Electronic Supplementary Information

Enhanced photoelectrochemical aptasensing platform based on exciton energy transfer between CdSeTe alloyed quantum dots and SiO_2@Au nanocomposites

Gao-Chao Fan,[a] Hua Zhu,[a] Qingming Shen,[c] Li Han,[a,c], Ming Zhao,[a] Jian-Rong Zhang*,[a,b] and Jun-Jie Zhu*,[a]

[a] *State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, PR China*

[b] *School of Chemistry and Life Science, Nanjing University Jinling College, Nanjing 210089, PR China*

[c] *Institute of Advanced Materials, Nanjing University of Posts and Telecommunications, Nanjing 210046, PR China*

[*] E-mail: jrzhang@nju.edu.cn; jjzhu@nju.edu.cn

Section 1: Experimental

Materials and Reagents. Titanium foil (99.7% purity, 0.127 mm thick), sodium borohydride ($NaBH_4$), Selenium powder (Se), Tellurium powder (Te), 3-mercaptopropionic acid (MPA), poly(diallyldimethylammonium chloride) (PDDA, 20 wt% in water), thrombin, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), tris(2-carboxyethyl)phosphine (TCEP), monoethanolamine (MEA), bovine serum albumin (BSA), hemoglobin (BHb), human IgG (HIgG) and glucose oxidase (GOD) were all obtained from Sigma-Aldrich (USA). Hydrofluoric acid (HF, 40 wt% in water), ammonium hydroxide ($NH_3 \cdot H_2O$, 25 wt% in water) and trisodium citrate dihydrate were obtained from Nanjing Chemical Reagent Co., Ltd. (China). SiO_2 nanoparticles (99.5%, 35±5 nm) were obtained from Aladdin reagent Inc. (Shanghai, China). Cadmium chloride ($CdCl_2 \cdot 2.5H_2O$), sodium hydroxide (NaOH) and chloroauric acid ($HAuCl_4 \cdot 4H_2O$) were purchased from Shanghai Chemical Reagent Co. (China). Ascorbic acid (AA) was purchased from Sinopharm Chemical Reagent Co., Ltd. (China). All other reagents were of analytical grade and used as received. All aqueous solutions were prepared with deionized water (DI water, 18 MΩ/cm), which was obtained from a Milli-Q water purification system. Tris-HCl buffer solution (pH 7.4, 10 mM) containing 0.1 M NaCl for preparation and hybridization of DNA stock solutions.

The oligonucleotides were ordered from Shenggong Bioengineering Co., Ltd. (Shanghai, China) with the following sequences: thrombin aptamer, 5'-GGTTGGTGTGGTTGG-3'; NH_2-DNA, 5'-NH_2-TTTTTCCAACCAC- 3'; SH-DNA, 5'- ACCAACCTTTTTT-SH-3'.

Apparatus. The microwave synthesis of quantum dots was performed on a WBFY-201 Microwave Oven equipped with atmospheric reflux device (Nanjing Keer Instrument Equipments Co. Ltd., Nanjing, China). Photoelectrochemical measurements were performed with a homemade photoelectrochemical system. A 500 W Xe lamp, with a spectral range of 200-2500 nm, was used as the irradiation source with the light intensity of 400 $\mu W \cdot cm^{-2}$ estimated by a radiometer (Photoelectric Instrument Factory of Beijing Normal University). Photocurrent was measured on a CHI 660D electrochemical workstation (Shanghai Chenhua Apparatus Corporation, China) with a three-electrode system: a modified TiO_2:N-NTs electrode with a geometrical area of 0.25 cm^2 as working electrode, a Pt wire as counter electrode and a saturated Ag/AgCl electrode as reference electrode. The UV-visible (UV-vis) absorption spectra were tested on a UV-3600 UV-visible spectrophotometer (Shimadzu, Japan). Photoluminescence (PL) spectra were recorded on a RF-5301PC spectrofluorophotometer (Shimadzu, Japan). Field-emission scanning electron microscopy (FE-SEM) was carried out on a Hitachi S-4800 scanning electron microscope (Hitachi Co., Japan) equipped with EX-250 Energy-dispersive X-ray spectroscopy (EDX, HORIBA Co., Japan). Transmission electron microscopy (TEM) was performed with a JEOL-2100 transmission electron microscope (JEOL, Japan).

Synthesis of CdSeTe AQDs. The synthetic procedure of water-soluble MPA-capped CdSeTe AQDs was based on our previously reported method with appropriate modifications.[1] After 50 mL of 5 mM $CdCl_2$ was mixed with 37 μL of MPA, 1 M NaOH was added to adjust its pH to 12.2 and bubbled with highly pure N_2 for 30 min. Then premixed solution of freshly prepared NaHTe and NaHSe with different Te/Se molar ratios were added into the $CdCl_2$ solution. The typical molar ratio of Cd^{2+}/Te^{2-}/MPA was 1/0.04/1.7. The mixture solution was stirred at low temperature (< 20 °C) for 1 h under N_2 protection, and then was treated with microwave irradiation at 400 W for 4 min. Finally, the CdSeTe AQDs solution was acquired.

Preparation of TiO_2:N-NTs/CdSeTe electrode. The TiO_2-NTs electrode was prepared by anodic oxidation of a pure titanium sheet. Prior to anodization, titanium sheets were mechanically polished with abrasive papers and rinsed with DI water, and then ultrasonically cleaned in acetone, ethanol and DI water for 10 min, respectively. The cleaned titanium sheet was anodized at 5 V in 0.5 wt % HF solution at room temperature for 20 min in a two-electrode system with a platinum electrode as the cathode. The as-prepared TiO_2-NTs electrode was immersed in 1 M $NH_3 \cdot H_2O$ solution for 12 h and then annealed at 450 ºC for 1 h in ambient air atmosphere to form the uniform anatase TiO_2:N-NTs electrode.[2] The CdSeTe film was assembled by alternately dipping the TiO_2:N-NTs electrode into a 0.5% PDDA solution and the as-obtained CdSeTe AQDs solution for 10 min, respectively. The film was carefully washed with DI water after each dipping step. The two-step dipping procedure was termed as “one coating”. The coating process was repeated four times and the TiO_2:N-NTs/CdSeTe electrode was prepared.

Synthesis of Au NPs. Au NPs were synthesized based on the reported method with some modifications.[3] Briefly, an aqueous solution containing 75 mL of DI water, 1.5 mL of 25 mM $HAuCl_4$ and 5 mL of 10 mM trisodium citrate was first prepared in a conical flask. Next, 1.5 mL of ice-cold, freshly prepared 0.1 M $NaBH_4$ solution was added into the above solution while stirring. The mixture solution turned pink immediately after adding $NaBH_4$, indicating particle formation. After stirred for 6 h at room temperature, the resulting Au NPs solution was obtained and kept in a refrigerator at 4 ºC for further use.

Preparation of SiO_2@Au/SH-DNA conjugates. In a typical process, 10 mg of purchased SiO_2 NPs were first dispersed into 3 mL of 5% PDDA aqueous solution at room temperature. After shaking for 3 h, the suspension was centrifuged and washed with DI water several times to remove unadsorbed PDDA. Subsequently, the PDDA coated SiO_2 NPs were resuspended into 3 mL of DI water followed by adding excessive of purified Au NPs into the suspension. After shaking for 2 h, the centrifugation/wash cycles were operated to remove unadsorbed Au NPs. The obtained SiO_2@Au NCs were resuspended into 1 mL of 10 μM SH-DNA which was activated with TCEP (10 mM) beforehand, and kept shaking for 12 h. The unbound SH-DNA was removed by centrifugation at 4 °C for 15 min twice, and then the SiO_2@Au/SH-DNA was acquired.

Fabrication of the aptasensor. NH_2-DNA was immobilized onto the TiO_2:N-NTs/CdSeTe electrode via the classic EDC coupling reaction between carbonyl groups on the surface of the MPA-capped CdSeTe AQDs and amino groups of the NH_2-DNA. Firstly, the CdSeTe AQDs modified electrode was activated by dropping 25 μL DI water containing 20 mM EDC and 10 mM NHS for 1 h at room temperature, followed by rinsing with Tris-HCl buffer (10 mM, pH 7.4) to remove the excess EDC and NHS. Then 20 μL of 1 μM NH_2-DNA was dropped onto the electrode surface and incubated at 4 °C overnight, and then the electrode was rinsed with Tris-HCl buffer to remove unbound NH_2-DNA. Next, the electrode was blocked with 20 μL of 1 mM MEA at room temperature for 1 h and rinsed with Tris-HCl buffer thoroughly. Subsequently, the electrode was successively incubated with 20 μL of 1 μM thrombin aptamer and SiO_2@Au/SH-DNA at 37 °C for 1 h, and rinsed with Tris-HCl buffer after each incubation step, which allowed NH_2-DNA and SH-DNA to hybridize with thrombin aptamer. After that, the resulting electrode was employed as a photoelectrochemical aptasensor and incubated with 20 μL of different concentrations of thrombin solutions at 37 °C for 1 h. Finally, the electrode was rinsed with Tris-HCl buffer and prepared for photoelectrochemical detection.

Photoelectrochemical measurement. Photoelectrochemical detection was carried out at room temperature in Tris-HCl buffer (0.1 M, pH 7.4) containing 0.1 M AA, which was served as a sacrificial electron donor during the photocurrent measurement. Light excitation of 460 nm was switched on and off every 10 s. The applied potential was 0.0 V. The AA electrolyte was deaerated by pure nitrogen for 15 min before photocurrent measurement.

Section 2: HRTEM images of the CdSeTe AQDs and Au NPs

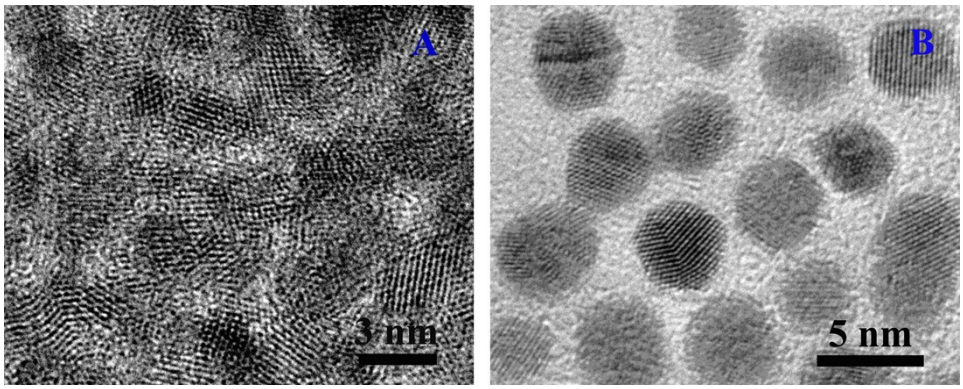

Figure S1. HRTEM images of the (A) CdSeTe AQDs and (B) Au NPs.

To confirm successful synthesis of the CdSeTe AQDs and Au NPs, HRTEM characterization was performed. Fig. S1-A and S1-B shows the HRTEM images of the prepared CdSeTe AQDs and Au NPs, respectively. The lattice fringes of the CdSeTe AQDs and Au NPs were clearly observed, and their average sizes of about 2.84 nm and 4.15 nm were obtained.

Section 3: EDX spectrum of the TiO_2:N-NTs electrode

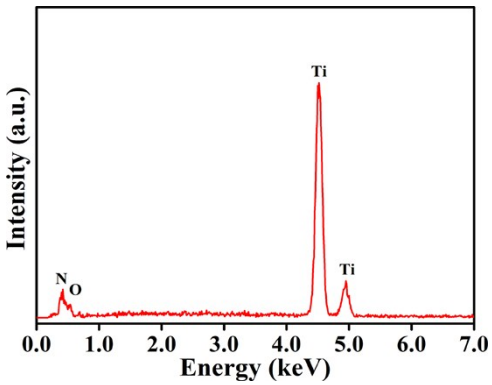

Figure S2. EDX spectrum of the prepared TiO_2:N-NTs electrode.

Section 4: Optimization of the Te/Se ratio in CdSeTe AQDs

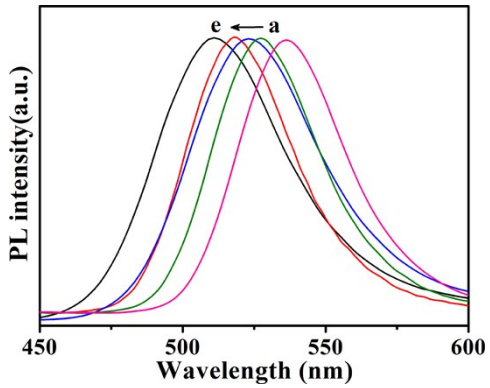

Figure S3. Normalized PL spectra of the CdSeTe AQDs with different Te/Se molar ratios after microwave radiated for 4 min: (a) 100/0, (b) 83/17, (c) 68/32, (d) 60/40, and (e) 51/49.

Fig. S3 shows the PL spectra of the CdSeTe AQDs with different Te/Se molar ratios after microwave radiated for 4 min. Curve a-d correspond to Te/Se molar ratios of 100/0, 83/17, 68/32, 60/40, and 51/49, respectively. The PL emission peaks of curve a to curve d were located at 536, 527, 522, 517, and 511 nm, respectively. As the plasmon absorption peak of the synthesized Au NPs was located at 515 nm, the CdSeTe AQDs with Te/Se molar ratio of 60/40 (PL emission peaks at 517 nm) were used as energy donors to ensure maximum spectral overlap.

Section 5: Optimization of the coating number of CdSeTe AQDs

As the thickness of the CdSeTe film could influence the photocurrent intensity of the TiO_2:N-NTs/CdSeTe electrode, the coating number of the CdSeTe AQDs was optimized. Fig. S4 exhibits the photocurrent intensity of the TiO_2:N-NTs/CdSeTe electrodes obtained undergoing different coating numbers of CdSeTe AQDs. As the coating numbers increased up to four, the thickness of

CdSeTe film increased progressively causing more and more light absorption, and the photocurrent intensity increased. After the coating number further increased, more and more surface recombination centers formed on excess CdSeTe AQDs, and therefore the photocurrent intensity decreased. Accordingly, four coating numbers of CdSeTe AQDs was selected to fabricate the sensing electrode in this work.

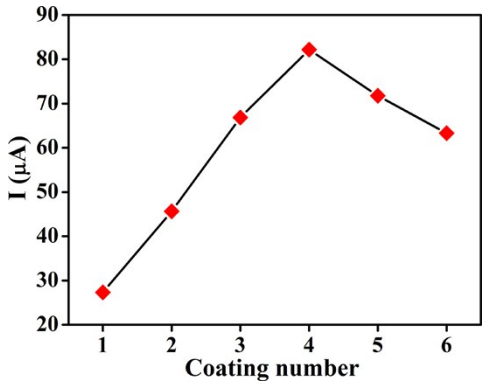

Figure S4. Effect of coating number of the CdSeTe AQDs on photocurrent intensity of the TiO_2:N-NTs/CdSeTe electrode.

Section 6: Concentration optimization of NH_2-DNA and thrombin aptamer

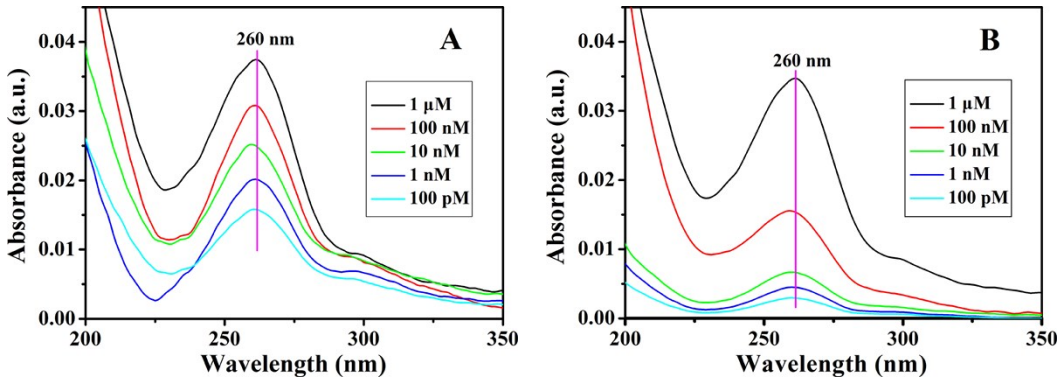

Figure S5. UV-vis absorption spectra of different concentrations of NH_2-DNA (A) before and (B) after incubation step.

In order to optimize the concentration of incubated NH_2-DNA, UV-vis absorption spectra of different concentrations of NH_2-DNA before and after incubation step were tested, and all of the absorption peaks located at 260 nm. As shown in Fig. S5, Part A represents before incubation, whereas Part B stands for after incubation. Compare with absorption intensity before incubation, very weak absorptions were fund after the TiO_2:N-NTs/CdSeTe electrode was incubated with 100 pM, 1 nM, and 10 nM NH_2-DNA. After 100 nM NH_2-DNA was incubated, the absorption began to evidently increase, which indicate that the 100 nM NH_2-DNA was exceed. Thus, it can be conclude that the immobilized concentration of NH_2-DNA on the TiO_2:N-NTs/CdSeTe electrode was in the range of 10 nM to 100 nM. In order to ensure maximum immobilization, the added bioprobes usually exceed 10-100 times. Thus the concentration of 1 μM NH_2-DNA was utilized in

the present work. As thrombin aptamer hybridized with NH_2-DNA according to 1:1 mole ratio, the concentration of 1 μM thrombin aptamer was also used in this work.

Section 7: Comparison of using SiO_2@Au NCs and Au NPs as energy acceptors

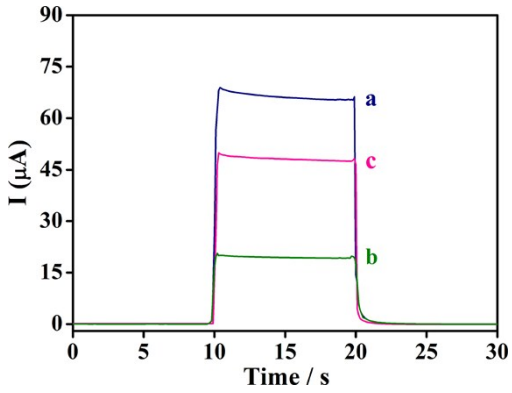

Figure S6. Photocurrent responses of the (a) TiO_2:N-NTs/CdSeTe/NH_2-DNA/MEA/aptamer electrode, after (b) SiO_2@Au/SH-DNA immobilization or after (c) Au/SH-DNA immobilization.

In order to demonstrate high SPR absorption efficiency of SiO_2@Au NCs, bare Au NPs were used as energy acceptors for comparison. As shown in Fig. S6, after SiO_2@Au NCs immobilized on the aptamer modified electrode, the photocurrent intensity significant decreased from curve a to curve b (ΔI=46.68 μA). Yet, the photocurrent intensity decreased moderately from curve a to curve c (ΔI=18.14μA) after Au NPs immobilization, which was only about 39% of that SiO_2@Au immobilization. Thus, the comparison results proved distinct advantages of the SiO_2@Au NCs as energy acceptors.

Section 8: Selectivity, reproducibility and stability of the aptasensor

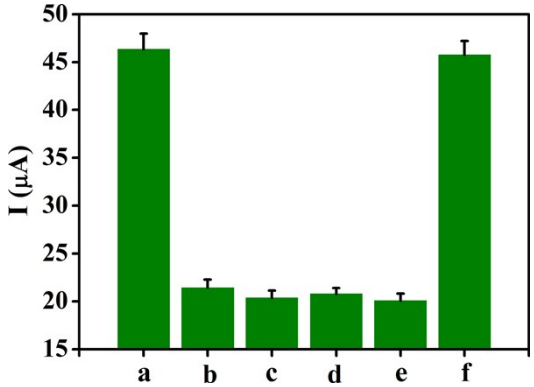

Figure S7. Photocurrent responses of the aptasensor to (a) 1 pM of thrombin, 10 pM of (b) BSA, (c) BHb, (d) HIgG and (e) GOD, and (f) their mixture. The error bars showed the standard deviation of five replicate determinations.

The selectivity of the designed photoelectrochemical aptasensor was evaluated by comparing

the photocurrent response to some representative interferents, including bovine serum albumin (BSA), hemoglobin (BHb), human IgG (HIgG) and glucose oxidase (GOD). As can be seen from Fig. S7, the photocurrent response to 10 pM of BSA, BHb, HIgG and GOD were much lower than that of 1 pM of thrombin, indicating negligible interference of these interferents. Besides, the photocurrent response to the mixed sample composed of 1 pM of thrombin, and 10 pM of BSA, BHb, HIgG and GOD was also investigated, and no significant difference of photocurrent response could be observed as compared to the result obtained in the presence of only 1 pM of thrombin. All these results demonstrated that the proposed aptasensor had a satisfactory specificity to thrombin detection.

The reproducibility of the aptasensor was assessed by both intra-assay and inter-assay relative standard deviation (RSD). Analyzed from the photocurrent responses of five replicate determinations, the intra-assay RSDs were 3.9%, 3.5%, and 3.2% towards 0.1, 1, and 10 pM of thrombin, respectively. The inter-assay RSD of 3.4%, 3.9%, and 3.6% were acquired via measuring the same samples with five electrodes fabricated independently under identical experimental conditions. The results suggested a satisfactory precision and reproducibility of the aptasensor.

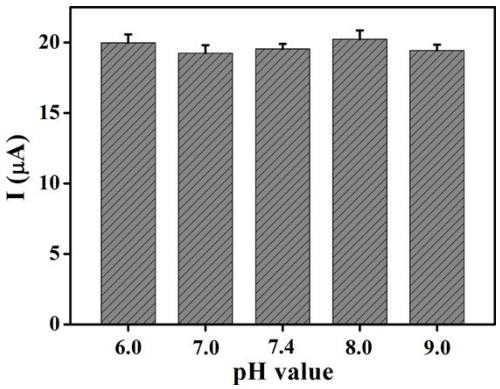

Figure S8. Photocurrent responses of the aptasensor after being incubated in Tirs-HCl buffer with different pH from 6.0 to 9.0. The error bars showed the standard deviation of five replicate determinations.

The stability of the aptasensor was estimated by the photocurrent change of the sensing electrode. The photoelectrochemical aptasensor was stored in a dark and humid environment at 4 °C before it was used for further measurement. After two weeks, the photocurrent intensity retained 93.6% value of its initial response, indicating its good storage stability. Besides, the stability of the aptasensor was also tested under different pH conditions. As shown in Fig. S8, the photocurrent intensity of the sensing electrode did not change after being incubated in Tris-HCl buffer with the variation of pH from 6.0 to 9.0, proving good pH stability of the designed aptasensor.

Section 9: References

1. L. Li, Y. Chen, Q. Lu, J. Ji, Y. Shen, M. Xu, R. Fei, G. Yang, K. Zhang, J. R. Zhang and J. J. Zhu, *Sci. Rep.*, 2013, **3**, 1529.
2. Y. K. Lai, J. Y. Huang, H. F. Zhang, V. P. Subramaniam, Y. X. Tang, D. G. Gong, L. Sundar, L. Sun, Z. Chen and C. J. Lin, *J. Hazard. Mater.*, 2010, **184**, 855−863.
3. N. R. Jana, L. Gearheart and C. J. Murphy, *Langmuir,* 2001, **17**, 6782−6786.