Article

Cite This: *Biomacromolecules* XXXX, XXX, XXX−XXX

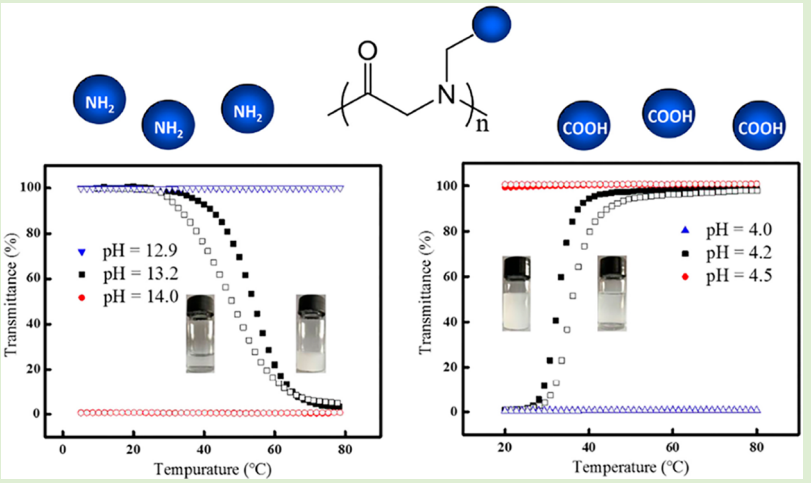
pubs.acs.org/Biomac

Charge-Determined LCST/UCST Behavior in Ionic Polypeptoids

Chao Xing, Zhekun Shi, Jiliang Tian, Jing Sun,* and Zhibo Li*

Key Laboratory of Biobased Polymer Materials, Shandong Provincial Education Department; School of Polymer Science and Engineering, Qingdao University of Science and Technology, Qingdao, 266042, China

Supporting Information



ABSTRACT: Stimuli-responsive polymers have received increasing interest for a variety of applications. Here, we report a series of unique charge-determined thermoresponsive polypeptoids synthesized by a combination of ring-opening polymerization and click chemistry. The LCST-type and UCST-type behavior is mainly dominated by the charge state on the side chain. Further, the phase transition temperature highly depends on the degree of polymerization, the side-chain architecture, the pH value, and so on. The obtained polypeptoid solutions exhibit good stability against temperature and salt concentration. To our knowledge, this report presents the first charge-determined LCST/UCST-type polymer from identical homopolymer backbone that displays a wide range of tunable cloudy points in aqueous media. We propose the hydrogen-bonding interaction plays a critical part on the solution behavior. These features make polypeptoids ideal candidates for highly designable stimuli-responsive polymeric materials.

INTRODUCTION

Thermoresponsive polymers, a class of promising smart materials, have received extensive interest for both fundamental research and applications in the past two decades.[1,2] In particular, water-soluble thermoresponsive polymers are capable of exhibiting a reversible phase transition to temperature in aqueous media.[3] They offer great potential for biomedical applications such as protein delivery, smart hydrogels, and surface science.[4] A majority of thermoresponsive polymers show typical lower critical solution temperature (LCST) behavior,[5−9] such as poly(*N*-isopropylacrylamide) (PNIPAM), OEG-grafted (meth)acrylates polymers, and polypeptides. The LCST-type polymer undergoes a phase transition from a hydrated state to a dehydrated state upon heating. In contrast, the reported polymers exhibiting upper critical solution temperature (UCST) behavior are relatively rare, such as poly(sulfobetaine) and poly(*N*-acryloylglycinamide).[10−15] The UCST-type polymer shows a miscibility gap at low temperature. In most cases, the UCST behavior is observed in organic solvents or organic/water mixtures. Only a few polymers were reported to display UCST behavior in aqueous solution, particularly under physiologic conditions.[11,16] Polymers that can exhibit both LCST and UCST behavior under mild and physiologic conditions have been barely reported.[17,18] It has been reported that copolymerization is one of the strategies by tuning the types and compositions of monomers.[13] A recent study shows that copolymers composed of *N*-acryloylglycinamide and diacetone acrylamine have either LCST- or UCST-type transitions, depending on the compositions, degree of polymerization (DP), polymer concentration, and so on.[19] A new generation of smart polymers with finely tunable LCST/UCST properties is desired.[20]

Polypeptoids are a promising class of peptidomimetic polymers based on an N-substituted glycine backbone.[21]

Special Issue: Biomacromolecules Asian Special Issue

Received: February 10, 2018
Revised: March 29, 2018

DOI: 10.1021/acs.biomac.8b00240
Biomacromolecules XXXX, XXX, XXX−XXX

They offer great advantageous properties for both fundamental research and applications in biotechnology. Due to the similarity in structure to polypeptides, the polypeptoids exhibit excellent biocompatibility and potential bioactivities.[22−24] The lack of inter- and intrachain hydrogen bonding and chirality in the main chain results in a potentially flexible backbone. The properties of polypeptoids are mainly dominated by the side-chain identity, which offers a unique and facile way to tailor the properties of the polymers by structural design of the side chains. Further, the polypeptoids are highly designable in terms of synthetic approaches. In addition to solid-phase method, the ring-opening polymerization (ROP) has emerged as an effective method to produce the polypeptoids with high molecular weights in high yields.[25−27] The postmodification of the well-defined polypeptoid precursors offer a convenient method to prepare polypeptoids with versatile functionalities.[28,29]

We have previously prepared a family of pegylated nonionic polypeptoids that exhibits lower critical solution temperature (LCST) behavior.[30] Unlike our previous study, we synthesized a family of ionic polypeptoids with tunable thermal-responsive property in this study. The synthetic method combines ring-opening polymerization (ROP) technique with thiol−ene/yne click chemistry. The obtained polypeptoids show either LCST-type or UCST-type behavior, depending on the side-chain charges. Moreover, the phase transition of the polypeptoids in both cases is pH-sensitive. The influence of the side-chain architecture, the degree of polymerization (DP), and the polymer concentration on the solution properties of the polypeptoids has been systematically investigated. We propose the phase transition temperature is highly dependent on the hydrogen bonding of the system. To our best knowledge, this is the first report of a charge-determined thermoresponsive polymer from an identical homopolymer backbone.

EXPERIMENTAL SECTION

Materials and Methods. Glyoxylic acid (50 wt % in H_2O) and allylamine (98%) were purchased from Shandong Xiya Chemical Co., Ltd. Progargylamine was purchased from Nantong Camry Chemical Co., Ltd. Benzylamine (99%), cysteamine hydrochloride (98%), mercaptoacetic acid (99%), di-*tert*-butyl dicarbonate, and 2,2-dimethoxy-2-phenylacetophenone (DMPA) were purchased from Aladdin reagent. Phosphorus trichloride and trimethylamine were purchased from Sinopharm Chemical Reagent Co., Ltd. 3-Mercaptopropionic acid was purchased from Adamas Reagent Co., Ltd. Dichloromethane (DCM), hexane, and tetrahydrofuran (THF) were purified by passing through activated alumina columns prior to use. The rest of chemicals were purchased from commercial suppliers, which were used without further purification unless otherwise stated. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AV500 FT-NMR spectrometer. Tandem gel permeation chromatography (GPC) was performed at 50 °C using an SSI pump connected to Wyatt Optilab DSP with 0.02 M LiBr in DMF as eluent at flow rate of 1.0 mL/min. The concentrations of GPC samples were ∼5 mg/mL. The molecular weights were calibrated against polystyrene (PS) standards. Fourier transform infrared (FTIR) spectra were recorded from 2000 to 1400 cm^{-1} on a Horiba−Jobin Yvon FluoroMax-4 spectrofluorometer. All the sample solutions were cast on KBr plates at different temperatures before measurement. We defined the cloud points (CPs) as the temperature at the transmittance of 50% during the heating process. All the CPs were measured by monitoring the transmittance of a 500 nm light beam through a quartz sample cell on a Shimadzu UV-2910 spectrometer. The heating/cooling rate is 1 °C/min. Circular dichroism spectra were recorded on an applied photophysics chirascan CD spectrometer. The solution was placed into a quartz cell with a path length of 0.1 cm. The electrophoretic mobility (μE) of the aggregates in dependence of pH was measured on a Malvern Zetasizer Nano ZS. Zeta-potentials (ζ) were calculated with the Smoluchowski's formula: $\zeta = \mu E\eta/\epsilon$, where η denotes the viscosity and ϵ the permittivity of the solution. Solutions were prepared by dissolving the polymer in water at desired concentrations, followed by titration of the initial solution to the desired pH using 1 M HCl or 1 M NaOH, respectively.

Synthesis of 2-(Allylamino)acetic Acid Hydrochloride (a). Glycoxylic acid solution (60 g) was mixed with 300 mL of CH_2Cl_2 in the reaction flask. A total of 15 mL of allylamine was added slowly, and the reaction was stirred for 6 h at room temperature. The CH_2Cl_2 was then removed by rotavapor. An aqueous solution of HCl (300 mL, 1.0 M) was added, followed by refluxing in 110 °C and stirring overnight. Brown solid was further purified by recrystallization in methanol/THF (30 mL/300 mL) at −10 °C overnight after water was removed by rotavapor. The resulting compound was obtained by filtered and dried under vacuum (14.1 g, 46.5% yield). ^{1}H NMR (500 MHz, D_2O) δ 5.81 (m, 1H), 5.43 (t, 2H), 3.79 (s, 2H), 3.64 (d, 2H).

Synthesis of 2-(Allyl(*tert*-butoxycarbonyl)amino)acetic Acid (b). A total of 12 g **a** was dissolved in 200 mL of water. A total of 21.4 g di-*tert*-butyl dicarbonate and 27 mL of triethylamine was then added and stirred overnight at room temperature. The mixture was washed by hexane (3 × 100 mL) to remove unreacted di-*tert*-butyl dicarbonate followed by adjusting pH to 3 with 4 M aqueous HCl; an aqueous phase was then extracted with ethyl acetate (3 × 100 mL). The supernatant was separated and washed with brine and dried with anhydrous Na_2SO_4. The white solid was collected after the solvent was removed by rotavapor (6.1 g, 71.7%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.77 (m, 1H), 5.16 (t, 2H), 3.98 (s, 2H), 3.88 (d, 2H), 1.43 (s, 9H).

Synthesis of *N*-Allyl *N*-Carboxyanhydride (Allyl-NCA, c). A total of 6 g **b** was dissolved in 250 mL of anhydrous CH_2Cl_2 under nitrogen in a 500 mL flask. A 2.5 mL aliquot of PCl_3 was then added dropwise to the reaction solution and the system was stirred for 3 h in an ice bath. After the solvent was removed under vacuum, the oil was extracted with 15 mL of CH_2Cl_2 and filtered. In a glovebox, further purification was performed from anhydrous THF/hexane. After 4−5 times dissolving/precipitating cycles, clear oil was obtained (2.2 g, 53.4%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.78 (m, 1H), 5.34 (d, 2H), 4.07 (s, 2H), 4.01 (d, 2H).

Synthesis of Poly(*N*-allyl glycine) (PNAG). In a typical procedure, the allyl-NCA (300 mg, 2.12 mmol) dissolved in 3 mL of anhydrous THF, followed by adding a stock solution of benzylamine (3.61 wt % in THF). The solution was stirred at 50 °C for 24 h with nitrogen protection, and the polymerization progress was monitored by FTIR. The solution was precipitated into an excess of hexane. The product was dried under reduced pressure to yield a white solid (74.2% yield). All the other polymers were prepared in a similar way according to the designed NCA to initiator ratio.

Poly(*N*-propargylglycine) (PNPG) was synthesized by following a published procedure.[28]

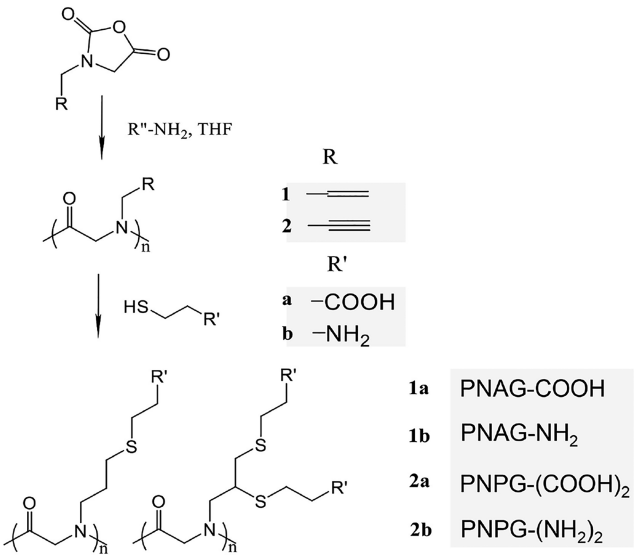
General Procedure for Modification with Cysteamine Hydrochloride/3-Mercaptopropionic Acid. Typically, cysteamine hydrochloride (1.19 g, 10.5 mmol) or mercaptoacetic acid (1.12 g, 10.5 mmol), PNAG (100 mg, [SH] = 1.05 mmol, [SH]/[C═C] = 10), and DMPA (13 mg, 0.053 mmol) were dissolved in 1 mL of DMF. The system was degassed and then irradiated with UV light at room temperature for 3 h. After dialyzed for 3 days and lyophilized, white solid was achieved (75.4% yield). All the other PNAG and PNPG were modified in a similar way.

RESULTS AND DISCUSSION

The *N*-propargyl *N*-carboxyanhydride (NPG-NCA) and *N*-allyl *N*-carboxyanhydride (Allyl-NCA) monomers were synthesized following reported methods (Scheme S1).[31] The chemical structure of the monomers was confirmed by ^{1}H NMR spectroscopy (Figures S1 and S2). The homopolymers were then synthesized by ring-opening polymerization (ROP) of both NCA monomers with a nucleophilic initiator benzylamine (Scheme 1). The polymerization was monitored by FTIR. The disappearance of two characteristic $\nu_{C=O}$ peaks of the

DOI: 10.1021/acs.biomac.8b00240
Biomacromolecules XXXX, XXX, XXX−XXX

Scheme 1. Synthetic Pathways of Polymerization of NPG-NCA and Allyl-NCA Monomers and Subsequent Radical Thiol Addition[a]



[a]Reaction conditions are described in the text.

monomer at 1790 and 1860 cm^{-1} was considered as complete conversion of NNCA monomers to polypeptoids.[32] All peaks of the obtained polypeptoids are well assigned in the ^1H NMR spectra, which confirm the chemical structures (Figures 1 and S2). A series of homopolypeptides with different degrees of polymerization (DPs) were then synthesized by varying the ratio of NCA to the initiator. The average DPs of PNPG and PNAG were in the range of 41−107 and 21−79, respectively. Table 1 summarized the molecular characteristics of both polymers $PNPG_n$ and $PNAG_n$, where the subscript n represents the average DP of both polymers. ^1H NMR spectroscopy was used to determine the molar ratio and molecular weight of the blocks. The DPs were obtained from the proton integral ratios of allyl group or propargyl group to the phenyl group. The GPC trace shows a narrow molecular weight distribution with

Table 1. Molecular Parameters of Homopolypeptoids

samples	feed ratio[a] (NCA/initiator)	n[b]	M_n[b] (kg/mol)	M_n[c] (kg/mol)	dispersity[c] ($Đ$)
$PNPG_{41}$	40	41	3.8	2.8	1.21
$PNPG_{83}$	80	83	7.8	4.1	1.27
$PNPG_{107}$	110	107	10.7	4.9	1.16
$PNAG_{21}$	20	21	1.8	4.8	1.31
$PNAG_{46}$	40	46	4.4	4.5	1.13
$PNAG_{55}$	60	55	5.8	5.2	1.10
$PNAG_{79}$	80	79	7.6	6.2	1.09

[a]Feed molar ratio of NCA/initiator. [b]Calculated from ^1H NMR spectra. [c]Determined from GPC; n represents the average DP of PNPG or PNAG.

dispersity ($Đ$) ≤ 1.31, indicating the well-controlled polymerization (Figure S3).

The polypeptoids were subsequently modified with thiol-terminated regents to yield functionalized polypeptoids (Scheme 1). PNAG was conjugated with cysteamine hydrochloride and mercaptoacetic acid to yield $PNAG-NH_2$ and PNAG-COOH, respectively. Figure 1b shows a typical ^1H NMR spectrum of $PNAG-NH_2$, where the protons of newly formed CH_2SCH_2 linkage at $\delta \sim 2.53-2.88$ ppm are visible. Further the protons of alkenyl group at 5.01−5.94 ppm entirely disappear, which indicates the virtually quantitative conversion of alkenyl group. The chemical structure of $PNAG-NH_2$ was further confirmed by FTIR, shown in Figure 2. A characteristic stretching band of at 3081 cm^{-1} ($\nu_{=C-H}$) is completely absent after the modification, confirming the quantitative conversion. The successful modification of PNAG with mercaptoacetic acid was also confirmed by ^1H NMR and FTIR (Figures 1a and 2). PNPG was modified in a similar way to yield $PNPG-(NH_2)_2$ and $PNPG-(COOH)_2$ with branched side chains, respectively. Similarly, the results suggest the successful modification (Figures S4 and S5). In a typical ^{13}C NMR spectrum, the absence of protons at 73.8−79.7 ppm suggests the complete conversion of the alkynyl groups (Figure S4).

All of the polypeptoids modified with mercaptoacetic acid can readily dissolve in alkaline solution. Due to the presence of

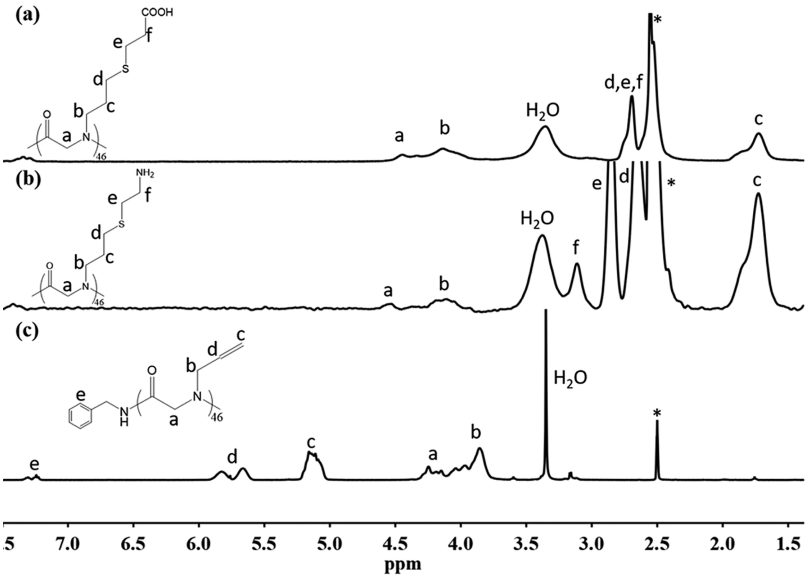


Figure 1. Representative ^1H NMR spectra of (a) $PNAG_{46}$-COOH; (b) $PNAG_{46}-NH_2$; (c) $PNAG_{46}$ in DMSO (* indicates DMSO).

DOI: 10.1021/acs.biomac.8b00240
Biomacromolecules XXXX, XXX, XXX−XXX

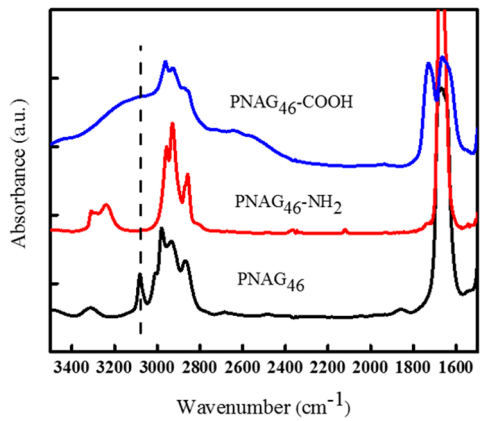


Figure 2. FTIR spectra of $PNAG_{46}$, $PNAG_{46}$-NH_2, and $PNAG_{46}$-COOH.

carboxylic acid groups on the side chains, the polymers are expected to show pH-responsive behavior in aqueous solution. The desired pH value was adjusted by HCl or NaOH solutions. At pH ≥ 4.5, a clear solution of $PNPG_{41}$-$(COOH)_2$ is obtained at a concentration of 2 mg/mL over the entire experimental temperature window (Figure 3a). As pH is decreased to 4.2, the solution appears cloudy at ambient temperature. This is due to the increasing content of protonated −COOH moieties that decrease the solubility of the polypeptoid.[33] Note that the poly(L-glutamic acid) analog was reported to show a pK_a of ∼4.3.[34] Upon heating, the polymer solution shows phase transitions from cloudy to clear. Figure 3a shows the transmittance increases from 0% to 100% as the temperature increases from 20 to 80 °C. We defined the cloud point (CP) as the temperature at the transmittance of 50% during the heating process, which was determined to be 35 °C in this case. During the cooling process, reversible phase transition is observed. The transmittance recovers completely to 0%, resulting in the $CP_{cooling}$ of 33 °C. This indicates a typical UCST behavior. The CP determined in the cooling ramp is slightly lower (2 °C) than that in the heating ramp. The hysteresis is possibly due to the overcooling during dehydration.[35] We have previously reported that pegylated polypeptoids show LCST behavior due to the presence of oligo(ethylene glycol) (OEG) groups.[30] Unlike previous results, herein we incorporated the carboxylic acid groups on the side chains, which largely enhance the polymer−polymer interactions via inter- and intrachain hydrogen bonding between amide groups on the backbone and −COOH groups on the side chain.[36] Upon heating, the phase transition occurred driven by thermally controlled reversible hydrogen bonding. This is not unexpected as the thermal-responsive property is dependent on many effects. It was previously reported that the zwitterionic groups can lead to the cluster formation that largely influences the thermal-responsive properties of the polypeptoid system.[37] Furthermore, the transition of *cis* backbone conformation to a mixture of *cis* and *trans* conformation is also related to the phase transition behavior.[38] FTIR was further used to explore the influence of hydrogen bonding on phase transition behavior (Figure S6). The characteristic band of −COOH at ∼3400 cm^{-1} (ν_{OH}) is significantly reduced at high temperature, indicating the hydrogen bonding formation between the polymer with water molecule. Further decreasing pH to 4.0 results in a turbid solution in the absence of the UCST behavior, due to the increased protonation degree of the −COOH groups. Interestingly, the UCST behavior is pH-responsive with extremely narrow transition of ΔpH = ∼0.2.

To quantify the percent protonation/deprotonation of the −COOH groups, we performed zeta-potential (ζ) measurement. The ζ percent based on the ζ (= −23.1 mV) of the solution at pH ≥ 7 is plotted as a function of pH (Figure S7). An abrupt increase is observed as pH ranges from 4 to 5. The percentages of ζ are observed to 6% and 97%, suggesting nearly complete protonation and deprotonation at pH 4.0 and 4.5, respectively. At pH 4.2, the ζ percent of 72% shows that the same amount of −COOH group is deprotonated. This indicates the slight variation in ζ percent can lead to the different thermal-responsive property. It is noteworthy that the previously reported poly(*N*-(2-carboxyethyl)glycine) with pendant COOH groups exhibit excellent water solubility.[39] Distinct from the reported one, $PNPG_n$-$(COOH)_2$ contains additional thioether groups on side chains with branched architecture, which are generated by thiol−yne click chemistry. The difference in structure varies the hydrophilic−hydrophobic balance of the system. All of these features result in the pH-dependent UCST-type thermoresponsive behavior in the polymer. Note that the polypeptoids barely show CD signals. This is due to the lack of chiral center, suggesting the absence of the secondary structure (data not shown).

The influence of the concentration on the solution properties of $PNPG_{41}$-$(COOH)_2$ samples was investigated. Generally,

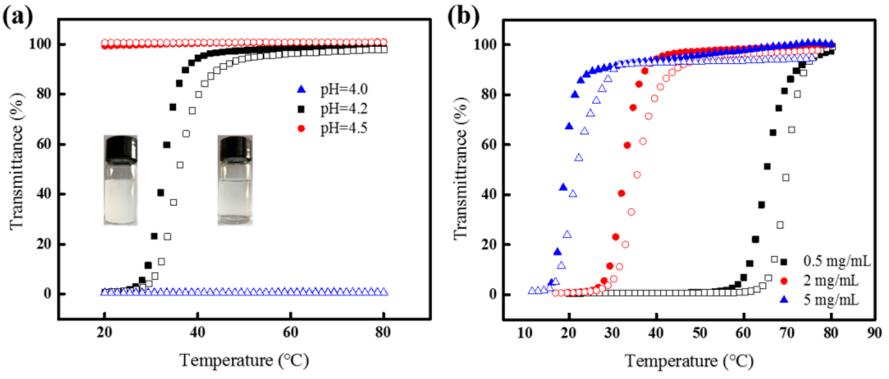


Figure 3. (a) Plots of transmittance as a function of temperature for aqueous solutions (2 mg/mL) of $PNPG_{41}$-$(COOH)_2$ at different pH. Filled symbol: cooling ramp; open symbol: heating ramp. (b) Plots of transmittance as a function of temperature for aqueous solutions of $PNPG_{41}$-$(COOH)_2$ at pH = 4.2 at different concentrations.

DOI: 10.1021/acs.biomac.8b00240
Biomacromolecules XXXX, XXX, XXX−XXX

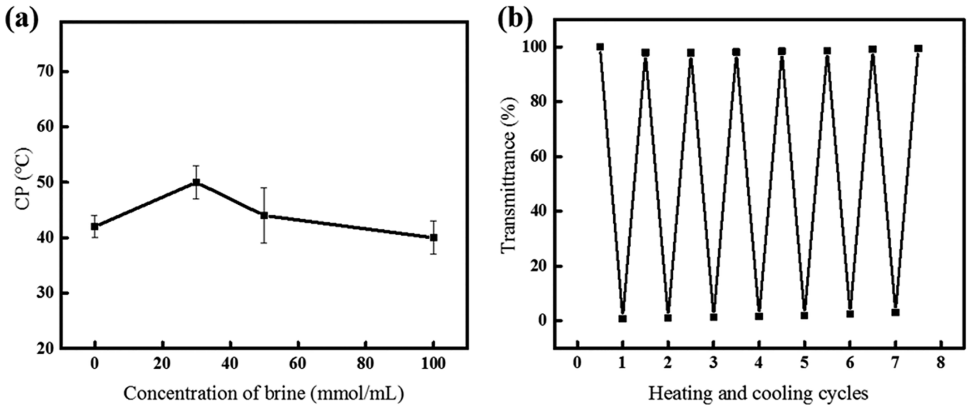


Figure 4. (a) Plots of CP as a function of NaCl concentration for $PNPG_{107}$-$(COOH)_2$ solution at a concentration of 0.5 mg/mL at pH 4.2. (b) Transmittance of $PNPG_{41}$-$(COOH)_2$ aqueous solution at a concentration of 0.5 mg/mL at pH 4.2 vs 8 heating and cooling cycles between 20 and 80 °C.

increasing the concentration results in lower CP (Figure 3b), which is different from the most reported UCST system.[16,19] The deviation is likely due to the charge state of the polymers. Considering the pH-sensitive property, slight variation of protonation degree may change the solubility of the polymer. We further investigated the addition of salt on the solution behavior of the polypeptoids. A typical plot of CP of $PNPG_{107}$-$(COOH)_2$ as a function of NaCl concentration show that the CP remains consistent as the NaCl concentration is increased from 0 to 100 mM (Figure 4a). This suggests that the solution is relatively stable to the salt, despite the presence of ionic groups. We also incorporated $CaCl_2$ at a concentration up of 50 mM into the system. Interestingly, the complex solution lacks UCST behavior over the entire experimental window. We attribute this to the coordination of −COOH groups in the polymer with Ca^{2+} ions, which disrupt the hydrogen bonding of the system (Figure S8).[40] This further confirms that the hydrogen bonding plays a vital part in the thermal-responsive property of the system. Moreover, the phase transition is recovered after 8 times heating and cooling run between 20 and 80 °C, suggesting good stability of the polypeptoid solution (Figure 4b).

We further systematically studied the temperature effect on the solution properties of $PNPG_n$-$(COOH)_2$ samples. A family of $PNPG_n$-$(COOH)_2$, with n ranging from 41 to 107 were prepared. We plotted the CP of $PNPG_n$-$(COOH)_2$ solutions versus DP of PNPG. It is observed that increasing the DP results in decreased CP (Figure 5). It was reported the longer polymer chain enables less hydrophobic residues exposed in aqueous media.[30] More of the deionized −COOH groups embedded in the longer polymer chain can reduce the aggregation of the polymers and increase the solubility of the polymer in aqueous solution. To probe the influence of the side chain architecture, we investigated the temperature effect on the solution properties of $PNAG_n$-COOH. Interestingly, the $PNAG_n$-COOH samples exhibit UCST-type behavior as well. Plotting the CP of $PNAG_n$-COOH solutions versus DP of PNAG shows a similar tendency. Note that the CP of $PNPG_n$-$(COOH)_2$ is ∼18 °C higher than that of $PNAG_n$-COOH at DP of ∼40 as the concentration and pH is fixed. This is possibly due to the stronger hydrogen bonding in the $PNPG_n$-$(COOH)_2$, suggesting that the molecular architecture plays a vital role on the CPs.

To systematically investigate the charge state on the solution behavior of the polymer, the polypeptoids were further

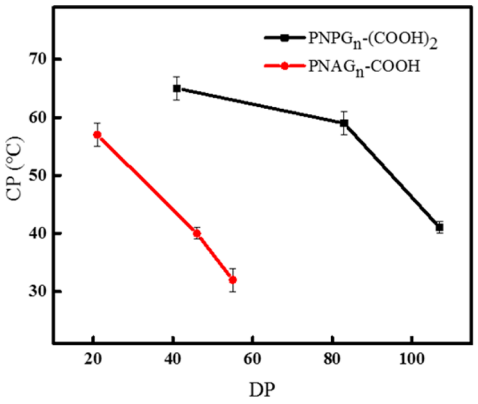


Figure 5. Plots of CPs as a function of DP for Bn-$PNPG_n$-$(COOH)_2$ and Bn-$PNAG_n$-COOH at a concentration of 0.5 mg/mL at pH 4.2.

modified with cysteamine to obtain $PNPG_n$-$(NH_2)_2$ and $PNAG_n$-NH_2. The obtained polymers are expected to show pH-responsive behavior due to the presence of amine groups on the side chains. In this case of $PNAG_n$-NH_2, the polymers are well soluble at pH ≤ 12.9 at a concentration of 10 mg/mL over the entire measurable temperature (Figure 6). Interestingly, unlike the reported poly(*N*-(2-aminoethyl)glycine) with pendant NH_2 groups with good water solubility,[39] the samples exhibit reversible phase transition from clear to cloudy upon heating with increasing pH to 13.2. This suggests a typical LCST behavior, consistent with the positively charged polypeptoids. It is conceivable that the pH effect on the solution behavior is extremely important. The incorporated amine groups on the side chains behave as the hydrogen bond donor and form strong interaction with water, which results in the LCST-type behavior. It is known that the UCST behavior is ascribed to the strong polymer−polymer and solvent−solvent interactions. Instead, the strong polymer−solvent interaction generally leads to the LCST behavior.[11,17] In this case, the hydrogen bonding between the amine groups and amide groups in $PNAG_n$-NH_2 is relatively weak as compared to $PNAG_n$-COOH, which results in the LCST behavior rather than UCST behavior. FTIR was used to determine the influence of hydrogen bonding on the phase transition behavior (Figure S6b). Similarly to positively charged polypeptoid, the broader characteristic band of amine groups at ∼3460 cm^{-1} (ν_{NH2}) at low temperature, indicative of the hydrogen bonding

DOI: 10.1021/acs.biomac.8b00240
Biomacromolecules XXXX, XXX, XXX−XXX

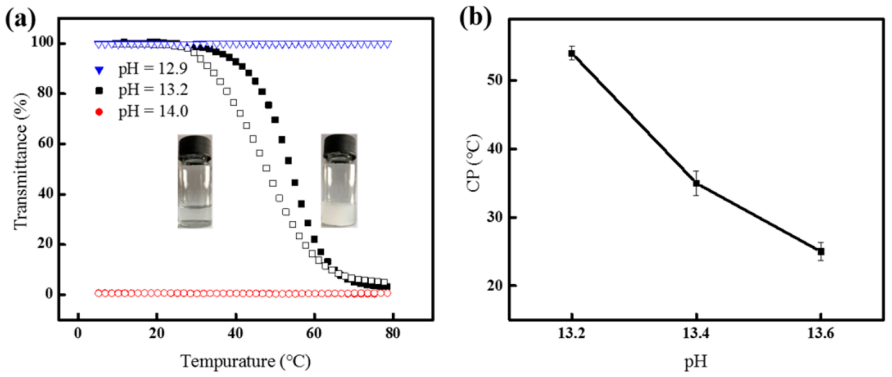


Figure 6. (a) Plots of transmittance as a function of temperature for aqueous solutions (10 mg/mL) of $PNAG_{79}$-NH_2 at different pH. Filled symbol: heating ramp; open symbol: cooling ramp. (b) Plots of CP as a function of pH for aqueous solutions (10 mg/mL) of $PNAG_{79}$-NH_2.

forming between the polymer and water molecule. As pH is increased from 13.2 to 13.6, the CP of $PNAG_n$-NH_2 decreases from 54 to 22 °C (Figure 6b). Further increasing pH to 14.0 results in the turbid solution without exhibiting the LCST behavior. We presume that this is due to the increased deprotonation degree of the polymer that reduces the solubility of the polymer. To confirm this, we performed zeta-potential (ζ) measurement. The ζ percent based on the ζ (= 20.4 mV) of the solution at pH $\geq$ 7 is plotted versus pH (Figure S7). An abrupt increase is observed in the pH range of 13−14. As pH increases from 13.2 to 13.6, the ζ percent decreases from 88% to 49%, suggesting increased deprotonation degree of the polymer. Further decreasing pH to 14.0 results in the ζ percent of 10%, consistent with the previous conclusion. Note that the $PNPG_n$-$(NH_2)_2$ with branched side chains lacks both LCST and UCST behavior in the experimental window, confirming the importance of the molecular architecture.

The solution property of $PNAG_n$-NH_2 was further systematically investigated. Decreasing the concentration results in the lower CP and incomplete recovery of transmittance of the solution, suggesting high concentration is essential for the LCST behavior (Figure S9). We also investigated the stability of the polypeptoid solution at a concentration of 10 mg/mL. The heating and cooling cycles were performed for 8 times between 20 and 80 °C. The phase transition is entirely recovered, suggesting good stability to temperature (Figure S10). Similar to the polymer with pedant carboxylic acid groups, the influence of salts on the CP is barely visible (Figure S11). Further, we synthesized a series of $PNAG_n$-NH_2 with different DP. All the samples show LCST-type behavior. A plot of the CP versus DP for the $PNAG_n$-NH_2 shows that the CP decreases with increasing DP, which is likely due to less solubility of longer molecular chains, irrespective of the presence of ionized groups (Figure 7).[41] Note that this differs from the results of polypeptoids with −COOH groups on the side chain. We attribute it to the different molecular interactions of the systems.

CONCLUSION

In conclusion, a family of ionic polypeptoids were synthesized by a combination of ROP and thiol−ene/yne click chemistry. The polypeptoids prepared in this work show reversible LCST-type or UCST-type behavior in aqueous media, which is fairly distinct from the previously reported positively and negatively charged polypeptoids and pegylated nonionic polypeptoids. The thermoresponsive behavior is highly dependent on the

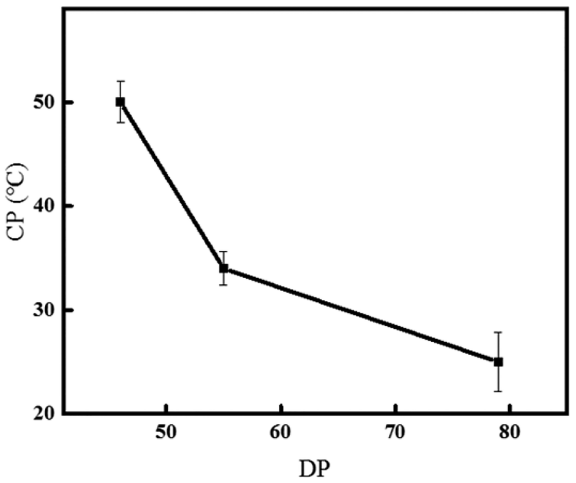


Figure 7. Plots of CP as a function of DP for aqueous solutions (10 mg/mL) of $PNAG_n$-NH_2 at pH 13.6.

side-chain charges. Further, the phase transition of the polypeptoids in both cases is pH-sensitive due to the presence of charge. A systematic study of the influence of the temperature and pH on the solution properties of the polypeptoids was performed. We propose the hydrogen bonding interaction plays an extremely critical part on the solution behavior.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biomac.8b00240.

Additional $^1H/^{13}C$ NMR data, FTIR spectra, GPC data, details of CPs, and turbidity measurements (PDF).

AUTHOR INFORMATION

Corresponding Authors

*E-mail: jingsun@qust.edu.cn.
*E-mail: zbli@qust.edu.cn.

ORCID

Jing Sun: 0000-0003-1267-0215
Zhibo Li: 0000-0001-9512-1507

Funding

This work was supported by National Natural Science Foundation of China (51503115, 21674054, 51722302, and

DOI: 10.1021/acs.biomac.8b00240
Biomacromolecules XXXX, XXX, XXX−XXX

21434008), Qingdao Innovation Leader Talent Program (third), and Taishan Scholars Program.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by National Natural Science Foundation of China (51503115, 21674054, 51722302, and 21434008), Qingdao Innovation Leader Talent Program (third), and Taishan Scholars Program.

■ REFERENCES

(1) Stuart, M. A.; Huck, W. T.; Genzer, J.; Müller, M.; Ober, C.; Stamm, M.; Sukhorukov, G. B.; Szleifer, I.; Tsukruk, V. V.; Urban, M. Emerging applications of stimuli-responsive polymer materials. *Nat. Mater.* **2010**, *9*, 101−113.

(2) Mura, S.; Nicolas, J.; Couvreur, P. Stimuli-responsive nanocarriers for drug delivery. *Nat. Mater.* **2013**, *12*, 991−1003.

(3) Roy, D.; Brooks, W. L.; Sumerlin, B. S. New directions in thermoresponsive polymers. *Chem. Soc. Rev.* **2013**, *42*, 7214−7243.

(4) Dimitrov, I.; Trzebicka, B.; Müller, A. H. E.; Dworak, A.; Tsvetanov, C. B. Thermosensitive water-soluble copolymers with doubly responsive reversibly interacting entities. *Prog. Polym. Sci.* **2007**, *32*, 1275−1343.

(5) Huang, J.; Heise, A. Stimuli responsive synthetic polypeptides derived from N-carboxyanhydride (NCA) polymerisation. *Chem. Soc. Rev.* **2013**, *42*, 7373.

(6) Lahasky, S. H.; Hu, X.; Zhang, D. Thermoresponsive Poly(α-peptoid)s: Tuning the Cloud Point Temperatures by Composition and Architecture. *ACS Macro Lett.* **2012**, *1*, 580−584.

(7) Xia, Y.; Yin, X.; Burke, N. A. D.; Stöver, H. D. H. Thermal Response of Narrow-Disperse Poly(N-isopropylacrylamide) Prepared by Atom Transfer Radical Polymerization. *Macromolecules* **2005**, *38*, 2275−2283.

(8) Vancoillie, G.; Frank, D.; Hoogenboom, R. Thermoresponsive poly(oligo ethylene glycol acrylates). *Prog. Polym. Sci.* **2014**, *39*, 1074−1095.

(9) Lutz, J. F.; Akdemir, Ö.; Hoth, A. Point by point comparison of two thermosensitive polymers exhibiting a similar LCST: is the age of poly(NIPAM) over? *J. Am. Chem. Soc.* **2006**, *128*, 13046−13047.

(10) Woodfield, P. A.; Zhu, Y.; Pei, Y.; Roth, P. J. Hydrophobically Modified Sulfobetaine Copolymers with Tunable Aqueous UCST through Postpolymerization Modification of Poly(pentafluorophenyl acrylate). *Macromolecules* **2014**, *47*, 750−762.

(11) Glatzel, S.; Laschewsky, A.; Lutz, J. F. Well-Defined Uncharged Polymers with a Sharp UCST in Water and in Physiological Milieu. *Macromolecules* **2011**, *44*, 413−415.

(12) Seuring, J.; Bayer, F. M.; Huber, K.; Agarwal, S. Upper Critical Solution Temperature of Poly(N-acryloyl glycinamide) in Water: A Concealed Property. *Macromolecules* **2013**, *45*, 374−384.

(13) Seuring, J.; Agarwal, S. First Example of a Universal and Cost-Effective Approach: Polymers with Tunable Upper Critical Solution Temperature in Water and Electrolyte Solution. *Macromolecules* **2012**, *45*, 3910−3918.

(14) Seuring, J.; Agarwal, S. Polymers with Upper Critical Solution Temperature in Aqueous Solution: Unexpected Properties from Known Building Blocks. *ACS Macro Lett.* **2013**, *2*, 597−600.

(15) Fu, W.; Luo, C.; Morin, E. A.; He, W.; Li, Z.; Zhao, B. UCST-Type Thermosensitive Hairy Nanogels Synthesized by RAFT Polymerization-Induced Self-Assembly. *ACS Macro Lett.* **2017**, *6*, 127−133.

(16) Shimada, N.; Ino, H.; Maie, K.; Nakayama, M.; Kano, A.; Maruyama, A. Ureido-derivatized polymers based on both poly(allylurea) and poly(L-citrulline) exhibit UCST-type phase transition behavior under physiologically relevant conditions. *Biomacromolecules* **2011**, *12*, 3418−3422.

(17) Zhu, Y.; Batchelor, R.; Lowe, A. B.; Roth, P. J. Design of Thermoresponsive Polymers with Aqueous LCST, UCST, or Both: Modification of a Reactive Poly(2-vinyl-4,4-dimethylazlactone) Scaffold. *Macromolecules* **2016**, *49*, 672−680.

(18) Wu, G.; Chen, S. C.; Zhan, Q.; Wang, Y. Z. Well-Defined Amphiphilic Biodegradable Comb-Like Graft Copolymers: Their Unique Architecture-Determined LCST and UCST Thermoresponsivity. *Macromolecules* **2011**, *44*, 999−1008.

(19) Sun, W.; An, Z.; Wu, P. UCST or LCST? Composition-Dependent Thermoresponsive Behavior of Poly(N-acryloylglycinamide-co-diacetone acrylamide). *Macromolecules* **2017**, *50*, 2175−2182.

(20) Boustta, M.; Colombo, P. E.; Lenglet, S.; Poujol, S.; Vert, M. Versatile UCST-based thermoresponsive hydrogels for loco-regional sustained drug delivery. *J. Controlled Release* **2014**, *174*, 1−6.

(21) Sun, J.; Zuckermann, R. N. Peptoid polymers: a highly designable bioinspired material. *ACS Nano* **2013**, *7*, 4715−4732.

(22) Klinker, K.; Schäfer, O.; Huesmann, D.; Bauer, T.; Capelôa, L.; Braun, L.; Stergiou, N.; Schinnerer, M.; Dirisala, A.; Miyata, K. Secondary Structure-Driven Self-Assembly of Reactive Polypept(o)ides: Controlling Size, Shape and Function of Core Cross-Linked Nanostructures. *Angew. Chem., Int. Ed.* **2017**, *56*, 9608−9613.

(23) Gangloff, N.; Ulbricht, J.; Lorson, T.; Schlaad, H.; Luxenhofer, R. Peptoids and Polypeptoids at the Frontier of Supra- and Macromolecular Engineering. *Chem. Rev.* **2016**, *116*, 1753.

(24) Knight, A. S.; Zhou, E. Y.; Francis, M. B.; Zuckermann, R. N. Sequence Programmable Peptoid Polymers for Diverse Materials Applications. *Adv. Mater.* **2015**, *27*, 5665.

(25) Zhang, D.; Lahasky, S. H.; Guo, L.; Lee, C. U.; Lavan, M. Polypeptoid Materials: Current Status and Future Perspectives. *Macromolecules* **2012**, *45*, 5833−5841.

(26) Fetsch, C.; Grossmann, A.; Holz, L.; Nawroth, J. F.; Luxenhofer, R. Polypeptoids from N-Substituted Glycine N-Carboxyanhydrides: Hydrophilic, Hydrophobic, and Amphiphilic Polymers with Poisson Distribution. *Macromolecules* **2011**, *44*, 6746−6758.

(27) Tao, X.; Du, J.; Wang, Y.; Ling, J. Polypeptoids with Tunable Cloud Point Temperatures Synthesized from N-Substituted Glycine N-Thiocarboxyanhydrides. *Polym. Chem.* **2015**, *6*, 3164−3174.

(28) Lahasky, S. H.; Serem, W. K.; Guo, L.; Garno, J. C.; Zhang, D. Synthesis and Characterization of Cyclic Brush-Like Polymers by N-Heterocyclic Carbene-Mediated Zwitterionic Polymerization of N-Propargyl N-Carboxyanhydride and the Grafting-to Approach. *Macromolecules* **2011**, *44*, 9063−9074.

(29) Secker, C.; Robinson, J. W.; Schlaad, H. Alkyne-X modification of polypeptoids. *Eur. Polym. J.* **2015**, *62*, 394−399.

(30) Tian, J. L.; Sun, J.; Li, Z. Biomimetic Pegylated polypeptoids with Thermoresponsive Properties. *Polymer* **2018**, *138*, 132−138.

(31) Robinson, J. W.; Secker, C.; Weidner, S.; Schlaad, H. Thermoresponsive Poly(N-C3 glycine)s. *Macromolecules* **2013**, *46*, 580−587.

(32) Guo, L.; Zhang, D. Cyclic poly(alpha-peptoid)s and their block copolymers from N-heterocyclic carbene-mediated ring-opening polymerizations of N-substituted N-carboxylanhydrides. *J. Am. Chem. Soc.* **2009**, *131*, 18072−18074.

(33) Murnen, H. K.; Rosales, A. M.; Jaworski, J. N.; Segalman, R. A.; Zuckermann, R. N. Hierarchical self-assembly of a biomimetic diblock copolypeptoid into homochiral superhelices. *J. Am. Chem. Soc.* **2010**, *132*, 16112−16119.

(34) Kukula, H.; Schlaad, H.; Antonietti, M.; Förster, S. The formation of polymer vesicles or "peptosomes" by polybutadiene-block-poly(L-glutamate)s in dilute aqueous solution. *J. Am. Chem. Soc.* **2002**, *124*, 1658−1663.

(35) Lutz, J. F.; Weichenhan, K.; Akdemir, Ö.; Hoth, A. About the Phase Transitions in Aqueous Solutions of Thermoresponsive Copolymers and Hydrogels Based on 2-(2-methoxyethoxy)ethyl Methacrylate and Oligo(ethylene glycol) Methacrylate. *Macromolecules* **2007**, *40*, 2503−2508.

(36) Takeshi, M.; Masashi, N.; Yasuhisa, F.; Keiji, M.; Masami, T.; Maeda, Y. Soluble−Insoluble−Soluble Transitions of Aqueous Poly-

DOI: 10.1021/acs.biomac.8b00240
Biomacromolecules XXXX, XXX, XXX−XXX

(N-vinylacetamide-co-acrylic acid) Solutions. *Langmuir* **2006**, *22*, 4336−4342.
(37) Du, P.; Li, A.; Li, X.; Zhang, Y.; Do, C.; He, L.; Rick, S. W.; John, V. T.; Kumar, R.; Zhang, D. Aggregation of cyclic polypeptoids bearing zwitterionic end-groups with attractive dipole-dipole and solvophobic interactions: a study by small-angle neutron scattering and molecular dynamics simulation. *Phys. Chem. Chem. Phys.* **2017**, *19*, 14388−14400.
(38) Ma, J.; Xuan, S.; Guerin, A. C.; Yu, T.; Zhang, D.; Kuroda, D. G. Unusual molecular mechanism behind the thermal response of polypeptoids in aqueous solutions. *Phys. Chem. Chem. Phys.* **2017**, *19*, 10878−10888.
(39) Nam, K. T.; Shelby, S. A.; Choi, P. H.; Marciel, A. B.; Chen, R.; Tan, L.; Chu, T. K.; Mesch, R. A.; Lee, B. C.; Connolly, M. D. Free-floating ultrathin two-dimensional crystals from sequence-specific peptoid polymers. *Nat. Mater.* **2010**, *9*, 454−460.
(40) Wang, Q.; Mynar, J. L.; Yoshida, M.; Lee, E.; Lee, M.; Okuro, K.; Kinbara, K.; Aida, T. High-water-content mouldable hydrogels by mixing clay and a dendritic molecular binder. *Nature* **2010**, *463*, 339−343.
(41) Tao, X.; Deng, Y.; Shen, Z.; Ling, J. Controlled Polymerization of N-Substituted Glycine N-Thiocarboxyanhydrides Initiated by Rare Earth Borohydrides toward Hydrophilic and Hydrophobic Polypeptoids. *Macromolecules* **2014**, *47*, 6173.

DOI: 10.1021/acs.biomac.8b00240
Biomacromolecules XXXX, XXX, XXX−XXX