

## DNA Hybridization Detection with Blue Luminescent Quantum Dots and Dye-Labeled Single-Stranded DNA

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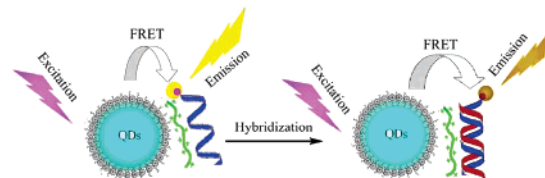
Quantum dots (QDs) have become an important photonic tool in the past two decades due to their unique properties, such as high chemical stability, resistance to photodegradation, and readily tunable optical properties.<sup>1–3</sup> QD colloids are often prepared using organometallic routes at high temperature.<sup>4–6</sup> The most widely used surface-capping ligands are trioctylphosphine/trioctylphosphine oxide (TOP/TOPO) and long-chain alkylamine. The resulting QDs are hydrophobic, and further chemical modifications are generally required for compatibility with biological applications. Since the first reports on the designs of hydrophilic QDs and QD–protein conjugates,<sup>1,2</sup> a number of surface functionalization schemes have been developed to make QDs water soluble and thus suitable for biological applications.<sup>3,7,8</sup> An alternative approach is to synthesize QDs directly in an aqueous medium. Since the original report on the aqueous synthesis of mercaptoethanol- and thioglycerol-capped CdTe QDs,<sup>9</sup> significant progress has been made in the preparation of thiol-capped CdTe QDs that exhibit very stable luminescence.<sup>10</sup>

Due to their tunable narrow-band emission and broad excitation spectra QDs are excellent donors for fluorescence resonance energy transfer (FRET)-based biosensors. Several sensor designs based on FRET between QDs and dye-labeled biomolecules have been developed.<sup>11–13</sup> For example, Mauro et al. designed a maltose-binding assay based on FRET between CdSe/ZnS QDs and dye acceptors.<sup>11</sup> A FRET TNT sensor was developed based on a hybrid QDs antibody fragment,<sup>12</sup> and Gill et al. used FRET in CdSe/ZnS–DNA conjugates to probe hybridization and DNA cleavage.<sup>13</sup> More recently, QD and FRET-based protease sensors to measure extracellular matrix metalloproteinase activity have been reported.<sup>14</sup>

Homogeneous DNA fluorescence assays are a particularly versatile way to detect hybridization, but generally, labeling of two nucleic acids or dual modification of the same strand is necessary to achieve sequence specificity. Here we report a simple sensing platform to evaluate specific hybridization based on the FRET between luminescent CdTe QDs and dye-labeled single-stranded DNA (ssDNA) probes through a cationic polymer acting as an electrostatic linker (Scheme 1). Unlike the FRET-based sensors mentioned above, in this design covalent immobilization of the probe molecules is not required, and DNA sequence specificity was achieved with minimal probe modification.

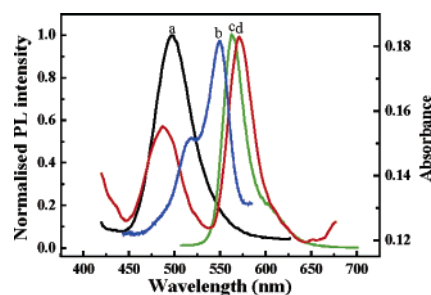
Water-soluble CdTe QDs were prepared in aqueous solution by using thioglycolic acid as the capping ligand.<sup>9</sup> After refluxing for 10 min, the resulting CdTe QD colloid was irradiated for 12 h with a 150-W xenon lamp at room temperature (see Supporting Information for details). During irradiation, the emission of CdTe QDs colloid progressively blue shifted, and the PL intensity increased due to the oxidation of Te atoms and the formation of a CdS shell. The negatively charged CdTe QDs were dissolved in a cationic polymer solution, poly(diallyldimethylammonium chloride) (PDADMAC), for 10 min to acquire positive surface charge. These positively charged CdTe QDs (CdTe<sup>+</sup>) had an emission peak at 497 nm with a fwhm of 45 nm (Figure 1).

**Scheme 1.** Principle of DNA Hybridization-Detection System Based on the QD/Cy3-labeled DNA FRET

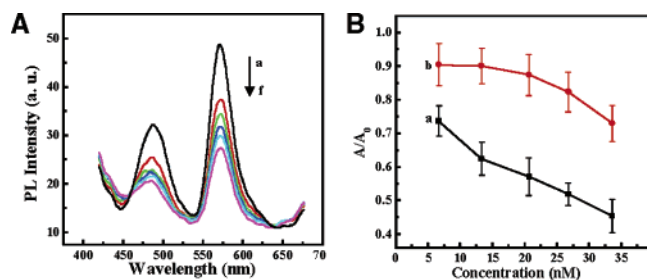


Cy3-labeled ssDNA (Cy3-DNA) was chosen as the acceptor. Although Cy3 is widely used for DNA labeling, it is rarely employed in FRET measurements with QDs because its emission peak overlaps with those of most commercially available QDs, and effective separation cannot be obtained. The absorption and emission spectra of Cy3-DNA in 100 mM NaCl and 50 mM sodium citrate (SSC buffer, pH 7.8) are shown in Figure 1. The emission of CdTe<sup>+</sup> partly overlaps with the absorption spectrum of Cy3, suggesting that efficient FRET between them can take place. There is a little overlap between CdTe<sup>+</sup> and Cy3 emission, allowing effective separation. Indeed, in a solution of CdTe<sup>+</sup> and Cy3-labeled ssDNA, excitation of CdTe<sup>+</sup> at 360 nm results in efficient FRET to Cy3 (Figure 1). (Note that there is negligible direct emission from Cy3 upon excitation at 360 nm.) There is a 10 nm red-shift in the Cy3 emission peak that can be explained by an increase in polarity in the vicinity of Cy3 due to the interaction with the cationic polymer.<sup>15,16</sup> It is interesting to note that this is accompanied by a 10-nm blue-shift of the CdTe<sup>+</sup> emission. In a control experiment, no FRET between negatively charged CdTe QDs and Cy3 was detected (see Figure 2 of Supporting information), suggesting the electrostatic interactions play a key role to ensure proximity between donor and acceptor. The calculated FRET efficiency is 92% according to  $E = 1 - F_{DA}/F_D$ , where  $F_{DA}$  and  $F_D$  are integrated fluorescence intensities of CdTe<sup>+</sup> in the presence or absence of the acceptor Cy3, respectively.<sup>17</sup>

In order to obtain high FRET efficiencies the excess of unbound cationic polymer PDADMAC in the CdTe<sup>+</sup> solution had to be minimized. An experiment was carried out by adding PDADMAC



**Figure 1.** Normalized spectra of (a) emission of CdTe<sup>+</sup> excited at 360 nm, (b) absorption of Cy3-DNA, (c) emissions of Cy3-DNA excited at 488 nm, and (d) emission of CdTe<sup>+</sup>/Cy3-DNA excited at 360 nm; All spectra were recorded in SSC buffer.



**Figure 2.** (A) Emission spectra of CdTe<sup>+</sup>/Cy3-DNA hybrid after hybridization with different concentrations of target. (a) 0 nM; (b) 6.7 nM; (c) 13.3 nM; (d) 20.2 nM; (e) 26.8 nM; (f) 33.6 nM. (B) Normalized fluorescence changes of Cy3-DNA after incubation with complementary (a) or non-complementary DNA (b) samples. A<sub>0</sub> and A are integrated areas of Cy3 before and after the incubation, respectively.

to a solution containing unmodified CdTe QDs and Cy3-DNA (see Figure 3 of Supporting information). Increasing the concentration of PDADMAC from  $5.8 \times 10^{-6}$  wt % to  $1.7 \times 10^{-5}$  wt % caused a small increase in the fluorescence intensity of Cy3, but when the concentration of PDADMAC reached  $4.1 \times 10^{-5}$  wt %, the fluorescence intensity of Cy3 began to decrease with a maximal FRET efficiency of 77%. This result implies that Cy3-DNA prefers to interact with free PDADMAC molecules in the solution. In solution, the polymer chains have a conformation of a flexible random coil, which facilitates their interaction with ssDNA through electrostatic and hydrophobic interactions.

To study how the conjugation of CdTe<sup>+</sup> and Cy3-DNA was affected by the presence of sample DNA, the hybrid of CdTe<sup>+</sup> and Cy3-DNA was incubated with different concentrations of complementary DNA at room temperature, and spectra were obtained after 30 min (Figure 2A). While the fluorescence intensity of Cy3 progressively decreased with increased concentration of complementary DNA (Figure 2A), the normalized integrated area showed a sample concentration-dependent decrease as shown in Figure 2B, indicating an increased distance between QDs and dsDNA. This may be explained by the more rigid DNA duplex structure as compared to ssDNA, which may increase the distance between polymer and dye, thus decreasing the FRET efficiency. Additionally, the increase in negative charge density due to the formation of a DNA duplex will increase the repulsive electrostatic forces between negatively charged CdTe QDs and DNA-Cy3, also resulting in larger distances and lower FRET efficiency.

After incubation with noncomplementary DNA, the fluorescence intensity of Cy3-DNA also decreases slightly (Figure 2B). This is probably due to the competition of added negatively charged noncomplementary DNA with Cy3-DNA in the CdTe<sup>+</sup>/Cy3-DNA duplex. It is clear, though, that noncomplementary DNA causes a much smaller change in the fluorescence intensity of Cy3 than corresponding concentrations of complementary DNA.

As noted above, the luminescent intensity of the CdTe<sup>+</sup>/Cy3-DNA complex generally decreased with increasing concentrations of DNA, largely independent of the general FRET behavior. In order to understand this phenomenon, the interaction between

CdTe<sup>+</sup> and ssDNA was investigated. It was found that the addition of complementary DNA caused the decrease in luminescent intensity of CdTe<sup>+</sup> in the presence of high concentration of DNA probe (see Figure 4 of Supporting information) probably due to aggregation of QDs caused by hybridization.

Longer sequences of DNAs were used to evaluate the general usefulness of this sensing platform. Generally, similar results were obtained (see Figures 5 and 6 of Supporting information).

In summary, a simple DNA-sensing platform was developed on the basis of the FRET between blue-luminescent CdTe QDs and dye-labeled ssDNA. A cationic polymer acts as an “electrostatic linker” to achieve efficient energy transfer from the QD donor to the dye acceptor. The differential interaction of single-stranded and double-stranded DNA with CdTe<sup>+</sup> results in differential changes of FRET efficiency, which is used here to recognize the hybridization event. This platform provides a homogeneous DNA assay that has all the advantages of a solution-based fluorescence detection method, but requires only minimal DNA modification.

**Acknowledgment.** We greatly thank the Royal Society of New Zealand Marsden Fund for financial support of this research.

**Supporting Information Available:** Synthesis and modification of CdTe QDs, general experimental procedure, interaction between CdTe<sup>+</sup> and ssDNA, measurement of the amount of bound Cy3-DNA, and detection of longer DNA. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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**Supporting Information for**  
**DNA Hybridization Detection with Blue Luminescent Quantum Dots**  
**and Dye-Labeled Single-Stranded DNA**

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**Materials**

Thioglycolic acid ( $\geq 98\%$ ),  $\text{CdCl}_2$  (99.99%), tellurium powder (99.997%),  $\text{NaBH}_4$  (95%), poly(diallyldimethylammonium chloride) (PDADMAC) (very low molecular weight  $<100000$ , 35 wt. % in  $\text{H}_2\text{O}$ ) and Ultrafree-MC Microcentrifuge filters (NMWL 100,000Da, PTHK polysulfone membrane) were obtained from Sigma-Aldrich. The DNAs were purchased from Alpha DNA, Canada and the sequences are as follows:

Cy3-labelled probes:

5'-GAT GAG TAT TGA TGC CGA-Cy3 (18 base);

5'-GAT GAG TAT TGA TGC CGA GAT GAG TAT TGA TGC CGA -Cy3 (36 base);

5'-GAT GAG TAT TGA TGC CGA GAT GAG TAT TGA TGC CGA GAT GAG  
TAT TGA TGC CGA-Cy3 (54 base),

plain probe:

5'-GAT GAG TAT TGA TGC CGA-3',

complementary targets:

5'-TCG GCA TCA ATA CTC ATC-3',

5'-TCG GCA TCA ATA CTC ATC TCG GCA TCA ATA CTC ATC -3',

5'-TCG GCA TCA ATA CTC ATC TCG GCA TCA ATA CTC ATC TCG GCA TCA  
ATA CTC ATC-3'

non-complementary: 5'- TAT GCT GGT GCG TCG CAC-3'

A quartz cell of 400  $\mu\text{L}$  volume was used for fluorescence measurement.

**Synthesis of CdTe QDs**

The synthesis of CdTe QDs was performed according to the reference<sup>1</sup> with some modification. First, NaHTe was prepared by adding 40 mg  $\text{NaBH}_4$  to a flask containing

46 mg tellurium powder and 2 ml Milli-Q water under nitrogen atmosphere. The reaction was kept on for several hours until all tellurium powder was dissolved.

0.092 g (0.5 mmol) of  $\text{CdCl}_2$  and 0.092 mg (1 mmol) of thioglycolic acid were dissolved in 100 mL Milli-Q water, followed by adjusting pH to 8.2 by addition of 1 M NaOH solution. The mixture was deaerated by  $\text{N}_2$  bubbling for 30 min. Then NaTeH solution (0.062 mmol) was quickly injected into the mixture under vigorous stirring, followed by refluxing the mixture for 10 min under open-air conditions.

The obtained CdTe colloid was irradiated using a 150 W xenon lamp with a water filter to attenuate the near infrared part of the spectrum. During irradiation, the PL intensity increased and the emission peak shifted towards the blue end of the spectrum, as shown in Figure 1. The irradiation promotes oxidation of unsaturated Te atoms which were identified as hole traps<sup>2</sup> and are known to be highly susceptible to oxidation<sup>3</sup>. The oxidation of Te atoms leads to a decrease in particle size which results in a blue shift of absorption and emission peaks. At the same time, the irradiation causes the decomposition of TGA with release of sulfur to form a CdS shell around the CdTe core<sup>4</sup>, resulting in the enhancement of the PL efficiency<sup>5</sup>. The formation of a CdS shell also greatly inhibits further oxidation of CdTe QDs. Note: Highly luminescent CdTe QDs colloid can also be obtained by direct irradiation without refluxing, but require longer irradiation times.

### **Modification of CdTe QDs**

200 mL of 2-propanol was added to as-prepared CdTe QDs colloid solution. CdTe QDs were precipitated from the solution and collected by centrifugation. The obtained CdTe QDs were first dried at room temperature under vacuum and then redissolved in 50 mL of PDADMAC solution (0.35 wt. %, pH 7.0) for 10 min. 150 mL of 2-propanol was added to precipitate the QDs. After centrifugation, the QDs were dissolved in 50 mL of Milli-Q water. The procedure was repeated to remove any unbounded polymer. The finally obtained positively charged CdTe QDs ( $\text{CdTe}^+$ ) were dispersed in 100 mL of SSC buffer (100 mM NaCl and 50 mM sodium citrate, pH 7.8) and used for the subsequent experiments.

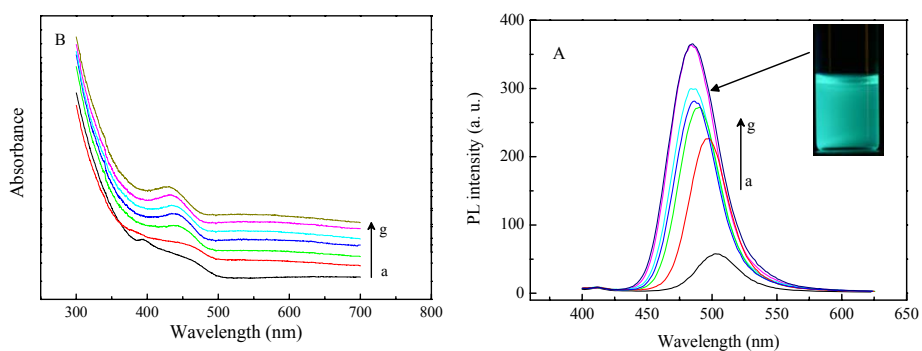
### **General Experimental Procedure**

5  $\mu\text{L}$  of Cy3-DNA (31.55  $\mu\text{M}$ ) was added to 295  $\mu\text{L}$  of SSC buffer solution containing 20  $\mu\text{L}$  of PDADMAC modified CdTe QDs and the mixture was kept for 5

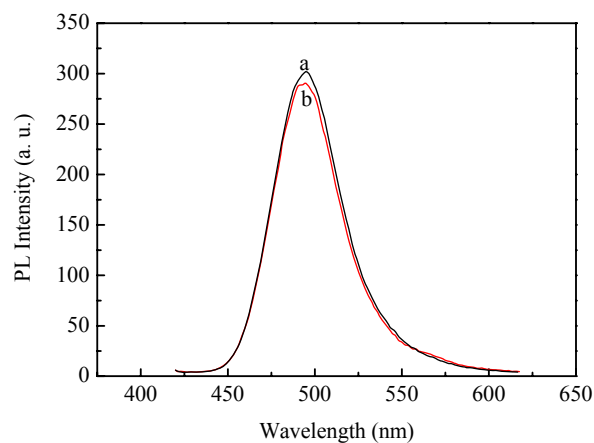
min to form Cy3-DNA/CdTe<sup>+</sup> hybrid before fluorescence measurement. For DNA sensing, 5  $\mu$ L of DNA sample solutions with different concentrations were added to the solution containing Cy3-DNA/ CdTe<sup>+</sup> hybrid prepared as described above and kept for half an hour at room temperature. SSC buffer containing 100 mM NaCl and 50 mM sodium citrate (pH7.8) was used throughout. All spectra were recorded with a PerkinElmer LS55 luminescence spectrometer at an excitation wavelength of 360 nm.

### Measurement of the Amount of Cy3-DNAs Bound with CdTe<sup>+</sup> QDs

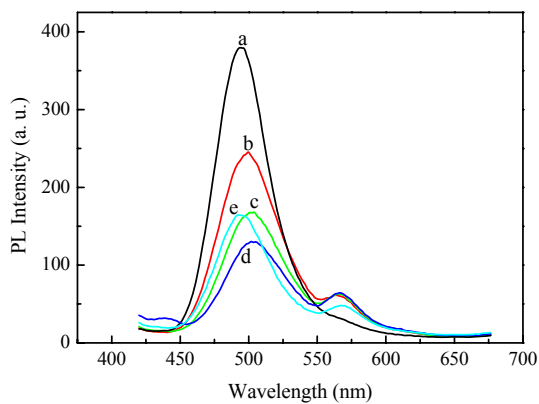
5  $\mu$ L of Cy3-DNA (31.55  $\mu$ M) was added to 295  $\mu$ L of SSC buffer solution containing 20  $\mu$ L of CdTe<sup>+</sup> QDs and the mixture was kept for 5 min. Then the mixture was transferred to an Ultrafree-MC Microcentrifuge filter (NMWL 100,000Da). Cy3-DNA/ CdTe<sup>+</sup> hybrid was separated from the solution by centrifugation at 7000 g for 8 min. The absorbance of the filtrate solution at 260 nm was measured by using a Shimadzu UV-1700 Spectrophotometer. The absorbance of 300  $\mu$ L of SSC buffer containing 5  $\mu$ L of Cy3-DNA (31.55  $\mu$ M) at 260 nm was also measured. The amount of Cy3-DNAs bound with CdTe<sup>+</sup> QDs was calculated by using the change in the absorbance. The result revealed that 0.132 nmol of Cy3-DNA (440 nM) was bound with the given amount of CdTe<sup>+</sup> QDs after adding of 5  $\mu$ L of Cy3-DNA (31.55  $\mu$ M). This also means that 84% of added Cy3-DNA was bound with CdTe<sup>+</sup> QDs.



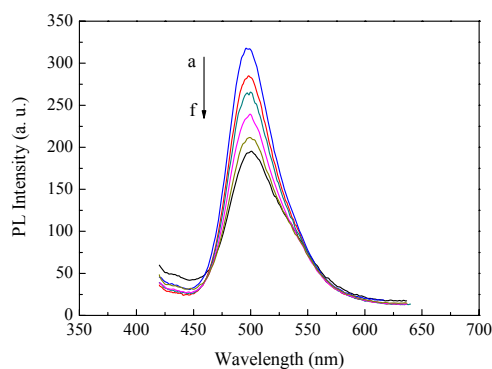
**Figure 1.** Emission spectra (A) and absorption spectra (B) of CdTe QDs colloid solutions during irradiation. (a) 0 h; (b) 2 h; (c) 6 h; (d) 8 h; (e) 10 h; (f) 12 h; (g) 14 h. Inset: photo of CdTe QDs colloid solution after irradiation of 14 h, excited at 365 nm by using a UV lamp.



**Figure 2.** Emission spectra of unmodified CdTe QDs excited at 360 nm. (a) without Cy3-DNA; (b) in the presence of Cy3-DNA ( $0.53 \mu\text{M}$  ).



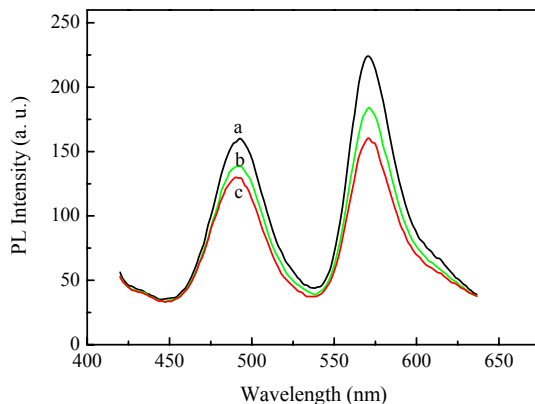
**Figure 3.** Emission spectra of the solution containing unmodified CdTe QDs and  $0.53 \mu\text{M}$  of Cy3-DNA after continuous addition of PDADMAC solution. Excitation wavelength is 360 nm. The volume of PDADMAC solution ( $3.5 \times 10^{-4}$  wt. %) added to the solution: (a)  $0 \mu\text{L}$ ; (b)  $5 \mu\text{L}$ ; (c):  $10 \mu\text{L}$ ; (d):  $15 \mu\text{L}$ ; (e)  $35 \mu\text{L}$ .



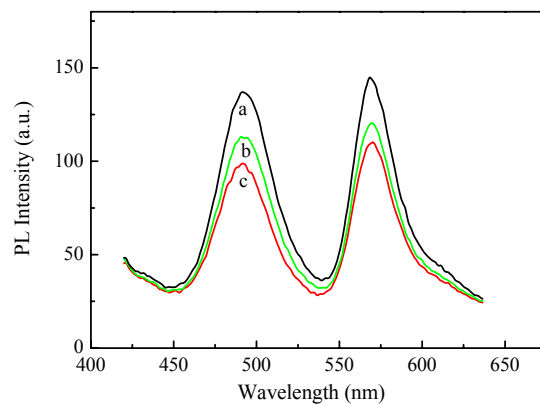
**Figure 4** Emission spectra of the solution containing  $\text{CdTe}^+$ ,  $0.528 \mu\text{M}$  probe without Cy3 labelling and different concentrations of complementary DNA. (a) 0 nM; (b) 6.7 nM; (c) 13.3 nM; (d) 20.2 nM; (e) 26.8 nM; (f) 33.6 nM.

#### Evaluation of the sensing approach for detection of longer DNA sequences

The effectiveness of the suggested DNA detection platform was additionally evaluated by using longer Cy3 labelled DNAs (36 base and 54 base) and corresponding complementary targets. Similar results to those discussed above were obtained, as shown in Figure 5 and 6. The FRET efficiencies are 83% and 79% for Cy3-DNA with 36 bases and 54 bases, respectively. The decrease of FRET efficiency with the increase of DNA length is probably due to the larger volume of longer sequences, which increases the average distance between QDs and the dye.



**Figure 5** Emission spectra of  $\text{CdTe}^+$  / Cy3-DNA (36 base) hybrid after hybridization with different concentration of corresponding target. a: 0 nM; b: 6.7 nM; c: 13.3 nM;



**Figure 6** Emission spectra of  $\text{CdTe}^+$  / Cy3-DNA (54 base) hybrid after hybridization with different concentration of corresponding target. a: 0 nM; b: 6.7 nM; c: 13.3 nM;

Reference:

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