Quantum Dots

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Quantum Dot Bioconjugation during Core–Shell Synthesis**

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Quantum dots (QDs)[1] have attracted tremendous interest in biological applications, such as bioimaging,[2,3] biolabeling,[4,5] and biosensing,[6,7] because of their advantages over organic fluorophores at high quantum yield (QY), size-tunable narrow emission, photostability, etc.[8] Many of the above applications entail water-soluble and biomolecule-conjugated QDs. To date, various strategies have been developed to obtain QD bioconjugates, either by cross-linking chemistry,[2,3,5] biotin–avidin interactions,[7,8] or ligand-exchange methods,[9,10] but these commonly require multiple steps to obtain the final products. Herein, we present a simple and robust one-step method for creating stable, water-soluble QD–biomolecule conjugates. We demonstrate the successful implementation of this strategy by using DNA molecules as a model system, but expect this could also be extended to other types of biomolecules.

This new strategy was devised so that direct capping of QDs with thiolated DNA oligonucleotides could be achieved during the formation of core–shell QDs. This in situ functionalization of QDs with DNA avoids the cross-linking chemistry or the second-step ligand exchange after core–shell formation. We demonstrate that a high DNA density on the QD surface is achieved and the high QY and stability of the QDs are preserved. Such prepared DNA-capped QDs could serve as excellent candidates for fabrication of biosensors and nanodevices.[11]

The process of QD functionalization with DNA is illustrated in Figure 1a. The starting oleylamine-capped CdSe QDs were prepared by our previously reported low-temperature synthesis method.[12–14] Coating of the CdSe core with a ZnS shell and capping of the surface with thiolated DNA oligonucleotides are achieved in a single-step one-pot reaction by mixing the oleylamine-capped QD core with Zn^{2+}, S^{2-}, and thiolated DNA all dissolved in dimethyl sulfoxide (DMSO).

We synthesized two different CdSe@ZnS core–shell QDs with the surface capped with different DNA strands (Figure 2): green QD ($\lambda_{Em} = 548$ nm) capped with a capture strand C1, denoted G-QD-C1, and red QD ($\lambda_{Em} = 617$ nm) capped with a capture strand C2, denoted R-QD-C2. These QDs are purified and finally dispersed in 0.5 × Tris/acetic acid/EDTA (TAE) Mg^{2+} buffer solution (see Experimental Section for details).

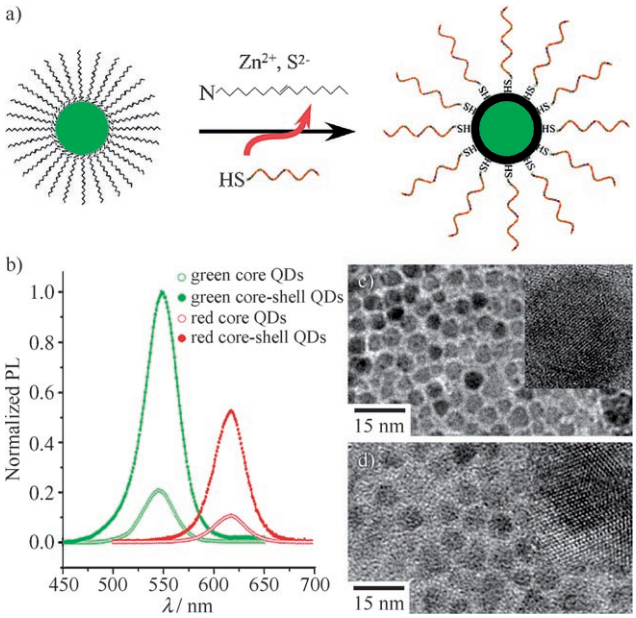

Figure 1. a) One-step in situ DNA functionalization of CdSe@ZnS core–shell QDs. b) Photoluminescence (PL) spectra of CdSe core QDs and the DNA-capped CdSe@ZnS core–shell QDs. Both the green and red QDs show a significant increase of the QY after growth of the ZnS shell and DNA capping, simultaneously. The intensities were normalized by green CdSe@ZnS core–shell QDs. c, d) TEM images of green and red core–shell QDs, respectively. Higher-magnification images of individual dots are shown in the insets.

The PL spectra of the QDs before and after the ZnS shell growth/surface capping are shown in Figure 1b. For measurement of the spectra, the oleylamine-capped CdSe QDs were dispersed in hexane solution and the obtained DNA-capped CdSe@ZnS core–shell QDs were dispersed in 0.5 × TAE Mg^{2+} buffer solution. As we demonstrated,[5,14] there were negligible spectral shifts before and after shell formation. The QYs of DNA-capped CdSe@ZnS QDs are significantly increased from those of the QD cores: 8.6 to 41.3% for the green QD and 4.4 to 21.7% for the red QD.[15] It is well-known that passivating CdSe core particles with a larger-bandgap layer of ZnS increases the QYs of QDs.[5,16,17] The significant increase in the QYs indicates that the core–shell structures are formed successfully. The excellent water solubility of the DNA-capped QDs also reveals that their surfaces are well-capped with DNA. Transmission electron microscopy (TEM) images (Figure 1c and d) show that both G-QD-C1 and R-QD-C2 have uniform sizes of (4.9 ± 0.2) and (6.9 ± 0.3) nm, respectively, and good crystalline structures (see inset images).

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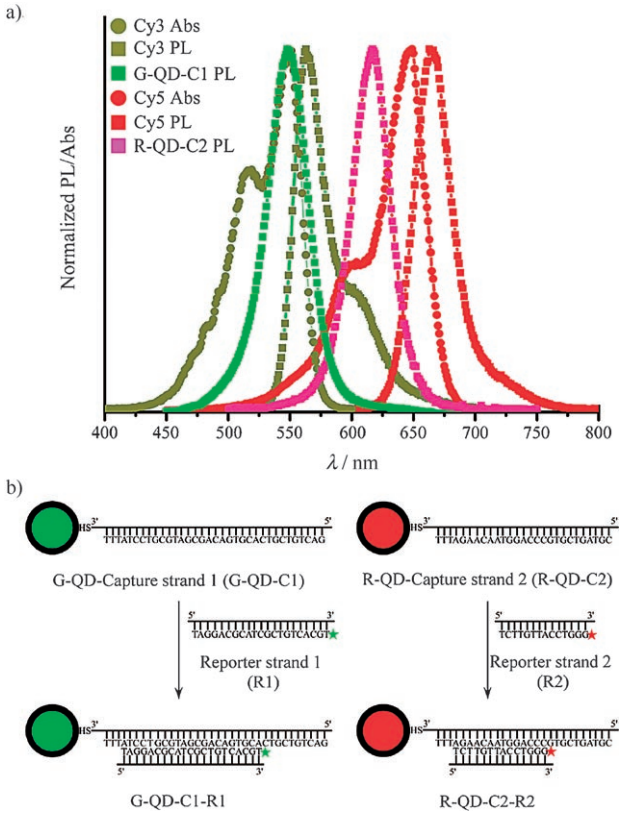

Figure 2. a) Absorbance (Abs) and emission (PL) spectra of Cy3 and Cy5, together with the emission spectra of the green and red QDs. Excellent spectral overlap of the emission of the G-QD with Cy3 and R-QD with Cy5 ensure efficient FRET between each donor–acceptor pair. b) Hybridization of the reporter strand R1 carrying Cy3 (green star) to its complementary strands on the G-QD-C1. Similarly, hybridization of reporter strand R2 carrying Cy5 (red star) to C2 capped on the R-QD-C2. To simplify, only one DNA strand is shown on each QD.

To verify that the prepared core–shell QDs were indeed capped with the thiolated DNA, fluorescent resonant energy transfer (FRET) from QDs to fluorescent dyes was investigated. We chose two dyes, Cy3 and Cy5, which have good spectral overlap with the green and red QDs, respectively (Figure 2a). Cy3 and Cy5 covalently conjugated to the 3′ end of the DNA reporter strands R1 and R2, which are complementary to the capture strands C1 and C2 on the surface of the G-QD-C1 and R-QD-C2, respectively (Figure 2b). Upon DNA hybridization between the capture probes and the reporter strands, the distances from the dyes to the center of the QDs were estimated to be 11.2 nm for Cy3 to G-QD and 9.8 nm for Cy5 to R-QD, assuming a fully extended DNA double helix.

In both QD–dye pairs, the QD acts as the donor and the dye acts as the acceptor. The distance R_0 between the donor and acceptor, at which the FRET efficiency is 50% for the two QD–dye pairs, has been calculated to be 5.97 and 5.39 nm,[6] respectively. Although the long distance between the donor and the acceptor leads to a very low FRET efficiency (ca. 2%) for a single QD–dye molecule pair, highly efficient energy transfer can be achieved by bringing multiple acceptors close to a donor.[18] If we successfully capped the QDs with the DNA strands in the one-step procedure, efficient FRET from the QDs (donor) to dyes (acceptors) could be expected upon DNA hybridization. Excitation at 365 nm was selected to minimize acceptor absorption and thus avoid the direct excitation of the acceptors.

Figure 3a and c show the PL spectra of the QD donor and dye acceptor in a titration series where increasing amounts of reporter strands were added. In all cases, raw PL spectra were

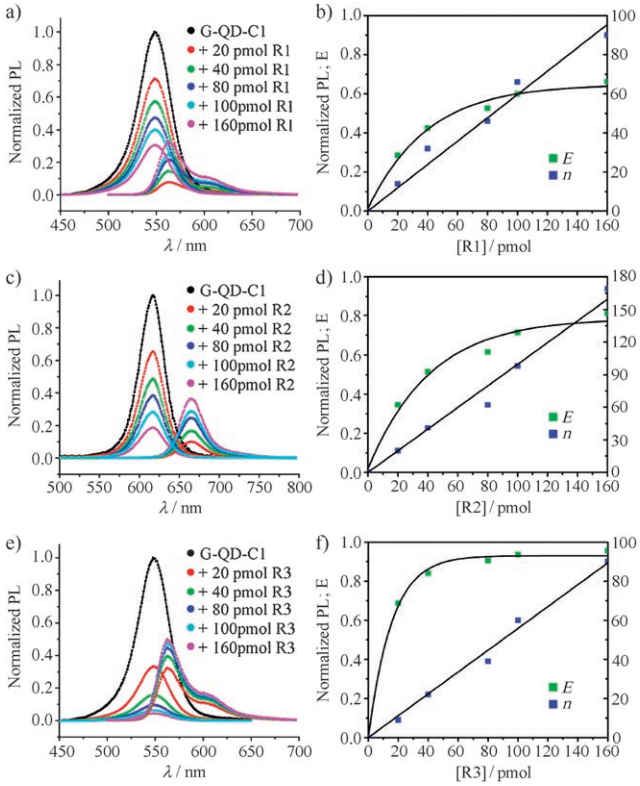

Figure 3. Evolution of the PL spectra from the donor and acceptor versus increasing acceptor concentration after deconvoluting the raw signal: a) G-QD-C1-R1, c) R-QD-C2-R2, e) G-QD-C1-R3. Experimental values for the FRET efficiency extracted from the donor PL loss (■) and the calculated number of acceptors per QD (■) versus acceptor concentration: b) G-QD-C1-R1, d) R-QD-C2-R2, f) G-QD-C1-R3.

deconvoluted to spectrally separate the signals characteristic of the QD and dye fluorophores.[6] The data clearly show that there is a progressive quenching of the QD emission and a systematic enhancement of the dye emission as the number of dye molecules surrounding the QDs increases. The FRET efficiency E, that is, the percentage drop of the QD PL intensity relative to the QD alone, and the average number n of hybridized dye molecules per QD were calculated through the Förster equation:[6] $E = nR_0^6/(r^6+nR_0^6)$.

Figures 3b and d depict that, as the amount of the reporter strand increases from 0 to 160 pmol in the solution, the FRET efficiency increases and the acceptor/QD ratio increases as well. For G-QD-C1, the number of reporter strands R1 hybridized to a single G-QD is 90 with a maximum FRET efficiency of 66%, and for R-QD-C2 the number of reporter strands R2 hybridized to a single R-QD is 169 with a

maximum FRET efficiency of 81%. The number of acceptors per QD is larger than the previously reported result, in which the QD–DNA sample was prepared through a ligand-exchange process.[19] This finding is consistent with our previous report indicating that the density of capping ligands on the QD surface obtained by the direct in situ functionalization method is much higher than that obtained by using the ligand-exchange method.[5]

The number of acceptors that can be captured per red QD is roughly twice that per green QD, which is also consistent with the fact that the calculated surface area of a red QD (ca. 150 nm^2) is double that of a green QD (ca. 75 nm^2) based on size analysis from TEM images. From this we can estimate the surface area that a DNA molecule covers on QDs as about 0.83–0.90 nm^2. This value is reasonable for the single-stranded DNA molecule, but for double-stranded DNA in the case of DNA hybridization, this DNA density may seem too crowded because the diameter of a DNA double helix is about 2 nm. However, there is a circa 2-nm spacer between the thiol end of the capture DNA to the point where DNA hybridization starts (Figure 2b), which makes the actual surface area of the QD more than doubled at the layer where DNA hybridization starts. It is therefore possible to accommodate up to 100 DNA double strands on a sphere with a radius of 5.5 nm.

From the above results, we conclude that the QDs were capped with the thiol-modified DNA probes during preparation, and that the dye-labeled DNA strands were specifically hybridized with the DNA capture probes on the QD surface. We also designed two other control experiments, shown below, to further confirm these conclusions.

On the basis of the Förster equation,[6] we know that the FRET efficiency depends on the distance between donor and acceptor. We designed a reporter strand R3 that carries Cy3 at the 5′ end such that hybridizing R3 with G-QD-C1 gives a distance between G-QD and Cy3 of about 7.5 nm, shorter than that in G-QD-C1-R1 (11.2 nm). A series of deconvoluted PL spectra of G-QD and Cy3 under different R3 concentrations is shown in Figure 3e, with the calculated FRET efficiency and dye/QD ratio in Figure 3f.

As shown in Figure 3e, with a shorter distance between the G-QD donor and Cy3 the maximum FRET efficiency increases dramatically to 95%, in comparison with ca. 66% in Figure 3a. This fits well with the Förster equation for a short donor–accepter distance. The calculated dye/QD ratio is close in the two different samples. This result further confirms that we successfully achieved DNA-capped QDs in the single-step reaction with our new strategy, and the number of DNA molecules per QD can be quantified by using FRET measurements.

The second control experiment was designed to clear the doubt that FRET might come from nonspecific binding of reporter strands to capture strands on the QD surface. We used thiolated T_{15} as the capture strand C3, and prepared G-QD-C3 and R-QD-C3 by the same strategy, in which C3 is not complementary to either reporter strand R1 (Cy3-labeled) or R2 (Cy5-labeled). The deconvoluted spectra of donor and acceptor from G-QD-C3-R1 and R-QD-C3-R2 are shown in Figure 4, and the spectra from the complementary G-QD-C1-R1 and R-QD-C2-R2 are also included for comparison.

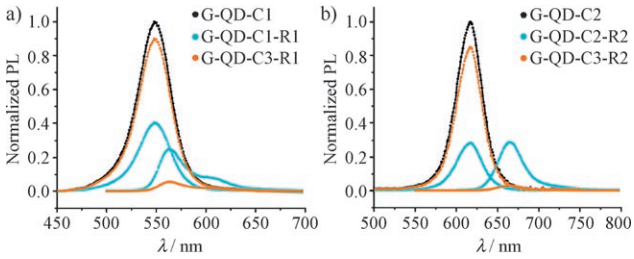

Figure 4. Evolution of PL spectra from the donor and acceptor after deconvoluting the raw signal from noncomplementary strands on the QDs. a) C3-R1 on green QD and b) C3-R2 on red QD. The complementary G-QD-C1-R1 and R-QD-C2-R2 at the same acceptor concentration (100 pmol) are also shown for comparison.

Figure 4 shows that there is a small FRET (ca. 10%) in both of the noncomplementary systems G-QD-C3-R1 and R-QD-C3-R2. This PL loss of the QDs can be explained by diffusion-driven dynamic quenching between QDs and dye acceptors that are freely moving in the solution.[20] However, in the complementary systems of G-QD-C1-R1 and R-QD-C2-R2, the observed FRET is significant with the same concentration of the reporter strands. This finding verified that the FRET in our study comes from the sequence-specific binding of DNA strands on the surface of the DNA-capped QDs.

In summary, we have described a facile strategy to achieve QD–DNA bioconjugation in a one-step reaction. DNA oligomers with a single thiol modification were used for the conjugation. We foresee that other types of biomolecules carrying a thiol or other ligands that can replace the initial core-capping molecules could be readily adapted to this strategy. For example, peptides containing cysteine residues would be a natural candidate to test, and could provide a broad range of QD bioconjugates for biomedical applications.

Experimental Section

Preparation of G-QD-C1 and R-QD-C2: Core CdSe QDs were prepared by our reported low-temperature methods.[13,14] The CdSe@ZnS core–shell QDs with thiolated DNA as the capping ligand were prepared as follows. In a typical reaction, P_2S_5 (0.01 mmol), mercaptopropyl acid (MPA; 10 μL), and butylamine (10 μL) were heated at 110°C for 20 min in DMSO (1.5 mL) in a sealed vial to dissolve the sulfide. In a separate vial, $ZnCl_2$ (0.05 mmol), MPA (10 μL), and butylamine (10 μL) were mixed in DMSO (1.5 mL) and heated to dissolve the $ZnCl_2$ in the same way. After cooling to 75°C, thiolated DNA (200 nmol, capture strand C1) in H_2O (20 μL) was added to the sulfide solution, which was kept at 75°C for 20 min. After cooling to room temperature, oleylamine-capped green-emitting CdSe core particles (1.5 mg) were dissolved in the P_2S_5 solution, which was then mixed with $ZnCl_2$ solution. The resulting mixture was heated at 70°C for 1 h in a conventional oven to yield the G-QD-C1 products. Red-emitting CdSe QDs in the P_2S_5 solution and capture strand C2 were used in the same protocol to produce R-QD-C2.

The obtained products were purified through a 100000 molecular weight cutoff (MWCO) Microcon centrifugal filter device to remove the free ligands and DMSO solvent, and then dissolved in 0.5 × TAE Mg^{2+} buffer solution (Tris, 20 mM; acetic acid, 10 mM; EDTA, 1 mM; and magnesium acetate, 6.25 mM; pH 8.0).

The sizes of the QD products were examined with a 2010F JEOL transmission electron microscope. The PL spectra were measured

with a PTI spectrofluorometer. Deconvolution of the composite signal was performed by assuming a linear superposition of PL signals with known spectral shapes, thus yielding individual spectra for the QDs and dye for each titration.

Hybridization of G-QD-C1 with R1, G-QD-C1 with R3, G-QD-C3 with R1, and R-QD-C2 with R2 and R-QD-C3-R2 was executed in 0.5 × TAE Mg^{2+} buffer solution, after sequentially annealing at 65 °C for 10 min, 45 °C for 10 min, and 37 °C for 10 min and cooling to room temperature.

The DNA strands were all obtained from www.idtdna.com, and purified by HPLC (strands modified with dyes) or 10% denaturing PAGE. The sequences of the DNA strands are listed as follows: Capture strand 1 (C1): 5′-GAC TGT CGT CAC GTG ACA GCG ATG CGT CCT ATT T-C_3-thiol (34 bases); Capture strand 2 (C2): 5′-CGT AGT CGT GCC CAG GTA ACA AGA TTT-C_3-thiol (27 bases); Capture strand 3 (C3): 5′-TTT TTT TTT TTT TTT-C_3-thiol (15 bases); Reporter strand 1 (R1): 5′-TAG GAC GCA TCG CTG TCA CGT-Cy3-3′ (a 21-base-pair plus 3T distance from the surface of G-QD to Cy3 when R1 hybridizes with C1); Reporter strand 2 (R2): 5′-TCT TGT TAC CTG GG-Cy5-3′ (a 14-base-pair plus 3T distance from the surface of R-QD to Cy5 when R2 hybridizes with C2); Reporter strand 3 (R3): 5′-Cy3-CGC TGT CAC GTG ACG ACA GTC-3′ (a 13-base single-strand distance from the surface of G-QD to Cy3 when R3 hybridizes with C1).

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