# The DNA-Mediated Formation of Supramolecular Mono- and Multilayered Nanoparticle Structures

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Herein, we report a new, noncovalent DNA-based strategy for controlling the stepwise growth of layered nanoparticle structures off of glass surfaces. Others have demonstrated how bifunctional organic molecules[1−4] or polyelectrolytes[5] can be used to controllably construct mono- and multilayered nanoparticle materials off of planar substrates. The attractive feature of using DNA as a particle interconnect is that, in principle, one can synthetically program interparticle distances, particle periodicities, and particle compositions through choice of DNA sequence. In addition to providing a new and powerful method for controlling the growth of nanoparticle-based architectures from solid substrates, this strategy also allows one to evaluate the relationship between nanoparticle aggregate size and both melting and optical properties of aggregate DNA-interlinked structures. An understanding of these two physical parameters and their relationship to materials architecture is essential for utilizing nanoparticle network materials, especially in the area of biodetection.

Recently, we invented a new method for colorimetrically detecting DNA with probes comprised of gold nanoparticles functionalized with hundreds of oligonucleotides.[6] The method involves the use of two probes that are complementary to a target sequence, Scheme 1A. When the probes encounter such target DNA, they are assembled into a network structure that results in a concomitant red-to-blue color change. This color change is due, in part, to the increased plasmon interactions of the particles within the DNA cross-linked network structure. In addition to being a very simple method for detecting DNA, this assay exhibits extraordinary selectivity when compared to other hybridization-based assays. The origin of this selectivity advantage derives from the extraordinarily sharp melting properties of these DNA-interconnected nanoparticle network materials. Indeed, normal melting for a 24 nucleotide duplex occurs over a 25 deg temperature range (Figure 2C), whereas the same set of sequences when used as linkers to form a nanoparticle network structure will melt over 6 deg.[6] An unanswered set of questions pertains to the factors that control the melting properties of these unconventional network structures. We had originally hypothesized that this sharp melting behavior was due to the following: (1) a cooperative effect involving thousands of cross-linked nanoparticles within the network structure and (2) the probing of a nanoparticle signature rather than a DNA signature in the monitoring of the melting process. However, with solution grown network structures, it is impossible to control aggregate size and systematically evaluate how increased cross-linking affects the melting properties of nanoparticle network structures. Herein, we show how our strategy for preparing supramolecular layered nanoparticle structures in stepwise fashion allows us to systematically address these issues.

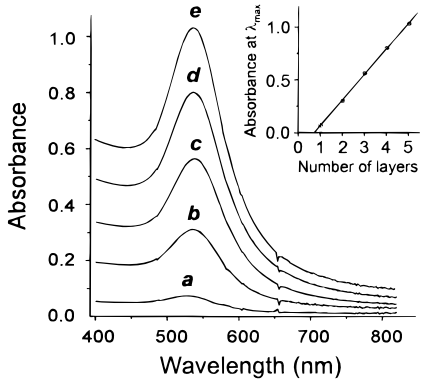
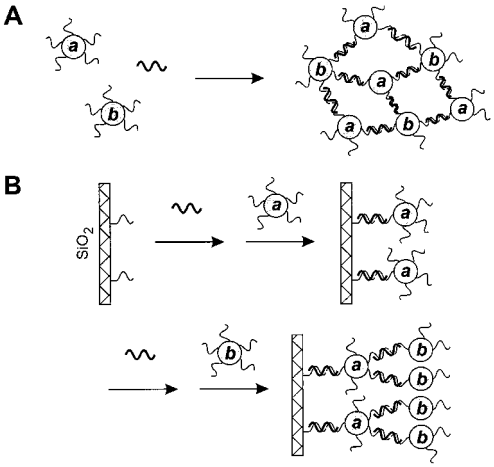



**Figure 1.** UV-visible spectra of alternating layers of Au nanoparticles ***a*** and ***b***, hybridized to an oligonucleotide-functionalized glass microscope slide via the complementary DNA linker **3**. Spectra represent assemblies with 1 (**a**, $\lambda_{max}$ = 524 nm), 2 (**b**, $\lambda_{max}$ = 529 nm), 3 (**c**, $\lambda_{max}$ = 532 nm), 4 (**d**, $\lambda_{max}$ = 534 nm) and 5 (**e**, $\lambda_{max}$ = 534 nm) layers. Spectra were measured directly through the slide. Inset: Absorbance of nanoparticle assemblies at $\lambda_{max}$ with increasing layers.

**Scheme 1**




**1** 5′ $HS(CH_2)_6O(PO_2^-)$O-CGC-ATT-CAG-GAT 3′
**2** 3′ $HS(CH_2)_3O(PO_2^-)$O-ATG-CTC-AAC-TCT 5′
**3** 5′ TAC-GAG-TTG-AGA-ATC-CTG-AAT-GCG 3′

The oligonucleotide-functionalized, 13-nm-diameter Au nanoparticles used to construct the multilayer assemblies have been described previously.[6a] Particles were treated with 5′-hexanethiol-capped oligonucleotide **1** and 3′-propanethiol-capped oligonucleotide **2** to yield particles ***a*** and ***b***, respectively. Glass slides were functionalized with 12-mer oligonucleotide **2** via the method of Chrisey et al.[7] To build nanoparticle layers, the substrates were

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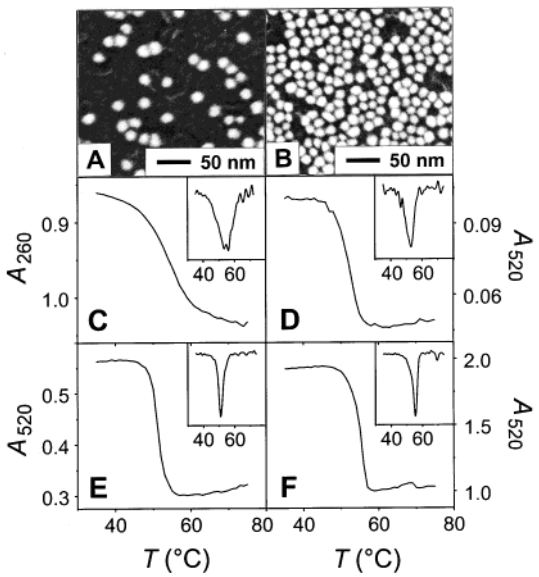


**Figure 2.** (A) FE-SEM of one layer of oligonucleotide-functionalized Au nanoparticles cohybridized with DNA linker to an oligonucleotide-functionalized, conductive indium−tin oxide (ITO) slide. The visible absorbance spectrum of this slide was identical with that of Figure 1a, indicating that functionalization and particle coverage on ITO is similar to that on glass. The average density of counted particles from 10 such images is ~800 particles/$\mu$m$^2$. (B) FE-SEM image of two layers of particles on the ITO slide. The average density of counted particles from 10 such images is ~2800 particles/$\mu$m$^2$. (C) Dissociation of a 0.5 $\mu$M solution of the oligonucleotide duplex (**1** + **2** + **3**) to single strands in 0.3 M NaCl, 10 mM phosphate buffer solution (pH 7), monitored by UV absorption at 260 nm. (D−F) Dissociation of 1 layer (D), 4 layers, (E) and 10 layers (F) of oligonucleotide-functionalized Au nanoparticles from glass slides immersed in 0.3 M NaCl, 10 mM phosphate buffer solution. Melting profiles were obtained by measuring the decreasing absorption at 520 nm through the slides with increasing temperature. Insets: First derivatives of the measured dissociation curves. The fwhm of these curves measure (C) 13.2 °C, (D) 5.6 °C, (E) 3.2 °C, and (F) 2.9 °C.

first immersed in a 10 nM solution of 24-mer linker **3** and allowed to hybridize with it for 4 h at room temperature, Scheme 1B. The substrates were washed with clean buffer solution and then hybridized with a 2 nM solution of particle ***a*** for 4 h at room temperature to attach the first particle layer. A second particle layer could be deposited onto the first one by similarly exposing the surface to solutions of linker **3** and particle ***b***. These hybridization steps could be repeated to deposit multiple, alternating layers of particles ***a*** and ***b*** onto the glass, each layer connected to the previous one by linker **3**. In the absence of linker, or in the presence of noncomplementary oligonucleotide, no hybridization of nanoparticles to the surface was observed.

Each hybridized nanoparticle layer imparted a deeper red color onto the substrate, and after 10 hybridized layers, the supporting glass slide appeared reflective and gold in color. Transmission UV−vis spectroscopy of the substrate was used to monitor the successive hybridization of nanoparticle layers to the surface, Figure 1. The low absorbance of the initial particle layer suggests that it seeded the formation of further layers, which showed a near linear increase in the intensity of the plasmon band with each additional layer.[8] The linearity of the absorbance increase after the generation of the initial particle layer indicates that the surface was saturated with hybridized nanoparticles with each successive application, Figure 1 (inset). This is supported by field-emission scanning electron microscope (FE-SEM) images of one (Figure 2A) and two (Figure 2B) particle layers on a surface, which show low particle coverage with one layer but near complete coverage with two layers. The $\lambda_{max}$ of the plasmon band for the multilayer assemblies shifts no more than 10 nm, even after 5 layers. The *direction* of this shift is consistent with other experimental[2b] and theoretical[9,10] treatments of Au nanoparticle aggregates. However, the *magnitude* of the shift is small compared to that previously observed for suspensions of oligonucleotide-linked Au nanoparticle networks, which show $\lambda_{max} > 570$ nm. This suggests that many more linked nanoparticles, perhaps hundreds or thousands, are required to produce the dramatic color change from red to blue observed for gold nanoparticle-based oligonucleotide probes.[6] Surface plasmon shifts for aggregated Au particles have been shown to be highly dependent on interparticle distance,[9,11] and the large distances provided by oligonucleotide linkers (8.2 nm for this system) significantly reduce the progressive effect of particle aggregation on the Au surface plasmon band.

The dissociation properties of the assembled nanoparticle multilayers were highly dependent upon the number of layers deposited. When the multilayer-coated substrates were suspended in buffer solution and the temperature raised above the $T_m$ of the linking oligonucleotides (53 °C), the nanoparticles dissociated into solution, leaving behind a colorless glass surface. Increasing or decreasing the pH (>11 or <3) or decreasing the salt concentration of the buffer suspension (below ~0.01 M NaCl) also dissociated the nanoparticles by dehybridizing the linking DNA. The multilayer assembly was fully reversible, and nanoparticles could be hybridized to and dehybridized from the glass substrates (e.g., three cycles were demonstrated with no detectable irreversible particle binding).

Significantly, while all of the surface bound nanoparticle assemblies dissociated above the $T_m$ of the linking oligonucleotides, the sharpness of these transitions depended on the size of the supported aggregate, Figure 2D−F. Surprisingly, the dissociation of the first nanoparticle layer from the substrate exhibits a transition (Figure 2D, fwhm of the first derivative = 5 °C) that is sharper than that of the same oligonucleotides without nanoparticles in solution, Figure 2C. As more nanoparticle layers are hybridized to the substrate, the melting transition of the oligonucleotide-linked nanoparticles becomes successively sharper (Figure 2E,F, fwhm of the first derivative = 3 °C), until it matches that of the large nanoparticle network assemblies found in solution.[6] These experiments confirm that more than two particles and multiple DNA interconnects are required to obtain the optimally sharp melting curves. They also show that the optical changes in this system are completely decoupled from the melting properties (i.e., small aggregates can give sharp transitions but still be red).

These observations are significant for the following reasons. (1) This is the first reported methodology for forming layered nanoparticle-based materials from DNA and designed hybridization events. (2) The strategy should be quite general and extendable to other nanoparticle sizes and compositions.[12] (3) The unexpected sharp melting properties of single particles hybridized to the glass support point to a possible route to developing chip-based detection formats for DNA with selectivities that are better than those of conventional probe systems.

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(8) For each successive nanoparticle layer formation, no additional absorbance was observed on exposure for longer times or to higher concentrations of either linker **3** or nanoparticle solution.

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