

Electronic Supplementary Material for

Photoelectrochemistry immunoassay of alpha-fetoprotein based on a SnO₂/In₂S₃ heterojunction and an enzyme-catalyzed precipitation strategy

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Reagents

Tin chloride pentahydrate ($\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$), citric acid (CA), indium chloride tetrahydrate ($\text{InCl}_3 \cdot 4\text{H}_2\text{O}$), chitosan (CS), cerium nitrate hexahydrate ($\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$), 3-amino-9-ethylcarbazole (AEC), L-cysteine (L-Cys), hydrogen peroxide (H_2O_2), methanol, hydrochloric acid (HCl), ascorbic acid (AA), sodium hydroxide (NaOH), potassium dihydrogen phosphate (KH_2PO_4) and dipotassium hydrogen phosphate (K_2HPO_4) were purchased from Sinopharm (Shanghai, China). 3-Aminopropyltriethoxysilane (APTES, $\geq 99.5\%$ purity), N-hydroxy succinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and glutaraldehyde (GLD) were purchased from Sigma-Aldrich. The above reagents are of analytical grade or higher purity.

AFP (Ag), AFP antibody and cytokeratin 19 fragment 21-1 (CYFRA21-1) were purchased from Shanghai Lingchao Biotechnology Co., Ltd. Immunoglobulin G (IgG), carcinoembryonic antigen (CEA) and procalcitonin (PCT) were purchased from Beijing Bioss Biological Co. The FTO conductive glass was obtained from Zhuhai Kaivo Optoelectronic Technology Co. The buffers used for PEC immunoassay and rinse were 0.100 M phosphate buffer (pH 7.40, 0.100 M KH_2PO_4 - K_2HPO_4) and 0.0100 M phosphate buffer, respectively. Milli-Q ultrapure water (Millipore, $\geq 18 \text{ M}\Omega \text{ cm}$) was employed throughout.

Instrumentation

The PEC tests were carried out on an electrochemical workstation (ZENNIUM, ZAHNER-elektrik GmbH & Co. KG, Germany) with a PEC unit (CIMPS, PP211, ZAHNER-elektrik GmbH & Co. KG, Germany). The xenon lamp (WLC02, ZAHNER-elektrik GmbH & Co. KG, Germany) with visible light filter (400-700 nm) was used as the light source (100 mW cm⁻²). Cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and Mott-Schottky plot (M-S plot) experiments were carried out on a CHI660E electrochemical workstation (Shanghai Chenhua Instrument Co., Ltd.). Scanning electron microscopy (SEM) characterization was performed on a FEI Helios NanoLab 600i scanning electron microscope with an Oxford energy dispersive X-ray spectroscopy (EDS) analysis detector for elemental analysis. TECNAI F-30 transmission electron microscopy (TEM) instrument was used for the ultramicroscopic characterization of materials. Ultima IV X-ray diffractometry (XRD) was used for physical phase analysis. The ultraviolet-visible diffuse reflectance spectra (UV-vis DRS, with BaSO₄ as a reference) was recorded on a UV-2600i UV-vis spectrophotometer. FT-IR spectrum was collected on a Shimadzu IRTracer 100 Fourier transform infrared (FT-IR) spectrophotometer (KBr pellet). UV-vis absorption spectra were collected on a UV-2600i ultraviolet-visible spectrophotometer. The immune-electrode was used as the working electrode at an applied bias of 0.05 V vs SCE, a Pt disk as the counter electrode and a saturated calomel electrode as the reference electrode.

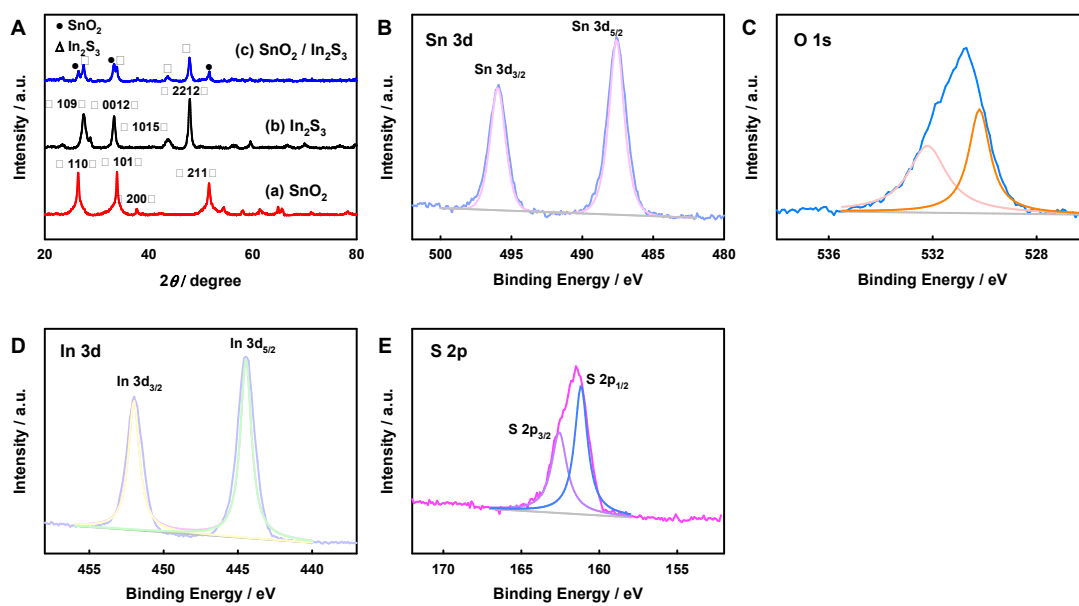


Fig. S1 (A) XRD patterns of SnO₂ (a), In₂S₃ (b) and SnO₂/In₂S₃ (c). XPS high-resolution spectra of (B) Sn 3d, (C) O 1s, (D) In 3d and (E) S 2p.

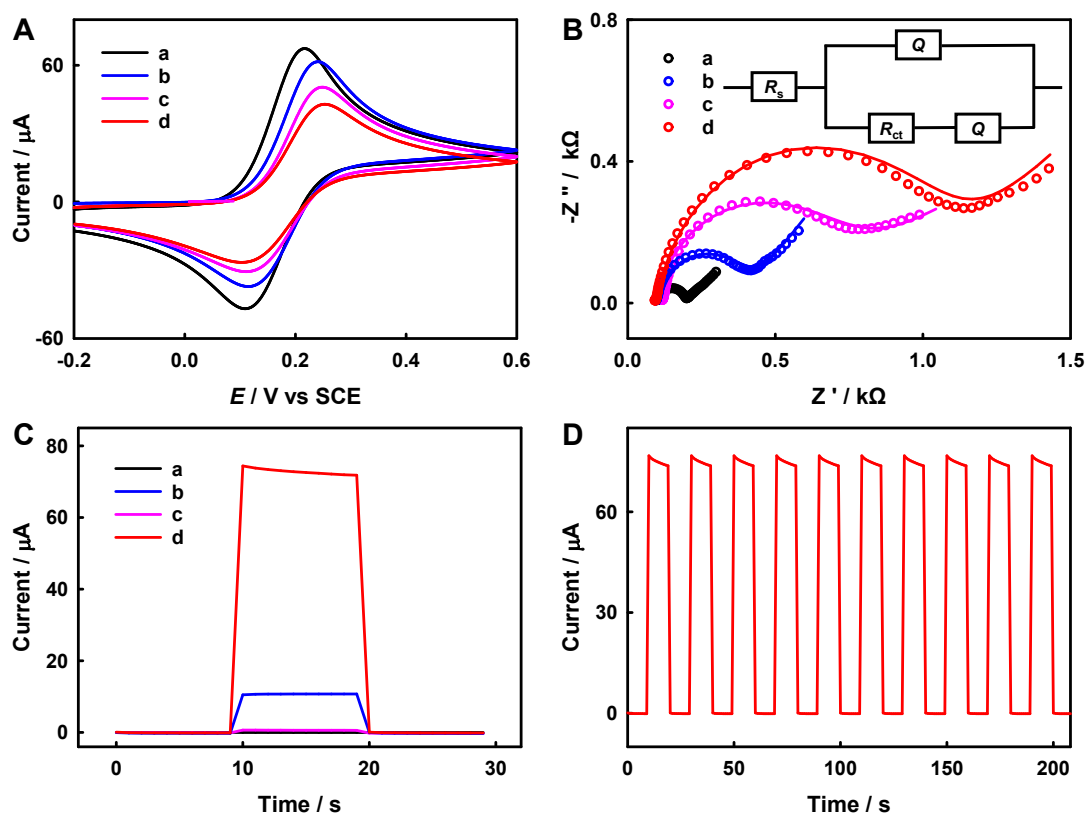


Fig. S2 (A) CV, (B) EIS, (C) photocurrent responses on (a) FTO, (b) $\text{In}_2\text{S}_3/\text{FTO}$, (c) SnO_2/FTO and (d) $\text{SnO}_2/\text{In}_2\text{S}_3/\text{FTO}$, and (D) Stability of $\text{SnO}_2/\text{In}_2\text{S}_3/\text{FTO}$ photoanode. CV scan rate: 50 mV s^{-1} . EIS experiment: $100 \text{ kHz} \sim 0.01 \text{ Hz}$, 0.20 V , 200 s resting time, circle: experimental value; curve: fitted to a modified Randles equivalent circuit (inset). Photocurrent experiment: PBS (pH 7.40) containing 0.05 M AA at 0.05 V vs SCE .

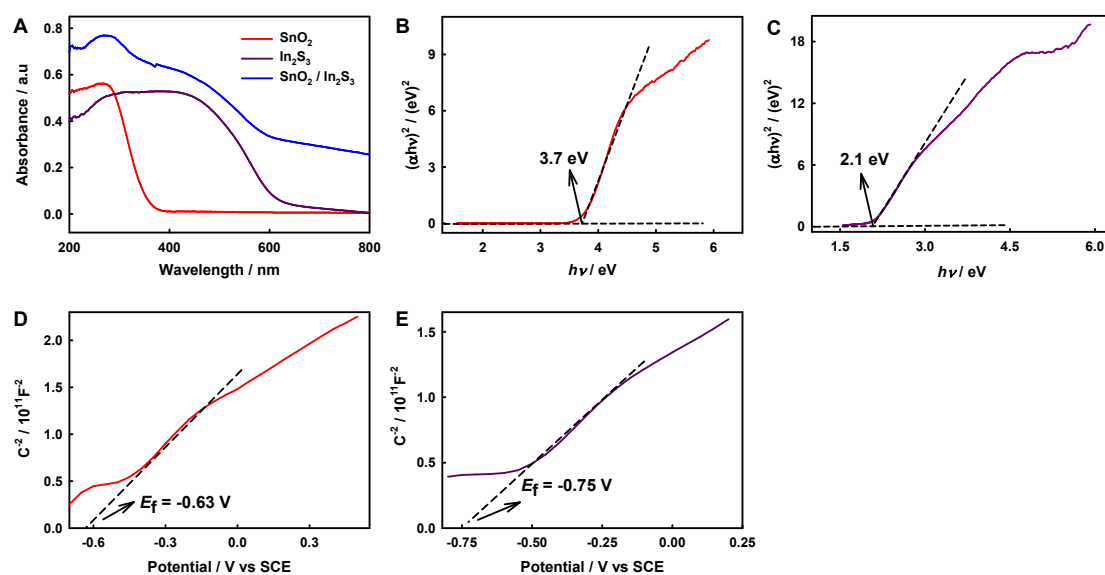


Fig. S3 (A) UV-vis DRS of SnO₂, In₂S₃ and SnO₂/In₂S₃. Tauc plots of (B) SnO₂ and (C) In₂S₃.

M-S plots of (D) SnO₂ and (E) In₂S₃. Mott-Schottky experiment: 0.2 M aqueous Na₂SO₄,

1000 Hz. The band gap (E_g) of a semiconductor material can be calculated according to the

UV-vis DRS and the Tauc equation¹, $ahv = A(hv - E_g)^n$, where a is the absorption coefficient,

h is Planck's constant (6.63×10^{-34} J s), ν is the incident photon frequency (s^{-1}), E_g is the

absorption band gap (in eV), A is a constant, and the parameter n is related to different types

of electronic transitions ($n = 1/2, 2, 3/2$ or 3 for directly allowed, indirectly allowed, directly

forbidden and indirectly forbidden transitions, respectively)². Both SnO₂ and In₂S₃ are direct

semiconductor^{3, 4}. $(ahv)^2$ is plotted against hv . From the intercept point at $y = 0$, it can be

deduced that the E_g values are 3.7 eV for SnO₂ and 2.1 eV for In₂S₃.

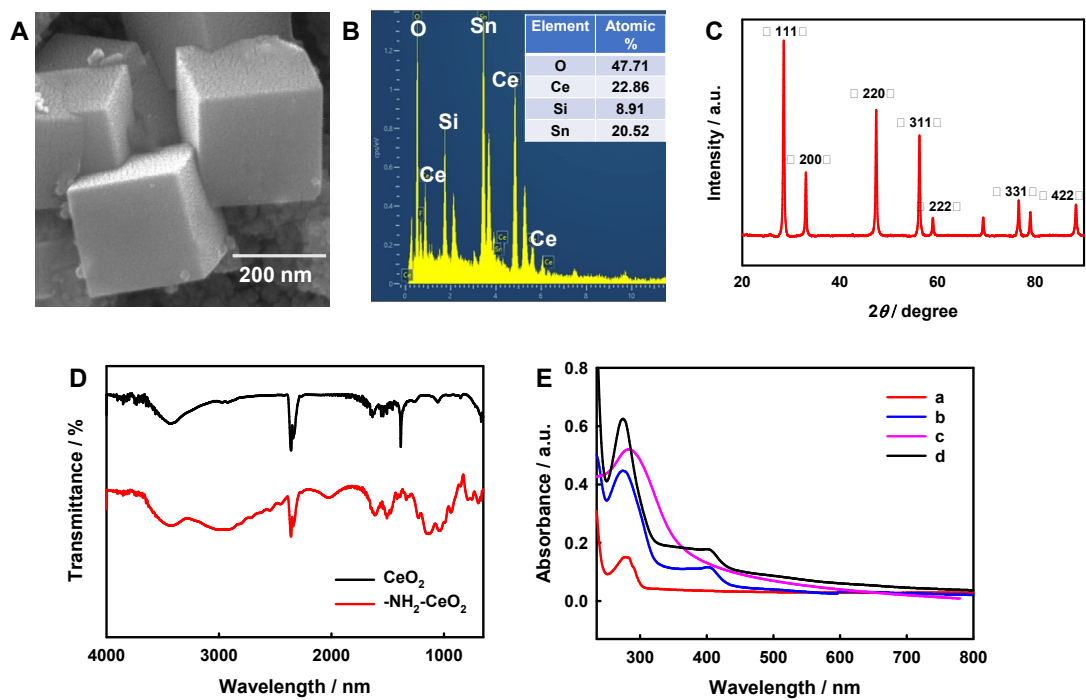


Fig. S4 (A) SEM image of CeO₂/FTO, (B) EDS image of CeO₂/FTO, (C) XRD image of CeO₂, (D) FT-IR image of CeO₂ and NH₂-CeO₂, and (E) UV-vis absorption spectra of (a) Ab₂, (b) HRP, (c) NH₂-CeO₂ and (d) HRP-CeO₂-Ab₂.

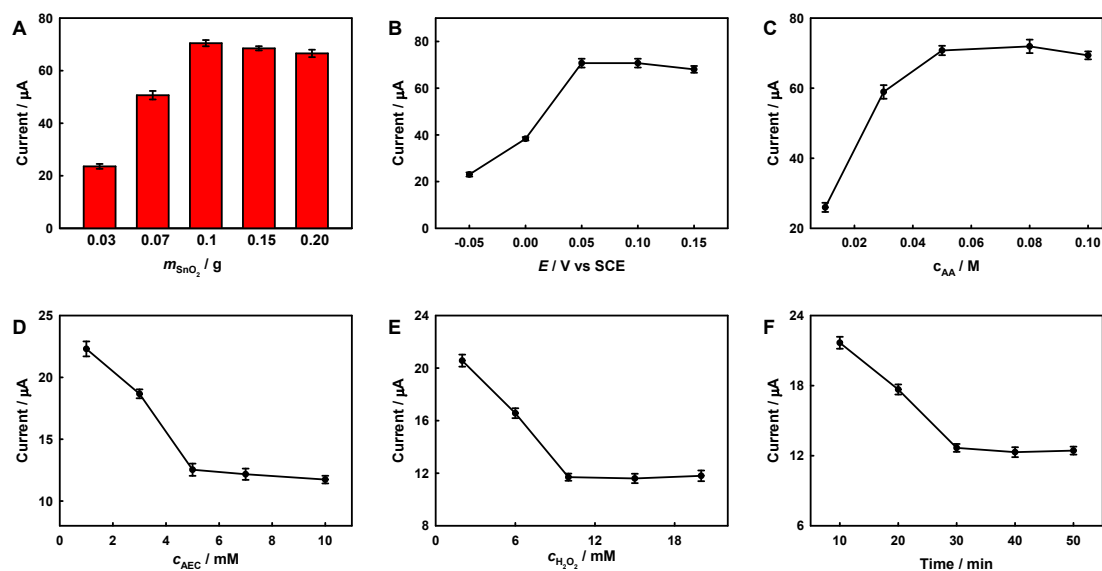


Fig. S5 Photocurrent responses using different mass of SnO_2 (A), as well as optimization of bias (B), AA concentration (C), AEC concentration (D), H_2O_2 concentration (E), and catalytic precipitation time (F). 500 pg mL^{-1} Ag. Here, in order to obtain the best performance of PEC analysis, we have studied the addition amount of SnO_2 (m_{SnO_2}), bias, AA concentration (c_{AA}), AEC concentration (c_{AEC}), H_2O_2 concentration ($c_{\text{H}_2\text{O}_2}$) and catalytic precipitation reaction time (t). With the increase of the amount of SnO_2 added, the photocurrent response is increased from 0.03 g to 0.1 g and then reaches the maximum and tends to be stable. The photocurrent increases with the bias shift from -0.05 V to 0.05 V and reaches the maximum and tends to be stable at 0.05 V or more positive bias. At 0.05 V bias, the background current is also good. When the concentration of AA increased from 0.02 M to 0.05 M, the photocurrent increased significantly. The photocurrent tends to be stable with $c_{\text{AEC}} \geq 5 \text{ mM}$ and $c_{\text{H}_2\text{O}_2} \geq 10 \text{ mM}$, and the catalytic precipitation reaction time reaches a stable level at 30 min. Therefore, the optimized optimal conditions are as follows: $m_{\text{SnO}_2} = 0.1 \text{ g}$, bias = 0.05 V vs SCE, $c_{\text{AA}} = 0.05 \text{ M}$, $c_{\text{AEC}} = 5 \text{ mM}$, $c_{\text{H}_2\text{O}_2} = 10 \text{ mM}$, and the catalytic precipitation reaction time = 30 min.

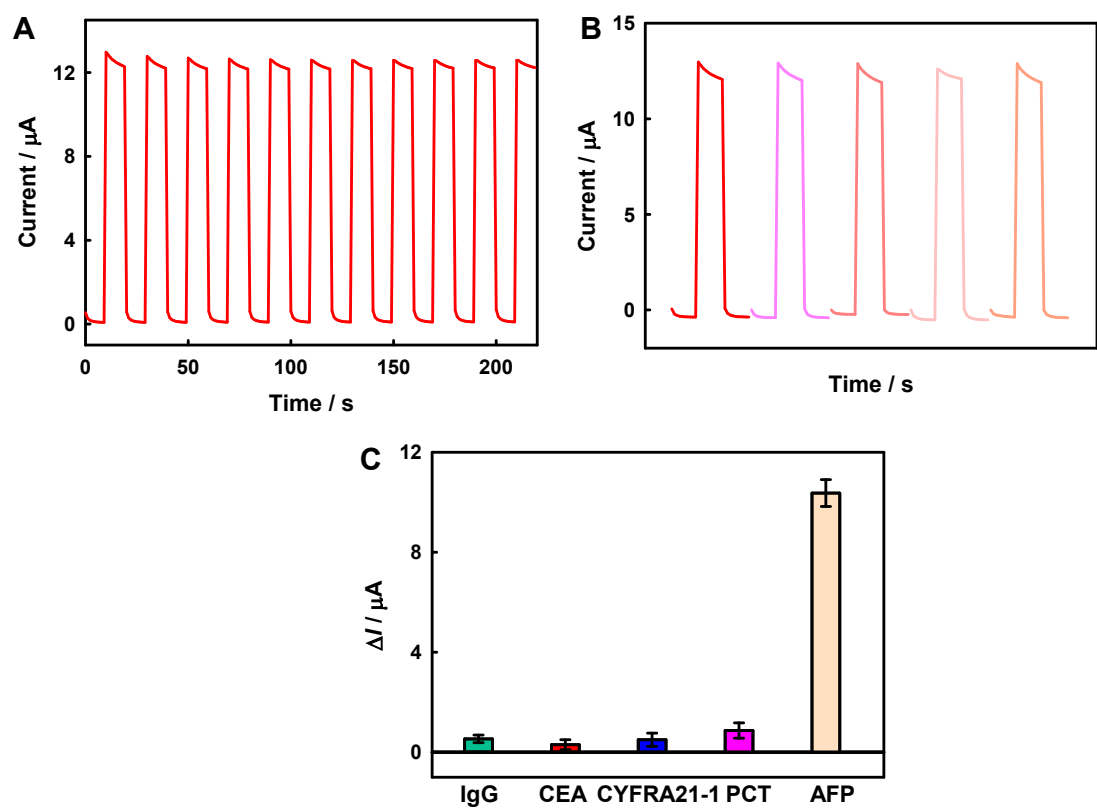


Fig. S6 (A) Stability and (B) reproducibility of the sandwich immune-electrode assay (500 pg mL^{-1}) with all interferent candidates each at a concentration of 50 ng mL^{-1} , $\Delta I = I_0 - I$, where I_0 is BSA/ Ab_1 /GLD/CS/ SnO_2 / In_2S_3 /FTO electrode, I is the photocurrent after treating the BSA/ Ab_1 /GLD/CS/ SnO_2 / In_2S_3 /FTO electrode with AFP or each of the potential interferents.

Table S1. Sensor performance comparison *

Technique	Linear range (ng mL ⁻¹)	LOD (pg mL ⁻¹)	Ref.
ECL	0.001-50	3.4	5
ECL	0.025-15	12	6
Colorimetry	10-1000	2.3	7
Electrochemisty	0.01-50	0.589	8
Electrochemisty	0.001-10000	1.09	9
FL	0.05-20	17.3	10
PEC	0.005-15	1.88	11
PEC	0.01-100	7	12
PEC	0.001-1000	0.82	13
PEC	0.05-100	50	14
PEC	0.0005-50	0.15	This work

* ECL: electrochemiluminescent; FL: Fluorescence.

Table S2. Detection of AFP in human serum samples by standard addition method ($n = 3$)*

Sample No.	Added (ng mL ⁻¹)	Measured (ng mL ⁻¹)	RSD (%)	Recovery (%)
1	1.00	0.920	3.3	92.0
2	0.100	0.0951	5.8	95.1
3	0.0100	0.0106	7.6	106

*Here, the healthy human serum samples were donated by Hunan Normal University Hospital and they are from male and female aged 18~23 with good physical quality. The samples were centrifuged at 5000 rpm for 10 min and diluted 20 times with 0.01 M PBS (pH 7.40), and AFP standard solution was added to 3 tubes of 1 mL diluted serum to make it final concentrations at 1.00 ng mL⁻¹, 0.100 ng mL⁻¹, and 0.0100 ng mL⁻¹. The PEC test was carried out under the optimal experimental conditions.

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