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CO_2-driven vesicles to micelle regulation of amphiphilic copolymer: random versus block strategy

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1. ^{1}H NMR characterizations

The copolymers were synthesized by reversible addition–fragmentation chain transfer polymerization (RAFT), using *macro*-PEO as a macromolecular chain transfer agent (CTA). The synthesis route is shown in Fig. 1, and their characterizations are shown in Figure S1–S4.

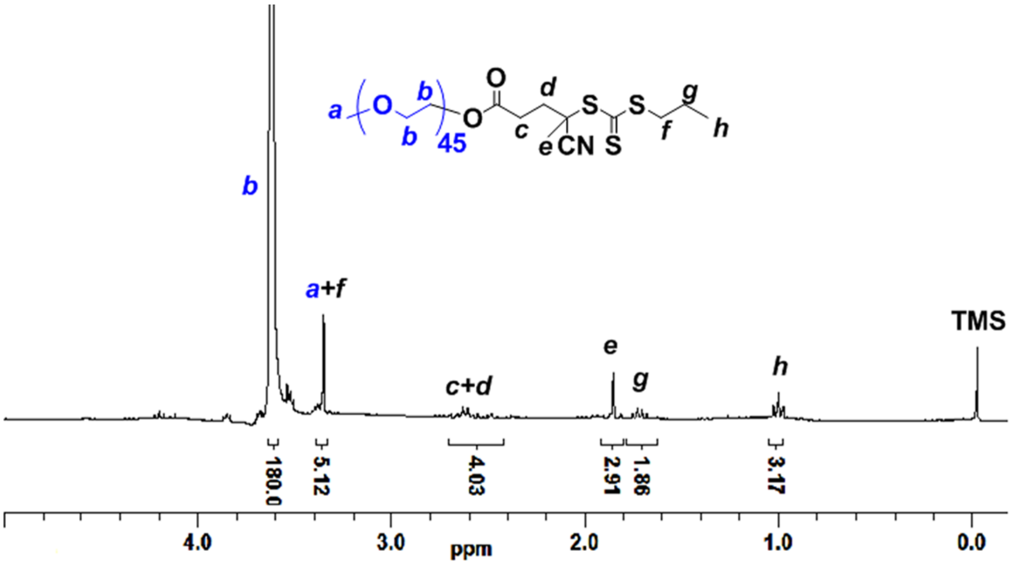

Fig. S1 ^{1}H NMR spectrum of *macro*–PEO_{45} in $CDCl_3$.

For random copolymer $\mathbf{P_r}$, PEO_{45}–*b*–($DEAEMA_{90}$–*r*–St_{66}), the polymerization degree is calculated according to equations (1) and (2) as follows:

$$\frac{\delta_b}{4} : \frac{\delta_h}{2} : \frac{\delta_l}{5} = DP_{PEO} : DP_{DEAEMA} : DP_{St} \qquad (1)$$

$$\frac{\delta_b}{4} = DP_{PEO} = 45 \qquad (2)$$

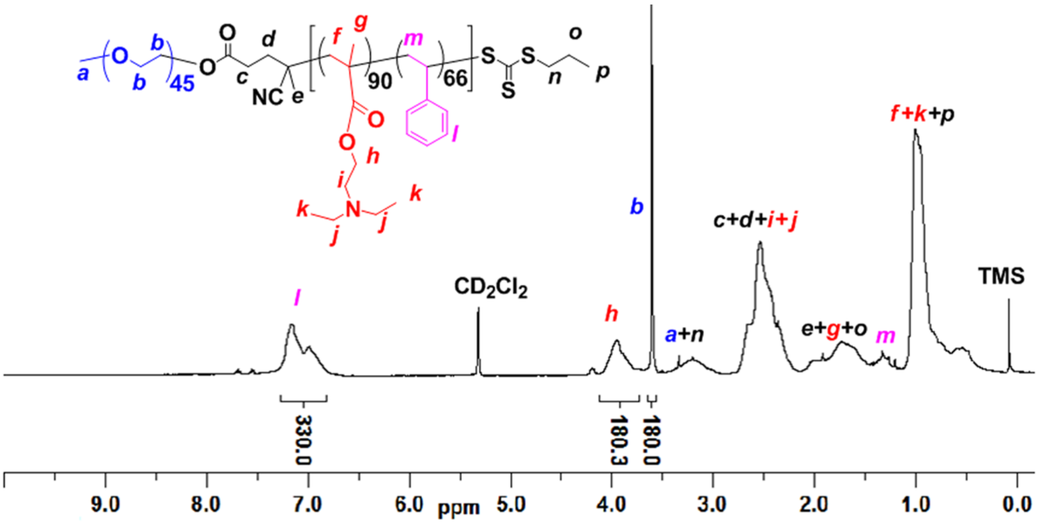

Fig. S2 ^{1}H NMR spectrum of random copolymer $\mathbf{P_r}$, PEO$_{45}$–*b*–(DEAEMA$_{90}$–*r*–St$_{66}$) in CD$_2$Cl$_2$.

For the diblock precursor and triblock polymer $\mathbf{P_b}$, PEO$_{45}$–*b*–DEAEMA$_{93}$–*b*–St$_{66}$, the polymerization degree is calculated according to the above equations (1) and (2) as well.

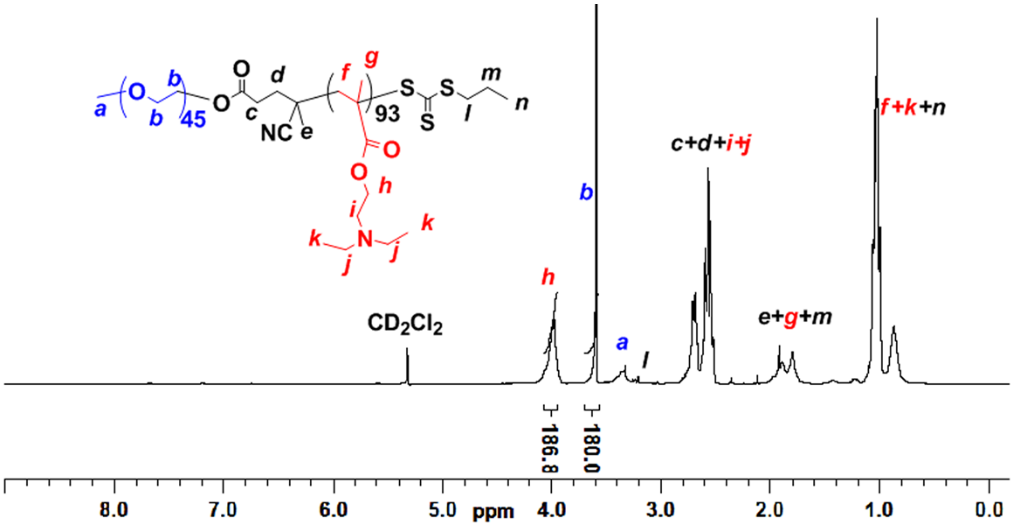

Fig. S3 ^{1}H NMR spectrum of diblock precursor PEO$_{45}$–*b*–DEAEMA$_{93}$ in CD$_2$Cl$_2$.

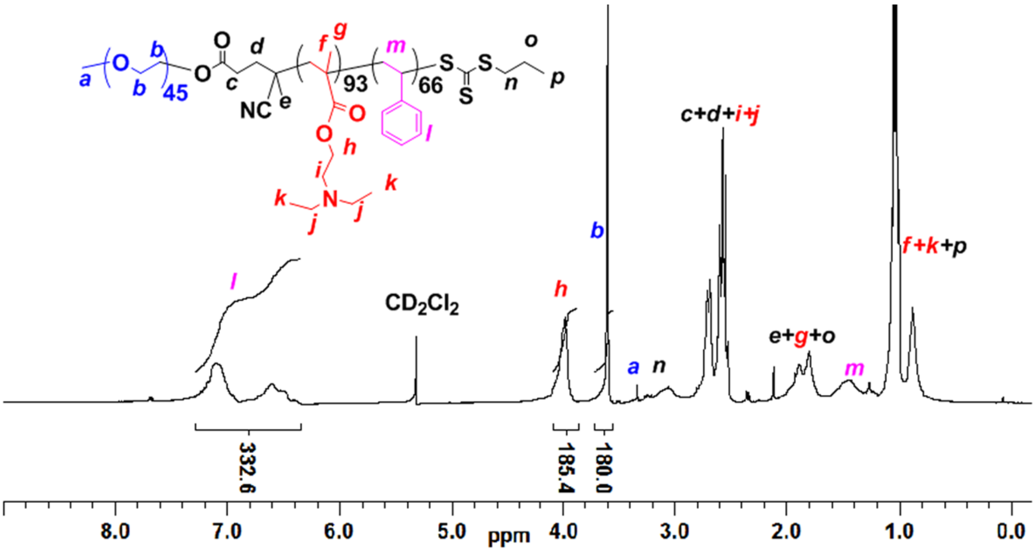

Fig. S4 ^1H NMR spectrum of triblock polymer $\mathbf{P_b}$, PEO_{45}–*b*–$DEAEMA_{93}$–*b*–St_{66} in CD_2Cl_2.

2. Calculation of average sequence length in random copolymer

The reactivity ratio of St (r_{St}) and DEAEMA (r_{DEAEMA}) can be determined by the Kelen-Tudos method, [1]

$$(\rho\text{-}1)/R = r_{St}\rho/R^2 - r_{DEAEMA} \qquad (3)$$

where R is defined as the molar feed ratio of St to DEAEMA, and ρ is defined as the molar ratio of St to DEAEMA in copolymer. For the random copolymer $\mathbf{P_r}$: R=0.2686, ρ=0.7333. So the average sequence length of St ($\bar{n}_{St}$) can be calculated according to equation (4) and (5) as follows:

$$p_{11} = \frac{r_{St}}{r_{St} + 1/R} \qquad (4)$$

$$\bar{n}_{St} = \frac{1}{1 - p_{11}} \qquad (5)$$

Similarly, the average sequence length of DEAEMA ($\bar{n}_{DEAEMA}$) can be calculated according to equation (6) and (7) as follows:

$$p_{22} = \frac{r_{DEAEMA}}{r_{DEAEMA} + R} \qquad (6)$$

$$\bar{n}_{\mathrm{DEAEMA}} = \frac{1}{1-p_{22}} \qquad (7)$$

Here using the r_{St}=0.22 and r_{DEAEMA}=0.42 from literature,[2] we can accordingly calculate the average sequence length of St and DEAEMA as:

$$\bar{n}_{St} = 1.06\ ;\ \bar{n}_{DEAEMA} = 2.56$$

It means that every one St monomeric unit locates in three DEAEMA units in general. The maximum protonated degree is only 35% for the random copolymer Pr, which means over 65% of DEAEMA units cannot be protonated. And considering 50% of the sequence length of DEAEMA is less than 3, the minimum sequence length of DEAEMA units that must be present for CO_2 response should be 3, implying only the DEAEMA units where the sequence length is over 3 can react with CO_2.

3. pKa measurement and protonated degree calculation of the DEAEMA moieties

To measure the pK_a of the diblock copolymers in aqueous solution, 5 mL polymer solution was titrated with 0.002 mol·L^{-1} hydrochloric acid calibrated by NaOH, while the pH was continuously monitored with the pH meter. The pH corresponding to the half of the equivalence was taken as the pK_a value,[3] pK_a=5.0 for the random copolymer $\mathbf{P_r}$, PEO_{45}–*b*–($DEAEMA_{90}$–*r*–St_{66}) and pK_a=6.7 for the triblock copolymer $\mathbf{P_b}$, PEO_{45}–*b*–$DEAEMA_{93}$ –*b*–St_{66}, as shown in Fig. S5.

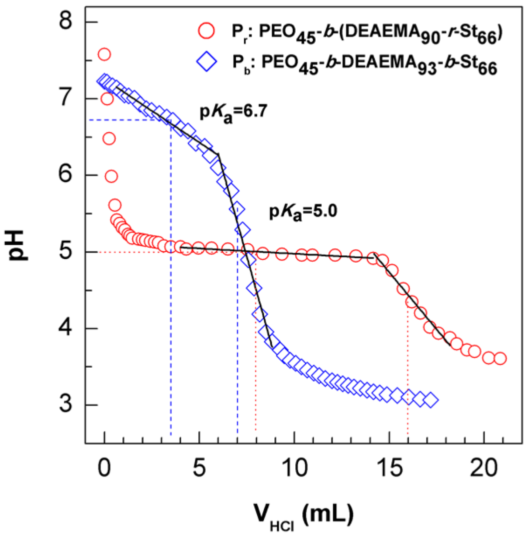

Fig. S5 Titration curve of vesicular solution (5.0 mL, 1.0 g·L^{-1}) with HCl (0.002 mol·L^{-1}).

The protonation degree (δ) of DEAEMA moieties was calculated according to the following equations (8)–(11):[3]

$$K_a = \frac{[\mathrm{PDEAEMA}][\mathrm{H}^+]}{[\mathrm{PDEAEMAH}^+]} \quad (8)$$

$$\delta = \frac{[\mathrm{PDEAEMAH}^+]}{[\mathrm{PDEAEMA}]+[\mathrm{PDEAEMAH}^+]} \quad (9)$$

$$\mathrm{pH} = -\log[\mathrm{H}^+] \quad (10)$$

$$\delta = \frac{1}{1+10^{\mathrm{pH}-\mathrm{p}K_a}} \quad (11)$$

Based on the pK_a and the different pH values of the polymer solution as function of CO_2 bubbling time (the flow rate was fixed at around 15 mL·min^{-1}), the protonation degrees of DEAEMA moieties in copolymers at different pH values were calculated. One can figure out that δ of DEAEMA moieties in $\mathbf{P_r}$ increases from 0.3% to approximately 35% after CO_2 bubbling for 20 min. For the triblock counterpart $\mathbf{P_b}$, the δ increases from 16% to 97% after CO_2 bubbling for 20 min.

4. Kinetically trap for $\mathbf{P_b}$ aggregates formation

For the triblock copolymer, the DLS measurement shows that the vesicles have a significant size expansion upon the stimulus of CO_2. Is it possible that the PS serves as kinetically trap for the vesicle formation? To clear this confusion, we formed the assemblies of $\mathbf{P_b}$ in water that has already been treated with CO_2 for 1 h, and then visualize the morphology with TEM. Though the aggregate is over stained, we can distinguish the lighter member and the dark volume, indicating as vesicles (Fig. S6a). Furthermore, the average diameter (D_h) detected with DLS is approximately 390 nm (Fig. S6b), which is much larger than that of $\mathbf{P_b}$ assemblies formed in water without CO_2 treatment (190 nm). So the size expansion in DLS measurement when CO_2 is bubbling might be caused by the kinetically trap of polystyrene block in formation of the self-assemblies of triblock copolymer $\mathbf{P_b}$.

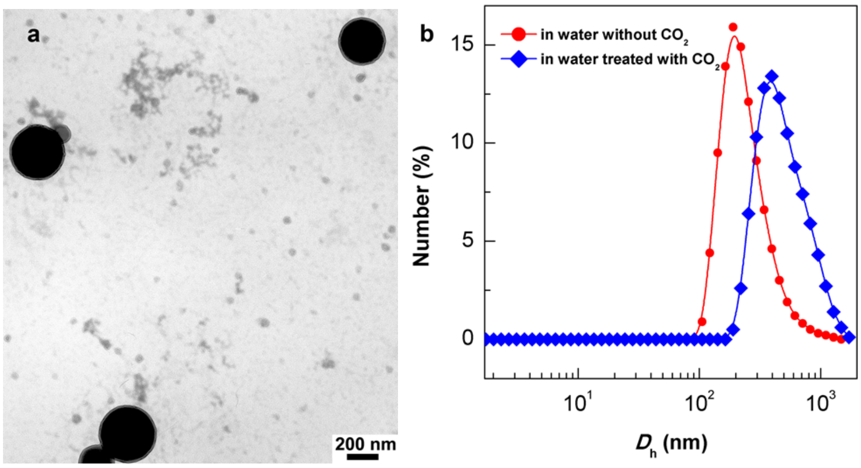

Fig. S6 TEM image (a) and DLS data (b) of the aggregate for $\mathbf{P_b}$ formed in water that has been treated with CO_2.

5. Analysis with the theory of hydrophilic volume fraction (f_{philic})

According to the concept proposed by Discher et al.[4,5], the morphology of polymer aggregates might be predicted with the hydrophilic volume fraction (f_{philic}). The block copolymer is expected to form spherical micelles when f_{philic} > 50%, worm-like micelles when 40% < f_{philic}< 50%, vesicles or otherlamellar structures for f_{philic}< 40%. In the present work the equation can be produced as follows:

$$f_{\mathrm{philic}} = \frac{M_{\mathrm{PEO}}/\rho_{\mathrm{PEO}} + M_{\mathrm{DEAEMA+}}/\rho_{\mathrm{DEAEMA+}}}{M_{\mathrm{PEO}}/\rho_{\mathrm{PEO}} + M_{\mathrm{DEAEMA}}/\rho_{\mathrm{DEAEMA}} + M_{\mathrm{St}}/\rho_{\mathrm{St}}} \times 100\% \qquad (12)$$

Where the M_{PEO}, $M_{\mathrm{DEAEMA+}}$, M_{DEAEMA}, and M_{St} are the mass of PEO, charged DEAEMA, total DEAEMA, and St; ρ_{PEO}, $\rho_{\mathrm{DEAEMA+}}$, ρ_{DEAEMA}, and ρ_{St} are the density of PEO, charged DEAEMA, total DEAEMA, and St. (ρ_{PEO} = 1.15 g·cm^{-3}, ρ_{St} = 1.06 g·cm^{-3}, $\rho_{\mathrm{DEAEMA+}} \approx \rho_{\mathrm{DEAEMA}}$ =1.19 g·cm^{-3})[6]

We calculated the value of f_{philic} for the random and triblock copolymer before and after reaction with CO_2 according to equation (12), and the results are listed in Table S1. Before CO_2 bubbling, it can be found that the f_{philic} is 8% for the random copolymer **P_r** and 18% for the block counterpart **P_b**, respectively. Both of them form vesicles, in agreement with the theory prediction. But after CO_2 bubbling, the aggregate of the random copolymer **P_r** should be vesicles for f_{philic}=30%, actually it appears as spherical micelles. For the block counterpart **P_b**, the aggregate is still vesicles rather than the predicted spherical micelles with f_{philic}=70%. So in the present work, this theory seems not fully suitable for the comprehension of the morphology after the treatment of CO_2 for both **P_r** and **P_b**.

Table S1 Protonation degrees (δ) of PDEAEMA in diversity copolymers

Sample	Before bubbling CO_2			After bubbling CO_2		
	pH	δ	f_{philic}	pH	δ	f_{philic}
P_r	7.50	0.3%	8%	5.27	34.9%	30%
P_b	7.42	16%	18%	5.12	97.4%	70%

6. Ultrasonic treatment of P_r

Considering the vesicle to spherical micelle morphologic transition of **P_r** is related with limited protonation of the DEAEMA moieties in the random structure, it is interesting to check whether an ultrasonic treatment of **P_r** can break up the aggregate to get higher protonated degree. Before the stimulus of CO_2, the **P_r** vesicular solution was ultrasonic treated for 30 min. Then the pH is monitored when CO_2 is bubbling. As shown in Fig. S7a, the pH decreases from 6.95 to 5.19 upon the treatment of CO_2. And the protonated degree (δ) is calculated according to the above-mentioned equation (11), showing a corresponding increase from 1% to 39% which is comparable with that of **P_r** without ultrasonic treatment (from 0.3% to 35%). Furthermore, TEM visualization indicates the aggregate before CO_2 bubbling is vesicle with an average diameter of 157 nm, and transform into spherical micelle with an average diameter of 43 nm (Fig. S7b and S7c), which is also similar with the morphologic change of $\mathbf{P_r}$ free of ultrasonic treatment (vesicle to spherical micelle). In short, the ultrasonic treatment has no influence for the protonated degree or the self-assemble morphology for the random copolymer $\mathbf{P_r}$.

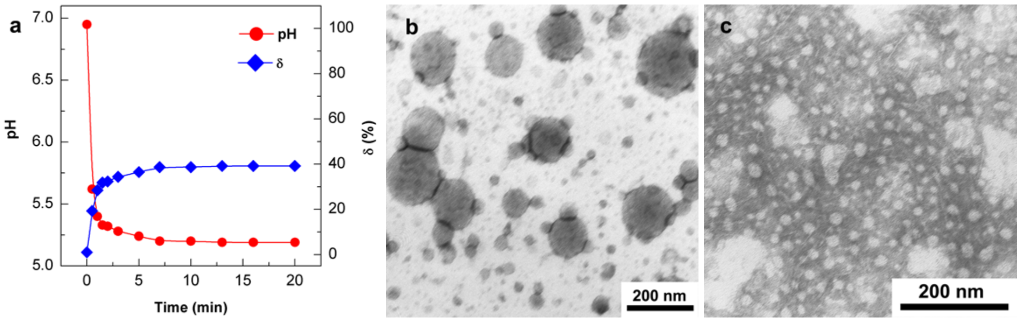

Fig. S7 The pH, protonated degree change (a) and TEM images (b, c, stained with 0.2 wt% phosphotungstic acid) of $\mathbf{P_r}$ aggregates after ultrasonic treatment of 30 min. (a): before CO_2 bubbling; (b) after CO_2 bubbling. Bars: 200 nm.

7. Reversibility of the morphologic transition

After the removal of CO_2 by bubbling N_2 for the aggregate of random copolymer $\mathbf{P_r}$, the intensity distribution shows two peaks, indicating the coexistence of large aggregates with D_h of approximately 300 nm and small aggregates with D_h of approximately 78 nm (Fig. S8a). The corresponding number distribution appears as two peaks as well, one with D_h of approximately 73 nm is much stronger than the other with D_h of approximately 295 nm, suggesting the assemblies of around 73 nm dominate the majority in this system. And TEM image shows that the majority of the assemblies are spherical micelles and a few vesicles (Fig. S8b), indicating the irreversible feature of the vesicle to micelle transition in the present work.

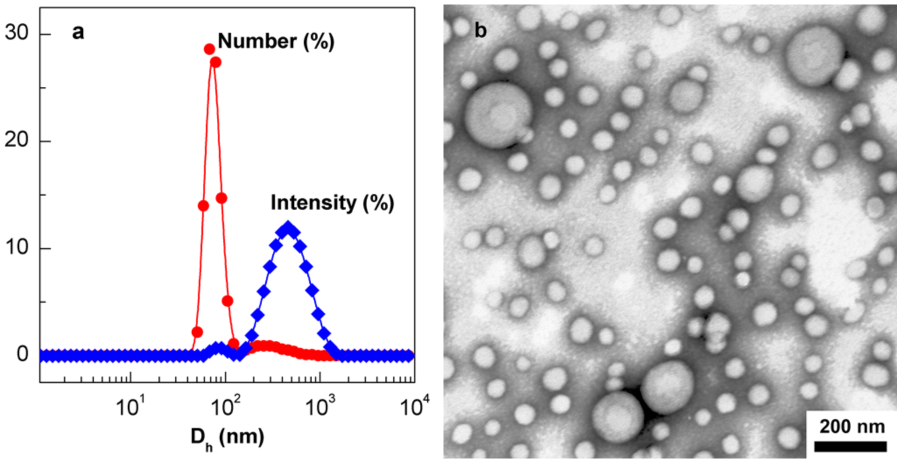

Fig. S8 DLS data (a) and TEM image (b) of $\mathbf{P_r}$ assemblies after removing CO_2.

Reference

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