Electronic Supplementary Information (ESI)

Simple Construction of $Cu_{2-x}S$:Pt Nanoparticles as Nanotheranostic Agent for Imaging Guided Chemo-Photothermal Synergistic Therapy of Cancer

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1. Experimental Section

1.1 Materials and Characterization

Copper (II) acetylacetonate (98%), oleylamine (C18 content 80−90%), polyvinylpyrrolidone (PVP Mw ≈ 58000), and H_2PtCl_6•$6H_2O$ were purchased from Aladdin Industrial Corporation (Shanghai, China). Sulfur powder (S, 99.5%), chloroform (99.0%), ethanol (99.7%), dichloromethane (99.0%), ether (99.0%), and cyclohexane (99.0%) were purchased from Beijing Chemical Regents. All the reagents were analytical grade and used directly without further purification. Powder X-ray diffraction (XRD) patterns were obtained on a D8 ADVANCE X-ray diffractometer with Cu Kα radiation (λ = 1.5418 Å) with an operation voltage and current maintained at 40 kV and 40 mA. Low-/high-resolution transmission electron microscopy (TEM) images were obtained with a FEI TECNAI G2 high-resolution transmission electron microscope operating with a field-emission gun operating at 200 kV. X-ray photoelectron spectroscopy (XPS) was obtained on a VG ESCALAB MKII spectrometer. Inductively couple plasma mass spectrometry (ICP-MS) was carried out on an ELAN 9000/DRC. Inductively couple plasma-optical emission spectrometry (ICP-OES) was taken on a PerkinElmer ICP instrument. Fourier transform infrared (FT-IR) spectroscopy was recorded on a PerkinElmer 580B IR spectrophotometer using the KBr pellet technique. The absorption spectra were recorded on a Shimadzu UV-3600 spectrophotometer.

1.2 Synthesis of $Cu_{2-x}S$ NPs

Briefly, 2 mmol of sulfur was dissolved in 12 mL of oleylamine, and stirred at 70 °C for 0.5 h in an oil bath. Subsequently, this solution was added dropwise into the mixture of chloroform solution (20 mL), oleylamine (5 mL) and $Cu(acac)_2$ (2 mmol). After stirring for another 0.5 h, the $Cu_{2-x}S$ NPs were isolated by centrifugation, washed two times with ethanol and chloroform, and re-dispersed in cyclohexane (40 mL) for later use.

1.3 Synthesis of $Cu_{2-x}S$:Pt(y) NPs

1 mmol of sulfur was dissolved in 3 mL of oleylamine, and rapidly injected into a cyclohexane solution of the as-synthesized $Cu_{2-x}S$ NPs (10 mL) at 70 °C and stirred

for 10 min in an oil bath. Then, the mixture of chloroform (4 mL), oleylamine (1 mL), and $H_2PtCl_6 \cdot 6H_2O$ (0.1, 0.2, 0.3, or 0.4 mmol) was added dropwise into the above solution and stirred at 70 °C for 0.5 h. The $Cu_{2-x}S$:Pt(y) NPs were obtained by centrifugation, washed with ethanol and cyclohexane, and re-dispersed in cyclohexane (20 mL) for later use.

1.4 Surface modification of $Cu_{2-x}S$:Pt(y) NPs

20 mL of $Cu_{2-x}S$:Pt(y) NPs cyclohexane solution was first dispersed in 80 mL of dichloromethane. Then, 1 g of PVP was added and the mixture was stirred at room temperature for 10 min. $Cu_{2-x}S$:Pt(y)/PVP NPs were precipitated by adding ether and re-dispersed in PBS solution. The resulting solution was centrifugation at high speed to remove large aggregates. Then, the obtained solution was transferred into dialysis bag and dialyzed against PBS solution for 72 h. Finally, the $Cu_{2-x}S$:Pt(y)/PVP NPs were dispersed in PBS solution and kept at 4 °C for later use.

1.5 Photothermal experiments

The temperature rise of $Cu_{2-x}S$:Pt(y)/PVP NPs PBS dispersions (1 mL) at various Cu concentrations (0, 12.5, 25, 50, 100, 200, and 400 ppm) upon NIR irradiation (808 nm, 2 W/cm^2) was measured every 2 seconds by a thermocouple microprobe.

1.6 *In vitro* cytotoxicity assessment

In vitro cytotoxicity of $Cu_{2-x}S$:Pt(0.3)/PVP NPs and $Cu_{2-x}S$/PVP NPs was evaluated by MTT assay of 4T1 murine breast tumor cells. 4T1 cells were provided by the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. 4T1 cells were seeded into 96-well cell culture plates (10^4 per well) at 37 °C and 5% CO_2 for 24 h. After 24 h of incubation, the DMEM was taken out from the wells, and the cells were washed for three times with PBS solution. $Cu_{2-x}S$:Pt(0.3)/PVP NPs and $Cu_{2-x}S$/PVP NPs solutions with various Cu concentrations (0, 1.6, 3.2, 6.3, 12.5, 25, 50, 100, 200 and 400 ppm) were added into the medium, respectively. Then, the cells were incubated at 37 °C and 5% CO_2 for 24 h. Thereafter, MTT (10 μL, 5 mg mL^{-1}) solutions was added into each well and the plate was incubated for 4 h at 37 °C and 5% CO_2 in order to form a purple formazan dye, which was dissolved in DMSO for 15 min after removing the medium. Finally, the enzyme-linked immunosorbent assay

reader was used to measure the optical absorbance of the colored solution at 570 nm. Each experiment was repeated for three times.

1.7 *In vitro* cellular uptake assay

4T1 murine breast tumor cells were seeded in 1 mL of medium in each well (3.5 cm in diameter) of a 6-well plate at the density of 10^5 cells per well. After 24 h of incubation at 37 °C, the medium was replaced with fresh medium containing $Cu_{2-x}S$:Pt(0.3)/PVP NPs or $Cu_{2-x}S$/PVP NPs with Cu concentration of 100, 200 and 400 ppm. After another 24 h of incubation at 37 °C, the medium was removed; the cells were washed three times with PBS (pH = 7.4) and trypsinized. Then, the cell suspensions were digested using 1 mL of concentrated $HNO_3$10% (*v/v*) and the intracellular Pt and Cu concentration were determined by ICP-MS.

1.8 *In vitro* photothermal ablation of cancer cells

4T1 murine breast tumor cells were seeded into 96-well cell culture plates (10^4 per well) at 37 °C and 5% CO_2 for 24 h. Then, the cells were treated with PBS solution containing $Cu_{2-x}S$:Pt(0.3)/PVP NPs or $Cu_{2-x}S$/PVP NPs with various Cu concentrations (0, 1.6,3.2, 6.3, 12.5, 25, 50, 100, 200, and 400 ppm). Then, the cells were irradiated by an 808-nm laser with a power density of 2 W cm^{-2} for 10 min. The cell viabilities were measured by MTT assays.

1.9 Live/Dead staining kit

4T1 murine breast tumor cells (4×10^4 cells per well) were placed in a 24-well plate. After incubation overnight, culture medium was removed and cells were washed three times with PBS. Then, 4T1 cells were treated with PBS, $Cu_{2-x}S$/PVP NPs PBS solution (25 ppm), $Cu_{2-x}S$:Pt(0.3)/PVP NPs PBS solutions with various Cu concentrations (6.3, 12.5, and 25 ppm) for 6 h, respectively, and then irradiated under 808 nm laser (2 W cm^{-2}) for 10 min. After incubation for 18 h, 4T1 murine breast tumor cells were washed with PBS and stained with calcein AM (2 μM) and propidium iodide (PI, 4 μM) for 2 h and observed using NikonTi-S fluorescence microscope (Tokyo, Japan), live cells showed green color and dead ones exhibited red color.

1.10 Animal Experiments

Female Kunming mice were obtained from the Laboratory Animal Center of Jilin University (China). All the mouse experiments were performed in compliance with the National Regulation of China the Care and Use of Laboratory Animals and all mice were handled under the protocol approved by the Institutional Animal Care and Use Committee of Jilin University. The tumor models were established by subcutaneous injection of H22 cells in the left axilla of each Kunming mouse. In addition, H22 cells were provided by the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences.

1.11 *In vitro and in vivo* X-ray CT imaging

PBS solutions of $Cu_{2-x}S$:Pt(0.3)/PVP NPs with different Cu concentrations (0, 0.38, 0.75, 1.5, and 3 mg/mL) were prepared for CT imaging. We performed X-ray CT imaging on 4T1 tumor-bearing mice by intravenous injection of $Cu_{2-x}S$:Pt (0.3)/PVP NPs (200 μL, 8.5 mg Cu/kg, n = 3) at indicated time points. CT imaging was performed on a Philips iCT 256 slice scanner operated at 120 kV and 300 mA, with a slice thickness of 0.9 mm.

1.12 *In vivo* photothermal effect

The tumor-bearing mice were injected intravenously with PBS solution containing $Cu_{2-x}S$:Pt(0.3)/PVP NPs at the same dose (200 μL, 8.5 mg Cu/kg). Then, the tumor was exposed to an 808 nm laser (2 W cm^{-2}) for 10 min and imaged by an IR thermal imaging camera at different time intervals (pre, 1 h, 2 h, and 1 d).

1.13 Biodistribution of $Cu_{2-x}S$:Pt(0.3)/PVP NPs

4T1 tumor-bearing mice were sacrificed at 1 h, 2 h, 1 d, 3 d, and 5 d, respectively, after intravenous injection of $Cu_{2-x}S$:Pt (0.3)/PVP NPs (200 μL, 8.5 mg Cu/kg, n = 3). After digesting the major organs (heart, liver, spleen, lung, and kidney), tumor, and feces with aqua regia solution for 6 h, the Cu and Pt amount per unit mass was quantified by ICP-MS.

1.14 *In vivo* chemo-photothermal synergistic therapy and histology examinations

The tumor-bearing mice (the tumor volume was about 200 mm^3) were randomly allocated into five groups (three mice per group): the tumor-bearing mice in [$Cu_{2-x}S$:Pt(0.3)/PVP NPs+NIR 1d] treatment group were injected intravenously with Cu_{2-}

$_x$S:Pt(0.3)/PVP NPs PBS solution (200 μL, 8.5 mg Cu/kg) and irradiated with 808 nm laser (2 W cm^{-2}, 10 min) after 24 h postinjection; the mice in [Cu_{2-x}S:Pt(0.3)/PVP NPs+NIR 1h] group were injected intravenously with Cu_{2-x}S:Pt(0.3)/PVP NPs PBS solution (200 μL, 8.5 mg Cu/kg) and then exposed to an 808 nm laser at 2 W cm^{-2} for 10 min after 1 h postinjection; the Cu_{2-x}S:Pt(0.3)/PVP group [only treated with Cu_{2-x}S:Pt(0.3)/PVP NPs], PBS group (only treated with PBS), and NIR group (only treated with 808 nm laser) were set as the control groups. The length and width of the tumors were measured by caliper every 2 days after treatments. The tumor volumes were calculated according to the formula $V = ab^2/2$ (a and b represent length and width of the tumor, respectively). Relative tumor volumes were calculated as V/V_0, where V_0 was the tumor volume when the treatment was initiated. We also collected the body weight data of the mice on a daily basis for 16 d. Then, the mice were killed 16 days after the treatments. The tumors were obtained from the above five groups. The major organs (heart, liver, spleen, lung, and kidney) taken out from [Cu_{2-x}S:Pt(0.3)/PVP NPs+NIR 1d], [Cu_{2-x}S:Pt(0.3)/PVP NPs+NIR 1h], and PBS group were stained with H&E for histological analysis.

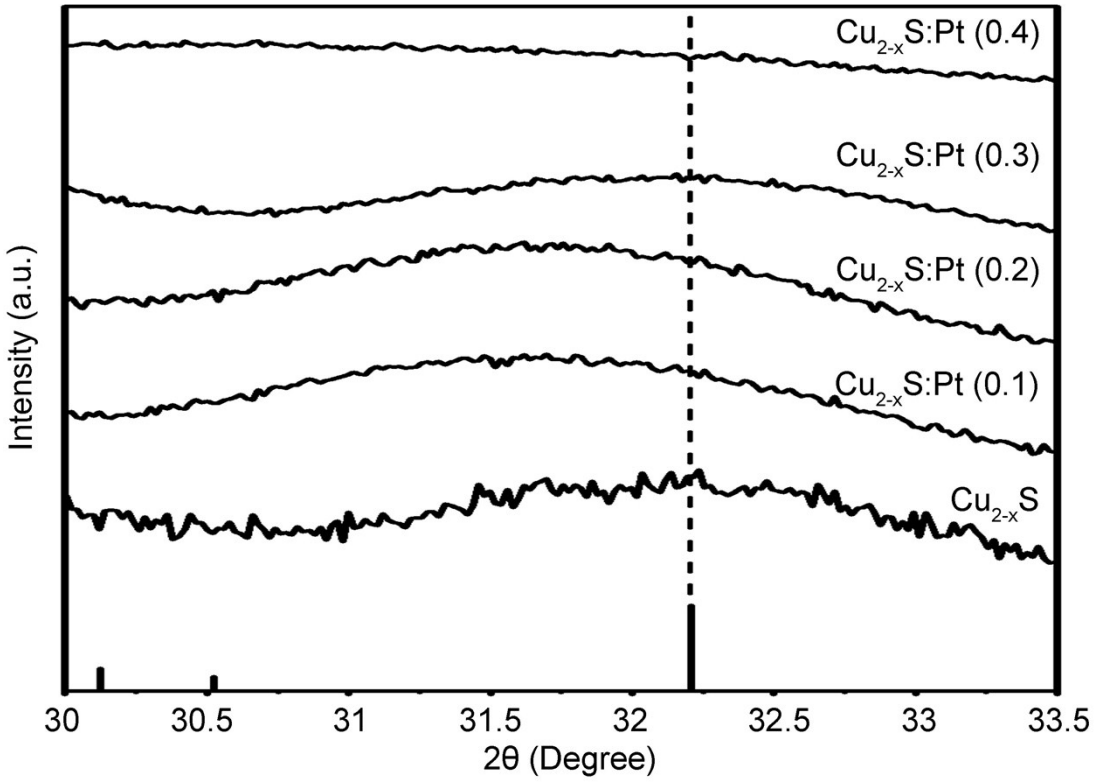

Fig. S1 The enlarged XRD pattern of $Cu_{2-x}S$:Pt(y) NPs (y = 0.1, 0.2, 0.3, and 0.4 mmol) and $Cu_{2-x}S$ NPs.

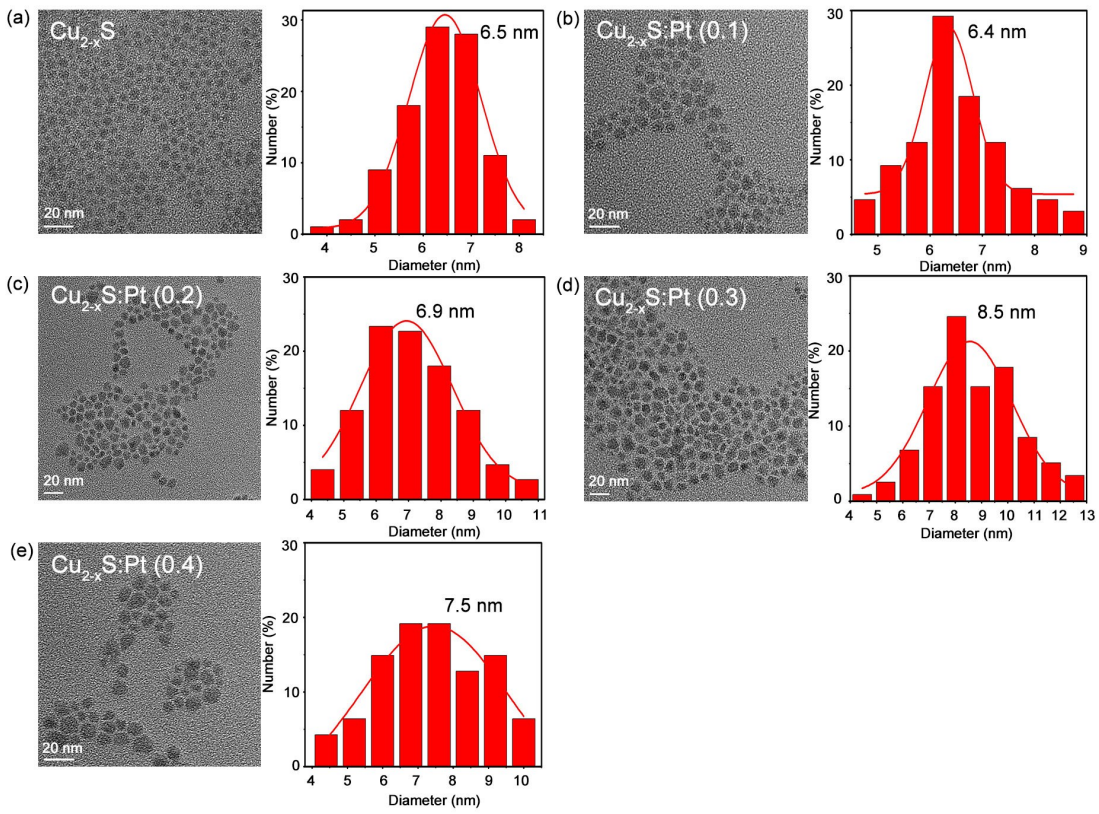

Fig. S2 TEM images and corresponding size distribution histograms of $Cu_{2-x}S$:Pt(y) NPs (y = 0.1, 0.2, 0.3, and 0.4 mmol).

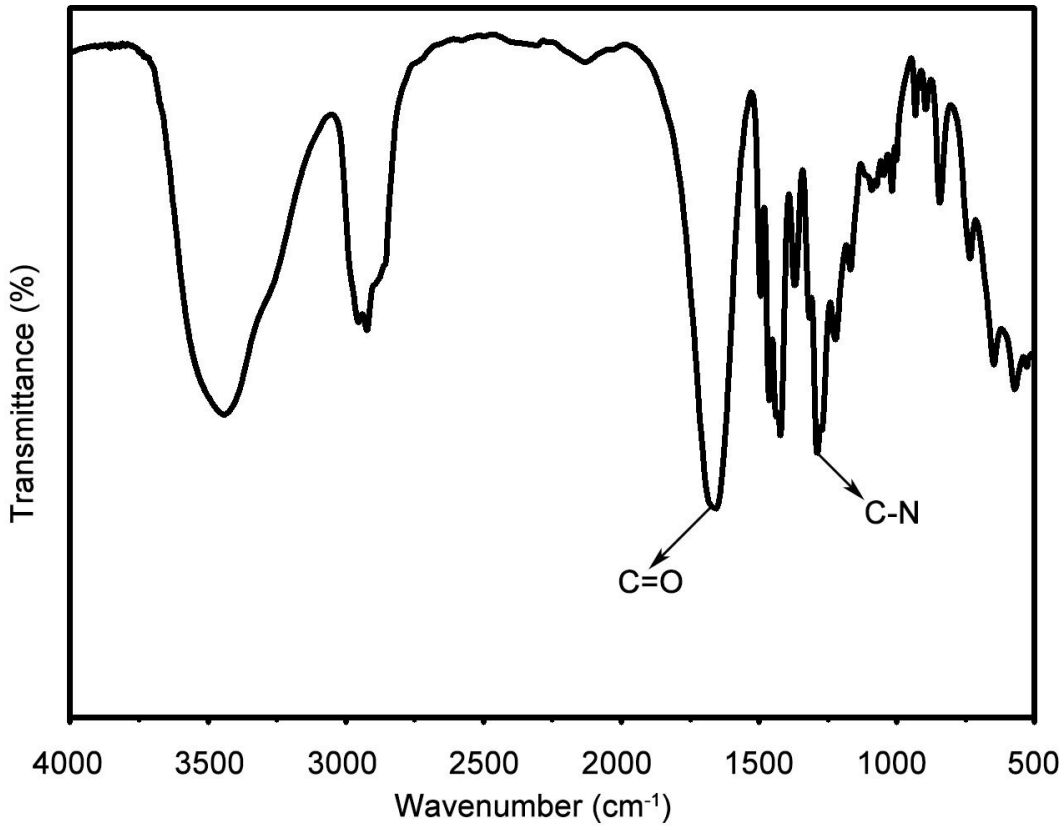

Fig. S3 FTIR spectroscopy of $Cu_{2-x}S$:Pt(0.3) /PVP NPs.

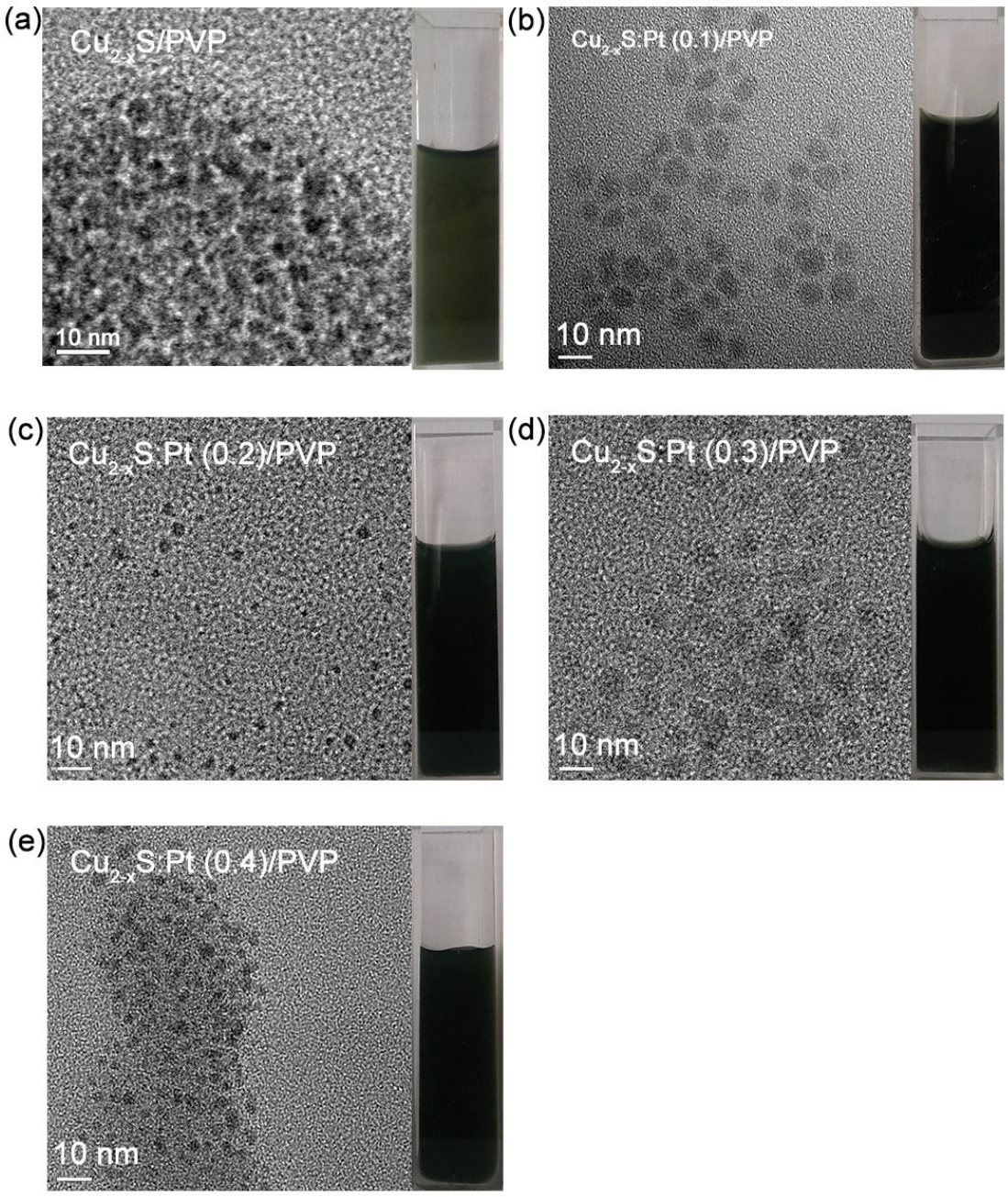

Fig. S4 TEM images and digital photographs of $Cu_{2-x}S$/PVP NPs and $Cu_{2-x}S$:Pt (y)/PVP NPs (y = 0.1, 0.2, 0.3, 0.4) dispersed in PBS with Cu concentration of 200 ppm.

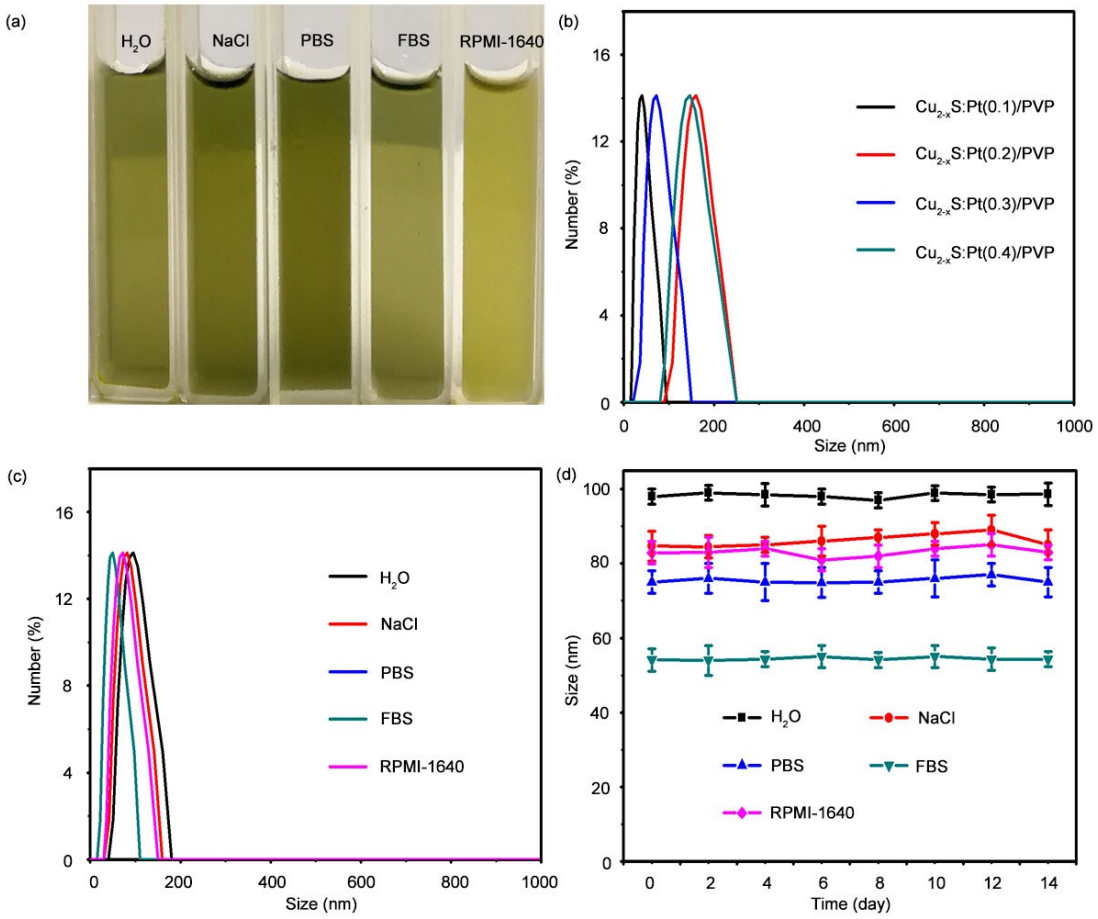

Fig. S5 (a) The photographs of $Cu_{2-x}S$:Pt(0.3)/PVP NPs (Cu concentration: 25 ppm) dispersed in water, 0.9% NaCl, PBS, FBS, and RPMI-1640; (b) Hydrodynamic size of the $Cu_{2-x}S$:Pt(y)/PVP NPs (y = 0.1, 0.2, 0.3, 0.4) dispersed in PBS; (c) Hydrodynamic size of $Cu_{2-x}S$:Pt(0.3)/PVP NPs dispersed in water, 0.9% NaCl, PBS, FBS, and RPMI-1640; (d) Hydrodynamic size changes of $Cu_{2-x}S$:Pt(0.3)/PVP NPs dispersed in water, 0.9% NaCl, PBS, FBS, and RPMI-1640 for 14 days.

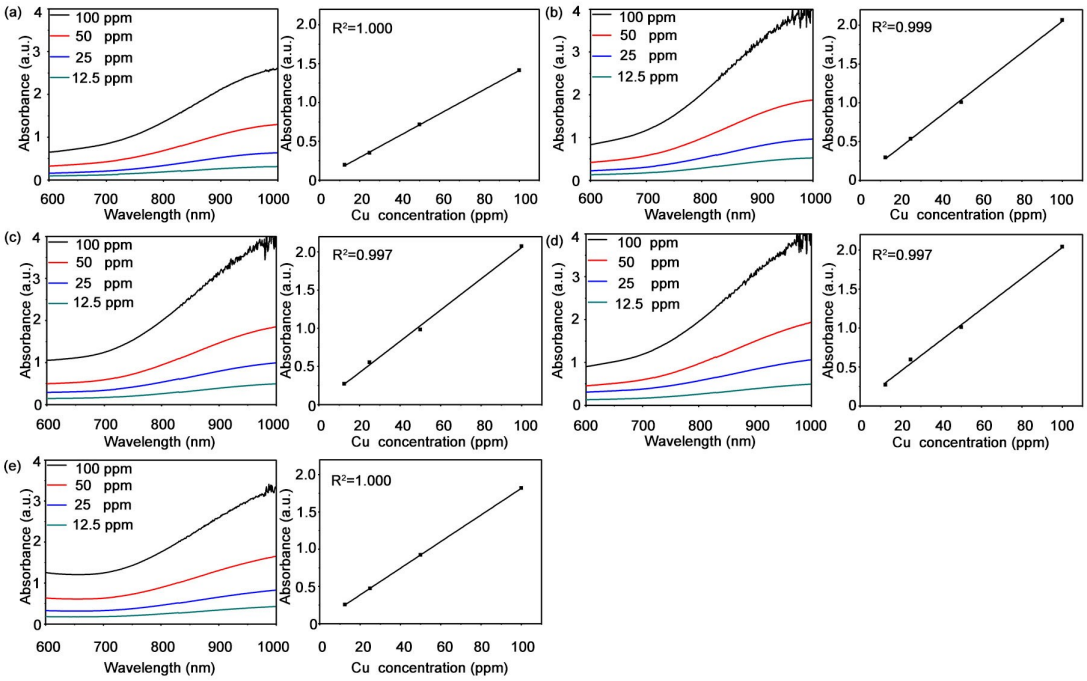

Fig. S6 The absorption spectra and the corresponding linear fitting plots of absorbance versus Cu concentration at 808 nm of $Cu_{2-x}S$/PVP NPs (a), $Cu_{2-x}S$:Pt(0.1)/PVP NPs (b), $Cu_{2-x}S$:Pt(0.2)/PVP NPs (c), $Cu_{2-x}S$:Pt(0.3)/PVP NPs (d), and $Cu_{2-x}S$:Pt(0.4)/PVP NPs (e).

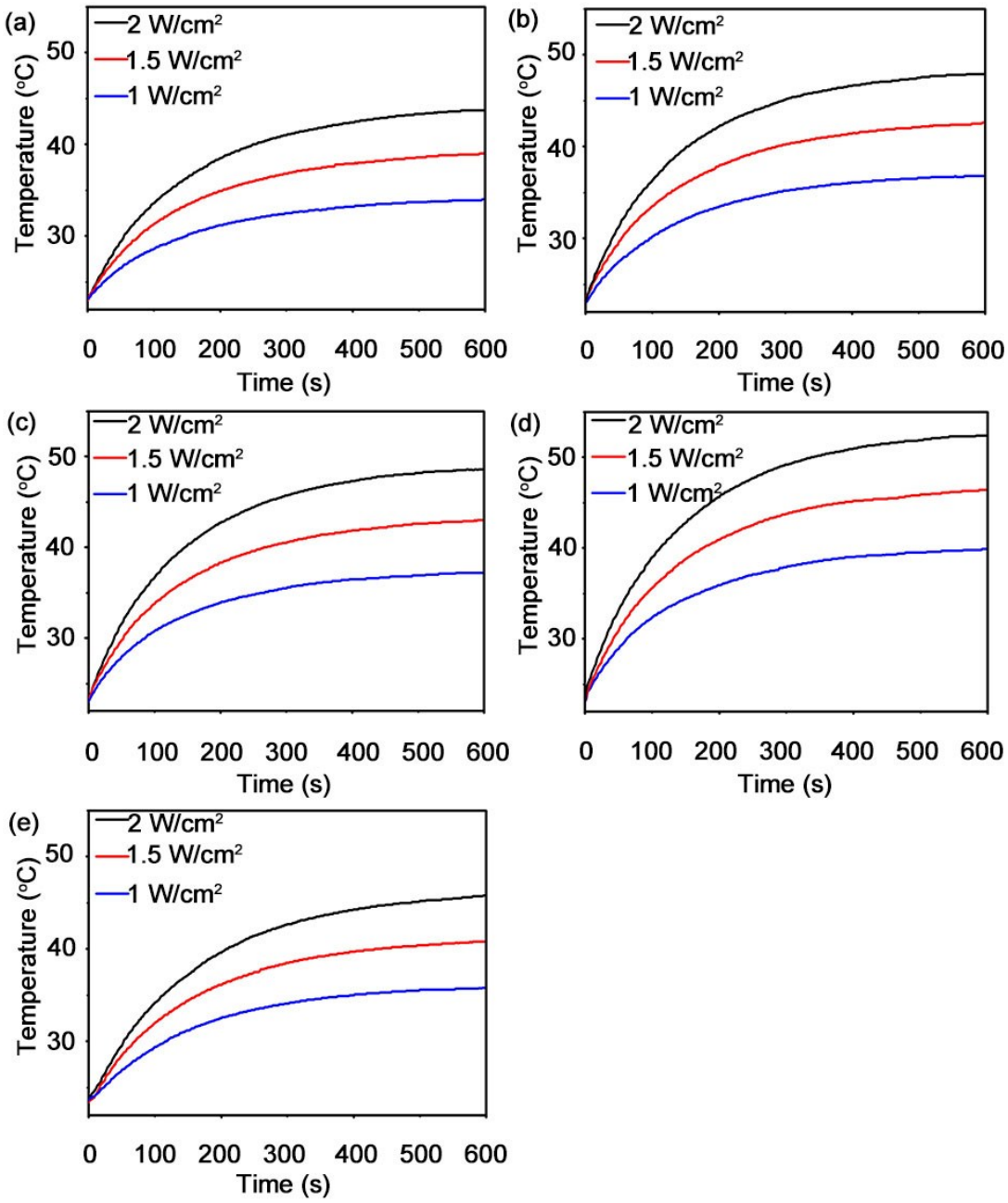

Fig. S7 Temperature elevation profiles of $Cu_{2-x}S$/PVP NPs (a), $Cu_{2-x}S$:Pt(0.1)/PVP NPs (b), $Cu_{2-x}S$:Pt(0.2)/PVP NPs (c), $Cu_{2-x}S$:Pt(0.3)/PVP NPs (d), and $Cu_{2-x}S$:Pt(0.4)/PVP NPs (e) at 50 ppm by 808 nm laser irradiation with different power density.

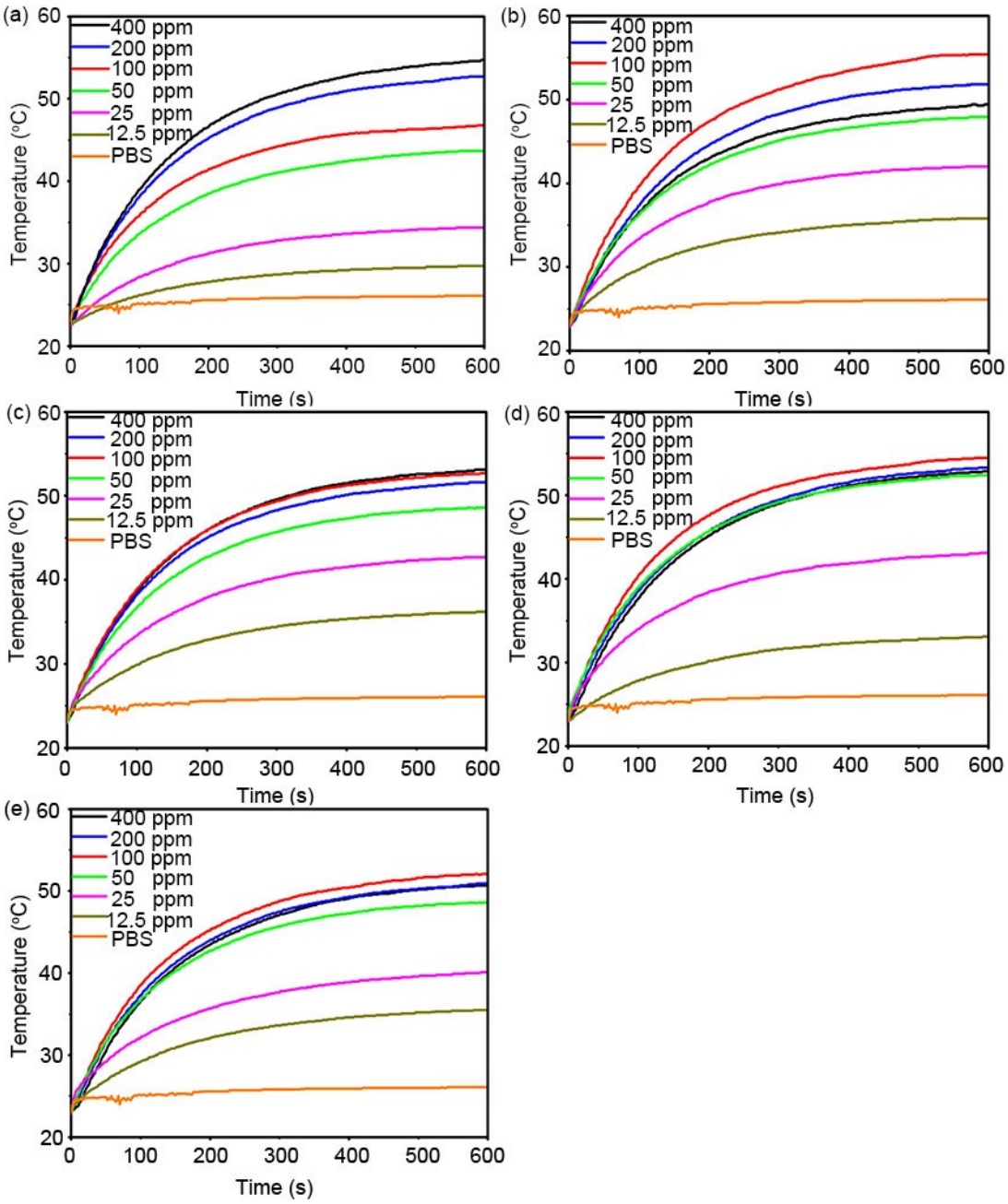

Fig. S8 Temperature elevation profiles of $Cu_{2-x}S$/PVP NPs (a), $Cu_{2-x}S$:Pt(0.1)/PVP NPs (b), $Cu_{2-x}S$:Pt(0.2)/PVP NPs (c), $Cu_{2-x}S$:Pt(0.3)/PVP NPs (d), and $Cu_{2-x}S$:Pt(0.4)/PVP NPs (e) at different concentrations upon 808 nm laser irradiation (2 W cm^{-2}, 10 min).

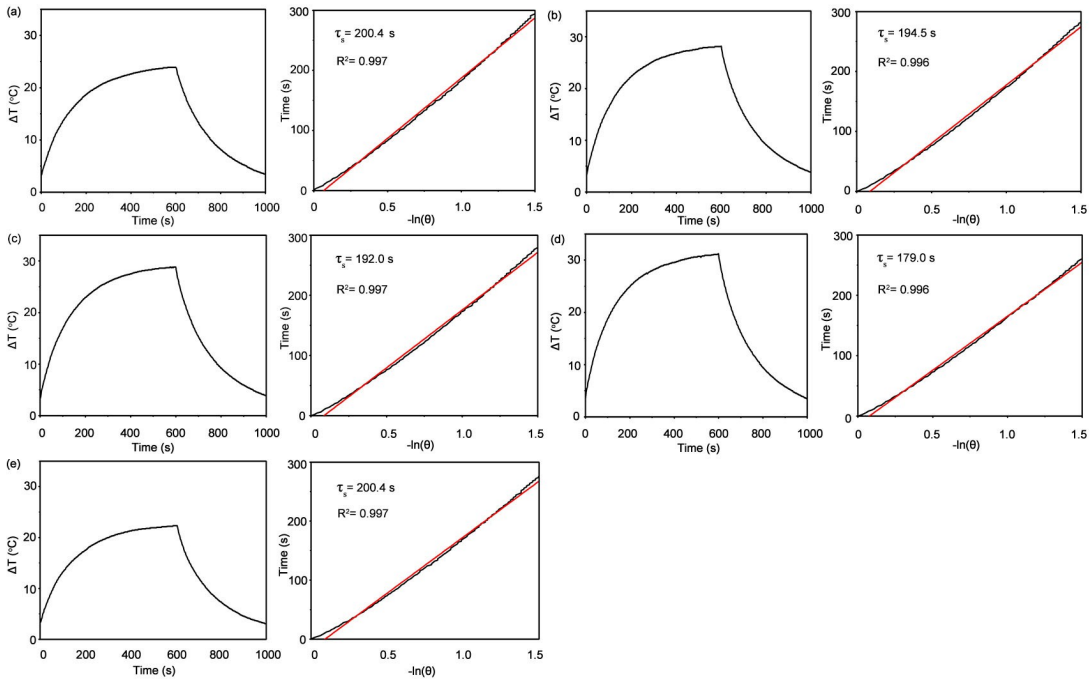

Fig. S9 The photothermal response profiles of $Cu_{2-x}S$/PVP NPs (a), $Cu_{2-x}S$:Pt(0.1)/PVP NPs (b), $Cu_{2-x}S$:Pt(0.2)/PVP NPs (c), $Cu_{2-x}S$:Pt(0.3)/PVP NPs (d), and $Cu_{2-x}S$:Pt(0.4)/PVP NPs (e) solutions upon 808 nm laser irradiation (2 W cm^{-2}, 10 min) and then the laser was shut off, and the corresponding plots of the cooling time versus –ln(θ) obtained from the cooling stage.

SI-1 Calculation for photothermal conversion efficiency:

The photothermal conversion efficiency (η) colud be calcuted by the following equations:

$$\eta = \frac{hS(T_{\max} - T_{surr}) - Q_{dis}}{I(1-10^{-A_{808nm}})} (1)$$

where h, S, T_{max}, T_{surr}, Q_{dis}, I, and A are heat transfer coefficient, irradiated area, the equilibrium temperature, the ambient temperature of the surroundings, the heat dissipation from the light absorbed by the quartzsample cell, the laser power density, and absorption at 808 nm, respectively. The value of hS is calculated by using the following equation (2) to (4):

$$hS = \frac{\sum m_i C_{p,i}}{\tau_S} \quad (2)$$

$$t = -\tau_s \ln\theta \quad (3)$$

$$\theta = \frac{T - T_{surr}}{T_{\max} - T_{surr}} \quad (4)$$

where m, C_p, t, τ_s are the mass of sample, the thermal capacity of sample, cooling time after irradiation, and the sample system time constant, respectively. The Q_{dis} was measured independently. The value of Q_{dis} is calculated by using the following equation:

$$Q_{dis}=hS(T_{max,PBS,}-T_{surr,PBS})$$

The T_{max}, T_{surr}, and τ_s was obtained from Fig. S8. The value of m and C_p are 1g and 4.2 J/(g•^{o}C). Therefore, the photothermal conversion efficiency (η) of $Cu_{2-x}S$:Pt(y)/PVP NPs and $Cu_{2-x}S$/PVP NPs at 808 nm could be calculated. Such as, $Cu_{2-x}S$/PVP NPs (27%), $Cu_{2-x}S$:Pt(0.1)/PVP NPs (30%), $Cu_{2-x}S$:Pt(0.2)/PVP NPs (31%), $Cu_{2-x}S$:Pt(0.3)/PVP NPs (37%), and $Cu_{2-x}S$:Pt(0.4)/PVP NPs (23%).

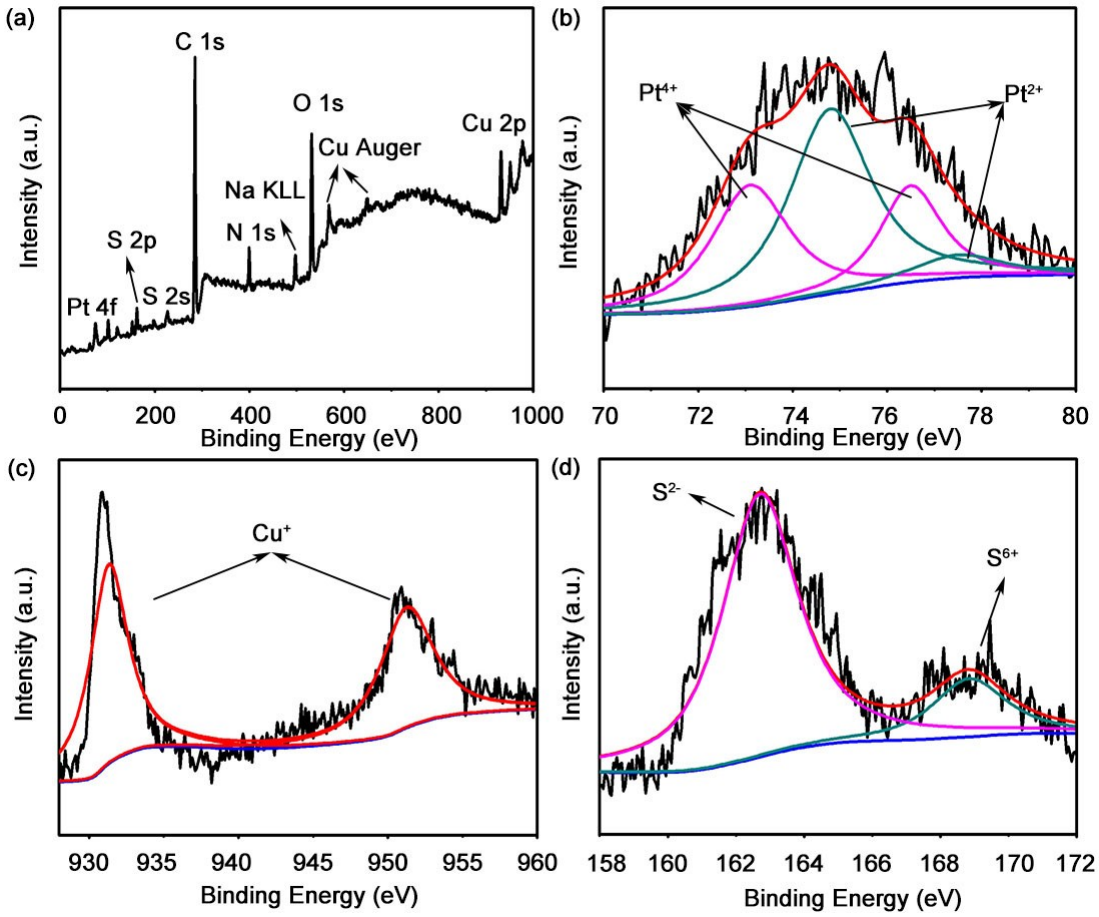

Fig. S10 (a) The full XPS spectrum of $Cu_{2-x}S$:Pt(0.3)/PVP NPs; The XPS spectrum of Pt 4f (b), Cu 2p (c), and S 2p(d).

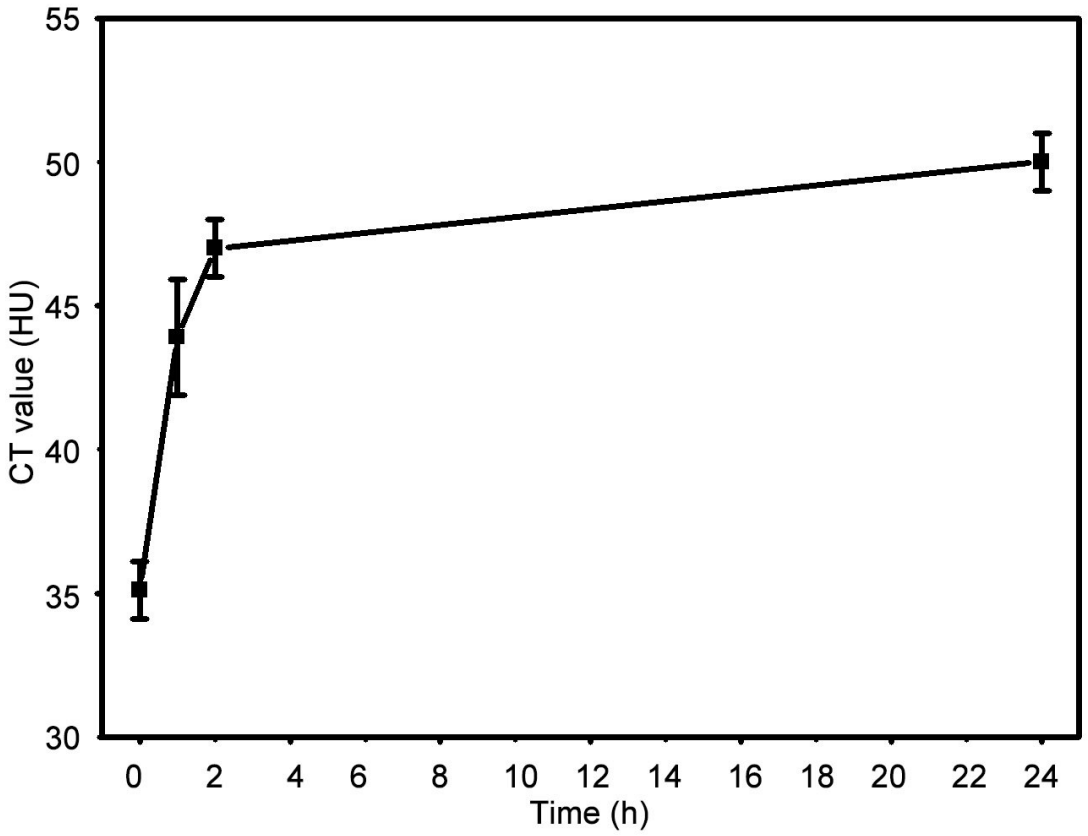

Fig. S11 HU values of tumor *in vivo* CT imaging after intravenous injection of $Cu_{2-x}S$:Pt(0.3)/PVP NPs (200 μL, 8.5 mg Cu/kg) measured at timed intervals.