Entropic Barriers, Frustration and Order: Basic Ingredients in Protein Folding

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We solve a model that takes into account entropic barriers, frustration, and the organization of a protein-like molecule. For a chain of size $M$, there is an effective folding transition to an ordered structure. Without frustration, this state is reached in a time that scales as $M^\lambda$, with $\lambda \approx 3$. This scaling is limited by the amount of frustration which leads to the dynamical selectivity of proteins: foldable proteins are limited to $\sim 300$ monomers; and they are stable in one range of temperatures, independent of size and structure. These predictions explain generic properties of in vivo proteins.

Proteins fold to a well defined three dimensional structure, usually referred to as the native state. However, two basic ingredients of these biomolecules oppose this ordering process. Namely, the large entropy associated with the many possible conformations, and the energetic frustration present in proteins. From a physical point of view, the interplay between order, entropy and frustration poses some fundamental questions as to what the mechanism of the folding process is: “Is it possible to rationalize folding as a relaxation process to thermodynamic equilibrium (as some experiments suggest [1])? Or “is some cell engineered —e.g. chaperon mediated [2]— mechanism needed to understand the folding process? In this paper, we address these questions by solving a protein-like model with all three aforementioned properties. We find that, indeed, under some well defined conditions kinetically foldable proteins can exist. Moreover, the mechanism reconcile very restrictive properties of in vivo globular proteins: their typical size is restricted to about 300 residues (or monomers) and they are stable in a unique range of temperatures!

One obstacle to folding, referred to as Levinthal’s “paradox” [3], has been discussed in several articles dealing with protein folding dynamics [4,5]. It relates to the fact that the time needed to find the native state by sampling at random the protein phase space is of the order of the age of the universe. Nonetheless, proteins fold in plying at random the protein phase space is of the order of the time needed to find the native state by sampling globular conformations, as in regimes II and III. Analytical approaches to understand the role of frustration have lead to the conclusion that random sequences of aminoacids should resemble spin-glasses [4b], and therefore show glassy dynamics. Hence, it has been argued that kinetically foldable sequences (i.e. those that fold fast, say, in a biological time scale) must somehow have minimum frustration [11a].

By the end of regime II, most of the native-like structure has already been acquired. As indicated in Fig. 1, this regime entails an entropy crisis in a rugged energy landscape. To unveil this process, we choose not to describe regime III, avoiding a detailed description of the native state. Thus, we model the folding process to a generic native-like structure. Given a well defined native structure, the microscopic model consists on (a) the definition of what a native-like state is, (b) the characterization of the space of conformations, and (c) the dynamics. In what follows, the Boltzmann constant has been set to one, and we work in adimensional units.

The energetics involve only short range pairwise interactions (paired monomers do not interact). A conformation with $M = 2N$ monomers has at most $N^2$ non-overlapping bonds. (a) There are $N$ native contacts defined as the set of $N$ distinct pairs of residues which are closest in space in the native conformation. All other possible contacts are defined as non-native bonds. Those structures with all $N$ native contacts formed are called native-like states. This definition does not uniquely determine a three dimensional structure, nevertheless this generic native-like state is expected to resemble the overall native structure. (b) We consider two energy scales: $-\varepsilon_N < 0$ for native bonds, and $\varepsilon_{NN}$ for non-native ones. As shown in Fig. 2A, conformations are classified according to their number of native $(i)$ and non-native $(j)$ bonds. Their energy is given by $E = -i\varepsilon_N - j\varepsilon_{NN}$, with $\varepsilon_N \geq \varepsilon_{NN} \geq 0$. The energy constant $\varepsilon_{NN}$ yields an attractive force between non-native bonds, giving rise to a frustrated energy landscape. The ratio $\Delta = \varepsilon_{NN}/\varepsilon_N$ is defined as the frustration parameter (see Fig. 2A).

To obtain the spectrum, we compute recursively the exact crosslinking coefficient $G(i,j)$, i.e. the number of different combinations of $i$ native and $j$ non-native bonds among $M$ distinguishable monomers.
\[ C(q) = \sum_{i,j=0}^{q} C(i,j) \delta_{i+j,q} = \frac{M!}{2^{q} q! (M-2q)!}, \]

where \( q = i + j \) is the total number of bonds formed. There is also a remanent entropy \( S(q) \) associated with the number of conformations that share the same set of paired monomers. For a conformation with, say, one bond \( S(q = 1) = S(l + l_1 + l_2) \), where \( l, l_1 \) and \( l_2 = M - l - l_1 - 2q \) are the length of the loop, and of the two free ends of the polypeptide chain. The entropy of loops and free ends is approximated by that of paired monomers. For a conformation with, say, one bond \( S_0(l) = l \ln w + w \) is a constant (see, e.g., Ref. [6]). This assumes that all bonded pair of monomers is equally likely, regardless to their separation along the chain. Hence, the whole conformational space of our model \( C_T \) can be enumerated as

\[ C_T = \sum_{q=0}^{N} \sum_{i+j=q} C(i,j) \exp[S_0(M-2q)] . \]  

Using (2), we obtain the exact partition function, and therefore all thermodynamic quantities. Fig. 2A shows a scheme of the spectrum for \( \Delta = 1/3 \), and sketches (c) the dynamics chosen to mimic the folding process (explained in detail in Fig. 2B).

Entropy plays a leading role on selecting folding pathways. In particular, noting that the likelihood of forming non-native bonds is much higher than the one of forming native ones, there is a natural tendency to prefer non-native states. In this sense, even with no energy barriers (\( \Delta = 0 \)), i.e. every single state is connected to the ground state by an energy decreasing pathway, non-native states form entropic barriers to folding [3].

The model has a zero temperature transition. As shown in Fig. 3A, the specific heat diverges (logarithmically) as \( M \to \infty \). However, proteins are finite, and for a finite size chain, there is an effective folding transition at \( T = T_f(M) \) (defined by the peak in the specific heat). The inset in Fig. 3A shows that for \( T > T_f \) most bonds are non-native, whereas for \( T < T_f \) the protein orders into a native-like state [3]. The best fit for \( T_f(M, \varepsilon_N, \Delta, w) \) and \( M = 40 - 1600, \varepsilon_N = 3, \varepsilon_{NN} = 0 - 3^\circ, w = 1 - 5 \) yields

\[ T_f(M, \varepsilon_N, \Delta) = 1.01 \varepsilon_N(1/\Delta)/\ln(M) + a/M, \]

where \( a (\simeq 2) \) depends on \( w \) and \( \Delta \), and is used as a fitting parameter for the leading correction-to-scaling term. For the aforementioned range of parameters, (3) deviates from the exact values of \( T_f \) (measured with four significant figures) by less than 1%! Note that the leading term in (3) is independent of \( w \). This can be understood because the folding pathways are mostly determined by the crosslinking coefficient \( C(i,j) \), whereas \( w \) acts as a non-specific entropic weight.

What happens with the dynamics? The time scale to reach equilibrium \( \tau \) is measured by fitting the exponential decay \( \exp(-t/\tau) \) of the long-time deviation from equilibrium of any correlation function. The time \( t \) is measured in updates of the master equation defined by the transition probabilities in Fig. 2B. Time scales are independent of the initial condition. To unveil the role of the entropic barriers we first calculate \( \tau \) with no frustration (\( \Delta = 0 \)). As shown in the inset of Fig. 3B, the equilibrium relaxation time near \( T_f \) diverges as \( M \to \infty \). The divergence of the peak in \( \tau \) scales as \( \tau_\infty \sim M^z \), with \( z = 3.8 \pm 0.25 \) (not shown). An striking observation is that the relaxation time to the native-like state \( (T < T_f) \) scales as \( \tau_0 \approx 0.45 M^\lambda \) with \( \lambda = 3.02 \pm 0.02 \), independent of \( w \) and temperature!

On adding frustration, a frustration limited folding time scale \( \tau_\Delta \) enters into the problem. Hence, as shown in Fig. 3B, below some temperature \( T_\Delta \) the ordered state is achieved in a time scale that diverges as \( T \to 0 \) as

\[ \tau_\Delta \approx 0.5 M^\lambda \exp(2 \varepsilon_N \Delta/T)/w^{4.0}, \]

with \( \lambda = 3.14 \pm 0.07 \). This expression combines both entropic barriers and the largest minimal energy barrier \( 2 \varepsilon_N \Delta \) on the landscape (see Fig. 2A). The dependence on \( w \) is mostly due to the normalization factor of the transition probabilities—likely an artifact of the dynamics.

For \( \Delta = 1/3 \), the peak in \( \tau \) diverges with an exponent \( z = 4.3 \pm 0.2 \) (not shown). More importantly, we find a small range of temperatures around \( T_\Delta = 0.3 \) where—indeed of size—the native-like state is reached in a time scale \( \sim \tau_0 \). Away from this temperature, proteins will either fold too slow \((T < T_\Delta)\) or they will not fold at all \((T > T_f)\). As far as we know, this is the first time a model has predicted the dynamical selection of a whole class of proteins in a well defined range of temperatures! This behavior is robust, however, the size of the stability region and \( T_\Delta \) have a smooth dependence on \( \Delta \) and \( w \). By further increasing \( \Delta \), \( \tau_\Delta \) takes over the relaxation dynamics, even at \( T > T_f \). Fig. 3B shows that already for \( \Delta = 2/3 \) there is no dynamical evidence for the order- ing transition at \( T_f(M) \). Substituting (3) \( T = T_f \) in (4) yields the frustration limited time scale at the transition

\[ \tau_\Delta \approx 0.5 M^\zeta/w^{4.0} \text{ with } \zeta = \lambda + 2\Delta/(1 - \Delta). \]

Thus, a native-like conformation is reached in a folding time scale \( \tau_f \approx \max\{\tau_\Delta, \tau_0\} \). Fast folding sequences will fold in \( \tau_f \approx \tau_0 \) ! Eq. 5 embodies the expected divergence of the folding time in the “glassy” limit \( \Delta \to 1 \). Indeed, as shown in Fig. 4, a small change in \( \Delta \) can lead to an enormous increase in \( \tau_f \).

Our results are in excellent agreement with simulations of random sequences of protein-like chains, where it has been shown that few sequences can fold, while most sequences do not—not even to native-like intermediates [3,4]. Fast folding in a limited range of temperatures has also been observed in Monte Carlo simulations of lattice models of proteins [12,15], and has been suggested
by Wolynes and collaborators [8,11a] in the context of a glass transition.

It can be argued that one update of the master equation should roughly correspond to $t_0 = 10^{-7}$ sec., i.e. time scale to diffuse over some few residues length. This leads to the conclusion that, even when there is no frustration, folding in a biological time scale of seconds is restricted to globular proteins with 300 or less residues (see Fig. 4). Smaller proteins with $M \approx 40$ may fold as fast as $10^{-3}$ sec. With frustration, proteins fold fast if $M \approx 300$ for $\Delta = 1/3$, and $M \approx 20$ for $\Delta = 1/2$. These limited sizes and time scales are reasonable when compared to those found in nature. Hence, we conclude that the typical ratio of free energies of a random (or non-native) bond to that of a native bond must be close to the typical ratio of free energies of a random (or non-native) bond to that of a native bond. Hence, we conclude that the relative free energies of non-bonded interactions should roughly correspond to the relative free energies of non-bonded interactions (see Fig. 4).

The overall relaxation must also involve regime III. The average time to escape from one low-energy native-like state to another will depend on the energy barrier separating them. The structural rearrangement of a native-like state may involve freeing a loop or, at most, a surface from its neighbors. Accordingly, the number of broken bonds should scale as $N^{1/3}$ for a loop, or $N^{2/3}$ for a surface. An estimate for the escape time can then be $t_0 \exp\left[\varepsilon_{NN}(aN)^{1/2}/T\right]$ see [7], smaller than $t_0$ for numbers like $a = 1/3$ and $T = 0.4$. This analysis points out to the conclusion that acquiring the native-like structural features is the rate limiting step of the folding process.

We have solved a non-sequential model of protein folding that focuses on the rearrangement of random conformations to close-to-native structures. The most striking predictions are that, regardless of the details of the model and native structure, folding is limited to a well-defined range of temperatures $T_\Delta \sim T \sim T_f$ and $\varepsilon/k_B T \sim 3-10$— and to globular proteins with $M \approx 300$ or less residues. Away from these limits, proteins do not fold. The model predicts a small (logarithmic) excess of heat at the ordering transition $T_f$. We expect $T_f$ and $T_\Delta$ to be rather close for kinetically foldable proteins [9b]. Hence, experimentally, it may be difficult to resolve the excess of heat from these two transitions. Entropic barriers determine the time needed to find a native-like state, which scales as $M^\lambda$, where $\lambda \approx 3$. Based on polymer dynamics insights, a similar exponent has already been predicted for the second stage of the folding kinetics [11,17]. The dynamics is governed by a multiplicity of folding pathways with non-native-like transients. The limitation to the aforementioned scaling time is the amount of frustration $\Delta$, defined as the relative (attractive) strength of non-native and native bonds. If the amount of frustration is too large ($\Delta > 1/3$), the time scale to reach the native-like state scales as $M^N \zeta$, where $\zeta = \lambda + 2\Delta/(1-\Delta)$. In this case, folding in a biological time scale is slow and restricted to very small protein sizes. We conclude that the acquisition of native-like structure is the rate limiting step of folding. Folding can be rationalized based on thermodynamic stability, at least that of native-like states. Whether overcoming the rather large energy barriers needed to differentiate these states requires the mediation of, say, chaperones remains uncertain.

We thank R. Baeza, N. Bralic, and V. Tapia for stimulating discussions. This work was supported in part by FONDECYT No. 3940016, and DIPUC.

FIG. 1. Scheme of the three stage folding kinetics. I.-Starting from a fully unfolded conformation R, the initial regime corresponds to a fast down-hill energy minimization process, where the ruggedness of the energy surface
plays almost no role. II.- Here, the mostly non-native contacts—i.e. bonds not present in the native state N—rearrange into native ones, reaching fairly stable and compact native-like structures. III.- The third regime corresponds to the search of the native state among a small set of close-to-native conformations separated by rather large energy barriers. These barriers are mostly due to the cooperativity required by excluded volume interactions to change structural features buried in the protein core.

FIG. 2. (A) Spectrum. Each level \([i, j]\) includes all possible distinct conformations with \(i\) native and \(j\) non-native number of bonds. Some typical numbers for \(M = 100\) and \(w = 5\) are shown in parenthesis. The slope \(\Delta\) measures the amount of energetic frustration in the model. Frustration is maximum when \(\Delta = 1\). As indicated by the solid and dotted lines, the spectrum already suggests a multiplicity of pathways for connecting the states. Note, e.g., the minimum energy barrier pathway connecting states \([0, N]\) and \([N, 0]\), which crosses over \(N - 1\) small barriers of size \(\varepsilon_{NN}\) and one of size \(2\varepsilon_{NN}\). (B) Transition probabilities for states \([i, j]\). From each state \([i, j]\) one can either form a new bond (non-local event) or break one (local event). As expected in a real physical situation, the entire temperature dependence is on the backward transition probabilities, and comes from the energy penalty of breaking a bond. The forward probabilities, however, depend on the likelihood of forming a new bond. Given that there are \(q\) bonds formed, the free sites can form \((M - 2q)\) new bonds. \(P_N\) (\(P_{NN} = 1 - P_N\)) is the probability of forming a native bond. Detailed balanced requires an extra factor \(k(q)\) proportional to the ratio of the number of conformations with \(q + 1\) bonds and that with \(q\) bonds. The probabilities are uniformly normalized such that the largest transition probability of the master equation is one half.

FIG. 3. (A) Thermodynamics for \(\varepsilon_N = 3\), \(\Delta = 1/3\), and \(w = 5\). Specific heat (fluctuations of the reduced energy \(\bar{E} = E/T\)) as a function of temperature, for \(M = 24, 50, 100, 200, 400, 800,\) and \(1600\). The dashed line shows the power-law divergence of the specific heat peak as \(T_f(M) \to 0\). The inset shows the average number of native and non-native bonds as a function of temperature. Axes are in adimensional units, and data is exact. Dotted line indicates \(T_f\) for \(M = 50\). (B) Dynamics. Scaled relaxation time for \(\varepsilon_N = 3\) and \(w = 5\) as a function of temperature. Inset shows the case \(\Delta = 0\): For \(T \lesssim 0.4\), the data for all values of \(M\) collapse to a constant (see \(\tau_0\) in text). Main figure shows the cases \(\Delta = 1/3\) and \(\Delta = 2/3\). Solid lines correspond to Eq. 4. The horizontal axes for \(\Delta = 2/3\) has been rescaled by a factor 1.15. The symbols denote \(M = 100 \times, 80 +, 50 \triangle, 30 \bullet, 20 \diamond,\) and \(10 \ast\). Error bars on \(\tau\) are less than 0.4%. Dotted lines are a guide to the eye.

FIG. 4. Scaling of the relaxation time to a native-like state as a function of size and frustration. Dotted line corresponds to the case with no frustration. Dashed line indicates a correspondence between time measured in updates and seconds.
Nr. of Non-Native bonds

\[ 0 \rightarrow 1 \rightarrow j \rightarrow N-2 \rightarrow N-1 \rightarrow N \]

Energy \( \varepsilon_N \)

- \( (10^{69}) \)
- \( (10^{71}) \)
- slope \( \Delta = 1/3 \)
- \( (10^{81}) \)
- \( (10^{80}) \)
- \( (10^{78}) \)
- \( (10^{77}) \)

\[ k(q) = \frac{C(q+1)}{C(q)w^2} \]

A

\[ \alpha_i \frac{\exp(-\varepsilon_N/T)}{q} \quad [i-1,j] \]

\[ \alpha_j \frac{\exp(-\varepsilon_{NN}/T)}{q} \quad [i,j-1] \]

\[ \alpha k(q)P_N \quad [i,j] \quad q = i+j \]

\[ \alpha k(q)P_{NN} \quad [i,j+1] \]
