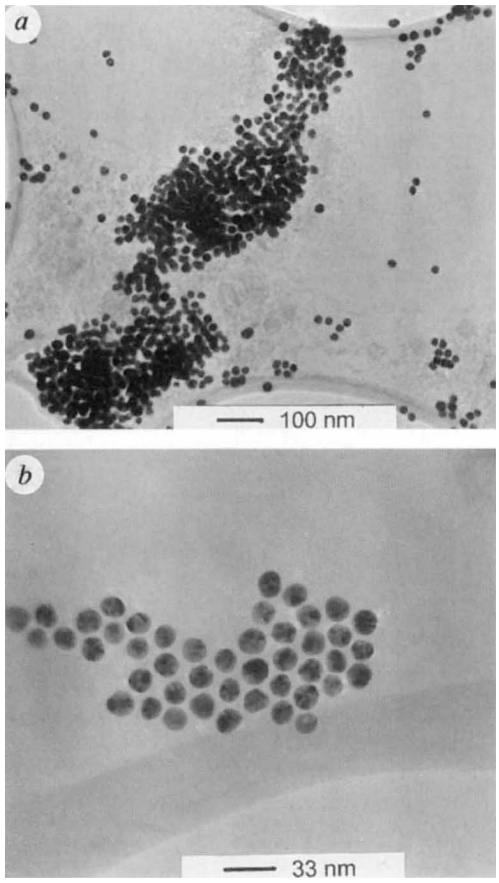

Fig. 4 TEM images of: *a*, an aggregated DNA/colloid hybrid material; *b*, a two-dimensional colloidal aggregate showing the ordering of the DNA-linked Au nanoparticles. Images were taken with a Hitachi 8100 Transmission Electron Microscope.

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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CORRESPONDENCE should be addressed to C.A.M. (e-mail: camirkin@chem.nwu.edu).

Organization of 'nanocrystal molecules' using DNA

A. Paul Alivisatos*, Kai P. Johnsson†, Xiaogang Peng*, Troy E. Wilson†, Colin J. Loweth†, Marcel P. Bruchez Jr* & Peter G. Schultz†

* Department of Chemistry, University of California at Berkeley, Berkeley, California 94720, USA and Molecular Design Institute, Lawrence Berkeley National Laboratory, Berkeley, California 94701, USA
† Howard Hughes Medical Institute, Department of Chemistry, University of California at Berkeley, Berkeley, California 94720, USA

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

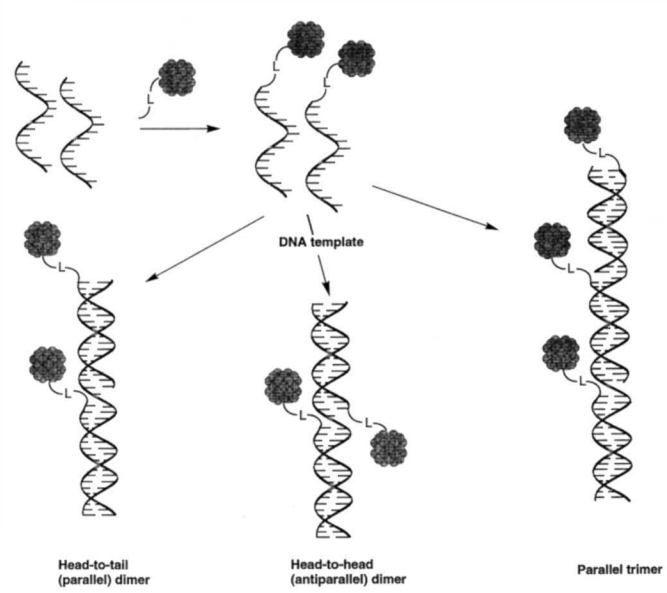

FIG. 1 Nanocrystal assembly based on Watson–Crick base-pairing interactions. Attachment of the inorganic nanocrystal (shaded) to either the 3′ or 5′ termini of the oligonucleotide 'codon' by linker L, permits the preparation of head-to-head dimers, head-to-tail dimers, and trimers, as shown schematically here and observed in the TEM images of Fig. 3.

METHODS. The 'codon' and template oligonucleotides were prepared using an Applied Biosystems Model 391 DNA Synthesizer. Sulphydryl groups were introduced at the 5′ or 3′ termini of 18-nucleotide (nt) synthetic oligonucleotides by capping with *S*-trityl-6-mercaptohexylphosphoramidite or by using 1-*O*-dimethoxytritylpropyldisulphide-1′-succinoyl support (Glen Research, Sterling, Virginia), respectively. The 5′-thiol group was detritylated using silver nitrate and dithiothreitol following the procedure of Zuckermann *et al.*[29]. Oligonucleotides thiolated at the 3′ terminus were oxidized using a 0.02 M iodine solution after each coupling step and cleaved from support with 0.05 M dithiothreitol during deprotection in concentrated NH_4OH. All oligonucleotides were purified by gel electrophoresis or reverse-phase high-performance liquid chromatography (HPLC). Concentrations were determined spectrophotometrically and by titration of free thiol using 5,5′-dithiobis(2-nitrobenzoic) acid. Thiol-modified oligonucleotides were stored at −20 °C in aqueous 50 mM dithiothreitol. Oligonucleotides were desalted on a Pharmacia Superdex 75 HR 10/30 column using 3 mM bis-Tris, 1 mM EDTA buffer, pH 6.5. Extinction coefficients were determined by combining standard extinction coefficients for the deoxynucleotide triphosphates (dNTPs). Sequences prepared for these experiments include: the 5′-thiol-terminated 18-nt oligomer (5′-HS-CAGTCAGGCAGTCAGTCA-3′) (oligo **1**); the 37-nt template (5′-TGACTGACTGCCTGACTGTTGACTGACTGCCTGACTG-3′) (template **2**); the 5′-thiol-terminated 18-nt oligomer (5′-HS-CTTGCACTAGTCCTTGAG-3′) (oligo **3**); the 3′-thiol-terminated 18-nt oligomer (5′-CAGTCAGGCAGTCAGTCA-SH-3′) (oligo **4**); the 37-nt template (5′-CTCAAGGACTAGTGCAAGTTGACTGACTGCCTGACTG-3′) (template **5**); the 56-nt template (5′-TGACTGACTGCCTGACTGTTGACTGACTGCCTGACTGTTGACTGACTGCCTGACTG-3′) (template **6**); and the unlabelled 18-nt oligomer (5′-CAGTCAGGCAGTCAGTCA-3′) (oligo **7**). Oligonucleotides, modified at either the 3′ or 5′ termini with a free sulphydryl group (1.5 μM) were coupled with a 10-fold excess of the monomaleimido–Au particles (Nanoprobes, Stony Brook, NY) in aqueous 20 mM NaH_2PO_4, 150 mM NaCl, 1 mM EDTA buffer, pH 6.5, containing 10% isopropanol at 4 °C for 24 h. The oligonucleotide–Au conjugates were then combined with the appropriate amounts of 37-nt single-stranded oligonucleotide templates and the resulting double-stranded complexes were purified from single-stranded precursors on a sizing column (Superose 12 10/30 in aqueous 5 mM NaH_2PO_4, 150 mM NaCl buffer, pH 6.5). The ratio of absorbances (A_{280}/A_{420}), determined spectrophotometrically, indicated that the complex had the desired stoichiometries of oligonucleotide and Au cluster.

structures, the assembly of which are controlled by highly selective, non-covalent interactions. For example, nucleic acids can fold into a variety of nanometre-sized structures including double helices, triplexes, knots, Holliday structures and polyhedra[27,28]. These structures are dictated by a network of specific hydrogen-bonding interactions involving the purine and pyrimidine bases. Consequently, by tagging individual nanocrystals with a DNA 'codon' (a single-stranded DNA of defined length and sequences), it should be possible to self-assemble nanocrystal molecules in two and three dimensions by specific base-pairing interactions with a designed single-stranded DNA template. Furthermore, a variety of higher-order nanocrystal molecules could be accessible by attaching nanocrystals of different sizes and compositions to unique 'codons' and assembling the 'codons' and template strands in different geometries.

To test the feasibility of this approach, we chose as initial targets the head-to-head (antiparallel) and head-to-tail (parallel) homodimers shown in Fig. 1. Gold (Au) clusters, 1.4 nm in diameter and passivated with water-soluble phosphine ligands (Nanoprobes, Stony Brook, New York), were used for these investigations. The particles contain one *N*-propylmaleimide substituent per cluster which can be selectively coupled to a sulphydryl group incorporated into the single-stranded DNA 'codon'. Oligonucleotides, modified at either the 3′ or 5′ termini with a free sulphydryl group, were coupled with an excess of monomaleimido–Au particles. The oligonucleotide–Au conjugates were then combined with appropriate mounts of 37-nucleotide single-stranded templates and the resulting double-stranded complexes were purified from single-stranded precursors on a sizing column. The retention times of the DNA–Au conjugates were significantly different from the retention times of the corresponding Au–oligonucleotide duplexes. The ratio of absorbances at

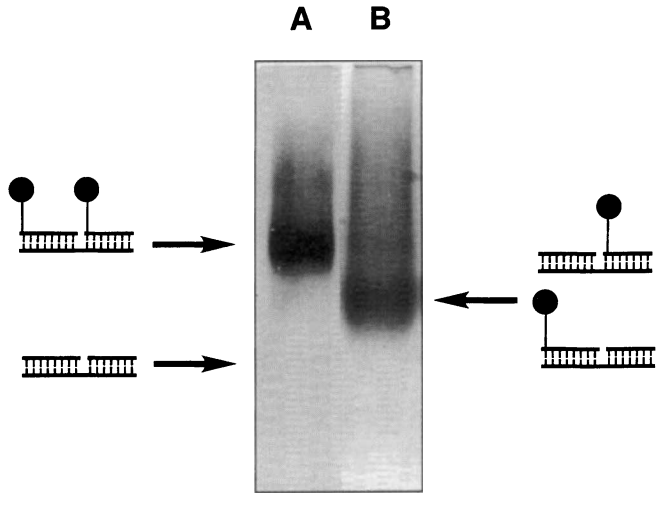

FIG. 2 Gel electrophoresis (10% acrylamide) results of DNA–Au complexes after purification by a sizing column; lane A, a 2 : 1 mixture of Au-labelled oligo **1** and the corresponding template **2**; lane B, a 1 : 1 mixture of Au-labelled oligo **1** and oligo **7** incubated with template **2**. The arrows indicate the retention times of the nucleic acid complexes containing two, one and zero Au clusters (indicated by the filled circles). Gel electrophoresis was accomplished using 10% crosslinked polyacrylamide gels[30] in 1× TBE buffer at 80 mV. The Au-labelled oligonucleotides were visualized by development with LI Silver stain (Nanoprobes, Stony Brook, NY), and the non-Au-labelled duplexes were visualized by treatment with ethidium bromide. The band representing the template **2** annealed to two oligo **7** fragments was visualized using ethidium bromide staining but not with the LI Silver stain.

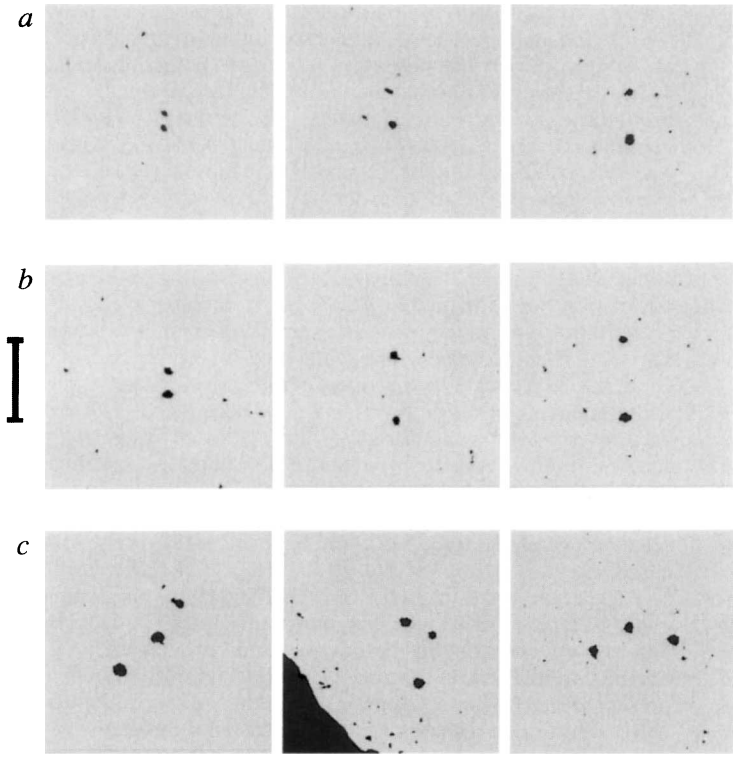

FIG. 3 Transmission electron microscopy (TEM) images of 1.4 nm Au–nucleic acid complexes. *a*, Head-to-head dimer sample in which a 2 : 1 ratio of oligo **1** was hybridized to the corresponding template **2**. From left to right are examples of the nearest, average and farthest dimers. The centre-to-centre distances range from 2.9 ± 0.6 to 10.2 ± 0.6 nm. *b*, Head-to-tail dimer sample in which a 1 : 1 ratio of oligo **3** and oligo **4** were hybridized to template **5**. From left to right are examples of the nearest, average and farthest dimers. The centre-to-centre distances in this example ranged from 2.0 ± 0.6 to 6.3 ± 0.6 nm. *c*, Equidistant trimer sample in which oligo **1** was hybridized to the trimeric template **6**. The curvature apparent in the trimer samples may reflect the flexibility of the linkers between the Au centres and the nucleic acids or bending in the DNA. Scale bar, 10 nm. The absorbances of Au–DNA samples used for imaging was approximately 5 mAU (where AU is absorbance units). A Jeol-100CX TEM instrument, operating at 80 kV, was used to image the samples which had been deposited on 400-mesh copper grids coated with ultra-thin (2–3 nm) holey carbon films. The carbon-coated grids were subjected briefly to an air plasma (30 seconds at 50 mtorr) followed by deposition of 10–15 μl of a 10 mg per ml solution of polylysine (reactive molecular mass 10–40,000, pH 7.5; Sigma Chemical, St Louis, Missouri). The grids were dried in air, solutions of the DNA–Au complexes were deposited and the excess solution was removed by wicking. Examination of a large number of nanocrystal–DNA complexes by TEM indicated that ~70% were consistent with the expected dimeric structures.

280 nm to that at 420 nm (A_{280}/A_{420}), determined, indicated that the complex had spectrophotometrically the desired stoichiometries of oligonucleotide and Au cluster. The complexes were also analysed by gel electrophoresis using ethidium bromide and silver staining to visualize the oligonucleotides and oligonucleotide–Au conjugates, respectively (Fig. 2). In the case of the dimeric complexes, individual bands were observed that were assigned to double-stranded complexes containing two, one or zero Au particles. These assignments were confirmed by competing the oligonucleotide–Au conjugate with free oligomer and analysing the pattern and intensities of the resulting bands (Fig. 2).

Transmission electron microscopy (TEM) was used to characterize the DNA–Au nanocrystal molecules. Dilute samples (20–30 nM) were imaged on ultra-thin carbon films coated with polylysine solution (10 mg per ml) to enhance binding of the oligonucleotides. Experiments using a double-stranded oligonucleotide–Au control showed that 90% of the Au particles are well separated, thereby excluding the possibility of aggregation due to TEM sample preparation. The TEM images (Fig. 3*a*, *b*) of the DNA–Au complexes were consistent with the expected dimeric structures. Modelling of the parallel and antiparallel dimers indicates that the centre-to-centre distances between the two Au particles can vary from 2.6 to 12.5 nm and 2.6 to 7.5 nm, respectively, due to the combined lengths of the linkers and the ligand shell. The distances observed under the TEM for the parallel and antiparallel dimers range from 2.9 ± 0.6 to 10.2 ± 0.6 nm and 2.0 ± 0.6 to 6.3 ± 0.6 nm, respectively. As additional evidence for our ability to chemically control nanocrystal molecules, we synthesized a trimeric parallel complex using a 56-nucleotide single-stranded DNA template (Fig. 3*c*).

These results demonstrate the potential of using oligonucleotides to self-assemble inorganic nanoparticles into aggregates that are discrete, well-defined, homogeneous and soluble. Further improvements in the methodology, particularly shorter and more rigid linkers and soluble, stable ligands for nanocrystals other than Au, are required before the full range of physical phenomena expected in coupled nanocrystal systems can be investigated. However, this approach should allow the chemical synthesis of complex mixtures of nanocrystals of differing sizes and compositions with the size of complete aggregate limited only by the degree of control over the nucleic acid template. □

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CORRESPONDENCE should be addressed to A.P.A. or P.G.S.