Electronic Supplementary Information (ESI)

Rational design of near-infrared platinum(II)-acetylide conjugated polymers for photoacoustic imaging-guided synergistic phototherapy under 808 nm irradiation

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Experimental Section

Materials

Unless otherwise stated, all raw materials were purchased and used without treatment. All solvents were purified and dried according to standard procedures before used. Cell culture reagents and fetal bovine serum (FBS) were supplied from Beyotime Biotechnology.

Instruments

The Bruker Ultra Shield Plus 400 MHz NMR instrument was used to measure the NMR spectra. The Bruker autoflex matrix assisted laser desorption ionization time of flight mass spectrometer was used to measure the molecular weight. The number-average molecular weight (M_n) and weight-average molecular weight (M_w) of the conjugated polymers were characterized in THF by gel permeation chromatography (polystyrene as standard) at 308 K. The UV-visible absorption spectra was collected on the Shimadzu UV-3600 UV-VIS-NIR spectrophotometer. The femtosecond transient absorption (fs-TA) spectra were performed by Newport Transient Absorption Spectrometer, in which a Spectra-physics Tsunami Oscillator (80 MHz, 800 nm) was used as the seed for a Spectra-Physics Spitre Regenerative amplier (1 kHz, 4 mJ). The transmission electron microscope (TEM, JEM-2100) was used to measure the morphology of nanoparticles. The dynamic light scattering (DLS) on Zetasizer Nanoseries (Nano ZS90) was used to measure the particle size. The Power Wave XS/XS2 microplate spectrophotometer was used for the methyl thiazolyl tetrazolium (MTT). Confocal luminescence imaging was performed using the Olympus IX81 laser scanning confocal microscope. Photoacoustic (PA) imaging was acquired with a LAZR instrument (Visualsonics, 2100 High-Resolution Imaging System). The NIR thermal imager (FLIR E40) was used to measure the photothermal imaging. The VLP-2000 laser power meter was used to measure the power density.

Preparation and characterization of CP-NPs

The solution of CPs in THF (1.0 mg/mL) was swiftly injected into the DSPE-mPEG$_{5000}$ aqueous solution (10.0 mg in 10 mL water) under continuous sonication. THF was removed by nitrogen blowing on the solution surface. The aqueous solution was filtered through a polyethersulfone (PES) syringe driven filter (0.44 μm) (Millipore), and was further centrifuged using a centrifugal filter and washed three times with deionized water. The concentrations of CP-NPs solutions were determined by UV-Vis

absorption according to their absorption coefficients. The morphology of CP-NPs was characterized by transmission electron microscopy (TEM). The hydrodynamic diameter was measured using dynamic light scattering (DLS).

Absorption spectra

Absorption spectra of monomer, free CPs and CP-NPs were measured on UV-Vis absorption spectrophotometer (UV2600, Shimadzu). The absorption of CPs was collected with 6 various concentrations, and plotted against the concentration. The concentrations of CP-NPs solutions were calculated as the slope of fitted line from linear regression.

Femtosecond transient absorption spectra

The measurement of fs-TA spectra was performed by Newport Transient Absorption Spectrometer, in which a Spectra-physics Tsunami Oscillator (80 MHz, 800 nm) was used as the seed for a Spectra-Physics Spitre Regenerative amplier (1 kHz, 4 mJ). An 808 nm semiconductor laser was purchased from Changchun New Industries Optoelectronics Technology Co., Ltd. The measurements were performed using a pump-probe setup (18SI80466 Rev.1, Newport). The samples were pumped with the pulses (120 fs, 1000 Hz) at 810 nm, which were generated by a femtosecond mode-locked Ti-sapphire laser (Spectra Physics). The probe pulses (400-800 nm) were generated by focusing a small portion (∼50 μJ) of the fundamental 810 nm laser pulses on a thin CaF2 plate.

***In vitro* photodynamic studies**

The singlet oxygen ($^1O_2$) generation of CP-NPs under 785 nm and 808 nm irradiation was measured using 1,3-diphenylbenzofuran (DPBF) as $^1O_2$ indicator. CP-NPs at different concentrations were mixed with of DPBF in ethanol under continuous stirring. Under 785 nm and 808 nm irradiation at 1.0 W cm^{-2}, the absorption spectra of DPBF at 416 nm were recorded. Subsequently, for singlet oxygen quantum yield (Φ_Δ) measurement, indocyanine green (ICG) was selected as a reference compound (Φ_Δ^{ICG} = 0.14). The solutions of CP-NPs (2.0 μg mL^{-1}) and ICG (1.0 μM) containing DPBF (80.0 μM) were irradiated at 808 nm (1.0 W cm^{-2}) for 300 s, respectively. The singlet oxygen quantum yield (Φ_Δ) was calculated by the relative approach according to the equation (1),

$$\Phi_{\Delta(x)} = \Phi_{\Delta(std)}(\frac{S_x}{S_{std}})(\frac{F_{std}}{F_x}) \quad (1)$$

where subscripts x and std designate the sample and ICG, respectively, S denotes the slope of the plots of the absorbance of DPBF (at 416 nm) vs the irradiation time, F represents the absorption correction factor, which is given by $F = 1\text{-}10^{-O.D.}$. (O. D. represents the optical density of the sample and ICG at 808 nm).

***In vitro* photothermal studies**

The different concentrations of CP3-NPs (2, 5, 10 and 20 μg mL^{-1}) were irradiated with 808 nm irradiation at 0.5, 1.0 and 1.5 W cm^{-2} for 6 min, respectively. Then, the temperature elevation was measured using a FLIR. To measure the photothermal conversion efficiency, CP3-NPs were exposed under 808 nm laser irradiation at 1.0 W cm^{-2}. When the temperature reached a plateau, the irradiation was removed for cooling down to room temperature. The temperature of the solution was recorded during this process. The photothermal conversion efficiency (η) was calculated according to the following two equations (2) and (3):

$$\eta = \frac{hS\Delta T_{max} - Q_s}{I(1 - 10^{-A_{808}})} \quad (2)$$

$$\tau_s = \frac{m_D C_D}{hS} \quad (3)$$

Where h is the heat transfer coefficient, S is the surface area of the container, and the value of hS is calculated from the Figure S12. The ΔTmax is the temperature change of CP-NPs aqueous solution at the maximum steady-state temperature, I is the laser power, A_{808} is the absorbance of the CP3-NPs at 808 nm, and Q_s expresses the heat associated with light absorption by the solvent. The variable 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.

Cell viability assessment

The cell lines HeLa (human cervical cancer) were purchased from the Institute of Biochemistry and Cell Biology, SIBS, CAS (China). The cells were cultured in Dulbecco's modified Eagle's medium and provided with 10% fetal bovine serum (FBS) at 37 °C with 5% CO_2. To measure the cytotoxicity of CP3-

NPs, MTT assays was taken on HeLa cells. They were seeded into a 96-well cell culture plate at 10^4/well, and were cultured at 100% humidity 37 °C with 5% CO_2 for 24 h. HeLa cells were incubated with different concentrations of CP3-NPs (5, 10, 15, 20 and 25 μg/mL) for 24 h. Finally, MTT (5 mg/mL) was added to each well and incubated for 4 h. To measure the phototoxicity of CP3-NPs, HeLa cells were incubated with CP3-NPs at the concentration of 15 μg mL^{-1} for 24 h, followed by 5 min irradiation at 0.1, 0.3, 0.5, 1 and 1.5 W cm^{-2}, respectively, under 808 nm. The inhibition of cell growth was calculated by the following formula: cell viability (%) = (mean of Abs. value of treatment group/mean Abs. value of control) × 100%.

***In vitro* singlet oxygen detection**

HeLa cells were incubated with CP3-NPs (15 μg mL^{-1}) or PBS at 37 °C and 100% humidity of 5% CO_2 for 24 h. Then the HeLa cells were treated with DCFH-DA (the commercial $^1O_2$ indicator) at 37 °C for 30 min, followed by irradiation for 5 min (808 nm, 1 W cm^{-2}). In the control group, the cells were incubated with CP3-NPs plus VC. Next, the cells were washed for three times and the fluorescence intensity was measured. The fluorescence from DCF was collected (λ_{ex} = 488 nm, λ_{em} = 500-540 nm).

Calcein-AM/PI assay

HeLa cells were cultured at 37 °C and 100% humidity of 5% CO_2 for 24 h. CP3-NPs (15 μg mL^{-1}) was added to the medium and cells were cultured for another 24 h. The cells were treated with 5 μL PI and 10 μL Calcein AM in dark for 15 min, and then irradiated with 808 nm laser (1 W cm^{-2}) for 5 min. The images were obtained by the confocal microscopy in green channel for AM (λ_{ex} = 488 nm, λ_{em} = 500-560 nm) and red channel for PI (λ_{ex} = 488 nm, λ_{em} = 600-680 nm).

***In vitro* PA studies**

PA images were acquired with a LAZR instrument (Visualsonics, 2100 High-Resolution Imaging System). A commercial Endra Nexus128 PA tomography system (Endra Inc., Ann Arbor, Michigan) was also used in this imaging. The PA signals with different concentrations of CP3-NPs were recorded at the laser wavelength of 810 nm.

Blood routine examination

To explore the hematologic index change, mice were intravenously injected with CP3-NPs (0.8 mg kg^{-1}). The whole blood was harvested from the orbital venous plexus on 1, 8 and 16 day post-injection, and subject to blood routine examination.

Animals tumor model

All animal experiments were performed in accordance with the NIH guidelines for the care and use of laboratory animals (NIH Publication no. 85-23 Rev. 1985) and approved by the Jiangsu Administration of Experimental Animals. All Hela tumor model was established from Jiangsu Keygen Biotech Co., Ltd. and used referring to the standard of the Laboratory Animal Center of Jiangsu Keygen Biotech Co., Ltd. When the tumor volume reached to about 120 mm^3, we began to conduct the following *in vivo* therapy experiments.

***In vivo* synergistic phototherapy**

To evaluate the therapeutic efficacy of CP3-NPs, the tumor-bearing Hela mice were injected with CP3-NPs at the doses of 0.8 mg kg^{-1} with PBS as the control (n = 4). Then, the tumors were exposed under 808 nm irradiation at 1.0 W cm^{-2} for 5 min or not after 6 h injection. The tumor size was measured using a vernier caliper during 16 days, followed by their body weight for subsequent 16 days. After the whole therapeutic process, all the mice were killed, the tumors and main organs including heart, liver, spleen, lung and kidney were collected.

Ex vivo histological staining

After the whole therapeutic process, the various tissues including tumor, heart, liver, spleen, lung and kidney were collected and fixed with 4% formaldehyde for hematoxylin and eosin (H&E) staining to analyze the potential side effect of CP3-NPs.

Synthesis

M1. 2,4-Dimethylpyrrole (0.62 g, 6.6 mmol) and 3,4-dioctylbenzaldehyde (1.09 g, 3 mmol) were dissolved in CH_2Cl_2 (20 mL) under nitrogen. Catalytic amount of trifluoroacetic acid (50 μL, 0.65 mmol) was dropped into the mixture. After 1 h of stirring under ice bath, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (0.68 g, 3 mmol) was added for additional 1 h stirring at room temperature. Then, triethylamine (NEt_3) (10 mL, 72 mmol) and boron trifluoride diethyl etherate ($BF_3{\cdot}Et_2O$) (10 mL, 80 mmol) were added. The reaction mixture was washed after 2 h of stirring at room temperature. The crude product was obtained after removal of deep red precipitate through the filtration. The filtrate was extracted using CH_2Cl_2 and then dried over anhydrous Na_2SO_4. The residue was purified by column chromatography for generating yield a red-orange solid (1.20 g, 69%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.96 (d, *J* = 5.6 Hz, 1H), 6.78 (d, *J* = 2.4 Hz, 1H), 6.76 (s, 1H), 5.98 (s, 2H), 4.04 (t, *J* = 2.4 Hz, 2H), 3.94 (t, *J* = 2.4 Hz, 2H), 2.55 (s, 6 H), 1.86 (t, *J* = 8.0 Hz, 2H), 1.80 (t, *J* = 8.0 Hz, 2H),1.19-1.37 (m, 20 H), 1.48 (s, 6 H), 0.88 (s, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.25, 149.70, 149.57, 143.24, 141.89, 131.76, 127.07, 121.05, 120.38, 113.68, 113.21, 69.42, 69.18, 31.85, 31.82, 29.46, 29.39, 29.29, 29.26, 29.21, 26.07, 25.97, 22.71, 22.69, 14.59, 15.47, 14.15, 14.13 ppm. MS (MALDI-TOF-MS): calcd for $C_{35}H_{51}BF_2N_2O_2$: 580.401 $[M+2H]^+$, found: 579.499.

M2. A round-bottomed flask was first charged with **M1** (1.16 g, 2 mmol), N-Iodosuccinimide (NIS) (1.12 g, 5 mmol), and $CHCl_3$ (20 mL). After the addition was complete, the solution was stirred 0.5 h at room temperature. The mixture was added anhydrous Na_2CO_3 and extracted with dichloromethane, the organic layer was dried over anhydrous Na_2SO_4. The residue was purified by column chromatography on silica gel eluting with petroleum ether/CH_2Cl_2 = 3:1. Eluent was removed by rotary evaporation for yielding red power (1.50 g, 90%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.98 (d, *J* = 8.0 Hz, 1H), 6.74 (d, *J* = 2.0 Hz, 1H), 6.72 (s, 1H), 4.05 (t, *J* = 6.4 Hz, 2H), 3.93 (t, *J* = 6.4 Hz, 2H), 2.64 (s, 6 H), 1.88 (t, *J* = 7.6 Hz, 2H), 1.80 (t, *J* = 7.6 Hz, 2H),1.23-1.35 (m, 20 H), 1.48 (s, 6 H), 0.88 (s, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.55, 149.99, 145.44, 141.62, 131.65, 126.67, 120.25, 113.82, 112.85, 69.50, 69.24, 31.84, 31.81, 29.45, 29.37, 29.29, 29.25, 29.18, 26.05, 25.95, 22.71, 22.69, 17.08, 16.02, 14.15 ppm. MS (MALDI-TOF-MS): calcd for $C_{35}H_{49}BF_2I_2N_2O_2$: 832.402 $[M]^+$, found: 831.106.

M3. A mixture of **M2** (998 mg, 1.2 mmol) and 4-(dimethylamino)benzaldehyde (804 mg, 5.4 mmol) was dissolved in toluene (60 mL). Acetic acid (3 mL) and piperidine (4.5 mL) were added in the system. The mixture was refluxed for 48 h using a Dean-Stark condenser. After consumption of all the starting

materials, the reaction mixture was cooled to room temperature. The crude product was extracted with CH_2Cl_2 and water, the organic layer was dried with anhydrous Na_2SO_4 and the solvent was removed by rotary evaporation. The residue was purified by column chromatography on silica gel eluting with petroleum ether/CH_2Cl_2 = 1:1 for generating a black solid (0.36 g, 27%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.76-7.72 (m, 3H), 7.57 (d, *J* = 8.8 Hz, 4H), 6.98 (d, *J* = 8.0 Hz, 1H), 6.78-6.74 (m, 3H), 6.7 (d, *J* = 8.8 Hz, 4H), 4.06 (t, *J* = 6.8 Hz, 2 H), 3.96 (t, *J* = 6.8 Hz, 2 H), 3.09 (s, 12 H), 1.88 (t, *J* = 4.0 Hz, 2H), 1.82 (t, *J* = 4.0 Hz, 2H), 1.53 (s, 6 H), 1.23-1.32 (m, 20 H), 0.88 (s, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 190.36, 154.35, 151.18, 149.81, 144.92, 139.49, 132.03, 129.34, 127.73, 125.19, 114.47, 113.74, 112.14, 111.01, 69.46, 69.25, 40.32, 40.11, 31.87, 31.83, 29.48, 29.40, 29.31, 29.28, 29.22, 26.08, 25.98, 22.73, 22.71, 17.54, 14.18 ppm. MS (MALDI-TOF-MS): calcd for $C_{53}H_{67}BF_2I_2N_4O_2$: 1094.761 $[M+2H]^+$, found: 1092.996.

M4. A mixture of **M3** (218 mg, 0.2 mmol), $Pd(PPh_3)_4$ (11.6 mg, 0.01 mmol) and CuI (7.6 mg, 0.04 mmol) were dissolved in distilled toluene (20 mL) and dry trimethylamine (40 mL). Trimethylsilylacetylene (78.4 mg, 0.8 mmol) was injected before the mixture was stirred for overnight. After the reaction was finished, the solvent was removed by rotary evaporation. The residue was purified by column chromatography on silica gel eluting with petroleum ether/CH_2Cl_2 = 1:2 for giving a dark brown solid (150 mg, 73%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.40 (d, *J* = 12.0 Hz, 2H), 7.62 (d, *J* = 15.2 Hz, 2H), 7.56 (d, *J* = 7.2 Hz, 4H), 6.96 (d, *J* = 8.8 Hz, 1H), 6.69-6.79 (m, 6H), 4.05 (t, *J* = 6.8 Hz, 2 H), 3.95 (t, *J* = 6.8 Hz, 2 H), 3.05 (s, 12 H), 1.87 (t, *J* = 4.0 Hz, 2H), 1.81 (t, *J* = 4.0 Hz, 2H), 1.56 (s, 6 H), 1.24-1.31 (m, 20 H), 0.88 (s, 6 H), 0.27 (s, 18 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 151.12, 139.24, 129.31, 125.47, 113.75, 112.12, 103.27, 99.92, 69.42, 40.29, 31.87, 31.83, 29.74, 29.47, 29.39, 29.28, 26.08, 22.72, 14.17, 13.19, 0.13 ppm.MS (MALDI-TOF-MS): calcd for $C_{63}H_{85}BF_2Si_2N_4O_2$: 1034.382 $[M+2H]^+$, found: 1033.354.

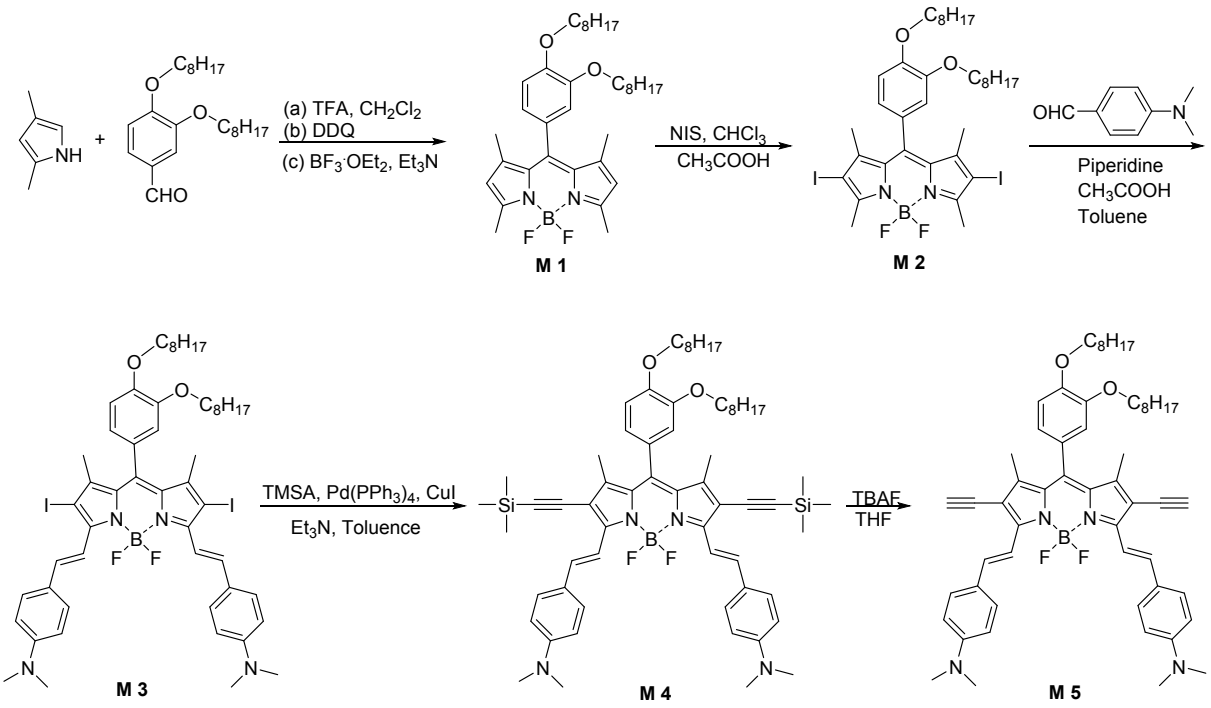
M5. **M4** (103 mg, 0.1 mmol) was dissolved in distilled THF (10 mL). Tetrabutylammonium fluoride (0.4 M in THF, 1 mL) was added dropwise at -78 °C under nitrogen. The mixture was stirred for 0.5 h. After the reaction was finished, the mixture was extracted with CH_2Cl_2 and water. The organic layers were dried over anhydrous Na_2SO_4 and the solvent was removed by reduced pressure. The residue was purified by column chromatography on silica gel eluting with petroleum ether/CH_2Cl_2 = 1:1 for yielding a brown solid (45 mg, 51%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.60 (d, *J* = 15.6 Hz, 1H), 8.50 (s, 1H), 8.3 (d, *J* = 18.0 Hz, 1H), 7.52-7.66 (m, 6H), 6.85 (d, *J* = 12.8 Hz, 2 H), 6.74 (d, *J* = 8.8 Hz, 4 H), 4.06

(t, *J* = 7.2 Hz, 2 H), 3.93 (t, *J* = 6.8 Hz, 2 H), 3.68 (s, 1 H), 3.61 (s, 1 H), 3.09 (s, 6 H), 3.04 (s, 6 H), 1.88 (t, *J* = 8.0 Hz, 2H), 1.80 (t, *J* = 6.0 Hz, 2H), 1.66 (s, 3 H), 1.55 (s, 3 H), 1.21-1.27 (m, 20 H), 0.88 (s, 6 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 152.69, 151.30, 149.56, 145.81, 139.12, 132.48, 129.54, 127.27, 125.33, 120.85, 113.73, 112.00, 111.45, 85.74, 78.59, 69.25, 40.04, 31.41, 29.23, 25.72, 22.47, 13.61, 12.90 ppm. MS (MALDI-TOF-MS): calcd for $C_{57}H_{69}BF_2N_4O_2$: 891.01 $[M+2H]^+$, found: 890.55.

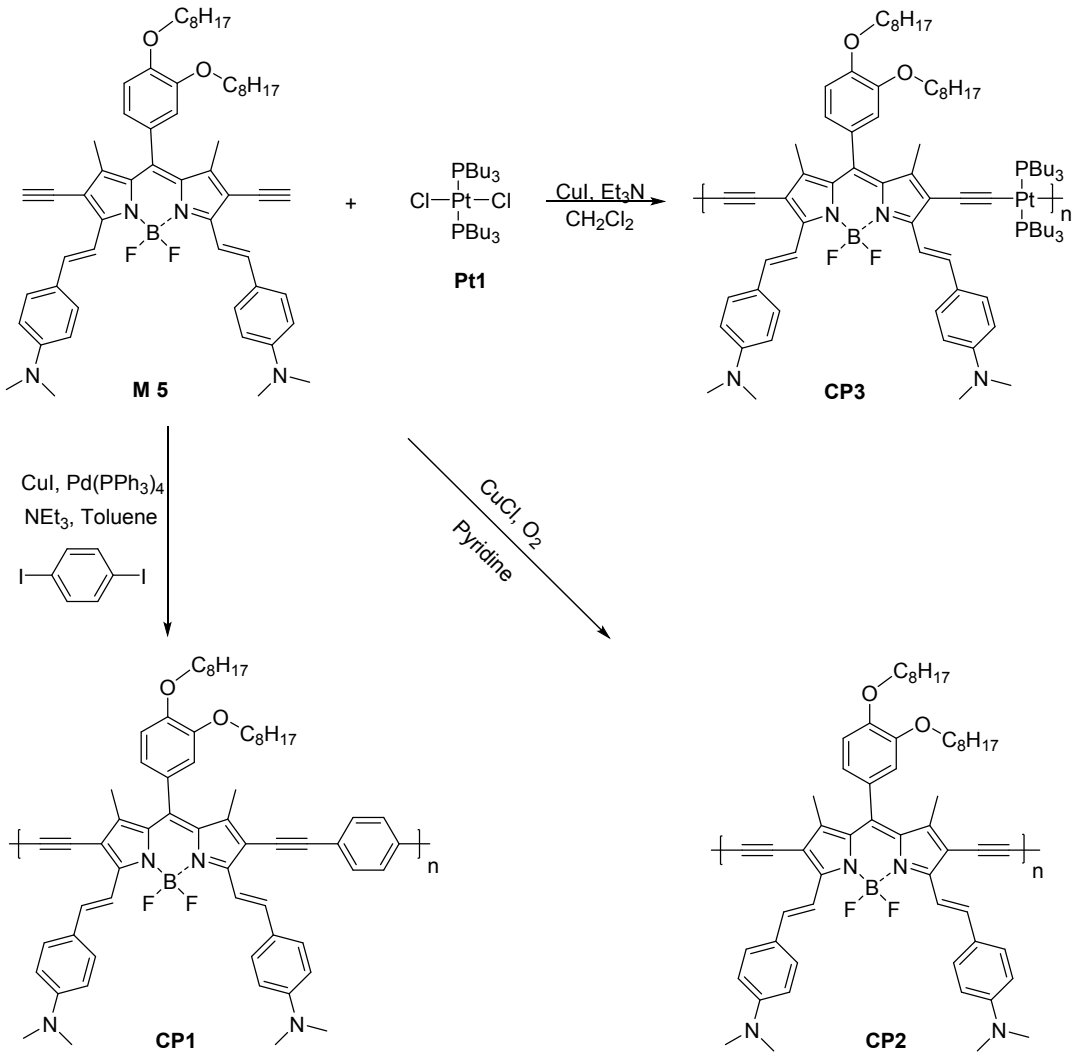
CP1. A mixture of **M5** (178 mg, 0.2 mmol), 1, 4-diiobenzene (66 mg, 0.2 mmol), $Pd(PPh_3)_4$ (11.6 mg, 0.01 mmol) and CuI (7.6 mg, 0.04 mmol) were dissolved in distilled toluene (10 mL). The dry trimethylamine (20 mL) was added into the mixture under N_2 atmosphere. The resultant mixture was refluxed for 24 h. After the reaction was finished, the mixture was filtered and the combined organic layer was dried over anhydrous Na_2SO_4. Then, the mixture was poured into a large excess of methanol. The precipitate was collected and washed with hexane. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.43-8.35 (1H, CH=CH), 7.76-7.56 (8H, ArH), 7.51-7.46 (1H, ArH), 7.24-7.18 (2H, CH=CH), 7.04-6.97 (1H, ArH), 6.87-6.71 (5H, ArH), 4.13-3.93 (4H, -CH_2), 3.31-2.91 (12H, -$N(CH_3)_2$), 2.10-2.02 (6H, CH_3 of BDP), 1.64-1.54 (14H, -CH_2), 1.38-1.24 (20H, -CH_2), 0.96-0. 82 (6H, CH_3).

CP2. **M5** (178 mg, 0.2 mmol) was added to pyridine (3 mL) and then CuCl (2 mg, 0.01 mmol) was added into the medium. The reaction mixture was stirred for overnight at room temperature while a flow of air was bubbled during the reaction progress. After the reaction was finished, the mixture was poured into a large excess of methanol. The precipitate was collected and washed with hexane. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.36 (2H, CH=CH), 7.73-7.47 (6H, ArH), 7.01 (1H, ArH), 6.89-6.59 (5H, ArH), 4.17-3.91 (4H, -CH_2), 3.20-2.71 (10H, -$N(CH_3)_2$), 2.10-1.96 (6H, CH_3), 1.72-1.66 (4H, -CH_2), 1.40-1.31 (20H, -CH_2), 0.95-0.86 (6H, CH_3).

CP3. **M5** (178.2 mg, 0.2 mmol), trans-$[PtCl_2(P(C_4H_9)_3)_2]$ (**Pt1**, 134 mg, 0.2 mmol), CuI (7.6 mg, 0.04 mmol) and Et_3N/CH_2Cl_2 (30 mL, 1:2, v/v) were charged in a pressure vessel under nitrogen. After stirring at room temperature overnight, the solvent was removed by rotary evaporation. The residue was first purified by column chromatography. After concentration of the dichloromethane solution, brown solid was collected by precipitation into methanol. Subsequent washing with methanol gave the polymer as brown solid (260 mg, 87.4%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.97-8.74 (2H, CH=CH), 7.71-7.50 (5H, ArH), 7.01-6.95 (1H, ArH), 6.87-6.79 (2H, ArH), 6.75-6.67 (3H, ArH), 4.08 (2H, -CH_2), 3.96 (2H, -CH_2), 3.12-2.96 (11H, -$N(CH_3)_2$), 1.70-1.46 (44H, -CH_2 of BDP and side chain of Bu_3), 1.44-1.17 (34H, -CH_2 of BDP and side chain of Bu_3), 0.96-0.71 (27H, -CH_2 of BDP and side chain of Bu_3).



Scheme S1. Synthetic route of BDP monomers.



Scheme S2. Synthetic route of **CP1**, **CP2** and **CP3**.

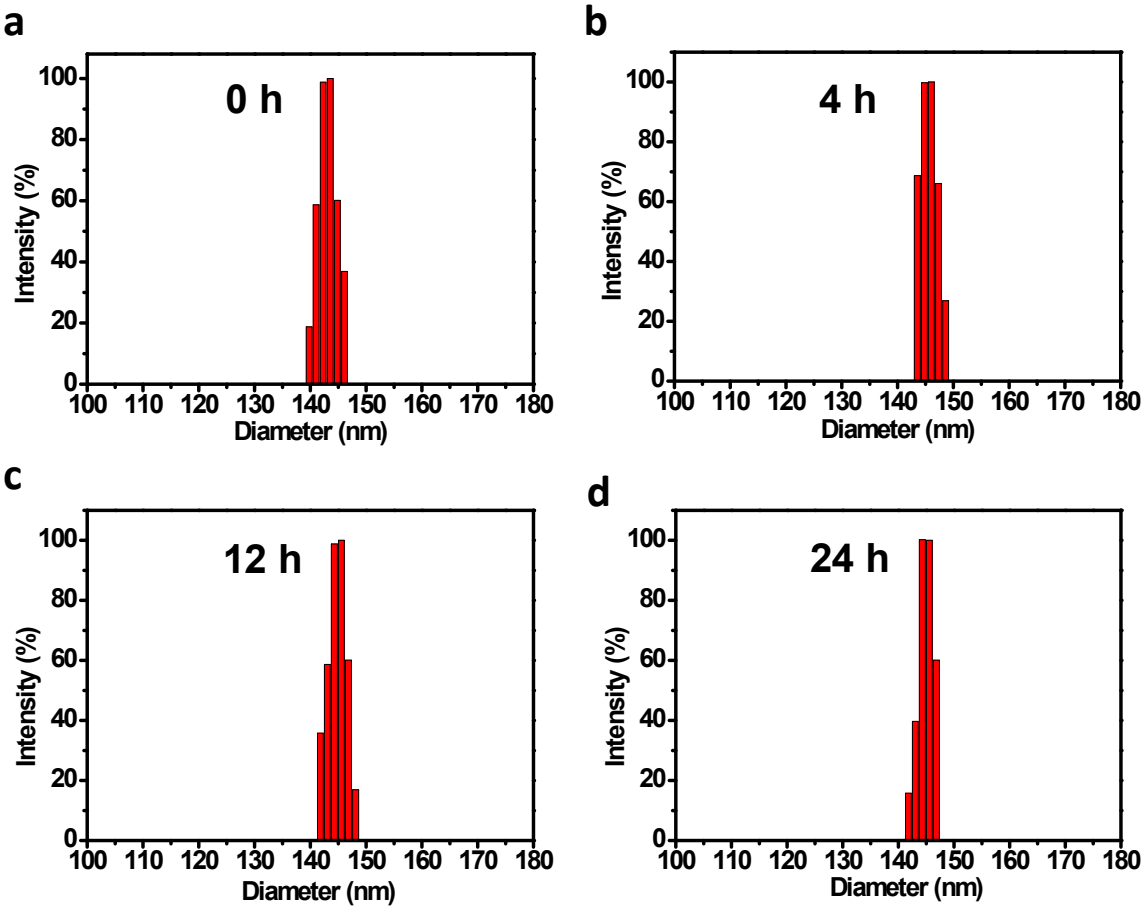


Fig S1. DLS data of CP3-NPs (2 μg mL^{-1}) after different incubation periods (a) 0 h; (b) 4h; (c) 12 h; (d) 24 h with serum. No obvious change in hydrodynamic sizes demonstrates the excellent physiological stability of CP3-NPs in biological environment.

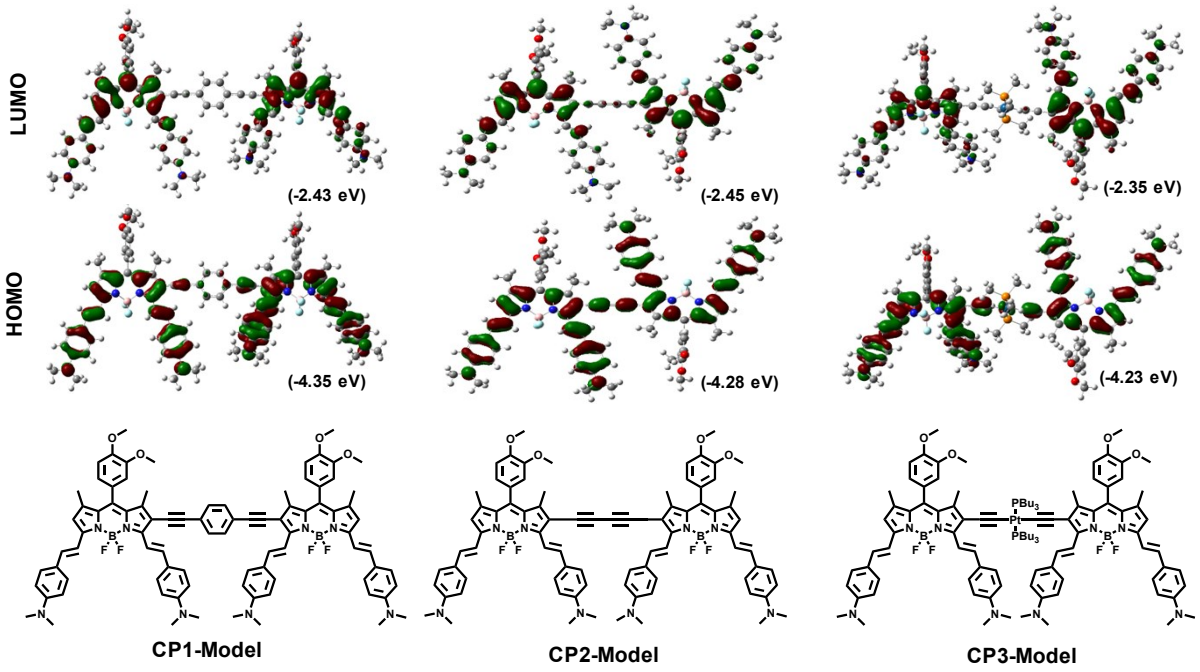


Fig S2. Highest occupied, and lowest unoccupied molecular orbitals of energy-minimized calculated (Gaussian) CPs-Model.

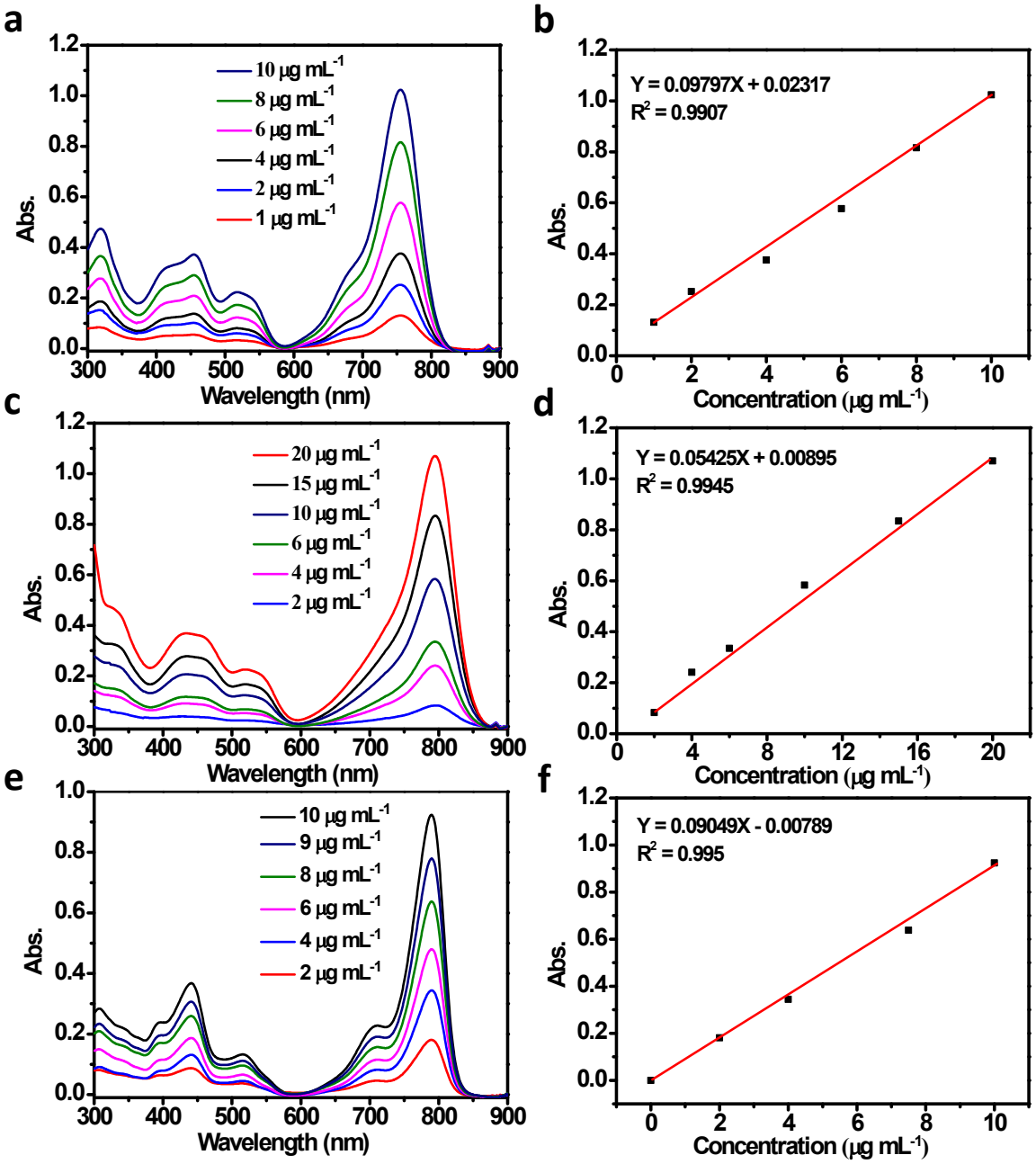


Fig S3. (a) UV-vis absorption spectra of **CP1** at various concentrations in $CHCl_3$. (b) The equation was calculated according to the maximal absorption of (a) with different concentration at 755 nm, respectively. (c) UV-vis absorption spectra of **CP2** at various concentrations in $CHCl_3$. (d) The equation was calculated according to the maximal absorption of (c) with different concentration at 795 nm, respectively. (e) UV-vis absorption spectra of **CP3** at various concentrations in $CHCl_3$. (f) The equation was calculated according to the maximal absorption of (e) with different concentration at 790 nm, respectively.

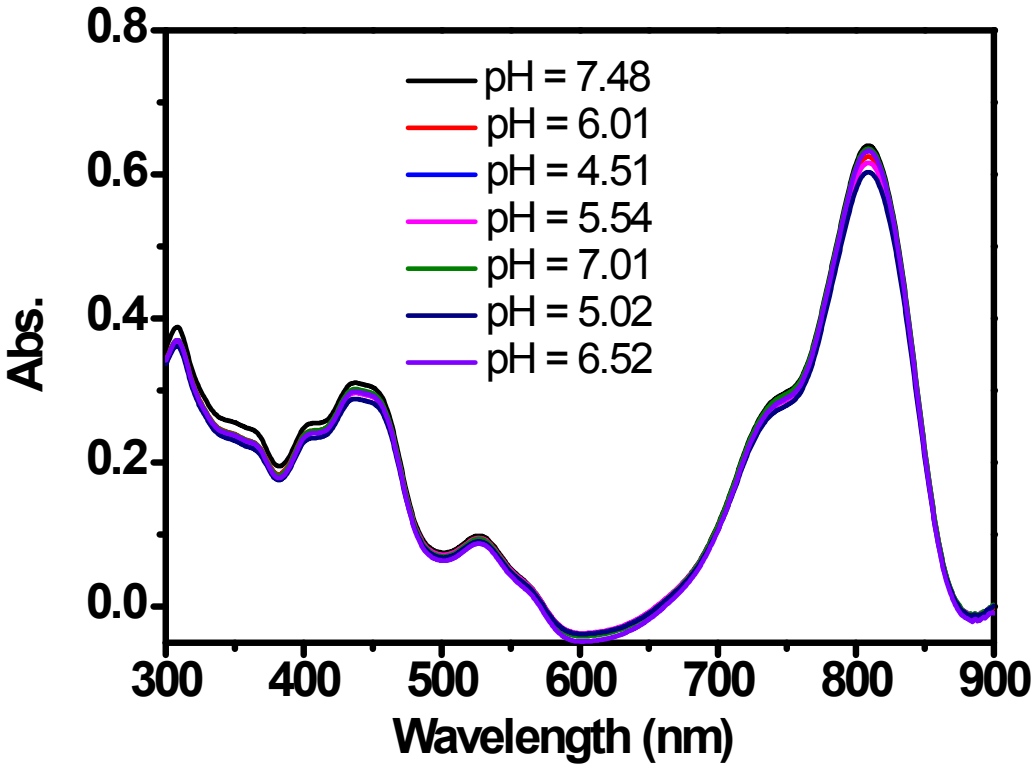


Fig S4. Absorption spectra of CP3-NPs at various pH PBS solution at the concentrations of 10 μg mL^{-1}.

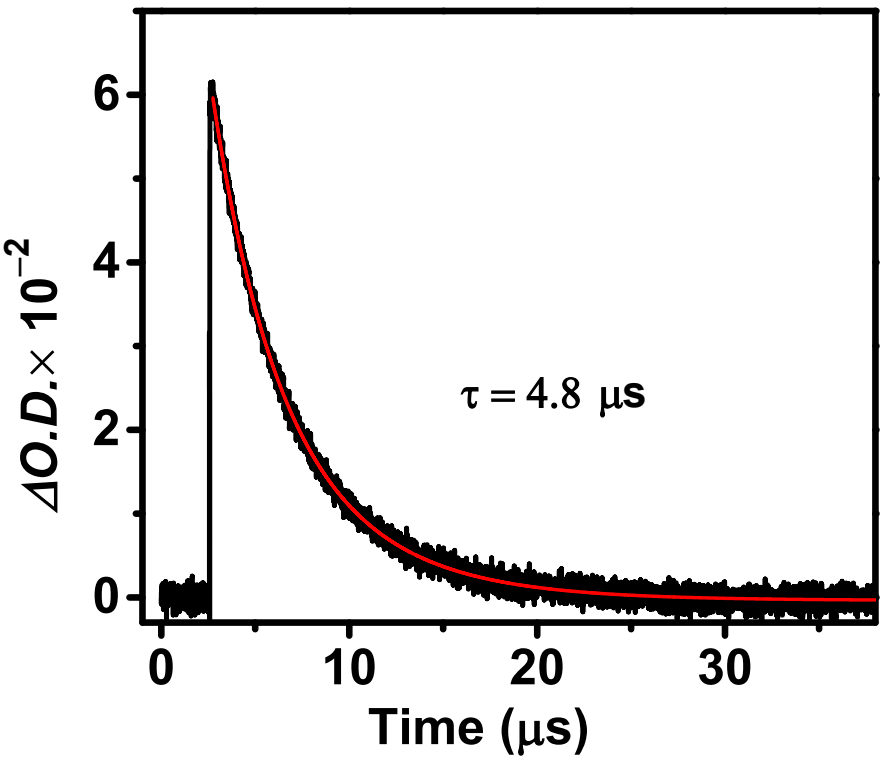


Fig S5. Decay trace of CP3-NPs at the excitation of 810 nm in the transient absorption measurement.

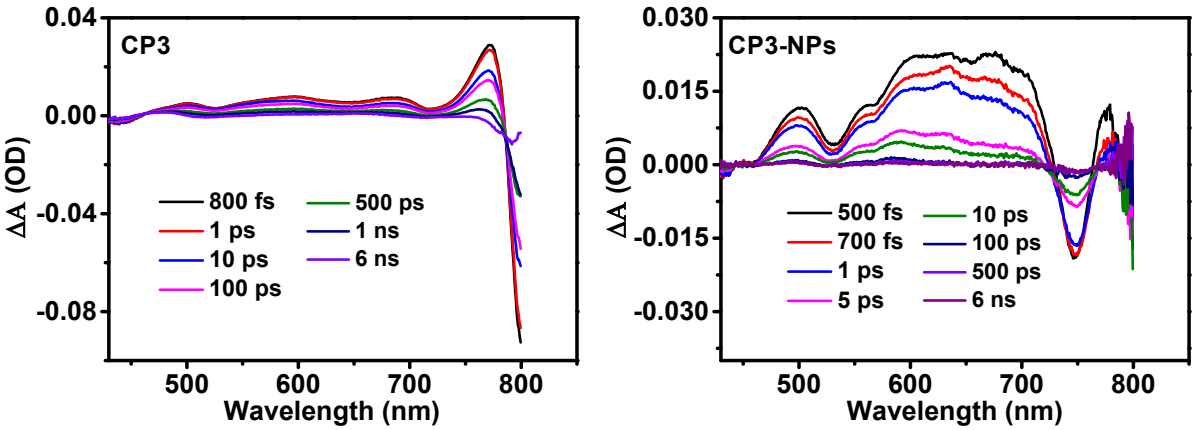


Fig S6. The fs-TA spectra of **CP3** and CP3-NPs obtained at selected delay times.

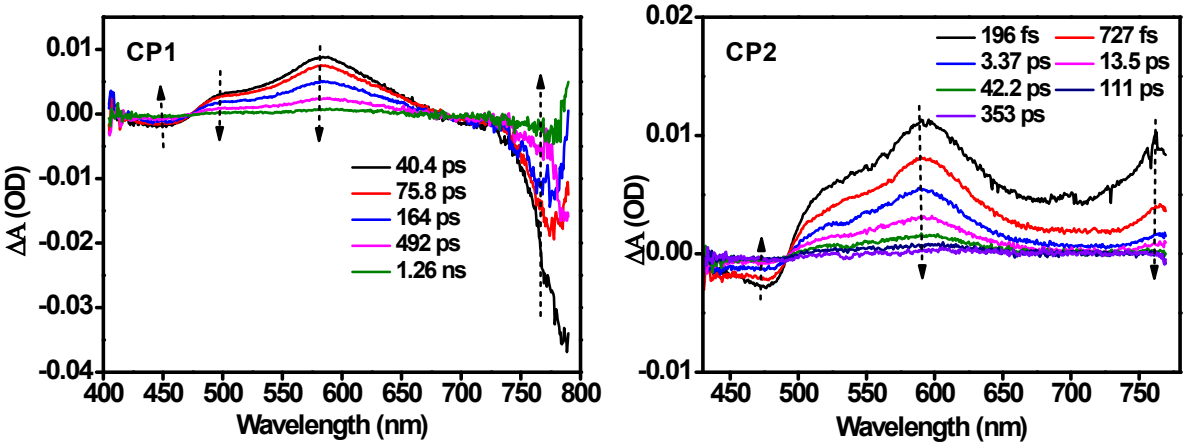


Fig S7. The fs-TA spectra of **CP1** and **CP2** obtained at selected delay times.

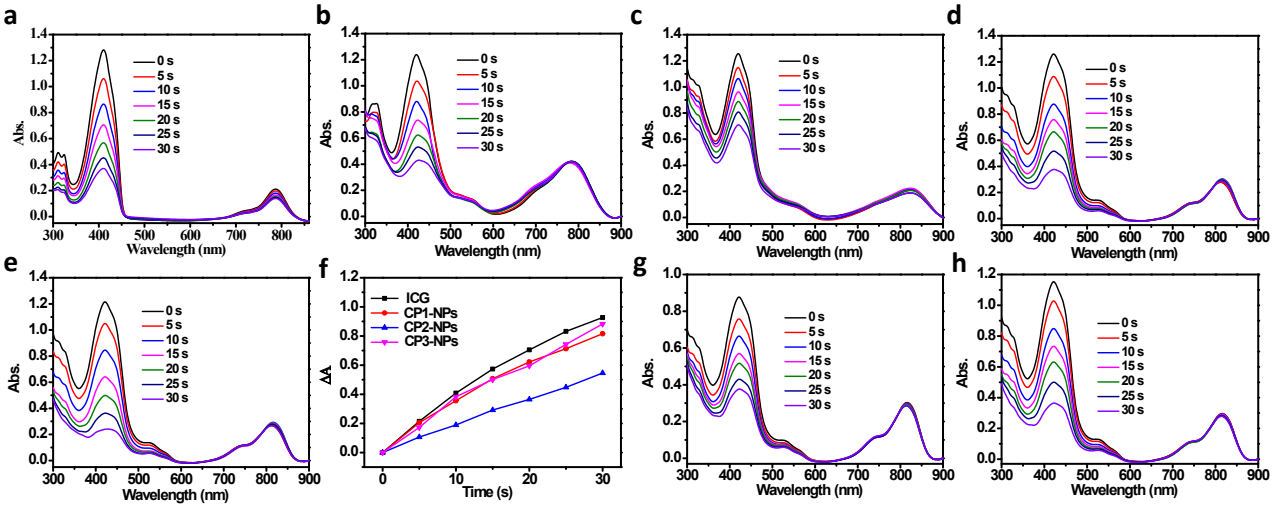


Fig S8. Absorption of DPBF with ICG (a), DPBF with CP1-NPs (b), DPBF with CP2-NPs (c), and DPBF with CP3-NPs (d) *vs* irradiation time in water, respectively (785 nm, 1 W cm^{-2}). (e) Absorption of DPBF with CP3-NPs under irradiation time in water (808 nm, 1 W cm^{-2}). (f) The spectra of absorption changes *vs* irradiation time. Absorption of DPBF with CP3-NPs under irradiation (g) (808 nm, 0.5 W cm^{-2}) and (h) (808 nm, 0.8 W cm^{-2}) time in water.

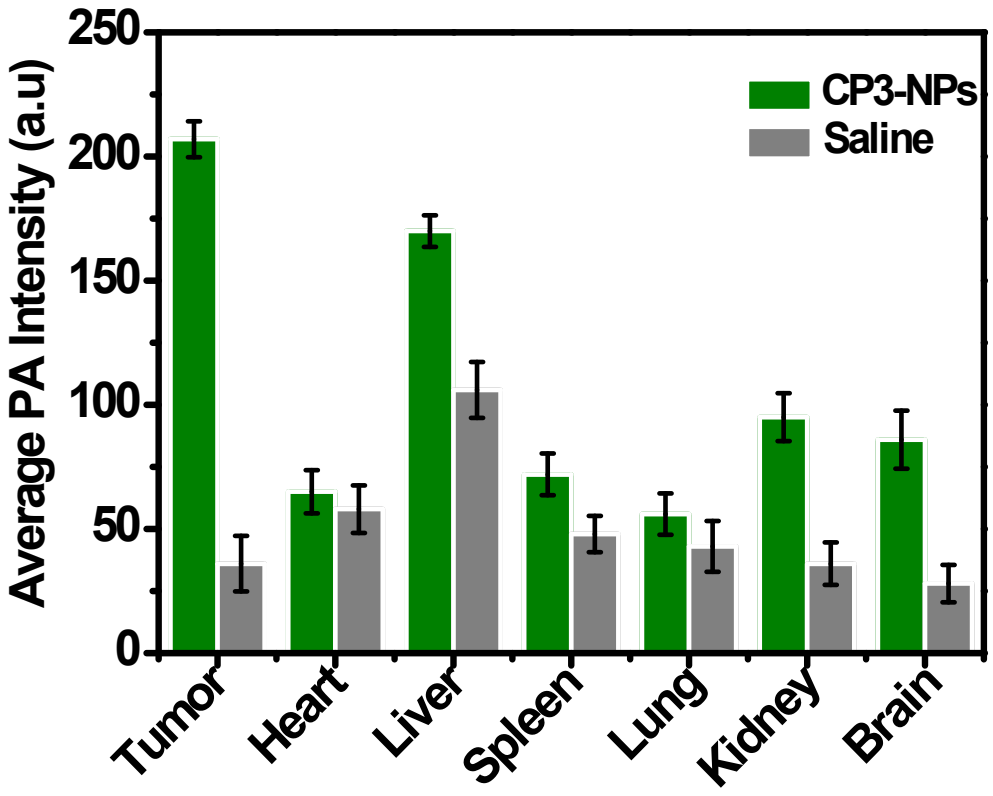


Fig S9. Ex vivo PA quantification of major organs of mice 6 h after injection of CP3-NPs or saline.

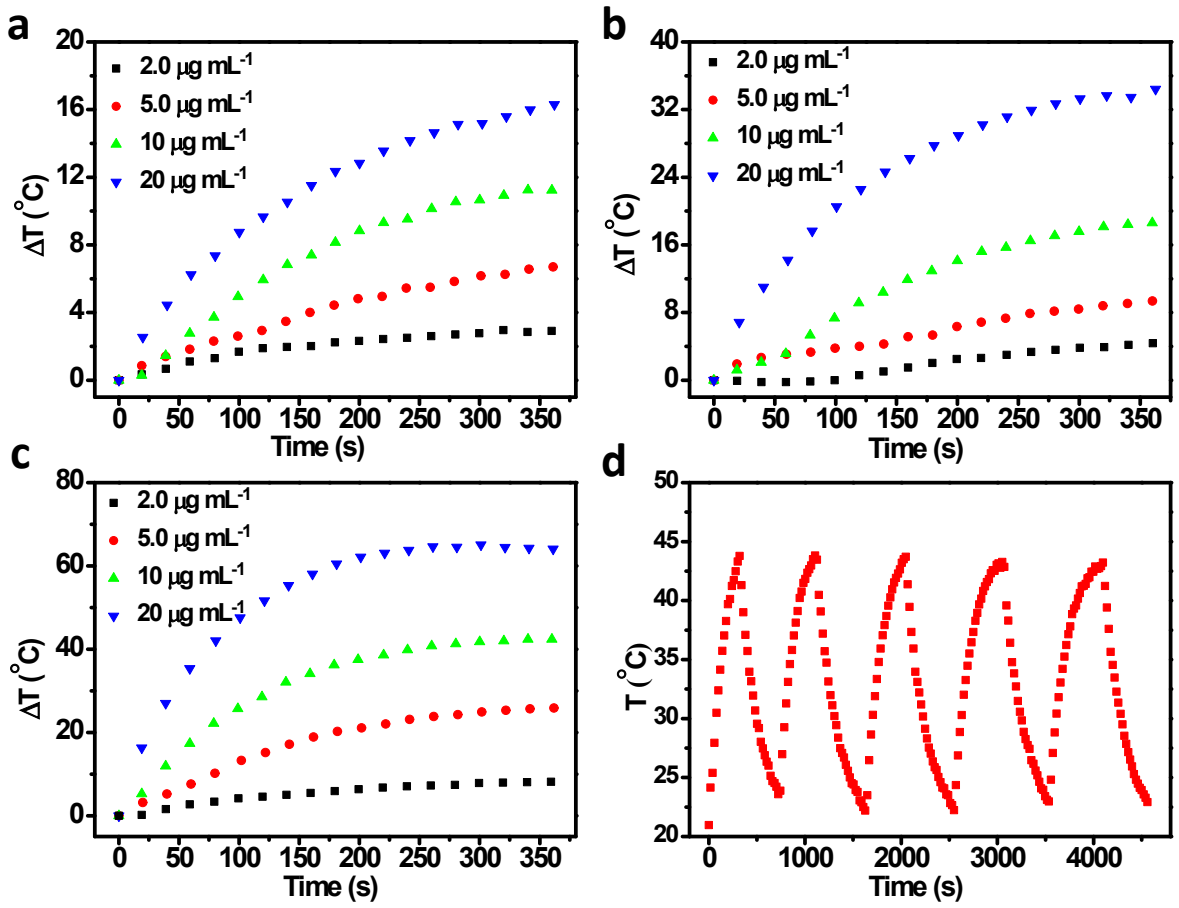


Fig S10. Temperature elevation of CP3-NPs at various concentrations under 808 nm irradiation at (a) 0.5 W cm^{-2}, (b) 1 W cm^{-2}, and (c) 1.5 W cm^{-2} for 6 min, respectively. (d) Temperature elevation of CP3-NPs under five irradiation/cooling cycles.

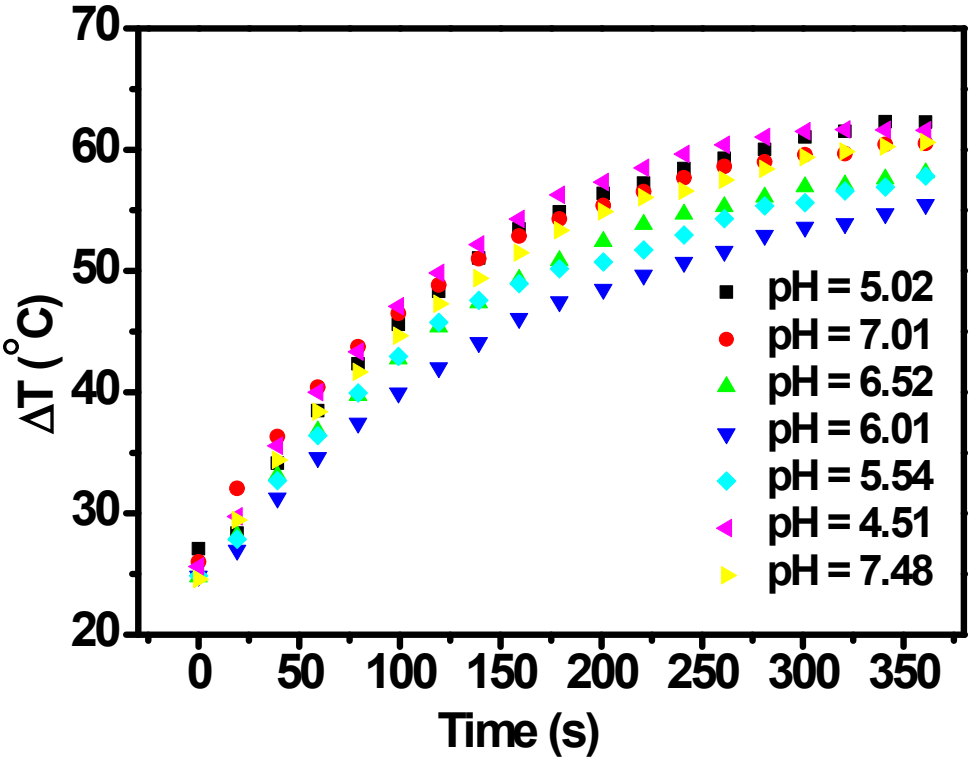


Fig S11. Temperature elevation of CP3-NPs at 10 μg mL^{-1} under 808 nm irradiation at 1.0 W cm^{-2} under various pH PBS solution.

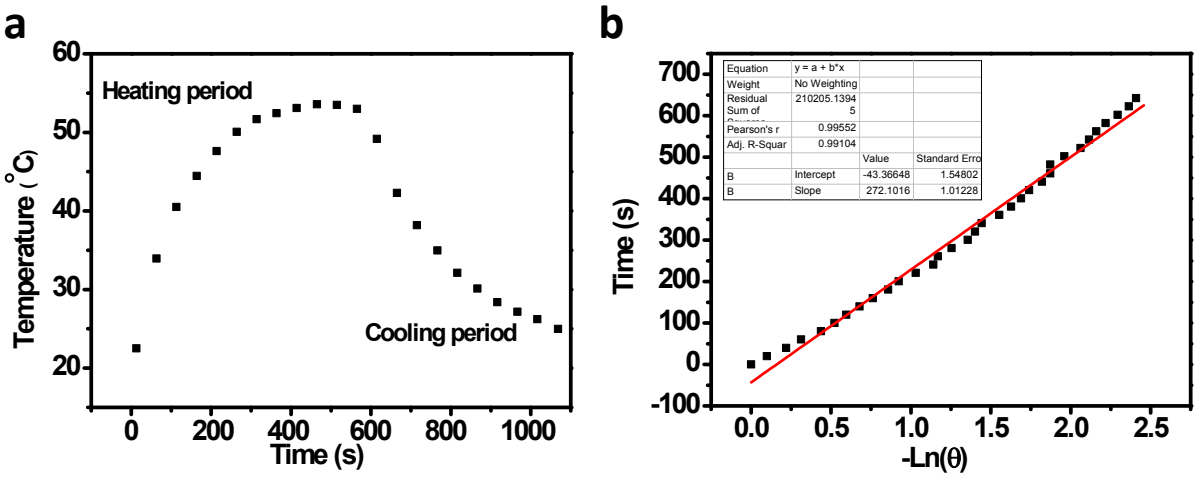


Fig S12. (a) Temperature elevation of CP3-NPs at the concentration of 10 μg mL^{-1} under 808 nm irradiation at 1 W cm^{-2} for 6 min, and subsequent cooling in the absence of irradiation. (b) Time constant for heat transfer is determined to be τ_s= 272 s by applying the linear time data from the cooling period versus negative natural logarithm of driving force temperature, which is obtained from the cooling stage of (a).

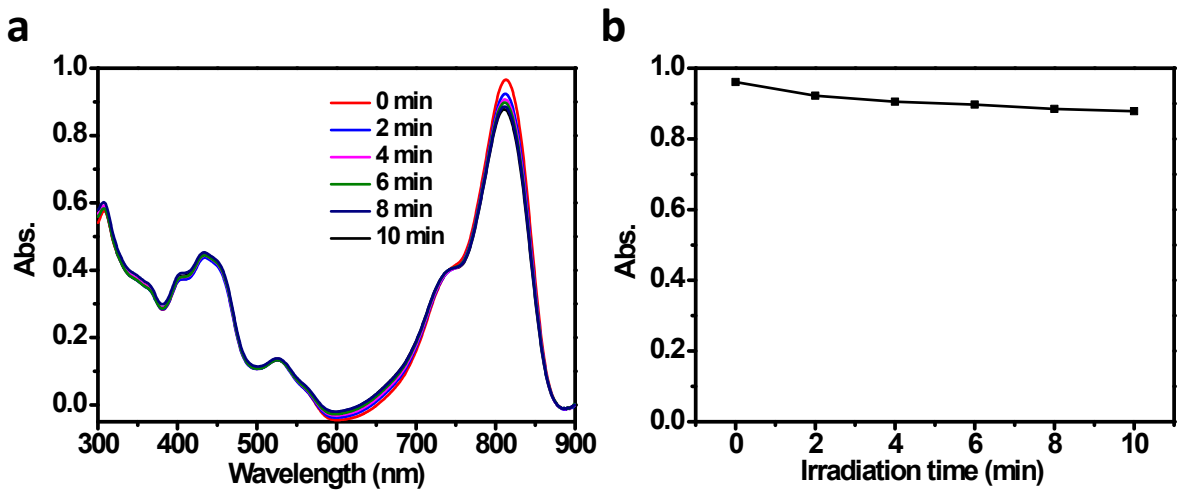


Fig S13. (a) Photostability of CP3-NPs under 808 nm irradiation at 1 W cm^{-2} for 2 min, 4 min, 6 min, 8min and 10 min, respectively. (b) Photostability of CP3-NPs at each maximum absorption peak.

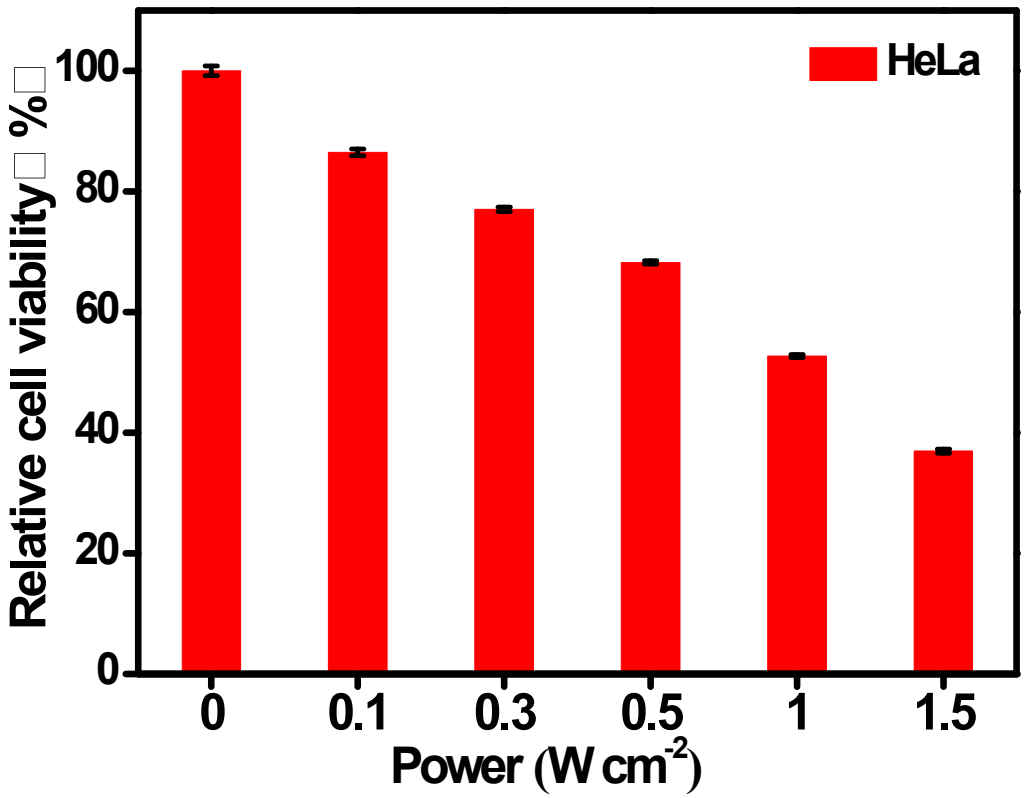


Fig S14. *In vitro* cell viability of Hela cells incubated with CP3-NPs at the concentration of 15 μg mL^{-1} under different power at 37 °C for 24 h.

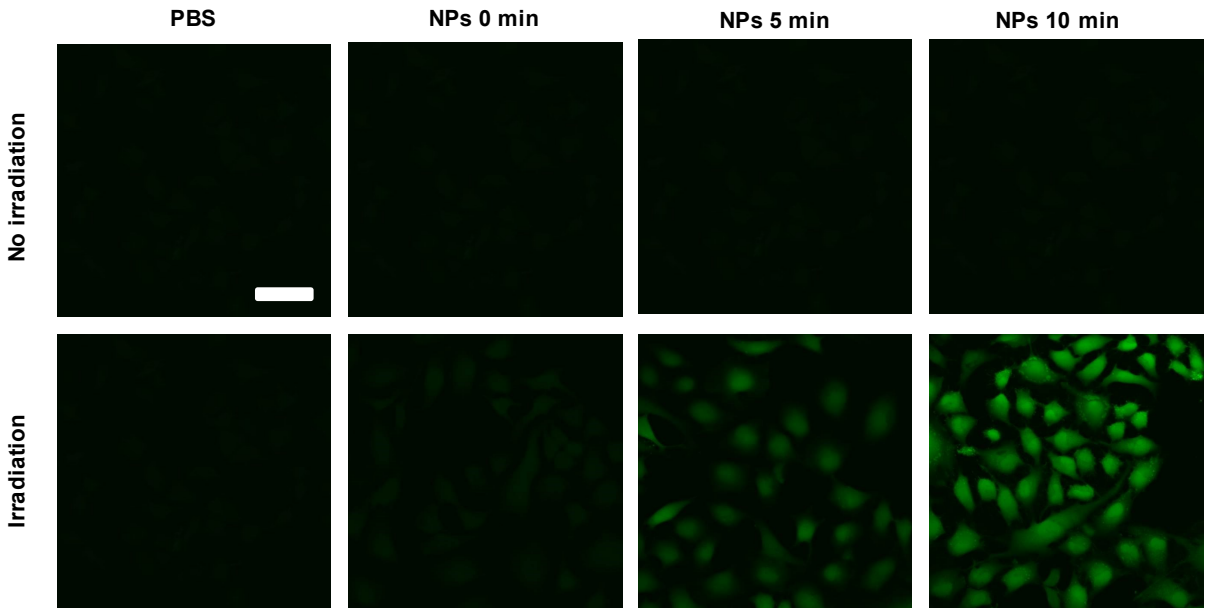


Fig S15. Fluorescent image of Hela cells treated with CP3-NPs for singlet oxygen detection at PBS and NPs with or without light irradiation using DCFH-DA staining (Scale bar, 100 μm). Cells were viewed 30 min later in green channel (λ_{ex} = 488 nm, λ_{em} = 500-540 nm).

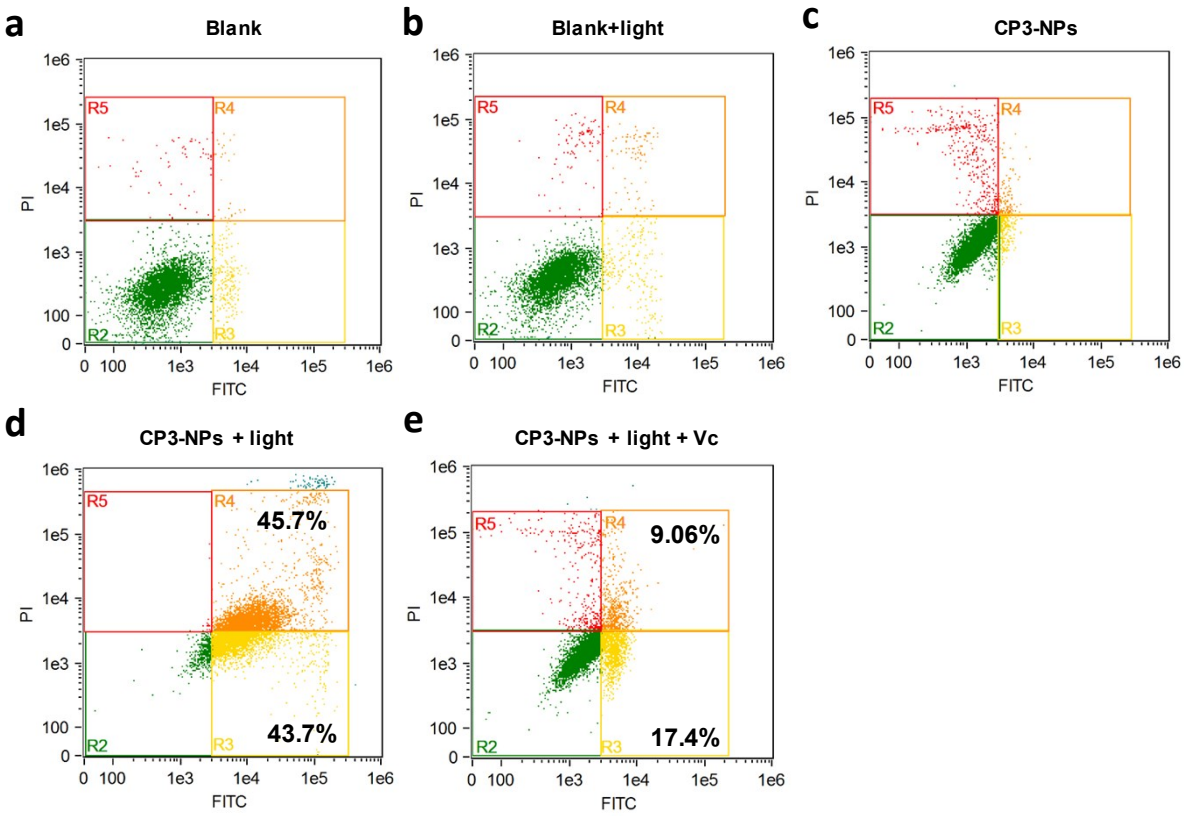


Fig S16. Flow cytometry quantification of annexin V-FITC- and PI-labeled HeLa cells. Cells were viewed in the green channel for annexin V-FITC (λ_{ex} = 488 nm, λ_{ex} = 500-560 nm) and red channel for PI (λ_{ex} = 488 nm, λ_{ex} = 600-680 nm).

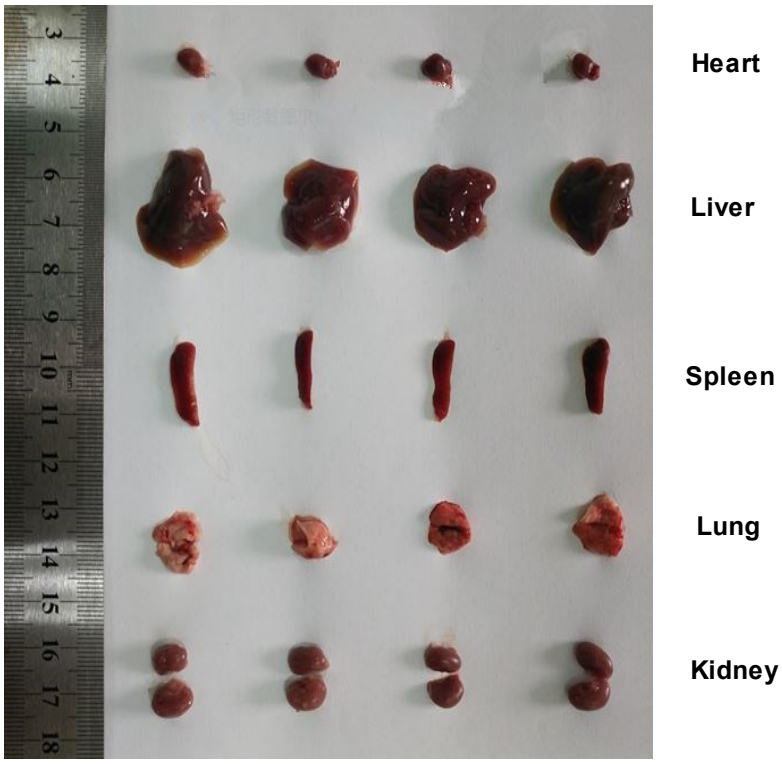


Fig S17. Various normal tissues harvested from the mice treated with CP3-NPs and PBS at the dose of 0.8 mg kg^{-1} under 808 nm irradiation on the tumors.

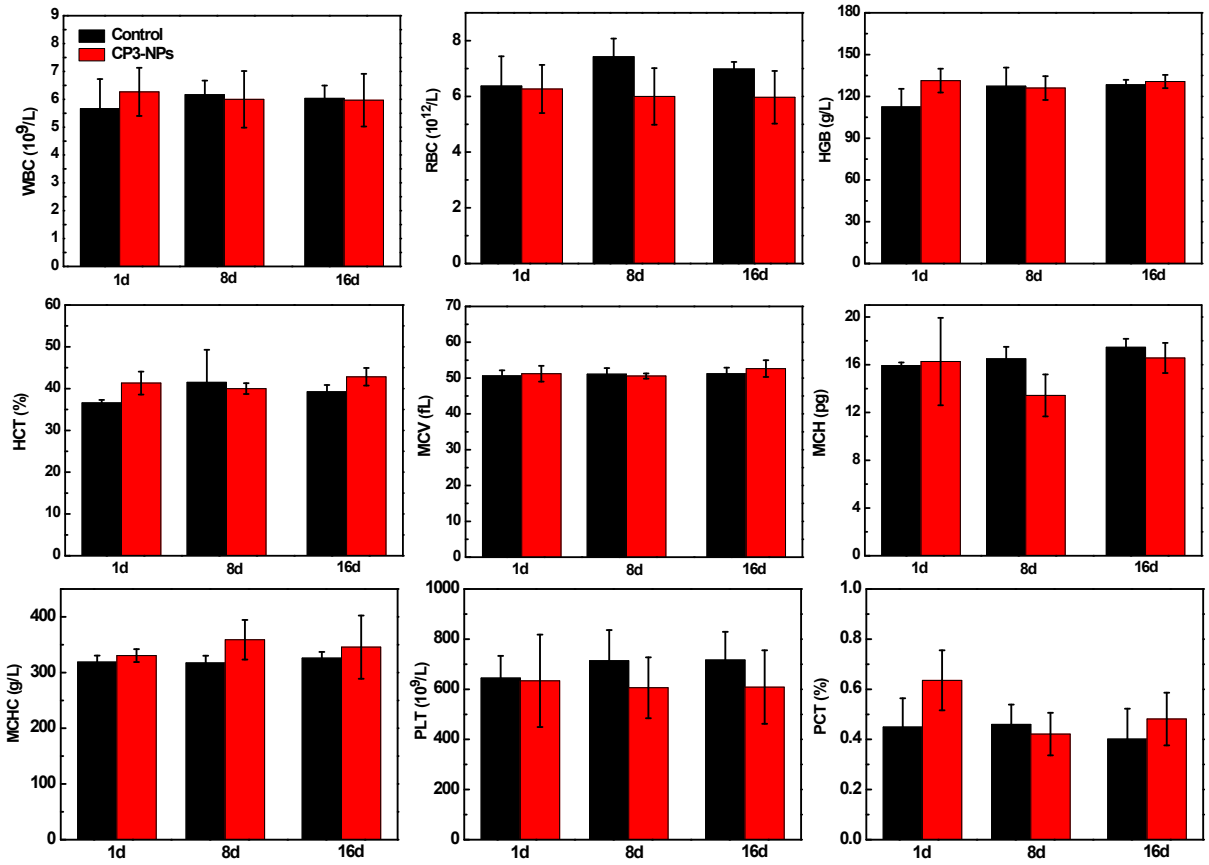
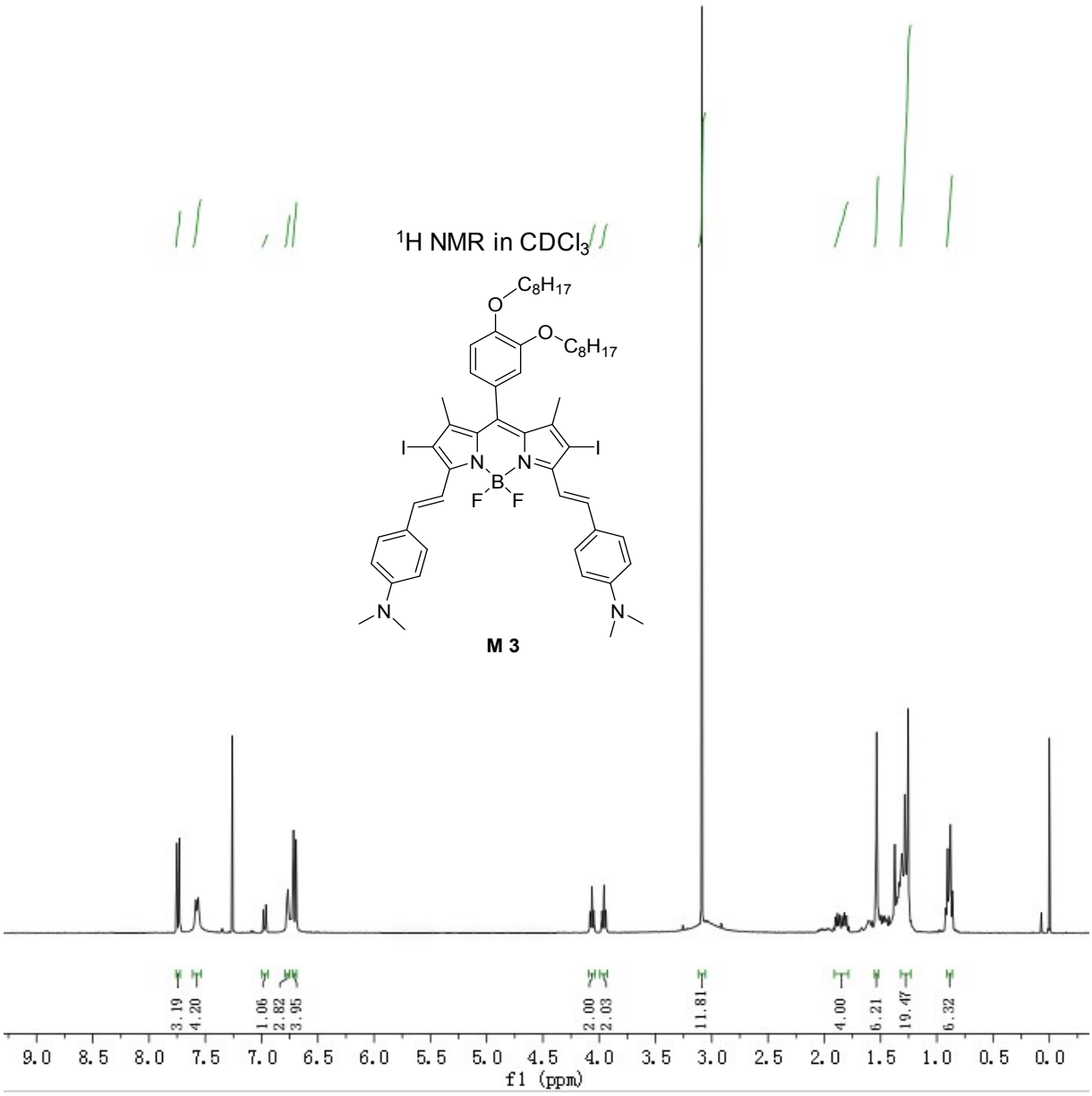
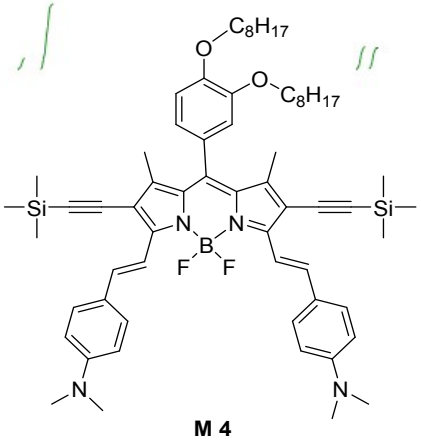


Fig S18. Hematology analysis from healthy and CP3-NPs treated mice performed at 1, 8 and 16 days (n = 3 mice/group). The indicators include white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT), plateletocrit (PCT). No significant difference was found between control and CP3-NPs treated groups. Error bars correspond to standard deviation.

The ^{1}H, ^{13}C NMR, and MS spectra of new compounds.

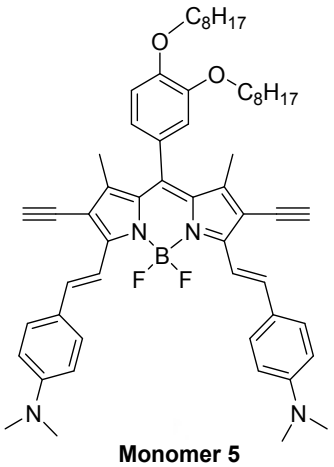


^{1}H NMR in $CDCl_3$



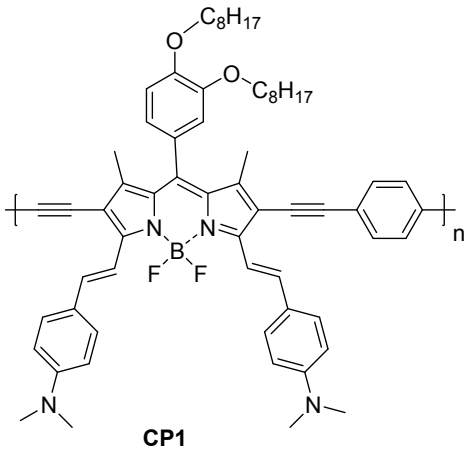
M 4

^{1}H NMR in $CDCl_3$



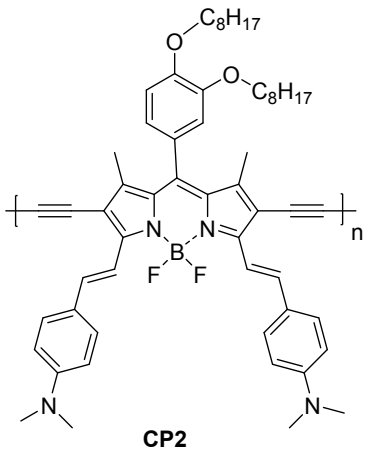
Monomer 5

^{1}H NMR in $CDCl_3$

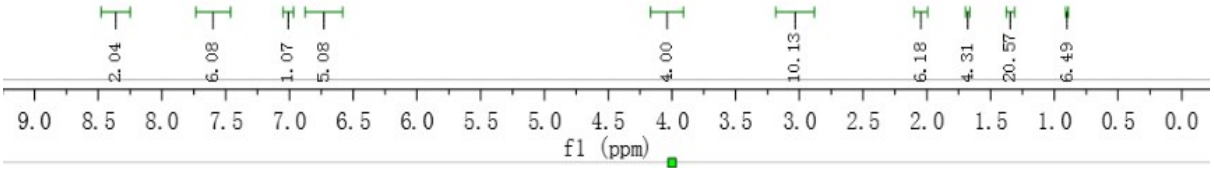


CP1

^{1}H NMR in $CDCl_3$



CP2



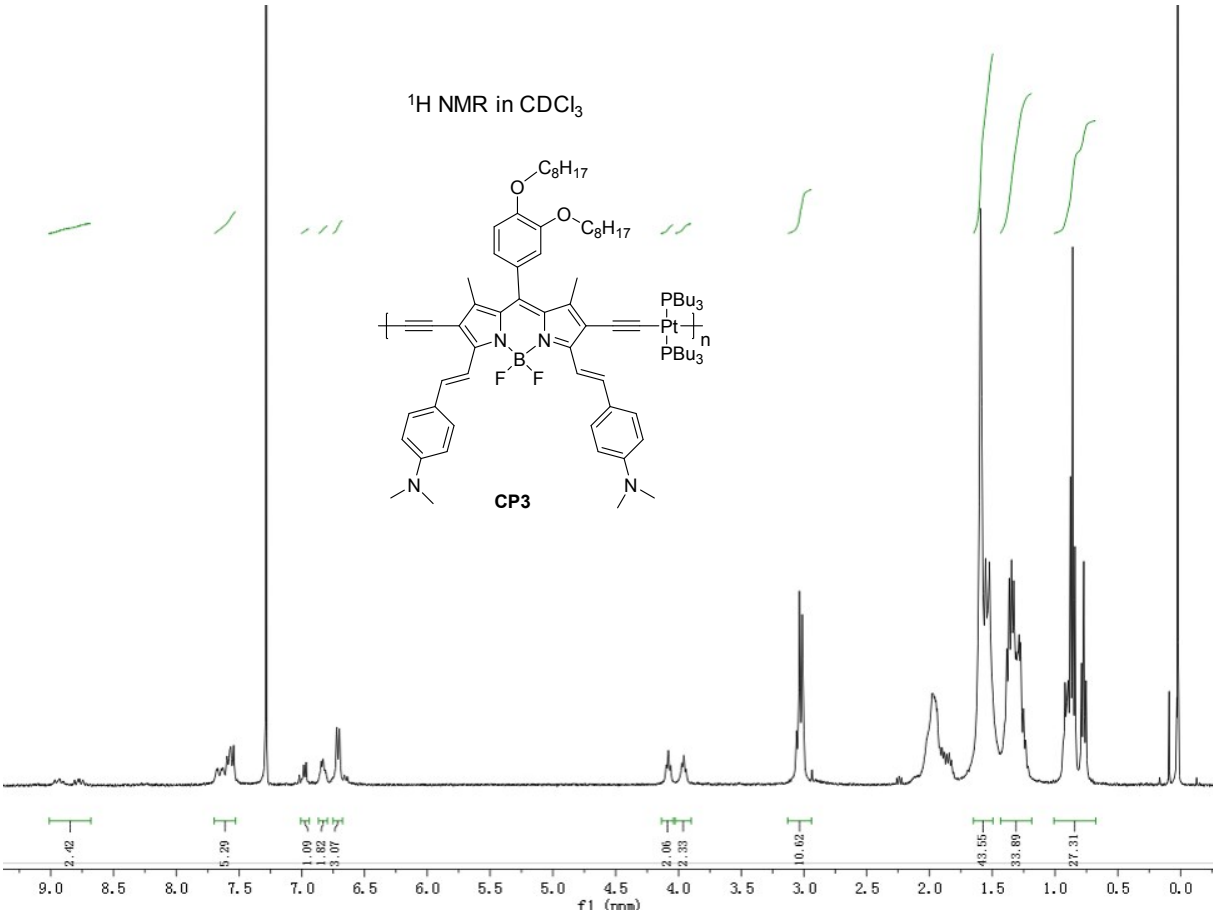
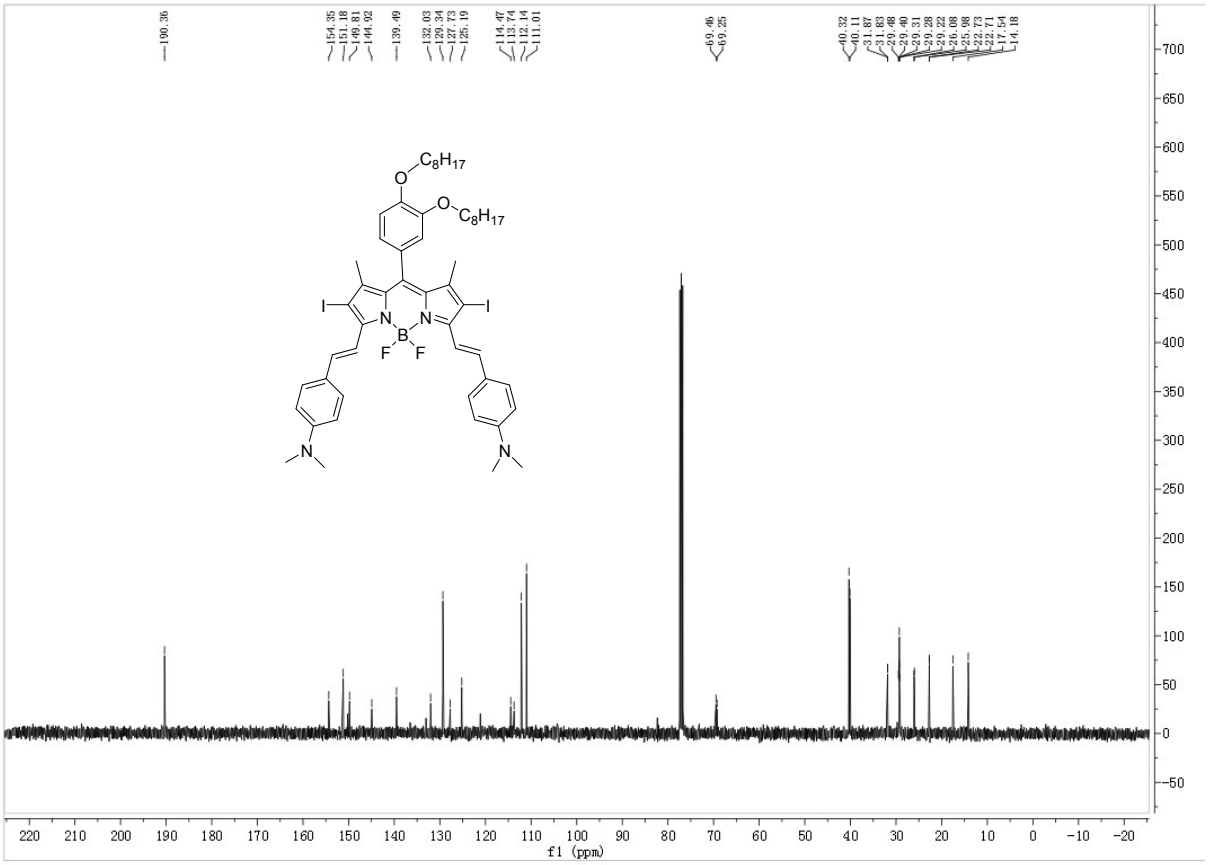
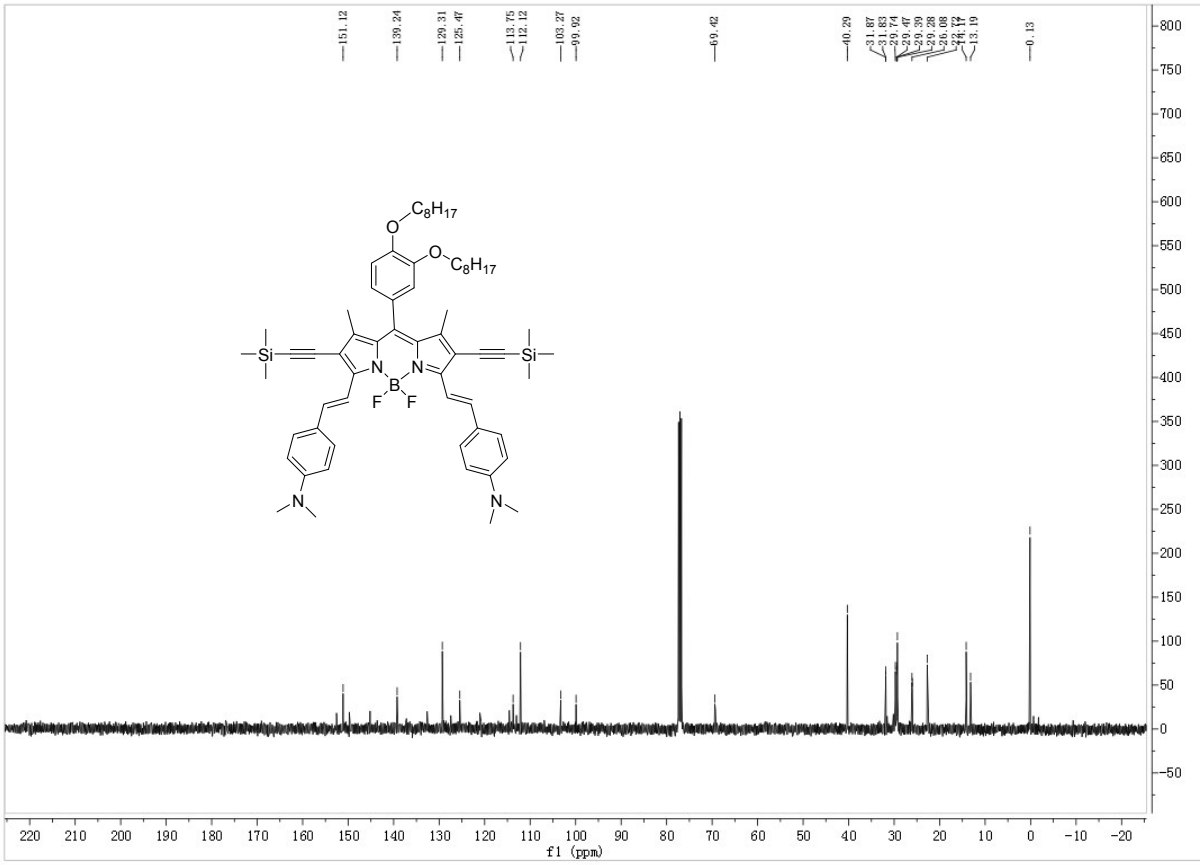


Fig S18. The ^{1}H NMR spectra of **M3-M5** and **CP1-CP3**.


190.36
154.35
151.18
149.81
144.92
139.49
132.03
129.34
127.73
125.19
114.47
113.74
112.14
111.01
69.46
69.25
40.32
40.11
31.87
31.83
29.48
29.40
29.31
29.28
29.22
26.08
25.98
22.73
22.71
17.54
14.18
C8H17
O
O
C8H17
I
I
N
B
N
F
F
N
N
f1 (ppm)


151.12
139.24
129.31
125.47
113.75
112.12
103.27
99.92
69.42
40.29
31.87
31.83
29.74
29.47
29.39
29.28
26.08
22.72
22.67
14.17
13.19
0.13
f1 (ppm)

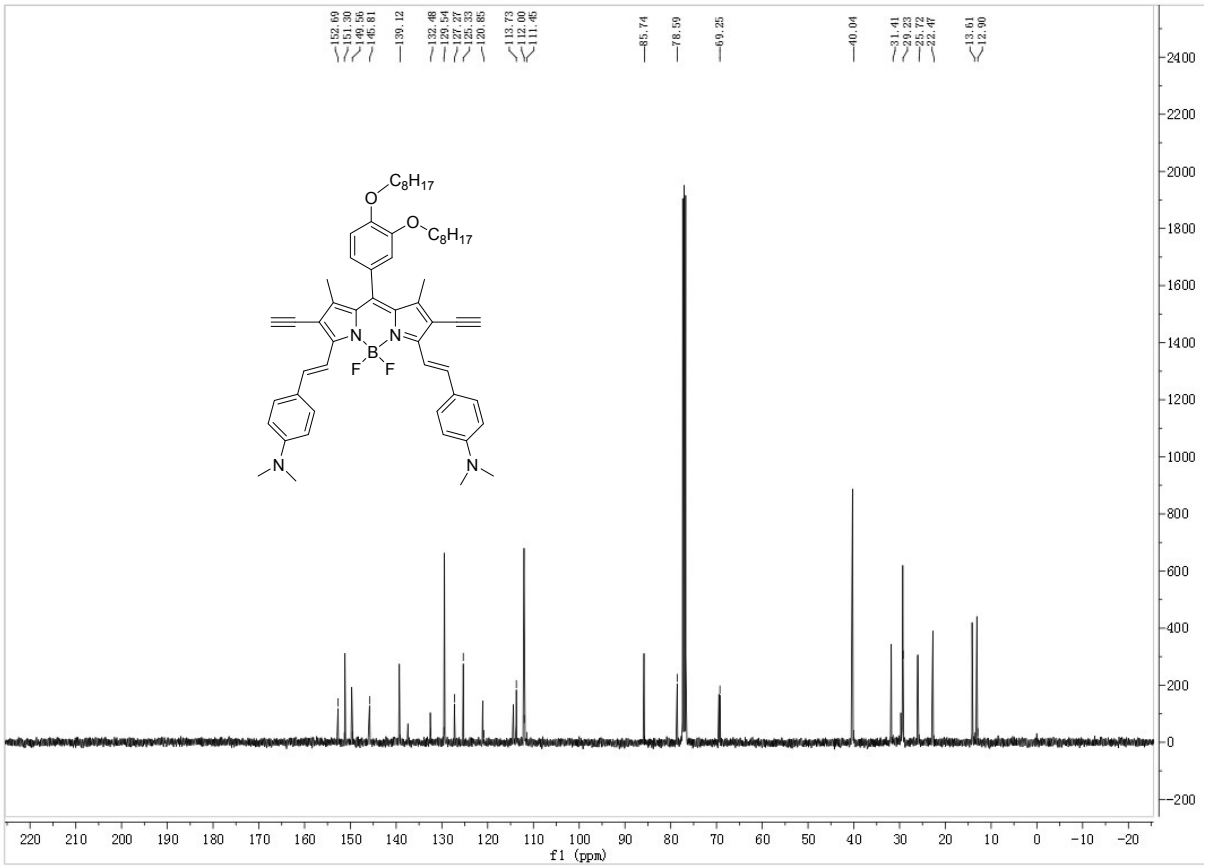
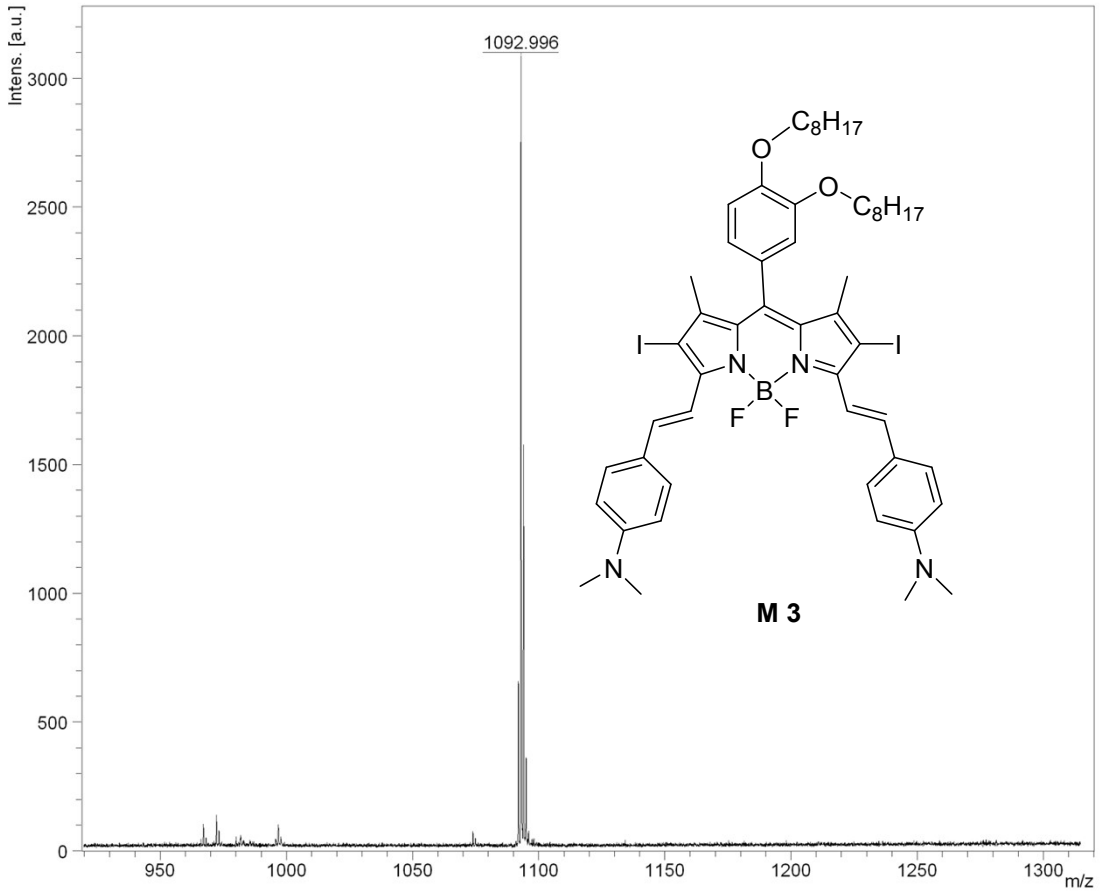


Fig S19. The ^{13}C NMR spectra of **M3**-**M5**.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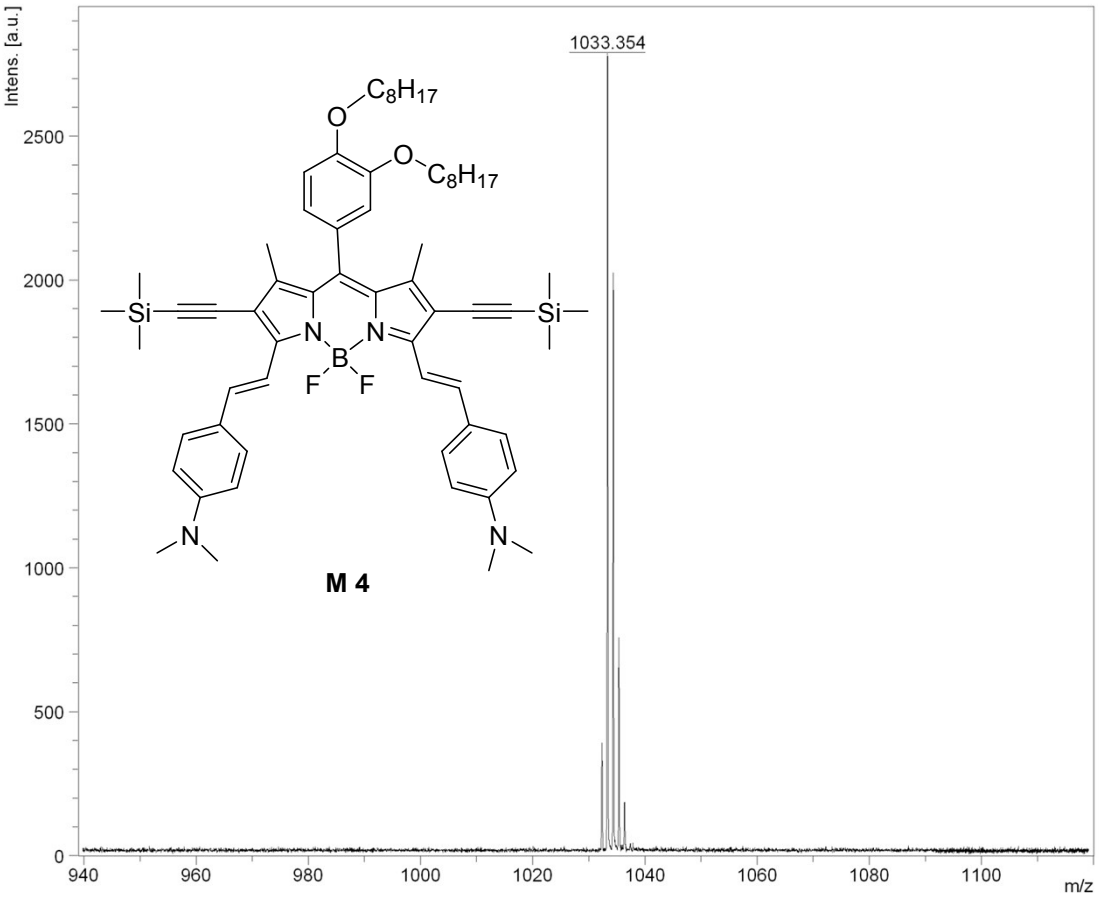
3000
2500
2000
1500
1000
500
0
950
1000
1050
1100
1150
1200
1250
1300
m/z
O
C8H17
O
C8H17
I
I
N
N
B
F
F
N
N

M 3


Intens. [a.u.]
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2000
1500
1000
500
0
940
960
980
1000
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1080
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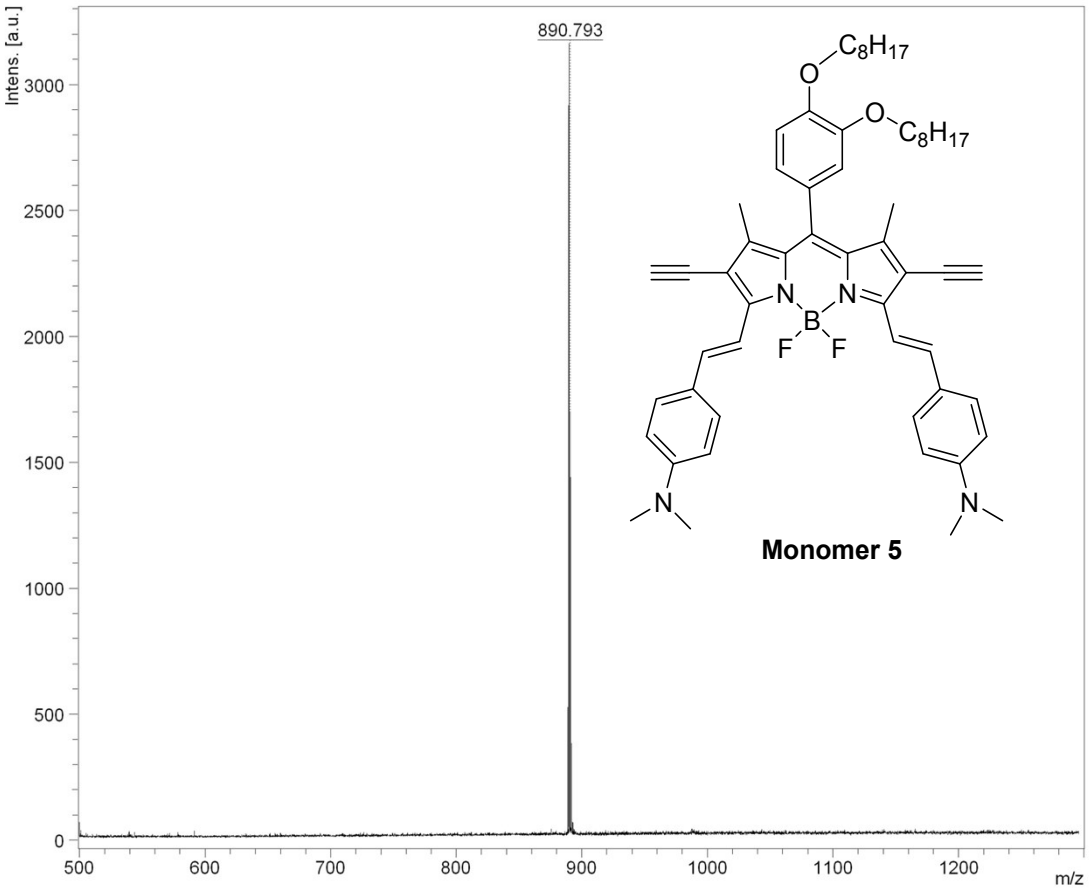

Fig S20. MALDI-TOF-MS spectra of **M3-M5**.