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# Compact quantum dot probes for rapid and sensitive DNA detection using highly efficient fluorescence resonant energy transfer

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## Abstract

We developed a simple method for quickly synthesizing compact quantum dot (QD)–DNA probes for sensitive DNA detection using fluorescence resonant energy transfer (FRET). The density of DNA probes on the QD surface was controlled to avoid steric hindrance and to promote rapid hybridization with target DNA molecules. The radius of the final QDs was only around 3 nm after applying the functional coating, enabling highly efficient energy transfer. It was demonstrated that nearly 70% transfer efficiency could be achieved with only a few DNA molecules on each QD and that the FRET-based DNA detection could be carried out within 10 min with a sub-nM detection limit. Theoretical analysis was also performed to confirm our results.

 Supplementary data are available from [stacks.iop.org/Nano/20/305502](http://stacks.iop.org/Nano/20/305502)

(Some figures in this article are in colour only in the electronic version)

## 1. Introduction

Semiconductor quantum dots (QDs) represent a revolution in the application of fluorophores for biological labeling when compared to traditional dye labels, because they are photo- and chemically stable and have high quantum yield. In addition, different fluorescence wavelengths, i.e., colors, can be achieved by simply varying the QD size; and all colors of QDs can be excited by a single excitation source, which makes it much easier to excite QDs than traditional fluorescent dyes that possess relatively narrow absorption bands [1–4]. These features render QDs extremely useful for multiplexed detection with high sensitivity. Because of their excellent optical properties, QDs have quickly emerged as a new class of fluorescent sensors for biological and biomedical applications [5–8]. Detection of biomolecules such as peptides [9], proteins [10, 11], and DNA [12, 13] has recently been demonstrated using QDs.

Fluorescence resonant energy transfer (FRET) is a widely used detection method for studying the interactions between

biomolecules. When the donor and the acceptor are in close proximity, the excitation energy is transferred from the donor to the acceptor, which may be indicative of the binding of two biomolecules, to which the donor and the acceptor are respectively attached. Due to their advantageous optical and chemical properties, and the ease of surface modification, QDs have become remarkable donors for FRET [14–18]. QD-based FRET has been demonstrated in many bioconjugate systems such as maltose-binding protein (MBP)–QD conjugates [18, 19] and streptavidin–QD conjugates [12].

However, the typical Förster distance is only around 4–7 nm for QD and dye pairs [16]. In current high quality water-soluble QDs, they are capped with amphiphilic polymer [20, 21], protein [18, 19], or silica [21, 22]. After encapsulation with these materials, the hydrodynamic radius of QDs increases to approximately 10–20 nm [23–25], much greater than the Förster distance. As a result, the energy transfer efficiency between a single QD and a single dye is only a few per cent [2]. While in recent QD–DNA systems,

**Table 1.** Sample names and DNA sequences.

Sample name	Sequence
DNA-1 (probe)	NH <sub>2</sub> C <sub>12</sub> -5'-AAC CAG AGA ACC GTA-3'
DNA-2 (probe)	NH <sub>2</sub> C <sub>12</sub> -5'-GAT GAG TCC TGA GTA ACC ACT CAT AGT TTA-3'
DNA-1C (target)	Rhodamine red-3'-TTG GTC TCT CGG CAT-5'
DNA-2C (target)	TEX red-3'-CTA CTC AGG ACT CAT TGG TGA GTA TCA AAT-5'
DNA-T (negative)	NH <sub>2</sub> C <sub>12</sub> -5'-TTT TTT TTT TTT TTT-3'
DNA-T2 (negative)	NH <sub>2</sub> C <sub>12</sub> -5'-TTT TTT TTT TTT TTT TTT TTT TTT TTT-3'
DNA-NC (negative)	Rhodamine red-3'-CGT ACT CAA GAT TCG-5'

high transfer efficiency (nearly 100%) was observed, a large number (50–100) of acceptor dyes and, hence, DNA probes were required on the QDs surface [26, 27], a constraint which significantly deteriorates the sensitivity of the QDs as a FRET-based biosensor. Moreover, the density of DNA on the QD surface strongly affects the efficiency of donor/acceptor hybridization [28]. It was found that at the lowest DNA probe density, essentially 100% of probes can be hybridized, whereas at a high DNA probe density the efficiencies drop to as little as ~10% due to steric hindrance [28]. As a consequence, long hybridization time and/or annealing are needed in DNA detection [26, 29].

Very recently Zhou *et al* reported synthesis of compact QDs where the capture DNA probes were directly and covalently coupled to the QD surface [30]. EG<sub>3</sub>COOH and EG<sub>3</sub>OH mixed at a predetermined ratio were coated on the QD surface to reduce the steric hindrance. With this method the shortest distance between the center of the QDs and dye could be approximately 5 nm (assuming fully extended QD coating molecules). Significantly improved energy transfer efficiency (~48%) at a low DNA/QD ratio and rapid DNA hybridization (~10 min) at room temperature were demonstrated. However, synthesizing EG<sub>3</sub>COOH and EG<sub>3</sub>OH ligands for QD capping was time-consuming and involved many complicated steps. In addition, due to the long molecules used in the QD coating, the size of the QDs was still relatively large.

In this work, we developed a quick and convenient method to fabricate compact covalently linked QD–DNA probes and subsequently used them for sensitive FRET-based DNA detection. The density of the DNA probes on the QDs was controlled by using the mixture of dihydrolipoic acid (DHLA) and 2-mercaptoethanol that are shorter in length than EG<sub>3</sub>COOH and EG<sub>3</sub>OH, and are commercially available. The final size of functionalized QD was only 2.8 nm in radius for green QDs and 3.3 nm in radius for orange QDs, respectively, the most compact donor QDs to our knowledge. We further demonstrated rapid detection of DNA (~10 min) with energy transfer efficiency of nearly 70% at a low DNA-to-QD ratio (<10) and with a sub-nM detection limit. Theoretical analysis was also performed to confirm our results.

## 2. Experimental section

### 2.1. Materials

Triethylphosphine oxide (TOPO, 90%), trioctylphosphine (TOP, 90%), cadmium oxide (99.5% pure), selenium powder (100 mesh, 99.99%), hexamethyldisilathiane ((TMS)<sub>2</sub>S),

hexadecylamine (HDA, 90%), diethylzinc (1.0 M in hexanes), dodecanoic acid (99%), 2-mercaptoethanol (99.0%), dihydrolipoic acid (DHLA, reduced, 98%), anhydrous toluene (99.8%), sodium bicarbonate, sodium borohydride (96%), sodium dodecyl sulfate (SDS, 98.5%), phosphate buffered saline, dimethylformamide (DMF, 99.8%), high-performance liquid chromatography (HPLC) grade methanol, chloroform, SSC buffer (20× concentrate), Tween 20 and 0.2 μm syringe filters were obtained from Sigma-Aldrich (St Louis, MO). Phosphate buffer (50 mM, pH 6.5) was obtained from Fisher Scientific (Pittsburgh, PA). 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), and *N*-hydroxysulfosuccinimide (Sulfo-NHS) were obtained from Pierce (Rockford, IL). NAP-5 columns were obtained from GE Healthcare (South Burlington, VT). Hybridization buffer and Superaldehyde substrates glass slides were obtained from TeleChem International Inc. (Sunnyvale, CA).

All DNA oligonucleotides used in experiments were of HPLC grade and were obtained from IDT (Coralville, IA). The DNA sample names and the sequences are given in table 1. Briefly, DNA-1 (15-base) and DNA-2 (30-base) served as the DNA probe. The 15-base DNA-1C (labeled with rhodamine red) and the 30-base DNA-2C (labeled with TEX red) served as the target and they were complementary to DNA-1 and DNA-2, respectively. The 15-base DNA-T, DNA-NC, and 30-base DNA-T2 were used as the negative control.

### 2.2. Quantum dot synthesis

CdSe/ZnS core–shell QDs were produced by a modification of the methods described previously [31, 32]. In a typical synthesis, 30 mg of CdO was mixed with 600 mg dodecanoic acid, 4 g of 99% TOPO, and 4 g of 90% HDA; these precursors were placed in a 3-neck flask, which was flushed with argon. This mixture was then heated to ~300 °C and held at that temperature until all CdO was dissolved, and then heat was removed. 180 mg Se dissolved in 2 ml TOP was swiftly injected into the reaction flask at the temperature appropriate to make the correct size QDs (lower temperatures correspond to smaller QDs). Immediately following this injection, 10 ml of toluene was injected to stop QDs growth. The QDs were then annealed at ~150 °C for 30 min. The QDs were then cooled and precipitated with anhydrous methanol, then re-suspended in 7 g of 90% TOPO. This mixture was heated to ~80 °C and flushed with argon again before 2.5 ml ZnS was added. The system was then raised to 180 °C for 30 min to grow the shell, and then annealed for 2.5 h at 120 °C. These QDs were then collected by precipitation with methanol and stored in

chloroform. In this study, we prepared two sizes of TOPO-QDs with green and orange emission spectra, respectively.

### 2.3. Preparation of OH/COOH-capped and COOH-capped QDs

In order to avoid the potential steric hindrance problem on QD–DNA probes, the density of the functional groups on QDs, to which DNA probes would subsequently be attached, should be controlled. To achieve this, hydroxyl groups (–OH) were used to partially block the QD surface. Briefly, the TOPO capped QDs were dried by evaporation. Then 0.6 ml DHLA and 0.9 ml 2-mercaptoethanol in a molar ratio of 1:4 was mixed in 1.4 ml of methanol and subsequently added to 130 mg of these QDs. The mixture was stirred overnight at 65 °C and the resulting water-soluble QDs were precipitated by adding 3 ml of chloroform. The aliquots were centrifuged at 14 000 rpm for 5 min and the supernatant was discarded. The precipitate was re-dissolved in 1 ml methanol and precipitated with 3 ml chloroform. After the second time of centrifugation at 14 000 rpm for 5 min, the supernatant was discarded and the QDs were dissolved in 1.6 ml of deionized water and filtered through a 0.2  $\mu\text{m}$  syringe filter to obtain OH/COOH-capped QDs in clear solution.

To obtain the negative control QDs, i.e., QDs fully coated with carboxyl groups, TOPO capped QDs were dried by evaporation, then to 80 mg of the QDs, 1 ml of DHLA and 0.5 ml of methanol were added. This mixture was stirred overnight at 65 °C and the resulting water-soluble QDs were then precipitated by adding 0.5 ml of 5 M NaOH and 3 ml of DMF. The residue was washed by methanol/DMF (v:v = 1:2) and then the resulting suspension was centrifuged at 14 000 rpm for 5 min. The supernatant was discarded and the QDs were dissolved in 1 ml of deionized H<sub>2</sub>O and filtered through a 0.2  $\mu\text{m}$  syringe filter to obtain DHLA-capped QDs in clear solution.

### 2.4. Preparation of covalently conjugated QD–DNA

EDC (2.5 mg) and sulfo-NHS (1.25 mg) dissolved in 60  $\mu\text{l}$  of phosphate buffer (25 mM, pH 6.5) was mixed with OH/COOH-capped QDs ( $\sim 3 \mu\text{M}$ ) and DHLA-capped QDs ( $\sim 6 \mu\text{M}$ ), respectively. After incubating for 15 min at room temperature, 60  $\mu\text{l}$  of 50  $\mu\text{M}$  amine-modified DNA probes were added to OH/COOH-capped QDs and 120  $\mu\text{l}$  of 50  $\mu\text{M}$  amine-modified DNA probes were added to DHLA-capped QDs. After 30 min at 30 °C, unreacted EDC/sulfo-NHS and excessive DNA were removed by a NAP-5 column with 1  $\times$  PBS and the purified QD–DNA probe conjugates were stored in a 4 °C dark room.

Using the above method, in our experiment, the following samples were prepared: green OH/COOH-capped QDs with emission at 536 nm conjugated with DNA-1 and with DNA-2 (QD536–DNA-1 and QD536–DNA-2, positive control), orange OH/COOH-capped QDs with emission at 589 nm conjugated with DNA-2 (QD589–DNA-2, positive control). Green OH/COOH-capped QDs conjugated with DNA-T (QD536–DNA-T, negative control), Orange DHLA-capped QDs conjugated with DNA-2 (QD589–DNA-2, negative control).

### 2.5. Glass slide assay

20  $\mu\text{M}$  5'-amino-modified unlabeled DNA-2C (positive control) or DNA-T2 (negative control) in 1  $\times$  PBS was spotted (2  $\mu\text{l}$  per spot) onto superaldehyde substrates slides with a micropipette and incubated for 10 min in a freshly prepared 0.25% aqueous solution of NaBH<sub>4</sub>, followed by washing for 1 min with a 0.2% aqueous solution of sodium dodecyl sulfate (SDS) and 2 min with distilled water. QD536–DNA-2 was mixed with an equal volume of 2  $\times$  PBS and was spotted (2  $\mu\text{l}$  each spot) onto a DNA-2C modified glass slide (positive control) and a DNA-T2 (negative control) modified glass slide with a micropipette, which was subsequently covered with a plastic coverslip to incubate for 30 min at 45 °C. The slides were washed for 2 min with 6  $\times$  SSC containing 0.2% Tween 20, 2 min with 6  $\times$  SSC, 1 min with 2  $\times$  SSC, and 1 min with distilled water before being dried by N<sub>2</sub> gas flow and stored at 4 °C until scanning by a Olympus IX70 microscope (color filter: ET-GFP (FITC/Cy2) from Chroma, exposure time: 660 ms).

### 2.6. Spectroscopy of hybridization of donor with acceptor

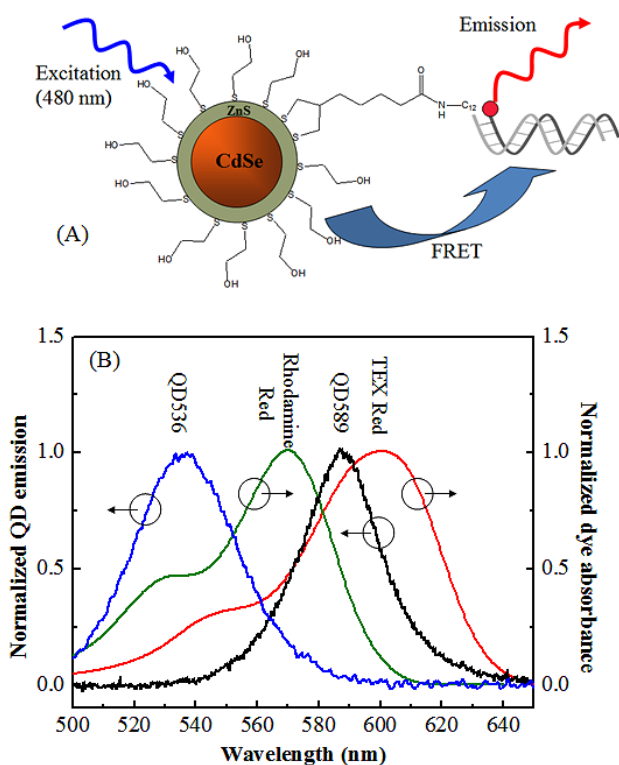
Hybridization of QD–DNA probes and dye-labeled targets took place by mixing them in an equal volume in a micro-quartz cuvette at room temperature. The total volume of the hybridization was 60  $\mu\text{l}$  and all of the hybridizations were executed in 1  $\times$  PBS buffer solution. A laser at 480 nm was used for QD excitation. The fluorescence spectra were recorded by a USB 4000 Miniature Fiber Optic spectrometer (Ocean Optics, FL) at a scan rate of 100 ms–10 s for post-analysis.

## 3. Results and discussion

The schematic of the covalently conjugated QD–DNA probes for FRET detection is illustrated in figure 1(A). The QD surface was coated with carboxyl groups and non-functional hydroxyls were used to minimize the steric hindrance problem. The DNA probes were covalently bonded to the QDs through a C<sub>12</sub> spacer. The target DNAs were labeled with dyes in a way that the dye was on the near end of the DNA strand. The normalized emission/absorbance spectra for the QD/dye pair used in experiments (QD536/rodamine red and QD589/TEX red) are presented in figure 1(B). The quantum yield, measured using rhodamine 6G in ethanol as a standard, was 15% approximately for both types of QDs. The size distribution estimated from the emission linewidth was 7.5% and 5% for QD536 and QD589, respectively.

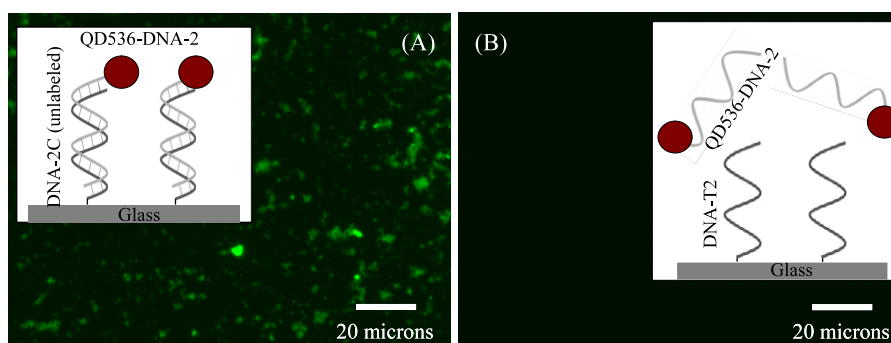
We used dynamic laser scattering (DLS) to characterize the size of the OH/COOH-capped QDs and the subsequent QD–DNA probe. It was found that the radius of QD536 and QD589 after the OH/COOH capping was approximately 2.85 nm and 3.35 nm, respectively, which was in agreement with the bare core/shell QD radius ( $\sim 1.7$  nm for Q536 and 2.3 nm for Q589) and the length of 2-mercaptoethanol and DHLA [33–35]. This is the minimum possible distance between the donor and acceptor.

After DNA probe conjugation, the QD–DNA radius became 3.78 nm for QD536–DNA-1 (15 bases) and 4.18 nm

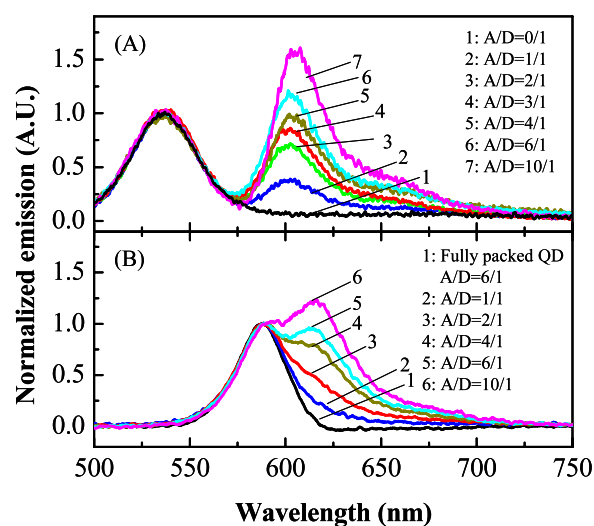


**Figure 1.** (A) Schematic of FRET between the QD donors and the dye acceptors. The QD surface is capped with the mixture of hydroxyl and carboxyl groups. DNA probes are covalently conjugated to the QDs. (B) Normalized emission and absorbance spectra for QD donors and dye acceptors used in the experiment.

for QD589–DNA-2 (30 bases), suggesting that DNA probes were successfully conjugated to the QDs, which was further verified by a glass slide assay shown in figure 2. However, considering the length of fully extended DNA-1 (–) of 6.8 nm (11.7 nm), the increase of 0.93 and 0.83 nm in the QD–DNA radius indicated that on average only 13% of the QD536 surface and 7% of the QD589 surface was covered with the DNA probes. Since functional carboxyl groups occupied 20% of the QD surface, the above results show that for short DNA probes (DNA-1, 15 bases) the majority of carboxyl groups were utilized whereas a much lower density of carboxyl groups participated in the conjugation of long DNA probes, which was in agreement with previous studies [36].

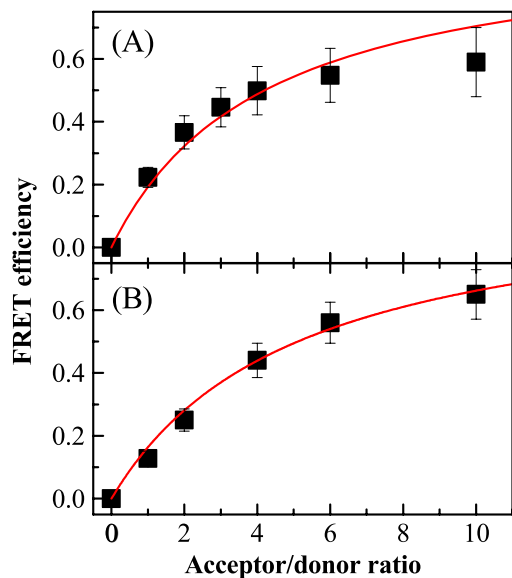


**Figure 2.** (A) Microscope image of QD536–DNA-2 hybridization with unlabeled DNA-2C immobilized on a glass slide. (B) Microscope image of the QD536–DNA-2 when the negative control (DNA-T2) was immobilized on a glass slide.



**Figure 3.** (A) Spectral evolution of the FRET between the donor (OH/COOH-capped QD536–DNA-1, concentration fixed at 100 nM) and acceptor (rhodamine red–DNA-1C) at various acceptor-to-donor ratios. (B) Curve 1: fully packed QD589–DNA-2 (100 nM) mixed with complementary TEX red–DNA-2C (600 nM). Curves 2–6: spectral evolution of the FRET between the donor (OH/COOH-capped QD589–DNA-2, concentration fixed at 100 nM) and acceptor (TEX red–DNA-2C) at various acceptor-to-donor ratios. In both (A) and (B), the emission of dye directly excited by the 480 nm laser is removed. Spectra are normalized to the peak of the corresponding QD emission. Incubation time for all curves was 30 min.

Figure 3 shows the normalized FRET spectra from OH/COOH coated QD536–DNA-1 and QD589–DNA-2 and their corresponding dye-labeled complementary target DNA. The details of how the FRET spectra were obtained are presented in supplementary data (available at [stacks.iop.org/Nano/20/305502](http://stacks.iop.org/Nano/20/305502)). With the increased acceptor-to-donor ratio the acceptor peak intensity increases, suggesting that more QD–DNA probes were hybridized with the target. As mentioned earlier, the density of the functional groups, hence the DNA probes, on the QD surface plays an important role in DNA hybridization. The OH/COOH coated QDs had low density of DNA probes on their surface, allowing for successful hybridization between probes and targets. As a control experiment, we designed and synthesized QD589 fully packed

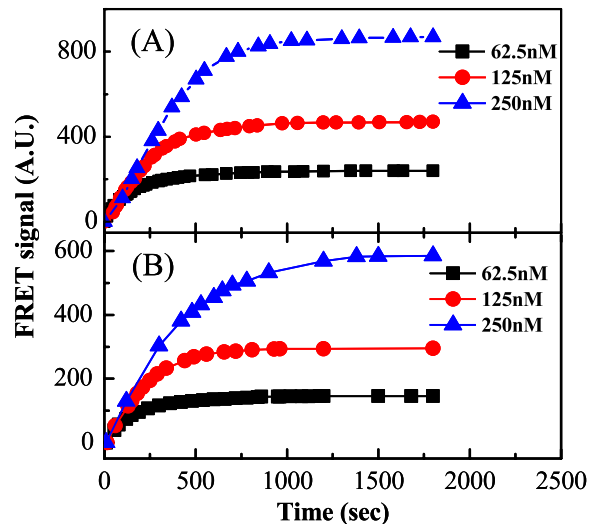


**Figure 4.** FRET efficiency as a function of the acceptor-to-donor ratio obtained from the corresponding curves in figure 3 (squares). (A) Donor: QD536-DNA-1. Acceptor: rhodamine red-DNA-1C. The solid line is the curve fit using equation (3) and  $R_0 = 3.7$  nm and  $r = 4.7$  nm. (B) Donor: QD589-DNA-2. Acceptor: TEX red-DNA-2C. The solid line is the curve fit using equation (3) and  $R_0 = 4.0$  nm and  $r = 5.2$  nm.

with DHLA (i.e., carboxyl groups), followed by coupling of 5'-C<sub>12</sub>-amine-modified 30-base DNA-2 on the QD589 surface. Curve 1 in figure 3(B) shows the FRET spectrum when TEX red-DNA-2C was added to the negative control QD589-DNA-2 probes at a 6:1 acceptor-to-donor ratio. It is nearly the same as the emission spectrum obtained with QD589 alone (see figure 1(B)), indicating that no hybridization occurred between probes and targets.

Figure 4 presents the FRET efficiency,  $E$ , using the FRET spectra in figure 3 and the equation  $E = 1 - I_D/(I_A + I_D)$ , where  $I_A$  and  $I_D$  are the integrated emission for the acceptor and donor, respectively (for details, see supplementary data (available at [stacks.iop.org/Nano/20/305502](http://stacks.iop.org/Nano/20/305502))). The calculated FRET efficiency was found to increase linearly with increased acceptor-to-donor ratio and reached 50% at approximately 4:1 ratio, meaning that on average every QD had four target DNA molecules (DNA-1C for figure 4(A) and DNA-2C for figure 4(B)) on its surface, assuming that all of the molecules were hybridized with the probes on the QD surface. 60%–70% transfer efficiency could be reached when the acceptor-to-donor ratio was 10:1, much lower than the acceptor-to-donor ratio reported in previous studies [26, 27]. Note that since the DNA linked to functionalized QD589 through a C<sub>12</sub> spacer, the distance between the DNA and the center of the QD536 and QD589 was approximately 4.7 nm and 5.2 nm, respectively, 1.85 nm larger than the smallest distance defined by OH/COOH-capped QDs. FRET efficiency can be even larger with a shorter or no spacer.

In order to estimate the number of hybridized DNAs on the QD536 (QD589) surface, we assume that a fully packed QD536 (QD589) of 4.7 nm (5.2 nm) in radius can



**Figure 5.** (A) Time dependent emission intensity at 600 nm resulting from the FRET between QD536-DNA-1 and rhodamine red-DNA-1C at an acceptor-to-donor ratio of 1:1. (B) Time dependent emission intensity at 625 nm resulting from the FRET between QD589-DNA-2 and TEX red-DNA-2C at an acceptor-to-donor ratio of 1:1.

accommodate 88 (108) double-stranded DNA molecules on its surface, considering that the size of a double-stranded DNA is about 2 nm [37]. As discussed earlier, only 13% (7%) of the surface was functional for the OH/COOH-capped QD536 (QD589), which results in a maximum of 11 (7–8) hybridized DNA molecules on the QD536 (QD589) surface on average, in agreement with the experimentally observed acceptor-to-donor ratio in figure 4, at which the FRET signal starts to saturate.

### 3.1. Calculation of Förster distance and FRET efficiency

The Förster distance,  $R_0$ , can be calculated using

$$R_0 = \left( \frac{9000(\ln 10)k_p^2 Q_D}{N_A 128\pi^5 n_D^4} I \right)^{1/6}, \quad (1)$$

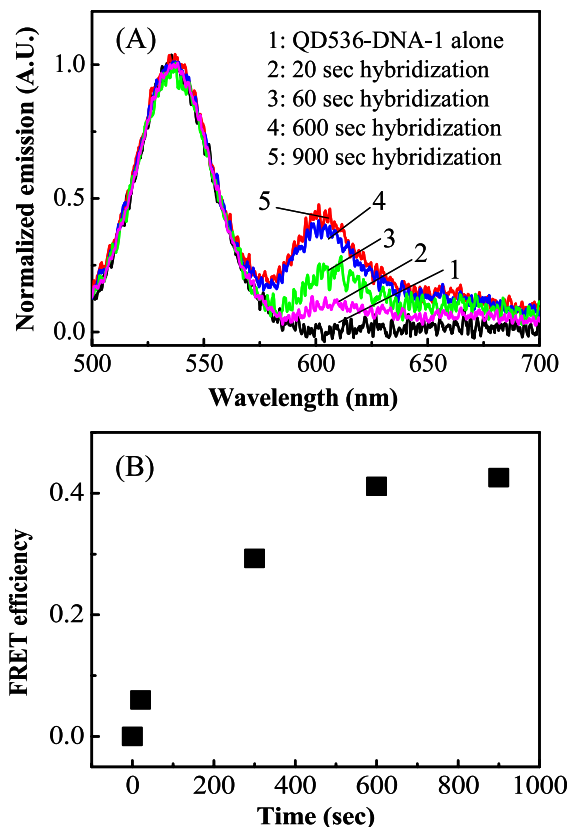
where  $k_p^2 = 2/3$  for randomly oriented dipoles,  $n_D (=1.33)$  is the buffer refractive index,  $N_A$  is Avogadro's number, and  $Q_D$  ( $\sim 15\%$ ) is the donor QD quantum yield.  $I$  is the spectral overlap between the donor emission and acceptor absorption and is defined as:

$$I = \int_0^\infty F(\lambda)A(\lambda)\lambda^4 d\lambda, \quad (2)$$

where  $F(\lambda)$  is the normalized donor emission spectrum and  $A(\lambda)$  is the acceptor extinction coefficient. Using the spectra in figure 1(B),  $R_0$  for QD536/rhodamine red and QD589/TEX red are calculated to be 4.5 nm and 5.0 nm, respectively. The energy transfer efficiency can be calculated using:

$$E = \frac{nR_0^6}{r^6 + nR_0^6}, \quad (3)$$

where  $n$  is the number of acceptor interacting with the donor and  $r$  is the distance between the acceptor and donor.



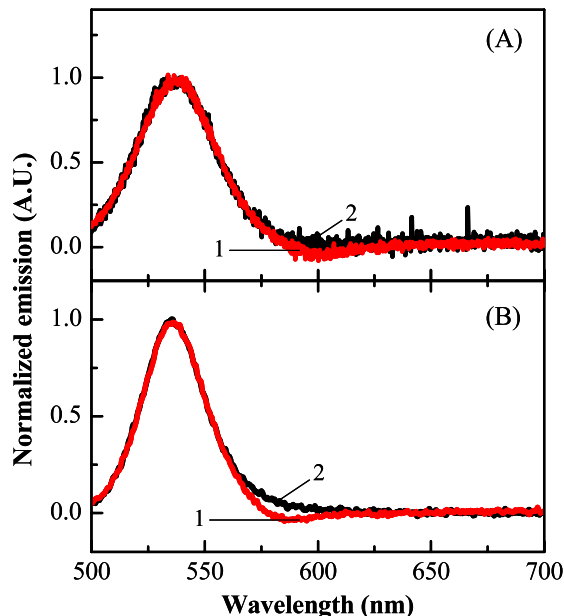
**Figure 6.** (A) Time dependent spectral evolution of the FRET between QD536–DNA-1 and rhodamine red–DNA-1C at a molar ratio of 1:3 with various incubation times. QD536 concentration was 1 nM. The emission of rhodamine red directly excited by the 480 nm laser is removed. All spectral are normalized to the QD536 emission peak. (B) The corresponding time dependent FRET efficiency.

The solid line in figure 4 plots the curve fit using equation (3) with  $R_0 = 3.7$  nm and  $r = 4.7$  nm for QD536 and  $R_0 = 4.0$  nm and  $r = 5.2$  nm for QD589. The Förster distance used here is smaller than their respective theoretically obtained values, i.e., 4.5 and 5.0 nm. This difference may reflect the fact that not all target DNA molecules were hybridized with the DNA probes on the QDs.

### 3.2. Rapid, specific, and sensitive DNA detection

Rapid, sensitive and specific DNA detection can be achieved through two mechanisms. First, OH/COOH-capped QDs were employed to obtain low density of DNA probes on the QD surface, which avoids steric hindrance and promotes rapid hybridization with target DNAs. Second, using small QDs enables high FRET efficiency even at low target DNA concentrations. Figure 5 depicts the evolution of the FRET signal with various QD–DNA probes and the corresponding targets, showing that the hybridization could be completed within 10–15 min at room temperature. Note that although in the experiment two different DNA lengths (15-base in figure 5(A) and 30-base in figure 5(B)) were used, the overall hybridization kinetics is quite similar.

To test the FRET detection limit of our QD–DNA probes, in figure 6, 3 nM target DNA (rhodamine red–DNA-1C)



**Figure 7.** Negative control experiments to evaluate non-specific binding between QD–DNA probes and the target DNA. (A) Curve 1: FRET spectrum of QD536–DNA-1 (100 nM) mixed with rhodamine red–DNA-NC (negative control, 100 nM) at an acceptor-to-donor ratio of 1:1 after 30 min incubation. Curve 2: the spectrum of QD536–DNA-1 alone. (B) Curve 1: FRET spectrum of QD536–DNA-T (negative control, 100 nM) mixed with rhodamine red–DNA-1C (100 nM) at an acceptor-to-donor ratio of 1:1 after 30 min incubation. Curve 2: the spectrum of QD536–DNA-T alone. In both (A) and (B), the emission of dye directly excited by the 480 nm laser is removed. All spectral are normalized to the corresponding QD emission peak.

could be detected within 10 min using 1 nM QD probes (QD536–DNA-1). Even at a low acceptor-to-donor ratio (3:1), approximately 45% FRET efficiency was obtained. Given such high FRET efficiency, sub-nanomolar detection is certainly possible. Using equation (3) with  $R_0 = 4.5$  nm,  $n = 3$ ,  $r = 4.7$  nm, FRET efficiency is estimated to be 69%, higher than the experimental observation. Such discrepancy is due to the fact that not all target DNA molecules were hybridized with the QD probes, leading to a lower actual acceptor-to-donor ratio on the QD surface, as discussed previously.

The QDs developed in this work exhibited high specificity towards to the target DNA and low non-specific binding. Figure 7 shows the nearly overlapped FRET spectra before and after non-complementary DNA molecules were added, indicating that no FRET occurred. The slight negative FRET signal around 590 nm where it is below the QD536 emission spectrum may be attributable to measurement errors in rhodamine red emission, which is subtracted to obtain the FRET spectrum.

## 4. Summary

We have synthesized very compact QD–DNA probes where the density of the probes on the QD surface was controlled avoid steric hindrance. The size of the functionalized QD was approximately 3 nm, which is the closest possible distance between the donor and the acceptor. Using the QDs developed

in this work, the DNA detection could be completed in 10 min with a sub-nM detection limit. Our QD–DNA probes can be used in the development of highly sensitive and specific sensors for applications in biomedical, homeland security, and life sciences.

## Acknowledgment

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