DNA-Modified Core−Shell Ag/Au Nanoparticles

YunWei Cao, Rongchao Jin, and Chad A. Mirkin*

Department of Chemistry and Center for Nanofabrication and Molecular Self-assembly Northwestern University, 2145 Sheridan Road Evanston, Illinois 60208

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In 1996, we reported a method for utilizing biomolecules, such as DNA, and their molecular recognition properties to guide the assembly of nanoparticle building blocks modified with complementary recognition elements into functional materials.[1] These materials have found wide application in the development of highly sensitive and selective diagnostic methods for DNA.[2] This material synthesis approach has been extended to a wide range of biomolecules, including peptides and proteins,[3] and a modest collection of nanoparticles, including gold and semiconductor quantum dots.[4−9] In each case, when a new nanoparticle composition is designed, new modification methods must be developed for immobilizing biomolecules on the surface of the particles of interest. This approach has been extensively utilized but with limited success. The methods for modifying gold nanoparticles have now been optimized and generalized for a wide range of particle sizes and surface compositions, including spheres and rods.[1,2,4,10] Gold particles are particularly easy to modify because they are often stabilized with a weakly binding layer of charged ligands (e.g., citrate) that can be replaced with molecules with chemical functionalities that bind more strongly (e.g., thiols, amines, and disulfides) to their surfaces than these ligands. The CdSe and CdS quantum dots have proven more difficult to modify because they have a surfactant layer that is very strongly bound to their surfaces and, consequently, difficult to displace.[5] No successful routes have been developed for creating stable oligonucleotide conjugates with silver nanoparticles, primarily because they tend to chemically degrade under conditions used to effect DNA hybrization. A major advance would be to devise a method for designing particles with the physical properties of a chosen nanoparticle composition but with the surface chemistry of gold. Herein, we report a low-temperature method for generating core−shell particles consisting of a core of Ag and a monolayer shell of Au that can be readily functionalized with oligonucleotides using the proven preparatory methods for pure gold particle oligonucleotide conjugates.[2d] Moreover, we show how this novel nanoparticle composition can be used to access a colorimetric detection system distinct from the pure gold system.[2a,d]

Ag nanoparticles are desired compositions for building blocks in material synthesis and as biological labels for two important reasons. (1) Ag particles exhibit a surface plasmon band between ∼390 and 420 nm, depending on the particle size;[11] this is a spectral regime that is distinct from that of Au (520−580 nm). (2) The extinction coefficient of the surface plasmon band for an Ag particle is approximately 4 times as large as that for an Au particle of the same size.[12] Therefore, Ag particles functionalized with DNA would provide not only an opportunity to tailor the optical properties of DNA/nanoparticle composite structures but also routes to new diagnostic systems that rely on the position and intensity of the surface plasmon band (e.g. colorimetric systems based on absorption or scattering, or SPR and SERS detection systems).

Experimentally, we have determined that Ag nanoparticles cannot be effectively passivated by alkylthiol-modified-oligonucleotides using the established protocols for modifying Au particles.[2] Indeed, Ag particles prepared via such methods irreversibly aggregate when heated in a solution with a salt concentration necessary to effect DNA hybridization (0.05 M NaCl). Herein, we use a core−shell approach to overcome this problem. In this approach, a thin Au shell was grown upon an Ag nanoparticle, forming a particle with an Au outer surface that can be easily modified with alkylthiol-oligonucleotides. In the first step, Ag nanoparticles were prepared by literature methods.[13] The particles were then passivated with Bis(*p*-sulfonatophenyl)-phenylphosphine (BSPP, 0.3 mM), purified by gradient centrifugation (collecting the primary fraction; ∼12 nm in diameter), and redispersed in Nanopure water. Gold shells, approximately one-monolayer thick, were grown on the surface of the Ag nanoparticles (0.32 nmol of Ag particles in 100 mL of 0.3 mM sodium citrate aqueous solution) by simultaneously treating them with $HAuCl_4$ and sodium borohydride via dropwise addition at 0 °C on the benchtop. The reduced gold has an affinity for the Ag surface, in part, because of its near-zero lattice mismatch.[14] The simultaneous dropwise addition of dilute Au precursors inhibits the formation of gold cluster nucleation sites by keeping the concentration of these gold-forming reagents ∼2 mM. After enough $HAuCl_4$ and $NaBH_4$ were added to the nanoparticles to produce one monolayer of Au on the particles (5% excess, calculated assuming 12-nm spheres: 0.8 mg of $HAuCl_4 \cdot 3H_2O$ and 3.7 mg of $NaBH_4$), the reaction was stopped, and 30 μmol of BSPP was added. Then, the Ag/Au core−shell nanoparticles were purified by centrifugation and redispersed in Nanopure water (12.4 nm in diameter particles, ($\sigma = 18\%$), Figure 1A). The Ag:Au ratio in these core−shell particles was determined to be 5.2:1 by energy-dispersive X-ray (EDX) microanalysis of the particles, Figure 1B. Such a ratio corresponds to an Au shell thickness of 3.1 ± 0.6 Å, which correlates with approximately one monolayer of Au atoms.

Significantly, the extinction spectrum of the core−shell particles is very similar to that for the citrate-stabilized pure Ag particles. The surface plasmon band of the Ag remains at the same wavelength but is dampened by about 10%, and the gold plasmon band is observed as a slight buckle at 500 nm. These spectral features provide strong evidence for gold shell growth. It should be noted that by using different procedures, others have prepared

* To whom correspondence should be addressed. E-mail: camirkin@chem.nwu.edu.

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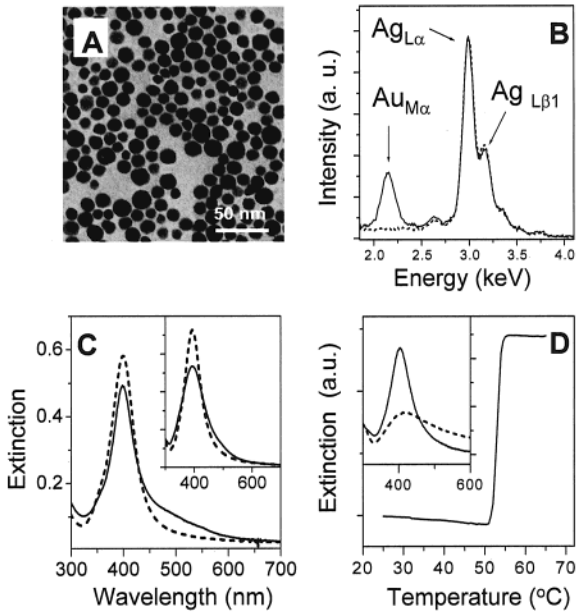

Figure 1. (A) TEM image of Ag/Au core−shell nanoparticles. (B) EDX spectra of Ag core particles (dotted line) and Ag/Au core−shell particles (solid line). L and M signify electron transitions into the L and M shell of the atoms, respectively, from higher states. (C) UV−vis spectra of Ag core (dotted line) and Ag/Au core−shell (solid line). The inset shows the calculated extinction spectra of Ag particles (dotted line) and Ag/Au core−shell particles (solid line). (D) Thermal denaturization curve of aggregates formed from hybridized oligonucleotide-modified Ag/Au core−shell particles in buffer solution (0.3 M NaCl and 10 mM phosphate buffer, pH = 7). The base sequences are given in Figure 2A. The inset shows the UV−vis spectra of dispersed oligonucleotide-modified Ag/Au core−shell particles (solid line) and aggregated (dotted line) oligonucleotide-modified Ag/Au core−shell particles formed via hybridization.

gold-coated silver nanoparticles.[15] However, those procedures lead to Ag/Au alloys;[15a] the extinction spectra of such particles exhibit characteristic red shifting and *broadening* of the plasmon resonance. Moreover, if one intentionally makes a solution of alloyed Ag/Au particles, they can be easily distinguished from core−shell particles with comparable Ag/Au ratios (see Supporting Information). Indeed, the core−shell Ag/Au nanoparticles prepared in this work retain the optical properties of the core with no observed red-shifting of the Ag plasmon band, Figure 1C. Using Mie theory, we calculated the extinction spectrum of a particle consisting of an 11.8 nm Ag core and a monolayer Au shell.[11] The calculated spectrum was almost superimposable with the experimentally measured spectrum of the particles, Figure 1C (inset).

The surface modification of these core−shell nanoparticles with 3′- and 5′-alkanethiol-capped oligonucleotides was accomplished using a procedure identical to the one used for 13-nm gold particles.[2d] Significantly, the oligonucleotide-modified particles exhibit the stability of their pure gold counterparts and can be suspended in 1 M NaCl solutions indefinitely. Note that oligonucleotide-modified Ag/Au alloy particles (Supporting Information) do not exhibit the stability of these oligonucleotide-modified core−shell particles and irreversibly aggregate under comparable conditions. Moreover, the core−shell particles undergo hybridization with complementary linking oligonucleotides to form aggregated structures with a concomitant darkening of the solution; however, no distinct color change is observable by the naked eye,

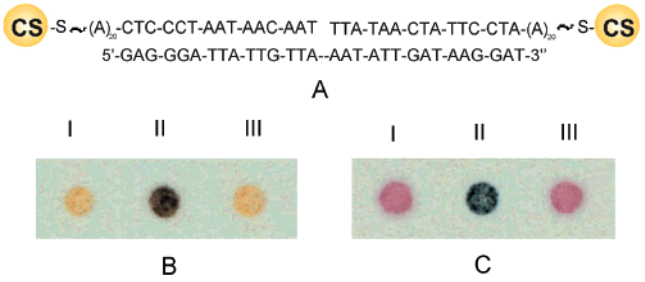

Figure 2. (A) Mercaptoalkyl-oligonuleotide-modified Ag/Au core−shell particles and polynucleotide target. CS: core−shell; alkyl:propyl (left) and hexyl(right). DNA spot test using: (B) 12.4-nm Ag/Au nanoparticle probes and (C)13-nm Au nanoparticle probes: (I) without target, (II) with target at room temperature, (III) with target at 58.0 °C, a temperature above the T_m (53.0 °C) of the hybridized DNA.

Figure 2A. Like their oligonucleotide-modified pure gold counterparts, the particles within these aggregate structures can be disassembled by heating the aggregates above the "melting temperature" (T_m) of the duplex linkers, Figure 1D. UV−vis spectroscopy shows a red-shifting and dampening of the plasmon resonance of the core−shell particles upon DNA-induced assembly, Figure 1D (inset).

The particle-assembly process induced by the complementary DNA also can be monitored on a C_{18}-reverse-phase alumina TLC plate, allowing for comparison with the pure gold system. With the core−shell particles, a distinct yellow-to-dark brown color change is observed upon particle assembly in the presence of complementary target, Figure 2B-I and 2B-II. Note that when the solution temperature is above the T_m of the DNA duplex linkers, a yellow spot is formed on the reverse phase alumina support, Figure 2B-III. When one compares the properties of these new Ag/Au core−shell probes with those derived from pure gold nanoparticles (with identical oligonucleotide sequences), Figure 2C, one realizes that the core−shell particles provide a route to a second colorimetric change distinct from the gold system that ultimately could be used for monitoring two different oligonucleotide targets in one sample. Such capabilities could be important for both research-based and clinical genomic assays where multicolor formats are essential.[16]

In conclusion, this paper is important for the following reasons. (1) It provides a straightforward method for preparing nanoparticles with the optical, and many of the physical, properties of silver but the stability of gold. (2) It shows how oligonucleotides, and presumably other biomolecules (e.g., proteins), can be used to modify the surfaces of such particles, thereby imparting useful biorecognition properties to them. (3) This approach could be generalized to prepare other particles such as Cu and Pt to create a series of core−shell particles with tailorable physical properties by virtue of choice of core but the surface chemistry and stability of the native, and oligonucleotide-modified, pure gold particles.

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Supporting Information Available: Synthesis of Ag/Au alloy particles, UV−vis spectra of Ag/Au alloy and core−shell nanoparticles, comparison of the stability of the oligonucleotide-modified Ag/Au alloy and core−shell nanoparticles in buffer solution (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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