-Supporting Information-

Immunogold Labeling-Induced Synergy Effect for Amplified Photoelectrochemical Immunoassay of Prostate-Specific Antigen

Wei-Wei Zhao, Xiao-Ya Dong, Jing Wang, Fen-Ying Kong, Jing-Juan Xu and Hong-Yuan Chen**

Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China.

Tel/ Fax: +86-25-83597294; E-mail: xujj@nju.edu.cn; hychen@nju.edu.cn

Experimental section

Materials.

The ITO slices (type N-STN-S1-10, China Southern Glass Holding Co., Ltd) were used as the working electrode. Total prostate-specific antigen (PSA), two mouse antihuman total PSA monoclonal antibodies, clone P27A10 (primary capture antibody, Ab_1) and clone P27B1 (secondary detection antibody, Ab_2), alpha-fetoprotein (AFP), and carcinoembryonic antigen (CEA) were purchased from Shuangliu Zhenglong Biochemical Lab (Chengdu, China). N-(3-dimethylaminopropyl)-N-ethyl-carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), Poly(diallyldimethylammonium chloride) (PDDA; 20%,w/w in water, MW = 200 000-350 000), bovine serum albumin (BSA), human serum albumin (HSA) , thioglycolic acid (TGA) were purchased from Sigma-Aldrich (St. Louis, MO). Tween 20 was purchased from Amresco. 4-chloro-1-naphthol (4-CN) was obtained from Tokyo Kasei Kogyo Co., Ltd (Japan). Ascorbic

acid (AA) was obtained from Sinopharm Chemical Reagent Co., Ltd (China). Other chemicals were of analytical reagent grade and used as received. 0.01 M PBS (pH 7.4) was used for the preparation of antigen(Ag) and antibody(Ab) stock solutions. The washing buffer solution was 0.01M PBS (pH 7.4) containing 0.05 % Tween 20. The blocking buffer was 0.01M PBS (pH 7.4) containing 3% (w/v) BSA. All aqueous solutions were prepared using ultra-pure water (Milli-Q, Millipore).

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 ITO electrode with geometrical area of 0.25 ± 0.01 cm^2 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 15 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. ξ-potential was acquired with a MALVERN (Nano-Z) instrument.

Fabrication of TGA-stabilized CdS QDs modified ITO electrode.

The utilized CdS QDs were synthesized according to the previous report with slight modification.[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, 5.5 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 1:1.1. 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℃ for further use.

ITO slices were cleaned by immersion in 2M boiling KOH solution solved in 2-propanol for 20 min, followed by washing copiously with water and dried at 120 ℃ for 2 h. The PDDA/CdS multilayer film was grown by alternately dipping of the cleaned ITO slices into a solution of 2% PDDA containing 0.5 M NaCl and the as-obtained CdS QDs solution for 10 min, respectively. The ξ-potential of the cationic polyelectrolyte PDDA and TGA-capped CdS QDs utilized in this work are determined as + 6.27 mV and -16.6 mV, respectively. This process was repeated 3 times to obtain desired photocurrent intensity. The films were carefully washed with doubly distilled water after each dipping step.

Synthesis of Au NPs and preparation of HRP-Au NP-Ab_2 bioconjugates.

Au NPs were prepared by adding 20 ml $HAuCl_4$ aqueous solution consisting 10 mg of gold to 170 mL boiling water in a flask. When the solution was boiling again, 10 mL of 1% sodium citrate solution was added while stirring vigorously. After continuous boiling for 30 min, it was allowed to cool down to room temperature. The average diameter of the prepared Au NPs was about 20 ± 2 nm. Au NPs were coated with Ab_2 and HRP according to a documented method.[3] At room temperature, 1.5 μL of 5.0 mg/mL Ab_2 and 3.0 μL of 5.0 mg/mL HRP were added in 1.0 mL of the Au NPs solution containing 0.04% trisodium citrate, 0.26 nM potassium carbonate. The mixture was then gently mixed for 2 h, blocked by 100 μL of 1% BSA solution for 30 min at room temperature, and thereafter centrifuged at 15,000 rpm for 20 min at 4 ℃. After centrifugation, the oiled drop was washed by washing buffer, recentrifugated and finally resuspended in 100μL of 1% BSA as the assay solution.

Immunoassay development.

Ab_1 was immobilized onto the PDDA/CdS modified ITO electrode *via* the classic EDC coupling reactions between COOH groups on the surface of the TGA-capped CdS QDs and the NH_2 groups of Ab_1. In detail, the CdS QDs modified electrode was activated by immersion in 1.0 mL of distilled water containing 20 mg EDC and 10 mg NHS for 1 h at room temperature,

followed by thoroughly rinsing with washing buffer to wash off the excess EDC and NHS. Subsequently, 25 μL of 0.5 mg/mL Ab_1 was spread onto the resulting electrode surface at 4 °C in a moisture atmosphere to avoid evaporation of solvent. After incubation for 16 h, the electrode was rinsed with the washing buffer to remove physically adsorbed Ab_1. The electrode was then blocked with 25 μL blocking solution for 2 h at 4°C to block non-specific binding sites and washed with the washing buffer thoroughly. Next, 25 μL of analyte antigen with different concentrations were dropped onto the Ab_1 modified electrodes for an incubation of 60 min at 37 ℃ followed by washing with washing buffer. After the binding reaction between Ab_1 and the antigen, the electrodes were allowed for labeling by additional incubation with 25 μL diluted Ab_2–Au NPs–HRP bioconjugates solution for 60 min, and again the electrodes were washed thoroughly with water to remove nonspecifically bound conjugations. To prepare the biocatalytic precipitation (BCP) solution, 4-CN was firstly dissolved in ethanol, and then the ethanolic stock solution was diluted with 0.1 M PBS, pH 7.4, to compose the solution that includes 1×10^{-3} M 4-CN and 2% (v/v) ethanol. Finally, the Ab_1-Ag-Ab_2-Au NPs-HRP modified electrodes were allowed for 10 min incubation at 25 ℃ in the obtained probe solution consisting of 1×10^{-3} M 4-CN and 1.5×10^{-4} M H_2O_2. Thereafter, the electrodes were rinsed with 0.01 M phosphate buffer, pH 7.4, and then introduced for the respective PEC measurements.

References.

[1] Wang, G. L.; Yu, P. P.; Xu, J. J.; Chen, H. Y. J. Phys. Chem. C. 2009, 113, 11142.
[2] Zhao, W. W.; Wang, J; Xu, J. J.; Chen, H. Y. Chem. Commun. 2011, 47, 10990.
[3] Cui, R. J.; Huang, H. P.; Yin, Z. Z.; Gao, D.; Zhu, J. J. Biosens. Bioelectron., 2008, 23, 1666.