Supporting Information

In Situ Cross-Linking of Vesicles in Polymerization-Induced Self-Assembly

Qingwu Qu,[‡] Guangyao Liu,[‡] Xiaoqing Lv, Baohua Zhang, and Zesheng An*

Institute of Nanochemistry and Nanobiology, College of Environmental and Chemical Engineering, Shanghai University, Shanghai 200444, China

Materials

Diacetone acrylamide (DAAM, 99%) was purchased from Sigma-Aldrich. 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (V-50, 97%), allylamine hydrochloride (98%), acryloyl chloride (97%), *N*,*N*-dimethylacrylamide (DMA, 99%), and sodium dodecyl sulfate (SDS, 99%) were purchased from J&K Scientific. 2,2′-Azobis(2-methylpropionitrile) (AIBN, CP), hexadecyl trimethyl ammonium bromide (HDTAB, 99%), and Tween-20 (CP) were purchased from Sinopharm Chemical Reagent. The liquid monomers were passed through a column of Al_2O_3 to remove the inhibitor prior to use. AIBN was recrystallized from methanol twice.

Characterization and Methods

Nuclear magnetic resonance (NMR) spectroscopy. NMR spectra were collected on a Bruker AV 500 MHz spectrometer and chemical shifts were reported using solvent residue as the reference.

Gel permeation chromatography (GPC). GPC was performed on a Waters Alliance e2695 GPC system, equipped with a Styragel guard column, a Waters Styragel HR3 (molecular weight range 5.0

$\times 10^2$–3.0×10^4 g mol^{-1}), a Waters Styragel HR4 (molecular weight range 5.0×10^3–6.0×10^5 g mol^{-1}), and a Waters Styragel HR5 (molecular weight range 5.0×10^4–4.0×10^6 g mol^{-1}). Detection was performed on a 2414 refractive index (RI) detector using *N*,*N*-dimethylformamide (HPLC grade, containing 1 mg mL^{-1} LiBr) as the eluent at a flow rate of 0.8 mL min^{-1}. The temperature of the columns was set at 65 °C and the temperature of the refractometer was set at 45 °C. Analysis of molecular weights and dispersities was performed using Empower 2 software against poly(methyl methacrylate) (PMMA) standards (molecular weight range 2.4×10^2–1.0×10^6 g mol^{-1}).

Dynamic light scattering (DLS). For dispersions at a concentration of 1% in surfactant challenge experiments, DLS was performed on a Malvern ZEN 3600 at a detection angle of 173°. For size analysis the particle dispersions were diluted to a concentration of 0.1% and measured on a Malvern Zetasizer ZS90 at a detection angle of 90°. Z-average diameter (D_h) and polydispersity (PDI) were automatically analyzed in the cumulant mode using the Malvern software.

Transmission electron microscopy (TEM). TEM was performed on a Jeol 200CX microscope operating at 200 kV. To prepare TEM samples, the colloid solutions were diluted to generate dispersions with a concentration of 0.1–0.2%, which were dropped onto carbon-coated copper grids and dried in air overnight.

Transmittance measurements. A Hitachi U-3010 UV-vis spectrometer was used for transmittance measurements at 600 nm for the vesicle dispersions (1%) used in surfactant challenge experiments.

DMF resistance studies

0.2 mL of the as-synthesized vesicle dispersions were diluted with 2 mL of DMF. The resulting dispersions were left for 1 h and were then used for DLS and TEM characterization. To re-disperse the vesicles from DMF back to water, the DMF dispersions were placed in a dialysis tube (MWCO 3 k) and dialyzed against water by changing water several times during one day. The re-dispersed vesicles with a concentration of ~ 0.7% were again subject to DLS and TEM characterization.

Surfactant challenge studies

The as-synthesized vesicle dispersions were diluted with water to make a final concentration of 1%. A certain amount of a surfactant was then added to make the surfactant concentration 1% in the vesicle dispersions. Transmittance was measured at 600 nm on a UV-vis spectrometer for the dispersions before and 20 h after addition of a surfactant. To remove surfactant, the dispersions with an added surfactant were dialyzed against water in a dialysis tube (MWCO 7 k) for one day by changing water several times. After dialysis, the concentration of vesicle dispersions was reduced to ~ 0.5%, which were used for DLS and TEM characterization.

Synthesis of Allyl Acrylamide (ALAM)

A stirred solution of allylamine hydrochloride (3.695 g, 0.039 mol) and Et_3N (8.393 g, 0.083 mol) in CH_2Cl_2 (50 mL) was cooled to 0 °C, followed by dropwise addition of acryloyl choride (3.754 g, 0.041 mol). The reaction mixture was gradually warmed to room temperature for another 24 h. The precipitated salt was removed by filtration. The filtrate was washed with 0.1 M HCl and saturated NaCl mixed solution. The combined organic layers were dried over $MgSO_4$ and the liquid was passed through a column of Al_2O_3 to remove the inhibitor. Removal of solvents and drying under vacuum gave the title compound.[1]

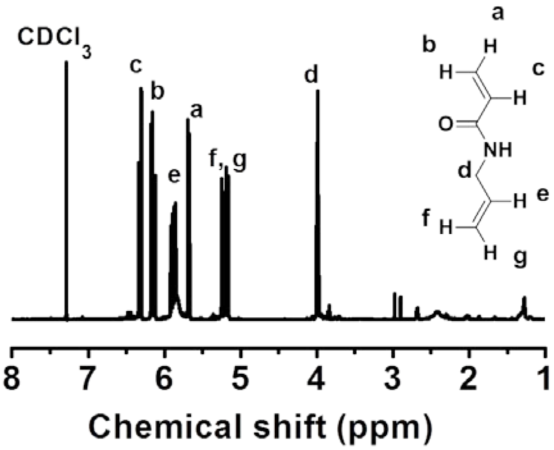


Figure S1. ^1H NMR spectrum of allyl acrylamide in $CDCl_3$.

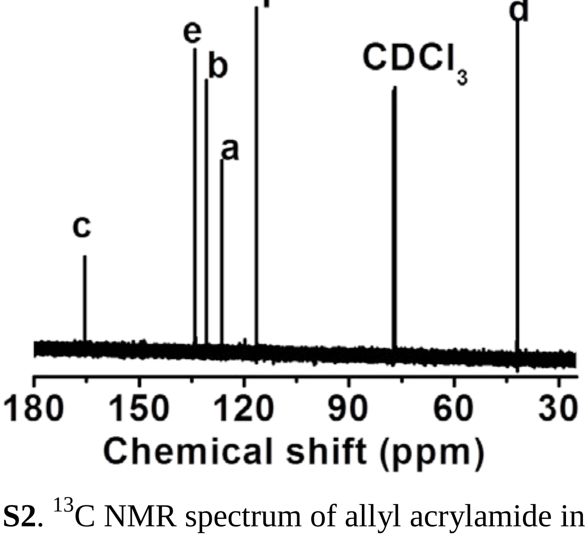
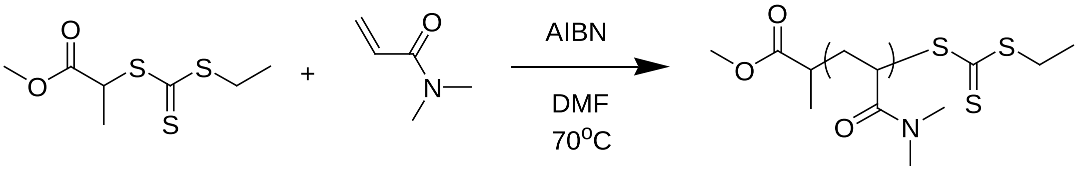


Figure S2. ^{13}C NMR spectrum of allyl acrylamide in $CDCl_3$.



Synthesis of PDMA$_{30}$ Macro-CTA

PDMA$_{30}$ Macro-CTA was synthesized according to a previously published protocol.[2] Specifically, 2-ethylsulfanylthiocarbonylsulfanyl-propionic acid methyl ester (0.431 g, 1.92 mmol) and *N*,*N*-dimethylacrylamide (7.612 g, 0.076 mol) were dissolved in *N*,*N*-dimethylformamide (DMF) (16.1 mL). The solution was degassed with nitrogen at 0 °C for 30 min and polymerization was initiated at 70 °C by injection of a degassed AIBN DMF solution (6.3 mg, 0.038 mmol). The polymerization was stopped at 2 h with 75% monomer conversion as determined by ^{1}H NMR spectroscopy. After purification by precipitation into ethyl ether and drying under vacuum, 4.23 g of a yellow solid was obtained in 52.6% yield. $M_{\mathrm{n,theory}}$ = 3.2 kg/mol, M_{n} = 4.08 kg/mol (GPC), $Đ$ = 1.10 (GPC). ^{1}H NMR (500 MHz, $CDCl_3$): 3.2-2.75 ppm (m, -$N(CH_3)_2$), 2.75-2.25 ppm (m, -(CO)$CHCH_2$-), 1.85-1.1 (m, -$CHCH_2$-).

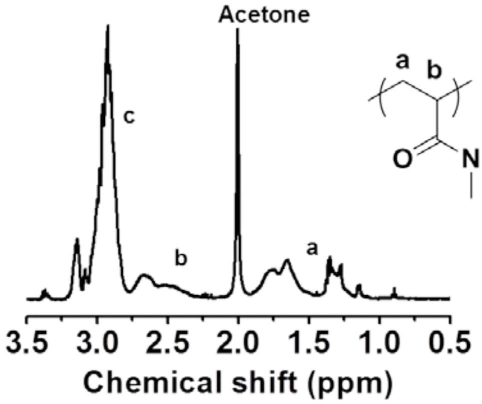


Figure S3. ^{1}H NMR spectrum of PDMA$_{30}$ in acetone-d_6.

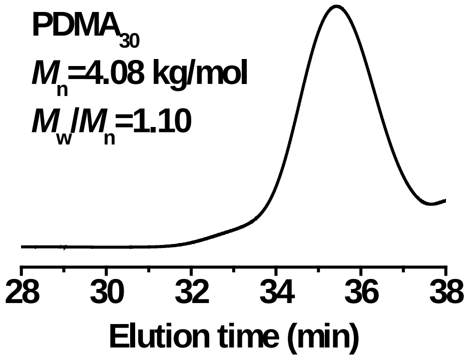
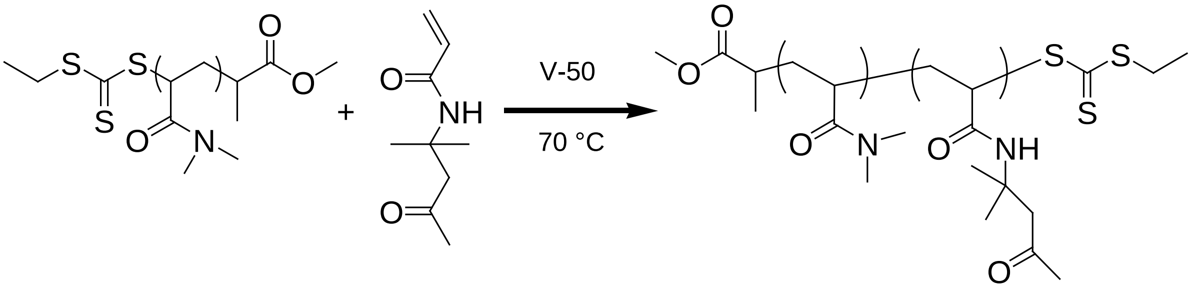


Figure S4. GPC trace of PDMA$_{30}$ (DMF, PMMA standards).



Synthesis of PDMA–*b*–PDAAM Linear Block Copolymer Vesicles

$PDMA_{30}$–*b*–$PDAAM_{276}$ linear block copolymer vesicles were synthesized using a previously reported protocol.[2] The total solids content was 20% and the molar ratio of $[PDMA_{30}]$/[DAAM]/[V-50] was controlled at 1/300/0.02. Macro-CTA $PDMA_{30}$ (0.064 g, 0.02 mmol) was dissolved in water (5.36 mL), to which were added DAAM (1.009 g, 5.96 mmol) and DMF (40 μL, internal standard). After the mixture was degassed with nitrogen in an ice/water bath for 30 min, it was immersed into a preheated oil bath at 70 °C. After the temperature was stabilized, a degassed V-50 solution (100 μL, 0.11 mg, 0.39 μmol) was injected via a microsyringe. The monomer conversion was 92% after 10 h of polymerization under protection of nitrogen (sample $\mathbf{V_{300\text{-}20\text{-}0}}$ in Table S2). Kinetic data for this synthesis is shown in Figure S5.

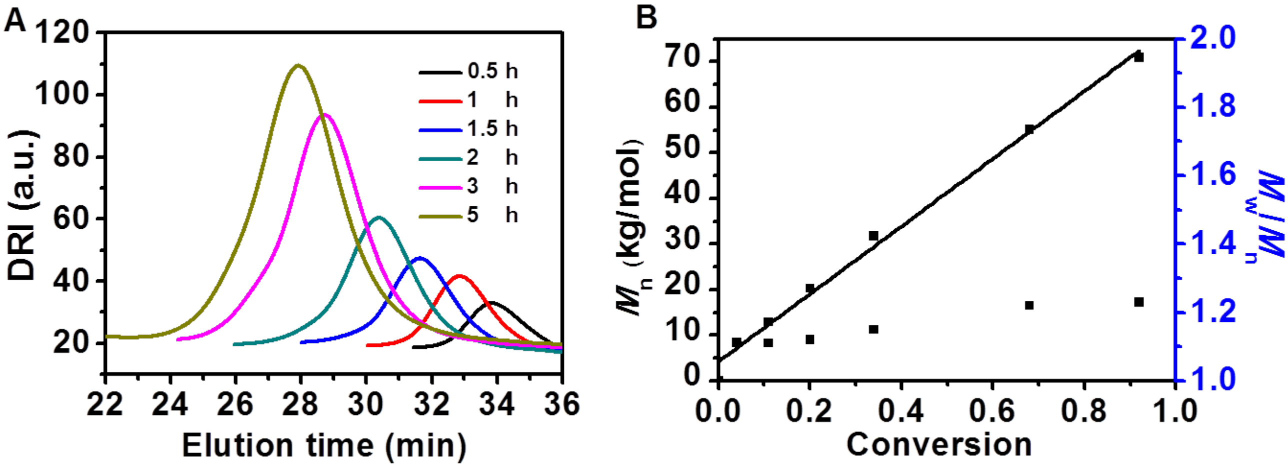
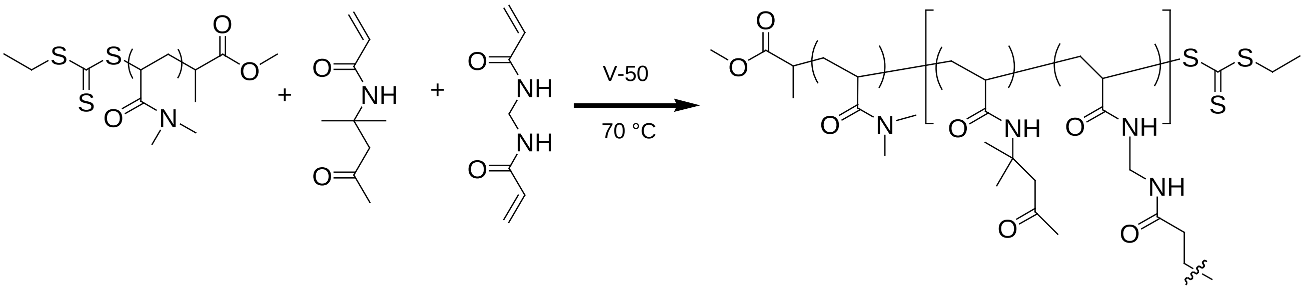


Figure S5. Kinetic data for the synthesis of $PDMA_{30}$–*b*–$PDAAM_{276}$ linear diblock copolymer vesicles ($\mathbf{V_{300\text{-}20\text{-}0}}$ in Table S2) via RAFT dispersion polymerization of DAAM at 70 °C in water ($[PDMA_{30}]$/[DAAM]/[V-50]=1/300/0.02, solids 20%). (A) Evolution of GPC traces with polymerization time, (B) dependences of number-average molecular weight (M_n) and dispersity (M_w/M_n) on monomer conversion.



Synthesis of Cross-Linked PDMA$_{30}$–*b*–P(DAAM$_{300}$–*co*–BIS$_x$) Block Copolymer Vesicles

In attempts to synthesize PDMA$_{30}$–*b*–P(DAAM$_{300}$–*co*–BIS$_x$) cross-linked vesicles, RAFT dispersion polymerization of DAAM was performed with varying amounts of BIS (1-3 mol% relative to DAAM). The total solids content was controlled at 20% and the molar ratio of [PDMA$_{30}$]/[DAAM]/[V-50] was 1/300/0.03. In a typical synthesis using 1 mol% BIS, BIS (0.0085 g, 0.055 mmol) was dissolved in water (4.96 mL) (*Attention should be paid to completely dissolve the floating flakes of BIS in order to get the correct BIS concentration!*), to which were added Macro-CTA PDMA$_{30}$ (0.059 g, 0.0183 mmol), DAAM (0.933 g, 5.513 mmol) and DMF (40 μL, internal standard). After the solution was degassed with nitrogen in an ice/water bath for 30 min, it was immersed into a preheated oil bath at 70 °C. After the temperature was stabilized, a degassed V-50 solution (100 μL, 0.150 mg, 0.00055 mmol) was injected via a microsyringe. The polymerization was allowed to continue for 10 h under protection of nitrogen (sample 1 in Table S1). The results for vesicle synthesis in the presence of varying amounts of BIS are summarized in Table S1.

Table S1. Summary for the Synthesis of PDMA$_{30}$–*b*–(PDAAM$_{300}$–*co*–BIS$_x$) Vesicles[a]

sample	**DP**[b]	**V-50**[c]	**BIS**[d] **(mol%)**	**solids**[e] **(%)**	**time**[f] **(h)**	**conv.**[g] **(%)**	***D*$_h$ (nm) (PDI)**[h]	**morphology**[i]
1	300	0.03	1	20	4	>99	543 (0.134)	vesicle
2	300	0.03	1.25	20	4	-	-	precipitate
3	300	0.03	1.5	20	10	-	-	gel
4	300	0.03	2	20	10	-	-	gel
5	300	0.03	3	20	2	-	-	gel

[a]Dispersion polymerization conditions: 70 °C, 500 rpm, water. [b]Target PDAAM DP. [c]Ratio of [V-50]/[PDMA$_{30}$]. [d]BIS feeding ratio relative to DAAM. [e]Total solids content (m$_{DAAM}$ + m$_{PDMA}$)/V$_{water}$). [f]Polymerization time. [g]Monomer conversion determined by ^{1}H NMR spectroscopy in DMSO-d_6. [h]Hydrodynamic diameter and DPI of vesicles obtained by DLS. [i]Morphology by TEM or visual observation .

Data for DMF Dissolution Studies of PDMA$_{30}$–*b*–P(DAAM$_{300}$–*co*–BIS$_3$) Cross-Linked Block Copolymer Vesicles

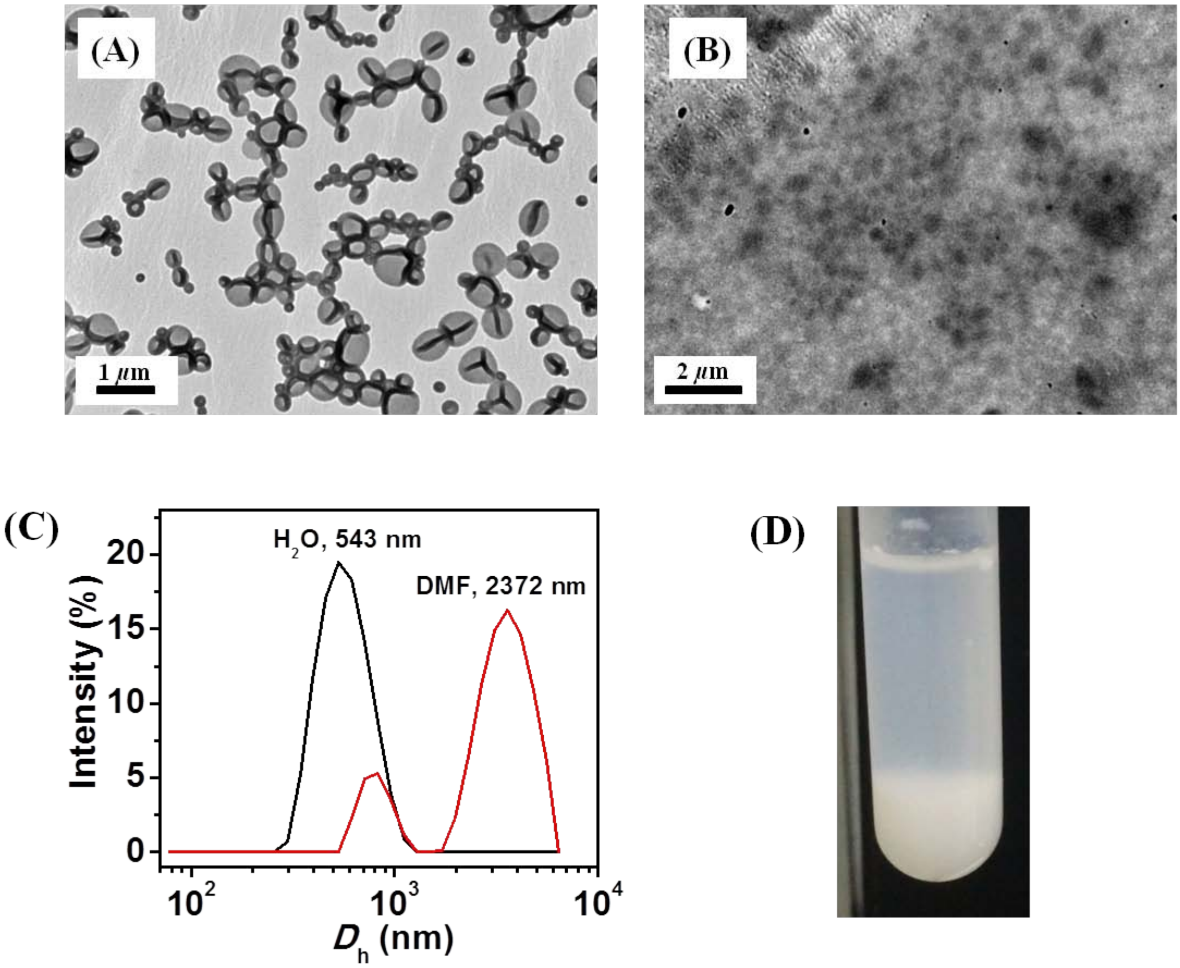


Figure S6. TEM micrographs of PDMA$_{30}$–*b*–P(DAAM$_{300}$–*co*–BIS$_3$) vesicles (1 mol% BIS) (sample 1 in Table S1): (A) prepared from aqueous dispersion; (B) prepared from DMF dispersion. (C) DLS diameter distributions measured in water and DMF. (D) Photograph for the solution showing precipitation after re-dispersion from DMF to water via dialysis. (*Please note all the TEM micrographs for DMF dispersions appeared fuzzy and had smeared surface, especially for vesicles with a low cross-linking density)*

Data for SDS Challenge Studies of PDMA$_{30}$–*b*–P(DAAM$_{300}$–*co*–BIS$_{3}$) Cross-Linked Block Copolymer Vesicles

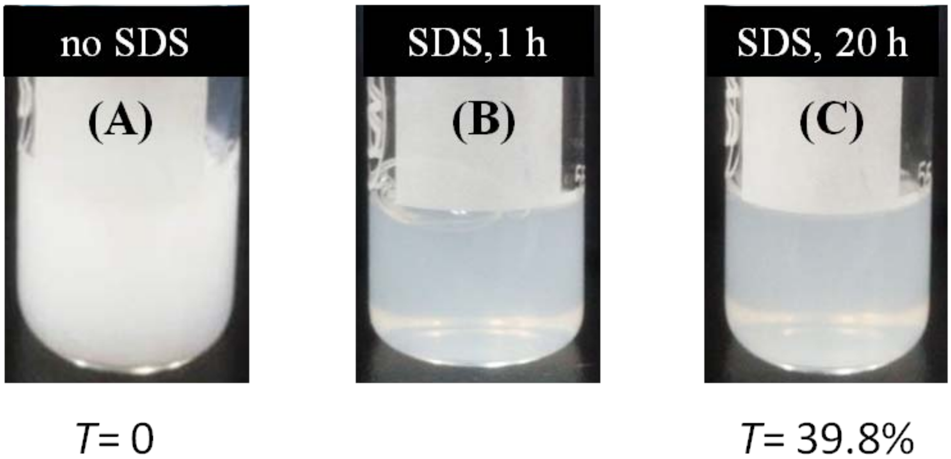
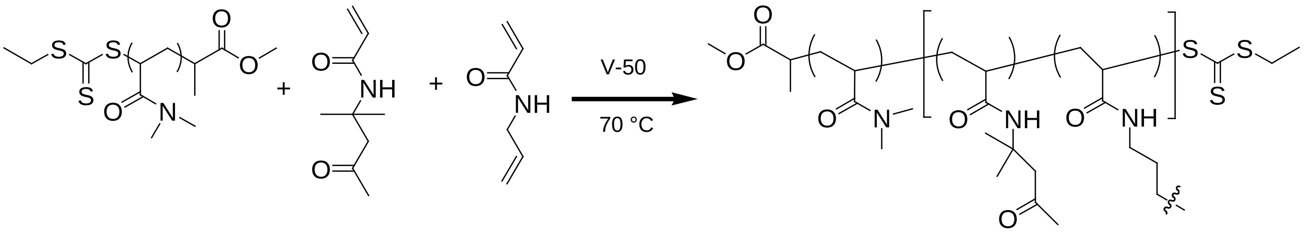


Figure S7. Photographs for 1% aqueous dispersions of PDMA$_{30}$–*b*–P(DAAM$_{300}$–*co*–BIS$_{3}$) vesicles (1 mol% BIS) (sample 1 in Table S1): (A) before addition of SDS (0 transmittance); (B) after addition of SDS (1% in solution) and being stirred for 1 h; (C) after addition of SDS (1% in solution) and being stirred for 20 h (39.8% transmittance).



Synthesis of PDMA$_{30}$–*b*–P(DAAM$_{300\text{-}400}$–*co*–ALAM$_x$) Cross-Linked Block Copolymer Vesicles

PDMA$_{30}$–*b*–P(DAAM$_{300\text{-}400}$–*co*–ALAM$_x$) cross-linked block copolymer vesicles were synthesized by RAFT aqueous dispersion polymerization in the presence of varying amounts of ALAM at 70 °C. The total solids content was controlled at either 10% or 20%, and the target DP of PDAAM was either 300 or 400. The molar ratio of [V-50]/[PDMA$_{30}$] was in the range of 0.02-0.2; *it was necessary to use a higher amount of V-50 to get high monomer conversions, when more ALAM was used or when polymerization was conducted at a lower solids content (10%).* In a typical synthesis (sample **V$_{300\text{-}20\text{-}3}$** in Table S2), PDMA$_{30}$ (0.079 g, 0.025 mmol) was dissolved in water (6.68 mL), to which were added DAAM (1.258 g, 7.43 mmol), ALAM (24.8 mg, 0.22 mmol) and DMF (60 μL, internal standard). After the solution was degassed with nitrogen in an ice/water bath for 30 min, polymerization was initiated by addition of a degassed V-50 solution (100 μL, 0.13 mg, 0.48 μmol) at 70 °C. For kinetic studies, aliquots were withdrawn from the reaction at pre-determined time intervals for GPC, DLS and NMR characterization. The results for vesicle synthesis in the presence of varying amounts of ALAM are summarized in Table S2.

Table S2. Summary for the Synthesis of PDMA$_{30}$–*b*–(PDAAM$_{300\text{-}400}$–*co*–ALAM$_x$) Vesicles[a]

sample[b]	**DP**[c]	**V-50**[d]	**ALAM**[e] **(mol%)**	**solids**[f] **(%)**	**time**[g] **(h)**	**conv.**[h] **(%)**	***D*$_h$ (nm) (PDI)**[i]	**morphology**[j]
V$_{300\text{-}20\text{-}0}$	300	0.02	0	20	5	92	499 (0.136)	vesicle
V$_{300\text{-}20\text{-}1}$	300	0.02	1	20	11	86	449 (0.141)	vesicle
V$_{300\text{-}20\text{-}2}$	300	0.02	2	20	10	95	430 (0.106)	vesicle
V$_{300\text{-}20\text{-}3}$	300	0.02	3	20	11	86	413 (0.172)	vesicle
V$_{300\text{-}20\text{-}4}$	300	0.02	4	20	6	-	-	precipitate
V$_{300\text{-}20\text{-}5}$	300	0.02	5	20	8	-	-	precipitate
V$_{400\text{-}10\text{-}5}$	400	0.2	5	10	2	>99	249 (0.043)	vesicle
V$_{400\text{-}10\text{-}7}$	400	0.2	7	10	5	-	-	precipitate

[a] Dispersion polymerization conditions: 70 °C, 500 rpm. [b] Subscript represents target DP, solids (%) and amount of ALAM (mol%) relative to DAAM. [c] Target PDAAM DP. [d] Ratio of [V-50]/[PDMA$_{30}$]. [e] ALAM feeding ratio relative to DAAM. [f] Total solids content (m$_{DAAM}$ + m$_{PDMA}$)/V$_{water}$). [g] Polymerization time. [h] Monomer conversion determined by ^{1}H NMR in DMSO-*d*$_6$. [i] Hydrodynamic diameter and DPI of vesicles obtained by DLS. [j] Morphology by TEM or visual observation.

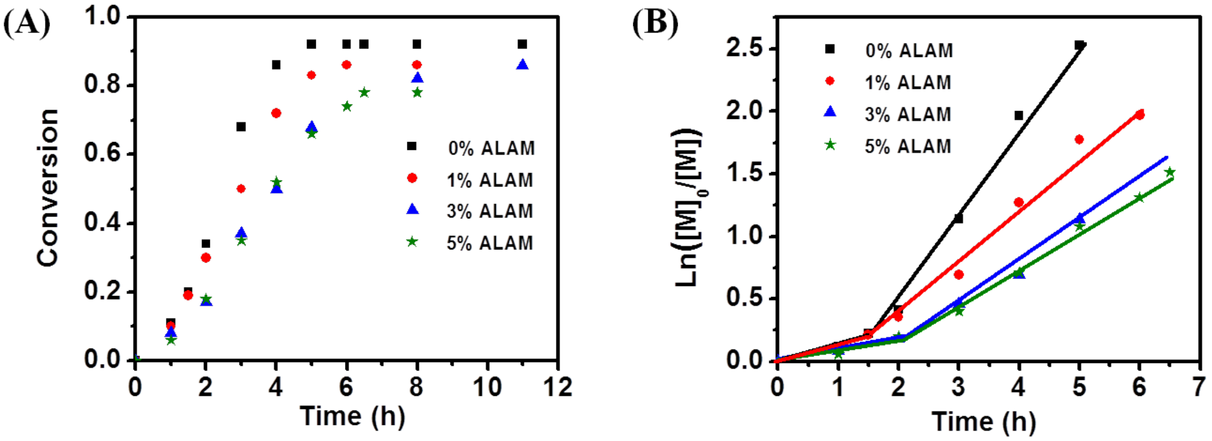


Figure S8. Dispersion polymerization data for the synthesis of $PDMA_{30}$–*b*–$P(DAAM_{300}$–*co*–$ALAM_x)$ vesicles in the presence of varying amounts of ALAM (mol% relative to DAAM), $[DAAM]/[PDMA_{30}]/[V\text{-}50]=300/1/0.02$, solids content 20%, 70 ^{o}C: (A) Evolution of DAAM conversion with polymerization time; (B) $\text{Ln}([M]_0/[M])$ vs polymerization time.

Table S3. Parameters for the Critical Self-Assembly Points in Dispersion Polymerization in the Presence of Varying Amounts of ALAM.[a]

ALAM[b] (mol%)	time[c] (h)	conv.[d] (%)	DP[e]	k_{p1}[f] (h^{-1})	k_{p2}[g] (h^{-1})
0	1.5	20	60	0.148	0.715
1	1.5	19	57	0.140	0.482
3	2	17	51	0.094	0.317
5	2	18	54	0.099	0.293

[a] Dispersion polymerization conditions corresponding those in Figure S8. [b] ALAM feeding ratio relative to DAAM. [c] Polymerization time reaching critical self-assembly point. [d] DAAM conversion at critical self-assembly point. [e] DP of PDAAM at critical self-assembly point. [f] Observed polymerization rate constant prior to critical self-assembly point. [g] Observed polymerization rate constant post critical self-assembly point.

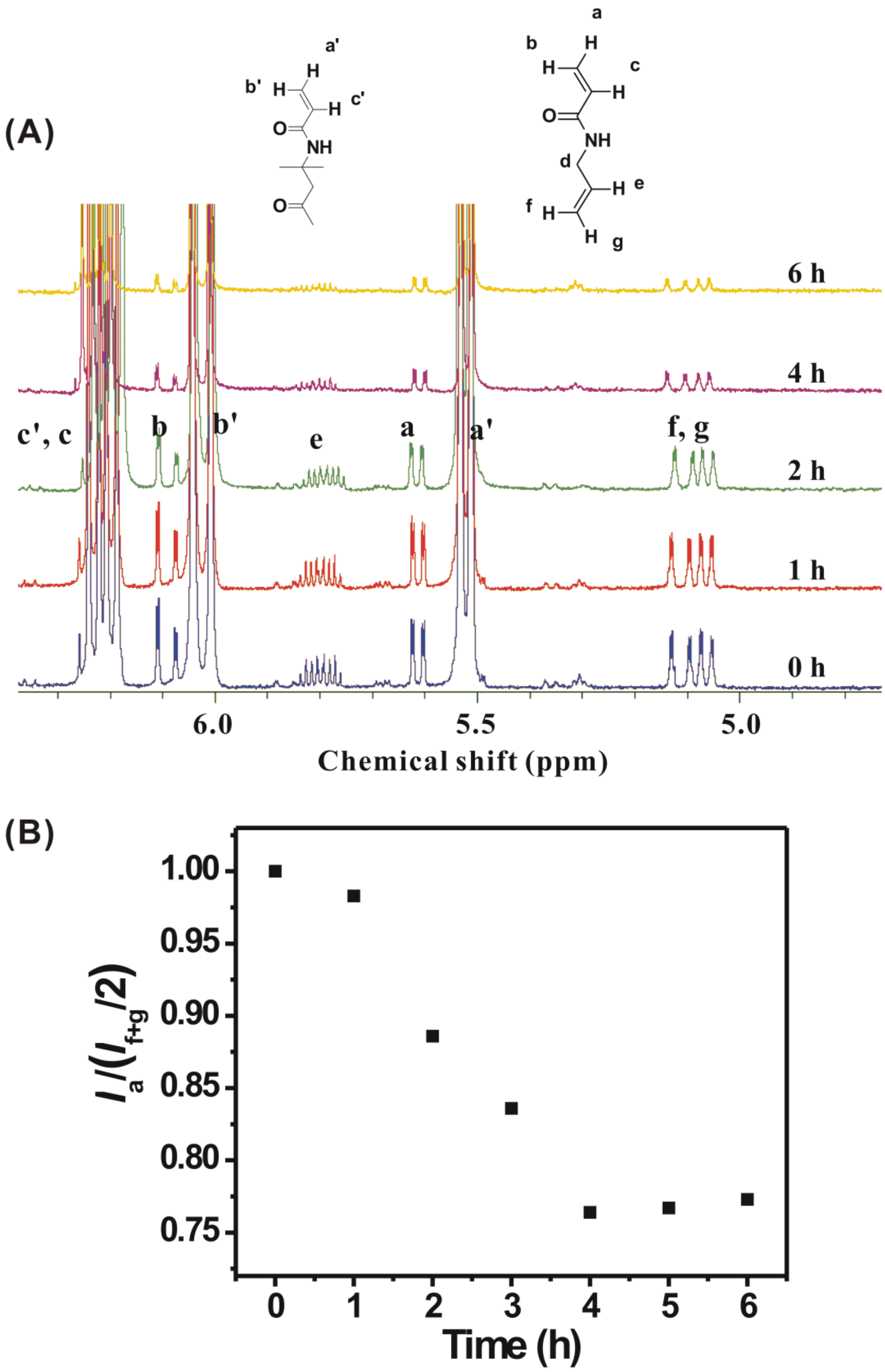


Figure S9. ^{1}H NMR spectra (A) and integral ratio of the two vinyl groups of ALAM during the dispersion polymerization of DAAM in the presence of 5 mol% ALAM, targeting DP 300, solids 20%, 70 °C . (***Figure S9*** *shows the acrylamido group polymerizes faster than the allyl group of ALAM. Please note after 4 h, it became difficult to quantify the signals of the double bonds due to their low quantities used and their conversions*)

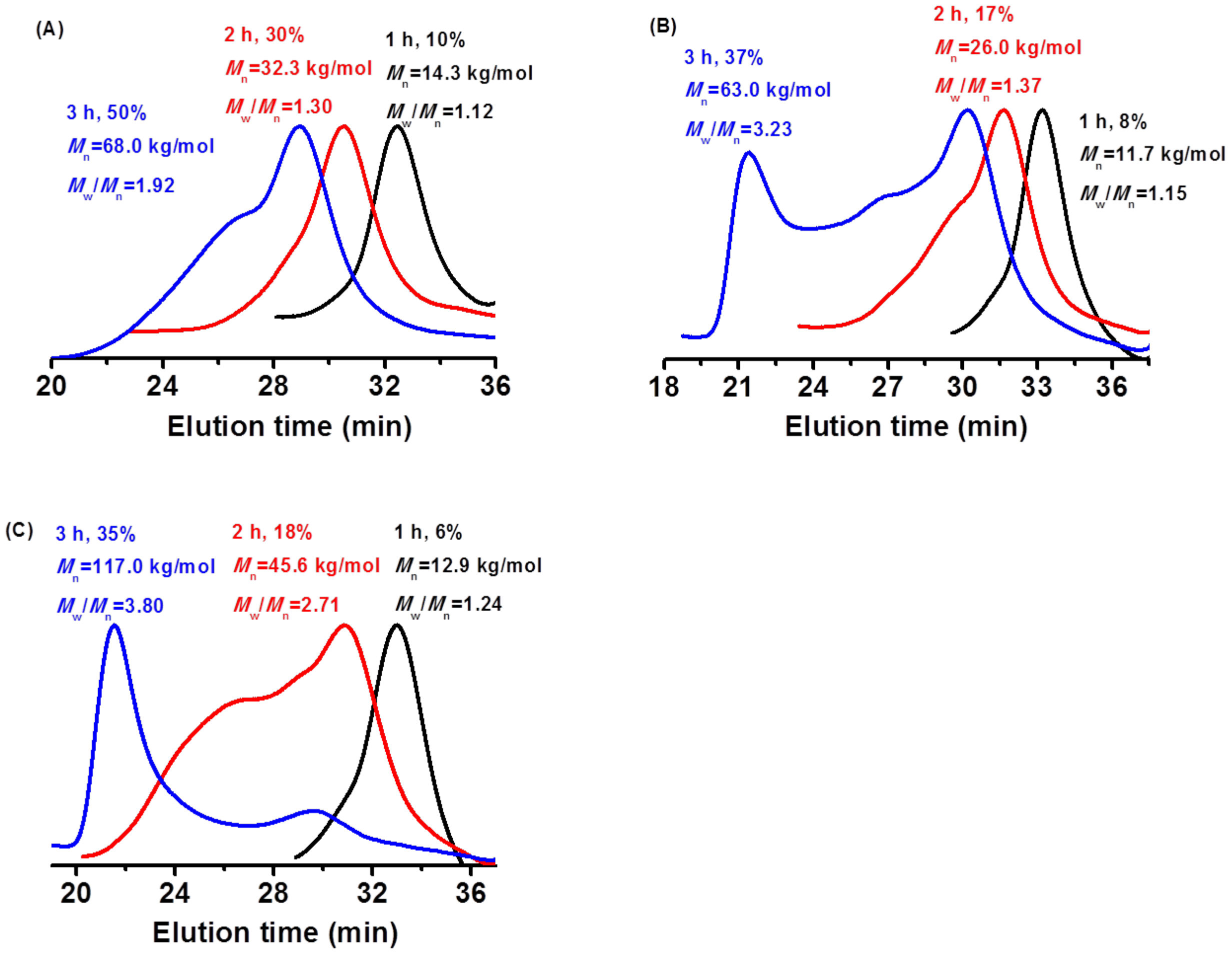


Figure S10. GPC traces during the synthesis of PDMA$_{30}$–*b*–P(DAAM$_{300}$–*co*–ALAM$_x$) vesicles ([DAAM]/[PDMA$_{30}$]/[V-50]=300/1/0.02, solids content 20%, 70 ^{o}C), in the presence of varying amounts of ALAM (relative to DAAM): (A) 1 mol%; (B) 3 mol%; (C) 5 mol% (*please note this synthesis finally formed viscous precipitate*).

Data for DMF Dissolution Studies of PDMA$_{30}$–*b*–PDAAM$_{300}$ Linear and PDMA$_{30}$–*b*–P(DAAM$_{300\text{-}400}$–*co*–ALAM$_x$) Cross-Linked Block Copolymer Vesicles

Table S4. DLS Results for Vesicle Dispersions in Water, DMF, and Re-Dispersed from DMF to Water via Dialysis.

vesicle code	***D*$_h$ (nm) (PDI)[a] (in water)**	***D*$_h$ (nm) (PDI)[a] (in DMF)**	**swelling ratio[b] (%)**	***D*$_h$ (nm) (PDI)[a] (DMF to water)**
V$_{300\text{-}20\text{-}0}$	499 (0.136)	12 (0.658)	-	1168 (poor quality, finally precipitate)
V$_{300\text{-}20\text{-}1}$	449 (0.141)	1255 (poor quality)	180	2314 (poor quality, finally precipitate)
V$_{300\text{-}20\text{-}2}$	430 (0.106)	904 (0.138)	110	485 (0.195)
V$_{300\text{-}20\text{-}3}$	413 (0.172)	826 (0.163)	100	443 (0.213)
V$_{400\text{-}10\text{-}5}$	255 (0.062)	541 (0.007)	112	284 (0.142)

[a] DLS analysis was performed on a Malvern nano ZS90 for 0.1% dispersions. [b] Swelling ratio=[(*D*$_h$ in DMF)-(*D*$_h$ in water)]/(*D*$_h$ in water), swelling ratio of vesicles cross-linked by 1 mol% BIS is 336.

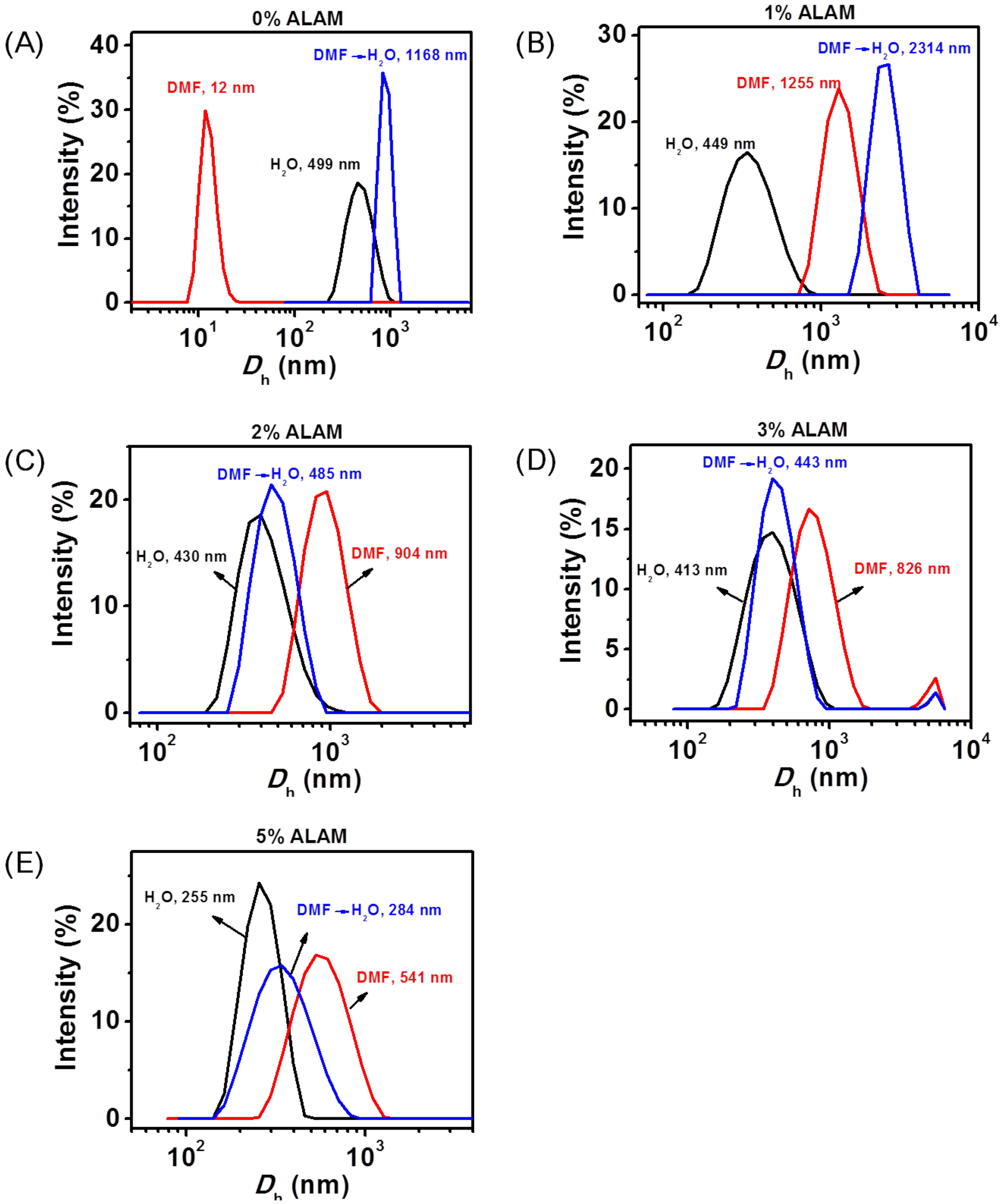


Figure S11. DLS intensity-average size distributions of linear PDMA$_{30}$–*b*–PDAAM$_{300}$ vesicles **V$_{300\text{-}20\text{-}0}$** (A) and cross-linked PDMA$_{30}$–*b*–P(DAAM$_{300\text{-}400}$–*co*–ALAM$_x$) vesicles **V$_{300\text{-}20\text{-}1}$** (B), **V$_{300\text{-}20\text{-}2}$** (C), **V$_{300\text{-}20\text{-}3}$** (D), **V$_{400\text{-}10\text{-}5}$** (E) in water, DMF, and re-dispersed from DMF to water via dialysis. (*These figures correspond to the data presented in Table S4*)

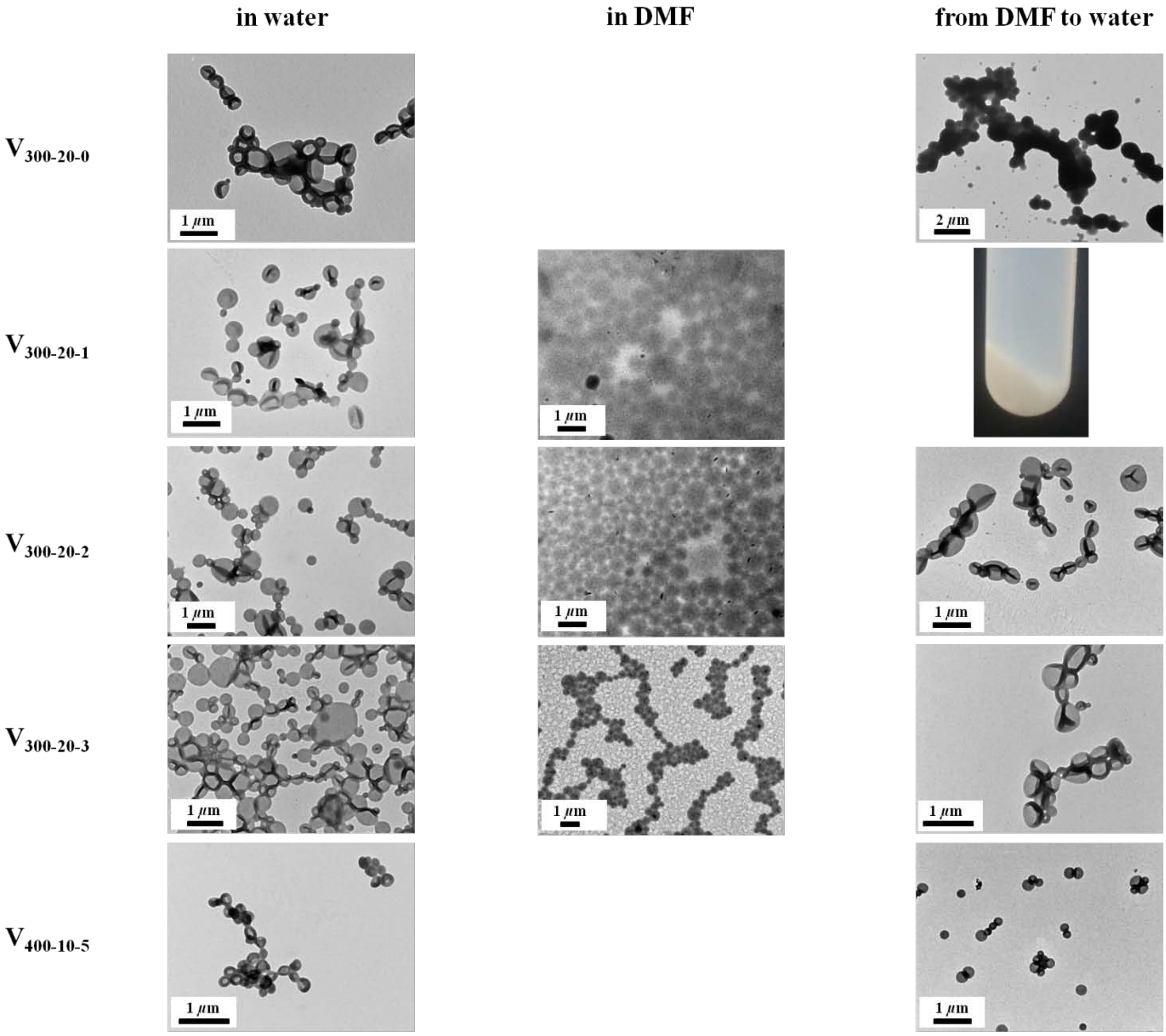


Figure S12. TEM micrographs (or solution photograph) for vesicle dispersions in water, DMF, and re-dispersed from DMF to water via dialysis.

Data for Surfactant Challenge Studies of PDMA$_{30}$–*b*–PDAAM$_{300}$ Linear and PDMA$_{30}$–*b*–P(DAAM$_{300\text{-}400}$–*co*–ALAM$_x$) Cross-Linked Block Copolymer Vesicles

Table S5. Effect of Adding Surfactants to Aqueous Dispersions (1.0 %) of Block Copolymer Vesicles.

vesicle code	surfactant	surfactant conc. (%)	D_h (nm) (PDI)[a]	T^b (%)
$V_{300\text{-}20\text{-}0}$	none	0	451 (0.138)	0
	SDS	0.5	380 (0.212)	0.8
		1	112 (0.564)	92.4
		10	144 (0.455)	97.3
	HDTAB	1	441 (0.181)	0
		10	466 (0.126)	0
	Tween-20	1	463 (0.157)	0
		10	414 (0.163)	0
$V_{300\text{-}20\text{-}1}$	none	0	473 (0.068)	0
	SDS	1	409 (0.132)	44.7
	HDTAB	1	454 (0.049)	0
	Tween-20	1	443 (0.191)	0
$V_{300\text{-}20\text{-}2}$	none	0	430 (0.106)	0
	SDS	1	-	1.2
$V_{300\text{-}20\text{-}3}$	none	0	431 (0.176)	0
	SDS	1	402 (0.184)	0.4
		10	402 (0.171)	0.4
	HDTAB	1	404 (0.228)	0
		10	404 (0.194)	0
	Tween-20	1	415 (0.204)	0
		10	398 (0.221)	0
$V_{400\text{-}10\text{-}5}$	none	0	247 (0.030)	0
	SDS	1	249 (0.002)	0
	HDTAB	1	243 (0.015)	0
	Tween-20	1	277 (0.085)	0

[a] DLS analysis conducted on a Malvern ZEN 3600 at a detection angle of 173° without dilution of the vesicle dispersions (1%). [b] Transmittance of the vesicle dispersions (1 %) measured at 600 nm by UV-vis spectroscopy.

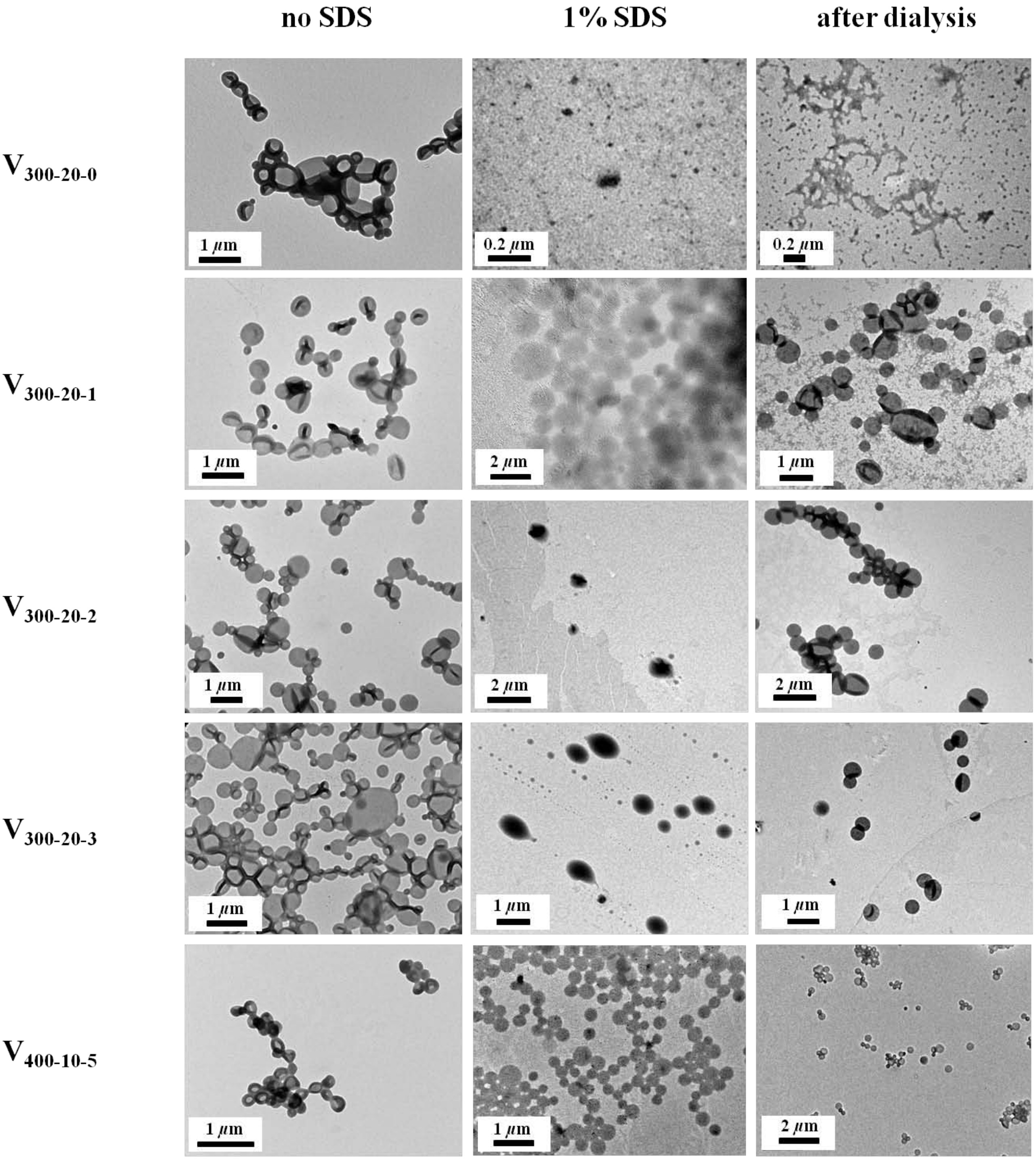


Figure S13. TEM micrographs for vesicle dispersions in water without SDS, in the presence of 1% SDS, and after dialysis in water to remove SDS.

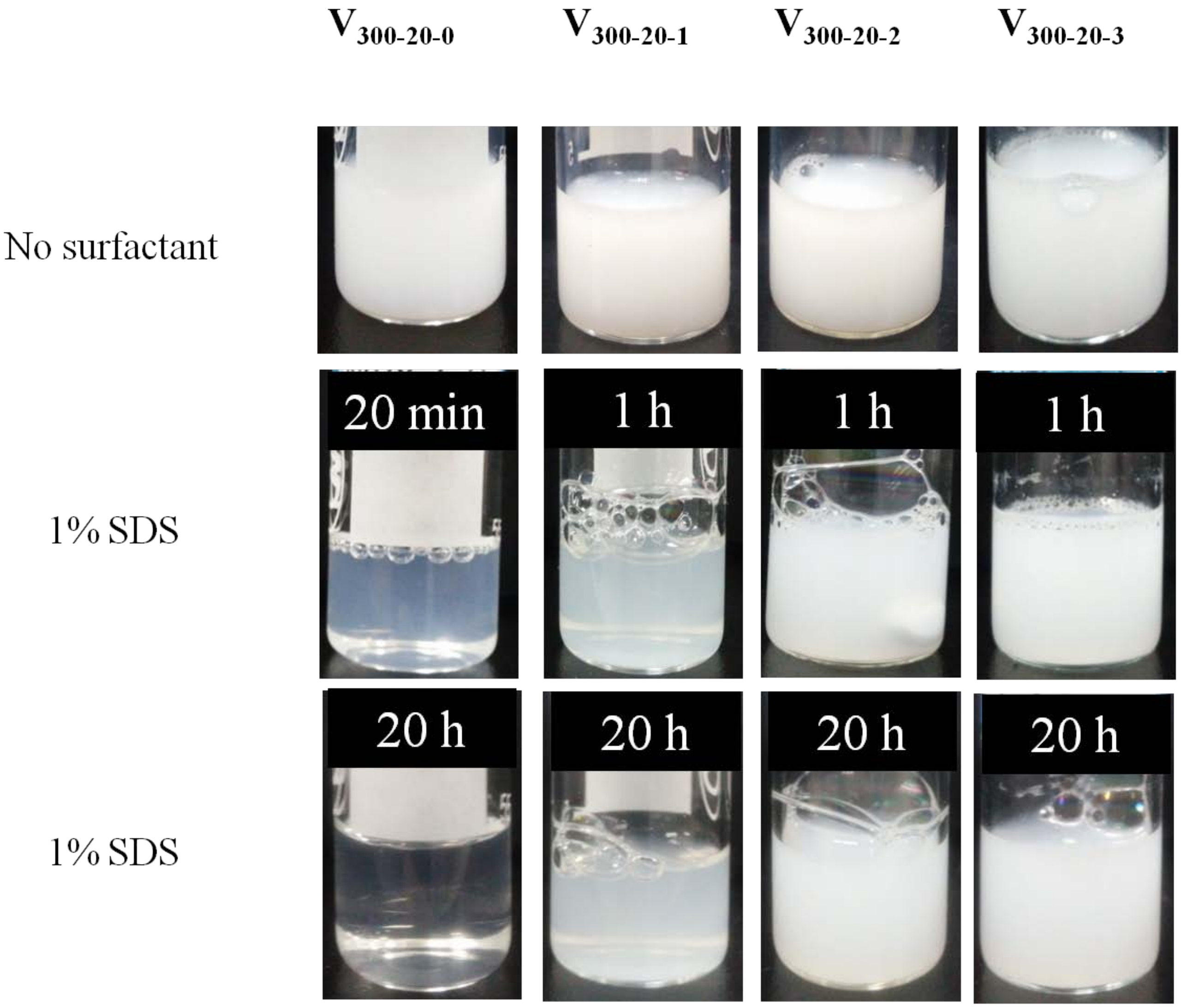


Figure S14. Photographs for vesicle dispersions (1%) in water without and in the presence of 1% SDS.

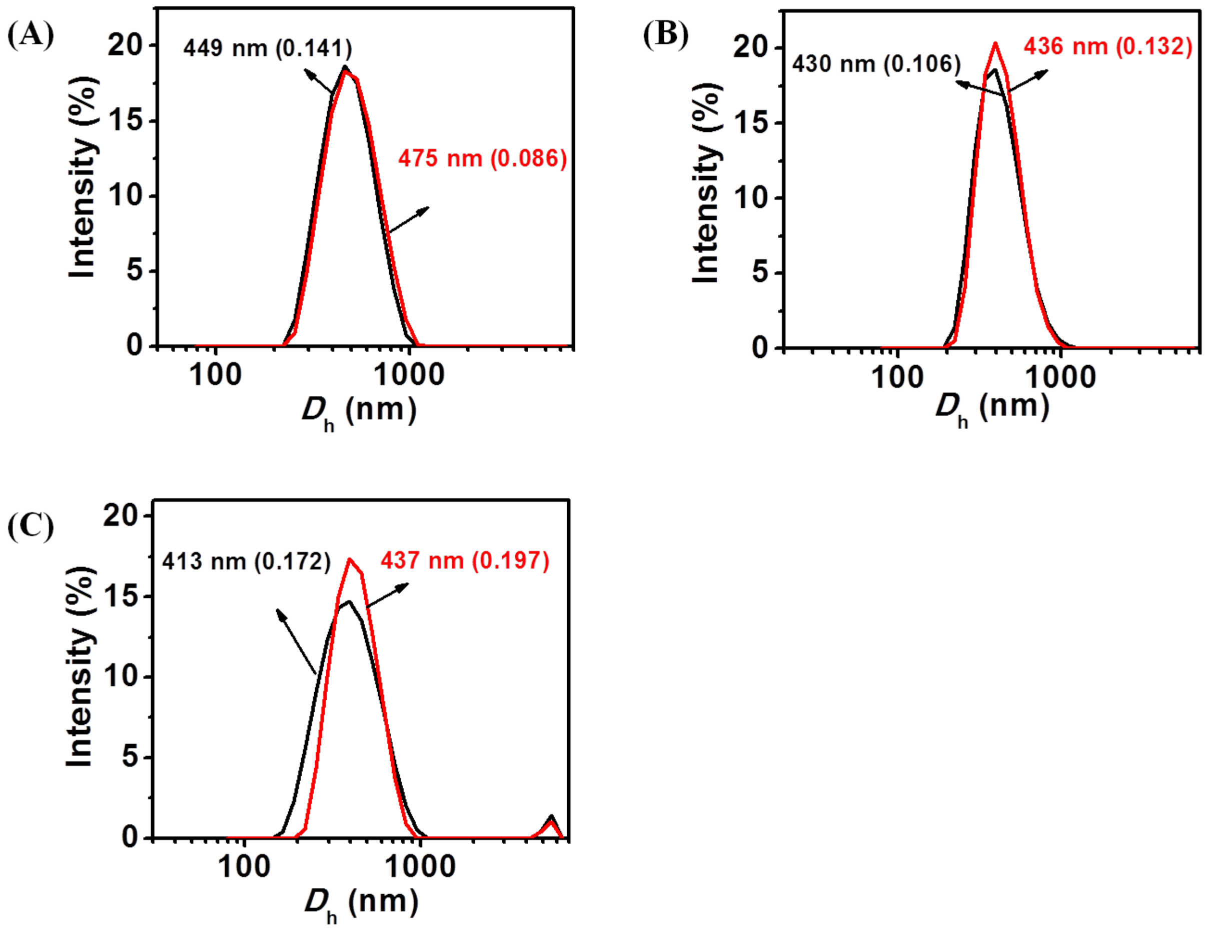


Figure S15. Intensity-average size distributions of $\mathbf{V}_{300\text{-}20\text{-}1}$ (A), $\mathbf{V}_{300\text{-}20\text{-}2}$ (B), and $\mathbf{V}_{300\text{-}20\text{-}3}$ (C) in as-synthesized dispersions (black line) and after 1% SDS treatment followed by dialysis (red line).

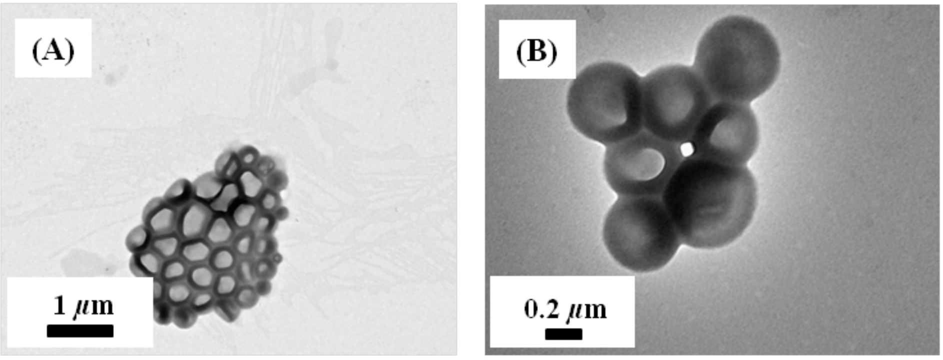


Figure S16. TEM micrographs for $\mathbf{V_{300\text{-}20\text{-}0}}$ dispersions (1%) in the presence of 10% HDTAB (A) and 10% Tween-20 (B). (*Analysis: The membrane thickness of the vesicles in the presence of 10% HDTBA or 10% Tween-20 is 96.3±1 nm or 102.5±1 nm, respectively, which are higher than the 75.0±1 nm for the vesicles in the absence of surfactant.*)

References

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(2) Zhou, W.; Qu, Q.; Xu, Y.; An, Z. *ACS Macro Letters* **2015**, *4*, 495.