Electronic Supplementary Information

Enhancing photoelectrochemical performance of $ZnIn_2S_4$ by Phosphorus doping for sensitive detection of miRNA-155

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Experimental section

Chemicals and materials

$In(NO_3)_3 \cdot xH_2O$ was bought from Shanghai Titan Scientific Co., Ltd. (Shanghai, China). Zinc nitrate ($Zn(NO_3)_2$), Ascorbic acid (AA), NaH_2PO_2 and L-cysteine were supplied by Chengdu Kelong Chemical Inc. (Chengdu, China). 6-mercaptohexanol (MCH) and Gold chloride ($HAuCl_4$) were acquired from Sigma-Aldrich (St. Louis, MO, USA).

$K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ were supplied by Beijing Chemical Reagent Co. (Beijing, China). Tris-hydroxymethylamino methane-hydrochloride (Tris) was supplied by Shanghai Roche Pharmaceutical Ltd. (Shanghai, China). Double-stranded specific nuclease (DSN) and 10×DSN master buffer were purchased from Axxora, LLC.(San Diego, CA, U.S.A.). 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM KCl and 140 mM NaCl were used to prepare the solution 20 mM Tris-HCl solution (pH 7.4). potassium ferricyanide and potassium ferrocyanide were dissolved in PBS solution to prepare $[Fe(CN)_6]^{3-/4-}$ solution (pH 7.4, 5.0 mM) .

The oligonucleotides sequences were supplied by Shanghai Sangon Biological

Engineering Technology and Services Co., Ltd. (Shanghai, China). The sequences were as follows:

HP1: 5'-SH-TTT TTG AGG CGC AGT CTA ACC CCT ATC ACG ATT AGC ATT AAT AGA CTG CG-3'

S1 : 5'-NH_2-TAG ACT GCG CCT C-3'

miRNA-155: UUA AUG CUA AUC GUG AUA GGG GU

miRNA-141: UAA CAC UGU CUG GUA AAG AUG G

miRNA-21: UAG CUU AUC AGA CUG AUG UUG A

miRNA-122: UGG AGU GUG ACA AUG GUG UUU G

Apparatus

The PEC tests were measured with a PEC workstation (Ivium, Netherlands) and carried out under optimal experimental conditions of 5 mL 0.1 M PBS solution (PH=7.0) containing different concentration of electron donor ascorbic acid (AA), the light-emitting diode (LED) lights source (460 nm) acted as excitation light source with switching off-on-off for 10–20-10 s under the potential of 0.0 V. Electrochemical impedance spectroscopy (EIS) was measured with a CHI 660e electrochemistry workstation (Shanghai Chenhua Instrument, China). The morphologies of the prepared nanomaterials were characterized by scanning electron microscopy (SEM, S-4800, Hitachi, Japan). A three-electrode system consist of a platinum wire, a Saturated Calomel Electrode (SCE, saturated KCl) and a glassy carbon electrode (GCE, Φ = 4 mm), which is the reference electrodeas, the counter electrode and the working electrode successively.

Synthesis of $ZnIn_2S_{4-x}P_x$

The $ZnIn_2S_4$ was synthesized according to a reported solvothermal method with some modifications. Typically 25 mL $In(NO_{3)\ 3}\cdot xH_2O$ aqueous solution (0.5 mmol/L) was mixed with 0.25 mmoL $Zn(NO_3)_2$, after stirring until a uniform suspension obtained, 5 mL and L-cysteine solution (0.05 mol/L) were then added into the as-prepared suspension under continuous stirring. Subsequently, the mixture was transferred into the Teflon-lined stainless-steel autoclave at 200 °C for 18 h. After the temperature was cooled down to room temperature, the precipitate was centrifuged and washed with ethanol three times. Finally, the product was dried at 70 °C for 12 h.

Finally, 5 mg of $ZnIn_2S_4$ and 100 mg of NaH_2PO_2 were placed at two separate positions in a ceramic crucible with the NaH_2PO_2 at the upstream side. The samples were heated at 400 °C for 1 h with Ar gas flowing. The final product was obtained.

Preparation of S1 modified SiO_2 (S1-SiO_2)

Firstly, 1.77 mL of TX-100, 7.5 mL of cyclohexane and 1.8 mL of 1-hexanol were mixed and stirred for 30 min under magnetic stirring. Then 30 μL TEOS was quickly added to the solution and stirred for 20 min. After that, $NH_3\cdot H_2O$ (25 %-28 %) was added dropwise with a total volume of 0.8 mL. The reaction system was then sealed and stirred for 16 h. Finally, the synthesized SiO_2 was washed repeatedly with ethanol and water. The preparation of S1-SiO_2 complex are as follows: 40 μL (3-aminopropyl) triethoxysilane solution (97 %) was mixed with 5 mL SiO_2 NPs solution and stirred overnight to obtain amination SiO_2 NPs (NH_2-SiO_2 NPs), and washed with ethanol. Then, 400 μL S1 DNA (50 μmol/L) and 10 mL NH_2-SiO_2 NPs solution were mixed with 3 μL glutaraldehyde (0.05 %) and stirred at room temperature for 1 h to obtain S1-SiO_2 complex.

Preparation of the PEC biosensor

Primarily, the bare GCE was polished with 0.3 μm alumina slurry and sonicated using ultrapure water to get mirror-like surface. Then, 20 μL of 0.03 mmol L^{-1} $ZnIn_2S_4P_X$ was coated on the electrode surface and dried at room temperature to form a photoactive film. Subsequently, the electrode was coated with Au NPs by electrotrolytic deposition under the potential of -0.2 V for 30 s. As shown in Scheme 1, the electrodes were incubated with 10 μL of 1 μM HP1 at 4 °C for 12 h and blocked with 10 μL of 0.1 mM HT at room temperature for 40 min. Then the solution of microRNA-155 (20 μL), 0.2 U DSN and 1×DSN master buffer were modified on the electrodes at 60 °C for 40 min. HP1 can be opened by target and a DNA-RNA duplex was generated. The DSN enzyme can cleave the DNA of the DNA-RNA duplex to release the remaining HP1 and reuse of target microRNA-155. Then, DSN stop solution (2 mL) was added to the mixture to denature DSN for 10 min. Subsequently, 20 μL SiO_2-S1 was droped onto the electrodes to hybrid with remaining HP1 at 37 °C for 2 h.

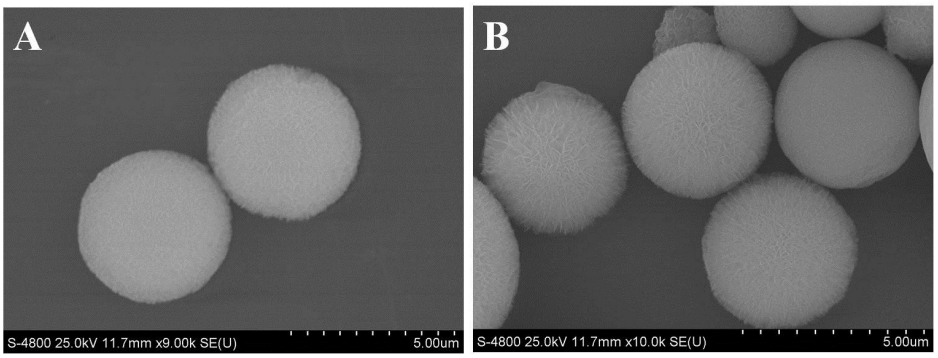

Fig. S1 SEM images of $ZnIn_2S_4$ (A) and $ZnIn_2S_{4-x}P_x$ (B).

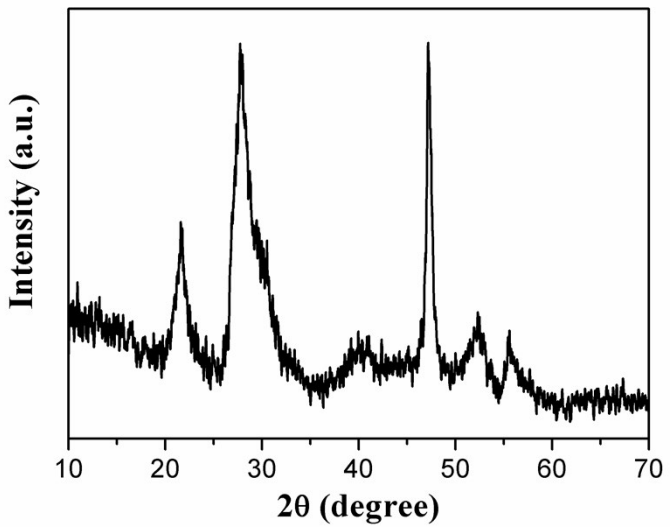

Fig. S2 XRD patterns of $ZnIn_2S_{4-x}P_x$.

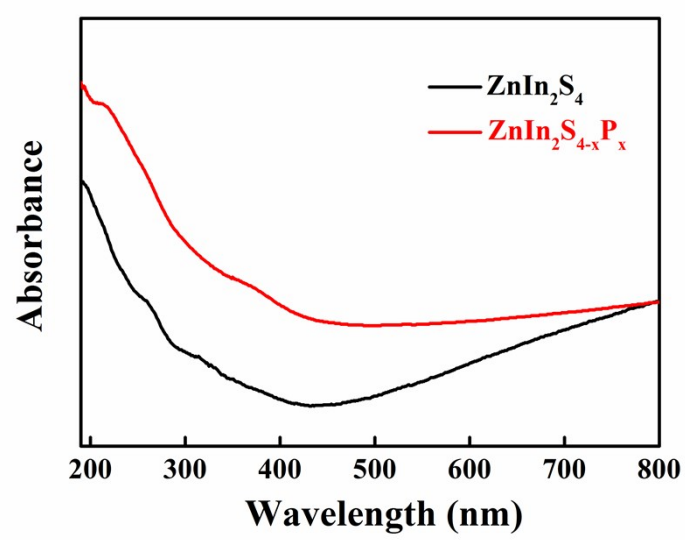

Fig. S3 UV−vis absorption spectrum of $ZnIn_2S_4$ and $ZnIn_2S_{4-x}P_x$.

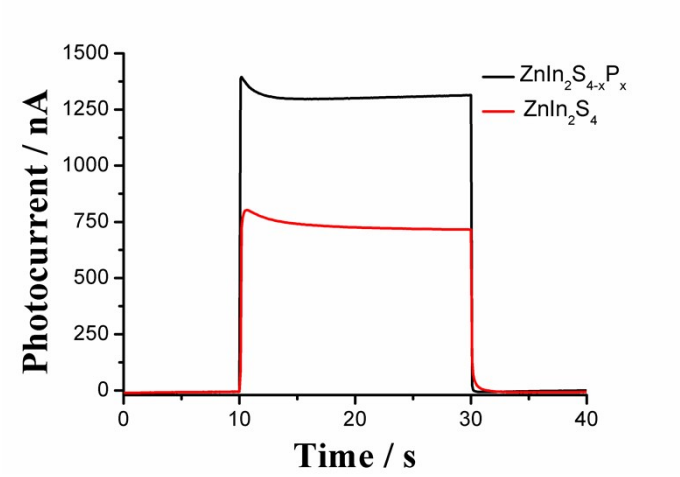

Fig. S4 PEC responses of the $ZnIn_2S_{4-x}P_x$ and $ZnIn_2S_4$ materials.

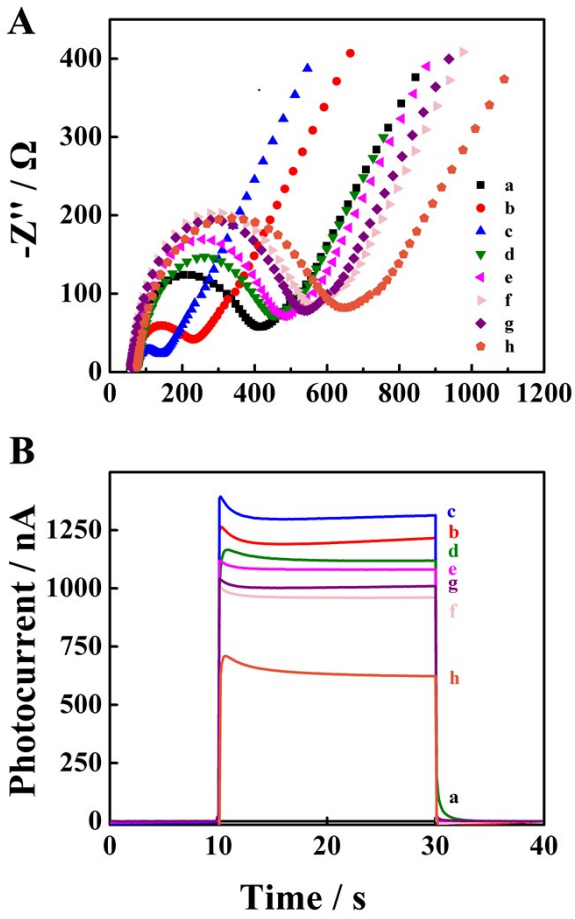

Fig. S5 (A) Typical EIS responses and (B) PEC responses of different modified electrodes: (a) bare GCE, (b) GCE/phosphatizing $ZnIn_2S_4$, (c) GCE/ $ZnIn_2S_{4-x}P_x$ /Au, (d) GCE/ $ZnIn_2S_{4-x}P_x$ /Au/HP1, (e) GCE/ $ZnIn_2S_{4-x}P_x$ /Au/HP1/MCH, (f) GCE/ $ZnIn_2S_{4-x}P_x$ /Au/HP1/MCH/ miRNA-155, (g) GCE/ $ZnIn_2S_{4-x}P_x$ / Au/ HP1/ MCH/miRNA-155/DSN, (h) GCE/ $ZnIn_2S_{4-x}P_x$ /Au/HP1/ MCH/miRNA-155/ DSN/ S1-SiO_2.

1.3 *Optimization of experimental conditions for the fabrication of biosensor*

The concentration of $ZnIn_2S_{4-x}P_x$ was considered as an important factor to affect the intensity of the photocurrent. As shown in Fig. S6A, the photocurrent signal increased at beginning and then decreased with the increasing of $ZnIn_2S_{4-x}P_x$ concentration. The maximum photocurrent reached when the concentration of $ZnIn_2S_{4-x}P_x$ was 0.03 M. Therefore, 0.03 M of $ZnIn_2S_{4-x}P_x$ was used in the further experiments. Ascorbic acid (AA) was used as an electron donor to improve the intensity of the photocurrent. Consequently, the effect of AA concentration was investigated. As shown in Fig. S6B, the photocurrent signal increased constantly with the increasing of AA concentration until 0.4M. Therefore, 0.4 M was confirmed as the optimal concentration and used in the following test.

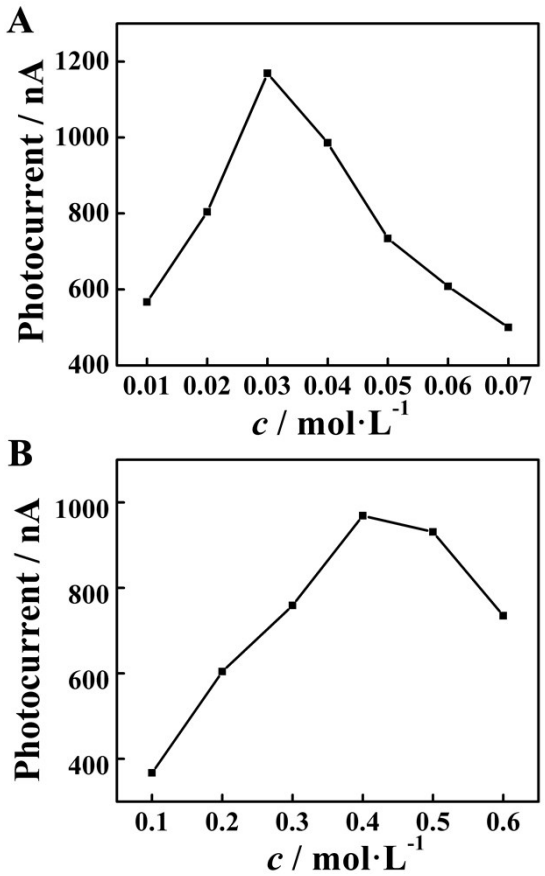

Fig. S6 The optimization of (A) $ZnIn_2S_{4-x}P_x$ and (B) AA concentration.

Table S1 Comparison of the different analytical approach for the detection of miRNA-155.

Analytical method	Linear range /pmol·L^{-1}	Detection limit /pmol·L^{-1}	Ref.
FL	50-1×10^{4}	11	[1]
DPV	2×10^{-3}–8	6×10^{-4}	[2]
EC	1×10^{-3}–100	1.4×10^{-4}	[3]
ECL	1-10^{7}	0.67	[4]
PEC	0.1–1×10^{4}	0.033	This work

Abbreviations: fluorescence (FL); differential pulse voltammetry (DPV); electrochemical (EC)

[1] Y. Borghei, M. Hosseini, M. R. Ganiali, *Microchimica Acta*, **2017**, 184, 2671-2677.

[2] Azimzadeh M., Rahaie M., Nasirizadeh N., Ashtari K., Naderi-Manesh H. *Biosensors and Bioelectronics*, **2016**, 77, 99-106.

[3] Hu T., Zhang L., Wen W., Zhang X., Wang S. Enzyme catalytic amplification of miRNA-155 detection with graphene quantum dot-based electrochemical biosensor. *Biosensors & Bioelectronics*, **2016**, 77, 451-456.

[4] F. Wang, C. Fu, C. Huang, N. Li, Y. Wang, S. Ge, J. Yu. *Biosensors and Bioelectronics*, **2020**, 150, 111917