

## Self-assembly and pH-Responsive Properties of Poly(L-glutamic acid-*r*-L-leucine) and Poly(L-glutamic acid-*r*-L-leucine)-*b*-Polysarcosine<sup>\*</sup>

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**Abstract** Polypeptides and polypeptoids were widely used as biomedical materials because of their good biocompatibility. In this work we reported a series of pH-responsive copolypeptides and polypeptide-polypeptoid block copolymers, *i.e.* random copolymers of L-glutamic acid (Glu) with L-leucine (Leu) [poly(Glu-*r*-Leu)s], as well as their block copolymers with polysarcosine (polySar). Well-defined poly(Glu-*r*-Leu)s with predictable compositions and molecular weights were synthesized by ring opening polymerization of corresponding *N*-carboxyanhydride monomers. We investigated the relationship between hydrophilicity-hydrophobicity transition and copolymer composition. With increasing Leu fraction, both the pH value of cloud point and the micellar size increased. Poly(Glu-*r*-Leu) with 60% Leu exhibited a cloud point at the pH of 5.0 to 6.0 the same as that in endosome and lysosome. Poly(Glu-*r*-Leu)-*b*-polySars assembled in phosphate buffer and performed pH-responsive morphology change from orbicular micelles at high pH to worm-like micelles at low pH. They were potential pH-responsive carriers for drug and gene delivery to enhance cargo release in cells.

**Keywords:**  $\alpha$ -Amino acid NCA; Random/block copolypeptide; pH-Responsive; Hydrophilicity-hydrophobicity transition.

## INTRODUCTION

Polypeptides have the same repeating units of natural  $\alpha$ -amino acids as proteins. They are biocompatible, biodegradable, and having stable high-ordered conformations<sup>[1]</sup>, as potential biomaterials used in drug and gene delivery<sup>[2–4]</sup>, tissue engineering<sup>[5]</sup>, and cell membrane penetration<sup>[6, 7]</sup>. Ring opening polymerization (ROP) of (protected)  $\alpha$ -amino acid *N*-carboxyanhydride (NCA) is the most promising synthetic approach for polypeptides, which enables good controls on desired molecular weights (MWs), narrow MW distributions, predictable compositions, complex topologies and functional end groups<sup>[8–10]</sup>.

Stimuli-responsive drug/gene delivery systems attract increasing attention in medical research because they can release cargoes on targeted location or time by a precisely designed trigger, resulting in maximal therapeutic activities and minimal negative side effects<sup>[11]</sup>. pH change is one of the most exciting triggers since different parts of cellules and tissues have different pH values. The pH values in blood circulation and extracellular environment of normal tissues are about 7.4, while extracellular pH of tumors is 6.5–7.2. Along endocytic pathway, pH is getting even lower. For instance, it is 5.0–6.5 in endosome and 4.5–5.0 in lysosome<sup>[2, 12]</sup>. Many pH-responsive carriers are reported based on this principle to identify normal tissues and tumors, or release cargoes before entering into lysosome and then decomposed<sup>[2, 3]</sup>.

Every unit of poly-L-glutamic acid (polyGlu) has a carboxyl group which is ionized at high pH, resulting in

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its water solubility as an extended coil conformation due to charge repulsion. When pH decreases, carboxyl groups are protonated. The polymer then adopts a helical conformation and becomes insoluble in water<sup>[3, 13, 14]</sup>. This phase-transition could be adjusted by copolymerization Glu with hydrophobic amino acids<sup>[15]</sup>.

L-Leucine (Leu) is a natural hydrophobic amine acid and its homopolymer adopts a helical conformation. Poly-L-leucine (polyLeu) is insoluble in water in any pH and most organic solvents<sup>[16]</sup>. Some properties of random copolymers of L-glutamic acid with L-leucine [poly(Glu-*r*-Leu)s] have been reported including helix-coil transition<sup>[17, 18]</sup>, adsorption dynamics at an oil/water interface<sup>[19]</sup>, and surface interactions with celluloses<sup>[20–22]</sup>. To the best of our knowledge, pH-dependent phase-transition of poly(Glu-*r*-Leu) has not been studied yet.

Polysarcosine (polySar) or poly-*N*-methylglycine is a kind of polypeptoids which have similar structure compared to polypeptides<sup>[23]</sup>. Being soluble and nonionic in aqueous solution in any pH<sup>[23–26]</sup>, it is highly biocompatible<sup>[23, 27]</sup>, biodegradable<sup>[28]</sup>, and protein resistant like poly(ethylene glycol) (PEG)<sup>[29, 30]</sup>. It is a competitive alternative of PEG in biomaterial syntheses<sup>[31]</sup>.

In this work, we investigated the dependence of hydrophilicity-hydrophobicity transition of poly(Glu-*r*-Leu) on polymer composition in order to optimize the copolymers with phase-transition at pH of 5.0–6.0 which matches the pH change in cellule endocytosis process. Self-assembly of block copolymers of poly(Glu-*r*-Leu) with polysarcosine is also investigated in different pH buffers.

## EXPERIMENTAL

### Materials

$\gamma$ -Benzyl-L-glutamate (BLG) (98%, CS-Pharm, China), L-leucine (99%, Adamas, China), sarcosine (98%, Sigma-Aldrich, America), triphosgene (99%, Adamas, China), trifluoroacetic acid (TFA) (99%, Acros, Belgium), HBr (33 wt% solution in glacial acetic acid, Acros, Belgium), methyl chloroformate (96%, Sinopharm, China), PBr<sub>3</sub> (98.5%, Sinopharm, China), trimethyl chlorosilane (99%, Adamas, China) were used as received. Tetrahydrofuran (THF) and hexane were refluxed over potassium/benzophenone ketyl before use. Dioxane was refluxed over sodium/benzophenone ketyl before use. Ethyl acetate and triethylamine were stirred over CaH<sub>2</sub> and distilled. *N,N*-dimethylformamide (DMF) was stirred over anhydrous BaO followed by vacuum distillation. Benzylamine was stirred over CaH<sub>2</sub> followed by vacuum distillation. CCl<sub>4</sub> was stirred over P<sub>2</sub>O<sub>5</sub> and distilled.

### BLG NCA, Leu NCA and Sar NCA

The synthesis of BLG and Leu NCA follows literatures<sup>[32–34]</sup>. In a typical procedure, BLG (10.2 g, 43.0 mmol) was suspended in 100 mL anhydrous THF. Triphosgene (4.80 g, 16.3 mmol) dissolved in 20 mL THF was added dropwise. The reaction was performed at 45 °C under argon atmosphere until a clear solution. The solution was poured into anhydrous hexane and stored overnight at –20 °C for complete crystallization. The crude product was recrystallized from ethyl acetate/hexane for three times. BLG NCA was obtained as white crystals after dried in vacuum (yield = 72%). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  = 7.31–7.22 (m, 5H, ArH), 6.89 (s, 1H, NH), 5.04 (s, 2H, ArCH<sub>2</sub>), 4.30 (t, 1H,  $\alpha$ -CH), 2.49 (t, 2H,  $\gamma$ -CH<sub>2</sub>), 2.20–1.97 (m, 2H,  $\beta$ -CH<sub>2</sub>).

Leu NCA (yield = 51%) was synthesized following the same procedure. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  = 7.33 (s, 1H, NH), 4.31 (dd, 1H,  $\alpha$ -CH), 1.81–1.70 (m, 2H, CH<sub>2</sub>), 1.67–1.57 (m, 1H,  $\gamma$ -CH), 0.92 (t, 6H, CH<sub>3</sub>).

Sar NCA was synthesized following literature with slight modification<sup>[35]</sup>. Sar (44.5 g, 0.500 mol) and sodium carbonate (26.5 g, 0.250 mol) were dissolved in 500 mL 2 mol/L NaOH solution. After cooled to 0 °C, methyl chloroformate (38.7 mL, 0.500 mol) was added dropwise with stirring, and continued to react for 1 h. The mixture was acidified to pH 2–3 by HCl. Then the product was extracted by ethyl acetate and concentrated to a viscous liquid. This liquid and trimethyl chlorosilane (69.7 mL, 0.550 mol) were dissolved in 700 mL anhydrous THF, and triethylamine (76.2 mL, 0.550 mol) was added dropwise with stirring. After reflux for 2 h, the solution was cooled to room temperature and diluted by 300 mL hexane. After filtration, the filtrate was concentrated in vacuum, and the silylation product was then isolated by distillation. The product was dissolved in 200 mL anhydrous CCl<sub>4</sub>, and PBr<sub>3</sub> (10.1 mL, 0.106 mol) was added dropwise with stirring. The mixture was

stirred at 50 °C for 2 h and 60 °C for 1 h. After cooling to 0 °C, the crystallized Sar NCA was isolated by filtration. The crude product was recrystallized from dioxane/CCl<sub>4</sub> to be white crystals and dried in vacuum (yield = 40%). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 500 MHz):  $\delta$  = 4.15 (s, 2H, CH<sub>2</sub>), 3.05 (s, 3H, CH<sub>3</sub>).

#### ***Poly(BLG-r-Leu)***

BLG NCA and Leu NCA were dissolved in anhydrous DMF (0.5 mol/L) in a certain ratio. Benzylamine (1.0 mol% of NCAs) was added to initiate the polymerization at room temperature for 72 h. The product was isolated by precipitation from methanol and dried in vacuum.

#### ***Poly(BLG-r-Leu)-b-PolySar***

The above-mentioned poly(BLG-r-Leu) reaction mixture was separated to several parts under argon. One part was precipitated from methanol for analysis. Other parts were injected into a DMF solution of Sar NCA with various ratios and continued to react for another 72 h at room temperature. The product was isolated by precipitation from diethyl ether and dried in vacuum.

#### ***Deprotection***

Poly(BLG-r-Leu) or poly(BLG-r-Leu)-b-polySar was dissolved in TFA and cooled in an ice-water bath. HBr solution was added and the reaction was performed for 4 h. After precipitation in diethyl ether, the residue was dissolved in DMF and dialyzed (MWCO 3500) against deionized water for 3 days. The suspension was freezing-dried.

#### ***pH-Dependent Phase Transition***

Phase transitions were determined by UV-Vis spectrum. Poly(Glu-r-Leu)s were dissolved in NaOH solution and diluted to the concentration of 1 mg/mL. The solutions were titrated by HCl to complete precipitation. Both pH values and transmittances at 600 nm were recorded at 37 °C. Then the solutions were titrated by NaOH back to complete dissolved, pH values and transmittances were recorded.

#### ***Assembly of Poly(Glu-r-Leu)***

Poly(Glu-r-Leu)s with low Leu contents were dissolved in 0.01 mol/L phosphate buffered saline (PBS) with pH of 7.4 directly. The samples with high Leu contents could not dissolve in PBS directly. They were suspended in PBS and a little amount NaOH was added until they dissolved completely. Then the solution was acidized back to 7.4 with HCl. The final concentration of all polypeptide solutions was 1 mg/mL.

#### ***Assembly of Poly(Glu-r-Leu)-b-PolySar***

Poly(Glu-r-Leu)-b-polySar was dissolved in NaOH solution with the concentration of 1 mg/mL, then dialyzed to 0.01 mol/L phosphate buffers with pH of 8.0 or 5.0. Finally, the solution was diluted to 0.67 mol/L for analysis.

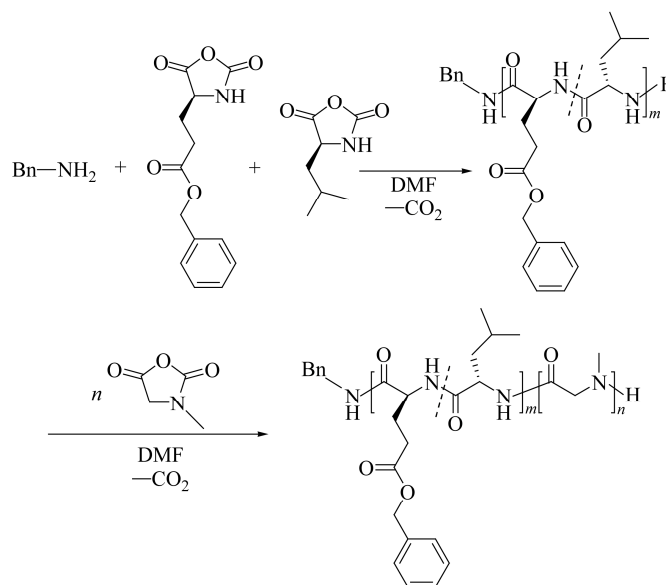
#### ***Measurements***

Molecular weights (MWs) and polydispersity indices (PDIs) were determined by size-exclusion chromatography (SEC) using a Waters 1515 system, equipped with a Waters 1515 isocratic high performance liquid chromatograph pump, a column of PLgel 5  $\mu$ m MIEXD-C and a Wyatt Optilab DSP interferometric refractometer. DMF containing 0.05 mol/L LiBr was used as the eluent with a flow rate of 1.0 mL/min at 60 °C. Commercial poly(methylmethacrylate)s (PMMA)s were used as calibration standards. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance DMX 500 spectrometer (<sup>1</sup>H: 500 MHz) or Avance-400 spectrometer (<sup>1</sup>H: 400 MHz) with CDCl<sub>3</sub>, CF<sub>3</sub>COOD or a mixture of CDCl<sub>3</sub> and CF<sub>3</sub>COOH of 2:1 (V/V) as solvent and tetramethylsilane (TMS) as internal reference. The hydrodynamic diameters of the micelles were measured by dynamic light scattering (DLS) at 37 °C using a particle size analyzer (Zetasizer Nano Series, Malvern Instruments). The measurements were made at a fixed angle at 90° and a wavelength of 657 nm. UV-Vis spectra were recorded on a UV-2550 spectrometer (Shimadzu). pH of aqueous solutions was measured by a pH meter (PHS-P, Shanghai Dapu). The morphologies of nanoparticles in aqueous solution were observed on an HT7700 TEM instrument with the accelerating voltage of 120 kV. The samples were prepared by placing the solutions on copper grids coated with organic film, stained with uranyl acetate aqueous solution and dried under ambient condition.

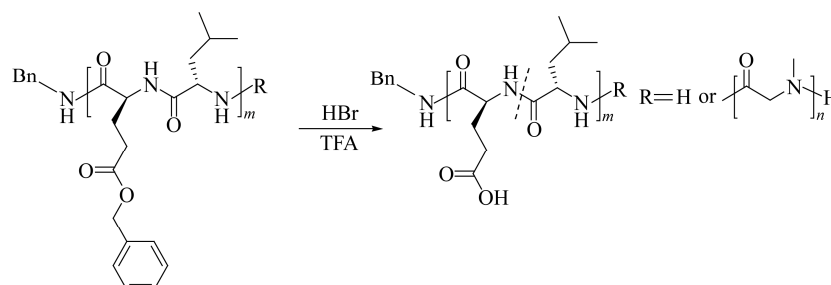
## RESULTS AND DISCUSSION

### Random Copolypeptides of L-glutamic Acid and L-leucine and Their pH-Responsive Phase Transition

As shown in Schemes 1 and 2, random copolypeptides of L-glutamic acid with L-leucine are synthesized by polymerization of BLG NCA and Leu NCA, and deprotection to remove benzyl group. Here, we simplify poly(BLG-*r*-Leu) and the corresponding poly(Glu-*r*-Leu) copolypeptides as PBL-*x* and PGL-*x*, respectively, where *x* represents the molar percentage of Leu units in feed.



**Scheme 1** Synthesis of poly(BLG-*r*-Leu) and poly(BLG-*r*-Leu)-*b*-polySar



**Scheme 2** Deprotection of poly(BLG-*r*-Leu) or poly(BLG-*r*-Leu)-*b*-polySar

Figure 1 summarizes the  $^1\text{H}$ -NMR spectra of homopolymer of BLG, copolymer poly(BLG-*r*-Leu) and deprotected poly(Glu-*r*-Leu). In poly(BLG-*r*-Leu) spectrum (Fig. 1B), the characteristic signals of both units are well-assigned. The signal  $\text{H}^b$  contributes to the methylene proton of benzyl group of BLG units, while  $\text{H}^e$  is the methyl group of Leu units. Their ratio determines the composition of poly(BLG-*r*-Leu) which is in good agreement with the feed ratio of two monomers. After deprotection (Fig. 1C), all the proton signals of benzyl group ( $\text{H}^a$  and  $\text{H}^b$ ) of BLG units disappear, indicating a complete deprotection, and the composition of polymers do not have any change. The percentages of Leu units in copolypeptides are summarized in Table 1.

According to SEC measurements (Fig. 2), PDIs of copolypeptides are narrow and keep unimodal peak when Leu content is low, while a high molecular weight shoulder appears and gets more obvious when Leu content increases. Because polyLeu is insoluble in DMF, polypeptides have stronger tendency to aggregate in DMF with higher Leu contents, which results in highly variable multi-modal appearance in SEC profiles. This

result is similar as that in other reports<sup>[18, 36, 37]</sup>. The MWs calibrated by PMMA standards are not absolute values because polypeptides perform special secondary structure in DMF differing from the random coil of PMMA, which is also depending on the compositions of copolypeptides<sup>[38]</sup>.

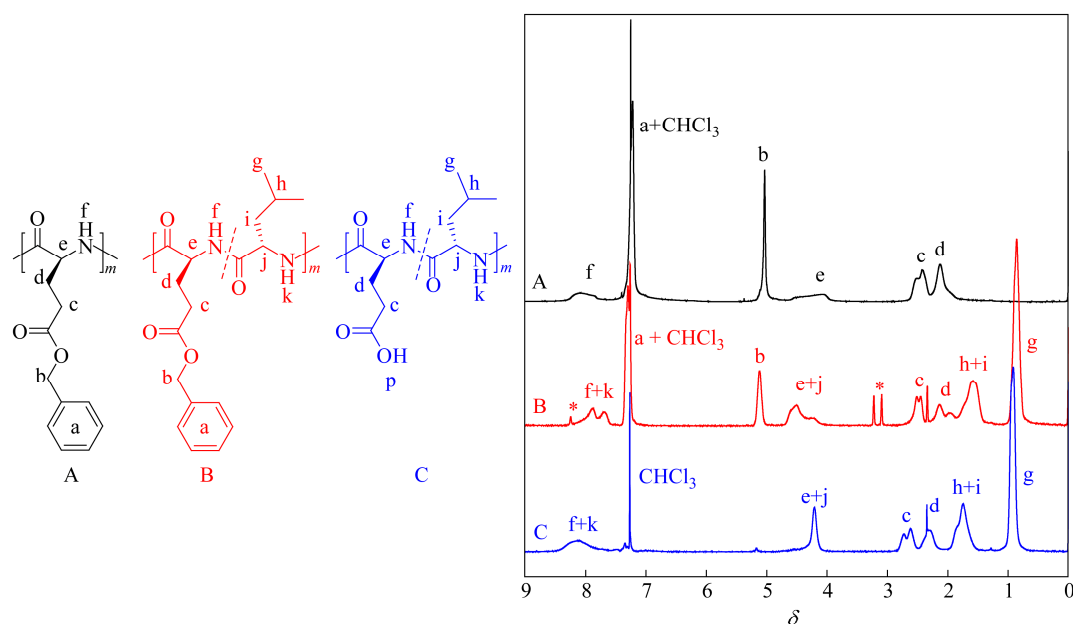


**Fig. 1**  $^1\text{H}$ -NMR spectra of polyBLG (A), poly(BLG-*r*-Leu) (B) and poly(Glu-*r*-Leu) (C) (\* DMF. The solvent is  $\text{CDCl}_3/\text{CF}_3\text{COOH}$  (2:1) mixture.)

**Table 1.** Characterization of poly(BLG-*r*-Leu) copolypeptides

| Sample | BLG:Leu in feed <sup>a</sup> | BLG:Leu in polymer <sup>a, b</sup> | Yield (%) | $M_w$ <sup>c</sup> (kDa) | PDI <sup>c</sup> |
|--------|------------------------------|------------------------------------|-----------|--------------------------|------------------|
| PBL-0  | 100:0                        | 100:0                              | 71        | 9.6                      | 1.26             |
| PBL-30 | 70:30                        | 71:29                              | 87        | 12.9                     | 1.29             |
| PBL-40 | 60:40                        | 58:42                              | 83        | 9.1                      | 1.32             |
| PBL-50 | 50:50                        | 49:51                              | 82        | 8.1                      | 1.30             |
| PBL-60 | 40:60                        | 37:63                              | 83        | 7.0                      | 1.28             |
| PBL-70 | 30:70                        | 30:70                              | 94        | 6.5                      | 1.30             |

<sup>a</sup> Molar ratio; <sup>b</sup> Calculated by  $^1\text{H}$ -NMR; <sup>c</sup> Measured by SEC

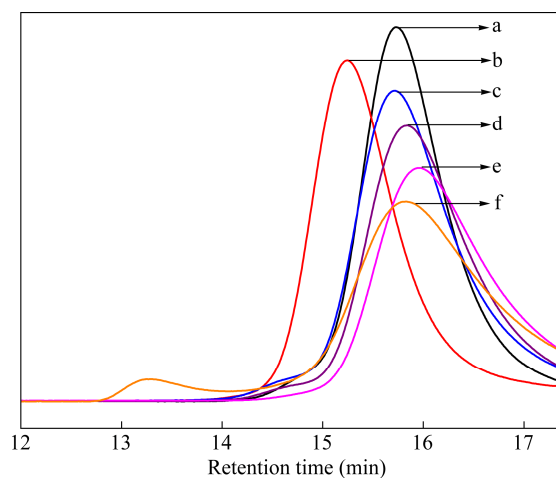


**Fig. 2** SEC traces of PBL-0 (a), PBL-30 (b), PBL-40 (c), PBL-50 (d), PBL-60 (e) and PBL-70 (f)

When PGL-*x* solutions are titrated from basic to acidic by HCl, light transmittances exhibit a sharp change responding to pH change (Fig. 3A and Table 2). The pH value of cloud point, defined as the point at 50% transmittance, increases when Leu fraction increases. The polyglutamic acid homopolymer, *i.e.* PGL-0, displays a very narrow transition pH window ( $< 0.5$ ) which is defined as the pH difference at 95% and 5% transmittance. The transition pH window gets broad with high Leu content. The precipitation of PGL-60 starts at a pH of 7.0 and completes at 4.3 with cloud point of 5.8. Its transition window is lower than 7.4, *i.e.* the pH value in blood circulation and extracellular environment of normal tissues. Thus, PGL-60 can keep soluble in blood and normal tissues and begin to aggregate and precipitate in tumors where extracellular pH is between 6.5 and 7.2. It would precipitate completely in endosome with pH of 5.0–6.5 and lysosome with pH of 4.5–5.0 (Fig. 3B). This pH-responsive transition of PGL-60 lightens its application as gene carriers<sup>[15]</sup>.

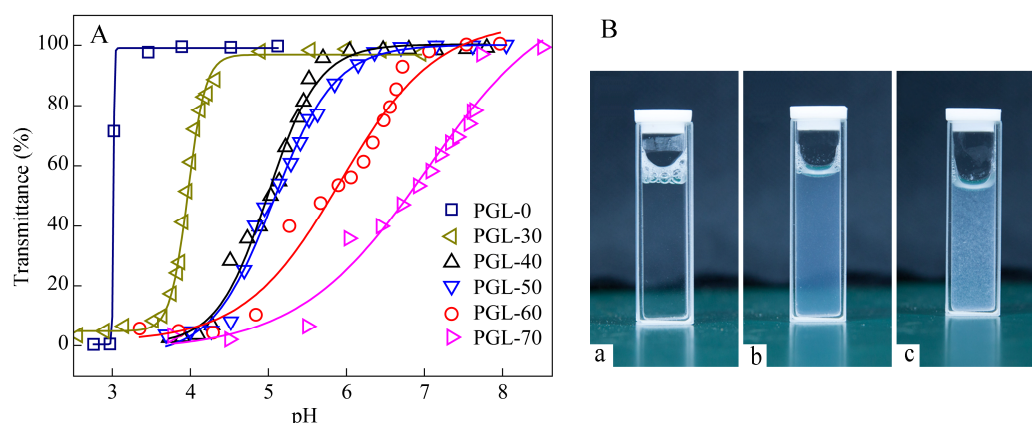


**Fig. 3** (A) pH-dependent light transmittance changes of PGL-*x* samples titrated from basic to acidic condition; (B) photographs of PGL-60 in water with various pH of 7.5 (a), 5.7 (b) and 4.9 (c)

**Table 2.** pH of cloud point and aggregation sizes of different PGL-*x*

| Sample | pH of cloud point during acidification <sup>a</sup> | pH of cloud point during basification <sup>a</sup> | Diameter <sup>a, b</sup> (nm) | PDI <sup>a, b</sup> |
|--------|-----------------------------------------------------|----------------------------------------------------|-------------------------------|---------------------|
| PGL-0  | 3.01                                                |                                                    |                               |                     |
| PGL-30 | 3.95                                                |                                                    |                               |                     |
| PGL-40 | 5.04                                                | 6.20                                               | 19                            | 0.239               |
| PGL-50 | 5.05                                                | 6.65                                               | 26                            | 0.192               |
| PGL-60 | 5.76                                                | 6.92                                               | 44                            | 0.237               |
| PGL-70 | 6.80                                                | 7.92                                               | 57                            | 0.208               |

<sup>a</sup> 1 mg/mL PGL-*x* samples; <sup>b</sup> DLS analysis in 0.01 mol/L PBS with pH of 7.4

After acidulated to complete precipitation, PGL-*x* solutions are titrated by NaOH back to be dissolved. It is confirmed that the pH-responsive phase transition is reversible (Fig. 4). The curves of phase transition move to higher pH as well as the cloud points (Table 2) indicating hysteresis of the phase transitions.

Amphiphilic poly(Glu-*r*-Leu)s aggregate slightly in PBS with pH of 7.4 to form nanoparticles. Measured by DLS at 37 °C (Table 2 and Fig. 5), the hydrodynamic diameters of PGL-*x* increase with the increasing ratio of Leu units. Especially, the diameter of PGL-60 is 44 nm, which is suitable for blood circulation<sup>[39]</sup>.

#### Block Polypeptide-Polypeptoids of Poly(Glu-*r*-Leu)-*b*-PolySar and Their pH-Responsive Self-assembly

As shown in Schemes 1 and 2, block polypeptide-polypeptoids are synthesized by sequence addition of Sar NCA after polymerization of poly(BLG-*r*-Leu) and deprotection to remove benzyl groups. PGL-70 is selected as hydrophobic segment containing 70% of Leu units with a cloud point pH of 6.80, and polySar with various lengths as hydrophilic segment. We simplify poly(BLG-*r*-Leu)-*b*-polySar and the corresponding deprotected poly(Glu-*r*-Leu)-*b*-polySar as PBLS-*y* and PGLS-*y*, respectively, where *y* represents the molar ratio of Sar to poly(BLG-*r*-Leu) in feed.

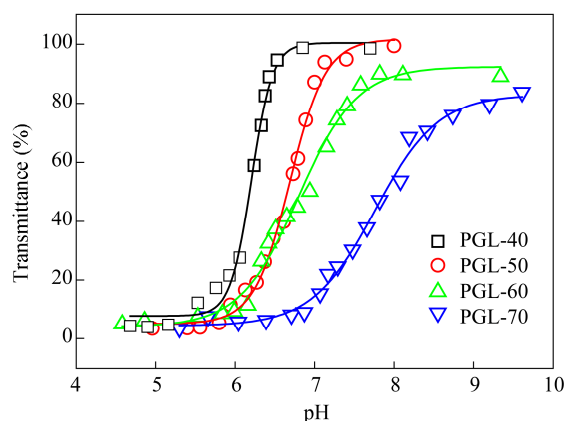


**Fig. 4** pH-dependent light transmittance changes of PGL-*x* samples titrated from acidic to basic condition

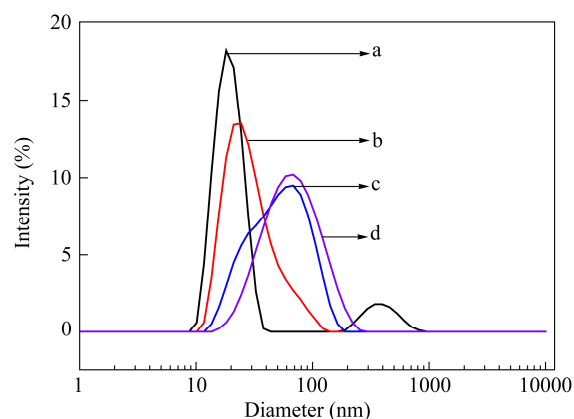


**Fig. 5** DLS profile of PGL-40 (a), PGL-50 (b), PGL-60 (c) and PGL-70 (d) in 0.01 mol/L PBS with pH of 7.4

Figure 6 compares the  $^1\text{H}$ -NMR spectra of poly(BLG-*r*-Leu), poly(BLG-*r*-Leu)-*b*-polySar and poly(Glu-*r*-Leu)-*b*-polySar. In poly(BLG-*r*-Leu)-*b*-polySar spectrum (Fig. 6B), the signal  $\text{H}^f$  contributes to the methyl proton of Sar units. We calculate compositions of poly(BLG-*r*-Leu)-*b*-polySar polymers by the ratio of the signals of  $\text{H}^b$ ,  $\text{H}^g$  and  $\text{H}^f$ , which are in good agreement with the feed ratio.

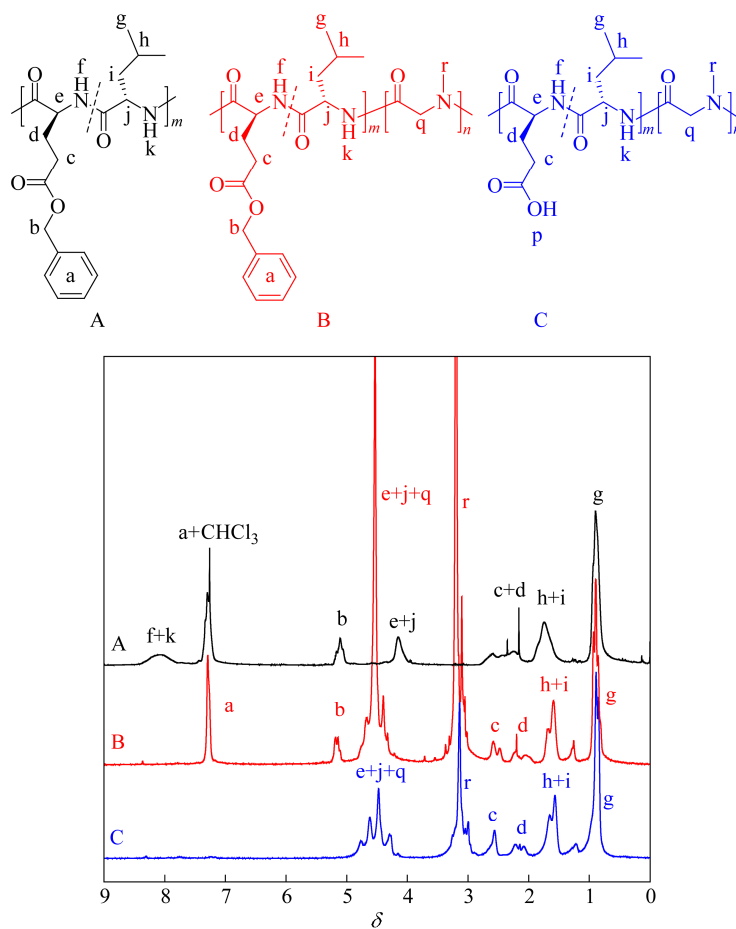


**Fig. 6**  $^1\text{H}$ -NMR spectra of poly(BLG-*r*-Leu) in  $\text{CDCl}_3/\text{CF}_3\text{COOH}$  (2:1) mixture (A), poly(BLG-*r*-Leu)-*b*-polySar in  $\text{CF}_3\text{COOD}$  (B) and poly(Glu-*r*-Leu)-*b*-polySar in  $\text{CF}_3\text{COOD}$  (C)

A quantitative deprotection of BLG units is confirmed by  $^1\text{H}$ -NMR spectra according to complete disappearance of benzyl protons of  $\text{H}^a$  and  $\text{H}^b$  (Fig. 6C). The Sar contents decrease differing from Glu and Leu (Table 3), indicating that polySar segments are degraded in acidic treatment in deprotection. The released low MW sarcosine oligomers are washed away in dialysis. This confirms that polypeptoid is not as stable as polypeptide in acidic environment<sup>[31]</sup>.

**Table 3.** Characterization of poly(BLG-*r*-Leu)-*b*-polySar and poly(Glu-*r*-Leu)-*b*-polySar

| Sample   | Sar:PBL-70<br>in feed <sup>a</sup> | BLG:Leu:Sar<br>in polymer <sup>a, b</sup> | Yield (%) | $M_w^c$<br>( $10^4$ Da) | PDI <sup>c</sup> | Glu:Leu:Sar after<br>deprotection <sup>a, b</sup> |
|----------|------------------------------------|-------------------------------------------|-----------|-------------------------|------------------|---------------------------------------------------|
| PGLS-200 | 200:1                              | 30:70:200                                 | 90        | 2.4                     | 1.50             | 30:70:44                                          |
| PGLS-400 | 400:1                              | 30:70:379                                 | 96        | 2.4                     | 1.68             | 30:70:111                                         |

<sup>a</sup> Molar ratio; <sup>b</sup> Calculated by  $^1\text{H}$ -NMR; <sup>c</sup> Measured by SEC

After copolymerization with Sar NCA, the SEC traces move to high MW range as a unimodal profile with the disappearance of the hump of PBL-70 aggregates (Fig. 7). Soluble as a random coil in DMF, long polySar chain makes polypeptide-polypeptoid more soluble and destroys the aggregations of polyLeu in random copolypeptide block. This result further confirms the mono-dispersity of poly(BLG-*r*-Leu)s.

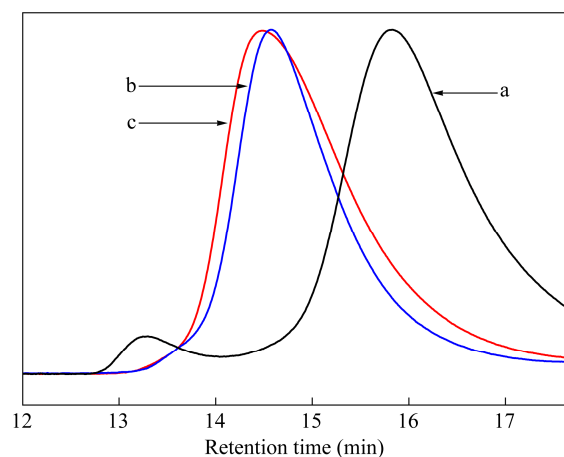


**Fig. 7** SEC profiles of PBL-70 (a), PBLS-200 (b) and PBLS-400 (c)

Poly(Glu-*r*-Leu)-*b*-polySar samples self-assemble in phosphate buffer, and their morphologies depend on pH values of their environment. PGLS-200 (Figs. 8a and 8b) forms orbicular micelles at pH of 8.0 with a diameter  $\sim 7$  nm. At pH of 5.0, they change to worm-like micelles with similar diameters. For PGLS-400 (Figs. 8c and 8d), the diameter of orbicular micelles at high pH is  $\sim 12$  nm, and changes to about 9 nm for congeries at low pH. At high pH of 8.0, poly(Glu-*r*-Leu) is partially soluble with assemblies because of high content of Leu acting as an amphiphilic core. The polySar acts as hydrophilic shell to stabilize micelles. At lower pH of 5.0, protonated poly(Glu-*r*-Leu)s are insoluble and increase the hydrophobic fractions in block polypeptide-polypeptoids. This transformation changes the curvature of nanostructure and makes micelles further assemble into worm-like structures<sup>[40]</sup>.

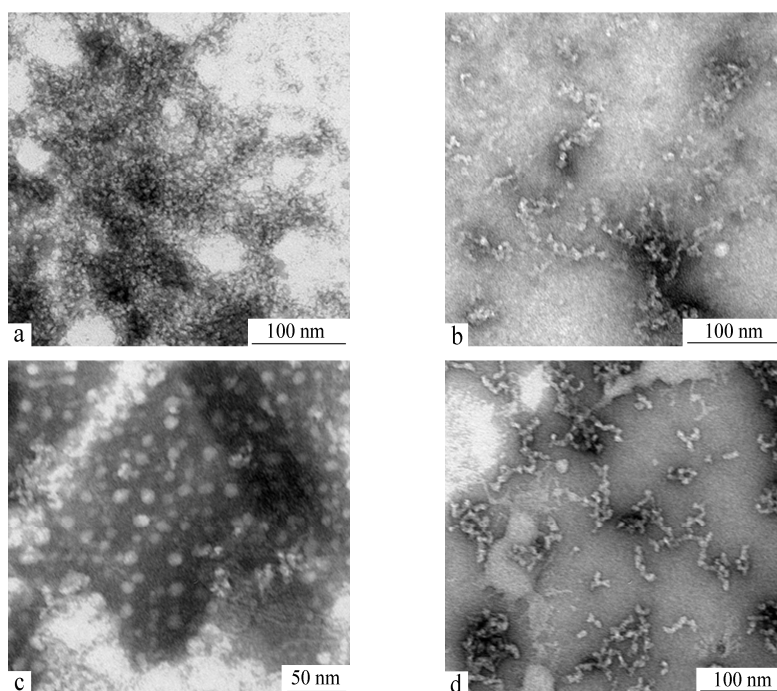


**Fig. 8** TEM of PGLS-200 at pH of 8.0 (a) and 5.0 (b), and PGLS-400 at pH of 8.0 (c) and 5.0 (d)

## CONCLUSIONS

We synthesized random copolypeptides of glutamic acid and leucine by ROP of NCA monomers and deprotection successively, and investigated the relationship between their hydrophilicity-hydrophobicity transitions and the Leu fractions. With the increasing molar ratio of Leu units, the pH of cloud point increased. When molar ratio of Leu was 60%, the cloud point of copolypeptide was 5.8 with a transition pH window from 7.0 to 4.3, and the diameter was ~44 nm at pH of 7.4.

We synthesized block copolymers of poly(Glu-*r*-Leu)-*b*-polySar by a consequence addition of NCAs. Poly(Glu-*r*-Leu)-*b*-polySar self-assembled to nanoparticles in phosphate buffer with a core of amphiphilic poly(Glu-*r*-Leu) and a shell of hydrophilic polySar. pH-responsive morphology changes were observed from orbicular micelles at high pH of 8.0 to worm-like micelles at low pH of 5.0.

According to pH-responsive phase transition and self-assembly, the natural amine acid-based polypeptides and polypeptide-polypeptoids are expected to circulate in blood system and perform hydrophilicity-hydrophobicity transition on the extracellular environment-endosome-lysosome way, and thus to be used as smart drug or gene carriers.

## REFERENCES

- 1 Deming, T.J., *Adv. Mater.*, 1997, 9(4): 299
- 2 Huang, J. and Heise, A., *Chem. Soc. Rev.*, 2013, 42(17): 7373
- 3 Wong, S., Shim, M.S. and Kwon, Y.J., *J. Mater. Chem. B*, 2014, 2(6): 595
- 4 Lu, H., Wang, J., Song, Z.Y., Yin, L.C., Zhang, Y.F., Tang, H.Y., Tu, C.L., Lin, Y. and Cheng, J.J., *Chem. Commun.*, 2014, 50(2): 139
- 5 Deming, T.J., *Prog. Polym. Sci.*, 2007, 32(8–9): 858
- 6 Shi, N.Q., Qi, X.R., Xiang, B. and Zhang, Y., *J. Control. Release*, 2014, 194: 53
- 7 Wang, F.H., Wang, Y., Zhang, X., Zhang, W.J., Guo, S.G. and Jin, F., *J. Control. Release*, 2014, 174: 126

- 8 Deming, T.J., *Nature*, 1997, 390(6658): 386
- 9 Peng, H., Chen, W.L., Kong, J., Shen, Z.Q. and Ling, J., *Chinese J. Polym. Sci.*, 2014, 32(6): 743
- 10 Mavroggiorgis, D., Bilalis, P., Karatzas, A., Skoulas, D., Fotinogiannopoulou, G. and Iatrou, H., *Polym. Chem.*, 2014, 5(21): 6256
- 11 He, C.L., Zhuang, X.L., Tang, Z.H., Tian, H.Y. and Chen, X.S., *Adv. Healthc. Mater.*, 2012, 1(1): 48
- 12 Huang, Y., Tang, Z.H., Zhang, X.F., Yu, H.Y., Sun, H., Pang, X. and Chen, X.S., *Biomacromolecules*, 2013, 14(6): 2023
- 13 Wang, J., Lu, H., Kamat, R., Pingali, S.V., Urban, V.S., Cheng, J.J. and Lin, Y., *J. Am. Chem. Soc.*, 2011, 133(33): 12906
- 14 Rodriguez-Hernandez, J. and Lecommandoux, S., *J. Am. Chem. Soc.*, 2005, 127(7): 2026
- 15 Xia, J.L., Chen, J., Tian, H.Y. and Chen, X.S., *Scientia Sinica Chimica*, 2010, 40(3): 255
- 16 Holowka, E.P., Pochan, D.J. and Deming, T.J., *J. Am. Chem. Soc.*, 2005, 127(35): 12423
- 17 Fasman, G.D., Lindblow, C. and Bodenheimer, E., *Biochemistry*, 1964, 3(2): 155
- 18 Bychkova, V.E., Gudkov, A.T., Miller, W.G., Mitin, Y.V., Ptitsyn, O.B. and Shpungin, I.L., *Biopolymers*, 1975, 14(8): 1739
- 19 Beverung, C.J., Radke, C.J. and Blanch, H.W., *Biophys. Chem.*, 1998, 70(2): 121
- 20 Soderquist, M.E., Gershman, H., Anderson, J.M. and Walton, A.G., *J. Biomed. Mater. Res.*, 1979, 13(6): 865
- 21 Helmus, M.N., Gibbons, D.F. and Jones, R.D., *J. Biomed. Mater. Res.*, 1984, 18(2): 165
- 22 Jayakumar, R., Murali, J., Koteeswari, D. and Gomathi, K., *J. Biochem.*, 2004, 136(4): 457
- 23 Tao, X.F., Deng, Y.W., Shen, Z.Q. and Ling, J., *Macromolecules*, 2014, 47(18): 6173
- 24 Fetsch, C., Grossmann, A., Holz, L., Nawroth, J.F. and Luxenhofer, R., *Macromolecules*, 2011, 44(17): 6746
- 25 Birke, A., Huesmann, D., Kelsch, A., Weilbaecher, M., Xie, J., Bros, M., Bopp, T., Becker, C., Landfester, K. and Barz, M., *Biomacromolecules*, 2014, 15(2): 548
- 26 Tao, X.F., Deng, C. and Ling, J., *Macromol. Rapid Commun.*, 2014, 35(9): 875
- 27 Luxenhofer, R., Fetsch, C. and Grossmann, A., *J. Polym. Sci., Part A: Polym. Chem.*, 2013, 51(13): 2731
- 28 Ulbricht, J., Jordan, R. and Luxenhofer, R., *Biomaterials*, 2014, 35(17): 4848
- 29 Ostuni, E., Chapman, R.G., Holmlin, R.E., Takayama, S. and Whitesides, G.M., *Langmuir*, 2001, 17(18): 5605
- 30 Zhou, M., Liu, H.W., Venkiteswaran, A., Kilduff, J., Anderson, D.G., Langer, R. and Belfort, G., *J. Mater. Chem.*, 2011, 21(3): 693
- 31 Heller, P., Birke, A., Huesmann, D., Weber, B., Fischer, K., Reske-Kunz, A., Bros, M. and Barz, M., *Macromol. Biosci.*, 2014, 14(10): 1380
- 32 Daly, W.H. and Poche, D., *Tetrahedron Lett.*, 1988, 29(46): 5859
- 33 Peng, H., Ling, J. and Shen, Z.Q., *J. Polym. Sci., Part A: Polym. Chem.*, 2012, 50(6): 1076
- 34 Peng, H., Ling, J., Zhu, Y.H., You, L.X. and Shen, Z.Q., *J. Polym. Sci., Part A: Polym. Chem.*, 2012, 50(15): 3016
- 35 Kricheldorf, H.R., von Lossow, C. and Schwarz, G., *Macromol. Chem. Phys.*, 2004, 205(7): 918
- 36 Guo, L. and Zhang, D., *J. Am. Chem. Soc.*, 2009, 131(50): 18072
- 37 Lahasky, S.H., Hu, X.K. and Zhang, D.H., *ACS Macro Lett.*, 2012, 1(5): 580
- 38 Huesmann, D., Birke, A., Klinker, K., Tuerk, S., Raeder, H.J. and Barz, M., *Macromolecules*, 2014, 47(3): 928
- 39 Maruyama, K., Yuda, T., Okamoto, A., Kojima, S., Suginaka, A. and Iwatsuru, M., *Biochim. Biophys. Acta.*, 1992, 1128(1): 44
- 40 Blanazs, A., Armes, S.P. and Ryan, A.J., *Macromol. Rapid Commun.*, 2009, 30(4–5): 267