

Supporting Information

Corona-Cross-Linked Polymer Vesicles Displaying a Large and Reversible Temperature-Responsive Volume Transition

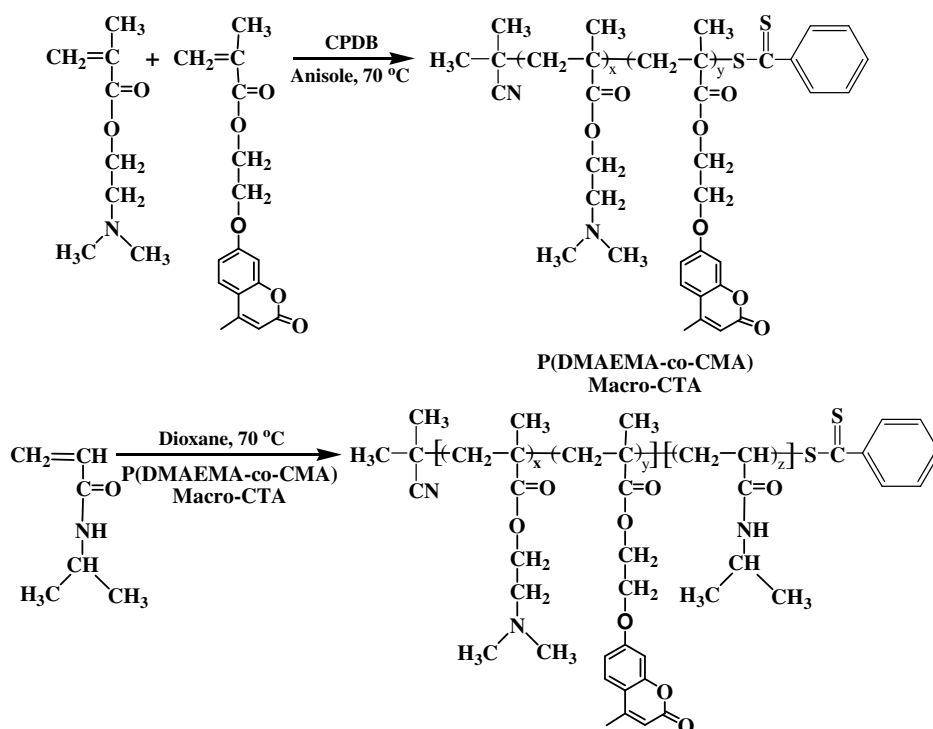
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1. Synthesis of Diblock Copolymers

1.1 Materials

All chemicals were purchased from Aldrich and used as received unless otherwise noted. N,N-dimethylaminoethyl methacrylate (DMAEMA, 98%) was passed through a basic aluminum oxide column and distilled under vacuum prior to use. N-isopropylacrylamide (NIPAM, 97%) was recrystallized from hexane. 2, 2'-Azobis(isobutyronitrile) (AIBN) was recrystallized twice from ethanol. 4-Methyl-[7-(methacryloyl)oxy]ethyl]oxy]coumarin (coumarin methacrylate, CMA) was synthesized using a method previously reported.¹ The chain transfer agent (CTA), 2-(2-cyanopropyl) dithiobenzoate (CPDB), was synthesized using a method in the literature.²



Scheme S1. Synthetic route to the diblock copolymer of P(DMAEMA-*co*-CMA)-*b*-PNIPAM.

1.2 Synthesis of P(DMAEMA-*co*-CMA) macro-chain transfer agent

The general procedure for RAFT polymerization followed previous reports.³⁻⁵ Using the sample of P(DMAEMA₄₉-*co*-CMA₃) as an example, the synthetic procedure is as follows. DMAEMA (3.14 g, 20 mmol), CMA (0.29 g, 1 mmol), CPDB (88 mg, 0.4 mmol) and AIBN (13 mg, 0.08 mmol) were dissolved in 4 mL anisole (99%, anhydride) in a 10 mL flask. The reaction mixture was degassed under vacuum for 10 min and refilled with nitrogen. The flask was placed in a pre-heated oil bath at 70 °C for 5 h. After polymerization, the solution was cooled down to room temperature. The polymer was precipitated in hexane three times and dried under vacuum for 24 h. From GPC measurements using polystyrene (PS) standards, the polymer sample has a M_n of 8800 g/mol and a polydispersity index (M_w/M_n) PDI= 1.09. From the ¹H NMR spectrum (in CDCl₃), the integrals of the resonance peaks of aromatic ring in CPDB (7.85 ppm), the side methylene groups of CMA and DMAEMA (at 4.3 and 4.0 ppm, respectively) yielded a NMR-based M_n =8200 g/mol and the estimated composition with 49 units of DMAEMA and 3 units of CMA.

1.3 Synthesis of the diblock copolymer P(DMAEMA-*co*-CMA)-*b*-PNIPAM

The synthetic route to the diblock copolymer is shown in Scheme 1. Using the sample of P(DMAEMA₄₉-*co*-CMA₃)-*b*-PNIPAM₇₄ as an example, the diblock copolymer synthesis was carried out using the following procedure. Macro-CTA of P(DMAEMA₄₉-*co*-CMA₃) (210 mg, 0.025 mmol), NIPAM (560 mg, 5 mmol) and AIBN (1 mg, 0.006 mmol) were dissolved in 2 mL of dioxane (99%, anhydride) in a 10 mL flask. The reaction mixture was degassed under vacuum for 10 min and refilled with nitrogen. The flask was then placed in a pre-heated oil bath at 70 °C for 1 h. The polymer was purified by precipitation in a mixture of ethyl ether and hexane (1/3, v/v) three times and dried under vacuum for 24 h. From GPC, the diblock copolymer has a M_n of 18400 g/mol and PDI= 1.18. The diblock copolymer composition was calculated from the ¹H NMR spectrum; the number of NIPAM units in the sample was estimated to be 74.

1.4 Synthesis of other block copolymers

A number of other block copolymers were synthesized using the same procedure (synthetic details not repeated here). These include a diblock copolymer whose PNIPAM block bears coumarin side groups and a sample whose PDMAEMA and PNIPAM blocks both contain coumarin side groups. These samples could be used to prepare wall-cross-linked vesicles as well as vesicles with both corona and wall cross-linked. The characteristics of all synthesized block copolymers are summarized in Table S1. An example of GPC curves and ¹H NMR spectra are shown in Fig. S1.

Table S1. Characteristics of synthesized block copolymers

Sample	$M_{n, GPC}$ (g/mol) <i>a</i>	$M_{n, NMR}$ (g/mol) <i>b</i>	PDI	Mole fraction of CMA in PDMAEMA	Mole fraction of CMA in PNIPAM
PDMAEMA ₄₇	8.1 k	7.4 k	1.08	0	0
P(DMAEMA ₄₉ - <i>co</i> -CMA ₃)	8.8 k	8.2 k	1.09	5.7%	0
PDMAEMA ₄₇ - <i>b</i> -PNIPAM ₇₅	18.7 k	15.9 k	1.16	0	0
PDMAEMA ₄₇ - <i>b</i> -P(NIPAM ₇₉ - <i>co</i> -CMA ₄)	24.3 k	17.5 k	1.18	0	5.2%
P(DMAEMA ₄₉ - <i>co</i> -CMA ₃)- <i>b</i> -PNIPAM ₇₄	18.4 k	16.7 k	1.18	5.7%	0
P(DMAEMA ₄₉ - <i>co</i> -CMA ₃)- <i>b</i> -P(NIPAM ₈₀ - <i>co</i> -CMA ₄)	19.7 k	18.7 k	1.17	5.7%	5.1%

^a Determined by GPC using polystyrene standards for calibration. ^b Molecular weight and block copolymer compositions calculated from ¹H NMR spectra in CDCl₃.

1.5 Preparation of vesicles and their photo-cross-linking

A sample of P(DMAEMA_{49-co}-CMA₃)-*b*-PNIPAM₇₄ (2 mg) was dissolved in distilled water (10 mL) at T < LCST of PNIPAM (~ 5 °C) for overnight. The solution was then filtered with a 200-nm pore filter (from Whatman). To form the vesicles, the solution was heated to 40 °C (>LCST of PNIPAM) and equilibrated for 10 min. The vesicle size was measured by DLS. For the photo-cross-linking reaction of coumarin, a vesicle solution (4 mL) was exposed to UV light of $\lambda > 310$ nm (~ 500 mW/cm² measured at 320 nm) for 15 min to obtain corona-crosslinked vesicles. Despite the large size, the dispersion of vesicles was stable for 2-3 days, and the aggregation occurred after longer times could easily be re-dispersed by stirring.

2. Characterizations

Gel permeation chromatography (GPC) measurements were performed on a Waters system equipped with a refractive index detector (RI 410) and a photodiode array detector (PDA 996). THF was used as the eluent at an elution rate of 1 mL min⁻¹, and polystyrene standards were used for calibration. ¹H NMR spectra were obtained with a Bruker spectrometer (300 MHz, AC 300). To characterize the thermo-responsive behavior, a Varian spectrometer (600 MHz, INOVA system) was used to record the spectra at different temperature. The photo-cross-linking of coumarin side groups was obtained by using a UV-vis spot curing system (Novacure) with a 320-500 nm filter generating UV light at $\lambda > 310$ nm (intensity at 320 nm is ~ 500 mW cm⁻²). For the photo-cleavage of cyclobutane rings (photo-de-cross-linking), a UV-C Air sterilizer lamp (1.25 W) peaked at $\lambda = 254$ nm was used at a distance of 5 cm to the solution. UV-vis spectra were taken using a spectrophotometer (Varian 50 Bio). Dynamic light scattering (DLS) experiments were carried out on a Brookhaven goniometer (BI-200) equipped with a highly sensitive avalanche photodiode detector (Brookhaven, BI-APD), a digital correlator (Brookhaven, TurboCorr) that calculates the photon intensity autocorrelation function $g^2(t)$, a helium-neon laser ($\lambda = 632.8$ nm), and a thermostat sample holder. The apparent hydrodynamic diameter (D_H) of vesicular aggregates was obtained by Cumulants and CONTIN analyses, and the light scattering intensity was measured at 90°. All initial solutions at the molecularly dissolved state were filtered with a 200-nm pore filter and, before the DLS measurement at each temperature, the solution was equilibrated for 10 min. Each DLS measurement was performed five times to calculate the average hydrodynamic diameter. The vesicle solution after cross-linking was separated by centrifugation using a Hettich D-78532 centrifuge at 6000 rpm for 60 min. The sizes of vesicles were examined using a Hitachi H-7500 transmission electron microscope (TEM) operating at 60 kV. Samples for TEM were prepared by casting 3-5 μ L of the vesicle solution on a carbon-coated copper grid, followed by drying at a predetermined temperature. In some cases (when mentioned in the text), TEM samples were stained with phosphotungstic acid (0.5 wt% aqueous solution) to increase the contrast. The encapsulation of a hydrophobic dye, Nile Red (NR), by the vesicles was investigated by using a fluorescence microscope (Leica DMRX). It was equipped with a filter for 515-560 nm excitation. The fluorescence microscope could also be used as a normal optical microscope for reflection observations. The steady-state fluorescence emission spectra of the vesicle solution equilibrated with NR LC were recorded using a fluorescence spectrophotometer (Varian Cary Eclipse) equipped with a single cell Peltier for controlled-temperature measurements.

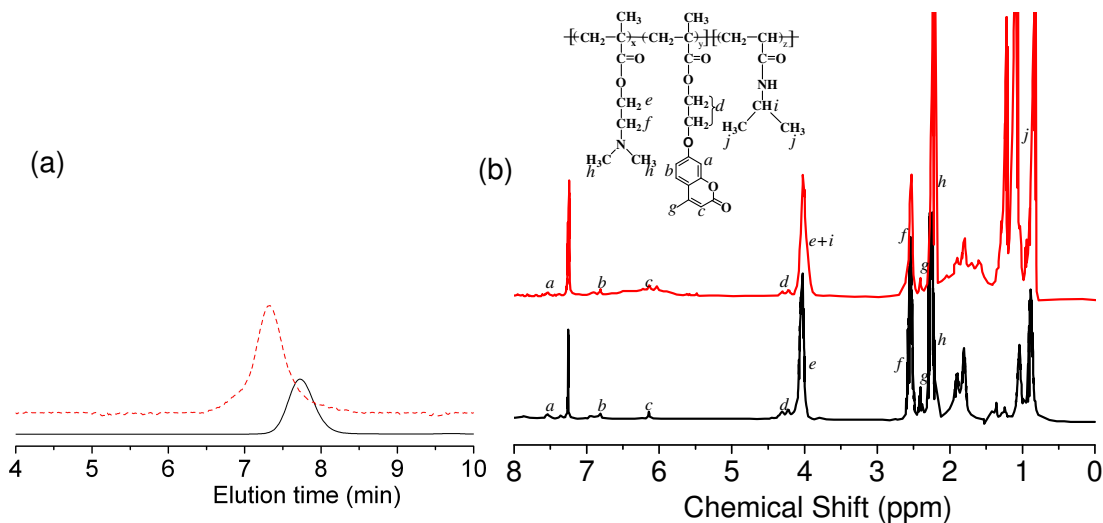


Figure S1. (a) GPC traces and (b) ¹H NMR spectra in CDCl₃ of the macro-chain transfer agent P(DMAEMA₄₉-co-CMA₃) (bottom, black) and the diblock copolymer P(DMAEMA₄₉-co-CMA₃)-b-PNIPAM₇₄ (top, red).

3. Thermo-responsive Behavior of the Diblock Copolymer in Aqueous Solution

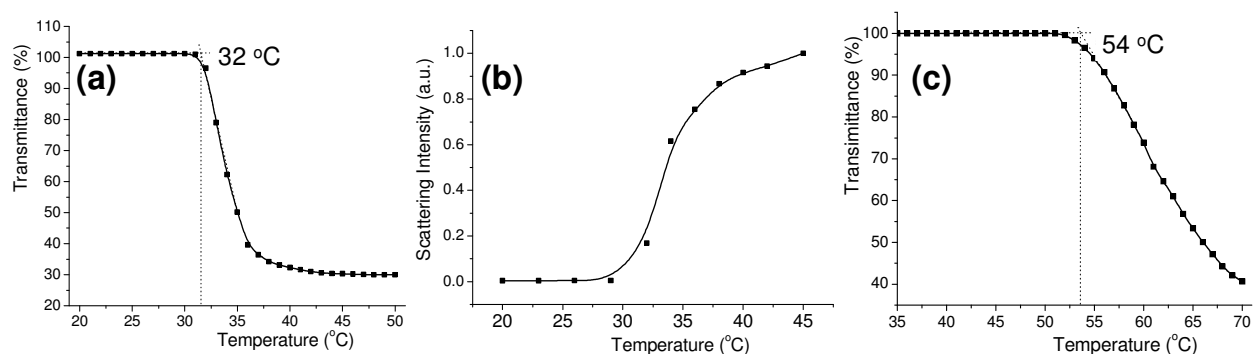


Figure S2. (a) Plot of transmittance (550 nm) vs. temperature for P(DMAEMA₄₉-co-CMA₃)-b-PNIPAM₇₄, (b) plot of light scattering intensity (measured at 90°) vs. temperature for P(DMAEMA₄₉-co-CMA₃)-b-PNIPAM₇₄, and (c) plot of transmittance vs. temperature for P(DMAEMA₄₉-co-CMA₃). All measurements were made with a polymer concentration of 0.2 mg mL⁻¹ and a heating rate of 0.5 °C/min. The LCST of the diblock copolymer is that of the PNIPAM block, while the rise in scattering intensity is due to the formation of vesicles. The P(DMAEMA₄₉-co-CMA₃) block shows a LCST at about 54 °C.

4. Variable-Temperature ¹H NMR and UV-Vis Spectra

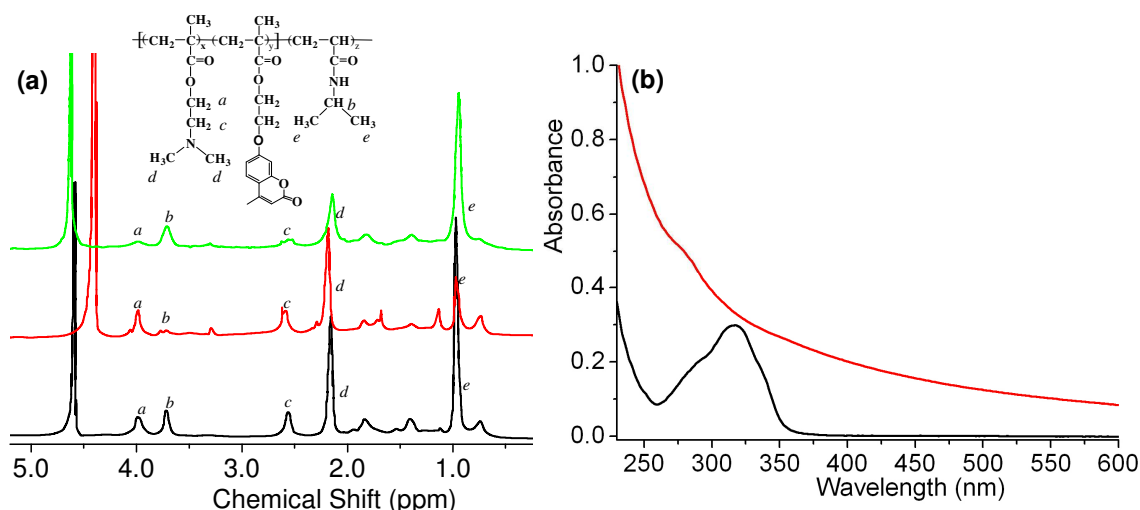


Figure S3. Shown in (a) are the ^1H NMR spectra of an aqueous solution of $\text{P}(\text{DMAEMA}_{49}\text{-co-CMA}_3)\text{-b-PNIPAM}_{74}$ at $20\text{ }^\circ\text{C}$ (initial solution) (bottom, black), at $40\text{ }^\circ\text{C}$ (middle, red), and at $20\text{ }^\circ\text{C}$ after exposure to UV light for cross-linking $40\text{ }^\circ\text{C}$ (top, green). The dehydration of PNIPAM at $40\text{ }^\circ\text{C}$, forming the vesicle membrane, is indicated by the reduced intensity of its resonance signals with respect to the signals of $\text{P}(\text{DMAEMA-co-CMA})$ (peaks *b* and *e* with respect to peaks *d*, in particular). When cooled back to $20\text{ }^\circ\text{C}$, PNIPAM chains are re-dissolved leading to the recovery of the resonance peaks. It is also clear that the photo-cross-linking reduced the intensity of peaks of $\text{P}(\text{DMAEMA-co-CMA})$ block. This suggests that the formation of coumarin dimers may bring down the LCST of this random copolymer, and that cross-linked $\text{P}(\text{DMAEMA-co-CMA})$ chains were also in an aggregated state at $40\text{ }^\circ\text{C}$. **The spectral differences as compared to Figure S1 (b) could be attributed to the use of different solvents (ref.5).** The effective photo-cross-linking in the solution can be seen from the UV-Vis spectra in (b). The initial solution at $20\text{ }^\circ\text{C}$ (black line) displays the absorption band of coumarin at $\sim 320\text{ nm}$. After exposure to UV light at $40\text{ }^\circ\text{C}$, the solution became highly turbid and the baseline went beyond the detection limit (spectrum not shown). After cooling back to $20\text{ }^\circ\text{C}$ (red line), the spectrum shows no absorption band of coumarin, indicating the complete photo-dimerization reaction; while the shape of the baseline is signature of wavelength-dependant light scattering, indicating the preservation of aggregates due to the cross-linking.

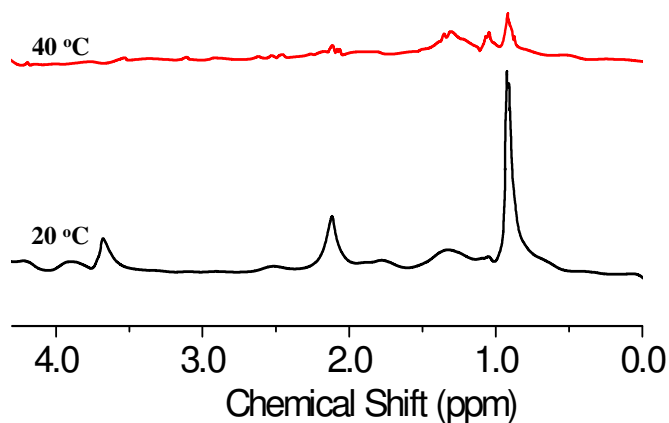


Figure S4. ^1H NMR spectra (in D_2O) of the vesicles collected by centrifugation. Both PNIPAM and cross-linked $\text{P}(\text{DMAEMA-co-CMA})$ chains are hydrated at $20\text{ }^\circ\text{C}$ (vesicle expansion) and dehydrated at

40 °C (vesicle contraction). We note that no spectral region of ppm>5 was shown in Figs. S3 and S4 because no coumarin signals could be detected in aqueous solution. This suggests that even with hydrated polymer chains, coumarin side groups could be in an aggregated state due to strong hydrophobic interactions.

5. More TEM Images

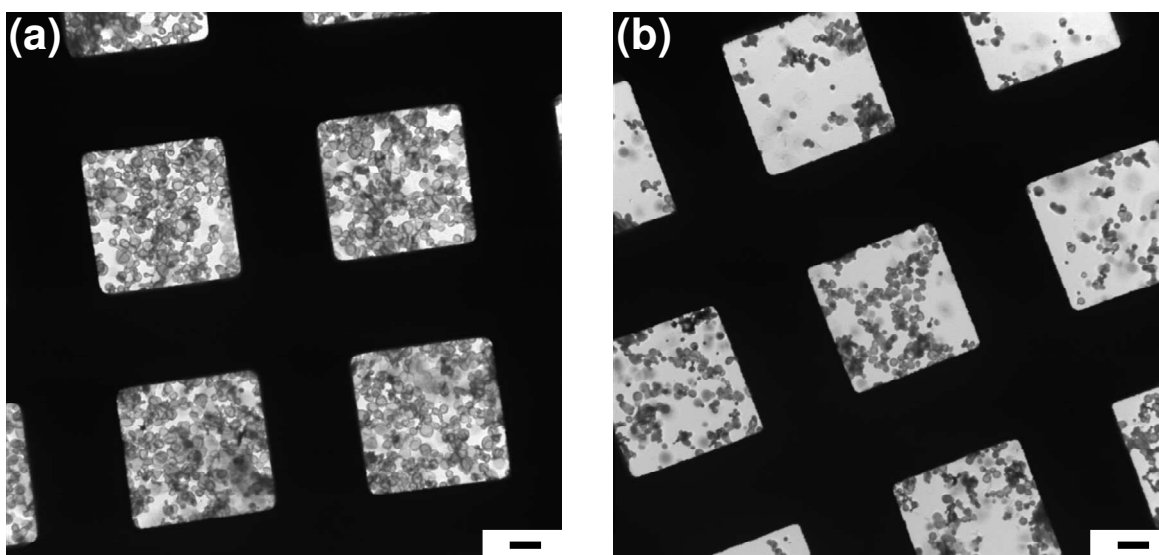


Figure S5. Unstained low-magnification (700 \times) TEM images of P(DMAEMA₄₉-*co*-CMA₃)-*b*-PNIPAM₇₄ vesicles deposited on a grid at 20 °C (a) and 40 °C (b), showing the large size difference. The scale bars are 10 μ m.

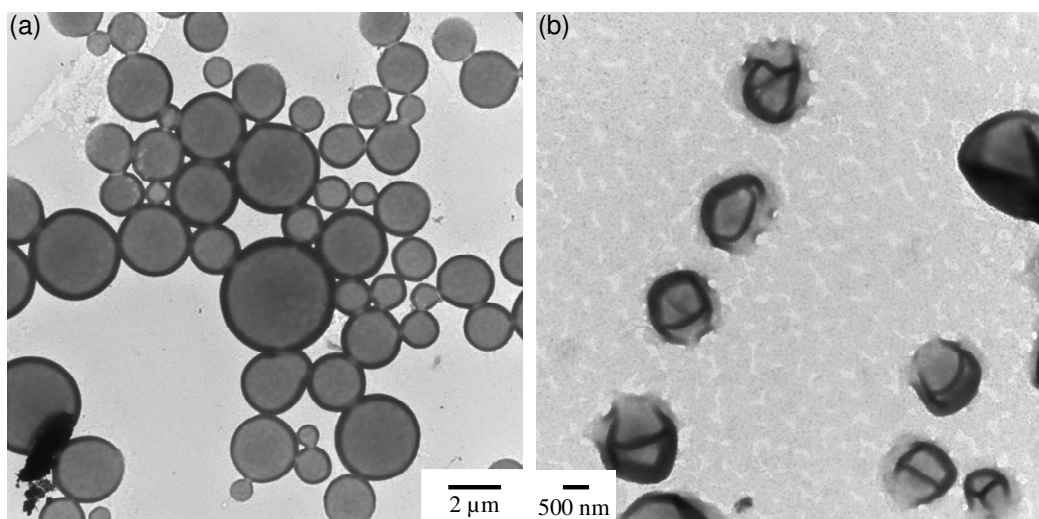


Figure S6. TEM images of P(DMAEMA₄₉-*co*-CMA₃)-*b*-PNIPAM₇₄ vesicles, stained with 0.5 wt% phosphotungstic acid solution, at 20 °C (a) and 40 °C (b) (note the different scale bars). **The staining allowed the membrane of vesicles to be better observed as compared to the unstained image in Figure 3a. Also, these images were recorded with a vesicular solution without being subjected to centrifugation,**

while the image in Figure 3a, showing a more uniform size of vesicles, was recorded from a vesicular solution after centrifugation.

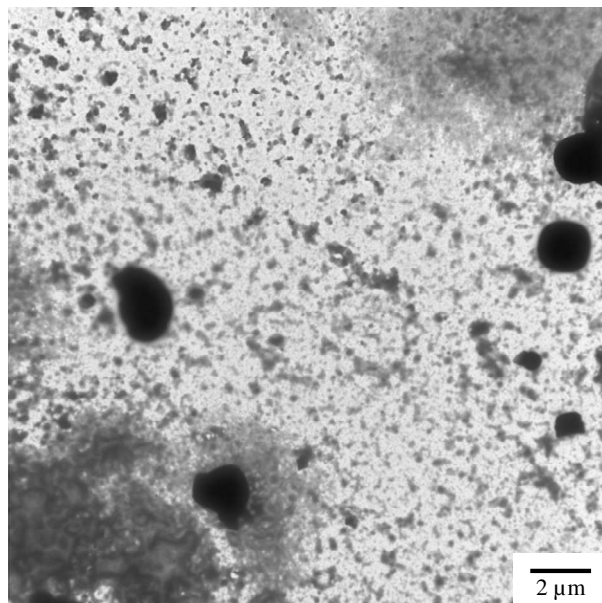


Figure S7. TEM image of a cast sample at 20 °C stained by phosphotungstic acid after photo-de-cross-linking of P(DMAEMA₄₉-*co*-CMA₃)-*b*-PNIPAM₇₄ upon $\lambda < 260$ nm UV exposure for 10 min. The disintegration of the vesicles is evident.

References

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