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Water-Soluble Polymer Brush-Substituted Squaraine NIR-II Dye for Efficient Photothermal Therapy

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1. Experiments

1.1 Materials

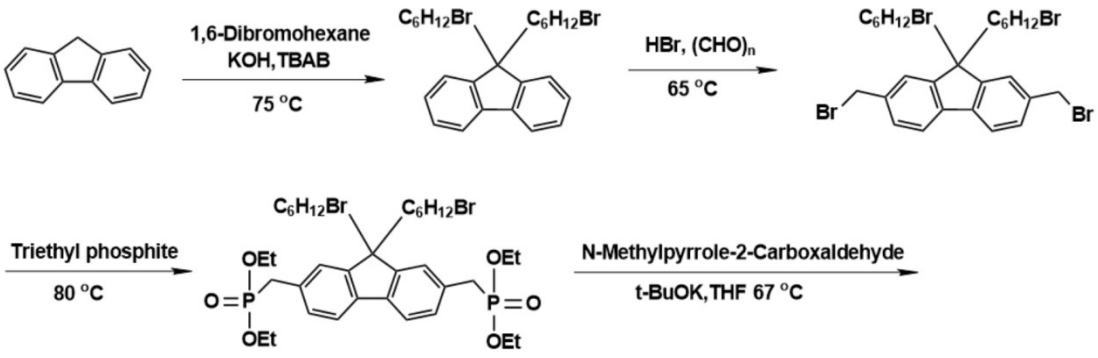
All reagents were obtained from commercial sources and used as received without further purification. The chicken used for NIR II depth imaging was purchased from a fresh supermarket around the school. MCF-7 cells were provided by the Shanghai Laboratory Animal Center, Chinese Academy of Science (SLACCAS). The Annexin V-FITC/propidium iodide (PI) cell apoptosis kit was obtained from KeyGen Biotech. Co., Ltd. (Nanjing, China). Dulbecco's modified Eagle's medium (DMEM, Gibco, U.S.) was obtained from Gene Tech Co. (Shanghai, China). All small animal experiments were carried out in accordance with the specifications of The National Regulation of China for Care and Use of Laboratory Animals, which have been approved by the Jiangsu Administration. Tumor-bearing mice (age5-6 weeks) were purchased from KeyGEN BioTECH. Co., Ltd (Nanjing, China) with pathogen-free feeding environment.

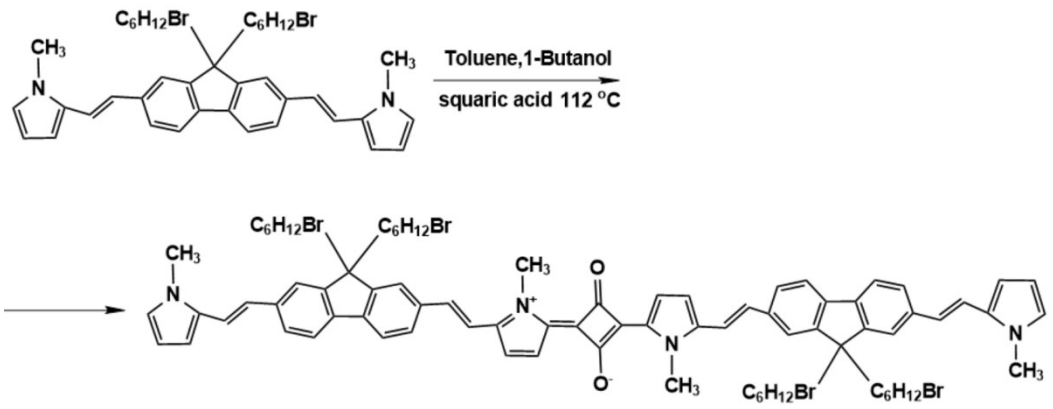
1.2 Instruments.

The ^{1}H NMR (400 MHz) spectra were measured by a Bruker Ultra Shield Plus 400 MHz spectrometer. Dynamic light scattering (DLS) analysis was performed with a commercial light scattering spectrometer (ALV/CGS-3; ALV, Langen, Germany). The morphology of the nanoparticles was investigated using an HT7700 transmission electron microscope. The UV–vis-NIR absorption spectra were recorded on a Shimadzu UV-3600 spectrophotometer. The fluorescence spectra were monitored by NIR-II spectroscopy (Fluorolog 3 Horiba) with an InGaAs NIR detector under 808 nm diode laser excitation at room temperature. The 808 nm laser was purchased from Changchun New Industries Optoelectronics Technology Co., Ltd.

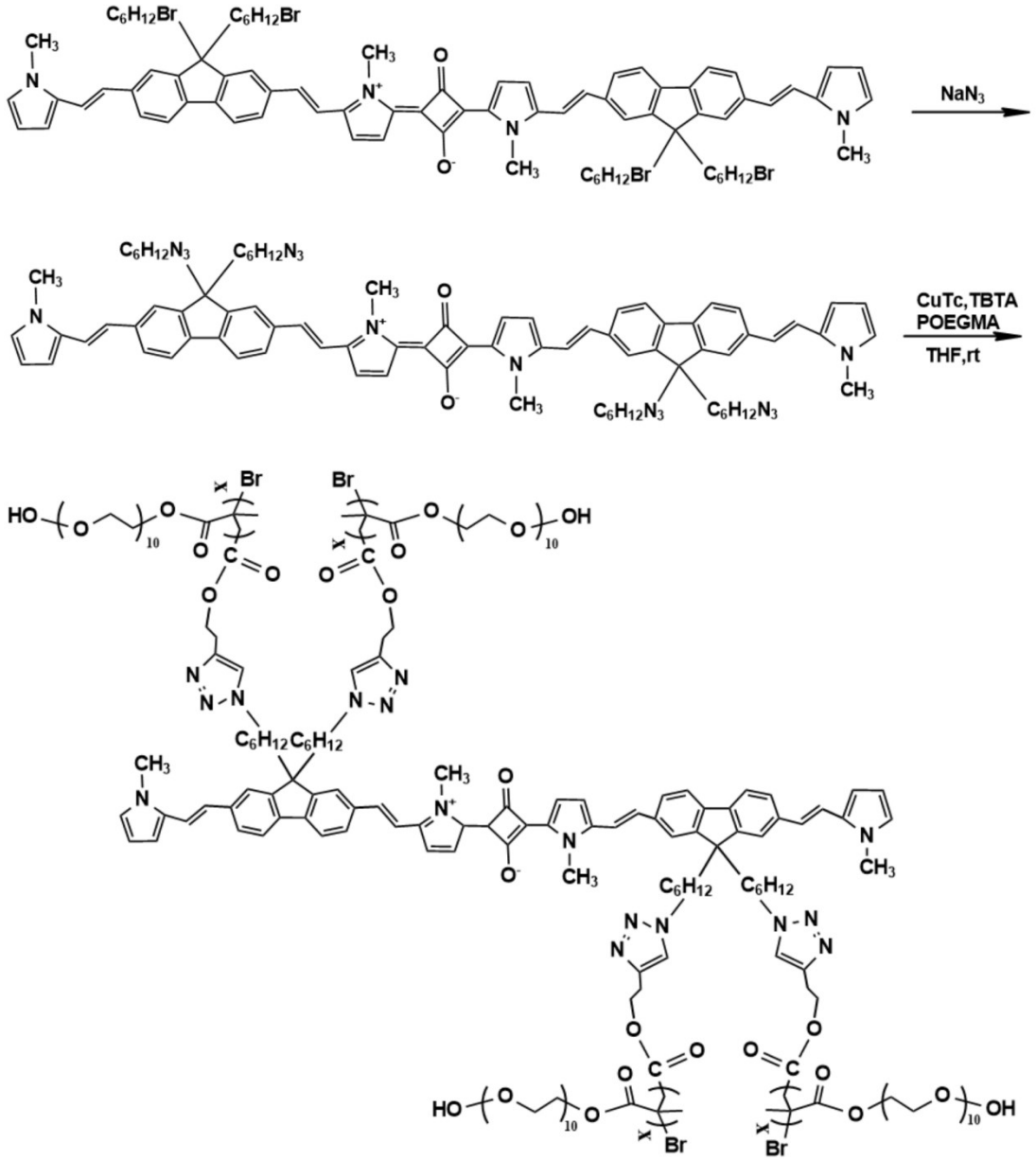
1.3 Synthesis of SQ and related intermediates

The synthetic routes toward related intermediates are shown in Scheme S1. The detailed procedures are described below:





Scheme S1 Synthetic routes to SQ.



Scheme S2 Synthetic routes to SQ-POEGMA.

(1) Synthesis of 1

Under nitrogen, fluorene (6.64 g, 20 mmol) and tetrabutylammonium bromide (1.6 g) were dissolved in 60 mL bromododecane, and then an aqueous solution of potassium hydroxide (50 wt%) was added. The mixed solution was stirred at ambient temperature for 30 minutes and then reacted at 75°C for 24 hours. After quenching the reaction mixture by the addition of distilled water, the crude compound was extracted with dichloromethane and washed with brine. The combined organic layers were dried over $MgSO_4$ and concentrated in a rotary evaporator. The crude compound was purified by column chromatography using hexane as the eluent to give the pure compound in a yield of 6.2 g (63%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.70 (d, J = 7.7 Hz 2H), 7.36 (m, 6H), 3.30 (t, *J* = 6.8 Hz, 4H), 2.00 (m, 4H), 1.67 (dt, *J* = 14.4, 6.9 Hz, 4H), 1.21 (p, *J* = 7.3 Hz, 4H), 1.09 (p, *J* = 7.3 Hz, 4H), 0.70 (m, 4H).

(2) Synthesis of 2

A mixture of compound 1 (2.94 g, 6 mmol), paraformaldehyde (1.8 g, 60 mmol), and 30% hydrobromidic acid in acetic acid (15 mL) was stirred at 65°C for 24 h. After the reaction mixture was cooled to room temperature, it was slowly poured into saturated sodium bicarbonate solution (100 mL). The mixture was extracted three times with dichloromethane (100 mL) and washed sequentially with water and saturated sodium bicarbonate solution and brine. The combined organic extracts were dried over anhydrous magnesium sulfate and filtered. After removal of the solvent, viscous liquid 2 was obtained by column chromatography with a yield of 65%. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.67 (d, J = 7.7 Hz, 2H), 7.42-7.32 (m, 4H), 4.63 (s, 4H), 3.30 (t, J = 6.8 Hz, 4H), 2.02-1.96 (m, 4H), 1.67 (dt, J = 14.4, 6.9 Hz, 4H), 1.22 (m, J = 14.8, 7.1 Hz, 4H), 1.09 (m, J = 14.8, 7.4 Hz, 4H), 0.68-0.58 (m, 4H).

(3) Synthesis of 3

The synthesis of compound 3 was prepared by the reaction of 2 (6.78 g, 10 mmol) with 5.0 mL of triethyl phosphite at 80°C for 10 h followed by the removal of the unreacted triethyl phosphite under reduced pressure. After cooling, the unreacted triethylphosphite was removed under vacuum, and the residue was passed through a short silica gel column using a mixture of hexane and acetone (v:v 10:1) to afford 7.32 g (yield 60%) of compound 3 as a viscous oil. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.58 (d, J=8.0 Hz, 2H), 7.23 (d, J = 5.6 Hz, 4H), 3.97 (dt, J = 15.5, 7.5 Hz, 8H), 3.27-3.17 (m, 8H), 1.97-1.88 (m, 4H), 1.61 (p, J = 6.9 Hz, 4H), 1.21 (t, J = 7.1 Hz, 12H), 1.13 (m, J = 7.6 Hz, 4H), 1.03 (m, J = 7.2 Hz, 4H), 0.56 (m, J = 7.9 Hz, 4H).

(4) Synthesis of 4

A solution of t-BuOK (1.68 g, 15 mmol) in anhydrous THF (10 mL) was added dropwise at ambient temperature under nitrogen to a mixture of 3 (3.96 g, 5 mmol) and the corresponding N-alkylpyrrole-2-carboxaldehyde (1.79 g, 10 mmol) in 20 ml THF. After the sample was refluxed for 12 h, the reaction mixture was cooled, and THF was removed under reduced pressure. The residue was neutralized with 5% hydrochloric acid and extracted with chloroform. The extraction was washed with water, saturated $NaHCO_3$, and brine and then dried over anhydrous magnesium sulfate. Evaporation of solvent afforded the crude product; it was purified by column chromatography with a mixture of hexane and ethyl acetate (3:1) as eluent, yield: 65%. 1H NMR (400 MHz, Chloroform-d) δ 7.67 (d, J = 7.7 Hz, 2H), 7.46-7.35 (m, 4H), 6.65 (s, 2H), 6.61 (s, 2H), 6.17 (m, 2H), 3.64 (s, 6H), 3.37 (m, 4H), 2.02-0.65 (m, 24H).

(5) Synthesis of SQ, 5

The synthesis of compound 4 (0.197 g, 0.3 mmol) and squaric acid (0.0171 g, 0.15 mmol) in n-butanol/methylbenzene (1:3, 50 mL) was refluxed at 112°C. The reaction mixture absorption spectra were frequently monitored, and the reaction was stopped when the absorption of higher molecular weight homologs (750 nm) started to appear. After the dark green reaction mixture was cooled, the n-butanol and methylbenzene were removed under reduced pressure to give a viscous solution. To remove the unreacted 4, the resultant dark green viscous solution was dissolved in dichloromethane, and the product was precipitated by adding light petroleum ether and washing with diethyl ether. After three reprecipitations and washing with petroleum ether and diethyl ether, the product was obtained at a 13% yield. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.87 (d, 2H), 7.69 (m, 4H), 7.59 (d, 2H), 7.49 (m, 4H), 7.15-6.96 (m, 6H), 6.69 (s, 2H), 6.56 (s, 2H), 6.20 (m, 2H), 4.39 (s, 6H), 3.78 (s, 6H), 3.30 (s, 8H), 2.06-0.68 (m, 24H).

1.4 Preparation of SQ NP and SQ-POEGMA

(1) Preparation of SQ NP

We dissolved SQ in tetrahydrofuran (THF) at a concentration of 0.5 mg mL^{-1} and dissolved F-127 in water at a concentration of 3.0 mg mL^{-1}. The ratio of SQ to F-127 was 1:15. Subsequently, the SQ organic solution was rapidly poured into the F-127 aqueous solution with sonication. The THF was then removed by complete evaporation in an airing chamber with stirring at 40°C. Finally, the green SQ NP aqueous solution was obtained. The tubular ultrafiltration modules were used to remove the excess F-127 aqueous solution until the SQ-NP aqueous solution was enriched to a concentration of 2.0 mg mL^{-1}.

(2) Preparation of SQ-POEGMA

Synthesis of SQ-N3, 6

Compound 5 (98 mg, 0.066 mmol) and sodium azide (50 mg, 0.75 mmol) were dissolved in DMF (10 mL) and heated for 12 h at 45°C. After that, a large amount of water was added, and then it was extracted twice with ethyl acetate. The combined organic phase was dried with $MgSO_4$ and evaporated in vacuo. The crude product was subjected to flash column chromatography on silica gel to afford a dark green solid (90 mg). ^{1}H NMR (500 MHz, Chloroform-d) δ 7.84 (d, 2H), 7.65 (m, 4H), 7.53 (d, 2H), 7.45 (m, 4H), 7.11-6.94 (m, 6H), 6.66 (s, 2H), 6.53 (s, 2H), 6.18 (s, 2H), 4.35 (s, 6H), 3.74 (s, 6H), 3.12 (m, 12H), 2.04-0.66 (s, 20H).

Synthesis of SQ-POEGMA,7

The dark green solid was dissolved in THF (5 mL), and w-alkynyl-PEG-hydroxyl PEG5000 (Mn=5000) (620 mg), tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) (3 mg), and copper (I) thiophene-2-carboxylate (CuTc) (6 mg) were added. The system was stirred at RT for 0.5 h and then filtered with diatomite. The solution was evaporated in vacuo.

2. Figures and discussion.

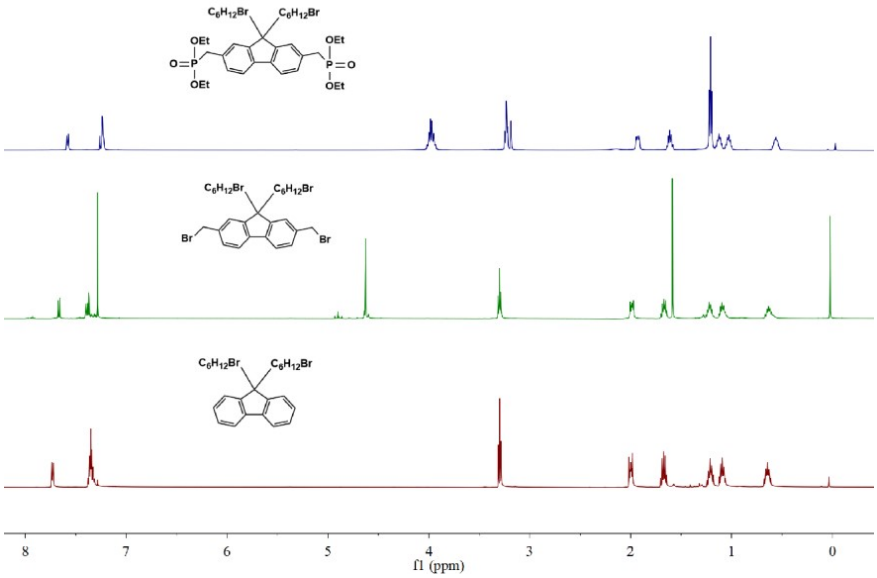


Fig. S1. ^{1}H-NMR spectra of M1-M3 in $CDCl_3$.

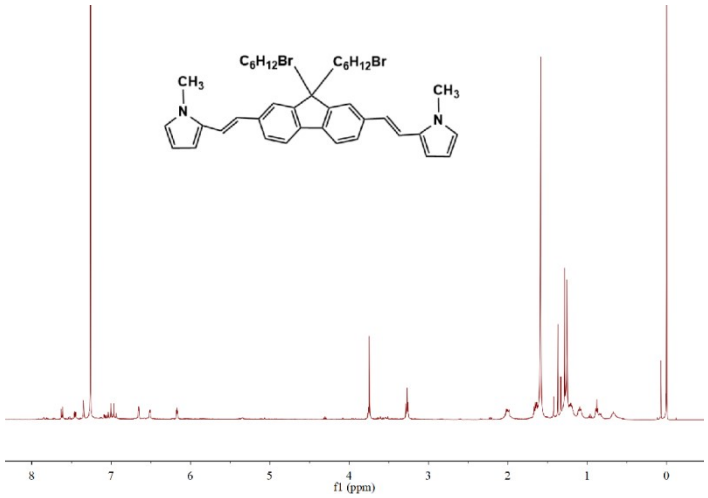


Fig. S2. ^{1}H-NMR spectra of M4 in $CDCl_3$.

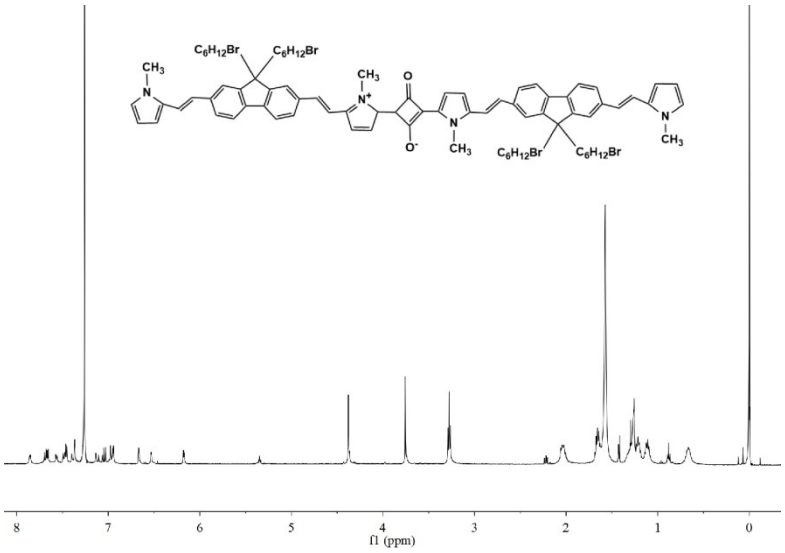


Fig. S3. ^{1}H-NMR spectra of SQ in $CDCl_3$.

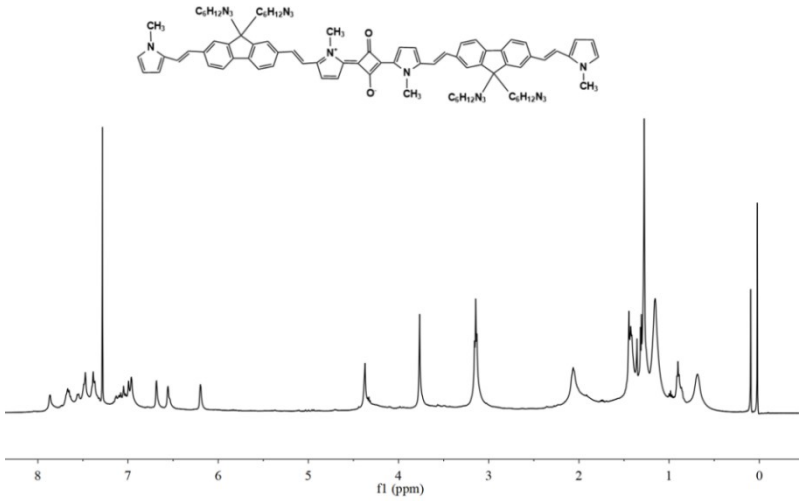


Fig. S4. ^{1}H-NMR spectra of SQ-N_3 in $CDCl_3$.

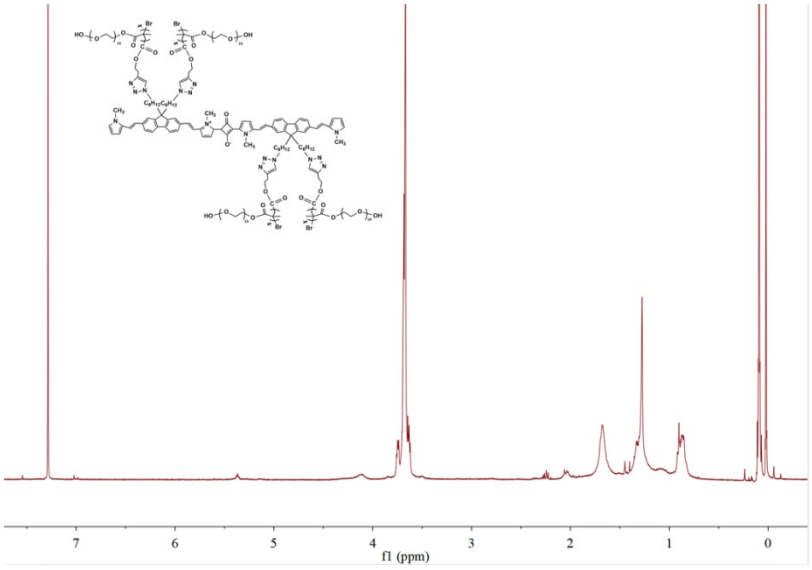


Fig. S5. ^{1}H-NMR spectra of SQ-POEGMA in $CDCl_3$.

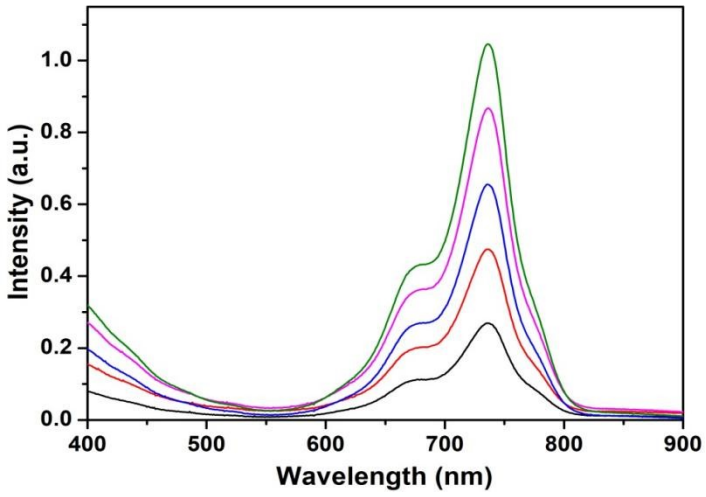


Fig. S6 Absorption spectra of SQ in THF

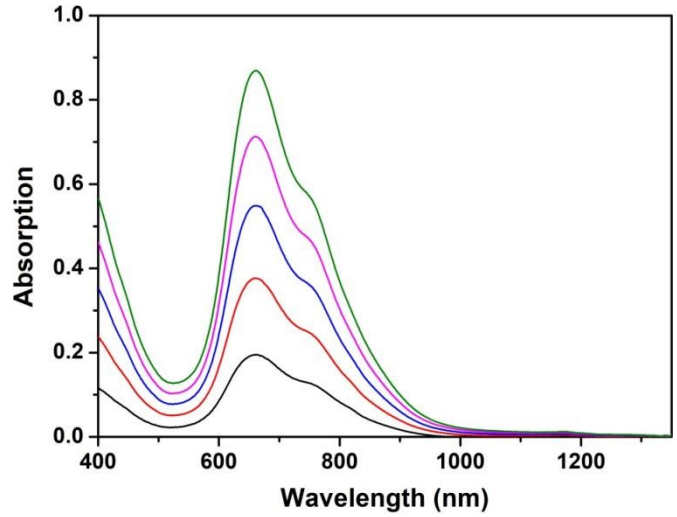


Fig. S7 Absorption spectra of SQ-NP in water.

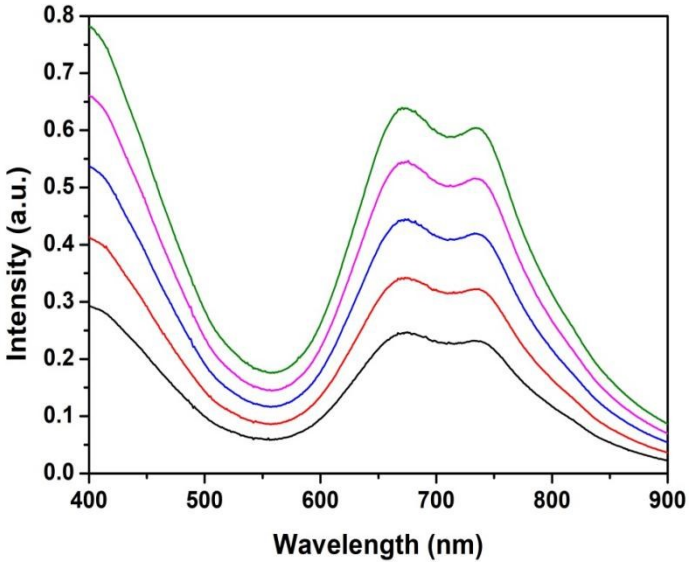


Fig. S8 Absorption spectra of SQ-POEGMA in aqueous solution.

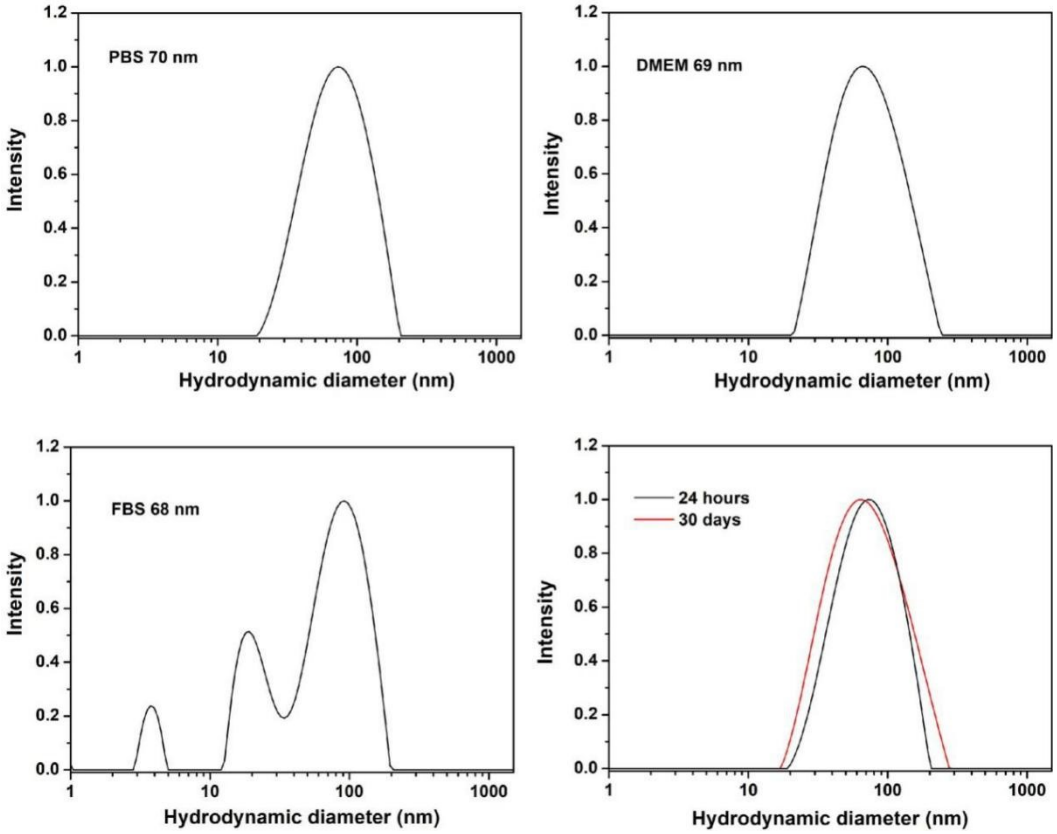


Fig. S9 DLS data of the SQ-NP in water, DMEM, FBS and PBS.

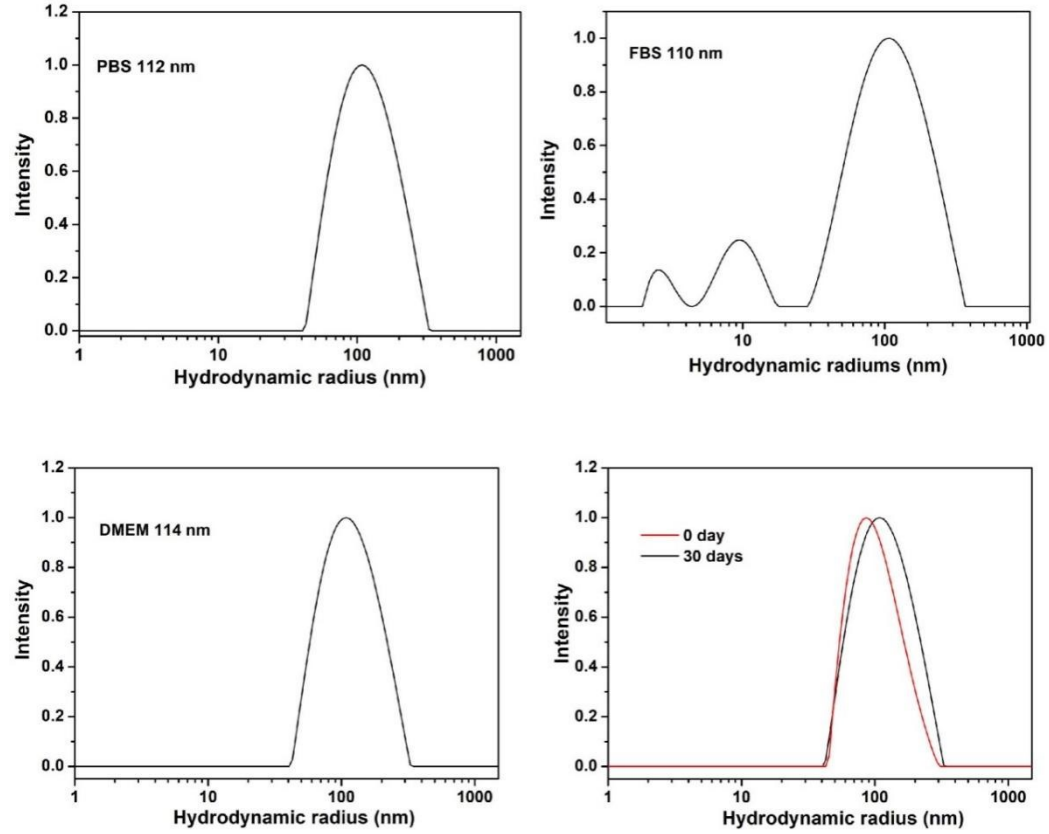


Fig. S10 DLS data of the SQ-POEGMA in water, DMEM, FBS and PBS

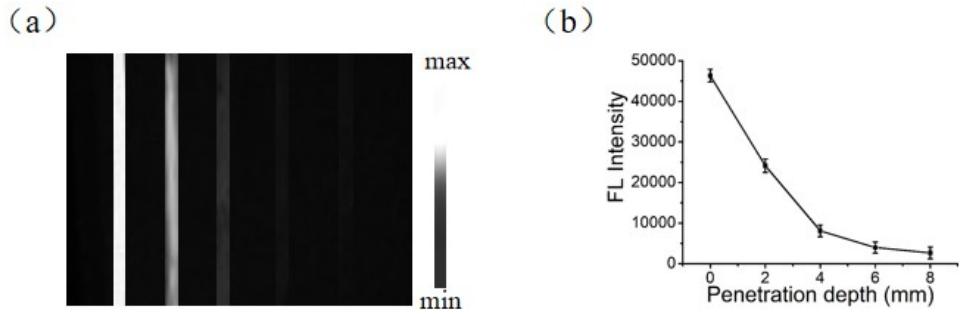


Fig. S11 (a) NIR-II fluorescence imaging images of SQ-POEGMA aqueous solution in chicken breasts of different thicknesses; (b) NIR-II fluorescence intensity of SQ-POEGMA aqueous solution at different tissue depths

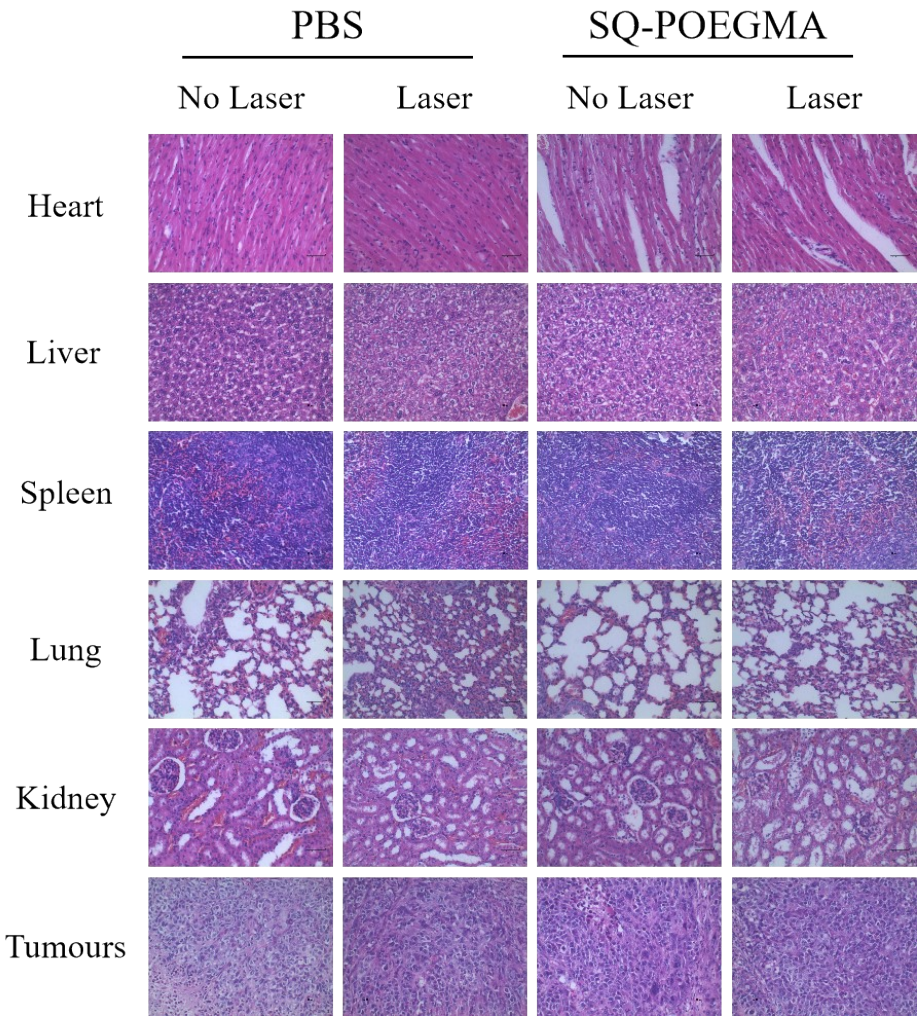


Fig. S12 HE-stained sections of different organs and tumors in MCF-7 tumor mice with and without treatment with laser irradiation (808 nm, 1.5 W/cm^2).