Supporting Information

Synthesis, thermoresponsivity and multi-tunable hierarchical self-assembly of multi-responsive (AB)$_m$C miktobrush-coil terpolymers

Xiaomin Zhu, Jian Zhang, Cheng Miao, Siyu Li and Youliang Zhao*

Suzhou Key Laboratory of Macromolecular Design and Precision Synthesis, Jiangsu Key Laboratory of Advanced Functional Polymer Design and Application, State and Local Joint Engineering Laboratory for Novel Functional Polymeric Materials, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou 215123, China

E-mail: ylzhao@suda.edu.cn (Y.Z.).

Abbreviations: compound micelle (CM), cylinder (C), degree of hydration (DH), degree of protonation (DPr), disk-like compound vesicle (DCV), disk-like micelle (DM), hydrated fraction (f_h), large compound micelle (LCM), maximum hydrated fraction ($f_{h,max}$), miktobrush-coil polymer (μBCP), multi-tunable hierarchical self-assembly (MHSA), nanoribbon (NR), nanosheet (NS), network (NW), pH-induced self-assembly (pHISA), polyhedron micelle (PM), sheet (S), thermo-induced self-assembly (TISA), vesicle (V).

Experimental

Materials

The chemicals were ordered from Sigma-Aldrich unless otherwise stated. 2-(Dimethylamino)ethyl methacrylate (DMAEMA, 97%) and *N,N*-dimethyl acrylamide (DMA, 97%) were passed through a basic alumina column before use, and *N*-isopropylacrylamide (NIPAM, 97%) was recrystallized from hexane/toluene mixtures to remove the inhibitor. CuBr (98%) was washed with acetic acid and ethanol, followed by vacuum drying. 2,2'-Azobis(isobutyronitrile) (AIBN, Macklin, 98%) was recrystallized twice from ethanol. 4-Vinylbenzyl chloride (VBC, Amar, 90%), D,L-homocysteine thiolactone hydrochloride (98%, Aladdin), 2-bromoisobutyric acid (Macklin, 98%), tris(2-dimethylaminoethyl)amine (Me$_6$TREN, 98%, TCI), ethanolamine (99%) and other chemicals with analytical grade were used directly. The organic solvents were purified using standard procedures. 2-(2-Cyanopropyl) dithiobenzoate (CPDB),[1] 4-vinylbenzyl 2-bromo-2-methylpropanoate (VBP)[2] and 2-maleimidyl-4-thiobutyrolactone (MTL)[3] were synthesized according to reference methods.

Synthesis of P(VBP-*co*-MTL)

To a Schlenk tube were added CPDB (44.2 mg, 0.20 mmol), VBP (1.42 g, 5.0 mmol), MTL (0.986 g, 5.0 mmol) and AIBN (3.3 mg, 0.02 mmol), and then *N,N*-dimethylformamide (DMF) was added to reach a total volume of 10 mL. After flushing with nitrogen for 20 min, the solution was subjected to polymerization in the sealed tube at 70 °C for 20 h. The polymer solution was repeatedly precipitated into diethyl ether, and P1 (1.25 g, 50% conversion) was obtained after vacuum drying. GPC and ^{1}H NMR analyses: $M_{n,GPC}$ = 4690 Da, Đ$_M$ = 1.15, and $M_{n,NMR}$ = 6000 Da. ^{1}H NMR (400 MHz, CDCl$_3$): δ 5.8-8.0 (m, Ph*H* of RAFT moiety and Ar*H* of VBP unit), 5.17 (s, ArC*H*$_2$O of VBP unit), 4.62 (s, C*H*COS of MTL unit), 3.31 (m, C*H*$_2$S), 0.7-3.0 (m, C*H*$_2$C*H* and C*H*$_3$ of VBP unit, C*H*C*H*CONC*H*C*H*$_2$ of MTL unit, and terminal C(C*H*$_3$)$_2$). FT-IR (ATR): 3459, 2933, 1774, 1702, 1517, 1451, 1388, 1273, 1203, 1157, 1108, 1055, 1009, 967, 936, 842, 762, 692 cm^{-1}.

Synthesis of P(VBP-*co*-MTL)-*b*-PDMA

To a Schlenk tube were added P1 (0.300 g, 0.050 mmol), DMA (0.991 mg, 10 mmol) and AIBN (1.6 mg, 0.010 mmol), and then DMF was added until the volume was 5.0 mL. The solution was degassed

with nitrogen for 20 min, followed by chain extension polymerization at 70 °C for 20 h. Most of the solvent was moved under reduced pressure, and the crude product was isolated by dissolving in THF and precipitating into hexane. After vacuum drying at 40 °C, P2 (1.13 g, 84% conversion) was obtained. GPC and ^{1}H NMR analyses: $M_{n,GPC}$ = 21600 Da, Đ$_M$ = 1.11, and $M_{n,NMR}$ = 22900 Da. ^{1}H NMR (400 MHz, CDCl$_3$): δ 5.8-8.0 (m, PhH of RAFT moiety and ArH of VBP unit), 5.18 (m, ArCH_2O of VBP unit), 4.65 (m, CHCOS of MTL unit), 3.32 (m, CH_2S), 2.75-3.22 (m, CH_3N of PDMA), 0.70-2.75 (m, CH_2CH of PDMA, CH_2CH and CH_3 of VBP unit, CHCHCONCHCH_2 of MTL unit, and terminal C(CH_3)$_2$). FT-IR (ATR): 3414, 2933, 1781, 1706, 1614, 1497, 1446, 1402, 1358, 1259, 1204, 1149, 1012, 1099, 1056, 936, 844 cm^{-1}.

Synthesis of P((VBP-*g*-PNIPAM)-*co*-MTL)-*b*-PDMA

Initially, P2 (0.382 g, 0.20 mmol of −Br functionality), NIPAM (9.05 g, 80 mmol), CuBr (28.7 mg, 0.20 mmol), Me$_6$TREN (46.1 mg, 0.20 mmol), and solvents (v_{DMF}:$v_{isopropanol}$ = 1:1) were added to a Schlenk tube under nitrogen, and the total volume was 20 mL. The reaction mixture was degassed with four freeze-pump-thaw cycles and polymerized at 60 °C for 12 h. Afterwards, the mixture was diluted with a large amount of THF, and then the copper salt was removed by passing through a short column of neutral alumina. After concentration, precipitation and vacuum drying, 1.07 g (7.6% conversion) of P(MTL-*co*-(VBP-*g*-PNIPAM))-*b*-PDMA with reactive end groups was obtained.

Subsequently, the reactive RAFT and ATRP moieties were subjected to end-capping reactions. The polymer was dissolved in acetonitrile (10 mL) bearing AIBN (27 mg, 0.16 mmol), and the solution was heated at 80 °C for 5 h to deactivate the RAFT moiety. After cooling down, BuSH (19.8 mg, 0.22 mmol) and triethylamine (22.3 mg, 0.22 mmol) were added to the solution under nitrogen. The thio-bromo click reaction was conducted at room temperature for 20 h. After removing the salt by centrifugation, P3 without reactive end groups was isolated by concentration, precipitation and vacuum drying. GPC-MALLS and ^{1}H NMR analyses: $M_{n,LS}$ = 63800 Da, Đ$_M$ = 1.12, and $M_{n,NMR}$ = 63600 Da. ^{1}H NMR (400 MHz, CDCl$_3$): δ 5.7-7.5 (m, ArH of VBP unit and CONH of PNIPAM), 5.02 (m, ArCH_2O), 4.68 (m, CHCOS of MTL unit), 4.00 (s, CHNH of PNIPAM), 3.32 (m, CH_2S), 2.75-3.22 (m, CH_3N of PDMA), 0.70-2.75 (m, CH_2CH of PDMA, CH_2CH and CH(CH_3)$_2$ of PNIPAM, CHCHCONCHCH_2 of MTL unit, CH_2CHAr, CH_3CH_2CH_2CH_2S, and C(CH_3)$_2$). FT-IR (ATR): 3429, 3290, 3075, 2968, 2935, 1776, 1707,

1631, 1540, 1457, 1383, 1256, 1152, 1061, 988, 930, 834 cm^{-1}.

Synthesis of P((VBP-*g*-PNIPAM)-*co*-(MTL-*g*-PDMAEMA))-*b*-PDMA copolymers

P3 (0.212 g, 0.040 mmol of thiolactone unit) and DMF solution bearing AIBN (0.13 mg, 0.80 μmol) was added to a glass tube, and the total volume was about 0.32 mL. To the solution was injected ethanolamine (4.9 mg, 0.080 mmol) under nitrogen. The aminolysis was conducted in the sealed tube at ambient temperature for 24 h. Afterwards, degassed DMAEMA (0.101 g, 0.64 mmol) was injected into the mixture, and the telomerization was performed at 65 °C for 20 h. Most of the solvent was removed under reduced pressure, and the crude product was isolated by repeated precipitation from THF solution into hexane. After vacuum drying, P4 (0.307 g, 92% conversion) was obtained as yellowish solid. Other toothbrushlike copolymers P5-P7 were synthesized according to similar procedures, in which the molar ratios of monomer to thiolactone unit were 25 (P5), 40 (P6) and 80 (P7), respectively. ^{1}H NMR (400 MHz, CDCl$_3$): δ 5.8-7.5 (m, Ar*H* of VBP unit and CON*H* of PNIPAM), 4.5-5.1 (m, ArC*H$_2$*O and C*H*CONH), 4.06 (s, C*H$_2$*O of PDMAEMA), 4.00 (s, C*H*NH of PNIPAM), 3.75 (s, NHCH$_2$C*H$_2$*OH), 3.48 (s, NHC*H$_2$*CH$_2$OH), 2.75-3.22 (m, C*H$_3$*N of PDMA), 0.70-2.75 (m, C*H$_2$*C*H* of PDMA, C*H$_2$*C*H* and CH(C*H$_3$*)$_2$ of PNIPAM, C*H$_2$*CC*H$_3$* and (CH$_3$)$_2$NC*H$_2$* of PDMAEMA, C*H*C*H*CO and CHC*H$_2$*CH$_2$S of maleimide unit, C*H$_2$*C*H*Ar, C*H$_3$*C*H$_2$*C*H$_2$*C*H$_2$*S, and C(C*H$_3$*)$_2$). FT-IR (ATR): 3416, 3284, 2953, 2829, 2781, 1788, 1721, 1630, 1552, 1458, 1387, 1246, 1154, 1025, 963, 840, 732 cm^{-1}.

Synthesis of P4'-P7' bearing poly(2-(*N*-oxide-*N,N*-dimethylamino)ethyl methacrylate) (PODEMA) segments by oxidation reaction

In a typical run, P6 (20 mg) was dissolved in 3% hydrogen peroxide solution (5 mL). The mixture was stirred at 25 °C overnight, followed by dialysis in deionized water to remove excess H$_2$O$_2$. The oxidized copolymer (denoted as P6') with PODMAEMA segments was recovered by freeze-drying. Other oxidized copolymers P4', P5' and P7' were synthesized according to similar procedures. P6': ^{1}H NMR (400 MHz, D$_2$O): δ 4.49 (s, C*H$_2$*O of PODEMA), 4.03 (m, NHCH$_2$C*H$_2$*OH), 3.87 (s, C*H*NH of PNIPAM), 3.75 (s, C*H$_2$*N of PODEMA), 3.63 (m, C*H*S), 3.57 (m, NHC*H$_2$*CH$_2$OH), 3.33 (s, C*H$_3$*N of PODEMA), 2.75-3.15 (m, C*H$_3$*N of PDMA), 0.60-2.75 (m, other C*H*, C*H$_2$* and C*H$_3$* of backbone, PDMA, side chains and end groups). FT-IR (ATR)：3376, 3287, 2929, 1719, 1637, 1551, 1459, 1388, 1243, 1152, 1046, 950, 890, 667 cm^{-1}.

Characterization

^{1}H (400 MHz) NMR spectra were measured on a Varian spectrometer using CDCl$_3$ (T = 25 ^{o}C) or D$_2$O (T = 25-60 ^{o}C). Apparent molar mass ($M_{n,GPC}$) and dispersity ($Đ_M$) of linear copolymers were measured on a Waters 1515 gel permeation chromatography (GPC) using three MZ-Gel SDplus columns at 40 °C, in which DMF acted as the eluent and PMMAs served as standard samples. The number-average molar mass ($M_{n,LS}$) and dispersity of toothbrushlike copolymers were determined by gel permeation chromatography with multiple angle laser scattering detector (GPC-MALLS) using DMF as an eluent, and $M_{n,LS}$ was obtained by a triple detection method. Fourier Transform Infrared (FT-IR) spectra were measured on a Bruker Vertex 70 spectrometer. To determine the cloud point of copolymer aqueous solutions, turbidity analysis was performed at 500 nm on a Shimadzu UV-3150 UV-vis spectrophotometer equipped with a thermoregulator. To determine apparent hydrodynamic diameter (D_h) and particle size distribution (PD) of copolymer assemblies, dynamic light scattering (DLS) analysis was performed at different temperatures using Zetasizer Nano-ZS from Malvern Instruments equipped with a 633 nm He-Ne laser using back-scattering detection. To check morphology of nanoparticles, transmission electron microscopy (TEM) images were measured using a Hitachi H-600 electron microscope, and scanning electron microscopy (SEM) images were obtained on a Hitachi S-4700 field emission SEM system.

References

1   J. Chiefari, Y. K. Chong, F. Ercole, J. Krstina, J. Jeffery, T. P. T. Le, R. T. A. Mayadunne, G. F. Meijs, C. L. Moad, G. Moad, E. Rizzardo and S. H. Thang, *Macromolecules,* 1998, **31**, 5559–5562.

2   W. X. Dai, X. M. Zhu, J. Zhang and Y. L. Zhao, *Chem. Commun*., 2019, **55**, 5709−5712.

3   T. Rudolph, P. Espeel, F. E. Du Prez and F. H. Schacher, *Polym. Chem*., 2015, **6**, 4240–4251.

Table S1 Influence of solution pH on degree of hydration (DH) of various groups of PNIPAM (CHNH and CH(CH_3)$_2$) and PDMAEMA (CH_2O, CH_2N and CH_3N) segments of P6 assemblies formed in D$_2$O at 25 °C

pH	DH(CH_2O)	DH(CHNH)	DH(CH_2N)	DH(CH_3N)	DH(CH(CH_3)$_2$)
3.0	0.130	0.839	0.456	0.695	0.881
7.8	0.502	0.936	0.557	0.658	0.938
10	0.175	0.847	0.530	0.506	0.893

Table S2 Influence of temperature on $I(T)/I(T_0)$ (T_0 = 25 °C) of various groups and mass fraction of hydrated polymer segments of P6 assemblies formed in D$_2$O at pH 3.0

T (°C)	$I(T)/I(T_0)$ (CH$_2$O)[a]	$I(T)/I(T_0)$ (CH)[b]	$I(T)/I(T_0)$ (CH$_2$NH$^+$)[a]	$I(T)/I(T_0)$ (CH$_3$NH$^+$)[a]	$I(T)/I(T_0)$ (CH$_3$)[b]	$f_{h,1}/f_{h,2}$[c]	$f_{h,max}$[d]
25	1	1	1	1	1	0.776	0.761
30	0.733	0.856	0.955	0.925	0.920	0.772	0.712
35	0.614	0.711	0.950	0.824	0.770	0.726	0.635
40	0.453	0.214	0.951	0.803	0.301	0.291	0.498
45	0.332	0.0804	1.21	1.05	0.196	0.145	0.557
50	0.137	0.0518	1.30	1.12	0.176	0.122	0.576
55	0.0055	0.0206	1.30	1.10	0.152	0.107	0.563
60	0	0	1.29	1.10	0.147	0.104	0.561

[a] $I(T)/I(25\ ^oC)$ of OCH$_2$CH$_2$NH$^+$(CH$_3$)$_2$ groups of PDMAEMA segments. [b] $I(T)/I(25\ ^oC)$ of CH(CH$_3$)$_2$ groups of PNIPAM segments. [c] Mass ratio of hydrated PNIPAM to hydrated PDMAEMA calculated from CH_3 (PNIPAM) and CH_3NH$^+$ (PDMAEMA). [d] Maximum mass fraction of hydrated PDMA, PNIPAM and PDMAEMA segments.

Table S3 Influence of temperature on $I(T)/I(T_0)$ (T_0 = 25 °C) of various groups and mass fraction of hydrated polymer segments of P6 assemblies formed in D_2O at pH 10

T (°C)	$I(T)/I(T_0)$ $(CH_2O)^a$	$I(T)/I(T_0)$ $(CH)^b$	$I(T)/I(T_0)$ $(CH_2N)^a$	$I(T)/I(T_0)$ $(CH_3N)^a$	$I(T)/I(T_0)$ $(CH_3)^b$	$f_{h,1}/f_{h,2}{}^c$	$f_{h,max}{}^d$
25	1	1	1	1	1	1.08	0.668
30	0.578	0.923	0.861	0.694	0.959	1.49	0.577
35	0.193	0.698	0.548	0.311	0.726	2.52	0.413
40	0	0.397	0.336	0.105	0.462	4.76	0.286
45	0	0.230	0.212	0.0562	0.234	4.50	0.210
50	0	0.108	0.136	0.0382	0.146	4.13	0.181
55	0	0.0759	0.0753	0.0259	0.0833	3.48	0.160
60	0	0.049	0.0471	0.0251	0.0619	2.67	0.154

[a] $I(T)/I(25$ °C) of $OCH_2CH_2N(CH_3)_2$ groups of PDMAEMA segments. [b] $I(T)/I(25$ °C) of $CH(CH_3)_2$ groups of PNIPAM segments. [c] Mass ratio of hydrated PNIPAM to hydrated PDMAEMA calculated from CH_3 (PNIPAM) and CH_3N (PDMAEMA). [d] Maximum mass fraction of hydrated PDMA, PNIPAM and PDMAEMA segments.
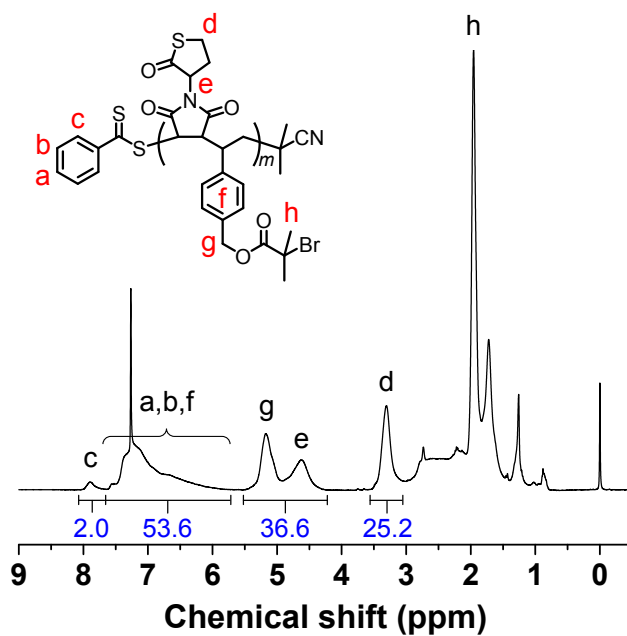
Fig. S1 ^{1}H NMR spectrum of P1 recorded in CDCl$_3$.
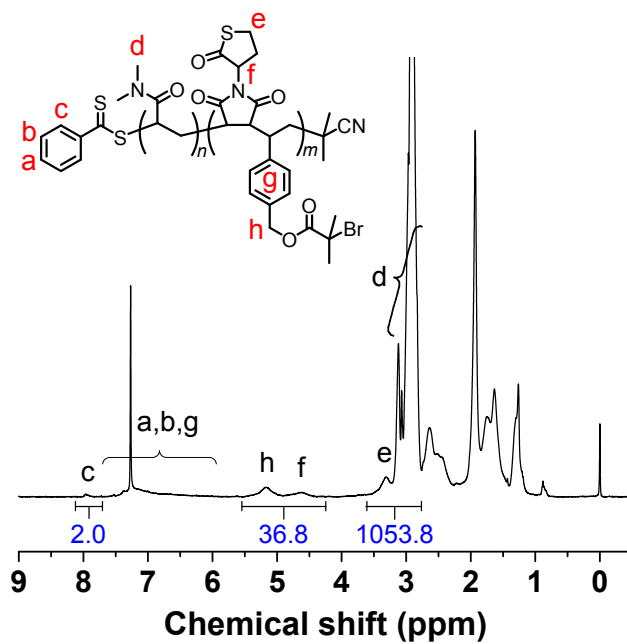
Fig. S2 ^{1}H NMR spectrum of P2 recorded in CDCl$_3$.
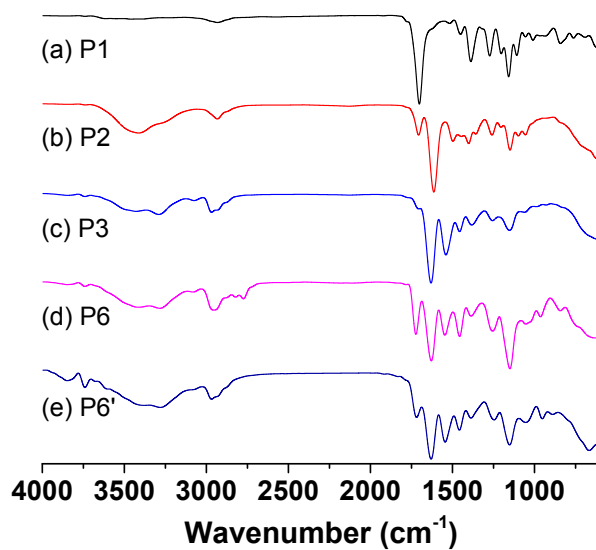
Fig. S3 FT-IR spectra of typical copolymers.
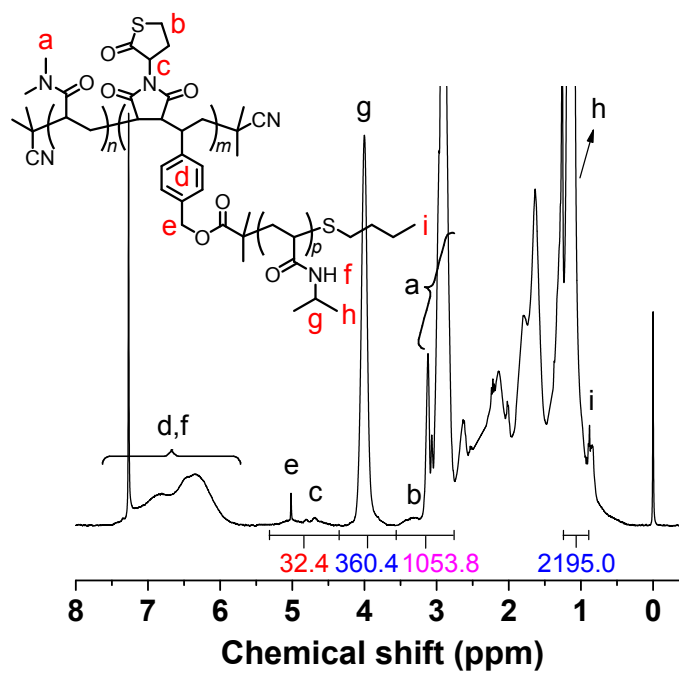
Fig. S4 ^{1}H NMR spectrum of P3 recorded in CDCl$_3$.
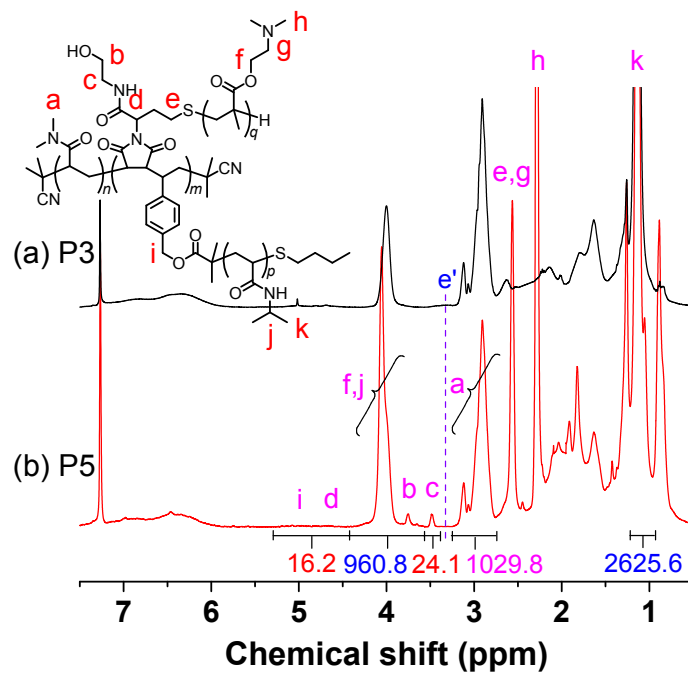
Fig. S5 Comparison on ^{1}H NMR spectra of P3 (a) and P5 (b) recorded in CDCl$_3$, in which e' denotes the signal of CH_2S (thiolactone unit of P3) at 3.32 ppm.
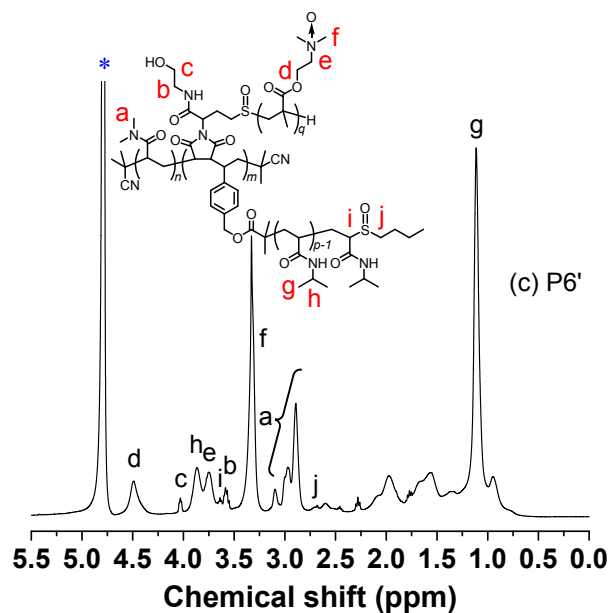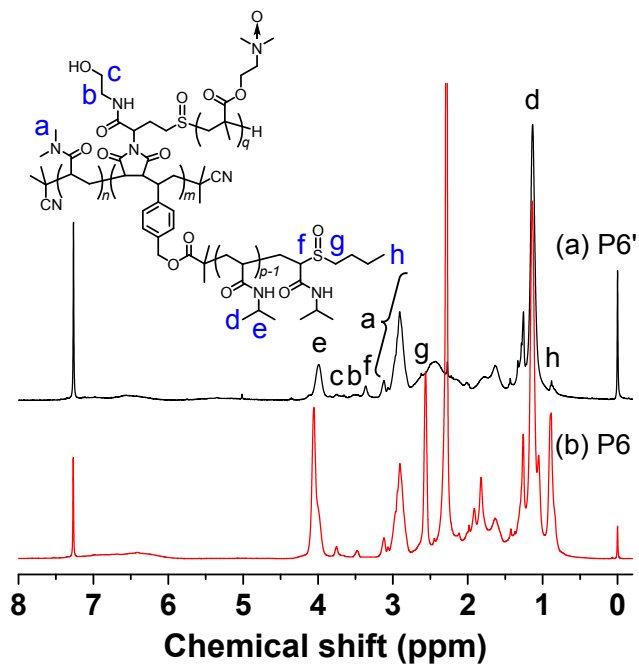
Fig. S6 Comparison on ^{1}H NMR spectra of P6' (oxidized P6, a, c) and P6 (b) recorded in CDCl$_3$ (a, b) or D$_2$O (*, c) at 25 °C, in which only sulfoxide groups were shown in chemical structure of P6' although the thioether moieties of P6 may be oxidized into both sulfoxide and sulfone groups.
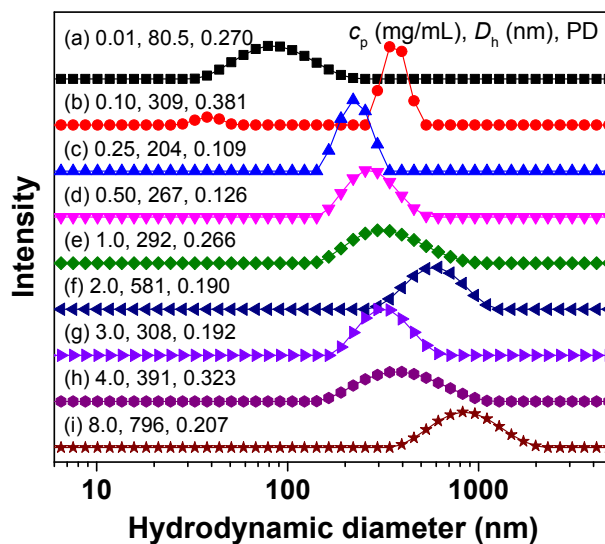
Fig. S7 Influence of polymer concentration on DLS plots of P6 solutions formed in deionized water at 25 °C.
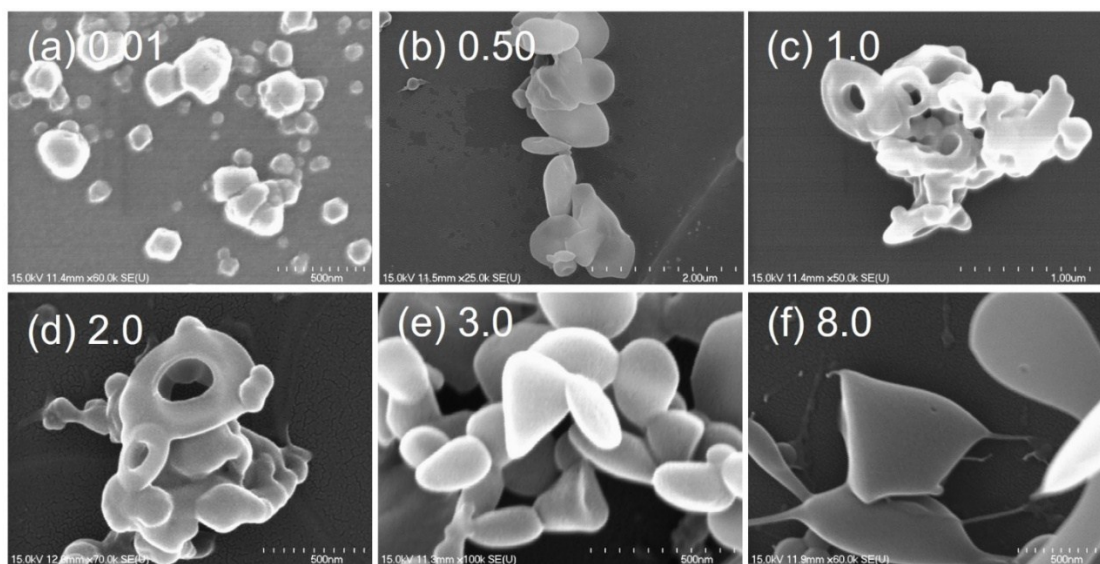
Fig. S8 Typical SEM images of P6 assemblies (c_p = 0.01-8.0 mg mL^{-1}) formed in aqueous solution at different concentrations.
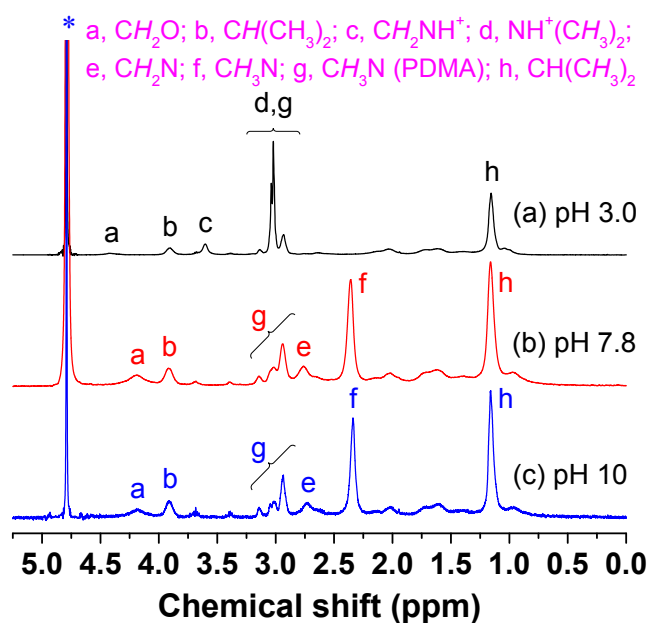
Fig. S9 ^{1}H NMR spectra of P6 recorded in D_2O ($c_p = 2.0$ mg mL^{-1}) with different pHs at 25 ^{o}C, in which typical signals of protonated PDMAEMA (a, c, d), PDMAEMA (a, e, f), PNIPAM (b, h), and PDMA (g) were labeled.
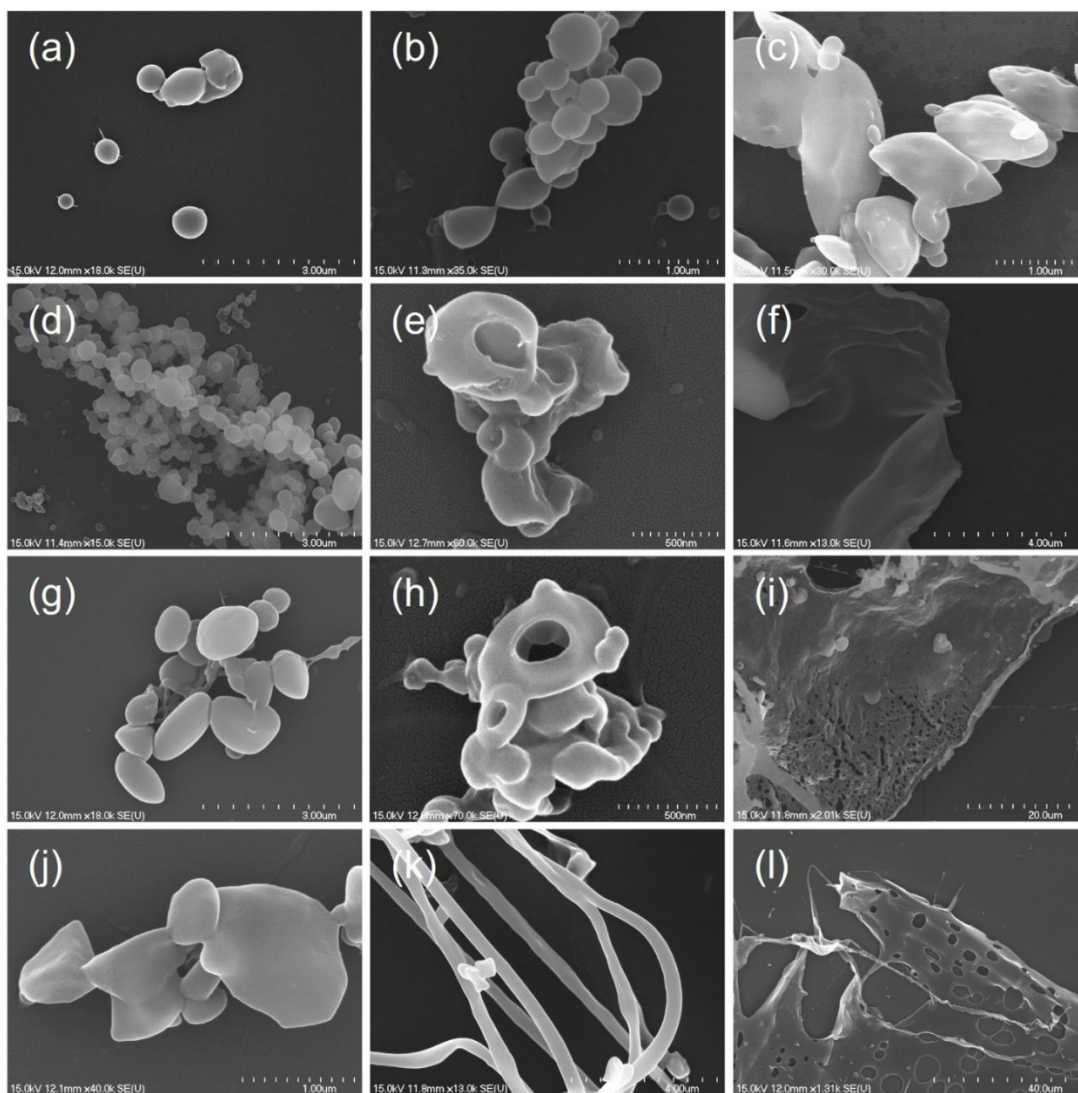
Fig. S10 Typical SEM images of copolymer assemblies formed from P4 (a-c), P5 (d-f), P6 (g-i) or P7 (j-l) in aqueous solution at different pHs (c_p = 2.0 mg mL^{-1}): pH = 3.0 (a, d, g, j), 7.5-7.8 (b, e, h, k), or 10 (c, f, i, l).
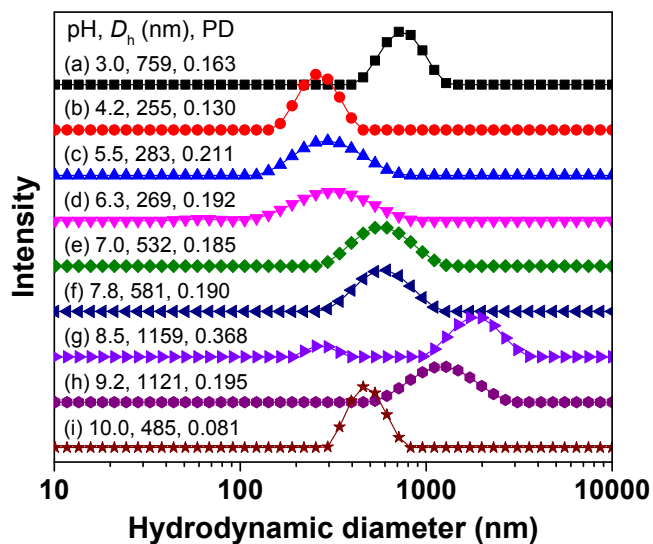
Fig. S11 Influence of pH on DLS plots of P6 aqueous solutions (c_p = 2.0 mg mL^{-1}) at 25 ^{o}C.
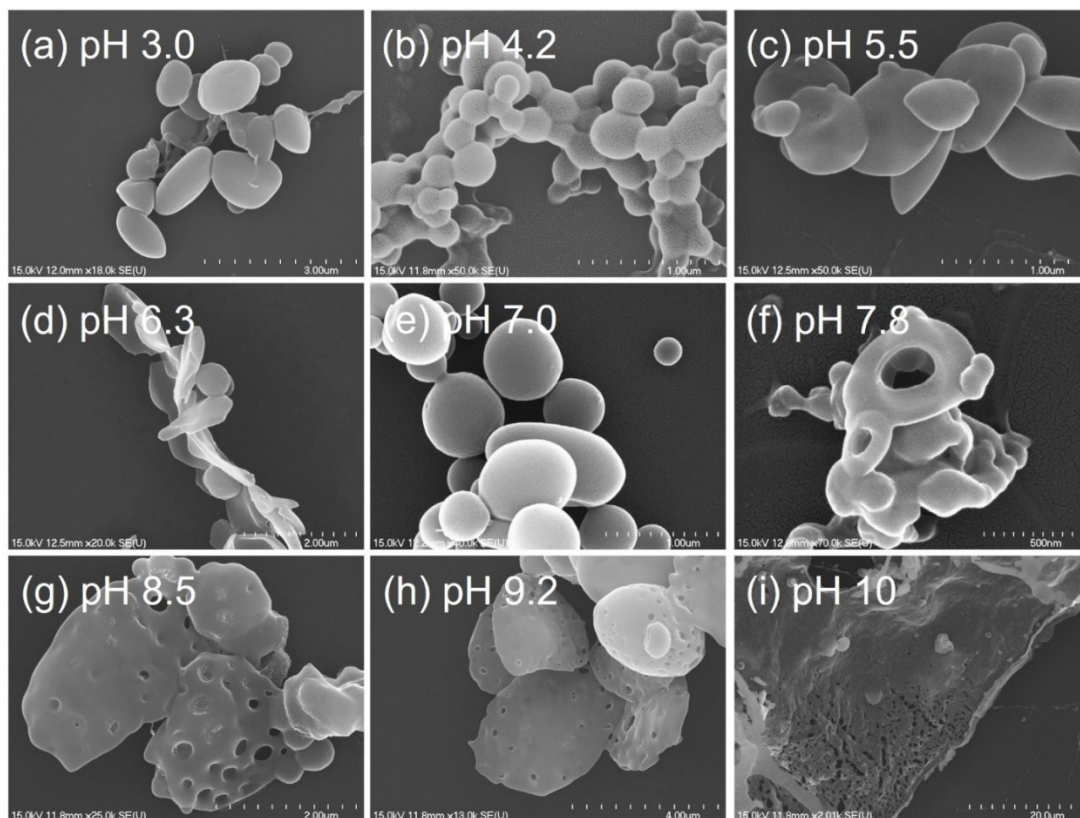
Fig. S12 Typical SEM images of P6 assemblies (c_p = 2.0 mg mL^{-1}) formed in aqueous solution at different pHs.
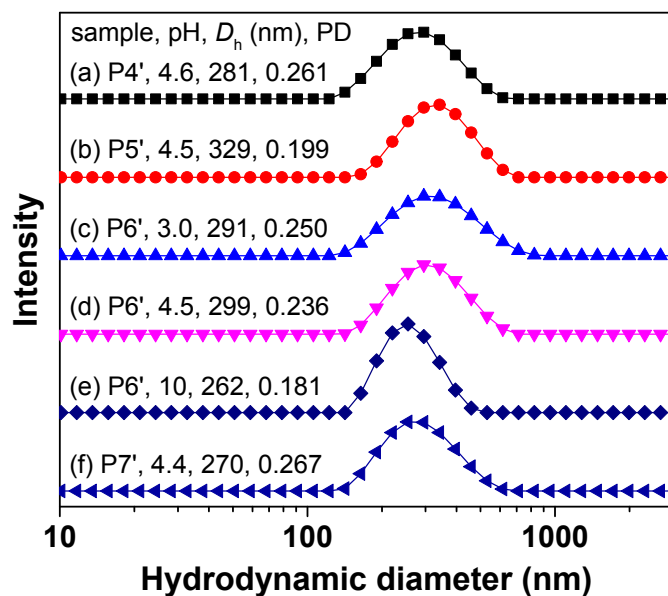
Fig. S13 DLS plots of copolymer solutions (c_p = 2.0 mg mL^{-1}) formed from oxidized copolymers in deionized water at different pHs at 25 ^{o}C.
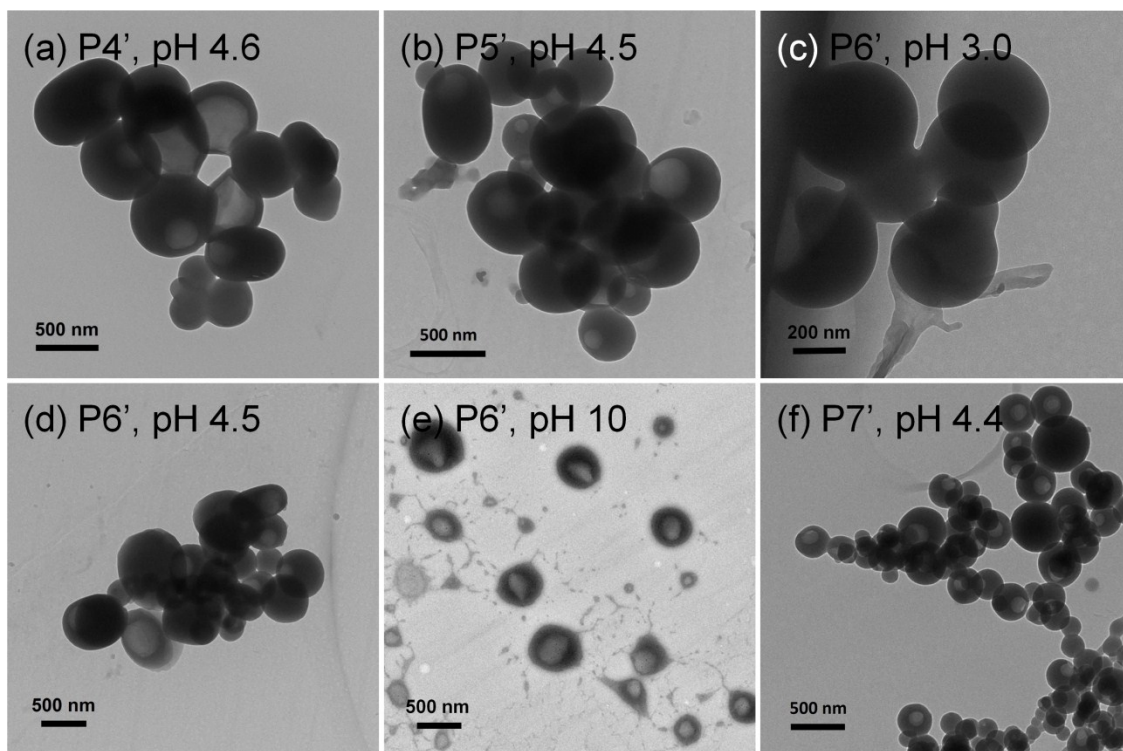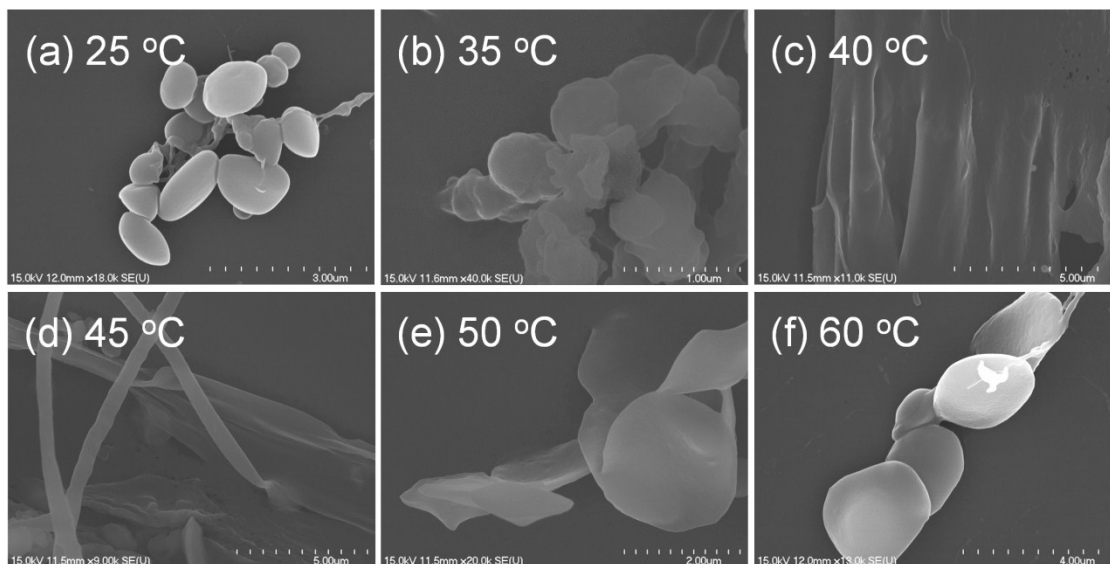

Fig. S14 TEM images of copolymer assemblies (c_p = 2.0 mg mL^{-1}) formed from oxidized copolymers in deionized water at different pHs at 25 ^{o}C.

Fig. S15 Influence of temperature on SEM images of P6 assemblies (c_p = 2.0 mg mL^{-1}) formed in aqueous solution at pH 3.0.
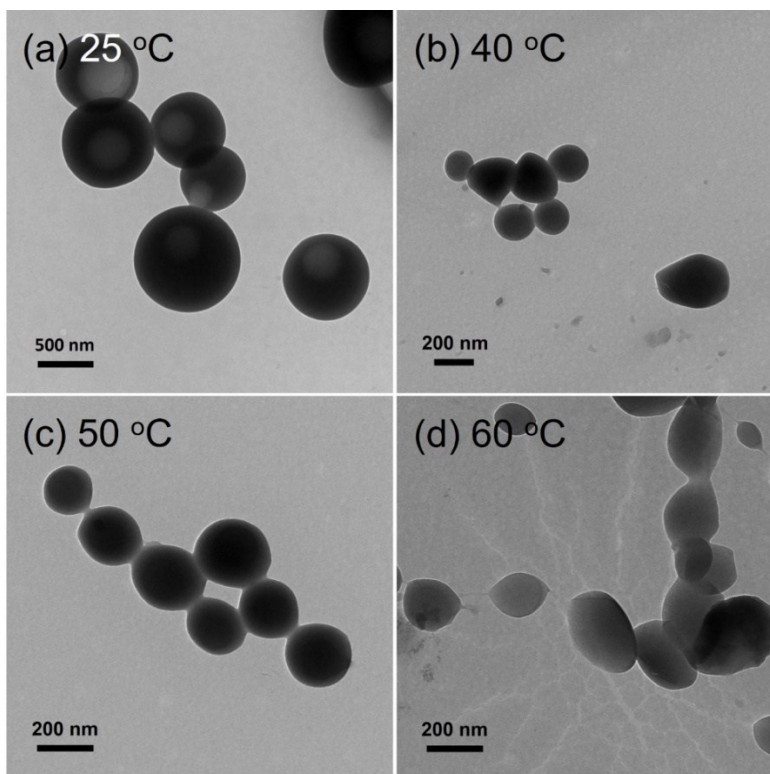

Fig. S16 Influence of temperature on TEM images of P6 assemblies (c_p = 2.0 mg mL^{-1}) formed in aqueous solution at pH 7.8.
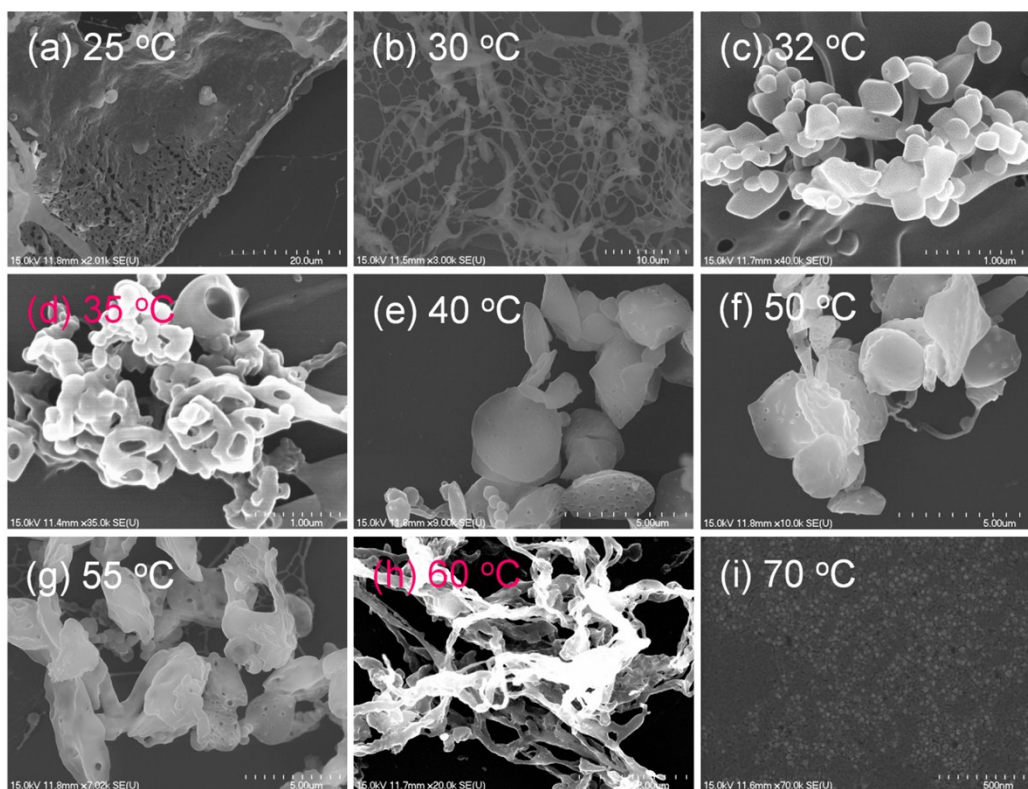
Fig. S17 Influence of temperature on SEM images of P6 assemblies (c_p = 2.0 mg mL^{-1}) formed in aqueous solution at pH 10.

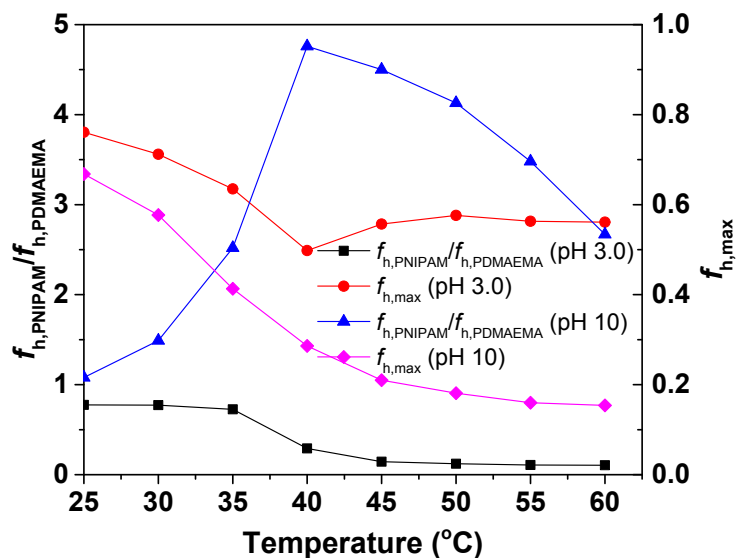**Fig. S18** Evolution of $f_{h,PNIPAM}/f_{h,PDMAEMA}$ and $f_{h,max}$ of P6 assemblies formed in D$_2$O at pH 3.0 and 10 with increasing temperature.