Supporting Information for

A covalent organic framework-based nanoreactor for enhanced photothermal therapy *via* inhibiting intracellular heat defense systems

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Content

1. Materials and instrumentations

1,3,5-Tris(4-aminophenyl)benzene (TPB) and 2,5-dimethoxyterephthaldehyde (BPTA) were purchased from Jilin Province Yanshen Technology Co., Ltd. ICG-N_3 were purchased from Xi'an rulxlb Biological Technology Co., Ltd. glucose oxidase were purchased from Shanghai yuanye Bio-Technology Co., Ltd. Fluorescein isothiocyanate isomer I and sodium L-ascorbate were purchased from Energy Chemical Co., Ltd. Hydrogen Peroxide Assay Kit and ATP Assay Kit were purchased from Beyotime Biotechnology Co., Ltd. Benzaldehyde were purchased from Aladdin Reagent Co., Ltd. All reactants were used as purchased without further purification. Acetonitrile, acetic acid, N, N-Dimethylformamide and copper sulfate pentahydrate were purchased from Sinopharm Chemical Reagent Co., Ltd. Ultra-pure water was prepared with an Aquapro System (18 MΩ). Cell Counting Kit-8 were purchased from MedChemExpress Co. Ltd. Phosphate-Buffered Saline (PBS), Dulbecco's Phosphate-Buffered Saline (DPBS), and Fetal bovine serum (FBS) was purchased from VivaCell (Shanghai, P. R. China). Dulbecco's Modified Eagle Medium (DMEM), Penicillin Streptomycin Mixtures (Pen-Strep), and Trypsin-EDTA Solution (0.25%) were purchased from HyClone Laboratories, Inc. Normocin was purchased from Invivogen (San Diego, CA, USA).

Fourier transform infrared (FT-IR) spectra were obtained in the 400-4000 cm^{-1} range using a Thermo Scientific Nicolet iS50 FT-IR Spectrometer equipped with diamond attenuated total reflection (ATR) module. Each spectrum was the average of 16 scans. Ultraviolet–visible (UV−vis) absorption spectra were recorded on a Shimadzu UV-2700 Double Beam UV−vis Spectrophotometer. Scanning electron microscopy (SEM) micrographs were recorded on a Hitachi SU8010 Scanning Electron Microscope. Transmission electron microscope (TEM) micrographs were recorded on a Hitachi HT7700 120 kV Compact-Digital Transmission Electron Microscope. 808 nm laser (FC-808-10W-MM) was purchased from Shanghai Xilong Optoelectronics Technology Co., Ltd. Powder X-ray diffraction (PXRD) patterns were obtained on a Rigaku SmartLab SE X-Ray Powder Diffractometer with Cu Kα line focused radiation (λ = 1.5405 Å) from 2θ = 2.00° up to 30.00° with 0.01° increment. Hydrodynamic particle size and zeta potential were measured using Malvern Zetasizer Nano ZS90 System. Laser scanning confocal fluorescence images were captured with a Leica TCS SP8 Confocal Laser Scanning Microscopy with an objective lens (×20). Glass bottom dishes were purchased from S3 Cellvis (Mountain View, CA, USA). Microplate assays were carried out on a Molecular Devices SpectraMax i3x Multi-Mode Microplate Detection System. ^{1}H NMR data were collected on an AM-400 spectrometer. Chemical shifts are reported in δ relative to TMS. ^{13}C CP-MAS solid-state NMR spectra were recorded on a MERCURY plus 400 spectrometer operating at resonance frequencies of 400 MHz. The surface

area was measured by the BET (Brunauer Emmer Teller) isotherms using N_2 adsorption at 77 K, this was done on the Micromeritics ASAP 2020 sorption/desorption analyzer.

2. Cell culture and laboratory animals

The MCF-7 (human breast adenocarcinoma cell line) was provided by Stem Cell Bank, Chinese Academy of Sciences (Shanghai, P. R. China), and cultured in DMEM supplemented with FBS (10%), Normocin (50 μg/mL), penicillin (100 U/mL) and streptomycin (100 μg/mL) in an atmosphere of CO_2 (5 vol%) and air (95 vol%) at 37°C.

Nude mice (BALB/c-nu, femina, aged 4 weeks, 10–25 g) were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd. Animal experiments were reviewed and approved by the Ethics Committee of Shandong Normal University, Jinan, P. R. China (approval number AEECSDNU 2022050). All the animal experiments complied with relevant guidelines of the Chinese government and regulations for the care and use of experimental animals.

3. Snthesis of materials

3.1 Synthesis of TPB-BPTA-COF (1)

2,5-Bis(2-propynyloxy)terephthalaldehyde was purchased from Jilin Province Yanshen Technology Co., Ltd. FT-IR (ATR, cm^{-1}): 3272 (s), 2884 (m), 2754 (w), 2130 (m), 1673 (s), 1487 (m), 1416 (s), 1381 (s), 1292 (m), 1210 (s), 1140 (m), 1022 (s), 878 (m), 716 (m), 691 (s). ^{1}HNMR (400 MHz, $CDCl_3$, 25 °C, TMS, ppm): δ 10.52 (s, 2H), 7.59 (s, 2H), 4.84 (s, 4H), 2.56 (s, 2H).

A mixture of 1,3,5-tris(4-aminophenyl)-benzene (23.985 mg, 0.0682 mmol), 2,5-Bis(2-propynyloxy)terephthalaldehyde (24.791 mg, 0.1023 mmol), acetonitrile (25 mL) and glacial acetic acid (2.7 mL) was stirred at 25 °C for 12 h. The particles were isolated by centrifugation and washed with acetonitrile three times to generate TPB-BPTA-COF as a yellow powder. FT-IR (ATR, cm^{-1}): 3290 (m), 3031 (w), 2918 (w), 2161 (w), 2121 (w) ,1615 (m), 1592 (m), 1505 (m), 1486 (w), 1444 (m), 1423 (s),1357 (w), 1289 (m), 1193 (m), 1148 (m), 1029 (m), 915 (w), 878 (w), 830 (s), 669 (w), 632 (w), 539 (w).

3.2 Synthesis of COF-ICG (2)

A mixture of **1** (1 mg, 0.07 mmol) and ICG-N_3 (1 mg, 0.0012 mmol) in DMF solution (2 mL). Under the protection of nitrogen, added 200 μL of sodium L-ascorbate (0.986 mg 0.005 mmol) and anhydrous copper sulfate (0.776 mg 0.003 mmol) mixture to it. The above mixture was stirred at 25 ℃ for 12 hours. The resulting solids were completely washed with DMF to remove unreacted ICG-N_3 and washed with EDTA aqueous solution(1 mol/L) to remove the copper. After centrifugation in DMF， the supernatant and all the washing solutions were collected to measure the reaction capacity and reaction efficiency. The reaction efficiency was according to the

following equations: ($A_{ICG\ before}$-$A_{ICG\ in\ supernatant}$)/$A_{ICG\ before}$ * 100%. The reaction capacity and reaction efficiency were calculated to be 920 μg/mg and 92.0%, respectively. **COF-ICG** was obtained as green solid. FT-IR (ATR, cm^{-1}): 3284 (m), 2926 (m), 1661 (s), 1592 (w), 1505 (m), 1486 (w), 1418 (s), 1289 (w), 1194 (m), 1148 (w), 1089 (m), 1011 (w), 924 (m), 830 (m), 665 (w).

3.3 Synthesis of GOx@COF-ICG (3)

A mixture of **2** (1 mg), glucose oxidase (100 μg) and DPBS (PH=7.4, 1 mL) was stirred at 25 ℃ for 12 hours. The resulting solids were completely washed with DPBS to generate **GOx@COF-ICG (3)**. FT-IR (ATR, cm^{-1}): 3290 (m), 2925 (m), 1660 (w), 1593 (w), 1506 (m), 1479 (w), 1418 (s), 1359 (m), 1290 (w), 1195 (m), 1137 (w), 1091 (s), 1033 (w), 1010 (m), 926 (m), 896 (w), 830 (m).

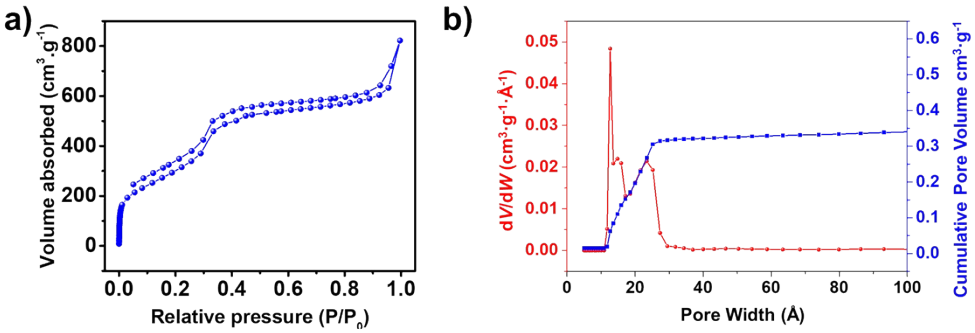

Fig. S1 N_2 adsorption isotherms and pore widths of **1**. The BET Surface Area of **1** is 821.1678 m^2/g.

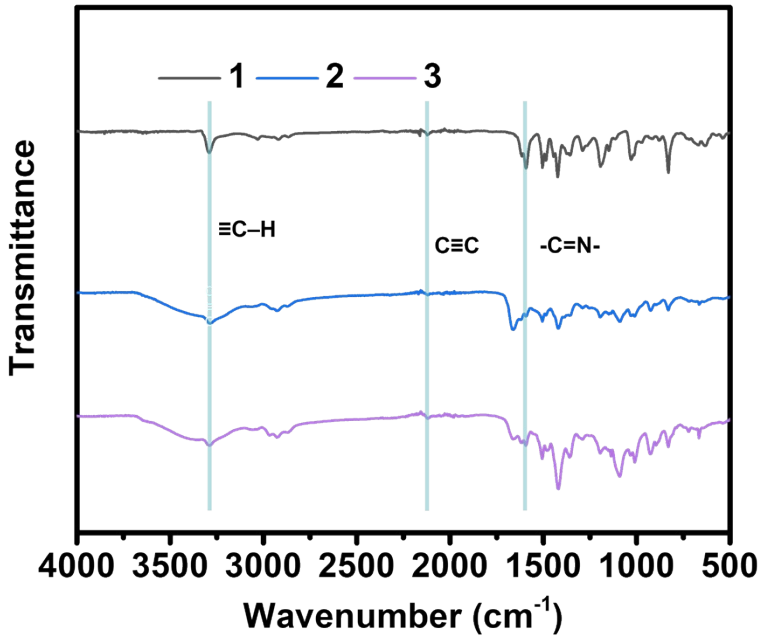

Fig. S2 IR spectra of **1**-**3**.

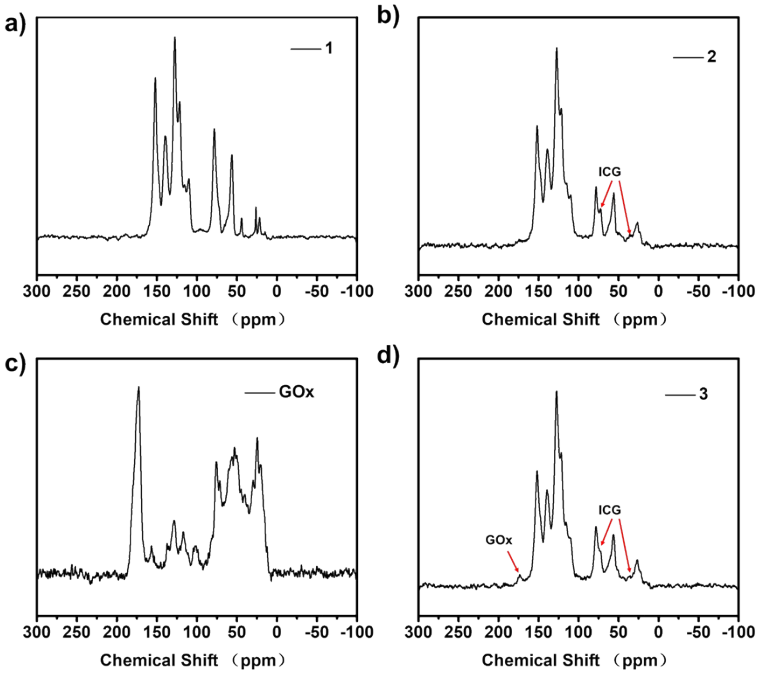

Fig. S3 ^{13}CNMR spectra of GOx and **1**-**3**.

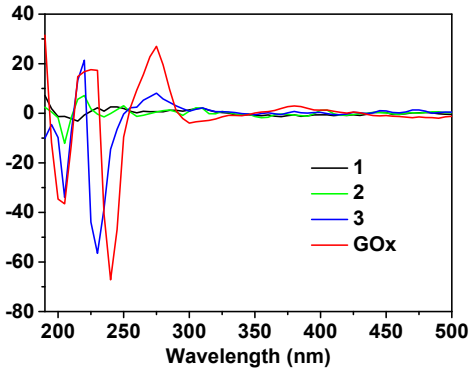

Fig. S4 CD spectra of GOx and **1**-**3**.

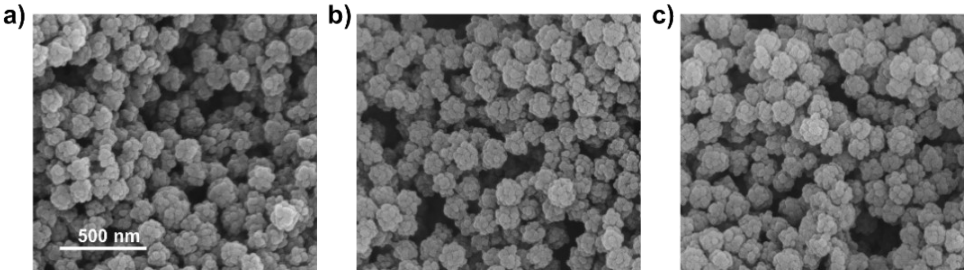

Fig. S5 SEM images of **1** (a) **2** (b), and **3** (c).

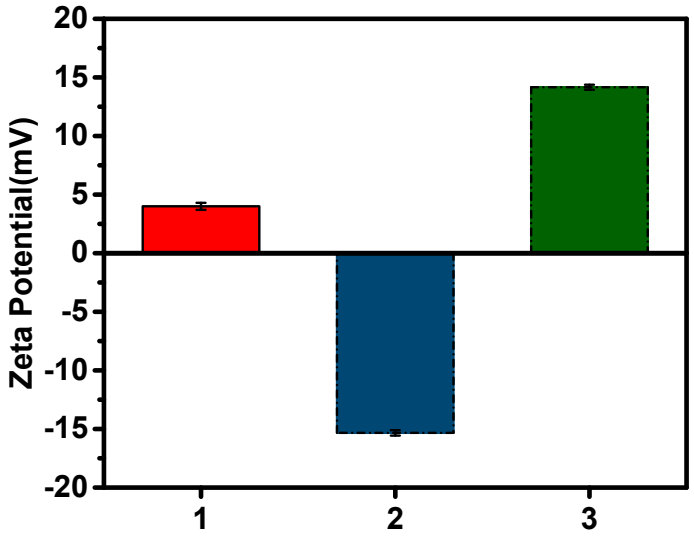

Fig. S6 The zeta potentials of **1** -**3**.

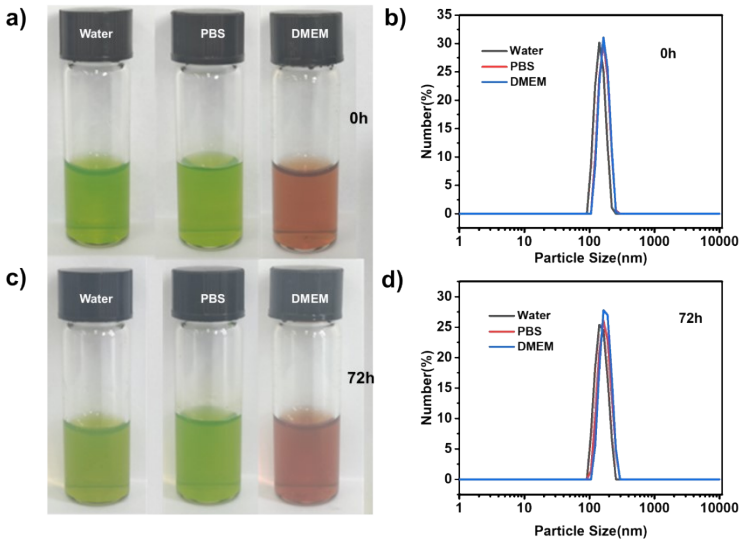

Fig. S7 The stability of **3** in different physiological solutions.

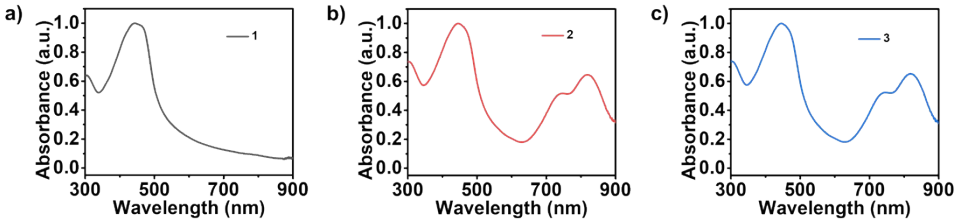

Fig. S8 The UV-vis absorption spectra of 1-3.

3.4 GOx-FITC bioconjugation.

GOx-FITC was prepared *via* mixing 10 mg GOx and 1 mg fluorescein isothiocyanate (FITC) in 2 mL of PBS solution and stirring for 24 hours and the unreacted dye was then removed by dialysis (MWCO: 10, 000 Da) against 1 × PBS (pH = 7.4) for 48 h. The GOx-FITC conjugates could be stored at 4°C for further use. The bioconjugation was confirmed by recording the spectrum with a fluorometer.

3.5 the loading capacity and loading efficiency of GOx.

GOx-FITC solutions with different concentrations (0, 1, 2, 4, 16 μg/mL) were prepared, then the fluorescence spectra (excitation at 488 nm) from 500-600 nm were recorded by a fluorescence spectrometer. The GOx-FITC@COF-ICG was synthesized using the method of preparing **3**. After centrifuged, the supernatant and all the washing solutions were collected to measure the loading capacity and loading efficiency. The loading capacity was calculated according to the following equations: $(M_{initial\ drug} - M_{drug\ in\ supernatant})/M_{nanoparticles}$. The loading efficiency was according to the following equations: $(M_{initial\ drug} - M_{drug\ in\ supernatant})/M_{initial\ drug} \times 100\%$. The loading capacity and loading efficiency were calculated to be 94.25 μg/mg and 94.25%, respectively.

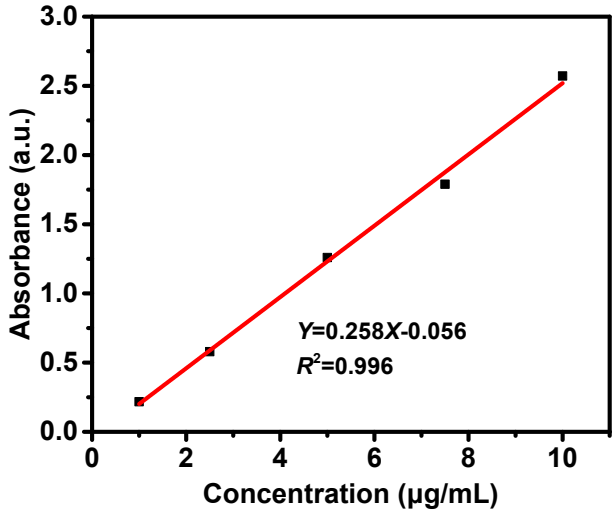

Fig. S9 Standard Curves of ICG-N_3.

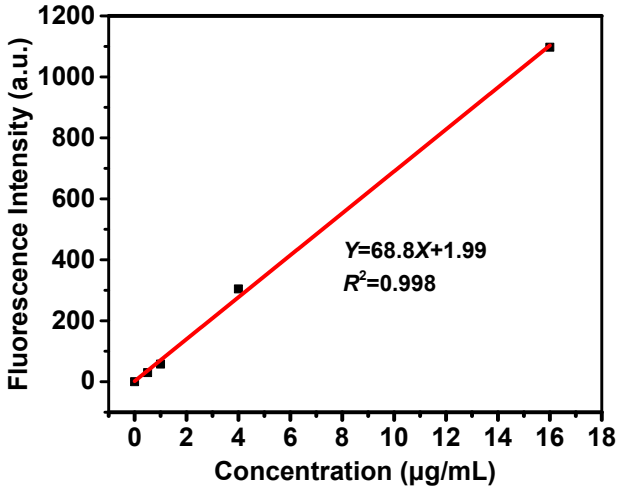

Fig. S10 Standard Curves of GOx-FITC

4. Detection of pH value

Different concentrations of **GOx** (0-100 μg/mL) and **3** solutions (0-100 μg/mL) were prepared, incubated with 10 mmol/L glucose for 3 hours, The pH changes caused by different concentrations of GOx and **3** catalyzed glucose were measured by pH meter.

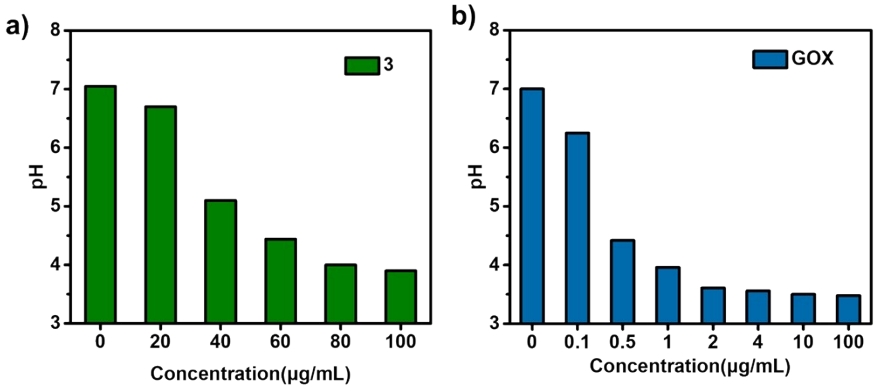

Fig. S11 The pH values of **3** and GOx solution with different glucose concentration.

5. Detection of H_2O_2

The relative concentration of H_2O_2 in vitro in different groups was obtained by the following method. The material was divided into five groups (1) water, (2) **2** (100 μL/mL), (3) **2** + laser (100 μL/mL), (4) **3** (100 μL/mL), (5) **3** (100 μL/mL) + laser, respectively, in glucose (10 mmol/L) solution. For the laser irradiation group, an 808 nm laser (1.4 W/cm^2) was used for 10 min. After 3 h of incubation at 37 °C, the relative content of H_2O_2 in vitro was determined using the hydrogen peroxide assay kit based on a ferrous oxidation-xylenol orange (FOX) assay and analyzed using a microplate reader.

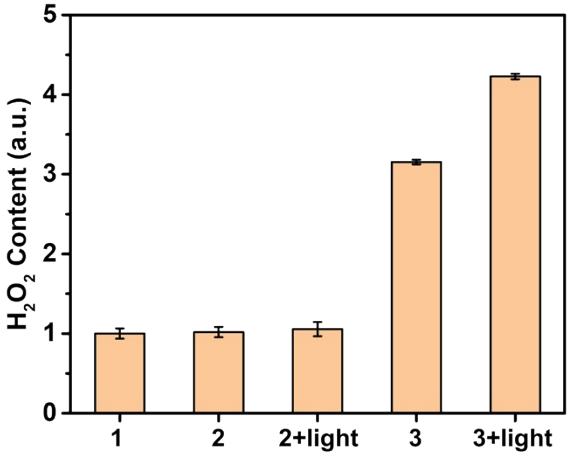

Fig. S12 The relative content of H_2O_2 for different formulations

6. Photothermal conversion efficiency

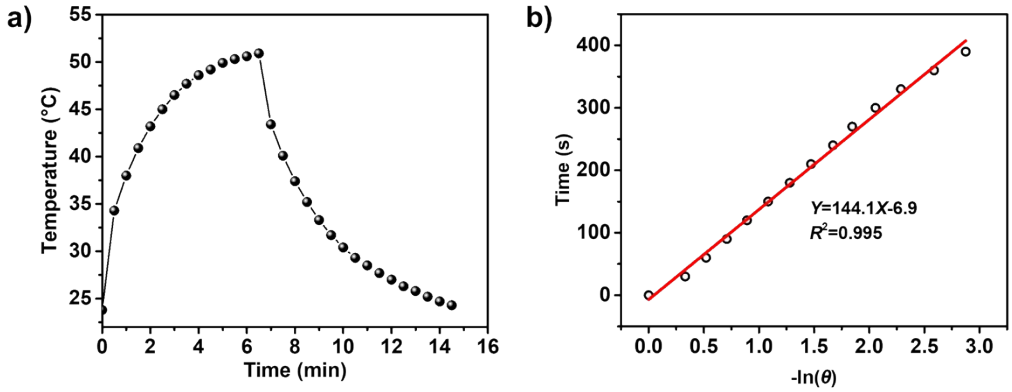

Fig. S13 (a) After 10 min of 808 nm laser irradiation (1.6 W/cm^2) and natural cooling to room temperature, **3** (100 μg/mL) temperature rise was observed. (B) T-(- ln θ) curve of natural cooling period.

The PBS dispersion of **3** (1 mL, 0-100 μg/mL) was added in a quartz dish and irradiated with an 808 nm laser (0-1.6 W/cm^2) for 10 min. Then, the laser was turned off to allow the dispersion to cool naturally. The temperature of the dispersion was recorded at 30 s intervals. The photothermal conversion efficiency was calculated according to the following formulas:

$$\eta = \frac{Q_s - Q_w}{I\left(1 - 10^{-A_{808}}\right)}$$

$$Q_s = hS\Delta T_{s,h}$$

$$Q_w = hS\Delta T_{w,h}$$

$$hS = \frac{mc}{\tau}$$

$$\tau = -\frac{dt}{dln\,\theta}$$

$$\theta = \frac{T_{t,c} - T_{min,c}}{\Delta T_{s,c}}$$

η, photothermal conversion efficiency; A_{808}, the absorption of solution at 808 nm; I, the power of the laser; $\Delta T_{s,h}$, the changed temperature of solution in the heating curve; $\Delta T_{w,h}$, the changed temperature of water in the heating curve; c, specific heat capacity of water; m, solution mass; τ, slope of t - (-ln θ)graph; t, time in the cooling curves; $\Delta T_{s,c}$, the changed temperature of solution in the cooling curve; $T_{min,c}$, the final temperature of solution in the cooling curve; $T_{t,c}$, the temperature of solution at different times in the cooling curve. According to the above formula, the photothermal conversion rate is 51.7%.

7. Cell uptake

Cells were seeded into glass bottom dishes and incubated overnight in a CO_2 incubator. After removal of the culture medium, the cells were incubated with DPBS dispersion of **3** (200 μL, 10 μg/mL) for 2 h in a CO_2 incubator, and washed with DPBS twice carefully. Then cells were incubated with Hoechst 33258 (200 μL, 10 μM) for an additional 1 h, and washed with DPBS twice. Finally, the laser scanning confocal fluorescence images were captured. The red images of **3** were excited by 561 nm light, and the emission wavelength range was collected at 600±20 nm. The blue images of nucleus were excited by 405 nm light, and the emission wavelength range was collected at 460±20 nm. Controls were conducted to make sure images were free of crosstalk.

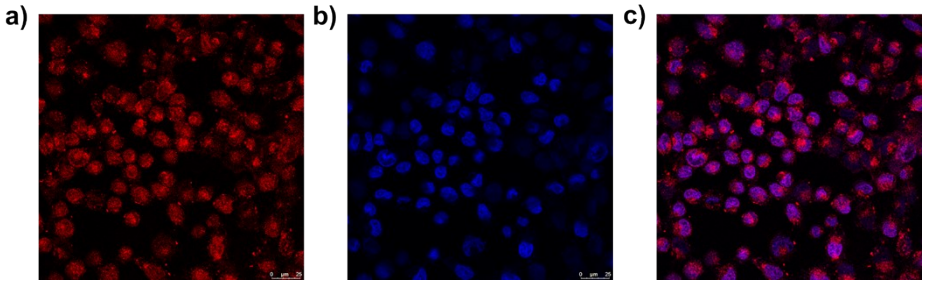

Fig. S14 Cell uptake of **3** in MCF-7 cells.

8. In vivo antitumor therapy

Cells were seeded into 96-well plates with a cell number of ~5k cells/well and incubated overnight in a CO_2 incubator. After removal of the culture medium, the cells were incubated with DPBS dispersion of **2** (100 μL, 0-100 μg/mL), or **3** (100 μL, 0-100 μg/mL) for 4 h in a CO_2 incubator. For PTT, the cells were exposed to 808 nm laser (1.6 W/cm^2, 10 min). After additional 24 h incubation, cck-8 (10 μL) and DMEM (100 μL) was added to each well and incubated for additional 3 h in a CO_2 incubator, followed by recording the absorbance at 450 nm.

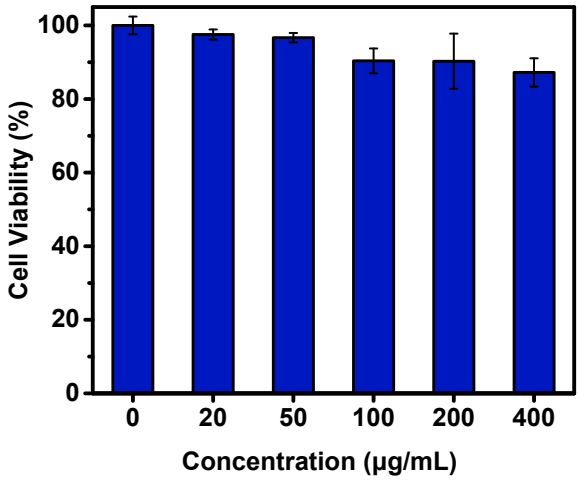

Fig. S15 CCK-8 assays of MCF-7 cancer cells incubated with different concentration of **2**.

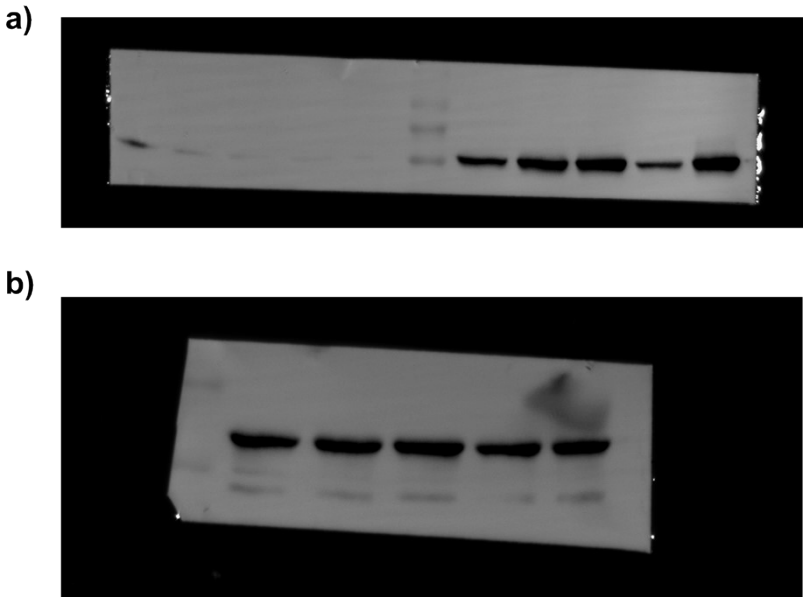

Fig. S16 Unedited original western blot images. (a) HSP70. (b) β-actin.

9. Live/dead cell staining assay

To detect the viable and dead cells, MCF-7 cells were cultured in confocal dishes overnight and divided into 4 groups: **2**, **2** + Light, **3**, **3** + Laser. The different nanoparticles (60 μg/mL, in terms of COF) in DMEM medium supplemented with 10% (v/v) fetal bovine serum (FBS) were added to the confocal dishes and incubated with 4 hours, respectively. For laser irradiation groups, 808 nm laser (1.0 W/cm^2) was utilized to irradiate the cells for 10 min. All the groups were further cultured for 12 hours. Finally, the cells were stained with Calcein AM (λ_{ex}=490 nm, λ_{em}=515 nm) / Propidium Iodide (PI) (λ_{ex}=535 nm, λ_{em}=617 nm) for 15 min and analyzed with CLSM.

10. H_2O_2 and ATP determination in vitro

To detect the H_2O_2 and ATP Relative content , MCF-7 cells were cultured in Petri dishes overnight, after removal of the culture medium, the cells were incubated with DPBS dispersion of the following 5 groups: (1) DPBS, (2) **2** (60 μL/mL, 5mL), (3) **2** (60 μL/mL, 5mL) + Light, (4) **3** (60 μL/mL, 5mL), (5) **3**(60 μL/mL, 5mL) + Laser for 4 h in a CO_2 incubator. For laser irradiation groups, 808 nm laser (1.0 W/cm^2) was utilized to irradiate the cells for 10 min. Then all the groups were further cultured for 24 hours in DMEM. Finally, Hydrogen Peroxide Assay Kit and ATP Assay Kitwas were used to determine the relative content of H_2O_2 and ATP in vitro and analyzed with SpectraMax i3x.

11. HSP-70 content in vitro

To detect the HSP-70 relative content in vitro, MCF-7 cells were cultured in Petri dishes overnight, after removal of the culture medium, the cells were incubated with DPBS dispersion of the following 4 groups: (1) **2** (60 μL/mL, 5mL), (2) **2** (60 μL/mL, 5mL) + Light, (3) **3** (60 μL/mL, 5mL), (4) **3** (60 μL/mL, 5mL) + Laser for 4 h in a CO_2 incubator. For laser irradiation groups, 808 nm laser (1.0 W/cm^2) was utilized to irradiate the cells for 10 min. Then all the groups were further cultured for 24 hours in DMEM .SDS-PAGE protein analysis HSP-70 relative content.

12. In vivo antitumor therapy

MCF-7 cancer cells (10^6 cells) suspended in DPBS (100 μL) were subcutaneously injected into the flanks of each mice to establish MCF-7 xenograft model. Length (L) and width (W) of the tumor were determined by digital calipers. The tumor volume (V) was calculated by the formula $V = 1/2 \times L \times W^2$. When the tumor size reached ~150 mm^3, the nude mice bearing MCF-7 tumors (n = 30) were randomly distributed into 5 groups. After intratumoral injection, the nude mice were feeding for 4 h, and for the treatment group, light treatment was performed on the tumor site. The mice continued to be fed for 14 days. The tumor volume and nude mouse body weight were recorded daily during the experimental period.

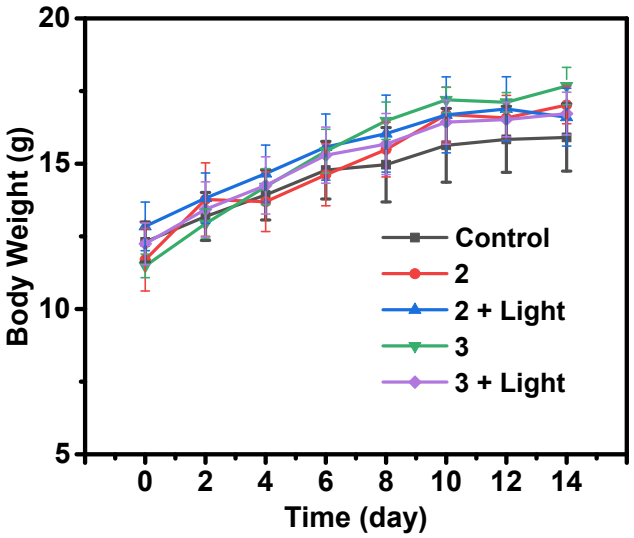

Fig. S17 Body weight of the mice in various groups during the treatment.

13. Histological analysis

After 14 days of the therapy, all mice were euthanized. Choice representative tumor bearing mice from different treatment groups, the tumor tissue and major organs (heart, liver, spleen, lung and kidney) were collected for H&E histological analysis.

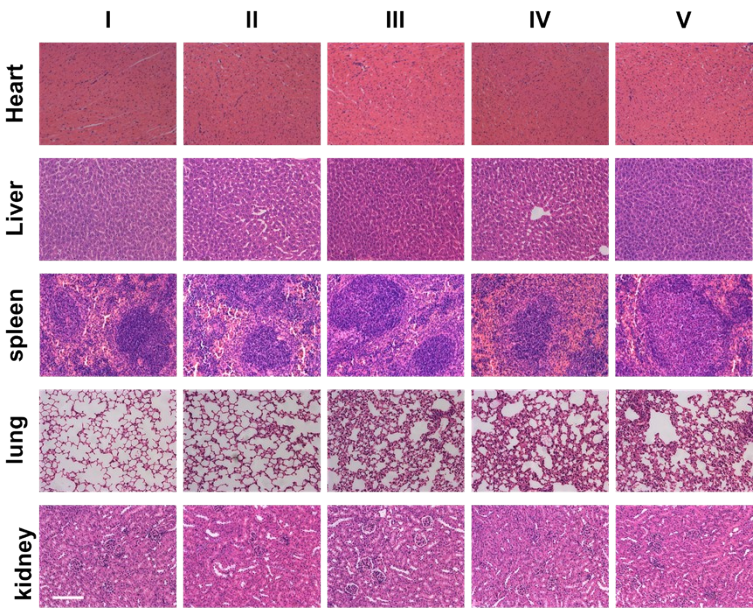

Fig. S18 H&E stained images of the major organs, including heart, liver, spleen, lung, and kidney. Scale bar, 100 μm.