Supplementary Information

Gold nanorods-paper electrode based enzyme-free electrochemical immunoassay of prostate specific antigen using porous zinc oxide spheres-silver nanoparticles nanocomposites as labels

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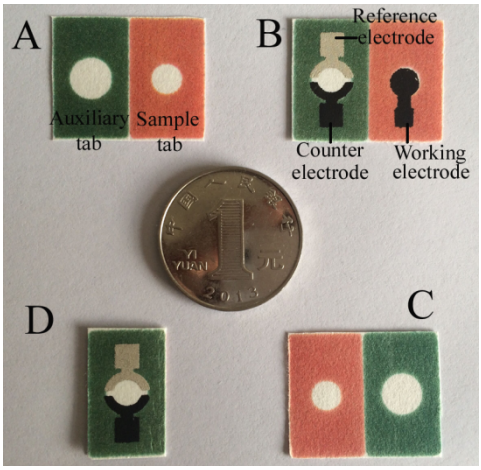
Preparation of the electrochemical origami device

Wax-screen-printing was used to build the origami EC device. The fabrication process consisted of wax-printing, baking the wax-patterned sheet, followed by screen-printing electrodes. Firstly, The shape of the hydrophobic barrier on the origami device, which contained a paper auxiliary zone (8 mm in diameter) on the auxiliary tab (12.0 mm × 20.0 mm), and a paper sample zone (6.0 mm in diameter) on the sample tab (12.0 mm × 20.0 mm), respectively (Scheme S1A), was designed using Adobe illustrator CS4. Then, the entire origami device could be produced in bulk on an A4 paper sheet using a commercially available solid-wax printer (Xerox Phaser 8560N color printer). Owing to the porous structure of paper, the melted wax could penetrate into the paper network to decrease the hydrophilicity of paper remarkably. Meanwhile, the unprinted area (paper auxiliary zone and paper sample zone) still maintained good hydrophilicity, flexibility, and porous structure and will not affect the further screen-printing of electrodes and modifications.

The electrode arrays consisted of a screen-printed Ag/AgCl reference electrode and carbon counter electrode on the paper auxiliary zone and a screen-printed carbon working electrode on the paper sample zone (Scheme S1B and S1C). Between the sample tab and auxiliary tab, the unprinted line (1 mm in width) was defined as the fold line, which could ensure that the paper sample zone on the sample tab was properly and exactly aligned to the auxiliary zone on the auxiliary tab after folding (Scheme S1D), due to the difference of flexibility between the printed and unprinted area after baking. After folding, the three screen-printed electrodes (working electrode, reference electrode, and counter electrode) will be connected once the paper EC cell has been filled with solution.



Scheme S1. The photo images of (A) the origami device, (B) One side of the device with the

screen-printed working, reference and counter electrode, (C) the other side of the device and (D) the origami device after folding down the sample tab below the auxiliary tab.

Preparation of PZS-AgNPs-Ab_2

The preparation procedure of the PZS-AgNPs-Ab_2 bioconjugate was as follows: 1.0 mg of the as-prepared PZS-AgNPs was dispersed into 1.0 mL of pH 7.4 PBS. Followed by ultrasonication for 5 min, 10 μL of Ab_2 (20 μg mL^{-1}) was added into the solution, and then the mixture was slightly vortexed for 4 h. Subsequently, free antibodies were removed by centrifugation and washing with PBS (pH 7.4) for several times to obtain the Ab_2 modified PZS-AgNPs. Next, the precipitate of PZS-AgNPs antibodies conjugates was dispersed with 1.0 mL of 1% BSA solution to block the nonspecific binding sites. Finally, the resultant PZS-AgNPs-Ab_2 were dispersed with PBS (pH 7.4) to a final volume of 1.0 mL and stored at 4 °C for later use.

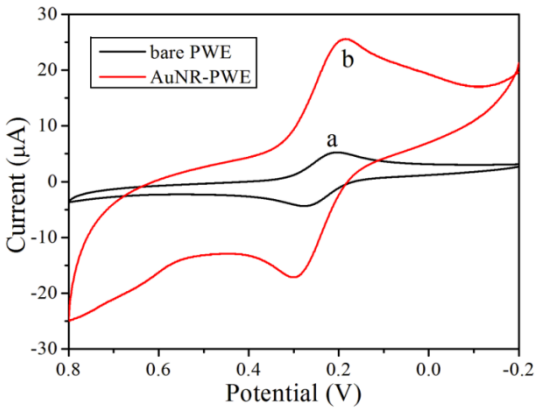


Fig. S1. CVs of (a) bare PWE and (b) AuNRs-PWE in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 0.5 M KCl.

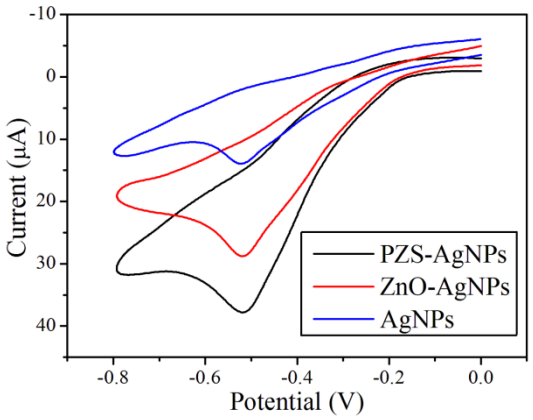


Fig. S2. CVs of the different immunosensors constructed with various signal label using 1.0 ng mL^{-1} PSA as model.

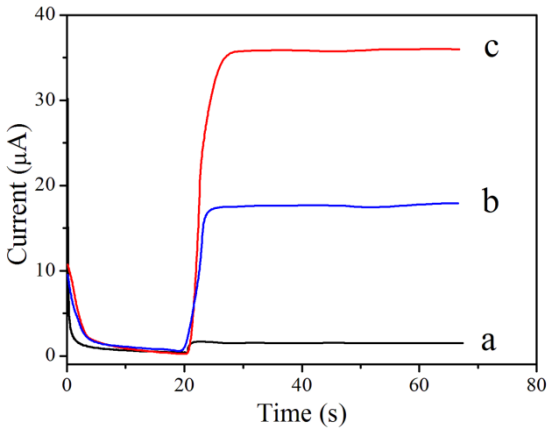


Fig. S3. Current responses of the immunosensor with different concentration of PSA: (a) 0 ng mL^{-1}, (b) 0.05 ng mL^{-1}, (c) 1.0 ng mL^{-1}.

Optimization of assay conditions

To achieve an optimal electrochemical signal, the experimental conditions were optimized. During the immunosensor preparation process, the same concentration of PSA (1.0 ng mL^{-1}) was used to fabricate the immunosensor. The temperature of the antigen-antibody reaction greatly affected the sensitivity of the immunosensor. In general, 37 °C, close to the normal temperature of the human body, was favorable for the antigen-antibody interaction. Considering the practical application hereafter, all the experiments were carried out at room temperature.

As we know, highly acidic or alkaline surroundings would damage the immobilized protein, especially in alkalinity. Fig. S4A showed the effects of pH on the current responses of the immunosensor. The currents increased steeply with the increase of pH from 4.1 to 7.4, and then decreased from 7.4 to 8.5. An optimal amperometric response was achieved at pH 7.4. Hence, pH 7.4 of PBS was selected as the electrolyte for PSA detection.

The incubation time was also an important parameter for both capture PSA and signal antibody on the electrode surface. As seen from Fig. S4B, the electrochemical response increased with increasing incubation time of PSA antigen, and then tended to a steady value after 40 min, indicating a thorough capturing of the antigens on the electrode surface. In the second immunoassay incubation step, the current showed the same changing tendency, and the response current reached a plateau at about 40 min. Longer incubation time did not improve the response. Therefore, 40 min was selected as the incubation time for determination of PSA in this study.

Because of the immobilization of antibodies on the PZS-AgNPs nanocarrier, the ratio of

antibodies and nanocarrier was an important factor on the response signal. To define the ratio of Ab_2 and PZS-AgNPs, we use different concentration of Ab_2 to label the carrier. It could be seen that with the augment of Ab_2 concentration at lower concentration, the signal intensity increased dramatically. Then the trend gradually slowed down till the Ab_2 concentration up to 20 μg mL^{-1} indicating the occurrence of the saturation concentration (Fig. S4C). Accordingly, 20 μg·mL^{-1} Ab_2 was used to label the nanocarrier.

To adequately release the catalytic efficiency of carrier PZS-AgNPs, the concentration of the added H_2O_2 in PBS (pH 7.4) should be optimized. Higher concentration of H_2O_2 might inhibit the catalysis, while the catalysis could not be completely embodied at lower concentration. As shown in Fig. S4D, one can see that catalytic current increased with increasing the concentration of H_2O_2, and the maximum response was achieved at 3.5 mM H_2O_2. Therefore, 3.5 mM H_2O_2 was selected as the optimal condition to detect PSA.

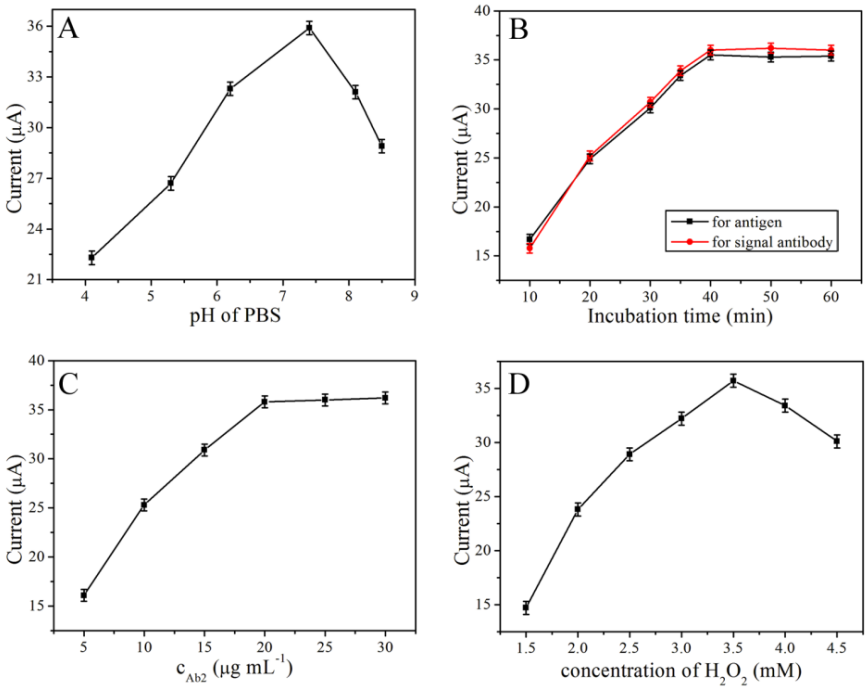


Fig. S4. Influence of (A) pH of PBS, (B) incubation time, concentration of (C) Ab_2 and (D) H_2O_2 on current response of the immunosensor. The data was recorded in PBS solution (pH 7.4) containing 3.5 mM H_2O_2 at -0.52 V. n = 11 for each point, error bars represent standard deviation standard deviation (SD).

The AuNRs modified paper electrode was fabricated at 95 °C for 3 h. With the increase of reaction time, the amount of AuNRs on paper electrode increased and the morphology of AuNRs became uniform. However, the longer reaction time changed the structure of AuNRs (Fig. S5).

Meanwhile, these paper electrodes were used to construct immunosensors under same condition using 1.0 ng mL^{-1} PSA, respectively. As shown in Fig. S6A, the current response increased with the increasing of reaction time, and the maximum response was achieved at reaction time 3 h. This may attributed to the AuNRs obtained at 3 h owned largest surface area and electronic transmission rate. Thus, 3 h was chosen as optimal reaction time to synthesize AuNRs modified paper electrode.

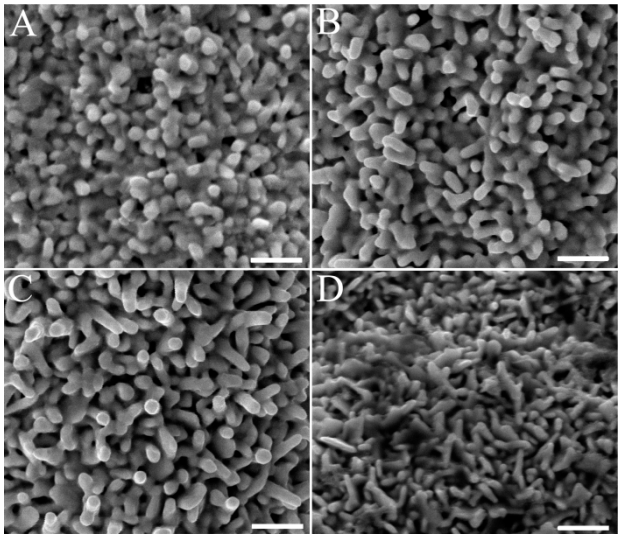


Fig. S5. SEM images of AuNRs modified paper electrode with different reaction: (A) 1 h, (B) 2 h, (C) 3 h, (D) 4 h. Scale bar = 500 nm.

AgNPs owned high catalytic activity toward H_2O_2 reduction. The content of AgNPs in PZS-AgNPs composites was adjusted by the addition mass of $AgNO_3$ and reaction time. As shown in Fig. S6B, the current response increased with the increasing of addition mass of $AgNO_3$ and then leveled off at 0.02 g of $AgNO_3$, which indicated a saturated loading AgNPs on PZS. Meanwhile, the current response showed the same changing tendency, and the current reached a plateau at reaction time of 3 h (Fig. S6C). Therefore, 0.02 g of $AgNO_3$ and reaction time of 3 h were selected to fabricate PZS-AgNPs composites.

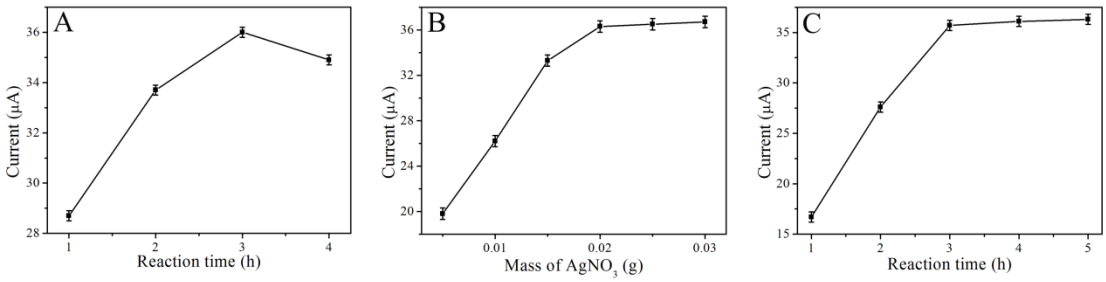


Fig. S6. Influence of (A) reaction time in fabrication of AuNRs-PWE, (B) additional mass of $AgNO_3$ in fabrication of PZS-AgNPs, (C) reaction time in fabrication of PZS-AgNPs on current

response of the immunosensor. n = 11 for each point, error bars represent standard deviation SD.

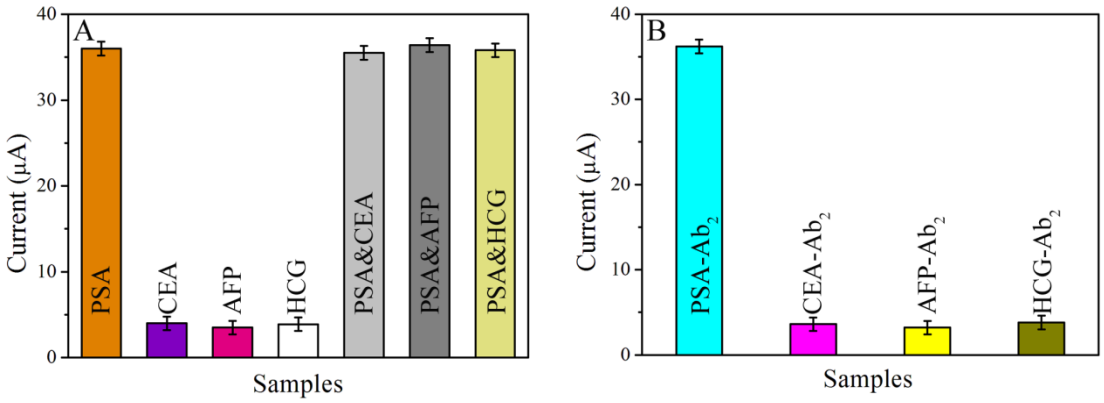


Fig. S7. The interfering effects of (A) sample matrix components and (B) signal antibodies on the current responses of the electrochemical immunosensor. n = 11 for each bar, error bars represent SD.

Table S1. Comparison of analytical properties of different immunoassays toward PSA

Electrode	Signal label	Linear range (ng mL^{-1})	Detection limit (ng mL^{-1})	References
MPS/ITO	SiO_2@C-dots	0.01-50	0.003	1
GE-CHIT/GCE	GOx-GNR	0.01-8	0.008	2
Au electrode	ferrocene-helix peptide	0.5-40	0.2	3
Au electrode	G-quadruplex-hemin DNAzyme	0.14-1400	0.14	4
AuNR-PWE	PZS-AgNPs	0.004-60	0.0015	This work

References

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