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Supporting Information

Intracellular microenvironment responsive PEGylated polypeptides nanogels with ionizable cores for efficient doxorubicin loading and triggered release

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1 Preparation of disulfide-core-cross-linked PEG-polypeptides nanogels

1.1 Preparation of mPEG-P(LG-*co*-LC) nanogels

The mPEG-P(BLG-*co*-LC) copolymers were synthesized through one-step ring-opening polymerization (ROP) of BLG NCA and LC NCA with mPEG-NH_2 as macroinitiator. Typically, BLG NCA (2.1 g, 8 mmol), LC NCA (292.3 mg, 1.0 mmol) and mPEG-NH_2 (1.0 g, 0.2 mmol) were dissolved in 50 mL of dry DMF in a flame-dry flask. The polymerization was performed at 25 °C for 3 d. Then, the solution was precipitated into excessive diethyl ether. The obtained product was further washed twice with diethyl ether and dried under vacuum at room temperature for 24 h (Yield: 85.3%). mPEG-P(LG-*co*-LC) was synthesized by removing the benzyl group from mPEG-P(BLG-*co*-LC). Briefly, mPEG-P(BLG-*co*-LC) was dissolved in dichloroethanoic acid (100 g L^{-1}) and 33 wt. % HBr solution in acetic acid was then added (20 mL for 1 g copolymer). After stirring for 1 h at 30 °C, the mixture was precipitated into diethyl ether (10 times volume of the reaction solution). The obtained product was further washed twice with diethyl ether and dried under vacuum at room temperature for 24 h (Yield: 82.3%). The disulfide-core-cross-linked mPEG-P(LG-*co*-LC) nanogels were prepared by directly dispersing the resultant products in PBS.

1.2 Preparation of mPEG-P(LL-*co*-LC) nanogels

mPEG-P(ZLL-*co*-LC) copolymers were synthesized similarly as mPEG-P(BLG-*co*-LC). In a typical polymerization, ZLL NCA (2.3 g, 8 mmol), LC NCA (292.3 mg, 1.0 mmol) and mPEG-NH_2 (1.0 g, 0.2 mmol) were dissolved in 50 mL of dry DMF in a flame-dry flask. The polymerization was performed at 25 °C for 3 d. Then, the solution was precipitated into excessive diethyl ether. The obtained product was further washed twice with diethyl ether and dried under vacuum at room temperature for 24 h (Yield: 80.3%). Then, mPEG-P(ZLL-*co*-LC) was dissolved in trifluoroacetic acid (100 g L^{-1}) and 33 wt. % HBr solution in acetic acid was then added (20 mL for 1 g copolymer). After stirring for 30 min at 25 °C, the mixture was precipitated into diethyl ether (10 times volume of the reaction solution). The obtained product was further washed twice with diethyl ether and dried under vacuum at room temperature for 24 h (Yield: 84.5%).

Table S1 Values of the intercept (a), slope (b) and correlation coefficient (R^2) for DOX release profiles.

Nanogel	pH	0 mM GSH			10 mM GSH		
		a	b	R^2	a	b	R^2
mPEG-P(LG-*co*-LC)-1	7.4	0.045	0.026	0.990	0.037	0.059	0.977
	5.5	0.046	0.032	0.981	0.025	0.128	0.974
mPEG-P(LG-*co*-LC)-2	6.8	0.049	0.026	0.975	0.060	0.089	0.982
	7.4	0.044	0.025	0.991	0.049	0.058	0.990
mPEG-P(LG-*co*-LC)-3	7.4	0.046	0.023	0.977	0.040	0.057	0.988
mPEG-P(LL-*co*-LC)-1	7.4	0.051	0.020	0.956	0.068	0.068	0.981
	5.5	0.062	0.021	0.933	0.062	0.113	0.972
mPEG-P(LL-*co*-LC)-2	6.8	0.052	0.020	0.959	0.046	0.077	0.992
	7.4	0.049	0.019	0.957	0.076	0.059	0.962
mPEG-P(LL-*co*-LC)-3	7.4	0.049	0.018	0.947	0.065	0.058	0.969

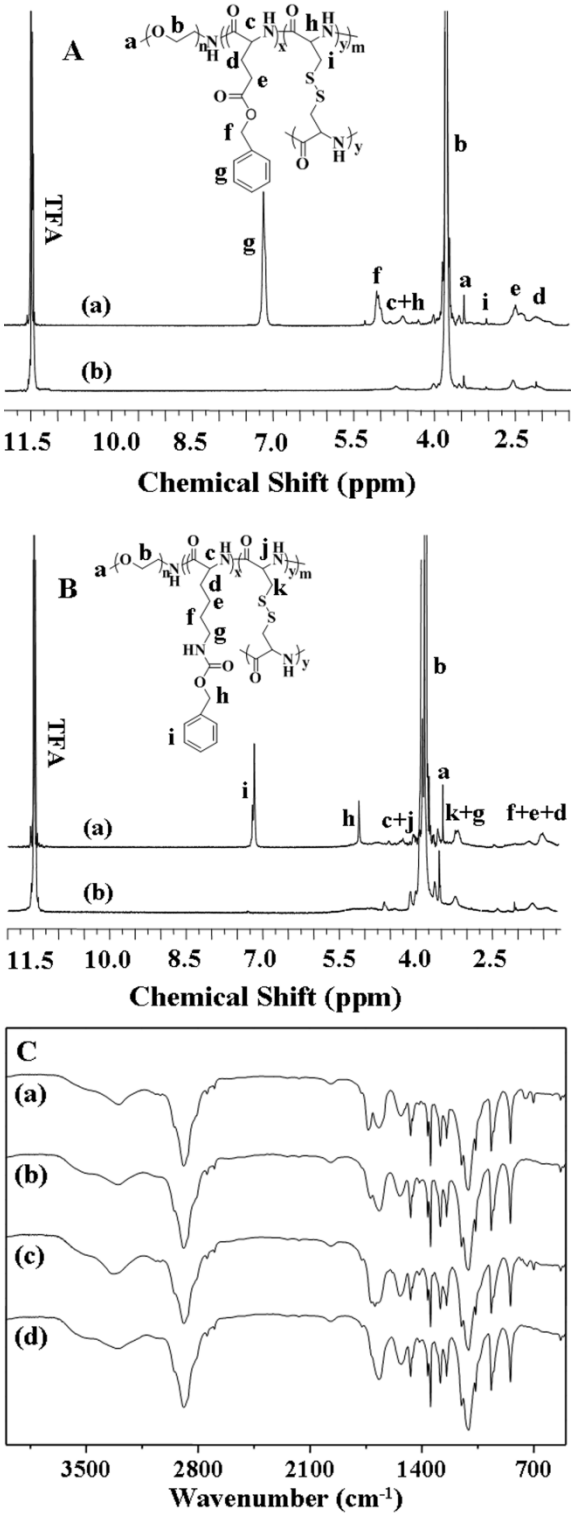

Fig. S1 ^{1}H NMR (in TFA-*d*) spectra of mPEG-P(BLG-*co*-LC)-2 (a) and mPEG-P(LG-*co*-LC)-2 (b) (A) and mPEG-P(ZLL-*co*-LC)-2 (a) and mPEG-P(LL-*co*-LC)-2 (b) (B), and FT-IR spectra of mPEG-P(BLG-*co*-LC)-2 (a), mPEG-P(LG-*co*-LC)-2 (b), mPEG-P(ZLL-*co*-LC)-2 (c) and mPEG-P(LL-*co*-LC)-2 (d) (C).

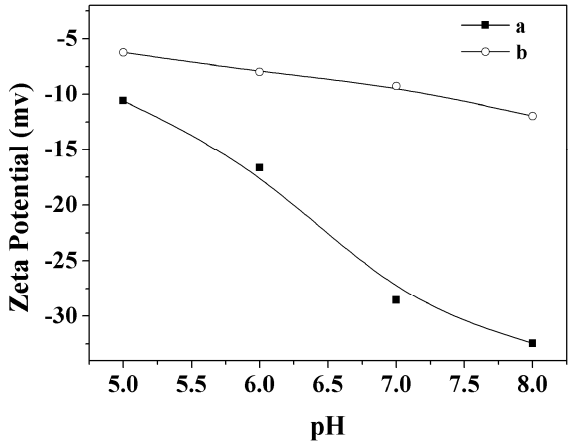

Fig. S2 ζ-potential of empty (a) and DOX-loaded mPEG-P(LG-*co*-LC)-2 (b) nanogels as a function of pH.

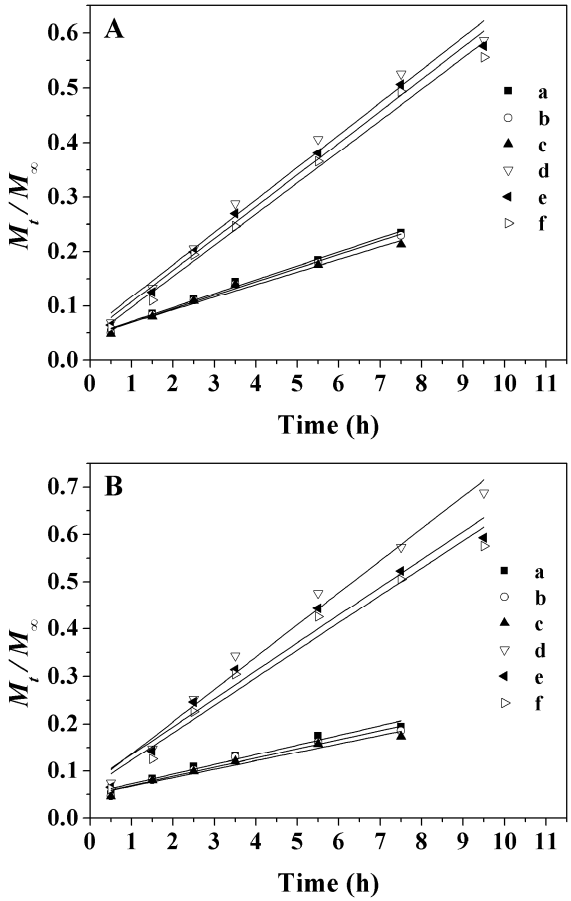

Fig. S3 Plots of M_t/M_∞ against t for DOX release from the DOX-loaded mPEG-P(LG-*co*-LC)-1 (a), -2 (b) and -3 (c) without GSH, and -1 (d), -2 (e) and -3 (f) with 10 mM GSH (A); mPEG-P(LL-*co*-LC)-1 (a), -2 (b) and -3 (c) without GSH, and -1 (d), -2 (e) and -3 (f) with 10 mM GSH (B) in PBS at pH 7.4, 37 °C. Data were presented as mean ± standard deviation (n = 3).

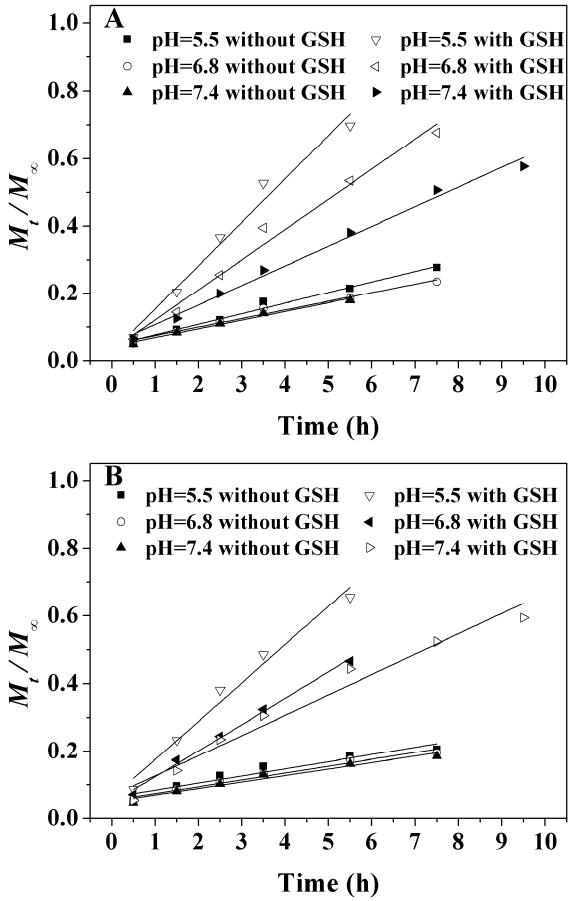

Fig. S4 Plots of M_t/M_∞ against t for DOX release from DOX-loaded mPEG-P(LG-*co*-LC)-2 (A) and mPEG-P(LL-*co*-LC)-2 (B) at pH 5.5, 6.8 and 7.4 without and with 10 mM GSH in PBS at 37 °C. Data were presented as mean ± standard deviation (n = 3).

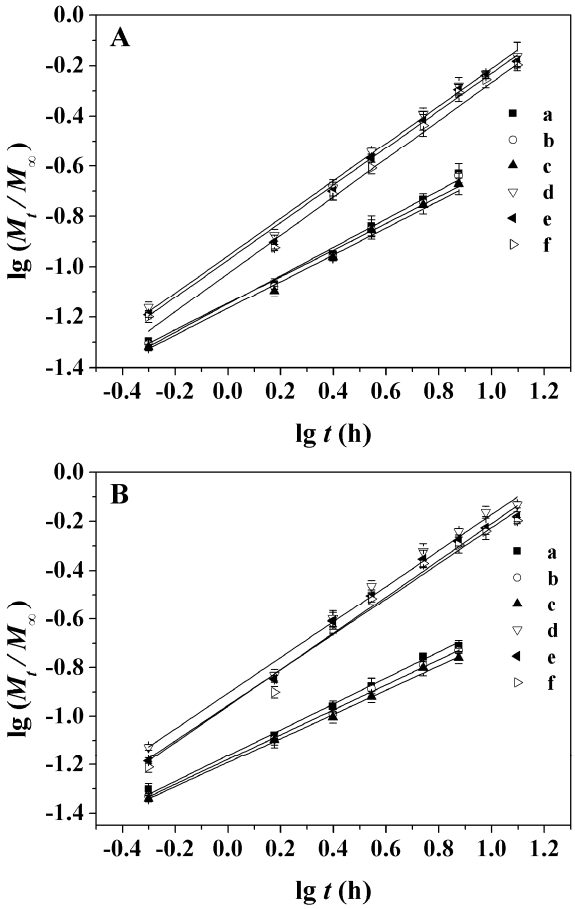

Fig. S5 Plots of lg (M_t/M_∞) against lg t for DOX release from the DOX-loaded mPEG-P(LG-*co*-LC)-1 (a), -2 (b) and -3 (c) without GSH, and -1 (d), -2 (e) and -3 (f) with 10 mM GSH (A); mPEG-P(LL-*co*-LC)-1 (a), -2 (b) and -3 (c) without GSH, and -1 (d), -2 (e) and -3 (f) with 10 mM GSH (B) in PBS at pH 7.4, 37 °C. Data were presented as mean ± standard deviation (n = 3).

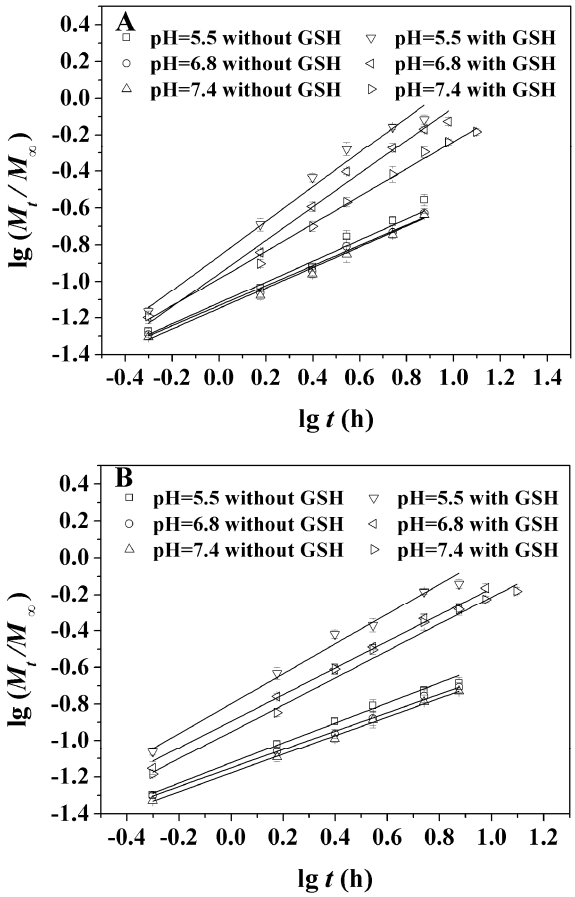

Fig. S6 Plots of lg (M_t/M_∞) against lg t for DOX release from DOX-loaded mPEG-P(LG-*co*-LC)-2 (A) and mPEG-P(LL-*co*-LC)-2 (B) at pH 5.5, 6.8 and 7.4 without and with 10 mM GSH in PBS at 37 °C. Data were presented as mean ± standard deviation (n = 3).

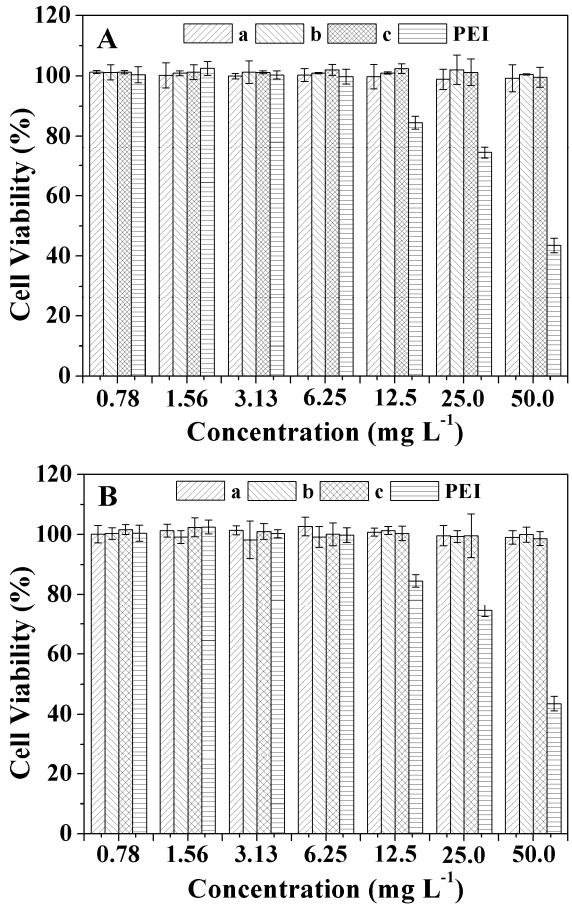

Fig. S7 Cell viabilities of HeLa cells incubated with mPEG-P(LG-*co*-LC)-1 (a), -2 (b), -3 (c) (A), and mPEG-P(LL-*co*-LC)-1 (a), -2 (b), -3 (c) (B) with PEI25K as control for 72 h. Data are presented as mean ± standard deviation (n = 6).

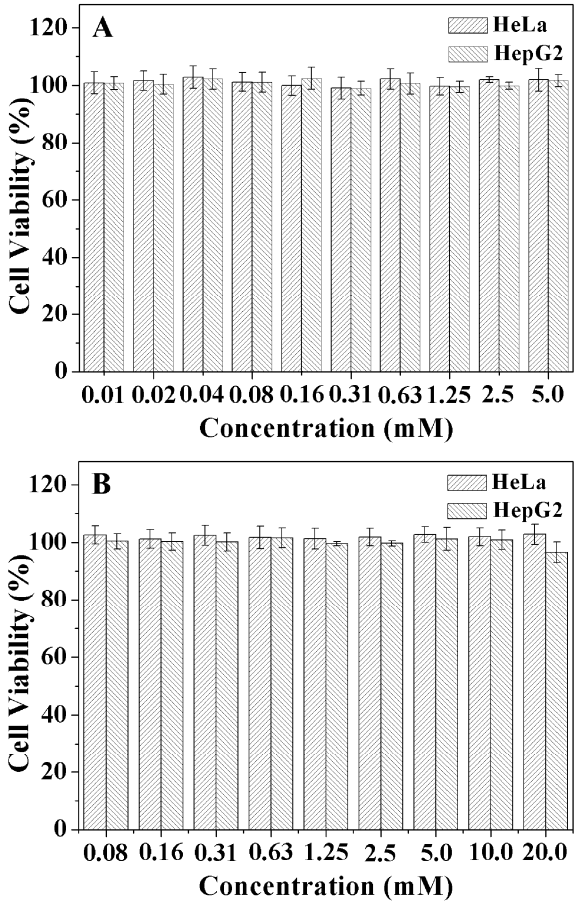

Fig. S8 Cell viabilities of HeLa (A) and HepG2 (B) cells incubated with BSO (A), and GSH (B) for 24 h. Data are presented as mean ± standard deviation (n = 6).

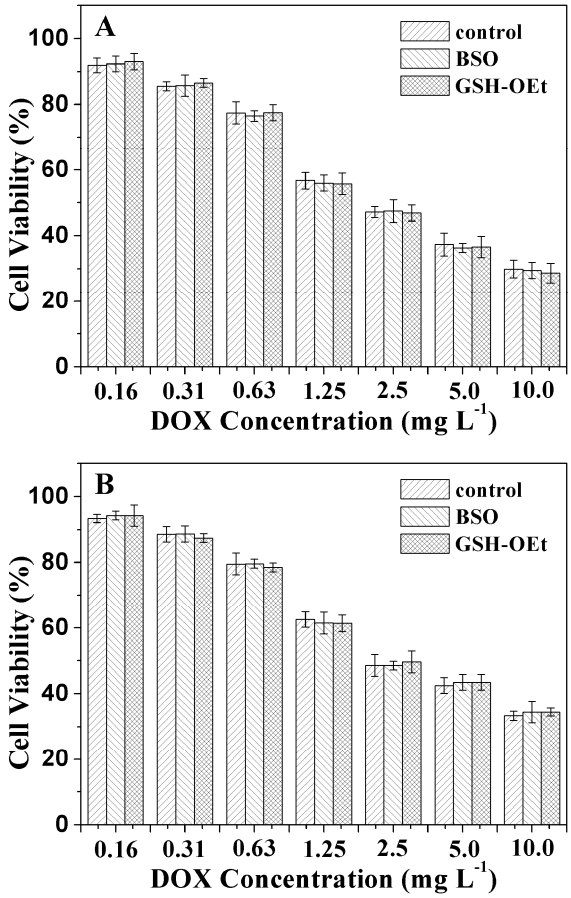

Fig. S9 Proliferation inhibitions towards HeLa (A) and HepG2 (B) cells incubated with free DOX at various concentrations for 24 h. The cells were pretreated with 0.5 mM BSO or 10 mM GSH. The nonpretreated cells were used as control. Data are presented as mean ± standard deviation (n = 6).