Influence of End-Block Dynamics on Deformation Behavior of Thermoresponsive Elastin-like Polypeptide Hydrogels

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ABSTRACT: End-block association dynamics are known to influence deformation behavior in block copolymer systems. The ability to obtain precisely sequence controlled systems can help inform this deformation behavior, which is often influenced by dispersity in sequence and molar mass. Elastin-like polypeptides (ELPs) are a class of protein that consists of a sequence of five amino acids (XPGVG) that thermoresponsively aggregate in solution. These ELPs can be used as end-blocks in triblock fusion proteins with coiled-coil associating midblock domains to result in dual-associating, network-forming materials. By modifying the standard glycine-containing ELP sequence (XPGVG) to instead contain alanine in the third position of the repeat sequence (XPAVG), it is possible to improve the properties of the material in both shear and extension. In extension at 50 °C, the alanine-containing triblock (A10P4A10) and the glycine-containing triblock (G10P4G10) have similar Young’s moduli. However, while G10P4G10 yields and breaks within 5 strain units, A10P4A10 plastically deforms to an ultimate strain at break of over 15 strain units and a tensile stress of almost 90 kPa. In shear, G10P4G10 exhibits a clear stress overshoot at less than one strain unit before plateauing at a steady-state shear stress of 0.43 kPa, while the shear stress of A10P4A10 monotonically increases to a shear stress of 48.4 kPa after 2.4 strain units. In shear, these differences in behavior correlate to rearrangement of G10P4G10’s nanostructure during viscous-dominated flow, while the nanostructure of A10P4A10 initially elastically deforms. These differences are a function of the relative network relaxation time scales, where the presence of the faster glycine-containing ELP end-block sequence decreases the network relaxation time such that it occurs on the same order of magnitude as the imposed flow.

INTRODUCTION

Associating block copolymer networks are found in a wide range of technologies from self-healing biomaterials in tissue engineering applications to enhanced oil recovery and pressuresensitive adhesives. In all of these applications, the materials are subjected to highly nonlinear flows, and a better understanding of the flow and deformation behavior is crucial to material design. For instance, in tissue engineering it can be necessary to deliver therapeutic agents via a syringe or other implement where the shear forces on the therapeutic could cause damage and/or loss of efficacy. The addition of associating networks to drilling fluids is often used to increase the areal sweep efficiency of the fluid. Improving the performance of these materials will be enabled by an increased understanding of how they deform.

The deformation behavior of block copolymers in shear has been extensively studied in the literature. Block copolymers are known to exhibit different mechanical behavior as a function of their morphology in both the melt and gel state, especially at longer times. Changing the relative rate of shear on the system results in different deformation behavior, where the presence of shear on time scales that are greater than the defect relaxation time results in affine network deformation. However, shear on the same time scale as the defect relaxation time results in layer sliding, which can lead to shear-induced formation of ordered structures. The differences in the particular mechanism of deformation and rearrangement of ordered structure have been used to help explain differences in elasticity in cubic micellar gels. Increased hindrance to flow results in increased elasticity in thermogels of synthetic block copolymers in water composed of a body-centered cubic (BCC) structure. The ability to study this behavior in monodisperse, well-characterized systems is a way to further enhance understanding of how end-block association and dissociation kinetics contribute to differences in deformation behavior.

Recent work has developed monodisperse protein block copolymers with thermoresponsive elastin-like polypeptide (ELP) end-blocks that can be used to better inform the polymer physics behind block copolymer response under...
deformation. The precise sequence of the ELPs is known to affect their mechanical properties with only small changes in chemical composition and solubility, providing an ideal model material to explore the role of end-block dynamics on block copolymer behavior. The effects of sequence changes to the classical (VPGVG)₄ ELP sequence on the thermoresponsive behavior of the ELP itself have been studied extensively, both to characterize the effects of substituting out the fourth valine with the other amino acids and to characterize the effects of substituting out the glycine in the third amino acid position. The particular effect of substituting a more hydrophobic amino acid like alanine for glycine in the third position results in a transition from type II β-turns to the tighter type I β-turns. This substitution slows the ELP dynamics such that solutions of only ELP form tough and thermoresponsive gels due to the formation of a kinetically arrested and phase-separated nanostructure. These ELP sequences can be combined with other moieties via cloned protein fusions or biocatalysis where the dynamics of the chosen ELP sequence can result in increases or decreases to properties like toughness or strain at break.

This ability to adjust the nonlinear mechanical response by making minor changes to the amino acid sequence can be used to better understand how copolymer end-block properties contribute to bulk mechanical properties in materials for specific applications. For instance, in adhesives the ability to attain precise control of chain dynamics is known to assist in consistency and reproducibility. Nonhydrogel adhesives tend to incorporate tackifiers or plasticizers to assist in increasing the value of the loss modulus and the value of tan δ. However, in hydrogel systems it is more difficult to add in tackifiers and plasticizers. Instead, it is necessary to engineer other aspects of the system to compensate. Thus, the ability to tune chain dynamics through amino acid sequence in nanostructured protein hydrogels can be used as a way to assist in better understanding of the nonlinear phenomena responsible for increased material performance.

In this work, a combination of start-up shear, nonlinear oscillatory shear, tensile tests, and rheo-SAXS in the flow-gradient (1–2) plane were used to investigate how changing end-block dynamics using amino acid sequence changes influences the deformation behavior for thermoresponsively reinforceable block copolymer gels. Protein-based triblock gels were prepared using two different thermoresponsive ELPs fused to midblock linker domains composed of soluble poly electrolyte chains alternating with self-associating α-helices. At temperatures below the transition temperature of the ELP end-block, the self-associating midblock (Scheme 1) forms a weak network that is reinforced above the transition temperature by the formation of micellar aggregates. Two specific ELP sequences are used in the thermoresponsive end-blocks—containing glycine and one containing alanine. This results in three different possible triblock proteins: G₁₀P₄G₁₀ (G-type), A₁₀P₄G₁₀ (AG-type), and A₁₀P₄A₁₀ (A-type) (Scheme 1). These three proteins were explored at temperatures around and above the transition temperatures of the end-blocks (Tₑ = 24.0 and 14.1 °C for the glycine-containing and alanine-containing ELPs, respectively) to elucidate the effects of microscopic changes to end-block sequence and dynamics on macroscopic behavior.

### METHODS

**Protein Expression.** ELP proteins were expressed in Escherichia coli (E. coli) using a home-built fermenter. A 50 mL overnight LB culture was used to inoculate a 5 L fermentation in Terrific Broth (TB) media at approximately 30 °C under Kanamycin selection (50 µg/mL) with 0.7 mL of antifoam (Sigma). The expression proceeded for 16–19 h without induction under active air flow (1 L/L culture/min). Approximately 3–5 h after inoculation, 5 mL of 80% wt% glycerol was added. Cells were resuspended in a magnesium-supplemented Tris (MENT) buffer (10 mM Tris, 100 mM NaCl, 1 mM EDTA, 5 mM MgCl₂, pH = 7.5) at approximately 30 g of wet cell mass per 100 mL of buffer and frozen at −80 °C overnight to help break down the cell periplasm. Lysis was performed using lysozyme (100 mg per 100 mL of resuspension) for 1–3 h followed by sonication and clarification. DNase I and RNase A were added to the clarified supernatant (approximately 2 mg of each per 100 mL of resuspension), which was then incubated for 2–3 h at 37 °C. Following this incubation, a concentrated solution of sodium acetate in MENT at pH = 5 was added to obtain final concentrations of either 100 mM (G₁₀P₄G₁₀ and A₁₀P₄G₁₀) or 370 mM (A₁₀P₄A₁₀) sodium acetate in MENT at pH = 5. These solutions then underwent two rounds of thermal cycling between 4 and 37 °C to remove further impurities. Following the final resuspension, the solutions were dialyzed against ultrapure Milli-Q water before purification using anion exchange in 6 M urea, 20 mM Tris, pH = 8 over HiTrap Q prepacked columns (GE Healthcare) by automated chromatography. After binding, the products were eluted using a gradient of 2 M NaCl from 0 to 8% over 20 column volumes, with a majority of the product eluting below 4%. The purified fractions were identified using SDS-PAGE, dialyzed against ultrapure water, and lyophilized (Figure S5-1).

Some of the A-type ELP triblocks were expressed using alternative expression conditions resulting in better expression yield. Instead, the expression was started at 30 °C and induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at an optical density of 1.0 ± 0.2 at 600 nm. Subsequently, the temperature was decreased to 20 °C. These
expressions proceeded for approximately 20 h after induction. Purification followed the steps described previously. The molecular weight and end-block fraction of the resultant proteins were determined in previous work and are summarized in Table S5.1,10

**Rheology.** Small- and large-amplitude oscillatory shear rheology was performed using a stress-controlled Anton-Paar MCR 702 rheometer in dual-mode (TwinDrive) mode outfitted with an environmental enclosure and Peltier heater. Linear rheology was performed in direct strain oscillation mode (DSO) at γ = 1%. Large-amplitude oscillatory shear was performed at 1, 10, 100, and 1000% strain at three different angular frequencies (ω = 0.0126, 0.126, and 1.26 rad/s). At each strain, the waveform was obtained following at least 10 periods of oscillation, and subsequent strains were performed following a 10 min wait period. Hydrogels were prepared at a concentration of 15% (w/v) and loaded into either a 10 or 25 mm cone-and-plate geometry at 4 °C. Both lower and upper geometries had sandblasted surfaces. To prevent dehydration, samples were sealed with mineral oil. Temperature ramps were performed at 1 °C/min, and experiments were performed at temperature following equilibration for at least 1 h. Step-shear experiments were performed at 0.002 s⁻¹ for 1200 s followed by 1200 s of relaxation.

**Rheo-SAXS.** Small-angle X-ray scattering measurements at controlled shear rates were performed at the S-1D-D beamline through the DuPont—Northwestern—Dow Collaborative Access Team at the Advanced Photon Source at Argonne National Lab using a custom shear cell with strain control but no force transducer. The shear cell itself consists of an annular cone-and-plate geometry with a 5° cone angle such that the X-ray beam passes through the shear cell before interacting with the sample on the downstream side. The shear cell contains two resistive heating elements on each of the sample sections. Temperature control below ambient can be achieved by circulating LN₂-chilled N₂. Measurements were performed with a gear reducer (consisting of the upper geometry, electrical assembly, motor, and gear reducer) to provide top-down access to the lower geometry. The resultant normalized birefringence was calculated as

\[
I_{BP} = \frac{1}{I_{open}} \left( \frac{1}{I_{dark}} (I_B - I_{dark}) - (I_{cross} - I_{dark}) \right)
\]

where \(I_{open}\) is the normalized birefringence signal (or power fraction), \(I_B\) is the birefringence signal of the sample, \(I_{dark}\) is the background detector signal when there is no incident laser, \(I_{cross}\) is the detector signal when the polarizer is in place and the laser is on, \(I_{cross}\) is the detector signal when the laser is on with no polarizer, and \(T_s\) is the sample transmission (\(= I_{cross}/I_{open}\)).

**RESULTS AND DISCUSSION**

**Effect of End-Block Sequence Changes on Mechanical Reinforcement.** Despite the fact that all three of the triblock ELPs used in this study were designed to be of equivalent molecular weights with equal end-block and midblock volume fractions (Scheme 1), the slower kinetics inherent to the alanine-containing ELP sequence has a substantial effect on the properties of the copolymers. Temperature sweeps (Figure 1) show that both the A- and G-type triblock ELPs mechanically transition around the same temperature as their end-block transition temperatures (14.1 and 24.0 °C, respectively),10 while the AG-type triblock ELP transitions roughly halfway between the two at approximately 20 °C. The A-type triblock undergoes a very sharp thermoresponsive transition and shows

![Figure 1. Storage moduli for all three proteins at 15% (w/v) in 100 mM phosphate buffer taken at a rate of 1 °C/min. Data were taken at ω = 100 rad/s and 1% strain.](Image)

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minimal increase in the storage modulus above 20 °C. In contrast, the AG-type and G-type gels have broader transitions and moduli that continue to increase until almost 40 °C. However, at 20 °C, the AG-type triblock has already started to transition, while the G-type triblock does not begin undergoing its mechanical transition until just above 20 °C. In their fully reinforced state at 50 °C, the modulus of the A-type triblock is roughly 1.5 times the moduli of the triblocks containing the glycine-rich ELP end-blocks.

Triblocks with alanine-containing end-blocks are orders of magnitude tougher in tension than the G-type triblock across all three temperatures (Figure 2a). Tensile tests were performed at 0.2 s⁻¹, corresponding to an angular frequency of 1.3 rad/s where the linear oscillatory shear data exhibits large differences between the three proteins as a function of both sequence and temperature (Figure 2 and Figure S1-1). The difference in toughness can be understood based on large differences in the shape of the tensile curves for each of the three materials. The G-type triblock could only be tested at 35 and 50 °C due to insufficient strength at 20 °C and in both cases underwent an overshoot in the stress before decaying toward a plastic deformation plateau stress prior to fracture at around 500% engineering strain. The AG- and A-type triblocks both reached higher extensions (around 1800 ± 33 and 2100 ± 54% engineering strain, respectively, in Figure 2b). While the stress in the A-type triblock continuously increases with increasing strain, reaching an ultimate stress of 127 ± 23 kPa depending on temperature, the AG-type triblock shows a plastic deformation plateau at lower stress around 20 kPa until the sample breaks. The combination of higher strain and stress at break results in the orders of magnitude higher toughness values for the A- and AG-type triblocks (Table 1 and Figure 2).

Increasing temperature results in increased toughness for the G- and AG-type triblocks, while the toughness of the A-type triblock decreases from 20 to 50 °C. This decrease happens despite strengthening associations for both glycine and alanine-containing end-blocks with increasing temperature because the midblock associations weaken upon heating. Because the glycine-containing end-block continues its aggregation transition over a broader temperature range that ends at higher temperatures, there is a larger increase in end-block aggregation to counterbalance the decrease in midblock aggregation. This leads to moderate increases in toughness with temperature. However, the A-type block has completed the transition by 20 °C, so the dominant effect is the softening of the midblocks, leading to a drop in toughness.

The strains at break exhibited by the A- and AG-type triblocks at 35 and 50 °C are very high for hydrogel materials, on the same order of magnitude as those recently reported for self-healing elastomers and chain extended coiled-coil associating networks. These high moduli cannot be explained using simple elastic network theories; when a theoretical fraction of elastically effective chains is calculated, it is greater than 1, suggesting an important contribution of the end-block domain packing to the modulus. Bao et al. saw tensile stresses as high as 200 kPa at similar extensions. However, the extension at break was a function of strain rate, indicating that the rate of extension relative to the properties of the associating groups is an important factor when designing tough, extensible materials. In this case the presence of alanine-containing end-blocks, which associate and dissociate slower than glycine-containing end-blocks, result in increased extension. At the same time, the presence of faster glycine-containing end-blocks in the AG-type triblock results in lower fracture stresses but achieves high extension with a long period of plastic draw. This rate dependence of mechanical properties is evident in linear oscillatory shear data (Figure 3), where the A-type triblock exhibits higher storage moduli across a frequency range spanning 5 orders of magnitude.

Structural Deformation Behavior during Start-Up Shear. Probes of structure evolution under deformation provide insight into how this minor change in polymer end-block leads to such large changes in material performance. While all three triblock proteins can be modeled as disordered spheres prior to flow (Supporting Information section 2), the sequence changes between these three proteins result in different behavior during deformation due to the differences in

![Image](Image60x497 to 300x606)

**Table 1. Tensile Properties of Proteins**

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<td>1.50 × 10⁻¹ ± 4.23 × 10⁵</td>
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<tr>
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the kinetic time scales of the two different types of end-blocks. The faster kinetics inherent to the glycine-containing end-block result in more viscous-like behavior at low strains that allows for structural alignment, while the slower kinetics inherent to the alanine-containing end-block does not allow for structural alignment on the time scale of the applied shear. When the G-type triblock in its reinforced state at 35 and 50 °C is sheared at 0.002 s⁻¹ for 20 min, the primary ring first elongates into the second and fourth quadrants of the flow-gradient plane (Figure 4 and Figure S2-5), where the first and second quadrants correspond to the location of the strain and compression axes, respectively, given the directionality of the shear flow (Figure 5b). This elongation occurs at a strain of approximately 50% and reflects the shear deformation of the domain spacing in real space.²⁸ By 100% strain, the ring intensity rearranges to form a 2-fold rotationally symmetric pattern with two intense peaks along the gradient direction. Above 100% strain, the intensity continues to redistribute and form four additional peaks of lower intensity around the primary ring. This intensity redistribution at high strains indicates structural alignment. At 20 °C where the G-type ELP has a more dominant viscous-like response in small amplitude oscillatory shear, the 2-D scattering pattern shows no visible deformation or alignment during shear (Figure 4b and Figure S2-1).

The A-type material shows substantially different behavior from the G-type triblock. The A-type, which has the slowest relaxation processes of the three triblocks, initially elongates into the second and fourth quadrants of the flow-gradient plane while shrinking in the first and third quadrants (Figure 4a, Figures S2-1 and S2-5). This occurs for all three temperatures and is similar behavior to the G-type triblock (Figure 4b). However, at strains greater than 100%, the intensity does not redistribute around the ring as it does for the G-type triblock. At 50 °C, the intensity undergoes additional rearrangement at higher strains. Unlike at 20 and 35 °C, the ring ultimately elongates along the gradient axis instead of at a 45° angle such that broad higher-intensity peaks form along the flow axis. This ring deformation corresponds to increased domain spacing in the flow direction which occurs shortly after the strain reaches 100%.

The AG-type triblock, where one end-block of the G-type is substituted with an alanine-containing end-block, exhibits behavior that is intermediate between the G and A-type triblocks (Figure 4, Figures S2-1 and S2-5). Similarly to the G-type, at low strain (γ < 100%), the scattering pattern initially

![Figure 3](image-url) **Figure 3.** (a) Storage and (b) loss moduli for all three proteins at 35 °C during linear oscillatory shear at 1% strain. All proteins were at 15% (w/v) in 100 mM phosphate buffer.

![Figure 4](image-url) **Figure 4.** (a) Transient 2-D scattering patterns for all three proteins at 35 °C as a function of increasing strain and (b) 2-D scattering patterns for all three proteins at all three temperatures following approximately 2.4 units of shear unless otherwise stated. All samples are 15% (w/v) in 100 mM phosphate buffer, and data were obtained during start-up of shear at a rate of 0.002 s⁻¹. The q-scale for all images is −0.2391 to 0.2391 nm⁻¹ in both the gradient and flow directions.

![Figure 5](image-url) **Figure 5.** Start-up of shear at 0.002 s⁻¹ for all three proteins at 35 °C and a concentration of 15% (w/v) in 100 mM phosphate buffer showing (a) the principal axis ratio as a function of strain, where (b) shows the location of the principal axes in q-space relative to the flow and gradient axes on the 2-D scattering pattern of A₁₀P₄G₁₀ after approximately 240 s of shear at 35 °C. The black dashed line in (a) corresponds to the predicted principal axis ratio for affine deformation per eq 1. For purposes of discussion, the first quadrant in q-space corresponds to the upper left of the 2-D scattering pattern.
Figure 6. Shear stress as a function of strain during start-up of steady shear at a rate of 0.002 s\(^{-1}\) for (a) G\(_{10}\)P\(_{4}\)G\(_{10}\), (b) A\(_{10}\)P\(_{4}\)G\(_{10}\), and (c) A\(_{10}\)P\(_{4}\)A\(_{10}\). All three proteins were at a concentration of 15% (w/v) in 100 mM phosphate buffer. The y-axes have been chosen to make the different behaviors clearer. For a more direct comparison, see Figure S1-7.

These differences in structural deformation behavior, where the intensity rearranges for the AG- and G-type triblocks, correlate with mechanical behavior. In shear, the rearrangement of intensity for the AG- and G-type triblocks corresponds to the presence of plastic draw in tension experiments. At the same time, the A-type triblock which appears to just elastically deform with strain does not exhibit plastic draw under tension. However, in steady shear the A- and G-type triblocks both exhibit stress overshoots prior to reaching steady-state stresses (Figure 6a). These overshoots occur on different time scales. Above the glycine-containing end-block’s transition temperature, the G-type triblock exhibits a stress overshoot at around 25% strain and reaches steady state well before 100% strain (Figure 6a). The shear stress of the A-type triblock instead yields around 25% strain such that the slope decreases but does not result in an overshoot until higher, temperature-dependent strains. The A-type triblock’s shear stress does not reach steady state until over 200% strain (Figure 6c and Figure S1-9). The AG-type triblock exhibits completely different behavior from either the A- or G-type triblocks (Figure 6b). At low strains, the shear stress of the AG-type triblock exhibits similar behavior to the A-type triblock. However, instead of undergoing an overshoot at higher strains, the shear stress monotonically increases with increasing strain. This increase coincides with the structural reorganization seen in scattering.

Both in shear and in quiescent conditions, structural reorganization and phase transitions in block copolymer micellar systems are known to occur via structural melting, layer sliding, or the presence of defects in the ordered structure. Analysis of the 2-D scattering patterns for the G-type under shear shows the development of six peaks around the primary ring at 35 and 50 °C (Figure 4, Figures S2-3 and S2-5). The presence of these six peaks could be indicative of the formation of a hexagonally close-packed (HCP) structure. However, while the 2-D scattering pattern of the A-type triblock (two A end-blocks) initially deforms, the peak intensity does not rearrange to form additional peaks during the applied strain. These two differences in deformation behavior indicate that at least one glycine-containing end-block is required to enable structural rearrangement. The AG-type triblock exhibits similar behavior to the G-type triblock at higher weight fraction. In previous work, the G-type triblock at 20 and 30% (w/v) exhibited similar transitions at roughly equivalent strains. However, increased strain resulted in the eventual formation of the 6-fold symmetry seen here in the G-type triblock at 15% (w/v) after low strains. It is likely that the presence of the

\[
\frac{q_{\text{peak, compression}}}{q_{\text{peak, strain}}} = \left( \frac{1 + \gamma + \gamma^2/2}{1 - \gamma + \gamma^2/2} \right)^{1/2}
\]
Figure 7. Normalized Lissajous–Bowditch curves for all three proteins at 35 °C showing (a) elastic and (b) viscous behavior as a function of increasing strain at 0.0126 rad/s. Black dashed lines represent crossover from more elastic-like behavior to more viscous-like behavior as a function of increasing strain. Gray regions represent more viscous-like behavior. All three proteins were at a concentration of 15% (w/v) in 100 mM phosphate buffer.

Figure 8. (a) 2-D scattering patterns for all three proteins approximately 1150 s following shear cessation unless otherwise specified. It should be noted that the precise times range from 1100 to 1220 s following shear cessation. (b) Principal axis ratio averaged between approximately 1000 and 1200 s following shear cessation for all three proteins as a function of temperature with the exception of G10P4G10 at 50 °C, for which data correspond to 550–740 s following shear cessation. The black dashed line represents the theoretical ratio for an isotropic system. Error bars correspond to either the standard deviation of the data points or the average of the error of the points, whichever was larger. (c) G10P4G10 approximately 1200 s following shear cessation at 35 °C showing the presence of 6-fold symmetry. The q-scale for all images is ~0.2391 to 0.2391 nm −1 in both the gradient and flow directions. All proteins are at a concentration of 15% (w/v) in 100 mM phosphate buffer.

One possible mechanism that is known to result in 6-fold symmetry, specifically in HCP cylinders, is layer sliding. Sliding layers occur when the imposed shear rate is on the order of the inverse relaxation time of system defects (γ*), while bulk deformation of the sample occurs when the rate of deformation is higher than γ*.
phenomena like shear banding. This supports the idea that the G-type triblock is likely undergoing layer sliding. The AG-type Lissajous patterns at high strain more closely resemble the G-type patterns, with clear loops at 20 °C and the presence of what could be loops at 35 and 50 °C (Figure 7b and Figure S1-3). In the A-type triblock, the viscous projection of the Lissajous curves shows evidence of overshoots in stress as a function of strain rate. In similar materials, the projections for the A-type in Figure 7b have been replicated using models that take into account structural changes during the oscillation. This particular projection corresponds to a model where the material of interest undergoes few structural changes over the course of the oscillation.

**Relaxation Behavior.** Upon cessation of shear, the elongated patterns present following approximately 2.4 strain units are able to relax. However, approximately 1100 s following shear cessation, only the G-type triblock approaches a principal axis ratio of around 1, indicating a nondeformed circular primary ring (Figure 8b). In addition to relaxing back to a nondeformed ring, the six peaks that developed in the scattering patterns of the G-type triblock during shear at 35 and 50 °C develop into a 6-fold symmetrical 2-D scattering pattern at long times following shear cessation. The development of this 6-fold symmetry is indicative of a hexagonal close-packed structure. The presence of principal axis ratios greater than 1 for the AG- and A-type triblocks is consistent with the slow dynamics of the A-type end-blocks hindering structural rearrangement. Qualitatively, the 2-D scattering patterns for both triblocks with alanine-containing ELPs remain unchanged up to 1200 s following shear cessation (Figure 8a). The AG- and A-type triblock primary rings are still deformed, and the principal axis ratio approximately 1100 s following shear behaves nonmonotonically with increasing temperature, although this effect is much larger for the A-type triblock. This degree of deformation retained in the primary ring is a consequence of the degree of alignment of achieved during shear.

While this deformation behavior does not represent a relaxation mechanism, it does reflect the increased time scale for relaxation for these two materials. The time scale of relaxation for all three of these proteins can be roughly calculated from double exponential fits to the relaxation in stress following shear (Figure 9b). This results in a long time scale and a short time scale such that the shorter time scale ranges from around 10 to 100 s and the longer time scale ranges from 500 to 10 000 s (Figure 9a and Figure S1-7).

The shorter relaxation time is tied to the differences in end-block transition temperatures. The AG and A-type triblock relaxation times increase only slightly with increasing temperature. However, the relaxation time of the G-type triblock (composed of only glycine-containing end-blocks that transition at around 24 °C) increases almost an order of magnitude from 20 to 35 °C. The relaxation time at 35 °C is then very similar to the relaxation time at 50 °C. At 35 and 50 °C, the shorter relaxation time is highest for the G-type triblock and lowest for the A-type triblock. Previous work has related the shorter relaxation time following cessation of shear to the coiled-coil exchange time. This is consistent with the fact that this relaxation time is very similar for all triblocks in the ordered state, but when the end-blocks are not associated (G-type at 20 °C), then the relaxation time grows somewhat shorter as the end-block is freed.

The longer relaxation time increases with each subsequent alanine-containing end-block substitution. The relaxation time nearly doubles from the G-type to the AG-type triblock and then more than doubles from the AG-type to the A-type triblock. The longer time constant is likely related to a slower molecular process like the pullout of a chain end, potentially corresponding to a terminal or defect relaxation time in the gels. This agrees well with the hypothesis that structural rearrangement, specifically for the G-type triblock, is occurring via layer sliding. The imposed shear rate (0.002 s$^{-1}$) is on the order of the inverse relaxation time of the defects for the G-type triblock, where the inverse of the longer relaxation time ranges from 0.0018 to 0.0011 s$^{-1}$. Conversely, the inverse of the longer relaxation times for the A- and AG-type triblocks is orders of magnitude slower than the imposed shear rate, and both systems exhibit high deformation ratios in shear.

The time scale for the development of 6-fold symmetry in the G-type triblock is temperature-dependent, occurring faster for the 35 °C sample. The specific structure cannot be verified without access to an additional scattering plane or flow birefringence measurements. It is possible that the 6-fold symmetry corresponds to spherical packing that facilitates the orientation of slip planes. At 35 and 50 °C, the micellar packing fraction of the G-type triblock in quiescent is around 0.51 ± 0.002, which is just below the critical packing fraction expected for cubic packing of spheres. It is also possible that the 6-fold symmetry corresponds to hexagonally packed cylinders oriented perpendicular to the direction of flow. Hexagonally packed cylinders have been seen for similar systems, and the perpendicular orientation of cylinders or lamellae relative to flow is not unprecedented. Both of these structures support the mechanism for structural reorganization. However, the presence of spheres prior to shear, the return to the same domain spacing following shear, and the quiescent volume fraction indicate that the 6-fold symmetry most likely corresponds to spherical packing.

**CONCLUSIONS**

Linear and nonlinear rheology were combined with transient scattering behavior under shear to quantify and describe the deformation behavior of nanostructured protein hydrogels under flow. Minor changes in the end-block amino acid sequence, where the standard ELP sequence (XPGVG) as substituted to include an alanine instead of a glycine in the third position, yield dramatic changes in the mechanical response of the materials, providing a model system for exploring the effect of end-block kinetics on block copolymer response. In
particular, it is shown that changing from G-type to A-type end-blocks leads to an increase in the toughness of the materials by 2 orders of magnitude and extension by 1 order of magnitude. The protein sequence corresponding to the weakest tensile and shear behavior deformed via rearrangement of disordered micelles to a hexagonally packed structure. This rearrangement was the result of increasingly viscous behavior with increasing strain and the formation of sliding layers, suggesting that the imposed shear rate was on the order of the inverse of the network defect relaxation time.

The more hindered end-block sequence, where both end-blocks contain the alanine-substituted ELPs, exhibited dominant elastic behavior at higher strains. This resulted in the development of an ellipse under flow in the scattering patterns that aligned along the compression axis with two broad peaks centered about the strain axis. This ellipse underwent further rotation at 50 °C to result in increased domain spacing along the shear axis about which the two broad peaks are centered. These differences in behavior occurred largely due to differences in end-block kinetics and end-block segregation strength, where the inverse of the defect relaxation time in the A-type triblock is orders of magnitude lower than the imposed shear rate.

Following shear, the relaxation behavior can be described using two characteristic relaxation times: a short time describing the contributions from the midblock micellar network that is on the order of 100 s and a longer time potentially representing the terminal relaxation time or the relaxation time of network defects ranging from 1000 to 10 000 s. The longer relaxation time is related to the kinetics of the associating end-blocks. These results show how changes in end-block dynamics can be used to adjust network relaxation times, which directly affects the response of network structure in nonlinear deformation. This enables tailoring the bulk mechanical properties for specific applications where the ability to increase the extensibility, maximum stress at break, or toughness of a material is important.

■ ASSOCIATED CONTENT

Supporting Information The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.macromol.8b00002.

Supplemental rheology, rheo-SAXS, tensile testing, birefringence, and protein characterization data (PDF)

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Notes
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