6-1-2016

Programming Molecular Association and Viscoelastic Behavior in Protein Networks

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ADVANCED MATERIALS

Supporting Information

for *Adv. Mater.*, DOI: 10.1002/adma.201506216

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Experimental Section

Plasmid construction Genes encoding the artificial proteins used in this study were created using a combination of gene synthesis (Genscript, Piscataway, NJ) and standard molecular cloning techniques. The artificial protein PEP was encoded on the pET15b plasmid (pET15b PEP) (Novagen, Madison, WI). All other proteins were encoded on pQE-80L plasmids (pQE-80L EPE, pQE-80L ERE, pQE-80L EPE L44A) (Qiagen, Valencia, CA). The complete amino acid sequence for each protein is given in Table S1.

Protein expression and purification Chemically competent BL21 *Escherichia coli* (New England BioLabs, Ipswich, MA) were transformed with plasmids encoding the artificial proteins EPE, ERE, and EPE L44A. Expression was carried out at 37 °C in Terrific broth containing 100 μg mL^{-1} ampicillin (BioPioneer, San Diego, CA). At an optical density at 600 nm (OD_{600}) of 0.9-1, expression was induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) (BioPioneer). The cells were harvested 4 hr after induction by centrifugation at 6000 *g*, 4 °C for 8 min. Cell pellets were subjected to two freeze-thaw cycles, resuspended in TEN buffer (10 mM Tris, 1 mM EDTA, 100 mM NaCl, pH 8.0) at a concentration of 0.5 g mL^{-1} and subjected to a final freeze-thaw cycle. The lysate was treated with 10 μg mL^{-1} DNaseI (Sigma, St. Louis, MO), 5 μg mL^{-1} RNaseA (Sigma), 5 mM $MgCl_2$, and 1 mM phenylmethylsulfonyl fluoride (Gold Biotechnology, Olivette, MO) while shaking at 37 °C, 250 rpm for 30 min. Cell lysis was completed by sonication with a probe sonicator (QSonica, Newton, CT).

The artificial proteins were purified based on the inverse temperature transition associated with elastin-like polypeptides (ELPs). To prevent chain extension of the target proteins by disulfide formation, 0.1% (v/v) β-mercaptoethanol (β-ME) (Sigma) was added to the lysate. The lysate was cooled to 4 °C and clarified by centrifugation at 39,000 *g*, 4 °C for 1 hr. To depress the lower critical solution temperature of the hydrophilic ELPs, sodium

chloride was added to the supernatant to a final concentration of 2 M. After shaking at 37 °C for 1 hr, aggregated proteins were pelleted by centrifugation at 39,000 *g*, 37 °C for 1 hr. The target proteins were extracted from the pellet with water containing 0.1% (v/v) β-ME overnight at 4 °C. This process was repeated twice but the β-ME was omitted. Instead, after the second and third temperature cycles, the proteins were reduced with 5 mM tris(hydroxypropyl)phosphine (THP) (Santa Cruz Biotechnology, Dallas, TX) at 4 °C. Residual salt and reducing agents were removed by desalting using Zeba 7K MWCO columns (Thermo Fisher Scientific, Waltham, MA) equilibrated with degassed distilled and deionized water (ddH_2O). The proteins were lyophilized and stored under argon at -80 °C. Purified proteins were characterized by SDS-PAGE and MALDI-TOF to confirm their purity and molecular weight. Typical yields of EPE and ERE were 200 mg L^{-1} and 100 mg L^{-1} of culture, respectively.

Expression of PEP from the pET15b plasmid requires the BL21(DE3) *E. coli* strain (Novagen) containing the T7 RNA polymerase. Protein expression was performed in Terrific broth containing 100 μg mL^{-1} ampicillin. Cells were grown at 37 °C until the OD_{600} reached 0.9-1.0. Protein expression was induced with the addition of 1 mM IPTG and proceeded for 5 hr, after which time the cells were harvested and lysed with 8 M urea. Cells suspended in 8 M urea were subjected to three freeze/thaw cycles followed by sonication. Clarified lysates were obtained by centrifugation and PEP was isolated by affinity chromatography with nickel nitriloacetic acid (NiNTA) resin (Qiagen) under denaturing conditions. The elution fractions containing purified PEP were combined, dialyzed against distilled water for 48 hr at 4 °C using a MWCO 12,000-14,000 membrane (Spectrum Laboratories, Rancho Dominquez, CA), and lyophilized. Yields of PEP were approximately 100 mg per liter of culture.

MALDI-TOF mass spectrometry Lyophilized proteins (EPE, ERE, EPE L44A, and PEP) were dissolved in ddH_2O at a concentration of 10 mg mL^{-1}. The protein solutions were mixed with sinapinic acid matrix (10 mg mL^{-1} in 6:3:1 water:acetonitrile:1% trifluoroacetic

acid) at ratios varying from 4:1 to 10:1 (matrix to protein). The mixtures were spotted on the MALDI sample plate and allowed to dry. Spectra were acquired on a Voyager DE Pro spectrometer (Applied Biosystems, Carlsbad, CA).

Ellman's assay Ellman's reagent, 5,5'-dithio-bis-(2-nitrobenzoic acid), (Sigma) was used to measure the concentration of free thiols [1]. Briefly, protein was dissolved at a concentration of 5 mg mL^{-1} in reaction buffer (100 mM sodium phosphate, 1 mM EDTA, pH 8.0). In a cuvette, the protein solution (125 μL) and Ellmans' reagent (50 μL, 5 mg mL^{-1} in reaction buffer) were added to the reaction buffer (2.5 mL). The reaction was incubated for 15 min and the absorbance at 412 nm was measured on a Cary 50 Bio UV/VIS spectrophotometer (Varian, Palo Alto, CA). The thiol concentration was calculated using the extinction coefficient 14,150 M^{-1} cm^{-1} [2].

Swelling measurements Swollen hydrogels were blotted with filter paper to remove excess buffer, weighed on an analytical balance (Mettler Toledo, Columbus, OH) to obtain the swollen mass, and placed in ddH_2O for 48 hr with several changes to remove salts. They were then transferred to microcentrifuge tubes, frozen with liquid nitrogen, and lyophilized to obtain the dry mass. The mass swelling ratio, Q_m, is equal to the swollen mass divided by the dry mass. The same procedure was followed for EPE and ERE gels swollen in PBS containing 8 M urea or 6 M guanidinium chloride.

Supplementary Text

Models for rheological data The dynamic storage moduli of ERE:EPE gels were fit to a Maxwell model for a viscoelastic solid using the curve fitting tool in MATLAB R2014a (Mathworks, Torrance, CA). In this model, the storage and loss moduli are given by the following expressions:

$$G'(\omega) = G_0 + G\frac{(\omega\tau)^2}{1+(\omega\tau)^2} \qquad (1)$$

$$G''(\omega) = G\frac{\omega\tau}{1+(\omega\tau)^2} \qquad (2)$$

where G_0 is the component of the storage modulus that is independent of the oscillation frequency, G describes the component of the storage modulus that varies with the oscillation frequency, and τ is the characteristic relaxation time.[3, 4] In the high frequency limit, the plateau storage modulus $G'(\infty)$ is equal to $G_0 + G$. In the low frequency limit, the plateau storage modulus $G'(0) = G_0$. The parameters G and τ determined by fitting the storage modulus to Eq. 1 were also used to evaluate the loss modulus by Eq. 2. The Maxwell model is only an approximation for the frequency-dependent behavior of ERE:EPE gels. The relaxations observed in the experimental data are broader than those predicted for a single Maxwell mode. Such broad relaxations in physical protein gels were better fit with a stretched exponential model by Tang *et al.*[5] However, this analysis still provides a useful method to quantify the plateau values and relaxation times in the dynamic storage moduli.

For stress relaxation experiments, the relaxation function, $G(t)$, was fit to a single exponential model,

$$G(t) = G\exp\left(-\frac{t}{\tau}\right) + G_\infty \qquad (3),$$

a double exponential model,

$$G(t) = G_1\exp\left(-\frac{t}{\tau_1}\right) + G_2\exp\left(-\frac{t}{\tau_2}\right) + G_\infty \qquad (4),$$

and a stretched exponential (or Kohlrausch-Williams-Watts, KWW) model,[5]

$$G(t) = G\exp\left(-\left(\frac{t}{\tau_{KWW}}\right)^{\beta}\right) + G_\infty \qquad (5).$$

In the stretched exponential model, the exponent β varies between 0 and 1. It can be used along with the parameter τ_{KWW} to calculate the mean relaxation time, $\langle\tau\rangle$,

$$\langle\tau\rangle = \frac{\tau_{KWW}}{\beta}\Gamma\left(\beta^{-1}\right) \qquad (6)$$

where $\Gamma(\beta^{-1})$ is the gamma function evaluated at β^{-1}.

Estimation of the theoretical molecular weight between cross-links (M_c) based on the protein sequences The calculation of the expected or theoretical molecular weight between cross-links in ideal ERE:EPE gels is based on the protein sequences and the molar ratio of each protein in the gelation mixture. In a fully cross-linked EPE gel, in which all chain ends are linked by PEG-4VS and all P midblocks participate in physical cross-links, the average molecular weight between cross-links is calculated as the average of the molecular weight of the segment N-terminal to the P domain and the segment C-terminal to the P domain. These chains are predominantly elastin-like repeats and PEG. The N- and C-terminal segments of EPE have molecular weights of 8763 Da and 7563 Da, respectively. Each arm of the 4-arm PEG vinyl sulfone has a molecular weight of 10,000/4 = 2500 Da.

$$M_c = (0.5)(8763 + 2500) + (0.5)(7563 + 2500) = 10{,}663 \text{ Da}$$

In a fully cross-linked ERE gel, the average molecular weight between cross-links is calculated as the molecular weight of the protein between the cysteine residues (18,058 Da) plus two PEG arms (2 x 2500 Da).

$$M_c = 18{,}058 + 5000 = 23{,}058 \text{ Da}$$

In 75:25, 50:50, and 25:75 gels, the average molecular weight between cross-links is calculated as the average of the result for EPE gels (10,663 Da) and the result for ERE gels (23,058 Da) weighted by the molar fraction of each protein in the cross-linking precursor. This assumes an equal reactivity and that all EPE chains form physical cross-links. The molar ratio of ERE and EPE is calculated from the molar masses of the two proteins. For 50:50 gels, this gives

$$\frac{(0.5 \text{ mL})\left(\frac{150 \text{ mg ERE}}{\text{mL}}\right)\left(\frac{\text{mmol ERE}}{18{,}476 \text{ mg ERE}}\right)}{(0.5 \text{ mL})\left(\frac{150 \text{ mg EPE}}{\text{mL}}\right)\left(\frac{\text{mmol EPE}}{21{,}464 \text{ mg EPE}}\right)} = 1.16 \frac{\text{mol ERE}}{\text{mol EPE}}$$

$$M_c = \frac{(1.16)(23{,}058) + (1)(10{,}663)}{1.16 + 1} = 17{,}338 \text{ Da}$$

Likewise for 75:25

$$\frac{(0.75 \text{ mL})\left(\frac{150 \text{ mg ERE}}{\text{mL}}\right)\left(\frac{\text{mmol ERE}}{18{,}476 \text{ mg ERE}}\right)}{(0.25 \text{ ml})\left(\frac{150 \text{ mg EPE}}{\text{mL}}\right)\left(\frac{\text{mmol EPE}}{21{,}464 \text{ mg EPE}}\right)} = 3.49 \frac{\text{mol ERE}}{\text{mol EPE}}$$

$$M_c = \frac{(3.49)(23{,}058) + (1)(10{,}663)}{3.49 + 1} = 20{,}273 \text{ Da}$$

and 25:75

$$\frac{(0.25 \text{ mL})\left(\frac{150 \text{ mg ERE}}{\text{mL}}\right)\left(\frac{\text{mmol ERE}}{18{,}476 \text{ mg ERE}}\right)}{(0.75 \text{ mL})\left(\frac{150 \text{ mg EPE}}{\text{mL}}\right)\left(\frac{\text{mmol EPE}}{21{,}464 \text{ mg EPE}}\right)} = 0.39 \frac{\text{mol ERE}}{\text{mol EPE}}$$

$$M_c = \frac{(0.39)(23{,}058) + (1)(10{,}663)}{0.39 + 1} = 14{,}095 \text{ Da}$$

These values are listed in column six of Table S4. The theoretical value of M_c decreases as the fraction of EPE is increased because more physical cross-links are expected to be present.

Estimation of the experimental molecular weight between cross-links (M_c) based on rubber elasticity theory

Affine approximation The experimental molecular weight between cross-links is calculated from the equilibrium swelling ratio and the high frequency plateau value of *G'*. For a network cross-linked in the presence of solvent[6]:

$$G = RT \frac{C_0}{M_c}\left(1 - \frac{2M_c}{M_n}\right)\left(\frac{\varphi}{\varphi_0}\right)^{1/3} \qquad (7)$$

where

G = shear modulus, kPa (taken here as the high frequency plateau storage modulus, G'_∞)

R = gas constant, 8314 kPa cm^3 mol^{-1} K^{-1}

T = temperature, K

C_0 = initial (preparation state) polymer concentration, g cm^{-3}

M_c = average molecular weight between cross-links, g mol^{-1}

M_n = number average molecular weight of the polymer chain, g mol^{-1}

φ_0, φ = initial (preparation state) polymer volume fraction, equilibrium swelling polymer volume fraction

End effects are assumed to be negligible in the end-linked networks, meaning that

$$\left(1-\frac{2M_c}{M_n}\right)\approx 1$$

and

$$G'_\infty = RT\frac{C_0}{M_c}\left(\frac{\varphi}{\varphi_0}\right)^{1/3} \qquad (8).$$

An equivalent version of Eq. 8 was used to estimate the molar concentration of elastically effective chains in end-linked PEG-*co*-peptide hydrogels swollen to equilibrium.[7]

The swollen polymer volume fractions are determined from the mass swelling ratio, Q_m, assuming a gel density of 1 g cm^{-3} (ie. mostly water) and a dry polymer density weighted by the mass fraction of protein and PEG-4VS (approximately 0.8 and 0.2, respectively). The density of the artificial proteins is taken to be that of elastin, 1.3 g cm^{-3}, and the density of 10,000 g mol^{-1} PEG is 1.2 g cm^{-3}.[8, 9]

$$\rho_{\text{polymer}} = (0.8)(\rho_{\text{protein}})+(0.2)(\rho_{\text{PEG}})$$

$$(0.8)(1.3\ \text{g/cm}^3)+(0.2)(1.2\ \text{g/cm}^3)=1.28\ \text{g/cm}^3$$

The swollen volume fraction is defined as the volume of polymer divided by the volume of the gel.

$$\varphi=\frac{V_{\text{polymer}}}{V_{\text{gel}}}=\frac{\frac{m_{\text{polymer}}}{\rho_{\text{polymer}}}}{\frac{m_{\text{gel}}}{\rho_{\text{gel}}}}=\frac{1}{Q_m}\frac{\rho_{\text{gel}}}{\rho_{\text{polymer}}} \qquad (9)$$

$$\varphi_{\text{EPE}}=\frac{1}{Q_m}\frac{\rho_{\text{gel}}}{\rho_{\text{polymer}}}=\frac{1}{13.5}\left(\frac{1}{1.28}\right)=0.058$$

$$\varphi_{\mathrm{ERE}} = \frac{1}{Q_m}\frac{\rho_{\mathrm{gel}}}{\rho_{\mathrm{polymer}}} = \frac{1}{19.7}\left(\frac{1}{1.28}\right) = 0.040$$

The volume fraction φ_0 in the preparation state prior to swelling can be calculated from the polymer (protein and PEG-4VS) concentration in the cross-linking reaction assuming that all chains are incorporated into the network.

$$C_0 = 15\ \mathrm{wt\%} = 150\frac{\mathrm{mg}}{\mathrm{mL}} = 0.150\frac{\mathrm{g}}{\mathrm{cm}^3}$$

$$\varphi_0 = C_0\frac{1}{\rho_{\mathrm{polymer}}} = 0.150\frac{\mathrm{g}}{\mathrm{cm}^3}\left(\frac{\mathrm{cm}^3}{1.28\ \mathrm{g}}\right) = 0.117$$

Alternatively, the dry polymer mass following lyophilization can be divided by the cross-linking volume (40 μL) to estimate the initial concentration of polymer in the network before swelling. This method does not include chains that were not incorporated into the network (ie. the sol fraction) but were instead lost during swelling. The typical dry mass measured after lyophilizing the hydrogels was 4.3-5 mg, indicating that 72-83% of the original 6 mg of polymer remained. This is consistent with the results of the non-reducing SDS PAGE analysis and Ellman's assay (Figure S4) suggesting a cyclic fraction of approximately 20%. Cyclic proteins should not be able to participate in covalent cross-linking and should be removed from the gel during swelling.

$$C_0 = \frac{m_{\mathrm{polymer}}}{V_0} \qquad (10)$$

$$C_{0,\mathrm{EPE}} = \frac{0.0043\ \mathrm{g}}{0.040\ \mathrm{cm}^3} = 0.1075\ \mathrm{g/cm}^3$$

$$C_{0,\mathrm{ERE}} = \frac{0.0049\ \mathrm{g}}{0.040\ \mathrm{cm}^3} = 0.1213\ \mathrm{g/cm}^3$$

$$\varphi_{0,\mathrm{EPE}} = C_{0,\mathrm{EPE}}\frac{1}{\rho_{\mathrm{polymer}}} = 0.1075\frac{\mathrm{g}}{\mathrm{cm}^3}\left(\frac{\mathrm{cm}^3}{1.28\ \mathrm{g}}\right) = 0.084$$

$$\varphi_{0,\mathrm{ERE}} = C_{0,\mathrm{ERE}} \frac{1}{\rho_{\mathrm{polymer}}} = 0.1213 \frac{\mathrm{g}}{\mathrm{cm}^3} \left(\frac{\mathrm{cm}^3}{1.28\ \mathrm{g}} \right) = 0.095$$

Using the high frequency storage moduli from the rheological experiments, the average molecular weight between cross-links can be determined by rearranging Eq. 8 to give Eq. 11.

$$M_c = RT \frac{C_0}{G'_\infty} \left(\frac{\varphi}{\varphi_0} \right)^{1/3} \tag{11}$$

$$M_{c,\mathrm{EPE}} = \left(8314 \frac{\mathrm{kPa\ cm}^3}{\mathrm{mol\ K}} \right) (298\ \mathrm{K}) \left(\frac{0.1075\ \mathrm{g/cm}^3}{13.5\ \mathrm{kPa}} \right) \left(\frac{0.058}{0.084} \right)^{1/3} = 17{,}353\ \mathrm{g/mol}$$

$$M_{c,\mathrm{ERE}} = \left(8314 \frac{\mathrm{kPa\ cm}^3}{\mathrm{mol\ K}} \right) (298\ \mathrm{K}) \left(\frac{0.1213\ \mathrm{g/cm}^3}{4.5\ \mathrm{kPa}} \right) \left(\frac{0.040}{0.095} \right)^{1/3} = 49{,}645\ \mathrm{g/mol}$$

Phantom network approximation In the phantom network approximation, the cross-links are not fixed in space as in the affine approximation but instead fluctuate. This decreases the free energy per chain and therefore decreases the modulus. In an ideal network, the phantom network modulus is related to the affine modulus from Eq. 7 through the cross-linker functionality, f.[10]

$$G_{\mathrm{phantom}} = \left(1 - \frac{2}{f} \right) G_{\mathrm{affine}} \tag{12}$$

The PEG-4VS cross-linker has a functionality of 4. The physical cross-links have a functionality of 5. The molar ratio of chemical to physical cross-links can be used to calculate an average cross-linker functionality, $\overline{f}$, which varies between $\overline{f}$ = 4.3 for EPE gels and $\overline{f}$ = 4 for ERE gels. In this case the prefactor in Eq. 12 becomes 0.53 for EPE gels and 0.5 for ERE gels. Including this adjustment in Eq. 11 gives M_c based on the phantom network model, which is approximately one-half of the value from the affine model.

The calculated values of M_c based on the affine approximation and the phantom network approximation are listed in Table S4 columns 4 and 5, respectively. Both models have been used previously to describe networks formed by cross-linking PEG macromers.[11-

[13] In these studies, the experimental data were best modeled by the phantom approximation at lower initial polymer volume fractions and the affine approximation at higher initial polymer volume fractions. With the exception of ERE gels, the theoretical values of M_c of the gels prepared in this work fall between the values calculated by the affine model and by the phantom network model. In ERE gels, the theoretical value is close to the value calculated by the phantom network model. One possible explanation for this observation is that the covalent cross-links fluctuate as modeled by the phantom approximation whereas the larger physical cross-links do not.

The estimation of M_c described here assumes that a perfect network is formed and that the chains between cross-links behave ideally, which is almost certainly not the case. Non-idealities such as loops, missed cross-links (chemical or physical), dangling chains, and entanglements likely exist in the gels. These effects might explain some of the discrepancies between the theoretical values of M_c and the calculated values of M_c.

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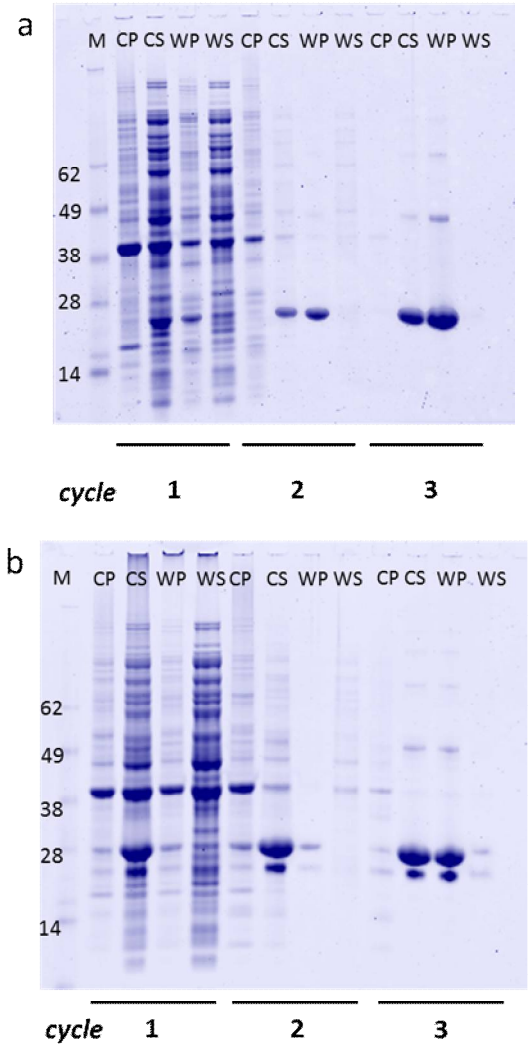
Table S1. Amino acid sequences for artificial proteins ERE, EPE, PEP, and EPE L44A.

Protein	**Sequence**
ERE	MRCSSHHHHHHVDGHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVP GVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPG VGELYAVTGRGDSPASSAPIATSVPGVGVPGVGVPGEGVPGVGVPGV GVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVG VPGVGVPGGLLDGPQGIWGQLECM
EPE	MRCSSHHHHHHVDGHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVP GVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPG VGELGSGLGSAPQMLRELQETNAALQDVRELLRQQVKEITFLKNTVM ESDASKLNTSVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGV PGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGGLLE CM
PEP	MKGSHHHHHHHVDGSGSGSGSGSGSGAPQMLRELQETNAALQDVRE LLRQQVKEITFLKNTVMESDASGSGSGSGSGSGLDGHGVGVPGVG VPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGV PGVGVPGVGVPGEGVPGVGVPGVGELYAVTGRGDSPASSAPIATSVP GVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPG VGVPGVGVPGVGVPGEGVPGVGVPGVGVPGGLLDGSGSGSGSGSGS GAPQMLRELQETNAALQDVRELLRQQVKEITFLKNTVMESDASGSGS GSGSGSGSGLEMHHHHHHK
EPE L44A	MRCSSHHHHHHVDGHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVP GVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPG VGELGSGLGSAPQMLRELQETNAA**A**QDVRELLRQQVKEITFLKNTVM ESDASKLNTSVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGV PGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGGLLE CM

Figure S1. SDS-PAGE of artificial proteins during inverse temperature cycling. ERE (a), EPE (b), and EPE L44A (c) were purified by three cycles of inverse temperature cycling. After each centrifugation step, samples of the supernatant and pelleted fractions were saved. The target proteins are expected to be soluble in the cold step (4 °C, low ionic strength) and insoluble in the warm step (37 °C, 2 M NaCl). For SDS-PAGE analysis, the proteins in the pelleted fractions were extracted in 8 M urea. The samples were boiled in SDS loading buffer with 2.5% (v/v) β-ME to reduce disulfide bonds. The gel was stained with colloidal blue stain (Life Technologies, Carlsbad, CA) to visualize proteins. After 3 cycles of cold and warm spins, the target proteins were successfully purified from the *E. coli* lysates.

CP: cold pellet WP: warm pellet
CS: cold supernatant WS: warm supernatant
M: SeeBlue protein marker (molecular weights in kDa)



C

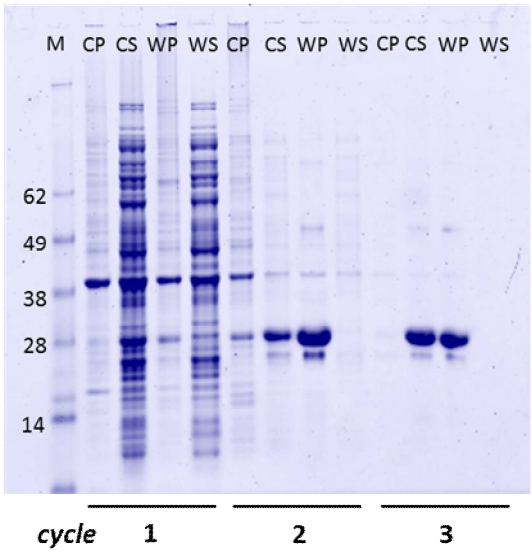
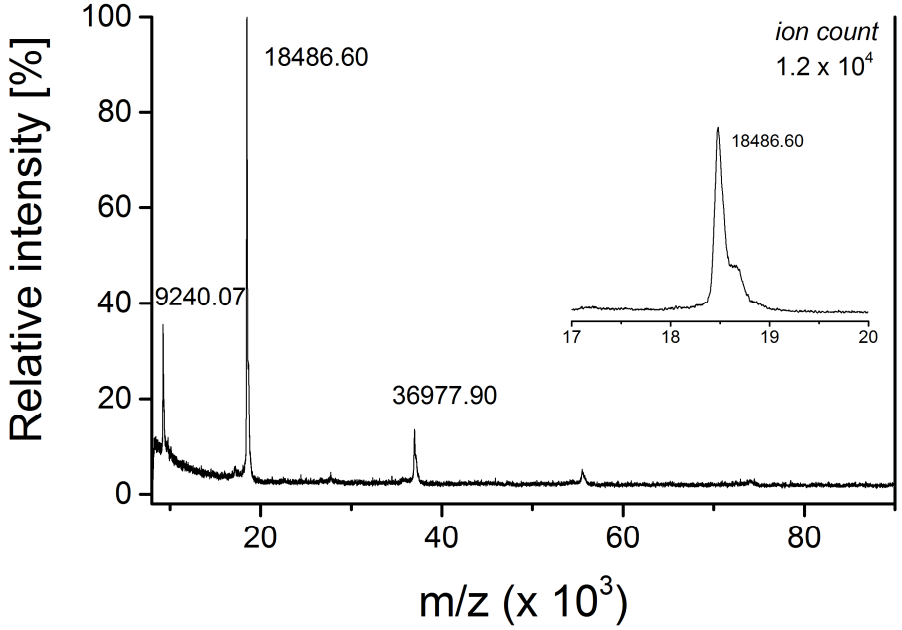


Figure S2. MALDI-TOF mass spectrometry of purified artificial proteins. (a) ERE (calculated 18474, observed 18487), (b) EPE (calculated 21465, observed 21464), (c) EPE L44A (calculated 21422, observed 21428). Peaks assigned to the doubly charged species and dimers are also labeled.

a



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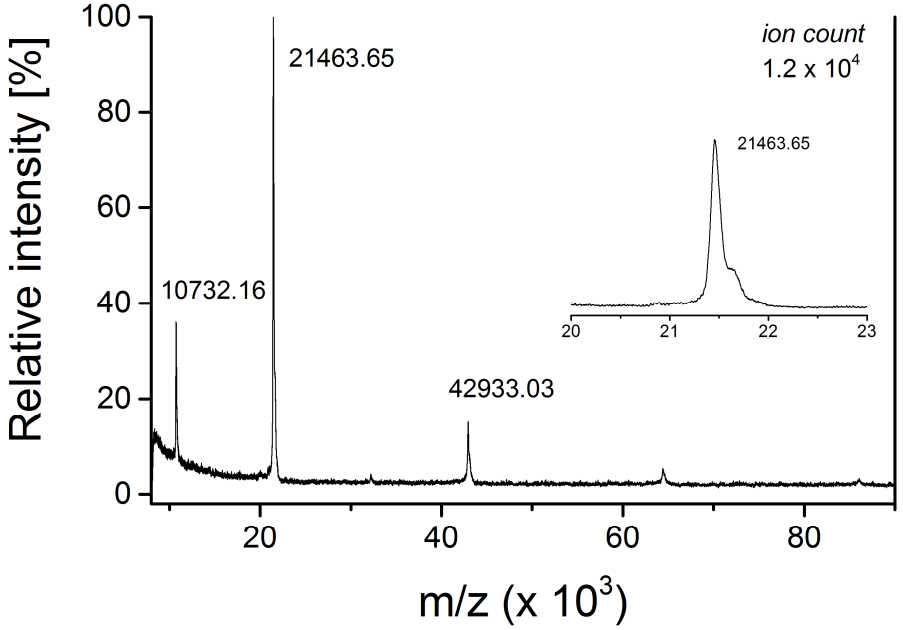

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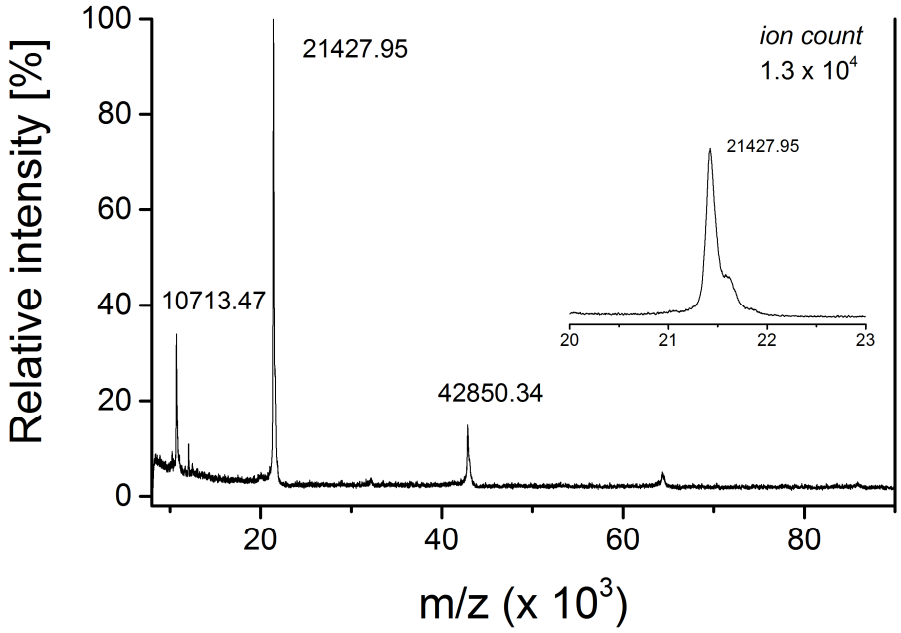

Relative intensity [%]
100
80
60
40
20
0
21427.95
10713.47
42850.34
ion count
1.3 x 10^4
21427.95
20
21
22
23
20
40
60
80
m/z (x 10^3)

Figure S3. Ellman's assay and non-reducing SDS-PAGE for purified artificial proteins. (a) Ellman's assay measures the concentration of free thiols in the protein preparations (n = 3, avg ± sd). The measured thiol concentration for each protein is approximately 80% of the expected concentration based on the amount of protein and assuming two cysteines per protein. SDS-PAGE performed on samples without reducing agent demonstrates that EPE, ERE (b) and EPE L44A (c) are primarily monomeric after desalting and lyophilization. However, approximately 20% of the monomeric protein is cyclized, consistent with the results of Ellman's assay in (a). The cyclized proteins run at a lower apparent molecular weight than the linear proteins and are absent in control lanes containing samples that were boiled in loading buffer containing 2.5% (v/v) β-ME as a disulfide reductant. The gels were stained with InstantBlue (Expedeon, San Diego, CA).

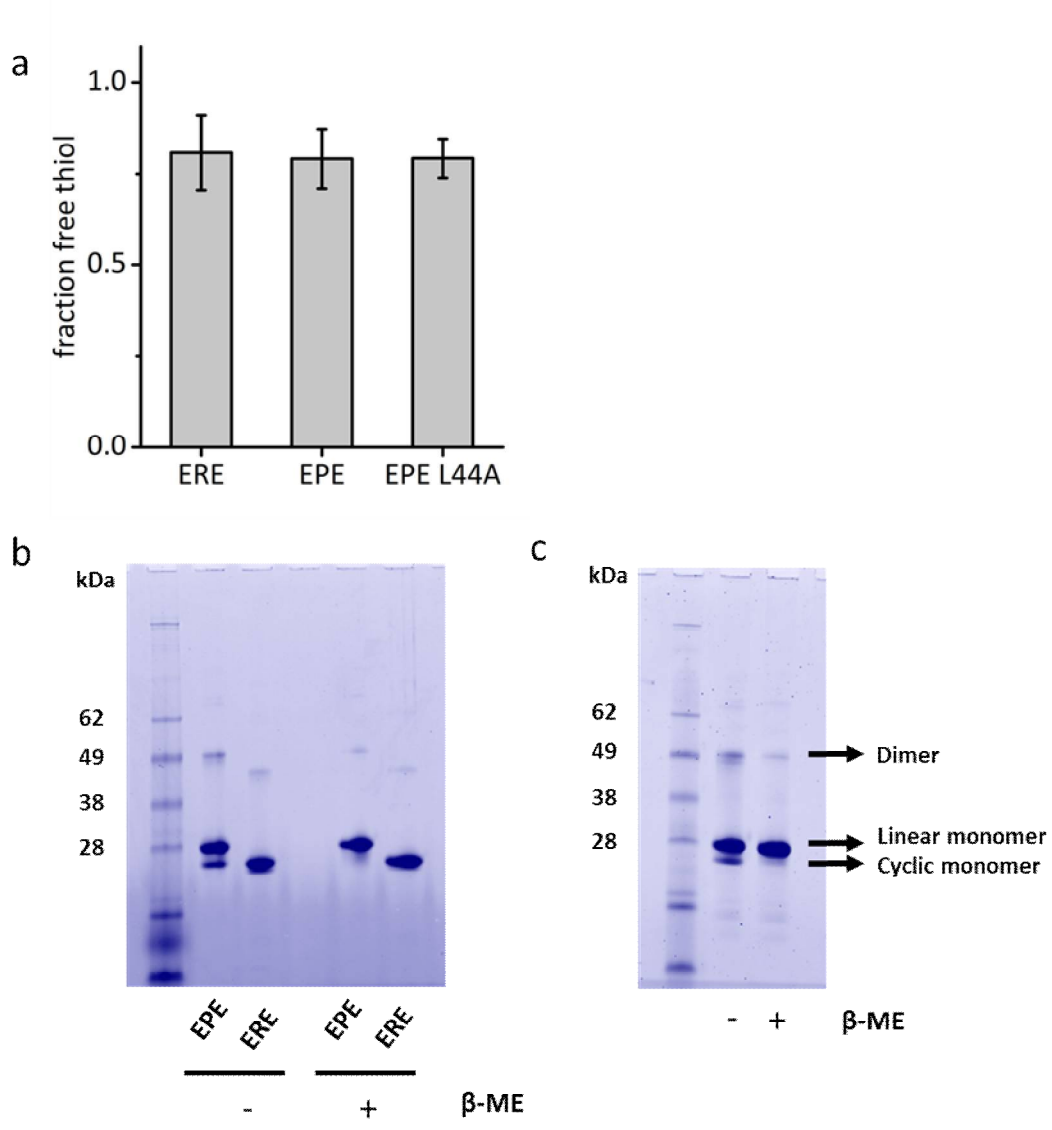
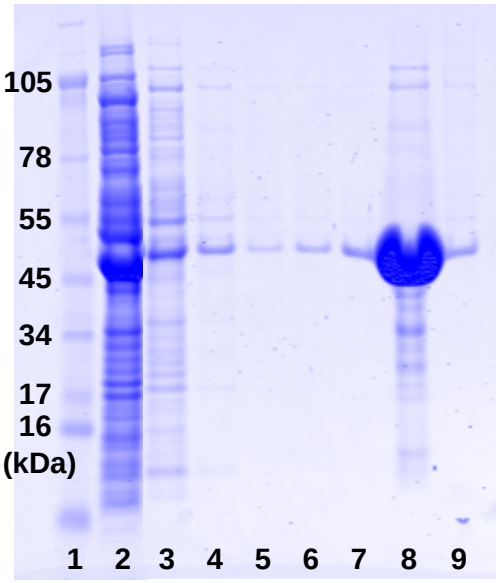


Figure S4. SDS-PAGE and MALDI-TOF for PEP. (a) SDS-PAGE showing the purification of PEP by NiNTA affinity chromatography. (1) Molecular weight marker. (2) Column flow through. (3-5) Column wash with 8 M urea, pH 8.0, 10 mM imidazole. (6-7) Column wash with 8 M urea, pH 6.5, 10 mM imidazole. (8-9) Eluted protein with 8 M urea, pH 3.5, 250 mM imidazole. (b) MALD-TOF spectrum of PEP (calculated 32047, observed 32060). Peaks assigned to the doubly charged species and dimers are also labeled.

a



b

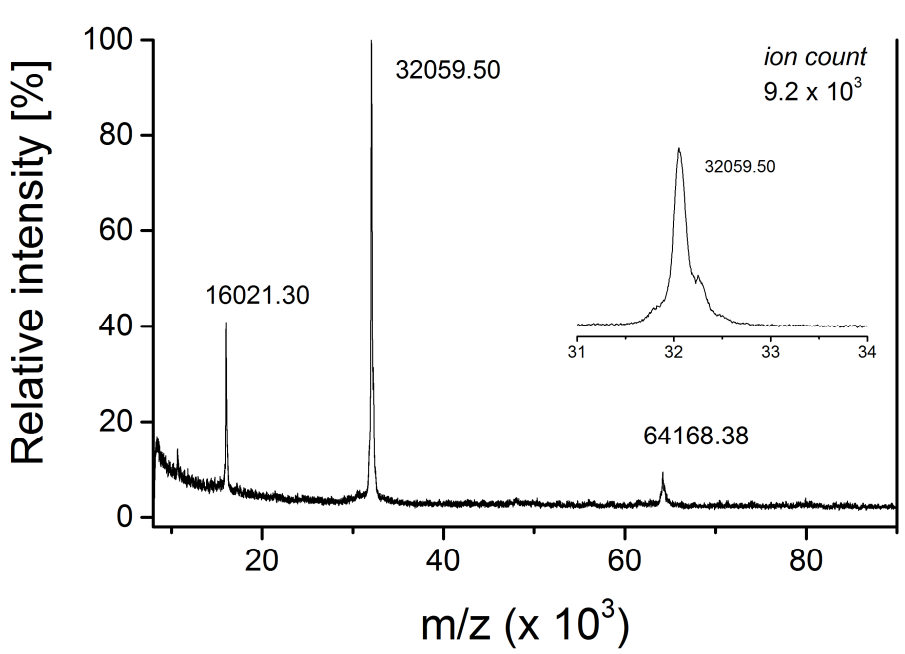


Figure S5. Dynamic moduli Maxwell fits for ERE:EPE hydrogels. Maxwell model fits (solid lines) and experimental data (filled symbols) are shown for hydrogels prepared from EPE, ERE, and mixtures of the two proteins. The parameters determined by fitting Eq. 1 to the data for *G'* in (a) were also used to evaluate the loss moduli by Eq. 2 in (b). Note that the relaxation is broader than expected for a single Maxwell mode.

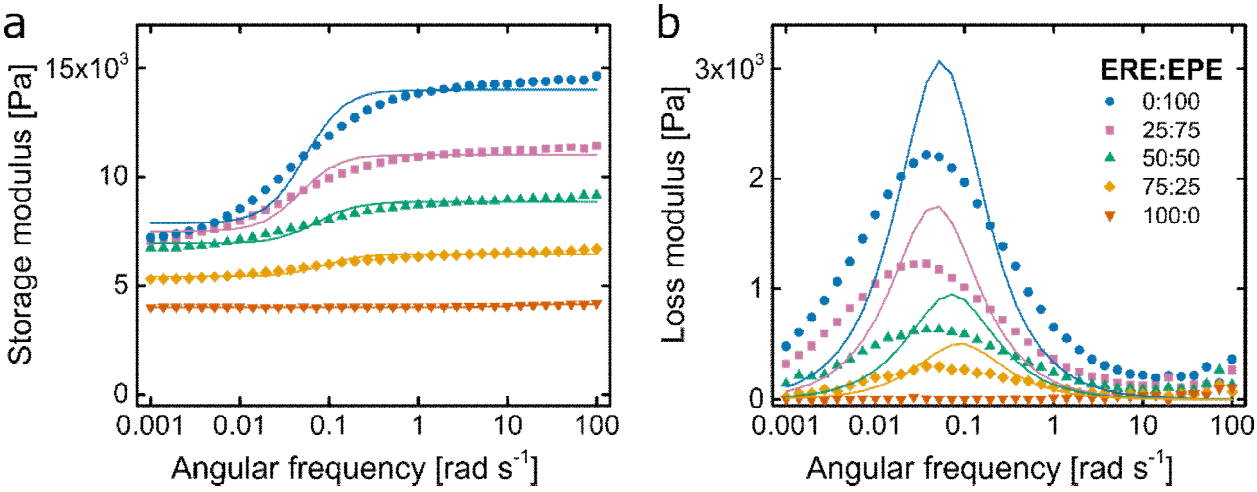


Table S2. Maxwell model parameters for ERE:EPE hydrogels. The experimental data were fit to Eq. 1 to obtain the parameters G, G_0, and τ. These parameters were then used to generate the curves shown in Figure S5 a and b and to evaluate the plateau moduli $G'(\infty)$ and $G'(0)$ in Figure 3c.

Composition (ERE:EPE)	***G* (Pa)**	***G*$_0$ (Pa)**	**τ (sec/rad)**	**$(2\pi)\tau$ (sec)**
100:0	135 ± 16	4391 ± 360	0.11 ± 0.02	0.66 ± 0.11
75:25	1023 ± 64	5467 ± 120	10.6 ± 1.4	66.8 ± 9.1
50:50	2087 ± 217	6144 ± 765	15.1 ± 1.0	94.9 ± 6.2
25:75	3528 ± 25	7334 ± 217	23.8 ± 2.2	149.5 ± 14.1
0:100	6517 ± 390	6901 ± 572	18.7 ± 0.8	114.0 ± 5.7

Figure S6. Relaxation function for an EPE hydrogel fit to single exponential, double exponential, and stretched exponential models. The relaxation function $G(t)$ is plotted against time for a 1% strain over the duration of 2 hours. The dashed lines are fits of the experimental data to a single exponential model (Eq. 3, blue), a double exponential model (Eq. 4, green) and a stretched exponential model (Eq. 5, orange). All of the models capture the short and long time plateau behavior, but the additional parameters in the double exponential model or stretched exponential model are required to fit the broadness of the relaxation.

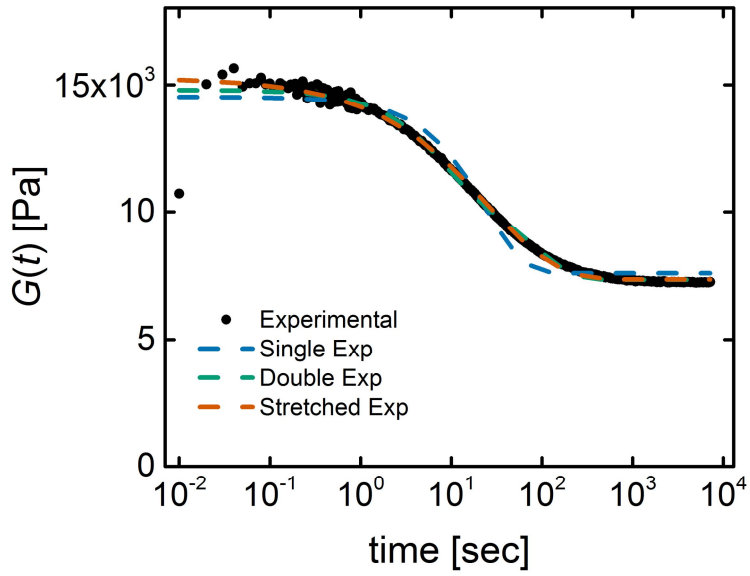


Figure S7. Stress relaxation experiments for ERE:EPE hydrogels. (a) Representative stress relaxation curves are shown for hydrogels prepared from EPE, ERE, and mixtures of the two proteins. The relaxation function $G(t)$ is plotted against time for a 1% strain over the duration of 2 hours. The dashed lines are fits of the experimental data to the stretched exponential model given in Eq. 5. (b) The stretched exponential fit was evaluated at the limits $t = 0$ and $t \rightarrow \infty$ to give $G(0)$ and $G(\infty)$, respectively ($n \geq 3$, avg ± sd). The relaxation function at these limits is in agreement with $G'(\infty)$ and $G'(0)$ from the frequency sweep experiments. The ERE hydrogel does not exhibit significant stress relaxation and was not fit to the stretched exponential model.

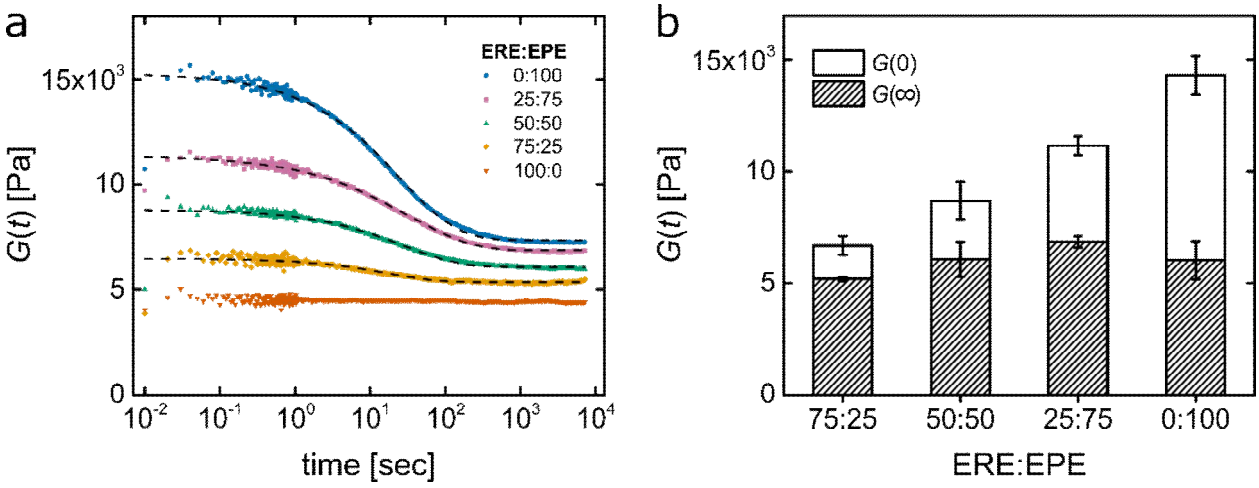


Table S3. Stretched exponential (KWW) parameters for stress relaxation experiments with ERE:EPE hydrogels. The experimental data were fit to Eq. 5 to obtain the parameters (G_∞, G, τ_{KWW}, and β). These parameters were used to generate the dashed curves in Figure S7a and to evaluate the plateau values of $G(t)$ shown in Figure S7b.

Composition (ERE:EPE)	G (Pa)	G_∞ (Pa)	τ_{KWW} (s)	$\langle\tau\rangle$ (s)	β
75:25	1480 ± 404	5188 ± 192	13.9 ± 3.7	49.3 ± 38.8	0.480 ± 0.179
50:50	2633 ± 291	6056 ± 1105	25.0 ± 2.5	47.8 ± 14.3	0.532 ± 0.074
25:75	4312 ± 155	6840 ± 349	35.3 ± 4.0	63.2 ± 8.3	0.534 ± 0.014
0:100	8171 ± 343	6020 ± 848	27.7 ± 4.4	47.4 ± 7.5	0.551 ± 0.011

Table S4. Calculated average molecular weight between cross-links (M_c). The swelling ratios (column 2) and high frequency plateau storage moduli (column 3) were used to compute the average molecular weight between cross-links by the affine approximation (column 4) and the phantom network approximation (column 5). The values are compared to the theoretical molecular weight between cross-links determined from the protein sequences (column 6). With the exception of the covalent ERE network, the theoretical values of M_c fall between the values calculated by the affine and phantom network models.

Composition (ERE:EPE)	Q_m	$G'(\infty)$ **(kPa)**	M_c **(kg/mol) (affine)**	M_c **(kg/mol) (phantom)**	M_c **(kg/mol) (sequence)**
100:0	19.7	4.5	49.6	24.8	23.1
75:25	18.1	6.5	33.8	17.2	20.3
50:50	16.1	8.2	28.4	14.7	17.3
25:75	14.7	10.9	20.9	11.0	14.1
0:100	13.5	13.4	17.3	9.3	10.7

Figure S8. Disruption of physical cross-linking in EPE hydrogels by buffer containing guanidinium chloride as protein denaturant. EPE was cross-linked with PEG-4VS and swollen to equilibrium in PBS containing 6 M guanidinium chloride (pH 7.4). The loss of physical cross-linking is similar to the behavior of EPE gels swollen in PBS containing 8 M urea.

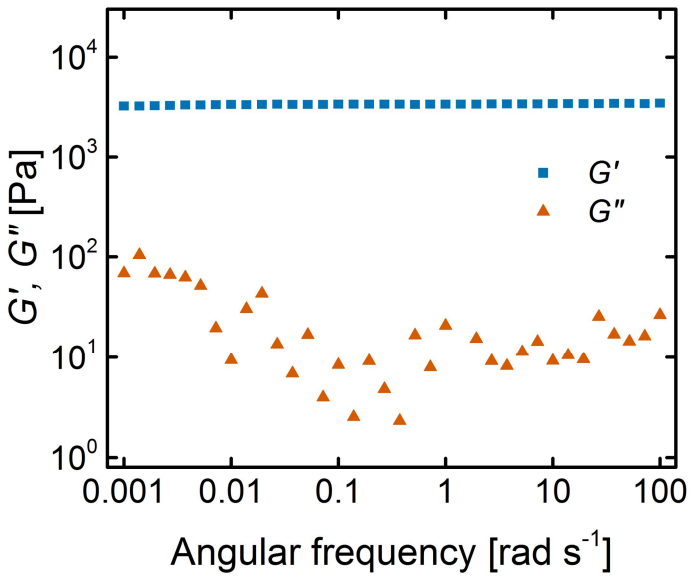


Figure S9. Linear rheology of PEP hydrogels. Small amplitude oscillatory shear rheology frequency sweeps for 7% (w/v) PEP in PBS, pH 7.4, 25 °C. The data were acquired at 1% strain amplitude (blue) and 10% strain amplitude (red). Both strain amplitudes are in the linear viscoelastic region, and the dynamic moduli are nearly identical under each condition. In the frequency sweep at 1% strain amplitude, however, the stress (and torque measured by the transducer) falls below the minimum value for accurate measurement. These data points are indicated by open symbols. For this reason, we have reported the results at 10% strain amplitude in Figure 2c.