Electronic Supplementary Information

Target protein induced cleavage of a specific peptide for prostate-specific antigen detection with positively charged gold nanoparticles as signal enhancer

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Experimental section

Chemicals and material

Nicotinamide adenine dinucleotide (NADH), bovine serum albumin (BSA, 96 – 99%), tris (2-carboxyethyl) phosphine (TCEP), hemoglobin (Hb) and gold chloride tetrahydrate ($HAuCl_4 \cdot 4H_2O$) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ were bought from Beijing Chemical Reagent Co. (Beijing, China). Cetyltrimethlammonium bromide (CTAB) and dithiobis (succinimidyl propionate) (DSP) were purchased from Tianjin Heowns Biochem LLS (Tianjin, China). Human Apolipoprotein A-1 (APO-A1), prostate-specific antigen (PSA), human alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) were purchased from Biocell Company (Zhengzhou, China). A specific peptide (CEHSSKLQLAK-NH_2) was provided by Shanghai Science Peptide Biological Technology Co., ltd. (Shanghai, China). Phosphate buffered solution (PBS, pH 7.4, 0.1 M) was prepared with 0.1 M Na_2HPO_4, 0.1 M KH_2PO_4 and 0.1 M $MgCl_2$. 20 mM Tris-HCl buffer (pH 7.4) was prepared with 140 mM NaCl, 1 mM $CaCl_2$, 5 mM KCl and 1 mM $MgCl_2$. All aqueous solutions were prepared with ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore). All chemicals were of analytical grade and used as received.

Apparatus

In the electrochemical experiments, cyclic voltammetric (CV) and electrochemical impedance spectroscopy (EIS) were performed on a CHI 660D electrochemistry workstation (Shanghai Chenhua Instrument, China) with a conventional three-electrode system including a bare or modified glassy carbon electrode (GCE, Φ= 4 mm) as working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum wire electrode as auxiliary electrode. The pH measurements were carried out with a pH meter (MP 230, Mettler Toledo, Switzerland).

Experimental measurements

CV of stepwise fabrication electrodes was performed in 2 mL $[Fe(CN)_6]^{3-/4-}$ solution (5.0 mM) containing 0.1 M KCl with a scanning potential range from -0.2 to 0.6 V at a scan rate of 100 mV s^{-1}. EIS was performed in 2 mL $[Fe(CN)_6]^{3-/4-}$ solution (5.0 mM) containing 0.1 M KCl with the frequencies swept from 0.1 to 105 Hz. The impedance spectras were plotted in the form of Nyquist plots, and the concentration of PSA could be quantified by the change of electron transfer resistance of the electrode.

The fabrication of the biosensor

Prior to use, bare GCE was polished carefully with 0.3 μm and 0.05 μm alumina powder and ultrasonically washed in ultrapure water and absolute ethanol for 3 minutes. The pretreated GCE was immediately then soaked in $HAuCl_4$ aqueous solution (1 *wt*%) and electrodeposited under the potential of -0.2 V for 30 s to introduce a layer of gold nanoparticles (depAu). Then, 15 μL DSP solution (2 mM) was dropped on the electrode and incubated at room temperature for 60 min. During the process, DSP was bound to depAu by Au-S bond. To reduce nonspecific adsorption, the modified electrode was incubated with 20 μL BSA solution (0.5 *wt*%) for 40 min. Following that, the specific peptide pre-incubated with TCEP (TCEP was used to prevent disulfide formation between peptides) was assembled on the electrode for the interaction between DSP and the amino of peptide. Then the prepared gold nanoparticles (AuNPs) with diameter about 13 nm according to the literature[1,2] were coated on the peptide decorated electrode. After that, the electrode was dipped into positively charged CTAB solution (7.4×10^{-2} M) containing NADH (4×10^{-4} M) and

$HAuCl_4$ (1.8×10^{-4} M) for 2h, in which AuNPs were capped with positively charged CTAB by electrostatic adsorption. Followed by washing with ultrapure water, the positively charged AuNPs/peptide/BSA/DSP/depAu/GCE biosensor was obtained. Ultimately, the obtained biosensor was stored at 4 °C when not in use.

Results and discussion

The electrochemical characterization of the stepwise modified electrode

To further confirm the assemble process of biosensor, EIS which has been proven as one of the most powerful tools for interfacial investigation was employed to monitor the fabrication process of modified electrode. Fig. S1 displayed the EIS of different electrode stages in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution. The bare GCE showed a very small semicircle (curve a), indicating a low transfer resistance. Owing to the conductivity of depAu, a smaller semicircle (curve b) was obtained after the electrochemical deposition of AuNPs (depAu). However, after the consecutive assembling of DSP, BSA and peptide, the resistances were increased with the stepwise increase of semicircle diameter (curves c-e), due to the electrode surface coated with above substances which act as barrier layers preventing $[Fe(CN)_6]^{3-/4-}$ from approaching the electrode surface. The resistance decreased after the electrode was incubated with AuNPs (curve f), indicating that the AuNPs could promote the electron transfer. The resistance further decreased after the electrode was treated in positively charged CTAB solution (curve g), owing to the attraction between positively charged AuNPs and negatively charged $[Fe(CN)_6]^{3-/4-}$, which promoted the electron transfer significantly. At last, the resistance (curve h) increased largely owing to the positively charged AuNPs signal enhancer on the peptide was cleaved by PSA. The results indicated that a sensing interface was effectively constructed.

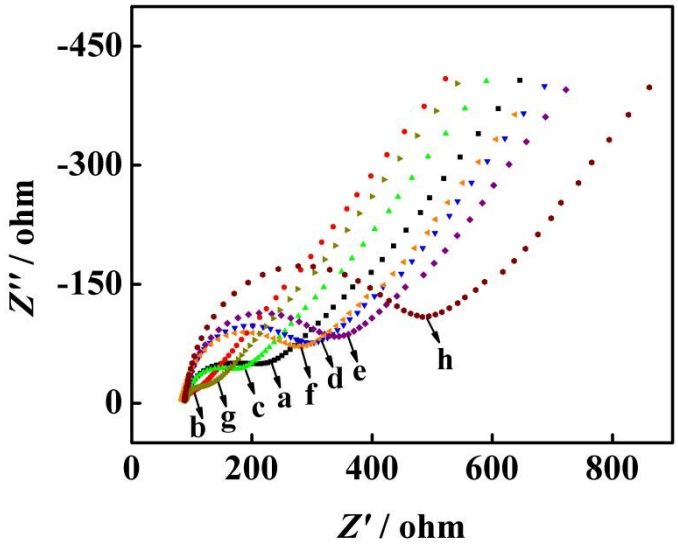

Fig. S1 EIS of bare GCE (a); depAu/GCE (b); DSP/depAu/GCE (c); BSA/DSP/ depAu/GCE(d); peptide/BSA/DSP/depAu/GCE (e); AuNPs/peptide/BSA/DSP/ depAu/GCE (f); positively charged AuNPs/peptide/BSA/DSP/depAu/GCE (g); positively charged AuNPs/ peptide/ BSA/ DSP/depAu/GCE after being incubated with PSA (1 ng·mL^{-1}) (h) in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution.

Optimization of the experimental conditions

In order to maximize the sensitivity and efficiency of the biosensor, the optimization for the peptide concentration and the incubation time of peptide were investigated by CV experiments in $[Fe(CN)_6]^{3-/4-}$ solution (5.0 mM) containing 0.1 M KCl. Fig. S2A showed the change of peak current intensity after the modified electrode incubated with different concentrations of peptide, respectively. The current peak intensity greatly increased as the peptide concentration elevated from 1.0 μM to 3.0 μM and reached a maximum at about 3.0 μM, indicating that the peptide concentration was saturated. Therefore, 3.0 μM was chosen as the appropriate concentration of peptide to prepare the biosensor.

The incubation time of the peptide is another important factor affecting the experimental performance of biosensor. Fig. S2B displayed the dependence of current intensity on incubation time of peptide. The current intensity sharply increased with the increasing of incubation time from 10 min to 40 min. When the incubation time was prolonged from 40 min to 70 min, the current intensity nearly kept stable.

Therefore, 40 min was adopted as the incubation time for 3.0 μM peptide in the following whole experiments to obtain the best results.

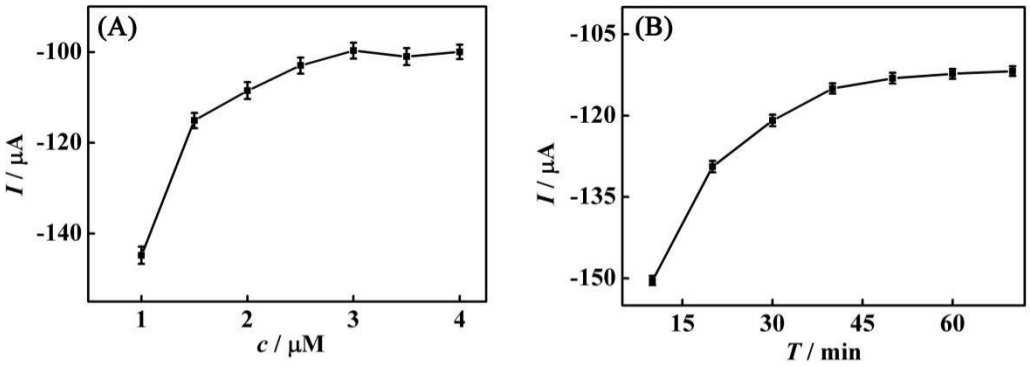

Fig. S2 The optimization of experimental parameters investigated by CV: (A) the optimum of peptide concentration and (B) incubation time of peptide immobilization.

Reproducibility and stability of the proposed biosensor

The reproducibility of biosensor was investigated by analysis of the same concentration of PSA (10 ng·mL^{-1}) using four electrodes prepared in the same conditions. As shown in Table S3, a relative standard deviation (RSD) of 2.78%was acquired. When the present biosensor was repeated for four measurements with 5 ng·mL^{-1} PSA, a RSD of 3.79% was obtained, suggesting the proposed biosensor has acceptable reproducibility.

The stability of biosensor was studied through measuring the EIS responses of as-prepared biosensor of long-term storage every day for a long time at 4°C. The biosensor retained 94% of its initial EIS value after 6 days storage, which indicated that the biosensor had a good stability.

Table S1 Comparisons of proposed biosensor with other detection methodologies in reproducibility and sensitivity.

Analytical method	Reproducibility	Detection limit	Linear range	Ref.
CV	5.8%	0.015 ng/mL	0.05-50 ng/mL	3
DPV	2.6%	0.02 ng/mL	0.2-40 ng/mL	4
SWV	6.1%	2 pg/mL	0.01-40 ng/mL	5
SWV	3.0%	0.2 ng/mL	0.5-40 ng/mL	6
ECL	5.47%	8 pg/mL	0.01-8 ng/mL	7
EIS	2.78%	0.06 pg/mL	0.2×10^{-3}-45 ng/mL	Our work

Abbreviation: Cyclic voltammetry (CV); Differentialpulse voltammetry (DPV); Square-wave voltammograms (SWV); Electrochemiluminescent (ECL); Electrochemical impedance spectroscopy (EIS).

Table S2 Performance compared with other "signal-off" biosensor for PSA detection.

Analytical method	Detection limit	Linear range	Ref.
Signal-off	0.038ng/mL	1.0×10^{-10}-8.0×10^{-9} g/mL	8
Signal-off	0.2 ng/mL	0.5-40 ng/mL	9
Signal-off	-----	10 pM-100 nM	10
Signal-on	0.06 pg/mL	0.2 pg/mL-45 ng/mL	Our work

Tabel S3 Reproducibility assays using four biosensors prepared in the same conditions.

Electrode number	1	2	3	4	RSD (%)
EIS response (ohm)[a]	141.96	140.8	138.78	142.36	2.78

[a]Using 10 ng·mL^{-1}PSA for assays.

Reproducibility assays using one biosensor for four measurements.

Measurement number	1	2	3	4	RSD (%)
EIS response (ohm)[b]	110.7	108.4	109.34	108.92	3.79

[b]Using 5 ng·mL^{-1}PSA for assays.

Table 1 Determination of PSA added in human serums (*n*=3) with the proposed biosensor

Sample number	Added/(ng·mL^{-1})	Found/(ng·mL^{-1})	Recovery/%	RSD/%
1	0.0010	9.654×10^{-4}	96.54	3.68
2	0.010	1.0267×10^{-2}	102.67	5.67
3	0.10	9.432×10^{-2}	94.32	2.32
4	1.0	1.0038	100.38	4.37

Table 2 Analytical results of PSA in clinical serum samples

Sample	1	2	3
CL method (ng·mL^{-1})	0.78	0.27	0.40
Proposed method(ng·mL^{-1})	0.81	0.26	0.42
Relative error (%)	3.8	-3.7	5.0

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