Supporting information

General information and methods

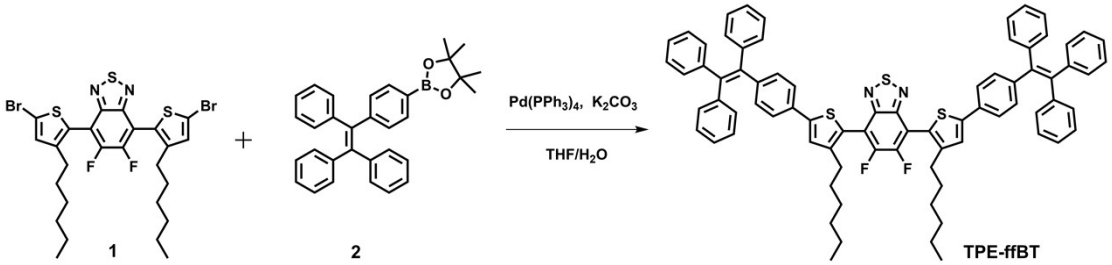
Materials

Monomer 1 was purchased from SunaTech Inc. Compound 2,[1] and 3[2] were synthesized according to literature methods. All chemicals were obtained from commercial sources and used without further purification unless otherwise specified. Murine 4T1 cancer cells were purchased from cell culture center of Institute of Basic Medical Sciences, Chinese Academy of Medical Science (Beijing, China). PBS buffer, DMEM (Dulbecco's modified Eagle medium), fetal bovine serum (FBS), Lysotracker blue and Mitotracker Green were purchased from Thermo Fisher Scientific (Shanghai, China). 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-methoxy(polyethylene glycol)-2000] (DSPE-PEG2000) was purchased from Ponsure (Shanghai, China). MTT was purchased from Tiangen Biotech (Beijing, China). Singlet Oxygen Sensor Green (SOSG) and Dihydrorhodamine 123 (DHR123) were purchased from Thermo Fisher Scientific (Shanghai, China). Reductant vitamin C (Vc) was purchased from TiTan (Shanghai, China). Chlorin e6 (Ce6) was purchased from Macklin (Shanghai, China). Propidium iodide (PI), Calcein AM were purchased from Thermo Fisher Scientific (Shanghai, China). DCFH-DA was purchased from Bidepharm (Shanghai, China). The female BALB/c mice (6-week-old) were purchased from China Boryxin Biotechnology Co. (hunan, China).

Measurements

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE NEO 500 spectrometer, using locking to the deuterated solvent ($CDCl_3$) and using tetramethylsilane (TMS) as an internal standard. High-resolution mass spectra (HRMS) were measured on Thermoscientific Q-Exactive. UV-vis absorption spectra were measured on a UV-2600i spectrophotometer (Shimadzu, Japan) by using a 1 cm glass cuvette. fluorescence emission spectra were collected on a F-7100 fluorescence spectrophotometer (Hitachi, China). Zeta potential measurements and particle size measurements were conducted on dynamic light scattering (NanoBrook, Brokhaven, USA). Confocal laser scanning microscope (CLSM) characterization was conducted with a confocal laser scanning biological microscope (TI2-CTRE, Nikon, Japan). In vitro animals fluorescence imaging was carried out by living imaging system (IVScope 8200, CliNX, China). The absorbance for MTT analysis was recorded on a microplate reader (Epoch, BioTek, Germany).

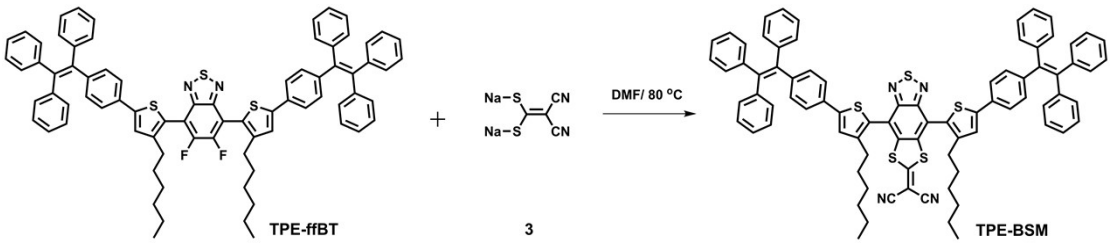
Synthetic procedures and characterization data for the compounds



Scheme S1 Synthetic route of TPE-ffBT.

Synthesis of TPE-ffBT. Monomer 1 (500 mg, 0.755 mmol, 1.0 equiv), monomer 2 (865 mg, 1.89 mmol, 2.5 equiv), $Pd(PPh_3)_4$ (87.2 mg, 0.076 mmol, 0.1 equiv) and K_2CO_3 (365 mg, 2.64 mmol, 3.5 equiv) were added to an 100 mL two-neck round-bottom flask

with a reflux condenser and stir bar. Nitrogen gas was allowed to flow through the headspace for 30 min. Then, THF/water mixtures (*v*/*v* = 5/1, 60 mL) was added and the reaction was heated to 70 °C. After no further conversion as monitored by thin-layer chromatography (TLC), the solution was diluted with dichloromethane and washed with water and brine before drying over $MgSO_4$. The crude reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (petroleum ether/dichloromethane, 2:1 to 1:2, v/v) to yield orange solid TPE-ffBT (577 mg, 0.495 mmol, 65.6%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37 (d, J = 7.9 Hz, 4H), 7.16 – 6.96 (m, 38H), 2.49 (t, J = 7.4 Hz, 4H), 1.62 – 1.54 (m, 4H), 1.23 – 1.13 (m, 12H), 0.77 (t, J = 6.3 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 146.19 (s), 145.24 (s), 143.58 (dd, J = 16.4, 2.8 Hz), 141.37 (s), 140.36 (s), 131.90 (s), 131.39 (d, J = 8.3 Hz), 128.03 – 127.48 (m), 126.82 – 125.96 (m), 124.98 (s), 124.68 (s), 122.62 (s), 77.29 (s), 77.03 (s), 76.78 (s), 31.51 (d, J = 12.8 Hz), 30.18 (d, J = 7.4 Hz), 29.77 (d, J = 13.9 Hz), 29.04 (s), 22.52 (s), 14.04 (s). HRMS (ESI) for $C_{78}H_{66}F_2N_2S_3$ $[M]^+$: calcd 1164.4356, found 1164.4388.



Scheme S2 Synthetic route of TPE-BSM.

***Synthesis of TPE-BSM*.** TPE-ffBT (300 mg, 0.257 mmol) and 3 (60.0 mg, 0.322 mmol, 1.25 equiv) were added to a vial with septa cap and stir bar, which was then backfilled with nitrogen. DMF (20.0 mL) was added and the vial was placed in a 80 °C bath, quickly converting the yellow solution to brown. After no further conversion as

monitored by TLC, the solution was diluted with dichloromethane and washed with water and brine before drying over $MgSO_4$. The crude reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (petroleum ether/dichloromethane, 2:1 to 1:2, v/v) to give bright orange-red solid TPE-BSM (262 mg, 80.3%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40 (d, J = 7.8 Hz, 4H), 7.31 (s, 2H), 7.20 – 6.99 (m, 36H), 2.55 – 2.34 (m, 4H), 1.57 (d, J = 9.1 Hz, 4H), 1.22 – 1.08 (m, 12H), 0.78 (dt, J = 13.5, 6.6 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 153.52 (s), 146.87 (d, J = 14.7 Hz), 145.17 (s), 144.06 (s), 143.71 – 143.36 (m), 141.54 (s), 140.42 – 139.80 (m), 132.03 (s), 131.71 – 131.17 (m), 128.07 – 127.58 (m), 126.89 – 126.28 (m), 125.36 – 124.84 (m), 122.54 (s), 112.09 (s), 77.30 (s), 77.04 (s), 76.79 (s), 31.46 (d, J = 11.4 Hz), 30.21 (d, J = 14.3 Hz), 29.75 (d, J = 7.4 Hz), 28.89 (d, J = 10.9 Hz), 22.50 (s), 14.04 (d, J = 8.2 Hz). HRMS (ESI) for $C_{78}H_{66}F_2N_2S_3$ $[M]^+$: calcd 1266.3891, found 1266.3905.

Experimental procedures.

Preparation of TPE-ffBT NPs, TPE-BSM NPs and Ce6-NPs

TPE-ffBT (1.0 mg) and DSPE-PEG_{2000}-COOH or DSPE-PEG_{2000} (2.0 mg) were fully dissolved in THF (1.0 mL). The mixture was quickly injected into 10.0 mL of deionized water under ultrasonic crusher with 25% power for 4 min, which was stirred in fume hood for two days. The crude NPs were further filtered through a membrane filter (diameter = 200 nm) for further usage. TPE-BSM NPs were prepared with the same procedure.

US-Triggered $^1O_2$ Generation of ffBT-TPE, BSM-TPE and Ce6:

First, to analyze the sonodynamic effect of the prepared ffBT-TPE、BSM-TPE, the SOSG as a $^1O_2$ detector was used, and Ce6 was used as the control. The obtained molecules were dissolved in DMF and the SOSG in methanol, respectively. Then SOSG was diluted by ultrapure water with the final concentration of 10.0 μM. The fluorescence intensity of SOSG solutions in the presence of ffBT-TPE, BSM-TPE or Ce6 was detected upon US irradiation (1.0 MHz, 1 W/cm^2) for different times. Afterwards, the fluorescence of SOSG was measured by a F-7100 fluorescence spectrophotometer.

To observe the sonodynamic effect of ffBT-TPE、BSM-TPE in DMF/water mixtures at different water (0%, 50%, 90%) fractions (f_w), the SOSG as a $^1O_2$ detector was used. ffBT-TPE and BSM-TPE solutions (different water fractions) containing SOSG detectors (10.0 μM) were then treated by US (1.0 MHz, 1 W/cm^2) for different times, followed with the fluorescence measurement by a F-7100 fluorescence spectrophotometer.

Light-triggered ROS generation observation:

To analyze the photodynamic effect of ffBT-TPE and BSM-TPE, DHR123 was employed as the as ROS probe, and RB was used as the control. The fluorescence intensity of DHR 123 (10.0 μM) in the presence of ffBT-TPE, BSM-TPE or RB solutions treated with white light (15.0 mW/cm^2) was monitored for 2 minutes. In addition, by comparing the fluorescence variation before and after the addition of

vitamin C (Vc), determined, the radicals generation of ffBT-TPE、BSM-TPE could be detected.

Cell cultures

4T1 cells were cultured in DMEM medium containing 10% FBS and antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin) in a humidified incubator with 5% CO_2 at 37 ℃.

Cell imaging

4T1 cells were grown overnight in cell cultural dish for 24 h, followed with the treatment of the obtained nanoparticles. The cells were further imaged by CLSM using different combination of excitation wavelength for each dye.

In vitro SDT evaluation

4T1 cells were seeded in 96-well plates at a density of 8×10^4 cells/mL. After 12 h of culture, different concentrations of TPE-ffBT NPs and TPE-BSM NPs were added and incubated at 37 ℃ for 12 h in dark. After US irradiation of 1.3 W/cm^2, 1MHz, 50% duty cycle for 8 min, the cells were further incubated at 37 ℃ for 12 h in dark. MTT assay was conducted as described before.

ROS generation measurement in vitro

4T1 cells were grown in a confocal imaging dish at 37 ℃. After incubation with medium containing 25.0 μM of TPE-ffBT NPs and TPE-BSM NPs for 20 h, the cells were treated with 10.0 μM of DCFH-DA for 30 min at 37 ℃ in the presence of US irradiation of 0.8 W/cm^2,1 MHz, 10% duty cycle for 30 s, followed with characterization by CLSM.

Live/dead cell imaging assay.

4T1 cells were grown in a confocal imaging dish at 37 ℃. After incubation with medium containing 25.0 μM TPE-ffBT NPs and TPE-BSM NPs for 20 h. After US irradiation (0.8 W/cm^2,1 MHz, 10% duty cycle for 30 s), the cells were stained with calcein-AM (5.0 μM) for 30 min, followed with CLSM characterization.

Antitumor efficacy of TPE-ffBT-NPs and TPE-BSM-NPs in vivo:

Female BALB/c mice (5 weeks old) were purchased from China Boryxin Biotechnology Co. (Hunan, China). All animal procedures have been approved through the Animal Ethics Committee of The University of South China according to the Regulations for the Administration of Affairs Concering Experimental Animals (Hunan Province, China). The mice were subcutaneously injecting 4T1 cells (1 × 107 cells) in PBS buffer. When the tumor size was about ~5 to 7 mm, the 4T1 cells-bearing mice mode were divided into 8 groups through intravenous injection of the corresponding agents:

I: Sterilized PBS without US (n=5)

II: Sterilized PBS with US (1.5 W /cm^2,1.0 MHz,50% duty cycle,) for 5 min (n=5)

III: TPE-ffBT NPs without US (n=5)

IV: TPE-ffBT NPs with US (1.5 W /cm^2,1.0 MHz,50% duty cycle) for 5 min (n=5)

V: TPE-BSM NPs without US (n=5)

VI: TPE-BSM NPs with US (1.5 W /cm^2,1.0 MHz,50% duty cycle) for 5 min (n=5)

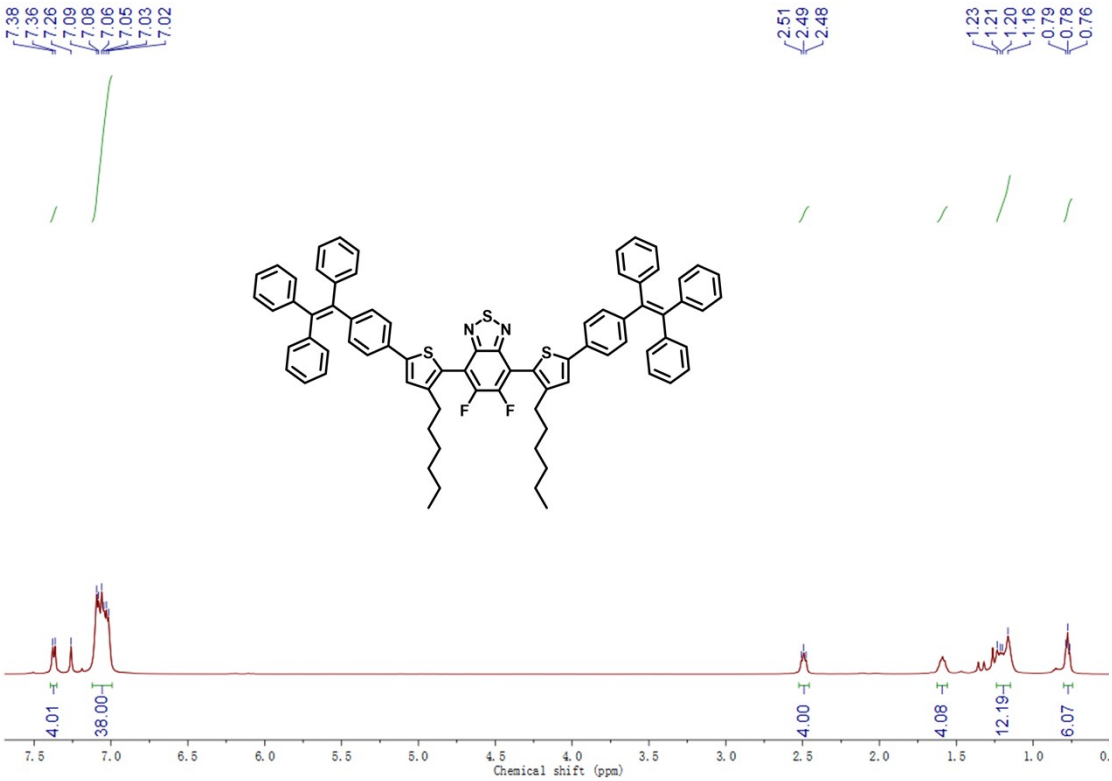


Fig. S1 ^{1}H NMR spectrum of TPE-ffBT.

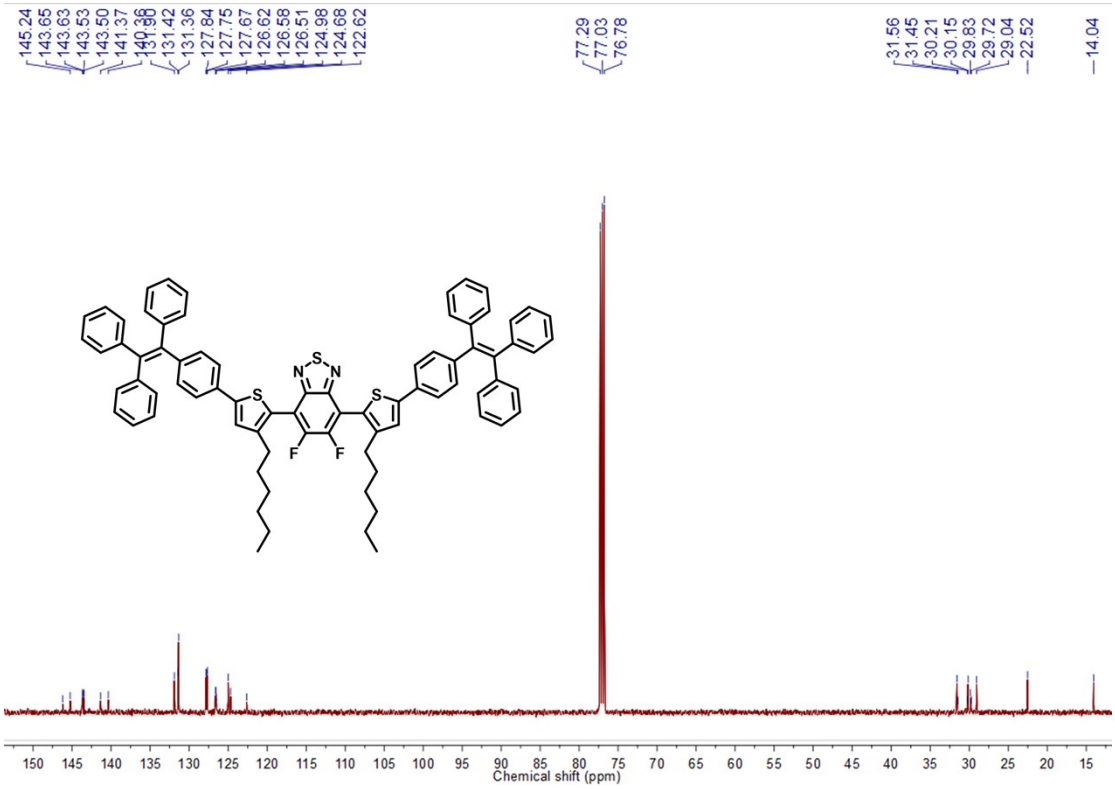


Fig. S2 ^{13}C NMR spectrum of TPE-ffBT.

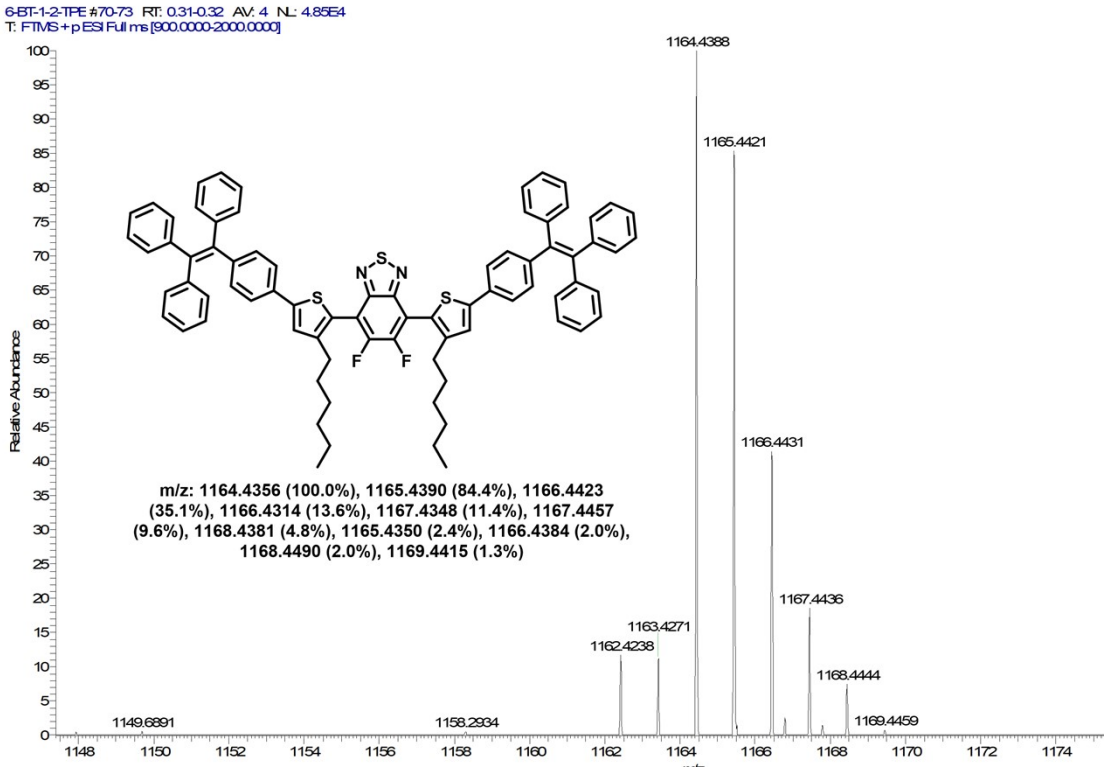


Fig. S3 HRMS spectrum of TPE-ffBT.

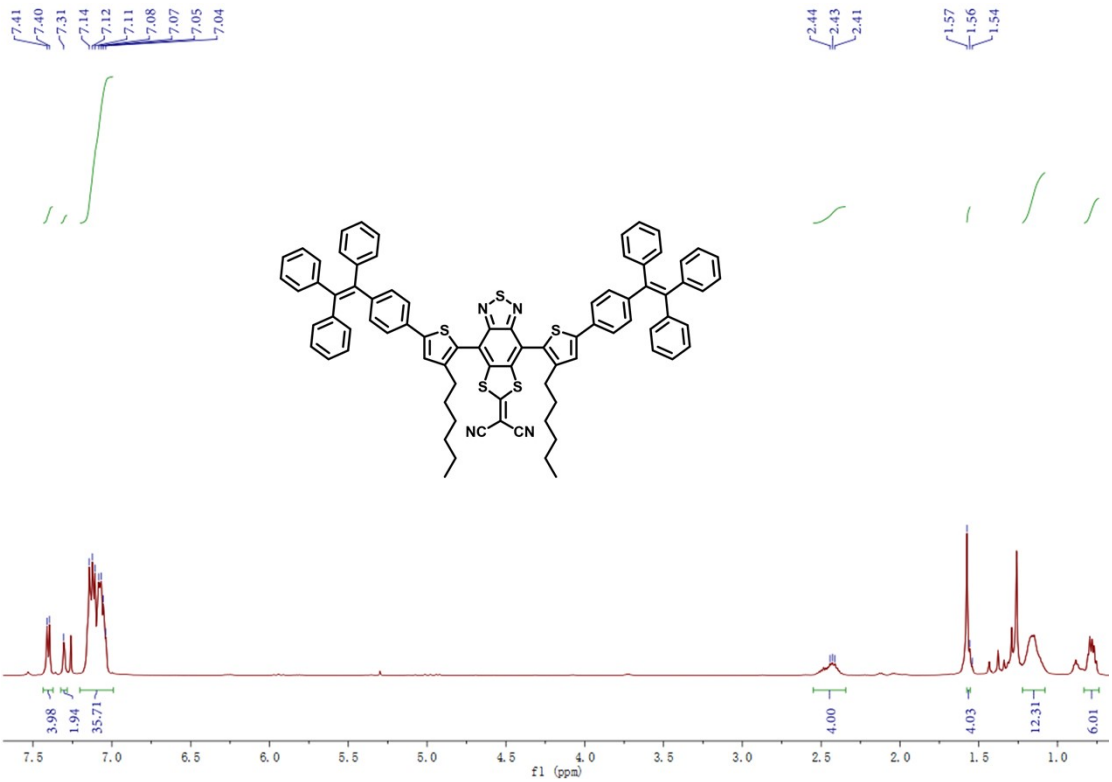


Fig. S4 ^{1}H NMR spectrum of TPE-BSM.

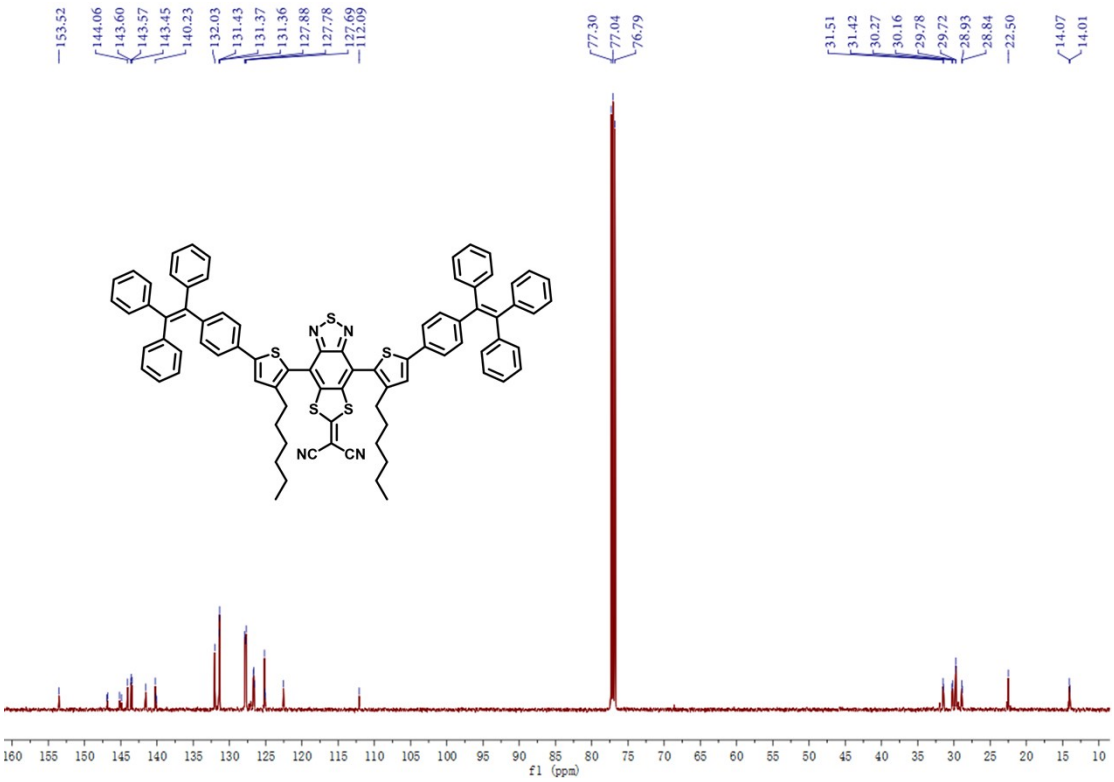


Fig. S5 ^{13}C NMR spectrum of TPE-BSM.

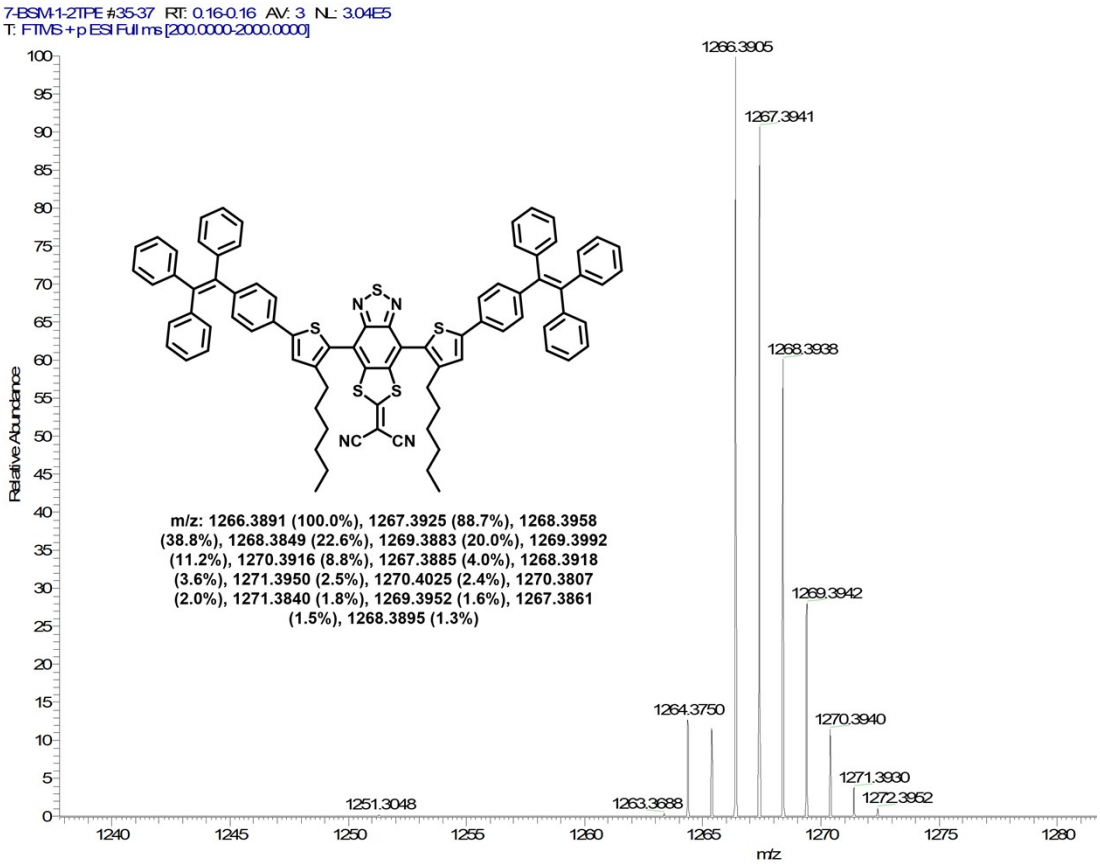


Fig. S6 HRMS spectrum of TPE-BSM.

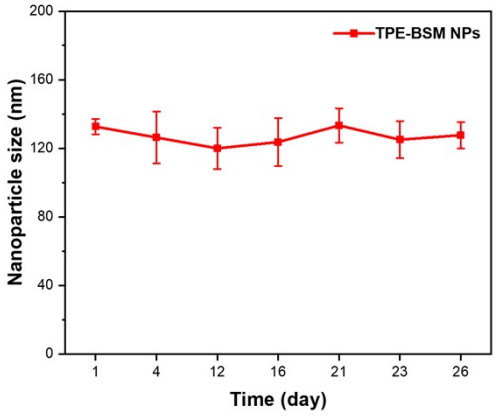


Fig. S7 Diameter variation of TPE-BSM NPs in water for 26 days.

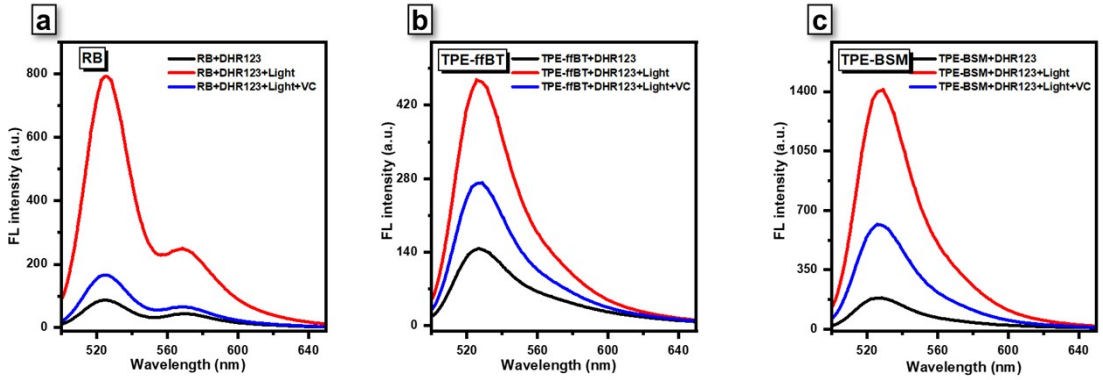


Fig. S8 Fluorescence intensity of DHR123 treated by RB (a), TPE-ffBT (b) and TPE-BSM (c) in the absence and presence of Vc under white light irradiation.

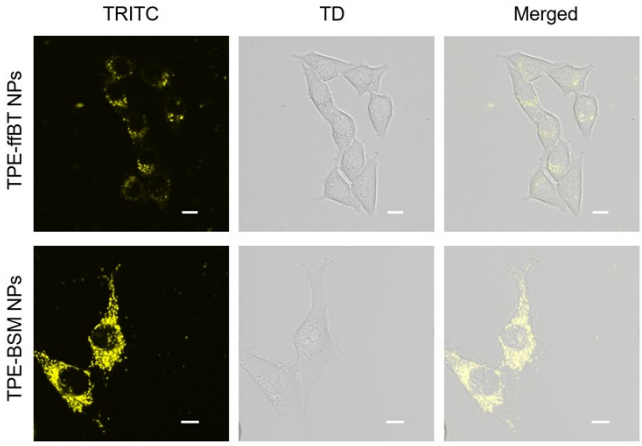


Fig. S9 CLSM images of 4T1 cells incubated with TPE-ffBT NPs and TPE-BSM NPs. Scar bar = 10 μm.

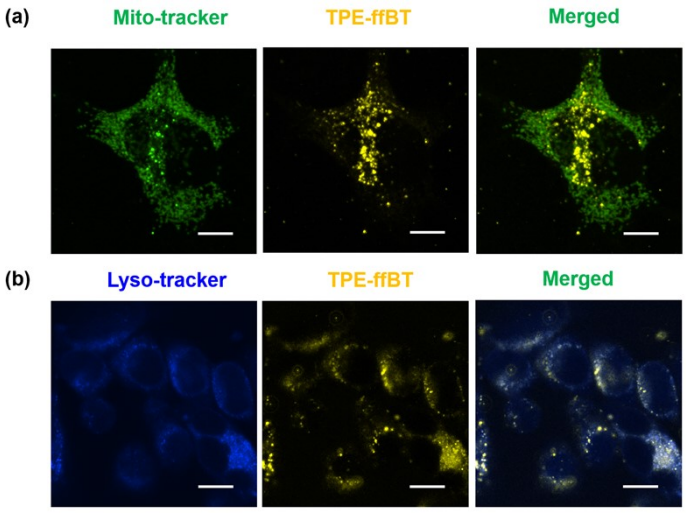


Fig. S10 Colocalization images of 4T1 cells co-stained by TPE-ffBT NPs with Lyso-tracker (a) and Mito-tracker (b). Scale bar = 10 μm.

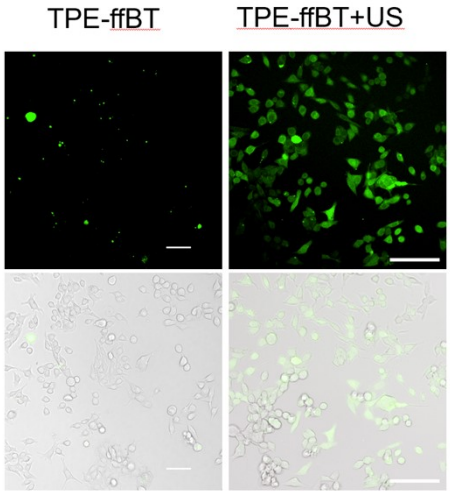


Fig. S11 Intracellular ROS generation of 4T1 cells in TPE-ffBT NPs group with different treatments. Scale bar = 10 μm.

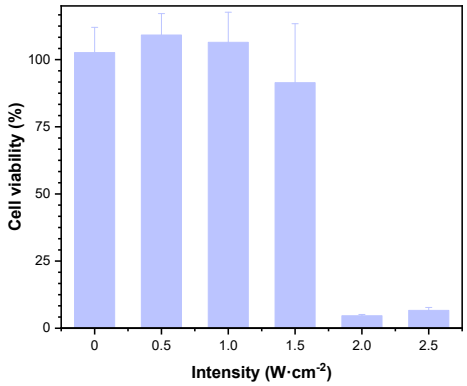


Fig. S11 Cell viability of 4T1 cells with different power of US (1 MHz, 50% duty cycle, 8 min).

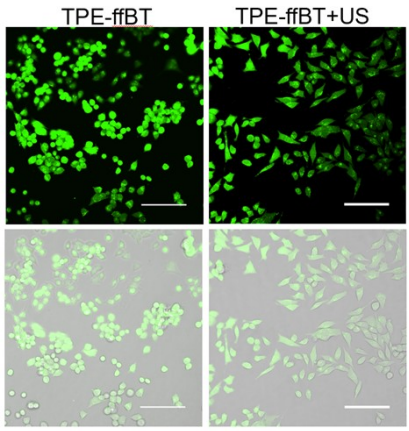


Fig. S13 Live/dead cell co-staining assays using calcein-AM for TPE-ffBT NPs with or without US irradiation. Scale bar = 10 μm.

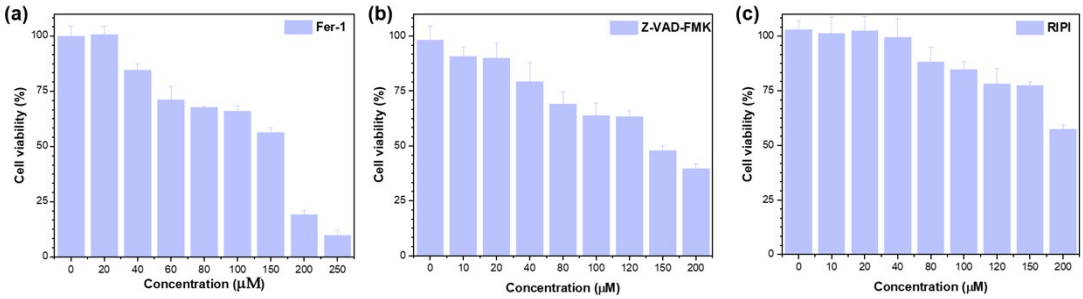


Fig. S14 Cell viability of 4T1 cells after the treatment with different concentration of Fer-1 (a), Z-VAD-FMK (b), RIPI (c).

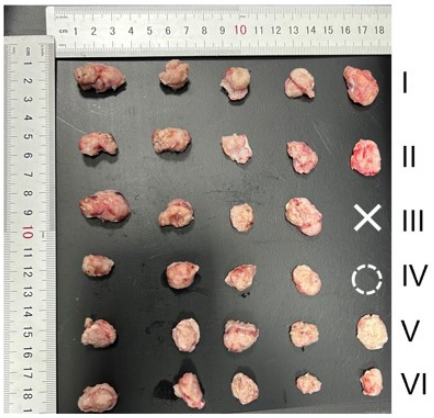


Fig. S15 Excised tumors after different treatments. I: Sterilized PBS without US. II: Sterilized PBS with US for 5 min. III: TPE-ffBT NPs without US. IV: TPE-ffBT NPs with US for 5 min. V: TPE-BSM NPs without US. VI: TPE-BSM NPs with US for 5 min.

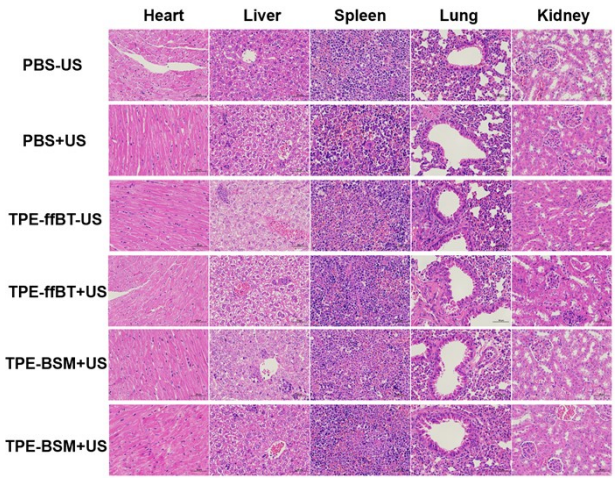


Fig. 16 H&E staining images of organs with different treatments.

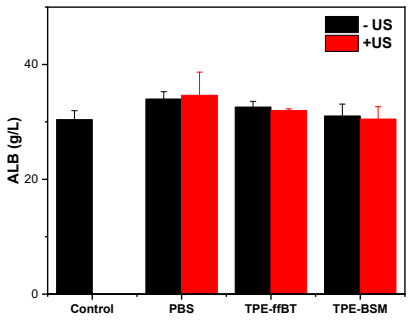


Fig. S17 (g) Levels of ALB in blood samples from mice with different treatments.

References

1. B. Li, Z. Li, T. Hu, Y. Zhang, Y. Wang, Y. Yi, F. Guo and L. C. Zhao, Highly Efficient Blue Organic Light-emitting Diodes from Pyrimidine-based Thermally Activated Delayed Fluorescence Emitters, *J. Mater. Chem. C.*, 2018, **6**, 2351-2359.
2. X. Gao, C. Di, Y. Hu, X. Yang, H. Fan, F. Zhang, Y. Liu, H. Li and D. B. Zhu, Core-Expanded Naphthalene Diimides Fused with 2-(1,3-Dithiol-2-Ylidene)Malonitrile Groups for High-Performance, Ambient-Stable, Solution-Processed n-Channel Organic Thin Film Transistors, *J. Am. Chem. Soc.*, 2010, **3**, 3697-3699.