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Electronic supplementary information

Chemoenzymatic synthesis of dual-responsive graft copolymers for drug delivery: long-term stability, high loading and cell selectivity

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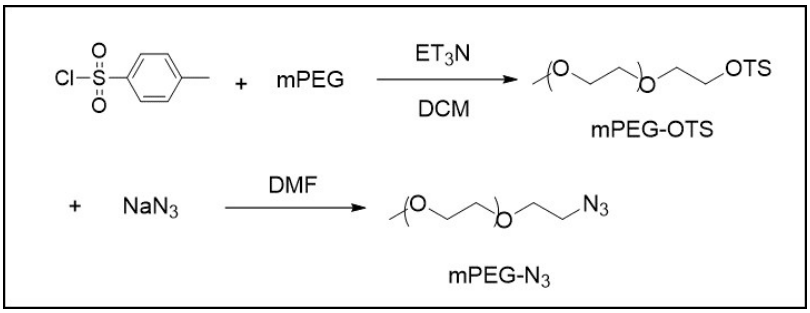
Methods

Synthesis of Azido end-functional poly(ethylene glycol) methyl ether (mPEG–N_3)



Schme. S1. Synthesis of azido end-functional mPEG–N_3.

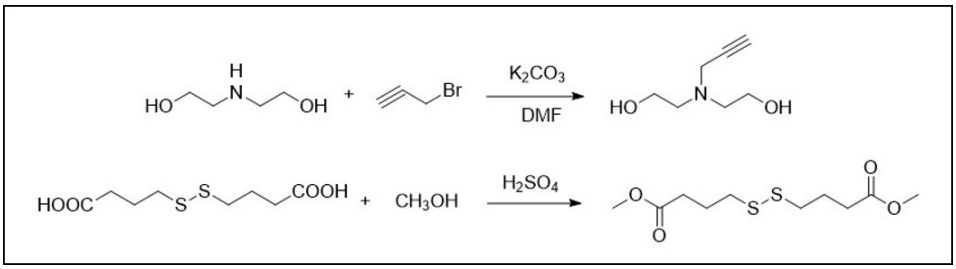
mPEG (Mn = 1000, 2000 Da) (10 mmol) and triethylamine (30 mmol) were added in 50 mL dichloromethane. Under magnetic stirring at room temperature for 24 h, The dichloromethane solution was washed successively by water (50 mL × 2), Brine (50 mL × 2), 1 M HCL (50 mL × 1), Saturated sodium bicarbonate solution (50 mL × 1), water (50 mL × 2) and dried over anhydrous magnesium sulfate, and filtered. Then crude product purified *via* excess adding of cold ether absolute, which finally dried at room temperature in a vacuum oven overnight after filtration to yield a white powder mPEG-OTS. mPEG-OTS (5 mM) was dissolved in 40 mL DMF and sodium azide (15 mM) were then added. The reaction was stirred at room temperature for 24 h. Then DMF was removed *via* vacuum evaporation, and 50 mL toluene was added with subsequently filtration to remove undissolved solid. The toluene solution was removed *via* vacuum evaporation.[1] Then product mPEG-N_3 precipitated by excess adding of cold ether absolute, which finally dried at room temperature in a vacuum oven overnight to yield a white powder. Yield of $mPEG_{1000}$-N_3 and $mPEG_{2000}$-N_3 are 76.1% and 78.3%.

$mPEG_{1000}$-N_3: ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ: 3.69–3.63 (m, 95H, methylene in mPEG), 3.38 (s, 3H, –OC*H*3 end group). ^{13}C NMR (100MH, $CDCl_3$) δ: 72.0, 70.5, 70.0, 59.0, 50.7.

$mPEG_{2000}$-N_3: ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ: 3.68–3.65 (m, 184H, methylene

in mPEG), 3.38 (s, 3H, –OC*H*3 end group). ^{13}C NMR (100MHz, $CDCl_3$) δ: 72.0, 70.6, 70.0, 59.0, 50.7.

Synthesis of various monomers



Schme. S2. Synthesis of *N*-propargyldiethanolamine and dimethyl 4,4′-dithiodibutyrate

Synthesis of *N*-propargyldiethanolamine.

Anhydrous K_2CO_3 (60 mmol) and Diethanolamine (30 mmol) in 50 mL DMF and the mixture was cooled in an ice bath. Once cooled propargyl bromide (36 mmol) was added. The reaction flask was let slowly let warm to 80 °C for 4 h. After removing the K_2CO_3 by filtering, and evaporated under reduced pressure.[2] The final product was obtained by silica gel column chromatography (CH_2Cl_2 to 95:5 CH_2Cl_2–MeOH) resulting in an orange liquid in 46.5% yield.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ: 3.67–3.64 (m, 4H, –NCH_2CH_2–), 3.49 (d, *J* = 2.3 Hz, 2H, –$NCH_2C{\equiv}CH$), 2.76–2.74 (m, 4H, –NCH_2CH_2–), 2.22–2.21 (t, *J* = 2.3 Hz, 1H, –$NCH_2C{\equiv}CH$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 78.4, 73.1, 59.2, 55.1, 42.3. HR-MS (ESI): Calcd for: $C_7H_{13}NO_2$: 144.1024 ([M + H]+), Found 144.1004 ([M + H]+).

Synthesis of dimethyl 4,4′-dithiodibutyrate

4,4’-Dithiodibutyric acid (20 mmol) were added in 100 mL methanol. Under magnetic stirring at 80 °C for 4 h , and the methanol evaporated under reduced pressure. The final product was obtained by silica gel column chromatography (4:1 petroleum ether–ethyl acetate), resulting in dimethyl 4,4′-dithiodibutyrate as an

colorless liquid in 91.2% yield. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 3.68 (s, 6H, C*H*$_3$O–), 2.72 (t, *J* = 6.9 Hz, 4H, –SC*H*$_2$CH$_2$CH$_2$–), 2.45 (t, *J* = 7.1 Hz, 4H, –SCH$_2$CH$_2$C*H*$_2$–), 2.06–1.99 (m, 4H, –SCH$_2$C*H*$_2$CH$_2$–). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ: 173.3, 51.6, 37.7, 32.3, 24.1. HR-MS (ESI): Calcd for: $C_{10}H_{18}O_2S_2$: 289.0545 ([M + Na]+), Found: 289.0539 ([M + Na]+).

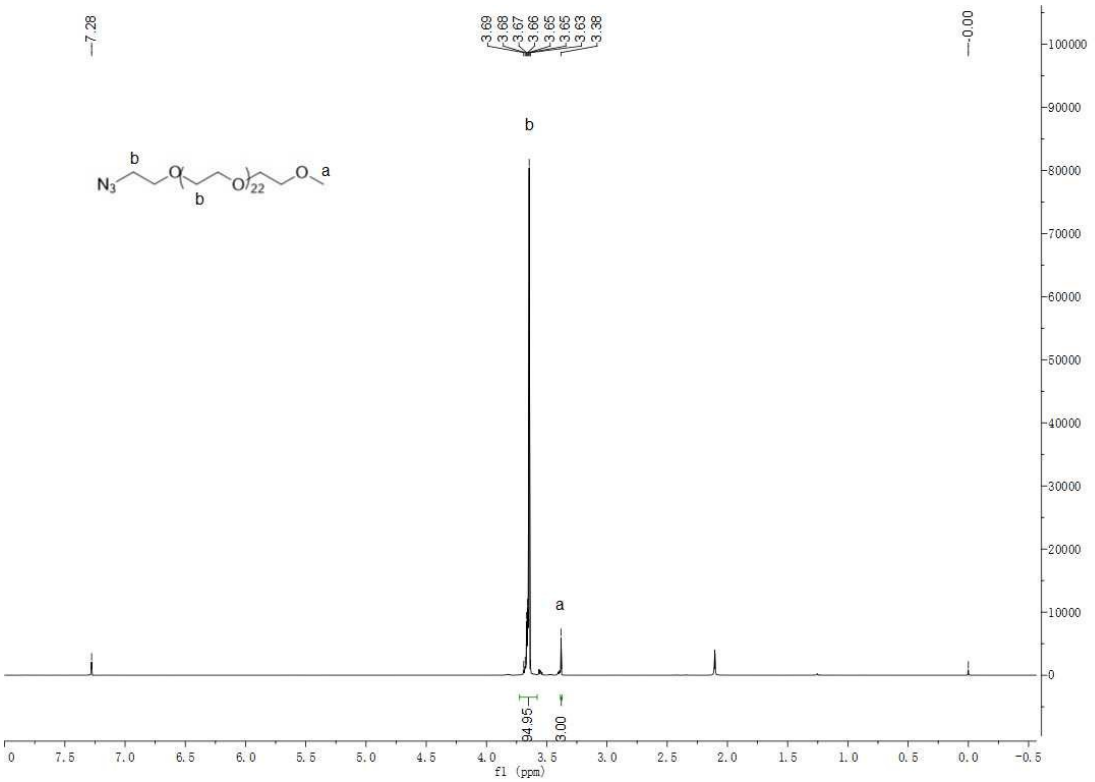


Fig. S1. ^{1}H NMR spectrum of $mPEG_{1000}$-N_3 in $CDCl_3$.

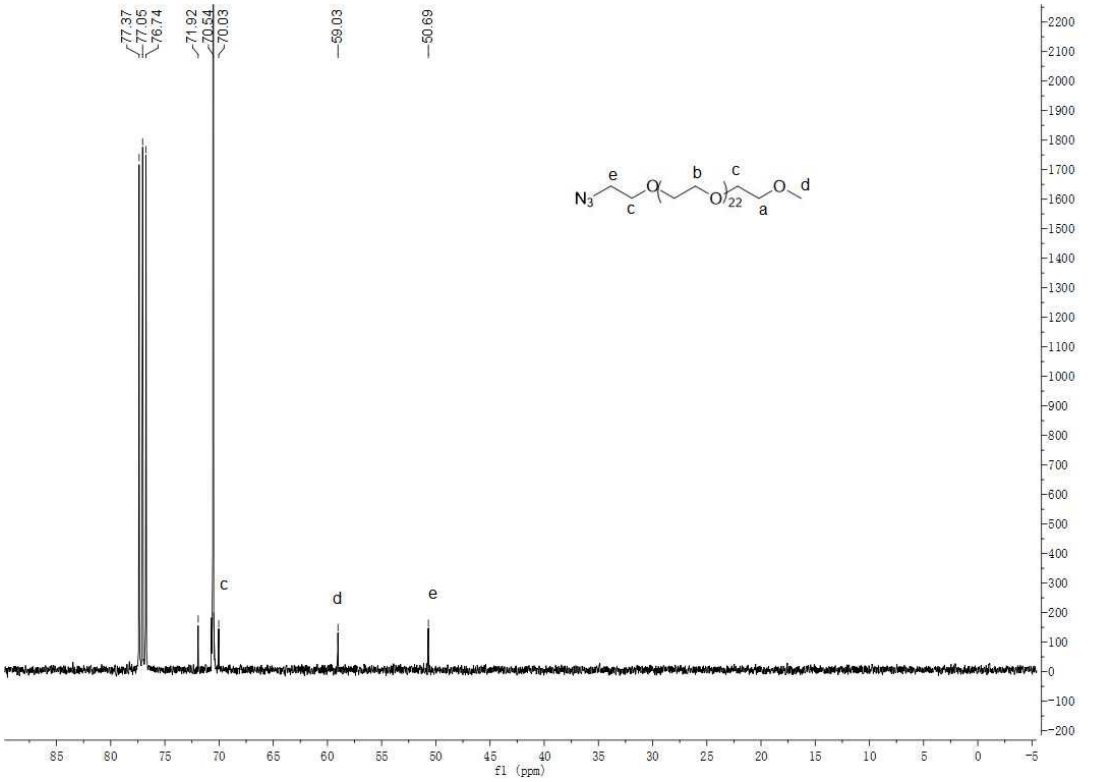


Fig. S2. ^{13}C NMR spectrum of $mPEG_{1000}$-N_3 in $CDCl_3$.

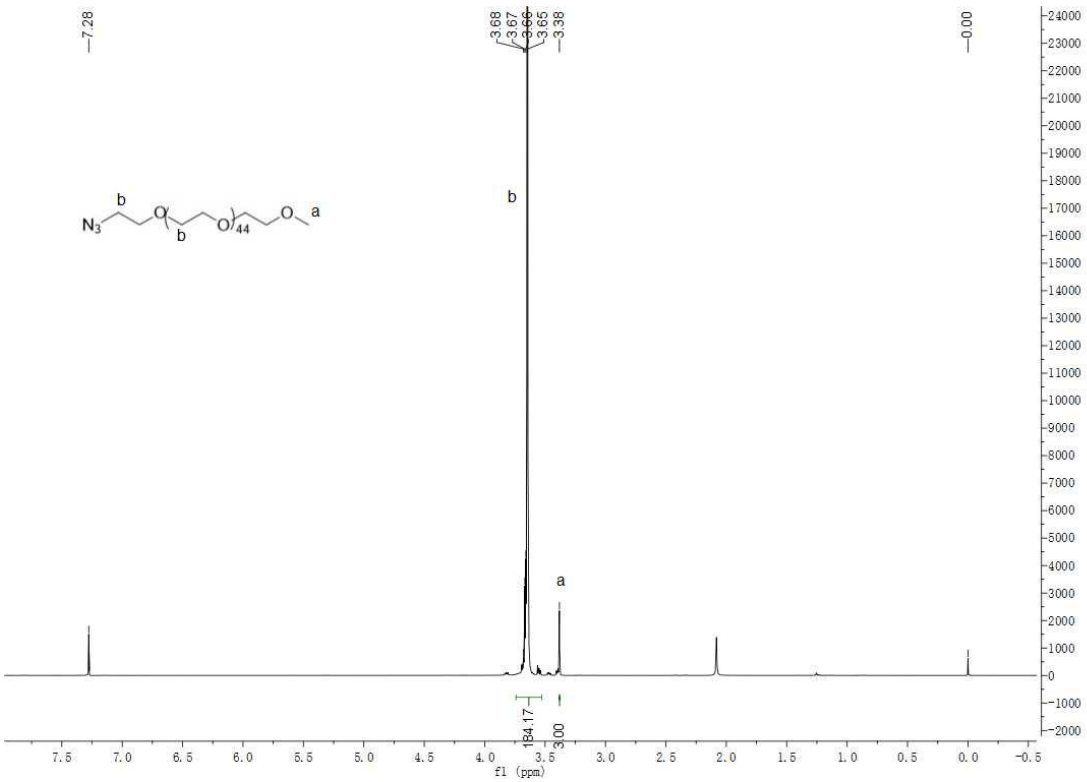


Fig. S3. ^{1}H NMR spectrum of $mPEG_{2000}$-N_3 in $CDCl_3$.

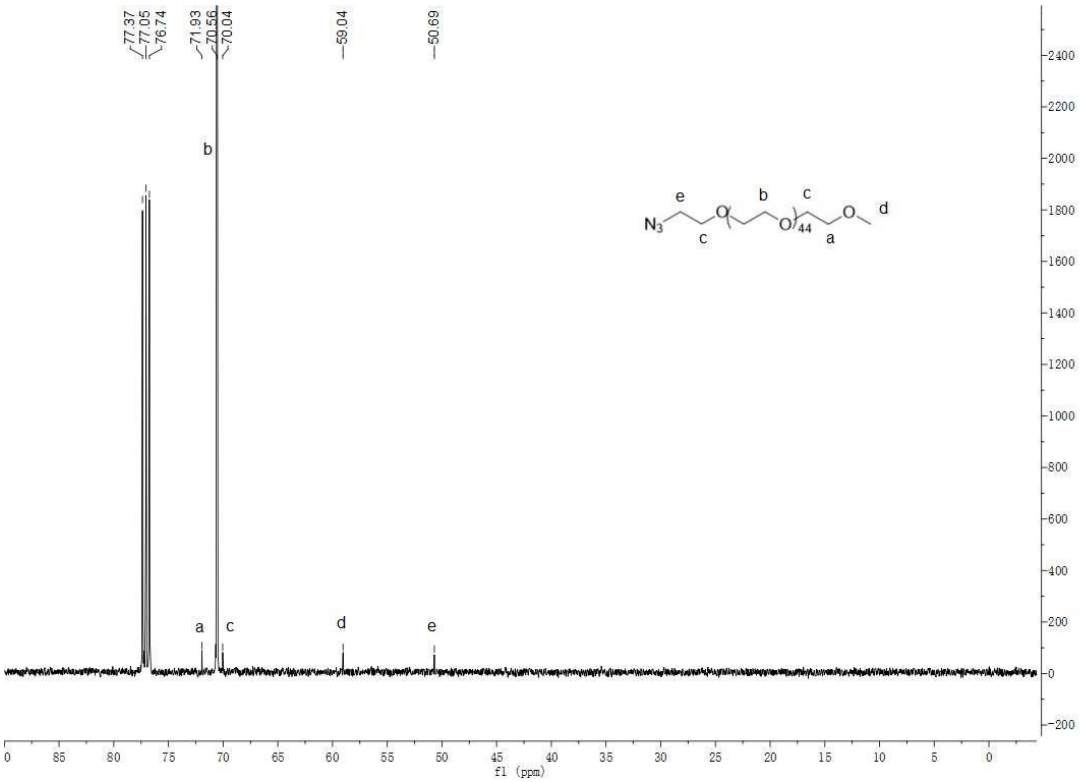


Fig. S4. ^{13}C NMR spectrum of $mPEG_{2000}$-N_3 in $CDCl_3$.

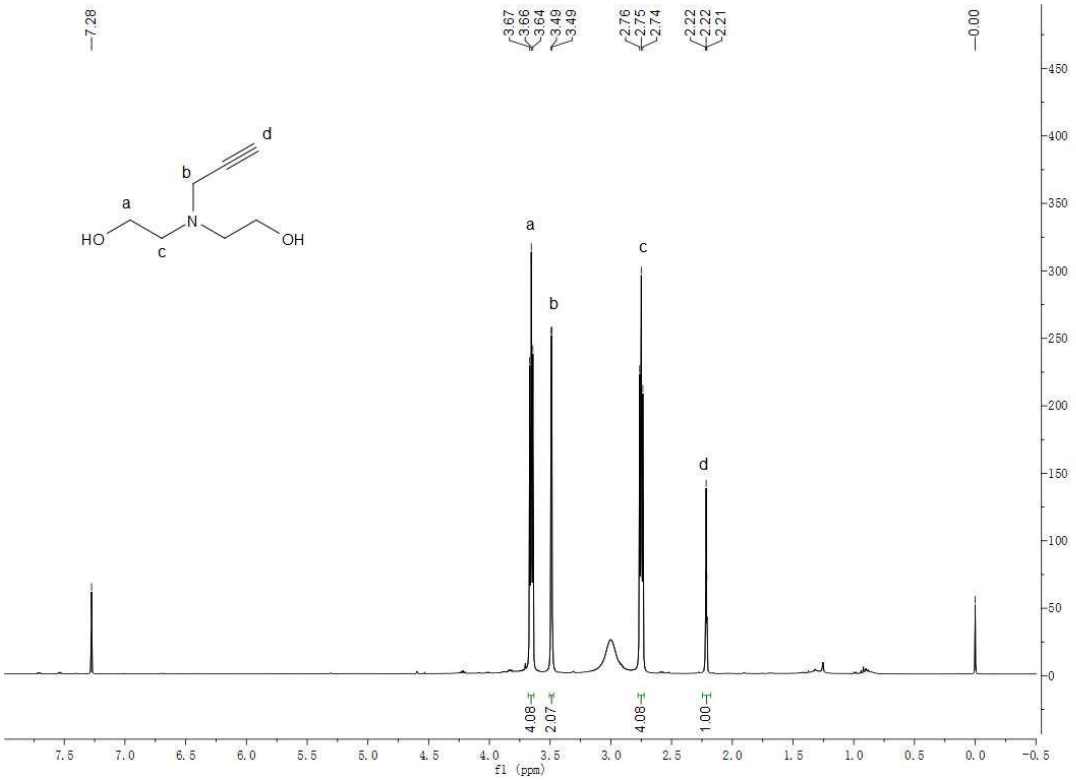


Fig. S5. ^{1}H NMR spectrum of *N*-propargyldiethanolamine in $CDCl_3$.

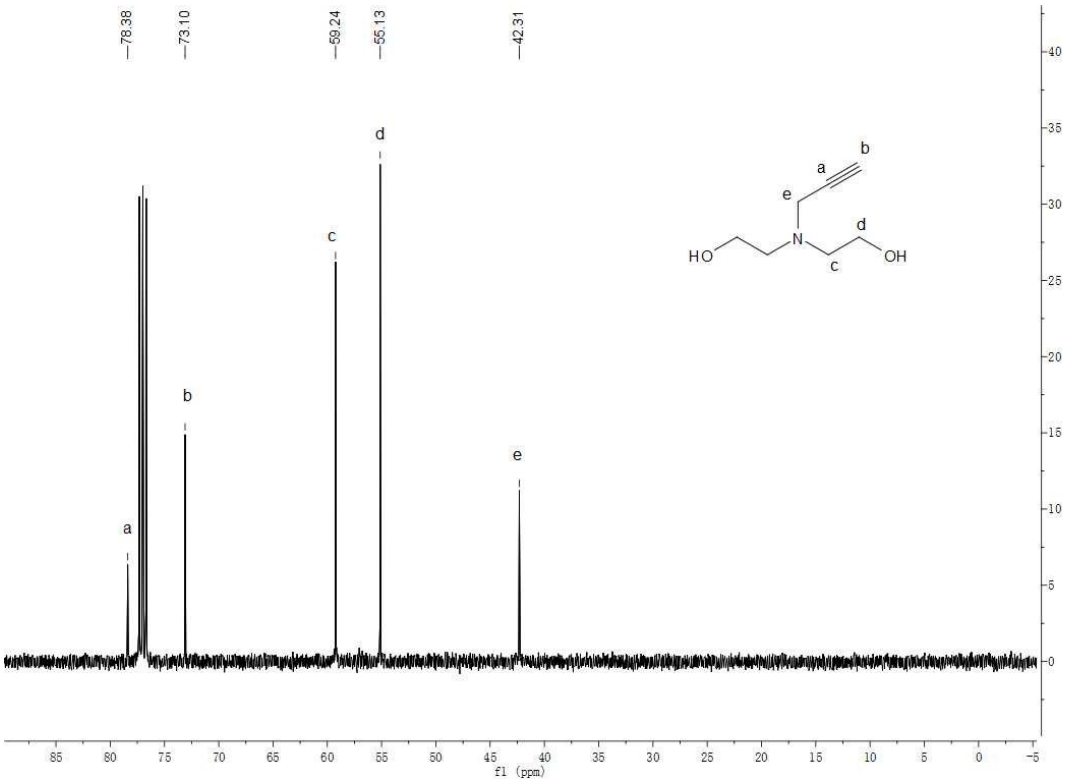


Fig. S6. ^{13}C NMR spectrum of *N*-propargyldiethanolamine in $CDCl_3$.

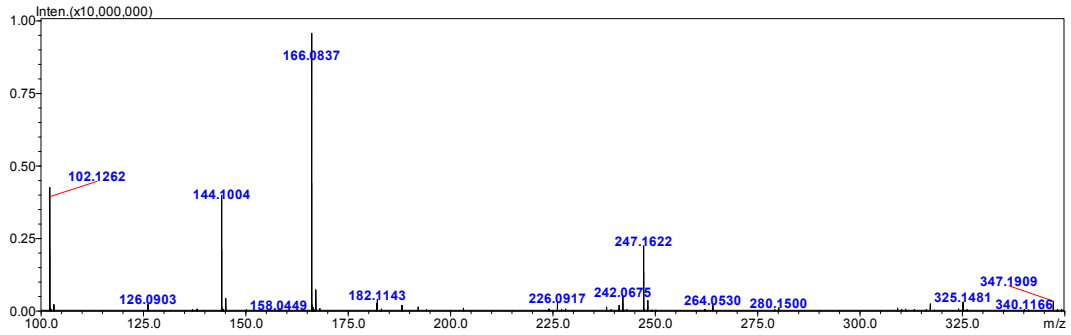


Fig. S7. HR-MS of *N*- propargyldiethanolamine.

Fig. S8. ^{1}H NMR spectrum of dimethyl 4,4′-dithiodibutyrate in $CDCl_3$.

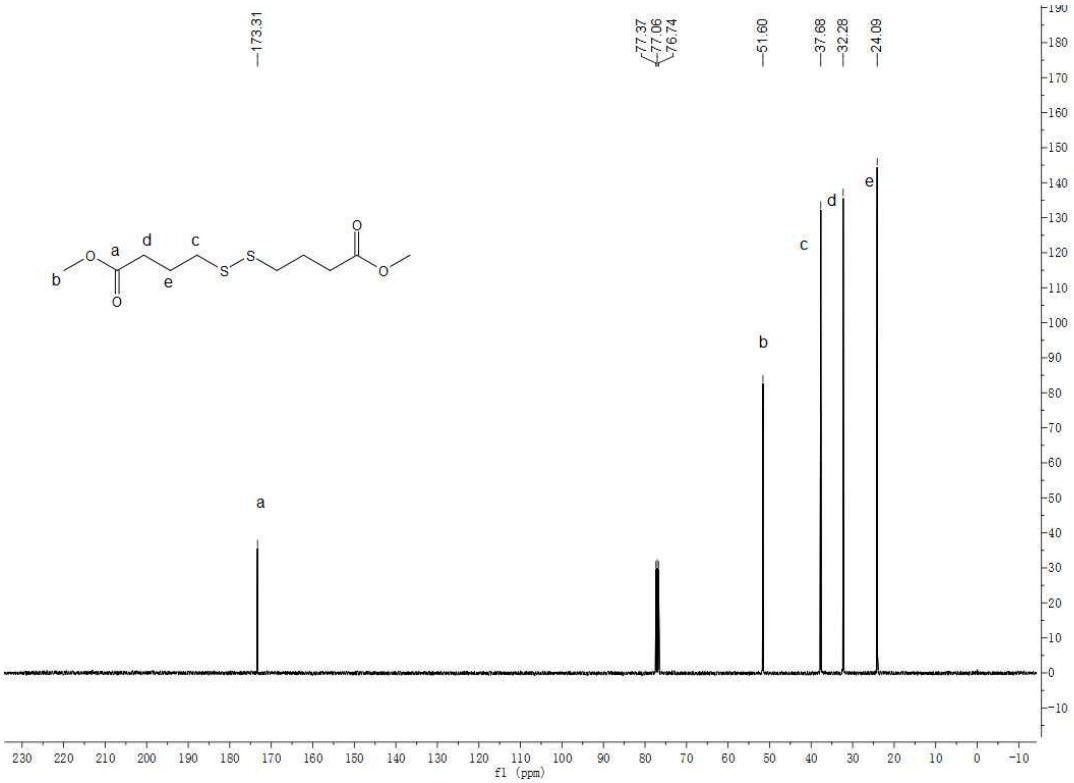


Fig. S9. ^{13}C NMR spectrum of dimethyl 4,4′-dithiodibutyrate in $CDCl_3$.

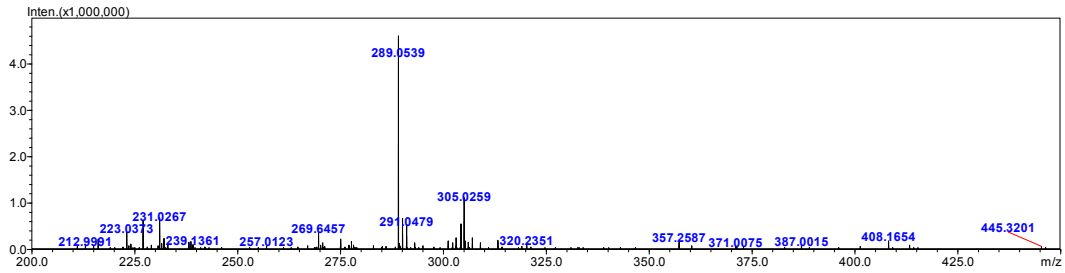


Fig. S10. HR-MS of dimethyl 4,4′-dithiodibutyrate.

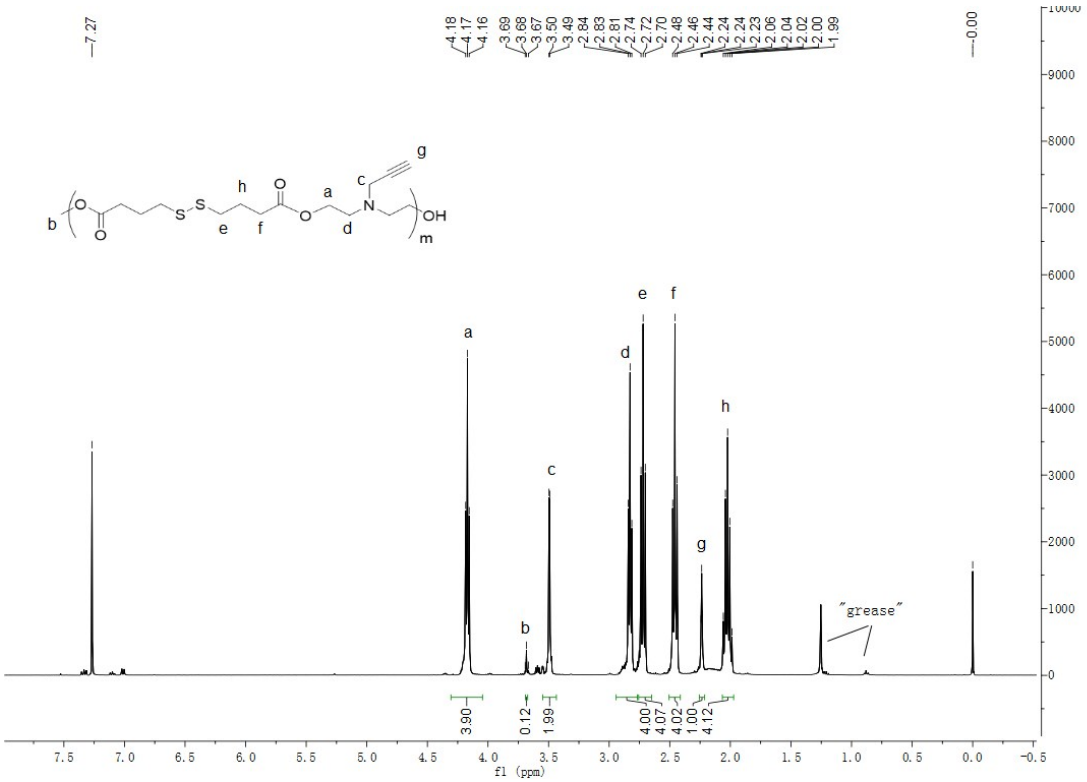


Fig. S11. ^{13}H NMR spectrum of PPD copolymers in $CDCl_3$.

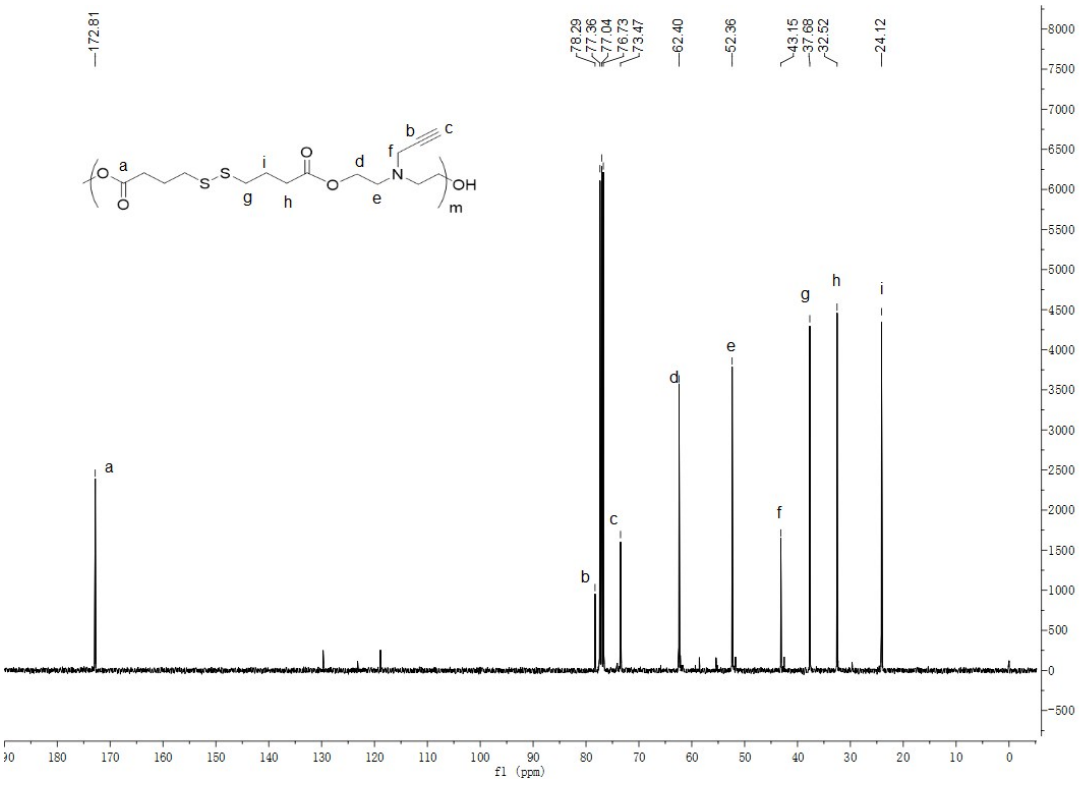


Fig. S12. ^{13}C NMR spectrum of PPD copolymers in $CDCl_3$.

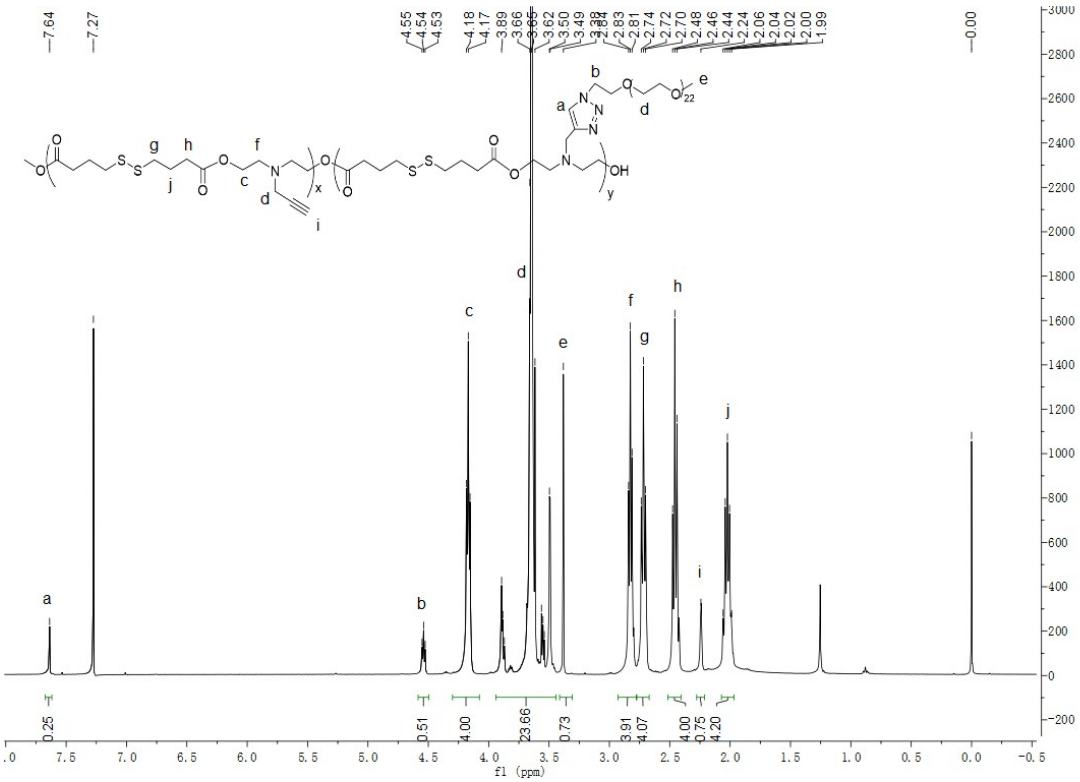


Fig. S13. ^{1}H NMR spectrum of PPD-*g*-mPEG$_{1000}$-25%in $CDCl_3$.

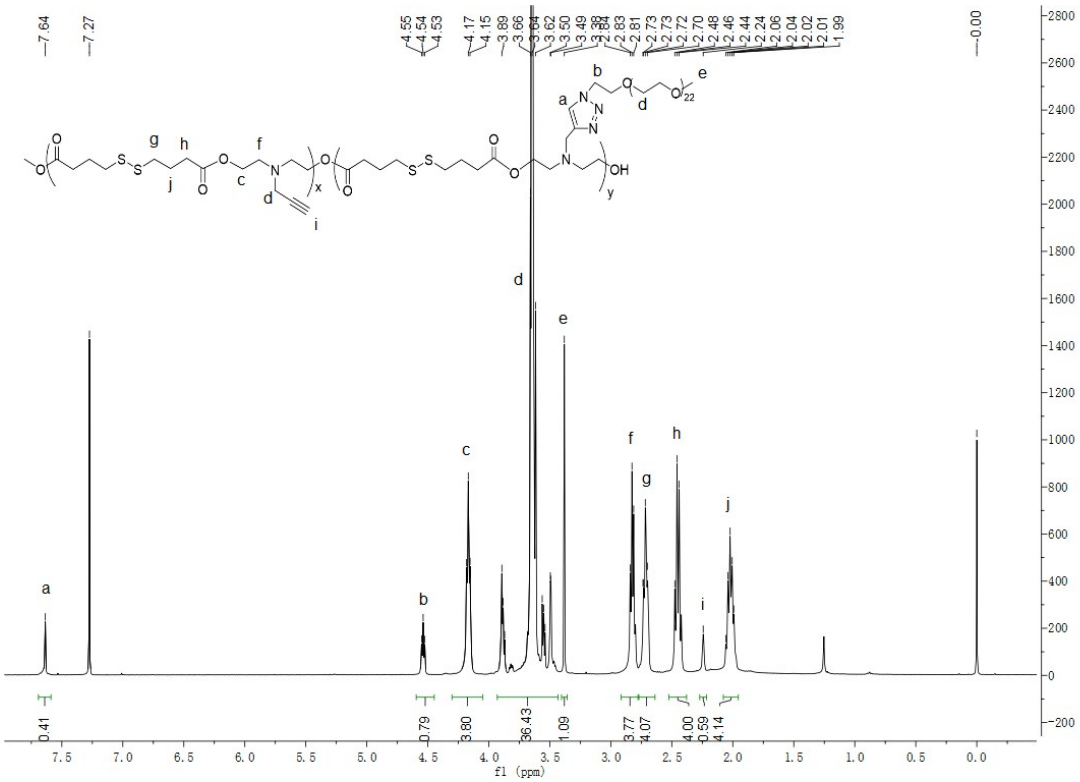


Fig. S14. ^{1}H NMR spectrum of PPD-*g*-mPEG$_{1000}$-41% in $CDCl_3$.

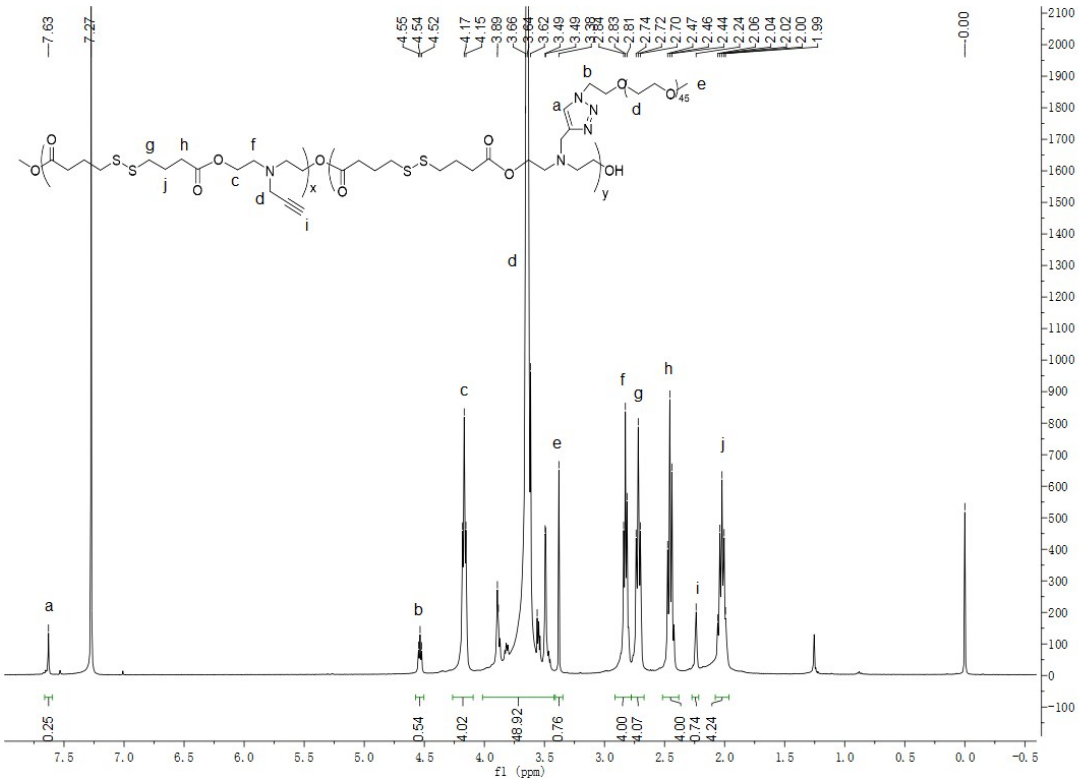


Fig. S15. ^{1}H NMR spectrum of PPD-*g*-mPEG$_{2000}$-25% in $CDCl_3$.

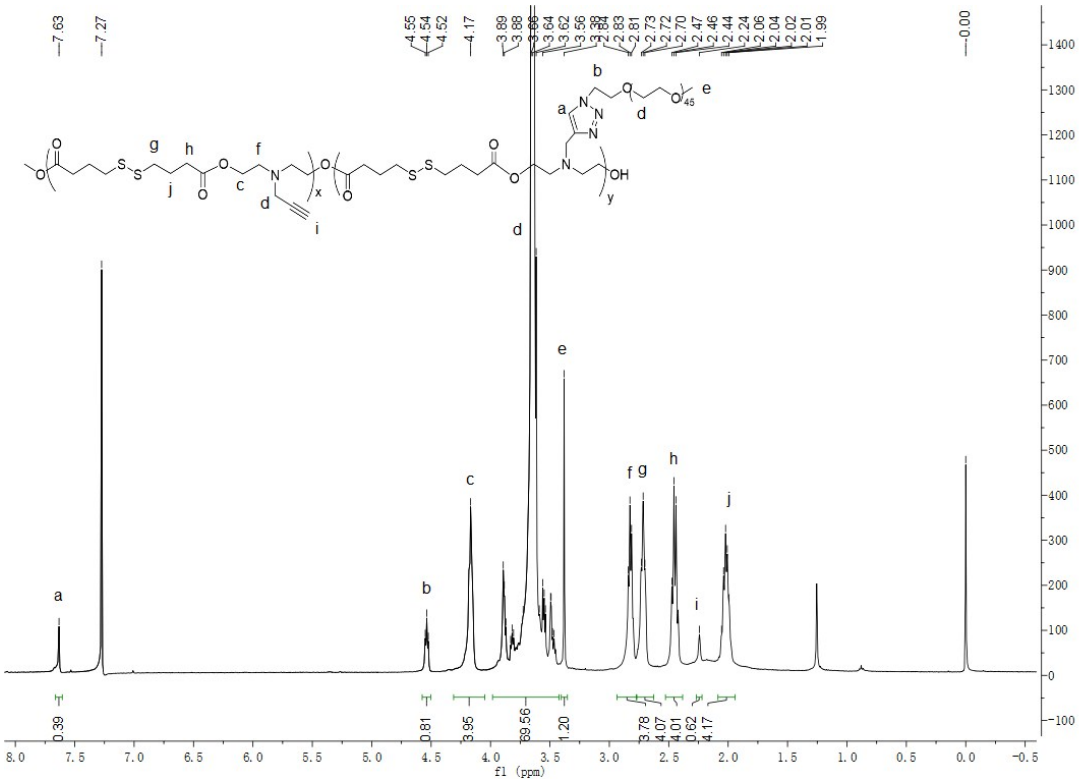


Fig. S16. ^{1}H NMR spectrum of PPD-*g*-mPEG$_{2000}$-39% in $CDCl_3$.

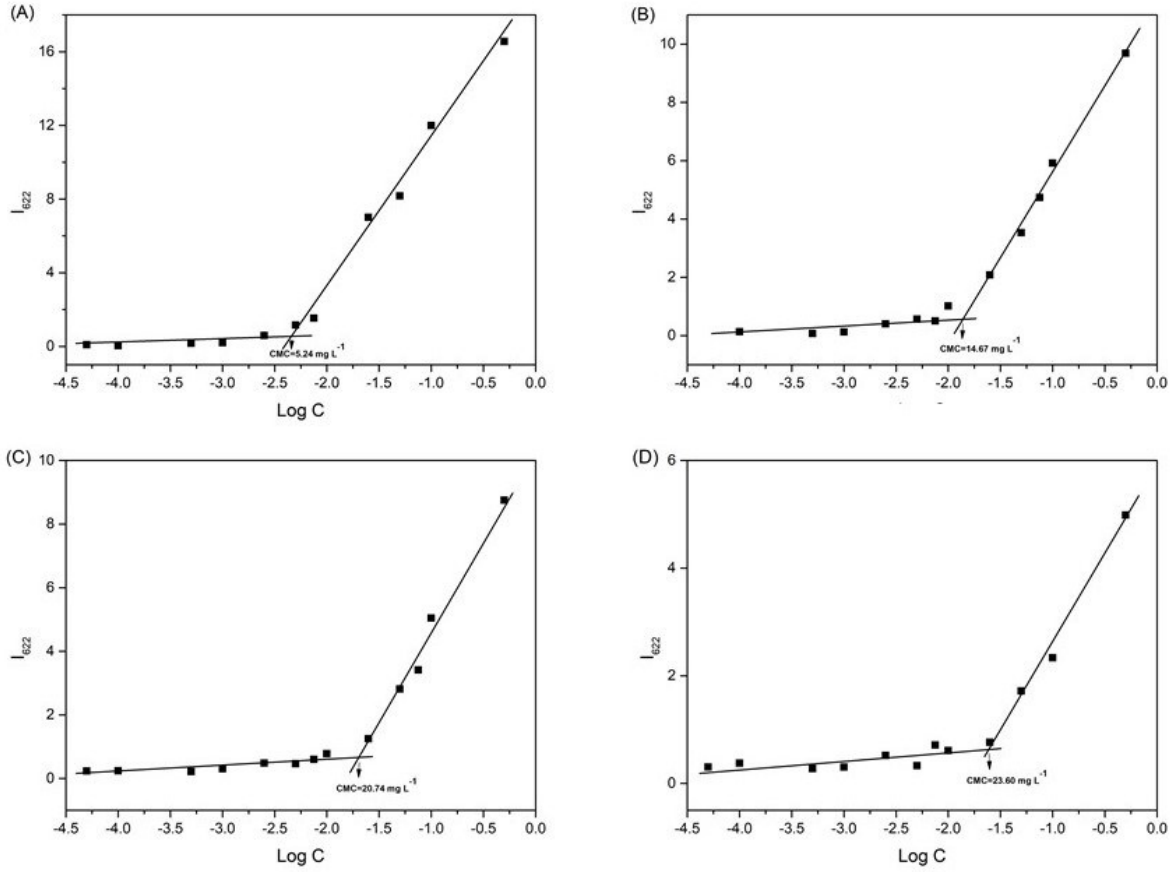


Fig. S17. Plots of the intensity ratio I_{622} from the nile red emission spectra versus the logarithm of the concentration of (A) PPD-*g*-mPEG$_{1000}$-25%; (B) PPD-*g*-mPEG$_{1000}$-41%; (C) PPD-*g*-mPEG$_{2000}$-25%; (D) PPD-*g*-mPEG$_{2000}$-39%.

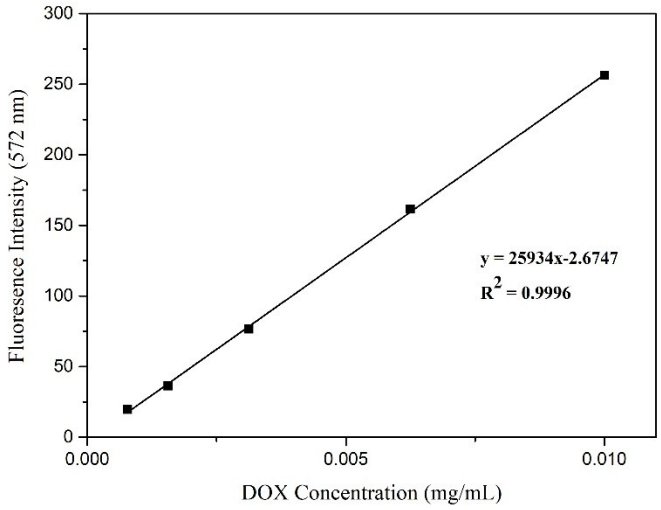


Fig. S18. The standard curve of DOX in DMF.

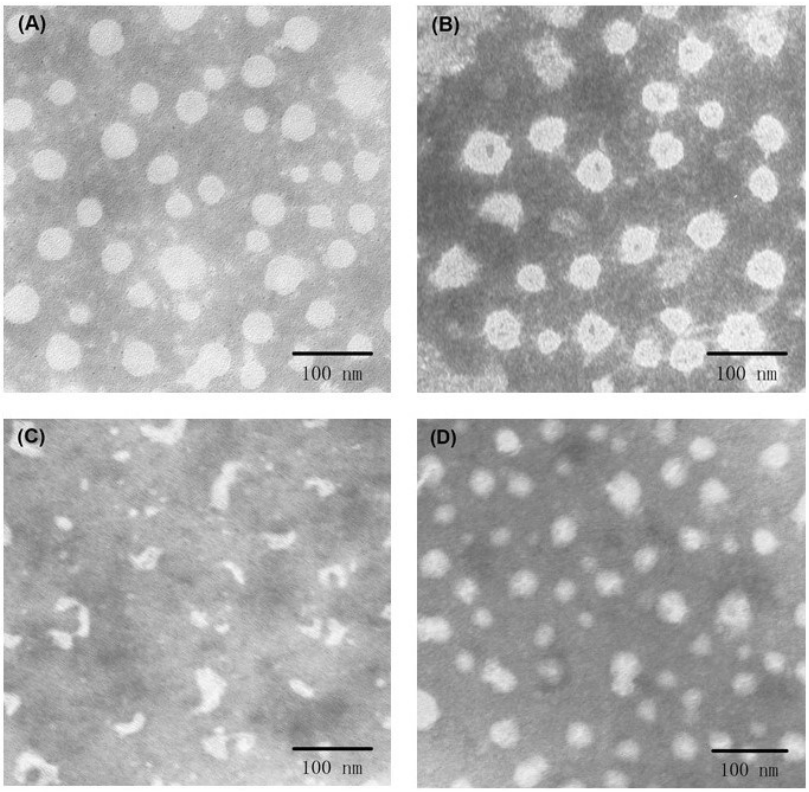


Fig. S19. TEM micrographs of DOX-loaded PPD-*g*-mPEG$_{1000}$-25% micelles under (A) pH 7.4 and (B) pH 5.5 with 10 mM GSH for 15 days, and DOX-loaded PPD-*g*-mPEG$_{1000}$-41% micelles under (C) pH 7.4 and (D) pH 5.5 with 10 mM GSH for 15 days. Scale bar = 100 nm.

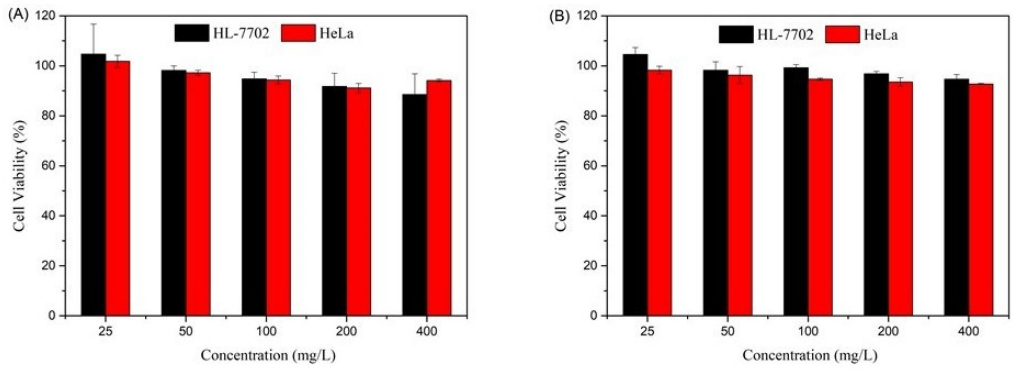


Fig. S20. *In vitro* cell cytotoxicity of (A) Blank PPD-*g*-mPEG$_{1000}$-25% micelles; (B) Blank PPD-*g*-mPEG$_{1000}$-41% micelles against HeLa cells and HL-7702 cells after 24 h incubation.

References

1. B. Y. Liu, W. X. Wu, Y. H. Liu, C. Jia, X. L. Yang, J. Li, N. Wang and X. Q. Yu, *Polym. Chem*., 2017, **8**, 5982–5987.

2. B. Boens, T. S. Ouk, Y. Champavier and R. Zerrouki, *Nucleos. Nucleo. Nucl.*, 2015, **34**, 500–514.