Electronic Supplementary Material (ESI) for Analytical Methods.


# Electrochemical immunosensor based on a multiple signal amplification strategy for highly sensitive detection of prostate specific antigen

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**Reagents and apparatuses**

Human PSA antibody ($Ab_1$, $Ab_2$) and antigen were purchased from Biocell Science Co., Ltd. (Shanghai, China). Bovine serum albumin (BSA, 96-99%) was bought from Sigma Reagent Co., Ltd. (St. Louis, MO, USA). Cobalt chloride ($CoCl_2 \cdot 6H_2O$), copper chloride ($CuCl_2 \cdot 2H_2O$) and 3-aminopropyl-triethoxysilane (APTES, 98%) were purchased from Shanghai Aladdin Chemistry Co., Ltd. (Shanghai, China). $HAuCl_4 \cdot 4H_2O$ was obtained from Sinopharm Chemical Reagent Shanghai Co., Ltd., China. Phosphate buffer saline (PBS) was prepared by using 1/15 mol $L^{-1}$ $Na_2HPO_4$ and 1/15 mol $L^{-1}$ $KH_2PO_4$ solution and used as an electrolyte for all electrochemical measurement. All other reagents are at analytical grade and used directly without further purification. Ultrapure water (18.25 MΩ, 25 ºC) was used throughout the study.

All electrochemical measurements were operated on a CHI760D electrochemical workstation (Chenhua Instrument Shanghai Co., Ltd., China). A saturated calomel electrode (SCE), a platinum wire electrode and a glassy carbon electrode (GCE) (4 mm in diameter) were used as reference, counter, working electrodes. The surface morphology of nanomaterial was observed using a JSM-6700F scanning electron microscopy (SEM). Transmission electron microscopic (TEM) image was obtained from a JEOL-1400 microscope (Japan). The presented elements of the nanocomposite were obtained using Energy Dispersive X-ray Spectroscopy (EDX).

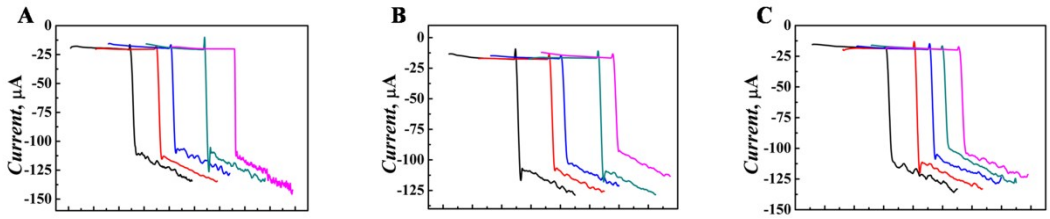


Fig. S1 The electrocatalytic current responses of the immunosensor after addition of $H_2O_2$ correspond to reproducibility, selectivity and stability.

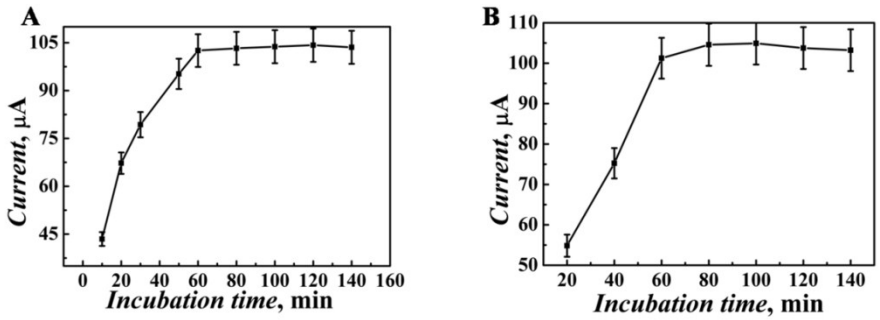


Fig. S2 Effect of incubation time between PSA and $Ab_1$ (A) and between PSA and $Ab_2$-Pd@Pt-APTES-$Cu_2O$@$Co_3O_4$ (B) on the electrocatalytic current response of the immunosensor for the detection of 1 ng $mL^{-1}$ PSA. Error bar=RSD (*n*=5).

Table S1 Assay results of clinical serum samples using the immunosensor and standard ELISA methods (ng/mL)

| Sample | 1 | 2 | 3 |
|---|---|---|---|
| Immunosensor | 0.83 | 1.29 | 2.32 |
| ELISA | 0.79 | 1.32 | 2.25 |
| Relative error (%) | 4.8 | -2.3 | 3.0 |

**Function of BSA**

In order to prove the effect of BSA on eliminating nonspecific binding, we operated a comparative experiment.

Firstly, the GCE was polished with 1.0, 0.3 and 0.05 μm alumina powder successively and cleaned thoroughly before use. Then, 6 μL of Au-CS-Gr solution was dropped on the electrode surface and dried at room temperature. Subsequently, 6 μL of 10 μg $mL^{-1}$ $Ab_1$ was added onto the electrode and incubated for 1 h. For comparison, after washing with PBS (pH=7.4), 3 μL of 1% BSA was modified on one electrode and another one was not added. Finally, 6 μL of 1.8 mg $mL^{-1}$ $Ab_2$-Pd@Pt-APTES-$Cu_2O$@$Co_3O_4$ dispersion was dropped onto the modified electrode and incubated for one more hour. Amperometric *i-t* curve was used to record the electrocatalytic current signal with the constant scanning potential at -0.4 V. 10 μL of 5 mmol $L^{-1}$ $H_2O_2$ was added in the PBS at pH=6.8 when the background current was stable. The experiment result was shown in Fig. S3. It exhibited very low current response when 3 μL of 1% BSA was added on the electrode (curve a). This phenomenon can be explained as BSA blocked the non-specific binding sites. $Ab_2$-Pd@Pt-APTES-$Cu_2O$@$Co_3O_4$ was hardly modified on the electrode. However, the electrode showed a significant current response without BSA added (curve b). This is caused by the adsorption of $Ab_2$-Pd@Pt-APTES-$Cu_2O$@$Co_3O_4$ onto the electrode surface and the substrate material. Therefore, BSA ensured specific binding of antigen to antibody by eliminating nonspecific binding.

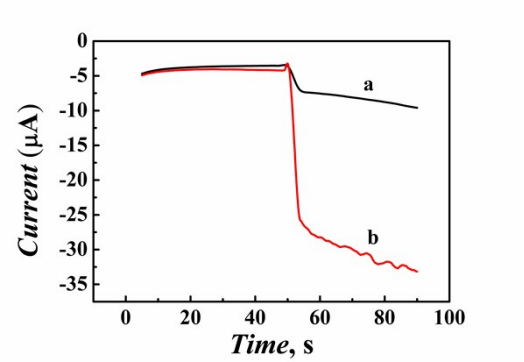


Fig. S3 The Amperometric *i-t* curve of the electrode with 3 μL of 1% BSA added (a) and without 3 μL of 1% BSA added (b).