Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.

Supplementary Information

Structural Optimization of Organic Fluorophores for Highly Efficient Photothermal Therapy

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Materials

2, 4-Dimethly-1H-pyrrole was purchased from Suzhou Boke Chemistry Co., Ltd.. 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone (DDQ) and 1-Pyrenecarboxaldehyde were purchased from Tianjin Seans Biochemical Technology Co., Ltd.. Trifluoroacetic acid (TFA) was purchased from Adamas Reagent Co., Ltd.. Triethylamine (TEA) and 1-Naphthaldehyde were brought from Shanghai Aladdin Biochemical Technology Co., Ltd.. Benzaldehyde, boron (tri) fluoride etherate, piperidine and acetic acid were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd.. Hoechst 33258 were purchased from Jiangsu KeyGEN Biotechnology Co., Ltd.. Trypsin was purchased by Beijing Solarbio Science & Technology Co., Ltd..

Characterization

^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were measured in Chloroform-d ($CDCl_3$) at room temperature by an AV-400 NMR spectrometer from Bruker. UV and Fluorescence were recorded on TU-1901 (Beijing Purkinje General Instrument Co., Ltd.) and Edinburgh Instrument FLS-920 spectrometer, respectively. Diameter, diameter distribution of the nanoparticles were determined by Malvern Zeta-sizer Nano for dynamic light scattering (DLS). The measurement was carried out at 25 ^{o}C and the scattering angle was fixed at 90^o. The morphology of NPs was measured by transmission electron microscopy (TEM) performed on a JEOL JEM-1011 electron microscope operating. Analytical balance (XS105DU) and Rainin Pipettes from METTLER TOLEDO were used to quantify solid and liquid respectively. Confocal laser scanning microscopy (CLSM) images were taken using a Zeiss LSM 700 (Zurich, Switzerland). Near infrared fluorescence (NIRF) imaging was obtained by Meatro 500 FL *in vivo* optical imaging system (Cambridge Research & Instrumentation, Inc. USA).

Experiments

Synthesis of Ph-BDP, Na-BDP and Py-BDP. Synthesis of BDP. Benzaldehyde (10 mmol) was dissolved in dried dichloromethane (DCM) followed by addition of 2,4-dimethyl-1H-pyrrole (22 mmol) under Nitrogen (N_2) protection, and then trifluoroacetic acid was added. After stirring for 12 h, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone (10 mmol) was added, and the stirring was continued for 2 h. Triethylamine (10 mL) and boron (tri) fluoride etherate (12 mL) were added slowly. The mixed solution was stirred for 2 h and washed with water. Then the organic layer was dried and concentrated on a rotary evaporator, and finally purified by silica gel column. The product was a yellow solid. Yield: 30%. ^{1}H nuclear magnetic resonance (^{1}H NMR) (400 MHz, Chloroform-d ($CDCl_3$)) δ= 7.51-7.45 (m, 3H), 7.30-7.26 (d, 2H), 5.98 (s, 2H), 2.56 (s, 6H), 1.37 (s, 6H).

Synthesis of Ph-BDP, Na-BDP and Py-BDP. BDP (0.5 mmol) and benzaldehyde, 1-naphthaldehyde or 1-pyrenecarboxaldehyde (1.5 mmol) were added in toluene (30 mL), followed by the addition of a small amount of piperidine and acetic acid. The mixed solution was heated to 120 ^{o}C for 2 h under the protection of N_2. The final product was purified by a silica gel column.

Preparation of NPs. Preparation of Ph-BDP NPs, Na-BDP NPs and Py-BDP NPs. Ph-BDP (2 mg) was dissolved in tetrahydrofuran (THF, 4 mL) and then dropwise added pure water (10 mL). The stirring was kept at a constant speed until the THF was completely evaporated. Ph-BDP NPs were obtained after dialysis and centrifugation. Na-BDP NPs and Py-BDP NPs were prepared as described above.

Preparation of Py-BDP@F127 NPs. Py-BDP@F127 NPs were prepared by encapsulating Py-BDP into Pluronic F127 micelles. Py-BDP (4 mg) was dissolved in THF (4 mL) and then dropwise added into aqueous solution of F127 (20 mg in 10 mL water). The stirring was kept at a constant speed until the THF was completely evaporated. Ph-BDP@F127 NPs was obtained after dialysis and centrifugation.

Singlet oxygen detection. The ability of molecules in N, N-dimethylformamide (DMF) to produce singlet oxygen ($^1O_2$) *in vitro* were tested by the changes of absorbance of 1,3-diphenylisobenzofuran (DPBF). DPBF solution was added in Ph-BDP (Na-BDP or Py-BDP) solution and the absorption spectra of the mixed solution were measured after a specific time of illumination with red light emitting diode (LED) light (27 mW cm^{-2}). The detection interval

was 20 s. The concentration of BDP was 2 μM.

The ability of NPs in phosphate buffer saline (PBS) to produce $^1O_2$ *in vitro* were tested by the changes of absorbance of 9,10-anthracenediyl-bi(methylene)-dimalonic acid (ABDA). ABDA solution was added in Ph-BDP NPs (Na-BDP NPs, Py-BDP NPs) solution and the absorption spectra of the mixed solution were measured after a specific time of illumination with red LED light (27 mW cm^{-2}). The detection interval was 1 min. The concentration of BDP NPs was 10 μM.

***In vitro* photothermal effects.** The photothermal response of NPs in water (200 μL) was recorded with laser irradiation. The PCE was calculated using the reported methods.[Rs1] All temperatures were recorded every 10 s.

Cell culturing. All cells were grown in Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with 10% heat-inactivated fetal bovine serum (FBS, GIBCO), 100 U Ml^{-1} penicillin and 100 μg mL^{-1} streptomycin (Sigma). All the cells were cultured in a humidified incubator at 37 °C with 5% CO_2.

Cellular uptake. Human cervical carcinoma (HeLa) cells were inoculated into 6-well culture plates. After 24 h of incubation, NPs (10 μM) were added to the cells. After incubation for predetermined time, the supernatant was removed and HeLa cells were washed with PBS. Subsequently, the cells were fixed in 4% paraformaldehyde. Then the nuclei were stained with Hoechst 33258. Finally, slides were viewed by using a confocal laser scanning microscope (CLSM) imaging system.

Cytotoxicity assays. In order to evaluate the phototoxicity of NPs *in vitro*, HeLa and mouse breast carcinoma (4T1) cells were tested for cell viability by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays. Cells were inoculated into 96-well culture plates. After 24 h of culture, the NPs of different concentrations were used to incubate the cells, and 4 parallel wells were used for each sample concentration. After incubation for 4 h, cells in light group were irradiated with a 685 nm laser (0.55 W cm^{-2}) for 5 min, and those in dark group were placed in the same environment without light for 5 min. After 24 h of incubation, 20 μL of MTT solution (5 mg mL^{-1}) was added to each well. After 4 h, the supernatant was sucked out and 150 mL of dimethyl sulfoxide (DMSO) was added. The plates were shocked for 3 min and the absorption value at 490 nm was detected by a Bio-Rad 680 microplate reader.

Animals and tumor model. All experiments were performed in compliance with the guidelines of Chinese Academy of Science Committee for Animal Use and Care. Chinese Academy of Science Committee approved the experiments in this work. Mouse cervical carcinoma (U14) cells were injected subcutaneously into mice. When the tumor volume was 100 mm^3, the mice were randomly divided into different groups, 4 mice in each group.

***In vivo* near infrared fluorescence (NIRF) imaging.** The Py-BDP@F127 NPs were injected into the mice by intravenous injection. Fluorescence images of mice at different injection times were obtained by *in vivo* optical imaging system.

***In vivo* assessment of antitumor efficacy.** The concentrations of intratumoral BDP NPs were 1.13 μmol Kg^{-1} (irradiation conditions: 685 nm laser, 0.55 W cm^{-2} for 10 min). The concentration of intravenous Py-BDP NPs was 3.39 μmol Kg^{-1} (irradiation conditions: 685 nm laser, 0.4 W cm^{-2} for 10 min).

Statistical Analysis. Data were analyzed with One-way ANOVA (SPSS statistical software, Version 17.0). It was considered statistically significant when $P < 0.05$.

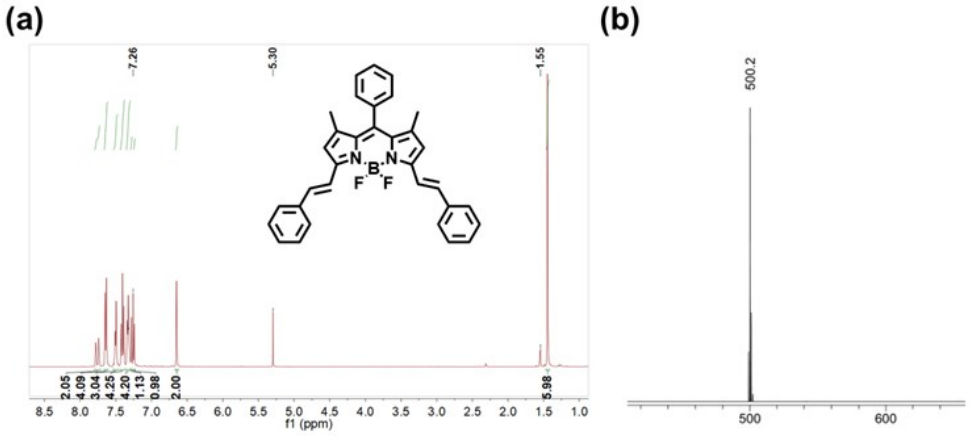

Fig. S1 ^{1}H NMR and MALDI-TOF MS spectra of Ph-BDP.

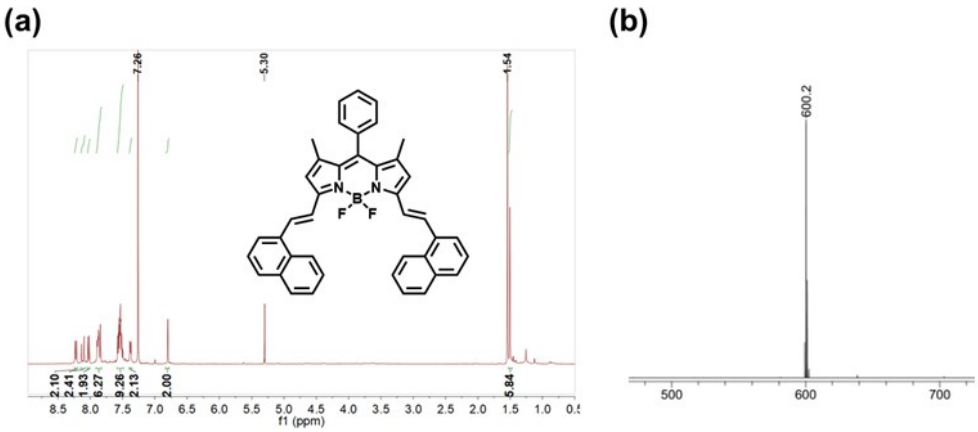

Fig. S2 ^{1}H NMR and MALDI-TOF MS spectra of Na-BDP.

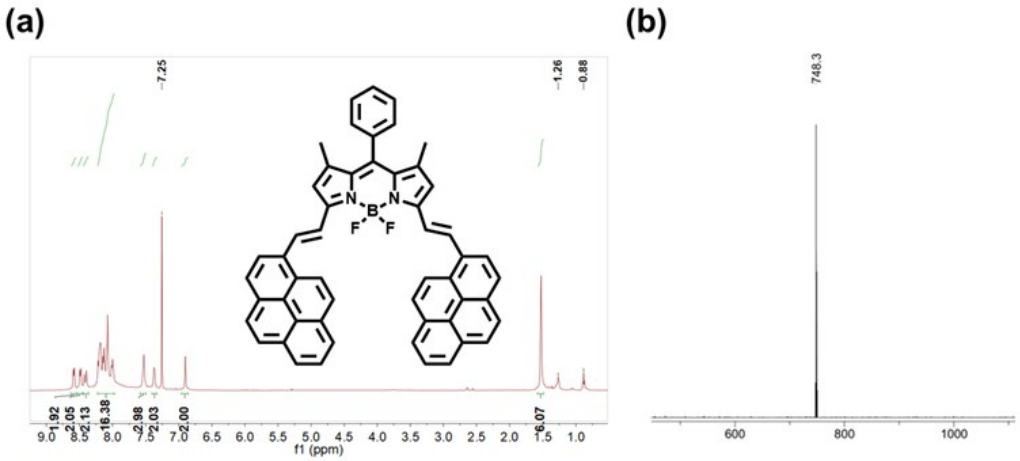

Fig. S3 ^{1}H NMR and MALDI-TOF MS spectra of Py-BDP.

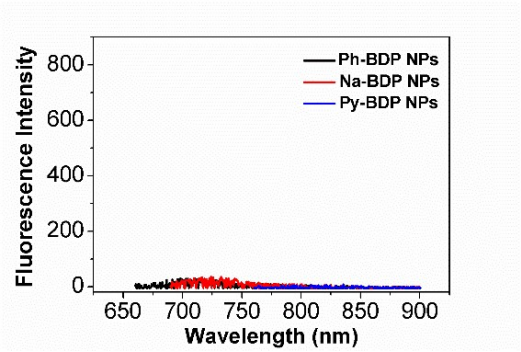

Fig. S4 The emission spectra of three BDP NPs in H_2O (10 μM).

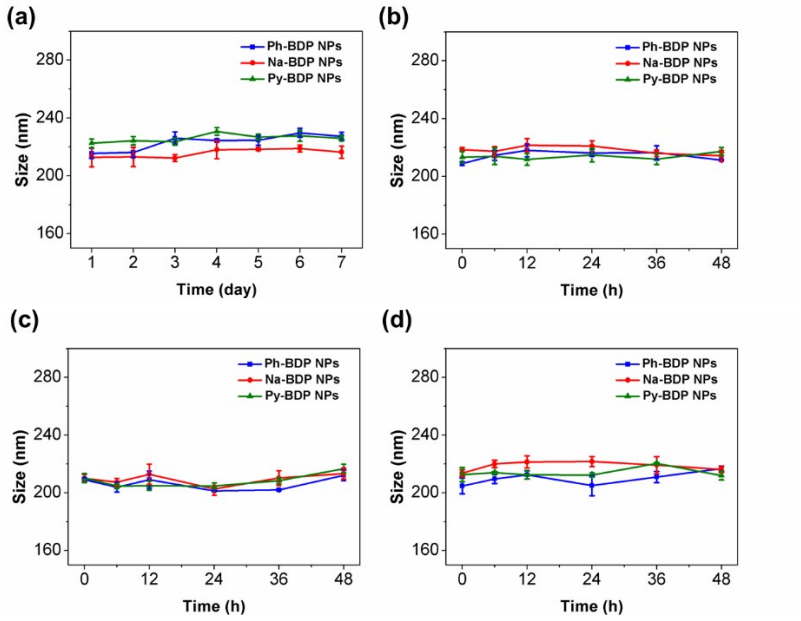

Fig. S5 Stability of three BDP NPs (10 μM) in (a) H_2O, (b) PBS, (c) PBS with 10% serum and (d) acid PBS (pH=6.8). The results are represented as mean ± SD (n=3). Error bars represent standard deviations of three independent experiments.

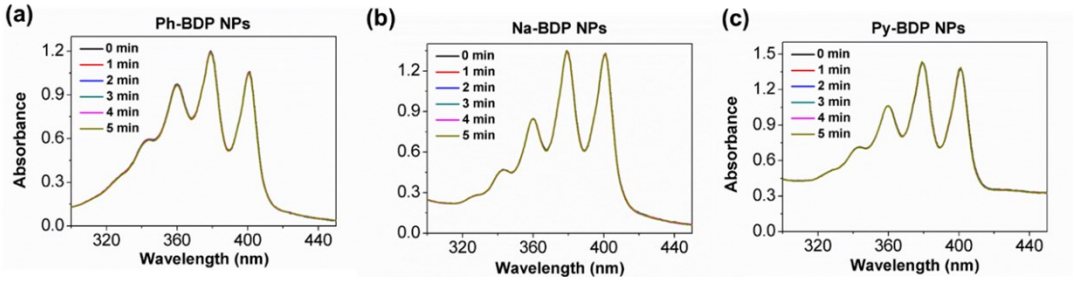

Fig. S6 Absorption spectra of ABDA with three NPs (10 μM) under red LED light irradiation (27 mW cm^{-2}) for different time.

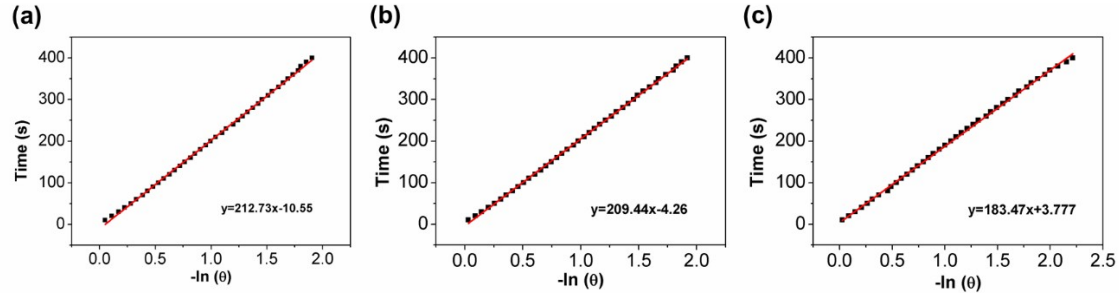

Fig. S7 Linear time data versus -ln (θ) obtained from the cooling period of (a) Ph-BDP NPs (25 μM), (b) Na-BDP NPs (25 μM) and (c) Py-BDP NPs (10 μM).

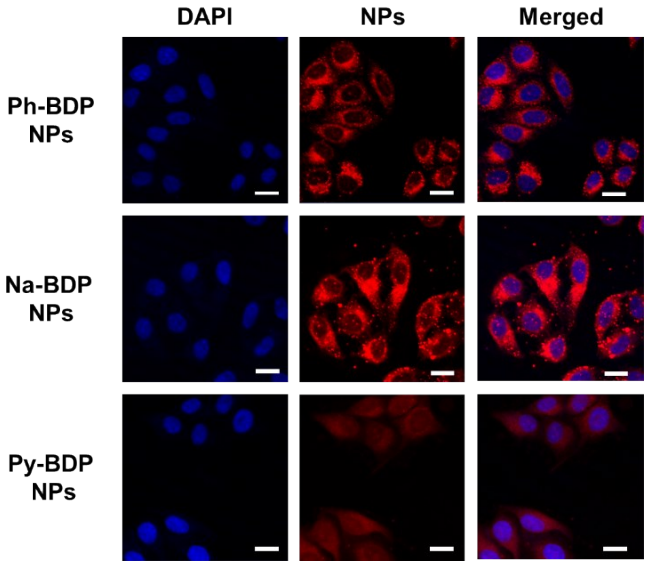

Fig. S8 CLSM images of three BDP NPs (10 μM) in HeLa cells (the endocytosis time was 4 h). Scale bar: 20 μm.

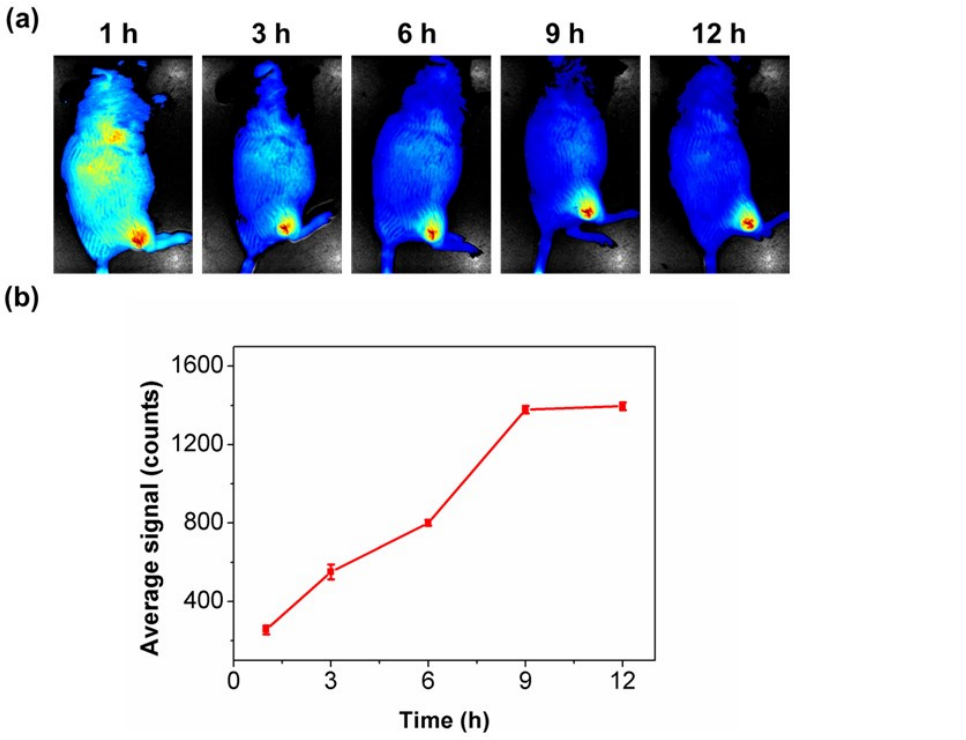

Fig. S9 (a) NIRF images of mice and (b) average signal of tumors after intravenous injection of Py-BDP@F127 NPs (0.17 mg mL^{-1}). The results are represented as mean ± SD (n=3). Error bars represent standard deviations of three independent experiments.

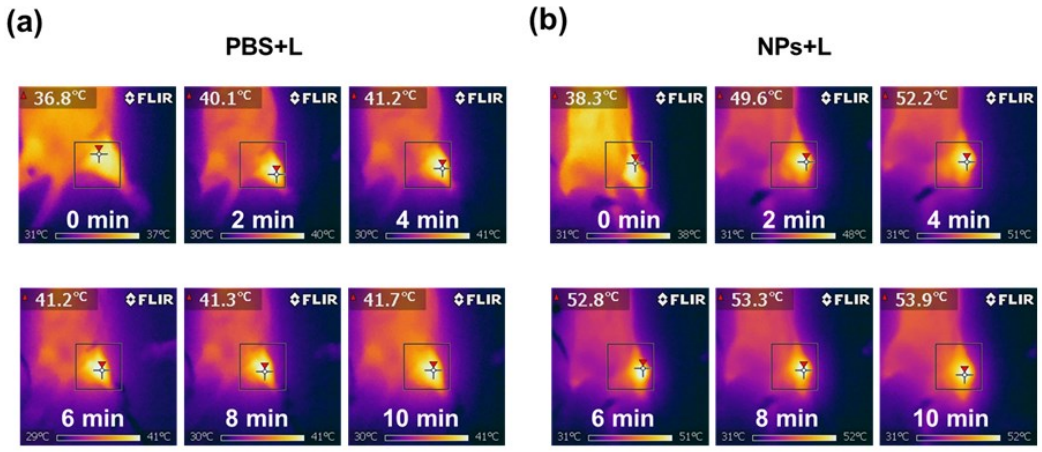

Fig. S10 Infrared thermal images of tumors injected with (a) PBS and (b) Py-BDP@F127 NPs under laser irradiation.

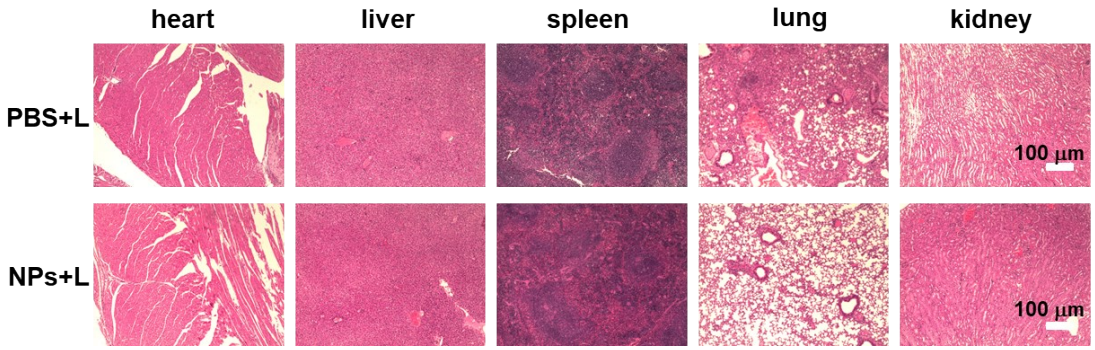

Fig. S11 H&E staining of major organs.

Reference

(S1) Li, C.; Zhang, W.; Liu, S.; Hu, X.; Xie, Z., Mitochondria-Targeting Organic Nanoparticles for Enhanced Photodynamic/Photothermal Therapy. *ACS Appl. Mater. Inter.* **2020**, 12, 30077-30084.