DNA-Directed Synthesis of Binary Nanoparticle Network Materials

Robert C. Mucic, James J. Storhoff, Chad A. Mirkin,* and Robert L. Letsinger*

Department of Chemistry, Northwestern University, Evanston, Illinois 60208

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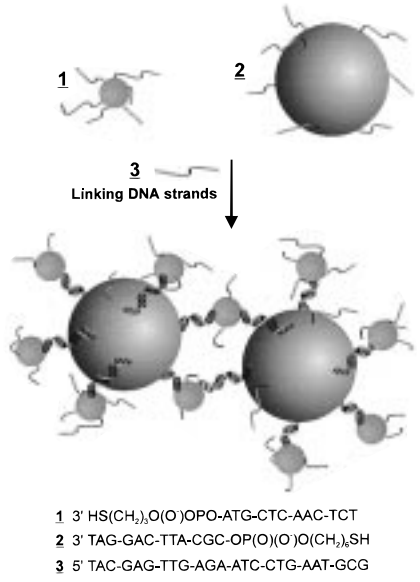
A major limitation in nanoparticle-based materials chemistry is the lack of suitable assembly methods for preparing extended two- and three-dimensional architectures with synthetically programmable building block and assembly parameters. Such parameters include the size, size distribution, shape, and chemical composition of the nanoparticle building blocks, the length and stability of the molecule-based particle interconnects, and the length scale of the extended architecture. To date, both covalent and noncovalent interconnect strategies have been used to assemble nanoscale inorganic building blocks into two- and three-dimensional macroscopic structures.[1−4] All of these strategies have advantages or disadvantages depending upon the targeted nanoparticle-based material.

We recently reported one of the more versatile noncovalent methods for assembling nanoparticles into network materials.[5−7] Our method takes advantage of nanoparticle building blocks functionalized with oligonucleotides and a linker oligonucleotide that is complementary to the modified nanoparticles of interest. Thus far, only one nanoparticle system, which exclusively involves oligonucleotide-functionalized 13-nm-diameter Au particles as the building blocks, has been extensively studied. Nevertheless, this initial one-component system (here, component refers to the type of particle) already has increased our understanding of particle−particle interactions on the nanometer-length scale and has led to the development of a new and highly selective colorimetric detection technology for oligonucleotides.[6,7] Herein, we show how this DNA-directed assembly strategy can be used to prepare binary (two-component) network materials comprising two different-sized oligonucleotide-functionalized nanoparticles, Scheme 1. Importantly, the proof-of-concept demonstrations reported herein suggest that this strategy could be extended easily to a wide variety of multicomponent systems where nanoparticle building blocks that vary in chemical composition or size are arranged in space on the basis of their interactions with complementary linking DNA. Because of its chemical origin, this strategy is not limited by physical particle size selection rules in forming extended structures; for example, we can prepare "anatural" structures where particle size ratios ordinarily would not be suitable for forming binary ab−ab structures (e.g., via sedimentation).[8]

Scheme 1



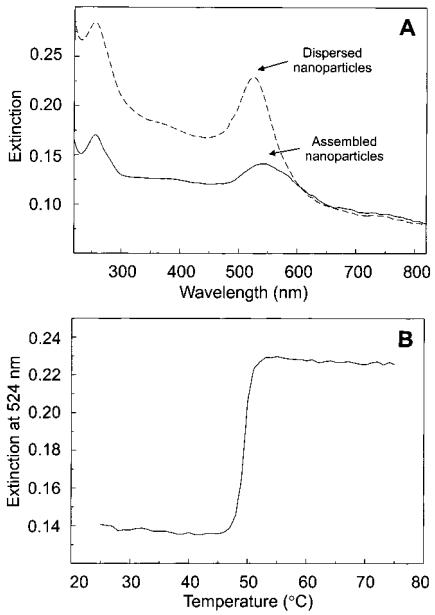


Figure 1. (A) UV−visible spectrum of a 8:1 mixture of **1**-modified 8-nm particles and **2**-modified 31-nm particles before (- - -) and after (—) linking with **3**. (B) Temperature vs extinction profile for the thermal denaturation of the binary network materials (monitored at 524 nm). The mixture of oligonucleotide-modified nanoparticles and linker oligonucleotide (680 μL) was diluted to 1.7 mL (using 0.3 M NaCl, 10 mM phosphate buffer at pH 7) in a quartz cuvette equipped with a stir bar. Measurements were performed by recording the spectra at 1 °C intervals with a hold time of 1 min at each degree while stirring at 700 rpm.

Experiments were carried out with citrate-stabilized colloidal gold particles averaging 8 and 31 nm in diameter.[9] Thiol-modified strands **1** and **2** were synthesized and immobilized on nanoparticle surfaces by previously described methods.[5−7] The 8-nm particles were treated with propanethiol-capped oligonucleotide **1**, and the

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(9) These particles were purchased from ICN Pharmaceuticals as 5- and 40-nm-diameter colloidal gold particles, but TEM analysis of them showed that their diameters were 8 ± 1 and 31 ± 3 nm, respectively. Particles were sized by scanning 3 by 4 in. TEM negatives into a computer using a flatbed scanner (Epson Expression 636) with subsequent analysis of ∼100 particles via graphics software (Corel Photo-Paint).

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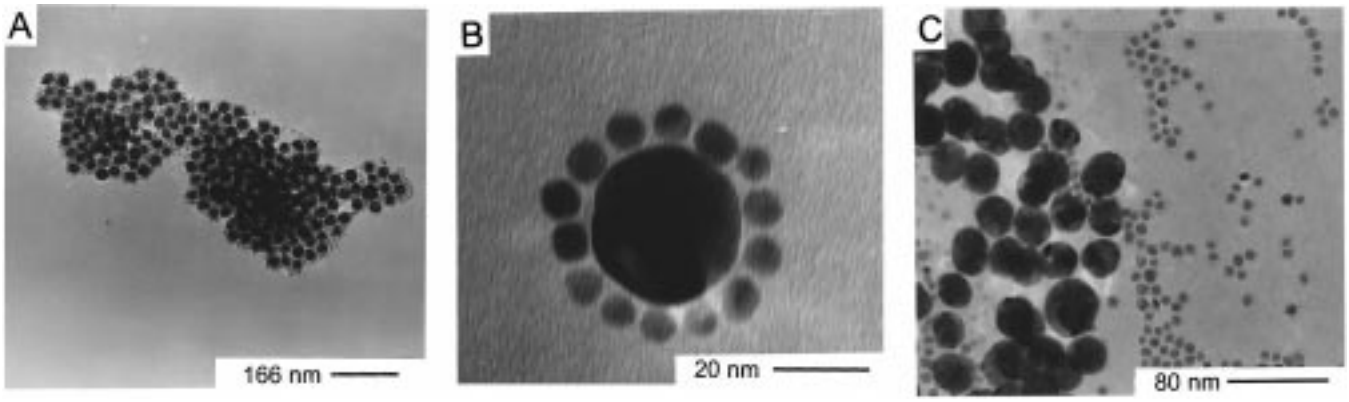

Figure 2. TEM images of the binary nanoparticle network materials supported on holey carbon grids: (A) an assembly generated from **1**-modified 8-nm particles, **2**-modified 31-nm particles, and linking oligonucleotide **3**; (B) a nanoparticle satellite structure obtained from the reaction involving 120:1 **1**-modified 8-nm particles/**2**-modified 31-nm particles and linking oligonucleotide **3**; and (C) **1**-modified 8-nm particles and **2**-modified 31-nm particles mixed together without linking oligonucleotide **3**. TEM was performed on a Hitachi H-8100 electron microscope operating at 200 kV. It is important to note that the nanoparticle aggregate structures observed may not *exactly* represent the structures in solution since the hybridization ability of the oligonucleotides may be affected by sample preparation and the TEM conditions. For example, we found that the small particles sometimes settle near the assembly from which they were originally linked, resulting in the appearance of an extra layer of small particles surrounding some of the large particles.

31-nm particles were modified with the hexanethiol-capped oligonucleotide **2**.[10] The size and optical properties of the particles did not significantly change upon modification with the oligonucleotides as evidenced by TEM and UV−visible spectroscopy. However, some irreversible aggregation did occur with the 31-nm particles. The aggregates were removed by centrifugation (30 s at 1000 rpm) to yield solutions consisting of discrete oligonucleotide-stabilized particles. Atomic emission spectroscopy was used to determine the concentrations of atomic gold in the nanoparticle solutions. From these values and the spectral data, the molar extinction coefficients for the 8- and 31-nm-diameter particles were found to be 7.5×10^7 M^{-1} cm^{-1} (ϵ at 522 nm) and 4.7×10^9 M^{-1} cm^{-1} (ϵ at 524 nm), respectively.[11] In contrast to their citrate-stabilized precursors, which immediately and irreversibly aggregate upon raising the NaCl concentration of the solution to 0.1 M, the oligonucleotide-modified particles show no evidence of aggregation even at 1 M NaCl.

For the first set of experiments, 50 pmol (40 μL) of oligonucleotide **3** was introduced into a cuvette containing 100 μL (0.60 pmol) of the 8-nm-diameter particles modified with **1** and 540 μL (0.075 pmol) of the 31-nm-diameter particles modified with **2**. This solution initially appeared pink, but after standing at room temperature for 24 h, the color faded. In the UV−visible spectrum of the solution, this change appears as a broadening and red shift in the particle surface plasmon resonance from 524 to 543 nm, Figure 1A. When solutions containing the assemblies are heated above the dissociation temperature of the hybridized oligonucleotides, the pink color of the solution and the surface plasmon band at 524 nm, characteristic of dispersed particles, reappear (Figure 1B). This "melting transition" was extremely sharp and reversible, which appears to be a common characteristic of all DNA-linked nanoparticle network structures studied thus far.[5−7] TEM images (Figure 2A) of the system in the assembled state indeed show extended aggregates of two different-sized particles with a (big particle/small particle) periodicity, as expected for linkages generated by oligonucleotide hybridization.

The structure of the assembly can be readily controlled as evidenced by a second type of experiment. In this case, the linking oligonucleotide **3** (24 μL, 30 pmol) was added to a solution containing a 120:1 ratio of modified 8-nm particles (280 μL, 1.68 pmol) and modified 31-nm particles (100 μL, 0.014 pmol). TEM images of the assemblies collected by centrifugation revealed clusters or "satellite structures" consisting of single 31-nm particles surrounded by many 8-nm particles, Figure 2B.[12] The satellite structures comprise ~95% of the sample with the remainder being 8-nm particles. Significantly, there was no evidence of 31-nm particles without neighboring 8-nm particles.

Control experiments performed by mixing the **1**-modified 8-nm particles (40 μL, 0.60 pmol) and the **2**-modified 31-nm particles (540 μL, 0.075 pmol) in the absence of linking oligonucleotide **3** did not result in a colorimetric change or the formation of extended periodic binary assemblies (Figure 2C). Under these conditions, randomly dispersed particles or aggregates, consisting of particles of the same size, are observed by TEM. The latter size-dependent, particle separation phenomenon has been noted by others, who have studied mixtures of two different sizes of colloidal particles.[13] The synthesis of the satellite structures depicted in Figure 2B is significant; since all of the structures generated from the DNA-modified nanoparticles are essentially living polymers, these two-component satellite structures form the basis for a new type of building block that could be incorporated into our DNA-driven particle assembly scheme giving entry into multicomponent nanostructured materials.

In conclusion, this communication shows how the molecular recognition properties of oligonucleotides can be used to direct the placement of two different particle types within extended particle assemblies. In the examples provided herein, the nanoparticle building blocks only differ in size, but this methodology also should be extendable to building blocks that differ in chemical composition (e.g., CdS, CdSe, Pt, and Ag).

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(10) In loading the 31-nm-diameter particles, an additional synthetic step was carried out, which enhanced their stability. After mixing the nanoparticles with the alkylthiol oligonucleotides, pyridine was added to a final concentration of 0.01 M. Following aging for 24 h in 0.1 M NaCl, the nanoparticles were collected and then redispersed in 0.3 M NaCl, 10 mM phosphate buffer at pH 7 (see ref 7).

(11) The molar concentration of particles was determined by comparing the number of gold atoms in a particle of known diameter to the total number of atoms in the solution. The number of atoms per particle was determined using the average size of the particle (determined by TEM) and the density of gold. Assuming spherical particles, the number of atoms in a 8- and 31-nm-diameter particle were estimated to be 15 800 and 920 000, respectively.

(12) TEM samples of the nanoparticle "satellite structures" were prepared by centrifuging the mixture for 4 min at 6000 rpm (Eppendorf model 5415C microcentrifuge) and then decanting the supernatant containing the unlinked 8-nm particles. Aqueous buffer was used to redisperse the oily precipitate. Then, a 10-μL sample of the solution was pipetted onto a holey carbon grid and the volume of the droplet was reduced by slowly wicking away the solution with a Kimwipe.

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