# High Density Glycopolymer Functionalized Perylene Diimide Nanoparticles for Tumor-targeted Photoacoustic Imaging and Enhanced Photothermal Therapy

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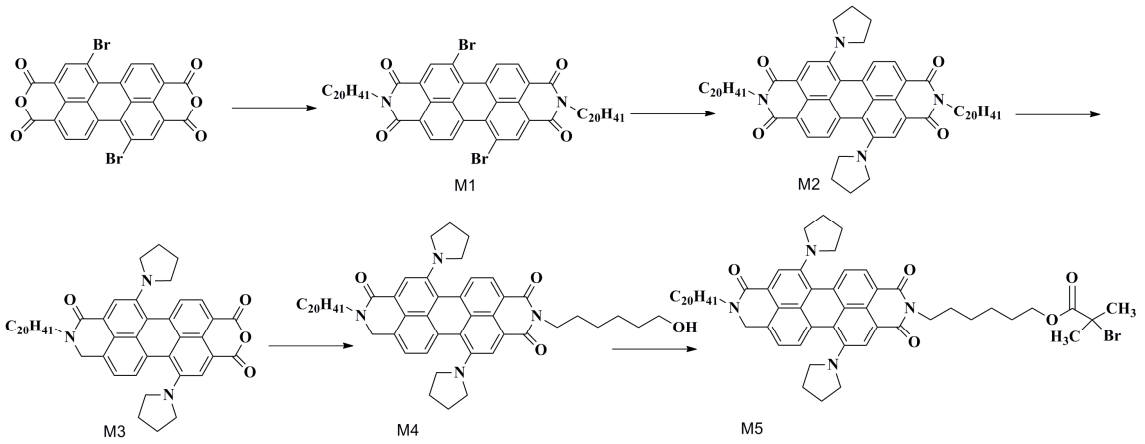


**Scheme. S1.** Synthetic route to Br-PDI (M5).

Preparation of M1:

5,12-dibromoanthra [2,1,9-def:6,5,10-d'e'f'] diisochromene -1,3,8,10- tetraone (0.85 g, 1.55 mmol), 2-n-Octyl-1-dodecylamine (1.53 g, 5.1 mmol), and acetic acid (5.0 g, 8.33 mmol) were dissolved in N-methyl-2-pyrrolidinone (NMP, 100 mL) and stirred at 85 ℃ under an argon atmosphere for about 12 h. After cooled to room temperature, poured the products into the aqueous $NaHCO_3$. Then the precipitate was separated by suction filtration, washed with deionized water until pH=7, finally dried under vacuum. The crude product was purified by silica gel column chromatography with $CH_2Cl_2$/petroleum ether (4:1, v/v) as eluent. The orange band was collected and M1 was obtained after evaporation of the solvent as a brown powder (Yield: 1.6 g, 90%).The regioisomeric 1,7- and 1,6-dibromoperylene bisimides 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, 69 H), 0.45 (t, 12 H) ppm. $^1$H NMR spectrum of M1 is presented in Figure S1.

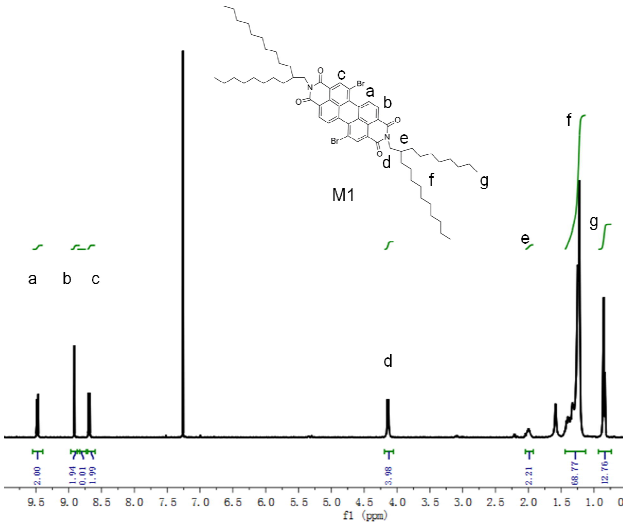


**Figure S1.** $^{1}$H-NMR spectra of M1 in $CDCl_3$.

Preparation of M2:

M1 (0.332 g, 0.3 mmol) was dissolved in 8 mL pyrrolidine, then heated to 55 ℃ under an argon atmosphere. After stirred for about 24 h then evaporated the solvents by rotary evaporators. The residue was purified by column chromatography on silica gel with $CHCl_3$ as eluent. The regioisomeric 1,7- and 1,6-dibromoperylene bisimides could be separated by column chromatography at this step. The green fraction was collected and after evaporation of the solvent, M2 was collected as a green powder (Yield: 0.248 g, 75%).$^{1}$H NMR (400 MHz, $CDCl_3$, δ): 8.28 (d, 4 H), 7.53 (s, 2 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, 2 H), 1.48 (m, 68 H), 0.63 (t, 12 H) ppm. $^{1}$H NMR spectrum of M2 is presented in Figure S2.

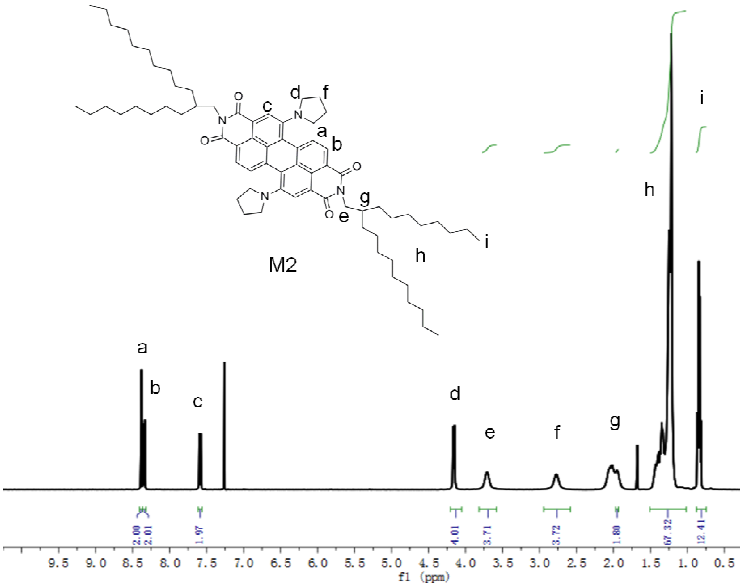


**Figure S2**. $^{1}$H-NMR spectra of M2 in $CDCl_3$.

Preparation of M3:

M2 (1.09 g, 1.00 mmol) and NaOH (4.68 g, 83.40 mmol) were dissolved in isopropanol (36.00 mL), then heated to reflux. After stirred for 0.5 h, the reaction mixture was poured into acetic acid (50.00 mL) and stirred over night at room temperature. After filtration, the precipitate was washed with excess water then MeOH. The crude product was purified by silica gel column chromatography with $CHCl_3$ as eluent. The green band was collected and M3 was obtained after evaporation of the solvent as a green powder (Yield: 1.6 g, 70%). $^{1}$H NMR (400 MHz, $CDCl_3$, δ): 8.45-8.32 (m, 4H), 7.46-7.53 (d, 2H), 3.85-3.65 (m, 4H), 2.85-2.65 (m, 2H), 2.05-1.95 (m, 4H), 1.48 (m, 34 H), 0.80 (t, 6 H) ppm. $^{1}$H NMR spectrum of M3 is presented in Figure S3.

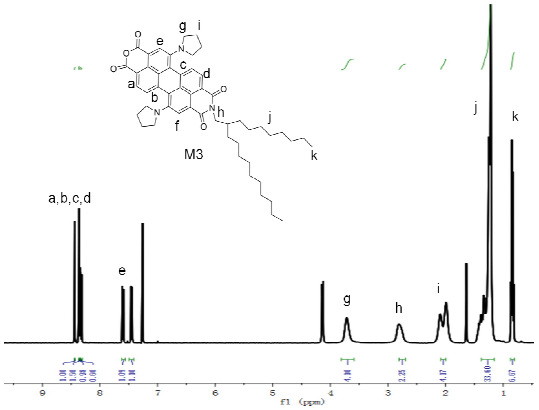


**Figure S3**. $^{1}$H-NMR spectra of M3 in $CDCl_3$.

Preparation of M4:

6-aminohexan-1-ol (0.16 g, 1.37 mmol) and M3(0.80 g, 1.04 mmol) were dissolved in N-methyl-2-pyrrolidinone (NMP; 100.00 mL) ,then heated to 85 °C under an argon atmosphere. After stirred for 2 h, the reaction mixture was poured into aqueous $NaHCO_3$ and then separated the mixture by suction filtration. After dried under vacuum, the residue was purified by column chromatography on silica gel with $CHCl_3$/ethyl acetate (3:1, v/v) as eluent. The reseda band was collected and M4 was obtained after evaporation of the solvent as a brown powder (Yield: 0.60 g, 75%). $^{1}$H NMR (400 MHz, $CDCl_3$, δ): 8.45-8.32 (m, 4H), 7.51 (d, 2H), 3.65-4.35 (m, 10H), 2.65-2.75 (m, 1H), 2.05-1.95 (m, 4H), 1.48 (m, 34 H), 0.80 (t, 6 H) ppm. $^{1}$H NMR spectrum of M4 is presented in Figure S4.

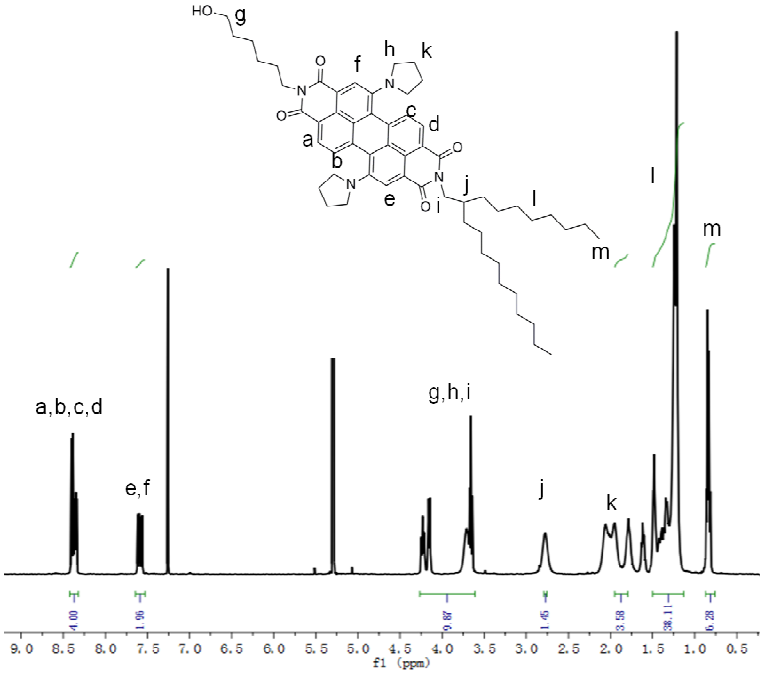


**Figure S4**. $^{1}$H-NMR spectra of M4 in $CDCl_3$.

Preparation of M5:

M4 (2 - (6 - hydroxyhexyl) – 9 - (2 - octyldodecyl) - 5,12 - di(pyrrolidin – 1 - yl)anthra[2,1,9 - def:6,5,10 - d'e'f']diisoquinoline - 1,3,8,10(2H,9H) – tetraone ,0.2 g, 0.21 mmol) , triethylamine (0.027 g, 0.27 mmol) was dissolved in DCM (40.00 mL, Anhydrous dichloromethane),then the reaction system was placed in an ice-water bath,2-bromo-2-methylpropanoyl bromide(0.07 g, 0.32 mmol) was slowly added to the flask, a white precipitate appeared. After the addition, the reaction was warmed to room temperature under an argon atmosphere. After stirred for 12 hours, the reaction mixture was washed with a large amount of $H_2O$ to remove salts and unreacted 2-bromo-2-methylpropanoyl bromide, then dried under vacuum,the residue was purified by column chromatography on silica gel with DCM / ethyl acetate(3:1, v/v) as eluent. The dark green band was collected and M5 was obtained after evaporation of the solvent as a brown powder (Yield: 0.16 g, 80%).$^{1}$H NMR (400 MHz, $CDCl_3$, δ): 8.45-8.32 (m, 4H), 7.61 (d, 2H), 4.21-4.25 (m, 6H), 3.75 (m, 4H), 2.85 (m, 4H),1.71-2.24(d,12H), 1.48 (m, 34 H), 0.80 (t, 6 H) ppm. $^{1}$H NMR spectrum of M5 is presented in Figure S5.

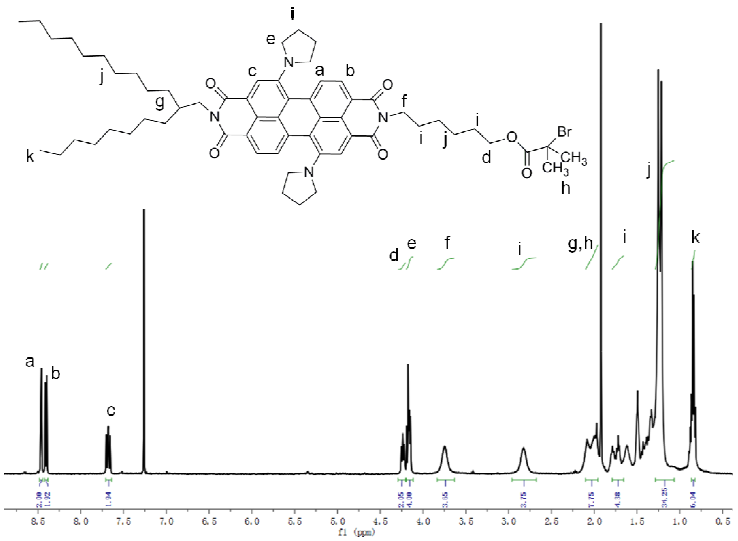

**Figure S5**. $^{1}$H-NMR spectra of M5 in $CDCl_3$.

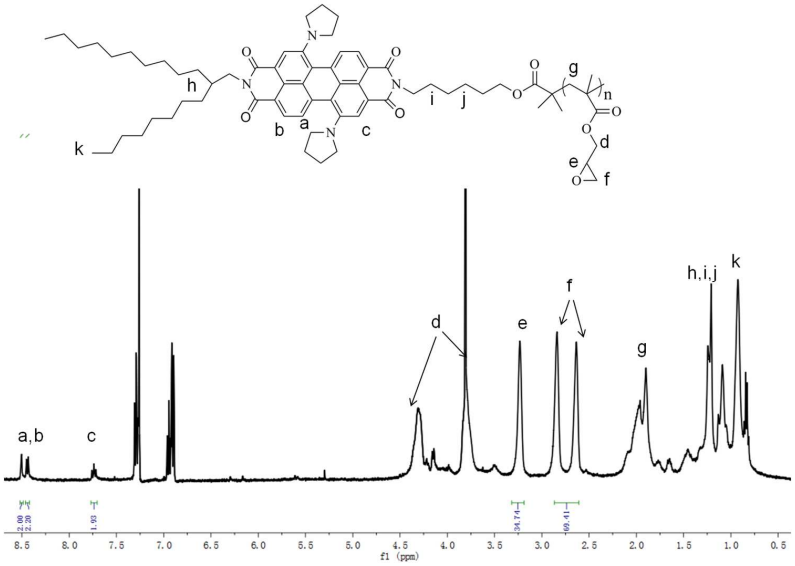

**Figure S6**. $^{1}$H-NMR spectra of PGMA-PDI in $CDCl_3$.

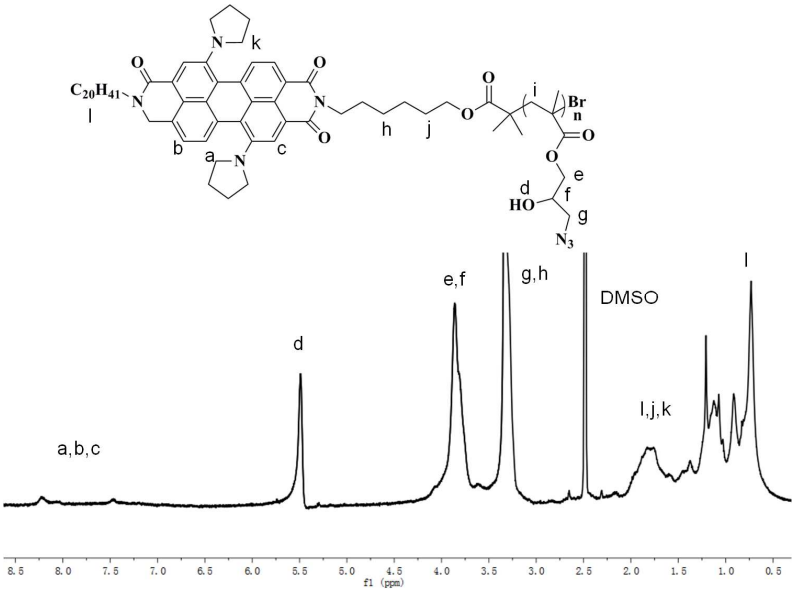


**Figure S7**. $^{1}$H-NMR spectra of PGMA-$N_3$-PDI in DMSO.

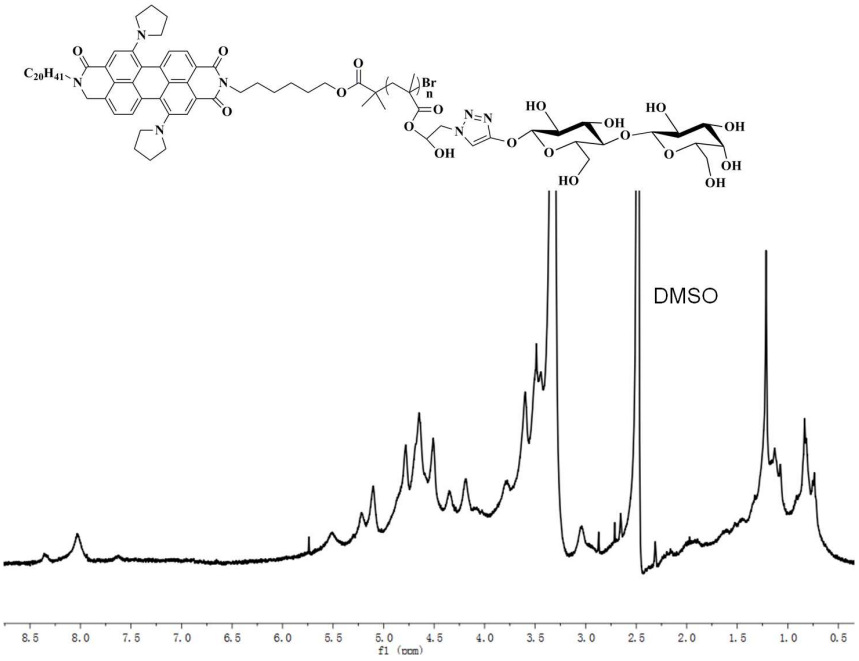


**Figure S8**. $^{1}$H-NMR spectra of PLAC-PDI in DMSO-$d_6$.

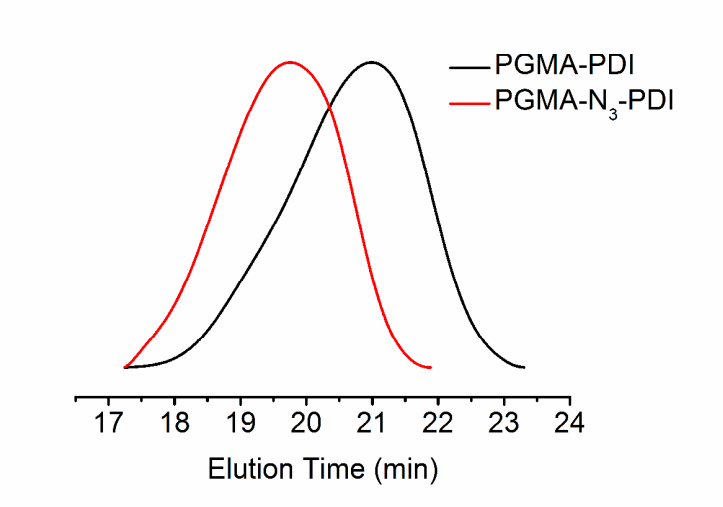


**Figure S9**. The gel permeation chromatography (GPC) of PGMA-PDI and PGMA-$N_3$-PDI.

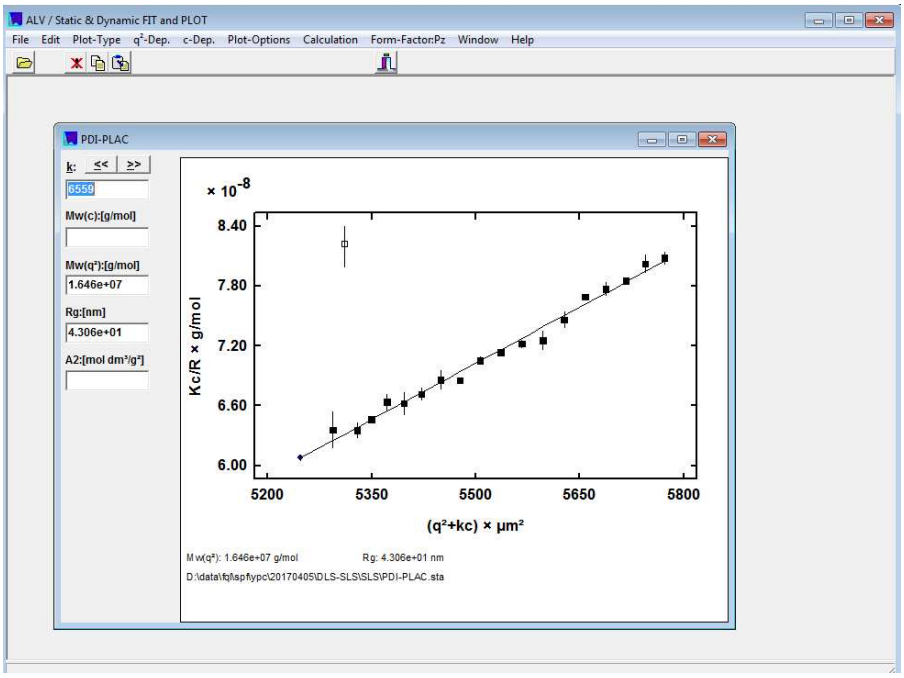


**Figure S10**. The molecular weight ($M_w$) of PLAC-PDI NPs measured by static light scattering.

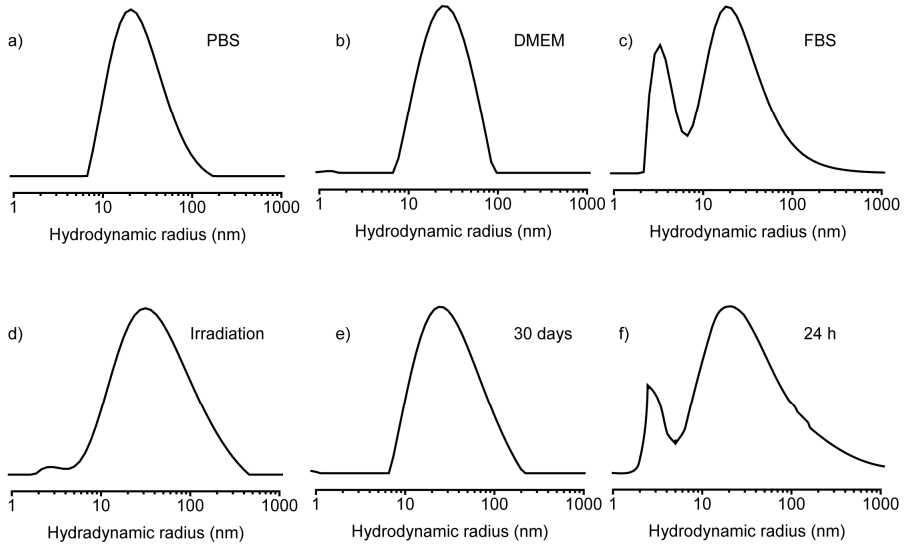


**Figure S11**. Dynamic light scattering (DLS) of PLAC-PDI NPs in PBS buffer (a), DMEM cell culture medium (b), fetal bovine serum (FBS) (c), after 30 min laser irradiation (d), after 30 days storage in dark (e), and after 24 h incubation in FBS (f).

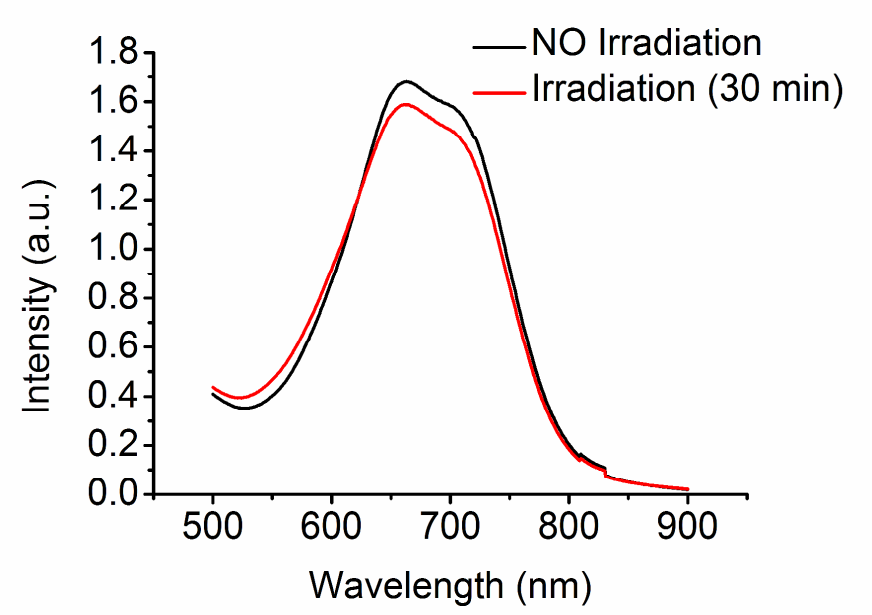


**Figure S12**. UV-vis-NIR spectra of PLAC-PDI NPs in water with laser irradiation for 30 min (730 nm laser with a power intensity of 500 mW $cm^{-2}$).

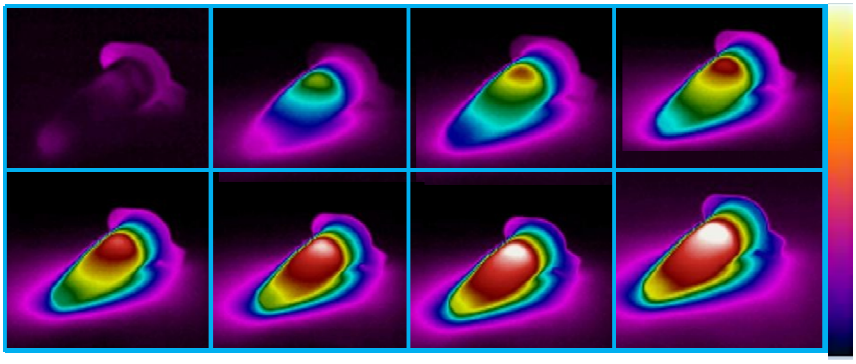

**Figure S13**. Real-time thermal images of PLAC-PDI NPs aqueous solution under laser irradiation for 0 min, 1 min, 3 min, 5 min, 7min, 9 min, 10 min, 11 min (730 nm, 500 mW $cm^{-2}$).

Based on the total energy balance for this system:

$$\sum_i m_i c_{p,i} \frac{dT}{dt} = Q_{NPs} + Q_s - Q_{loss} \quad (1)$$

where m and Cp are the mass and heat capacity of solvent (water), respectively. T is the solution temperature.

QNPs is the photothermal energy input by PLAC-PDI NPs:

$$Q_{NPs} = I(1 - 10^{-A_\lambda})\eta \quad (2)$$

where I is the laser power, Aλ is the absorbance of PLAC-PDI NPs at the wavelength of 730 nm, and η is the conversion efficiency from the absorbed light energy to thermal energy. Qs is the heat associated with the light absorbance of the solvent, which is measured independently to be 25.2 mW using pure water without PLAC-PDI NPs.

Qloss is thermal energy lost to the surroundings:

$$Q_{loss} = hA\,\Delta T \quad (3)$$

where h is the heat transfer coefficient, A is the surface area of the container, and ΔT is the temperature change, which is defined as T-Tsurr (T and Tsurr are the solution temperature and ambient temperature of the surroundings, respectively).

At the maximum steady-state temperature, the heat input is equal to the heat output, that is:

$$Q_{NPs} + Q_s = Q_{loss} = hA\,\Delta T_{max} \quad (4)$$

where ΔTmax is the temperature change at the maximum steady-state temperature. According to the Equation.2 and Equation.4, the photothermal conversion efficiency (η) can be determined:

$$\eta = \frac{hA\,\Delta T_{max} - Q_s}{I\,(1 - 10^{-A_\lambda})} \quad (5)$$

In this equation, only hA is unknown for calculation. In order to get the hA, we herein introduce $\theta$, which is defined as the ratio of ΔT to ΔTmax:

$$\theta = \frac{\Delta T}{\Delta T_{max}} \quad (6)$$

Substituting Equation.6 into Equation.1 and rearranging Equation.1:

$$\frac{d\theta}{dt} = \frac{hA}{\sum_i m_i c_{p,i}}\left[\frac{Q_{NPs} + Q_s}{hA\,\Delta T_{max}} - \theta\right] \quad (7)$$

When the laser was shut off, the $Q_{NP}s + Qs = 0$, Equation.7 changed to:

$$dt = -\frac{\sum_i m_i c_{p,i}}{hA}\frac{d\theta}{\theta} \quad (8)$$

Integrating Equation.8 gives the expression:

$$t = \frac{\sum_i m_i c_{p,i}}{hA}(-\ln\theta) \quad (9)$$

Thus, hA can be determined by applying the linear time data from the cooling period vs –lnθ (Figure 3c,d). Substituting hA value into Equation.5, the photothermal conversion efficiency (η) of PLAC-PDI NPs can be calculated.

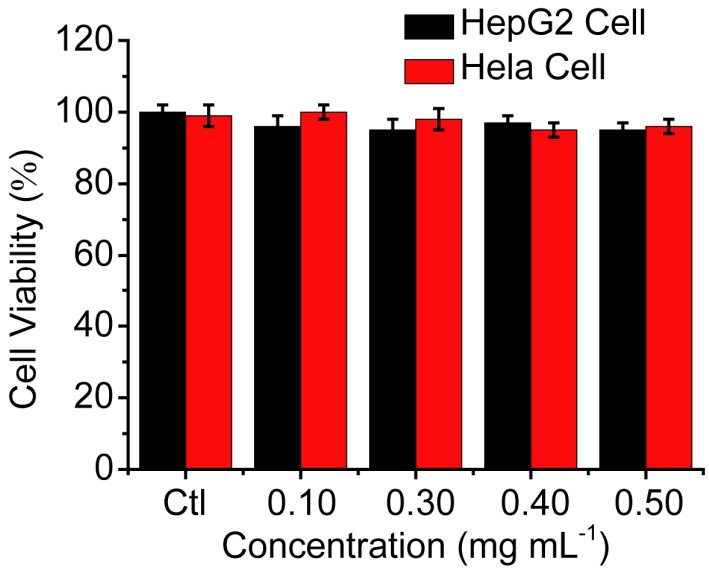


**Figure S14**. Viability of HepG2 cells and Hela cells after being incubated with various concentrations (0, 0.10, 0.30, 0.40, 0.50 mg $mL^{-1}$) of PLAC-PDI NPs for 24 h without laser irradiation.

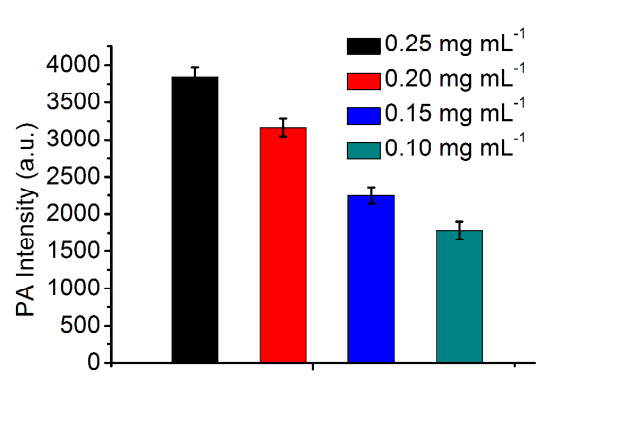


**Figure S15**. The PA intensity of HepG2 cells after 4 h incubated with different concentrations PLAC-PDI NPs.

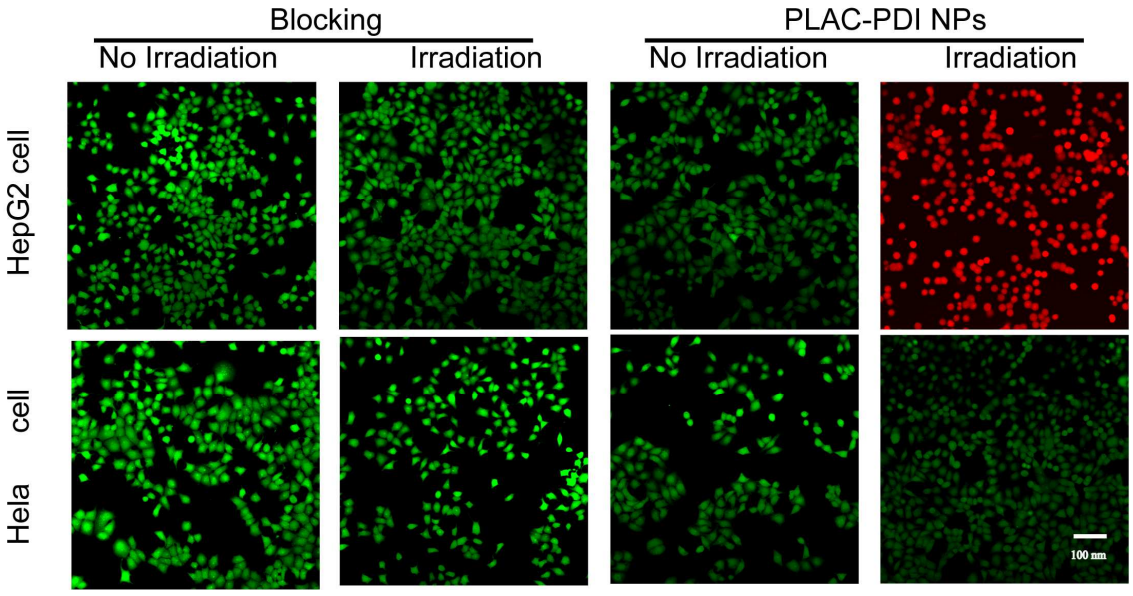


**Figure S16**. Fluorescence images of calcein-AM/PI co-stained. Incubation for 4 h, with exposure to 730 nm laser at the power densities of 500 mW $cm^{-2}$ for 10 min.

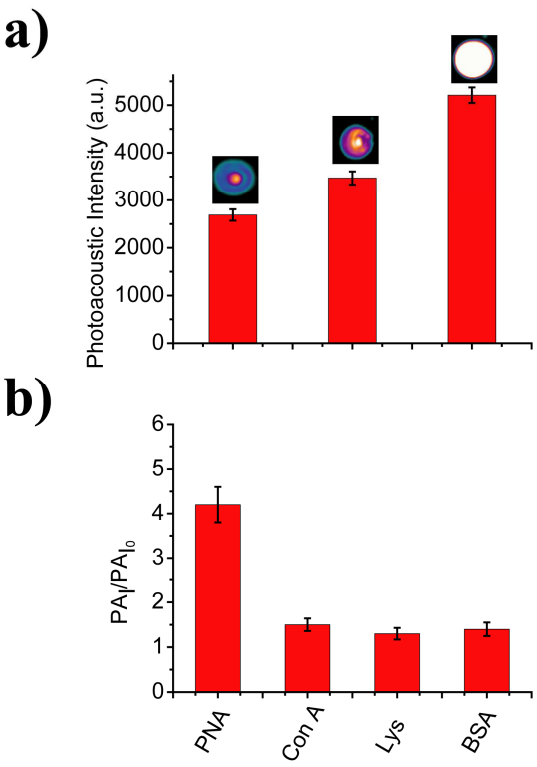


**Figure S17**. a) The PA intensity of PLAC-PDI NPs (0.5 mg $mL^{-1}$) in the presence of 0, 5, and 10 μg peanut agglutinin (PNA). b) PA intensity change of PLAC-PDI NPs in the

presence of 10 μg different proteins (where I and $I_0$ are the PA intensity of PLAC-PDI NPs in the presence and absence of a protein).

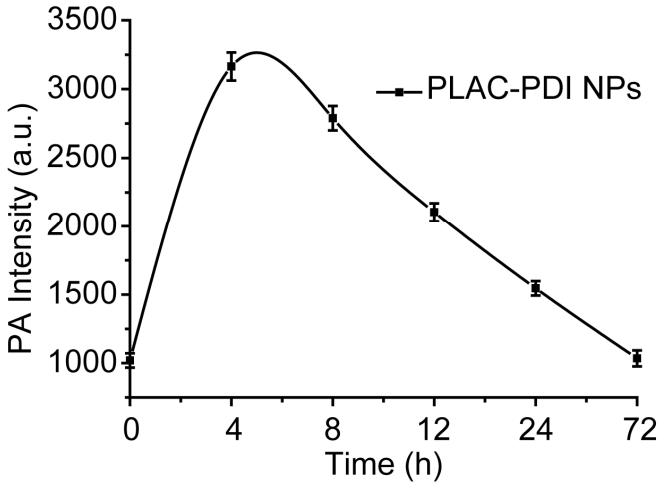


**Figure S18.** Real-time monitoring of the PA intensity changes of blood after injection of PLAC-PDI NPs.

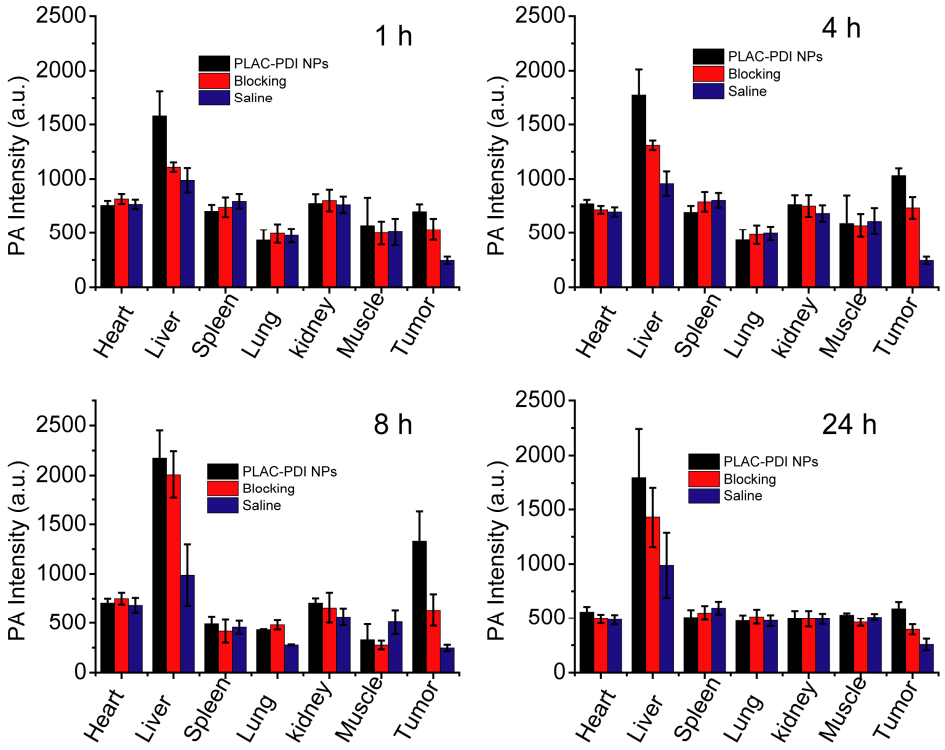


**Figure S19**. The PA signal intensity of main organs at 1 h, 4 h, 8 h, and 24 h post-injection of PLAC-PDI NPs (pre-injection without/with lactose) and saline (mean ± SD, n = 3).