Why Do Proteins Look Like Proteins?

Hao Li, Robert Helling, Chao Tang, and Ned Wingreen
NEC Research Institute, 4 Independence Way, Princeton, New Jersey 08540
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Protein structures in nature often exhibit a high degree of regularity (secondary structures, tertiary symmetries, etc.) absent in random compact conformations. We demonstrate in a simple lattice model of protein folding that structural regularities are related to high designability and evolutionary stability. We measure the designability of each compact structure by the number of sequences which can design the structure, i.e., which possess the structure as their nondegenerate ground state. We find that compact structures are drastically different in terms of their designability; highly designable structures emerge with a number of associated sequences much larger than the average. These structures are found to have “protein like” secondary structure and even tertiary symmetries. In addition, they are also thermodynamically more stable than ordinary structures. These results suggest that protein structures are selected because they are easy to design and stable against mutations, and that such a selection simultaneously leads to thermodynamic stability.

Natural proteins fold into unique compact structures despite of the huge number of possible configurations [1]. It has been established since Anfinsen that for most single domain proteins, the information coded in the amino-acid sequence is sufficient to determine the three dimensional folded structure, which is the minimum free energy structure [2]. It is evident that protein sequences must be selected by nature such that they fold into unique three dimensional structures. Since folding maps sequences to structures, it is quite natural to ask whether selection principles also apply to nature’s choice of structures. Protein structures often exhibit a high degree of regularity, e.g., rich secondary structures (α helices, β sheets) and sometimes even striking tertiary symmetries, which are absent in random compact structures. What is the origin of these regularities? Does nature select special structures for design? What are the underlying principles governing the selection of structures?

In this letter, we report our recent results from a simple model of protein folding which suggest some answers to the above questions. We focus on the properties of each individual compact structure, by finding out the sequences which have the given structure as their non-degenerate ground state. We show that the number of sequences $N_S$ associated with a given structure $S$ differs drastically from structure to structure, and that preferred structures emerge with $N_S$ much larger than the average. These special structures are “protein like” with secondary structures and symmetries, and are thermodynamically more stable than ordinary structures.

Our results are derived from a minimal model of protein folding, which we believe captures the essential ingredients of the problem. In this model, a protein is represented by a self-avoiding chain of beads placed on a discrete lattice, with two types of beads used to mimic polar (P) and hydrophobic (H) amino acids [3]. A sequence is specified by a choice of monomer types at each position on the chain, $\{\sigma_i\}$, where $\sigma_i$ could be either H or P, and $i$ is a monomer index. A structure is specified by a set of coordinates for all the monomers $\{r_i\}$. The energy of a sequence folded into a particular structure is given by short range contact interactions,

$$H = \sum_{i<j} E_{\sigma_i \sigma_j} \Delta(r_i - r_j),$$

where $\Delta(r_i - r_j) = 1$ if $r_i$ and $r_j$ are adjoining lattice sites but $i$ and $j$ are not adjacent in position along the sequence, and $\Delta(r_i - r_j) = 0$ otherwise. Depending on the types of monomers in contact, the interaction energy will be $E_{HH}$, $E_{HP}$, or $E_{PP}$, corresponding to H-H, H-P, or P-P contacts respectively (see Fig.2).

The above simple model has some justification in nature. It is known that the major driving force for protein folding is the hydrophobic force [4]. The tendency of amino acids to avoid water drives proteins to fold into a compact shape with a hydrophobic core, and such a force is effectively described by a short range contact interaction. Although there are twenty different types of amino acids in nature, quantitative analysis of real protein data reveals that they fall into two distinct groups (H or P) according to their affinities for water [5]. There is also experimental evidence that certain proteins can be designed by specifying only this HP pattern of the sequence [6].

We choose the interaction parameters in Eq. (1) to satisfy the following physical constraints: 1) compact shapes have lower energies than any non-compact shapes; 2) hydrophobic (H) monomers are buried as much as possible, expressed by the relation $E_{PP} > E_{HP} > E_{HH}$, which lowers the energy of configurations in which H’s are hidden from water; 3) different types of monomers tend to segregate, expressed by $2E_{HP} > E_{PP} + E_{HH}$. Conditions 2) and 3) were derived from our analysis of the real protein data contained in the Miyazawa-Jernigan matrix of inter-residue contact energies between different types of amino acids [3].
We have studied the model on a three dimensional cubic lattice and on a two dimensional square lattice. We focus on the designability of each compact structure. Specifically, we count the number of sequences $N_S$ which have a given compact structure $S$ as their unique ground state. This requires identification of the minimum energy compact conformations of each sequence. Since all compact structures have the same total number of contacts, we can freely shift and rescale the interaction energies, leaving only one free parameter. Throughout this paper, we choose $E_{HH} = -2.3$, $E_{HP} = -1$ and $E_{PP} = 0$ which satisfy conditions 2) and 3) above. The results are insensitive to the value of $E_{HH}$ as long as both these conditions are satisfied.

As a result of this complete enumeration, we obtain all possible sequences which “design” a given structure, i.e., have that structure as their unique ground state. We denote by $N_S$ the number of sequences associated with a structure $S$. In this way, the number $N_S$ is a measure of the designability of a given structure, and we have this information for all compact structures.

A surprising result is that compact structures differ drastically in terms of their designability. There are structures that can be designed by an enormous number of sequences, and there are “poor” structures which can only be designed by a few or even no sequences. For example, the top structure can be designed by 3794 different sequences ($N_S = 3794$), while there are 4256 structures for which $N_S = 0$. The number of structures having a given $N_S$ decreases monotonically (with small fluctuations) as $N_S$ increases (see Fig. 1(a)). There is a long tail to the distribution. Structures contributing to the tail of the distribution have $N_S >> N_S = 61.72$, where $N_S$ is the average number. We call these structures “highly designable” structures. The distribution is very different from the Poisson distribution which would result if the compact structures were statistically equivalent. For a Poisson distribution with a mean $N_S = 61.72$, the probability of finding even one structure with $N_S > 120$ is already $1.76 \times 10^{-6}$.

We observe that highly designable structures have certain secondary structures absent in random compact structures. We examine the compact structures with the ten largest $N_S$, and find that all have parallel running lines folded in a regular way (see Fig. 2 for a typical example). The number of straight lines (three amino acids in a row) found in these structures is 8 or 9, while the average structure has only 5.4 straight lines.

To make sure that the above results are not artifacts of small size ($3 \times 3 \times 3$ cube), we have also done systematic studies of size dependence in two dimensions (the study of larger structures in 3D is not practical due to limits of computing power). We have studied systems of sizes $4 \times 4$, $5 \times 5$, $6 \times 5$, and $6 \times 6$ on a 2D square lattice. For systems of sizes $6 \times 5$ and $6 \times 6$, a random sampling of sequences is performed. To compare systems of different sizes, appropriate rescaling of the axes is necessary. We choose bin sizes for $N_S$ to be proportional to $N_S$, and rescale the number of structures by a factor proportional to the total number of structures. For the $6 \times 5$ and $6 \times 6$ cases, to make sure that the random sampling of sequences produces a reliable distribution, we double the number of sequences until a fixed distribution is reached.

We find that the systems of different sizes in 2D all have the same qualitative behavior as that found in 3D. In each case, we find that there are highly designable structures which stand out. For the $6 \times 5$ and $6 \times 6$ systems where the total numbers of structures are sufficiently large to produce smooth distributions, we find that the two distributions have nearly identical shapes (see Fig. 1(b)). We find that the tail of the 2D distribution can be fitted by an exponential function (see insert

![FIG. 1. (a): Histogram of number of structures with a given number of associated sequences $N_S$ for 3D $3 \times 3 \times 3$ case, in a log-log plot. (b): Histogram of number of structures with a given $N_S$ for 2D $6 \times 5$ (filled triangle) and $6 \times 6$ (open square) case, in a log-log plot. The bin size and rescaling of $y$ axis are explained in the text. Insert: same data in a semi-log plot.](image-url)
to Fig. 1(b)). In contrast the tail in the 3D case falls off slightly slower than exponential.

Similar to the 3D case, we observe that the highly designable structures in 2D also exhibit secondary structures. In the 2D $6 \times 6$ case, as the surface to interior ratio approaches that of real proteins, we find several interesting features. Specifically, we find that the highly designable structures often have bundles of pleats and long strands, reminiscent of $\alpha$ helices and $\beta$ strands in real proteins; in addition, some of the highly designable structures have tertiary symmetries (see Fig. 2 for a typical structure).

A striking property of the highly designable structures is that they are, on average, thermodynamically more stable than other structures. The stability of a structure can be characterized by the average energy gap $\overline{\delta_S}$, averaged over the $N_S$ sequences which design the structure. For a given sequence, the energy gap $\delta_S$ is defined as the minimum energy required to change the ground state structure to a different compact structure. For the 3D $3 \times 3 \times 3$ structures, we find that there is a strong correlation between the number of sequences $N_S$ and the average gap $\overline{\delta_S}$ (see Fig. 3). Highly designable structures have average gaps much larger than those of structures with small $N_S$, and there is a sudden jump in $\overline{\delta_S}$ for structures with $N_S \approx 1400$. The number of structures with large gaps is 60. The abrupt jump in $\overline{\delta_S}$ is somewhat unexpected compared to the smooth distribution of $N_S$. Such an abrupt transition provides a useful way of differentiating the special, highly designable structures from the ordinary ones. According to this distinction, highly designable structures are only a small fraction (0.12%) of all the compact structures.

The fact that highly designable structures are more stable than other structures can be understood qualitatively in the following way. Consider a particular sequence associated with a highly designable structure $S$. A mutation of the sequence may change the energy of the structure $S$ as well as those of the competing structures. If the gap is large, it is less probable that the energies of the competing structures will shift below that of the structure $S$. Thus the structure $S$ is likely to stay as the ground state of the mutant. Therefore, a large gap is

![Fig. 2. Structures with largest number of $N_S$ for 3D $3 \times 3 \times 3$ case (top) and 2D $6 \times 6$ case (bottom). The sequences are one of the $N_S$ possible sequences. Beads colored black are of H type, and beads colored light grey are of P type. Two beads are considered to be in contact if they are nearest neighbors but not connected by the backbone.](image)

![Fig. 3. Average gap of 3D $3 \times 3 \times 3$ structures plotted against $N_S$ of the structures.](image)
likely to correlate with a large number of sequences \( N_S \) which design the structure.

An important approach in studying real protein structures is to study mutation effects and homologous sequences (sequences related by a common ancestor in the past). In our simple model, we call the \( N_S \) different sequences that design the same structure “homologous”. We have analyzed mutation patterns of the homologous sequences for highly designable structures. The analysis reveals phenomena similar to those observed in real proteins. For example, we find sequences with no apparent similarities (with different types of monomer at more than half of the sites) which can design the same structure. We also find some sites are highly mutable while some sites are highly conserved. The conserved sites for a given structure are generally those sites with the smallest or largest number of sides exposed to water. Fig. 4 shows the probability \( P_P \) of finding a P monomer at a particular site, calculated for the structure with largest \( N_S \) for the 3D \( 3 \times 3 \times 3 \) case and the 2D \( 6 \times 6 \) case (structures shown in Fig. 2). For the 3D case, we find sites which are perfectly conserved with \( P_P = 0 \) and \( P_P = 1 \).

\[
S = \sum_{sites} \left[ -P_P \ln(P_P) + (P_P - 1) \ln(1 - P_P) \right].
\]

\( N_{est} \) is a good order-of-magnitude estimate for \( N_S \). For example, for the top structure in 3D \( 3 \times 3 \times 3 \) case, \( N_{est}/N_S \approx 3.5 \). However, for the less designable structures, \( N_{est} \) drastically overestimates \( N_S \). The large deviation starts at \( N_S \approx 1400 \), at the boundary between large gap and small gap structures. This indicates that for highly designable structures, the mutations are roughly independent, while they are highly correlated for other structures.

Although our results for the 3D case were derived for small structures (\( 3 \times 3 \times 3 \)), we believe similar results hold for much larger structures. There is evidence to this effect from recent studies of design on larger structures by Yue and Dill using a similar model. In a few cases studied by Yue and Dill, they found that sequences with a small ground state degeneracy corresponded to structures with certain protein-like secondary structures and tertiary symmetries. In light of our findings, we believe that such protein-like structures are the highly designable structures with large \( N_S \). This interpretation is different from that of Yue and Dill, who suggest that having minimal degeneracy is enough to produce protein-like secondary structure and tertiary symmetries. From our results, we know that it is possible to find sequences to uniquely design even “poor” structures. It is the requirement that \textit{many} sequences design a particular structure which leads to protein-like secondary structures and tertiary symmetries.

Although the detailed structures of real proteins are determined by many factors, e.g., hydrogen bonding, shapes of the amino acids, etc., our results from the simple model suggest that there is a principle of design and evolutionary stability which should play a crucial role in the selection of protein structures, i.e., real protein structures must be highly designable and mutable. Since highly designable structures are also more stable, such a selection principle solves the thermodynamic stability problem simultaneously. From an evolutionary point of view, highly designable structures are more likely to be picked through random selection of sequences in the primordial age, and they are stable against mutations.

Our proposed principle of selection based on designability and mutability should have important corollaries in protein structure prediction and design. If in fact nature only selects highly designable structures, then structure prediction algorithms should limit the search of the conformational space to these special structures, which could be only a tiny fraction of the total number of possible structures. In fact, a quite successful algorithm
for structure prediction has been developed recently, by using the templates from known protein structures \[10\]. Our study lends theoretical support to such an approach. Further improvement depends on finding practical ways of identify highly designable structures.

In conclusion, we find that there is a small fraction of compact structures which are highly designable and mutable. These preferred structures often have protein-like secondary structure and even tertiary symmetries. We find that highly designable structures are also more stable thermodynamically. These results suggest that high designability and evolutionary stability should play a crucial role in the selection of protein structures, and that such a selection principle leads to thermodynamic stability at the same time.

An important question to ask is what is the kinetic accessibility of these structures, and are there any other selection principles imposed by the kinetics? It is likely that the highly designable structures are also easier to fold into, due to the large gap in their excitation spectrum \[11\]. We have performed successful preliminary folding simulations for some highly designable structures. A more systematic study of kinetics, including ordinary structures is underway.

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* Present address: The second Institute for Theoretical Physics, DESY/University of Hamburg, Hamburg, Germany.

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