A DNA-based method for rationally assembling nanoparticles into macroscopic materials

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COLLOIDAL particles of metals and semiconductors have potentially useful optical, optoelectronic and material properties[1–4] that derive from their small (nanoscopic) size. These properties might lead to applications including chemical sensors, spectroscopic enhancers, quantum dot and nanostructure fabrication, and microimaging methods[2–4]. A great deal of control can now be exercised over the chemical composition, size and polydispersity[1,2] of colloidal particles, and many methods have been developed for assembling them into useful aggregates and materials. Here we describe a method for assembling colloidal gold nanoparticles rationally and reversibly into macroscopic aggregates. The method involves attaching to the surfaces of two batches of 13-nm gold particles non-complementary DNA oligonucleotides capped with thiol groups, which bind to gold. When we add to the solution an oligonucleotide duplex with 'sticky ends' that are complementary to the two grafted sequences, the nanoparticles self-assemble into aggregates. This assembly process can be reversed by thermal denaturation. This strategy should now make it possible to tailor the optical, electronic and structural properties of the colloidal aggregates by using the specificity of DNA interactions to direct the interactions between particles of different size and composition.

Previous assembly methods have focused on the use of covalent 'linker' molecules that possess functionalities at opposing ends with chemical affinities for the colloids of interest. One of the most successful approaches to date[5] has involved the use of gold colloids and well established thiol adsorption chemistry[6,7]. In this approach, linear alkanedithiols were used as the particle linker molecules. The thiol groups at each end of the linker molecule covalently attach themselves to the colloidal particles to form aggregate structures. The drawbacks of this method are that the process is difficult to control and the assemblies are formed irreversibly. Methods for systematically controlling the assembly process are needed if the materials properties of these unusual structures are to be exploited fully.

Our oligonucleotide-based method allows the controlled and reversible assembly of gold nanoparticles into supramolecular structures. Oligonucleotides offer several advantages over non-biological-based linker molecules. For example, discrete sequences of controlled length and with the appropriate surface binding functionality may be prepared in an automated fashion with a DNA synthesizer. In this way, the molecular recognition properties of the oligonucleotides may be used to trigger the colloidal self-assembly process. The interparticle distances and stabilities of the supramolecular structures generated by this method can be controlled through the choice of oligonucleotide sequence and length, solvent, temperature and supporting electrolyte concentration.

Others also have recognized the utility of DNA for the preparation of new biomaterials and nanofabrication methods. Previous researchers have focused on using the sequence-specific molecular-recognition properties of oligonucleotides to design impressive structures with well defined geometric shapes and sizes[8–18]. The chemistry proposed here focuses on merging the chemistry of DNA with the chemistry of inorganic colloidal materials. In addition to generating materials with properties that are hybrids of their DNA and colloidal precursors, the union of metal-colloid and DNA chemistry offers significant opportunities relative to the construction of pure DNA materials. As noted by Seeman[19], 'the theory of producing DNA [structures] is well ahead of experimental confirmation. It is much easier to design a [structure] than it is to prove its synthesis.' An advantage of the DNA/colloid hybrid materials reported herein is that the assemblies can be characterized easily by transmission electron microscopy (TEM) and/or atomic force microscopy (AFM) as well as spectroscopic methods conventionally used with DNA.

Our approach to using oligonucleotides for the controlled assembly of gold nanoparticles into aggregate macroscopic structures is outlined in Fig. 1. First, 13-nm-diameter Au particles are prepared[2,20]. These particles form a dark red suspension in water, and like thin-film Au substrates[21], they are easily modified with oligonucleotides, which are functionalized with alkane thiols at their 3′ termini. In a typical experiment, one solution of 17 nM (150 μl) Au colloids is treated for 24 h with 3.75 μM (46 μl) 3′-thiol-TTTGCTGA, and a second solution of colloids is treated with 3.75 μM (46 μl) 3′-thiol-TACCGTTG. Note that these oligonucleotides are non-complementary. After treatment with the thiol-capped oligonucleotides, the two colloidal Au solutions are combined, and because of the non-complementary nature of the oligonucleotides, no reaction takes place. A beneficial consequence of capping the colloids with these oligonucleotides is

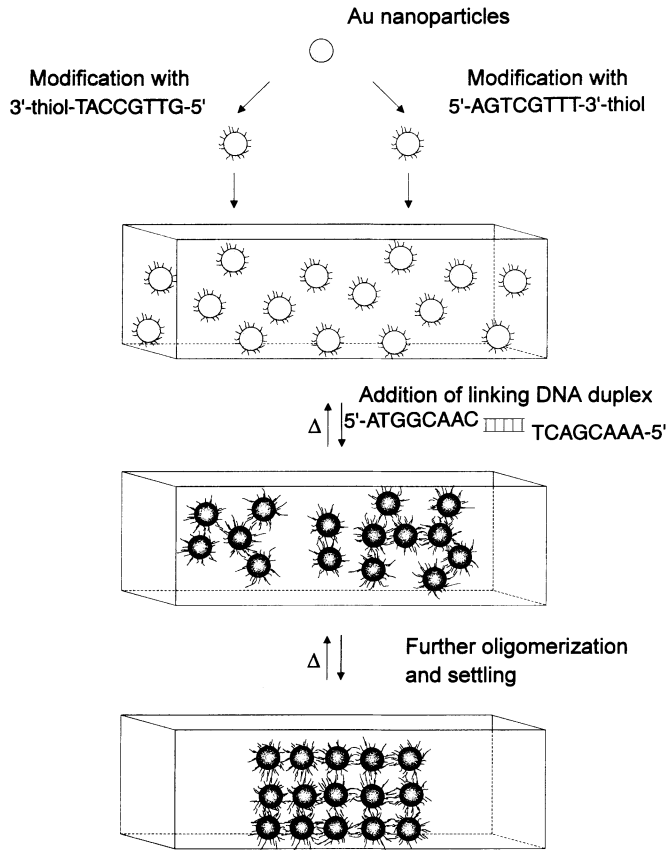

FIG. 1 Scheme showing the DNA-based colloidal nanoparticle assembly strategy (the hybridized 12-base-pair portion of the linking duplex is abbreviated as ▭▭▭▭). If a duplex with a 12-base-pair overlap but with 'sticky ends' with four base mismatches (5′-AAGTCAGTTATACGCGCTAG and 3′-ATATGCGCGATCAAATCACA) is used in the second step, no reversible particle aggregation is observed. The scheme is not meant to imply the formation of a crystalline lattice but rather an aggregate structure that can be reversibly annealed. Δ is the heating above the dissociation temperature of the duplex.

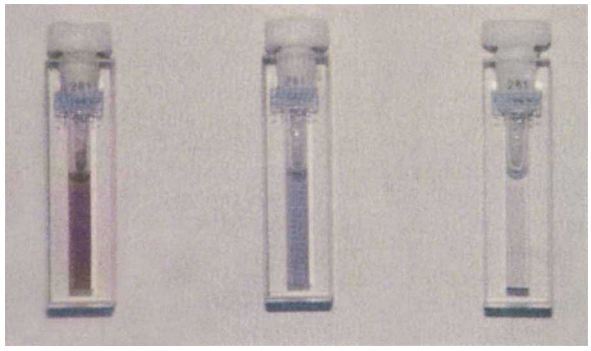

FIG. 2 Cuvettes with the Au colloids and the four DNA strands responsible for the assembly process. Left cuvette, at 80 °C with DNA-modified colloids in the unhybridized state; centre, after cooling to room temperature but before the precipitate settles; and right, after the polymeric precipitate settles to the bottom of the cuvette. Heating either of these cool solutions results in the reformation of the DNA-modified colloids in the unhybridized state (shown in the left cuvette).

that they are much more stable than bare Au colloids to increased salt concentration and temperature. When heated or in a solution of high salt concentration (0.1 M NaCl), bare colloids undergo irreversible particle-growth reactions that result in their precipitation. In contrast, the DNA-modified Au nanoparticles reported here are stable at elevated temperatures (80 °C) and in aqueous 0.1 M NaCl solutions for days, presumably because their DNA-modified surfaces prohibit them from getting close enough to undergo particle growth. This is important because high salt concentrations are needed for the DNA hybridization events depicted in Fig. 1.

In the next step of the assembly scheme, a duplex consisting of 5′-ATGGCAACTATACGCGCTAG and 3′-ATATGCGCGATCTCAGCAAA (the duplex has a 12-base-pair overlap (underlined), containing 8-base-pair sticky ends, which are complementary to the 8-base-pair oligonucleotides that are covalently attached to the Au colloids; Fig. 1) is added to the dark red solution. The solution is then diluted with aqueous NaCl (to 1 M) and buffered at pH 7 with 10 mM phosphate, conditions which are suitable for hybridization of the oligonucleotides. Significantly, an immediate colour change from red to purple is observed and a precipitation reaction ensues. Over the course of several hours, the solution becomes clear and a pinkish-grey precipitate settles to the bottom of the reaction vessel (Fig. 2). Presumably, the free ends of the 'linking' duplex bind to the complementary oligomers anchored to the gold, thereby crosslinking the colloids, which ultimately results in the formation of the pinkish-grey polymeric DNA–colloid precipitate. To verify that this process involved both the DNA and colloids, the precipitate was collected and resuspended (by shaking) in 1 M aqueous NaCl buffered at pH 7. Then, a temperature/time dissociation experiment was performed by monitoring both an optical absorption dependent on hybridization of DNA (260 nm) and one dependent on the degree of colloid aggregation (700 nm), Fig. 3*a*. As the temperature is cycled between 0 and 80 °C, which is 38 °C above the dissociation temperature (T_m) for the DNA-duplex ($T_m = 42$ °C), there is an excellent correlation between the optical signatures for both the colloids and DNA. In the absence of DNA, the ultraviolet–visible spectrum for the naked Au colloids is much less temperature-dependent (Fig. 3*b*). There is a substantial optical change when the polymeric DNA–colloid precipitate is heated above its melting point. The clear solution turns dark red as the polymeric biomaterial dehybridizes to generate the unlinked colloids which are soluble in the aqueous solution. This process is very reversible as evidenced by the temperature traces in Fig. 3*a*. In a control experiment designed to verify that this process was due to oligonucleotide hybridization, a duplex with four base-pair mismatches in each of the 'sticky' ends of the linkers (step 2 in Fig. 1) did not induce the reversible particle aggregation process.

Further evidence of the polymerization/assembly process comes from TEM studies of the polymeric precipitate (Fig. 4). TEM images of the colloids linked with hybridized DNA show large assembled networks of the Au colloids (Fig. 4*a*). Naked Au colloids do not aggregate in this manner under comparable conditions, but rather undergo particle-growth reactions[2]. Note that there is no evidence of colloid particle growth as the hybridized colloids seem to be remarkably regular in size with an average diameter of 13 nm. With TEM, because of the superposition of layers, it is difficult to assess the degree of order for three-dimensional aggregates. But smaller-scale images of single-layer, two-dimensional aggregates provide more compelling evidence of the self-assembly process (Fig. 4*b*). This figure shows close-packed assemblies of the aggregates with uniform particle separations ~60 Å. This distance is somewhat shorter than the maximum spacing (95 Å) expected for colloids connected by rigid DNA hybrids with the selected sequences. But because of the nicks in the DNA duplex, these are not rigid hybrids and are quite flexible. It should be noted that, in principle, this is a variable that can be controlled by reducing the system from four overlapping strands to three (thereby reducing the number of nicks) or by using triplexes instead of duplexes.

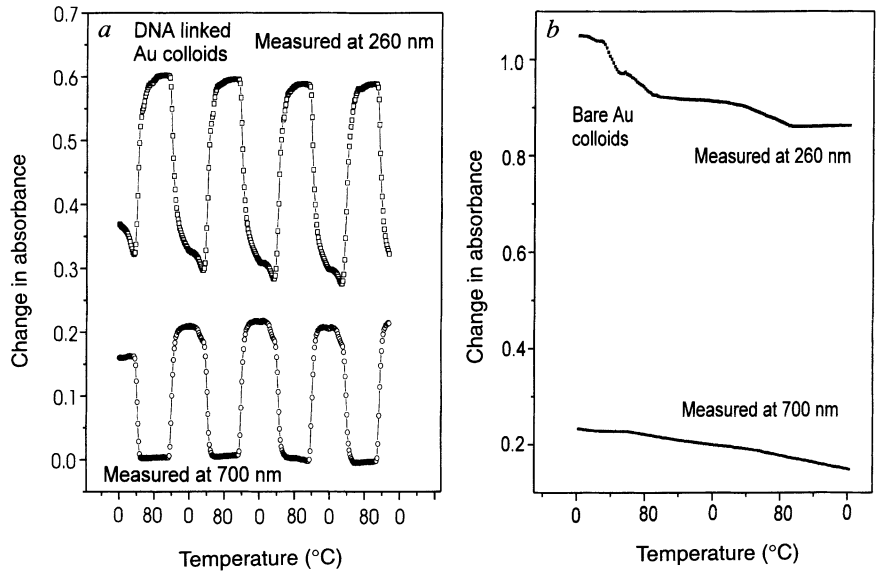

FIG. 3 *a*, Absorbance versus temperature/time profile for DNA/colloid hybridized materials. At low temperatures the Au colloids aggregate owing to the hybridization of 'linking' DNA. At high temperature (80 °C), the colloids dehybridize and form a dark red solution (see Fig. 1 and Fig. 2). The temperature versus time profile shows that this is a reversible process. *b*, Results of same procedure shown in *a*, but applied to an aqueous solution of unmodified Au colloids (5.1 nM, same concentration as in *a*).

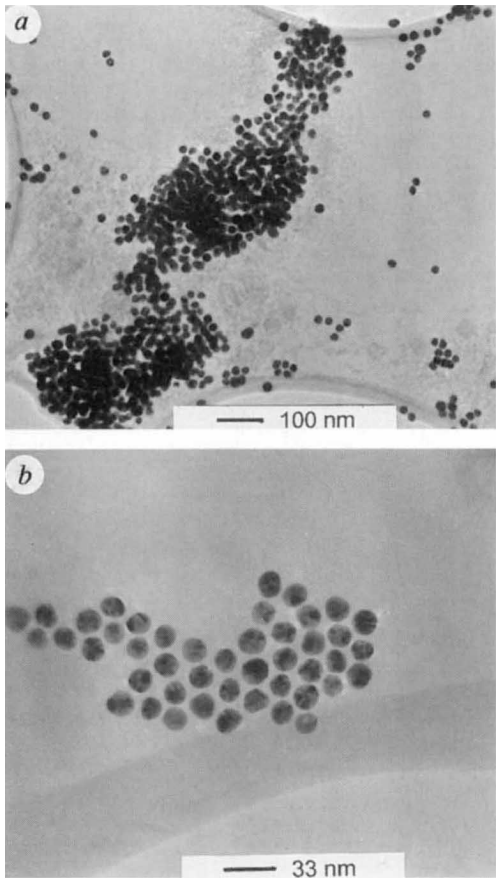

Fig. 4 TEM images of: *a*, an aggregated DNA/colloid hybrid material; *b*, a two-dimensional colloidal aggregate showing the ordering of the DNA-linked Au nanoparticles. Images were taken with a Hitachi 8100 Transmission Electron Microscope.

This work gives entry into a new class of DNA/nanoparticle hybrid materials and assemblies, which might have useful electrical, optical and structural properties that should be controllable through choice of nanoparticle size and chemical composition, and oligonucleotide sequence and length. We note that it should be possible to extend this strategy easily to other noble-metal (for example, Ag, Pt)[22] and semiconductor (for example, CdSe and CdS)[23,24] colloidal nanoparticles with well established surface coordination chemistry. Our initial results bode well for the utility of this strategy for developing new types of biosensing and sequencing schemes for DNA. The Au colloidal particles have large extinction coefficients for the bands that give rise to their colours (Fig. 2). These intense colours, which depend on particle size and concentration and interparticle distance, make these materials particularly attractive for new colorimetric sensing and sequencing strategies for DNA. □

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Organization of 'nanocrystal molecules' using DNA

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PATTERNING matter on the nanometre scale is an important objective of current materials chemistry and physics. It is driven by both the need to further miniaturize electronic components and the fact that at the nanometre scale, materials properties are strongly size-dependent and thus can be tuned sensitively[1]. In nanoscale crystals, quantum size effects and the large number of surface atoms influence the, chemical, electronic, magnetic and optical behaviour[2–4]. 'Top-down' (for example, lithographic) methods for nanoscale manipulation reach only to the upper end of the nanometre regime[5]; but whereas 'bottom-up' wet chemical techniques allow for the preparation of monodisperse, defect-free crystallites just 1–10 nm in size[6–10], ways to control the structure of nanocrystal assemblies are scarce. Here we describe a strategy for the synthesis of 'nanocrystal molecules', in which discrete numbers of gold nanocrystals are organized into spatially defined structures based on Watson-Crick base-pairing interactions. We attach single-stranded DNA oligonucleotides of defined length and sequence to individual nanocrystals, and these assemble into dimers and trimers on addition of a complementary single-stranded DNA template. We anticipate that this approach should allow the construction of more complex two- and three-dimensional assemblies.

Previous approaches towards the preparation of coupled quantum dots include co-colloids of cadmium selenide–zinc oxide (CdS–ZnO; ref. 11) and cadmium sulphide–silver iodide (CdS–AgI; ref. 12). In addition, small molecule crosslinking agents have been used to synthesize aggregates of Au (ref. 13) and cadmium sulphide linked to titanium oxide (CdS–TiO_2; ref. 14) as well as discrete dimers of cadmium selenide (CdSe; ref. 15). Finally, the collective properties of nanocrystals have been investigated using organic monolayers[16–22] and crystallization[23–26] to generate ordered arrays of inorganic quantum dots. It remains an open question whether self-assembly methods can be employed to generate complex sequences of nanocrystals.

Biological systems are characterized by remarkably complex