Macromolecular Bioscience
www.mbs-journal.de

# Tunable, Functional Diblock Copolypeptide Hydrogels Based on Methionine Homologs

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**The preparation of new diblock copolypeptide hydrogels derived from homologs of L-methionine, that is, L-homomethionine and L-6-(methylthio)-L-norleucine is described. Compared to L-methionine residues, use of L-methionine homologs allow improved copolymerization with L-leucine residues to give well-defined block copolypeptides. These copolypeptides are subsequently modified using robust thioether alkylation reactions employing a variety of functional epoxides, which yield samples capable of forming transparent, self-healing hydrogels in water. The facile variation of different functional epoxides for postpolymerization modification is found to allow predictable functionalization and tuning of hydrogel properties by the modification of simple precursors.**

## 1. Introduction

There is a need for mechanically tunable, degradable hydrogels that contain diverse functionality for applications as carriers for drug delivery and as scaffolds for tissue repair.[1–3] Our group has developed a family of diblock copolypeptide hydrogels (DCH) to address these needs,[4] and have reported formulations that can be charged,[5] non-ionic,[6] or thermoresponsive[7] with tunable mechanical properties. Hydrophobically assembled DCH contain long, hydrophilic segments with disordered conformations, and shorter hydrophobic segments with either $\alpha$-helical or $\beta$-sheet ordered conformations, for example, poly(L-lysine-HCl)$_{180}$-*block*-poly(L-leucine)$_{20}$, $K_{180}L_{20}$, where the conformation directed assembly of ordered hydrophobic segments in water forms tape-like assemblies that branch and entangle to give 3D hydrogel networks.[4,5] However, the functionality of most DCH formulations are based on the specific amino acid monomers used in their preparation, for example, lysine or glutamic acid,[5] requiring use of different monomers to prepare DCH with different functionality. It would be useful to have a means to readily introduce different chemical functionalities into DCH using diblock copolypeptides based on a small set of amino acid monomers.

The objective of this study was the preparation of diblock copolypeptides that utilize homologs of L-methionine as precursors to a variety of different functional DCH (**Scheme 1**).[8] L-Methionine residues are emerging as versatile components of biomimetic polypeptide materials since they can be readily modified in different ways with both high selectivity and high conversion.[9,10] These modifications also mimic natural L-methionine biochemical processes, that is, oxidation and alkylation reactions.[10] For example, L-methionine sulfoxide residues in proteins are a natural product of oxidative stress within cells and tissues,[11] and hydrophobic poly(L-methionine), M, can likewise be oxidized to give non-ionic, hydrophilic, and minimally toxic poly(L-methionine sulfoxide), $M^O$.[12] Alkylation of L-methionine residues using a variety of reagents also mimics the natural products S-methyl-L-methionine and S-adenosyl-L-methionine,[13,14] and provides a means to convert hydrophobic L-methionine to hydrophilic poly(S-alkyl-L-methionine sulfonium), $M^R$, functionalized polypeptides.[10] Our group has developed and utilized these methods to prepare L-methionine-based functional copolypeptide vesicles[15] and hydrogels.[6]

The preparation of L-methionine-based DCH was recently reported, where L-methionine residues were either oxidized to non-ionic sulfoxides, or alkylated to give methyl sulfonium derivatives.[6] One challenge in the development of these materials was copolypeptide synthesis, where the long M segments required for hydrogel formation were found to aggregate during polymerization, which in turn compromised the addition of the hydrophobic poly(L-leucine), L, blocks. Consequently, the resulting modified copolypeptides, $M^O{}_{170}L_{23}$ and $M^R{}_{170}L_{24}$ were unable to form good hydrogels.[6] Our solution was to incorporate ≈10 mol% L-alanine residues during methionine polymerization, giving poly(L-methionine-*stat*-L-alanine) segments that did not aggregate, thus facilitating copolypeptide synthesis, for example, $(M^OA)_{170}L_{24}$ and $(M^RA)_{170}L_{23}$, and enabling DCH formation.[6]

Here, we report an alternative strategy utilizing L-methionine homologs instead of L-methionine for preparation of DCH. The advantage of these homologs, L-homomethionine and L-6-(methylthio)-L-norleucine (Scheme 1), is that they can be used to prepare long homopolypeptide segments without aggregation,[8] eliminating the need to incorporate a

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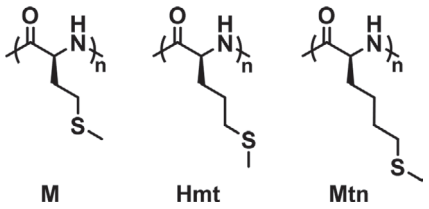
**DOI: 10.1002/mabi.201900243**




**Scheme 1.** Structures of homologous thioether containing polypeptides: poly(L-methionine), M; poly(L-homomethionine), Hmt; and poly(6-(methylthio)-L-norleucine), Mtn.

comonomer such as L-alanine. We also wanted to evaluate if these homologs provide a means to adjust DCH properties via their differences in side-chain length. These homologs possess the same thioether functionality found in L-methionine, and so it was expected they could be modified using the same alkylation or oxidation methods.[10] To form DCH, the thioether groups in L-homomethionine and L-6-(methylthio)-L-norleucine copolypeptides were alkylated using a variety of epoxides, giving the corresponding sulfonium derivatives bearing different functional groups.[9] We have studied the ability of these copolypeptides to form hydrogels, and the effects of side-chain length and epoxide functionality on hydrogel properties. These results provide a starting platform for the development of self-healing, tunable copolypeptide hydrogels prepared from simple precursors that can possess a range of different functionality.

## 2. Experimental Section

### 2.1. Materials and Methods

Ambient temperature reactions were performed at ≈22 °C. Hexanes and tetrahydrofuran (THF) were degassed by sparging with $N_2$, then dried by passing through alumina columns. All other solvents were used as received from Fisher Scientific. Dialysis was performed using deionized water (18.2 MΩ-cm) prepared by passing in-house deionized water through a Millipore Milli-Q Biocel A10 unit, and regenerated cellulose dialysis tubing was obtained from Spectrum Labs (Rancho Dominguez, CA). For other experiments, in-house deionized water was used. NMR spectra were recorded on a Bruker AV400 instrument with chemical shifts reported relative to the solvent signal. Infrared spectra of solution samples were collected using a Perkin Elmer 1605 FTIR Spectrophotometer. Tandem gel permeation chromatography/light scattering (GPC/LS) was performed on an SSI Accuflow Series III liquid chromatograph pump equipped with Wyatt DAWN EOS LS and Optilab rEX refractive index (RI) detectors. Separations were achieved using xStream $H_2O$ 500 Å Jordi Labs 5 µm mixed bed column with 0.5 wt% potassium trifluoroacetate in HFiP as the eluent at 30 °C. All GPC/LS samples were prepared at concentrations of 10 mg mL$^{-1}$. Amino acid *N*-carboxyanhydrides (NCAs)[16] including L-homomethionine-*N*-carboxyanhydride (Hmt NCA) and 6-(methylthio)-L-norleucine-*N*-carboxyanhydride (Mtn NCA),[8] copolypeptide $K_{180}L_{30}$,[5] PEG-NCO,[17] and non-commercial epoxides ($EG_1$, $EG_3$ and $N_3$)[9,18] were prepared using previously described procedures.

### 2.2. Experimental Procedures

#### *2.2.1. Representative Synthesis of $Hmt_{180}L_x$ Block Copolypeptides: $Hmt_{180}L_{30}$*

In a dinitrogen-filled glovebox, Hmt NCA (400 mg, 2.1 mmol) was dissolved in anhydrous THF (25 mg mL$^{-1}$). To the stirred solution was added $(PMe_3)_4Co$ (0.80 mL of a 50 mM solution in THF) via syringe.[16] After 2 h, an aliquot (20 µL) was removed for FTIR analysis that confirmed all of the NCA had been consumed. Another aliquot (0.20 mL) of the polymerization solution was removed and reacted with $mPEG_{44}$-NCO (70 µL, 3.0 equiv. per Co, 40 mg mL$^{-1}$ in THF) to yield end-capped polymer for molecular weight analysis by $^1$H NMR.[17] From the remaining polymerization reaction mixture, a sample (17 mL, 2.1 mmol Hmt residues) was removed, and to this was added L-leucine NCA (Leu NCA; 1.1 mL, 0.35 mmol, 50 mg mL$^{-1}$ in THF). After complete consumption of the second NCA was confirmed by FTIR, the reaction mixture was removed from the glove box and the copolymer was precipitated into $H_2O$ (10 mL), washed three times with $H_2O$ (15 mL), and then isolated by lyophilization to obtain the product copolypeptide (310 mg, 89% yield). $^1$H NMR (400 MHz, trifluoroacetic acid [TFA-*d*], 25 °C): $\delta$ 4.76–4.63 (m, 1H), 2.73–2.54 (m, 2H), 2.26–2.10 (m, 3H), 2.08–1.86 (m, 2H), 1.85–1.56 (m, 5H), 1.03–0.86 (m, 6H).

#### *2.2.2. Representative Synthesis of $Mtn_{180}L_x$ Block Copolypeptides: $Mtn_{180}L_{30}$*

The procedure followed was identical to the synthesis of $Hmt_{180}L_x$ copolymers, except that for the initial segment an equivalent amount of Mtn NCA was used in place of Hmt NCA. $^1$H NMR (400 MHz, TFA-*d*, 25 °C): $\delta$ 4.73–4.55 (m, 1H), 2.71–2.51 (m, 2H), 2.25–2.06 (m, 3H), 1.99–1.77 (m, 2H), 1.76–1.61 (m, 5H), 1.60–1.41 (m, 2H), 1.02–0.84 (m, 6H).

#### *2.2.3. Diblock Copolypeptide Alkylation*

The thioether groups in diblock copolypeptides were alkylated with epoxides using previously reported procedures.[9] Alkylations typically gave greater than 98% functionalization of thioether groups, and isolated yields above 95%.

#### *2.2.4. Alkylation of $Hmt_{180}L_{30}$ Using Epoxide Mixtures*

For mixed alkylations, the ratio of alkylating agents, glycidyl *n*-butyl ether, nBu-epoxide, glycidyl azide, $N_3$-epoxide, and 2-(2,5,8,11-tetraoxadodecyl)oxirane, $EG_3$-epoxide, were varied to determine the relative rate of alkylation due to steric hindrance differences. In general, alkylating agents were combined in a vial (0.61 mmol, 10 equiv. per each Hmt residue). To the epoxide mixture was added $Hmt_{180}L_{30}$ (10 mg, 0.061 mmol of Hmt residues) and acetic acid (AcOH; 0.40 mL). The reaction was stirred for 72 h at 37 °C, then transferred to 2000 molecular weight cutoff (MWCO) dialysis tubing. The sample was dialyzed against 3 mM HCl (aq) for 48 h, then $H_2O$ for 24 h with

dialysate changes three times daily. The product copolypeptide was isolated as a white solid by lyophilization. The mol% $EG_3$ was determined by comparing the unobscured 2H peak of nBu (1.65 ppm) to the 3H peak of Hmt (3.06 ppm) and subtracting the resulting mol% nBu from 100. $^1$H NMR (400 MHz, TFA-*d*, 25 °C): $\delta$ 4.89–4.65 (m, 1H), 4.64–4.48 (m, 1H), 4.02–3.29 (m, 29H), 3.19–2.94 (m, 3H), 2.40–1.92 (m, 4H), 1.61–1.54 (m, 2H), 1.44–1.31 (m, 4H), 1.01–0.83 (m, 9H).

#### 2.2.5. Synthesis of 2,5,8,11-Tetraoxatetradec-13-yne ($EG_3$-alkyne)

A solution of triethylene glycol monomethyl ether (1.0 mL, 6.1 mmol) in anhydrous (anh.) THF (30 mL) was prepared and stirred under $N_2$. To this solution was added NaH (60% in mineral oil, 370 mg, 9.1 mmol) and NaI (45 mg, 0.30 mmol), and then propargyl bromide (80% in toluene, 1.0 mL, 9.1 mmol). The mixture was stirred for 16 h at room temperature, then concentrated. The crude product was dispersed in water (60 mL) and extracted into ethyl acetate (EtOAc; 3 × 40 mL), and then concentrated to give a yellow oil (1.0 g, 85% yield). Spectra were in accordance with data reported in literature.[19]

#### 2.2.6. Synthesis of 5(6)-Carboxamide-N-(propargyl)-fluorescein

5(6)-Carboxyfluorescein (0.25 g, 0.66 mmol) was dissolved in anh. *N,N*-dimethylformamide (10 mL). To the solution was added anh. *N,N*-diisopropylethylamine (0.58 mL, 3.3 mmol) followed by propargylamine (0.13 mL, 0.11 mmol). Next, *N,N,N′,N′*-tetramethyl-*O*-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (0.38 g, 1.0 mmol) was added in one portion. The foil-wrapped reaction was stirred for 16 h, then concentrated under vacuum to give a thick oil. The crude product was purified using column chromatography using 73:2:25% (EtOAc: methanol:hexanes). The recovered solute was triturated three times with $H_2O$ and lyophilized to give a yellow solid (210 mg, 77% yield). Spectra were in accordance with that reported in literature.[20]

#### 2.2.7. Procedure for CuAAC Conjugations

Example procedure for conjugation of alkyne functionalized fluorescein probes to hydrogels. In a foil-wrapped vial, $(R\text{-}Hmt)_{180}L_{30}$ (R = $N_3$:$EG_3$ in a 5:95 ratio (prepared using mixed epoxide alkylation procedure), 10 mg, 0.025 mmol), 5(6)-carboxamide-*N*-(propargyl)-fluorescein (0.60 mg, 0.00014 mmol), and sodium ascorbate (2.6 mg, 0.013 mmol) were combined in 0.50 mL $H_2O$. In a separate vial, $CuSO_4 \cdot 5H_2O$ (0.70 mg, 0.0026 mmol) and *N,N,N′,N″,N″*-pentamethyldiethylenetriamine (1.1 µL) were dissolved in 0.25 mL $H_2O$. Solutions were stirred under $N_2$ (g) for 1.0 h before combining in foil-wrapped vial. The reaction was stirred under $N_2$ (g) for 24 h, then transferred to 2000 MWCO tubing. The product was dialyzed against 0.50 mM disodium EDTA (aq) for 48 h, 3 mM HCl (aq) for 48 h, and then water for 24 h with dialysate changed four times daily. Product was isolated by lyophilization (11 mg, 98% yield, yellow-orange solid).

#### 2.2.8. Hydrogel Formulation

The desired amount of lyophilized copolypeptide was weighed out in a 1/2-dram glass vial. To this solid was added Milli-Q $H_2O$, 25 mM NaCl (aq) or 1x PBS (aq) to achieve the desired final copolypeptide concentration, and the sample was allowed to form a hydrogel. All further studies were performed on hydrogels prepared at least 16 h in advance of analysis.

#### 2.2.9. Rheology Measurements

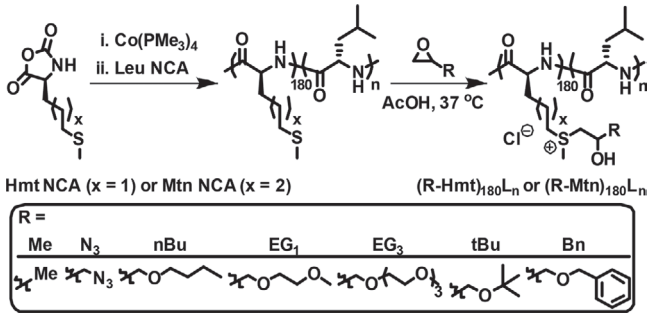
Dynamic rheology analyses were conducted on an Anton Paar Physica MCR 301 rheometer using an 8 mm diameter parallel plate geometry. Oscillatory strain amplitude sweeps (0.0–5.0) were conducted at fixed frequency (1 Hz, or 6.3 rad $s^{-1}$) to establish the linear regime. Next, oscillatory frequency sweeps ($\omega$ = 1.0–100 rad $s^{-1}$) were conducted to measure $G'$ (storage modulus) and $G''$ (loss modulus) versus frequency. Samples were allowed to rest for several minutes between analyses to allow sample recovery. Analysis of hydrogel recovery (self-healing) consisted of two alternating conditions: large amplitude oscillation to break down the gel structure (strain amplitude of 5.0 at 1 Hz) for 250 s, followed by linear small-deformation oscillation to monitor recovery of mechanical strength (strain amplitude of 0.10 at 1 Hz) for 250 s.

#### 2.2.10. Laser Scanning Confocal Microscopy of Fluorescently Labeled Hydrogel

The laser scanning confocal microscopy hydrogel image (3.0 wt% in DI water) was taken on a Leica TCS-SP1 MP-inverted confocal and multiphoton microscope equipped with an argon laser (476 and 488 nm blue lines), a diode (DPSS) laser (561 nm yellow-green line). The fluorescein labeled hydrogel sample was visualized on a glass slide with a spacer between the slide and the cover slip (double-sided tape) allowing the hydrogel to be minimally disturbed during focusing. A Z-slice thickness of 0.78 µm was used. Sample imaging was performed at the Advanced Light Microscopy/Spectroscopy Center (ALMS) at the UCLA California NanoSystems Institute (CNSI).

## 3. Results and Discussion

The preparation of L-homomethionine and L-6-(methylthio)-L-norleucine-based diblock copolypeptides utilized the corresponding *N*-carboxyanhydride (NCA) monomers of these amino acids, Hmt NCA and Mtn NCA, respectively (**Scheme 2**), that were recently reported.[8] Hmt NCA and Mtn NCA were separately polymerized using $Co(PMe_3)_4$ initiator in THF to give homopolypeptide segments of ≈180 residues in length (Scheme 2; Table S1 and Figures S1 and S2, Supporting Information).[16] To each Hmt or Mtn segment was added Leu NCA in different amounts to give diblock copolypeptides with L segments ≈20 or 30 residues in length. These parent copolypeptides were obtained in high overall yields with low dispersities, and compositions that closely matched expected values




**Scheme 2.** Synthesis of hydrogel forming diblock copolypeptides.

(Table S1, Supporting Information). These fully hydrophobic copolypeptides there then separately reacted with one of six different functional epoxides using established methods[9] to give the corresponding amphiphilic Hmt or Mtn sulfonium containing samples (R-Hmt)$_{180}$L$_n$ and (R-Mtn)$_{180}$L$_n$, respectively (Scheme 2). Using excess epoxide (≈3–10 equivalents per thioether group), the conversion of thioether groups to S-alkyl sulfonium groups was found to be greater than 98%, quantified using $^1$H NMR spectroscopy (see Supporting Information). All sulfonium containing block copolypeptide derivatives were converted to chloride ion salts by dialysis against aqueous HCl, followed by purification by dialysis against DI water. Samples were isolated as fluffy white solids after lyophilization.

Samples of (R-Hmt)$_{180}$L$_{20}$ and (R-Hmt)$_{180}$L$_{30}$, where R = Me, EG$_1$, EG$_3$, nBu, tBu, and Bn, were separately dissolved in DI water at 3.0 wt% of copolypeptide, and all were found to form self-supporting, transparent hydrogels, which were expected to possess branched, tape-like fibrillar networks as observed for all other DCH (**Figure 1**).[4,21] Rheological analysis of these samples revealed that both L segment length and side-chain functionality, R, affected hydrogel mechanical properties (**Figure 2**). All samples with longer L$_{30}$ segments were found to give hydrogels with greater stiffness (i.e., increased storage modulus *G′*) compared to the corresponding L$_{20}$ samples, as expected from prior studies on amphiphilic DCH.[5] Samples with more hydrophilic side-chain groups (i.e., R = Me, EG$_1$, EG$_3$) formed

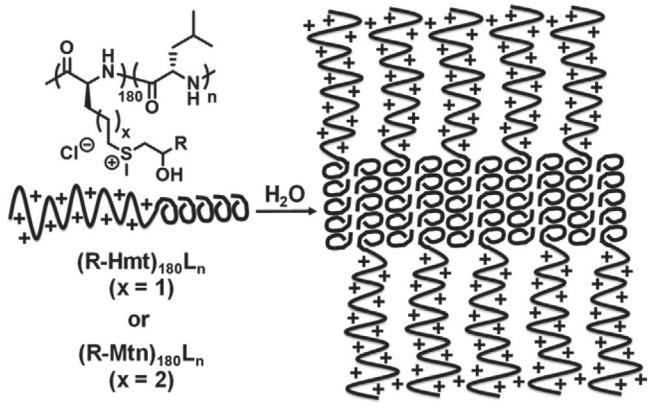



**Figure 1.** Schematic showing proposed self-assembly of amphiphilic (R-Hmt)$_{180}$L$_n$ and (R-Mtn)$_{180}$L$_n$ in water into fibrils that branch and entangle to form hydrogel networks.

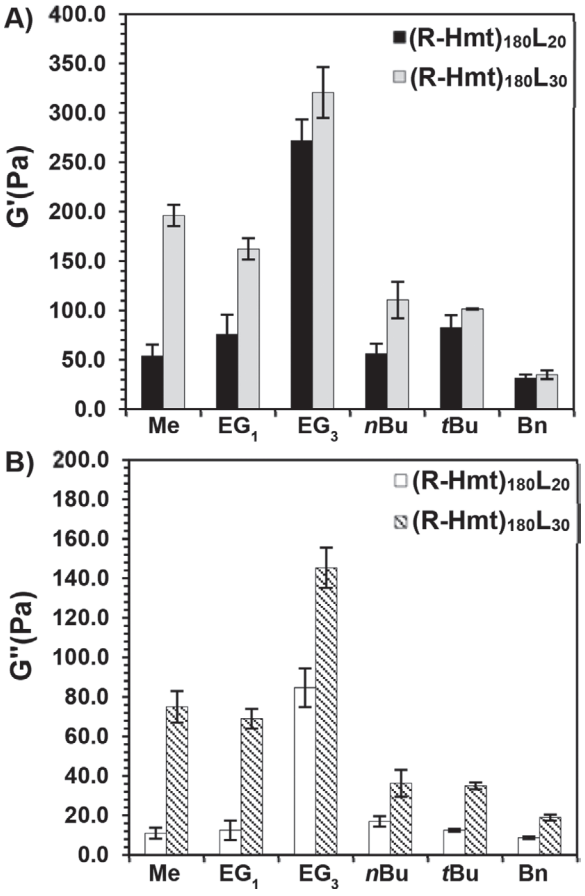



**Figure 2.** Rheology data for 3.0 wt% (R-Hmt)$_{180}$L$_n$, *n* = 20 or 30, hydrogels in DI water for different side-chain substituents, R. A) Storage modulus, *G′* (Pa), of (R-Hmt)$_{180}$L$_{20}$ (black) and (R-Hmt)$_{180}$L$_{30}$ (gray). B) Loss modulus, *G″* (Pa), of (R-Hmt)$_{180}$L$_{20}$ (white) and (R-Hmt)$_{180}$L$_{30}$ (striped). All *G′* and *G″* values are measured at a frequency of 1 Hz and strain amplitude of 0.10, and are averages of triplicate runs with bars indicating standard deviations.

stiffer hydrogels compared to samples containing more hydrophobic side-chain groups (i.e., R = nBu, tBu, Bn). It was somewhat surprising that the R-Hmt segments containing these hydrophobic side-chains were hydrophilic enough to promote hydrogel formation at all. It appears the presence of charged sulfonium ions and adjacent hydroxyl groups in the side-chains derived from epoxide alkylation is sufficient to overcome the hydrophobic influences of these R groups, albeit at the cost of weaker hydrogel formation. Due to superior hydrogel formation with the (R-Hmt)$_{180}$L$_{30}$ samples, subsequent studies were focused on samples with longer L$_{30}$ segments.

To compare samples containing different side-chain lengths, the mechanical properties of (R-Hmt)$_{180}$L$_{30}$ samples were compared with the corresponding series of (R-Mtn)$_{180}$L$_{30}$ samples at 3.0 wt% in DI water. All (R-Mtn)$_{180}$L$_{30}$ samples were found to form transparent hydrogels, and rheological analysis revealed that (R-Mtn)$_{180}$L$_{30}$ hydrogels possessed similar stiffness to corresponding (R-Hmt)$_{180}$L$_{30}$ hydrogels, but were overall weaker especially for samples with more hydrophilic side-chains (**Figure 3**). The trends in *G′* as functions of different R groups were found to be similar between both sets of hydrogels. The

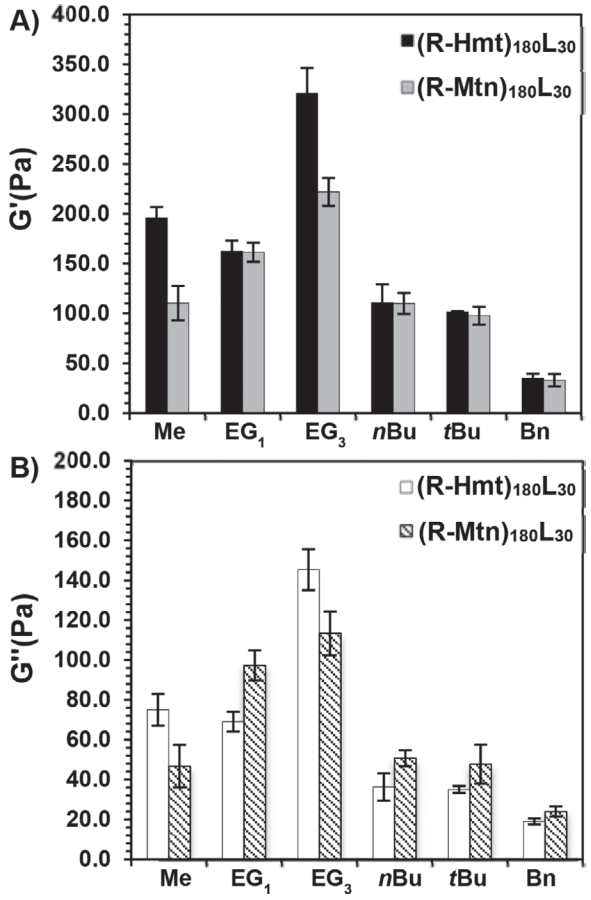



**Figure 3.** Rheology data for 3.0 wt% $(R\text{-}Hmt)_{180}L_{30}$ and $(R\text{-}Mtn)_{180}L_{30}$ hydrogels in DI water for different side-chain substituents, R. A) Storage modulus, *G′* (Pa), of $(R\text{-}Hmt)_{180}L_{30}$ (black) and $(R\text{-}Mtn)_{180}L_{30}$ (gray). B) Loss modulus, *G″* (Pa), of $(R\text{-}Hmt)_{180}L_{30}$ (white) and $(R\text{-}Mtn)_{180}L_{30}$ (striped). All *G′* and *G″* values measured at a frequency of 1 Hz and strain amplitude of 0.10, and are averages of triplicate runs with bars indicating standard deviations.

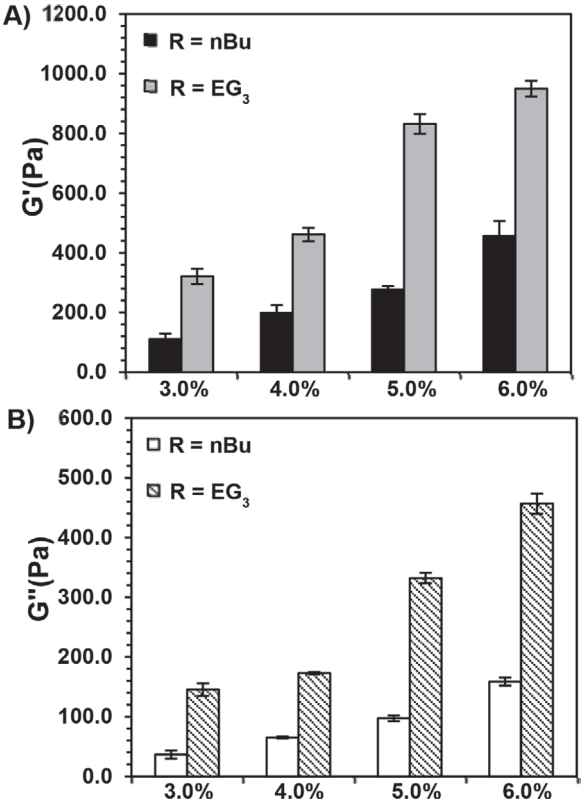



**Figure 4.** Rheology data for different concentrations (wt%) of $(R\text{-}M^H)_{180}L_{30}$ hydrogels in DI water, R = nBu or $EG_3$. A) Storage modulus, *G′* (Pa), when R = nBu (black) and R = $EG_3$ (gray). B) Loss modulus, *G″* (Pa), when R = nBu (white) and R = $EG_3$ (striped). All *G′* and *G″* values are measured at a frequency of 1 Hz and strain amplitude of 0.10, and are averages of triplicate runs with bars indicating standard deviations.

increased hydrophobic side-chain length in $(R\text{-}Mtn)_{180}L_{30}$ versus $(R\text{-}Hmt)_{180}L_{30}$ appears to have a modest weakening effect on hydrogel stiffness, especially for the samples where R = Me and $EG_3$. This side-chain length effect is also seen by comparison to previously reported DCH, $(M^MA)_{170}L_{27}$, containing methylated methionine residues.[6] At 3.0 wt% in DI water, *G′* for $(Me\text{-}Mtn)_{180}L_{30}$, $(Me\text{-}Hmt)_{180}L_{30}$, and $(M^MA)_{170}L_{27}$ hydrogels were 110, 195, and 230 Pa, respectively.[6] While these DCH possess similar mechanical properties overall, stiffness was found to decrease with increasing side-chain lengths. For all samples studied, those with the greatest EG content, R = $EG_3$, always gave the stiffest hydrogels at equivalent copolypeptide concentration, likely due to the high water solubility of the $EG_3$ groups that help solubilize the polypeptide chains.[22] However, variation of the R groups, even to substituents with much less hydrophilicity, also resulted in hydrogel formation and provides a convenient means to adjust hydrogel stiffness.

The versatility of thioether alkylation methodology is not limited to uniform modification of polypeptides, but also allows statistical incorporation of different R groups on individual chains. To illustrate the potential of this strategy, samples of $Hmt_{180}L_{30}$ were alkylated with mixtures containing varying ratios of $EG_3$-epoxide and nBu-epoxide, yielding fully modified $(R\text{-}Hmt)_{180}L_{30}$ samples with $EG_3$ content varying from 29 to 90 mol% (Table S2, Supporting Information). Rheology data for these samples at 3.0 wt% in DI water, including pure $(EG_3\text{-}Hmt)_{180}L_{30}$ and $(nBu\text{-}Hmt)_{180}L_{30}$ for comparison (Figure S3, Supporting Information), show that even small percentages of nBu content can be used to finely tune hydrogel properties. Beyond side-chain modification, copolypeptide concentration was also found to be a convenient means to independently adjust hydrogel stiffness (**Figure 4**). Similar to amphiphilic DCH,[5] increased sample concentrations in DI water resulted in increased hydrogel stiffness for all samples studied. Both $(EG_3\text{-}Hmt)_{180}L_{30}$ and $(nBu\text{-}Hmt)_{180}L_{30}$ hydrogels remained transparent at all concentrations.

To study the effect of solution ionic strength on hydrogel properties, $(EG_3\text{-}Hmt)_{180}L_{30}$, $(EG_3\text{-}Mtn)_{180}L_{30}$, and a $K_{180}L_{30}$ (K = poly(L-lysine·HCl))[5] sample for comparison were each dissolved at 3.0 wt% in either DI water, 25 mM NaCl (aqueous) or 1x PBS buffer (aqueous, ≈150 mM ionic strength). The $K_{180}L_{30}$ sample forms a stiffer hydrogel compared to the $(EG_3\text{-}Hmt)_{180}L_{30}$ and $(EG_3\text{-}Mtn)_{180}L_{30}$ samples, and similar to earlier findings was found to weaken significantly in the

**Table 1.** Average storage modulus, $G'$, for $K_{180}L_{30}$, $(R\text{-}Hmt)_{180}L_{30}$, and $(R\text{-}Mth)_{180}L_{30}$ hydrogels in different media.

| Solvent | Hydrogel stiffness, $G'$ [Pa][a)] | | |
|---|---|---|---|
| | $K_{180}L_{30}$ | $(R\text{-}Hmt)_{180}L_{30}$ | $(R\text{-}Mtn)_{180}L_{30}$ |
| DI water | 1423 | 321 | 222 |
| 25 mM NaCl | 797 | 115 | 71 |
| pH 7.4 PBS | 623 | 94 | 47 |

[a)]All hydrogels were prepared at 3.0 wt% in DI water, 25 mM NaCl, or pH 7.4 PBS. R = $EG_3$. All $G'$ values were measured at a frequency of 1 Hz and strain amplitude of 0.10, and are averages of triplicate runs.

presence of added ions,[4] with $G'$ decreasing more than 50% in 1x PBS (**Table 1**; Figure S4, Supporting Information). The $(EG_3\text{-}Hmt)_{180}L_{30}$ and $(EG_3\text{-}Mtn)_{180}L_{30}$ samples showed similar weakening in increased ionic strength media, yet all samples remained transparent hydrogels under these conditions. In charged DCH, it is believed that added salt weakens these hydrogels by both screening like charges on the polyelectrolyte segments thereby weakening repulsive interactions that influence self-assembly, and by increasing hydrophobic attraction leading to decreased hydration of the copolypeptide assemblies.[4]

Amphiphilic DCH possess self-healing properties that allow rapid recovery of hydrogel mechanical properties after being broken down under high shear.[5] To see if the new hydrogels reported here also possess the ability to self-heal, the mechanical recovery of a $(EG_3\text{-}Hmt)_{180}L_{30}$ hydrogel after breakdown under high strain was studied (**Figure 5**). Exposure of a 3.0 wt% $(EG_3\text{-}Hmt)_{180}L_{30}$ in water to high-amplitude oscillatory strain resulted in complete loss of elastic properties and liquid like behavior ($G'' > G'$). Upon removal of high-amplitude strain, the sample was found to rapidly (i.e., <10 s) recover its elastic properties without any detectable hysteresis, which is a hallmark feature of DCH.[5,6] This self-healing behavior is beneficial for minimally invasive applications requiring delivery of hydrogel depots via small catheter or syringe needle, where the shear thinning properties of the hydrogel allow it to liquefy during injection and then quickly recover elastic network properties and form a hydrogel deposit upon exiting the needle.[6,23]

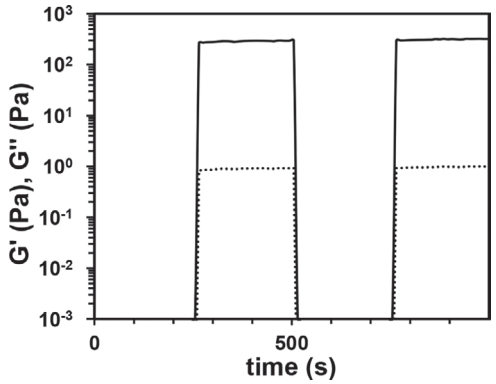



**Figure 5.** Storage modulus ($G'$ [Pa], solid line) and loss modulus ($G''$ [Pa], dotted line) for a 3.0 wt% $(EG_3\text{-}Hmt)_{180}L_{30}$ hydrogel as a function of stepwise large-amplitude oscillatory strain in DI water. For the initial 250 s, the hydrogel was broken down at a large strain amplitude of 5 at 1 Hz. This was followed by low strain linear recovery at a small strain amplitude of 0.10 at 1 Hz for 250 s. This cycle was then repeated to demonstrate hydrogel self-healing after breakdown.

To illustrate the ability to incorporate chemically reactive functionality into the $(R\text{-}Hmt)_{180}L_{30}$ hydrogels, glycidyl azide, $N_3$-epoxide, was also used for thioether alkylation to highlight the potential for secondary functionalization of hydrogels. Azide groups are well known to undergo copper-catalyzed azide-alkyne cycloaddition (CuAAC) reactions with alkynes to selectively install functional modifications such as targeting ligands and fluorescent labels under mild conditions in aqueous media.[24] To illustrate the potential of this strategy, a sample of $Hmt_{180}L_{30}$ was alkylated with a mixture of $EG_3$-epoxide and $N_3$-epoxide, to yield a fully modified $(R\text{-}Hmt)_{180}L_{30}$ sample with $EG_3$ content of 95 mol% and $N_3$ content of 5 mol% (see Supporting Information). In a proof of concept study, $EG_3$-alkynes were efficiently grafted onto the azide groups of the $N_3$ functionalized $(R\text{-}Hmt)_{180}L_{30}$ sample in water. Rheological analysis of this sample before and after CuAAC conjugation with $EG_3$-alkyne at 2.5 wt% in DI water showed that there was a small improvement in hydrogel stiffness after conjugation of the hydrophilic $EG_3$ groups (Figure S5, Supporting Information). Finally, an alkyne functionalized fluorescein probe was also conjugated to the $N_3$ functionalized $(R\text{-}Hmt)_{180}L_{30}$ sample using CuAAC which allowed labeling of the hydrogel for fluorescence imaging (Figure S6, Supporting Information).

## 4. Conclusions

We have described the preparation of new DCH derived from homologs of L-methionine, that is, L-homomethionine and L-6-(methylthio)-L-norleucine, that are self-healing and allow fine tuning of properties by choice of side-chain length and by reaction with different functional epoxides. This methodology was found to eliminate the need for use of comonomers in the preparation of thioether containing DCH, and allows for the preparation of a diverse range of functional hydrogels from simple precursors via high yield postpolymerization thioether alkylation. These results provide a starting platform for the development of self-healing, tunable copolypeptide hydrogels for potential use as depots for local delivery of bioactive molecules.

## Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

## Acknowledgements

This work was supported by the NSF under award No. CHE-1412367.

## Conflict of Interest

The authors declare no conflict of interest.

## Keywords

block copolymer, hydrogel, methionine, polypeptide

Received: July 8, 2019
Revised: August 10, 2019
Published online:

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