Protein Foldability and Designability: General Physics and Pretty Chemistry

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Abstract

Making use of a simplified model for protein folding, it can be shown that conformations which are particularly stable when their energy is minimized with respect to amino acid sequence (in the sense that they display a large energy gap to the lowest structurally dissimilar conformation), aside from leading to fast folding, are highly designable (in the sense that many sequences target onto it in the folding process). These results are quite general, do not depend on the particular symmetries displayed by the compact conformation chosen as native, and can be obtained making use of a large class of contact energies. On the other hand, the design of sequences which fold onto native conformations displaying secondary structures and eventually tertiary symmetries, is a difficult task requiring a delicate tuning of the contact energies.
A basic property of “wild type” proteins is that they fold on short call. A second one is that they can accommodate numerous mutations that are neutral with respect to structure changes. Another is that they display, in the native conformation, secondary structures and tertiary symmetries, features that define their biological specificity [4].

Single domain notional proteins must display a large energy gap $\delta$ between the native state and the bulk of misfolded conformations that are structurally dissimilar to the native state (calculated making use of a Monte Carlo formalism), in keeping with the fact that fast folding is tantamount to a value of the “order parameter” $\xi = \delta/\bar{U} \gg 1$, where $\bar{U}$ is the average value of the contact energies among the amino acids. In cases where $\bar{U} = 0$, the order parameter to be used is $\xi = \delta/\sigma$, where $\sigma$ is the dispersion of the interaction energies [2–9]. The designed sequence which in the native conformation fulfills $\xi \gg 1$, allows for billions of mutations leading to sequences which have the native conformation as their non-degenerate ground state with energies lying inside the gap and, more importantly, which fold to the native state in a time of the order of that associated with the folding of the designed sequence [10].

To design a sequence which folds fast and which is highly resilient to point mutations is easily achieved making use of a large class of contact energies among the amino acids, and does not require the native conformation to display “wild-type” secondary or tertiary structures [5,11–20].

It is not difficult either to derive contact energies which select, as the most designable conformations, those displaying “wild-type” secondary structures [21]. What is very difficult, requiring a detailed tuning of the contact energies, is to achieve that the associated sequences fold, let alone whether they do it fast or slow.

To illustrate the above points we report, in what follows, results obtained making use of a lattice model [2–9], where the differences between the amino acids are manifested in pairwise interaction energy of variable magnitude and sign, depending on the identity of the interacting amino acid. The configurational energy is
\[ E = \frac{1}{2} \sum_{i,j}^N U_{m(i),m'(j)} \Delta(|\vec{r}_i - \vec{r}_j|), \]  

\{\vec{r}\} being the set of coordinates of all of the monomers describing a chain conformation and \( U_{m,m'} \) is the effective interaction energy between monomers \( m \) and \( m' \). The quantity \( \Delta(|\vec{r}_i - \vec{r}_j|) \) is a contact function. It is equal to one if sites \( i \) and \( j \) are at unit distance (lattice neighbours) not connected by a covalent bond, and zero otherwise. In addition, it is assumed that on-site repulsive forces prevent two amino acids from occupying the same site simultaneously, so that \( \Delta(0) = \infty \).

We start considering the case of proteins displaying a large value of the parameter \( \xi \). A particular realization of this situation is provided by a 20-letter amino acid chain, composed of 36 monomers and designed to fold into the native conformation shown in Fig. 1a. The quantities \( U_{m,m'} \) used here correspond to the contact energies obtained from a statistical analysis of real proteins data and were taken from Table 6 of ref. \[22\] ("disordered" M-J contact energies (MJ)\(_d\)). In this case, the average value of the contact energies is \( \bar{U} = 0 \), the corresponding standard deviation \( \sigma \equiv 0.3 \). The 36-mer chain denoted as \( S_{36} \) and designed by minimizing, for fixed amino acid composition, the energy of the native conformation with respect to the amino acids sequence is shown in Fig.1b. In the units we use (\( RT_{room} = 0.6 \) kcal/mol), the energy of \( S_{36} \) in its native conformation is \( E_{\text{nat}} = -16.5 \) and the value of the gap \( \delta = 2.5 \), yielding a value for the order parameter \( \xi = 8.3 \). Making use of Monte Carlo (MC) simulations it is found that at the temperature \( T = 0.28 \), optimal from the point of view of allowing for the accumulation of statistically representative samples of the different simulations, and at the same time leading to a consistent population of the native conformation (\( \approx 10\% \)), the sequence \( S_{36} \) folds into the native conformation in \( 8 \cdot 10^5 \) MC steps.

The characterization of the role played by the different amino acids in the folding process of \( S_{36} \) have been carried out in ref. \[23\] (cf. also \[24\]), by using mutations. It was found that the 36 sites of the native conformation (see Fig.1a) can be classified as "hot" (red bead, numbered 6, 27, and 30), "warm" (yellow beads numbered 3, 5, 11, 14, 16 and 28), and
“cold” (the rest of the beads, coloured green) sites. On average, mutations on the 27 "cold" sites yield sequences that still fold to the native structure (neutral mutations), although the folding time is somewhat longer than for \( S_{36} \). Sequences obtained from mutations on the six warm sites fold, as a rule, to a unique conformation, sometimes different but in any case very similar to the native one. Mutations on the three hot sites lead, in general, to complete misfolding (denaturation) of the protein. These results were found not to depend on the possible structures displayed by the native conformations, nor on any three-dimensional properties of the native conformation, aside from the general requirement of being compact. In fact, making use of the \((MJ)_d\) contact energies, any compact structure is an equally good native. Similar results as those discussed above were obtained making use of a random force with the same average and standard deviation of the \((MJ)_d\) contact energies, as well as with the Go model \[12\].

Because of the large value of \(\delta\), and the many possible sites available to introduce neutral mutations, there are of the order of \(10^9 - 10^{10}\) sequences \(S'_{36}\) obtained from \(S_{36}\) through single- and multiple- amino acid concentration conserving mutations (swapping of amino acids) which have an energy, when calculated in the native conformation, lying within the gap \(\delta\). The sequences \(S'_{36}\) fold into the native conformation shown in Fig. 1a, in times of the order, although somewhat longer, than that associated with the folding of \(S_{36}\) \[10\]. These results indicate that the native structure, which, as mentioned above, can be any compact structure, is highly designable.

A revealing example of the difficulties met in trying to design fast folding, highly designable proteins with "wild type"-like secondary structures is provided by the results of ref. \[21\] (cf. also \[25\]), where a complete enumeration and energy calculation of all compact configurations of 2 letters chains containing 27 monomers was presented. The designability of each compact structure was measured by the number of sequences \(S_{27}\) that can design the structure, that is, sequences that posses the structure as their non-degenerate ground state. It was found that highly designable conformations posses "protein-like" secondary structures and even tertiary symmetries, and are thermodynamically more stable than other
conformations.

In keeping with the fact that the basic test a notional protein should pass to qualify as such is to fold to the native structure in a "short time" (typically of the order of $10^5 - 10^6$ MC steps), we have calculated the dynamics of a number of the sequences designed by Li et al. [21]. We have found that neither the sequences associated with the poorly-designable (cf. Fig.2(c)) nor with the highly-designable (cf. Fig.2(a)) conformations fold. Such sequences are thus unlikely candidates for coding functional proteins.

The reason for these results is to be found on the fact that all the structures of the two letter chain display a gap $\delta$ which is negative. In fact elongated, poorly designable and thus structurally dissimilar configurations exist (cf. Fig.2(b)), sampled by the Monte Carlo simulations which, for a given amino acid composition have an energy which is lower than that associated with the original, highly designable configuration. Defining the energy gap $\delta$ as the energy difference between the native and the lowest structural dissimilar compact configuration, leads to a positive value of $\delta$ which is of the order of the average contact energies, and consequently to an “order parameter” $\xi$ of the order of 1.

At the “microscopic” level we have found that all the configurations of the two letter 27mer chains display a very large number of "hot" sites. In fact, essentially half of all sites are "hot" sites (cf. Fig.2). In other words, introducing single point mutations in the most designable structures of ref. [21] one finds that one, out of two, leads to protein denaturation (i.e., in the present case, makes unstable the native structure), a behaviour not observed in “wild-type” single domain proteins. In fact, the strategy of multi-domain proteins, where the folding of each domain is controlled by a small number of strongly interacting amino acids, is used here to keep in place secondary structures on a single domain protein.

A possible way to design a viable notional protein making use of the 3D, 27-monomer chains of ref. [21] could be to modify the contact energies used in this paper, eventually introducing an angle dependence (cf. e.g. ref. [20]) so as to selectively change the value of $\delta/\bar{U}$. That is, to force the ratio $(\delta/\bar{U})_{\text{non-design}}$ associated with the non-designable structures to remain at the present value (of the order of unity), while boosting the ratio $(\delta/\bar{U})_{\text{design}}$
characterizing the designable structures to become of the order of 10.

In keeping with this discussion we report results of MC simulations carried out for the twenty letter 27mer making use of contact energies taken from Table 5 of ref. [22] ("ordered" Miyazawa-Jerningan contact energies (MJ)). Also of a U-matrix containing 210 matrix elements of the form $U_{ij} = h_{ij} + \eta$, where $h_{ij}$ can be $-1, 0$ or $1$ according to the hydrophobicity or polarity of the ith and jth residues, while $\eta$ is a random number taken from a Gaussian distribution centered around zero and with standard deviation 0.34 [27]. In these cases it is found that not all compact conformations can act as native configurations. In fact, one has to carefully choose the ratio between local and non-local contacts to obtain natives in which the designed sequence displays a large value of $\xi$. Examples are shown in Fig.3, where the corresponding conformations are associated with values of $\xi$ equal to 6.9 (Fig.3(a)), 3.8 (Fig.3(b)) and 0.2 (Fig.3(c)). Although these conformations do not display secondary structures typical of "wild-type" proteins as discussed in ref. [21], they provide examples of the possible links which, appropriately chosen contact energies may create between selected 3D conformations and high entropy regions of the sequence space (large gap $\delta$), that is between structure and foldability.

One can conclude that stability, designability and fast folding are basic physical properties characterizing notional proteins, properties which depend only on the large number of degrees of freedom displayed by these system in sequence space, while the presence of secondary structures and tertiary symmetries are “pretty” expressions of detailed chemistry.

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FIGURES

FIG. 1.  (a) The conformation of the 36–monomers chosen as the native state in the design procedure. Each amino acid residue is represented as a bead occupying a lattice site. The design tends to place the most strongly interacting amino acids in the interior of the protein where they can form most contacts. The strongest interactions are between groups D, E and K (compare to b), the last one being buried deep in the protein (amino acid in site 27). (b) Designed amino acid sequence $S_{36}$. The color plots in figs. 1, 2 and 3 were obtained by using the graphic program of ref. [28].
FIG. 2.  (a) Most designable conformation of a 27 monomer chain composed of two types of amino acids, namely hydrophobic (H) and polar (P) to which are associated the interaction energies $E_{HH} = -2.3$, $E_{HP} = -1.0$ and $E_{PP} = 0$ \cite{21}. The corresponding sequence which minimizes this configuration with a fixed number of H and P residues (13 and 14 respectively) is $S_1 \equiv$ HPPHPHHPHPPHPHPPHPHPPHHH, and displays an energy gap $\delta = 2.6$ to the lowest fully-compact structurally different configuration. Note however that non-compact conformations with energy lower than the energy of the native state have been found. Changes in the monomer sites plotted as red dots, correspond to the hot mutations discussed in the text. Mutations in these sites increase the energy of the target structure by an amount of the order or greater than the so called energy gap $\delta$ which, in this case, is of the order of the standard deviation of the interaction energies ($\sigma = 0.9$). (b) Starting from a random, elongated configuration of the sequence denoted $S_1$, the chain compacts but does not fold, in the sense that it reaches different conformations which, within the statistic accumulated (500 coils followed through $10^8$ MC steps), it never targets into the most designable ("native") configuration shown in (a). Of all the random configurations targeted in the folding process by the sequence $S_1$, the one shown here is one, among many, which displays an energy which is lower than the most designable configuration shown in (a). (c) Example of the so called 'less-designable' conformation of Li et al. \cite{21}, The sequence $S_2 \equiv$ PPNNNHPPNHPPNHPPNHPPNHNP is found to minimize the energy of this conformation. Starting from random, elongated, configurations of this sequence the chain compacts but, again, never folded, in the sense explained in (b).
FIG. 3. Examples of 27mers compact conformations. Sequence energy has been minimized for each of them making use of a matrix of the form $U_{ij} = h_{ij} + \eta$, where the Hermitian matrix $h_{ij}$ is composed of four blocks of elements -1, 0, 0 and 1 respectively, while $\eta$ is a random number taken from a Gaussian distribution having average value zero and standard deviation 0.34. The corresponding standard deviation of the matrix $U_{ij}$ is then 0.65. The designed sequences $S_{27}$ displays, in the native conformation, values of the order parameter $\xi = 6.9$ (a), $\xi = 3.8$ (b) and $\xi = 0.2$ (c), respectively. In keeping with these results, configurations (a) and (b) contain only 15% and 7% of "hot" sites (red beads) respectively, while almost 70% of all sites are "hot" in configuration (c). While the sequences $S_{27}(\xi)$ associated with the conformations (a) and (b) fold (in times of the order of $10^6$ MC steps), the sequence associated with the conformation (c) does not. Consequently, the compact conformation (c) does not qualify as a native conformation.
