Three-Dimensional BODIPY-Iron(III) Compound with Promoted H_2O_2-Response for NIR-II Photoacoustic Imaging Guided Chemodynamic/Photothermal Therapy

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MATERIALS AND METHODS

1. Materials and apparatus

All of chemicals were purchased from Adamas-beta Co. Ltd., and were used without further purification unless otherwise state. The synthesis of BODIPY were according to reported literature.[1] ^{1}H NMR and ^{13}C NMR spectra were measured on a Varian Mercury Plus 400 spectrometer with tetramethylsilane as the internal standard. Absorption and photoluminescent emission spectra were measured in DMF or aqueous solution using UV-3600 plus spectrophotometer (SHIMADZU) and LUMINA fluorescence spectrophotometer (Thermo Scientific), respectively. The single crystal data collection was performed on a Bruker APEX-II CCD detector using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). The structure was solved by direct method using SHELXS-2014 and refined against F^2 using SHELXL-2014. Hydrogen atoms were fixed geometrically and refined isotropically. The size and scale were measured via transmission electron microscopy (TEM, JEM-2010FEF) and dynamic light scattering (DLS, Malvern Zeta Sizer).

2. Synthesis of BDP-4OH

Under N_2 atmosphere, to dried *N,N*-dimethylformamide (10 mL), BODIPY (0.324 g, 1mmol), 3,4-dihydroxybenzalhyde (0.552 g, 4 mmol), ice acetic acid (0.5 mL) and piperidine (0.5 mL) were added. The mixture was then heated to 120 ^{o}C and kept at this temperature for 1 hour. When cooling to room temperature, the reaction mixture was poured into deionized water, and filtered via Buchner funnel, and washed with deionized water several times. The crude was purified via column chromatography (silica gel, ethyl acetate/dichloromethane = 1:1) to

afford black product (0.35 g) with 63% yield. ^{1}H NMR (400 MHz, DMSO-d6) δ 9.53 (s, 2H), 9.34 (s, 2H), 7.59-7.56 (m, 2H), 7.44-7.34 (m, 4H), 7.28 (d, J = 16.8 Hz, 2H), 7.10 (s, 2H), 6.95-6.90 (m, 5H), 6.83 (d, J = 8.0 Hz, 2H), 1.40 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d6) NMR (101 MHz,) δ 152.75, 148.19, 148.18, 146.36, 146.35, 141.65, 137.92, 137.90, 134.92, 132.87, 129.66, 128.95, 128.47, 128.45, 121.26, 118.50, 116.59, 115.48, 113.57, 14.69.

3. The preparation of BDP-Fe NPs or BDP-Cu NPs

To a solution of BDP-4OH (11 mg, 0.02 mmol) and $FeCl_3 \cdot 6H_2O$ (5.4 mg, 0.02 mmol) or $Cu(OAc)_2 \cdot H_2O$ (4.0 mg, 0.02 mmol) in *N,N*-dimethylformamide (4 mL), 50 μL triethylamine was added, then the mixture was stirred at room temperature for 2 hours. The mixture was slowly dropt into the vigorous stirring deionized water (40 mL). After that, the solution was dialyzed against water by a 3kDa cut off membrane.

To improve the stability, surface functionalization of the above nanoparticles were performed. Under sonication, poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (44 mg) was added into the above solution. The as-prepared solution was stored at room temperature under atmosphere.

4. The determination of metal content

Firstly, 0.1 mL of BDP-Fe NPs solution (200 μg/mL) was dissolved in excess aqua regia, and then diluted to 10 mL. Secondly, the ferric content in BDP-Fe NPs was determined by inductively coupled plasma mass spectrometry (ICP-MS), the ferric concentration in the diluted solution was 11.64 μg/L, and the ferric content was calculated to be 5.5 wt% in BDP-Fe NPs. In theory, the ratio of Fe(III) to catechol was 1:3, so the theoretic ferric content was 6.2 wt%.

5. Fenton reaction properties of BDP-Fe NPs

To investigate the Fenton reaction properties of BDP-Fe NPs, o-phenylenediamine (OPD), using as indicator, was catalytically oxidized by hydrogen peroxide in the presence of BDP-Fe NPs. Then the OPD aqueous solution (1mM, 1.0 mL), H_2O_2 (1 mM, 0.2 mL), BDP-Fe (200 μg/mL, 0.2 mL) and deionized water (0.6 mL) were added into the cuvette, recording the variation of absorption at different times.

6. Photothermal experiments of BDP-Fe NPs.

Both the solution of BDP-4OH-Fe NPs (100 μg/mL) and deionized water were irradiated by 730 nm laser (0.8 W/cm^2). The variation of temperature was recorded by an FLIR thermal camera. Setting deionized water as control group, BDP-4OH-Fe NPs aqueous solution was irradiated for 10 minutes, and then cooled down naturally. The photothermal conversion efficiency (η) was determined according to the following equations:

$$\eta = \frac{hS\Delta T_{max} - Q_s}{I(1 - 10^{-A})}$$

$$\tau_s = \frac{m_D c_D}{hS}$$

Where h is the heat transfer coefficient, S is the surface area of the container, the value of hS is calculated from the Figure S8. The T_{max} is the temperature change of BDP-4OH-Fe NPs aqueous solution at the maximum steady-state temperature, I is the laser power, A is the absorbance of the BDP-4OH-Fe NPs aqueous solution at 730 nm. Q_s expresses the heat associated with light absorption by the solvent. The τ_s is the sample-system time constant, and m_D and c_D are the mass and heat capacity (4.2 J/g) of the deionized water used as the solvent.

Photothermal stability. BDP-4OH-Fe NPs aqueous solution (100 μg/mL) was irradiated for 10 minutes (730 nm, P = 0.8 W/cm^2), and then cooled down naturally for 10 minutes, and this process was repeated 5 cycles. The variation of temperature was recorded by an FLIR thermal camera.

Concentration-dependent photothermal property. BDP-Fe NPs aqueous solution with different concentration 20, 40, 60, 80 and 100 μg/mL was irradiated by 730 nm laser (0.8 W/cm^2) for 10 minutes, and the variation of temperature was recorded by an FLIR thermal camera.

7. Cell culture

HeLa cells were cultured in fresh Dulbecco's Modified Eagle's Medium (DMEM) containing 10% inactivated fetal bovine serum (FBS) and 1% (penicillin and streptomycin) under a humidified atmosphere with 5% CO_2 and 95% air at 37 °C.

8. Live/dead cell staining assay

HeLa cells were seeded on 6-well plates at a density of 1×10^5 cells per well and cultured in incubator for 24 h. After that, the culture medium was replaced the fresh DMEM with BDP-Fe NPs (30 μg/mL) for 8 h under dark conditions in the incubator.

Then the cells were irradiated with 730 nm laser (0.80 W/cm^2) for 5 min. After the treatment, the cells were further cultured for 4 hours, then rinsed with PBS and stained by using calcein acetoxymethyl ester (calcein AM) and propidium iodide (PI), the residual dyes were washed out by PBS for three times. The fluorescence microscope was applied to observe the green fluorescence of calcein AM and red fluorescence of PI indicating live and dead cells, respectively.

9. MTT assay

HeLa cells were seeded into two 96-well plates with 200 μL fresh DMEM and incubated the cells in a humidified atmosphere. After 24 hours, the medium was replaced by the fresh DMEM with different concentrations of BDP-Fe NPs (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 and 55 μg/mL) with/without 100 μM H_2O_2 for 8 h under dark conditions in the incubator, the number of the well is five for each group. Then, The plates of BDP-Fe NPs and BDP-Fe NPs+H_2O_2 was irradiated by 730 nm laser (P = 0.8 W/cm^2) for 5 minutes per well, the other plates were still kept in incubator. After that, all the plates were incubated for another 8 hours, then, the MTT solution (20 μL, 5 mg/mL) was added to each well and incubated for another 4 h. Finally, 150 μL of DMSO was added to dissolve the purple precipitate. The absorption intensity was measured at the optical densities (O.D) of 492 nm with a microplate reader. The mean cell viability and standard deviation for the parallel five wells for each concentration were calculated. Cell viability values were calculated by the following formula: Cell viability (%) = absorbance of experimental group/ the absorbance of control group ×100%.

10. Nude mouse model

All animal experiments were approved and guided by School of Pharmaceutical Science, Nanjing Tech University, in compliance with relevant laws and guidelines. The nude mice (four weeks aged, 16-18 g weight) were purchased from Comparative Medicine Centre of Yangzhou University (Permit number: SCXK(Su)2012-0004). The HeLa tumors were generated by subcutaneous injection with 100 μL of PBS containing 4×10^6 cells on the left front leg of the mice. When the tumor volumes approached 100-150 mm^3, the mice were used to carry out the following *in vivo* experiments.

11. In vivo PA imaging

For *in vivo* PA imaging, the BDP-Fe NPs aqueous solution (200 μg/mL, 100 μL) were intravenously injected into mice for PA imaging scans under 700 nm laser irradiation (P = 0.8 W/cm^2). PA imaging of the mice before BDP-Fe NPs injection was also taken as the control.

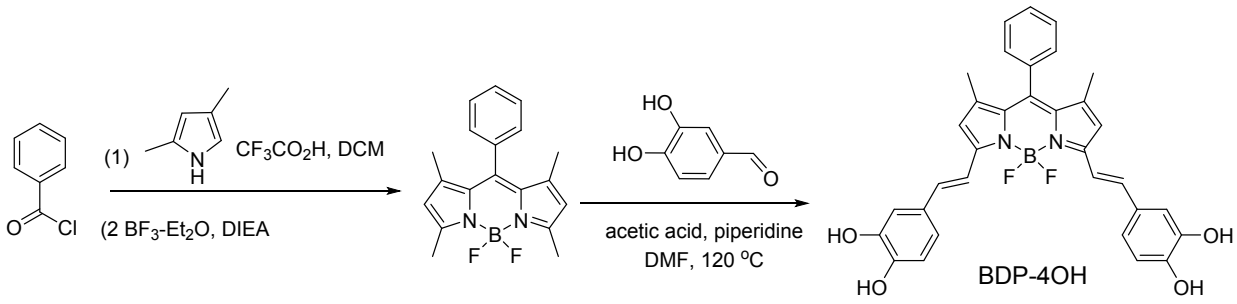
12. In vivo photothermal imaging

For *in vivo* photothermal imaging, the saline (100 μL)) and BDP-Fe NPs aqueous solution (200 μg/mL, 100 μL) were intravenously injected into mice for photothermal imaging under 700 nm laser irradiation. The variation of temperature and images were recorded by an FLIR thermal camera.

13. Synergistic CDT and PTT *in vivo*

The HeLa tumor bearing mice were randomly divided into three groups (saline, BDP-Fe NPs, and BDP-Fe NPs + laser, n = 6/group). The mice of saline group were injected with normal saline (100 μL), the mice of BDP-Fe NPs, and BDP-Fe NPs + laser were injected with BDP-Fe NPs aqueous solution (200 μg/mL, 100 μL). After 4 h of injection, the solid tumors of BDP-Fe NPs with laser groups were irradiated for 10 min with 730 nm laser (0.8 W/cm^2). The body weight and tumor volumes of each group were recorded in every two days by a digital scale and caliper, respectively. The tumor volume was calculated by formula of length × width× width/2.

One day after irradiation, one mouse from each group was sacrificed and the tumor tissues were collected for hematoxylin and eosin (H&E) staining to further evaluate the therapeutic effect of each group. To check the *in vivo* biocompatibility of the BDP-Fe NPs, after 15 days, the mice of each group were randomly chosen and euthanized to collect organs (including the heart, liver, spleen, lung, and kidney) for H&E staining.



Scheme S1. Synthetic routine of BDP-4OH.

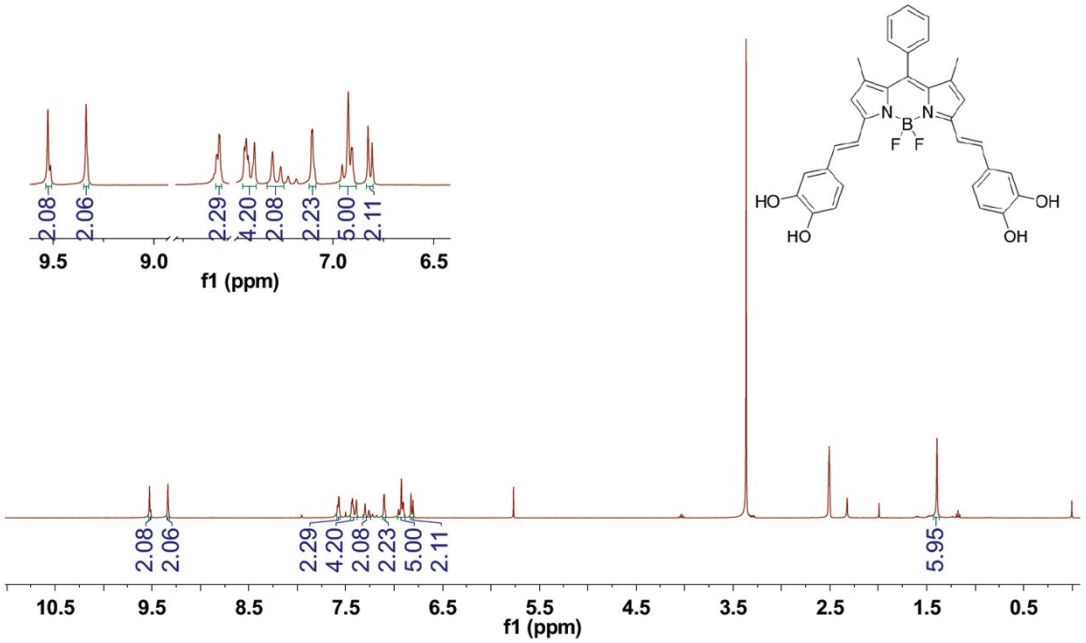


Figure S1. ^{1}H NMR spectrum of BDP-4OH.

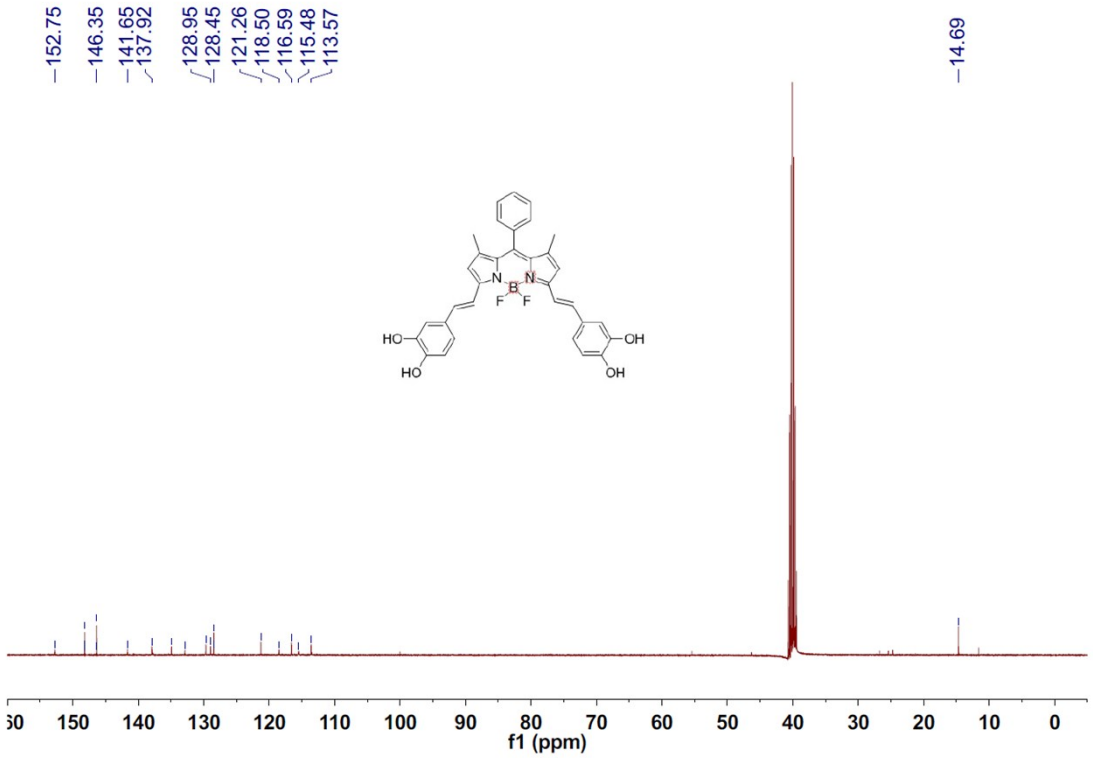


Figure S2. ^{13}C NMR spectrum of BDP-4OH.

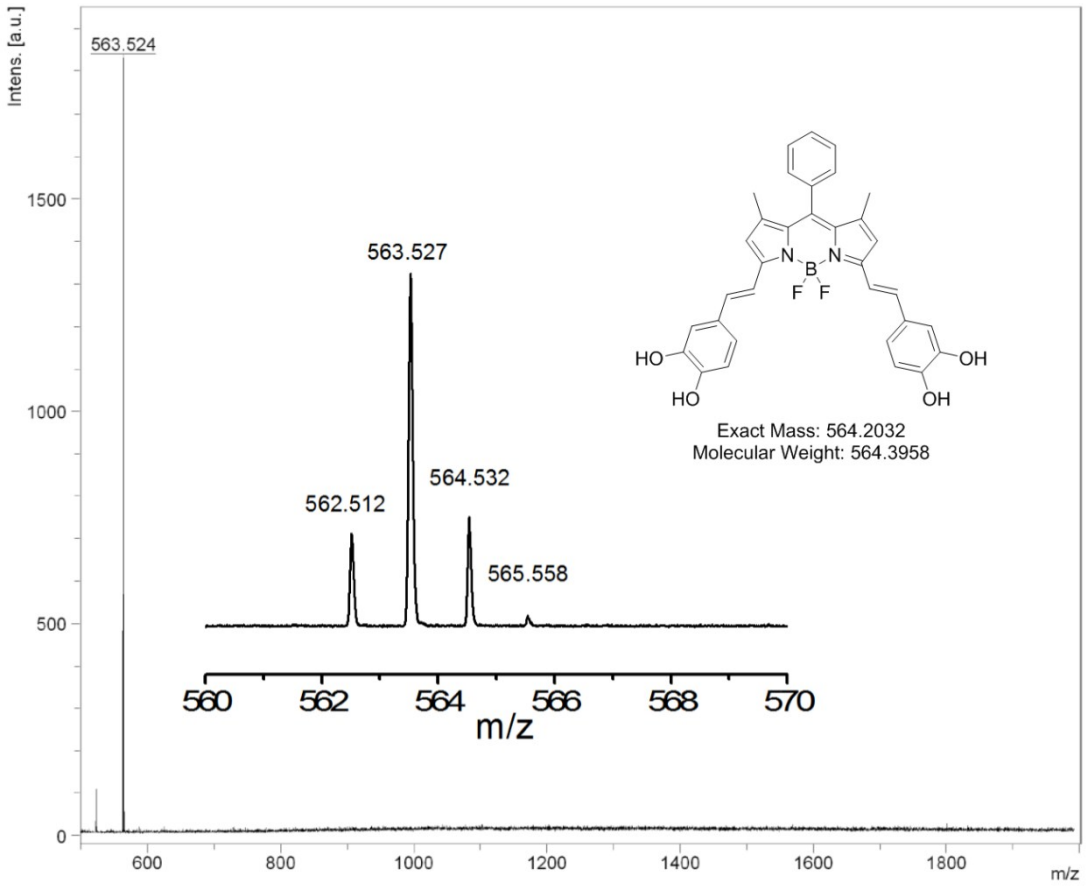


Figure S3. MALDI-TOF mass spectrum of BDP-4OH.

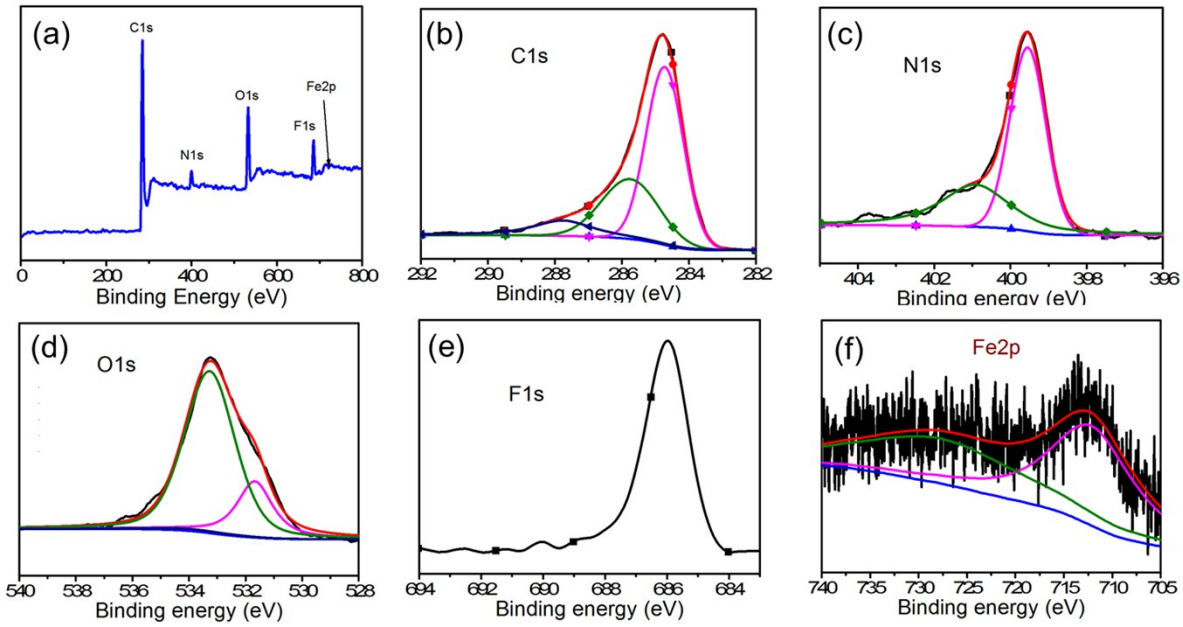


Figure S4. (a-f) Represented C1s, N1s O1s, F1s and Fe2p XPS spectra, respectively.

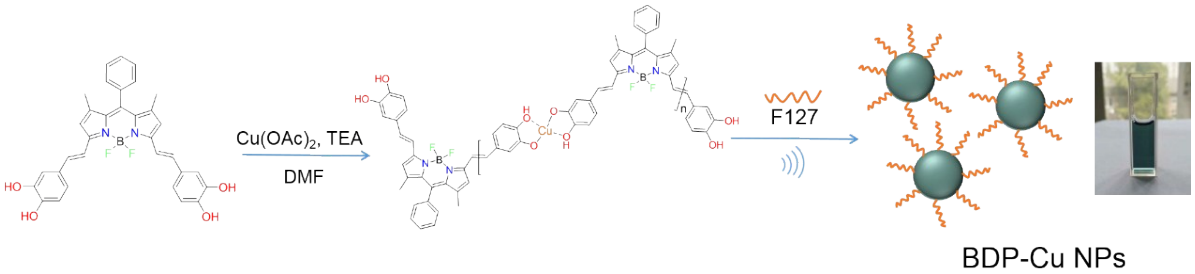


Figure S5. The preparation of BDP-Cu NPs with 1D structure.

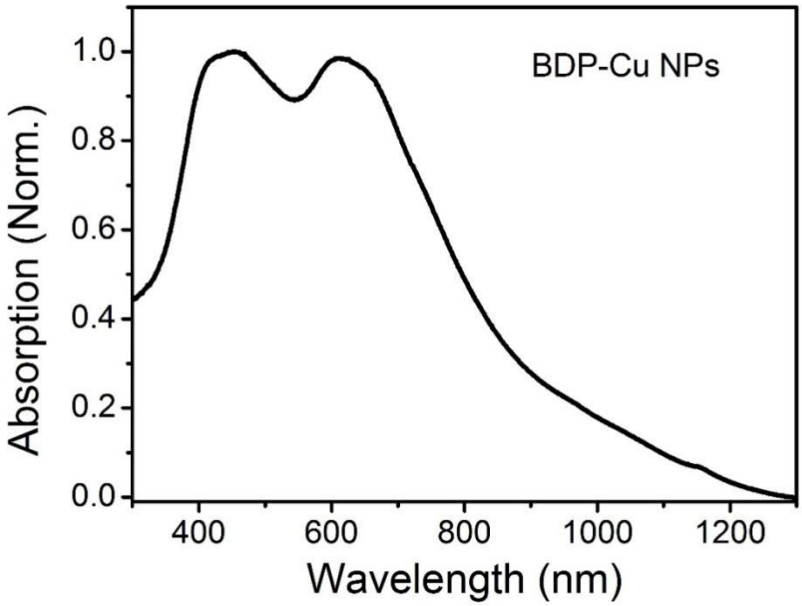


Figure S6. The absorption spectrum of aqueous BDP-Cu NPs.

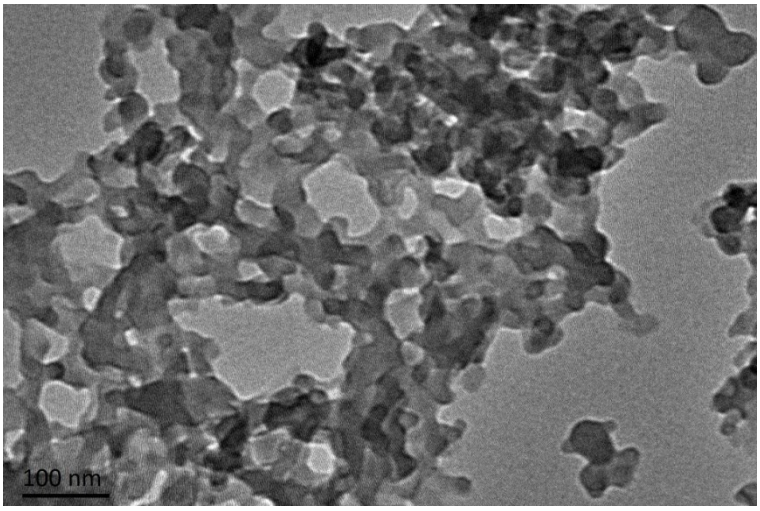


Figure S7. TEM image of BDP-Cu NPs.

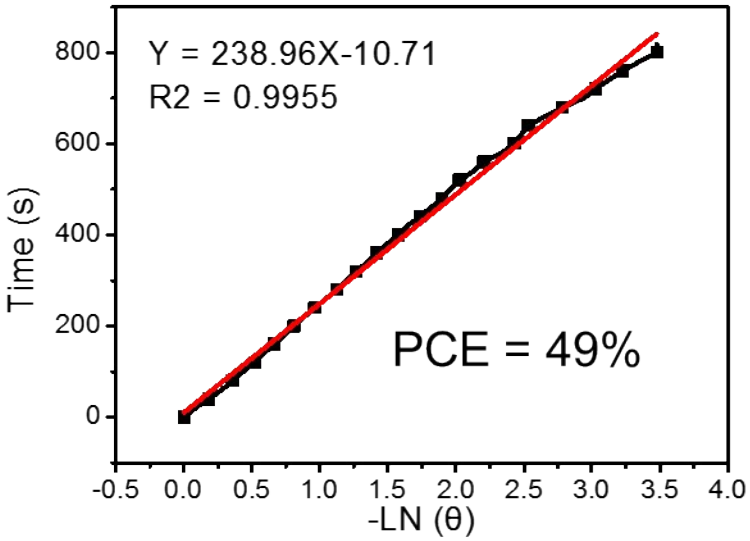


Figure S8. Plot of time versue - LN(θ) of BDP-Fe NPs, θ is driving force of temperature.

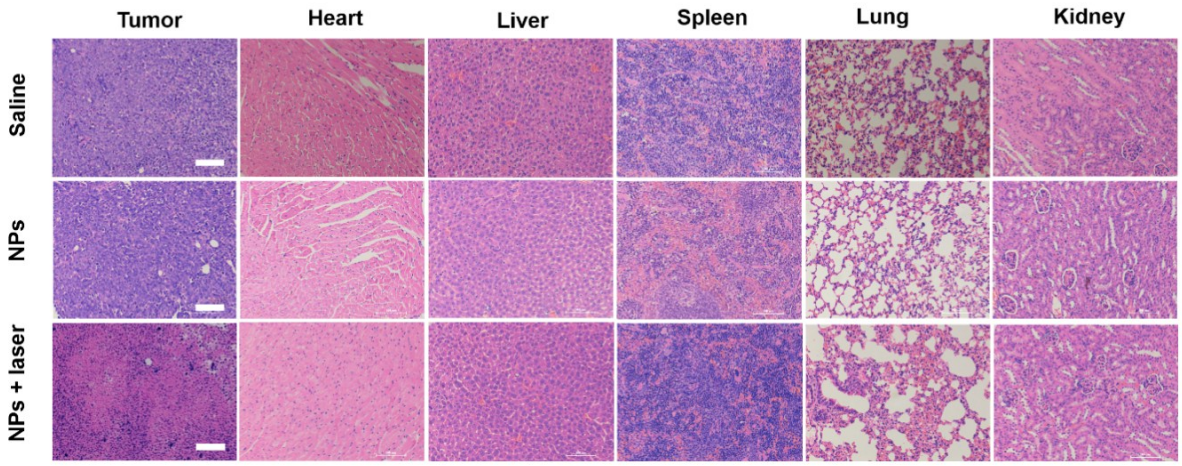


Figure S9. H&E stained images of major organs (heart, liver, spleen, lung, and kidney) for different groups after 14 days treatment and H&E staining of tumor slice 24 hours post treatments. Scale bar: 100 μm.

Table S1. Crystal parameters of BDP-4OH.

name	BDP-4OH
CCDC No.	1950349
formula	$C_{39}H_{38}BF_2N_3O_5$
fw[g/mol]	677.53
crystal colar	black
crystal size [mm]	0.15*0.12*0.09
T [K]	296
lattice type	triclinic
space group	*P*-1
a [Å]	11.152(2)
b [Å]	12.967(3)
c [Å]	13.780(3)
α [°]	113.934(4)
β [°]	105.531(5)
γ [°]	98.396(5)
V [Å^3]	1679.9(6)
Z	2
ρ_{calcd} [g/cm3]	1.139
F(000)	712
absorption coefficient [mm^{-1}]	0.096
measured	3184
observed	5565
θ range [°]	2.26~26.25
R1	0.0991
ωR2	0.1891
completeness	0.939
S	1.104

Reference:

(1) Zou, J.; Yin, Z.; Ding, K.; Tang, Q.; Li, J.; Si, W.; Shao, J.; Zhang, Q.; Huang, W.; Dong, X. BODIPY Derivatives for Photodynamic Therapy: Influence of Configuration versus Heavy Atom Effect. *ACS Applied Materials & Interfaces* **2017**, *9*, 32475-32481.