Pathways in Two-State Protein Folding

Audun Bakk and Johan S. Høye
Department of Physics, Norwegian University of Science and Technology, NTNU, N–7034 Trondheim, Norway

Alex Hansen∗ and Kim Sneppen†
International Centre for Condensed Matter Physics, University of Brasília, CP 04513, 70919–970 Brasília–DF, Brazil

Mogens H. Jensen
Niels Bohr Institute, Blegdamsvej 17, DK-2100 Ø, Denmark

(January 9, 2014)

The thermodynamics of proteins indicate that folding/unfolding takes place either through stable intermediates or through a two-state process without intermediates. The rather short folding times of the two-state process indicate that folding is guided. We reconcile these two seemingly contradictory observations quantitatively in a schematic model of protein folding. We propose a new dynamical transition temperature which is lower than the thermodynamic one, in qualitative agreement with in vivo measurement of protein stability using E.coli. Finally we demonstrate that our framework is easily generalized to encompass cold unfolding, and make predictions that relate the sharpness of the cold and hot unfolding transitions.

PACS: 05.70.Jk, 82.20.Db, 87.15.By, 87.10.+e

Proteins fold to a uniquely defined ground state, and do this in spite of the astronomical number of possible states. This paradox, usually attributed to Levinthal, is further sharpened in view of the fact that there is thermodynamic evidence for the folding transition behaving nearly as for a two-state system for a large sub-class of single domain proteins [1–4]. One would think that an on-off process would exclude the possibility of guiding, and indeed simple guiding predicts a first order phase transition which is far softer than experimentally found for the two-state class of proteins. The purpose of this letter is to quantify the degree of guiding that is compatible with the observed two-state folding process. We do this through generalizing a hierarchical protein model introduced earlier in Ref. [5]. In this model guiding dominates the dynamics of the folding process, which in this frame is defined through the Monte Carlo (MC) procedure applied when simulating the stochastic behavior of the model. We find here that guiding can dominate the dynamics of folding and still maintain the thermodynamic behaviour as that of a two-state process.

Another prediction of our scenario is that the cold unfolding transition [6,2] should exhibit a sharpness close to that of the hot unfolding transition. To our knowledge there is no experimental studies of the sharpness of the cold unfolding transition.

The van’t Hoff relation [3],

\[ \Delta H = \alpha k T_c^2 \frac{\Delta C}{Q}, \]

provides a powerful way to quantify the sharpness of a first order phase transition taking place at \( T_c \). It relates the enthalpy difference between the two phases, \( \Delta H \) to the height of the heat capacity peak, \( \Delta C \) and latent heat of the transition \( Q \) with a proportionality factor \( \alpha \). A smaller \( \alpha \) corresponds to a sharper transition. When the transition is two state, \( \alpha = 4 \), and when the transition has a large number of equally stable intermediates, \( \alpha = 12 \). For the single domain proteins, ribonuclease, lysozyme, chymotrypsin, cytochrome c and myoglobin, Privalov and Khechinasvili [1] find experimentally

\[ \alpha = 4.2 \]

(2)

to within 5 % accuracy, demonstrating that these transitions are very nearly two-state. Other small proteins like \( \alpha \)-lactalbumin, RNase H, barnase and cyt c have known metastable intermediates [5].
The two extremes of protein folding is spanned by respectively the two-state and a multiple state “zipper”-like description of the process. We sketch the model and its parametrization briefly here. The relevant degrees of freedom (conformational angles) are modeled through binary variables \( \psi_i \). They either locally match the ordered structure: \( \psi_i = 1 \), or they do not: \( \psi_i = 0 \). Guiding is imposed through the series of inequalities

\[
\psi_i \geq \psi_{i+1}.
\]

The variables \( \psi_i \) alone cannot describe the degrees of freedom that become liberated when a portion of the protein is not matching the local structure of the native state. In order to take these extra degrees of freedom into account, a second, independent set of variables, \( \xi_i \), is introduced. For simplicity, these variables are assigned the values 1 or \( 1 - \xi \). The Hamiltonian is

\[
H = -\sum_{i=1}^{N} \psi_i \xi_i,
\]

with the constraints in effect. The interpretation of the terms in this Hamiltonian is that when there is a local match, \( \psi_i = 1 \), there is an energy cost of \( E \) to change the \( \xi_i \) variable. When there is no match, there is no energy cost associated with changing \( \xi_i \) — it “flaps” freely.

We note that for any finite value of \( E \), the protein may change structure locally due to change in \( \xi_i \) even in the parts of the protein where \( \psi_i = 1 \). In order to simplify the analysis, we assume \( E \) to be sufficiently large compared to any other energy scale in the system — in particular \( kT \), where \( T \) is the temperature — so that the \( \xi_i \) variables never take the value \( 1 - \xi \) when \( \psi_i = 1 \).

We may define a set of binary, unconstrained variables \( \varphi_i \), taking the values zero or one such that

\[
\psi_i = \varphi_1 \cdots \varphi_i.
\]

In particular, \( \psi_1 = \varphi_1 \). In the limit when \( E \to \infty \), the Hamiltonian becomes

\[
H_{p1} = -\varphi_1 - \varphi_1 \varphi_2 - \varphi_1 \varphi_2 \varphi_3 + \cdots - \varphi_1 \varphi_2 \cdots \varphi_N,
\]

where there are no additional constraints. The role of the variables \( \xi_i \) is now played by the degeneracy present in \( \varphi_i \).

One can easily show that \( H \) has a first order phase transition at \( T_c = 1/\log(2) \) where the ordered phase \( \{\varphi_i\} = \{1111 \cdots 1\} \) with energy \( U = -N \) melts to a disordered structure with energy \( U \approx 0 \). Thus \( \Delta H = Q = N \) and \( \Delta C = N^2 \log(2)/2 \) leading to \( \alpha = 12 \). On the other hand if we only consider a rescaled last term

\[
H_{p2} = -N \varphi_1 \varphi_2 \cdots \varphi_N
\]

then one also obtains a phase transition at \( T_c = 1/\log(2) \), with \( \Delta H = Q = N \) but with \( \Delta C = N^2 \log(2)/4 \). Thus in this case \( \alpha = 4 \). There is no guiding in the Hamiltonian since the ground state, \( \{1111 \cdots 11\} \), is one out of the \( 2^N \) possible states, while all the other \( 2^N - 1 \) states are degenerate.

We define time in the model based on the MC method. The values of \( \varphi_i \) are chosen or changed randomly, and acceptance of each choice depends upon the usual Boltzmann factor due to any energy shift connected to this. Time advances by one unit for every attempted update of the \( \varphi_i \) variables. We note, however, that the dynamics of an MC procedure may be different from the actual dynamics of a given Hamiltonian, although properties at thermal equilibrium are properly represented.

The average folding time measured as the typical number of states visited before finding the ground state is widely different in the two models. For the true two-state model the average folding time is \( 2^N/2 \). For the guided system governed by the ground state is found in a time growing as \( N^2 \) when \( T \) is below \( T_c \). To reconcile that a large class of proteins behave as a two-state system with the necessity of being able to reach the ground state in a reasonable time, we now study a combination of the two Hamiltonians and

\[
H_{p} = \lambda_p H_{p1} + (1 - \lambda_p) H_{p2}.
\]

This Hamiltonian has a transition at \( T_c = 1/\log(2) \) for all values of \( \lambda_p \). The behaviour can be parametrized by the smallest \( n \) for which \( \varphi_{n+1} = 0 \). For a given temperature the partial free energy of states characterized by \( n \) is \( F(n) = n (T \log(2) - \lambda_p) - \delta_{n,N} (1 - \lambda_p) N \). In Fig. 1 we show \( F(n) \) schematically for different temperatures \( T \). Each \( F(n) \) exhibits a jump at \( n = N \) corresponding to the energy gain \( N(1 - \lambda_p) \) for reaching the ground state. At low \( T \), \( F(n) \) is monotonically decreasing, reflecting a fast folding kinetics where the typical folding time grows as \( N^2 \).
At an intermediate $T = T_G = \lambda_p / \log(2)$ all $n < N$ are equally probable. For $T$ in the interval between $T_G$ and $T_c$ the intermediate states are unstable (see Fig. 3) — i.e. they form a barrier between the folded and denatured state — and the folding time scale exponentially with both $T$ and $N$. At a higher $T = T_c = 1/\log(2)$ the folded state becomes unstable, and the protein melts ($n \approx 0$). The fact that the free energy landscape changes with $T$ means that two-state folding around $T_c$ is compatible with guiding and fast folding at low $T$.

Fig. 2 shows the van’t Hoff coefficient $\alpha$ as a function of $\lambda_p$ on the unit interval based on direct calculation of the partition function. One observes that increasing $\lambda_p$ — i.e. increasing the guiding — leads to increasing $\alpha$ and thus a softening of the transition. As $N$ is increased, the regime where $\alpha$ is very close to 4 is expanded towards higher values of $\lambda_p$. For example, with the experimental observation of $\alpha = 4.2$, and assuming $N = 10$, $\lambda_p$ is close to zero while for $N = 100$, $\lambda_p$ is approximately 0.7. Thus, in this latter case, 70% of the energy difference between the unfolded and folded states sits in the guiding, and still $\alpha$ is very close to the value indicating the folding process to be essentially a two-state process.

We now discuss the fact that large $N$ allows for more guiding without destroying the two-state nature of the transition. To understand this we note that any $\lambda_p < 1$ in fact define a virtual phase transition at $T = T_G < T_c$. At $T_G$ the protein would melt if it were not due to the additional gain in binding energy when the ground state is reached. This virtual transition is not seen directly in equilibrium thermodynamics, but strongly influences the dynamic behaviour in the temperature range between $T_G$ and $T_c$: In this intermediate regime the protein is a two-state system where occasionally melting implies a long waiting time with many partial refreeze attempts. Due to fluctuations a system with small $N$ can be partly refrozen also above the virtual transition, and thus $\alpha$ depends on system size as shown in fig. 2.

Experimentally, if one are dependent on dynamics one presumably measure $T_G$ as the transition temperature, while for experiments based on thermodynamics it would be $T_c$. For fast living organisms such as _E.coli_ the overall status of fraction of unfolded proteins can be monitored by the level of chaperone DnaK [10,11]. For temperatures between 13 and 37 C the DnaK per _E.coli_ cell raises slowly from 4000 to 6000, whereafter it raises sharply to $\sim 8500$ at 42 C and $\sim 18000$ at 46 C [12,13]. At 50 C the _E.coli_ dies. This may be taken as an indication that in the temperature interval above 37 C the typical proteins need help in the folding process. But as the cell is able to sustain life up to about 50 C, the typical proteins must have some stability up to this higher temperature. This is reminiscent of the behaviour of our model, with a $T_G$ of about 37 C, an exponentially slow folding of proteins, necessitating the help of chaperones, for higher temperatures and a $T_c$ of the order of 50 C [14].

The above considerations can be extended to include a more realistic scenario where the protein is reacting with water. Following ref. 3 we parametrize this through water variables $w_1, w_2, ..., w_N$, taking values $\xi_{\text{min}} + s\Delta$, $s = 0, 1, ..., g - 1$. Here, $\Delta$ is the spacing of the energy levels of the water-protein interactions. We quantify the coupling to the water by a combination of the Hamiltonians

$$H_{w1} = (1 - \varphi_1)w_1 + (1 - \varphi_1\varphi_2)w_2 + ... + (1 - \varphi_1\varphi_2 \cdots \varphi_N)w_N ,$$

and

$$H_{w2} = (1 - \varphi_1\varphi_2 \cdots \varphi_N)(w_1 + \cdots + w_N) ,$$

to form the total Hamiltonian

$$H = \lambda_p H_{p1} + (1 - \lambda_p)H_{p2} + \lambda_w H_{w1} + (1 - \lambda_w)H_{w2} .$$

(Here it may be noted that $H_{w2}$ may introduce _non-local_ interactions between distant units, when the terms are interpreted using the variables $\psi_i$ and $\xi_i$.) When $\lambda_p = \lambda_w = 1$ we are back to the Hamiltonian defined in ref. 3 whereas when $\lambda_p = \lambda_w = 0$ we are facing a two-state Hamiltonian. In Fig. 3 we display the heat capacity curves for these two extremes. The system is folded in its ground state between the cold unfolding transition at $T = 1.2$ and the hot unfolding transition at $T = 4.7$ As also quantified by the van’t Hoff coefficients, we see that the Hamiltonian without guiding gives a phase transition which is about a factor 3 sharper for both the cold and the hot unfolding transitions. Also in terms of temperature, these transitions are much more separated than in real systems where the freezing of water will look much more like “absolute zero”. The present model as it stands is not able to account for this.

In Fig. 4 we investigate systematically the van’t Hoff coefficient $\alpha$ as function of $\lambda_p$ and $\lambda_w$ for the hot (Fig. 4 a) and the cold (Fig. 4 b) transition. As is evident, $\alpha$ is similar but somewhat larger for the hot than for the cold transition. As a consequence, the cold transition transition is slightly sharper. We are not aware of any experimental measurements of the van’t Hoff coefficient for the cold transition. Such a measurement will, however, in practice be hampered as the cold transition is mainly seen experimentally at pH-values where it is close to the hot transition.
Finally, we note the distinct feature of the cold transition $\alpha$ when $(\lambda_p, \lambda_w) \approx (1, 0)$ where it drops to a value below 4. We will show in Fig. 1 that this is a remnant of the phase transition that would have appeared at $T = T_c = 1/\log(2)$ if the system were not coupled to the water.

We summarize by noting that in this protein model, it is easy to reconcile the thermodynamics of a two-state system with the dynamics of a guided system, as this can be done by diminishing either $\lambda_p$ and/or $\lambda_w$ from the value one. The dynamical consequence of the hereby masked guiding is a folding times that is dramatically reduced when temperature moving away from the transition temperature.

We note as final consequence of our model that good folders can be viewed as random sequences of folding steps of which the last have a particularly favorable binding energy thereby securing two state cooperativity.

A.B. thanks the Norwegian Research Council for financial support. A. H. and K. S. thank F.A. Oliveira and H.N. Nazareno for warm hospitality and the I.C.C.M.P. for support during our stay in Brazil. We thank G. Zocchi for countless discussions.

---

[1] P.L. Privalov and N.N. Khechinasvili, J. Mol. Biol. 86, 665 (1974).
[2] P.L. Privalov in Protein Folding, ed. T.E. Creighton (Freeman, San Francisco, 1992).
[3] R.L. Baldwin and G.D. Rose, TIBS 24, 26 (1999).
[4] R.L. Baldwin and G.D. Rose, TIBS 24, 77 (1999).
[5] A. Hansen, M.H. Jensen, K. Sneppen and G. Zocchi, Europhys. J. B 6, 157 (1998).
[6] B.L. Chen and J.A. Schellman, Biochem. 28, 685 (1989).
[7] J.A. Schellman, J. Phys. Chem. 62 1485 (1958).
[8] K.A. Dill, S. Bromberg, K. Yue, K.M. Fiebig, D.P. Yee, P.D. Thomas and H.S. Chan, Protein Science 4, 561 (1995).
[9] K. Binder, ed., Applications of the Monte Carlo Method in Statistical Physics (Springer Verlag, Berlin, 1987).
[10] C.A. Gross, Cellular and Molecular Biology (ASM Press, 1382, 1996).
[11] K.B. Arnvig, S. Pedersen and K. Sneppen, Nordita preprint 1999.
[12] S.L. Herendeen, R. A. VanBogelen and F. C. Neidhardt, J. Bacteriol. 139, 185 (1979).
[13] S. Pedersen, P.L. Bloch, S. Reeh and F.C. Neidhardt, Cell, 14, 179 (1978).
[14] A. Hansen, M. H. Jensen, S. Pedersen and K. Sneppen, in preparation.
[15] A. Bakk, J.S. Haye, A. Hansen, K. Sneppen and M.H. Jensen, in preparation.

---

FIG. 1. A schematical drawing of the partial free energy $F(n)$ as function of the level of folding $n$ for for different temperatures $T$.

FIG. 2. The van’t Hoff coefficient $\alpha$ as a function of $\lambda_p$ for $N = 10$ and 100.

FIG. 3. Heat capacity curves for $N = 50$ system with and without guiding, i.e. with $\lambda_p = \lambda_w = 1$ respectively $\lambda_p = \lambda_w = 0$. The parameters for the water variables are $\epsilon = -3.1$, $\Delta = 0.04$ and $g = 350$.

FIG. 4. van’t Hoff coefficient $\alpha$ for a) hot respectively b) cold transition for $N = 100$ system. Other parameters are as in Fig. 2.
Figure 1

A. Bakk, J.S Hoye, A. Hansen, K. Sneppen and M.H. Jensen

*Pathways in Two-State Protein Folding*
Figure 2

A. Bakk, J.S Hoye, A. Hansen, K. Sneppen and M.H. Jensen

*Pathways in Two-State Protein Folding*
Figure 3
A. Bakk, J.S Hoye, A. Hansen, K. Sneppen and M.H. Jensen
Pathways in Two-State Protein Folding
Figure 4a

A. Bakk, J.S Hoye, A. Hansen, K. Sneppen and M.H. Jensen

Pathways in Two-State Protein Folding
Figure 4b

A. Bakk, J.S Hoye, A. Hansen, K. Sneppen and M.H. Jensen

*Pathways in Two-State Protein Folding*