Supporting Information

Novel Water-Soluble Phthalocyanine-Based Organic Molecule for Effective NIR Triggered Dual Phototherapy of Cancer

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General materials characterization:

Materials and methods: ^{1}H NMR spectra were measured on the AVANCEIIIHD 500MHz spectrometer (USA) with tetramethylsilaneas the internal standard. MALDI-TOF massspectra were recorded on the Kratos AXIMA-CFR Kompact MALDI mass spectrometer with anthracene-1,8,9-triol as the matrix. The optical characteristics were investigated by UV−vis absorption spectra (TU1901 UV−vis spectrophotometer, Beijing PuXi general instrument co., LTD, China). Photothermal temperature was recorded by an IR thermal camera (E50, IRS Systems). All reagents and solvents, unless otherwise specified, were obtained from Aldrich (USA) and Acros (Belgium), and were used as received. All reactions were carried out using Schlenk techniques under a nitrogen atmosphere.

1,4,8,11,15,18,22,25-Octaoctyloxy-2,3,9,10,16,17,23,24-octa-(3-pyridyloxy)phthalocyanine ($H_2Pc(OC_6H_{13}OPy)_8$). $(OC_6H_{13}OPy)_2PN$ (1.4 g, 2.5 mmol) was dissolved in 10 mL dry n-pentanol and the mixture was heated at 100 ℃ for 5 min. Under nitrogen atmosphere, lithium (0.05 g, 7.5 mmol) was added, and then the mixture was refluxed for 2 h. After cooling to room temperature, solvent was removed by vacuum evaporation and the crude product was purified by column chromatography using silica gel with dichloromethane and ethyl acetate as the eluents to obtain the green powder. Yield: 160 mg (11 %). ^{1}H NMR (300 MHz, $CDCl_3$, d): 8.48–8.45 (m, 8H), 8.34–8.31 (m, 8H), 7.43–7.40 (m, 8H), 7.28–7.23 (m, 8H), 4.89–4.84 (t, 16H), 1.84–

1.75 (m, 16H), 1.28 – 1.12 (m, 80H), 0.83 – 0.78 (t, 24H). MS (MALDI-TOF): m/z: 2285.2 $[M + H]^+$. Elemental analysis calcd: C 71.49%, H 7.50%, N 9.81%; found: C 71.47%, H 7.45%, N 9.88%.

Zn-1. A solution of **$H_2Pc(OC_6H_{13}OPy)_8$** (100 mg, 0.05 mmol) and zinc acetate (16 mg, 0.09 mmol) in 15 mL dry DMF was heated to reflux for 5 h. After cooling to room temperature, solvent was removed by vacuum evaporation, the crude product was purified by alumina oxide chromatography with dichloromethane and ethyl acetate as the eluents to obtain the green powder. Then the product (80 mg, 0.03 mmol) was dissolved in 20 mL dry chloroform. Under nitrogen atmosphere, methyl iodide (120 mg, 0.84 mmol) was added, and the solution was stirred and heated to reflux for 2 h. After cooling to room temperature, the solvent was removed by vacuum evaporation, the crude product was purified by recrystallization with methanol and chloroform to obtain green powder. Yield: 113 mg (72%). ^{1}H NMR (300 MHz, DMSO-d6, d): 9.37–9.36 (m, 8H), 8.96–8.94 (m, 8H), 8.68–8.65 (m, 8H), 8.25– 8.20 (m, 8H), 4.95–4.91 (t, 16H), 4.38 (s, 24H), 1.74– 1.69 (m, 16H), 1.24–1.08 (m, 80H), 0.87 – 0.82 (t, 24H). MS (MALDI-TOF): m/z: 743.9 $[M]^{4+}$. Elemental analysis calcd: C 49.65%, H 5.55%, N 6.43%; found: C 49.62%, H 5.59%, N 6.41%.

Cu-1. A solution of **$H_2Pc(OC_6H_{13}OPy)_8$** (100 mg, 0.05 mmol) and copper acetate (18 mg, 0.09 mmol) in 15 mL dry DMF was heated to reflux for 5 h. After cooling to room temperature, solvent was removed by vacuum evaporation, the crude product was purified by alumina oxide chromatography with dichloromethane and ethyl acetate as the eluents to obtain the black green powder. Then the product (80 mg, 0.03

mmol) was dissolved in 10 mL dry chloroform. Under nitrogen atmosphere, methyl iodide (120 mg, 0.83 mmol) was added, and the solution was stirred and heated to reflux for 2 h. After cooling to room temperature, the solvent was removed by vacuum evaporation, the crude product was purified by recrystallization with methanol and chloroform to obtain green powder. Yield: 112 mg (71%). Elemental analysis calcd: C 49.65%, H 5.55%, N 6.43%; found: C 49.61%, H 5.58%, N 6.40%.

Photothermal evaluation:

The solutions of Cu-1 with differentconcentrations (5.0, 10.0 and 20.0 μg/mL) were continuously exposed to a 730 nm laser with a power density of 1.0 W/cm^2. The temperature was measured every 40 s and stopped until the temperature nearly reached a plateau. Every sample was irradiated for 25 min and allowed to cool down to the room temperature, which was counted as one cycle, PBS served as the control group. The photothermal conversion efficiency of Cu-1 was calculated with the following equation:

$$\eta = \frac{hS(T\,max - Tsurr) - Qs}{I(1 - 10^{-A_{730}})}$$

where h is the heat transfer coefficient, S is the surface area of the container, T_{max} is the maximum temperature of the Cu-1, and T_{surr} is the temperature of the surrounding. I is the laser power, A_λ is the absorbance of Cu-1 at 730 nm, Q_s is the heat change of the solvent, and η is the photothermal conversion efficiency.

Photodegradation of 1,3-Diphenylisobenzofuran (DPBF):

The DPBF was used for evaluating the ROS produce ability of Cu-1. Typically, 5 μL

of a DPBF/DMSO solution (10×10^{-3} M) was added to 1 mL Tris-buffer (10×10^{-3} M, pH = 7.4) containing Cu-1 (10 μg/mL). The mixture solution was irradiated under a 730 nm laser (1.0 W/cm^2) for different time. The characteristic UV-vis absorption spectrum of the DPBF was measured at 414 nm to determine the generation of ROS.

Intracellular ROS Assay:

A conventional ROS indicator DCFH-DA was also employed to detect the ROS generation of Cu-1 in solution. HeLa cells (1×10^5 cells/well) were seeded on coverslips on a 24-well plate and incubated for 24 h. The medium was replaced with complete DMEM medium containing Cu-1. After incubation for 4 h, DCFH-DA was added according to the standard protocol provided by the suppliers. After 10 min incubation, cells were washed twice with PBS and irradiated by the 730 nm laser at the power density of 1.0 W/cm^2 for 10 min. Then the medium was replaced with PBS for measurement by PL instrument in a dark room. The fluorescence of 2′,7′-dichlorofluorescein triggered by ROS under 730 nm laser irradiation was measured. The PL spectrawere obtained with excitation at 488 nm and emission was collected from 490 to 600 nm.

Cell Cytotoxicity Assay:

HeLa cells were incubated in high glucose medium of DMEM containing 10% heat-inactivated FBS and 2% penicillin-streptomycin (PS) at 37 °C and 5% CO_2 in the incubator.The HeLa cells were incubated into 96-well plates for 24 h to allow cell

attachment. The DMEM media were discard the cells were rinsed twice with PBS (10 × 10^{-3} M, pH = 7.4). Subsequently, the cells were incubated in DMEM containing Cu-1 with different concentrations for 4 h. The cells were treated with 730 nm laser irradiation for 20 min. The DMEM medium was replaced with 20 μL 0.5 mg/ml MTT and after 4 h the MTT solution was replaced with 150 μL DMSO solution. Cell viability was measured at 490 nm by colorimetric assay (RaytoRT-2100C, Shenzhen, China). Cells without irradiation in medium were used as control.

Cell labeling:

HeLa cells were seeded in a 6-well plate, preincubated for 12 h. Then incubated with Cu-1 for 6 h. The cells were treated with 730 nm laser irradiation for 20 min and washed with PBS three times. Next the cells were stained with PI and calcein AM for 15 min and washed with PBS three times. The fluorescence microscopy (Olympus, BX51) was used to observe the state of cells. Cells with PBS were used as control.

***In Vivo* Photothermal Therapy:**

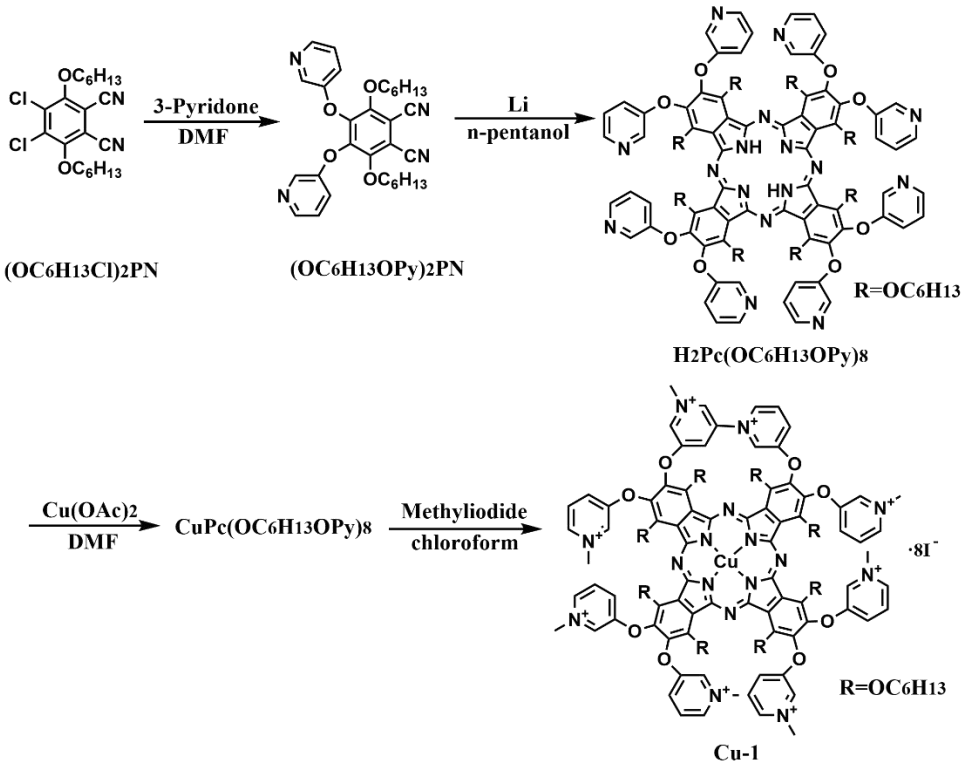
This study was performed in strict accordance with the NIH guidelines for the care and use of laboratory animals (NIH Publication No. 85-23 Rev. 1985). The animal use and care protocol were reviewed and approved by the Ethics Committee of the Harbin Medical University (approval No. HMUIRB-2008-06). All experiments were conformed to the Guide for Care and Use of Laboratory Animals. Female BALB/c mice (6-8 week old, 18 to 20 g body weight) were purchased from the 2nd Affiliated Hospital of Harbin Medical University Center (Harbin, China), maintained under pathogen-free conditions and allowed free access to sterile food and water. The tumor

model was established by injecting 1×10^6 4T1 cells into the right flank of each mouse. Tumor nodules were allowed to grow to a volume of ~100 mm^3 before treatments. The tumor bearing mice were randomized into four therapy groups (n = 5 per group): PBS (10×10^{-3} M, pH = 7.4), a 730 nm laser (1.0 W/cm^2) irradiation, Cu-1 without or with a 730 nm laser (1.0 W/cm^2) irradiation. Tumor length and width were measured with calipers, and the tumor volume was calculated using the equation $V = (a\times b^2)/2$, in which a was the length and b was the width. The tumor volume of each mouse were measured every two days.

Calculation of molar absorption coefficient:

$$\varepsilon = \frac{A}{bc}$$

where ε is the molar absorption coeffcient, A is the absorbance, b is the solution layer thickness (1 cm), and c is the molar concentration (mol/L).



Scheme S1 The synthetic route of Cu-1.

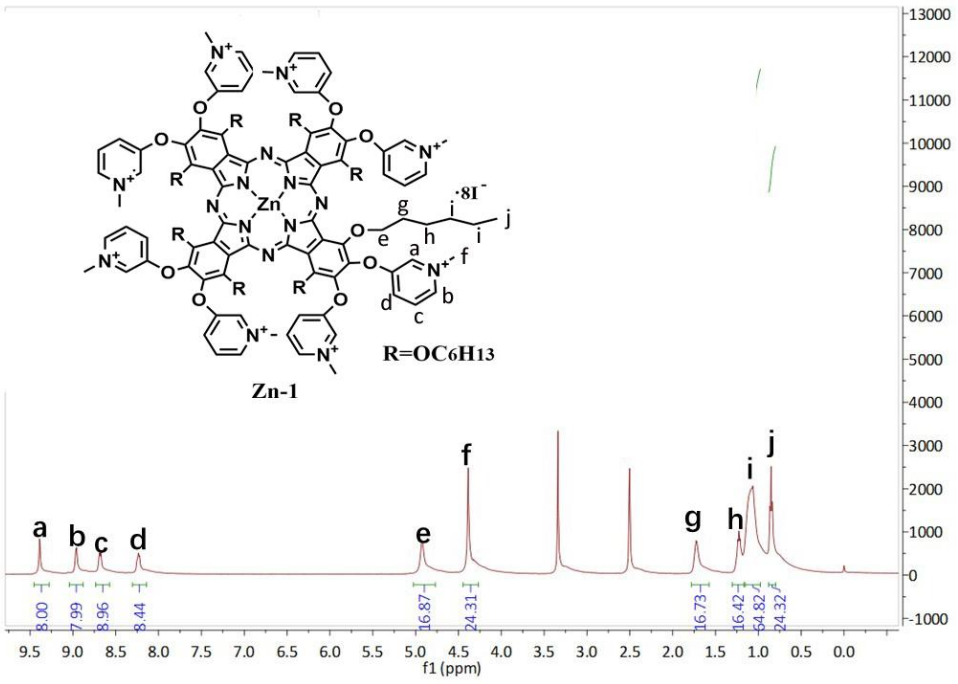


Figure S1 ^{1}H NMR spectrum of Zn-1 in DMSO.

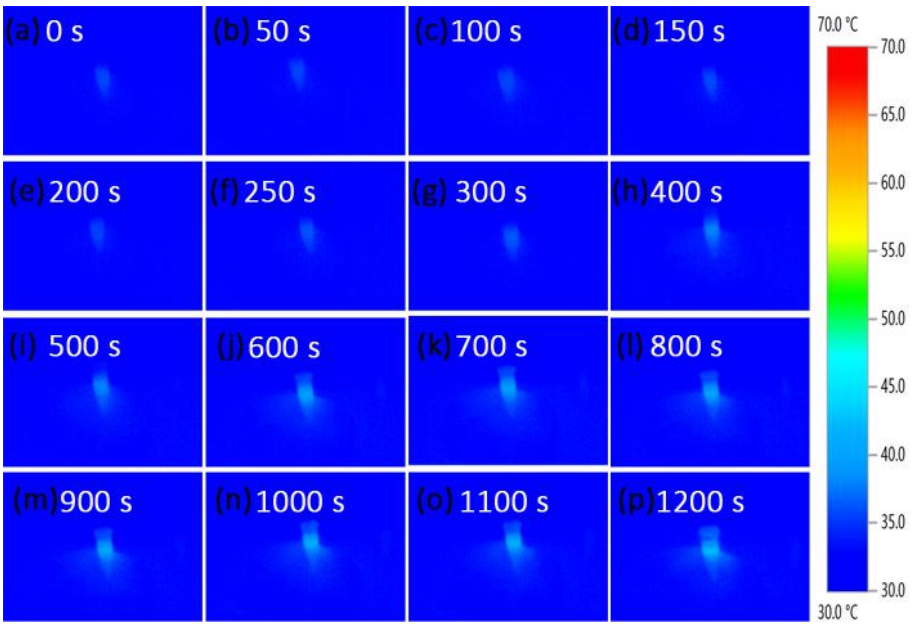


Figure S2 Thermal imaging of PBS under 730 nm laser irradiation (1.0 W/cm^2) for different times.

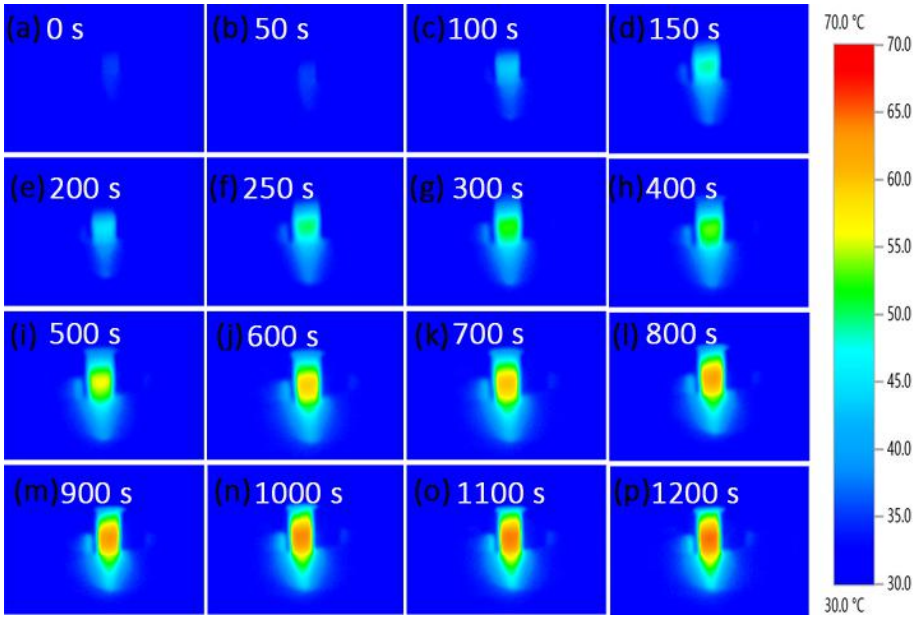


Figure S3 The photothermal heating curves of Cu-1 solutions ($C_{Cu\text{-}1}$= 5.0 μg/mL) under 730 nm continuous laser irradiation at the power density of 1.0 W/cm^2 for different times.

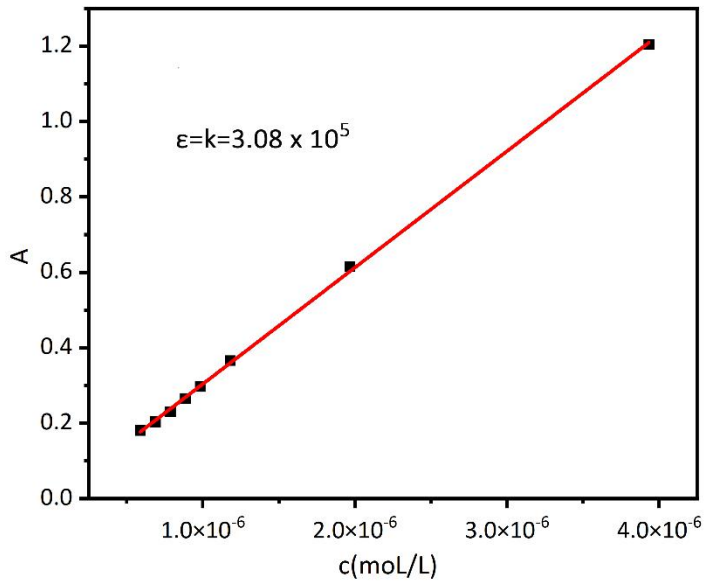


Figure S4 Calculation of molar absorption coefficient.

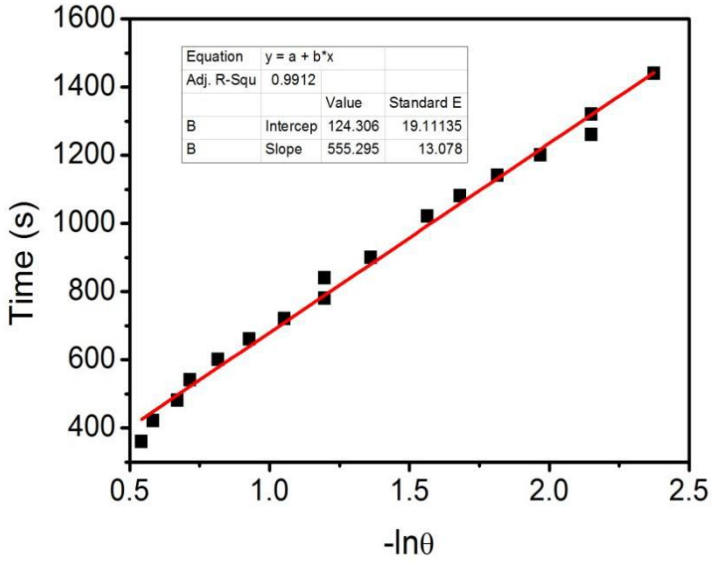


Figure S5 Linear time data versus -ln(θ) obtained from the cooling period of NIR laser off.

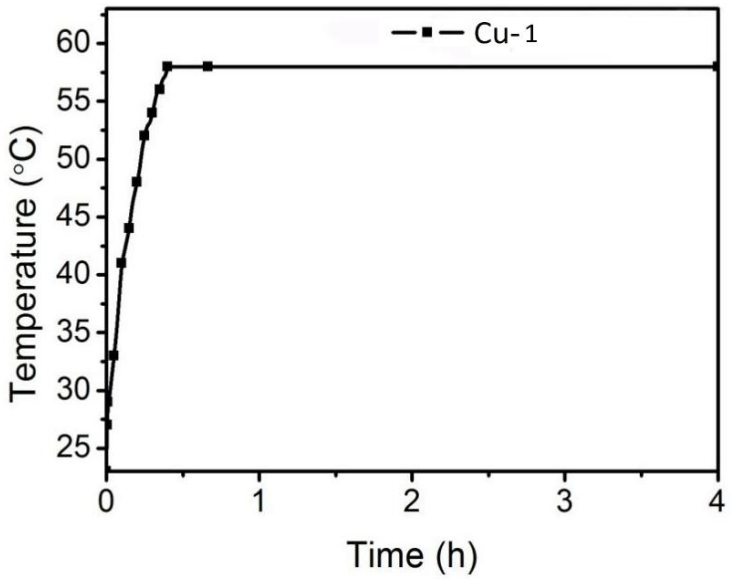


Figure S6 The photothermal heating curve of Cu-1 solutions (C_{Cu-1}= 5.0 μg/mL, 1.0 W/cm^2) under 730 nm continuous laser irradiation for 4 h.

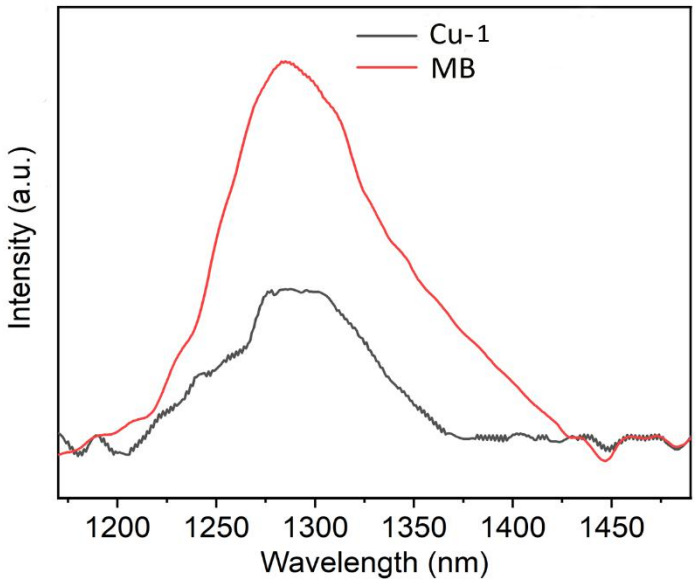


Figure S7 $^1O_2$ emission at ~1270 nm induced by the commercial MB and Cu-1 in ethanol under excitation with a 730 nm laser.

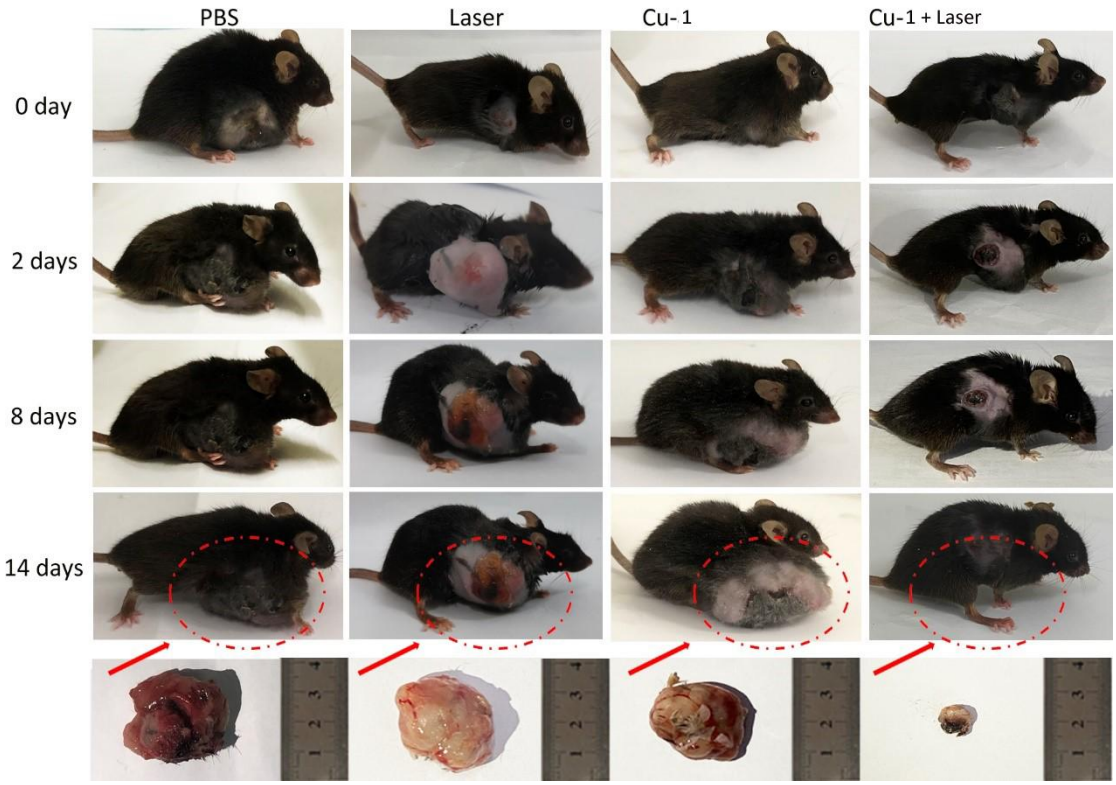


Figure S8 The photothermal heating curve of Cu-1 solutions (C_{Cu-1}= 40.0 μg/mL, 1.0 W/cm^2) under 730 nm continuous laser irradiation for 4 h.