Electronic Supplementary Information

Split-type photoelectrochemical immunoassay for sensitive quantification of carcinoembryonic antigen based on target-induced in-situ formation of Z-type heterojunction

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EXPERIMENTAL SECTION

Material and reagent. CEA antigen (D111404), CEA antibody (D19400, cAb), CEA antibody (D194003, dAb), and 96-well microtiter plate were acquired from Sangon Biotech Co., Ltd. (Shanghai, China). Zinc chloride ($ZnCl_2$), indium chloride ($InCl_3$), and thioacetamide (TAA) were purchased from Aladdin (Shanghai, China). Gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), and bovine serum albumin (BSA) were acquired from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other reagents were used as received without further purification.

Apparatus. Scanning electron microscopy (SEM; SU8100, Hitachi Instruments, Japan), transmission electron microscopy (TEM; Tecnai G2 F20, FEI Co., USA), X-ray photoelectron spectroscopy (XPS; Scientific ESCALAB 250, USA), and UV-visible diffuse reflectance spectrum (DRS; Cary 7000, Agilent Technologies, Inc) were used to characterize the as-prepared materials in this work. All photoelectrochemical measurement processes were performed on a CHI760E electrochemical workstation (Chenhua Instruments, China).

Synthesis of $ZnIn_2S_4$ nanoflower and gold nanoparticles-decorated $ZnIn_2S_4$ nanocomposites. The hierarchical $ZnIn_2S_4$ was synthesized by a solvothermal treatment according to previous literature.[1] Initially, $ZnCl_2$ (10 mmol), $InCl_3$ (20 mmol) and TAA (80 mmol) were added sequentially into 50-mL deionized water. After forming a homogeneous solution under continuous stirring, it was poured into a Teflon-lined stainless-steel autoclave of 100 mL capacity and heated at 160 °C for 6 h. After natural cooling, the samples were washed several times with deionized water and ethanol and then placed in a drying oven at 60 °C for 8 h.

Next, gold nanoparticles-functionalized $ZnIn_2S_4$ (AuNPs/$ZnIn_2S_4$) nanocomposites were obtained based on the $NaBH_4$-reducing method. In brief, 100 mg of the as-prepared $ZnIn_2S_4$ powder was dispersed in 20 mL of H_2O and ultrasonicated for 50 min. Thereafter, $HAuCl_4$ aqueous solution (2.0 mL, 20 mM) was quickly added to the solution. After being stirred in the dark overnight, a freshly prepared $NaBH_4$ solution (10 mL, 20 mM) was added dropwise into the above mixture, and the reaction proceeded under vigorous stirring for 3 h. Upon completion of the reaction, the product was collected by centrifugation, washed several times with water and ethanol, and finally dried at 50 °C.

Synthesis of silver nanoparticles and immunoprobe bioconjugates. Silver nanoparticles (AgNPs) were prepared as follows according to the literature.[2] Initially, 10 mL of 1.0 mM $AgNO_3$ aqueous solution was slowly dropped into 30 mL $NaBH_4$ aqueous solution (2.0 mM) under vigorous stirring in an ice bath. Then, the mixture was continuously stirred until the color turned from bright yellow to brownish yellow, and the temperature of the solution rose to room temperature. Finally, the obtained silver colloids were stored at 4 °C in darkness for further use.

The synthetic process of detection antibody-AgNPs conjugates was as follows.[3] Before labeling, AgNPs were adjusted to pH 9.0-9.5 by using Na_2CO_3 solution to avoid precipitation upon protein introduction. After that, the detection antibody (200 μL, 0.5 mg mL^{-1}) was injected into the above AgNPs solution (2.0 mL). Then, the resulting suspension was incubated overnight at 4 °C under the gently shaking condition. Afterwards, the mixture was centrifuged, and the obtained precipitate, dAb-AgNPs, was dispersed in 2.0 mL of PBS (pH 7.4) containing 1.0 wt% BSA and 0.5 wt% Tween 20.

Preparation of capture antibody-coated microplate. Prior to the immunoreaction, a capture antibody-coated microplate was prepared by injecting 50 μL of cAb (10 μg mL^{-1}) into a blank 96-well microplate. After being incubated overnight at 4 °C, the wells were washed three times by using PBS. Then, 300 μL of blocking buffer (10 mM PBS containing 1.0 wt% BSA, pH 7.4) for 60 min at 37 °C to eliminate the nonspecific adsorption. Following washing three times with PBS, the cAb-coated microplate was obtained and stored in a 4 °C refrigerator for further use.

Preparation of AuNPs/$ZnIn_2S_4$-modified FTO electrode. Primarily, the FTO glass electrode was ultrasonically cleaned in water, ethanol, and water in sequence, and then was dried at 60 °C. Next, 30 μL of AuNPs/$ZnIn_2S_4$ suspension (1.0 mg mL^{-1}, dispersed in water) was dropped onto the FTO with a fixed area of 0.28 cm^2, and dried in an oven at 50 °C.

Photoelectrochemical (PEC) measurement. The PEC measurements were carried out in 0.1 M Na_2SO_4 under the irradiation of a 300 W Xe lamp (PLS-SXE300, Perfectlight, Beijing, China) on an electrochemical workstation with a classical three-electrode system including a modified FTO working electrode, a Pt-wire counter electrode, and a saturated calomel reference (SCE) electrode. The constant potential was set at 0 V, and current-time (i-t) curve method was used in the whole PEC experiments.

ADDITIONAL RESULTS AND DISCUSSION

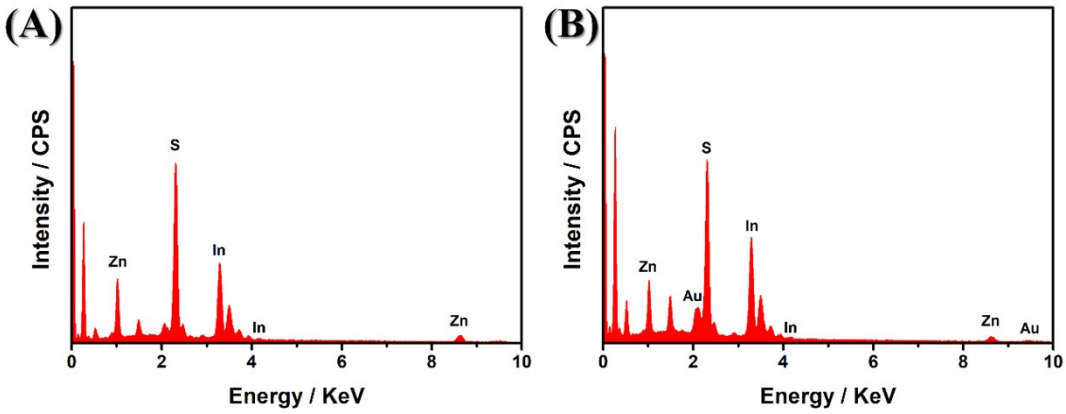

Fig. S1. EDS spectrum of (A) pure $ZnIn_2S_4$ sample and (B) AuNPs/$ZnIn_2S_4$ nanohybrids.

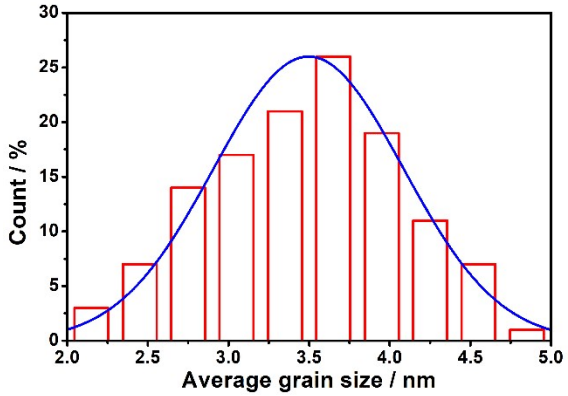

Fig. S2. Grain size distribution measured from inset of Fig. 1G by Nano measurer 1.2.

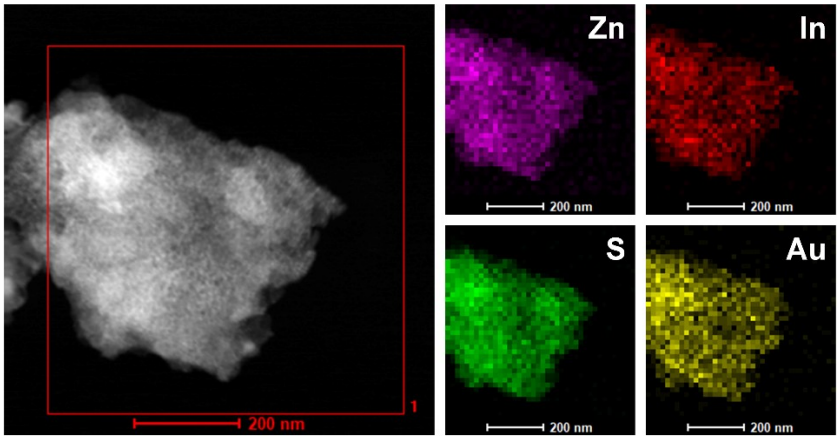

Fig. S3. EDS elemental mapping of AuNPs/$ZnIn_2S_4$.

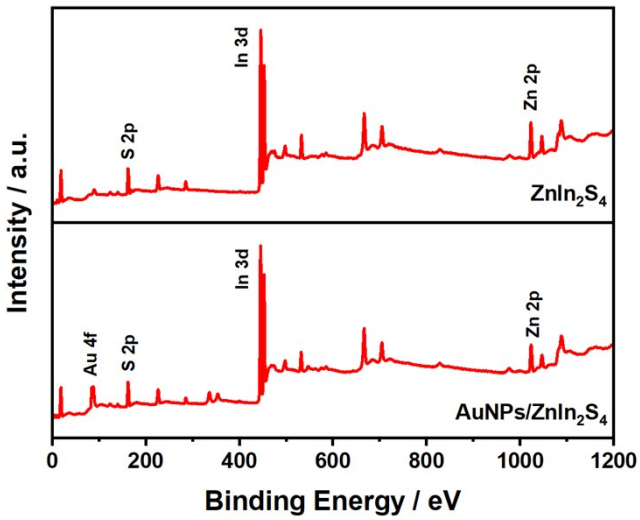

Fig. S4. The full XPS spectra of $ZnIn_2S_4$ and $AuNPs/ZnIn_2S_4$ samples.

The band gaps of these as-prepared materials were acquired through $(\alpha h\nu)^2$-hv elation fitting according to UV-DRS plots, which could be estimated from the equation: $(\alpha h\nu)^2 = A (h\nu - E_g)$, where α, h, ν, A, and E_g are the absorption coefficient, Planck constant, light frequency, the constant, and band gap energy, respectively.

The valence band and conduction band potentials of synthesized samples were calculated according to the following empirical formulas (1) and (2):

$$E_{CB} = \chi + E_c - E_g/2 \quad \cdots\cdots (1)$$

$$E_{VB} = E_{CB} + E_g \quad \cdots\cdots (2)$$

Where E_c is a constant relative to a standard H electrode, E_c = -4.5 eV; and χ is the geometric mean of the absolute electronegativity of the atoms in the semiconductor, the χ values of $ZnIn_2S_4$ and Ag_2S are 4.86 eV and 3.94 eV, respectively.[4]

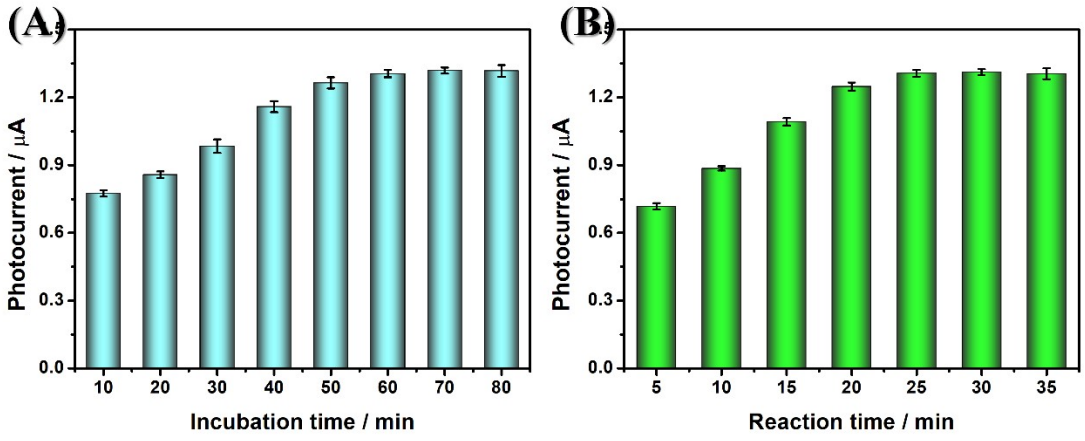

Fig. S5. Influence of (A) incubation time of the CEA antigen-antibody and (B) the ion-exchange reaction time on the photocurrent of PEC biosensor (1.0 ng mL^{-1} CEA used in all cases).

Table S1 Comparison of analytical properties of different methods toward target CEA

Method	Materials	Linear range	LOD	Reference
Fluorescence	Carbon Dots	0.5 - 1 ng mL^{-1}	0.3 ng mL^{-1}	5
Fluorescence	FeO_x@C@CS	0.05 - 7.5 pg mL^{-1}	0.025 pg mL^{-1}	6
Enzyme linked immunosorbent assay	-	0.5 - 60 ng mL^{-1}	174 pg mL^{-1}	7
Electrochemistry	Fe_3O_4@SiO_2	0.001 - 80 ng mL^{-1}	0.0002 ng mL^{-1}	8
Electrochemistry	CSH	0.1 pg - 80 ng mL^{-1}	0.03 pg mL^{-1}	9
Electrochemistry	Cu-Au/CNTs-CSs	0.025 - 25 ng mL^{-1}	0.5 pg mL^{-1}	10
Colorimetric	Cu_2O/Pt nanoparticles	0.1 - 80 ng mL^{-1}	0.026 ng mL^{-1}	11
Colorimetric	MoS_2	0.001 - 10 ng mL^{-1}	0.11 pg mL^{-1}	12
Chemiluminescence	Fe_3O_4@SiO_2	0.01 - 20 ng mL^{-1}	3.0 pg mL^{-1}	13
Surface enhanced Raman scattering	Gold nanoparticles	0.005 - 2 ng mL^{-1}	20 pg mL^{-1}	14
Surface enhanced Raman scattering	Gold nanostars	0.1 - 500 ng mL^{-1}	0.05 ng mL^{-1}	15
CVG-AFS/ICP-MS	CdTe	0.5 - 20 ng mL^{-1}	0.2 ng mL^{-1}	16
Photoelectrochemistry	AuNPs/$ZnIn_2S_4$	0.01 - 20 ng mL^{-1}	7.3 pg mL^{-1}	This work

FeO_x@C@CS: monodisperse spindle-like magnetic copper silicate; CSH: copper silicate hollow spheres; CNTs: Carbon nanotubes; CSs: carbon spheres; CVG-AFS/ICP-MS: chemical vapor generation-atomic fluorescence spectrometry/inductively coupled plasma-mass spectrometry.

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