Electronic Supplementary Information

Cu(I) complexes with aggregation-induced emission for enhanced photodynamic antibacterial application

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Experimental section

1. Materials

Ultrapure water (18.25 MΩ·cm) was used throughout the whole experiment. All glassware was washed with aqua regia, rinsed with copious ethanol and water, and dried in an oven before use. *para*-mercaptobenzoic acid (*p*-MBA) was purchased from TCI (Shanghai) Chemical Industry Development Co., Ltd. Copper (II) sulfate pentahydrate ($CuSO_4·5H_2O$), sodium hydrate (NaOH), methanol, N, N-dimethylformamide (DMF), glutathione (GSH), nitrogen (N_2) and oxygen (O_2) gas were purchased from Sinopharm Chemical Reagent Co., Ltd. Ethanol was purchased from Tianjin Fuyu Fine Chemical Co., Ltd (China). Glycerol and polyethylene glycol 10000 (PEG) were purchased from Tianjin BASF Chemical Co., Ltd. Acetone was purchased from Yantai Far East Fine Chemical Co., Ltd. Chitosan was purchased from Adamas Reagent Co., Ltd. Polyvinyl alcohol 1788 (PVA) was purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd. 2,7-

dichlorodihydrofluorescein diacetate (DCFH-DA) dye and dimethyl sulfoxide (DMSO) were ordered from Sigma Aldrich (China). Phosphate buffer solution (PBS), Luria-Bertani (LB) nutrient agar and LB broth were purchased from Qingdao Hope Biotechnology Co., Ltd. Standard strains of *Staphyloccocus aureus* ATCC 6538 (*S. aureus*), and *Escherichia coli* ATCC 25922 (*E. coli*) were purchased from China General Microbiological Culture Collection Center. Standard strains of Methicillin-resistant *Staphyloccocus aureus* ATCC 43300 (MRSA) were purchased from Mingzhou Biotechnology Co., Ltd. L929 mouse fibroblast cells were purchased from American Type Culture Collection. All materials were used as received without further purification.

2. Instruments

The digital photos were taken on the Cannon EOS 800D digital camera. The ultraviolet-visible (UV-vis) absorption spectra and the optical density at 600 nm (OD_{600}) of bacterial cells were acquired on a Shimazu UV-1800 spectrophotometer. The steady-state photoluminescence (PL) spectra were obtained on a PerkinElmer LS-55 fluorescence spectrometer. The molecular formula of Cu(I)-*p*-MBA complexes was analyzed on a Bruker Impact II (electrospray ionization mass spectrometry) ESI-MS system operating in negative ion mode. Dynamic light scattering (DLS) and Zeta (ζ) potential tests were conducted on a Zetasizer Nano ZS (Malvern Co., England) system. The X-ray photoelectron spectrum (XPS) was obtained on an ESCALAB MK II Axis Uitra Dld system using Al Kα as an exciting source. Transmission electron microscope (TEM) images and energy dispersive X-ray (EDX) element mapping results of PCuA were acquired on the JEOL JEM 2100F microscope with an operating voltage of 200 kV. The PL decay profile was gained on a transient photoluminescence spectrometer (FLS-1000, Edinburgh Instruments Ltd.). UV-vis diffuse reflectance spectrum (DRS) was recorded on a U-3900 spectrophotometer with an integration

sphere. The morphologies of bacteria were observed on a scanning electron microscope (SEM, JSM-6700F).

3. Synthesis

3.1 Synthesis of dispersive Cu(I)-*p*-MBA complexes

Firstly, 0.8 mL *p*-MBA solution (50 mM, in ethanol) was mixed with 4 mL ultrapure water. After that, aqueous solution of $CuSO_4 \cdot 5H_2O$ (0.2 mL, 100 mM) was introduced into the system and stirred for 10 min, followed by the addition of NaOH solution to adjust the pH to ~9.0 under stirring condition. Dispersive Cu(I)-*p*-MBA complexes solution was formed after stirring of 5 min.

3.2 Synthesis of AIE-featured Cu(I)-*p*-MBA complexes in mixed solvent

Firstly, the *p*-MBA (6.2 mg, 40 μmol) was added to the mixed solvent (4 mL) of water and ethanol (or other less polar solvents such as methanol, glycerol, DMF, and acetone) with different volume fractions. After that, aqueous solution of $CuSO_4 \cdot 5H_2O$ (0.2 mL, 100 mM) was introduced into the reaction system and stirred for 12 h at pH = ~3.0. The AIE-featured Cu(I)-*p*-MBA complexes with different aggregation states were fabricated. The synthesis of AIE-featured Cu(I)-GSH complexes is the same as that of the Cu(I)-*p*-MBA complexes in mixed solvent except for the replacement of the *p*-MBA (6.2 mg, 40 μmol) with GSH (0.75 mL, 66.67 mM).

3.3 Synthesis of AIE-featured PEG-condensed Cu(I)-*p*-MBA complexes (PCuA)

Firstly, the *p*-MBA solution (0.8 mL, 50 mM, dissolved in ethanol) was added to a flask containing 4 mL ultrapure water and 0.1 g PEG. After that, aqueous solution of $CuSO_4 \cdot 5H_2O$ (0.2 mL, 100 mM) was introduced into the reaction system and stirred for 12 h at pH = ~3.0, followed by stirring at 80 ^{o}C for 1 h. The AIE-featured PCuA with stable aggregated structures was fabricated. The PCuA was isolated through centrifugation and freeze-drying, and the as-obtained PCuA in solid

state was used for further antibacterial assays. The synthesis of AIE-featured PVA-condensed or chitosan-condensed Cu(I)-*p*-MBA complexes are the same as that of the PCuA except for the replacement of PEG with PVA or chitosan and none of heat treatment. Control experiment without the addition of polymers was performed under the same conditions.

4. Antibacterial experiments

Gram-positive *S. aureus,* gram-negative *E. coli,* and drug resistant MRSA were used as bacteria models to evaluate the broad-spectrum bactericidal activity of the PCuA. All the glassware, medium solution, and reagents used were sterilized in an autoclave at 121 °C and 103 kPa of pressure for 30 min before the antibacterial experiments. All tests were performed under sterile conditions. The visible light was provided by a commercial 300 W xenon lamp with an optical cut-off filter ($\lambda > 400$ nm, standard sunlight of 100 mW/cm^2).

Bacterial culture: The bacterial cells were cultured in LB medium in an orbital shaker at 37 °C for 24 h, subsequently centrifuged at 8000 rpm for 8 min to remove the metabolites, and finally diluted with PBS solution (0.01 M, pH 7.4) to prepare the bacterial suspension with the $OD_{600} = 0.1$ (equivalent to the bacteria concentration of ~10^7 CFU·mL^{-1}).

In a typical antibacterial test, the PCuA (10.75 mg, solid state) as antibacterial agent was added to 5 mL bacteria suspension ([Cu] = 0.4 mM in the medium), and then stirred for 60 min under different conditions: 1) in darkness; 2) visible light irradiation; 3) visible light irradiation with O_2 bubbling. In addition, control antibacterial tests with dark or light irradiation ($\lambda > 400$ nm, standard sunlight of 100 mW/cm^2) in the absence of antibacterial agents were performed by performing the same operation.

Bacterial counts: The bacterial suspension is diluted 1000 times. Afterwards, 100 μL of the

diluted bacterial suspension was pipetted and spread on fresh agar plates, and the plates were subsequently cultured at 37 °C for 24 h. Finally, the colonies growing on plates were counted to confirm the number of viable bacteria after incubation for calculating the antibacterial activities.

5. ROS assay

The generated ROS levels by the PCuA were measured by using DCF dye as an indicator, which is produced through the oxidation of DCFH-DA by ROS. In brief, 20 μL DMSO solution of DCFH-DA (1 mM) was added into 5 mL of freshly prepared sample solution under vigorous agitation. After reaction of 30 min, the PL spectra of the sample solution were measured with the photo-excitation at 488 nm. The ROS yield was reflected by the PL intensity at 525 nm.

6. Cytotoxicity experiments

6.1 Cell viability assay

The cell viability was evaluated by cell proliferation kit (MTT) with the L929 cells as a cell model. L929 cells were first seeded into a 96-well plate at a concentration of 1×10^4 cells/well in 100 μL of DMEM culture medium supplemented with 10% FBS, 100 μg/mL penicillin, and 100 μg/mL streptomycin at standard culture conditions (37 °C, 5% CO_2). PCuA or $CuSO_4$ with different concentrations were put into wells for 12 h of cell post-seeding. Ultrapure water and Tween 20 were used as negative control and positive control, respectively. 20 μL of MTT solution was added into the wells and allowed to continue to culture in the incubator for 4 h. Afterwards, the MTT solution was discarded, and DMSO was added to the wells. After stationary culture for 30 min, the optical density of the supernatants in the 96-well plate was measured at 570 nm using a Tecan Spark® multimode microplate reader to confirm the number of viable cells. Each experiment was repeated three times.

6.2 Live/Dead viability assay

Cytotoxic effect of L929 cell lines for PCuA was assessed using Live/Dead viability for mammalian cells according to the manufacturer's protocol. Briefly, cells were grown on 96-well plates and treated for 24 h. Staining was done using a 150 μL dual fluorescence staining solution consisting of calcein acetoxymethyl ester (2.0 μM) and ethidium homodimer (4.0 μM). Excitation/emission wavelengths of both fluoresceins were set at 494 nm/517 nm for calcein acetoxymethyl ester and 528 nm/617 nm for ethidium homodimer. Samples images were captured using a fluorescence microscope under 40× magnification for further analysis by the cellSens data analysis software.

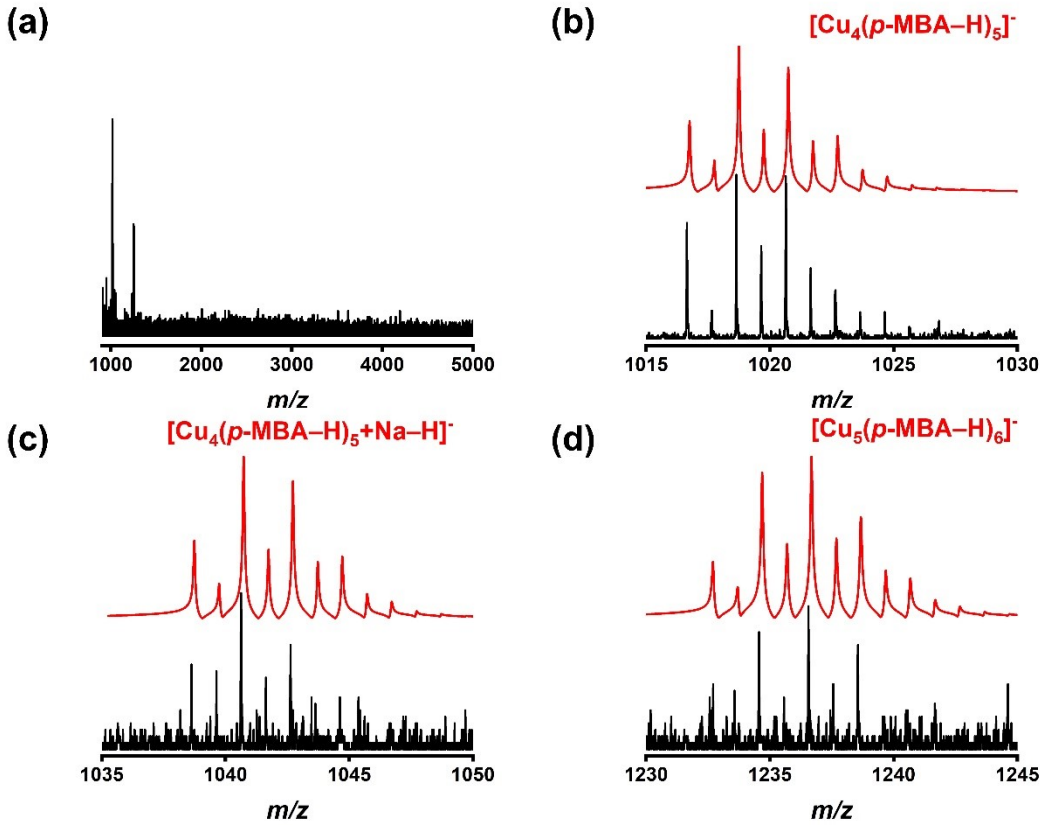

Figure S1. ESI-MS of Cu(I)-*p*-MBA complexes in a broad *m/z* range of 900 ~ 5000. Experimentally acquired (black curve) and theoretically simulated (red curve) isotope patterns of (b) $[Cu_4(p\text{-MBA} - H)_5]^-$, (c) $[Cu_4(p\text{-MBA} - H)_5 + Na - H]^-$ and (d) $[Cu_5(p\text{-MBA} - H)_6]^-$ oligomers. Note: the "*p*-MBA – H" represents the *p*-MBA ligand with deprotonated thiol group (-S) coordinating with Cu(I); "+ Na – H" represents the Na^+ that replaces the proton of the surface carboxyl group (-COOH) of *p*-MBA.

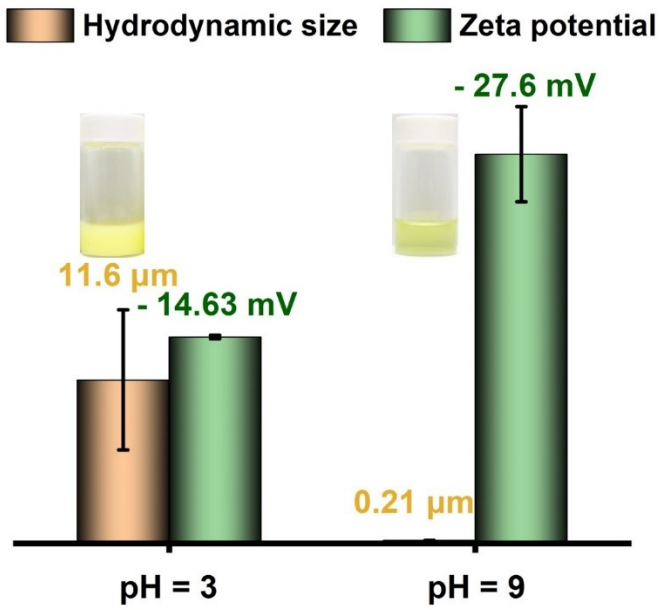

Figure S2. Hydrodynamic size and ζ of Cu(I)-*p*-MBA complexes dispersed in water at pH = 3 and 9. The insets show the images of Cu(I)-*p*-MBA complexes solution at pH = 3 and 9.

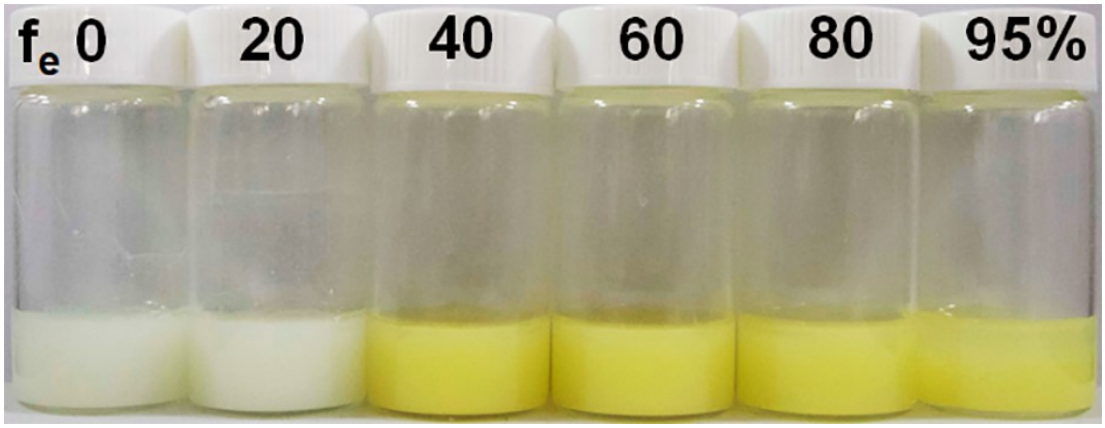

Figure S3. Digital photos of Cu(I)-*p*-MBA complexes solution at pH = ~3.0 in mixed solvents of ethanol/water with different $f_{ethanol}$ under visible light irradiation.

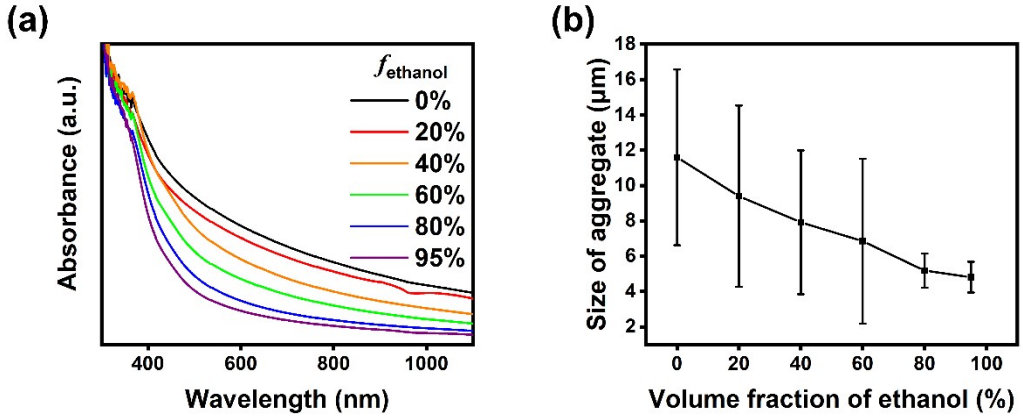

Figure S4. (a) UV-vis absorption spectra of Cu(I)-*p*-MBA complexes ([Cu] = 0.4 mM) in mixed solvents of ethanol/water with different $f_{ethanol}$. (b) Hydrodynamic size of the aggregates of oligomeric Cu(I)-*p*-MBA complexes at different $f_{ethanol}$ measured by DLS.

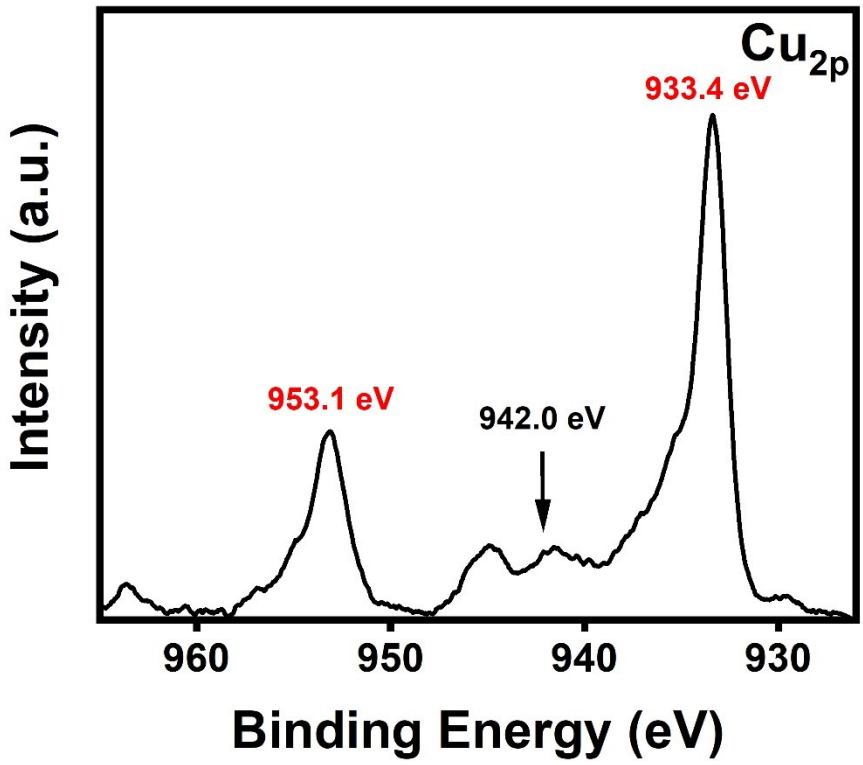

Figure S5. XPS of Cu_{2p} component in the Cu(I)-*p*-MBA complexes.

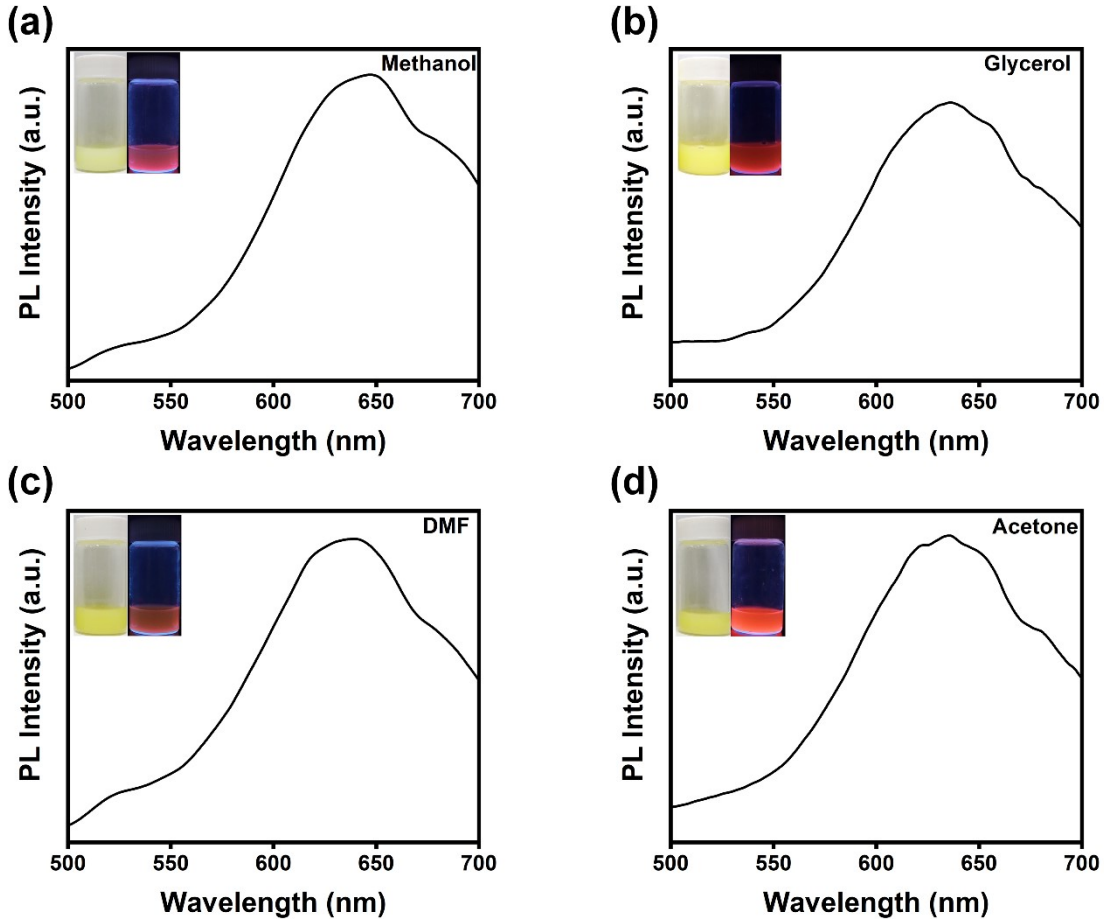

Figure S6. PL emission spectra (λ_{ex} = 365 nm) of Cu(I)-*p*-MBA complexes aggregates ([Cu] = 4 mM) in mixed solvents of (a) water/methanol, (b) water/glycerol, (c) water/DMF, and (d) water/acetone with a fixed volume fraction of 40% water. Inset: digital photos of corresponding suspension taken under visible (left items) and UV (right items) light irradiation.

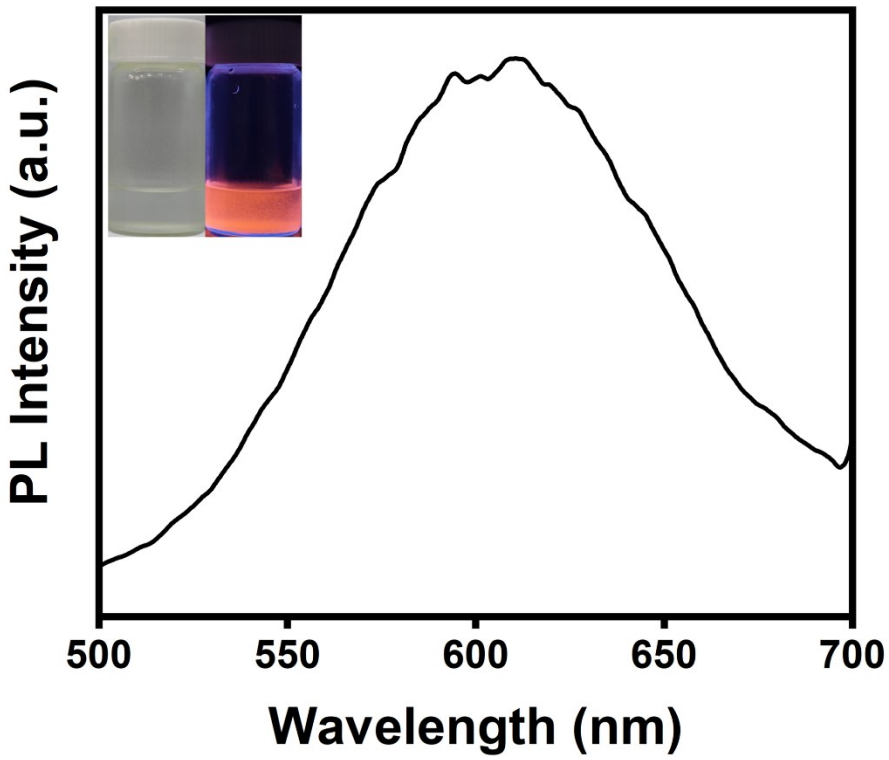

Figure S7. PL emission spectrum (λ_{ex} = 365 nm) of Cu(I)-GSH complexes aggregate ([Cu] = 1 mM) in a mixed solvent of water/ethanol ($f_{ethanol}$ = 80 %). Inset: digital photos of corresponding suspension taken under visible (left item) and UV (right item) light irradiation.

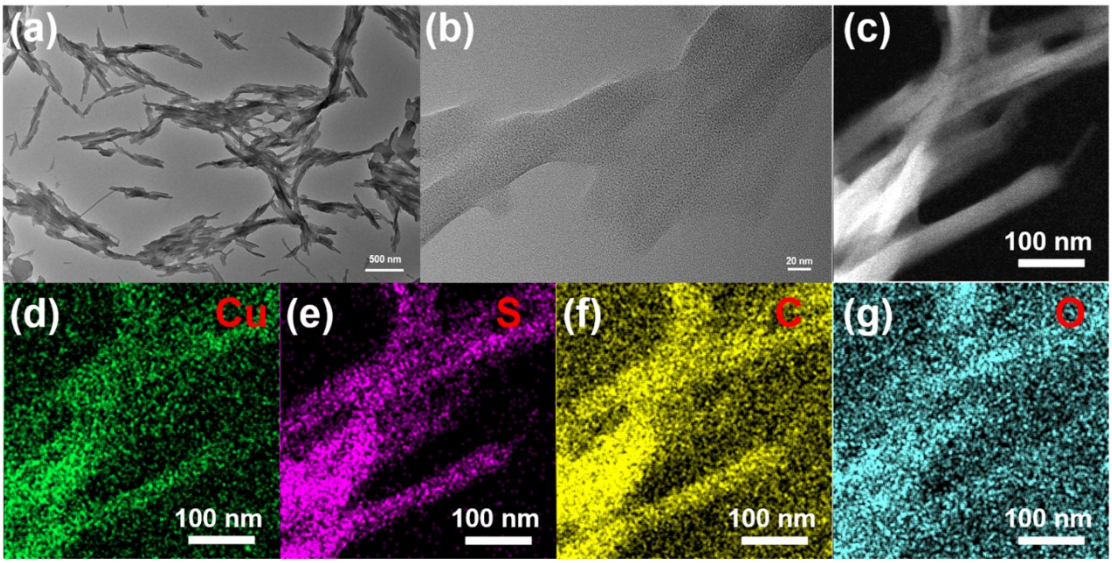

Figure S8. (a, b) TEM images of the PCuA with different magnifications. (c) EDX mapping image of the PCuA and (d-g) the corresponding elemental mappings images of Cu, S, C and O elements.

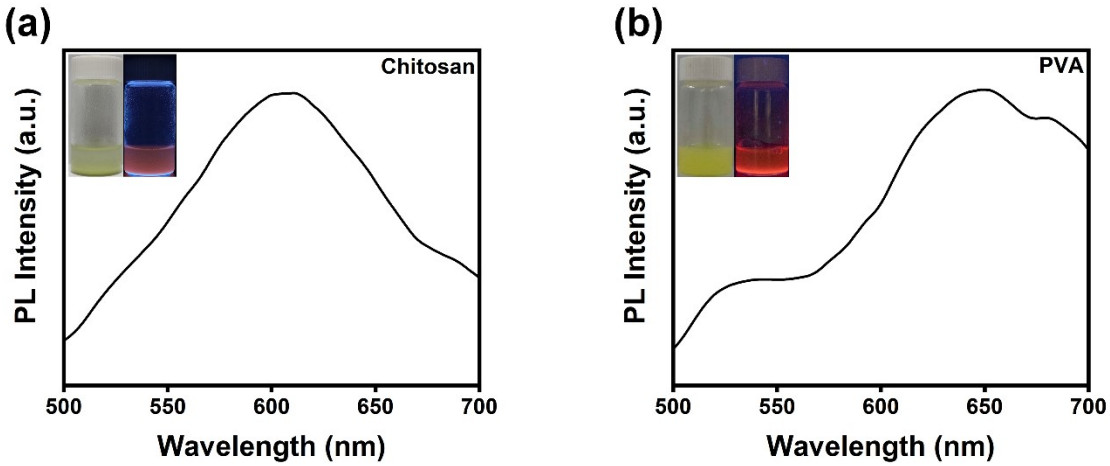

Figure S9. PL emission spectra (λ_{ex} = 365 nm) of the (a) chitosan-condensed and (b) PVA-condensed Cu(I)-*p*-MBA complexes aggregates in water. Inset: digital photos of corresponding samples taken under visible (left item) and UV (right item) light irradiation.

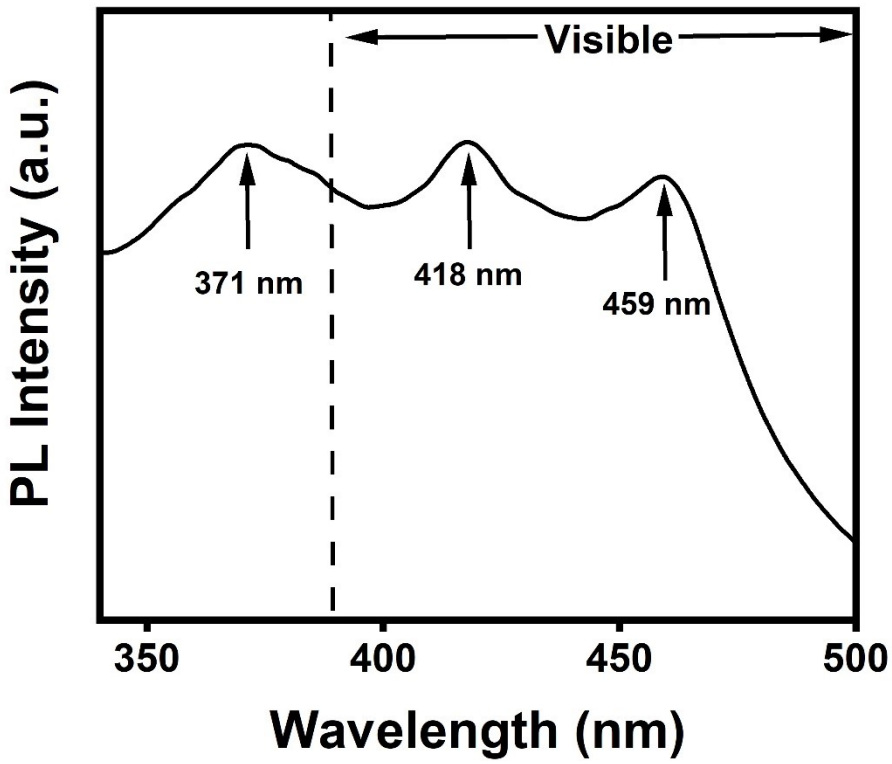

Figure S10. The PL excitation spectrum (λ_{em} = 640 nm) of the PCuA sample.

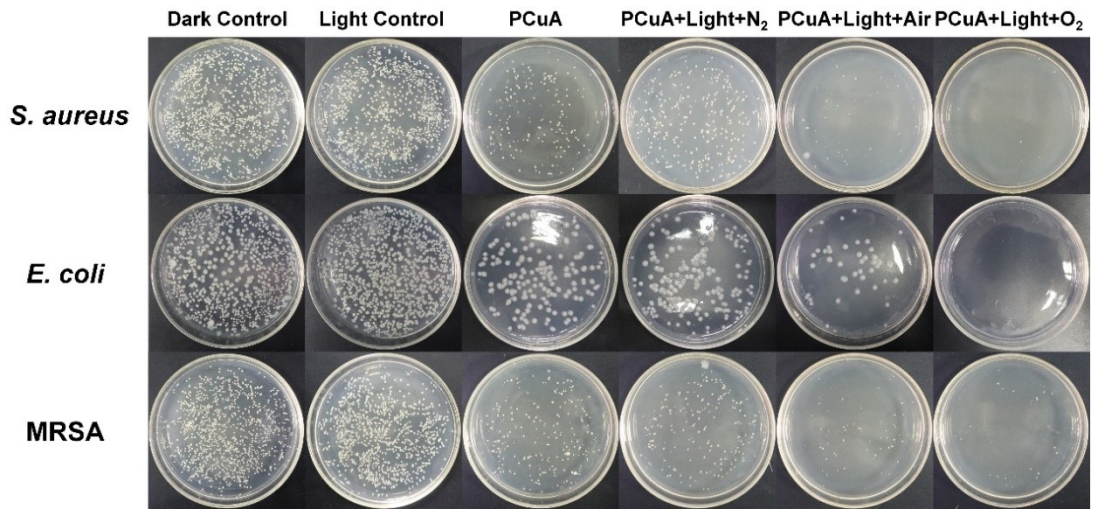

Figure S11. Bacterial colony photos of gram-positive *S. aureus*, gram-negative *E. coli* and drug-resistant MRSA treated with the PCuA ([Cu] = 0.4 mM) under different conditions for 60 min.

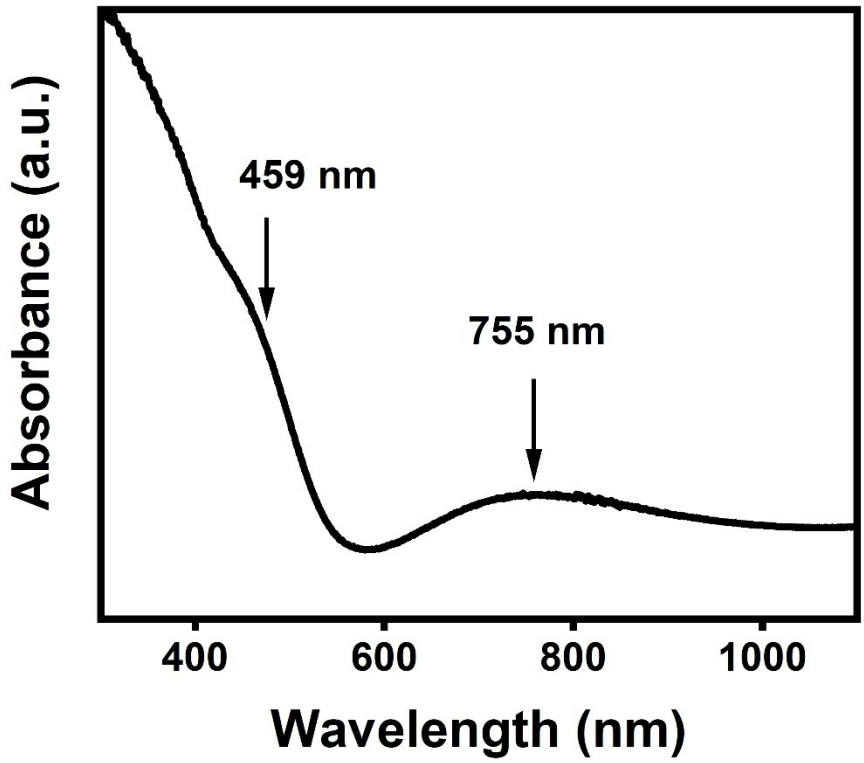

Figure S12. UV-vis DRS of the PCuA.

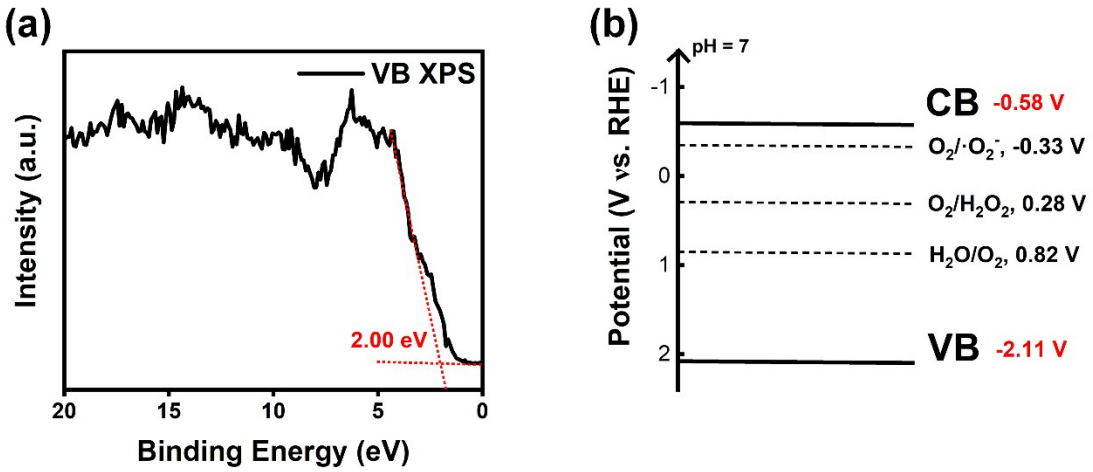

Figure S13. (a) VB-XPS and (b) energy level diagram of the PCuA.

Supplementary Note I: The VB XPS (Figure S10a) shows that the valence band (VB) of the PCuA is at 2.00 eV, which could be converted to 2.11 V vs RHE based on the formula $E_{RHE} = \varphi + E_{VB\text{-}XPS} - 4.44$ eV (here RHE denotes reversible hydrogen electron, and the φ (work function) = 4.55 eV). In addition, we transferred the UV-vis DRS of the PCuA to Tauc plot, and acquired the band gap of approximately 2.69 eV for the PCuA. Therefore, the position of the CB of the PCuA is determined to be – 0.58 V vs RHE, which is lower than the redox potential for electrons to reduce O_2 to ROS ($\cdot O_2^-$ and H_2O_2). $O_2 + e^- \rightarrow \cdot O_2^-$, - 0.33V vs. RHE, pH = 7. $O_2 + 2e^- + 2H^+ \rightarrow H_2O_2$, + 0.28 V vs. RHE, pH = 7. [1]

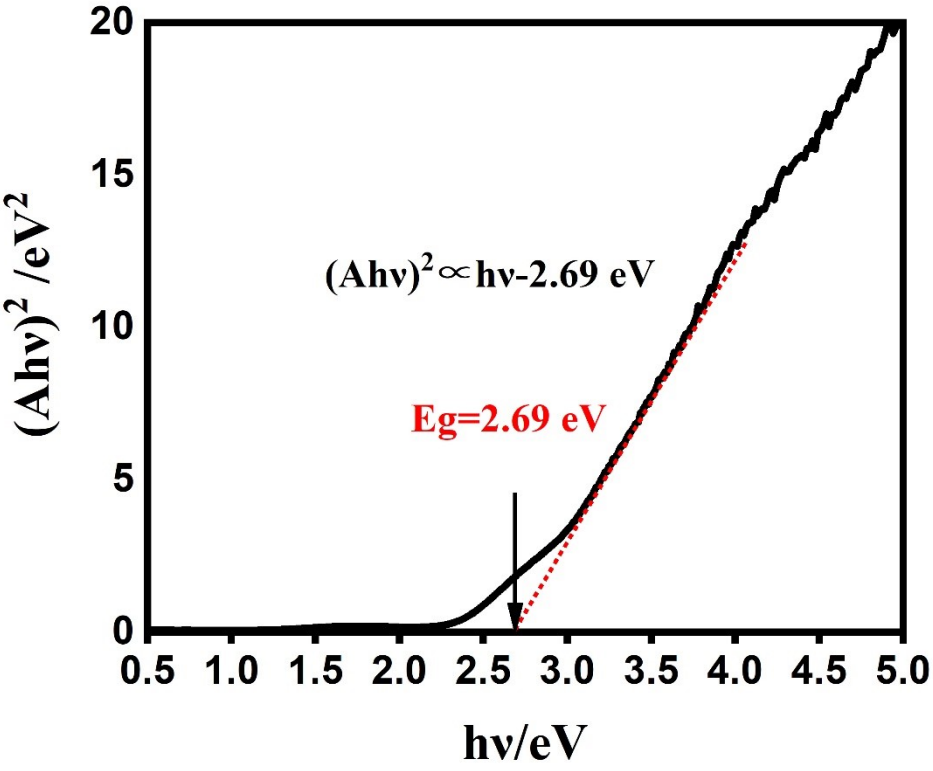

Figure S14. The Tauc plot of the PCuA obtained by converting the UV-vis DRS data shown in Figure S12.

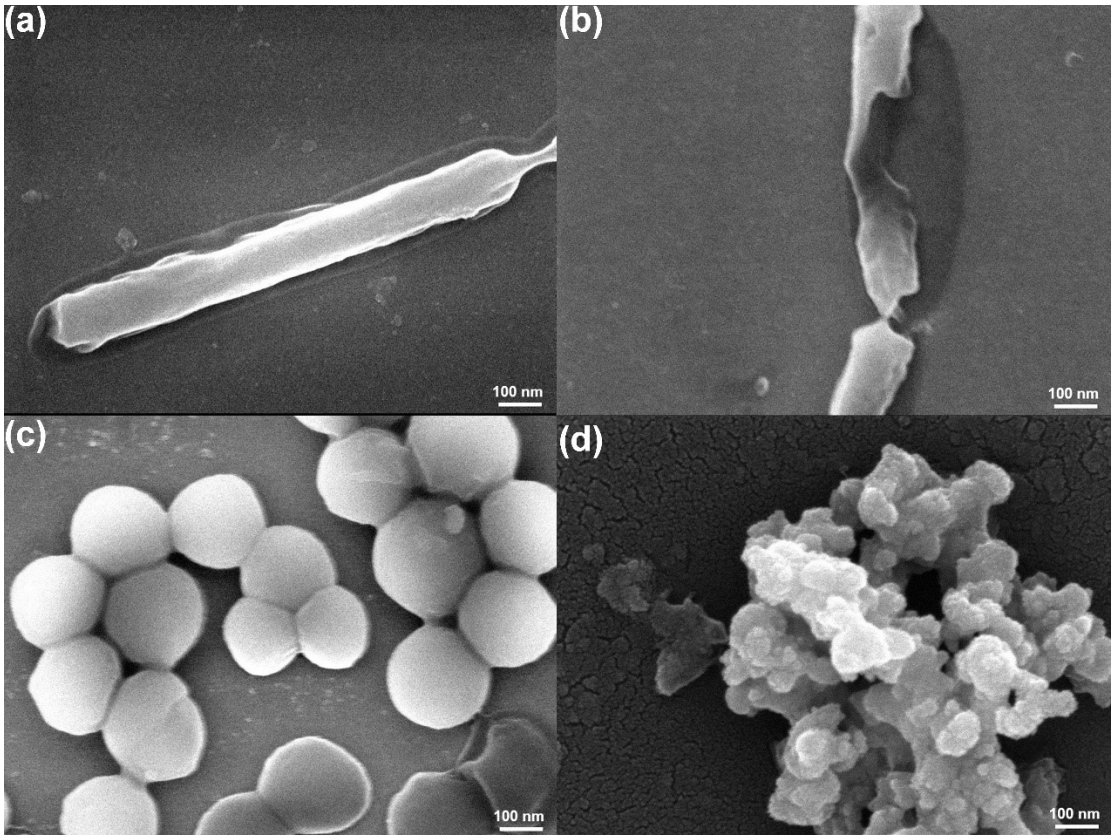

Figure S15. SEM images of *E. coli* (a, b) and *S. aureus* (c, d) before (a, c) and after (b, d) the treatment of PCuA under visible light irradiation.

Reference

1. H. Zhu, Q. Xue, G. Zhu, Y. Liu, X. Dou and X. Yuan, *J. Mater. Chem. A*, 2021, **9**, 6872-6880.