Supporting Information

Self-Assembly of Hybrid Photosensitizer for Synergistically Enhanced Photodynamic/Photothermal Therapy

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1. *Reagents and chemicals*

The Octvinyl-POSS was obtained from Hybrid Plastics (USA). Fetal bovine serum (FBS) was purchased from Gibco (Life Technologies). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), penicillin-streptomycin solution, 9,10-Anthracenediyl-bis(methylene)dimalonic acid (ABDA) and 2',7'-Dichlorofluorescin diacetate (DCFH_DA) were purchased from Sigma-Aldrich. Dulbecco's modified essential medium (DMEM) and PBS were commercial products from KenGEN Biotech Inc. All other chemicals and reagents were purchased from Sinopharm Chemical Reagent Co., Ltd without further purification. All reactions were performed in an inert gas atmosphere.

2. *Instrumentation and methods*

We collected the NMR signals on a Bruker NMR instrument (Ultra Shield Plus 400 MHz). Mass spectra were obtained from a Bruker MALDI-TOF mass spectrometer (autoflex III). The gel permeation chromatography (GPC) analysis was performed on a Shim-pack GPC-80X column (standard: polystyrene, eluent: tetrahydrofuran). The characterization of fourier transform infrared spectra (FT-IR) were executed on an IR Spectrometer (Spectrum Two, Perkin Elmer) by using potassium bromide and the scanned ranges of diffuse reflectance spectra were set from 4000 to 500 cm^{-1} wavenumbers. UV-Vis absorption spectra were recorded on a UV-3600 Shimadzu UV-Vis-Nir spectrophotometer in 10 mm path quartz cell. Meanwhile, the photoluminescence spectra of samples were measured on a Shimadzu RF-5301PC spectrophotometer. We acquired the hydrodynamic sizes on Brookhaven Zeta PALS with 90° collecting optics of He-Ne 633 nm laser. Laser confocal scanning microscope (CLSM) images were obtained from Olympus Fluoview 1000 (Olympus, Japan). The 635 nm continuous-wave semiconductor laser was generated by (MDL-D-635-2W, Changchun New Industries Optoelectronics Tech Co., Ltd, Changchun, China), and the laser power density was recorded by a power-meter PM121D and S310C (Thorlabs, USA). The thermal images were recorded on a thermal imaging camera of FOTRIC

220 series (FOTRIC 225, Nottingham PI Allen, TX, USA), and raw data were processed by using AnalyzIR software (FOTRIC, Nottingham PI Allen, TX, USA).

3. Synthesis details

Synthesis of $(Vinyl)_7$-POSS-alkyne

$(Vinyl)_7$-POSS-OH and $(Vinyl)_7$-POSS-alkyne were synthesized according to our previous literature.[1] $(Vinyl)_7$-POSS-OH could be afforded by using the octavinyl POSS reacted with Trifluoromethanesulfonic acid (TfOH), and it was further esterified with 4-pentynoic acid to yield the $(Vinyl)_7$-POSS-alkyne. Briefly, to a 125 mL round-bottomed flask was added $(Vinyl)_7$-POSS-OH (1.3 g, 2.0 mmol), 4-pentynoic acid (236 mg, 2.4 mmol), and DMAP (50 mg, 0.4 mmol). Then 25 mL of dried DCM was added to dissolve all the reactants at 0 °C. DIPC (378 mg, 3.0 mmol) was added into the mixture dropwise before being kept at R.T. overnight. After simple filtration, the organic solvent was removed under reduced pressure. And the residue was further purified on silica gel by using hexanes/dichloromethane (V/V = 1/1) as the eluent affording the product as a white powder in 88% yield. ^{1}H NMR (400 MHz; $CDCl_3$; Me_4Si): 6.14-5.90 (m, 21H), 4.28-4.23 (t, 2H), 2.52-2.48 (m, 4H), 1.96 (s, 1H), 1.23-1.19 (t, 2H). ^{13}C NMR (100 MHz; $CDCl_3$; Me_4Si): 171.63, 137.14, 128.54, 68.96, 60.98, 33.42, 14.32, 13.07.

Synthesis of 2-(thiophen-2-yl)-4H-thieno[3,2-b]pyrrole

The 2-(thiophen-2-yl)-4H-thieno[3,2-b]pyrrole was acquired according to previous literature.[2] The obtained pyrrole derivative (750 mg, 2.68 mmol) and KOH (601.4 mg, 10.72 mmol) were dispersed in 20 mL ethylene glycol, dissolved by heating and further refluxed for 2h under inert atmosphere. After cooling to room temperature, the solution was quenched with H_2O, followed by extracted with dichloromethane, and the organic layer was collected and evaporated. The resulting crude mixture was purified by column chromatography on silica gel (dichloromethane as the eluent) to afford the desired product as light yellow solid (467 mg, 80% yield). ^{1}H NMR (400 MHz; $CDCl_3$; Me_4Si):

8.22 (s, 1H), 7.19-7.15 (m, 2H), 7.07 (s, 1H), 6.94-6.90 (d, 1H), 7.02-7.00 (m, 1H), 6.99-6.97 (t, 1H), 6.65-6.64 (t, 1H).

Synthesis of BODIPY

2-(thiophen-2-yl)-4H-thieno[3,2-b]pyrrole (205 mg, 1 mmol) and 4- (2-azidoethoxy) benzaldehyde (95 mg, 0.5 mmol) were added into a 100 mL Schlenk flask under argon, and they were dissolved by 50 mL anhydrous dichloromethane. One drop of trifluoroacetic acid (TFA) was added to the mixture and they were stirred at R.T. overnight. 118 mg DDQ (0.5 mmol) was added into the system which was further reacted for one hour. Afterwards, 8 mL of triethylamine (Et_3N) and 8 mL of BF_3•OEt_2 were successively added. After reacting overnight, this reaction mixture was quenched with water and filtered. The filtrate was washed thoroughly with water and brine, dried over anhydrous $MgSO_4$, filtered, and evaporated under vacuum. The BODIPY compound was afforded as a metallic green solid in 71% yield by using silica gel column chromatography (dichloromethane and hexane, V/V = 2/1). ^{1}H NMR (400 MHz; $CDCl_3$; Me_4Si): 7.58-7.56 (d, 2H), 7.42-7.39 (m, 4H), 7.35 (s, 2H), 7.10-7.07 (m, 4H), 6.85 (s, 2H), 4.24-4.22 (t, 2H), 3.66-3.64(t, 2H). ^{13}C NMR (100 MHz; $CDCl_3$; Me_4Si): 160.09, 158.27, 151.15, 141.00, 137.75, 132.51, 128.46, 128.00, 127.03, 126.53, 119.34, 114.58, 108.94, 67.23, 50.09, 47.19, 8.77. MS (MALDI−TOF, m/z): calcd. 629.54 Da, found 630.77 Da.

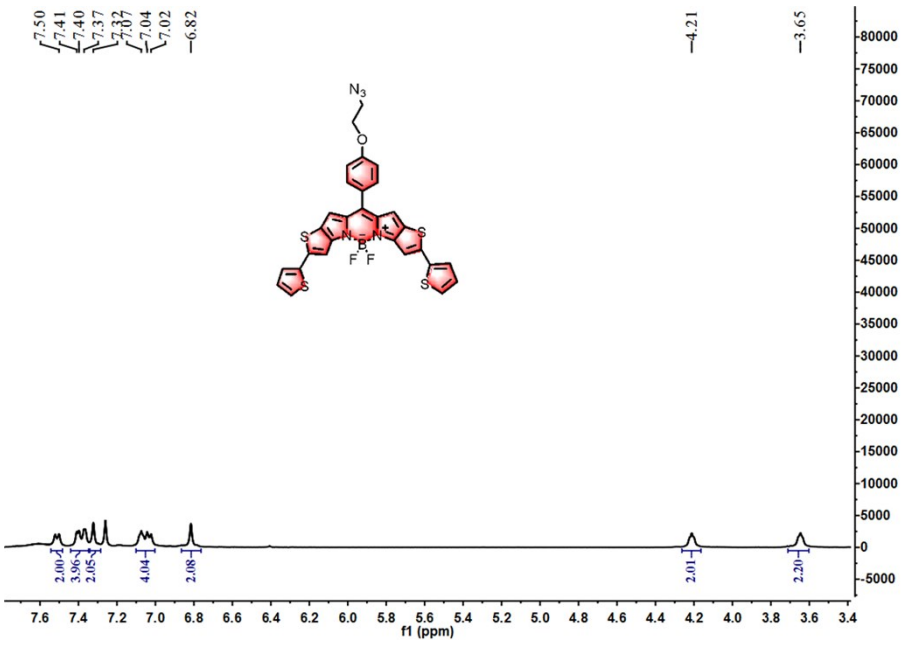

Fig. S1. The ^{1}H NMR spectrum of BODIPY.

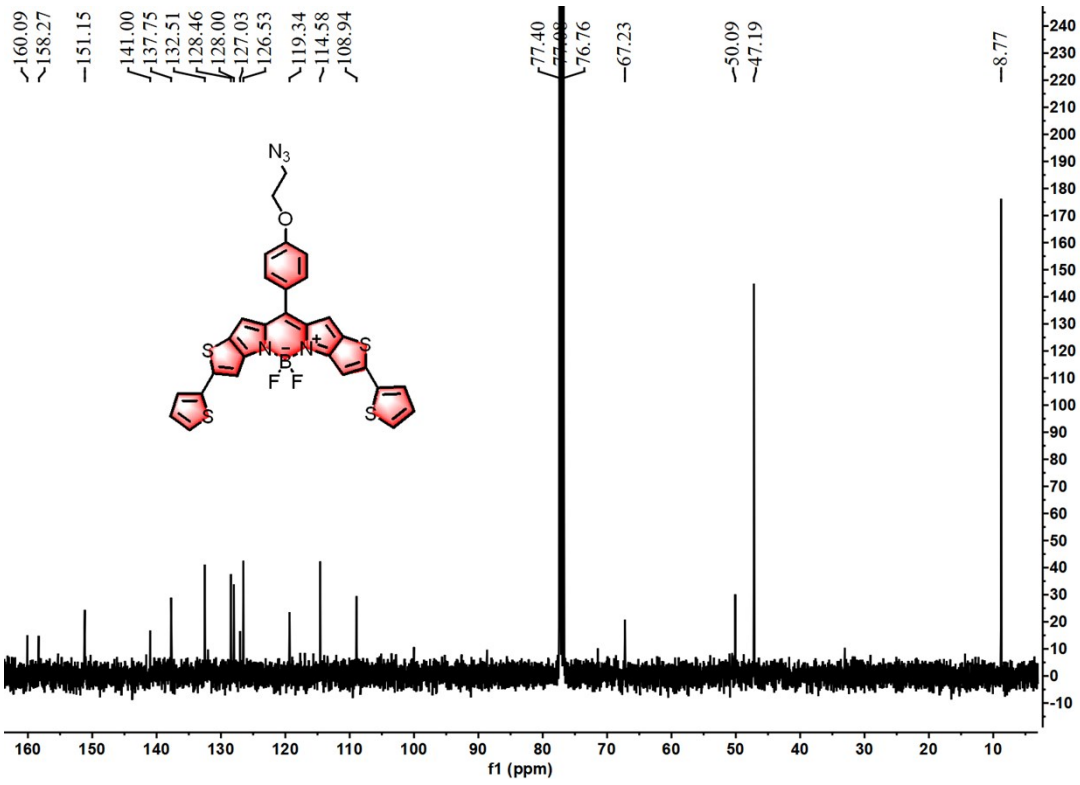

Fig. S2. The ^{13}C NMR spectrum of BODIPY.

Synthesis of Br-BODIPY

BODIPY (56.7 mg, 0.09 mmol), NBS (33.6 mg, 0.189 mmol) and 25 mL anhydrous THF were loaded in 50 mL flask. It was stirred in dark at R.T. overnight. The reaction was quenched by adding 5 mL sodium thiosulfate aqueous solution (2 mol/L), washed with water, and extracted with chloroform. The organic layer was dried and then distilled to afford the crude product. The pure product **Br-BODIPY** was isolated from chloroform/hexane by recrystallization (63 mg, yield: 89%). ^{1}H NMR (400 MHz; $CDCl_3$; Me_4Si): 7.57-7.55 (m, 2 H), 7.42-7.39 (m, 3 H), 7.35 (s, 1 H), 7.10-7.05 (m, 4 H), 6.83-6.86 (t, 2 H), 4.26-4.24 (t, 2 H), 3.69-3.67(t, 2 H). MS (MALDI−TOF, m/z): calcd. 786.86 Da, found 786.46 Da.

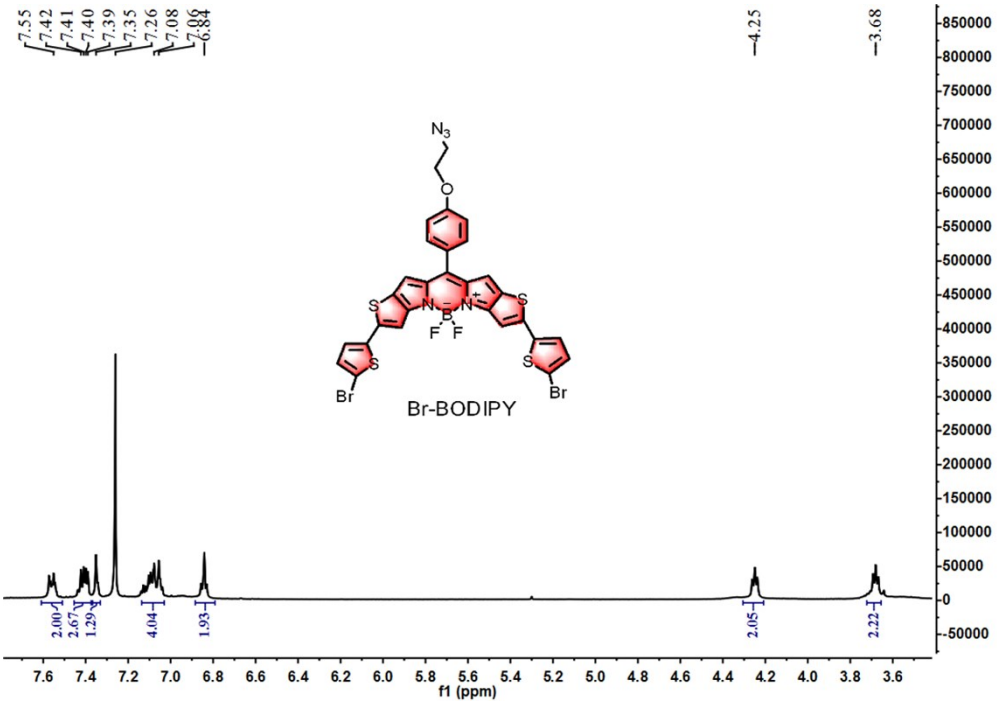

Fig. S3. The ^{1}H NMR spectrum of Br-BODIPY.

Synthesis of Br-BODIPY-POSS

Br-BODIPY-POSS was also obtained according to typical CuAAC "click" reaction procedure. To a 100 mL Schlenk flask were added Br-BODIPY (66 mg, 0.084 mmol), $(Vinyl)_7$-POSS-alkyne (75.3 mg, 0.1 mmol), CuBr (2.8 mg, 0.02 mmol) and fresh distilled THF (35 mL). The resulting solution was degassed according to the freeze-pump-thaw cycles method strictly, then PMDETA (58.1 mg, 0.2 mmol) was

added via syringe. The mixiture was further degassed by one cycle and allowed to stir for 24 h at R.T. The solvent THF was evaporated under vacuum and the mixture was purified by silica gel chromatography using dichloromethane: ethyl acetate (4:1) as the eluent. After removal of the solvent and refined with chloroform/hexane, Br-BODIPY-POSS was afforded as a dark green solid 97 mg, yield: 75%. ^{1}H NMR (400 MHz; $CDCl_3$; Me_4Si): 8.08 (s, 1H), 7.58-7.57 (m, 2 H), 7.42-7.39 (m, 3 H), 7.35 (s, 1 H), 7.10-7.05 (m, 4 H), 6.83-6.86 (t, 2 H), 6.13-5.86 (m, 21 H), 4.79-4.78 (t, 2 H), 4.47-4.46 (t, 2 H), 4.24-4.22 (t, 2 H), 3.15-3.12 (t, 2 H), 2.75-2.71 (t, 2H), 1.46-1.42 (t, 2H). MS (MALDI−TOF, m/z, Figure S4): found 1517.04, calcd. 1516.84.

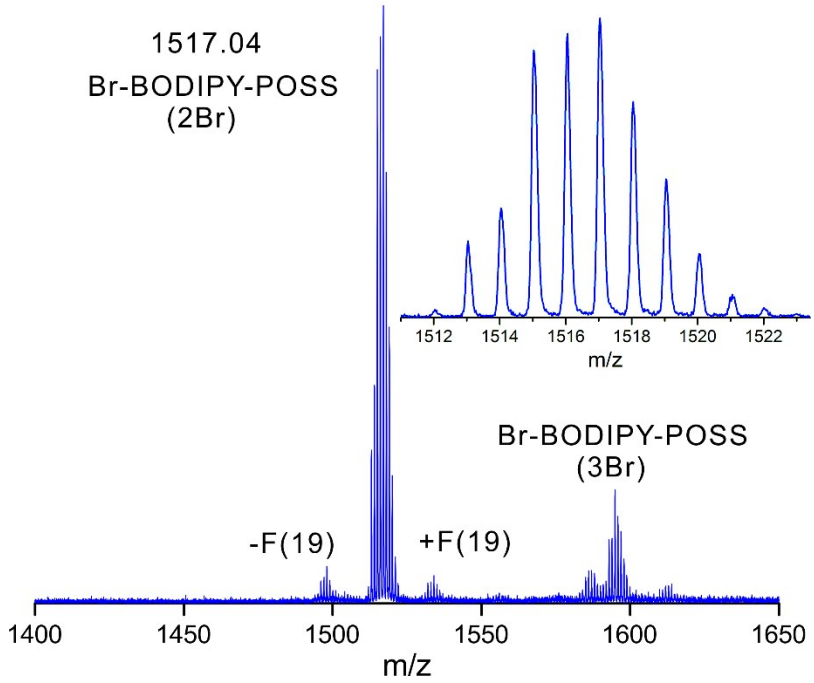

Fig. S4. The MALDI-TOF spectrum of Br-BODIPY-POSS, the inset image is the enlarge part of spectrum from 1510 to1525.

Synthesis of Br-BODIPY-POSS-PEG$_{2000}$ (BBPP)

Br-BODIPY-POSS (50 mg, 0.033 mmol), SH-PEG$_{2000}$ (462 mg, 0.231 mmol), DMPA (1 mg, 0.039 mmol), and anhydrous chloroform (125 mL) were loaded into a 250 mL flask. The chloroform solution was bubbled with argon for 30 min, then irradiated under a UV 365 nm lamp for 30 min. The solvent was removed and the residue was re-

dissolved with 20 mL methanol/water (v/v = 1:9). The mixture was dialyzed against methanol/water (v/v = 1:9) by using 5k Da molecular weight cutoff dialysis membrane for 2 times in 24 h, and further dialyzed against Milli-Q water for 3 times in 36h. Finally, the solution in dialysis tube was lyophilized and given the final product BBPP as fluffy green solid in 65% yield. ^{1}H NMR (400 MHz; $CDCl_3$; Me_4Si): 8.08 (s, 1H), 7.72-7.71 (m, 2H), 7.53-7.51 (d, 3H), 7.34 (m, 2H), 7.12-7.10 (m, 2H), 6.98-6.96 (m, 2H), 6.79 (s, 1H), 6.10-5.90 (m, 11H), 5.33 (t, 2H), 5.11 (t, 2H), 4.77 (t, 2H), 4.44 (t, 2H), 3.45-3.80 (m, PEG, centered in 3.63 ppm).

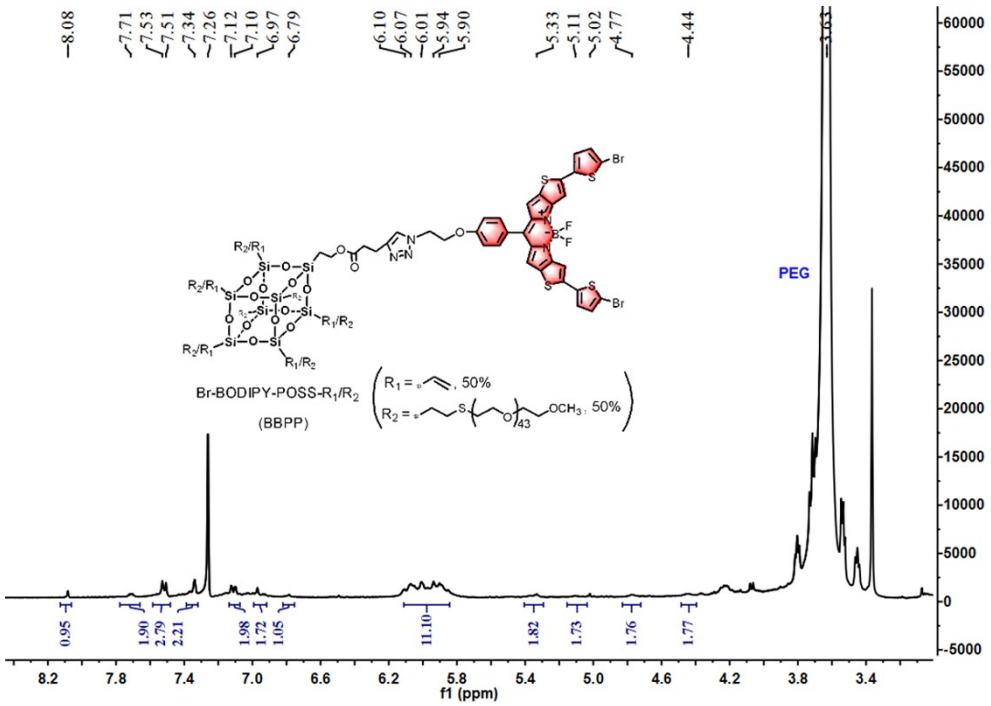

Fig. S5. The ^{1}H NMR spectrum of Br-BODIPY-POSS-PEG_{2000} (BBPP).

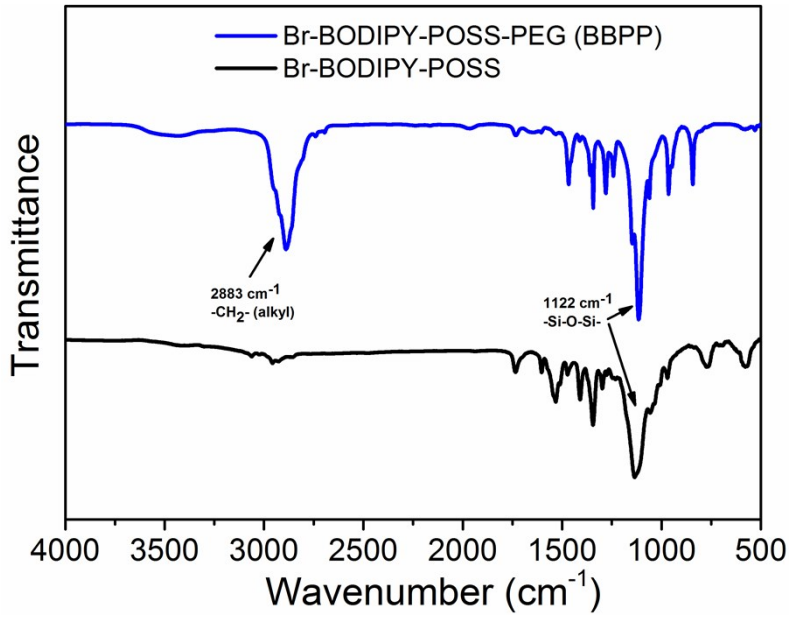

Fig. S6. The FTIR spectra of BBPP and Br-BODIPY-POSS.

Synthesis of Br-BODIPY-PEG$_{2000}$ (BBP)

Br-BODIPY (20 mg, 0.0253 mmol), PEG$_{2000}$-alkyne (62 mg, 0.0301 mmol), CuBr (1mg, 0.007 mmol) and fresh distilled THF (50 mL) were added to a 100 mL Schlenk flask. The system was degassed by freeze-pump-thaw cycles for three times, then 17.6 mg PMDETA (0.506 mmol) was added via syringe and stirred at room temperature for 24 h. After reacting for 24 h, the volatile was removed and the residue was purified by silica gel chromatography using dichloromethane and ethyl acetate (V/V = 1/1) as the eluent. After removal of the eluent, the crude product **BBP** was re-dissolved in 15 mL water and dialyzed against Milli-Q water with 2k Da molecular weight cutoff dialysis membrane for 3 times in 72 h. The solution in dialysis tube was lyophilized and give the final product green fluffy solid 60.6 mg in 86% yield. ^{1}H NMR (400 MHz; $CDCl_3$; Me_4Si): 7.82 (s, 1H), 7.54-7.52 (m, 2H), 7.41-7.37 (m, 3H), 7.32 (s, 1H), 7.11-6.69 (m, 4H), 6.80 (s, 2H), 4.82-4.80 (t, 2H), 4.70 (t, 2H), 4.47-4.44(t, 2H), 3.80-3.78 (m, 2H), 3.80-3.43 (m, PEG, centered in 3.62 ppm). MS (MALDI−TOF, m/z, Figure S8).

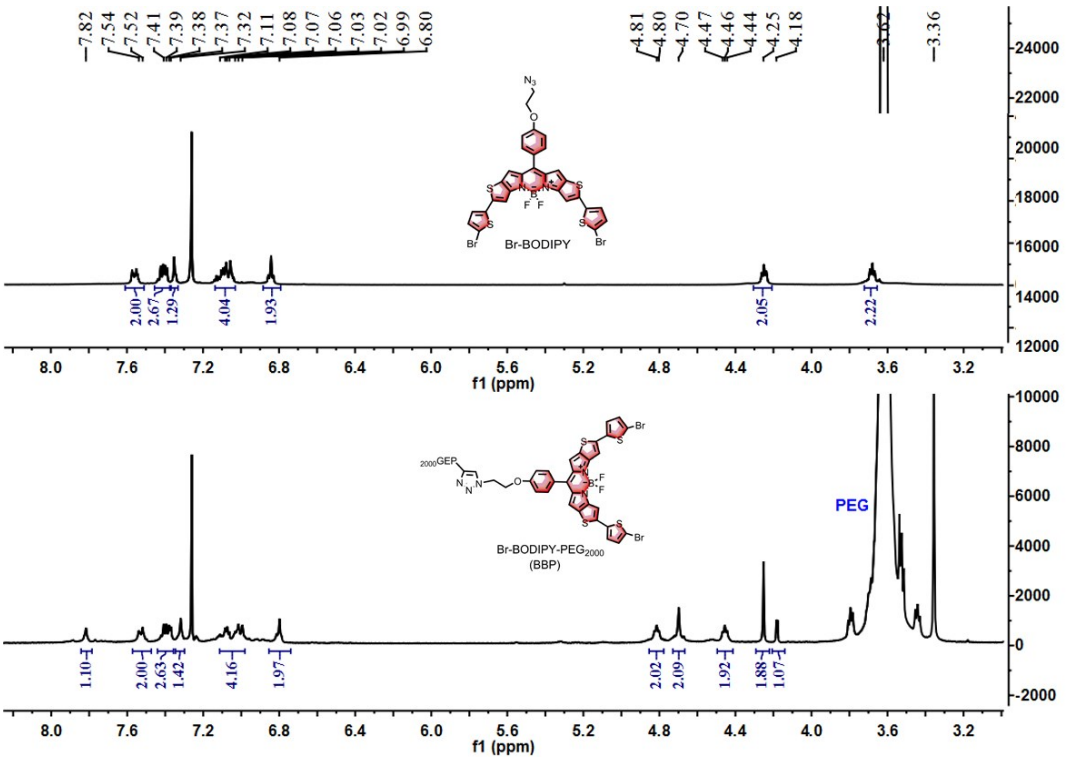

Fig. S7. The ^{1}H NMR spectra of Br-BODIPY and Br-BODIPY-PEG.

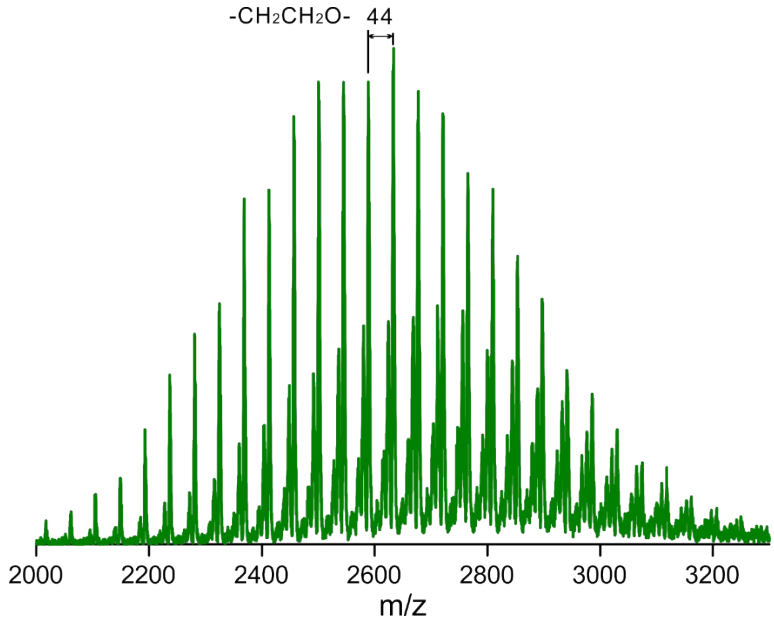

Fig. S8. The MALDI-TOF spectrum of Br-BODIPY-PEG (BBP).

4. Quantification of singlet oxygen generation

We use a commercial $^1O_2$ indicator ABDA to trap the $^1O_2$ and methylene blue (MB) as the standard photosensitizer with $^1O_2$ quantum yield (Φ_{MB}) of 0.52.[3, 4] The singlet oxygen quantum yields were measured in diluted solutions. An oxygen-saturated solution of photosensitizer containing 20 μM ABDA was prepared in the dark. The absorption of the $^1O_2$ indicator (ABDA) at 378 nm were recorded at 1 min intervals after exposing to 635 nm laser (power rate: 80 mW·cm^{-2}). The $^1O_2$ quantum yield of samples were calculated by a relative method using the following formula:

$$\Phi_X = \Phi_{MB} * \frac{K_X * F_{MB}}{K_{MB} * F_X}$$

Where subscripts X and MB refer to the sample and MB, respectively. *K* stands for the slope of the fitting line of the absorbance of ABDA versus irradiation time. *F* represents the absorption correction factor, which could be calculated by $F = 1 - 10^{-OD}$, ODs are the optical density of samples and MB at 635 nm.

5. *Photothermal Conversion Efficiency*

Photothermal conversion efficiency was calculated by recording the temperature change of the sample as a function of time under continuous irradiation of 635 nm laser (0.5 W·cm^{-2}) until the solution reached a steady-state temperature. The photothermal conversion efficiency (η) was calculated as the following Equation [1][5, 6]:

$$\eta = \frac{hS(T_{max} - T_s) - Q_0}{I_0(1-10^{-A})}$$

Where *h* stands for the heat transfer coefficient, *S* is the surface area of the container, T_{max} (53.3°C) stands for the maximum steady-state temperature, T_s is the ambient temperature of environment (23.5°C). Q_0 represents the heat dissipation from the light absorbed by the solvent and the quartz sample cell, I_0 is the incident laser power (0.5 W·cm^{-2}), and the *A* is the absorbance of the sample at 635 nm ($A_{635\ nm}$ BBPP = 2.09, Figure S9). the value of *hS* is derived from the following Equation [2]:

$$hS = \frac{m_d C_d}{\tau_s}$$

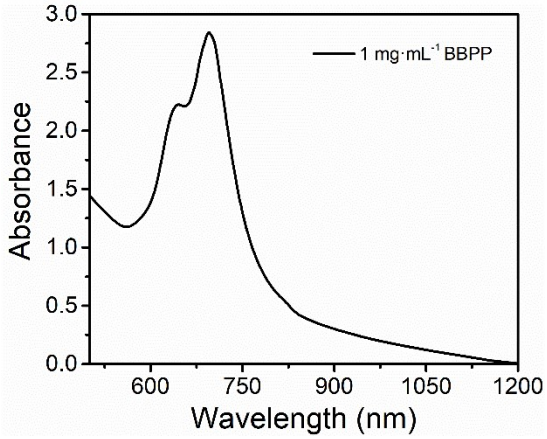

Fig. S9. The UV-vis absorbance spectra of 1 mg·mL^{-1} BBPP.

Where τ_s is the time constant for heat transfer of the system, it can be calculated by the following equation [3]:

$$\tau_s = - \frac{t}{ln\theta}$$

Where θ is the dimensionless driving force and t is the time, and it can be calculated as the following equation [4]:

$$\theta = \frac{T - T_s}{T_{max} - T_s}$$

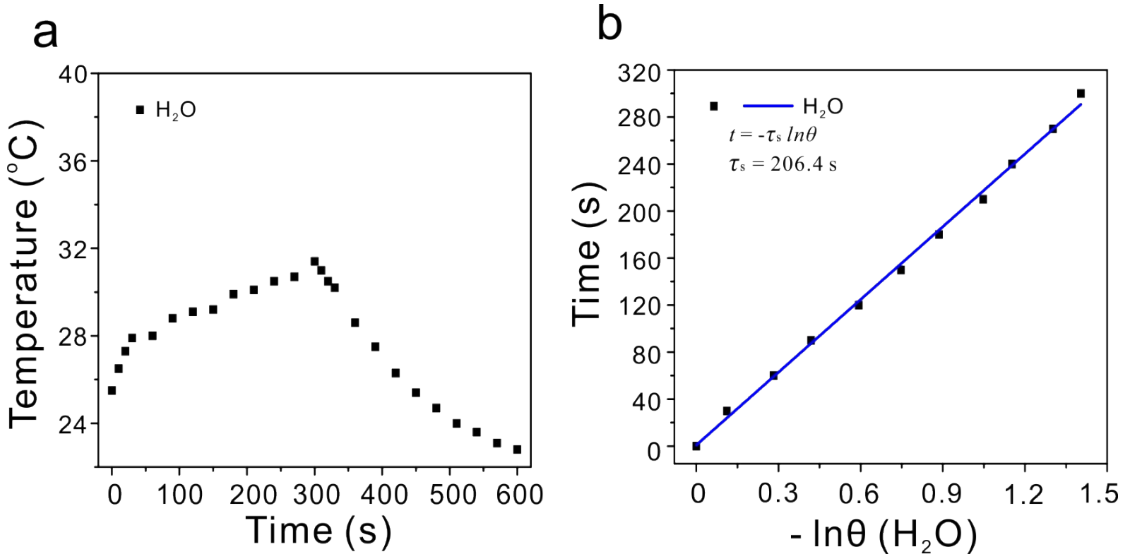

Fig. S10. (a) Temperature evolution of H_2O during heating (300 s) and cooling (300 s). (b) thermal equilibrium time constant revealed by the function of time data versus negative natural logarithm during the cooling period.

Q_0 is the heat dissipation of water and the quartz sample cell during the laser irradiation, so it could be calculated as the Equation [5] below:

$$Q_0 = \frac{m_d C_d \left(T_{max}(H_2O) - T_s\right)}{\tau_s(H_2O)}$$

Where $T_{max}(H_2O)$ is 31.4°C and $\tau_s(H_2O)$ is 206.4, m_d is the mass 0.2 g, C_d is the heat capacity 4.2 $J \cdot g^{-1}$; thus Q_0 was calculated to be 0.0464 W. And τ_s(BBPP) was determined as 158.6 s in Figure 4d, so hS can be calculated as 0.0053 W. Based on the Equation [1] and obtained data, the photothermal conversion efficiency (η) of BBPP was determined to be 30.2%.

According to the temperature evolution of BBP, the photothermal conversion efficiency (η) of BBP could be determined as 34.5%.

6. *In Vitro Photodynamic/Photothermal Therapy*

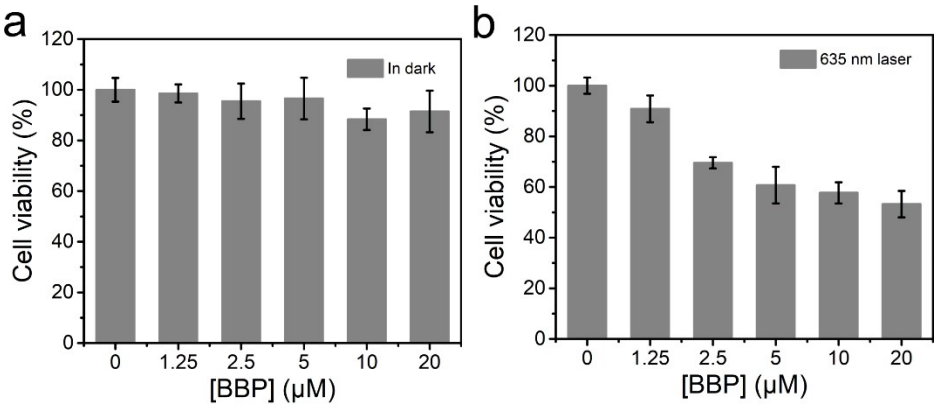

Fig. S11. Cell viability of HeLa cells treated with BBP (a) without and (b) with 635 nm laser irradiation for 4 min at the power density of 250 $mW \cdot cm^{-2}$.

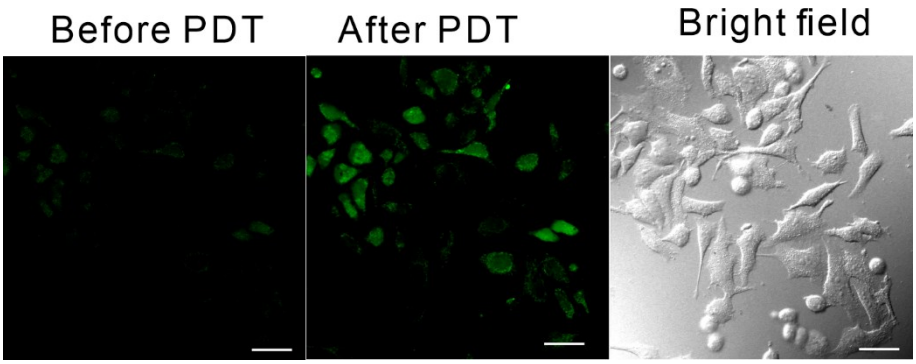

Fig. S12. The images of intracellular singlet oxygen induced by BBP upon irradiation in HeLa cells indicated by DCFH-DA staining. Scale bars: 40 μm.

7. In Vivo Photodynamic/Photothermal Therapy

All female nude mice (Balb/c) in experiments were purchased from OG Pharmaceutical. Co. Ltd (Nanjing, China). All animal experiments were conducted according to the protocols approved by OG Pharmaceutical Co. Ltd.

BALB/c mice bearing HeLa tumor were randomly divided into 4 groups, (n=6) And subjected to the following treatment: saline + laser, BBP + laser, BBPP, and BBPP + laser, respectively. For BBP or BBPP, 100 μL of photosensitizer was intravenously injected into the tumor-bearing mice, followed by laser irradiation at 20h post-injection. After different treatments, the tumor sizes and body weights were monitored every other day for 16 d. The tumor volumes were determined by V=0.5* length*(width)*(width), in which the length and width represent the greatest longitudinal diameter and greatest transverse diameter, respectively.

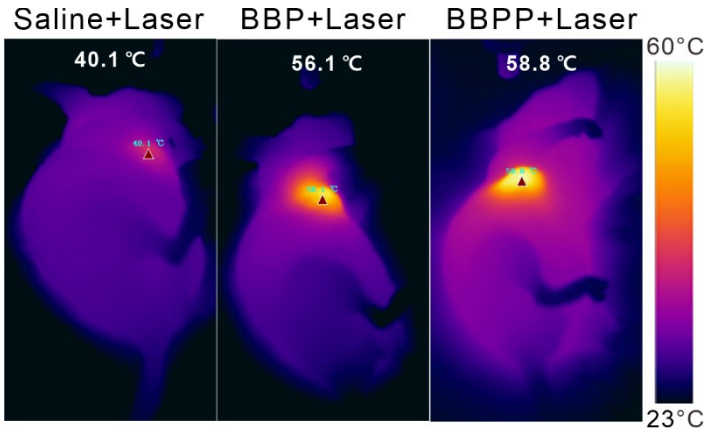

Fig. S13. Typical IR thermal images of tumors of HeLa tumor-bearing mice after intravenously injection of saline, BBP and BBPP upon 635 nm laser irradition (0.5 W·cm^{-2}).

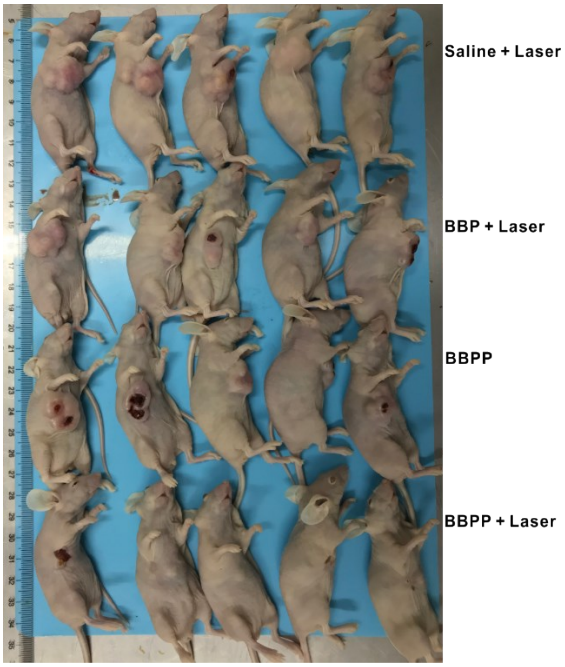

Fig. S14. Represensitive photographs of mice from groups as indicated at day 16 after the treatment.

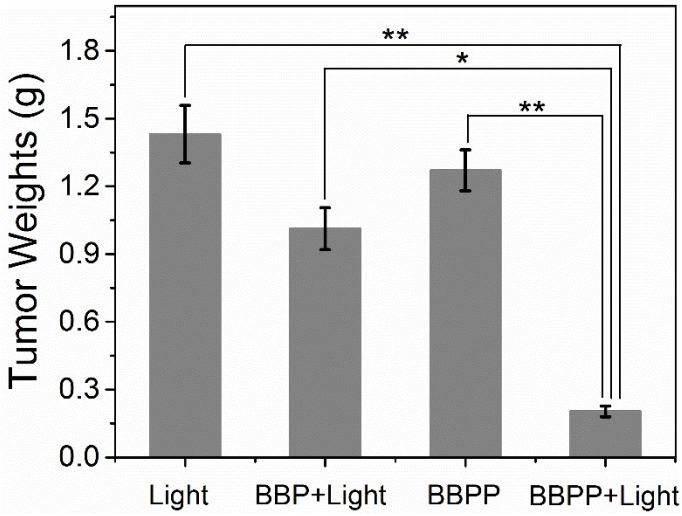

Fig. S15. Weights of tumors collected from groups as indicated at day 16 after treaments.

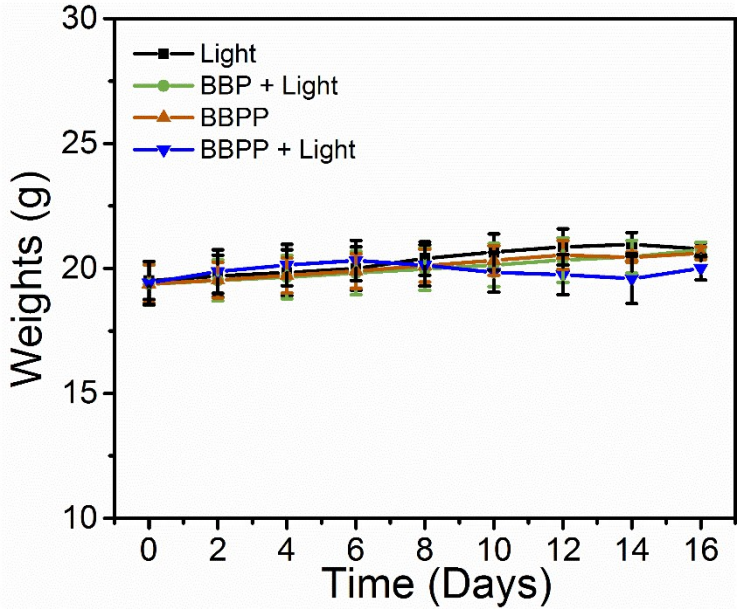

Fig. S16. Body weight profiles of mice from day 0 to day 16.

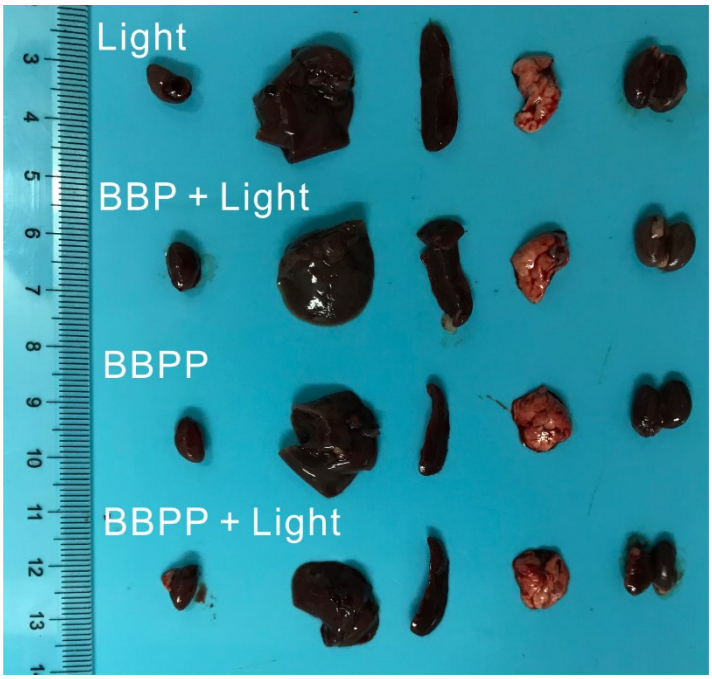

Fig. S17. Photographs of major organs collected from four groups of mice after the mice were sacrificed. From left to right: heart, liver, spleen, lung and kindey.

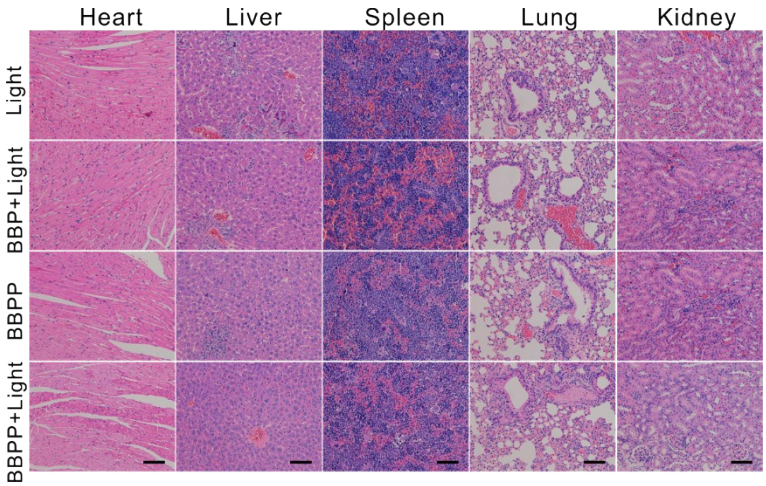

Fig. S18. H&E staining of heart, liver, spleen, lung and kidney slices collected different group as indicated at day 16 after treatment. Scale bars: 100 μm.

References

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