Electronic Supplementary Information

An ultrasensitive "on-off-on" photoelectrochemical aptasensor based on signal amplification of fullerene/CdTe quantum dots sensitized structure and efficient quenching with manganese porphyrin

Mengjie Li, Yingning Zheng, Wenbin Liang, Yali Yuan, Yaqin Chai* and Ruo Yuan*

Experimental section

Materials and reagents

3-mercapzopropionic acid (3-MPA), manganese porphyrin (MnPP), fullerene and L-cysteine (L-Cys) were bought from J&K Scientific Ltd. (Beijing, China). Hemoglobin (Hb), thrombin (TB), hexanethiol (HT), cadmium chloride ($CdCl_2$) and sodium tellurite (Na_2TeO_3) were bought from Sigma Chemical Co. (St. Louis. MO, USA). $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ were obtained from Beijing Chemical Reagent Co. (Beijing, China). N-hydroxy succinimide (NHS) and N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) were obtained from Shanghai Medpep Co., Ltd. (Shanghai, China). Sodium borohydride ($NaBH_4$) and sodium citrate were obtained from Chengdu KeLong Co., Ltd. (Chengdu, China). Tris-hydroxymethylaminomethane-hydrochloride (Tris) was obtained from Shanghai Roche Pharmaceutical Ltd. (Shanghai, China). Ascorbic acid (AA) was bought from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 0.1 M KCl, 0.1 M

KH_2PO_4 and 0.1 M Na_2HPO_4 were used to prepare 0.1 M phosphate buffered solution (PBS, pH 7.0). 20 mM Tris-HCl solution (pH 7.4) consisting of 1 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM KCl and 140 mM NaCl was utilized for preparing DNA solutions. The oligonucleotides sequences were bought from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China). The sequences were listed as follows:

Complementary thrombin binding aptamer (CTBA): 5′-NH_2-$(CH_2)_6$-CCA ACC ACA CCA ACC-3′;

Thrombin binding aptamer (TBA): 5′-GGT TGG TGT GGT TGG AGA AGA AGG TGT TTA AGT A-3′;

S_1: 5′-AGG GCG GGT GGG TGT TTA AGT TGG AGA ATT GTA CTT AAA CAC CTT CTT CTT GGG T-3′;

S_2: 5′- TGG GTC AAT TCT CCA ACT TAA ACT AGA AGA AGG TGT TTA AGT TGG GTA GGG CGG G-3′

Other chemicals used throughout this work were of analytical grade. Ultrapure water was employed for preparing solutions in this work.

The human real serum samples used for preliminary application of the PEC aptasensor were obtained from the Ninth People's Hospital of Chongqing (Chongqing, China), which were isolated from the whole blood by the centrifugation at 3000 rpm for 20 min and kept frozen at -80 °C for further use. This study protocol was performed in compliance with the relevant laws and institutional guidelines, and also approved by the local Institutional Review Board (IRB). Besides, informed consent for preliminary application with these samples was obtained.

Apparatus

The photoelectrochemical (PEC) measurements were carried out with a PEC

workstation (Ivium, Netherlands). Electrochemical impedance spectroscopy (EIS) measurements were realized with a CHI 660e electrochemistry workstation (Shanghai Chenhua Instrumission, China). The morphologies of the prepared nanomaterials were characterized by scanning electron microscopy (SEM, S-4800, Hitachi, Japan) with the voltage of 20 kV. UV-visible (UV-vis) absorption spectrums were carried out on UV-2450 UV-vis spectrophotometer (Shimadzu, Tokyo, Japan). A three-electrode system composed of a platinum wire as the counter electrode, an Ag/AgCl (saturated KCl) as the reference electrode and a glassy carbon electrode (GCE, Φ = 4 mm) as the working electrode.

Synthesis of CdTe quantum dots (CdTe QDs) and nano-C_{60}

The CdTe QDs were synthesized according to the previously reported method with minor modification.[1] Firstly, 0.074 g $CdCl_2$ was dissolved in ultrapure water (100 mL). Subsequently, under magnetic stirring, 0.10 g sodium citrate, 0.002 g Na_2TeO_3, 66 μL MPA and 0.20 g $NaBH_4$ were added into the above solution, respectively. After refluxing under condensation at 110 °C for 10 h, the mixture solution was centrifuged and rinsed carefully for three times with ethanol, and the obtained CdTe QDs was redispersed in 20 mL ultrapure water. Finally, the prepared CdTe QDs was stored at 4 °C in the dark for further use. For preparation of nano-C_{60}, briefly, appropriate amount of fullerene powder was placed in 4 mL toluene with ultrasonication until homogeneous purple solution appeared. The obtained nano-C_{60} solution was kept at 4 °C for further use.

Construction of PEC aptasensor

Prior to modification, the bare GCE was polished with alumina slurry and rinsed

carefully with ultrapure water for a mirror-like surface. Subsequently, 5 μL nano-C_{60} and 10 μL CdTe QDs were then dropped onto the pretreated electrodes, respectively. After drying at 37 °C, the electrodes were immersed into 20 μL PBS (pH 7.0) consisting of 20 mM NHS and 10 mM EDC for activating the carboxyl groups of CdTe QDs at room temperature for 1 h. Subsequently, the electrodes were incubated with 20 μL 1 μM CTBA at room temperature for 40 min *via* classic EDC/NHS coupling reaction. Then, 20 μL 1 μM TBA were dropped onto the modified electrodes and incubated at room temperature for 2h to hybridize with CTBA. After blocking with 20 μL 0.1 mM HT for 40 min, the obtained electrodes were incubated with 25 μL mixture solution containing 10 μL 1 μM S_1, 10 μL 1 μM S_2 and 5 μL 50 μM MnPP at 37 °C for 2 h to trigger the hybridization chain reaction (HCR) for immobilizing massive MnPP on the electrodes. At last, the TB with different concentrations were dropped onto the modified electrodes at room temperature for 40 min. The modified electrodes were thoroughly cleaned with ultrapure water after every modified step to remove the non-chemisorbed species.

PEC Measurement

The PEC aptasensor was analyzed at room temperature in 4 mL PBS (pH 7.0, 0.1 M) containing 0.1 mM AA, which was employed as a sacrificial electron donor during the PEC measurement. The LED lamp was served as the irradiation source and switched off-on-off for 10 s-20 s-10 s under 0.0 V potential.

Results and discussion

Characterization of the different nanomaterials

As shown in Fig. S1A, the as-prepared nano-C_{60} was monitored with SEM. The

typical SEM image of nano-C_{60} presented a well-defined globular structures, implying that the nano-C_{60} was successfully prepared. The sizes and morphologies of the as-synthesized CdTe QDs were characterized by high-resolution transmission electron microscopy (HRTEM). As shown in Fig. S1B, CdTe QDs were well dispersed and separated, which presented lattice plans of the particles with the size about 4 nm.

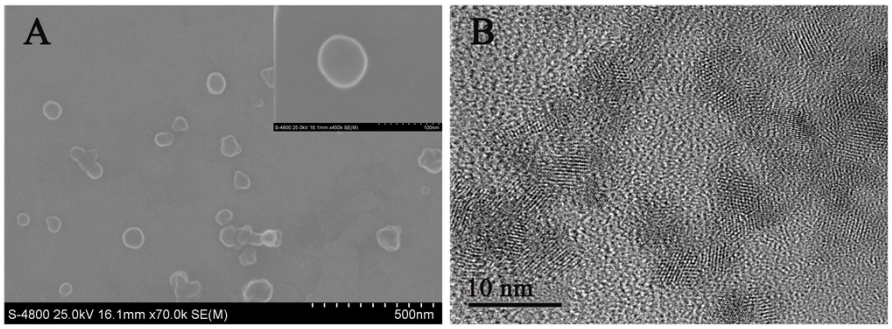

Fig. S1 SEM image of nano-C_{60} (A) and HRTEM image of CdTe QDs (B). The insert of (A) showed a partially enlarged SEM image of nano-C_{60}.

Typical UV-vis absorption spectrums characterization was also employed to further confirm the nano-C_{60}, CdTe QDs and nano-C_{60}/CdTe QDs. As displayed in Fig. S2, three strong optical absorption peaks for nano-C_{60} (curve a) were observed at 218, 262, and 337 nm, respectively. The characteristic absorption peak for CdTe QDs was at about 568 nm (curve b). Comparing to nano-C_{60} (curve a) and CdTe QDs (curve b), the nano-C_{60}/CdTe QDs spectrum (curve c) contained the characteristic absorption peaks of each individual component with a slight red shift which could be ascribed to the effect of residual solvent during the preparation of CdTe QDs.

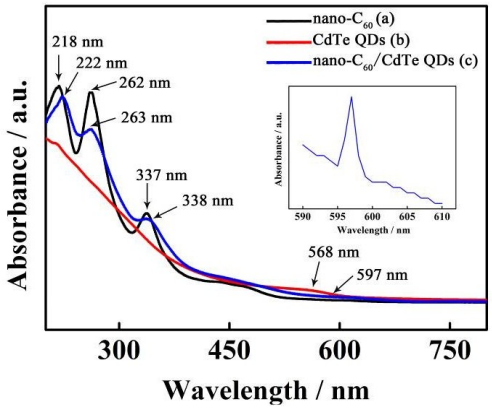

Fig. S2 Typical UV-vis absorption spectrums of (a) nano-C_{60}, (b) CdTe QDs, (c) nano-C_{60}/CdTe QDs. The insert showed a magnification of nano-C_{60}/CdTe QDs in the wavelength range of 590-610 nm.

Electron impedance spectroscopy (EIS) characterization of the PEC aptasensor

To confirm the proposed PEC aptasensor was successfully fabricated, EIS measurement was employed to characterize the stepwise modification process of the electrode. As shown in Fig. S3, after nano-C_{60} was coated onto the GCE surface, the charge-transfer resistance (R_{et}) increased (curve b) compared to that of bare GCE (curve a), because the uniform film of nano-C_{60} could block the electron transfer. When CdTe QDs was dropped on the modified electrode, the R_{et} increased sequentially (curve c) mainly due to the carboxyl groups of CdTe QDs, which could repel the transfer of the negatively charged probe ($[Fe(CN)_6]^{3-/4-}$). After the stepwise assembling of CTBA, TBA and HT, the R_{et} increased consecutively (curve d, e and f, respectively). After the mixture solution containing S_1, S_2 and MnPP was immobilized on the modified electrode surface, a remarkable decrease of the R_{et} was observed (curve g), since the massive MnPP embedded in DNA duplex performed excellent conductivity. The results suggested that a sensing interface was effectively

constructed.

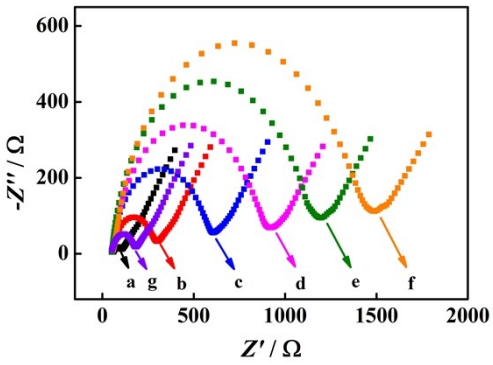

Fig. S3 EIS responses of (a) bare GCE, (b) nano-C_{60}/GCE, (c) CdTe QDs/nano-C_{60}/GCE, (d) CTBA/CdTe QDs/nano-C_{60}/GCE, (e) TBA/CTBA/CdTe QDs/nano-C_{60}/GCE, (f) HT/TBA/CTBA/CdTe QDs/nano-C_{60}/GCE, (g) S_1-S_2-MnPP/HT/TBA/CTBA/CdTe QDs/nano-C_{60}/GCE. The EIS measurement was performed in 2 mL PBS (pH 7.0, 0.1 M) containing 0.1 M KCl and 5.0 mM $[Fe(CN)_6]^{3-/4-}$ which acted as redox probe with a scan rate of 100 mV/s.

PEC characterization of the aptasensor

As shown in Fig. S4, photocurrent characterization for the proposed PEC aptasensor was carried out. The photocurrent closing to zero was observed from the bare GCE (curve a). When nano-C_{60} was modified on the GCE, the photocurrent enhanced remarkably (curve b), indicating the excellent photoelectric activity of nano-C_{60}.[2] Subsequently, when CdTe QDs was immobilized on the modified electrode, the photocurrent (curve c) increased to about 2.5-fold higher than that of the nano-C_{60} modified electrode owing to the commendable sensitization effect of CdTe QDs towards nano-C_{60}. After the modified electrode was incubated with CTBA, TBA and HT, the photocurrent decreased successively (curve d, e and f, respectively), which might be attributed to the relatively poor charge transfer of oligonucleotides

sequences and small organic molecules. And then, when the modified electrode was incubated with the mixture solution containing S_1, S_2 and MnPP, the photocurrent decreased dramatically (curve g), since the MnPP embedded in DNA duplex could efficiently quench the photocurrent response of nano-C_{60}/CdTe QDs. Finally, a marked increase of the photocurrent was observed (curve h) with the addition of TB (10 pM), it could be ascribed to that the formation of aptamer-TB complex made the release of MnPP from the modified electrode surface.

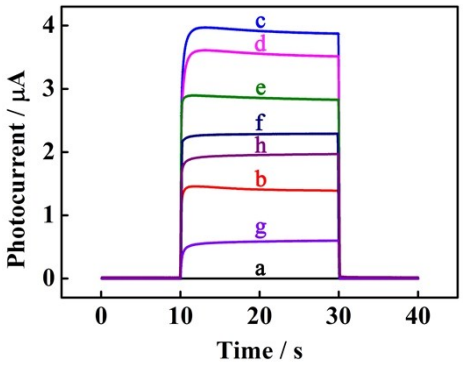

Fig. S4 PEC responses for each immobilized step: (a) bare GCE, (b) nano-C_{60}/GCE, (c) CdTe QDs/nano-C_{60}/GCE, (d) CTBA/CdTe QDs/nano-C_{60}/GCE, (e) TBA/CTBA/CdTe QDs/nano-C_{60}/GCE, (f) HT/TBA/CTBA/CdTe QDs/nano-C_{60}/GCE, (g) S_1-S_2-MnPP/HT/TBA/CTBA/CdTe QDs/nano-C_{60}/GCE, (h) TB/ S_1-S_2-MnPP/HT/TBA/CTBA/CdTe QDs/nano-C_{60}/GCE. The PEC measurement was carried out in 4 mL PBS (pH 7.0, 0.1 M) containing 0.1 mM AA.

Influence of MnPP on PEC signal

To investigate the quenching effect of quencher towards PEC signal, MnPP with various concentrations were employed for constructing PEC aptasensor in our work. As shown in Fig. S5, it was clear to obverse that a dramatic decrease for photocurrent when the concentration of MnPP was varied from 0 μM to 50 μM. Besides, with the

increasing of the concentration of MnPP from 50 μM, the photocurrent decreased gently. More inspiringly, a 82% quenching percentage for photocurrent was found when the concentration of MnPP was 50 μM. This phenomenon not only revealed the fact that MnPP could be considered as an efficient signal quencher towards nano-C_{60}/CdTe QDs, but also indicated that the optimal concentration of MnPP for signal quenching was 50 μM.

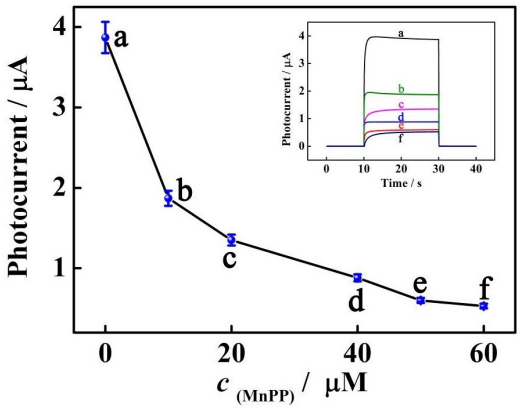

Fig. S5 The influence of MnPP with various concentrations on PEC signal. The insert showed the corresponding PEC signal with different MnPP concentrations in 4 mL PBS (pH 7.0, 0.1 M) containing 0.1 mM AA: (a) 0 μM, (b) 10 μM, (c) 20 μM, (d) 40 μM, (e) 50 μM, (f) 60 μM.

Condition optimization

To obtain superior sensing performance, experimental optimizations including the volume ratio of CdTe QDs to nano-C_{60}, the incubation time for HCR, the concentration of AA and the irradiation wavelength were investigated in our work. The effect of the volume ratio of CdTe QDs to nano-C_{60} on this PEC aptasensor was firstly investigated. As expected in Fig. S6A, the photocurrent increased obviously with increasing the volume ratio from 0.33 to 2 and came to decrease when the volume ratio was larger than 2. Thus, the 2:1 volume ratio between CdTe QDs and

nano-C_{60} was employed for this PEC aptasensor. The optimization of the incubation time for HCR was carried out in the range from 0.5 h to 3.5 h. As illustrated in Fig. S6B, the photocurrent decreased sharply with increasing the incubation time for HCR from 0.5 to 2 h and trended to level off, which indicated that the optimal incubation time for HCR was 2 h. Furthermore, the concentration of AA was optimized to obtain a higher PEC response. As shown in Fig. S6C, the photocurrent increased remarkablely with increasing the concentration of AA, and then decreased gently when the concentration of AA came to 0.1 mM. Accordingly, 0.1 mM AA was selected for optimal concentration of AA in the detection electrolyte. Moreover, the irradiation wavelength was also explored in our work. As illustrated in Fig. S6D, the photocurrent at 590 nm (column d) wasn't greater than any other column. Therefore, 590 nm was the optimal choice of the irradiation wavelength in this PEC aptasensor.

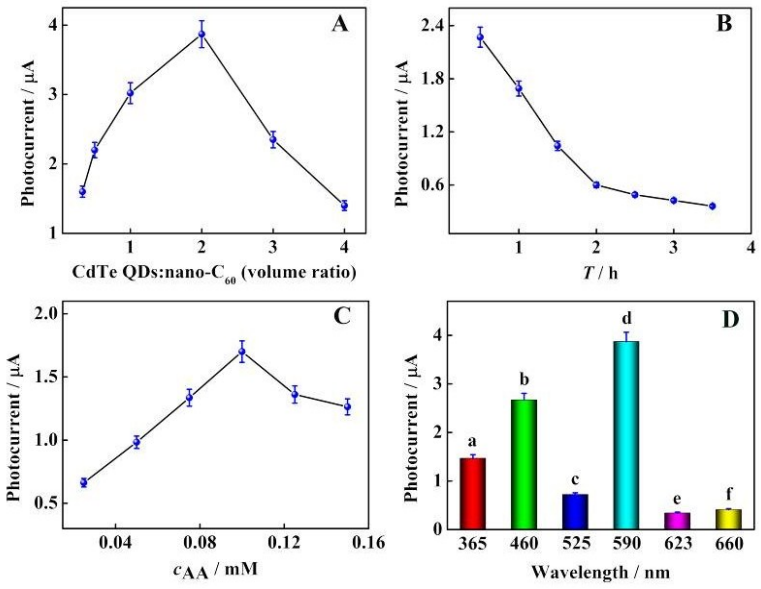

Fig. S6 The optimization for (A) the volume ratio of CdTe QDs to nano-C_{60}, (B) the incubation time for HCR, (C) the concentration of AA and (D) the irradiation wavelength in detection solution on the photocurrent responses of the PEC aptasensor.

Selectivity and stability of the PEC aptasensor

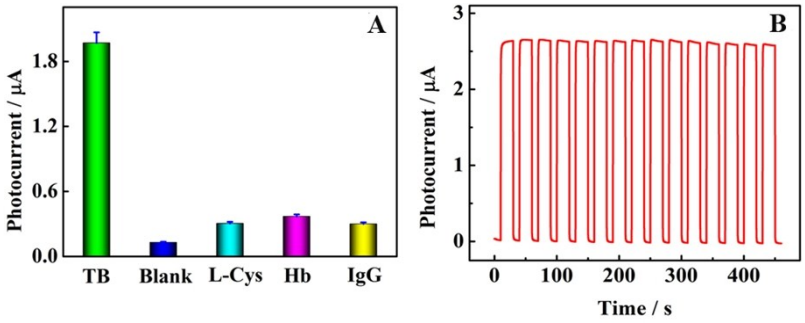

Fig. S7 (A) Selectivity of the PEC aptasensor with different targets: TB (0.01 nM), L-Cys (1 nM), Hb (1 nM) and IgG (1 nM). (B) Stability of the PEC aptasensor incubated with 1 nM TB under periodic off-on-off light for 15 cycles.

Table S1 Comparison of the TB detection with other detection methodologies

Analytical method	Detection limit	Linear range	Ref.
PEC	0.3 fM	1 fM~10 nM	***Our work***
PEC	2.8 fM	10 fM~50 pM	3
CL	80 pM	0.25 nM~5 nM	4
ECL	0.1 nM	1 nM~150 nM	5
UV-vis	1.5 pM	2.5 pM~6.2 nM	6
SV	1 nM	10 nM~10 μM	7
fluorescence	31.3 pM	62.5 pM~187.5 pM	8
DPV	5 pM	5 pM~1 nM	9

Abbreviations: chemiluminescence (CL); electrochemiluminescent (ECL); UV-vis absorbance measurements (UV-vis); stripping voltammetry (SV); differential pulse voltammetry (DPV).

Table S2 Detection of TB added in human serum samples with the proposed PEC aptasensor

Sample number	Added (nM)	Found (nM)	Recovery (%)

1	2.00	1.90	95.00
2	2.00×10^{-1}	2.03×10^{-1}	101.50
3	1.00×10^{-2}	1.01×10^{-2}	101.00
4	5.00×10^{-4}	5.40×10^{-4}	108.00
5	1.00×10^{-5}	0.94×10^{-5}	94.00

References

1 M. N. Ma, Y. Zhuo, R. Yuan and Y. Q. Chai, *Anal. Chem.*, 2015, **87**, 11389.

2 C. G. Hu, J. N. Zheng, X. Y. Su, J. Wang, W. Z. Wu and S. S. Hu, *Anal. Chem.*, 2013, **85**, 10612.

3 F. Xu, Y. C. Zhu, Z. Y. Ma, W. W. Zhao, J. J. Xu and H. Y. Chen, *Chem. Commun.*, 2016, **52**, 3034.

4 Z. Jiang, T. T. Yang, M. Y. Liu, Y. L. Hu and J. Wang, *Biosens. Bioelectron.*, 2014, **53**, 340.

5 P. Zhao, L. F. Zhou, Z. Nie, X. H. Xu, W. Li, Y. Huang, K. Y. He and S. Z. Yao, *Anal. Chem.*, 2013, **85**, 6279.

6 Y. Huang, J. Chen, S. L. Zhao, M. Shi, Z. F. Chen and H. Liang, *Anal. Chem.*, 2013, **85**, 4423.

7 E. Suprun, V. Shumyantseva, T. Bulko, S. Rachmetova, S. Rad'ko, N. Bodoev and A. Archakov, *Biosens. Bioelectron.*, 2008, **24**, 825.

8 H. X. Chang, L. H. Tang, Y. Wang, J. H. Jiang and J. H. Li, *Anal. Chem.*, 2010, **82**, 2341.

9 S. F. Liu, Y. Lin, L. Wang, T. Liu, C. B. Cheng, W. J. Wei and B. Tang, *Anal. Chem.*, 2014, **86**, 4008.