Protein structures and optimal folding emerging from a geometrical variational principle

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Novel numerical techniques, validated by an analysis of barnase and chymotrypsin inhibitor, are used to elucidate the paramount role played by the geometry of the protein backbone in steering the folding to the correct native state. It is found that, irrespective of the sequence, the native state of a protein has an exceedingly large number of conformations with a given amount of structural overlap compared to other compact artificial backbones; moreover the conformational entropies of unrelated proteins of the same length are nearly equal at any given stage of folding. These results are suggestive of an extremality principle underlying protein evolution, which, in turn, is shown to be associated with the emergence of secondary structures.

The rapid and reversible folding of protein-like heteropolymers into their thermodynamically stable native state [1] is accompanied by a huge reduction in conformational entropy [2]. Evidence has been accumulating for an achievement of the entropy reduction through a folding funnel which favors the kinetic accessibility of the native state [3]. Some fundamental questions remain, however, unanswered. What makes proteins特殊 compared to random heteropolymers? What guides the folding of a protein? Is it the sequence that is fundamental or its native structure?

In this letter, we examine these issues and focus on the special role played by the native structure of proteins, with no input of information regarding amino acid sequences. The study is carried out through a novel theoretical probe for the conformation space of proteins: a measure of the density of alternative conformations (DAC) having a given overlap or percentage of contacts in common with a fixed native structure. We demonstrate with studies on chymotrypsin inhibitor (2ci2) and barnase (1a2p) that the DAC provides key information on the folding nucleus [10]. An analysis of the DAC for real protein structures and for artificially generated decoy ones suggests that an extremal principle is operational in nature, which maximizes the DAC at intermediate overlap, providing a large basin of attraction [4] for the native state and promoting the emergence of secondary structures.

Operationally, our study consists of the determination of the number of alternative structures which have a given structural similarity to a putative native state. The structural similarity between the native structure and an alternative one is defined as the percentage of common native contacts in the alternative conformation. It is well known that such a measure is a good coordinate characterizing the folding process [11]. Following standard practice, two residues are defined to be in contact if the distance between their \( C_\alpha \) atoms is less than 6.5 Å. In an unbiased study, conformations that differ slightly should not be considered distinct. To avoid this problem, we perform a coarse-graining of the configurational degrees of freedom by adopting the discretization approach introduced by Covell and Jernigan [14], where the \( C_\alpha \)'s occupy sites on a suitably oriented FCC lattice (of edge 3.8 Å). This discretization does not distort the peptide angles and the position of the coarse-grained \( C_\alpha \)'s differ from the true ones by typically less than 1 Å RMSD [15]. For proteins of about 100 residues, the contact maps of the real and FCC coarse-grained contacts maps are virtually identical.

The generation of alternative conformations was carried out using a Monte Carlo procedure. A starting conformation was successively modified by displacing the \( C_\alpha \)'s to unoccupied positions of the FCC lattice. The move of an amino acid to an unoccupied site is allowed only if the new conformation satisfies certain constraints of steric overlap and peptide geometry. These constraints (any two non-consecutive residues cannot be closer than 4.65 Å due to excluded volume effects and the peptide bond is not stretched beyond 5.37 Å) were determined after carrying out an FCC coarse-graining of several proteins of intermediate length (≈ 100 residues) and enforced in the generation of alternative protein-like conformations.

In order to minimize the effects of correlation between successively generated structures, we typically discarded 50 elementary moves before accepting each new conformation. A newly generated conformation was accepted with the usual Metropolis rule according to the change in the Boltzmann weight: \( e^{\Delta /k_B T} \), where \( \Delta \) is the change in contact overlap and \( T \) is a fictitious temperature. By choosing \( T \) appropriately, one can readily generate alternative conformations with a desired average contact overlap, \( \bar{q} \). At a given temperature, the true number of alternative structures with overlap \( q \) is proportional to the number of states with overlap \( q \) obtained in the simulation multiplied by the Boltzmann weight. On undoing the Boltzmann bias, it is possible to recover the
true density of states in a region around $\bar{q}$. In order to obtain the density of states for all values of overlap, we performed Monte Carlo samplings at different temperatures and then used standard deconvolution procedures [7].

We begin with the backbones of the chymotrypsin inhibitor (2ci2) and barnase (1a2p) and generated alternative structures with a not too large overlap (18) (≈ 40%) for each of them. It turned out that the most frequent contacts shared by the native conformation of 2ci2 with the alternative ones involved the helical-residues 30-42 (see top Fig. 3) and the rarest ones pertained to interaction between the helix and $\beta$-strands and between the $\beta$-strands themselves. This is in excellent agreement with the studies of Fersht et al. [20,21], which demonstrated the formation of the helix at early stages of the folding. A different behaviour (see bottom Fig. 3) was found for barnase, where, again, for overlap of ≈ 40%, we find many contacts pertaining to the nearly complete formation of helix 1 (residues 8-18), a partial formation of helix 2, in particular bonds between residues 26-29 and 29-32 as well as several non-local contacts bridging the $\beta$-strands, especially residues 51-55 and 72-75. This picture is fully consistent with the experimental results obtained in ref. 22.

This provides a sound $a$ posteriori justification that the main features of the folding of a protein can be followed from a study of the DAC. Remarkably, the method discussed above relies entirely on structure-related properties and suggests that the features of the folding funnel are determined by the geometry of the “bare” backbone, while the finer details, of course, depend on the specific well-designed sequence.

We now turn to an analysis of three proteins of length 51 (1hcg, 1hja and 1sgp) which have nearly the same number of native contacts (≈ 83). For each structure, we calculated the DAC with the constraint that the total number of contacts in the alternative structures do not exceed 88 to avoid excessive compactness. In order to assess whether the DAC associated with naturally occurring proteins had special features, we generated three decoy compact conformations of the same length and number of contacts, but with different degrees of short and long range contacts (in sequence separation). These decoys (subject to the aforementioned “physical constraints”) were generated with a simulated annealing procedure to find the structure with the highest overlap with a target contact matrix. By tuning the number of short-range versus long-range entries in the target random contact matrix, we generated three structures with different degree of compactness and local geometrical regularity.

The plots of the DAC are shown in Fig. 4. A striking feature of the curves is that, for intermediate overlap, the DAC of the real proteins is enormously larger than that of the decoys (note the logarithmic scale) and suggests that naturally occurring conformations have a much larger number of entryway structures than random compact conformations. Furthermore, for very high values of the overlap, the steepness of the protein curves is much larger than those of the decoys, showing that the reduction in the conformational entropy is also correspondingly higher. This translates into the existence of a funnel with a very large basin and steep walls. Another significant feature is the good collapse of the protein curves. We have verified that this feature also obtains for 1bd0 and 2pk4 which each have 80 residues and 140 and 146 contacts respectively. A simple explanation for the curve collapse could be that the density of states for real proteins is “extremal”, in that it is close to the maximum possible value for intermediate values of the overlap.

The importance of the locality of contacts for folding kinetics was highlighted recently by Plaxco et al. [24] who found a correlation between folding rate and contact order, defined as the average sequence separation of contacts normalized to the total number of contacts and sequence length. With reference to Fig. 4, the contact order value for protein 1hcg, 1 hja and 1sgp is 0.139, 0.214 and 0.204 respectively. For the decoy structures, it is 0.424, 0.222 and 0.179 for the curves denoted by open squares, pentagons and hexagons, respectively. The lowest curve in the figure is indeed associated with an unusually high contact order in accord with the findings of Plaxco et al. [24].

A ubiquitous feature of protein structures is the existence of secondary structure motifs [24,25]. We have carried out some simple investigations to assess whether a correlation exists between the extremality of the DAC curve and the emergence of secondary-structure-like motifs.

We considered a space of contact maps [17], within which each of the residues interacted with the same number of other residues, $n_c$ (typically $n_c = 5$, as in the average case of a protein with about 100 residues and a cutoff distance of 6.5 Å). This space contains both maps corresponding to real structures and unphysical ones. Furthermore, to mimic the effects of the rigidity and geometry of the peptide bond, we disallowed contacts between residue $i$ and the four neighboring residues along the sequence $i-2$, $i-1$, $i+1$ and $i+2$.

In this context, the maximization of the density of states corresponds to finding the target matrix with the highest number of matrices sharing a given fraction of its contacts. Although it is difficult to solve this problem, for arbitrary values of the overlap, it is relatively easy to generate matrices with an overlap close to the maximum value, $\bar{q}_{\text{max}}$ (for a $L \times L$ matrix, $\bar{q}_{\text{max}} = L \cdot n_c$). To enumerate all matrices with overlap $\bar{q}_{\text{max}} - 2$, one first identifies a pair of non-zero entries in the target matrix $\bar{m}$: $\bar{m}_{i,j} = \bar{m}_{k,l} = 1$. Then it is necessary to check whether entries $\bar{m}_{i+1,j-1}$, $\bar{m}_{k+1,l+1}$ are both “free” (i.e. equal to zero) and do not correspond to forbidden contacts (e.g. between $i$ and $i+1$). If this is so, the old pair of entries (and their symmetric counterpart) are set to zero, and the new ones to 1. By considering, in turn, all possible pairs of non-zero entries one can generate all matrices of overlap.
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interactions between amino acids.

for protein design and the determination of the effective secondary structures. Our procedure ought to be useful for the generation of alternative conformations necessary for the selection of naturally occurring folds of proteins which, are suggestive of an extremality principle underlying the cant value of the reaction coordinate \([18]\). These results of structural overlap compared to other compact artificial backbones. Strikingly, by studying the conformational entropy of a backbone it is possible to identify the fold-

In summary, novel numerical techniques are used to elucidate the paramount role played by the geometry of the protein backbone in providing a large basin of attraction to the native state. It is found that, irrespective of the sequence, the native state of a protein has an exces-

\[ g_1(x) = \sum_i m_{i,i+x}; \quad g_2(x) = \sum_i m_{i,x-i} \quad (1) \]

which show peaks in correspondence with the sequence separation of residues involved in \(\alpha\)-helices and parallel \(\beta\)-sheets (\(g_1\)) or antiparallel \(\beta\)-sheets (\(g_2\)).

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\begin{enumerate}
\item C. Anfinsen, Science, 181, 223 (1973).
\item M. Karplus and D. L. Weaver, Nature, 260, 404-406 (1976); Protein Science, 3, 650-668 (1994).
\item O.B. Ptitstyin, FEBS Lett, 285, 176-181 (1991).
\item H.S. Chan and K. A. Dill, Journal of Chemical Physics, 99, 2116-2127 (1994).
\item J.D. Bryngelson, J.N. Onuchic, N.D. Socci and P.G. Wolynes, Proteins: Structure, Functions and Genetics, 21, 167-195 (1995).
\item P.E. Leopold, M. Montal and J.N. Onuchic, Proc. Natl. Acad. Sci. USA, 89, 8721-8725 (1992).
\item J. N. Onuchic, P.G. Wolynes, Z. Luthey-Schulten and N.D. Socci, Proc. Natl. Acad. Sci. USA, 92, 3626-3630 (1995).
\item H. Nymeyer, A. E. Garcia and J.N. Onuchic, Proc. Natl. Acad. Sci. USA, 95, 5921-5928 (1998).
\item K. A. Dill and H. S. Chan, Nature Structural Biology, 4, 10-19 (1997).
\item J.D. Bryngelson and P.G. Wolynes, Proc. Natl. Acad. Sci. USA, 84, 7524-7528 (1987).
\item N. Go, Macromolecules, 9, 535 (1976).
\item C. J. Camacho and D. Thirumalai, Proc. Natl. Acad. Sci. USA, 90, 6369-6372 (1993).
\item A. Sali, E. Shakhnovich and M. Karplus, Nature, 369, 248-251 (1994).
\item D. G. Covell and R. Jernigan, Biochemistry, 29, 3287 (1990).
\item B. H. Park and M. Levitt, J. Mol. Biol., 249, 493-507 (1995).
\item A. M. Ferrenberg and R. H. Swendsen, Phys. Rev. Lett. 63, 1195 (1989).
\item The significance of the overlap is measured against the typical overlap of any two compact-like conformations, which is about 10-20% (consistent with the unfolding simulations of ref. [19]).
\item T. Lazaridis and M. Karplus, Science, 278, 1928 (1997).
\item A. R. Fersht, Proc. Natl. Acad. Sci. USA, 92, 10869 (1995).
\item L. S. Itzhaki, D. E. Otzen and A. R. Fersht, J. Mol. Biol., 254, 260 (1995).
\item A. Matouschek, L. Serrano, A. R. Fersht, J. Mol. Biol. 224, 819 (1992).
\item K. M. Plaxco, K. T. Simons and D. Baker, J. Mol. Biol. 277, 985 (1998).
\item T. E. Creighton, Proteins: Structures and Molecular Properties, New York: W.H. Freeman, 1993.
\item H. Li, R. Helling, C. Tang and N. Wingreen, Science, 273, 666 (1996)
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FIG. 1. Ribbon plot (obtained with RASMOL) of 2ci2 (top) and barnase (bottom). The residues involved in the 12 [16] most frequent contacts of alternative structures with overlap \( \approx 40\% \) with the native conformations are highlighted in black. The majority of these coincide with contacts that are formed at the early stages of folding.

FIG. 2. Distribution of sequence separation of contacts common in alternative conformations for 2ci2 and 1a2p. The most frequent contacts in 2ci2 have a small sequence separation (3-4) and pertain to helix formation. 1a2p shows a very different behaviour with several contacts with very large sequence separation.

FIG. 3. Density of states for proteins for 1sgp (filled squares), 1hja (filled pentagons) and 1hcg (filled hexagons). Curves for artificial decoy structures are denoted by the open symbols.

FIG. 4. The upper [lower] triangle shows a target contact matrix with \( L = 60 \) that has a large [intermediate] number of contact maps with an overlap of \( \bar{q}_{\text{max}} - 2 \) contacts.
FIG. 5. Correlation functions (see equation 1) for an optimal target matrix of length 60 and for protein 3elix.