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HIGHLIGHT

The conundrum of gel formation by molecular nanofibers, wormlike micelles, and filamentous proteins: gelation without cross-links?

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The term *gel* is central to many scientific fields, including polymer science, biophysics, and supramolecular chemistry. In polymer science, a gel is said to be formed when polymer chains are linked into a permanent three-dimensional (3-D) network by *cross-links* that are either chemical bonds or strong physical associations. Linear chains in the absence of such cross-links are expected to form a network that is only defined by topological (entanglement) interactions between the chains; accordingly, such a network is expected to show viscoelastic rather than gel-like, rheology. On the other hand, many systems consisting of extended nanoscale fibers/chains (*e.g.*, supramolecular organo- and hydro-gels, wormlike micelles, and protein filaments like F-actin) do exhibit the rheology of permanent gel networks, even in the absence of putative cross-links. We argue here that linear fibers can indeed form gels through their topological interactions alone, *i.e.*, without cross-links, provided the fibers are sufficiently *long*

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(contour length much larger than cross-sectional size), sufficiently *stiff* (worm-like or rod-like) and temporally persistent, *i.e.*, "*unbreakable*". This hypothesis is supported by recent experimental and theoretical studies. In particular, we review recent experiments on wormlike micelles that show a smooth rheological transition from viscoelastic (relaxing) to gel (non-relaxing) behavior upon varying temperature.

1. Introduction

Gels are everywhere around us – many foods (*e.g.*, Jello, ketchup), consumer products (*e.g.*, toothpaste) and industrial products (*e.g.*, adhesives) are gels.[1,2] Indeed, the gel state is arguably the most fundamental state of living matter since the cytoplasm in eukaryotic cells is typically a gel as is the extracellular matrix around cells.[3–5] Given the ubiquity and importance of gels, it is perhaps surprising that the gel state has remained so poorly understood from a fundamental structural standpoint. What are the constituent aspects of these materials that are fundamental for their 'gel-like' elasticity? This question is the focus of this article.

Several decades ago, Flory addressed this question and provided a useful classification scheme for gels.[6] The prototypical Flory gel is formed through the cross-linking of molecular units, more or less randomly distributed in space, to form a three-dimensional (3-D) network, as in the example of vulcanized rubber. This 'percolation model' has since become the accepted paradigm for gels.[7] Recently, however, attention has been increasingly placed on "molecular gels",[8] which are formed by the supramolecular assembly of small organic molecules,[9–13] surfactants,[14] peptides[15,16] or globular proteins[17–19] into highly extended fibers. Although a rheologist would certainly recognize such materials as gels, an examination of their microstructure seems to indicate that a cross-linked network does not typically exist! These observations clearly challenge the conventional Flory definition of gelation in terms of cross-linked networks. They also raise the practical question: if cross-links are not required, then what physical characteristics of these fibrous materials gives rise to their gel-like elastic response and how do we then make such gels by design?

The rest of this paper is organized as follows. First, we describe the 'canonical' cross-linked Flory gels as a reference point for our discussion. Then, we expand our discussion to molecular gels and show that these do not generally fit into the conventional Flory network picture. Finally, we offer a new microstructural picture of the latter gels.

2. Gels as cross-linked networks

Two canonical types of polymer gels are shown schematically in Fig. 1. First, we describe a *chemical* gel obtained by free-radical polymerization in water of a monomer, which in this example is acrylamide (AAm), combined with a multifunctional cross-linker, bis-acrylamide (BIS) (Fig. 1a).[20] Such polyacrylamide (PAAm) gels are the workhorses of protein separation *via* gel electrophoresis.[3] The figure shows linear segments of PAAm being connected by BIS cross-links into a 3-D network that pervades the sample volume. The molar ratio of BIS : PAAm sets the cross-link density of the gel. Note that all the bonds in this gel, *i.e.*, those within a chain segment as well as the cross-links between segments, are strong covalent bonds. We then move on to a *physical* gel obtained by combining the biopolymer, sodium alginate with calcium (Ca^{2+}) ions.[21] Such gels are frequently used as matrices for cell culture and tissue engineering. The structure of this gel (Fig. 1b) involves the so-called "egg-box junctions" – these are junction zones between alginate chains where Ca^{2+} ions are electrostatically complexed to more than one chain. Thus, the cross-links in this gel are based on physical associative interactions, rather than covalent bonds.

The above two gels share many similarities: both have chains connected into sample-spanning 3-D networks and in both cases the cross-links are "permanent", *i.e.*, long-lived or even time-invariant. In rheological parlance, the gel does not relax; equivalently its structural relaxation time τ (and low-shear viscosity η_0) are practically infinite. A qualitative rheological test for a gel is to place the sample in an inverted vial – if the sample is able to hold its weight for very long periods of time upon vial inversion, it indicates a finite yield stress and hence a gel.[22] The standard quantitative rheological test on a gel is performed in the frequency domain (oscillatory shear), and the commonly measured properties are the elastic or storage modulus G' and the viscous or loss modulus G'' as functions of frequency ω.[1,2] A fully developed gel shows $G' > G''$ for several decades of ω, with the ratio of $G' : G''$ being 10 or higher.[23,24] Moreover, the elastic modulus G' exhibits a plateau at low ω, extending to timescales at least on the order of seconds (for an example, see Fig. 4a later).[23,24] Such an elastic response to deformation reflects the presence of the polymer network that stores the deformation energy over long timescales.[1,2]

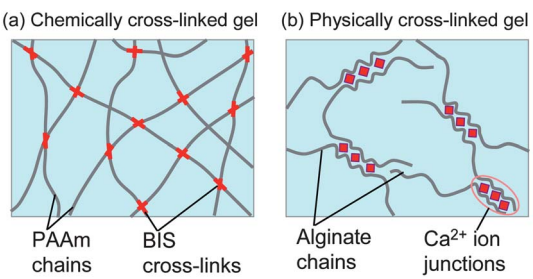

Fig. 1 Schematics of polymer hydrogels. (a) Chemically cross-linked gel by free-radical polymerization of the monomer AAm in the presence of BIS cross-linker. (b) Physically cross-linked gel by combining alginate and Ca^{2+} ions; here, the ions complex with adjacent chains along junction zones ("egg-box" junctions). In both (a) and (b), the chains are permanently linked into a 3-D network that extends throughout the volume.

3. Viscoelastic polymer solutions

In contrast to gels, there are many materials in which the elastic response is observed merely as a transient phenomenon. The classic example is that of solutions of a linear, high-molecular-mass polymer at a concentration within the semidilute regime. Such polymer solutions show a classic *viscoelastic* response in the frequency domain, with elastic behavior at short timescales (high ω) but viscous behavior at long timescales (low ω).[1,2] That is, at high ω, G' exhibits a plateau and is larger than G'' whereas at low ω, both moduli depend strongly on ω and $G'' > G'$ (for an example, see Fig. 4b below). The structural implication of the relaxation observed at low ω is that the polymer chains are not permanently constrained by their neighbors, unless cross-links of the type shown in Fig. 1 are added to entrap the chains.[2]

The interchain topological interactions between long flexible polymers are usually termed "entanglements".[25] The de Gennes[26] and Doi–Edwards[27] tube models of entanglement envision that a given chain is confined by surrounding chains into a harmonic tube defined along its contour. These models heuristically suggest that the escape of chains from their tubes by a snake-like motion or "reptation" dictates the timescale for stress relaxation. The main prediction of these physically attractive and simple models is that the relaxation time τ scales as a large power, typically $\tau \sim M^3$ of the polymer molecular mass M.[2,27] It is important to appreciate that τ is expected to remain finite for a viscoelastic material such as a polymer solution, while it is infinite for a gel. This raises an important question: if τ becomes extremely large due to the formation of long polymer-like chains by self-assembly, could we then have a gel for all practical purposes?

4. Molecular gels

Now, we turn our attention to molecular gels that arise by the self-assembly of small molecules.[8] A typical system involves a low-molecular-mass organic molecule (gelator) in a solvent. Hundreds of such gelators are now known, including both organogelators that can gel a variety of organic solvents[9,11] and hydrogelators that can gel water.[10] Initially, the gelator is dissolved in the solvent at a high temperature to produce a thin solution (sol). Upon cooling below a characteristic temperature T_{gel}, the sol is converted into an elastic gel that can hold its weight under tube inversion.[22] Rheological studies confirm the elastic nature of these gels, *i.e.*, the moduli G' and G'' are typically found to be frequency-independent.[9–11] Electron micrographs of these gels usually reveal a network of fibers (Fig. 2) and the term "self-assembled fibrous network (SAFIN)" is sometimes used in this context.[8] The diameter of the fibers is usually in the nanoscale range, whereas their lengths often exceed tens of microns, *i.e.*, the fibers are very long relative to their thickness. Evidently, the fibers are formed by the supramolecular assembly of gelator molecules along the axial dimension. But, as noted above for polymer solutions, the mere presence of fibers or chains is not sufficient for gel formation. So why are these materials gels rather than being viscous or viscoelastic fluids?

A similar scenario also exists for filamentous gels of peptides[15,16] and proteins.[17–19] In the case of filamentous cytoskeletal proteins such as actin or microtubules, the building blocks are themselves globular folded macro-molecules. For example, in the case of actin, the building blocks are globules of G-actin, a protein of molecular mass around 30 000 and a spherical size of ~5 nm diameter.[3,4] A solution of G-actin in water at room temperature is a thin sol. Under specific conditions of salt concentration, temperature, *etc.*, G-actin self-assembles into filaments of F-actin having diameters of about 30 nm and lengths of many microns.[3,4] In turn, the sample is converted into an elastic gel.[17–19] Although a large number of cross-linking proteins are known for F-actin,[3,4] gel formation occurs even in their absence.[17–19] So once again we are faced with the question of why these protein filaments form a gel rather than a viscoelastic solution.

To explain gel formation in molecular or protein gels, researchers often draw an analogy to cross-linked polymers (*i.e.*, the Flory model[6]) and insist that cross-links *must* exist. That is, there must be some mechanism by which fibers are constrained at junction points.[9,12,13] Three such scenarios can be naturally expected (Fig. 3): (i) attractions at junctions; (ii) junction zones or bundling; and (iii) jamming at junctions. The first possibility (Fig. 3a) is that when adjacent fibers cross at a junction point, they could experience a weak attractive interaction that essentially amounts to a cross-link. This interaction could be the same one that led to fiber growth in the first place or it could be an interaction specific to the interfacial chemistry of the fiber. The second scenario suggests that fibers could sometimes undergo higher-order supercoiling into axial bundles. Such bundling or twisting over zones (portions of adjacent chains) could essentially amount to cross-linking, as shown in Fig. 3b (and akin to Fig. 1b). Lastly, when fibers are rigid and growing, fiber ends could 'jam' into the bodies of adjacent fibers (Fig. 3c), resulting in a branched network. Alternately,

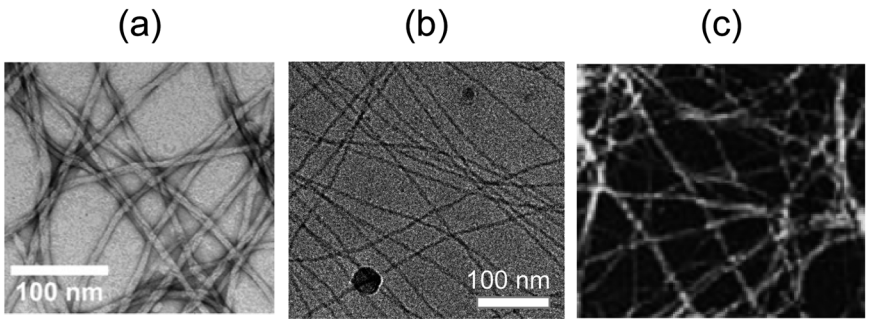

Fig. 2 Micrographs of three distinct classes of molecular gels. (a) TEM image of a hydrogel formed by the organic molecule Fmoc-F5-phenylalanine. Reprinted with permission from ref. 32. (b) Cryo-TEM image of a wormlike micellar gel based on 50 mM of the zwitterionic surfactant EDAB. Similar images are shown in ref. 33. (c) TEM image of the network of the filamentous protein F-actin at a concentration of 1 μM. Reprinted with permission from ref. 19. Copyright 2008, American Institute of Physics.

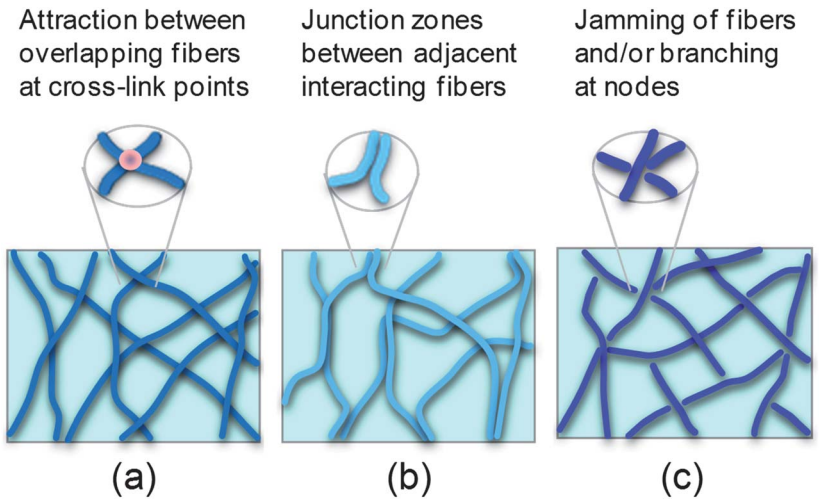

Fig. 3 Three scenarios by which cross-linking could occur in molecular gels. (a) When fibers overlap, they could experience a weak attraction at the point of overlap; (b) adjacent fibers could twist or bundle over small overlapping zones; (c) fibers could jam into each other at distinct nodes, or alternately, these nodes could serve as branch points. In all cases, the net result is that the cross-link points serve to immobilize the fibers and thereby give rise to a connected network of fibers.

branched fibers could arise due to secondary nucleation of grains with new orientations at the interface of the fiber, a process that ultimately leads to spherulitic growth.[28,29] In either case, these branch points would certainly constrain the fibers and could thus account for gel behavior.

While each of the above scenarios is plausible, and may occur in specific gels, there are also reasons to question whether cross-links can account *generally* for gels formed by supramolecular assembly. We now consider the evidence against the above scenarios for cross-links. First, regarding attractive interactions at junctions (Fig. 3a), direct evidence for these has not been forthcoming, despite the large number of studies on gels. Moreover, many gelators are amphiphilic molecules[10] that tend to arrange in such a way that the interior of a fiber is solvophobic while its exterior is solvophilic – if so, it is difficult to see why the solvophilic exterior surfaces of adjacent fibers should experience any strong attractions. Regarding junction zones or bundling (Fig. 3b), there are some published studies on gels where such zones are clearly revealed in micrographs,[30,31] but this is by no means universally true. Lastly, regarding spherulitic growth, it is seen in gels where the fibers are strongly crystalline,[30] but many gels have amorphous fibers,[14] and even when the fibers have ordered structure, they are often flexible rather than rigid, which may preclude simple jamming. Thus, it is not obvious *a priori* that a cross-linking based picture provides a comprehensive description of molecular gels.

To conclude this section, we discuss the micrographs shown in Fig. 2, which were chosen to be representative of various types of supramolecular gels. The first (Fig. 3a) is a TEM image of fibers in a hydrogel formed by the organic molecule, Fmoc-F5-phenylalanine.[32] The second (Fig. 3b) is a cryo-TEM micrograph of wormlike micelles in a hydrogel formed by a zwitterionic C22-tailed surfactant (more about this system in Section 5).[33] The third (Fig. 3c) is an electron micrograph of F-actin fibers formed *in vitro* by addition of salt to G-actin in the absence of other crosslinking proteins.[19] In each case, we can see long fibers that may be considered semi-flexible, *i.e.*, not coiled up like a 'random coil' but still showing some local curvature that fluctuates along the fiber so that the fibers are not rods either. No fiber ends are visible, which seems to rule out jamming, and significant branching of the fibers is also not evident. Junction zones or bundling cannot be ruled out in (a) and (c), but these features cannot be frequent, if they occur at all. The striking point is that although these are very different types of molecular gels, the fibers and their network actually appear to have a similar structure. In each case, the fibers seem to form an entangled mesh defined by topological interactions rather than a directly connected physical network – much like a bowl of spaghetti! (As will be noted later, the topological interactions between such fibers cannot strictly be termed "entanglements" in the way they are understood for flexible polymers, but for now we will use the terms interchangeably.) So what role do entanglements or topological interactions play in these networks? Can they explain the network elasticity, *i.e.*, the gel behavior? This is the focus of the remainder of this paper.

5. Wormlike micelles: gels *vs.* solutions

One class of soft materials that bridge the gap between entangled polymers and molecular gels are those containing wormlike micelles.[34–36] Aqueous wormlike micelles are typically composed of a relatively long-tailed cationic surfactant such as cetyl (C_{16}) trimethylammonium bromide (CTAB) and a salt such as sodium chloride (NaCl).[35,36] Surfactant concentrations are typically around 60 mM or about 3 mass%. In such mixtures, the surfactant molecules self-assemble into long cylindrical micelles, with diameters around 2–3 nm and average contour lengths exceeding 500 nm. These are called wormlike or threadlike or polymer-like micelles ("worms" for short) and their existence has been proven by a host of techniques including cryo-TEM.[36] Such worms entangle and form transient networks, much like polymers, and in turn, these solutions typically show viscoelastic behavior, *i.e.*, have a finite relaxation time.[34–36]

Wormlike micelle rheology can often be fitted to a Maxwell model with just a *single* relaxation time τ.[34] This is perhaps the simplest possible model for a viscoelastic fluid.[1,2] When this rheological behaviour was discovered, it was considered surprising because worms are highly polydisperse in their contour length and polydispersity typically leads to a spectrum of relaxation times rather than a single one. This aspect was explained by Cates as follows.[34] While worms are indeed long chains like polymers, they differ from polymers in that the chains are held by weak, non-covalent interactions. Surfactant molecules can thus be constantly exchanged between micelles,

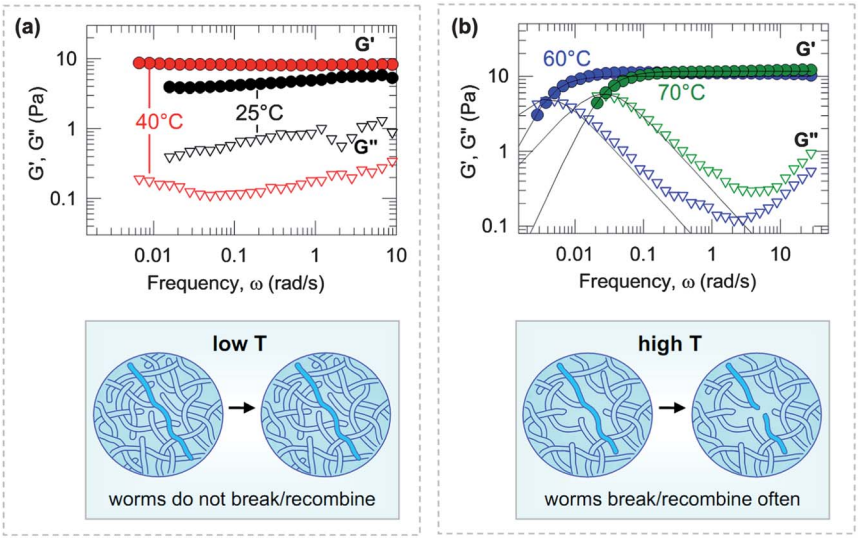

Fig. 4 EDAB worms at a 50 mM concentration can exhibit gel-like rheology at low temperatures (a) or viscoelastic rheology at higher temperatures (b) (data replotted from ref. 33). The elastic modulus G' is shown as filled circles while the viscous modulus G'' is shown as unfilled triangles. In (a), the moduli are nearly independent of frequency, corresponding to a gel-like response with an infinite relaxation time. The schematic suggests that the worms under these conditions form an entangled network and are temporally persistent, *i.e.*, they break and recombine very slowly. In (b), the dynamic moduli intersect at low frequencies, which implies a viscoelastic response with a finite relaxation time. The lines through the plots are fits to a single-τ Maxwell model. The schematic suggests that the worms in these cases are shorter, but still entangled; however, they break and recombine much more quickly (on a timescale of milliseconds), as shown by highlighting a single worm that breaks into two. The latter process provides a route for stress relaxation and accounts for the transition from gel to viscoelastic sol (having a finite relaxation time).

causing worms to frequently break and recombine (shown schematically in Fig. 4b). For this reason, worms are also referred to as "living" or "equilibrium" polymers. Worms can relax an applied stress by this breaking mechanism, which has a characteristic timescale called the breaking time t_{br} associated with it. In comparison, polymer chains, which feature strong covalent bonds between monomers, are effectively "unbreakable". Cates noted that when breaking occurs much more rapidly than the overall structural relaxation, *i.e.*, when $t_{br} \ll \tau$, corresponding to the "fast-breaking limit", the overall relaxation process would be Maxwellian, *i.e.*, there would be a single τ.[34,37] Experiments have confirmed the basic aspects of this theory.[34,36] Although the breaking time t_{br} of worms is a relatively difficult parameter to measure, reports of this quantity for Maxwellian worms formed by C16-tailed surfactants have found t_{br} to be on the order of 0.01 to 0.1 s.[38,39] These low values lend credence to the idea that worms exhibiting Maxwellian relaxation correspond to the fast-breaking limit.

In Fig. 4, we present data on wormlike micelles showing a response that is quite different from the above picture. The data is from a study by Kumar *et al.*[33] on a sample containing 50 mM of the surfactant erucyl dimethyl amidopropyl betaine (EDAB). This surfactant has a long erucyl tail (C22 with a *cis* unsaturation between the 8th and 9th carbons), and it is zwitterionic, with both cationic and anionic moieties in its headgroup. The weak overall charge on the head allows EDAB to form worms in water even in the absence of salt. Fig. 4 shows dynamic frequency spectra (G' and G'' *vs.* ω) at temperatures of 25 and 40 °C (Fig. 4a) and 60 and 70 °C (Fig. 4b). The data at 25 and 40 °C are characteristic of gels, *i.e.*, G' and G'' are nearly *independent of* ω and $G' > G''$ over the entire ω range. Note that the data at 40 °C go down to 0.007 rad s^{-1}, which is near the practical limit for commercial rheometers. Thus, this sample is clearly a gel at low temperatures by standard rheological criteria,[23,24] and this finding is further corroborated by data from steady-shear rheology and creep measurements,[33] all of which reveal a true yield stress. On the other hand, the data at 60 and 70 °C reflect a viscoelastic solution, *i.e.*, $G' > G''$ at high ω whereas $G'' > G'$ at low ω. The lines through the plots in Fig. 4b are fits to a single-τ Maxwell model and the values of τ are 250 s at 60 °C and 60 s at 70 °C.[33]

It is unusual that wormlike micelles can show gel-like rheology, rather than the expected viscoelastic response. Also, it is notable that worms can behave as a gel at low temperatures and transform gradually into a viscoelastic solution at higher temperatures. This is in contrast to most molecular gels, which as noted in Section 4 remain as gels until a characteristic temperature T_{gel}, whereupon they "melt" into a sol (thin viscous liquid).[9,11] Here, we instead find a smooth progression from an initial gel to a highly viscoelastic sol and thereafter a sol of lower viscosity. How can we explain these results and what does it imply for the nature of the gel state? Before continuing, we note that similar gel-like behavior of wormlike micelles has now been seen in at least three other systems to our knowledge: (a) another C22-tailed zwitterionic surfactant;[40] (b) a C22-tailed cationic surfactant;[41] and (c) a C22-tailed anionic surfactant[42] (in the latter two cases, worms were formed in the presence of salt). Thus, a common factor in gel-like worms is that the surfactant has a much longer tail (C22) compared to typical worms (C16 or shorter tails).

One might wonder if the gel-like EDAB worms are structurally different from conventional worms. However, no distinct structural features have been found using a number of techniques including SANS and cryo-TEM.[33] A cryo-TEM image of EDAB worms was shown earlier in Fig. 2b and additional images can be found in the Kumar *et al.*[33] paper. No unusual features are evident from these images, *i.e.*, there is no indication of junction zones, bundling or branching. Superficially, these images look just like those of any other conventional viscoelastic worms.[36] The worms are simply long and entangled. Why then are these materials gel-like?

Our hypothesis is that the gel-like rheology is a consequence of the EDAB worms having breaking times that are much, much longer than typical worms due to the longer surfactant tail length. This is suggested by the schematic in

Fig. 4a. For a worm to break, individual surfactants have to leave that worm and bind to an adjacent one. To do so, these surfactant molecules must pass through water. However, when the tail is long (C22), it is unfavorable for the surfactant to make contact with water. Note that the concentration of surfactant in equilibrium with micelles equals its critical micelle concentration (cmc).[43] The cmc of a C22 surfactant is about 10^3 times lower than that of a C16 surfactant.[33] In turn, this means that the breaking of a C22 worm is much less favored than that of C16 worms, *i.e.*, the breaking time t_{br} will be much higher than τ. If breaking was entirely prohibited (*i.e.*, if the worms were "unbreakable"), they would behave essentially like long, polydisperse polymers. In that case, τ would be related *via* a large exponent to the contour length of the worms L,[2,27,34] *i.e.*, $\tau \sim L^3$ in the reptation model. C22 worms like those of EDAB are also likely to be extremely long compared to C16 worms. One reason is that the lower cmc means that long micelles of EDAB are formed at lower concentrations.[33] Also, the longer surfactant tail has been shown to give rise to a higher end-cap energy for the worms, an effect that also implies the growth of longer worms.[41] In sum, we suggest that the gel behavior of worms formed by EDAB (and other C22-tailed surfactants) worms emerges from three important physical characteristics of these assemblies: the worms are very long relative to their cross-section; they are semi-flexible (worm-like); and they are relatively "unbreakable", *i.e.*, they are persistent on long timescales. In such cases, the topological interactions ("entanglements") between the worms are strongly manifested, and these interactions, rather than cross-links *per se* are hypothesized to be sufficient to convert the fluid into a gel.

How to explain the transition from gel to viscoelastic solution with increasing temperature? We suggest a combination of two factors: first, worms have been shown to undergo an exponential decrease in their contour length L as temperature increases.[34,41] Thus, the structural relaxation time should drop precipitously with temperature (since $\tau \sim L^3$). Second, surfactant diffusion becomes more rapid at higher temperatures, and so an EDAB molecule will be able to pass from one worm to another while reducing the time of unfavorable contact of its long hydrocarbon tail with water (as suggested by the schematic in Fig. 4b). In turn, the breaking time should also decrease with temperature.[38,39] The combination of these two effects is evidently enough to cause a transition from a low-T gel regime to a high-T viscoelastic regime where the overall relaxation time is finite.

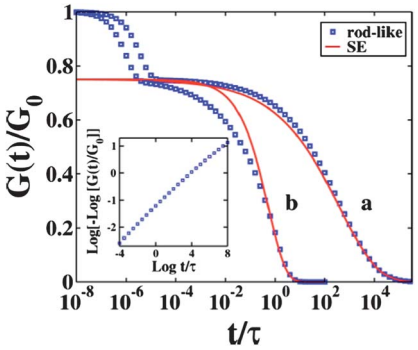

Fig. 5 Predictions of $G(t)$ for (a) long, unbreakable rods; and (b) living (frequently breaking) rods formed by self-assembly of monomers in an equilibrium polymerization model. In both cases, the relaxation is a multistep process with the slower step being a stretched exponential (SE) (eqn (1)). The solid lines are SE fits with β about 0.3 and 0.6 for (a) and (b), respectively. Figure reproduced with permission from ref. 44. Copyright 1998 American Chemical Society.

6. Relaxation of fiber networks: theory

We have used recent experimental results on wormlike micelles to define a minimal set of conditions for gelation in the absence of cross-links. In this context, it is worthwhile to discuss theoretical studies that can shed light on this matter. First, we consider a recent study by Stukalin *et al.*[44] on the relaxation of long polydisperse rods (equilibrium polymers) formed by the reversible self-association of monomers. It examined the scenario where the exchange time of the monomers with one another in solution (analogous to t_{br} above) became very long, *i.e.*, the rods, once formed, were persistent and almost unbreakable. The rods were taken to be in dilute solution, so that interchain interactions were eliminated. Fig. 5 shows the shear stress relaxation $G(t)$ of the rods under the above conditions. Two distinct relaxation processes are evident: a short time near-exponential relaxation followed by a long time stretched exponential relaxation of the form given by:[44]

$$G(t) = G_0 \exp\left[-\left(\frac{t}{\tau_T}\right)^{\beta}\right] \qquad (1)$$

The origin of the stretched exponential relaxation comes from the polydispersity of the rods, *i.e.*, the fact that shorter rods relax more quickly than longer rods. The stretching exponent β for the persistent rods in the above plot is around 0.3. β decreases from 1 with increasing rod length and plateaus at the above value for long persistent rods. Conversely, Fig. 5 also shows the $G(t)$ for long, living rods (*i.e.*, those with frequent monomer exchanges), and it exhibits a β around 0.6. Note that the high temperature limit $\beta = 1$ would be indicative of Maxwellian relaxation. Also, for persistent rods, the relaxation time τ_T within the stretched exponential, *i.e.*, the terminal relaxation time, grows exponentially high with increasing rod length (see Fig. 4 of Stukalin *et al.*). Thus, the simple growing of long persistent rods is evidently sufficient by itself to both increase τ_T to astronomical values, as well to create a stretching of the relaxation (reduce β).

A large τ_T is not enough to explain the onset of elasticity; for that we must account for the topological interactions between chains ("entanglements"). In our view, topological and packing interactions between the chains are governed by locally repulsive excluded volume interactions.[45,46] These interactions arise when the chains are considerably long relative to their cross-section and the interactions are strongly influenced by chain stiffness. Specifically, chains with a larger cross-sectional size (and thereby a higher stiffness) are predicted to show stronger topological interactions, and this has also been confirmed experimentally. In turn, the scaling exponent relating the relaxation time τ to chain length L is predicted to substantially increase upon going from flexible to stiff chains (from $\tau \sim L^3$ to $\tau \sim L^5$).[47] Numerous experiments have confirmed such a large increase in the scaling exponent for semi-flexible and stiff polymers.[48–50] Thus, topological interactions should be much more long-lived when the chains are worm-like (semi-flexible) or rod-like (rigid) as opposed to flexible structures.

Incidentally, semiflexible or stiff chains are less likely to form loops and knots – so the term "entanglement" is arguably a misnomer for the topological interactions between worm-like or rodlike chains or fibers. Still, the term is effective in conveying the idea of an interaction dictated by topological constraints, and the analogy once again is to the interactions between individual strands of spaghetti in a bowl.

7. Implications: what makes a gel?

We return to the fundamental question that motivated this paper: how can chains/filaments/fibers form a permanent gel? Certainly, gels can be formed by introducing permanent cross-links between the fibers, as envisioned by Flory, but we argue that it is also possible to have gels in which there are no such cross-links. The point of this paper is that a gel-like collective response can arise purely by topological interactions ("entanglements") of long fibers at semidilute concentrations. The requirements for gelation by "entanglement" are suggested to be the following: the fibers must be:

- *Long* (contour length of the fiber must be much larger than the cross-sectional dimension),
- *Stiff* (worm-like or rod-like), which is often realized when the fiber cross-sectional size is large,[4] and
- *Temporally persistent, or "unbreakable"* (*i.e.*, incapable of relaxing by molecular exchange/chain scission events).

We can also begin to rationalize why certain entangled chain/fiber systems form gels while others do not. Flexible polymers in solution do not form gels because the chains are not stiff enough or long enough (to have lengths in the micron range, the polymer molecular mass must be in excess of several million). Conventional surfactant worms can be sufficiently long and stiff, but they will not give gels if the worms break rapidly, since these breaking events provide an active channel for structural relaxation. As shown by Fig. 4, gel-like worms are found only when the surfactant tail length is long (C22) and the temperature is low, which satisfies the condition of long breaking times and thus temporal persistence of long worms. In the case of nanofibers formed by the assembly of organo- or hydro-gelator molecules, the fibers are usually persistent in shape as well as relatively stiff due to their large thickness (tens to hundreds of nanometers). Thus, gelation by entanglements requires only that the fibers be long relative to their cross-sectional dimension and sufficiently concentrated for large excluded volume interactions to be prevalent. Evidently, this condition is often met in practice, as seen from Fig. 2a. Finally, actin filaments are long and stiff enough to form entangled gels; evidently, the filaments in the gel regime must also be temporally persistent, *i.e.*, the timescales for these filaments to lose or add monomers must be quite slow relative to the relaxation timescale. In summary, the mere presence of long, semi-flexible fibers or chains at moderate concentrations can be enough to form a gel. Gel behavior can be rationalized on the basis of topological constraints ("entanglements") between the fibers; distinct cross-links are not necessary.

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