# SUPPORTTING INFORMATION

## Gel electrophoresis characterization

We employed gel electrophoresis to characterize as-prepared QD-DNA-biotin-STV nanoprobe.[1-2] When QDs were modified with dually labeled DNA, the conjugate migrated more slowly through gels than QDs alone (Figure S1) due to the retarded electrophoretic mobility. STV binding further led to obvious band retardation since the size and molecular weight of the conjugate were significantly enhanced. Control experiments by mixing QDs with ssDNA-biotin without sulfur modification (lane 4 in Figure S1) and mixing QD-DNA-biotin with BSA (lane 5 in Figure S1) did not show any retardation of the bands, suggesting that minimal non-specific binding occurred at the surface of QDs.

## ELISA characterziation

The functional property (binding affinity) of the QD-DNA-biotin conjugate was evaluated with enzyme-linked immunosorbent assay (ELISA). QD-DNA-biotin was immobilized to extravidin wells and then HRP-conjugated STV was employed to label and visualize the binding process, which showed a characteristic blue color and the absorbance at 595 nm. Significantly, with the increase of QD-DNA-biotin concentrations, the color arising from the HRP-based catalysis intensified (Figure S2). In negative control experiments, either QDs with HRP-STV or HRP-STV alone led to minimal absorption at 595 nm, suggesting little non-specific adsorption of HRP-STV to ELISA wells. These data clearly showed that biotin tags remained to be functional at the surface of QDs for further STV binding.

## EXPERIMENTAL SECTION

**Materials and Chemicals.** DNA employed in this study was purchased from Sangon (Shanghai, China). The sequence of the dually labeled DNA is HS-5'-$T_{30}$-3'-biotin. Tellurium powder (99.9%), $CdCl_2$ (99.9%), agarose, bovine serum albumin (BSA), STV, extravidin and HRP-STV were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tween 20 and 3-mercaptopropionic acid (MPA, 98%) were purchased from Fluka. Carcinoma embryonic antigen (CEA) and alpha-fetoprotein (AFP) were purchased from Industries International (Concord, USA). Anti-CEA monoclonal antibodies (mAbs, C1300-02) and anti-CEA biotinylated mAbs (C1300-7A) were purchased from USBIO. Sylgard184 were purchased from Dow Corning Co. Ltd (Shanghai, China). Silyated slides (aldehyde activated) were obtained from CEL Associates (Pearland, USA). Silicon patterns were fabricated in Shanghai Institute of Microsystem and Information Technology (SIMIT), Chinese Academy of Sciences.

Monodispersed, aqueous phase synthesized, CdTe/CdS core-shell QDs (aqQDs) capped with MPA were obtained *via* microwave irradiation as described in our

previous work.[3] The maximum emission wavelength of QDs was 593 nm. Such aqQDs are highly stable in PBS buffer (150 mM, pH 7.3) at 4°C, with minimal change in the photoluminescence observed during several months.

**Modification of QDs with DNA.** DNA was attached to QDs *via* ligand exchange. Briefly, 1.2 nmol QDs was mixed with 12 nmol thiolated DNA with the biotin tag. An appropriate calculated amount of 10 × Tris buffer (0.1 M Tris·HCl, 1 M NaCl, pH 7.6) was then added to obtain a final buffer concentration of 1 × Tris buffer (10 mM Tris·HCl, 0.1 M NaCl, pH 7.6). The solution was mixed and stored overnight at 25 ºC overnight in order to allow the complete exchange of the thiol group MPA associated with at the surface of QDs with the thiolated oligonucleotides. Free oligonucleotides were then removed. The DNA modified particles were separated from the free oligonucleotides by ethanol precipitation. That is, 600 μL of ethanol was added to the mixture to precipitate the QD-DNA-biotin conjugate. After being centrifuged at 15,000 rpm for 30 min, the supernatant was discarded and the pellet was re-dispersed by 200 μL 1 × Tris buffer. After sonication, 600 μL of ethanol was added and the solution was further centrifuged at 15,000 rpm for 30 min. The pellet obtained was dissolved in PBS buffer (150 mM, pH 7.3) to a desired concentration for measurement.

**Binding of STV to DNA modified QDs.** Conjugate of QD-DNA-biotin (0.5 nmol) was added to the solution of STV (10 nmol). The mixture was incubated at 25 °C for 30min with gentle shaking. Unbound STV was removed by ultrafiltration using 300 KDa Nanosep centrifugal devices by centrifugation at 5000 rmp for 10 min, and the lower phase containing free STV was discarded. The QD-DNA-biotin-STV conjugate in the upper phase was diluted by 500 μL PBS buffer, and further centrifugated at 5000 rmp for 10 min. This washing step was repeated twice. The conjugate was re-dispersed in PBS and then stored at 4 °C in a dark until use.

**Fluorescence measurements.** All fluorescence spectra were recorded with a fluorescence spectrometer (Hitachi F4500, Japan). The emission spectra (500-700 nm range) were recorded under a fixed excitation wavelength of 350 nm at a scan rate of 2 nm/s. The slot widths of the excitation and emission were both 2.5 nm.

**Gel electrophoresis.** QDs, the conjugates of QD-DNA-biotin and QD-DNA-biotin-STV were diluted in loading buffer and were run in 10 mM $K_2HPO_4$ buffer on a 1% agarose gel for 1.5 h. The gels were illuminated with an ultraviolet transilluminator and the fluorescence of the QDs was imaged *via* a gel imaging system.

**AFM measurements.** A sample of 10 μL was dropped onto the mica surface. After 1 min incubation at room temperature, the mica was rinsed with distilled water three times, and evaporated under a nitrogen stream. AFM in the tapping mode were performed in air using a Nanoscope IIIa microscope (Veeco Instruments, Santa Barbara, CA).

**Characterization of QD-DNA-biotin conjugates with ELISA.** Extravidin diluted with coating buffer was added to wells of a 96-well plate (100 μL/well). The plate was incubated overnight at 37 °C and then washed with washing buffer (PBS, containing 0.1% Tween-20). Binding sites not occupied by the coating protein were

then blocked by the blocking buffer (100 μL/well) for 2 h at room temperature. The plate was washed as before, and then incubated with test samples (QD-DNA-biotin and QDs) of serial dilutions (100 μL/well) for 1.5 hours at 37 $^{o}$C. After washing, HRP-STV diluted to 1: 1000 was added (100 μL/well) and the plate was incubated for 1 h at room temperature. Subsequently, the plate was washed and TMB substrate solution (100 μL/well) added. After incubation with shaking for 30min, the absorption values were recorded at 595 nm using a microplate reader.

**Immunoassay of CEA using a microfluidic protein chip.** CEA was detected using QD-DNA-biotin-STV as a fluorescent probe and a microfluidic protein chip previously reported in our work.[4] Briefly, the capture protein (the first mAbs, 0.5 mg/mL) was patterned on silylated slides through microchannels in the first polydimethylsiloxane (PDMS) layer. After incubated at 37 $^{o}$C for 1 h, the microchannels were rinsed with washing buffer (PBS, containing 0.1% Tween-20) and then the first PDMS piece was removed. The unoccupied sites were blocked with blocking buffer (150 mM PBS, pH 7.4, 5% BSA) for 1 h. The second PDMS piece, with the direction of its channels perpendicular to the first one, was then placed over the patterned area. Then a range of concentrations (50 fM – 50 nM, dissolved in PBS (150 mM, pH 7.4, 2% BSA)) of CEA solutions was passed through the second PDMS layer. After incubation at 37 °C for 1 h and repeated rising steps, biotinylated mAbs (0.2 mg/mL) was introduced to the cross areas where they bound to the target protein. After a rinsing step that removed unbound Abs, the conjugate of QD-DNA-biotin-STV was used to probe the binding process. After the removal of the second PDMS piece, the chip was scanned using a GenePix 400B microarray scanner (Axon Instruments) equipped with optical filters. Signals were excited at 532 nm and collected at 550~600 nm.

References

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2. W. J. Parak, T. Pellegrino, C. M. Micheel, D. Gerion, S. C. Williams and A. P. Alivisatos, *Nano Lett.* **2003**, 33-36.
3. Y. He, H. T. Lu, L. M. Sai, W. Y. Lai, Q. L. Fan, L. H. Wang and W. Huang, *J. Phys. Chem.* **2006**, 13370-13374.
4. J. Yan, M. Hu, D. Li, Y. He, R. Zhao, X. Jiang, S. Song, L. Wang and C. Fan, *Nano Res.* **2008**, 490-496.

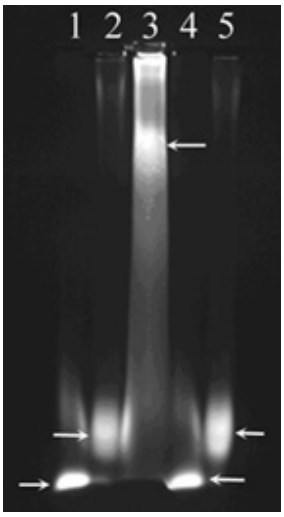


**Figure S1.** Characterization of QD conjugates by 1% agarose gel electrophoresis. The luminescence image was captured under UV illuminated. Lane 1: QDs; lane 2: QD-DNA-biotin; lane 3: QD-DNA-biotin-STV; lane 4: QDs conjugated with DNA-biotin without sulfur modification as a negative control; lane 5: QD-DNA-biotin conjugated with BSA as a negative control.

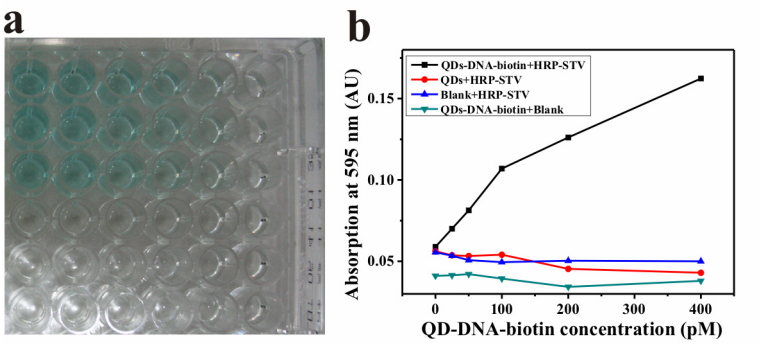


**Figure S2.** ELISA experiments in a 96-well plate. (a) Top three rows: three parallel experimental groups. QD-DNA-biotin was incubated in extravidin coated wells, and then HRP-STV was added. The concentrations of QD-DNA-biotin were 0, 25 pM, 50 pM, 100 pM, 200 pM, 400 pM from left to right. Bottom three rows: negative control groups. Row D: QDs were incubated in extravidin coated wells and HRP-STV was added. The concentrations of QDs were 0, 25 pM, 50 pM, 100 pM, 200 pM, 400 pM from left to right. Row E: PBS was in extravidin coated wells and HRP-STV was added. Row F: QD-DNA-biotin was in extravidin coated wells, while HRP-STV was not added. (b) The absorption was recorded at 595 nm using a micro platereader.

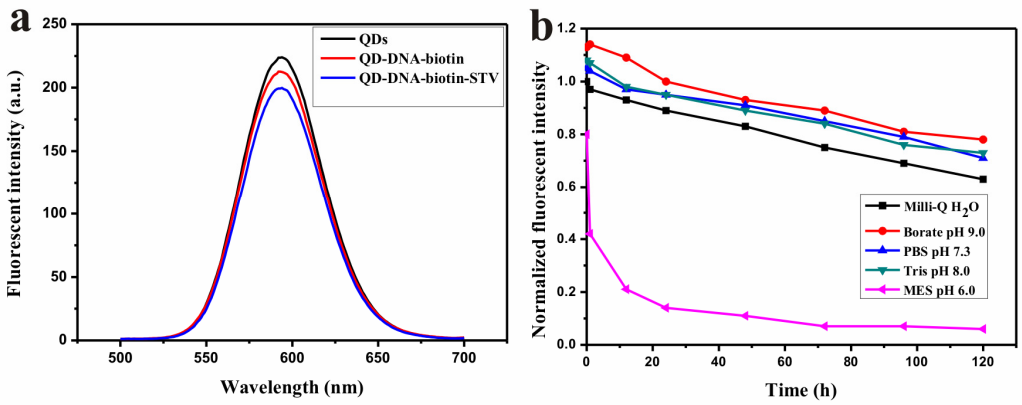


**Figure S3.** (a) Fluorescent spectra of QDs before (black) and after conjugation with the bridge DNA (red) and then with STV (blue). Spectra were recorded under identical experimental conditions at a QD concentration of 200 nM with the excitation wavelength at 350 nm. (b) The PL change of QD-DNA-biotin-STV when stored at 4 °C in dark in several different buffers. The fluorescent intensities were recorded at QDs concentration of 200 nM.

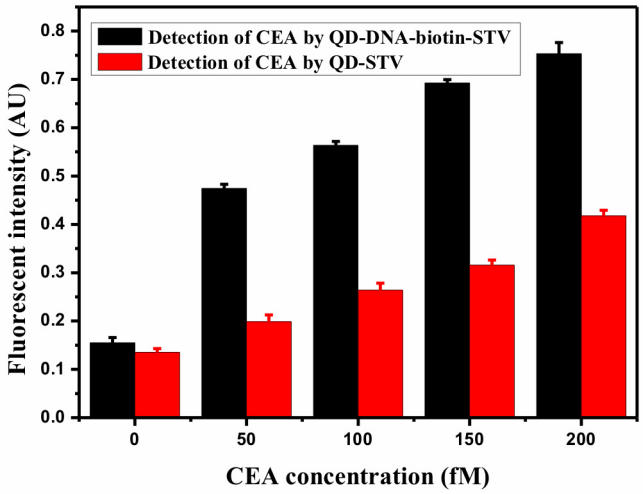


**Figure S4.** Comparison of the detection capability of QD-DNA-biotin-STV and QD-STV. The QD-STV conjugate was prepared *via* covalent crosslinking. The detection was carried out in the same microfluidic protein chip. The LODs were 50 fM and 200 fM (S/N≥3), respectively.

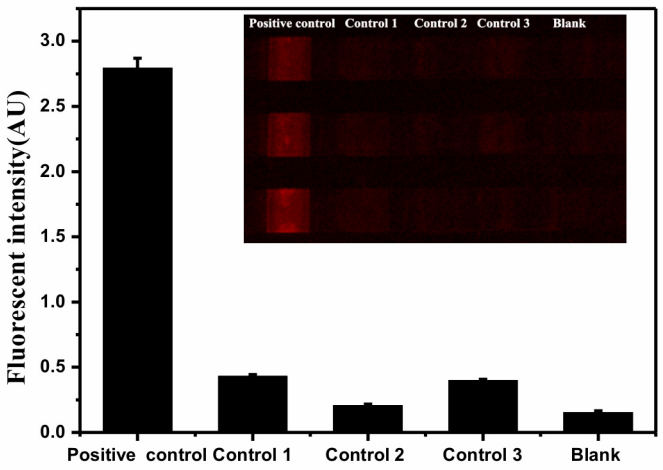


**Figure S5.** Study of non-specific binding of this QD-DNA-biotin-STV probe in protein chip. Positive control: CEA was detected with QD-DNA-biotin-STV. Control 1: AFP was detected with QD-DNA-biotin-STV. Control 2: CEA was detected with QD-DNA-biotin. Control 3: CEA was detected with QD-DNA-biotin-STV, but anti-CEA mAbs was substituted for anti-CEA biotin-mAbs. The concentrations of CEA and APF were 50 pM.