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Evolution of mechanics in α -helical peptide conjugated linear- and star-block PEG

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Abstract

We have designed a peptide conjugated poly-ethylene glycol (PEG) bioconjugate system that allows us to examine intra- and inter-molecular dynamics of gelation. We measure the kinetics of gelation for end-functionalized linear- and star-architectures, and we correlate the gelation behavior with the molecular structure and self-association. The 23-amino acid peptide sequence is known to form a coiled-coil structure as a function of the solution's electrolyte concentration, and the two topologies of the PEG are peptide end-functionalized to examine formation of supramolecular assemblies. Subsequently, microrheology is used to evaluate the dynamics of self-assembly and the gelation time-scales. This study shows that the dynamics of peptide folding and assembly for linear-PEG conjugated systems yield a percolated network, but the star-PEG conjugated systems yield discrete assemblies and remain viscous. The results suggest that the degree of intra- and inter-molecular folding defines the critical gel behavior of the supramolecular system.

Introduction

Several mechanically relevant proteins in nature (ie. collagen, elastin, and resilin) use periodically sequenced structural motifs to dynamically mediate both intra- and inter-molecular interactions, where the non-covalent nature of association allows organisms to access a greater range of structural and targeting possibilities¹ In contrast to the highly specific binding commonly found in biomolecular binding (ie. Antigen-antibody complexes), the resulting non-equilibrium supramolecular structures of these periodically sequenced proteins are a consequence of the dynamics of association. We examined the outcome of intra- and inter-molecular associations using a model system characterized at the molecular scale by examining the ensemble average formation of secondary structure and at the macroscopic scale by examining the rheological properties.

Our model system emulates work from Petka and Tirrell, where the polymer-peptide bioconjugate demonstrates how self-assembling peptides can serve as junction points that can be designed with an on/off switch for tunable mechanical properties². In their work, an

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ABA triblock PEG derivative yields a thermoreversible gel, where 'A' is a tunable α -helical peptide and 'B' is a PEG spacer. These peptides self assemble into coiled-coil dimers by following rules of heptad periodicity³. The pH sensitive glutamic acid residues on the peptide can be protonated, and the protonated state is responsible for driving the assembly of dimers and the stability of coiled-coil bundles. The dimers can also form crosslinks as a result of cooling. Therefore, heating or increasing the pH disassembles the polymer network to thermodynamically stable viscous state^{4, 5}. In our work, we look beyond intermolecular interaction between peptide-polymer triblock constructs by comparing the behavior of similar tri-block architectures and star-block architectures in order to explore the dynamics of intra- and inter-molecular assembly for gel formation.

Larsen and Furst have quantitatively investigated intermolecular dynamics of self-assembly and the liquid-solid transition (gel point) of cross-linked peptide gels using the 20-amino acid MAX1 peptide⁶. This peptide consists of two β -sheet forming legs composed of an alternating sequence of valines and lysines with a central tetrapeptide region with a high β -turn propensity. The gelation in this system occurs by the intermolecular interactions of these hairpins due to hydrogen bonding and attractive hydrophobic forces between β -sheets. The β -sheets form a self-assembled network that leads to the formation of gels on a time-scale of several hours⁷. Using microrheology, they are able to quantitatively determine a continuous increase in elasticity over time by a decrease of mean square displacement (MSD) of fluorescent microspheres^{6, 8}. Moreover, microrheology can be used to define the dynamics of peptide self-assembly and gelation for the β -sheet forming systems⁹, where pH can be used to stimulate reversible self-assembly^{7, 10}.

In this study, we use the microrheological techniques for measuring the MSD and dynamic evolution of rheological properties in model peptide-polymer bioconjugates that are designed to exhibit both intra- and inter-molecular helix self-assembly. We have designed the peptide sequence EKAELKKLLKAWKLLLEKAEK based on previous research aimed at rationally controlling surface activity as a function of electrolyte concentration¹¹. The peptide's charge distribution is designed to promote both parallel and anti-parallel assembly by 'overcharging' the peptide in the center-domain of the sequence^{12, 13}. Figure 1 summarizes our molecular designs as well as our central hypothesis in the development of supramolecular helical bundles. Our results highlight the importance of molecular architecture in the critical gelation times as well as the ability to form elastic networks.

Experimental

Materials

All amino acids for peptide synthesis as well as HBTU and Wang resin were purchased from Anaspec. Solvents and reagents for peptide synthesis and isolation including dichloromethane (DCM), N,N-dimethylformamide (DMF), N-methylmorpholine, trifluoroacetic acid (TFA), methyl tert-butyl ether (MTBE) and toluene were obtained from Fisher Scientific. Piperidine was obtained from Sigma-Aldrich. Homobifunctionalized carboxylated telechelic linear- and star-PEG derivatives of varying molecular weights including linear PEG 2,000, linear PEG 5,000, 4-arm PEG 10,000, and 4-arm PEG 20,000 were all purchased from JenKem Technology USA. Deionized water (18 M Ω , Direct-Q,

Millipore, Billerica, MA) along with reagent alcohol (90:5:5 vol % (ethanol:methanol:isopropanol) was used to clean all glassware prior to experiments. Pluronic F108 was donated from BASF Global. Red fluorescent 1.0 micron spheres were purchased from Invitrogen. Peptide synthesis was carried out on a Protein Technologies Inc. PS3 peptide synthesizer.

Peptide and PEG Conjugate Synthesis

The sequence EKA EKLLKKLLKAW EKLL EKA EK was synthesized through Solid Phase Peptide Synthesis (SPPS). This procedure is described elsewhere^{14, 15}. The peptide is grown sequentially one amino acid residue at a time on support resin. Wang resin is a hydroxymethyl-based colloidal particle with hydroxyl sites that are exposed upon swelling in DMF for attachment to the carboxyl end of the first amino acid via ester linkage with the hydroxyl site on the resin. The second residue onward was synthesized sequentially on a PTI-PS3 (Gyros Protein Technologies) through 9-fluorenylmethyloxycarbonyl (Fmoc) deprotection chemistry. Peptide synthesis was confirmed by matrix assisted laser desorption/ionization (MALDI) characterization (Figure S1). Immediately following synthesis, the peptide bound resin was deprotected and conjugated to either the linear or star PEG polymers. Carboxylated functionalized ends allowed for ease of conjugation with the same Fmoc chemistry used for peptide synthesis. The PEG-peptide conjugation step proceeded for five days in a 50% (v/v) DCM/DMF solution. Subsequently, the resin is washed with DMF and DCM, followed by cleavage from the resin with a 90% (v/v) TFA/DI water mixture for two hours. The polymer is precipitated from the TFA/water mixture with cold MTBE, then isolated by centrifugation, redispersion in DI water followed by freeze drying.

Kaiser Test

Our approach to determine the extent of the tethering chemistry for the peptide-PEG constructs was based on a ninhydrin test in conjunction with UV-vis spectroscopy. We perform an absorbance measurement for the presence of free amines. Two drops of each a 5% (w/v) Ninhydrin in ethanol and 0.001M Potassium Cyanide (KCN) in pyridine were added to 2.00 μ l of 300 mg/ml untethered Fmoc deprotected peptide in a 20 ml scintillation vial idle for five minutes. The ninhydrin test gives a blue color upon detection of free amines. The absorbance of blue was measured at 570 nm, after quenching with 3 ml DI water. The absorbance of the peptide was normalized against the linear and star constructs to determine the relative extent of reaction for the peptide-PEG tethering chemistry (Figure S2 and Table S1).

Circular Dichroism

We used CD to determine the ensemble average secondary structure of peptides. Protein secondary structure is measured in the 190–250 nm range through a 1 mm cylindrical quartz cell containing a solution of the peptide at 1.0 mg/ml. Mean residue ellipticity is determined using the following formula $[\theta]_{\text{MRE}} = 100\theta / Cln = [\text{deg cm}^2 \text{ dmol}^{-1}]$, where θ is the instrument output in millidegrees, C is the molar concentration, l is the path length in cm, and n is the number of amino acid residues in the peptide sequence.

Microrheology

We use passive microrheology to characterize the macroscopic mechanical transition of the peptide-PEG system. Briefly, we track the mean squared displacement (MSD) of the brownian motion of probe particles. Our procedure for multiple particle tracking follows the protocol developed by Larsen and Furst⁶. In order to circumvent aggregation issues at high salt concentrations, a pretreatment step is used to surface functionalize the probe particles with a steric barrier of F108 pluronic. We follow a protocol developed by Kim and Crocker¹⁶.

The polymer solutions were prepared in 3.5M NaCl along with the pluronic treated tracking particles. All solutions for microrheology are prepared at the same mole concentration 3mM. The particles are imaged at 63x using an oil immersion lens on an inverted fluorescent microscope. The Brownian motion of the probe particles are tracked for 300 frames at 30Hz using Matlab algorithms that were modified from the Dufresne, Kilfoil and Blair labs¹⁷⁻¹⁹.

Results and discussion

Circular Dichroism (CD) spectra for the peptide is shown as a function of the ionic strength in figure 2a. A 1.0 mg/ml solution of our untethered peptide in pure DI water the peptide shows a spectrum that correlates to a secondary structure that is 77% random coil, with a minimum at 201 nm. Least squared fitting shows that the peptide in DI water has 23% α -helical structure. Our model peptide sequence consists of a primary structure that contains eight Lysines(+) and five Glutamic Acids(-), resulting in a net +3 charge. The charge density generates an electrostatic repulsion that inhibits folding into a stable α -helical conformation despite the high propensity of the amino acids to form an α -helix. Increasing the electrolyte concentration screens the intra- and intermolecular electrostatic repulsion, resulting in a secondary structure transition to an α -helical conformation, with two minima that develop around 208 nm and 222 nm.

The helicity of the peptide was found to be 63% at 3.5M NaCl. Similar helicities were obtained for PEG tethered peptides (Figure S3). Moreover, the transition as a function of salt concentration can be shown by plotting the molar ellipticity (MRE) at 222 nm. Figure 2b shows the changing degree of helicity as a function of salt concentration, where lower MRE values at 222 nm indicates increased helicity. Based on work from the Woolfson lab, the amphiphilic nature of the hydrophilic and hydrophobic faces of the peptide in its ordered structure leads to the formation of coil-coiled bundles²⁰. Moreover, based on the primary sequence periodicity of the hydrophobic and hydrophilic residues, our peptide sequence fits a type II heptad repeat pattern (HHxxHHx) that allows us to predict that our peptides form tetrameric helical bundles^{21, 22}.

To examine the macroscopic gel properties, we apply microrheology. One-point probe particle tracking microrheology is often used to quantify the viscous and elastic moduli of a material, but for this study, we use the technique to examine the critical gelation dynamics. For both linear- and star-block PEG architectures, we observe diffusive behavior upon exposure to the electrolyte solution. These linear- and star-peptide functionalized PEGs are

initially viscous without a percolated network. Figure 3 includes a line with a slope equal to one, highlighting the viscous nature bioconjugated system at early times.

A key observation from our study is the timescale required for the transition from the sol to the gel regime for the linear peptide amphiphilic PEG 2,000 and PEG 5,000. For the linear Peptide-PEG 2,000-Peptide, measurements are obtained daily with viscoelasticity transitioning over the course of days. Measurements for the linear Peptide-PEG 5,000-Peptide are taken every twenty minutes with the sol-gel transition taking place over the course of a few hours. Percolation theory²³ tells that as the slopes in figure 3a and 3b decrease, a percolated polymer cluster aggregate develops. This causes the probes to become increasingly subdiffusive. Over the experimental time, both the linear Peptide-PEG 2,000-Peptide and linear Peptide-PEG 5,000-Peptide transition from a viscous to an elastic polymer, shown by slopes on both curves figure 3a and 3b that dampen to $0.2 \mu\text{m}^2/\text{s}$ for linear Peptide-PEG 2,000-Peptide and $0.0001 \mu\text{m}^2/\text{s}$ for the linear Peptide-PEG 5,000-Peptide implying elastic behavior.

In contrast to the linear Peptide-PEG constructs, the 4-arm star-block Peptide-PEG 10,000 and 20,000 architectures in figure 3c and 3d does not show an apparent transition from diffusive to elastic gel materials as a function of time. The slopes of all subsequent data sets remain consistent at a value of one over the duration of study²⁴. This result is in contrast to our original hypothesis and numerous findings in literature^{25–27} that attribute higher diffusion constants and stronger rheological properties for crosslinking star polymers. The results from this work highlight that intra-molecular crosslinking can inhibit macroscopic gelation rate^{28, 29}.

We know from percolation theory that the polymer liquid/gel crossover correlates with increasing polymer cluster size. Measurements (Figure S4) using dynamic light scattering (DLS) were taken to confirm that our star constructs initially form small clusters with no increase in size over time. In conjunction with the microrheology for increased gelation from Figure 3b, it was only the linear peptide-PEG 5,000 conjugate that showed increasing cluster size; an indicator of percolated network formation. The designed star bioconjugates with single carbon atom centered structures did not grow and maintained a constant cluster size, demonstrating that these molecules were prone to molecular self-association.

Work from Vlassopoulos et al. mirrors our findings with results of a thermodynamic study on a 3-armed polybutadiene structure with functionalized ends that drive molecular self-assembly³⁰. In this study, x-ray scattering was used to measure the presence of heterogeneous clusters, resolving structures with functionalized ends akin to our star bioconjugates. These experiments showed that the 3-armed architectures led to self-aggregation due to intramolecular folding as opposed to intermolecular self-assembly. The result is a drop in the storage modulus G' (room temperature) for temperature-dependent rheology studies. Structures with only one functionalized end resulted in more intermolecular junctions. Additionally, 3-armed polymers with telechelic functionalized ends on two of the arms yielded a linear type structure. These structures self-assembled into supramolecular architects and gave in the highest degree of intermolecular polymerization, resulting in the highest temperature stability for rheology experiments³¹.

This behavior is analogous to our own findings with the 4-arm star-block Peptide-PEGs where for this particular construct we attribute that the proximity of the helices confined in a single star-block molecule has the greatest potential to form intramolecular self-assemblies. In other words, the intra-molecular formation of our helical bundles will inhibit the inter-molecular assembly with adjacent structures. Furthermore, we observe that our 4-arm star-block PEG architectures in figure 3c and 3d maintain a constant diffusive slope over the 10-day experimental period. The 3-armed polybutadiene systems show behavior analogous to our findings, where the crossover from the viscous to the elastic regime is due to competing intermolecular and intramolecular self-assemblies.

Since the slope of the MSD data for the 4-arm star-block Peptide-PEG constructs in figures 3c and 3d maintain a value of one over a 10-day period, we can determine the solution viscosity using the Stokes-Einstein equation^{32, 33}. We found that the viscosity of the bulkier star Peptide-4 arm-PEG 20,000 is initially 4.51 cp and increases to 48.03 cp. In contrast, the star Peptide-4 arm-PEG 10,000 is initially 3.28 cp and increases to a final value of 24.50 cp. The observed increased viscosity for both star polymers gives clues into the dynamics of self-assembly. We can conclude that the intermolecular self-assemblies are occupying a greater volume fraction of the sample, but these assemblies are insufficient to cross the percolation threshold required for the crossover into the viscoelastic regime^{34, 35}.

We observe a higher viscosity for the star Peptide-4 arm-PEG 20,000 bioconjugate over the star Peptide-4 arm-PEG 10,000, and we also observe a faster transition to the viscoelastic regime for the linear Peptide-PEG 5,000-Peptide bioconjugates over the linear Peptide-PEG 2,000-Peptide. Furthermore, we did a comparison of viscosities for our star Peptide-4 arm-PEG 10,000 and star Peptide-4 arm-PEG 20,000 in figure 3c and 3d with PEG viscosity data obtained by others³⁶. Gonzalez-Tello et al used PEG-8000 mixtures at higher concentrations (10 wt%) to obtain viscosities of 8.9 cp at room temperature. In contrast, our MSD data yielded an average viscosity of 12.42 cp for star Peptide-4 arm-PEG 10,000 and 22.15 cp for star Peptide-4 arm-PEG 20,000 by using the Stokes-Einstein equation. Comparing these findings for our star Peptide-4 arm-PEG 10,000 bioconjugate to the literature data, we observe larger viscosities for similar sized molecules at a third of the mass fraction. This observed increased viscosity implies that increased intermolecular interactions due mainly to peptide self-assembly³⁷ form cross-linked networks in solution rather than simply PEG entanglement. In our study, we keep the molar concentration of the PEG bioconjugates constant. Therefore, we expect to observe a greater viscosity for the higher MW PEG molecules because of the higher mass fraction, 44.2 g/L for star Peptide-4 arm-PEG 20,000 and 30 g/L for star Peptide-4 arm-PEG 10,000. We have also shown that without telechelic crosslinking, none of the four PEG backbones cross into the viscoelastic gel regime, however differences in viscosity are observed. Using the Stokes-Einstein relation³², linear PEG 2,000 and 5,000 have calculated viscosities of 1.52 and 1.97 cp respectively, while star PEG 10,000 and 20,000 results in viscosities of 2.84 and 3.28 cp. Higher viscosities are observed for larger and bulkier PEG molecules at identical 50 mg/ml mass concentrations (Figure S5).

The superposition curves shown in figure 4 are constructed by shifting the individual MSD curves in figure 3 horizontally with respect to time and vertically with respect to MSD. In all

cases, the initial MSD data for all four constructs 4a – 4d has a slope equal to $\ln(r^2(\tau))/\ln\tau = 1$, that defines the diffusive viscous pregel. In each figure, the MSD curves are multiplied horizontally by a relaxation time shift factor 'a', and vertically by a creep compliance shift factor 'b' to overlay the previous MSD curve to a best-fit line. The time-cure superposition shown in figure 4a for the bioconjugate construct linear Peptide-PEG 2,000-Peptide transitions from a viscous pregel into a viscoelastic sol over 10-days. All slopes have an upward shape that indicates increasing viscoelasticity with properties in line with that of a sol rather than a gel, which is consistent with the dynamics of the pre-gel master curve. Figure 4a shows that a sol-gel transition has not been reached during this time window^{6, 38}.

The linear Peptide-PEG 5,000-Peptide bioconjugate is shown in Figure 4b. The time-cure superposition begins with a pregel master curve with a slope equal to one. Subsequent data curves are superimposed onto this curve until there is a divergence in the data, where the sample reaches the sol-gel transition. The material dynamics have changed and a new postgel master curve is constructed with subsequent data superimposed in reverse, leading to a pregel-postgel crossover that determines the critical sol-gel transition point. In the linear Peptide-PEG 5,000-Peptide case, the transition takes place after six hours. The difference in critical gel rates can be attributed to the rapidly increasing number of intermolecular peptide crosslinking ends for the linear Peptide-PEG 5,000-Peptide bioconjugate.

The similar structure between the linear Peptide-PEG 5,000-Peptide and the linear Peptide-PEG 2,000-Peptide bioconjugated constructs led to expectations for a sol-gel transition for both cases, but this was not the case. Again, we believe this is a result of the constant mole fractions in both samples, where measurements were conducted at a constant number of available crosslinks, but the linear Peptide-PEG 5,000-Peptide occupies a greater volume in solution compared to the linear Peptide-PEG 2,000-Peptide. Despite the fact that the linear Peptide-PEG 5,000-Peptide has the same peptide mole concentration, it will also have a greater number of PEG-based entanglements.

In contrast, the star bioconjugate constructs star Peptide-4 arm-PEG 10,000 and star Peptide-4 arm-PEG 20,000 in figures 4c and 4d, have similar dynamics. The subsequent superposition behaves linearly with a slope that remains equal to 1 throughout. This data shows us that in both cases the star constructs remain as viscous liquids. This is evidence for peptide end-groups forming coiled-coils in a predominantly intramolecular fashion, with peptides self-assembling on adjacent arms in the same molecule. Self-assembly in this manner inhibits the formation of a percolated gel network.

The relaxation time and creep compliance shift factors represented by a and b, respectively, are plotted against time in Figure 5. For the linear Peptide-PEG 5,000-Peptide bioconjugate shown in figure 5b, the pregel a and b shift factors diverge after a critical gelation time 't_c' of 386 minutes. This is a clear indicator of our critical region and the sol-gel transition. For the linear Peptide-PEG 2,000-Peptide shown in figure 5a, the relaxation time and MSD shift are both linear and do not diverge at any point along the 10-day measured time frame. Both the star Peptide-4 arm-PEG 10,000 and star Peptide-4 arm-PEG 20,000 bioconjugates, figure 5c and 5d, show no change in viscoelastic properties for the star constructs in either time or

MSD shifts. Again, we attribute this to the formation of intramolecular coiled-coil bundle formations that dominate the intermolecular bonding for the 4-arm star-block constructs.

Figure 6, shows the critical gelation time for linear Peptide-PEG 5,000-Peptide. This is achieved by the determination of the dynamic scaling exponents. Percolation theory highlights that as the probability (p) for random molecular associations increases that form individual clusters resulting in divergence in viscosity until the gel point is reached when $p = p_C$. Near this point an infinite cluster grows as the individual polymer clusters merge. Furthermore at this region the longest relaxation time ' τ_L ' exhibits power law behavior such that $a \sim \tau_L \sim e^y = ((1/2t - t_C^{1/2})/t_C)^y \sim ((1/2p - p_C^{1/2})/p_C)^y$ where ' y ' is the relaxation critical scaling component. Likewise the analogous holds true for steady-state creep compliance ' J_e^0 ' and $b \sim 1/J_e^0 \sim e^z = ((1/2t - t_C^{1/2})/t_C)^z \sim ((1/2p - p_C^{1/2})/p_C)^z$, where ' z ' is the creep compliance critical scaling component. The scaling exponents are found from the logarithmic slope of the distance from the critical gel time against a and b shift factors from figure 5³⁹. For ' t_C ' = 386 min, we have calculated a value for the relaxation time exponent $y = 2.61$ and the creep compliance exponent $z = 0.86$.

The critical relaxation exponent ' n ' is calculated by $n = z/y$. This returns a value for the viscoelastic exponent $n = 0.33$. The discontinuity that generates the viscoelastic exponent rises as a result of the formation of the infinite percolation cluster. A value of n below the theoretical Zimm limit of 0.5 for entangled polymers with strong hydrodynamic interactions is indicative of a highly cross-linked system. This tells us that for the linear polymer system under study a percolation type of self-assembly could be achieved at lesser molecular concentrations^{23, 38, 40–42}.

We believe the linear-chains are flexible along the PEG backbone so that the molecules can be modeled as a freely-jointed chain model, or Rouse-like chain dynamics, following the tube model for one dimension polymer motion. In contrast, no critical exponent was able to be determined for the star constructs as the molecular flexibility does not allow these constructs to be compatible with the entangled tube model proposed by McLeish⁴³. In our star-constructs, we believe intramolecular entanglements are dominant over intermolecular interactions. Therefore, the star systems would be better modeled by a Comb-branched model^{44, 45}.

Conclusions

We were able to demonstrate that for this system the gelation of peptide polymers arise from a combination of non-covalent self-assembly and polymer entanglement, where the monomeric architecture determines the mechanics. This behavior can inform the development of tunable gels through the formation of non-covalent 'crosslinks'. Our peptide-polymer bioconjugate designs examine the role of intra- and intermolecular interactions in the stabilization of a viscoelastic biomaterial. Moreover, we examine the dynamics of intramolecular interactions in the stabilization of secondary structure, but these intramolecular of interactions can also inhibit the formation of network self-assembly in our system. Finally, we show how branching in periodically sequenced polypeptides can influence the formation of intra- and inter-molecular forces that are responsible for the

evolution of crosslinked elastic systems. On the other hand, linear peptide-polymer bioconjugates can self-assemble intermolecularly forming a percolating network. Future experiments will examine the dynamics with various salts along the Hofmeister series that enhance or decrease peptide solubility that has an effect on the entropy of the peptide-polymer solution. Higher system entropies lead to increased molecular randomness and decreases polymer crosslinking and entanglement. Studying such a system allows control over the kinetics of gelation, useful for biological application⁴⁶.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. PEG-peptide bioconjugates. (a) The chemical structures of four bioconjugates. (b) Intramolecular (top) and intermolecular (bottom) interactions that can define gelation dynamics.

Figure 2.

Circular Dichroism. (a) Mean residue ellipticity as a function of [NaCl]. Blue represents the no NaCl in solution. Red, green, purple, and teal are increasing in [NaCl] 0.1M, 1.0M, 2.0M and 3.5M (b) Mean residue ellipticity at 222 nm as a function of ionic strength. A decrease in MRE represents increased helicity.

Figure 3.

Particle MSD is plotted as a function of time for each of the tethered Peptide-PEG linear and 4-arm star-block polymer alpha helical folded constructs at 3.5M NaCl in Fig. 3 (a–d). In each figure, the individual curves represent the measured MSD and the monitored gelation process as a function of time on a log-log plot taken over the course of days.

Figure 4.

MSD superposition from the data in Fig. 3. for linear peptide constructs (4a. & 4b.) as well as star peptide constructs (4c. & 4d.) Starting with the initial diffusive MSD data, subsequent curves are shifted by shift factors 'a' and 'b' horizontally [$a\tau$] and vertically [$b\langle r^2(\tau) \rangle$] that demonstrate changes in viscoelastic properties over long times.

Figure 5.

In 5a. – 5d. blue is the time (a) shift factor and red is the MSD (b) shift factor used for the superposition in figure 4 plotted as a function of time. Fig 5a and 5b represent shifts for linear Peptide-PEG-Peptide constructs while 5c and 5d represent 4-arm star-block Peptide-PEG constructs. The critical gel point is determined by large shift in either a or b.

Figure 6.

Linear Peptide-PEG 5,000-Peptide bioconjugate relaxation time ('a' blue) and MSD ('b' red) shift factors are plotted against the distance from the critical gel point for fitting to determine the critical scaling exponents y and z , and the critical relaxation exponent n .