Supporting Information

Rational design of nonlinear crystalline-amorphous-responsive terpolymers for pH-guided fabrication of 0D-3D nano-objects

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Experimental

Materials

All the starting chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. unless otherwise stated. Styrene (St, 99%, Meryer) was purified by successive washing with 5% NaOH aqueous solution and water, drying over $MgSO_4$ and distillation under reduced pressure, *tert*-butyl acrylate (*t*BA, 99%, Alfa-Aesar) was passed through a basic alumina column to remove the inhibitor, and ε-caprolactone (CL, 99%, Sigma-Aldrich) was distilled from CaH_2 under reduced pressure. 2,2′-Azobis(isobutyronitrile) (AIBN, 99%) was recrystallized twice from ethanol, and CuBr (98%) was purified by stirring in acetic acid and washing with ethanol before vacuum drying. 2,2-Bis(hydroxymethyl)propionic acid (97%, TCI), *N*,*N*′-dicyclohexylcarbodiimide (DCC, 95%, Macklin), 4-dimethylamino pyridine (DMAP, 97%, Macklin), stannous octoate ($Sn(Oct)_2$, 97%, Sigma-Aldrich), *N,N,N′,N″,N″*-pentamethyldiethylenetriamine (PMDETA, 99%, Aladdin), and other reagents with analytical grade were used as received. The organic solvents were dried and distilled according to standard procedures. 4-Cyanopentanoic acid dithiobenzoate (4-CPDB),[1] 2,2,5-trimethyl-(1,3)-dioxane-5-carboxylic acid (TDCA),[2] 2-(3-hydroxypropoxy)-6-methyl- benzaldehyde (HMBA),[3] and *N*-(2-(2-bromoisobutyroxy))ethyl maleimide (BEMI)[4] were synthesized and purified according to literature procedures.

Synthesis of FCHP

First, 3-(2-formyl-3-methylphenoxy)propyl 2,2-dihydroxymethylpropionate (FDHP) was synthesized by combination of esterification and deprotection. To a 250 mL of round-bottom flask was added HMBA (2.37 g, 12.2 mmol), TDCA (2.12 g, 12.2 mmol), DMAP (0.147 g, 1.2 mmol) and dry acetone (50 mL), and then about 50 mL of acetone solution containing DCC (2.70 g, 13.1 mmol) was slowly added to the solution cooled with ice water bath. Afterwards, the reaction was further conducted at ambient temperature for 24 h. After filtration, the solution was concentrated, and then THF (50 mL) was added to dissolve the crude product. The acid-catalyzed deprotection of ketal group was conducted by slow addition of 1.0 M HCl aqueous solution (13 mL) into the THF solution, followed by stirring at room temperature for 4 h. After reaction, saturated $NaHCO_3$ aqueous solution was added to quench the reaction, followed by concentration to remove THF. Dichloromethane (DCM, 150 mL) was added to the aqueous solution to extract the product, and then the organic phase was subjected to repeated washing with water and drying over $MgSO_4$. After concentration, the yellow liquid FDHP (3.08 g, 9.92 mmol) was obtained in 81.3% yield.

Second, esterification between FDHP and 4-CPDB was conducted to generate FCHP. To a 250 mL of round-bottom flask was added FDHP (2.06 g, 6.64 mmol), DCC (1.65 g, 8.0 mmol), DMAP (0.081 g, 0.66 mmol) and dry acetone (50 mL), and then about 30 mL of acetone solution bearing 4-CPDB (1.85 g, 6.62 mmol) was slowly added to the solution during 0-5 ^{o}C. Afterwards, the reaction was further conducted at 25 ^{o}C overnight. After filtration, the solution was collected and concentrated. The subsequent purification via flash column chromatography eluting with petroleum ether / ethyl acetate (2:1, *v*/*v*) mixtures gave the red solid FCHP (1.32 g, 2.31 mmol) in 34.9% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.66 (s, ArC*H*O, 1H), 7.91 (d, *J* 7.6, Ph*H*, 2H), 7.57 (t, *J* 7.2, Ph*H*, 1H), 7.32-7.45 (m, Ph*H* and Ar*H*, 3H), 6.82 (m, Ar*H*, 2H), 3.8-4.4 (m, CH_2OCO and ArOCH_2CH$_2$CH_2, 6H), 3.70 (d, *J* 5.2, CH_2OH, 2H), 2.3-2.8 (m, ArCH_3 and CH_2CH_2CO, 7H), 2.20 (m, ArOCH$_2$CH_2CH$_2$, 2H), 1.92 (s, CH_3, 6H), 1.22 (s, CH_3, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 222.33 (*C*=S), 192.11 (Ar*C*HO), 174.20, 171.65 (*C*=O), 162.31 (Ar*C*), 144.59 (Ph*C*), 142.28 (Ar*C*), 134.64 (Ar*C*), 133.24 (Ph*C*), 128.73 (Ph*C*), 126.81 (Ph*C*), 124.56 (Ar*C*), 123.59 (Ar*C*), 118.56 (*C*N), 110.02 (Ar*C*), 66.43, 65.09, 61.94 (*C*H$_2$O), 48.34, 45.81 (*C*CH$_3$), 33.46, 29.84, 28.70 (*C*H$_2$), 24.32 (*C*H$_3$), 21.58 (Ar*C*H$_3$), 17.72 (*C*H$_3$).

Synthesis of P1

To a Schlenk tube was added FCHP (0.228 g, 0.40 mmol), AIBN (6.6 mg, 0.040 mmol) and St (5.0 g, 48 mmol), and then dry toluene was added until the total volume was 12 mL. The solution was

subjected to degassing by bubbling with N_2 for 20 min, followed by polymerization at 60 °C for 16 h. The polymer solution was concentrated and precipitated from THF solution into cold methanol. After vacuum drying at 40 °C, 1.29 g (monomer conversion: 21.2%) of linear PSt_{26} (P1) was obtained as red powders. GPC and ^{1}H NMR analyses: $M_{n,GPC}$ = 3120 Da, $Đ_M$ = 1.09, and $M_{n,NMR}$ = 3280 Da (DP_{NMR} = 26). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.66 (s, C*H*O), 6.0-8.0 (m, Ph*H* of PSt, and terminal Ph*H* and Ar*H*), 4.5-5.0 (m, C*H* of terminal St unit), 3.75-4.45 (m, ArOCH_2 and CH_2OCO of end group), 3.62 (s, CH_2OH), 2.57 (s, ArCH_3), 0.7-2.5 (m, other C*H*, CH_2 and CH_3 originating from end group and PSt).

Synthesis of P2

To a Schlenk tube was added P1 (0.656 g, 0.20 mmol), St (3.13 g, 30 mmol), BEMI (0.116 g, 0.40 mmol) and AIBN (3.3 mg, 0.020 mmol), and then dry toluene was added until the total volume was 10 mL. After degassing via bubbling with N_2 for 20 min, the chain extension polymerization was conducted at 60 °C for 15 h. The polymer solution was precipitated into cold methanol, and then the precipitation from THF solution into methanol was repeated twice. After vacuum drying, 1.40 g (total monomer conversion: 22.9%) of linear triblock copolymer PSt_{26}-*b*-P(St_x-*co*-$BEMI_2$)-*b*-PSt_{30-x} (P2) was obtained as red powders. GPC and ^{1}H NMR analyses: $M_{n,GPC}$ = 6500 Da, $Đ_M$ = 1.10, and $M_{n,NMR}$ = 6980 Da (the numbers of monomer units were 56 for St unit and 2 for BEMI unit). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.66 (s, C*H*O), 6.0-8.0 (m, Ph*H* of St unit, and terminal Ph*H* and Ar*H*), 4.5-5.0 (m, C*H* of terminal St unit), 3.75-4.45 (m, ArOCH_2, and CH_2OCO of end group and BEMI unit), 3.62 (s, CH_2OH), 3.44 (m, CH_2N of BEMI unit), 0.7-3.1 (m, other C*H*, CH_2 and CH_3 originating from end group, BEMI unit and St unit).

Synthesis of P3

Cyclic triblock copolymer PSt_{26}-*b*-P(St_x-*co*-$BEMI_2$)-*b*-PSt_{30-x} (P3) was prepared by UV-induced Diels-Alder reaction at room temperature. A low-pressure mercury lamp was used to trigger the ring-closure reaction, in which the dose was around 100 μW cm^{-2}. In a typical run, P2 (20 mg) and DCM (500 mL) was added to a round-bottom flask, and the solution was subjected to UV irradiation for 15 h. Evaporation of the solvent allowed to recycle the solvent and isolate P3 as yellow solid powders. According to a similar procedure, fresh P2 and recycled DCM were used to repeatedly generate P3 until the total amount was about 240 mg. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.0-7.8 (m, Ph*H* of St unit, and Ph*H* and Ar*H* of linkage), 5.54 (m, ArC*H*OH), 4.34 (m, ArOCH_2), 3.80-4.26 (m, CH_2OCO of linkage and BEMI unit), 3.74 (m, ArCH_2), 3.62 (s, CH_2OH), 3.44 (m, CH_2N of BEMI unit), 0.7-3.2 (m,

other C*H*, CH_2 and CH_3 originating from linkage, BEMI unit and St unit).

Synthesis of P4 and P7

End group modification and ATRP were combined to generate starlike PSt_{26}-*b*-P(St_x-*co*-(BEMI-P*t*BA_{20})$_2$)-*b*-PSt_{30-x} (P4), followed by ATRP of *t*BA to achieve quasi-twin-tail tadpole-like *c*-[PSt_{26}-*b*-P(St_x-*co*-(BEMI-P*t*BA_{19})$_2$)-*b*-PSt_{30-x}] (P7). For synthesis of P4, radical-induced reaction using excess AIBN was performed to deactivate dithiobenzoate-bearing P2 in order to avoid potential effect of RAFT moiety on ATRP. The solution of P2 and 10-fold AIBN in toluene was heated at 85 ^oC for 3 h, followed by concentration, precipitation and vacuum drying to give deactivated P2. Afterwards, deactivated P2 (0.345 g, 0.10 mmol of bromide functionality), *t*BA (1.28 g, 10 mmol), CuBr (14.3 mg, 0.10 mmol) and PMDETA (17.3 mg, 0.10 mmol) were added to a Schlenk tube under nitrogen, and degassed acetone was added to reach a total volume of 2.5 mL. The contents were subjected to three freeze-vacuum-thaw cycles, followed by polymerization at 60 ^oC for 12 h. The catalyst was removed by dilution and passing through a short alumina column. The polymer solution was concentrated, and the polymer was isolated by repeated precipitation from THF solution to hexane. After vacuum drying, 0.604 g (monomer conversion: 20.2%) of P3 was obtained. Similarly, ATRP of *t*BA initiated with P3 macroinitiator was conducted to prepare P7.

P4: GPC and ^{1}H NMR analyses: $M_{n,GPC}$ = 10500 Da, $Đ_M$ = 1.13, and $M_{n,NMR}$ = 12000 Da. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.66 (s, C*H*O), 6.0-7.5 (m, Ph*H* of St unit, and terminal Ar*H*), 3.7-4.5 (m, ArOCH_2, CH_2OCO of end group and BEMI unit, and terminal C*H*Br), 3.62 (s, CH_2OH), 3.40 (m, CH_2N of BEMI unit), 0.6-3.2 (m, other C*H*, CH_2 and CH_3 originating from end group, BEMI unit, St unit and P*t*BA).

P7: GPC and ^{1}H NMR analyses: $M_{n,GPC}$ = 9120 Da, $Đ_M$ = 1.10, and $M_{n,NMR}$ = 11900 Da. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.0-7.8 (m, Ph*H* of St unit, and Ph*H* and Ar*H* of linkage), 5.53 (m, ArC*H*OH), 3.7-4.5 (m, ArOCH_2, ArCH_2, CH_2OCO of linkage and BEMI unit, and terminal C*H*Br), 3.64 (s, CH_2OH), 3.41 (m, CH_2N of BEMI unit), 0.6-3.1 (m, other C*H*, CH_2 and CH_3 originating from linkage, BEMI unit, St unit and P*t*BA).

Synthesis of P5 and P8

CL polymerization using P4 as a macroinitiator was adopted to prepare starlike terpolymer PCL_{30}-*b*-PSt_{26}-*b*-P(St_x-*co*-(BEMI-P*t*BA_{20})$_2$)-*b*-PSt_{30-x} (P5), and ROP of CL initiated with P7 afforded tadpole-linear diblock terpolymer *c*-[PSt_{26}-*b*-P(St_x-*co*-(BEMI-P*t*BA_{19})$_2$)-*b*-PSt_{30-x}]-*b*-PCL_{28} (P8). In a typical

run, to a Schlenk tube was added P4 (0.300 g, 0.025 mmol), CL (0.101 g, 0.88 mmol) and $Sn(Oct)_2$ (4.9 mg, 0.012 mmol) under nitrogen, and the dry toluene was added to reach a total volume of 0.88 mL. The contents were subjected to three freeze-vacuum-thaw cycles, followed by polymerization at 100 °C for 20 h. The product was purified by precipitation into hexane and vacuum drying, and P5 (0.392 g) was obtained in 91.1% conversion. P8 was synthesized according to similar procedures using P7 as a macroinitiator.

P5: GPC and ^{1}H NMR analyses: $M_{n,GPC}$ = 13800 Da, $Đ_M$ = 1.12, and $M_{n,NMR}$ = 15400 Da. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.66 (s, C*H*O), 6.0-7.5 (m, Ph*H* of St unit, and terminal Ar*H*), 3.7-4.5 (m, ArOCH_2, CH_2OCO of end group and BEMI unit, terminal C*H*Br and CH_2O of PCL), 3.65 (m, terminal CH_2OH), 3.41 (m, CH_2N of BEMI unit), 0.5-3.1 (m, other C*H*, CH_2 and CH_3 originating from end group, BEMI unit, St unit, P*t*BA and PCL).

P8: GPC and ^{1}H NMR analyses: $M_{n,GPC}$ = 12600 Da, $Đ_M$ = 1.11, and $M_{n,NMR}$ = 15100 Da. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.0-7.8 (m, Ph*H* of St unit, and Ph*H* and Ar*H* of linkage), 5.46 (m, ArC*H*OH), 3.7-4.5 (m, ArOCH_2, ArCH_2, CH_2OCO of linkage and BEMI unit, terminal C*H*Br and CH_2O of PCL), 3.65 (m, CH_2OH), 3.40 (m, CH_2N of BEMI unit), 0.6-3.2 (m, other C*H*, CH_2 and CH_3 originating from linkage, BEMI unit, St unit, P*t*BA and PCL).

Synthesis of PAA-bearing P6 and P9 via selective hydrolysis

In a typical run, to a glass tube was added P5 (0.15 g), trifluoroacetic acid (TFA, 80 mg) and DCM (3.0 mL), and the solution was stirred at room temperature for 24 h. After concentration and vacuum drying, P5 (0.13 g) was obtained. Similarly, P9 was prepared by hydrolysis using P8 as a raw material.

Self-assembly using different ratios of water to DMF

The solvent switch method was adopted to prepare P6 and P9 assemblies formed in aqueous solution. Take self-assembly using volume ratio of 1:3 (DMF/water) for example. Copolymer (10 mg) was initially stirred in DMF (5 mL) overnight to form the pre-assembled solution, and then deionized water (15 mL) was slowly added to the solution under vigorous stirring. The mixture was further stirred for 8 h, and the resultant solution was transferred into a dialysis membrane tubing (MWCO 3000). After dialysis against deionized water for 30 h, the resultant copolymer assemblies had a polymer concentration (c_p) of 0.5 mg mL^{-1}. The solution pH was 5.8 (for P6 assemblies) and 5.9 (for P9 assemblies). Afterwards, dilute HCl and NaOH aqueous solutions were carefully added to the solution to reach the predetermined pH. Before sample preparation, the copolymer assemblies formed at

different pHs were stirred overnight in closed vials.

Characterization

The apparent number-average molar mass ($M_{n,GPC}$) and dispersity ($Đ_M$) of various polymers were measured on a TOSOH HLC-8320 gel permeation chromatography (GPC) with three TSKgel SuperMultipore HZ-M columns at 40 °C, in which THF with a flow rate of 0.35 mL min^{-1} was used as the eluent and PSt homopolymers with low dispersity acted as standard samples. ^1H (400 MHz) NMR spectra were measured on a Varian spectrometer at 25 °C using $CDCl_3$, DMF-d_6 or mixtures of D_2O and DMF-d_6. Differential scanning calorimetry (DSC) was measured on Q200 DSC using a heating rate of 10 K min^{-1}. To measure hydrodynamic diameter (D_h), particle size distribution (PD) and Zeta potential of copolymer assemblies, dynamic light scattering (DLS) analysis was conducted at 25 °C using Zetasizer Nano-ZS from Malvern Instruments equipped with a 633 nm He–Ne laser using back-scattering detection. To determine the morphology of copolymer assemblies, transmission electron microscopy (TEM) images were measured using a Hitachi H-600 electron microscope with an acceleration voltage of 120 kV, and scanning electron microscopy (SEM) images were measured using a Hitachi S-4700 field emission SEM system, in which freeze-drying was used to prepare the samples.

References

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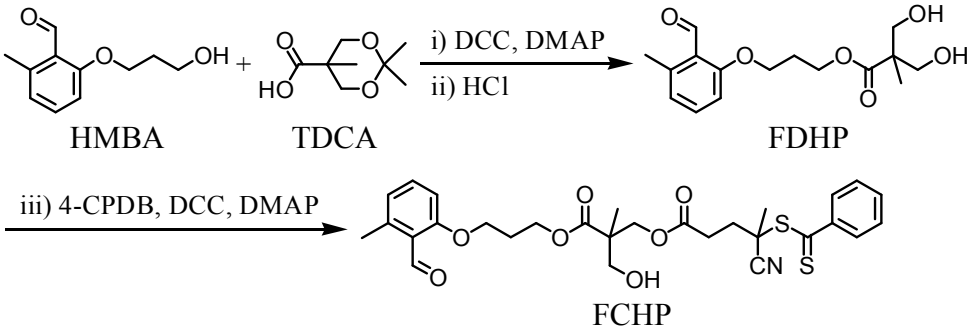

Scheme S1 Synthetic routes to FCHP.

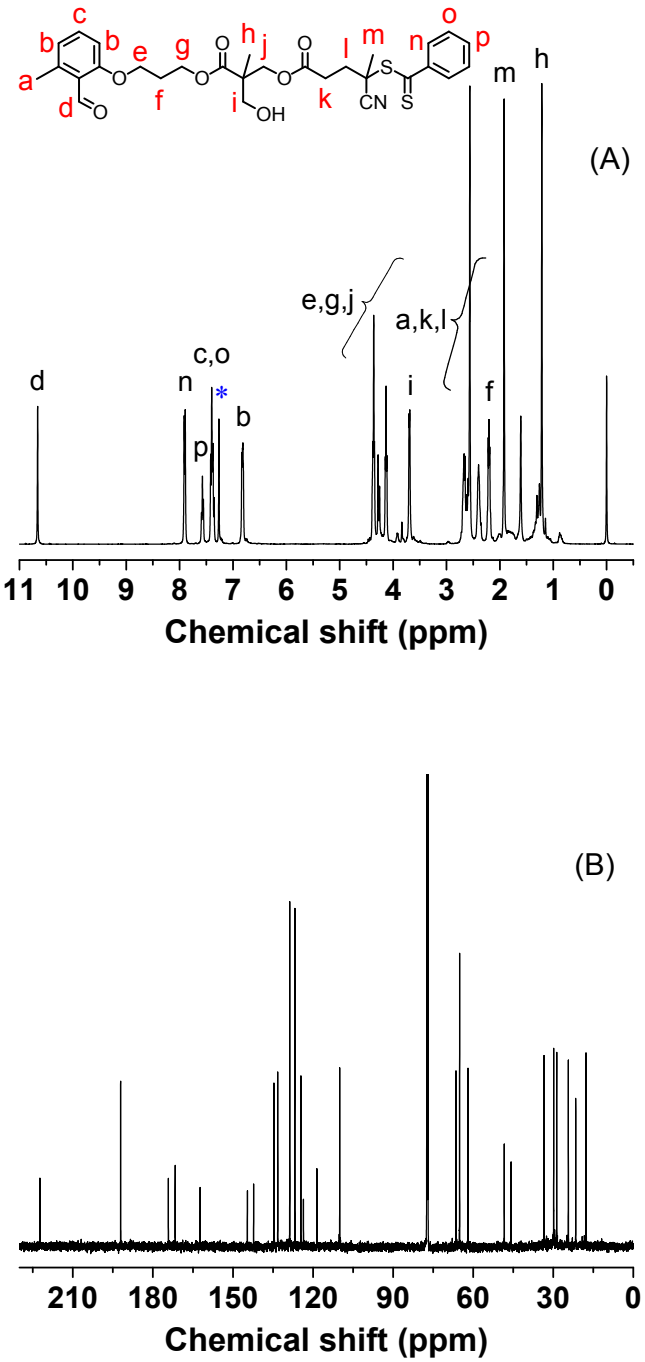

Fig. S1 ^{1}H (A) and ^{13}C (B) NMR spectra of FCHP recorded in $CDCl_3$ (*).

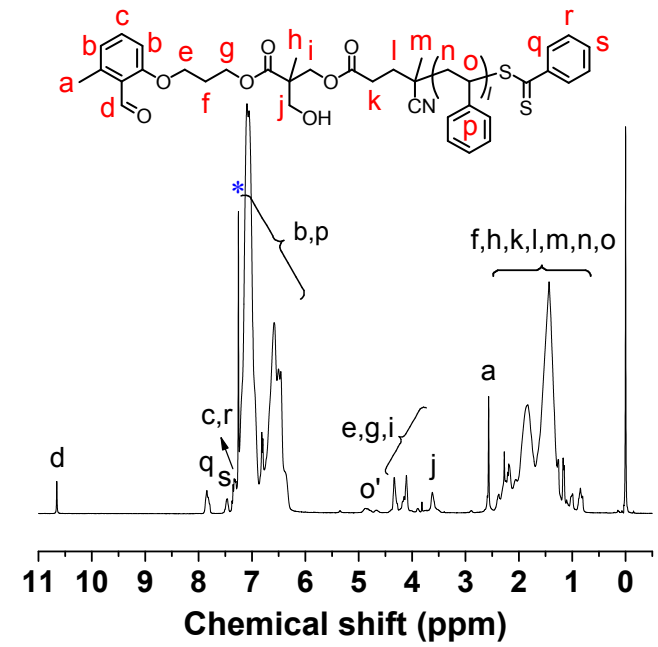

Fig. S2 ^{1}H NMR spectrum of P1 recorded in $CDCl_3$ (*), in which o' denotes terminal C*H*S.

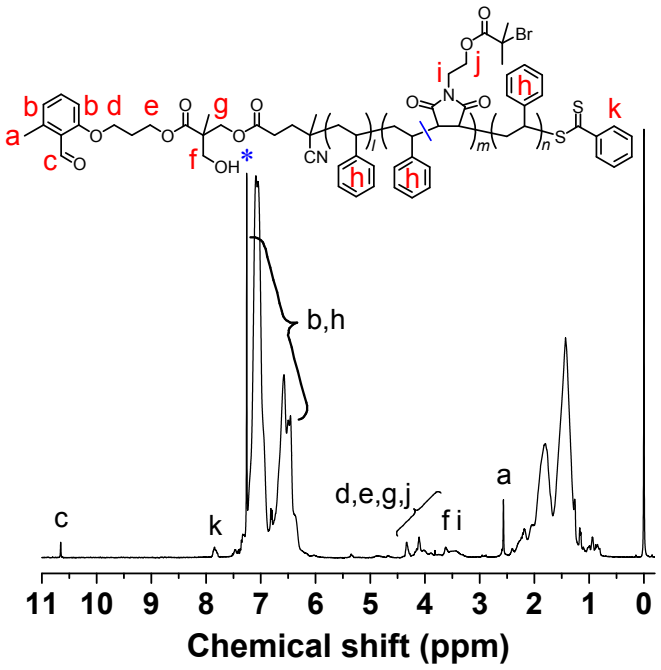

Fig. S3 ^{1}H NMR spectrum of P2 recorded in $CDCl_3$ (*).

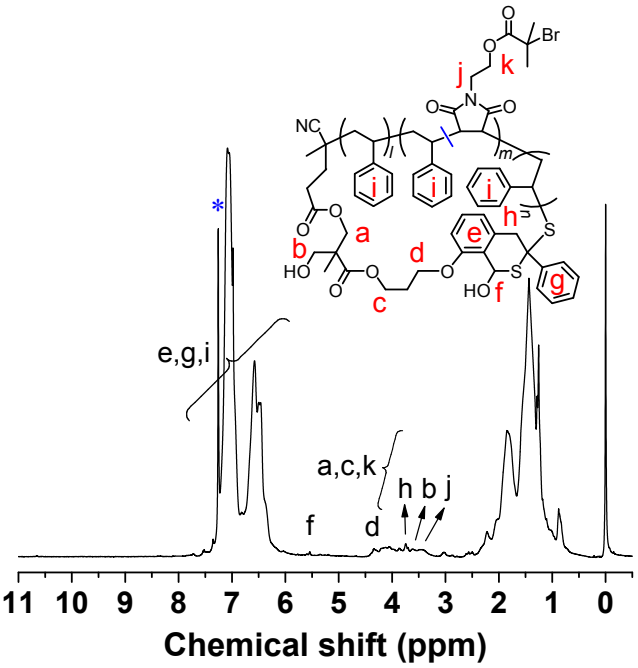

Fig. S4 ^{1}H NMR spectrum of P3 recorded in $CDCl_3$ (*).

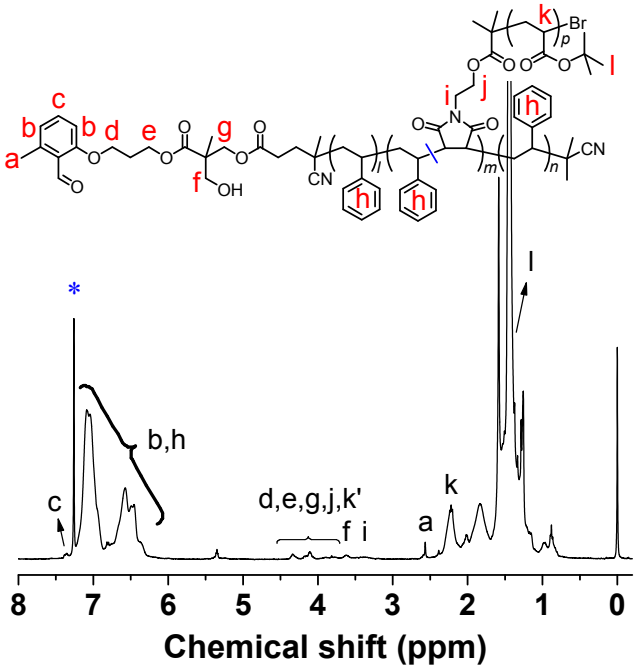

Fig. S5 ^{1}H NMR spectrum of P4 recorded in $CDCl_3$ (*), in which k' denotes terminal C*H*Br at 4.0-4.2 ppm.

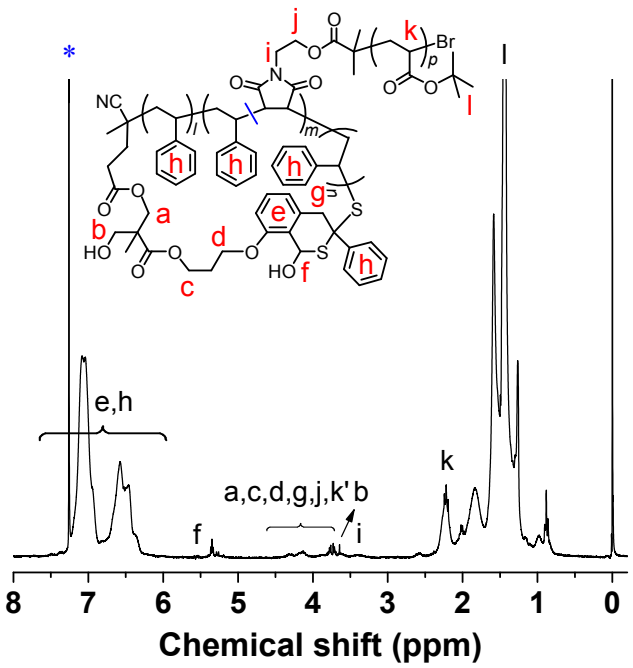

Fig. S6 ^{1}H NMR spectrum of P7 recorded in $CDCl_3$ (*), in which the signal of terminal C*H*Br (k') appears at 4.0-4.2 ppm.

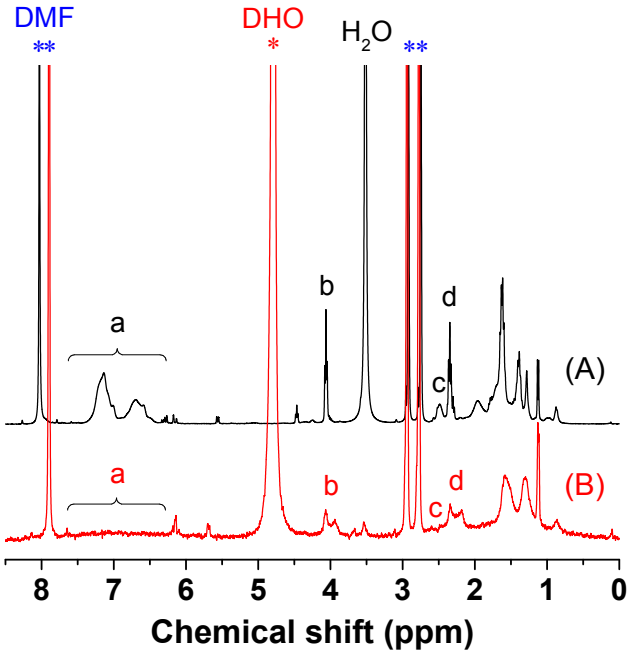

Fig. S7 ^{1}H NMR spectra of P6 recorded in DMF-d_6 (A, c_p = 4.0 mg mL^{-1}) and DMF-d_6/D_2O mixtures (1:3 (*v*/*v*), B, c_p = 1.0 mg mL^{-1}) at 25 °C, in which various signals were Ph*H* of PSt (a), CH_2O of PCL (b), C*H*CO of PAA (c) and CH_2CO of PCL (d).

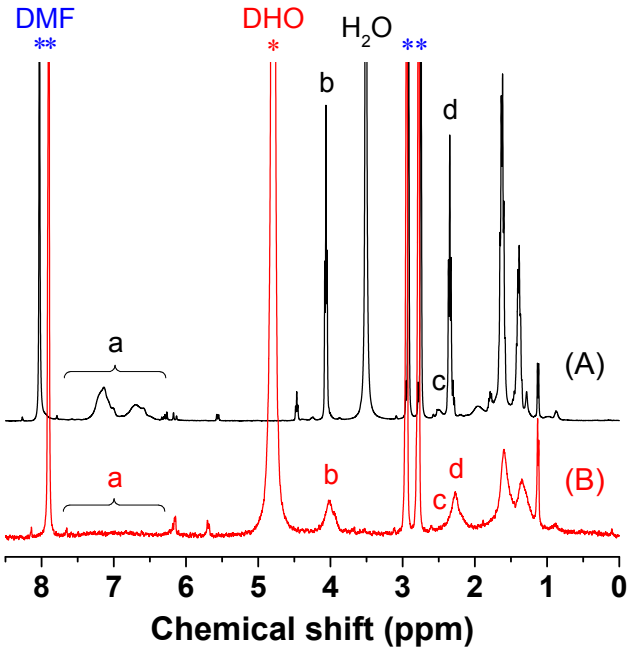

Fig. S8 ^{1}H NMR spectra of P9 recorded in DMF-d_6 (A, c_p = 4.0 mg mL^{-1}) and DMF-d_6/D_2O mixtures (1:3 (*v*/*v*), B, c_p = 1.0 mg mL^{-1}) at 25 °C, in which various signals were Ph*H* of PSt (a), C*H*$_2$O of PCL (b), C*H*CO of PAA (c) and C*H*$_2$CO of PCL (d).

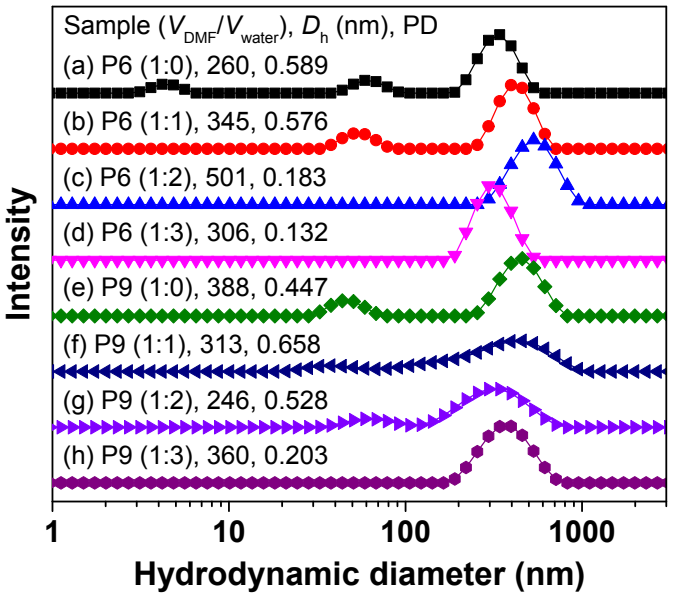

Fig. S9 DLS plots of copolymer assemblies (c_p = 0.5 mg mL^{-1}) formed from P6 and P9 in DMF or mixtures of DMF and H_2O.

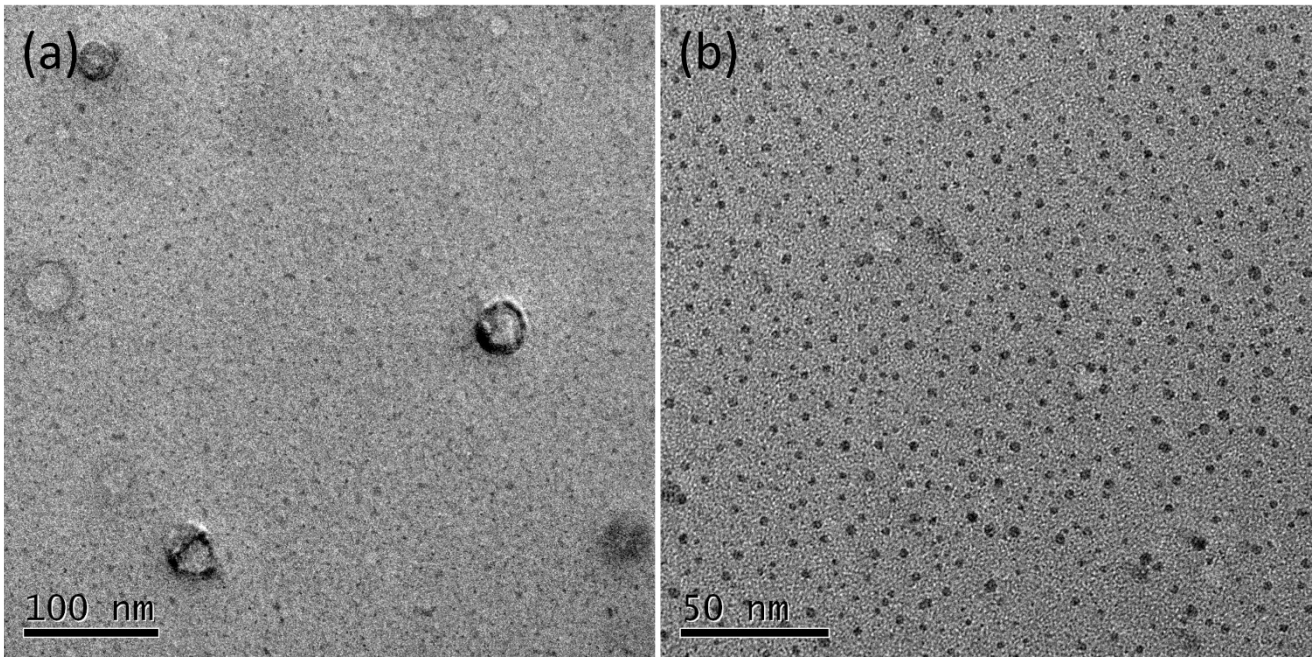

Fig. S10 Typical TEM images of P6 assemblies (c_p = 0.5 mg mL^{-1}) formed in DMF.

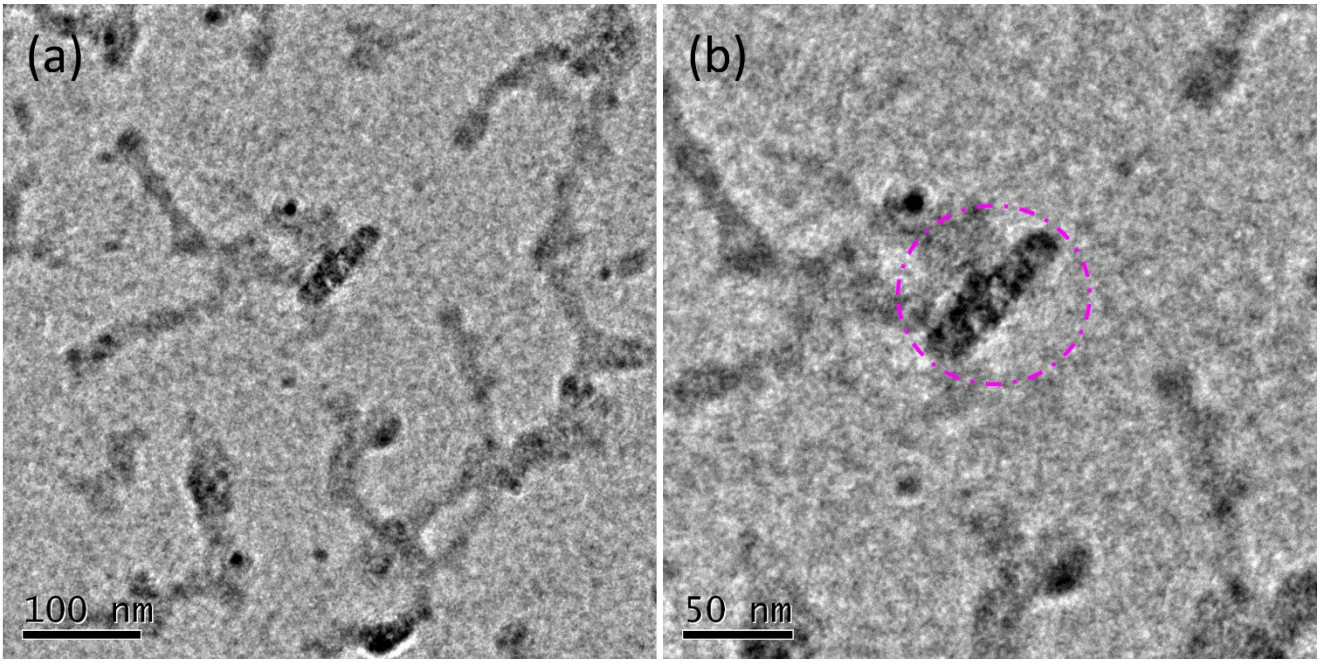

Fig. S11 TEM images (stained with RuO_4) of P9 assemblies (c_p = 0.5 mg mL^{-1}) formed in DMF, and they are expanded images of Fig. 6e.

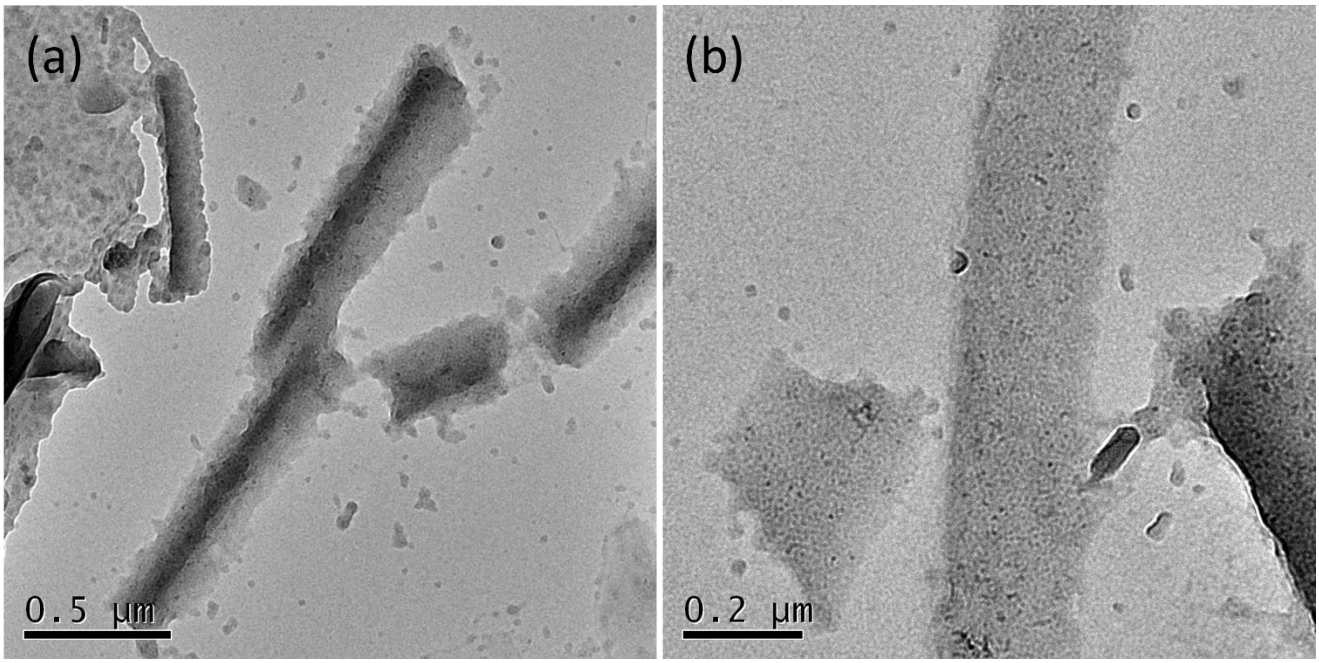

Fig. S12 Typical TEM images (stained with RuO_4) of P9 assemblies (c_p = 0.5 mg mL^{-1}) formed in DMF/water mixtures (1:3, *v*/*v*), followed by dialysis to remove DMF.

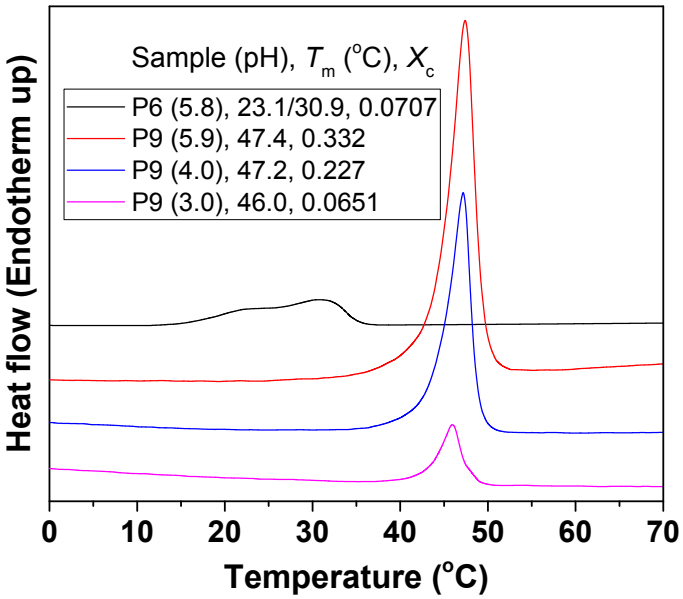

Fig. S13 DSC traces of P6 and P9 assemblies formed at different pHs (c_p = 0.5 mg mL^{-1}), in which the samples were isolated by freeze-drying.

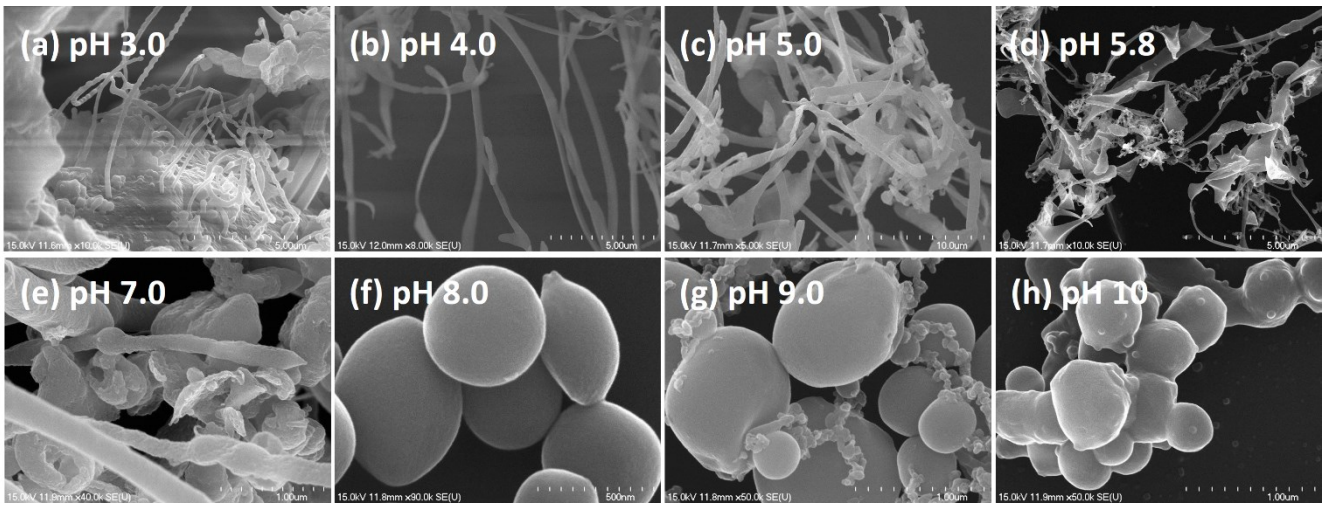

Fig. S14 Typical SEM images of P6 assemblies (c_p = 0.5 mg mL^{-1}) formed at different pHs.

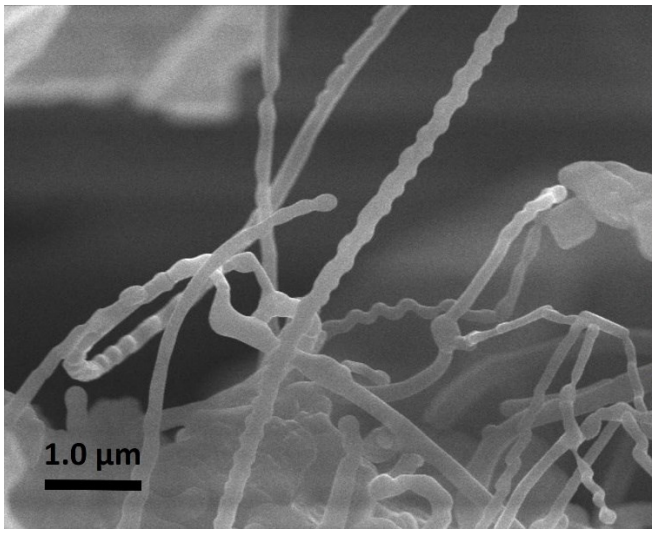

Fig. S15 Expanded SEM image of P6 assemblies (c_p = 0.5 mg mL^{-1}) formed at pH 3.0.

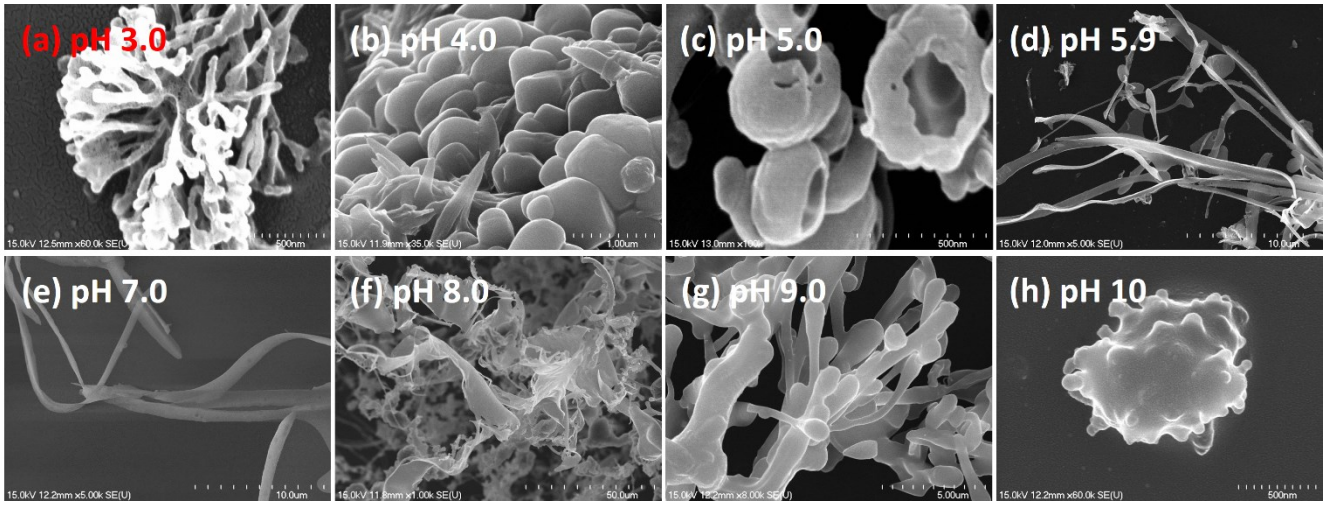

Fig. S16 Typical SEM images of P9 assemblies (c_p = 0.5 mg mL^{-1}) formed at different pHs.

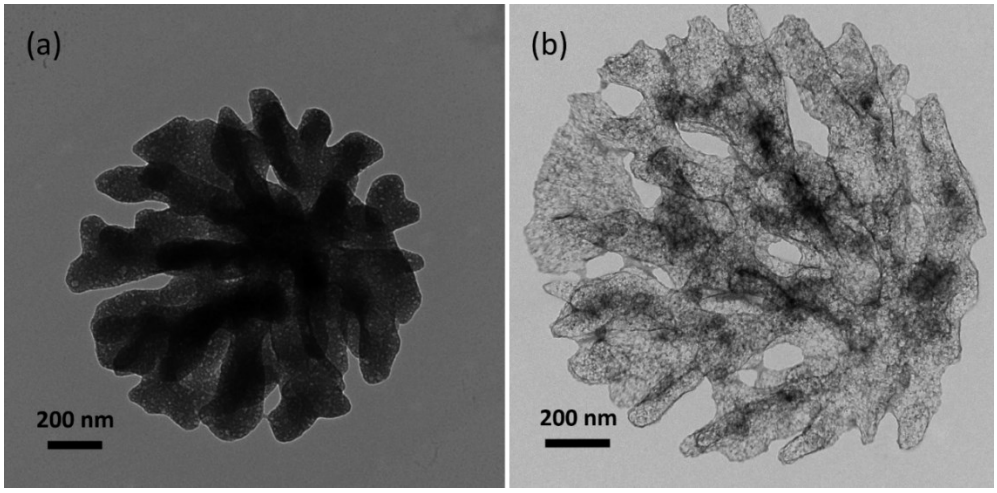

Fig. S17 Influence of low (a) and high (b) intensity of electron beam on morphologies of dendritic vesicles (c_p = 0.5 mg mL^{-1}) formed from P9 at pH 3.0 for 24 h.

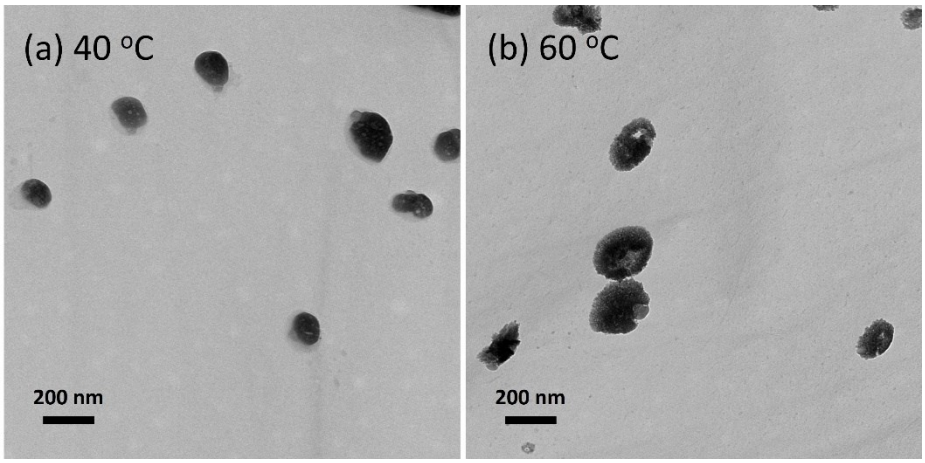

Fig. S18 Dependence of TEM images of P9 assemblies on heating treatment (t = 2 h), in which the original assemblies (c_p = 0.5 mg mL^{-1}) were formed in aqueous solution at pH 3.0 for 24 h.