Supporting Information

for

Supramolecular micellar drug delivery system based on multi-arm block copolymer for highly effective encapsulation and sustained-release chemotherapy

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1. Synthesis and characterization of functional monomers

1.1 Synthesis of BEP. To a stirred solution of 3-buten-1-ol (7.64 g, 106 mmol) and triethylamine (11.7 g, 116 mmol) in 200 mL of anhydrous THF at 0 °C were dropwise added a solution of COP (14.25 g, 100 mmol) in 50 mL of anhydrous THF, and the reaction mixture was allowed to stir for 12 h. After complete conversion of COP, as confirmed by TLC, the reaction mixture was filtered. The concentrated filtrate was distilled under reduced pressure to obtain a light yellow and viscous liquid (130 °C, 100 Pa). The yield was ~97%.

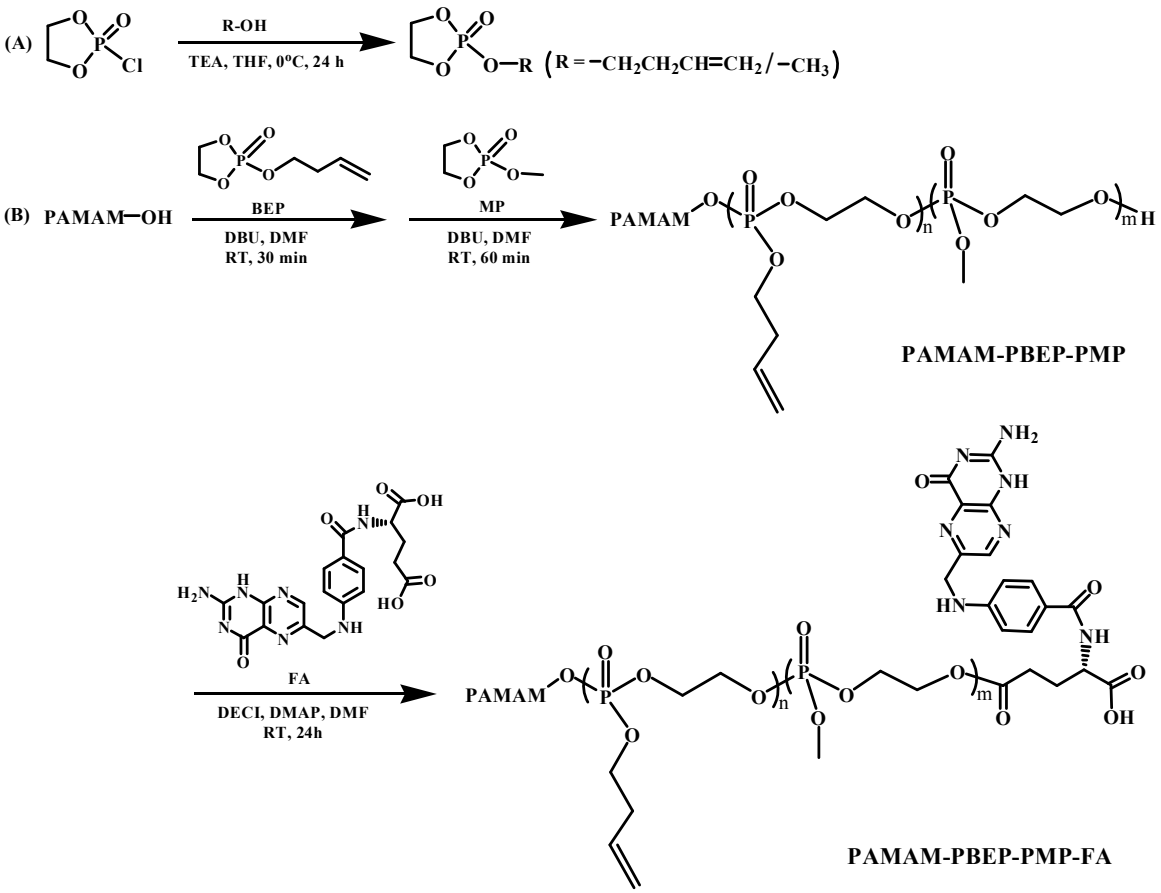
1.2 Synthesis of MP. A solution of COP (14.25 g, 100 mmol) in 50 mL of anhydrous THF was dropwise added into a stirred solution of methanol (3.85 g, 120 mmol) and triethylamine (12.14 g, 120 mmol) in 200 mL of anhydrous THF at 0 °C. The reaction mixture was allowed to stir for 12 h. After complete conversion of COP, as confirmed by TLC, the reaction mixture was filtered. The concentrated filtrate was distilled under reduced pressure to obtain a transparent and viscous liquid (94 °C, 350 Pa) with a yield of 98%.

1.3 Characterization of BEP and MP. The cyclic functional phosphoester monomers, 2-butenyl phospholane (BEP) and 2-methoxy phospholane (MP), were synthesized via nucleophilic substitution reaction between 2-chloro-1,3,2-dioxaphospholane-2-oxide (COP) and monohydric alcohols. As shown in the ^{1}H NMR spectra in Figure S1A, the resonances appeared at δ 3.83~0.90 ppm (peak c), δ 2.31~2.38 ppm (peak d), δ 5.75~5.85 ppm (peak e), and δ 5.03~5.16 ppm (peak f) are attributed to the protons of butenyl protons of BEP. In Figure S1C, the characteristic signal (peak b) assigned to the methoxyl protons of MP. From the ^{31}P NMR spectra in Figure S1B and D, the characteristic peaks of BEP and MP were 16.88 ppm and 17.89 ppm, respectively. In addition, FTIR results (Figure S2) together with NMR spectra confirmed the chemical structure of BEP and MP monomers.

2. Synthesis and characterization of multi-arm block copolymer

2.1 Synthesis of PAMAM-PBEP-PMP. A solution of BEP and a given amount of PAMAM in anhydrous dimethyl formamide were transferred into a flame-dried 25-mL shell vial equipped with a rubber septum and a stirring bar. At 25 °C, a solution of a given amount of DBU in anhydrous dimethyl formamide was injected into the vial via syringe, while being maintained under a nitrogen gas atmosphere. The feed ratio of the ingredients was shown in Table S1. After being stirred for 30 min, a solution of MP in anhydrous dimethyl formamide was injected into the vial, and sequentially stirring for 60 min. Afterwards, the reaction vial was unstoppered, and then a solution of acetic acid (excess) in dimethyl formamide was added into the reaction mixture to quench the reaction. The resulting mixture was purified by precipitation from dimethyl formamide into diethyl ether for three times and was then dried under vacuum to the copolymer (abbreviated as PAMAM-PBYP-PMP), as a light yellow highly viscous liquid in 75% yield. ^{1}H NMR and FTIR spectroscopy were used to testify the structure of prepared copolymer. GPC was used to determine the molecular weight and its distribution.

2.2 FTIR characterization of PAMAM-PBEP-PMP-FA. The broad band around 3255 cm^{-1} was attributed to the stretching vibration of the N-H, O-H and C-H of PAMAM-PBEP-PMP-FA. The characteristic adsorption band at 1511 cm^{-1} was assigned to the stretching vibration of C=C of the aromatic ring in FA. In addition, the band at 1682 cm^{-1} was corresponded to C=O in amide linkage of FA molecules. Furthermore, the band at 1610 cm^{-1} showed an eater linkage between FA and PMP, indicating the successful formation of PMP-FA conjugates.



Scheme S1. Design of multi-arm block copolymer. (A) Functional modification of cyclic phosphoester monomers via nucleophilic substitution reaction. (B) Sequential ring-opening polymerization of functional phosphoester monomers with hydroxyl-terminated poly(amido amine) dendrimer (PAMAM-OH) as an initiator.

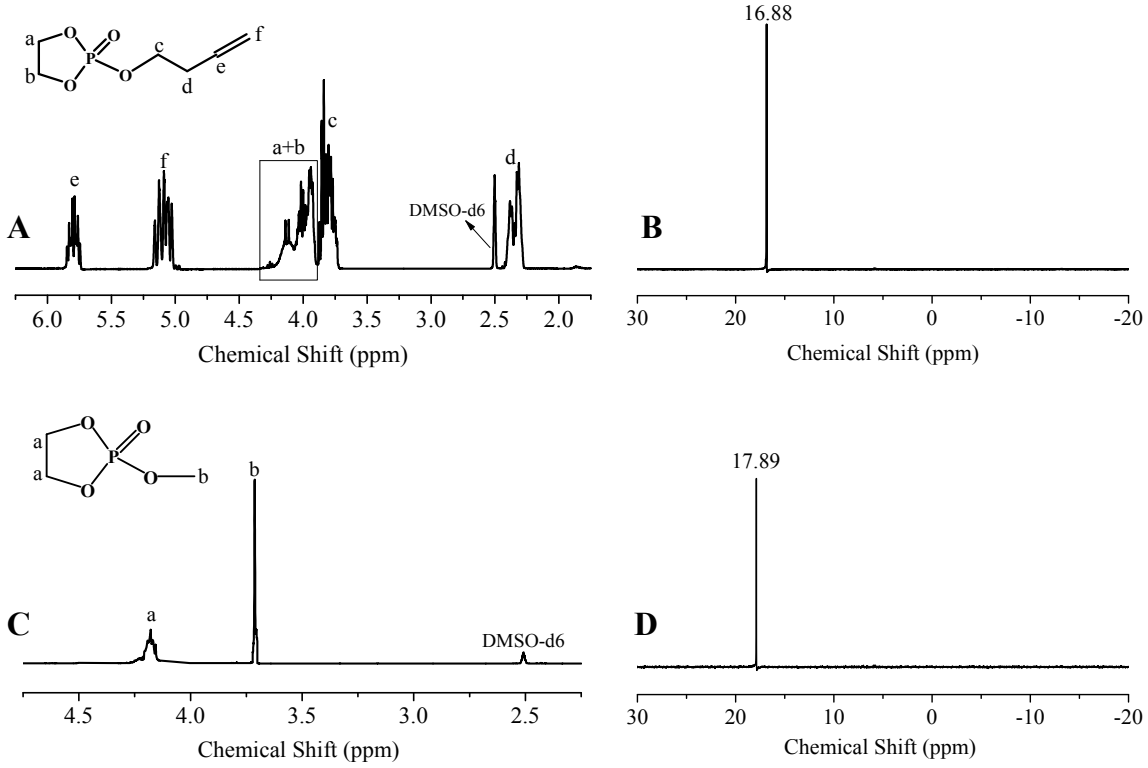


Figure S1. NMR spectra (400 MHz) of functional monomers in DMSO-d6 at room temperature: ^{1}H-NMR (A, C) and ^{31}P-NMR (B, D) spectra of BEP (A, B) and MP (C, D).

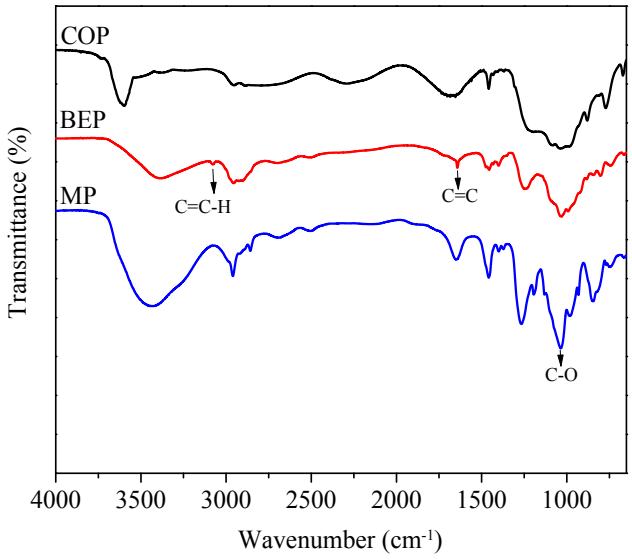


Figure S2. FTIR spectra of COP, BEP and MP.

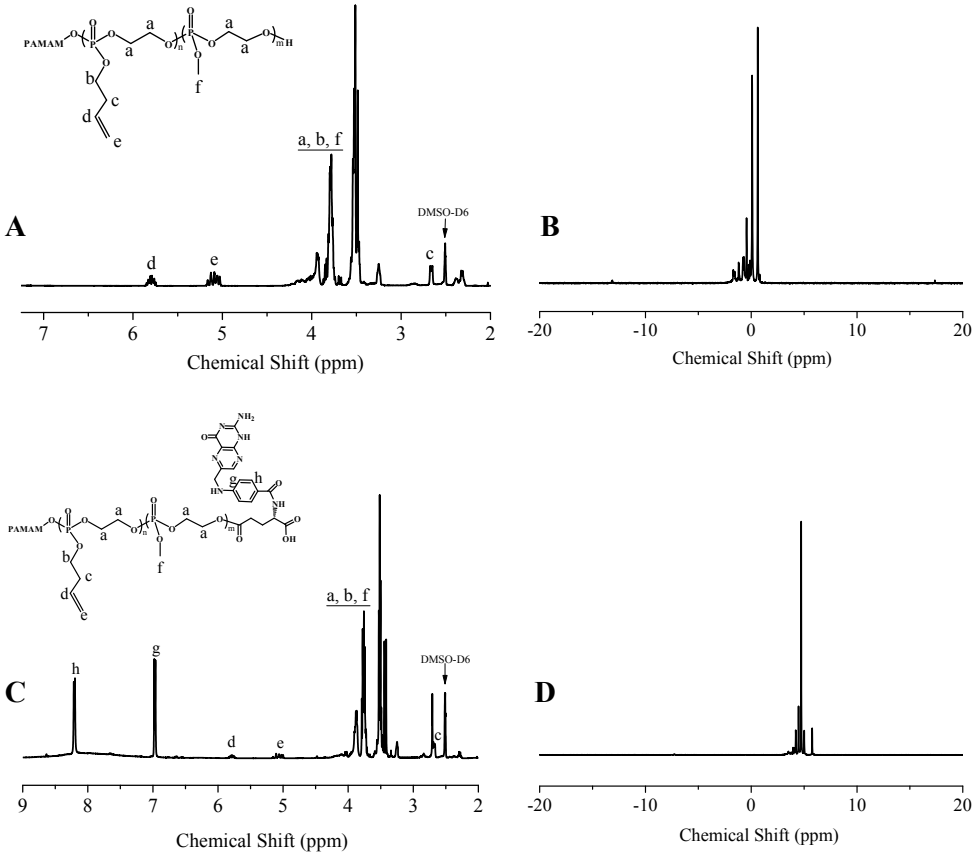


Figure S3. NMR spectra (400 MHz) of copolymers in DMSO-d6 at room temperature: ^{1}H-NMR (A, C) and ^{31}P-NMR (B, D) spectra of PAMAM-PBEP-PMP (A, B) and PAMAM-PBEP-PMP-FA (C, D).

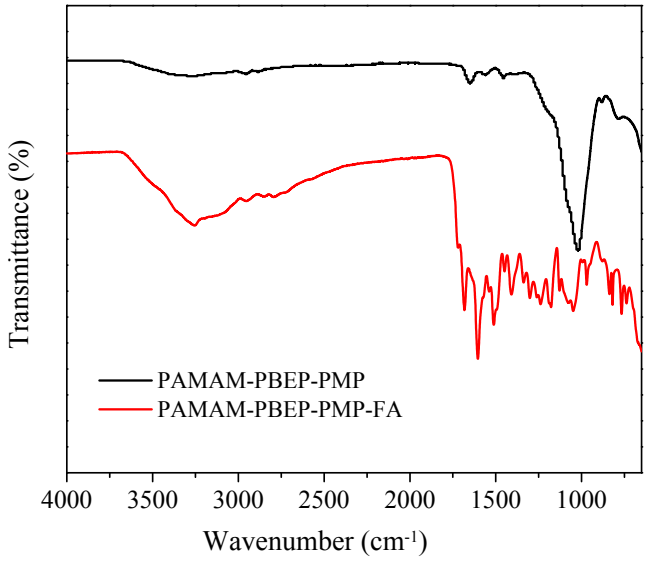


Figure S4. FTIR of PAMAM-PBEP-PMP and PAMAM-PBEP-PMP-FA

3. Preparation and characterization of self-assembled supramolecular micelles

3.1 Preparation of DOX-loaded supramolecular micelles. To prepare DOX-loaded polymeric micelles, the copolymers and DOX·HCl were firstly dissolved in DMF, respectively. Next, several drops of TEA were added into DOX·HCl solution to neutralize HCl for converting hydrophilic DOX·HCl to hydrophobic DOX. Then, the DOX solution and copolymer solution were mixed and stirred for 0.5 h. The feed weight ratio of DOX to the copolymer was 3:10. After that, ultrapure water was added dropwise into the above mixed solution under constant stirring. After 3 h of continuous stirring, DMF and free DOX were removed by dialysis against ultrapure water using a 2000 Da cut-off cellulose membrane. The final product was filtered through a 0.45 μm membrane filter, followed by lyophilization.

3.2 Characterization of self-assembled supramolecular micelles. Sizes and morphologies of micelles were tested and observed by DLS and TEM. Loading content and efficiency of DOX in micelles were measured by UV-vis spectrometry at 483 nm after the disruption of micelles and the solubilisation of DOX in dimethyl sulfoxide. DOX amount was determined according to the standard curve (Figure S3), and then drug loading content (DLC) and drug loading efficiency (DLE) were calculated following the below formula:

DLC % = (amount of DOX in nanocarrier / amount of DOX-loaded nanocarrier) × 100% ····· (1)

DLE % = (amount of DOX in nanocarrier / amount of DOX added) × 100% ····· (2)

Bovine serum albumin (BSA) was used as a model protein to test the protein adsorption of micelles. Amount of absorbed proteins was also detected by UV-vis spectrometry following the standard curve of BSA (Figure S4), and then calculated following the below formula:

Protein adsorption (%) = (amount of BSA in nanocarrier / total amount of BSA in solution) × 100%

..... (3)

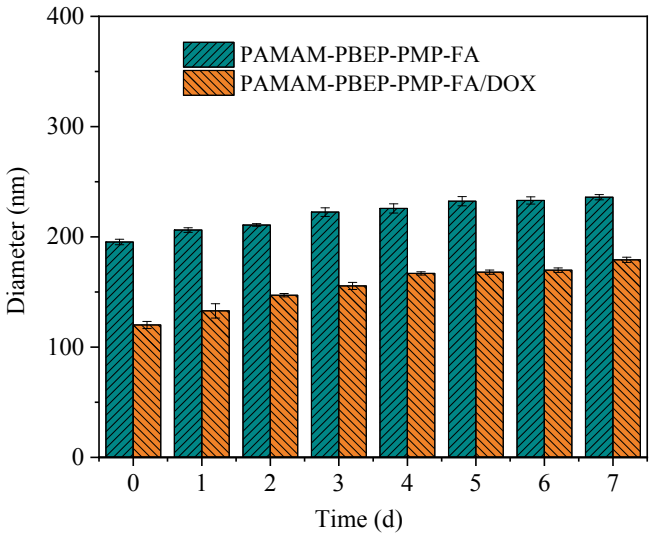


Figure S5. Stability analysis of micelles with/without loading DOX in bovine serum albumin (BSA) solution.

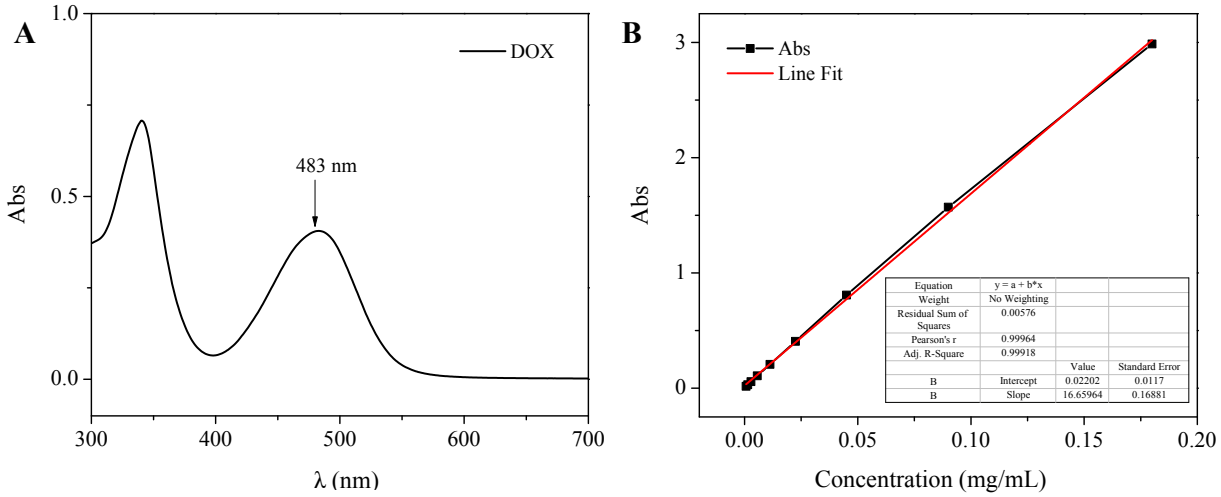


Figure S6. A) UV absorption curve of DOX mesured by UV-vis spectrometer. B) The standard curve of DOX with a correlation function between absorbance and concentration.

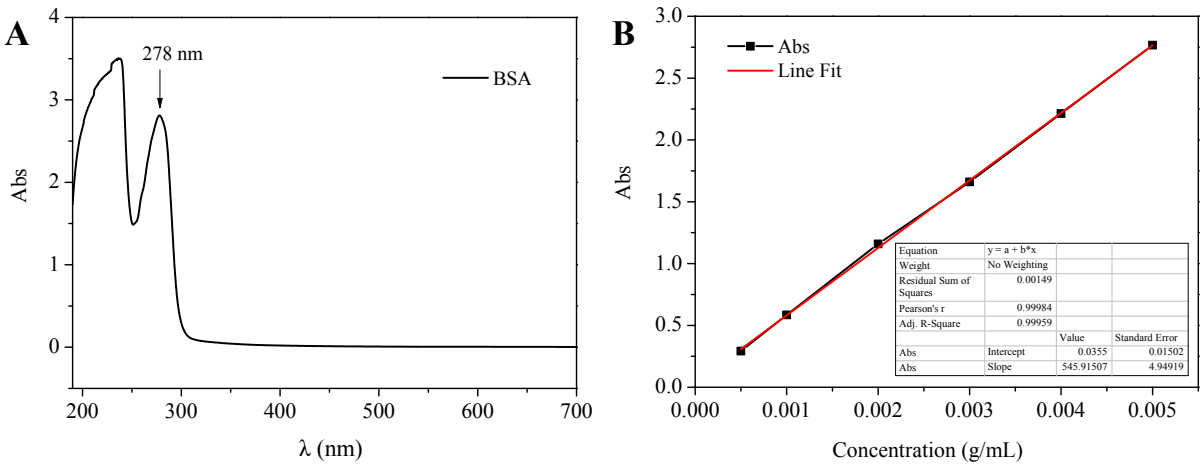


Figure S7. A) UV absorption curve of BSA mesured by UV-vis spectrometer. B) The standard curve of BSA with a correlation function between absorbance and concentration.

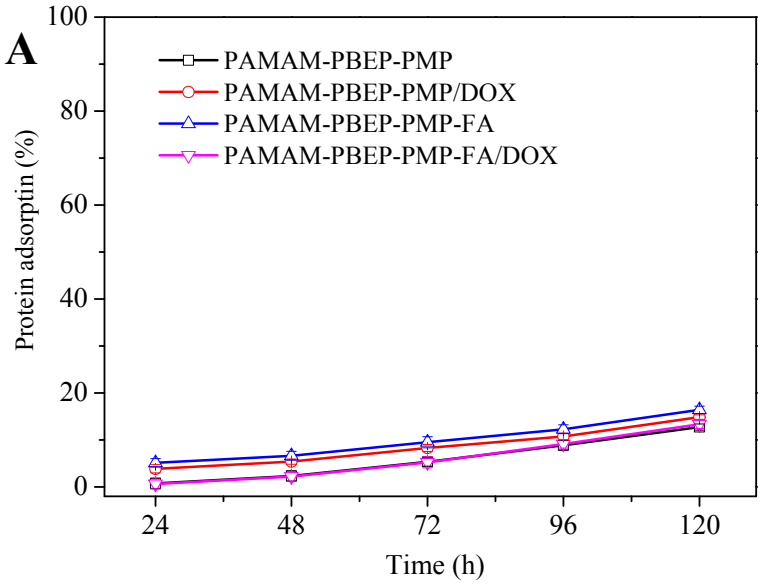


Figure S8. Protein adsorption of micelles incubated in BSA solution. The micelles were incubated in BSA solution for 120 h at 37 °C, mixture solutions were taken out every 24 hours and centrifuged for 5 min, and then supernatants were used for quantitative measurement of residual protein amounts by UV-vis at 278 nm.

Table S1. The mol ratio of PAMAM, BEP, MP and DBU

Samples	$n_{[OH]}:n_{[BEP]}:n_{[MP]}:n_{[DBU]}$	PAMAM	BEP	MP	DBU
		n [mmol]	n [mmol]	n [mmol]	n [mmol]

PAMAM-PBEP-PMP-1	1:12:40:1.5	0.056	5.375	17.92	0.672
PAMAM-PBEP-PMP-2	1:18:40:1.5	0.037	5.375	11.84	0.440
PAMAM-PBEP-PMP-3	1:24:40:1.5	0.034	6.450	10.88	0.408
PAMAM-PBEP-PMP-4	1:30:40:1.5	0.029	6.99	9.28	0.352
PAMAM-PBEP-PMP-5	1:36:40:1.5	0.028	8.06	8.96	0.336

Table S2. Molecular weight and distribution of polymers

Samples	**M_n**	**M_w**	**M_w/M_n**
PAMAM	1440		
PAMAM-PBEP-PMP	60990	65520	1.07
PAMAM-PBEP-PMP-FA	65130	73030	1.12

Table S3. Drug loading content (DLC) and drug loading efficiency (DLE) of nanoparticles

Samples	DLC%	DLE%
PAMAM-PBEP-PMP/DOX	21.47%	91.20%
PAMAM-PBEP-PMP-FA/DOX	20.66%	86.80%

Table S4. pH value changes of release media before and after DOX release

Release media	Before release	After release	D-value
pH 7.4	7.38	7.66	+ 0.28
pH 6.0	6.01	6.21	+ 0.2
pH 5.0	4.79	5.04	+ 0.25

pH 5.0 + PDE I	4.79	4.97	+ 0.18

Table S5. Hydrodynamic diameters of supramolecular micelles determined by DLS

Samples	Diameter(nm)	PDI
PAMAM-PBEP-PMP	184	0.34
PAMAM-PBEP-PMP/DOX	112	0.30
PAMAM-PBEP-PMP-FA	195	0.46
PAMAM-PBEP-PMP-FA/DOX	120	0.37

Table S6. Sizes of PAMAM-PBEP-PMP-FA/DOX micelles after DOX release

Samples	pH 7.4		pH 6.0		pH 5.0		pH 5.0 + PDE I	
	R_h(nm)	PDI	R_h(nm)	PDI	R_h(nm)	PDI	R_h(nm)	PDI
PAMAM-PBEP-PMP-FA/DOX	91.8	0.48	95.7	0.57	98.9	0.65	200.7	0.60

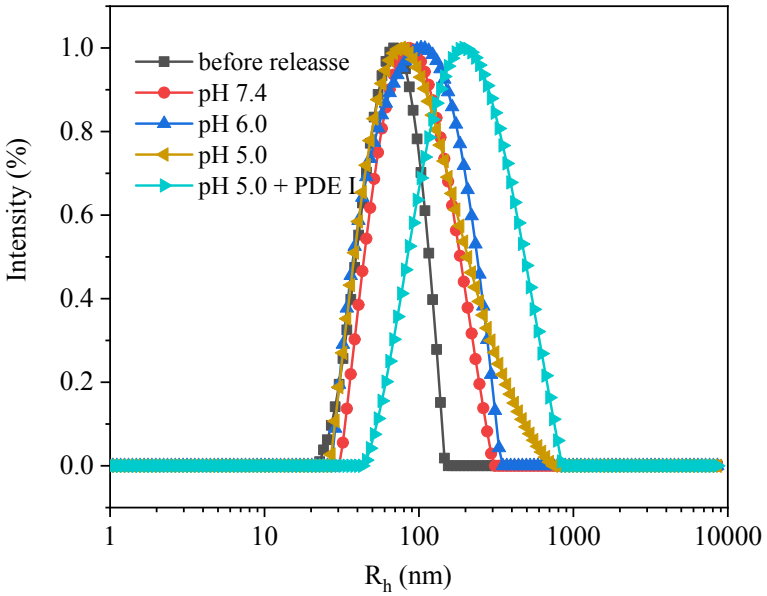


Figure S9. DLS traces of DOX-loaded micelles before and after DOX release

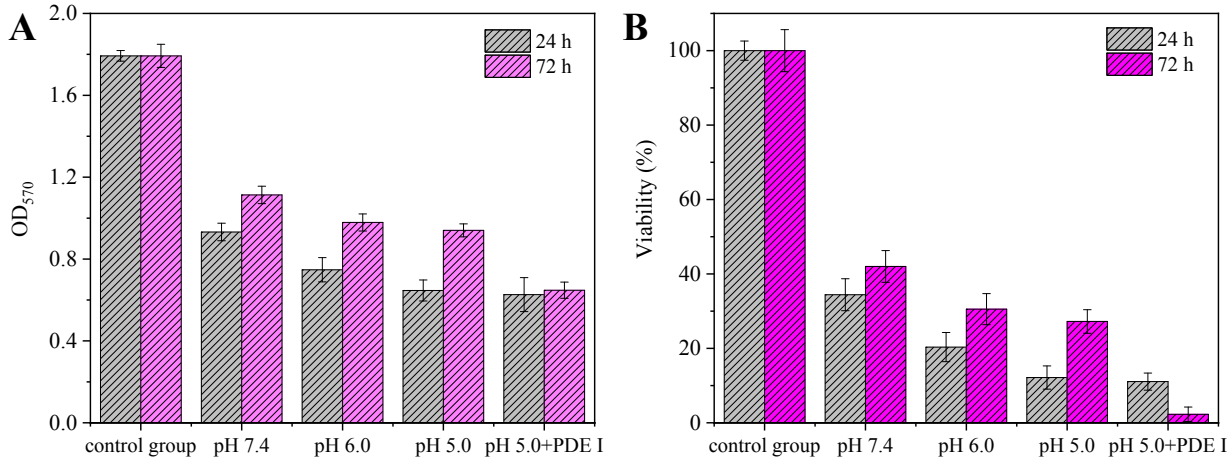


Figure S10. *In vitro* cytotoxicity of DOX-loaded supramolecular micelles against L929 cells at 24 h and 72 h incubation after DOX release for 312 h. Micelles group released in pH5.0+PDE I medium showed stronger toxicity against L929 cells, which demonstrated more DOX were released in simulated cellular microenvironment.

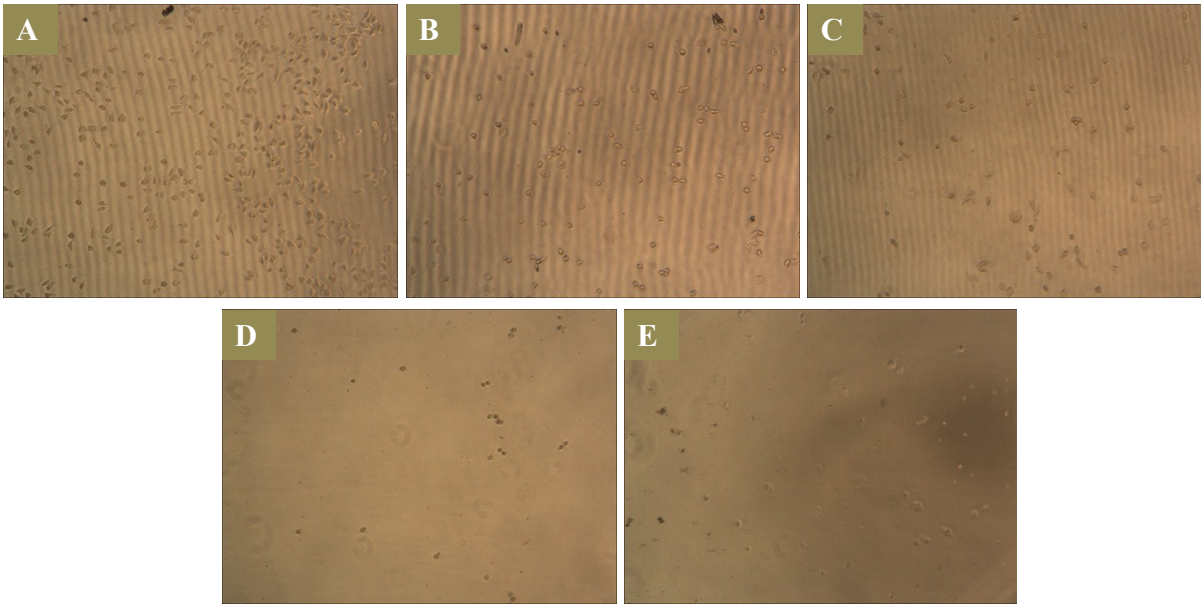


Figure S11. Inverted microscope photos of L929 cells after 72 h of incubation in different released micelles: (A) Control group (B) pH 7.4 (C) pH 6.0 (D) pH 5.0 (E) pH 5.0+PDE I

Figure S12. Photos of HepG2-tumor-bearing nude mice at the end of treatment (24st day)

Table S7. Tumor weight of HepG2-tumor-bearing nude mice at the end of treatment (24st day)

Samples	Tumor weight (g)					Average tumor weight (g)
PBS	3.3599	2.9519	2.4097	2.0249	1.9185	2.5330
Free DOX	2.4278	2.0769	2.1216	2.2432	0.9316	1.9602
PAMAM-PBEP-PMP/DOX	1.9264	1.0968	0.9892	1.5193	1.0837	1.3231
PAMAM-PBEP-PMP-FA/DOX	0.9963	0.8412	0.6969	0.8746	0.8165	0.8451

Table S8. Inhibition ratio of treatment groups in tumor volume and tumor weight

Samples	Inhibition ratio (%)	
	Tumor volume	Tumor weight
PBS	0	0
Free DOX	22.94	22.61
PAMAM-PBEP-PMP/DOX	46.67	47.77
PAMAM-PBEP-PMP-FA/DOX	62.43	66.64