

University of Warwick institutional repository: <http://go.warwick.ac.uk/wrap>

This paper is made available online in accordance with publisher policies. Please scroll down to view the document itself. Please refer to the repository record for this item and our policy information available from the repository home page for further information.

To see the final version of this paper please visit the publisher's website. Access to the published version may require a subscription.

Author(s): P. F. N. Faisca and R. C. Ball

Article Title: Thermodynamic control and dynamical regimes in protein folding

Year of publication: 2002

Link to published article:

<http://dx.doi.org/10.1063/1.1466833>

Publisher statement: Copyright (2002) American Institute of Physics. This article may be downloaded for personal use only. Any other use requires prior permission of the author and the American Institute of Physics. The following article appeared in Faisca, P. F. N. and Ball, R. C. (2002) Thermodynamic control and dynamical regimes in protein folding, *Journal of Chemical Physics*, **116** (16), 7231 and may be found at <http://dx.doi.org/10.1063/1.1466833>.

# Thermodynamic control and dynamical regimes in protein folding

P.F.N. Faisca<sup>1,2</sup> and R.C. Ball<sup>1</sup>

<sup>1</sup>*Department of Physics, University of Warwick, Coventry CV4 7AL, U.K.*

<sup>2</sup>*CFMC, Av. Prof. Gama Pinto, 2, 1649-003 Lisboa Codex, Portugal*

Monte Carlo simulations of a simple lattice model of protein folding show two distinct regimes depending on the chain length. The first regime well describes the folding of small protein sequences and its kinetic counterpart appears to be single exponential in nature, while the second regime is typical of sequences longer than 80 amino acids and the folding performance achievable is sensitive to target conformation. The extent to which stability, as measured by the energy of a sequence in the target, is an essential requirement and affects the folding dynamics of protein molecules in the first regime is investigated. The folding dynamics of sequences whose design stage was restricted to a certain fraction of randomly selected amino acids shows that while some degree of stability is a necessary and sufficient condition for successful folding, designing sequences that provide the lowest energy in the target seems to be a superfluous constraint. By studying the dynamics of under annealed but otherwise freely designed sequences we explore the relation between stability and kinetic accessibility. We find that there is no one-to-one correspondence between having low energy and folding quickly to the target, as only a small fraction of the most stable sequences were also found to fold relatively quickly.

87.14.Ee; 87.15.Aa

## I. INTRODUCTION

In the early sixties, the Nobel Laureate Christian Anfinsen showed through in vitro experiments that denatured proteins can refold to their original native structure in the absence of any catalyst [1]. This suggested that protein folding (PF) can be a spontaneous, first-order process, and that the only information required for the protein sequence to fold correctly is the sequence itself. Thus, in Anfinsen's perspective, sequence is the only determinant of the rates and mechanisms of folding. Soon after these discoveries, Levinthal pointed out that a random search of the conformational space, as implied by Anfinsen's thermodynamic hypothesis, could not explain the time scale of folding as observed in Nature [2]. To bypass this paradox, Levinthal proposed a kinetic view, that proteins must fold through some directed process, whose nature could involve for instance, the existence of folding pathways. The latter do not necessarily imply a fixed sequence of events in folding, nor do they require the existence of observable folding intermediates.

In the late 1980's, Bryngelson and Wolynes [3] introduced the concept of energy landscape—the free energy as a function of protein conformation—as an attempt to reconcile the kinetic and thermodynamic views of PF. The 'topology' of the landscape characterizes the folding kinetics through the existence of folding pathways. In Fig. 1 we show a cross section of the energy landscape of a hypothetical random heteropolymer: as a consequence of the existence of numerous local energy minima these sequences tend to behave as highly frustrated systems. Nevertheless, it has been shown that a very small frac-

tion of random sequences are able to stably fold to what can be considered their native states (a deep energy minimum of the energy landscape), in a biologically acceptable timescale [4].

Dill and co-workers [5] proposed that a stable, fast folding protein sequence must satisfy two essential requirements: thermodynamic stability meaning the existence of a deep global minimum in the energy landscape and kinetic accessibility meaning the existence of a basin of attraction sloping toward that minimum. This basin of attraction, first proposed by Leopold *et al* [6] has become one of the most important concepts in protein folding dynamics and is commonly known as the folding funnel.

In 1993, Shakhnovich and Gutin [7] developed a design method with the purpose of creating protein like sequences, that is, sequences that fold fast and stably to their respective native structures. The method is based on the thermodynamic stability requirement, and was inspired by the behavior displayed by that very small fraction of protein like random sequences. Shakhnovich [9] claims that a thermodynamic driven sequence selection actually solves the kinetic problem as well, which in turn suggests a correlation between thermodynamic stability and kinetic accessibility. This design method has been widely used to study the folding dynamics of protein sequences whose length ranges from a few to at least 80 beads in simple lattice models.

Is it possible to fold longer protein chains ( $> 80$  amino acids) using the thermodynamic stability as the unique driver of sequence design? This open question was the starting point and initial motivation for the present paper. As in previous studies, Monte Carlo simulations of a simple lattice model were used to tackle the problem. In the present case we particularly focus on the effects of native state structure on the folding dynamics.

This paper is organized as follows. Sec. II reviews the model as well as the numerical methods used to sample

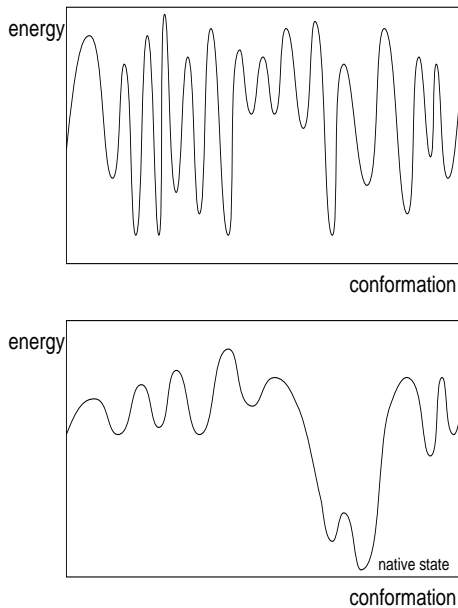


FIG. 1. Energy landscape of a random heteropolymer (upper figure) and a protein landscape exhibiting a folding funnel.

both conformational and sequence space. In Sec. III the numerical results are presented. We start by studying the dependence of the folding time on temperature and then explore the extent to which the former is a sequence specific kinetic property. Then, we present evidence for the existence of two distinct dynamical regimes in PF. Finally, for sequences which to fold in accordance to what we call the first regime, we explore the effects of stability on folding dynamics and also how it correlates with kinetic accessibility. In Sec. IV we make some final comments and conclusions.

## II. MODELS AND METHODS

### A. Folding in conformational space

Part of the contribution of computational physics to protein folding includes several findings obtained in the scope of simple lattice models. Most generally, these models consider a coarse-grained description of the protein, reduced to its backbone structure through a 'bead & stick' representation. Each bead is ascribed a certain chemical identity representing an amino acid type, while each stick stands for the peptide bond that covalently connects amino acids along the polypeptide chain. This structure is allowed to move in a three dimensional infinite lattice, subjected to excluded volume constraints and exploring the conformational space in accordance with a kink-jump dynamics obeying the restriction that no bond length changes (Fig. 2). The energy of a conformation is given by the contact Hamiltonian

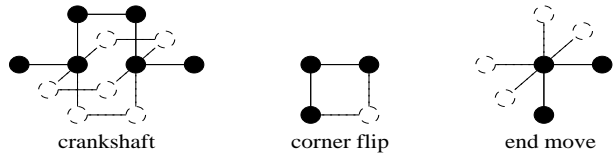


FIG. 2. Move set used to generate the dynamics.

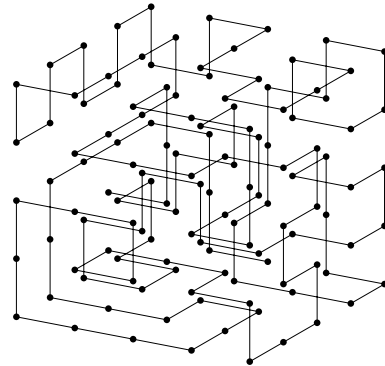


FIG. 3. Example of a 125 bead long target found by homopolymer relaxation.

$$H(\{\sigma_i\}, \{\vec{r}_i\}) = \sum_{i>j}^N \epsilon(\sigma_i, \sigma_j) \Delta(\vec{r}_i - \vec{r}_j), \quad (1)$$

where  $\{\sigma_i\}$  represents an amino acid sequence,  $\sigma_i$  standing for the chemical identity of bead  $i$ , while  $\{\vec{r}_i\}$  is the set of bead coordinates that define the conformation in question. The contact function  $\Delta$  equals 1 if beads  $i$  and  $j$  are in contact but not covalently linked and is 0 otherwise. We follow many previous studies in taking the interaction parameters  $\epsilon$  from the  $20 \times 20$  Miyazawa-Jerningan matrix, derived from the distribution of contacts in native proteins [11]. Eq. (1) defines an energy function in conformational space whose graph is the energy landscape. The energy landscape is explored via the Metropolis Monte Carlo (MC) acceptance rule. This means that downhill transitions (that lower the energy) are accepted with probability unity and uphill transitions with probability proportional to the Boltzmann factor. Thus if  $\Gamma_A$  and  $\Gamma_B$  are two distinct conformations, and  $\Delta H = H_A - H_B$  is the energy difference between the states defined by these conformations,

$$P_{\Gamma_A \rightarrow \Gamma_B} = \begin{cases} 1 & \text{if } \Delta H < 0 \\ \exp[-\Delta H/k_B T] & \text{if } \Delta H \geq 0, \end{cases} \quad (2)$$

where  $P$  is the transition probability,  $T$  the temperature and  $k_B$  the Boltzmann constant.

## B. Design in sequence space

The first step at the design stage is the choice of the target structure. A target is a maximally compact and otherwise arbitrary structure such as the one shown in Fig. 3. Maximally compact structures maximize the number of contacts for a certain chain length and therefore minimize the degeneracy of the Hamiltonian (1). The goal of the design process is, given a target, to find a sequence that folds to it efficiently, and we follow Shakhnovich and Gutin [7] in attempting this by seeking the sequence with the lowest possible energy in the target state, as given by Eq. (1). For this purpose, target's co-ordinates are quenched and the energy of Eq. (1) must be annealed with respect to the sequence variables. This naturally leads to the idea of simulated annealing in sequence space; starting from a random initial sequence, transitions between different sequences are successively attempted—different sequences are generated by randomly permutating pairs of beads—along with a suitable annealing schedule. If  $S_A$  and  $S_B$  are two different sequences the transition probability,  $P_{S_A \rightarrow S_B}$ , comes given Eq. (2) with the temperature replaced by  $T_i = \alpha T_{i-1}$  and  $0 < \alpha < 1$ . This optimization procedure has been coined simulated annealing in sequence space. It was the first successful procedure to design protein sequences.

## III. IN VIRTUO RESULTS

### A. Finding the optimal folding temperature

For each number of monomers  $N = 27, 36, 48, 64, 80$  and 100 we found 5 different maximally compact target structures by homopolymer relaxation. This method is an efficient way to systematically find kinetically accessible maximally compact structures and was previously used by Abkevich *et al* in [8] for 36 bead long targets. Then, for each target we prepared a set composed of 30 sequences by applying the Shakhnovich method as previously outlined. Next, we went on to determine the optimal folding temperature,  $T_{fold}$ . For this purpose, a designed sequence at each  $N$  was randomly selected and subjected to MC folding simulations at several temperatures. For  $N = 27, 36, 48$  and 64 a set of 50 MC runs was performed for each temperature and the folding time  $t$  was taken as the value of the mean first passage time (FPT) to the target averaged over the 50 MC runs. Results plotted in Fig. 4(a) show that there is an optimal folding temperature,  $T_{fold}$ , where folding to the target structure proceeds relatively fast. However, away from this optimum, both at higher and lower temperatures the process gets increasingly slower. In the first case the protein sequence tends to behave like a random heteropolymer rapidly fluctuating between unfolded states. In the second case the folding kinetics gets slower because there is a high probability for the chain to get trapped

into metastable states and folding entails overcoming the corresponding energy barriers [12].

For  $N = 80$  and  $N = 100$  the number of successful folding runs per each studied temperature, was only one half of the attempted total. For this reason, we choose  $T_{fold}$  as the temperature where the highest ratio of folding success could be observed.

It has been claimed that the folding time and temperature are both sequence specific parameters [13]. To investigate this issue, five 48 bead long sequences were randomly selected (one sequence per target) and their folding behavior was studied over a temperature range as shown in Fig. 4(b). These show that whilst folding to the target can be quite target and (or) sequence dependent, the optimal folding temperature is close to a self-averaging quantity in our simulations. It should be emphasised that our sequence design preserved overall chemical composition, in contrast to earlier work [13] which for unrestricted binary alphabet sequences found the optimal temperature to be more sequence dependent.

### B. Dependence of the folding probability on folding time: evidence for two folding regimes.

We have explored the time dependence of folding for the different chain lengths  $N$  each at their respective optimal folding temperature  $T_{fold}(N)$ . Specifically we report in Fig. 5 the probability  $P_{fold}(t)$  of the chain having visited its target conformation after time  $t$ . A first look at the graph suggests that for  $N$  up to 64 the curves appear to be functionally similar. A scaling factor of the form  $t' = (N'/N)^\alpha t$  translates in the logarithmic plot to a shift

$$\log t' = \log t + \alpha \log \frac{N'}{N}. \quad (3)$$

Taking  $\alpha = 5$  (and  $N' = 48$ ) the shifted curves  $N = 27, 36, 48$  and 64 superimpose well as shown in Fig. 6. For  $N \geq 80$  this superposition regime breaks down and the asymptotic value of  $P_{fold}(t)$  decreases quite considerably. Fig. 7 shows that the break in the folding behaviour is associated with the onset of target-dependence of the folding curves, where we have computed  $P_{fold}(t)$  separately for each of the five targets at both  $N = 64$  and  $N = 100$ . For  $N = 64$  all the targets exhibit a similar functional dependence of  $P_{fold}$  on  $\log(t)$ . All appear consistent with asymptotic values of  $P_{fold} \rightarrow 1$  and also the dispersion of the folding time is small. However, for  $N = 100$  four out of the five targets have apparent asymptotics  $P_{fold} \leq 1$  and there is considerably larger dispersion of the folding time.

One possible explanation for this change in behaviour which we have ruled out, is that for  $N \geq 80$  the optimal folding temperature might become a target sensitive parameter. To test this hypothesis, we randomly selected

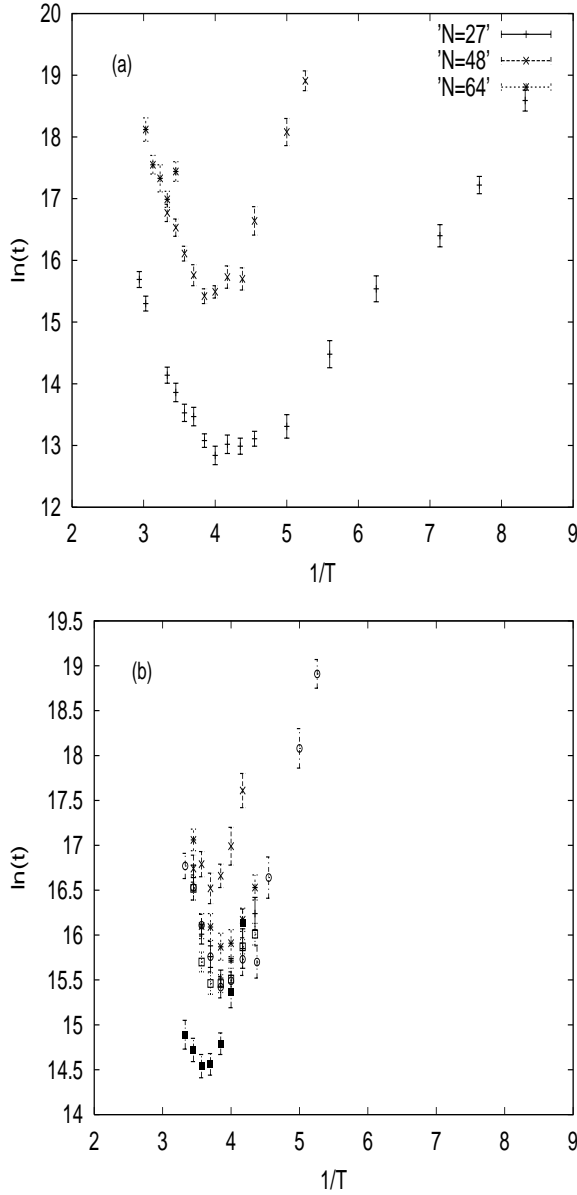


FIG. 4. (a) Dependence of the folding time  $t$  on the inverse temperature  $1/T$  for several values of the chain length. Note that as  $N$  increases the temperature range where folding can be observed narrows. Fig. 4(b) shows the same dependence for six different 48 bead long sequences trained to the same target.

five 100 bead long sequences (one per each target), and ran 20 folding simulations per each value of the temperature in a certain temperature range. Fig. 8 shows how foldicity, defined as the fraction of successful folding runs over the total number of attempted runs, changes with inverse temperature for each target. Except for target 5, all the others exhibit a maximum value of foldicity at what we considered the optimal folding temperature. Target 5 marginally shows a shallow minimum and is in any case the fastest and most successful to fold in our

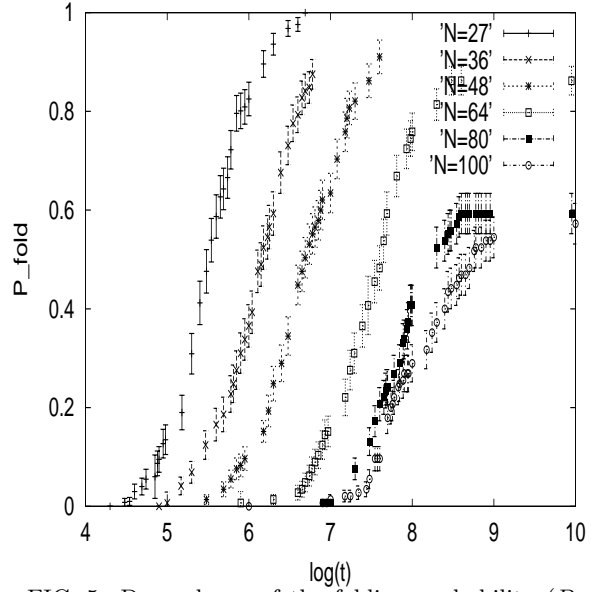


FIG. 5. Dependence of the folding probability ( $P_{fold}$ ) on  $\log(t)$ . For each curve 150 simulations were used distributed across the available targets and sequences.  $P_{fold}$  was calculated as the number of folding simulations which ended up to time  $t$  normalized to the total number of runs.

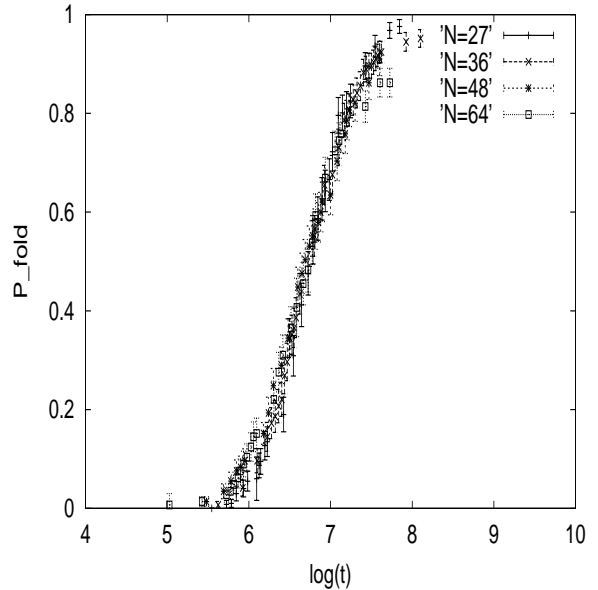


FIG. 6. Shifted curves for  $P_{fold}$  vs  $\log(t)$  for  $N = 27$ ,  $N = 36$  and  $N = 64$ . These curves were obtained by shifting the folding times by a factor of  $5 \log(48/N)$ .

previous results (Fig. 7(b)), so we can safely rule out target sensitive optimal folding temperature as the cause of target sensitive folding performance.

A primary conclusion that can be drawn from these results is that for  $N \geq 80$  folding dynamics becomes target selective, certain targets being more kinetically accessible than others. Having analysed only five targets per chain length, we are not in a position to characterise quantitatively the resulting distribution of behaviour. Nevertheless, the emergence of this new dynamical feature is a clear indication that for  $N \geq 80$  thermodynamic stability is not the dominant drive in folding, and in particular it does not solve the kinetic accessibility problem as it has been previously suggested.

### C. Folding kinetics-dependence of the folding time ( $t$ ) on the chain length ( $N$ )

The folding time  $t$  is a kinetic property that measures how fast a protein sequence folds into its native state from an initial unfolded coil. It is known that the fastest simple, single domain protein folds a million time faster than the slowest [10]. However, and despite this broad kinetic spectrum, there seems to be a general consensus among biochemists that protein kinetics falls into two main general classes. The analysis of experimental data collected in the course of the last ten years has put forward the theory that proteins smaller than 100 amino acids are committed to follow a two-state (or single exponential) kinetics. The transition state is the only kinetically important intermediate and conformational searching is the only factor limiting folding speed. Bigger proteins, on the other hand generally fold in agreement with a mutiexponential kinetics. The latter often involves fast collapse into kinetic traps and subsequent slower barrier climbing out of the traps. This generates an overall process characterised by the existence and accumulation of more than one important kinetic intermediate. Our results are in a broad agreement with this scenario, and the plot of  $(1 - P_{fold})$  in Fig. 9 supports identifying our regime for  $N < 80$  with single exponential kinetics.

Recall from Fig. 6, that tuning  $\alpha$  to 5 in Eq. (3) nicely superimposes the curves of  $P_{fold}$  vs  $\log t$  for  $N$  up to 64. This suggests that in our first regime a scaling law of the type  $t \approx N^5$  appropriately describes the dependence of the folding time,  $t$ , with the chain length,  $N$ . The plot of  $\ln t$  vs  $\ln N$  in Fig. 10 confirms (with a significant correlation of 0.99) an exponent of 5.27.

Note that for  $N \geq 80$  the mean FPT does not yield a correct estimate of the folding time because the value of  $P_{fold}$  does not tend to one in the limit of large  $t$ . Therefore we can only analyse the dependence of  $t$  on  $N$  for  $N \leq 80$ .

A scaling law of the form  $t \approx N^4$  was suggested by a previous estimate by Gutin *et al* [12] from folding simulations to targets which are not maximally compact structures. The fact that these targets have a higher kinetic

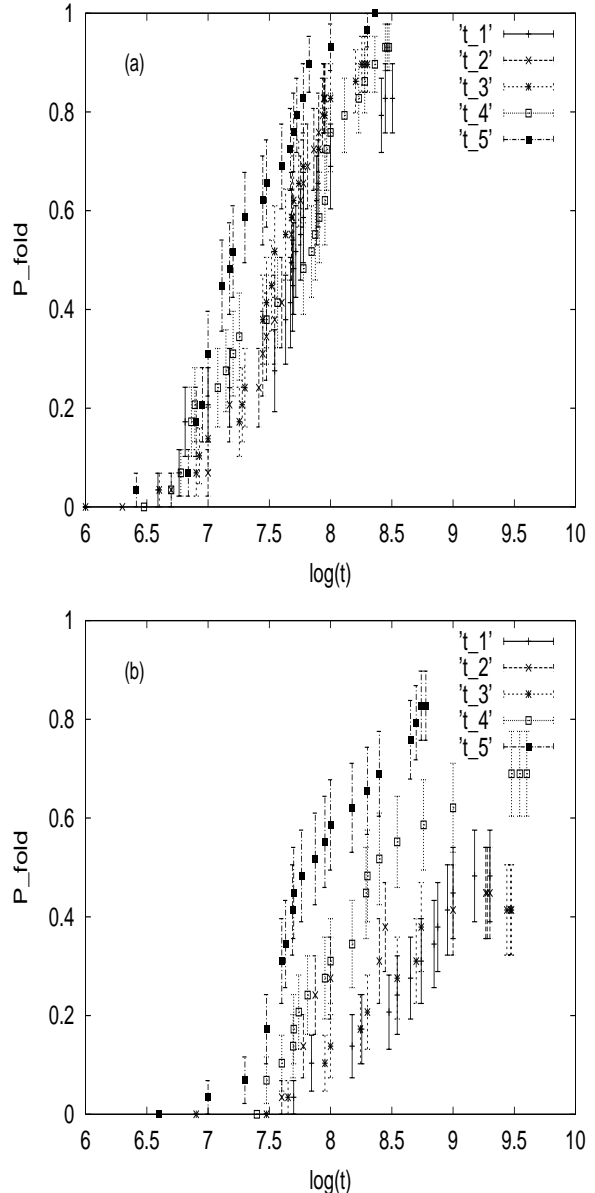


FIG. 7. Separate contribution of each one of the 64 (Fig. 7(a)) and 100 (Fig. 7(b)) bead long targets for the dependence of the folding probability ( $P_{fold}$ ) on  $\log(t)$ .  $P_{fold}$  was calculated as the number of folding simulations which ended up to time  $t$  normalized to the total number of runs.

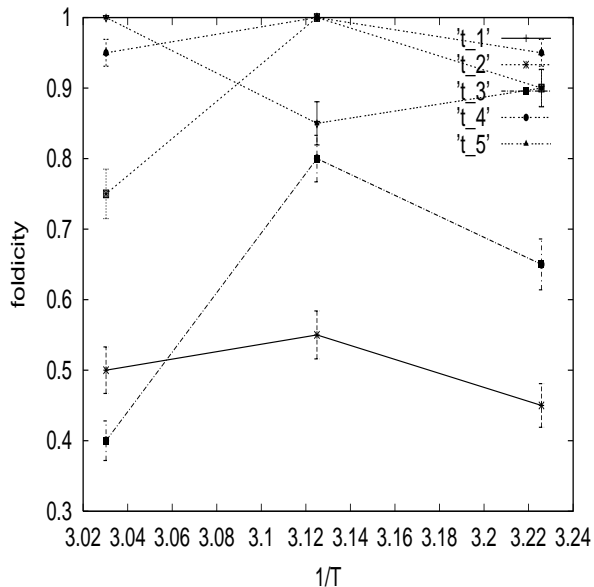


FIG. 8. Dependence of foldicity on the inverse temperature,  $1/T$  for each of the 100 bead long targets. Except for target 5, all the other targets exhibit a maximum value of foldicity at what we considered to be the optimal folding temperature.

accessibility than the ones we considered might explain the weaker dependence obtained for the folding time on the chain length.

#### D. Effects of stability on folding dynamics

In this section we explore the extent to which stability, as measured by the sequence energy in the target affects the folding performance of proteins in our first regime. For this purpose we studied the MFPT to five target states of several 48 bead long sequences whose training was handicapped by fixing a priori a certain fraction  $r_{fix}$  of the beads. We designed three sets of 30 sequences per target, each set corresponding to an  $r_{fix}$  of 0.01, 0.17 and 0.25 respectively. This biases the design procedure to sequences higher in energy as  $r_{fix}$  increases allowing us to explore an energy range of  $\Delta E \approx 5$ . The MC folding simulations were again performed at  $T_{fold}$  and proceeded up to  $n_s = 9 \times 10^8$  MC steps or until folding was observed. Firstly we analyse the effects of stability on foldicity. In Fig. 11, the main plot shows how foldicity changes with  $r_{fix}$ , while in the inner plot, the averaged mean sequence energy is plotted against the same parameter. It can be seen that foldicity is insensitive to raising the energy up to an average value of  $E \approx -19$  (corresponding to  $r_{fix} = 0.17$ ) but above this threshold it sharply decreases. We should stress however that  $E \approx -19$  is still well below the threshold heteropolymer energy,  $E_C$  [9], for this specific chemical composition  $\langle E_C \rangle = -7.3849952 \pm 1.7438$  where the average is taken over the five considered targets.

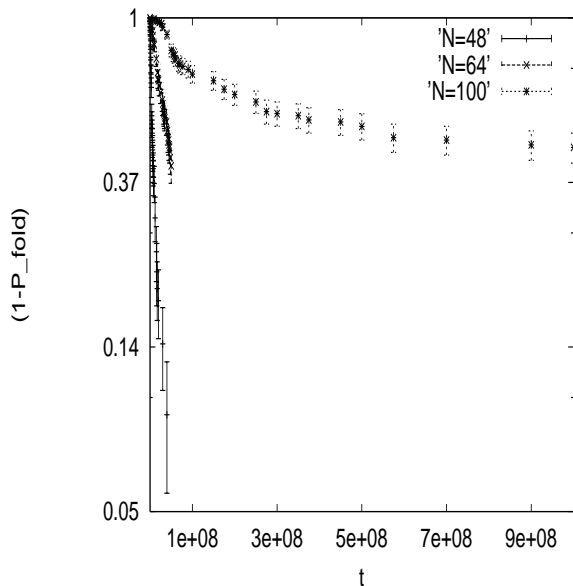


FIG. 9. Evidence for a single exponential kinetic regime for  $N = 48$  and  $N = 64$  but not 100.

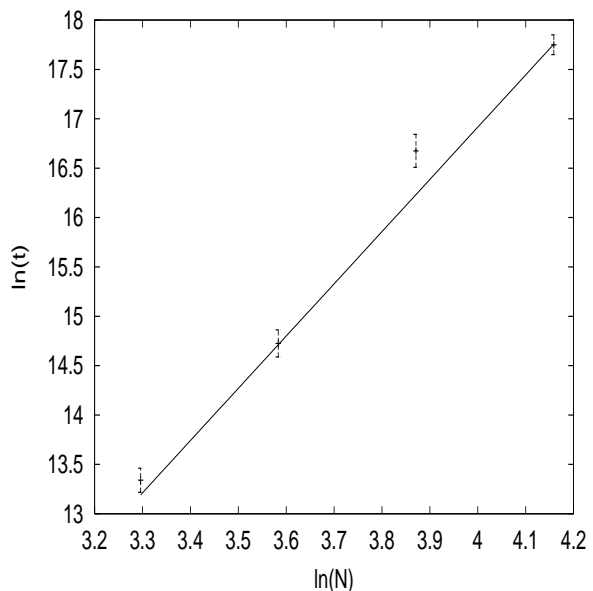


FIG. 10. Dependence of the folding time  $t$  on the chain length  $N$  for proteins that follow a two-state kinetics. For  $N = 27, 36, 48$  and  $64$  we computed the folding time as the mean FPT averaged over 150 simulation runs distributed over the five considered targets. We stress that the use of different targets to compute this plot does not smear the result because there is not target selectivity in this folding regime.

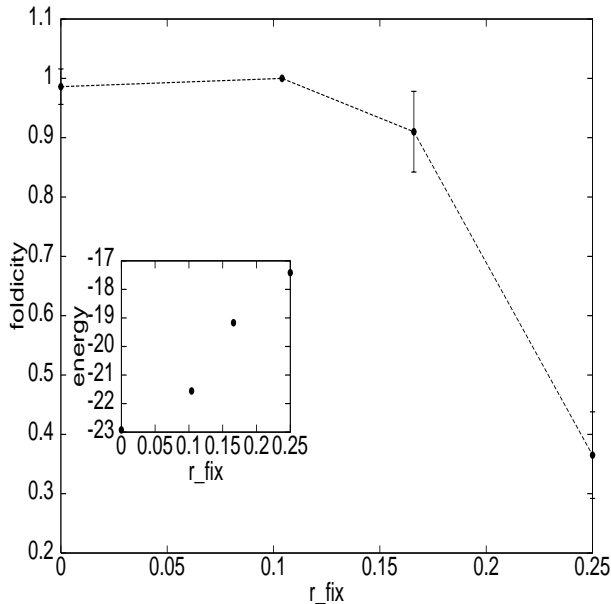


FIG. 11. Dependence of foldicity on the fraction of fixed monomers,  $r_{fix}$  (main plot) for  $N = 48$ . The inner plot shows the dependence of the mean averaged sequence energy with  $r_{fix}$ .

Fig. 12 shows the folding probability *vs* time for each value of  $r_{fix}$ . It can be seen that while the  $r_{fix} = 0.17$  curve is only shifted from the  $r_{fix} = 0$  and  $r_{fix} = 0.1$  curves (which translates in a slower folding dynamics), for  $r_{fix} > 0.17$  the folding regime clearly breaks away. Curiously, the curves corresponding to  $r_{fix} = 0$  and  $r_{fix} = 0.10$  nicely superpose. In energetic terms this translates into a break in the folding regime for energies higher than  $E \approx -19$ .

Taken together these results suggest that some degree of stability is a sufficient condition for folding, controlling and efficiently driving the dynamics of small protein sequences. This in turn agrees with the scenario of folding being essentially a downhill process to the native state (energy minimum). However, designing sequences that provide the lowest energy in the target seems to be a superfluous constraint.

### E. Stability and kinetic accessibility

The picture we can draw from the results presented so far is that for sequences whose length does not exceed 80 amino acids, thermodynamically oriented design ensures successful folding to the targets. As previously stated, it has been claimed [12] that not only do these sequences fold stably (in the sense of allowing a high target average time occupancy), but they also fold quicker, which suggests a correlation (at least to some extent) between stability and kinetic accessibility.

Fig. 13 shows an accessibility-stability plot; accessibility is measured by the folding time  $t$  (averaged mean FPT

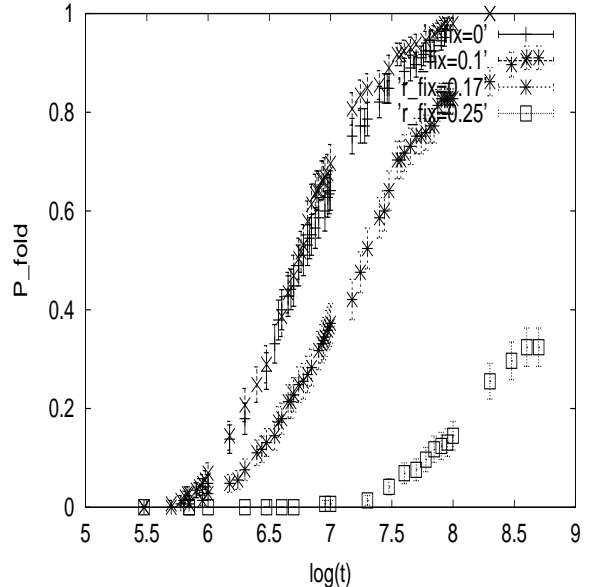


FIG. 12. Dependence of the folding probability,  $P_{fold}$ , on  $\log(t)$  for several values of the fraction of fixed monomers,  $r_{fix}$ .

over 10 simulation runs) and each point represents a 48 bead long sequence with energy  $E$  that folds to the target in time  $t$ . Sequences constrained by different values of  $r_{fix}$  are distinguished.

The graph strongly indicates that the quickest folders are not necessarily the most stable sequences. In a previous report, Fink and Ball [15], [16], [17], showed through the study of a convenient ensemble of 27 bead long sequences, that in the region of sufficient thermodynamic stability, the latter is in conflict with optimal accessibility, and that a significant increase in kinetic performance will be achieved if a marginal increase in the target's energy is allowed.

In order to investigate how stability and accessibility correlate for 48 bead long protein sequences, we prepared an ensemble of  $\approx 1000$  sequences, but instead of freezing all of them, some were annealed to some temperature different from zero. This allows us to scan a representative fraction of the accessibility-stability phase space. Results are plotted Fig. 14, where once again we are taking  $t$  as the averaged FPT over 10 simulation runs. The graph shows that the connection between stability and accessibility, is not that of a simple correlation. In particular, it can be seen that although the quickest folders appear in an energy range of high stability ( $-22 < E < -23$ ), the most stable sequences do not show highest accessibility.

## IV. CONCLUSIONS

The assumption that 'target structure' could be an important parameter in the dynamics of protein folding, and its subsequent introduction in the folding simula-



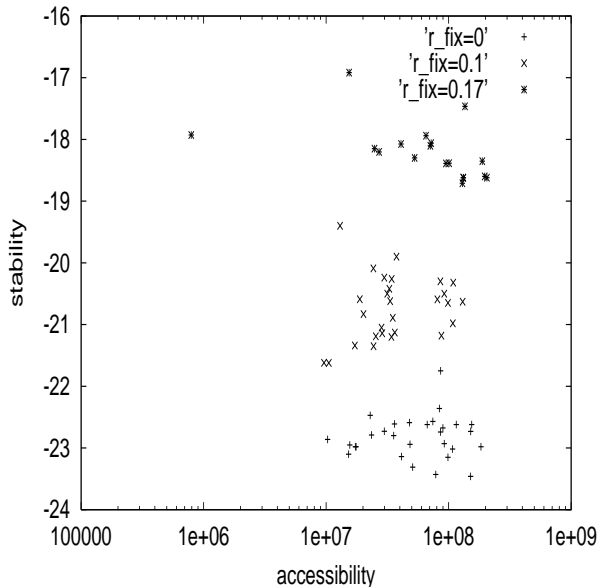


FIG. 13. Accessibility-stability plot for the 48 bead long sequences whose design stage was handicapped by fixing a priori a certain number of beads.

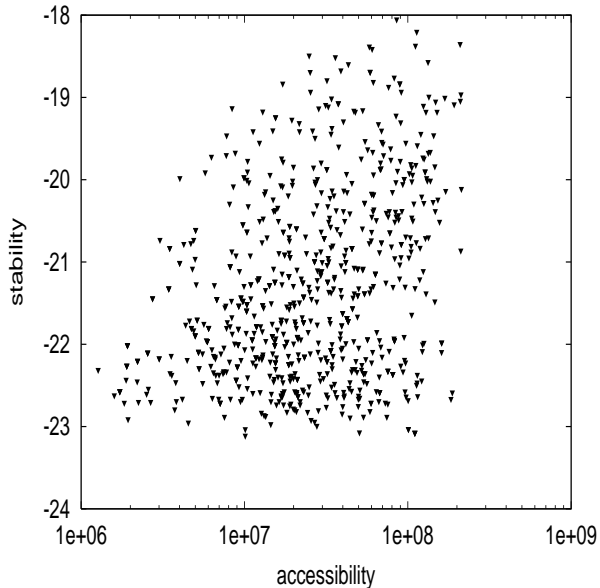


FIG. 14. Accessibility-stability scatter plot for an ensemble of  $\approx 1000$  48 bead long sequences.

tions of a simple lattice model, made it possible to discriminate between two distinct regimes in the dynamics. The first regime well describes the folding of small protein molecules, and its kinetics appears to be single exponential. In this case, conformational search must be the only factor limiting folding speed. Folding time scales with chain length as  $t \approx N^5$  in this regime. On the other hand, the folding of protein sequences bigger than 80 amino acids appears to be target sensitive with prone to a dynamics which we might interpret as the falling in kinetic traps strongly delaying folding to the target.

The extent to which stability, as measured by the sequence energy in the target, controls folding of proteins that fall in the first regime was investigated. Results agree with the idea that for small protein molecules, stability is a necessary and sufficient condition for successful folding. However, designing sequences for minimal energy in the target conformation appears to be superfluous.

The controversial claim that the most stable sequences are also the quickest folders was investigated. Notwithstanding the fact that this is a delicate issue given the considerable dimension of sequence space and the difficult task of suitably sampling it, our results (taken from 1000 sequences) strongly indicate that the correlation between stability and accessibility is essentially small.

As a general conclusion we can say that there is much more than thermodynamics in protein folding. In particular for long protein chains it is evident that target geometry matters and thermodynamic factors are not the sole determinant of folding performance.

There is a growing idea among biologists, that structural factors are a key point in protein folding dynamics. In this context and in the scope of lattice models, it is urgent to test the correlation that biochemists find between CO (contact order—the average sequence separation among contacting residue pairs) and folding rates.

## ACKNOWLEDGMENTS

The authors would like to thank Dr. Thomas M. Fink for helpful suggestions. P.F.N. Faisca would like to thank Programa Praxis XXI for financial support.

- 
- [1] C. Anfinsen, *Science* **181**, 223 (1973)
  - [2] C. Levinthal, *J. Chem. Phys.* **65**, 44 (1968)
  - [3] J. Bryngelson, P. Wolynes, *Proc. Natl. Acad. Sci. USA* **84**, 7524 (1987)
  - [4] A. Sall, E. I. Shakhnovich and M. Karplus, *Nature* **369**, 248 (1994)
  - [5] K. A. Dill and S. Chen, *Nature Struct. Biol.* **4**, 10 (1997)

- [6] P. E. Leopold, M. Montal and J. N. Onuchic, Proc .Natl. Acad. Sci. USA **89**, 8721 (1992)
- [7] E. I. Shakhnovich, A. M. Gutin, Proc .Natl. Acad. Sci. USA **90**, 7195 (1993)
- [8] V. I. Abkevich, A. M. Gutin and E. I. Shakhnovich, J. Mol. Biol. **252**, 460, (1995)
- [9] E. I. Shakhnovich, Phys. Rev. Lett. **72**, 3907 (1994)
- [10] K. W. Plaxco, K. T. Simmons, I. Ruczinski and D. Baker, Biochemistry **39**, 11177 (2000)
- [11] S. Myazawa and R. Jernigan, Macromolecules **18**, 534 (1985)
- [12] A. M. Gutin, V. I. Abkevich and E. I. Shakhnovich, Phys. Rev. Lett. **77**, 5433 (1996)
- [13] N. D. Socci and J.N. Onuchic, J. Chem. Phys. **101**, 1519 (1994)
- [14] H.S.Chen, K.A. Dill, Proteins: Structure, Function and Genetics **30**, 2 (1998)
- [15] T. M. Fink and R. C. Ball, Physica D **107**, 199 (1997)
- [16] T. M. Fink and R. C. Ball, submitted to Phys. Rev. Lett. (2000)
- [17] T. M. Fink and R. C. Ball, submitted to Phys. Rev. Lett. (2000)