Electronic Supporting Information

A Surface-Adaptive Nanocarrier to Prolong Circulation Time and Enhance Cellular Uptake

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1. Materials

Cis-aconitic anhydride was purchased from Sigma-Aldrich. Doxorubicin-HCl (DOX·HCl) was supplied by Jingyan Chemicals Corporation (Shanghai, China). α-Methoxy-ω-aminopoly(ethylene glycol) (CH_3O-PEG_{113}-NH_2; M_w = 5000; M_w/M_n = 1.05) was purchased from Aladdin and used after dried under vacuum. ε-(benzyloxycarbonyl)-L-lysine N-carboxyanhydride (Lys(Z)-NCA) were synthesized by the Fuchs-Farthing method using bis(trichloromethyl) carbonate (triphosgene) according to ref 1. Fluorescein isothiocyanate (FITC), Hexane-1,6-dioldiacrylate (HDD, 99%), and 4,4′-trimethylene dipiperidine (TDP, 97%) were purchased from J&K and used as received. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl; ≥98%, Fluka), N-hydroxysuccinimide (NHS; ≥97%, Fluka), trifluoroacetic acid, hydrogen bromide (HBr; 45% in acetic acid) was purchased from Sigma-Aldrich. Sulfo-cyanine 5 NHS ester and Cyanine 5.5 NHS ester were purchased from Lumiprobe Co. (Florida, USA). HepG2 (human liver hepatocellular carcinoma cell line) was cultured in RPMI-1640 medium. The medium was supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), all cell culture media and supplies were ordered from Gibco (Gibco Corporation, Grand Island, NY, USA). The BALB/c mice were purchased from the Laboratory Animal Center of The Academy of Military Medical Sciences (Beijing, China).

2. Synthesis of cis-aconityl-Doxorubicin

Cis-aconityl-doxorubicin (CA-DOX) was synthesized as shown in Fig. S1 according to some previous reports.[2] In brief, DOX·HCl (100 mg, 0.172 mmol) was dispersed in 60 ml of 0.1 M carbonate buffer(pH 8.7) and stirred for 30 min. Cis-aconitic anhydride(100 mg, 0.641mmol) was added slowly to the suspension at 0 °C, the pH value was maintained at 8.5-8.7 with ice-cold NaOH (0.5 M) throughout the process, the mixture was turning transparent gradually. After 20 min of reaction, the temperature was adjusted to 25 °C (30 min). The reaction mixture was re-cooled to 0 °C, and acidified by addition of ice-cold HCl (1 M) till heavy precipitation. The product was collected by centrifugation for 10 min at 8000 rpm (4 °C), then suspended in appropriate distilled water and recovered by lyophilisation. A bright red power was obtained (yield 56%).

As shown in Fig. S5, a high purity CAD was obtained through calculating the integration ratio (about 3.0) of characteristic peaks (a, 3 H) of the anthracene protons of DOX and two peaks (b, 1 H) around 6.55 ppm of the protons of -COC*H*= in the cis- and trans-forms of CAD. ESI-MS was performed for investigating the mass value of CAD. Due to the loss of a carbon dioxide molecule under testing condition, m/z calculated for $[M\text{-}CO_2\text{-}H]^-$ and $[M\text{-}CO_2\text{-}2H+Na]^-$ were 654.1808 and 676.1618 respectively (Fig. S6), which is consistent with previous literature.[1]

3. Synthesis of polymer conjugates

3.1 PEG-*b*-P(Lys-*co*-LysDox) (PEG-*b*-P(Lys/CA-Dox))

PEG-*b*-P(Lys-*co*-LysDox) was synthesized as shown in Fig. S2. Poly(ethylene glycol)-*b*-poly(L-lysine)(PEG-*b*-PLys) was prepared as described previously.[3] Briefly, Lys(Z)-NCA(0.98 g, 3.2 mmol) was dissolved in 30 ml of DMF and polymerized by addition of MeO-PEG_{113}-NH_2 (2.0g, 0.4 mmol) with the terminal primary amino group as the initiator. The reaction mixture was stirred for 3 days at 35 °C under a dry argon atmosphere. Then solvent was evaporated under reduced pressure. The resulting product was dissolved in 15 ml of $CHCl_3$ and then precipitated into excessive diethyl ether to obtain PEG-*b*-PLys(Z) (yield 90%). Deprotection of Z group in PEG-*b*-PLys(Z) was carried out by addition of HBr (33 wt.% in HOAc, 2 mL) to the solution of PEG-*b*-PLys(Z) (2.0 g) in 20 ml CF_3COOH for 2 h at 0 °C. The reaction mixture was precipitated into excessive cold diethyl ether. The precipitation was dissolved in DMF and purified by filtering through a 0.22 μm Millipore filter. The filtrate was precipitated in excessive diethyl ether to

remove the residual CF_3COOH and obtain PEG-*b*-PLys (yield 60%). Then the product was dried at room temperature under vacuum. PEG-*b*-PLys was characterized by ^1H NMR. As shown in Fig. S7, the degree of polymerization (DP) of Lys was estimated to be 7 by calculating the peak integration ratio of -OCH_2CH_2- protons of PEG at 3.59 ppm and -NHC*H*CO- protons of PLys at 4.32 ppm.

CAD was conjugated to PEG-*b*-PLys by acylation of the pendent primary amino groups of PEG-*b*-PLys using EDC/NHS as coupling agent. CAD (30 mg, 0.042 mmol) was dissolved in 10 ml of DMF, EDC·HCl (40 mg, 0.208 mmol) and NHS (10 mg, 0.086 mmol) were added to the solution. The mixture was stirred for 4 hours at 0 °C, then PEG-*b*-PLys (30 mg, 4μmol) was added, followed by addition of 20 μl TEA. The reaction mixture was warmed gradually to ambient temperature and kept in the dark for further 24 hours. Subsequently, the whole solution was transferred to a dialysis bag (MWCO 3500) and dialyzed against distilled water for 3 days. PEG-*b*-P(Lys-*co*-LysDox) was obtained by lyophilisation. PEG-*b*-P(Lys-*co*-LysDox)$_7$ was characterized by ^1H NMR. As shown in Fig. S8, the peaks around 8.0 ppm which is the characteristic peaks of the anthracene protons of DOX indicate that DOX were conjugated to polymers successfully.

Then hydrophilic dye Cy5 was conjugated to PEG-*b*-P(Lys-*co*-LysDox) was synthesized by acylation of the pendent primary amino groups using EDC/NHS as coupling agent for ex vivo imaging.

3.2 Synthesis of 2-aminoethylacrylate

2-aminoethylacrylate was synthesized as described in Fig. S3.

2-Boc-Aminoethanol (5.0 g, 31 mmol) and TEA (8.64 ml, 62 mmol) were dissolved in 150 ml of CH_2Cl_2 and cooled to 0 °C. A solution of acryloyl chloride (5.6 g, 62 mmol) in CH_2Cl_2 (50 ml) was added dropwise under a dry argon atmosphere. The reaction mixture was stirred for 2 h and warmed slowly to room temperature for further 1 h. 50 ml of saturated sodium bicarbonate solution was added, the aqueous phase was extracted with 50 ml of CH_2Cl_2 twice. The organic phase was collected and dried with Na_2SO_4, followed by concentration under reduced pressure. 2-Boc-aminoethylacrylate was purified by silica gel column chromatography using ether acetate/petroleum ether (1/2, v/v) as an eluent to obtain a yellowish solid (yield 93%).^1H NMR ($CDCl_3$) δ (ppm): 1.45 (a, 9 H), 3.43 (c, 2 H), 4.22 (d, 2 H), 4.76-4.78 (b, 1 H), 5.86 (e, 1 H), 6.14

(e, 1 H), 6.40 (e, 1 H). Afterward, a Boc deprotection reaction was performed by addition of 20 ml CF_3COOH to the solution of 2-Boc-aminoethylacrylate (2.0 g, 9.3 mmol) in CH_2Cl_2 (20 ml) for 24 h at 30 °C, then the solvent was removed under reduced pressure, the crude product was purified by dissolving in appropriate methanol and evaporating under vacuum twice at 25 °C. A dark yellow oil was obtained (yield 90%).^{1}H NMR ($CDCl_3$) δ (ppm): 3.20 (c, 2 H), 4.25 (d, 2 H), 4.76-4.78 (b, 1 H), 6.05 (e, 1 H), 6.22 (e, 1 H), 6.49 (e, 1 H)

3.3 PAE-*b*-P(Lys-*co*-LysDox) (PAE-*b*-P(Lys/CA-Dox))

PAE-*b*-P(Lys-*co*-LysDox) was synthesized as shown in Fig. S4.

PAE-*b*-PLys was prepared by deprotection of benzyl group of PAE-*b*-PLys(Z), which was synthesized by a Michael-type addition polymerization of PLys(Z) monoacrylate, HDD and TDP. PLys(Z) monoacrylate was synthesized through the ROP of Lys(Z)-NCA monomer with 2-aminoethylacrylate as initiator, followed by termination by acetic anhydride. Lys(Z)-NCA (2.3 g, 7.5 mmol) was dissolved in 20 ml of DMF, then a solution of $TFA^{-}\cdot NH_3^{+}$ terminated 2-aminoethylacrylate (146 mg, 0.62 mmol) and TEA (86 µl, 0.62mmol) in 10 ml of DMF was added. The reaction was stirred for 3 days at 35 °C and terminated by addition of acetic anhydride (266 µl, 2.8 mmol), then the solvent was evaporated under reduced pressure, followed by dissolution of the crude product in 15 ml of $CHCl_3$ and precipitation in excessive cold diethyl ether. The PLys(Z) monoacrylate was dried under vacuum at room temperature for 24 h (yield 75%). The DP of Lys(Z) was calculated to be 8 by comparing of the peak integration of -C*H*=CH_2 protons of 2-aminoethylacrylate around 6.0 ppm and -OCH_2ph protons of PLys(Z) at 5.0 ppm as shown in Fig. S9. PLys(Z) (1.0 g, 0.48mmol) monoacrylate was dissolved in DMF (10 ml), then HDD (2.27 g, 10mmol) were added, followed by addition of TDP (2.36 g, 11mmol) in $CHCl_3$ (5 ml). After stirring for 2 days at 80 °C, the solvent was removed under reduced pressure. The crude product was dissolved in $CHCl_3$ and precipitated in cold diethyl ether to obtain PAE-*b*- PLys(Z) (yield 80%). PAE-PLys(Z) was characterized by ^{1}H NMR, as shown in Fig. S10, PDI is about 1.62 (GPC using DMF as eluent). Similar to the deprotection of benzyl group in PEG-*b*-PLys(Z), PAE-*b*-PLys(Z) (2.0 g) was dissolved in 20 ml CF_3COOH, 2 mL of HBr (33 wt.% in HOAc) was added, then the reaction mixture was stirred vigorously for 2 h at 0 °C and precipitated into excessive cold diethyl ether. The crude product was dissolved in DMF and precipitated in excessive diethyl

ether twice to obtain PAE-*b*-PLys (yield 72%). Moieties of PAE segment was hydrolyzed by CF_3COOH and HBr. the DP of PAE after deprotection was calculated to be 22 as shown in Fig. S11. A unimodal peak was detected by GPC using H_2O as eluent.

The similar synthetic route with PEG-*b*-P(Lys-*co*-LysDox) was employed to prepare PAE-*b*-P(Lys-*co*-LysDox). In briefly, CAD was dissolved in DMF, EDC and NHS was added, the reaction mixture was kept in an iced bath for 4 h, then PAE-*b*-PLys and TEA were added. After 24 h at room temperature, the product was purified by dialyzing against distilled water for 3 days. PAE-*b*-P(Lys-*co*-LysDox) conjugate was obtained by lyophilisation. PAE_{22}-*b*-P(Lys-*co*-$LysDox)_8$ were characterized by ^1H NMR (Fig. S12).

4. Determination of DOX content of the polymer conjugates

The amount of DOX was calculated according to hydrolysis method reported previously.[4] PEG-*b*-P(Lys-*co*-LysDox) (1 mg) and PAE-*b*-P(Lys-*co*-LysDox) (1 mg) were dispersed in 4 ml of distilled water separately. The DOX of the conjugates was hydrolyzed by addition of 0.9 ml of HCl (2.2 M) to the solution of polymer conjugates (0.2 ml). 1 ml of methanol was added to dissolve the hydrolysis product doxorubicinone, then the reaction mixture was incubated for 2 h at 50 °C. after cooling to room temperature, quantitation of the generated doxorubicinone was conducted by HPLC (LC-20AT, Shimadzu, Kyoto, Japan). A Hypersil ODS C18 column (5μm, 25 cm x 4.6 mm) was used at 30 °C. The mobile phase was acetonitrile/0.01 M KH_2PO_4/acetic acid (55:45:0.27 by volume) and the injection volume was 20 μl at a flow rate of 1.0 ml/min, the detected UV absorption wavelength was 490 nm. The number of DOX of polymer conjugates was estimated by measuring the relevant peak area using free DOX as standard. The number of DOX of polymer conjugates was estimated to be 3.2 (PEG_{113}-*b*-P($Lys_{3.8}$-*co*-$LysDox_{3.2})_7$) and 4.0 (PAE_{22}-*b*-P(Lys_4-*co*-$LysDox_4)_8$) by HPLC method as described above, and the Dox content of the obtained PEG_{113}-*b*-P($Lys_{3.8}$-*co*-$LysDox_{3.2})_7$ and PAE_{22}-*b*-P(Lys_4-*co*-$LysDox_4)_8$ were 21.4 wt.% and 17.8 wt.% respectively.

5. Investigation of the property of PAE

In our study, PAE based on HDD and TDP was utilized as the pH responsive block. Poly (β-amino ester) was synthesized by a Michael-type addition of HDD (10.0 mmol) and TDP (10.5 mmol) at 55 °C for 3 days similar to the synthetic route of PAE-*b*-PLys(Z). PAE (10 mg) was added to 2 ml of HCl (1 M), then pH value was adjusted to 4.0. Transmittance and pH value of the

solution was recorded with the addition of NaOH (0.25 M). Similarly, PAE-*b*-P(Lys-*co*-LysDox) (3 mg) was suspended in 2 ml of HCl (1 M), then pH value was immediately adjusted to 4.0. pH value of the solution was recorded with the addition of NaOH (0.25M), this measurement should be conducted quickly. Below PAE's pK_b, PAE was soluble and positively charged. As shown in Fig. S15a, with addition of 0.25 M NaOH, pH value of the solution of PAE was maintained at 6.7 with a slight change, which was considered as the pK_b of PAE. When pH is above 6.5, the transmittance of the solution was decreasing gradually to be zero as shown in Fig. S15b. In addition, we investigated the pK_b of PAE_{22}-*b*-P(Lys_4-*co*-$LysDox_4$)$_8$, as shown in Fig. S16, with addition of NaOH, pH value of the solution was maintained at 6.75 with a slight change. This result implied that PAE in the copolymer exhibited the same pH responsive property with PAE homopolymer.

6. Preparation of micelles

The idea ratio of hydrophilic/hydrophobic on the surface of micelle with prolonged blood circulation was 1:1 according to our previous research.[2] Mixed shell micelles (MSMs) were prepared as follow, PEG_{113}-*b*-P($Lys_{3.8}$-*co*-$LysDox_{3.2}$) (1 mg) (MW=8075 g/mol) and PAE_{22}-*b*-(Lys_4-*co*-$LysDox_4$) (Mw=13340 g/mol) (0.90 mg) were dissolved in 1.5 ml of DMF in which the weight ratio of PEG segment to PAE segment was 1:1. The solution was added dropwise to 10 ml of water (pH 6.0) at 30 s intervals under stirring with a magnetic bar. The solution of micelle was stirred for 1 h, PAE collapsed on the core after the pH value was adjusted to 7.4. The micelle solution was dialyzed against deionized water for 2 days, followed by ultrafiltration before use. The single PEGylated micelles (PEGSMs) were prepared with similar procedure. Briefly, PEG_{113}-*b*-P($Lys_{3.8}$-*co*-$LysDox_{3.2}$) (2 mg) was dissolved in DMF, and added dropwise to 5 ml of deionized water. The micelle was stirred for 24 h at room temperature. The micelle solution was dialyzed against deionized water for 2 days and concentrated by ultrafiltration before use.

The average hydrodynamic diameters of MSMs were measured to be around 41 nm (pH 6.5) and 46 nm (pH 7.4) with narrow size distributions by DLS as shown in Fig. S13a and S13b. The TEM images revealed that the obtained MSMs were spherical and the sizes were around 45 nm (inset in Fig. S13a and S13b). It implicated that the collapse of PAE on the micelle core did not substantially change the size and shape of micelles. The PEGSMs had a narrow size distribution around 40 nm and a spherical morphology as shown in Fig. S14.

7. *In vivo* blood clearance kinetics assay

The animal studies were performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals (Tianjin, revised in June 2004) and adhered to the Guiding Principles in the Care and Use of Animals of the American Physiological Society.Standard curves of the FITC-labeled micelle solution (MSMs and PEGSMs) was established before administration. The BALB/c mice (n = 3) were injected intravenously via the tail vein with FITC-labeled micelle at a dose of 20 mg/kg, respectively. At predetermined time point (10 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, and 24 h), blood samples were collected into heparinized tubes. The blood samples were centrifuged at 4000 rpm for 10 min to remove the blood cells, the plasma fractions were collected and measured by a fluorescence spectrometer (excitation at 490 nm and emission at 520 nm). The amount of the micelle remaining in blood was determined according to the standard curves. The percent injected dose (%ID) was calculated by comparing the amount of micelle remaining in blood with the injected dose. Blank samples from mice with the administration of physiological saline in the same way were analyzed as the back-ground fluorescence of the plasma.

MSMs had significantly longer elimination half-lives ($t_{1/2}$) and mean residence time (MRT) in comparison to PEGSMs ($p < 0.01$). The area under the blood concentration versus time curve (AUC) was significantly increased from 239.9 μg·h·ml^{-1} for PEGSMs to 1070.8 μg·h·ml^{-1} for MSM ($p < 0.05$). MSMs can achieve a lower clearance (CL) compared to PEGSMs ($p < 0.05$) (as shown in Fig. S15 and Table S2)

8. The cellular uptake

HepG2 cells were seeded into 96-well plates at a density of 0.6×10^4 cells per well in 100 μL RPMI-1640 medium/PBS. After an incubation of 24 hours, the culture medium of each well was replaced with 500 μL of fresh medium with different pH values (pH 7.4 and 6.5), then the free DOX and the DOX-conjugated micelles (PEGSMs and MSMs) were added to each well. After 2 hours further incubation, the culture medium was removed and cells were washed three times with 500 μL PBS buffer. The cellular uptake of micelles was observed with an inverted fluorescence microscope (DMI6000B, Leica, Wetzlar, Germany).

For quantitative analysis of cellular uptake, HepG 2 cells were seeded into 96-well plates at a density of 0.6×10^4 cells per well in 100 μL RPMI-1640 medium/PBS. After an incubation of 24

hours, the culture medium of each well was replaced with 500 μL of fresh medium with different pH values (pH 7.4 and 6.5), then PBS (as control) and the DOX-conjugated micelles (PEGSMs and MSMs) were added to each well. After 2 hours further incubation, the culture medium was removed, cells were washed three times with 500 μL PBS buffer and detached by 0.02% (w/v) EDTA and 0.25% (w/v) trypsin solution, and then dispersed in 0.25 mL of PBS for flow cytometric measurement. Cells treated with PBS were used as control.

The highest mean fluorescence intensity of HepG2 cells incubated with MSMs at pH 6.5 (Fig. S18) also confirmed the enhanced cellular uptake of MSMs at tumor environment.

9. pH responsiveness of MSMs core

The mixed shell micelles (MSMs) solution (2 ml) was filtrated through a 0.45 μm Millipore filter to a clean scintillation vial, and then 1 ml of 0.1 M citrate buffer solution (pH 5.0) (150 mM NaCl) was added through a 0.45 μm Millipore filter. Hydrodynamic diameter distribution of micelle and light intensity were recorded by DLS at fixed time intervals.

MSMs-Cy5.5 was prepared as follow, MSMs solution (0.2 mg/ml, 10 ml) was incubated with Cy5.5 NHS ester (100 μg dissolved in 1 ml of DMF) at 37 °C. After 24 hours, the whole solution was dialyzed against DMF/water (5 % v/v) to remove the unreacted Cy5.5 for 24h, then dialyzed against PBS solution (pH 7.4, 10mM) for another two days. The obtained MSMs were concentrated by ultrafiltration. 2ml of MSMs-Cy5.5 was added into 4 ml of 0.1 M citrate buffer solution (pH 5.0) at 37 °C. At fixed time, the fluorescence of the solution was measured (excitation at 695nm).

As shown in Fig. S19, two size distributions were detected at 1 h, where the larger one around 120 nm should be attributed to swollen MSMs while the smaller one around 20 nm could be ascribed to small aggregates because of the dissociation of MSMs. Over time, continued swollen MSMs increased the size of larger distribution and dissociation of MSMs decreased the scattering intensity of smaller distribution. This was consistent with the diminishing light intensity of MSMs solution (Fig. S20) as the contribution of smaller nanoparticles to light intensity was much lower than that of larger ones. The dissociation of MSMs was also investigated by the homofluorescence resonance energy transfer (homoFRET) assay based on Cy5.5 as reported by Gao.[5] When the covalently bound (through amido bond to PLys) hydrophobic Cy5.5 stayed in the micelle core, the fluorescence was quenched by homoFRET. As shown in Fig.21, after the pH of MSM solution

was adjusted to 5.0, the remarkably increased fluorescence intensity (on state) revealed that polymers labeled with Cy5.5 were separated from the MSMs and MSMs core was dissociated. Thus the MSMs could rapidly release DOX in response to intracellular pH.

10. *In vitro* drug release under different pH condition

1 ml of micelle solution (pH 5.0, 6.5 and 7.4) (the content of DOX was 100 μg) was transferred to dialysis bag (MWCO 3500), and then each dialysis bag was immersed in 40 ml of 0.1 M citrate buffer solution with the same pH value and incubated in shaker (200 rpm, 37 °C). To simulate blood flow, at fixed time intervals, 4 ml of dialysis fluid was taken out for fluorescence measurement (excitation at 490nm) and an equal volume of fresh buffer was added in. The amount of released DOX was determined by measuring the height of emission peak (590 nm) using free DOX as standard. The release experiments were conducted in triplicate, and the results presented are the average data.

11. Cell viability

The cytotoxicity of the micelles (PEGMs, MSMs) were determined against HepG2 cells at different pH values (pH 7.4 and 6.5) by MTT assay. In the MTT assay, HepG2 cells were seeded into 96-well plates at a density of 4000 cells per well in 500 μL RPMI-1640 medium/PBS (pH 7.4). After 24 h incubation, half of the wells were adjusted to pH 6.5. PEGSMs and MSMs solution were added to each well with different DOX concentrations (0.01, 0.1, 1, 5, 10 μg/mL). The saline solution was used as control. After 2 h further co-incubation, the culture medium was removed and replaced with fresh medium. After 24 hours, 25 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) (5 mg/ml) was added to each well and the mixture was incubated for another 4 h, then 150 μL of DMSO was added to dissolve the obtained blue formazan crystals. The absorbance was measured at a wavelength of 570 nm and the viability was expressed as the percentage of the control.

12. *ex vivo* Imaging of tumor accumulation

HepG2 tumor-bearing nude mice (BALB/c mice) were used for tumor accumulation studies. When the tumors reached about 200 mm^3 two weeks later, the mice were randomly divided into two groups and intravenously injected by PEGSMs/Cy5 and MSMs/Cy5 at same PEG-P(Lys-LysDox-LysCy5) (synthesized as described in the supporting information) dose. At 1h, 6h, 24h post-injection, the mice were sacrificed, and then tumors as well as the major organs were

harvested. *ex vivo* imaging was conducted by the Kodak IS *in vivo* FX imaging system.

13. Characterization

^{1}H NMR spectra were recorded on a Varian UNITY-plus 400 M NMR spectrometer at room temperature with tetramethylsilane (TMS) as an internal standard. Electron spray ionization (ESI) mass spectrometry was performed on a VG-ZAB-HS spectrometer (VG Company, England using the Fast Atom Bombardment (FAB) method. The number-average molecular weight (M_n) and weight-average molecular weight (M_w) were measured by gel permeation chromatography (GPC) at 25 °C with a Waters 1525 chromatograph equipped with a Waters 2414 refractive index detector. GPC measurements were carried out using THF, DMF and H_2O as eluents with a flow rate of 1.0 mL/min, respectively. Polystyrene and PEG standards were used for calibration. Dynamic light scattering (DLS) experiments at a 90° scatter angle were performed on a laser light scattering spectrometer (BI-200SM) equipped with a digital correlator (BI-9000AT) at 636 nm at required temperature. All samples were obtained by filtering through a 0.45 μm Millipore filter into a clean scintillation vial. Transmission electron microscopy (TEM) measurements were performed using a Philips T20ST electron microscope at an acceleration voltage of 100 kV. To prepare the TEM samples, the sample solution was dropped onto a carbon-coated copper grid and dried slowly at required temperature. The zeta potential values were measured on a Brookheaven ZetaPALS (Brookheaven Instrument, USA), using phosphate buffer (PB) solution (0.01 M) with a pH range from 5.0 to 7.4 as the background buffer. The instrument utilizes phase analysis light scattering at 37 °C to provide an average over multiple particles. The turbidity test of PAE was performed by the determination of transmittance under the detection of UV-vis spectrophotometer (Purkinje General, China).

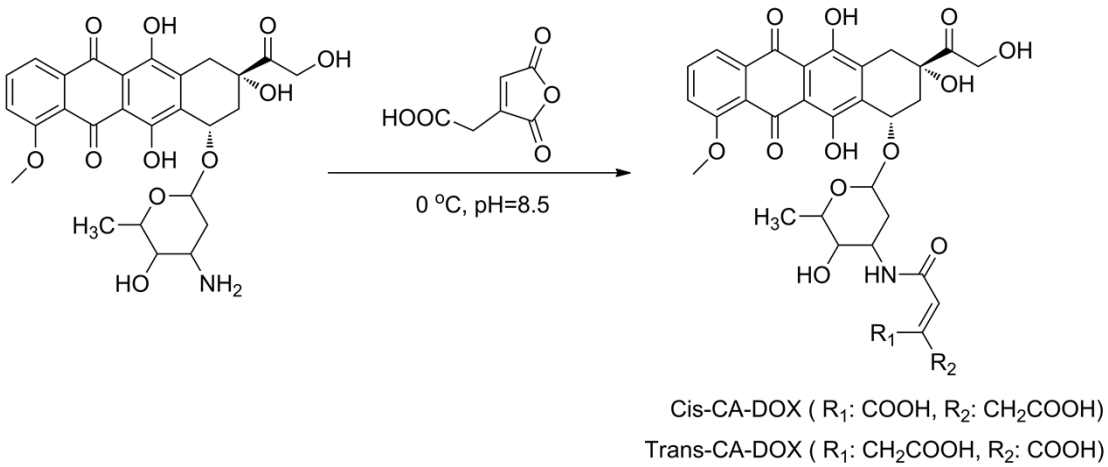

Fig. S1. Synthesis of CA-DOX.

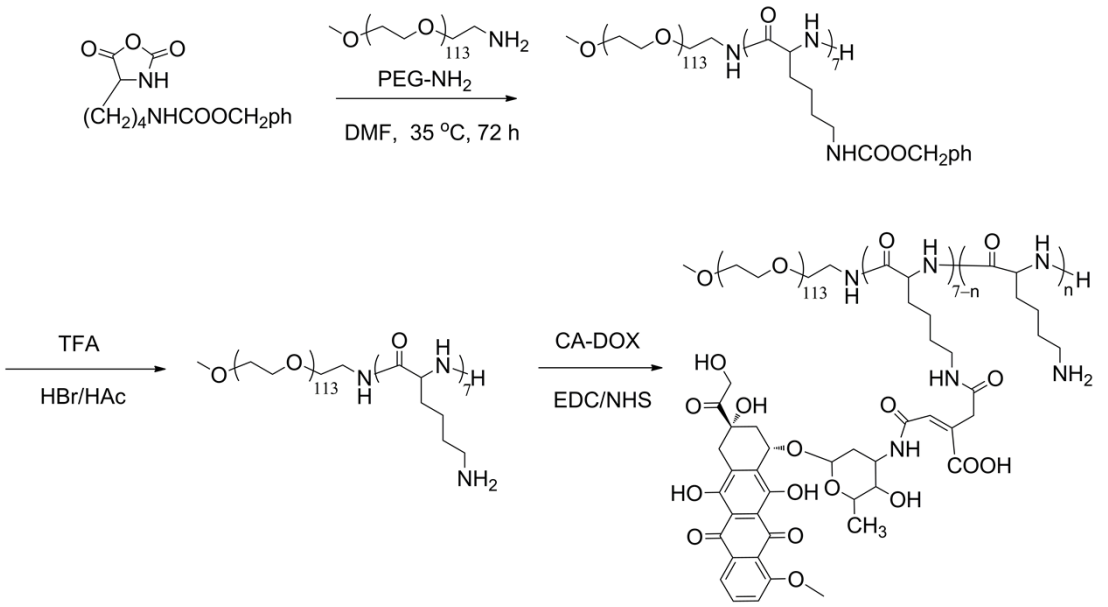

Fig. S2. Synthesis of PEG-*b*-P(Lys-*co*-Lys-CA-DOX).

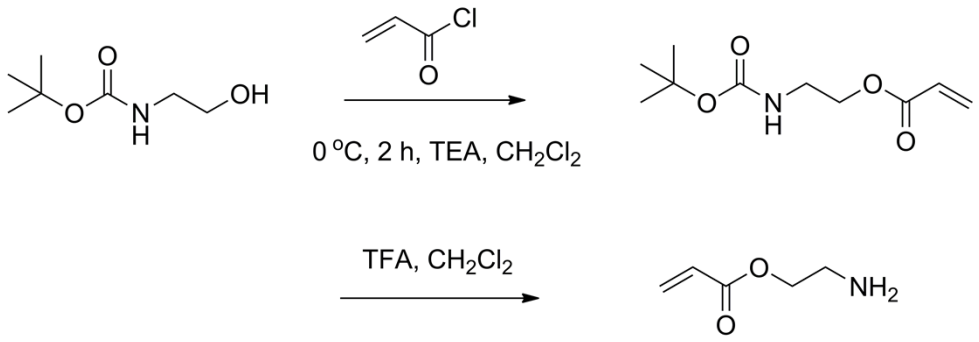

Fig. S3. Synthesis of 2-aminoethylacrylate.

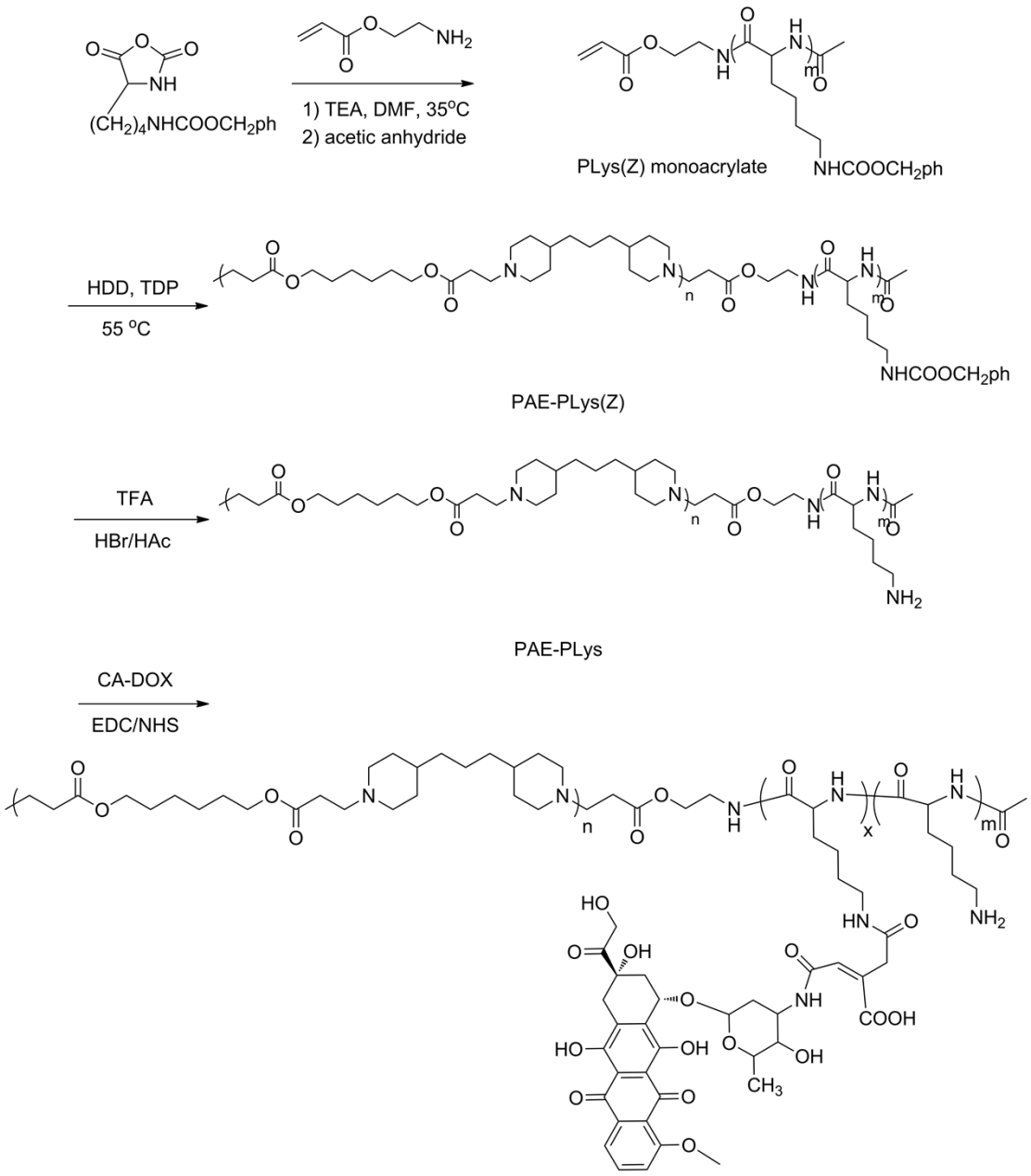

Fig. S4. Synthesis of PAE-*b*-P(Lys-*co*-Lys-CA-DOX).

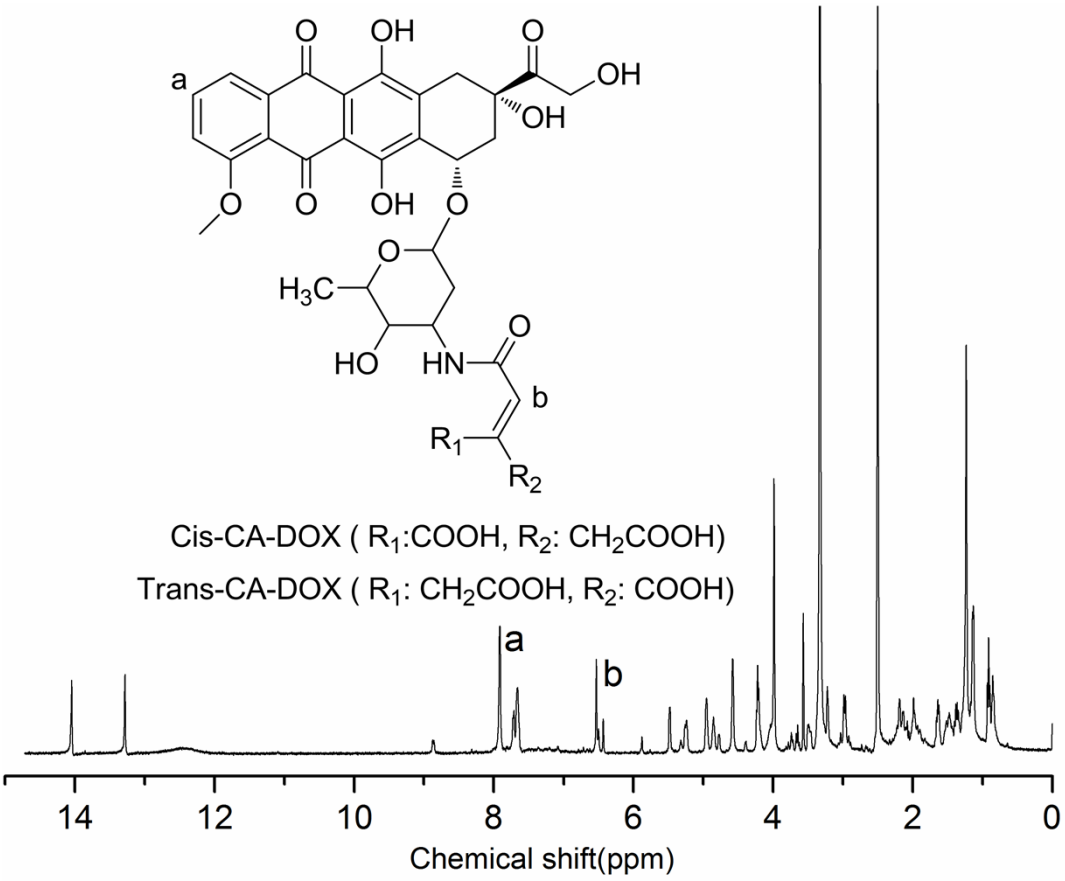

Fig. S5. [1] H NMR spectra of CAD in DMSO-d_6.

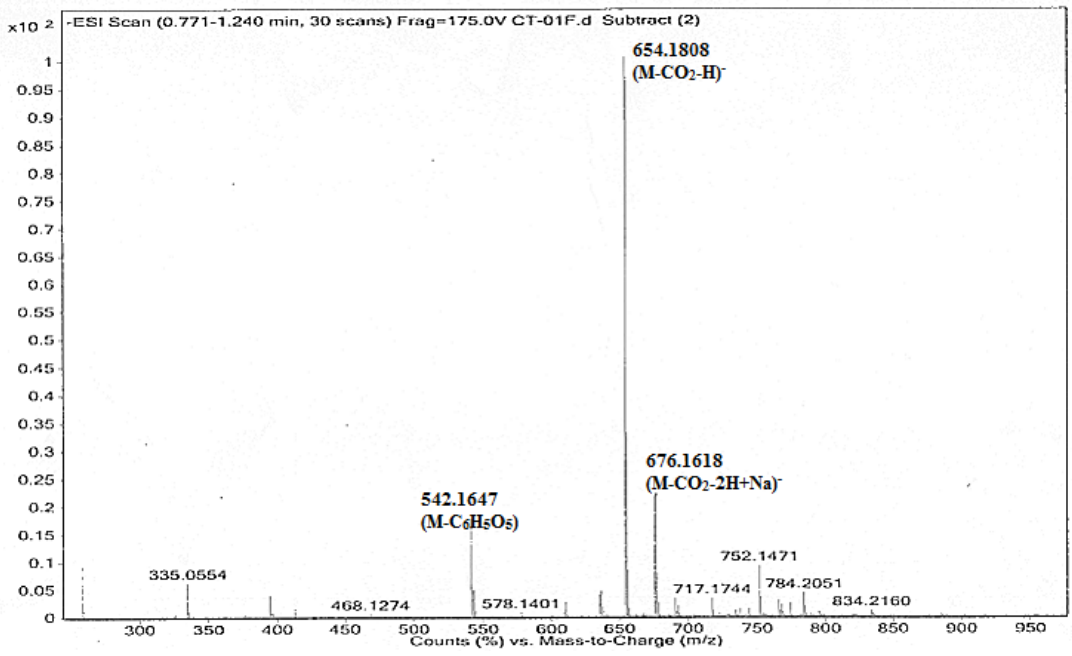

Fig. S6. ESI-MS spectra of CAD.

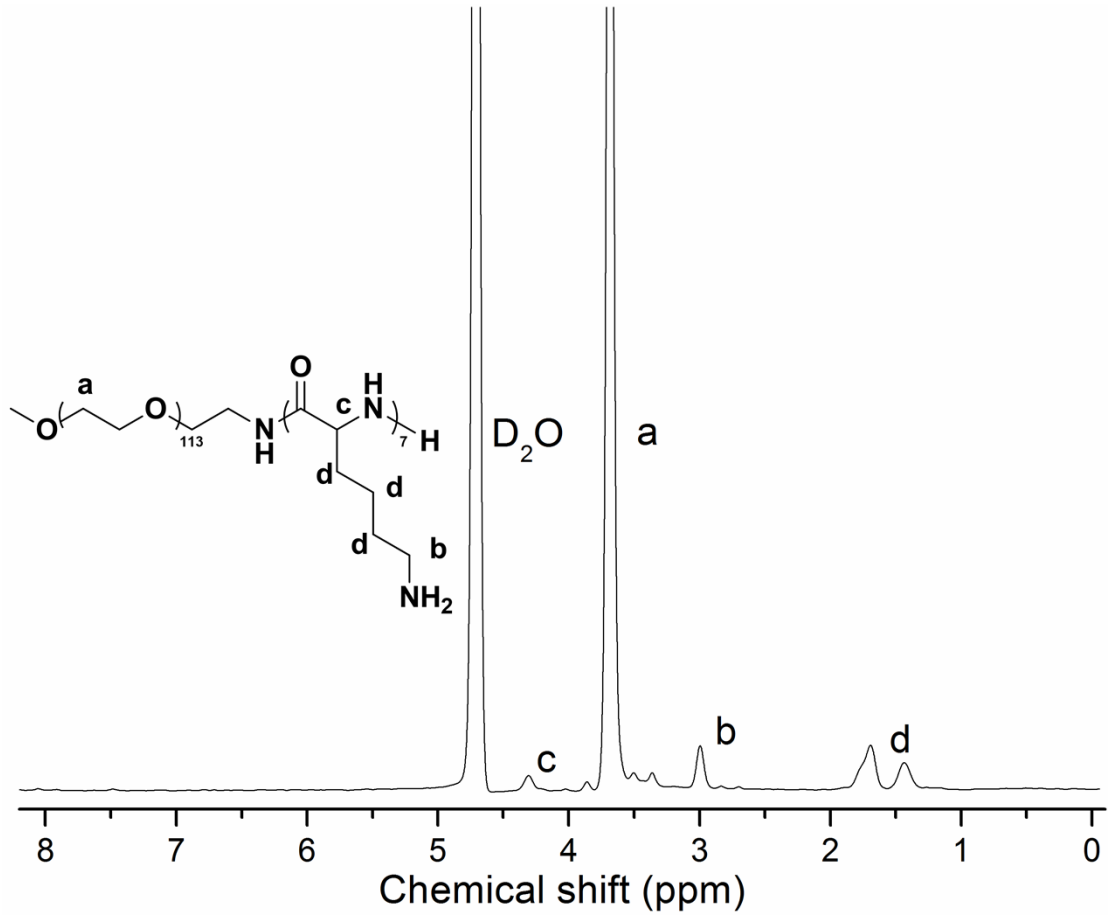

Fig. S7. ^{1}H NMR spectra of PEG_{113}-*b*-$PLys_{7}$ in D_2O.

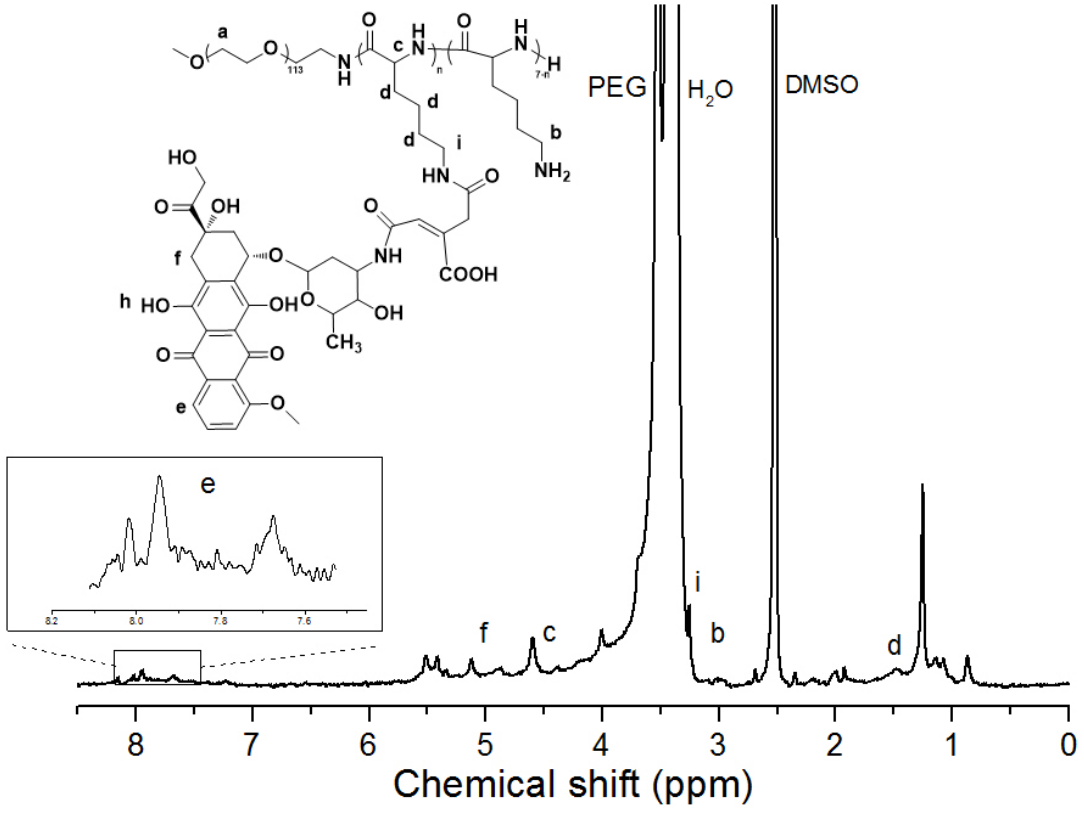

Fig. S8. ^{1}H NMR spectra of PEG_{113}-*b*-P($Lys_{2.2}$-*co*-$LysDox_{1.8}$)$_4$ in DMSO-d_6.

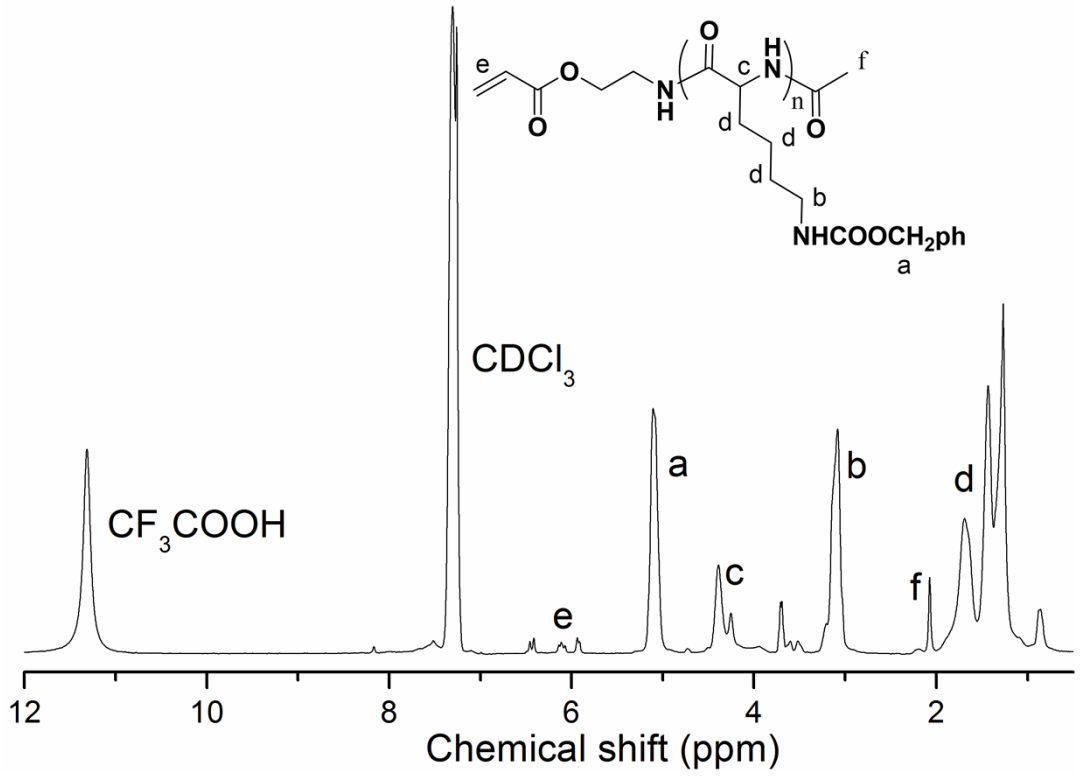

Fig. S9. ^{1}H NMR spectra of PLys(Z)$_8$ monoacrylate in $CDCl_3$ with addition of CF_3COOH.

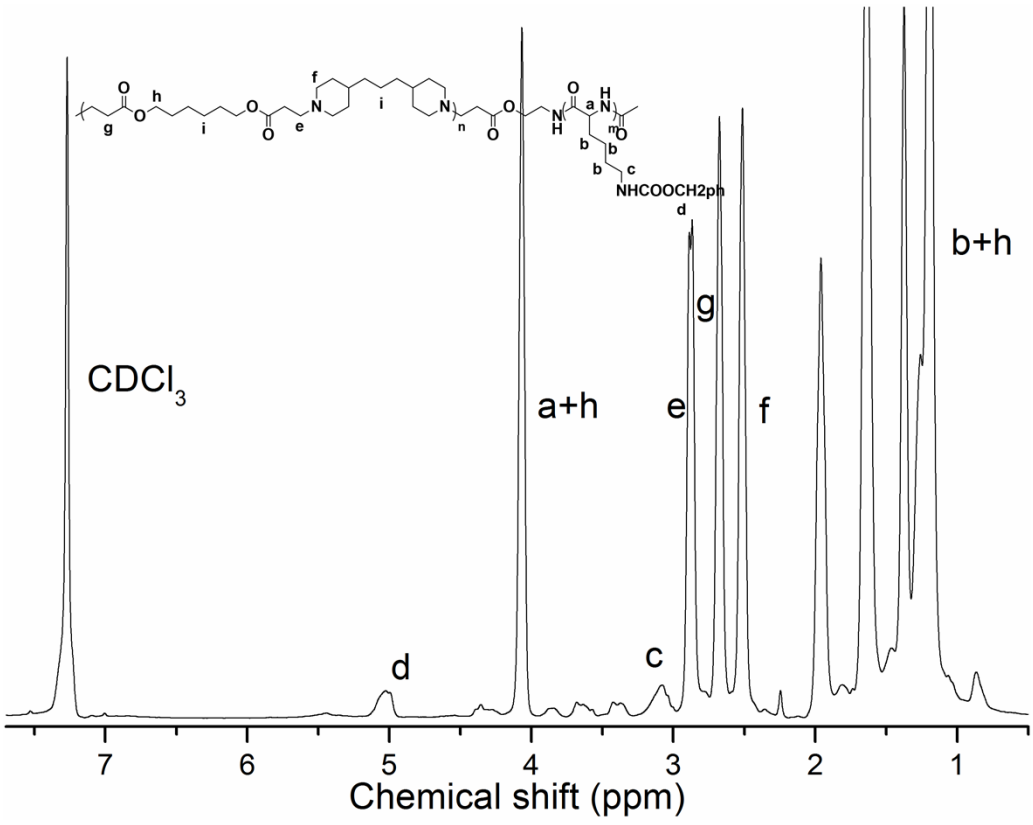

Fig. S10. ^{1}H NMR spectra of PAE-*b*-PLys(Z)$_8$ in $CDCl_3$.

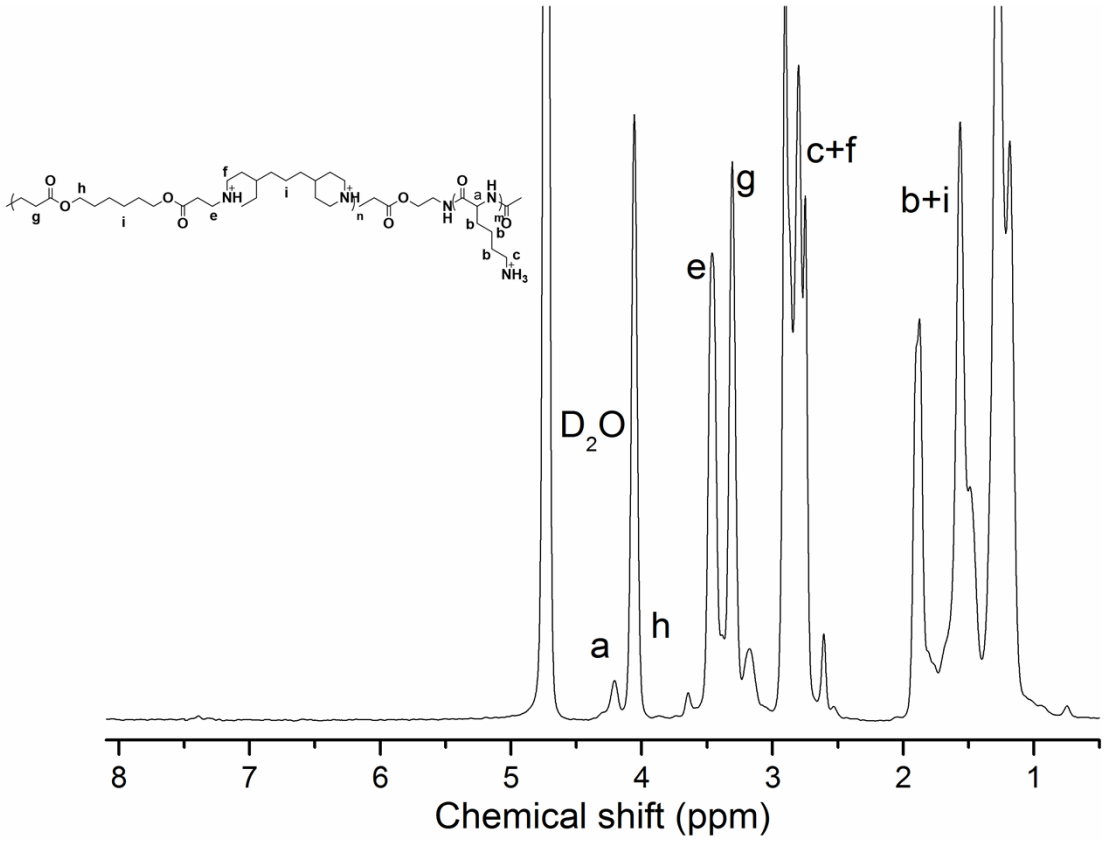

Fig. S11. ^{1}H NMR spectra of PAE_{22}-*b*-$PLys_8$ in D_2O with addition of DCl.

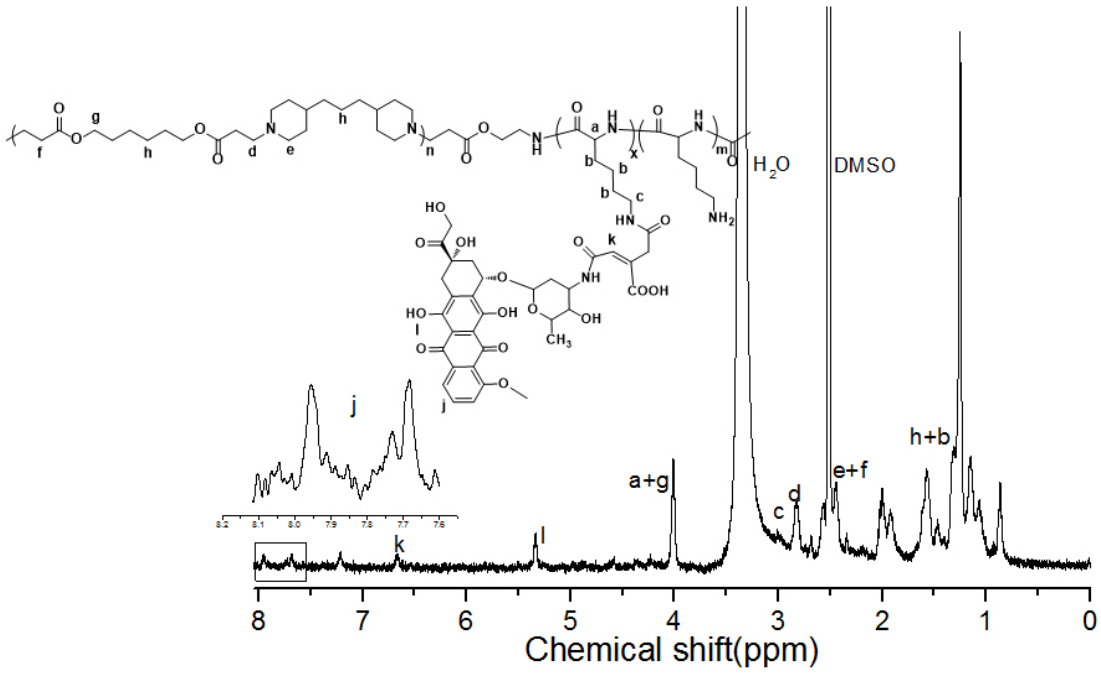

Fig. S12. ^{1}H NMR spectra of PAE_{22}-*b*-P(Lys_4-*co*-$LysDox_4$) in DMSO-d_6.

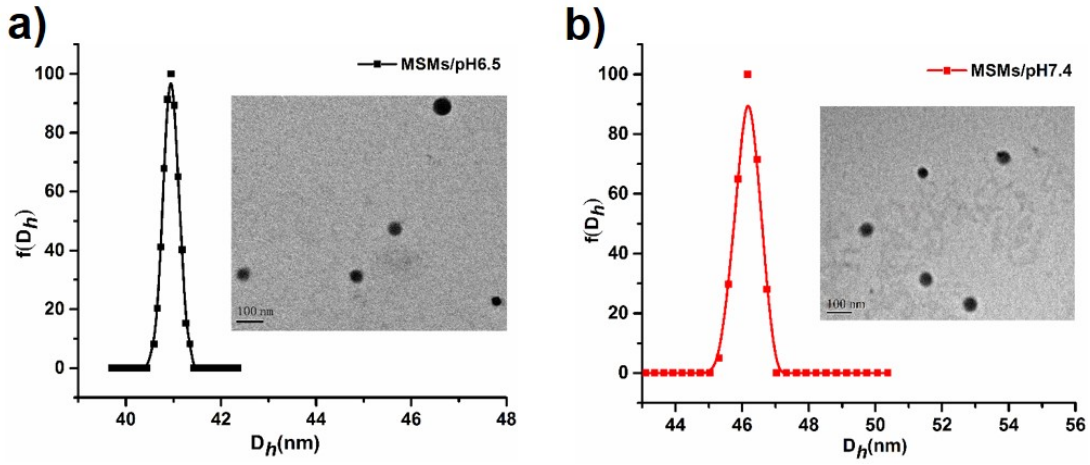

Fig. S13. Hydrodynamic diameter distribution and TEM images (inset) of MSMs at pH 6.5 (a) and at pH 7.4 (b) in 0.1 M phosphate buffer solution.

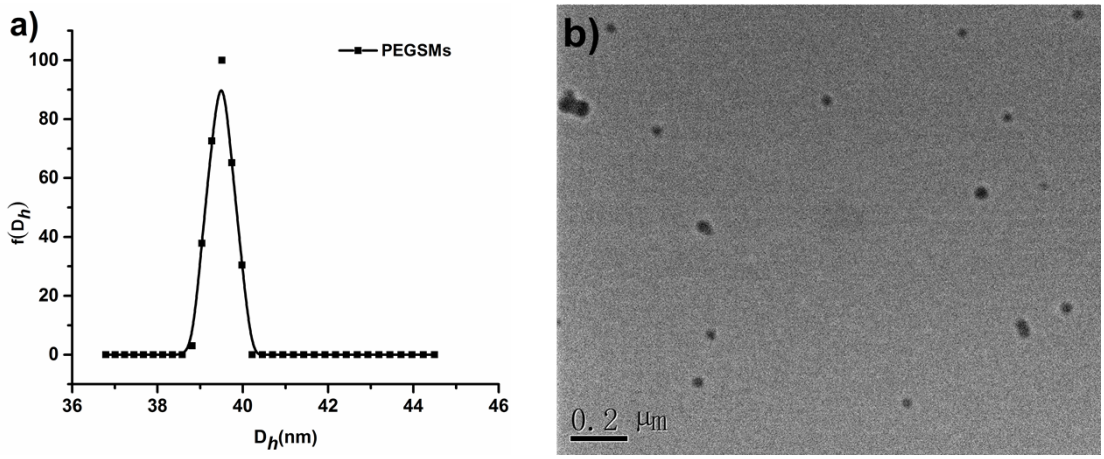

Fig. S14. (a) Hydrodynamic diameter distribution and (b) TEM images of PEGSMs at pH 7.4.

Table S1. Diameters and zeta potential of PEGSMs and MSMs.

Micelles	pH 7.4		pH 6.5	
	Diameters (nm)	Zeta potential (mv)	Diameters (nm)	Zeta potential (mv)
PEGSMs	39.5±1	-3.08±0.39	40.5±1	-4.06±0.78
MSMs	46.2±1	-3.17±0.89*	41.1±1	4.92±0.45

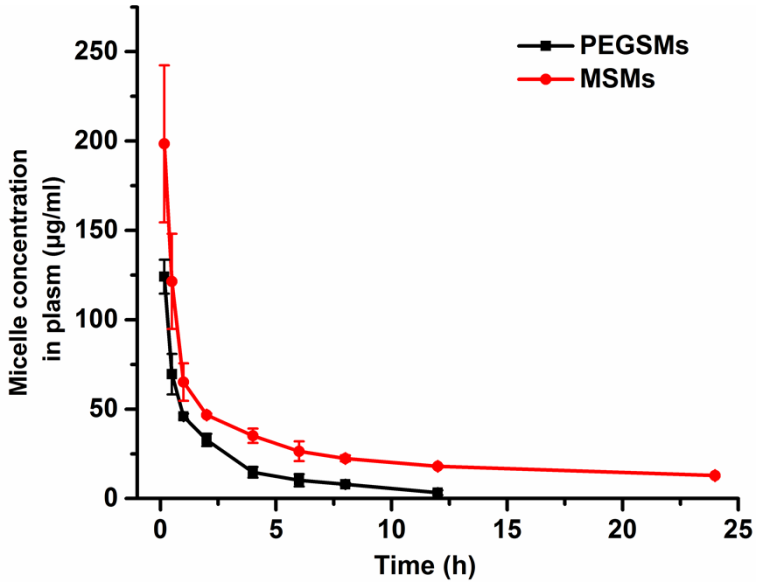

Fig. S15. The concentration of PEGSMs and MSMs in plasma after intravenous injection into BALB/c mice.

Table S2. Pharmacokinetic Parameters Analysis of PEGSMs and MSMs Injected into BALB/c Mice.

Micelles	$t_{1/2}$ (h) [a]	$AUC_{(0\text{-}inf)}$ (g·h·ml^{-1}) [b]	CL (mL·kg^{-1}·h^{-1}) [c]	MRT (h) [d]
PEGSM	8.8±0.43	239.9±39.07	82.66±12.578	2.9±0.41
MSM	15.6±1.51**	1,070.8±163.08*	18.47±2.586*	7.9±0.24**

[a] elimination half-life of micelles in the bloodstream. [b] The area under the blood concentration versus time curve. [c] Clearance. [d] Mean residence time. All values are reported as mean ±SD. ** p<0.05; * p<0.01 significant difference compared to PEGSM group.

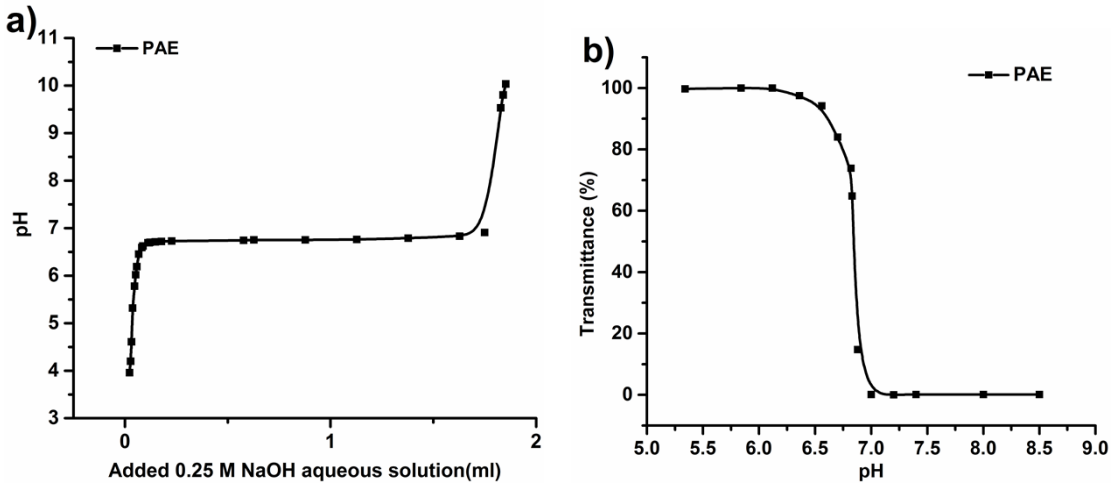

Fig. S16. (a) Titration curves of PAE. (b) Turbidity curves of PAE.

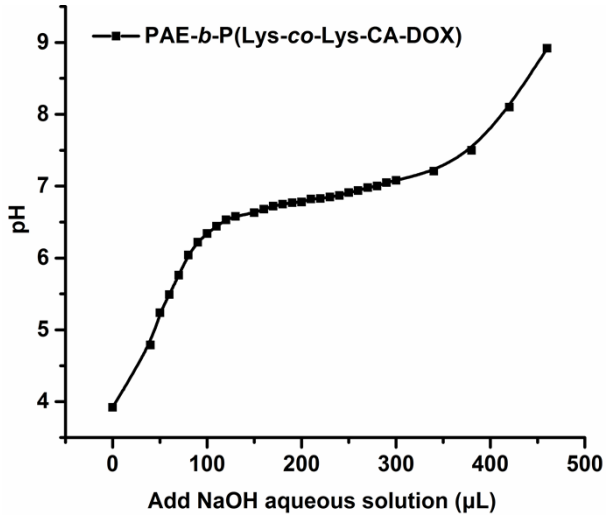

Fig. S17. Titration curves of PAE-*b*-P(Lys-*co*-Lys-CA-DOX).

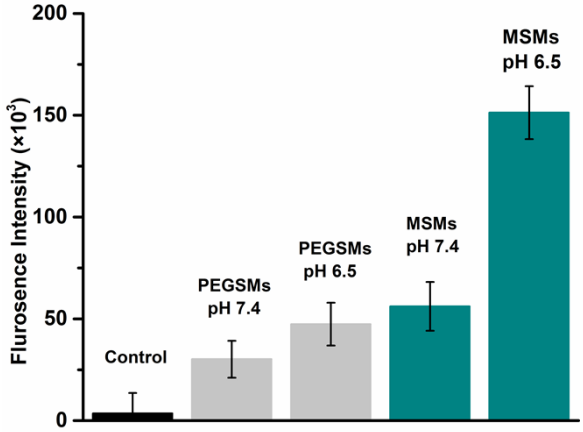

Fig. S18. Quantitative analysis of *in vitro* cellar uptake from flow cytometry results.

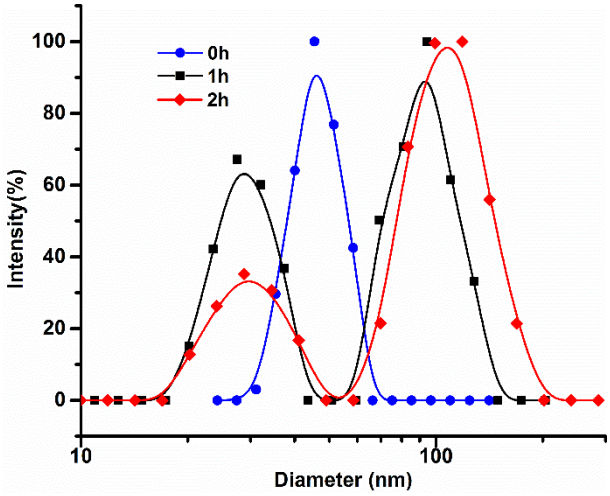

Fig. S19. Variation of size distribution of MSM at endosomal pH vs. time.

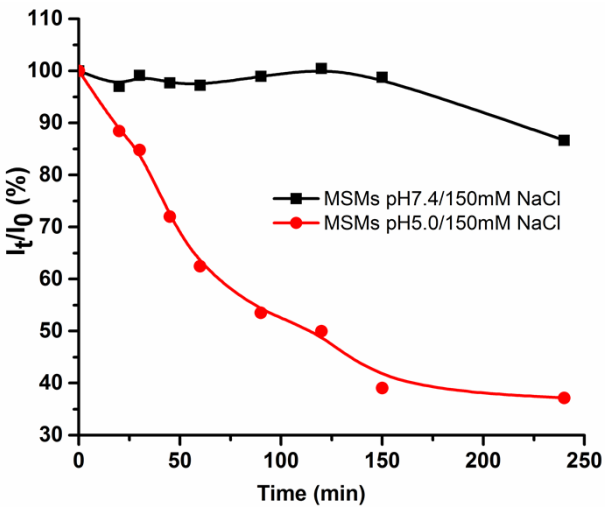

Fig. S20. Relative scattering light intensity of MSMs at different pH condition.

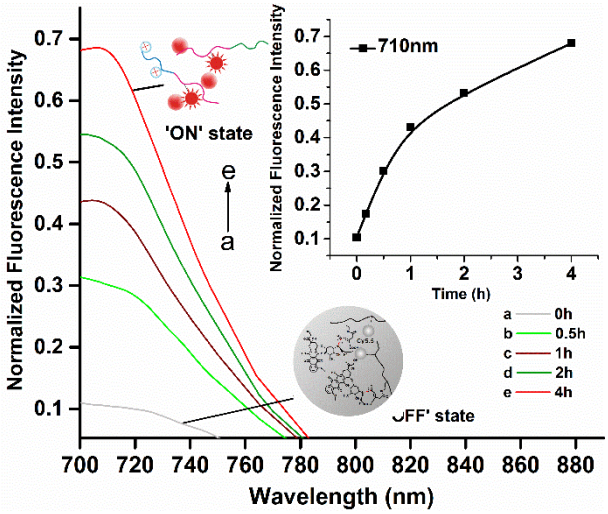

Fig. S21. Homofluorescence resonance energy transfer (homoFRET) assay (variation of fluorescence intensity at the wavelength of 710nm inset) at pH 5.0.

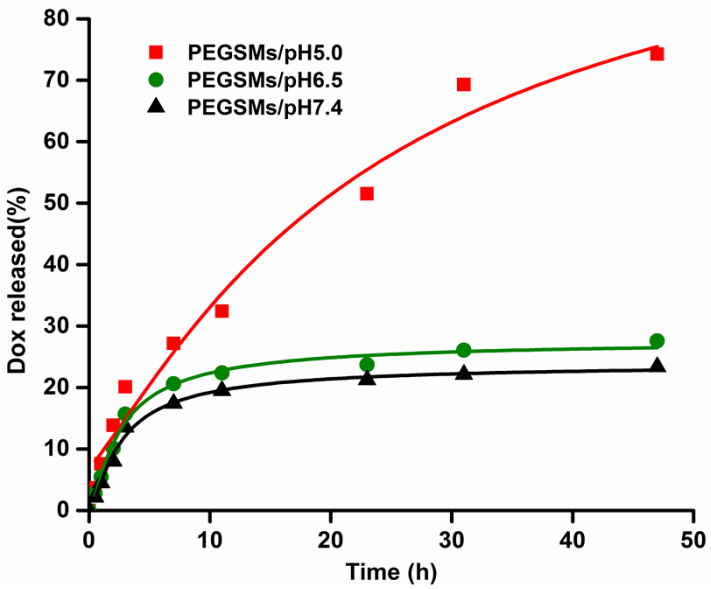

Fig. S22. *In vitro* release profiles of DOX from PEGSMs in 0.1 M citrate buffer solution under different pH condition.

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