Supplementary Material (ESI) for Faraday Discussions

Electronic Supporting Information

Development of smart nanoparticle-aptamer sensing technology

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Experimental Section

Materials and Methods

Chemicals and Reagents

CdSe/ZnS core/shell quantum dots (first exciton peaks at 525 and 585 nm) were purchased from Evident Technologies Inc (Troy, New York, USA). They were supplied in toluene solutions capped with trioctylphosphine oxide (TOPO) ligands with average crystal core diameters of 3.4 and 5.0 nm. *N,N'*-dicyclohexylcarbodiimide (DCC) was purchased from Acros Organics (Fisher Scientific, Loughborough, UK). 3-mercaptopropionic acid (>99%), 2-mercaptoethanol (>99%), DL-lipoic acid (>98%), dithiothreitol (DTT, >98%), bromoacetylbromide (98%), diethyl iminodiacetate (98%), trifluoroacetic acid (>99%), tris base, NaCl, Na_2HPO_4, $Na_2S.9H_2O$ (>98%), $ZnCl_2$ (>99%) and other chemicals and reagents were all purchased from Sigma-Aldrich (Dorset, UK). All chemicals and reagents were used as received without further purification unless otherwise stated. Solvents were obtained from Fisher Scientific (Loughborough, UK) and used as received. All buffers were prepared with ultra-pure water obtained from a Milli-Q water-purification system (resistance > 18 $M\Omega.cm^{-1}$) unless otherwise stated. Chromatography TLC was performed with commercial silica gel plates with fluorescent backing. The plates were visualised by fluorescence under UV 254 nm, and/or by iodine staining. Flash column chromatography was performed using 230–400 mesh silica gel purchased from Breckland Scientific Supplies (Thetford, UK).

Instruments:

NMR Spectroscopy Spectra were recorded by NMR Service of Cambridge University Chemical Laboratory, using Bruker Avance 500 Cryo Ultrashield (500MHz for ^1H, 125MHz for ^{13}C) or

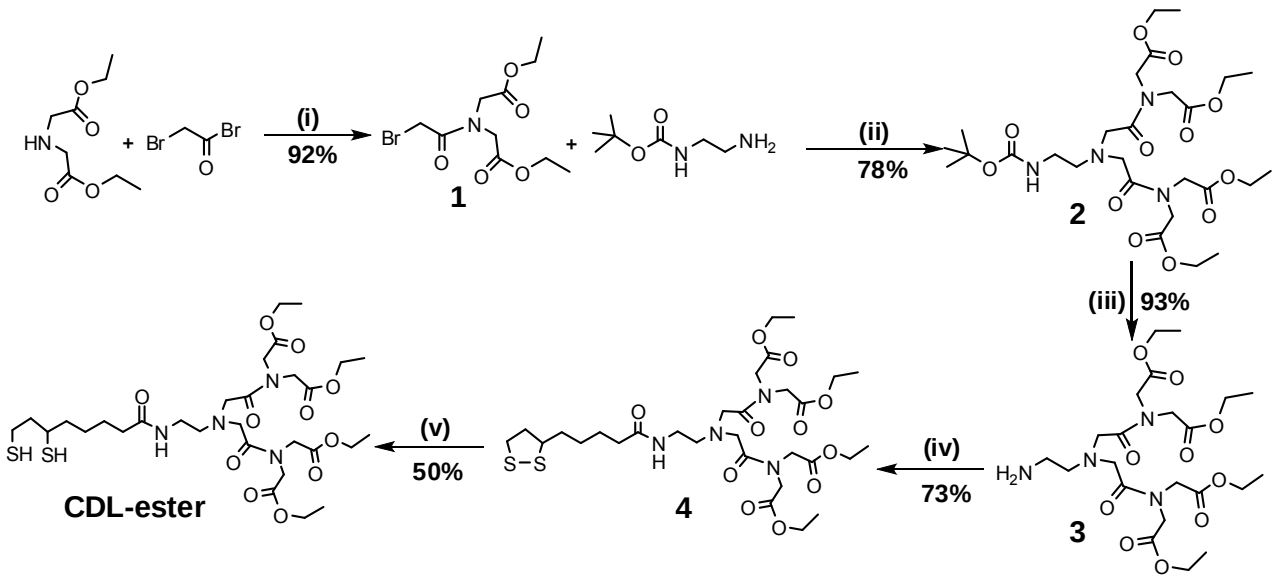
Bruker Avance 400 QNP (400MHz for 1H, 100MHz for ^{13}C) spectrometer in $CDCl_3$. All chemical shifts are reported in ppm. All coupling constants are reported in Hertz. Attached proton tests were performed for all 1H-NMR to determine the proton multiplicity of each resonance.

Mass Spectroscopy data were obtained using Agilent 1100 Series liquid-chromatography equipment coupled to Waters LCT Premier time-of-flight mass spectrometer. Samples were dissolved in 1:1 (v/v) water/acetonitrile, and ionised by positive ESI. The specified accuracy is 5 ppm.

UV-Vis Spectroscopy: UV-Vis spectra were acquired on a Varian Cary 300 Bio Spectrophotometer and a black-walled quartz cuvette with 1 cm path length. Samples were scanned from 400 nm to 800 nm in 1nm increment at a rate of 600 nm min^{-1}. All scans were conducted in dual-beam mode with the spectral band width set to 2 nm. All data reported were corrected for solvent absorption.

Fluorescence Spectroscopy: Fluorescence measurements were either taken on an Aminco-Bowman Series 2 Luminescence spectrometer (Sim-Aminco Spectronic Instruments Inc, Rochester, NY) or a Varian Cary Eclipse fluorescence spectrometer on a 1 cm path length quartz cuvette. An excitation and emission bandwidth of 4 nm was used. The samples were excited at 350 nm to record the fluorescence emission spectra. The photomultiplier detector voltage was set to 800 V.

Synthesis of the chelating dendritic ligand (CDL)



Scheme S1. Schematic procedures of the synthesis of the chelating dendritic ligand (**CDL**).

- **(i)** EtOAc/0 ^{o}C, then reflux 30 min;
- **(ii)** K_2CO_3, DMF, 60 ^{o}C, 24 h;
- **(iii)** TFA, DCM, RT, 4 h;
- **(iv)** DMAP, DCC, DCM, 0 ^{o}C, 1.5 h, RT, 24 h;
- **(v)** DTT, aq. K_2CO_3, H_2O/EtOH, RT, 10 min.

The synthesis of the CDL ligand was carried out following a reported literature procedure.[1]

Compound (**1**): Bromoacetylbromide (0.11 mol) was added dropwisely into a solution of diethyl iminodiacetate (10 g, 0.052 mol) in 40 mL ethyl acetate under stirring cooled over an ice-bath over 15 mins. The reaction mixture was then allowed to warm up to RT and refluxed for a further 30 min. The solvent and excess of bromoacetylbromide were removed under reduced pressure. Then chloroform was added to the residue and the resulting organic layer was washed with aqueous 5% $NaHCO_3$ solution and then with saturated brine. The organic phase was separated and dried with anhydrous Na_2SO_4 and the solvent was removed under reduced pressure to give a light brown liquid (**1**) in 98% yield. ESI-MS (*m*/*z*): 357 ($M + H^+$). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.24 (m, 6H, CH_3), 4.17 (m, 10H, CH_2).

Compound **2**: *N*-boc-ethylenediamine (0.970 g, 6.05 mmol), compound **1** (3.740 g, 12.1 mmol), anhydrous potassium carbonate (1.659 g, 12.0 mmol) and DMF (13 mL) were mixed together and stirred vigorously under nitrogen at 60 ^{o}C for 24 hrs. The solid in the reaction mixture was filtered off, and the solvent was removed under reduced pressure (2 mmHg, 40 ^{o}C, 30 min). The residue was dissolved in ethylacetate (60 mL). The solution was then washed with water (3 × 10 mL), brine (3 × 10 mL) and dried over $MgSO_4$. The solvent was removed to give a crude product, which was further purified by flash column chromatography (methanol/chloroform 1:25), giving the desired carbamate (compound **3)** (2.93 g, 78%) as a viscous yellow liquid. TLC (MeOH/$CHCl_3$ 1:25) R_f 0.21. ^{1}H-NMR (400MHz): δ (ppm) 5.73 (t, br, J = 4.5, 1H, H_9), 4.32 (s, 4H, H_{14}), 4.21 (q, J = 7.2, 4H, H_{16}), 4.17 (q, J = 7.1, 4H, H_{16}), 4.12 (s, 4H, H_{14}), 3.49 (s, 4H, H_{12}), 3.18 (q, J = 5.1, 2H, H_{10}), 2.73 (t, J = 5.7, 2H, H_{11}), 1.43 (s, 9H, H_x), 1.28 (t, J = 7.1, 6H, H_{17}), 1.25 (t, J = 7.1, 6H, H_{17}). ^{13}C-NMR (100 MHz): δ (ppm) 170.79 (C, C_{13}), 169.05 (C, C_{15}), 169.02 (C, C_{15}), 156.31 (C, C_z), 78.69 (C, C_y), 61.61 (CH_2, C_{16}), 61.24 (CH_2, C_{16}), 55.86 (CH_2, C_{12}), 53.52 (CH_2, C_{11}), 49.80 (CH_2, C_{14}), 48.20 (CH_2, C_{14}),38.04 (CH_2, C_{10}),28.38 (CH_3, C_x), 14.08 (CH_3, C_{17}). HR-MS: calcd m/z for $C_{27}H_{46}N_4O_{12}Na$ $(M + Na)^+$ 641.3010, found 641.3011.

Compound **3:** A mixture of compound **2** (1.126 g, 1.820 mmol), trifluoroacetic acid (5.5 mL) and dichloromethane (5.5 mL) was stirred under a nitrogen atmosphere for four hours at 25 ˚C. The solvent was removed. Aqueous solution (1 M) of sodium carbonate was added to neutralise the mixture, which was then extracted with chloroform (4×10 mL). The organic extract was washed with brine (1×15 mL) and dried with magnesium sulphate. The solvent was removed to give the crude compound **3** (0.882 g, 93%) as a viscous yellow liquid. The crude product was used for the

next step without purification. TLC (MeOH/$CHCl_3$ 1:25) R_f 0.05. ^{1}H-NMR (400 MHz): δ (ppm) 4.31 (s, 8H, H_{14}), 4.17 (q, J = 7.1, 8H, H_{16}), 3.46 (s, 4H, H_{12}), 2.80 (m, 4H, H_{10}, H_{11}), 1.21 (t, J = 7.2, 12H, H_{17}). ^{13}C-NMR (100 MHz): δ (ppm) 171.07 (C, C_{15}), 169.01 (C, C_{13}), 61.20 (CH_2, C_{16}), 56.26 (CH_2, C_{11}), 50.13 (CH_2, C_{12}), 48.21 (CH_2, C_{14}), 39.20 (CH_2, C_{10}), 14.08 (CH_3, C_{17}). HR-MS: calcd m/z for $C_{22}H_{38}N_4O_{10}Na^+$ $(M + Na^+)^+$ 541.2486, found 541.2518.

***Coupound* 4:** A solution of lipoic acid 5 (0.308 g, 1.49 mmol), amine **4** (0.773 g, 1.49 mmol) and 4-dimethylaminopyridine (0.017 g, 1.6 mmol) in dichloromethane (3 cm_3) was cooled to 0 ˚C under a nitrogen atmosphere. To this mixture a solution of dicyclohexylcarbodiimide (0.339 g, 1.64 mmol) in dichloromethane (3 mL) was added dropwisely over 5 min. The reaction mixture was stirred for 1.5 hour at 0 ˚C, then warmed to 25 ˚C and stirred for 24 hours. The precipitate formed was filtered out. The reaction mixture was washed with brine and dried with magnesium sulphate. The solvent was removed to give a crude product, which was then purified by flash column chromatography (MeOH/$CHCl_3$ 1:25), giving compound **4** (0.78 g, 73%) as a viscous yellow liquid. TLC (MeOH/$CHCl_3$ 1:25) R_f 0.19. $_1$H-NMR (400 MHz): δ (ppm) 7.49 (s, br, 1H, H_9), 4.25 (s, 8H, H_{14}), 4.20 (q, J = 7.3, 8H, H_{16}), 4.14 (s, 4H, H_{12}), 3.58 (m, 1H, H_3), 3.26 (q, J = 5.0, 2H, H_{10}), 3.13 (m, 2H, H_1), 2.79 (t, J = 5.2, 2H, H_{11}), 2.46 (m, 1H, H_2), 2.25 (t, J = 7.5, 2H, H_7), 1.91 (m, 1H, H_2), 1.70 (m, 4H, H_4, H_6), 1.47 (m, 2H, H_5), 1.27 (t, J = 7.2, 12H, H_{17}). ^{13}C-NMR (100 MHz): δ (ppm) 173.42 (C, C_8), 171.15 (C, C_{15}), 168.78 (C, C_{13}), 61.35(CH_2, C_{16}), 56.45 (CH, C_3), 55.45 (CH_2, C_{11}), 52.91 (CH_2, C_{12}), 48.28 (CH_2, C_{14}), 40.18 (CH_2, C_2), 38.40 (CH_2, C_{10}), 37.21 (CH_2, C_1), 36.13 (CH_2, C_4), 34.61 (CH_2, C_7), 28.93 (CH_2, C_5), 25.38 (CH_2, C_6), 14.12 (CH_3, C_{17}). HR-MS: calcd m/z for $C_{30}H_{50}N_4O_{11}S_2Na$ $(M + Na)^+$ 729.2815, found 729.2847.

CDL-ester: A mixture of compound **4** (0.396 g, 0.560 mmol), aqueous potassium carbonate solution (0.5 mL, 1 M), dithiothreitol (0.167 g, 1.05 mmol), ethanol (2 mL) and water (5 mL) was stirred vigorously for 10 mins. The solvents of the reaction mixture was removed under reduced pressure on a rotary evaporator, and then extracted with chloroform (3 × 5 mL). The organic extract was purified by flash column chromatography (methanol/chloroform 1:25). **CDL-ester** was collected as a viscous colourless liquid (0.200 g, 50%). TLC (MeOH/$CHCl_3$ 1:25) R_f 0.17. ^{1}H-NMR (400 MHz): δ (ppm) 7.43 (s, br, 1H, H_9), 4.24 (s, 8H, H_{14}), 4.19 (q, J = 7.2, 8H, H_{16}), 4.13 (s, 4H, H_{12}), 3.57 (s, br, 2H, H_{10}), 2.92 (m, 1H, H_3), 2.69 (m, 2H, H_1), 2.23 (t, J = 7.4, 2H, H_7), 1.88 (m, 1H, H_2), 1.78-1.39 (m, 7H, H2, H_4–H_6), 1.34 (t, J = 8.0, 1H, H_a), 1.29 (d, J = 7.3, 1H, H_b), 1.26 (t, J = 7.2, 12H, H_{17}). ^{13}C-NMR (100 MHz): δ (ppm) 172.97 (C, C_8), 170.89 (C, C_{15}), 168.54 (C, C_{13}),

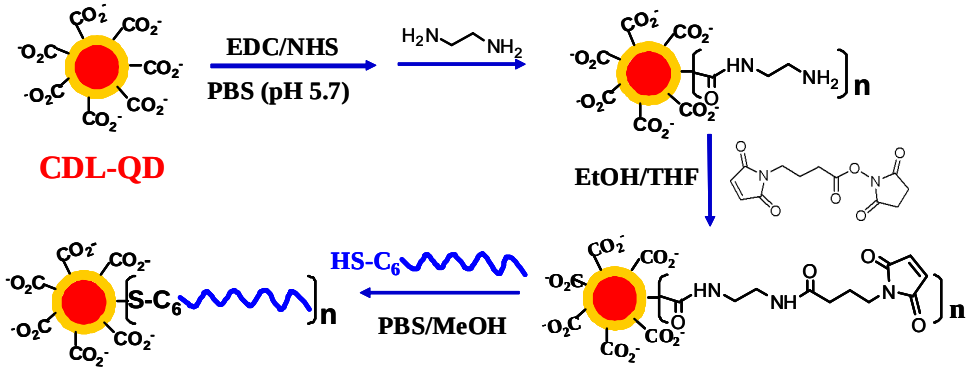
61.40 (CH_2, C_{16}), 55.38 (CH_2, C_{11}), 52.68 (CH_2, C_{12}), 48.31 (CH_2, C_{14}), 42.77 (CH_2, C_2), 39.40 (CH, C_3), 39.09 (CH_2, C_{10}), 38.82 (CH_2, C_4), 36.21 (CH_2, C_7), 26.72 (CH_2, C_5), 25.27 (CH_2, C_6), 22.27 (CH, C_1), 14.13 (CH_3, C_{17}). HR-MS: calcd m/z for $C_{30}H_{52}N_4O_{11}S_2Na$ ($M + Na^+$) 731.2972, found 731.2980.

Preparation of water-soluble QDs by ligand exchange

The ligand exchange reactions of the QDs with functional thiols were carried out following our earlier procedures.[2] Briefly, the TOPO capped CdSe/ZnS core/shell QD was precipitated by adding ~1 mL of the toluene solution to ~10 mL of ethanol, and the resulting mixture was then centrifuged at 14,000 rpm for 5 mins. The clear supernatant was discarded. The pellet was dissolved in ~0.5 mL toluene and precipitated by ethanol followed by centrifugation and discard of clear supernatant another time. This process is to remove any uncapped free TOPO ligand that may interfere with the ligand exchange reaction. The pellet was then dissolved in 1 mL $CHCl_3$, into which 1 mL ethanol solution of the functional thiol ligands (1 M for MPA, 40 mM for CDL-ester) and tetramethyl-ammonium hydroxide (2 mole equivalent of ligands) was added. The resulting mixed solution was refluxed for 4 hrs under a N_2 atmosphere. After the solution has been cooled down to RT, the solvent was removed under reduced pressure. The solid was then dissolved in ~0.5 mL EtOH, and $CHCl_3$ was added to precipitate the MPA capped QDs followed by centrifugation at 14,000 rpm for 10 mins. The dissolving, precipitation and centrifugation process was repeated three times to remove any uncapped free ligand. The pellet was air dried and then dissolved in ~ 2 mL of MilliQ water to make the stock solution.

A slightly different procedure was used to make **CDL** capped QDs. After the initial ligand exchange and removal of solvents as above, the solid was dissolved in $CHCl_3$ and hexane was added to precipitate the CDL-ester capped QD and then centrifuged at 14,000 rpm for 10 mins to separate the QD from free ligands. The pellet was re-dissolved in $CHCl_3$, precipitated by addition of hexane and centrifuged twice to completely remove uncapped free ligands. The ethyl esters of BD ligand was hydrolysed to carboxylic acids by dissolving the pellet in a mixed solution of 1 mL EtOH and 1 mL of 1M K_2CO_3 aqueous solution, the resulting solution was refluxed for 2 hrs to obtain a precipitate. The precipitate was separated from the solution by centrifugation, and the pellet was dissolved in minimum amount of water (~0.2 mL) and precipitated by adding EtOH followed by centrifugation at 14,000 rpm for 5 mins. The process was repeated three times and finally the pellet was dissolved in 2 mL of pure water to obtain the stock QD solution.

Preparation of CDL-QD-DNA conjugate sensors.



Scheme S2. Schematic showing the preparation route for CDL-QD-DNA conjugate sensor. The CDL-QD was first activated by EDC/NHS in phosphate buffer (pH 5.7), which then react with 1,2-ethylenediamine (EA, 1M) to transform some of the carboxylic acids on the CDL-QD to amines, which then reacts with 4-maleimidobutyric acid-NHS ester, a hetero-bifunctional cross linker in mixed solution of EtOH/THF, to introduce free maleimide groups, which finally reacts with a thiolated capture DNA to make the CDL-QD-DNA conjugate.

Reference:

1) Wang, Y. A.; Li, J. J.; Chen, H.; Peng, X. *J. Am. Chem. Soc*. **2002**, *124*, 2293

2) Zhou, D.J.; Ying, L.; Hong, X.; Hall, E. A. H.; Abell, C.; Klenerman, D. *Langmuir* **2008**, *24*, 1659.

3) Product Information, Evident Technologies Inc.

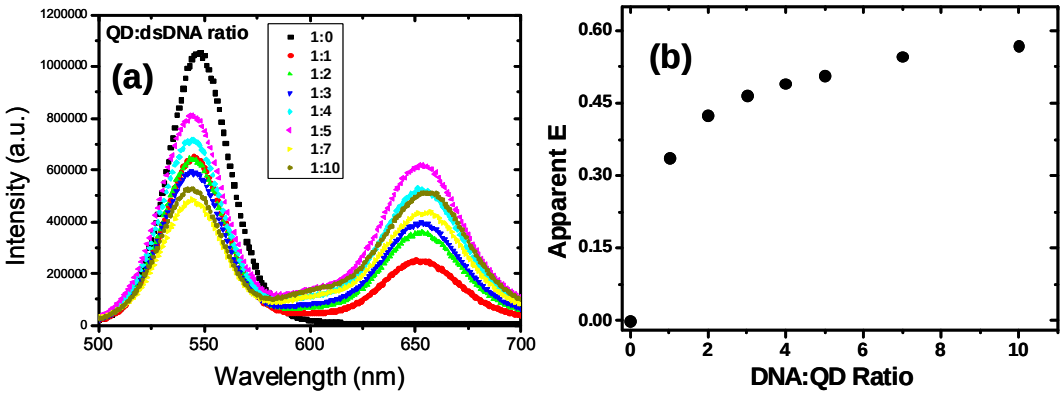


Figure S1. (**a**) Fluorescence spectra of a series of self-assembled DHLA-QD-TBA/TBA-C samples, each containing 100 nM QD in 1 x PBS. The samples were excited at 450 nm. (**b**) Plot of the apparent FRET efficiency *E* v.s. the DNA:QD ratios.

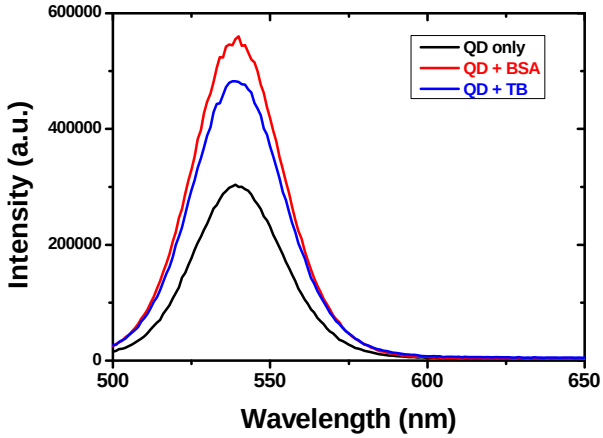


Figure S2. Fluorescence spectra of the DHLA-QD only (black), DHLA-QD + BSA (red) and DHLA-QD + TB (blue) in PBS buffer (10 mM phosphate, 150 mM NaCl, pH 7.2) excited at 450 nm. The QD concentrations were all 20 nM and the protein concentrations were 100 nM.

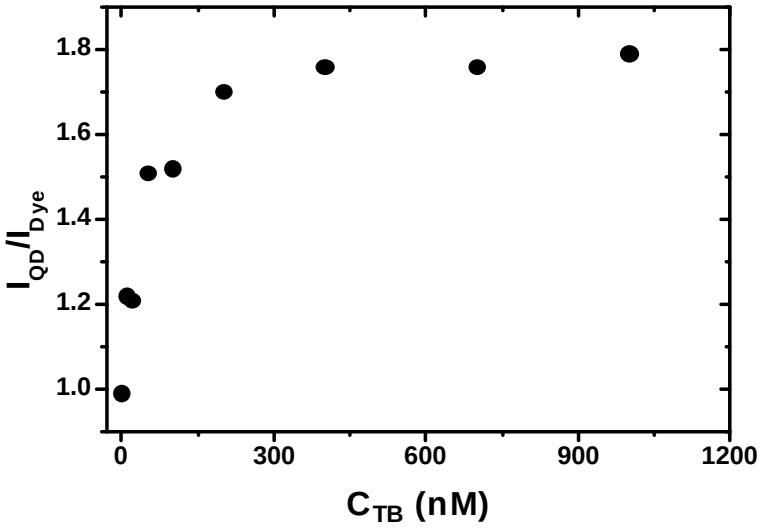


Figure S3. A ratiometric analysis (QD to Atto-647N fluorescence intensity ratio, I_{QD}/I_{Dye} *v.s.* TB concentration) of the detection of unlabelled TB using a self-assembled QD-TBA/TBA-C conjugate.

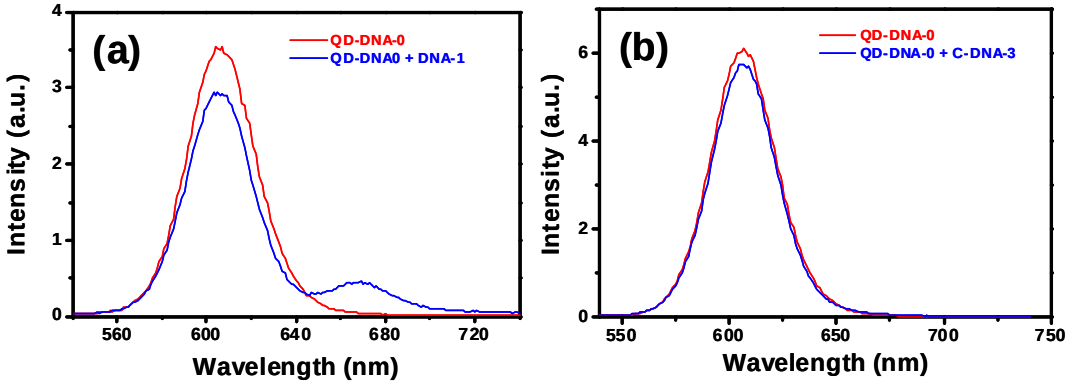


Figure S4. (**a**) Fluorescence spectra of the CDL-QD-$(DNA\text{-}0)_n$ conjugate before (red line) and after (blue line) being mixed with complementary DNA-1 (3'-labelled with Alexa 647 dye). (**b**) Fluorescence spectra of the CDL-QD-$(DNA\text{-}0)_n$ conjugate before (red line) and after (blue line) being mixed with a non-complementary DNA-NC (3'-labelled with Alexa 647). All experiment was performed in PBS buffer (10 mM phosphate, 100 mM NaCl, pH 7.4) with 50 nM QD-$(DNA\text{-}0)_n$ and 500 nM DNA-1 or DNA-NC. The sequences of the three DNAs are:

DNA-0: HS-C_6H_{12}- 5'-ATC GCA AGA ATT CCA TGA CCA ACC TGC ACT-3'
DNA-1: Alexa-647- 3'-TAG CGT TCT TAA GGT ACT GGT TGG ACG TGA-5'
DNA-NC: 5'-CAA GCC ATT GTA GTC CCG CAA CAC ACT CGA-3′-Alexa-647

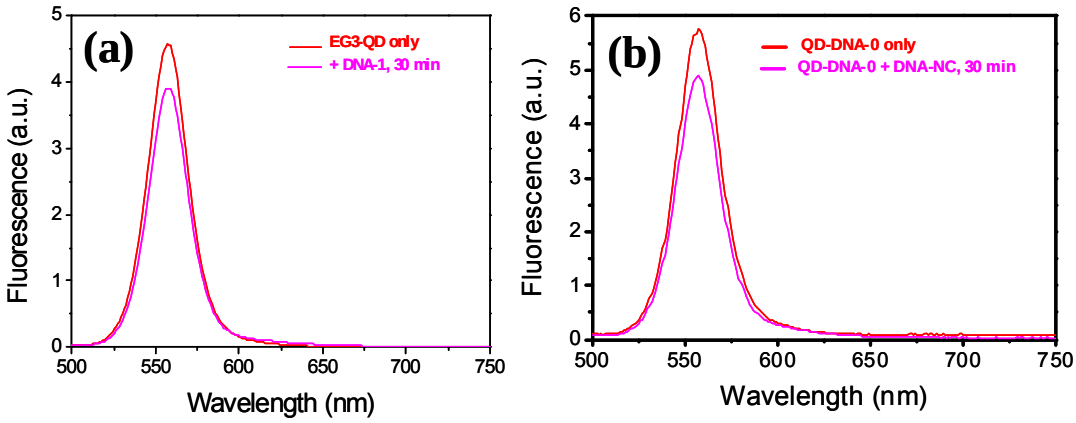


Figure S5. Control experiments. (**a**) Fluorescence spectra of the EG_3-QD (with no capture DNA-T attached) before (red) and after (pink) mixed with DNA-1 (Alexa 594 labeled) in PBS for 30 mins. (**b**) Fluorescence spectra of the QD-DNA-T conjugate before (red) and after (pink) mixed with an Alexa 594 labeled non-complimentary DNA (DNA-NC) in PBS for 30 mins. All experiments were done at 100 nM QD and 220 nM DNA.

The DNA sequences are:

DNA-T $H_2NC_6H_{12}$-5'-CAT AAA AGA GCT CCA TAT CCA ACC TGC ACG-3'

DNA-C **Alexa 594**-3'-GTA TTT TCT CGA GGT ATA GGT TGG ACG TGC-5'

DNA-NC1 3'-AAT CAG GGA TTT ACG TGC ACG ACA CAC ACT-5'-**Alexa 594**

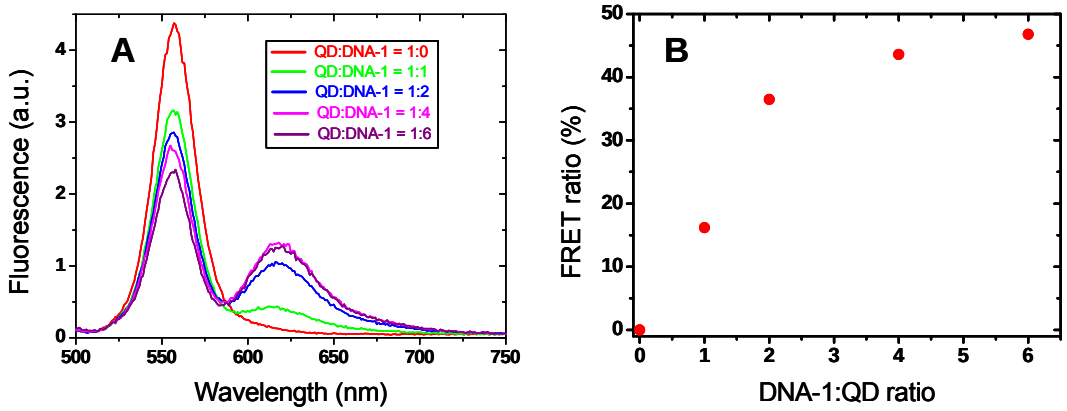


Figure S6. (**A**) Fluorescence spectra of the QD-DNA-T conjugate after hybridization with DNA-1 at different molar ratios. All experiments were carried out in PBS with 100 nM QD-DNA-T conjugate excited at 445 nm. (**B**) A plot of the apparent FRET efficiency *versus* the DNA-1:QD ratios.