Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information

Strain-stiffening gels based on latent crosslinking†

Yen H. Tran,[a] Matthew J. Rasmuson,[a] Todd S. Emrick,[b] John Klier*[a] and Shelly R. Peyton*[a]

[a]*Department of Chemical Engineering, University of Massachusetts-Amherst, Amherst, MA 01003-9303, USA.*
[b]*Department of Polymer Science and Engineering, University of Massachusetts-Amherst, Amherst, MA 01003-9303, USA.*
** E-mail: klier@umass.edu; speyton@ecs.umass.edu*

Synthesis of HEMA-LA. HEMA-LA monomer was prepared following the previously reported method.[34] Briefly, 2-hydroxyethyl methacrylate (HEMA) (4.70 mL, 38.8 mmol, Sigma-Aldrich), lipoic acid (LA) (8.00 g, 38.8 mmol, Sigma-Aldrich), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) (11.15 g, 58.2 mmol, Fisher Scientific), and 4-(dimethylamino)pyridine (DMAP) (4.74 g, 38.8 mmol, Sigma-Aldrich) were dissolved in 100 mL distilled dichloromethane (DCM, Sigma-Aldrich) in a dry round-bottom flask. The reaction mixture was stirred at room temperature for 24 h under nitrogen. The reaction was sequentially washed with 1 M $HCl_{(aq)}$ (Fisher Scientific), saturated $NaHCO_{3(aq)}$ (Fisher Scientific), and brine solutions (Fisher Scientific). The organic layers were collected and dried over anhydrous $MgSO_4$ (Fisher Scientific), filtered, and concentrated by rotary evaporation, giving HEMA-LA monomer as a yellow oil (8.5 g, 70% yield). ^{1}H NMR spectrum was recorded at 400 MHz on Avance400 spectrometer. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.06 (s, 1H), 5.36 (s, 1H), 4.26 (s, 4H), 3.5 (m, 1 H), 3.11 (m, 2H), 2.40 (m, 1H), 2.3 (t, 2H), 1.87 (s, 3H), 1.35−1.70 (m, 8H).

Characterization of gels

Swelling studies. The swelling volume ratio Q is defined as the ratio of the sample volume at swelling equilibrium V to the initial volume V_0. Swelling equilibrium is defined after gels sit in a swelling solution for 5 days. The weight fraction of polymer (polymer content) of swollen gels r was determined as the ratio of the dry weight of the sample W_d to its swollen weight W_s:

$$r = \frac{W_d}{W_s} \qquad (1)$$

Other solvents such as dimethylformamide (DMF) or tetrahydrofuran (THF) could be also used to make gels.

Determination of mesh size. To determine an approximate average mesh size of gel network, swollen gels were weighed, fully lyophilized, and weigh again. The average mesh size, ξ, of the gels was

determined as a function of HEMA-LA concentration according to the Flory theory as modified by Canal and Peppas:[54]

$$\xi = {v_{2,s}}^{-1/3}\left(\bar{r}^2\right)^{1/2} \quad (2)$$

where $v_{2,s}$ is the swollen volume fraction of polymer and $\left(\bar{r}^2\right)^{1/2}$ is the average end-to-end distance of the crosslinker.

Thiol concentration measurement. The available thiol concentration in reduced gels was monitored using adapted Ellman's assay. Briefly, a reaction buffer containing 0.1 M sodium phosphate (Fisher Scientific) and 1 mM EDTA (Fisher Scientific), pH 8.0 was prepared. An Ellman's reagent solution was prepared by dissolving 4 mg of Ellman's reagent (Thermo Scientific) in 1 mL of reaction buffer. Cysteine hydrochloride monohydrate (Sigma-Aldrich) was used as the standard. A set of seven cysteine standards was prepared as follows:

Table S1: Cysteine standards for Ellman's assay

Standard	Volume of reaction buffer (mL)	Amount of cysteine	Final concentration (mM)
1	5.00	1.317 mg	1.50
2	0.2	1 mL of Standard 1	1.25
3	0.4	0.8 mL of Standard 1	1.00
4	0.6	0.6 mL of Standard 1	0.75
5	0.8	0.4 mL of Standard 1	0.50
6	1.0	0.2 mL of Standard 1	0.25
7	1.2	0	0.0

A small portion of gel after being crushed was used as sample whose thiol concentration was ensured to be in the working range of the standard curve (0.1-1.0 mM). A volume of 250 µL of each standard was added to separate test tubes. For gel samples, an additional volume of reaction buffer was added to each gel portion so that the total volume was 250 µL. In each test tube of standards or gel samples, 50 µL of Ellman's reagent solution and 2.5 mL of reaction buffer were added. The mixture

was mixed and incubated at room temperature for 15 min, followed by absorbance measurement at 412 nm using BioTek Synergy H1 microplate reader.

Statistical analysis

Statistical analysis was performed using Prism v5.04 (GraphPad Software). Data are reported as mean ± standard deviation, unless otherwise noted. Statistical significance of the difference between pairs of means was evaluated by computing *P*-values with unpaired Student's *t* test (with Welch's correction as necessary). When one-way ANOVAs were performed, the Tukey post-test was used to determined significance of pairwise differences. $P \leq 0.05$ is denoted with *, ≤ 0.01 with **, ≤ 0.001 with ***, and ≤ 0.0001 with ****; $P > 0.05$ is considered not significant.

Supplemental Results

Esterification of HEMA with LA, using EDC coupling, gave HEMA-LA monomer as a yellow oil (Fig. S1a). HEMA-LA was characterized by ^{1}H NMR spectroscopy at 400 MHz (Bruker 400) noting the vinyl protons at 5.4 and 6.1 ppm, and the characteristic signals from lipoic acid moiety in the alkyl region (~2.5 ppm) of the spectrum (Fig. S1b, c).

Thiol concentration was quantified using Ellman's test with a typical calibration curve between the absorbance reading and thiol concentration (Fig. S2a). The reduction time from disulfides to thiols was optimized by varying the reaction time from 2-10 h and thiol concentration was measured immediately after each run. Also, two groups of gels were prepared in which one group was the swollen gels sitting in swelling solution for 5 days and the other was the swollen gels that underwent vacuum drying step for 48 h at room temperature. The result showed that the swollen gels had slightly higher free thiol production compared to the vacuum dried gels. It was determined that the optimal reduction time is 6 h by simply using swollen gels (Fig. S2b). The reduction yield was also determined for each group of gels in all 3 sets (Fig. S2c). The reduction yield was defined as the amount (%) of thiols reduced. It was calculated by dividing the actual thiol concentration after the reduction by the theoretical thiol concentration. The relative, empirical thiol concentration was determined by performing an Ellman's test

for gels immediately after the reduction. The theoretical thiol concentration was calculated from HEMA-LA concentration in the gels (i.e., 1 M of HEMA-LA is equivalent to 2 M of thiols, theoretically). The swelling behavior of reduced gels was also studied as defined in Equation (1). Fig. S2d-f showed that reduced gels from all 3 sets exhibited deswelling over time post-reduction. The deswelling occurred after about 1 day after the reduction for Sets I and II (both composed of short co-monomer M1) and about 2 days for Set III (composed of long M1). This deswelling behavior indicated that thiol crosslinking occurred over time after the reduction. Moreover, the increase in gel stiffness as shown in Fig. 4 proved that this additional crosslinking was from inter-strand disulfide formation instead of cyclic disulfide reformation. Fig. S2g showed that the average mesh sizes of gels ranged from 12.0 ± 0.4 Å to 13 ± 0.2 Å for Set I (short CL/short M1), 19.6 ± 0.5 Å to 26.6 ± 0.3 Å for Set II (long CL/short M1), and 10.4 ± 0.9 Å to 16.8 ± 0.5 Å for Set III (short CL/long M1). While the CL concentration in all gels were the same, these results validated that the average mesh size of network composed of longer CL is longer than that of network composed of shorter CL. When compared Sets I and III, the average mesh sizes of Set I gels were slightly smaller due to shorter M1. Interestingly, within each set, while the total polymer content was the same for each gel group, the mesh size decreased with increasing HEMA-LA concentration (i.e., A<B<C). This could be explained as the addition of hydrophobic HEMA-LA resulted in a decrease in the maximum swelling as well as in the organization of solvent molecules within gel structure.

Fig. S3 showed Young's modulus of gels in Sets I and III as a function of time post-reduction. Note that these results were used to calculate the stiffness increase in Fig. 4.

Stress, quantified by the compression force applied on different gels, is plotted as a function of applied strain and time post-reduction (Fig. S4). At the same strain, stress required increased over time for all gels in Sets I and II (Fig. S4a-f). Due to the steric shielding effect of long M1 in Set III, no significant increase in stress over time was observed (Fig. S4g-i). These results were correlated with the increase in stiffness shown in Fig. 4.

Fig. S5 showed changes in Young's modulus of gels under 3 h strain in Sets I and III. Similar to Set I (Fig. 5), gels in Set I exhibited strain-stiffening behavior as the inter-chain crosslinking resulted in faster strain-induced stiffening compared to spontaneous crosslinking in the unstrained network (Fig.

S5a-c). However, only small increases in stiffness were observed in all gel groups in Set III (Fig. S5d-f) due to shielding effect of long M1.

To confirm that strain stiffening was due to inter-chain disulfide crosslinks rather than other types of interactions such as chain entanglement or electrostatic attraction, 2 control gel groups were used to perform strain induction. In control group 1, gels I-C, II-C, and III-C were used (structures and compositions given in Fig. 1b, c) but no reduction was performed. In this case, cyclic disulfides were remained and no free thiols available during strain induction. In control group 2, gel without latent crosslinking sites was composed of PEGDMA M_n 750 as crosslinker and HEA as the only monomer. This control gel contained 0.129 M of CL and 1.68 M of HEA, giving the molar ratio of CL:monomer to be 1:13. This gel was denoted as gel II-D. Since no disulfides were present in the gel, no reduction was necessary. After strain induction, there were no changes in stiffness in both groups of gels (Fig. S6), indicating that no other types of interactions such as chain entanglement or hydrogen bonding occurred in such network. This also confirmed that the strain stiffening behavior in our gels was due to inter-chain disulfide formation during strain application.

Copolymer structure can be determined by the reactivity ratios of the two monomers, r_1 for HEMA-LA and r_2 for HEA, which relate the preference of a monomer to reacting with itself or with the other monomer.[55] We estimated the monomer reactivity ratios using the Fineman-Ross linearization method using the following equation:[56]

$$\frac{F}{f}(f-1) = r_1\left(\frac{F^2}{f}\right) - r_2 \qquad (3)$$

where F is the molar ratio of the monomers in the feed and f is the molar ratio of the monomers in the copolymer, which were calculated using ^{1}H NMR. The plot of $F(f-1)/f$ versus F^2/f gives a straight line whose slope is r_1 and intercept is $-r_2$. Different copolymer compositions were prepared to generate this plot (Fig. S7). The reactivity ratios of HEMA-LA and HEA were determined to be 0.792 and 1.21, respectively. The type of copolymer formed can be determined using the reactivity ratios as explained by Smith and Klier.[57] Briefly, if both ratios are approximately equal to 1, the two monomers have equal reactivities and a random copolymer is formed. With the calculated reactivity ratios of HEA and HEMA-

LA both close to 1 rather than other extreme cases, the synthesized copolymer was in random distribution.

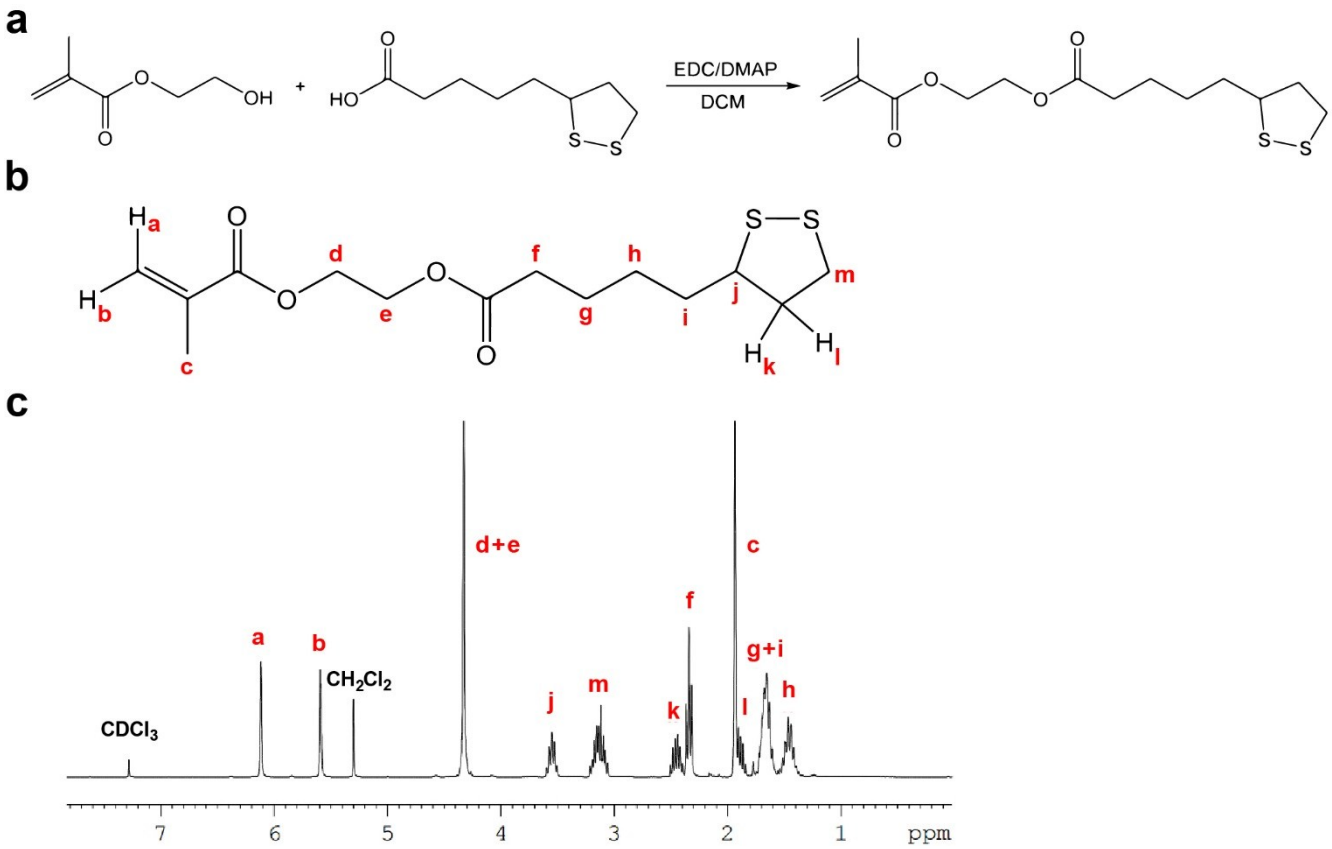

Fig. S1 Synthesis of HEMA-LA. a) Homopolymerization of HEMA-LA by RAFT reaction between HEMA and LA using EDC as coupling agent and DMAP as catalyst in DCM. b) Chemical structure of HEMA-LA. c) ^{1}H NMR spectrum of HEMA-LA.

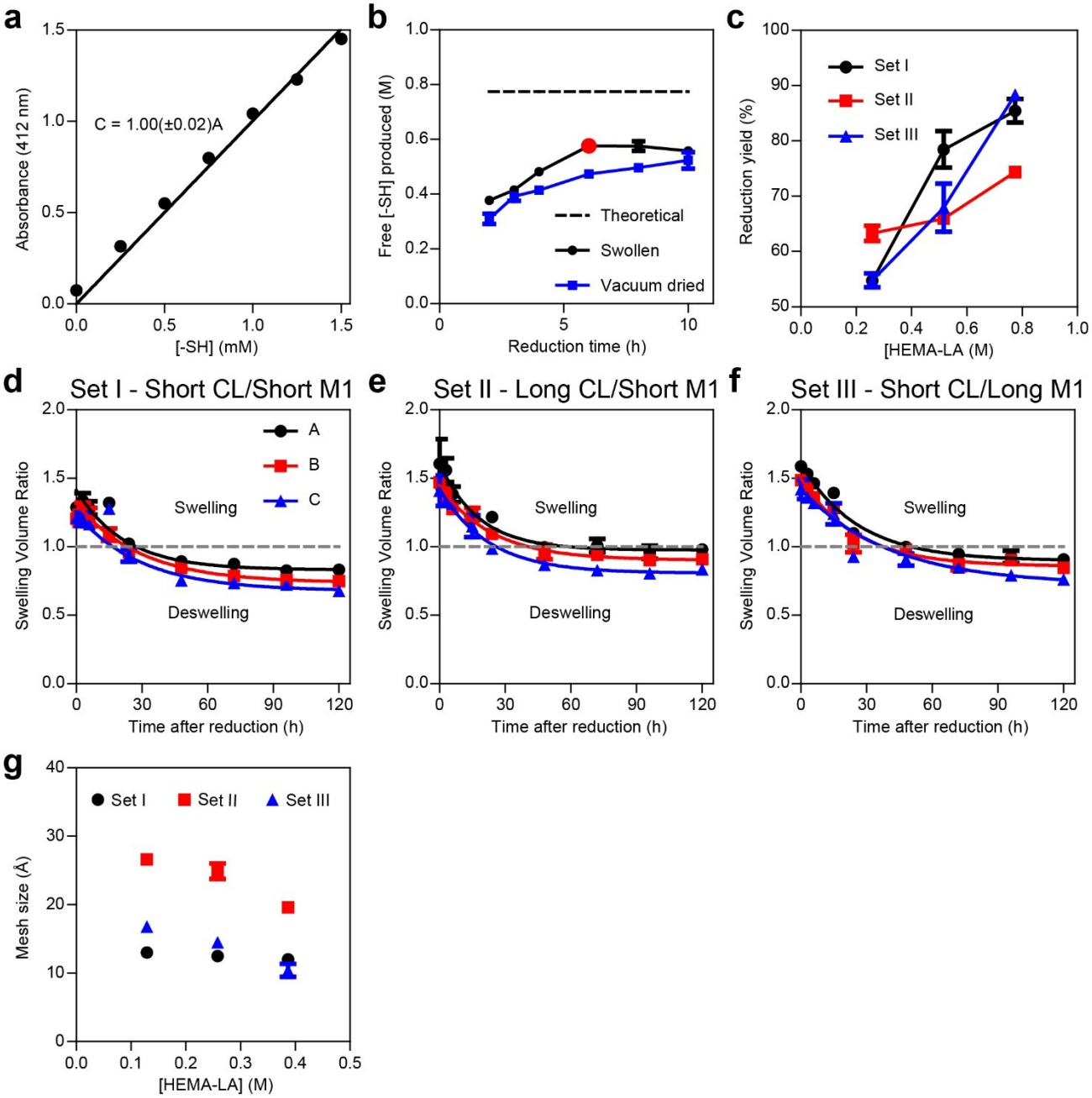

Fig. S2 Characterization of strain-stiffening gels. a) Calibration of Ellman's reagent using cysteine standard to quantify thiol concentration. b) Optimization of reduction time using II-C gels with two different methods. c) Reaction yield after 6-hour disulfide reduction for different gel sets and gel groups. d-f) De-swelling behavior of HEMA-LA gels in 1:4 ethanol:DMSO as a function of time after reduction of different gel structures. d) Set I (short CL/short M1). e) Set II (long CL/short M1). f) Set III (short CL/long M1). g) Average mesh size of gels as a function of HEMA-LA concentration.

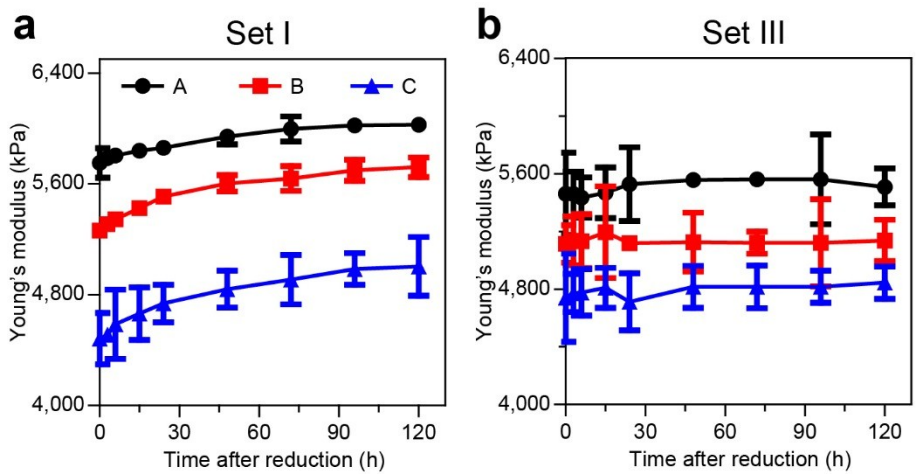

Fig. S3 Young's modulus as a function of time post-reduction of a) Set I and b) Set III.

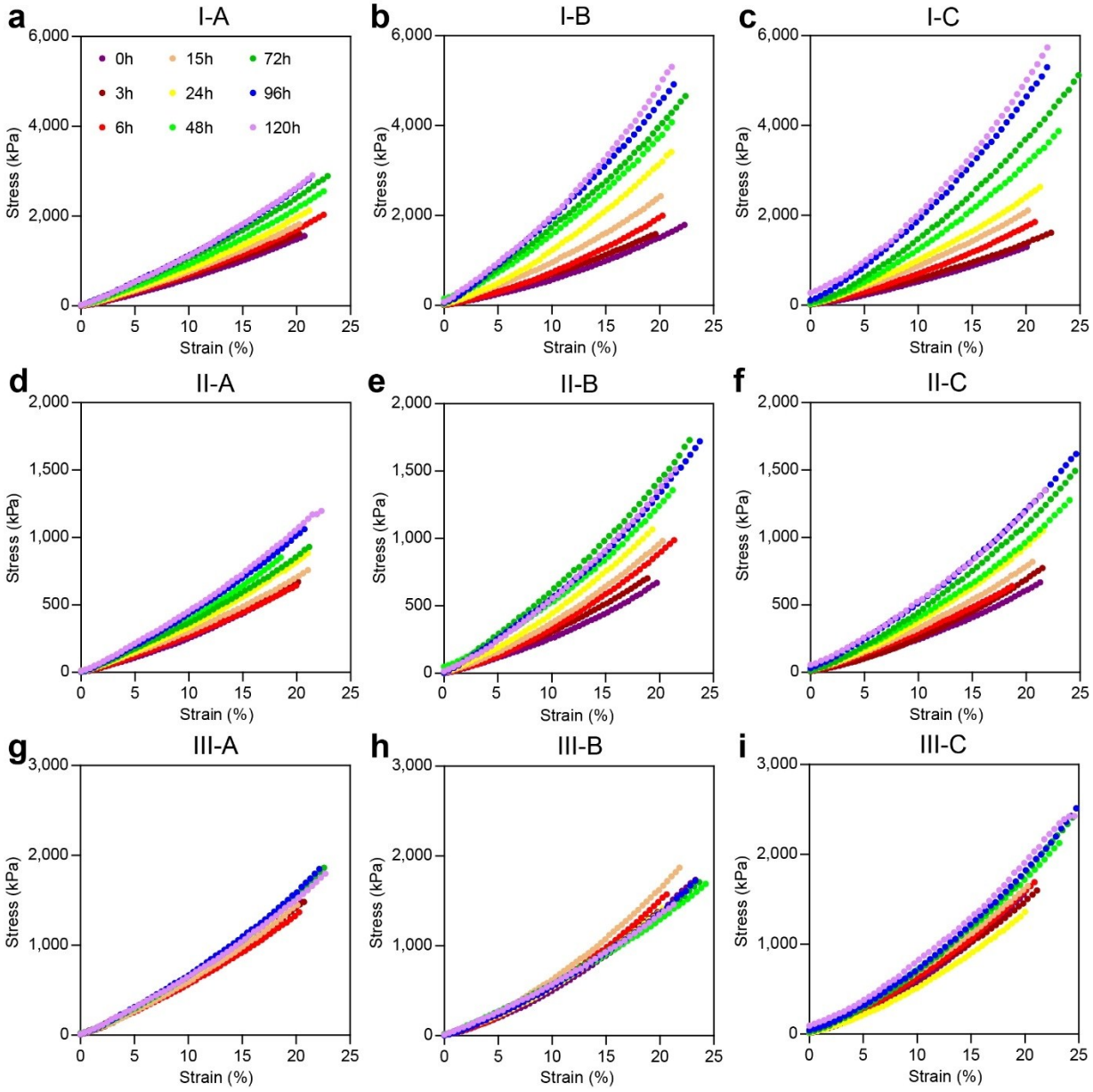

Fig. S4 Stress-strain behavior during spontaneous crosslinking at different times after reduction of different gel structures. a-c) Set I (short CL/short M1). d-f) Set II (long CL/short M1). g-i) Set III (short CL/long M1).

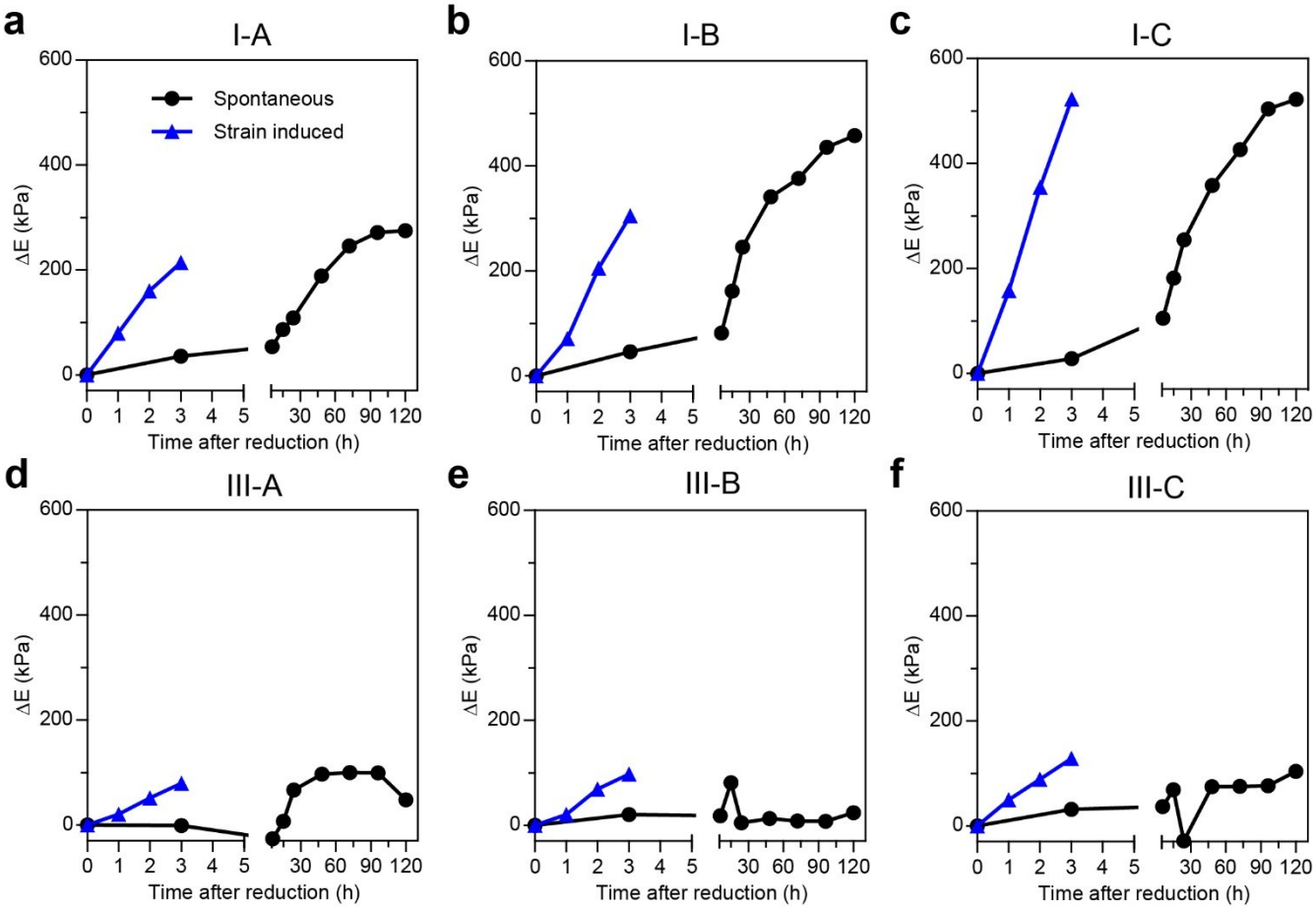

Fig. S5 Change in Young's modulus post-reduction under strain (blue) for 3 hours compared to spontaneous state (black) of a-c) Set I gels, and d-f) Set III gels.

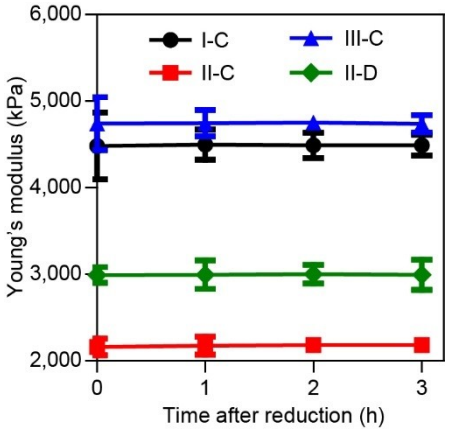

Fig. S6 Young's modulus post-reduction under strain for 3 hours of control gels I-C, II-C, and III-D where no reduction was performed, and II-D where no HEMA-LA was present ([CL]:[M1] = 1:13).

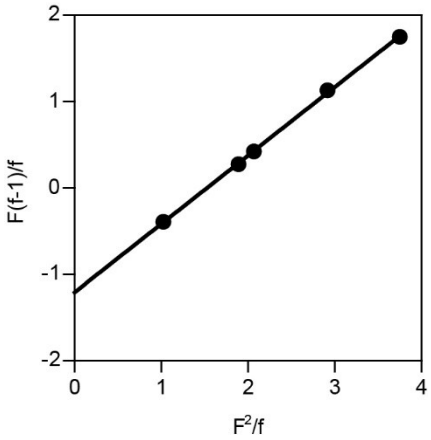

Fig. S7 Determination of monomer reactivity ratios of (HEA/HEMA-LA) copolymerization using Fineman-Ross linearization method.

References

54. T. Canal and N. A. Peppas, *J. Biomed. Mater. Res. A*, 1989, **23**, 1183-1193.
55. A. Habibi, E. Vasheghani-Farahani, M. A. Semsarzadeh and K. Sadaghiani, *Polym. Int.*, 2003, **52**, 1434-1443.
56. M. Fineman and S. D. Ross, *J. Polym. Sci.*, 1950, **5**, 259-265.
57. B. L. Smith and J. Klier, *J. Appl. Polym. Sci.*, 1998, **68**, 1019-1025.