Electronic Supplementary Material (ESI) for Polymer Chemistry.

Synthesis of indocyanine green functionalized comblike poly(aspartic acid) derivatives for enhanced cancer cell ablation by targeting endoplasmic reticulum

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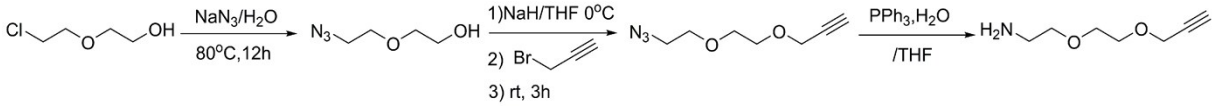
Materials.

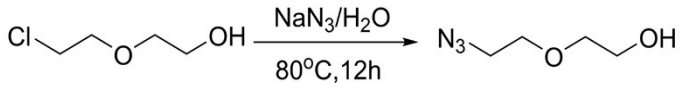
Phosphoric acid, sulfolane, L-aspartic acid, 2-(2-chloroethoxy)ethanol, sodium hydride, triphenylphosphine, methoxypoly(ethylene glycol) (Mn~1000), p-toluenesulfonyl, ethyl iodide, 1-Bromo-6-chlorohexane, sodium iodide and Cremophor® EL were obtained from Aladdin (Shanghai, China). NaCl, Na_2SO_4, $MgSO_4$, NH_4Cl, sodium hydroxide, HCl, diethyl ether, methanol, ethanol, ethyl acetate, tetrahydrofuran, dichloromethane, pyridine, trimethylamine, acetone, dimethyl formamide, acetonitrile, acetic anhydride were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Mesitylene and 1,1,2-trimethylbenz[e]indole were purchased from Energy Company (Shanghai, China). Propargyl bromide was purchased from Tokyo Chemical Industry (Shanghai, China). Glutaconaldehyde dianil hydrochloride was purchased from J&K Chemical Ltd. (Shanghai, China). Paclitaxel (PTX) was purchased from Adamas Reagent, Ltd. (Shanghai, China). Poly-ε-lysine was obtained from Zhengzhou BAINAFO Bioengineering Co., LTD. 2',7'-Dichlorofluorescin diacetate (DCFH-DA), bisbenzimide H 33342 trihydrochloride (Hochest 33342), DMSO-d6, $CDCl_3$, McCoy's 5A medium, minimum essential medium (with non-essential amino acids), phosphate buffered saline (PBS, 1 X), penicillin-streptomycin (10,000 units penicillin and 10 mg streptomycin/ml), and trypsin-EDTA solution (2.5 g porcine trypsin and 0.2 g EDTA·4Na per liter of Hanks' balanced salt solution with phenol red) were obtained from Sigma-Aldrich Co. LLC. (Shanghai, China). Fetal bovine serum (FBS) was purchased form GIBCO BRL (Grand Island, NY). ER tracker green (DiOC6(3)) was purchased from KeyGEN BioTECH (Jiangsu, China). Cell Counting Kit-8 and Annexin V, FITC Apoptosis Detection Kit were purchased from Dojindo (Shanghai, China). Deionized water was used in all experiments. All other chemicals were available commercially and used without further purifications.

Synthetic details

Part I: Synthesis of 2-(2-(prop-2-ynyloxy)ethoxy)ethanamine

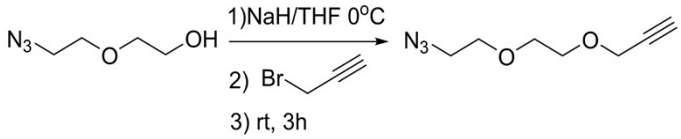


1.1 Synthesis of 2-(2-azidoethoxy)ethanol



2-(2-chloroethoxy)ethanol (6.20 g, 50 mmol, 1.0 equiv.) and NaN_3 (6.50g, 100 mmol, 2.0 equiv.) were dissolved in deionized water (25 ml). The reaction mixture was heated to 80 °C and stirred under N_2 atmosphere for 12 hours. After cooling to room temperature, the solution was extracted with diethyl ether (5×20 ml). Then organic phases were combined and washed with saturated NaCl, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to yield 2-(2-azidoethoxy)ethanol (6.00 g, 45.8 mmmol, 92 %). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 3.83 – 3.74 (m, 2H), 3.74 – 3.67 (m, 2H), 3.67 – 3.58 (m, 2H), 3.42 (t, J = 4.9 Hz, 2H), 1.95 (s, 1H).

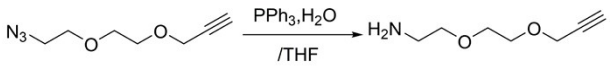
1.2 Synthesis of 3-(2-(2-azidoethoxy)ethoxy)prop-1-yne [1]



NaH (3.66g, 91.6 mmol, 2.0 equiv. 60% dispersion in mineral oil) was added to 250 ml round-bottom flask and rinsed with THF for three times to remove mineral oil, then THF (100 ml) was added, the suspension was cooled down to 0 °C. 2-(2-azidoethoxy)ethanol (6.0 g, 45.8 mmol, 1.0 equiv.) was slowly added to the suspension (hydrogen were generated). In the resulting slurry, propargyl bromide (6.54 g, 54.9 mmol, 1.20 equiv.) was added slowly, then reaction mixture was gradually warm up to room temperature and stirred for additional 3 hours. The reaction slurry turned brownish after reaction was finished. The reaction was then re-cooled to 0 °C, water (6 ml) was slowly added to the reaction mixture, solvent was evaporated under reduced pressure. Saturated NH_4Cl (20 ml) was added and the resulting slurry was extracted with CH_2Cl_2 (5×20 ml). The organic phases were combined and dried with anhydrous

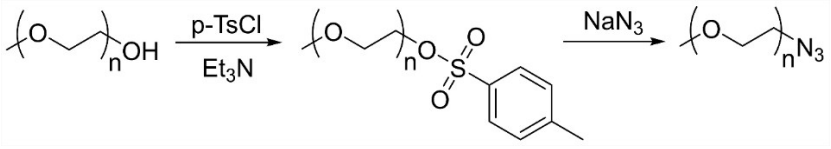
Na_2SO_4, filtered and evaporated under reduced pressure. Crude product was purified by silica gel chromatography (CH_2Cl_2/CH_3OH, 95/5, v/v) to yield 3-(2-(2-azidoethoxy)ethoxy)prop-1-yne as a light brown oil (4.88 g, 28.8 mmol, 63 %). ^1H NMR (400 MHz, $CDCl_3$, δ): 4.22 (d, J = 2.4 Hz, 2H), 3.78 – 3.65 (m, 6H), 3.41 (t, J = 5.0 Hz, 2H), 2.44 (t, J = 2.3 Hz, 1H).

1.3 Synthesis of 2-(2-(prop-2-ynyloxy)ethoxy)ethanamine

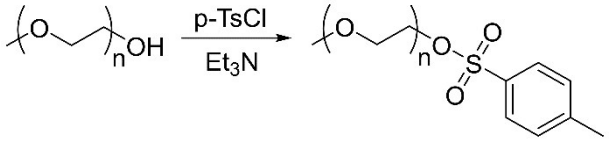


3-(2-(2-azidoethoxy)ethoxy)prop-1-yne (3.08 g, 18.2 mmol, 1.0 equiv.) was dissolved in THF (50 ml), triphenylphosphine (5.25g, 20.0 mmol, 1.1 equiv.) and H_2O (665 μL, 2.0 equiv.) were added to the reaction mixture, and stirred at room temperature for 10 hours. After reaction was completed, 1 M HCl (54 ml) was added to acidify the solution, and THF was evaporated under reduced pressure, unreacted triphenylphosphine and byproduct triphenylphosphine oxide were extracted by EtOAc. The resulting aqueous solution was basified by adding 1 M NaOH (64.8 ml), and extracted with CH_2Cl_2 for 5 times. The combined organic layer was washed with saturated NaCl, dried under anhydrous $NaSO_4$. Then filtered and evaporated under reduced pressure to yield 2-(2-(prop-2-ynyloxy)ethoxy)ethanamine as light yellow oil (1.68 g, 13.3 mmol, 64.5 %). ^1H NMR (400 MHz, $CDCl_3$, δ): 4.21 (d, J = 2.2 Hz, 2H), 3.71 (dd, J = 5.7, 3.2 Hz, 2H), 3.65 (dd, J = 5.8, 3.1 Hz, 2H), 3.52 (t, J = 5.2 Hz, 2H), 2.87 (t, J = 5.2 Hz, 2H), 2.47 (t, J = 2.4 Hz, 1H), 1.47 (s, 2H).

Part II: ***Synthesis of azido modified mPEG (mPEG-N_3, Mn~1000)***



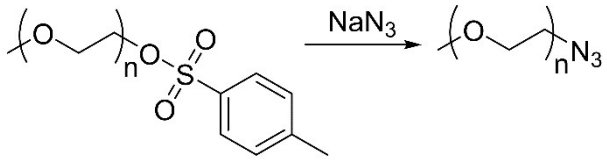
2.1 Synthesis of mPEG-OTs



Methoxypoly(ethylene glycol) (25.0 g, 25.0 mmol, Mn~1000) was heated to 80 ^oC under vacuum with stirring for 2 h to remove traces of water, when cooled to room

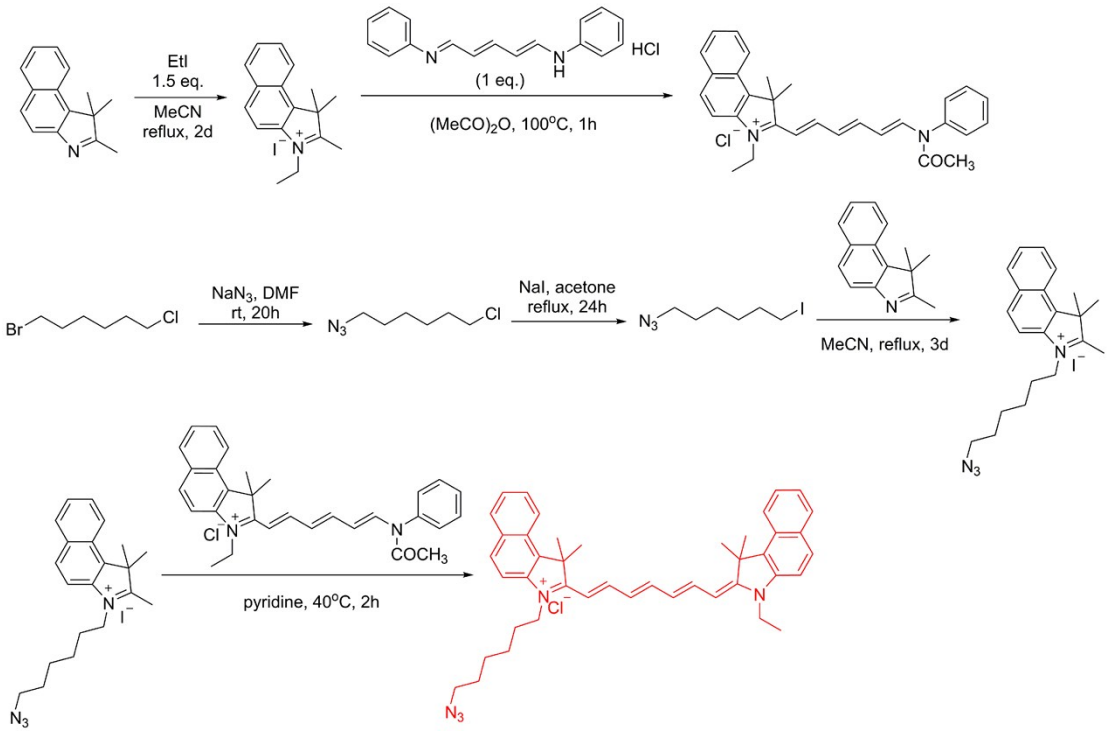
temperature, DCM (150 ml) and trimethylamine (8.71 ml, 62.5 mmol) were added, then the reaction was cooled to 0 °C, p-toluenesulfonyl (7.9 g, 40 mmol) was added. After addition was completed, the reaction was gradually warm to room temperature, and allowed for further reaction for 20 h. The reaction mixture was washed with 10 % NaOH and excess saturated NaCl successively, and dried with $NaSO_4$. The $NaSO_4$ was filtered out and the filtrate was evaporated under reduced pressure to yield crude product mPEG-OTs (28.8 g, 99 %) as light yellow solid.

2.2 Synthesis of azido modified mPEG

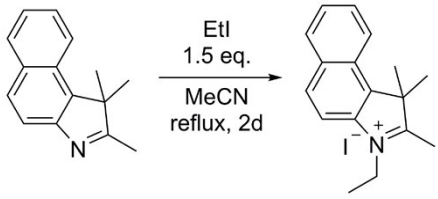


The obtained mPEG-OTs (28.8g) was dissolved in DMF (80 ml), then NaN_3 (2.44 g, 37.5 mmol, 1.50 equiv.) was added and stirred at 85 °C for 12 h, and the solvent was evaporated under reduced pressure. DCM was added and insoluble solids were filtered out, the filtrate was washed with saturated NaCl, then DCM was evaporated under reduced pressure. Water was added and the aqueous solution was extracted with ether to remove low molecule PEG, then extracted with DCM (4×20 ml), the combined organic layer was dried with $NaSO_4$, filtered and evaporated under vacuum to obtain mPEG-N_3 (22.57 g, 86.7 %).

Part III: Synthesis of N_3 modified indocyanine green

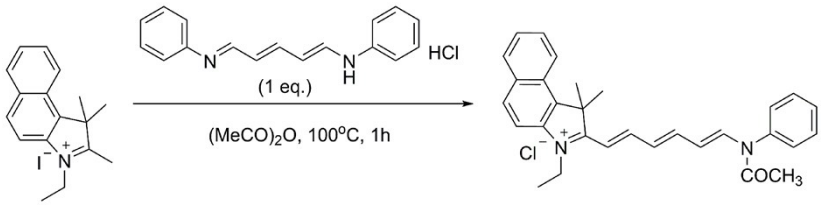


3.1 Synthesis of 1,1,2-Trimethyl-3-ethylbenz[e]indolium iodide salt[2]



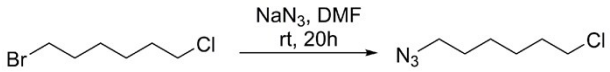
1,1,2-Trimethylbenz[e]indole (4.19 g, 20.0 mmol) was dissolved in acetonitrile (150 ml), then ethyl iodide (4.68 g, 30 mmol, 1.5 equiv.) was added in this solution and reflux for 2 days under N_2 atmosphere. After reaction completed, the resulted transparent dark green solution was evaporated under vacuum and the residue was repeatedly washed with ether to obtain compound 1,1,2-Trimethyl-3-ethylbenz[e]indolium iodide salt as light grey solids (6.15 g, 16.8 mmol, 84.2 %). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.18 – 8.01 (m, 3H), 7.85 (d, J = 8.9 Hz, 1H), 7.75 (t, J = 7.6 Hz, 1H), 7.68 (t, J = 7.5 Hz, 1H), 4.89 (q, J = 7.4 Hz, 2H), 3.25 (s, 3H), 1.88 (s, 6H), 1.69 (t, J = 7.4 Hz, 3H).

3.2 Synthesis of 2-[6-(N-Phenyl-N-acetylamino)-1,3,5-heptatrienyl]-1,1-di-methyl-3-ethyl-1H-benz[e]indolium chloride salt:



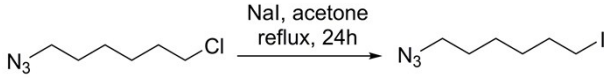
1,1,2-Trimethyl-3-ethylbenz[e]indolium iodide salt (3.65 g, 10.0 mmol) and glutaconaldehyde dianil hydrochloride (2.85 g, 10.0 mmol) were dissolved in acetic anhydride (73 ml), and the reaction mixture was stirred at 100 °C for 1 h under N_2 atmospehres. Excess acetic anhydride was evaporated under vacuum, the product was washed with excess ether and water, dissolved in ethanol and reprecipitated in ether, then dried under vacuum to obtain 2-[6-(N-Phenyl-N-acetylamino)-1,3,5-heptatrienyl]-1,1-di-methyl-3-ethyl-1H-benz[e]indolium chloride salt as dark purple solid (5.33 g, 95 %). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.24 – 8.12 (m, 2H), 8.05 (m, 2H), 7.94 (m, 1H), 7.70 (m, 2H), 7.62 (m, 2H), 7.55 (m, 3H), 7.17 (d, J = 6.5 Hz, 2H), 7.05 – 6.91 (m, 1H), 5.43 (dd, J = 13.6, 11.5 Hz, 1H), 4.89 (q, J = 7.1 Hz, 2H), 1.98 (s, 8H), 1.68 (s, 3H), 1.58 (t, J = 7.1 Hz, 3H). MALDI-TOF (m/z): $[M\text{-}Cl^-]^+$ calcd for $C_{43}H_{48}N_5$, 634.39; found, 634.33.

3.3 Synthesisi of 1-azido-6-chlorohexane[3]



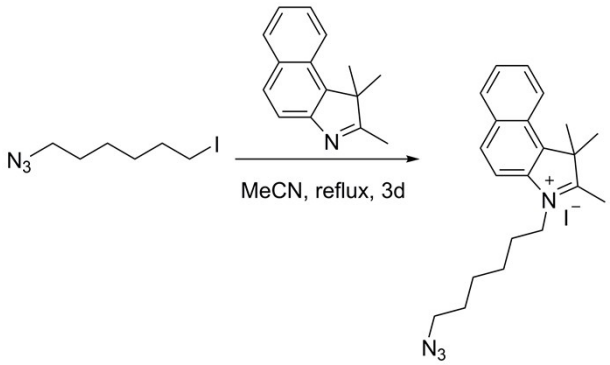
1-Bromo-6-chlorohexane (3.99 g, 20.0 mmol) was dissolved in DMF (25 ml), and then NaN_3 (1.30 g, 20.0 mmol) was added in the reaction mixture and the resulted heterogeneous mixture reacted for 20 h at room temperature. After reaction completed, H_2O (25 ml) was added and the resulting mixture was extracted with ether (40 ml×3). The combined organic phase was washed with water and saturated NaCl successively and dried with $MgSO_4$. After filtration, the filtrate was evaporated to give compound 1-azido-6-chlorohexane (3.13 g, 19.4 mmol, 97 %) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 3.55 (t, *J* = 6.6 Hz, 2H), 3.28 (t, *J* = 6.9 Hz, 2H), 1.84 – 1.74 (m, 2H), 1.67 – 1.57 (m, 2H), 1.53 – 1.36 (m, 4H).

3.4 Synthesis of 1-azido-6-iodohexane[3]



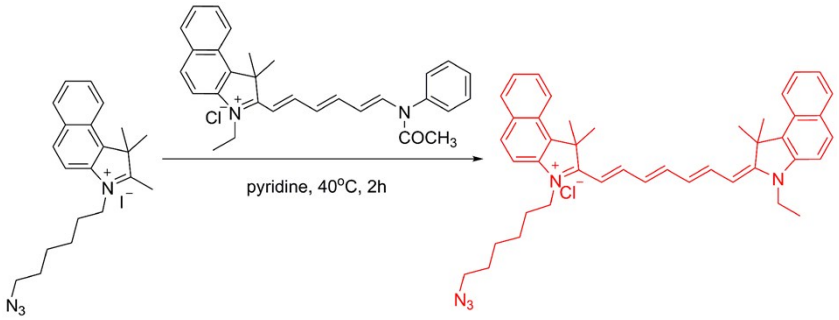
Azido-6-chlorohexane (3.00 g, 18.6 mmol) was dissolved in acetone (90 ml), then NaI (5.58 g, 37.2 mmol) was added in above solution, the heterogeneous mixture was refluxed under N_2 for 24 h, then concentrated under vacuum, and water (40 ml) was added. The aqueous solution was extracted with EtOAc (40 ml×3), the combined organic phase was washed with water and saturated NaCl successively, then dried with $MgSO_4$. After filtration, the filtrate was evaporated to give compound 1-azido-6-iodohexane (4.18 g, 16.5 mmol, 88.7 %) as light yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$) 3.28 (t, *J* = 6.9 Hz, 2H), 3.20 (t, *J* = 6.9 Hz, 2H), 1.90-1.74 (m, 2H), 1.70-1.56 (m, 2H), 1.55-1.34 (m, 4H).

3.5 Synthesis of 1,1,2-Trimethyl-3-(3-Azidohexyl)-benz[e]indolium iodide salt



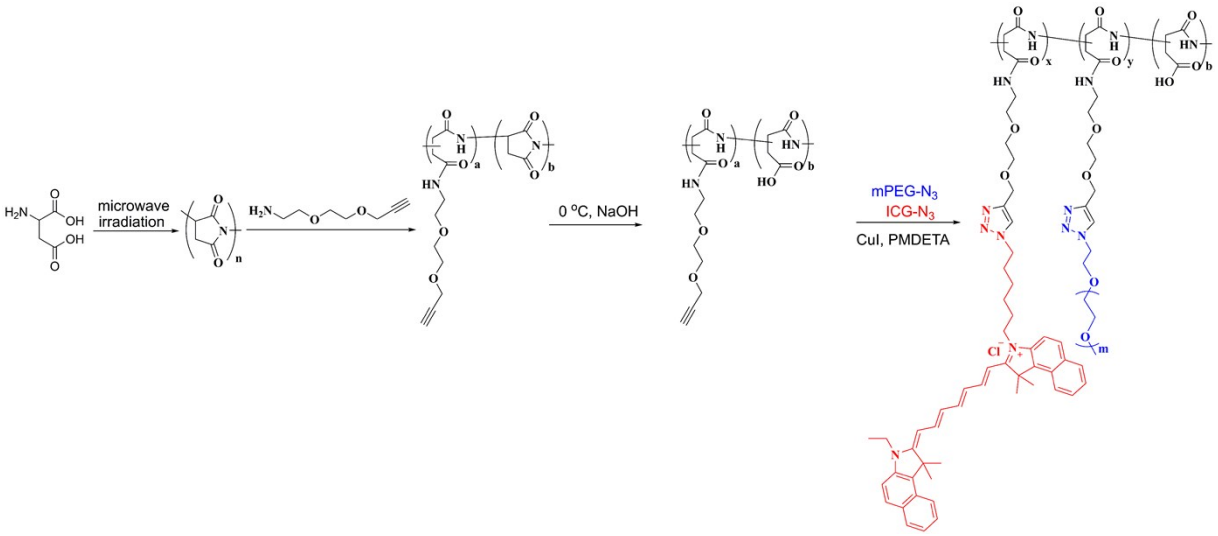
1,1,2-trimethylbenz[e]indole (2.09 g, 10.0 mmol) was dissolved in acetonitrile (30 ml), and then 1-azido-6-iodohexane (3.80 g, 15 mmol, 1.5 equiv.) was added in this solution and kept at 85 ^{o}C for 3 days under N_2 atmosphere. After reaction completed, the resulted transparent dark green solution was evaporated under vacuum and the residue was precipitated in ether. The resulted solid was repeatedly washed with ether to obtain compound 1,1,2-Trimethyl-3-(3-Azidohexyl)-benz[e]indolium iodide salt as light green soild (4.16 g, 90 %). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.17 – 8.01 (m, 3H), 7.82 (d, J = 8.9 Hz, 1H), 7.76 (t, J = 7.5 Hz, 1H), 7.69 (t, J = 7.5 Hz, 1H), 4.85 (t, J = 7.7 Hz, 2H), 3.29 (t, J = 6.5 Hz, 2H), 3.23 (s, 3H), 2.12 – 1.97 (m, 2H), 1.89 (s, 6H), 1.67 – 1.54 (m, 4H), 1.49 (m, 2H).

3.6 Synthesis of 2-[7-(1,3-Dihydro-1,1-dimethyl-3-ethylbenz[e]indolin-2-ylidene)-1,3,5-heptatrienyl]-1,1-dimethyl-3-(3-Azidohexyl)-1H-benz[e]indolium chloride salt (N_3 modified indocyanine green, abbreviated as ICG-N_3):

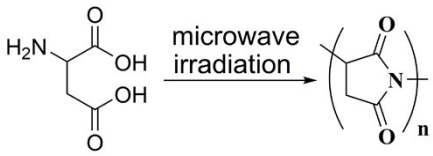


In pyridine (3 ml) solution of 1,1,2-Trimethyl-3-(3-Azidohexyl)-benz[e]indolium iodide salt (231 mg, 0.500 mmol), 2-[6-(N-Phenyl-N-acetylamino)-1,3,5-heptatrienyl]-1,1-di-methyl-3-ethyl-1H-benz[e]indolium chloride salt (236 mg, 0.500 mmol) was added, and the resulted solution was stirred at 40 °C for 2 h under nitrogen. After removal of the solvent under vacuum, the residue was precipitated by adding excess ether, the resulted green solid was purified by silica gel chromatography (CH_2Cl_2/CH_3OH, 95/5, v/v) to give azido modified ICG (ICG-N_3) as purple solids with metallic luster. ^{1}H NMR (400 MHz, DMSO- d_6) δ): 8.26 (d, J = 8.5 Hz, 2H), 8.07 (s, 6H), 7.81 (t, J = 12.8 Hz, 1H), 7.72 (d, J = 8.9 Hz, 2H), 7.65 (t, J = 7.6 Hz, 2H), 7.50 (t, J = 7.4 Hz, 2H), 6.59 (t, J = 12.5 Hz, 2H), 6.43 (dd, J = 13.8, 5.8 Hz, 2H), 4.44 - 4.06 (m, 4H), 1.92 (s, 12H), 1.76 (s, 2H), 1.61 - 1.25 (m, 9H). MALDI-TOF (m/z): $[M\text{-}Cl^-]^+$ calcd for $C_{43}H_{48}N_5$, 634.39; found, 634.33.

Part IV: Synthesis of PAsp-g-(PEG-ICG)

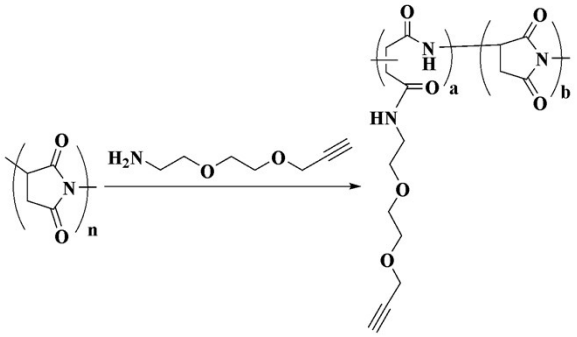


4.1 Synthesis of Polysuccinimide (PSI)



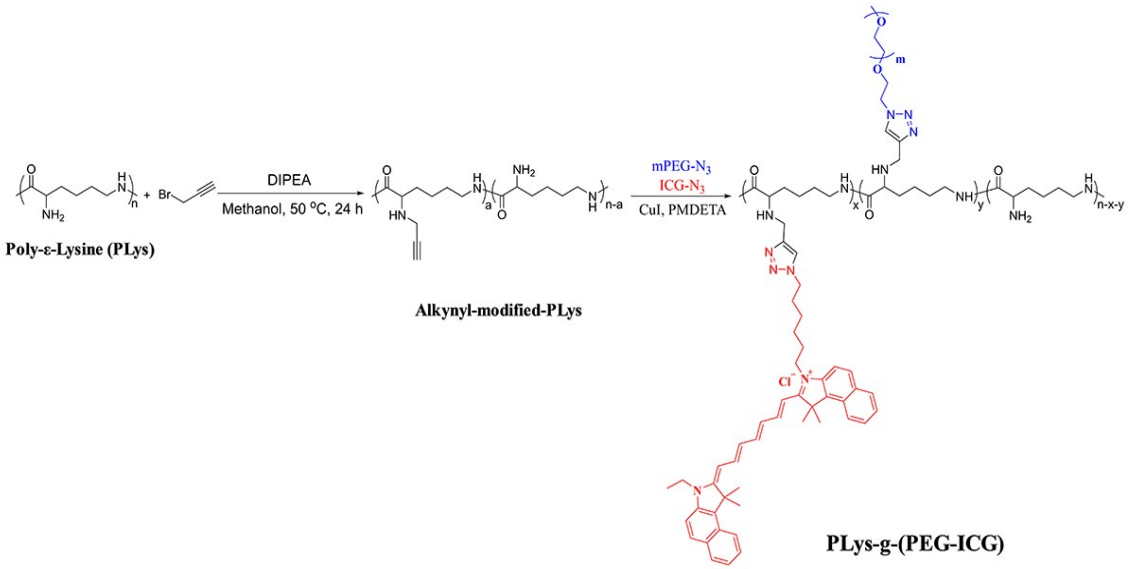
Phosphoric acid (277 mg, 2.40 mmol, 85 wt % in water) was dissolved in sulfolane (180 μL) in 35 ml pressurized vessels, L-aspartic acid (2.00 g, 15.0 mmol) were grounded into powder and mixed with the above solution. Mesitylene (5 ml) were added into the mixture, the sealed vessels was placed in automated microwave sample preparation system (DISCOVER CEM), then the vessel was heated to 190 °C under 150 W microwave irradiation and held at this temperature for 20 min. After cooled to room temperature, the resulted pink solid was dissolved in DMF (3 ml), insoluble solid was filtrated, then ethanol was added to the filtrate, and the white precipitate was obtained and washed with water until it was neutral. The resulting solid was dried under vacuum to obtain PSI (1.19 g, 12.2 mmol, 81.7 %), ^{1}H NMR (400 MHz, DMSO-d_6, δ): 5.27 (s, 1H), 3.20 (s, 1H), 2.70 (s, 1H).

4.2 Synthesis of Alkynyl modified PSI



Polysuccinimide (1.00 mmol, 97.1 mg) were dissolved in DMF (0.4 ml), and then 2-(2-(prop-2-ynyloxy)ethoxy)ethanamine (143.2 mg, 1.0 mmol, 1.0 equiv.) were added in above solution. The reaction mixture was stirred at room temperature for 12 h. After reaction completed, the resulting solution was precipitated by adding excess ether, the precipitate was isolated by centrifugation and dissolved in DMF (0.2 ml). After that the excess ether was added again, the precipitated was washed with ether repeatedly and dried under vacuum to obtain alkynyl modified polysuccinimide (PSI-g-alkynyl) 200 mg.

Part V: Synthesis Scheme of PLys-g-(PEG-ICG)



[1] A. X. Zhang, R. P. Murelli, C. Barinka, J. Michel, A. Cocleaza, W. L. Jorgensen, J. Lubkowski, D. A. Spiegel, *J. Am. Chem. Soc.* **2010**, *132*, 12711.

[2] T. Hirata, H. Kogiso, K. Morimoto, S. Miyamoto, H. Taue, S. Sano, N. Muguruma, S. Ito, Y. Nagao, *Bioorg. Med. Chem.* **1998**, *6*, 2179.

[3] S. Helmy, F. A. Leibfarth, S. Oh, J. E. Poelma, C. J. Hawker, J. Read de Alaniz, *J. Am. Chem. Soc.* **2014**, *136*, 8169.

TABLE

Table S1. DLC and EE of different PTX loaded PAsp-g-(PEG-ICG) micelles

	Polymer (mg)	PTX added (mg)	TDLC (wt %)	DLC (wt %)	EE (%)
micelle-20	10.15	2.56	20.1	16.4	81.3
micelle-25	9.95	3.41	25.5	23.9	93.6
micelle-30	10.16	4.31	29.8	28.2	94.8

Note: TDLC refer to theoretical drug loading capacity, DLC refer to actual drug loading capacity, EE refer to entrapment efficiency.

FIGURES

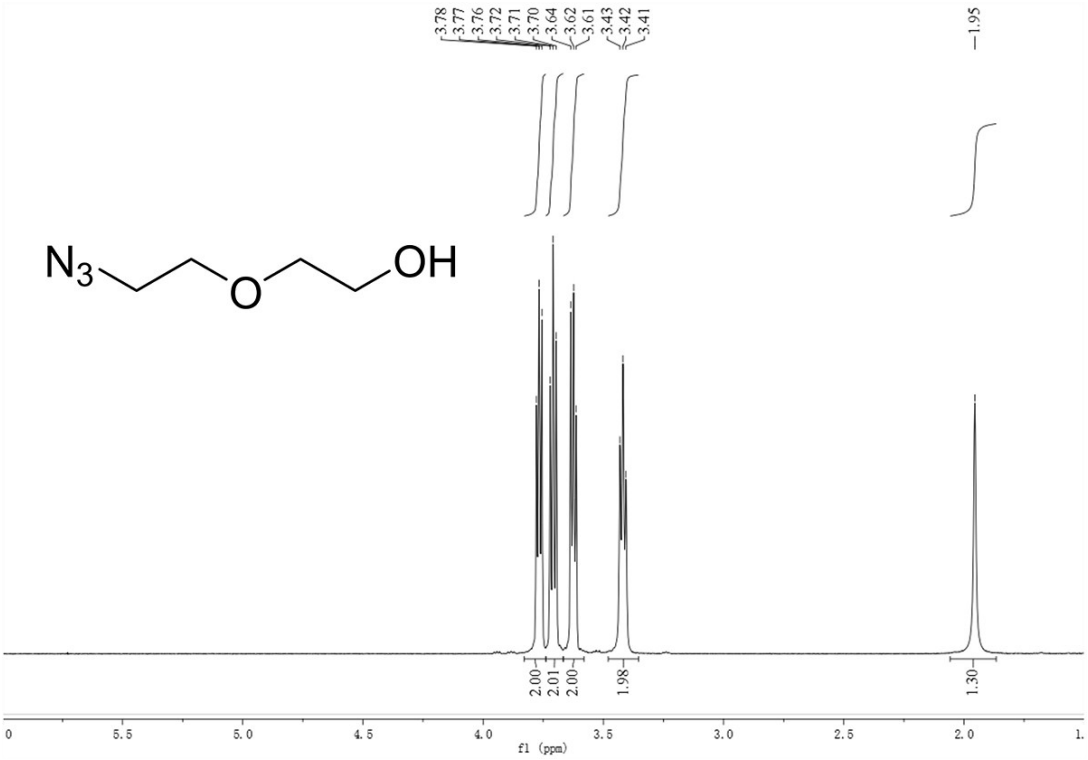


Figure S1. ^{1}H NMR of 2-(2-azidoethoxy)ethanol.

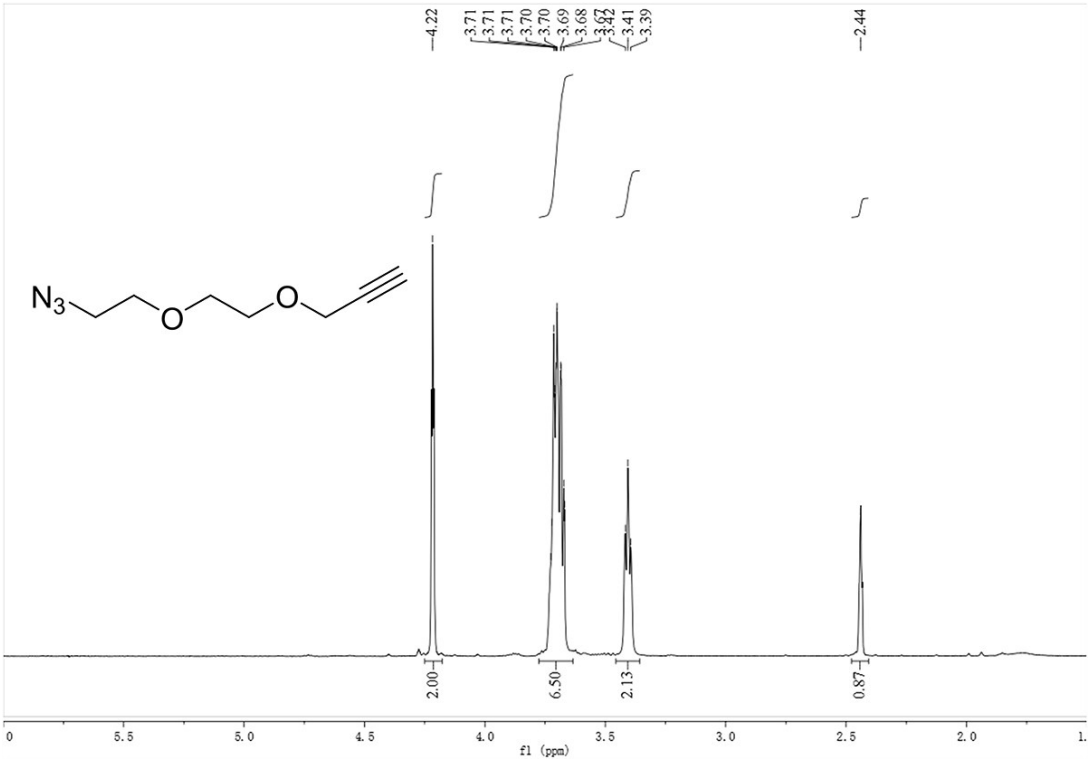


Figure S2. ^{1}H NMR of 3-(2-(2-azidoethoxy)ethoxy)prop-1-yne.

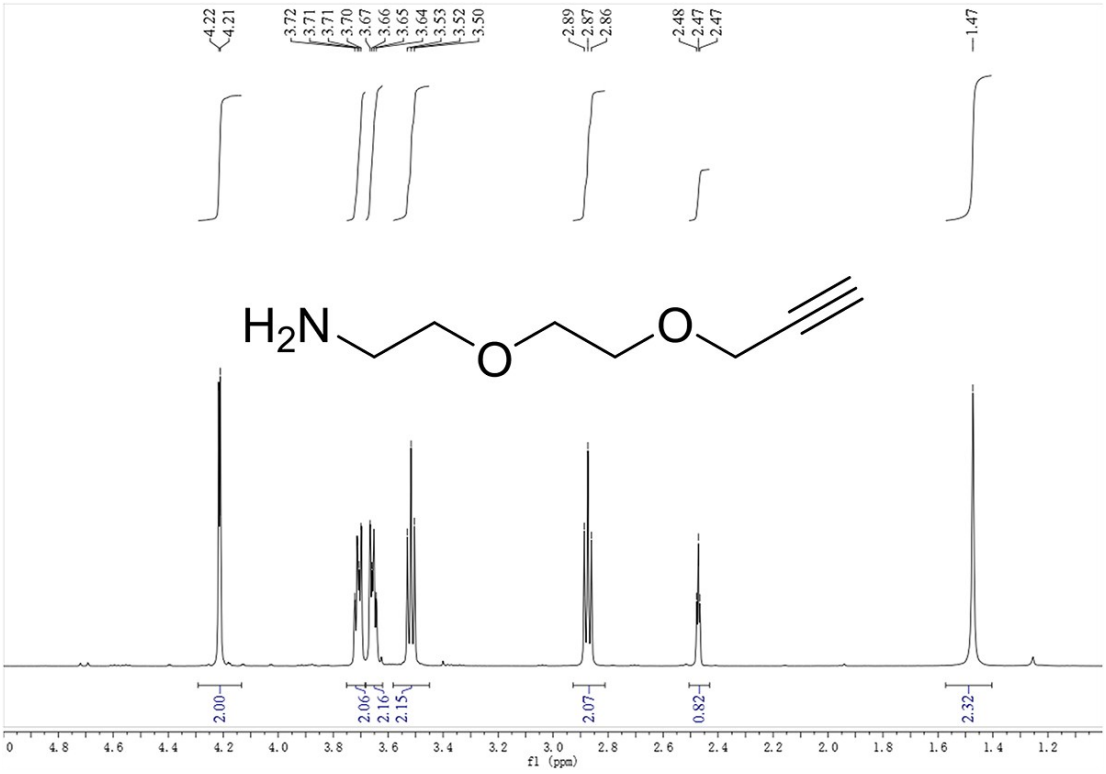


Figure S3. ^{1}H NMR of 2-(2-(prop-2-ynyloxy)ethoxy)ethanamine.

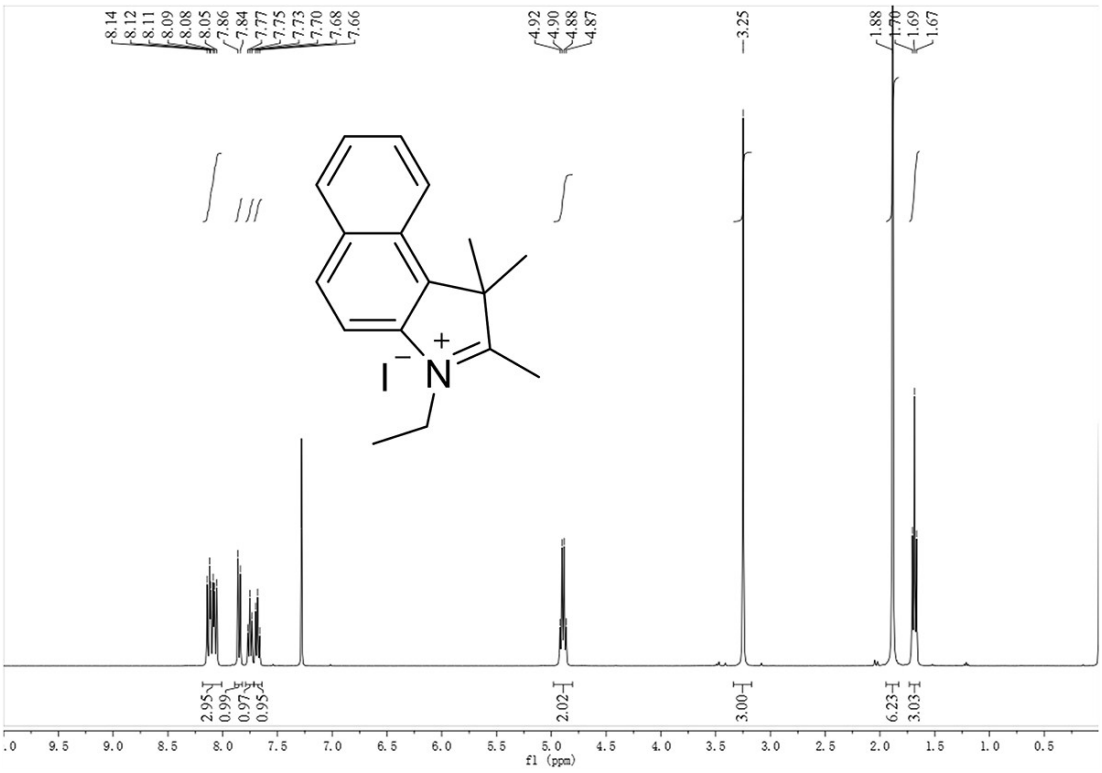


Figure S4. ^{1}H NMR of 1,1,2-Trimethyl-3-ethylbenz[e]indolium iodide salt.

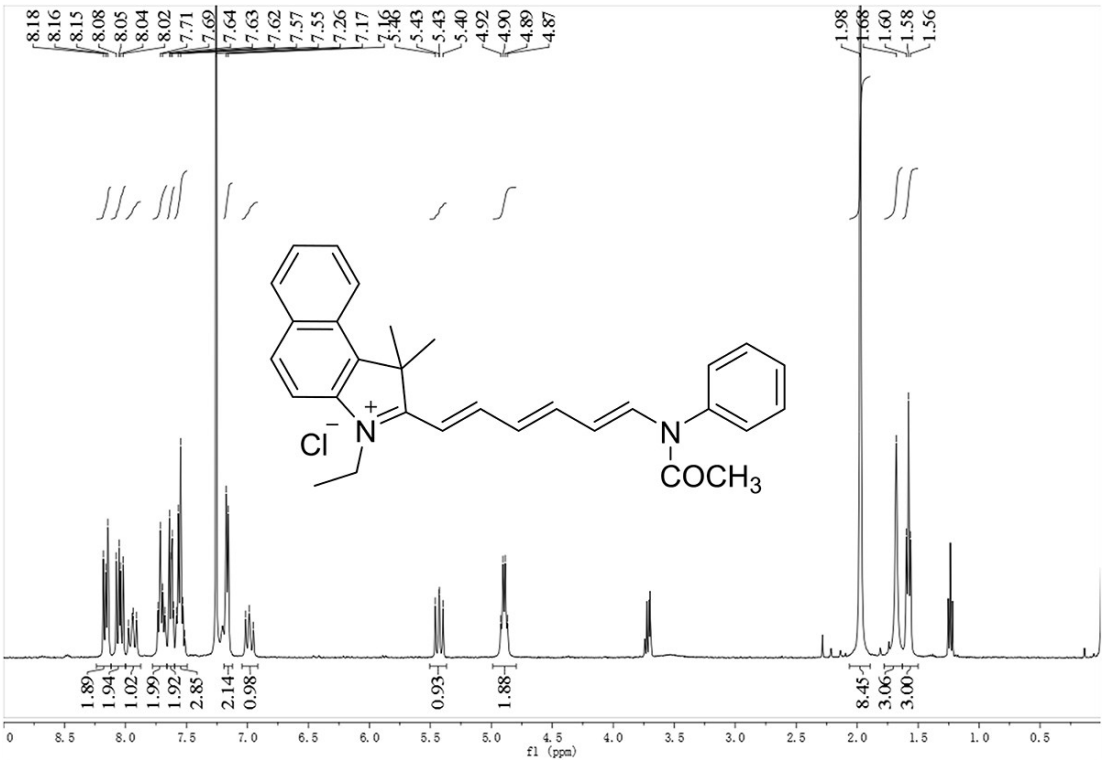


Figure S5. ^{1}H NMR of 2-[6-(N-Phenyl-N-acetylamino)-1,3,5-heptatrienyl]-1,1-dimethyl-3-ethyl-1H-benz[e]indolium chloride salt.

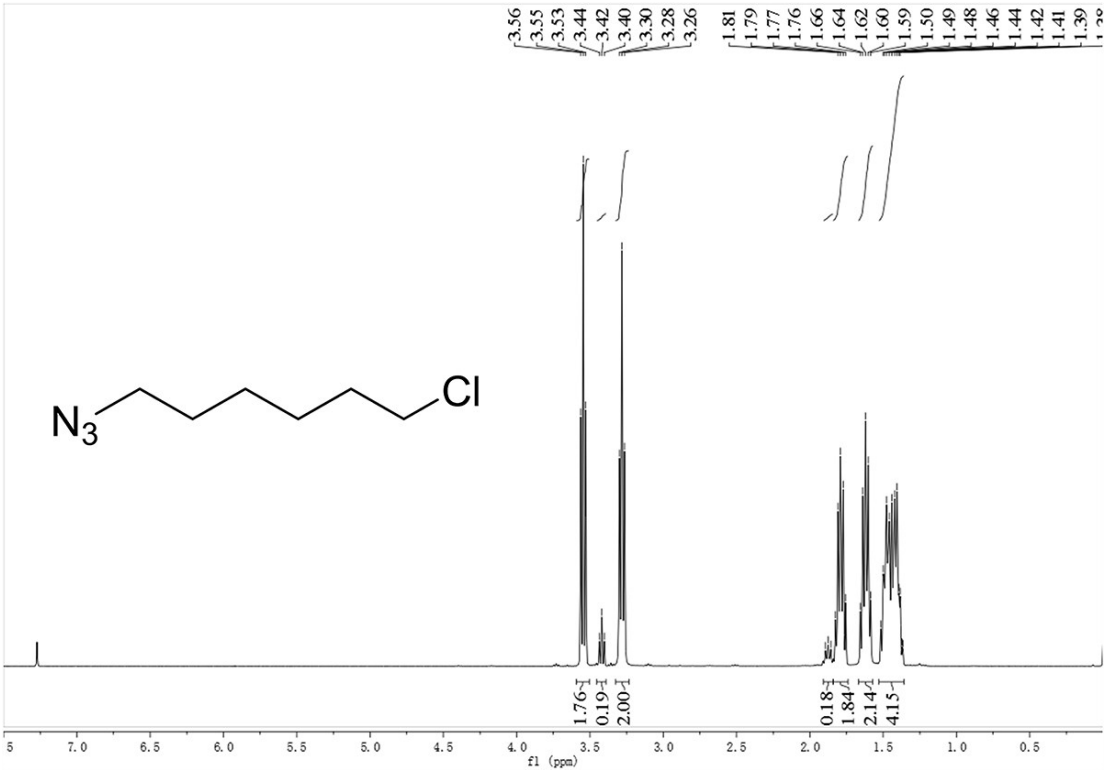


Figure S6. ^{1}H NMR of 1-azido-6-chlorohexane.

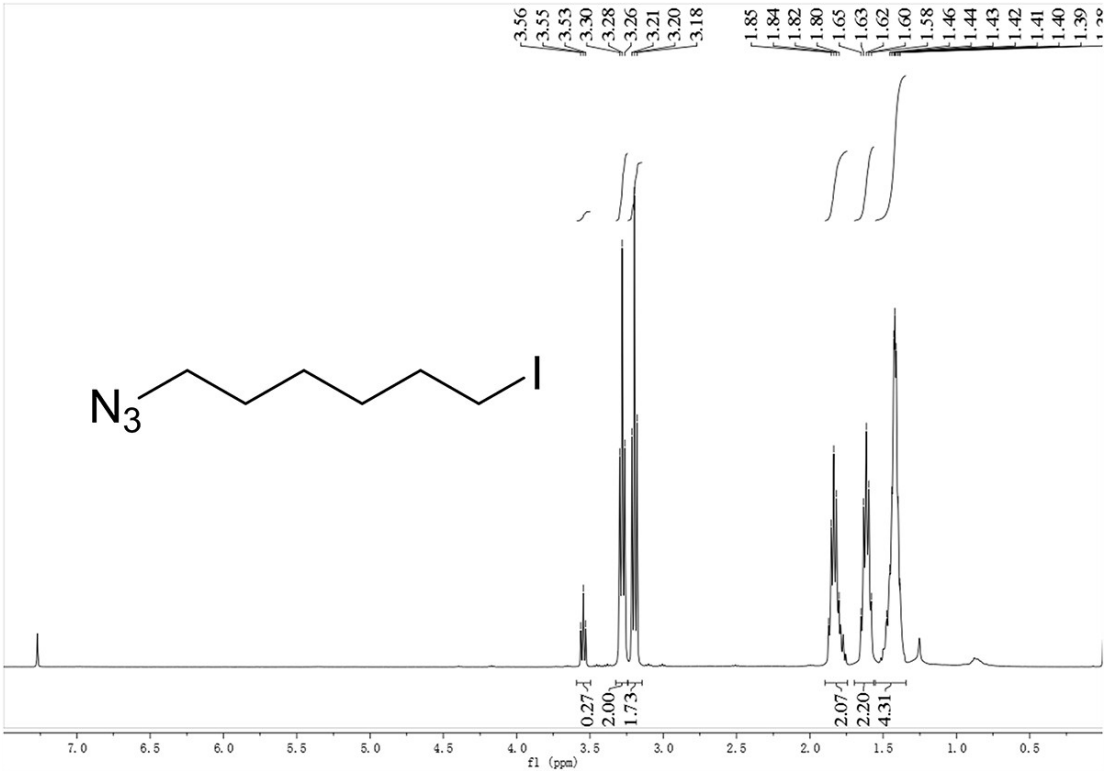


Figure S7. ^{1}H NMR of 1-azido-6-iodohexane.

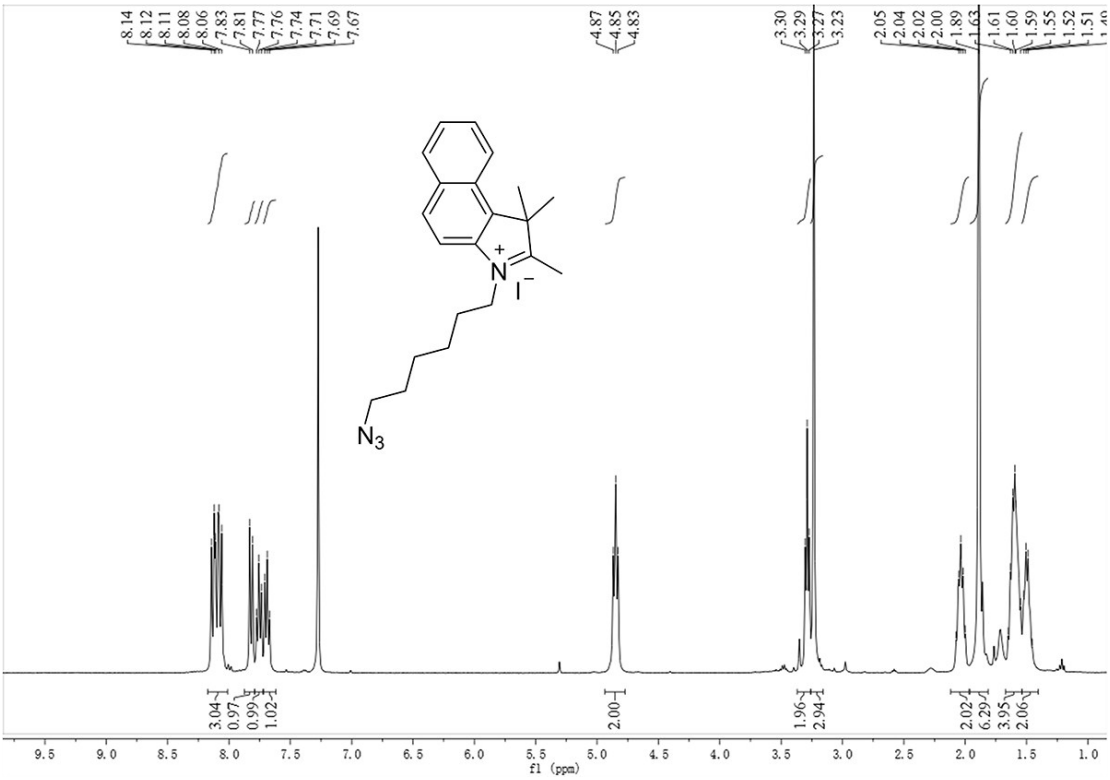


Figure S8. ^{1}H NMR of 1,1,2-Trimethyl-3-(3-Azidohexyl)-benz[e]indolium iodide salt.

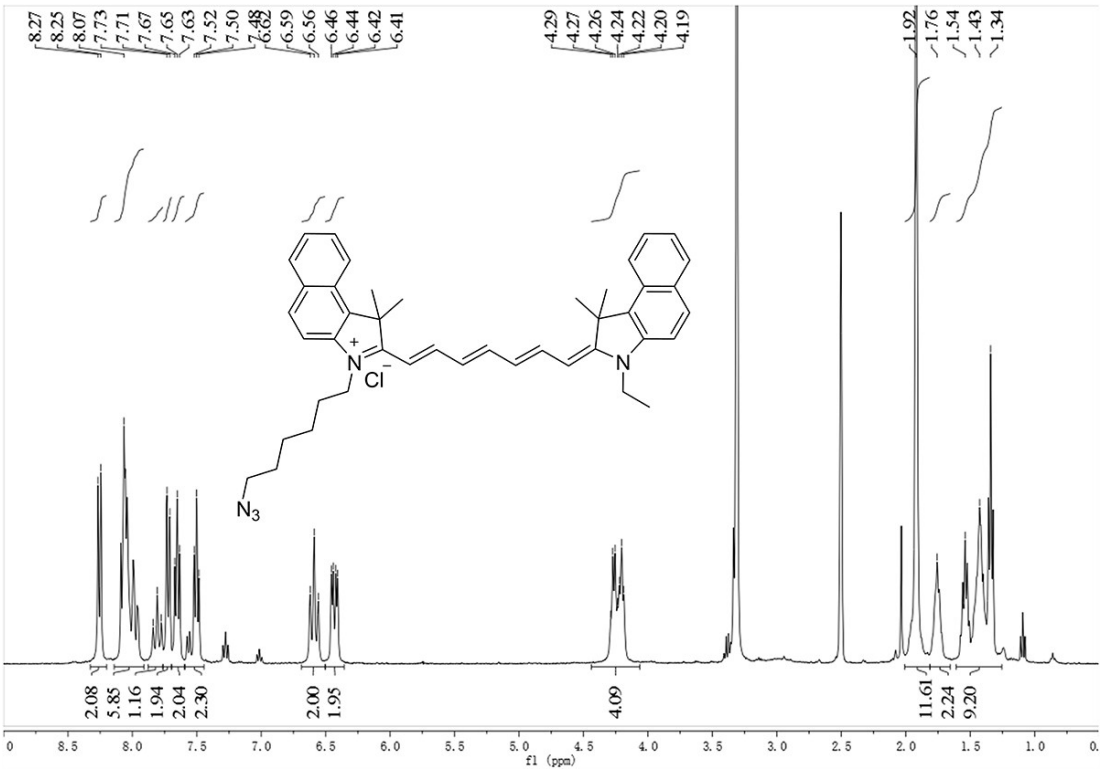


Figure S9. ^{1}H NMR of 2-[7-(1,3-Dihydro-1,1-dimethyl-3-ethylbenz[e]indolin-2-ylidene)-1,3,5-heptatrienyl]-1,1-dimethyl-3-(3-Azidohexyl)-1H-benz[e]indolium chloride salt (ICG-N_3).

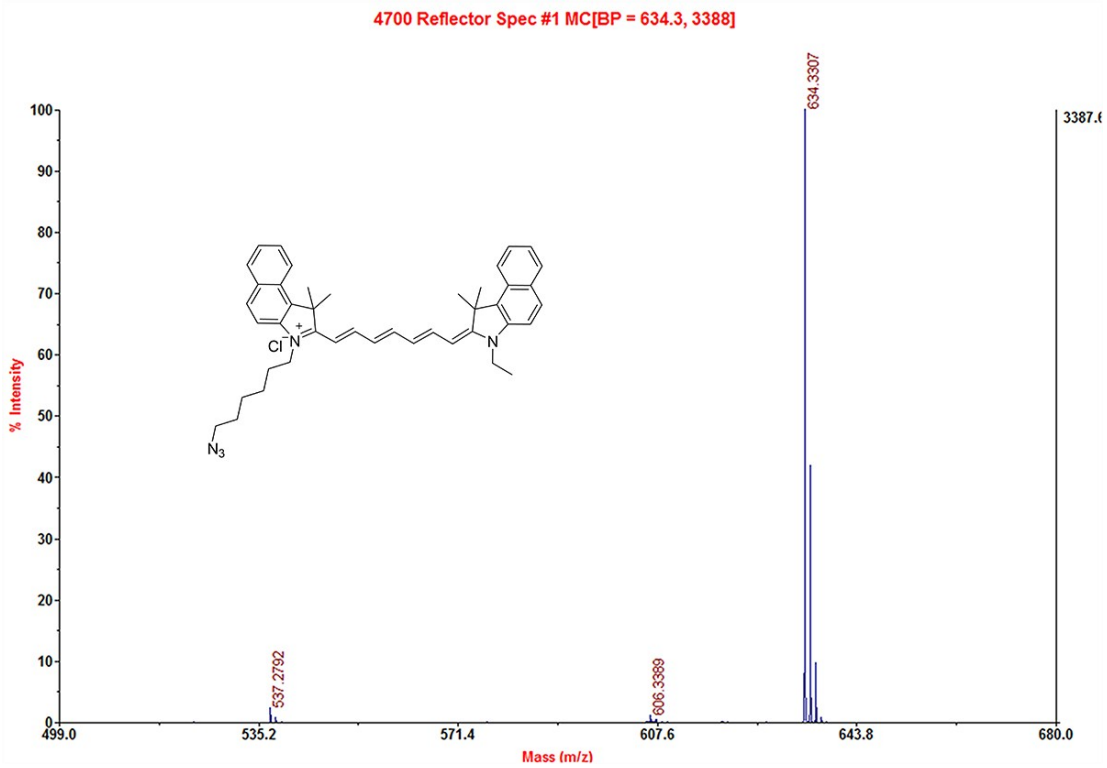


Figure S10. Mass analysis of ICG, MALDI-TOF (m/z): $[M\text{-}Cl^-]^+$ calcd. for $C_{43}H_{48}N_5$, 634.39; found, 634.33. ICG-N_3 0.5mg/ml in CH_3CN; Matrix: saturated DCTB in CH_3CN, samples:matrix (v/v) 1:5.

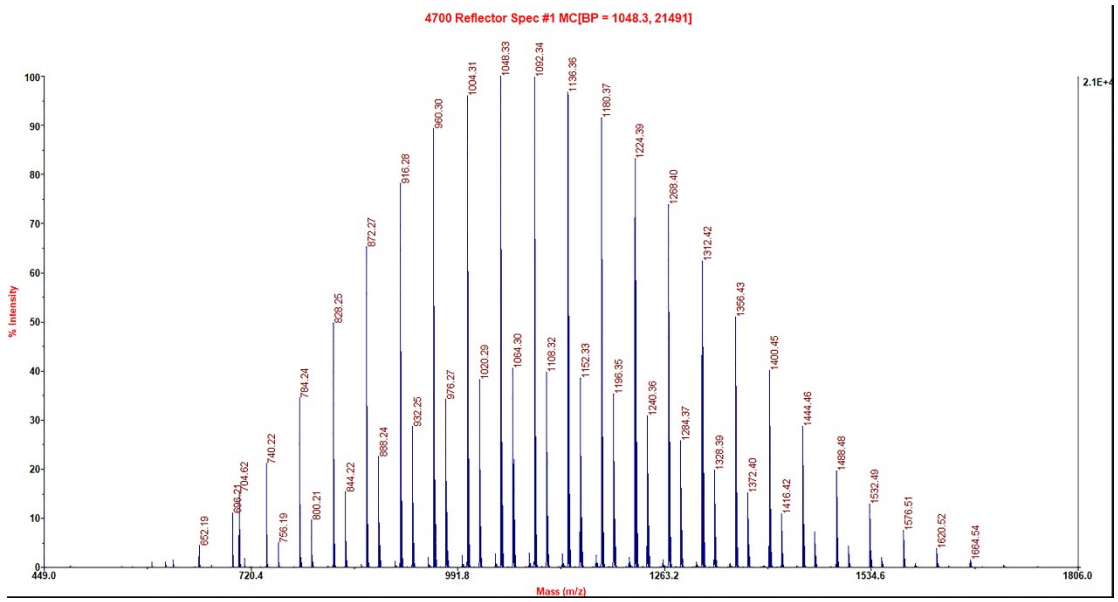


Figure S11. Mass analysis of mPEG-N_3, mPEG-N_3 0.5 mg/ml in CH_3CN; Matrix: saturated DCTB in CH_3CN, samples:matrix (v/v) 1:5. Mn, 1133.35, Mw, 1161.74, PDI=1.03.

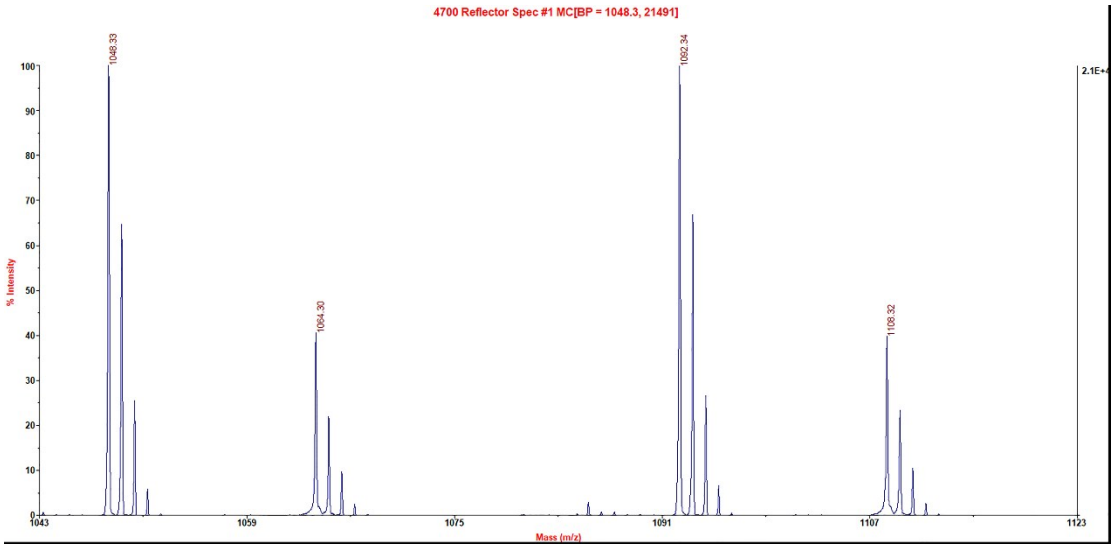


Figure S12. Mass analysis of mPEG-N_3, enlarged picture of Figure S11. MALDI-TOF (m/z): $[M+Na]^+$ calcd. for $CH_3(OCH_2CH_2)_{22}N_3$, 1048.33; found, 1048.47. MALDI-TOF (m/z): $[M+K]^+$ calcd. for $CH_3(OCH_2CH_2)_{22}N_3$, 1064.44; found, 1064.30.

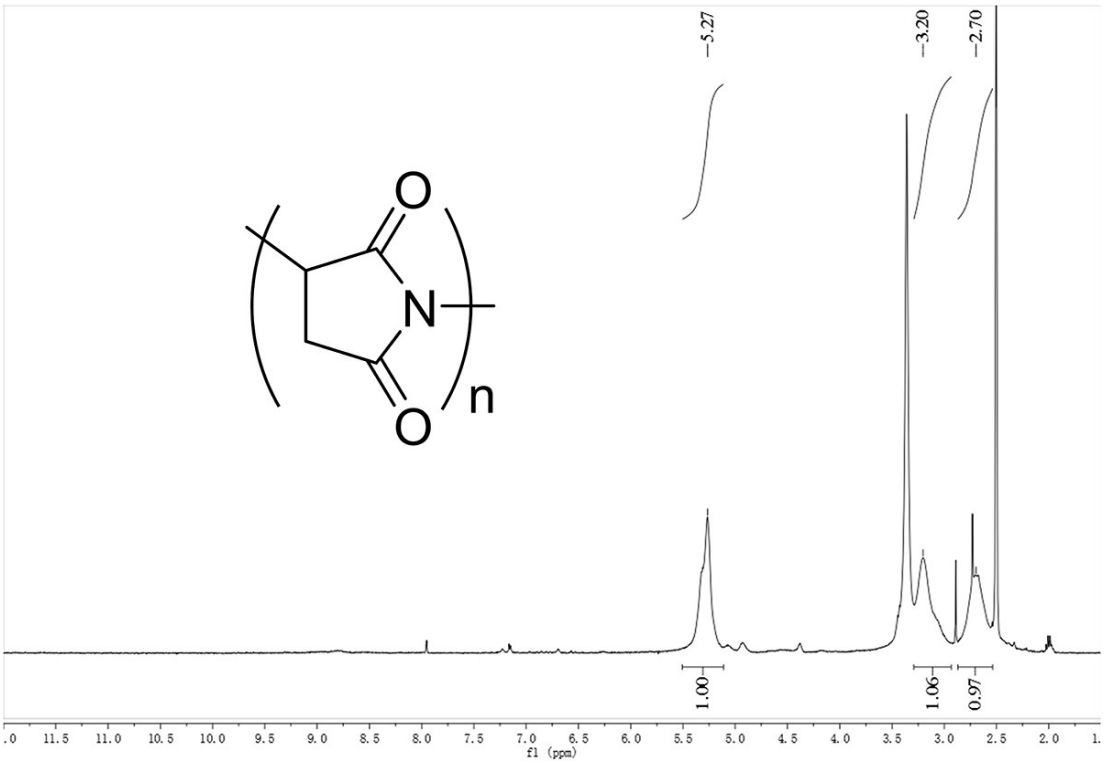


Figure S13. ^{1}H NMR of PSI.

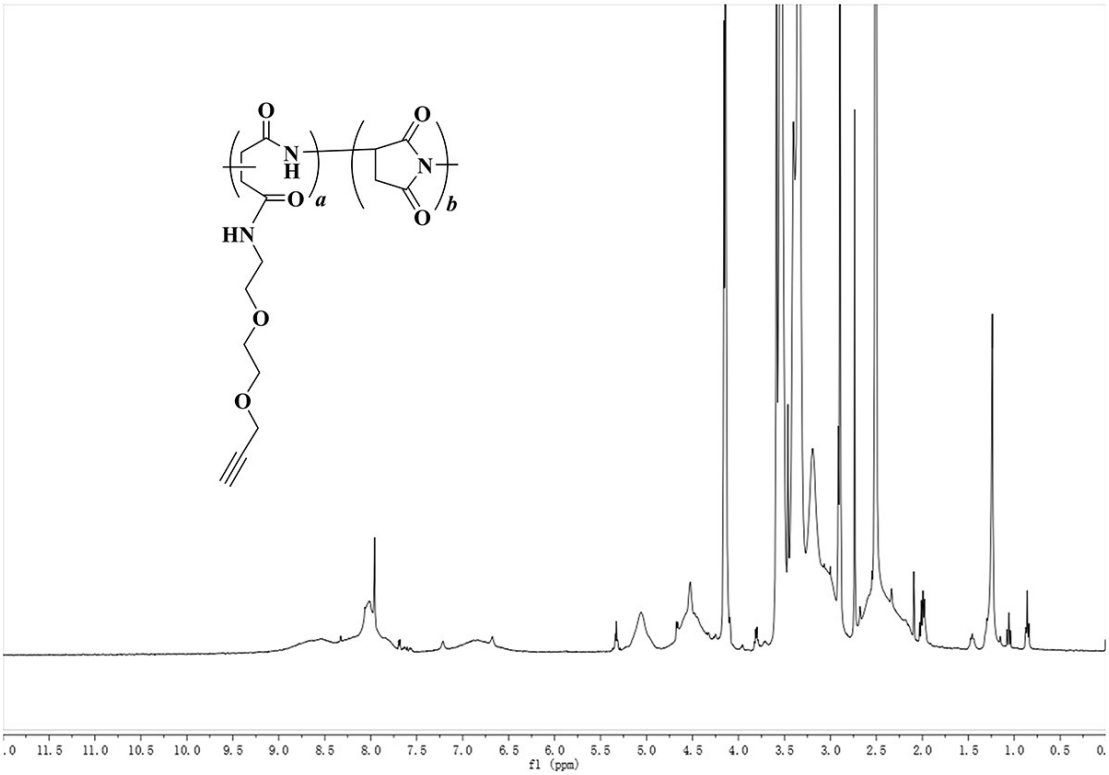


Figure S14. ^{1}H NMR of alkynyl modified PSI.

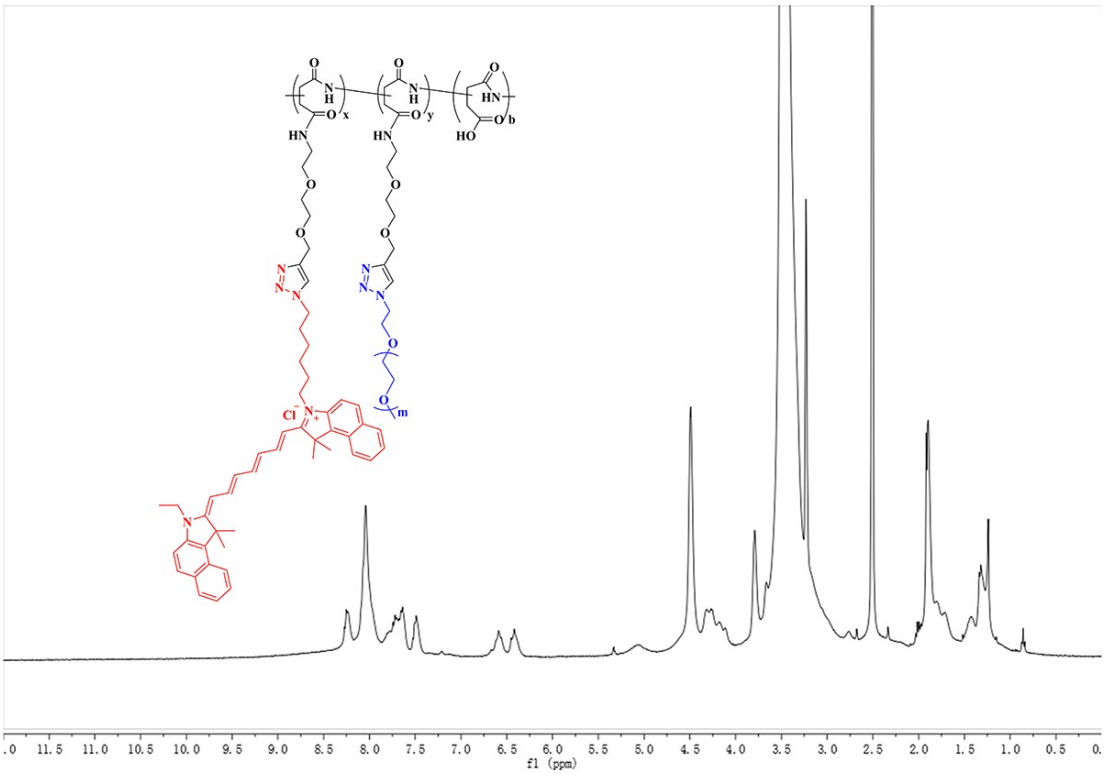


Figure S15. ^{1}H NMR of PAsp-*g*-(PEG-ICG).

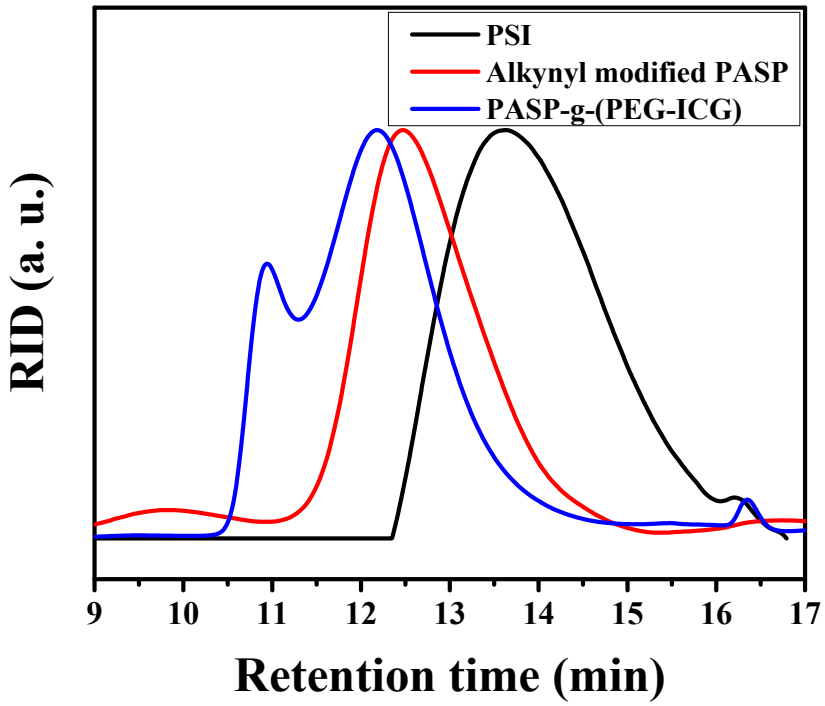


Figure S16. GPC analysis of PSI, Alkynyl modified PASP and PASP-g-(PEG-ICG), PSI: Mw=7.5×10^3, Mn=4.8×10^3, PDI=1.5; Alkynyl modified PASP: Mw=1.3×10^4, Mn=2.1×10^4, PDI=1.6; PASP-g-(PEG-ICG): Mw=5.6×10^4, Mn=2.5×10^4, PDI=2.2.

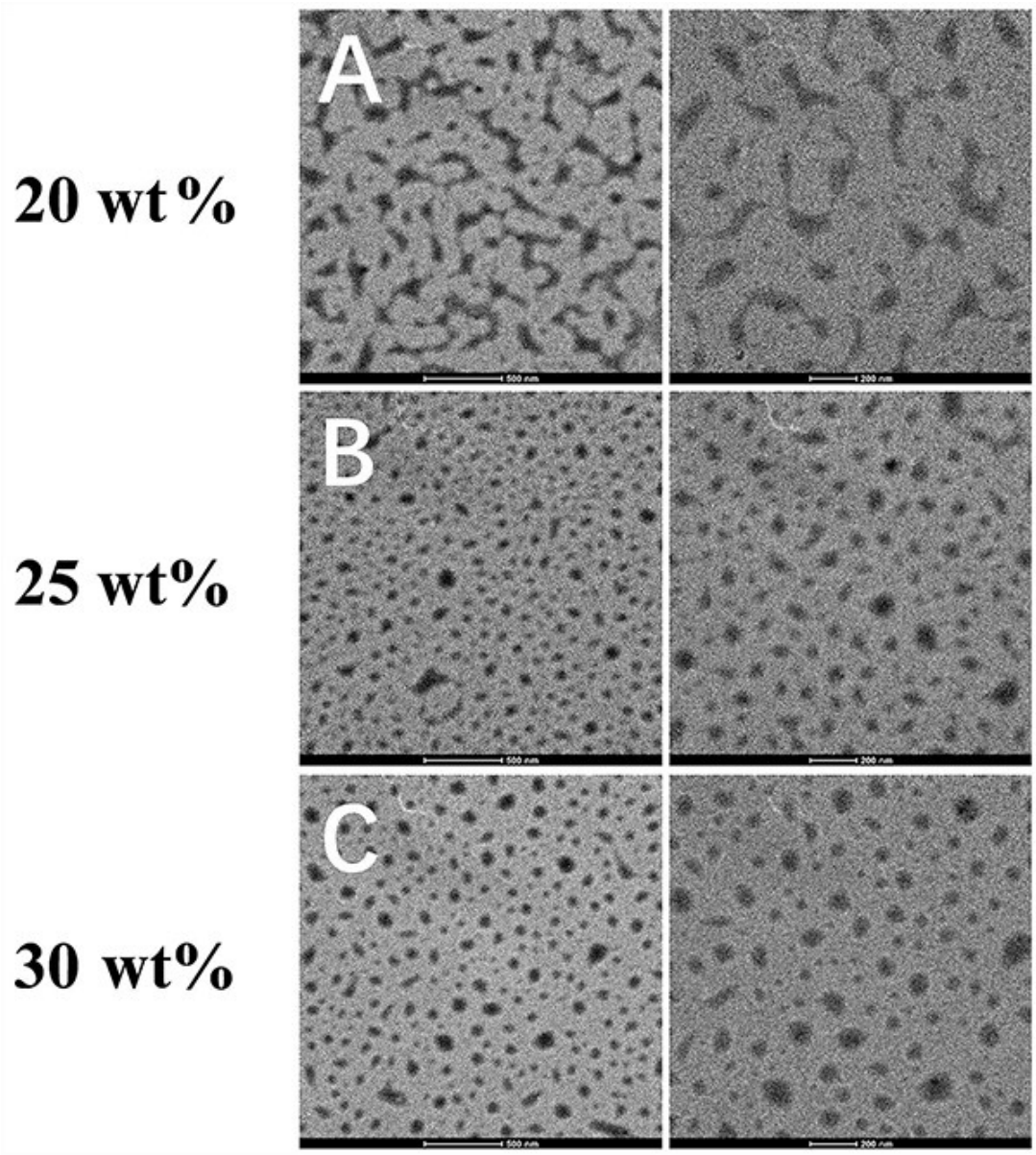


Figure S17. TEM images of PTX@PASP-g-(PEG-ICG) micelles with drug loading capacity of (A) 16.4 %, (B) 23.9 %, (C) 28.2 %, respectively.

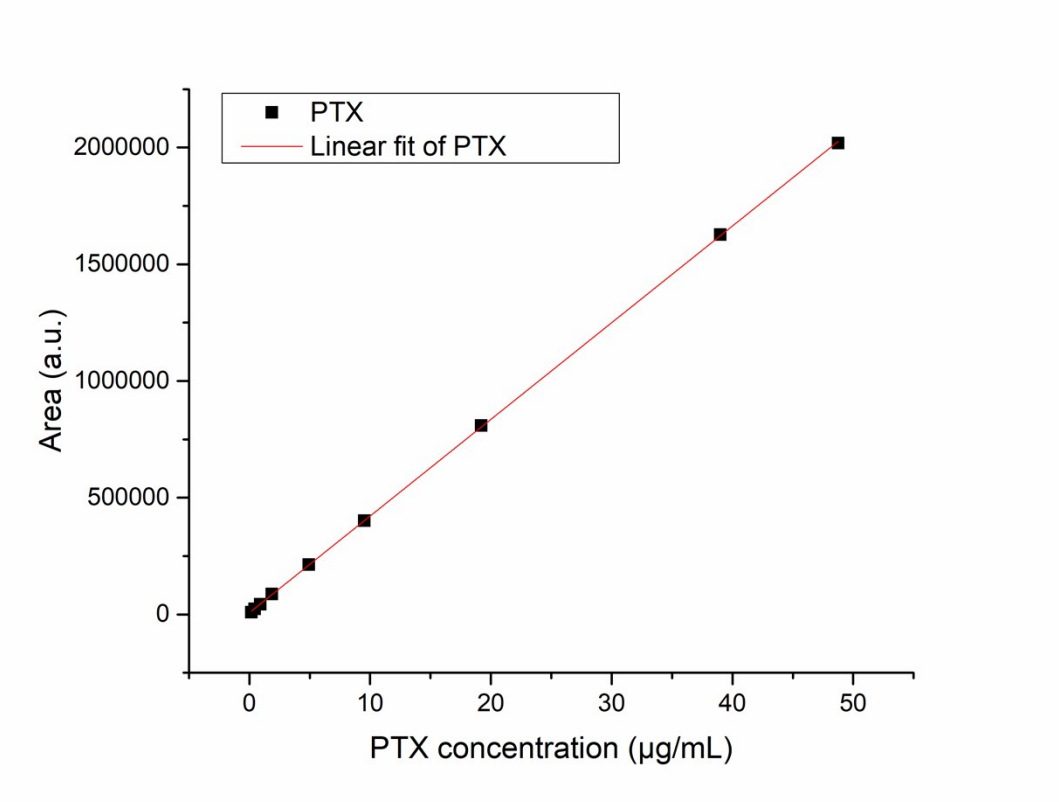


Figure S18. Standard curve of PTX, detection wavelength at 227 nm, mobile phase acetonitrile/H_2O=4:1, A=41414c+6959, adj. R^2=0.99996.

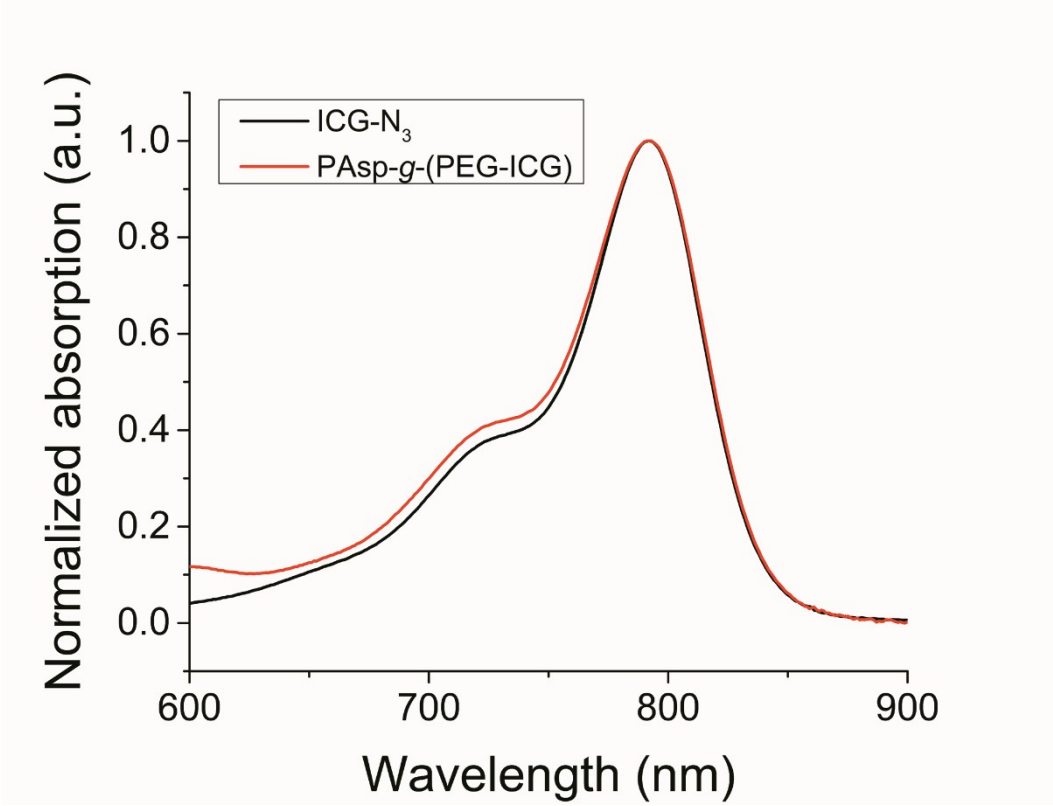


Figure S19. Absorption curve of ICG-N_3 and PAsp-*g*-(PEG-ICG) in DMSO.

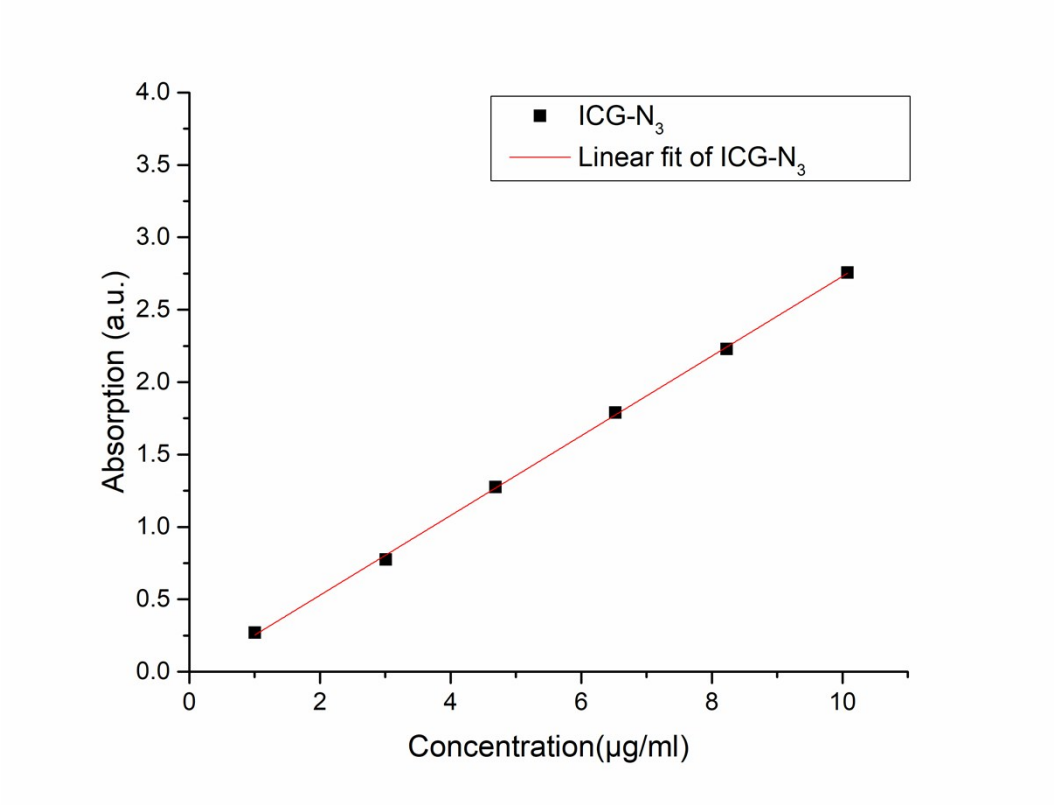


Figure S20. Standard curve of ICG-N_3 in DMSO (792 nm), A=0.2752c-0.022, adj. R^2=0.9995.

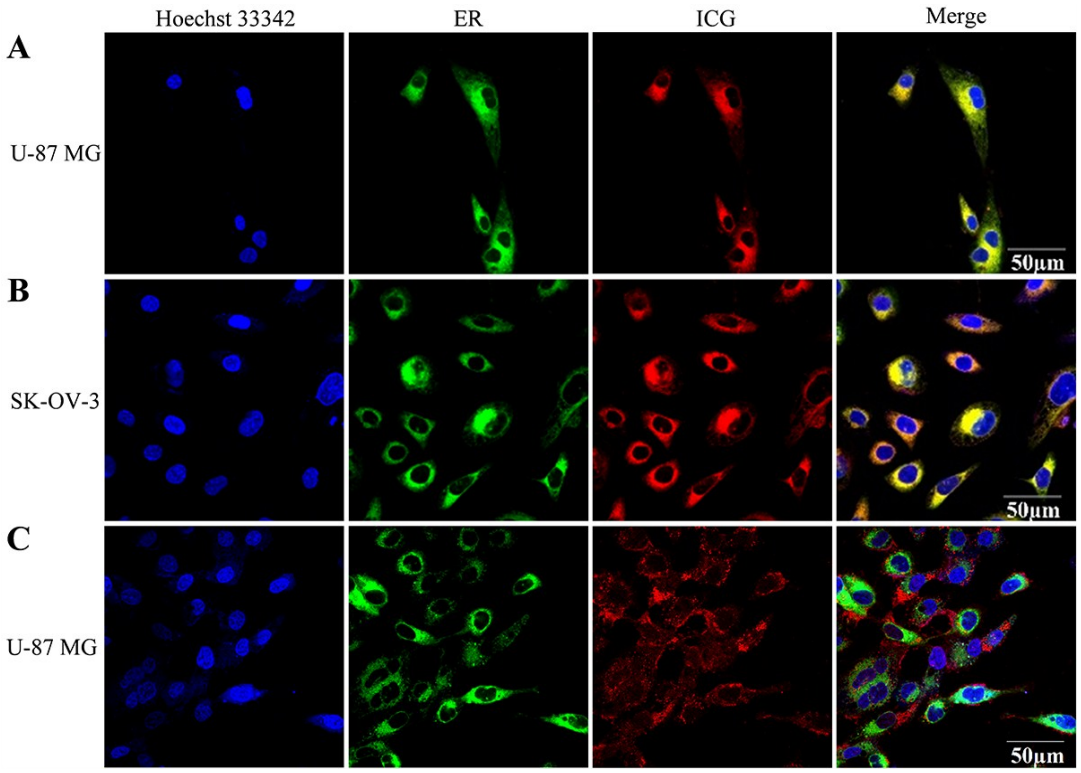


Figure S21. Subcellular distribution of PTX@PAsp-g-(PEG-ICG) micelle. (A, B) The distribution of PTX@PAsp-g-(PEG-ICG) micelle in U-87 MG and SK-OV-3 cells. (C) The distribution of PTX@PLys-g-(PEG-ICG) micelle in U-87 MG cells. Nucleus and ER of (A) U-87 MG cells and (B) SK-OV-3 cells (C) U-87 MG were stained with Hoechst 33342 and ER tracker green, respectively.

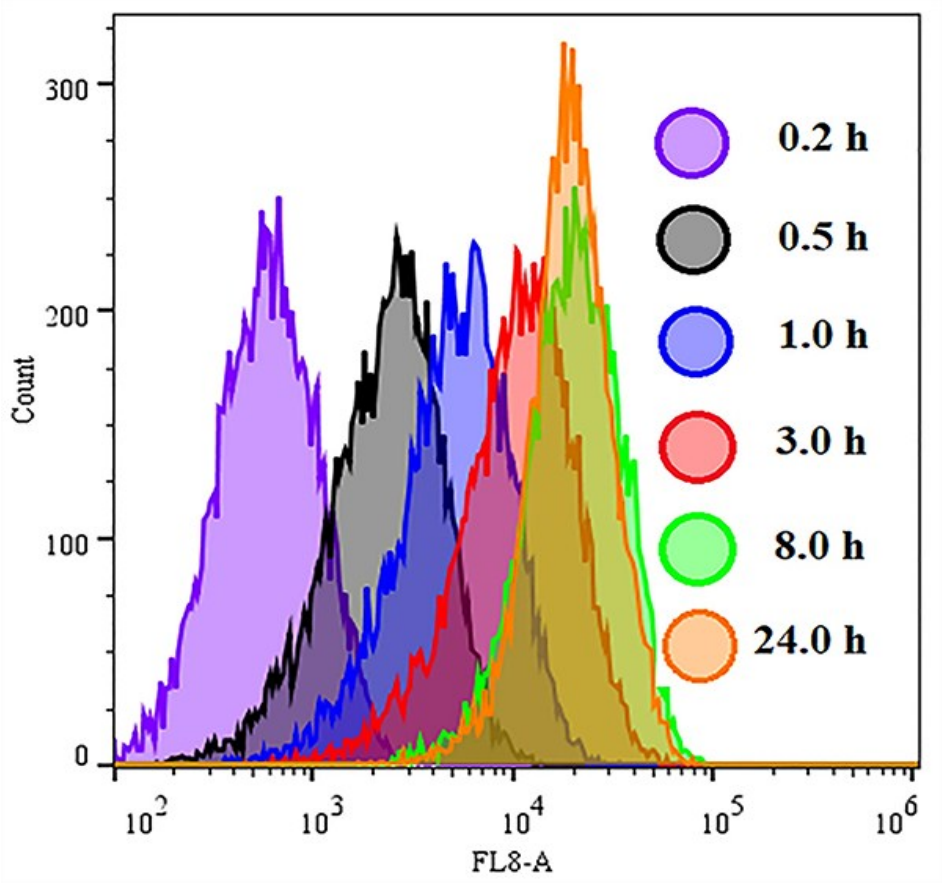


Figure S22. Fluorescence intensity in U-87 MG cells incubated with PTX@PASP-g-(PEG-ICG) micelle (1 μg/ml) at different time intervals determined by flow cytometry (Ex=638 nm, Em=755 nm LP).

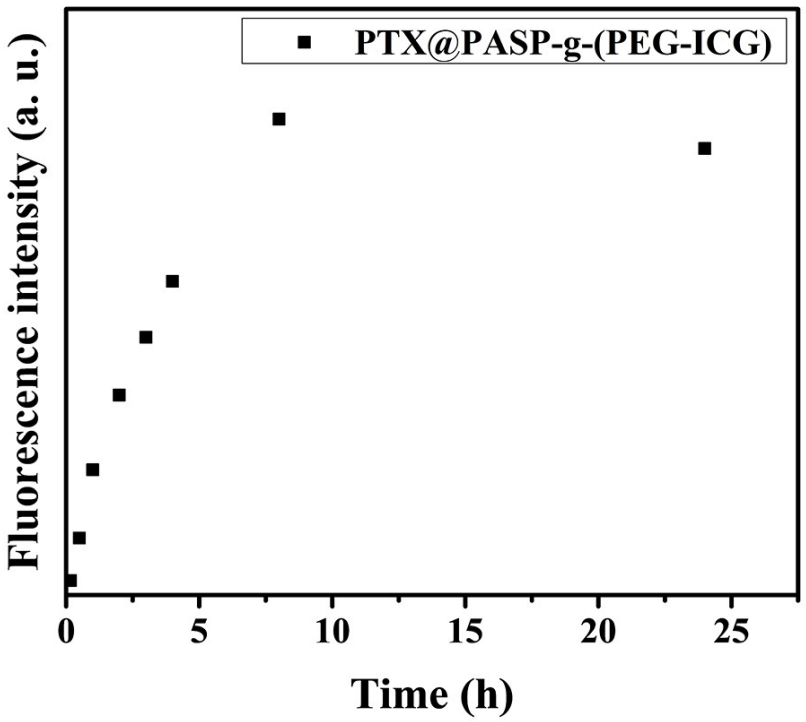


Figure S23. Fluorescence intensity in U-87 MG cells incubated with PTX@PASP-g-(PEG-ICG) micelle (1 μg/ml) at different time intervals determined by flow cytometry (Ex=638 nm, Em=755 nm LP).

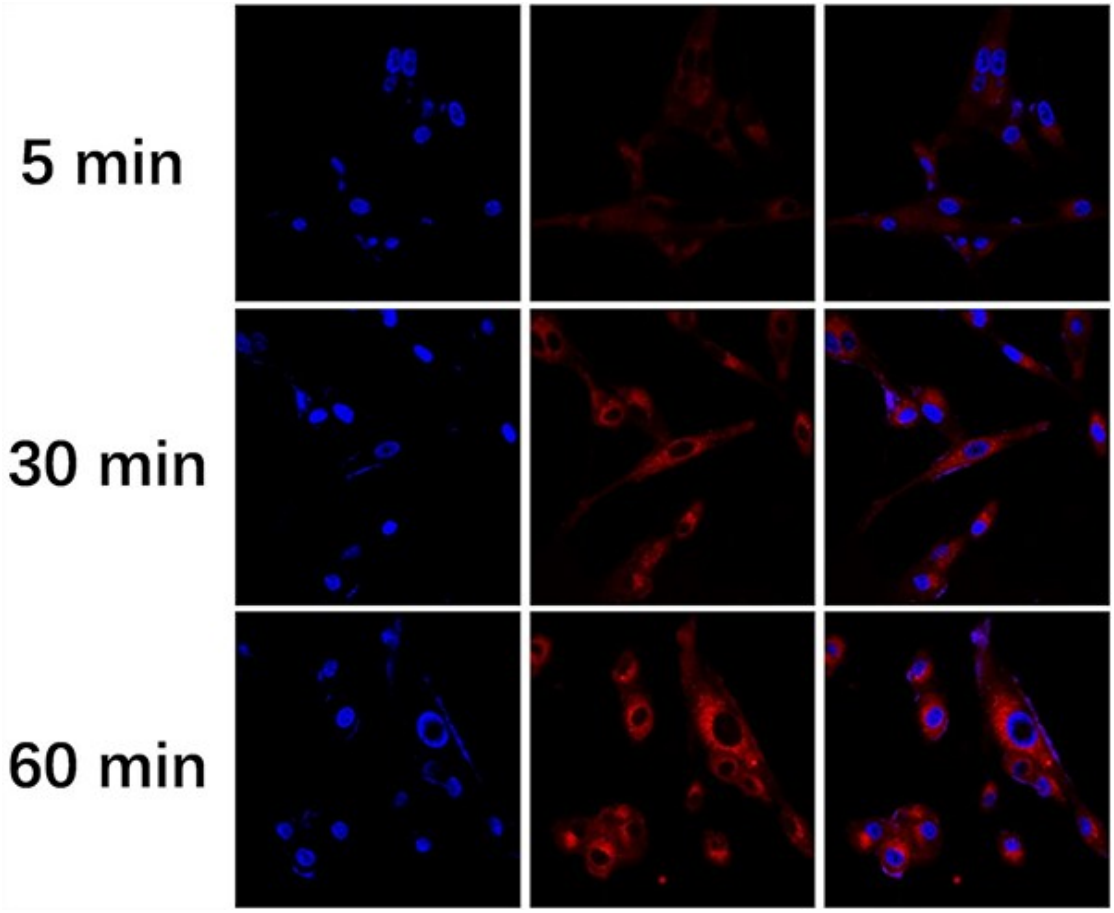


Figure S24. Fluorescence intensity changes of PTX@PAsp-g-(PEG-ICG) micelles in U-87 MG cells as determined by confocal laser scanning microscopy (Ex=635 nm, Em=668 nm LP).

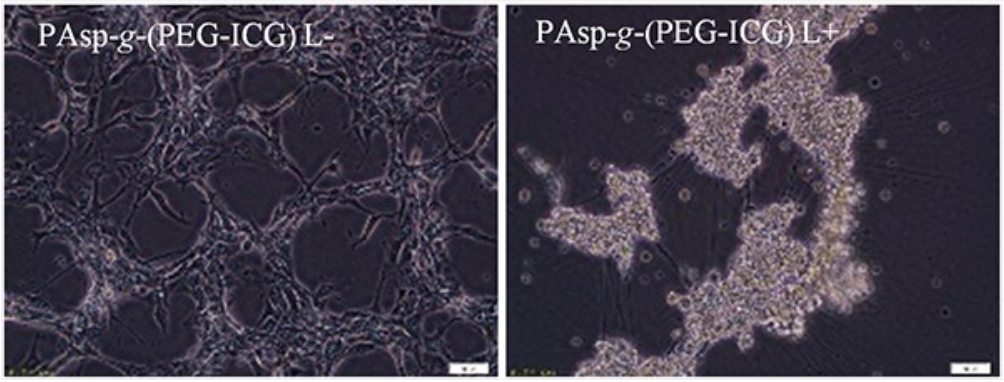


Figure S25. The morphology of U-87 MG cells incubated with culture medium, PAsp-*g*-(PEG-ICG) micelle with/without laser irradiation (0.2 W/cm^2, 785 nm, 5 min).

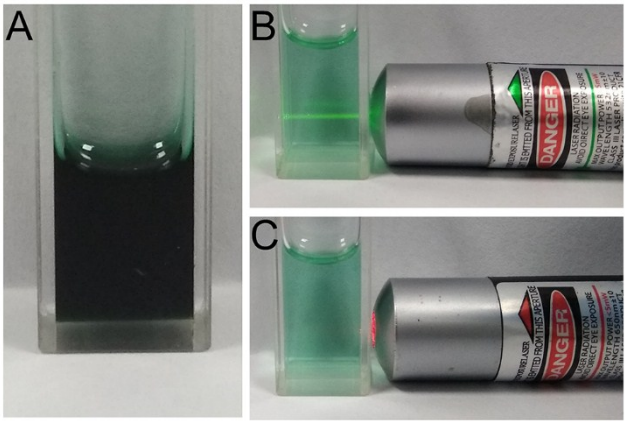


Figure S26. Tyndall effect of PTX@PAsp-g-(PEG-ICG) micelles. (A) PTX@PAsp-g-(PEG-ICG) micelles aqueous solution (PTX 2.0 mg/ml, DL 30%), which showed good stability. (B) (C) Tyndall effect of PTX@PAsp-g-(PEG-ICG) micelles (PTX 50 μg/ml, DL 30%) using 488 nm or 633 nm laser as an incident light, respectively.

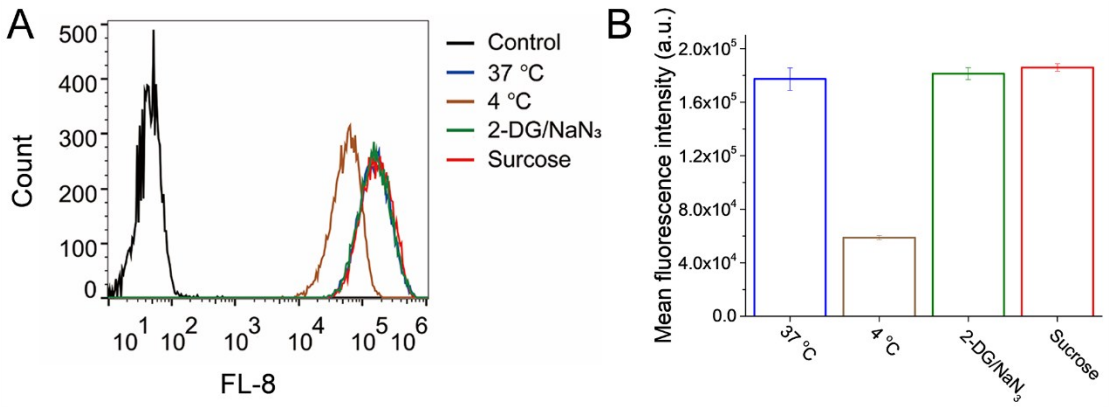


Figure S27. Uptake of PTX@PAsp-*g*-(PEG-ICG) into SK-OV-3 cells under different inhibitory conditions. (A) Fluorescence intensity of PTX@PAsp-*g*-(PEG-ICG) in SK-OV-3 cells pretreated with different inhibitors as determined by flow cytometry. (B) Quantitative analysis of fluorescence intensity in SK-OV-3 cells.

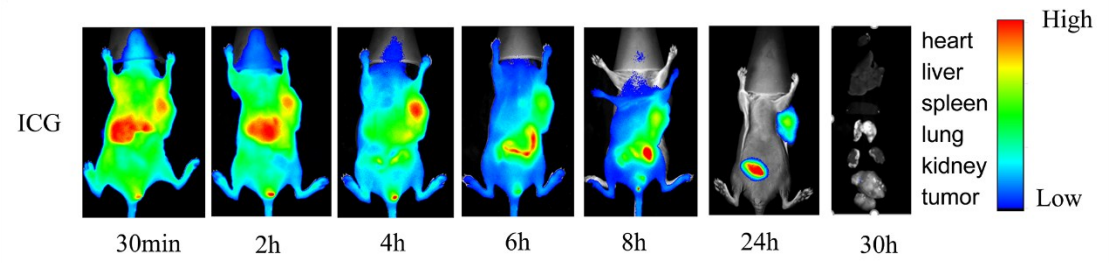


Figure S28. In vivo biodistribution images of free ICG (5 mg/kg) in U-87 MG tumor-bearing mice after intravenous injection at different time intervals. (Ex=760 nm, Em=830 nm, exposure time=2 s).

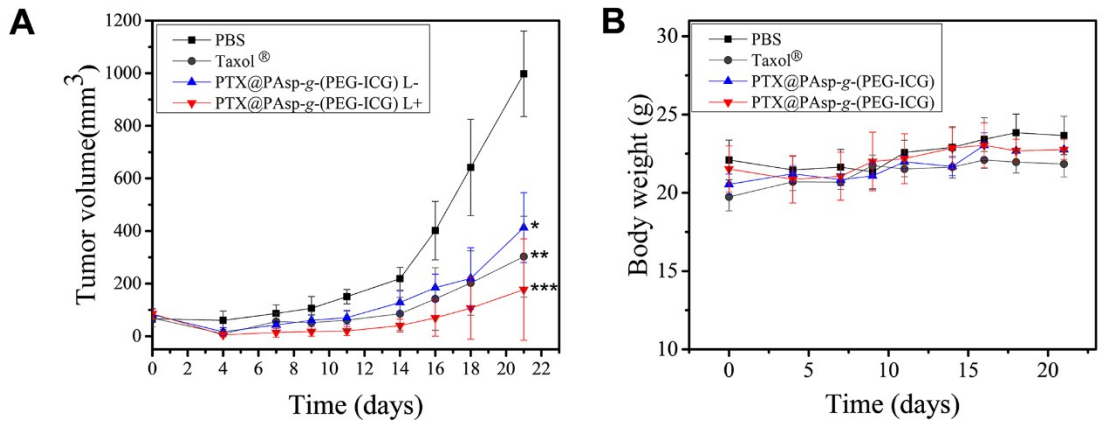


Figure S29. *In vivo* application of PTX@PAsp-g-(PEG-ICG) micelles. (A) Taxol and PTX@PAsp-g-(PEG-ICG) micelles (PTX 1 mg/mL, 10 mg/kg) were administrated intravenous for all mice (n=5) at day 0, L+ group were anaesthetized and irradiated with 785 nm laser (2 W/cm^2, 5 min) at 24 h after administration. * $p<0.5$, ** $p<0.01$, *** $p<0.001$ (B) Mice body weight curves of different groups of U-87 MG tumor-bearing mice after treatment.