Electronic Supporting information (ESI)

A quantum dot-intercalating dye dual-donor FRET based biosensor

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Materials and Methods

Chemicals and reagents

CdSe/ZnS core/shell QD (EviDot ED-C11-TOL-0540, emission peak ~540 nm, crystal diameter ~ 3.4 nm) was purchased from Evident Technologies (Troy, New York, USA). The QD was supplied as toluene solution capped with TOP (trioctyl phosphine) and TOPO (trioctyl phosphineoxide) ligands, denoted as TOPO-QD. *N*-(3-dimethylaminopropyl)-*N*-ethyl-carbodiimide hydrochloride (EDC. HCl, 99%), N-hydroxysuccinimide (NHS, 98%), sodium bicarbonate, glutathione, DNAse and RNAse free NaCl, Na_2HPO_4, thrombin and all other chemicals and reagents were all purchased from Sigma-Aldrich (Dorset, UK). Solvents were obtained from Fisher Scientific (Loughborough, UK) and used as received. All DNAs (TBA, DNA1, DNA2，DNA3，DNA3-SM and DNA-NC) together with their respective modification and fluorophore labelling were purchased commercially from IBA GmbH. They have been PHLC purified (for TBA) or double-HPLC purified (for all other fluorophore labelled DNAs) by the supplier. They were uses as received without further purification. 10 × PBS buffer (100 mM phosphate, 1.50M NaCl, pH 7.4) and phosphate buffer (20 mM phosphate, pH = 5.57) were prepared with ultra-pure MilliQ water (resistance ~18 $M\Omega.cm^{-1}$) and adjusted to the required pH by using NaOH or HCl.

Preparation of glutathione modified QD

Glutathione capped QD was prepared by following our previously established procedures.[1,2] Briefly, TOPO-QD (80 nmol) in toluene were precipitated by EtOH. The precipitate was dissolved in chloroform (1 ml), into which was added 200 μl GSH solution containing 28.4 mg

GSH and 20 mg KOH in MeOH. The mixture was shaken for 30 min, and a precipitate was formed. After centrifugation, the clear supernatant was discarded and the precipitate was washed with MeOH 3 times to remove any uncapped GSH. The precipitate was finally dissolved in pure water to form a bright green stock solution and was stored in a fridge at 4 ^{o}C till use, denoted as QD-GSH. Its concentration was determined from its absorbance at respective exciton peak by using the Beer-Lambert law following the previously established method.[1] Compared to the parent TOPO-QD, the quantum yield the QD-GSH showed only a small decrease of ~8% (from 71% to 65%).

Preparation of covalently coupled QD-DNA conjugate

QD-DNA conjugation was performed by following our previous procedures.[3] Briefly, EDC (30 mg) and NHS (15 mg) were dissolved in 200 μL phosphate buffer (20 mM, pH = 5.57) into which was added 2.0 nmole of QD-GSH (223 μL, 8.98 μM), and the resulting solution was sonicated for 20 s. The mixture was allowed to stand at room temperature for 1 h to activate the carboxylic acid groups in QD-GSH into active NHS esters. After that, the solution was centrifuged (at 21,000 × g for 3 mins), and the clear supernatant was discarded. The obtained precipitate pellet was washed carefully with water (2 × 100 μL). After that, the QD pellet was added with TBA (200 μM 150 μL, 30 nmole), and $NaHCO_3$ buffer (50 mM, pH = 9, 100 μL) and thoroughly mixed, sonicated and then was allowed to stored overnight at 4℃. The resulting solution was then added EtOH (400 uL) and stored in a freezer for 1 hour. After that, the solution was centrifuged and the clear supernatant which contained the unreacted TBA was carefully removed (checked with an UV lamp to ensure it did not contain any QDs). The pellet was carefully washed with a mixed solution of EtOH (400 uL), water (100 uL) and 50 mM $NaHCO_3$ (100 uL), and an absorption spectrum was taken on the combined clear supernatant/wash and the absorbance at 260 nm was used to calculate the amount of DNAs not conjugated to the QD, which gave a value of ~ 20 nmol. Therefore there were 10 nmol of the TBA conjugated to the QD-GSH (2 nmol), so the DNA-QD copy numbers attached to each QD was estimated to be 5, denoted as QD-TBA_5. We found that the copy number of TBA attached to each QD varied from batch to batch, but generally in the range of 4-5 using the method described above. Finally, the pellet was dissolved in 0.5 mL of pure water to obtain a clear QD-TBA_5 stock solution and stored in the dark at 4°C till use. A second batch was also prepared where

the average number of TBA strands conjugated to each QD was found to be 4, denoted as QD-TBA_4.

Hybridization of DNA probes

The total volume of the hybridization reaction solution was kept constant at 400 μL, with a fixed final QD concentration in PBS. The reaction was carried out in batches under identical conditions, where 40 μL of the 10 × PBS, the calculated amount of water, DNA probes, and the required amount of the QD-TBA_4 conjugate were sequentially added and thoroughly mixed. The hybridization reaction was carried out for 30 mins at room temperature (ca 20 ^{o}C) before measurement.

Fluorescence measurement

Fluorescence spectra were recorded on a Spex Fluoro Max-3 fluorescence spectrophotometer. The emission spectra (480–720 nm range) were recorded under a fixed excitation wavelength of 450 nm, which corresponds to the minimum absorption (λ_{min}) of the dye acceptor, Atto 647N, to minimize the direct excitation of the acceptor dye. The spectra were corrected from the direct dye excitation background, although weak, using the same dye labeled DNA as reference. The data were analysed using the Origin software (version 8.5).

UV-Vis spectra

The absorption spectra of the QD-TBA conjugates were measured on a Cary 50 Bio UV-vis spectrophotometer (Varian Inc., CA, USA). A spectral range of 220–600 nm was recorded at a medium scan rate at a slit width of 2 nm with a quartz cuvette. All spectra were corrected with the background absorption of the corresponding blank buffer (EtOH/water/50 mM $NaHCO_3$ 4:1:1) using the same cuvette.

Reference

1) Y. Zhang, H. Y. Zhang, J. Hollins, M. E. Webb and D. J. Zhou, *Phys. Chem. Chem. Phys*., 2011, **13**, 19427-19436.
2) H. Y. Zhang, P. G. Stockley and D. J. Zhou, *Faraday Discuss*., 2011, **149**, 319-332.
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Table S1. Comparison of the sensitivity and specificity of some QD-FRET based DNA sensors using fluorescence spectroscopy, and some other DNA sensors using direct electrochemical, surface plasmon resonance (SPR) and quartz crystal microbalance (QCM) readout.

Detection System	Target/length	Specificity	LOD (nM)	Ref
QD-BRET	DNA/22 mer	?	20	[1]
QD-FRET	DNA/19 mer	< 2	40 (1 μM QD)	[2]
QD-FRET	DNA/24 mer	< 2	12 (0.06 μM QD)	[2]
QD-FRET	DNA/25 mer	~3	200	[3]
QD-FRET	DNA/18-32 mer	2-3	~5	[4]
QD-FRET	DNA/30 mer	34	0.5	[5]
QD-EB-dual donor FRET	DNA/12 mer	SNP possible	0.5	This work
Electrochemical	DNA/18 mer	?	59 nM	[6]
Electro impedance	DNA/18 mer	?	~1 nM	[7]
Electrochemical	DNA/30 mer	?	43 nM	[8]
Direct SPR	DNA/16mer	?	10 nM	[9]
Direct QCM	DNA/509 mer	?	10 nM	[10]

BRET: bioluminescence resonance energy transfer.

LOD: Limit of detection.

Specificity: FRET ratio between complementary and non-complementary DNA target. None of the previous QD-FRET sensors limited above has demonstrated SNP discrimination ability.

Reference

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[2] W. R. Algar and U. J. Krull, *Anal. Chim. Acta,* 2007, **581**, 193.

[3] J. Lee, Y. Choi, J. Kim, E. Park and R. Song, *ChemPhysChem*, 2009, **10**, 806.

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[8] A. Erdem, et al., *Electroanalysis* 2007, **19**, 798

[9] L. He, et al., *J. Am. Chem. Soc.*, 2000, **122**, 9071.

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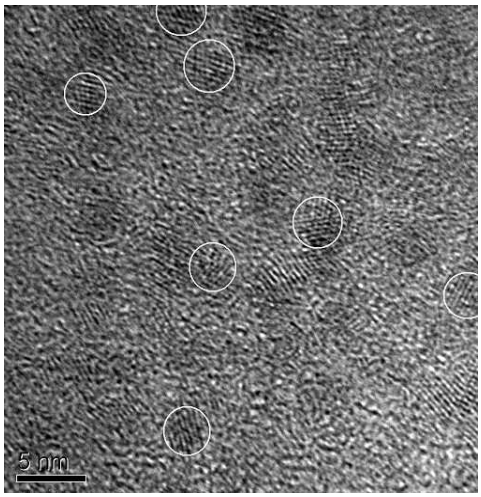

Fig. S1. A typical TEM image of the GSH-QD. The QD crystal lattice can be clearly seen, suggesting that these QDs are highly crystalline, but the particle borders could not be identified clearly. A few white circles drawn on each QD crystals were used to indicate the rough diameters of the QD particles, which gave a rough QD diameter of about 3.4 nm, consistent with the product description from the manufacture (*e.g.* crystal diameter ~ 3.4 nm). This suggests that ligand exchange with GSH did not change the QD size significantly.

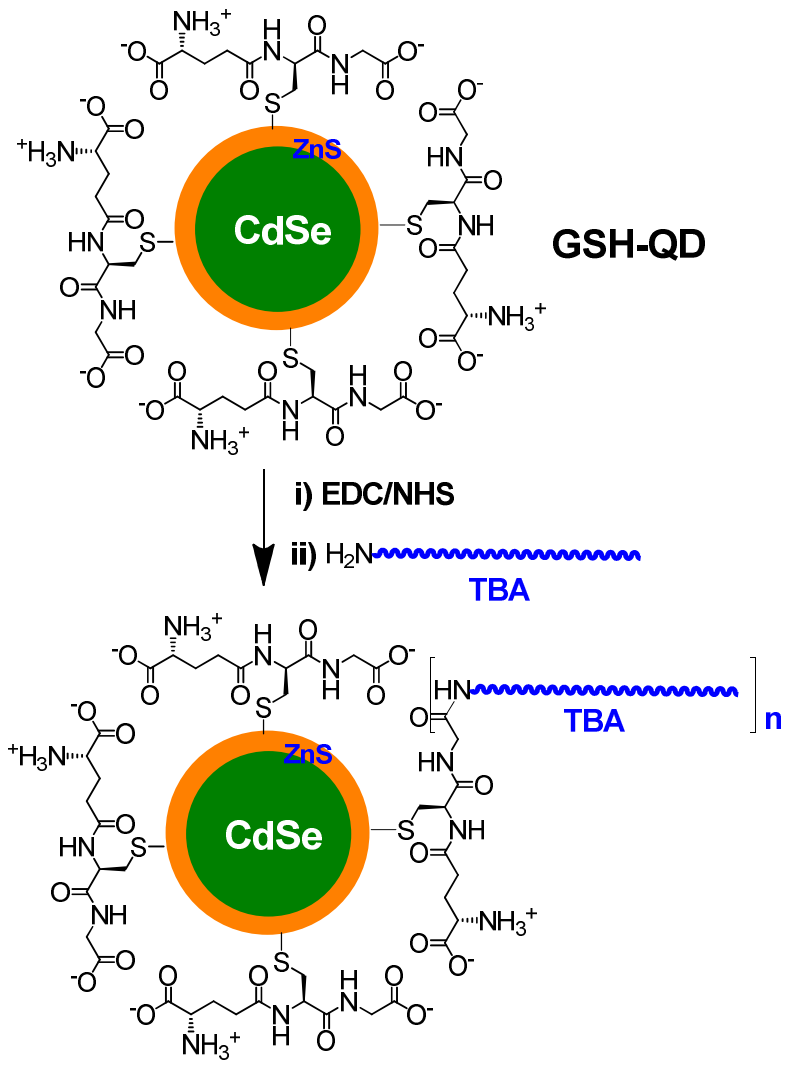

Fig. S2: (Top) Schematic structure of a GSH-capped CdSe/ZnS core/shell QD. GSH molecule binds to the QD surface mainly *via* its thiolate group coordinating to the Zn^{2+} ion on the ZnS shell, leaving other functional groups in its two arms (two -COOH and one -NH_2 groups) exposed on the surface. The COOH groups are negatively charged while the NH_2 group is positively charged at neutral pH, providing the QD with good water-solubility and electrostatic stabilization. The two arms form a relatively dense surface capping on the QD surface that can resist non-specific adsorption of DNA.

(Bottom), the COOH group was activated by EDC/NHS to form an activated NHS ester, which then readily reacts with the amine group of the TBA strand to form covalently conjugated QD-$(TBA)_n$ conjugate which was then used for DNA and protein sensing in this study.

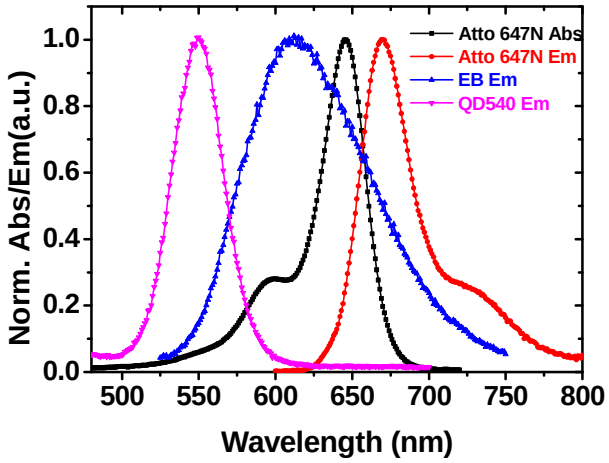

Fig. S3: Spectroscopic characterization of the QD emission (**pink**), EB emission (**blue**), and Atto-647N absorption (**black**), Atto647N emission (**red**).

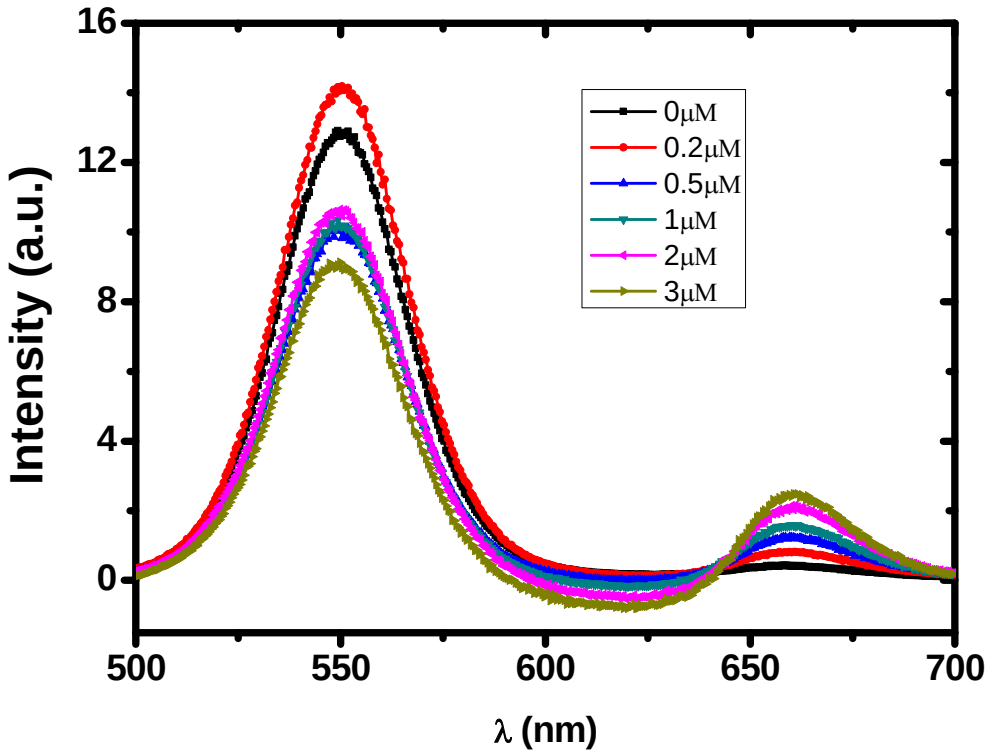

Fig. S4: Corrected fluorescence spectra (spectra shown in **Fig. 2A** in the main text after subtraction of direct EB excitation). The sub-zero emission intensity over the 580-640 nm range of the spectra at higher EB concentrations (*e.g.* 1-3 μM) is due to the significant FRET between EB to Atto-647N, which leads to over correction for EB direct excitation emission.

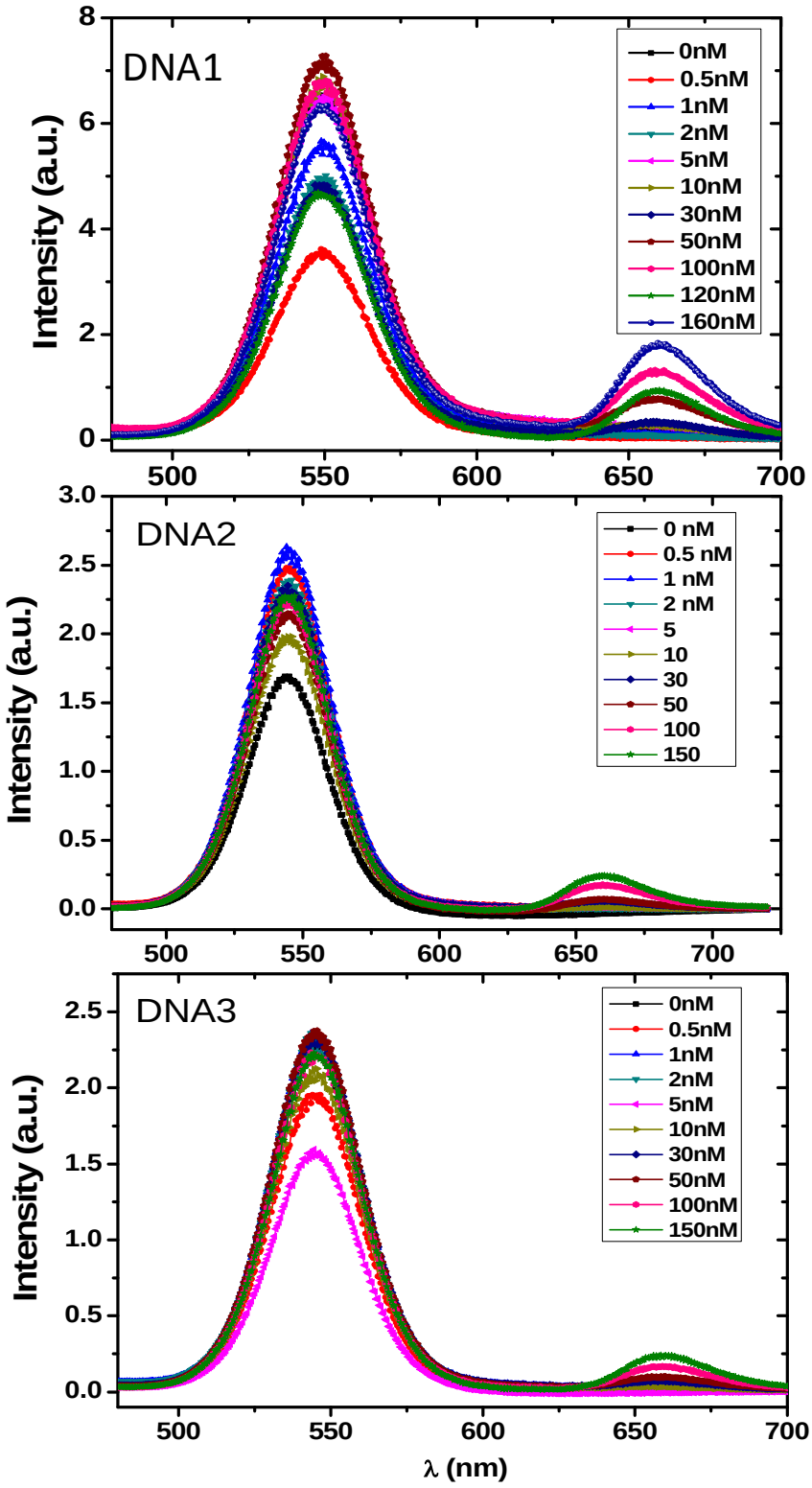

Fig. S5. Fluorescence spectra of the QD-TBA_5-EB system (30 nM QD-TBA_5 + 0.6 μM EB) after hybridization with different concentration of DNA1 (top), DNA2 (middle), and DNA3 (bottom) in PBS. From top to bottom at QD peak fluorescence, the DNA concentrations are 160/150, 120, 100, 50, 30, 10, 5, 2, 1, 0.5 and 0 nM, respectively.

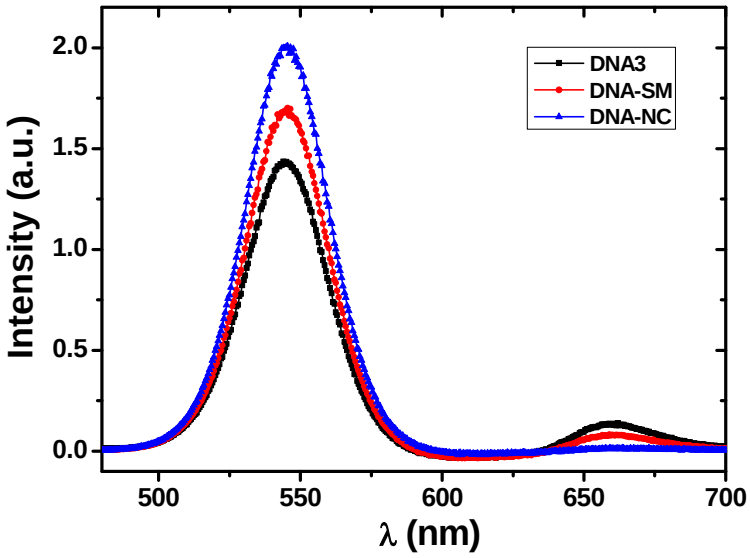

Fig. S6. Fluorescence spectra of the QD-TBA_5 (20 nM) after hybridization with 80 nM of different DNAs in the presence of 0.6 μM EB PBS. **Black**: full complementary target DNA3; **red**: single-base mismatch target DNA3-SM; **blue**: non-complementary target DNA-NC. All spectra have been corrected for the corresponding (EB + DNA target) background fluorescence.

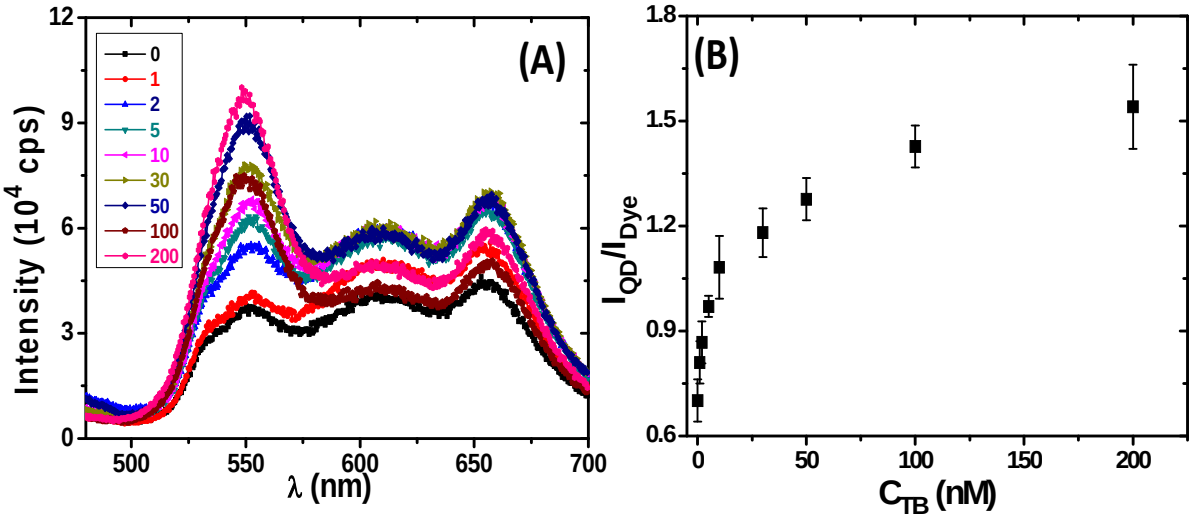

Fig. S7. (A) Fluorescence spectra showing the detection of thrombin (TB) by the dual-donor FRET sensor (10 nM QD-$(TBA/DNA2)_5$ + 0.6 μM EB). From top to bottom at QD fluorescence peak at 550 nm, TB concentrations are 200, 100, 50, 30, 2, 10, 5, 2, 1 and 0 nM, respectively. **(B)** A plot of the I_{QD}/I_{Dye} ratio as a function of TB concentration, error bars represent standard deviations of two parallel independent experiments.