Supporting Information

pH-Responsive Vesicles Based on A Hydrolytically Self-Crosslinkable Copolymer

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Figures S1-S9 and Experimental Section

Figure S1 GPC traces of PEO_{43}-*b*-P(DEA_x-*stat*-$TMSPMA_y$) copolymers and the initial PEO-Br macro-initiator.

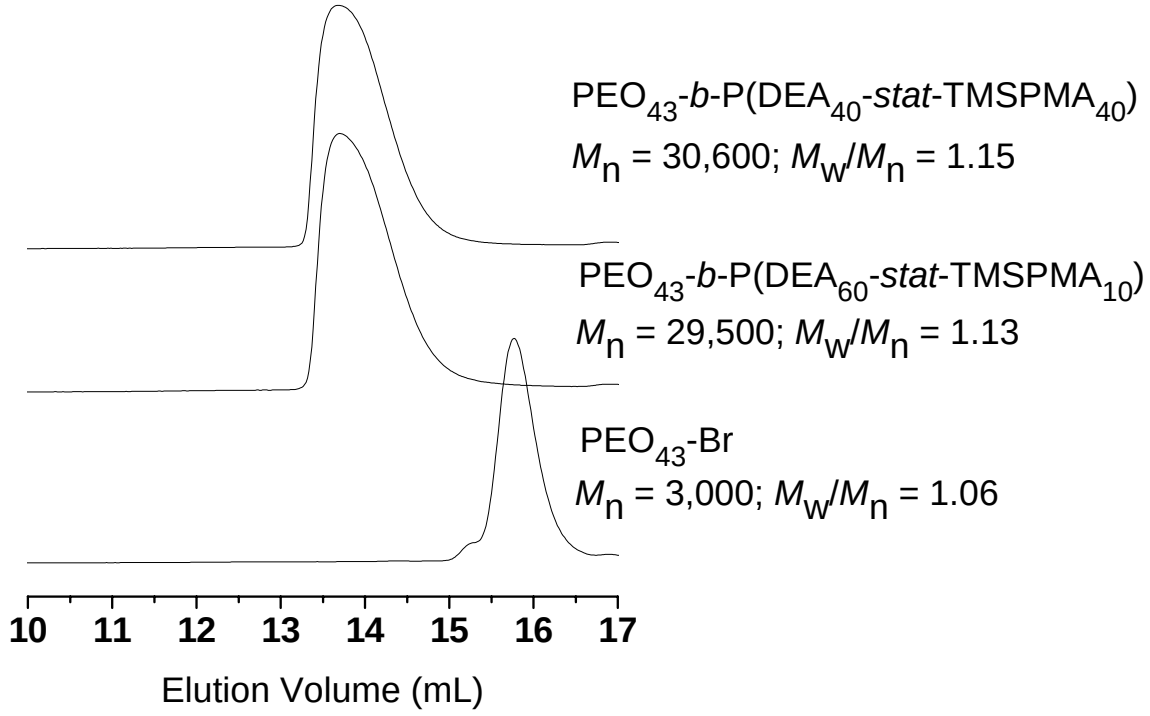


Figure S2 (See overleaf) Assigned ^1H NMR spectra of PEO_{43}-*b*-P[DEA_{40}-*stat*-$TMSPMA_{40}$] (40 mg) in (A) $CDCl_3$ (1.0 mL); (B) d_8-THF, 1.0 mL); (C) after adding 2.0 mL of D_2O into 1.0 mL of d_8-THF for 37 min. (note the appearance of a CH_3OD signal due to the hydrolysis of trimethoxysilyl groups); (D) after a reaction time of 122 h (note that the peak of CH_3OD becomes sharper); (E) vesicles at pH 2, note the appearance of the weak, broadened peak assigned to the DEA residues with in the vesicle wall at around 1.05 ppm. Addition of DCl led to protonation of the DEA residues within the vesicle walls, which increased the vesicle permeability (compare spectra D and E).

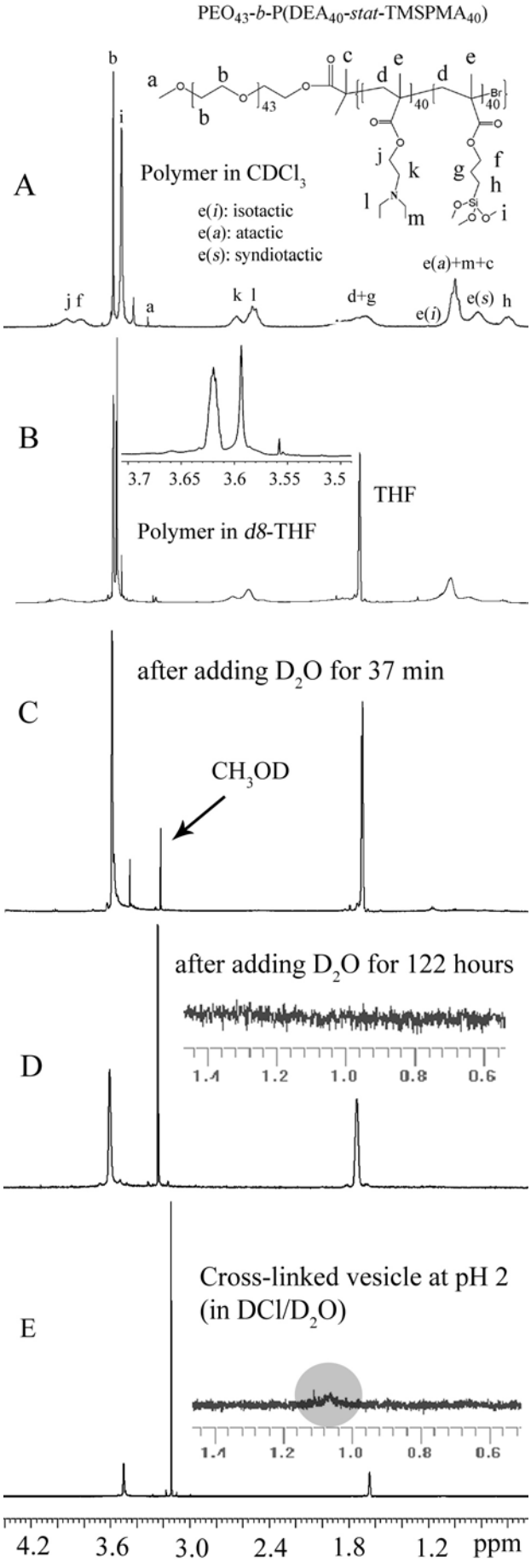

PEO43-b-P(DEA40-stat-TMSPMA40)
A
Polymer in CDCl3
e(i): isotactic
e(a): atactic
e(s): syndiotactic
B
Polymer in d8-THF
THF
C
after adding D2O for 37 min
CH3OD
D
after adding D2O for 122 hours
E
Cross-linked vesicle at pH 2
(in DCl/D2O)
4.2
3.6
3.0
2.4
1.8
1.2
ppm

Figure S3 (A) Kinetic plots for the hydrolysis of the 3-(trimethoxysilyl)propyl methacrylate residues within the walls of vesicles formed by a PEO_{43}-*b*-P(DEA_{40}-*stat*-$TMSPMA_{40}$) copolymer at a 40.0 g/L of C_{ini} in a THF/water solution at 20 ^{o}C and pH 7. (B) Corresponding conversion vs. time plots for the hydrolysis of $–OCH_3$ groups to release CH_3OD.

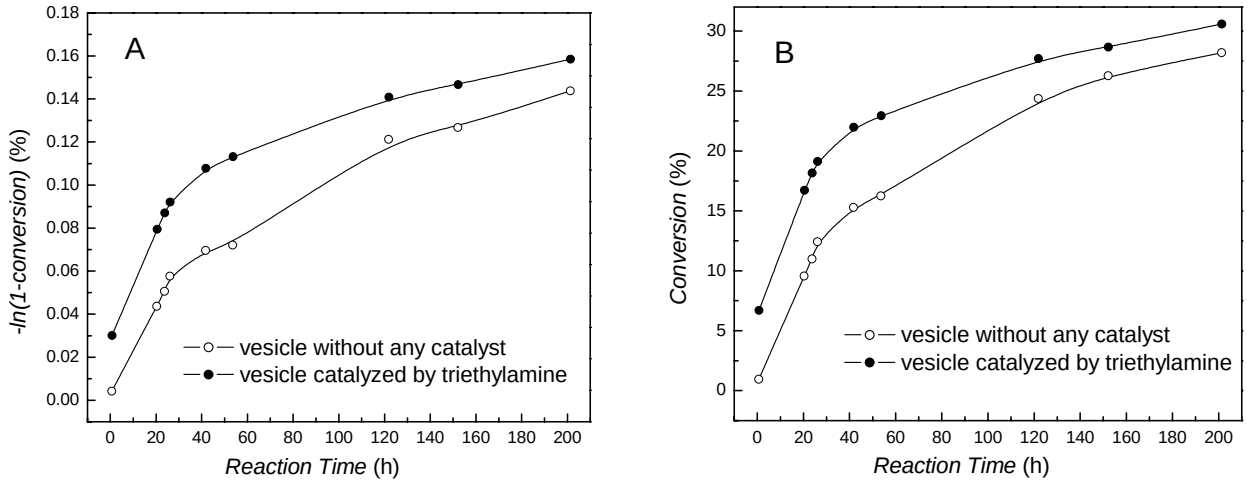


Figure S4. TEM images of vesicles prepared without any triethylamine (TEA) catalyst. The corresponding TEM image of vesicles prepared in the presence of TEA is shown in Figure 1A.

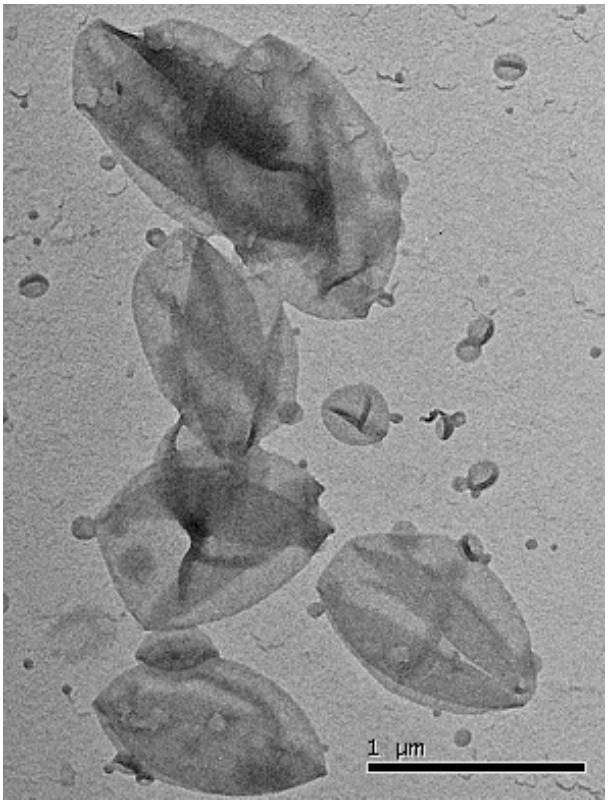


Figure S5 TEM images of vesicles prepared without any added catalyst. The vesicles were examined by TEM after a PEO_{43}-*b*-P(DEA_{40}-*stat*-$TMSPMA_{40}$) copolymer solution in 1:2 THF: water was stirred for 10 h at 20 ^{o}C. The initial copolymer concentration in THF prior to dilution with water at pH 7 was 120 g/L. Note the existence of some elongated vesicles due to increasing the initial copolymer concentration in THF.

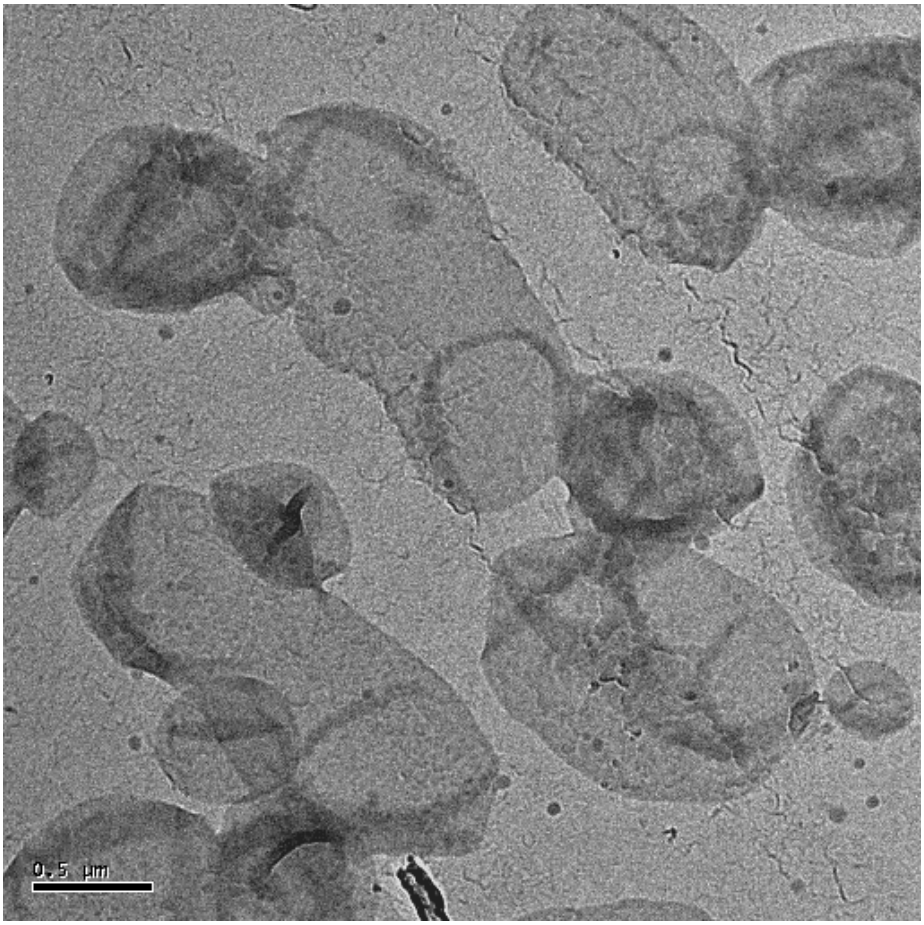
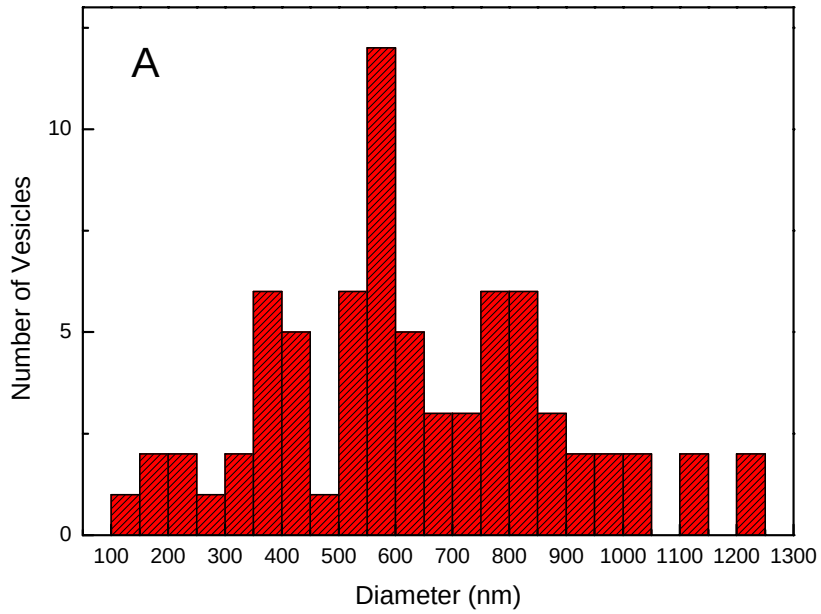


Figure S6 Vesicle particle size distributions: (A) calculated from 74 vesicle images shown in Figure 1A, the mean diameter is 630 ± 250 nm; (B) calculated from 100 vesicles prepared at an initial copolymer concentration of 10.0 g/L and a water content of 52.9 wt. %. In both cases the copolymer was PEO_{43}-*b*-P(DEA_{40}-*stat*-$TMSPMA_{40}$). The mean vesicle diameter was 260 ± 80 nm.



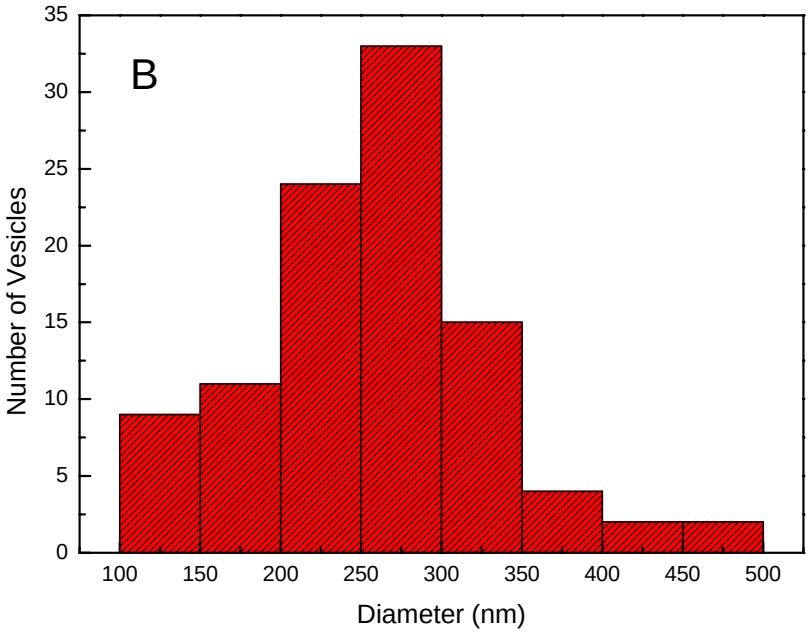


Figure S7 Low magnification TEM image of vesicles prepared from PEO_{43}-*b*-P[DEA_{40}-*stat*-$TMSPMA_{40}$] copolymer decorated with gold nanoparticles under the same conditions as those shown in Figure 1B). The vesicles before decoration are the same as those shown in Figure 1A. Note that this image supports the hypothesis that the gold nanoparticles are only formed *within* the vesicle walls, and not in the surrounding aqueous solution.

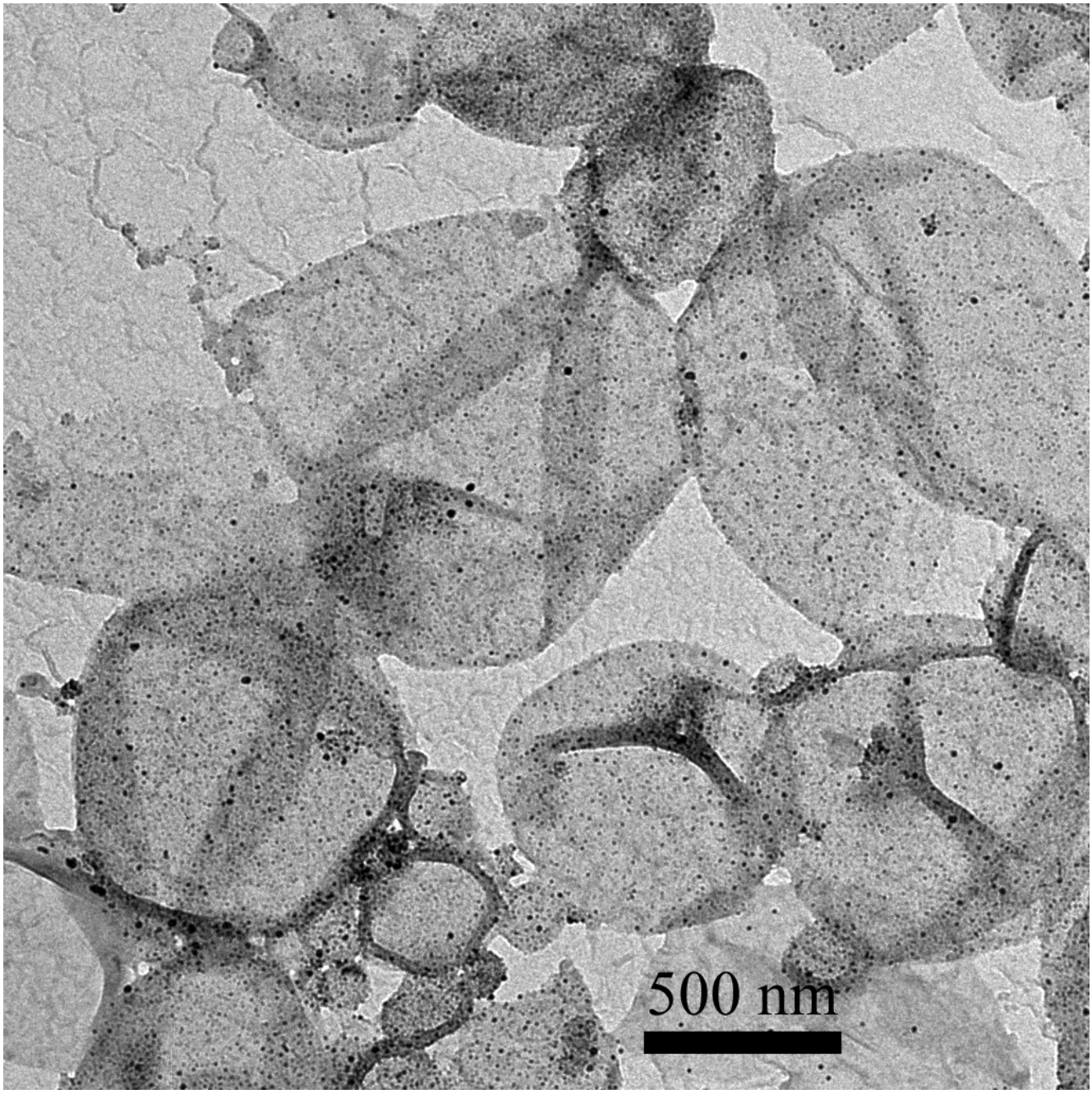


Figure S8　A high magnification TEM image of a gold nanoparticle-decorated vesicle wall of a single vesicle. This image was recorded from the same vesicles as those shown in Figure S7.

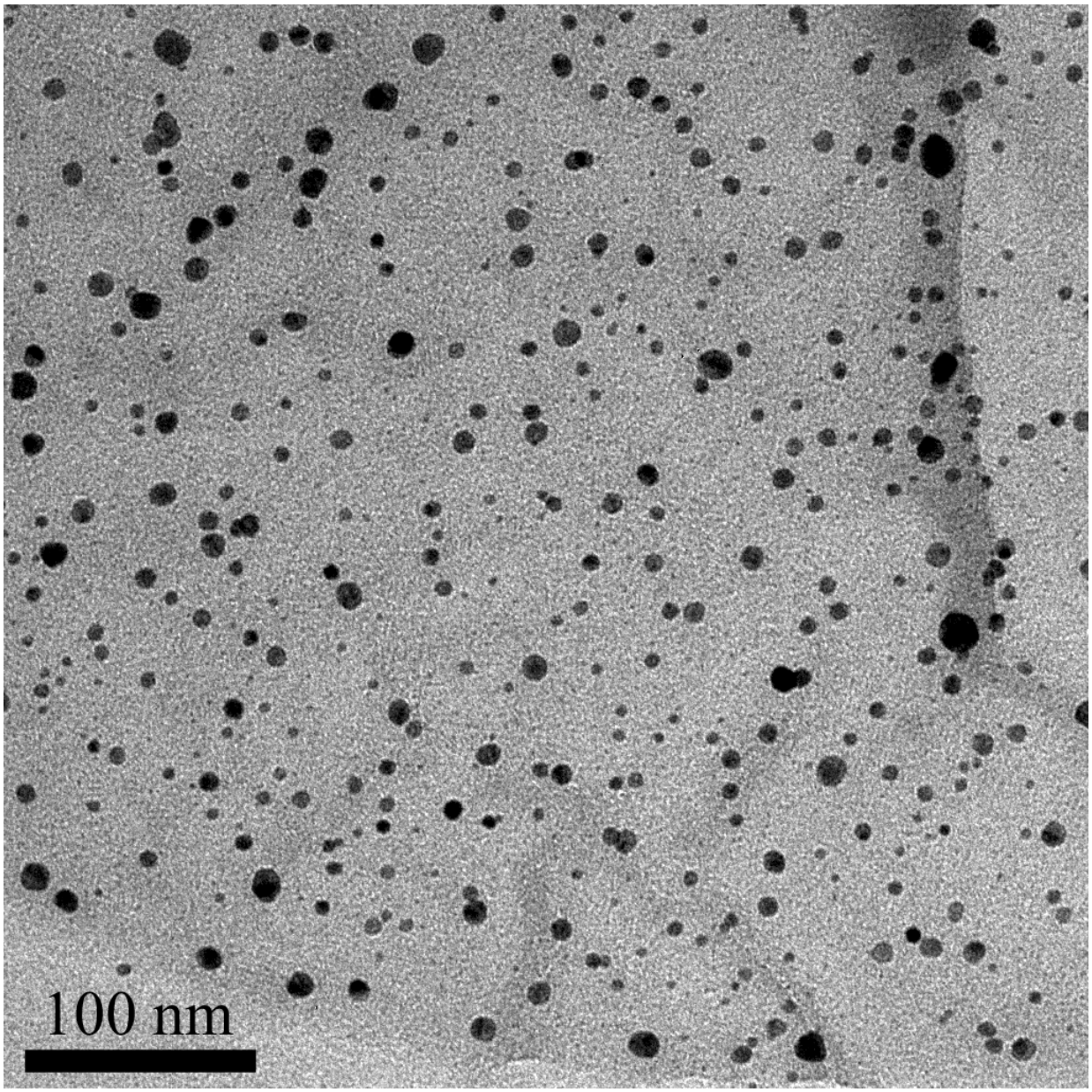
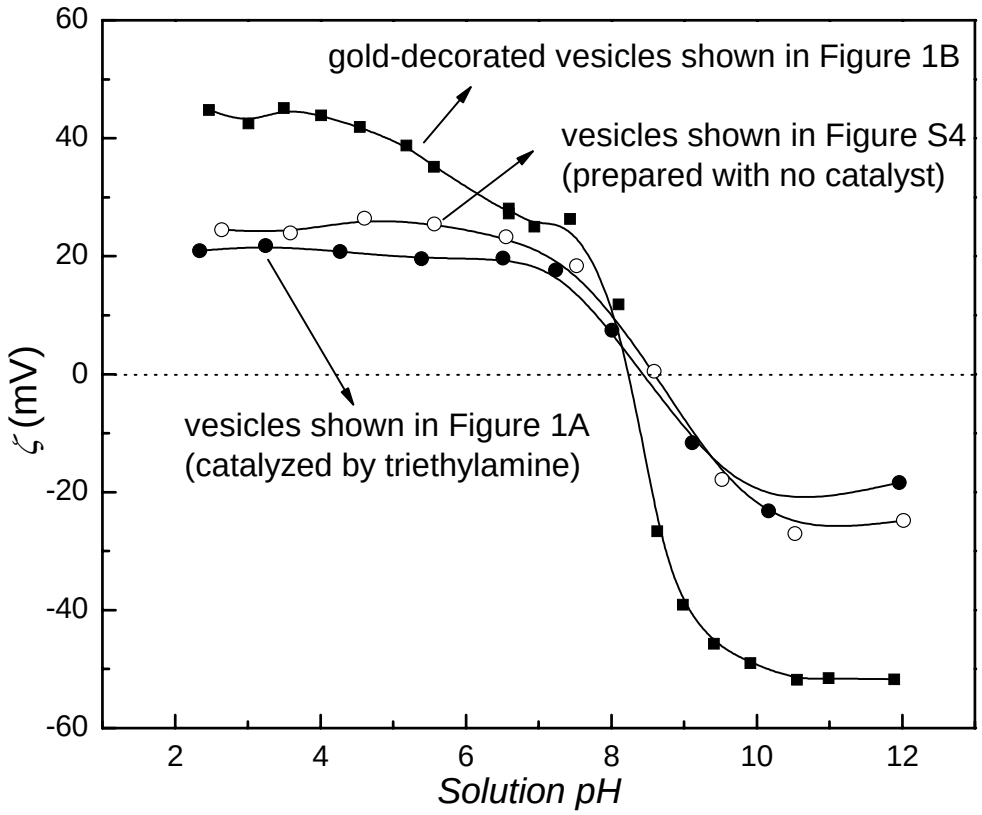


Figure S9 Aqueous electrophoresis (zeta potential) data obtained for vesicles prepared from a PEO_{43}-*b*-P[DEA_{40}-*stat*-$TMSPMA_{40}$] diblock copolymer as a function of pH.



Experimental Section

Materials. Both TMSPMA and DEA monomer were purchased from Aldrich, dried over CaH_2 overnight, and distilled under reduced pressure prior to use. Anhydrous methanol was purchased from Aldrich and refluxed over magnesium. Anhydrous tetrahydrofuran (THF; Aldrich) was dried by refluxing in the presence of sodium and distilled prior to use. $CDCl_3$ was dried over CaH_2 and distilled prior to use. Poly(ethylene oxide) methyl ether (MeO-PEO-OH; M_n ca. 1,900; M_w/M_n = 1.10; purchased from Alfa) was dried azeotropically using anhydrous toluene to remove water. Triethylamine, Cu(I)Br, 2,2'-bipyridine (bpy), 2-bromoisobutyryl bromide and other reagents were purchased from Aldrich and used as received.

Characterization. The molecular weight distributions of the copolymers were assessed at 30 °C using a Polymer Laboratories PL-GPC50 Integrated GPC system equipped with a Polymer Laboratories pump, a PLgel 5μm MIXED-C column (300 × 7.5 mm), a WellChrom K-2301 refractive index detector, a viscometry detector and a PD 2020 light scattering detector. The calibration was carried out using six poly(methyl methacrylate) standards with M_p values ranging from 1,310 to 211,400. The eluent was THF and the flow rate was 1.0 mL/min. The data were processed using Cirrus GPC offline GPC/SEC software (version 2.0).

^{1}H NMR spectra were recorded using Bruker DRX250 (250 MHz) or DRX 500 (500 MHz) spectrometers, with $CDCl_3$ (dried with CaH_2 and distilled prior to use), CD_3OD, d_8-THF and D_2O/CD_3OD, D_2O/d_8-THF, $D_2O/$ d_8-THF/DCl, as solvents at room temperature. The copolymer compositions were determined from ^{1}H NMR spectra in dry $CDCl_3$. The rate of hydrolysis was studied by monitoring the increase in the peak integral due to the CH_3OD product, using the integrated signal assigned to the PEO block as an internal standard.

TEM images were obtained using a Philips CM100 electron microscope operating at 100 kV and equipped with a LaB6 gun and a Gatan 1K x 1K digital camera. To prepare TEM samples, 5 μL of diluted vesicle solution was dropped onto a carbon-coated copper grid, and the water droplet was allowed to evaporate slowly under ambient conditions.

DLS studies of 0.02 g/L aqueous vesicle solutions were carried out over a range of pH using 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 angle was fixed at 90°. The data were processed by cumulant analysis of the experimental correlation function and analyzed using the Stokes-Einstein equation, assuming that the vesicles could be treated as dilute, non-interacting hard spheres.

Zeta potential studies were conducted at 25 °C using a ZETASIZER Nano series instrument (Malvern Instruments) equipped with a multi-purpose autotitrator (MPT-2) and Dispersion Technology Software (version 4.00).

Fluorescent experiments were carried out to assess the pH-sensitive permeability of the vesicle walls using a commercial stopped-flow apparatus (SFM-300; Bio Logic Science Instruments), which comprised a fluorescent detector, an ALX-220 Arc lamp power supply, a PMS-250 output filter, an MPS-60 microprocessor unit, and an MOS-450 unit. The data were processed using the Bio-Kine 32 software (version 4.25). For more detailed information about this instrument please see the www.bio-logic.info website. The general protocol for this experiment was as follows. The vesicle solution used in this experiment was diluted from a vesicle solution under the following conditions: The C_{ini} of PEO_{43}-*b*-P(DEA_{40}-*stat*-$TMSPMA_{40}$) copolymer in THF was 120 mg/mL and the final volume ratio of THF: water was 1:2. Triethylamine (0.1 wt. %) was added after 1 h of vesicle formation. Three syringes were set up, containing 10 mL aqueous vesicle solution (0.50 g/L; 3.6 x 10^{-4} mol copolymer/L solution) in the R1 position, 10 mL pure water in the R2 position, and 10 mL aqueous Rhodamine B solution (1.0 x 10^{-3} mol/L) in the R3 position. First just the vesicle solution alone was evaluated; these experiments yielded a horizontal straight line around $I/I_0 = 0$, which indicated no fluorescence decay occurred on the time scale of the measurements. Second, the solutions in the R3 (Rhodamine B) and R2 (pure water at pH 2, 7 or 12) positions were mixed (at 3:1 and also at other ratios) in a series of control experiments. Again, only horizontal straight lines were obtained and no significant decrease in fluorescence intensity was observed. Finally, after careful cleaning, the R1 (vesicle solution) and R3 (Rhodamine B) positions were selected so as to mix these solutions. The mixing ratio of vesicle solution to Rhodamine B solution was chosen to be 3:1, which is equivalent to a 1:1 molar ratio of copolymer to dye molecule. Other mixing ratios were also evaluated and in all cases about 700 µL of mixed solution was maintained within the cell. The total flow rate was set at 7 mL/s and the temperature was held constant at 24.0 °C. The kinetics of permeation of the Rhodamine B dye through the vesicle membrane walls were monitored by recording the fluorescence intensity ratio, I/I_0, vs. time.

Syntheses of PEO-Br Macro-initiators. The PEO-Br macro-initiator was prepared by the reaction of MeO-PEO-OH with excess 2-bromoisobutyryl bromide in the presence of triethylamine. First, MeO-PEO-OH (20.00 g, 0.0105 mol) was dissolved in 100 mL toluene at 80 ^{o}C. After azeotropic distillation (to remove traces of water), anhydrous THF (250 mL) was added into the reaction flask, followed by triethylamine (2.75 mL, 0.020 mol). 2-Bromoisobutyryl bromide (2.50 mL, 0.020 mol) dissolved in anhydrous THF (50 mL) was finally added dropwise

over 2 h to the solution at 20 ^{o}C. The solution was stirred for two days and then treated with activated charcoal. After filtration, the filtrate was collected and the solvent was removed by rotary evaporation. The crude product was dissolved in dichloromethane and then poured into water with vigorous stirring for 1 h. The organic phase was collected and the water phase was extracted twice using dichloromethane. The combined organic solution was further washed with 1.0 M HCl and 1.0 M NaOH successively, dried over anhydrous $MgSO_4$ and the solvent was then removed using a rotary evaporator. The product was dissolved in 10 mL of dichloromethane and precipitated into 500 mL diethyl ether. This dissolution/precipitation cycle was repeated two more times. Yield: ~80%. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.93 (s, 6H, $BrC(CH_3)_2$), δ 3.38 (s, 3H, OCH_3), δ 3.64 (s, 4H, OCH_2).

Copolymerization of TMSPMA and DEA by ATRP. A literature method reported by Du and Chen was modified as follows.[1] In a typical synthesis, a Schlenk flask with a magnetic stir bar and a rubber septum was charged with Cu(I)Br (35.7 mg, 0.250 mmol), PEO_{43}-Br macro-initiator (0.50 g, 0.250 mmol) and bpy (78.1 mg, 0.500 mmol) and deoxygenated three times. Deoxygenated TMSPMA (2.43 mL, 10.2 mmol), DEA (2.03 mL, 10.1 mmol), and methanol (5 mL) were added to this flask. The statistical copolymerization was carried out at 20 °C for 48 h. Finally, the diblock copolymer solution was transferred to a silica column by syringe to remove the spent ATRP catalyst.

Preparation of Vesicles. To prepare the copolymer vesicles, 2.0 mL water was added dropwise to 1.0 mL of copolymer solution in THF over a 10 min. period by syringe with continuous stirring. In some cases approximately 3.0 μL of triethylamine catalyst was added to the copolymer solution to accelerate the hydrolytic cross-linking reaction after vesicles had been formed for 1 h. However, the addition of triethylamine was not essential for vesicle preparation.

Loading Gold Nanoparticles into Vesicle Walls. The diluted cross-linked aqueous vesicle solutions prepared from PEO_{43}-*b*-P(DEA_{40}-*stat*-$TMSPMA_{40}$) (light blue appearance) were mixed with aqueous $HAuCl_4$ at various $HAuCl_4$ / DEA molar ratios (ranging from 1:20 to 1:2). The color of the copolymer solution ranged from light yellow (Au: N atomic ratio = 1:20) to yellow (Au: N atomic ratio =1:2), depending on the amount of added $HAuCl_4$. After stirring the vesicle solution for 0.50 h, an aqueous solution of sodium borohydride

($NaBH_4/HAuCl_4$ molar ratio = 1.0) was added. The solution immediately became either light wine red (if originally light yellow), or wine red (if originally yellow), indicating the reduction of the Au(III) anions to Au(0). Black deposits of *precipitated* gold were observed if the $HAuCl_4$ / DEA molar ratio exceeded 1:2.

(1) Du, J. Z.; Chen, Y. M. *Macromolecules* **2004**, *37*, 6322-6328.