Cold and Warm Denaturation of Hydrophobic Polymers.

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We introduce a polymer model where the transition from swollen to compact configurations is due to interactions between the monomers and the solvent. These interactions are the origin of the effective attractive interactions between hydrophobic amminoacids in proteins. We find that in the low and high temperature phases polymers are swollen, and there is an intermediate phase where the most favorable configurations are compact. We argue that such a model captures in a single framework both the cold and the warm denaturation experimentally detected for proteins. Some consequences for protein folding are discussed.

Modeling polymers and polymer collapse, beyond being a challenge of great theoretical interest, is extremely important for many different applications\textsuperscript{1}. In particular, in connection to the protein folding problem, the collapse of polymers has gained a special status in statistical physics. The simplest models of proteins describe them as polymers whose building monomers (amminoacids) can be of two different types, either hydrophobic (H) or polar (P) (such a simplification was introduced in the so-called HP model\textsuperscript{2}). The protein folds through attractive H-H interactions. (Homo)Polymers with attractive monomer-monomer interactions are known to undergo a phase transition from a high-temperature swollen phase to a low-temperature compact one. The presence of attractive interactions between hydrophobic amminoacids justifies then the collapse of the protein below the folding temperature. In particular, trying to maximize the number of H-H contacts, most hydrophobic monomers will be found in the core of the protein, in agreement with experimental observations\textsuperscript{3}.

It is important to understand the origin of the attractive H-H interaction: as their name suggests, hydrophobic amminoacids do not like being in contact with water. As a consequence, hydrophobic amminoacids in solution tend to aggregate in order to minimize the area exposed to water. This tendency to aggregate can be modeled as an attractive interaction. In proteins, hydrophobic amminoacids try to hide away from water, burying themselves in the core of the protein (whose surface is mainly made of polar, hydrophilic amminoacids).

At temperatures much lower than the folding transition, the HP model (and models derived from it) predicts that proteins stay in a compact state, with a greater and greater probability to be in their ground state. This principle has also been used to classify different amminoacid sequences as good or bad folders (from a thermodynamical point of view) according to e.g. the gap between the ground state and the first excited states, or the uniqueness of the ground state on a compact configuration\textsuperscript{4}.

Recently, nonetheless, there has been a growing evidence for the so-called cold denaturation of proteins: at low temperatures proteins such as β-Lactoglobulin A, myoglobin, apomyoglobin, Ribonuclease A, and Escherichia Coli’s HPr\textsuperscript{5} unfold to a swollen configuration\textsuperscript{6}. Cold denaturation is assumed to be a general property of all globular proteins\textsuperscript{7}. These experimental findings are clearly incompatible with the predictions of the HP model.

In this Letter we propose a mechanism driving the collapse of a polymer, mimicking the nature of the hydrophobic effect. Furthermore, we find within a single framework both a cold and a warm collapse transitions, in agreement with experimental observations for hydrophobic amminoacids. The model is built on the present understanding of the microscopic organization of the water/hydrophobic molecules system.

Hydrophobic molecules are essentially non-polar entities, weakly, if at all, interacting with water molecules. Therefore, there is no true repulsion between water and hydrophobic molecules. We can get an insight in the energetics of water/hydrophobic molecules systems from experiments. In the case of pentane\textsuperscript{8} (hydrocarbons are in general hydrophobic), one finds an enthalpy of transfer $\Delta H_{a\to s}$ from the aggregate phase to the aqueous solution phase that is strongly temperature dependent: at low temperatures $\Delta H_{a\to s} < 0$, and at high temperatures $\Delta H_{a\to s} > 0$, showing that at low temperatures the solution phase is energetically more favourable than the aggregate one; at high temperatures the situation reverses.

Microscopically, a hydrophobic molecule in solution displaces water molecules that, at a low enough temperature, build an ice-like cage around the hydrophobic molecule, giving origin to a structure that is energetically more favorable than bulk, liquid water\textsuperscript{9}. This energetic gain gives the major contribution to the minimization of the free energy, and as a consequence the system tries to maximize the number of cages by exposing as many hydrophobic molecules as possible, hence the cold denaturation of proteins and $\Delta H_{a\to s} < 0$. At higher temperatures water around hydrophobic molecules can no more
form the cages, but due to the steric constraints imposed by the molecule, they cannot even fully exploit the energetically favorable hydrogen bonding of bulk, liquid water. As a result, disordered water molecules around hydrophobic amminoacids are energetically less favorable than bulk water ($\Delta H_{\text{m-water}} > 0$): proteins try therefore to hide their hydrophobic parts in their core. This is what is usually referred to as the hydrophobic effect, and it is the consequence of a complex interplay between energy minimization and entropy maximization. Unfortunately, the detailed behavior of water at low temperatures is still far from being well understood from a microscopic point of view; the nature of the hydrogen bond itself is still a matter of controversy and of deep investigations. Therefore it is still not possible to give a detailed description of the hydrophobic amminoacid-water organization and of the energies associated to the cages and to the disordered configurations.

We model the polymer as a self-avoiding walk (SAW) on a lattice. In every lattice site (except those occupied by the polymer) there is a Potts variable with $q$ states (labelled for convenience from 0 to $q - 1$), representing a group of water molecules in $q$ different collective states. We associate the state $q = 0$ to the cage configuration, energetically favorable when water is in contact with the polymer, and the remaining $q - 1$ states to disordered, unfavorable, configurations. The Hamiltonian of the system is

$$H = \sum_{i=1}^{N} \sum_{j,n,i} \epsilon \left( - J \delta_{s_i,0} + K (1 - \delta_{s_i,0}) \right).$$

(1)

The first sum runs over the $N$ monomers of the polymer. The second primed sum runs over the water occupied nearest neighbors of each monomer. The interaction constants $J$ and $K$ (both positive) represent respectively the energies of the cage configurations and of the disordered ones with respect to bulk water. There are no monomer-monomer interactions.

Some kind of water-protein interaction with a similar description of the water degrees of freedom was already proposed in [10] with the same motivations. Yet, in that model, the water-protein interaction was introduced in a “mean field” fashion, and moreover protein folding was described by collective hierarchical variables hiding the microscopic description, that we believe fundamental to give qualitative, but also quantitative, predictions.

Starting from (1) we can write the partition function of the system as

$$Z_N = \sum_C Z_N(C)$$

(2)

where $Z_N(C)$ is the partition function associated to a single configuration $C$. It is important to observe that the maximum number of water sites in contact with the polymer is $M = 2(d - 1)N + 2$ for a hypercubic lattice in $d$ dimensions. For general polymer configurations the number of contacts is smaller than $M$. Nonetheless, all of these $M$ water sites must be taken into account in order to give the correct weight to all the $Z_N(C)$’s. Moreover, the way Hamiltonian (1) has been written implies that a single water site can be counted more than once. It is then possible to write a fairly simple expression for the configuration partition function:

$$Z_N(C) = q^{n_0(C)} \prod_{l=1}^{z} Y_l(C)$$

(3)

with

$$Y_l(C) = \left( (q - 1) e^{-\beta K} + e^{\beta J} \right)^{n_l(C)}$$

(4)

where $n_l(C)$ is the number of water sites with $l$ polymer contacts and $z$ is the coordination number of the lattice. As usual, $\beta = 1/k_B T$ and we take $k_B = 1$. Analogously, it is possible to write similar expressions (even though a little more complicated) for the internal energy $U(C)$ of a configuration (and then calculate the internal energy $U = \sum_C U(C)$).

We analyze the thermodynamic behavior of the system in $d = 2$ by means of exact enumeration techniques on the Manhattan lattice for polymers of length up to $N = 25$ monomers. The Manhattan lattice is a two-dimensional lattice on which rows (columns) are alternately left/right (up/down) oriented. The physics of polymer collapse in the well-known case of attractive monomer-monomer interactions is the same on the Manhattan lattice as on regular two-dimensional lattices (there is still some debate on non-universalities in the values of some exponents [11][12]). We are therefore confident that our exact enumeration on the Manhattan lattice describes the correct behavior of the polymer on regular Euclidean lattices.

We calculated the specific heat per monomer of the system as the derivative of $U$ with respect to the temperature $T$, see Fig.1. We used $K/J = 2$ and $q = 16000$ (both $K$ and the temperature can be normalized with respect to $J$). In Fig.1 two peaks of the specific heat appear, corresponding to temperatures $T_C$ and $T_W$. By direct inspection of the values of the configuration partition function in eq. (3), we find that below $T_C$ the most probable configurations are swollen, maximizing the number of water-polymer contacts. Between $T_C$ and $T_W$ the polymer collapses, and the most probable configurations are the compact ones (in the particular cases of $N = 16, 25$ we verified that they correspond to square configurations). Finally, for $T > T_W$ the weight of compact and swollen configurations becomes comparable and the warm denaturation takes place.

A number of questions arise for this problem: as it can be seen from Fig.1 the low-temperature peak in the specific heat is stable against the length of the polymer, a
clear indication of a true phase transition. On the contrary, the high-temperature specific heat peak seems to flatten as the length of the polymer is increased, indicating that the high temperature denaturation transition could disappear as the length of the polymer increases. This scenario needs further investigations. Moreover, the behavior of the two peaks as a function of $K/J$ is such that they coalesce when $K/J = 1$. The height of the warm denaturation peak depends also on $q$, increasing as $q$ increases, as shown in Fig.2 (as a consequence, the flattening of the warm denaturation peak depends on the interplay between $q$ and $N$). Also the cold and warm denaturation temperatures depend on $q$. In the inset in Fig.2 we temptatively show that the dependence of $T_W$ on $q$ follows a power-law: the best fit, quite stable adding points on both extrema ($q$ small and large) is $T_W \simeq 0.22 + 1.47q^{-0.17}$.

Hydrophobic amminoacids are modeled through an effective mutual attractive interaction. In this way the solvent degrees of freedom can be neglected. Here we show that the Hamiltonian in eq.(1) and the corresponding configuration partition function in eq.(3) give rise to an effective monomer-monomer attractive interaction. Let us consider the two polymer configurations $C$ and $C'$ represented in Fig.3. Configuration $C'$ is characterized by having a monomer-monomer contact, as opposed to configuration $C$. If we forget about the solvent, and we attribute the difference of the two partition functions $Z(C)$ and $Z(C')$ to a monomer-monomer interaction energy $\epsilon$, we can write

$$\epsilon = -\frac{1}{\beta} \ln \frac{Z(C')}{Z(C)} \quad \text{(5)}$$

The energy $\epsilon$ is represented in Fig.3 as a function of the temperature. The low and high temperature limits can be easily computed as

$$\epsilon \simeq 2J \quad T \rightarrow 0$$
$$\epsilon \simeq -2 \left( \frac{K - J + K}{q} \right) \quad T \rightarrow \infty \quad \text{(6)}$$

The effective monomer-monomer interaction energy is therefore repulsive for small temperatures, and attractive for high temperatures, mimicking an effective attractive H-H interaction. Yet, through this model, it is extremely clear that the hydrophobic attractive interaction is present only when hydrophobic monomers are in contact with water. We can picture a situation where only a few monomers are hydrophobic. In this case they try to hide in the core of some globule. Within the core there are no more interactions between them, if not residual ones such as Van der Waals forces. As a consequence, in the core of a protein, the hydrophobic interaction can not justify the maximization of the number of contacts, neither it can discriminate between the native state of the protein and the molten globule state, where the hydrophobic protein core is believed to be in an amorphous state.

The absence of interactions in the core of the collapsed polymer is the cause of the weakness of the warm denaturation transition: for a polymer of $N$ monomers, the energy of compact configurations grows like $N^{1/2}$, since it is just a perimeter effect. On the contrary, we can expect an exponential number of ways to open up the compact polymer, and therefore the entropy grows like $N$. On larger and larger length scales the entropic term dominates and compact configurations are not stable anymore. On the contrary, cold denaturation is a true transition: again the entropy grows like $N$, but in this case the energy is extensive too.

In conclusion, we have introduced a model Hamiltonian for the collapse of a polymer interacting with the solvent, but without monomer-monomer interactions. Such a model mimics (in an extremely simplified way) the behavior of hydrophobic polymers in water: as a result we find the presence of both a cold and a warm denaturation temperatures, as experimentally found for proteins (whose folding is believed to be induced by hydrophobic interactions). Between $T_W$ and $T_C$ the polymer collapses to compact configurations. Indeed the model tries to capture some of the essential physics of the water-hydrophobic amminoacids interaction at a microscopic level, and as byproduct (necessary for consistency), it justifies the presence of an effective attractive H-H interaction at high temperatures. Yet, this model rises some questions about the reliability of using an attractive H-H interaction also in the core of proteins (where water is absent) at odds with models presently in use. This model further shows that the solvent degrees of freedom can, and should, be taken into account in the formulation of protein models, at least in a simplified way. In particular it calls for a much better understanding of the physics of water around hydrophobic amminoacids. We are presently working on larger computer simulations of this model for polymers embedded in lattice different from the Manhattan one. We are also considering slight modifications of this prototype model to take into account a better description of the water degrees of freedom and of their energetics. Nevertheless, we believe to have pointed out the essential ingredients to describe in a single framework the processes of cold and warm denaturation.

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![FIG. 1. Specific heat of the system for different polymer lengths $N = 16,20,25$. Here $K/J = 2$ and $q = 16000$. The cold and warm denaturation temperatures, $T_C$ and $T_W$, are marked with arrows.](image1)

![FIG. 2. Specific heat vs. temperature for $K/J = 4$ and different values of $q$, namely $q = 2^n \cdot 1000$ with $n = 0, \ldots, 8$, and $N = 16$. In the inset the cold denaturation temperatures (+) are shown vs. $q$ (they have already been shifted by the constant term 0.22); the solid line is the fit $T_W - 0.22 \approx 1.47q^{-0.17}$.](image2)

![FIG. 3. Two different configurations differing only for a monomer-monomer contact. These configurations are used to calculate $\epsilon$ as in Fig.3. The water sites that changed their number of contacts from $C$ to $C'$ are indicated with their number of contacts.](image3)
FIG. 4. Values of the effective monomer-monomer interaction energy as computed from (5) and the configurations in Fig.2. The ratio $K/J = 2$ and the values of $q = 16000, 2000, 1000, 100, 50, 10$ have been used. The transition between repulsive and attractive energies becomes more and more abrupt as $q$ increases.