Cold and Hot Denaturation of Polysoaps

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ABSTRACT: A simple phenomenological model predicting the hot and cold denaturation of polysoaps is presented. Polysoaps are flexible water-soluble polymers that incorporate, at intervals, covalently bound amphiphiles. In water, these self-assemble into intrachain micelles, thus giving rise to hierarchical self-organization. This structure is predicted to denature, to disassemble, both upon heating and upon cooling. It is possible to narrow the stability range of the intrachain structure by increasing the length of the spacer chain joining the amphiphilic monomers. A similar erect may influence the stability range of protein−polymer conjugates and certain glycosylated proteins.

I. Introduction

Proteins undergo both cold and hot denaturation.[1] The native protein unfolds to a random coil configuration upon both heating and cooling. A similar effect is expected for polysoaps. In water, at intermediate temperatures, the polysoaps self-assemble into a hierarchical intrachain structure. This structure is expected to "denature" outside the temperature stability range; *i.e.,* the self-assembled structure can be destroyed by heating as well as by cooling. In distinction to proteins, it is possible to control the width of the stability range of the "folded" state of polysoaps. In the following we present a highly simplified theoretical model of this effect. The two main results are (i) the identification of the appropriate molecular design parameter and (ii) the dependence of the stability range on it. In addition to the fundamental interest in mimicking the denaturation behavior of proteins, the effect is also of practical importance. For example, it is relevant to applications such as the control of salt deposition in water systems.[2]

Polysoaps are flexible, hydrophilic polymers that incorporate, at intervals, m covalently bound amphiphile.[3] In water, the amphiphilic monomers self-assemble into intrachain micelles. The intrachain micelles consist of two region.[4] The inner region is similar to a "free micelle" of free, unpolymerized amphiphiles. It comprises a dense core formed by the hydrophobic tails of the amphiphiles. The polar or ionic head groups straddle the core−water boundary. This region is surrounded by a corona of swollen loops formed by the spacer chains joining the aggregated amphiphiles (Figure 1). The number of intrachain micelles formed by a single polymer depends on m and on the aggregation number of the intrachain micelles at equilibrium, p_{eq}. In the following we focus on the case of long polysoaps, $m/p_{eq} \gg 1$, that form a string of *spherical* intrachain micelles. We assume that all the polymerized amphiphiles are micellized.[5] Altogether one may distinguish a three-level hierarchy in the organization of the chain. The sequence of monomers may be considered as a primary structure. The intrachain micelles define a secondary structure. For short chains, $m/p_{eq} \approx 1$, there is no higher level of organization. On the other hand, for long chains, $m/p_{eq} \gg 1$, a ternary

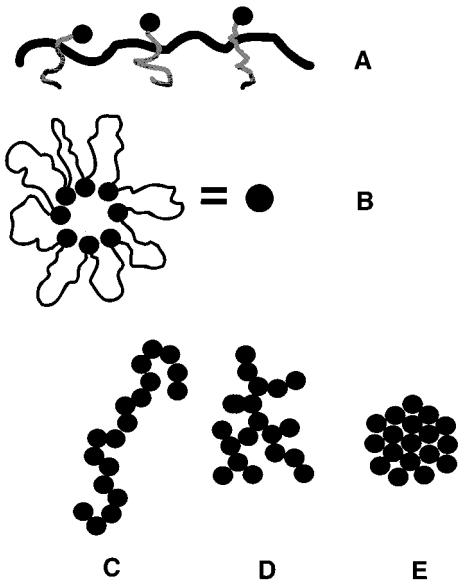

Figure 1. Polysoaps comprising of amphiphilic monomers joined by flexible, hydrophilic spacer chains exhibit a hierarchy of intrachain self-organization. The monomer sequence (A), the "primary structure", is imposed by the synthesis. The polymerized amphiphiles self-assemble into intrachain micelles (B), thus giving rise to a secondary structure. In long polysoaps a tertiary level of self-organization is associated with the configurations of the string of micelles. Among the possible configurations are linear string (C), branched string (D), and spherical globule (E).

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level of organization is associated with the configurations of the string of micelles. Three extreme scenarios may be envisioned (Figure 1): a linear string of micelles, a branched string,[4] and a spherical globule of close-packed micelles.[6] The third, globular, state is thermodynamically favored. This is because of bridging attractions due to exchange of amphiphiles between different micelles. Such exchanges result in spacer chains that bridge different micelles, thus giving rise to an attraction. Nevertheless, other "ternary" configurations may well occur as metastable states. This is because configurational rearrangements require repartitioning of the amphiphiles between different intrachain micelles. In turn, this requires a temporary expulsion of the hydrophobic tails out of the micellar cores, thus giving rise to free energy barriers.

The stability range of self-ssembled polysoaps and of free micelles formed by unpolymerized amphiphiles are closely related. The critical micelle concentration, cmc, of ionic surfactants typically exhibits a shallow but clear minimum at a temperature $\tilde{T}_m$ which depends on the surfactant and the ionic strength of the solution.[7-9] This is traceable to a shallow minimum in the transfer free energy of the hydrophobic tails from water into the micellar core, $-\delta$.[8] As we shall argue, this results in a corresponding maximum in the core−water surface tension, γ. This feature also gives rise to the hot and cold denaturation of the secondary/tertiary structure of polysoaps. The equilibrium structure of "free micelles" reflects a balance between two main contributions to the free energy per amphiphile.[10] First is the surface free energy associated with the interface between the hydrophobic core and the surrounding aqueous medium. This term favors micellar growth. Together with δ, it is also responsible for the minimum displayed by the cmc at $\tilde{T}_m$. The second contribution reflects the repulsive interactions between the head groups. This penalty term opposes micellar growth. A second penalty term comes into play in the case of intrachain micelles formed by polysoaps.[4] It is due to the crowding of the coronal chains and the consequent increase in the number of monomer−monomer contacts. The relative importance of the coronal penalty grows with n, the length of the hydrophilic spacers that join the polymerized amphiphiles. The aggregation number, p_{eq}, decreases as n increases. Eventually, when n is large enough the formation of intrachain micelles is repressed altogether. As was noted earlier, the predicted hot and cold denaturation of polysoaps is due to the surface term. The coronal penalty, on the other hand, allows for the tuning of the stability range. It shrinks as n increases.

For simplicity we limit the discussion to aqueous solutions of high ionic strength. This eliminates long-range electrostatic interactions from our considerations. The discussion is also limited to monovalent ions. In addtion, we focus on monodispersed polysoaps with well-defined n and m. Water is assumed to be an athermal good solvent for the hydrophilic chain segments. Finally, we confine the analysis to the case of ionic amphiphilic monomers that do not adsorb onto the hydrophilic segments. In section II we will summarize the phenomenological model describing intrachain micelles for a given temperature, T, and ionic strength. The T dependence of this model will be considered in section III. Two ingredients are involved. One is the free energy of the electric double layer surrounding the micelles. The second is a phenomenological T dependence of δ and γ. We will attempt to place the denaturation behavior of polysoaps in perspective in section IV, where we consider broader similarities between polysoaps and proteins. In particular, we note that a coronal contribution may allow the tuning of the stability range of protein−polymer conjugates and of certain carbohydrate-derivatized proteins.

II. Model of Intrachain Micelles

It is natural to use micelles formed by the free, unpolymerized surfactants as a reference system for intrachain micelles. Our discussion is thus based on a simple phenomenological model for free micelles.[10] Within this model the free energy per amphiphile, in a micelle with an aggregation number p, $\tilde{\epsilon}_p$, is comprised of three terms. The first is due to the interfacial free energy of the core−solvent boundary. This term favors aggregation so as to minimize the interfacial free energy per amphiphile, γa. Here γ is the core−water surface tension and a is the surface area per head group. This term is opposed by the repulsive interactions between the head groups. The number of binary contacts between one head group and its neighbors is proportional to the surface density of the head groups, $1/a$. The associated term is thus K/a, where K, for a given T and ionic strength, is a constant. The third term is the transfer free energy of the hydrophobic tail from water into the micellar core, $-\delta$. Altogether $\tilde{\epsilon}_p \approx \gamma a + K/a - \delta$ and minimization with respect to a yields $\tilde{\epsilon}_{p_o} \approx 2\gamma a_o - \delta$, where $a_o \approx (K/\gamma)^{1/2}$ is the optimal area per head group. It is thus possible to rewrite $\tilde{\epsilon}_p$ as $\tilde{\epsilon}_p \approx \gamma a_o(a/a_o + a_o/a) - \delta$. Note that within this model the configurational free energy of the hydrophobic tail is neglected.[11] For intrachain micelles it is necessary to supplement the free energy per surfactant by a fourth contribution.[4] This is a penalty term allowing for the coronal free energy per amphiphile, F_{corona}. The aggregation forces the spacer chains to fold back and form loops. Since the area per head group is rather small, the loops crowd each other. The resulting increase in the number of monomer−monomer interactions gives rise to F_{corona}. F_{corona} supplements the head group repulsion in opposing micellar growth. An intrachain micelle comprised of p polymerized surfactants is surrounded by p loops consisting each of n monomers. We mostly focus on polysoaps such that the coronal dimension, H, is large in comparison to the radius of the core, r_{core}. As a result, the overall micelle size is $r_{micelle} \approx H$. The micellar corona is then similar to a star polymer with $2p$ arms each consisting of $n/2$ monomers; *i.e.*, the corresponding star is obtained by cutting each loop in half. The characteristics of the starlike corona, dimensions, concentration profile, and free energy per arm, are well described by the Daoud−Cotton model.[12-15] F_{corona} and H, as given by this model, are[16]

$$H/b \approx p^{1/5}n^{3/5};$$
$$F_{corona}/kT \approx p^{1/2} \ln(r_{core} + H)/r_{core} \approx p^{1/2} \text{ in } n \quad (1)$$

where b is a characteristic monomer size.

The equilibrium structure of an intrachain micelle corresponds to a minimum of the free energy per polymerized surfactant[4] $\epsilon_p = \tilde{\epsilon}_p + F_{corona} \approx \gamma a_o[(a/a_o) + (a_o/a)] + F_{corona} - \delta$. To proceed it is necessary to express F_{corona} in terms of the area per head group, a, and the volume of the hydrocarbon tail, v. For a spherical micelle incorporating p amphiphiles $r_{core}^3 \approx pv$ and r_{core}^2

$\approx pa$, thus leading to $p \approx v^2/a^3$. Upon defining $p_o \approx v^2/a_o^3$ we may rewrite F_{corona} as $F_{corona}/kT \approx p_o^{1/2}(a_o/a)^{3/2} \ln n$. Introduction of the dimensionless variable $u = (a_o/a)^3 = p/p_o$ allows us to express ϵ_p as

$$\epsilon_p \approx \gamma a_o(u^{-1/3} + u^{1/3} + \kappa u^{1/2}) - \delta \tag{2}$$

Here $\kappa \approx kTp_o^{1/2} \ln n/\gamma a_o > 0$ is a dimensionless parameter measuring the relative importance of F_{corona} and the head groups repulsion for $a = a_o$. When $\kappa \ll 1$, the coronal contribution is negligible. In this case the surface free energy, $u^{-1/3}$, is comparable to the head group penalty, $u^{1/3}$. Accordingly $u_{eq} \approx 1$ and the micelles retain the characteristics, a_o and p_o, of micelles formed by free surfactants. In this limit the micellar size, $r_{micelle}/b \approx p_{eq}^{1/5}n^{3/5}$, is

$$r_{micelle}/b \approx p_o^{1/5}n^{3/5} \tag{3}$$

In the opposite limit, $\kappa \gg 1$, the coronal penalty is dominant. The driving term, $u^{-1/3}$, is comparable to the coronal penalty, $\kappa u^{1/2}$, and $u_{eq} \approx \kappa^{-6/5}$ or

$$p_{eq} \approx p_o\kappa^{-6/5} \approx p_o^{2/5}(\gamma a_o/kT \ln n)^{6/5} \tag{4}$$

The micellar size in this limit is

$$r_{micelle}/b \approx p_o^{2/25}n^{3/5}(\gamma a_o/kT \ln n)^{6/25} \tag{5}$$

For the thermodynamically stable, golobular state $R_g \approx (m/p_{eq})^{1/3}r_{micelle}$ is the span of the chain. Thus, for $\kappa \ll 1$

$$R_g/b \approx N^{1/3}n^{4/15}p_o^{-2/15} \tag{6}$$

while for $\kappa \gg 1$

$$R_g/b \approx N^{1/3}n^{4/15}p_o^{-4/75}(\gamma a_o/kT \ln n)^{-4/25} \tag{7}$$

where $N \approx nm$ is the polymerization degree of the polysoap. In the denatured state, the intrachain micelles are fully dissociated. Accordingly, the Flory radius of the denatured chain, assuming that water is an athermal good solvent for the hydrophilic backbone, is

$$R_F/b \approx N^{3/5} \tag{8}$$

In other words, the configuration of the denatured polysoap is that of a simple flexible chain.

III. Cold and Hot Denaturation of Polysoaps

The discussion presented in section II utilizes two primary features of the simple micellar model. First, the free energy per aggregated surfactant, ϵ_p, attains its minimum at the optimal area per head group, a_o. The second is the associated free energy scale, γa_o. In the $\kappa \ll 1$, only the position of the minimum of ϵ_p, $p \approx p_o$, is of relevance. On the other hand, the functional form of ϵ_p determines the precise expressions for p_{eq} and R_g in the $\kappa \gg 1$ limit. However, the qualitative behavior reflects the two features noted above. Up to this point the derivation of $\tilde{\epsilon}_p$ and the precise nature of K and γ are not important. This is no longer the case when we attempt to understand the T dependence of the intrachain self-assembly. To this end we need to know the leading T dependence of both K and γ. With this in mind we identify K/a with the electrostatic free energy per surfactant, F_E.[8] Obtaining a rigorous expression for F_E involves a solution of the appropriate Poisson−Boltzmann equation. A simpler route is available in the case considered, that of high ionic strength. F_E arises because of the electric double layer surrounding the micellar core. The characteristic length of the double layer is the Debye length, κ_D^{-1}. The total electric free energy, pF_E, is equal to the electrostatic energy of a capacitor comprising two concentric, conducting spheres of radii r_{core} and $r_{core} + \kappa_D^{-1}$. For simplicity we limit the discussion to monovalent ions and head groups. In this case the charge on the inner sphere, on the surface of the core, is $Q = \pm pe$ and $\kappa_D^{-1} = (\epsilon kT/e^2c)^{1/2}$, where e is the electronic charge, ϵ is the dielectric constant of solvent, and c is the electrolyte concentration. The potential difference between the two spheres is $V = (Q/\epsilon)[(1/r_{core}) - 1/(r_{core} + \kappa_D^{-1})] \approx Q\kappa_D^{-1}/\epsilon r_{core}^2$. The corresponding energy, $QV/2$, is thus $pF_E \approx Q^2\kappa_D^{-1}/\epsilon r_{core}^2$. Since $r_{core}^2 \approx pa$, $F_E \approx e^2\kappa_D^{-1}/\epsilon a$. Comparison to K/a yields

$$K \approx \frac{e^2\kappa_D^{-1}}{\epsilon} \approx K'(kT)^{1/2} \tag{9}$$

where $K' \sim c^{-1/2}$.

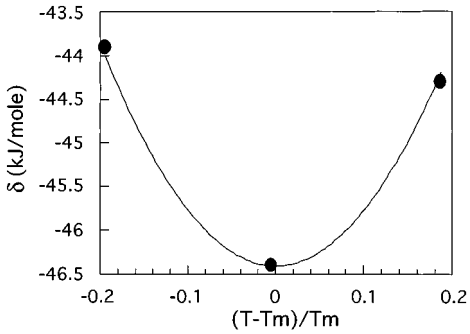

Figure 2. Plot of the transfer free energy, δ, as a function of the tempreature, T. Data for CTAB were obtained from ref 8. The minimum of $\delta(T)$ is attained at $T_m = 370$ K. The solid line represents the best fit of the data to eqs 10 and 11 with the best fit parameters of $n_m b^2\gamma_{oo} = -46$ kJ/mol and $n_m b^2\gamma' = -64$ kJ/mol.

This brings us to the T dependence of γ, the surface tension of the hydrocarbon core, and δ, the transfer free energy. A complete theoretical argument leading to the T dependence of these is not available at this point. There is a wide consensus concerning two ingredients.[17] First, the van der Waals interactions between water and apolar solutes are favorable. Second, the introduction of apolar moities perturbs the extended network of hydrogen bonds formed by the water molecules. The resulting rearrangements are associated with an entropy loss. Having said that, we will propose purely phenomenological expressions for the T dependence of γ and δ. These are proposed on the basis of the following observations: (i) The cmc of ionic surfactants typically exhibits a shallow minimum at a temperature $\tilde{T}_m$, which varies with the amphiphile and the ionic strength.[9] In turn, this is attributed to a corresponding weak minimum in $-\delta$. In the following $\delta(T)$ is obtained from the measured cmc data *via* the dressed micelle model[8] (Figure 2). Note that the minimum in $-\delta$ occurs at $T_m \neq \tilde{T}_m$. (ii) δ of unbranched hydrocarbons varies linearly with the number of methylene groups.[8,10] In view of these two points we base our phenomenological

treatment on the following two assumptions. First, δ is roughly related to γ as

$$\delta \approx \gamma b_m{}^2 n_m \tag{10}$$

where $b_m{}^2$ is the surface area of a methylene group and n_m is their number. Second, the functional form of γ is

$$\gamma \approx \gamma_{oo} - \gamma'\left(\frac{T - T_m}{T_m}\right)^2 \tag{11}$$

This amounts to an expansion of γ in powers of $(T - T_m)/T_m$, truncated at the quadratic term. γ_{oo} is the maximal value of γ, at $T = T_m$, and γ' is the coefficient of the quadratic term in the expansion. For tetradecyltrimethylamonium bromide (CTAB) $\gamma_{oo} = 0.0235$ J/m^2 and $\gamma' = 0.0326$ J/m^2. The preceding discussion suggests that γ and δ, as obtained from the "dressed micelle" model, are attributes of the hydrophobic tails alone. In reality, this is not the case. T_m varies with the size of the counterion. Furthermore, the observed trends are different for cationic and anionic amphiphiles.[9] The T dependence of K, as obtained above, cannot produce such variations. F_E does not distinguish between anioinic and cationic micelles. Nor does it allow for the size of the counterions.The observed behavior is ascribed to differences in the solvation behavior of the ions. In the framework of our highly simplified model the solvation contribution is incorporated into δ and γ, primarily *via* the experimentally observed $\tilde{T}_m$.

Having specified the T dependence of K, γ, and δ, we are now in a position to discuss the stability range of the intrachain micelles. In this case there is no involvement of the translational entropy of the amphiphiles and thus no cmc. Instead we will find critical values of T and n for which the micelle formation is repressed. We may identify these by invoking the condition $p_{eq} \approx 1$.[18] It is first necessary to incorporate (9)−(11) into the simple micelle model. As before, $a_o \approx (K/\gamma)^{1/2}$. At $T = T_m$, $a \equiv a_{oo} \approx (K'/\gamma_{oo})^{1/2}(kT_m)^{1/4}$ and thus

$$a_o \approx a_{oo}\left(\frac{T}{T_m}\right)^{1/4}\left[1 - \frac{\gamma'}{\gamma_{oo}}\left(\frac{T - T_m}{T_m}\right)^2\right]^{-1/2} \tag{12}$$

Using $p \approx v^2/a^3$ we define $p_{oo} \approx v^2/a_{oo}{}^3$ leading to

$$p_o \approx p_{oo}\left(\frac{T_m}{T}\right)^{3/4}\left[1 - \frac{\gamma'}{\gamma_{oo}}\left(\frac{T - T_m}{T_m}\right)^2\right]^{3/2} \tag{13}$$

In the limit of $\kappa \ll 1$ this allows us to obtain the range of stability by invoking $p_o \approx 1$ thus leading to

$$p_{oo}{}^{-2/3}\left(\frac{T}{T_m}\right)^{1/2} \approx 1 - \frac{\gamma'}{\gamma_{oo}}\left(\frac{T - T_m}{T_m}\right)^2 \tag{14}$$

For the case of interest, $p_{oo} \gg 1$, the denaturation temperature is specified by

$$T/T_m \approx 1 \pm (\gamma_{oo}/\gamma')^{1/2} \tag{15}$$

However, the control parameters, p_{oo}, γ', and γ_{oo}, do not vary with the architecture of the polysoap. Varying n has a negligible effect on the denaturation temperature when $\kappa \ll 1$. The situation is different in the opposite limit, of $\kappa \gg 1$, where $p_{eq} \approx p_o\kappa^{-6/5}$ as given by (4). In this case, the $p_{eq} \approx 1$ requirement leads to $\ln n \approx \gamma v^{2/3}/kT$. Defining $\ln n_{oo} \approx \gamma_{oo} v^{2/3}/kT_m$, we find

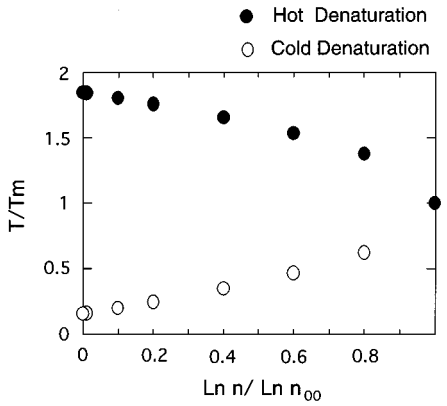

Figure 3. Plot of T/T_m *vs* $\ln n/\ln n_{oo}$. Data were obtained for γ' and γ_{oo} of CTAB (Figure 2). The filled circles indicate the hot denaturation and the open circles refer to the cold denaturation. The cold and hot denaturation temperatures are equally but oppositely displaced from T_m. The magnitude of the displacement increases as n decreases.

$$\frac{\ln n}{\ln n_{oo}}\frac{T}{T_m} \approx 1 - \frac{\gamma'}{\gamma_{oo}}\left(\frac{T - T_m}{T_m}\right)^2 \tag{16}$$

n_{oo} is the minimal length of a spacer chain that will prevent intrachain self-assembly when γ attains it maximal value, *i.e.*, at $\gamma = \gamma_{oo}$. This in turn leads to

$$\frac{T}{T_m} \approx 1 - \frac{\gamma_{oo}}{2\gamma'}\frac{\ln n}{\ln n_{oo}} \pm \frac{\gamma_{oo}}{2\gamma'}\left[\frac{4\gamma'}{\gamma_{oo}}\left(1 - \frac{\ln n}{\ln n_{oo}}\right) + \left(\frac{\ln n}{\ln n_{oo}}\right)^2\right]^{1/2} \tag{17}$$

where $\ln n < \ln n_{oo}$. The higher temperature corresponds to hot denaturation, while the lower one corresponds to cold denaturation (Figure 3). In the limit of $\gamma' \gg \gamma_{oo}$, this reduces to

$$\frac{T}{T_m} \approx 1 \pm [(1 - \ln n/\ln n_{oo})\gamma_{oo}/\gamma']^{1/2} \tag{18}$$

For $n = n_{oo}$ there is no difference between the hot and cold denaturation temperatures, $T = T_m$, since n is just large enough to destabilize the micelles. As n decreases, the hot and cold denaturation temperatures are equally but oppositely displaced from T_m.

IV. Synthetic Polymers, Proteins, and Polysoaps: A Perspective

There are important differences between the expected denaturation behavior of polysoaps and the observed behavior for proteins. For example, the denaturation of proteins can involve equilibrium intermediates between the native, folded protein and the fully unfolded state.[19] Such an intermediate state is referred to as a molten globule. It is a dense object possessing a fully developed secondary structure, α-helices, and hydrogen-bonded β-sheets, but lacking the final tertiary structure; *i.e.*, the relative positions of the atoms differ from those found in the native state. This difference is attributed to "melting" of the tight packing of the side chains of the amino acids.[1,19] This feature clearly has no counterpart in polysoaps.

In spite of the differences, the hot and cold denaturation of polysoaps resembles the corresponding effect

in proteins. As we shall discuss, this is not the only similarity between the two systems. A number of "coarse grained" features are common to both. These limited commonalties are of interest nevertheless. They suggest that polysoaps might occupy an intermediate position between two extensively studied classes of polymers: "conventional synthetic polymers" and proteins.[20] This is a noteworthy situation because the overlap between the two fields is rather small. The limited overlap reflects a number of features that distinguish proteins from typical synthetic polymers. Proteins may incorporate up to 20 chemically different monomers, *i.e.*, amino acids. By contrast, synthetic polymers are typically formed from one to three different monomer species. The amino acids undergo complicated directional interactions involving hydrophobic and electrostatic interactions as well as hydrogen bonds. While the interactions in proteins are typically highly directional, the behavior of synthetic polymers can be often rationalized in terms of isotropic contact interactions. Moreover, the chemical sequence and the molecular weight of each protein are uniquely defined. Such control is, at present, impossible for synthetic polymers. It was only attained by biological synthesis of artificial proteins.[21] The study of proteins focuses on their behavior in aqueous media or in anisotropic apolar systems, *i.e.*, lipid membranes. By contrast, synthetic polymers are often insoluble in water. Most important, flexible synthetic polymers behave as structureless random coils, while proteins fold into unique three-dimensional structures.

As we have stressed previously, the similarities between polysoaps and proteins involve "coarse grained" behavior, *i.e.*, generic features that are independent of the detailed structure of the proteins. As we shall argue later, these similarities are nevertheless useful. These include the following. First, polysoaps are expected to undergo a hierarchical intrachain self-assembly.[4,6] The resulting structure does not uniquely specify the relative positions of most monomers, as is the case in proteins. However, the formation of well-defined, organized domains is nevertheless reminiscent of the self-assembly encountered in protein. Second, in both systems the free energy surface corresponding to the configurational phase space is rugged. Proteins exhibit such ruggedness because of frustration on account of the constraints imposed by the chemical sequence.[1] In homopolysoaps, incorporating a single type of amphiphile, the ruggedness reflects kinetic barriers to interconversion between different configurations.[6] Frustration is, however, likely to play a role in the case of heteropolysoaps incorporating different and incompatible amphiphiles. Third, free amphiphiles are expected to cause an "unfolding" of the self-assembled polysoaps.[4,6] This is reminiscent of the surfactant-induced denaturation of proteins. In both systems the fully denatured state is of a chain decorated by adsorbed micelles.[22] Fourth, polysoaps form physical gels due to the formation of mixed micelles incorporating amphiphiles from different chains.[3] A similar gelation mechanism is encountered in proteins.[23,24] Fifth, the stretching elasticity of the muscle protein Titin exhibits a weak plateau at intermediate extensions.[25−27] This observation was interpreted in terms of a coexistence between folded and unfolded domains. A similar force profile was predicted for polysoaps.[28,29] In both cases the underlying physics involves unidimensional coexistence subject to a field. Finally, as discussed in this article, polysoaps, like proteins, are expected to undergo both cold and hot denaturation.

The resemblance between protein folding and micellization was already noted by Tanford.[30] As he pointed out, this similarity is superficial. This is, in part, because micelles of free, unpolymerized amphiphiles dissolve upon dilution. Clearly, proteins do not exhibit such behavior. For this reason, polysoaps, with their intrachain micelles, are better suited as a model system for proteins. In polysoaps, as in proteins, the self-assembly does not depend on the concentration because the amphiphilic moieities are incorporated into a chain. Additionally, the similarities listed above between polysoaps and proteins may be traced to the following origins. First, many amino acids have nonpolar, aliphatic side chains. The amphiphilic nature of such amino acids[30] makes for a basic similarity between proteins and polysoaps. Second, water is the principal solvent for both systems, and water structure near nonpolar groups is a major contributing factor to their self-assembly.

It is also important to note the clear differences between the two families of polymers. Proteins are dense objects. Their interior contains densely packed α-helices and the hydrogen-bonded antiparallel regions of β-sheets. With the exception of a few flexible loops observed infrequently on the protein surface,[31] there is no counterpart to the micellar corona. Second, the architecture of the polysoaps allows for complete segregation of hydrophilic and hydrophobic components in the self-assembled structures. On the other hand, the chemical sequence of proteins often enforces the "burial" of hydrophilic monomers in hydrophobic domains. Finally, the architectural richness of proteins, the number of different monomers, and their complicated interactions result in uniquely folded state. In marked contrast, the "folded" state of polysoaps is highly degenerate and far simpler.

Are the similarities between polysoaps and proteins of possible use? Can they be utilized to attain a better understanding of proteins? The usefulness of these similarities can be measured by the utility of resulting conjectures concerning proteins. Two such conjectures come to mind. The first is related to the recent results, discussed earlier, concerning the extension of Titin. As noted, the force law of both Titin and polysoaps exhibits a plateau attributed to unidimensional coexistence. The extension of polysoaps is affected by the presence of free amphiphiles. Increasing their concentration causes the width of the plateau to shrink with a corresponding increase in the associated tension. Eventually, the secondary structure is fully unfolded and the plateau disappears altogether. The force law thus exhibits features reminiscent of a critical point.[32] This suggests that a similar effect may occur upon stretching Titin, for example, in the presence of denaturing agents such as amphiphiles. Some support for this conjecture is given by experimental results. Studies of surfactant-induced unfolding of undeformed proteins exhibit similar features to those predicted for polysoaps. The hydrophobic domains unravel with increasing concentration of surfactants and this process is associated with the formation of micelles on the hydrophobic segments.[22] Certain results concerning the extension behavior of DNA may, possibly, lend further support. A plateau in the force law was also observed for stretched DNA.[33,34] The plateau was attributed, as in the case of polysoaps,

to unidimensional coexistence.[33,35] Furthermore, the plateau disappeared altogether in the presence of an excess of an intercalating agent that acts as a denaturant.[33] While this is suggestive of the conjectured scenario, it is important to note the distinctive features of this case. The self-assembly of DNA is largely driven by hydrogen bonding and by the stacking of nucleotide bases, rather than by hydrophobic interactions. Furthermore, the unidimensional coexistence invoked in order to rationaIize the DNA force law involves two different "folded" forms of DNA.

The second conjecture concerns the cold denaturation of proteins. The study of this effect is impeded by the fact that the cold denaturation often occurs below the freezing temperature of water.[36] It is thus necessary to resort to experiments involving supercooled, dust-free solutions. Alternatively, it is possible to study cold denaturation in the presence of chemical denaturants.[37−39] Our analysis of the polysoaps case suggests another approach. Namely, using the coronal penalty to narrow the thermal stability range of derivatized proteins and, in particular, to increase the cold denaturation temperature above the freezing point of water. Two alternative routes allow us to pursue this approach. The first involves artificially modified proteins. One might conjugate hydrophilic polymers with proteins, *i.e.*, by coating the protein surface with a corona of chemically grafted chains.[40] Protein−polymer conjugates based on poly(ethylene oxide) were extensively studied for medical purposes.[41] Such conjugates retain their biological activity *in vivo*, attesting to their stability at body temperature. In certain cases, high grafting densities were reported.[42] One would expect the cold denaturation of the protein−polymer conjugates to occur at higher temperatures as the coronal penalty increases, *i.e.*, as the grafting density or the molecular weight of the grafted chains increases. To our knowledge the thermal stability of protein−polymer conjugates, as compared to that of the bare proteins, has not been studied to date. The second possible avenue involves biologically produced carbohydrate-derivatized proteins. Such proteins typically carry few terminally attached hydrophilic carbohydrate chains.[43] These attached sugars are often short and branched and therefore rigid structures. However, some proteins expressed in yeast bear carbohydrate polymers comprised of between 50 and 100 monomers.[43] The highest degree of derivatization is found in glycoaminoglycans, or GAGs, consisting of a core protein domain, which is extensively glycosylated.[44] In such sytems the coronal penalty discussed in this paper is expected to be relevant. In particular, one may anticipate a narrowing of their thermal stability range as compared to that of the bare proteins.

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References and Notes

(1) See: Creighton, T. E., Ed. In *Protein Folding*; Freeman: New York, 1994.
(2) Hann, W. M.; Robertson, S. T.; Bardsely, J. H. IN *Intl. Water Conf.*, paper 93-59, Pittsburgh, 1993.
(3) (a) Bader, H.; Dorn, K.; Hupferand, B.; Ringsdorf, H. *Adv. Polym. Sci.* **1985**, *64*, 1. (b) Gros, L.; Ringsdorf, H.; Scupp, H. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 305. (c) Laschewsky, A. *Adv. Polym. Sci.* **1995**, *124*, 1. (d) Hogen-Esch, T. E.; Amis, E. *Trends Polym. Sci.* **1995**, *3*, 98. (e) Glass, J. E., Ed. In *Polymers in Aqueous Media: Performance Through Association*; American Chemical Society: Washington, DC, 1989. (f) Glass, J. E., Ed. In *Hydrophilic Polymers: Performance with Enviromental Acceptance*; American Chemical Society: Washington, DC, 1996.
(4) Borisov, O. V.; Halperin, A. *Langmuir* **1995**, *11*, 2911.
(5) This assumption is not valid for very large m's because the mixing entropy of dissociated amphiphiles and micelles favors dissociation.[29,32] The thermal stability of a single micelle is not affected by this effect. For simplicity we ignore it.
(6) Borisov, O. V.; Halperin, A. *Macromolecules* **1996**, *29*, 2612.
(7) Goddard, E. D., Ananthapadmanabhan, K. P., Eds. In *Interactions of Surfactants with Polymers and Proteins*; CRC Press: Boca Raton, FL, 1993.
(8) Evans, D. F.; Wennerstrom, H. *The Colloidal Domain*; VCH: New York, 1994.
(9) Kresheck, G. C. In *Water*; Franks, F., Ed.; Plenum: New York, 1975, Vol. 4, Chapter 2.
(10) Israelachvili, J. N. *Intramolecular and Surface Forces*, 2nd ed.; Academic Press: London, 1991.
(11) See, for example, in: *Micelles, Microemulsions, Membranes and Monolayers*; Gelbart, W., Ben-Shaul, A., Roux, D., Eds.; Springer-Verlag: New York, 1993.
(12) Daoud, M.; Cotton, J. P. *J. Phys. (Paris)* **1982**, *43*, 531.
(13) Birshtein, T. M.; Zhulina, E. B. *Polymer* **1984**, *25*, 1453.
(14) Halperin, A.; Tirrell, M.; Lodge, T. P. *Adv. Polym. Sci.* **1990**, *100*, 31.
(15) Halperin, A. In *Soft order in Physical Systems*; Bruinsma, R., Rabin, Y., Eds.; NATO ASI series, Series B, Physics; Plenum: New York, 1994; Vol. 323.
(16) Within this model the corona is envisioned as a concentric array of shells of close-packed screening blobs. Each arm contributes a single blob to each shell. Thus, a shell of radius r and area r^2 consists of $2p$ blobs of cross section ξ^2 thus leading to $\xi \approx r/p^{1/2}$. Since $\xi \sim \phi^{3/4}$ the concentration profile is $\phi \sim r^{-4/3}$. H is determined by the requirement of monomer conservation $n \approx \int_{R\mathrm{core}}^{R\mathrm{core}+H}(\xi/b)^{5/3}\xi^{-1}\,\mathrm{d}r$, where $(\xi/b)^{5/3} \approx g$ is the number of monomers in a blob of size ξ and b is a typical monomer size. The free energy per arm, as given by the kT per blob ansatz, is $F_{\mathrm{corona}}/kT \approx \int_{R\mathrm{core}}^{R\mathrm{core}+H}\xi^{-1}\,\mathrm{d}r$. F_{corona} and H are thus specified by (1).
(17) Blokzijl, W.; Engberts, J. B. F. N. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1545.
(18) One may use different criteria. The choice does not affect the qualitative features of the predicted behavior. Since we use scaling arguments, $p_{\mathrm{eq}} \approx 1$ corresponds to micelles having a small aggregation number. It is indicative of the approach of full dissociation.
(19) Bychkova, V. E.; Ptitsin, O. *Chemtracts Biochem. Mol. Biol.* **1993**, *4*, 133.
(20) Random copolymers may also be considered as occupying such an intermediate position. However, this system is closer to proteins than to homopolymers and its description is, accordingly, more difficult. The theoretical description of random copolymers as model proteins is reviewed by: Karplus, M.; Shakhnovich, E. In *Protein Folding*; Creighton, T. E., Ed.; Freeman: New York, 1994.
(21) Tirrell, J. G.; Tirrell, D. A. *Curr. Opinion Solid State Mater. Sci.* **1996**, 1, 407.
(22) Turro, N. J.; Lei, X.-G.; Ananthapadmanabhan, K. P.; Aronson, M. *Langmnir* **1995**, *11*, 2525.
(23) Ross-Murphy, S. B., Burchard, W., Eds. In *Physical Networks—Polymers and Gels*; Elsevier Applied Science: London, 1990.
(24) Djabourov, M. *Contemp. Phys.* **1988**, *29*, 273.
(25) Kellermayer, M. S. Z.; Smith, S. B.; Granzier, H. L.; Bustamante, C. *Science* **1997**, *276*, 1112.
(26) Rief, M.; Gautel, M.; Oesterhelt, F.; Fernandez, J. M.; Gaub, H. *Science* **1997**, *276*, 1109.
(27) Tskhovrebova, L.; Trinick, J.; Sleep, J. A.; Simmons, R. M. *Nature* **1997**, *387*, 308.
(28) Borisov, O. V.; Halperin, A. *Europhys. Lett.* **1996**, *34*, 657.
(29) Borisov, O. V.; Halperin, A. *Macromolecules* **1997**, *30*, 4432.
(30) Tanford, C. *The Hydrophobic Effect*, 2nd ed.; Wiley: New York, 1980.
(31) Creighton, T. *Proteins*, 2nd ed.; W. H. Freeman: New York, 1993.
(32) Borisov, O. V.; Halperin, A. *Phys. Rev. E* **1998**, *57*, 812.
(33) Cluzel, P.; Lebrun, A.; Heller, C.; Lavery, R.; Viovy, J.-L.; Chatenay, D.; Caron, F. *Science* **1996**, *271*, 792.

(34) Smith, S. B.; Cui, Y.; Bustamante, C. *Science* **1996**, *271*, 795.
(35) Marko, J. F. *Phys. Rev. E* **1997**, *55*, 1758.
(36) Privalov, P. L.; Griko, Y. V.; Venyaminov, S. Yu.; Kutyshenko, V. P. *J. Mol. Biol.* **1986**, *190*, 487.
(37) Griko, Y. V.; Privalov, P. L. *Biochemistry* **1992**, *31*, 8810.
(38) Chen, B. L.; Schellman, J. A. *Biochemistry* **1989**, *28*, 685.
(39) Privalov, P. L. *Crit. Rev. Biochem. Mol. Biol.* **1990**, *25*, 281.
(40) Harris, J. M., Ed. In *Poly(ethylene glycol) Chemistry*; Plenum Press: New York, 1992.
(41) Katre, N. V. *Adv. Drug Delivery Rev.* **1993**, *10*, 91.
(42) Keating, M. J.; Holmes, R.; Lerner, S.; Ho, D. H. L *Leuk. Lymphoma* **1993**, *10*, 153.
(43) Fukuda, M., Hindsgaul, O., Eds. *Molecular Glycobiology*; ILR Press: New York, 1994.
(44) Alberts, B.; Bray, D.; Lewis, J.; Raff, M.; Roberts, K.; Watson, J. *The Molecular Biology of the Cell*; Garland Publishing: New York, 1983.

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