Linking Rates of Folding in Lattice Models of Proteins with Underlying Thermodynamic Characteristics

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Abstract

We investigate the sequence-dependent properties of proteins that determine the dual requirements of stability of the native state and its kinetic accessibility using simple cubic lattice models. Three interaction schemes are used to describe the potentials between nearest neighbor non-bonded beads. We show that, under the simulation conditions when the native basin of attraction (NBA) is the most stable, there is an excellent correlation between folding times $\tau_F$ and the dimensionless parameter $\sigma_T = (T_0 - T_F)/T_0$, where $T_0$ is the collapse temperature and $T_F$ is the folding transition temperature. There is also a significant correlation between $\tau_F$ and another dimensionless quantity $Z = (E_N - E_{ms})/\delta$, where $E_N$ is the energy of the native state, $E_{ms}$ is the average energy of the ensemble of misfolded structures, and $\delta$ is the dispersion in the contact energies. An approximate relationship between $\sigma_T$ and the $Z$-score is derived, which explains the superior correlation seen between $\tau_F$ and $\sigma_T$. For two state folders $\tau_F$ is linked to the free energy difference (not simply energy gap, however it is defined) between the unfolded states and the NBA.

I. INTRODUCTION

Natural proteins reach their native conformation in biologically relevant time scale of about a second or less starting from an ensemble of denatured conformations [1]. The native state of proteins is also stable (albeit marginally) under physiological conditions [1]. The underlying energy landscape of random sequences is far too rugged [2–4] to be navigated in biologically relevant time scale. Thus, it is believed that protein sequences have evolved so that the dual requirements of stability and kinetic accessibility of their native states are simultaneously satisfied. An important question that arises from this observation is: What are the sequence dependent properties of proteins that govern their foldability? The sequences that satisfy the above stated dual requirements are considered to be foldable, and hence are biologically competent. This and related questions have attracted considerable theoretical attention over the last several years [2–13]. Minimal protein models [2–11,13], which capture some but not all the energetic balances in proteins, are particularly suited to provide a detailed answer to the question posed here.

There have been three proposals in the literature, which have attempted to identify the characteristics of sequences that give some insight into the foldability. Below we briefly
describe the three criteria following the order of their appearance in the literature:

1. By using the random energy model (REM) as a caricature of proteins it has been suggested \cite{4,14,15} that foldable sequences have large values of $T_F/T_{g,eq}$ where $T_F$ is the folding transition temperature and $T_{g,eq}$ is an equilibrium glass transition temperature, which in the original REM model is associated with the temperature at which the entropy vanishes. It has been subsequently realized that in order to utilize this criterion in lattice models $T_{g,eq}$ has to be replaced by a kinetic glass transition temperature \cite{16}.

2. Theoretical considerations and lattice and off-lattice model simulations show that for optimal foldable sequences the collapse transition temperature is relatively close to $T_F$ \cite{9–11,17,18}. In other words, sequences that fold extremely rapidly have small values of

$$\sigma_T = \frac{T_F - T_F}{T_\theta} \tag{1}$$

where $T_\theta$ is the temperature at which the polypeptide chain makes a transition from the random coil state to a set of compact conformations. The characteristic temperatures $T_\theta$ and $T_F$ are **equilibrium properties** that can be altered by not only changing the external conditions, but also by mutations \cite{10,11}. The collapse transition temperature is, in principle, measurable from the temperature dependence of the radius of gyration, which can be measured using small angle X-ray scattering (or neutron scattering) experiments. We have shown that, depending on the values of $\sigma_T$, the very nature of the folding kinetics can be dramatically altered \cite{11,17}. In particular sequences for which $\sigma_T \approx 0$ (here the process of collapse and the acquisition of the native state is **indistinguishable**) fold by two state kinetics. Such sequences are also stable over a wider variety of external solvent conditions. On the other hand, sequences, for which $\sigma_T$ is relatively large, exhibit more complicated kinetics \cite{9,10}.

3. Finally it has been argued that the ”necessary and sufficient” conditions for a sequence to be foldable is that there be a large energy gap (with dimensions kcal/mol) or the native state be a ”pronounced” minimum in energy \cite{19}. The validity of this criterion, even for lattice models, has been questioned in several articles \cite{11,12,13,20,21}.

In general, sequences which fold rapidly (small values of $\sigma_T$) are most easily generated by performing some sort of optimization in sequence space. One popular way of getting optimized sequences is to minimize the dimensionless $Z$-score \cite{22,23} defined as

$$Z = \frac{E_N - E_{ms}}{\delta} \tag{2}$$

where $E_N$ is the energy of the native state, $E_{ms}$ is the average energy of the misfolded (or partially folded) states, and $\delta$ is the dispersion in the contact energies. The purpose of this paper is to investigate if the folding rates are correlated with the $Z$-score in a manner similar to the correlation between folding times $\tau_F$ and $\sigma_T$ \cite{3,11,12,17,18}. We show, using a database of several lattice models of proteins, that there is a significant correlation between $\tau_F$ and the **dimensionless quantity** $Z$-score. The correlation, however, is not as strong as that
seen between $\tau_F$ and $\sigma_T$ at least in these models. The rest of paper is organized as follows. In section II we present the models and the computational protocol. In section III the stability of the native state under the simulation conditions is established. The correlations between $\tau_F$ and $\sigma_T$ and the $Z$-score are also discussed. We also establish an appropriate relationship between $\sigma_T$ and the $Z$-score. The paper is concluded in section IV with some additional remarks.

II. METHODS

A. Lattice Models of Proteins

We model a protein sequence as a self-avoiding walk on a cubic lattice with the spacing $a = 1$. A conformation of a polypeptide chain is given by vectors $\{\vec{r}_i\}, i = 1, 2...N$. The value of $N$ for three sequences is 36 and for the remaining nineteen sequences, $N = 27$. If, two nonbonded beads $i$ and $j$ $(|i - j| \geq 3)$ are nearest neighbors on a lattice, i.e., $|\vec{r}_i - \vec{r}_j| = a$, they form a contact. The energy of a conformation is given by the sum of interaction energies $B_{ij}$ associated with the contacts between beads

$$E = \sum_{i<j} \Delta(|\vec{r}_i - \vec{r}_j| - a)B_{ij},$$

(3)

where $\Delta$ is unity, when $|\vec{r}_i - \vec{r}_j| = a$ and is zero, otherwise. We have used three forms for the contact matrix elements $B_{ij}$ which mimic the diversity of interactions between various amino acids. Sixteen sequences with $N = 27$ in this study have the contact matrix elements $B_{ij}$ obtained from a Gaussian distribution

$$P(B_{ij}) = \frac{1}{\sqrt{2\pi B}} \exp\left(-\frac{(B_{ij} - B_0)^2}{2B^2}\right)$$

(4)

where $B_0$ is the average attraction interaction and the dispersion $B$ gives the extent of diversity of the interactions among beads. The energies for these sequences are measured in terms of $B$ which is set to unity; $B_0$ is taken to be $-0.1$. We will refer to this interaction scheme as the random bond (RB) model. For three other sequences with $N = 27$ and one sequence with $N = 36$ $B_{ij}$ are taken from Table III of ref. [24]. We will refer to this interaction scheme as the KGS model. A modified form of the Miyazawa-Jernigan potentials is used for two $N = 36$ sequences [25]. We will denote this interaction scheme as the MJ model.

Fifteen RB 27-mer sequences used in this study are taken from our previous work [11]. An additional RB 27-mer sequence was included in our database during the course of this work to expand the range of $\sigma_T$ values. Of the sixteen sequences nine have maximally compact native states, while the remaining seven sequences have non-compact native structures. Three KGS 27-mer sequences have identical maximally compact native structures. Similarly three 36-mer sequences have identical maximally compact native conformations. The native conformation of 36-mer sequences is shown in Fig. (1a).

For each sequence we perform Monte Carlo simulations (for details, see ref. [11]) and determined $T_\theta$ and $T_F$ using multiple histogram technique [26], which is described in the
context of protein folding elsewhere [27,29]. Briefly, $T_\theta$ is associated with the peak of the specific heat $C_v$ as a function of temperature. Such estimates of $T_\theta$ coincide with the peak in the derivative of the temperature dependence of the radius of gyration $<R_g>$ [27]. The folding transition temperature is obtained from the peak of the fluctuations in the overlap function, $\Delta \chi$ [9]. These methods have been successfully used to obtain the two characteristic equilibrium temperatures for lattice, off-lattice, and all-atom models of proteins [9,11,28,29]. In Fig. (1b) we show the temperature dependence of $\Delta \chi$, $C_v$, and $d <R_g>/dT$ for the sequence whose native conformation is shown in Fig. (1a). From the peaks of these plots we get $T_F = 0.80$ and $T_\theta = 1.14$, so that $\sigma_T$ (see Eq. (1)) for this sequence is 0.30.

We also computed the Z-score (see Eq. (2)) for the twenty two sequences. The values of $E_{ms}$ were calculated as $E_{ms} = c <B>$, where $c$ is the number of contacts in the misfolded structures and $<B>$ is the average contact energy for a particular sequence. The dispersion $\delta$ is determined from $\delta^2 = <B^2> - <B>^2$, where $<B^2>$ is the average of the square of contact energies. In general, $c$ is equal to the number of contacts in the native state which for maximally compact structures is 28 for $N = 27$ and 40 for $N = 36$. The kinetic simulations are done at sequence dependent temperatures [11,19], which were determined by the condition $<\chi(T_s)> = \alpha$. This criterion for choosing $T_s$ allows several sequences to be compared on equal footing regardless of topology and the nature of interaction potentials used. The value of $\alpha = 0.21$ is chosen so that $T_s < T_F$ for all sequences. This ensures that the native conformation or more precisely the native basin of attraction is the most dominant at $T = T_s$.

The folding times are calculated from the time dependence of the fraction of unfolded molecules, $P_u(t)$ [17]. The function $P_u(t)$ may be computed from the distribution of first passage times. Operationally for every sequence an ensemble of initial denatured conformations (obtained at $T > T_\theta$) is generated. For each initial condition the temperature is reduced to $T_s$ and the dynamics is followed till the first passage time is reached. Typically we generated between 200 to 500 independent trajectories in order to reduce the statistical error in determining $\tau_F$ to about 5%.

### III. RESULTS

(a) **Stability of the native basin of attraction:** Due to the discrete nature of spatial and energetic representation the native state of lattice models of proteins is a single microstate. In the coarse grained energy representation every term in the energy function has Ising like discreteness (see Eq. (3)). Since these models represent a coarse grained caricature of proteins it is useful to define a native basin of attraction (NBA). This is necessary because the idea that the native state is a single microstate is clearly unphysical. The native basin of attraction has a volume associated with it. The larger such a volume is the smoother one expects the underlying energy landscape to be. The probability of being in the NBA is defined as [29]

$$P_{NBA}(T) = \frac{\sum_i \delta(\chi_i \leq \chi_{NBA}) \exp \frac{E_i}{kT}}{\sum \exp \frac{E_i}{kT}}$$

where $\chi_{NBA}$ is the value of the overlap function at the folding transition temperature $T_F$, $E_i$ is the energy of the conformation $i$, and $\chi_i$ is the corresponding value of the overlap. The
overlap function is defined as
\[ \chi = 1 - \frac{1}{N^2 - 3N + 2} \sum_{i \neq j, j \pm 1} \delta(r_{ij} - r_{ij}^N), \] (6)
where \( r_{ij} \) is the distance between the \( i \) and \( j \) beads and \( r_{ij}^N \) is the distance between the same beads in the native conformation. According to Eq. (5) all conformations with overlaps less than \( \chi_{NBA} \) map onto the NBA, which implies that a steepest descent quench would directly lead these conformations to the NBA. The above definition of NBA is physically appealing. For all the sequences \( T_F \), obtained from the peak of fluctuations in the overlap function, nearly coincides with \( T_F \) determined using \( P_{NBA}(T_F) = 0.5 \).

In order to demonstrate the stability of the NBA we have calculated \( P_{NBA}(T_s) \) for all the sequences. Recall that the sequence dependent temperatures at which the simulations are performed are chosen so that \( < \chi(T_s) > = 0.21 \). In Fig. (2a) we show \( P_{NBA}(T_s) \) for the twenty two sequences. For all the sequences the probability of being in the NBA exceeds 0.5, which implies that for the simulation conditions employed here the stability of the native state is automatically ensured. Among the nineteen 27-mer sequences fifteen are exactly the same ones as reported in our previous work \[11\], and the temperatures of the simulations are identical to those used in our earlier studies. Thus, even in our earlier work the stability of the NBA at the simulation temperatures has been guaranteed.

(b) Dependence of folding times on \( \sigma_T \): The folding times for the 22 sequences considered here have been computed using methods described in detail elsewhere \[17\]. In Fig. (2b) we plot the dependence of \( \tau_F \) on \( \sigma_T \). This figure clearly shows that the folding times correlate extremely well with \( \sigma_T \) under conditions when the NBA is stable (see Fig. (2a)). A relatively small change in \( \sigma_T \) can lead to a dramatic increase in folding times. For example, an increase in \( \sigma_T \) by a factor of four results in three orders of magnitude increase in \( \tau_F \). This figure clearly shows that the dual requirements of thermodynamic stability of the NBA and kinetic accessibility of the NBA are satisfied for the sequences with relatively small values of \( \sigma_T \). This verifies the foldability principle which states that fast folding sequences with stable native states have \( T_F \approx T_\theta \) \[30\].

There are two important points concerning the results presented in Fig. (2b): (1) The excellent correlation shown in Fig. (2b) should be considered as statistical i.e., we expect to find such dependence of \( \tau_F \) on \( \sigma_T \) only if a number of sequences over a range of \( \sigma_T \) is examined. This implies that it is not possible to predict the relative rates of folding for sequences whose \( \sigma_T \) values are close. Such sequences are expected to fold on similar time scales. (2) Foldable sequences with small values of \( \sigma_T \) reach the NBA over a wider range of external conditions than those with moderate values of \( \sigma_T \). Since not all naturally occurring proteins fold rapidly it follows that there are proteins with moderate values of \( \sigma_T \) that reach their NBA by complex kinetics \[34,39\]. This involves, in addition to the direct pathway to the native state, off-pathway processes involving intermediates. Such sequences reach the NBA by a kinetic partitioning mechanism \[10,30\].

(c) Relationship between \( \tau_F \) and Z-score: The folding times as a function of the Z-score for our database of sequences are shown in Fig. (3a). It is clear that there is a significant correlation between the two. However, the correlation here is not as good as that in Fig. (2b). The plausible reasons are given in the next subsection in which the relationship between Z-score and \( \sigma_T \) is explored. It is tempting to think that because the numerator of
Z-score is some measure of the so-called stability gap. One can conclude that folding rates are linked just to $E_N - E_{ms}$. We show below that this is not the case.

(d) **Relationship between Z-score and $\sigma_T$:** The significant correlations between the folding times and $\sigma_T$ and Z-score suggest that there might be a relationship between these two dimensionless quantities. We arrive at an approximate relationship between the two which also explains the reasons for the superior correlation between $\tau_F$ and $\sigma_T$.

The rationale for using $\sigma_T$ as a natural criterion that satisfies the dual requirements of stability and kinetic accessibility is the following \[30\]. The transition from compact states to the native state at $T_F$ is usually first order, and neglecting the entropy associated with the native state $T_F$ is approximately given by \[18\]

$$T_F \approx \frac{\left|\delta E_{SG}\right|}{S_{NN}}$$

(7)

where $\delta E_{SG}$ is roughly the stability gap and $S_{NN}$ is the entropy of the (non-native) states whose average energy is roughly $|\delta E_{SG}|$ above the NBA. If there is considerable entropy associated with the NBA this has to be subtracted from the denominator of Eq. (7). Since our arguments do not really depend on this, we ignore it here. The transition from the random coil states to the collapsed states occurs at $T_\theta \approx D/k_B$, where $D$, is the driving force, that places the hydrophobic residues in the core and the polar residues in the exterior creating an interface between the compact molecule and water.

The entropy of the intervening non-native states is a function of $D$. Consider the case of large $D$. In this case the polypeptide chain will undergo a non-specific collapse into one of the exponentially large number of compact conformations. This renders $S_{NN}$ extensive in $N$, where $N$ is the number of amino acid residues in the polypeptide chain. This makes $T_F$ very low even for moderate sized proteins. In the opposite limit, when $D$ is small, there is not enough driving force to create a compact structure. Here again $S_{NN}$ is extensive and as a result $T_F$ becomes low. Thus an optimum value of $D$, which reflects a proper balance of local interactions (leading to secondary structures) and long range interactions (causing compaction and formation of tertiary structure) is necessary \[31\] so that $S_{NN}$ be small enough. This would make $T_F$ as large as possible without exceeding the bound $T_\theta$. Thus, optimizing $\delta E_{SG}$, $S_{NN}$, and $D$ (hence $T_\theta$) leads to small values of $\sigma_T$, which therefore emerges as a natural parameter that determines the folding rates and stability.

Consider the spectrum of states of protein-like heteropolymers. It has been suggested, using computational models \[32\] and theoretical arguments \[15\], that generically the spectrum of states consists of the NBA separated from the non-native states by $\delta E_{SG}$. Above the manifold of non-native states one has the ensemble of random-coil conformations. A lower limit of the energy separating the random coil conformations and the non-native compact structures is $\delta$, which is the dispersion in the energy of the non-native states. (In lattice models $\delta$ is associated with the dispersion in the contact energies). Thus $k_B T_\theta > \delta$. Assuming$^1$ that the density of non-native structures in the energy range $-\delta/2 \leq E - \delta E_{SG} \leq \delta/2$.

$^1$Notice that the arguments do not depend on the precise form of the density of states. All we require is that the density of misfolded states has the functional dependence so that $S_{NN} = \ldots$
is $\Omega_{NN} \simeq (E - \delta E_{SG})^\alpha$ (where $\alpha$ is an even integer) we get $S_{NN} \approx k_B \ln(\delta/\delta_0)$, where $\delta_0$ is a sequence dependent constant. From these arguments it follows that

$$\frac{T_F}{T_0} < \frac{|Z|}{S_{NN} k_B} \approx \frac{|\delta E_{SG}|}{\delta} C \ln \frac{1}{\delta_0}$$

(8)

if $\delta E_{SG}$ is identified with $E_N - E_{ms}$. Thus, maximizing the ratio $T_F/T_0$ (or minimizing $\sigma_T$) is approximately equivalent to minimizing the ratio $Z/(S_{NN}/k_B)$. It is perhaps the neglect of the entropy of the non-native states that leads to the poorer correlation between $\tau_F$ and $Z$-score as compared to correlation between $\tau_F$ and $\sigma_T$ (see Figs. (2b) and (3a)).

(e) Linking $\tau_F$ to various definitions of the energy gap: Since the numerator of the $Z$-score is an estimate of $\delta E_{SG}$, it might be tempting to conclude that there is relationship between $\tau_F$ and the associated energy gap. In the context of minimal models of proteins a number of definitions of the "energy gap" have been proposed. It is useful to document these definitions:

1. **Standard Energy Gap:** The time honored definition of the energy gap for any system (not consisting of fermions) is $\Delta_F = E_N - E_1$, where $E_N$ is the energy of the ground (native) state and $E_1$ is that of the first excited state. This definition is usually deemed inappropriate for protein-like lattice models because a flip of one of the beads can lead to a trivial structural change especially for non-compact native states. Such structures would belong to the NBA (see Eq. (5)). In real models in water, even a casual flip of a residue could involve substantial solvent rearrangements, resulting in significant energy (or enthalpy) penalty.

2. **Compact Energy Gap:** The compact energy gap is $\Delta_{CS} = E_{CSN} - E_{CS1}$ [19], where $E_{CSN}$ and $E_{CS1}$ are the energies of the native state and the first excited state respectively. The superscript $CS$ indicates that these conformations are restricted to the ensemble of maximally compact conformations. It has been shown that the ground states of many sequences are non-compact [11]. Furthermore, in several cases the lowest energy of the maximally compact conformation is much greater than those of the manifold of non-compact non-native structures [11]. The correlation between $\tau_F$ and $\Delta_{CS}$ is, at best, poor (see Fig. (22) of ref. [11]).

3. **Stability gap:** The notion that $\delta E_{SG}$ should play an important role in determining both the stability and the folding rates is based on sound physical arguments [4]. We believe that its close relation to $T_F$ makes the stability gap a very useful physical concept.

4. **Z-score gap:** This is defined as $\Delta_Z = E_N - E_{ms}$, which is the numerator of Eq. (2). This is closely related to $\delta E_{SG}$, and for practical purposes may be identical. The precise value for $E_{ms}$ depends on the given sequence, and even the practitioner. The great

$k_B \ln \Omega_{NN}$ be positive. For example, if $\Omega_{NN}(E) \sim \exp(E - \delta E_{SG})$, then $S_{NN} \sim k_B \ln[\text{sh}(\delta/2\delta_0)]$, where $\delta_0$ is a suitable constant.
utility of the Z-score is that one can use it as a technical device to assess the efficiency of threading algorithm or for generating sequences that are good folders [22,23]. For these two purposes the precise values of $E_{ms}$ do not appear to be very important. Since $E_{ms}$ can be altered freely the definition of $\Delta Z$ is somewhat ambiguous. In this article we have used the same definition of $E_{ms}$ for all sequences, and this allows us to assess the efficiency of the Z-score in determining folding kinetics.

We have tested the relationship between $\tau_F$ and $\Delta Z$ using the database of 22 sequences. A plot of the folding time for the 22 sequences as a function of $\Delta Z$ is given in Fig. (3b). We see a very poor correlation between $\tau_F$ and $\Delta Z$ for all sequences included in our database. There appears to be a link between $\tau_F$ and $\Delta Z$ for the three 27-mer sequences with the KGS potentials but none for the three 36-mer sequences. The number of sequences with the KGS interaction scheme and the modified MJ scheme is too small for meaningful trend to be established. However, it is clear that the overall correlation between $\tau_F$ and $\Delta Z$ is poor.

(e) Probing the correlation between $\tau_F$ and the free energy of stability: The various energy gaps described above do not adequately correlate with $\tau_F$. A plausible reason could be that the energy gaps ignore the entropy of the chain in the denatured states. Here, we explore the idea that the free energy of stability of the native state itself could be an indicator of foldability. Consider a large number of two state folders. In this case only the NBA and the ensemble of unfolded states which have very little overlap with the native state are significantly populated. From a physical point of view, the appropriate equilibrium quantity that could correlate with the folding rates is the free energy difference between the two states. We consider the free energy of stability defined for two state folders (with small values of $\sigma_T$) as

$$\Delta F_{U-N} = -k_B T_s \ln K(T_s)$$

with the equilibrium constant

$$K(T_s) = \frac{P_{NBA}(T_s)}{1 - P_{NBA}(T_s)}$$

where $T_s$ is the simulation temperature and $P_{NBA}(T)$ is given in Eq. (5). In experiments $\Delta F_{U-N}$ should be replaced by $\Delta G_{H_2O}$ which gives the stability of the native state in the limit of zero denaturant concentration.

In order to examine the dependence of $\tau_F$ on $\Delta F_{U-N}$ we singled out the two state folders from our database. For these sequences we computed $\Delta F_{U-N}$ using Eq. (9). In Fig. (4) we plot $\tau_F$ as a function of $\Delta F_{U-N}$. We do find important correlation which approaches the quality of that shown in Fig. (2b). For the two state folders it is clear that $\Delta F_{U-N}$ is a good estimate of $k_B T_F$ and hence $T_\theta$ (since $\sigma_T$ is small). Thus the correlation seen in Fig. (4) is not entirely unexpected.

IV. CONCLUSIONS

The variations in folding times for a variety of sequences under conditions when the native basin of attraction is the most populated can be understood in terms of $\sigma_T$ (see Eq. 
Thus, the simultaneous requirements of thermodynamic stability and kinetic accessibility are satisfied for sequences for which $\sigma_T$ is small. Such sequences are foldable over a broad range of external conditions.

It might be tempting to conclude that computation of $T_\theta$ and $T_F$ for lattice models requires exhaustive simulations. This is not the case. In order to get reasonably accurate estimate of $T_\theta$ and $T_F$ by multiple histogram method we find that, for most sequences, between 8 to 10 trajectories each with about 50 millions of Monte Carlo steps are sufficient. By comparison, reliable determination of folding kinetics time scales requires a few hundred trajectories at various temperatures. Thus, $\sigma_T$ is a useful criterion for designing fast folding sequences. By contrast, notice that when a Z-score optimized sequence is generated, its thermodynamics (as well as kinetics) is \textit{a priori} unknown. A separate set of simulations has to be performed at various temperatures in order to obtain its thermodynamics.

There is a significant correlation between Z-score and the rates of folding. This correlation is not nearly as good as the one between $\tau_F$ and $\sigma_T$. The connection between $\tau_F$ and the Z-score suggests that this could arise because the entropy of the non-native states (or more precisely the entropy difference between the non-native states and the native basin of attraction) is not taken into account in the Z-score. More importantly, the Z-score does not appear to be easily measurable making its experimental validation difficult, if not impossible.

There appears to be no useful predictive relationship between the various energy gaps and the folding times. In seeking a correlation involving energetics and entropy of the unfolded and folded states we have found that for two state folders the \textit{free energy of stability of the native state with respect to unfolded states} correlates well with the folding times. Note that this quantity includes the entropies of the NBA and the unfolded states. The correlation between the folding time and $\sigma_T$, and with the free energy of stability for two state folders can be verified experimentally.

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Fig. (1) (a) The conformation of the native state of a 36-mer MJ sequence. This sequence is SQKWLERGATRIADGDLPVNGTYFSCKIMENVHPLA, where we have used the one letter representation of the amino acids. This conformation is the lowest energy conformation in the native basin of attraction. (b) Temperature dependence of the fluctuations in the overlap function \(\Delta \chi\) (solid line), specific heat \(C_v\) (dotted line), and the derivative of the radius of gyration with respect to temperature \(d < R_g > /dT\) (dashed line) for the sequence whose native state is displayed in Fig. (1a). The scale for \(C_v\) and \(d < R_g > /dT\) is given on the right. The collapse temperature \(T_\theta\), obtained from the larger peak of specific heat \(C_v\) curve, is found to be 1.14. It is seen that \(T_\theta\) is very close to the temperature at which \(d < R_g > /dT\) reaches maximum (at 1.19). The two peaks in \(d < R_g > /dT\), with the smaller one coinciding with the location of the maximum in \(\Delta \chi\), suggest that from a thermodynamic viewpoint a three state description is more appropriate for this sequence. The value of \(T_F\), which is associated with the peak of \(\Delta \chi\), is 0.80. Therefore, for this sequence collapse and folding transition temperatures are separated by a large interval, and \(\sigma_T\) (=0.30) is consequently large. For this sequence the value of \(T_F\) obtained from the condition \(P_{NBA}(T_F) = 0.5\) is 0.79, which nearly coincides with the peak position of \(\Delta \chi\). In majority of the sequences we only observe one peak in \(C_v\) and \(d < R_g > /dT\). Hence, it is necessary to introduce independent order parameters to determine \(T_F\).

Fig. (2) (a) The values of the probability of being in the native basin of attraction \(P_{NBA}\) at the sequence dependent simulation temperatures \(T_s\) for the database of 22 sequences considered in this study. The horizontal dotted line corresponds to \(P_{NBA} = 0.5\). This figures shows that at the simulation temperatures \(P_{NBA}\) exceeds 0.5 which implies that the stability criterion is automatically satisfied. (b) Plot of the folding times \(\tau_F\) as a function of \(\sigma_T\) for the 22 sequences. This figures shows that under the external conditions when the NBA is the most populated there is a remarkable correlation between \(\tau_F\) and \(\sigma_T\). The correlation coefficient is 0.94. It is clear that over a four orders of magnitude of folding times \(\tau_F \approx \exp(-\sigma_T/\sigma_0)\) where \(\sigma_0\) is a constant. In both panels the filled and open circles are for the RB and KGS 27-mer models, respectively. The open squares are for \(N = 36\).

Fig. (3) (a) The dependence of \(\tau_F\) on the Z-score. There is a significant correlation between the folding times and the Z-score. Since the scales for the Z-score depend on both the interaction scheme and the length of the sequence it is hard to fit the data for all 22 sequences. If we restrict ourselves to the 16 sequences in the RB model, we find that the correlation coefficient is 0.70, which is not nearly as good as in Fig. (2b). (b) Plot of \(\tau_F\) as function of \(\Delta_Z\) which is the energy gap that appears in the numerator of Eq. (2). This figure clearly shows that there is no correlation between \(\tau_F\) and the \(\Delta_Z\). The various symbols are the same as in Fig. (2b).

Fig. (4) This figure shows, for the two state folders only, the dependence of \(\tau_F\) on the free energy of stability \(\Delta F_{U-N}\) of the NBA with respect to denatured states. Notice that \(\Delta F_{U-N}\) is not an energy gap. It includes the entropies of the folded and unfolded states explicitly and is obtained from the equilibrium constant between the unfolded states and the NBA at the simulation temperature \(T_s\).
