

Supporting Information

Bioetching-Induced Visualized-Organic Photoelectrochemical Transistor Dual-Signal Mode Sensor for Alkaline Phosphatase Detection

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1. Materials and Apparatus

1.1 Materials.

Zinc acetate dihydrates ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$), alkaline phosphatase (ALP, ≥ 10 DEA units/mg, derived from bovine intestinal mucosa), glucose oxidase (GOx), immuno-globulin G (IgG), horseradish peroxidase (HRP), pyrophosphatase (PPase), bovine serum albumin (BSA), and sodium thiophosphate (TP) was purchased from Shanghai Macklin Biochemical Technology Co., Ltd. Graphene oxide (GO) dispersion was purchased from Jiangsu Xianfeng Nanomaterials Technology Co., Ltd. (Nanjing, China) Indium nitrate ($\text{In}(\text{NO}_3)_3 \cdot 4\text{H}_2\text{O}$), cadmium chloride hemi(pentahydrate) ($\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$), glycine (Gly), thioacetamide (TAA), ethylene glycol (EG) and ethanol were provided by Sinopharm Chemical Reagent Co., Ltd. (China). Serum samples from healthy adult volunteers were provided by Affiliated Hospital of Jiangsu University (Zhenjiang, China). Phosphate-buffered saline (PBS) of different pH were prepared by mixing 0.1 M stock solutions of NaH_2PO_4 and Na_2HPO_4 at specific ratios, and the washing buffer was prepared by 0.1 M PBS (pH 7.4). Then the Prussian blue solution was composed of 0.05 M $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.05 M $\text{K}_3[\text{Fe}(\text{CN})_6]$ and 5 mM HCl. All the chemicals used in the experiments were used directly without further purification, and all reagent solutions were prepared with deionized (DI) water.

1.2 Apparatus

The morphologies of nanomaterials and the elemental composition of the sample were observed by scanning electron microscopy (SEM, JSM-7800F, Japan) equipped with energy-dispersive spectrometer (EDS) and transmission electron microscopy (HR-TEM, JEOL JEM-AFM, Japan). X-ray diffractometer equipped with (Bruker, Germany) high-intensity $\text{Cu K}\alpha$ ($\lambda = 1.5406$) radiation was used to record the X-ray diffraction (XRD) pattern. The absorption spectrum was tested by UV-vis spectrophotometer (UV2550, Shimadzu, Japan) from 200 to 1000 nm with BaSO_4 as reflectance sample. X-ray photoelectron spectroscopy (XPS) measurements were measured on Thermo Scientific K-Alpha X-ray photoelectron spectrometer with $\text{Al K}\alpha$ X-rays as the excitation source. The photoluminescence (PL) spectra of the samples

were obtained at room temperature with a FLUOROMAX-4C-TCSPC. Raman spectra were obtained on a Fisher Laser Microscope Raman Spectrometer (DXR, Thermo, USA). The photoelectrochemical (PEC), electrochemical impedance spectroscopy (EIS), and cyclic voltammograms (CV), Mott-Schottky (M-S) measurements were performed using a conventional three-electrode system (CHI 660E, Chen Hua Instruments Co., Ltd. Shanghai, China). All the electrochemical measurements used Xe lamp (PLS-SXE 300C (BF) Beijing perfect Light Technology Co., Ltd.) as the light irradiation source for the whole detection.

2. Experimental Section

2.1 Synthesis of ZnIn₂S₄ (ZIS)

The preparation of ZIS was based on previous reports with some modifications.^{1, 2} Typically, 73.2 mg of Zn(CH₃COO)₂·2H₂O, 195.3 mg of InCl₃·4H₂O and 200 mg TAA were dissolved in a 10 mL of EG. The above mixture was magnetically stirred at room temperature for 1 h until well dispersed. The homogeneous mixture was then transferred to a Teflon-lined stainless-steel autoclave and reacted at 160 °C for 16 h. At the end of the reaction, the product was centrifuged at 8000 rpm for 5 minutes and washed alternately with water and ethanol to remove water-soluble and fat-soluble impurities. The washed product was transferred to a vacuum drying oven at 60 °C for 12 h. Finally, the solid product was ground into powder form and collected.

2.2 Synthesis of NRGO and ZIS/NRGO

ZIS/NRGO composites were synthesized by a one-step hydrothermal method. Firstly, 3.525 mL of GO dispersion with 0.0976 g of Gly was added to 10 mL of EG and stirred well. Then 73.2 mg of Zn(CH₃COO)₂·2H₂O, 195.3 mg of InCl₃·4H₂O and 200.0 mg TAA were added to the above solution and well dispersed. The homogeneous mixture was then transferred to a Teflon-lined stainless-steel autoclave and reacted at 160 °C for 16 h. At the end of the reaction, the product was centrifuged at 8000 rpm for 5 min and washed alternately with water and ethanol to remove water-soluble and fat-soluble impurities. The washed product was transferred to a vacuum drying oven at 60 °C for 12 h. Finally, the solid product was ground into powder form and collected. The

preparation of NRGO was like that of ZIS/NRGO, except that only GO and Gly are added to the EG solution.

2.3 Preparation of electrochromic (EC) regions

First, the FTO electrode (1 cm × 3 cm) was sequentially washed with toluene, acetone, ethanol, and ultrapure water. Subsequently, the surface was immersed in a 1 M 1:1 (v/v) ethanol/NaOH solution for 15 min to activate it. Afterward, the surface was rinsed with ultrapure water and dried with N₂. A 100 mL solution containing 0.05 M FeCl₃, 5 mM HCl, and 0.05 M K₃Fe(CN)₆ was prepared as the electrodeposition solution. A three-electrode system consisting of an Ag/AgCl electrode, a Pt wire electrode, and FTO was used to carry out the electrodeposition of Prussian blue using the constant potential deposition method. The electrodeposition parameters were set at a voltage of 0.3 V and a time of 75 s.

2.4 Preparation of channel device of the OPECT sensor

Firstly, screen printing device specifications were customized from Qing Dao Technology Co., Ltd. as designed, in which the length and width of the channel were 6 mm*0.2 mm. Conductive carbon paste was printed on polyethylene glycol terephthalate (PET) substrate to make source electrode and drain electrode. Then the PEDOT:PSS was printed on the top of the channel to form the layer of thin film. Last, these devices were sealed with polyethylene plastic (PE) film for later use.

2.5 Fabrication of visualized-OPECT dual-signal mode sensor for ALP activity detection

Fabrication of electrochromic regions: The F-doped SnO₂ (FTO, 1 cm × 3 cm) electrodes were ultrasonically cleaned with toluene, acetone, ethanol, and deionized water, respectively, before use, followed by drying with nitrogen. The electron injection region of the FTO was masked and the substrate was placed in an electrodeposition solution containing 0.05 M FeCl₃·6H₂O, 0.05 M K₃[Fe(CN)₆], and 5 mM HCl. PB was deposited in the electrochromic region using a constant voltage deposition method (0.3 V, 75 s), with the working electrode as the FTO substrate and the counter electrode as the Pt wire, and the reference electrode was an Ag/AgCl electrode, and the area was rinsed with ultrapure water at the end of the deposition. Subsequently, the substrate was

placed in an oven at 60 °C overnight to immobilize the PB on the electrode surface.

Fabrication of the FTO/ZIS/NRGO/Cd²⁺: The FTO electrode was then modified with ZIS/NRGO (FTO/ZIS/NRGO) by drop-coating in 20 μL of a ZIS/NRGO solution (2 mg·mL⁻¹). The as-prepared FTO/ZIS/NRGO electrode was incubated in 1 mM Cd²⁺ solution at 25 °C for 24 h, and the FTO/ZIS/NRGO/Cd²⁺ electrode was obtained. Finally, the prepared electrodes were stored in a refrigerator for further use.

ALP activity detection: To assess ALP activity, 30 μL of varying ALP concentrations (0.001 to 50 U·L⁻¹, 1 to 250 U·L⁻¹) and 30 μL of 10 mM TP were combined in a 96-well plate, followed by incubation at 37 °C for 1 h. Subsequently, the resulting solution was applied to the FTO/ZIS/NRGO/Cd²⁺ electrode and incubated at 37 °C for an additional 1 h to yield the FTO/ZIS/NRGO/CdS electrode. The ALP-catalyzed reaction was examined for optimal time and TP concentration (Fig. S1).

2.6 Electrical measurements

OPECT devices were tested in the electrolyte of PBS solution (0.1 M, pH 7.4). For transfer characteristics (I_{DS} - V_G), the channel current I_{DS} was measured as a function of gate voltage V_G under a constant drain voltage $V_{DS} = -0.2$ V. The channel current I_{DS} as a function of time (I_{DS} -time) was measured under constant gate and drain voltages ($V_{DS} = -0.2$ V, $V_G = 0$ V) under illumination.

For the traditional PEC and EC measurements, the PBS solution (0.1 M, pH 7.4) was also used as the electrolyte. The EC measurements was performed under Xe lamp illumination. The photocurrent was measured with a three-electrodes system (a modified FTO electrode as the working electrode, a saturated calomel electrode as the reference electrode, and a Pt wire as the counter electrode) at a constant potential of 0 V. The photovoltage, electrochemical impedance spectroscopy (EIS) and Mott-Schottky were also tested using the same three-electrode system. The EIS tests were recorded in 0.1 M KCl solution containing Fe(CN)₆^{3-/4-} (5 mM) in a frequency range from 0.1 Hz to 100 kHz; The open circuit potential was used as a DC bias potential, on which amplitude of the AC potential was 5 mV. All the modified FTO electrodes ensure the same quality of material in the same working area.

2.7 ALP activity detection in serum samples

The serum samples used in this experiment were provided by volunteers from the Hospital of Jiangsu University. Before use, they were centrifuged at 3000 rpm for 20 min to remove the precipitate in the serum. The supernatant was diluted 1000 times, and ALP standard samples (0.1, 0.5 and 10 U·L⁻¹) were spiked. OPECT and EC detection of ALP activity in serum samples was like that in the buffer.

3. Results and Discussion

The UV-Vis diffuse reflectance spectroscopy (DRS) in Fig. S1A indicates the successful introduction of NRGO, effectively broadening the absorption range of ZIS. Furthermore, the corresponding Tuac plot (Fig. S1B) was obtained using the formula: $(\alpha h\nu)^n = A(h\nu - E_g)$, where h and A represent Planck's constant and the proportionality constant, respectively.³ The energy band gap (E_g) of ZIS was calculated to be 2.05 eV. In the XPS-VB spectrum (Fig. S1C), the valence band (VB) of ZIS was determined to be 1.34 eV. When considering the corresponding photocurrent signals, which exhibited a significant improvement for CdS-containing photocurrent signals (16.86 μA), a possible electron transfer mechanism is proposed, as illustrated in Fig. S1D. Upon photostimulation, photogenerated electrons in CdS and ZIS are transferred from the VB to the conduction band (CB). Subsequently, the electrons in the CB of CdS can be transferred to the VB of ZIS and recombine with the photogenerated holes, forming the Z-Scheme heterojunction.⁴ In addition, as a conductive carrier, NRGO can effectively improve the transfer efficiency of photogenerated electrons.⁵ Finally, the photogenerated electrons are transferred into the FTO, producing a positive anodic photocurrent.⁶ In this case, the lifetimes of the photogenerated carriers (electrons and holes) are extended, and the redox reactions in solution significantly suppress the reverse reactions, improving the charge separation efficiency.⁷ Additionally, ZIS/NRGO/CdS does provide abundant active sites, which are favorable for light harvesting, and the tight binding between the interfaces shortens the distance of charge transfer.⁸ These factors ultimately result in a significant enhancement of the photocurrent signal.

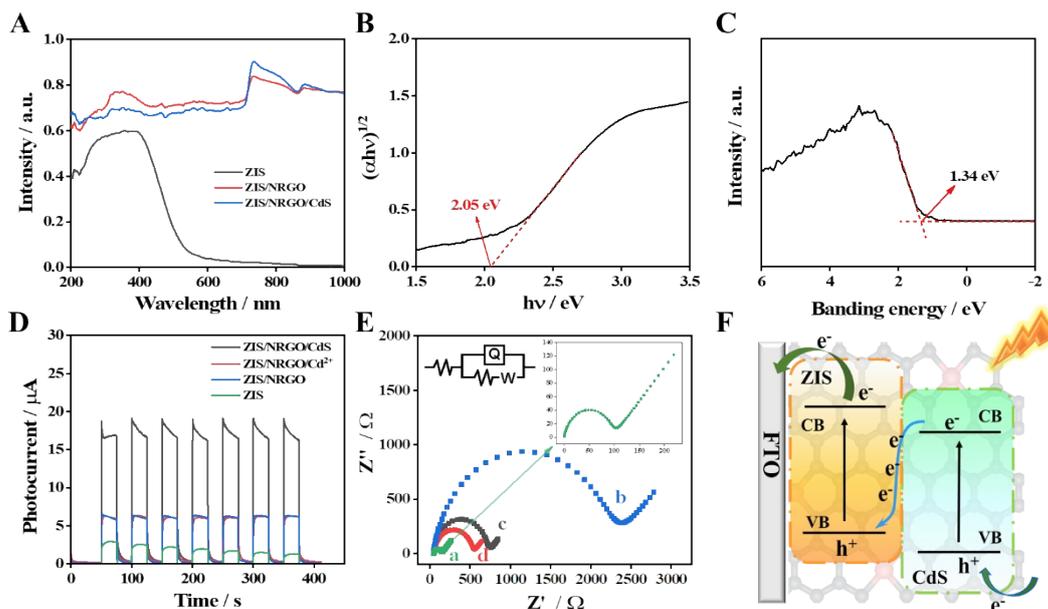


Fig. S1 (A) UV–vis DRS of ZIS, ZIS/NRGO, and ZIS/NRGO/CdS. (B) Tauc and (C) XPS valence band spectra of ZIS. (D) Photocurrent of ZIS, ZIS/NRGO, ZIS/NRGO/Cd²⁺, and ZIS/NRGO/CdS in 0.1 M PBS (pH 7.4). (E) EIS of the bare FTO (a), ZIS/FTO (b), ZIS/NRGO/FTO (c), and ZIS/NRGO/CdS/FTO (d). (F) Z-Scheme ZIS/NRGO/CdS photogenerated charge transfer processes within heterojunction.

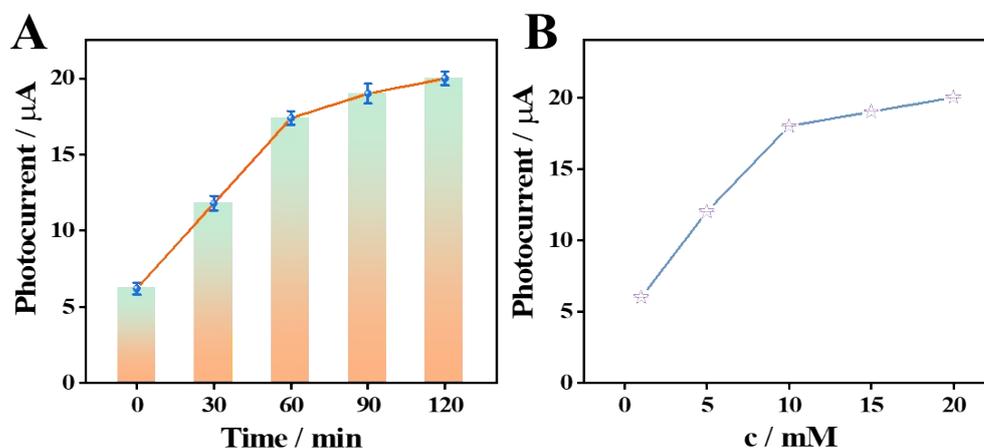


Fig. S2 (A) Optimization of ALP catalytic reaction time and (B) the TP concentration based on photocurrent response at 37 °C.

As shown in Fig. S2A, the optimised time for the ALP-catalysed reaction was 60 min, the temperature was 37 °C, ALP activity was fixed at 10 U·L⁻¹ and TP concentration was fixed at 10 mM. As shown in Fig. S2B,

the concentration of TP was optimised to 10 mM for subsequent biosensor applications, the activity of ALP was fixed at 10 U·L⁻¹ and the reaction conditions were 37 °C for 60 min.

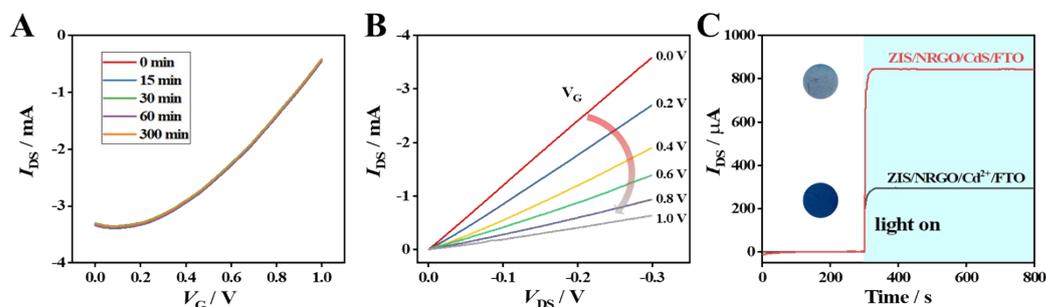


Fig. S3 (A) The transfer characteristics (I_{DS} - V_G , $V_{DS} = -0.2$ V) of the OPECT device with an Ag/AgCl gate electrode. (B) The output characteristics (I_{DS} - V_{DS}) of the OPECT device with a Ag/AgCl gate electrode. (C) I_{DS} response of ZIS/NRGO/Cd²⁺/FTO and ZIS/NRGO/CdS/FTO under illumination.

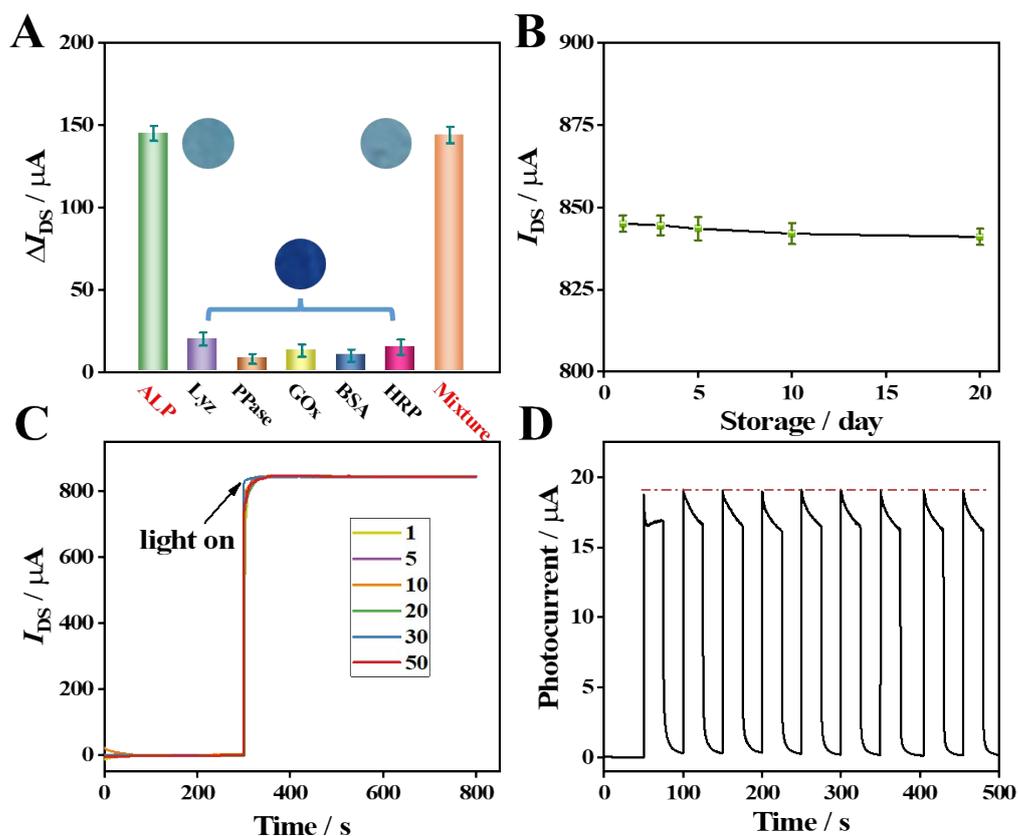


Fig. S4 (A) The selectivity of the visualized-OPECT dual-signal mode sensor. (B) The long-term storage stability of the OPECT sensor. (C) The repeatability for gate

electrodes and channel in OPECT device. (D) The stability evaluation of gate electrode for 1000 s.

Long-term storage stability (Fig. S4B): After the gate electrodes were modified with CdS and stored them in the refrigerator (4 °C) for 1, 3, 5, 7, 10 and 20 days. Then, the gate electrodes were taken out and conducted OPECT test. After 50 times tested, the I_{DS} signal did not change significantly showing good repeatability.

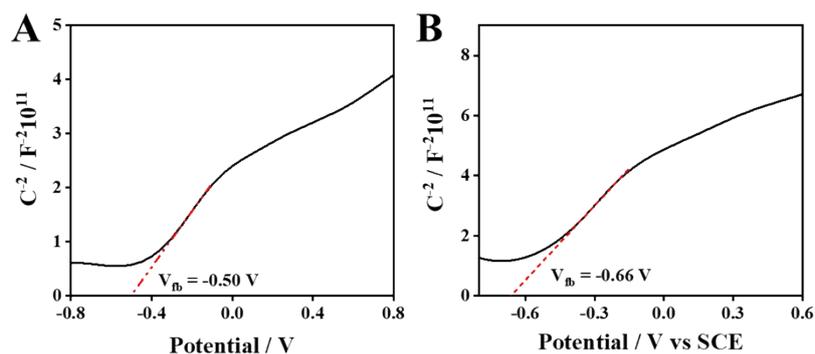


Fig. S5 Mott-Schottky plots of (A) CdS and (B) ZIS.

Table S1. Comparison with other detection methods for detecting ALP.

Methods	Liner range (U·L ⁻¹)	Detection limit (U·L ⁻¹)	Refs.
Electrochemiluminescent and electrochemical dual-mode biosensor	0.01-100	0.0076	9
PhotoATRP signal amplification electrochemical biosensor	10-150	100	10
OPECT sensor	0.005-15	0.0012	11
PEC sensor	50-1000	42.1	12
PEC sensor	0.01-500	0.005	13
OPECT sensor	0.001-50	0.00082	This work

Table S2. Recovery studies in serum samples

Added (U·L⁻¹)	Found (U·L⁻¹)	Recovery (%)	RSD (%)
0.1	0.108	108.3	2.39
0.5	0.505	101.1	3.27
10	9.983	99.8	1.54

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