Programmed Assembly of DNA Functionalized Quantum Dots

Gregory P. Mitchell, Chad A. Mirkin,* and
Robert L. Letsinger
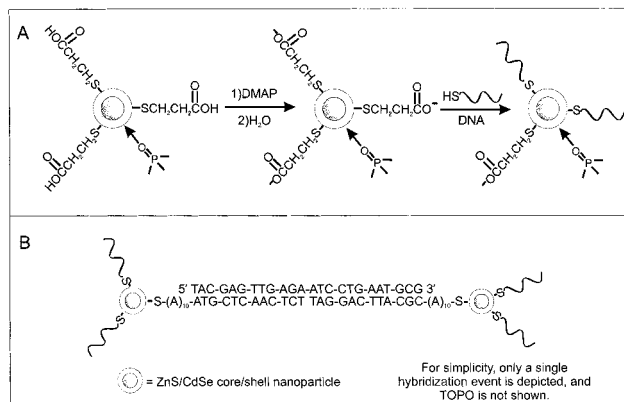
*Department of Chemistry, Northwestern University
2145 Sheridan Rd., Evanston, Illinois 60208*

Received May 19, 1999

Synthetic methodologies for semiconductor quantum dots (QDs) have improved greatly in recent years, and for some materials, most notably CdSe, monodisperse samples of predetermined size can now be prepared with relative ease.[1] As a result, the unique electronic and luminescent properties of these particles have been studied extensively,[2] potentially paving the way for QDs to be employed in diverse technologies, such as light-emitting diodes[3] and nonradioactive biological labels.[4] However, many applications will require that the particles be arranged spatially on a surface or organized into three-dimensional materials.[5] Moreover, the ability to organize one or more types of nanoparticles into ordered structures[6] would allow for the construction of completely new types of hybrid materials with new and potentially interesting and useful properties. Unfortunately, precise control over extended structure, particularly for multicomponent materials, has remained elusive.

DNA is the ideal synthon for programming the assembly of nanoscale building blocks into periodic two- and three-dimensional extended structures, and our group previously demonstrated that it can be utilized effectively to guide the construction of *macroscopic* network assemblies of Au nanoparticles.[7] These assemblies already have been utilized to develop an entirely new colorimetric detection scheme for DNA[7b] and offer promise in other areas, including catalysis,[8] Raman spectroscopy,[9] and nanoscale electronics.[3] Importantly, the many attributes of DNA, which include ease of synthesis, extraordinary binding specificity, and virtually unlimited programmability by virtue of nucleotide sequence, can be exploited for the use of QD assembly. However, modification of QDs with DNA has proven to be much more difficult than for Au nanoparticles. Citrate-stabilized gold particles are ideally suited for direct surface substitution reactions, since the citrate−Au interaction is relatively weak. Indeed, alkylthiol

---

* To whom correspondence should be addressed. E-mail: camirkin@chem.nwu.edu.

(1) Methods for preparing QDs, see: (a) Murray, C. B.; Norris, D. J.; Bawendi, M. G. *J. Am. Chem. Soc.* **1993**, *115*, 8706. (b) Hines, M. A.; Guyot-Sionnest, P. *J. Phys. Chem.* **1996**, *100*, 468.

(2) For a general report, see: (a) Alivisatos, A. P. *J. Phys. Chem.* **1996**, *100*, 13226 and references therein. Also see: (b) Klein, D. L.; Roth, R.; Kim, A. K. L.; Alivisatos, A. P.; McEuen, P. L. *Nature* **1997**, 699. (c) Kuno, M.; Lee, J. K.; Dabbousi, B. O.; Mikulec, F. V.; Bawendi, M. G. *J. Chem. Phys.* **1997**, *106*, 9869. (d) Nirmal, M.; Dabbousi, B. O.; Bawendi, M. G.; Macklin, J. J.; Trautman, J. K.; Harris, T. D.; Brus, L. E. *Nature* **1996**, *383*, 802.

(3) (a) Schlamp, M. C.; Peng, X.; Alivisatos, A. P. *J. Appl. Phys.* **1997**, *82*, 5837. (b) Dabbousi, B. O.; Bawendi, M. G.; Onitsuka, O.; Rubner, M. F. *Appl. Phys. Lett.* **1995**, *66*, 1316.

(4) (a) Bruchez, M.; Moronne, M.; Gin, P.; Weiss, S.; Alivisatos, A. P. *Science* **1998**, *281*, 2013. (b) Chan, W. C. W.; Nie, S. M. *Science* **1998**, *281*, 2016.

(5) Vossmeyer, T.; Jia, S.; Delonno, E.; Diehl, M. R.; Kim, S.-H.; Peng, X.; Alivisatos, A. P.; Heath, J. R. *J. Appl. Phys.* **1998**, *84*, 3664.

(6) Murray, C. B.; Kagan, C. R.; Bawendi, M. G. *Science* **1995**, *270*, 1335.

(7) (a) Mucic, R. C.; Storhoff, J. J.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1998**, *120*, 12674−12675 (the extinction coefficient for ∼13 nm Au nanoparticles was measured using the technique reported here). (b) Storhoff, J. J.; Elghanian, R.; Mucic, R. C.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1998**, *120*, 1959. (c) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J. *Nature* **1996**, *382*, 607. (d) Storhoff, J. J.; Mirkin, C. A. *Chem. Rev.* **1999**, *99*, 1849. (e) Storhoff, J. J.; Mucic, R. C.; Mirkin, C. A. *J. Clust. Sci.* **1997**, *8*, 179.

(8) *Clusters and Colloids*; Schmid, G., Ed.; VCH: Weinheim, 1994.

(9) Freeman, R. G.; Grabar, K. C.; Allison, K. J.; Bright, R. M.; Davis, J. A.; Guthrie, A. P.; Hommer, M. B.; Jackson, M. A.; Smith, P. C.; Walter, D. G.; Natan, M. J. *Science* **1995**, *267*, 1629.
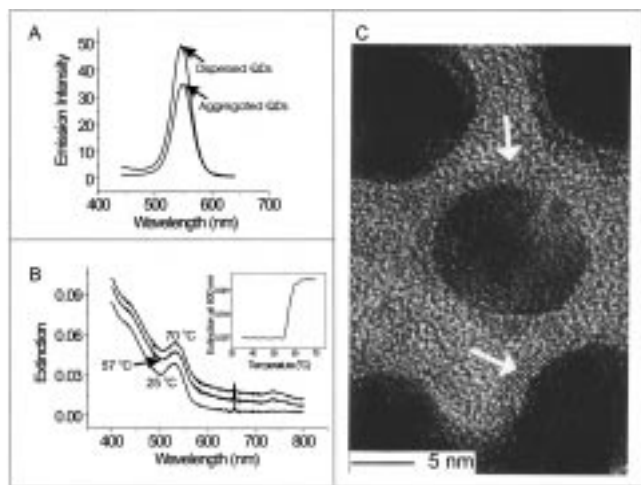
Scheme 1

For simplicity, only a single hybridization event is depicted, and TOPO is not shown.

◯ = ZnS/CdSe core/shell nanoparticle

functionalized DNA can be used to replace surface bound citrate to generate stable particles with surface bound oligonuleotides susceptible to hybridization with complementary oligonucleotides or particles. On the other hand, the common methods for preparing highly luminescent CdSe QDs yield materials that are coated with a mixture of trioctylphosphine oxide (TOPO) and trioctylphosphine (TOP). As a result, these QDs are soluble only in nonpolar solvents, making them difficult to functionalize with highly charged DNA strands by direct reaction. We have overcome this difficulty and report in this communication the first successful modification of semiconductor nanoparticles with single stranded DNA, the generation of DNA-linked QD assemblies, and a preliminary account of the optical properties of these novel structures. It should be noted that others, in elegant studies, have looked at the interactions between QDs and duplex DNA,[10] but these studies do not make use of the sequence specific binding properties of DNA to direct the assembly of extended QD structures. In other work, Alivisatos et al. have shown how gold nanoparticles can be arranged on a single oligonucleotide template.[10c]

The lack of water solubility of the QDs hinders the approach of directly substituting surface-bound TOPO with the water soluble alkylthiol-capped oligonucleotides. Therefore, we have used 3-mercaptopropionic acid to initially passivate the QD surface and act as a pH trigger for controlling water solubility and subsequent oligonucleotide surface immobilization. Other approaches have used adsorbates with carboxylic acids to covalently attach protein structures to the QDs or silica layers on QDs to increase their water solubility.[4a,b]

An excess of 3-mercaptopropionic acid (0.10 mL) reacted immediately with a suspension of ∼20 mg of TOP/TOPO stabilized CdSe/ZnS QDs in 1.0 mL of *N,N*-dimethylformamide to form propionic acid functionalized QDs.[1b] Unlike the TOP/TOPO stabilized native QDs, these particles exhibit a characteristic ν_{CO} band at 1710 cm^{-1} for the surface bound propionic acid. Although they were practically insoluble in water, their solubility could be significantly enhanced by deprotonating the surface bound mercaptopropionic acid sites with 4-(dimethylamino)-pyridine (DMAP). The QDs then dispersed readily in water, producing orange solutions that are stable for up to a week at room temperature.

Particles prepared in this manner displayed indefinite aqueous stability after they were soaked in a solution of either 3′-propylthiol- or 5′-hexylthiol-terminated 22mer DNA (1.0−2.0 ODs/mL) for 24−40 h, Scheme 1A. Moreover, the colloid

---

(10) (a) Coffer, J. L.; Bigham, S. R.; Li. X.; Pinizzotto, R. F.; Rho, Y. G.; Pirtle, R. M.; Pirtle, I. L. *Appl. Phys. Lett.* **1996**, *69*, 3851. (b) Mahtab, R.; Rogers, J. P.; Singleton, C. P.; Murphy, C. J. *J. Am. Chem. Soc.* **1996**, *118*, 7028. (c) Alivisatos, A. P.; Johnsson, K. P.; Peng, X. G.; Wilson, T. E.; Loweth, C. J.; Bruchez, M. P.; Schultz, P. G. *Nature* **1996**, *382*, 609.

Figure 1. (A) Fluorescence spectra comparing dispersed and aggregated QDs, with an excitation at 400 nm. The samples were prepared identically, except for the addition of complementary "linker" DNA to the sample denoted "aggregated" and an equal volume and concentration of noncomplementary DNA to the sample denoted "dispersed". (B) Visible spectra of QD/QD assemblies at different temperatures before, during, and after "melting". Only selected temperatures, which illustrate major changes in nanoparticle assembly dissociation, are shown. The inset displays a temperature vs extinction profile for the thermal denaturation of the assemblies. (C) HR-TEM image of a portion of a hybrid Au/QD assembly. The lattice fringes of the QDs, which resemble fingerprints, appear near each Au nanoparticle.

remained strongly fluorescent, with a sharp [full width at half-maximum (fwhm) = 33 nm], symmetrical emission at 546 nm (indicative of a ~3.2 nm CdSe core).[1a] The colloids were then separated from excess DNA by dialysis against phosphate-buffered saline (see Supporting Information for details).

Two different QD conjugates were prepared via this protocol and stored in an aqueous solution of 0.3 M NaCl, 10 mM sodium phosphate buffer (pH 7), and 0.01% sodium azide, Scheme 1A. One was modified with a 22mer, comprised of a propylthiol functionality at the 3′ end, a 12mer capture sequence, and an intervening 10 base (all A) spacer (**1**). The other employed a 5′-hexylthiol-terminated sequence, also with a 10A spacer, and a 12mer capture sequence which was noncomplementary with the 3′-propylthiol sequence (**2**).[7b] When approximately equal quantities of these solutions[11] were mixed and then combined with the complementary linking 24mer sequence (60 pmol), QD assemblies formed within 20−30 min at room temperature, Scheme 1B. Faster linking took place when the mixture was frozen and then allowed to thaw. The clusters generated were not large enough to settle out of solution, but could be separated by centrifugation at relatively low speeds (10000 rpm for 10 min), as compared with the unlinked particles (30000 rpm for 2−3 h). The hybridization was accompanied by a decrease in integrated fluorescence intensity by an average of 26 ± 6% (adjusted for changes in absorbance at the excitation wavelength of 320 nm), and a ~2 nm red shift of the emission maximum, presumably due to cooperative effects between QDs, Figure 1A. Interestingly, Bawendi et al. noticed a similar, albeit slightly larger, red shift when comparing the fluorescence of close-packed QDs and widely separated dots isolated in a frozen matrix.[6a] These changes in the fluorescence spectra may be an indication of excimer formation between QDs, but the exact nature of such a complex is still largely a matter of speculation. As expected, no aggregation was

observed when the "linker" was missing or not complementary,[12] or when either one of the two types of particles was absent. The "melting" behavior of the DNA also can be monitored by observing the electronic absorption spectra of the aggregates as a function of temperature, Figure 1B (T_m = 57 °C, when monitored at 600 nm), demonstrating conclusively that DNA has been immobilized on the QD surface and that hybridization is responsible for the assembly process. The transition also is extremely sharp when compared with DNA alone (fwhm of the respective first derivatives: 4.0 °C vs 9 °C), which is consistent with the formation of an aggregate structure with multiple DNA links per particle.[7c] An increase in extinction was observed upon denaturation, most likely because of a screening effect whereby particles in the interior of the assemblies are prevented from absorbing light by the surrounding QDs.

With DNA-functionalized QDs in hand, the construction of hybrid assemblies made from multiple types of nanoparticle building blocks now becomes feasible. When a mixture of 3′-propylthiol-terminated DNA-modified ~13 nm Au nanoparticles and 5′-hexylthiol-terminated DNA-modified QDs (or 5′ Au and 3′ QDs) is combined with the 24mer target strand and then frozen, a reddish purple precipitate forms and is visible when the solution thaws. Again, no aggregation behavior was observed unless both types of particles and a complementary target were present. High-resolution TEM images of these assemblies show a network of Au nanoparticles interconnected by multiple QDs, which can be identified by their lattice fringes, Figure 1C. These assemblies clearly exhibit an "A-B" structure, which demonstrates the role that DNA plays in forming them. The hybrid metal/semiconductor system can be studied by fluorescence, as described above, or by electronic absorption spectroscopy by following the changes in the surface plasmon resonance of the Au nanoparticles, Figure 1D. The large molar extinction coefficient associated with the plasmon band of the ~13 nm Au particles (2.8×10^8 M^{-1} cm^{-1})[7a] provides an exquisitely sensitive probe with which to monitor hybrid aggregate dehybridization. Therefore, a "melting" experiment can be performed on a sample of the hybrid material that is one tenth the concentration of the pure QD sample. Similar to the pure QD system described above, a sharp (fwhm of the first derivative = 4.5 °C) melting transition occurs at 58 °C for the hybrid system when monitored at 525 nm (λ_{max} for the dispersed gold particles).

The results described in this communication establish that the immobilization of DNA onto QD surfaces has been achieved and that these particles can now be used in combination with complementary oligonucleotides and other DNA-functionalized particles to form pure QD or mixed Au/QD nanoparticle structures. The successful modification of semiconductor QDs with DNA has significant implications for materials research, and the door is now open for more extensive inquiries into the luminescent, electronic, and chemical properties of these unique building blocks as they are incorporated into new and functional *multicomponent* nanostructured materials.

Supporting Information Available: Experimental details, UV/vis spectra of Au/QD assemblies, and fluorescence spectra of QDs before and after assembly formation (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA991662V

(11) Quantities used were as follows: 3′-propylthiol-terminated DNA modified QDs, 200 μL, optical density at 530 nm = 0.224; 5′-hexylthiol-terminated DNA-modified QDs, 200 μL, optical density at 530 nm = 0.206.

(12) The noncomplementary sequence: 5′-CTA-CTT-AGA-TCC-GAG-TGC-CCA-CAT-3′.