Supplementary Materials

A redox-activatable biopolymer-based micelle for the sequentially enhanced mitochondria-targeted photodynamic therapy and hypoxia-dependent chemotherapy

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Materials and methods

Materials: Hyaluronic acid was purchased from TCI Development Co. Ltd (Shanghai, China). Cysteamine hydrochloride, 1-ethyl-3 (3-dimethylaminopropyl) carbodiimide (EDC), Hydroxysuccinimide (NHS) and L-carnitine were obtained from Sigma Aldrich (St. Louis, MO, USA). Dithiodipridine, acetic acid, 3-mercaptopropionic acid, silica gel (200-300 mesh), 2-nitroaniline, cyanamide solution, 4-nitrophthalimide, pyrene, ammonium molybdate tetrahydrate, zinc acetate, sodium sulfide ($Na_2S.9H_2O$), urea, hydrogen peroxide, sodium hydroxide (NaOH), N, N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO) were purchased from Aladdin Biochemical Technology Co. Ltd (Shanghai, China).

Synthesis of HA-SH. To synthesize HA-SH, the hyaluronic acid (200 mg, 0.5 mmol) was dissolved in 40 mL of PBS at the pH 6.8 under stirring. Subsequently, EDC (287.6 mg) and NHS (202.7 mg) were added into the solution and stirred for another 2 h. Furthermore, cysteamine hydrochloride (337.8 mg) were added into the mixture solution and reaction would last another 24 h. Afterwards the crude product was further purified through dialysis (3000 Da, MWCO, Millipore). To avoid the oxidation of thiol groups, the dialysis was carried out at low temperature of 4℃ at the pH value of 5.0. After dialyzing for 2 days, the obtained product was lyophilized and denoted as HA-SH.

Synthesis of 3-(2-pyridyldithio) propanoic acid. The synthesis protocol of the 3-(2-pyridyldithio)propanoic acid was adapted from a previous study. [1] 2,2'-dithiodipyridine (3.8 g) was dissolved into 10 mL ethanol solution and then 0.4 mL of glacial acetic acid was added into mixture solution. Afterward, 3-mercaptopropionic acid (0.9 g) was added dropwise into the mixture solution and the reaction would continue at room temperature for 20 hours under stirring. The mixture was then transferred to the rotating vacuum evaporator to remove the solvents, and the temperature was set to 40-45℃. The dried product was further purified with silica-gel column chromatography with dichloromethane/methanol mixture (3: 2) containing 4 wt% of acetic acid. At last, the mixture solution was evaporated, washed with ethyl alcohol and evaporated again to remove the excess acetic acid in the product. The final product was denoted as Py-s-s-COOH.

Synthesis of zinc phthalocyanine (ZnPc). The synthesis protocol of the zinc phthalocyanine was adapted from a previous study. [2] Here urea (10 g), 4-nitrophthalimide (3.8 g), and ammonium molybdate (0.05 g) were collected and transferred to the three neck round bottom flask and then heated to 160℃. Then, zinc acetate (0.9 g) was added to the melted mixture and reacted until the color of the mixture solution changed liquid to blue-violet. Furthermore, the product was washed with 1 M hydrochloric solution at 95℃ for 1 hour, filtered and extensively washed with deionized water. Next, the mixture solution was boiled with 1 M of sodium hydroxide solution for 1 hour and washed until the filtered water reached the neutral pH. Finally, the synthetic product was collected and denoted as nitrozinc phthalocyanine.

To obtain zinc phthalocyanine (ZnPc), the nitrozinc phthalocyanine (0.8 g) and sodium sulfide nonahydrate (2.9 g), DMF (20 mL) were reacted at 60℃ for 1.5 h

under nitrogen. Next, the products were washed using water and filtered. The purified product was a dark green solid, which was denoted as zinc phthalocyanine.

Synthesis of TPZ (3-amino-1,2,4-benzotriazine-1,4-dioxide). To obtain 3-amino-1,2,4-benzotriazine-1-oxide, the 2-nitroaniline (20 g) was added dropwise into 18 mL of a 50% monocyandiamide solution and continuously stirred at 50 °C for 15 min. Subsequently, the mixture was heated to 100 °C, reacted for 1.5 h until it separated into two layers. Next, sodium hydroxide (18 mol/mL) was added dropwise into the mixture solution and eventually stirred at 100 °C for 12 h. After the reaction was completed, a viscous suspension was collected, dispersed in the water and coled down to ambient temperature. Then, the precipitate was extensively washed with water and ethyl acetate thrice. Afterward, a yellow solid was obtained and denoted as 3-amino-1,2,4-benzotriazine-1-oxide.

The obtained 3-amino-1,2,4-benzotriazine-1-oxide (4.4 g) and glacial acetic acid (220 mL) were mixed and stirred at 50 °C to form suspension. Then, 30% hydrogen peroxide was added into the suspension and the mixture was allowed to react for another 10 hours in the dark. Once this step was completed, the color of solution became pompadour, then the mixture was evaporated under vacuum and recrystallized with ethanol to obtain red crystals, which was denoted as 3-amino-1,2,4-benzotriazine-1,4-dioxide.

Synthesis of ZnPc-S-S-Py. To synthesize ZnPc-S-S-Py, 3-(2-pyridyldithio) propanoic acid was dissolved into the DMF (10 mL), followed by EDC (15 mg) and NHS (9 mg). The reaction would last for 30 min to activate the carboxyl groups of the 3-(2-pyridyldithiol) propanoic acid. Furthermore, ZnPc (100 mg) was added into the mixture and stirred for another 24 h. Next, the remaining solvent was evaporated and the product was further purified by washing with ethyl alcohol and rotary-evaporation. The obtained product was denoted as ZnPc-S-S-Py.

Synthesis of HA-S-S-ZnPc. To prepare HA-S-S-ZnPc, HA-SH (200 mg) and ZnPc-S-S-Py (437 mg) were added into mixture of PBS (100 mL) and DMF (100 mL) upon sonication. After that, the mixture solution was incubated for 24 h under stirring. Raw product was dried by high vacuum and washed thrice using DMF to remove the unreacted ZnPc-S-S-Py and by-product of pyridine. Finally, the cotton-like product with color of dark green denoted as HA-S-S-ZnPc. The loading ratio of ZnPc in HA-S-S-ZnPc was determined according to the UV/vis spectroscopy based on the standard curve of ZnPc (λ = 720 nm).

Synthesis of HA-S-S-ZnPc-Lc. To fabricate HA-S-S-ZnPc-Lc, Lc (83 mg) was firstly added into 10 mL of PBS solution containing 98.7 mg of EDC and 78.8 mg of NHS. After stirring for 2 h, 200 mg of HA-S-S-ZnPc was added into the above solution and the mixture was stirred for another 24 h. The mixture solution was dialyzed against deionized water (3000Da, MWCO, Millipore) to remove unreacted reactants. The final product was denoted as HA-S-S-ZnPc-Lc.

Synthesis of HA-S-S-ZnPc-Lc micelles. To synthesize the HA-S-S-ZnPc-Lc micelles, 10 mg HA-S-S-ZnPc-Lc was added into the mixture of water (20 mL) and THF (20 mL) upon ultrasonication. The mixture solution was dried under vacuum to

remove the excess THF, followed by lyophilized to remove the water in the mixture solution. The product was denoted as HA-S-S-ZnPc-Lc.

Critical micelle concentration. To investigate the critical micelle concentration (CMC) of HA-S-S-ZnPc-Lc micelle, pyrene was dissolved into acetone and then the solution was diluted to 6×10^{-6} M with deionized water. The mixture solution was evaporated and the HA-S-S-ZnPc-Lc at different concentrations (1×10^{-4} to 1 mg/mL) was added into the above solution and dispersed upon sonication. Afterwards, the concentration of pyrene was measured until it reached about 6×10^{-7} M. Next, the mixture solution was incubated it at 55°C for 1 h and equilibrated at room temperature overnight in the dark. Finally, pyrene fluorescence spectra were detected by fluorescence spectrophotometer at 390 nm by referring to the intensity ratio of I338/I333.

Preparation the drug-loaded micelle of HA-S-S-ZnPc-Lc@TPZ. To obtain HA-S-S-ZnPc-Lc@TPZ, 10 mg of HA-S-S-ZnPc-Lc was dispersed into the mixture solution containing water (5 mL) and THF (5 mL) upon sonication. Subsequently, 10 mg of TPZ was dispersed in the mixture solution containing water (5 mL) and THF (5 mL) and added dropwise to the above solution. The mixture was allowed to react for another 12 h under stirring condition. Afterwards, the mixture solution was dried under vacuum to remove the excess THF, followed by dialyzing against deionized water to remove unloaded TPZ. The content of TPZ in HA-S-S-ZnPc-Lc@TPZ was determined according to the UV/vis spectroscopy based on the standard curve TPZ (λ= 470 nm).

Preparation of FITC labeled HA-S-S-ZnPc-Lc. To obtain FITC-labeled sample series, 10 mg of HA-S-S-ZnPc-Lc and 10 mg of FITC was dissolved in the mixture solution containing water (20 mL) and THF (20 mL) and the FITC would then react with the ZnPc moieties for fluorescent labeling. The reaction continued under stirring at room temperature for 24 h in the dark. The product was purified through dialysis against deionized water (1000Da, MWCO, Millipore). The product was denoted as HA-S-S-ZnPc-Lc-FITC. FITC labeled HA-S-S-ZnPc was prepared using a similar method.

Preparation of Cy5 labeled HA-S-S-ZnPc-Lc. To prepare Cy5-labeled sample series, 5 mg of Cy5 was added in the mixture solution containing 2 mL of water and 2 mL of DMF. Subsequently, EDC (1.019 mg) and NHS (0.6 mg) was added into above solution and the reaction was performed under stirring for 2 h. After stirring for 2 h, the mixture solution was added dropwise into the mixture solution containing 20 mg HA-S-S-ZnPc-Lc, 20 mL of DMF and 20 mL of deionized water. Then the mixture solution was incubated for 24 h under stirring condition. Finally, the mixture solution was dialyzed against deionized water via dialysis membrane (1000Da, MWCO, Millipore). The final product was denoted as HA-S-S-ZnPc-Lc-Cy5.

GSH-responsive TPZ release from HA-S-S-ZnPc-Lc@TPZ micelles. The GSH-responsive release profile of TPZ was investigated under GSH. Briefly, dialysis bag contained of HA-S-S-ZnPc-Lc@TPZ micelles in PBS (5 mg/mL) was dipped in beaker glass containing GSH in the PBS (10 mM), along with continuous shaking at 37° C in the dark. At different time intervals, 2 mL of PBS was collected and 2 mL of

fresh PBS was supplemented at the same time. The concentration of TPZ was quantified by UV-vis spectrophotometer at 450 nm. As for the control, the HA-S-S-ZnPc-Lc@TPZ release profile was also quantified in the PBS solution.

Cell culture. The 4T1 breast cancer cells were purchased from American Type Culture Collection (ATCC). The cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS, Gibco), penicillin (100 U/mL) and streptomycin (100 μg/mL) at 37°C) and continuously injected with 5% CO_2. The medium was refreshed twice a day.

Characterization of cellular uptake and intracellular distribution of FITC-labeled HA-S-S-ZnPc-Lc@TPZ micelles. For the analysis of the cell uptake and distribution, 4T1 and HUVECs with initial density of 1×10^5 cells/mL was seeded into the culture dish. After the cell confluency reached about 70-80%, the medium was replaced with fresh medium with and without HA (5 mg/mL) and further incubated for 2 h. Next, the culture medium was replaced by fresh ones with or without FITC-labeled HA-S-S-ZnPc-Lc@TPZ (200 μg/mL) and incubated for another 2 h. The cells were then washed with PBS, fixed with paraformaldehyde at 4°C for 30 min, and again washed thrice with PBS, then observed using confocal microscope. **Flow cytometric analysis for cellular uptake of the nanosamples:** To analyze the cellular uptake of HA-S-S-ZnPc-Lc, 4T1 and HUVEC cells was seeded into 6-well plate with an initial density of 1×10^6 unit/mL and cultured for 24 h, the original culture medium was removed with fresh ones containing HA-S-S-ZnPc-Lc-FITC (200 μg/mL) and further incubated for 2h or 4h. After that, the cells were washed twice with PBS, and subsequently detached using Trypsin. The detached cells were washed twice by centrifugation. Finally, the cell uptake was investigated by flow cytometry (Beckman Coulter).

Assessment on the mitochondrial-targeting capability of micelles. The mitochondrial targeting capability of micelles were analyzed for the evaluation of the cancer selectivity. Briefly, 4T1 cells was seeded into the confocal dish with the initial density 1×10^5 cells/mL and incubated for 24 h. After culturing for 24 h, the culture media was refreshed with medium containing HA-S-S-ZnPc-Lc-FITC and HA-S-S-ZnPc-FITC and further cultured for 12 h. Then, the cells were washed thrice with PBS, stained with Mito Tracker Red and observed using confocal microscope.

Evaluation of intracellular hypoxia and ROS level in tumor cells. The hypoxia and ROS level in tumor cells were investigated using oxidative stress/hypoxia detection kit (Enzo Life Sciences, USA). Briefly, 4T1 cells was seeded into the confocal dish until the cells reached confluence about 80%. Afterward, the original culture medium was replaced with fresh medium with and without HA-S-S-ZnPc-Lc@TPZ and further cultured for 6 h. Subsequently, the hypoxia staining dye were added into each sample and further incubated for 30 min. Next, the cells were washed with PBS twice and irradiated using NIR (720 nm, 200 mW/cm^2, 5 min). Finally, the 4T1 cells were washed with PBS twice and the fluorescence imaging was observed under confocal microscope.

In vitro cytotoxicity assay. To evaluate the in vitro toxicity of the HA-S-S-ZnPc-Lc@TPZ micelles combined with NIR treatment to 4T1 cells, the original cell

medium was replaced with fresh medium containing PBS, TPZ (2, 4, 6, 8, 10 μg/mL), HA-S-S-ZnPc (50, 100, 150, 200, 250 μg/mL), HA-S-S-ZnPc-Lc (50, 100, 150, 200, 250 μg/mL), HA-S-S-ZnPc@TPZ (50, 100, 150, 200, 250 μg/mL), HA-S-S-ZnPc-Lc@TPZ (50, 100, 150, 200, 250 μg/mL). The treated samples were further incubated for 24 h. After culturing for 24 h, the samples were irradiated with NIR (720 nm, 200 mW/cm^2,10 min) and incubated for another 30 min, while the sample without NIR irradiation was used as a control. At last, the cell viability was determined by MTT assay kit (Solarbio Science & Technology Co. Ltd, China).

Western blot assays: To determine the cytosolic release of cytochrome c, 4T1 cells were seeded into 6-well plate at an initial density of 1×10^6 cells/mL and cultured for 24 h. Subsequently, the culture media were replaced by fresh ones containing PBS, TPZ, HA-S-S-ZnPc-Lc, HA-S-S-ZnPc-Lc@TPZ and further incubated for 24 h. After that, the cells were treated by NIR irradiation (200 mW/cm^2, 10 min) and further incubated for 30 min. Subsequently, the cytosol protein was fractionated using a cytosol/ mitochondria fractionation kit (Invitrogen) according to the protocol provided by the manufacturer. After that, the cytosol protein was quantified by electrophoresis using BCA protein kit (Beyotime) and subjected to 12% SDS-containing polyacrylamide (SDS-PAGE) gel. The proteins were then transferred from the gel onto PVDF membrane (Immobilon P, Millipore) and blocked by primary and secondary antibodies. The blots were visualized by a molecular imager Versa Doc MP 4000 system (Bio-Rad).

Flow cytometry analysis of cell apoptosis. To analyze the ratio of cell apoptosis after treatment with HA-S-S-ZnPc-Lc@TPZ+NIR, the apoptosis levels were detected using Annexin V-FITC/PI Apoptosis Detection Kit (Invitrogen) with 3 replicates for statistical analysis. First, the 4T1 cells with an initial density of 1×10^6 cells/mL was seeded into 6-well plate and cultured for 24 h. Second, the original culture medium was removed with fresh ones containing PBS, TPZ (8 μg/mL) HA-S-S-ZnPc (200 μg/mL), HA-S-S-ZnPc-Lc (200 μg/mL), HA-S-S-ZnPc-Lc@TPZ(200 μg/mL) and further incubated for 24 h. After that, the cells were treated by NIR irradiation (200 mW/cm^2, 10 min) and further incubated for 30 min. At last, the cells were collected by centrifugation at 1000 rpm and the apoptosis ratios were investigated using Annexin V-FITC/PI Apoptosis Detection Kit (Invitrogen, USA).

Live-dead cell staining. To investigate the cell survival and death ratio, 4T1 cells were seeded into the confocal dish. After the confluency of the cells reached about 80%, the original culture medium was replaced by the fresh ones containing PBS, TPZ (8 μg/mL), HA-S-S-ZnPc (200 μg/mL), HA-S-S-ZnPc-Lc (200 μg/mL), HA-S-S-ZnPc-Lc@TPZ (200 μg/mL). The treated samples were further incubated for 24 h. After culturing for 24 h, the samples were irradiated with NIR (720 nm, 500 mW/cm^2, 10 min) and incubated for another 30 min, while the sample without NIR irradiation was used as a control. Subsequently, the cells were stained with Calcein AM/Propidium Iodide (PI) for 15 min, washed twice with PBS and observed using confocal microscope (LSM 510 Metanlo, Zeiss Co., Germany).

In vivo tumor model. Animal experiments were carried out in under the Animal Management Rules of the Ministry of Health of the People's Republic of China (Document No. 55, 2001) and in accordance with the protocol approved by Laboratory Animals of Chongqing Medical University. For the 4T1 cancer model, female nude mice with average weight of 21.5±1g were purchased from the Chongqing Medical University (Chongqing, China) and the mice were housed in the animal facility of SPF. To develop metastatic breast cancer model, the left flanks of mice were disinfected with 70% ethanol and injected with 1×10^7 4T1 mammary carcinoma cells suspended in 100 μL of PBS. To ensure the mice were maintained in the healthy condition, their body weight was monitored twice a day.

In vivo photodynamic therapy. The 4T1 bearing-tumor mice were randomly assigned to different treatment groups and the treatment was performed when the tumor volumes reached about 200 mm^3. The treatment setup was divided as follows: Group 1: PBS; Group 2: PBS+NIR; Group 3: TPZ+NIR; Group 4: HA-S-S-ZnPc-Lc+NIR, Group 5: HA-S-S-ZnPc-Lc@TPZ+NIR. The TPZ (2 $mg\cdot kg^{-1}$), HA-S-S-ZnPc-Lc (50 mg kg^{-1}) and HA-S-S-ZnPc-Lc@TPZ (50 mg kg^{-1}) were intravenously injected into each mouse and the mice were housed for 24 h. After injection for 24 h, the 4T1 tumor-bearing nude mice in Group 2, Group 3, Group 4 and Group 5 were exposed to NIR (720 nm, 500 $mW\cdot cm^{-2}$, 10 min). The tumor-bearing mice then were treated every day, while their tumor volumes change and body weight were recorded every two days. The tumor length (marked as L) and width (marked as W) was measured with a digital vernier caliper every two days and the tumor volume (marked as V) was calculated according to the formula $V=LW^2/2$. Twenty-one days post treatment, the tumor tissues and major organs (heart, liver, spleen, kidney, lung) were harvested for subsequent analysis. Then, the samples were fixed by formalin at 4°C for 24 h, embedded in the paraffin and sectioned for hematoxylin and eosin (H&E) and TUNEL staining.

In vivo fluorescence imaging. To investigate the fluorescence distribution in the mice, 4T1 bearing-mice were intravenously injected with the Cy5 (5mg/kg) and Cy5 labeled micelles of HA-S-S-ZnPc-Lc-Cy5 with the same equivalent Cy5 dose. After that, in vivo fluorescence imaging of the whole-body was carried out using IVIS Spectrum In Vivo Imaging System (Perkin Elmer, USA) at certain time intervals (0 h, 2 h, 6 h, 12 h, 24 h, 48 h, 72 h, 96 h). After injection for 24 h or 96 h, the mice were sacrificed and the main organs including heart, liver, spleen, lung, kidneys and brain were imaged.

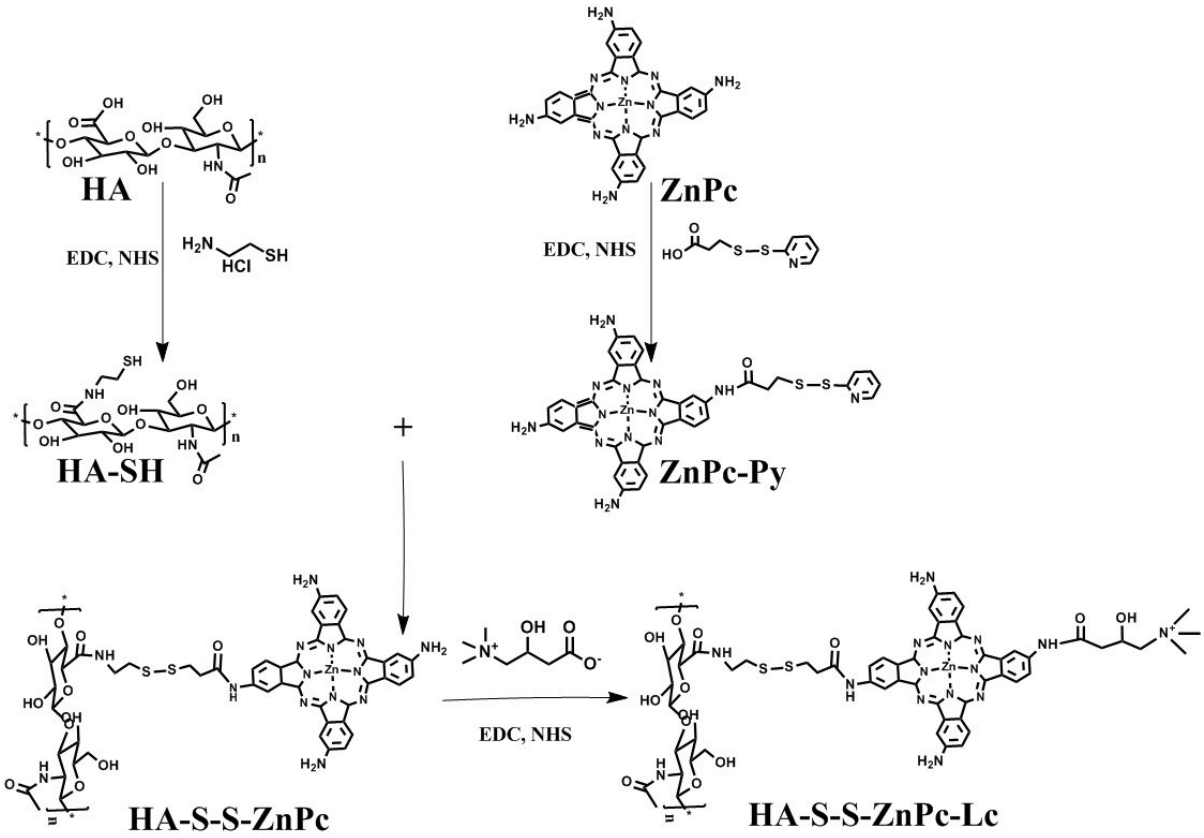
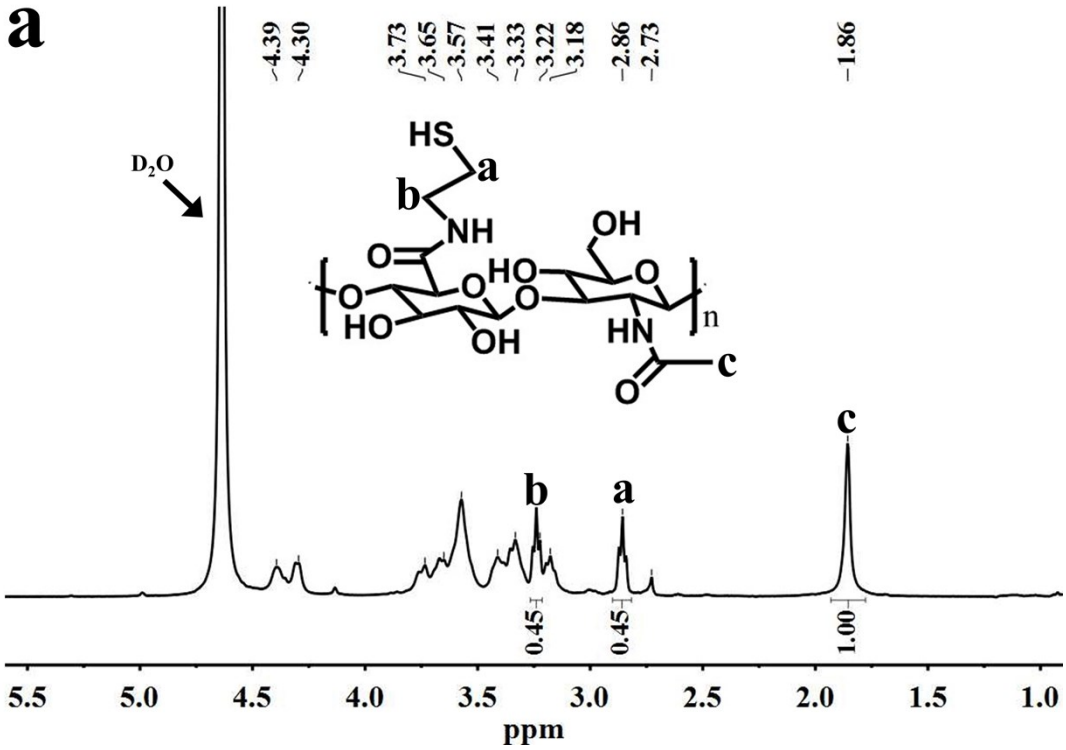


Figure S1. Synthetic route of HA-S-S-ZnPc-Lc biopolymer.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a
b
NH
O
OH
HO
O
n
HN
c
b
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c
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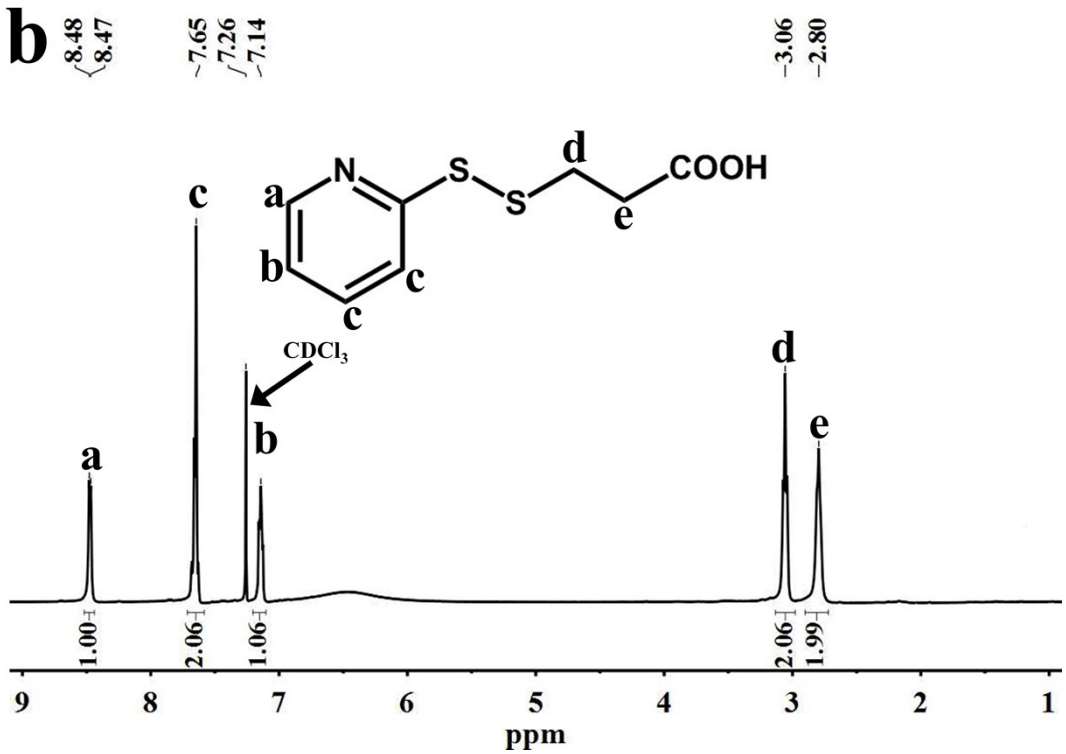
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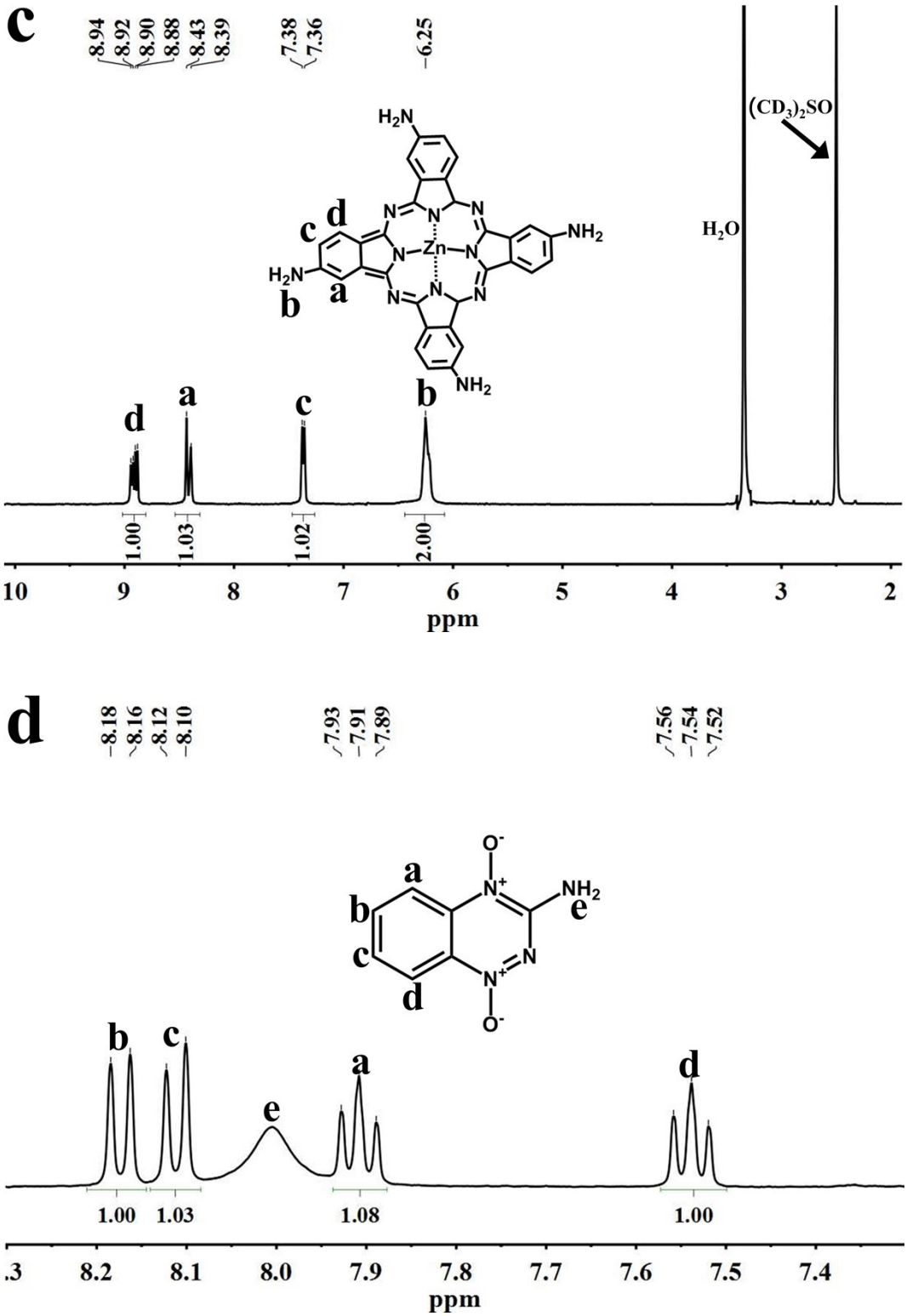


Figure S2. [1]H NMR spectra of (a): HA-SH, (b) Py-S-S-COOH, (c) ZnPc and (d) TPZ.

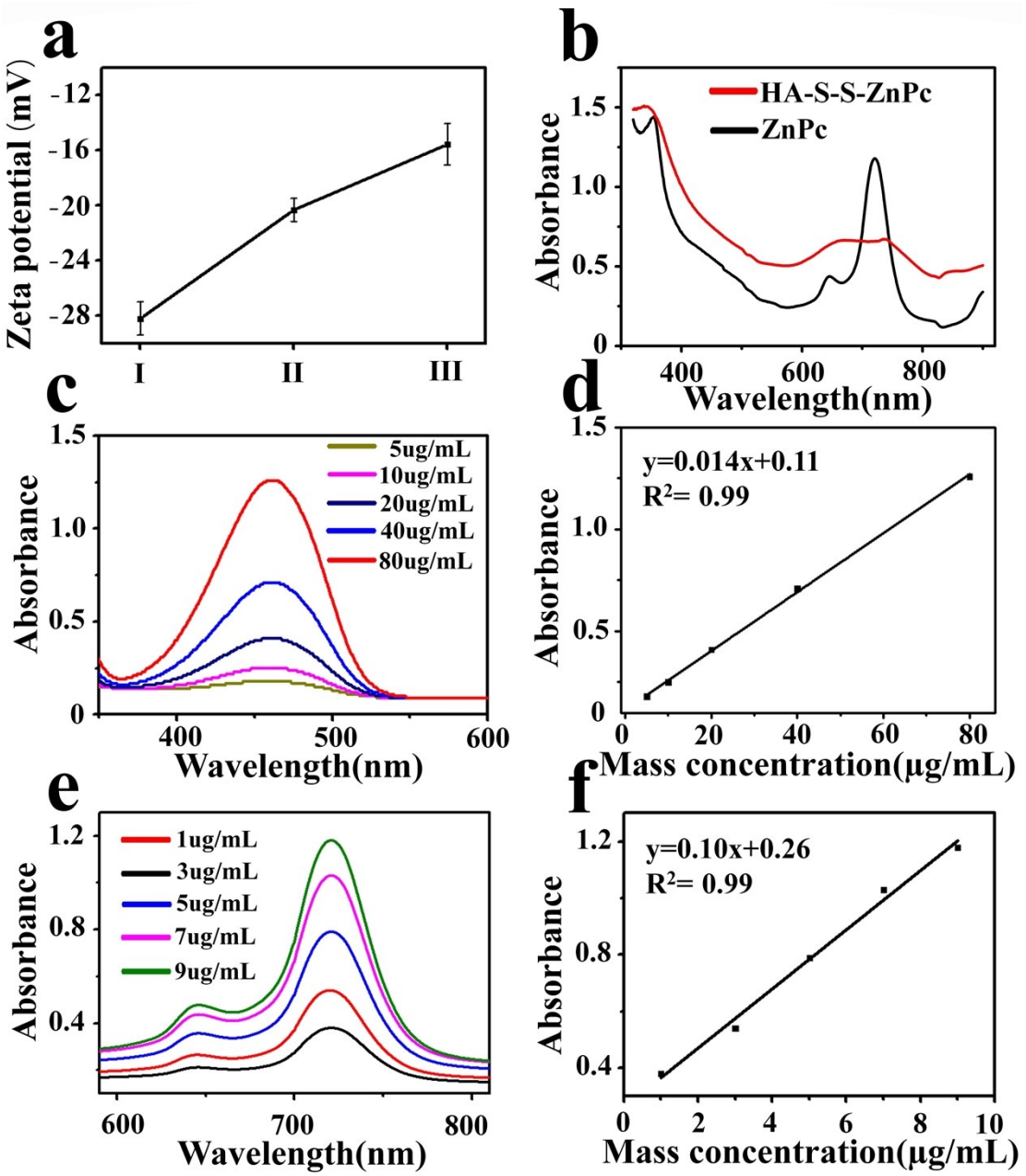


Figure S3. (a) Zeta potential changes throughout the synthetic process: (I) HA, (II) HA-S-S-ZnPc, (III) HA-S-S-ZnPc-Lc. (b) The UV/Vis absorption spectra of ZnPc (in DMSO) and HA-S-S-ZnPc (in water). (c) The UV/Vis absorption spectra of TPZ at different concentrations. (d) The standard curve of TPZ at different concentrations. (e) The UV/Vis absorption spectra of ZnPc at different concentrations. (f) The standard curve of ZnPc at different concentrations.

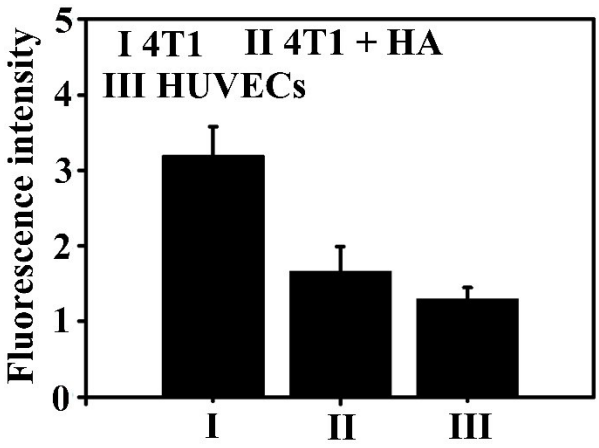


Figure S4. The quantitative analysis of micelle uptake under different conditions. (I) 4T1 cells after incubation with HA-S-S-ZnPc-Lc-FITC. (II) CD44-blocked 4T1 cells after incubation with HA-S-S-ZnPc-Lc-FITC. (III) HUVECs cells treated with HA-S-S-ZnPc-Lc-FITC.

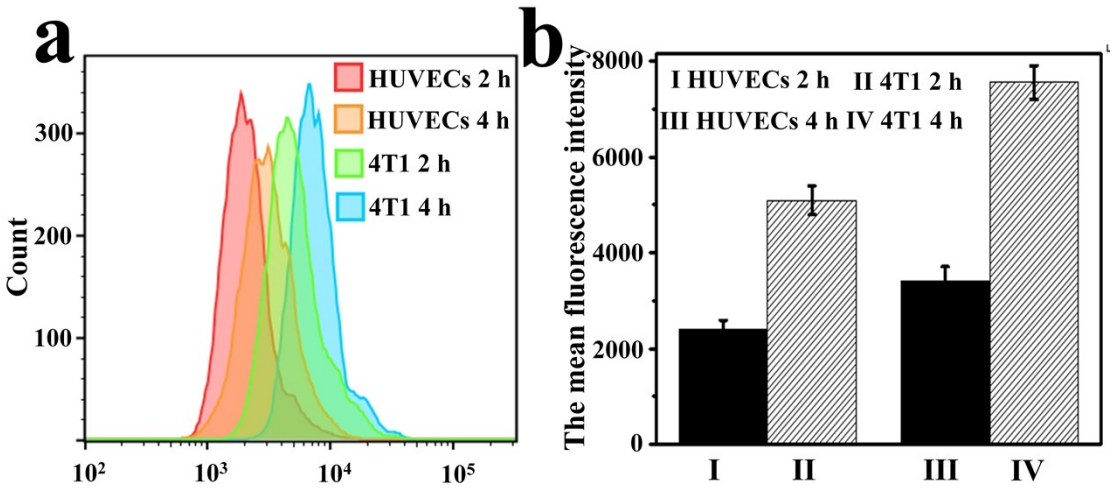


Figure S5. (a) Flow cytometric analysis on the endocytic uptake of FITC-labeled micelles by 4T1 and HUVECs cells after 2 h and 4 h of incubation. (b) Quantitative analysis of the flow cytometric data on the uptake amount of FITC-labeled micelles.

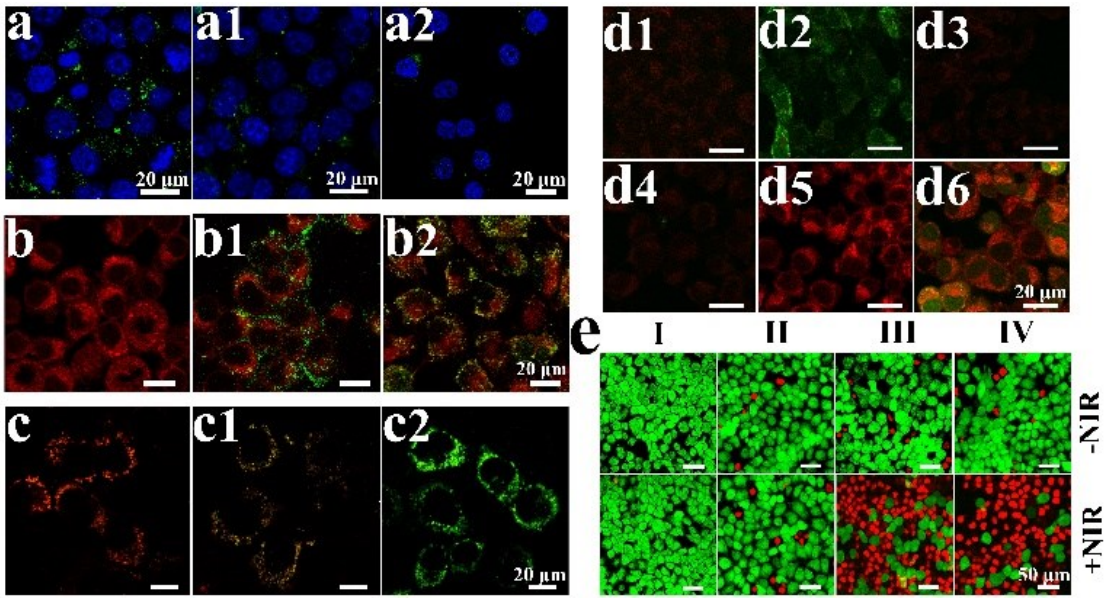


Figure S6. (a) CLSM images of 4T1 cells after incubation with HA-S-S-ZnPc-Lc-FITC. (a1) CLSM images of CD44-blocked 4T1 cells after incubation with HA-S-S-ZnPc-Lc-FITC. The cells were pretreated with HA (5 mg/mL) for competitive CD44 binding. (a2) CLSM images of HUVECs cells treated with HA-S-S-ZnPc-Lc-FITC. Scale bars: 20 μm. (b) CLSM images showing tumor mitochondria (red fluorescence) and the intercellular distribution of FITC-labeled biopolymers (green fluorescence) after treatment with PBS (b), HA-S-S-ZnPc-FITC (b1) and HA-S-S-ZnPc-Lc-FITC (b2). CLSM images of 4T1 cells stained with JC-1 to assess the mitochondrial damage after treatment with PBS (c), HA-S-S-ZnPc+NIR (c1) and HA-S-S-ZnPc-Lc+NIR (c2). (d) CLSM images on the changes in the intracellular ROS and hypoxia levels in 4T1 cells after treatments with PBS (d1), ROS inducer (d2), HA-S-S-ZnPc-Lc (d3), PBS+NIR (d4), hypoxia inducer (d5), HA-S-S-ZnPc-Lc + NIR (d6). The ROS inducer and hypoxia inducer were added as positive control. (e) Live/dead cell imaging of 4T1 cells after treatment with (I) PBS, (II) TPZ, (III) HA-S-S-ZnPc-Lc and (IV) HA-S-S-ZnPc-Lc@TPZ micelles with or without laser irradiation.

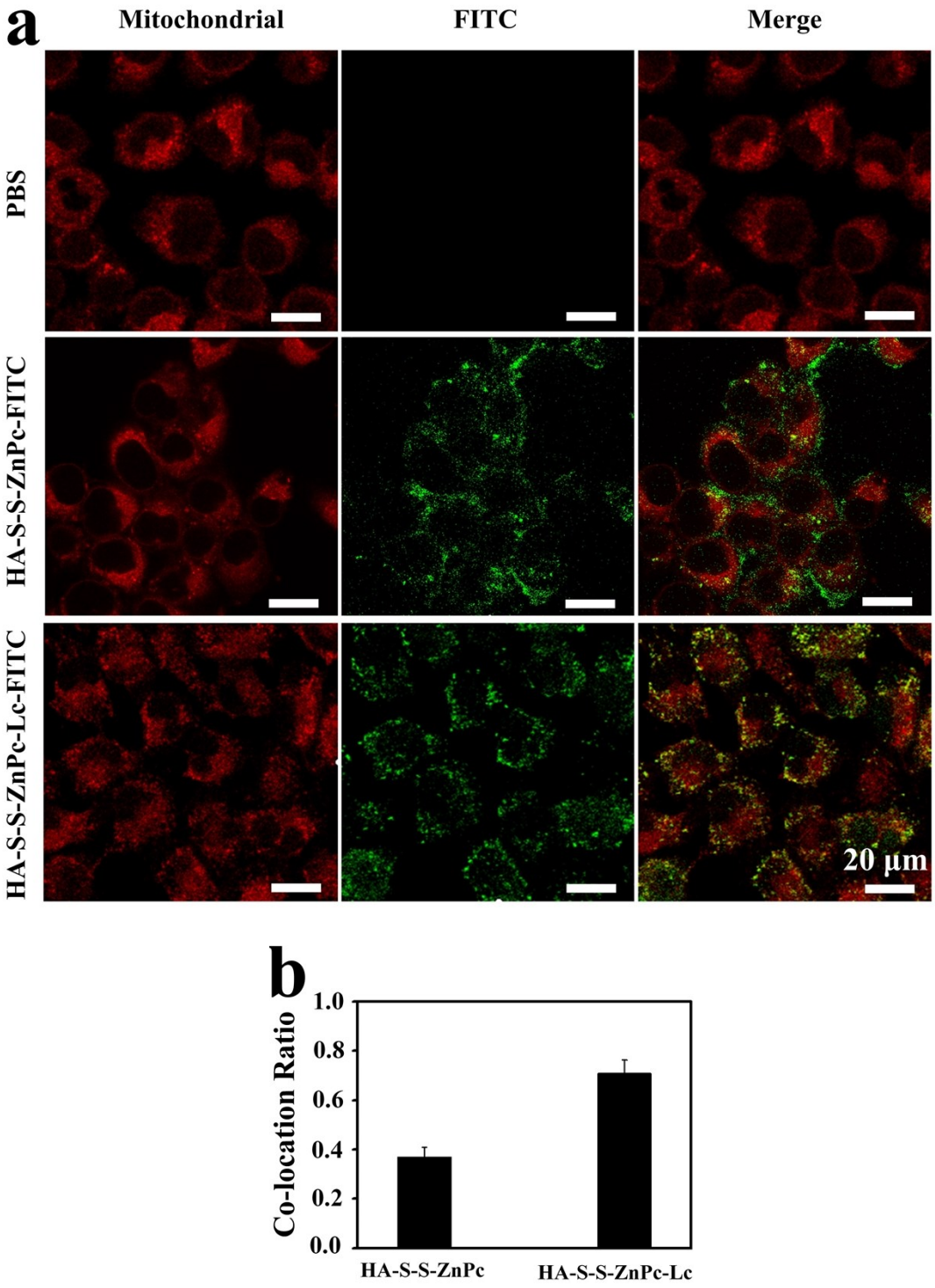


Figure S7. (a) Fluorescence images of the 4T1 cells after incubation with PBS, HA-S-S-ZnPc-FITC and HA-S-S-ZnPc-Lc-FITC (200μg/mL) for 24 h. (b) Fluorescence colocalization analysis on the mitochondrial targeting ability of the ZnPc-Lc.

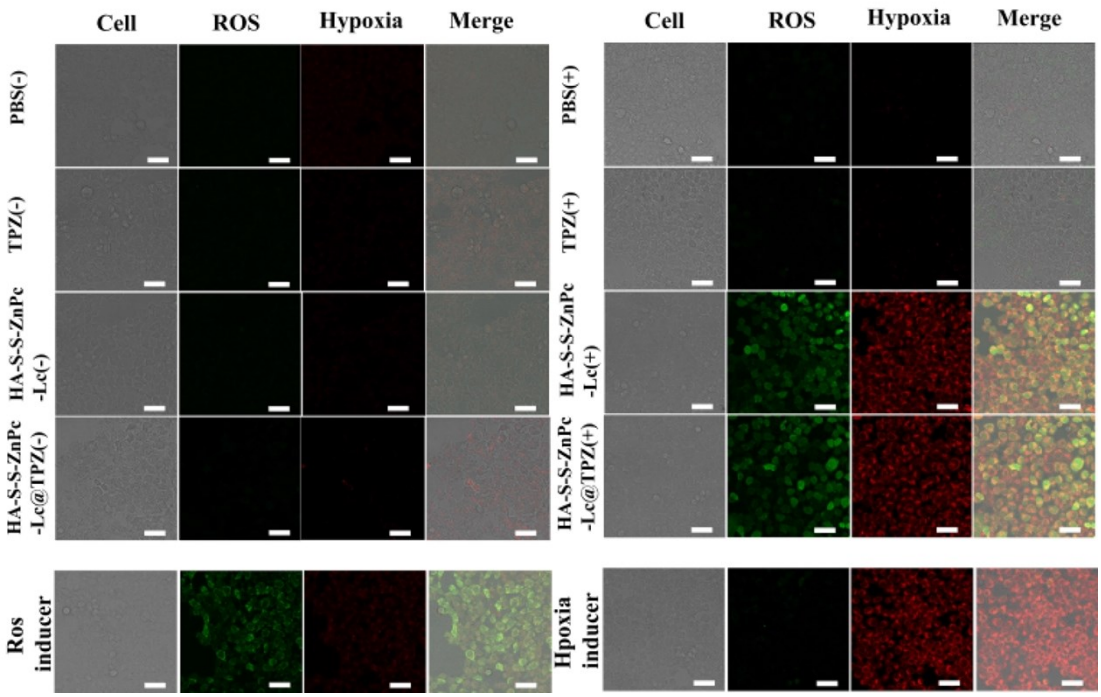


Figure S8. The ROS/hypoxia levels in 4T1 cells after treatment with PBS, TPZ, HA-S-S-ZnPc-Lc, HA-S-S-ZnPc-Lc@TPZ, PBS+NIR, TPZ+NIR, HA-S-S-ZnPc-Lc+NIR, HA-S-S-ZnPc-Lc@TPZ+NIR, hypoxia inducer (DFO) and ROS inducer (Pyo).

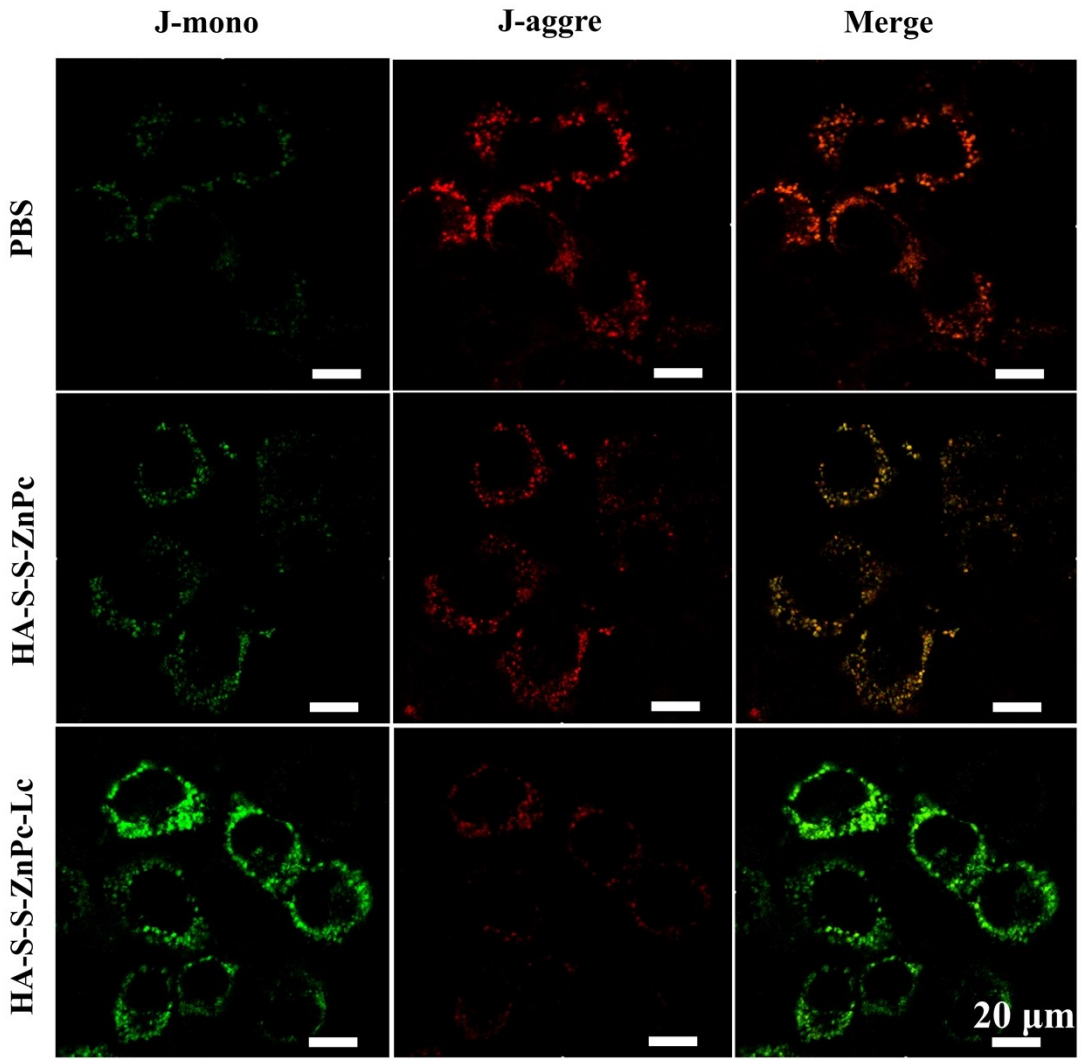
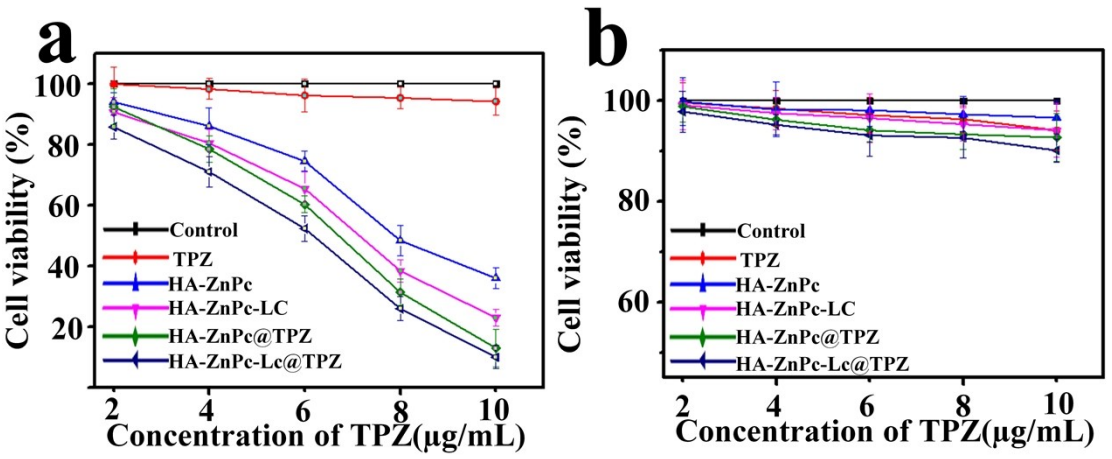


Figure S9. CLSM images of the mitochondrial membrane depolarization after treatment with PBS, HA-S-S-ZnPc, HA-S-S-ZnPc-Lc (200μg/mL) for 24h with NIR irradiation for 10 min at 200 mW/cm^2.



c

Group A Vs Group B		Concentration of TPZ (μg/mL)				
A	B	2	4	6	8	10
HA-S-S-ZnPc-Lc	HA-S-S-ZnPc-Lc@TPZ	*	*	*	**	**
HA-S-S-ZnPc	HA-S-S-ZnPc@TPZ	*	*	**	**	**
HA-S-S-ZnPc@TPZ	HA-S-S-ZnPc-Lc@TPZ	*	*	*	*	n.s
HA-S-S-ZnPc	HA-S-S-ZnPc-Lc@TPZ	*	*	***	***	***

Figure S10. (a) and (b) The 4T1 cell viability after the 24 h incubation with PBS, TPZ, HA-S-S-ZnPc, HA-S-S-ZnPc@TPZ, HA-S-S-ZnPc-Lc and HA-S-S-ZnPc-Lc@TPZ micelles with or without NIR irradiation (200 mW/cm^2, 10 min). (c) Statistical analysis on the tumor inhibition efficacy of different groups under NIR irradiation. (*P<0.05, **P<0.01, ***P<0.001).

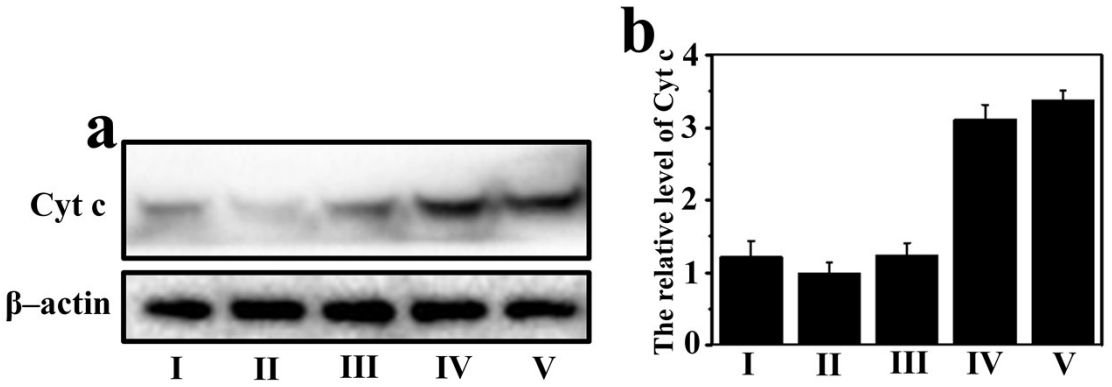


Figure S11. (a) Western blot showing the cytosolic level of cytochrome c (cyt c) after the 24 h incubation with PBS (I), PBS+NIR (II), TPZ+NIR (III), HA-S-S-ZnPc-Lc+NIR (IV) and HA-S-S-ZnPc-Lc@TPZ+NIR (V). (b) Western blotting assay for the quantitative analysis of cytochrome c release.

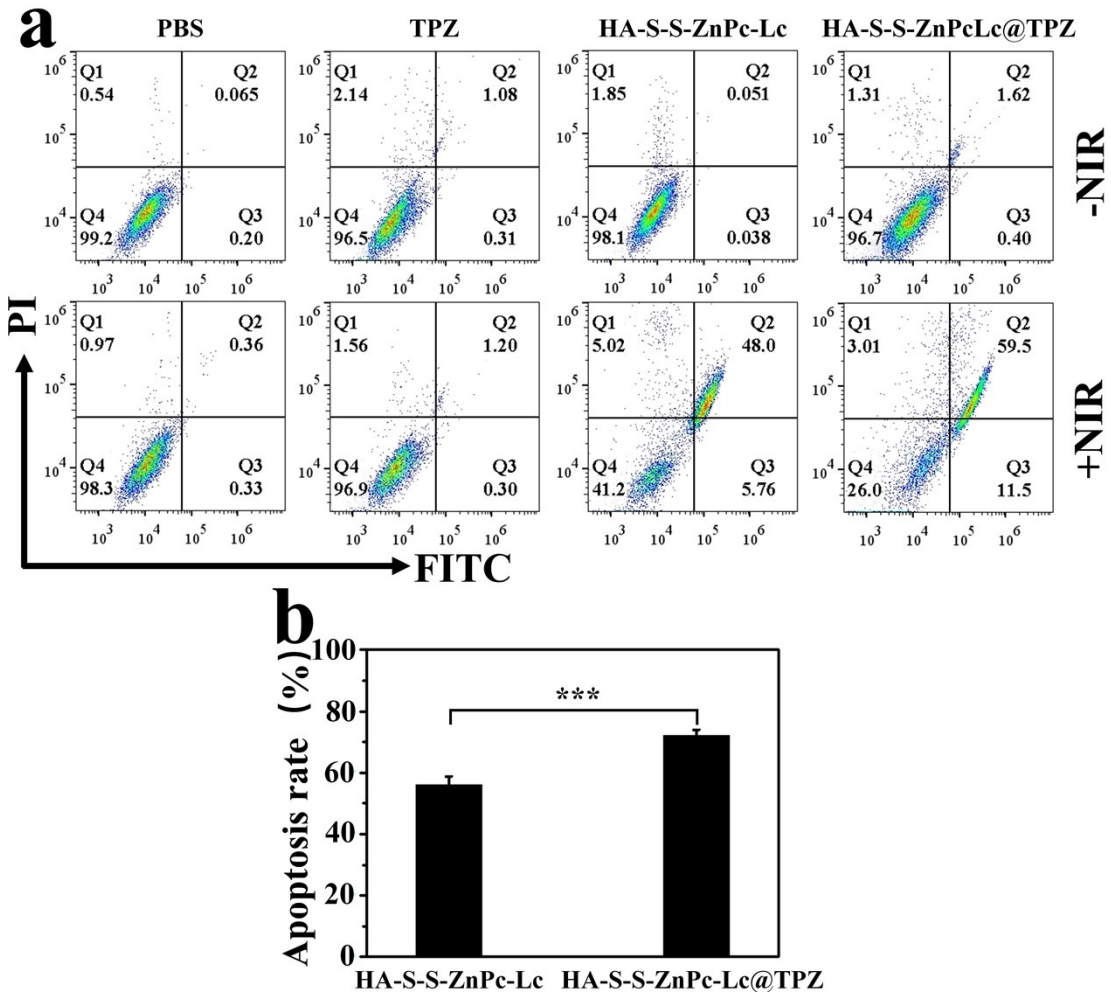


Figure S12. Cellular apoptosis levels in sample groups treated by PBS, TPZ HA-S-S-ZnPc-Lc, HA-S-S-ZnPc-Lc@TPZ micelles with or without NIR irradiation. (b) Flow cytometric analysis on apoptosis rate of 4T1 cells after incubation with HA-S-S-ZnPc-Lc and HA-S-S-ZnPc-Lc@TPZ (200μg/mL) for 24h with NIR irradiation for 10 min at 200 mW/cm^2. The asterisk symbol indicates the significance level (***$p < 0.001$) (n=3).

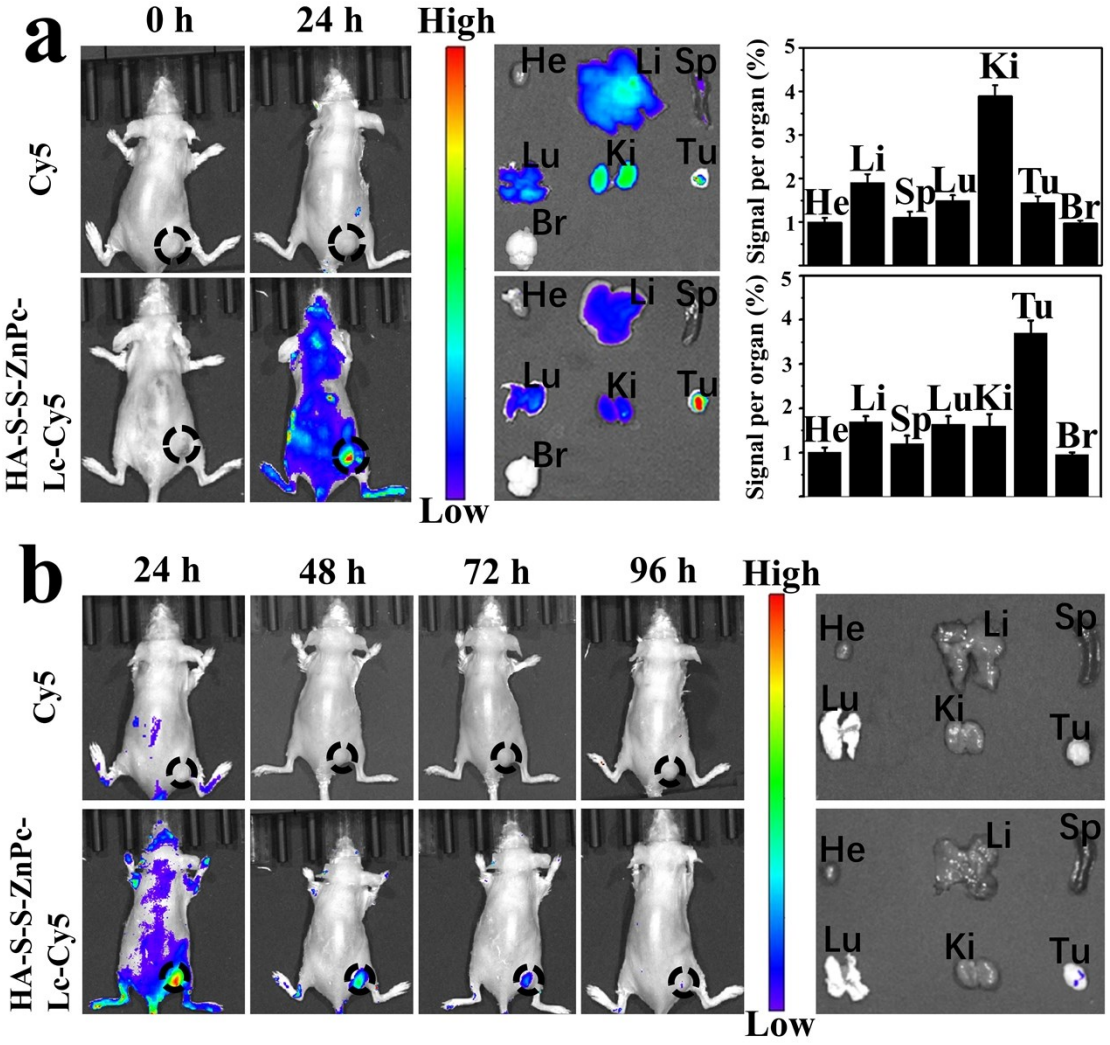


Figure S13. (a) The distribution of Cy5 and Cy5-labeled micelles in tumor-bearing mice after tail vein injection. Images in the mid column show the fluorescence distribution in different organs at 24 h post injection and images in the right column are the corresponding statistical analysis results. (b) Changes of Cy5 fluorescence of Cy5 and Cy5-labeled micelles in tumor-bearing mice after tail vein injection at different times. Images in the right column show the fluorescence distribution in major organs at 96 h post injection.

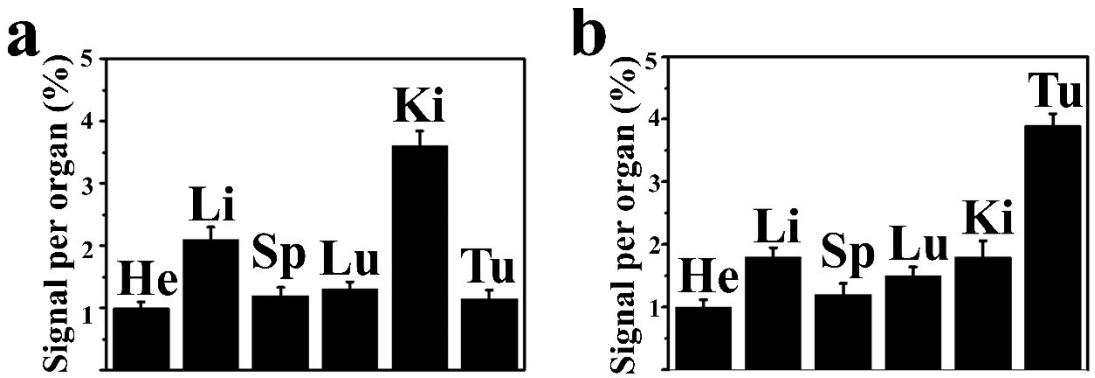


Figure S14. Fluorescence distribution in major organs after 24 h post injection. (a) Cy5 and (b) Cy5-labeled micelles.

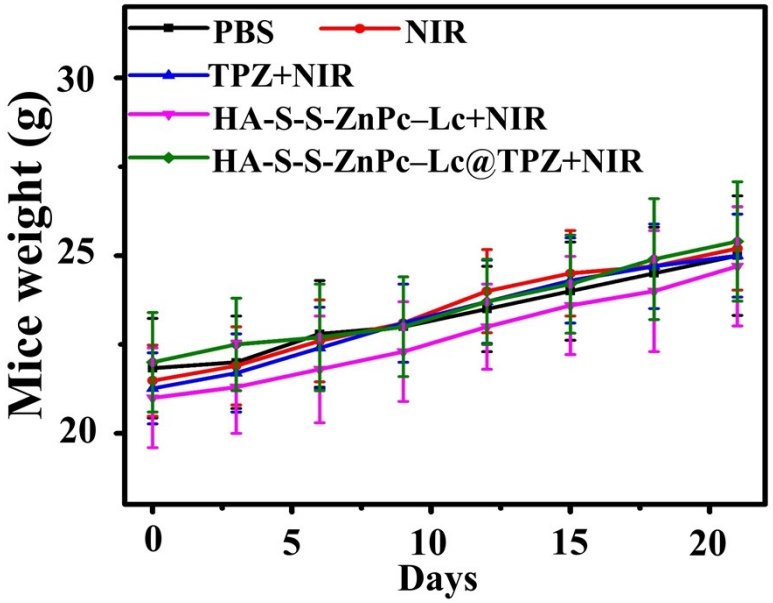


Figure S15. Changes in average mouse body weight after various treatments for 21 days.

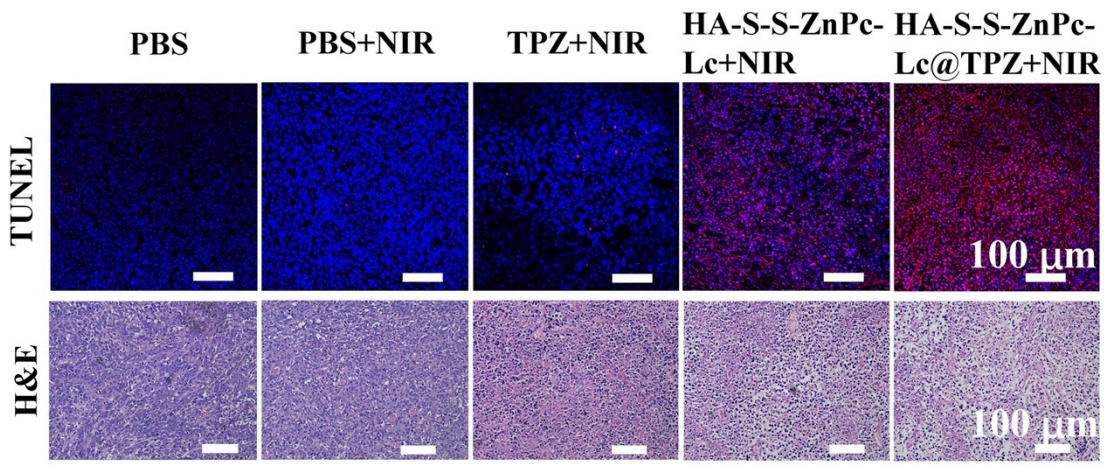


Figure S16. Histological analysis of tumor slices by H&E and TUNEL staining after treatments with PBS, PBS+NIR, TPZ+NIR, HA-S-S-ZnPc-Lc+NIR and HA-S-S-ZnPc-Lc@TPZ+NIR.

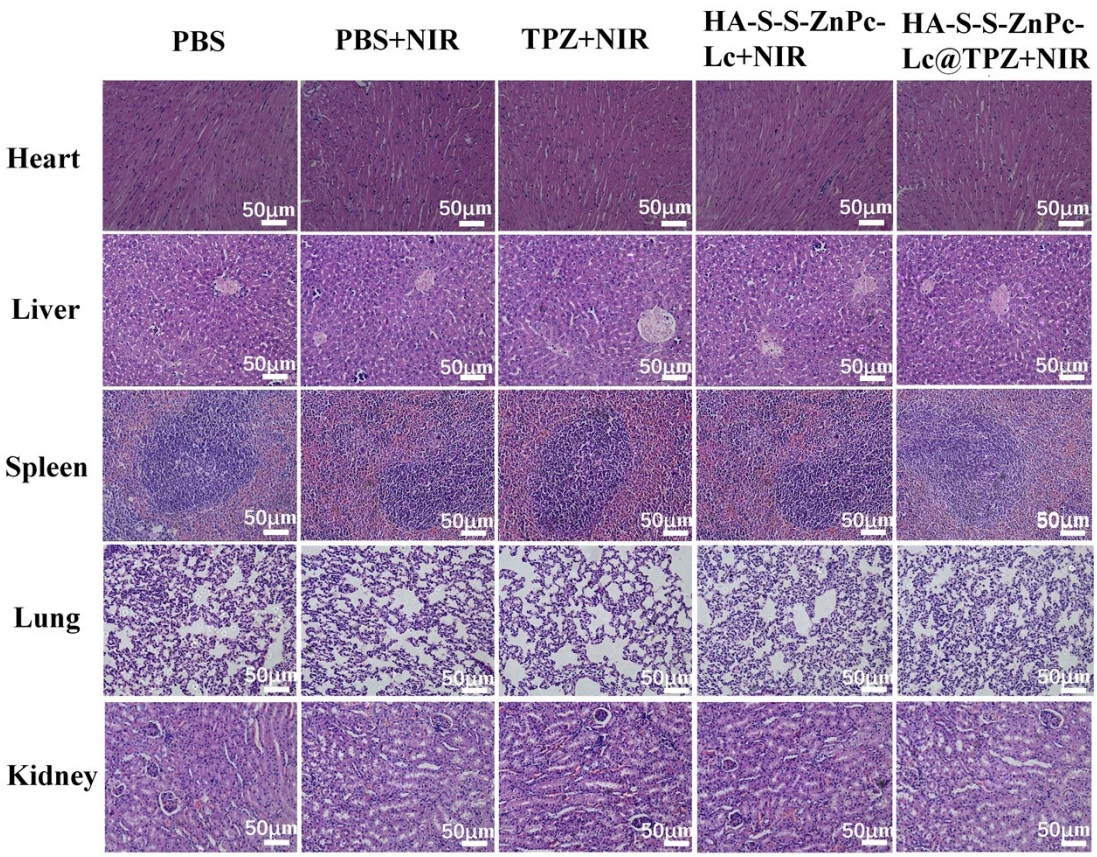


Figure S17. H&E analysis of major mouse organs (heart, liver, spleen, lung and kidney) after the treatment of PBS, PBS+NIR, TPZ+NIR, HA-S-S-ZnPc-Lc+NIR and HA-S-S-ZnPc-Lc@TPZ+NIR.