SUPPORTING INFORMATION FOR
Chemical-chemical redox cycling amplification strategy in self-powered photoelectrochemical system: A proof of concept for signal amplified photocathodic immunoassay

Jing-Lu Lv, a Bing Wang, a Xiao-Jing Liao, a Shu-Wei Ren, b Jun-Tao Cao, *a and Yan-Ming Liu* a

a College of Chemistry and Chemical Engineering, Institute for Conservation and Utilization of Agro-bioresources in Dabie Mountains, Xinyang Normal University, Xinyang 464000, China
b Xinyang Central Hospital, Xinyang 464000, China

* To whom correspondence should be addressed.
* E-mail: liuym9518@sina.com; jtc011@163.com
Tel & fax: +86-376-6392889
Contents

Part 1: Experimental ................................................................. S3
Part 2: Scheme 1B ................................................................. S6
Part 3: XPS of the photoanode ................................................. S6
Part 4: SEM, XRD, and UV-vis absorption spectrum of the photocathode .... S7
Part 5: Optimization of experimental conditions .......................... S7
Part 6: SEM and EDX images of Ag/CuInS$_2$ on photocathode ........ S8
Part 7: XPS spectra of the CuInS$_2$ and Ag/CuInS$_2$ ...................... S9
Part 8: EIS and PEC characterization of the chemical-chemical redox cycling amplification .................. S10
Part 9: Selectivity ................................................................. S11
Part 10: Table S1 .................................................................. S11
Part 11: Table S2 ............................................................... S11
Part 12: Table S3 ............................................................... S12
Part 13: Reference ............................................................... S12
Part 1: Experimental

Materials. IL-6 and anti-IL-6 polyclonal antibody were purchased from Shanghai Linc-Bio Science Co., Ltd. (Shanghai, China). Human serum albumin (HSA), bovine serum albumin (BSA), and human IgG were bought from Shanghai Solarbio Bioscience & Technology Co., Ltd (Shanghai, China). ALP and 4-aminophenyl phosphate (APP) monosodium salt hydrate were ordered from Apollo Scientific Ltd. (Manchester, UK). 4-aminophenol (AP), β-nicotinamide adenine dinucleotide reduced dipotassium (NADH), and ascorbic acid (AA) were obtained from Sigma-Aldrich (St. Louis, MO). TaCl₅, Bi(NO₃)₃·5H₂O, CuCl₂, InCl₃, and glutaraldehyde (GLD) were provided by Shanghai Macklin Biochemical Co., Ltd (China). AgNO₃, chitosan (CS) powder (from crab cells, 85% deacetylation), Na₂S·9H₂O, Tween-20, aminopropyltriethoxysilane (APTES), and SiO₂ nanoparticles (99.5%, 30 nm) were from Aladdin Reagent Inc. (Shanghai, China). All chemicals were of analytical grade. The aqueous solutions were prepared with ultrapure water (18.2 MΩ·cm) from Aike water treatment solution provider (China).

Phosphate buffer solution (PBS, 0.01 M, pH 7.4) was used for the preparation of the antibody and antigen solution. The attenuation and usage of ALP were performed in Tris-HCl (0.01 M, pH 8.0) including 5.0 mM MgCl₂ and 0.2 mM ZnCl₂. The Tris-HNO₃ buffer (pH 9.0) for enzymatic reaction contained 50 mM Tris and 10 mM Mg(NO₃)₂, and the pH value was regulated by HNO₃.

Apparatus. The cyclic voltammogram (CV), and electrochemical impedance spectroscopy (EIS) were obtained on a RST5200 electrochemical workstation (Zhengzhou Shiruisi Technology Co., Ltd., China) with a three-electrode system in 5.0 mM K₃[Fe(CN)₆]/K₄[Fe(CN)₆] (1:1) mixture containing 0.1 M KCl. The morphologies of the samples were observed with a field emission scanning electron microscope (SEM, Hitachi, S-4800, Japan). X-ray photoelectron spectra (XPS) images were recorded on a K-Alpha X-ray photoelectron spectrometer (Thermo Fisher Scientific Co., USA). Transmission electron microscopy (TEM) images were
taken on a FEI Tecnai G2 F20 electron microscope operated at 200 kV. The UV-vis absorption spectra were recorded on a PerkinElmer Lambda 950 UV–Vis spectrophotometer (Shimadzu, Japan). Powder X-ray diffraction (XRD) was recorded on a Rigaku Smartlab 9KW X-ray diffractometer (Japan, Cu Kα radiation, λ = 1.5406 Å).

The self-powered detection device was constructed by using a two-compartment cell with a Nafion 117 membrane as separator. The Bi₂S₃/Bi₃TaO₇ heterojunction-based photoelectrode and CuInS₂-based photoelectrode were separately dipped into the two compartments of the detection device served as photoanode and photocathode, respectively. The CHI660E electrochemical workstation (Shanghai Chenhua Apparatus Corporation, China) was used to record the photocurrent responses produced by the two-electrode system and a white light with a spectral range from 400 to 700 nm from a 3 W LED lamp was utilized as stimulus light to irradiate both the photoanode and the photocathode. The distance between the light source and the electrode surface was 10 cm and the stimulus light was switched on and off every 10 s. The external voltage was 0.0 V.

Preparation of the Bi₂S₃/Bi₃TaO₇ heterojunction-based photoanode. Bi₃TaO₇ nanoparticles (NPs) and Bi₂S₃ nanorods (NRs) were first prepared according to the literatures. The Bi₂S₃/Bi₃TaO₇ heterojunction was prepared by directly mixing Bi₃TaO₇ NPs with Bi₂S₃ NRs according to a certain proportion in water and then ultrasonication for 30 min. To fabricate the photoanode, the bare electrodes were ultrasonicated for 10 min in acetone solution, ultrapure water, ethanol solution and ultrapure water, respectively, and then dried at 120 °C for 2 h. 20 μL of the above mentioned Bi₂S₃/Bi₃TaO₇ suspension (Bi₃TaO₇ : Bi₂S₃ = 1:3, 3 mg/mL) was then dropped onto the surface of the cleaned ITO electrode with an exposed geometric area of 0.25 cm², and then dried in an oven at 60 °C. Finally, the Bi₂S₃/Bi₃TaO₇/ITO electrode was acquired and used as photoanode.

Preparation of the CuInS₂ microflowers-based photocathode. CuInS₂ was synthesized by a hydrothermal method. Typically, 40 mL of ethylene glycol solution containing 0.03 M CuCl₂ (copper source), 0.03 M InCl₃ (indium source), and 0.12 M...
thiourea (sulfur source), was transferred to a 50 mL polytetrafluoroethylene lined autoclave. After reaction for 24 h at 200 °C, the black precipitates of CuInS₂ micro-flowers were acquired. The precipitates were washed several times with ultrapure water and ethanol, and then dried at 60 °C overnight. To fabricate the photocathode, 6 mg/mL of the CuInS₂ solution (20 μL) containing 1wt% CS and 1% acetic acid was spread on the surface of the cleaned ITO electrode with an exposed geometric area of 0.25 cm² and the photocathode was fabricated after drying the modified electrode at 60 °C.

**Preparation of the Anti-IL-6-SiO₂-ALP.** The anti-IL-6-SiO₂-ALP probe was synthesized as follows. Firstly, the SiO₂ was aminated with APTES, and then functionalized with aldehyde group with GLD. Secondly, 400 μL anti-IL-6 (15 μg/mL) and 400 μL ALP (30 μg/mL) were pre-mixed and added to 400 μL aldehyde group functionalized SiO₂ solution. After 1 h reaction at 37 °C under gently stirring, the mixture was centrifuged and dispersed in 400 μL PBS. Thirdly, 0.01 M PBS solution (pH 7.4) containing 1% (w/v) BSA was used to block nonspecific binding sites for 1 h at 37 °C. Subsequent to centrifugation and washing, the anti-IL-6-SiO₂-ALP probe was redispersed in 400 μL PBS and stored at 4 °C before use.

**PEC measurements.** The PEC analysis was carried out in a split-type mode. The AP obtained from the enzyme catalytic reaction in the 96-well plate was immediately transferred into the Tris-HNO₃ buffer containing 1.0 mM AgNO₃ and 1.0 mM NADH on the CuInS₂/ITO-based photocathode. The redox cycling process on the photocathode was achieved for 10 min incubation. After rinsing the photocathode with Tris-HNO₃ buffer and water thoroughly, the electrode was assembled with the photoanode to construct the self-powered device for PEC measurements. The PBS (pH 7.4, 0.01 M) and that containing 0.1 M AA was used as the detection solution in the photocathodic and photoanodic cells, respectively. **In the PEC measurements, the stimulus light was switched on and off every 10 s.**
Part 2: Scheme 1B

Scheme 1B. Photogenerated electron-hole transfer process of the PEC system.

Part 3: XPS of the photoanode

The surface composition and oxidation state of various elements in the prepared Bi$_2$S$_3$/Bi$_3$TaO$_7$ heterojunction were further studied by XPS measurement. It can be observed from the entire XPS spectrum that the product is mainly composed of Bi, Ta, O, S, C elements (Figure S1A). In Figure S1B, the predominant peaks at 158.18 eV and 163.50 eV are identified at Bi 4f$_{7/2}$ and Bi 4f$_{5/2}$, respectively, confirming the existence of Bi$^{3+}$, meanwhile the binding energy at 160.81 eV is responsible for the S 2p$_{3/2}$ of S$^{2-}$. In Figure S1C, the peaks at 28.08 eV and 25.18 eV are ascribed to Ta 4f$_{3/2}$ and Ta 4f$_{7/2}$ of Ta$^{5+}$, respectively. Consequently, the successful synthesis of Bi$_2$S$_3$/Bi$_3$TaO$_7$ heterojunction is further verified.
**Part 4: SEM, XRD, and UV-vis absorption spectrum of the photocathode**

**Figure S2.** (A) SEM images of CuInS$_2$ at low and high (inset in A) magnification; (B) XRD pattern; and (C) UV-vis absorption spectra of CuInS$_2$.

**Part 5: Optimization of experimental conditions**

As the photoactive material for the photoanode construction, the Bi$_2$S$_3$/Bi$_2$TaO$_7$ heterojunction in terms of the mass ratio and the concentration was firstly optimized. Figure S3A displays the photocurrent responses of 2.0 mg/mL Bi$_2$S$_3$/Bi$_2$TaO$_7$ heterojunction at different mass ratio of Bi$_2$TaO$_7$ to Bi$_2$S$_3$. It can be seen that the photocurrent response of Bi$_2$S$_3$/Bi$_2$TaO$_7$ reached the highest value at the mass ratio of 1:3 ($m_{\text{Bi}_2\text{TaO}_7}$:$m_{\text{Bi}_2\text{S}_3}$). Then, the PEC behaviors of Bi$_2$S$_3$/Bi$_2$TaO$_7$ at different concentrations ($m_{\text{Bi}_2\text{TaO}_7}$:$m_{\text{Bi}_2\text{S}_3}$ = 1:3) were investigated. As shown in Figure S3B, the maximum PEC response was observed at the concentration of 3 mg/mL. Therefore, 3
mg/mL of Bi$_2$S$_3$/Bi$_2$TaO$_7$ heterojunction at the mass ratio of 1:3 (m$_{Bi_3TaO_7}$:m$_{Bi_2S_3}$) was selected for the subsequent photoanode preparation.

The concentration of CuInS$_2$ for photocathode construction was also studied. It can be seen from Figure S3C that the concentration of CuInS$_2$ at 6.0 mg/mL could achieve the highest photocurrent responses compared to the other concentrations of CuInS$_2$. So, 6.0 mg/mL CuInS$_2$ was used for the subsequent photocathode construction.

Fig. S3. PEC responses of Bi$_2$S$_3$/Bi$_2$TaO$_7$/ITO in PBS solution including 0.1 M AA with (A) various mass ratios of Bi$_3$TaO$_7$ to Bi$_2$S$_3$ at 2.0 mg/mL: 1:1 (curve a); 1:2 (curve b); 1:3 (curve c); 1:4 (curve d); 1:5 (curve e), and (B) varied concentrations: 1.0 mg/mL (curve a); 2.0 mg/mL (curve b); 3.0 mg/mL (curve c); 4.0 mg/mL (curve d); 5.0 mg/mL (curve e). (C) Photocurrent responses of the CuInS$_2$/ITO cathode prepared with different concentrations of CuInS$_2$ suspension, varied concentrations: 4.0 mg/mL (curve a); 5.0 mg/mL (curve b); 6.0 mg/mL (curve c); 7.0 mg/mL (curve d); 8.0 mg/mL (curve e). In PEC measurements, the stimulus light was switched on and off every 10 s.

**Part 6: SEM and EDX images of Ag/CuInS$_2$ on photocathode**

The presence of Ag on the CuInS$_2$ was characterized by SEM and EDX measurement, respectively. The Figure S4B exhibits the Ag particles were decorated on the CuInS$_2$ surface, indicating that Ag nanoparticles were successfully deposition on the CuInS$_2$. The EDX image of the CuInS$_2$ (Figure S4C), indicating that the sample was mainly composed of Cu, In, and S elements. Figure S4D display that the chemical composition of the Ag/CuInS$_2$, the result demonstrates that Ag element existed on the CuInS$_2$. 
**Fig. S4.** SEM images and the corresponding energy-dispersive X-ray (EDX) spectra of CuInS$_2$/ITO photocathode before (A, C) and after Ag (B, D) deposition, respectively.

**Part 7: XPS spectra of the CuInS$_2$ and Ag/CuInS$_2$**

The chemical state of Ag on the CuInS$_2$ was also studied by XPS analysis. The survey spectra in Figure S5A indicate the presence of Cu, In, and S elements in the CuInS$_2$ and Ag/CuInS$_2$. Moreover, the diffraction peak of Ag could be clearly observed in the Ag/CuInS$_2$, which consistent with EDX images. The XPS spectra of Ag 3d state contained two peaks at 368.16 eV and 374.16 eV should be assigned to 3d$_{5/2}$ and 3d$_{3/2}$, respectively (Figure S5B), demonstrating the existence of Ag (0). These results could prove the Ag was successfully deposited on CuInS$_2$ surface. As shown in Figure S5C and Figure S5C', the peaks at 444.47 eV and 452.04 eV correspond to the In 3d$_{5/2}$ and In 3d$_{3/2}$, respectively. The XPS survey of CuInS$_2$ is seen to be very similar to those for Ag/CuInS$_2$, indicating that the decoration of Ag nanoparticles onto CuInS$_2$ cannot lead to an evident change in the elemental oxidation state. It can be deduced that there is no possible reaction between the Ag$^+/Ag$ and CuInS$_2$. 

---

*S9*
Fig. S5. XPS spectra of the CuInS$_2$ and Ag/CuInS$_2$: (A) the full survey spectrum; (B) Ag 3d; (C and C') In 3d; and (D and D') S 2p.

Part 8: EIS and PEC characterization of the chemical-chemical redox cycling amplification

Fig. S6. (A) EIS and (B) photocurrent responses of the CuInS$_2$/ITO electrode before and after redox cycling process.
Part 9: Selectivity

![Selectivity graph]

**Fig. S7.** Selectivity of the PEC system. The concentrations of IL-4, IL-8, IL-18, HSA, hIgG, IL-6 are $1.0 \times 10^{-10}$, $1.0 \times 10^{-10}$, $1.0 \times 10^{-10}$, $4.0 \times 10^{-2}$, $1.0 \times 10^{-2}$, and $1.0 \times 10^{-10}$ g/mL, respectively.

**Part 10: Table S1** The comparison of the proposed method with the previously reported methods for IL-6 detection.

<table>
<thead>
<tr>
<th>Analytical methods</th>
<th>Linear ranges (pg mL$^{-1}$)</th>
<th>Detection limits (pg mL$^{-1}$)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anodic PEC method</td>
<td>500 - 10000</td>
<td>500</td>
<td>5</td>
</tr>
<tr>
<td>Anodic PEC method</td>
<td>10 - 60000</td>
<td>3.4</td>
<td>6</td>
</tr>
<tr>
<td>Cathodic PEC method</td>
<td>0.1 - 500000</td>
<td>0.037</td>
<td>7</td>
</tr>
<tr>
<td>Integrating photoanode</td>
<td>5 - 500000</td>
<td>1.8</td>
<td>8</td>
</tr>
<tr>
<td>with photocathode</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Integrating photoanode</td>
<td>0.05 - 10000</td>
<td>0.02</td>
<td>This work</td>
</tr>
<tr>
<td>with photocathode</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Part 11: Table S2 Analytical results of the proposed method for IL-6 in human serum samples.

<table>
<thead>
<tr>
<th>Serum Samples No.</th>
<th>This work (pg mL(^{-1}))</th>
<th>RSDs (%, n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.170</td>
<td>5.5</td>
</tr>
<tr>
<td>2</td>
<td>0.186</td>
<td>4.8</td>
</tr>
<tr>
<td>3</td>
<td>0.603</td>
<td>6.1</td>
</tr>
<tr>
<td>4</td>
<td>1.230</td>
<td>7.6</td>
</tr>
<tr>
<td>5</td>
<td>1.905</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Part 12: Table S3 Recovery of IL-6 in human serum samples.

<table>
<thead>
<tr>
<th>Serum samples No.</th>
<th>Found (pg mL(^{-1}))</th>
<th>Added (pg mL(^{-1}))</th>
<th>Total found (pg mL(^{-1}))</th>
<th>Recovery (%)</th>
<th>RSDs (%, n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.01</td>
<td>0.180</td>
<td>110.0</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.263</td>
<td>93.0</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>1.174</td>
<td>100.4</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>2.001</td>
<td>96.0</td>
<td>7.9</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.905</td>
<td>1.00</td>
<td>2.765</td>
<td>86.0</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>12.218</td>
<td>103.13</td>
<td>4.7</td>
<td></td>
</tr>
</tbody>
</table>

Part 13: Reference


