Electronic Supplementary Information

A photoelectrochemical biosensor based on methylene blue sensitized Bi_5O_7I for sensitive detection of prostate specific antigen

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1. Materials and Regents

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Ethylene glycol, KI and ethanol were purchased from Sinopharm chemical Reagent Co., Ltd. (Shanghai, China). Bismuth nitrate ($Bi(NO_3)_3·5H_2O$) was obtained from Shenyang Reagent Plant No. 5. Methylene blue (MB) was purchased from Shanghai Aladdin Industrial Corporation (Shanghai, China). Hexanethiol (HT)、30% hydrogen peroxide (H_2O_2) and gold chloride tetrahydrate ($HAuCl_4·4H_2O$) were obtained from Sigma Chemical Co. (St. Louis. MO, USA). N-hydroxy succinimide (NHS) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) were obtained from Shanghai Medpep Co.,Ltd. (Shanghai, China). Prostate-specific antigen (PSA) was purchased from Biocell Biotech Co,. Ltd. (Zhengzhou, China). The DNA oligonucleotides in the experiment were obtained from Sangon Biological Engineering Technology and Services Co., Ltd. The relevant DNA sequences used in the experiment were shown below:

Table S1. Sequences used in the experiment

Name	Sequence (5'→3')
Capture DNA	SH-CGCGCCATCAAATAGC
PSAA	NH_2-$(CH_2)_6$-TTAATTAAAGCTCGCCATCAAATAGC
S1	TCTGGCTATTTGATGGCGTATT-$(CH_2)_6$-NH_2

2. Apparatus

The measurements of cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were performed on a CHI 760E electrochemistry workstation (Shanghai Chenhua Instrument, China). Scanning electron microscopy (SEM) images

were determined by a Hitachi S-4800 high resolution scanning electron microscope (Hitachi, Japan). X-ray photoelectron spectroscopy (XPS) were determined by a Thermo Scientific K-Alpha+ X-ray photoelectron spectrometer (Thermo Fay, USA). Transmission electron microscope (TEM) were determined by a FEI Tecnai G2 F20 high resolution transmission electron microscope (FEL Co., Ltd., USA). Uv-visible (UV-vis) absorption spectrum was adopted by UV-2450 UV-vis spectrophotometer (Shimadzu, Tokyo, Japan).

3. Synthesis of Bi_5O_7I Microspheres

Floral Bi5O7I was synthesized by hydrothermal method with minor modification on the basis of reference.[1] Firstly, 1.38 g $Bi(NO_3)_3 \cdot 5H_2O$ was dissolved in 25 mL ethylene glycol and stirred at room temperature for 30 min, denoted as solution A. Then, 0.498 g KI was dissolved in 25 mL ethylene glycol and stirred at room temperature for 30 min, denoted as solution B. After that, the solution B was slowly poured into the solution A under stirring and continued to stir for another 1 h at room temperature. The well-stirred solution was then transferred to an 80 mL Teflon-lined stainless-steel autoclave and reacted at 160 ℃ for 12 h. When the reaction was completed, the resulting precipitate was collected, washed with deionized water and ethanol for several times and dried at 80 ℃ for 12 h. Finally, the resulting precipitate was heated at 500 ℃ for 2 h to obtain a white solid powder.

4. Target Conversion Process

The conversion process of the target was described in detail in Scheme 1A. The 100 μL carboxyl-modified magnetic beads were washed with PBS 7.0 for three times

for magnetic separation and redispersed in 2 mL PBS solution for later use. Subsequently, 50 μL 0.04 M EDS and 50 μL 0.01 M NHS were mixed with 500 ?L magnetic beads solution and oscillated at 4 ℃ for 1 h. Then, 100 μL 2.5 μM PSAA was added into the above solution and stirred slowly for 12 h at 4 ℃. Finally, 100 μL 2.5 μM S1 was mixed evenly with the above solution and stirred the solution for 2 h at room temperature, namely the S1-PSAA-MB. PSA-PSAA-MB and S1 were obtained by magnetic bead separation after the mixture was incubated at 37 ℃ for 2 h with the volume ratio of 1:1 to PSA.

5. Preparation of PEC Biosensor

The detailed steps for constructing the biosensor were described in Scheme 1B. Firstly, the glassy carbon electrode (GCE) was polished with α-Al_2O_3 powder, then cleaned with deionized water and ethanol to obtain a smooth mirror shape. Then, 5 μL 2 mg/mL Bi_5O_7I was incubated on the treated electrode and dried at 37 ℃ for 20 min to form a uniform film on the electrode. Next, the modified electrode was immersed in 1% $HAuCl_4$ aqueous solution to obtain the gold nanoparticles layer (Dep Au) by electrodeposition under a constant potential of -0.2 V for 15 s. Subsequently, the capture DNA (2 μM, 15 μL) was immobilized on the surface of the electrode via Au-S bonds at 4 ℃ for 12 h. After that, to block the nonspecific binding sites, the modified electrode was incubated with 10 μL 0.1 mM HT for 40 min at room temperature. Then, 10 μL of obtained S1 solution was incubated on the modified electrode for 2 h at 37 ℃, which could hybridize with capture DNA to form dsDNA. Finally, 10 μL 2.5 μM MB was incubated on the resulted electrode for 2 h at room

temperature.

6. PEC Measurements

The PEC test experiments were conducted at 5 mL of PBS solution (0.1 M, pH 7.4) and 50 μL of H_2O_2 was dispersed in the solution as electron donor. The PEC signal for each test was measured under an excitation wavelength (λ = 365 nm) and equipped with a light-emitting diode lamp as the excitation light source switched from "off-on-off" for 10 s-20 s-10 s under 0.0 V potential.

9. Optimization of Experimental Conditions

In order to achieve the best detection performance of the designed biosensor, the experimental conditions were optimized. The constructed PEC biosensor was related to the concentration of MB and the incubation time of MB. As shown in the Figure S1A, PEC signal gradually increased with the increase of MB concentration, and PEC signal reached its maximum when the concentration of MB increased to 2.5 μM. Therefore, 2.5 μM was selected as the optimal concentration of MB. As illustrated in Figure S1B, the PEC signal changed with the incubation time of MB and increasing from 30 min to 120 min while slightly decreasing from 120 min to 180 min. Therefore, 120 min was selected as the optimal incubation time of MB.

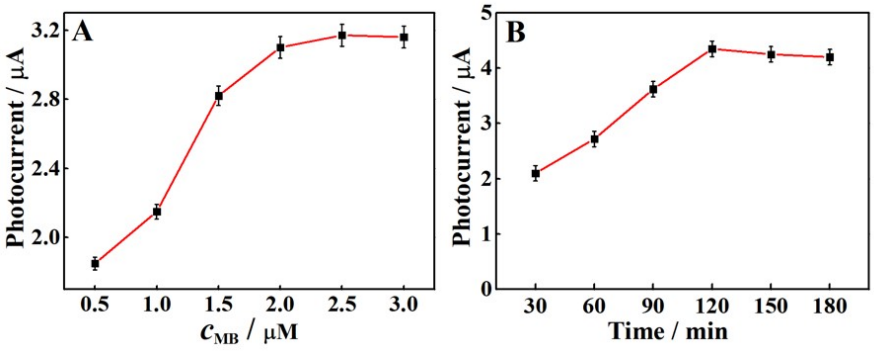

Figure S1. The condition optimization of (A) the concentration of MB and (B) the incubation time of MB.

Table S2. The comparison of various detection methods of PSA

Detection technologies	Detection range	Detection limit	Reference
fluorescent	10 pg/mL-10 ng/mL	9.2 pg/mL	2
fluorescent	3 ng/mL-10 ng/mL	1.07 pg/mL	3
electrochemical	5 pg/mL-50 ng/mL	0.78 fg/mL	4
electrochemical	5 pg/mL-10 ng/mL	0.76 pg/mL	5
electrochemiluminescence	1 pg/mL-300 ng/mL	0.2 fg/mL	6
electrochemiluminescence	0.05 pg/mL-50 ng/mL	0.034 ng/mL	7
photoelectrochemical	5 pg/mL-20 ng/mL	1.5 pg/mL	8
photoelectrochemical	0.1 pg/mL-100 ng/mL	10.06 pg/mL	9
photoelectrochemical	0.1 fg/mL-0.1 ng/mL	0.047 fg/mL	***this work***

10. Real Sample Analysis of Serum Samples

To evaluate its feasibility, the biosensor was applied to the detection of PSA in human serum samples. Different concentrations of CEA (10 fg/mL, 100 fg/mL, 1 pg/mL, 10 pg/mL) were added to the serum samples diluted 50 times for further detection. As displayed in Table 2, the recoveries ranged from 97.5% to 103.2%, indicating the accuracy and reliability of this method, and it had a good application prospect in diagnosis and medical treatment.

Table S3. Determination of PSA in Human Serum with this Proposed Biosensor.

Sera sample	Added concentration	Obtained concentration	Recovery / %	RSD / %
1	10 fg/mL	9.95 fg/mL	99.5	1.5
2	100 fg/mL	103.2 fg/mL	103.2	4.0
3	1 pg/mL	1.007 pg/mL	100.7	4.1
4	10 pg/mL	9.75 pg/mL	97.5	3.2

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