Supporting Information

Self-Assembly of Semiconducting-Plasmonic Gold Nanoparticles with Enhanced Optical Property for In Vivo Photoacoustic Imaging and Therapy

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Materials and Characterization.

Methoxy-poly(ethylene glycol)-thiol (PEG-SH) with a molecular weight of 5 kDa was purchased from Laysan Bio, Inc. (Arab, AL). Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$) was obtained from Alfa Aesar (Haverhill, MA). N,N,N',N',N''-pentamethyl diethylenetriamine (PMDETA), pentafluorophenylmethacrylate (PFPMA), N-methyl-2-pyrrolidinone, pyrrolidine, isopropanol, trifluoroacetic acid, styrene, copper(I) bromide washed by acetic acid and methanol and sodium citrate (99%), 2, 2'-dithiobis [1-(2-bromo-2-methyl-propionyloxy)]ethane (DTBE), 2-n-octyl-1-dodecylamine, and 1,7-dibromoperylene-3,4,9,10-tetracarboxylic acid anhydride were purchased from Sigma-Aldrich (St. Louis, MO). Styrene was passed through a basic alumina column to remove the inhibitor and dried over CaH_2, then distilled in vacuum. Gold nanoparticles were synthesized according to our previous report.[1] All the other chemical reagents were obtained from commercial suppliers and used without further purification.

UV-vis absorption spectra were recorded by using a SHIMADZU UV-2501 spectrophotometer. Transmission electron microscopy (TEM) images were obtained on a JEOL TEM 2010 electron

microscope at an acceleration voltage of 100 kV. Scanning electron microscopy images were obtained on a Hitachi S-4800 FESEM. ^{1}H NMR spectra were obtained on Bruker AV300, using $CDCl_3$ as the solvent. Gel permeation chromatography (GPC) was measured on a Shimadzu HPLC system using THF as the eluent, and the molecular weight was calibrated with polystyrene standards. DSC analysis of copolymers was carried out using a Shimadzu Differential Scanning Calorimeter (DSC-60, Columbia, USA) with samples under a nitrogen atmosphere to determine the glass transition temperature (Tg) and melting temperature (Tm). DSC measurements were performed at a heating rate of 10 °C/min in the temperature range of -100 °C to 100 °C and sequential cooling back to -100 °C at a cooling rate of 50 °C/min, and then the sample was heated at a rate of 10 °C/min again. Dynamic light scattering experiments were carried out on Malvern Zetasizer Nano-ZS (Worcestershire, UK) at 25 °C.

Synthesis of Dibromo-N,N'-di(2-octyldodecyl)-3,4,9,10-tetracarboxylic diimide (2): A suspension of dibromoperylene-3,4,9,10-tetracarboxylic dianhydride (1) (1.7 g, 3.10 mmol), 2-n-octyl-1-dodecylamine (3.06 g, 10.2 mmol), and propionic acid (15 g, 24.99 mmol) in N-methyl-2-pyrrolidinone (200 mL) was stirred at 85 °C under N2 for 8 h. After cooling down the mixture to 25 °C, it was poured into 1 N HCl, and then washed with deionized water until pH 7, and dried under vacuum. The crude product was purified by silica gel column chromatography with CH2Cl2/haxane (5:1, v/v, Rf = 0.4) as the eluent. Compound 2 was obtained after evaporation of the solvent as a brown powder (3.20 g, 90%). 1H NMR (400 MHz, CDCl3): δ = 9.50 (m, 2 H), 8.94 (d, 2 H), 8.71 (s, 2 H), 4.26–4.19 (m, 4 H), 1.79–1.70 (m, 4 H), 1.50–1.43 (m, 66 H), 1.00 (t, 12 H) ppm. HRMS: calcd: C64H88Br2N2O4 [M + H]+ 1106.5101; found: 1106.5132.

Synthesis of 1,7-di(pyrrolidin-1-yl)-N,N'-di(2-octyldodecyl)-3,4,9,10-tetracarboxylic Diimides (3): A mixture of 2 (331.8 mg, 0.3 mmol) and pyrrolidine (8 mL) was heated to 55 °C under N2. The reaction mixture was kept at 55 °C for about 36 h, after that, the solvent was evaporated. It was then washed with water and methanol for 3 times respectively, to give a green solid after the solvent was removed. The residue was purified by column chromatography on silica gel with CHCl3 (Rf = 0.43) as the eluent. The regioisomeric 1,7- and 1,6-dibromoperylene bisimides could be separated by column chromatography at

this step. The 1,6-dibromoperylene bisimide is the blue fraction. 3 was collected as a green powder (248 mg, 75%). 1H NMR (400 MHz, CDCl3): δ = 8.28 (d, 4 H), 7.53 (s, 4 H), 4.25–4.19 (t, 4 H), 3.67 (m, 4 H), 2.65–2.51 (m, 4 H), 2.03–1.72 (m, 12 H), 1.48 (m, 66 H), 1.00 (t, 12 H) ppm. HRMS: calcd: C72H104N4O4 [M + H]+ 1088.8111; found: 1088.8134.

Synthesis of 1,7-di(pyrrolidin-1-yl)-N-(2-octyldodecyl)-3,4:9,10-tetracarboxylic acid-3,4-anhydride-9,10-imide (4): A solution of 2 (2.18 g, 2.00 mmol) and KOH (9.78 g, 16.72 mmol) in tert butyl alcohol (72 mL) was heated to reflux at 85 °C. After stirring for 1 h, the reaction mixture was poured into the mixture of acetic acid and HCl and stirred over night at room temperature. After filtration, a large amount of water and methanol used to wash the precipitate. The crude product was purified by silica gel column chromatography with CH2Cl2 (Rf = 0.35) as the eluent. The green band was collected and 4 was obtained after evaporation of the solvent as a green powder (1.2 g, 70%). 1H NMR (400 MHz, CDCl3) 8.45-8.32 (m, 4H), 7.46-7.53 (d, 2H), 4.22 (d, 2H), 3.85-3.65 (m, 4H), 2.85-2.65 (m, 4H), 2.25-1.95 (m, 8H), 1.48 (m, 33 H), 0.80 (t, 6 H) ppm. HRMS: calcd: C52H63N3O5 [M + H]+ 809.4876; found: 809.4844.

Synthesis of 1,7-di(pyrrolidin-1-yl)-N-(2-octyldodecyl)-N'-6-hexan-1-amine-3,4,9,10-tetracarboxylic Diimides (5): A mixture of 4 (404.74 mg, 0.50 mmol), N-boc-1,6-hexanediamine (118.97 mg, 0.550 mmol), and imidazole (50 g) was heated at 130 °C for 3 h. The resulting mixture was poured into water (150 mL). The precipitate was separated by suction filtration, washed with deionized water until pH 7. The residue was subjected to silica gel column chromatography with CH2Cl2 as the eluent. 5 was obtained after evaporation of the solvent as a green powder. And then the green powder was dissolved in anhydrous CH2Cl2, and 5 mL trifluoroacetic acid (TFA) was dropped slowly into the solution. The color of the reaction solution changed from green to red immediately after addition of TFA. The solution was stirred over night at room temperature. The solvent and excess TFA were evaporation. Then 10 mL water was added to the solid and the suspension was treated for 30 min under sonication. The product was filtered off and dried under vacuum to obtain a green solid. The crude product was purified by silica gel column chromatography (CH2Cl2/methanol = 6:1 as eluent) (461.61 mg, 90%). 1H NMR

(400 MHz, CDCl3): 8.23-8.34 (m, 4 H), 7.32-7.49 (m, 2 H), 4.15-4.22 (t, 4 H), 3.65-3.65 (S, 4H), 3.01-3.09 (m, 4 H), 1.85-2.05 (m, 14 H), 1.21-1.66 (m, 41 H), 0.80-0.91 (t, 9 H) ppm. MALDI-TOF MS: calcd: C58H77N5O4 [M+H]+ 909.60; found: 910.260.

Synthesis of SH-PS–co-PPFPMA Copolymers: Precursor copolymers were synthesized using atom transfer radical polymerization (ATRP) following the similar procedures previously reported5. Briefly, styrene (1.70 g, 8 mmol), PFPMA 3.5 g (13.8 mmol), PMDETA (21 μL, 0.1 mmol) and DTBE (0.5 g, 0.1 mmol) were charged into a glass tube. Then anisole was added to dissolve the monomers and initiator. After three cycles of freeze-pump-thaw to remove the oxygen, CuBr (14 mg, 0.1 mmol) was added into the polymerization tube under nitrogen atmosphere, and the tube was sealed in vacuum. The polymerization was carried out at 100 °C for 8 h. After polymerization, the reaction mixture was diluted with 5 mL THF, and passed through a neutral Al2O3 column to remove the catalyst. The THF solvent was removed by rotary evaporator. The residue was precipitated by methanol to give a white solid. After synthesis, the polymers were characterized by 1H NMR, gel permeation chromatography (GPC), and differential scanning calorimetry (DSC). All the NMR spectra were obtained in CDCl3 using tetramethylsilane (TMS) as the internal reference on a Varian 400 MHz 1H NMR spectrometer.

Synthesis of SH-PS–co-PPPDI Copolymers: For the synthesis of PDI-functionalized S-S-PS–PPDI copolymers, 90 mg (1.26 μmol) of S-S-PS–PPFPMA was dissolved in 10 mL of THF, and then 100 mg (110 μmol) of PDI monomer was added. The mixture was stirred for 24 h at 50 °C under nitrogen. Functionalized copolymers were precipitated into cold methanol and dried under vacuum. 150 mg of colorless powders was obtained.

Preparation of Amphiphilic Au@PPDI/PEEG: Amphiphilic Au@PPDI/PEG was prepared by coadsorption of PEG-SH and SH-PS-co-PPDI onto AuNP through the Au-S bond through a ligand exchange method. In brief, a solution of PEG (25 mg) and HS-PS-co-PPDI (20 mg) in DMF (2 mL) was slowly added into 50 mL of the original 15 nm Au NPs (4.5 nM) in DMF. After the solution was stirred for 24 h, Au@PPDI/PEG NPs were purified by centrifugation (10000 g, 20 min). The obtained amphiphilic Au@PPDI/PEG NPs were dissolved in chloroform at 4 °C for further use.

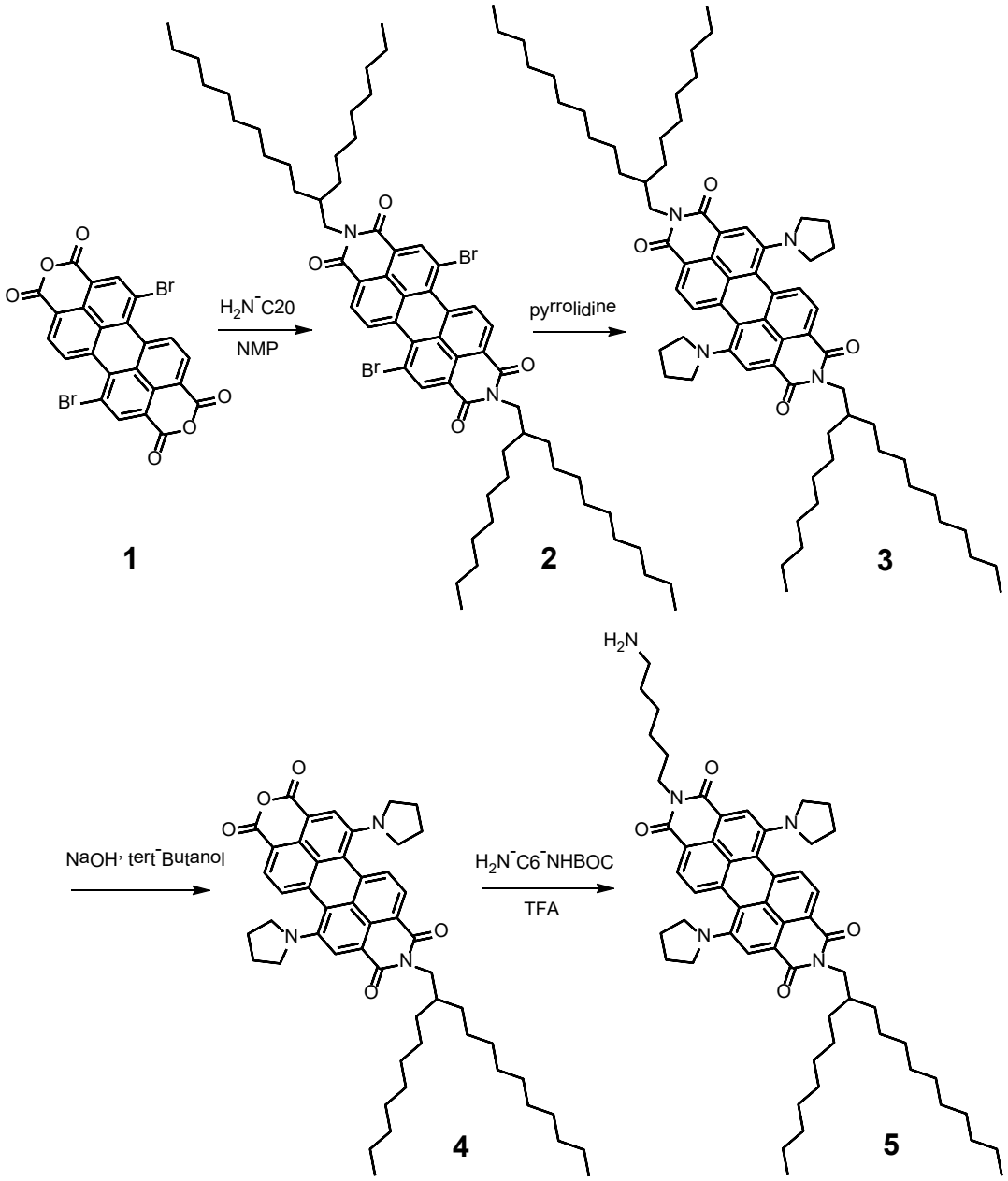
Photothermal Property of the Au@PPDI/PEG Vesicle: A 0.5 mL hybrid vesicles solution in a 2 mL plastic tube was exposed to NIR laser for 5 min at different power densities. The laser should irradiate the entire solution surface. The infrared thermal images and temperature variation of the Au@PPDI/PEG vesicle solutions were tested through a SC300 infrared camera (FLIR). The mixture of PDI NP and Au@PMMA/PEG aqueous solutions and PBS as control groups were also irradiated with NIR laser for the same time.

Photoacoustic Imaging of the Tumor: In vivo PA imaging of hybrid Au@PPDI/PEG vesicles was performed in a U87MG tumor xenograft model. All in vivo experiments were approved by the animal care and use committee of the NIH Clinical Center. The U87MG tumor was prepared by by inoculating 1×106 U87MG cancer cells in 100 μL PBS into the right shoulder of the nude mice under anesthesia. After the tumor growth for about two weeks, the tumor volume reached about 70 mm3. And then, 200 μL hybrid vesicle solution in PBS (500 μg/mL) was injected into the tumor-bearing mice intravenously. Afterwards, the whole tumor region was scanned by using the VisualSonics Vevo 2100 LAZR system equipped with a 40 MHz, 256-element linear array transducer.

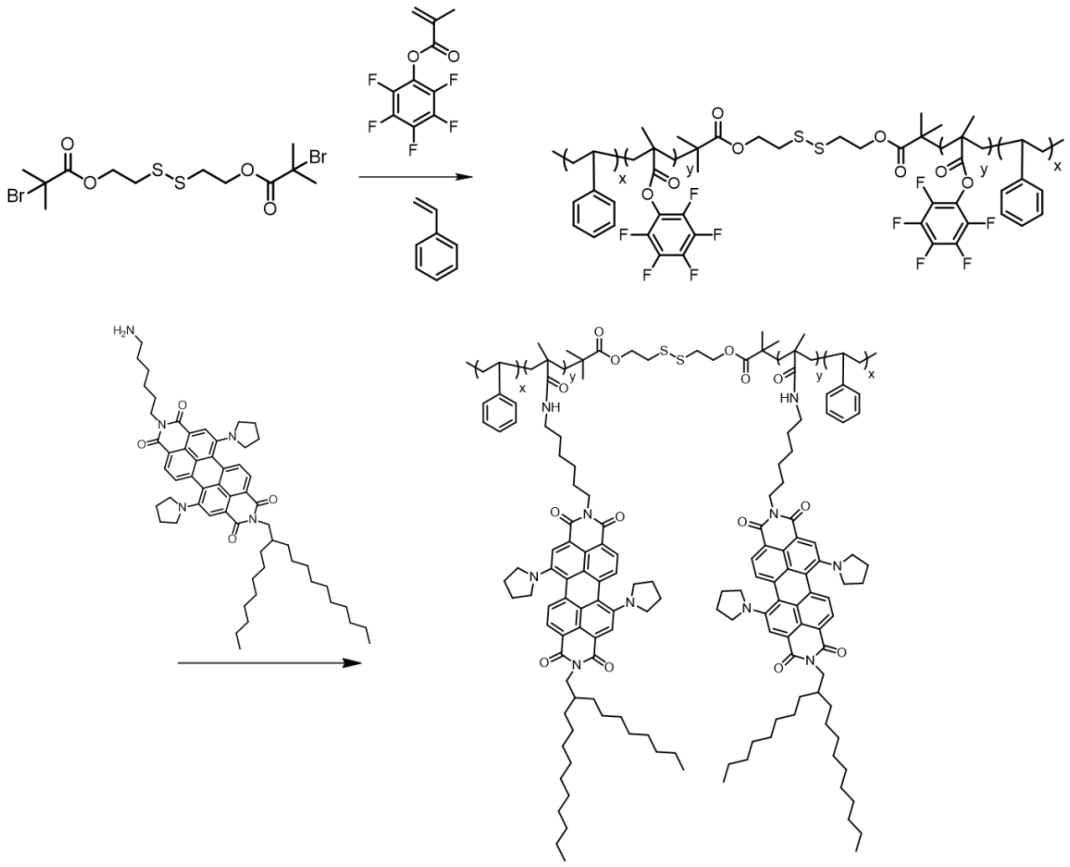
In Vivo Cancer Therapy: For the cancer therapy, 200 μL hybrid Au@PPDI/PEG vesicle solution (500 μg Au/mL) in PBS was injected intravenously into the U87MG tumor-bearing mice when the tumor volume reached about 70 mm^3 (after two weeks inoculation). At 30 h pots-injection of the vesicle, the whole tumor was exposed to NIR laser at 808 nm for 5 min at a power density of 0.3 W/cm^2. During laser irradiation, thermal images and average temperature of the tumor region were recorded and examinzed by using an infrared camera. After photothermal therapy of the tumor, a caliper was used to measure the size of the tumor at various time points.

References

1. Song J, Cheng L, Liu A, Yin J, Kuang M, Duan H. Plasmonic vesicles of amphiphilic gold nanocrystals: self-assembly and external-stimuli-triggered destruction. *J Am Chem Soc.* 2011;133:10760–63.



Scheme S1. Synthesis of PDI monomer.



Scheme S2. Synthetic route of S-S-PS-PPDI copolymer.

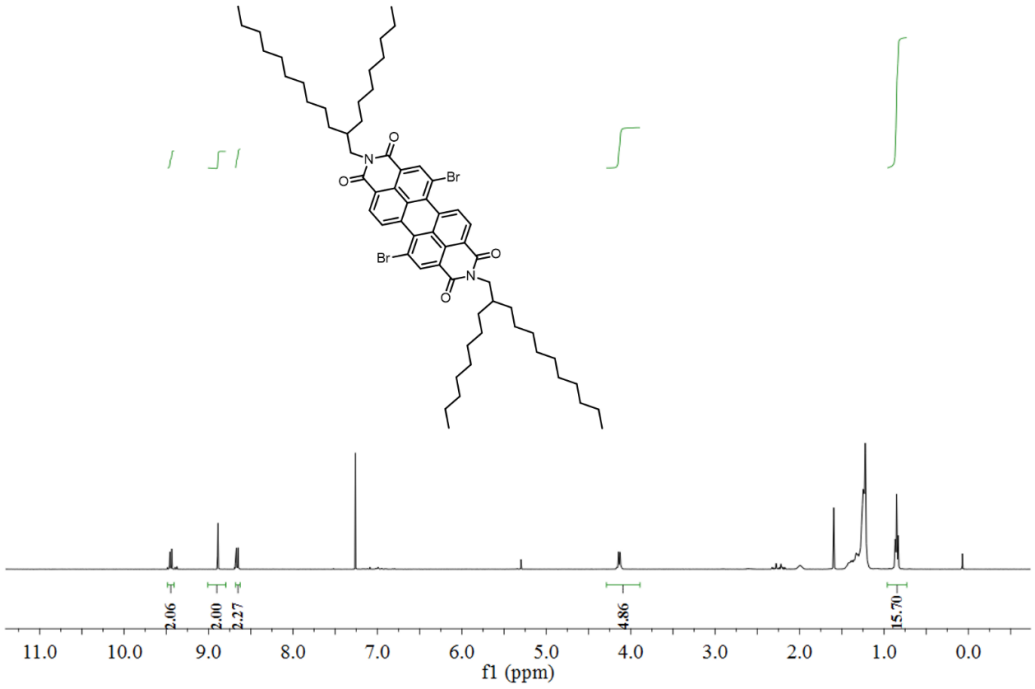


Figure S1. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, room temperature) of **2**.

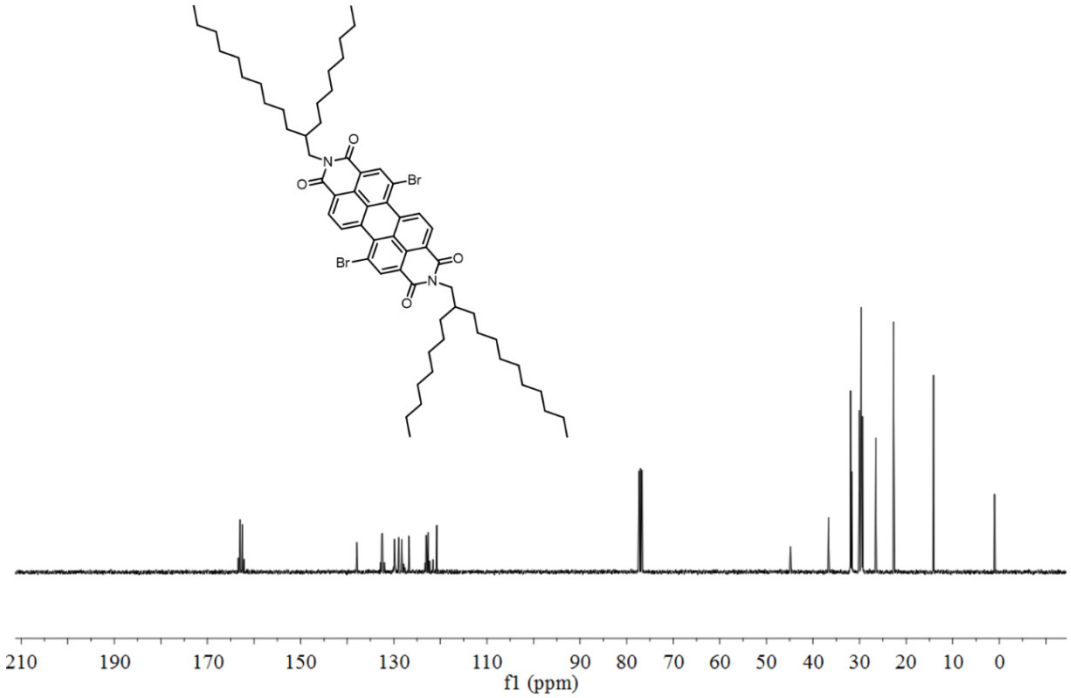


Figure S2. ^{13}C NMR spectrum (100 MHz, $CDCl_3$, room temperature) of **2**.

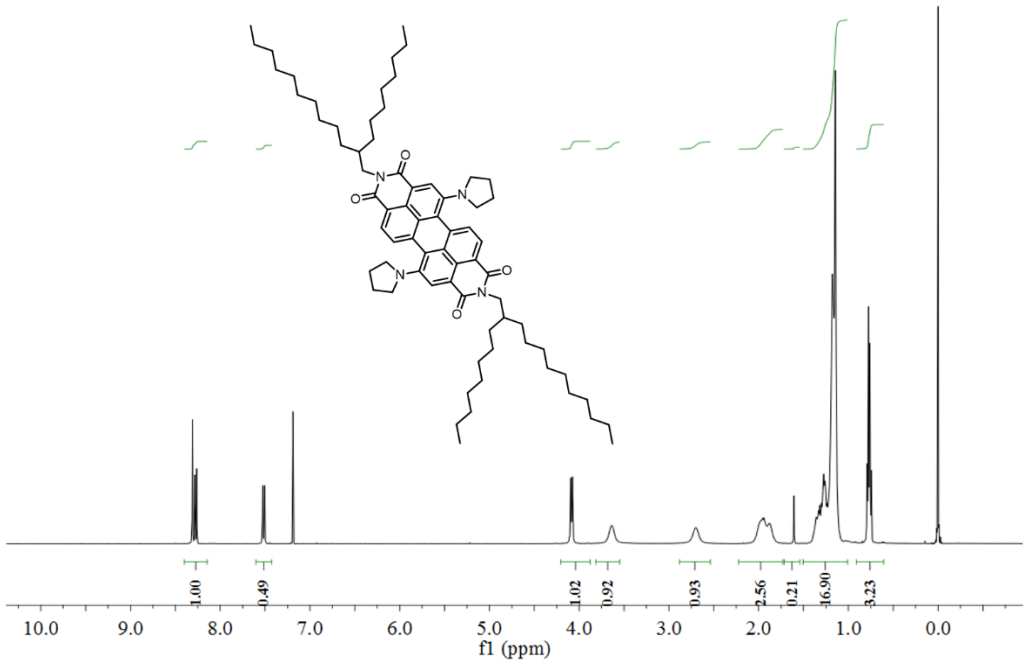


Figure S3. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, room temperature) of **3**.

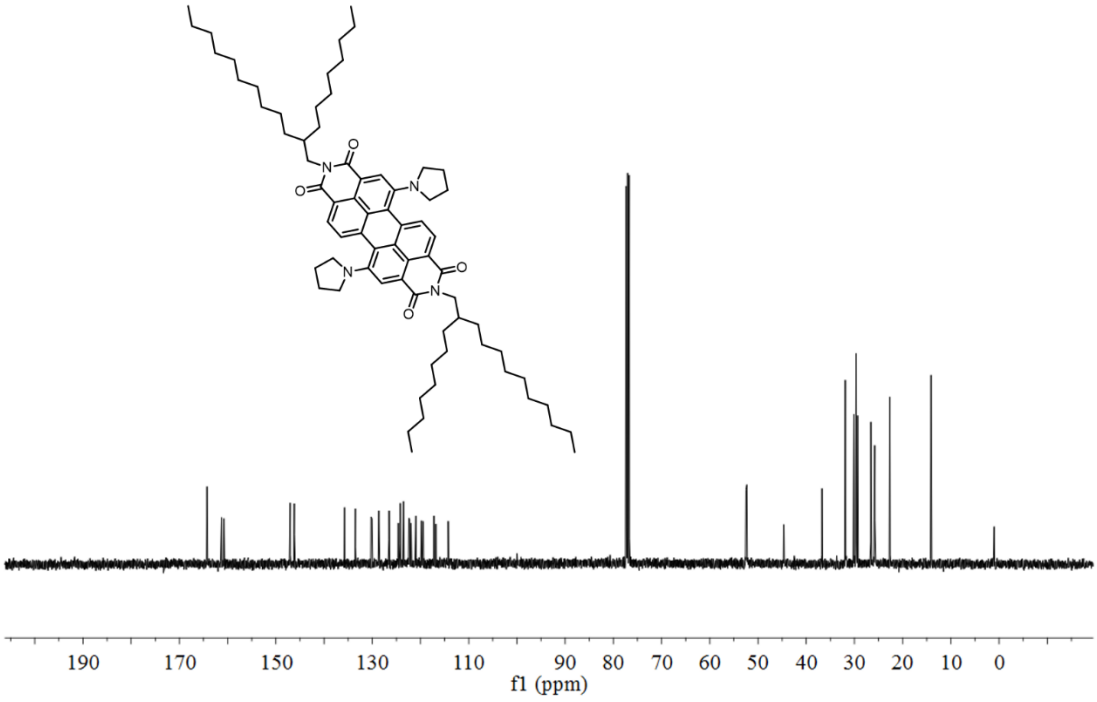


Figure S4. ^{13}C NMR spectrum (100 MHz, $CDCl_3$, room temperature) of **3**.

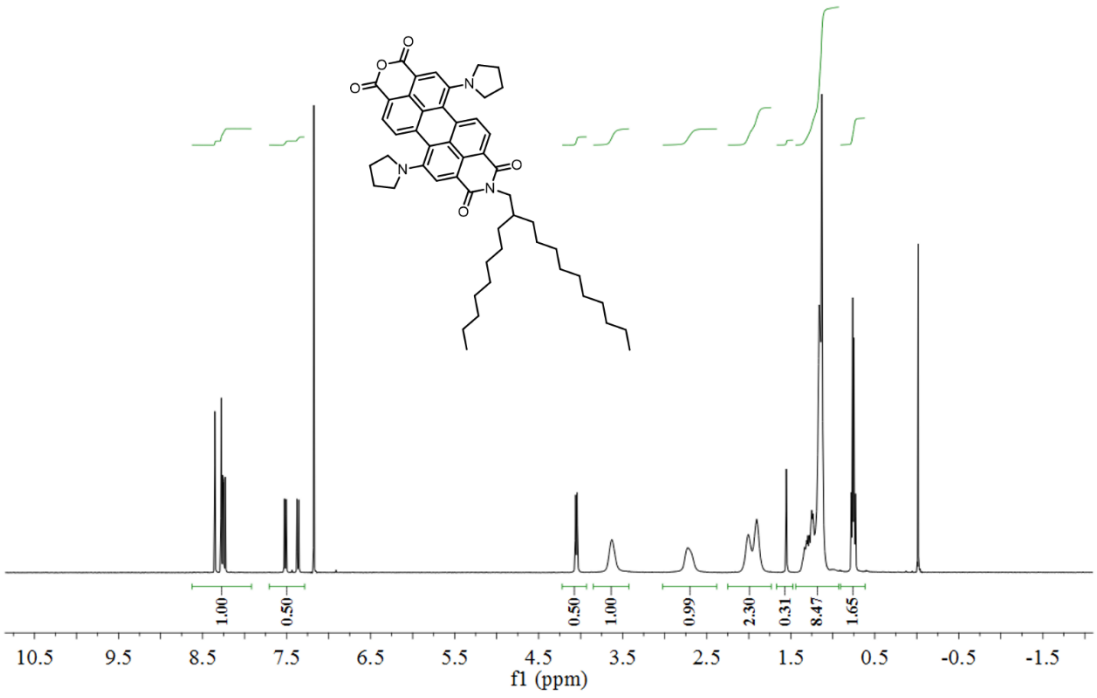


Figure S5. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, room temperature) of **4**.

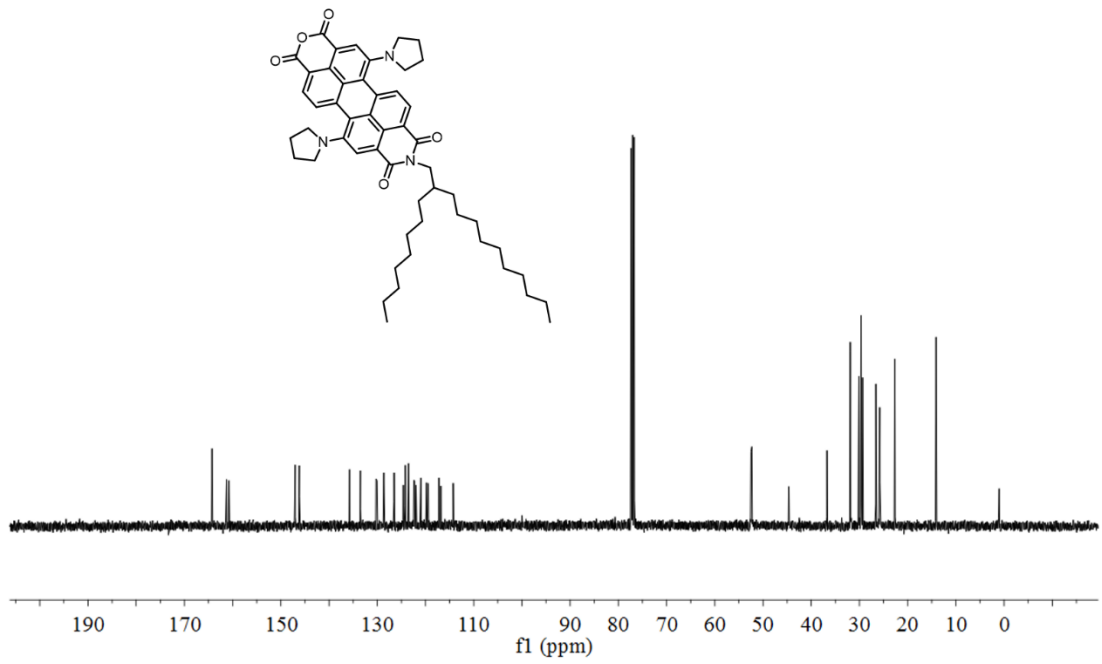


Figure S6. ^{13}C NMR spectrum (100 MHz, $CDCl_3$, room temperature) of **4**.

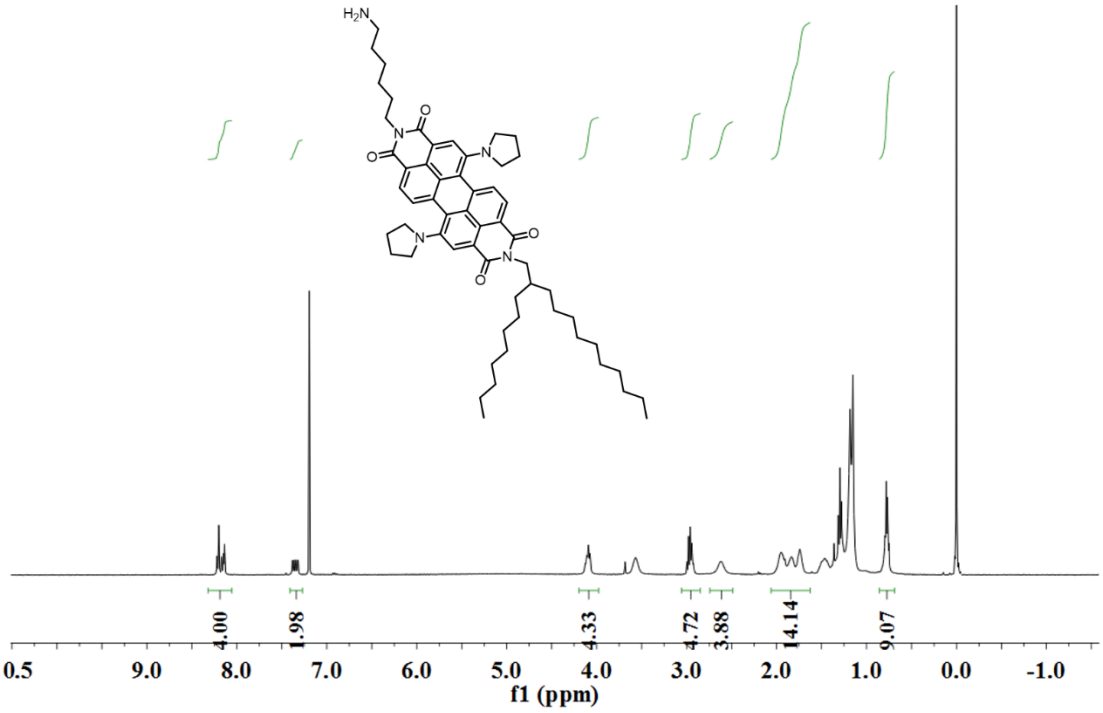


Figure S7. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, room temperature) of **5**.

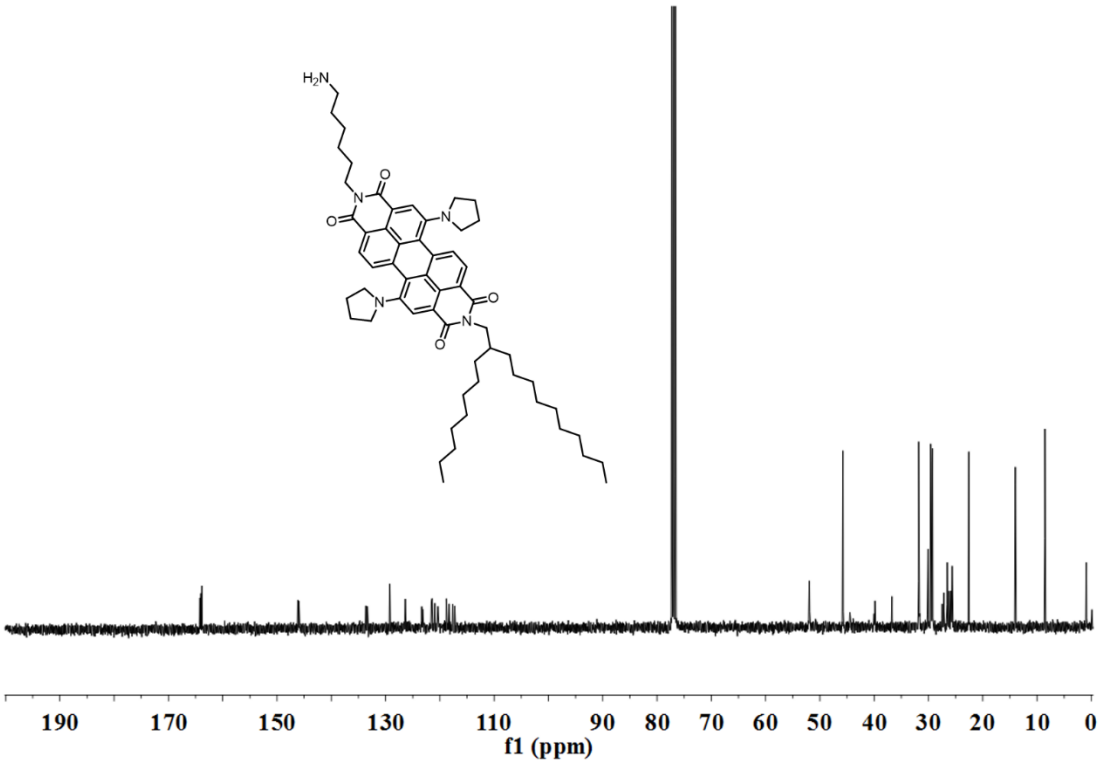


Figure S8. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, room temperature) of **5**.

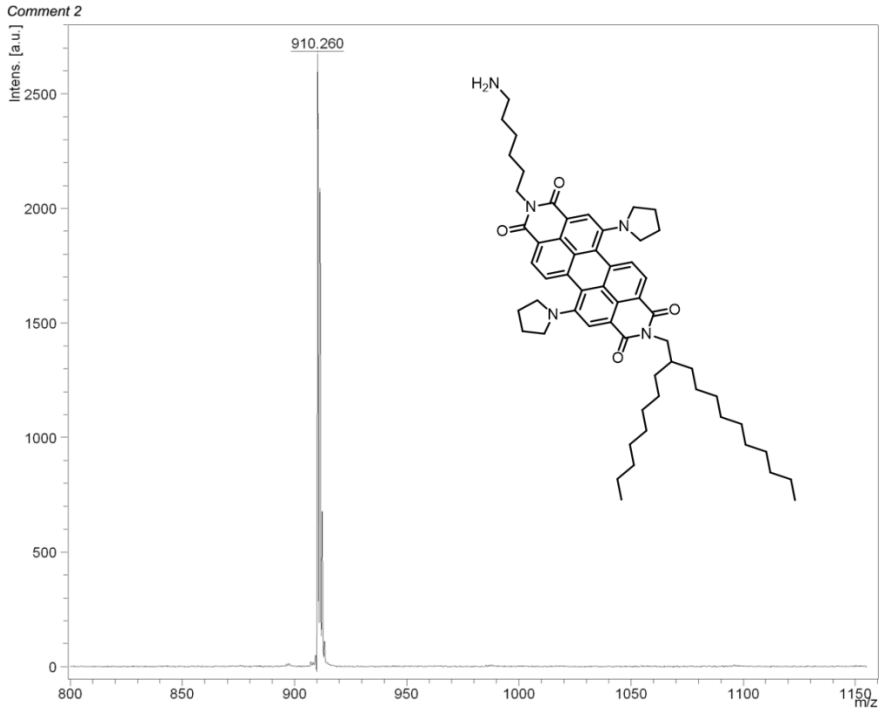


Figure S9. MALDI-TOF mass spectrum of **5**.

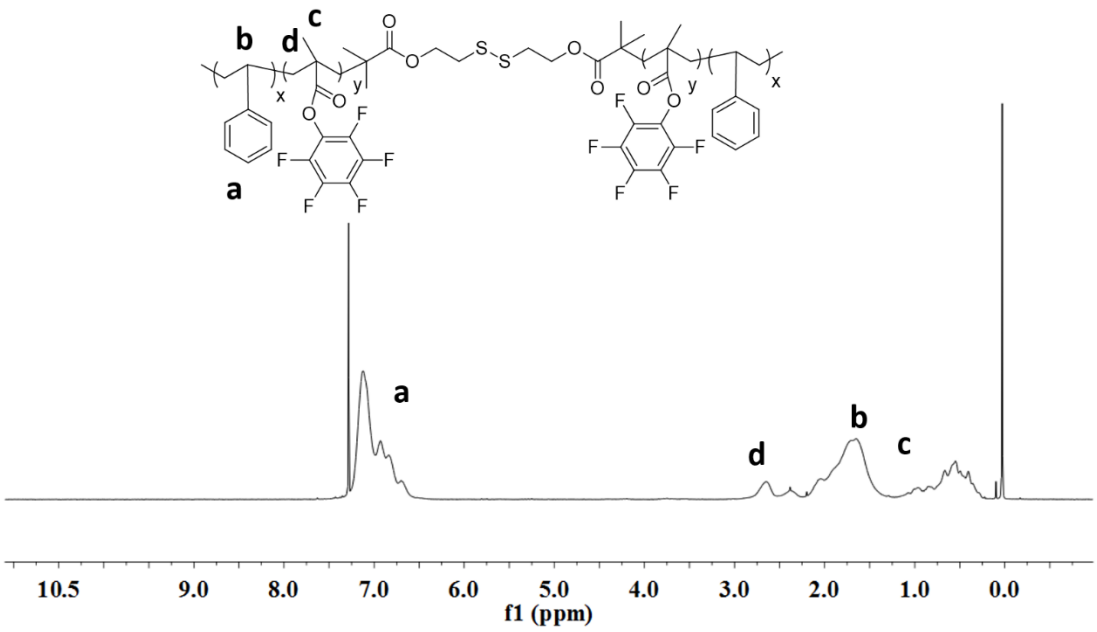


Figure S10. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of S-S-PS-PPFPMA.

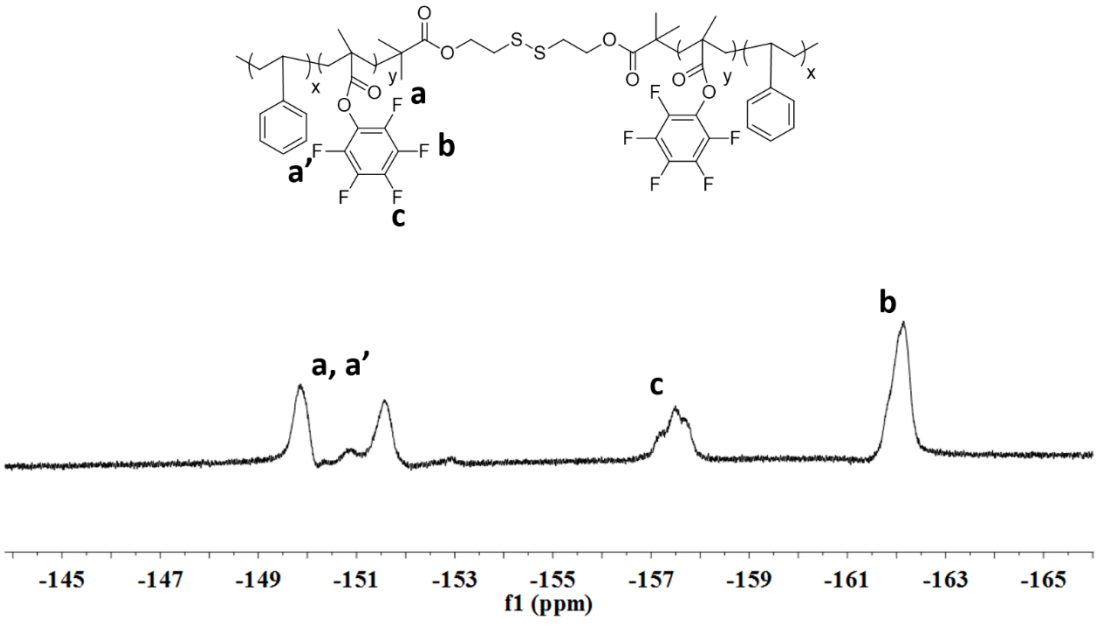


Figure S11. ^{19}F NMR spectrum (400 MHz, $CDCl_3$) of S-S-PS-PPFPMA.

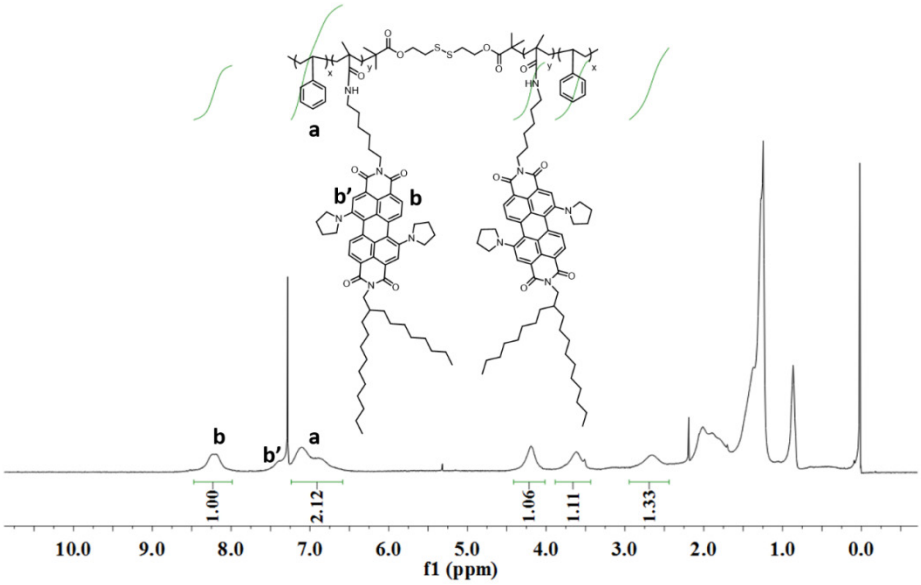


Figure S12. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of S-S-PS-PPDI.

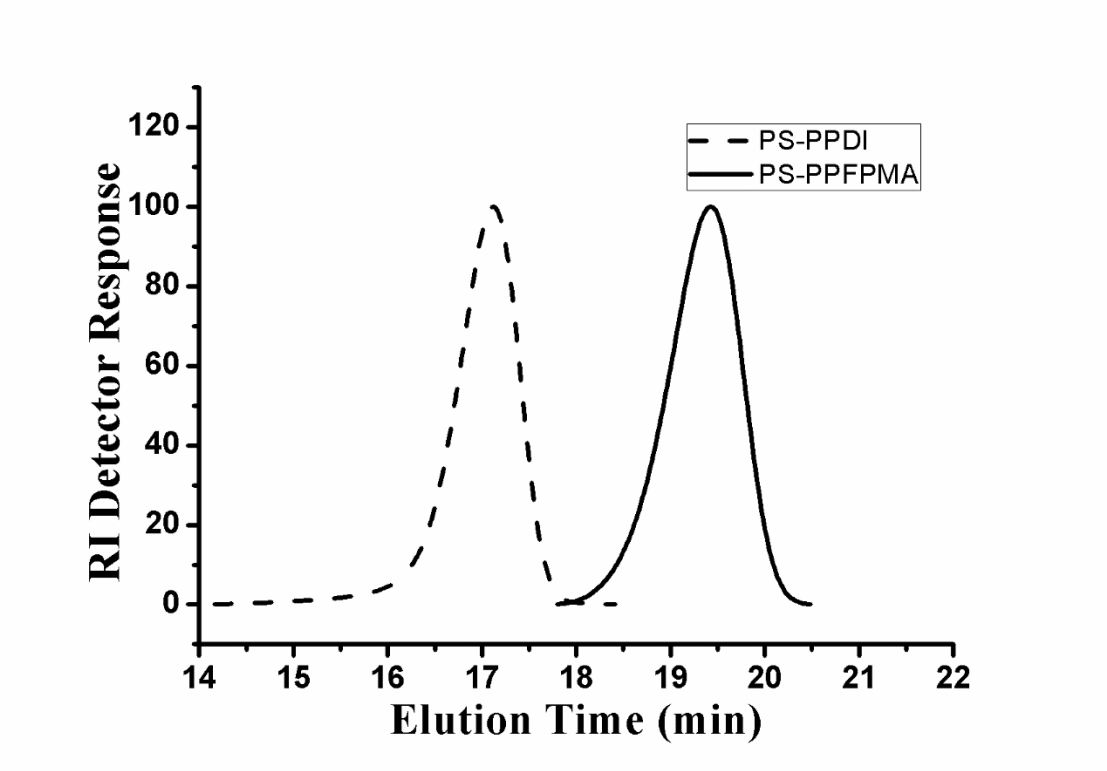


Figure S13. GPC curves of PS-PPDI and PS-PPFPMA.

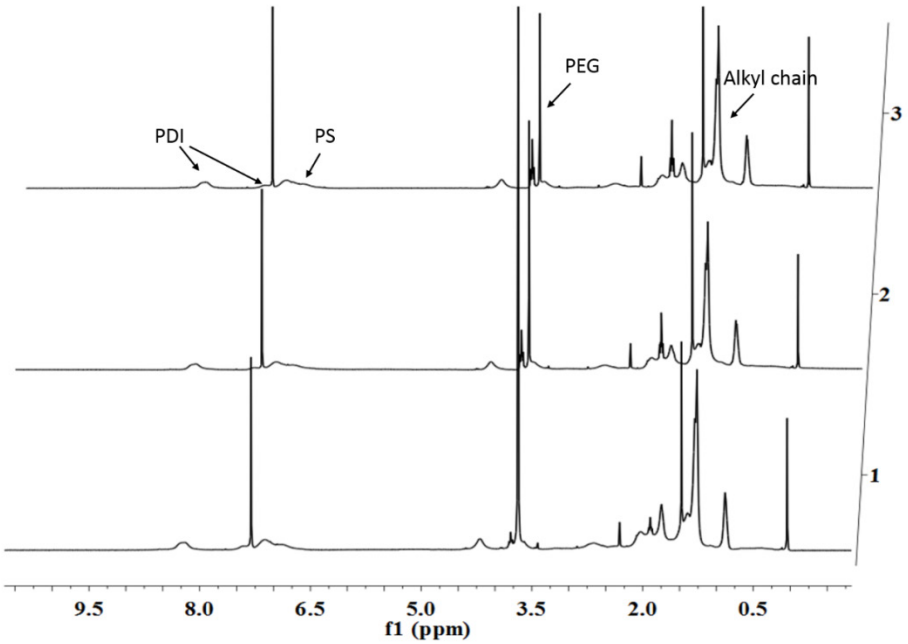


Figure S14. ^{1}H NMR spectra (400 MHz, $CDCl_3$) of Au@PPDI/PEG with different PPDI to PEG ratios: 1:1 (1); 2:1 (2); 3: 1 (3).

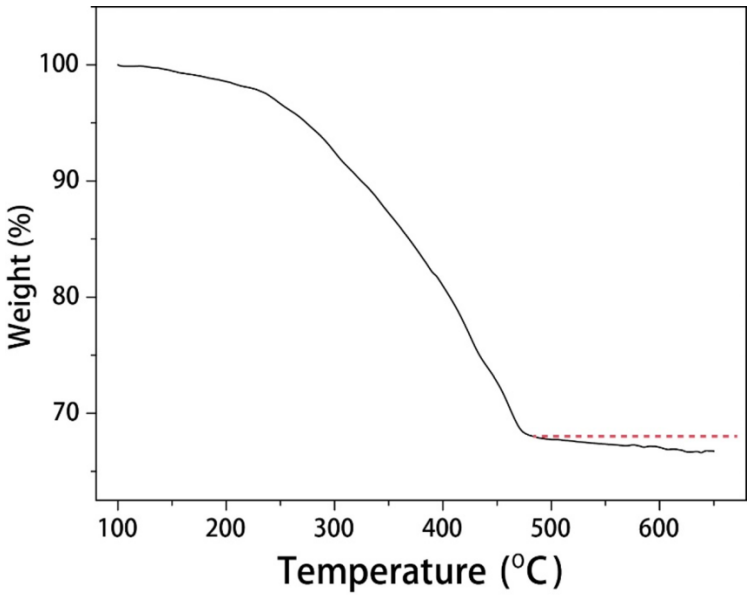


Figure S15. TGA analysis of the Au@PPDI/PEG vesicles.

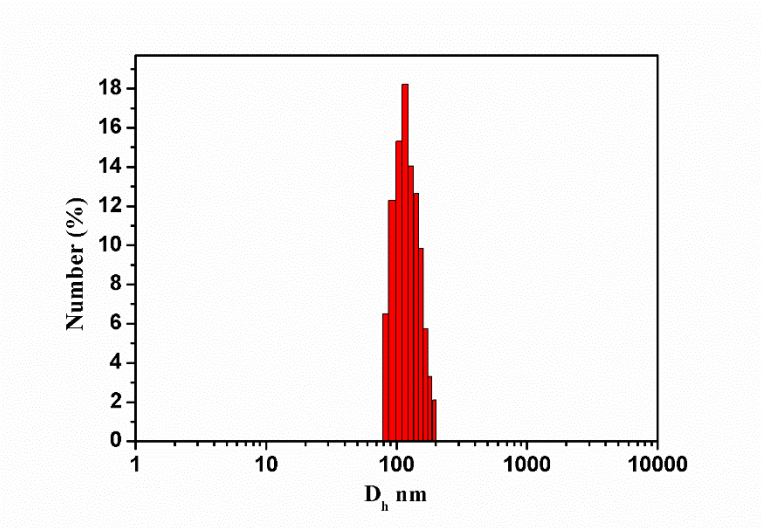


Figure S16. Hydrodynamic diameter distribution of the Au@PPDI/PEG vesicles.

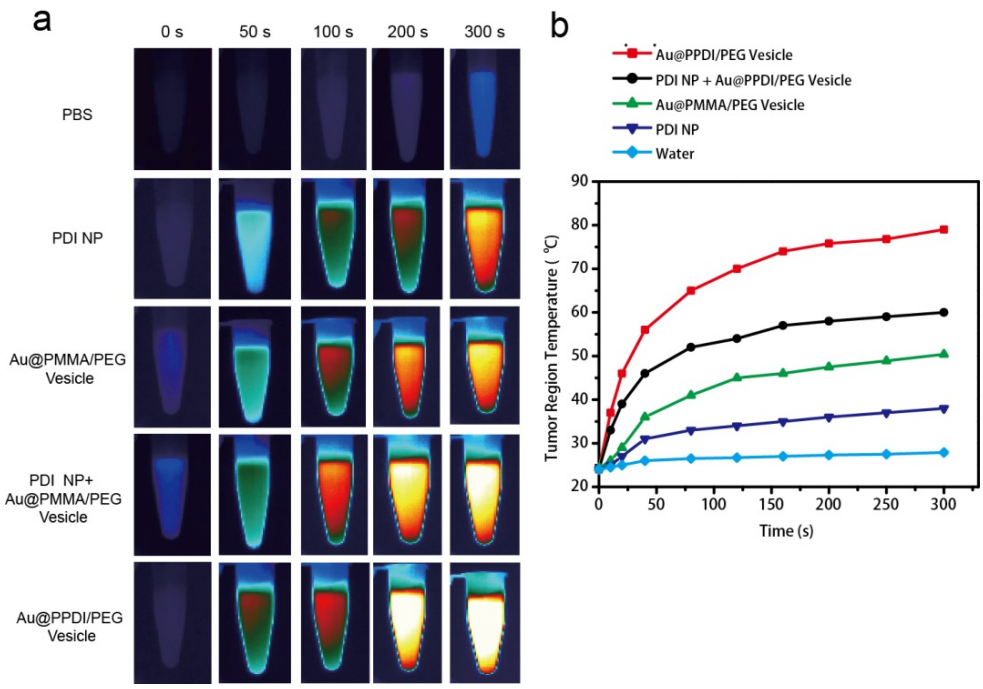


Figure S17. Thermographic images (a) and temperature changes of the samples irradiated with NIR laser at a power density of 0.3 W/cm^2.

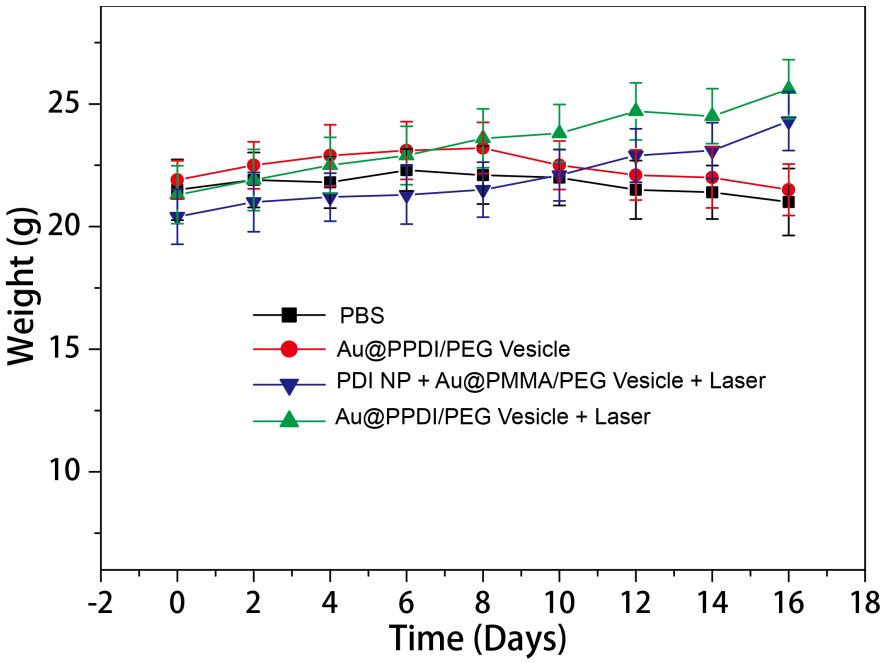


Figure S18. The body weight changes of U87MG tumor bearing mice as a function of time after different treatments