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Research Article

Competitive Supramolecular Associations Mediate the Viscoelasticity of Binary Hydrogels

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ABSTRACT: Supramolecular polymers are known to form strong and resilient hydrogels which can take up large amounts of water while exhibiting ease of processing and self-healing. They also possess similarities with networks of biological macromolecules. The combination of these features makes supramolecular polymers ideal candidates for studying mechanisms and consequences of self-assembly, which are relevant to biological materials. At the same time, this renders investigations of mixed hydrogels based on



different supramolecular compounds necessary, since this substantially widens their applicability. Here, we address unusual viscoelastic properties of a class of binary hydrogels made by mixing fibrillar supramolecular polymers that are formed from two compounds: 1,3,5-benzene-tricarboxamide decorated with aliphatic chains terminated by tetra(ethylene glycol) (BTA) and a 20 kg/ mol telechelic poly(ethylene glycol) decorated with the same hydrogen bonding BTA motif on both ends (BTA-PEG-BTA). Using a suite of experimental and simulation techniques, we find that the respective single-compound-based supramolecular systems form very different networks which exhibit drastically different rheology. More strikingly, mixing the compounds results in a nonmonotonic dependence of modulus and viscosity on composition, suggesting a competition between interactions of the two compounds, which can then be used to fine-tune the mechanical properties. Simulations offer insight into the nature of this competition and their remarkable qualitative agreement with the experimental results is promising for the design of mixed hydrogels with desired and tunable properties. Their combination with a sensitive dynamic probe (here rheology) offer a powerful toolbox to explore the unique properties of binary hydrogel mixtures.

■ INTRODUCTION

Ever since their discovery,^{1,2} supramolecular polymers have received a great deal of attention because of the reversibility and dynamic nature of the secondary bonds that govern their self-organization. These dynamic bonds dictate the macroscopic properties and enable applications in various fields ranging from biomaterials to microelectronics.³⁻⁵ Supramolecular chemists have worked for years on fine-tuning the molecular structure to obtain macroscopic properties that for a long time were reserved for macromolecules only.⁶ On the other hand, a grand challenge from the standpoint of materials design is the fundamental understanding of the dynamic assembly of supramolecular gelators and the role of competitive associations which may often be present.

In conventional synthetic or biological polymers, where monomeric constituents are connected through covalent bonds, their physical properties such as viscoelasticity can be tuned by blending different polymers, adding molecular solvents, or by copolymerization of two or more different monomers. An additional possibility with ample implications in tailoring their properties is introducing physical interactions (e.g., hydrogen bonds) which lead to the formation of dynamic networks. For example, interacting polymer blends or copolymers typically exhibit a non-monotonic dependence of segmental dynamics and viscosity on composition.⁷⁻¹¹ This has important consequences on the tunability of the mechanical^{12–14} and thermal properties (e.g., gelation temperature).¹⁵ For materials based on supramolecular polymers, the additional challenge and at the same time design parameter is the fact that the viscoelasticity of their transient networks depends on the average lifetime of the secondary interactions associated with their self-assembly. The most widely explored example of this category of molecules is wormlike micelles where the relaxation time of the material is related to the breaking time of the associating units.¹⁶ Thus, the relaxation time of the materials can be tuned by controlling the length of the polymer as well as the association strength. A mixture comprising an entangled aqueous solution of wormlike micelles and hydrophobically modified polymers was also

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Scheme 1. Chemical Structures of BTA (Left) and the Synthetic Scheme for BTA-PEG-BTA (Right)



Figure 1. (a) Linear viscoelastic spectra of the pure components, BTA (blue) and BTA-PEG-BTA (red) and two characteristic compositions of their mixtures, BTA/BTA-PEG-BTA 50/50 (purple) and BTA/BTA-PEG-BTA 75/25 (green) at 5 wt %. Closed circles correspond to the storage moduli, while open squares correspond to loss moduli. The lines represent the dynamic moduli obtained from creep measurements, which are inverted to yield the storage (solid lines) and loss (dashed lines) moduli (see Methods). (b) Loss factor $(tan(\delta))$ as a function of the frequency. The relaxation processes are shown with arrows (see text). Solid lines represent $tan(\delta)$ from creep measurements.

found to exhibit a non-monotonic dependence of the plateau modulus and terminal relaxation time on composition, due to the synergistic interactions of the two components.^{17,18} Along the same lines, entangled wormlike micelles mixed with short telechelic chains formed a transient network with monotonic dependence of viscoelastic properties on composition.¹⁹ It should be noted, however, that in these surfactant micelle composites the motifs for self-assembly are different (surfactant molecule and hydrophobic unit in a covalently bonded chain in the presence of salt ions¹⁷ or telechelic polymer bridging the surfactant micelles¹⁹).

Recent advances in the chemistry of supramolecular polymers have led to the use of supramolecular copolymerization as a means to tailor the properties of the supramolecular polymers by carefully balancing the secondary interactions, inspired by the approaches in covalent polymer synthesis.²⁰ In an attempt to control the dynamics of the monomers, Thota et al.²¹ copolymerized 1,3,5-benzene-tricarboxamide (BTA) derivatives with linear and dendritic ethylene glycol sidechains. The sterically hindered dendritic monomers stabilized the supramolecular polymers by slowing down the dynamics of the BTA monomers. Using the concept of sterically hindered

monomers copolymerized with monomers forming long polymers, Appel et al.²² achieved control over the length of 1D fibrillar assemblies. Others have successfully studied how the properties of amphiphilic block copolymers affect the gelation of supramolecular compounds and the mechanical properties of the resultant materials. Using telechelic polymer chains having one or both ends functionalized with the same supramolecular motif, the hydrogels exhibited an unusual viscoelastic behavior that varied non-monotonically with the fraction of the moiety.^{23–25} However, there is a strong need to obtain a molecular-level understanding on how such properties can arise in supramolecular binary hydrogels.

Here, we address the formidable challenge to rationally combine two different supramolecular compounds, which are based on the same hydrogen bonding motif BTA, to produce responsive materials with tunable properties. We approach this bioinspired challenge by focusing on synthetic hydrogels. In particular, we prepare self-assembled 1D fibers based on two compounds, BTA^{26-28} and a telechelic poly(ethylene glycol) (PEG) with both ends functionalized with BTA (BTA-PEG-BTA), and explore the viscoelastic properties of their mixtures at different ratios. These mixtures form stable hydrogels whose

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viscoelastic properties and structure are investigated by means of shear rheology and cryogenic transmission electron microscopy (cryo-TEM). Complementary coarse-grained molecular dynamics (MD) simulations elucidate the interplay of two different interactions between the compounds and its implications on the dynamics of the mixed networks. We find a non-monotonic dependence of viscoelasticity on the composition of the mixture, which points to the importance of competitive supramolecular associations as a molecular design tool for making hydrogels with desired and tunable properties.

RESULTS AND DISCUSSION

The synthesis of the BTA that assembles into large aspect ratio supramolecular polymers is based on strategies reported previously²⁶ (see details in the Supporting Information, SI). This small molecule contains three aliphatic chains with tetra(ethylene glycol) at their periphery (Scheme 1). We designed a new supramolecular telechelic compound that features the same BTA motif attached to the two ends of a linear PEG (Mn = 20 kDa). To synthesize BTA-PEG-BTA, the small molecule BTA was first tosylated and subsequently azidated. The monoazide product was isolated from the resulting mixture by column chromatography. The reaction with dialkyne functionalized PEG yielded the telechelic BTA-PEG-BTA on the multigram scale (Scheme 1, Figures S1–S10 of the SI).

Using oscillatory shear rheology, we have characterized the mechanical properties of the two pure components (here described as BTA and BTA-PEG-BTA) and their mixtures at several mixing ratios. The total concentration of the hydrogels, for both pure components and mixtures, was always kept at 5 wt %. We observe that the linear viscoelastic spectra of these materials depend non-monotonically on the mixing ratio. The frequency-dependent storage (G') and loss (G'') moduli, obtained over a large range of frequencies for the pure components as well as the mixtures, are shown in Figure 1 and in Figures S11-S13, and the respective complex viscosities in Figure S14. The viscoelastic spectrum of the pure BTA selfassembled network is characterized by an extended entanglement-like plateau region followed by an almost Maxwellian relaxation, reminiscent of that of living polymers (blue symbols and lines in Figure 1a, Figure S11).^{16,29–31} The plateau modulus (G_p) has a low value (10 Pa) and extends over almost 7 decades, indicating a persistent network made by highly entangled supramolecular polymers. On the other hand, the pure BTA-PEG-BTA telechelic system exhibits a more complex viscoelastic response (red symbols and lines in Figure 1a, Figure S12). The high-frequency plateau modulus exceeds that of pure BTA hydrogel by almost three decades, and there is a fast moduli crossover, with G'' > G', afterwards. This BTA-PEG-BTA system exhibits a slow relaxation process before eventually reaching terminal flow below 0.01 rad/s, much before that of the BTA-based network. By estimating the volume fraction occupied by the PEG only, we consider an equivalent solution of pure PEG linear chains that has a molar mass of 20 kg/mol. The dilution effect is so marked that no entanglements would be formed for such a solution. However, the molar mass needed for a pure PEG network (at the same volume fraction) to exhibit the same high-frequency plateau modulus as the BTA-PEG-BTA system and relaxation time, is calculated to be 1100 kg/mol (see SI for details; Figure S12). Clearly, the PEG chain by itself is not long enough to produce the viscoelastic spectrum of BTA-PEG-BTA as displayed in

Figure 1a. The mesh size calculated from the high-frequency modulus using the theory of rubber elasticity is 8 nm, a value very close to the radius of gyration of 20 kg/mol PEG in water (about 9 nm). Based on this, we attribute this mode to the relaxation of the PEG strands in the system. Furthermore, we have conducted dynamic light scattering measurements (Figures S15, S16) in the dilute regime (0.3 wt %), i.e., very different from the concentration regime where the properties of the networks were investigated (Figures 1,2, and 6 below),



Figure 2. Plateau modulus for the high-frequency (fast) and low-frequency (slow) relaxation modes presented in the viscoelastic spectra of the mixtures of Figure 1a, plotted against the weight fraction of the telechelic component BTA-PEG-BTA. The values were extracted at the minima of the loss factor $tan(\delta)$. Lines are drawn to guide the eye.

to show that the BTA-PEG-BTA self-assembles in the dilute regime. The distribution of relaxation times obtained from the Laplace transformation of the intermediate scattering function, using the CONTIN routine,³² shows a unimodal distribution with an apparent hydrodynamic radius of approximately 50 nm, which is larger than the size of the single PEG chain of 20 kg/mol as mentioned above, or a unimer of BTA-PEG-BTA (or spherical micelle as discussed below). The presence of the latter cannot be excluded, but there are certainly very few since they are not resolved by DLS. Therefore, self-assembly is confirmed and the supramolecular interactions are responsible for the observed behavior and the two hydrogels exhibit markedly distinct viscoelastic signatures.

The binary hydrogels formed from BTA and BTA-PEG-BTA (see Methods for details) show the emergence of two different modes of relaxation (Figure 1 and Figure S13) which ultimately yield two plateau moduli. The two relaxation processes are clearly visible in Figure 1b, as minima in the loss factor as a function of frequency. Interestingly, the longest relaxation time, crudely obtained from the low-frequency moduli crossover or the onset of terminal slopes of moduli with frequency, varies with composition. This affects the viscosity in an unexpected manner, because the viscosities of the mixtures are much higher than those of the two pure components, disobeying normal mixing rules³³ (Figure \$14). The nonlinear response of the two hydrogels and their mixtures to large-amplitude oscillatory shear confirms their dramatically different dynamics. The BTA-based network exhibits a two-step yielding process and thixotropic response, in contrast to the BTA-PEG-BTA system that is reversible with one yielding process. Interestingly, the mixtures exhibit only one yielding process, reversibility, and a much lower yield strain than either of the pure components. Both the yield stress



Figure 3. Cryo-TEM images of the pure components and different mixtures. In (a) are the fibers formed from the pure BTA and in (e,f) is the micellar fluid formed from the pure BTA-PEG-BTA. Images at different compositions are shown: (b) BTA/BTA-PEG-BTA 90/10; (c) BTA/BTA-PEG-BTA 75/25; (d) BTA/BTA-PEG-BTA 50/50 mixture with white arrows indicating short fibers. In images (a-d) the scale bar is 100 nm; in (e) and (f), it is 50 nm. The concentrations are low for the images (a-d): 582 μ M for image (a), 250 μ M for images (b,c), 500 μ M for image (d), and refer to nondilute regime (2.2 mM) for images (e.f).

and yield strain exhibit a non-monotonic dependence on composition. Details are provided in the SI (Figure S17). We note, however, that the nonlinear response requires a separate dedicated study which is beyond the scope of the present investigation.

We now discuss the non-monotonic dependence of the plateau modulus on the composition of the mixture (here represented by the weight fraction of the telechelic component BTA-PEG-BTA), as shown in Figure 2. Similar findings have been observed by the group of Messersmith,³⁴ who used similar materials for tissue engineering. In their work, they observe the non-monotonicity of mechanical properties with mixing ratio but do not elucidate its origin. Here, we try to shed light as to why this non-monotonicity is observed in such systems.^{23,25,34}

To rationalize these findings, we first examined whether the supramolecular structures formed at different mixing ratios can provide insights into the variations in moduli and dynamics. Specifically, a pertinent question is whether the lengths of the supramolecular structures (which will ultimately dictate the relaxation time of the network) as well as the characteristic size of the network (which will ultimately dictate the shear modulus) vary with mixing ratio, and therefore, we performed cryo-TEM experiments at concentrations below 1 wt %, i.e., well below the concentration used in the rheological measurements, since in these conditions, the much lower viscosity of the samples facilitates the blotting step needed in the sample preparation. Indeed, one can observe a nonmonotonic evolution of the characteristic lengths, starting from Figure 3a which depicts the network formed by the pure BTA even in these more dilute solutions (due to the long size of the fibers). It is evident that the network comprises very long, persistent fibers as also observed by Leenders et al.²⁴ The estimated average mesh size of the pure BTA network at a concentration of 582 μ M is approximately 50 nm. This value is consistent with the viscoelastic data (obtained at higher concentrations), since by applying the theory of networks of semiflexible chains and subsequently using the dilution law for

the mesh size,³⁵ we obtain a similar value of 53 nm (for a detailed description of this calculation, see Figure S18 and relevant discussion). On the other hand, the telechelic BTA-PEG-BTA forms a micellar fluid, rather than an entanglementlike polymer network (Figure 3e,f and Figure S19), which yields an elastic response at higher frequencies due to the transient bridges that can be formed by one BTA-PEG-BTA molecule bridging two micelles. In this case, at a concentration similar to the one used for rheological measurements (~5 wt %), many spherical micelles are observed (5-10 nm in size, Figure 3f) along with some elongated objects that are most likely due to bridging between the spherical micelles (Figure 3e). This is similar to what has been reported for amphiphilic triblock copolymer solutions³⁶⁻³⁸ as well as for similar supramolecular systems.^{25,34} The contrast in these images originates from the (aggregated) hydrophobic parts of the BTA-PEG-BTA, because the electron density of the hydrated PEG-chain is very similar to the surrounding water. Subsequently, we prepared several binary samples (<1 wt %) by adding BTA-PEG-BTA to the BTA polymers in solution. Upon increasing the amount of BTA-PEG-BTA (Figure 3b,c,d and Figure S20), we observe that the resulting supramolecular fibers become progressively shorter. When 10 mol % of BTA-PEG-BTA is present (Figure 3b), it is not possible to distinguish single fibers anymore, but instead, we observe smaller strands between network junctions. The presence of 25 mol % of BTA-PEG-BTA (Figure 3c) results in a clear decrease in the length of the fibers, and multiple polymer ends are visible. In the 50/50 mixture, some short fibers (<100 nm) can be observed along with micelles of similar size as in Figure 3e,f. These observations suggest that in the mixtures there is a competition between fiber formation (by BTA) and bridging micelles (by BTA-PEG-BTA), where the BTA units of both components mix. A larger fraction of BTA-PEG-BTA will result in more bridges between micelles, but at the same time, it will decrease the length of the fibers due to steric effects, i.e., steric hindrance because of the large PEG part of BTA-PEG-BTA that prevents the stacking of many BTA cores on top of one another. The same or similar changes in the average size of the assembled structures and the bridging between the fibers can occur at higher concentrations, and we propose that they may be at the origin of the non-monotonic rheological behavior observed. The cartoons of Figure 4 illustrate the



Figure 4. Cartoon representations of the supramolecular networks that are formed in the studied hybrid systems in nondilute regime, with the hydrophobic part of both molecules colored in gray, the BTA in cyan, and the BTA-PEG-BTA in red. The PEG-chains of BTA-PEG-BTA that form bridges are colored orange to be easily distinguishable. Several parts of the networks are enlarged to show individual aggregates (top insets), and interactions between aggregates (bottom insets). In the pure BTA network, (a) there is no bridging between the fibers due to the absence of BTA-PEG-BTA and the network is formed only by entanglements of the long fibers. The BTA-PEG-BTA (d) does not form a cohesive network but loosely connected micelles. The mixtures with BTA/BTA-PEG-BTA 90/10 (b) and 50/50 (c) are characterized by cross-linking between the fibers, which increases when the fraction of BTA-PEG-BTA increases (c), showing PEG bridges as orange lines (bottom insets of b,c). Steric hindrance due to the PEG polymer (detailed in top inset of c) results in shorter fibers with increasing amounts of BTA-PEG-BTA.

changes in the network structure of the binary hydrogels diagnosed by the rheological measurements (Figure 1a) and supported by the cryo-TEM images at lower concentrations (Figure 3).

We have further investigated the molecular origin of the non-monotonic dependence of the zero-shear viscosity on mixture composition (see Figure S14) that resulted from the mixing of the two compounds, and in particular the interplay between the two different interactions. Recent observations indicated that the telechelic PEG molecules increase the exchange rate of low-molar-mass hydrogelators when they are mixed into the supramolecular polymers formed by the small molecules.³⁹ The large hydrophilic PEG polymer makes the structures more dynamic or, in other words, decreases the association strength of the small molecules. Consistent with

these results, hydrogen/deuterium exchange mass spectrometry (HDX-MS) experiments show that the association between BTAs decreases in the presence of BTA-PEG-BTA (for a detailed description see Figure S21 and Table S1).⁴⁰ Since the association between BTA monomers decreases, they become more dynamic with increasing amounts of BTA-PEG-BTA; hence, the average length of the supramolecular polymers should be reduced, as confirmed by the images from cryo-TEM (Figure 3). However, the value of the viscosity in the mixtures is not determined by the length of the assembly alone, but rather by the complex network resulting from mixing. Actually, the viscosity values of the mixtures rich in BTA (in terms of mass fraction) are much larger than those of pure BTA, since the modulus increases by 2 orders of magnitude, even though the respective relaxation time in most mixtures is shorter as compared to pure BTA (Figure 1). On the other hand, the mixtures where BTA-PEG-BTA is in excess, exhibit a larger viscosity value, reflecting their much longer relaxation time compared to pure BTA-PEG-BTA.

To test the idea that the structural changes illustrated in Figure 4 can be at the origin of the rheological behaviors observed upon varying the relative composition of the binary gels, the experimental system was modeled as a mixture of two different coarse-grained associating units (beads of diameter dwith different association strengths), and an angular potential was applied that dictates the angle formed by three bonded beads depending on the size of the beads using an approach previously developed for colloidal gels⁴¹⁻⁴³ (for more information, see SI and Figure S22). By changing the association strength, the model comprises two species that, as pure components, self-assemble respectively into a percolated network of semiflexible fibers (I) and small aggregates between which bonds can break and reform easily (II). Figure 5 shows snapshots from the configurations obtained through self-assembly in the molecular dynamics simulations (see SI for more information) for the pure components (Figure 5a and d) and for the mixed systems (Figure 5b and c) in the nondilute regime and at the same relative compositions, as suggested in Figure 4 (here the composition in the numerical simulation samples is expressed in terms of the number fraction of associating units that belongs to the strongly associating component I). The snapshots show only portion of the simulation box to make distinctive features visible (see scale bar corresponding to 4d in Figure 5) and demonstrate how the model self-assembled structures, while not including all the complexity of the experimental system, well capture the essential ingredients of the association in the pure components and mixed systems. Furthermore, as discussed in the following, having used nonequilibrium molecular dynamics to compute the viscoelastic spectra of the pure components, we could also establish the correspondence between the rheological behavior in simulations and experiments, and therefore used the model to gain insight into the rheological response of the mixed systems. With small amounts of the weaker component (II), we observe additional bridges between the fibers of the main network (Figure 5b), formed by the strongly associating units. Once the fraction of the weakly associating units is increased, the bridging of the fibers is more pronounced and a more tightly connected network seems to develop (Figure 5c). The networks of the mixtures shown in Figure 5 are reminiscent of double or interpenetrating networks investigated in different contexts,44,45 which, however, are usually permanently cross-



Figure 5. Simulation snapshots of structures self-assembled in the molecular dynamics simulations at conditions similar to those of **Figures 1** and 4 (nondilute concentrations). Gray indicates the strongly associating (entangled) units (pure BTA fibrillar network), while orange indicates the weakly associating units (BTA-PEG-BTA micellar fluid). The entangled network with long fibers formed from the pure model BTA is shown in (a), and the small aggregates obtained from pure model BTA-PEG-BTA in (d). The structure in (b) is obtained with a ratio 90/10, defined by the number fraction of pure BTA units, where the micelles of BTA-PEG-BTA form additional connections in the first network and (c) corresponds to a 50/50 mixture with even more connections and effectively shorter fibers. In all subfigures, the scale bar is 4*d*i, with *d*i the diameter of the beads that are used as associating units in the model.

linked, rubber-like materials with extra reversible interactions such as metal—ligand complexes. Here, instead, all the interactions in our materials are transient, which makes the system very dynamic.

From the above combination of experimental observations and simulations (which are further elaborated below), the emerging picture indicates that the rheological response of the mixture originates from the fact that the aggregating units of one component can be embedded in the network of the fibers of the other component, yielding enhanced mechanical properties when their amount is increased; however, when the relative composition is such that the network of the former component becomes too sparse, it will not be further "reinforced". This picture is described consistently in Figures 1, 2 (rheology), 3 (cryo-TEM), 4 (schematic illustration), and 5 (simulation snapshots), which suggest wider implications of the present findings, beyond the specific hydrogel mixture, as discussed below.

All structures were sheared in silico^{42,46} to obtain the viscoelastic spectra of the simulated networks which are shown in Figure 6a. To convert the compositions of the mixtures in the model, expressed in terms of the number fraction of the component I associating units, into a weight fraction as used for the experiments, we have to consider that in the real material the self-assembling units of the pure component II are heavier and have a bigger linear size (~4 times bigger). Considering the same proportion of two components as in the experiments, we obtain an equivalent weight fraction for each numerical composition, for both components I and II (see also the Methods section and the SI). Here and in the following, we use the converted weight fraction $w_{\rm II}$, expressed in terms of component II, consistently with the experimental data of Figure 2, to rationalize the non-monotonic dependence of the rheological response on the mixture compositions. The spectrum for the pure component II (in red) exhibits two characteristic relaxation modes, indicated by the red arrows, similar to the pure BTA-PEG-BTA network. The simulations indicate that the high-frequency mode corresponds to the relative motion of the nearest-neighboring units in each micelle (or aggregate), while the low-frequency mode corresponds to the relaxation of the whole aggregate (see SI). In the mixed networks, G' and G'' vary non-monotonically with increasing the weight fraction w_{II} , in analogy to the mixing ratio in the experiments. To map the simulation time scales to the experimental results, we compare the corresponding highermode relaxation with the experiments and estimate that the highest $\omega \tau_0$ in the simulations should correspond to 10^2 rad/s. By analogy, the range of $[10^{-4}-1] \omega \tau_0$ in the simulations should then correspond to $[10^{-2}-10^2]$ rad/s for ω in the experiments. This allows a fair comparison between the



Figure 6. (a) Viscoelastic spectra obtained from numerical simulations, for the model mimicking pure BTA and for the model mimicking pure BTA-PEG-BTA, as well as for the mixed networks. Closed symbols indicate the storage modulus (G') and open symbols the loss modulus (G''). Here and in the following plots, error bars are smaller than the symbol size used (see SI). The spectrum for the pure component I is shown in blue and has a low-frequency plateau in the storage modulus (indicated by a blue arrow), corresponding to the presence of entanglements in the pure BTA network. (b) The low-frequency plateau modulus G_p has a non-monotonic dependence on the mixing ratio w_{ID} as also found in experiments. G_p has a maximum $w_{II,Max} \sim 0.25$. This dependence of G_p on w_{II} can be directly associated with the change in the fraction of units forming entanglements and bridges between the fibers in mixed networks, as shown in Figure 7a).



Figure 7. Fraction p(z) of units in the two components with a specific coordination number (z) as a function of w_{II} . (a) Fraction of particles forming fibers (z = 2). (b) Fraction of particles forming cross-links (z = 3). The units with z = 2 are those that only participate in fibers (component I) or small linear aggregates (component II). The units with z = 3 in component I correspond to entanglements, while units with z = 3 in component I correspond to bridges between the fibers of component I in the mixed network. We use blue circles for the fraction in component I, red squares for the fraction in component II, and black diamonds for their sum for a specific z.

experimental and simulated viscoelastic spectra (Figures 1a and 6a, respectively).

In Figure 6, the moduli are plotted using the reduced units of the simulation model, i.e., ε/d^3 , where ε is the interaction strength between units of component I and d is the diameter of the coarse-grained associating units. To convert the modulus units to real units, we consider that the modulus at crossover frequency of the pure component II in the simulations is approximately equal to that of the pure BTA-PEG-BTA in the experiment, i.e., we can set $10^{-1} \varepsilon/d^3 \approx 10^3$ Pa (see Figures 1a and 6b). This would correspond, for example, to $d \approx 10$ nm and $\varepsilon \approx 10kT$ (considering $kT \approx 10^{-20}$ J, with k being Boltzmann's constant and T the absolute temperature). For the size of coarse-grained units, $d \approx 10$ nm seems also a reasonable length-scale, since the average mesh size of the pure BTA network in the experiment is about 7 nm (see eq 3 in SI) and the average mesh size of the entangled network of component I in the simulation is about 2d.⁴¹ With this conversion, the values of the moduli obtained in the simulations of the model systems would vary between 1 Pa and 10⁴ Pa, which is consistent with the experiments (see Figures 1a and 4).

From the analysis of the linear viscoelastic spectra obtained by simulations, we extract the dependence of the moduli on the fraction of components. As shown in Figure 6b, the plateau modulus from simulations exhibits a non-monotonic behavior with the mixing ratio as well. Indeed, the plateau modulus reaches a maximum at 0.25 weight fraction. While the simple coarse-grained model used in the simulations (see SI) cannot capture the full chemical complexity of the experimental system, the results obtained do capture qualitatively the rheological response of the mixtures, and therefore give the opportunity to investigate its microscopic origin, offering a potentially powerful tool for designing materials described with this kind of interactions. In fact, more information regarding the non-monotonicity can be obtained from the simulations by assessing how the additional bridges formed by the BTA-PEG-BTA in the pure BTA network vary with the mixing ratio. We do this by computing the number of first neighbors of particles (coordination number) in the simulation box, for each particle, and track how many of them have a coordination number of 2 (meaning that they are embedded in a fiber) or coordination number of 3 (i.e., forming entanglements or bridges) as we vary the mixing ratio. In Figure 7a, we show the total number

of particles with a coordination number of 3 normalized by the total number of particles and the same is done for their presence in each component separately. The fraction of units forming entanglements and bridges between the fibers increases with increasing $w_{\rm II}$ and has a maximum at $w_{\rm II,max} \sim$ 0.25, where the maximum in the modulus is observed. When further increasing w_{II} , component II (pure BTA-PEG-BTA) plays a dominant role in the structure of the mixture and there are fewer and fewer fibers available from component I (pure BTA) to form additional bridges. As a consequence, the modulus of these mixed networks is mainly (or only) due to the entanglements in component I, which progressively decrease with increasing w_{II} (see Figure 7a). Consistently, with increasing w_{II} , the fraction of units forming fibers of component I continuously decreases (Figure 7b), while the respective fraction for component II that goes into small aggregates (i.e., that do not form bridges with component I) continuously increases. One can see from the plot that the latter increase faster with w_{II} for $w_{II} > w_{II, max}$ and therefore, the sum of the units that do not participate in entanglements or bridges has a non-monotonic dependence on w_{II} . At large w_{II} , the mixture consists mainly of small linear aggregates of component II, which reduces the modulus.

These results elucidate well how the competitive association of the BTA-PEG-BTA micelles in the pure BTA fibrillar network depends on the relative composition and therefore controls the resulting non-monotonic dependence of the viscoelastic response on the mixing ratio.

Lastly, we briefly discuss the observation that in the experimental spectra there are two main modes dictating the dynamics (see Figure 1a). It is beyond the scope of this study to interpret these in detail, and this will be addressed in the future. Here, we limit the discussion to simple observations and speculate that the complexity in the dynamics of the system originates from the fact that such systems have been shown to have a distribution of monomer exchange times. Put more simply, in such systems there is always an exchange of monomers between supramolecular polymers; however, because the self-assembly is not perfect, there is local disorder in the structures. There are defects along the fibers, which lead to "exchange hot-spots" along the fibers²⁸ that cause a distribution of exchange times. It is reasonable to assume that this is also true for the BTA-PEG-BTA molecule; thus,

one may think of a situation where there are some bridges that open much faster than the rest, and this could explain why in the mixtures there are two dominant modes. If we use the two predominant models for the dynamics of associating polymers, the supramolecular wormlike model¹⁶ and the sticky reptation model,⁴⁷ once a bridge is lost the fiber is free to reptate, but it can also break while it reptates, which may or may not lead to more bridges to be released, and so on and so forth. This centipede-like response⁴⁸ would lead to a very complex time distribution which is beyond our present capabilities to model.

In conclusion, by using state-of-the-art chemistry and a powerful combination of dynamic experiments (rheology) and simulations, we have proposed a two-component hydrogel system, comprising two different supramolecular molecules based on the same hydrogen bonding motif, that exhibits a non-monotonic mechanical and structural response by changing the mixing ratio. Our study sheds light on why such two-component supramolecular systems, based on the same motif with different internal structure, forming long topologically entangled fibrils (BTA) and short bridged fibrils (BTA-PEG-BTA), can exhibit a non-monotonic evolution of mechanical properties upon mixing. This bears similarities to wormlike micelles mixed with hydrophobically modified polymers¹⁷ and differs from wormlike micelles mixed with amphiphilic chains;¹⁹ however, both systems are very different from the present binary hydrogel. With the help of simulations, we have shown that the competitive interactions (making the structure more dynamic and essentially weakening, on average, the association between units), as well as the varying connectivity of the network formed from component I and of its organization in space with varying the mixture composition, lead to the non-monotonicity in the modulus. By simulating these systems in the simplest possible way as a mixture of two species, one strongly associating into network forming (entangled) semiflexible fibers and one weakly associating into small micelles, we are able to reproduce the main rheological features and the non-monotonic evolution of the viscoelastic shear modulus with mixing ratio. Our results illustrate how a counterintuitive increase in the modulus can result from the addition of a weakly interacting supramolecular motif to a more stable entangled network of polymers. The fact that this feature is captured by a simple coarse-grained model suggests that the association strength and the ability to create additional bridges can be the main characteristics to focus on for material design in a variety of different chemical compounds, although more work will be needed in this direction (for example, to examine the role of the internal flexibility of the motif). In particular, our findings point to the fact that not only the interactions between the single associating units, but also their changing organization in space with changing relative composition, play a role in the emerging mechanics of binary and multicomponent gels. There is an interesting analogy with mixtures of colloidal particles of different sizes and interactions, where the smaller particles can act as depletants,^{49,50} or mixtures of microgels with tunable interaction sites where one can tailor the self-assembled structure formed.^{51,52} Along the same lines, it has been shown that the organization in space of binary colloidal dispersions where the particles are coated with DNA, can be tailored by varying the specific motifs on the surface of the particles (where the interactions can vary with relative composition due to different organization in space).⁵³ Hence, the approach developed here, combining macroscopic (rheology) and

microscopic (cryo-TEM) experiments with appropriate coarse-grained simulations, can elucidate the origin of the reported non-monotonicity in viscoelastic properties and help identify which ingredients are the most important for material design and help further guide the chemical synthesis or the exploration of new compounds. With this in mind, the present work could inspire new ways to carefully tune the properties of multicomponent gels based only on the binding strength of the units, e.g., in mixed networks based on peptides, block copolymers, or DNA origami rods with telechelic connectors, for the design of biomaterials,^{34,53} or to understand similar processes in nature, e.g., the cross-linking of macromolecular Factin chains, composed of G-actin monomers, by actin-binding proteins, such as α -actinin.^{54,55} The noncovalent interactions of these proteins promote their hierarchical organization into kinetically trapped gel-like materials in cytosol. Intriguingly, assemblies of actin filaments have been observed at exceedingly low molar ratios of cross-linker protein, such as 1:90 in α actinin/G-actin.

METHODS

Sample Preparation. The concentration of all hydrogels, pure components and mixtures, was always kept at 5 wt %. The dry monomers and the appropriate amount water were placed in a vial along with a Teflon-coated magnetic stirring bar and were shaken until a hazy suspension started to form. The vial was then immersed in a water bath at 80 °C sitting on a hot plate with magnetic stirring mechanism. The vials were sealed with Teflon tape to ensure that no water from the bath entered the vial while the preparation protocol was ongoing. The vials remained in the bath for 15 min with short intervals (~10 s) of vortexing and were then left to equilibrate overnight at room temperature. The next day, a strong gel was formed that could sustain its weight (vial inversion test).

Rheology. We performed rheological measurements in an Anton-Paar (Austria) Physica MCR-501 rheometer, operating in the strain-controlled mode, equipped with a Peltier unit for temperature control at 37 °C, which also constitutes the bottom plate, and a cone geometry. All metallic components were stainless steel. We used three different cones, 8 ($\alpha = 1^{\circ}$) mm, 12.5 ($\alpha = 1^{\circ}$) mm, and 25 mm ($\alpha = 2^{\circ}$) to increase the torque signal when needed. Samples were loaded at 15 °C to avoid evaporation during the loading procedure. An aluminum sealing ring was placed around the sample to create a silicon oil (PDMS) bath, covering the lateral surface of the sample exposed to air to prevent evaporation. Several measurements were conducted a priori in order to ensure the nondiffusivity of the stagnant oil into the hydrogels and the negligible extrafriction due to the presence of the sealing fluid. After successful loading and thermal equilibration, we performed a mechanical rejuvenation test to erase the mechanical history of the sample due to the hydrogel preparation and loading in the measuring stage (see SI and Figure S23). We have used two different rejuvenation protocols. One consists of a fixed high strain (nonlinear regime) and fixed oscillatory frequency (1 rad/s) for a given time until full liquid-like behavior of the system is reached (typically 300 s). The second protocol consists of a fixed oscillatory frequency (10 rad/s) applied on the material while the strain amplitude is varied from small to large values, well into the nonlinear regime. Afterward, the aging kinetics were observed and measurements were done after the sample has reached its mechanical steady-state (Figure S23). Creep measurements are step-stress experiments, typically used to

study materials with solid-like or very slow flow response. When the applied stress is within the linear viscoelastic regime, then the creep response (compliance) can be converted into dynamic moduli through the relaxation/retardation spectra.⁵⁶ Here, the creep compliance J(t) was converted to $G'(\omega)$ and $G''(\omega)$ by means of the NLReg software, based on a generalization of the Tikhonov regularization method.⁵⁷

Simulations. We have studied the properties of the binary hydrogel mixture by means of coarse-grained Molecular Dynamics (MD) simulations for N particles with position vectors $\{\mathbf{r}_i\}$, $i = 1, \dots, N$, and total potential energy $\mathbf{U}(\mathbf{r}_i)$ involving two- and three-body terms. The two-body term is a Lennard-Jones like potential which consists of a repulsive core and a narrow attractive well, controlling the bond strength. The three body term limits the coordination number and confers angular rigidity to the interparticle bonds^{41,43,58} (see Figure S22). All simulations have been performed using a version of LAMMPS⁵⁹ suitably modified by us to include this interaction potential. Here, we considered two distinct types of particles (associating units) whose interactions have the same form but differ in the value of the parameter that determines the relative strength of the attractive potential well. The latter is important, as by changing it, one can mimic the association, respectively, in the pure BTA and BTA-PEG-BTA experimental systems, as described in the Results section. Further details can be found in the SI (section VIII and VIII A).^{41-43,46,58} The starting point for all calculations is always a configuration of $N = N_1 + N_2$ particles (N_1 with interactions corresponding to component I and N_2 for the component II) initially prepared at $\frac{kT}{\varepsilon} = 5 \times 10^{-3}$ (where ε is the energy unit used in all simulations) leading to stable self-assembled structures that depend on the interaction parameters. Each configuration is carefully equilibrated using an optimized protocol. All the data refer to mixtures consisting of N =32 000–64 000 particles in a cubic simulation box of size L =54d. For each data point, we have run simulations over 5 independently generated samples and used sample-to-sample fluctuations to obtain error bars. In all plots, error bars are smaller than the symbol size used. The self-assembled structures are then quenched to $\frac{kT}{\epsilon} \simeq 0$ by means of dissipative dynamics to perform the rheological tests, which use a shearing protocol based on nonequilibrium molecular dynamics and a computational scheme, inspired by a recently developed experimental technique,⁶⁰ to obtain the full linear viscoelastic spectrum by applying an optimally windowed chirp (OWCh) signal.⁴⁶ Further information can be found in the SI (section VIII B).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acscentsci.0c00279.

Details on the synthetic process, the rheological measurements, and protocols and the MD simulations (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Lehn, J.-M. Perspectives in Supramolecular Chemistry—From Molecular Recognition towards Molecular Information Processing and Self-Organization. *Angew. Chem., Int. Ed. Engl.* **1990**, *29* (11), 1304–1319.

(2) Aida, T.; Meijer, E.W. Supramolecular Polymers, We've Come Full Circle. *Isr. J. Chem.* **2020**, *60*, 33.

(3) Aida, T.; Meijer, E. W.; Stupp, S. I. Functional Supramolecular Polymers. *Science* **2012**, 335 (6070), 813–817.

(4) Webber, M. J.; Appel, E. A.; Meijer, E. W.; Langer, R. Supramolecular Biomaterials. *Nat. Mater.* **2016**, *15* (1), 13–26.

(5) Du, X.; Zhou, J.; Shi, J.; Xu, B. Supramolecular Hydrogelators and Hydrogels: From Soft Matter to Molecular Biomaterials. *Chem. Rev.* **2015**, *115* (24), 13165–13307.

(6) Sijbesma, R. P.; Beijer, F. H.; Brunsveld, L.; Folmer, B. J. B.; Hirschberg, J. H. J. K.; Lange, R. F. M.; Lowe, J. K. L.; Meijer, E. W. Reversible Polymers Formed from Self-Complementary Monomers Using Quadruple Hydrogen Bonding. *Science* **1997**, *278* (5343), 1601–1604.

(7) Coleman, M.; Painter, P. Hydrogen Bonded Polymer Blends. Prog. Polym. Sci. 1995, 20 (1), 1-59.

(8) Yang, Z.; Han, C. D. Rheology of Miscible Polymer Blends with Hydrogen Bonding. *Macromolecules* **2008**, *41* (6), 2104–2118.

(9) Cai, H.; Ait-Kadi, A.; Brisson, J. Dynamic Rheological Analysis of a Miscible Blend Showing Strong Interactions. *Polymer* **2003**, *44* (5), 1481–1489.

(10) Miyata, H.; Yamaguchi, M.; Akashi, M. Structure and Viscoelastic Properties of Amorphous Ethylene/1-Hexene Copolymers Obtained with Metallocene Catalyst. *Polymer* **2001**, *42* (13), 5763–5769.

(11) Carella, J. M.; Graessley, W. W.; Fetters, L. J. Effects of Chain Microstructure on the Viscoelastic Properties of Linear Polymer Melts: Polybutadienes and Hydrogenated Polybutadienes. *Macromolecules* **1984**, *17* (12), 2775–2786.

(12) Aamer, K. Rheological Studies of PLLA-PEO-PLLA Triblock Copolymer Hydrogels. *Biomaterials* **2004**, *25* (6), 1087–1093.

(13) Breedveld, V.; Nowak, A. P.; Sato, J.; Deming, T. J.; Pine, D. J. Rheology of Block Copolypeptide Solutions: Hydrogels with Tunable Properties. *Macromolecules* **2004**, *37* (10), 3943–3953.

(14) Pham, Q. T.; Russel, W. B.; Thibeault, J. C.; Lau, W. Micellar Solutions of Associative Triblock Copolymers: The Relationship between Structure and Rheology. *Macromolecules* **1999**, 32 (15), 5139–5146.

(15) He, Y.; Lodge, T. P. Thermoreversible Ion Gels with Tunable Melting Temperatures from Triblock and Pentablock Copolymers. *Macromolecules* **2008**, *41* (1), 167–174.

(16) Cates, M. E. Reptation of Living Polymers: Dynamics of Entangled Polymers in the Presence of Reversible Chain-Scission Reactions. *Macromolecules* **1987**, 20 (9), 2289–2296.

(17) Couillet, I.; Hughes, T.; Maitland, G.; Candau, F. Synergistic Effects in Aqueous Solutions of Mixed Wormlike Micelles and Hydrophobically Modified Polymers. *Macromolecules* **2005**, *38* (12), 5271–5282.

(18) Gouveia, L. M.; Müller, A. J. The Effect of NaCl Addition on the Rheological Behavior of Cetyltrimethylammonium P-Toluenesulfonate (CTAT) Aqueous Solutions and Their Mixtures with Hydrophobically Modified Polyacrylamide Aqueous Solutions. *Rheol. Acta* **2009**, *48* (2), 163–175.

(19) Nakaya-Yaegashi, K.; Ramos, L.; Tabuteau, H.; Ligoure, C. Linear Viscoelasticity of Entangled Wormlike Micelles Bridged by Telechelic Polymers: An Experimental Model for a Double Transient Network. *J. Rheol.* **2008**, *52* (2), 359–377.

(20) Besenius, P. Controlling Supramolecular Polymerization through Multicomponent Self-Assembly. J. Polym. Sci., Part A: Polym. Chem. 2017, 55 (1), 34–78.

(21) Thota, B. N. S.; Lou, X.; Bochicchio, D.; Paffen, T. F. E.; Lafleur, R. P. M.; van Dongen, J. L. J.; Ehrmann, S.; Haag, R.; Pavan, G. M.; Palmans, A. R. A.; Meijer, E. W. Supramolecular Copolymerization as a Strategy to Control the Stability of Self-Assembled Nanofibers. *Angew. Chem., Int. Ed.* **2018**, *57* (23), 6843– 6847.

(22) Appel, R.; Fuchs, J.; Tyrrell, S. M.; Korevaar, P. A.; Stuart, M. C. A.; Voets, I. K.; Schönhoff, M.; Besenius, P. Steric Constraints Induced Frustrated Growth of Supramolecular Nanorods in Water. *Chem. - Eur. J.* 2015, 21 (52), 19257–19264.

(23) Kieltyka, R. E.; Pape, A. C. H.; Albertazzi, L.; Nakano, Y.; Bastings, M. M. C.; Voets, I. K.; Dankers, P. Y. W.; Meijer, E. W. Mesoscale Modulation of Supramolecular Ureidopyrimidinone-Based Poly(Ethylene Glycol) Transient Networks in Water. J. Am. Chem. Soc. 2013, 135 (30), 11159–11164.

(24) Leenders, C. M. A.; Mes, T.; Baker, M. B.; Koenigs, M.. M. E.; Besenius, P.; Palmans, A. R. A.; Meijer, E. W. From Supramolecular Polymers to Hydrogel Materials. *Mater. Horiz.* **2014**, *1* (1), 116–120. (25) Noteborn, W. E. M.; Zwagerman, D. N. H.; Talens, V. S.; Maity, C.; van der Mee, L.; Poolman, J. M.; Mytnyk, S.; van Esch, J. H.; Kros, A.; Eelkema, R.; Kieltyka, R. E. Crosslinker-Induced Effects on the Gelation Pathway of a Low Molecular Weight Hydrogel. *Adv. Mater.* **2017**, *29* (12), 1603769.

(26) Leenders, C. M. A.; Albertazzi, L.; Mes, T.; Koenigs, M. M. E.; Palmans, A. R. A.; Meijer, E. W. Supramolecular Polymerization in Water Harnessing Both Hydrophobic Effects and Hydrogen Bond Formation. *Chem. Commun.* **2013**, *49* (19), 1963.

(27) Bochicchio, D.; Pavan, G. M. Effect of Concentration on the Supramolecular Polymerization Mechanism via Implicit-Solvent Coarse-Grained Simulations of Water-Soluble 1,3,5-Benzenetricarboxamide. J. Phys. Chem. Lett. **2017**, 8 (16), 3813–3819.

(28) Bochicchio, D.; Salvalaglio, M.; Pavan, G. M. Into the Dynamics of a Supramolecular Polymer at Submolecular Resolution. *Nat. Commun.* **2017**, 8 (1), 1 DOI: 10.1038/s41467-017-00189-0.

(29) Khatory, A.; Lequeux, F.; Kern, F.; Candau, S. J. Linear and Nonlinear Viscoelasticity of Semidilute Solutions of Wormlike Micelles at High Salt Content. *Langmuir* **1993**, *9* (6), 1456–1464.

(30) Cates, M. E.; Candau, S. J. Statics and Dynamics of Worm-like Surfactant Micelles. J. Phys.: Condens. Matter **1990**, 2 (33), 6869– 6892.

(31) Louhichi, A.; Jacob, A. R.; Bouteiller, L.; Vlassopoulos, D. Humidity Affects the Viscoelastic Properties of Supramolecular Living Polymers. J. Rheol. 2017, 61 (6), 1173–1182.

(32) Provencher, S. W. CONTIN: A General Purpose Constrained Regularization Program for Inverting Noisy Linear Algebraic and Integral Equations. *Comput. Phys. Commun.* **1982**, *27* (3), 229–242.

(33) Irving, J. B. Viscosities of Binary Liquid Mixtures: A Survey of Mixture Equations; Report No. 630; National Engineering Laboratory: East Kilbridge, Glasgow, U.K., 1977.

(34) Cheng, J.; Amin, D.; Latona, J.; Heber-Katz, E.; Messersmith, P. B. Supramolecular Polymer Hydrogels for Drug-Induced Tissue Regeneration. *ACS Nano* **2019**, *13* (5), 5493–5501.

(35) MacKintosh, F. C.; Käs, J.; Janmey, P. A. Elasticity of Semiflexible Biopolymer Networks. *Phys. Rev. Lett.* **1995**, 75 (24), 4425–4428.

(36) Annable, T.; Buscall, R.; Ettelaie, R.; Whittlestone, D. The Rheology of Solutions of Associating Polymers: Comparison of Experimental Behavior with Transient Network Theory. *J. Rheol.* **1993**, 37 (4), 695–726.

(37) Watanabe, H.; Sato, T.; Osaki, K.; Yao, M.-L.; Yamagishi, A. Rheological and Dielectric Behavior of a Styrene-Isoprene-Styrene Triblock Copolymer in Selective Solvents. 2. Contribution of Loop-Type Middle Blocks to Elasticity and Plasticity. *Macromolecules* **1997**, 30 (19), 5877–5892.

(38) Watanabe, H.; Matsumiya, Y.; Sawada, T.; Iwamoto, T. Rheological and Dielectric Behavior of Dipole-Inverted (SIS) $_p$ -Type Multiblock Copolymers: Estimates of Bridge/Loop Fractions for Respective I Blocks and Effect of Loops on High Extensibility of Bridges. *Macromolecules* **2007**, 40 (19), 6885–6897.

(39) Hendrikse, S. I. S.; Wijnands, S. P. W.; Lafleur, R. P. M.; Pouderoijen, M. J.; Janssen, H. M.; Dankers, P. Y. W.; Meijer, E. W. Controlling and Tuning the Dynamic Nature of Supramolecular Polymers in Aqueous Solutions. *Chem. Commun.* **2017**, *53* (14), 2279–2282.

(40) Lou, X.; Lafleur, R. P. M.; Leenders, C. M. A.; Schoenmakers, S. M. C.; Matsumoto, N. M.; Baker, M. B.; van Dongen, J. L. J.; Palmans, A. R. A.; Meijer, E. W. Dynamic Diversity of Synthetic Supramolecular Polymers in Water as Revealed by Hydrogen/Deuterium Exchange. *Nat. Commun.* **2017**, *8* (1), 15420.

(41) Colombo, J.; Del Gado, E. Self-Assembly and Cooperative Dynamics of a Model Colloidal Gel Network. *Soft Matter* **2014**, *10* (22), 4003.

(42) Colombo, J.; Del Gado, E. Stress Localization, Stiffening, and Yielding in a Model Colloidal Gel. J. Rheol. 2014, 58 (5), 1089–1116.

(43) Bouzid, M.; Gado, E. D. Mechanics of Soft Gels: Linear and Nonlinear Response. In *Handbook of Materials Modeling: Applications: Current and Emerging Materials*, Andreoni, W.; Yip, S., Eds.; Springer International Publishing: Cham, 2018; pp 1–29. DOI: 10.1007/978-3-319-50257-1_129-1.

(44) Gong, J. P.; Katsuyama, Y.; Kurokawa, T.; Osada, Y. Double-Network Hydrogels with Extremely High Mechanical Strength. *Adv. Mater.* **2003**, *15* (14), 1155–1158.

(45) Filippidi, E.; Cristiani, T. R.; Eisenbach, C. D.; Waite, J. H.; Israelachvili, J. N.; Ahn, B. K.; Valentine, M. T. Toughening Elastomers Using Mussel-Inspired Iron-Catechol Complexes. *Science* **2017**, 358 (6362), 502–505.

(46) Bouzid, M.; Keshavarz, B.; Geri, M.; Divoux, T.; Del Gado, E.; McKinley, G. H. Computing the Linear Viscoelastic Properties of Soft Gels Using an Optimally Windowed Chirp Protocol. *J. Rheol.* **2018**, *62* (4), 1037–1050.

(47) Leibler, L.; Rubinstein, M.; Colby, R. H. Dynamics of Reversible Networks. *Macromolecules* **1991**, *24* (16), 4701–4707.

(48) Baeza, G. P.; Dessi, C.; Costanzo, S.; Zhao, D.; Gong, S.; Alegria, A.; Colby, R. H.; Rubinstein, M.; Vlassopoulos, D.; Kumar, S. K. Network Dynamics in Nanofilled Polymers. *Nat. Commun.* **2016**, 7 (1), 11368.

(49) Bergman, M. J.; Gnan, N.; Obiols-Rabasa, M.; Meijer, J.-M.; Rovigatti, L.; Zaccarelli, E.; Schurtenberger, P. A New Look at Effective Interactions between Microgel Particles. *Nat. Commun.* **2018**, 9 (1), 5039.

(50) Truzzolillo, D.; Marzi, D.; Marakis, J.; Capone, B.; Camargo, M.; Munam, A.; Moingeon, F.; Gauthier, M.; Likos, C. N.; Vlassopoulos, D. Glassy States in Asymmetric Mixtures of Soft and Hard Colloids. *Phys. Rev. Lett.* **2013**, *111* (20), 208301.

(51) Månsson, L. K.; de Wild, T.; Peng, F.; Holm, S. H.; Tegenfeldt, J. O.; Schurtenberger, P. Preparation of Colloidal Molecules with Temperature-Tunable Interactions from Oppositely Charged Microgel Spheres. *Soft Matter* **2019**, *15* (42), 8512–8524.

(52) Månsson, L. K.; Immink, J. N.; Mihut, A. M.; Schurtenberger, P.; Crassous, J. J. A New Route towards Colloidal Molecules with Externally Tunable Interaction Sites. *Faraday Discuss.* **2015**, *181*, 49–69.

(53) Geerts, N.; Schmatko, T.; Eiser, E. Clustering versus Percolation in the Assembly of Colloids Coated with Long DNA. *Langmuir* **2008**, *24* (9), 5118–5123.

(54) Pelletier, O.; Pokidysheva, E.; Hirst, L. S.; Bouxsein, N.; Li, Y.; Safinya, C. R. Structure of Actin Cross-Linked with α -Actinin: A Network of Bundles. *Phys. Rev. Lett.* **2003**, *91* (14), 148102.

(55) Lieleg, O.; Claessens, M. M. A. E.; Bausch, A. R. Structure and Dynamics of Cross-Linked Actin Networks. *Soft Matter* **2010**, *6* (2), 218–225.

(56) Ferry, J. D. Viscoelastic Properties of Polymers; Wiley: New York, 1980.

(57) Honerkamp, J.; Weese, J. A Nonlinear Regularization Method for the Calculation of Relaxation Spectra. *Rheol. Acta* **1993**, 32 (1), 65–73.

(58) Del Gado, E.; Kob, W. A Microscopic Model for Colloidal Gels with Directional Effective Interactions: Network Induced Glassy Dynamics. *Soft Matter* **2010**, *6* (7), 1547.

(59) Plimpton, S. Fast Parallel Algorithms for Short-Range Molecular Dynamics. J. Comput. Phys. 1995, 117, 1.

(60) Geri, M.; Keshavarz, B.; Divoux, T.; Clasen, C.; Curtis, D. J.; McKinley, G. H. Time-Resolved Mechanical Spectroscopy of Soft Materials via Optimally Windowed Chirps. *Phys. Rev. X* **2018**, *8* (4), 041042.