

Compartmentalization of ABC triblock copolymer single-chain nanoparticle via coordination driven orthogonal self-assembly

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Electronic Supplementary Information

Synthesis of PHPMA-*b*-PHisAM

PHPMA was first synthesized as follows. HPMA monomer (5.05 g, 35.26 mmol), CEP chain transfer agent (0.11 g, 0.42 mmol) and SPTP initiator (33.8 mg, 0.11 mmol) were dissolved in a mixture of methanol and water (20:80, wt./wt.; 5.06 g) in a 50 mL flask. The solution was adjusted to pH 3.5 using 5.0 M hydrochloric acid. The flask was immersed into water bath at 25°C. The solution was bubbled with argon gas in the dark for 1.5 h, and irradiated with visible light for 1.5 h (¹H NMR: 73% conv). The reaction solution was diluted in 5.0 mL methanol. Polymer was precipitated from large excess of acetone, and dried in vacuum oven at 25°C. Yield: 3.36 g, 92%; ¹H NMR: DP = 60, namely PHPMA₆₀; SEC: M_n = 9.7 kDa, PDI = 1.11. Next, PHPMA₆₀ extended with HisAM monomer at 1.0 M HisAM and $[\text{HisAM}]_0/[\text{PHPMA}_{60}]_0/[\text{SPTP}]_0 = 65:1:0.25$ in water at pH 3.5 under visible light for 45 min (¹H NMR: 91% conv.). Solution was dialyzed against water at pH 3.5 in a membrane (MWCO = 3.5 kDa) and lyophilized to afford the product (yield: 0.75g, 85%). Fig. S1a shows that the proton signals are discernible, which suggest excellent aqueous solubility. The degree of polymerization of PHPMA (DP_{PHPMA}) of 60 and that of PHisAM ($\text{DP}_{\text{PHisAM}}$) of 56 were quantified based on Equation S1-S2, in which I_d , I_{s+t} , I_{c+h+i} are integral signals of d in HPMA units, s, t at RAFT chain-ends (insert), and c in HPMA and h, i in HisAM units. It thus termed PHPMA₆₀-*b*-PHisAM₅₆. SEC analysis revealed well-defined PHPMA₆₀-*b*-PHisAM₅₆ at M_n =16.9 kDa and PDI=1.09 (Fig. S1b).

$$\text{DP}_{\text{HPMA}} = \frac{4 \times I_d}{I_{s+t}} \quad (\text{S1})$$

$$\text{DP}_{\text{HisAM}} = \frac{I_{c+h+i} - 2 \times I_d}{I_{s+t}} \quad (\text{S2})$$

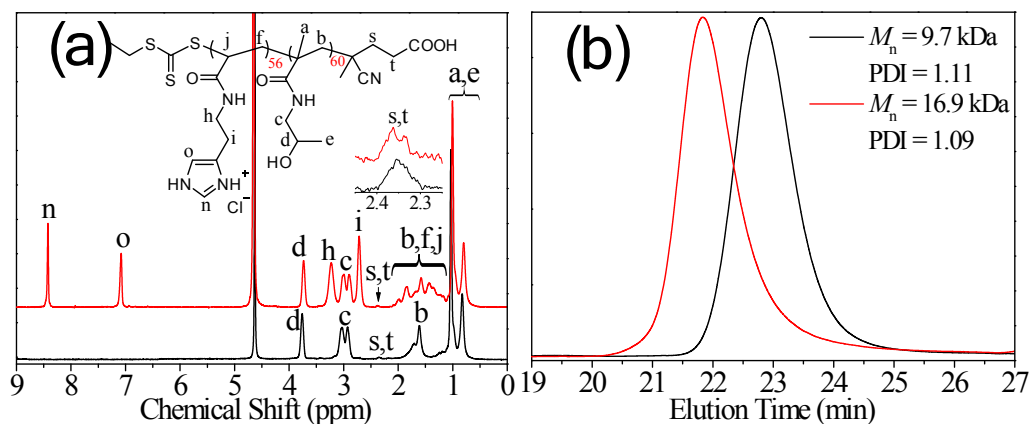


Fig. S1 (a) ¹H NMR spectra and (b) SEC traces of PHPMA₆₀ (black) and PHPMA₆₀-*b*-PHisAM₅₆ (red).

CONTIN analysis of correlation functions recorded by DLS

The CONTIN method¹⁻³ was used to analyse the normalized electric field-field time correlation function $g^{(1)}(q, \tau)$, which is related to the measured intensity-intensity time correlation function $G^{(2)}(q, \tau)$ as determined by DLS using Siegert relationship (eq. S3), in which A is the measured baseline, β is the coherent factor and $|g^{(1)}(q, \tau)| = \exp(-\Gamma \cdot \tau)$. The correlation function $G^{(2)}(q, \tau)$ could result in a line width distribution $G(\Gamma)$. For dilute system, $G(\Gamma)$ can be converted to a translational diffusion coefficient distribution $G(D)$ by $\Gamma = D \cdot q^2$, where D is translational diffusion coefficient and q is the magnitude of scattering wave vector, $q = 4\pi n_0 / \lambda_0 \sin(\theta/2)$, where n_0 is the refractive index of the solvent, λ_0 is the wavelength of laser in a vacuum and θ is the scattering angle. Then, the hydrodynamic diameter ($D_{h, DLS}$) of the particles can be calculated from D via Stokes–Einstein equation. DLS measurements also provide information on the particle-size distribution in solution from a plot of $\Gamma G(\Gamma)$ versus $D_{h, DLS}$.

$$G^{(2)}(q, \tau) = A \times (1 + \beta \times |g^{(1)}(q, \tau)|^2) \quad (S3)$$

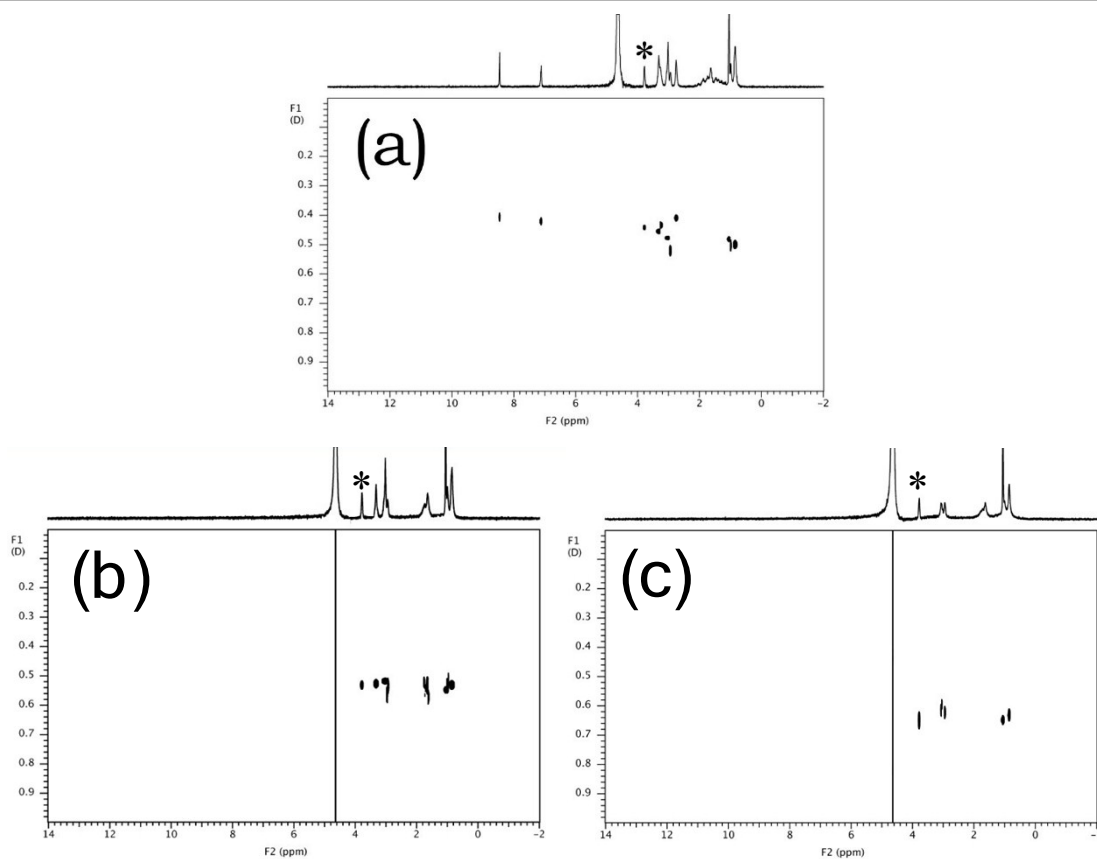


Fig. S2 NMR DOSY spectroscopy for the analysis of diffusion coefficient (D_{DOSY}) values of P-3 at pH 3.0 (a), 4.4 (b) and 6.4 (c).

Analysis of Diffusion Coefficient of P-3 using DOSY Experiment

Fig. S2 shows that the resonance in HPMA units at $\delta = 3.9$ ppm (star) is discernible in the solution during neutralization. Thus, it was used as an indicative to analyse the pH-labile diffusion coefficient (D_{DOSY}) value of P-3.

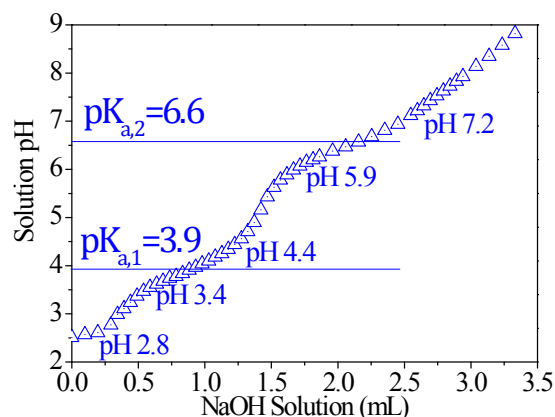


Fig. S3 Potentiometric titration plot of **P-3** (1.0 mg/mL in water, $[\text{Cu(II)}]/[\text{HisAM}+\text{AEMA}] = 1/4$). It shows the isolated buffer regions within pH 3.4–4.4 and 5.9–7.2.

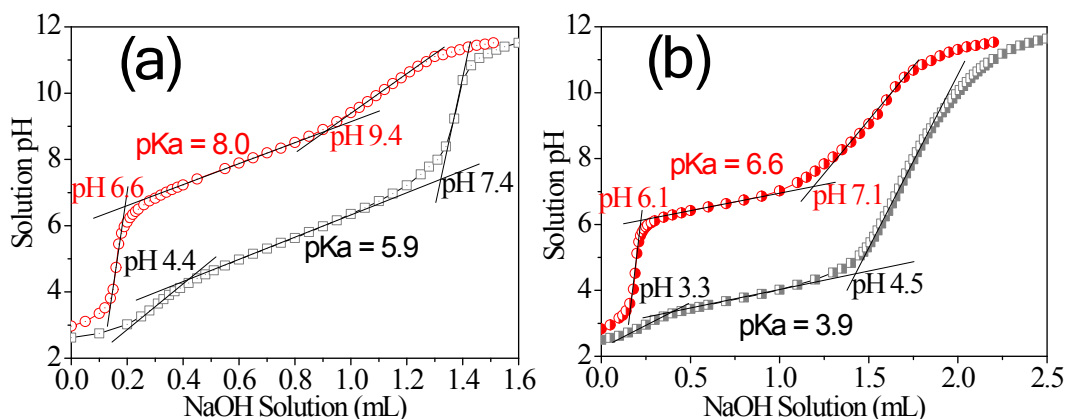


Fig. S4 Potentiometric titration plots of **P-1** (black; 4.2 mg/mL, $[\text{HisAM}] = 11.2 \text{ mM}$) and **P-2** (red; 3.6 mg/mL, $[\text{AEMA}] = 11.6 \text{ mM}$) in the absence (a) and the presence (b) of copper ions at $[\text{Cu(II)}]/[\text{HisAM}] = [\text{Cu(II)}]/[\text{AEMA}] = 1/4$. Different from the overlapped buffer regions in the absence of copper ions shown in Fig. S4a, coordination led to buffer regimes of pH 3.3–4.5 (PHisAM) and 6.1–7.1 (PAEMA) (Fig. S4b).

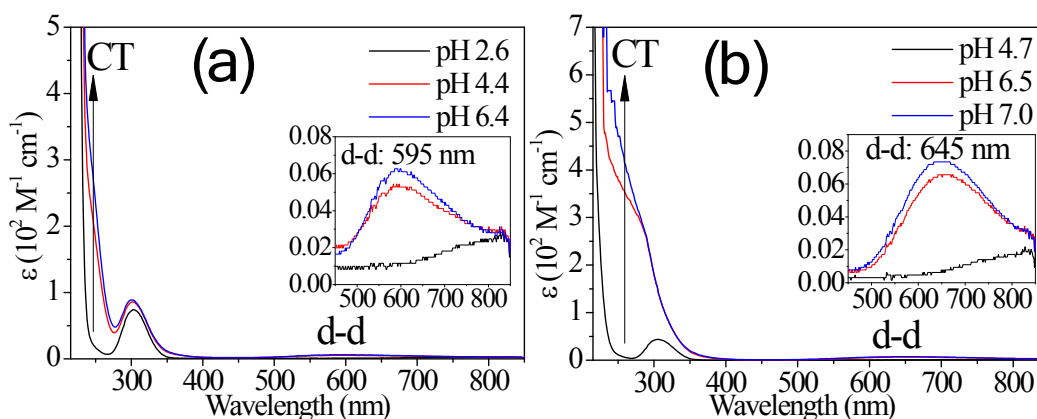


Fig. S5 UV-vis spectra of (a) **P-1** and (b) **P-2** (2.0 mg/mL in water, $[\text{Cu(II)}]/[\text{HisAM}]=[\text{Cu(II)}]/[\text{AEMA}]=1/4$) at labelled pH values. Herein, the Cu(II) -PHisAM complex band locates at $\lambda_{\text{max}} = 595 \text{ nm}$ but that of Cu(II) -PAEMA complexes at 645 nm .

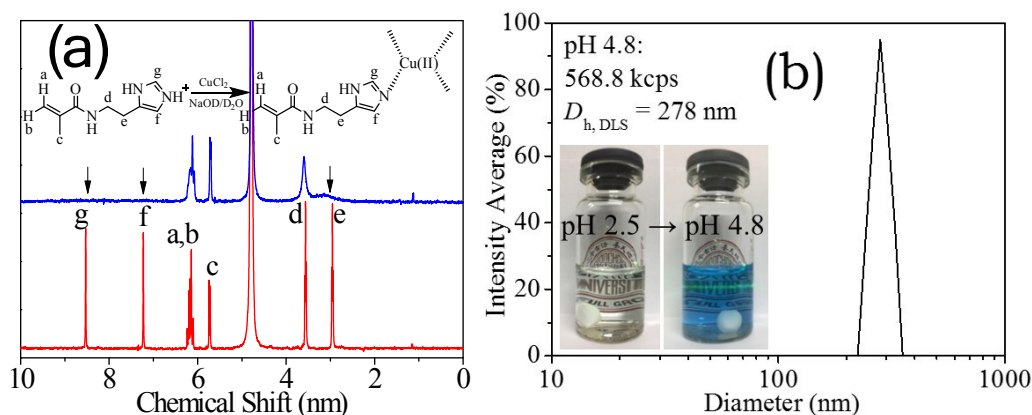


Fig. S6 (a) ^1H NMR spectra of HisAM monomer (2.0 mg/mL in water) in the absence (red) and presence (blue) of copper ions at $[\text{Cu(II)}]/[\text{HisAM}]=1/4$ and pH 4.8. (b) DLS profiles of the solution after neutralization from pH 2.5 to 4.8.

Fig. S6a shows that the resonances in imidazole ring and neighbouring spacer (arrows) disappeared because of Cu(II)-coordination. This coordination induced phase transition, as judged by observable laser beam across the bluish solution (Fig. S6b, inset), dramatically increased DLS intensity, and detected nanoparticle size at a $D_{h, \text{DLS}} = 278 \text{ nm}$ (Fig. S6b).

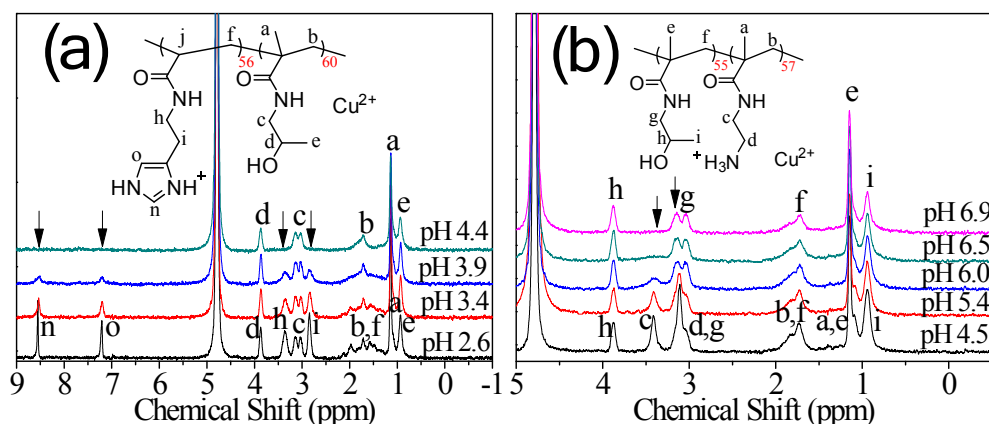


Fig. S7 ^1H NMR spectra of (a) **P-1** and (b) **P-2** (2.0 mg/mL in D_2O , $[\text{Cu(II)}]/[\text{HisAM}] = [\text{Cu(II)}]/[\text{AEMA}] = 1/4$) at labelled pH values.

Fig. S7a shows that signals in PHisAM block diminished at 3.4 and vanished at pH 4.4, which suggest that PHisAM block collapsed over pH 3.4-4.4. In contrast, those in PAEMA block decreased at pH 5.4 and vanished at pH 6.9 (Fig. S7b). The pH values of the PAEMA-folding are clearly higher than those of the former.

Theoretical size and the reduction of P-3 single-chain nanoparticle

Theoretical size of a completely compacted polymer chain into spherical nanoparticle, was evaluated using eq. S4,⁴ in which $D_{h, \text{theo}}$ is the diameter, M_n is the number-average molecular weight and copper ions (e.g. $M_n=34.5\text{kDa}$ of metal-folded **P-3**), ρ is the density (1.10 g/cm^3), and N_A is Avogadro's number. Accordingly, the $D_{h, \text{theo}}$ value of 4.6 nm of the metal-folded **P-3** was assessed. The expected size shrinkage after intermolecular crosslinking into model SCNP is given by $R = R_0(1 - x)^{0.6}$,⁵ where x is a fraction of crosslinked groups. Accordingly, an expected shrinkage of 52% in the diameter was assessed.

$$D_{h, \text{theo}} = \left(\frac{6M_n}{\pi\rho N_A} \right)^{1/3} \quad (\text{S4})$$

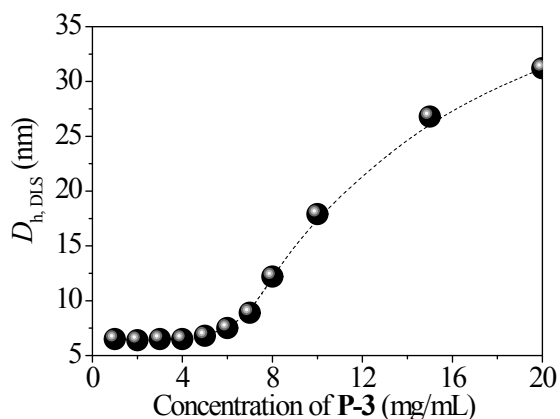


Fig. S8 Concentration dependent $D_{h,DLS}$ values of **P-3** in water at $[Cu(II)]/[HisAM+AEMA] = 1/4$ and pH 6.4. The result illustrates that the intramolecular self-assembly occurred below 5 mg/mL.

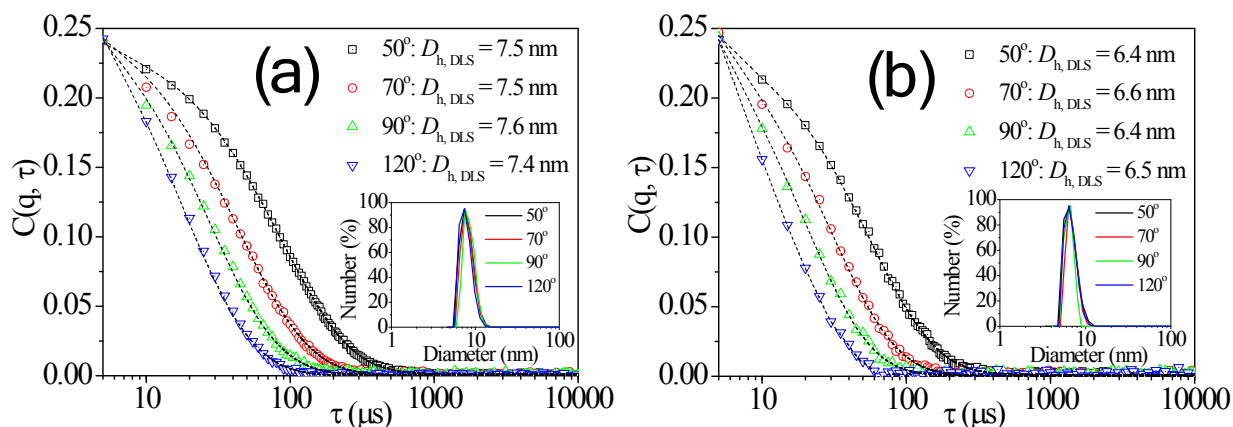


Fig. S9 Angle-dependent correlation functions and CONTIN plots (*insert*) of **P-3** (2.0 mg/mL, $[Cu(II)]/[HisAM+AEMA]=1/4$) in water at pH 4.8 (a) and 6.2 (b), respectively.

Table S1. Cross-Sectional Sizes of Line-Labelled SCNP Representatives that are shown in Left Image of Fig. 6

Sample	1-1	1-2	2-1	2-2	3-1	3-2	4-1	4-2	Average
Height (nm)	1.8	1.8	1.9	1.7	1.7	1.8	1.9	1.7	1.8 ± 0.1
Width (nm)	16.9	18.3	15.6	15.8	16.9	18.5	15.6	16.3	16.7 ± 1.8

Reference

1. T. B. Liu, *J. Am. Chem. Soc.*, 2002, **124**, 10942-10943.
2. A. Z. Niu, D. J. Liaw, H. C. Sang and C. Wu, *Macromolecules*, 2000, **33**, 3492-3494.
3. S. W. Provencher, *Comput. Phys. Commun.*, 1982, **27**, 229-242.
4. L. Oria, R. Aguado, J. A. Pomposo and J. Colmenero, *Adv. Mater.*, 2010, **22**, 3038-3041.
5. J. A. Pomposo, J. Rubio-Cervilla, A. J. Moreno, F. Lo Verso, P. Bacova, A. Arbe and J. Colmenero, *Macromolecules*, 2017, **50**, 1732-1739.