Coiled-Coil Hydrogels: Effect of Grafted Copolymer Composition and Cyclization on Gelation

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ABSTRACT: A mean-field theoretical approach was developed to model gelation of solutions of hydrophilic polymers with grafted peptide motifs capable of forming associates of coiled-coil type. The model addresses the competition between associates engaged in branching and cyclization. It results in relative concentrations of intra- and intermolecular associates dependent on associate strength and motif concentration. The cyclization probability is derived from the model of equivalent Gaussian chain and takes into account all possible paths connecting the interacting motifs. Examination of the association−dissociation equilibria, controlled by the equilibrium constant for association taken as input information, determines the fractions of inter- and intramolecularly associated motifs. The gelation model is based on the statistical theory of branching processes, and in combination with the cyclization model, it predicts the critical concentration delimiting the regions of gelled and liquid states of the system. A comparison between predictions of the model and experimental data available for aqueous solutions of poly[*N*-(2-hydroxypropyl)methacrylamide] grafted with oppositely charged pentaheptad peptides, CCE and CCK, indicates that the association constant of grafted motifs is four orders of magnitude lower than that of free motifs. It is predicted that at the critical concentration of each motif of about 6×10^{-7} mol/cm^3, about half of the motifs in the associated state are engaged in intramolecular bonds.

Introduction

Coiled-coil hydrogels are hybrid hydrogels that contain synthetic polymer chains and covalently bound peptide segments capable of forming reversible coiled-coil associates. The native coiled-coils are involved in important biological functions. They are naturally present in a variety of proteins such as transcription factors, cytoskeletal proteins, motor proteins, and viral fusion proteins. The interaction surface of helical chains contains hydrophobic residues, such as leucine arranged in the so-called leucine zipper. The formation or dissociation of the coiled-coil superhelix is sensitive to several stimuli, such as pH, ionic strength, and temperature. Therefore, coiled-coil hydrogels have a high potential for drug delivery.[1−4] Several of such hydrogels were synthesized and studied, in which motifs were built in as blocks of a multiblock copolymer[5−10] or grafts of a linear hydrophilic polymer.[11] Heterodimers are formed when motif A can form and associate with only motif B. To ensure the formation of heterodimers, electrostatic attractors have been placed on A and B motifs, a method extensively used in the design of heterodimeric, heterotrimeric, and heterotetrameric coiled-coils (cf., e.g., refs 12−14).

The aim of this article is to formulate a mean-field model for the sol−gel transition of aqueous solutions of polydisperse copolymers composed of a hydrophilic copolymer backbone and grafted peptide motifs associating into coiled-coils. In particular, the article analyzes the self-assembly of two graft copolymers; one carrying motifs (grafts) A and the other carrying motifs B. Following mixing, coiled-coil associates are formed exclusively by the association of motifs A with B into heterodimers AB. Pairs of chains A and B form intermolecular associates or a sequence of them. All additional connections of the same pairs by associates are considered to be intramolecular, and they close a cycle. The model permits the prediction of the relationship between the structure (composition) of copolymers and their self-assembly into hydrogels. Only a single-phase system was considered. The results of modeling were compared with experiments.

Formation of Physical Gels. The behavior of graft copolymers profoundly differs from that of free grafts in that (1) the grafted system is polyfunctional with functionality distribution, (2) the formation of cyclic structures is relatively strong and depends on concentration, (3) the binding strength may be affected by attachment to a polymer structure, (4) the mechanism of cluster growth and gel formation depends on concentration (initial polymer coils and clusters overlap or do not overlap), and (5) phase separation can occur. First, let us briefly review the facts that are important for model formulation. This summary will be concerned with (a) association−dissociation rates and equilibria of coiled-coils, (b) the formation of physical gels and their transformations, and (c) competing branching and cyclization.

Association−Dissociation of Coiled-Coils: Dynamics and Equilibria. The kinetics and equilibria of formation and dissociation of coiled-coils are relatively well understood (cf., e.g., refs 12−23). The attractive forces between motifs A and B forming heteroassociates A−B are relatively strong. It is assumed that separated motifs A and B exist as disordered coils; in the associate, they exist in the helical forms. The formation of the coiled-coil is a two-state transition between disordered and helical forms, and the formation of the associate with motifs still in (partially) disordered states seems to be the rate-determining step. The equilibrium constants for dissociation or association ($K_{\mathrm{dis}} = 1/K_{\mathrm{as}}$) depend on the number and structure of heptads of the motifs.

The conclusion from these studies is that the second-order rate constants for association are at least on the order of 10^6 $\mathrm{M}^{-1}\mathrm{s}^{-1}$, with the half time at concentration 100 μM, $t_{1/2} = 10^{-2}$ s. These high rates approached the regime of encounter control in reaction kinetics. If we consider the first-order dissociation rates on the order of 10^{-1} s^{-1}, then the half time for dissociation,

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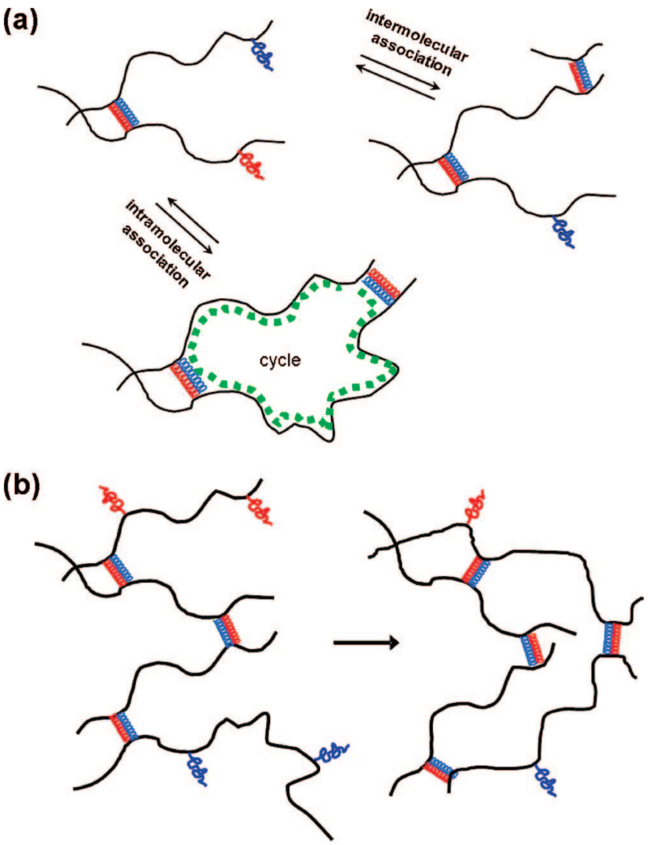
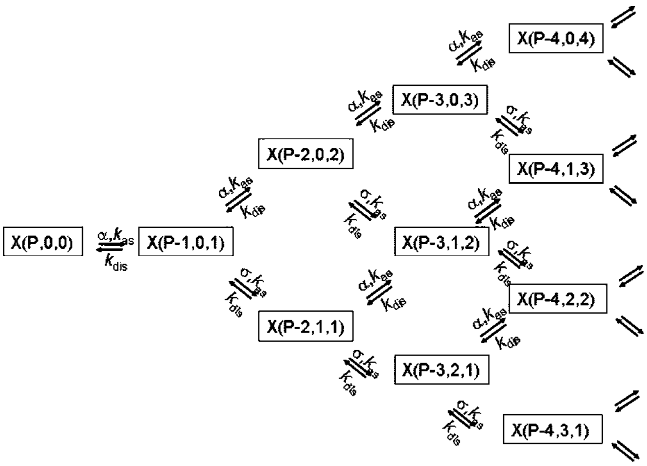


Figure 1. (a) Coiled-coil inter- and intramolecular association of motifs and (b) longer distance (secondary) cyclization.

$t_{1/2}$, is on the order of 10 s. The equilibrium constants for association, K_{as}, vary in the range of 10^8-10^{12} mol/cm^3. As we will show later, it is evident that attachment of motifs to polymer chains slows down the association of motifs by several orders of magnitude and lowers the strength of the associates (cf., also ref 24).

Formation of Physical Gels and Their Transformation by External Forces. A characteristic property of physical gels is the reversibility of cross-links, so that at equilibrium the chains connecting cross-links are fully relaxed. This is valid for any kind of strain, including swelling. If solvent (water) is added to the gel, more swollen gel with relaxed chains or a viscous liquid is eventually obtained. A two-phase system is the other alternative. The rates of rearrangement of physical cross-links' and network chains' relaxation can be very different.[25] The cross-link reorganization can be very fast. In polymer solutions, polymer−polymer contacts can be considered to be very mobile cross-links. Alternatively, the cross-link rearrangement is very slow, and the gel resembles a permanent (covalent) network. Gelatin is a good example: it behaves as a permanent gel, but in fact it is a very high-viscosity fluid with an extended low-frequency plateau.[25,26] Tirrell et al.[27] analyzed relaxations of networks from triblock copolymers composed of leucine zipper end-blocks and polyelectrolytic random coil midblock. The leucine zippers form cross-links of tetrameric associates. The mechanical network relaxation (or creep) changed in parallel with the lifetime of coiled-coil. The exchange of strands in coiled-coil associates was slower by a factor of 5−10 compared with the macroscopic mechanical relaxation time, and thus the cross-link rearrangement was rate determining for system dynamics. Associative thickener, a diblock copolymer with hydrophilic and hydrophobic blocks, is another example of a transient network. Micelles composed of the hydrophobic blocks are the physical cross-links.[25,28−30]

Scheme 1. Evolution of States of Building Units of a Monodisperse *P*-mer Containing *P* Motifs[a]



[a] States of building units X(ω, σ, α) are characterized by the number of motifs in ω-, σ-, and α-states; k_{as} and k_{dis} are, respectively, rate constants for association and dissociation.

Whether the polymer coils initially overlap ($c_m > c^*$) or do not overlap ($c_m < c^*$) depends on the initial concentration of the motif (c_m). In the first case, the free motifs of polymer clusters interact with motifs of other clusters as well as their own motifs. For $c_m < c^*$, the formation of dimers of polymer coils carrying A and B motifs is apparently fast, but the majority of the associations occur intramolecularly. Only free motifs in the cluster periphery can take part in the intermolecular association; the motifs buried inside cannot be accessed. Eventually, the system reaches the percolation threshold, and a macrogel, possibly heterogeneous, results. This initial part resembles gelation by free-radical nonlinear copolymerization.[31,32]

Irrespective of the cluster growth mechanism and the possible temporary heterogeneity, at equilibrium reached after a long time, the final states are relatively simple: (1) a single-phase gel or liquid, (2) a two-phase system with liquid−liquid, liquid−gel, and gel−gel phases, or (3) a three-phase system under invariant conditions (at constant pressure). The three-phase equilibrium is possible according to Gibbs phase rule, $f = C + 2 - P$, where f denotes degrees of freedom, C is the number of components, and P is the number of phases. In polymer systems, three-phase equilibria have been predicted and studied.[34−36] All equilibria are of liquid−liquid type, and whether a phase is a liquid or a gel depends on whether at the given concentration the percolation limit is exceeded or not. In equilibrium, the coexisting phases should be homogeneous because of the minimization of the interface energy.

Theoretical Approaches to Physical Gelation. The mean-field thermodynamic theoretical approach to polymer systems in which strong physical bonds can give rise to gelation and possibly to phase separation was developed by Semenov and Rubinstein et al.,[37−42] F. Tanaka et al.,[43−45] and Erukhimovich et al.[46,47] The polymer chains are composed of segments involved in weak interactions but also contain groups that strongly interact with themselves (stickers), forming a physical bond that is much stronger than the weak bonds. The free energy of the system polymer with stickers−solvent is an additive function of nonsticker and stickers interactions. Using standard thermodynamic procedures, the phase separation limit, phase volume, and concentration ratios can be calculated. We calculated the gelation threshold from polymer structure connectivity by applying the Flory−Stockmayer criteria. Semenov and Rubinstein correctly argued that gelation was not a thermodynamic transition and that beyond the gel point the gel was not acyclic. This is in line with Flory's model, which is based on cycle rank buildup in the gel.[48] Within this mean-field approach, other mechanisms of cross-link formation (stickers associated into spherical, cylindrical, or disklike micelles) were also considered.[40−42] Erukhimovich's model considers the sol−gel transformation to be a first-order thermodynamic transition coupled to phase separation due to ring closure in the gel. This result is not supported by experiment: reversible physical or chemical gelation is not associated with phase separation, but under certain conditions, phase separation can occur before or beyond the gel point or even coincide with the gel point. Gelation and phase separation are well described by mean-field theories, such as those of refs 37−42, considering gelation to be a topological singularity. The approach presented below is based on similar principles and deals explicitly with pregel cyclization and functionality distributions as main factors affecting the critical concentration for gelation in addition to the bond strength.

Several computer simulations have been developed. A coarse grain model employing lattice Monte Carlo simulation was used to model gelation of gelatin (stickers introduced along polymer chains).[49] The stickers formed associates of three to simulate the formation of triple-helical sequences. After each step, the system was equilibrated. Choosing association energies for the sticker, the helix content and chain connectivity were calculated, and the network connectivity parameter was related to equilibrium modulus. Similarly, the association−dissociation equilibria in solutions of Gaussian chains with stickers were simulated by using the Monte Carlo method on lattice.[50,51] The system was equilibrated through a combination of reptation moves and chain insertions and removals. Near the gel point, the distribution turned bimodal, and the largest cluster was the gel (cf., off-space Monte Carlo simulation of chemical cross-linking[53]). The authors have found very good agreement with the mean-field treatment. However, it is known that 3-D space simulation involving various preset degree-of-polymerization and composition distributions is difficult. This speaks in favor of a mean-field treatment. The simulation of the effect of stress on mechanical behavior of physical networks is interesting.[53] Molecular dynamics models for the gelation of block copolymer systems by other mechanisms (formation of micelles or microbundles) were described by Guo and Lijten.[54]

For most physical gels, the threshold concentration for gelation is rather low, and the competition between inter- and intramolecular bonds cannot be neglected. In fact, in almost all recent theoretical approaches, mean-field and simulations in space, intramolecular cross-linking was considered (loops, flower micelles) at least verbally or by introducing a cyclization term; however, this hardly allowed for quantification. Also, numerical results are available.[50] For the covalent network, the cyclization phenomenon is better understood and quantification is possible. For the not-too-large extent of cyclization, the concept of Kuhn's equivalent chain was used by Jacobson and Stockmayer[55] for the calculation of the probability that two functional groups on the same molecule meet in the reaction volume. Several theoretical approaches to ring-chain competition for linear as well as branched polymers[56−64] were developed. For branching systems, it is, for instance, the "rate" theory[57,58] or "spanning tree" method.[59−64]

Development of the Model

Modeling was carried out in three steps:

Module 1 deals with the probabilities of cycle formation relative to the formation of an intermolecular associate for the synthetic copolymers carrying the motifs. These probabilities serve as input for the formulation of the association−dissociation rates and equilibria involving inter- and intramolecular associates (Module 2). The output of Module 2, balances between inter- and intramolecular associates dependent on concentration, serves as input for Module 3, where the problem of connectivity is treated to find the conditions for the gel point. The treatment of cyclization in multifunctional polydisperse systems is a complex problem, and to keep the length of the article reasonable, it is presented in the Supporting Information.

Inter- versus Intramolecular Association. The chains are copolymers of nonfunctional units and units carrying motifs. Chains having motifs of type A interact with chains having motifs of type B. The motifs form associates AB. In addition to intermolecular association, association between A and B motifs already connected by a sequence of bonds (cyclization) is possible (Figure 1). The situation is complicated for several reasons: (1) because of the existence of compositional distribution, there exists a distribution of path lengths connecting A and B motifs, and (2) the components A and components B are polydisperse, which also somewhat affects the extent of cyclization. The full treatment of the cyclization problem is given in the Supporting Information. In the approach to cyclization developed here, some simplifying assumptions have been made: (1) The distribution of monomer units with and without motifs is random. (2) For the purpose of the calculation of the extent of cyclization, the chains are taken to be monodisperse of degree of polymerization, P; in the polydisperse system, this P is considered to be the number-average, P_n. (3) Only "primary" cyclization (Figure 1a) is considered, not the "secondary" cyclization (Figure 1b), but its possible contribution is estimated. (See the Supporting Information.) (4) The motifs exist in three states: unreacted (ω-state), ring closing (σ-state), and branching (α-state). The number fractions of these states are ω, σ, and α, respectively; the states are distributed among the motif units randomly, respecting the fact that a motif in the σ-state cannot exist on a chain without any motif in the α-state.

The output from the cyclization part serving as input for Module 2 is the cyclization probability per copolymer units, C_{sP}. It corresponds to the case in which any unit of copolymer A can form an intramolecular bond to any unit of one copolymer B already attached to A intermolecularly (Figure 1b). C_{sP} is the concentration of the complementary motifs in the reaction volume around the selected motif and can thus be compared with the concentration of motifs supplied by other (nonattached) chains.

Association−Dissociation Equilibria. The phenomenon of gelation is modeled by the application of the statistical branching theory (next section). This theory works with building units in different states distinguished by the number of various types of (associative) bonds the building unit has to other units. In our case, the building units are the grafted copolymer molecules bearing motifs in nonassociated and associated states. The states of motifs in associates are further distinguished by the number of motifs engaged in intermolecular or intramolecular associates. Intermolecular association contributes to structure growth leading to gelation, but the intramolecular association does not. Cross-linking of the system of copolymer chains to degree-of-polymerization and compositional distributions and involving cyclization is the most complex case of applied branching theory. To illustrate the complexity of the case partially, we describe the association of A with B P-mers by a set of equilibria shown in Scheme 1. The system is transformed by inter- and intramolecular association and dissociation of associates. The resulting set of concentrations of units in different states is a direct input in the branching theory in its spanning-tree approximation version.

The processes in Scheme 1 are described by differential equations for the time change in concentration of building units

$$\begin{aligned}
\frac{d[\mathrm{A}(P,0,0)]}{\mathrm{d}t} &= -k_{\mathrm{as}}P[\mathrm{A}(P,0,0)]c_{\mathrm{B}\omega} + k_{\mathrm{dis}}[\mathrm{A}(P-1,0,1)] \\
\frac{\mathrm{d}[\mathrm{A}(P-1,0,1)]}{\mathrm{d}t} &= k_{\mathrm{as}}P[\mathrm{A}(P,0,0)]c_{\mathrm{B}\omega} - k_{\mathrm{dis}}[\mathrm{A}(P-1,0,1)] \\
&\quad - k_{\mathrm{as}}(P-1)[\mathrm{A}(P-1,0,1)]c_{\mathrm{B}\omega} + 2k_{\mathrm{dis}}[\mathrm{A}(P-2,0,2)] \\
&\quad - k_{\mathrm{as}}(P-1)[\mathrm{A}(P-1,0,1)]C'_{\mathrm{sP}} + 2k_{\mathrm{dis}}[\mathrm{A}(P-2,1,1)] \\
\frac{\mathrm{d}[\mathrm{A}(P-2,0,2)]}{\mathrm{d}t} &= k_{\mathrm{as}}(P-1)[\mathrm{A}(P-1,0,1)]c_{\mathrm{B}\omega} - \\
&\qquad 2k_{\mathrm{dis}}[\mathrm{A}(P-2,0,2)] \\
&\quad - k_{\mathrm{as}}(P-2)[\mathrm{A}(P-2,0,2)]c_{\mathrm{B}\omega} + 3k_{\mathrm{dis}}[\mathrm{A}(P-3,0,3)] \\
&\quad - k_{\mathrm{as}}(P-2)[\mathrm{A}(P-2,0,2)]C'_{\mathrm{sP}} + 3k_{\mathrm{dis}}[\mathrm{A}(P-2,1,2)] \\
\frac{\mathrm{d}[\mathrm{A}(P-2,1,1)]}{\mathrm{d}t} &= k_{\mathrm{as}}(P-1)[\mathrm{A}(P-1,0,1)]C'_{\mathrm{sP}} - \\
&\quad 2k_{\mathrm{dis}}[\mathrm{A}(P-2,1,1)] - k_{\mathrm{as}}(P-2)[\mathrm{A}(P-2,1,1) \qquad (1)
\end{aligned}$$

The quantity $c_{\mathrm{B}\omega}$ is the concentration of free motifs B in the system, and $C_{\mathrm{s}P}$ is the concentration of B groups effective in cyclization (see below) supplied by units already connected to the unit under consideration. In equilibrium, the right-hand sides of the equations are equal to zero, and $k_{\mathrm{as}}/k_{\mathrm{dis}} = K_{\mathrm{as}}$. We have the i^2 algebraic equations for $i^2 + i$ variables (i is defined by X(P-i, *, *)), but if the series is truncated when the concentrations [X(*, *, *)] are sufficiently small, then we can get the solution by consecutive substitutions. For application to the case discussed here, the problem of polydispersities must be solved.

Therefore, we will try to find a simpler solution in which average fractions of motifs ω-, σ-, and α-states dependent on system variables are found and a certain (random) distribution among the motif units is assumed.

The following quantities will be used: (1) The degree of conversion of motifs into associates, ξ_{X}, (X = (A, B)); only associates AB are formed. For the stoichiometric system (equal number of moles of motif A and B in the system), $\xi_{\mathrm{A}} = \xi_{\mathrm{B}}$. For the nonstoichiometric system, $\xi_{\mathrm{A}} \neq \xi_{\mathrm{B}}$. (2) The fraction of motifs in the free state is equal to $\omega_{\mathrm{X}} = 1 - \xi_{\mathrm{X}}$, the intramolecularly associated state $\sigma_{\mathrm{X}} = \xi_{\mathrm{X}}s$, and the intermolecularly associated state, $\alpha_{\mathrm{X}} = \xi_{\mathrm{X}}a$; $\omega_{\mathrm{X}} + \sigma_{\mathrm{X}} + \alpha_{\mathrm{X}} = 1$, $s + a = 1$. (3) The molar concentration of motifs X in solution, c_{mX} (mol/cm^3). (4) The molar fraction of monomer units in the copolymer bearing motif X, x_{X}. (5) The degree of polymerization of the grafted chain, P_{X}. (6) The constants k_{as} and k_{dis} are assigned to the rate of association of motifs and dissociation of associates, respectively. Their ratio is equal to the association constant $K_{\mathrm{as}} = k_{\mathrm{as}}/k_{\mathrm{dis}}$. (7) The set of summed cyclization probabilities, $C_{\mathrm{s}P}$, derived in the Supporting Information for the given degree of polymerization P, is the input information.

$C_{\mathrm{s}P}$ is the probability that two motifs A and B residing on already connected A and B chains, respectively, meet in a reaction volume element dV = dx dy dz. It has the meaning and dimension of the concentration [mol/cm^3] of the motifs.

As we have already discussed, the intramolecular associate can only be formed between A and B motifs residing on chains already connected by an intermolecular associate. The competition between intra- and intermolecular association is limited to the subsystem excluding chains without any motif engaged in an associate. Under the assumption of random distribution of states of motifs, the fraction of chains without any associated motif is $(1 - x_{\mathrm{X}}\xi_{\mathrm{X}})^{P_x}$ and that of chains with at least one associated motif is equal to $1 - (1 - x_{\mathrm{X}}\xi_{\mathrm{X}})^{P_x}$, where ξ_{X} is the fraction of associated motifs and x_{X} is the fraction of motif-bearing units in the chain.

The cyclization probability, $C_{\mathrm{s}P}$, was derived for all possible ring closure paths between any pair of units of the A chain and the topologically neighboring B chain assuming the validity of the conformational statistics of equivalent Gaussian chain (cf., e.g., refs 53−61) and respecting the presence of rigid rodlike associates in the sequences of monomer units closing the cycle. It reads (cf., eqs A-9 and A-10 in the Supporting Information)

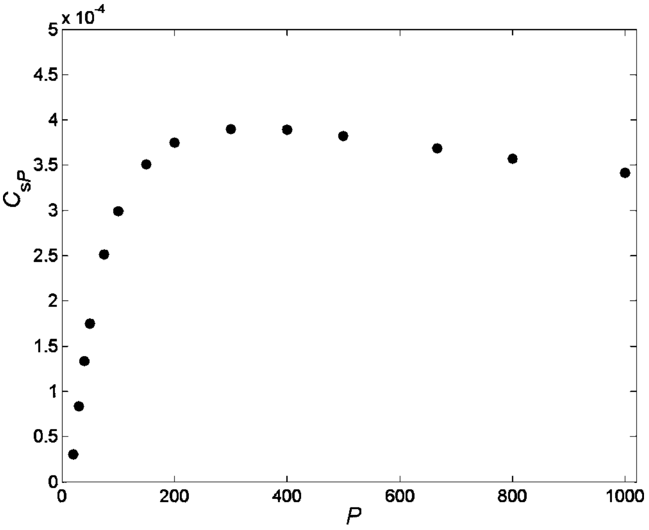


Figure 2. Summed cyclization probability, $C_{\mathrm{s}P}$, [mol/cm^3] as a function of the degree of polymerization of copolymer chains, P; length of superhelix bridge, d_{cc} = 2.5 nm, number of monomer units in the statistical segment, Q = 3, length of the monomer unit, l_{m} = 0.32 nm; number of monomer units in the smallest possible ring, i_0 = 10 (the smallest cyclic structure is composed of 2 superhelices connected by 10 monomer units).

$$C_{\mathrm{s}P} = \sum_{i=i_0}^{2(P-1)} \left(\frac{3}{2\pi i l_{\mathrm{m}}^2 Q}\right)^{-3/2} \exp\left(-\frac{3}{2 i l_{\mathrm{m}}^2 Q} 2 d_{\mathrm{cc}}^2\right) (A_{\mathrm{rn}})_i / N_{\mathrm{Avo}} \qquad (2)$$

In this equation, the combinatorial term $(A_{\mathrm{rn}})_i$ is the number of possible paths of length i-monomer units; i extends from i_0 to $2(P - 1)$ (cf., eq A-9 in the Supporting Information); l_{m} is the length of the monomer unit, Q is the number of monomer units in the segment of equivalent Gaussian chain, and N_{Avo} is Avogadro's number equal to 6.022×10^{23} molecules/mol. The exponential term cares for the presence of the coiled-coil rigid rod. It expresses the reduction of ring closing probability. By introducing a rod, the chain ends are separated by the distance, d_{cc}, from each other. The factor of 2 expresses the fact that there are two rigid-rod elements in the cycle and that their orientations are mutually independent ($d_{2\mathrm{cc}} = \sqrt{2}d_{\mathrm{cc}}$). The dependence of $C_{\mathrm{s}P}$ on the degree of polymerization of the chains, P, is shown in Figure 2 for the set of parameters indicated.

Equation 2 was derived for monodisperse copolymers; for the polydisperse gelling systems, P was considered to be equal to the number-average degree of polymerization, P_{n}. The Supporting Information contains further refinements of the cyclization model and quantification of the effect of approximations in the derivation of the model for $C_{\mathrm{s}P}$. The refinements are concerned with the inclusion of the effect of polydispersity of real chains, the effect of possible steric obstructions on the formation of new associates when some already exist, and the consideration of cyclization involving topologically more distant chains.

Rates and Equilibria of Inter- and Intramolecular Association in Solution. In this part, the rates of association and dissociation per motif are formulated by employing the mass action law. In equilibrium, the concentrations of intra- and intermolecular associates, respectively, are constant.

Rates of Intramolecular Association and Dissociation. A motif (e.g., motif A) is found in the system with probability equal to its molar concentration, c_{mA}. It is in the ω-state with probability ω_{A}. To consider cyclization, it should be selected from the subset of units that already have at least one motif in the α-state

$$S_{\mathrm{X}} = 1 - (1 - x_{\mathrm{X}} + x_{\mathrm{X}}\omega_{\mathrm{X}})^{P_{\mathrm{X}}} = 1 - (1 - x_{\mathrm{X}}\xi_{\mathrm{X}})^{P_{\mathrm{X}}}$$

The target unit for ring closure is a B chain. The cyclization probability, $C_{\mathrm{s}P}$, was derived for the case in which any monomer unit of copolymer A can form a cyclic structure with any monomer unit of copolymer B. In reality, only motif units in ω-states interact. Therefore, $C_{\mathrm{s}P}$ must be multiplied by the factor $x_{\mathrm{A}}\omega_{\mathrm{A}}x_{\mathrm{B}}\omega_{\mathrm{B}}$. Moreover, the A chain under consideration can have more than one partner B chain. The partners are connected to it by α-associates. The number of such additional partners is equal to $(P - 1)x_{\mathrm{X}}\alpha_{\mathrm{X}}$. The rate of dissociation of intramolecular associates is proportional to the concentration of σ-associates considering again the subsystem with one or more motifs in the α-state. In each primary cycle, there are two associates, one inter- and one intramolecular; the σ-associate disappears by cleavage of any of the two associates in the cycle (factor 2). Therefore, for intramolecular association and dissociation of associates

$$\begin{aligned}\frac{\mathrm{d}c_{\mathrm{A}\sigma}}{\mathrm{d}t} &= k_{\mathrm{as}}c_{\mathrm{mA}}\omega_{\mathrm{A}}S_{\mathrm{A}}x_{\mathrm{A}}\omega_{\mathrm{A}}x_{\mathrm{B}}\omega_{\mathrm{B}}C_{\mathrm{s}P_{\mathrm{A}}}[1 + (P_{\mathrm{A}} - 1)x_{\mathrm{A}}\alpha_{\mathrm{A}}] - \\ &\qquad 2k_{\mathrm{dis}}c_{\mathrm{mA}}S_{\mathrm{A}}\sigma_{\mathrm{A}} \\ \frac{\mathrm{d}c_{\mathrm{B}\sigma}}{\mathrm{d}t} &= k_{\mathrm{as}}c_{\mathrm{mB}}\omega_{\mathrm{B}}S_{\mathrm{B}}x_{\mathrm{B}}\omega_{\mathrm{B}}x_{\mathrm{A}}\omega_{\mathrm{A}}C_{\mathrm{s}P_{\mathrm{B}}}[1 + (P_{\mathrm{B}} - 1)x_{\mathrm{B}}\alpha_{\mathrm{B}}] - \\ &\qquad 2k_{\mathrm{dis}}c_{\mathrm{mB}}S_{\mathrm{B}}\sigma_{\mathrm{B}}\end{aligned} \tag{3}$$

Rates of Intermolecular Association and Dissociation. The motif A selected in the subset can react with motifs B on all B molecules, including those with all free motifs. Dissociation is proportional to the concentration of the respective associate present in the same subset

$$\begin{aligned}\frac{\mathrm{d}c_{\mathrm{A}\alpha}}{\mathrm{d}t} &= k_{\mathrm{as}}c_{\mathrm{mA}}S_{\mathrm{A}}\omega_{\mathrm{A}}c_{\mathrm{mB}}\omega_{\mathrm{B}} - k_{\mathrm{dis}}c_{\mathrm{mA}}S_{\mathrm{A}}\alpha_{\mathrm{A}} \\ \frac{\mathrm{d}c_{\mathrm{B}\alpha}}{\mathrm{d}t} &= k_{\mathrm{as}}c_{\mathrm{mB}}S_{\mathrm{B}}\omega_{\mathrm{B}}c_{\mathrm{mA}}\omega_{\mathrm{A}} - k_{\mathrm{dis}}c_{\mathrm{mB}}S_{\mathrm{B}}\alpha_{\mathrm{B}}\end{aligned} \tag{4}$$

For equilibrium, the rates of inter- and intramolecular association are equal to the respective rates of dissociation

$$\begin{aligned}K_{\mathrm{as}}\omega_{\mathrm{A}}^{2}x_{\mathrm{A}}x_{\mathrm{B}}\omega_{\mathrm{B}}C_{\mathrm{s}P_{\mathrm{A}}}[1 + (P_{\mathrm{A}} - 1)x_{\mathrm{A}}\alpha_{\mathrm{A}}] - 2\sigma_{\mathrm{A}} = 0 \\ K_{\mathrm{as}}\omega_{\mathrm{B}}^{2}x_{\mathrm{B}}x_{\mathrm{A}}\omega_{\mathrm{A}}C_{\mathrm{s}P_{\mathrm{B}}}[1 + (P_{\mathrm{B}} - 1)x_{\mathrm{B}}\alpha_{\mathrm{B}}] - 2\sigma_{\mathrm{B}} = 0\end{aligned} \tag{5}$$

$$\begin{aligned}K_{\mathrm{as}}\omega_{\mathrm{A}}\omega_{\mathrm{B}}c_{\mathrm{mB}} - \alpha_{\mathrm{A}} = 0 \\ K_{\mathrm{as}}\omega_{\mathrm{A}}\omega_{\mathrm{B}}c_{\mathrm{mA}} - \alpha_{\mathrm{B}} = 0\end{aligned} \tag{6}$$

where $K_{\mathrm{as}} = k_{\mathrm{as}}/k_{\mathrm{dis}}$ is the equilibrium constant for association.

For the nonstoichiometric system

$$c_{\mathrm{mA}}/c_{\mathrm{mB}} = r_{\mathrm{A}},\ \ \alpha_{\mathrm{B}} = r_{\mathrm{A}}\alpha_{\mathrm{A}},\ \ \sigma_{\mathrm{B}} = r_{\mathrm{A}}\sigma_{\mathrm{A}},\ \ 1 - \omega_{\mathrm{B}} = r_{\mathrm{A}}(1 - \omega_{\mathrm{A}})$$

For the sake of simplicity and in accordance with experimental result, we consider the stoichiometric system, $r_{\mathrm{A}} = 1$, $\alpha_{\mathrm{A}} = \alpha_{\mathrm{B}}$, $\sigma_{\mathrm{A}} = \sigma_{\mathrm{B}}$, $P_{\mathrm{A}} = P_{\mathrm{B}}$, $x_{\mathrm{A}} = x_{\mathrm{B}}$, $c_{\mathrm{mA}} = c_{\mathrm{mB}}, = c_{\mathrm{m}}$, and so on.

By substituting α from eq 6 into eq 5, one gets an equation that can be solved numerically with respect to ω

$$\omega^{5}K_{\mathrm{as}}^{2}x^{3}c_{\mathrm{m}}(P - 1)C_{\mathrm{s}P} + \omega^{3}K_{\mathrm{as}}x^{2}C_{\mathrm{s}P} + 2\omega^{2}K_{\mathrm{as}}c_{\mathrm{m}} + 2\omega - 2 = 0 \tag{7}$$

The values of α and σ are then obtained from eq 6 and the balance equation

$$\alpha = K_{\mathrm{as}}\omega^{2}c_{\mathrm{m}},\ \ \sigma = 1 - \omega - \alpha \tag{8}$$

Gelation by Association and Network Structure Buildup. In equilibrium at low concentrations, the distribution of finite associative clusters of grafted chain exists. All paths of units connected by associates are finite. At a certain critical concentration of motifs, $(c_{\mathrm{mX}})_{\mathrm{crit}}$, one of the paths becomes infinite, which corresponds to the gel point. At still higher concentrations, the number of grafted chains in the infinite structure (gel) increases, the connectivity (number of infinite paths within the gel) increases, and the gel gets stronger. This is a similar picture as that for covalent networks formed as a result of a kinetic process, but the connectivity is transient and subject to external perturbations. The advantage of the reversible system is the equilibration of structures, whereas in covalent systems, the structure is affected by the formation history. The number of intermolecular associates that is necessary for obtaining an infinite path depends on the number of motifs per chain, the associate strength (K_{as}), and other parameters discussed in the preceding section. For network buildup from functional copolymers, the distributions of degrees of polymerization, copolymer composition, and states of motifs are of great importance.

The method we have used for the description of gelation and network formation is the statistical branching theory similar to that used for permanent networks. The approach to cycle formation is based on the spanning-tree approximation. The motifs closing cycles (σ-state) are considered to be free but inactive, unable to interact with partners' three motifs to form new associates. In the graph-theoretical language, a spanning tree of a graph with cycles is a treelike graph where all vertices are connected by a minimum number of edges. In our case, the edges represent the intermolecular associates. Some examples of applications of the theory of branching processes to important systems including biomaterials can be found in refs 32, 33, and 65−71. The distributions in this system are described as follows: (1) The distribution in the degrees of polymerization is defined by the number-fraction generating function, $N(Z) = \sum_{i=1}^{\infty} n_i Z^i$. In our calculations, we have used the most probable distribution, for which the number fraction $n_i = (1 - q)q^{i-1}$; $q = 1 - 1/P_n$. For components A and B

$$N_{\mathrm{A}}(Z_{\mathrm{A}}) = \frac{(1 - q_{\mathrm{A}})Z_{\mathrm{A}}}{1 - q_{\mathrm{A}}Z_{\mathrm{A}}},\ \ N_{\mathrm{B}}(Z_{\mathrm{B}}) = \frac{(1 - q_{\mathrm{B}})Z_{\mathrm{B}}}{1 - q_{\mathrm{B}}Z_{\mathrm{B}}} \tag{9}$$

(2) The distribution in composition (copolymer units without and with motifs). For the random distribution of monomer units in an i-mer, the generating functions C of variables ξ_{A} and ξ_{B} are calculated by

$$\begin{aligned}C_{\mathrm{A}i}(\xi_{\mathrm{A}}) &= (1 - x_{\mathrm{A}} + x_{\mathrm{A}}\xi_{\mathrm{A}})^{i},\ \ C_{\mathrm{B}i}(\xi_{\mathrm{B}}) = (1 - x_{\mathrm{B}} + x_{\mathrm{B}}\xi_{\mathrm{B}})^{i} \\ C_{\mathrm{A}i}(\xi_{\mathrm{A}}) &= \sum_{k=0}^{i} \frac{i!}{k!(i - k)!}(1 - x_{\mathrm{A}})^{i-k}x_{\mathrm{A}}^{k}z_{\mathrm{AB}}^{k}\end{aligned} \tag{10}$$

(3) The distribution of the states of motifs in the dissociated state (ω-state) engaged in intramolecular (σ-state) and in intermolecular (α-state) associates; pgf B

$$\begin{aligned}B_{\mathrm{A}}(\omega_{\mathrm{A}}, \sigma_{\mathrm{A}}, \alpha_{\mathrm{A}}z_{\mathrm{AB}}) &= \sum_{\substack{r,s,t=0 \\ r+s+t=i}}^{i} b_{\mathrm{A}}(r, s, t)z_{\mathrm{AB}}^{\mathrm{t}} \\ B_{\mathrm{B}}(\omega_{\mathrm{B}}, \sigma_{\mathrm{B}}, \alpha_{\mathrm{B}}z_{\mathrm{BA}}) &= \sum_{\substack{r,s,t=0 \\ r+s+t=i}}^{i} b_{\mathrm{B}}(r, s, t)z_{\mathrm{BA}}^{\mathrm{t}}\end{aligned} \tag{11}$$

where $b(r, s, t)$ is the probability of finding a building unit (copolymer chain) having $r + s + t = i$ monomer units bearing the motif, of which r, s, and t are, respectively, in ω-, σ-, and α-states. The motifs in the α-state are important for branching (structure propagation), and thus the pgf variable z is assigned to them. The coefficients $b(r, s, t)$ are, in fact, normalized concentrations $[X(r, s, t)]$ of Scheme 1 (cf., preceding section). As explained before, this exact approach has not been sufficiently developed as of yet, and an approximation for the distribution of B will be used.

The basic information on the branching system is stored in the probability generating function, F_{0n}, which is a sum of components A and B (n_A and n_B are molar fractions)

$$F_{0n}(\mathbf{z}) = n_A F_{0An}(z_{AB}) + n_B F_{0Bn}(z_{BA}) \qquad (12)$$

To incorporate distributions, successive substitutions are performed

$$F_{0An}(z_{AB}) = N_A(C_A(B_A(z_{AB}))), \quad F_{0Bn}(z_{BA}) = N_B(C_B(B_B(z_{BA}))) \qquad (13)$$

These equations can be decoded as follows: Each i-mer of the degree-of-polymerization distribution (eq 9) has a distribution in the composition described by eq 10; the selected i-mer containing k motifs has distribution of states given by pgf B; one particular arrangement has t motifs in the α-state.

Proceeding in the standard way, the pgf's for the number of additional motif-bearing monomer units in the α-state on chain units already associated with some other chain are obtained by differentiation and renormalization as

$$F_A(z_{AB}) = \left(\frac{\partial N_A}{\partial C_A}\frac{\partial C_A}{\partial B_A}\frac{\partial B_A}{\partial z_{AB}}\right) \Big/ \left[\frac{\partial N_A}{\partial C_A}\frac{\partial C_A}{\partial B_A}\frac{\partial B_A}{\partial z_{AB}}\right]_{z_{AB}=1}$$

$$F_B(z_{BA}) = \left(\frac{\partial N_B}{\partial C_B}\frac{\partial C_B}{\partial B_B}\frac{\partial B_B}{\partial z_{BA}}\right) \Big/ \left[\frac{\partial N_B}{\partial C_B}\frac{\partial C_B}{\partial B_B}\frac{\partial B_B}{\partial z_{BA}}\right]_{z_{BA}=1} \qquad (14)$$

The gel point is determined by the condition

$$\left[\frac{\partial F_A}{\partial z_{AB}}\frac{\partial F_B}{\partial z_{BA}}\right]_{z_{AB}=z_{BA}=1} = 1 \qquad (15)$$

From eq 15, one can get critical values of α_A and α_B or other parameters, and from a combination with relations of the preceding section, one can get the critical concentration. For the particular distributions selected here (cf.,eqs 9–11),

$$F_{0An}(z_{AB}) = \frac{(1-q_A)(1-x_A+x_A B_A(z_{AB}))}{1-q_A(1-x_A+x_A B_A(z_{AB}))} \qquad (16)$$

Let us analyze the branching pgf $B(z)$: In the first approximation, the distribution of states is assumed to be random.

$$B_{Ai}(z_{AB}) = (\omega_A + \sigma_A + \alpha_A z_{AB})^i = (1 - \xi_A + \xi_A(s_A + a_A z_{AB}))^i \qquad (17)$$

In the right-hand-side part of eq 17, the distribution is expressed by the fraction of all units involved in associates, ξ, and the fraction of those engaged in ring closing and branching associates, s and a, respectively. In the power expansion of $(\omega_A + \sigma_A + \alpha_A z_{AB})^i$, the logically nonexisting terms (those states of chains in which motif bearing units are present in the σ-state without any unit being in the α-state) are given by $(\omega_A + \sigma_A)^i - \omega_A^i$. In passing from F_{0An} to F_A, these terms are automatically eliminated during differentiation with respect to the variable z_{AB}. Therefore, from eq 16 using the standard procedure shown in eq 14, one gets

$$F_A(z_{AB}) = \frac{(1-q_A)^2}{(1-q_A\{x_A[1-\xi_A+\xi_A(1-a_A+a_A z_{AB})]+1-x_A\})^2} \qquad (18)$$

The expressions for the B component are analogous.

The gel point is defined by the equality defined by the relation (eq 15), which gives

$$\frac{(1-q_A)(1-q_B)}{4q_A q_B x_A x_B \xi_A \xi_B a_A a_B} = \frac{1}{(P_{Aw}-1)(P_{Bw}-1)x_A x_B \xi_A \xi_B a_A a_B} = 1 \qquad (19)$$

For components A and B of equal distributions, $q_A = q_B = q$, $x_A = x_B = x$, $\xi_A = \xi_B = \xi$, $a_A = a_B = a$

$$(\xi a)_{\mathrm{crit}} = \frac{1-q}{2qx} = \frac{1}{(P_w - 1)x} \qquad (20)$$

where P_w is the weight-average degree of polymerization of grafted copolymer chains. This result is very simple and understandable. The product $\xi a = \alpha$ is the conversion of motifs to intermolecular associates. The critical concentration of motifs A or B, $(c_{mX})_{\mathrm{crit}}$, is obtained by numerical solution after combination with eqs 6 and 7. The dependences of $(c_{mX})_{\mathrm{crit}}$ on system parameters are discussed in the Discussion and Comparison with Experimental Data section.

To analyze the effect of random distribution of states (eq 17), namely, the generation of logically nonexistent terms, we modified the distribution in the following way: The power expansion of random distribution of states (eq 17) for P-mer reads

$$(1-\xi+\xi z)^P = (1-\xi)^P + P(1-\xi)^{P-1}\xi z + \frac{P(P-1)}{2}(1-\xi)^{P-2}\xi^2 z^2 + ... + \binom{P}{k}(1-\xi)^{P-k}\xi^k z^k + ... + \xi^P z^P \qquad (21)$$

if cyclization is involved and only motifs engaged in intermolecular associates are counted as active $z \rightarrow s + az$, where s and a are (as above) fractions of motifs in intra- and intermolecular associates, respectively. Because in the alternating type of association "intra cannot exist without inter", the distribution (eq 21) should be modified as follows

$$(1-\xi)^P + P(1-\xi)^{P-1}\xi z + \frac{P(P-1)}{2}(1-\xi)^{P-2}\xi^2 z(s+az) + ... + \binom{P}{k}(1-\xi)^{P-k}z(s+az)^{k-1} + ... + \xi^P z(s+az)^{P-1} = \vartheta(z)(1-\xi+\xi(s+az))^P - (1-\xi)^P(\vartheta(z)-1) \qquad (22)$$

where $\vartheta(z) = z/(s + az)$. Equation 22 can be transformed into the probability generating function for the number of intermolecular bonds issuing from a building unit

$$F_{0n}(z) = \vartheta(z)(1-\xi+\xi(s+az))^P - (1-\xi)^P(\vartheta(z)-1) \qquad (23)$$

and the gel point condition reads

$$F'(1) = \frac{2(1-a)P\xi a + P(P-1)\xi^2 a^2 - 2a(1-a)[1-(1-\xi)^P]}{(1-a)(1-(1-\xi)^P) + P\xi a} = 1 \qquad (24)$$

For $a = 1$ (all associates are intermolecular, no cyclization), equation (24) reduces to that known for cross-linking of primary chains

$$(P-1)\xi a = 1 \qquad (25)$$

The critical values of conversion of motifs in associates, ξ_{crit}, calculated using distribution (eq 22) and expressed by eq 23 and the gel point condition (eq 24) with that of eq 21 and critical condition (eq 24) were compared in the range of P from 100 to 1000. One finds that for $a = 0.5$ (50% associates intramolecular), the difference in ξ_{crit} amounts to about 5–7%, and for $a = 0.1$ (~90% associates intramolecular), it increases to 25–30%.

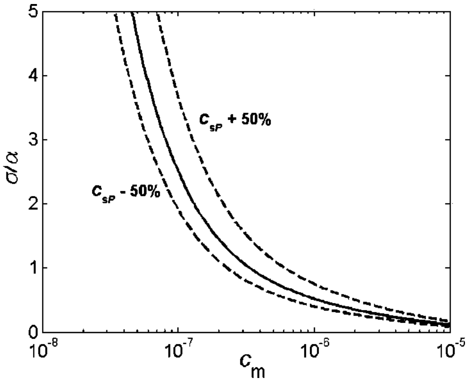


Figure 3. Dependence of the ratio of motifs engaged in intra(σ)- and inter(α)-molecular associates dependent on the concentration of motifs [mol/cm^3]; $P = 660$, $K_{as} = 10^5$ cm^3/mol, $C_{sP} = 3.69 \times 10^{-4}$ mol/cm^3; dashed curves for values of $C_{sP} + 50\%$ and $C_{sP} - 50\%$.

Because this difference is not significant, we applied the random distribution of states of motifs.

Discussion and Comparison with Experimental Data

Connecting biological motifs into interdependent multiplets by attaching them to a flexible polymeric chain brings a new dimension to manipulation with system connectivity. Connectivity determines the growth of associates, gelation, and temporary gel properties. It is not only the associative bond strength but also the wastage of its connective ability through forming cyclic structures that determines the physical state and system's performance in application. A new feature is brought into the behavior of biohybrids by a certain fuzziness of ensembles of synthetic polymers through distributions in their composition, number of units, topology, number of motifs, associative power, and so on. These distributions are important and may profoundly affect the expected outcome.

In this contribution, we have offered a mean-field approach to gelation of hydrophilic polymers with grafted peptides capable of forming helical associates as temporary cross-links. Let us repeat that we have dealt only with single-phase systems. The model has been developed for the so-called heterodimeric system in which motif A on one chain can associate with motifs B on the other chain. The simulation of structural changes occurs in a three-step procedure: (a) treatment of the formation of inter- and intramolecular bonds, (b) association−dissociation equilibria, and (c) increase in structure connectivity up to gelation.

In the first module, the competition between intra- and intermolecular bonds (ring-chain competition) is analyzed. Because intramolecular associates do not contribute to the growth of clusters of associated chains and thus to the gelation, this is an important issue. We have decided not to place the issue of modeling of cyclization in the main body of the article but as Supporting Information to keep the length of the presentation reasonable and to focus on gelation.

The general conclusion is that cyclization is promoted by dilution of the system, by increasing the degree of polymerization of chains, and by increasing the fraction of monomer units bearing motifs. Regarding the correctness of the values of the cyclization probability, C_{sP}, we refer to the analysis made in the Supporting Information. This value can be increased by mainly considering cyclization with chains in more distant generations, and it can be decreased by obstructions in the case of chains with more numerous motifs engaged in associates. We estimated that this deviation would hardly exceed 30% of C_{sP}. Figure 3 shows the effect of even larger deviations (±50%). Within this wide range, c_m varies from (2 to 6) $\times 10^{-7}$ mol/cm^3. This is not much with respect to experimental observation of critical concentration. In general, the ratio σ/α increases with decreasing concentration, with the decrease being dependent on the association constant and the degree of polymerization.

In the simulation of association−dissociation equilibria (second module), we expected that the values of K_{as} or K_{dis} could come from "outside", that is, from numerous data in the literature. However, they are related to free motifs, and no information was available for grafted systems. Tirrell's work[27] indicates that associations and dissociations in polymer hybrid systems are much slower. Our conjecture is that K_{as} can also be lower by a few orders of magnitude compared with the free peptide motifs. The second postulate is that the same value of K_{as} is applicable to coiled-coils connecting the chains inter- and intramolecularly because no strain is expected to exist in the rings. Equilibrium is reached when the rates of association and dissociation are equal, and this allows us to find the fractions of motifs engaged in intermolecular associates, intramolecular associates, and free motifs, respectively,α, σ, and ω. The results, when combined with module 3, give the critical concentration to reach the gel point. It shows that for stronger association, the critical concentrations necessary to reach gelation are substantially lower and cyclization is much stronger.

Let us show the results of simulation and compare them with experimental data. In the Utah laboratory, the hybrid hydrogel systems based on *N*-(2-hydroxypropyl)methacrylamide copolymers were synthesized. They consisted of the hydrophilic polymer backbone and a pair of oppositely charged peptide grafts. Two distinct pentaheptad peptides (CCE and CCK) were anticipated to create a dimerization motif and serve as physical cross-linkers by the formation of antiparallel heterodimeric coiled-coils.[11] The number-average degree of polymerization, P_n, was equal to 670, and the fraction of units with motifs, x, was 0.034. The critical concentration for gelation was found to be ~0.1 wt %, which corresponded to $c_{mA} = c_{mB} = c_m = \sim 6 \times 10^7$ mol/cm^3. For the grafted copolymer of this study, the growth of clusters connected with coiled-coil dimers was monitored by quasielastic light scattering (QELS).[24] In 1 wt % copolymer solution, the formation of clusters was much slower than that of free motifs. The changes observed in QELS resembled branching in homogeneous chemically cross-linked hydrogels. The shape of the intensity autocorrelation function of the stretched exponential changed to power law dependence, which is characteristic of gelation, after 8 h. At this concentration, the network buildup by association continued, and more coherent gel was formed. No signs of phase separation were observed at any studied concentrations.

The simulation shows that the critical concentration of the motifs, $(c_m)_{crit}$, depends on the degree of polymerization of grafted chains as well as on the association constant, K_{as}. (See Figures 4 and 5.)

Figures 4 and 5 clearly show that the critical concentration of motifs is a robust function of the association constant and does not depend much on the extent of cyclization. Therefore, for the system investigated experimentally, K_{as} must be in the range of 5×10^4 to 1×10^5 cm^3/mol; values above 10^5 cm^3/mol would be out of the range of experimental values of c_m. The attachment of motifs to long polymer chains considerably limits their mobility and reorientation capability. At these values of K_{as}, the σ/α ratio is on the order of unity (cf., Figure 3, where for $K_{as} = 10^5$ cm^3/mol, it is equal to 0.8). As we have seen, the value of σ is not very important for the critical concentration, but it would influence the pregel cluster size and strength of the gel beyond the gel point. Theoretical or experimental data on cyclization in these polydisperse polymeric systems are not available. For a much simpler but somewhat similar system, it was found by lattice simulation that of all bonds formed at percolation threshold, 30% were intramolecular.[52]

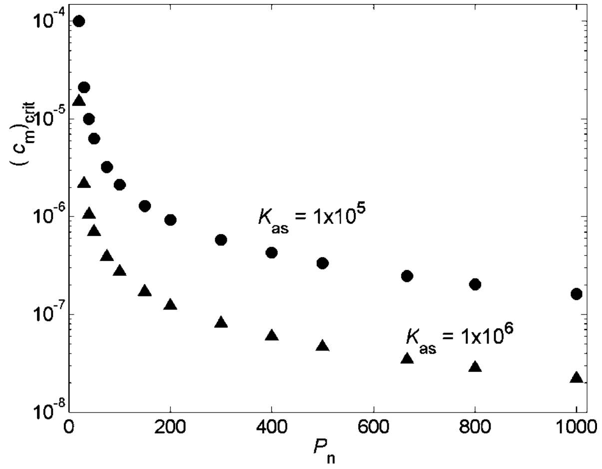


Figure 4. Dependence of critical concentration of motifs [mol/cm^3] on number average degree of polymerization of grafted chains for values of $K_{as} = 1 \times 10^{-5}$ and 1×10^{-6} cm^3/mol.

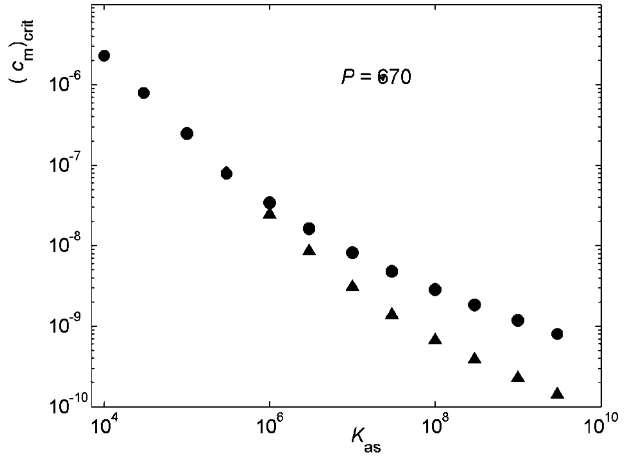


Figure 5. Dependence of critical concentration of motifs at the gel point [mol/cm^3] on the values of the association constant, K_{as} [cm^3/mol]; $P_n = 670$; ●, calculated for standard value of $C_{sP=670} = 3.69 \times 10^{-4}$ mol/cm^3; ▲, for obstructed cyclization ($\tau = 0.1$) giving $C_{sP=670} = 2.5 \times 10^{-5}$ mol/cm^3.

More support for a low value of K_{as} comes from slow dynamics of structural evolution measured by QELS.[24] Our rough estimate is that the rate constant for association could decrease from approximately 10^5 to 10 M^{-1}s^{-1}, and the dissociation constant could be on the order of 10^{-2} s^{-1}, that is, 10^{-5} mol/cm^3, which gives $K_{assoc} \approx 10^5$ cm^3/mol. This means that the rates of association and dissociation of motifs in the grafted polymers are controlled by relaxation of the chains and by steric constraints. Not only the motifs encounter but also the reorganization into the superhelical coiled-coils determine the rates.

The appropriateness of the mean-field concept of the problem should be checked by proving that grafted chains in solution overlap. The critical concentration was found[11] to be about 0.1 wt %, which corresponds to molar concentration of motifs of about 6×10^{-7} mol/cm^3. The hydrodynamic radius of a grafted polymer molecule measured by QELS[24] is ~110 nm, and one polymer molecule bears about 20 motifs. This means that the critical overlap concentration, c^*, is $\sim 10^{-8}$ mol/cm^3, by more than an order of magnitude lower than the critical concentration. Therefore, the overlap of grafted chains seems to be guaranteed. In the literature, one finds electron micrographs of highly swollen chains prepared by freeze-drying technique. The morphology is of the open-cell and channel-like structure, as formed by phase separation of the excess of solvent in the gel. We do not think that this morphology was generated during association by phase separation but that it was generated as a result of freeze drying. The morphology is similar to that of gelatin gels after the freezing cycle.[71]

Concluding this comparison, we can say that from results obtained with grafted polymer of degree of polymerization $P_n = 670$, association–dissociation occurs in the single-phase system, it is several orders of magnitude slower than that for free motifs, and the probable value of the association constant is on the order of 10^5 cm^3/mol (5×10^4 to 1×10^5). The ratio of intra- to intermolecular associates (σ/α) amounts to about 0.8, that is, about one intramolecular associate per one intermolecular associate. The fraction of motifs engaged in intermolecular associates is equal to 0.022 (which corresponds to the classical condition of one motif in intermolecular associate per P_w), and that in inter- and intramolecular associates is equal 0.040, which means that 96% of motifs are in the free (ω) state at the critical concentration. When the binding constant is increased while P is kept constant, the same number of intermolecular associates is necessary to reach the gel point (0.022), but the critical concentration decreases, and the fraction of motifs engaged in intramolecular associates increases. Lower concentration and more intramolecular binding can lead to preferential formation of intramolecularly cross-linked dimers and oligoassociates. This will drive the system to the microgelation regime and a violation of the condition of work with average concentration characteristic for the mean-field approach. Lowering P will increase the critical concentration and also lower cyclization; the dynamics is expected to be faster, which will be manifested by an increase in K_{as}. A decrease in the fraction of motifs, x, at the same P will help in the transformation of motifs to intermolecular associates, but the gel will be weaker and the response to external stimuli causing dissociation of associates can be smaller.

This brings us to the question of what more the model developed here can offer. So far, we have been mainly concerned with the critical concentration and the most probable values of the association constant. Further on, the branching theory elaborated within module 3 can be used for the calculation of equilibrium degree-of-polymerization averages of clusters in the subcritical region and beyond the gel point; it offers the fraction of sol and concentration of elastically active strands in the temporary network. The possibility of obtaining experimental values depends on whether relaxation of weak physical bonds can be well separated from dissociation of strong physical bonds, the coiled-coil associates. Beyond the gel point, the definition of cyclic bond must be changed because by its nature, a gel inevitably contains many cyclic structures.

So far, in model calculations, we have been concerned with stoichiometric systems, that is, those with equal initial concentrations of motifs A and B. The alternating character of association offers manipulation of the critical concentration and gel properties by varying the molar ratios of A and B motifs. For instance, the model can predict the A to B molar ratio above or below, which the system cannot gel at any concentration. The off-stoichiometry can be generated in several ways: different amounts of A and B grafted polymers of nearly the same composition or the same amount of A and B grafted polymers of different composition. The prediction of recipes for the preparation of two liquid preassociates having subcritical excess of A and B motifs that will gel fast upon mixing is also possible by adaptation of our approach. Therefore, the model can serve as a guide in exploring new strategies in drug delivery.

Conclusions

The mean-field theory of gelation of solutions of polymer chains A and B with grafted protein motifs capable of forming coiled-coil heterodimeric associates was developed. It is based on association−dissociation equilibria as balances between the rates of association and dissociation of motifs. The associates are distinguished as being intermolecular and intramolecular. The fractions of intramolecular associates relative to intermolecular associates are controlled by cyclization probability. In the cyclization probability, all possible ways of closing a cycle between a pair of A and B chains are considered. The association−dissociation equilibria are then coupled to a statistical branching theory to get information on structure growth: size of clusters in the pregel region, condition for gelation, and postgel evolution of the gel structure. The system characteristics depend on the equilibrium constant of association, the degree of polymerization, and composition distributions of the grafted copolymer. In this contribution, we focused on the critical conditions at the gel point and compared the modeling results with experiment. The main conclusions are: (1) The association constant for a coiled-coil motif that is chemically bonded to a copolymer chain of degree of polymerization of about 700 decreases by a factor of 10^4 from $K_{as} = 10^9$ cm^3/mol for free, not-bonded motif to 10^5 cm^3/mol. This is due to steric reasons. (2) The ratio of motifs engaged in intra- and intermolecular associates is approximately 1:1. (3) At the critical concentration of motifs, 6×10^{-7} mol/cm^3, and at higher concentrations, the systems undergo neither microgelation nor phase separation.

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Supporting Information Available: Details of the cyclization probability, the cycle closure probability, and the refinement of the cyclization model. This material is available free of charge via the Internet at http://pubs.acs.org.

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