Supporting Information

Fluorescence Resonance Energy Transfer between a Quantum Dot Donor and a Dye Acceptor Attached to a DNA

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Experimental Details

Materials

3-Mercaptopropionic acid (99%, MPA), tetramethylammonium hydroxide, 1 M Tris·HCl buffer (pH 7.6), high purity NaCl (DNAnase and RNAnase free), methanol, chloroform, and other chemicals and reagents were purchased from Sigma-Aldrich (Dorset, UK) and used as received unless otherwise stated. Toluene solution of trioctylphosphine oxide (TOPO) capped CdSe/ZnS core/shell QD (TOPO-QD, peak emission ~553 nm) was purchased from Evident Technologies (New York, USA). The TOPO-QD, soluble in nonpolar solvents, was rendered water-soluble by ligand exchange with MPA following a literature procedure.[1-2] Briefly, to a 0.5 mL toluene solution of the TOPO-QD (5 mg/mL) was added 2 mL of chloroform, into which 2 mL of MPA in methanol (30 mM, pH adjusted to ~10 by tetramethylammonium hydroxide) was added dropwisely. The mixture was stirred for 30 min at room temperature, then 5 mL water was added. After shaking, most of the QDs were transferred into the aqueous layer, suggesting the successful formation of MPA capped QD (MPA-QD). The aqueous layer was separated from the organic layer. The obtained MPA-QD was either directly coupled to thiolated DNA or coupled to the DNA after removal of the free MPA ligand.

Removal of free MPA from MPA-QD

The volume of aqueous phase obtained above was reduced to ~ 0.2 mL under reduced pressure on a rotary evaporator, and then 5 mL ethanol was added to precipitate the MPA-

QD. The resulting solution was centrifuged at 14,000 rpm for 20 min. The clear solution on the top (non-fluorescent when irradiated with a UV light) was discarded, and a red pellet at the bottom of the centrifuge tube was collected. The pellet was washed, sonicated and centrifuged at 14,000 rpm for 20 min twice to remove the excess of unbound MPA (MPA is soluble in ethanol). The resulting MPA-QD pellet was finally dissolved in MilliQ water, sonicated, and then stored at 4 °C. The MPA-QD is quite stable under such storage conditions, with no observable changes in fluorescence property for 3 months.

Double HPLC purified single-stranded (ss) DNAs were purchased from IBA GmbH (Göttingen, Germany). Hybridised double-stranded (ds) DNA were prepared by heating a mixed solution of the two complementary strands in 1:1 molar ratio in Tris buffer (10 mM Tris.HCl, 100 mM NaCl, pH 7.6) to 90º, then slowly cooled down to room temperature over 2 hrs.[3] The sequence of the duplex DNA is as follows:

HSC_6H_{12}-5′-CAT AAA AGA GCT CCA TAT CCA ACC TGC ACG-3′
3′-GTA TTT **T**CT CGA GGT ATA GGT TGG ACG TGC-5′

where the base ***T*** shown as a red letter is labelled with an Alexa 594 fluorophore.

Preparation of QD-DNA conjugate

DNA was conjugated to the QD following a literature procedure.[4] Briefly, to a solution of MPA-capped QD (50 μL, 6.8 μM) was added a certain amount of the duplex DNA to obtain preset DNA to QD molar ratios. After thorough mixing, the solution was stored at 4 ºC in a fridge for 2 days. A calculated amount of 10 × Tris buffer (0.1 M Tris·HCl, 1M NaCl, pH 7.6) was then added to obtain a final buffer concentration of 1 × Tris buffer (10 mM Tris·HCl, 0.1M NaCl, pH 7.6). The solution was mixed and stored at 4 ºC for a further 2 days to ensure the efficient conjugation of the DNA to the QD. Then 1 mL ethanol was added to each sample to precipitate the QD-DNA conjugates. The resulting solution was centrifuged at 14,000 rpm for 20 min, and the clear solution that was colourless and non-fluorescent when irradiated with a UV lamp was discarded. This suggested that neither DNA nor QD was removed from the sample, and the conjugation efficiency of the DNA to the QD must close to unity. Then 50 μL 1 × Tris buffer was added to each sample to dissolve the pellet. After sonication, 1 mL of ethanol was added and the solution was further centrifuged at 14,000 rpm for 20 min. The pellet obtained was dissolved in 1 × Tris buffer to a desired concentration for measurement.

UV-Vis spectra

The absorption spectra of the QD-DNA conjugates were measured on a Cary 300 Bio UV-vis spectrophotometer (Varian Inc., CA, USA). A spectral range of 360-800 nm was

recorded at a scan rate 600 nm/min at a slit width of 2 nm. The spectral background was corrected with blank 1 × Tris buffer using the same cuvette.

Fluorescence spectra

All fluorescence spectra were recorded on an Aminco-Bowman Series 2 Luminescence spectrometer (Sim-Aminco Spectronic Instruments Inc, Rochester, NY). The emission spectra (500 – 800 nm range) were recorded under a fixed excitation wavelength of 450 nm at a scan rate of 2 nm/s. An excitation and emission bandwidth of 4 nm was used. For QD-DNA conjugates, where the DNA was labeled with the Alexa 594 fluorophore, the fluorescence spectra was corrected from direct excitation of the dye by using a dye-labelled DNA as reference. Quantum yield of the QD was measured by using Rhodamine-6G in ethanol (95% under 480 nm excitation) as reference. The optical densities of the QD and Rhodamine-6G solutions used were 0.05 at 480 nm. Since the fluorescence quantum yield of QD in the QD-DNA conjugates is dependent on the DNA to QD ratio, the approximate FRET efficiency was estimated using $E = I_A/(I_A + I_D)$ rather than using donor quenching method, where I_D and I_A are donor and acceptor fluorescence intensities respectively.

Atomic force microscopy

Freshly prepared ultra-flat template stripped gold (TSG) surfaces were used as the substrates.[5] Firstly, a positively charged self-assembled monolayer of 6-mercaptohexyl-*N*-pyridinium bromide (MHP·Br) was coated onto the TSG surface, ensuring the effective immobilisation of the negatively charged QD-DNA conjugates. Then 5 μL of the QD-DNA conjugates was applied to the TSG surface, and left for 1 hr. The surfaces were thoroughly rinsed with MilliQ water, and blown dry with N_2 before images were taken.

All AFM experiments were carried out on a Digital Instrument (Veeco, Santa Babara, CA) Dimension 3100 AFM with a Nanoscope IV controller in tapping mode at 24 ± 1 ºC at a scan rate of 0.8-1 Hz. Ultra-sharp MikroMasch silicon cantilevers (NSC15 series, 125 μm long, tip radius < 10 nm, spring constant ~ 40 N/m, resonant frequency ~300 KHz) were used. Topographic images were taken under light tapping just enough to obtain a stable image to minimize the effect of tip compression of the features. All images were captured in 512 × 512 pixels per image and analyzed with the Nanoscope image analyzing software (version 5.12r3) using first order flattening.[5]

Single-molecule FRET measurements

A home-built dual-channel confocal fluorescence microscope was used to detect freely-diffusing single molecules.[6,7] The QD donor was excited by an Argon ion laser (Model 35LAP321-230, Melles Griot) with 20 μW at 488 nm. The donor and acceptor fluorescence were collected simultaneously as the QD-DNA conjugates diffuse through the laser focus, giving coincident green and red fluorescence bursts, through an oil-immersion objective (Apochromat ×60, NA1.45, Nikon), separated by a dichroic mirror (585DRLP, Omega Optical), filtered by longpass and bandpass filters (510ALP and 545AF75 for the donor channel, 560ALP and 645AF75 for the acceptor channel, all filters are purchased from Omega Optical) and detected separately by two photon-counting modules (SPCM-AQR14, Perkin Elmer). The outputs of the two detectors were recorded by two computer-implemented multi-channel scalar cards (MCS-PCI, EG&G). Sample solutions of 1 nM QDs in DNA/QD conjugates were used to achieve single-molecule detection. This concentration is much higher than what is typically needed because most QDs in DNA/QD conjugates are in dark states (the quantum yield of the MPA-QD is ~ 15%). All samples were diluted in a Tris buffer (10 mM Tris, 100 mM NaCl, pH 7.6) containing 0.01% Tween 20 to reduce surface absorption. A threshold of 50 counts per ms bin for the sum of the donor and acceptor fluorescence signals was used to differentiate single molecule bursts from the background. Background from buffer solution, direct excitation and signal crosstalk from donor to the acceptor were measured using appropriate corresponding control samples and they were subtracted from fluorescence burst. FRET efficiencies, E, of each burst were calculated according to $E = n_A/(n_A + \gamma n_D)$, where n_A and n_D are the acceptor and donor counts respectively. $\gamma = (\phi_A\eta_A)/(\phi_D\eta_D)$ is a factor accounting for the difference in the quantum yields of the donor and acceptor, ϕ_A and ϕ_D, and detection efficiencies, η_A and η_D, for the acceptor and donor channels, respectively. It has been found that the ratio of detection efficiency $\eta_A/\eta_D = 1$ and the quantum yield of quantum dot ϕ_D varies with the DNA/QD ratio in the bulk measurement. Therefore, we assume $\gamma = 1$ and only approximate FRET efficiency can be obtained.

References

(1) Wuister, S. F.; Swart, I.; van Driel, F.; Hickey, S. G.; Donega, C. D. *Nano Lett.* 2003, *3*, 503.
(2) Zhou, D. J.; Bruckbauer, A.; Abell, C.; Klenerman, D.; Kang, D.-J. *Adv. Mat.* 2005, *17*, 1243.
(3) Zhou, D. J.; Sinniah, K.; Abell, C.; Rayment, T. *Langmuir* 2002, *18*, 8278.
(4) Mitchell, G. P.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* 1999, *121*, 8122.
(5) Zhou, D. J.; Bruckbauer, A.; Ying, L. M.; Abell, C.; Klenerman, D. *Nano Lett.* 2003, *3*, 1510.
(6) Ying, L. M.; Green, J. J.; Li, H. T.; Klenerman, D.; Balasubramanian, S. *Proc. Natl. Acda. Sci. U.S.A.* 2003, *100*, 14629.
(7) Li, H. T., Zhou, D. J., Browne, H., Balasubramanian, S.; Klenerman, D. *Anal. Chem. 2004*, *76*, 4446.

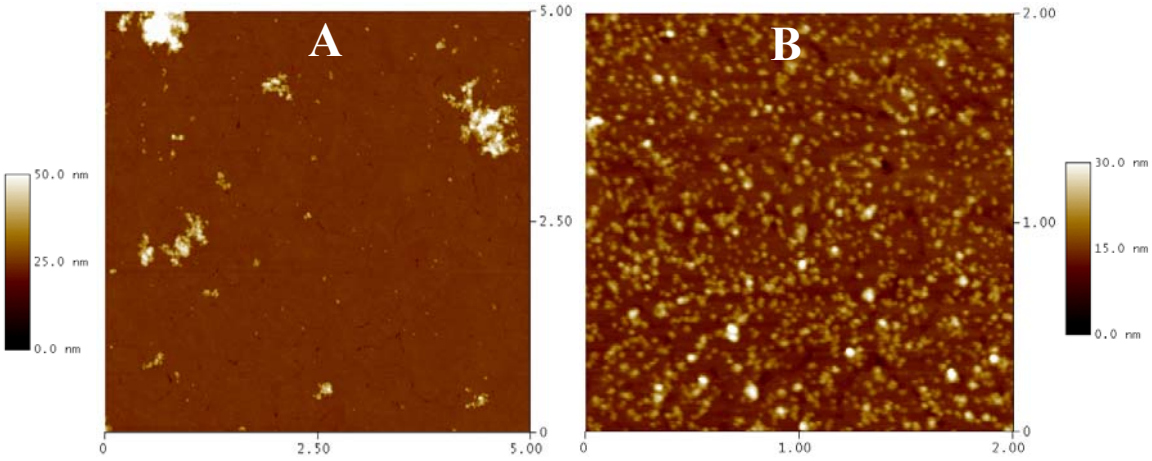

Figure S1. Tapping mode AFM topographic images of the QD-DNA conjugates at DNA:QD ratios of 0.25 (**A**, left scale bar) and 2 (**B**, right scale bar). The image sizes are 5 × 5 μm^2 for **A** and 2 × 2 μm^2 for **B**. The samples were deposited on freshly prepared ultra-flat template stripped gold surface modified with a self-assembled monolayer 6-mercaptohexyl-*N*-pyridinium.

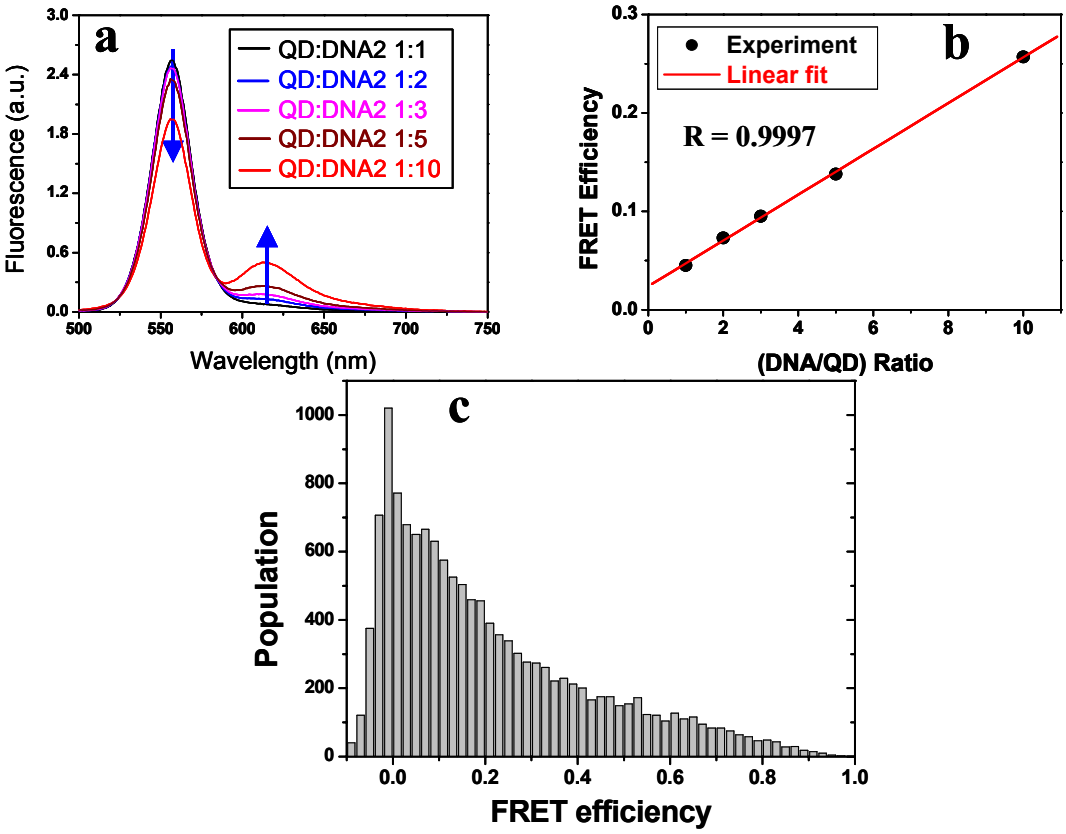

Figure S2. (**a**) Fluorescence spectra of the QD-DNA conjugates prepared without the removal of the free MPA at different ratios of the dye-labelled DNA to QD. The blue arrows show the progressive changes of the fluorescence as the DNA:QD ratio increases from 1:1 to 1:10. (**b**) Plot of the FRET efficiency obtained from the bulk measurement versus the DNA:QD ratios. (**c**) A representative single-molecule FRET histogram obtained from a QD-DNA conjugate at the DNA to QD ratio of 1:1.

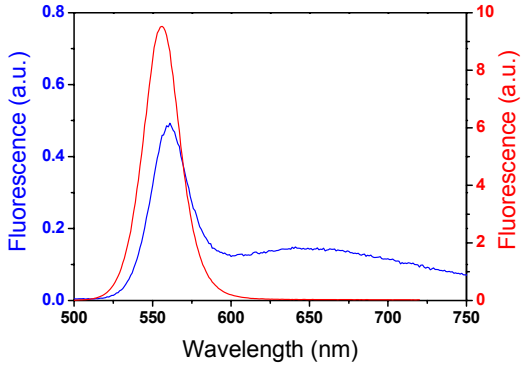

Figure S3. Fluorescence spectra of the MPA-QD (red line, right scale) and the QD-DNA conjugate at a DNA:QD ratio of 4 (blue line, left scale). Non-labelled DNA, with the same sequence as the labelled being used in the FRET studies, was used here. Both spectra were recorded under the identical experimental conditions at a QD concentration of 1 μM excited at 450 nm. It is clear that the QD fluorescence was significantly reduced when conjugated with 4 DNA molecules, and surface defect emission is also clearly seen.