Electronic Supplementary Material (ESI) for Nanoscale.
This journal is © The Royal Society of Chemistry 2023

Supporting Information

Hollow $Cu_{2-x}Se$-based Nanocatalysts for Combined Photothermal and Chemodynamic Therapy in the Second Near-Infrared Window

Qianqian Gao [a, #], Xuelu He [a, #], Lulu He [a, c, #], Jie Lin [a, b, c], Le Wang [a, c], Yujiao Xie [a, c], Aiguo Wu [a, b, c *], Juan Li [a, b, c *]

[a] *Ningbo Key Laboratory of Biomedical Imaging Probe Materials and Technology, Zhejiang International Cooperation Base of Biomedical Materials Technology and Application, Chinese Academy of Sciences (CAS) Key Laboratory of Magnetic Materials and Devices, Ningbo Cixi Institute of Biomedical Engineering, Zhejiang Engineering Research Center for Biomedical Materials, Ningbo Institute of Materials Technology and Engineering, Chinese Academy of Sciences, Ningbo, 315201, China*

[b] *University of Chinese Academy of Sciences, Beijing 100049, P.R. China*

[c] *Advanced Energy Science and Technology Guangdong Laboratory, Huizhou, 516000, China*

[#]These authors are equally contributed.

Corresponding author: Aiguo Wu (aiguo@nimte.ac.cn)

Juan Li (lij@nimte.ac.cn)

Experimental Details

Chemicals.

All chemicals and reagents used were of analytical grade and without any further purification. Copper chloride dihydrate, polyvinylpyrrolidone (PVP K30, MW = 40000 Da), ascorbic acid (AA), and copper sulfate pentahydrate were obtained from Sinopharm Chemical Reagent Co., Ltd. Selenium powder (~100 mesh), sodium hydroxide, sodium citrate dihydrate and sodium borohydride were purchased from Aladdin Chemistry Co., Ltd. Hydroxylamine solution was purchased from Sigma-Aldrich Chemical Co. Ltd. Deionized (DI) water obtained from a Milli-Q water system was used for all the experiments.

Characterization.

X-ray powder diffraction (XRD, D8 Advance) and field-emission scanning electron microscope (SEM, Regulus 8230) were used for the determination of crystal structure and morphology. Transmission electron microscope (TEM, JEOL-2100F) was used for recording transmission electron microscope images. Further elemental mapping images and selected area electron diffraction (SAED) patterns and corresponding X-ray energy dispersive X-ray energy spectroscopy (EDS) spectra were performed by high-resolution transmission electron microscope (Talos, F200x). The electron binding energy and surface chemical composition were performed with an X-ray photoelectron spectrometer (XPS, Axis Ultra DLD). The surface areas of the samples were determined by Bruner-Emmett-Taylor method (BET, ASAP 2020M).

Synthesis Methods.

Cube Cu_2O. 0.0853 g of $CuCl_2 \cdot 2H_2O$ and 0.6 g of PVP were dissolved into 20 mL of DI water under stirring, then 0.32 g of NaOH was added. After stirring for 10 min, 0.1769 g of AA was added. The products were collected by centrifugation and washed several times with deionized water and ethanol.

Octahedron Cu_2O. 35 mg of $CuCl_2 \cdot 2H_2O$, 1.1 g of PVP and 0.01 g of sodium citrate

dihydrate were dissolved into 20 mL of DI water under stirring. 0.16 g of NaOH was added into the above mixture and kept stirring for another 30 minutes at room temperature. Then followed by the addition of 0.2111g of AA, the solution was transferred into a 50℃-oil bath, holding for 1.5 hours.

Sphere Cu_2O. In a mixed solution containing 0.01 M $CuSO_4 \cdot 5H_2O$ (25 mL) and PVP (0.2 g), a solution prepared from pre-mixing 0.1 M hydroxylamine solution (5 mL) and 1.5 M NaOH aqueous solution (5 mL) was added. Then the above solution was stirred for 10 minutes at room temperature.

Hollow Cube, Octahedron and Sphere $Cu_{2-x}Se$. Firstly, selenium source (NaHSe solution) was prepared by mixing 0.03 g of Se powder and 0.05 g of $NaBH_4$ into 15 mL DI water under N_2 atmosphere at room temperature for 40 min. For the formation of hollow $Cu_{2-x}Se$ nanoparticles, the as-prepared selenium source was added into the above cube, octahedron and sphere Cu_2O suspensions and stirred for 4 h. The prepared $Cu_{2-x}Se$ nanoparticles were purified by repeated centrifugation (11000 rpm, 10 min), washed with DI water and ethanol.

$Cu_{2-x}Se$@CQ and $Cu_{2-x}Se$@CQ@NPY. CQ and octahedron $Cu_{2-x}Se$ were dissolved in DMSO at a mass ratio of 5:1, and the mixture was stirred at room temperature for 24 h. Further, the DSPE-PEG-COOH (8 mg/mL) was activated by a mixture of EDAC (1.52 mg/mL) and NHS (0.67 mg/mL) in DI water for 4 h with an ice bath. Then, 2 mL of NPY (44 mg/mL in water) solution was added into the above mixture and then stirred for 16 h at room temperature. The resulting product was purified by dialyzing against water using a membrane (MWCO: 3500 Da, Minnesota Ming and Manufacturing Company, U.S.A.) for 3 days. The as-obtained $Cu_{2-x}Se$@CQ nanoparticles were added into DSPE-PEG-NPY (DI water, 5 mL) at a mass ration of 1:4 and stirred for 12 h in an ice bath.

Photothermal Performance.

To systematically evaluate the differences in the photothermal performance of cube, octahedron and sphere $Cu_{2-x}Se$ nanoparticles, the aqueous solutions of $Cu_{2-x}Se$ at different concentrations (0, 10, 20, 40, 80 and 120 μg/mL) were irradiated with a 1064

nm NIR laser (5 min, 1.0 W/cm^2). The temperature of solutions was recorded every ten second.

Photothermal Conversion Efficiency Calculation.

To calculate the photothermal conversion efficiency (η), the $Cu_{2-x}Se$ aqueous solutions with three morphologies (40 μg/mL) were irradiated for 10 min, then the laser was turn off to let the samples cool down naturally. The photothermal conversion efficiency (η) was calculated according to the following formulas:

$$\eta = \frac{hS(T\max - T\mathrm{sur}) - Q\mathrm{dis}}{I(1 - 10^{-A1064})} \quad (1)$$

Where T_{max} is the equilibrium temperature after 10-min irradiation, T_{Sur} is the ambient temperature, Q_{dis} stands for the heat dissipation by the test cell, I is the1064 nm laser power (1.0 W/cm^2), and A_{1064} is the absorbance of the solution at 1064 nm. h is the heat transfer coefficient, S is the surface area of the cell, and the value of hS is determined according to the equation (2):

$$hS = \frac{m\mathrm{D}c\mathrm{D}}{\xi\mathrm{s}} \quad (2)$$

Where m_D is the mass of the sample (1 g), C_D is the heat capacity of the solution (4.2 J/g), and ξs is the time constant of the system.

Chemodynamic Property.

To evaluated the Fenton catalytic property of $Cu_{2-x}Se$, methylene blue (MB) was used as a chemical probe to detect the generation of ·OH radicals. The experiment was divided into four groups: (i) MB; (ii) MB+H_2O_2; (iii) MB+$Cu_{2-x}Se$ (iv) MB+H_2O_2+$Cu_{2-x}Se$. Briefly, $Cu_{2-x}Se$ (0 or 40 μg/mL) with different morphologies or H_2O_2 (0 or 1.0 mM) was added into MB solution (10 μg/mL) and stirred for 30min at 25℃. The absorbance of MB at 664 nm in PBS was detected, and the whole reaction was carried out in a dark environment. In addition, to evaluate the effect of temperature on the generation of ·OH radicals, the temperature was increased to 45°C which could simulate the hyperthermic environment of PTT effect.

Cell Culture.

Human esophageal squamous cell line K150 and human breast cancer cell line MCF-7 were obtained from Shanghai Zhong Qiao Biotechnology, and cultured in DMEM culture containing 1% (v/v) penicillin, 1% (v/v) streptomycin, and 10% (v/v) fetal bovine serum at 37 °C in 5% CO_2.

***In Vitro* Cytotoxicity Evaluation.**

The standard MTT assay was used to evaluate cell viabilities. K150 or MCF-7 cells were seeded into the 96-well plate at a density of 1.0×10^5 cells/well and incubated overnight and then cultured with different conditions.

To study the CDT therapy effect of cube, octahedron and sphere $Cu_{2-x}Se$, K150 cells were then cultured with $Cu_{2-x}Se$ at different concentrations (0, 5, 10, 20, 30, 40, 50 μg/mL) and H_2O_2 (50 μM) incubated for another 12 h. To study the PTT/CDT synergetic therapy effect *in vitro*, K150 cells were then treated with $Cu_{2-x}Se$ at different concentrations (0, 5, 10, 20, 30, 40, 50 μg/mL) and H_2O_2 (50 μM) for 2 h, followed by a 1064 nm laser irradiation (5 min, 1.0 W/cm^2) and incubated for another 12 h.

MCF-7 cells were incubated to assess the therapy effect against tumor cells *in vitro* by $Cu_{2-x}Se$@CQ@NPY. MCF-7 cells were seeded into 96-well plates overnight, and incubated with $Cu_{2-x}Se$@CQ@NPY with different concentrations (0, 5, 10, 20, 30, 40, 50 μg/mL) for 24 h. Besides, $Cu_{2-x}Se$@CQ@NPY with different concentrations (0, 5, 10, 20, 30, 40, 50 μg/mL) and respectively treated with H_2O_2 or NIR or H_2O_2+NIR were incubated for 24 h.

Cellular Uptake.

MCF-7 cells were seeded in cell dishes at a density of 1.0×10^5 cells/dish and cultured overnight. Then the cells were incubated with fresh DMEM containing Rho B labelled $Cu_{2-x}Se$@CQ@PEG or $Cu_{2-x}Se$@CQ@NPY and further cultured for 4 h. After that, cells were fixed by using 4% formaldehyde for 15 min, followed by staining the cells nuclei by Hoechst 33342 for 10 min and imaged by laser scanning confocal microscope (LSCM).

Intracellular ROS Detection.

The DCFH-DA was used as an indicator to detect the generation of active oxygen species. MCF-7 cells were seeded in 35 mm confocal dishes at a density of 1.0×10^5 cells/dish and cultured overnight. The dishes were divided into three groups: (i) Control; (ii) $Cu_{2-x}Se$; (iii) $Cu_{2-x}Se$@CQ@NPY. The groups were incubated with DMEM culture medium containing $Cu_{2-x}Se$ or $Cu_{2-x}Se$@CQ@NPY (50 μg/mL) for 12 h, the cells were incubated with DCFH-DA (500μL, 10 μM) for 20 min. After that, the cells were washed with PBS twice carefully to remove excess probe, then imaged with LSCM.

Autophagy Staining Assay.

The monodansylcadaverine (MDC) was used as a fluorescence probe to detect the generation of autophagosome. MCF-7 cells were seeded in 35 mm confocal dishes at a density of 1.0×10^5 cells/dish and cultured overnight. The dishes were divided into three groups: (i) Control; (ii) CQ; (iii) $Cu_{2-x}Se$@CQ@NPY. The groups were incubated with DMEM culture medium containing CQ (10 μg/mL) or $Cu_{2-x}Se$@CQ@NPY (50 μg/mL) for 8 h, then the cells were incubated with MDC for 20 min. After that, the cells were washed with assay buffer twice carefully to remove excess probe, then imaged with LSCM.

Live/Dead Cell Staining Assay.

MCF-7 cells were seeded in confocal dishes at a density of 1.0×10^5 cells/dish and cultured overnight. The dishes were divided into five groups: (i) Control; (ii) Control + 1064 nm; (iii) $Cu_{2-x}Se$@CQ@NPY + 50 μM H_2O_2; (iv) $Cu_{2-x}Se$@CQ@NPY + 1064 nm; (v) $Cu_{2-x}Se$@CQ@NPY + 50 μM H_2O_2 + 1064 nm. The group (iii) ~ (v) were incubated with DMEM culture medium containing $Cu_{2-x}Se$@CQ@NPY (50 μg/mL) for 6 h, and then treated with 50 μM H_2O_2 and/or a 1064 nm laser (1.0 W/cm^2, 10 min). After additional 12 h incubation, the cells were incubated with Calcein-AM (Ex = 488, Em = 520 ± 20 nm) for 20 min and PI (Ex = 525, Em = 620 ± 20 nm) for 10 min, then imaged with LSCM.

Tumor Model.

Female Balb/C nude mice aged 5 weeks were bought from Changzhou Cavens Model Animal Co., Ltd. (Changzhou, China). Then, MCF-7 cells (1.2×10^7 cells in 100 μL PBS) were subcutaneously implanted into the right hind leg of the mice to establish the xenograft tumors. When the tumor reached the appropriate volume, the *in vivo* experiments were conducted. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Ningbo University and approved by the Animal Ethics Committee of Ningbo University (Ningbo, China, Permit NO. SYXK (Zhe) 2019-0005).

***In Vivo* PTT/CDT Therapy.**

When the tumor volume got around 100 mm^3, the mice were randomly divided into four groups (n = 5): (i) PBS only, (ii) PBS+laser, (iii) $Cu_{2-X}Se$@CQ@NPY, and (iv) $Cu_{2-X}Se$@CQ@NPY+laser. Mice in the group iii and iv were intravenously injected with $Cu_{2-x}Se$@CQ@NPY at a dose of 5 mg/kg. After that, NIR laser was carried out at the group ii and iv after 24 h of injection (1.0 W/cm^2, 5 min). The tumor temperature changes and the corresponding IR thermal images were monitored by IR thermal camera (Fortric 225). The body weight and relative tumor volume were recorded every two days. The tumor volume (V) = (tumor length) × $(\text{tumor width})^2 / 2$. After 14 days, the tumor tissues and the major organs including heart, liver, spleen, lung and kidney of mice from different groups were collected to conduct the hematoxylin and eosin (H&E) staining.

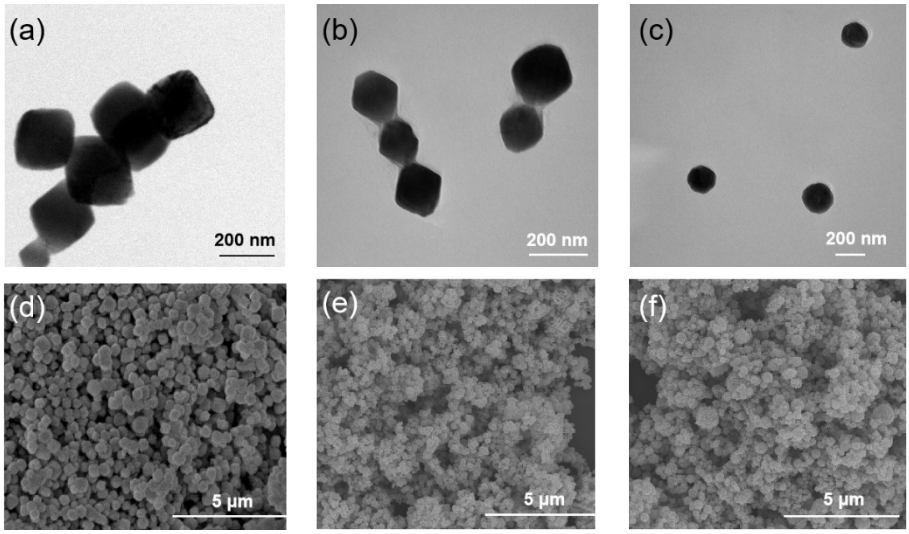

Figure S1. TEM images of (a) cube, (b) octahedron and (c) sphere Cu_2O nanoparticles. SEM images of (d) cube, (e) octahedron and (f) sphere Cu_2O nanoparticles.

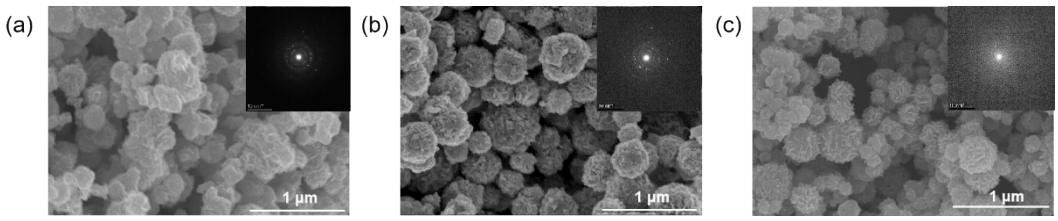

Figure S2. SEM images of (a) cube, (b) octahedron and (c) sphere $Cu_{2-x}Se$ nanoparticles. Insets are SAED patterns of $Cu_{2-x}Se$ nanoparticles with three morphologies.

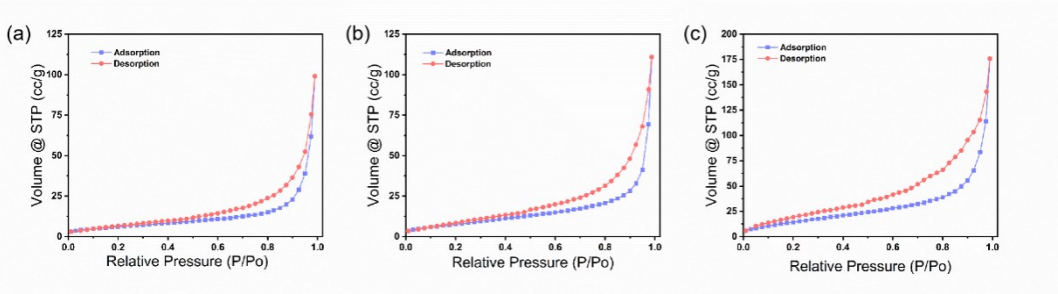

Figure S3. N_2 adsorption-desorption isotherms of (a) cube, (b) octahedron and (c) sphere $Cu_{2-x}Se$ nanoparticles.

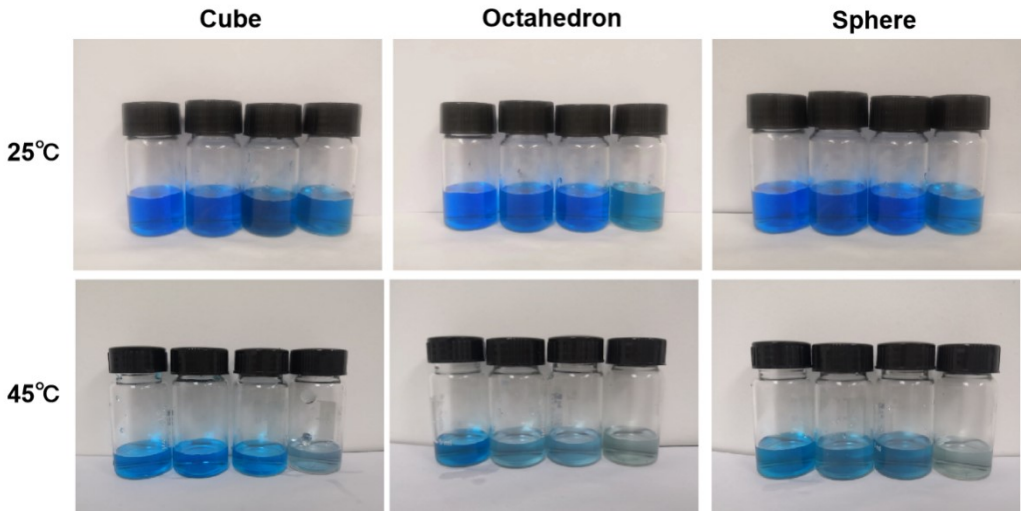

Figure S4. Comparison of the decrease absorption of MB induced by $Cu_{2-x}Se$ nanoparticles with three morphologies at 25 ℃ and 45 ℃. For each picture from left to right is (i) MB; (ii) MB+H_2O_2; (iii) MB+$Cu_{2-x}Se$ (iv) MB+H_2O_2+$Cu_{2-x}Se$.

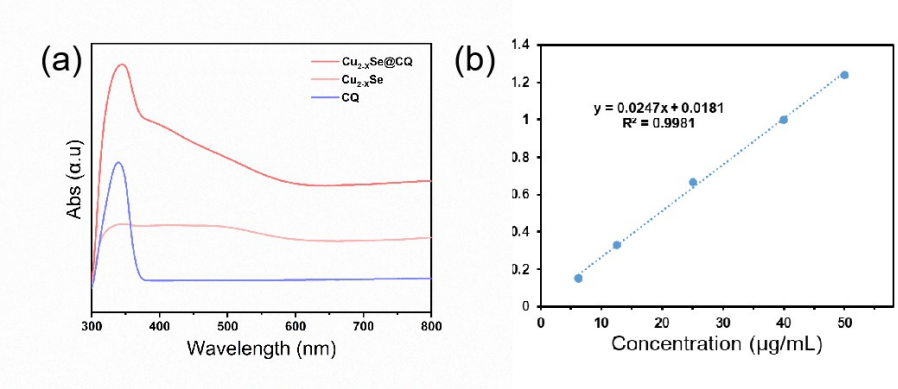

Figure S5. (a) UV-vis spectra of CQ, $Cu_{2-x}Se$, and $Cu_{2-x}Se$@CQ in DSMO. (b) Standard curve of CQ in DMSO.

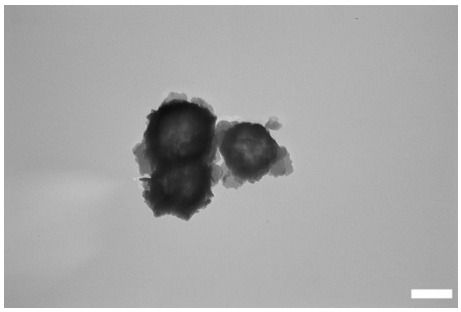

Figure S6. TEM images of $Cu_{2-x}Se$@CQ@NPY. The scar bar is 200 nm.

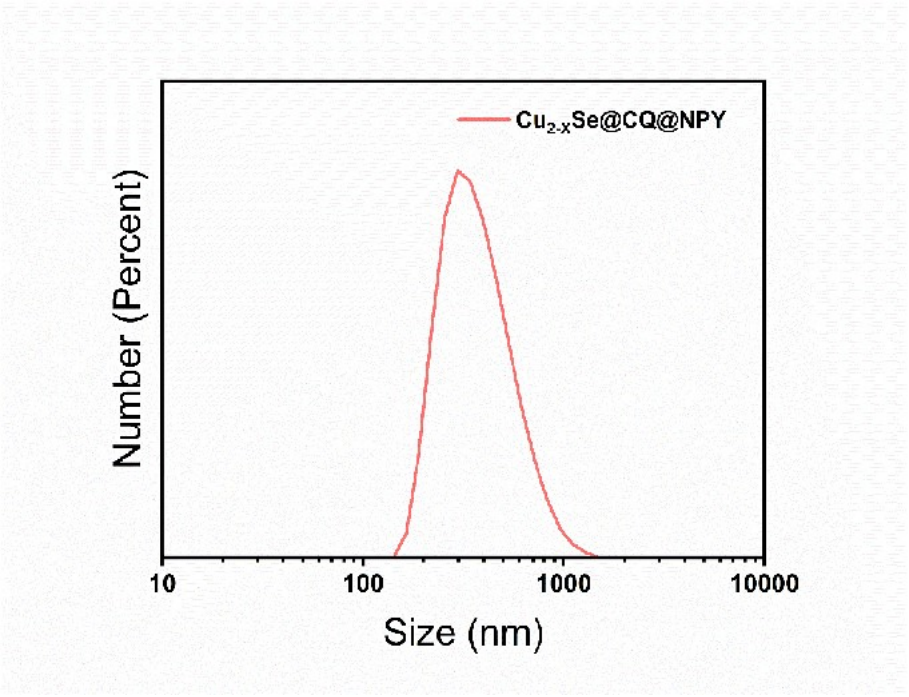

Figure S7. DLS size profile of $Cu_{2-x}Se$@CQ@NPY.

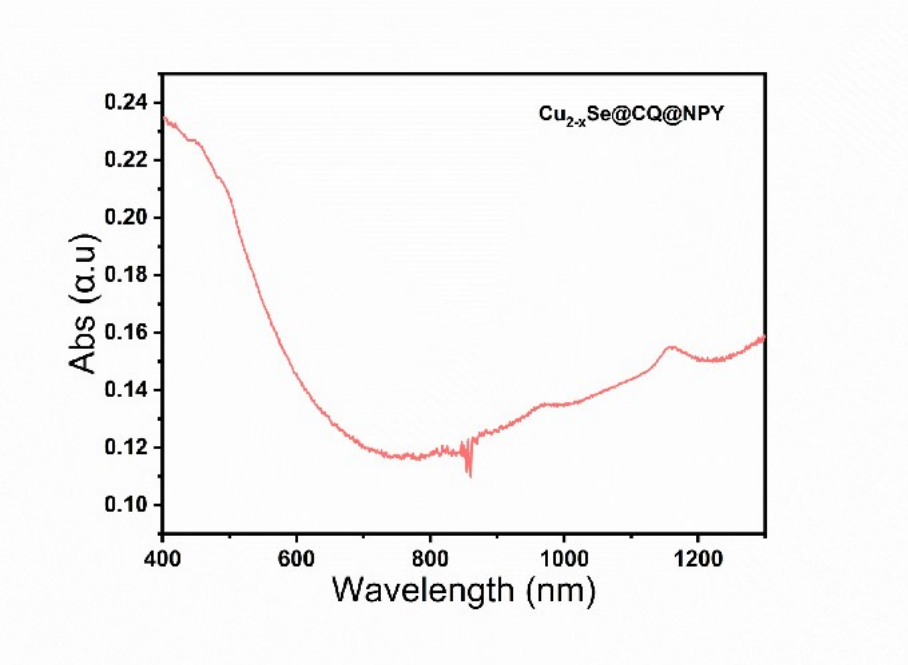

Figure S8. Vis-NIR absorption spectra of $Cu_{2-x}Se$@CQ@NPY.

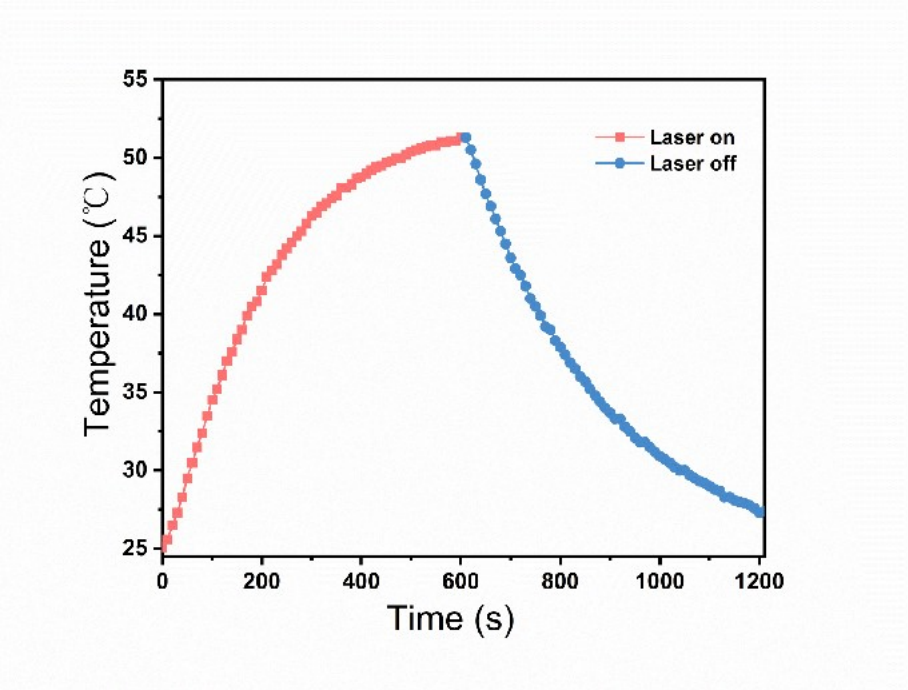

Figure S9. Temperature variation curves of $Cu_{2-x}Se$@CQ@NPY aqueous solution (40 μg/mL) during one heating-cooling cycle (1064 nm laser irradiation, 1.0 W/cm^2).

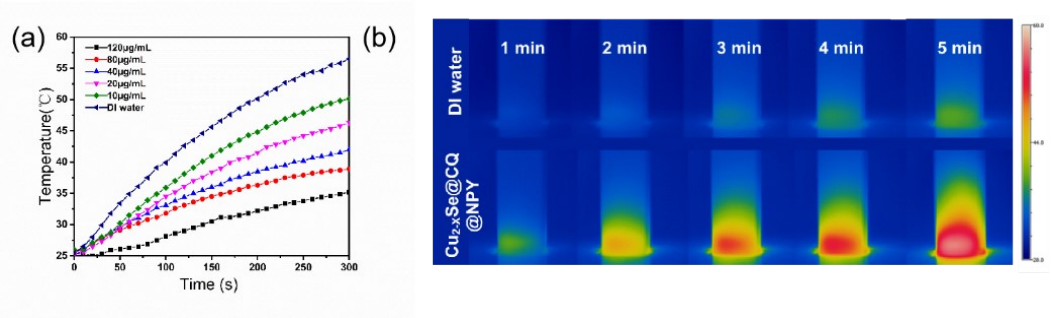

Figure S10. (a) Temperature variations of different concentration of $Cu_{2-x}Se$@CQ@NPY aqueous solutions and (b) Thermal images of $Cu_{2-x}Se$@CQ@NPY aqueous solutions (40 μg/mL) under 1064 nm laser irradiation (1.0 W/cm^2).

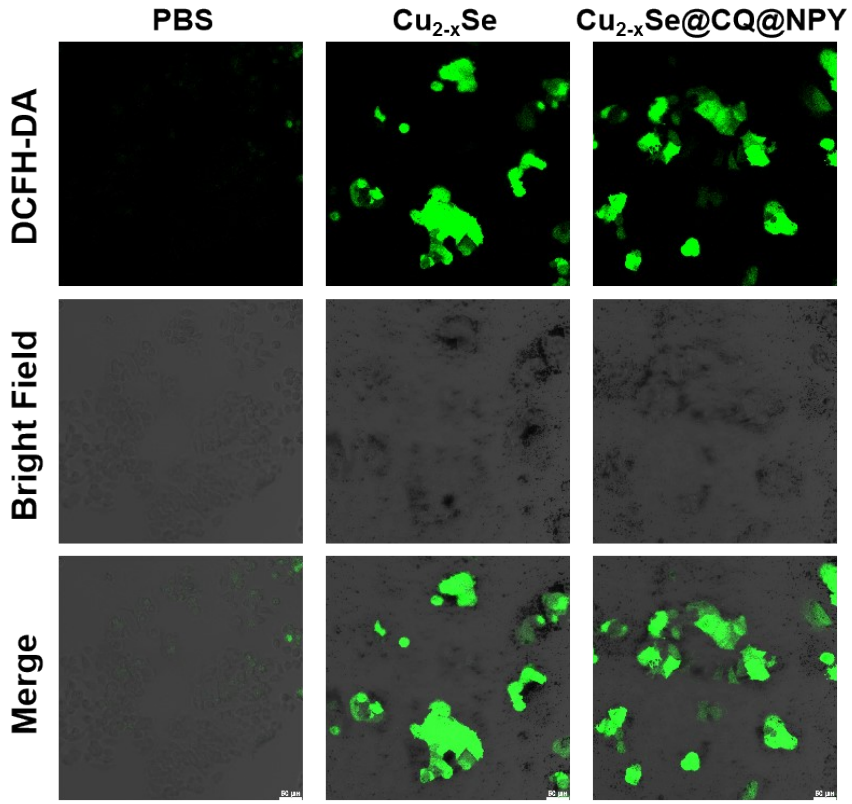

Figure S11. Detection of ROS production using DCFH-DA in different conditions by confocal laser scanning microscopy. The scale bar is 50 μm.

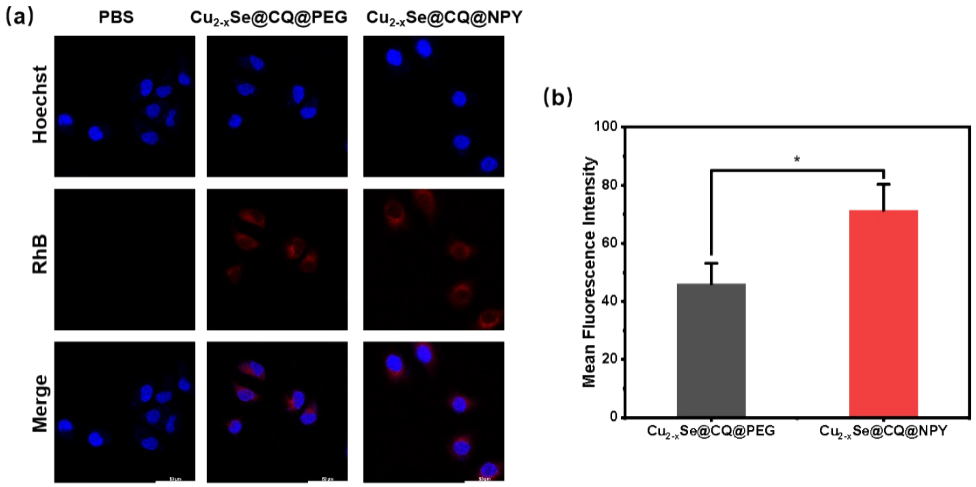

Figure S12. (a) Confocal laser scanning microscopy images of MCF-7 cells incubated with RhB labelled $Cu_{2-x}Se$@CQ@PEG or $Cu_{2-x}Se$@CQ@NPY (50 μg/mL) for 4 h; (b) The mean fluorescence intensity (MFI) analyzed by ImageJ software was presented as the mean ± SD (n = 5). *$p < 0.05$. The scale bar is 50 μm.

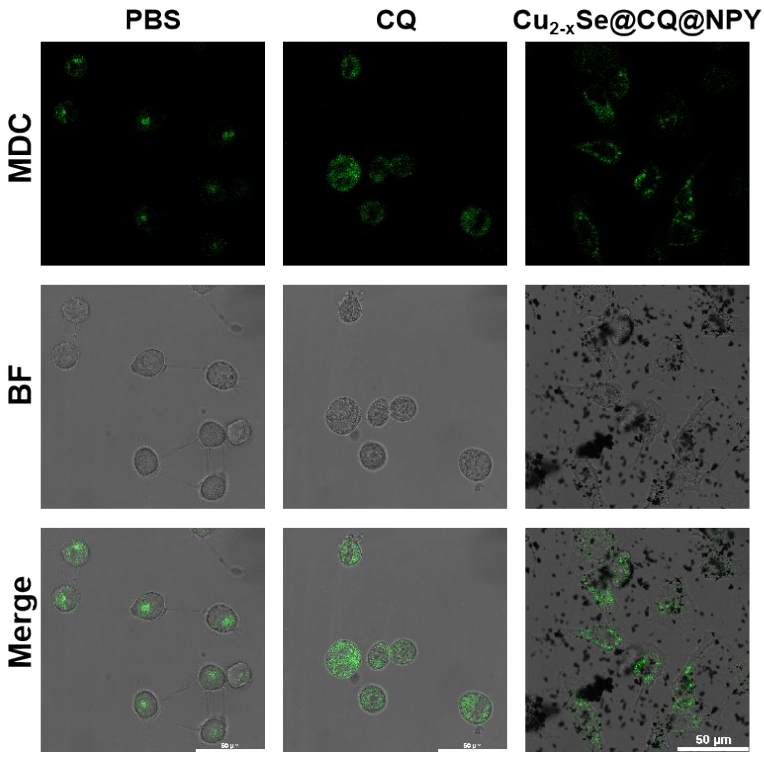

Figure S13. CLSM images of MCF-7 cells incubated with CQ or $Cu_{2-x}Se$@CQ@NPY (50 μg/mL) for 8 h and then stained with monodansylcadaverine (MDC). The scale bar is 50 μm.

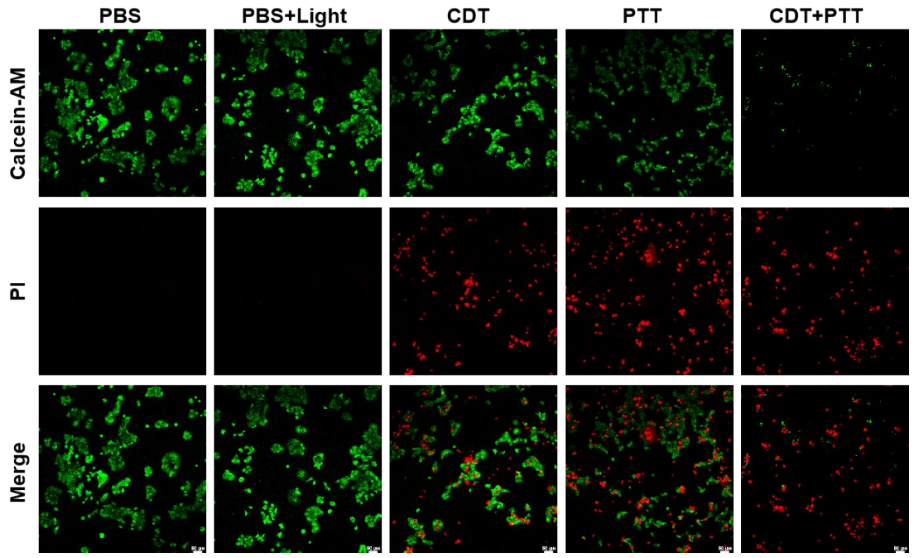

Figure S14. Confocal laser scanning microscopy images of MCF-7 cells stained with calcein acetoxymethyl ester (Calcein-AM) and propidium iodide (PI) after various treatments. The scale bar is 50 μm.

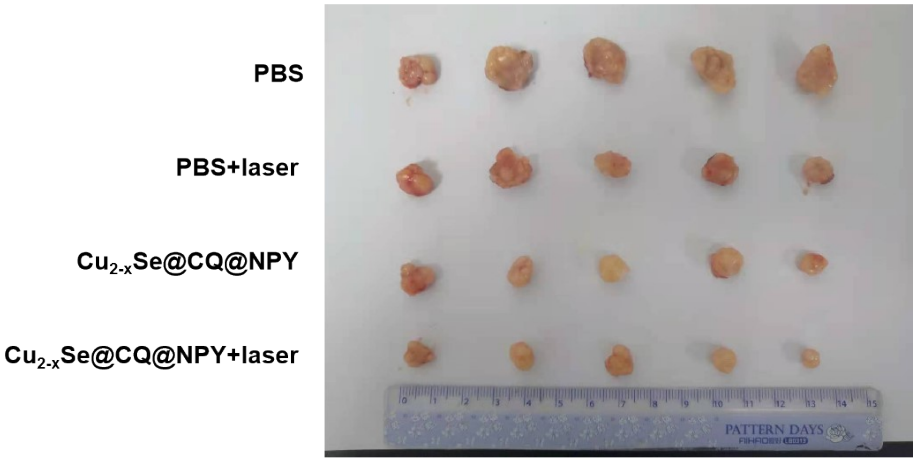

Figure S15. The photographs of the excised tumors from mice.

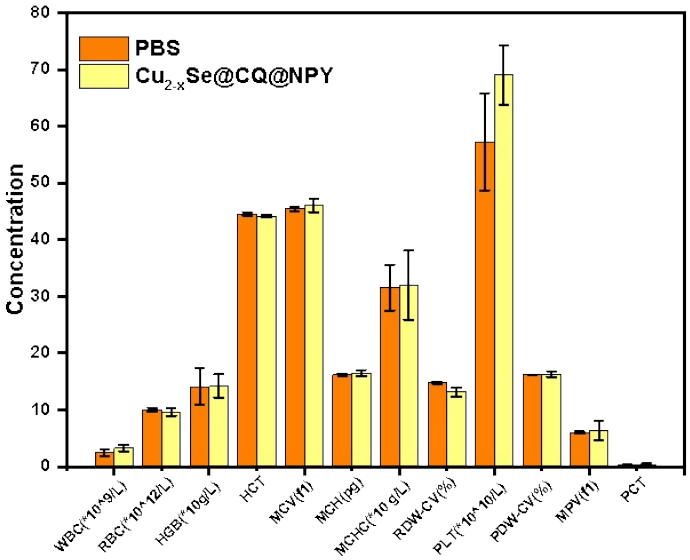

Figure S16. Blood routine indicators including white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin to determine blood drug concentration (MCHC), red blood cell distribution width (RDW), platelets (PLT), platelet distribution width (PDW), mean platelet volume (MPV) and packed cell volume (PCT) treated with or without $Cu_{2-x}Se$@CQ@NPY nanoparticles.

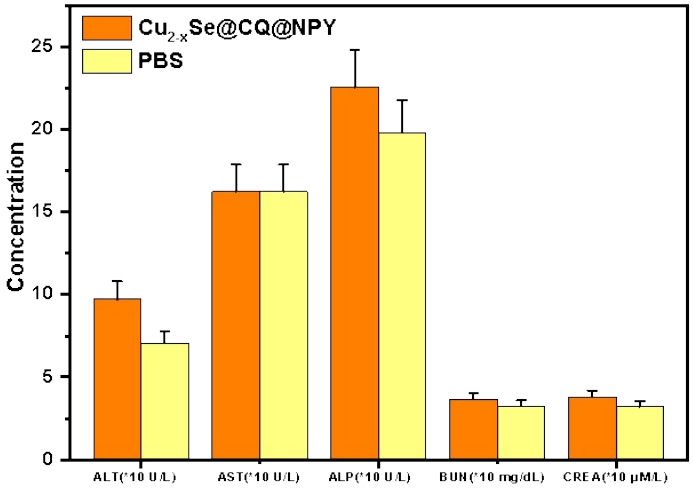

Figure S17. Liver function indicators including alanine aminotransferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), and renal function markers including blood urea nitrogen (BUN) and creatinine (CREA) treated with or without $Cu_{2-x}Se$@CQ@NPY nanoparticles.