(A)** and after one week of storage in a dark container **(B)**. *(bottom)* TEM images of TAS-NP solution immediately after synthesis **(C)** and after one week of storage in a dark container **(D)**. The images are taken with a direct magnification of 100,000x; the bars in the lower left corners of the images are 20 nm. Histogram analysis of core size from TEM imaging analysis is included as part of the Supporting Information.

Figure 2. A) UV-Vis spectra of CS-NP solution (a), a monolayer of CS-NPs on 3-APTMS-modified glass (b), and a multi-layer, dithiol-linked film of CS-NPs (c). **B)** UV-Vis spectra of TAS-NP solution (a), a monolayer of TAS-NPs on 3-APTMS-modified glass (b), and a multi-layer (approximately eight layers), dithiol-linked TAS-NP film fifty days after construction (c).

Figure 3. A) Film growth or assembly trends for hexanedithiol-linked (a), nonanedithiol-linked (b), and PL-linked (c) TAS-NP films. Note: Error bars of dithiol-linked data are smaller than their markers. (**B**) UV-Vis spectra of a PL-linked TAS-NP film during assembly with increasing dip cycles.