

Homopolymer Vesicles Triggered by Side-Chain Hydration

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Electronic Supplementary Information

Abstract The formation of homopolymer vesicles and the identification of their self-assembly mechanism have been challenging issues in the field of polymer self-assembly. Using complementary circular dichroism (CD), Fourier transform infrared (FTIR) and low-field nuclear magnetic resonance (LF-NMR) spectroscopies, in this study we show that the higher hydrophilic ability of side-chains is a key factor for the formation of homopolymer vesicles from homo poly(*N*-carbobenzoxy-L-lysine) (PZlys), in which the vesicular structures are confirmed by scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM) measurements. Poly(γ -benzyl-L-glutamate-co-*N*-carbobenzoxy-L-lysine) [P(BLG-co-Zlys)] containing different molar ratios of BLG to Zlys units with the same peptide backbone as PZlys are used as the controls. The copolymers can only form micelles under the same conditions, due to the more hydrophobic nature of their side-chains. These results show that the specific side-chain structure of the polypeptides plays a fundamental role in the self-assembly of homopolymers. These findings can be useful to understand how the hydrophilicity of the side chains of amino acid residues on the surface of a protein affects its structures.

Keywords Polypeptide; Vesicle; Self-assembly; Hydrophobic; Micelle

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INTRODUCTION

Polymer vesicles, being among the most important self-assembled polymer structures, have attracted considerable attention in the recent years, due to their potential applications in fields ranging from nano- to biotechnology.^[1–5] For instance, polymer vesicles self-assembled from amphiphilic block copolymers through non-covalent interactions (such as hydrophilic/hydrophobic, electrostatic, hydrogen bonding and π - π interactions) have been extensively explored over the past decades, with applications ranging from simulated cell membranes to biological delivery systems.^[6–15] Recently, with the development of new synthesis and polymerization technologies, amphiphilic homopolymer vesicles have also attracted significant interest owing to their versatility.^[16–18] Despite the rapidly growing interest in amphiphilic homopolymers, the formed nanostructures are still not well-defined and the driving force of the homopolymer self-assembly process remains unclear.

Amphiphilic homopolymers contain both hydrophobic and hydrophilic moieties and can assemble into different nanostructures such as vesicles, toroid, necklaces, nanoworms and discs.^[19–26] After the pioneering work on the self-assembly of

amphiphilic homopolymers by Thayumanavan *et al.*,^[27–29] different types of homopolymers have been prepared and their self-assemblies have been investigated.^[6–15,20,21] Because of the ambiguous interface between the hydrophobic and hydrophilic domains in amphiphilic homopolymers, the investigation of their self-assemblies is more complicated than that of block copolymers. Vesicles formed from amphiphilic block polymers possess a hydrophobic membrane and hydrophilic interior, in which bilayer and interdigitated structures driven by hydrophobic effects are the two basic packing modes in polymer vesicle membranes.^[17] However, Du and co-workers confirmed that amphiphilic homopolymer-based vesicles have a complicated ‘gradient membrane’ structure.^[30,31] Mazzier and co-workers reported that novel fullerene (C₆₀) end-capped poly(γ -benzyl-L-glutamate) (PBLG) homopolymers were able to self-assemble in water, creating toroidal or vesicular nanostructures, and the experimental results were confirmed by molecular dynamics simulations.^[15] Nevertheless, very limited attention has been devoted to the interaction between hydrophilic and hydrophobic units in amphiphilic homopolymers, which may be due to the lack of appropriate and effective characterization methods. More recently, our group used a combination of Fourier-transform infrared (FTIR) and low-field nuclear magnetic resonance (LF-NMR) techniques to confirm the formation of vesicles driven by the hydrated backbone of metal-carbonyl homopolymers with ionized end groups.^[32] The molecular-level identifica-

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tion of these interactions will support a deeper understanding of the assembly mechanism of the present systems.

Herein, we report the preparation of homopolypeptide vesicles by solution self-assembly of poly(*N*-carbobenzoxy-L-lysine) (PZlys) homopolypeptide possessing hydrophobic and hydrophilic side-chains. The combination of CD, FTIR and LF-NMR measurements confirm that the side-chains of PZlys, as the hydrophilic component, play an important role in the formation of vesicles. Furthermore, the random copolypeptides poly(γ -benzyl-L-glutamate-co-*N*-carbobenzoxy-L-lysine) [P(BLG-co-Zlys)] with a lower content of hydrophilic side-chains are found to only assemble into micelles.

EXPERIMENTAL

General Considerations

N-carbobenzoxy-L-lysine (Zlys) and L-glutamate were purchased from Sinopharm Chemical Reagent Co. γ -Benzyl-L-glutamate (BLG), *N*-carbobenzoxy-L-lysine-*N*-carboxyanhydrides (Zlys-NCA) and γ -benzyl-L-glutamate-*N*-carboxyanhydrides (BLG-NCA) were synthesized according to the method described in the literature.^[33] Dialysis bags (Green Bird, 3500 molecular weight cut-off) and ultrapure water (18 k Ω) were used in the experiments. Methanol, *n*-butylamine, tetrahydrofuran (THF) and dimethylformamide (DMF) were purchased from Sinopharm Chemical Reagent Co. and purified by conventional procedures if needed.

The molecular weights and polydispersity (M_w/M_n ; M_w = weight-average molecular weight; M_n = number-average molecular weight) of PZlys and P(BLG-co-Zlys) were recorded on a gel permeation chromatography (GPC) equipped with two Mixed-B columns (Polymer Laboratory Corp. pore size = 10 μ m; column size = 300 $\times$ 7.5 mm) and a refractive index detector (Perkin-Elmer Series 200) using DMF (0.01 mol·L⁻¹ LiBr) as the eluent at 40 °C with a flow rate of 1.0 mL·L⁻¹. The column system was calibrated by a set of mono-dispersed standard PMMA.

¹H-NMR spectra were recorded on a Bruker Avance 500 MHz using CDCl₃ + 12% TFA as the solvent and tetramethylsilane (TMS) as the internal reference.

Fourier transform infrared (FTIR) spectra were recorded on a Nicolet iS50 spectrometer in Attenuated Total Reflectance (ATR) mode. The wavenumber was set from 400 cm⁻¹ to 4000 cm⁻¹. The THF/ water solutions were prepared by adding various amount of D₂O into the solution of PZlys or P(BLG-co-Zlys) in THF (1.0 mg·mL⁻¹, 500 μ L). The spectra were quickly recorded after dripping the solutions on the ATR crystal. The reason for using D₂O is that the vibration peak of D₂O is more than 2000 cm⁻¹, which has little effect on the characteristic peak values (1650–1750 cm⁻¹) of amide groups of polypeptides.^[34]

Dynamic light scattering (DLS) was performed on a Malvern Zetasizer NANO ZS3600 with a 4.0 mW laser (λ =633 nm). All the measurements were conducted at 20 °C with a scattering angle of 90°.

Circular dichroism (CD) spectra were measured over the wavelength range between 190 and 280 nm using a 0.1 cm quartz cell on a JASCO J-1500-150 CD spectrometer (JASCO, Japan). The sample solutions were prepared by adding various amount of ultrapure water into the solution of PZlys or

P(BLG-co-Zlys) in THF (1.0 mg·mL⁻¹, 500 μ L). The mean residue molar ellipticity of each polypeptide was calculated on the basis of the measured apparent ellipticity by following the literature-reported formulae: ellipticity ([θ] in deg·cm²·dmol⁻¹) = (millidegrees $\times$ mean residue weight)/(path length in millimeters $\times$ concentration of polypeptide in mg·mL⁻¹).^[35]

The scanning electron microscopy (SEM) measurement was performed using a field emission microscope (Gemini 450) with a low accelerating voltage of 2 kV. The transmission electron microscopy (TEM) observation was carried out on a JEM-2100F JEOL TEM microscope with an accelerating voltage of 200 kV. AFM images were obtained by multimode atomic force microscopy (Dimension 3100, Veeco), employing the tapping mode. The sample solutions were dropped on clean silicon wafers and allowed to dry naturally at room temperature. Before SEM observations, the samples were sputtered by gold.

Low field nuclear magnetic resonance (LF-NMR) analysis was performed at 25 °C by means of a VTMR20-analyst NMR spectrometer. T_2 was measured using the Carr-Purcell-Meiboom-Gill sequence (CPMG). T_2 measurements were performed with a τ -value (time between 90 pulse and 180 pulse) of 800 μ s.^[36] Data were acquired as 8 scan repetitions. The repetition time between two succeeding scans was 5 s. The LF-NMR relaxation curve was fitted to a multi-exponential curve with the MultiExp Inv Analysis software (Niumag Electric Corporation, Suzhou, China) using the inverse Laplace transform algorithm.^[32]

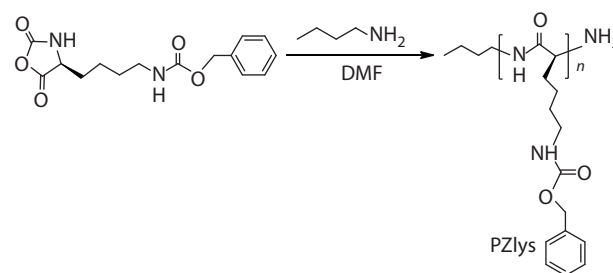
Synthesis of Homopolypeptide

Poly(*N*-carbobenzoxy-L-lysine) (PZlys)

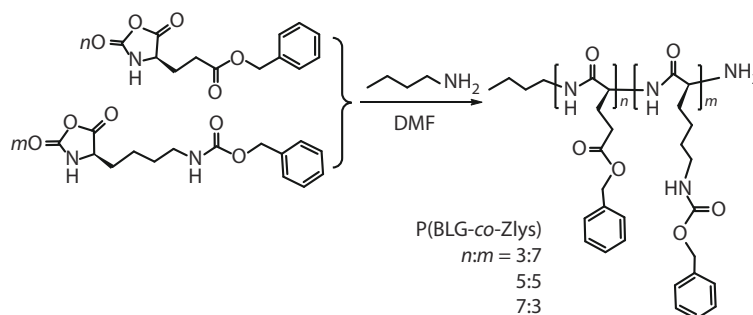
PZlys was synthesized by the ring-opening polymerization (ROP) of Zlys-NCA using *n*-butylamine as an initiator (Scheme 1) according to a similar procedure reported in the literature.^[37] Briefly, Zlys-NCA (2.0 g, 6.54 mmol), 6.4 μ L of *n*-butylamine and 20 mL of dry DMF were transferred into a dry Schlenk flask under a nitrogen atmosphere. After 3-days of polymerization, the solution was precipitated into an excess amount of methanol, filtered and dried at room temperature in a vacuum oven for 48 h. Yield: 80.5%.

Synthesis of Random Copolypeptides Poly(γ -benzyl-L-glutamate-co-*N*-carbobenzoxy-L-lysine) [P(BLG-co-Zlys)]

P(BLG-co-Zlys) random copolypeptides were synthesized by ring-opening polymerization (ROP) of Zlys-NCA and BLG-NCA using *n*-butylamine as an initiator (Scheme 2). The synthesis processes of P(BLG-co-Zlys) were similar as that of PZlys. Typically, Zlys-NCA (0.85 g, 2.77 mmol), BLG-NCA (0.73 g, 2.77



Scheme 1 Synthetic route to Pzlys.



Scheme 2 Synthetic route to P(BLG-co-Zlys) ($n:m$ means molar ratio).

mmol), 2.7 μL of *n*-butylamine and 15 mL of dry DMF were put into a dry Schlenk flask under a nitrogen atmosphere. The molar ratio of BLG-NCA to Zlys-NCA was 5:5. After 3-days of polymerization, the solution was precipitated into an excess amount of methanol, filtered and dried at room temperature in a vacuum oven for 48 h. Yield: 82.3%. The other two P(BLG-co-Zlys) samples were synthesized by using the same method through controlling the BLG-NCA to Zlys-NCA molar ratio of 3:7 or 7:3. The three P(BLG-co-Zlys) samples with BLG-NCA to Zlys-NCA molar ratios of 3:7, 5:5 and 7:3 were designated as P1, P2 and P3, respectively.

Self-assembly of Homopolypeptide PZlys and Random Copolypeptides P(BLG-co-Zlys) in Solution

The self-assembled aggregate solutions were prepared according to the following method. Briefly, PZlys or P(BLG-co-Zlys) was dissolved in THF with a concentration of about 1.0 mg/mL. The ultrapure water was added dropwise at a rate of 0.8 $\mu\text{L/s}$ to PZlys or P(BLG-co-Zlys) solution with vigorously stirring until the volume ratio of THF to water was 1:9. Subsequently, the solutions were dialyzed against water for 12 h to remove all organic solvents. All the experimental procedures including the processes of water addition and dialysis were performed at 25 $^{\circ}\text{C}$.

RESULTS AND DISCUSSION

Synthesis and Characterization of Polypeptides

The homopolypeptide PZlys and copolypeptides P(BLG-co-Zlys) with different molar ratios of BLG to Zlys units were synthesized via ROP using *n*-butylamine as an initiator (see Schemes 1 and 2). The degrees of polymerization (DP) of both PZlys and P(BLG-co-Zlys) were calculated to be approximately 80 through the corresponding $^1\text{H-NMR}$ (see Fig. S1 and Fig. S2 in the electronic supplementary information, ESI). By controlling the molar ratio of the monomers BLG-NCA to Zlys-NCA, three copolypeptide samples P(BLG-co-Zlys) with different BLG to Zlys unit molar ratios (3:7, 5:5 and 7:3) and the same DP values, can be synthesized and are denoted as P1, P2 and P3, respectively. Based on the $^1\text{H-NMR}$ measurements, the actual molar ratios of the monomer unit BLG to Zlys in copolypeptides P(BLG-co-Zlys) were almost equal to the theoretical molar ratios of the monomers BLG-NCA to Zlys-NCA (3:7, 5:5 or 7:3, see Table S1 in ESI) within the experimental error. The data from the $^1\text{H-NMR}$ and gel permeation chromatography (GPC) measurements (see Figs. S1 and S2 in ESI) are listed in Table S1 (in ESI), which confirmed the successful synthesis of PZlys and P(BLG-co-Zlys).

Self-assembly of Polypeptides

The PZlys and P(BLG-co-Zlys) aggregates were prepared by dialysis in THF/water (1/9, V/V) solution. A combination of SEM, TEM and AFM techniques was employed to examine the morphologies and structures of PZlys and P(BLG-co-Zlys) aggregates. An obvious collapse can be clearly observed in the SEM image shown in Fig. 1(a), reflecting the hollow structure of PZlys aggregates.^[16] Fig. 1(b) shows the typical TEM image, in which the real electron transmittance chart (Fig. 1c) related to the red scan-line (inset in Fig. 1b) is consistent with the same shape as the electron transmittance simulation chart of a hollow sphere, demonstrating the vesicular morphology of PZlys aggregates.^[19,31,38] The AFM image in Fig. 1(d) further confirms the vesicular structure of PZlys aggregates,^[39,40] as the uneven curve shown in Fig. 1(e) clearly prove the collapsed vesicular structure. Dynamic light scattering (DLS) measurement was carried out to evaluate the size of the PZlys vesicles in aqueous solution.^[41,42] The hydrodynamic diameter of the PZlys vesicles is 258.1 nm, with a low polydispersity index (PDI) of 0.18 (Fig. 1f and Fig. S3 in ESI). This size is significantly larger than the number-averaged diameter of the vesicles determined from the TEM (222.0 nm) and AFM (250.0 nm) (Fig. 1e) measurements. The possible reason for this discrepancy is that the DLS data is a reflection of vesicle aggregation in aqueous solution.^[30] The electron transmittance chart in Fig. 1(c) plotted along the red scan line in the inset of Fig. 1(b) suggests that the membrane thickness of PZlys vesicles was approximately 14.0 nm.^[16,31,38] However, the copolypeptides P(BLG-co-Zlys) (i.e., P1, P2 and P3) with the same peptide backbone as PZlys appear to self-assemble into micelles, due to the absence of features denoting structural collapse in the SEM images shown in Figs. 2(a)–2(c). Solid spheres are only observed in the TEM image (Fig. 2d), with a hydrodynamic diameter of around 250.2 nm estimated through the DLS analysis (Fig. 2f and Fig. S3 in ESI), and the electron transmittance chart in Fig. 2(e) is different from that of the PZlys vesicles (Fig. 1c), clearly demonstrating the solid sphere structure of the P2 micelles.^[16] The AFM images shown in Fig. S4 (in ESI) further confirms the micellar morphology of P2 aggregates. The comparison of the chemical structures of PZlys and P(BLG-co-Zlys) suggests that their backbones are same, while their side-chains are different. Therefore, the interaction between water molecules and the side-chains may be the reason for the different assembly behaviors of PZlys and P(BLG-co-Zlys).

Mechanism of Self-assembly

Based on the specific electronic transitions of the amide groups of the peptide backbone upon irradiation with circularly

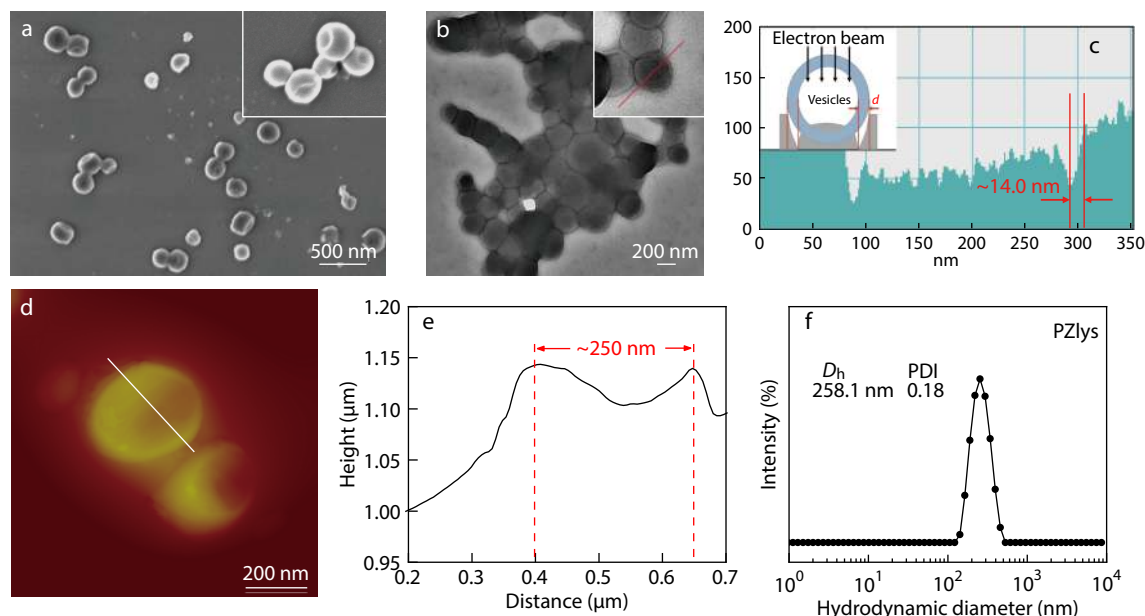


Fig. 1 (a) SEM and (b) TEM images of PZlys vesicles by self-assembly of the PZlys homo-polypeptide; (c) The real electron transmittance chart related to the red scan-line in (b), suggesting that the actual membrane thickness of vesicle is about 14.0 nm, and the inset is an electron transmittance simulation graph of a hollow sphere, where d is the membrane thickness; (d) AFM image of PZlys vesicles; (e) The diameter of a vesicle is about 250.0 nm, as the white line indicated in (d); (f) DLS curve of PZlys vesicles.

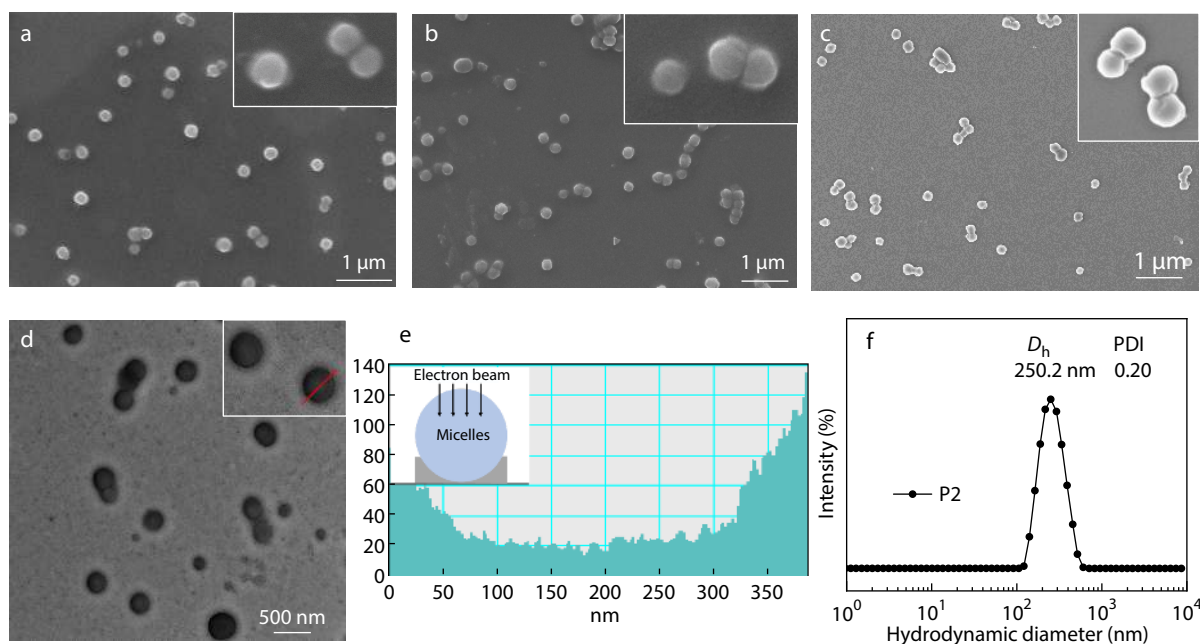


Fig. 2 SEM images: (a), P1; (b), P2 and (c), P3. (d) TEM image of P2 micelles and (e) the electron transmittance chart related to the red scan-line in (d) and the inset is an electron transmittance simulation graph of a solid sphere; (f) DLS curve of P2 micelles.

polarized UV light, circular dichroism (CD) is often used to evaluate the secondary structure of polypeptides in aqueous solutions.^[43,44] As shown in Fig. 3, two broad negative Cotton effects around 209 and 221 nm (originating from $n-\pi^*$ characteristic transitions) are obviously observed with increasing amount of water, suggesting that both PZlys and P(BLG-co-Zlys) adopt a stable α -helical conformation in THF/water solutions,^[44–47] and the nature of their side-chains

does not affect their α -helical conformation structures. Upon gradually increasing the amount of water from 0 μL to 30 μL , the $\theta_{221}/\theta_{209}$ ratios of PZlys and P(BLG-co-Zlys) remain almost unchanged and are always higher than 0.9, indicating their higher helical packing density.^[48,49] The results also show that water has almost no effect on the α -helical conformations formed by intramolecular hydrogen bonds between the peptide backbones of PZlys or P(BLG-co-Zlys), indicating that

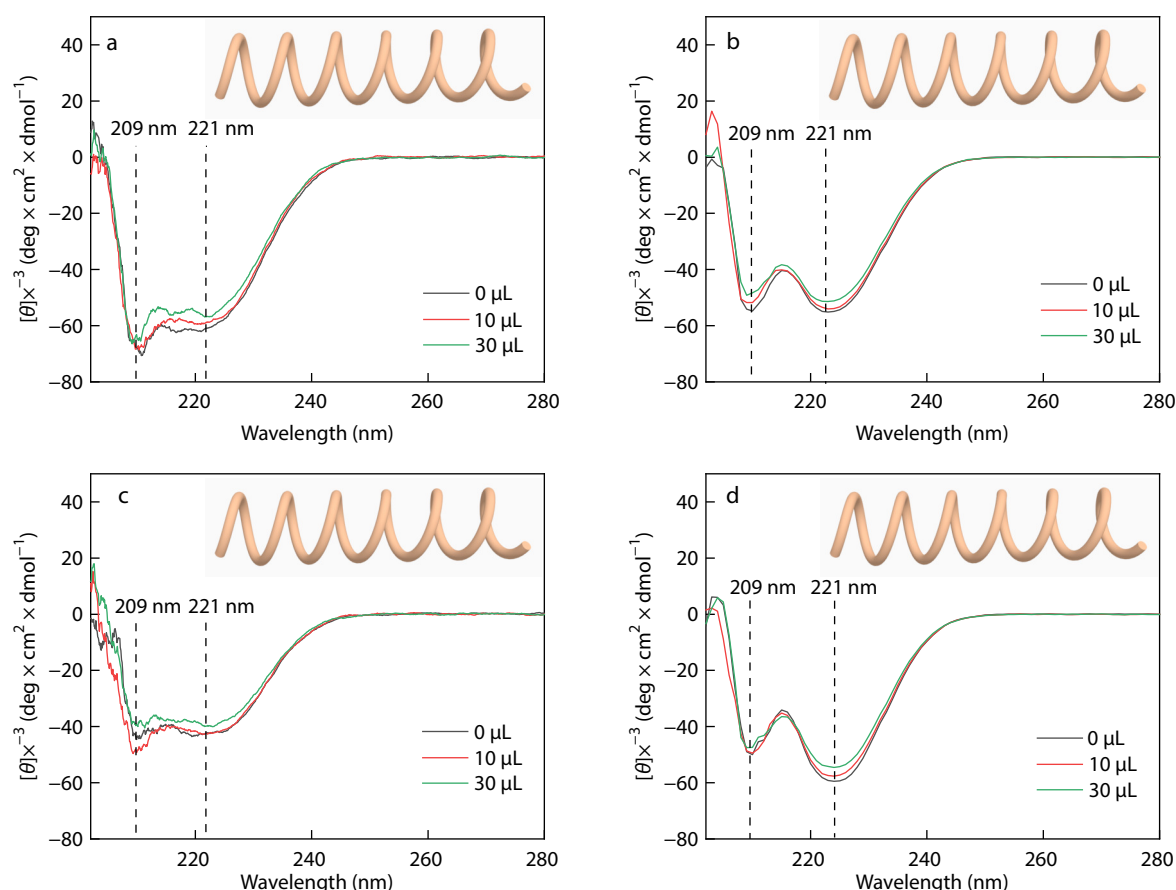


Fig. 3 CD spectra of (a) PZlys, (b) P1, (c) P2 and (d) P3 in THF with the addition of different amounts of water. The inset images mean the α -helical conformations of polypeptides.

the polypeptide backbone is hydrophobic.

To further investigate the interaction of water with the PZlys and P(BLG-co-Zlys) chains in solution at a molecular level, we first analyzed the FTIR spectra of PZlys and P(BLG-co-Zlys) in THF (1.0 mg/mL, 500 μ L) in the presence of various amounts of water (0–30 μ L were used to avoid aggregation). As shown in Fig. 4, the bands at 1651 and 1543 cm^{-1} for PZlys and P(BLG-co-Zlys) including P1, P2 and P3 attributed to the amide I and amide II bands remain at the same positions, respectively, suggesting that neither the amide groups in the PZlys backbone nor those in the P(BLG-co-Zlys) backbone interact with water molecules during the water addition.^[50,51] On the other hand, the appearance of the peaks at 1651 and 1543 cm^{-1} indicates that both PZlys and P(BLG-co-Zlys) adopt a stable α -helical conformation in THF/water solution, as shown by the CD measurements. The bands at 1725 cm^{-1} (Fig. 4a) and 1720 cm^{-1} (Figs. 4b and 4c) are assigned to the $-\text{C}(\text{O})\text{O}-$ stretching vibrations of urethane bonds.^[52–54] The stretching vibration of the $-\text{C}(\text{O})\text{O}-$ ester groups in the side-chains of P2 and P3 appears at 1730 cm^{-1} (Figs. 4c and 4d). As shown in Fig. 4(a), a shoulder band at 1703 cm^{-1} related to a hydrogen bonded carbonyl urethane group can be observed when the water amount is 0 μ L.^[55] Upon adding water amount from 0% to 30 μ L, the shoulder band at 1703 cm^{-1} adjacent to the band at 1725 cm^{-1} become gradually stronger (Fig. 4a), which implies that the ester carbonyls in the ureth-

ane bonds of the PZlys side-chains are hydrated. In addition, the shoulder band at 1703 cm^{-1} is still observed in the FTIR spectra of P1 with the BLG to Zlys unit molar ratio of 3:7 (Fig. 4b), and barely visible in those of P2 (Fig. 4c) and P3 (Fig. 4d) with increasing the BLG to Zlys unit molar ratio from 5:5 to 7:3 under the same water content conditions (Fig. 4), indicating that water molecules interact only with the ester carbonyl groups in the urethane bonds and not with the $-\text{C}(\text{O})\text{O}-$ ester groups in the side-chains of P(BLG-co-Zlys). Therefore, it is reasonable to conclude that PZlys is able to interact with a large amount of water molecules through side-chain hydration in aqueous solution, while the water interaction of P(BLG-co-Zlys) is weaker, due to the reduced number of hydratable groups such as urethane.

The side-chains of PZlys are more hydrophilic than those of P(BLG-co-Zlys), which may be due to the different polarity of the urethane ($-\text{NHC}(\text{O})\text{O}-$) and ester ($-\text{C}(\text{O})\text{O}-$) groups. It is well-known that lone-pair electrons of nitrogen can be delocalized to the carbonyl oxygen in the urethane group, and the former is easier to be protonated at the carbonyl oxygen in comparison with the ester group,^[56] this would lead to a stronger hydrophilicity of the urethane group than the ester group. Hence, LF-NMR measurements with a precise relaxation time (T_2) corresponding to the water motions were carried out to further explore the interaction of water molecules with the side-chains of PZlys and P2.^[36] Functional groups

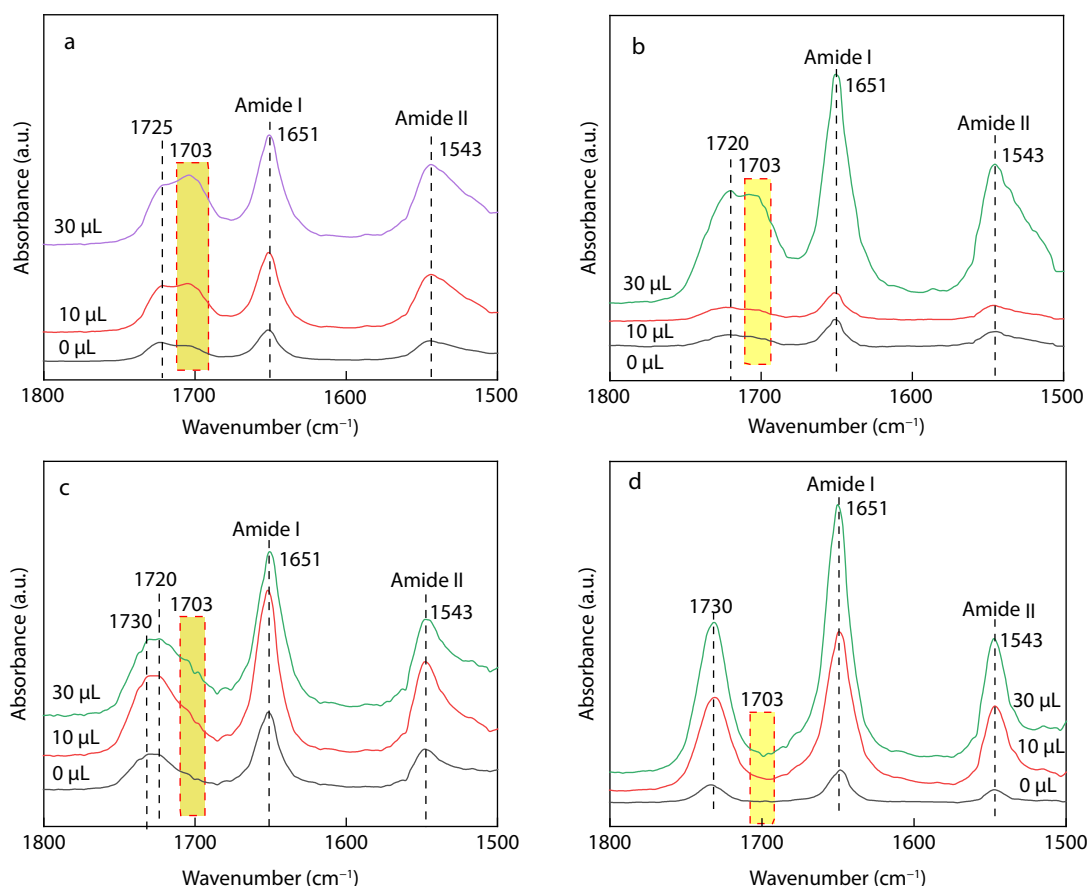


Fig. 4 FTIR spectra of (a) PZlys, (b) P1, (c) P2 and (d) P3 in THF with the addition of different amounts of water.

that hydrate water with a shorter T_2 relaxation time usually possess a higher polarity, because the larger binding forces between water and the function group can reduce the relaxation time for the excited water molecules to return to the ground state.^[57,58] In theory, T_2 values of 0.1–100 ms and 1000–10000 ms should be assigned to bound (T_{2b}) and bulk water (T_{2w}), respectively.^[59–62] Two peaks can be observed in Figs. 5(a) and 5(b), including a weak peak at 0.1–100 ms (T_{2b}) and a strong one at 1000–10000 ms (T_{2w}). Based on the FTIR and CD results discussed above, the T_{2b} value of 0.1–100 ms attributed to bound water originates from the water molecules bound to the polar groups of side-chains, rather than the amide groups of the PZlys and P(BLG-co-Zlys) backbones. On the other hand, the T_{2b} value of the PZlys solution is smaller than that of P2 in the presence of both 10 and 20 μL of water, suggesting that the hydrophilicity of the side-chain groups of PZlys is stronger than that of P2.^[32] It is suggested that the polarity of the urethane group is stronger than that of the ester group,^[56] and the addition of ester groups in P(BLG-co-Zlys) reduces the hydrophilicity of their side-chains, compared with those of PZlys. Therefore, it can be concluded that the side-chains with the urethane groups are hydrophilic in the case of PZlys, resulting in the formation of vesicle-like aggregates. However, the lower number of hydrophilic side-chains reduces the side-chain hydration ability of P(BLG-co-Zlys), which will form micelle-like aggregates in aqueous solution, driven by hydrophobic interactions.

Overall, the CD, FTIR and LF-NMR results show that the urethane groups in the side-chains of PZlys form hydrogen bonds with water molecules, giving rise to vesicle-like self-assemblies, and further stabilized by the interaction between amino groups and water molecules along the periphery.^[63] Moreover, it is well known that PZlys chains with α -helical conformation can be modeled by a compact rodlike structure, with a radius of 0.23 nm and a length of 0.15 nm per repeating unit (*N* ϵ -carbobenzoxyl-L-lysine herein).^[44] Given the DP of 80 for PZlys and a length of 0.15 nm per *N* ϵ -carbobenzoxyl-L-lysine unit of the α -helix, the length of PZlys can be calculated to be about 12 nm, which is consistent with the membrane thickness of the PZlys vesicles measured from TEM ($\sim$ 14 nm), except for the contribution of the side-chains. The PZlys chains with rigid α -helical conformation are found in an interdigitated side-by-side arrangement in the vesicle membrane, oriented toward the center of the vesicle, as shown in Scheme 3. It can be inferred that the membrane structure is homogeneous (due to the rigid α -helical conformation of PZlys revealed by the CD and FTIR results) and contains water (based on the FTIR and LF-NMR data), this is different from the complex ‘gradient membrane’ structure reported by Du and co-workers.^[30,31] On the other hand, when the content of the hydrophilic side chains is reduced, their hydrophilicity also decreases. The interaction between side-chain and water is relatively weak, resulting in a higher interfacial energy at the hydrophobic–hydrophilic boundary. Therefore, P(BLG-co-Zlys)

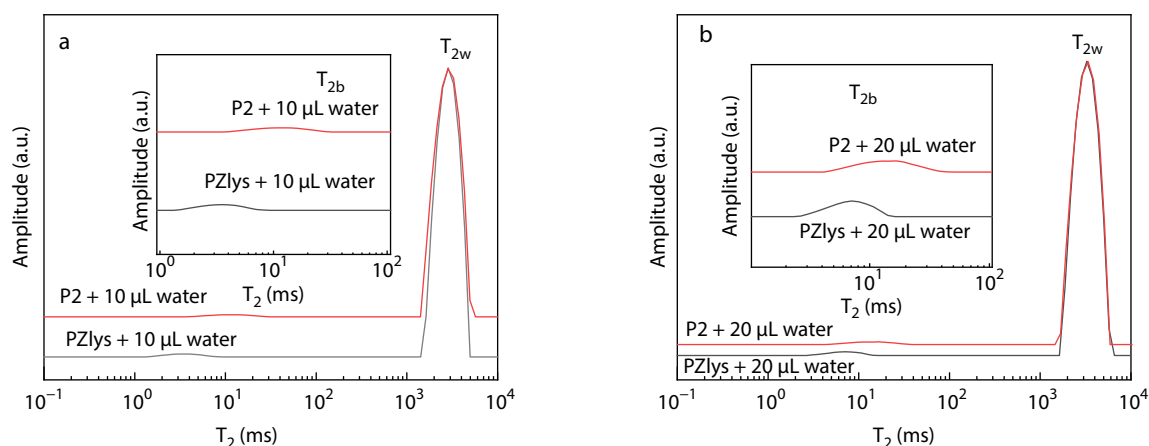
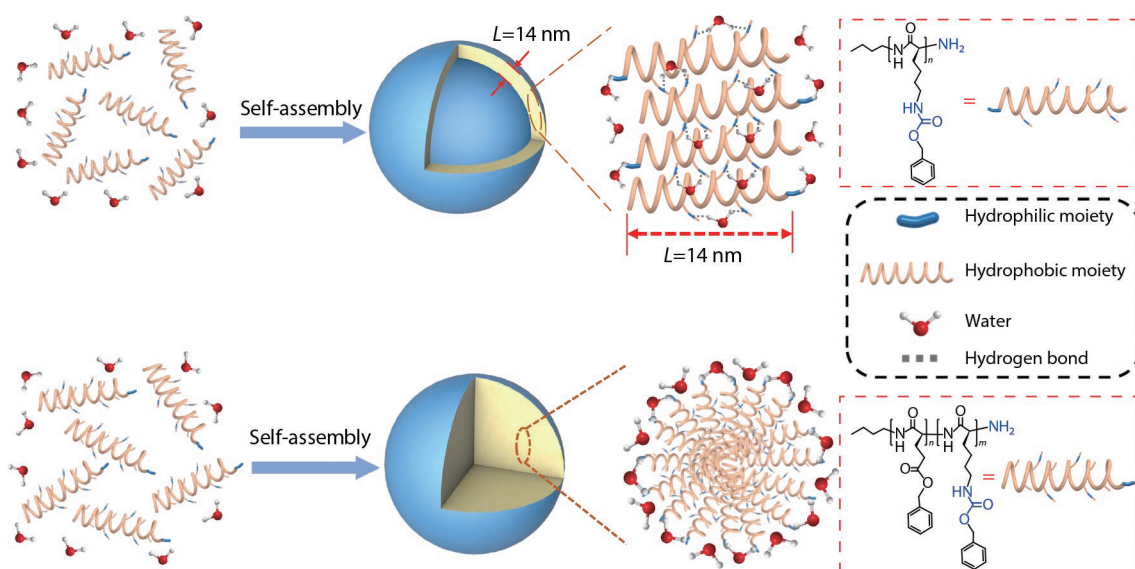


Fig. 5 LF-NMR spectra of PZlys and P2 in THF with the addition of different amounts of water: (a) 10 μL , (b) 20 μL .



Scheme 3 Schematic illustration of PZlys vesicles and P(BLG-co-Zlys) micelles formation. The membrane thickness (L) of PZlys vesicle is 14.0 nm.

self-assembles into micelles (Scheme 3) to minimize the interfacial energy.^[64]

CONCLUSIONS

Homopolymer PZlys and copolymers P(BLG-co-Zlys) were successfully synthesized by ROP using *n*-butylamine as an initiator. SEM, TEM and AFM measurements reveal that PZlys homopolymer synthesized by ROP of Zlys-NCA can self-assemble into a vesicular structure. CD, FTIR, and LF-NMR data show that the higher hydration ability of the side chains is a key factor for the formation of PZlys vesicles, as the urethane bonds of the side chains can form hydrogen bonds with water. However, P(BLG-co-Zlys), with the same peptide backbone and fewer hydrophilic side chains, can only form micelles under the same conditions. Overall, these results show that PZlys homopolymer has the same ability to assemble in a vesicle-like morphology as amphiphilic block copolymer, and the specific side-chain structures of the polypeptides play an important role in the assembly of homopolypeptides. These

findings can be useful to understand how the hydrophilicity of the side groups of amino acid residues on the surface of a protein affects its structures.

NOTES

The authors declare no competing financial interest.

Electronic Supplementary Information

Electronic supplementary information (ESI) is available free of charge in the online version of this article at <http://doi.org/10.1007/s10118-022-2784-3>.

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