Emergence of stable and fast folding protein structures

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Abstract

The number of protein structures is far less than the number of sequences. By imposing simple generic features of proteins (low energy and compaction) on all possible sequences we show that the structure space is sparse compared to the sequence space. Even though the sequence space grows exponentially with $N$ (the number of amino acid residues) we conjecture that the number of low energy compact structures only scales as $\ln N$. This implies that many sequences must map onto countable number of basins in the structure space. The number of sequences for which a given fold emerges as a native structure is further reduced by the dual requirements of stability and kinetic accessibility. The factor that determines the dual requirement is related to the sequence dependent temperatures, $T_\theta$ (collapse transition temperature) and $T_F$ (folding transition temperature). Sequences, for which $\sigma = (T_\theta - T_F)/T_\theta$ is small, typically fold fast by generically collapsing to the native-like structures and then rapidly assembling to the native state. Such sequences satisfy the dual requirements over a wide temperature range. We also suggest that the functional requirement may further reduce the number of sequences that are biologically competent. The scheme developed here for thinning of the sequence space that leads to foldable structures arises naturally using simple physical characteristics of proteins. The reduction in sequence space leading to the emergence of foldable structures is demonstrated using lattice models of proteins.

I. INTRODUCTION

The primary building blocks of proteins are $\alpha$-helices (one dimensional ordered structures), $\beta$-sheets (contain two dimensional order), and loops of varying bending rigidity. From these seemingly simple building blocks (referred to as elements of secondary structure) complex three dimensional topological structures emerge [1]. The number of distinct topological folds is suspected to be about a thousand - a relatively small number [3]. The emergence of such structures, which should also be (under folding conditions) kinetically accessible on
biologically relevant time scale of about one second, raises a number of conceptually interesting questions. Two such questions we address in this article are: (a) From the dense sea of sequence space how do viable protein structures emerge? (b) Among the possible candidate sequences for proteins, all of which can fold into a specific (or closely related) structures, what factors determine the kinetic accessibility of the native (or ground) state?

Both questions require elaboration. Consider the first question. The number of possible sequences for a protein with $N$ amino acids is $20^N$ which, for $N = 100$, is approximately $10^{130}$. The number of folds in natural proteins, which are low free energy compact structures, is clearly far less than the number of possible sequences. The first question (posed in the previous paragraph) is connected with the dramatic reduction that occurs from the dense sea of sequence space to the countable number of viable protein structures when some physical restrictions are placed on the nature of acceptable protein structures. This question can be quantitatively answered using simple lattice models.

The second question concerns the thermodynamic stability and kinetic accessibility of the native state of a candidate sequence with a well defined low energy structure. A sequence must have the desired topological structure which is thermodynamically (at least marginally) stable under folding conditions. Typically, the native structures of single domain proteins are stable (with respect to unfolded state) by about $(8 - 25)k_BT$ or $(5 - 15)\text{kcal/mol}$ [1]. Furthermore, the putative native conformation must be kinetically accessible on biologically relevant time scales of less than about $1\text{s}$. This dual requirement of the stability and kinetic accessibility sharply restricts the number of sequences that can be candidate proteins. Using general theoretical arguments and computations we will give a simple criteria, in terms of sequence dependent properties, that can discriminate between sequences that satisfy the dual requirement and those that do not.

II. FROM DENSE SEQUENCE SPACE TO LOW DENSITY STRUCTURE SPACE

As mentioned in the Introduction the sequence space is extremely dense in the sense that the number of possible sequences goes as $20^N$. Clearly only an extremely small fraction of the sequences encodes protein structures. A quantitative understanding of the mapping between sequences and structures may be obtained using lattice models of proteins. It is worth recalling a couple of physical characteristics of folded states of natural proteins. In general, proteins in their native states are relatively compact. The dense interior of protein structures is largely made up of hydrophobic residues. With these two restrictions on the native structures we will show that, although the number of sequences is astronomically large, the number of compact low energy structures (protein-like) is considerably smaller. This would imply that for many sequences the low energy compact structures could be nearly the same. In other words, the basins of attraction in the structure space are rare enough so that a large number of sequences map on to precisely one basin. This plausibility, which we demonstrate using lattice models of proteins, naturally explains the emergence of greatly limited number of structures from the sea of sequence space [4].

We use lattice models of proteins to illustrate the mapping from sequence space to structure space. Lattice models of proteins are highly simplified coarse grained representations of polypeptide chains. Several aspects of protein topology, such as secondary structures and loops, are not clearly reproduced in such simplified caricatures of proteins. Nevertheless,
studies from several groups \cite{3-7} over the last decade illustrate that certain general aspects of folding of proteins can be, at least, qualitatively gleaned using simple models. They are especially suited for answering conceptual issues such as the ones posed in this article.

In the coarse grained representation of proteins each amino acid is represented as a bead. Thus, the polypeptide chain becomes a set of connected beads. The identity of the amino acid is specified by the precise interactions with other beads when they are near neighbors in a lattice. This coarse grained representation may be thought of as an $\alpha$-carbon representation of the polypeptide chain that roughly mimics the tertiary interactions in proteins. Later in this article we will use a slightly more realistic lattice model that involves an approximate description of side chains. With this simple model the following interactions in a polypeptide chain are taken into account: (i) connectivity of the chain describing the backbone; (ii) excluded volume effects which prevent the various amino acid residues from occupying the same lattice site; (iii) approximate interactions between residues that are far away along the sequence but are near each other spatially, i.e., tertiary interactions that confer the globular topology of proteins.

The positions of $N$ beads $\vec{r}_i$, $i = 1, 2, ..., N$ represent the conformation of the polypeptide chain. For concreteness we choose a cubic (or square) lattice with lattice spacing $a$. The energy of a given conformation is taken to be

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

where $\delta_{r,a}$ is the Kronecker delta function. The matrix elements $B_{ij}$ give an estimate of the strength of the tertiary interactions between the residues $i$ and $j$, when they are near neighbors on the lattice. In this model, the sequence is specified by the matrix elements $B_{ij}$. We will consider two forms for the contact interaction matrix to ensure that the conclusions are robust to changes in the potential function.

**2D HP model:** In order to probe the nature of the structure space in proteins we first consider relatively short chains in dimension $d = 2$. In this example, there are only two kinds of amino acid residues, namely hydrophobic (H) and polar (P). Various aspects of the resulting HP model (two letter code) introduced by Dill and coworkers \cite{8} can be investigated by exact enumeration for short enough chains ($N \lesssim 25$ or so). In this model a sequence is given by the nature of the amino acid residue at a given position. For example, HPHPH, is an example of a sequence for $N = 5$. There are $2^N$ total sequences for a given $N$. In the HP model the matrix $B$ is 2x2 and its elements are $B_{HH} = -\epsilon$ and all others are zero. Despite the simplicity of the model it is not exactly solvable because of the chain connectivity and excluded volume effects.

Given a sequence, all possible conformations and their energies may be computed for $N \lesssim 25$ in $d = 2$. We are particularly interested in compact structures (CSs) and the subset of CSs with minimum energies (MECSs) \cite{9}. The minimum energy compact structures (MECSs), which have protein-like properties, require that the ground states have H residues surrounded by a large number of hydrophobic residues as is topologically allowed. Clearly, because of restrictions due to connectivity and self-avoidance not all sequences can have ground state topologies that satisfy this crucial protein-like criterion. From this argument we expect that the structure space is considerably more sparse than the sequence space of proteins.
This can be made quantitative using lattice models. For the HP model there are two relevant parameters, namely, the total number of beads $N$ and $N_H$, which is the number of hydrophobic residues. The compact structures (CSs) are the ones with the largest number of nearest neighbor contacts. The MECSs are obtained from the set of CSs and are identified to be those with the lowest energy. For a given value of $N$ and $N_H$ we generated all possible \( \binom{N}{N_H} \) sequences. Exact enumeration of all CS is possible for $N < ∼ 22$ using the Martin algorithm [10]. For $N = 23$ to 26 computations of the number of CSs and MECSs were done using a finite number (≈ $10^4$) of randomly selected sequences [9].

The number of CSs in its most general form may be written as [11]

$$C_N(\text{CS}) \approx Z^N_1 N^{d-1} N^{\gamma_c-1},$$

(2)

where $\ln Z$ is the conformational free energy (in units of $k_B T$), $Z_1$ is a surface fugacity term, $d$ is the spatial dimension, and $\gamma_c$ measures possible logarithmic corrections to the free energy. We found that the appropriately averaged series (see [9] for details) for $C_N(\text{CS})$ is smooth and no evidence for surface fugacity term is observed. More importantly, there appears to be no logarithmic correction to the free energy, i.e., $\gamma_c \approx 1$ [9]. The value of $\ln Z$ is consistent with the mean field prediction that is $Z \approx q/e$, where $q$ is the lattice coordination number. Thus, for all practical purposes $C_N(\text{CS}) \approx (q/e)^N$ [12], which shows that the number of compact structures grows exponentially with $N$.

Protein-like structures are not only compact but also have low energy (we assume that the entropy associated with the native state is negligible) [1]. With this in mind, we have computed the number of compact structures that have the lowest energy, i.e., MECSs. From the viewpoint of evolution, it can be argued that the time scale for mutations is so long that for all practical purposes the sequences can be considered to be quenched. Accordingly, we have calculated the quenched average $C_{N,H}(\text{MECS}) = \exp[\ln c_{N,H}(\text{MECS})]$, where the averaging is performed over all possible sequences and $c_{N,H}$ is the number of MECS for a given sequence. This number has a minimum at $N_{H}^{\text{min}} \approx 0.6$ [3], which implies that in general protein-like sequences should be slightly more hydrophobic. Analysis of PDB structures indeed shows that the average fraction of hydrophobic residues is about 0.55 [13]. The most significant finding of this work is that $C_{N,H}^{\text{min}}$ does not appear to grow with $N$. This has prompted us to conjecture that $C_{N,H}^{\text{min}} \approx \ln N$.

There are implications of the spectacular finding that the number of MECSs, which have protein-like characteristics, is very small and does not grow significantly with the size of the polypeptide chain. The sequence space is extremely large even with the restriction that the fraction of hydrophobic residues is 0.6. Nevertheless, we find that the number of possible low energy compact structures is extremely small. This implies that many sequences must map onto a given basin of attraction corresponding to one MECS. In other words, the number of sequences with a given fold has to be sufficiently large. Our results do not specify the density of sequences for every possible low energy compact structure or a likely protein fold. Presumably, this depends on the topology and the exact potentials used. We believe that the central result on the mapping to the low density of structure space compared to the sequence space is robust and independent of specific potentials or the underlying lattice.

Random Bond Model: One might argue that the HP model is too simple because it does not adequately capture the diversity of interactions in proteins which is thought to be responsible for specificity. In order to test the generality of the results (mapping of many sequences to
In natural proteins the fraction of hydrophobic residues changes. We set $\Delta$ to be constant and equal to 1. The choice of $B_0$, which controls the fraction of hydrophobic interactions, is important. We expect that the value of $B_0$ should determine the size of conformational space of minimum energy structures (MESs) defined below. In order to check the variations in our results we used several values for the hydrophobic parameter $B_0$, namely, 0, -0.1, -2. The fraction of hydrophobic residues $\lambda_H$ can be related to the hydrophobic parameter $B_0$ as follows. Since it is natural to attribute negative $B_{ij}$ to those between hydrophobic residues and positive ones to those between hydrophilic residues, one can specify the boundary energies $B_H$ and $B_P$ ($B_H = -B_P$) in such a way that the energies $B_{ij}$ below $B_H$ corresponds to hydrophobic interactions and the energies above $B_P$ pertain to hydrophilic interactions. The values of $B_{ij}$ occurring between $B_H$ and $B_P$ are associated with mixed interactions. If the number of hydrophobic and hydrophilic residues is $N_H$ and $N_P$, respectively, $(N_H + N_P = N)$, the fraction of hydrophobic energies $\lambda$ among $B_{ij}$ is roughly $(N_H/N)^2 = \lambda_H^2$. This fraction (i.e., $\lambda$) can be also obtained by integrating Eq. (3) from minus infinity to the energy $B_H$. The relationship between $\lambda_H$ and $B_0$, thus, is given by $\lambda_H \simeq \sqrt{\lambda} = \sqrt{\int_{-\infty}^{B_H} P(B_{ij})dB_{ij}}$. The precise values of $B_H$ and $B_P$ can be determined if one considers the case with $B_0 = 0$, for which $N_H = N_P$. The result is $B_H = -0.675$. Clearly, when $B_0 = 0$ one half of all residues is hydrophobic and the others are hydrophilic. When $B_0 = -2$, the fraction of hydrophobic residues $\lambda_H$ increases to about 94 percents, i.e., the sequences are largely made up of hydrophobic residues. The choice of $B_0 = -0.1$ is motivated by the observation that in natural proteins the fraction of hydrophobic residues $\lambda_H$ is about 0.55.

We used the Martin algorithm, which ensures exhaustive enumeration of all self-avoiding conformations, to explore the conformational space of the polypeptide chain of a given length. In order to reduce the sixfold symmetry on the cubic lattice we fixed the direction of the first monomeric bond in all conformations. The remaining conformations are related by eightfold symmetry on the cubic lattice (excluding the cases when conformations are completely confine to a plane or straight line). To decrease further the number of conformations to be analyzed the Martin algorithm was modified to reject all conformations related by symmetry.

We define MES as those conformations, whose energies lie within the energy interval $\Delta$ above above the lowest energy $E_0$. Several values for $\Delta$ were used to ensure that no qualitative changes in the results are observed. We set $\Delta$ to be constant and equal to 1.2 (or 0.6) (definition (i)). We have also tested another definition for $\Delta$, according to which $\Delta = 1.3|E_0 - tB_0|/N$, where $t$ is the number of nearest neighbor contacts in the ground state (definition (ii)). It is worth noting that in the latter case $\Delta$ increases with $N$. Both definitions yield equivalent results. Using these definitions for $\Delta$, we computed $C(MES)$ as a function of the number of residues $N$.

The computational technique involves exhaustive enumeration of all self-avoiding conformations for $N \leq 15$ on cubic lattice. In doing so we calculated the energies of all conformations according to Eq. (1), and then determined the number of MES. Each quantity, such as
the number of MES, $C(MES)$, the lowest energy $E_0$, the number of nearest-neighbor contacts $t$ in the lowest energy structures, is averaged over 30 sequences. Therefore, when referring to these quantities, we will imply their average values. To test the reliability of the computational results an additional sample of 30 random sequences was generated. Note that in the case of $C(MES)$ we computed the quenched averages, i.e., $C(MES) = \exp[\ln[\langle c(MES) \rangle]]$, where $c$ is the number of MES for one sequence.

The number of MES $C(MES)$ is plotted as a function of the number of residues $N$ in Fig. (1a) for $B_0 = 0$ and $\Delta = 0.6$. A pair of squares at given $N$ represents $C(MES)$ computed for two independent runs of 30 sequences each. For comparison, the number of self-avoiding walks $C(SAW)$ and the number of CS $C(CS)$ are also plotted in this figure (diamonds and triangles, respectively). The most striking and important result of this graph is the following: As expected on general theoretical grounds, $C(SAW)$ and $C(CS)$ grow exponentially with $N$, whereas the number of MES $C(MES)$ exhibits drastically different scaling behavior. There is no variation in $C(MES)$ and its value remains practically constant within the entire interval of $N$ starting with $N = 7$. We find (see Fig. (1)) that $C(MES) \approx 10^1$. This result further validates our earlier finding for two dimensional model. These results suggest that $C(MES)$ grows (in all likelihood) only as $\ln N$ with $N$.

It is interesting to compare the changes in $C(MES)$ with respect to $B_0$. In Fig. (1b) we show the results for $C(MES)$ for $B_0 = -0.1$. This figure demonstrates clearly that there is no qualitative difference between $B_0 = 0$ and $B_0 = -0.1$ as far as the behavior of $C(MES)$ is concerned. On an average $C(MES)$ at $B_0 = -0.1$ differs from that at $B_0 = 0$ by only 19%. In general, $C(MES)$ decreases as $B_0$ is lowered. Similarly, we find that just as for $B_0 = 0$ and $-0.1$ the dependence of $C(MES)$ on $N$ for $B_0 = -2.0$ is unchanged (data not shown). These observations prompt us to suggest that the conjecture that $C(MES) \sim \ln N$ may be generic.

It is well known that the native states of proteins are compact but are not maximally so. The number of maximally compact low energy structures $C(MECS)$ is clearly a function of $B_0$ which is the driving force for collapse of the chain. We find that for $B_0 = 0$ on an average only 7% of MES are maximally compact. The corresponding result for $B_0 = -0.1$ is 10%. For $B_0 = -2.0$ we find that there is a dramatic increase (not unexpectedly) in the fraction of MECS. Depending on the precise value of $\Delta$ the fraction of MECS is either 76% ($\Delta = 1.2$) or 86% ($\Delta = 0.6$). In either case, we find that the vast majority of MES are also maximally compact.

These additional computations on 3D RB model reaffirm our earlier conclusions that the number of low energy compact structures at best increases with $N$ as $\ln N$. This explains quantitatively the drastic reduction in going from the sequence space to the structure space. It is also worth emphasizing that the low energy structures (protein-like) are not maximally compact which is in accord with recent study.

3D HP model: The calculations described above suggest that upon imposing minimal restrictions on the structures (compactness and low energies) the structure space becomes sparse. As suggested before this must imply that each basin of attraction (corresponding to a given MES) in the structure space must contain numerous sequences. The way these sequences are distributed among the very slowly growing number (with respect to $N$) of MES, i.e., the density of sequences in structure space, is an important question. This was beautifully addressed in the paper by Li et. al (LWC) [15]. They considered a three dimensional ($N = 27$)
cubic lattice. By using HP model and restricting themselves to only maximally compact structures as putative native basins of attractions (NBA) they showed certain basins have much larger number of sequences. In particular, they discovered that one of the NBAs serves as a ground state for 3794 (total number is $2^{27}$) sequences and hence was considered most designable. The precise density of sequences among the NBAs is clearly a function of the interaction scheme. These calculations and the arguments suggested earlier [9] point out that since the number of NBA for the entire sequence space is small it is likely that proteins could have evolved randomly. In particular, it is possible that many of the naturally occurring folds correspond to basins of attraction in the structure space so that many sequences have these folds as the native conformations, i.e., these are highly designable structures in the language of LWC [15]. These ideas have been further substantiated by Lindgard and Bohr [16], who showed that among maximally compact structures there are only very few folds that have protein-like characteristics. These authors also estimated using geometrical characteristics and stability arguments that the number of distinct folds is on the order of a thousand. All of these studies confirm that the density of the structure space is sparse. Thus, each fold can be designed by many sequences. From the purely structural point of view nature does have several options in the sense that many sequences can be "candidate proteins". However, there is also evolutionary pressure to fold rapidly [17] (i.e., a kinetic component to folding). This requirement further restricts the possible sequences that can be considered as proteins, because they must satisfy the dual criterion of reaching a definite fold on a biologically relevant time scale.

III. FOLDABILITY

The emergence of structures, even preferred ones, may be understood in terms of simple models described in the previous section. From a biological viewpoint there are two equally significant criteria that a protein-like sequence must satisfy: (a) A given sequence must fold reversibly to the stable native state; (b) The native state must be kinetically accessible on a biologically relevant time scale $t_B$ (for many small proteins $t_B$ is several ms [18]). The dual criterion should be satisfied for a sequence to be biologically competent. Sequences that satisfy the dual criterion are termed foldable. (We do not consider here sequences that can be made to reach the native conformation using molecular chaperones.)

We have proposed that there is a sequence dependent quantity that can be used to discriminate between fast and slow folding sequences [19,20]. This foldability criterion can be described in terms of the underlying characteristic temperatures that determine the "phases" of the polypeptide chain. It is well known that at sufficiently high temperatures proteins are random coil-like and are in the same universality class as self-avoiding walks. At the collapse transition temperature, $T_\theta$, a transition to compact structures takes place. This is analogous to the collapse process that occurs in homopolymers when the solvent quality is altered.

The collapse transition in proteins may be first order for the following reasons. Proteins (especially small ones) are highly designed. This means that the hydrophobic residues are arranged along the chain so that topological frustration (incompatibility of low energy structures on small and long length scales) is minimized. The collapse occurs upon burial of hydrophobic residues which, in aqueous medium, requires some free energy cost. This may
render the transition first order. It is likely that the order may be altered upon addition of certain co-solvents.

A polypeptide chain that has a unique ground state is made up of several types of amino acid residues. The presence of these types of residues allows for a discrimination among all the compact structures. This implies that there is another transition at a lower temperature $T_F$ below which the folded native state is stable. The folding transition temperature $T_F$ is normally determined using an order parameter that distinguishes between the unfolded state (or a stable intermediate) and the native state. The transition at $T_F$ is, in general, first order.

By using theoretical arguments and many numerical simulations we have proposed that the foldability index

$$\sigma = \frac{(T_\theta - T_F)}{T_\theta}$$  \hspace{1cm} (4)

determines the foldability of a given sequence. We have unambiguously demonstrated that sequences with small values of $\sigma$ fold efficiently in a such a way that collapse and folding are almost synchronous [24]. Such sequences are highly optimized so that they can fold efficiently over a wide range of temperatures. On the other hand, sequences for which the foldability index has intermediate values reach the native conformation by following complicated kinetics [20]. In these cases only a fraction of the initial population of molecules reaches the native conformation efficiently while the remaining fraction gets trapped in metastable intermediates. Clearly such sequences are not as well optimized and hence they are able to reach the native conformation only over a relatively narrow temperature range. If the foldability index is relatively large (> 0.7 or so) they do not reach the native conformation on biologically relevant time scale. The folding of such sequences might require molecular chaperones. It is surprising that the various anticipated mechanisms for protein folding can be classified in terms of an experimentally measurable foldability index $\sigma$.

We will illustrate the foldability principle using the 3D cubic lattice model with side chains [21]. To this end, we select two 15-mer sequences referred to as A and B with dramatically different thermodynamic and kinetic properties. (Both of the sequences employ Kolinski-Godzik-Skolnick interaction potentials for $B_{ij}$ [22].) The first sequence, A, the ground state of which is displayed in Fig. (2a), is a two-state folder [21]. The collapse temperature $T_\theta$ inferred from the location of the maximum in the specific heat $C_v$ is equal to 0.27 (in reduced temperature units). Alternatively, one may determine the collapse temperature from the temperature dependence of the radius of gyration $<R_g>$ assuming that at $T_\theta$ the derivative $d <R_g>/dT$ is maximal. This gives the same value of $T_\theta = 0.27$. This confirms, in contrast to erroneous claims in the literature, that $T_\theta$ is the collapse transition temperature, i.e., it is the temperature at which there is a dramatic reduction in $<R_g>$. Since we are dealing with finite systems such transitions are not sharp. Thus, the large change in $<R_g>$ from the unfolded random coil state to compact conformations occurs over a temperature interval $\Delta T_\theta$. The smaller $\Delta T_\theta$ becomes the sharper the collapse transition would be.

The folding transition temperature $T_F$ is defined as the temperature at which the fluctuations in overlap function $<\chi>$ reaches a maximum [20]. We find that for sequence A $T_F = 0.26$. Therefore, the value of the parameter $\sigma$ (Eq. (4)) for this sequence is 0.04. The cooperativity of folding transition may be inferred from the dimensionless parameter [21].
\[ \Omega_c = \frac{T_{\text{max}}^2}{\Delta T} \frac{d < \chi >}{dT}, \]

where \( T_{\text{max}} \approx T_F \) is the temperature at which \( d < \chi > /dT \) is maximum and \( \Delta T \) is the full width at half maximum of \( d < \chi > /dT \) transition in an infinite system \( \Omega_c \rightarrow \infty \). Thus, \( \Omega_c \) is a quantitative measure to which the folding transition is cooperative. For finite sized proteins \( \Omega_c \) would not be very large. Analysis of experimental data suggests that \( 5 \leq \Omega_c \leq 100 \) for typical proteins that undergo a two-state transition \[21\]. For sequence A we find \( \Omega_c = 5.3 \), which given the small size of a model protein suggests a highly cooperative two-state transition.

The characteristic temperatures for sequence B (see Fig. (2b) for the ground state) are as follows. The collapse transition takes place at \( T_\theta = 0.44 \), at which \( C_v \) has a peak (or 0.48 if the location of the maximum in \( d < R_g > /dT \) is considered). The folding temperature \( T_F \) is found to be 0.30. Thus, sequence B has the value of \( \sigma = 0.32 \). It is important to point out that the cooperativity index \( \Omega_c \) for sequence B is significantly smaller being equal to 2.0.

According to the foldability principle the fast folding sequences, i.e., those which fold thermodynamically and kinetically by a cooperative two-state transition, are characterized by small values of \( \sigma \). In contrast, sequences, which fold via intermediates and display low cooperativity of folding, have large \( \sigma \). Thus, we expect that sequence A should be a fast (i.e., the native state is reached rapidly) two-state folder, while B is likely to be a slow folder with well populated intermediate(s). The data given above indicate that sequence A for which \( \sigma = 0.04 \) folds almost three times more cooperatively (as measured by \( \Omega_c \)) than B does. The lower cooperativity of folding of B is related to the presence of intermediates. Indeed, the thermal distribution of \( \chi \) values \( h(\chi) \) at the folding transition is bimodal for A since only two states, folded, \( \mathbf{F} \), and unfolded, \( \mathbf{U} \), are significantly populated at \( T_F \) (Fig. (3a)). In contrast, \( h(\chi) \) for sequence B has several dominant peaks, of which the middle one located at \( \chi \sim 0.3 \) is attributed to an equilibrium intermediate (Fig. (3b)).

Kinetics: The analysis of folding kinetics of sequences A and B further highlights the dramatic differences in their folding properties. In order to calculate the folding time \( \tau_F \) we computed the distribution of first passage times \( \tau_{1i} \) from hundreds of individual folding trajectories. The first passage time \( \tau_{1i} \) is the first time a given trajectory labeled \( i \) reaches the native conformation starting from initially unfolded state. If the distribution of \( \tau_{1i} \) is known it is straightforward to calculate the fraction of unfolded molecules \( P_u(t) \) which have not yet reach the native state at time \( t \). The profiles of \( P_u(t) \) can be accurately fit with exponentials that provides a reliable method for computing \( \tau_F \) as \( \tau_F = \int_0^\infty P_u(t)dt \). We have already calculated the time dependence of the radius of gyration \( <R_g(t)> \) averaged over multiple folding trajectories.

For sequence A we found that the function \( P_u(t) \) decays exponentially over a wide temperature range \( T \geq 0.85T_F \). For example, Fig. (4) shows that at \( T = 0.94T_F \) \( \tau_F = 2.07 \times 10^6 \) MCS and folding is two-state, i.e., \( P_u(t) \sim (1 - e^{-t/\tau_F}) \). The time dependence of the radius of gyration reveals two collapse stages - the initial extremely rapid burst phase followed by a more gradual chain compaction (Fig. (4)). Consequently, \( <R_g(t)> \) is well fit with the sum of two exponentials with the times scales \( \tau_1 = 0.00831 \times 10^6 \) MCS and \( \tau_2 = 0.698 \times 10^6 \) MCS. We interpret \( \tau_2 \) as the overall time scale for compaction, i.e., \( \tau_2 = \tau_c \), while the first time scale \( \tau_1 = \tau_{bc} \) is identified with the burst phase. From this, we obtain that the ratio of
folding and collapse time scales $\tau_F/\tau_c$ is 3.0.

One of the questions of great current experimental interest \cite{24,25} is the precise relationship between the dynamics of chain collapse (as measured by the decay of $< R_g(t) >$ with the characteristic time $\tau_c$) and the time for acquisition of the native conformation, namely, $\tau_F$. It has been suggested that non-specific collapse of the chain (this happens for sequences with large $\sigma$) inevitably slows down folding \cite{26}. Sequence A collapses rapidly and the subsequent folding is also fast. This seemingly puzzling observation may be understood in terms of theoretical arguments \cite{26} and earlier computations on lattice models without side chains. We had shown that as long as the initial collapse leads to native-like structure \cite{19,26} (i.e., this is a specific collapse), then subsequent folding can be rapid. This is precisely what takes place in sequence A. Recent experiments have also come to similar conclusions \cite{24,25}. The ratio $\tau_F/\tau_c$ will depend on $\sigma$ and external conditions, but for sequences which undergo specific collapse we expect $\tau_F/\tau_c < 10$.

Sequence B folds on dramatically longer time scales. For example, $\tau_F$ at $T = T_F$ is $106 \times 10^6$ MCS. For comparison, for sequence A $\tau_F$ at $T_F$ is only $1.84 \times 10^6$ MCS. Therefore, sequence A folds 60 times faster than B. This finding is in accord with the values of $\sigma$, which for A and B are 0.04 and 0.32, respectively. This exercise illustrates the validity of the foldability principle based on $\sigma$: The eight-fold difference in $\sigma$ values between these sequences results in sixty-fold difference in their folding times. Clearly, the description of folding of these two sequences is given here for illustration purposes and full validation of the foldability principle requires a study of much larger database of sequences. This has been done in a number of papers \cite{20,27}, in which exhaustive examination of many sequences, both for lattice and off-lattice models, offered a reliable statistical proof of the foldability principle.

The foldability criterion, illustrated using two sequences A and B, gives the factor that determines the sequences that are biologically competent. From the mapping of sequence space to structure space we found that a large number of sequences can have similar topology for the native conformation. However, the requirement of stability and kinetic accessibility of the native conformation further restricts the number of biologically competent sequences. It is remarkable that a single sequence dependent thermodynamic parameter $\sigma$ (which is experimentally measurable) controls the foldability of protein sequences.

**IV. CONCLUSIONS**

In this article we have used simple models to show how stable (under folding conditions) and fast folding protein structures could have evolved. By merely studying the mapping between sequence space and structure space (with the focus on minimum energy structures) it is easy to understand why only a small fraction of sequences can have protein-like characteristics. The importance of using low energy as a natural selection scheme has also been emphasized by Abkevich et. al \cite{28}. As pointed out by these authors it is likely that in pre-biotic evolutionary process the simple requirement of solubility in water may have inhibited the synthesis of sequences that are highly hydrophobic. The arguments developed here and elsewhere \cite{9} show that indeed the optimal fraction of hydrophobic residues should not be significantly greater than 0.5. Such an optimal value not only ensures the stability of folded structures in water, but also greatly reduces the number of minimum energy struc-
tured. For biological viability the dual requirement of stability and kinetic accessibility is vitally important. The intrinsic sequence dependent parameter $\sigma$ (see Eq. (1)) determines foldability of sequences. By this two step analysis we are able to rationalize how through evolutionary pressure nature may have solved the Levinthal paradox in sequence space, i.e., the navigation of sequence space to synthesize functional proteins. The functional requirement, namely, that certain residues have precise positions in the folded state of proteins may further reduce the number of sequences that are biologically competent. We represent the reduction scheme in Fig. (5). The number of generations required for a random sequence to evolve to functionally competent proteins is an interesting questions. In principle, this can be addressed using simple caricatures of proteins [29].

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Fig. (1) Scaling of the number of MES \( C(\text{MES}) \) (squares) is shown for two values of the hydrophobic parameter \( B_0 \), 0 (panel (a)) and -0.1 (panel (b)) and \( \Delta = 0.6 \). Data are obtained for the cubic lattice. The pairs of squares for each \( N \) represent the quenched averages for different samples of 30 sequences. The number of compact structures \( C(\text{CS}) \) and self-avoiding conformations \( C(\text{SAW}) \) are also displayed to underscore the dramatic difference of scaling behavior of \( C(\text{MES}) \) and \( C(\text{CS}) \) (or \( C(\text{SAW}) \)). It is clear that for both values of \( B_0 \) \( C(\text{MES}) \) remains flat, i.e. it grows no faster than \( \ln N \).

Fig. (2) Native structures of sequences A and B are shown in the panels (a) and (b), respectively. Dark grey side chains indicate hydrophobic residues, whereas light grey ones represent hydrophilic amino acids. Both of the conformations are compact with well-defined hydrophobic core. Figure is generated using program RasMol.\[23\].

Fig. (3) Thermally weighted distributions of states \( h(\chi) \) quantified using the overlap function \( \chi \) at \( T = T_F \) for sequences A (panel (a)) and B (panel (b)). The profiles \( h(\chi) \) are strikingly different. In the upper panel only two states, \( \chi \sim 0.6 \) (unfolded) and \( \chi < 0.2 \) (folded), are populated and consequently \( h(\chi) \) is bimodal. The profile \( h(\chi) \) for B indicates the existence of the third populated intermediate state at \( \chi \sim 0.3 \). Accordingly, sequences A and B are classified as two- and three-state folders, respectively.\[23\].

Fig. (4) The time dependence of the normalized radius of gyration \( <R_g(t)> \) and the fraction of unfolded molecules \( P_u(t) \) for sequence A at \( T = 0.94 T_F \). Data are averaged over 100 (for \( <R_g(t)> \)) or 600 (for \( P_u(t) \)) trajectories. \( P_u(t) \) decays exponentially with the time scale \( \tau_F = 2.07 \times 10^6 \) MCS. The approach of \( <R_g(t)> \) to equilibrium is well described by two exponentials with the times scales \( \tau_{bc} = 0.083 \times 10^6 \) MCS and \( \tau_c = 0.698 \times 10^6 \) MCS, where \( \tau_{bc} \) corresponds to extremely rapid burst phase and \( \tau_c \) manifests more gradual final compaction. The ration \( \tau_F/\tau_c \) is approximately 3.0.\[23\].

Fig. (5) Schematic illustration of the stages in the drastic reduction of sequence space in the process of evolution to functionally competent protein structures.
Fig. 1a

Fig. 1b
Fig. 4
Fig. 5