Directional supracolloidal self-assembly via dynamic covalent bonds and metal coordination

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Electronic Supporting Information (ESI)

Synthesis of poly(2-aminoethylmethacrylamide hydrochloride)-*block*-poly(2-hydroxypropylmeth-acrylamide) (PAEMA-*b*-PHPMA)

2-Hydroxypropylmethacrylamide (HPMA)[1] and 4-cyano-4-ethylsulfanylthiocarbonylsulfanylpentanoic acid (CEP)[2] were synthesized according to literature procedures. 2,4,6-Trimethylbenzoyldiphenylphosphine oxide (TPO, 97%) was purchased from Runtec Chem. 2-Aminoethylmethacrylamide hydrochloride (AEMA) was synthesized according to the procedures described in our recent article.[3] Firstly, HPMA (2.86 g, 20.0 mmol), CEP (70.1 mg, 0.267 mmol), TPO (23.2 mg, 67 μmol), and butoxyethanol (1.76 g) was dissolved in a 25 mL flask. Water (2.05 g) was added in solution, and then adjusted to pH 3.4 using a 5.0 M hydrochloric acid. The flask was capped with rubber septa, and immersed in a water bath at 25°C. The solution was bubbled with argon gas in the dark for 60 min, and then irradiated with visible light for 60 min. ^{1}H NMR: 75% conversion. Polymer was obtained by precipitation from excess of acetone and dried in vacuum oven at 30°C overnight. Yield: 2.01 g, 94%. ^{1}H NMR: DP_{HPMA}=56; SEC: M_n=14.2 kDa, M_w/M_n=1.08. This polymer was thus termed as $PHPMA_{56}$. Secondly, $PHPMA_{56}$ (0.273 g, 33 μmol), TPO (2.9 mg, 8.3 μmol), and 1.50 g DMF were dissolved in a 25-mL flask. AEMA (0.543 g in 1.50 g water, 3.3 mmol) was charged in this flask. The solution was adjusted to pH 3.4 using hydrochloric acid (5.0 M). This flask was capped with rubber septa, and then immersed in a water bath at 25°C. The solution was bubbled with argon gas for 60 min, and irradiated with visible light for 1 h. ^{1}H NMR: 50% conversion. The solution was dialyzed against water using a dialysis membrane (MWCO=1.0 kDa), and then freeze-dried in a Labconco Freezone 2.5L freeze-drier. Yield: 0.42 g, 77%. ^{1}H NMR: DP_{HPMA}=56, DP_{AEMA}=57; SEC: M_n=30.6 kDa, M_w/M_n=1.07. The block copolymer was termed as $PAEMA_{57}$-*b*-$PHPMA_{56}$.

The synthetic procedures for $PAEMA_{47}$-*b*-$PHPMA_{247}$ were the same as those described above, except for synthesis of $PHPMA_{247}$ at $[HPMA]_0$:$[CEP]_0$=300 and $[HPMA]_0$:$[TPO]_0$=1200, and then the synthesis of $PAEMA_{47}$-*b*-$PHPMA_{247}$ at $[AEMA]_0$:$[PHPMA_{247}]_0$=100 and $[AEMA]_0$:$[TPO]_0$=400.

Polymer Characterization

The degree of polymerisation (DP) and the unit ratio of block copolymer were assessed by ^{1}H NMR on an INOVA 400 MHz NMR instrument under scanning for 32 times at 25°C. The number-average molecular weights (M_n) and polydispersity indices (PDI, M_w/M_n) of PAEMA-*b*-PHPMA was assessed by size exclusion chromatography (SEC) with a PL-GPC220 system that was fitted with a refractive index detector and a set of columns that consisted of two PLGel 10 μm MIXED-B columns and one PLGel 5 μm MIXED-D column. The columns were eluted with DMF that contained 0.01 M LiBr. Polystyrene standards (PS, Agilent, 3.37 ~ 6035.0 kDa) were used for the calibration. The calibration and analysis were performed at a flow rate of 1.0 mL/min at 80°C. The polymer (40 mg) was dissolved in methanol (0.4 g) in a 10 mL vial. Methyl acrylate (10-fold excess to AEMA unit), triphenylphosphine (100-fold excess to CEP chain-ends) and TEA (2-fold excess to AEMA units) was added in this vial, and stirred at 25°C for 24 h. After evaporation under reduced pressure, the solution was diluted with 3.0 mL DMF, and then filtered using a 0.22-μm PVDF filter.

As shown in *top scheme* of Fig. S1, the copolymer was synthesized *via* aqueous RAFT polymerisation under visible light irradiation at 25°C. As shown in Fig. S1a, except for **H**OD impurities of deuterium oxide at δ=4.79 ppm, other impurities were not detected. The integral I_d:I_c:I_a:I_{b+e} of the bottom spectrum equals to a 1:2:2:6 within analysis errors, which was consistent with the proton ratio of PHPMA. Moreover, the signals *g* and *h* of CEP chain-ends were discernible at δ=2.4-2.6 ppm (*insert*). The signals were selected to assess DP value of PHPMA according to Equation S1, in which I_d and I_{g+h} were the integrations of signals C**H**(CH_3)OH in HPMA units and C**H**$_2$C**H**$_2$COOH chain-ends.

$$DP_{HPMA} = \frac{4 \times I_d}{I_{g+h}} \qquad \text{(S1)}$$

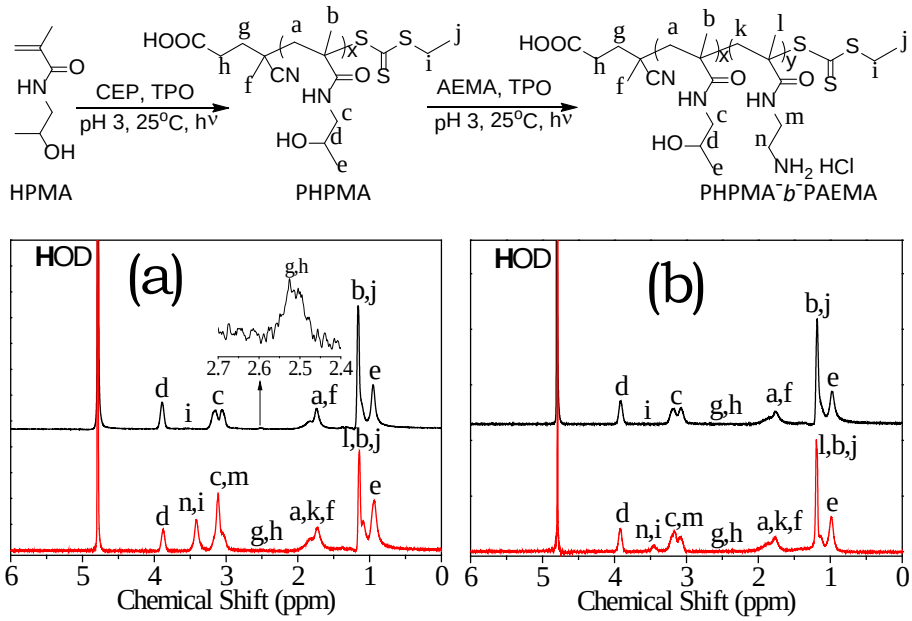

Fig. S1 (*Top*) Schematic illustration for synthesis of PAEMA-*b*-PHPMA via aqueous RAFT polymerisation under visible light irradiation at 25°C; (*bottom*) ^{1}H NMR spectra of (**a**) $PHPMA_{56}$ (black) and $PAEMA_{57}$-*b*-$PHPMA_{56}$ (red), and (**b**) $PHPMA_{247}$ (black) and $PAEMA_{47}$-*b*-$PHPMA_{247}$ (red).

The intact molecular structures of these copolymers were confirmed by ^{1}H NMR studies. The copolymer unit ratios were assessed according to Equations S1-2, in which I_d is integration of signal C**H**(CH_3)OH in HPMA units, I_{c+m+n} is the integrations of signals CONHC**H**$_2$ in HPMA units and CONHC**H**$_2$C**H**$_2$$NH_2$HCl in AEMA units.

$$DP_{AEMA} = \frac{I_{c+m+n} - 2 \times I_d}{4 \times I_d} \times DP_{HPMA} \qquad \text{(S2)}$$

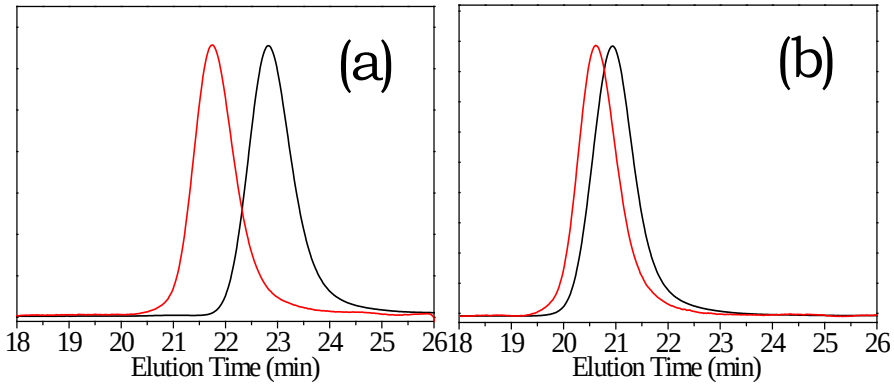

Fig. S2 SEC traces of (**a**) $PHPMA_{56}$ (black) and $PAEMA_{57}$-*b*-$PHPMA_{56}$ (red), and (**b**) $PHPMA_{247}$ (black) and $PAEMA_{47}$-*b*-$PHPMA_{247}$ (red)

The molecular weights and polydispersity indices were assessed by SEC. To circumvent absorption of NH_3^+-motifs on the surface of SEC column materials,[4] these groups were converted into tertiary amines by the addition with methyl acrylate according to procedures described by Armes and co-workers.[5, 6] In addition, triphenylphosphine was added to circumvent the recombination of thiol chain-ends formed by hydrolysis of trithiocarbonate.[7] ^{1}H NMR studies demonstrate that the reaction was complete after stirring the reaction solution at 25°C for 28 h.

As shown in Fig. S2, their SEC traces are unimodal and symmetrical, and shift to higher molecular weight sides after chain extension. These results demonstrate the well-controlled polymerisation. Well-defined copolymers was thus obtained. $PAEMA_{57}$-*b*-$PHPMA_{56}$ (^{1}H NMR: HPMA/AEMA=56/57, SEC: M_n=30.6 kDa, PDI=1.07); $PAEMA_{47}$-*b*-$PHPMA_{247}$ (^{1}H NMR: AEMA/HPMA=47/247, SEC: M_n=85.3 kDa, PDI=1.09).

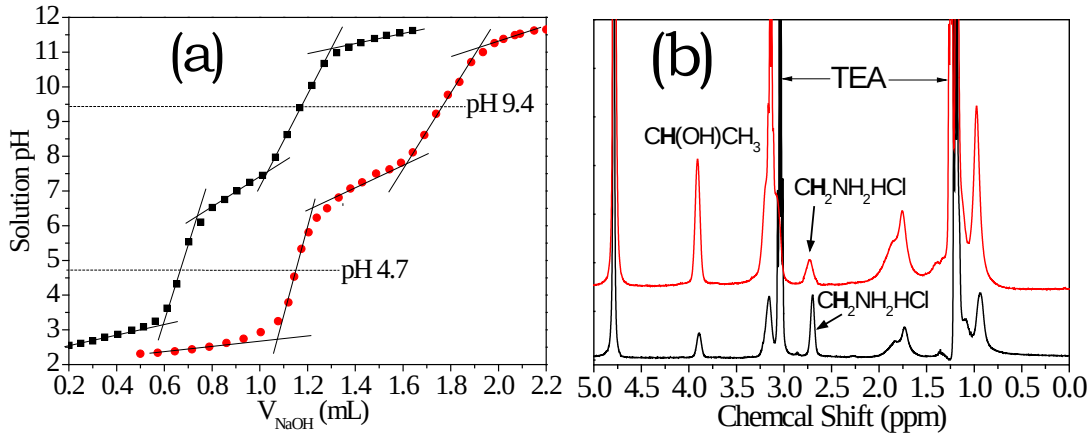

Fig. S3 (**a**) acid-base titration plots and (**b**) ^{1}H NMR spectra of the copolymers in deuterium oxide at pH 9.5 at 25°C. Copolymers: PAEMA$_{57}$-*b*-PHPMA$_{56}$ (black) and PAEMA$_{47}$-*b*-PHPMA$_{247}$ (red).

According to acid-base titration plots (Fig. S3a), the amine motifs have been ionized in acidic solutions over pH<4.7 and deionized in alkali solutions over pH>9.4. Moreover, these copolymers were molecularly dissolved in water, as indicated by the transparent solutions and completely discernible proton signals in D_2O at pH 9.5 (Fig. S3b). Moreover, signal C**H**$_2$NH$_2$HCl at 3.4 ppm up-field shifted to 2.69 ppm due to the conversion into non-ionic reactive NH$_2$ groups. Excellent solubility at pH 9.5 was verified by integral $I_{3.90}/I_{2.69}$=1/2 for PAEMA$_{57}$-*b*-PHPMA$_{56}$, and $I_{3.90}/I_{2.69}$=5/2 for PAEMA$_{47}$-*b*-PHPMA$_{247}$ (*red*), in which $I_{3.90}/I_{2.69}$ is the integral ratio of signals C**H**(OH)CH$_3$ in HPMA at 3.90 ppm and CH$_2$C**H**$_2$NH$_2$ in AEMA at 2.69 ppm.

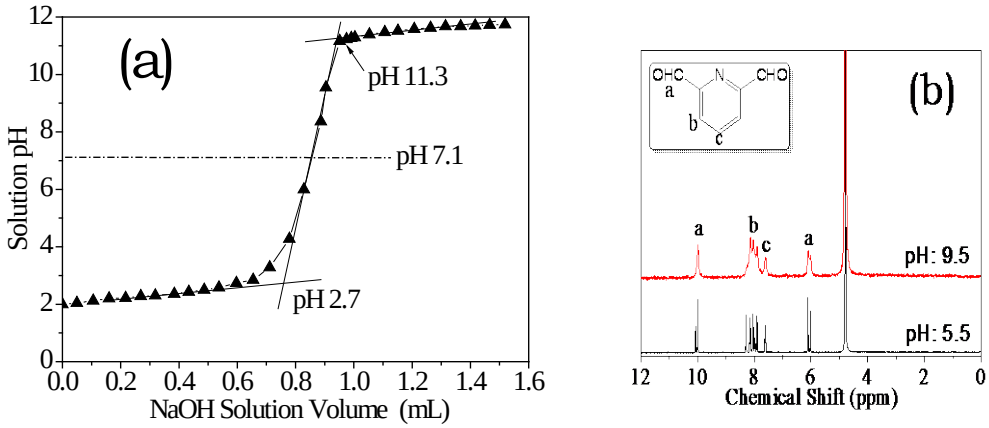

Fig. S4 (**a**) acid-base titration plot and (**b**) ^{1}H NMR spectra of pyridine-2,6-dicarbaldehyde (PDCA) in deuterium oxide at pH 5.5 and 9.5, respectively.

The titration result indicated a weak alkali nature of PDCA compound (Fig. S4a). As shown in Fig. S4b, the proton signals exhibit narrow and sharp peaks in the solutions at pH 5.5 and 9.5, suggesting that the compound was completely hydrated in water.

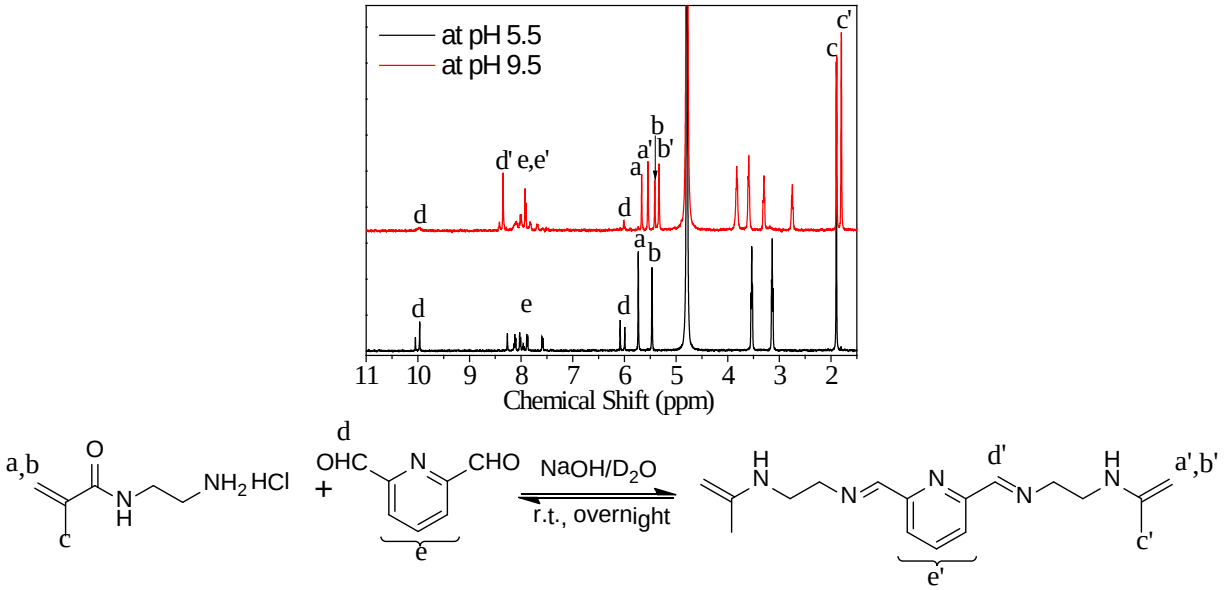

Fig. S5 ^{1}H NMR spectra of the solutions of AEMA and PDCA ([AEMA]$_0$/[PDCA]$_0$=2,1.50 g/L AEMA in D_2O) at pH 5.5 and 9.5, respectively, after stirring at ambient temperature overnight; *bottom*: schematic illustration for the reaction of dynamic imine conversion.

As shown in Fig. S5, signals at δ = 6.0 and 9.9 ppm (C**H**O in PDCA)[8, 9] are discernible at pH 5.5 and 9.5. However, the signals decreased upon alkalization from pH 5.5 to pH 9.5. Moreover, signal *d'* at δ=8.36 ppm (C**H**=N) appeared in solution at pH 9.5. These results suggest the conversion into dynamic imine bonds at pH 9.5. 68% conversion was achieved in this basic solution, as assessed by the integral $I_{a'}/I_{a+a'}=I_{b'}/I_{b+b'}=I_{c'}/I_{c+c'}=0.68$.

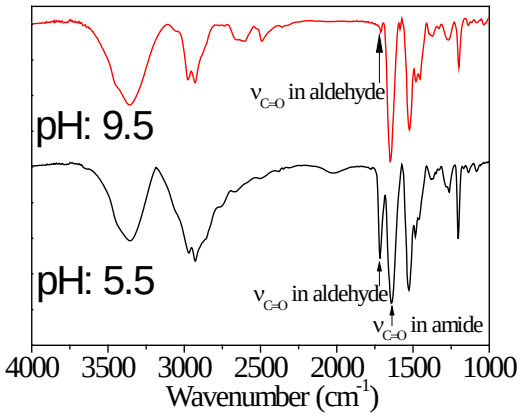

Fig. S6 FTIR spectra of the dried films of the mixture of PAEMA$_{57}$-*b*-PHPMA$_{56}$ and PDCA obtained by casting the reaction solutions ([AEMA]$_0$/[PDCA]$_0$=2, 1.50 g/L polymer in H_2O, pH was adjusted by TEA, stirred at 25°C overnight) on CaF_2 tablets and dried under reduced pressure.

As shown in Fig. S6, the amide carbonyl peak at 1643 cm^{-1} maintained constant either at pH 5.5 or 9.5. Nevertheless, aldehyde carbonyl peak of PDCA at 1717 cm^{-1} decreased remarkably upon alkalization of the solution to pH 9.5. These results demonstrate that the reaction of PDCA has been essentially complete, which was quite akin to small-molecule imine conversion as observed by FTIR spectroscopy.[10, 11]

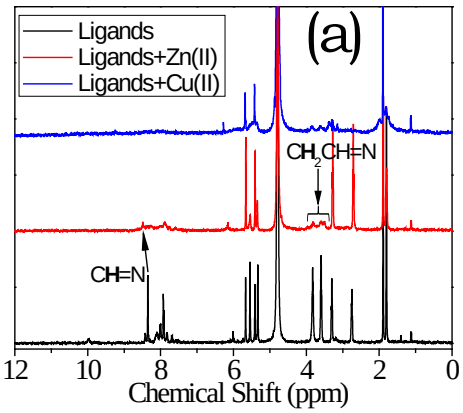

Fig. S7 ^{1}H NMR spectra of reaction solution of small molecules (black, $[AEMA]_0/[PDCA]_0$=2, 1.50 g/L AEMA in D_2O at pH 9.5, at 25°C), and the solutions after addition of zinc acetate (red) and copper acetate (blue) at a $[Mt(II)]_0/[ligand]_0$=0.3.

As shown in Fig. S7, the signal at δ=8.33 ppm (C**H**=N) has been remarkably attenuated and shifted to 8.49 ppm, and the signals at δ=3.83 and 3.6 ppm (C**H**$_2$CH=N) were also attenuated after Zn(II)-coordination. These results suggest that the coordination induced desolvation of their imine derivatives. In contrast, the signals of C**H**=N and C**H**$_2$CH=N virtually disappeared after Cu(II)-coordination. These results suggest that the Cu(II)-complexes were more hydrophobic than Zn(II)-complexes in water.

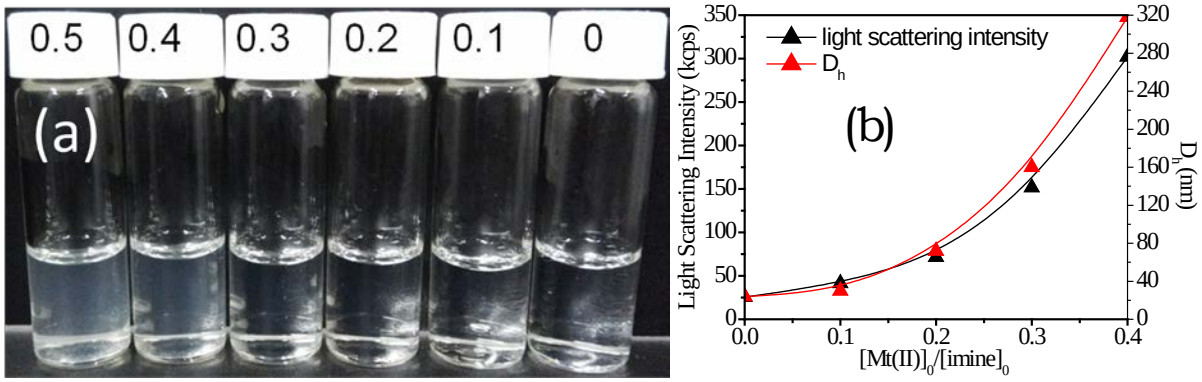

Fig. S8 (**a**) The solution images and (**b**) the variation of light scattering intensity (*black*) and D_h (*red*) of PDCA-conjugated $PAEMA_{57}$-*b*-$PHPMA_{56}$ micelles (0.75 mg/mL of the initial block copolymer) and the solutions upon addition of zinc ions varying $[Zn(II)]_0/[ligand]_0$ from 0 to 0.1, 0.2, 0.3, 0.4, and 0.5. Herein, the same results were obtained after the solutions were maintained at ambient temperature for 5 months.

As shown in Fig. S8a, the solution of micelles changed from transparent to misty upon increasing $[Zn(II)]_0/[ligand]_0$ to 0.4 and 0.5, suggesting the self-assembling of micelles due to coordination with zinc ions. As shown in Fig. S8b, the light scattering intensity increased slightly on increase of the ratio from 0.1 to 0.2, in which the overall sizes increase from D_h=30 nm to 72 nm. However, the light scattering intensity increased significantly on increase of the ratio to 0.4, and the apparent overall sizes significantly increased to D_h=318 nm. More importantly, their nanostructures were highly stable, as indicated by essentially the constant light scattering intensities and D_h values after these solutions was maintained at ambient temperature for five months.

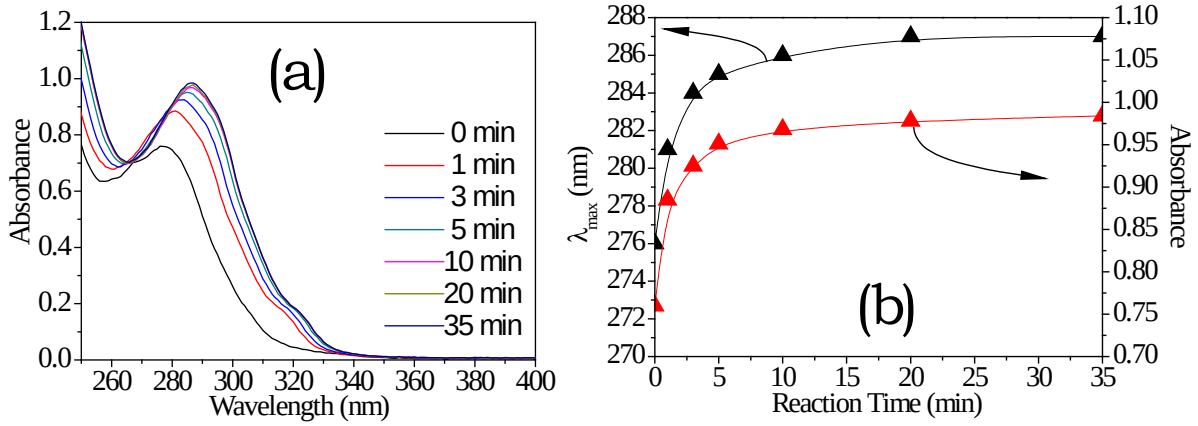

Fig. S9 (**a**) The evolution of UV spectrum of the reaction solution of $PAEMA_{57}$-*b*-$PHPMA_{56}$ (75 mg/L in water) on simultaneous imine conversion and coordination at a $[AEMA]_0$:$[PDCA]_0$:$[Zn(II)]_0$=2:1:0.3 at 25°C; (**b**) the variation of characteristic wavelength (λ_{max}, *black*) and the absorbance (*red*).

As shown in Fig. S9a, the peak at λ_{max} = 275 nm has shifted to 286 nm and the absorbance increased immediately after adjusting the solution to pH 9.5, suggesting that imine conversion has occurred. A weak shoulder over λ=310~330 nm appeared shortly in 1 min, indicating the coordination of their imine motifs with zinc ions. The reactions were finalized very shortly, i.e., within ~ 6 min (Fig. S9b).

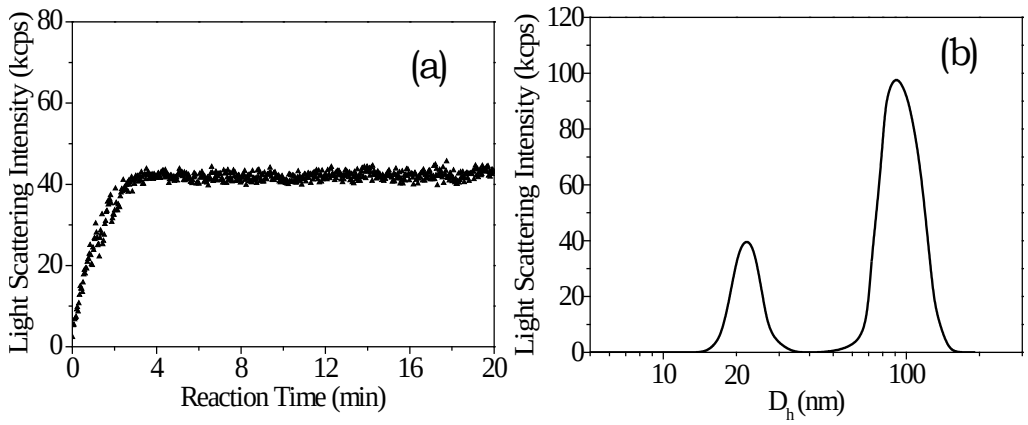

Fig. S10 (**a**) the evolution of light scattering intensity of $PAEMA_{57}$-*b*-$PHPMA_{56}$ (0.75 mg/mL in water) on simultaneous imine conversion and coordination at $[AEMA]_0$:$[PDCA]_0$:$[Zn(II)]_0$=2:1:0.3 at 25°C; (**b**) size distribution profiles of aggregates after equilibrated at ambient temperature for 2 days.

As shown in Fig. S10a, the light scattering intensity is rather low (1.8 kcps) in acidic solution, suggesting that the copolymer is soluble in water. However, the light scattering intensity increased dramatically to a 42.5 kcps after the alikalization to pH 9.5, and levelled off shortly in 3 min. These results suggest that the copolymer has phase-separated from water. Thereafter, the light scattering intensity maintained constant. After the equilibration at ambient temperature for 2 days, the aggregates with a D_h=89 nm and a μ_2/Γ^2=0.295 were assessed by DLS studies (Fig. S10b).

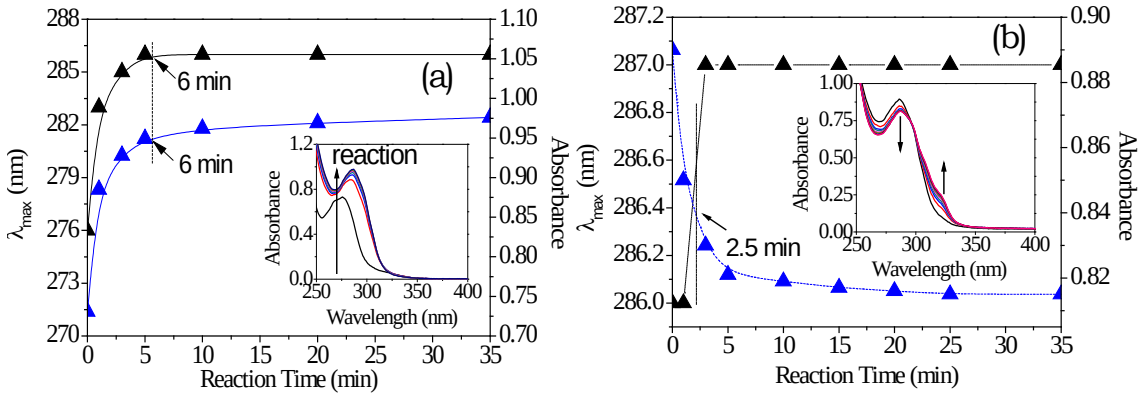

Fig. S11 The variation of characteristic wavelength (λ_{max}, black) and absorbance (blue) of PAEMA$_{47}$-*b*-PHPMA$_{247}$ (0.225 mg/mL) on conjugation with PDCA at a [AEMA]$_0$/[PDCA]$_0$=2 in water at 25°C (**a**) and then coordination with zinc ions at [Zn(II)]$_0$/[ligand]$_0$=0.3 (**b**). Insert: the evolution of their UV spectra.

As shown in Fig. S11, the imine conversion was equilibrated shortly in 6 min, and coordination was finalized in 2.5 min.

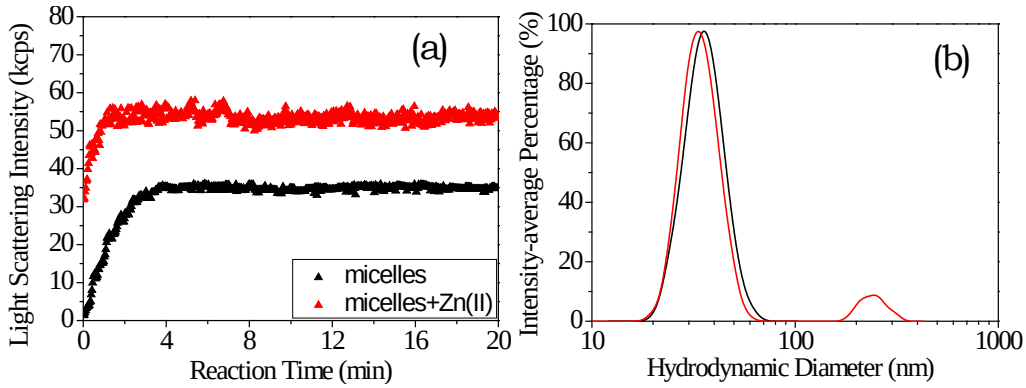

Fig. S12 (**a**) the evolution of light scattering intensity of PAEMA$_{47}$-*b*-PHPMA$_{247}$ (0.225 mg/mL) on conjugation with PDCA at [AEMA]$_0$/[PDCA]$_0$=2 in water at 25°C (black), and then coordination with zinc ions at [Zn(II)]$_0$/[ligand]$_0$=0.3 (red); (**b**) the size distribution profiles of PDCA-conjugated micelles (black) and those after the coordination with zinc ions (red).

As shown in Fig. S12a, the light scattering intensity increased rapidly and levelled off shortly in 4 min, suggesting that PDCA-conjugation led to rapid phase-separation from water. The light scattering intensity increased rapidly and then levelled off shortly in 2 min after addition of zinc ions, suggesting that these micelles were coordinated with zinc ions immediately.

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