Electronic Supplementary Information

CdSe/ZnS Core Shell Quantum Dots Based FRET Binary Oligonucleotide Probes for Detection of Nucleic Acids

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Materials:

1-Ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrodrochloride (EDC) was obtained from Sigma-Aldrich. Qdot® ITK™ Carboxyl Quantum Dots (605 nm) was obtained from Invitrogen (Oregon, USA). The oligonucleotides (Cy5-DNA, amino-DNA and target DNA), whose sequences were based on sensorin mRNA, were synthesized on a DNA Synthesizer (Expedite 8909, Applied Biosystems, Oregon, USA) by standard solid-phase phosphoramidite chemistry (Glen Research) or obtained from Integrated DNA Technologies. The sequences of the oligonucleotide probes and targets were shown in Scheme 2.

Synthesis of 5'-amino labeled DNA:

The 5'-amino labeled DNA was prepared on a DNA synthesizer (Expedite) at 1μmole synthesis scale and purified by using a oligonucleotide purification cartridge (OPC) followed by HPLC. The purified product was identified by MALDI TOF mass spectrometry.

Synthesis of Cy5-DNA conjugates:

The purified 5'-amino labeledDNA was dissolved in 1ml of 0.1 M $NaHCO_3/Na_2CO_3$ buffer (pH=8-9), and 3 mg of Cy5-NHS was dissolved in 500 µL DMF. The two solutions were mixed and stirred overnight in the dark at room temperature. The mixture was purified using OPC to collect the corresponding fraction, which was further purified by HPLC. Cy5-DNA was identified by MALDI TOF mass spectrometry.

HPLC conditions for purification:

Waters HPLC (Delta 600) with reverse-phase column (150 X 4.6 mm Xterra C18 column, Waters), mobile phase: A, 8.6 mM triethylamine/100 mM hexafluoroisopropyl alcohol in water (pH=8.1); B, methanol. Elution was performed with 100% A isocratic over 10 min, followed by a linear gradient of 0-50% B for 20mins and then B isocratic over another 20 min.

Synthesis of QD-DNA conjugates:

The carboxylic groups on the surface of QD (Qdot® ITK™ 605) were activated with EDC and allowed to react with 3'-amino labeled DNA. 6.4 pmol of QD was mixed with 2.08 nmol of EDC and 0.256 nmol of 3'amino-labled DNA in 85 µL of H_2O. The mixture was allowed to react for 3 h at room temperature. Excess DNA and unreacted EDC were immediately removed from the carboxylic QD-DNA conjugate by spin filtration with Amicon Ultra 30,000 MWCO spin filters (Millipore). The reaction mixture was spun at 14,000 rpm for 1 min and the retentate was suspended in 400 µL water after the flow-through was discarded. The final product was suspended in water and assessed by Agilent 2100 Bianalyzer (Agilent).

Figure S1 shows gel electrophoresis traces of free QD (before attaching DNA), free DNA which was used for preparation of QD-DNA conjugates, and the synthesized QD-DNA conjugate. All three fractions showed distinct bands. As expected, QD-DNA showed a band with reduced mobility compared to free DNA.

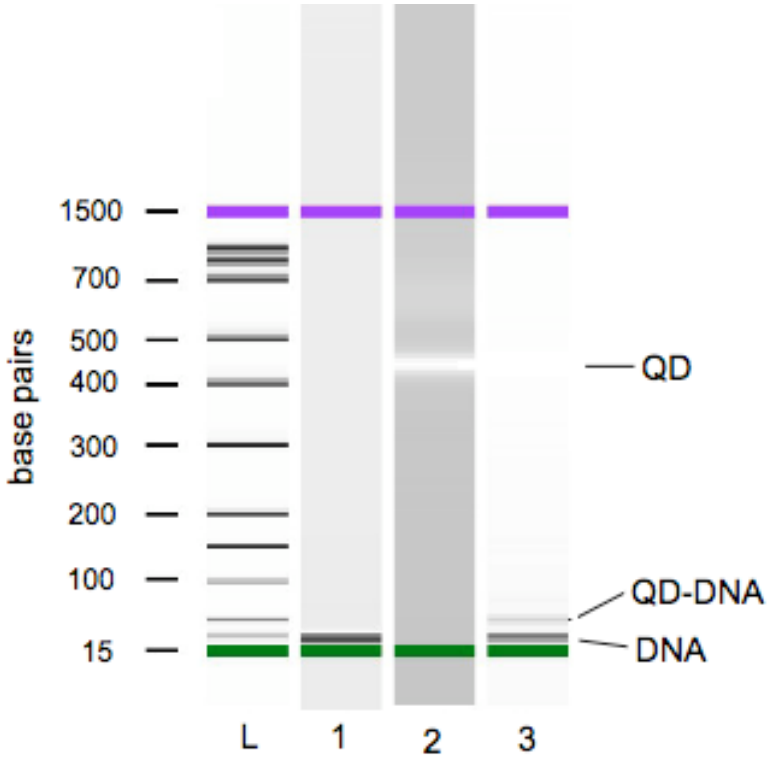

Fig. S1 Gel electrophoresis of DNA used for synthesis to attach to QDs (1), free QD (Qdot® ITK™ Carboxyl Quantum Dots 605) (2) and QD-DNA (3).

The optimum ratio of Cy5-DNA to QD-DNA in binary probes was found to be 25 : 1, which is based on fluorescence titration experiments with varying ratios of QD-DNA to Cy5-DNA in the presence of target DNA. This suggests that in the order of 25 DNA strands are attached to one QD particle. However, it can not be excluded that small amounts of free DNA, which is not covalently linked to QD, is present in the QD-DNA sample.

Fluorescence measurements:

Steady-state fluorescence was recorded at room temperature on SPEX Fluorolog-3 spectrometer FL3-22 (Horiba Jobin Yvon Inc., Edison, New Jersey) using quartz cuvettes with path length of 4x4 mm. Time-resolved experiments were performed on an OB920 single-photo counting spectrometer (Edinburgh Analytical Instruments, U.K.) with a PicoQuant 460 nm pulsed LED or 659 nm diode laser as excitation source.

Förster distance between QD605 (donor) and Cy5 acceptor:

The Förster distance (R_0) is defined as the donor – acceptor distance with 50% energy transfer efficiency and is calculated from eqn S1.

$$R_0 = (BQ_D I)^{1/6} = \left(\frac{9000(\ln 10)k_p^{\,2}Q_D}{N_A 128\pi^5 n_D^{\,4}} I\right)^{1/6} \qquad \text{(S1)}$$

Where Q_D is the fluorescence quantum yield of the donor (QD605) in the absence of acceptor, Q_D = 0.55. I is the integral of the spectra overlap between acceptor (Cy5) absorption and donor (QD) fluorescence (Fig. S2). N_A is Avogadro's number. $k_p^{\,2}$ is a factor describing the relative orientation of the transition dipoles of donor and acceptor. We use $k_p^{\,2}$ = 2/3 for randomly oriented dipoles. n_D is the refractive index of the aqueous buffer solution (1.4).

The spectral integration and calculations were performed using FRETView, a web-based program (N. Stevens, J. Dyer, A. A. Marti, M. Solomon and N. J. Turro, FRETView: a computer program to simplify the process of obtaining fluorescence energy transfer parameters, *Photochem. Photobiol. Sci.,* 2007, **6**, 909-911). The estimated Förster distance in our FRET system is R_0 = 6.9 nm.

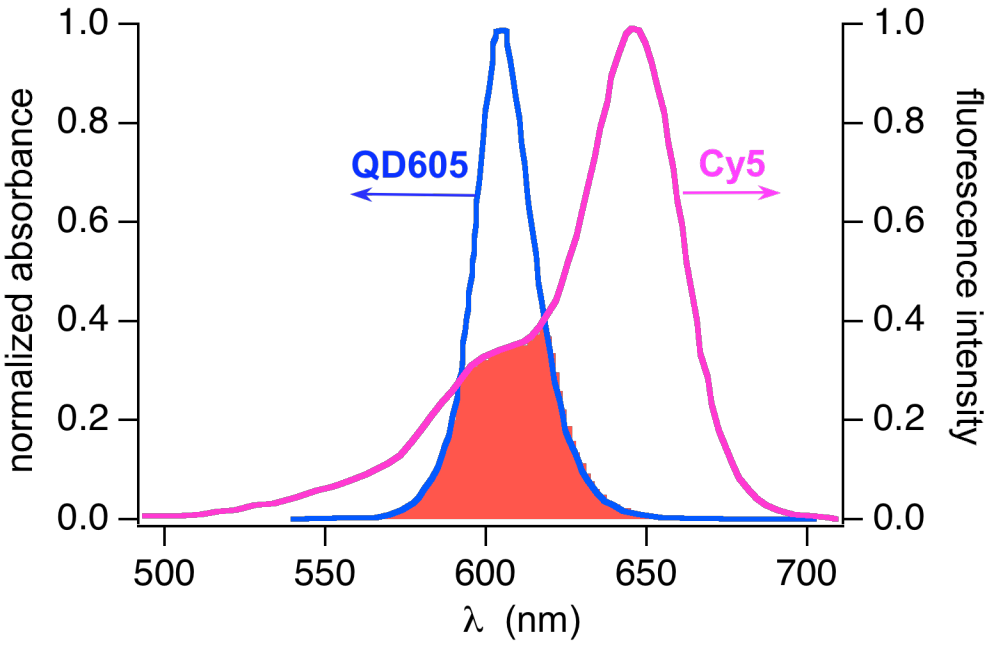

Fig. S2 Normalized donor absorbance (blue) and acceptor fluorescence (pink). The red area represents the spectral overlap. Molar absorptivity of the acceptor (Cy5) at the maximum: 250,000 $M^{-1}cm^{-1}$ (647 nm).

Hybridization of QD-DNA and Cy5-DNA with different targets:

The hybridization of target DNA with the QD-DNA conjugate and Cy5-DNA was carried out in Tris buffer (10 mM Tris-HCl, 100 mM NaCl, 1 μM EDTA, pH 7.4). 2.4 pmol of QD was used for QD-DNA conjugate preparation according to the method above, 0.28 nmol Cy5-DNA was added, a fluorescence spectrum was taken. Then 0.04 nmol target DNA was added for the hybridization. The total volume of the hybridization reaction solution was kept constant at 300 μL. 0.04 nmol of three different targets with different base distances were studied under identical condition. The hybridization reaction was carried out at room temperature for 5h before the fluorescence spectrum was taken.

Hybridization kinetics of QD-DNA and Cy5-DNA with target DNA:

For kinetic studies, the required amount QD-DNA conjugate and Cy5-DNA solution in Tris buffer was prepared and transferred to a fluorescence cuvette, and a fluorescence spectrum was taken. 0.04 nmol target DNA was then added and quickly mixed with a micropipette. Steady-state fluorescence spectra were then recorded after different reaction times. Figure S2 shows the time-dependent spectral evolution of the FRET between QD-DNA and Cy5-DNA with hybridization time. When the probes were free in solution, mainly emission from the QD was observed. In the present of target, QD and Cy5 were bought into close proximity, a condition favorable for FRET. Gradually, QD emission at 605 nm decreased and Cy5 emission at 670 nm increased through FRET as the hybridization was clearly observed. However, a complete hybridization process required about 5 h at which point no higher Cy5 emission signal was produced. The long hybridization time maybe due to the high density DNA conjugate on the QD.

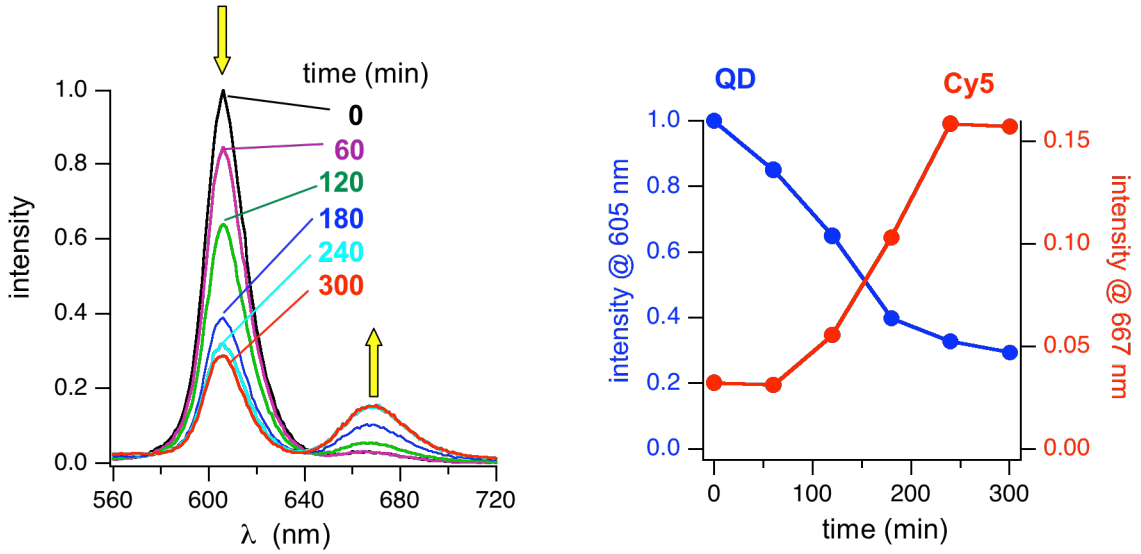

Fig. S3 The time-dependent spectral evolution of the FRET between QD-DNA (0.2 μM) and Cy5-DNA (5 μM) with hybridization time with target T2 (5 μM). Left: fluorescence spectra with excitation at 460 nm. Right: fluorescence intensities of QD at 605 nm (blue) and Cy5 at 667nm (red).

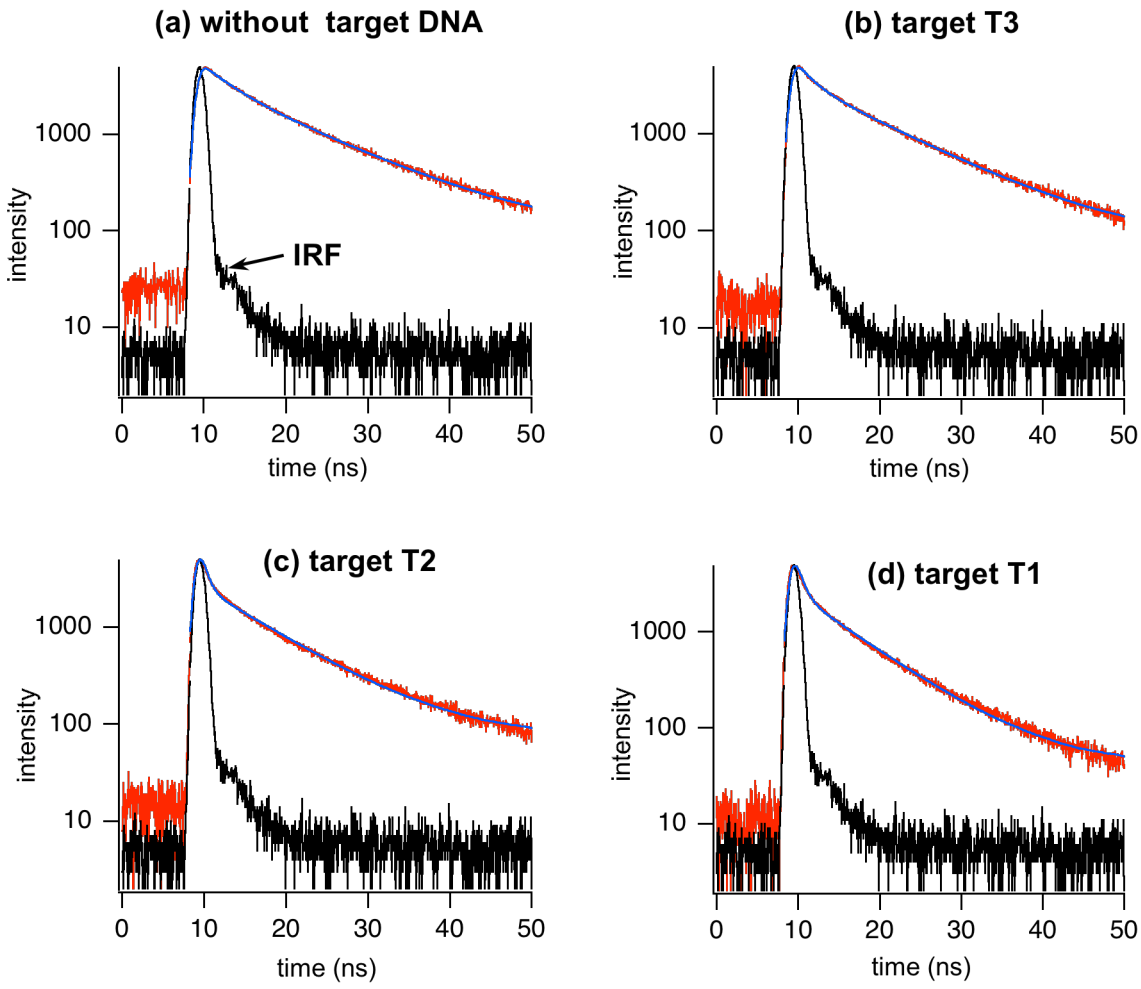

Fig. S4 Decay traces (red lines) of Cy5 fluorescence (monitored at 605 nm) after excitation with light pulses (460 nm) of aqueous buffer solutions of QD-DNA (0.2 μM) and Cy5-DNA (5 μM) in the absence (a) and presence (b-d) of target DNA (5 μM). IRF (black lines): instruments response function. Biexponential fits (blue lines) of decay traces.

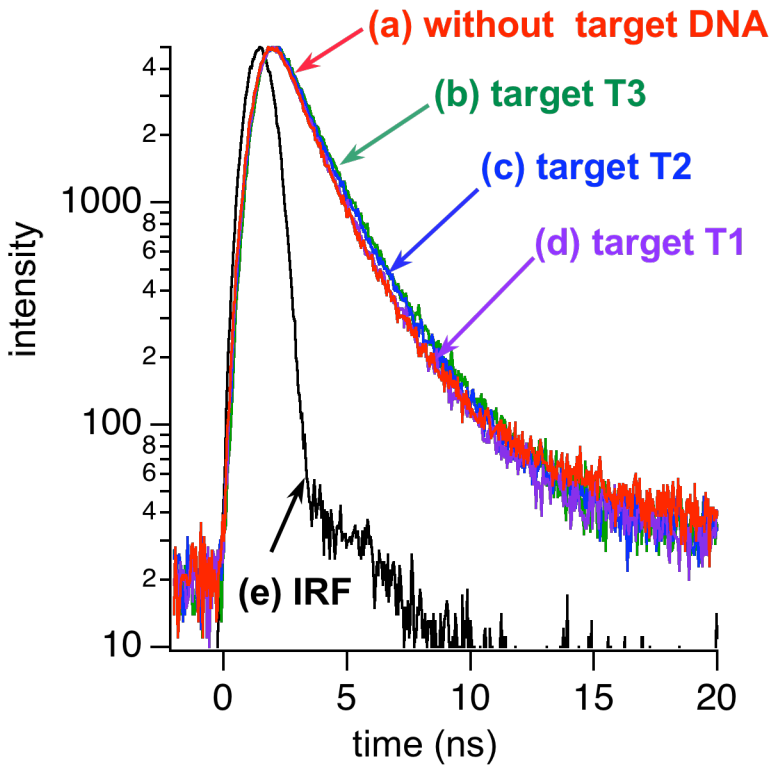

Fig. S5 Decay traces of Cy5 fluorescence (monitored at 667 nm) after excitation with light pulses (460 nm) of aqueous buffer solutions of QD-DNA (0.2 μM) and Cy5-DNA (5 μM) in the absence (a) and presence (b-d) of target DNA (5 μM). IRF: instruments response function (e).

The decay traces in the presence of target (b-d) show a slight delay compared to the fluorescence of Cy5 in the absence of target. This small delay is consistent with rapid energy transfer from OD to Cy5. However, the expected delay in fluorescence rise time of Cy5 in the presence of target due to energy transfer can not be resolved under our experimental conditions because of the relatively slow instruments response function (e). In addition, the small fraction of fluorescence generated from direct excitation of Cy5 at 460 nm causes further complications in the rise time kinetics.