SUPPORTING INFORMATION FOR

In Situ Electron Donor Consumption Strategy for Photoelectrochemical Biosensing of Protein Based on Ternary $Bi_2S_3/Ag_2S/TiO_2$ NTs Arrays

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Section 1: Experimental

Chemicals. The oligonucleotides were ordered from Sangon Biotech Co., Ltd. (Shanghai, China) with the following sequences: NH_2-Apt, 5'-NH_2-$(CH_2)_6$-TTT TAT ACC AGC TTA TTC AAT T-3'; 5'-SH-$(CH_2)_6$-TTT TAA TTG AAT AAG C-3'. AAO from Cucurbita sp. (EC 1.10.3.3) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). CEA was from Zhengzhou Immuno Biotech Co., Ltd. (Zhengzhou, China). Human serum albumin (HSA), human IgG (hIgG), and bovine serum albumin (BSA) were from Shanghai Solarbio Bioscience & Technology Co., Ltd. (see bio Biotechnology). SiO_2 nanoparticles (99.5%, 30 nm), aminopropyltriethoxysilane (APTES), silver nitrate ($AgNO_3$), bismuth nitrate [$Bi(NO_3)_3 \cdot 5H_2O$] and sodium sulfide ($Na_2S \cdot 9H_2O$) were from Aladdin Reagent Inc. (Shanghai, China). Gold nanoparticles (Au NPs) were prepared according to our previous work[1] AA, glutaraldyhyde (GLD) (50% aqueous solution) and chitosan powder (CS) were from Sinopharm Chemical Reagent Co., Ltd. (China). Methanol and ethanol were from Tianjin Yongda Chemical Reagent Co., Ltd. (Tianjin, China). Phosphate buffer solution (PBS, pH 5.6 or 7.4) was freshly prepared before use. All other reagents were of analytical grade and employed as received. All aqueous solutions were made using pure water. (18.25 MΩ·cm, Kangning water treatment solution provider, China).

Apparatus. All PEC experiments were carried out in a homemade three-electrode system with a modified TiO_2 NTs electrode as working electrode, a Pt-wire as counter electrode, and a Ag/AgCl electrode as reference electrode. The PEC signals were measured with a CHI660E electrochemical workstation (Shanghai Chenhua Apparatus Corporation, China) and a PEAC 200A PEC reaction instrument (Tianjin Aidahengsheng Science-Technology Development Co., Ltd., China). Scanning electron microscopy (SEM) images were obtained by S-4800 (Hitachi, Tokyo, Japan).

Construction of $Bi_2S_3/Ag_2S/TiO_2$ NTs electrode. TiO_2 NTs were synthesized by anodic oxidation treatment according to our previous report.[1] The Ag_2S was deposited on the as-prepared TiO_2 NTs by the successive ionic layer adsorption and reaction

(SILAR) means.[2] At first, the film of TiO_2 NTs was dipped in the 0.05 M $AgNO_3$ ethanol solution for 1 min, then rinsed with ethanol and dried. Subsequently, the film was immersed in 0.05 M Na_2S methanol solution for another 1 min, and washed with methanol as well as dried. This procedure was repeated 4 cycles. The obtained film was called Ag_2S/TiO_2 NTs. The formation of Bi_2S_3 on Ag_2S/TiO_2 NTs was also carried out by SILAR method. The Ag_2S/TiO_2 NTs film was steeped in 0.004 M $Bi(NO_3)_3$ ethanol solution and 0.26 M Na_2S aqueous solution for 10 min, respectively, after each solution, the film was taken out and thoroughly rinsed with respective solvent and dried. The obtained film was called $Bi_2S_3/Ag_2S/TiO_2$ NTs and utilized as the electrode of the biosensor. The same process was used to prepare Ag_2S/TiO_2 NTs and Bi_2S_3/TiO_2 NTs.

Preparation of the probe of Apt-SiO_2-AAO. To improve the combination with NH_2-Apt and AAO, SiO_2 was firstly functionalized by APTES before combination. Secondly, 1.5 mL of aminated SiO_2 was mixed with 0.5 mL GLD solution (2.5 wt %), and then stirred for 6 h at room temperature. Next, the obtained mixture was washed by centrifugation with ethanol and water to remove the unreacted GLD. The obtained aldehyde-functionalized SiO_2 was redissolved in 1 mL PBS (0.01 M, pH 7.4). Subsequently, NH_2-Apt and AAO were immobilized on the aminated SiO_2 through cross-linking by GLD. 400 μL NH_2-Apt (0.20 μM) and 400 μL AAO (0.20 mg mL^{-1}) were added to 400 μL acquired aldehyde-functionalized SiO_2 solution and stirred mildly for 1 h at 37 °C. To remove unconjugated NH_2-Apt as well as AAO, the resulted solution was centrifuged and washed several times with PBS, and then redispersed in 400 μL PBS. After that, 80 μL BSA (1.0 wt %) was devoted to block the nonspecific binding sites for 1 h at 37 °C. At last, after washing, the product was dissolved in 400 μL PBS and stored at 4 °C for future use.

Fabrication of the biosensor. In brief, Au NPs were modified on $Bi_2S_3/Ag_2S/TiO_2$ NTs electrode by CS for 1 h under 4 °C. Then, the SH-DNA was immobilized on the electrode for 1 h at 37 °C. Subsequently, 0.1% BSA was immobilized on the electrode for 0.5 h at 37 °C. Next, the Apt-SiO_2-AAO was anchored onto the electrode for 1 h

at the same temperature. After that, the biorecognition between CEA and NH_2-Apt was performed on above-mentioned condition. After each step, the electrode was carefully washed with 0.01 M PBS. Finally, the constructed biosensor was stored under 4 °C for further application. PEC detection was performed in PBS (pH 7.4, 0.01 M) including 0.1 M AA. The white light with a spectral range from 400 to 700 nm from a 3 W LED lamp was utilized as stimulus light and switched on and off every 10 s, the experiment process lasted for 100s.

Section 2: Characterization of AuNPs

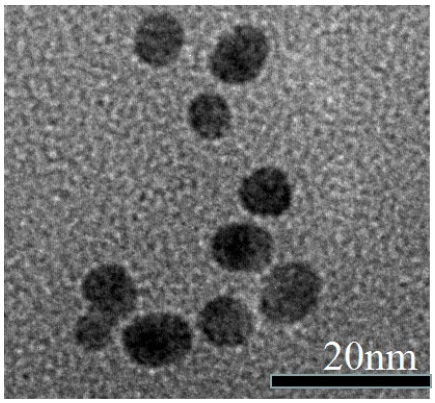

Fig. S1 TEM image of AuNPs.

The morphologies of Au NPs was characterized with TEM. As demonstrated in Fig. S1, the Au NPs have a quasi-spherical structure with a diameter of about 6.4 nm. The result implys that Au NPs were successfully synthesized.

Section 3: Optimization of experimental conditions

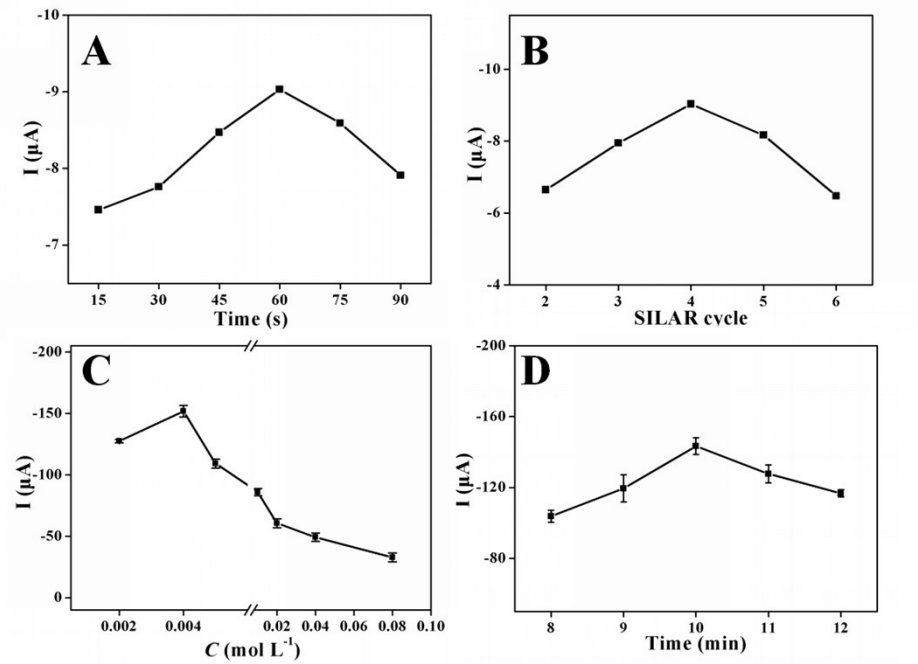

Fig. S2 Effects of (A) depositing time and (B) loading cycles of Ag_2S as well as (C) concentration and (D) depositing time of Bi_2S_3 on photocurrent responses.

The optimum conditions for the construction of $Bi_2S_3/Ag_2S/TiO_2$ NTs electrode were investigated in terms of the depositing time of Ag_2S as well as Bi_2S_3, the loading cycles of Ag_2S and the concentration of Bi_2S_3. As shown in Fig. S2A and B, the photocurrent intensities of Ag_2S reach a maximum value when the depositing time and cycles of Ag_2S achieve 60 s and 4 cycles, respectively. Correspondingly, this optimal case is chosen for modification of Ag_2S. Fig. S2C and D depict the photocurrent intensities responsed to the different concentrations and the depositing time of Bi_2S_3, respectively. Accordingly, 0.004 M with 10 min is selected for decorating Bi_2S_3 on the electrode in the following experiments.

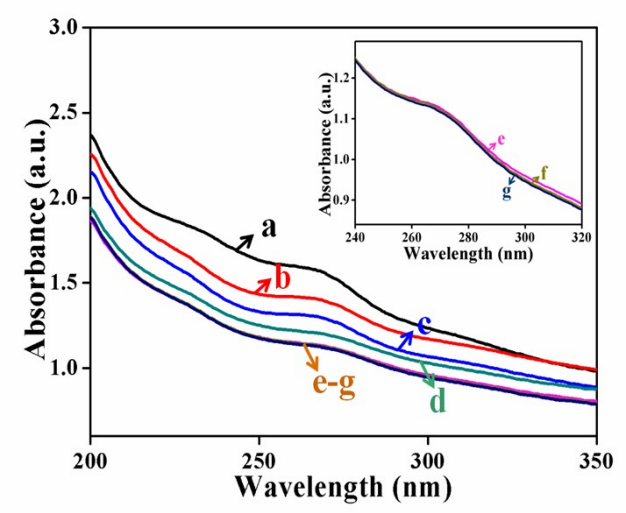

Fig. S3 UV-vis absorption spectra of various probes of Apt-SiO_2-AAO consisted of a certain amount of Apt and different concentrations of AAO: (a) 0.00 mg mL^{-1} AAO; (b) 0.05 mg mL^{-1} AAO; (c) 0.10 mg mL^{-1} AAO; (d) 0.15 mg mL^{-1} AAO; (e) 0.20 mg mL^{-1} AAO; (f) 0.25 mg mL^{-1} AAO; (g) 0.30 mg mL^{-1} AAO.

To evaluate the loading capacity of aptamer and AAO, we firstly fixed the amount of aptamer by optimizing concentration of the aptamer, and according to the experimental results, 0.20 μM of aptamer was used. Then, we explored the loading capacity of AAO by UV-vis absorption spectra of various probes of Apt-SiO_2-AAO consisted of a fixed amount of aptamer and different concentrations of AAO. As shown in Fig. S3, by comparison with absorption intensity of different probes of Apt-SiO_2-AAO, the absorption intensity obviously decreased with the AAO concentration increasing until 0.20 mg mL^{-1}. When the concentration exceeded 0.20 mg mL^{-1}, the absorption intensity gives no obvious change. The results indicated that the AAO concentration of 0.20 mg mL^{-1} achieved a saturated binding of AAO on the SiO_2 nanoparticle surface. Therefore, 0.20 mg mL^{-1} of AAO was used in preparation of the probe of Apt-SiO_2-AAO.

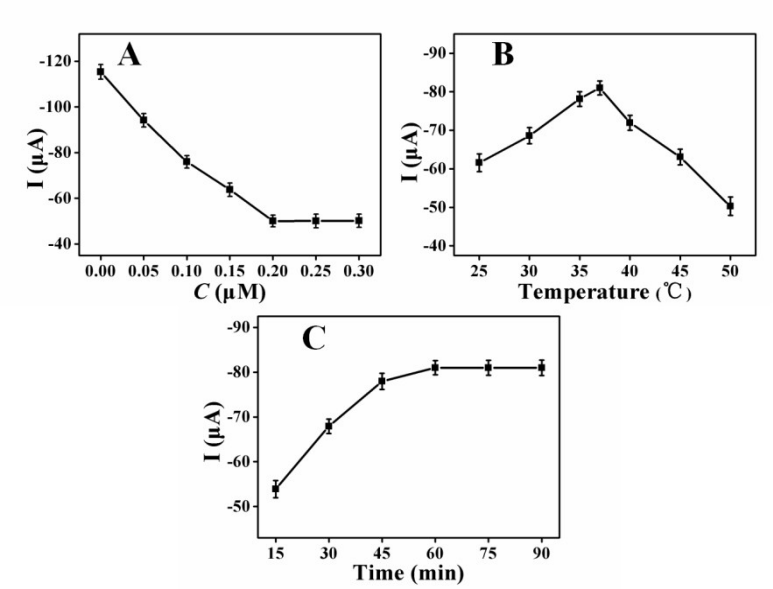

Fig. S4 Effects of (A) the concentration of the aptamer, (B) incubation temperature and (C) incubation time on photocurrent responses. Working solution: 0.01 M PBS (pH 7.4) including 0.1 M AA.

We have studied the conditions of the reaction between aptamer and target protein in terms of concentration of the aptamer, incubation temperature and incubation time. The effect of concentration of the aptamer ranged from 0.00 to 0.30 μM was tested. As shown in Fig. S4A, the photocurrent intensity decreased with the rising of concentration from 0.00 to 0.20 μM and then did not produce further signal change. Therefore, the optimal concentration of the aptamer is 0.20 μM. The effect of incubation temperature ranged from 25 ℃ to 50 ℃ was tested. As shown in Fig. S4B, the photocurrent intensity increased with the rising of temperature from 25 ℃ to 37 ℃ and then decreased from 37 ℃ to 50 ℃. Therefore, the best binding temperature for aptamer-CEA interaction is 37 ℃. To screen the proper incubation time on the photocurrent response, the incubation time from 15 min to 90 min was examined. As seen from Fig. S4C, the photocurrent intensity gradually enhanced with the increasing incubation time until 60 min and the incubation time longer than 60 min did not produce further signal change. So, 60 min was chosen.

Section 4: Selectivity and stability of the biosensor.

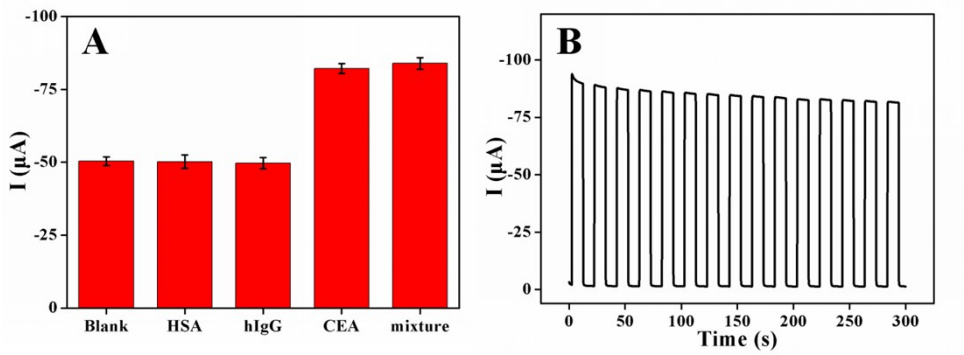

Figure S5. (A) Selectivity of the PEC biosensor for CEA detection; (B) Time-based photocurrent response of the biosensor incubation with 1 ng mL^{-1} CEA.

The selectivity of the present system was investigated by assay of two typical interfering proteins including HSA and hIgG. As demonstrated in Figure. S5A, the PEC signals of the interfering proteins are comparable to that in the blank test. Besides, the photocurrent response of the mixture involving 10 mg mL^{-1} HSA, 1 mg mL^{-1} hIgG and 0.1 ng mL^{-1} CEA was also studied, and no obvious difference of PEC response could be observed compared to the case of 0.1 ng mL^{-1} CEA. Thus, this proposed sensing strategy exhibits good performance for distinguishing CEA against other interfering proteins.

Meanwhile, the stability of the PEC response of such biosensor was also explored. As exhibited in Figure S5B, the photocurrent intensities of the biosensor could be observed barely obvious change for 300 s. Within 2 weeks of storage at 4 °C, the PEC signal of the biosenor still is stable and no apparent change. All the results suggest a good stability of the as-prepared PEC biosensor.

Section 5:

Table S1. Analytical results of the proposed PEC biosensor and ROCHE ECL method for CEA in human serum samples.

Serum samples	This work (ng mL^{-1})	ROCHE ECL (ng mL^{-1})	Relative errors (%)	RSD (%) (n = 3)
1	2.47	2.34	5.6	4.0
2	1.50	1.45	3.4	5.6
3	2.35	2.26	4.0	5.1
4	9.42	9.04	4.2	2.5
5	7.38	7.81	-5.5	5.0
6	6.66	6.88	-3.2	4.5
7	7.99	8.23	-2.9	5.8

Section 6:

Table S2. Recovery of CEA in human serum samples.

Serum samples	Found (ng mL^{-1})	Added (ng mL^{-1})	Total found (ng mL^{-1})	Recovery (%)	RSD (%) (n = 3)
1	1.45	0.01	1.46	100.0	4.0
		0.10	1.56	110.0	4.7
		1.00	2.43	98.0	3.4
2	8.23	0.01	8.24	100.0	4.6
		0.10	8.32	90.0	2.3
		1.00	9.18	95.0	3.1

Section 7: Reference:

1 B. Wang, Y. X. Dong, Y. L. Wang, J. T. Cao, S. H. Ma and Liu, Y. M, *Sens. Actuators B,* 2018, **254**, 159-165.

2 L. Y, Cheng, H. M. Ding, C. H. Chen and N. N. Wang, *J. Mater. Sci.: Mater. Electron.,* 2016, **27**, 3234-3239.