Supporting Information

pH-sensitive Vesicles Based on A Biocompatible Zwitterionic Copolymer

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Table S1, Figures S1-S13 and Experimental Section

Table S1. The properties of PMPC-*b*-PDPA diblock copolymers prepared by ATRP

entry	Copolymer composition by ^{1}H NMR in D_2O/DCl at pH 2	$M_{n,GPC}$	M_w/M_n	TEM Morphologies
1	$PMPC_{25}$-*b*-$PDPA_{70}$	35,200	1.08	vesicles + micelles
2	$PMPC_{25}$-*b*-$PDPA_{120}$	55,000	1.25	vesicles
3	$PMPC_{25}$-*b*-$PDPA_{160}$	74,000	1.23	vesicles

GPC analyses of copolymers 1-3 were conducted in a 3:1 chloroform/methanol mixture in the presence of 5 mM LiBr using poly(methyl methacrylate) standards.

Figure S1. Typical ^{1}H NMR spectrum of $PMPC_{25}$-*b*-$PDPA_{160}$ copolymer in DCl/D_2O (pH 2).

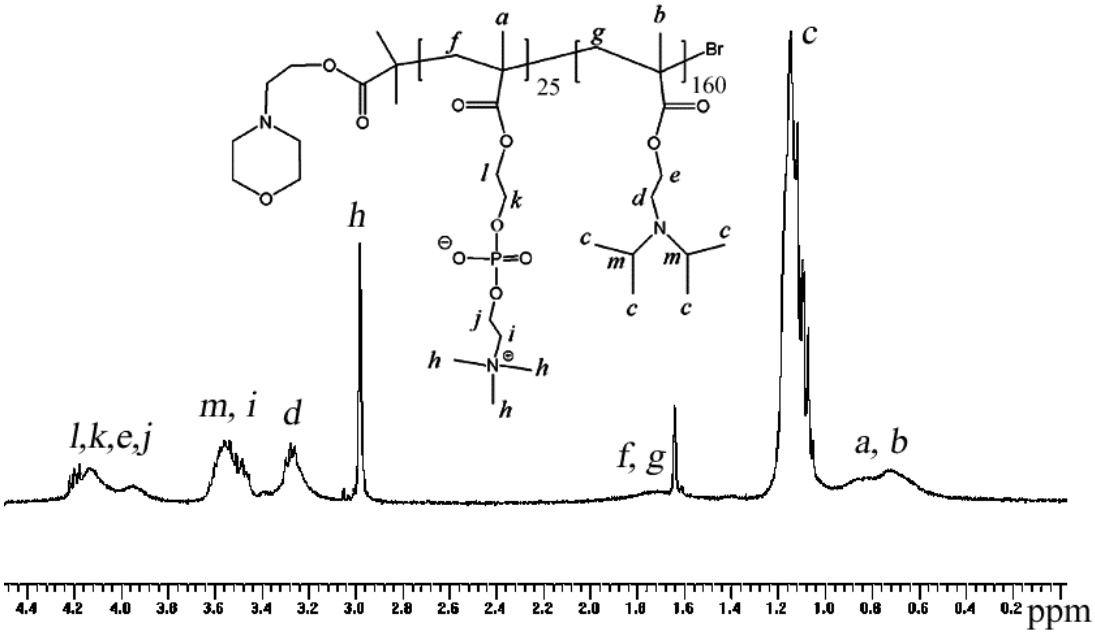


Figure S2. Relationship between apparent diffusion coefficient (D_{app}) and scattering vector (q) for vesicles prepared from the $PMPC_{25}$-*b*-$PDPA_{120}$ diblock copolymer (the initial copolymer concentration at pH 2 was 1.0 g/L and the final pH was 7.4). This indicates that the vesicles have spherical morphologies of low polydispersity.

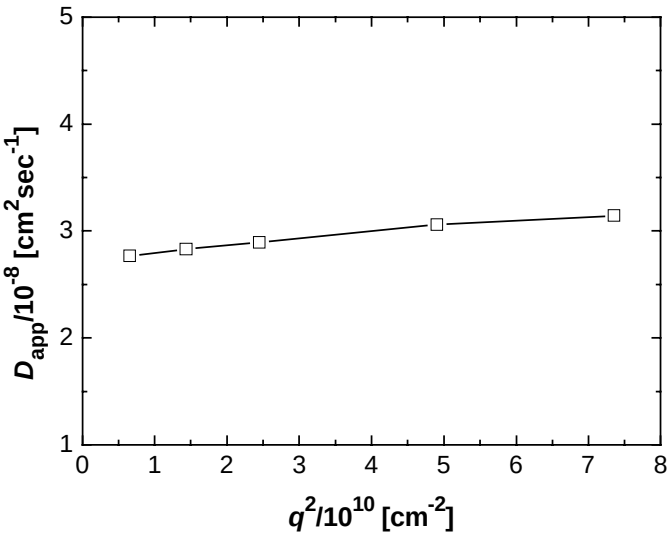


Figure S3. TEM images of ill-defined micellar aggregates (A) and well-defined vesicles (B-D) obtained at different solution pH using the $PMPC_{25}$-*b*-$PDPA_{120}$ diblock copolymer (the initial copolymer concentration at pH 2 was 1.0 g/L). According to DLS studies, the ill-defined micelles formed at pH 6 (see image A) have a mean intensity-average diameter of 156 nm and a broad polydispersity of 0.36. Thus this image shown is merely illustrative, rather than truly representative of the micelle size distribution. Larger micelles (some with diameters of hundreds of nanometers) are not shown here.

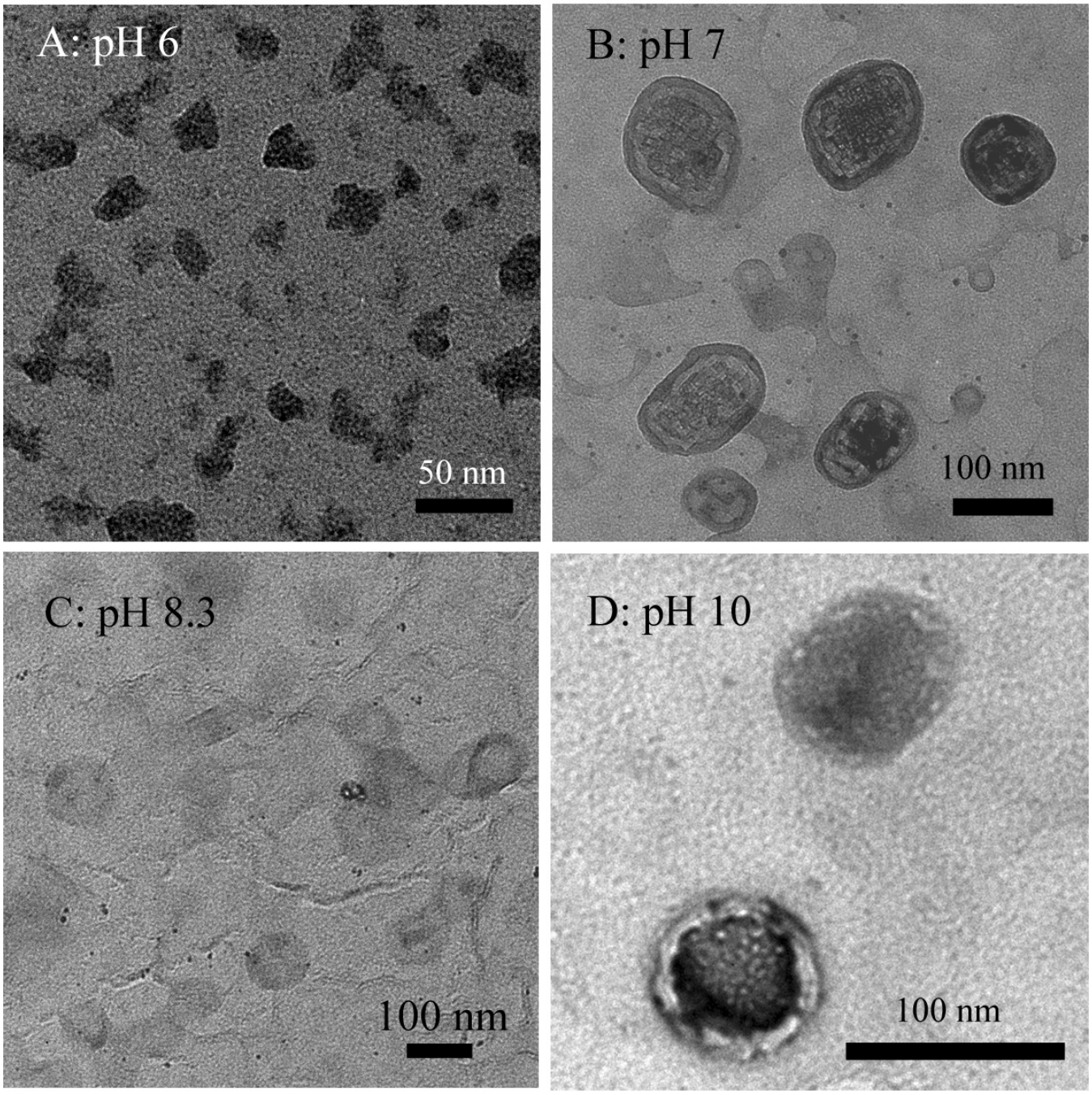


Figure S4 TEM images of vesicles prepared using the $PMPC_{25}$-*b*-$PDPA_{160}$ diblock copolymer: (A) without staining; (B) after staining the PMPC chains with an ethanolic solution of uranyl acetate. The mean vesicle wall thickness shown in A is 37 ± 4 nm. The initial copolymer concentration at pH 2 was 1.0 g/L and the final solution pH was 11.45.

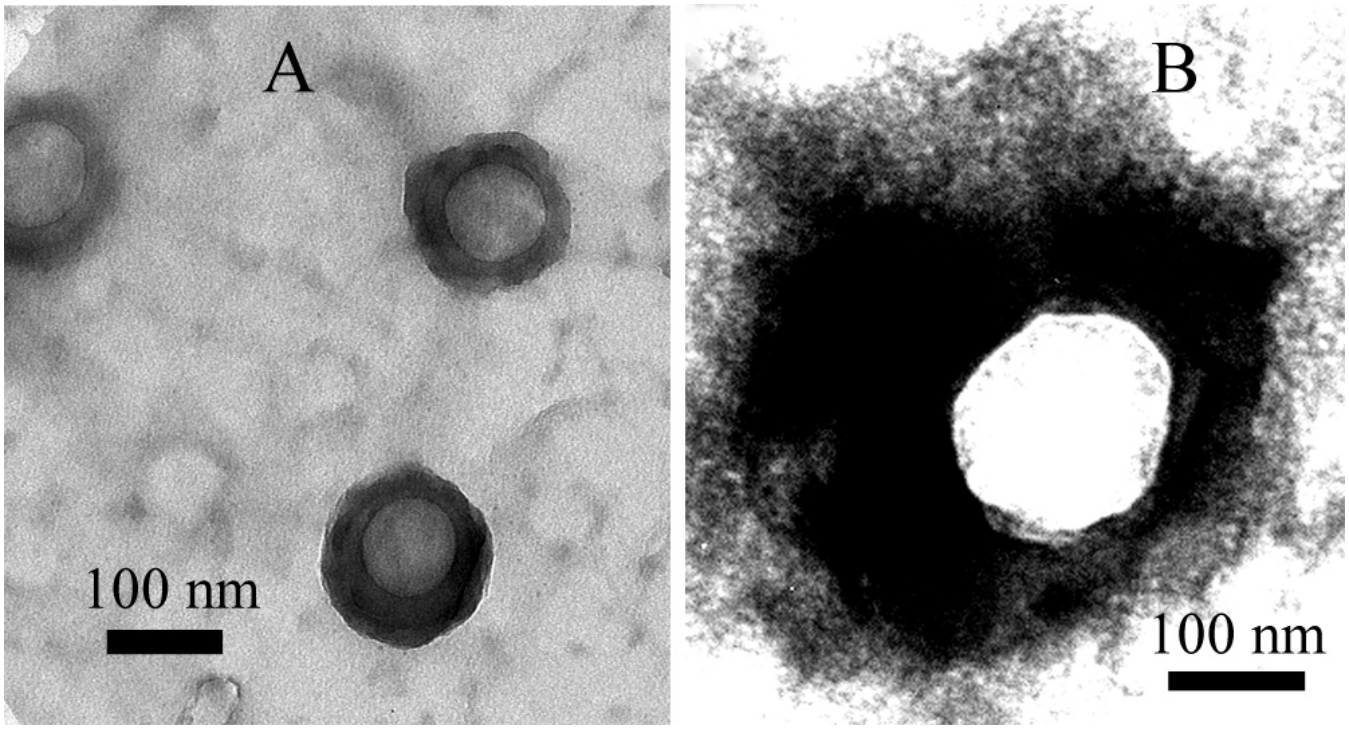


Figure S5 Low magnification TEM images of the vesicles shown in

Figure S4A above. This confirms that a relatively uniform vesicle size distribution is obtained.

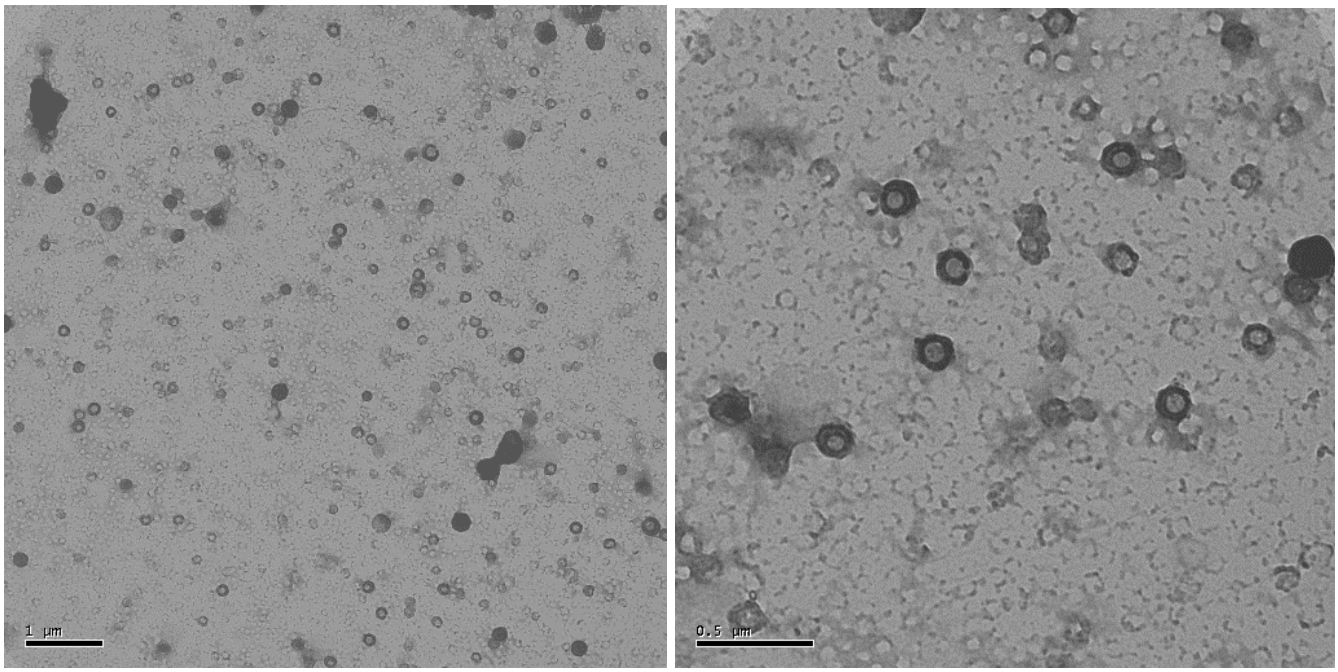


Figure S6. Plot of ln(t) vs ln(N), where t is the wall thickness of vesicles and N is the mean degree of polymerization of the PDPA block. The calculated result is $t = 0.067N^{1.168}$.

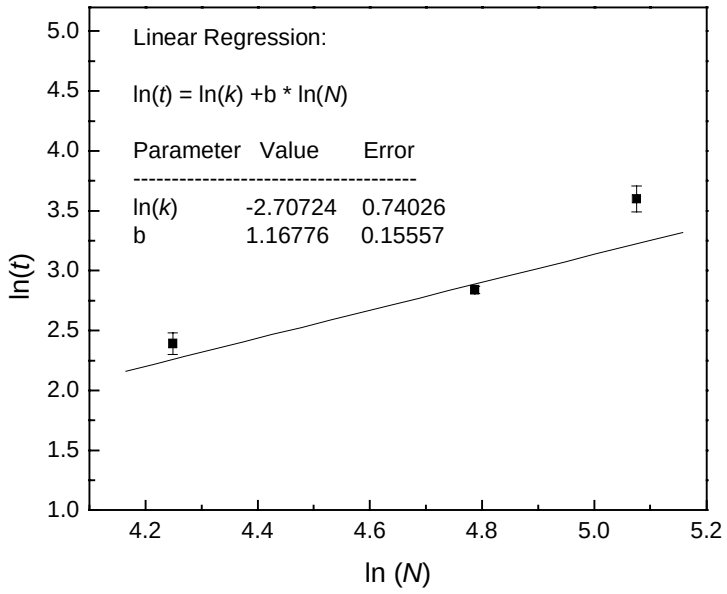


Figure S7. TEM images of vesicles and micelles formed by the $PMPC_{25}$-*b*-$PDPA_{70}$ diblock copolymer at pH 7.4 (the initial copolymer concentration at pH 2 was 1.0 g/L).

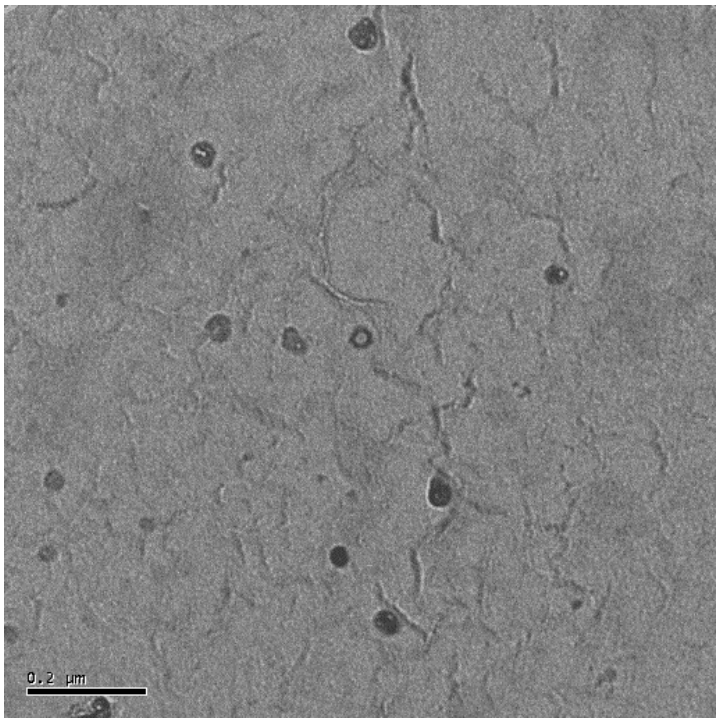


Figure S8. Digital photographs of aqueous solutions comprising the $PMPC_{25}$-b-$PDPA_{120}$ diblock copolymer at three initial copolymer concentrations at pH 2 (the final pH in each case is around 7.0).

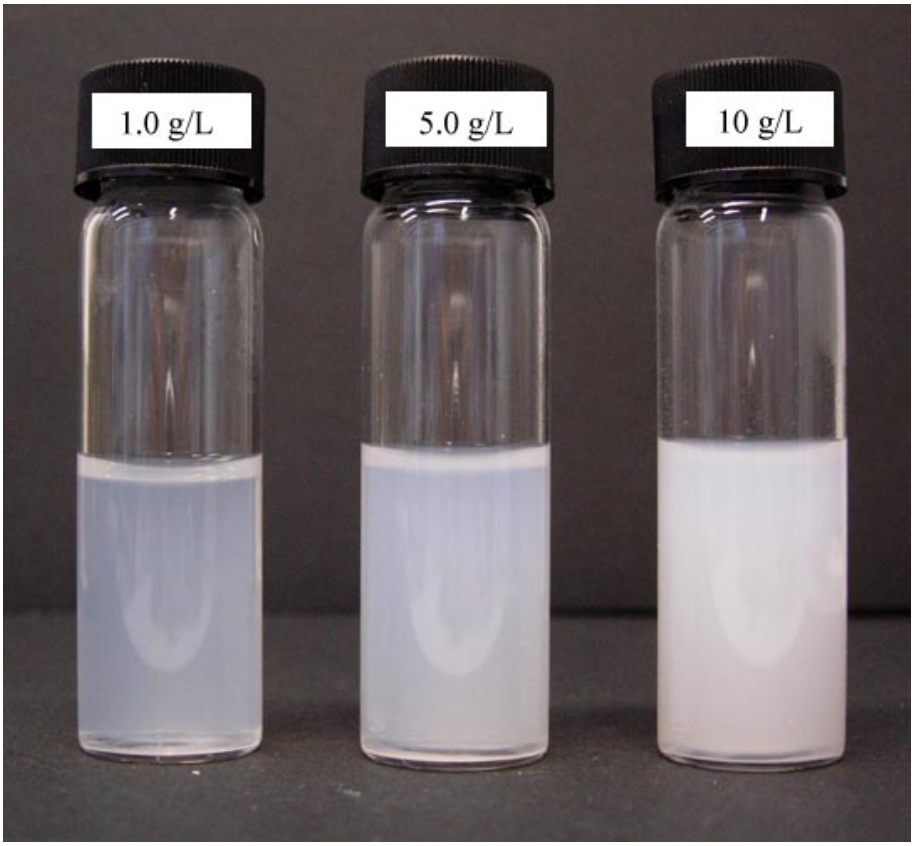


Figure S9. TEM images of complex aggregates and vesicles prepared from the $PMPC_{25}$-*b*-$PDPA_{120}$ diblock copolymer at pH 7.4 (the initial copolymer concentration at pH 2 was 5.0 g/L).

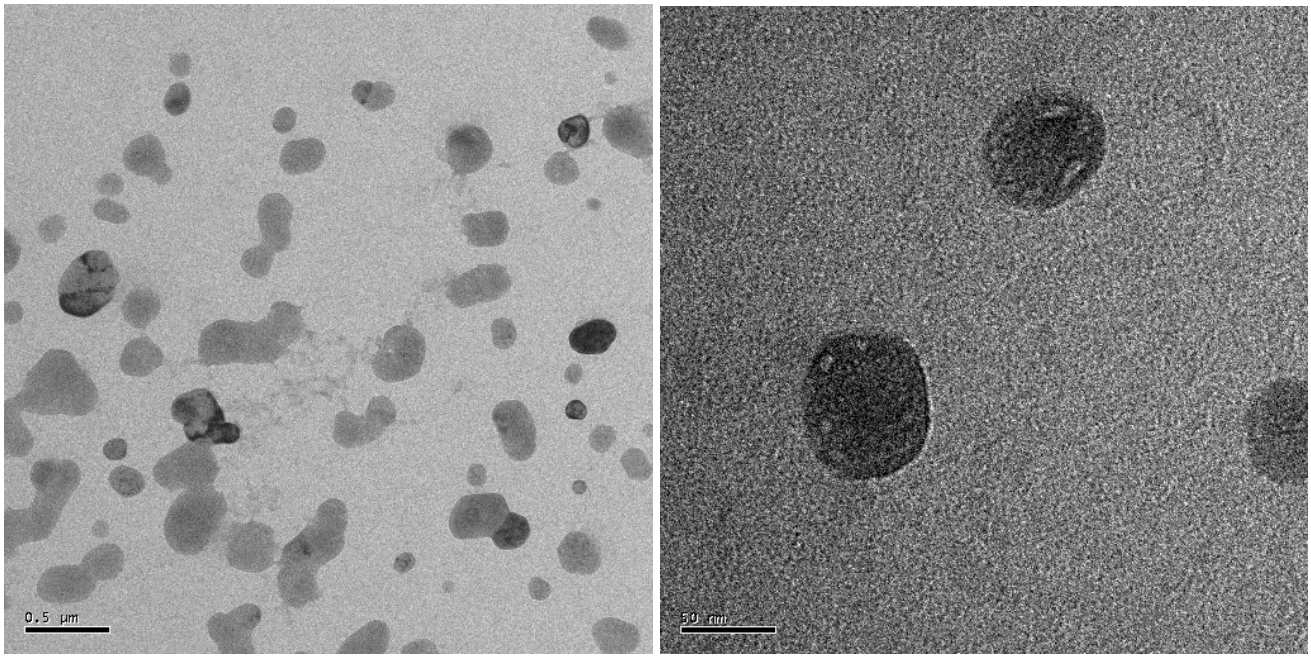


Figure S10. TEM images of complex aggregates prepared from an aqueous solution of $PMPC_{25}$-*b*-$PDPA_{120}$ diblock copolymer at pH 6.86 (the initial polymer concentration at pH 2 is 10.0 g/L).

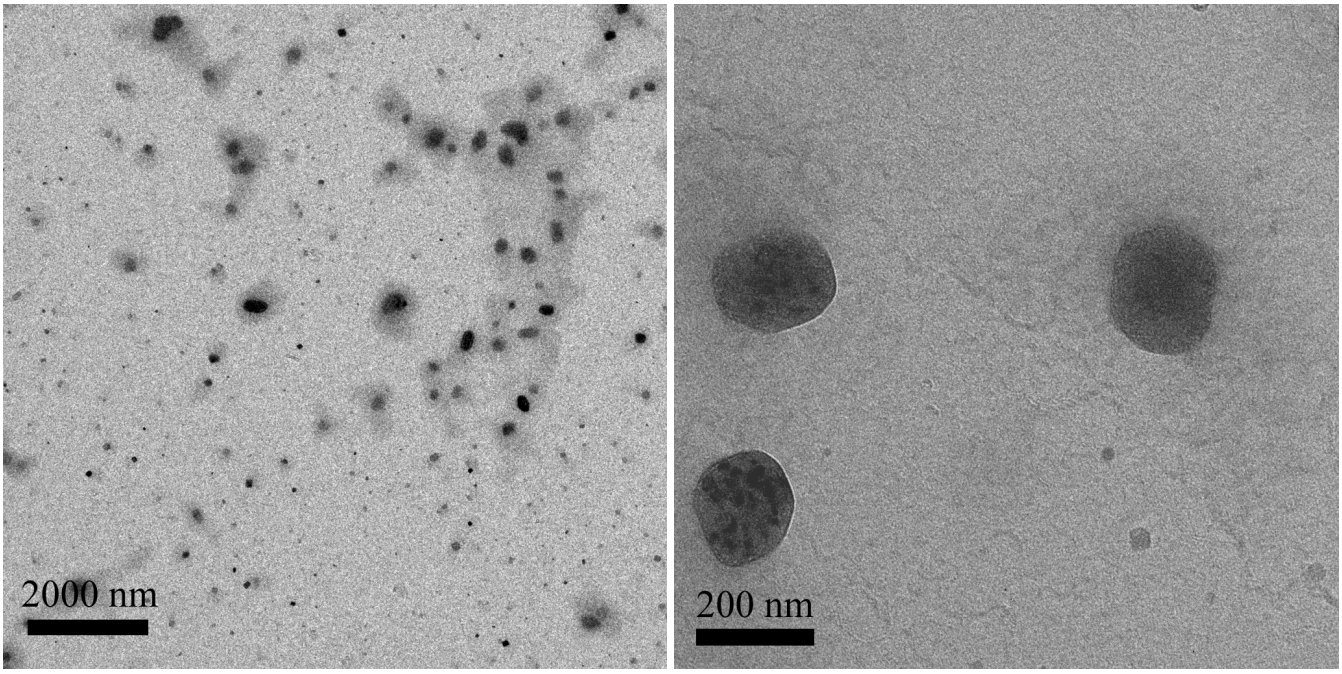


Figure S11. Schematic representation of the synthesis of gold nanoparticle-decorated vesicles.

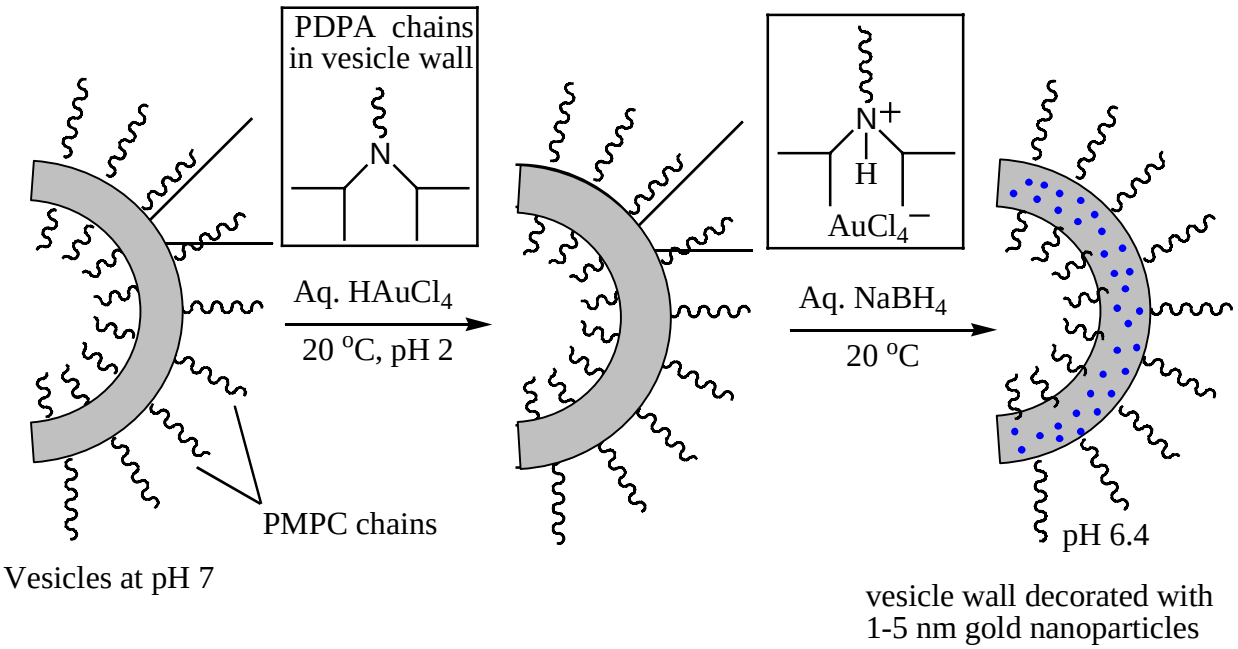
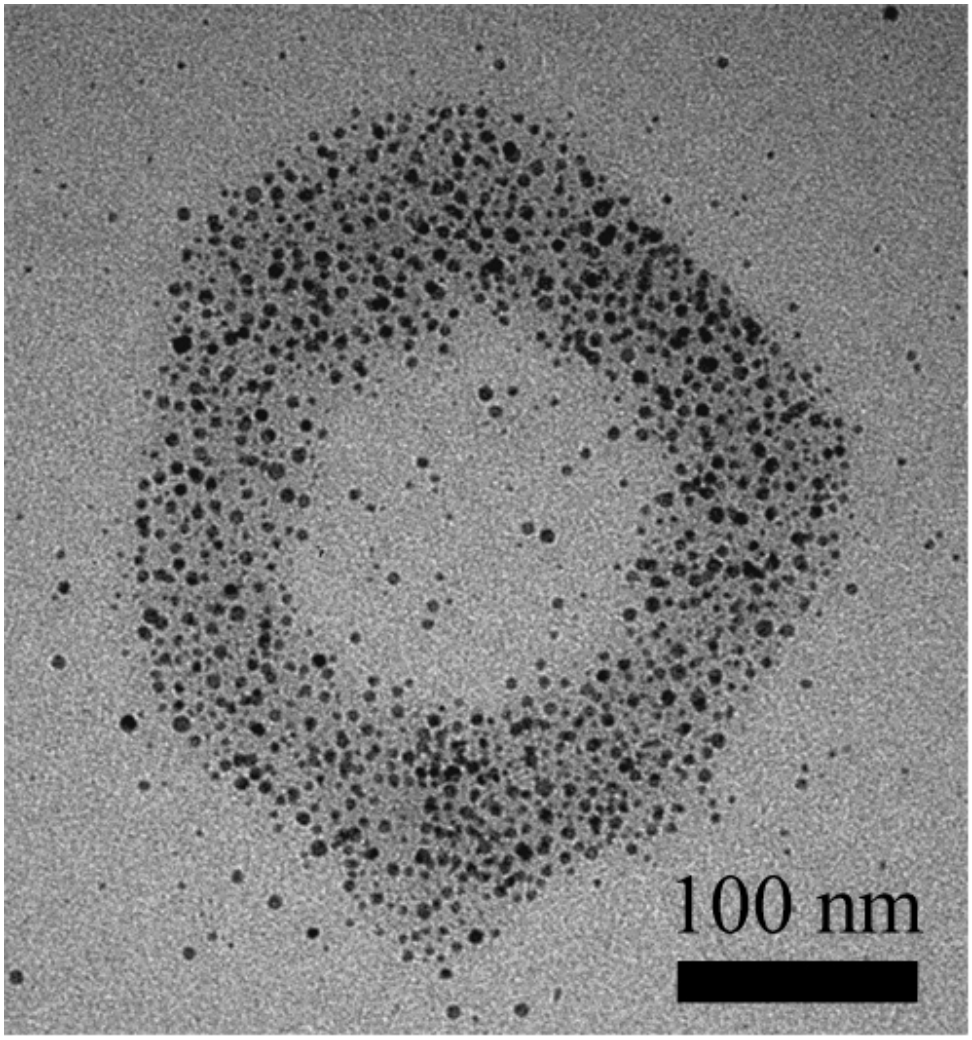


Figure S12. Magnified image of the vesicle shown in Figure 1B. The vesicles were prepared using the $PMPC_{25}$-*b*-$PDPA_{120}$ copolymer (the initial copolymer concentration at pH 2 was 1.0 g/L; immediately prior to the addition of $HAuCl_4$ the solution pH was 7.4; after $NaBH_4$ reduction the final solution pH was 6.4; the Au:DPA molar ratio was 1:40).


100 nm

Experimental Section

Materials. 2-(Methacryloyloxy)ethyl phosphorylcholine (MPC; > 99 %) was kindly donated by Biocompatibles, UK. 2-(Diisopropylamino)ethyl methacrylate (DPA) was purchased from Scientific Polymer Products (USA). Copper(I) bromide (CuBr; 99.999%), 2,2-bipyridine (bpy), methanol and isopropanol were purchased from Aldrich and were used as received. The silica used for removal of the ATRP copper catalyst was column chromatography grade silica gel 60 (0.063-0.200 mm) purchased from E. Merck (Darmstadt, Germany). 2-(*N*-Morpholino)ethyl 2-bromo-2-methylpropanoate (ME-Br) initiator was synthesized according to a previously reported procedure.[1] Doxorubicin (Dox) was purchased from Zhejiang Hisun Pharmaceutical Co Ltd (China). Dialysis tubing was purchased from Medicell International Ltd. with a molecular weight cut-off of 12,000-14,000.

Characterization. The M_n and M_w/M_n values of the three PMPC-PDPA diblock copolymers were assessed by gel permeation chromatography (GPC). The GPC set-up comprised a Polymer Labs PLgel 5 μm Mixed 'C' column operating at 40 ^oC in combination with a refractive index detector. The eluent was a 3:1 chloroform: methanol mixture at a flow rate of 1.0 ml min^{-1} and calibration was carried out using five near-monodisperse poly(methyl methacrylate) standards. The data were processed by Cirrus GPC offline GPC/SEC software.

^{1}H NMR spectra were recorded using a Bruker DRX250 (250 MHz) spectrometer at ambient temperature using either D_2O/DCl, CD_3OD or D_2O as solvents.

Transmission electron microscopy (TEM) images were obtained using a Philips CM100 electron microscope operating at 100 kV equipped with a LaB6 gun and a Gatan 1 K x 1 K digital camera. To prepare TEM samples, 5 μL of a diluted aqueous vesicle solution was placed on a carbon-coated copper grid, and the water droplet was allowed to evaporate under ambient conditions. Alternatively, a TEM grid coated with a thin aqueous vesicle solution film was quickly transferred into a pre-cooled flask, then frozen using liquid nitrogen. Then this sample was freeze-dried with the flask being placed in an ice bath. The latter protocol was preferred if room temperature exceeded 20 ^oC.

Dynamic light scattering (DLS) studies of aqueous vesicles over a range of solution pH were carried out on a Brookhaven Instruments Corp. BI-200SM goniometer equipped with a BI-9000AT digital correlator using a solid-state laser (125 mW, λ = 532 nm). The scattering angle was usually fixed at 90° but for angular dependence measurements it was varied from 30° to 150°. The data were processed by cumulants analysis of the experimental

correlation function and vesicle diameters were calculated from the computed diffusion coefficients using the Stokes-Einstein equation.

UV-visible absorption spectra were recorded using a Perkin Elmer UV-visible spectrophotometer. Data were processed using UV WinLAB software (v 2.85.04).

PMPC-b-PDPA diblock Copolymers by ATRP. In a typical ATRP procedure, a Schlenk flask with a magnetic stir bar and a rubber septum was charged with Cu(I)Br (25.6 mg, 0.178 mmol) and MPC (1.32 g, 4.46 mmol). ME-Br initiator (50.0 mg, 0.178 mmol) and bpy ligand (55.8 mg, 0.358 mmol) were dissolved in methanol (2 mL), and this solution was deoxygenated by bubbling N_2 for 30 minutes before being injected into the flask using a syringe. The [MPC]: [ME-Br]: [CuBr]: [bpy] relative molar ratios were 25: 1: 1: 2. The reaction was carried out under a nitrogen atmosphere at 20 °C. After 65 minutes, deoxygenated DPA (6.09 g, 28.6 mmol) and methanol (7 mL) mixture were injected into the flask. After 48 h, the reaction solution was diluted by addition of isopropanol (about 200 mL) and then passed through a silica column to remove the catalyst.

Preparation of Vesicles. The following protocol was adopted. Copolymer (20.0 mg) was dissolved into dilute aqueous HCl (pH 2; 20 mL; the initial copolymer concentration was 1.0 g/L) and stirred for 1 h. Then the pH was slowly adjusted over a 20 minute period from pH 2 to pH 5 using 0.50 M aqueous NaOH and then from pH 5 to pH 6.4 using 0.05 M aqueous NaOH over the same time scale. Finally, the solution pH was adjusted from 6.4 to 7.4 by slow addition of 0.001 M aqueous NaOH over 30 minutes. The final copolymer concentration after pH adjustment was about 0.8 g/L.

In situ Production of Gold Nanoparticles within the Vesicle Walls. Vesicle solutions prepared using the $PMPC_{25}$-*b*-$PDPA_{120}$ copolymer were mixed with aqueous $HAuCl_4$ at various $HAuCl_4$/DPA molar ratios (from 1:80 to 1:40). After stirring these solutions for half an hour, an aqueous solution of sodium borohydride (1:1 molar ratio relative to the amount of $HAuCl_4$ used) was added. The solutions immediately became colored, ranging from light wine red to light yellow depending on the $HAuCl_4$/DPA molar ratio.

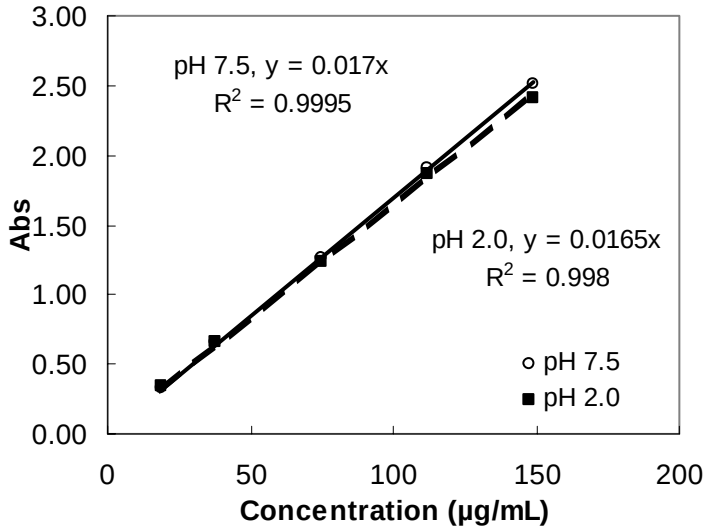
Drug Loading and Elution Experiment. Two loading protocols were examined in order to compare the difference between diffusional loading of the vesicles with loading by pH-induced encapsulation. The first loading protocol involved mixing 5.0 mL of a 1.00 g/L vesicle solution, prepared as described above, with 0.137 mL of a 18.25 g/L Dox solution for a target loading of 50 % Dox within the vesicles. This mixture was allowed to stand for 48 h to allow equilibrium to be attained. The Dox-vesicle mixture was subsequently dialyzed against 200 mL of a 0.9 % saline buffer at pH 7.5 for 15 h prior to an elution experiment. The Dox content in the dialyzed vesicle solution was determined at pH 2 (the solution pH was adjusted by adding HCl) using a Perkin-Elmer Lamda 25 UV spectrophotometer to compare the absorbance of this solution at 483 nm with a calibration curve constructed from aqueous Dox solutions of known concentration (see Figure S13). The Dox concentration determind after dialysis was 0.081 g/L.

A second loading method involved mixing 20 mL of a 1.00 g/L $PMPC_{25}$-b-$PDPA_{120}$ diblock copolymer with 0.548 mL of a 18.25 g/L Dox solution at pH 2 for a target loading of 50% Dox within the vesicles. Following the vesicle preparation protocol described above, the pH was gradually raised to pH 7.4, and the solution was allowed to stand for 48 h prior to dialysis. The Dox-vesicle mixture (5.0 mL) was dialyzed against 200 mL of 0.9% saline buffer at pH 7.5 for 15 h to remove excess Dox from the solution. The final Dox concentration in the vesicle solution after dialysis was 0.136 g/L as determined by visible absorption spectrophotometry using the calibration curve procedure described above.

A control solution without any PMPC-*b*-PDPA diblock copolymer was prepared by simply adding 0.137 mL of an 18.25 g/L Dox solution to 5.0 mL of water. This sample was not dialyzed prior to elution. The elution experiments were carried out immediately after the Dox-vesicle samples were dialyzed. 3.0 mL of Dox solution was added to the dialysis tubing either in the presence or absence of the aqueous vesicle solution and dialyzed against 50 mL of 0.9 % saline buffer at pH 7.5 and 20^{o}C. The elution bottle was kept agitated using an IKA® KS 260 Basic Shaker at 100 rpm. After suitable time intervals of typically 30-60 minutes, 5.0 mL of saline was periodically removed to determine the Dox concentration at 483 nm by visible absorption spectrophotometry using the calibration curve shown in Figure S13. This extract was replaced by the addition of 5.0 mL of fresh saline and the new Dox concentration was recalculated. After certain time intervals, the entire 50.0 mL volume of

saline in the external solution (i.e. outside the dialysis tubing) was removed and replaced by the same volume of fresh saline so as to maintain the chemical potential gradient for inter-membrane transport of the Dox.

Figure S13. Calibration curves of Dox determined in aqueous saline solution at pH 2.0 and pH 7.5 as measured at 483 nm by visible absorption spectrophotometry.



(1) Robinson, K. L.; Weaver, J. V. M.; Armes, S. P.; Diaz Marti, E.; Meldrum, F. C. *J. Mater. Chem.* **2002**, *12*, 890.