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Facile control of the self-assembled structures of polylysines having pendent mannose groups *via* pH and surfactant†

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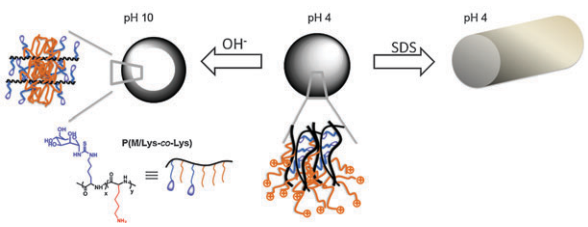
A mannose-modified polylysine was synthesized, the self-assembly of which in aqueous solution led to the formation of spherical micelles, vesicles and rod-like micelles in a controlled manner by simply changing the solution pH and adding a surfactant to the solution.

Amphiphilic copolymers are known to form a variety of molecular assemblies in solution, the morphology and structure of which depend not only on the polymer structure, composition and topology, but also on the nature of solvents and various environmental factors. Micelles and vesicles formed in aqueous media by amphiphilic copolymers are among the most interesting and widely studied assembled structures due to their applications in controlled and targeted drug delivery.[1–5] Considering the biodegradability, biocompatibility, tunable functionality and defined secondary structures of polypeptide, self-assembly of polypeptide-based block copolymers in aqueous solution has been the subject of many recent researches.[6–9] Among these copolymers, polylysine-based block copolymers have been demonstrated to be particularly interesting, mainly due to the controllable manipulation of the secondary structure and amphiphilicity of polylysine in response to an environmental stimulus (such as pH, surfactants, and temperature).[10–15] Since synthetic glycopolymers have shown enhanced interactions with cells, and found potential applications in cell sensing, drug delivery, and others,[16–18] synthesis and self-assembly of glycopolypeptide have recently attracted a great deal of attention.[19–21] However, these block copolymers are relatively difficult to synthesize, and manipulation of morphological transformation of the self-assembled structures from a single copolymer is hard. We present here a simple approach for the synthesis of a polylysine-based glycopolymer by simple modification of polylysine with mannose. This simple polymer is capable of self-assembling into a variety of structures by simply changing the pH of the solution and adding surfactant to the solution (see Scheme 1).

Copolymers **1** and **2** used for this study (see structure in Scheme 1) were synthesized by post-polymerization modification of poly(L-lysine) (PLys) (Scheme 2). Two PLys (DP = 32 or 61, determined by NMR) were obtained by ring-opening polymerization (ROP) of ε-benzyloxycarbonyl-L-lysine *N*-carboxyanhydride (Z-Lys NCA), followed by removal of the protective groups. 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl isothiocyanate (AcM-NCS) was then allowed to react with the PLys in DMF to afford P(AcM/Lys-*co*-Lys). In the ^{1}H NMR spectrum of P(AcM/Lys-*co*-Lys), the methylene protons adjacent to the amino groups (H_c, 2.98 ppm) shifted to 3.35 ppm ($H_{c'}$) after formation of the thiourea bonds (see ESI, Fig. S1).† Comparison of the relative integration of peaks at 2.98 ppm to the methyl protons in the acetyl groups at 1.95–2.10 ppm allowed us to calculate the substitution degree of mannose group, which could be controlled by the feed ratio of AcM-NCS to polylysine. Deacetylation of P(AcM/Lys-*co*-Lys) was then carried out in basic methanol aqueous solution. After dialysis (water/cellulose tube, MW cutoff 1000 g/mol) and freeze-drying, two copolymers, P(M/Lys-*co*-Lys), were obtained, whose structures were confirmed by FTIR, ^{1}H NMR and ^{13}C NMR spectra, and the substitution degree of the mannose group is 16% for copolymer **1** and 23% for copolymer **2** (see ESI, Fig. S2, S3 and S4).†

As a glycopolymer, P(M/Lys-*co*-Lys) is supposed to be highly water-soluble in an acidic conditions because PLys dissolves in water below pH 10 as a weak cationic polyelectrolyte.[22] However, when we dissolved P(M/Lys-*co*-Lys) directly in water (0.1 mg/mL) at pH 4, we found the formation of spherical micelles as evidenced by TEM and AFM (Fig. 1a and d), the diameters of which were about 10–15 nm for copolymer **1**. Similar spherical micelles were also observed for copolymer **2**, with a slight increase of diameters. We measured the CD spectra (see ESI, Fig. S5)† of copolymer **1** at pH 6.25 in water, and detected one positive band at 217 nm, suggesting that copolymer **1** existed as random coil as for unmodified PLys. Therefore, the introduction of pendent mannose groups (16% relative to the total lysine units) has very little effect on the secondary structure of PLys (see ESI, Fig. S6).† Schlaad and co-workers recently reported that a hydrophilic



Scheme 1 Schematic diagram showing the morphological transition among spherical micelles, vesicles and rod-like micelles formed by P(M/Lys-*co*-Lys) [copolymer **1** (x = 5, y = 27) and copolymer **2** (x = 14, y = 47)] in aqueous solution by changing the pH and adding SDS.

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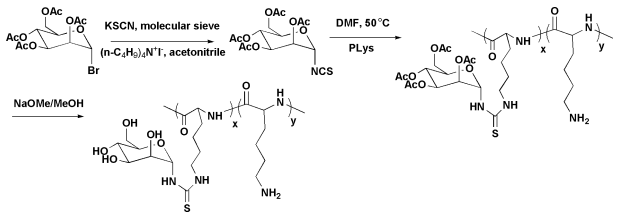

Scheme 2 Synthesis of P(M/Lys-*co*-Lys).

glycopolyamide homopolymer can self-assemble into spherical vesicles and nanofibers through intermolecular hydrogen bonding between amide and glucose units.[23] In our case, introduction of pendant mannose groups and thiourea groups to PLys should also be responsible for the micellar structure formation. Our copolymers contain thiourea groups, which have proven useful in constructing hydrogen bonding receptors.[24–26] The relatively acidic thiourea NH protons have a strong hydrogen-bond donor capability.[27] In order to know whether the thiourea is involved in hydrogen bonding formation between polymers, we measured the ^{13}C NMR spectrum of copolymer **1** in the presence of urea (see ESI, Fig. S7),†and found that the C-1 signal obviously shifted downfield, while other carbon signals of the mannose unit changed only slightly. C-1 is the carbon adjacent to the thiourea group, therefore, the observed shift variation presumably indicates that the hydrogen bonds contributed by thiourea groups dissociate after adding urea. The micellar aggregation was probably driven by the intermolecular hydrogen bonds from the thiourea N–H to the amide C=O on the PLys backbones, which mainly affect the chemical shift of the C-1 signal.[25,26] The proposed drawing at pH 4 in Scheme 1 just shows that the intermolecular hydrogen bonds are necessary for the formation of the micellar structures, however, the detailed structure of the micelles needs further investigation.

When the pH of the solution was adjusted to higher than 10, vesicles with diameters of 45–80 nm were observed for both

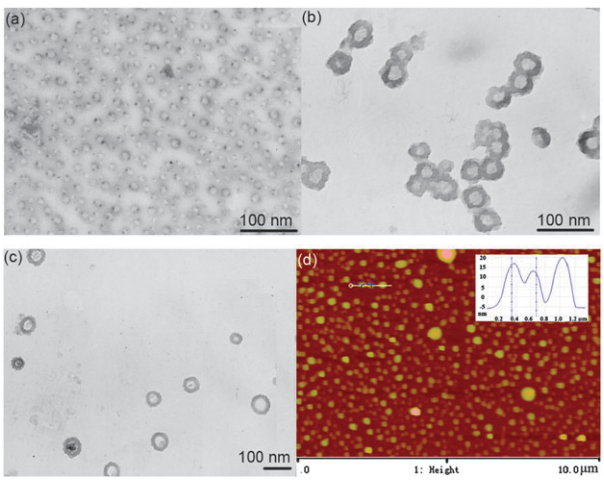

Fig. 1 TEM images of copolymer **1** and **2** at different pHs. (a) Copolymer **1**, pH = 4, (b) copolymer **1**, pH > 10, (c) copolymer **2**, pH > 10. (d) AFM images of copolymer **1** at pH = 4. Inset: Height information of copolymer **1** spherical micelles. The initial copolymer concentration is 0.1 mg/mL. Uranyl acetate was used as a staining agent.

copolymers. The wall thickness of the vesicle was 12 nm for copolymer **1** and 20 nm for copolymer **2** (Fig. 1b and c). The CD spectrum at this pH shows that the copolymer adopts an α-helix conformation (see ESI, Fig. S5),† which is due to the formation of intramolecular hydrogen bonding among PLys backbones. Because the spherical micelles built upon hydrogen bonds at lower pH are dynamic structures,[28] the stronger hydrogen bonding among PLys backbones drives the morphology change from micelles to vesicles. Pendant mannose units remain water-soluble, thus contributing to the stabilization of the vesicles in solution. To confirm this assumption, we measured the ^1H NMR of the vesicular solution in D_2O (see ESI, Fig. S3);† it was found that the signal appeared at 2.99 ppm ($-CH_2-NH_3^+$) in neutral D_2O shifted to 2.72 ppm in basic conditions, and at the same time the α-methine proton signals of PLys (CO*CH*NH, at 4.30 ppm) almost completely disappeared. These results indicated that the chain mobility of the PLys segments was greatly reduced, the PLys helix should exist in the hydrophobic vesicle wall, while the mannose groups are on the vesicle surfaces.[11]

The formation of vesicles was further ascertained by studying the container property with calcein as a probe.[29] An aqueous solution of calcein-loaded vesicles was prepared by mixing 5 mM calcein with 0.1 mg/mL copolymer **2** at pH 2, then the pH was adjusted to higher than pH 10 with aqueous NaOH, finally the solution was dialyzed against pH 10.83 carbonate buffer for 16 h. For these dye-loaded vesicles, the calcein concentration was 55 μM, and the loading efficiency was estimated to be 17%. The release of calcein was monitored by measuring the absorbance at 497 nm. The vesicle solution showed a much slower elution profile (see ESI, Fig. S8),† indicating a significantly retarded release of the calcein at this pH due to its entrapment within the vesicles. Moreover, if the initial calcein concentration was increased to three times higher, a more rapid release was observed. As expected, a control experiment in the absence of any vesicles revealed a more rapid dye elution.

We further confirmed that mannose groups do locate on the surface of the vesicles. It is well-known that mannose moieties have strong and specific interactions with Concanavalin A (*Con A*).[30] Soon after a *Con A* solution was added to the above vesicle solution at pH 10, the turbidity of the solution initially increased quickly and reached a plateau within 10 min (see ESI, Fig. S9),† a similar phenomenon was observed for other sugar-covered aggregates.[31]

When the pH was continuously increased up to 12, precipitation of the copolymer was observed for both samples. These precipitates could hardly dissolve in any common solvents, such as THF, DMF, DMSO. The FTIR spectrum (see ESI, Fig. S10)† of the lyophilized precipitates shows a strong band at 1628 cm^{-1} and a weaker band at 1686 cm^{-1}, indicating the formation of antiparallel *β*-sheet conformation of the peptide chains.[32]

Besides pH, adding anionic surfactants could also induce the change of self-assembled morphologies of P(M/Lys-*co*-Lys). When adding SDS into the copolymer solution (0.2 mg/mL) at pH 4, we found changes of both the secondary structure of the copolymer main chain and the self-assembled structures; the

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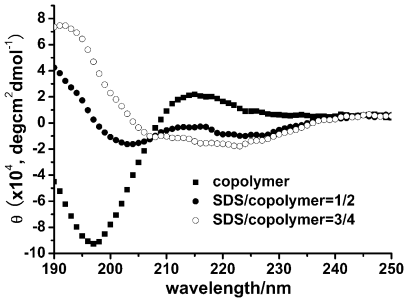

Fig. 2 CD spectra of copolymer **2** solution with or without SDS at pH 4. The ratio is the molar ratio of SDS to free primary amine groups of the copolymer.

SDS/lysine unit ratio played an important role. As can be seen in Fig. 2, with increasing the SDS/lysine unit ratio, the conformation of the copolymer changes gradually from random coil to β-sheet, where the CD spectrum has one minimum at 215 nm and a maximum at 195 nm. This conformation change is consistent with those observed for PLys in solution or tethered on surface, which was ascribed to the decreased electrostatic interaction among PLys chains and enhanced hydrophobic interaction among the bound SDS molecules. [33–35] Interestingly, when examining the solution by TEM (Fig. 3) and AFM (see ESI, Fig. S11),† we found the formation of rod-like micelles at a SDS/lysine unit ratio up to 0.75. We suppose that the SDS-induced β-sheet formation may not only break the intermolecular hydrogen bonding, but also lead to an increase in both the size of the core and the charge density of the corona of the original spherical micelle.[36] As a result, spherical micelles are transformed to rod-like micelles.

In summary, we demonstrated that a simple mannose-modified polylysine can self-assemble into spherical micelles, vesicles and rod-like micelles in aqueous solution *via* simply changing pH or adding a surfactant. The transformation between different morphologies was due to the formation of hydrogen bonding, secondary structure transition, and the change of hydrophobicity. Vesicle formation from such a simple polymer may be very promising for constructing cell-recognized drug deliver systems.

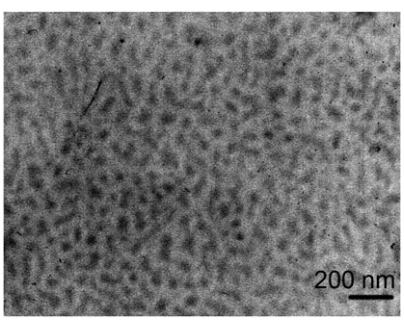

Fig. 3 TEM image for aggregate prepared from 0.2 mg/mL copolymer **2** aggregate in pH = 4 aqueous solution by adding 1 mM SDS with SDS/lysine unit ratio = 3/4. Uranyl acetate was used as the staining agent.

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