Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.

Supplementary Information

Copper depletion combined with photothermal therapy suppresses breast cancer

Chaonan Li,[ab] Dengyuan Hao,[ab] Xue Wang,[c] Tingting Sun*[a] and Zhigang Xie*[ab]

[a] State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, P. R. China

[b] School of Applied Chemistry and Engineering, University of Science and Technology of China, Hefei 230026, P. R. China

[c] Physical Examination and Health Management Department, China-Japan Union Hospital of Jilin University, Changchun, Jilin 130033, P. R. China

Materials

Succinic anhydride, 1-hydrcxybenzotrizole (HOBt) and triethylamine (TEA) were bought from Shanghai Aladdin Biochemical Technology Co., Ltd.. N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide (EDC•HCl) was bought from Beijing Innochem Co., Ltd.. $MPEG_{2000}$-DSPE was purchased from Shanghai Ponsure Co., Ltd.. 6 well and 96 well cell culture plate were bought from NEST Biotechnology Co., Ltd.. Cell viability (live/dead cell staining) assay kit, Hoechst 33258 and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Jiangsu KeyGEN Biotechnology Co., Ltd.. 100 mm cell and tissue culture dishes were purchased from Guangzhou Jet Bio-Filtration Co., Ltd..

Characterizations

^1H NMR spectra were measured in $CDCl_3$ at room temperature by an AV-400 NMR spectrometer from Bruker. Absorption and fluorescence spectra were recorded on Pgeneral TU-1901 and Edinburgh Instrument FLS-920 spectrometer, respectively. Diameter and diameter distribution of the nanoparticles were determined by Malvern Zeta-sizer Nano for dynamic light scattering (DLS). The measurement was carried out at 25 ^oC and the scattering angle was fixed at 90^o. The morphology of the nanoparticles was measured by transmission electron microscopy (TEM) performed on a JEOL JEM-1011 electron microscope operating at an acceleration voltage of 100 kV. 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).

Experiments

Synthesis of TBDP-COOH. TBDP (synthesized according to the previously reported method,[1] 0.1 mmol) and succinic anhydride (0.5 mmol) were solved in DMF and stirred at reflux under N_2 protection. After stirring for 24 h,, the solution was concentrated. The final product was purified by a silica gel column. The product was dark green solid. Yield: 45%.

Synthesis of PY-NH_2. The Synthesis of PY-NH_2 according to the reported method.[2]

Synthesis of PY-TBDP. TBDP-COOH (0.05 mmol) and PY-NH_2 (0.05 mmol) were dissolved in dry CH_2Cl_2, then EDC•HCl (0.075 mmol), HOBt (0.075 mmol) and TEA (0.075 mmol) were added in the mixed solution. After 24 h of stirring at room temperature, the solution was concentrated. The final product was purified by a silica gel column. The product is dark green solid. Yield: 40%.

Detection of chelated metal ions. Different concentrations of copper ion solutions were added to the PY-TBDP solution (10 μM in DMF), and the emission spectra of the mixed solutions were measured. Other metal ions were added into the PY-TBDP solution (10 μM in DMF), and the emission spectra of the mixed solution were measured. All metal ion solutions were prepared and used immediately. The Cu^+ solution was prepared with a certain concentration of cuprous bromide solution.

Preparation of NPs. PY-TBDP or TBDP (3 mg) was solved in THF (4 mL) and then slowly dropped into pure water containing $MPEG_{2000}$-DSPE (15 mg in 10 mL pure water). Stir at a constant speed until the THF was completely evaporated. PY-TBDP NPs and TBDP NPs was obtained by dialysis and centrifugation.

***In vitro* photothermal effects.** The photothermal response of NPs in water (200 μL) was recorded with laser irradiation (685 nm laser, 0.49 W cm^{-2}, 5 min) and then shut off. The photothermal conversion efficiency was calculated using the reported methods.[3] 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), and the culture medium was replace every day. All the cells were cultured in a humidified incubator at 37°C with 5% CO_2.

Cellular uptake. 4T1 cells were inoculated into 6-well culture plate. After 24 h of incubation, NPs (30 μM, diluted with DMEM) were added to the cells. After different incubation time, the supernatant was removed and the cells were washed with PBS. Subsequently, the cells were immersed in 4% paraformaldehyde fix solution (1 mL) to each well at room temperature. After 10 min, cells were washed twice with PBS. The nuclei were then stained with Hoechst 33258. Slides were mounted and viewed using a confocal laser scanning microscope imaging system.

Cytotoxicity assays. In order to evaluate the cytotoxicity of PY-TBDP NPs *in vitro*, 4T1, HeLa and L929 cells were tested for cell viability by MTT assay. Cells were inoculated into 96-well culture plate. After 24 h of culture, the NPs of different concentrations were used to incubate the cells and four parallel holes were used for each sample concentration. After 24 or 48 h of incubation, 20 μL of MTT solution (5 mg mL^{-1}) was added to each orifice plate. After 4 h, the supernatant was sucked out and 150 mL of DMSO was added. The orifice plate was shocked for 3 min and the absorption value at 490 nm was detected by a micro-plate reader.

Phototoxicity test: cells were incubated with PY-TBDP NPs for 4 h and then irradiated with laser (685 nm laser, 0.49 W cm^{-2}, 5 min).

Live and dead cell staining assays. In order to visualize the phototoxicity of PY-TBDP NPs *in vitro*, 4T1 cells were stained with PI and calcein-AM. First, cells were incubated with PY-TBDP NPs of different concentration. Second, cells in light group were irradiated with a 685 nm laser at 0.49 W cm^{-2} for 5 min and in dark group were placed in the same environment without light for 5 min. After 24 h of incubation, the cells were stained with calcein-AM and PI at room temperature for 30 min. Subsequently, the staining solution was sucked out and cells were washed by PBS. Finally, the cells were imaged by a Nikon C1si laser scanning confocal microscopy. In the picture, green represented living cells and red represented dead cells.

Animals and tumor model. All animal experiments have been approved (Approved No. 20220005) by the Ethics Committee of Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, and carried out according to Animal management regulations approved by the State Science and Technology Commission of the People's Republic of China and Instructive notions with respect to caring for laboratory animals approved by the Ministry of Science and Technology of the People's Republic of China. 4T1 cells were injected subcutaneously into mice. The mice were randomly divided into different groups with 4 mice in each group. Subsequently, intravenous injection of NPs (4 μmol kg^{-1}) were given to the treatment group. 6 h after the third intravenous injection, the mice in the light (L) group were irradiated with laser (0.4 W cm^{-2}).

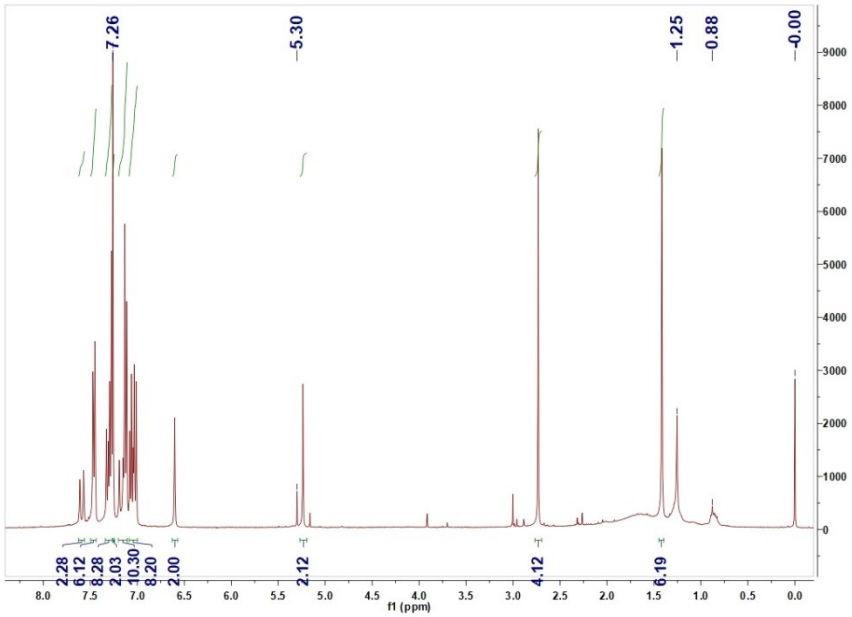

Fig. S1 ^{1}H NMR spectrum of TBDP-COOH.

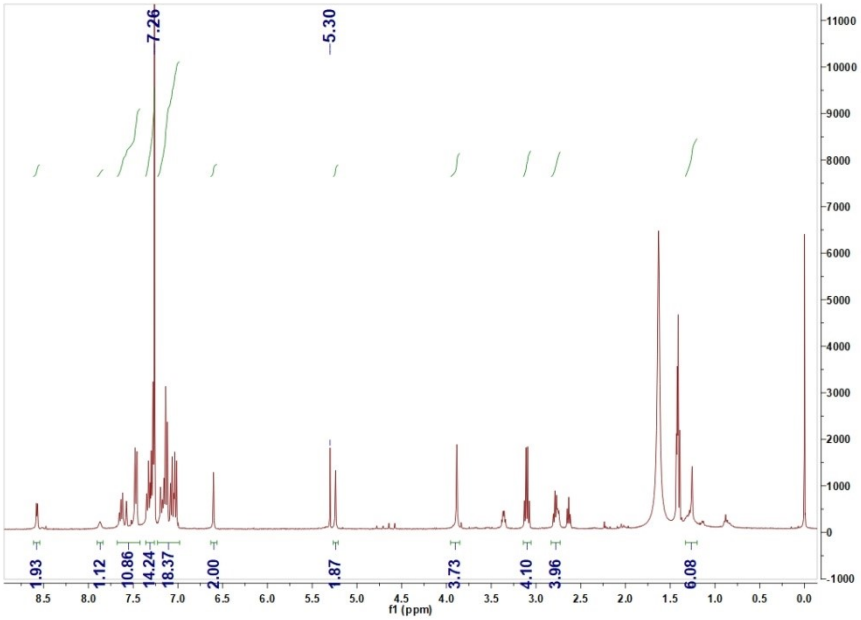

Fig. **S2** ^{1}H NMR spectrum of PY-TBDP.

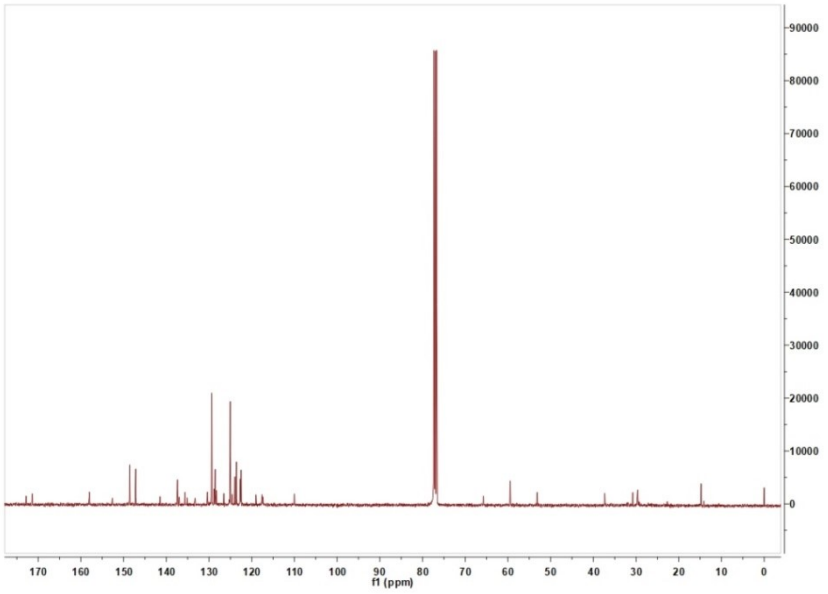

Fig. S3 ^{13}C NMR spectrum of PY-TBDP.

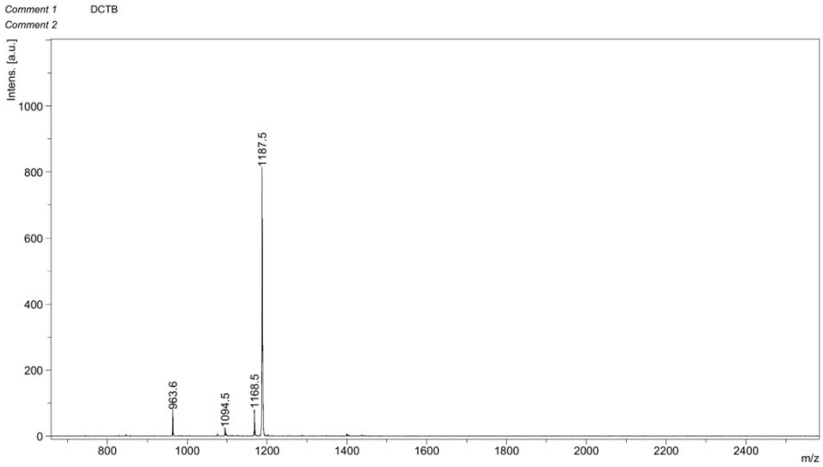

Fig. **S4** The MS spectrum of PY-TBDP.

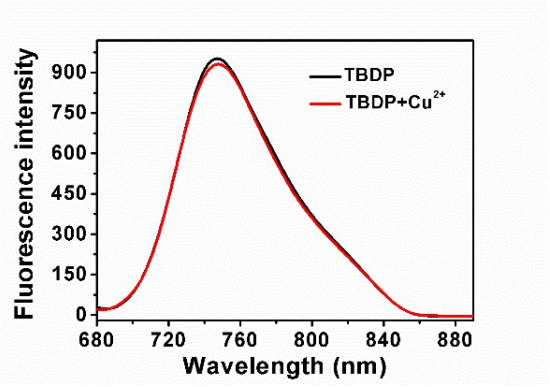

Fig. **S5** The emission spectra of TBDP (10 μM) in DMF without and with Cu^{2+} (10 μM).

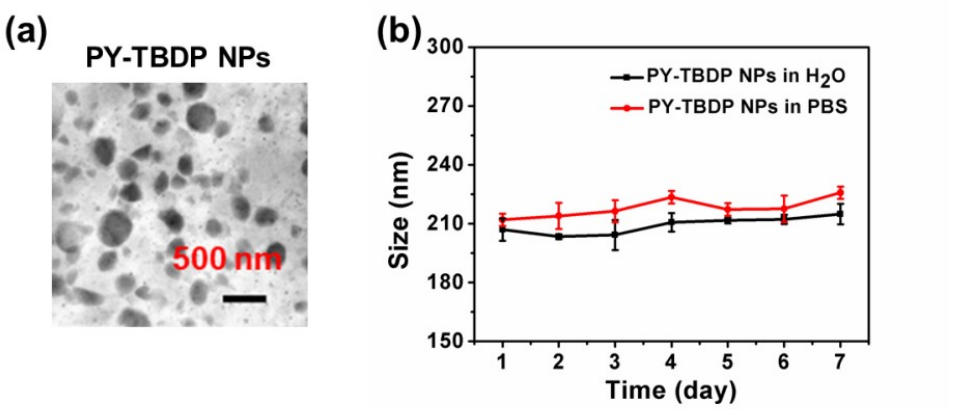

Fig. **S6** (a) TEM image of PY-TBDP NPs. (b) Stability of PY-TBDP NPs in H_2O and phosphate buffer solution (PBS).

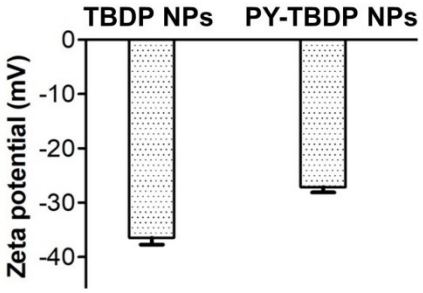

Fig. **S7** The surface zeta potential of TBDP NPs and PY-TBDP NPs.

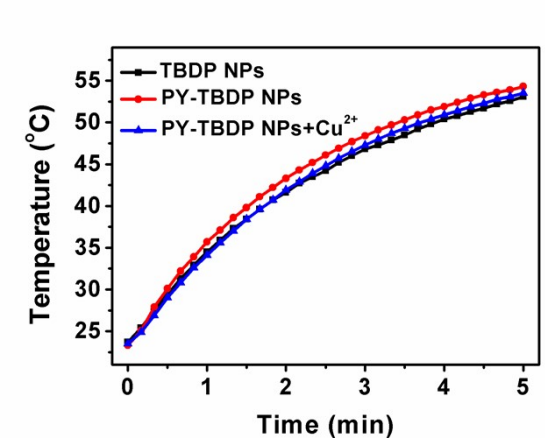

Fig. **S8** The temperature variation curves of TBDP NPs, PY-TBDP NPs and PY-TBDP NPs with Cu^{2+} under irradiation (685 nm laser, 0.49 W cm^{-2}).

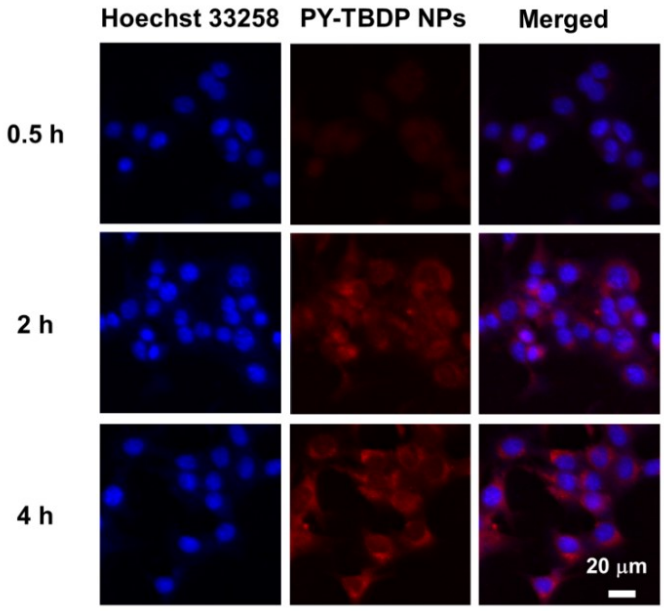

Fig. **S9** CLSM images of 4T1 cells with incubation of PY-TBDP NPs (30 μM) at 37°C for 0.5, 2 and 4 h.

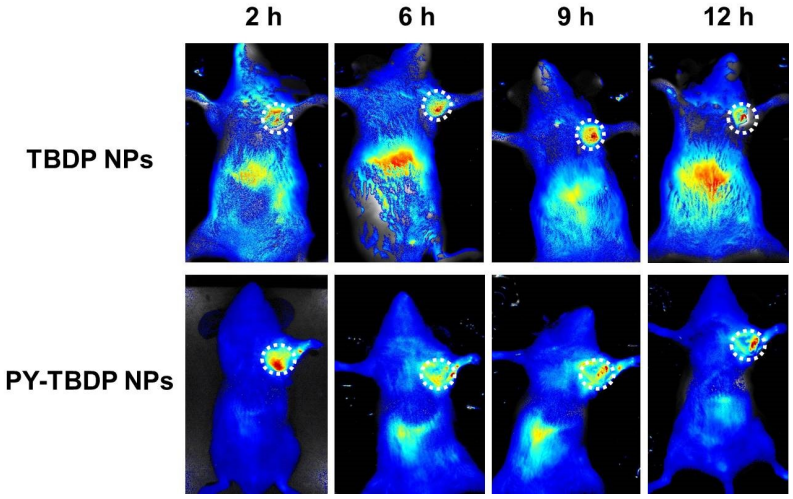

Fig. **S10** Fluorescence images (the white dotted circles indicate the tumor region) of the mice after tail vein injection of TBDP NPs and PY-TBDP NPs for different times.

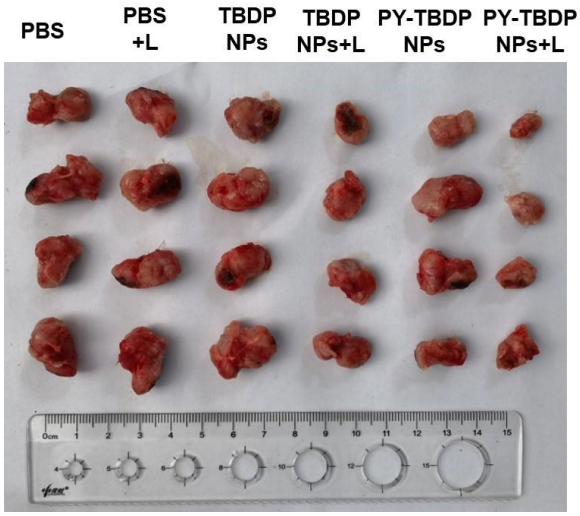

Fig. **S11** Tumor photos of the mice on the 18th day of treatment.

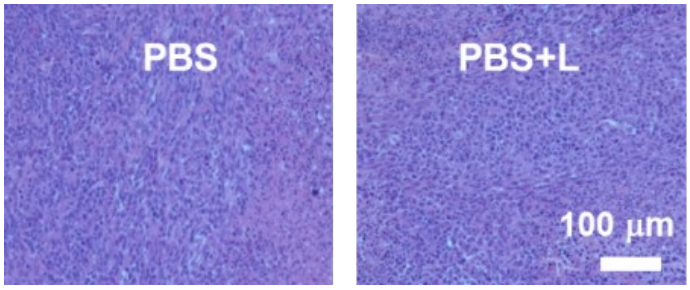

Fig. **S12** H&E staining of the tumors of the mice in the groups of PBS and PBS+L.

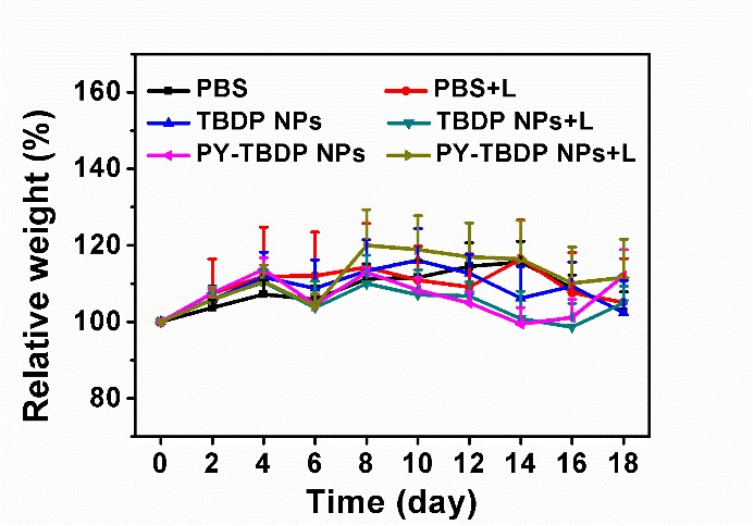

Fig. **S13** Changes of body weights of the mice during different treatments.

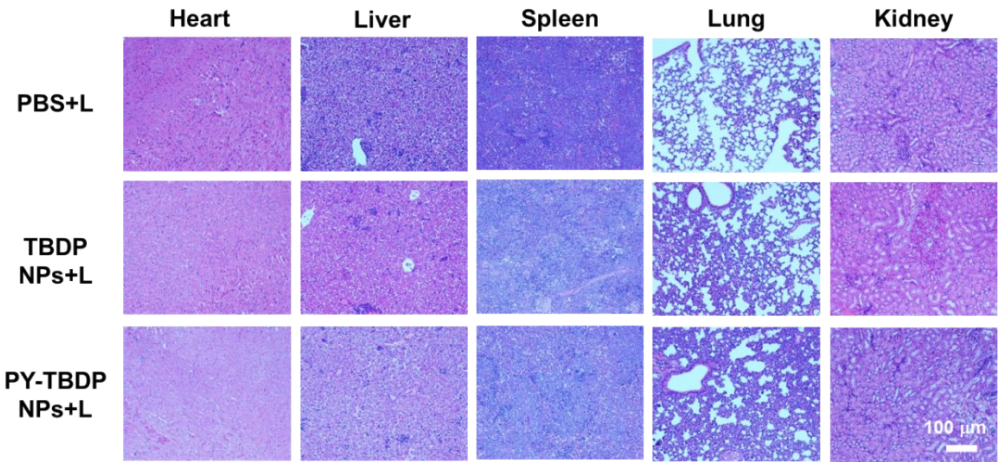

Fig. **S14** H&E staining of the main organs of the mice in the groups of PBS, TBDP and PY-TBDP.

References

1 C. Li, W. Lin, S. Liu, W. Zhang and Z. Xie, Self-destructive PEG-BODIPY nanomaterials for photodynamic and photothermal therapy, *J. Mater. Chem. B*, 2019, **7**, 4655-4660.

2 A. J. Surman, C. S. Bonnet, M. P. Lowe, G. D. Kenny, J. D. Bell, É. Tóth and R. Vilar, A pyrophosphate-responsive gadolinium(III) MRI contrast agent, *Chem. Eur. J.*, 2011, **17**, 223-230.

3 C. Li, W. Zhang, S. Liu, X. Hu and Z. Xie, Mitochondria-targeting organic nanoparticles for enhanced photodynamic/photothermal therapy, *ACS Appl. Mater. Interfaces*, 2020, **12**, 30077-30084.