Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Antibacterial metal-phenolic nanosheets as a smart carrier for controlled release of epirubicin hydrochloride

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1. General Information

1.1 Chemicals. TA were obtained from Aladdin Chemical Co. Ltd., cupric sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) was purchased from Tianjin Guangfu Fine Chemical Research Institute, Epirubicin Hydrochloride was purchased from Shanghai yuanye Bio-Technology Co., Ltd. Phosphate-buffered saline (PBS, tablet) and glutathione was purchased from Beijing Solarbio Science & Technology Co., Ltd. 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) was purchased from Shanghai Aladdin Biochemical Technology Co. Ltd. All other reagents and solvents were of reagent grade. The kidney cells of African green monkey (Vero) were obtained from Northwest Minzu University (Lanzhou, China). *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) were used in our experiments which obtained from the CAS Key Laboratory of Chemistry of Northwestern Plant Resources, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences. Live/Dead viability assay kit was purchased from Abnova Company. Ultrapure water was obtained by a water purification system, which was purchased from Shanghai Laikie Instrument Co., Ltd.

1.2 Materials characterization. The morphologies of Cu-TA nanosheets were investigated on an FEI-Tecnai G2 Transmission Electron Microscope (TEM) and JSM-6701F Scanning electron microscope. Brunauer-mmett-Teller (BET) surface areas and porosity for the Cu-TA was acquired by using a N_2 sorption instrument (Micromeritics, ASAP 2020). The X-ray diffraction measurements of Cu-TA were performed by a PHILP X'Pert PRO, using Cu Kα (λ = 1.542 Å) irradiation (40 kV, 40 mA) in the range of 2θ = 5-80°. Fourier Transform Infrared Spectrometer (FT-IR) was conducted by a Nexus 870 Fourier Transform Infrared Spectrometer (Thermo Fisher, USA). X-ray photoelectron spectroscopy (XPS) analysis was carried out using an ESCALAB 250Xi X-ray photoelectron spectrometer (Thermo Fisher scientific, USA). Thermogravimetric analyses (TGA) were performed on a STA 449C thermal analysis system with a nitrogen atmosphere at a heating rate of 10 °C/min from room temperature to 800 °C. Zeta potential and hydrodynamic size changes of Cu-TA before and after the EPI loading were recorded by laser dynamic scattering instrument (Zetasizer Nano 3600, UK). The thickness of Cu-TA was measured on an atomic

force microscope (AFM, Dimension ICON, Bruker). Fluorescence images on cells and coatings were obtained on an Olympus Fluoview 1000 confocal laser scanning microscope (Olympus, Tokyo, Japan). Fluorescence spectra of EPI were determined by Perkin-Elmer LS-55 fluorescence spectrophotometer with a slit width of 5.0 and 5.0 nm for excitation and emission. The content of Cu in Cu-TA and released samples was determined by Atomic Absorption Spectrometer (Jena ContrAA700). Imaging mass spectra were performed an imaging mass spectrometer (iMScope TRIO Shimadzu, Kyoto, Japan). Data were collected at 25 μm intervals. For each sample pixel, the surface was laser-irradiated with 80 shots (1000 Hz repetition rate, 10 μm laser diameter, 38 laser intensity). The detector voltage of the microchannel plate detector was 1.87 kV, while the sample voltage was 3.5 kV. Electron spin resonance (ESR) spectra were measured on a Bruker A300 spectrometer.

1.3 Disassembly experiments of Cu-TA

The about 10 mg of Cu-TA powders in 1.5 mL of PBS buffer (pH 7.4, pH 5.0), acetic acid-ammonium acetate buffer (pH 3.0) or about 5 mg of Cu-TA powders in 10 mL of 10 mM GSH (pH 7.4) were incubated in a thermostated shaker bath at 37 °C for the desired time. The remaining Cu-TA nanosheet were separated from the solution by centrifugation, washed with deionized water several times. The remaining nanosheets were dried under vacuum and weighed to record the residual weight of Cu-TA.

1.4 Live-dead assay of Cu-TA

Vero cells were seeded in a 24-well plate (1×10^6 cells per well) and incubated for one day. The old media were changed with the media containing the 5 and 50 μg/mL of Cu-TA and incubated at 37 °C in a humidified environment of 5% CO_2 for 24 h. The media were removed and 0.5 mL of a mixture of Calcein AM and propidium iodide (PI) was added to each well and incubated at 37 °C in a humidified environment of 5% CO_2 for 30 min. Both fluorochromes can be excited at 488 nm but emit green (λ_{Em} = 518 nm, live cells) and red fluorescence (λ_{Em} = 615 nm, dead cells), respectively. Then, the image of the stained cells was acquired by confocal laser scanning microscope.

1.5 Antibacterial activity assay.

The antibacterial activity of Cu-TA nanosheets was investigated through the minimum inhibitory concentration (MIC) test, inhibition zone and standard plate count method against *E. coil* and *S. aureus*. The bacteria were cultured in Lysogeny broth culture media for 24 h at 37 °C and then diluted with Lysogeny broth culture media to a final concentration of approximately 10^7 colony-forming units (CFU) mL^{-1} by measuring the optical density at 600 nm (OD_{600}) of suspension.

The antibacterial activity of Cu-TA was firstly tested by the minimum inhibitory concentration (MIC), which was determined using the microtiter broth dilution method. 100 μL of both bacterial dispersions (10^7 CFU mL^{-1}) was added in each well of 96-well plate, followed by the addition of a series of two-fold dilution of Cu-TA samples (100 μL). The plate was incubated at 37 °C for 24 h. the bacteria viability were determined by measuring the OD_{600} using the SpectraMax® Absorbance Reader (Molecular Devices, USA).

For inhibition zone test, about 20 mL of fresh nutrient agar (1.5%) was poured into Petri dish and allowed to solidify. The bacterial suspension (1.0 mL, 10^7 CFU mL^{-1}) of *S. aureus* and *E. coli* was uniformly coated on the agar surface with a Oxford cups (8 mm for outside diameter) on the agar surface, respectively. The Cu-TA dispersion (2 mg/mL, 100 μL) was injected into the Oxford cups and the dishes were incubated at 37 °C for 24 h.

The shake flask method uses *S. aureus* and *E. coli* with a concentration of 10^5-10^6 CFU/mL after calibration with a microplate reader with a wavelength of 600 nm. Subsequently, 100 and 10 mg of CuTA powders were added to conical flasks with a sealing membrane containing 50 mL of each bacterial solution, respectively. All conical flasks were incubated for 0-12 h (2, 4, 6 and 12 h) using an oscillating incubator (37 °C, 100 rpm). After that, 100 μL of bacterial liquid was taken out at specific times and diluted 10-fold and 100-fold in 24-well plates. Then, 100 μL of the diluted bacterial solution was smeared on agar plates. All agar plates were put in an incubator at 37 °C until colonies appeared. Finally, the number of bacterial colonies

on the agar plates was counted with a software of ImageJ and compared with the control to obtain the Elimination rate.

1.6 Cellular uptake

Hep G2 cells were seeded in six-well culture plate and cultured in Dulbecco's modified eagle media (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) at 37 °C in 5% CO_2/air atmosphere. The cells were then treated with 10 μg/mL of free EPI and Cu-TA/EPI nanodrug, respectively, at 37 °C for another 6 h. Then the medium was removed, and cells were washed with sterile PBS three times to remove any free material in the wells. 1 mL of 4% formaldehyde was added to each well to fix the cells at room temperature for 10 min. Finally, the cells were washed with PBS buffer before capturing images by using confocal laser scanning microscope and excitated at 488 nm.

1.7 *In Vitro* cytotoxicity evaluation of Cu^{2+} and the extract of Cu-TA

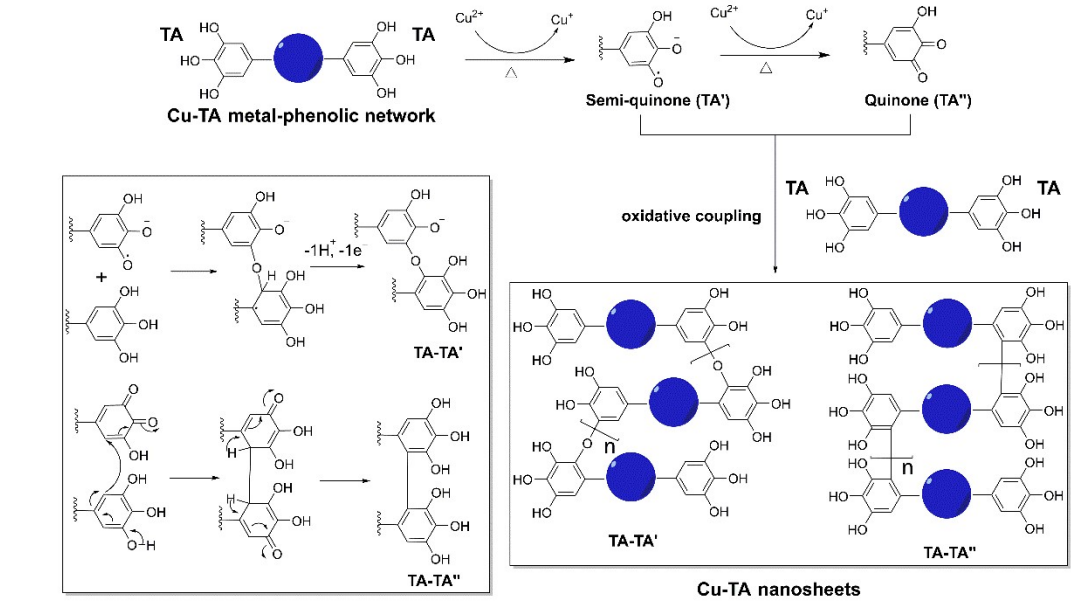
$CuSO_4 \cdot 5H_2O$ were used to evaluate the *in vitro* cytotoxicity of Cu^{2+} against Hep G2 cells. Hep G2 cells were incubated with 0, 0.1, 1, 5, 10, 25, 50 μg/mL of Cu^{2+} for 24 h and a MTT assay was performed to determine the cell viability.

Cu-TA nanosheets were soaked in PBS (pH 7.4) with concentrations of 2 mg/mL. After 12 h, 5 μL of extract was drawn for the test involving Vero cells and a MTT assay was performed to determine the cell viability.

1.8 Detection of *in Vitro* ·OH production

ESR spectra were used to detect *in vitro* ·OH production by Cu-TA (50 μL, 20 μg/mL) at different pH conditions (pH 7.4, 5.0), with or without premixed H_2O_2 (50 μL, 50 mM). After 3 min, a 5,5-dimethyl-1-pyrroline-N-oxide (DMPO, 50 μL, 10 mM) solution was added. Immediately thereafter, the solution was transferred to a quartz capillary tube and ESR signals were detected at room temperature.

2. Supplementary Schemes, Figures and Tables



Proposed oxidative coupling mechanism of Cu-TA network

Scheme S1. Proposed formation mechanism of Cu-TA nanosheets by Cu^{2+}-mediated oxidative coupling assembly of Cu-TA coordination complexes network.

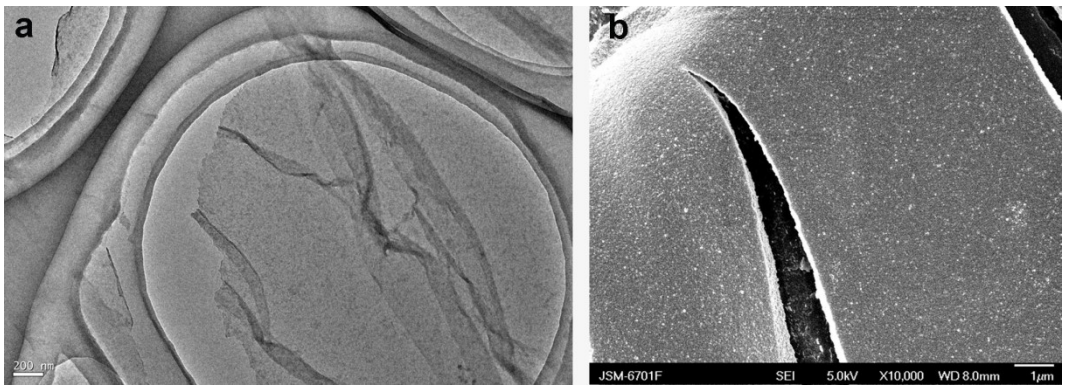


Figure S1. (a) TEM, (b) SEM image of TA samples prepared under the same procedures of Cu-TA only without introducing Cu^{2+}.

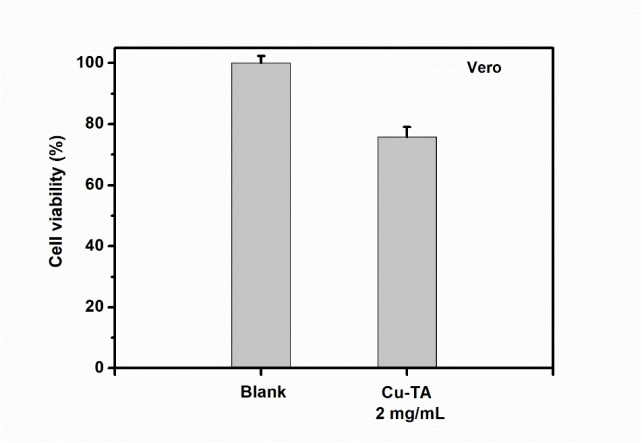


Figure S2. Cytotoxicity assay of Vero cells after 24 h of incubation with the extract of Cu-TA (2 mg/mL) nanosheet.

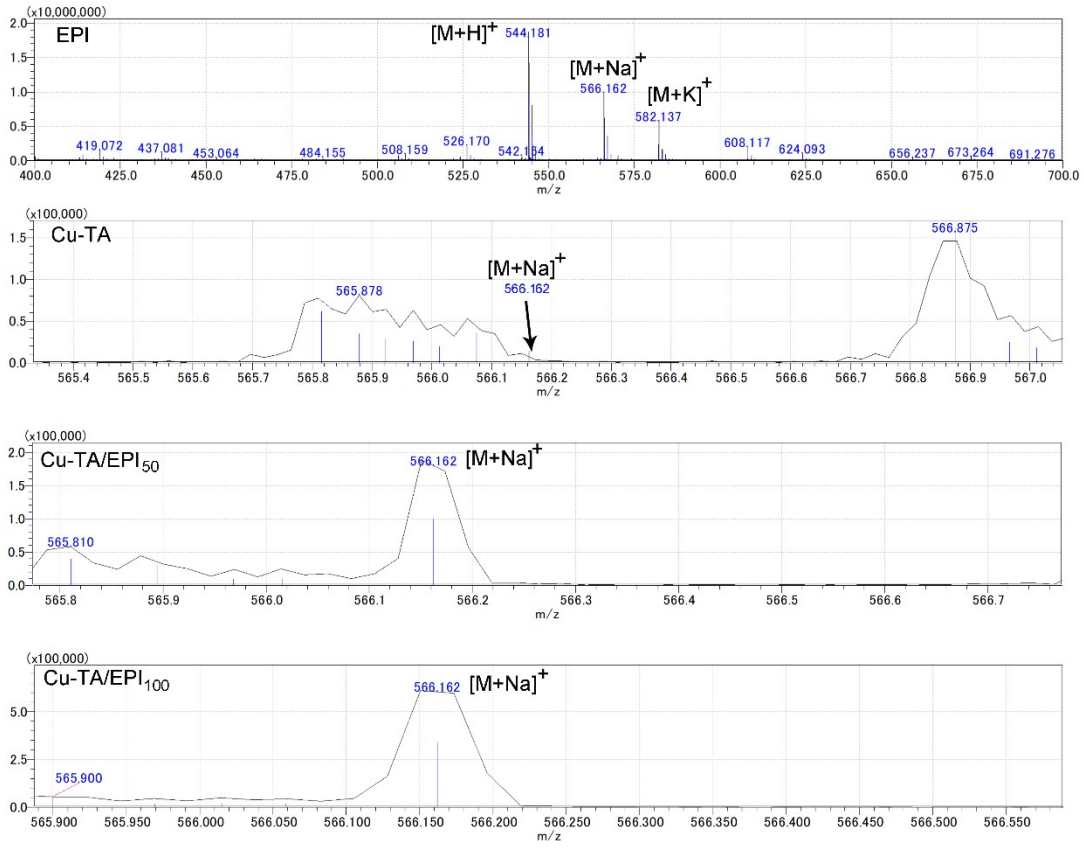


Figure S3. The MALDI-TOF-MS spectrum acquired from EPI (1 mg/mL, 3 μL), Cu-TA (2 mg/mL, 3 μL) and Cu-TA/EPI (2 mg/mL, 3 μL) using CHCA matrix in positive ionization mode. Cu-TA/EPI_{50} and Cu-TA/EPI_{100} represents the 50 or 100 μg of EPI was used in preparing of Cu-TA/EPI nanodrug with same amount of Cu-TA (200 μg).

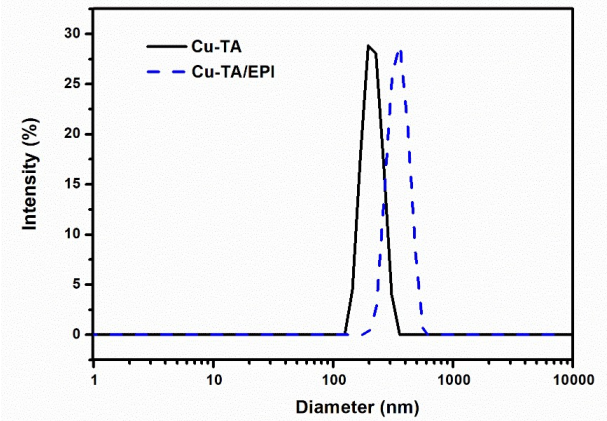


Figure S4. The dynamic laser light scattering (DLS) characterization of Cu-TA and Cu-TA/EPI. For all samples, the concentration is 0.05 mg/mL.

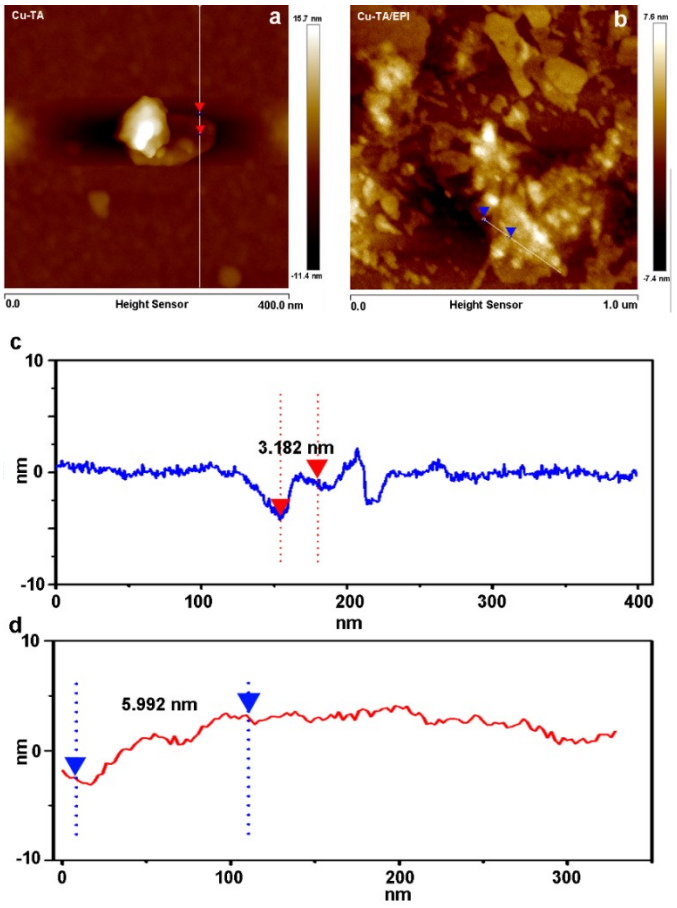


Figure S5. AFM images of Cu-TA (a) and Cu-TA/EPI (b). The height profiles of Cu-TA (c) and Cu-TA/EPI (d).

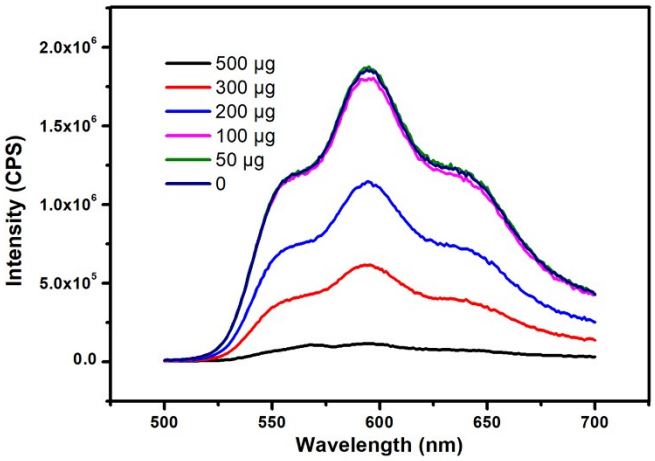


Figure S6. Fluorescence emission spectra of EPI solution (λ_{Ex} = 476 nm) after treated with increasing amounts of Cu-TA nanosheets from 0-500 µg.

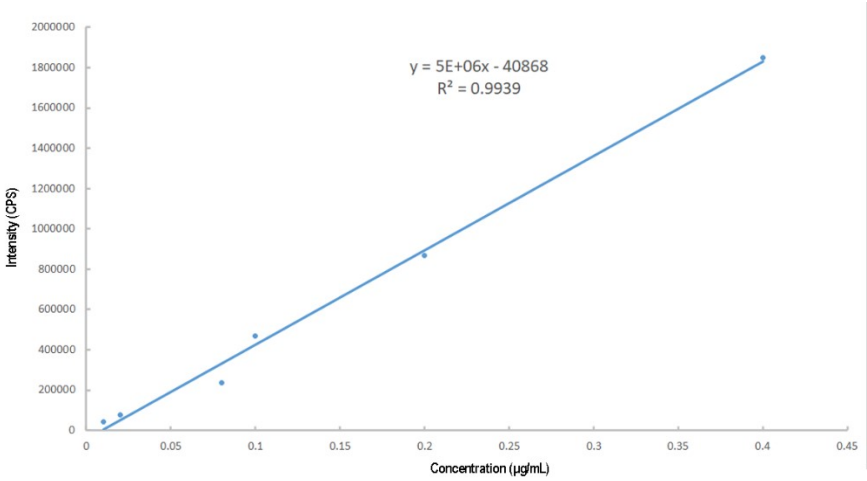


Figure S7. Calibration curve of EPI in water determined by fluorescence emission spectra (λ_{Ex} = 476 nm, λ_{Em} = 596 nm).

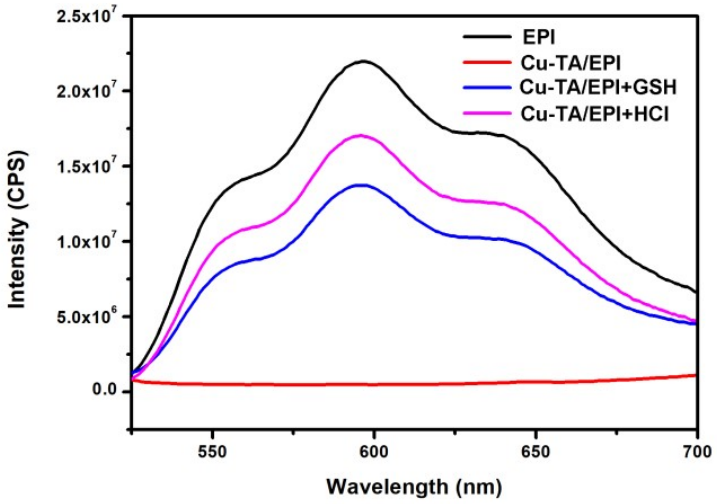


Figure S8. Fluorescence emission spectra of EPI solution, Cu-TA/EPI nanodrug solution (0.1 mg/mL) before and after introducing HCl (0.1 M, 10 μL) or GSH (10 mM).

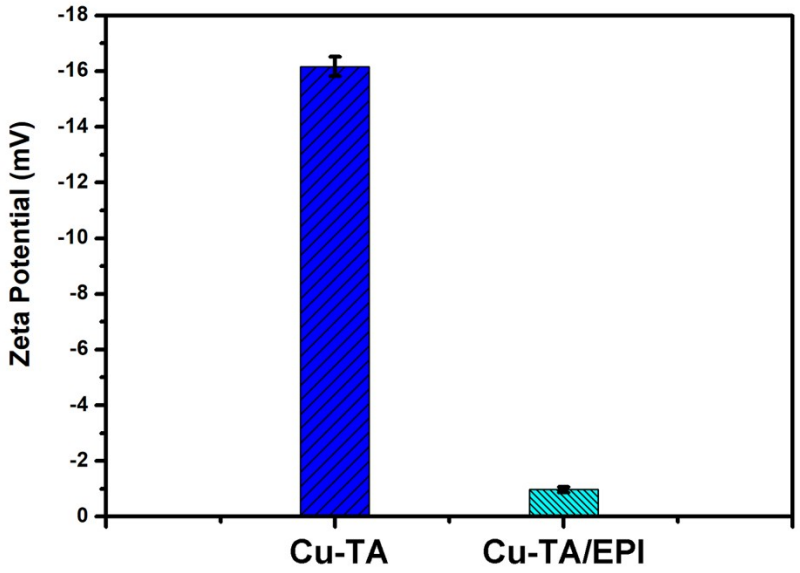


Figure S9. Zeta potential of Cu-TA and Cu-TA/EPI. For all samples, the concentration is 0.1 mg/mL.

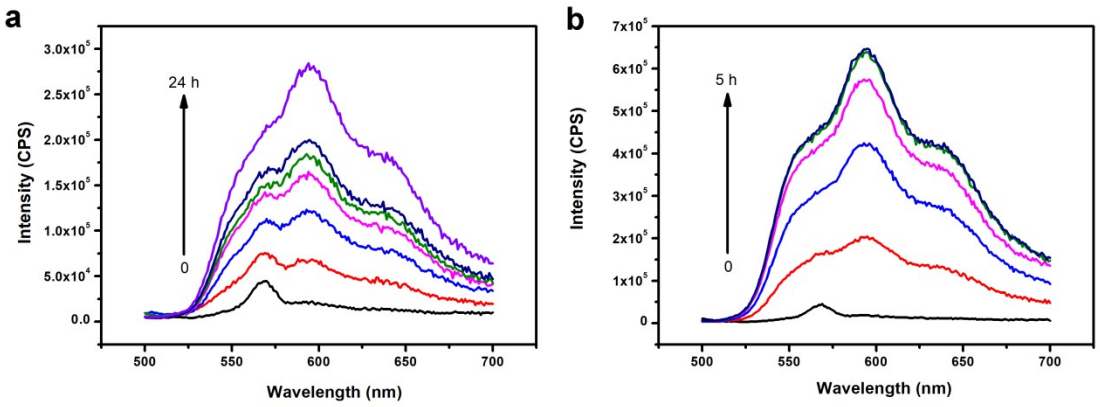


Figure S10. Time-dependent fluorescence emission spectra of EPI release after incubating Cu-TA/EPI in the PBS at pH 5.0 (a) and pH 7.4 containing 10 mM of GSH (b) at different time.

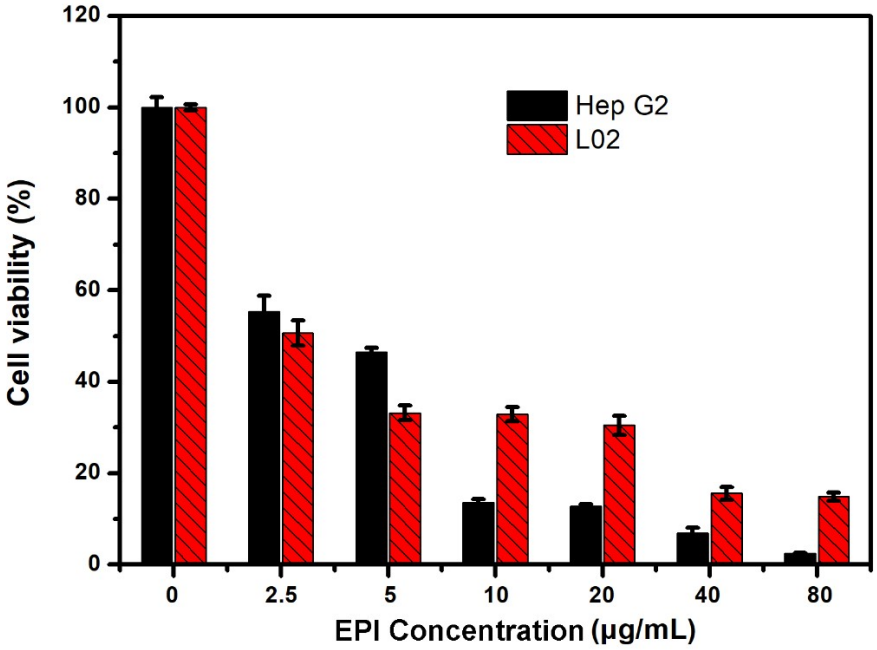


Figure S11. Cytotoxicity assay of Hep G2 and L02 cells after 24 h of incubation with Cu-TA/EPI

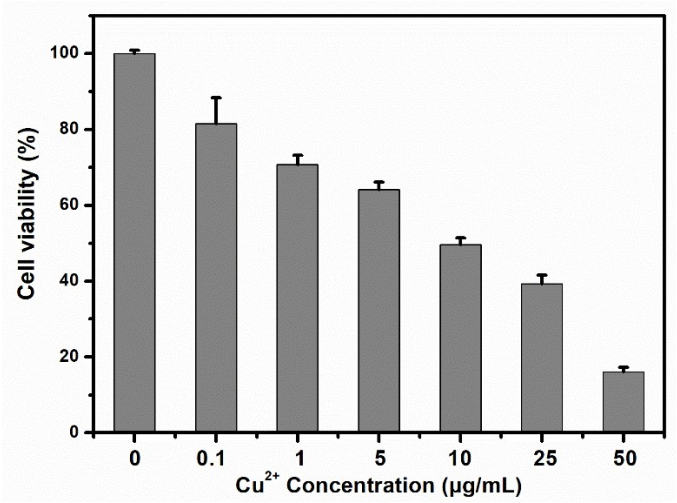


Figure S12. Cytotoxicity assay of Hep G2 after 24 h of incubation with Cu^{2+}.

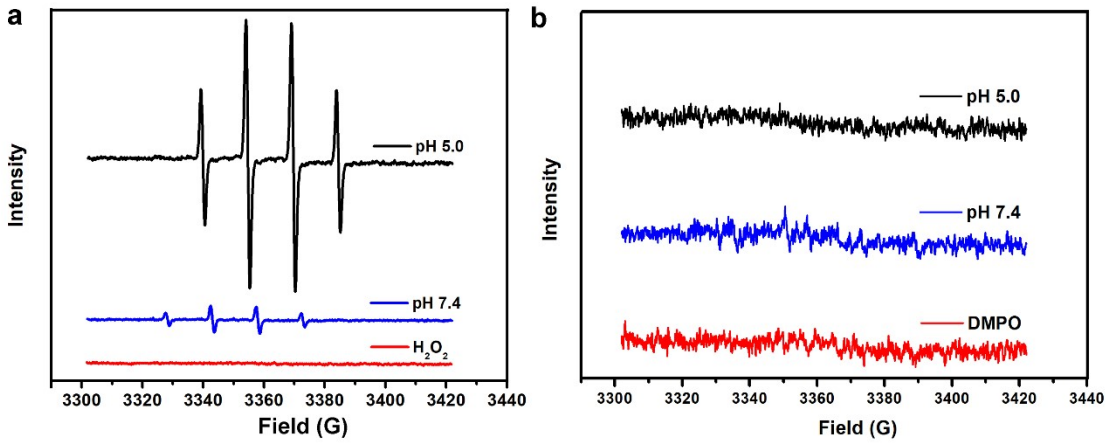


Figure S13. ESR signals detected under different conditions. (a) H_2O_2 only; Cu-TA+ H_2O_2, pH 7.4; Cu-TA+ H_2O_2, pH 5.0. (b) DMPO only; Cu-TA, pH 7.4, without H_2O_2; Cu-TA, pH 5.0, without H_2O_2.

Table S1 Contents of C, O, and Cu in Cu-TA nanosheets from XPS characterization

Element	C	O	Cu
Atomic concentration	34.47%	47.09%	18.44%