Communication

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J. Am. Chem. Soc., **2004**, 126 (35), 10832-10833• DOI: 10.1021/ja046747x • Publication Date (Web): 13 August 2004

J|A|C|S
COMMUNICATIONS

Published on Web 08/13/2004

Discrete Nanostructures of Quantum Dots/Au with DNA

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Assemblies of colloidal nanocrystals have received considerable attention in recent years due to their potential for producing functional materials with novel electronic, magnetic, and optical properties, which are desirable for applications in biological imaging and detection.[1] Experiments in which nanocrystals have been induced to form extended aggregates by the pairing of biological macromolecules (DNA, antibodies, etc.) have been very successfully exploited in new detection schemes.[2] If it proves possible to precisely control the number, composition, and distance of nanoparticles in a grouping, it may be possible to extend the earlier work and to create a more powerful set of biological detection schemes. However, it remains a challenge to synthesize discrete nanostructures, especially structures with a greater complexity than dimers and trimers.[3] In this communication, we demonstrate the synthesis of precise groupings of CdSe/ZnS core/shell semiconductor quantum dots (QDs) with Au nanoparticles. The structures obtained have one QD in the center and a discrete number of Au nanocrystals (one to seven) attached to it. The nanostructures are generated through hybridization of complementary DNA bound to the QD and Au and subsequent purification using gel electrophoresis.

QDs are useful biological labels because of their broad excitation spectra and their narrow and size tunable emission spectra, as well as their photostability.[4] With improved synthesis of monodisperse nanocrystal samples and bioconjugation methods to functionalize them, [5] QDs have begun to be applied in biological experiments and have shown advantages over traditional organic dyes. For example, X. Wu et al. reported multiplexed labeling to distinguish different parts of a single cell by simultaneously exciting different colored QDs;[6] D. Gerion et al. used them in room-temperature SNP human genotyping and pathogen detection;[7] and B. Dubertret et al. injected QDs into *Xenopus* embryos and followed embryonic development up to the late tadpole stage.[8] In addition to these advances, some recent work has shown that the fluorescence of QDs can be enhanced and blinking of individual dots (random intermittency of the fluorescence) may be reduced by putting QDs in the vicinity of Au surfaces;[9] this suggests that a structure consisting of a colloidal QD surrounded by Au nanoparticles may possess improved properties over QDs alone. Here, by putting Au nanoparticles around QD using DNA as the scaffolding material, we can control the distance between the Au and the QD as well as the number of Au nanocrystals around the central QD. The complexity and control reported here are considerably higher than in our previous reports of DNA-directed nanocrystal assemblies.[3] Moreover, DNA in the structures is readily manipulated and modified by a large number of enzymes,[10] which should give them further processibility. Hence, they hold great promise not only as more effective bioprobes but also for the fundamental understanding of the physical interactions between QDs and Au nanoparticles.

Au nanocrystals conjugated to one single strand of DNA were prepared and purified using gel electrophoresis. This technique was developed previously in our group and has been used to group Au particles into dimers and trimers.[3b,11] The use of Au nanoparticles containing only one DNA excludes cross-linking among particles in subsequent synthesis steps even in very concentrated solutions, thus ensuring high yields for the designed structures. The QD−DNA conjugates were prepared by direct association of biotinylated DNA (Integrated DNA Technologies, Coralville, IA) to streptavidin-coated QDots (Quantum Dot Corporation, Hayward, CA). The conjugation is very efficient because of the very high association constant between streptavidin and biotin.[12] In a typical synthesis, 26.6 pm biotin−DNA was added to 70 μL of 0.19 μM colloidal QD solution with a NaCl concentration of 100 mM and rocked for 3 or 4 h to form the QD−DNA conjugates. Then, an equimolar amount of Au−1 DNA conjugates with a concentration around 0.1 μM (the magnitude varies with each extraction from gel electrophoresis) was added to the QD−DNA conjugates. Afterward, the sample was left to rock overnight, allowing for DNA hybridization. Different groupings of nanoparticles were separated using gel electrophoresis. A typical gel image of the assemblies formed by 10 nm Au, QD 605-streptavidin, and 100mer DNA is represented in Figure 1a. The 1.6% agarose gel was run in 0.5x tris−borate−EDTA buffer at 6.7 V/cm for 1.5 h. The same gel is shown under both UV illumination (left panel) and white light illumination (right panel) to show QD and Au, respectively. Discrete bands are apparent and can be assigned to QD−DNA and Au−1 DNA conjugates, QD with one Au ($QD(Au)_1$), QD with two Au ($QD(Au)_2$), QD with three Au ($QD(Au)_3$), and so on. In general, the eye can easily differentiate seven or eight bands, but only bands correlated to structures with up to 4 Au around the central QD appear in the figure. A more detailed gel electrophoresis image is shown in Figure 1b that compares the mobility of all related samples. QD−DNA conjugates (lane 5) and Au−1 DNA conjugates (lane 7) in incubation buffer have similar mobilities in the gel. The addition of Au particles around the colloidal QDs increases the size and reduces the mobility so that the assemblies migrate more slowly in a gel (lane 3 and 4, the same sample). The more Au around the central QD, the slower the mobility. The grouping of QD and Au can only be a result of hybridization of complementary DNA, since neither the mixture of free QD and free Au (lane 1) nor the mixture of QD and Au with noncomplementary DNA (lane 2) gives discrete bands in the gel.

Figure 2a−d shows the TEM images of the first four nanostructure bands in the gel. To extract samples from the gel, the band was first cut, and then the gel slice was crushed and left at room temperature overnight in a small amount of TBE buffer. The images show one, two, three, and four Au around the central QD particle, as expected. Although the distance between QD and Au in a real structure should be roughly the same since short duplex DNA acts like a rigid rod, in the images they seem to vary because the images are two-dimensional projections of three-dimensional structures. A computer program was written to extract quantitative data from the TEM images. Figure 2e represents the statistical results for nanostructures of $QD(Au)_2$ and $QD(Au)_3$. Since Au particles have much higher contrast and are clearer in a TEM image than the QDs,

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10.1021/ja046747x CCC: $27.50

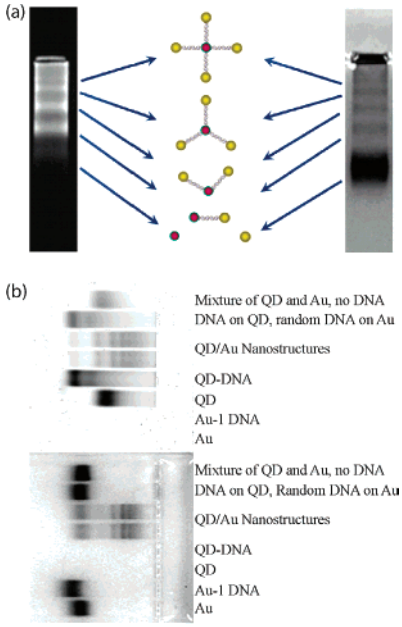

Figure 1. Gel electrophoresis migration pattern of QD/Au nonostructures. The same gel is illuminated under UV (to see QDs by fluorescence, left panel) and under white light (to represent Au through absorption, right panel). Discrete bands correspond to different numbers of Au (illustrated by cartoon) bound to the QDs through DNA hybridization. (b) Electrophoresis mobility of different samples. The top panel shows QDs. The bottom panel shows Au. Discrete bands only appear when QDs and Au have complementary DNA on them, which rules out nonspecific binding among QDs, Au, and DNA.

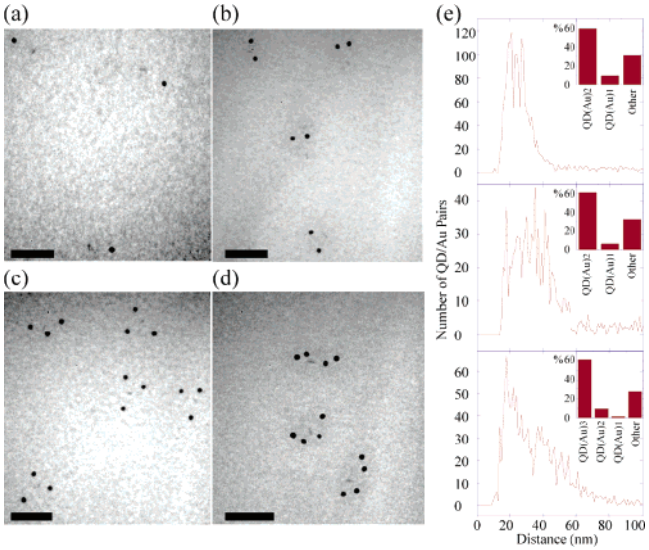

Figure 2. TEM images of discrete nanostructures of QDs/Au extracted from corresponding bands after gel electrophoresis. (a) $QD(Au)_1$. (b) $QD(Au)_2$. (c) $QD(Au)_3$. (d) $QD(Au)_4$. The scale bar is 100 nm. (e) Structure populations and pair distribution functions of $QD(Au)_2$ with 50mer DNA (top), and 100mer DNA connected $QD(Au)_2$ (middle), and $QD(Au)_3$ structures (bottom) based on quantitative analysis of the TEM image of corresponding samples.

the structure populations are calculated on the basis of Au particles in structures vs the total number of Au particles. The statistical analysis on several hundred Au nanoparticles shows that the yields for designed structures are 59.5% for $QD(Au)_2$ with 50mer DNA, 60.8% for $QD(Au)_2$, and 61.5% for $QD(Au)_3$ with 100mer DNA, as shown in the inset of Figure 2e. Pair distribution functions showing distances between all QD/Au pairs in each image for these three samples (Figure 2e) are also consistent with DNA-directed assembly. Pair distances reflect the radii of the QDs and Au nanoparticles, the thickness of coating layers, and the length of DNA linkers. Note that the maximum distances observed vary with the length of the DNA connecting the nanoparticles.

In conclusion, discrete nanostructures of QDs surrounded by different numbers of Au have been prepared through hybridization of attached DNA and purified by gel electrophoresis. Spectroscopic measurements on both ensemble and single-molecule scales are currently underway to investigate their optical properties. Rationally designed structures such as these open new possibilities for researching novel nanoparticle properties and for developing more efficient nanoprobes.

Acknowledgment. C.M.M. is grateful for a Howard Hughes Medical Institute Predoctoral Fellowship. This work was supported by NIH National Center for Research Resources through the University of California, Los Angeles, subaward agreement 0980GCD709 through the U.S. Department of Energy under Contract No. DE-AC03-76SF00098, and by DOD Advanced Research Project Agency under award No. 066995. H.Y. acknowledges financial support from the Laboratory Directed Research and Development Program through Lawrence Berkeley National Laboratory (DE-AC03-76SF00098) and from a startup fund through University of California at Berkeley.

Supporting Information Available: Close-up TEM images of discrete nanostructures better showing both QD and Au (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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JA046747X