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Supplementary Information for

Near-infrared Absorbing Amphiphilic Semiconducting Polymers for Photoacoustic Imaging

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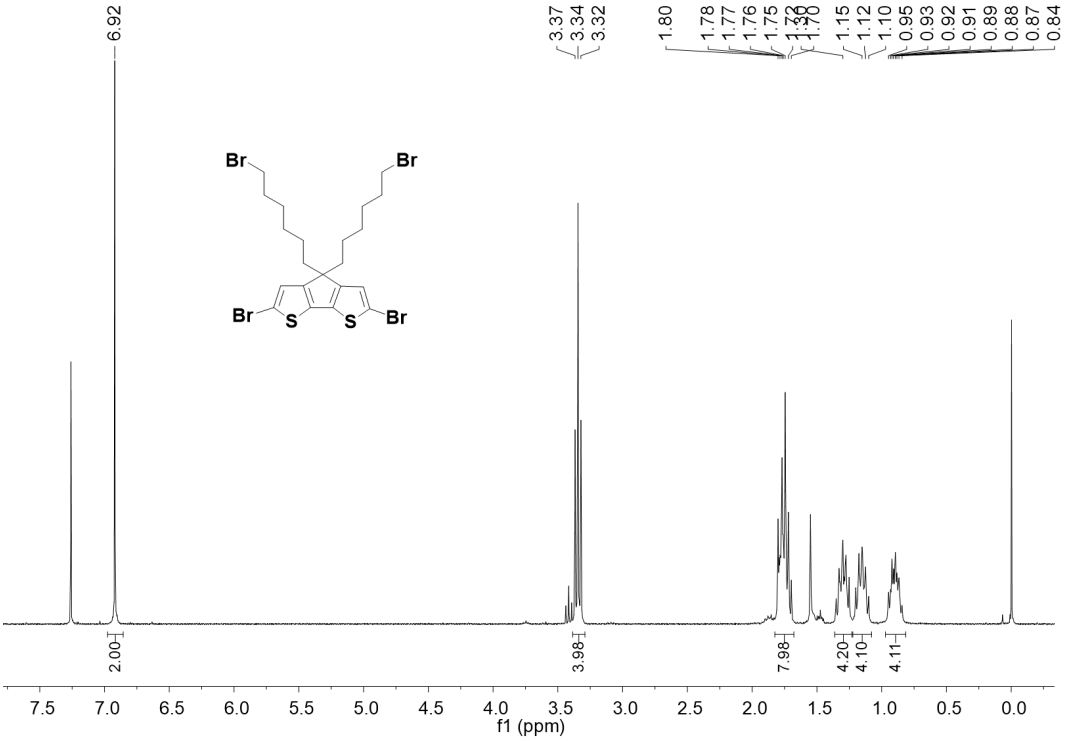

Figure S1. ^{1}H NMR spectra of monomer 1 in $CDCl_3$.

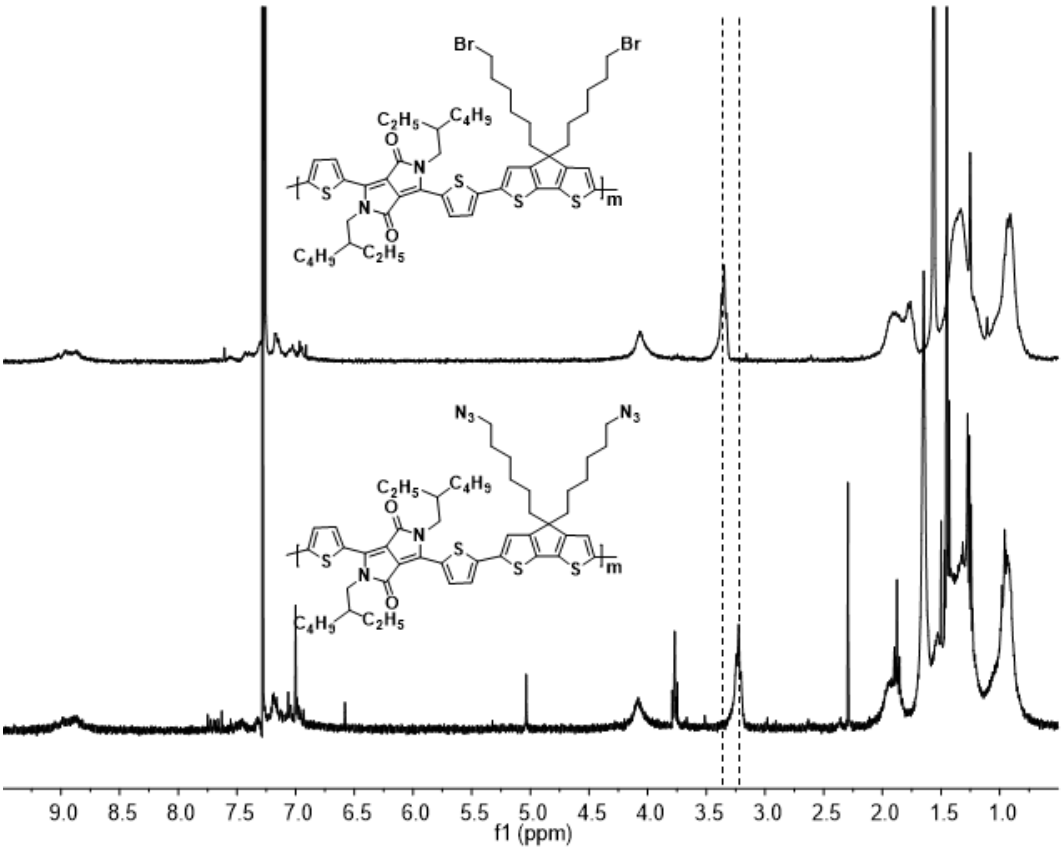

Figure S2. ^{1}H NMR spectra of PCD-Br (top) and PCD-N_3 (bottom) in $CDCl_3$.

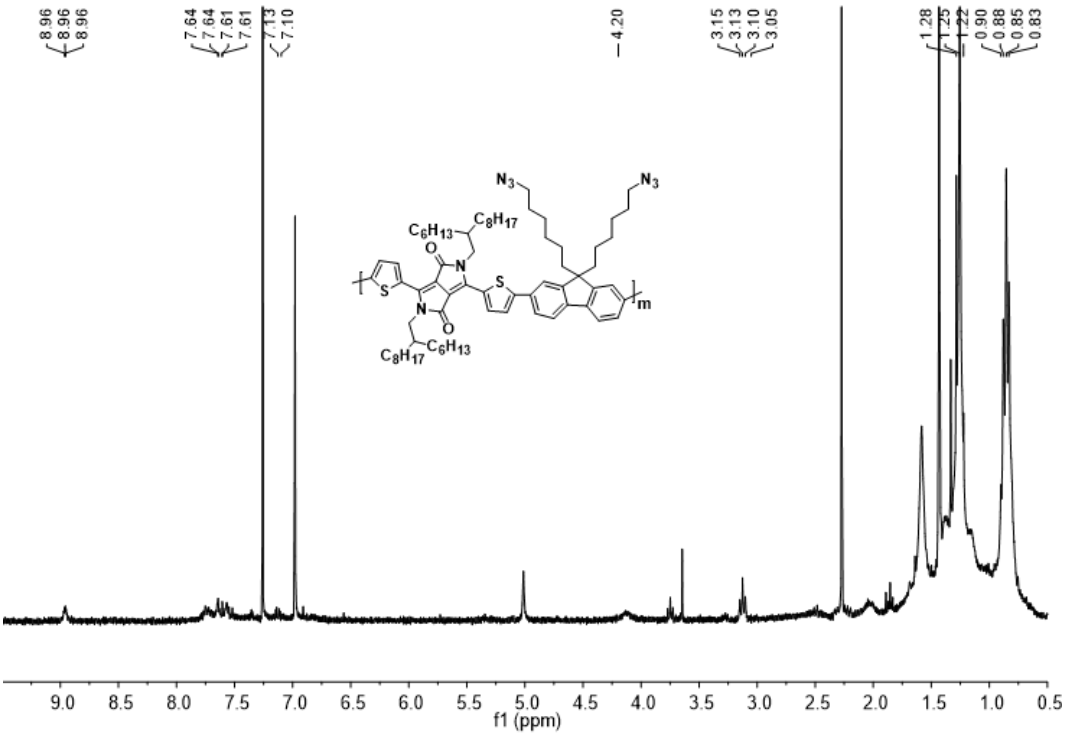

Figure S3. ^{1}H NMR spectra of PFD-N_3 in $CDCl_3$.

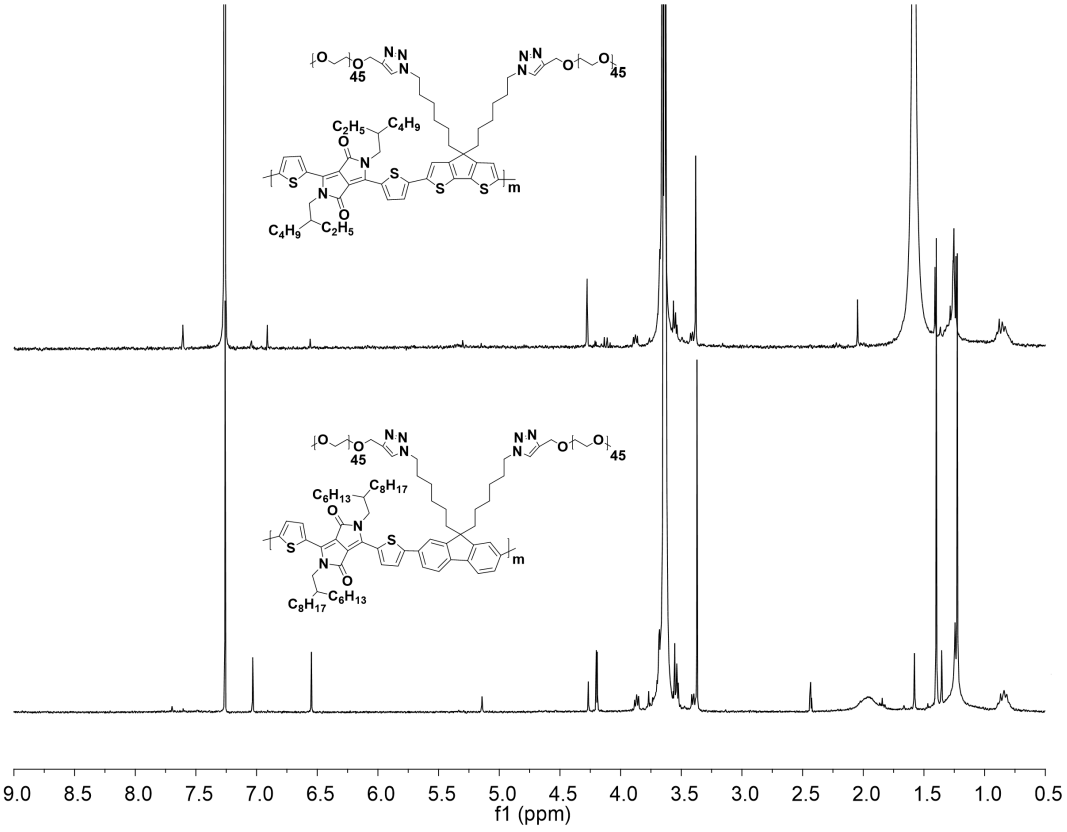

Figure S4. ^{1}H NMR spectra of PCD-PEG (top) and PFD-PEG(bottom) in $CDCl_3$.

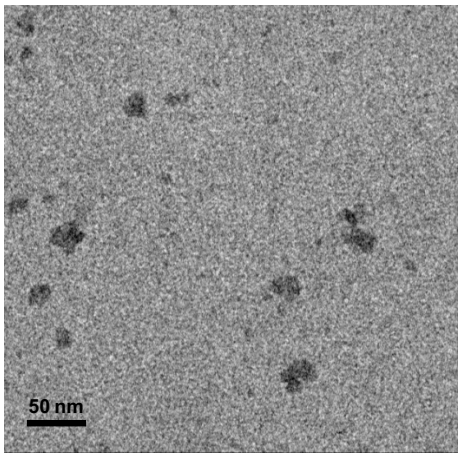

Figure S5. Representative TEM image of PFD-PEG.

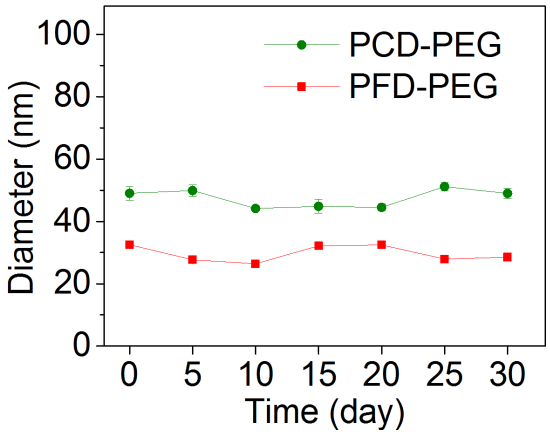

Figure S6. DLS data of PCD-PEG and PFD-PEG as a function of time incubated with PBS (pH = 7.4). Error bars represent the standard deviations of three separate measurements (n = 3).

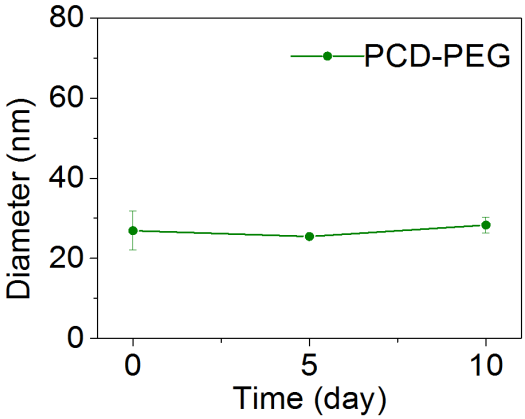

Figure S7. DLS data of PCD-PEG as a function of time incubated with FBS (pH = 7.4). Error bars represent the standard deviations of three separate measurements (n = 3).

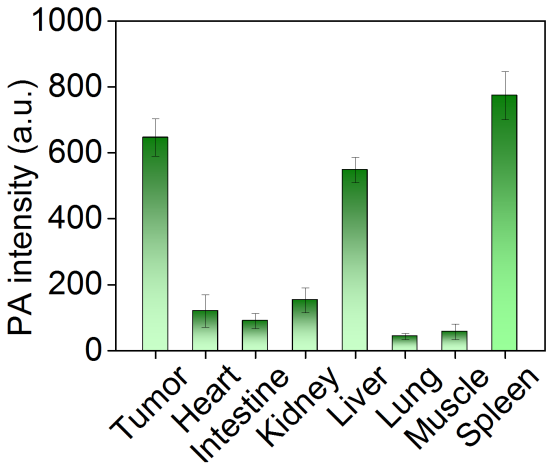

Figure S8. *Ex vivo* quantification of PA of major organs from mice (n = 3) 32 h after systemic administration of PCD-PEG.

Experimental sections

Chemicals

All chemicals were purchased from Sigma-Aldrich unless otherwise mentioned. 2,5-Bis(2-ethylhexyl)-3,6-bis(5-(trimethyl-stannyl)-thiophen-2-yl)pyrrolo[3,4-c]pyrrole-1,4(2H,5 H)-dione and 3,6-bis(5-bromothiophen-2-yl)-2,5-bis(2-hexyldecyl)pyrrolo[3,4-c] pyrrole-1,4(2H,5H)-dione were purchased from Luminescence Technology Corp. Poly(ethylene glycol) methyl ether alkyne (methoxy-PEG-alkyne, M_n = 2000) were purchased from J&K Scientific Ltd. 2,7-Bis[9,9(-bis(6-bromohexyl)-fluorenyl)-4,4,5,5-tetramethyl-[1.3.2] dioxaborolane was synthesized according to the previous literature.[1]

Characterization

DLS was performed on a Malvern Nano-ZS Particle Sizer. TEM images were captured from a JEM 1400 transmission electron microscope with an accelerating voltage from 40 to 120 kV. ^{1}H NMR spectra were recorded using a Bruker Advance II 300MHz NMR, $CDCl_3$ was used as the solvent. GPC results were obtained by Shimadzu LC-VP system with polystyrenes as the standard

and high purity of THF as the eluent. UV-vis spectra were obtained from a Shimadzu UV-2450 spectrophotometer.

Synthesis of 2,6-dibromo-4,4-bis(6-bromohexyl)-4H-cyclopenta[2,1-*b*:3,4-*b*']dithiophene

2,6-Dibromo-4H-cyclopenta-[2,1-b:3,4-b']dithiophene (100 mg, 0.30 mmol), freshly prepared 50% aq. NaOH (6.4 mL), and phase transfer catalyst tetrabutylammoniumiodide (20 mg) were added to a 50 ml round bottom flask. The flask was degassed by applying freeze–thaw cycles to remove air, followed by 1.6-Dibromohexane (0.3 mL, 0.75 mmol) addition via syringe (degassed) and the mixture was carried out at 75 °C continuously for 45 min. The reaction mixture was cooled to room temperature and extracted with ethyl acetate for one times. The organic layer was washed with water and dried over anhydrous sodium sulfate. The solvent was removed under vacuum, and the crude was purified via column chromatography over silica/hexane. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 6.92 (2 H, s), 3.34 (4 H, t, J = 6.8), 1.76 (8 H, dd, J = 18.8, 9.5, 4.6), 1.37 -1.23 (4 H, m), 1.22 -1.08 (4 H, m), 0.97 - 0.81 (4 H, m).

Synthesis of PCD-Br

2,6-dibromo-4,4-bis(6-bromohexyl)-4H-cyclopenta[2,1-b:3,4-b']dithiophene (60 mg, 0.082 mmol),

2,5-Bis(2-ethylhexyl)-3,6-bis(5-(trimethylstannyl)-thiophen-2-yl)pyrrolo[3,4-c]pyrrole-1,4(2H,5H)-dione (70.7 mg, 0.082 mmol), $PdCl_2(PPh_3)_2$ (7 mg, 0.011 mmol) and 2,6-di-tert-butylphenol (1.2 mg, 5.7 mmol) were added to a 50 ml Schlenk tube. The Schlenk tube was degassed with vacuum-argon cycles to remove air. Then, toluene (5 mL) was added to the tube, which was charged with argon through a freeze−pump−thaw cycle for three times. The polymerization reaction was carried out at 100 °C under vigorous stirring for 2 h. The resulting mixture was

poured into methanol and the dark-green precipitate was filtered. The polymer was purified by extraction using methanol, acetone, hexane and chloroform sequentially. The residue was collected and dried under vacuum. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.95 (2 H, s), 7.45 (2 H, d, J =92.8), 7.02 (4 H, dd, J= 48.7, 30.8), 4.07 (4 H, s), 3.36 (4 H, d, J 6.5), 1.82 (12 H, d, J =37.1), 1.29 (24 H, d, J =24.2), 0.92 (12 H, d, J =7.5).

Synthesis of PFD-Br

3,6-Bis(5-broMo-2-thienyl)-2,5-bis(2-hexyldecyl)pyrrolo[3,4-c]pyrrole-1,4(2H,5H)-dione (126 mg, 0.139 mmol), 2,7-Bis[9,9(-bis (6-bromohexyl)-fluorenyl)-4,4,5,5-tetramethyl-[1.3.2] dioxa borolane (100 mg, 0.139 mmol), $Pd(PPh_3)_4$ (5.5 mg, 0.052 mmol) and K_2CO_3 (244.98 mg, 1.39 mmol) were placed in a 50 mL Schlenk tube. Then a mixture of water (2.5 mL) and toluene (5 mL) with methyltrioctylammonium chloride (1 mg) were added to the reaction tube, and the reaction vessel was degassed by freeze-pump-thaw circles for three times. The mixture was vigorously stirred at 100 °C for 2 h and then the solvent was removed under reduced pressure. The obtained solid was re-dissolved by excess dichloromethane and washed with water for three times. The organic phase was concentrated and precipitated into excess methanol. The obtained solid was washed three times by methanol and then dried under vacuum for 24 h to afford the PFD-Br. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.96 (2 H, s), 7.62 (8 H, d, J =10.7), 4.38-3.97 (4 H, m), 3.29 (4 H, t, J= 7.1), 2.15-1.77 (6 H, m), 1.25 (64 H, t, J =9.3), 0.87 (12 H, dd, J =14.1, 6.5).

General Procedure for PCD-N_3 and PFD-N_3

PCD-Br or PFD-Br (10 mg) was dissolved into a mixture of THF (6 mL) and DMF (3 mL). Then sodium azide (2 equiv to bromide group of PCD-Br or PFD-Br) was added into the solution. The reaction was carried out at room temperature overnight. After that the solvent was removed under

reduced pressure, an excess of dichloromethane was added to the residue. The resulting solution was washed three times with water and the organic phase was collected. The obtained solution was then concentrated and precipitated into excess methanol to obtain solid which was washed with methanol for three times. The obtained solid was dried under vacuum overnight to obtain PCD-N_3 or PFD-N_3. PCD-N_3: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.91 (2 H, d, J =33.6), 7.79 -7.29 (2 H, m), 7.09 (2 H, d, J =43.3), 4.07 (4 H, s), 3.21 (4 H, d, J= 6.2), 1.78-1.46 (12 H, m), 1.28 (22 H, dd, J =28.7, 11.5), 1.11-0.68 (16 H, m). PFD-N_3: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.96 (2 H, s), 7.62 (8 H, d, J =10.7), 4.38 -3.97 (4 H, m), 3.13 (4 H, t, J= 7.1), 2.15-1.77 (6 H, m), 1.25 (64 H, t, J =9.3), 0.87 (12 H, dd, J =14.1, 6.5).

General Procedure for PCD-PEG and PFD-PEG

PCD-PEG and PFD-PEG polymers were prepared by using copper(I)-catalyzed alkyne-azide cycloaddition (CAAC) reaction. Briefly, PCD-N_3 or PFD-N_3 (3 mg) was dissolved into a mixture of THF (6 mL) solution. CuBr (2 equiv to azide group of PCD-N_3 or PFD-N_3), N,N,N',N'',N''-pentamethyldiethylenetriamine (PMDETA) (10 equiv to azide group of PCD-N_3 or PFD-N_3) and methoxy-PEG-alkyne (M_n = 2000, 2 equiv to azide group of PCD-N_3 or PFD-N_3) were added into previous solution subsequently. The reaction was carried out at room temperature under nitrogen atmosphere for 48 h. After that the solvents were removed under reduced pressure and the remaining residue was dissolved into water. The resulting solution was dialysis against DI water to remove the salt and excess methoxy-PEG-alkyne in the system. The PCD-PEG or PFD-PEG polymers were obtained after lyophilization. PCD-PEG: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.87-6.68, 4.27-4.12, 3.88, 3.65, 3.55, 3.38, 2.05, 1.45 -1.13, 0.87. PFD-PEG: ^{1}H NMR (300

MHz, $CDCl_3$, δ): 7.79-6.92, 4.35-4.10, 3.92-3.81, 3.74-3.58, 3.58-3.50, 3.43-3.35, 1.91, 1.63 -1.24, 0.86.

Cell Culture and Cytotoxicity Assay

The in vitro cytotoxicity was measured using [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) viability assay in human cervical carcinoma cell line HeLa. The 4T1 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum in a humidified environment containing 5% CO_2 and 95% air at 37 °C. 4T1 cells were seeded in 96-well plates (Costar, IL, U.S.A.) at an intensity of 3×10^4 cells/mL. After 24 h incubation, the medium was replaced by fresh medium containing PCD-PEG or PFD-PEG suspensions at different concentrations (10, 25, 50, 75, 100, 125 μg mL^{-1}) and the cells were then incubated for 24 h. After the designated time intervals, MTS reagent was added into cell culture medium in 1 to 10 volume ratios for cell incubation. 3 h later in an incubator, UV measurement (490 nm) was taken and normalized against untreated samples to the cell viability.

***In Vitro* Photothermal Studies.**

PBS (1×, pH 7.4) containing PCD-PEG or PFD-PEG (200 μL, 18 μg mL^{-1}) was exposed to laser irradiation at 808 nm (1 W cm-2). The temperature was monitored using a photothermal camera (FLIR T420) every 20 s until reaching maxima after approximately 6 min. After the laser exposure, the temperature was continuously monitored every 20 s for 6 min. The heating and cooling were repeated five times to test the photothermal stability of PCD-PEG or PFD-PEG.

***In Vitro* PA Instrumentation**

An optical parametric oscillator, OPO (Continuum, Surelite), pumped by a Q-switched 532 nm Nd:YAG laser was used as the excitation source. OPO can generate tunable laser pulses within 680-920 nm wavelength range with 5 ns pulse duration, 100 mJ $pulse^{-1}$ energy at 10 Hz repetition rate. The solution containing samples were placed inside a low-density polyethylene (LDPE) tube with an inner diameter (ID) of 0.59 mm and outer diameter (OD) of 0.78 mm. The sample containing LDPE tube, and the single-element ultrasound transducer, UST (V323-SU / 2.25 MHz, 13 mm active area, and 70% nominal bandwidth, Panametrics) were immersed in water medium for coupling of PA signals to UST. The LDPE tube was irradiated with wavelengths ranging from 680 - 920 nm with 10 nm increment. Respective PA signals were collected using the UST and these signals were subsequently amplified with a gain of 50 dB, and band pass filtered (1-10 MHz) by a pulser / receiver unit (Olympus-NDT, 5072PR). Finally, the output signals from the pulser/ receiver unit was digitized with a data acquisition card (GaGe, compuscope 4227) operated at 25 MHz and the acquired signals were stored in the computer. Peak-to-peak voltage of the PA signals was then normalized with the laser energy at each wavelength and were plotted against the wavelength to generate the PA spectrum.

Tumor Mouse Model

All animal experiments were performed in compliance with the Guidelines established by the Institutional Animal Care and Use Committee (IACUC), Sing Health. To establish tumor models in six-week-old female nu/nu mice, two million 4T1 cells suspended in 50 mL of 50% v/v mixture of Matrigel in supplemented DMEM (10% fetal bovine serum, 1% pen/strep (100 U ml^{-1} penicillin and 100 μg mL^{-1} streptomycin) were injected subcutaneously in the shoulders of the

mouse. Tumors were grown until a single aspect was ~8 mm (approximately 10-15 days) before used for in vivo imaging experiments.

***In Vivo* PA Imaging of Tumor**

4T1 tumor xenografted nude mice were anesthetized using 2% isoflurane in oxygen, and a catheter was applied to the tail vein. The mice were placed in the Endra Nexus128 PA imaging system, and were scanned to determine the endogenous signal of tumors at 790 nm before systemic administration with PCD-PEG (140 μg in 200 μL) (n=3) or saline (200 μL) (n=3) through catheter. Data was acquired through a continuous model that took 12 s to obtain one data set. At different time point (0 h, 8 h, 24 h and 32 h), the PA signal of mice was measured. For vivo PA imaging, mice were sacrificed by CO_2 asphyxiation, and organs were embedded in agar phantom and acquired immediately with Endra Nexus128 PA imaging system. Three-dimensional PA image was reconstructed off-line using data acquired from all 128 transducers at each view. The reconstructed raw data was analyzed using OSiriX software.

Reference

1 K. Pu, Z. Fang, B. Liu, *Adv. Funct. Mater.* 2008, **18**, 1321.