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Multidentate-Protected Colloidal Gold Nanocrystals: pH Control of Cooperative Precipitation and Surface Layer Shedding

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S Supporting Information

ABSTRACT: Colloidal gold nanocrystals (AuNCs) with broad size tunability and unusual pH-sensitive properties have been synthesized using multidentate polymer ligands. Because they contain both carboxylic functional groups and sterically hindered aliphatic chains, the multidentate ligands can not only reduce gold precursors but also stabilize gold nanoclusters during nucleation and growth. The "as-synthesized" AuNCs are protected by an inner coordinating layer and an outer polymer layer and are soluble in water and polar solvents. When the solution pH is lowered by just 0.6 units (from 4.85 to 4.25), the particles undergo a dramatic cooperative transition from being soluble to insoluble, allowing rapid isolation, purification, and redispersion of the multidentate-protected AuNCs. A surprising finding is that when a portion of the surface carboxylate groups are neutralized by protonation, the particles irreversibly shed their outer polymer layer and become soluble in nonpolar organic solvents. Furthermore, the multidentate polymer coatings are permeable to small organic molecules, in contrast to the tightly packed self-assembled monolayers of alkanethiols on gold. These insights are important in regard to the design of "smart" imaging and therapeutic nanoparticles that are activated by small pH changes in the tumor interstitial space or endocytic organelles.

Colloidal gold nanocrystals (AuNCs) are a class of "plasmonic" nanostructures with size-dependent electronic, optical, and chemical properties.[1] They are currently the subject of intense research and development for applications in biodiagnostics,[2] surface-enhanced Raman scattering (SERS),[3] photothermal therapy,[4] and in vivo delivery of therapeutic and imaging agents.[5] Unlike semiconductor quantum dots (QDs), which often contain cadmium or other toxic elements,[6] colloidal gold has been used to treat rheumatoid arthritis in humans for over 20 years and is believed to be largely nontoxic and safe.[7] At present, two major approaches are widely used for the chemical synthesis of high-quality gold nanoparticles. The first approach is based on the method of Turkevich and Frens,[8] in which sodium citrate is used as a reducing agent and stabilizing ligand. While the particle size can be controlled by the gold precursor/citrate molar ratio, the resulting NCs are not well protected and are prone to aggregation upon storage or exposure to salts. The second approach is based on the method of Brust and Schiffrin,[9] in which alkanethiols are used to stabilize gold particles in a two-phase emulsion process. This method yields monolayer-protected gold clusters that are highly stable, but the available range of nanoparticle sizes is limited to only 1−5 nm.[9,10]

Here we report a new strategy based on the use of multidentate polymer ligands to synthesize protected AuNCs with broad size tunability and pH-sensitive surface properties. The carboxylic acid functional groups of the polymer ligands are able to coordinate with the surface atoms of the NCs and behave in the same manner as common capping ligands.[8] However, the large number of functional groups in the polymer considerably alters the nucleation and growth kinetics of the NCs relative to that for the monovalent ligands used previously.[8−10] Each polymer molecule has ∼14 carboxylic groups available for binding to the NC surface [see the Supporting Information (SI)]. This multidentate effect significantly increases the binding affinity of the ligand and results in an extremely stable surface coating. The surface coating also contains a large number of extended alkyl chains that are able to interact with the hydrophobic side chains of the free polymer, leading to a thick hydrophobic middle layer and a hydrophilic outer layer (exposed carboxylic acid groups). This allows highly stable AuNCs with tunable sizes of 5−150 nm to be prepared. As discussed in more detail below, the multidentate-protected NCs can be isolated, purified, and redispersed, practically behaving as chemical reagents.

As shown in Figure 1a, immediately after synthesis, the AuNCs are protected by an inner coordinating layer and an outer polymer layer and are soluble in water and polar solvents. Transmission electron microscopy (TEM) revealed a thick polymer coating around the AuNCs (Figure 1c and Figure S2 in the SI). When a portion of the surface carboxylate groups are neutralized at a lower pH, the NCs irreversibly shed their outer polymer layer and become soluble in nonpolar organic solvents such as chloroform. After the loss of this outer polymer layer, the NCs are encapsulated by a single layer of the multidentate ligands, and their hydrodynamic size decreases by 15−20 nm (Figure 1b). This large size decrease indicates that the inner coordinating layer (formed by multidentate binding) is much tighter than the outer polymer layer (consisting of a hydrophobic layer and exposed carboxylic acid groups). We found that two conditions must be met for this shedding of the outer layer to occur: (i) the surface charges must be neutralized at acidic pH (below the pK_a value of acetic acid) and (ii) the charge-neutralized NCs must be exposed to a nonpolar solvent such as chloroform. Once the outer layer is lost, the NCs are soluble only in nonpolar solvents and cannot be redissolved in water or polar solvents unless excess polymer ligands are added. This irreversible behavior rules out the possibility of a single loose polymer layer, which would be expected to reversibly expand and contract in response to pH changes.

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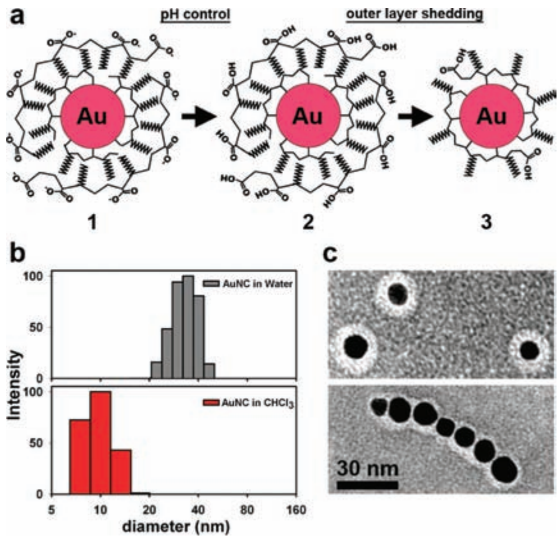

Figure 1. (a) Schematic illustration (not to scale) of multidentate-protected AuNCs with pH-sensitive properties, including solubility control by surface charge modification (**1** → **2**) and outer layer shedding (**2** → **3**). (b) Dynamic light scattering data showing a dramatic change in the hydrodynamic size of the AuNCs after shedding of the surface layer. (c) TEM images showing (top) a thick polymer coating (white) surrounding water-soluble AuNCs and (bottom) a much thinner polymer shell after outer layer shedding in chloroform. The chain-shaped arrangement of nanoparticles was an artifact caused by drying on the TEM grid.

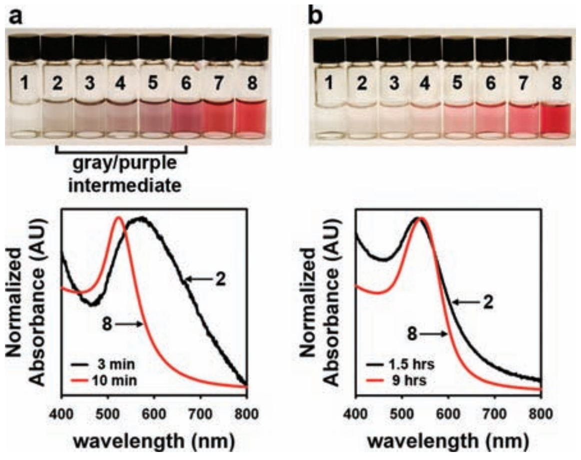

Figure 2. Comparison of AuNC nucleation and growth kinetics observed using traditional monovalent or multidentate polymer ligands. (a) Color photograph (top) and absorption spectra (bottom) at different reaction times during the citrate reduction procedure. (b) Color photograph (top) and absorption spectra (bottom) at different reaction times during AuNC synthesis in the presence of multidentate ligands. The labels 2 and 8 in the absorption spectra correspond to vial numbers in the photos. The reaction time for completion was ~9 h for the multidentate procedure, which is > 50-fold longer than that (10 min) of the citrate procedure.

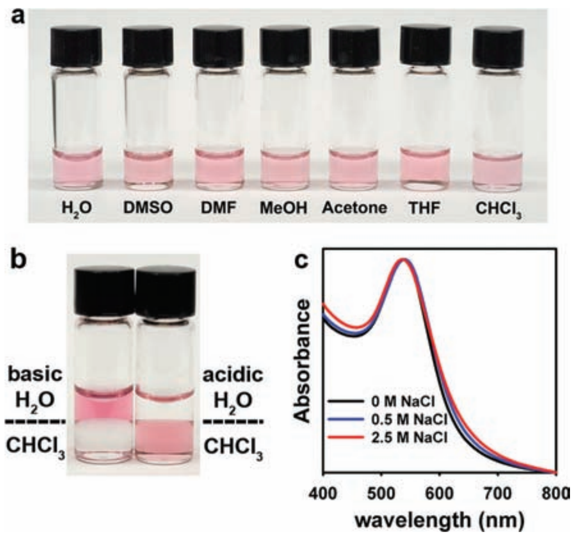

Figure 3. Broad solubility and stability of multidentate-polymer-coated AuNCs. (a) Color photograph showing stable AuNCs dissolved in a broad range of solvents. (b) Color photograph showing extraction of AuNCs into the aqueous phase at basic pH and into the organic phase (chloroform) at acidic pH. (c) Absorption spectra in the presence of 0.1−2.5 M NaCl.

As shown in Figure 2, the use of multidentate polymer ligands gives rise to novel nucleation and growth mechanisms in comparison with those for monovalent ligands. With the traditional citrate method, the AuNC nucleation and growth process undergoes a transient stage in which the solution initially becomes clear (reduction to atomic Au clusters) and subsequently progresses to a gray intermediate before developing the characteristic ruby-red color of gold colloids (Figure 2a). This was confirmed by monitoring the absorbance, which first shows a broad absorption spectrum (black curve) that rapidly becomes blue-shifted and narrows to form the surface plasmon resonance (SPR) peak typical of monodisperse AuNCs (red curve). The transient intermediate is noticeably absent when the NCs are prepared using the multidentate polymer ligand (Figure 2b), indicating that the polymer-induced NC formation proceeds through different nucleation and growth mechanisms:[11] the AuNCs slowly appear and increase in concentration as the synthesis progresses. Absorption spectra measured during NC growth illustrate the appearance and gradual red-shifting of the SPR peak (Figure 2b).

An important finding is that the NC growth rate is reduced by nearly 2 orders of magnitude relative to that reported for monovalent ligands.[8−10] The increased binding affinity of the multidentate ligand certainly reduces the rate at which elemental Au atoms are added to the growing particles, but this effect cannot completely account for the large difference in kinetic rates. Further experimental studies showed that using similar polymers *without hydrophobic alkyl chains* reduced the growth kinetics by only 1 order of magnitude. Thus, the additional growth retardation is due to steric hindrance by the multidentate polymer coating, as observed in the synthesis of other NCs.[12] This is an important insight because slower growth kinetics allows better control of the AuNC size. The size can also be tuned by changing the ratio of polymer ligands to Au atoms, as in the synthesis by citrate reduction.[8] Using these two strategies, we succeeded in preparing multidentate-protected AuNCs with a wide range of sizes (5−150 nm with ~10% variation) (Figures S3 and S4).

The multidentate-protected AuNCs are immediately soluble in water and polar solvents such as dimethylformamide and acetone (Figure 3a). However, adding an aqueous solution of AuNCs to a nonpolar solvent such as chloroform results in two separate phases, with the negatively charged AuNCs remaining in the aqueous phase (Figure 3b). As discussed above, when the surface charge is reduced at lower pH, the AuNCs rapidly shed their outer polymer layer,

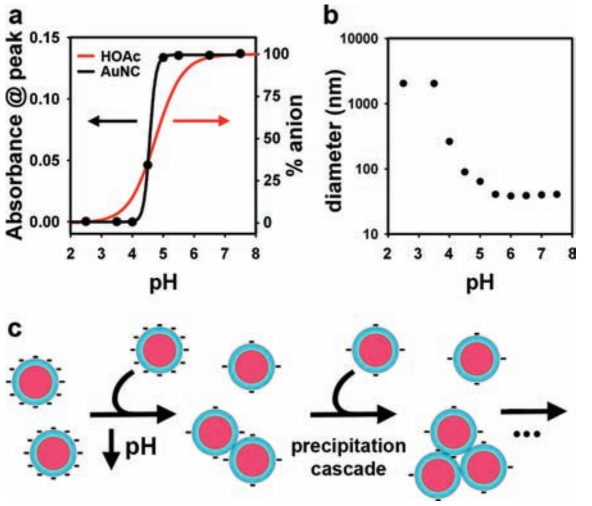

Figure 4. pH-triggered cooperative transition in solubility as measured by precipitation of multidentate-protected AuNCs. (a) Plots of AuNC solubility (black) and the degree of carboxylic acid deprotonation (red) as a function of pH. The solubility curve closely mirrors the titration curve of acetic acid but is much sharper because of a cooperative effect (see the text). (b) Hydrodynamic diameter as a function of pH, showing the formation of nanoparticle aggregates at lower pH. (c) Schematic illustration of pH-triggered precipitation of nanoparticles. Surface carboxylate ions are protonated as the pH decreases, resulting in a decrease in electrostatic repulsion and eventual precipitation from solution.

exposing hydrophobic alkyl chains. This process can be used to transfer AuNCs with a wide range of sizes into a variety of nonpolar solvents. Furthermore, in contrast to citrate-stabilized particles that aggregate at salt concentrations of 10 mM or higher, the polymer-coated AuNCs are highly resistant to salt-induced aggregation at concentrations as high as 2.5 M (shown by the SPR peak in Figure 3c). The particles also exhibit high stability in serum solutions, suggesting that the polymer-coated AuNCs are likely to be well-suited for biodiagnostic applications under complex conditions.

As shown in Figure 4a, the protected NCs are sensitive to pH and can be precipitated and isolated under acidic conditions. The precipitated NCs can subsequently be purified and redissolved by raising the solution pH to 6−7. Significantly, nanoparticle precipitation takes place in a pH range that is much narrower than the titration curve of carboxylic acid (Figure 4b). On the basis of the fitted experimental data, we estimate that 95% of the particles are soluble at pH 4.85, while only 5% are soluble at pH 4.25. This sharp transition in solubility is triggered by a pH change of only 0.6 units. If it is assumed that the acid−base equilibrium closely follows the titration curve, this solubility transition corresponds to a 40% change in carboxyl neutralization [from 35% neutral carboxyl groups (−COOH) at pH 4.85 to 75% neutral groups at pH 4.25].

It is worth noting that Mirkin and co-workers[13] reported a strongly cooperative effect for DNA-conjugated gold nanoparticles, leading to very sharp "melting" curves and improved specificity for discrimination of single-base mismatches. The observed cooperativity is believed to arise from a dependence of the oligo melting temperature on the local salt concentration (localized counterions that are needed to neutralize the charges on oligonucleotides).[13a] Here we believe that cooperative precipitation arises from a "magnifying" process in which extensive nanoparticle aggregation is triggered by small changes in surface charge and hydrophobicity (Figure 4c). Both electrostatic and hydrophilic/hydrophobic interactions are likely to be important. At pH values above the pK_a of the carboxylic acids, most of the functional groups are deprotonated, and the charged nanoparticles are stabilized by electrostatic repulsion (Figure S5). As the pH approaches the pK_a, a portion of these carboxylic acid groups become protonated, losing their charges and becoming more hydrophobic. When the threshold where the diminishing electrostatic repulsion is no longer able to counteract the van der Waals and hydrophobic interactions is reached, a precipitation cascade and the sharp transition seen in the stability curve occur. Recent work by Gao and co-workers[14] showed a similar cooperative effect in which small pH changes are able to disrupt the thermodynamic stability of self-assembled polymer nanoparticles, triggering the release of encapsulated imaging or therapeutic agents. Designed for targeting specific endocytic organelles in living cells, their nanoparticle probes showed a signal response (fluorescence intensity change) of as much as 50-fold when the pH was changed by only 0.25 units.

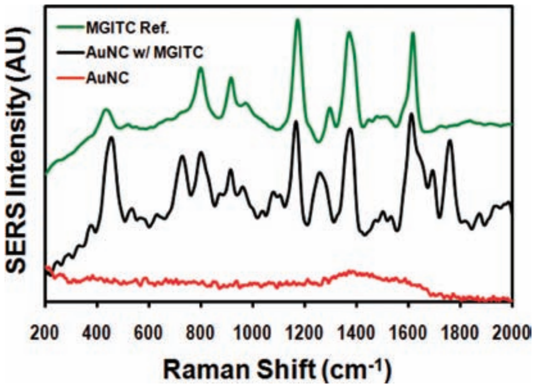

Figure 5. SERS studies of 60 nm multidentate-protected AuNCs demonstrating that small reporter molecules are able to move across the multidentate polymer coating and adsorb on the gold surface: (red) negative control (multidentate-coated AuNCs without the reporter dye); (black) SERS spectra of multidentate-protected AuNCs after the addition of MGITC; (green) standard SERS nanoparticles encoded with MGITC. λ_{ex} = 633 nm; laser power = 3 mW; integration time = 1 s.

Finally, the multidentate polymer coatings were found to be permeable to small molecules such as organic dyes. This is surprising because TEM showed that the polymer coatings are fairly thick with few or no defects (Figure 1c). Unlike small monovalent ligands such as alkanethiols that are able to pack closely on gold surfaces,[15] it is possible that the random adsorption of multidentate polymers on the growing NC could leave "empty" patches on the particle surface. These regions are protected by the overall corona of the surrounding polymers but are available to bind small molecules that can diffuse through the polymer coating. Experimental evidence comes from SERS studies in which the reporter molecules had to move across the polymer coating and then adsorb on the AuNC.[3] Previous work showed that AuNCs with a diameter of 60−70 nm are optimal for surface Raman enhancement (at 633−647 nm excitation).[16] We thus optimized the polymer concentration and reaction time of the synthesis procedure to prepare AuNCs with an average diameter of 60 nm. After the addition of a Raman reporter dye [malachite green isothiocyanate (MGITC)], the AuNCs exhibited strong SERS signals (Figure 5), closely matching the reference spectrum obtained from SERS particles prepared using previously reported methods.[16b] Several new peaks were observed from the multidenetate-coated particles, likely as a result of surface enhancement of the strongly adsorbed carboxylate groups and the polymer backbone. However, these peaks were absent in the control spectrum, and further studies are needed to determine the molecular origins of these peaks.

In summary, we have developed a new strategy based on the use of multidentate polymer ligands to synthesize colloidal gold nanocrystals with broad size tunability and pH-sensitive properties. The "as-synthesized" AuNCs are protected by an inner coordinating layer and an outer polymer layer and are broadly soluble in water and polar solvents. The protected AuNCs have been shown to undergo a pH-dependent transition in solubility, allowing them to be rapidly isolated, purified, and redispersed. When a portion of the surface carboxylate groups are neutralized at lower pH, the particles can further shed their outer polymer layer and become soluble in nonpolar organic solvents in an irreversible manner. In contrast to tightly packed monolayers of alkanethiols on gold, the multidentate polymer coatings are permeable to small organic molecules, allowing the preparation of spectrally encoded SERS nanoparticles for medical diagnostic and imaging applications. Such stable and permeable surface coatings are also expected to be important in nanoparticle-based catalysis, in which the reactants must diffuse in through the polymer layer and the products must diffuse out.

■ ASSOCIATED CONTENT

S **Supporting Information.** Detailed procedures and additional data. This material is available free of charge via the Internet at http://pubs.acs.org.

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