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# Cold and warm swelling of hydrophobic polymers

Paolo De Los Rios[1] and Guido Caldarelli[2]
[1]Institut de Physique Théorique, Université de Lausanne, 1015 Lausanne, Switzerland
[2]INFM Sezione di Roma 1, Dipartimento di Fisica, Università La Sapienza, Piazzale Aldo Moro 2, 00185 Roma, Italy

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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 amino acids 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 thermosensitive polymers and for proteins.

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Modeling polymers and polymer collapse, beyond being a challenge of great theoretical interest, is extremely important for many different applications [1]. In particular, in connection with the protein folding problem, the collapse of polymers has gained a special status in statistical physics [2]. Existing models of proteins are based on the golden rule that the driving force of protein folding and of protein stability is hydrophobicity [2,3]: it has been observed that most of the hydrophobic amino acids of globular proteins in their native conformation are sheltered in the core, hidden from water by a shell of polar amino acids. Such behavior is consistent with the well known general tendency of hydrophobic molecules in water to aggregate to reduce as much as possible their contact surface with water.

Usually, hydrophobicity is modeled through attractive effective interactions between hydrophobic molecules, which are a reminder of the free energy difference between solute and aggregate states. The latters are more stable and are therefore characterized by a lower free energy that can be ascribed to an effective attraction between molecules (averaging over the water degrees of freedom). The phase diagram of polymers with attractive monomer-monomer interactions has been extensively reported in the literature (see [1] Chap. 8, and references therein), with a high temperature phase where polymers are swollen, well described as self-avoiding walks (SAWs), and a low temperature phase where polymers are compact. Yet it is known from experiments that the free energy difference $\Delta F$ between solute and aggregate states of hydrophobic molecules is not a monotonically increasing function as temperature is lowered. Instead, $\Delta F$ reaches a maximum at some temperature $T_m$ below which it begins to decrease [4,5]. A clear example of this behavior is represented by thermosensitive polymers, such as poly ($N$-isopropylacrylamide) (used in hydrogels), which are swollen at low temperatures and collapse on heating [6]. Such a behavior is also typical of globular proteins, which in some cases have even been seen to denature at low temperatures (*cold denaturation*) [7]. This rich phenomenology is clearly incompatible with the simple modeling of hydrophobicity as an attractive interaction, but points to the complexity of the physics of water around hydrophobic molecules.

Recently, some new understanding of hydrophobicity both at an effective and at a molecular level is emerging (although the main ideas date back even to 1945 [8]). Water around hydrophobic molecules can organize in so called clathrate structures: icelike cages that are energetically favorable with respect to bulk liquid water because of an increased degree of hydrogen bonding. Since there are a few ordered cage configurations, an entropy loss is associated with them. Water molecules can be in disordered configurations as well, with a reversed entropic and energetic balance with respect to the cage configurations. At an effective level this behavior has been captured by Muller and by Lee and Graziano (MLG) with a bimodal description of the energy of water in the shell around hydrophobic molecules [9,10]. The MLG model is better described pictorially [see Fig. 1(a)]. It is characterized by eight parameters (namely, the four energies $E_{ob}, E_{db}, E_{os}, E_{ds}$, and the four degenerations $q_{ob}, q_{db}, q_{os}, q_{ds}$) that can be obtained phenomenologically, by fitting the model to experimental data (actually, it is possible to choose one energy and one degeneration as references, so that the true number of parameters can be reduced

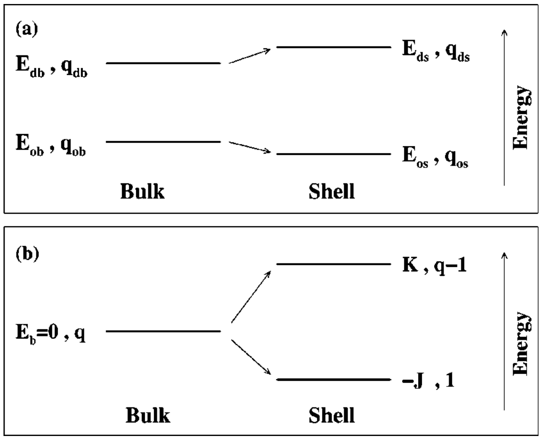

FIG. 1. Bimodal effective models. Panel (a): MLG model, with bimodal energy distributions for both bulk and shell water molecules. The lower levels represent ordered groups of water molecules, the higher levels disordered ones. The order of energies and of degeneracies, as obtained from experiments, is $E_{ds} > E_{db} > E_{ob} > E_{os}$ and $q_{ds} > q_{db} > q_{ob} > q_{os}$ ($ds$ = disordered shell, $os$ = ordered shell, $db$ = disordered bulk, $ob$ = ordered bulk). Panel (b): the simplified bimodal energy distribution, with just three free parameters.

to six). Interestingly, such a bimodal description was also derived from a molecular model of water by Silverstein, Haymet, and Dill [11]. From a theoretical model point of view, the MLG model has too many free parameters. Without loss of generality, we reduce the number of parameters to three ($J$, $K$, and $q$), using the model shown in Fig. 1(b), where there is no longer a gap between the bulk states and both bulk and shell water molecules are characterized by a single degeneration parameter $q$.

Recently some models trying to describe cold denaturation have been proposed, and indeed the solvent has to be taken into account somehow [12]; yet in such models monomers do not appear explicitly, rather they are described as a set of hierarchical variables. Here we model the polymer conformations as self-avoiding walks on a lattice. All monomers are hydrophobic. On every lattice site (except those occupied by the polymer) there is a Potts variable with $q$ states (labeled for convenience from 0 to $q-1$), representing a group of water molecules in $q$ different collective states. We associate the state $s=0$ with the cage configuration, energetically favorable when water is in contact with the polymer, and the remaining $s=1,\ldots,q-1$ states with disordered, unfavorable, configurations. The Hamiltonian of the system is

$$H=\sum_{\langle j,H\rangle}[-J\delta_{s_j,0}+K(1-\delta_{s_j,0})]. \tag{1}$$

The sum runs over the water sites that are nearest neighbors of some hydrophobic ($H$) monomer. There are no monomer-monomer interactions.

Starting from Eq. (1) we can write the partition function of the system as $Z_N=\Sigma_C Z_N(C)$, where $Z_N(C)$ is the partition function associated with 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 a general polymer configuration, instead, 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. It is then possible to write a fairly simple expression for the configuration partition function:

$$Z_N(C)=q^{n_0(C)}[(q-1)e^{-\beta K}+e^{\beta J}]^{n_1(C)}, \tag{2}$$

where $n_0(C)$ is the number of bulk water sites and $n_1(C)$ is the number of shell water sites (shell meaning in contact with a monomer); $z$ is the coordination number of the lattice. As usual, $\beta=1/k_BT$ and we take $k_B=1$.

We analyze the thermodynamic behavior of the system in $d=2$ by means of exact enumeration techniques on the square lattice for polymers of length up to $N=25$ monomers (corresponding to 5 768 299 665 distinct configurations). A finite-size scaling analysis is difficult for such short chains; yet it is possible to classify the polymer configurations according to $n_1$ (the perimeter): for SAWs of $N$ monomers, the fraction of configurations of perimeter $n_1$ is well approximated by a Poisson distribution of the form

$$P_N(n_1)\sim e^{-\delta(N-1)}\frac{[\delta(N-1)]^{2N+2-n_1}}{(2N+2-n_1)!} \tag{3}$$

with $\delta\sim0.75$. Analogous results are not new in SAW physics: a Poisson distribution of the SAW configurations as a function of the number of monomer-monomer contacts was already found in [13] (and indeed the perimeter $n_1$ and number of monomer-monomer contacts are somehow related, since each monomer-monomer contact reduces the perimeter by two units). The smallest allowed value of $n_1$ in Eq. (3) is of the order of $n_{1,min}\sim2\sqrt{\pi}\sqrt{N}$, assuming that the smallest perimeter is attained by compact circular configurations. Using Eq. (3) we have been able to extend our study up to $N\sim100$, so that a proper finite-size scaling analysis is possible [14].

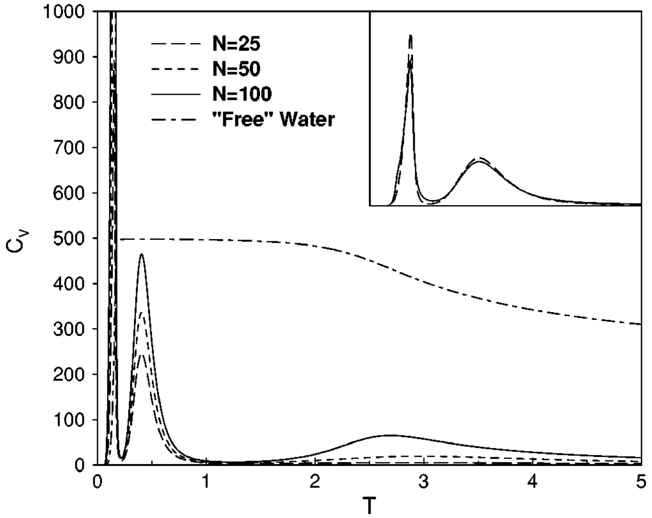

FIG. 2. Heat capacity for polymer lengths $N=25,50,100$ using the extrapolated Poisson distribution. Here $K/J=2$ and $q=1000$. The dashed line represents the average number of water sites that are not in contact with the polymer. In the inset, we plot the heat capacity for $N=25$ from exact enumeration (solid line) and using the Poisson distribution (dashed line).

We calculated the specific heat of the system as $C_v=dU/dT$, where $U$ is the internal energy of the system. We show the results for $K/J=2$ and $q=1000$ (both $K$ and the temperature can be normalized with respect to $J$). The behavior of the system does not change much for different $K/J$ ratios and values of $q$. First, we checked that the specific heat for a polymer of $N=25$ monomers does not change significantly using either the exact enumeration data, or the Poisson distribution Eq. (3) (see the inset of Fig. 2). We are therefore confident that Eq. (3) captures the correct physics of the problem, and in Fig. 2 we show the heat capacity for $N=25,50,100$, and the number of water sites that are not in contact with the polymer (we recall that their maximum number is $2N+2$). Three peaks of the specific heat appear. The one at low temperature corresponds to an increase of the number of ''free'' water sites: correspondingly, we identify it with a collapse transition, where the polymer goes from a stretched configuration to a compact one, reducing its perimeter. As can be seen from Fig. 3(a) the low temperature peak

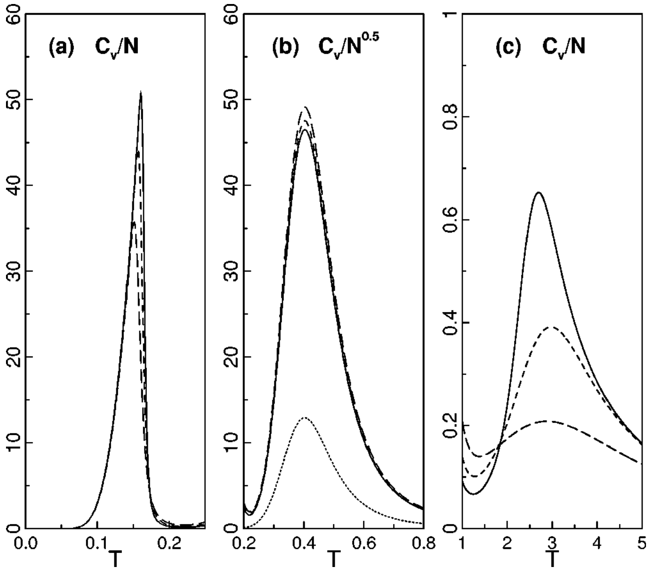

FIG. 3. (a) Specific heat for the low temperature peak of Fig. 2: the peak height is slightly diverging as $N$ increases, suggesting a first order, or weak second order, transition. (b) Collapse of the intermediate temperature heat capacity peak in Fig. 2: the proportionality to $\sqrt{N}$ is evident, as well as the proportionality to the single perimeter site specific heat. (c) Monomer specific heat for the high temperature peak in Fig. 2: the peak height is diverging for increasing $N$, suggesting a second order phase transition, as found for the $\theta$-point model. The symbols are as in Fig. 2.

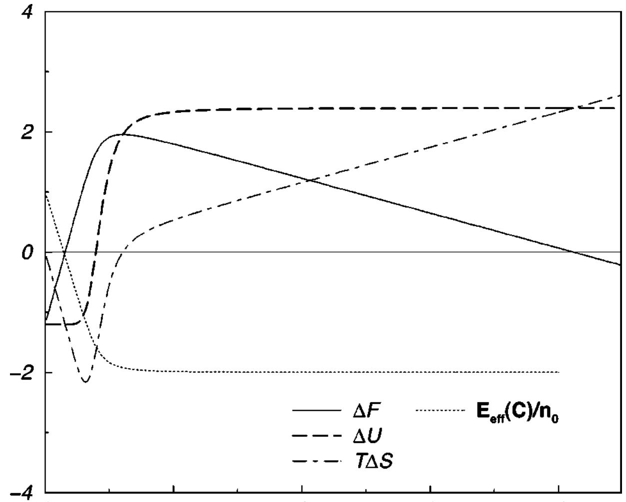

FIG. 4. Free energy, enthalpy, and entropy of unfolding from Eq. (4). We have chosen $q=1000$ and the connective constants $\mu_{SAW}$ and $\mu_{HW}$ for a two-dimensional square lattice [1], and $K/J=2$. The effective energy per ''free'' water site is also shown.

grows as fast as or even faster than $N$, a clear indication of its phase transition nature. The intermediate peak, instead, scales as $\sqrt{N}$ [see Fig. 3(b)]: it disappears in the thermodynamic limit. Moreover, it is easily seen [see the dotted line in Fig. 3b)] that it corresponds to the specific heat peak of the single perimeter water site: the peak height is proportional to $\sqrt{N}$ because it is proportional to the perimeter of the collapsed polymer. The polymer reopens at the third peak of the heat capacity, and the height of this peak grows faster than $N$ [see Fig. 3(c)]: we believe that this transition corresponds to the usual $\theta$-model transition of self-interacting polymers (a true grand-canonical treatment of the present model is under study) [1].

A further important test for the model introduced here is a (rough) determination of $\Delta F$, the free energy difference between swollen and compact states of the polymer. Actually, the definitions of compact and swollen states are poor, so we make a simplifying assumption: compact states are described by Hamiltonian walks (HW; Hamiltonian walks pass through all the sites of the lattice) and swollen states by SAWs. Moreover, we assume that the typical partition function for SAWs is given by Eq. (2) with an average number of water-polymer contacts $\tilde{n}_1 \sim 1.2N$ as obtained from exact enumeration, and that the number of water sites in contact with a HW of $N$ monomers is proportional to $\sqrt{N}$, the perimeter of compact conformations. With these approximations, we can write the free energies per monomer for both HWs and SAWs in the thermodynamic limit [which suppresses the perimeter term; see also the finite-size scaling discussion above and Fig. 3(b)] as

$$f_{HW} = -2T\ln q - T\ln \mu_{HW},$$

$$\begin{aligned} f_{SAW} = {} & -T\tilde{n}_1 \ln[(q-1)e^{-\beta K} + e^{\beta J}] \\ & -T(2-\tilde{n}_1)\ln q - T\ln \mu_{SAW}, \end{aligned} \tag{4}$$

from which the free energy difference follows as

$$\begin{aligned} \Delta f = f_{SAW} - f_{HW} = {} & -2T\tilde{n}_1 \ln\left(\frac{(q-1)e^{-\beta K} + e^{\beta J}}{q}\right) \\ & -T\ln\frac{\mu_{SAW}}{\mu_{HW}}, \end{aligned} \tag{5}$$

where $\mu_{SAW} \sim 2.63$ and $\mu_{HW} \sim 1.47$ are the connecting constants of SAWs and HWs on the square lattice, obtained from exact enumerations and mean-field treatments, respectively (the number of SAW and HW configurations of length $N$ scales as $\mu_{SAW}^N$ and $\mu_{HW}^N$, respectively) [1]. From Eq. (5) we can also derive the denaturation enthalpy $\Delta h$ and entropy $\Delta s$. They are shown in Fig. 4. As can be seen, $\Delta f$ is negative at both low and high temperatures, consistent with cold and warm denaturation. The full behaviors of $\Delta h$ and $T\Delta s$ qualitatively reproduced in Fig. 4 are typical of the calorimetry of hydrophobic molecules and proteins.

As we already recalled, the hydrophobic interaction is usually modeled through an effective attractive interaction, giving raise to the well-known $\theta$ model. In this way the solvent degrees of freedom can be traced out. Here we show that the Hamiltonian (1) and the corresponding configuration partition function (2) give rise to an effective interaction that is close to, yet not identical to, a monomer-monomer interaction. Indeed, assuming that the configuration partition function $Z(C)$ is given by a Boltzmann weight $\exp[-\beta E_{eff}(C)]$, we can write

$$E_{eff}(C) = -\frac{1}{\beta}\ln Z(C) = \frac{1}{\beta}\ln\left[\left(1-\frac{1}{q}\right)e^{-\beta K}+\frac{1}{q}e^{\beta J}\right]n_0(C), \tag{6}$$

where we used the relation $n_1 = M - n_0$; a further contribution to Eq. (6), independent of the configuration, has been omitted. The effective energy $E_{eff}(C)$ (represented by the dotted line in Fig. 4) is therefore proportional to the number $n_0$ of water sites that do not touch the polymer. In turn, $n_0$ is related to the number of monomer-monomer contacts: every monomer-monomer contact contributes two units of $n_0$; yet other factors, such as turns and kinks, contribute to $n_0$. The present model is thus close yet not equivalent to a $\theta$ model with a temperature dependent monomer-monomer interaction. Recently Trovato *et al.* [15] introduced a model where the hydrophilicity/hydrophobicity of the monomers is an annealed variable (hence their effective mutual interaction is temperature dependent), finding results similar to the ones of this work, although without any reference to the microscopic physics of the water-monomer system. From Fig. 4 it is clear that at high temperature the effective energy is negative, mimicking an attractive *hydrophobic* interaction, but at low temperatures it becomes positive: it results in an effective repulsive interaction leading to cold denaturation. Indeed, a temperature dependent effective interaction, with a behavior similar to that depicted in Fig. 4, could be introduced directly from the beginning, reproducing cold denaturation. Of course, a model with some explicit or semiexplicit description of the solvent degrees of freedom has the advantage that effective interactions can be directly calculated so that the temperature dependence of the effective parameters has a sounder basis. Actually, the definition of effective interactions is limited not only by their temperature dependence, but also by their context dependence: the energy of three-monomer configurations is not, in general, the sum of three two-monomer interactions. These effects emerge from our model even if it is not the best arena to work out their details: molecular simulations of water-solute systems have been carried out [16], showing indeed the presence of nontrivial many-body effects. For proteins, the context dependence is even more dramatic, due to the many different amino acid species, both polar and nonpolar, large and small.

In conclusion, we have introduced a model Hamiltonian for the collapse of a polymer interacting with the solvent, but without explicit 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 cold and warm denaturation temperatures, as experimentally found for some proteins [7] and for some broadly used thermosensitive homopolymers such as poly ($N$-isopropylacrylamide) [6], and we reproduce the known calorimetric behavior of these systems. The model tries to capture some of the essential physics of the water-hydrophobic molecule interaction at a microscopic level, and it is close (yet not exactly equivalent) to a $\theta$ model with a temperature dependent monomer-monomer interaction.

We are presently working on larger Monte Carlo simulations of this model for longer polymers, for a full assessment of the phase diagram of the model. 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. In particular, a full introduction of the MLG model would be welcome (and preliminary results show consistency of the results using both models represented in Fig. 1). Yet it is important to stress the goal of this model, which is not a detailed, quantitative reproduction of the phase diagram of hydrophobic polymers, but rather to propose a way of modeling hydrophobic polymers in solution that is able to reproduce *qualitatively* the known calorimetric data. We are also working on applications of similar approaches to protein models, finding results in agreement with protein calorimetry [17].

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[1] C. Vanderzande, *Lattice Models of Polymers* (Cambridge University Press, Cambridge, 1998), and references therein.

[2] K.F. Lau and K.A. Dill, Macromolecules **22**, 3986 (1989); H.S. Chan and K.A. Dill, Phys. Today **46**(2), 24 (1993).

[3] W. Kauzmann, Adv. Protein Chem. **14**, 1 (1959).

[4] K.P. Murphy, P.L. Privalov, and S.J. Gill, Science **247**, 559 (1990).

[5] P.L. Privalov and S.J. Gill, Adv. Protein Chem. **39**, 191 (1988); G.I. Makhatadze and P.L. Privalov, J. Mol. Biol. **213**, 375 (1990); **232**, 639 (1993); P.L. Privalov and G.I. Makhatadze, *ibid.* **232**, 660 (1993); G.I. Makhatadze and P.L. Privalov, Adv. Protein Chem. **47**, 307 (1995), and references therein.

[6] C. Wu and S. Zhou, Phys. Rev. Lett. **77**, 3053 (1996); C. Wu and X. Wang, *ibid.* **80**, 4092 (1998).

[7] See, e.g., N.C. Pace and C. Tanford, Biochemistry **7**, 198 (1968); P.L. Privalov, Yu. V. Griko, S. Yu. Venyaminov, and V.P. Kutyshenko, J. Mol. Biol. **190**, 487 (1986); Y.V. Griko and P.L. Privalov, Biochemistry **31**, 8810 (1992); J. Zhang, X. Peng, A. Jonas, and J. Jonas, *ibid.* **34**, 8631 (1995); E.M. Nicholson and J.M. Scholtz, *ibid.* **35**, 11 369 (1996); for a general review see P.L. Privalov, Crit. Rev. Biochem. Mol. Biol. **25**, 181 (1990), and references therein.

[8] H.S. Franks and M.W. Evans, J. Chem. Phys. **13**, 507 (1945); see also T.E. Creighton, *Proteins. Structures and Molecular Properties* (W.H. Freeman & Company, New York, 1993), pp. 157–161, and references therein.

[9] N. Muller, Acc. Chem. Res. **23**, 23 (1990).

[10] B. Lee and G. Graziano, J. Am. Chem. Soc. **22**, 5163 (1996).

[11] K.A.T. Silverstein, A.D.J. Haymet, and K.A. Dill, J. Chem. Phys. **111**, 8000 (1999).

[12] A. Hansen, M.H. Jensen, K. Sneppen, and G. Zocchi, Eur. Phys. J. B **10**, 193 (1999).
[13] J. Douglas, C.M. Guttman, A. Mah, and T. Ishinabe, Phys. Rev. E **55**, 738 (1997).
[14] A preliminary analysis of this model can be found in P. De Los Rios and G. Caldarelli, e-print cond-mat/9903394, and in P. Bruscolini and L. Casetti, Phys. Rev. E **61**, R2208 (2000).
[15] A. Trovato, J. van Mourik, and A. Maritan, Eur. Phys. J. B **6**, 63 (1998).
[16] P.L. San Biagio, D. Bulone, V. Martorana, M.B. Palma-Vittorelli, and M.U. Palma, Eur. Biophys. J. **27**, 183 (1998).
[17] P. De Los Rios and G. Caldarelli, Phys. Rev. E **62**, 8449 (2000).