Communication

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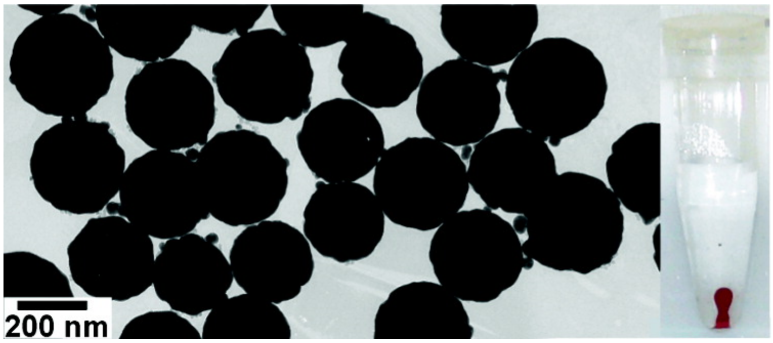
J. Am. Chem. Soc., **2005**, 127 (44), 15362-15363• DOI: 10.1021/ja055056d • Publication Date (Web): 15 October 2005



Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

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Three-Layer Composite Magnetic Nanoparticle Probes for DNA

Savka I. Stoeva, Fengwei Huo, Jae-Seung Lee, and Chad A. Mirkin*

Department of Chemistry and International Institute for Nanotechnology, Northwestern University,
2145 Sheridan Road, Evanston, Illinois 60208

Received July 26, 2005; E-mail: chadnano@northwestern.edu

Gold nanoparticles functionalized with oligonucleotides are important reagents in many highly sensitive and selective DNA and protein detection schemes.[1] They are also key building blocks in several assembly schemes that take advantage of the chemically programmable, sequence-specific hybridization properties of nucleic acids.[1a,2] The ubiquitous use of gold nanoparticles in biodetection, as opposed to other compositions, stems from the existence of well-established straightforward methods for the functionalization of the gold surface with ligands containing thiol or disulfide groups, the cooperative binding properties of the probes, their intense optical properties, and their catalytic properties.[1,3] Each of these properties leads to a selectivity or sensitivity advantage over conventional probes.

Magnetic particles also are attractive materials that have been widely exploited in biology for diagnostic and therapeutic applications.[4] While methods for the synthesis of magnetic particles with control over size and shape exist,[5] their surface functionalization with biomolecules often requires elaborate synthetic schemes.[6] A major advance would be to develop a synthetic method that leads to composite materials that have both the stability, surface chemistry, and optical properties of gold and the magnetic properties of superparamagnetic particles. Core−shell approaches to realizing such probe structures have met with limited success. Direct coating of magnetic particles with gold is a difficult task due to the dissimilar nature of the two surfaces. Methods that are based on the synthesis of one of the compositions in the presence of the other have led to structures with minimal interface contact between gold and the magnetic particle.[7]

Herein, we describe a novel method for the synthesis of composite particles with a gold surface, silica core, and magnetic inner layer. We further demonstrate that the three-layer magnetic nanoparticles can be functionalized with DNA following synthetic methods for pure gold nanoparticles[8] and reversibly assembled into macroscopic aggregates using complementary linking oligonucleotides. Significantly, the aggregate structures exhibit sharp melting profiles when heated above the melting temperature of the duplex DNA linkers, which predetermines their utility in high selectivity DNA detection schemes.[1c]

The synthetic approach for the three-layer magnetic nanoparticle probes utilizes positively charged amino-modified SiO_2 particles as templates for the electrostatic assembly of negatively charged 15 ± 1 nm superparamagnetic, water-soluble Fe_3O_4 nanoparticles (Figure 1). The $SiO_2-Fe_3O_4$ particles electrostatically attract 1−3 nm Au nanoparticle seeds that act in a subsequent step as nucleation sites for the formation of a continuous gold shell around the $SiO_2-Fe_3O_4$ particles upon $HAuCl_4$ reduction.[9]

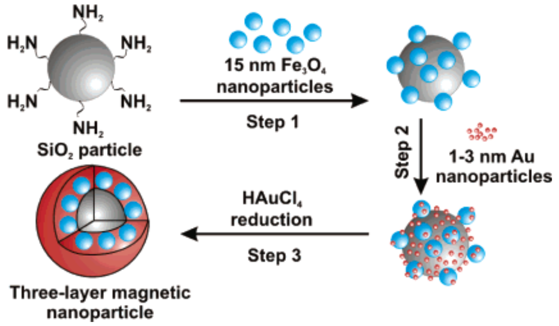

Figure 1. Synthetic scheme for the preparation of the three-layer magnetic nanoparticles.

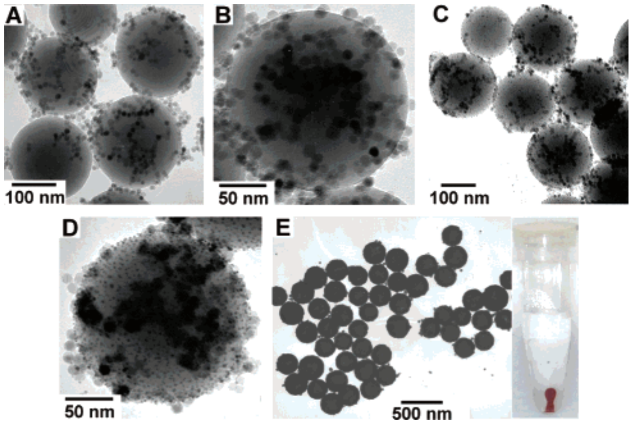

Figure 2. TEM images of colloids after each synthetic step. (A and B) SiO_2 particles covered with silica-primed Fe_3O_4 nanoparticles ($SiO_2-Fe_3O_4$). (C and D) SiO_2 particles covered with silica-primed Fe_3O_4 nanoparticles and heavily loaded with Au nanoparticle seeds ($SiO_2-Fe_3O_4-Au_{seeds}$). (E) Three-layer magnetic nanoparticles synthesized in a single step process from particles presented in (C) and (D). Note the uniformity of the gold shell. The inset shows the three-layer magnetic nanoparticles drawn to the wall with a magnet. The superparamagnetic property was characterized by SQUID (see Supporting Information).

The Fe_3O_4 nanoparticles are synthesized in hexane by a high-temperature seed-mediated growth process developed by Sun et al.[5a] The hydrophobic Fe_3O_4 particles are easily transferred into aqueous media with tetramethylammonium 11-aminoundecanoate, a bipolar surfactant. The Fe_3O_4 nanoparticles are stabilized and made more hydrophilic by reacting them with a sodium silicate solution (see Supporting Information).[10] This results in a thin primer layer of silica on the particle surface.[10] Silica-primed Fe_3O_4 colloids are stable for months and have a negative net interfacial charge at pH 7 (average ζ-potential is −35 mV). Silica-primed Fe_3O_4 colloids have a strong propensity to electrostatically interact at pH 7 with positively charged amino-modified SiO_2 particles (average ζ-potential is +50 mV). Figures 2A and B are TEM images of 200 nm amino-modified SiO_2 particles (G. Kisker GbR) densely loaded with 15 nm silica-primed Fe_3O_4 nanoparticles. The $SiO_2-Fe_3O_4$ particles are overall positively charged (average ζ-potential is +20 mV), and they electrostatically attract negatively charged 1−3 nm Au nanoparticles synthesized in advance by reduction of $HAuCl_4$ with tetrakis(hydroxymethyl)phosphonium chloride (THPC) (Figures 2C and D). The TEM images of extensively washed $SiO_2-Fe_3O_4-Au_{seeds}$ particles reveal a dense surface coverage of both Fe_3O_4 and

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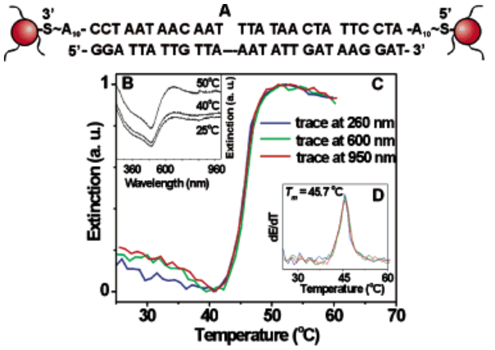

Figure 3. (A) Oligonucleotides used in the melting experiments. (B) UV−vis spectra of hybridized DNA-modified three-layer magnetic nanoparticles (25 °C) and dispersed particles (50 °C). (C) Thermal denaturation curves of aggregates formed from hybridized DNA-modified three-layer magnetic nanoparticles. (D) First derivatives of the “melting” curves shown in (C).

Au particles. EDX characterization shows that the particles contain the three diagnostic elements of the precursors, Fe, Si, and Au. The Fe_3O_4 particles determine the magnetic properties of the composite particles, and the Au seeds act as nucleation centers for the deposition of a continuous gold shell around the particles. The gold shell is grown by $HAuCl_4$ reduction with formaldehyde and results in composite three-layer nanoparticles with smooth gold surfaces (Figure 2E). The particles are indeed magnetic and quickly move to the sidewall of a reaction vessel when exposed to a bar magnet (Figure 2E, inset). Note that, because of the density of the magnetic particles within the three-layer composite structure, these composite materials move to the reaction vessel walls at a rate approximately 30 times faster than that of the unmodified Fe_3O_4 particles. Significantly, the experimental conditions for the composite particle synthesis have been optimized to allow the uniform gold shell growth to be performed in a single step (see Supporting Information). The final composite magnetic colloid exhibits a broad plasmon band above 600 nm, appears red in color due to light scattering, but looks green in color based upon transmitted light.

To evaluate the chemical characteristics and hybridization properties of these composite nanoparticles in the context of a bioanalysis experiment, we functionalized two different batches of identical particles with 3′- and 5′-thiol-modified DNA, respectively, following procedures used to modify pure Au nanoparticles (Supporting Information).[8] Importantly, the DNA-modified three-layer magnetic nanoparticles can be repeatedly centrifuged and redispersed in buffer solution (0.1 M NaCl, 0.005% Tween 20, 10 mM phosphate buffer, pH = 7.4) without aggregation. Moreover, the DNA-modified three-layer magnetic nanoparticles assemble into macroscopic aggregates upon hybridization with complementary linking oligonucleotides (Figure 3A) in the same way as pure gold nanoparticles. The three-layer magnetic nanoparticles can be reversibly released from the aggregates by raising the temperature above the melting point (T_m) of the duplex DNA linkers. This process is accompanied by changes in the UV−vis spectra (Figure 3B) and can be experimentally monitored by measuring the extinction of the solution as a function of temperature (Figure 3C). Significantly, the melting process is characterized by sharp melting transitions (fwhm ∼ 3.5 °C) resulting from the cooperative melting effect observed for aggregates of nanoparticles heavily functionalized with DNA.[8b]

In conclusion, this paper describes a novel method for the synthesis of composite particles with the optical and chemical properties of DNA-functionalized gold nanoparticles and the superparamagnetic properties of Fe_3O_4 nanoparticles. These structures exhibit the highly cooperative binding properties that are key to high selectivity biodiagnostic systems based upon nanoparticle probes,[1] and researchers now can begin to evaluate how magnetism can be used to facilitate target isolation and hybridization in the context of chip-based assays that rely on nanoparticle probes. In principle, the synthetic procedure can be extended to the preparation of various particle sizes using different-sized SiO_2 template particles and other compositions where electrostatic assembly can be utilized to generate layered structures analogous to the ones presented herein.

Acknowledgment. C.A.M. acknowledges the Air Force Office of Scientific Research, the Defense Advanced Research Projects Agency, NSF, Doris Duke Foundation, and NIH for support of this research. C.A.M. is also grateful for support from a NIH Director’s Pioneer Award.

Supporting Information Available: Detailed procedures for Fe_3O_4 and the three-layer magnetic nanoparticle probe synthesis. TEM, UV−vis, ζ-potential, and SQUID measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA055056D