-Supporting Information-

Folding-based Photoelectrochemical Biosensor: Binding-induced Conformation Change of Quantum Dots-tagged DNA probe for Mercury(Ⅱ) Detection

Materials and Apparatus

DNA Oligonucleotides were acquired from Sangon Biotechnology Company, Ltd. (Shanghai, China) and contained the following sequences: 5'- SHCTTGTTTCTCCCCCCTGTTTCTTG-NH_2-3' (MSO). 6-Mercapto-1-hexanol (MCH), tris (hydroxymethyl) aminomethane (Tris), tris (2-carboxyethyl) phosphinehydrochloride (TCEP) and ascorbic acid(AA) were obtained from Sigma Aldrich. $CdCl_2 \cdot 2.5H_2O$ was obtained from Shanghai Jinshan Tingxin Chemical Plant. $Na_2S \cdot 9H_2O$ was obtained from Shanghai Lingfeng Chemical Reagent Co., LTD(Shanghai, China). Phosphate buffer solution (PBS, pH 7.4) is prepared from $Na_2HPO_4 \cdot 12H_2O$ and NaH_2PO_4. A stock solution of the MSO probe was prepared at a concentration of 100 μM in 10 mM Tris-HCl buffer solution (pH7.4) and was stored frozen. A solution of 10 mM Tris-HCl (pH 7.4) was used to dilute the stock solution when needed. A stock solution of Hg^{2+} ions was prepared from $Hg(NO_3)_2$ at a concentration of 1 mM. All the other chemicals were of analytical grade. All aqueous solutions were prepared with ultrapure water (Milli-Q, Millipore). Gold substrates were prepared on Ti sheets. Ti sheets were mechanically polished with different abrasive papers and rinsed in distilled water and then ultrasonically cleaned in a dilute HF solution for 5 min, followed by rinsing with water and then acetone for 10 min. Then the clean Ti sheets were coated with an Au layer (60 nm) by Sputter apparatus.

Photoelectrochemical (PEC) measurements were performed with a homemade PEC system. A 500 W Xe lamp equipped with monochromator was used as irradiation source to produce the monochromatic illuminating light on the front of the electrode. Photocurrent was measured on a CHI 750a electrochemical workstation (Shanghai Chenhua Apparatus Co., China) with a three-electrode system: a modified Au electrode with geometrical circular area (radius-0.5 cm) as the

working electrode, a Pt wire as the counter electrode and a saturated Ag/AgCl electrode as the reference electrode. All the photocurrent measurements were performed at a constant potential of 0 V (versus Ag/AgCl). A 0.1 M PBS containing 0.1 M AA was used as the blank solution for photocurrent measurements, which was degassed by highly pure nitrogen for 10 min before PEC experiments and then kept over a N_2 atmosphere for the entire experimental process. Transmission electron microscopic images (TEM) were performed with a JEOL model 2000 instrument operating at 200 kV accelerating voltage. UV-vis absorption spectra were acquired with a Shimadzu UV-3600 UV/vis spectrophotometer.

Synthesis of TGA-stabilized CdS QDs

The utilized CdS QDs were synthesized according to the previous report.[1,2] Briefly, 250 μL of TGA was added to 50 mL of 1.0×10^{-2} M $CdCl_2$ aqueous solution, N_2 was bubbled throughout the solution to remove O_2 for 30 min at 110℃. During this period, 1.0 M NaOH was added to adjust the pH of the above solution to the desired value of 11. Then, 5 mL of 0.1 M Na_2S aqueous solution was injected into this solution to obtain TGA-capped water-soluble CdS QDs and the reaction mixture was refluxed under N_2 atmosphere for 4 h. This procedure produced CdS QDs with a Cd to S (Cd/S) ratio of 1:1. Finally, the desired TGA-stabilized CdS QDs were obtained and then diluted with the same volume of water and stored in a refrigerator at 4℃ when not in use.

For further activation, the obtained CdS QDs solution was treated according to a documented method with slight modification.[3] Firstly, the aqueous solution was thoroughly washed by isopropanol and centrifuged at 8000 rpm for 10 min. Then the terminal carboxylic acid groups on the QD surfaces were activated by EDC (25 mg), and NHS (12 mg) mixed in 10mM PBS buffer (pH 7.4, 20 mL) for 2 h with continuous gentle mixing.

Assembly of QDs-MSO-Au conjugates and Hg^{2+} detection

The MSO probes were immobilized on gold coated Ti sheets through thiol-gold interactions. Firstly, the Au surface was incubated with the solution of the MSO molecules (1 μM in 10mM PBS, pH7.4) for 12 h at room temperature, then immersed in MCH solution (1mM in 10mM PBS) at the same pH for about 30 minutes to remove the nonspecifically attached DNA and simultaneously to eliminate the steric hindrance among attached DNA molecules. After removal of MCH by thoroughly rinsed with 10mM PBS solution, the amino-terminated surface was

immersed into the freshly activated CdS QDs solution for 10 h at 4℃. Then the electrode was rinsed with 10 mM PBS buffer (pH 7.4) to wash off the excess CdS QDs.

The obtained QDs-DNA-Au assembly was served as the work electrode in the following photoelectric chemical analysis and the initial photocurrent signal is measured in 0.1 M PBS solution containing 0.1 M AA. Next, 25 μL of analyte Hg^{2+} with different concentrations were dropped onto the QDs-MSO modified electrodes for an incubation of 60 min in moist environment at 37 ℃. Thereafter, the electrodes were rinsed with 10 mM PBS, pH 7.4, and then introduced for the respective PEC measurements.

EIS measurement.

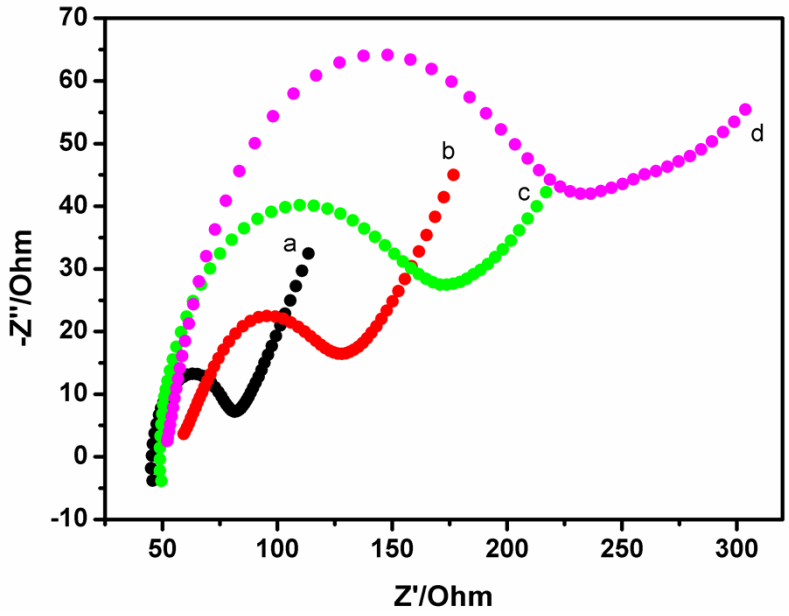

Figure S1 EIS of (a) bare Au electrodes (b) after MSO probe immobilization and MCH blocking, (c) after CdS QDs anchoring (d)after combining the Hg^{2+} corresponding to 500 pM. The EIS measurements are carried out in 0.1 M KCl containing 5.0 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1). The frequency range was between 1 and 100 000 Hz with an applied voltage of 5 mV.

As an effective tool for characterizing the interfacial properties of electrodes, EIS was also utilized to monitor the fabrication process. As shown in Figure S1, for the bare Au electrode, the impedance spectrum exhibited a small semicircle (curve a). Comparing curves a and b, one can see that after the MSO probe is covalently immobilized on the electrode surface, the electron transfer resistance R_{et} increases remarkably. Following subsequent stepwise immobilization of QDs and the Hg^{2+} capture, it increased gradually (curves c−d), turning out the successful assembling of sensing elements on the electrode surface. In curve c, poor conductive performance

of the single-strand leads to the increase of resistance. Although the folding conformation by T–Hg^{2+}–T interaction can partly neutralize negative charges on the electrode surface, the QDs layer progressively obstruct the mass transport and electron transfer of the electrochemical probe to the electrode surface by elevating the hindrance effect and final insulating effect, with an obvious resistance increase in curve d.

XPS element analysis

Table S1 The analysis of element percentage content. "Au-MSO" corresponds to the blank sample without cross-linking of QDs, while ''Au-MSO-CdS QDs'' stands for the CdS QDs modified one .

Name	% Conc.	
	Au-MSO	Au-MSO-CdS QDs
C 1s	45.70	44.32
O 1s	29.79	27.00
N 1s	4.60	5.79
P 2p	4.89	3.57
S 2p	1.69	1.64
Au 4f	13.33	17.51
Cd 3d	0	0.17

Stability of the Hg^{2+} transducer

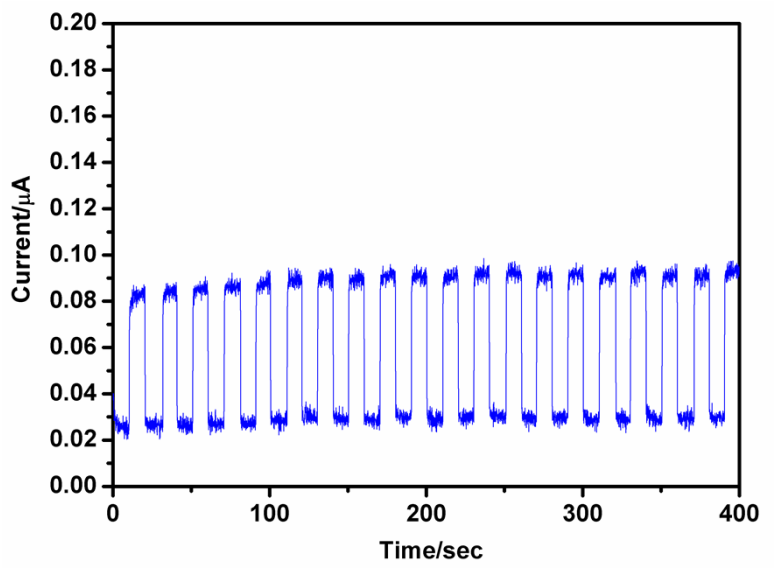

Fig. S2 Photocurrent stability of the proposed Hg^{2+} transducer to 300 pM Hg^{2+}

References.

[1] Zhao, W. W.; Wang, J; Xu, J. J.; Chen, H. Y. Chem. Commun. 2011, 47, 10990.

[2] Zhao, W. W.; Ma, Z. Y.; Yu, P. P.; Dong, X. Y.; Xu, J. J.; Chen, H. Y. Anal. Chem. 2012, 84, 917

[3] Liu, H. Y. ; Xu, S. M. ; He, Z. M. ; Deng, A. P. ; Zhu, J. J. Anal. Chem.2013,85,3385