Supplementary Information

Synergistic Dual-pH Responsive Copolymer Micelles for Impdiment of Premature Drug Release in Blood and Motivation of the Intracellular Delivery Efficiency

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Synthesis of mPEG-b-P(CL-co-BMPCL).

The mPEG-P(CL-co-BMPCL) was synthesized by ring-opening polymerization (ROP) of ε-caprolactone and BMPCL in the presence of mPEG as an initiator and $Sn(Oct)_2$ as a catalyst according to our previous report[1]. Briefly, mPEG (1.0 g, 0.2 mmol) was dissolved in 3 mL toluene. Then ε-caprolactone (1.53 g, 13.4 mmol), BMPCL (0.167 g, 0.6 mmol) and stannous octoate were added to the reaction mixture under dry nitrogen and sealed. The system was stirred at 130 ◦ C for 12 h and then precipitated with excess diethyl. The resulting white solid was purified by precipitating with diethyl ether for three times. The mPEG-P(CL-co-BMPCL) product was dried in vaccumn for 24 h at room temperature

Synthesis of (2,2-dimeth yl-1,3-dioxolane-4-yl) methyl acrylate (DDMA).

The preparation of DA was carried out by the reaction of 1,2-isopropylideneglycerol (8.4 g, 65 mmol), methacryloylchloride (8.2 g, 90 mmol) and triethylamine (9.1 g, 90 mmol) in anhydrous CH_2Cl_2 (50 mL) in an ice bath for 15 h. The reaction solution was filtered and sequentially washed with 0.1 N HCl, 0.1 N NaOH and distilled water. The filtrate was concentrated by rotary evaporation. The product was purified by silica gel chromatography[2]. ^{1}H-NMR characterization of DDMA was shown in Figure. S1.

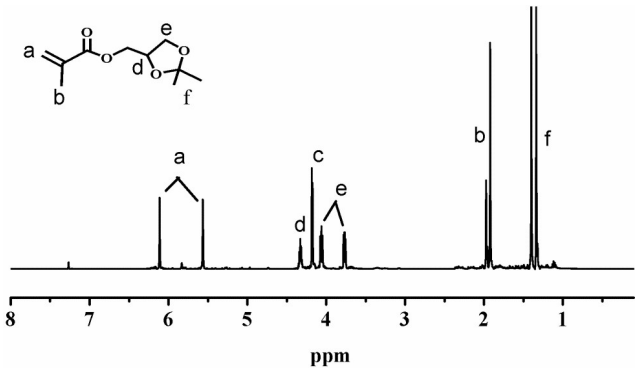

Fig. S1. ^{1}H-NMR characterization of DDMA

Table S1. Stability of blank and DOX-loaded mPEG-ros-P(CL-co-DCL) NPs

Copolymers	Blank NPs				DOX-loaded NPs			
	Size (nm)[a]	PDI [a]	Size (nm)[b]	PDI[b]	Size (nm)[a]	PDI [a]	Size (nm)[b]	PDI [b]
mPEG-b-(PCL-*g*-PDA_{30})	93 ± 4	0.10	100 ± 5	0.13	125 ± 4	0.12	122 ± 5	0.12
mPEG-b-(PCL-*g*-P(DA_{30}-*co*-$DMAEMA_{10}$))	105 ± 6	0.11	110 ± 6	0.12	116 + 4	0.11	110 ± 6	0.13
mPEG-b-(PCL-*g*-P(DA_{30}-*co*-$DMAEMA_{15}$))	128 ± 4	0.13	127 ± 7	0.12	127 + 3	0.12	126 ± 4	0.12
mPEG-b-(PCL-*g*-P(DA_{30}-*co*-$DMAEMA_{20}$))	140 ±3	0.12	142 ± 5	0.11	135 + 6	0.11	146 ± 6	0.11

[a]Determined using Laser particle size analyzer at 25°C in PBS (10 mM, pH 7.4).

[b] Determined using Laser particle size analyzer at 25°C in PBS (10 mM, pH 7.4) containing 10% (v/v) FBS .

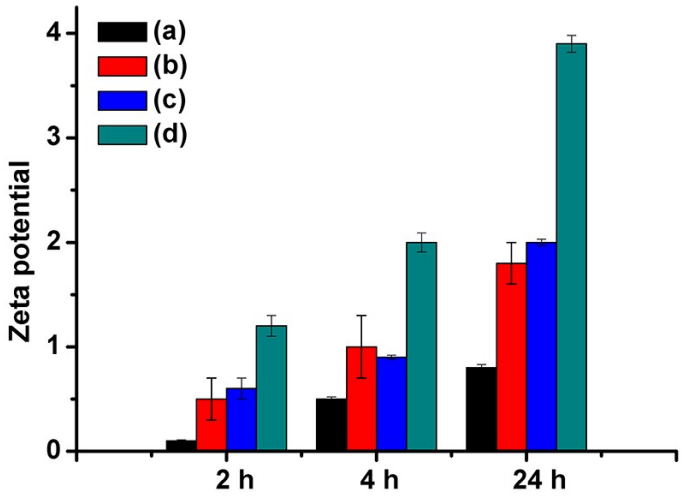

Fig. S2. The zeta potential of the freeze-dried micelles dispersed in H_2O at pH 5.0. mPEG-b-(PCL-g-PDA_{30}) (a), mPEG-b-(PCL-g-P(DA_{30}-co-$DMAEMA_{10}$)) (b), mPEG-b-(PCL-g-P(DA_{30}-co-$DMAEMA_{15}$)) (c) and mPEG-b-(PCL-g-P(DA_{30}-co-$DMAEMA_{20}$)) (d)

Table S1 Physiochemical characteristics of mPEG-b-(PCL-g-P(DA-co-DMAEMA)) micelles with FRET probes (DiO and DiI).

Copolymers	Loading content (%, w/w)		Diameters[a]	PDI[a]
	DIO	DiI		
mPEG-b-(PCL-g-PDA_{30})	0.60	0.61	101 ± 3	0.118
mPEG-b-(PCL-g-P(DA_{30}-*co*-$DMAEMA_{10}$))	0.63	0.62	125 ± 5	0.126
mPEG-b-(PCL-g-P(DA_{30}-*co*-$DMAEMA_{15}$))	0.66	0.65	132 ± 7	0.121
mPEG-b-(PCL-g-P($DA_{3}0$-*co*-$DMAEMA_{20}$))	0.74	0.73	140 ± 6	0.122

[a]Loading content of payloads were measured using UV absorbance
[b]Mean diameter and polydispersity were determined by DLS

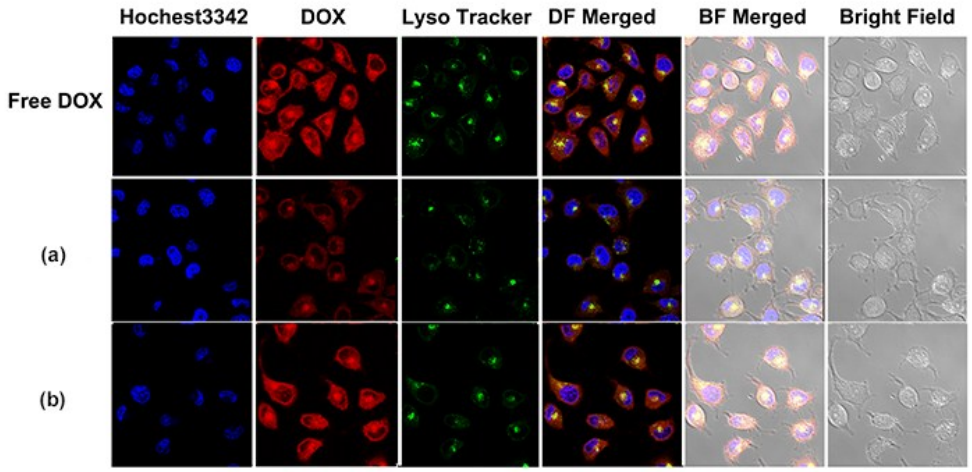

Fig. S3 Representative Fluorescence microscopy images of MCF-7 cells incubated with DOX-loaded

mPEG-b-(PCL-g-PDA_{30}) (a) and mPEG-b-(PCL-g-P(DA_{30}-co-$DMAEMA_{20}$)) (d) micelles and free DOX (10 μg/mL) for 12 h (scale bars, 25mm).

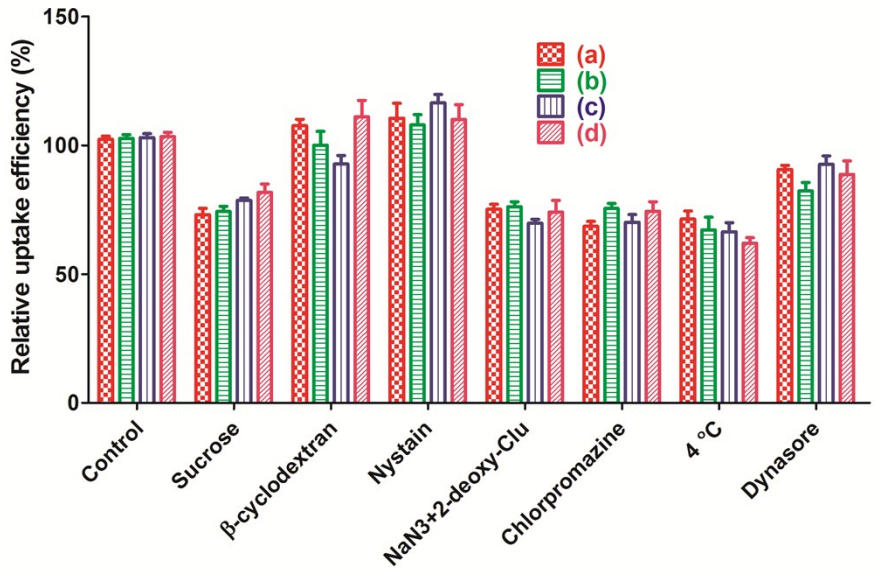

Fig. S4. Endocytosis inhibition of DOX-loaded mPEG-b-(PCL-g-PDA_{30}) (a), mPEG-b-(PCL-g-P(DA_{30}-co-$DMAEMA_{10}$)) (b), mPEG-b-(PCL-g-P(DA_{30}-co-$DMAEMA_{15}$)) (c), and mPEG-b-(PCL-g-P(DA_{30}-co-$DMAEMA_{20}$)) (d) micelles in MCF-7 cells using various endocytosis inhibitors.

Reference

[1] Deng H, Zhang Y, Wang X, Cao Y, Liu J, Liu J, et al. Balancing the stability and drug release of polymer micelles by the coordination of dual-sensitive cleavable bonds in cross-linked core. Acta biomaterialia. 2015;11:126-36.

[2] Zhang D, Zhang H, Nie J, Yang J. Synthesis and self‐assembly behavior of pH‐responsive amphiphilic copolymers containing ketal functional groups. Polymer International. 2010;59:967-74.