Phase diagram of a semiflexible polymer chain in a $\theta$ solvent: application to protein folding

by

S.Doniach +, T. Garel, and H.Orland*

Service de Physique Théorique†

CE-Saclay, 91191 Gif-sur-Yvette Cedex, France

“Submitted for publication to J. Chem.Phys.”

Saclay, SPhT/95-119

Short title: Phase diagram of a protein chain.

PACS: 87.10; 61.40; 64.70

+ usually at: Dept. Applied Physics, Stanford University, Stanford CA 94305
* Also at Groupe de Physique Statistique, Université de Cergy-Pontoise, 95806 Cergy-Pontoise Cedex, France
† Laboratoire de la Direction des Sciences de la Matière du Commissariat à l’Energie Atomique
ABSTRACT

We consider a lattice model of a semiflexible homopolymer chain in a bad solvent. Beside the temperature $T$, this model is described by (i) a curvature energy $\varepsilon_h$, representing the stiffness of the chain (ii) a nearest-neighbour attractive energy $\varepsilon_v$, representing the solvent (iii) the monomer density $\rho = \frac{N}{\Omega}$, where $N$ and $\Omega$ denote respectively the number of monomers and the number of lattice sites. This model is a simplified view of the protein folding problem, which encompasses the geometrical competition between secondary structures (the curvature term modelling helix formation) and the global compactness (modeled here by the attractive energy), but contains no side chain information. By allowing the monomer density $\rho$ to depart from unity one has made a first (albeit naive) step to include the role of the water. In previous analytical studies we considered only the (fully compact) case $\rho = 1$, and found a first order freezing transition towards a crystalline ground state (also called the native state in the protein literature).

In this paper, we extend this calculation to the description of both compact and non-compact phases. The analysis is done first at a mean-field level. We then find that the transition from the high temperature swollen coil state to the crystalline ground state is a two-step process for which:(i) there is first a $\theta$ collapse transition towards a compact “liquid” globule, and (ii) at low temperature, this “liquid” globule undergoes a discontinuous freezing transition. The mean-field value of the $\theta$ collapse temperature is found to be independent of the curvature energy $\varepsilon_h$. This mean-field analysis is improved by a variational bound, which confirms the independence of the $\theta$ collapse temperature with respect to $\varepsilon_h$. This result is confirmed by a Monte Carlo simulation, although with a much lower value of the $\theta$ temperature. This lowering of the collapse transition allows the possibility (for large $\varepsilon_h$) of a direct first order freezing transition, from a swollen coil to the crystalline ground state. For small values of $\varepsilon_h$, the mean-field two-step mechanism remains valid.

In the protein folding problem, the “liquid” compact phase is likely to be related to the “molten globule” phase. The properties of this model system thus suggest that, even though side chain disordering is not taken into account, disordering of the backbone of a protein may still be a sufficient mechanism to drive the system from the native state into the molten globule state.
1. INTRODUCTION

The thermodynamic equilibrium states of small globular proteins in solution depend on solvent conditions, including: temperature, pH, salt concentration and denaturant concentration.

Roughly speaking, there are two ways to go from a fully unfolded swollen coil state to the native state: through a single first order folding transition, or through an intermediate collapsed phase [1]. The nature of the transition from an unfolded state to this phase, which may be generically called a molten globule [2,3], depends in detail on the specific protein and solvent conditions, but often has the character of a continuous \(\theta\)-collapse. The subsequent transition to the native state is generally first order in character. Note that, in a finite system such as a protein, the use of the expression “phase transition” should be interpreted as “strongly cooperative phenomenon.”

In order to elucidate the balance of enthalpic and entropic terms leading to this phase diagram we have previously represented the protein folding process using a highly simplified homopolymer model, originally due to Flory [4]. In this model the tendency to form local order which may have 1-or 2-dimensional character (\(\alpha\) helices or \(\beta\) sheets) is complemented by a tendency to global compactness representing the effect of hydrophobic forces. The properties of this model were previously studied numerically by Kolinsky et al [5]. We have previously given an analytical mean field treatment of the model [6,7] which was restricted to the fully compact phases and yielded a first order freezing transition towards a native-like structure.

In this paper we extend this model by (i) allowing for the presence of vacancies (ii) assigning a finite attractive energy \(\varepsilon_v\) between (nonconsecutive) neighbouring monomers. This generalization enables us to study the full phase diagram of the model by the method of mean field theory, including the high temperature disordered coil state. From a more (bio)chemical point of view, points (i) and (ii) above are a first step towards a proper treatment of the solvent properties.

We report elsewhere the results of numerical studies which yield information about the structural character of the partially folded states of this model [8].

Besides its application for proteins, this model is clearly of interest to describe the melting or crystallization of semi-flexible polymers [4]: in this case, intermediate compact
phases are likely to possess orientational order [9,10].

The organization of this paper is as follows. In section 2, we recall for completeness our previous study of fully compact phases, through the formalism of Hamiltonian paths. The method is applied to the case of 1-dimensional structures which may be thought of as helix-like (sheets may be treated in a similar way). The extended model, also restricted to the case of helix-like structures, is presented at a mean-field level in section 3, together with its resulting phase diagram. In section 4, we derive an upper bound for the free energy, which strongly suggests that the collapse temperature is indeed independent of the curvature energy. Using previous determinations of the $\theta$ temperature, we obtain a modified phase diagram, which is then checked numerically by Monte Carlo simulations. Connections with other approaches are considered in the conclusion.

2. SECONDARY STRUCTURES IN A COMPACT PHASE

In this section, we shall describe how one can study, in terms of a very simplified and schematic homopolymer model the thermodynamics of secondary structures in a compact phase. Before doing so, let us reconsider the problem of the collapsed chain on a lattice, initially without introducing any tendency to secondary structure formation (i.e. for zero stiffness).

2.1 Hamiltonian paths and the compact phase

We consider a $d$-dimensional hypercubic lattice of $N = L^d$ sites, and its associated Hamiltonian paths representing the conformations of a polymer with $N - 1$ links. We recall that a Hamiltonian path (HP) is a path which visits all sites of the lattice once and only once. In this approach, to compute the entropy of the collapsed but disordered phase means to evaluate the number of Hamiltonian paths on the lattice. For simplicity, we consider closed paths, but, as is well known in polymer theory [11], (i.e. in the large $N$ limit), boundary conditions play only a subdominant role in terms of the free energy.

Thus we define the partition function $Z$ by:

$$Z = \sum_{\{HP\}} 1 \quad (2.1)$$

Note that $Z$ is nothing but the total number of HP. One may rewrite (2.1) as:

$$Z = \lim_{n \to 0} \frac{1}{n} \frac{\int \prod_{\{\vec{r}\}} d\vec{\varphi}(\vec{r}) e^{-A_G} \prod_{\{\vec{r}\}} \frac{1}{2} \varphi^2(\vec{r})}{\int \prod_{\{\vec{r}\}} d\vec{\varphi}(\vec{r}) e^{-A_G}} \quad (2.2.a)$$
where
\[ A_G = \frac{1}{2} \sum_{\bar{r}, \bar{r}'} \tilde{\varphi}(\bar{r}) (\Delta_{\bar{r}\bar{r}}')^{-1} \tilde{\varphi}(\bar{r}') \]  \hfill (2.2.b)

In eq.(2.2), \( \varphi(\bar{r}) \) is a \( n \)-component field attached to each point \( \bar{r} \) of the lattice, and the operator \( \Delta_{\bar{r}\bar{r}}' \) is 1 if \( \bar{r} \) and \( \bar{r}' \) are nearest-neighbour, and 0 otherwise. The denominator is a simple Gaussian integral, equal to \( \exp(-\frac{n}{2} \log \det \Delta_{\bar{r}\bar{r}}') \). It is equal to one in the limit \( n \to 0 \), and will be omitted henceforth.

To prove the identity between (2.2) and (2.1), namely that (2.2) indeed counts all HP, we use Wick’s theorem. At each site \( \bar{r} \), one attaches a factor \( \frac{1}{2} \tilde{\varphi}^2(\bar{r}) \), which can be viewed graphically as one line coming into and one line coming out of site \( \bar{r} \). The elementary contraction (see eq. (2.2.b)) reads:

\[ \overline{\varphi_\alpha(\bar{r}) \cdot \varphi_\beta(\bar{r}')} = \delta_{\alpha\beta} \Delta_{\bar{r}\bar{r}}', \]  \hfill (2.3)

where \( \alpha \) and \( \beta \) denote any of the \( n \)-components of the field \( \tilde{\varphi}(\bar{r}) \). Since eq.(2.3) conserves the index \( \alpha \), each connected part of the path yields a factor \( n \) through the summation over \( \alpha \). To deal with the one chain problem (or with a single connected path), one must therefore extract the term proportionnal to \( n \), hence the factor \( \lim_{n \to 0} \frac{1}{n} \) in eq.(2.2.a). By doing so \([9]\), paths with more than one connected part, which have higher powers of \( n \), are cancelled, which proves the identity of (2.1) and (2.2).

The exact evaluation of (2.2) is out of reach, and we restrict ourselves to a simple approximation, namely the Saddle-Point Method (SPM). Minimizing the exponent of the numerator of (2.2.a) with respect to \( \tilde{\varphi}(\bar{r}) \) yields:

\[ \sum_{\bar{r}'} \Delta_{\bar{r}\bar{r}}^{-1} \tilde{\varphi}(\bar{r}') = \frac{2\tilde{\varphi}(\bar{r})}{\tilde{\varphi}(\bar{r})^2} \]  \hfill (2.4)

If we assume periodic boundary conditions on the lattice, it is legitimate to look for a homogeneous solution to (2.4). We further break the \( O(n) \) symmetry by choosing \( \tilde{\varphi} \) in a given direction, say 1, and take the solution as:

\[ \tilde{\varphi}(\bar{r}) = (\varphi, 0, \ldots) \]  \hfill (2.5.a)

where \( \varphi \) is a constant, independent of space. Using (2.4) we get \( \varphi^2 = 2q \), where \( q = 2d \) is the coordination number of the lattice. In the SPM, the partition function (2.4) reads:

\[ Z = \left( \frac{q}{e} \right)^N \]  \hfill (2.5.b)
where $e = \exp(1) = 2.718 \ldots$. This result [12] is in excellent agreement with numerical simulations and exact results, on various lattices, even in low dimensions. This is due to the fact that fluctuations around the SPM are indeed small [2,12].

Equation (2.5.b) shows that in the limit of zero stiffness the compact phase has a sizeable conformational entropy $S/N = \log(q/e)$, and thus no definite three-dimensional shape: a polymer globule (compact phase) is a statistical mixture of an exponentially large number of conformations. (We remind the reader that these results are strongly dependent on the boundary conditions, assumed here to be periodic). To sum up, we have modeled the collapsed phase of a single polymer chain below the $\theta$ point by lattice Hamiltonian paths. In this approach, one loses track of the relation between the spatial degrees of freedom and the curvilinear abscissa along the chain.

2.2) Secondary structure in the compact phase

Following Flory [4], we may think of each link of the Hamiltonian path as representing a helical turn. Since hydrogen-bonds have a tendency to favor long helices, that is to align the links of our model, we attribute an energy penalty $\varepsilon_h$ to the breaking of an helix, that is whenever the Hamiltonian path makes a turn (corner). This model of a homopolymer with finite stiffness has attracted a lot of attention in the theory of polymer melting [6,9,10]. The partition function of the system, at inverse temperature $\beta = \frac{1}{T}$, reads

$$\mathcal{Z} = \sum_{\{HP\}} e^{-\beta \varepsilon_h N_c\{H\}}$$  \hspace{1cm} (2.6)

where $\{HP\}$ denotes the ensemble of all Hamiltonian paths, and $N_c\{H\}$ denotes the number of corners present in path $H$. Following the previous section, we introduce on each lattice site $\vec{r}$ and for each direction $\alpha = 1, \ldots, d$ an $n$-component real field $\vec{\varphi}_\alpha (\vec{r})$. The partition function $\mathcal{Z}$ can be rewritten:

$$\mathcal{Z} = \lim_{n \to 0} \frac{1}{n} \int \prod_{\vec{r}} \prod_{\alpha=1}^{d} d\vec{\varphi}_\alpha (\vec{r}) e^{-A_G} \prod_{\vec{r}} \left[ \frac{1}{2} \sum_{\alpha=1}^{d} \vec{\varphi}_\alpha^2 (\vec{r}) + e^{-\beta \varepsilon_h} \sum_{1 \leq \alpha < \gamma \leq d} \vec{\varphi}_\alpha (\vec{r}) \cdot \vec{\varphi}_\gamma (\vec{r}) \right]$$  \hspace{1cm} (2.7.a)

with

$$A_G = \frac{1}{2} \sum_{\alpha=1}^{d} \sum_{\vec{r}, \vec{r}'}\vec{\varphi}_\alpha (\vec{r}) \cdot (\Delta^\alpha_{\vec{r}\vec{r}'} \cdot)^{-1} \cdot \vec{\varphi}_\alpha (\vec{r}')$$  \hspace{1cm} (2.7.b)
where the operator \( \Delta^\alpha_{\bar{r}\bar{r}'} \), is 1 if \( \bar{r} \) and \( \bar{r}' \) are nearest-neighbour in direction \( \alpha \), and 0 otherwise. Note again that we have omitted the denominator in (2.7.a), since it goes to unity when \( n \to 0 \).

In order to prove the equivalence of (2.6) and (2.7), we will use again Wick’s theorem: we define, from (2.7.b), the elementary contraction:

\[
\varphi^{(u)}_\alpha (\bar{r}) \cdot \varphi^{(v)}_\gamma (\bar{r}') = \delta_{uv} \delta_{\alpha\gamma} \Delta^\alpha_{\bar{r}\bar{r}'},
\]  

(2.8)

where \( u \) and \( v \) are component indices of \( \varphi_\alpha (\bar{r}) \), running from 1 to \( n \).

Expanding the product over \( \{\bar{r}\} \) in (2.7.a), we must choose at each site \( \bar{r} \) either a term of the form \( \frac{1}{2} \varphi^2_\alpha (\bar{r}) \) or one of the form \( e^{-\beta \varepsilon h} \varphi_\alpha (\bar{r}) \cdot \varphi_\gamma (\bar{r}) \), corresponding respectively to a path going straight through \( \bar{r} \) in direction \( \alpha \), or to a path making a turn at \( \bar{r} \), from direction \( \alpha \) to direction \( \gamma \). By contracting the fields according to (2.8), we construct a sum over all self avoiding compact closed paths with appropriate weights, with an additional factor \( n \) (due to the summation over component index \( u \)) for each closed loop. As above, we extract the single chain contribution by taking the limit \( n \to 0 \). This concludes the proof.

The mean field equations (saddle point on (2.7.a)) read:

\[
\sum_{\bar{r}', \bar{r}'} (\Delta^\alpha_{\bar{r}\bar{r}'})^{-1} \varphi_\alpha (\bar{r}') = \frac{\varphi_\alpha (\bar{r}) + e^{-\beta \varepsilon h} \sum_{\gamma(\neq \alpha)} \varphi_\gamma (\bar{r})}{\frac{1}{2} \sum_{\alpha'} \varphi^2_{\alpha'} (\bar{r}) + \frac{1}{2} e^{-\beta \varepsilon} \sum_{\gamma' \neq \alpha} \varphi_{\alpha'} (\bar{r}) \cdot \varphi_{\gamma'} (\bar{r})}
\]  

(2.9)

Assuming periodic boundary conditions, the isotropic homogenous mean-field solution takes the form \( \varphi_\alpha (\bar{r}) = \varphi \) for any \( \alpha = 1, ..., d \) and \( \bar{r} \). From (2.9), we obtain:

\[
\varphi^{(1)} = \sqrt{\frac{4}{d}}
\]  

(2.10.a)

At this mean-field level, the free energy per site reads:

\[
f = -T \log \left( \frac{q(\beta)}{e} \right)
\]  

(2.10.b)

with

\[
q(\beta) = 2 + 2(d - 1) e^{-\beta \varepsilon h}
\]  

(2.10.c)

and \( e = 2.71828... \). Note that \( q(\beta) \) plays the role of an effective coordination number .
The phase transition of the model described by eq. (2.7) has been studied in detail in reference [6]. The ground state consists of a bundle–of–sticks like conformation taking the form of straight paths which make turns on the surface. This solution, which has \( f = 0 \) in the large \( N \) limit, is not obtained from the saddle point equation (2.9), which is not surprising for a structure which is essentially one dimensional. It must nevertheless be considered, since it is clearly the lowest energy state of the chain. Within our approximations, a first order phase transition must then occur at a temperature \( T_F \) for which \( f = 0 \), yielding an effective coordination number \( q(\beta) \) equal to \( e \). For \( d = 3 \), \( T_F = 0.58 \varepsilon_h \). Below this temperature, the free energy remains equal to zero.

Physically, there is a competition between the entropy gain of making turns, and the corresponding energy loss. At high temperature, the corners are mobile in the bulk, leading to a liquid like structure, whereas at low temperature, the system is frozen, in stretched walks, with the corners expelled on the surface. The two regions are separated by a phase transition at \( T_F \).

The average length of a helix is given by

\[
\ell = \frac{N\varepsilon_h}{U(\beta_F)} \tag{2.11}
\]

where \( U(\beta) = -\frac{\partial}{\partial \beta} \log Z \) is the internal energy. Just above the freezing point, in \( d = 3 \), the average secondary structure persistence length is equal to \( \ell_F = 3.78 \), and is of \( \mathcal{O}(\ell = N^{1/3}) \) in the low temperature phase. Note that in this very simplified picture \( \ell_F \) corresponds to a typical number of amino-acids per \( \alpha \)-helix of the order of 15, since one link corresponds to a helical turn, that is 3.6 amino acids.

Since the density of corners is extensive (i.e. \( \mathcal{O}(N) \) for \( T > T_F \)), and drops to zero (i.e. \( \mathcal{O}(N^{1-1/d}) \)) for \( T < T_F \), the freezing transition is a first order in the thermodynamic limit.

This model can be easily extended to incorporate the existence of \( \beta \)-sheets [7]: the physics of the latter model is very similar to that of the previous model (2.2), namely, a liquid-like high temperature phase with no definite \( \beta \)-sheet structure, and a low temperature frozen phase, consisting of stacks of parallel \( \beta \)-sheets.
3. VACANCIES AND GEOMETRICAL FRUSTRATION: A MEAN-FIELD TREATMENT

We wish now to explore the full phase diagram of the protein chain, including its disordered (denatured) coil state. For the sake of simplicity, we will generalize the approach of section 2 to the case of helix-like structures (again, the extension to the β-sheet case is straightforward). We consider a $d$-dimensional hypercubic lattice of $\Omega = L^d$ sites, and a polymer chain of $N$ monomers, each monomer being at a lattice site. Following the preceding discussion, we attribute an energy penalty $\varepsilon_h$ for each corner (i.e., when two consecutive links are not aligned). Furthermore, we now introduce a finite Van der Waals attractive energy $\varepsilon_v$ for each pair of non consecutive monomers separated by one lattice spacing. We emphasize once more that this model aims at a simplified description of the solvent conditions.

The partition function reads

$$Z_N = \sum_{(n_r=0,1)} \sum_{\{\mathcal{H}_N\}} e^{-\beta \varepsilon_h N_c} e^{-N \beta \varepsilon_v} e^\beta \varepsilon_v \sum_{\mathcal{F}} n_{\mathcal{F}} \sum_{\mathcal{F}'} \delta(\sum_{\mathcal{F}} n_{\mathcal{F}} - N) \tag{3.1}$$

where $n_r$ denotes the occupation number of lattice site $\mathcal{F}$, and $\{\mathcal{H}_N\}$ denotes now a self avoiding path of $N$ monomers on the lattice. The constant factor $e^{-N \beta \varepsilon_v}$ in eq.(3.1) ensures that one does not count the attractive energy between consecutive monomers. Following section 2, it is easy to reformulate this problem by introducing at each site $\mathcal{F}$ of the lattice (i) $n$-components fields $\varphi_\alpha(\mathcal{F})$, with $\alpha = 1, \ldots, d$ (ii) a scalar field $\psi_\mathcal{F}$ needed to perform the Hubbard-Stratanovich transformation on the occupation numbers $n_\mathcal{F}$. Introducing a fugacity $K$ per monomer, the grand canonical partition function $Z(K)$ may be written:

$$Z(K) = \lim_{n \to 0} \frac{1}{n} \int \prod_\mathcal{F} d\varphi_\mathcal{F} \prod_{\alpha=1}^d d\varphi_\alpha(\mathcal{F}) e^{-A_G} \prod_\mathcal{F} \left[ 1 + Ke^{-\beta \varepsilon_v} e^{\beta \varepsilon_v} \psi_\mathcal{F} B_\mathcal{F} \right] \tag{3.2.a}$$

with

$$A_G = \frac{1}{2} \sum_{\mathcal{F},\mathcal{F}'} \psi_\mathcal{F} (\Delta_{\mathcal{F}\mathcal{F}'})^{-1} \psi_\mathcal{F}' + \frac{1}{2} \sum_{\alpha=1}^d \sum_{\mathcal{F},\mathcal{F}'} \varphi_\alpha(\mathcal{F}) \cdot (\Delta_{\mathcal{F}\mathcal{F}'})^{-1} \cdot \varphi_\alpha(\mathcal{F}') \tag{3.2.b}$$

and

$$B_\mathcal{F} = \left[ \frac{1}{2} \sum_{\alpha=1}^d \varphi_\alpha^2(\mathcal{F}) + e^{-\beta \varepsilon_h} \sum_{1 \leq \alpha < \gamma \leq d} \varphi_\alpha(\mathcal{F}) \cdot \varphi_\gamma(\mathcal{F}) \right] \tag{3.2.c}$$
it is easy to check, using the approach of section 2, that $Z_N$ in (3.1) is the coefficient of $K^N$ in eq.(3.2); we may alternatively write

$$N = \frac{\partial \log Z(K)}{\partial \log K}$$ (3.3)

Our strategy is now straightforward: we evaluate (3.2) via an isotropic saddle point method and periodic boundary conditions ($\vec{\varphi}_\alpha(\vec{r}) = \bar{\varphi}$; $\psi_\vec{r} = \psi$). With these hypothesis, eq.(3.2.a) gives

$$\log Z(K) = \Omega \left[ -\frac{1}{2} \left( \frac{1}{2d} \psi^2 + \frac{d}{2} \bar{\varphi}^2 \right) + \log(1 + K e^{-\beta \varepsilon_v} e^{\sqrt{\beta \varepsilon_v} \psi} B) \right]$$ (3.4)

where $B$ is now space independent and given by (3.2.c). Breaking the $O(n)$ symmetry as in section 2, the saddle point equations reads

$$\frac{\psi}{(2d)} = \frac{(d\varphi^2)}{2} \frac{K \sqrt{\beta \varepsilon_v} e^{-\beta \varepsilon_v} e^{\sqrt{\beta \varepsilon_v} \psi} (1 + (d - 1) e^{-\beta \varepsilon_h})}{D}$$ (3.5.a)

$$\frac{d\varphi}{2} = \frac{(d\varphi)}{2} \frac{K e^{-\beta \varepsilon_v} e^{\sqrt{\beta \varepsilon_v} \psi} (1 + (d - 1) e^{-\beta \varepsilon_h})}{D}$$ (3.5.b)

with

$$D = 1 + \frac{(d\varphi^2)}{2} \frac{K e^{-\beta \varepsilon_v} e^{\sqrt{\beta \varepsilon_v} \psi} (1 + (d - 1) e^{-\beta \varepsilon_h})}{D}$$ (3.5.c)

Equation (3.3) in turn gives

$$N = \Omega \frac{(d\varphi^2)}{2} \frac{K e^{-\beta \varepsilon_v} e^{\sqrt{\beta \varepsilon_v} \psi} (1 + (d - 1) e^{-\beta \varepsilon_h})}{D}$$ (3.5.d)

Introducing the monomer density $\rho = \frac{N}{\Omega}$, we have from equations (3.5)

$$\varphi^2 = \frac{4}{d} \rho$$ (3.6.a)

$$\psi = 2d \sqrt{\beta \varepsilon_v} \rho$$ (3.6.b)

$$D = \frac{1}{(1 - \rho)}$$ (3.6.c)

leading to a free energy per monomer:

$$f = -\frac{T}{N} \log Z_N = -\frac{T}{N} \log \left( \frac{Z(K)}{K^N} \right)$$ (3.7.a)
We finally obtain

\[ f = -T \log \left( \frac{q(\beta)}{e} \right) + T \frac{(1 - \rho)}{\rho} \log(1 - \rho) + \varepsilon_v - d\varepsilon_v \rho \]  

(3.7.b)

This equation is reminiscent of a Flory-Huggins formula [13] for a single chain: the first term represents the entropy of the collapsed chain (see eq.(2.10.b)), the second term accounts for the entropy of vacancies, and the last two terms represent the attractive energy. Note that, we work here with the free energy per monomer rather than with the free energy per site.

As discussed in section 2, the fully stretched (or crystalline) phase makes turns at the “surface”, among the vacancies; this phase is not obtained in the saddle point approximation. Its free energy per monomer is easily obtained as

\[ g = -(d-1) \varepsilon_v \]  

(3.8)

since \( \varepsilon_v \) is the energy between two neighbouring non consecutive monomers. Note that for density of vacancies \( \rightarrow 0 \), i.e. \( \rho = 1 \), the analysis of section 2 is recovered: one may also argue that the limit \( \varepsilon_v \rightarrow \infty \) in equation (3.7.b) implies that the phase with \( \rho = 1 \) corresponds to the minimum of the free energy \( f \).

Since our study encompasses the study of the high temperature phase as well, it is also interesting to consider the value of the free energy \( f \) for the limit \( \rho = 0 \), that we call \( f_0 \). We get

\[ f_0 = -T \log q(\beta) + \varepsilon_v, \]  

(3.9)

that is the free energy of the swollen coil, which corresponds, in our approximations, to the entropy of a Brownian walk on a lattice with temperature dependent connectivity.

We now consider the phase diagram implied by the study of equations (3.7.b) (3.8), and (3.9).

Broadly speaking, one should compare the free energies per monomer \( f \) and \( g \). A first order freezing of the type described previously will occur when \( g \leq f \). We have chosen to present our result in the \((t,x)\) plane, with \( t = \frac{T}{\varepsilon_v} \) and \( x = \frac{\rho}{\varepsilon_v} \). Beside the extremal points \( \rho = 0 \) and \( \rho = 1 \), we have to consider the solution \( \rho = \rho^* \) with

\[ \left( \frac{\partial f}{\partial \rho} \right)_{\rho = \rho^*} = 0 \]  

(3.10.a)
yielding

\[
\frac{\log (1 - \rho^*)}{\rho^*} + 1 + \rho^* \frac{d}{t} = 0
\]  

(3.10.b)

(a) the \( \theta \) collapse transition

From eq.(3.10.b), one may get a non-zero density \( \rho^* \), below the \( x \)-independent \( \theta \) temperature \( t_\theta = 2d \), i.e. \( t_\theta = 6 \) for \( d = 3 \).

(b) the freezing transition

One has to compare \( f(\rho^*) \) and \( g \), where \( \rho^* \) is given by eq.(3.10.b). It is easy to see that for \( x \to \infty \), we have at the transition \( \rho^*_\infty = 0.5 \), and \( t_\infty = \frac{d}{4 \log 2 - 2} \), yielding \( t_\infty \approx 3.88 \) for \( d = 3 \). For \( x \to 0 \), we recover the result of section 2, namely \( \rho^*_0 = 1 \) and \( t_0 = x/\log \left( \frac{2(d-1)}{(e-2)} \right) \), i.e. \( t_0 \approx 0.58x \) for \( d = 3 \).

Note that in the mean-field treatment of our model, it is not possible to obtain a direct transition from the high temperature phase to the crystalline ground state. This transition would occur for \( f_0 = g \), and the resulting temperature is lower than \( t_\theta \), as seen from eqs(3.8) and (3.9). The fact that one has to go through a dense intermediate phase can be traced back to the vacancy entropy in eq (3.7.b). The resulting phase diagram is shown in Figure 1. We now study corrections to the mean-field picture.

4. VARIATIONAL BOUNDS AND MONTE CARLO SIMULATIONS: THE MODIFIED PHASE DIAGRAM

The \( \theta \) collapse transition is a 2nd order transition, and is thus not very accurately described by mean-field methods, whereas the freezing transition being first-order may be quite well described within this framework. For example, we find too high a value of the \( x \)-independent collapse temperature \( (t_\theta = 6 \) for \( d = 3 \)). Numerical estimates [19] give \( t_\theta \approx 3.64 \) for \( x = 0 \), which may imply a modification of the phase diagram of figure 1.

A variational bound to the free energy may be easily obtained. Indeed, from equation (3.2.c) we have (using Schwarz inequality):

\[
B_{\vec{r}} = \frac{d}{2} (1 - e^{-\beta \varepsilon_h}) \left( \frac{1}{d} \sum_{\alpha = 1}^{d} \varphi_{\alpha}^2 (\vec{r}) \right) + \frac{e^{-\beta \varepsilon_h}}{2} \left( \sum_{1 \leq \alpha \leq d} \varphi_{\alpha} (\vec{r}) \right)^2
\]

\[
\geq \frac{q(\beta)}{2d} \left( \frac{1}{d} \sum_{1 \leq \alpha \leq d} \varphi_{\alpha} (\vec{r}) \right)^2
\]

(4.1.a)
where \( q(\beta) \) is the effective connectivity of the lattice, as given by (2.10.c). From (4.1.a) we get the bound

\[
Z \geq \left( \frac{q(\beta)}{2d} \right)^N Z_\theta(\epsilon_v, T)
\]

(4.1.b)

where \( Z_\theta(\epsilon_v, T) \) is the partition function of a chain with no curvature energy, with collapse energy \( \epsilon_v \). We first note on this equation that the r.h.s. displays a collapse transition at the \( \theta \) temperature of the "pure" system, that is the system with no stiffness. This result is consistent with the result of section 3.

As mentioned above, the "pure" \( \theta \) point has been calculated numerically in three dimensions, leading to a temperature much lower than the mean-field estimate \( (t_\theta \simeq 3.64 \text{ vs. a mean-field estimate of } t_\theta = 6) \). This effect is partly due to the fact that the \( \theta \) point is a second order phase transition, which is dominated by fluctuations, which are not well taken into account at the mean-field level.

On the other hand, the freezing transition is a first order transition, and thus is not driven by large fluctuations. The mean-field approximation is then much more reliable.

If we take these two facts seriously, we obtain the phase diagram represented on figure 2. One qualitative modification of the phase diagram is the possibility of a direct freezing transition, without passing through a \( \theta \) collapse. Indeed, defining the critical value \( x_c \) as the intersection between the line of the \( \theta \) collapse \( (t = 3.64) \) and the freezing curve, we see that for:

\[ x < x_c: \text{the transition to the native (crystalline) state proceeds through a } \theta \text{ collapse, followed by a freezing transition. There is a whole region in temperature, where the system is collapsed, but with no definite secondary structure, and this may be identified with a "molten globule" state.} \]

\[ x > x_c: \text{the freezing transition occurs at a higher temperature than the } \theta \text{ collapse, and thus the system will directly freeze, from the swollen coil to the native state.} \]

Using equations (3.7.b), (3.8), and (3.10) together with the three dimensional numerical value of \( t_\theta \), we find \( x_c \simeq 15 \).

We have checked these predictions numerically by performing Monte Carlo simulations. We have used a Monte Carlo growth method [20], which has proven both fast and reliable for calculations of thermodynamical properties of random chains. We grow pop-
ulations of $M = 20000$ chains of length $N = 200$, for various values of $t = T/\varepsilon_v$ and $x = \varepsilon_h/\varepsilon_v$. There is of course a degradation of the statistics in the frozen phase, and we have thus restricted the parameter $x$ to 3. The numerical results are plotted on figure 2. Each calculated point is represented by a star. We see that the agreement is quite good, and seems to confirm the theoretical phase diagram predicted above.

5. CONCLUSIONS

We have studied a simplified homopolymer model to describe the interplay between compactness and local order in a protein chain. In a mean-field treatment of this model, the native state can be reached only after a $\theta$ collapse transition towards a liquid globule (the collapse transition temperature being independent of the stiffness (or curvature) energy). In this globule, helix–like secondary structures progressively grow and ultimately freeze. These results are not capable of representing the traditional two-state coil $\leftrightarrow$ native renaturation transition for globular proteins.

We have argued that this disagreement may be partially explained by the mean-field treatment. Using more reliable estimates of the three dimensional $t_{\theta}$ temperature [19], we have used variational bounds, together with Monte Carlo calculations, to show that (i) $t_{\theta}$ is indeed independent of $\varepsilon_h$ (ii) the phase diagram may be qualitatively modified for large enough values of $x = \varepsilon_h/\varepsilon_v$, allowing for a unique transition from the coil towards the native state. In our model, we get a very high value for the critical $x_c$, separating the two transitions regime from the unique “coil-native state renaturation transition” ($x_c \simeq 15$). This value, which shows that a large portion of the phase diagram is occupied by a compact liquid or molten globule phase, is certainly not realistic for globular proteins.

Finally, we think that dealing with an homopolymer model may be inappropriate to describe the renaturation transition of globular proteins. The latter has been modeled in a simplified theoretical approach by Finkelstein and Shakhnovich [14,15] and by Alonso, Dill and Stigter [16]. Transitions of this type have also been studied analytically in a random heteropolymer model [17]. Such two-state transitions have their origin in the heteropolymer nature of proteins for which hydrophobic residues become segregated within the core of the protein, while hydrophilic residues are in contact with solvent.

Our results show that the homopolymer model has a molten globule like thermodynamic phase. Although the model has no side chains and is hence incapable of including
the effects of side chain entropy on the native ↔ molten globule transition, it does reveal another entropic contribution which has not been considered in this context by previous workers: the entropy of backbone disordering.

Thus, even though side chain rotational entropy is undoubtedly important in the molten globule state, we have shown that backbone disordering may be capable of driving a first order transition, and hence that side chain entropy need not necessarily be the driving force for the transition. Thus backbone disordering provides a complementary mechanism to the discussion of Bromberg and Dill [18], who argue that the onset of side chain disordering may not be sufficiently sharp to drive the native ↔ molten globule transition.

Last but not least, it is clear that our results pertain to the case of semi-flexible polymers. The intermediate compact phase is then likely to possess orientational (e.g. nematic) order [9,10].
FIGURE CAPTIONS

Figure 1: Mean field phase diagram in the \((t, x)\) plane. The solid line denotes the continuous \(\theta\) transition from the coil (C) to the liquid globule (G). The dashed line denotes the first order freezing transition to the native state (N).

Figure 2: Numerical three dimensional phase diagram in the \((t, x)\) plane. The solid line denotes the continuous \(\theta\) transition from the coil (C) to the liquid globule (G). The dashed line denotes the first order freezing transition to the native state (N). The stars show the results of the Monte Carlo calculations. The multicritical point corresponds to \((t_c \simeq 3.6, x_c \simeq 15)\).
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