Designability of lattice model heteropolymers

G. Tiana$^1$, R. A. Broglia$^{1,2}$ and D. Provasi$^1$

$^1$ Dipartimento di Fisica, Università di Milano e INFN Sezione di Milano, via Celoria 16, 20133 Milano, Italy

$^2$ The Niels Bohr Institute, Blegdamsvej 17, 2100 Copenhagen, Denmark

(February 1, 2008)

Abstract

Protein folds are highly designable, in the sense that many sequences fold to the same conformation. In the present work we derive an expression for the designability in a 20 letter lattice model of proteins which, relying only on the Central Limit Theorem, has a generality which goes beyond the simple model used in its derivation. This expression displays an exponential dependence on the energy of the optimal sequence folding on the given conformation measured with respect to the lowest energy of the conformational dissimilar structures, energy difference which constitutes the only parameter controlling designability. Accordingly, the designability of a native conformation is intimately connected to the stability of the sequences folding to them.

I. INTRODUCTION

Even a quick look at the set of known proteins (PDB database) reveals a striking feature. While there are tens of thousands of protein sequences, they only assume some thousands folds. In other words, proteins are highly designable. This concept can be quantified by measuring the number of sequences that fold uniquely into a particular structure.
With the use of a simple 20 letter lattice model of protein folding it has been shown that the whole issue of estimating the number of sequences that fold to the same conformation is reduced to enumerate how many of them have native energy lying below a threshold $E_c$, the energy which any sequence with the same composition displays in the conformation structurally dissimilar to the native conformation.

The aim of the present paper is to provide a reliable, analytic expression for $n$, which we shall show increases exponentially with the gap $\delta$ between the native energy of the optimal sequence $E_n$ and the threshold energy $E_c$. This functional form is found to be universal, as it emerges from the Central Limit Theorem. We have furthermore found that while the parameters defining $n$ depend on the interaction matrix, they are independent of the particular choice made for the native structure or for the optimal sequence.

In Sections 2 and 3 we briefly review the 20 letters lattice model of proteins in general and the question of protein designability in particular. The quantitative, analytic answer to the question of how many mutations a designed protein tolerates is given in Section 4. The conclusions are collected in Section 5.

II. LATTICE MODELS

A useful theoretical approach to study protein folding is provided by a simplified lattice model, where the protein is a string of beads that is arranged on a cubic lattice. The configurational energy of a chain of $N$ monomers is given by

$$E = \frac{1}{2} \sum_{i,j}^N U_{m(i),m(j)} \Delta(|\vec{r}_i - \vec{r}_j|),$$

(1)

where $U_{m(i),m(j)}$ is the effective interaction potential between monomers $m(i)$ and $m(j)$, $\vec{r}_i$ and $\vec{r}_j$ denote their lattice positions and $\Delta(x)$ is the contact function. In Eq. (1) the pairwise interaction is different from zero when $i$ and $j$ occupy nearest–neighbour sites, i.e., $\Delta(a) = 1$ and $\Delta(na) = 0$ for $n \geq 2$, where $a$ indicates the step length of the lattice. In addition to these interactions, it is assumed that on–site repulsive forces prevent two amino acids to occupy the same site simultaneously, so that $\Delta(0) = \infty$. 

2
We shall consider throughout a 20–letters representation of protein sequence where \( U \) is a \( 20 \times 20 \) matrix. A possible realization of this matrix is given in ref. [6] (Table 5 and 6), where it was derived from frequencies of contacts between different amino acids in protein structures. The employment of a \( 20 \times 20 \) matrix ensures that the threshold energy \( E_c \) is well defined, depending only on the interaction matrix elements and on the composition of the protein in terms of amino acids. The model we study here is a generic heteropolymer model which has been shown to reproduce important generic features of protein folding thermodynamics and kinetics, independent on the particular potential chosen [10,11]. However, in using such an approach, one should keep in mind that the labelling of amino acids (spherical beads all of the same size and with no side chain) is generic too and may be no obvious relation between those labels and labels for real amino acids.

Good–folder sequences are characterized by a large gap \( \delta = E_c - E_n \) (compared to the standard deviation \( \sigma \) of the contact energies) between the energy of the designed sequence in the native conformation \( E_n \), and the lowest energy of the conformations structurally dissimilar to the native conformation [1,2,3,4,19]. In other words, good folders are associated with a normalized gap \( \xi = \delta / \sigma \gg 1 \), quantity closely related to the z–score [12]. For example, the 36mer sequence listed in the caption to Fig. 1 and called \( S_{36} \) in the literature [13,14,15,16,17,18], designed by minimizing the energy in the target (native) conformation with respect to amino acid sequence for fixed composition has, in the units considered here (\( RT_{room} = 0.6 \text{ kcal/mol} \)), an energy gap \( \delta = 2.5 \) and thus a sufficiently large value of \( \xi \) (= 2.5/0.3 \( \approx 8.33 \)) so as to ensure fast folding. In fact, Monte Carlo simulations carried out at the temperature \( T = 0.28 \) of 3000 36mers with energies, in the native conformation, lying inside the gap fold in times \( \leq 7 \times 10^7 \) MC steps [19] (For caveats see ref. [20]). In particular \( S_{36} \) folds in \( 6.5 \times 10^6 \) MC steps.

It has been also shown that most of the thermodynamical [18] and dynamical [13,19] behaviour of designed proteins is controlled by only \( 5 - 10\% \) of the sites. As a consequence, making mutations in these sites, which are called "hot" in ref. [18], one destroys, as a rule, the ability the protein has to fold (denaturation). On the other hand, the effects of substitutions
in any other site (that can be regarded as "cold") are small, leading to neutral mutations.

III. DESIGNABILITY WITH 20 LETTERS MODELS

While twenty letters heteropolymers capture the essential components of real proteins, it is hardly possible to enumerate all sequences which have a given conformation as their non–degenerate ground state. Accordingly, it is not possible to calculate the exact designability of protein conformations. To bypass this problem, we shall determine designability from energetic considerations, using a strategy which relies on the fact that all sequences which have an energy lower than $E_c$ fold on short call, in any case in times which are much shorter than that associated with the random search [5].

Any sequence of a given length $N$ (e.g. $N = 36$) can be obtained making $m \leq N$ mutations (i.e. substitution of an amino acid in a given site with a different one) in the minimum energy sequence (e.g. $S_{36}$ in the case of Fig. 1(a)). Consequently, the designability of a conformation can be found starting from the minimum energy sequence, counting how many mutations lay within the gap $\delta = E_c - E_n$. If $\Delta E$ is the change in the energy of the native state produced by a mutation, $p_m(\Delta E)$ the energy distribution probability associated with $m$ mutations and $n_{m}^{\text{tot}}$ the total number of sequences that can be produced by introducing $m$ mutations in the minimum energy sequence, designability can be defined as

$$n = \sum_{m=1}^{N} n_m,$$  \hspace{1cm} (2)\hfill

$$n_m = n_{m}^{\text{tot}} \int_{0}^{\delta} p_m(\Delta E) \, d(\Delta E).$$  \hspace{1cm} (3)\hfill

So far, we have done nothing more than expressing the problem in another way, since to know the spectrum of mutation energies of the optimal sequence one has again to enumerate all sequences. In fact, it looks like as if we have made things even worse, in that now one has to find the optimal sequence, which is a non–trivial problem, and also has to ensure that $E_c$ does not change with mutations.
We shall show in the following that the distribution of mutation energies does not depend on the particular structure or on the particular sequence chosen (provided that $E \ll E_c$) nor on the contact energy matrix used to design the protein, but only on its composition and on the number of contacts (or the length, if it is fully compact). This observation leaves room to approximations. In fact, if one is able to find an approximate expression for $p(\Delta E)$, such an expression will hold for all model proteins of the same length. Furthermore, the knowledge of the sequence associated with the global minimum of energy $E_n$ is not necessary (because all sequences have the same spectrum of mutations), only the value of $E_n$ is required. Consequently, even if the optimal sequence cannot be known without a full enumeration of all sequences, it is allowed to use any other sequence with energy $E \approx E_n$, introducing in this way only an error in the integration boundaries (and not on the function to be integrated). It is then possible to calculate designability of a structure from Eq. (2–3) using an approximate distribution $p(\Delta E)$ and an approximate value of $\delta$.

The most conservative way to calculate the number of sequences which fold to a conformation is then to use a distribution $p_m(\Delta E)$ found only by swappings between the residues of the optimal sequence, as in such a way the composition is conserved and $E_c$ does not change. On the other hand, since there are also sequences with different composition folding to the same conformation, one is also forced, in principle, to calculate the number $n$ associated with pointlike mutations. The values found from swapping of amino acids and from pointlike mutations can be viewed as the lower and the upper limit to designability, respectively.

In Figs. 2(a) and 2(b) we display the unnormalized energy distribution probabilities associated with two composition–conserving and with two pointlike mutations of $S_{36}$ (the integral of these distributions being the total number of sequences). Each of these curves can be well fitted by the sum of two Gaussians, whose means are $\Delta E_2^2 = 1.2$ and $\Delta E_2^2 = 3.0$ (Fig. 2(a), composition conserving case) and $\Delta E_2^2 = 1.1$ and $\Delta E_2^2 = 3.6$ (Fig. 2(b), pointlike mutations case). Standard deviations are $\sigma_2 = 0.7$ and $\sigma_2 = 1.0$ (Fig. 2(a) ) and $\sigma_2 = 0.7$ and $\sigma_2 = 1.1$ (Fig. 2(b) ). The behaviour of these two distributions seems very alike, except
for the fact that the area below the composition-conserving curve is much smaller than that below the pointlike mutations curve. This is because much fewer mutations can be made in the first than in the second case and, consequently, the associated Gaussian behaviour is less well defined.

The overall structure of the curves shown in Fig. 2 can be understood from the fact that the average value of $\Delta E$ for "cold" sites is 0.65 and for "hot" sites is 2.87 \[18\]. Accordingly, the low-energy peak can be associated with two mutations in "cold" sites, while the high-energy peak can be associated with a mutation in a "cold" site and a mutation in a "hot" site. The contribution from mutations in two "hot" sites leads to an enhancement of the high energy tail of the curve. Concerning the Gaussian behaviour, we note that the energies associated with the 19 possible mutations on a given "cold" site are uncorrelated. In other words, one has to pay an energy $\Delta E_2/2 \approx 0.6$ (concerning the factor $1/2$ one is reminded of the fact that $\Delta E_2$ gets contributions from two mutations) to remove the wild-type residue, reflecting the fact that it has been optimized. Second, one has to introduce a new residue in the niche left by the wild-type residue. The Gaussian shape of the distribution suggests that the niche is neutral with respect to the new residue and that the new interactions are merely random. To be more precise, the change in energy $\Delta E$ upon mutations is the difference between the energy needed to remove the original residue (which is roughly constant and assumes two different values for cold and for hot sites) and the sum of a number of contact energies associated with the new residue, energies which can be considered as random numbers. Being the sum of random numbers, the energy associated with the new residue is forced to respect the Central Limit Theorem, and consequently its distribution approaches a Gaussian function. Of course, an exact Gaussian distribution could be reached only if the number of nearest neighbours of each site were infinite (while in a cubic lattice this is, at most, five). On the other hand, the fact that $p_m(\Delta E)$ can be accurately fitted by Gaussian distributions (cf., e.g., Fig. 2(b)) testifies to the fact that we are not far from the conditions in which the Central Limit Theorem holds.

While the hypothesis that "cold" mutations give raise to Gaussian-like peaks is quite
grounded, due to the uncorrelateness of the energy contributions of "cold" sites, it is unlikely that the Central Limit Theorem works properly for "hot" sites, whose energy contributions are correlated [19]. In order to calculate the degree of designability of a protein conformation, we only need to know the contributions from cold sites and, consequently, we don’t need to better characterize the peaks associated to "hot" sites.

We have found that the distribution of mutation energies are rather universal functions. Examples of such a behaviour are shown in Fig. 3, where 2–pointlikes mutations spectra $p_2(\Delta E)$ associated with low energy 36mer sequences optimized (making use of the MJ elements of Table 6) on three different conformation (cf. Figs. 1(a)–1(c) ) and with three sequences designed on the same conformation (Fig. 1(a) ) are displayed. Similar results have been obtained for chains of different lengths. Furthermore, using different $20 \times 20$ interaction matrices lead to the same Gaussian behaviour of $p_2(\Delta E)$, although the mean values and the standard deviations are different. This is again a consequence of the Central Limit theorem. This can be seen from Fig. 4, where we display the function $p_2(\Delta E)$ associated with two pointlike mutations on $S_{36}$ (cf. Fig. 1), but making use this time of the interaction matrix elements listed in Table 5 of ref. [6]. Because the average change in energy upon mutations in cold sites is zero, while that in hot sites is 0.35, it is easy to identify the peaks associated with two cold mutations ($\overline{\Delta E}_2 = 0$ and $\sigma_2 = 0.34$), with one cold and one hot mutations ($\overline{\Delta E}_2 = 0.35$ and $\sigma_2 = 0.02$), and with two hot mutations ($\overline{\Delta E}_2 = 0.70$ and $\sigma_2 = 0.22$).

Summing up, the function $p_2(\Delta E)$ associated with chains of different length and sequence as well as designed on different native conformations overlap quite nicely, suggesting that the spectrum of both composition conserving and non–conserving mutations is universal. On the other hand, the actual value of $\overline{\Delta E}_2$ and $\sigma_2$ characterizing the different peaks of the energy distribution probability depend on the matrix used to describe the contact energies among the amino acids.

The univerality of the energy distribution probability is in agreement with the interpretation of the main peaks of the spectrum of mutations of a designed protein in term of "cold" and "hot" sites. In fact, the properties of the "hot" sites are rather homogeneous,
their contribution to the mutation spectrum being universal. Assuming furthermore that
the interactions associated to the mutations in "cold" sites are random, the resulting energy
distribution is Gaussian (and so universal), and its standard deviation depends only on the
interaction matrix, while its average value depends on the degree of optimization of the
wild-type monomer. This, in turn, can be approximated by the degree of optimization of
the whole chain (measured by the energy gap $\delta$) divided by its length, a quantity which
is essentially constant for long chains \[24\] (for example, in the case of $S_{36}$ this number is
$2.5/36 = 0.07$).

IV. HOW MANY GOOD FOLDERS?

The basic idea to calculate the designability of a model protein, as we have discussed
above, is to find a simple approximation to the universal distribution of energies associated
with mutations onto the optimal sequence, integrate this distribution up to the gap $\delta$ and
normalize this result to the total number of mutations that one can make (cf. Eqs. (2–3) ).
As a consequence of this, designability turns out to depend only on the length of the protein
(through the total number of mutations) and on the gap $\delta$.

In order to calculate $n$ using Eq. (2–3) we have first to know the total number $n_{m}^{tot}$ of
sequences that can be obtained by making $m$ sequence–conserving mutations (swappings)
in the optimal sequence. This number can be obtained by counting the number of ways one
can select $m$ sites, multiplied by the number of permutations of these sites which move all
the $m$ residues. That is

$$n_{m}^{tot} = \binom{N}{m} P_0(m),$$

where $P_i(m)$ is the number of ways one can permute $m$ sites in such a way that only $i$
positions are kept fixed. From the relation

$$m! = P_0(m) + P_1(m) + P_2(m) + \ldots + P_{m-2}(m) + 1$$

it is possible to extract the expression for $P_0$,
\[ P_0(m) = m! - \sum_{k=1}^{m-2} \binom{m}{k} P_0(m-k) - 1. \]  

For large \( m \), one can use the Stirling approximation for the factorials in Eqs. (4–6), and keep only the largest exponent term in the sum (saddle point approximation), obtaining 
\[ n_m^{tot} \approx \exp(\alpha m). \]  
The constant \( \alpha \) can be determined from the relation 
\[ n_N^{tot} = e^{\alpha N} = N!, \]  
which for \( N = 36 \) leads to \( \alpha = 2.66 \).

To proceed further in the calculation of \( n \), one needs to find a simple approximation to \( p_m(\Delta E) \). For this purpose, we shall express the energy distribution of an arbitrary number of mutations as a convolution of functions \( p_2(\Delta E) \) associated with the swapping of two amino acids. The validity of this approximation rests on the ansatz that every couple of mutations affect the energy of the native state independently of the other couple of mutations. This approximation is expected to work also for large values of \( m \), where the probability of mutating neighbouring sites is not negligible, because the contact energy associated with the mutated residues are in any case random quantities with average zero (cf. the discussion in the previous section). Within this scenario, the number of folding sequences displaying \( 2m \) mutations and whose energy in the native conformation lies inside the energy gap can be written as

\[
n_{2m} \approx n_{2m}^{tot} \int_0^\delta dE \int_{-\infty}^{+\infty} d\Delta E_1 d\Delta E_2 \cdots d\Delta E_{m-1} \times \\
p_2(\Delta E_1) p_2(\Delta E_2) \cdots p_2(\Delta E_{m-1}) p_2(\Delta E - \Delta E_1 - \Delta E_2 - \cdots - \Delta E_{m-1}).
\]  

Making use of the energy distribution probability associated with an amino acid swapping (composition conserving mutations) or with two point-like mutations (composition non-conserving mutations) one obtains the lower and the upper limit of the designability of a conformation.

In what follows we shall essentially discuss the case of composition conserving mutations. If \( \delta \) is lower than the peak associated with mutations in ”hot” sites (as in the case of the sequence \( S_{36} \) where \( \delta = 2.5 \), cf. Fig. 2), one should convolute only the peak of \( p_2(\Delta E) \) associated with mutations in ”cold” sites. Exploiting the fact that the convolution of
$m$ Gaussian distributions, of the form $\exp((\Delta E - \overline{\Delta E}^2)^2/2(\sigma^2)^2)$ is a Gaussian distribution with average $\overline{\Delta E}^2_m = m\overline{\Delta E}^2$ and standard deviation $\sigma^2_{2m} = \frac{m}{2}\sigma^2$, it is possible to write

$$n_{2m} \approx n^\text{tot}_{2m}(2\pi m^2\sigma^2)^{-1/2} \exp \left(\frac{-(\Delta E^2)}{2(\sigma^2)^2}m\right) \times$$
$$\times \int_0^\delta d\Delta E \exp \left(-\frac{\Delta E^2}{2m(\sigma^2)^2} + \frac{\overline{\Delta E}^2\Delta E}{2(\sigma^2)^2}\right).$$  \hspace{1cm} (8)

For $m \gg \delta/(2\sigma^2)^{1/2}$ (in the case of S$_{36}$ this condition means $m \gg 2$) one can neglect the first exponential factor in the integral, in which case the integration can be carried out analytically, leading to

$$n_m \approx n^\text{tot}_m(\pi m^2\sigma^2/2)^{-1/2} \exp \left(-\frac{\overline{\Delta E}^2}{4(\sigma^2)^2}m\right) \frac{2(\sigma^2)^2}{\overline{\Delta E}^2} \left(\exp \left[\frac{\overline{\Delta E}^2}{2(\sigma^2)^2}\delta\right] - 1\right)$$  \hspace{1cm} (9)

where the substitution $2m \to m$ has been made. This equation tells us that designability increases exponentially with the gap $\delta$. In other words, the number of sequences folding to a (compact) conformation is determined only by the gap associated with the minimum energy sequence.

We have shown that the concepts of designability (i.e. number of sequences folding to a given conformation) and foldability (i.e., thermodinamical stability of the sequences with low energy on the given conformation, expressed by the gap $\delta$) are intimately connected by Eq. (8). If a protein is stable in its native conformation, such native conformation is necessarily highly designable. Vice versa, if a conformation is highly designable, there exist sequences with a large gap folding to it.

To give a numerical evaluation of protein conformations, we make use of Eq. (8) in the form

$$n = \sum_{m=1}^{N} \frac{k}{m} \exp \left[\left(\alpha - \frac{\overline{\Delta E}^2}{4(\sigma^2)^2}\right)\frac{m}{m}\right],$$  \hspace{1cm} (10)

where $k$ does not depend on $m$ and, for the case of the structure displayed in Fig. 1(a), assumes the value $k = 17$ (in keeping with the fact that $\delta = 2.5$, $\overline{\Delta E}^2 = 1.2$ and $\sigma^2 = 0.7$). In the case in which $\alpha > \overline{\Delta E}^2/4(\sigma^2)^2$, which in the case of the 36mer under discussion implies
α > 0.73, one can keep only the largest term in the above sum. Within this approximation one can write $n \approx e^{1.90 \times 36} = 0.6 \cdot 10^{30}$, a number to be compared with $n_{36}^{\text{tot}} = 3.72 \cdot 10^{41}$.

One can mention, for the sake of completeness, that the number of sequences within the gap obtained by pointlike mutations (which is the upper limit to designability), is well fitted by the function $4 \exp(5m)$, while the total number of sequences is $19^m \left(\frac{N}{m}\right)$.

V. CONCLUSIONS

The degree of designability of a given conformation depends exponentially on the energy gap $\delta$. Since the number of folding sequences is given by the integral of a universal function (the mutation energy distribution) carried up to $\delta$, a quantity which also determines the thermal stability of the designed protein, one can conclude that designability and thermal stability are strongly interconnected. In other words, sequences displaying large gaps are both thermally stable and highly designable. Even sequences displaying, in the native conformation, a small gap fold on short call and share (in the compaction process) the conserved contacts leading to local elementary structures and to the (post–critical) folding nucleus \cite{19,26}. Consequently, it is possible to obtain from them, through composition–conserving mutations, other sequences folding to the same native conformation and displaying a large gap. In other words, any sequence able to fold fast, folds to a highly designable conformation.

We have estimated that there are of the order of $10^{30}$ sequences folding to a compact 36mer conformation, over a total of $10^{41}$. This is only the lower limit, but let us assume that it describes well the typical degree of designability of the designed protein. Is this number small or big? The answer to this question has, of course, important implications from the evolutionary point of view. If good folders were distributed homogeneously in the space of sequences (like in the case of RNA \cite{27}) the important parameter would be their density, that is $10^{-11}$. This number would be very low, preventing sequences from moving along neutral pathways (which are collections of sequences folding to the same conformation and differing by single mutations). Such a scenario is very unfavourable for evolution. The situation is
however quite different for proteins. In fact, it has been shown [28] that good folders group themselves in clusters and superclusters, giving rise to a quite an inhomogeneous landscape. Consequently, the relevant parameter which measures the designability of a conformation is the total number of sequences which conserve, in any way, the energy gap. This number ($> 10^{30}$) is very large in particular in keeping with the fact that over a life span of the order of 60 mutations occur in the genome of each person [29].
FIGURES

FIG. 1. Three conformations used as natives in the present study. Sequence $S_36$, which is a good folder onto structure (a), is SQKWLERGATRIADGDLVPNGTYFSCKIMENVHPLA.

FIG. 2. Energy distribution for 2 composition-conserving (a) and pointlike (b) mutations. The parameters of the Gaussian fit (dotted line) are given in the text.

FIG. 3. The distribution of energies associated to two composition-conserving mutations made on three sequences designed on three different 36mers conformations. (b) The spectrum obtained making two composition-conserving mutations on three sequences designed on the same conformation (the one displayed in Fig. 2.4). The values of the energy gap are $\delta = 2.5$ (dotted curve), $\delta = 1.6$ (solid line) and $\delta = 1.3$ (dashed line).

FIG. 4. The distribution $p(\Delta E)$ associated with two pointlike mutations for the structure displayed in Fig. 1(a) when the monomers interact with the matrix listed in Table 5 of ref. 8 (instead of Table 6). The dashed line is the Gaussian fit obtained with the least-square method.
REFERENCES

[1] E. I. Shakhnovich, Phys. Rev. Lett. 72, 3907 (1994)

[2] V. I. Abkevich, A. M. Gutin and E. I. Shakhnovich, J. Chem. Phys 101, 6052 (1994)

[3] R. Goldstein, Z. Luthey–Schulten, P. Wolynes, Proc. Natl. Acad. Sci. USA 89, 4918 (1992)

[4] A. Sali, E. Shakhnovich and M. Karplus, J. Mol. Biol. 235, 1614 (1994)

[5] R. A. Broglia, G. Tiana, H. E. Roman, E. Vigezzi and E. I. Shakhnovich, Phys. Rev. Lett. 82, 4727 (1999)

[6] S. Miyazawa and R. Jernigan, Macromolecules 18, 534 (1985)

[7] N. Go, Int. J. Peptide Prot. Res. 7, 313 (1975)

[8] K. F. Lau and K. Dill, Macromolecules 22, 3986 (1989)

[9] E. I. Shakhnovich, A. M. Gutin, J. Chem. Phys. 93 5967 (1989)

[10] E. I. Shakhnovich, Curr. Opin. Struct. Biol. 7, 29 (1997)

[11] E. I. Shakhnovich, Folding and Design 1, R50 (1996)

[12] J. Bowie, R. Luthey–Schulten, D. Eisenberg, Science 253, 164 (1991)

[13] G. Tiana and R. A. Broglia, J. Chem. Phys. 114, 2503 (2001)

[14] V. I. Abkevich, A. M. Gutin and E. I. Shakhnovich, J. Chem. Phys. 101, 6052 (1994)

[15] V. Abkevich, A. Gutin and E. Shakhnovich, Biochemistry 33, 10026 (1994)

[16] N. Socci, W. Bialek, J. Onuchic, Phys. Rev. E 49 (1994) 3440

[17] D. Klimov and D. Thirumalai, Phys. Rev. Lett 76 (1996) 4070

[18] G. Tiana, R. A. Broglia, H. E. Roman, E. Vigezzi and E. I. Shakhnovich, J. Chem. Phys. 108, 757 (1998)
[20] Strictly speaking, the Monte Carlo algorithm was to designed to study equilibrium properties of systems with many degrees of freedom [21]. Nonetheless, it has been shown [22] that, being equivalent to solving the Fokker–Planck Equation for diffusion in a potential, it can be helpful also in studying the kinetical properties of complex systems, provided that the Fokker–Planck approximation is valid (i.e., moves are local and the potential changes smoothly on the diffusion length scale). Furthermore, Rey and Skolnick have shown [23] that the folding trajectories obtained with Monte Carlo simulations are consistent with those obtained with real Molecular Dynamics calculations.

[21] N. Metropolis, A. W. Rosenbluth, M. N. Rosenbluth, A. H. Teller and E. Teller, J. Chem. Phys. 21, 1087 (1953)

[22] K. Kikuchi, M. Yoshida, T. Maekawa and H. Watanabe, Chem. Phys. Lett. 196, 57 (1992)

[23] J. Rey and J. Skolnick, Chem. Phys. 158, 199 (1991)

[24] This is equivalent to assume that different degrees of optimization are mainly due to "cold" sites, while "hot" sites are always optimized at the same degree.

[25] In the case of pointlike mutations this cannot be done, because the associated $p_2(\Delta E)$ is not zero for negative values of the energy, so that energies within the gap can be obtained from mutations in "hot" sites, compensated by mutations with negative energy values in other sites.

[26] R. A. Broglia, G. Tiana, S. Pasquali, H. E. Roman, E. Vigezzi, Proc. Natl. Acad. Sci. USA 95, 12930 (1998)

[27] P. Schuster and W. Fontana, Physica D 133, 427 (1999)

[28] G. Tiana, R. A. Broglia and E. Shakhnovich, Proteins 39, 244 (2000)

[29] T. E. Creighton, Proteins, J. Freeman and Co., New York (1993)
