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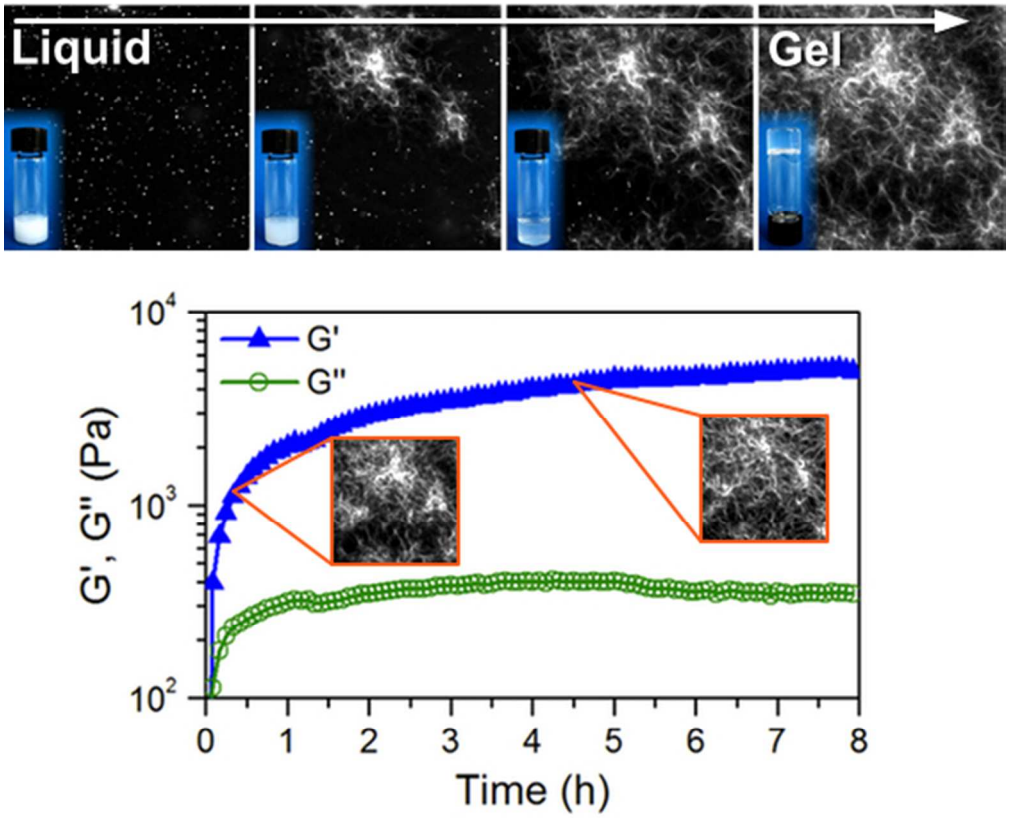

Fmoc-diphenylalanine gels provide experimental evidence for the formation of equilibrium gels predicted in simulations of patchy particles.
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ARTICLE

Evidence for Equilibrium Gels of Valence-Limited Particles

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We explore the formation and structure of gels produced from solutions of the aromatic dipeptide derivative molecule fluorenylmethoxycarbonyl-diphenylalanine (Fmoc-FF) in dimethyl sulfoxide (DMSO). Mixing these solutions with water results in the self-assembly of Fmoc-FF molecules into space-filling fibrous networks, exhibiting mechanical properties characteristic of gels. Using confocal fluorescence microscopy, we observe the gel transition in situ and find that, upon the addition of water, the solution undergoes a rapid transition to a non-equilibrium state forming ~ 2 μm spheres, followed by the formation of fibers 5–10 nm in diameter, nucleating at a sphere surface and expanding into the solution as the remaining spheres dissolve, extending the network. The gel aging process is associated with the network becoming increasingly uniform through apparent redissolution/reaggregation of the Fmoc-FF molecules, corresponding to the observed increase in the elastic modulus to a plateau value. We demonstrate that this increase in uniformity and elastic modulus can be expedited by controlling the temperature of the system, as well as that these gels are thermally reversible, further indicating that the system is in equilibrium in its fibrous network state. X-ray scattering information suggests that the packing of the molecules within a fiber is based on π–π stacking of ß-sheets, consistent with models proposed in the literature for similar systems, implying that each particle (molecule) possesses a limited number of interaction sites. These observations provide experimental evidence that these low molecular weight gelator molecules can be considered valence-limited "patchy" particles, which associate at low enough temperature to form equilibrium gels.

Introduction

Low molecular weight gelator molecules suspended in a liquid can form gels at very low mass fractions (< 1 wt%) due to highly specific interactions that result in the formation of fibers that branch, entangle and fill space.[1] A specific class of gelators associated with aromatic short peptide derivatives possessing an N-terminal fluorenyl-9-methoxycarbonyl (Fmoc) group self-assemble into strongly anisotropic structures with high aspect ratios, creating fibrous networks that have long relaxation times and display large elastic moduli and yielding behavior under an applied load.[2-16]

An example representative dipeptide, Fmoc-diphenylalanine (Fmoc-FF, Figure 1), forms molecular gels when fiber formation is triggered by a change in the pH or by the addition of water to a solution of Fmoc-FF in dimethyl sulfoxide (DMSO), upon which the molecules undergo a self-assembly process into a space-filling fibrous network. The building blocks of these fibers are individual molecules experiencing specific anisotropic interactions, associating primarily through π–π stacking and hydrogen bonding; thus, these materials differ significantly from polymeric networks, in which chemically crosslinked polymer chains give rise to solid-like properties.[17] Physical gelation occurs in colloidal systems typically associated with particles of tens or hundreds of nanometers in size undergoing aggregation due to short-range attractions, which have been widely studied for spherical particles and centrosymmetric interactions.[18] Hence, dipeptide-based molecular gels are also distinctive from classic particulate gels, as the particles (molecules) are of sub-nanometer size, the interactions are highly anisotropic, and the resulting aggregates are highly structured.

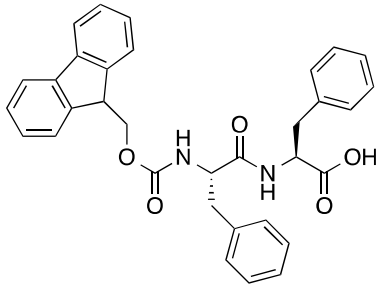

Figure 1. Chemical structure of Fmoc-diphenylalanine.

Attractions between particles that have finite volumes have long been associated with first order gas-liquid phase transitions. More recently, these same concepts have been applied to mixtures of molecules or particles in a solvent where the potential of mean force between solute molecules is mediated by the solvent. The result is predictions of equilibrium liquid-liquid and liquid-crystal phase transitions driven by solute attractions. Applied initially to solutes with isotropic attractions, these studies explained the experimental observations of liquid-liquid phase separations that are metastable as compared to a liquid-crystal phase,[19-21] providing confidence in treating mixtures through the classical McMillan-Mayer theory.[22] In this approach, for the purposes of determining equilibrium thermodynamic properties, the mixture is treated as a one-component system where the solute experiences solvent mediated interactions. The power of this approach is that simulations and theory are greatly simplified as the apparatus of the statistical mechanics of single component systems can be transferred to colloidal suspensions and molecular mixtures in a straightforward manner.

An example can be found in simulations of the aggregation behavior of "patchy" particles capable of forming a limited number of bonds, which were done on single component systems but can be applied to describe structure and aggregation properties of particles in solution.[18,23,24] These simulations were done to explore the effects of reducing the number of bonds a particle can make on the conditions giving rise to a gas-liquid phase transition and demonstrated that as the number of bonds the particles can make drops below 3, attractions no longer produce a gas-liquid (or for molecules in solution, a liquid-liquid) phase transition. As the valence of the particles drops towards 3, the temperature (kT/ε where kT is the product of Boltzmann's constant and the absolute temperature and ε is the contact value of the strength of attraction) at the critical point drops and the volume fraction at the critical point decreases to very low volume fraction. This behavior opens up a large volume fraction vs. strength of attraction space where liquids are thermodynamically stable. In this region, as the strength of attraction increases, particle diffusivity slows until relaxation times approach those associated with gels. In these slowly diffusing limits, simulations display extended but fluctuating structures that spread to fill space.[18,23,24] Iso-diffusivity lines become very sensitive to strength of attraction at low volume fraction and insensitive to strength of attraction at high volume fractions. In these systems, long mechanical relaxation times are associated with slow diffusion of the solute and when the solute diffuses sufficiently slowly, the system is said to have formed a gel.

Dynamic localization theory, developed initially for particles with centrosymmetric interactions but extended to particles with anisotropic shape, describes gelation is a similar language. In this theory, gelation is not a discontinuous change in behavior, but is best characterized by a smooth yet rapid change in relaxation times for density fluctuations that takes place over a narrow range of temperature or solute volume fraction.[25-27] These increases in relaxation times are associated with collective processes that result in particles being localized by nearest neighbors. This localization is described in terms of a dynamical potential of mean force that characterizes the rate of density fluctuation decay. The dynamical potential is determined from the equilibrium properties of the system thus again emphasizing that localization is a dynamical transition, not an equilibrium phase separation. This model and the simulation work on valence limited particles indicate that gelation can occur in thermodynamically stable systems where the strength of attraction and volume fraction reach values where relaxation times increase rapidly with changes in either variable.

Self-assembling peptide-based systems offer possibilities in designing new materials with unique structural, optical, mechanical and biological properties based on the molecular composition and due to the variety of structures into which these molecules will assemble (helices, coiled-coils, ß-sheets, ß-hairpins, etc.).[28-30] These architectures can be utilized to form nanostructured hydrogels from peptide solutions, offering numerous potential applications in tissue engineering, drug delivery, nanofabrication, and biosensing.[30] However, the origin of the gel transition of peptide-based materials, as well as the relationship between the composition, structure, and mechanical properties of these molecular gels, remain poorly understood. The mechanism by which the relaxation times of these materials are dramatically increased are important to understanding shelf life and stability of the formed structures. If gels formed from these molecules are nonequilibirum, they will continuously evolve towards an equilibrium state, which may have a completely different form (e.g., a liquid in equilibrium with compact crystals) with subsequent and perhaps dramatic changes to mechanical properties.

Using Fmoc-FF gels as a model system, we have previously established a range of conditions under which a solution of the peptide molecule in DMSO forms a molecular gel when mixed with water.[31] The driving force for the gel transition is inferred from the hydrophobic nature of the Fmoc-FF molecules and their ability to hydrogen bond. Rigid gels ($G' \sim 10^5$ Pa) are formed at low Fmoc-FF volume fractions ($\phi < 1\%$). The solutions move from low viscosity liquids to materials with substantial moduli and long relaxation times over small changes in Fmoc-FF or water concentration enabling the definition of a well-defined line of gel transition in a plot of water concentration as a function of ϕ. Over a wide range of conditions, the gel elastic modulus follows a universal scaling with volume fraction ($G' \sim \phi^{2.5}$). We hypothesize that these peptide-based molecular gels can be considered equilibrium gels, corresponding to simulation predictions in which "patchy" particles with a limited coordination number for possible bond formation reduce the tendency of the system to phase separate.

Here, we investigate the relationship between the structure and mechanical properties of Fmoc-FF molecular gels using optical, scattering, and rheological techniques. We show that prior to gelation, upon addition of water to a DMSO solution of Fmoc-FF, there is first an instantaneous precipitation to a temporary non-equilibrium state, followed by a rearrangement into a fibrous network. We demonstrate that the aging process of these molecular gels is associated with a tendency of the system to evolve towards a steady state through the structural rearrangements into an increasingly uniform network. These structural changes are correlated with growth in elastic modulus to a plateau value. We find that in liquid samples at concentrations right below the gel point, fibrous precursors are formed, and that above the gel point, increasing Fmoc-FF concentration results in increased diameter of gel fibers. Finally, we investigate the effects of temperature on the system and show that, in addition to previously reported mechanical reversibility,[10,14,31] these gels are thermoreversible. These results suggest that the when attractions are sufficiently strong relative to the average thermal energy in the system, Fmoc-FF molecules in solution aggregate and evolve towards a uniform distribution of fibers.

Experimental Section

Materials. Fmoc-diphenylalanine (Fmoc-FF) in solid powder was purchased from Bachem (Bubendorf, Switzerland) and used without further purification. Dimethyl sulfoxide (99.5%) obtained from Sigma-Aldrich was used as a solvent. Nile Blue fluorescent dye was purchased from Sigma-Aldrich. All samples were prepared with deionized water (resistivity 16.7 MΩcm).

Gel Preparation. The mass of required solid Fmoc-FF was measured on a scientific electronic scale with 0.001 g precision. The measured amounts of the peptide were dissolved in DMSO at selected concentrations, after which the samples were mixed for 1–2 minutes until the solid was fully dissolved in the solvent and a clear solution was obtained. Gelation of the samples was triggered by the addition of water at various ratios in order to obtain the desired final concentrations. For preparation at elevated temperatures, the Fmoc-FF/DMSO and water were preheated using a hotplate or water bath and then mixed in a vial. Cooling was performed by conditioning the sample to room temperature or by immersing the sample into an ice water bath for more pronounced temperature differences.

Confocal Fluorescence Microscopy. A small amount (~ 40 ppm) of a fluorescent dye (Nile Blue) was added to the solution of the peptide in DMSO. Immediately upon mixing the solution with water, 50 µL of the mixture was transferred into a glass bottom dish (MatTek Corp.) and covered with a lid. The sample was loaded onto a Zeiss LSM 700 confocal microscope and images were taken using a 63x magnification oil immersion objective at 639 nm excitation wavelength. Varying the amount of dye between 20–100 ppm did not qualitatively affect the gel structure.

Transmission Electron Microscopy (TEM). Gel samples were prepared as described previously. Immediately upon the addition of water to the solution of Fmoc-FF in DMSO, the sample was lightly mixed and a 15 µL droplet was transferred onto a glass surface, before the onset of gel transition. A 400 mesh carbon coated copper grid (SPI Supplies) was placed on the sample droplet for 30 s and the excess liquid was removed. The grid was then washed and placed on a 15 µL droplet of 7% uranyl acetate solution for 60 s, after which the excess stain was removed. The sample grid was covered to minimize water evaporation stored overnight. TEM images were collected using a JEOL 2010 LaB6 transmission electron microscope operating at 200 kV.

Rheology Measurements. Rheology experiments were performed on a TA Instruments DHR-3 rheometer with a Peltier plate and solvent trap. For elastic modulus measurements at room temperature, a 4º/40 mm cone geometry was used. Evaporation of water from the sample was minimized using the instrument's solvent trap setup, in which a small well in the rotating cone is filled with a low viscosity mineral oil and the blades of the solvent trap cover are immersed into the well, thereby creating a thermally stable vapor barrier around the sample. Fmoc-FF/DMSO solutions were prepared as previously described. As soon as water was added to the solution in a vial, the sample was mixed lightly and 1.2 mL of the mixture was quickly transferred onto the rheometer. The rotating element was lowered into the measurement position while the sample was still in liquid form (before the onset of gel transition), after which the experiment was started immediately. The evolution elastic and viscous moduli with time was measured at 25 ºC, at an oscillating stress (1–10 Pa) and frequency (1 Hz) in the linear viscoelastic region. For measurements at higher temperatures, where melting of the gel sample was required, a cup and bob geometry was used (15 mm radius cup and 14 mm radius DIN rotor). The liquid Fmoc-FF/DMSO/H2O mixture was loaded into the cup, after which the rotor was lowered, the solvent trap cover was placed, and the measurement was commenced. The temperature was controlled via the TRIOS software interface (TA Instruments).

Small Angle and Wide Angle X-Ray Scattering. Scattering studies were performed at beamline 5-ID-D of the Advanced Photon Source at Argonne National Laboratory, equipped with two 2-D CCD detectors for simultaneous SAXS and WAXS collection. Samples were loaded into cells sealed with Kapton tape from both sides with 1 mm spacing in between. The beam was operated at a wavelength of 1.24 Å and scans were taken in quadruplicate and averaged. The data was processed using Irena SAS analysis macros written for Igor Pro v6.22A software.

Results and Discussion

Preparation methods of gels from Fmoc-peptides generally fall into one of two categories:[14] (1) a number of studies have followed a gel preparation technique that involves the dissolution of the peptide molecule in an aqueous solution at elevated pH and then adjusting the pH of the solution by addition of glucono-δ-lactone (GdL)[4,5] or by acid titration at elevated temperature, followed by cooling overnight;[6-9] (2) the other preparation method is based on dissolving the peptide in a solvent and triggering the gelation process by adding water to the solution at room temperature, creating a three-component (peptide/solvent/water) system.[10-13] Here we focus on the second preparation method and study Fmoc-FF/DMSO/H_2O as a model molecular gel.

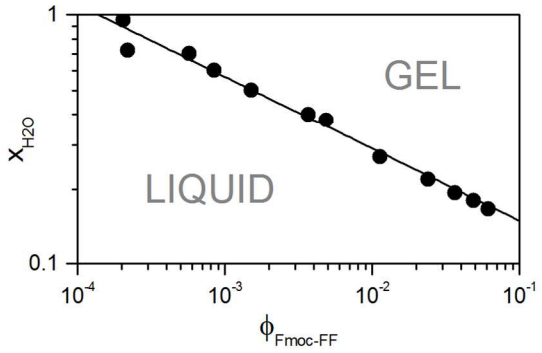

Figure 2. Gel line for Fmoc-FF/DMSO/water gels obtained by titration experiments. $\phi_{Fmoc\text{-}FF}$ represents the volume fraction of Fmoc-diphenylalanine in the gel; x_{H2O} is the final water concentration in the gel (by volume).

The conditions at which the solution gels depend on the particle volume fraction (Fmoc-FF volume fraction) and strength of attraction (controlled by water concentration).[31] For purposes of clarity, that phase diagram is reproduced here (Figure 2). The solution of Fmoc-FF in DMSO is a clear liquid, which when mixed with water undergoes rapid precipitation leading initially to a turbid white mixture, followed by a gradual shift (< 5 min) to a transparent gel (Figure 3), or a clear liquid at water concentrations below the gel point. The opacity of the

intermediate stage indicates the presence of structures of sizes large enough to scatter light.

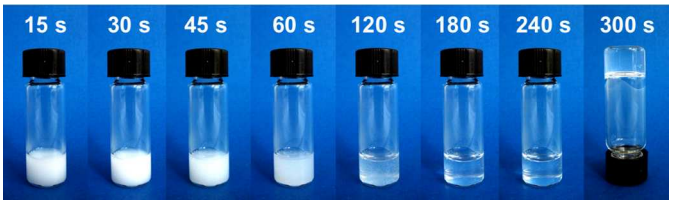

Figure 3. Change in appearance of an Fmoc-FF gel sample with time.

Using fluorescence confocal microscopy, the gelation process can be observed in situ from the moment of mixing the peptide solution with water (Figure 4). Introducing water into the system "shocks" the initially well dispersed hydrophobic Fmoc-FF molecules, causing a rapid shift (< 1 s) into a transient non-equilibrium state, composed of spherical clusters of diameters of ~ 2 μm (Figure 4a). This rapidly formed solid phase then transforms in a slower process (< 5 min) into a fibrous network. Rapidly growing fibers are nucleated from a limited number of spheres while the surrounding spheres dissolve and are attracted to the fibers, further extending the network (see Supplementary Information for confocal microscopy video of the gelation process†). Similar behavior has also been observed for another dipeptide molecule, Fmoc-leucine-glycine (Fmoc-LG).[12] These observations indicate that the fibrous structure represents a free energy minimum for the dipeptide molecules. Over longer times (> 30 min), the fibers continue to evolve with the overall network becoming increasingly uniform. This slow (several hours) restructuring of the network occurs through redistribution of molecules by a dissolution/reaggregation process that extends and creates branches in fibers. During this process, the sizes of the thicker fibers are reduced and new, thinner connections can be seen forming between large fibrous clusters (Figure 4a). The final diameters of the filaments, estimated to be between 5 and 10 nm from TEM images (Figures 4b and 4c), are smaller than the wavelength of visible light, and, as a consequence, the resulting bulk gel has a transparent appearance. Thus, the aging process of Fmoc-FF gels is associated with the tendency of approaching to a steady state corresponding to a uniformly distributed fibrous network. The system remains stable in its gelled state for an indefinite period of time (gels formed in our lab have been stable beyond 4 years). The slow approach to a uniform gel is correlated with the temporal evolution of the elastic modulus, which shows an initial jump related to the percolation of the fibrous network, followed by a slow increase to a finite steady-state plateau value over several hours (Figure 5).

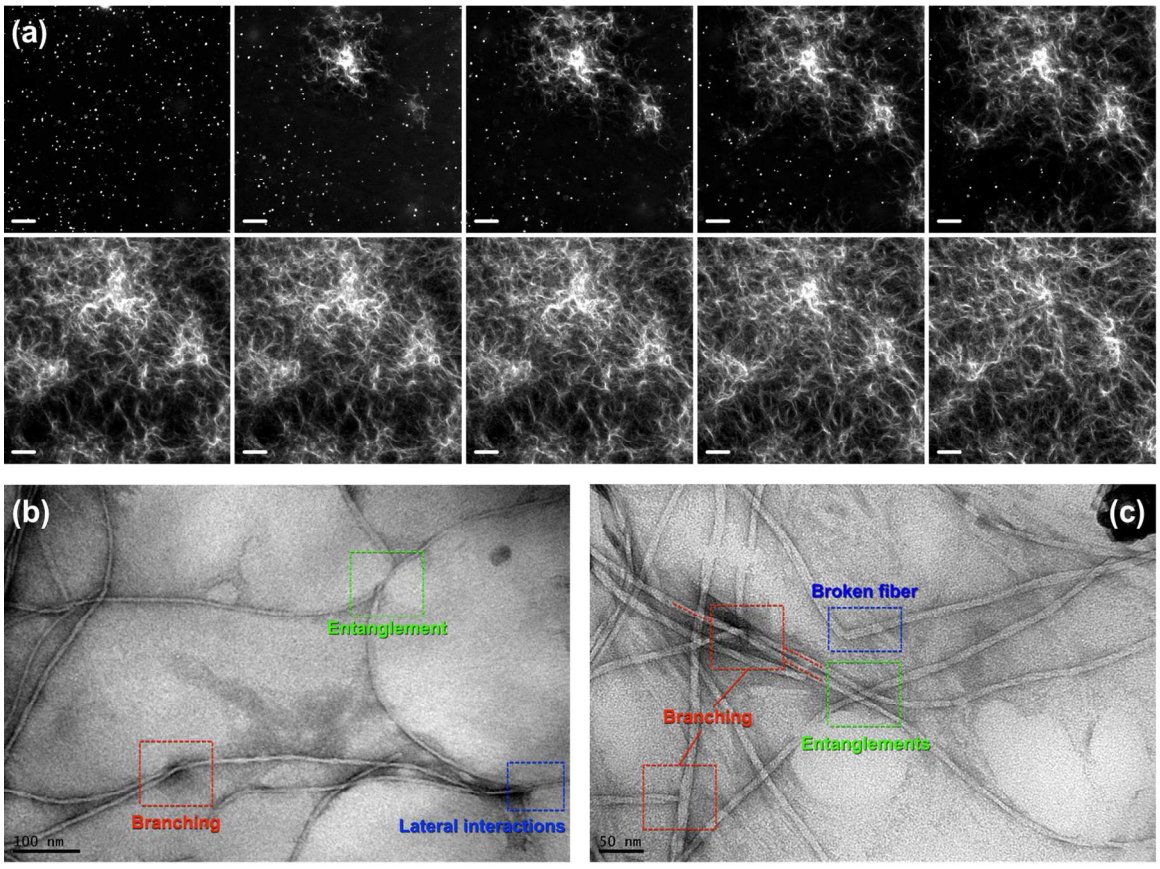

Figure 4. (a) Fluorescence confocal microscopy images taken at different times of the gel transition (top: 0, 30, 40, 50 s; bottom: 300 s, 1, 2, 3, 4 h) from the point of mixing (scale bar represents 10 μm); **(b)** and **(c)** TEM micrographs showing higher magnifications of the fibrous structure including branching, entanglements, and lateral interactions (the characteristic fiber diameters are estimated to be on the order of 5–10 nm).

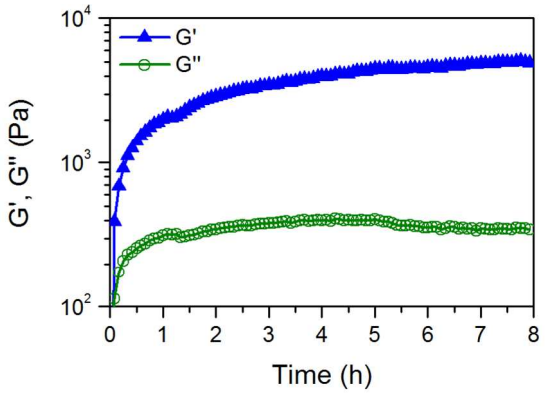

Figure 5. Temporal evolution of the elastic modulus of an Fmoc-FF gel ($\phi_{Fmoc\text{-}FF}$ = 0.005, x_{H2O} = 0.5. After the initial increase associated with the gel transition (t < 5 min), the modulus continues to grow over a period of several hours until a constant plateau value is reached.

The evolution of the structure in Fmoc-FF gels is distinctively different from that typically observed for particulate gels where, when a gel is formed from an arrested liquid-liquid phase separation by quenching into the spinodal,[18] gel aging is accompanied by the lowering of the system's free energy by decreasing surface area, which can be considered an Ostwald ripening process.[17] The particle-rich and particle-poor domains thus become larger, promoting the phase separation process, which can ultimately lead to a collapse of the network.[32] In an Fmoc-FF gel, the increase in network uniformity results in a steady-state structure, providing long-term stability (years). This spontaneous increase in surface area during Fmoc-FF gel formation and aging is evidence that gelation in this system upon increasing attractions does not result in crossing the spinodal or crystallization boundary.

The formation of elongated, uniformly distributed structures can be understood as resulting from the formation of extended, equilibrium, slowly relaxing structures composed of particles experiencing "patchy" interactions, described by Zaccarelli and Sciortino.[18,23,24] As mentioned in the introduction, their simulations show that when particles can only form a low number of bonds, extended structures that relax slowly are formed at low temperatures. The slow diffusion of particles within these entangled and branched structures results in long material relaxation times characteristic of gels. When the number of bonds a particle can form decrease towards 3, liquid-liquid phase separation is lost and iso-diffusivity lines corresponding to long relaxation times extend to very low volume fractions where the particles form increasingly extended structures.

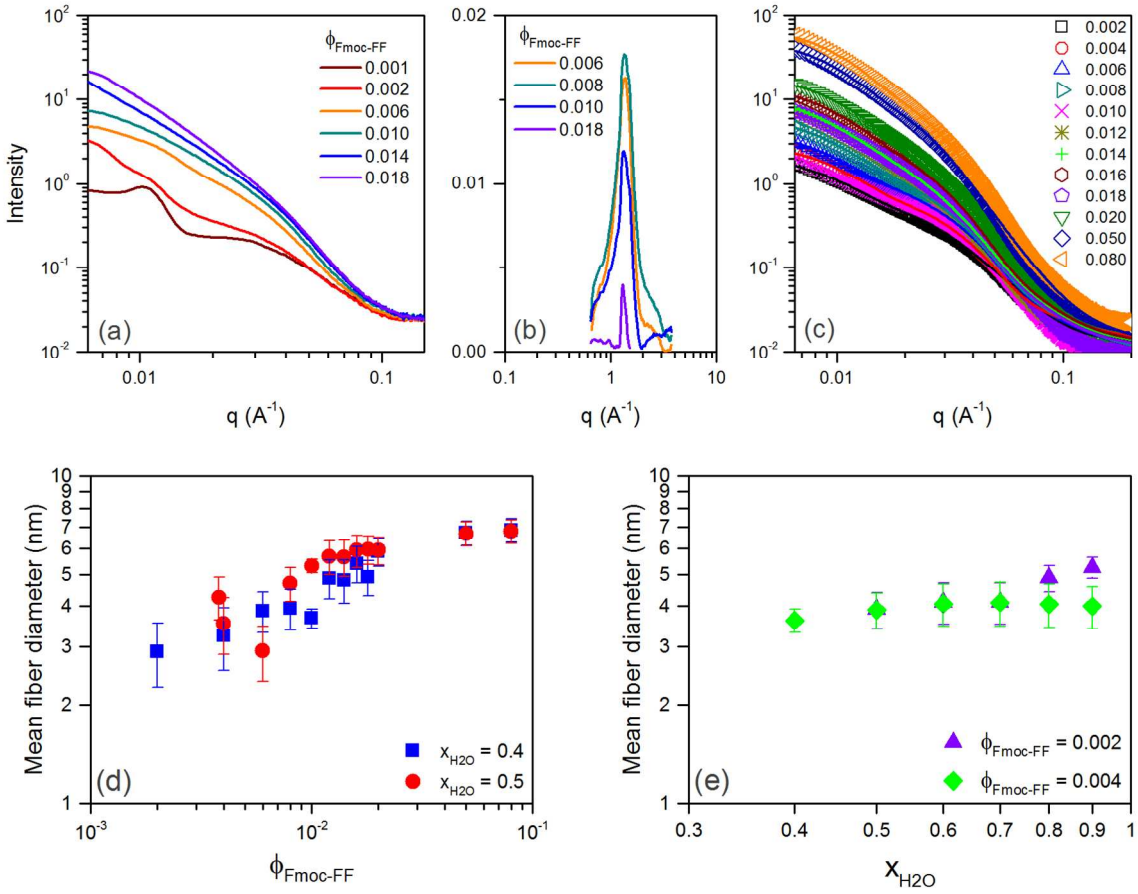

Figure 6. (a) Small angle X-ray scattering from Fmoc-FF gels of different Fmoc-FF volume fractions (at 50% v/v water concentration); **(b)** Wide angle X-ray scattering data after subtracting solvent and water signals; **(c)** Cylindrical form factor fits of gel scattering curves; **(d)** Mean fiber diameters estimated from curve fits for various Fmoc-FF volume fractions and **(e)** water concentrations.

For a pH-triggered Fmoc-FF/H_2O/NaOH/HCl gel system, Ulijn *et al.* proposed a structural model based on π–π stacking interactions between the fluorenyl groups of anti-parallel ß-sheets in which the phenylalanine groups of two molecules are associated through hydrogen bonding, creating a fiber of 3 nm in diameter.[7] In this model, an Fmoc-FF molecule would have a coordination number of 3 (resulting from one molecule forming a ß-sheet with another, and π–π stacking of the fluorenyl group with one molecule above and one below), which suggests the possibility of the formation of an equilibrium gel at the observed low volume fractions.

In order to analyze the structure for an Fmoc-FF/DMSO/H_2O system, small angle (SAXS) and wide angle X-ray scattering (WAXS) spectra of samples of different compositions (Figure 6) were obtained at the 5-ID-D beamline of the Advanced Photon Source at Argonne National Laboratory. At water concentration of 50% (v/v) and Fmoc-FF volume fraction of $\phi_{Fmoc\text{-}FF} = 0.001$, which is below the gel point ($\phi_{Fmoc\text{-}FF} = 0.002$), the mixture is liquid and at small angles the scattering shows that small objects ~ 60 nm in size ($q \sim 0.02$ Å^{-1}) are formed (Figure 6a). As the Fmoc-FF volume fraction is increased and the gel point is crossed ($\phi_{Fmoc\text{-}FF} \geq 0.002$), characteristic branched fibrous networks scattering patterns are obtained, with slopes at high q featuring fractal dimensions ~ 1.1–1.3. The existence of precursors to gelation was also confirmed by confocal fluorescence microscopy; at a composition below the gel point, fibrous clusters can be observed (Figure 7a), while at the gel point, fibers connect the clusters (Figure 7b). The size of the observed clusters is however much larger than 60 nm, suggesting that the scattering is probing structures inside the flocks as opposed to the entire clusters. At large scattering angles, subtracting the solvent and water scattering from the raw data of samples above the gel point revealed a peak at 1.3 Å^{-1} corresponding to a spacing of 0.48 nm (Figure 6b), which is characteristic of ß-sheet π–π stacking.[7,8] Due to the strong scattering of DMSO, other potential spacing-indicating peaks were not attainable. However, the evidence of ß-sheet spacing indicates that in an Fmoc-FF/DMSO/H_2O system the packing of molecules inside the fibers is possibly similar to the model proposed by Ulijn *et al.*[7]. The formation of ß-sheets that are further π–π stacked suggests the interactions of each Fmoc-FF molecule can be approximated as corresponding to three neighboring molecules, providing support for the formation of gels from valence limited particles as proposed by Zaccarelli and Sciortino.[18, 23, 24]

The SAXS curves for gel samples of different compositions were fit using a cylindrical fiber form factor with the length/diameter aspect ratio set to ~1000 (Figure 6c). The mean fiber diameters estimated from the fits were found to be weakly dependent on water concentration and range from 3 nm (matching the size predicted by Ulijn's model) to 7 nm (Figures 6d and 6e). This suggests that with increasing Fmoc-FF volume fraction the scattering curves are obtained from laterally associating fibers (Figure 4b) or that the fibers grow in diameter, implying that the molecular packing could depend on the composition of the system.

The self-healing properties of Fmoc-FF gels, i.e. ability to rebuild to their original strength after yielding at high enough shear stress has been reported[10,14,31] and is another indication that the system is in equilibrium. We have further explored the behavior of the Fmoc-FF/DMSO/water system when thermal changes are imposed. When a solution of Fmoc-FF in DMSO is mixed with water at elevated temperatures, the gelation process can be halted. In the experiments reported here, at higher temperatures the system initially forms the temporary nonequilibrium state in which the molecules form 2 µm spheres (opaque white appearance), which rapidly transitions into a clear liquid solution (Figure 8). Increasing the temperature lowers the strength of attraction between the molecules relative to the average thermal energy in the system, as well as the surface tension of the hydrophobic Fmoc-FF in contact with water, resulting in a solution of nonassociated Fmoc-FF molecules. When the sample cools, the solution begins turning opaque white (Figure 8), i.e., the molecules start forming spheres again, the gelation proceeds through network formation and there is a transition to a transparent gel. Similarly, if the temperature of a fully gelled sample is increased, the gel will melt, forming the colorless, clear solution, which rebuilds structure when the sample temperature is again lowered. These experiments demonstrate that the Fmoc-FF gels are thermoreversible and at sufficiently high attractions (low temperature) the fibrous network is the equilibrium state.

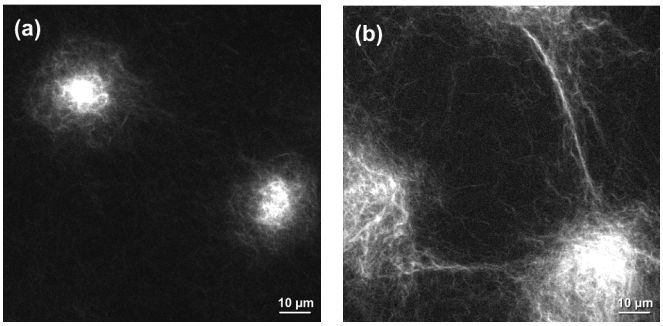

Figure 7. Confocal microscopy images of Fmoc-FF/DMSO/H_2O at compositions: **(a)** below the gel point; **(b)** at the gel point.

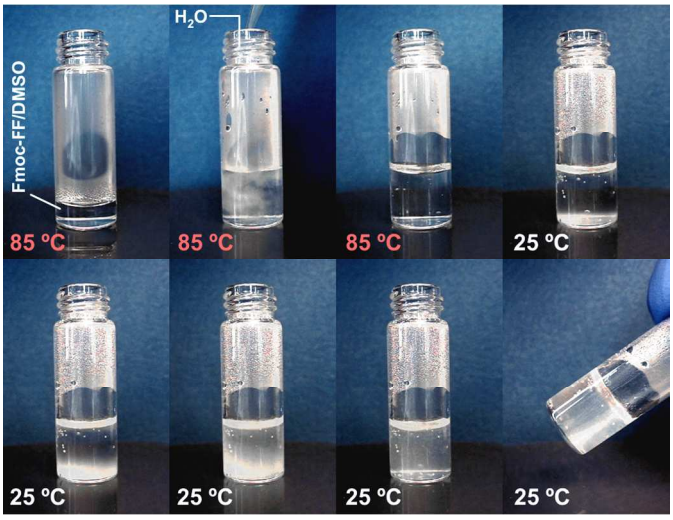

Figure 8. Effect of temperature on the Fmoc-FF/DMSO/H_2O system.

Confocal fluorescence microscopy carried out on temperature-cycled samples shows that repeated heating and cooling results in a finer network and smaller pore size (Figure 9). The rheological signature of these changes is shown in Figure 10, where, as the temperature is increased to the melting point of the gel and reduced back to 25 ºC, there is a

corresponding decrease and increase in the elastic modulus. Increasing the temperature of the system lowers diffusion time and enables more rapid rearrangements that lead to a uniform structure. Thus, when the network formation occurs at an elevated temperature, the Fmoc-FF molecules are capable of faster redistribution and, upon returning to room temperature, the subsequent elastic modulus is higher.

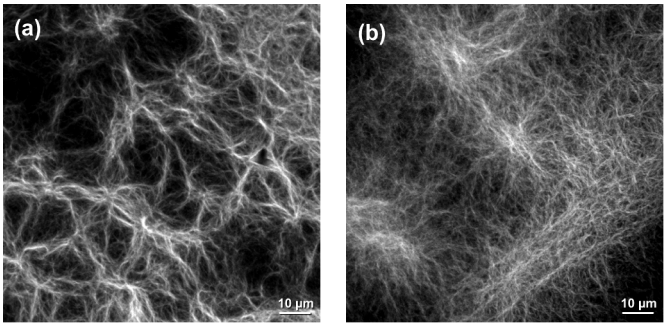

Figure 9. Confocal microscopy images of Fmoc-FF gel: **(a)** at room temperature; **(b)** after heating and cooling.

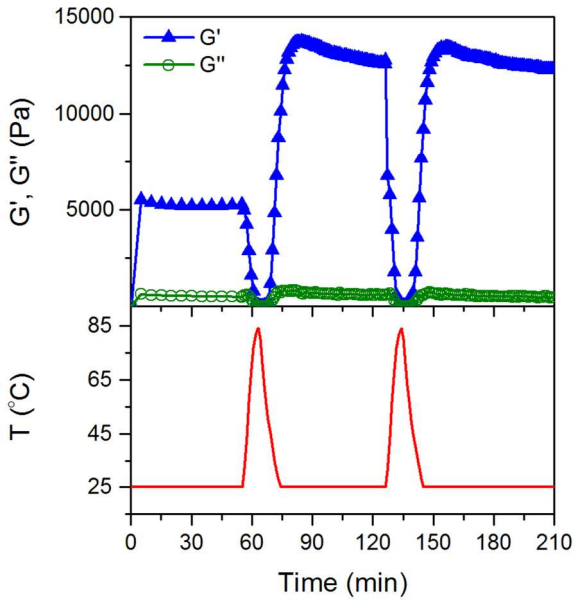

Figure 10. Changes in the elastic modulus resulting from melting and rebuilding of the gel structure.

Conclusions

At room temperature, hydrophobic Fmoc-FF molecules are readily soluble in DMSO, having a solubility point above 50 wt%. Upon addition of water, the solution undergoes a rapid transition to a non-equilibrium state forming ~ 2 µm spheres, which results in the solution taking on an opaque white appearance. If the system is below the gel point, the solution returns to being clear and transparent, although SAXS data suggests that as the gel line is approached these equilibrium solutions form ~ 60 nm structures. Above a water concentration sufficient to produce a gel, the dissolution of the metastable spheres is accompanied by a somewhat slower formation of a fibrous network, apparently nucleating at a sphere surface and expanding into the solution. In the late stage (t > 5 min), the network is composed of fibers with diameters of ~ 5–10 nm that continuously dissolve and reform as the uniform gel structure is established and sustained, leading to a steady state plateau elastic modulus. X-ray scattering data suggests that the packing of the Fmoc-FF molecules within a fiber are consistent with stacking of ß-sheets through π–π interactions between fluorenyl groups, similar to the model proposed by Ulijn *et al.*[7] For this type of packing, an Fmoc-FF molecule has 3 bonding sites (patches), which are expected from the simulations to result in equilibrium extended structures at low volume fractions.

As observed visually and rheologically, the process of forming a uniform fibrous network can be expedited by controlling the temperature. At sufficiently high temperature, Fmoc-FF gels melt and, upon cooling, reform the dynamic fibrous structures. These observations not only demonstrate thermal reversibility in addition to previously reported mechanical reversibility, but also indicate that the steady state of a uniform distribution of fibers can be achieved by two distinct routes suggesting a state point is being approached at long times, and that in the gelled state the system approaches equilibrium composed of a fibrous network. These gelled states can thus be interpreted within the framework of the simulation work of Zaccarelli and Sciortino,[18,23,24] who predict that gels featuring elongated structures can be formed without an intervening phase separation for particles with a limited number of attractive patches.

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