ADVANCED MATERIALS

Supporting Information

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Part A. Supplementary synthesis route

Compound 3. Allyl bromide (24.2 g, 0.2 mol) was added dropwise over 2 h to a stirring solution of pentaerythrotol 2 (68.0 g, 0.5 mol) in NaOH (9.6 g in 200 mL). Then the reaction was heated to 70 °C for 8 h. The mixture was diluted with water (100 mL) and extracted with EA (150 mL×5). The combined organic layer was dried over anhydrous Na_2SO_4, concentrated under vacuum and purified by column chromatography on silica gel (PE/EA = 1/1) to give compound 3 as clear oil (14.1 g, 42% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.82-5.92 (m, 1H), 5.18-5.28 (m, 2H), 3.97 (d, *J* = 8.0 Hz, 2H), 3.76 (s, 3H), 3.68 (s, 6H), 3.44 (s, 2H).
Compound 4. Under an atmosphere of argon, a solution of compound 3 (7.0 g, 40.0 mmol) in DMF (50 mL) was added dropwise into a suspension of NaH (5.8 g, 240.0 mmol) in DMF (100 mL) in an ice bath. After stirring for 30 min, a solution of $mPEG_7Tos$ (79.0 g, 160.0 mmol) in DMF (100 mL) was added and the resulting mixture was stirred at 60 °C for 24 h. Then DMF was evaporated under reduced pressure. The crude was purified by column chromatography on a silica gel (CH_2Cl_2 /MeOH = 10/1) to give alcohol compound 4 as a clear oil (38.4 g, 84% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.84-5.89 (m, 1H), 5.24 (d, *J* = 8.0 Hz, 1H), 5.12 (d, *J* = 4.0 Hz, 1H), 3.93 (s, 2H), 3.56-3.65 (m, 88H), 3.44 (s, 4H), 3.38 (s, 9H).
Compound 5. A mixture of $CHCl_3$: CH_3CN : H_2O (1:1:1.5, 210 mL) was added to compound 4 (37.7 g, 33.0 mmol), $NaIO_4$ (42.4 g, 198.0 mmol) and ruthenium(III)chloride hydrate (66.0 mg, 0.33 mmol) at 0 ºC. The mixture was stirred at room temperature for 3 h. Then the mixture was added to water and extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous Na_2SO_4, concentrated under vacuum and purified by column chromatography on a silica gel (CH_2Cl_2 /MeOH = 8/1) to give compound 5 as a clear oil (25.3 g, 66% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.04 (s, 2H), 3.$_{54}$-3.65 (m, 86H), 3.47 (s, 6H), 3.38 (s, 9H).
Compound 6. A solution of pentaerythritol 2 (68.1 g, 0.5 mol) in DMSO (100 mL) was heated to 80 ºC, then aqueous NaOH (4.0 g in 9 mL H_2O) was added to one portion, and *tert*-butyl acrylate (76.9 g, 0.6 mol) was added to the solution dropwise. The mixture was vigorously stirred overnight at 80 ºC. After cooling, the solution was extracted with EA. The combined organic phase was dried over anhydrous Na_2SO_4, concentrated under vacuum and purified by column chromatography on a silica gel (PE/EA = 1/1) to give compound 6 (46.2 g, 35% yield) as a clear oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.67 (t, *J* = 8.0 Hz, 2H), 3.65 (s, 6H), 3.52 (s, 2H), 3.10 (s, 3H), 2.50, 2.49(t, *J* = 8.0 Hz, 2H), 1.46 (s, 9H).

Compound 7. Triphenylphosphine (59.0 g, 225.0 mmol), 4 Å molecular sieves (15.0 g) and THF (150 mL) was added to a stirred suspension of compound 6 (13.2 g, 50.0 mmol). Then DIAD (45.5 g, 225.0 mmol) was added dropwise to the mixture at 0 ºC. The reaction mixture was then stirred for an additional 20 min. Then, perfluoro-tert-butanol (53.0 g, 225.0 mmol) was added to one portion, and the resulting mixture was stirred at 45 ºC for 48 h in a sealed vessel. The reaction mixture was added to water (100 mL) and extracted with EA (100 mL × 3). The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4 and concentrated through rotary evaporation. The residue was subjected to silica gel chromatography (PE/EA = 10/1) to give compound 7 (26.2 g, 57% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.04 (s, 6H), 3.64 (t, *J* = 8.0 Hz, 2H), 3.42 (s, 2H), 2.45 (t, *J* = 8.0 Hz, 2H), 1.44 (s, 9H); ^{19}F NMR (376 MHz, $CDCl_3$) δ −73.64.
Compound 8. Trifluoroacetic acid (63.9 g, 560.0 mmol) was added to a stirring solution of compound 7 (25.7 g, 28.0 mmol) and anisole (4.5 g, 42.0 mmol) in CH_2Cl_2 (200 mL) and the reaction mixture was stirred at room temperature for 4 h. Then, the mixture was concentrated under vacuum. The residue was purified by column chromatography on a silica gel (PE/EA = 3/1) to give compound 8 as a white solid (21.7 g, 90% yield). ^{1}H NMR (400 MHz, CD_3OD) δ

4.14 (s, 6H), 3.70 (t, *J* = 8.0 Hz, 2H), 3.47 (s, 2H), 2.54 (t, *J* = 8.0 Hz, 2H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -71.17.

Compound 9. Concentrated H_2SO_4 (4.0 mL) was added to a stirring solution of compound 8 (21.5 g, 25.0 mmol) in MeOH (100 mL). After refluxing for 8 h, the mixture was neutralized with saturated sodium bicarbonate solution. The mixture was added to water (100 mL) and extracted with EA (100 mL × 3). The combined organic phase was dried over anhydrous Na_2SO_4 and concentrated through rotary evaporation. The residue was purified by column chromatography on a silica gel (PE/EA = 10/1) to give compound 9 as a white solid (20.8 g, 95% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.03 (s, 6H), 3.67-3.70 (m, 5H), 3.41 (s, 2H), 2.55 (t, *J* = 8.0 Hz, 2H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -73.61.

Compound 10. Compound 9 (21.0 g, 24.0 mmol) was dissolved in MeOH (100 mL) and ethylenediamine (30 mL). The mixture was refluxed for 48 h. Then the mixture was evaporated and the residue was purified by column chromatography on a silica gel (CH_2Cl_2/MeOH = 10/1) to give compound 10 as a clear oil (15.8 g, 73% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 4.15 (s, 6H), 3.71 (t, *J* = 8.0 Hz, 2H), 3.47 (s, 2H), 3.26 (s, 2H), 2.73 (t, *J* = 8.0 Hz, 2H), 2.48 (t, *J* = 8.0 Hz, 2H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -71.16.

Compound 11. Under an atmosphere of argon, EDC (6.46 g, 33.7 mmol) was added to a stirring solution of HOBt (4.6 g, 33.7 mmol) and Fmoc-Boc-lys-OH (10.5 g, 22.5 mmol) in DMF (100 mL) at 0 °C. After 20 min, compound 10 (13.5 g, 15.0 mmol) in DMF (50 mL) was added to one portion and the reaction mixture was stirred at 45 ºC for 12 h. The reaction mixture was washed with brine (200 mL) and extracted with EA (150 mL × 4). The combined organic layer was dried over anhydrous Na_2SO_4, concentrated under vacuum and purified by column chromatography on a silica gel (PE/EA = 2/1) to give compound 11 as a white solid (13.0 g, 64% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 7.82 (d, *J* = 4.0 Hz, 2H), 7.69 (q, *J* = 4.0 Hz, 2H), 7.41 (t, *J* = 8.0 Hz, 2H), 7.33 (t, *J* = 8.0 Hz, 2H), 4.38-4.44 (m, 2H), 4.24 (t, *J* = 8.0 Hz, 2H), 4.12 (s, 6H), 4.0 (q, 4.0 Hz, 1H), 3.65 (t, *J* = 8.0 Hz, 2H), 3.41 (s, 2H), 3.29 (d, *J* = 4.0 Hz, 2H), 3.04 (t, *J* = 8.0 Hz, 2H), 2.42 (t, *J* = 8.0 Hz, 2H), 1.73-1.80 (m, 1H), 1.60-1.68 (m, 1H), 1.28-1.50 (m, 15H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -71.11.

Compound 12. Trifluoroacetic acid (21.7 g, 190.0 mmol) was added to a stirring solution of compound 11 (12.9 g, 9.5 mmol) and anisole (1.5 g, 14.3 mmol) in CH_2Cl_2 (100 mL) and the reaction mixture was stirred at room temperature for 4 h. Then, the mixture was concentrated under vacuum. The residue was purified by column chromatography on a silica gel (CH_2Cl_2/MeOH = 8/1) to give compound 12 as a white solid (11.2 g, 94% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 7.70 (d, *J* = 4.0 Hz, 2H), 7.56 (t, *J* = 4.0 Hz, 2H), 7.29 (t, *J* = 8.0 Hz, 2H), 7.21 (t, *J* = 8.0 Hz, 2H), 4.27-4.35 (m, 2H), 4.12 (t, *J* = 8.0 Hz, 1H), 4.00 (s, 6H), 3.92 (q, *J*=4.0 Hz, 1H), 3.53 (t, *J* = 8.0 Hz, 2H), 3.29 (s, 2H), 3.18 (s, 4H), 1.67–1.75 (m, 1H), 1.49-1.60 (m, 3H), 1.27–1.37 (m, 2H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -71.15.

Compound 13. Under an atmosphere of argon, EDC (2.8 g, 14.4 mmol) was added to a stirring solution of HOBt (1.9 g, 14.4 mmol) and compound 5 (11.1 g, 9.6 mmol) in DMF (50 mL) at 0 ºC. After 20 min, compound 12 (10.0 g, 8.0 mmol) was added to one portion at room temperature and the reaction mixture was stirred at 45 °C for 12 h. Then the reaction mixture was washed with brine (200 mL) and extracted with CH_2Cl_2 (150 mL, four times). The combined organic layer was dried over anhydrous Na_2SO_4, concentrated under vacuum and purified by column chromatography on a silica gel (CH_2Cl_2/MeOH = 10/1) to give compound 13 as a colorless oil (15.0 g, 78% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 4.0 Hz, 2H), 7.63 (d, *J* = 4.0 Hz, 2H), 7.40 (t, *J* = 8.0 Hz, 2H), 7.31 (t, *J* = 8.0 Hz, 2H), 4.35-4.45 (m, 2H), 4.21 (t, *J* = 8.0 Hz, 2H), 4.04 (s, 6H), 3.89 (s, 2H), 3.53-3.65 (m, 90H), 3.44 (d, *J* = 4.0 Hz, 6H), 3.37-3.39 (m, 13H), 2.90 (s, 2H), 2.40 (d, *J* = 4.0 Hz, 2H), 1.26–1.69 (m, 6H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -73.61.

Compound 14. Piperidine (6 mL) was added to a solution of compound 13 (7.2 g, 3.0 mmol) in DMF (30 mL). The mixture was stirred at room temperature for 4 h. Then, the DMF was removed under reduced pressure. The residue was purified by column chromatography on a silica gel (CH_2Cl_2/MeOH = 5/1) to give compound 14 as a colorless oil (5.4 g, 83% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.20 (d, *J* = 8.0 Hz, 2H), 4.18-4.31 (m, 1H), 4.05 (s, 6H), 3.90 (s, 4H), 3.56-3.66 (m, 85H), 3.38-3.45(m, 22H), 2.88 (s, 4H), 2.45 (t, *J* = 8.0 Hz, 2H), 1.41–1.85 (m, 6H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -73.58.

Compound 1 (Bodipy). Under an atmosphere of argon, EDC (345.0 mg, 1.8 mmol) was added to a stirring solution of HOBt (243.0 mg, 1.8 mmol) and compound 21 (387.0 mg, 0.6 mmol) in DMF (20 mL) at 0 ºC. After 20 min, HRfPEG (3.9 g, 1.8 mmol) was added to one portion at room temperature and the reaction mixture was stirred at 45 ºC for 24 h. The reaction mixture was washed with brine (200 mL) and extracted with CH_2Cl_2 (150 mL×3). The combined organic layer was dried over anhydrous Na_2SO_4, concentrated under vacuum and purified by column chromatography on a silica gel (CH_2Cl_2/MeOH = 10:1) to yield compound 1 as a green oil (2.08 g, 70% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 8.07-8.12 (m, 8H), 7.34-7.49 (m, 8H), 7.07-7.09 (m, 4H), 4.04 (s, 4H), 3.89 (s, 4H), 3.$_{54}$-3.65 (m, 186H), 3.42-3.44 (m, 20H), 3.37 (s, 18H), 1.90-1.95 (m, 2H), 1.69-1.78 (m, 2H), 1.53-1.56 (m, 4H), 1.34-1.37 (m, 4H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -71.34; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 171.1, 170.6, 167.8, 159.4, 132.3, 131.8, 129.3, 128.6, 125.4, 120.0 (q, *J*=291.0 Hz), 115.0, 71.9, 71.3, 71.0, 70.5, 70.4, 70.3, 70.2, 69.9, 67.6, 67.2, 66.1, 65.5, 59.0, 52.7, 46.1, 45.3, 39.7, 39.5, 38.0, 36.3, 31.8, 29.2, 22.6; MS (MALDI-TOF) calculated for $C_{196}H_{288}BF_{56}N_{11}NaO_{68}^+$ $[M+Na]^+$ 4981.9, found 4981.8.

Part B. Supplementary experimental details

1. Chemicals and reagents

Perfluorohexane was purchased from J&K (Shanghai, China). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum, dihydrochloride (DAPI) and carbon-H_2DCFDA were obtained from ThermoFisher Scientific (Waltham, MA, USA). The cell counting kit-8 (CCK-8) was supplied by Dojindo (Kumamoto, Japan). The *in situ* cell death detection kit and calcein-AM (CA) were purchased from Sigma-Aldrich (Shanghai, China). Propidium Iodide (PI) was obtained from Biolegend (San Diego, USA). Hoechst 33342 was provided by Beyotime (Jiangsu, China). Hypoxyprobe-1 Plus Kits were purchased from Hypoxyprobe (Burlington, MA). Singlet Oxygen Sensor Green® was perchased from ThermoFisher Scientific (Shanghai, China).

2. Synthesis of the nanoemulsion

Due to the limit of space, the methodology of BODIPY synthesis was moved into supplementary information. The nanoemulsion PFH@PEG-F_{54}-BODIPY was synthesized by the ultrasonic emulsification method. Specifically, the BODIPY amphiphile was dissolved in deionized water (2.5 mL, 0.1 mg/mL). Then, 75 μL PFH was gradually added into the BODIPY solution under sonication (100 W) over an ice bath for 6 min to form homogeneous nanoemulsions. To avoid contamination, the BODIPY solutions was filtered through a 220-nm pore membrane, and the ultrasonic probe was rinsed with 75% alcohol. To perform phototherapy *in vivo*, the nanoemulsion was oxygenated by inserting a 1 mL tip that connected to an oxygen pump (O_2 flow rate = 1 L/min) for 5 min.

3. Detection of singlet oxygen *in vitro*

First, 100 μL samples and 10 μL SOSG (50 mM) were mixed in a black 96-well plate (Corning®). After irradiation using a 660 nm laser at a power density of 200 mW/cm^2, the oxidized SOSG was quantified by measuring the fluorescence intensity (Ex/Em: 504/525 nm) using a multimode plate reader (EnSpire™, Perkin Elmer). The concentration of oxidized SOSG was positively correlated with the concentration of singlet oxygen. All operations were performed in the dark to avoid quenching of the BODIPY. The experiments for each group were run in triplicate.

A375 cells were cultured in DMEM (10% fetus bovine serum) and seeded into a 12-well plate at a density of 1.4×10^5 per well. After incubation for 24 h in a cell-culture incubator, the culture medium was replaced with 1 mL fresh culture medium. Then, PEG-F_{54}-BODIPY or PFH@PEG-F_{54}-BODIPY was added into the wells (5 μM BODIPY). The cells were further incubated for 12 h at 37 °C and 5% CO_2. After washing once with PBS, the cells were incubated with carboxy-H_2DCFDA (25 μM) for 30 min. Tthe cells were then washed again with PBS and irradiated with a 660 nm laser at a power density of 200 mW/cm^2 for 5 min per well. Then, the cells were fixed with 4% formaldehyde polymer for 10 min. Finally, the cells were replaced with 1 mL PBS. The fluorescence emission spectrum of carboxy-DCF (Ex/Em = 495/529 nm) was immediately captured on a confocal fluorescence microscope (TCS-SP5 II; Leica Biosystems).

4. Cellular uptake

A375 cells were cultured in DMEM (10% fetus bovine serum) and seeded in 12-well plates at a density of 1.4×10^5 per well. After incubation for 24 h in the incubator, the medium was replaced with 1.4 mL fresh culture medium. PFH@PEG-F_{54}-BODIPY and PEG-F_{54}-BODIPY were then added into the wells (10 μM BODIPY). After 2 h of incubation, cells were fixed with 4% paraformaldehyde and stained with DAPI (4 μM). Afterwards, the

cells were washed and observed using a laser scanning confocal microscope (TCS SP5 II, Lecia, Wetzlar, Germany).

5. Flow cytometry

The A375 cells were seeded with a density of 5 x 10^5 per well in 12-well plates. After incubation for 24 h, the medium was replaced with 1.4 mL fresh culture medium. Then, 700 ml PBS, PEG-F_{54}-BODIPY or PFH@PEG-F_{54}-BODIPY (2 μM of BODIPY) was added to each well, as appropriate. The cells were further incubated for 0.5, 1, 2, 6 and 24 h at 37 °C in an atmosphere containing 5% CO_2. After washing once with PBS, the cells were treated with trypsin and resuspended in 400 μl PBS for flow cytometry analysis (FACS-Calibur, BD Corp.). Red BODIPY fluorescence was collected on the FL4 channel. Data were obtained and analyzed using CELL QUEST and FLOWJO programs.

6. *In vitro* phototoxicity

A375 cells were cultured in DMEM (10% fetus bovine serum) and seeded into 96-well plates at a density of 1 × 10^4 cells per well. After incubation for 24 h in the incubator, cells were washed once with PBS. The cells were incubated with PFH@PEG-F_{54}-BODIPY and PEG-F_{54}-BODIPY (0, 0.1, 0.25, 0.5, 1, 2.5, 5 and 10 μM BODIPY) for 12 h at 37 ºC under the same conditions. To evaluate the cytotoxicity, the cells of each group were rinsed twice with PBS. A standard CCK-8 assay was carried out to evaluate the cell viability. To evaluated the *in vitro* PDT effect, cells of each group were also rinsed twice with PBS. Then the cells were irradiated using a 660 nm laser at a power density of 200 mW/cm^2 for 5 min. After irradiation, cells were incubated for another 24 h. The dark control group was kept under identical conditions except for irradiation. To evaluate the influence of laser power, A375 cells in a 96-well plate were incubated with PFH@PEG-F_{54}-BODIPY and PEG-F_{54}-BODIPY (5 μM BODIPY) for 12 h before the irradiation using a 660 nm laser (50, 200, 500, 1000 mW/cm^2). After irradiation, cells were incubated for another 24 h. Subsequently, the standard CCK-8 assay was carried out to evaluate the cell viability. To stain live and dead cells, the cells of each group were incubated with calcein AM (4 μM) and propidium iodide (4 μM) for 30 min, respectively. Cellular fluorescence was then examined using a Nikon Eclipse Ti inverted fluorescence microscope (Nikon Canada, Mississauga, Canada).

7. Animals

Female athymic BALB/c nude mice aged 4–6 weeks were purchased from Guangdong Medicinal Laboratory Animal Center (Guangzhou, China) and were used in accordance with the regulations of the Animal Ethical and Welfare Committee of Shenzhen University (AEWC-SZU). To develop a subcutaneous xenograft model, 5 × 10^6 human A375 cells were subcutaneously injected into the right flank of 6−7 weeks old athymic nude mice (Harlan). The mice were used for PAI and PDT when tumours reached ~80 mm^3, and used for FL/MR imaging when tumours reached ~200 mm^3. During the experiment, animals were monitored daily or once on alternate days for any clinically relevant abnormalities. If any animal was diseased, badly hurt or unable to eat due to factors unrelated to the treatment, the experiment performed on this animal was paused. If any animal was moribund due to treatment-associated toxicity or tumour over-growth (tumour length ≥ 20 mm), it was euthanized by overdose of anesthetics (5.0 v/v% chloral hydrate) and its survival time was recorded. This implementation of the protocol was approved by the AEWC-SZU.

8. Fluorescence imaging

For *in vitro* experiments, 200 μL of PFH@PEG-F_{54}-BODIPY or PEG-F_{54}-BODIPY of different concentrations was added into a 96-well plate for fluorescence scanning. For *in vivo*

experiments, 200 μL of PFH@PEG-F_{54}-BODIPY or PEG-F_{54}-BODIPY (corresponding to 0.4 μmol/kg) was intravenously injected into the A375 tumour-bearing mice. The fluorescent scans were recorded on an IVIS Spectrum system (Caliper Life Sciences, Hopkinton, MA). Fluorescence imaging was performed using a 675-nm excitation and a 720-nm emission filter.

9. Photoacoustic imaging. For *in vitro* experiments, 100 μL of PFH@PEG-F_{54}-BODIPY or PEG-F_{54}-BODIPY of different concentrations (from left to right: 0.1, 0.2, 0.3 and 0.4 mM BODIPY) were added into PCR or imaging tubes to perform photoacoustic imaging. For *in vivo* experiments, 200 μL PFH@PEG-F_{54}-BODIPY or PEG-F_{54}-BODIPY (corresponding to 0.4 μmol/kg) was intravenously injected into the A375 tumour-bearing mice. PAI was performed using a Vevo 2100 LAZR system (VisualSonics, Inc., New York, NY) equipped with a 40 MHz, 256-element linear array transducer on tumours. A 685-nm excitation filter was used.

10. Magnetic resonance imaging. For *in vitro* experiments, 3 mL of PFH@PEG-F_{54}-BODIPY or PEG-F_{54}-BODIPY (80 mg/mL BODIPY, 5 v/v% PFH) was diluted to different concentrations (from left to right: 16, 8, 4, 2, 1 mM) and then added into the imaging tube to perform the MRI imaging. For *in vivo* experiments, the A375 tumour-bearing mice were anesthetized by isoflurane and 100 μL of PFH@PEG-F_{54}-BODIPY was intratumourally injected into the tumour of A375-tumour bearing mice. MR imaging was conducted before and 1 h after the injection. All MRI experiments were performed using a Bruker 9.4 T MR scanner (Bruker Avance 400, Ettlingen, Germany).

11. *In vivo* phototherapy. Mice bearing A375 melanoma tumours were randomized into six groups including: 1) Saline group; 2) Saline plus laser group; 3) BODIPY plus laser group; 4) PFH@PEG-F_{54}-BODIPY group; 5) PFH@PEG-F_{54}-BODIPY plus laser group; 6) Repeated PDT group. For PDT treatment, mice were first *i.v.* injected with PFH@PEG-F_{54}-BODIPY or PEG-F_{54}-BODIPY (BODIPY dose: 2 μmol/kg) according to our previously developed methods 24 h in advance. Then, mice of groups 3, 5 and 6 were *i.v.* injected with 200 μL of nanoemulsion (30 μL of PFH). For hyperoxic breathing, mice were equipped with oxygen masks flowed with pure oxygen for 30 min. For US treatment, a US probe was applied to the surface of the tumour site for 30 min during hyperoxic breathing (1 MHz, 500 mW/cm^2). Afterwards, to conduct the PDT treatment, mice of groups 3 and 5 were irradiated using a 660 nm laser at a power density of 200 mW/cm^2 for 30 min. Mice of groups 2 and 6 received multiple treatments on days 1, 6, and 11. After various treatments, the lengths and widths of the tumours were measured every 2 days by using of a digital caliper for a period of 15 days. The tumour volume was calculated according to the following formula: volume = width^2 × (length/2).

12. *Ex vivo* histological staining. A375 tumour-bearing mice were sacrificed at 24 h after laser irradiation, and the tumours and major organs of mice in groups 1-5 were collected and fixed with paraformaldehyde or cryosectioned for hematoxylin-eosin (H&E) staining or terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) staining, respectively.

13. Statistical analysis. Data represent the mean ± s.d. Statistical differences were calculated with an unpaired two-tailed Student's t-test or one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison *post-hoc* test using GraphPad Prism 7.0 (GraphPad software). P values < 0.05 were considered statistically significant (**P*<0.05, ***P*<0.01 and

***P<0.001). Variances were similar between groups, as determined by the F test using GraphPad Prism 7.0. No statistical method was used to pre-determine sample size.

Part C. Supplementary characterization data of the PEG-F_{54}-BODIPY amphiphile

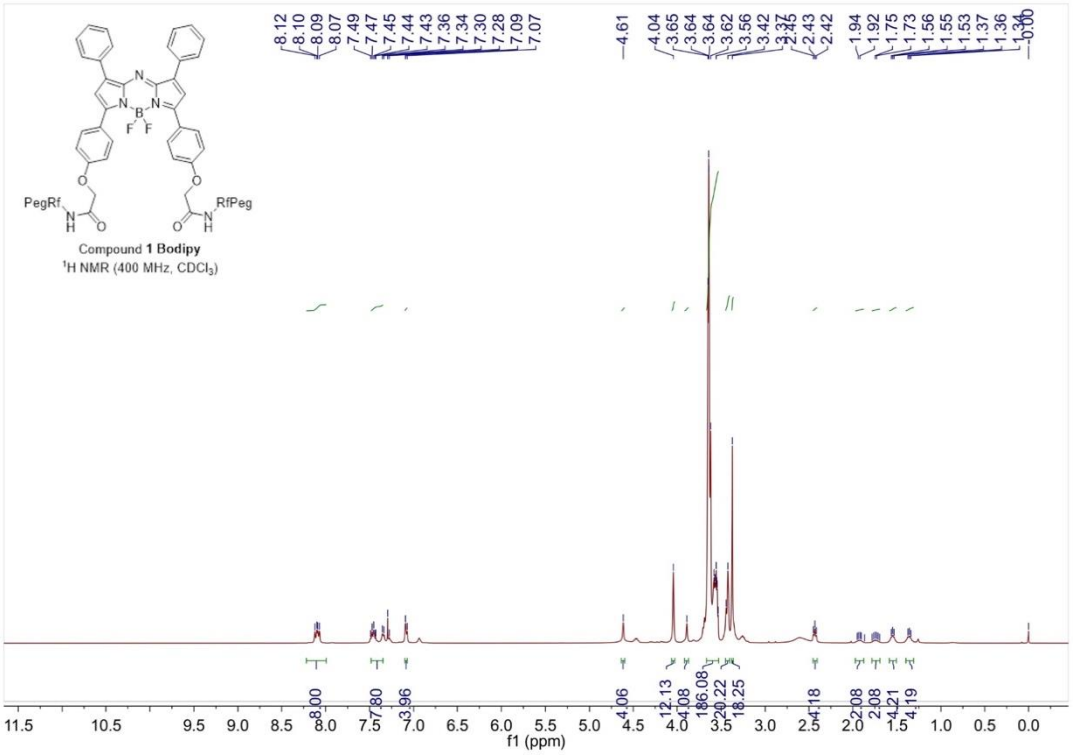

Figure S1. ^{1}H-NMR Characterizations.

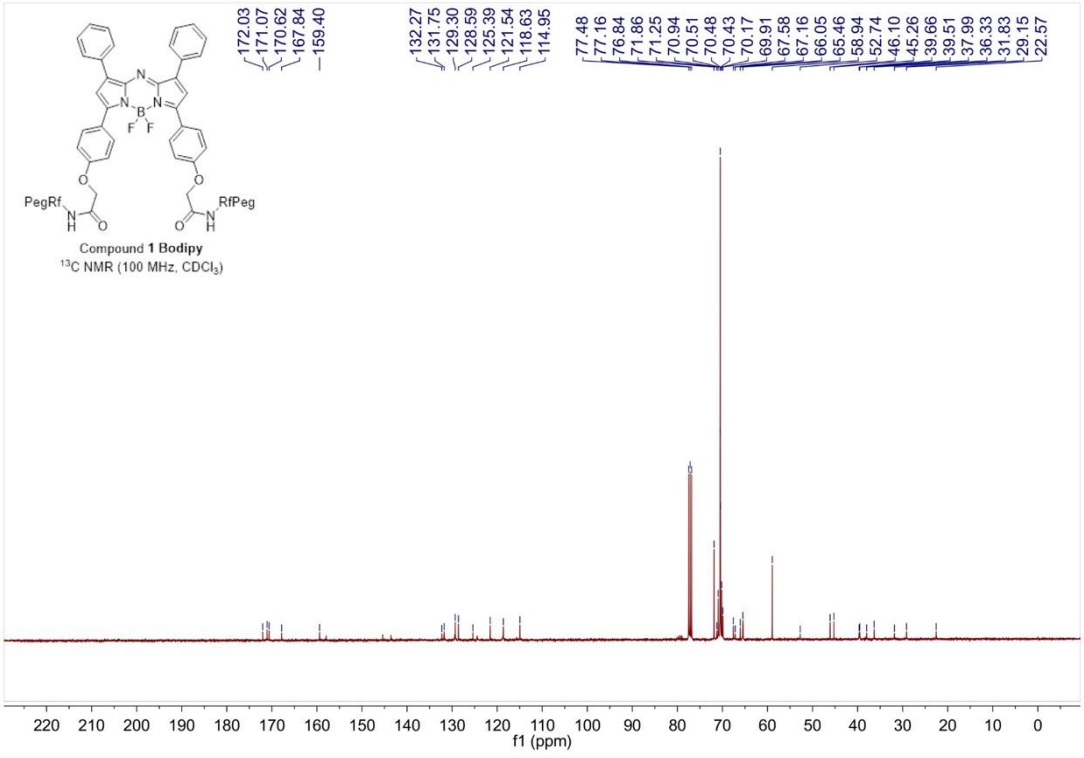

Figure S2. ^{13}C-NMR of Characterizations.

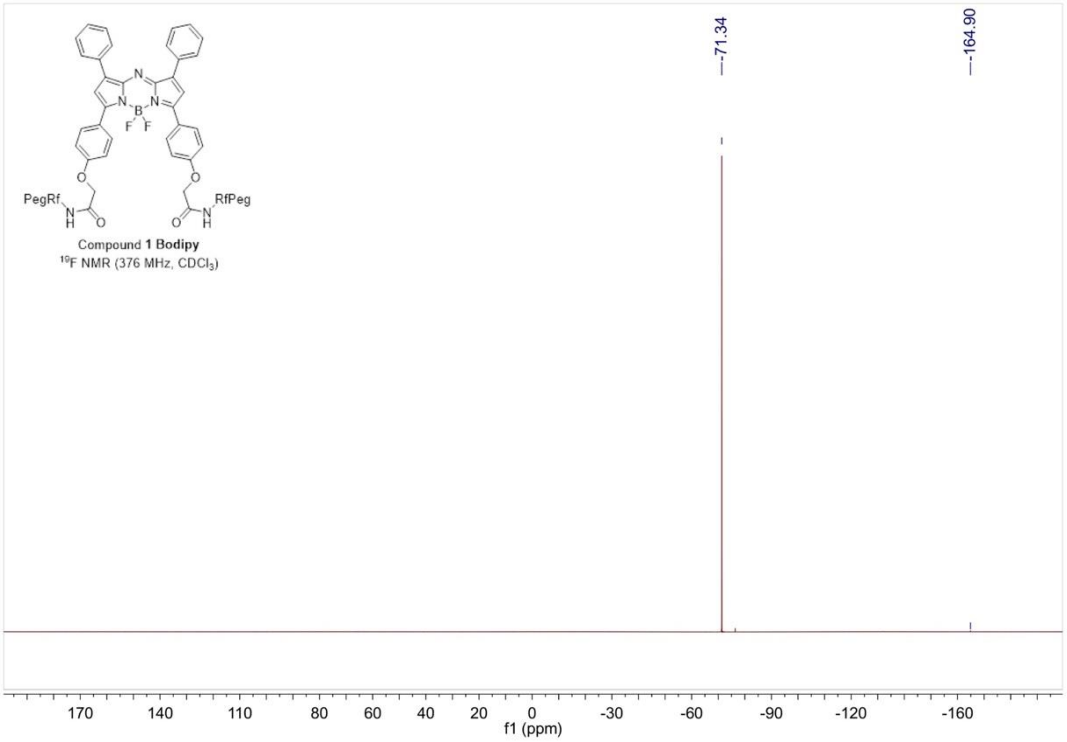

Figure S3. ^{19}F-NMR of the PEG-F_{54}-BODIPY amphiphile.

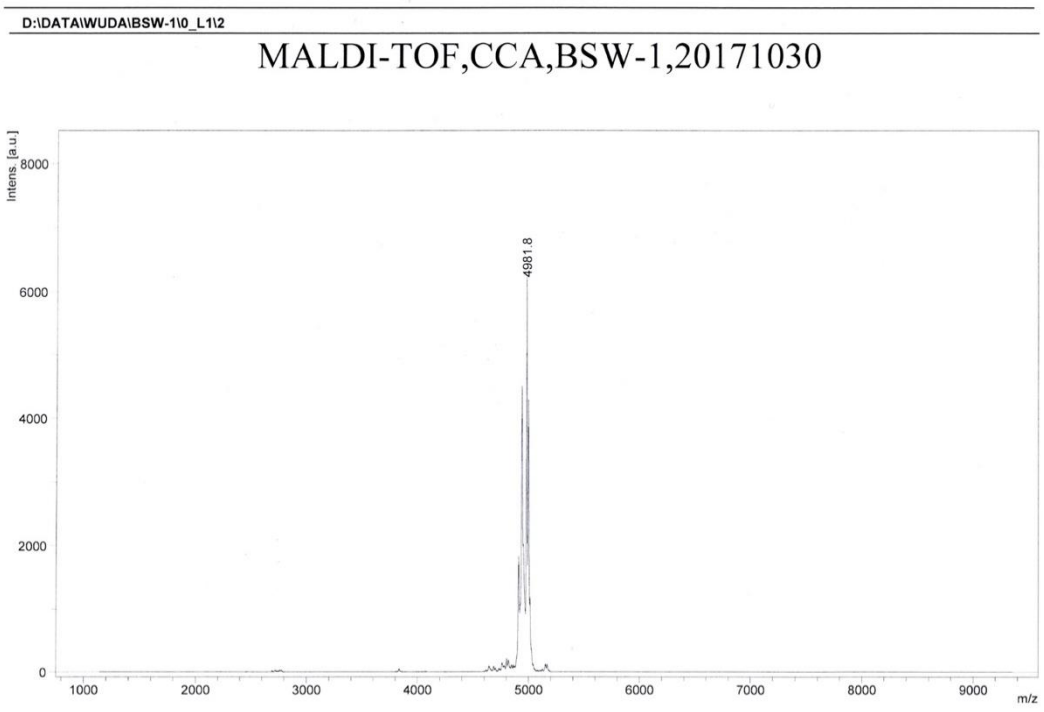

Figure S4. The MALDI-TOF mass spectrum (MS) of the PEG-F_{54}-BODIPY amphiphile.

Part D. Supplementary *in vitro* and *in vivo* data of PFH@PEG-F_{54}-BODIPY

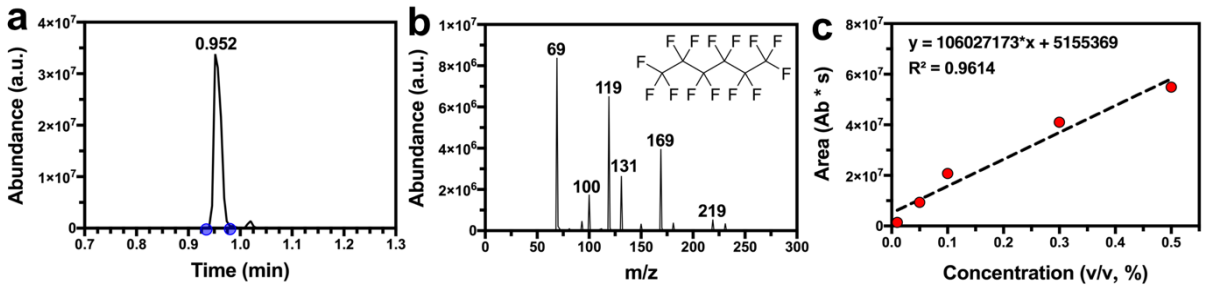

Figure S5. The perfluorohexane (PFH) content analysis. a) Gas chromatography (GC) and b) MS of PFH@PEG-F_{54}-BODIPY. c) The correlation of PFH concentrations and peak area of GC. The solvent of PFH is trichlorotrifluoroethane.

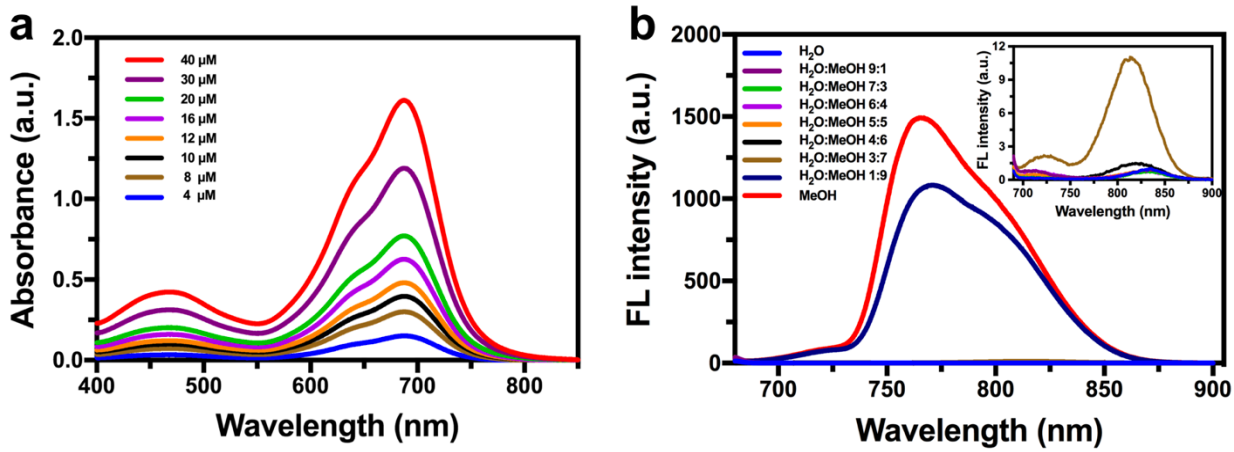

Figure S6. The fluorescence quenching of the PEG-F_{54}-BODIPY amphiphile. a) UV-Vis-NIR absorption spectra of BODIPY of different concentrations. b) Fluorescence emission spectra (right) of BODIPY in the mixed solvent of H_2O and methanol with various ratios.

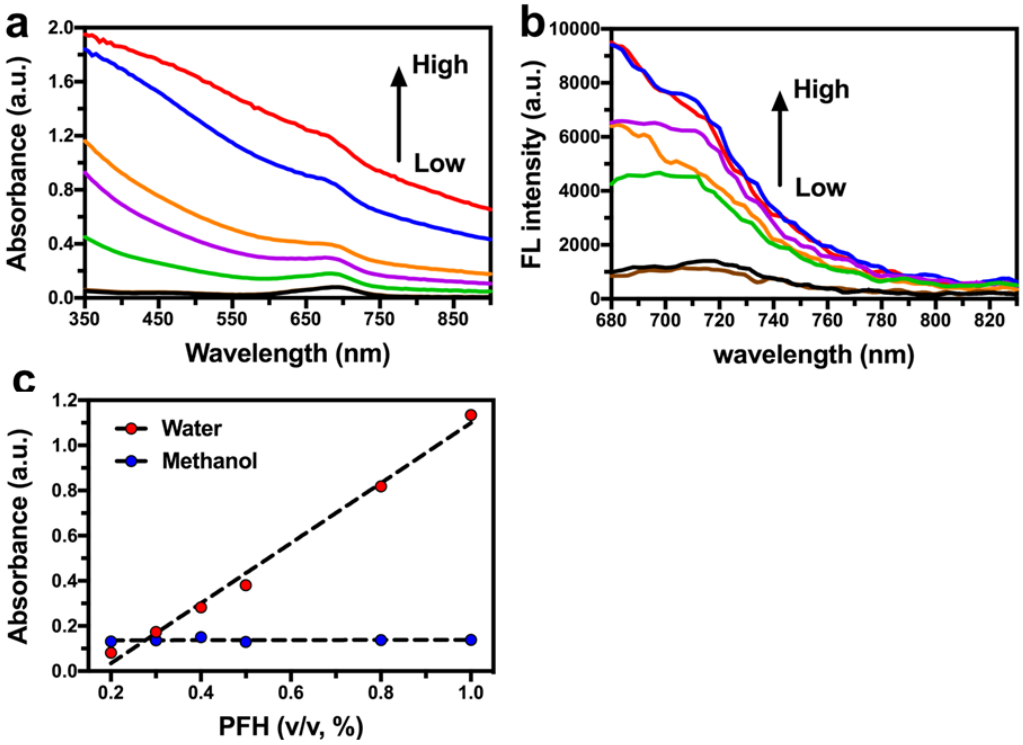

Figure S7. The role of PFH in the self-quenching relief of PFH@PEG-F_{54}-BODIPY. a) UV-Vis-NIR absorption spectra, b) fluorescence emission spectra and c) The correlation of absorbance value of PFH@PEG-F_{54}-BODIPY at 685 nm and the volume percent of PFH.

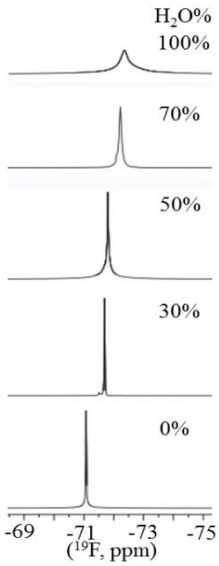

Figure S8. The architecture-dependent ^{19}F NMR signals of the PEG-F_{54}-BODIPY amphiphile. The BODIPY polymer was dissolved in the mixed solvent of H_2O and methanol with various ratios.

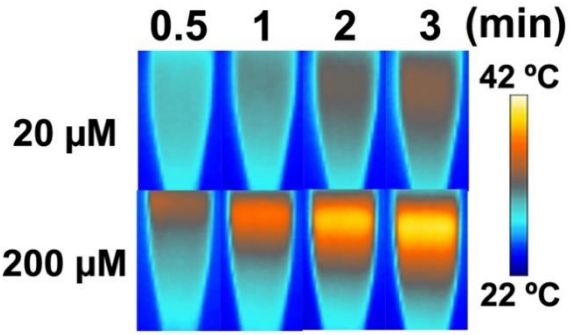

Figure S9. Heat generation of PFH@PEG-F_{54}-BODIPY. Thermal images of PFH@PEG-F_{54}-BODIPY aqueous solution (200 μL) was monitored by a thermal camera under irradiation using a 660 nm laser at the power density of 200 mW/cm^2 for 3 min. The concentration of BODIPY was 20 and 200 μM, respectively.

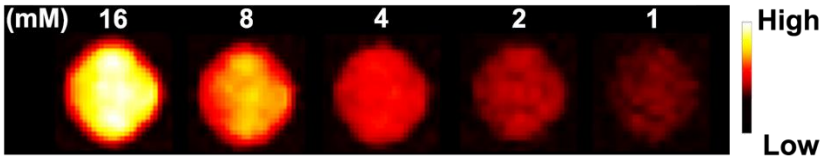

Figure S10. ^{19}F MRI of PEG-F_{54}-BODIPY. The color bar indicated a range from 0 to 2.5 × 10^5 (a.u.).

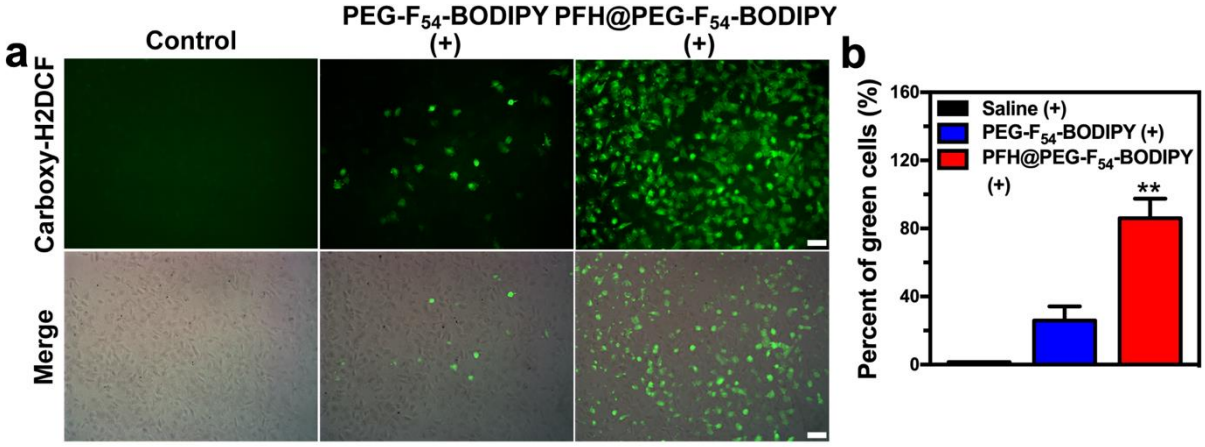

Figure S11. ROS generation *in vitro*. a) Fluorescence images and b) quantitative analysis of carboxy-H_2DCFDA stained A375 tumour cells after 12 h incubation with fresh medium, PEG-F_{54}-BODIPY or PFH@PEG-F_{54}-BODIPY exposed to a 660 nm laser at the power density of 200 mW/cm^2 for 5 min (5 μM BODIPY). Scale bar represents 50 μm. Values are the means ± s.d. (n = 3; **P < 0.01 versus BODIPY + Laser, two-sided Student's t-test).

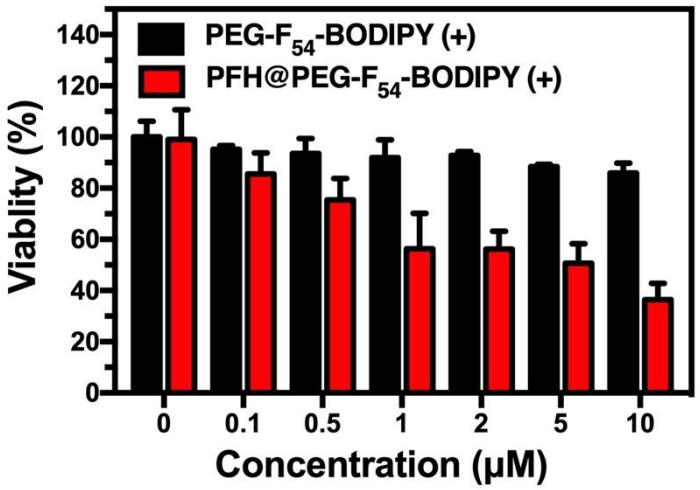

Figure S12. Relative viability of hypoxic A375 tumour cells treated with PFH@PEG-F_{54}-BODIPY or PEG-F_{54}-BODIPY at various doses after irradiation with a 660 nm laser at a power density of 200 mW/cm^2 for 5 min.

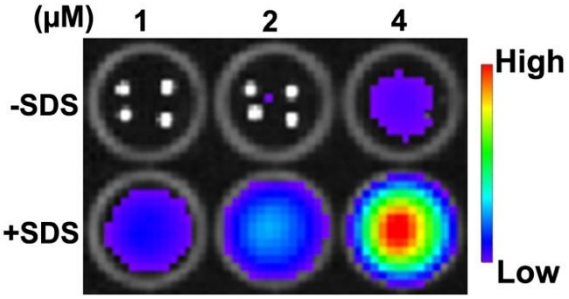

Figure S13. Fluorescence quenching of PFH@PEG-F_{54}-BODIPY in blood. Representative Near-infrared fluorescence signal of PFH@PEG-F_{54}-BODIPY in blood solutions in the absence and in the presence of SDS (10 mM).

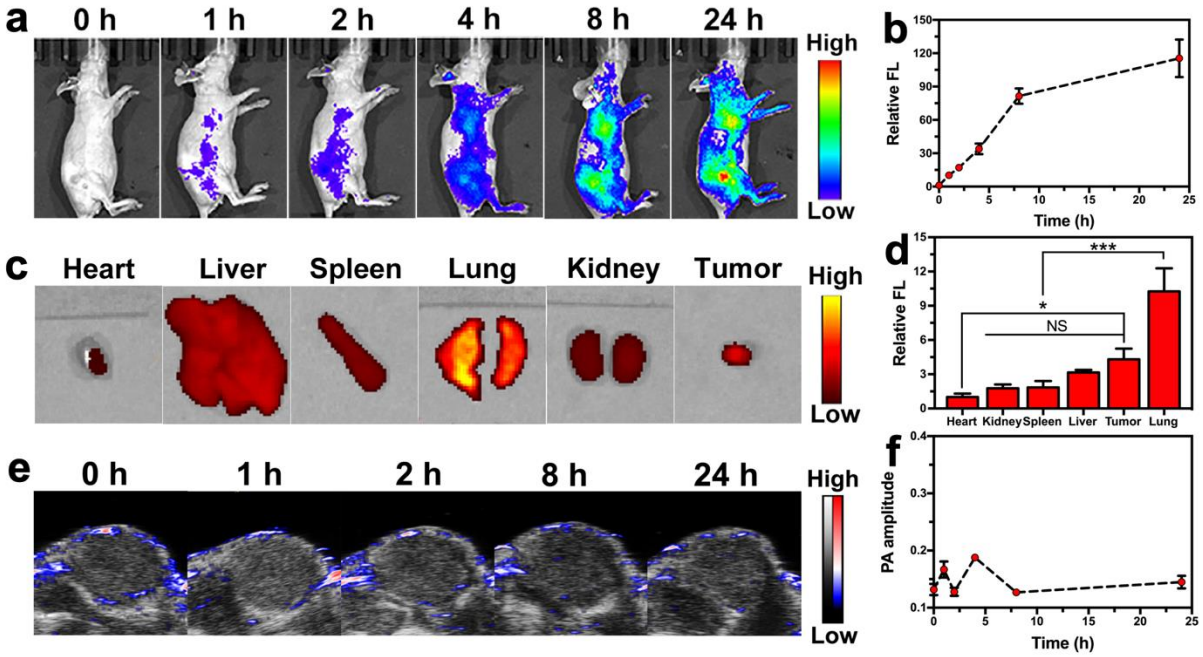

Figure S14. ***In vivo*** **FL/PA imaging of the PEG-F_{54}-BODIPY amphiphile.** a) Representative Near-infrared imaging and b) fluorescence quantification of the A375 tumour accumulation of BODIPY after intravenous injection of PEG-F_{54}-BODIPY. c) Representative Near-infrared imaging and d) fluorescence quantification of major organs of the A375 tumour-bearing mice 24 h after *i.v.* injection of PEG-F_{54}-BODIPY. e) Representative photoacoustic imaging and f) photoacoustic quantification of the tumour after intravenous injection of PFH@PEG-F_{54}-BODIPY. Values are the means ± s.d. (n = 3, ***P<0.001 for tumour compared to other organs using one-way analysis of variance (ANOVA) with Tukey's post hoc test).

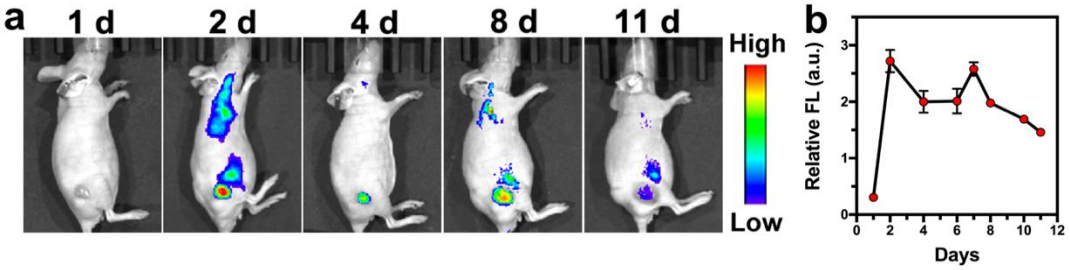

Figure S15. Long tumour retention of PFH@PEG-F_{54}-BODIPY. a) Near-infrared imaging and b) fluorescence quantification of the A375 tumour accumulation of BODIPY after intravenous injection of PFH@PEG-F_{54}-BODIPY for 11 days.

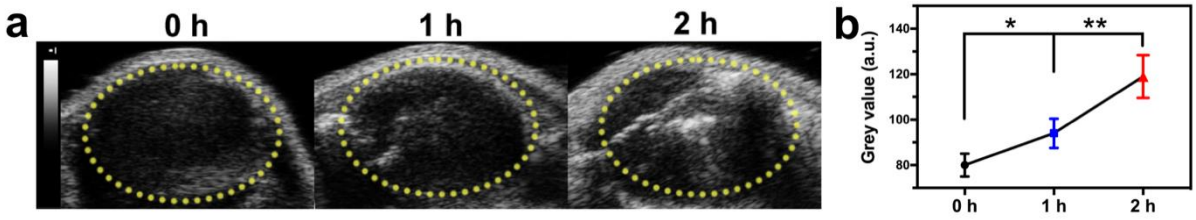

Figure S16. B-mode ultrasound images of A375 tumours 0, 1 and 2 h after intratumoural injection of PFH@PEG-F_{54}-BODIPY.

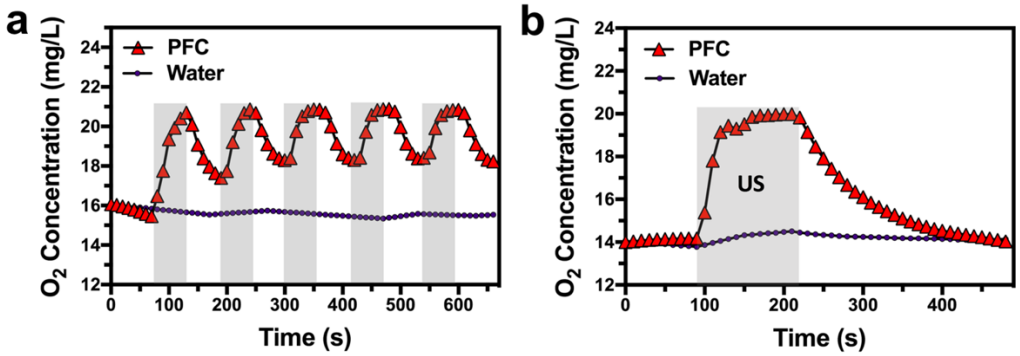

Figure S17. The oxygen storage capability of PFH. Changes of dissolved O_2 concentrations in deoxygenated pure water without or with addition of oxygen-loaded nanoemlusion. US treatment was applied on these solutions within the indicated period.

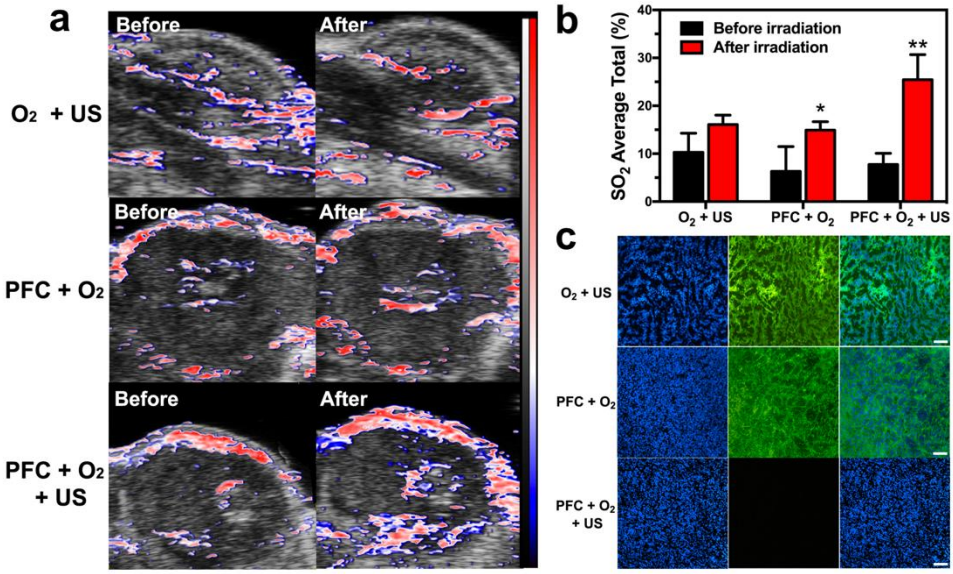

Figure S18. Tumor oxygenation mediated by PFH. a) PA imaging of A375 tumors to determine tumor oxygenation status by measuring the ratios of oxygenated hemoglobin (λ = 850 nm) and deoxygenated hemoglobin (λ = 750 nm) before and 30 min after indicated treatments. b) Quantification of the oxyhemoglobin saturation in the tumor. c) Representative immunofluorescence images of tumor slices stained by the Hypoxyprobe.

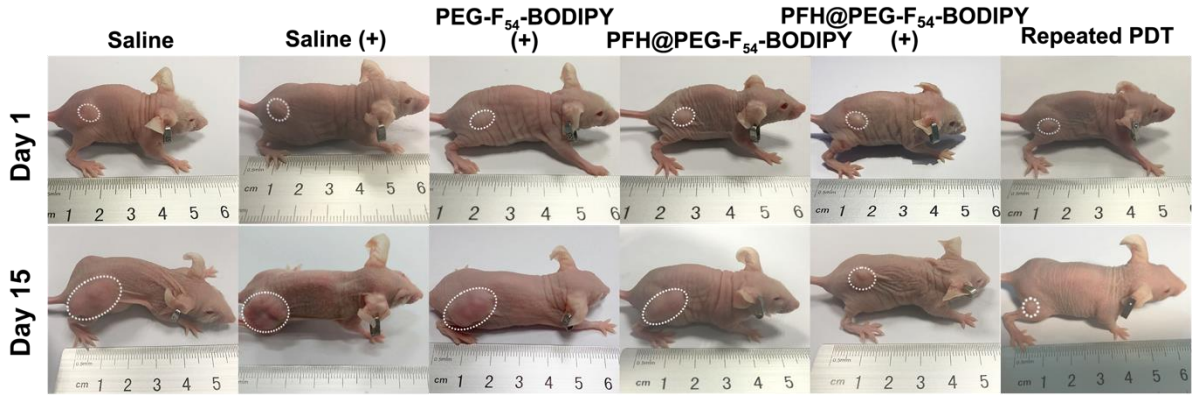

Figure S19. The photographs of mice right before and 15 days after different treatments.

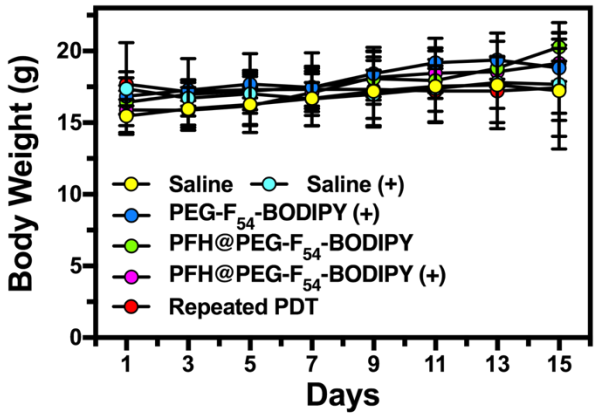

Figure S20. Body weight change. Body weight change of nude mice bearing A375 melanoma cancer xenograft receiving different treatments in the *in vivo* PDT experiment. BODIPY dose: 2 μmol/kg. (n = 7 for PFH@PEG-F_{54}-BODIPY plus laser group; n=5 for other groups)

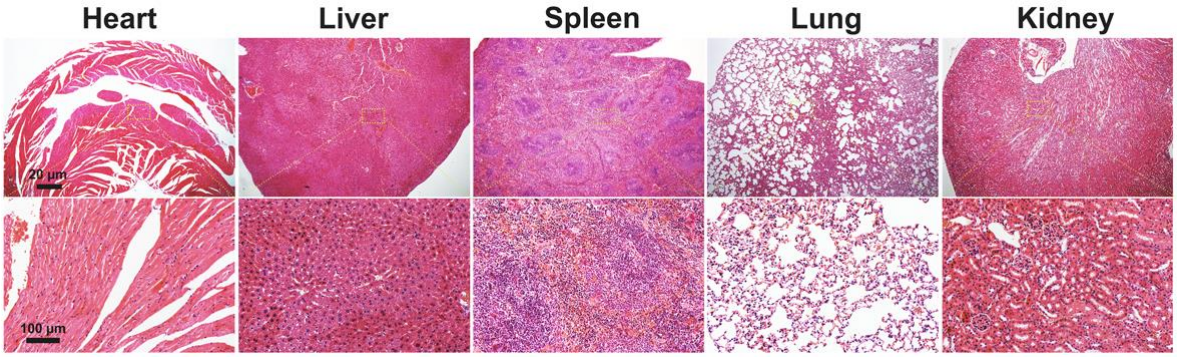

Figure S21. Normal tissue toxicity of PFH@PEG-F_{54}-BODIPY. Representative Hematoxylin and eosin (H&E) staining images for cellular morphology from major organs of healthy nude mice. The mice were *i.v.* injected with PFH@PEG-F_{54}-BODIPY and sacrificed 15 days after injection. BODIPY dose: 2 μmol/kg.

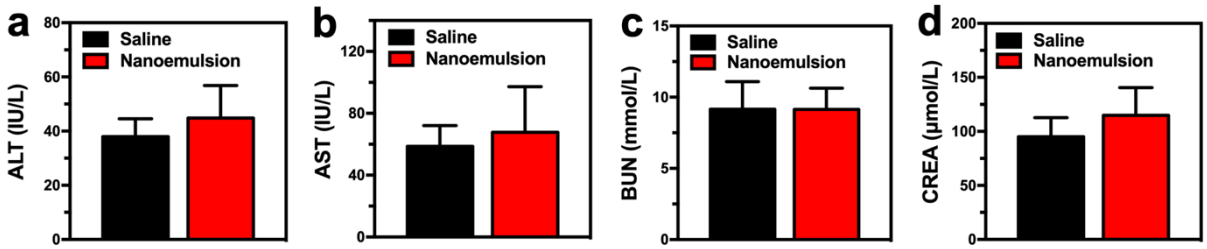

Figure S22. Serum chemistry. a) Glutamic-pyruvic transaminase (ALT), b) glutamic oxalacetic transaminase (AST), c) urea nitrogen (BUN) and d) creatinine (CREA) concentrations for liver and kidney functions of healthy nude mice 15 days after i.v. injection of PFH@PEG-F_{54}-BODIPY. BODIPY dose: 2 μmol/kg.

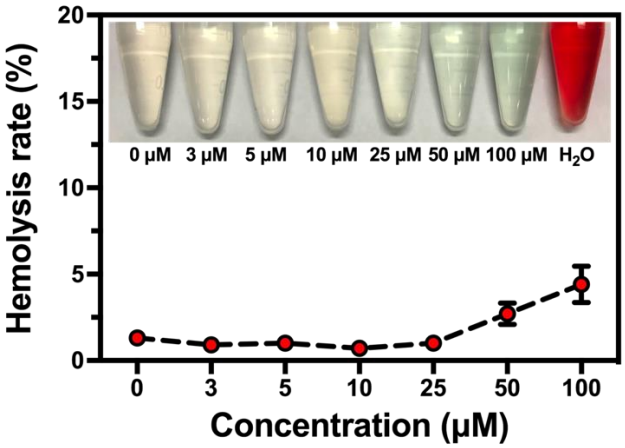

Figure S23. Hemolytic activity of PFH@PEG-F_{54}-BODIPY in blood. The Red blood cells (RBCs) were isolated from serum by centrifugation of the mixture containing 0.5 mL blood sample and 1 mL PBS solution at 4500 rpm for 3.5 min. PBS was used to wash the RBCs five times and dilute the purified cells to 5 mL. Then certain volume of diluted RBCs suspension (0.3 mL) was added to quadruple volume of PBS solution with different concentrations of PFH@PEG-F_{54}-BODIPY (0 to 100 μM). The mixtures were vortexed and kept to stand for 3 h at room temperature. Samples were then centrifuged to measure the absorbance of the supernatants at $_{54}$1 nm by an UV–vis spectroscopy. RBCs treated with deionized water and PBS were set as positive and negative controls.

Table S1. Conditional Singlet Oxygen Quantum Yields[a]

Photosensitizers	**Φso[1]**
Ce6	0.65
PFH@PEG-F_{54}-BODIPY	0.57
PEG-F_{54}-BODIPY	0.16

[a]These data were measured conditionally and involved approximations, do not use as the standard

Table S2. T_1 and T_2 of PFH@PEG-F_{54}-BODIPY

	T_1 (ms)	**T_2 (ms)**
BODIPY	375.35	28.15
PFH	1040	180.6

Table S3. Signal to noise (S/N) ratio of FL/PA/US trimodal imaging mediated by PFH@PEG-F_{54}-BODIPY and PEG-F_{54}-BODIPY

Signal to noise ratio	**PFH@PEG-F_{54}-BODIPY**		**PEG-F_{54}-BODIPY**	
	Before	**After**	**Before**	**After**
FLI	1.4	14.7	1.04	3.51
PAI	0.915	2.75	0.819	0.875
USI	0.898	2.39	0.977	0.9

Supplementary References

1 Ho, C.J., Balasundaram, G., Driessen, W., McLaren, R., Wong, C.L., Dinish, U.S., Attia, A.B., Ntziachristos, V., and Olivo, M. (2014). Multifunctional photosensitizer-based contrast agents for photoacoustic imaging. Sci. Rep. *4*, 5342.

2 Song, X., Feng, L., Liang, C., Yang, K., and Liu, Z. (2016). Ultrasound triggered tumor oxygenation with oxygen-shuttle nanoperfluorocarbon to overcome hypoxia-associated resistance in cancer therapies. Nano Lett. *16*, 6145-6153.