Electronic supplementary information

Novel hybrid vesicles co-assembled from cationic lipid and PAAc-g-mPEG with pH-triggered transmembrane channels for controlled drug release

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Experimental Section

Materials. Preparation and characterization of poly(*N*-acryloxysuccinimide) (PNAS) and mPEG-NH_2 were described in detail in our previous studies [1-4]. Didodecyldimethylammonium bromide (DDAB) was supplied by Aldrich and used as received. Doxorubicin hydrochloride (DOX) was obtained from Seedchem. Deuterium solvents used in ^{1}H-NMR measurements were obtained from Cambridge Isotope. Deionized water was produced from Milli-Q Synthesis (18 MΩ, Millipore). All other chemicals were reagent grade and used as received.

Copolymer Synthesis. Grafting reaction of PNAS (10 %, w/v) with mPEG-NH_2 (10 mol% with respect to the NAS residues), catalyzed by triethylamine, in DMF was carried out at 60 ^{o}C under stirring for 72 h. This was followed by the fully hydrolysis of the unreacted NAS moieties in the graft copolymer into the AAc residues by the

addition of excess tris buffer (pH 7.4), as shown schematically in Fig. S1. The copolymer solution was then subjected to dialysis (Cellu Sep MWCO 6000-8000) against deionized water for 7 days to remove DMF and N-hydroxysuccinimide. This was followed by diafiltration (Millipore, Labscale TFF System equipped with Pellicon XL membrane Biomax-10 MWCO 10000) to effectively eliminate unreacted mPEG-NH_2. The final product was then collected by lyophilization. The composition of the graft copolymer was determined by ^{1}H-NMR, using DMF in a sealed capillary as an external standard. The calibration curve was established by the relative signal integrals at δ 3.6 ppm of mPEG with eight concentrations in DMSO-d_6 to the signal integral of DMF at δ 8.0 ppm. Fig. S2 illustrates the ^{1}H-NMR spectrum of the graft copolymer (10.0 mg/mL) in DMSO-d_6 at 20 ^{o}C. The composition was thus determined by a mass balance based on the characteristic signal integral of mPEG at 3.6 ppm in combination with the calibration curve.

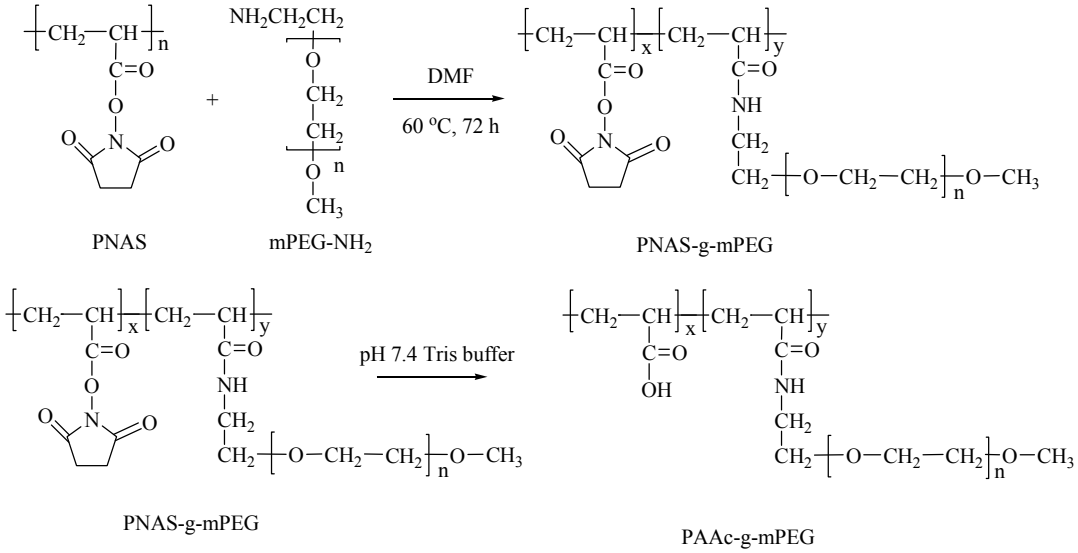

Fig. S1 Synthetic route and the chemical structure of the resultant graft copolymer.

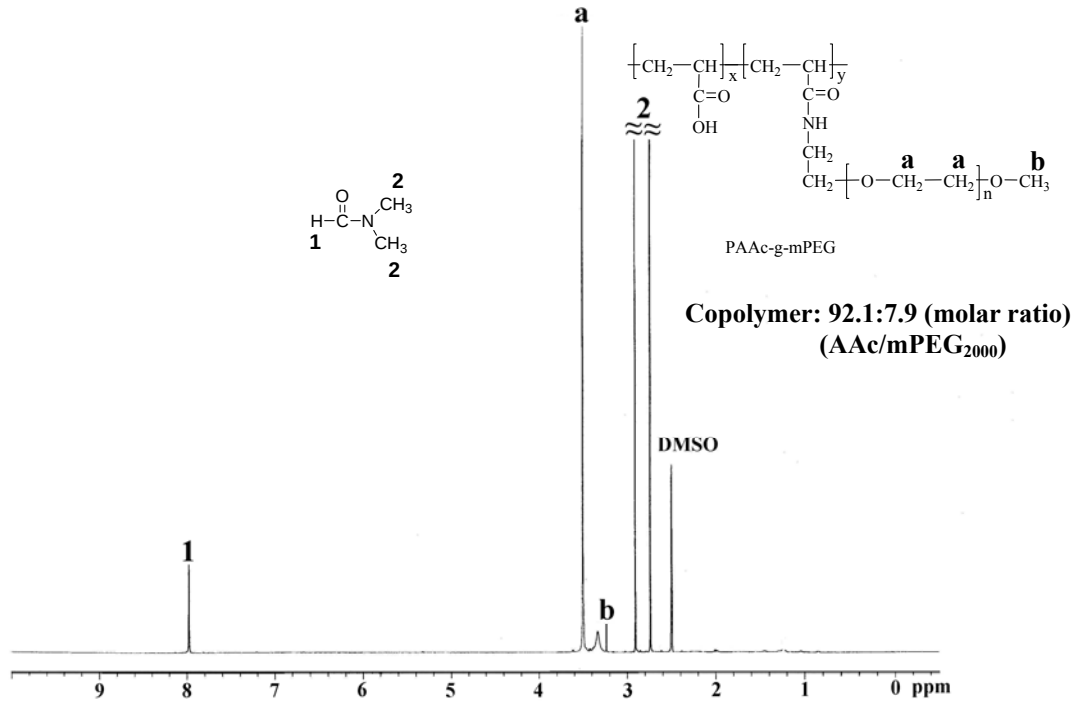

Fig. S2 ^{1}H-NMR spectrum of the graft copolymer in DMSO-d_6 at 20 ^{o}C, using the DMF signal at 8.0 ppm as an external reference.

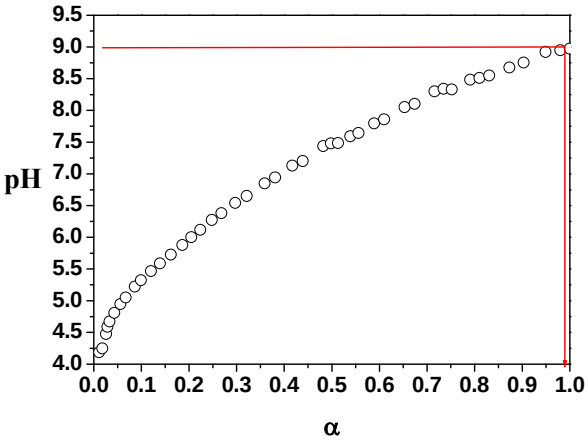

Fig. S3 Relationship between pH and ionization degree (α) of the AAc residues of the graft copolymer at 20 ^{o}C. The potentiometric titration of the graft copolymer in water with an aqueous NaOH solution (0.024 M) was conducted on a Mettler Toledo DL53 autotitration system equipped with DG 101-SC pH electrode under stirring.

Fluorescence Characterization. Aliqouts (10 μL) of N-phenyl-2-naphthylamine (PNA) in ethanol (1.0×10^{-3} M) were evaporated in vials and 1.0 mL of aqueous solutions (suspensions) of the graft copolymer (2.0 mg/mL), DDAB vesicles or copolymer/DDAB complex assemblies was then added separately. PNA at a constant concentration of 1.0×10^{-5} M within the samples was employed as a hydrophobic probe in the fluorescence measurements. Fluorescence characterization was achieved by measuring the maximum wavelength (λ_{max}) of fluorescence emission of PNA in the above samples [5]. The excitation was performed at 294 nm and the emission spectra were recorded in the wavelength range from 250 to 550 nm on a Hitachi F-7500 fluorescence spectrometer.

Table S1. λ_{max} values of the fluorescence emission of PNA alone in the aqueous phase identical to the medium used for the vesicle preparation, aqueous solutions (suspensions) of graft copolymer, DDAB vesicles, and copolymer/DDAB complex asemblies at pH 8.9 at 20 ^{o}C.

Sample	λ_{max} of PNA (nm)
Blank aqueous medium	436
Copolymer solution	433
DDAB vesicles	409
Copolymer/DDAB complex assemblies	411

TEM Examination. The **s**ample was prepared by placing a few drops of aqueous suspension of complex assemblies on a 300-mesh copper grid covered with carbon and allowed to stand at 20 °C for 20 s. Excess solution on the grid was gently removed with absorbent paper, followed by negative staining of the sample with a uranyl acetate solution (5.0 wt %) for 20 s. The sample was then dried at 20 °C for 2 days. The TEM image was attained on a JEOL JEM-1200 CXII microscope operating at an accelerating voltage of 120 kV.

Dynamic and Static Light Scattering (DLS and SLS) Measurements. The mean hydrodynamic particle diameters (D_h) and particle size distributions of complex assemblies in aqueous media of different pH were determined by a Brookhaven BI-200SM goniometer equipped with a BI-9000 AT digital correlator using a solid-state laser (35 mW, λ = 637 nm) detected at a scattering angle of 90^o, using the cumulant analysis method. The experimental results reported herein represent an average of at least triplicate measurements.

For determining the R_g/R_h values of complexed assemblies at different pH, the mean R_h was attained by DLS on a Brookhaven BI-200SM goniometer using a solid-state laser (35 mW, λ = 637 nm) at 90^o. The CONTIN algorithm was employed for data analysis in order to confirm the absence of bimodal particle size distribution of the complex assemblies with much enhanced reliability [6,7]. The observed angular independence of the apparent diffusion coefficients of the hybrid assemblies from DLS measurements as illustrated by a linear relationship between the relaxation frequency (Γ) and the square of the scattering vector (q^2) in Fig. S4 confirms the colloidal particles in spherical shape [8]. The R_g of complex assemblies was determined by the angular dependent measurements of the light scattering intensity. The partial Zimm plot of the reciprocal of the scattering intensity (I_{ex}^{-1}) versus the square of scattering vector (q^2) was used for quantitative determination of the R_g value [8]. The assembly suspension was passed through a 0.45 μm filter at 20 °C prior to DLS/SLS measurements.

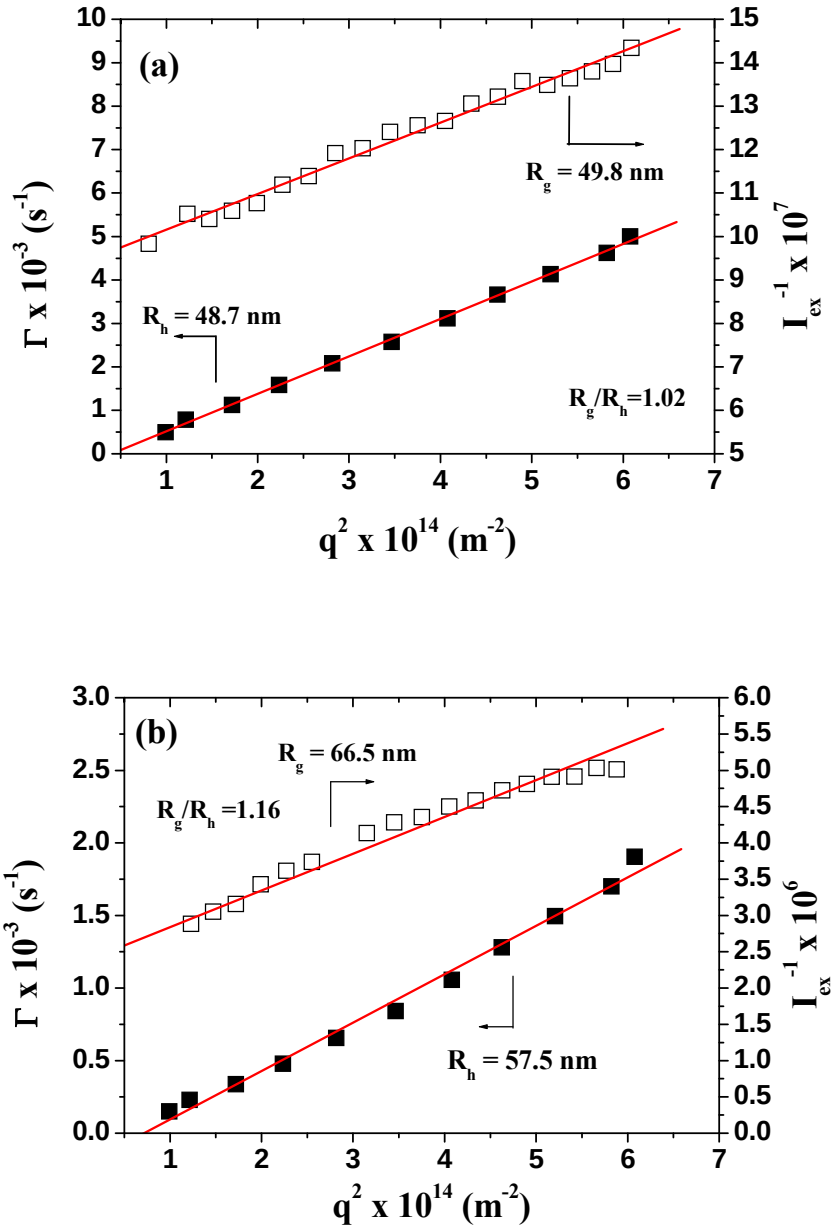

Fig. S4 Angular dependent DLS/SLS measurements achieved on copolymer/DDAB complex vesicles ($Z_{+/-}$ = 1.0) at pH (a) 8.9 and (b) 5.0.

^{1}H-NMR Characterization. The graft copolymer was dissolved in D_2O to a final concentration of 2.0 mg/mL and then the solution pD was adjusted to 8.9 by NaOD. The preparation of copolymer/DDAB complex vesicles in D_2O was then performed by the same procedure described in the text. The ^{1}H-NMR was conducted on a Varian

Unity Inova-600 at 600 MHz without sample spinning. The pulse width of 4.9 μs with a relaxation delay of 2.0 s was used. During ^{1}H-NMR measurements, DMF in a sealed capillary was coaxially placed in the sample tube as an external standard, and its signal at δ 8.45 ppm was selected as a reference resonance for evaluating the chemical shifts and integrals of feature signals of the copolymer and DDAB in the complex assemblies in D_2O (pD 8.9). The sample was equilibrated at 20 ^{o}C for 30 min prior to measurements.

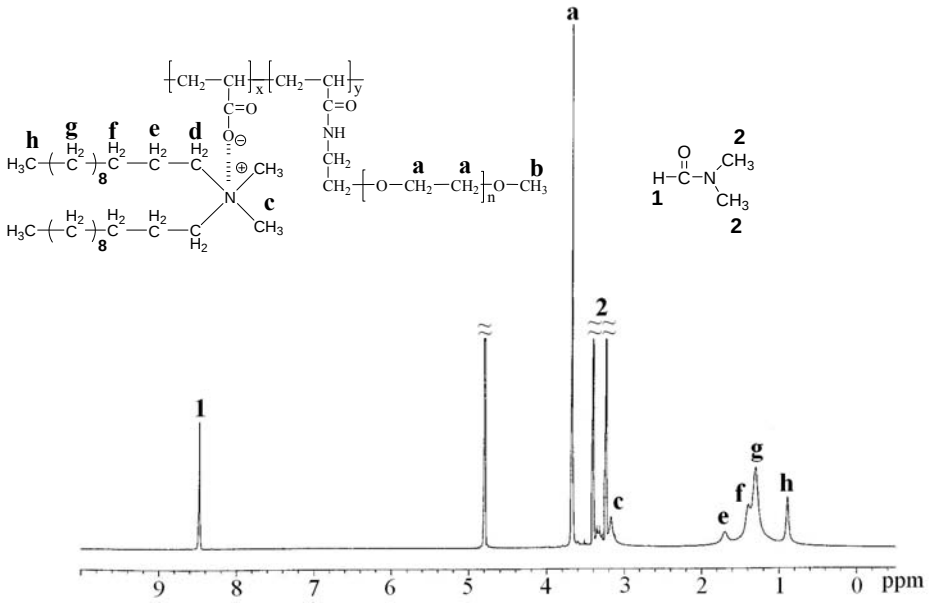

Fig. S5 ^{1}H-NMR spectrum of copolymer/DDAB complex vesicles ($Z_{+/-}$ = 1.0) in D_2O (pD 8.9) at 20 ^{o}C.

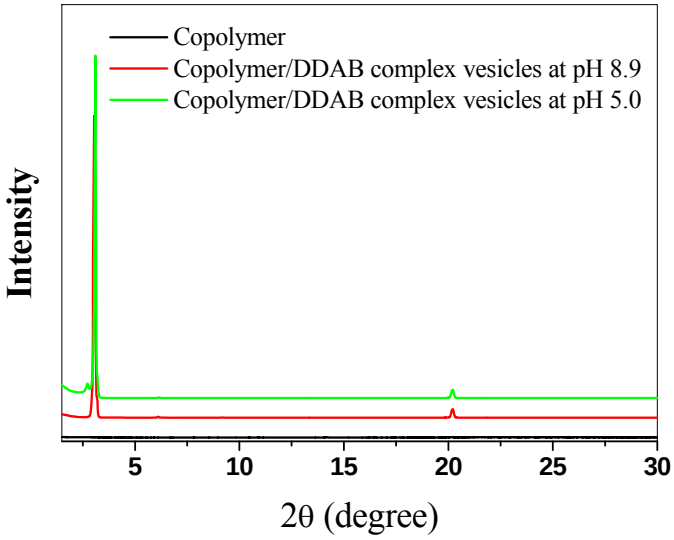

Fig. S6 Wide-angle X-ray diffraction (WXRD) profiles of the graft copolymer (black line) alone and copolymer/DDAB complex vesicles at pH 8.9 (red line) and 5.0 (green line), respectively. Self-supported cast films for the WXRD studies were obtained from spreading the complex assembly solutions on clean glass slides. WXRD spectra were obtained from a Rigaku diffractometer D/MAX2000 equipped with a rotating anode (Cu K_α radiation) operating at 40 kV and 30 mA. The diffraction data were collected at 2θ between 1.5° and 30° in a fixed mode with a scanning speed of 2 degree/min with step intervals of 0.01°.

Table S2. DLS data of homoPAAc/DDAB complex assemblies (developed at pH 8.9, $Z_{+/-}$ = 0.6) in aqueous solutions of pH 8.9, 7.4 and 5.0.

Sample	pH	D_h (nm)	PDI	Intensity (kcps)
homoPAAc/DDAB	8.9	262	0.04	296
assemblies	7.4	264	0.06	289
	5.0	264	0.11	299

Preparation and Studies of Cumulative DOX Release from Drug-Encapsulated Copolymer/DDAB Hybrid Vesicles. The graft copolymer was dissolved in aqueous solution of DOX (9.2×10^{-4} M) at pH 8.9 to a final concentration of 2.0 mg/mL. The

copolymer/DOX aqueous solution was then added into the vial deposited with a dry DDAB film on the inner surface and the solution was stirred at 20 ^{o}C for 24 h to obtain the DOX-loaded complex vesicle dispersions. Prior to the release study, the aqueous suspension of DOX-loaded copolymer/DDAB assemblies was dialyzed (Cellu Sep MWCO 12000-14000) against tris buffer solution of pH 7.8 (I = 0.01 M) for 2 days to remove free DOX species. The DOX-loaded copolymer/DDAB assemblies were then fully dissolved by the addition of DMF at a ratio of DMF/H_2O = 9/1 (v/v). The amount of DOX originally encapsulated was quantitatively determined by the fluorescence measurement on a Hitachi F-7500 fluorescence spectrometer equipped with a thermostat cell unit. The excitation was performed at 480 nm and the emission spectra were recorded in the range from 500 to 700 nm. The calibration curve was established by the fluorescence of DOX with different concentrations in the DMF/H_2O solution. Drug loading efficiency (DLE) was calculated according to the following formula:

DLE (%) = (weight of loaded DOX/weight of DOX in feed) × 100 %.

The release behavior of DOX from complex vesicles was examined by the dialysis technique. The DOX-loaded copolymer/DDAB vesicle dispersion (2.0 mL) was subjected to dialysis (Cellu Sep MWCO 12000-14000) against the buffer solutions of pH 7.4 and 5.0 (50 mL), respectively. The DOX release experiment was carried out by withdrawing 1.0 mL of external solution (pH 5.0 or 7.4) at preset time intervals and the concentration of DOX was determined by fluorescence measurements using the pertinent calibration curve of DOX with various concentrations in the aqueous solution of either pH 5.0 or 7.4.

References

1 Y. H. Hsu, W. H. Chiang, C. H. Chen, C. S. Chern and H. C. Chiu, *Macromolecules*, 2005, **38**, 9757.

2 Y. H. Hsu, W. H. Chiang, M. C. Chen, C. S. Chern and H. C. Chiu, *Langmuir*, 2006, **22**, 6764.

3 W. H. Chiang, Y. H. Hsu, T. W. Lou, C. S. Chern and H. C. Chiu, *Macromolecules*, 2009, **42**, 3611.

4 W. H. Chiang, Y. H. Hsu, C. S. Chern and H. C. Chiu, *J. Phys. Chem. B*, 2009, **113**, 4187.

5 M. Arotcarena, B. Heise, S. Ishaya and A. Laschewsky, *J. Am. Chem. Soc.*, 2002, **123**, 3787.

6 K. Chen, S. K. Ballas, R. R. Hantgan, D. B. Kim-Shapiro, *Biophys J* 2004, **87**, 4113.

7 W. Schartl, editor. Light scattering from polymer solutions and nanoparticle dispersions. Heidelberg, Germany: Springer-Verlag; 2007 [Chapter 4].

8 A. E. Smith, X. Xu, S. E. Kirkland-York, D. A. Savin and C. L. McCormick, *Macromolecules*, 2010, **43**, 1210.