Supporting Information

Carrier-free water-soluble porphyrin nanoparticles as highly effective photosensitizers for photodynamic therapy

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Content

1. Synthesis

Three porphyrin derivatives (T0, T1 and T2) were synthesized according to the published procedures.[S1] The specific synthetic routes are shown in Scheme S1.

1.1 Preparation of 7, 12-Bis(1-bromoethyl)-3, 8, 13, 17-tetramethylporphyrin-2, 18-dipropanoic acid (**1**)

Under nitrogen atmosphere, Hemin chloride (2.0184 g, 3.10 mmol) and 33 *wt*.% hydrogen bromide in acetic acid solution (30 mL) were added into a 100 mL of flask and stirred at 25 ℃ for 18 h in the dark. After the reaction, the excess acetic acid solution was removed under reduced pressure, and then dissolved in acetone (10 mL) and transferred to anhydrous ether (500 mL) to obtain the purplish-black precipitate. The resulting purplish-black products after filtration were collected without purification and dried in vacuum overnight (2.1677 g, yield 96.64%).

1.2 Preparation of 7, 12-Bis [1-(2-(2-hydroxyethoxy) ethoxy)] ethyl-2, 18-dipropionate (2-hydroxyethoxy) ethyl ester-3, 8, 13, 17-tetramethylporphyrin (**T0**)

Under nitrogen atmosphere, compound **1** (2.1677 g, 3.00 mmol) and diethylene glycol (20 mL, 210.7 mmol) were taken into a 100 mL of flask. After the compound **1** was completely dissolved, 0.25 mL of H_2SO_4 was added drop by drop, followed by ultrasonic reaction for 30 min, and then stirred for 24 h at 25 ℃ in the dark. After the reaction, the saturated $NaHCO_3$ solution (5 mL) was added to neutralize the remaining H_2SO_4. Then, the mixture was poured into 250 mL of *n*-butanol and washed by 250 mL of brine solution and deionized water, respectively, to remove the residual diethylene glycol. The organic layer was dried over anhydrous sodium sulfate and evaporated to obtain the reddish-brown oil-like crude product, which was purified via column chromatography on silica gel (ethyl acetate : methanol = 4 : 1) to achieve the pure reddish-brown oil **T0** (0.6437 g, yield 22.56%).

T0: ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 10.63 – 10.53 (m, 2H, *meso*-H), 10.13,10.12 (s, 2H, *meso*-H), 6.23 – 6.14 (m, 2H, OC<u>H</u>), 4.45 (t, J = 7.7 Hz, 4H, <u>CH_2</u>CH_2COO), 4.19 – 4.08 (m, 4H, COO<u>CH_2</u>CH_2), 3.98 – 3.88, 3.87 – 3.81, 3.80 – 3.74, 3.69 – 3.67, 3.62 – 3.53 (m, 16H, CHO<u>CH_2</u><u>CH_2</u>O<u>CH_2</u><u>CH_2</u>OH), 3.72, 3.70 (s, 6H, <u>CH_3</u>), 3.67, 3.66 (s, 6H, <u>CH_3</u>), 3.36 – 3.31 (m, 4H, CH_2<u>CH_2</u>COO), 3.30 – 3.21, 3.04 –

2.79 (m, 12H, $COOCH_2\underline{CH_2}O\underline{CH_2CH_2}OH$), 2.30 (d, J = 6.6Hz, 6H, $CH\underline{CH_3}$), -3.74 (s, 2H, pyrrole-H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 173.40, 173.36, 139.28 – 136.44 (16C, C pyrrole), 98.84, 98.48, 96.97, 96.62, 73.82, 72.53, 72.50, 72.44, 71.95, 71.90, 71.86, 70.86, 68.78, 68.72, 68.65, 68.44, 63.59, 63.17, 61.74, 61.05, 60.93, 60.88, 36.99, 36.94, 29.72, 25.41, 25.08, 21.89, 11.85, 11.77, 11.69, 11.63. FTIR (cm^{-1}): 3381, 3310, 2972, 2917, 2866, 1727, 1648, 1540, 1449, 1413, 1371, 1347, 1292, 1268, 1228, 1166, 1120, 1060, 990, 945, 886, 835, 790, 767, 724, 708, 693, 677. HRMS (ESI, positive): calcd for $C_{50}H_{70}N_4O_{14}$ $[M+H]^+$: 951.4961. Found: 951.4970. UV-vis: λ_{max}, nm: 390, 503, 535, 568, 620. Elemental Anal. Calcd for $C_{50}H_{70}N_4O_{14}$ (%): C, 63.14; H, 7.42; N, 5.89. Found: C, 63.04; H, 7.39; N, 5.90. HPLC purity: 96.46% (t_R = 5.50 min). (t_R: retention time)

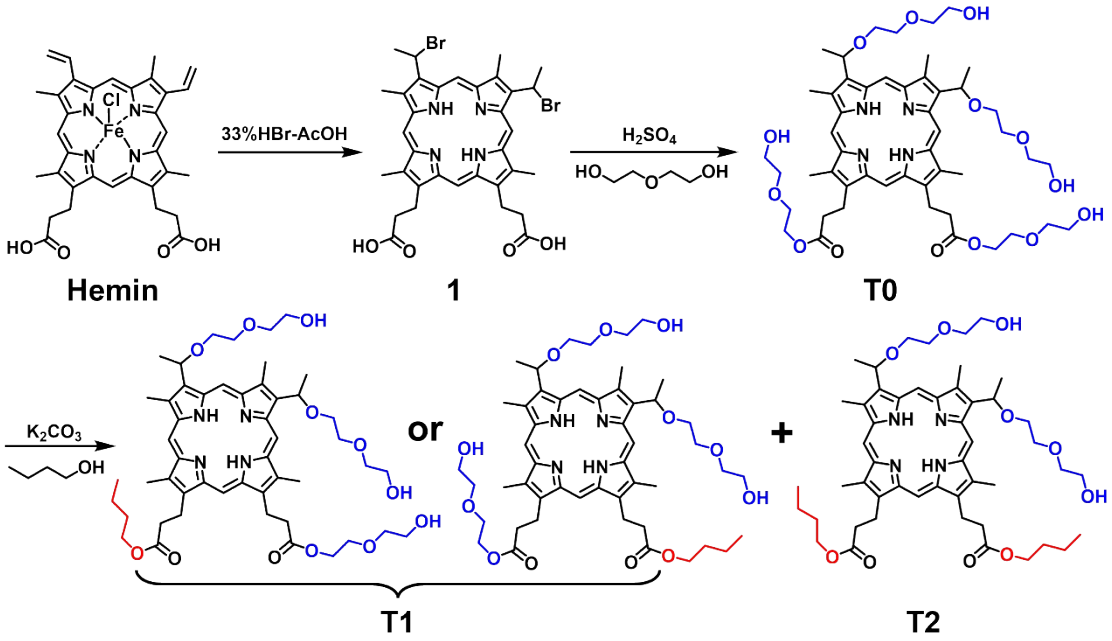
1.3 Preparation of 7, 12-Bis [1-(2-(2-hydroxyethoxy) ethoxy)] ethyl-2 (or 18) -dipropionate (2-hydroxyethoxy) ethyl ester-18 (or 2) -dipropionate butyl ester-3, 8, 13, 17-tetramethylporphyrin (**T1**) and 7, 12-Bis [1-(2-(2-hydroxyethoxy) ethoxy)] ethyl-2, 18-dipropionate butyl ester-3, 8, 13, 17-tetramethylporphyrin (**T2**)

Under nitrogen atmosphere, **T0** (0.5822 g, 0.612 mmol) and 40 mL of *n*-butanol were taken into a 100 mL of flask. After **T0** was completely dissolved, K_2CO_3 (0.6021 g, 4.36 mmol) was added and then stirred for 3.5 h at 50 ℃ in the dark. After the reaction, 10 mL of hydrochloric acid solution (2.24 mol/L) was added to neutralize the remaining K_2CO_3. Then, the mixture was poured into 250 mL of ethyl acetate and washed by 250 mL of brine solution and deionized water, respectively. The organic layer was dried over anhydrous sodium sulfate and evaporated to obtain the reddish-brown oil-like crude product, which was purified via column chromatography on silica gel (ethyl acetate : methanol = 9 : 1) to achieve the pure reddish-brown oil **T1** (second chromatographic band, 0.3024 g, yield 53.76%) and **T2** (first chromatographic band, 0.1632 g, yield 30.06%).

T1 : ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 10.67 – 10.51 (m, 2H, *meso*-H), 10.13, 10.11 (s, 2H, *meso*-H), 6.23 – 6.18 (m, 2H, O$\underline{CH}$), 4.50 – 4.41 (m, 4H, $\underline{CH_2}CH_2COO$), 4.18 – 4.08 (m, 4H, $COO\underline{CH_2}CH_2$), 3.96 – 3.89, 3.88 – 3.83, 3.80 – 3.78, 3.70 – 3.68, 3.64 – 3.53 (m, 16H, $CHO\underline{CH_2CH_2}O\underline{CH_2CH_2}OH$), 3.72, 3.71 (s, 6H, $\underline{CH_3}$), 3.67, 3.66

(s, 6H, $\underline{CH_3}$), 3.35 – 3.27 (m, 4H, $CH_2\underline{CH_2}COO$), 3.25 – 3.14, 2.98 – 2.68 (m, 6H, $COOCH_2\underline{CH_2}O\underline{CH_2CH_2}OH$), 2.30 (d, J = 6.6 Hz, 6H, $CH\underline{CH_3}$), 1.53 – 1.45 (m, 2H, $CH_2\underline{CH_2}CH_2CH_3$), 1.24 – 1.17 (m, 2H, $CH_2CH_2\underline{CH_2}CH_3$), 0.74 (t, J = 7.4 Hz, 3H, $CH_2CH_2CH_2\underline{CH_3}$), -3.74 (s, 2H, pyrrole-H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 173.43, 173.24, 139.74 – 136.88 (16C, C pyrrole), 98.91, 98.13, 96.99, 96.53, 73.83, 73.78, 72.53, 72.41, 71.78, 71.69, 71.44, 70.86, 68.75, 68.67, 64.62, 63.59, 61.78, 37.16, 36.99, 30.58, 29.73, 25.45, 21.91, 19.01, 13.59, 11.87, 11.80, 11.74, 11.65. FTIR (cm^{-1}): 3406, 3310, 2956, 2917, 2864, 1728, 1665, 1610 1451, 1418, 1371, 1347, 1292, 1268, 1227, 1163, 1120, 1061, 988, 945, 903, 886, 865, 835, 795, 741, 731, 708, 692, 678. HRMS (ESI, positive): calcd for $C_{50}H_{70}N_4O_{12}$ $[M+H]^+$: 919.5068. Found: 919.5070. UV-vis: λ_{max}, nm: 395, 503, 536, 570, 627. Elemental Anal. Calcd for $C_{50}H_{70}N_4O_{12}$ (%): C, 65.34; H, 7.68; N, 6.10. Found: C, 65.38; H, 7.71; N, 6.02. HPLC purity: 95.37% (t_R = 6.26 and 6.36 min).

T2: ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 10.60 – 10.50 (m, 2H, *meso*-H), 10.12, 10.11 (s, 2H, *meso*-H) , 6.24 – 6.16 (m, 2H, O$\underline{CH}$), 4.42 (t, J = 7.8 Hz, 4H, $\underline{CH_2}CH_2COO$), 4.12 – 4.04 (m, 4H, $\underline{CH_2}CH_2CH_2CH_3$), 3.97 – 3.88, 3.87 – 3.82, 3.81 – 3.75, 3.69 – 3.67, 3.64 – 3.54 (m, 16H, $CHO\underline{CH_2CH_2}O\underline{CH_2CH_2}OH$), 3.72, 3.70 (s, 6H, $\underline{CH_3}$), 3.67, 3.66 (s, 6H, $\underline{CH_3}$), 3.30 (t, J = 7.9, 4H, $CH_2\underline{CH_2}COO$), 2.29 (d, J = 6.6 Hz, 6H, $CH\underline{CH_3}$), 1.51 – 1.45 (m, 4H, $CH_2\underline{CH_2}CH_2CH_3$), 1.23 – 1.16 (m, 4H, $CH_2CH_2\underline{CH_2}CH_3$), 0.73 (t, J = 7.4 Hz, 6H, $CH_2CH_2CH_2\underline{CH_3}$), -3.70 (s, 2H, pyrrole-H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 173.32, 173.30, 139.72 – 136.14 (16C, C pyrrole), 98.61, 98.29, 96.97, 96.43, 73.85, 73.79, 72.54, 72.50, 72.46, 72.42, 70.86, 68.66, 64.58, 61.85, 61.80, 37.17, 30.59, 29.75, 29.36, 27.25, 25.43, 25.38, 25.30, 22.73, 21.90, 19.01, 14.17, 13.59, 11.87, 11.79, 11.73, 11.66. FTIR (cm^{-1}): 3422, 3310, 2956, 2926, 2866, 1728, 1665, 1607 1451, 1418, 1378, 1348, 1268, 1228, 1162, 1088, 1063, 988, 944, 903, 887, 833, 790, 741, 734, 708, 692, 678. HRMS (ESI, positive): calcd for $C_{50}H_{70}N_4O_{10}$ $[M+H]^+$: 887.5170. Found: 887.5175. UV-vis: λ_{max}, nm: 398, 503, 535, 572, 624. Elemental Anal. Calcd for $C_{50}H_{70}N_4O_{10}$ (%): C, 67.70; H, 7.95; N, 6.32. Found: C, 67.63; H, 7.88; N, 6.41. HPLC purity: 98.33% (t_R = 7.32 min).



Scheme S1. Synthesis routes of T0, T1 and T2.

2. Figures and Tables

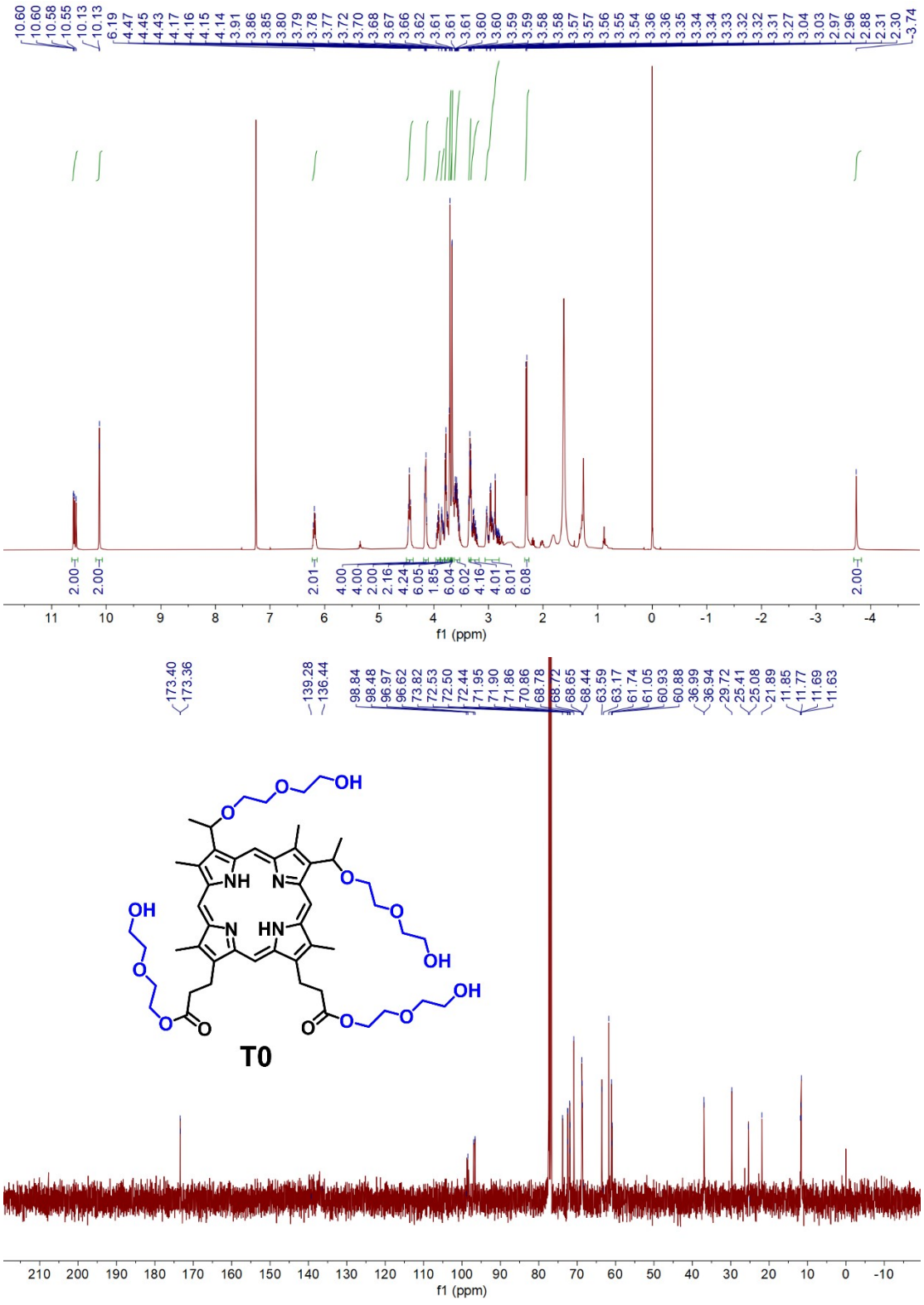


Figure S1. ^{1}H (top) and ^{13}C (bottom) NMR spectra of **T0** in deuterated chloroform ($CDCl_3$).

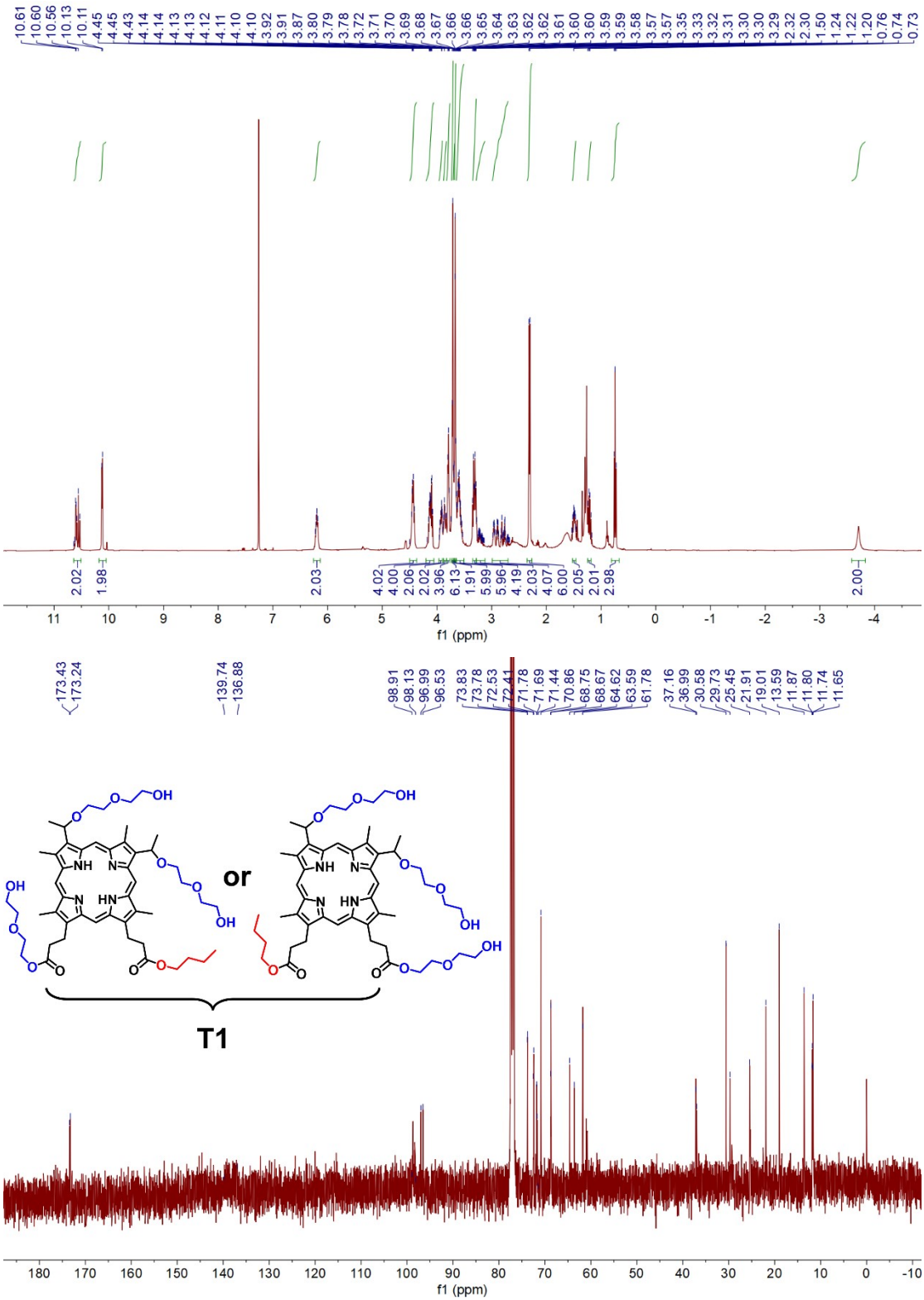


Figure S2. ^{1}H (top) and ^{13}C (bottom) NMR spectra of **T1** in $CDCl_3$.

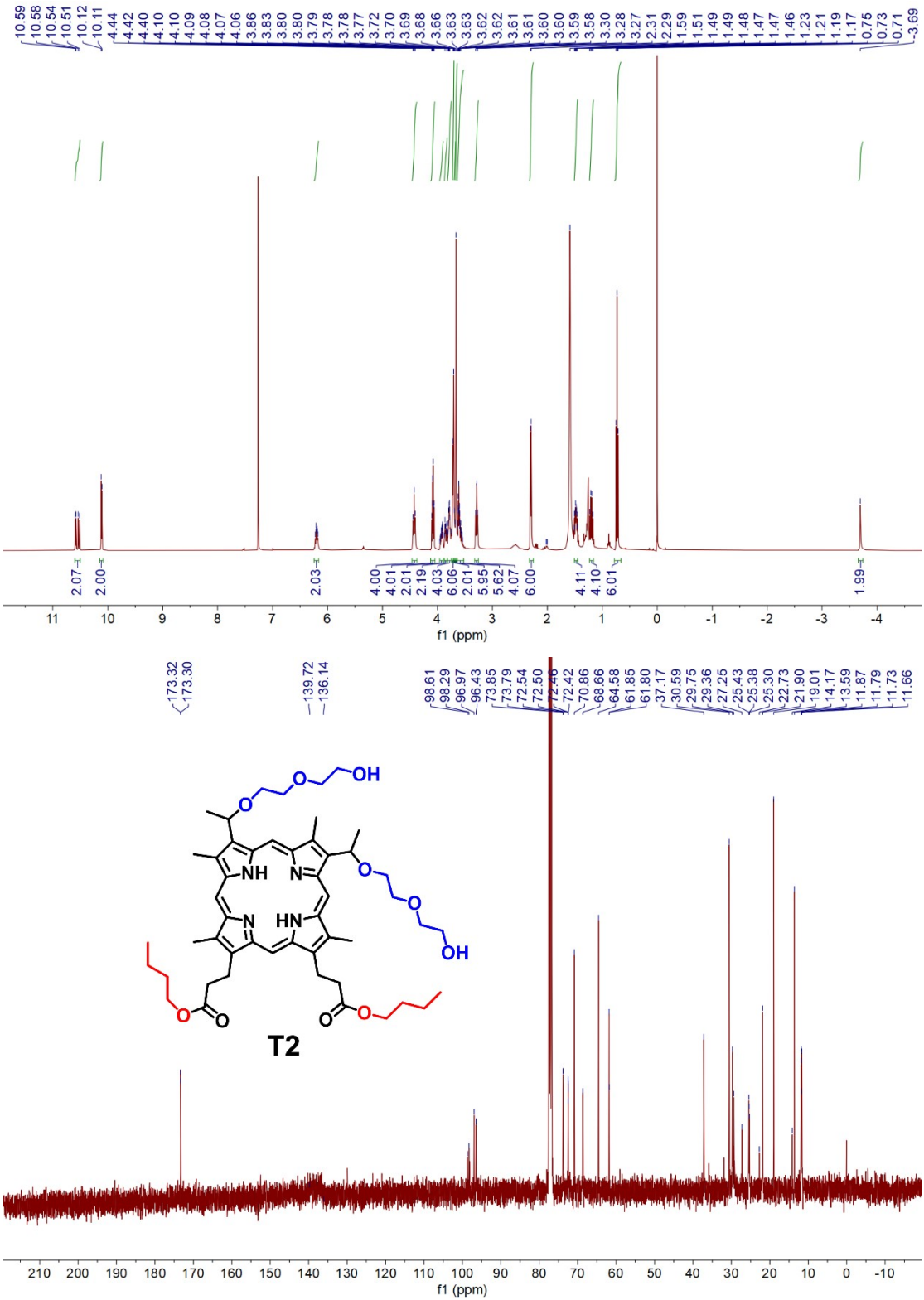


Figure S3. ^{1}H (top) and ^{13}C (bottom) NMR spectra of **T2** in $CDCl_3$.

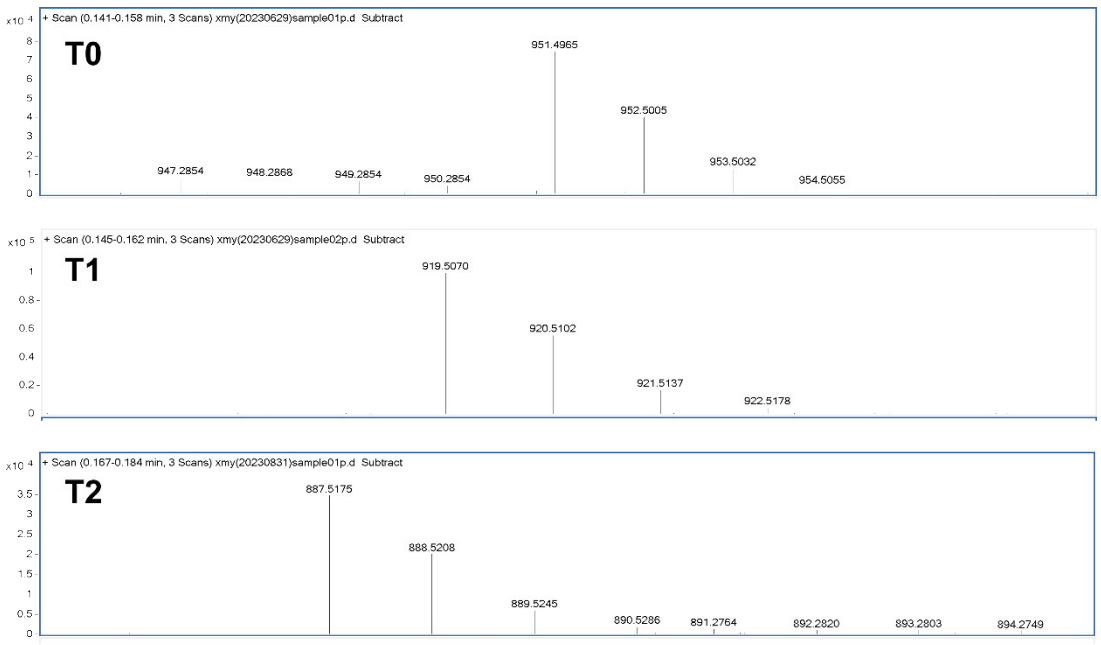


Figure S4. HRMS spectra of **T0**, **T1** and **T2**.

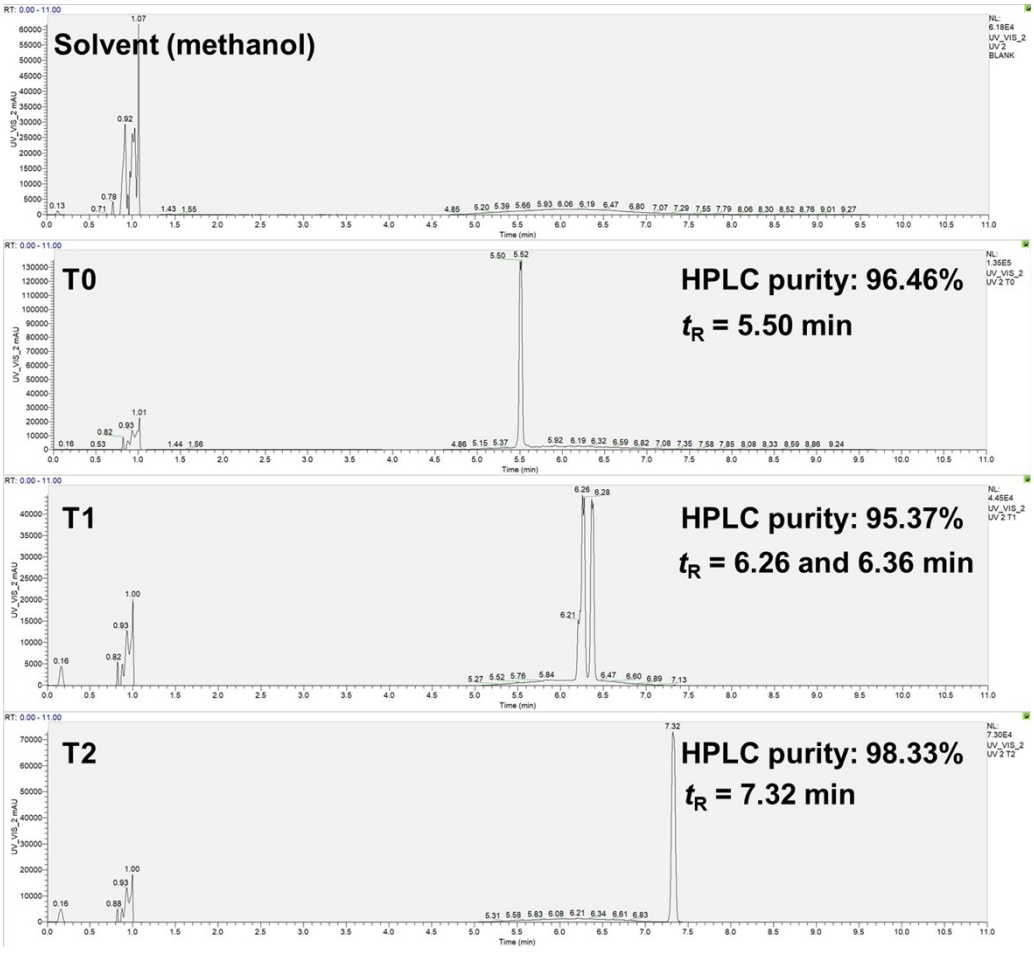


Figure S5. HPLC analysis of the solvent (methanol), **T0**, **T1** and **T2**.

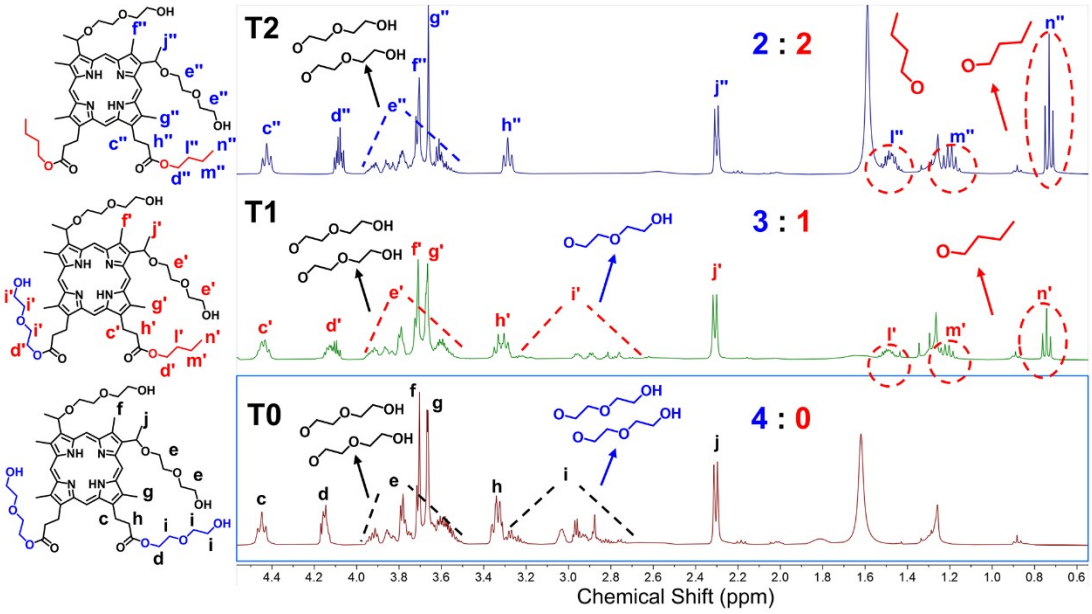


Figure S6. Local magnification of the ^{1}H NMR spectra of T0, T1 and T2.

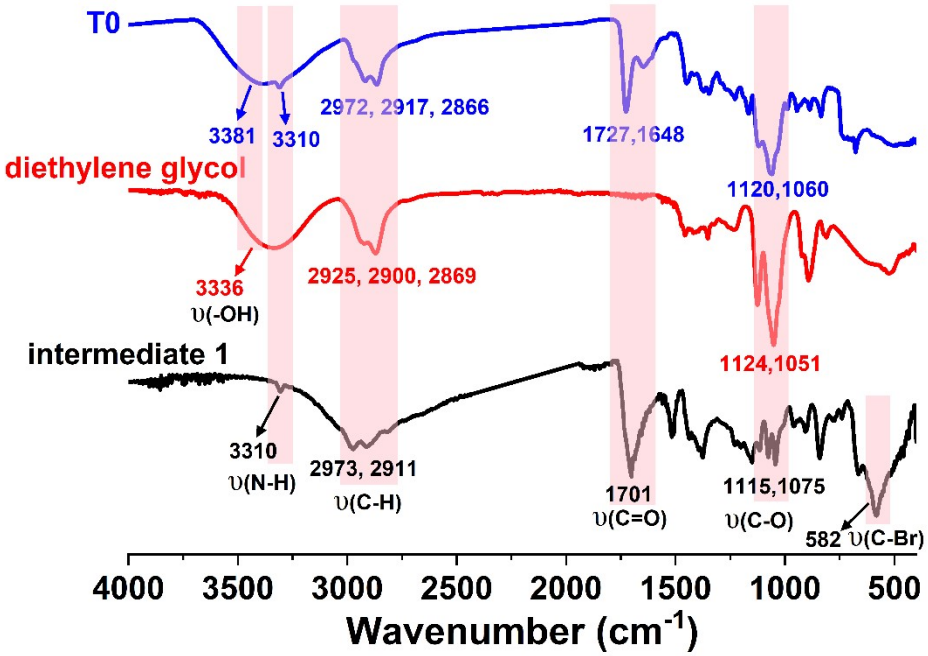


Figure S7. FTIR spectra of intermediate 1, diethylene glycol and **T0**.

Table S1. DLS data on the particle size stability of T0, T1 and T2 NPs for 1 day and 7 days, and their stability in the PBS buffer solutions with different pH values (pH = 5 – 9).

	T0 NPs		T1 NPs		T2 NPs	
Group	D_h (nm)	PDI	D_h (nm)	PDI	D_h (nm)	PDI
Day 1	65.3 ± 0.8	0.026 ± 0.023	135.0 ± 1.6	0.080 ± 0.007	210.1 ± 1.9	0.025 ± 0.017
Day 7	64.9 ± 2.2	0.034 ± 0.017	134.9 ± 0.3	0.104 ± 0.008	211.5 ± 4.5	0.009 ± 0.007
pH = 5	64.1 ± 4.0	0.030 ± 0.012	134.4 ± 0.6	0.071 ± 0.030	208.9 ± 6.5	0.030 ± 0.029
pH = 6	65.1 ± 3.0	0.035 ± 0.031	135.3 ± 3.9	0.139 ± 0.017	213.0 ±1.9	0.062 ± 0.031
pH = 7	65.5 ± 3.0	0.043 ± 0.019	134.8 ± 1.2	0.108 ± 0.011	212.7 ± 0.9	0.069 ± 0.007
pH = 8	65.5 ± 1.9	0.036 ± 0.024	134.8 ± 2.2	0.142 ± 0.055	212.9 ± 1.2	0.074 ± 0.011
pH = 9	66.3 ± 4.6	0.039 ± 0.008	135.3 ± 1.7	0.105 ± 0.065	212.7 ± 0.9	0.072 ± 0.006

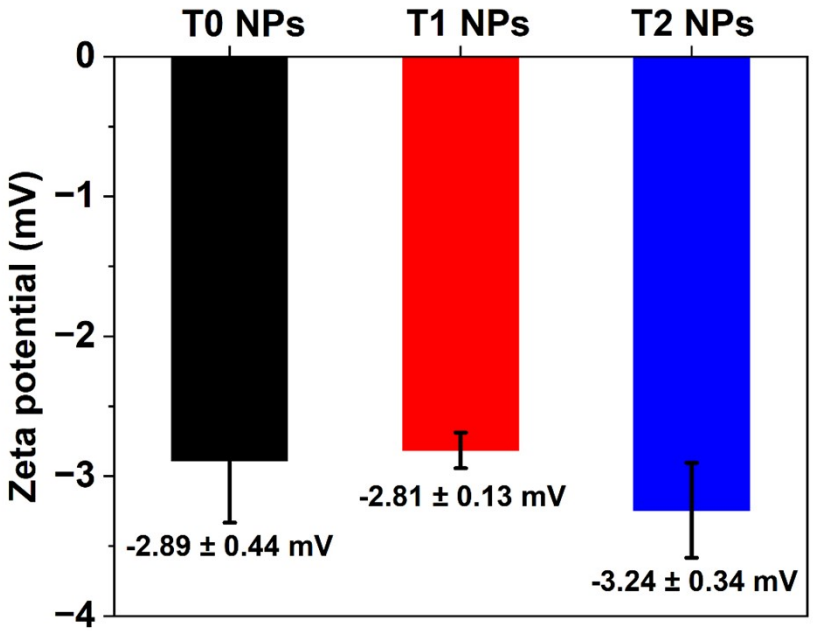


Figure S8. Zeta potential of **T0, T1** and **T2 NPs**.

Table S2. Photophysical data of **T0**, **T1** and **T2 NPs** in H_2O and RPMI-1640 medium.

Solvents	Samples	$\lambda_{max,\ abs}$ (nm)	Q-bands (nm)	$\lambda_{max,\ em}$ (nm)
H_2O	T0 NPs	390	503, 537, 570, 621	618
	T1 NPs	395	503, 536, 570, 627	620
	T2 NPs	398	503, 535, 572, 624	653
RPMI-1640 medium	T0 NPs	396	502, 536, 569, 623	620
	T1 NPs	401	501, 534, 569, 624	627
	T2 NPs	403	501, 533, 570, 625	627

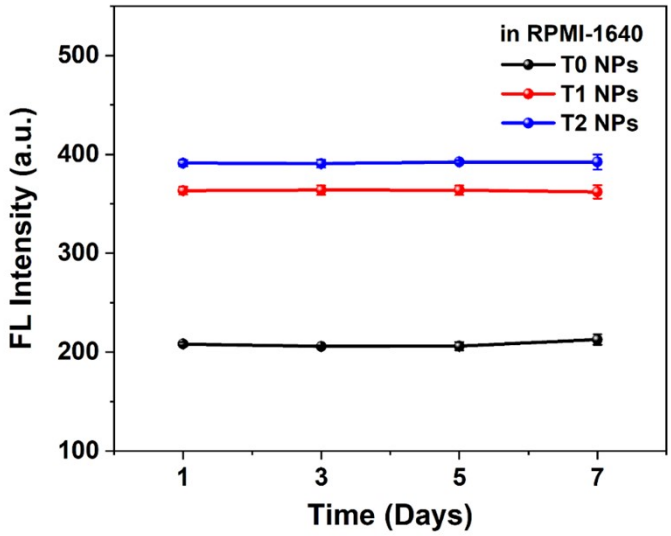


Figure S9. Fluorescence stability of T0, T1, and T2 NPs in RPMI-1640 medium for a week. The data are taken from the fluorescence intensity at the maximum emission wavelength. The concentration of T0, T1, and T2 NPs was 10 μM, and the excitation wavelength was set at 390 nm.

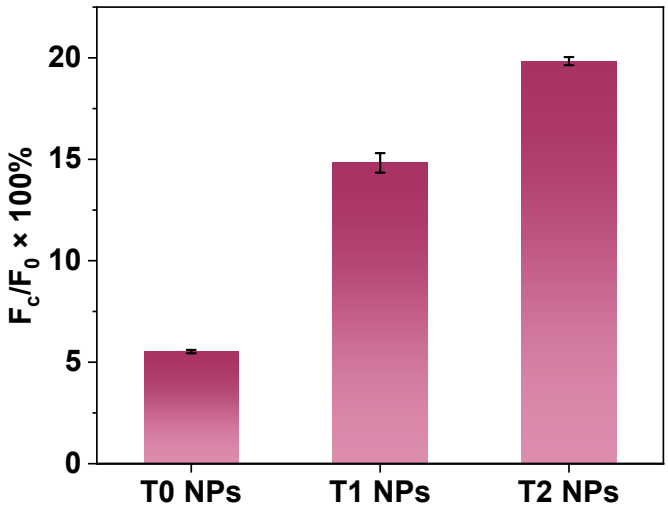


Figure S10. The fluorescence intensity ratio (F_c/F_0) of T0, T1 and T2 NPs. F_c is the intracellular fluorescence intensity at 625 nm after 3 h co-incubation with RPMI-1640 medium containing 2 μM of T0, T1, and T2 NPs. F_0 is the fluorescence intensity of RPMI-1640 medium containing 2 μM of T0, T1, and T2 NPs at 625 nm.

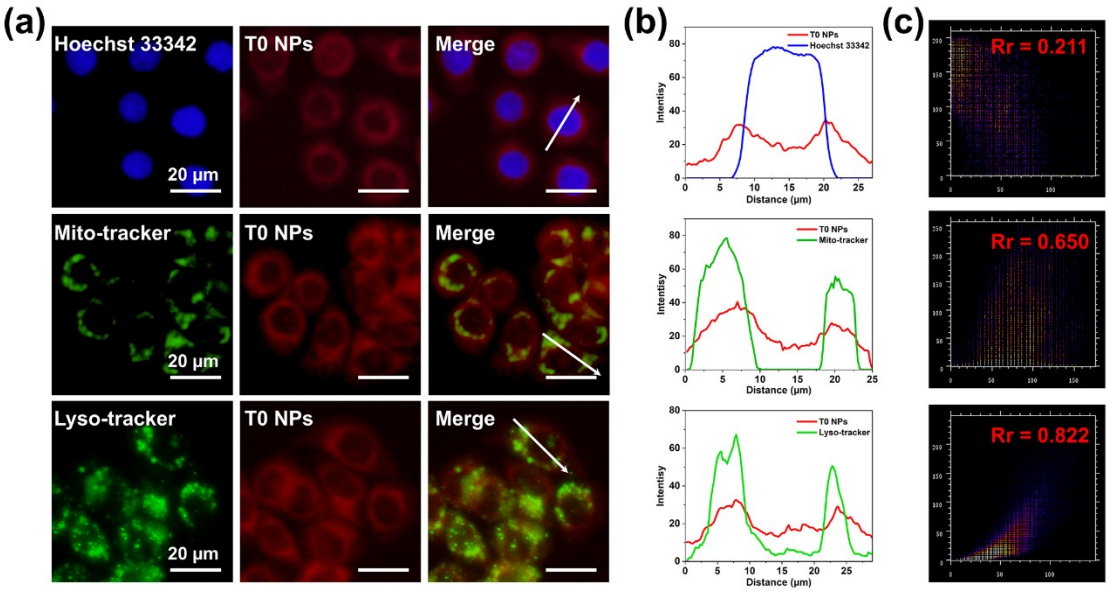


Figure S11. (a) Subcellular localization of T0 NPs in HT-29 cells. (b) Comparison of the fluorescence intensity profiles of probes and T0 NPs. Scale bar: 20 μm. (c) Pearson's correlation coefficients calculated based on (a).

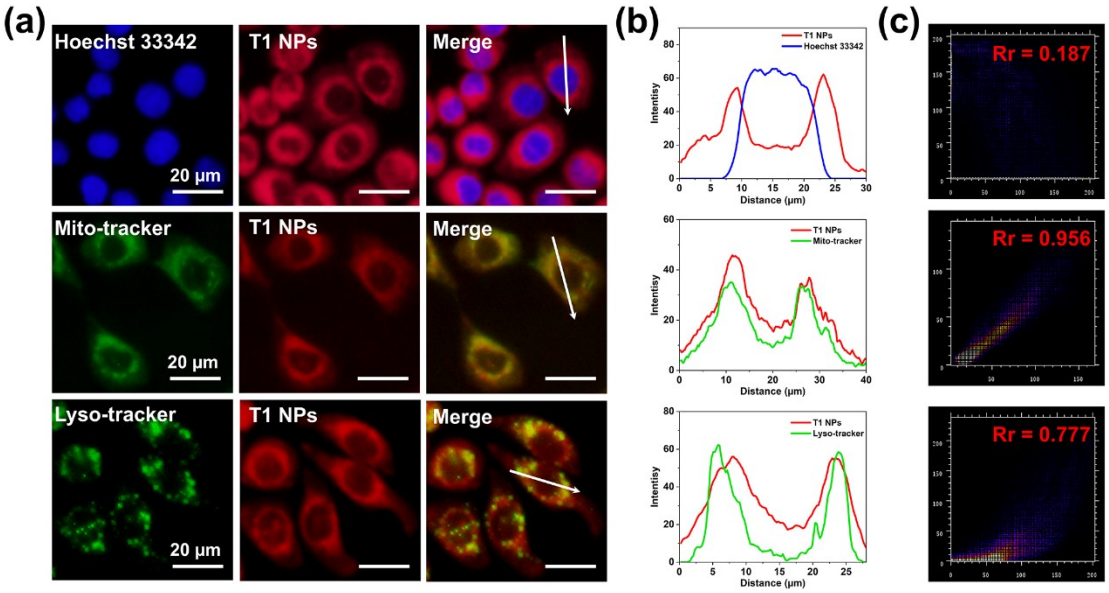


Figure S12. (a) Subcellular localization of T1 NPs in HT-29 cells. (b) Comparison of the fluorescence intensity profiles of probes and T1 NPs. Scale bar: 20 μm. (c) Pearson's correlation coefficients calculated based on (a).

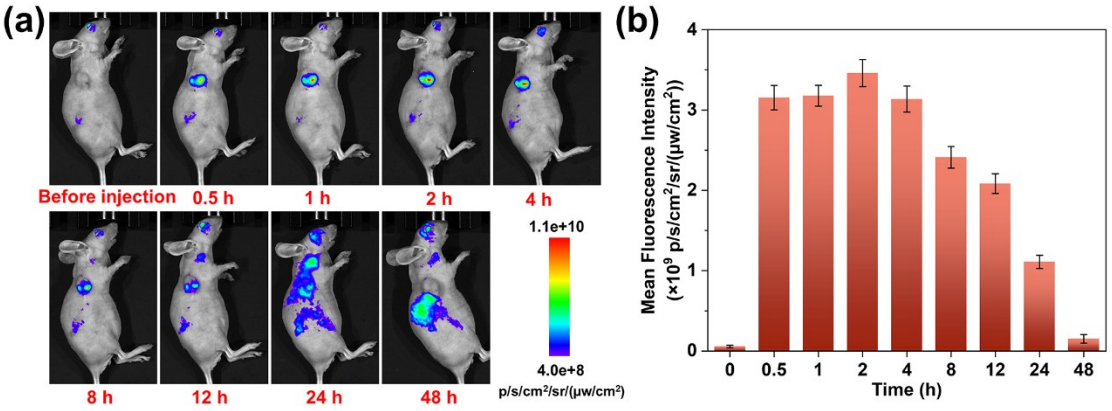


Figure S13. (a) Fluorescence images of HT-29 tumor-bearing mice at different times after T2 NPs injection (20 mg kg^{-1}, 100 μL), and (b) the statistical data at the tumor site.

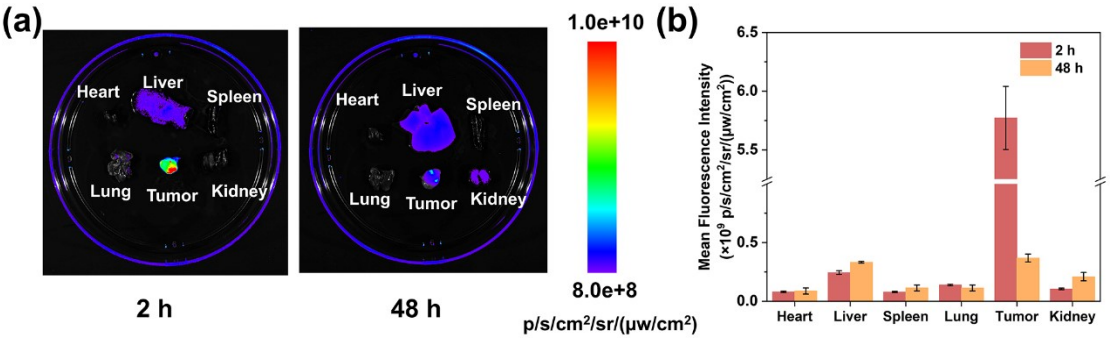


Figure S14. (a) T2 NPs distribution in the major organs and tumors of mice collected at 2 h and 48 h post injection, and (b) the corresponding statistical data.

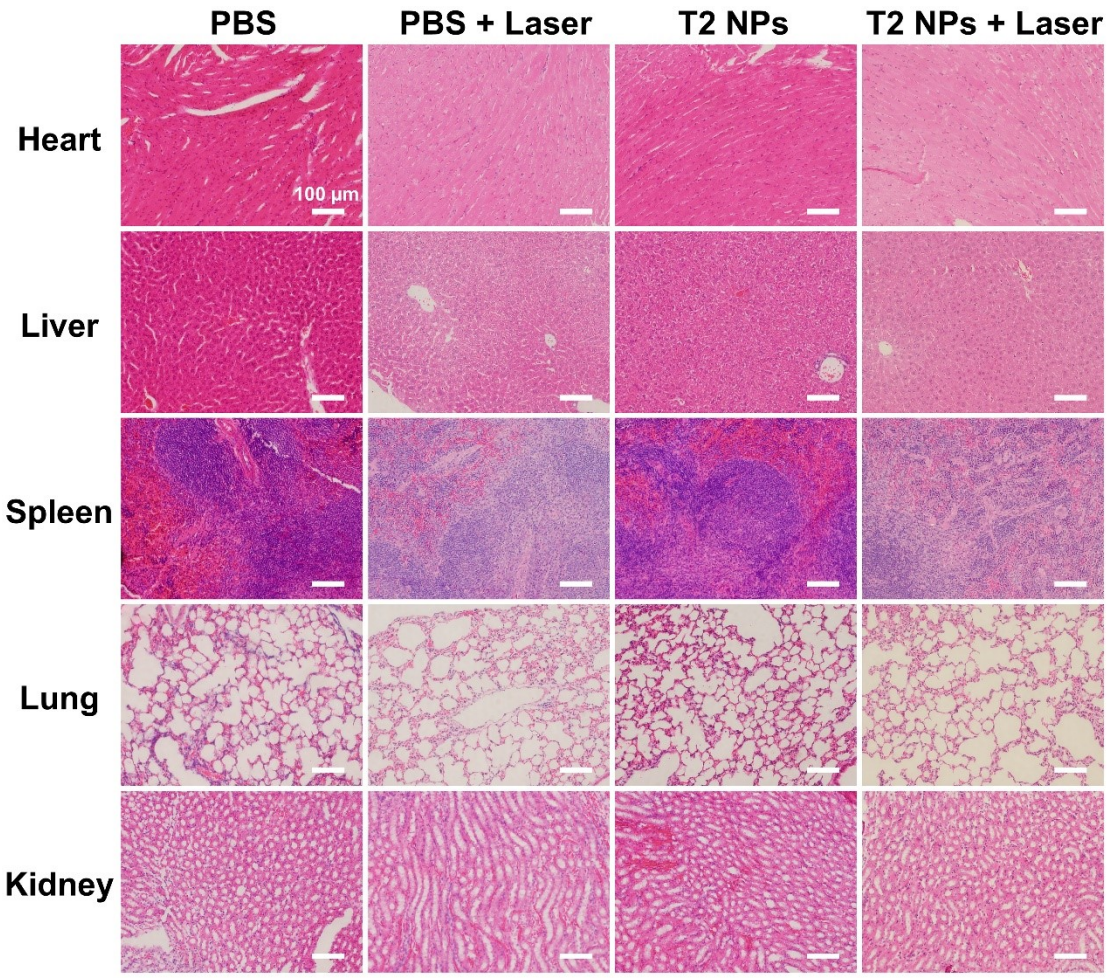


Figure S15. H&E staining images of the major organs (heart, liver, spleen, lung and kidney) of mice in the different treatment groups. Scale bar: 100 μm.

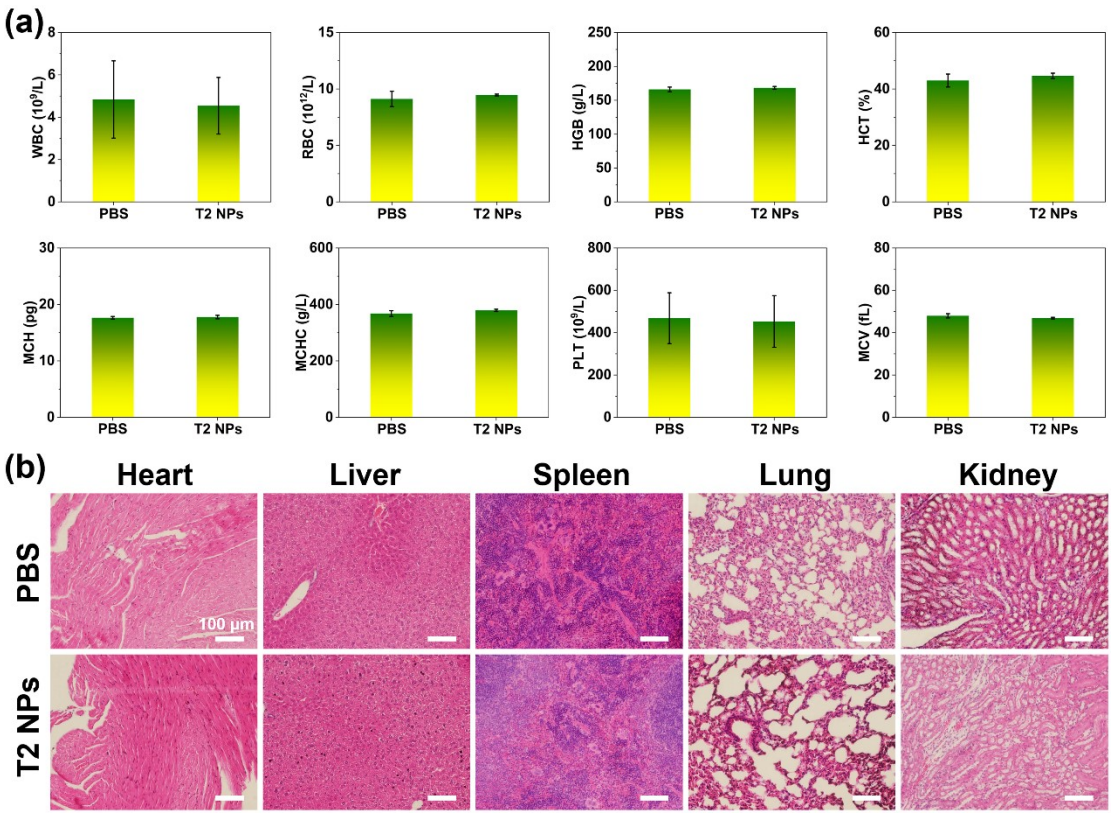


Figure S16. Toxicological tests of T2 NPs in healthy mice. (a) Blood routine data (n = 3) and (b) H&E staining images of the major organs of mice on the 5th day after intravenous injection of T2 NPs (20 mg kg^{-1}, 100 μL) and PBS (100 μL). Scale bar: 100 μm.

3. References

S1 J. Chen, C. Zhang, W. Nie, L. Liu, Q. Zhou and W. Cao, *Anal. Methods*, 2025, **17**, 4351-4358.