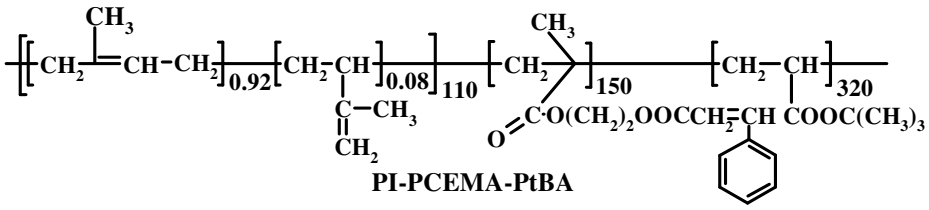
Supporting Information[1] for Hierarchical Interfacial Assembly of ABC Triblock Copolymer

Jiwen Hu, Guojun Liu*, and Gabriel Nijkang

Experimental Section

Materials. Decahydronaphthalene (DN, mixture of *cis* and *trans*, 98%, Aldrich), trifluoroacetic acid, (TFA, 99+%, Adlrich) and *N*, *N*-dimethylformamide (99.8+%, Aldrich), methanol (99.8+%, Fisher Scientific), methylene chloride (99.5+%, Fisher Scientific), osmium tetroxide (EM Sciences), uranyl acetate (EM Sceinces) were used as received. PI-PCEMA-PtBA was synthesized following procedures reported before.[2, 3] The molecular properties of the triblock are summarized in Table 1, where SEC and LS stand for size-exclusion chromatography and light scattering, respectively. The symbols M_w and M_n denote the weight- and number-average molecular weights and dn_r/dc denotes the specific refractive index increment of the polymers. Symbols n_w, m_w, and l_w represent the weight-average numbers of repeat units for PI, PCEMA, and PtBA, respectively.



PI-PCEMA-PtBA

Table 1. Molecular properties of PI-PCEMA-PtBA.

SEC M_w/M_n	dn_r/dc (mL/g) in THF	LS $10^{-4}M_w$	$n/m/l$	n_w	m_w	l_w
1.30	0.148	8.6	1.00:1.31:2.83	110	150	320

Preparation of Cylindrical Micelle-like Aggregates. PI-PCEMA-PtBA, 0.010 g, was mixed with 10 mL of DN in a 25-mL round-bottomed flask wrapped in aluminum foil. After sealing with a rubber septum, the mixture was bubbled by N_2 for 5 min. The mixture was stirred at 600 rpm with a magnetic stirring bar at 60±2 °C for 3 d before it was centrifuged at 2800 rpm (1550 *g*) for 10 min to settle a trace amount of undissolved solid.

Preparation of Spherical Micelle-like Aggregates. PI-PCEMA-PtBA, 5.6 mg, was dissolved in 1.0 mL of methylene chloride. To the solution was then added dropwise 5.0 mL of DN. The resultant solution was equilibrated for 2 h under stirring before N_2 was bubbled for 2 h to remove methylene chloride.

Ribbon Cages. A CM or SM solution was heated to 52±2 °C in a sealed round-bottom flask before MeOH, at one third the volume of DN, was added over 1 min under stirring at 800 rpm. To monitor the ribbon cage formation process, samples were taken 5, 30, and 120 min after methanol addition. They were immediately aspirated on carbon-

coated copper grids, dried under evacuation, and stained by RuO_4 for TEM analyses. We determined that the sample morphologies at 52±2 ^oC did not change noticeably between 1 and 7 d and were invariably ribbon cages.

Flowery Superaggregates at RT. To a CM or SM solution at 22±2 ^oC in a sealed round-bottom flask was added in 1 min under stirring at 800 rpm MeOH at one third the volume of DN. To follow the superaggregate formation process, samples were taken 5, 30, and 120 min after methanol addition. They were immediately aspirated on carbon-coated copper grids, dried by evacuation, and stained by RuO_4 for TEM analyses. We determined that the sample morphologies at 22±2 ^oC did not change noticeably between 1 and 7 d and were invariably flowery aggregates.

TEM Measurements. A Hitachi-7000 instrument operating at 75 kV was used to obtain TEM images. Micelle-like aggregates and superaggregates were directly sprayed onto carbon-coated copper grids from DN or DN/MeOH using a home-built device. The sprayed specimens were dried under vacuum at RT for 2 h before staining by RuO_4 vapor for 2 h for TEM observation.

AFM Measurements. The substrate used was of prime grade silicon wafer with orientation (100) purchased from Silicon Quest International. MAs and superaggregates were directly sprayed onto silicon. Tapping-mode atomic force microscopy (AFM) analyses were carried out on a Veeco multimode instrument equipped with a Nanoscope IIIa controller. The tips used were of the Nanosensors^{TM} NCHR-SPL type. The images were obtained using a free tip oscillation amplitude A_0 of 60 nm and a set point amplitude ratio R_{sp} of ~90%.

PI-PCEMA-PtBA Cylinder Crosslinking and Assembly. Cylindrical micelles of the triblock in DN, 1.5 mL at 1.0 mg/mL, was irradiated in a UV cell under magnetic stirring by a UV beam that had passed through a 270-nm cut-off filter from a 500-W mercury lamp for 0.5 h to crosslink the PCEMA core. This DN solution, 1.2 mL, was then transferred into a round-bottom flask and sealed by a rubber septum and heated to 52±2 ^oC. To this DN solution was then added 0.40 mL of methanol dropwise within 1 min and the resultant mixture was stirred for 2 d before aspiration for TEM analysis.

Solubility Test of Methanol in DN and DN in Methanol. Methanol, 1.0 mL, was added into 3.0 mL of DN. The mixture was sealed and stirred at 800 rpm at 22±2 and 52±2 ^oC for 1h. This was followed by stopping the stirring and leaving the cloudy solvent mixture to phase separate at the respectively temperatures for 1 h. Samples were taken from the bottom DN-rich and top MeOH-rich phases by a syringe equipped with long needle and injected into $CDCl_3$ for ^1H NMR analysis of the compositions in the two phases.

Cryogenic Transmission Electron Microscopy (Cryo-TEM). A drop of a ribbon cage solution was dispensed on a copper TEM grid covered by a holey Formvar film. The excess solution was quickly blotted away by a piece of filter paper before the grid was immersed into liquid propane cooled at ~90 K to vitrify the ribbon cage solution film. This was followed by the transfer of the grid into a liquid nitrogen reservoir and its mounting on a cryogenic sample holder. TEM observations were performed at -173 ^oC on a JEM-2010 CRYO-TEM operating at 200 kV equipped with a Gatan 655 cryoholder and a high-resolution cooled Gatan 832 CCD digital camera.

Preparation of Fluoresceinamine-tagged PI-PCEMA-PtBA or PI-PCEMA-P(tBA-AA-Fl). To attach fluoresceinamine to the tiblock copolymer, less than 3 mol%

of the tBA units were hydrolyzed.[4] In an example run, 100 mg of PI-PCEMA-PtBA was dissolved in 1.75 mL CH_2Cl_2. Triethylsilane, 15.75 μL, and trifluoroacetic acid, 87.7 μL, were then added to the mixture. The mixture was stirred for 4 min before 15 mL of MeOH/H_2O (9/1 v/v) was rapidly added to it to quench the reaction and precipitate out the polymer. The polymer was washed five times by 5 mL of 9/1 v/v MeOH/H_2O mixture before drying in a vacuum oven.

Fluoresceinamine was reacted with the acrylic acid groups by amidization.[5] In an example run, 90 mg of PI-PCEMA-P(tBA-AA) was dissolved in 2 mL of anhydrous DMF in a 50 mL round bottom flask. To the solution was then added 2 mL of anhydrous DMF containing 4 mg of fluoresceinamine, 4.5 mg of 1-hydroxybenzotriazole, and 6.3 mg of *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride. The mixture was wrapped in aluminum foil and magnetically stirred overnight. The mixture was then precipitated into 15 ml MeOH/H_2O (9/1 v/v) to recover the fluoresceinamine-labeled polymer. The precipitate was re-dissolved in 3 mL DMF and precipitated into MeOH/H_2O again. This last step was repeated until no fluoresceinamine was detected in the supernatant by UV measurements. The precipitate was then dried in a vacuum oven.

Fluorescence Microscopy Studies. The fluoresceine-tagged triblock PI-PCEMA-P(tBA-AA-Fl) was handled analogously as PI-PCEMA-PtBA to yield flower-like superaggregates at room temperature. To ensure the preparation of larger Pickering emulsion droplets, the magnetic stirring speed was dropped from the regularly-used 800 rpm to 400 rpm. The flowery aggregate solution freshly prepared at 22±2 ^{o}C by stirring polymer in DN/MeOH for 3 d was sealed between a glass slide and cover slip with epoxy resin cured at room temperature for 1 h. Fluorescence microscopy studies of such droplets were performed using a Nikon Eclipse TE2000-U inverted microscope.

Results and Discussion

Ribbon Cages. At 55±2 ^{o}C the dominant species 2 h after MeOH addition into DN solutions of either spherical or cylindrical micelles were ribbon cages as observed by TEM after sample aspiration on carbon-coated copper grids and solvent evaporation. Figure 1 shows more TEM images of ribbon cages formed from CMs.

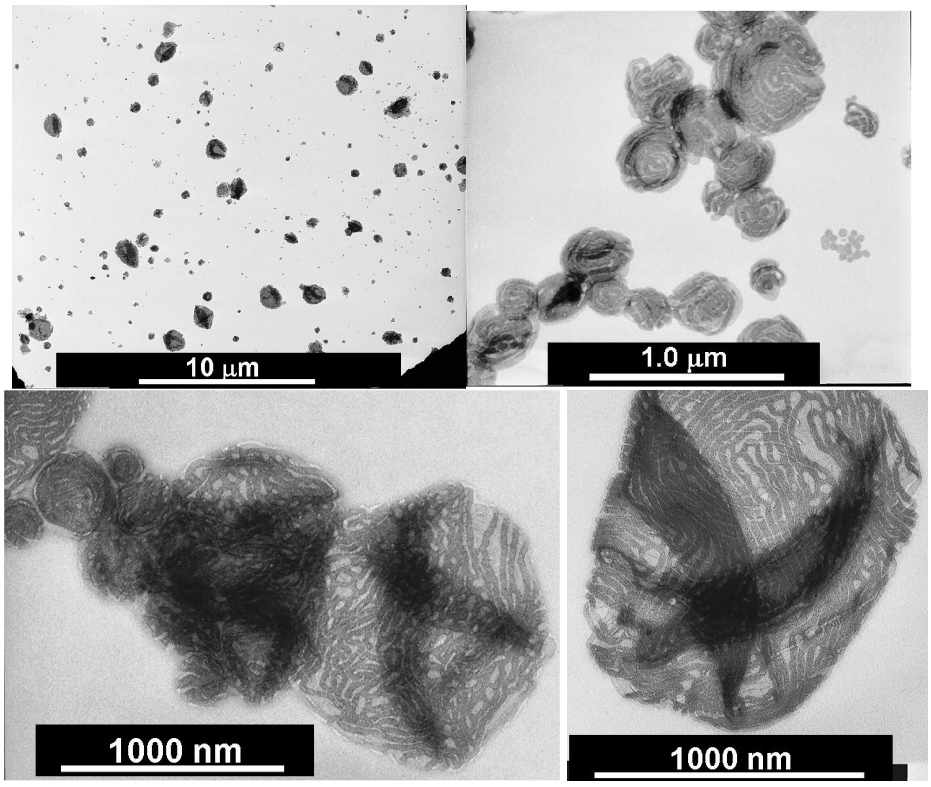


Figure 1. TEM images of ribbon cages from CMs at 52±2 ^{o}C 3 d after MeOH addition.

Figure 2 shows TEM images of ribbon cages formed from SMs at 52±2 ^{o}C.

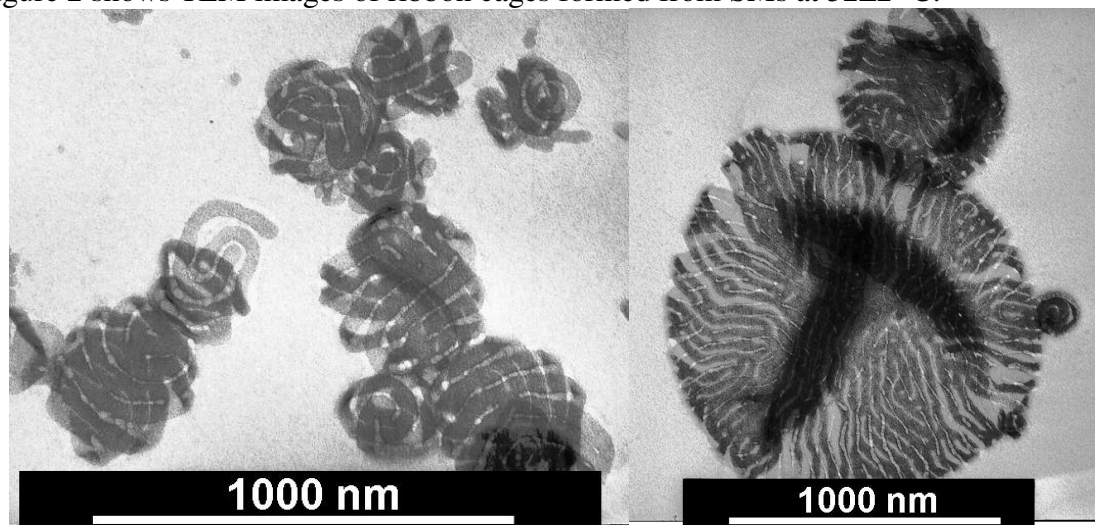


Figure 2. TEM images of ribbon cages from SMs at 52±2 ^{o}C.

To see the structure of the ribbon cages in their native state, we also attempted cryo-TEM. Figure 3 shows a low-magnification cryo-TEM image for the RIBBON CAGES. For the large size of the ribbon cages, few of them fell into the holes of the cellulose supporting film. The image shows, however, clearly that ribbon cages existed.

Unfortunately, most of them seemed to exist in a deflated state mostly like due to methanol evaporation during the blotting stage of specimen preparation.

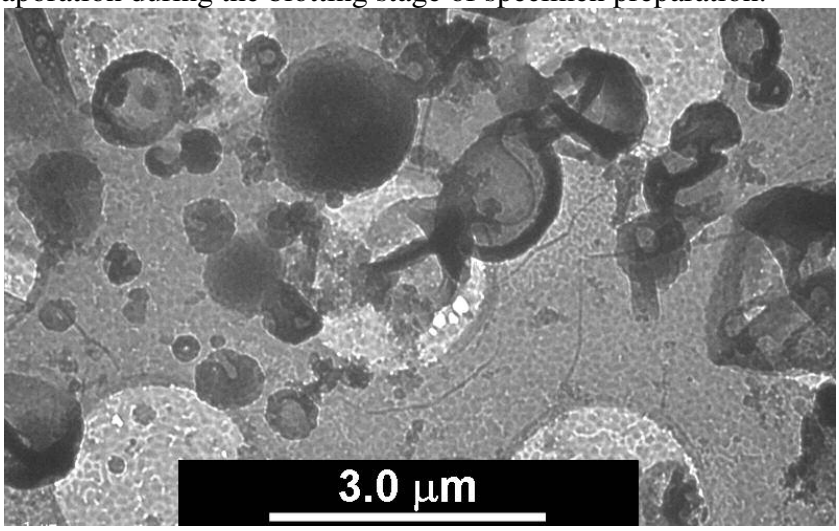


Figure 3. A low magnification image of ribbon cages from cryo-TEM.

Figure 4 shows cryo-TEM images of two ribbon cages at higher magnifications. In the left image one can discern the presence of some ribbons on the surface of the deflated ribbon cage. We also see some white spots which were most likely due to electron beam damage. The contrast is definitely much lower for the lack of staining than that seen in the routine TEM images of Figures 1 and 2 of the main text. The droplet on the right contained probably crystallized methanol in the core and we cannot see the content in the particle center. The presence of bumpy polymer-like structures on the surface of the particle is evident.

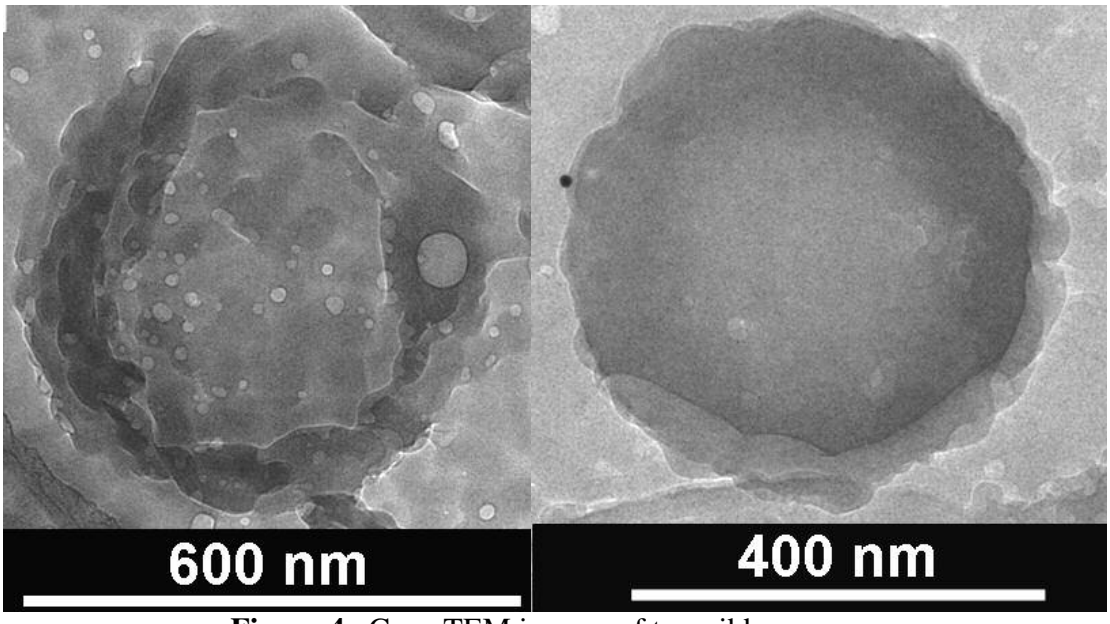


Figure 4. Cryo-TEM images of two ribbon cages.

Ribbon Cage Formation Kinetics at 55±2 °C **from CMs.** Figure 5 shows more room temperature TEM images of precursors to ribbon cages from CMs at 52±2 °C.

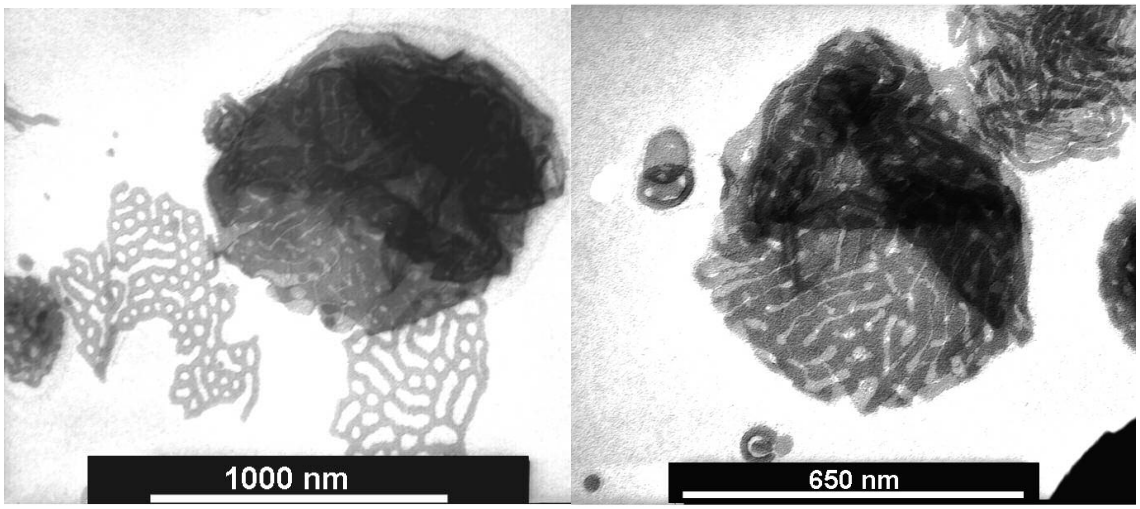


Figure 5. TEM images of precursors to ribbon cages from CMs 30 min (left) and 120 min (right) after MeOH addition.

Kinetics of SM Assembly at RT. Figure 6 shows room-temperature TEM images of superaggregates from SMs 30 and 120 min after MeOH addition.

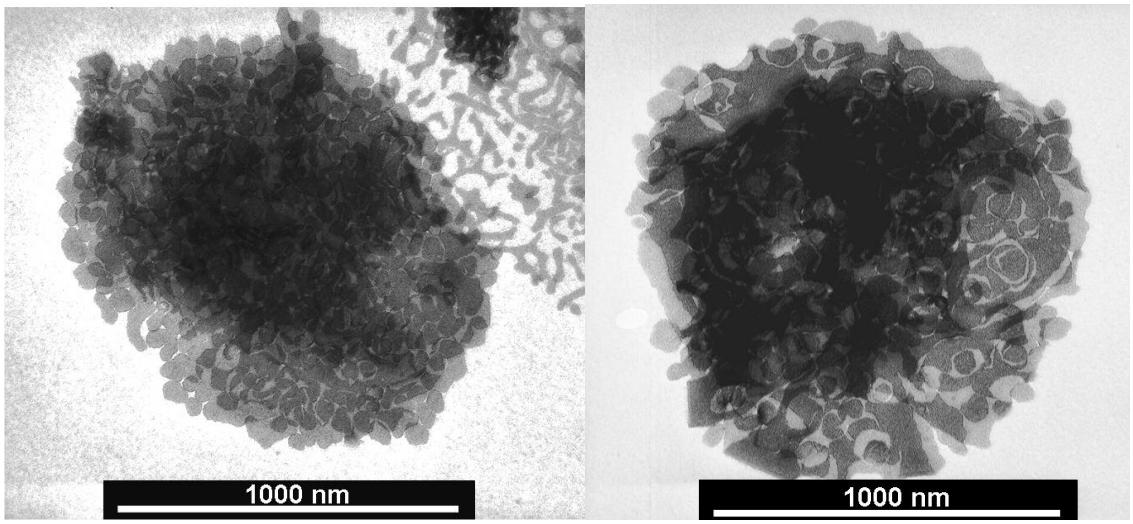


Figure 6. TEM images of superaggregates from SMs 30 min (left) and 120 min (right) after MeOH addition.

More TEM image of the Flower-like Superaggregates. The flower-like aggregates are more diversified in appearance. To offer readers a better feel, Figure 7 shows more TEM images of some of the super-aggregates.

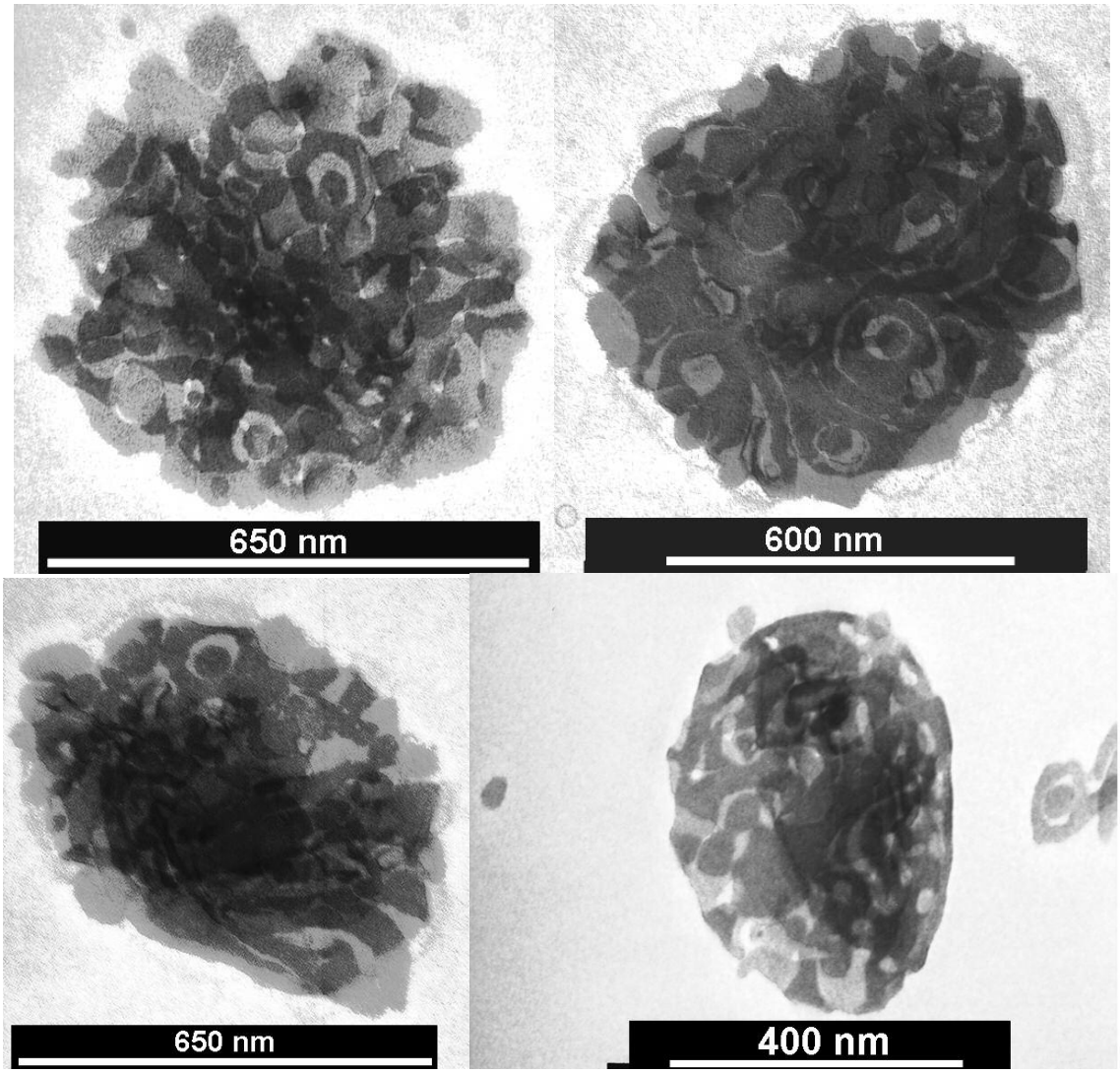


Figure 7. More TEM images of flowery superaggregates from SMs 2 d after MeOH addition into DN.

Fluorescence Microscopic Images. Figure 8 shows a fluorescence microscopic image of flowery superaggregates from PI-PCEMA-P(tBA-AA-Fl). Since only the rims of the aggregates emit fluorescence, this suggests unambiguously the assembly of the triblock at the DN/MeOH interface and at the interface only. It should be pointed out that the thin assembled PI-PCEMA-P(tBA-AA-Fl) layer was seen by fluorescence microscopy because the blooming thickness of the layer was much larger than the physical thickness.

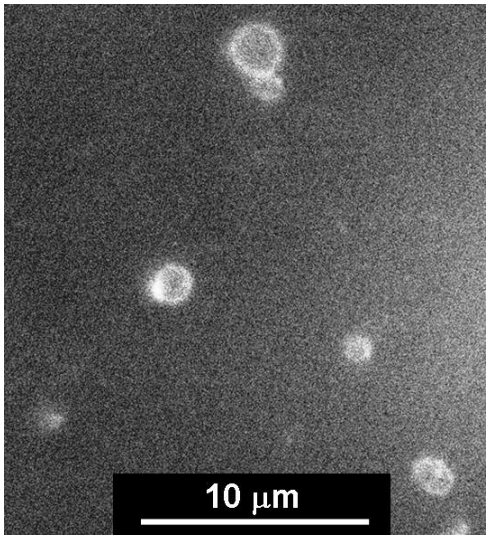


Figure 8. Fluorescence microscopy image of flowery aggregates of PI-PCEMA-P(tBA-AA-Fl).

AFM Images of the Superaggregates. Figure 9 shows topography images for one ribbon cage and one flowery superaggregate formed 2 d after MeOH addition from CMs and SMs at 52±2 and 22±2 °C, respectively. The structures all appear deflated due to solvent evaporation.

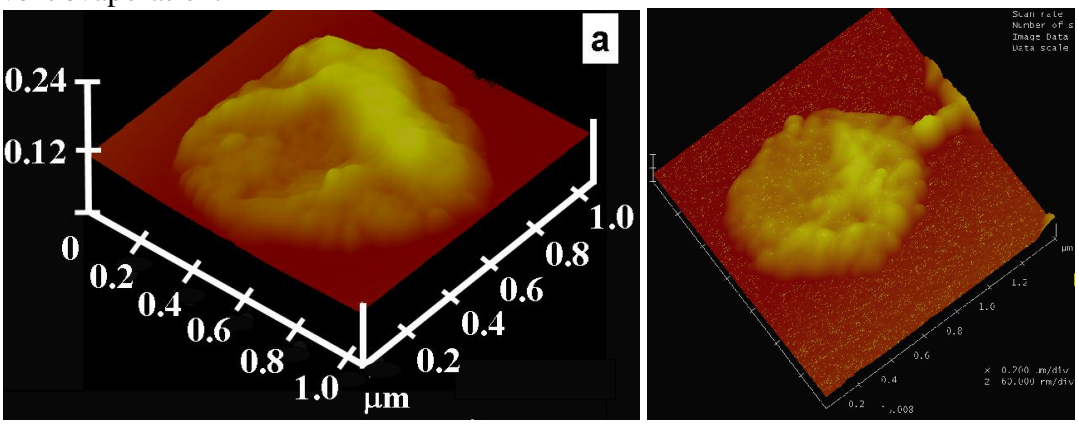


Figure 9. Topography images of one ribbon cage and one flowery superaggregate. The samples were derived from the CM and SM precursors, respectively.

Assembly of Crosslinked PI-PCEMA-PtBA Nanocylinders. We photocrosslinked the PCEMA core of the CMs to a CEMA double bond conversion of 15% in DN and then added methanol to such a solution. The crosslinked cylindrical MAs did not assemble as well as the pristine micelles probably due to the inability of the cylinders to undergo fission and fusion. Figure 10 shows a TEM image of assembled crosslinked PI-PCEMA-PtBA cylinders.

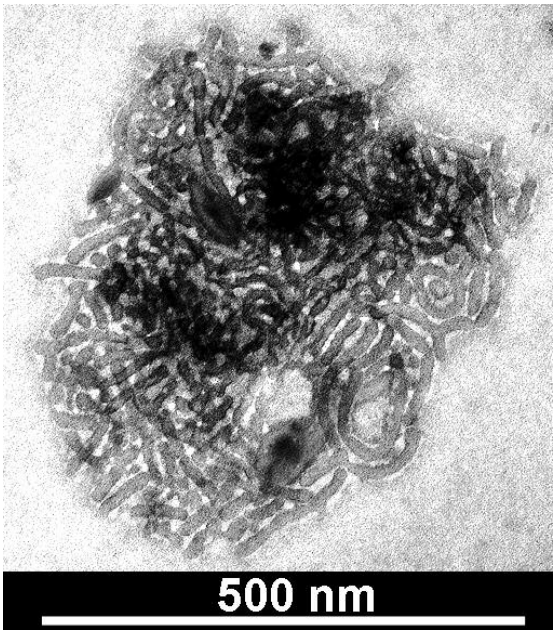


Figure 10. TEM image of superaggregates from crosslinked PI-PCEMA-PtBA cylinders 2 d after MeOH addition into DN.

Solubility of Methanol in DN and DN in Methanol. Our ^{1}H NMR analyses of DN- and MeOH-rich phases at different temperatures yielded the solubility values for DN in MeOH and MeOH in DN (Table 1). The compatibility between MeOH and DN evidently improved and thus γ_{12} decreased at the higher temperature.

Table 1. Solubility values of DN in MeOH and MeOH in DN at different temperatures.

Temperature (^{o}C)	**DN in methanol Molar ratio (volume ratio)**	**Methanol in DN Molar ratio (volume ratio)**
22±2	1/31 (1/7.8)	1/15 (1/60.2)
52±2	1/23 (1/5.9)	1/5 (1/19.4)

ρ_{DN}= 0.881g/cm^3, ρ_{MeOH}=0.792g/cm^3

PtBA Chain Distribution in the Ribbons of the Ribbon Cages. We made a huge effort trying to determine the PtBA chain distribution in the corona of the nanoribbons in the ribbon cages. This involved crosslinking the PCEMA core of the nanoribbons, hydrolyzing the PtBA chains, staining the resultant PAA chains with uranyl acetate, and ultrosonicating the ribbon cages to break them partially into nanoribbons. Figure 9 show a TEM image of such ribbons. This image suggests that the PtBA chains filled the cracks between the PCEMA ribbons in agreement with results from AFM and TEM (AFM did not see any cracks between the PCEMA ribbons but TEM did because PtBA was not stained by RuO_4).

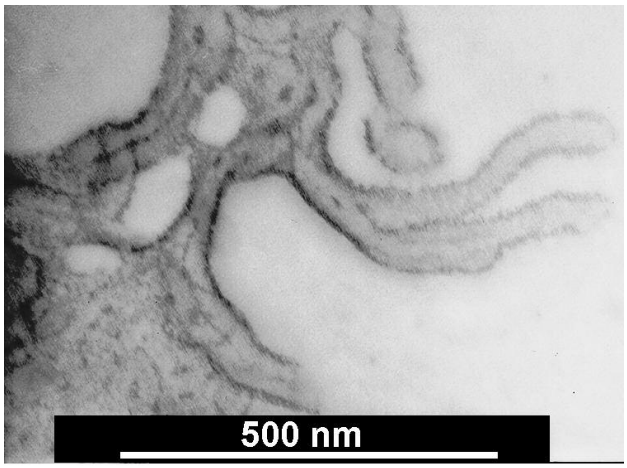


Figure 11. TEM image of nanoribbons of ribbon cages after PCEMA crosslinking, PtBA hydrolysis, and PAA staining by uranyl acetate.

Since only PtBA is soluble in methanol, we suspect that the PtBA and PI coronal chains are packed as illustrated in Figure 12.

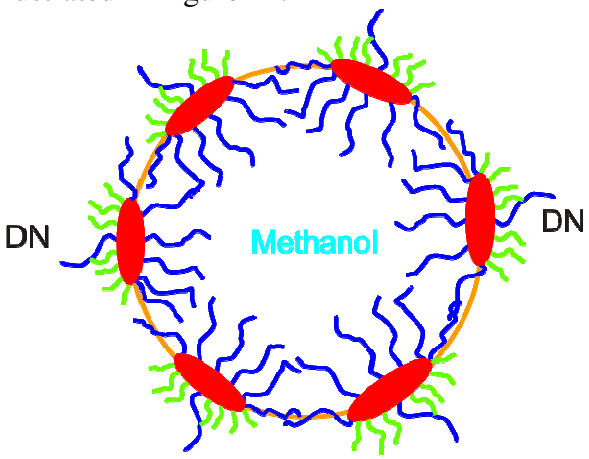


Figure 12. Schematic cross-sectional view of coronal chain packing in a PI-PCEMA-PtBA nanoribbon formed at 52±2 ^{o}C. The shorter green PI chains stretch probably exclusively into the DN phase and most of the longer blue PtBA chains stretch into the methanol phase and the DN/MeOH interface. The collapsed red PCEMA chains anchor at the DN/MeOH interface.

References

1. **Russell, J. T.; Lin, Y.; Boker, A.; Su, L.; Carl, P.; Zettl, H.; He, J. B.; Sill, K.; Tangirala, R.; Emrick, T.; Littrell, K.; Thiyagarajan, P.; Cookson, D.; Fery, A.; Wang, Q.; Russell, T. P.** ***Angew. Chem. In. Ed. Engl.*** **2005, *44*, 2420-2426.**
2. **Stewart, S.; Liu, G.** ***Angew Chem Int Ed Engl*** **2000, *39*, 340-344.**
3. **Stewart, S.; Liu, G. J.** ***Chem. Mater.*** **1999, *11*, 1048-1054.**
4. **Zheng, R. H.; Wang, J. D.; Liu, G. J.; Jao, T. C.** ***Macromolecules*** **2007, *40*, 7601-7608.**
5. **Liu, G.; Yan, X.; Li, Z.; Zhou, J.; Duncan, S.** ***J. Am. Chem. Soc.*** **2003, *125*, 14039-45.**