

Electronic Supporting Information

AgCu@CuO Aerogels with Peroxidase-Like Activity and Photoelectric Response for Sensitive Biosensing

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Materials and reagents

Sodium phosphate buffer solution (PBS, 0.02 M, pH 7.4) was supplied by Wuhan Boster Biological Technology., Ltd (Wuhan, China). Xanthine oxidase (XOD) was bought from Jiangsu luye biotechnology Co. Ltd. Hypoxanthine (HX) was ordered from Beijing Xinjingke Biotechnology Co., Ltd (Beijing, China). 4-chloro-1-naphthol (4-CN) was purchased from Shanghai meryer Chemical Technology Co., Ltd (Shanghai, China). 3,3',5,5'-tetramethylbenzidine (TMB) were obtained from Shanghai Aladdin Bio-Chem Technology Co., Ltd. Silver nitrate (AgNO_3), Copper nitrate trihydrate ($\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$), Sodium borohydride (NaBH_4 , 98%, powder), Sodium dihydrogen phosphate dihydrate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$), Disodium hydrogen phosphate dodecahydrate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$), hydrogen peroxide (H_2O_2), absolute ethanol were purchased from Singpharm Chemical Reagent Co., Ltd (Shanghai, China). ITO slices were ordered from Foshan Yuanjingmei Glass Co., Ltd.

Apparatus.

Scanning electron microscopy (SEM) was performed with a Quanta JEM-6700F (FEI, United States). X-ray diffraction patterns (XRD) analysis was carried out on a D8 ADVANCE (Bruker, Germany). Transmission electron microscopy (TEM) images were recorded on a Titan G260-300 (Thermo Fisher, USA). X-ray photoelectron spectroscopy (XPS) was measured on a Thermo ESCALAB 250XI (UIVAC-PHI Co.). UV-vis spectra were obtained by a multimode reader (Tecan Spark, Switzerland). Electrochemical impedance spectroscopy (EIS) was performed on a CHI 660E electrochemical workstation (Shanghai Chenhua Apparatus Corporation, China) with a three-electrode system in 1 M KCl solution containing 5 mM $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$ (1:1) mixture as an electrolyte at a frequency range of 1 Hz-10⁵ kHz. The content of each element in the sample was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) (Agilent 8800).

Preparation of AgCu hydrogels

Typically, 1 mmol AgNO_3 and 1 mmol $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ were dissolved in 80 mL deionized water individually. 30 mL of AgNO_3 and $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ mixture solution with a certain feed ratio of metal ions was heated to 60 °C followed by the injection of 4 mL of fresh prepared NaBH_4 solution (0.5 M) as reducing agent under vigorous stir for about 1 min. Then, the AgCu hydrogels were formed on the

bottom of the bottle after being kept still for 6 h at 60 °C. The as-prepared hydrogels were washed thoroughly with water and dispersed in 4 mL ultrapure water by sonication and preserved hermetically in the refrigerator (4 °C) for further use.

Fabrication of AgCu@CuO aerogel-based photocathode

The AgCu aerogel-based photocathode was fabricated by a drop-casting method. Before the modification, the indium tin oxide (ITO) slices ($1.0 \times 2.0 \text{ cm}^2$, $10 \text{ } \Omega/\text{square}$) were ultrasonically washed with distilled water and ethanol for 15 min, respectively, and dried at 60 °C. A droplet of 10 μL of AgCu hydrogels dispersion was cast on the ITO substrate with a circular-shaped opening of 0.3 cm in diameter. Then, the ITO slices were transferred to an alumina crucible pot with an annealing treatment at 300 °C for 1 h in the air (ramp rate = $2.5 \text{ } ^\circ\text{C}\cdot\text{min}^{-1}$). The obtained AgCu@CuO aerogel-based photocathodes were stored for further use.

Photoelectrochemical measurement

The PEC measurements were performed on the standard three-electrode electrochemical measurement system (CHI 842D, China) including a reference electrode (saturated Ag/AgCl), a counter electrode (Pt wire), and a working electrode (ITO slices). A Xenon lamp source (500 W, CHF-XM-500W, Beijing Perfectlight Technology Co., Ltd.) was utilized as the light source with a light switch. The intensity of the light was maintained at $100 \text{ mW}\cdot\text{cm}^{-2}$ by adjusting the distance between the light source and the photoelectrochemical cell. A 420 nm cutoff filter above was placed in front of the xenon lamp to filter out the UV light. The PEC activities of the AgCu@CuO aerogel-based photoelectrodes were examined by transient photocurrent response in phosphate buffer saline (pH 7.4) containing $7 \text{ } \mu\text{L}$ H_2O_2 (30 %) at a constant potential of 0 V under chopped light irradiation.

Electrochemical impedance spectroscopy Nyquist plots

The electrochemical impedance spectroscopy (EIS) was recorded on the standard three-electrode electrochemical measurement system (CHI 660e, China) including a reference electrode (Hg/HgCl₂ electrode filled with saturated potassium chloride aqueous solution as the reference electrode), a counter electrode (Pt wire), and a working electrode (ITO slices) at open circuit potential in 0.1 M KCl solution containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ with the frequency during the measurement range from 1

Hz to 10^5 Hz.

PEC measurements of XOD activity

PEC measurements were performed with a typical three-electrode glass cell with a quartz window. I-t curves were acquired in 15 mL of phosphate buffer saline (PBS, 20 mM, pH 7.4) at a constant potential of 0 V (vs. Ag/AgCl). Firstly, 20 μ L of 0.5 mM hypoxanthine (Hx) and 20 μ L of XOD of various concentrations in PBS (1 mM, pH 7.4) were mixed in 96-microwell plates and incubated for 20 min at 37 °C. After that, 20 μ L of 1 mM 4-CN in ethylalcohol and 40 μ L of PBS (10 mM, pH 5) were added to the mixture of XOD and Hx. 10 μ L of the above mixture was dropped on the surface of the as-prepared AgCu@CuO aerogel-decorated ITO. After being incubated for 20 min at 37 °C, the AgCu@CuO aerogel-based photocathodes were washed with water for the following i-t measurements.

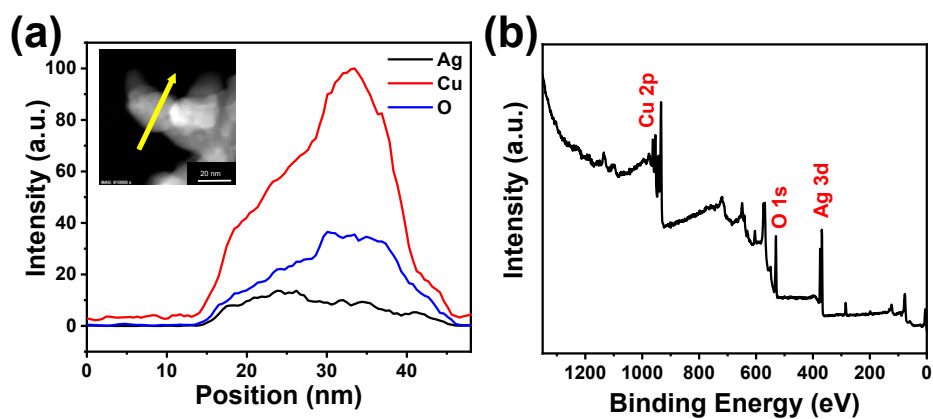


Figure S1. (a) EDX line scan profiles and (b) XPS spectrum of the prepared AgCu@CuO aerogels.

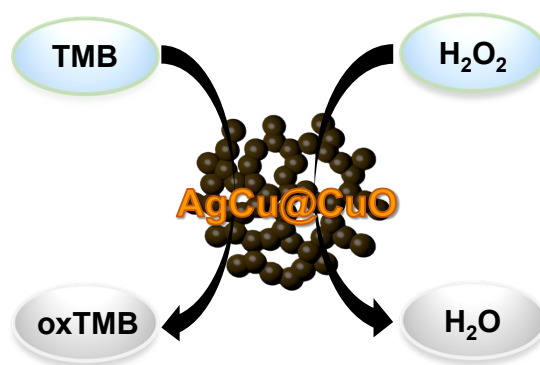


Figure S2. Schematic illustration of the peroxidase-like activity of AgCu@CuO aerogels.

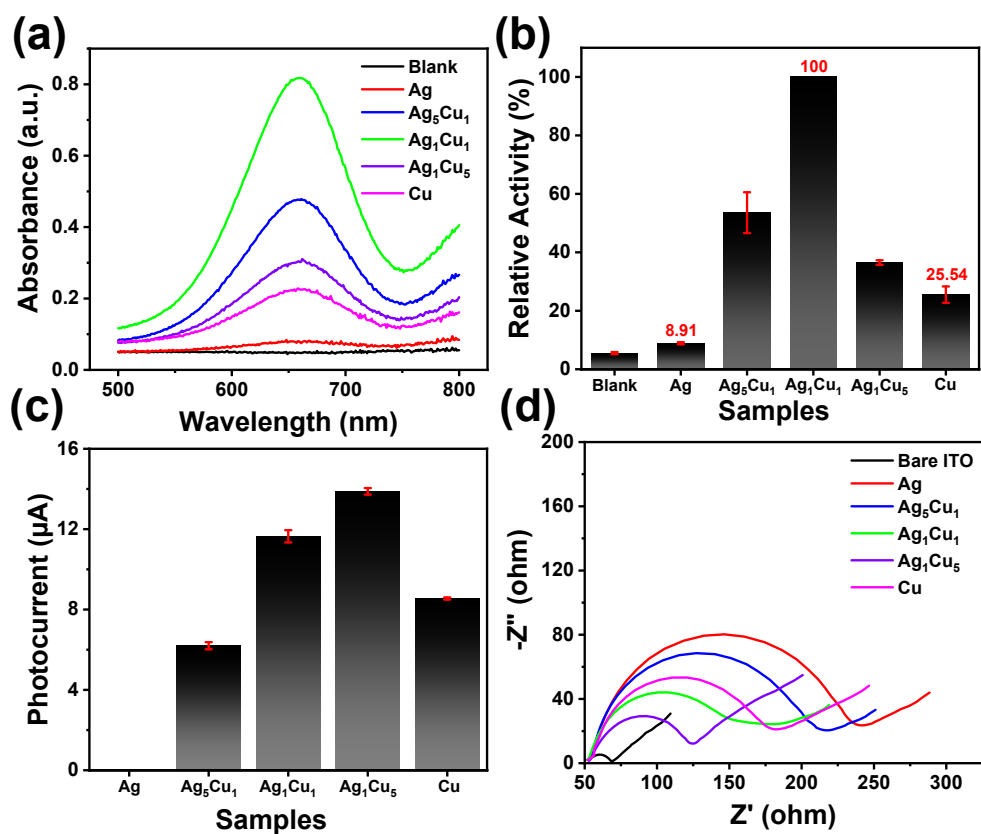


Figure S3. (a) UV-vis absorption spectra, (b) Relative peroxidase-like activity (c) Photocurrents, and (d) EIS Nyquist plots of AgCu@CuO aerogels decorated electrodes with different ratios of Ag to Cu.

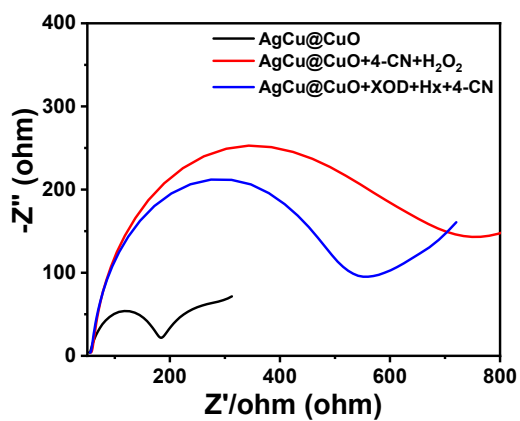


Figure S4. EIS Nyquist plots of pristine AgCu@CuO aerogels, AgCu@CuO + 4-CN + H₂O₂ and AgCu@CuO + XOD + Hx + 4-CN.

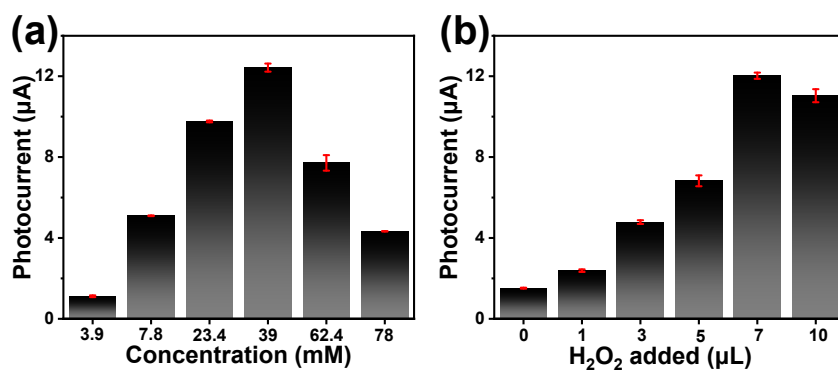


Figure S5. (a) Photocurrents of AgCu@CuO aerogels decorated electrodes of different metal concentrations, (b) Photocurrents of AgCu@CuO decorated electrodes with different amounts of H₂O₂ added in the electrolyte.

The concentration of the AgCu@CuO aerogels dropped on the electrodes was 39 mM, and the volume of the H₂O₂ added in the electrolyte was 7 μL, the electrode presented the highest photocurrent.

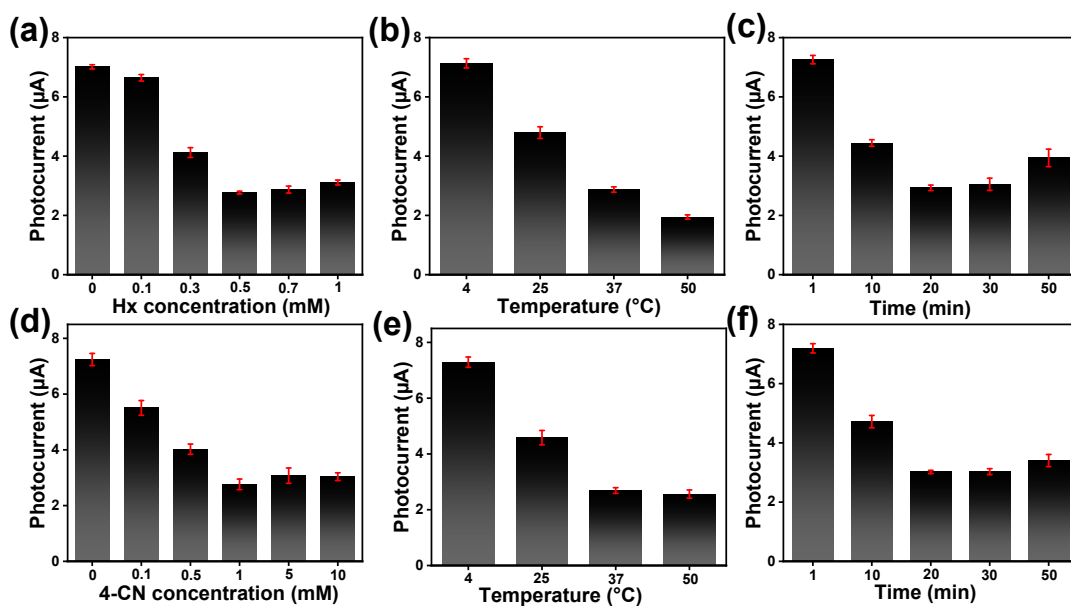


Figure S6. Optimization of (a) Hx concentration, (b) incubating temperature and (c) incubating time of the mixture of XOD and Hx, (d) 4-CN concentration, (e) incubating temperature, and (f) incubating time of the electrodes.

The concentration of Hx, the incubating temperature, and incubating time of XOD and Hx were ultimately selected to be 0.5 mM, 37 °C, and 20 min, respectively. Similarly, the concentration of 4-CN, the reaction temperature and reaction time of the mixture of 4-CN and H₂O₂ produced by XOD and Hx with AgCu@CuO aerogels were optimally selected to be 1 mM, 37 °C and 20 min, respectively.

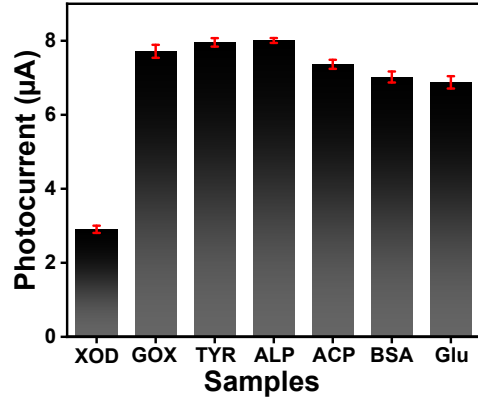


Figure S7. Selectivity of the AgCu@CuO aerogel-based photoelectrodes for XOD detection.

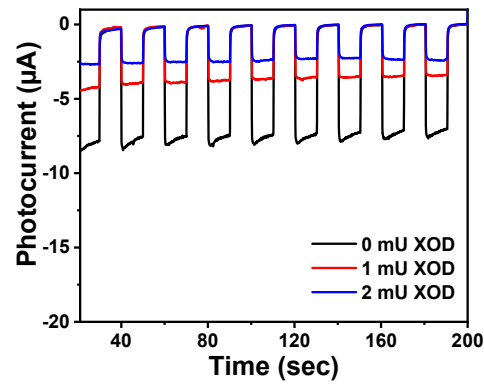


Figure S8. Stability of the AgCu@CuO aerogel-based photoelectrodes for XOD detection.

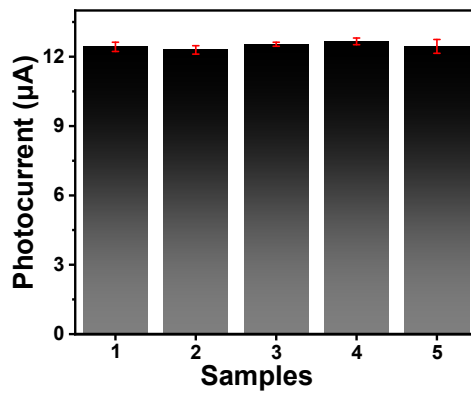


Figure S9. Repeatability of the photocurrent generated by the AgCu@CuO decorated ITO electrodes.

Table S1. Comparison of different methods for XOD activity determination

Methods	Materials	Linear range	LOD	Ref
Fluorescence	Naphthaliide	0 - 15 U•mL ⁻¹	0.7 U•mL ⁻¹	S1
Photoelectrochemical	TiO ₂	0.01 - 15 mU•mL ⁻¹	-----	S2
Fluorescence	Zr based metal-organic framework	0.2 - 40 U•L ⁻¹	0.004 U•L ⁻¹	S3
Phosphorescent	Mn-doped ZnS quantum dots	0 - 5 U•L ⁻¹	0.02 U•L ⁻¹	S4
Photoelectrochemical	AgCu@CuO aerogels	100 nU•mL ⁻¹ - 1 mU•mL ⁻¹	65.23 nU•mL ⁻¹	This work

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