Supplementary Information

Application of CuS-functionalized ZnO nanoflakes for paper-based photoelectrochemical immunoassay using an in situ electron donor producing strategy

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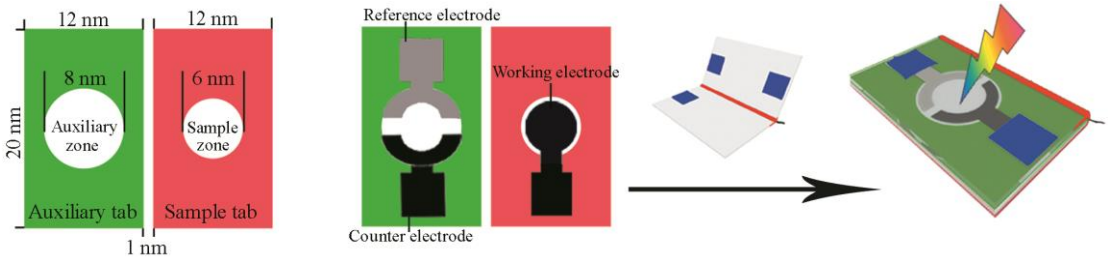

Scheme S1 Schematic representation of the construct and assay procedures for this origami device.

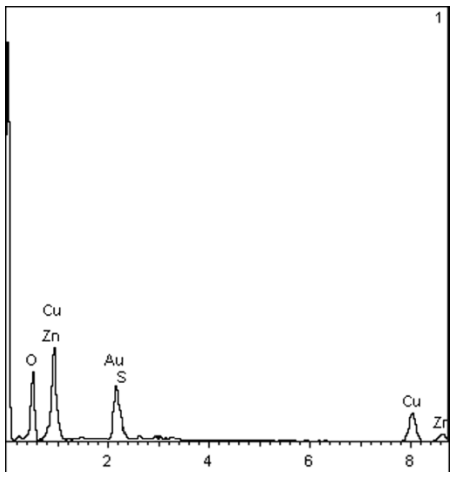

Fig. S1 EDS of the CuS/ZNF modified Au-PWE.

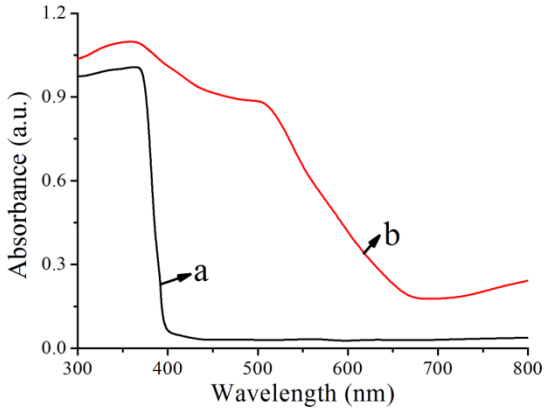

Fig. S2 UV-vis absorption spectra of (a) ZNF arrays and (b) CuS/ZNF arrays.

Effect of concentration of H_2O_2 on photocurrent response

H_2O_2 was chosen as electron donor to facilitate the generation of stable photocurrent. Changing the concentration of H_2O_2, different photocurrent was obtained. The photocurrent responses were improved with the increase of H_2O_2 concentration and a maximum current response was obtained at concentration of 5.0 mM (Fig. S3). Thus,

5.0 mM H_2O_2 was used to characterize the photocurrent response.

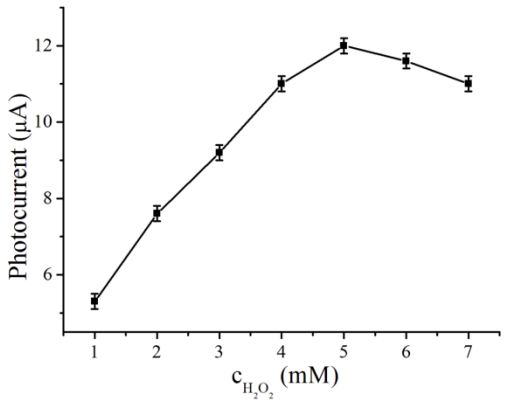

Fig. S3 The effect of concentration of H_2O_2 on photocurrent response.

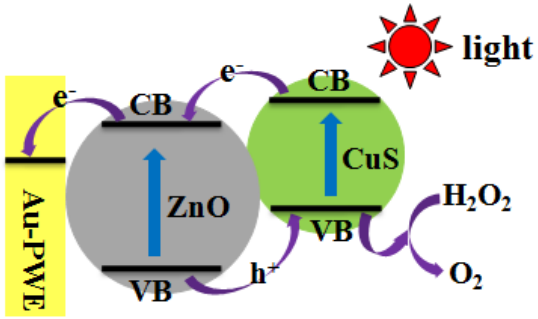

Fig. S4 The photocurrent generation mechanism of the immunosensor.

Optimization of experimental conditions

The temperature of the antigen-antibody reaction also greatly affected the sensitivity of the immunosensor. In general, 37 °C, i.e. close to the normal temperature of the human body, is favorable for the antigen-antibody interaction. Considering the practical application hereafter, all the experiments were carried out at room temperature (25 °C).

The incubation time was an important parameter for both capturing CEA on the electrode surface and specifically recognizing GOx-NPG-Ab_2. The effects of incubation time for antigen on the photocurrent intensity were investigated as follows: over a longer incubation time, the photocurrent response increased quickly and reached its maximum value at 45 min (Fig. S5A), indicating a thorough capturing of the antigens on the electrode surface. In the second immunoassay incubation step, the photocurrent intensity showed the same changing tendency, and the current reached a

plateau at about 45 min (Fig. S5B). To shorten the assay time, an incubation time of 45 min was selected for further study.

GOx was introduced into the immunosensor to catalyze glucose hydrolysis to produce electron donor of H_2O_2. Thus, the catalytic reaction time of GOx towards glucose hydrolysis has great influence on the sensitivity of the fabricated biosensor. As shown in Fig. S5C, the photocurrent intensity increased sharply during the catalytic time from 0 to 30 min and then tended toward a stable value, indicating the absolute catalytic reaction. Therefore, 30 min was selected as the optimized catalytic time for PEC experiments to obtain relatively larger photocurrent and consequently better sensitivity of the proposed biosensor.

In this work, the fabricated PEC biosensor for CEA detection was based on visible light irradiation. The effects of light wavelength on the photocurrent response of biosensor were investigated. As shown in Fig. S5D, the photocurrent intensity increased as the exciting wavelength increased from 420 nm to 510 nm, and then a slightly decreasing photocurrent was observed upon further increase of light wavelength. Thus, 510 nm was chosen as the external excitation wavelength in the following PEC experiments with an external light source.

The applied potential was an important parameter for producing the photocurrent. As shown in Fig. S5E, with an increase of potential from 0 to 0.4 V, the photocurrent increased accordingly. Obviously, the photocurrent at 0 V was 85% of that at 0.4 V, showing enough sensitivity for PEC detection of CEA. Meanwhile, the low applied potential was beneficial to the elimination of interference from other reductive species that coexisted in the real samples. Thus, 0 V was selected as the applied potential for the determination of CEA.

Because of the co-immobilization of GOx and Ab_2 on the NPG carrier, the ratio of GOx and Ab_2 (GOx/Ab_2) was the most important factor on the photocurrent response signal. As shown in Fig. S5F, the optimal photocurrent response was obtained at the ratio of 50/1. The increase of the GOx/Ab_2 ratio could increase the total loading amount of GOx, which participated in the enzymatic catalysis reaction and boosted the H_2O_2 generation. However, the reducing amount of Ab_2 in the immunoassay may

decrease coupling efficiency to the captured CEA at the electrode surface, which may result in a decreased response. Therefore, 50/1 molar ratio of GOx and Ab_2 was selected for the preparation of recognition elements.

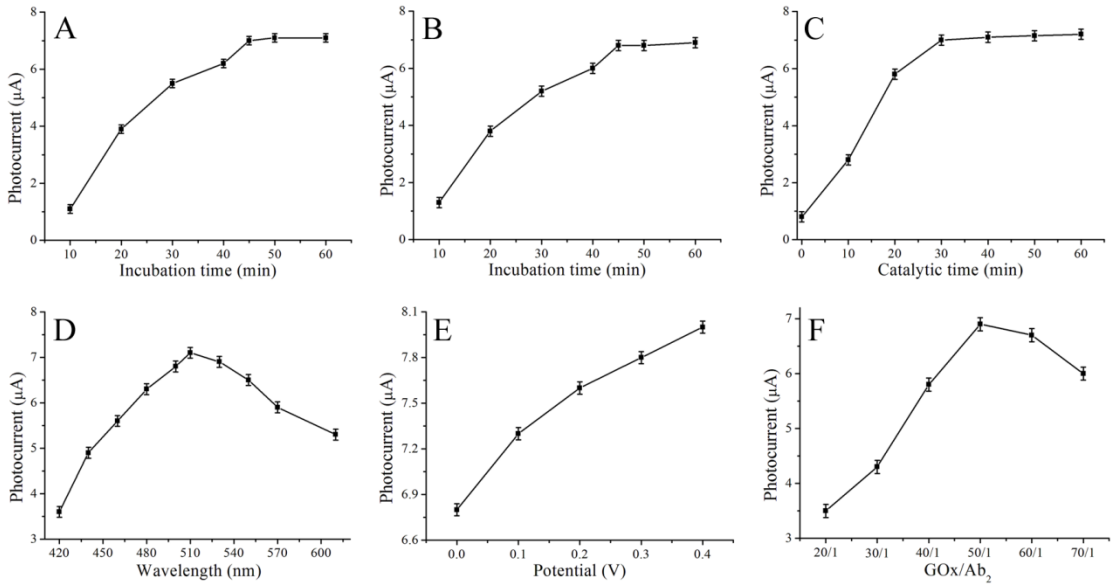

Fig. S5 The effect of (A) incubation for CEA, (B) incubation time for signal label, (C) catalytic time, (D) wavelength, (E) potential and (F) the ration of GOx to Ab_2 on photocurrent responses.

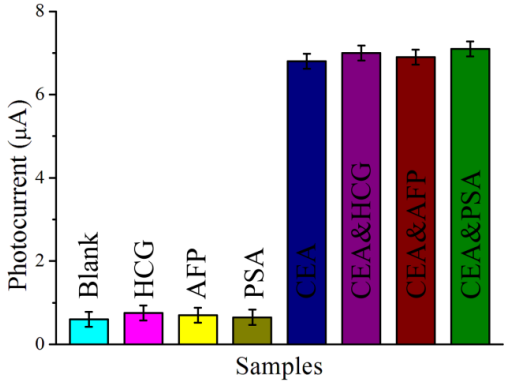

Fig. S6 The interfering effects of sample matrix components on the current responses.

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

Measurement protocol	Linear range (ng mL^{-1})	Detection limit (pg mL^{-1})	References
Electrochemiluminescent	0.02-80	6.8	1
PEC	0.05-20	10	2
PEC	0.005-20	1.8	3
Capacitive	0.01-10	10	4
Electrochemical	0.01-80	2.36	5
Chemiluminescence	0.1-40	/	6
PEC	0.002-100	0.7	This work

Table S2 Assay results of clinical serum samples using the proposed and referenced method.

Samples	1	2	3	4	5	6
Proposed method (ng mL^{-1})[a]	0.09	0.56	2.11	5.24	6.74	8.26
Reference method (ng mL^{-1})	0.085	0.58	2.19	5.12	6.53	8.51
Relative error (%)	5.88	-3.45	-3.65	2.34	3.22	-2.64

[a] The average value of seven successive determinations.

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