Supporting Information for:

Impact of Semiconducting Perylene Diimide Nanoparticle Size on Lymph Node Mapping and Cancer Imaging

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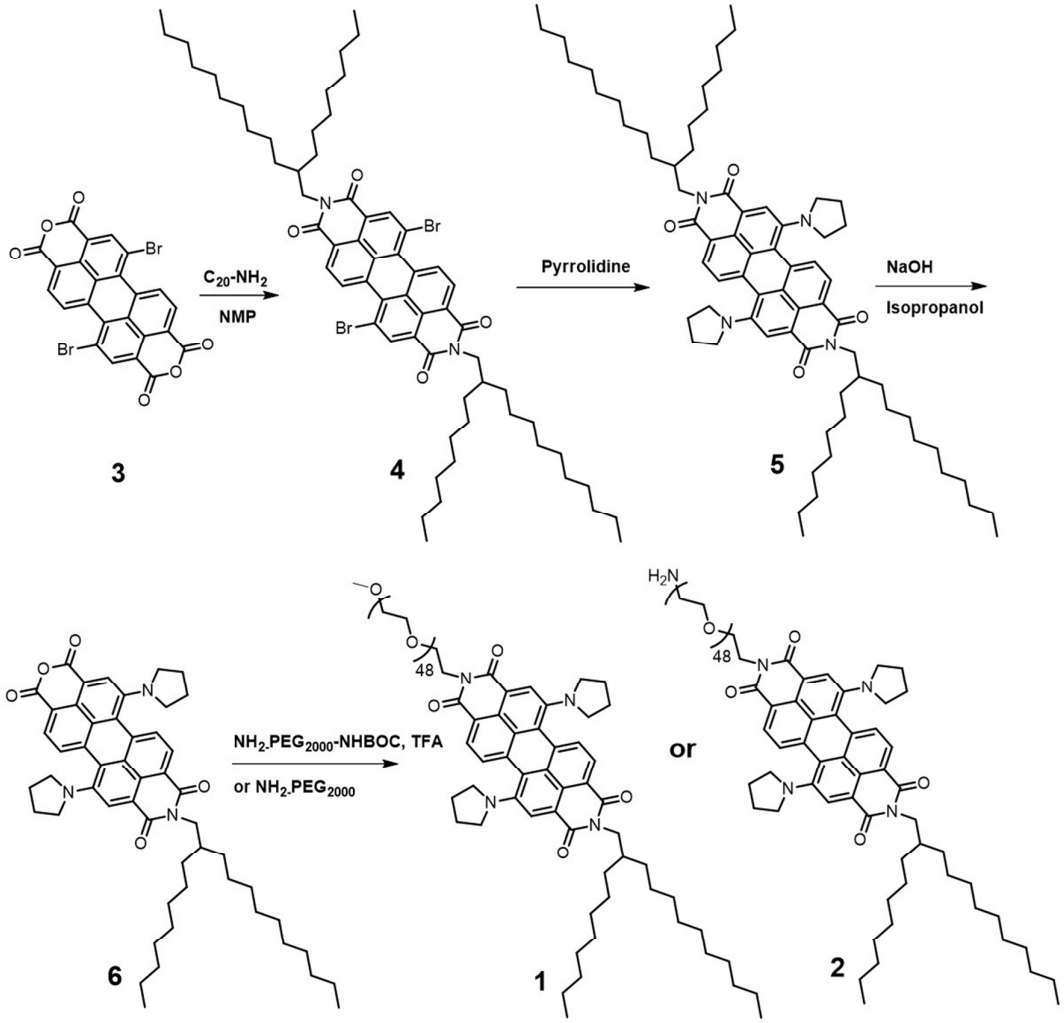
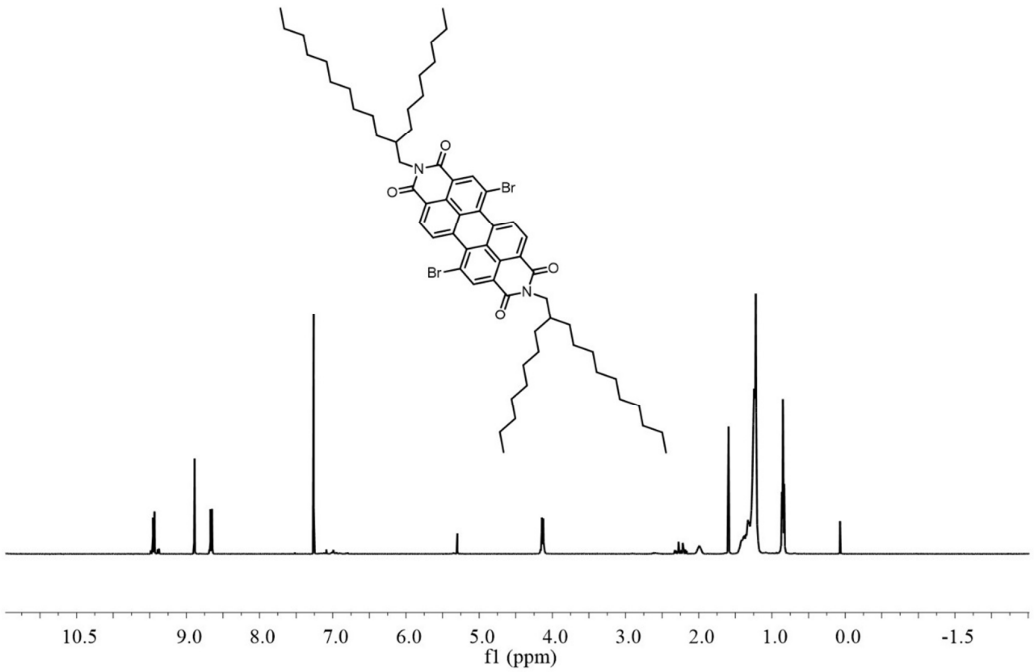
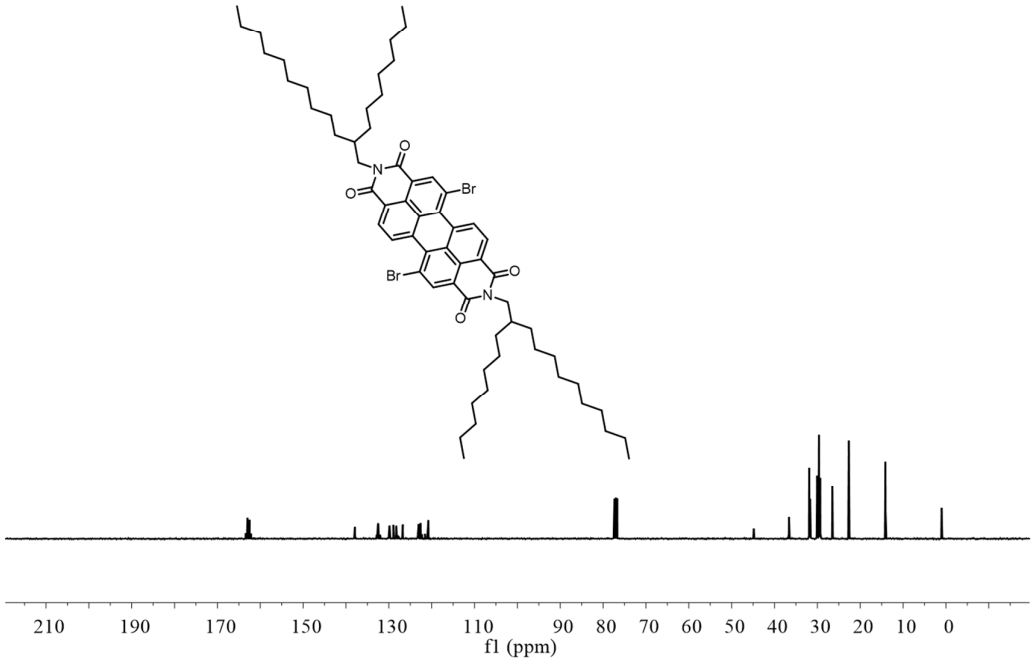
Contents:



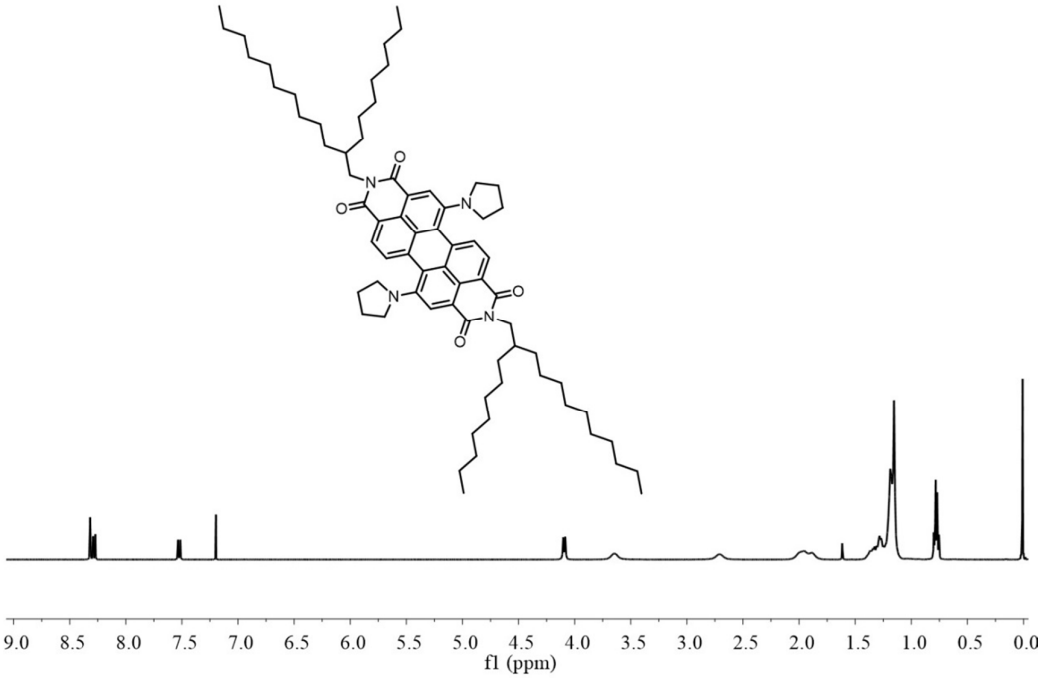
Scheme S1. Synthetic route of the two PDI amphiphilic molecules (Structure 1 and 2).



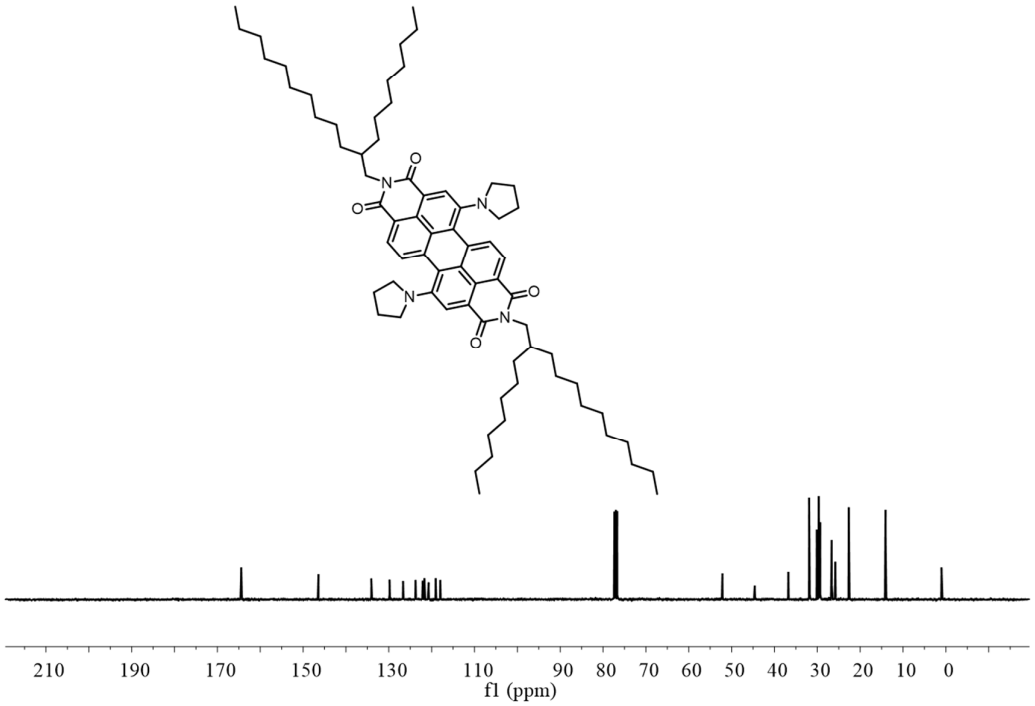
Supporting Figure S1. ^{1}H NMR spectrum (400 MHz, chloroform-*d*, room temperature) of **4**.



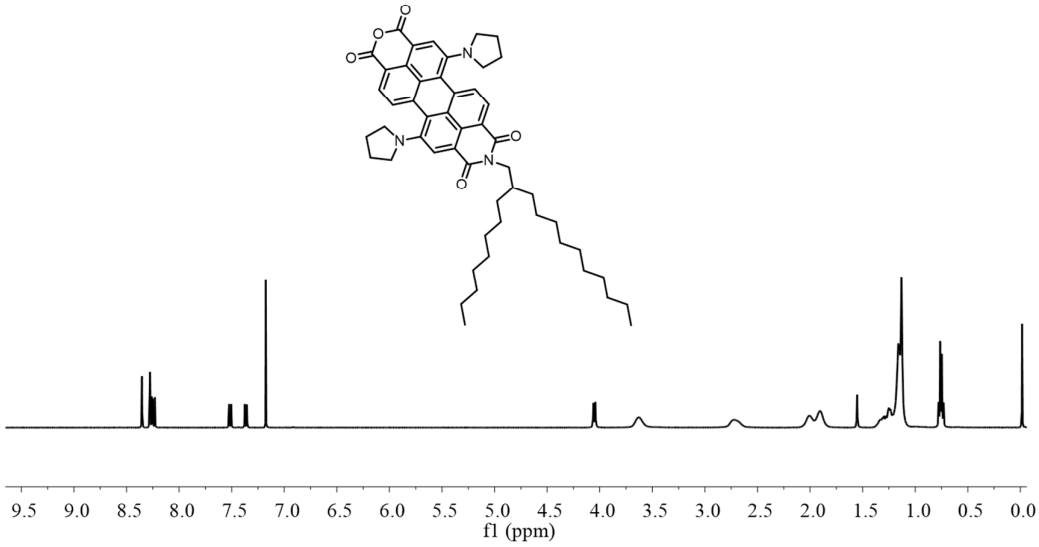
Supporting Figure S2. ^{13}C NMR spectrum (100 MHz, chloroform-*d*, room temperature) of **4**.



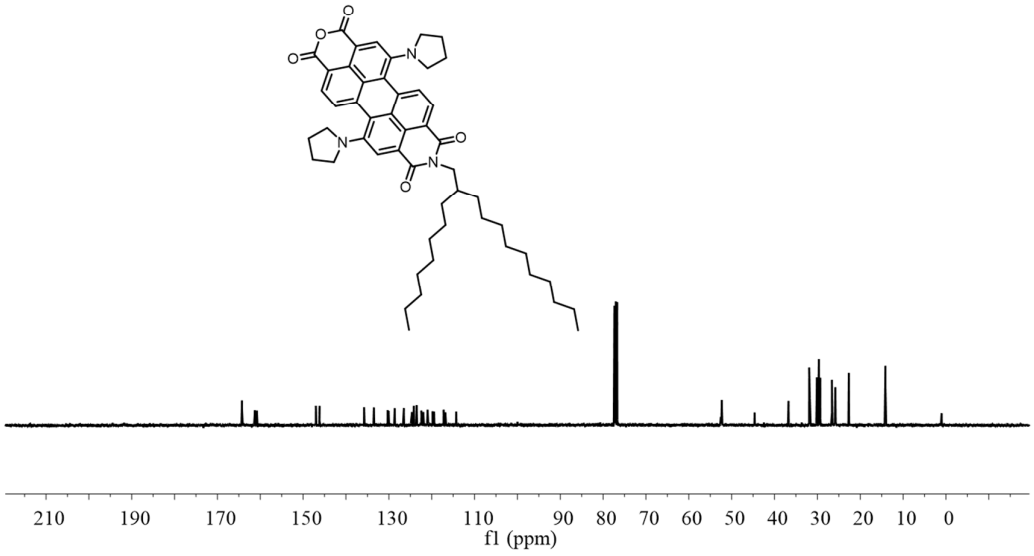
Supporting Figure S3. ^{1}H NMR spectrum (400 MHz, chloroform-*d*, room temperature) of **5**.



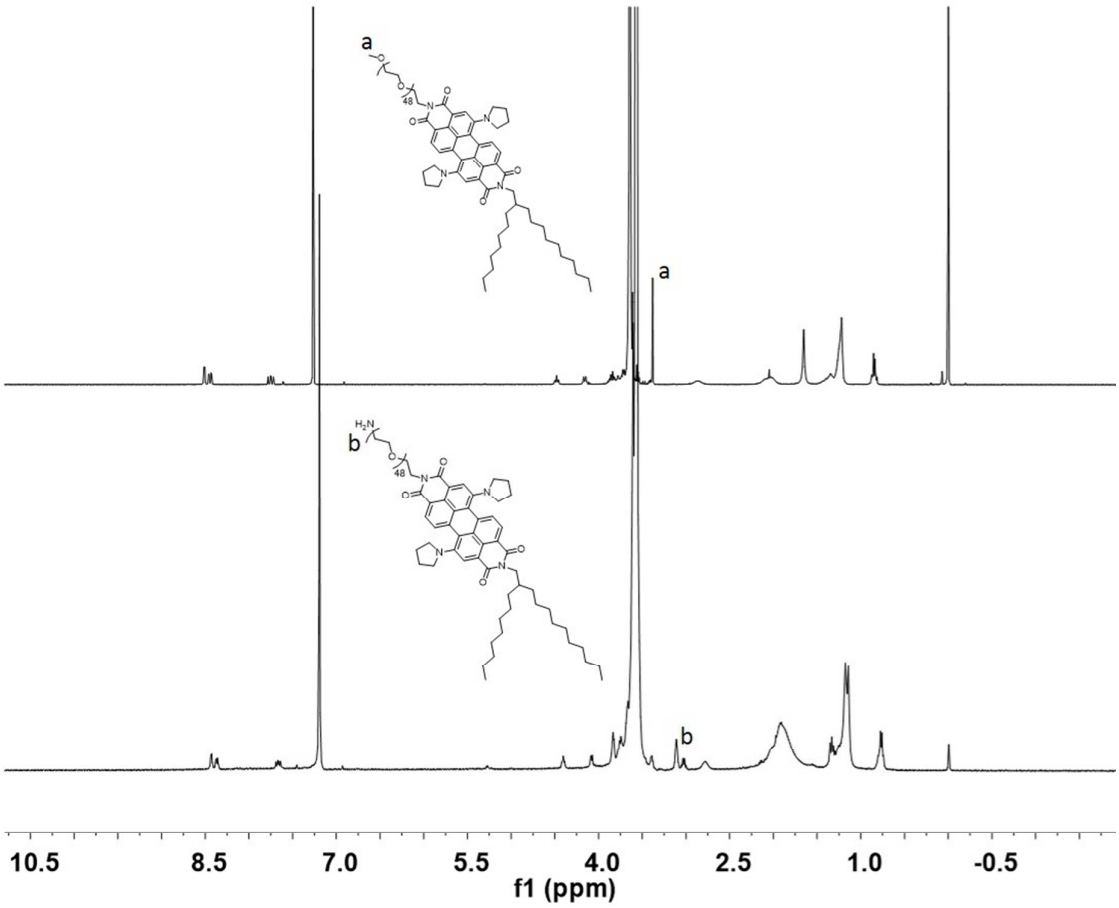
Supporting Figure S4. ^{13}C NMR spectrum (100 MHz, chloroform-*d*, room temperature) of **5**.



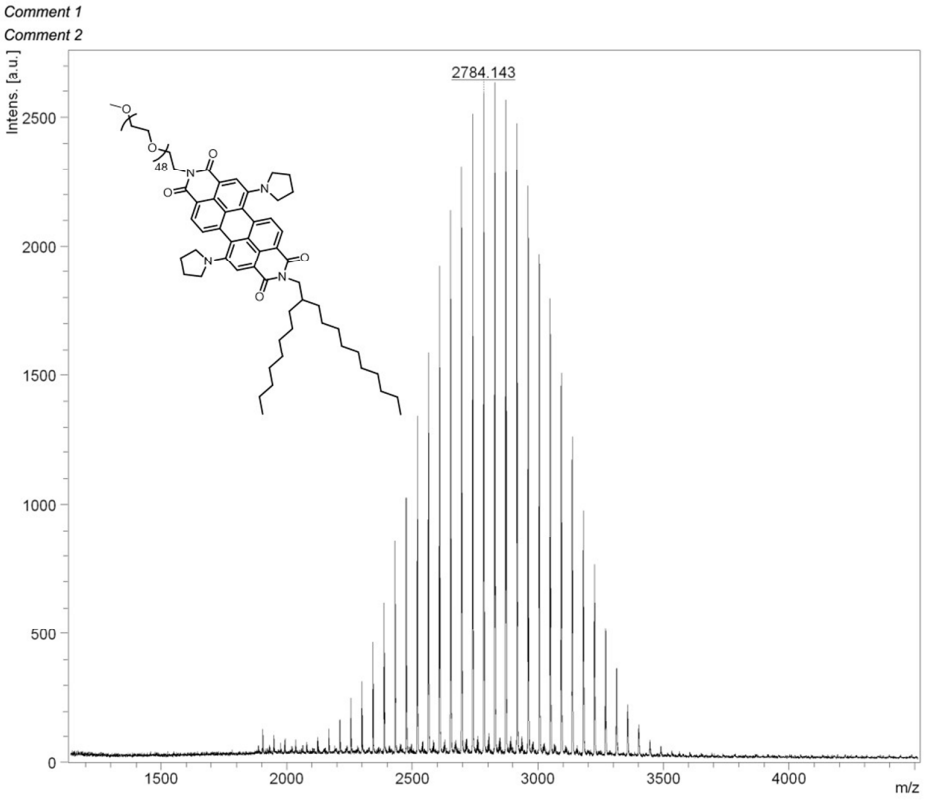
Supporting Figure S5. ^{1}H NMR spectrum (400 MHz, chloroform-*d*, room temperature) of **6**.



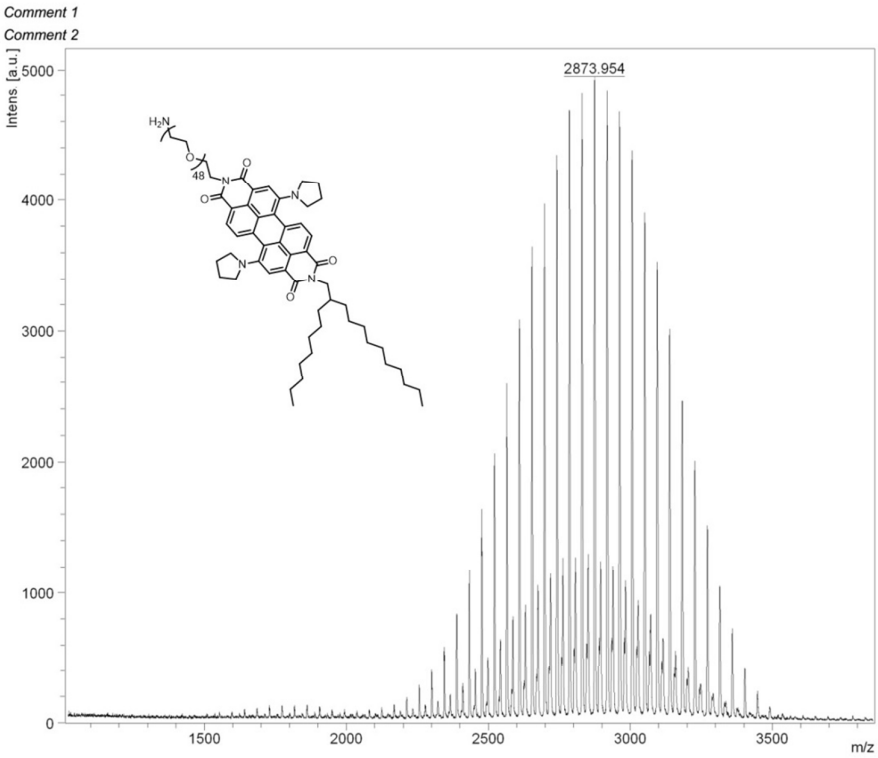
Supporting Figure S6. ^{13}C NMR spectrum (100 MHz, chloroform-*d*, room temperature) of **6**.



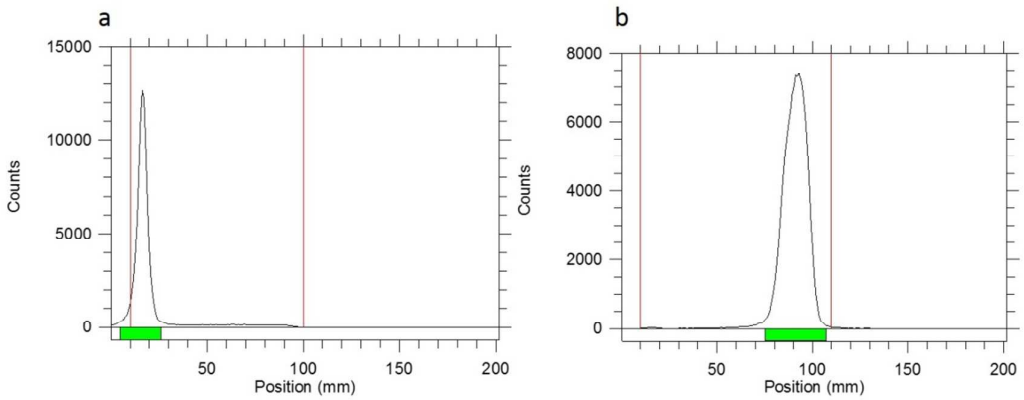
Supporting Figure S7. ^{1}H NMR spectra of methoxyl-peg-PDI (**1**) and NH_2-peg-PDI (**2**).



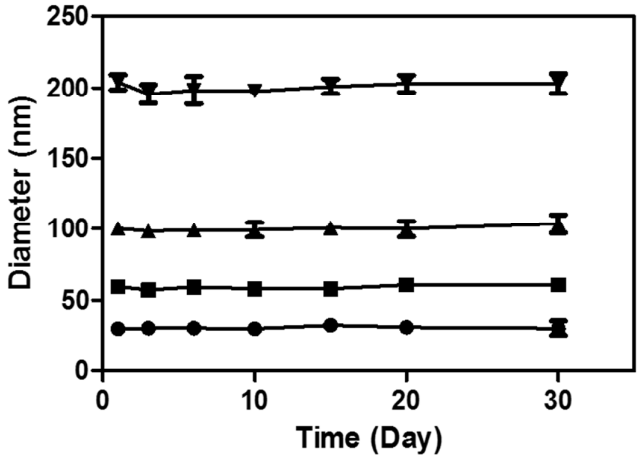
Supporting Figure S8. MALDI-TOF mass spectrum of methoxyl-peg-PDI (**1**).



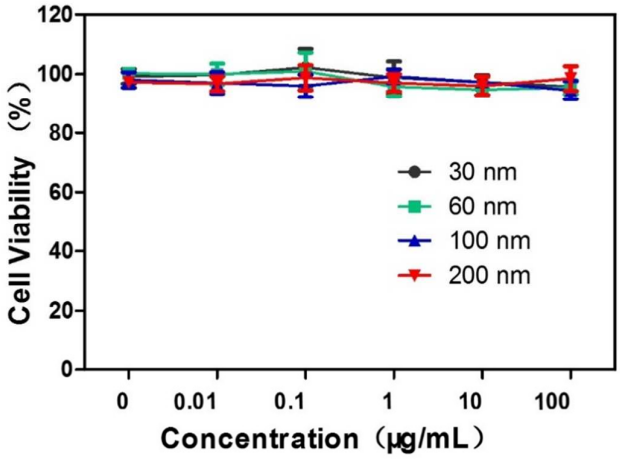
Supporting Figure S9. MALDI-TOF mass spectrum of NH_2-peg-PDI (**2**)



Supporting Figure S10. Radio instant thin-layer chromatography (ITLC) analyses of ^{64}Cu-labeled PDI NPs (a) and $^{64}CuCl_2$ aqueous solution (b).

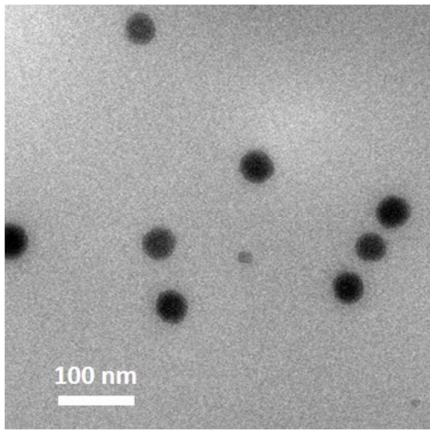


Supporting Figure S11. Dynamic light scattering measurement of the four kinds of PDI NP solutions after incubation for 30 days.

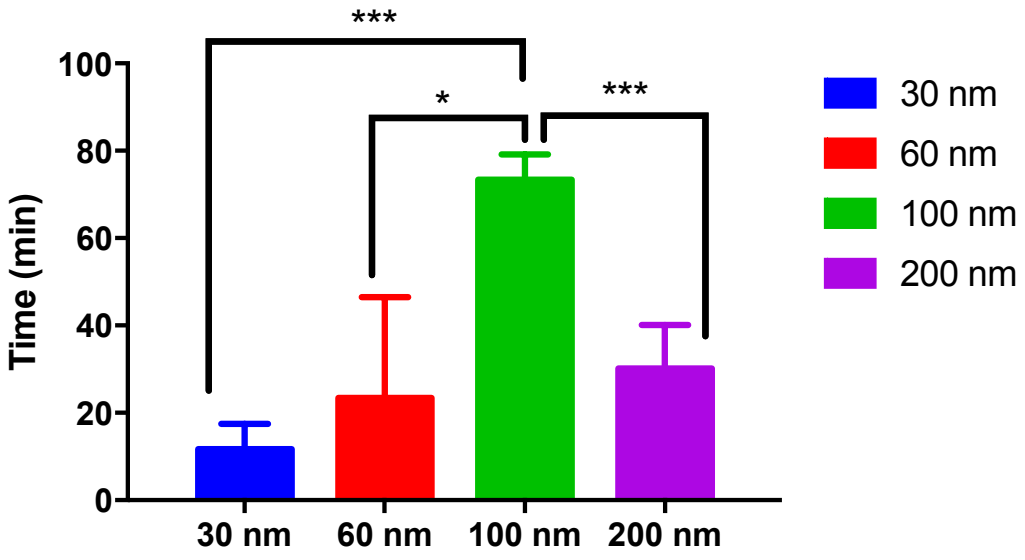


Supporting Figure S12. In vitro cytotoxicity studies of different size PDI NPs. In vitro viability of NIH-3T3 cells treated with PDI NP solutions in PBS at concentrations of 0, 0.01, 0.1, 1, 10 and 100 μg/mL for 24 h. The percentage cell viability of treated

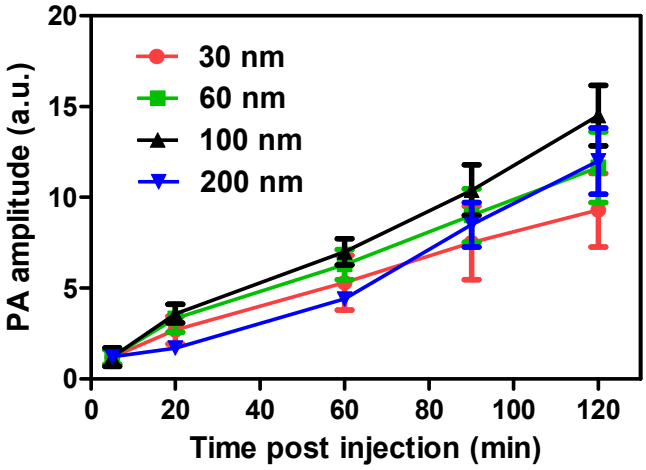
cells is calculated relative to that of cells treated with the same volume of PBS (viability was arbitrarily defined as 100%). Error bars represent standard deviations of three separate measurements.



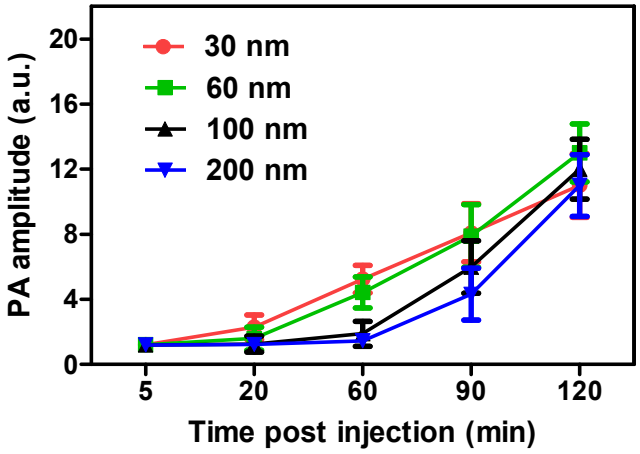
Supporting Figure S13. TEM images of 60 nm PDI NPs after laser irradiation.



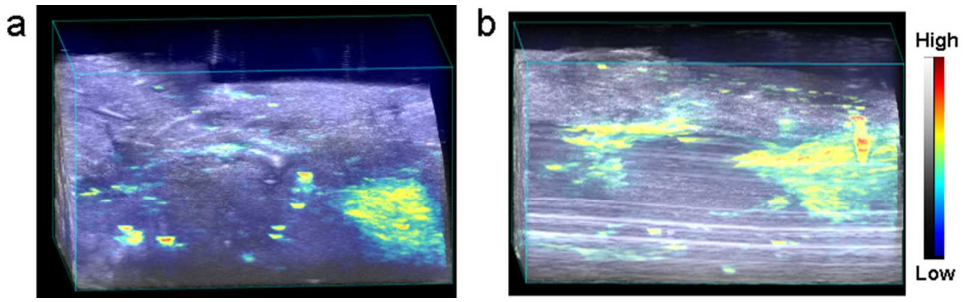
Supporting Figure S14. Comparison of the time interval of PET imaging with four sized NPs to differentiate sciatic and popliteal LN. *p < 0.05 and ***p < 0.001.



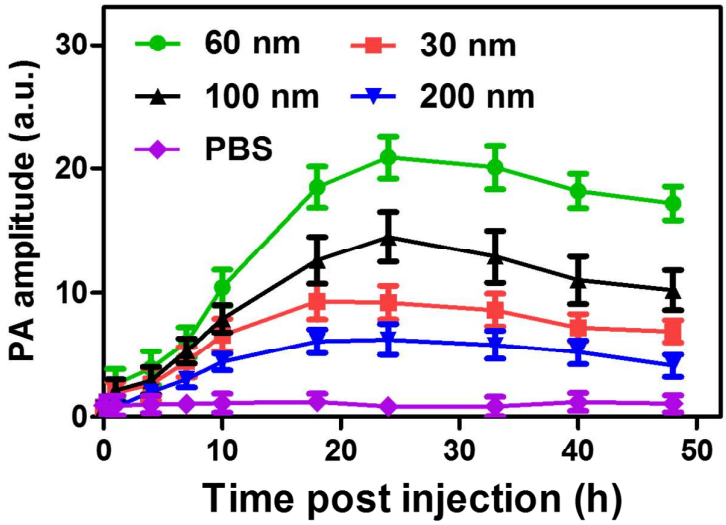
Supporting Figure S15. Photoacoustic amplitude of the popliteal LN region at different times after treatment with different sized PDI NPs.



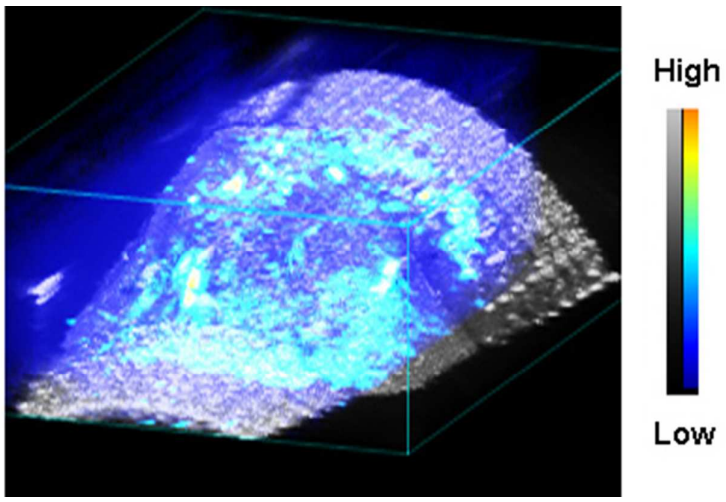
Supporting Figure S16. Photoacoustic amplitude of the sciatic LN region at different times after treatment with different sized PDI NPs.



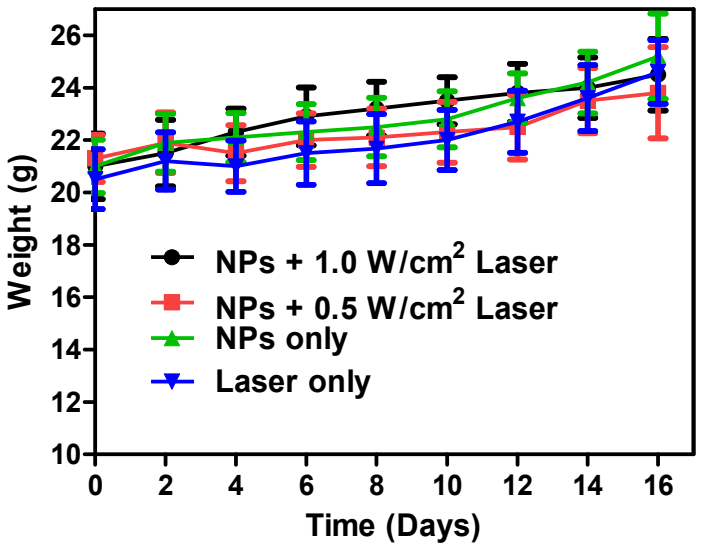
Supporting Figure S17. 3D photoacoustic images of the SLNs can give clear positional information after injection of 100 nm PDI NPs at 20 min (a) and 120 min (b) post-injection.



Supporting Figure S18. Average photoacoustic amplitude of the U87MG tumor region at different times after intravenous injection of different sized PDI NPs in PBS.



Supporting Figure S19. 3D PA images of the tumor treated with 60 nm PDI NPs at 24 h post-injection.



Supporting Figure S20. Body weight changes as a function of time for various treatment groups.

Experimental Section

Synthesis of 4:

A suspension of **3**[1] (0.85 g, 1.55 mmol), 2-n-Octyl-1-dodecylamine (1.53 g, 5.1 mmol), and acetic acid (5 g, 8.33 mmol) in *N*-methyl-2-pyrrolidinone (NMP; 100 mL) was stirred at 85 °C under N_2 for 8 h. After cooling the mixture to room temperature, it was poured into aqueous 1 N HCl and the precipitate was separated by suction filtration, washed with deionized water until pH 7, and dried under vacuum. The

crude product was purified by silica gel column chromatography with CH_2Cl_2/petroleum ether (4:1, v/v, *R*f = 0.5) as eluent. The orange band was collected and **4** was obtained after evaporation of the solvent as a brown powder (1.69 g, 91%). The regioisomeric 1,7- and 1,6-dibromoperylene diimide could not be separated by column chromatography. ^{1}H NMR (400 MHz, $CDCl_3$): 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. for $C_{64}H_{88}Br_2N_2O_4$ [M + H]+ 1106.5101; found 1106.5132.

Synthesis of 5:

A mixture of **4** (331.8 mg, 0.3 mmol) and 8 mL pyrrolidine was heated to 55 °C under N_2. The reaction mixture was kept at 55 °C for about 24 h and then the solvents were evaporated using rotary evaporation. The residue was purified by column chromatography on silica gel with $CHCl_3$ (Rf = 0.42) as eluent. The regioisomeric 1,7- and 1,6-dibromoperylene diimide could be separated by column chromatography at this step. The green fraction was collected and after evaporation of the solvent, **5** was collected as a green powder (248 mg, 75 %). ^{1}H NMR (400 MHz, $CDCl_3$): 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. for $C_{72}H_{104}N_4O_4$ [M + H]+ 1088.8111; found 1088.8134.

Synthesis of 6:

A solution of **5** (1.09 g, 1.00 mmol) and NaOH (4.68 g, 83.40 mmol) in isopropanol (36 mL) was heated to reflux. After stirring for 0.5 h, the reaction mixture was poured into acetic acid (50 mL) and stirred over night at room temperature. After filtration, the precipitate was washed with a large amount of H_2O, then MeOH. The crude product was purified by silica gel column chromatography with $CHCl_3$ (*R*f = 0.3) as eluent. The green band was collected and **6** was obtained after evaporation of the solvent as a green powder (0.60 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) 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. for $C_{52}H_{63}N_3O_5$ [M + H]+ 809.4876; found 809.4844.

Synthesis of 1, 2:

A mixture of **6** (404.74 mg, 0.50 mmol), (Me-PEG-NH_2 for synthesis of compound 1, which is in the same method with NH_2-PEG-PDI), tBOC-NH-poly(ethylene glycol)-NH_2 (1.155 g, 0.550 mmol), zinc acetate dihydrate (21.9 mg, 0.1 mmol), and imidazole (30 g) were heated at 140 °C for 3 h. The resulting mixture was poured into deionized water (150 mL). The precipitate was separated by suction filtration, and

washed with deionized water until reaching pH 7. The residue was purified by silica gel column chromatography (CH_2Cl_2, then MeOH/CH_2Cl_2=2/1, then MeOH /ethyl acetate = 1/1). The green powder was collected and **5** was obtained after evaporation of the solvent as a green powder (1.017 g, 70%). **5** was dissolved in a mixture of $CHCl_3$ (10 mL) and trifluoroacetic acid (10 mL) stirring overnight at room temperature. After evaporation of the solvent, the resulting solution was then precipitated in diethyl ether and then green powder **2** was obtained by filtering and further dried under vacuum.

References

(1) Würthner. F.; Stepanenko, V.; Chen, Z. J.; Saha-Möller, C. R,; Kocher, N.; Stalke, D. Preparation and Characterization of Regioisomerically Pure 1,7-Disubstituted Perylene Bisimide Dyes. *J. Org. Chem.* **2004**, *69*, 7933-7939.