Supplementary Information

Ultra-sensitive photoelectrochemical aptasensor based on a $CdIn_2S_4/ZnSnO_3$ composite for the detection of adenosine triphosphate

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1. Experimental Section

1.1 Materials and Reagents

Indium chloride ($InCl_3$), thioacetamide (C_2H_5NS), stannic chloride pentahydrate ($SnCl_4·5H_2O$), ferric chloride hexahydrate ($FeCl_3·6H_2O$), manganous chloride tetrahydrate ($MnCl_2·4H_2O$), sodium acetate (CH_3COONa), 3-aminopropyltriethoxysilane (APTES), guanosine triphosphate (GTP), potassium chloride (KCl), sodium chloride (NaCl), potassium ferricyanide ($K_3Fe(CN)_6$), and L-ascorbic acid (AA) were purchased from Shanghai McLean Biochemical Technology Co., Ltd. Cadmium chloride ($CdCl_2$), 3,3'-diaminobenzidine (DAB), adenosine triphosphate (ATP), uracil triphosphate (UTP), and cytidine triphosphate (CTP) were purchased from Shanghai Titan Technology Co., Ltd. Sodium hydroxide (NaOH) was

purchased from Shanghai Runjie Chemical Reagent Co., Ltd. Zinc acetate dihydrate ($Zn(CH_3COO)_2·2H_2O$) was purchased from Anhui Zesheng Technology Co., Ltd. Potassium ferrocyanide trihydrate ($K_4Fe(CN)_6·3H_2O$) was purchased from Tianjin Guangfu Fine Chemical Research Institute. Hydrogen peroxide (H_2O_2) and sodium dihydrogen phosphate dihydrate ($NaH_2PO_4·2H_2O$) were purchased from Sinopharm Chemical Reagent Co., Ltd. Bovine serum albumin (BSA), N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), and disodium hydrogen phosphate (Na_2HPO_4), and all DNA sequences were purchased from Shanghai Sangon Biotech Co., Ltd. Indium tin oxide (ITO) electrodes were purchased from South China Xiangcheng Technology Co., Ltd.The sequence of bases used are shown below:

cDNA: 5'-SH-C_6-ACT ATG ACC TTC CTC CGC AAT-3'

aptamer: 5'-COOH-ACC TGG GGG AGT ATT GCG GAG GAA GGT CAT AGT-3'

1.2 Apparatus

PEC performance testing and electrochemical impedance spectroscopy (EIS) testing were conducted using a CHI 660D electrochemical workstation (CHI Instruments, China). The electrolyte for EIS consisted of 0.01 M phosphate-buffered saline (PBS, pH 7.4) containing 0.1 M KCl and 2 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1). The frequency range spanned from 100 kHz to 0.01 Hz, with an AC potential amplitude of 5 mV peak-to-peak. A conventional three-electrode system was employed, comprising a modified ITO working electrode, an Ag/AgCl (saturated KCl) reference electrode, and a platinum wire counter electrode. A full-spectrum xenon lamp (Nanjing Yan'an Special Lighting Factory, China) served as the light source. Scanning electron microscopy (SEM) was performed using an S-4800 microscope (Hitachi, Japan). Transmission electron microscopy (TEM) was conducted using a JEM-2100 microscope (JEOL, Japan). X-ray photoelectron spectroscopy (XPS) measurements were carried out using an ES-CAL AB 250 spectrometer (Tecfuse, Inc., USA). Ultraviolet-visible (UV-Vis) spectroscopy measurements were conducted using a UV-3600 spectrometer (Shimadzu Corporation, Japan).

1.3 Detection system of PEC aptasensor

Photocurrent signals were measured in a 0.1 M PBS (pH 7.4) solution containing 0.1 M ascorbic acid (AA), with the applied voltage set to 0 V. After each modification step, the electrode surface was washed with Tris/EDTA buffer (containing 10 mM Tris-HCl and 1 mM EDTA, pH 7.8-8.2) to remove any unbound material. The electrode surface was then dried with nitrogen gas. The working area of the ITO electrode was 0.45 cm^2. The light source was activated every 20 s. When the electrode surface was exposed to light energy, a photocurrent was generated.

1.4 Preparation of $ZnSnO_3$

First, add 2.1 g of $SnCl_4 \cdot 5H_2O$ and 0.329 g of $Zn(CH_3COO)_2 \cdot 2H_2O$ to a 0.2 M NaOH solution. Stir the mixture for 1 h, then sonicate it for an additional 1 h. Next heat the mixture at 160 °C for 11 h. The resulting product should be washed alternately with water and ethanol, centrifuged, and then dried at 70 °C for 10 h. After grinding, white $ZnSnO_3$ nanoparticles were obtained.[59]

1.5 Preparation of $CdIn_2S_4$ and CIS/ZSO

Disperse a specified mass of $ZnSnO_3$ nanoparticles ultrasonically in 30 mL of water. Add 0.0916 g of $CdCl_2$ and 0.2212 g of $InCl_3$ sequentially to the resulting suspension. Ultrasonicate for 10 min to obtain solution A. Next, dissolve 0.15 g of C_2H_5NS in 20 mL of water to obtain solution B. Slowly add solution B to solution A while sonicating for 1 h. Finally, heat the mixed solution at 180 °C for 12 h. The centrifuged product was washed alternately with water and ethanol, then dried at 80 °C in a vacuum oven for 12 h to obtain the CIS/ZSO composite material. The mass ratios of the prepared composite materials were 0.1, 0.2, 0.3, 0.4 and 0.5. Using the same method, pure $CdIn_2S_4$ was prepared without the addition of $ZnSnO_3$.[60]

1.6 Preparation of $MnFe_2O_4$-NH_2

$MnFe_2O_4$ was prepared according to previously reported methods.[61] First, 3.6 g of

CH_3COONa was ultrasonically dissolved in 40 mL of ethylene glycol. Subsequently, 0.4925 g of $MnCl_2 \cdot 4H_2O$ and 1.35 g of $FeCl_3 \cdot 6H_2O$ were added to the solution. The resulting mixture was heated at 200 °C for 8 h. After cooling, the product was washed with water and ethanol, centrifuged, dried at 60 °C, and stored aside for later use.

The method for preparing $MnFe_2O_4$-NH_2 involves dispering 30 mg of $MnFe_2O_4$ ultrasonically in a mixed solution of 28.5 mL ethanol and 1.5 mL water. Subsequently, 1.8 mL of APTES is added dropwise to the solution, followed by ultrasonication for 1 h and heating at 75 °C for an additional hour. Finally, the product is washed and dried.

2. Result and discussion

2.1 XRD patterns of $MnFe_2O_4$-NH_2

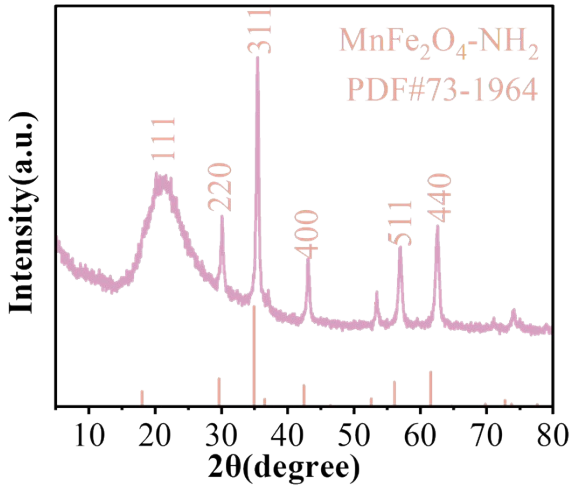

Fig. S1 XRD patterns of $MnFe_2O_4$-NH_2.

2.2 The peroxidase-like property of $MnFe_2O_4$-NH_2

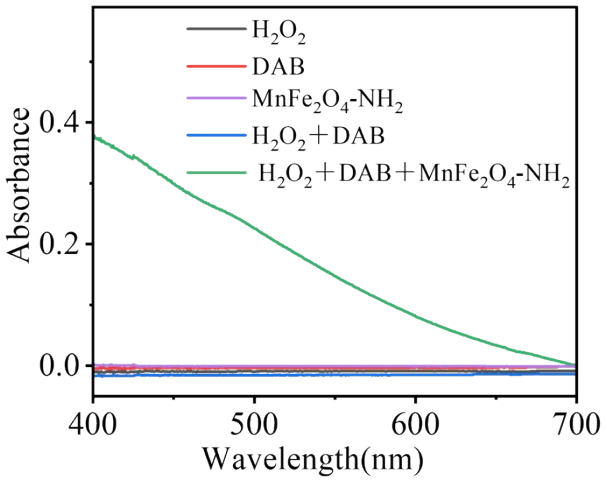

Fig. S2 Ultraviolet absorption spectra of H_2O_2, DAB, $MnFe_2O_4$-NH_2, H_2O_2 + DAB and H_2O_2 + DAB + $MnFe_2O_4$-NH_2.

2.3 Optimization of experimental conditions

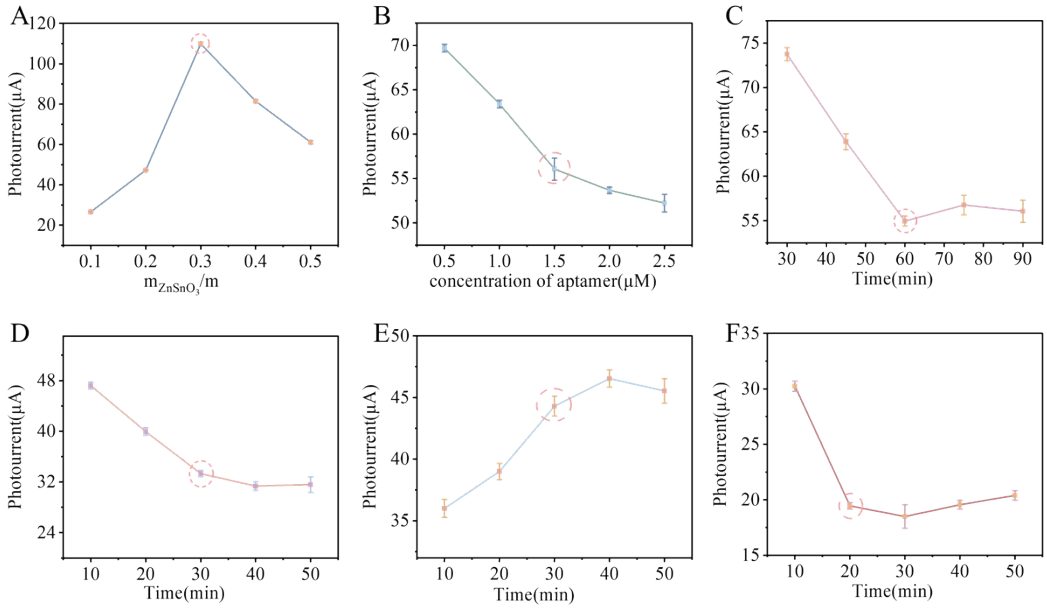

Fig. S3 Effect of experimental conditions on the photocurrent response of the PEC aptasensor platform: (A) Mass ratio of $ZnSnO_3$ to CIS/ZSO, (B) Concentration of the aptamer, (C) Incubation time for aptamers, (D) Incubation time for $MnFe_2O_4$-NH_2, (E) ATP binding time to aptamers, (F) Time required for catalytic precipitation.

2.4 Table S1

Incubation Time (min)	Signal (μA)	Increment per 10 min (%)
10	36.00	/
20	39.00	8.33
30	44.30	13.59
40	46.54	5.06

Table S1 Photocurrent signal and signal gain efficiency at different incubation times for ATP-aptamer binding.

The optimization of incubation time aims to achieve a balance among signal intensity, detection speed and practical application. We selected 30 min as the optimal condition based on the following analysis. First, according to the quantitative calculation of signal gain efficiency (Table S1), the signal increments per 10 min are 8.33% (10–20 min), 13.59% (20–30 min) and 5.06% (30–40 min), respectively. The binding between ATP and the aptamer enters a slow-growth stage after 30 min, and further prolonging incubation brings only limited signal improvement. Second, choosing 30 min highlights the advantage of rapid detection, which is more suitable for rapid screening and on-site analysis.

2.5 Table S2

Method	Linear range	Limit of detection	Reference
Nanopore sensor	20 pM-5 nM	3.9 pM	62
Electrochemistry	0.1 nM-100 nM	0.11 nM	63
Fluorescence	197 pM-197 nM	0.57 nM	64
PEC	5.0 pM-10 nM	3.2 pM	65
PEC	2.0 pM-197 nM	0.65 pM	This work

Table S2 Comparison of several different methods for detecting ATP

2.6 Table S3

Sample	Added(ng/ml)	Found(ng/ml)	Recovery(%)	RSD(%)
1	0.0000	0.0010	/	3.1492
2	0.1000	0.0984	97.4000	5.8028
3	1.0000	1.0005	99.9500	2.1746
4	10.0000	10.7844	107.8340	2.4429

Table S3 Analysis of ATP in authentic samples