Electronic Supplementary Information

Construction of a molecular beacon based on two-photon excited fluorescence resonance energy transfer with quantum dot as donor

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Experimental Details

Reagents and Instrumentations

Hydrophobic CdSe-ZnS QDs were synthesized in our laboratory. Streptavidin (SA) was purchased from Amresco. Biotinylated DNA strand with ROX (5′-ROX-TAC GAG GTA AAA GGC TCT CTC CCT GTC GTA-biotin-3′), complementary target DNA (5′-CAG GGA GAG AGC CTT TTA CC-3′), non-complementary target DNA (5′-GAC GCA CTG AAT CAG CTC TC-3′), and single-base mismatched target DNA (5′-CAG GGA GAG TGC CTT TTA CC-3′) were purchased from Shanghai Sangon Biological Engineering Technology & Service Co., Ltd. (Shanghai, China). Normal rabbit serum was the product of Zhongshan Golden Bridge Biotechnology Co., Ltd (China). Mercaptoacetic acid (MAA) was purchased from Sinopharm Chemical

Reagent Co. (Shanghai, China). Other reagents were obtained from Sigma. All the solutions were prepared in ultrapure water with a resistivity of 18.2 MΩ.cm (purified by Milli-Q system from Millipore China Ltd).

For two-photon excitation experiments, all samples were excited at 800 nm by a mode-locked Ti:Sapphire femtosecond pulsed laser (Chameleon Ultra I, Coherent Inc.) with a pulse width of 140 fs at a repetition rate of 80 MHz. The anti-Stokes photoluminescence was recorded on a DCS200PC Photon Counting with single-photon sensitivity through an Omni-λ5008 monochromator (Beijing Zolix Instruments Co., Ltd). One-photon excited fluorescence was measured on a Shimadzu RF-5301 fluorescence spectrophotometer with an excitation of 388 nm. Absorption measurements were conducted on a Shimadzu UV2550 UV-vis spectrophotometer. Gel electrophoresis experiment was performed with a DYCP-32B electrophoresis cell equipped with a DYY-5 electrophoresis power supply (Beijing Liuyi Instrument Factory). Settings used were 0.5% agarose in 1 × TAE buffer for 15 min at 120 V. The image was recorded by the Bio-Rad ChemiDoc XRS system.

Preparation of SA-QDs

Nearly monodisperse hydrophobic CdSe-ZnS core-shell QDs were synthesized and rendered water-soluble with mercaptoacetic acid (MAA) to facilitate the conjugation with SA according to a procedure reported by this group.[1] Subsequently, SA was covalently linked to MAA-QDs with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) as crosslinker. Briefly, water-soluble MAA-QDs were dispersed

in 0.1 M phosphate buffer, pH 6.8, at a final concentration of 1 μM. EDC dissolved in the same buffer at a concentration of 0.5 mg/mL was added, and the mixed solution was kept at room temperature for 30 min allowing for activation. With gentle shaking, a 10-fold molar excess of SA was added to the above solution. After incubating for 2~4 h at room temperature, the mixture was purified by ultrafiltration using an Amicon Ultra-4 centrifugal filter device with a MW cutoff of 30 kDa (Millipore Corp.) at 4 °C. Finally the purified products were collected and stored at 4 ºC for further use.

The conjugation efficiency was estimated as follows: the concentration of QDs was determined from the absorption spectrum according to the method reported by Peng et al.[2] Since QDs has a contribution to the absorbance at 280 nm, the concentration of SA was calculated from the absorbance at 280 nm after subtracting the contribution of QDs, using the molar extinction coefficient of 2×10^5 M^{-1} cm^{-1}.[3] Then the SA-to-QD ratio was calculated accordingly.

Determination of the Fluorescence Quantum Yield of MAA-QDs

The fluorescence quantum yield of MAA-QDs was determined by the relative optical method using rhodamine 6G ($\eta = 0.95$ in ethanol) as the reference,[4] according to the following equation:[5]

$$\eta_s = \frac{A_r I_s n_s^2}{A_s I_r n_r^2} \eta_r \qquad \text{(S1)}$$

The subscripts *s* and *r* represent the sample (MAA-QDs) and the reference (rhodamine 6G) respectively. η is the fluorescence quantum yield, *A* is the absorbance that was controlled below 0.1 for both substances in the experiment, *I* represents the integrated emission intensity and *n* is the refractive index of the solvent. In practice, the measurement is slightly more complicated. It was realized by measuring the integrated fluorescence intensities over a range of concentrations. And then the quantum yield was calculated according to a published procedure, which was deduced from equation S1, where *Grad* represents the gradient of the plot of absorbance versus integrated fluorescence intensity:

$$\eta_s = \frac{Grad_r n_s^2}{Grad_s n_r^2}\eta_r \qquad \text{(S2)}$$

Determination of the Two-Photon Absorption Cross Section of MAA-QDs

The two-photon absorption (TPA) cross-section of MAA-QDs was detected using the two-photon induced fluorescence method. MAA-QDs was diluted with ultrapure water to a concentration of 1.8×10^{-7} M. Rhodamine B in methanol (1×10^{-6} M) was used as the reference with the reported value of 150 GM (1 GM = 1×10^{-50} cm^4 s $photon^{-1}$) around 800 nm.[6]

$$\delta_s = \frac{S_s \eta_r \phi_r C_r}{S_r \eta_s \phi_s C_s}\delta_r \qquad \text{(S3)}$$

Similar to the calculation of fluorescence quantum yield, subscripts *s* and *r* denote the sample and the reference molecules, respectively. *S* represents the integrated

emission intensity of two-photon excited fluorescence, η is the fluorescence quantum yield, C is the concentration of the solution, and ϕ is the overall fluorescence collection efficiency of the experimental apparatus.

Construction of the Molecular Beacon

The molecular beacon with QDs as donor was constructed in aqueous buffer containing 20 mM Tris-HCl, 0.1 M NaCl, pH 8.0. Briefly, the ROX-tagged biotinylated DNA strand was prediluted to the concentration of 1×10^{-5} M with 20 mM Tris-HCl (0.1 M NaCl, pH 8.0.). Hairpin structure was formed by heating to 95 °C for 15 min followed by cooling to room temperature. Under room temperature, increasing amounts of the as-prepared hairpin structure were added to the solution of 60 nM SA-QDs in assay buffer. After brief incubation, FRET between QDs and ROX induced by the SA-biotin binding was investigated. The fluorescence emissions were measured under TPE (with 800 nm excitation) and OPE (with 388 nm excitation) modes.

Examination on the Performance of the Molecular Beacon

The specificity of the QDs-based molecular beacon was investigated, firstly in aqueous buffer, by recognizing complementary, non-complementary and single-base mismatched target DNA strands. The ROX-tagged biotinylated DNA and all three single-stranded target DNAs were prediluted to the same concentration of 1×10^{-5} M with 20 mM Tris-HCl (0.1 M NaCl, pH 8.0). Then ROX-tagged biotinylated DNA

was mixed thoroughly with an equal volume of one of the three target DNAs and allowed to hybridize at 95 °C for 15 min. The mixtures were cooled down to room temperature, and then SA-QDs (concentration fixed at 60 nM) was added to capture the biotinylated hybrids. After brief incubation, fluorescence detection was performed under TPE and OPE modes.

The applicability of the two-photon excited MB probe to complicated biological sample matrix was examined through recognizing complementary target DNA in spiked serum medium. Normal rabbit serum was used as received without any purification, and was 10-fold diluted with assay buffer. A similar experimental procedure as above was followed. The experiments were also conducted with the OPE model for comparison.

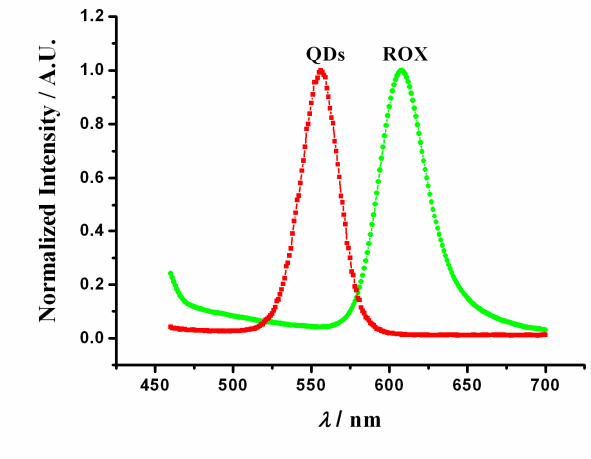

Fig. S1. The normalized emission spectra of donor SA-QDs and acceptor ROX.

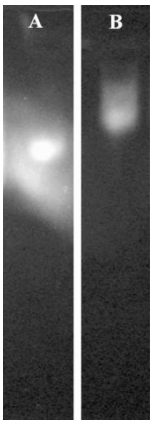

Fig. S2. Agarose gel electrophoresis characterization of MAA-QDs (lane A) and SA-QDs (lane B).

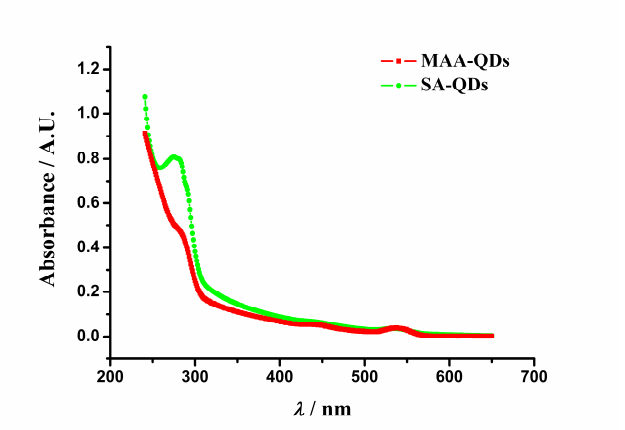

Fig. S3. The UV-Vis absorption spectra of MAA-QDs and SA-QDs.

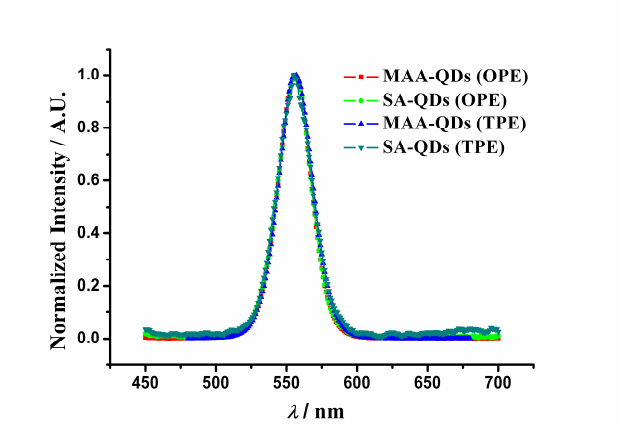

Fig. S4. The normalized fluorescence spectra of MAA-QDs and SA-QDs under OPE at 388 nm and TPE at 800 nm, respectively.

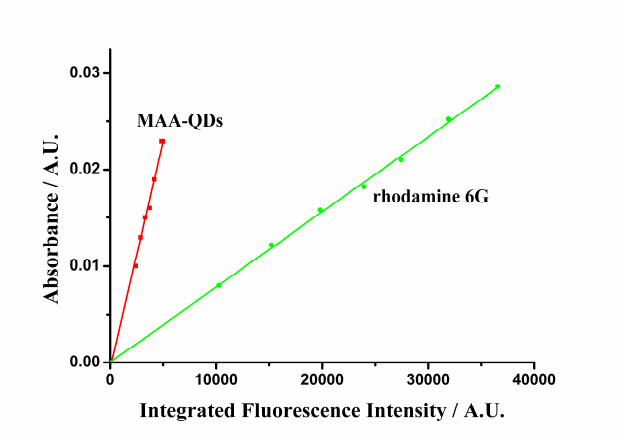

Fig. S5. The linear plot of the absorbance versus the integrated fluorescence intensity for MAA-QDs and rhodamine 6G. Excitation wavelength was fixed at 502 nm.

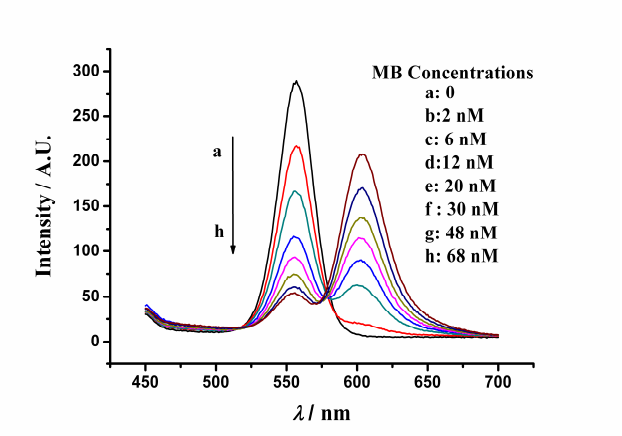

Fig. S6. One-photon fluorescence titration of SA-QDs (35 nM) with the biotinylated hairpin structure tagged with ROX. The fluorescence was excited at 388 nm and measured in assay buffer.

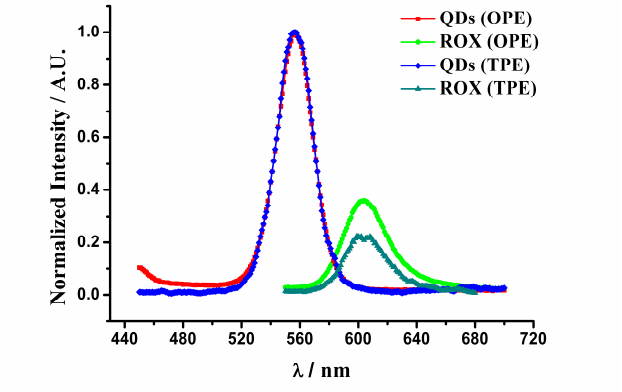

Fig. S7. The normalized direct excitation fluorescence spectra of pure QDs (donor) and ROX (acceptor) under one-photon excitation (at 388 nm, concentrations of QDs and ROX were both 35 nM) and two-photon excitation (800 nm, concentrations of QDs and ROX were both 60 nM).

References

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