Role of Side-Chain Molecular Features in Tuning Lower Critical Solution Temperatures (LCSTs) of Oligoethylene Glycol Modified Polypeptides

Eric G. Gharakhanian[a], Timothy J. Deming[a,b]*

[a] *Department of Chemistry and Biochemistry, University of California Los Angeles, Los Angeles CA 90095-1569, USA*

[b] *Department of Bioengineering, University of California Los Angeles, Los Angeles CA 90095-1600, USA*

* Corresponding author:

TJD: Department of Bioengineering, University of California Los Angeles, 5121 Engineering 5, Los Angeles CA 90095-1600, USA Tel. +1 310 267-4450

e-mail: demingt@seas.ucla.edu (T.J. Deming)

Abstract

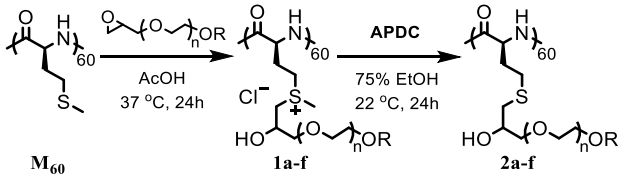
A series of thermoresponsive polypeptides has been synthesized using methodology that allowed facile adjustment of side-chain functional groups. The lower critical solution temperature (LCST) properties of these polymers in water were then evaluated relative to systematic molecular modifications in their side-chains. It was found that in addition to the number of ethylene glycol repeats in the side-chains, terminal and linker groups also have substantial, and predictable effects on cloud point temperatures (T_{cp}). In particular, we found that the structure of these polypeptides allowed for inclusion of polar hydroxyl groups, which significantly increased their hydrophilicity and decreased the need to use long oligoethylene glycol repeats to obtain LCSTs. The thioether linkages in these polypeptides were found to provide an additional structural feature for reversible switching of both polypeptide conformation and thermoresponsive properties.

Introduction

Polymers that respond to temperature in solution, especially in aqueous media, have received much attention for a variety of applications such as stimulus-responsive assemblies, and as materials for potential use in medicine.[1-3] Double hydrophilic block copolymers containing a thermoresponsive segment, i.e. possessing a lower critical solution temperature (LCST), are able to transform from solutions in water into hydrogels[4,5] or suspensions of nanoparticles[6,7] upon heating to above the LCST. In recent years there has been considerable development of new polymers that possess LCSTs in water, primarily based on repeats bearing short oligoethylene glycol (OEG) side-chains.[8] Initial efforts in this area focused on polymethacrylates and polyacrylates containing OEG side-chain groups,[9-12] and now this motif has been used to prepare other types of thermoresponsive polymers, such as OEG containing polypeptides.[13-23]

Thermoresponsive polypeptides are desirable compared to other polymers since they can degrade in living systems, which is advantageous for biological and medical applications. OEG containing thermoresponsive polypeptides have been prepared using a variety of methods, using different core amino acid residues, and also with a wide range in number of ethylene glycol (EG) repeats and means of their attachment to different residues.[13-23] While many thermoresponsive polypeptides have been described that possess LCSTs, there is limited understanding of how the molecular features of different side-chain structures affect solution properties. For most thermoresponsive polypeptides, LCST is mainly adjusted by variation of the number of side-chain EG repeats, with less attention given to the components of different linkages.[13-23] Hence, it can be difficult to understand the differences in thermoresponsive properties of OEG containing polypeptides prepared using different amino acids and side-chain linkages. Here, we have utilized a recently developed synthetic methodology that allows facile modification of polypeptide side-chains[24,25] to prepare a series of thermoresponsive OEG containing polypeptides, where the number of EG repeats, EG terminal groups, and linkage groups were all systematically varied in order to obtain insights on how these molecular features affect LCST behavior (Figure 1). Using this family of polypeptides with side-chain diversity, we show that adjustment of each of these molecular features can be used to predictably adjust LCST,

allowing for an understanding of how different molecular components influence polypeptide solution properties.



Sample	n	R	1 (% yield)	2 (% yield)
a	1	H	100	98
b	1	Me	97	80
c	1	Ac	94	97
d	2	Me	95	81
e	2	Et	92	99
f	3	Me	90	99

Figure 1. Synthesis of OEG functionalized polypeptides. $\mathbf{M_{60}}$ alkylated with OEG epoxides to provide sulfoniums, **1a-f**. Sulfoniums were demethylated to afford **OEG-Hcy**, **2a-f**. Yields are of isolated, fully functionalized polypeptides.

Experimental Section

Materials and Methods

Reactions at elevated temperature were controlled using a Corning PC 420D thermostated hotplate equipped with a thermocouple probe. AcOH (Fisher), Ac_2O (Fisher), CH_2Cl_2 (Fisher), 30% $H_2O_{2(aq)}$ (Fisher), TEA (Fisher), **APDC** (Acros), 70% mCPBA (Acros), 2-allyloxyethanol (Sigma-Aldrich), HCOOH (Sigma-Aldrich), OEG-monoalkyl ethers (Sigma-Aldrich) and epichlorohydrin (Alfa Aesar) were used as received. THF was degassed by sparging with N_2 and dried by passing through alumina columns. OEG-Epoxides, except 2-acetoxyethyl glycidyl ether (details below) have been previously reported and were prepared by known procedures.[24,26-28] 2-methoxyethyl chloroformate was prepared by a reported method.[29] Thin-layer chromatography was performed with EMD gel 60 F254 plates (0.25 mm thickness) and visualized using a UV lamp or permanganate stain. Silicycle Siliaflash G60 silica (60-200 μm) was used for flash chromatography. Dialysis was performed using deionized water (18.2 MΩ-cm), purified by passing in-house deionized water through a Millipore Milli-Q Biocel A10 unit. Regenerated cellulose dialysis tubing obtained from Spectrum Labs. Cloud point

temperature measurements were recorded on an HP 8453 spectrophotometer equipped with an Agilent 8909A temperature controller. CD spectra were recorded on either an Olis DSM 10 spectrophotometer or a JASCO J-715 spectrophotometer, using a 0.1 cm path length quartz cell. Elevated temperature CD spectra were obtained on the JASCO J-715 spectrophotometer using a water-jacketed cuvette holder heated by a PolyScience circulator. NMR spectra were recorded on a Bruker AV400 instrument with chemical shifts reported relative to residual solvent signal. ESI-MS was performed using a Waters LCT Premier spectrometer. IR spectra were recorded on a JASCO FT/IR-4100 spectrometer. Abbreviations: glacial acetic acid (AcOH), acetic anhydride (Ac_2O), Ammonium pyrrolidinedithiocarbamate (**APDC**), deuterated trifluoroacetic acid (d-TFA), ethyl acetate (EtOAc), hexafluoroisopropanol (HFiP), *meta*-chloroperoxybenzoic acid (mCPBA), pyridine (py), tetrahydrofuran (THF) and trimethylamine (TEA).

General Synthetic Procedures

Poly(L-methionine)$_{60}$, $\mathbf{M_{60}}$

Prepared by our previously reported method.[24] L-Methionine N-carboxyanhydride, L-Met NCA was polymerized with $Co(PMe_3)_4$ using a 20:1, monomer to initiator ratio.[30,31] The degree of polymerization (DP) was determined by endcapping a small aliquot from the polymerization mixture with 2 kDa PEG-isocyanate ($CH_3(OCH_2CH_2)_{45}N=C=O$) followed by ^{1}H NMR analysis.[24] Found DP = 58, designated as $\mathbf{M_{60}}$.

Poly(DL-methionine)$_{60}$, *rac*-$\mathbf{M_{60}}$

Prepared analogously to $\mathbf{M_{60}}$ using DL-Met NCA. Found DP = 56, designated as ***rac*-$\mathbf{M_{60}}$**. ^{1}H NMR (400 MHz, d-TFA, 25 ^{o}C): 4.81 (m, 1H), 2.64 (m, 2H), 2.36-1.89 (br m, 5H).

2-acetoxyethyl glycidyl ether

A solution of 2-(allyloxy)ethyl acetate[32] (1.0 g, 6.9 mmol, 1 eq) in CH_2Cl_2 (25 mL) was cooled on an ice bath. mCPBA (2.6 g, 10.4 mmol, 1.5 eq) was added in one portion. The mixture was allowed to warm to room temperature and stirred for 48 h. The reaction was quenched on an ice bath with 10% Na_2SO_3 (13 mL) and Na_2CO_3 (11 mL). The mixture was stirred for 5 min and transferred to a separatory funnel using EtOAc (30 mL) to

complete the transfer. The organic phase was partitioned, and washed with sat. aqueous $NaHCO_3$ (30 mL) followed by brine (30 mL). The organic extract was dried over Na_2SO_4 and concentrated by rotary evaporation. The residue was purified by flash chromatography (50% EtOAc/hexanes) to provide 2-acetoxyethyl glycidyl ether (0.79 g, 71 % yield) as a colorless liquid. R_F = 0.40; 50% EtOAc/Hexanes.

^{1}H NMR (400 MHz, $CDCl_3$, 25 ^{o}C): 4.23 (t, *J* = 4.9 Hz, 2H), 3.82 (dd, *J* = 11.7, 2.9 Hz, 1H), 3.17 (m, 2H), 3.43 (dd, *J* = 11.7, 6.0 Hz, 1H), 3.16 (m, 1H), 2.80 (dd, *J* = 5.0, 4.2 Hz, 1H), 2.61 (dd, *J* = 5.0, 2.7 Hz, 1H), 2.09 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 ^{o}C): δ 170.9, 71.8, 69.2, 50.7, 44.0, 20.8. ESI-MS *m/z* = 182.9794 $[M+Na]^+$ (calcd 183.0633 for $C_7H_{12}O_4Na$).

M_{60} Alkylation

M_{60} was alkylated with OEG-epoxides (3 eq per Met residue) in AcOH at 37 °C, by our previously reported method, to provide **1a-f** and **4a**.[24]

M_{60} Sulfonium Demethylation

M_{60} sulfonium derivatives (**1a-f, 4a**) were demethylated with **APDC** (5 eq per sulfonium residue) in 75% EtOH according to our previously reported procedure.[25]

Modification of OEG-HCy Polymers

Poly(*S*-(2-acetoxy-4,7,10,13-tetraoxatetradecyl)-L-homocysteine), 3a

A solution of **2f** (6.0 mg, 0.020 mmol OH-groups, 1 eq) in THF (0.50 mL) was treated with Ac_2O (19 µL, 0.20 mmol, 10 eq) followed by TEA (28 µL, 0.20 mmol, 10 eq). The mixture was allowed to stand 20 h at 22 ^{o}C. The reaction mixture was transferred to a 2 kDa MWCO dialysis bag and dialyzed against H_2O (48 h, 6 H_2O changes). The retentate lyophilized, to provide **3a** (6.1 mg, 90% yield).

^{1}H NMR (400 MHz, D_2O, 25 ^{o}C): 5.37-5.03 (br m, 1H), 4.48-4.11 (br m, 1H), 4.11-3.49 (br m, 14H), 3.41 (s, 3H), 3.18-2.46 (br m, 4H), 2.46-1.81 (br m, 5H).

Poly(*S*-(2-(((2-methoxyethoxy)carbonyl)oxy)-4,7,10,13-tetraoxatetradecyl)-L-homocysteine), 3b

A solution of **2f** (6.0 mg, 0.020 mmol OH-groups, 1 eq) in THF (0.50 mL) was treated with 2-methoxyethyl chloroformate (24 μL, 0.20 mmol, 10 eq) followed by pyridine (17 μL, 0.20 mmol, 10 eq). The product was purified and isolated analogously to **3a**, to provide **3b** (7.7 mg, 99% yield).
^{1}H NMR (400 MHz, D_2O, 25 ^oC): 5.20-4.91 (br m, 1H), 4.56-4.08 (br m, 3H), 4.05-3.19 (br m, 22H), 3.11-2.58 (m, 6H), 3.11-2.58 (br m, 4H), 2.47-1.99 (br m, 2H).

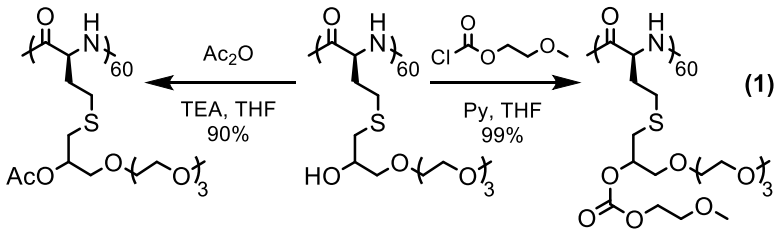
Poly(*S*-(2-hydroxy-4,7-dioxaoctyl)-L-homocysteine sulfoxide), 5a

2b (9.5 mg, 0.038 mmol thioether groups, 1 eq) was dissolved in HFiP (0.75 mL). The solution was treated with 30% aqueous H_2O_2 (11 μL, 0.10 mmol, 2.8 eq), vortexed briefly and allowed to stand for 16 h. The reaction mixture was quenched with 10% Na_2SO_3 (75 μL), transferred to a 2 kDa MWCO dialysis bag and dialyzed against H_2O (24 h, 4 H_2O changes). The retentate lyophilized, to provide **5a** (9.6 mg, 95% yield).
^{1}H NMR (400 MHz, D_2O, 25 ^oC): 4.59-4.19 (br m, 2H), 3.94-3.18 (br m, 13H), 2.75-2.26 (br m 2H). ATR-FTIR: 1650, 1542, 1100, 1033 cm^{-1}.

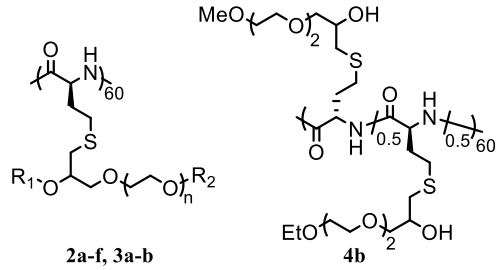
Poly(*S*-(2-hydroxy-4,7-dioxaoctyl)-L-homocysteine sulfone), 5b

2b (10.1 mg, 0.041 mmol thioether groups, 1 eq) was suspended in HCOOH (0.50 mL). The mixture was cooled to 8 ^oC and treated with 30% aqueous H_2O_2 (19 μL, 0.19 mmol, 5 eq), then allowed to stir at room temp for 16 h. The reaction mixture was quenched with 10% aqueous $NaHSO_3$ (0.1 mL), transferred to a 2 kDa MWCO dialysis bag and dialyzed against 3 mM aqueous HCl (48 h, 6 H_2O changes) followed by H_2O (24 h, 3 H_2O changes). The retentate lyophilized, to provide **5b** (10.9 mg, 96% yield).
^{1}H NMR (400 MHz, D_2O, 25 ^oC): 4.60-4.49 (br m, 1H), 4.38-4.25 (br m, 1H), 3.90-3.27 (br m, 9H), 3.27-2.93 (br m, 4H), 2.50-2.10 (br m, 2H). ATR-FTIR: 1651, 1550, 1284, 1115 cm^{-1}.

Results and Discussion



A series of poly(OEG-alkylated-L-homocysteine)$_{60}$ derivatives, **OEG-Hcy** (**2a-2f**) were prepared using a recently developed two-step process from poly(L-methionine)$_{60}$, $\mathbf{M_{60}}$, via its alkylation using functionalized epoxides in acetic acid, followed by demethylation using ammonium pyrrolidine dithiocarbamate (**APDC**) (Figure 1).[24,25] This methodology allowed us to rapidly and efficiently synthesize a systematic series of OEG functionalized polypeptides, which contained an unprecedented level of side-chain diversity (Figure 1). In these samples, the number of EG repeats was varied from 1 to 3, and the EG terminal groups were also varied to include H, Ac, Me, and Et. To further increase diversity, samples of polypeptide **2f** were modified at the hydroxyl groups in the linker between EG and amino acid into acetate (**3a**) and 2-methoxyethylcarbonate (**3b**) derivatives (eq 1). An equimolar statistical copolymer of **2d** and **2e** (**4b**, see SI eq S1) was also prepared for analysis. All of the non-ionic **OEG-Hcy** samples described above were found to adopt predominantly α-helical conformations in deionized water at 22 °C (except for water insoluble **2c**, which was measured in MeOH at 22 °C), as determined by circular dichroism (CD) spectroscopy (see SI Figure S1)



Polypeptide	n	R_1	R_2	T_{cp} (°C)
2a	1	H	H	--[a]
2b	1	H	Me	33
2c	1	H	Ac	NA[b]
2d	2	H	Me	53
2e	2	H	Et	28
2f	3	H	Me	76
3a	3	Ac	Me	39
3b	3	EC[c]	Me	41
4b	2	H	Me/Et[d]	40

Figure 2. Cloud point temperatures (T_{cp}) of **OEG-Hcy** polypeptides. T_{cp} determined by heating polymer samples (3.0 mg/mL) at a rate of 1 °C/min while recording transmittance (500 nm). T_{cp} was the temperature where 50 % transmittance was observed. a) No T_{cp} detected, polymer fully soluble from 20 to 80 °C. b) not applicable, polymer insoluble in H_2O down to 5 °C. c) EC = ($CH_3OCH_2CH_2OC(O)$-). d) equimolar statistical copolymer.

We envisioned that most of these **OEG-Hcy** derivatives would be thermoresponsive similar to OEG containing polypeptides reported by ourselves and other workers.[13-23] The significance of this new series of polymers is the incorporation of precise side-chain structural modifications, which enable systematic study of the effects of different functionalities on the properties of the materials. Figure 2 shows the results obtained from analysis of aqueous solutions of all the different **OEG-Hcy** homopolymers at concentrations of 3.0 mg/ml. Cloud point temperatures (T_{cp}) were determined at 50% transmittance by monitoring solution transmittance as a function of temperature, and were used to approximate the equilibrium LCST values.[19] Since chain length variation and polymer concentration are well known to affect T_{cp} values,[1-3,8] all samples were identical in length, being prepared from the same stock of $\mathbf{M_{60}}$.

As can be seen in Figure 2, all samples, except fully water soluble **2a** and water insoluble **2c**, showed a T_{cp} in water, which varied widely depending on number of EG repeats, as well as the nature of both the terminal and linker groups. These T_{cp} were found to be reversible with minimal hysteresis, and polymers remained α-helical above T_{cp} (see SI Figure S2). To study the effect of salts on T_{cp}, solutions of **2e** were examined in the presence of different Hofmeister anions. Anions were varied since they are known to have more substantial effects on polymer thermoresponsive properties compared to cations (Figure 3).[33] Different salt concentrations affected the cloud point temperatures of polymer **2e** in ways similar to observations made on other thermoresponsive polymer systems,[19,33,34] and allow for predictable tuning of T_{cp} in different ionic media. Note that none of the polycationic precursor polymers **1a**-**1f** showed a T_{cp} in water, and were fully water soluble due to their polyelectrolyte nature.[24]

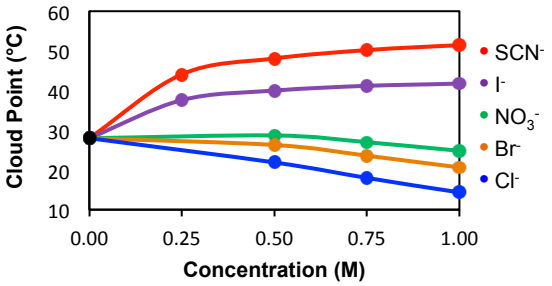


Figure 3. Cloud point temperatures (T_{cp}) for **2e** (3.0 mg/mL) in aqueous solutions containing different concentrations of Hofmeister salts (counterion = Na^+).

In order to better understand the origins of the differences in T_{cp} values for the samples in Figure 2, we examined trends in T_{cp} as individual molecular features were varied. Samples **2b**, **2d**, and **2f**, differ only in that the number of side-chain EG repeats increased from 1 to 3, which resulted in commensurate increases in T_{cp} of *ca*. 20 °C per EG residue (Figure 4a), similar to the increases observed in OEG containing polymethacrylates.[9-12] Variation in number of EG repeats is the most common method used to adjust T_{cp}, since OEG units enhance water solubility at lower temperatures via H-bonding interactions with solvent that favor mixing, but these H-bonding interactions are disrupted at elevated temperatures, resulting in an LCST.[8] Beyond variation of EG repeats, we observed that the nature of linker and EG terminal groups, R_1 and R_2 from Figure 2, respectively, also had significant effects on T_{cp}.

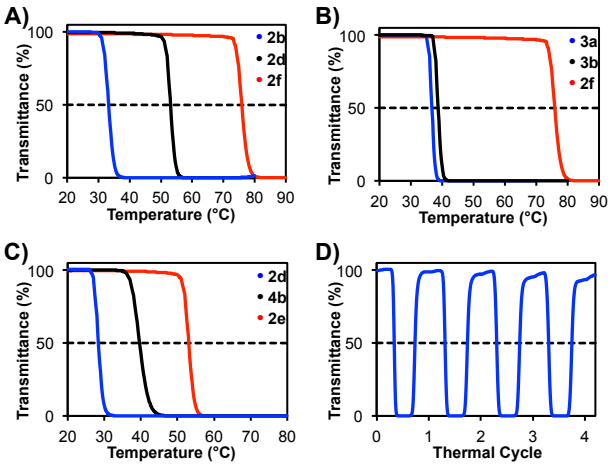


Figure 4. (A) Heating curves for methyl terminated **OEG-Hcy** polymers as number of OEG repeats increased from 1 (**2b**) to 2 (**2d**) to 3 (**2f**). (B) Change in T_{cp} upon conversion

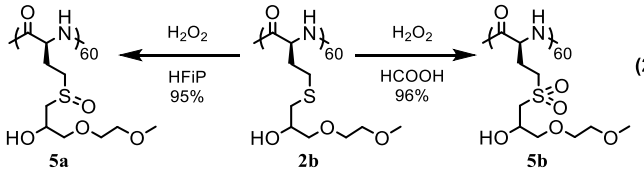
of the side-chain alcohol in **2f** to the acetate ester (**3a**) or 2-methoxyethyl carbonate (**3b**). (C) Comparison of methyl (**2d**) and ethyl (**2e**) terminated **OEG-Hcy** polypeptides with the equimolar Me/Et terminated statistical copolymer (**4b**). (D) Reversibility of thermal transition of **2b** with repeated cycling between 15 and 45 °C. All measurements performed with polypeptide (3.0 mg/mL) in H_2O with heating or cooling rates of 1 °C/min (2 °C/min for panel D).

Samples with different linker groups (R_1), which included hydroxyl (**2f**), acetate (**3a**) and 2-methoxyethylcarbonate (**3b**), were also found to possess a range of T_{cp} values (Figure 4b). Both carbonate and ester functionalities were found to greatly lower T_{cp} compared to the parent hydroxyl group, which has much greater ability to H-bond, both as donor and acceptor, to water solvent. The similarity in T_{cp} between **3a** and **3b** may be explained by the higher polarity of the ester group being counterbalanced by a less polar carbonate that also includes a solubilizing EG group. This series of samples shows that the hydroxyl group in the linker of **2f** provides a substantial enhancement in water solubility as evidenced by the increase in T_{cp} of *ca*. 36 °C over the other samples. Polar hydroxyl groups have been introduced previously in thermoresponsive statistical copolymers as a means to increase T_{cp}.[9,12,15] However, no other homopolymers with hydroxyl groups in each side-chain are known to possess an LCST in water, as high hydroxyl group density typically results in chains being fully soluble in water regardless of temperature.[9,12,15] The unique localization of hydroxyl groups in our samples within the linker region, as opposed to the side-chain terminus, may be the reason why hydroxyl containing **OEG-Hcy** polypeptides possess LCSTs. Supporting this hypothesis, sample **2a**, which contains an additional hydroxyl group at the side-chain terminus, was found to be fully water soluble with no LCST (Figure 2).

The effect of the EG terminal groups (R_2) on T_{cp} was also studied with samples **2d**, **2e**, and **4b**, where R_2 was either Me, Et, or a 1:1 statistical mixture of Me and Et. As the groups became more hydrophobic, the polymers became less water soluble, and T_{cp} values decreased (Figure 4c). The statistically grafted copolymer **4b** showed that terminal groups can be mixed to obtain a single, reversible transition at an intermediate T_{cp} value. Slight broadening of the thermal transition for this statistical copolymer compared to the

homopolymers may be due to small differences in comonomer distribution among individual copolymer chains. Physical blends were also prepared of sample **2e** with **2d** or **2f**, which upon heating showed the presence of distinct T_{cp} for each polymer component (see SI Figure S3).[35] These data suggest that statistical functionalization of individual chains is necessary to obtain a single, average T_{cp}, while physical blending retains the characteristics of the individual components. These principles are potentially useful for fine adjustment of T_{cp} values, as well as preparation of sequentially thermoresponsive blends and block copolymers.[35]

The above data show that **OEG-Hcy** polymers are a robust platform whose thermoresponsive properties can be adjusted predictably through variation of three distinct side-chain molecular features. Another important structural characteristic of **OEG-Hcy** polymers is their stable α-helical conformation, also found in other thermoresponsive polypeptides,[13,19] which allows for sharp thermal transitions with excellent reversibility over many heating/cooling cycles (Figure 4d). Thermoresponsive polypeptides with disordered or less stable α-helical conformations can adopt β-sheet conformations above T_{cp}, which leads to irreversible phase separation of the polymers.[13,17,20] The α-helical conformations of **OEG-Hcy** are also an important reason why these polymers possess lower LCSTs with fewer EG repeats compared to disordered polypeptides. The lack of conformational freedom in the α-helical chains leads to small entropy of mixing with water, which facilitates their phase separation at lower temperatures. By comparison, analogs of α-helical thermoresponsive polypeptides that possess disordered conformations, which have much greater entropy of mixing with water, are fully water soluble and do not have LCSTs.[13] Similar behavior was also observed here for a sample of **2b** prepared from racemic poly(DL-methionine) (i.e. ***rac*-2b**, see SI Figures S1 and S4).



(2)

Since chain conformations of **OEG-Hcy** polymers affect whether or not they have LCSTs in water, we utilized oxidation of the thioether linkages in these polymers as a means to alter both chain conformation and side-chain polarity (eq 2). As we previously

reported,[19,36,37] oxidation of thioether groups in poly(alkyl-L-homocysteine)s to sulfoxides results in a transition from α-helical to disordered conformations, and further oxidation to sulfones results in reversion to stable α-helical conformations. As shown by example with **2b**, these oxidation induced conformational changes, as measured using CD spectroscopy, also occur in the **OEG-Hcy** polypeptides (Figure 5a). Examination of the water solubility for the sulfoxide (**5a**) and sulfone (**5b**) derivatives of **2b** as a function of temperature showed that both have good solubility and neither polymer has a LCST (Figure 5b). The disordered conformation of **5a** likely improves solubility of this sample compared to **2b**, however the increased polarity of both the sulfoxide and sulfone groups in **5a** and **5b** also significantly increases their water solubility, such that the helicity of **5b** does not lead to recovery of an LCST. Overall, oxidation of thioether groups in **OEG-Hcy** polymers is an effective means to switch off their LCST properties. Since sulfoxides can also be reduced back to thioether groups under mild conditions,[19,36,37] interconversion between these two states can be envisioned as a means to reversibly switch **OEG-Hcy** polymers between thermoresponsive and fully water soluble states.

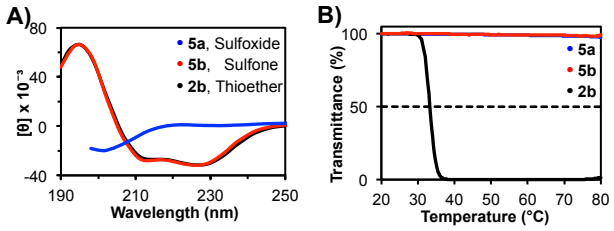


Figure 5. Effects of sulfur oxidation on chain conformation and thermoresponsive behavior of **OEG-Hcy** derivatives. (A) CD spectrum of thioether (**2b**) shows an α-helical conformation. Oxidation to the sulfoxide (**5a**) shows a disordered conformation, and further oxidation to the sulfone (**5b**) restores the α-helical conformation. All data were recorded in H_2O at 0.5 mg/mL, 20 °C. For **5b** no data were recorded below 198 nm due to sulfoxide absorption. **2b** and **5b** were found to be 84% and 86% α-helical, respectively. (**B**) **2b** shows a T_{cp} in water, but the more hydrophilic (**5a, 5b**) and disordered (**5a**) derivatives do not.

Conclusions

A series of new thermoresponsive polypeptides with systematic side-chain diversity has been reported and their LCST properties were evaluated relative to different molecular

modifications in their side-chains. In addition to LCST adjustment due to variation of EG repeats, we found that terminal and linker groups can also have substantial, and predictable effects on T_{cp}. In particular, we have found that **OEG-Hcy** structures allowed for inclusion of polar hydroxyl groups into homopolymers, which significantly increased their hydrophilicity and decreased the need to use long OEG repeats to obtain LCSTs. The thioether linkages in these polypeptides provided an additional structural feature for reversible switching of both polypeptide conformation and thermoresponsive properties. Overall, these **OEG-Hcy** polymers possess a number of side-chain molecular features that can be readily incorporated and manipulated to adjust T_{cp} and chain conformation, thus making this system attractive for applications requiring highly tunable thermoresponsive polymers.

Acknowledgements

This work was supported by the NSF under MSN 1412367.

Supporting Information Available.

CD spectra and NMR data for all new compounds, and additional figures and equations.

References

(1) Roy, D.; Cambre, J. N.; Sumerlin, B. S. Future perspectives and recent advances in stimuli-responsive materials. *Prog. Polym. Sci.* **2010**, *35*, 278-301.

(2) Jochum, F. D.; Theato, P. Temperature- and light-responsive smart polymer materials. *Chem. Soc. Rev.* **2013**, *42*, 7468-7483.

(3) Shen, Y.; Fu, X.; Fu, W.; Li, Z. Biodegradable stimuli-responsive polypeptide materials prepared by ring opening polymerization. *Chem. Soc. Rev.* **2015**, *44*, 612-622.

(4) Park, M. H.; Joo, M. K.; Choi, B. G.; Jeong, B. Biodegradable thermogels. *Acc. Chem. Res.* **2012**, *45*, 424–433.

(5) Chassenieux, C.; Tsitsilianis, C. Recent trends in pH/thermo-responsive self-assembling hydrogels: from polyions to peptide-based polymeric gelators. *Soft Matter* **2016**, *12*, 1344-1359.

(6) Randolph, L. M.; Chien, M.-P.; Gianneschi, N. C. Biological stimuli and biomolecules in the assembly and manipulation of nanoscale polymeric particles. *Chem. Sci.* **2012**, *3*, 1363-1380.

(7) Elsabahy, M.; Wooley, K. L. Design of polymeric nanoparticles for biomedical delivery applications. *Chem. Soc. Rev.* **2012**, *41*, 2545-2561.

(8) Lutz, J.-F. Polymerization of oligo(ethylene glycol) (meth)acrylates: Toward new generations of smart biocompatible materials. *J. Polym. Sci. A Polym. Chem.* **2008**, *46*, 3459-3470.

(9) Han, S.; Hagiwara, M.; Ishizone, T. Synthesis of thermally sensitive water-soluble polymethacrylates by living anionic polymerizations of oligo(ethylene glycol) methyl ether methacrylates. *Macromolecules* **2003**, *26*, 8312–8319.

(10) Zhao, B.; Li, D.; Hua, F.; Green, D. R. Synthesis of thermosensitive water-soluble polystyrenics with pendant methoxyoligo(ethylene glycol) groups by nitroxide-mediated radical polymerization. *Macromolecules* **2005**, *38*, 9509–9517.

(11) Lutz, J.-F.; Hoth, A. Preparation of ideal PEG analogues with a tunable thermosensitivity by controlled radical copolymerization of 2-(2-methoxyethoxy)ethyl methacrylate and oligo(ethylene glycol) methacrylate. *Macromolecules* **2006**, *39*, 893–896.

(12) Laloyaux, X.; Fautre, E.; Blin, T.; Purohit, V.; Leprince, J.; Jouenne, T.; Jonas, A.M.; Glinel, K. Temperature-responsive polymer brushes switching from bactericidal to cell-repellent. *Adv. Mater.* **2010**, *22*, 5024-5028.

(13) Chen, C.; Wang, Z.; Li, Z. Thermoresponsive polypeptides from pegylated poly-L-glutamates. *Biomacromolecules* **2011**, *12*, 2859-2863.

(14) Cheng, Y.; He, C.; Xiao, C.; Ding, J.; Zhuang, X.; Chen, X. Versatile synthesis of temperature-sensitive polypeptides by click grafting of oligo(ethylene glycol). *Polym. Chem.* **2011**, *2*, 2627-2634.

(15) Chopko, C. M.; Lowden, E. L.; Engler, A. C.; Griffith, L. G.; Hammond, P. T. Dual responsiveness of a tunable thermo-sensitive polypeptide. *ACS Macro Lett.* **2012**, *1*, 727-731.

(16) Shen, J.; Chen, C.; Fu, W.; Shi, L.; Li, Z. Conformation-specific self-assembly of thermo-responsive poly(ethylene glycol)-*b*-polypeptide diblock copolymer. *Langmuir* **2013**, *29*, 6271–6278.

(17) Fu, X.; Shen, Y.; Fu, W.; Li, Z. Thermoresponsive oligo(ethylene glycol) functionalized poly-L-cysteine. *Macromolecules* **2013**, *46,* 3753–3760.

(18) Zhang, S.; Fu, W.; Li, Z. Supramolecular hydrogels assembled from nonionic poly(ethylene glycol)-*b*-polypeptide diblocks containing OEGylated poly-L-glutamate. *Polym. Chem.* **2014**, *5*, 3346–3351.

(19) Kramer, J. R.; Deming, T. J. Multimodal switching of conformation and solubility in homocysteine derived polypeptides. *J. Amer. Chem. Soc.* **2014,** *136*, 5547–5550.

(20) Ma, Y.; Fu, X.; Shen, Y.; Fu, W.; Li, Z. Irreversible low critical solution temperature behaviors of thermal-responsive OEGylated poly(l-cysteine) containing disulfide bonds. *Macromolecules* **2014**, *47*, 4684–4689.

(21) Kapetanakis, A. Heise, A. Thermoresponsive glycopolypeptides with temperature controlled selective lectin binding properties. *Eur. Polym. J.* **2015,** *69*, 483-489.

(22) Yan, J.; Liu, K.; Zhang, X.; Zhang, A. Dynamic covalent polypeptides showing tunable secondary structures and thermoresponsiveness. *J. Polym. Sci. A Polym. Chem.* **2015**, *53*, 33-41.

(23) Zhang, S.; Alvarez, D. J.; Sofroniew, M. V.; Deming, T. J. Design and synthesis of nonionic copolypeptide hydrogels with reversible thermoresponsive and tunable physical properties. *Biomacromolecules* **2015**, *16*, 1331-1340.

(24) Gharakhanian, E. G.; Deming, T. J. Versatile synthesis of stable, functional polypeptides via reaction with epoxides. *Biomacromolecules* **2015**, *16*, 1802-1806.

(25) Gharakhanian, E. G.; Deming, T. J. Chemoselective synthesis of functional homocysteine residues in polypeptides and peptides. *Chem. Commun.* **2016**, *52*, 5336-5339.

(26) Kharasch, M. S.; Nudenberg, W. Condensation of epichlorohydrin with ethylene glycol: some new polyfunctional derivatives. *J. Org. Chem.* **1943**, *8*, 189-193.

(27) Jungk, J. S.; Moore, J. A.; Gandour, R. D. Efficient synthesis of C-pivot lariat ethers. 2-(Alkoxymethyl)-1,4,7,10,13,16-hexaoxacyclooctadecanes. *J. Org. Chem.* **1983**, *48*, 1116-1120.

(28) Toan, V. V.; Valet, A.; Hayoz, P. Polyoxyalkylene substituted and bridged triazine, benzotriazole and benzophenone derivatives as UV absorbers. U.S. Patent 6,369,267 B1, April 9, 2002.

(29) Ashburn, H. G.; Collett, A. R.; Lazzell, C. L. β-Alkoxy ethyl esters of chlorocarbonic and carbamic acids. *J. Am. Chem. Soc.* **1938**, *60*, 2933-2934.

(30) Deming, T. J. Facile synthesis of block copolypeptides of defined architecture. *Nature* **1997**, *390*, 386-389.

(31) Deming, T. J. Cobalt and iron initiators for the controlled polymerization of α-amino acid-N-carboxyanhydrides. *Macromolecules* **1999**, *32*, 4500-4502.

(32) Harding, J. S.; Owen, L. N. Dithiols. Part XIV. The alkaline hydrolysis of acetylated non-vicinal hydroxy-thiols. *J. Chem. Soc.* **1954**, 1536-1545.

(33) Zhang, Y.; Furyk, S.; Bergbreiter, D. E.; Cremer, P. S. Specific ion effects on the water solubility of macromolecules: PNIPAM and the Hofmeister series. *J. Amer. Chem. Soc.* **2005**, *127*, 14505-14510.

(34) Deyerle, B. A.; Zhang, Y. Effects of Hofmeister anions on the aggregation behavior of PEO-PPO-PEO triblock copolymers. *Langmuir* **2011**, *27*, 9203-9210.

(35) Karesoja, M.; Karjalainen, E.; Hietala, S.; Tenhu. H. Phase separation of aqueous poly(2-dimethylaminoethyl methacrylate-block-N-vinylcaprolactams). *J. Phys. Chem. B* **2014**, *118*, 10776-10784.

(36) Rodriguez, A. R.; Kramer, J. R.; Deming, T. J. Enzyme-triggered cargo release from readily prepared copolypeptide vesicles. *Biomacromolecules* **2013**, *14*, 3610-3614.

(37) Kramer, J. R.; Deming, T. J. Glycopolypeptides with a redox triggered helix to coil transition. *J. Amer. Chem. Soc.* **2012**, *134*, 4112-4115.

For Table of Contents use only