Four-states phase diagram of proteins.

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Abstract.

A four states phase diagram for protein folding as a function of temperature and solvent quality is derived from an improved 2-d lattice model taking into account the temperature dependence of the hydrophobic effect. The phase diagram exhibits native, globule and two coil-type regions. In agreement with experiment, the model reproduces the phase transitions indicative of both warm and cold denaturations. Finally, it predicts transitions between the two coil states and a critical point.

Understanding the physical mechanism underlying protein folding remains one of the main open problems of contemporary theoretical biophysics. The interplay between protein-protein and protein-solvent interactions that drive the folding of the polypeptide may be partly investigated using full atomistic representations. Computer simulations at this level of detail are shown for instance to provide crucial information about the stability of the proteins around its native structure. Such calculations are however very time consuming and not appropriate for characterizing the large conformational space of multimeric chains, which is a crucial step toward understanding the folding problem. [1].

This has led to the emergence of alternative approaches, such as the use of simpler coarse grained models. Among these, the lattice model is probably the most popular and efficient model that allows a wide sampling of the conformational space of a given polypeptide chain [2]. Accordingly, the 16-mer placed on a two dimensional lattice has often been used to this end. [3–5] Such a chain is long enough to capture fundamental mechanism of protein folding and short enough to allow the calculation of partition function by a full enumeration in reasonable computer times.

Over a decade ago, Dinner et al. [3] used such a model to derive the three-states phase diagrams of 16mers for different chain sequences as a function of temperature and average attraction between monomers. Coil, globule and native states were all obtained but the model failed to reproduce the well known cold denaturation. This transition, from the native to the coil state, upon lowering the temperature, consists in the loss of the order of the chain [6].

It was indeed later shown that the accuracy of the potential describing the interactions with the solvent is crucial [7]. We have recently proposed a refinement of the coupling model...
that explicitly includes a temperature dependent so-called hydrophobic effect in a solvation free energy contribution [8]. This model, considering the same 16-mer chain, predicts the existence of the coil and a native state, the warm and cold denaturation transition but produces no globule states. Despite this last shortcoming the coupling models were shown to be consistent with all-atom molecular dynamics simulations of a short peptide solvated in water [9]. In the recent litterature, other models dealing with the cold denaturation have also been proposed [10].

In this paper, we extend on previous calculations and propose a more comprehensive model of the hydrophobic effect that reproduces a four states phase diagram with both the warm and cold denaturation transitions. In the model, all the links between two adjacent nods of the lattice are taken into account (see an example in fig.1). The effective hamiltonian of conformation $m$ is given by:

$$H_{\text{eff}}^{(m)}(T) = E_{\text{intr}}^{(m)} + F_{\text{solv}}^{(m)}(T)$$  \hspace{1cm} (1)

The intrachain interaction energy for each conformation $m$ is described as in Dinner et al. [3]:

$$E_{\text{intr}}^{(m)} = \sum_{i>j} B_{ij} \Delta_{ij}^{(m)}$$  \hspace{1cm} (2)

where $B_{ij}$ is the specific interaction between residue sites $i$ and $j$ and $\Delta_{ij}^{(m)}$ equals 1 if $i$ and $j$ are in contact and 0 otherwise. Monomer-monomer interactions $B_{ij}$ are real numbers selected randomly from a normalized probability density - Gaussian distribution - with a standard deviation $\sigma = 2$. One single conformation, noted Nat, is selected at random among the more maximally compact structures, and considered as the native conformation of the sequence. Nat has 9 intrachain contacts. In the spirit of the Go-model [11], the corresponding values of interactions are described by the 9 smallest values of $B_{ij}$.

The free energy of solvation for each conformation may be written as a sum of two contributions:

$$F_{\text{solv}}^{(m)}(T) = \sum_{i=1}^{N} n_i^{(m)} f_i(T) + 2 n_s^{(m)} f_s(T)$$  \hspace{1cm} (3)

Where $n_i^{(m)}$ and $n_s^{(m)}$ are respectively the number of solvent sites surrounding residue $i$ and the total number of solvent-solvent contacts. $f_i(T)$ is the specific free energy of a solvent cell in interaction with residue $i$ and $f_s(T)$, that of a neat solvent cell.

Fig. 1 – One conformation, of a 16 monomers chain (filled circles) on a two-dimensional lattice. The thick solid lines represent the covalent bonds and the springs the intrachain contacts. The solvent sites are depicted as squares each of which is divided into four solvent cells (triangles). Solvent-solvent interactions involve two adjacent solvent cells (clear triangles) whereas a solvent-monomer bond involves a monomer and a nearest solvent cell (grey triangles).
Taken the extended structures (without any intrachain contacts) as the free energy reference, the effective hamiltonian may be rewritten as a summation of effective couplings between monomers (see the example of figure 2):

$$\mathcal{H}_{\text{eff}}^{(m)}(T) = \sum_{i>j}^{N} B_{ij}^{\text{eff}}(T) \Delta_{ij}^{(m)} \quad \text{with} \quad B_{ij}^{\text{eff}}(T) = B_{ij} - f_i(T) - f_j(T) + 2f_s(T) \quad (4)$$

Recently, Silverstein et al. [12] gave a description of the hydrophobic effect in terms of two energy spectra that best fits their simulation data. These results exhibits a low degenerated, narrow, (respectively high degenerated, extended,) spectra for neat water (respectively for aqueous solution with a non polar solute). Here, this physical picture is reduced further. The energy spectra of the solvent in interaction with monomer $i$ consists of $N_s$ energy values $B_i^{(j)}$, ($j = 1, N_s$) selected from a Gaussian distribution with standard deviation $\sigma$, while the energy spectrum of the neat solvent is given by a unique level, $N_s^\alpha$-fold degenerated, of energy $B_s$. Small values of $B_s$ models bad solvent and large values good solvent. Extending on our previous model, [8] we introduce here an extra parameter $\alpha$, representing the degeneracy ratio between the bulk and the first shell solvent cells. As the total degeneracy of the latter is higher than that of the former [12], one has $\alpha < 1$ and these degeneracies being related to the number of solvent configurations $N_s$ is a large number.

Accordingly, the free energies associated with the neat solvent and that of solvation of each monomer $i$ are respectively given by:

$$f_s(B_s, T) = B_s - \alpha T \ln N_s \quad (5)$$

$$f_i(T) = -T \ln z_i(T) \quad (6)$$

where $z_i(T)$ is the partition function of the solvent around monomer $i$. For large values of $N_s$, it may be written using a continuous formalism as:

$$z_i(T) = N_s \int_{B_{i \text{min}}}^{\infty} n(B_i) \exp \left( -\frac{B_i}{T} \right) dB_i \quad (7)$$

where $n(B_i)$ is the normalized Gaussian distribution truncated at $B_{i \text{min}} = \min_j B_i^{(j)}$, specific to each residue:

$$n(B_i) = \begin{cases} 0 & \text{if} \quad B < B_{i \text{min}} \\
\exp \left( -\frac{B_i^2}{2\sigma^2} \right) & \text{if} \quad B \geq B_{i \text{min}} \end{cases} \quad (8)$$
Equation (6) may therefore be rewritten as:

$$z_i(T) = N_s \exp \left(\frac{\sigma^2}{2T^2}\right) \frac{\text{erfc} \left(\frac{B_{\min}}{\sigma \sqrt{2}} + \frac{\sigma^2}{2T^2}\right)}{\text{erfc} \left(\frac{B_{\min}}{\sigma \sqrt{2}}\right)}$$

(9)

The density of probability that the smallest value of the $N_s$ set, chosen at random with a Gaussian distribution, be $B_{\min}$, is:

$$G_{N_s}(B_{\min}) = N_s \frac{g(B_{\min})}{\sigma \sqrt{2\pi}} \frac{\text{erfc} \left(\frac{B_{\min}}{\sigma \sqrt{2}}\right)}{\text{erfc} \left(\frac{B_{\min}}{\sigma \sqrt{2}} + 1\right)}$$

(10)

where $g(B_{\min})$ is the density of probability to select $B_{\min}$ and $P(x \geq B_{\min})$ the probability to draw a value $x$ larger than $B$. Thus, for each residue $i$, $B_{\min}^i$ is selected from the probability density:

$$G_{N_s}(B) = N_s \frac{\exp \left(-\frac{B^2}{2\sigma^2}\right)}{\sqrt{2\pi}} \left(\frac{1}{2} \text{erfc} \left(\frac{B}{\sigma \sqrt{2}}\right)\right)^{N_s-1}$$

(11)

The state of the chain under each set of conditions is determined from statistical equilibrium averages. For an observable $X^{(m)}$, the average over peptide structures may be defined as:

$$\langle X(B_s, T) \rangle = \sum_{m=1}^{\Omega} X^{(m)} P_{eq}(B_s, T)$$

(12)

with

$$P_{eq}(B_s, T) = \exp \left(-\frac{\mathcal{H}^{(m)}_{eq}}{T}\right) \sum_{m=1}^{\Omega} \exp \left(-\frac{\mathcal{H}^{(m)}_{eq}}{T}\right)$$

(13)

This expression allows the estimate of the chain entropy, $S_{ch}(B_s, T) = -\langle \ln P_{eq} \rangle$, the compactness of the peptide, defined as the average of $N_{ch}^{(m)} = \frac{1}{2} \sum_{i>j} N_{ij}^{(m)}$ where $\sum_{i>j} N_{ij}^{(m)}$ is the number of intra-chain contacts of structure $m$, and the order of the peptide, defined as the average of $Q^{(m)}$, the pairwise contact overlap of the structures with the native conformation $\left(Q^{(m)} = \frac{1}{2} \sum_{i>j} N_{ij}^{(m)} \Delta_{ij}^{nat}\right)$. The number of contacts of the more maximally compact structures (i.e. 9 for the specific chain length studied here), appears in the two above averages in order to normalized them to 1.

For a 16-mer chain model on a two dimensional lattice, the total number of structures is $n_{tot} = 802075$ among which $n_{ext} = 116579$ have zero contact. For given values of $B_s$ and $T$, the point state in the phase diagram is determined by $\langle Q \rangle$, $\langle N_{ch} \rangle$ and $S_{ch}$. When $\langle Q \rangle > 0.66$, the peptide is considered in the Native phase. When $\langle Q \rangle < 0.66$ and $\langle N_{ch} \rangle$ is 0.66, only some compact structures are relevant, and the chain is in the so-called globule state. When $\langle N_{ch} \rangle < 0.66$ and $S_{ch} = \ln n_{ext}$, the peptide is mainly in the extended conformation and the phase is coil type II. Last, when $\langle N_{ch} \rangle < 0.66$ and $S_{ch} > \ln n_{ext}$, almost all chain structures have a non zero probability to occur. This state is referred to as coil-type I.

By setting the model parameters to $\sigma = 0$ and $\alpha = 1$, the temperature dependence of the hydrophobic effect is effectively removed. Under such conditions, the corresponding phase diagrams is similar to that determined by Dinner et al. [3]. On the other hand, the two states phase diagram where the warm and cold denaturation are present [8] may be obtained by setting $\sigma = 2$, $\alpha = 0.5$ and $N_s = 10^5$.

For discussing the four state phase diagram, we set, in the following, the model parameters to $\sigma = 2$, $\alpha = 0.9$ and $N_s = 10^5$. The results are mildly sequence dependant. We therefore
select a particular sequence, and investigate its corresponding phase behavior. \( \langle N_c \rangle \), \( \langle Q \rangle \) and \( S_{ch} \) are displayed in fig.3 as a function of \( B_s \) and \( T \). Several qualitative features are directly observed from the 3-d plots. These may be classified depending on \( B_s \) as follow:

For \( B_s < -7.5 \), the \( \langle Q \rangle \) plots indicate that the peptide is in the native phase at low temperature and in denatured phase at high temperature. Depending on the \( B_s \) value, the transitions in \( \langle Q \rangle \) and \( \langle N_c \rangle \) take place at different temperatures, noted hereafter \( T_w \) and \( T_{ex} \) respectively (i.e. \( \langle Q \rangle (B_s, T_w(B_s)) = 0.66 \) and \( \langle N_c \rangle (B_s, T_{ex}(B_s)) = 0.66 \)). As for \( T > T_{ex} \), the chain entropy becomes an increasing function of temperature (up to \( \ln n_{tot} \)), one may identify three regions corresponding to the following phases: a coil type I phase for temperatures above \( T_{ex} \), a globule phase between \( T_w \) and \( T_{ex} \), and a native state below \( T_w \).

For \( -7.5 < B_s < -2.5 \), in addition to the states described above, transitions toward denatured states (\( \langle Q \rangle \to 0 \)) take place at low temperatures. Such transitions, occurring at temperatures \( T_c \) that depend on \( B_s \), represent cold denaturation. Below \( T_c \), the chain entropy is constant and equals \( \ln n_{ext} \) which indicates that the low temperature region corresponds to the coil type II state.

For \( B_s \) values above -2.5, \( \langle Q \rangle \), \( \langle N_c \rangle \) are very small. The chain is always in a coil state, regardless of the temperature. These values of \( B_s \) are therefore indicative of good solvation. Different states are however observed as shown from the \( \langle N_c \rangle \) and \( S_{ch} \) plots (fig.4). At low temperature, the compactness is rigourously null and the chain entropy equals \( \ln n_{ext} \), indicating that the peptide is in coil type II state. As the temperature increases, so does the entropy until reaching \( \ln n_{tot} \) and the chain is in coil type I phase. To better delineate the
frontier between the coil type I and coil type II regions, we have estimated numerically \( T \frac{dS}{dT} \), the contribution of the chain to the heat capacity of the system as a function of temperature. For \( B_s < 2.0 \), these contributions undergo a maxima at \( T = T_d \), which is a signature of a first order disordered-disordered transition between the two coil phases. For \( B_s > 2.0 \), the peak is no longer observed.

The previous results are summarized in the phase diagram reported in fig.5. For the particular sequence considered here, four states are distinguished. The native, globule and coil-type I phases coexist at the triple point: \((B_s, T) = (-3.4, 1.40)\) and the native, coil-type
I and coil-type II phases coexist at \((B_s, T) = (-2.4, 0.72)\). A critical point is observed at \((B_s, T)_c = (2.0, 4.0)\). Thus, moving along the \(B_s\) and \(T\) axes, transitions from Coil Type I and Coil Type II without crossing any peak in the heat capacity are allowed. Very small and smooth variations in \(S_{ch}\), \(\langle Q \rangle\) and \(\langle N_c \rangle\) occur on these ways. This confirms that Coil Type I and Coil Type II are two phases of the extended state, which implies that warm and cold denaturations are, indeed, transitions toward the same extented state. The existence of a hypothetical supercritical phase for \(B_s > 2.0\) or \(T > 4.0\) is not clear. The nature of the set of structures relevant in such an speculative region should be investigated by the detailed study of effective hamiltonian spectra as function of the temperature.

Last, in the simulations performed with \(\alpha = 0.5\) [8], the Globule and Coil Type I phases disappear leaving only the warm and the cold transitions between Coil Type II and Nat.

In summary, we have shown that the suitable solvation model presented in this paper allows to calculate, for the first time, a four-state phase diagram of a peptide chain. One would need, however, to elucidate the physical meaning of all the model parameters \(N_s\), \(\sigma\) and \(\alpha\) and their relative values for the 20 natural amino acids for a complete understanding of the mechanism responsible for protein folding. Last, it must be understood that similar phase diagrams are obtained if the same value of \(B_{i}^{\text{min}}\) is affected to every residue. However, we choose to select one value of \(B_{i}^{\text{min}}\) for each residue in order to model the specificity of the hydrophobicity of each monomer of the protein.

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