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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 in dependence 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 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 by 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 motifs in associated state is engaged in intramolecular bonds.

Keywords

gel; coiled-coil; cyclization; branching; gelation; equilibrium

INTRODUCTION

Coiled-coil hydrogels are hybrid hydrogels, which 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 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 high potential for drug delivery.[1–4] Several 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

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motif A can form associate only with motif B. To ensure 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 paper 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 paper analyzes the self-assembly of two graft copolymers; one carrying motifs (grafts) A and the other motifs B. Following mixing, coiled-coil associates are formed exclusively by 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 *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 the grafted system is (1) *polyfunctional* with *functionality distribution*, (2) *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), (5) *phase separation* can occur. First let us briefly review the facts important for model formulation. This summary will be concerned with (a) association-dissociation rates and equilibria of coiled-coils, (b) formation of physical gels and their transformations, (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 (partly) disordered states seems to be the rate-determining step. The equilibrium constants for dissociation or association ($K_{dis} = 1/K_{as}$) 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 of the order of $10^6 \, \text{M}^{-1}\text{s}^{-1}$ with the halftime at concentration 100 µM, $t_{1/2} = 10^{-2} \, \text{s}$. These high rates approached the regime of encounter control in reaction kinetics. If we consider the first order dissociation rates of the order of $10^{-1} \, \text{s}^{-1}$, the halftime for dissociation $t_{1/2}$ is of the order of 10 s. The equilibrium constants for association, K_{as}, vary in the range of 10^8–10^{12} mol/cm³. As we will show later, it is evident that attachment of motifs to polymer chains slows down 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 reversibility of crosslinks, so that at equilibrium, the chains connecting crosslinks 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 are eventually obtained. A two-phase system is the other alternative. The rates of

rearrangement of physical crosslinks and network chains relaxation can be very different.[25] The crosslink reorganization can be very fast. In polymer solutions, polymer-polymer contacts can be considered as very mobile crosslinks. Alternatively, the crosslink rearrangement is very slow and the gel resembles a permanent (covalent) network. Gelatin is a good example: it behaves as permanent gel, but in fact it is 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 crosslinks 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 to the macroscopic mechanical relaxation time: thus, the crosslink rearrangement was rate determining for system dynamics. Associative thickener, a diblock copolymer with hydrophilic and hydrophobic blocks, is another example of transient networks. Micelles composed of the hydrophobic blocks are the physical crosslinks.[25,28–30]

It depends on the initial concentration of the motif (c_m) whether the polymer coils initially overlap ($c_m > c^*$) or do not overlap ($c_m < c^*$). In the first case, the free motifs of polymer clusters interact with motifs of other clusters as well as its own motifs. For $c_m < c^*$, the formation of dimers of polymer coils carrying A and B motifs is apparently fast, but 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 non-linear copolymerization.[31–32].

Irrespective of cluster growth mechanism and possible temporary heterogeneity, at equilibrium reached after a long time, the final states are relatively simple: (1) single-phase gel or liquid, (2) a two-phase system with liquid-liquid, liquid-gel, and gel-gel phases, or (3) at invariant conditions (at constant pressure) three-phases can be in equilibrium. The three-phase equilibrium is possible according to Gibbs phase rule, $f = C + 2 - P$, where f denotes degrees of freedom, C number of components and P 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 co-existing phases should be homogeneous because of 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 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, much stronger than the weak bonds. The free-energy of the system polymer with stickers - solvent is an additive function of non-sticker and stickers interactions. Using standard thermodynamic procedures, the phase separation limit, phase volume and concentration ratios can be calculated. The gelation threshold is calculated 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 build-up in the gel.[48] Within this mean-field approach, other mechanisms of crosslink formation (stickers associated into spherical, cylindrical, or disk-like micelles) were also considered.[40–42] Erukhimovich's model considers the sol-gel transformation as a first order thermodynamic transition coupled with 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 describable by mean-field theories as those of Refs.37‾42 considering gelation a topological singularity. The approach presented below is based on similar principles and deals explicitly with pre-gel 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 for modeling of gelation of gelatin (stickers introduced along polymer chains).49 The stickers formed associates of three to simulate 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 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 turns bimodal and the largest cluster is the gel (cf. off-space Monte-Carlo simulation of chemical crosslinking53). 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. Interesting is the simulation of the effect of stress on mechanical behavior of physical networks.53 Molecular dynamics models for gelation of block copolymer systems by other mechanism (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 crosslinking was considered (loops, flower micelles), at least verbally, or by introducing a cyclization term which however, hardly allowed for quantification. Also, numerical results are available.[50] For covalent network, the cyclization phenomenon is better understood and quantification is possible. For not too large extent of cyclization, the concept of Kuhn's equivalent chain was used by Jacobson and Stockmayer[55] for 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:
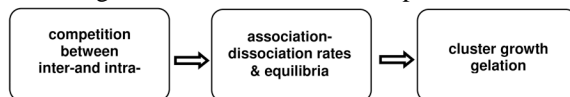
Module 1 deals with the probabilities of cycle formation relative to formation of an intermolecular associate for the synthetic copolymers carrying the motifs. These probabilities serve as input for formulation of the association-dissociation rates and equilibria involving inter- and intramolecular associates (Module 2). Output of Module 2, balances between inter- and intramolecular associates in dependence on concentration, serves as input of Module 3 where the problem of connectivity is treated to find the conditions for the gel point. 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 form of Supporting Information.

Inter- vs. intramolecular association

The chains are copolymers of non-functional 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 (Fig. 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, (2) the components A and components B are polydisperse which also somewhat affects the extent of cyclization. Full treatment of the cyclization problem is given in 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 calculation of extent of cyclization the chains are taken as monodisperse of degree of polymerization P; in polydisperse system this P is considered to be the number-average, P_n

3. only "primary" cyclization (Fig. 1a) is considered, not the "secondary" cyclization (Fig. 1b) but its possible contribution is estimated (see Supporting Information)

4. the motifs exist in three states: unreacted (ω-state), ring closing (σ-state), branching (α-state). The number fractions of these states are ω, σ, α, respectively; the states are distributed among the motif units randomly, respecting the fact that a motif in σ-state cannot exist on chain without any motif in 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 when any unit of the copolymer A can form an *intra*molecular bond to any unit of one copolymer B already attached to A intermolecularly (Fig. 1b). C_{sP} has the meaning of concentration of the complementary motifs in the reaction volume around the selected motif and can be thus compared with concentration of motifs supplied by other (non-attached) chains.

Association-dissociation equilibria

The phenomenon of gelation is modeled by application of the statistical branching theory (next section). This theory works with building units in different states distinguished by the numbers of various types of (associative) bonds the building unit is bonded to other units. In our case, the building units are the grafted copolymer molecules bearing motifs in non-associated and associated states. The states of motifs in associates are further distinguished by the numbers of motifs engaged in intermolecular or intramolecular associates. Intermolecular association contributes to structure growth leading to gelation but the intramolecular association does not. Crosslinking of the system of copolymer chains with degree-of-polymerization and compositional distributions and involving cyclization is the most complex case of applied branching theory. To illustrate partially the complexity of the case, we describe 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

$$\frac{d[\mathrm{A}(P,0,0)]}{dt} = -k_{as}P[\mathrm{A}(P,0,0)]c_{\mathrm{B}\omega} + k_{dis}[\mathrm{A}(P-1,0,1)]$$

$$\frac{d[\mathrm{A}(P-1,0,1)]}{dt} = k_{as}P[\mathrm{A}(P,0,0)]c_{\mathrm{B}\omega} - k_{dis}[\mathrm{A}(P-1,0,1)]$$
$$-k_{as}(P-1)[\mathrm{A}(P-1,0,1)]c_{\mathrm{B}\omega} + 2k_{dis}[\mathrm{A}(P-2,0,2)]$$
$$-k_{as}(P-1)[\mathrm{A}(P-1,0,1)]C'_{sP} + 2k_{dis}[\mathrm{A}(P-2,1,1)]$$

$$\frac{d[\mathrm{A}(P-2,0,2)]}{dt} = k_{as}(P-1)[\mathrm{A}(P-1,0,1)]c_{\mathrm{B}\omega} - 2k_{dis}[\mathrm{A}(P-2,0,2)]$$
$$-k_{as}(P-2)[\mathrm{A}(P-2,0,2)]c_{\mathrm{B}\omega} + 3k_{dis}[\mathrm{A}(P-3,0,3)]$$
$$-k_{as}(P-2)[\mathrm{A}(P-2,0,2)]C'_{sP} + 3k_{dis}[\mathrm{A}(P-2,1,2)]$$

$$\frac{d[\mathrm{A}(P-2,1,1)]}{dt} = k_{as}(P-1)[\mathrm{A}(P-1,0,1)]C'_{sP} - 2k_{dis}[\mathrm{A}(P-2,1,1)]$$
$$-k_{as}(P-2)[\mathrm{A}(p-2,1,1)]c_{\mathrm{B}\omega} + 3k_{dis}[\mathrm{A}(P-3,1,2)]$$
$$-k_{as}(P-2)[\mathrm{A}(P-2,1,1)]C'_{sP} + 3k_{dis}[\mathrm{A}(P-3,2,1)]$$

$$\vdots \tag{1}$$

The quantity $c_{\mathrm{B}\omega}$ is concentration free motifs B in the system and C_{sP} is the concentration of B groups effective in cyclization (see below) supplied by units already connected with the unit under consideration. In equilibrium, the right hand sides of equations are equal to zero, and $k_{as}/k_{dis} - K_{as}$. 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 $[\mathrm{X}(*,*,*)]$ are sufficiently small, 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 in dependence on system variables is found and a certain (random) distribution among the motif units is assumed.

The following quantities will be used:

- the degree of conversion of motifs into associates, ξ_X, (X = (A,B)); only associates AB are formed. For "stoichiometric" system (equal number of moles of motif A and B in the system), $\xi_A = \xi_B$, for non-stoichiometric system $\xi_A \neq \xi_B$

- the fraction of motifs in free state is equal to $\omega_X = 1 - \xi_X$, intramolecularly associated state $\sigma_X = \xi_X s$, and intermolecularly associated state, $\alpha_X = \xi_X a$; $\omega_X + \sigma_X + \alpha_X = 1$, $s + a = 1$

- the molar concentration of motifs X in solution c_{mX} (mol/cm^3)

- the molar fraction of monomer units in the copolymer bearing motif X, x_X

- the degree of polymerization of the grafted chain, P_X

- 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 $K_{as} = k_{as}/k_{dis}$

- the set of summed cyclization probabilities, C_{sP}, derived in the Supporting Information for the given degree of polymerization P is the input information.

C_{sP} is the probability that two motifs A and B residing on already connected A- and B-chains, respectively, meet in a reaction volume element $dV = dxdydz$. It has the meaning and dimension of concentration [mol/cm^3] of 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 −

$x_X\xi_X)^{P_x}$ and that of chains with at least one associated motif is equal to $1 - (1 - x_X\xi_X)^{P_x}$ 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_{sP} was derived for all possible ring closure paths between any pair of units of A-chain and topologically neighboring B-chain assuming validity of the conformational statistics of equivalent Gaussian chain (cf., e.g., refs.53⁻61) and respecting presence of rigid rod-like associates in the sequences of monomer units closing the cycle. It reads (cf. eq. (A-9) and eq. (A-10) of Supporting Information)

$$C_{sP} = \sum_{i=i_o}^{2(P-1)} \left(\frac{3}{2\pi i l_m^2 Q}\right)^{-3/2} \exp\left(-\frac{3}{2i l_m^2 Q}2d_{cc}^2\right)(A_{rn})_i/N_{Avo}$$

(2)

In this equation, the combinatorial term $(A_{rn})i$ is the number of possible paths of length i-monomer units; i extends from i_0 to $2(P-1)$ (cf., eq. (A-9) of 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 the Avogadro number equal to 6.022×10^{23} molecules/mole. The exponential term cares for the presence of 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. $(d_{2cc} = \sqrt{2}d_{cc})$. The dependence of C_{sP} on the degree of polymerization of chains, P, is shown in Fig. 2, for set of parameters indicated.

Equation (2) was derived for monodisperse copolymers, for the polydisperse gelling systems P was considered 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 derivation of the model for C_{sP}. The refinements are concerned with inclusion of the effect of polydispersity of real chains, effect of possible steric obstructions to formation new associates when some already exist, and 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 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 having already at least one motif in α-state

$$S_X = 1 - (1 - x_X + x_X\omega_X)^{P_X} = 1 - (1 - x_X\xi_X)^{P_X}.$$

The target unit for ring closure is a B chain. The cyclization probability, C_{sP}, was derived for the case when 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_{sP} must be multiplied by the factor $x_A\omega_A x_B\omega_B$. 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 $(P-1)x_X\alpha_X$. The rate of dissociation of intramolecular associates is proportional to concentration of σ-associates considering again the subsystem with one or

more motifs in 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*

$$\frac{dc_{A\sigma}}{dt} = k_{as} c_{mA} \omega_A S_A x_A \omega_A x_B \omega_B C_{sP_A} [1+(P_A-1)x_A\alpha_A] - 2k_{dis} c_{mA} S_A \sigma_A$$
$$\frac{dc_{B\sigma}}{dt} = k_{as} c_{mB} \omega_B S_B x_B \omega_B x_A \omega_A C_{sP_B} [1+(P_B-1)x_B\alpha_B] - 2k_{dis} c_{mB} S_B \sigma_B$$

(3)

***Rates of inter*molecular 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 concentration of the respective associate present in the same subset.

$$\frac{dc_{A\sigma}}{dt} = k_{as} c_{mA} S_A \omega_A c_{mB} \omega_B - k_{dis} c_{mA} S_A \alpha_A$$
$$\frac{dc_{B\sigma}}{dt} = k_{as} c_{mB} S_B \omega_B c_{mA} \omega_A - k_{dis} c_{mB} S_B \alpha_B$$

(4)

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

$$K_{as} \omega_A^2 x_A x_B \omega_B C_{sP_A} [1+(P_A-1)x_A\alpha_A] - 2\sigma_A = 0$$
$$K_{as} \omega_B^2 x_B x_A \omega_A C_{sP_B} [1+(P_B-1)x_B\alpha_B] - 2\sigma_B = 0$$

(5)

$$K_{as} \omega_A \omega_B c_{mB} - \alpha_A = 0$$
$$K_{as} \omega_A \omega_B c_{mA} - \alpha_B = 0$$

(6)

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

For the non-stoichiometric system

$$c_{mA}/c_{mB}=r_A, \quad \alpha_B=r_A\alpha_A, \quad \sigma_B=r_A\sigma_A, \quad 1-\omega_B=r_A(1-\omega_A)$$

For the sake of simplicity and in accordance with experimental result, we consider stoichiometric system, $r_A = 1$, $\alpha_A = \alpha_B$, $\sigma_A = \sigma_B$, $P_A = P_B$, $x_A = x_B$, $c_{mA} = c_{mB}$, $= c_m$, etc.

Substituting α from eqs (6) Into eqs. (5), one gets an equation which can be solved numerically with respect to ω.

$$\omega^5 K_{as}^2 x^3 c_m(P-1)C_{sP} + \omega^3 K_{as} x^2 C_{sP} + 2\omega^2 K_{as} c_m + 2\omega - 2 = 0$$

(7)

The values of α and σ are then obtained from eq. (6) and balance equation

$$\alpha=K_{as}\omega^2 c_m, \sigma=1-\omega-\alpha$$

(8)

.

Gelation by Association and Network Structure Build-up

In equilibrium at low concentrations, 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, $(c_{mX})_{crit}$, one of the paths gets "infinite" which corresponds to the gel point. At still higher concentrations, the number of grafted chains in the infinite structure (gel) increases and the connectivity (number of infinite paths within the gel) increases and the gel gets stronger. This is a similar picture as 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 necessary for obtaining an infinite path depends on the number of motifs per chain, associate strength (K_{as}), and other parameters discussed in the preceding section. For network build-up 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 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 as free but inactive, unable to interact with free motifs to form new associates. In the graph-theoretical language, a spanning tree of a graph with cycles is a tree-like graph where all vertices are connected by 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.[32,33,65−71]

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

$$N_A(Z_A) = \frac{(1 - q_A)Z_A}{1 - q_A Z_A}, N_B(Z_B) = \frac{(1 - q_B)Z_B}{1 - q_B Z_B}$$

(9)

2. the distribution in composition (copolymer units without and with motifs). For random distribution of monomer units in an i-mer, the generating functions C of variables ξ_A, ξ_B

$$C_{Ai}(\xi_A) = (1 - x_A + x_A\xi_A)^i, C_{Bi}(\xi_B) = (1 - x_B + x_B\xi_B)^i$$
$$C_{Ai}(\xi_A) = \sum_{k=0}^{i} (1 - x_A)^{i-k} x_A^k z_{AB}^k$$

(10)

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

$$B_A(\omega_A, \sigma_A, \alpha_A z_{AB}) = \sum_{\substack{r,s,t=0 \\ r+s+t=i}}^{i} b_A(r,s,t) z_{AB}^t$$

$$B_B(\omega_B, \sigma_B, \alpha_B z_{BA}) = \sum_{\substack{r,s,t=0 \\ r+s+t=i}}^{i} b_B(r,s,t) z_{BA}^t$$

(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 α-state are important for branching (structure propagation) and thus the pgf variable z is assigned to it. 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 yet, and approximation for the distribution 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 an B (n_A, n_B are molar fractions):

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

(12)

To incorporate distributions, successive substitutions are performed

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

(13)

These equations can be decoded as follows: Each i-mer of the degree-of-polymerization distribution (eq. (9)) has a distribution in 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 α-state.

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

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

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

(14)

The gel point is determined by the condition that

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

(15)

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

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

(16)

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

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

(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 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 non-existing terms (those states of chains in which motif bearing units are present in σ-state without any unit being in α-state) are given by $(\omega_A + \sigma_A)^i - \omega_A^i$. In passing from $F_{0 An}$ to F_A, these terms are automatically eliminated during differentiation with respect to the variable z_{AB}. Thus, from eq. (16) using the standard procedure shown in eq. (14), one gets

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

(18)

The expressions for the B-component are analogous.

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

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

(19)

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

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

(20)

where P_w is 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, $(c_{mX})_{crit}$, is obtained by numerical solution after combination with eq. (6) and eq. (7). The dependences of $(c_{mX})_{crit}$ on system parameters are discussed in the Discussion section.

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

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

(21)

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

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

(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

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

(23)

and the gel point condition reads

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

(24)

For $a = 1$ (all associates are intermolecular, no cyclization), equation ( ) reduces for that know for crosslinking of primary chains

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

(24)

The critical values of conversion of motifs in associates, ξ_{crit}, calculated using distribution (22) and expressed by eq. (23) and gel point condition (24) with that of eq. (21) and critical condition (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%, for a = 0.1 (~90% associates intramolecular), it increases to 25–30%. Because this difference is not significant we were applying 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 systems 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, units. topology, number of motifs, their associative power, etc. 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 crosslinks. Let us repeat that we have dealt only with single-phase systems. The model has been developed for so-called heterodimeric system in which motif A on one chain can associate with motifs B on the other chain. Simulation of structural changes occurs in a three-step procedure: (a) treatment of formation of inter- and intramolecular bonds, (b) association-dissociation equilibria, and (c) increase of structure connectivity up to gelation.

In the first module, the *competition between intra- and intermolecular bonds* (ring-chain competition) is analyzed. Since 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 degree of polymerization of chains and by increasing fraction of monomer units bearing motifs. Regarding the correctness of the values of cyclization probability, C_{sP}, we refer to the analysis made in Supporting Information. This value can be increased by consideration mainly of cyclization with chains in more distant generations and 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 ($\pm 50\%$). Within this wide range, c_m varies from 2×10^{-7} to 6×10^{-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, the decrease being dependent on the association constant and 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", i.e., 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 also K_{as} can 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, ω. The results, when combined with module 3, give as 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 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 crosslinkers by the formation of antiparallel heterodimeric coiled-coils.[11] The number-average degree of polymerization P_n was equal to 670; the fraction of units with motifs, *x*, was 0.034. The critical concentration for gelation was found to be about 0.1 wt.-% which corresponded to about $c_{mA} = c_{mB} = c_m = 6 \times 10^7$ 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, formation of clusters was much slower than that of free motifs. The changes observed in QELS resembled branching in homogeneous chemically crosslinked hydrogels. The shape of the intensity autocorrelation function of stretched exponential changed to power law dependence which is characteristic of gelation, after 8 h. At this concentration, the network build-up 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. Fig.4 and Fig.5)

Figure 4 and Figure 5 show clearly that the *critical concentration* of motifs is a robust function of the association constant and does not depend much on the extent of cyclization. Thus, for the system investigated experimentally, K_{as} must be in the range $5\times10^4 - 1\times10^5$ cm^3/mol; values above 10^5 cm^3/mol would be out of range of experimental values of c_m. 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 of the order of unity; cf. Fig. 3 where for $K_{as} = 10^5$ cm^3/mol, it is equal to 0.8. As we have seen, the value of σ it is not much important for the critical concentration, but it would influence the pre-gel 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 systems it was found by lattice simulation that of all bonds formed at percolation threshold, 30% were intramolecular.[52]

Another 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 fall down from approx. 10^5 to 10 M^{-1} s^{-1} and the dissociation constant could be of the order of 10^{-2} s^{-1}, i.e. 10^{-5} mol/cm^3, which gives $K_{assoc} \approx 10^5$ cm^3/mol. This means that the rate 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 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 about 6×10^{-7} mol/cm^3. The hydrodynamic radius of a grafted polymer molecules measured by QELS[24] is about 110 nm and one polymer molecule bears about 20 motifs. This means that the critical overlap concentration, c^*, is about 10^{-8} mol/cm^3, by more than an order of magnitude lower than the critical concentration. Thus, 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 freezing cycle.[72]

Concluding this comparison, we can say that from results obtained with grafted polymer of degree of polymerization $P_n = 670$, association/dissociation occur in single-phase system, that it is by several orders of magnitude slower than for free motifs, and that the probable value of the association constant is of the order of 10^5 cm^3/mol ($5\times10^4 . - 1\times10^5$). The ratio of intra to intermolecular associates (σ/ α) amounts to about 0.8. i.e., 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 1 motif in intermolecular associate per P_w), in inter- and intramolecular ones is equal 0.040, which means that 96% of motifs are in free (ω) state at the critical concentration. When the binding constant is increased keeping P 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 crosslinked dimers and oligoassociates. This will drive the system to the microgelation regime and violation of the condition of work with average concentration characteristic for the mean-field approach. Lowering of P will increase the critical concentration and also lower "cyclization"; the dynamics is expected to be faster which will be manifested by an increase of K_{as}. A decrease of the fraction of motifs, x, at the same P, will help in transformation of motifs into 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 what more the model developed here can offer. So far we were concerned mainly 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 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 to obtain 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 contains inevitably many cyclic structures.

So far, in model calculations, we were concerned with "stoichiometric" systems, i.e., those with equal initial concentrations of motifs A and B. The alternating character of association offers manipulation with 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 A and B grafted polymers of different composition. Prediction of recipes for preparation of two liquid pre-associates having subcritical excess of A and B motifs respectively, which will gel fast upon mixing, is also possible by adaptation of our approach. Thus, the model can serve as guidance 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 intermolecular and intramolecular. The fractions of intramolecular associates relative to intermolecular ones 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 with 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 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 chemically bonded to a copolymer chain of degree of polymerization about 700 decreases by a factor 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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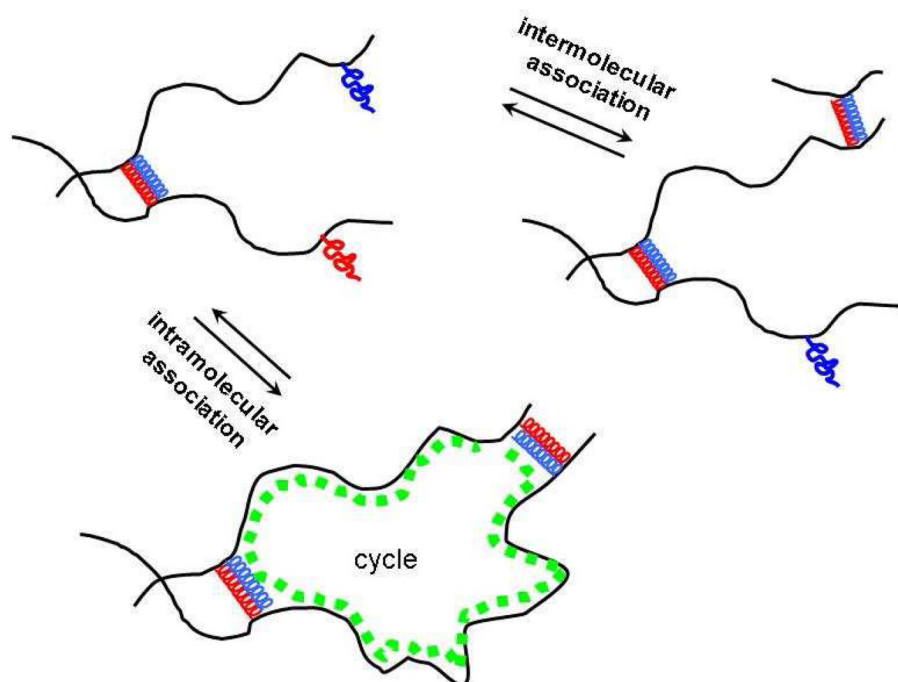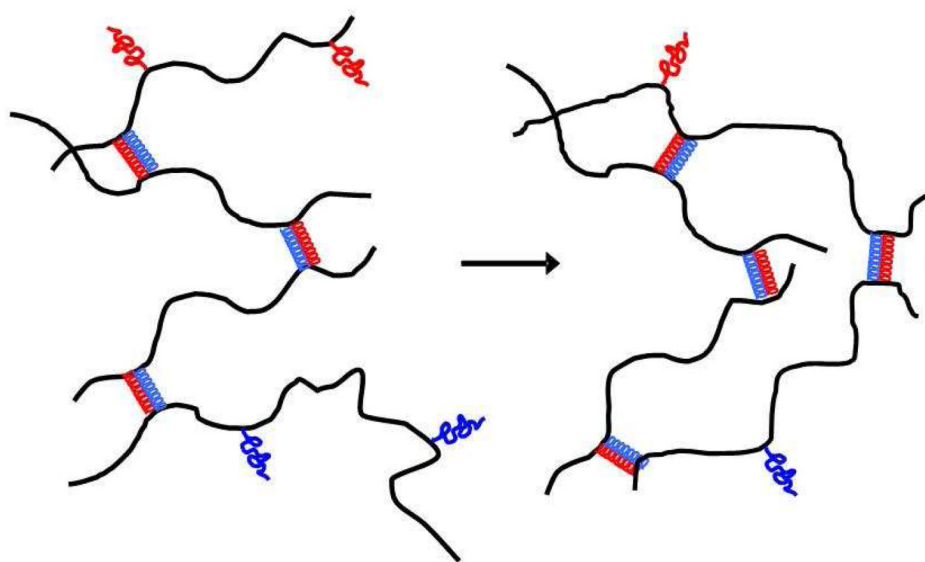
Figure 1a.

Figure 1b.

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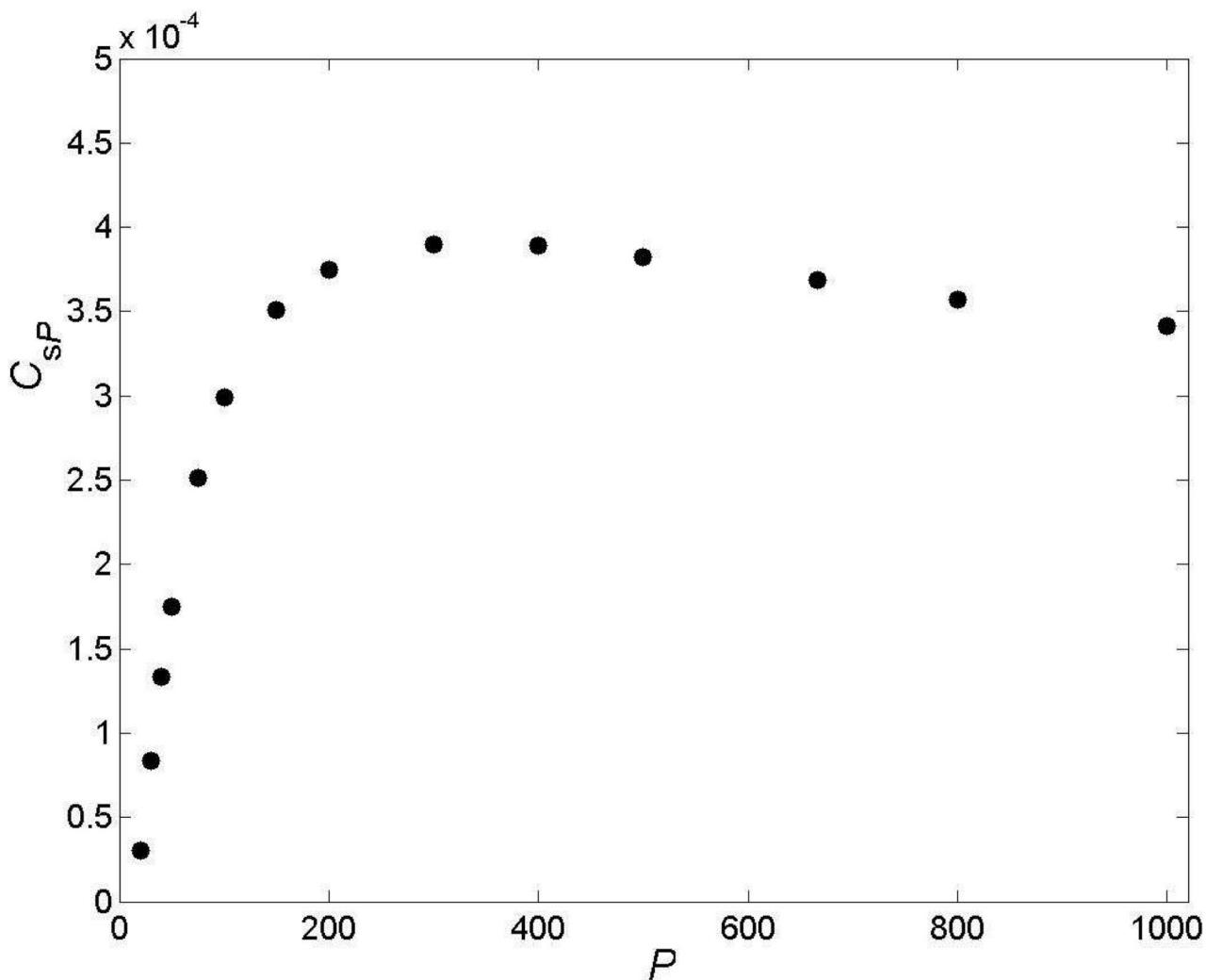Longer distance (secondary) cyclization.

Figure 2.
Summed cyclization probability, C_{sP}, [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 monomer unit $l_m = 0.32$ nm, the number of monomer units in the smallest possible ring $i_0 = 10$ (the smallest cyclic structure is composed of two superhelices connected by 10 monomer units).
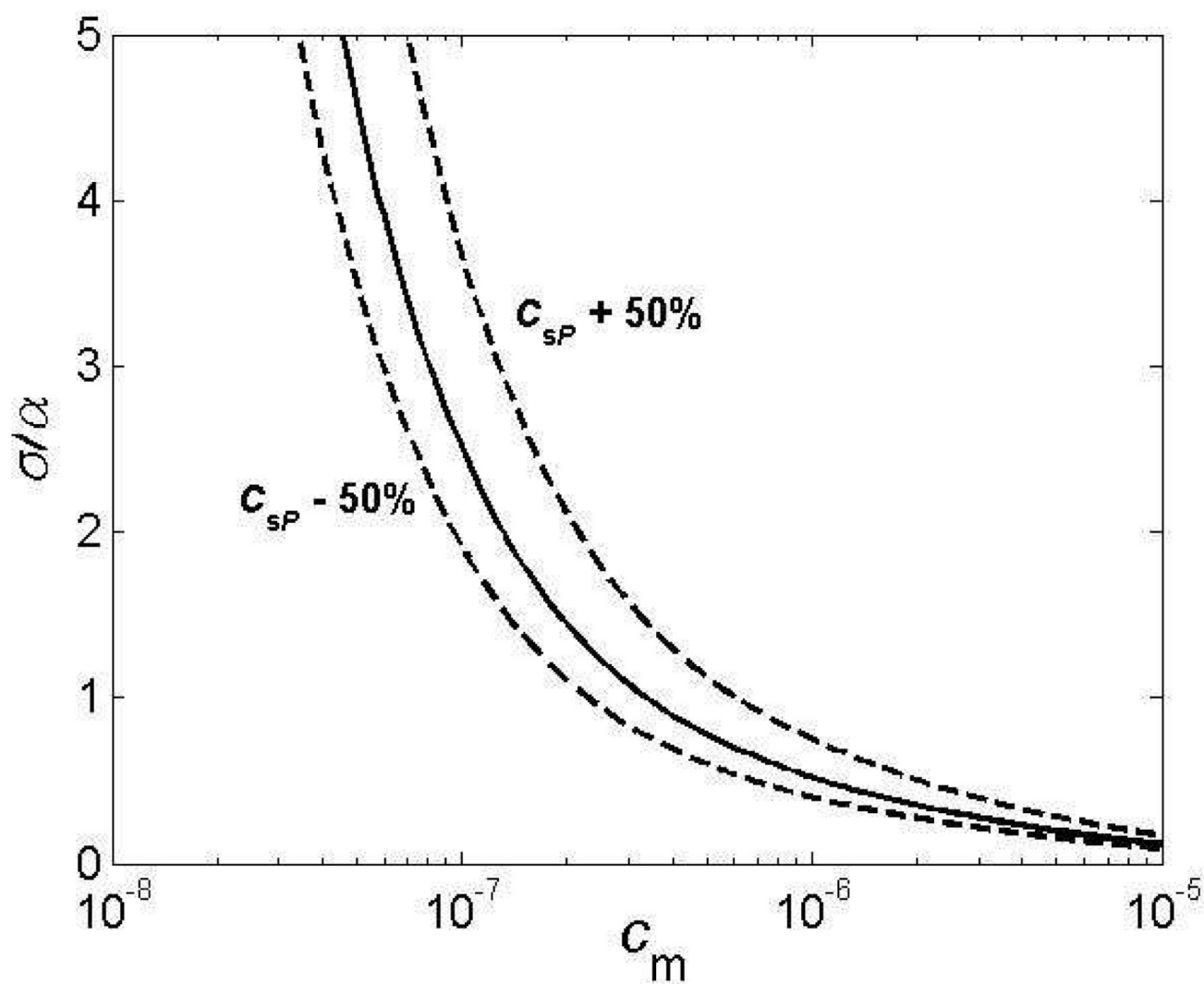
Figure 3.
Dependence of the ratio of motifs engaged in intra(σ)- and inter(α)-molecular associates in dependence on concentration of motifs [mol/cm^3]; $P = 660$, $K_{as} = 10^5$ cm^3/mol, $C_{sP} = 3.69 \times 10^{-4}$ mol/cm^3; dashed curves for values of C_{sP} + or − 50%
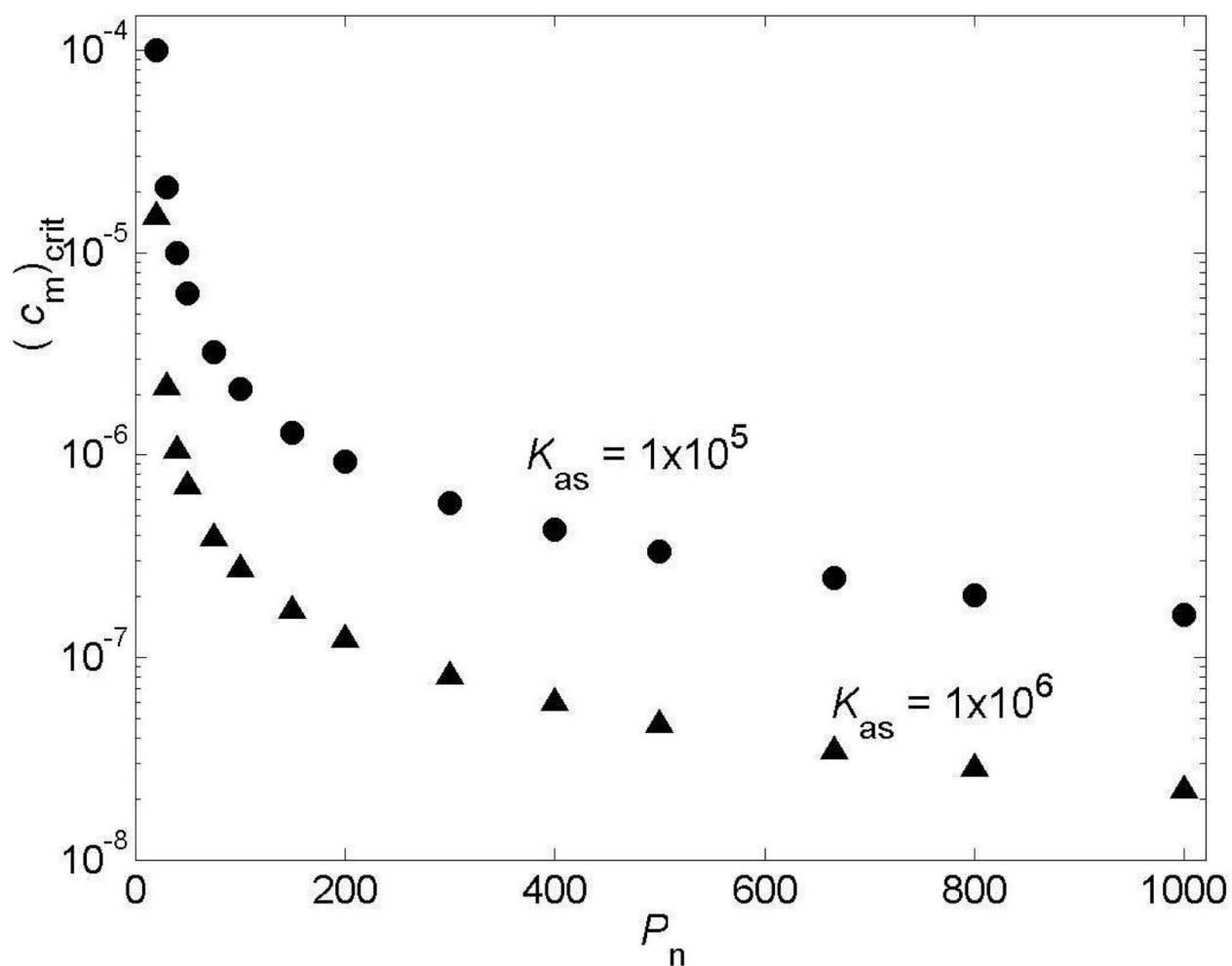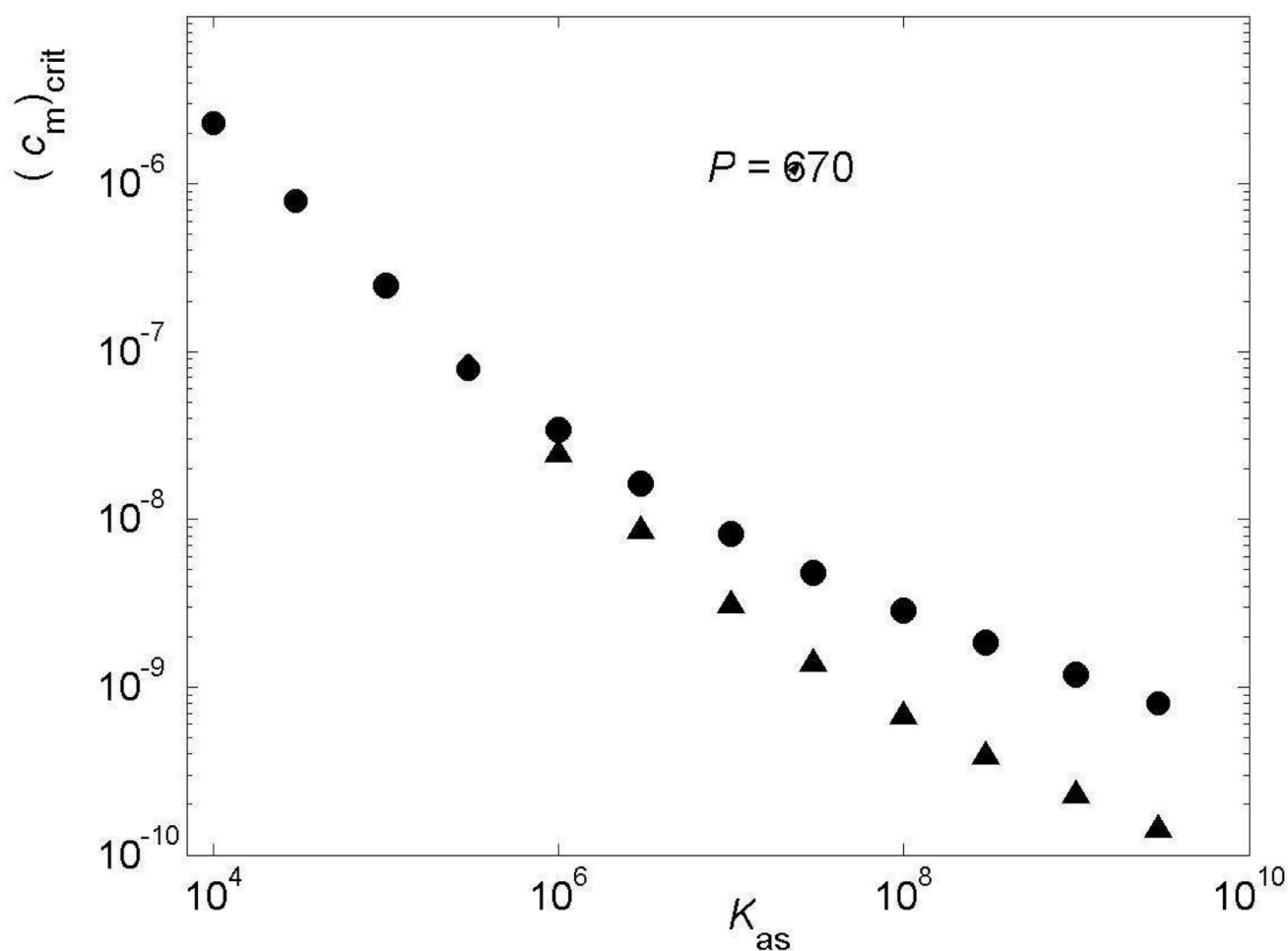
Figure 4.
Dependence of critical concentration of motifs [mol/cm3] on number average degree of polymerization of grafted chains for values of $K_{as} = 1 \times 10^{-5}$ and 1×10^{-6} cm^3/mol.

Figure 5.
Dependence of critical concentration of motifs at the gel point [mol/cm3] on the values of the association constant, K_{as} [cm^3/mol]; $P_n = 670$, full circles calculated for standard value of $C_{sP=670} = 3.69 \times 10^{-4}$ mol/cm^3; full triangles for obstructed cyclization ($\tau = 0.1$) giving $C_{sP=670} = 2.5 \times 10^{-5}$ mol/cm^3.
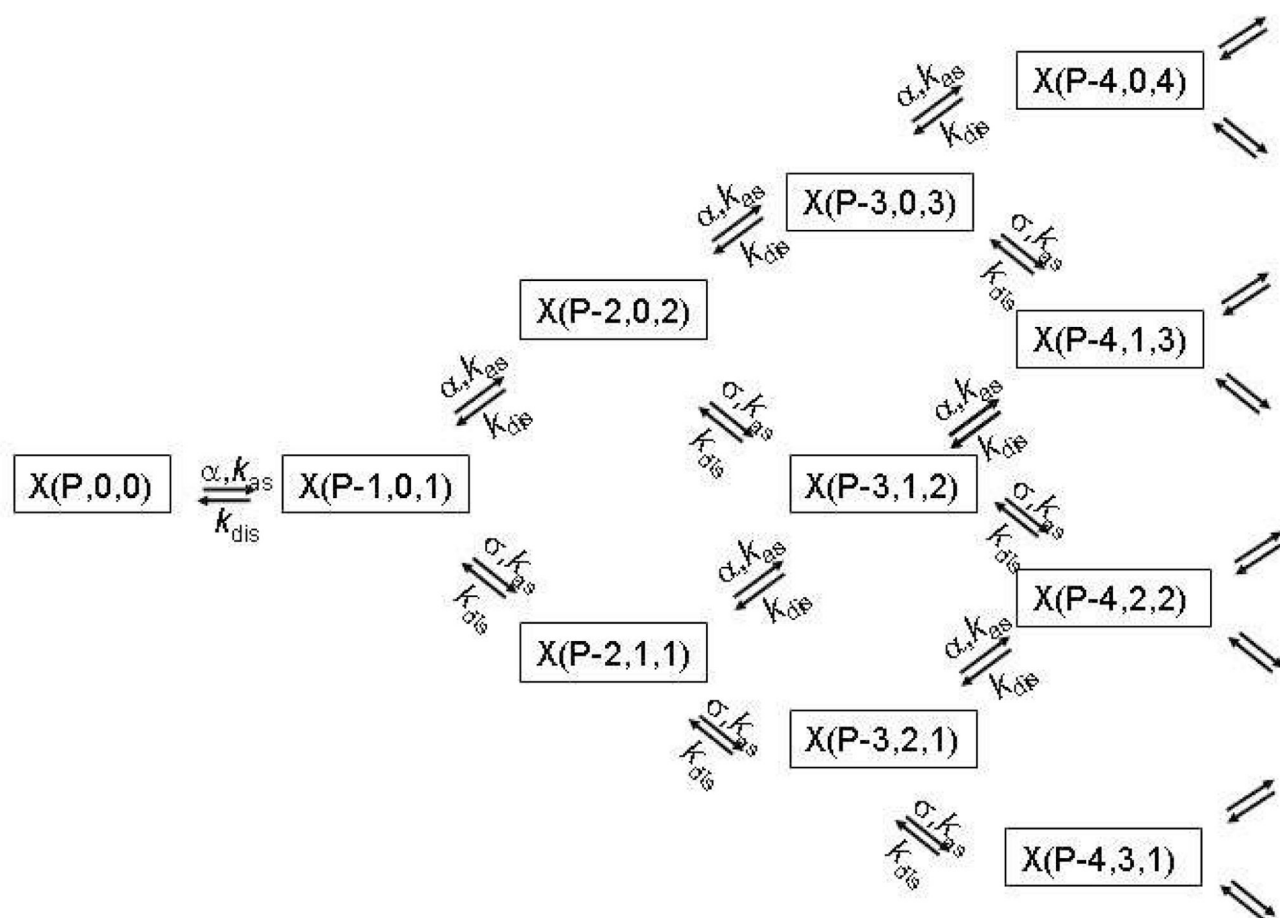
Scheme 1.
Evolution of states of building units of a monodisperse *P*-mer containing *P* motifs. The states of building units $X(\omega, \sigma, \alpha)$ are characterized by the number of motifs in ω-, σ-, and α-states; k_{as} and k_{dis} are, respectively, rate constants for association and dissociation.