Supplementary Information

Constructing Hierarchical Superstructures of Patchy Micelles and Modulation of Their Supracolloidal Assembly Pathway

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Experimental section

Materials

Commercially available chemicals were used as received unless otherwise stated. Styrene (>99%), 4-vinylpyridine (4VP, 95%), 2-cyano-2-propyl dodecyl trithiocarbonate (CPTTC, 97%), , 1,4-dibromobutane (99%), *N,N*-dimethylacetamide (DMAc, 99%), tetrahydrofuran (THF, 99%), dimethylsulfoxide (DMSO, 99.7%), methanol, ethanol,1, 4-dioxane (99.8%) and deuterium oxide (D_2O, 99.9%) were purchased from Sigma-Aldrich. Tris(2-phenylpyridinato)iridium(III) ($Ir(ppy)_3$, >95%) was purchased from TCI Chemicals. Styrene and 4VP were dried over CaH_2 and purified by vacuum distillation prior to polymerization.

Synthesis of PS-b-P4VP block copolymers

A series of polystyrene-*b*-poly(4-vinylpyridine) (PS-*b*-P4VP) block copolymers listed in Table 1 were synthesized via PET-RAFT polymerization. The P4VP block was first synthesized and subsequently used as a macro chain transfer agent (macro-CTA) for the synthesis of the PS block. A representative synthetic procedure for PS(85)-*b*-P4VP(15) is described below. For the synthesis of the P4VP macro-CTA, 4VP (4.0 g, 38 mmol) CPTTC (50 μL, 0.15 mmol) and $Ir(ppy)_3$ (0.5 mg, 0.76 μmol) were dissolved in DMSO (4.0 g) in a 20 mL vial equipped with a stirrer bar and a septum cap. After purging with N_2 for 15 min, the vial was placed in a blue LED light chamber (λ = 435 nm, 8.4 W) equipped with a stir plate

and an air circulator to minimize temperature changes induced by illumination. Polymerization was allowed to proceed upon irradiation of blue light and the progress of polymerization was monitored by ^{1}H NMR spectroscopy of reaction aliquots. After reaching the desired conversion, the light was turned off to stop polymerization. The mixture was diluted using methylene chloride (~ 30 mL) and poured into n-hexane (~ 1000 mL). The resulting precipitant was collected by vacuum filtration. After repeating the precipitation process twice, the precipitate was collected and dried in a vacuum oven at 50 °C overnight, yielding P4VP macro-CTA powder (2.2 g). For the subsequent synthesis of PS(85)-*b*-P4VP(15), the obtained P4VP macro-CTA (0.10 g) was combined with styrene (6.0 g, 0.058 mol), DMSO (9.0 g), and $Ir(ppy)_3$ (0.5 mg, 0.76 μmol) in a 20 mL vial equipped with a stirrer bar and a septum cap. After purging with N_2 for 15 min, polymerization was allowed to be proceed using the same protocol for P4VP macro-CTA synthesis. After reaching the desired conversion, the polymerization was terminated by turning off the light. The resulting mixture was diluted in methylene chloride (~ 10 mL) and poured into n-hexane (~ 1000 mL). The resulting precipitate was collected by vacuum filtration. After repeating the precipitation process twice, the resulting precipitant was collected and dried in a vacuum oven at 50 °C overnight, yielding PS(85)-*b*-P4VP(15) as a light-yellow powder(0.27 g).

Formation of PS-b-P4VP spherical micelles with the crosslinked cores

PS-*b*-P4VP (0.05 g) was dissolved in chloroform (12.5 g) using a 20 mL vial equipped with a stirrer bar. When the PS-*b*-P4VP was completely dissolved in chloroform, toluene (5.0 g), a selective solvent for the PS block, was injected into the solution dropwise using a syringe pump at 20 mL/h. Chloroform was then removed by rotary evaporation and a small amount of toluene is added to make up the total weight of the solution to 5.0 g, yielding a 1.0 wt% solution of PS-*b*-P4VP spherical micelles in toluene. This solvent-exchange route via chloroform was adopted because direct dissolution of PS-*b*-P4VP in toluene resulted in non-uniform micelles with low reproducibility. To crosslink the P4VP cores of the spherical micelles, 1,4-dibromobutane (0.84 μL, 7.1 μmol, 0.5 molar ratio to 4VP units) was added to the 1.0 wt% solution of PS-*b*-P4VP micelles, and the mixture was stirred at 50 °C for 2 days.

Preparation of patchy micelles

0.01 g of the 1.0 wt% core-crosslinked PS-*b*-P4VP spherical micelle solution in toluene was transferred into a 5 mL vial. Subsequently, 1.0 g of anhydrous DMF was added to the vial. The dispersion was kept for 1 min, yielding a 0.01 wt% solution of PS-*b*-P4VP patchy micelles dispersed in DMF.

Hierarchical colloidal assembly of patchy micelles

1.0 g of a 0.01 wt% dispersion containing PS-*b*-P4VP patchy micelles in DMF was filtered through a 0.2 μm syringe filter, and an aliquot (0.8 g) was transferred into a 5 mL vial. A DI water/DMF mixture (0.8 g, 12 wt% water) was then added using a syringe, adjusting the final water content to 6 wt%. The resulting dispersion was incubated at 30 °C without agitation for 10 min (for PS(149)-*b*-P4VP(15), 5 min) to obtain colloidal clusters including colloidal dimers, or for 30 min to obtain ladder-like linear structures.

Transformation of two-patch micelles into single-patch micelles

Using a dispersion containing patchy micelles of PS(67)-*b*-P4VP(15) or PS(49)-*b*-P4VP(8), the same procedure described above was repeated, except that amount of the 12 wt% DI water/DMF mixture was reduced to 0.56 g to set the water content of the final dispersion to 5 wt%, where colloidal assemblies of patchy micelles were rarely observed. The resulting dispersion was incubationed at 30 °C without agitation for 10 min.

Modulation of the pathway of colloidal assemblies

To induce the formation of supracolloidal chains from a dispersion containing patchy micelles of PS(49)-*b*-P4VP(8), the same procedure described above was repeated, except that 0.8 g of a 50 wt% DI water/DMF mixture was added to set the water content of the final dispersion to 25 wt%. The resulting dispersion was incubated at 30 °C without agitation for 1 day.

Reversibility of patch-number reconfiguration and supracolloidal assembly

To examine the reversibility of patch-number reconfiguration and supracolloidal assembly, two sets of experiments were performed using PS(49)-*b*-P4VP(8) patchy micelles. In the first set, 1.0 g of the 0.01 wt% dispersion containing patchy micelles of PS(49)-*b*-P4VP(8) in DMF was diluted with a 10 wt% DI water/DMF mixture to set the water content of the dispersion to 5 wt%, at which point the patchy micelles were transformed into single-patch micelles. In the second set, the same procedure was repeated using a 12 wt% DI water/DMF mixture to set the water content to 6 wt%, and the resulting dispersion was incubated at 30 °C without agitation for 30 min to form ladder-like linear structures. In both sets, water was subsequently removed by vacuum evaporation until the total mass of the dispersion reached 0.8 g, and anhydrous DMF (0.2 g) was added to restore the total mass to 1.0 g.

Synthesis of gold nanoparticle arrays

Dispersions of colloidal dimers or ladder-like linear structures of PS(49)-*b*-P4VP(8) were drop-cast onto carbon-coated TEM grids, and excess solvent was blotted with filter paper. The grids were dried in ambient condition overnight. The dried grids were then immersed in an aqueous solution containing 10 mM $HAuCl_4 \cdot 3H_2O$ and 0.1 wt% HCl for 10 min. After loading the gold precursors, excess solution was blotted with filter paper, and the polymer templates were removed by oxygen plasma treatment (60 W, 20 s), yielding arrays of gold nanoparticles on the grids. For the control experiment, drop-cast colloidal dimers of PS(49)-*b*-P4VP(8) were subjected to the same oxygen plasma treatment without prior immersion in the $HAuCl_4$/HCl solution.

Characterizations

^{1}H nuclear magnetic resonance (NMR) spectra were acquired using a Varian NMR System operating at 500 MHz. Gel permeation chromatography (GPC) was performed on an Agilent 1260 Infinity GPC system equipped with a PL gel 5-μm Mixed-D column and a differential refractive index detector, using DMF containing LiBr (0.01 M) as the eluent at a flow rate of 1.0 mL/min. Dynamic light scattering (DLS) analysis was carried out with a DLS-8000 instrument (Otsuka Electronics) using a He–Ne laser (632 nm) at a fixed scattering angle

of 90°. Scanning electron microscopy (SEM) was conducted using a Carl Zeiss SUPRA 55VP (2 kV) and a Hitachi S-4300 (15 kV). Transmission electron microscopy (TEM) was performed on a Hitachi H-7600 microscope operating at 100 kV. TEM samples were prepared by dropping a diluted solution (less than 0.01 wt%) of micelles or assemblies onto a carbon-coated TEM grid, blotting excess solvent with filter paper, and drying in ambient condition for overnight. The TEM samples were exposed to RuO_4 or I_2 vapors to selectively stain PS and P4VP domains, respectively. Cryo-TEM imaging was performed to verify the formation of ladder-like linear structures in solution. The dispersion containing ladder-like linear structures of PS(49)-*b*-P4VP(8), prepared by adding water to 6 wt% and incubating at 30 °C for 30 min, was used as the sample. Cryo-EM grids (Quantifoil R1.2/1.3 200 Cu with 2 nm carbon film) were glow-discharged for 10 s. A 3 μL droplet of the sample was applied to the grid using a Vitrobot Mark IV (Thermo Fisher Scientific, CMCI in Seoul) and blotted for 2 s. After plunge-freezing in liquid ethane, images were collected on a 200 kV cryo-TEM Glacios (Thermo Fisher Scientific) equipped with a Falcon 4 detector (Thermo Fisher Scientific) at a total electron dose of 10–20 $e^{-}/Å^2$.

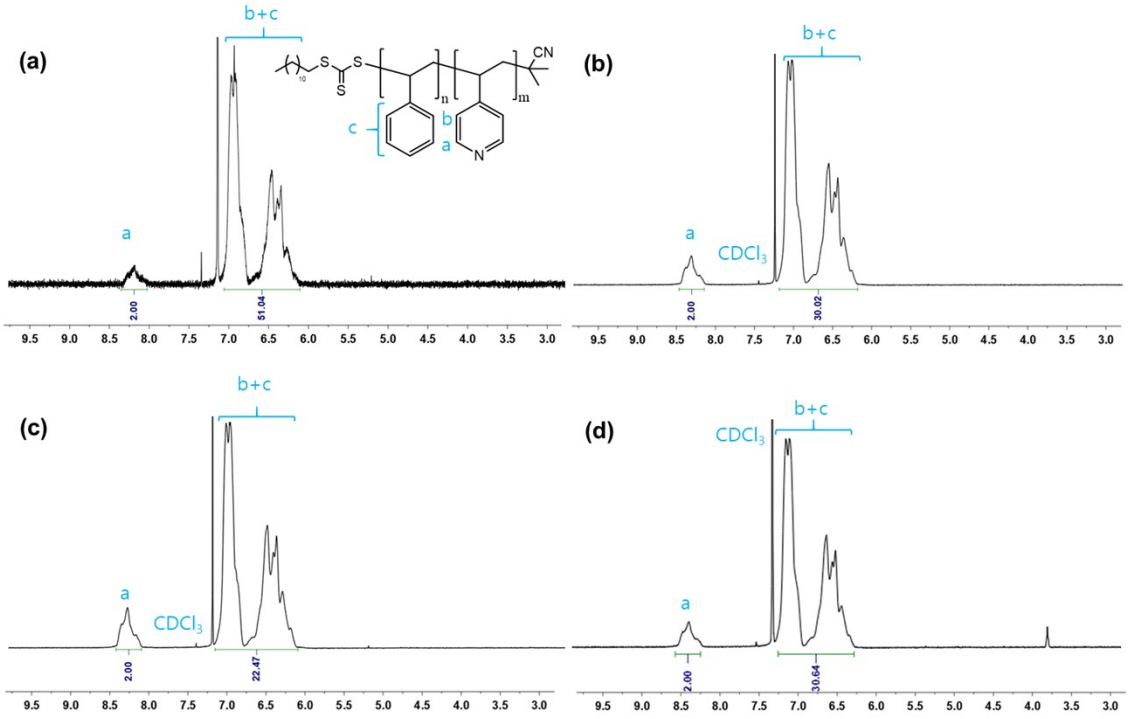


Fig. S1 ^{1}H NMR spectra of PS-*b*-P4VPs: (a) PS(149)-*b*-P4VP(15); (b) PS(85)-*b*-P4VP(15); (c) PS(67)-*b*-P4VP(15); (d) PS(49)-*b*-P4VP(8).

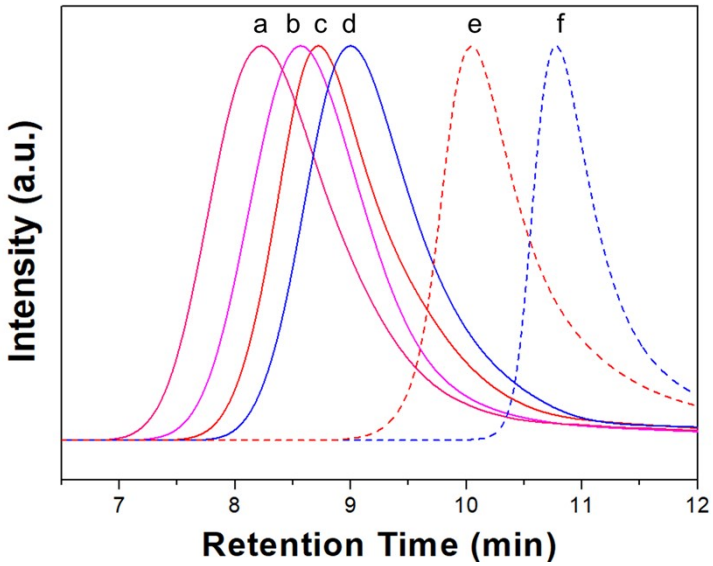


Fig. S2 GPC profiles: (a) PS(149)-*b*-P4VP(15); (b) PS(85)-*b*-P4VP(15); (c) PS(67)-*b*-P4VP(15); (d) PS(49)-*b*-P4VP(8); (e) P4VP(15); (f) P4VP(8).

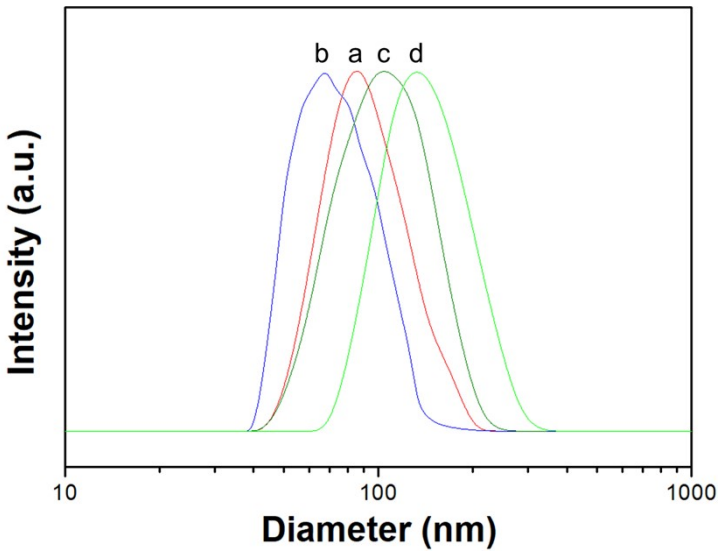


Fig. S3 DLS curves of PS(85)-*b*-P4VP(15): (a) toluene (D_h = 97 nm); (b) DMF (D_h = 74 nm); (c) H_2O/DMF 6 wt%, 10 min (D_h = 99 nm); (d) H_2O/DMF 6 wt%, 30 min (D_h = 142 nm). D_h denotes hydrodynamic diameter.

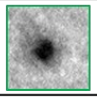
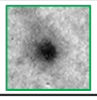
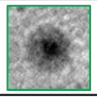
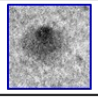
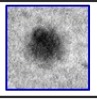
Solvent	Toluene	THF	1,4-Dioxane	DMAc	DMF
δ ($MPa^{1/2}$)	18.2	18.6	20.5	22.7	24.8
Dilution ×100					

Table. S1 Micelles of PS(85)-*b*-P4VP(15) prepared by 100-fold dilution of the toluene solution of spherical micelles into each solvent, with solvents having various solubility parameters (δ). Green and blue outlines indicate spherical micelles and patchy micelles, respectively. All the images were obtained after I_2 staining. The size of each TEM image is 100 nm × 100 nm.

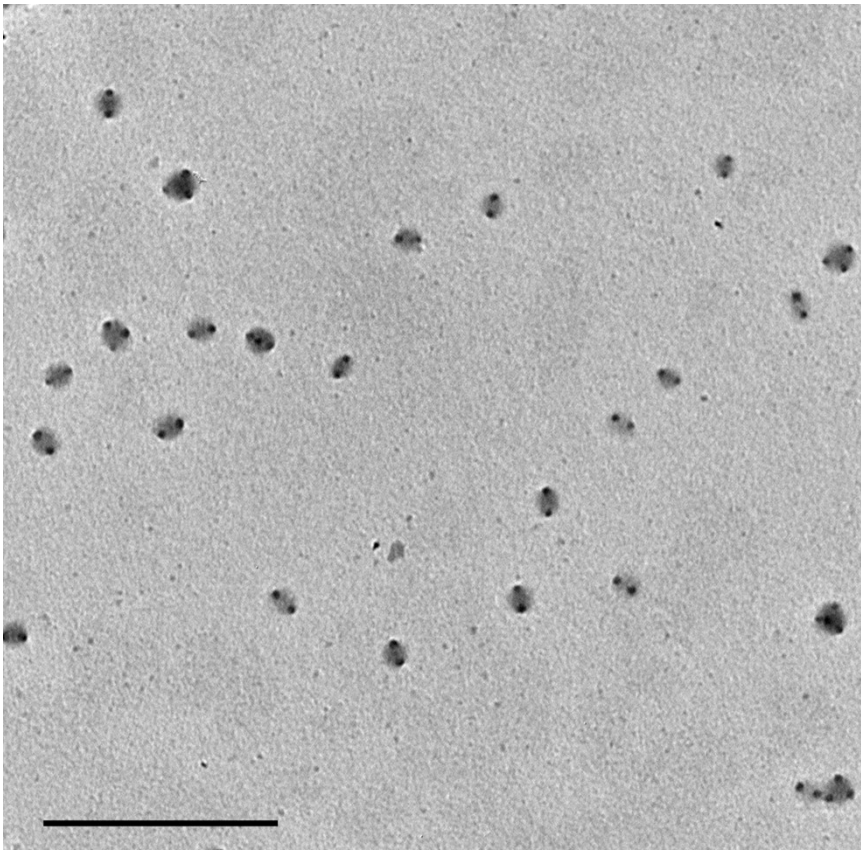


Fig. S4 Large-area TEM image of dimers of patchy micelles of PS(85)-*b*-P4VP(15). The image was obtained after I_2 staining. The scale bar is 500 nm.

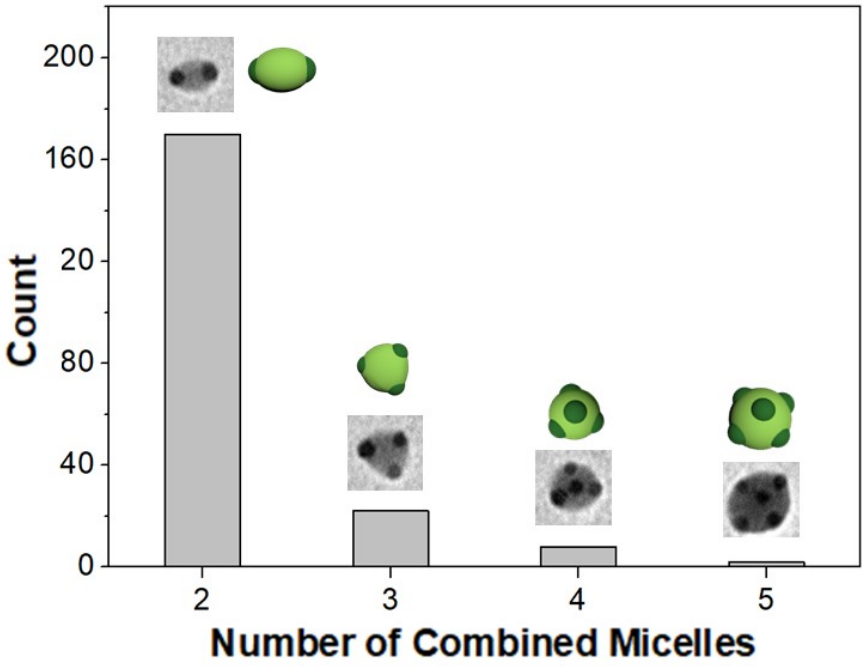


Fig. S5 Histogram of populations of the assemblies from PS(85)-*b*-P4VP(15) consisting of different numbers of patchy micelles. Each TEM image (100 nm × 100 nm), which was obtained after I_2 staining, of the assembly is given on the corresponding bar.

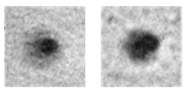
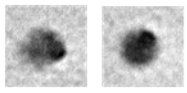
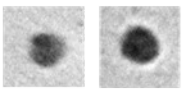
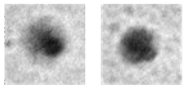
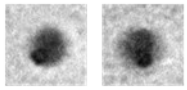
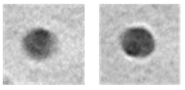
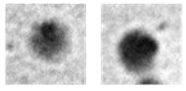
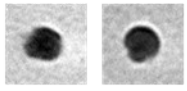
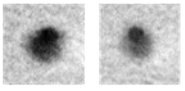
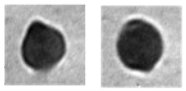
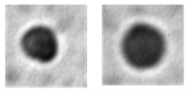
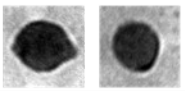
Solvent	Methanol	DMSO	Ethanol
δ (MPA ½)	29.7	26.4	26.2
6 wt%			
15 wt%			
30 wt%			
50 wt%			

Table. S2 Supracolloidal assembly behavior of PS(85)-*b*-P4VP(15) patchy micelles in DMF upon addition of polar solvents observed after 10 min (left) and 30 min (right) of incubation. Blue and orange outlines indicate patchy micelles and colloidal dimers, respectively. The size of each TEM image is 100 nm × 100 nm.

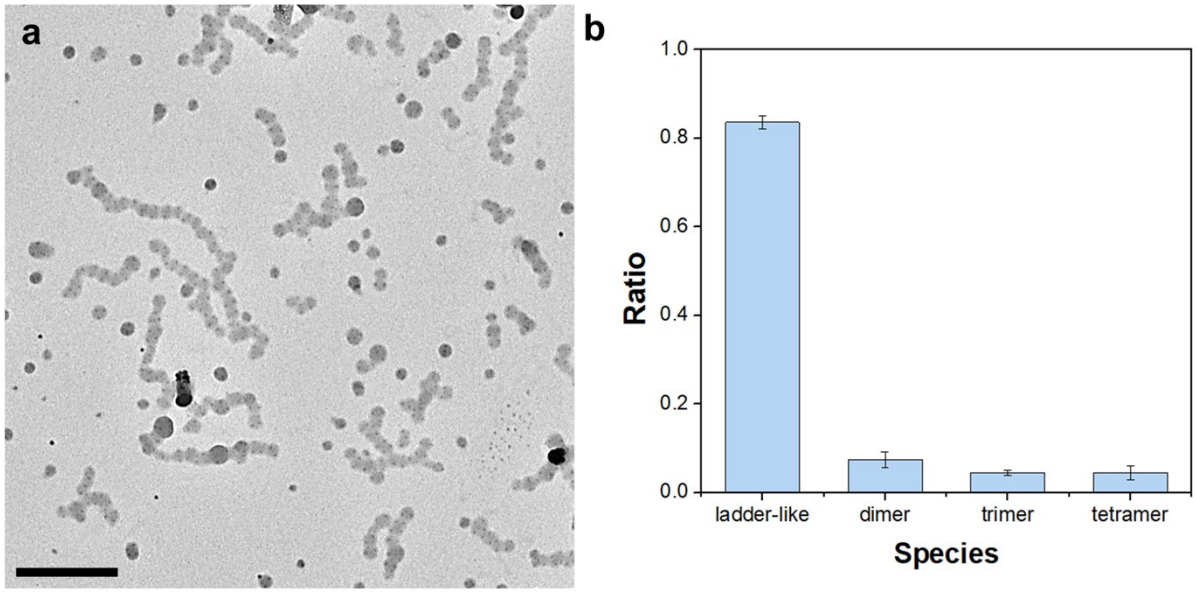


Fig. S6 (a) Large-area TEM image of ladder-like linear structures formed from PS(85)-*b*-P4VP(15) patchy micelles at 6 wt% water content after 30 min of incubation. The image was obtained after I_2 staining. The scale bar is 500 nm. (b) Histogram showing the ratio of each species based on the total number of P4VP cores counted. More than 300 cores were counted in each of three independent measurements, with error bars indicating standard deviations.

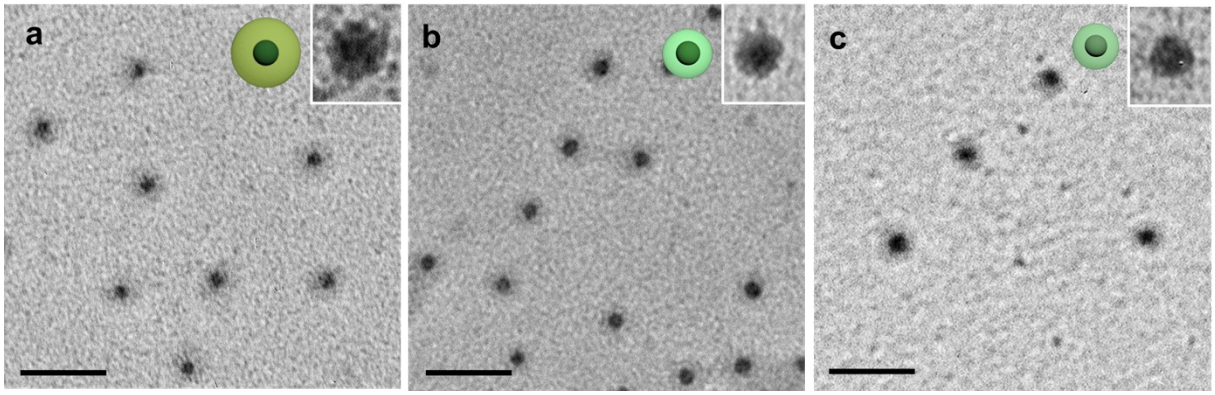


Fig. S7 TEM images of spherical micelles: (a) PS(149)-*b*-P4VP(15); (b) PS(67)-*b*-P4VP(15); (c) PS(49)-*b*-P4VP(8). The scale bars are 100 nm. All the images were obtained after I_2 staining. The insets are RuO_4-stained images at the same scale.

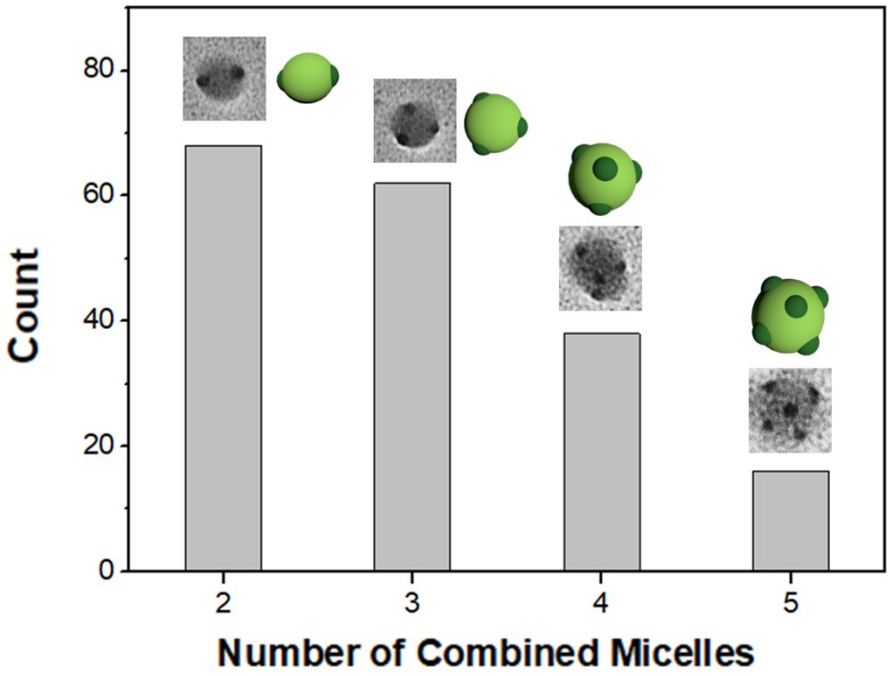


Fig. S8 Histogram of populations of the assemblies from PS(149)-*b*-P4VP(15) consisting of different numbers of patchy micelles. Each TEM image (120 nm × 120 nm), which was obtained after I_2 staining, of the assembly is given on the corresponding bar.

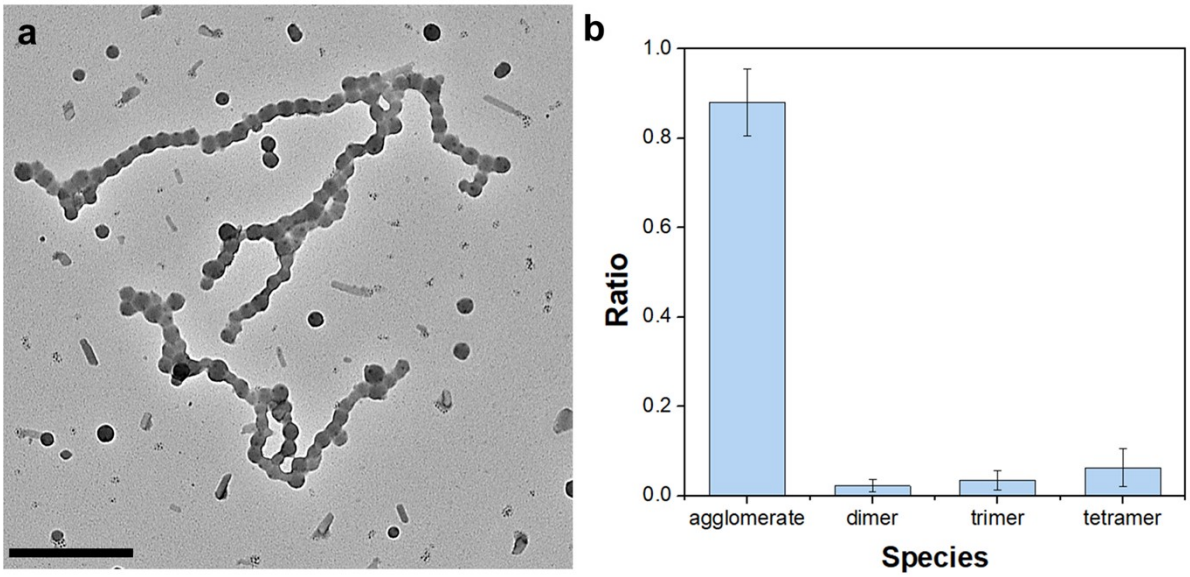


Fig. S9 (a) Large-area TEM image of disordered agglomerates formed from PS(149)-*b*-P4VP(15) patchy micelles at 6 wt% water content after 30 min of incubation. The image was obtained after I_2 staining. The scale bar is 500 nm. (b) Histogram showing the ratio of each species based on the total number of P4VP cores counted. More than 300 cores were counted in each of three independent measurements, with error bars indicating standard deviations.

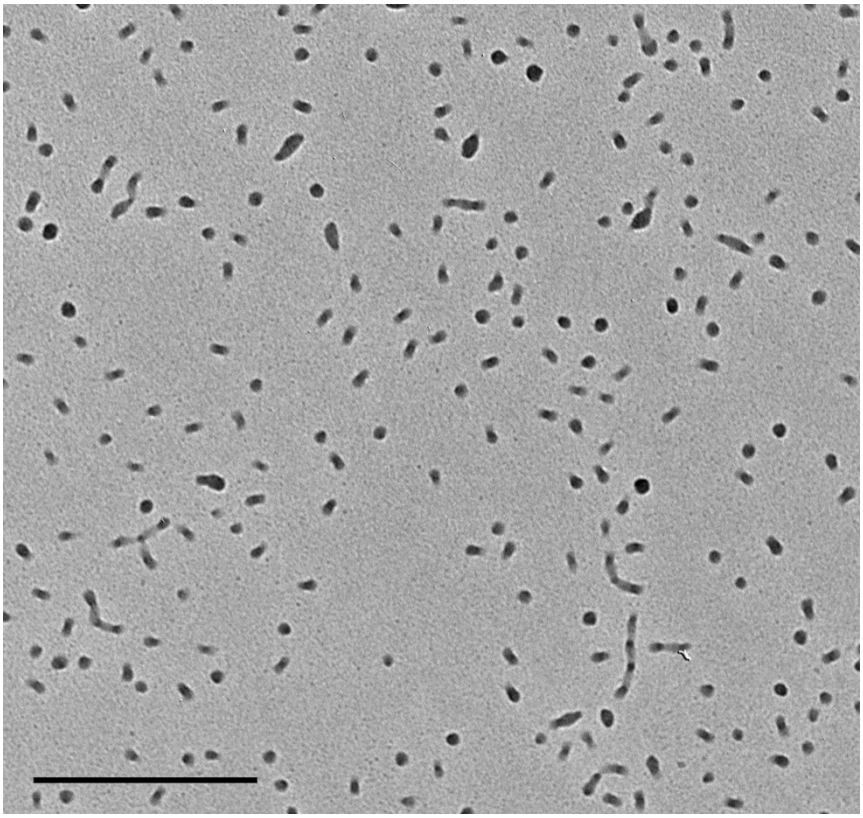


Fig. S10 Large-area TEM image of single- and two-patch micelles of PS(67)-*b*-P4VP(15). The scale bar is 500 nm.

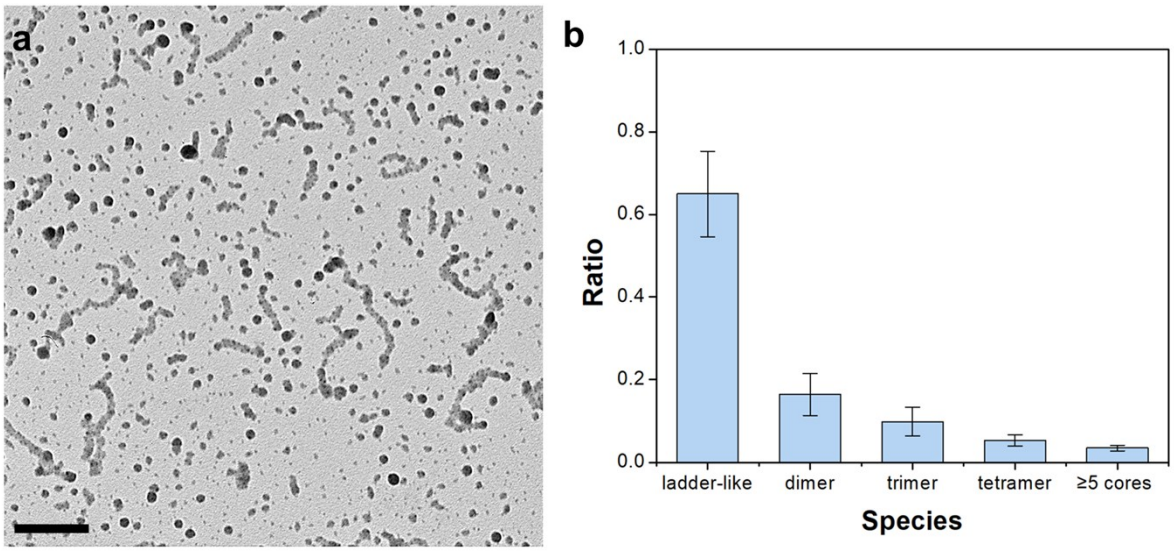


Fig. S11 (a) Large-area TEM image of ladder-like linear structures formed from PS(67)-*b*-P4VP(15) patchy micelles at 6 wt% water content after 30 min of incubation. The image was obtained after I_2 staining. The scale bar is 500 nm. (b) Histogram showing the ratio of each species based on the total number of P4VP cores counted. More than 300 cores were counted in each of three independent measurements, with error bars indicating standard deviations.

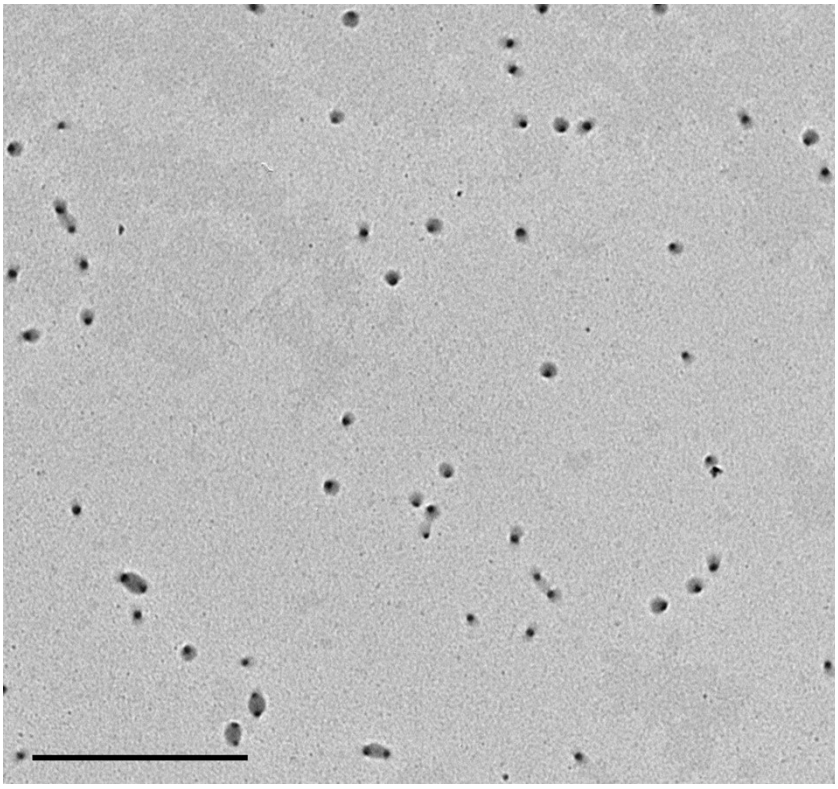


Fig. S12 Large-area TEM image of single- and two-patch micelles of PS(67)-*b*-P4VP(15) after adding water. The image was obtained after I_2 staining. The scale bar is 500 nm.

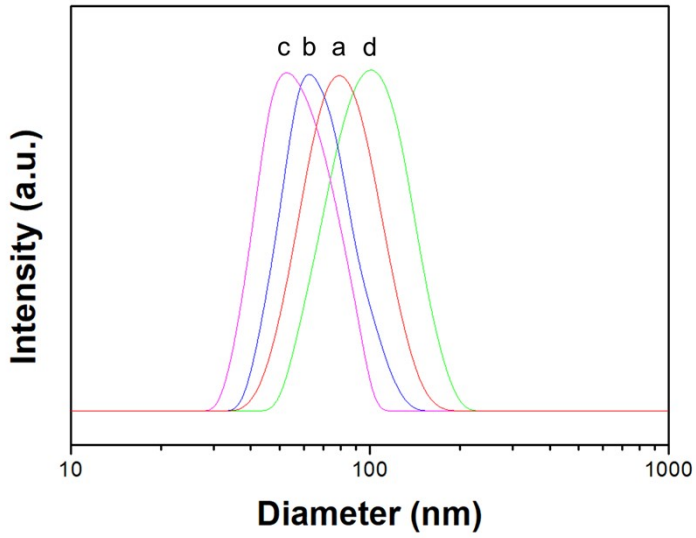


Fig. S13 DLS curves of PS(67)-*b*-P4VP(15): (a) toluene (D_h = 86 nm); (b) DMF (D_h = 66 nm); (c) H_2O/DMF 5 wt% (D_h = 55 nm); (d) H_2O/DMF 6wt%, 30 min (D_h = 102 nm).

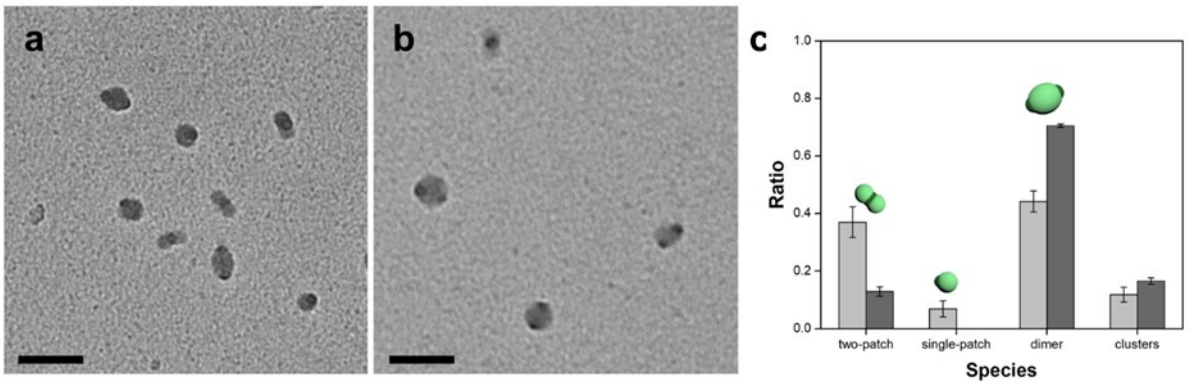


Fig. S14 TEM images of PS(67)-*b*-P4VP(15) after the addition of water (6 wt%), obtained (a) 1 min and (b) 10 min after water addition. (c) Histogram showing the population ratios of two-patch micelles, single-patch micelles, colloidal dimers, and trimers or higher-order clusters at 1 min (grey) and 10 min (black). More than 100 cores were counted in each of three independent measurements, with error bars indicating standard deviations. All the images were obtained after I_2 staining. The scale bars are 100 nm.

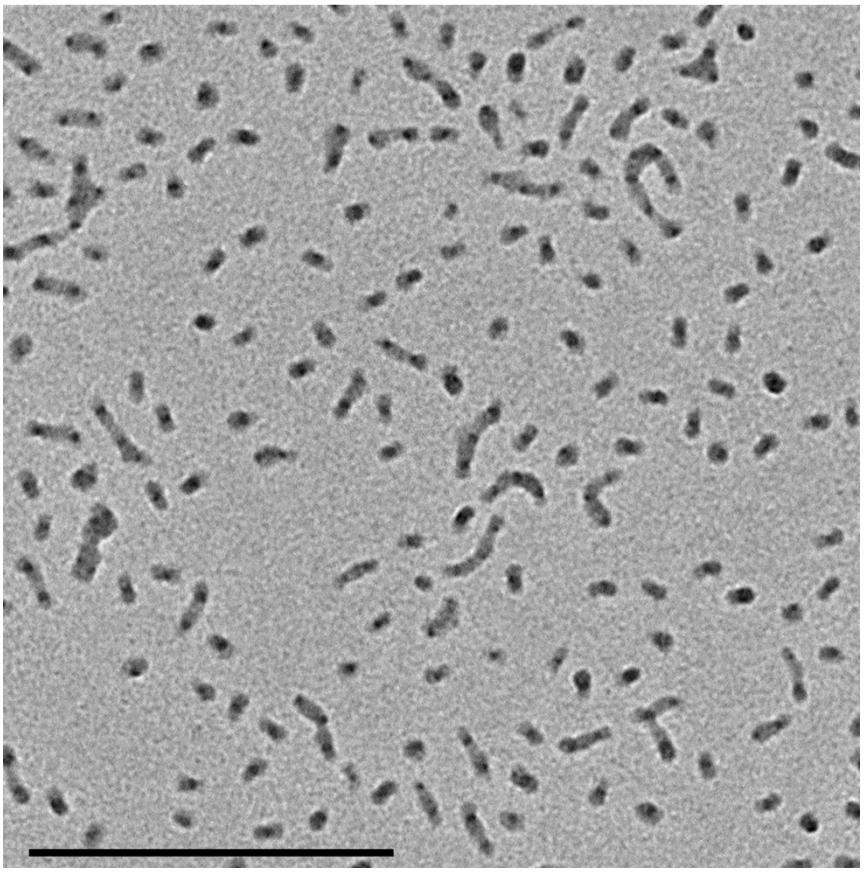


Fig. S15 Large-area TEM image of single- and two-patchy micelles of PS(49)-*b*-P4VP(8). The image was obtained after I_2 staining. The scale bar is 500 nm.

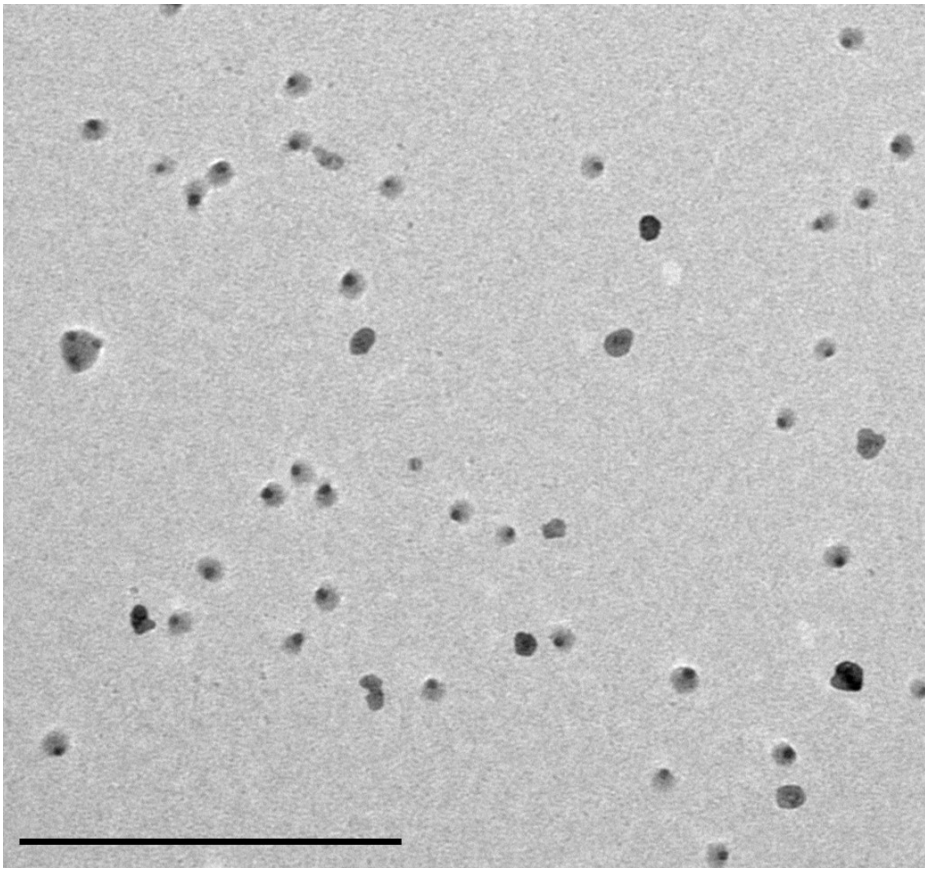


Fig. S16 Large-area TEM image of single- and two-patch micelles of PS(49)-*b*-P4VP(8) after adding water. The image was obtained after I_2 staining. The scale bar is 500 nm.

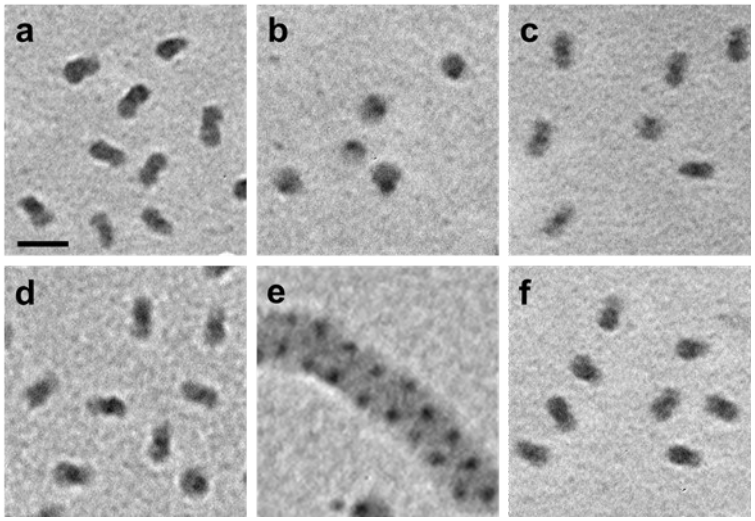


Fig. S17 Reversibility of patch number reconfiguration and supracolloidal assembly of PS(49)-*b*-P4VP(8) patchy micelles. (a, d) Two-patch micelles in DMF. (b) Single-patch micelles after adding water to 5 wt%. (c) Recovered two-patch micelles after removing water from (b) by vacuum evaporation. (e) Ladder-like linear structures after adding water to 6 wt% and incubating for 30 min. (f) Recovered two-patch micelles after removing water from (e) by vacuum evaporation. The scale bar is 50 nm.

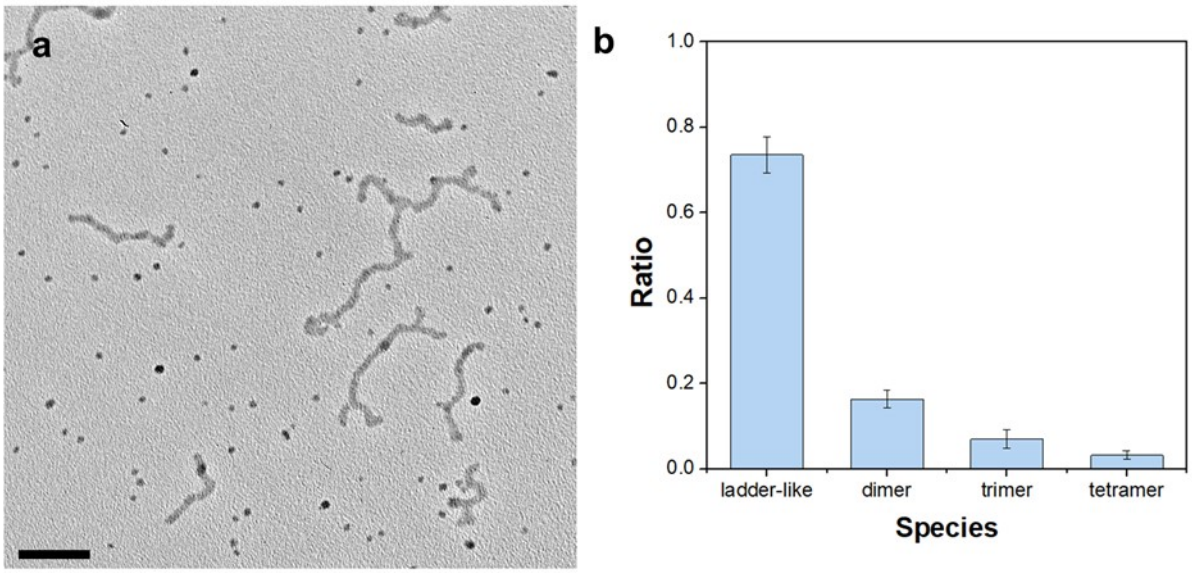


Fig. S18 (a) Large-area TEM image of ladder-like linear structures formed from PS(49)-*b*-P4VP(8) patchy micelles at 6 wt% water content after 30 min of incubation. The image was obtained after I_2 staining. The scale bar is 500 nm. (b) Histogram showing the ratio of each species based on the total number of P4VP cores counted. More than 300 cores were counted in each of three independent measurements, with error bars indicating standard deviations.

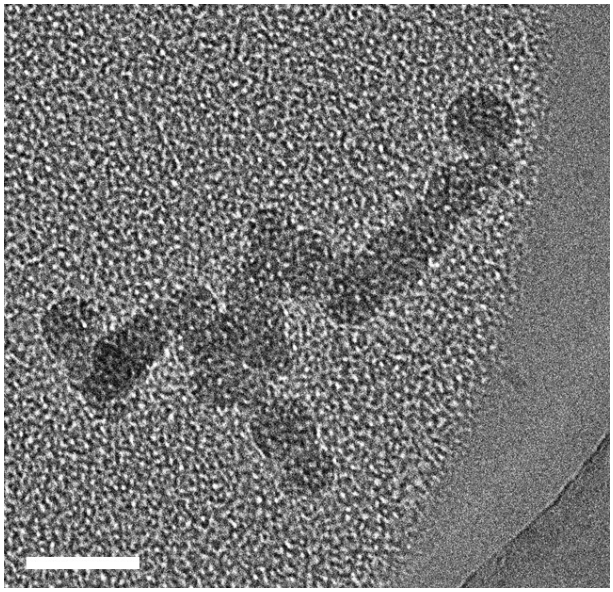


Fig. S19 Cryo-TEM image of a ladder-like linear structure obtained from PS(49)-*b*-P4VP(8) patchy micelles at 6 wt% water content after 30 min of incubation. The scale bar is 100 nm.

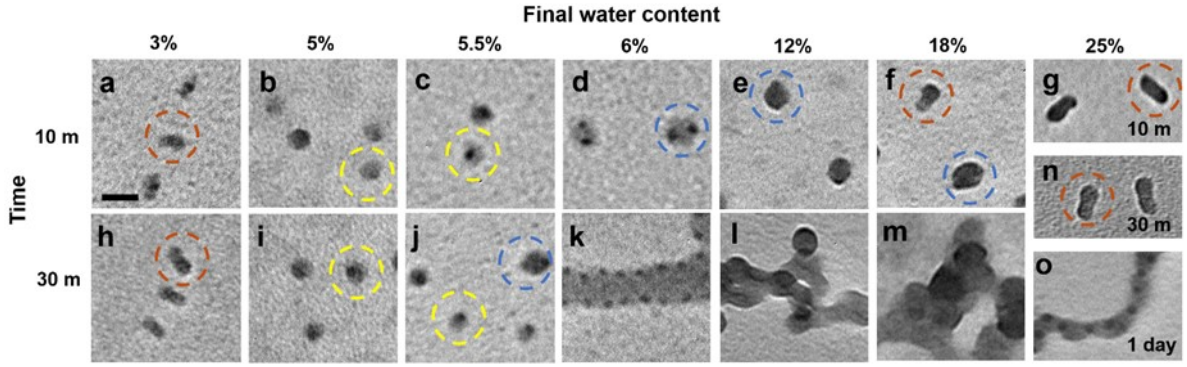


Fig. S20 TEM images of PS(49)-*b*-P4VP(8) patchy micelles in DMF/H_2O at different water contents after 10 min (top row) and 30 min (bottom row) of incubation, with the result at 25 wt% after 1 day additionally shown. Brown, yellow, and blue dashed circles indicate two-patch micelles, single-patch micelles, and colloidal dimers, respectively. The scale bar is 50 nm and applies to all images.

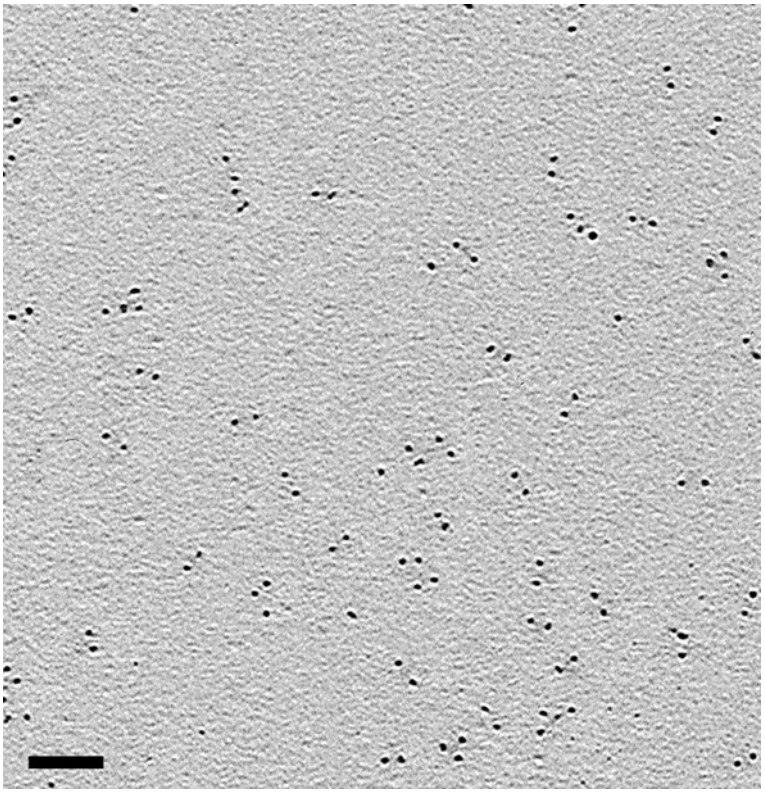


Fig. S21 Large-area TEM image of paired Au nanoparticles obtained using colloidal dimers of PS(49)-*b*-P4VP(8) as templates. The scale bar is 100 nm.

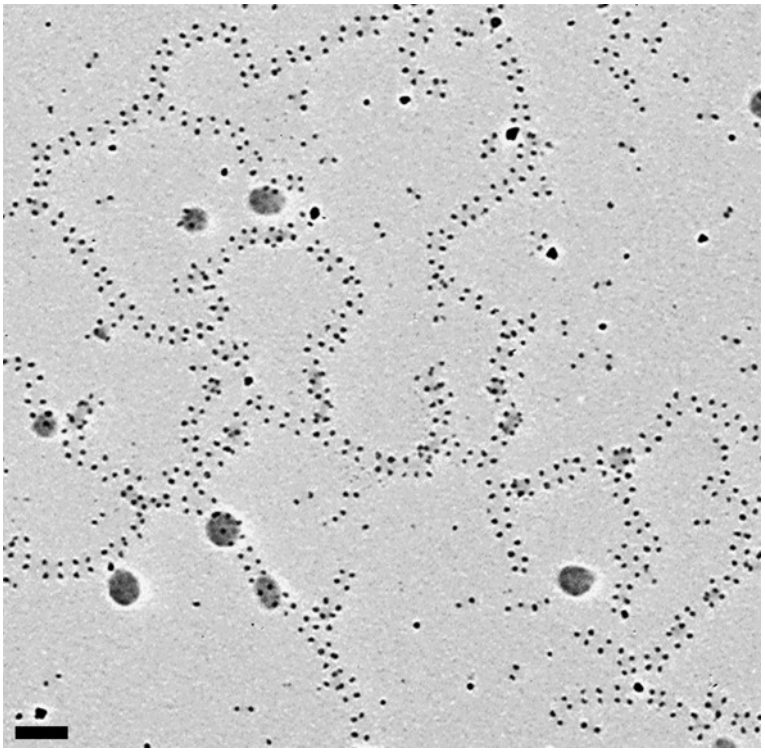


Fig. S22 Large-area TEM image of parallel twin arrays of Au nanoparticles obtained using ladder-like linear structures of PS(49)-*b*-P4VP(8) as templates. The scale bar is 100 nm.