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On folding and form: Insights from lattice simulations

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Abstract

Monte Carlo simulations of a Miyazawa-Jernigan lattice-polymer model indicate that, depending on the native structure's geometry, the model exhibits two broad classes of folding mechanisms for two-state folders. Folding to native structures of low contact order is driven by backbone distance and is characterised by a progressive accumulation of structure towards the native fold. By contrast, folding to high contact order targets is dominated by intermediate stage contacts not present in the native fold, yielding a more cooperative folding process.

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INTRODUCTION

Advances in experimental techniques and the use of computational models have shown that most small (from ~ 50 -120 amino acids), single domain proteins fold via two-state kinetics, without observable folding intermediates and with a single transition state associated with one major free energy barrier separating the native from the unfolded conformations [1, 2, 3, 4, 5]. In addition it is also well known that two-state proteins, with similar chain lengths, exhibit a remarkably wide range of folding rates, folding in microseconds to seconds [6, 7, 8]. Understanding what makes some proteins such incredibly fast folders will shed light into the underlying folding mechanism.

The energy landscape theory predicts that the landscape's ruggedness plays a fundamental role in the folding kinetics of proteins: The existence of local energy minima, that act as kinetic traps, is responsible for the overall slow and, under some conditions (as the temperature approaches the glass transition temperature), glassy dynamics. However, and as pointed out by Du *et al.* [9], another equally important aspect of the folding dynamics is related to the geometry of protein chain conformations. Indeed, both chain connectivity and (steric) excluded volume impose restrictions on the number of allowable conformations a polypeptide chain can adopt and these geometrical constraints play a significant role in determining the folding pathways that are kinetically accessible.

A quantitative measure of geometric complexity, the so-called relative contact order, CO , was introduced in 1998 by Plaxco *et al.* [8]: The CO is a simple, empirical parameter measuring the average sequence separation of contacting residue pairs in the native structure relative to the chain length of the protein

$$CO = \frac{1}{LN} \sum_{i,j}^N \Delta_{i,j} |i - j|, \quad (1)$$

where $\Delta_{i,j} = 1$ if residues i and j are in contact and is 0 otherwise; N is the total number of contacts and L is the protein chain length. A strong correlation ($r = 0.94$) was found between the CO and the experimentally observed folding rates in a set of 24 non-homologous single domain proteins [10] suggesting a topology-dependent kinetics of two-state folders. Results obtained by two of us [11] in the context of a simple Miyazawa-Jernigan (MJ) lattice polymer model [11] showed a significant correlation ($r = 0.70 - 0.79$) between increasing CO and the longer logarithmic folding times. In a more recent study Jewett *et al.* [12] found a similar

correlation ($r = 0.75$) for a 27-mer lattice polymer modeled by a modified Gō-type potential. These results support the empirical correlation found between contact order and the kinetics of two-state folders.

In this paper we investigate whether the geometry of the native structure does, or does not, promote different folding processes, eventually leading to different folding times, in the context of the MJ lattice-polymer model and Monte Carlo (MC) folding simulations. Although lattice models are not capable of describing the full complexity of real proteins they are non-trivial and thus may capture some fundamental aspects of protein folding kinetics [13]. The native structures considered in our study were selected on the basis of their different contact order parameters. The CO is clearly not the only way to quantify the native structure’s geometry but the empirical finding that the CO correlates well with the folding rates of real proteins strongly motivates its use for the purposes of the present work.

The paper is organized as follows: Section II reviews the model and methods used in the lattice simulations. In section III the numerical results are presented. We start with a preliminary study emphasizing the gross distinctive features observed between the folding dynamics to low and to high- CO structures. Subsequently we make a more detailed analysis of the folding dynamics associated with the low- CO and high- CO native structures that exhibit respectively the lowest and the highest folding times in order to highlight specific traits of the respective folding processes. In section IV we make some final remarks and summarize our conclusions.

MODEL AND METHODS

We consider a simple three-dimensional lattice model, based on a bead and stick representation, of a protein molecule. In such a coarse grained model a bead represents an amino acid and the unit length stick stands for the peptide bond that covalently connects amino acids along the polypeptide chain. The chains consist of $N = 48$ beads interacting via short-range interactions described by the contact Hamiltonian

$$H(\{\sigma_i\}, \{\vec{r}_i\}) = \sum_{i>j}^N \epsilon(\sigma_i, \sigma_j) \Delta(\vec{r}_i - \vec{r}_j), \quad (2)$$

where $\{\sigma_i\}$ represents an amino acid sequence, σ_i standing for the chemical identity of bead i , while $\{\vec{r}_i\}$ is the set of bead coordinates defining each conformer. The contact function Δ

is 1 if beads i and j are in contact but not covalently linked and is 0 otherwise. We follow previous studies [14, 15, 16, 17, 18] by taking the interaction parameters ϵ from the 20×20 MJ matrix, derived from the distribution of contacts in native proteins [19].

The folding simulations follow the standard MC Metropolis algorithm [20] and, in order to mimic protein movement, we use the kink-jump MC move set, including corner flips, end and null moves as well as crankshafts [21].

Each MC run starts from a randomly generated unfolded conformation (typically with less than 10 native contacts) and the folding dynamics is traced by following the evolution of the fraction of native contacts, $Q = q/Q_{max}$, where $Q_{max} = 57$ and q is the number of native contacts at each MC step. The folding time, t , is taken as the first passage time (FPT), that is, the number of MC steps that corresponds to $Q = 1.0$.

NUMERICAL RESULTS

Targets

The distribution of the relative contact order parameter over a population of 500 target geometries, folding to fill a simple cuboid, was found via homopolymer relaxation [11] and exhibits CO values that span the intervals centered around $CO = 0.13$ and $CO = 0.26$. To investigate the effects of CO on the folding dynamics we selected from our target pool the three lowest- CO and the three highest- CO maximally compact structures as the targets of our protein model.

A preliminary analysis of the folding dynamics

For each target, a set of 100 proteinlike sequences was prepared using the Shakhnovich and Gutin design method [14]. The averaged trained sequence energy, $\langle E \rangle$, and its standard deviation, σ , are shown in Table I, where the targets are ordered with increasing CO .

The folding dynamics was studied at the so-called optimal folding temperature, the temperature that minimizes the folding time, taken as the value of the mean FPT to the target averaged over the 100 MC runs [11]. Note that the high- CO targets are associated with folding times that are systematically larger than those associated with the low- CO targets.

Indeed, in this 6-element target set, contact order and folding times correlate well ($r = 0.84$). The simulated range of folding rates is, however, much narrower than that observed in real proteins (≈ 5 orders of magnitude); the simulated kinetics is typical of this type of models and thus it appears to be a limitation of the lattice polymer model as well as of some continuum, off lattice, models that exhibit similar behaviour [5].

In order to trace conformational changes we used the so-called contact map [22]. The contact map, \mathbf{C} , is an $N \times N$ symmetric matrix with elements, $C_{ij} = 1$, if beads i and j are in contact (but not covalently linked) and zero otherwise. In addition to containing the relevant information on the protein's structure (total number of contacts, specification of each contact and respective range) the contact map representation provides a straightforward way to compute the frequency, $\omega_{ij} = t_{ij}/t$, with which a native contact ij occurs in a MC run, t_{ij} being the total number of MC steps where $C_{ij} = 1$ and t the folding time. We have grouped the contacts into two classes, based on their frequency: If $\omega \geq 0.5$ the contact is long-lived, while short-lived contacts are those with a frequency $0.4 \leq \omega < 0.5$. We have focussed on the contacts that contribute to the folding process and thus excluded from the analysis contacts with small or marginal lifetimes.

We computed the mean frequency of each native contact $\langle \omega_{ij} \rangle$, averaged over 100 simulation runs, and report the results on Table I. We note that in the low- CO set, the fraction of native contacts with a significant lifetime, is approximately twice as large as the corresponding fraction in the high- CO set. In both sets, however, most of the long-lived contacts are local (a contact is local if the contacting beads are separated by less than 10 units of backbone distance), possibly due to the local nature of the kink-jump dynamics move set.

By contrast, the fraction of short-lived contacts is similar in both target sets; naturally the number of long-range (LR) contacts, contributing to Q , is clearly larger in the high- CO target set. The number of non-native contacts, N_{nat} , with a marginal lifetime ($\omega \geq 0.10$) is, as expected, larger in the high- CO target set.

These results indicate that the fraction of long-lived native contacts is higher in chains folding to low- CO targets and that, regardless of target geometry, the dynamics appears to be dominated by local contacts as these are the most frequent. Nevertheless, the appearance of a few long-lived LR contacts in both target sets suggests that they may play a role in the folding dynamics of these proteins.

Contact order and structural organization towards the native fold

In this section a detailed study of the folding dynamics exhibited by targets T_1 and T_5 is investigated. Targets T_1 and T_5 have considerably different geometries, as suggested by their contact order, and display the lowest and the highest observed folding times. Therefore they are good candidates to highlight the role of the native structure’s geometry (if any) on the folding dynamics. In particular, we investigate whether specific structural changes towards the native fold may be identified, for a given native structure’s geometry.

In Fig. 1 we plot the frequency, ω_{ij} , with which a native contact ij appears in the folding simulations of six randomly chosen sequences trained for targets T_1 and T_5 respectively. The major features observed for each target in different runs suggest a trend for the folding dynamics of target T_1 that is markedly different from that observed for target T_5 . In what follows we will investigate this difference.

Figures 2(a) and (b) show the *frequency maps* of targets T_1 and T_5 respectively. Each square represents an element $C_{ij} = 1$ of the contact map matrix, that is, a native contact ij , whose mean frequency, $\langle w_{ij} \rangle$, averaged over 100 MC runs, falls in a certain range indicated by the different colours. The frequency maps clearly identify the two model structures T_1 and T_5 and exhibit their different geometries. It is possible to identify a pattern in the colour distribution of target T_1 , which is not present in the frequency map of target T_5 , suggesting that the mean frequency of a native contact decreases monotonically with increasing contact distance in the low- CO target.

Let the backbone frequency, $\langle \omega_{|i-j|} \rangle$, be the mean frequency $\langle \omega_{ij} \rangle$ averaged over the number of contacts in each interval of backbone separation as defined in Table II. In Figs. 3(a) and 3(b) we plot the backbone frequency as a function of the distance, $|i - j|$ for the targets of the low- CO and the high- CO sets respectively. While for all low- CO targets $\langle \omega_{|i-j|} \rangle$ decreases monotonically with increasing contact distance, confirming the trend observed in the T_1 frequency map, for the high- CO targets no such trend is observed. One possible explanation, that we have ruled out, is that of a (negative) correlation between the frequency of a contact and its energy. In particular, one might expect the most stable contacts, those with the lowest energy, to be the most frequent. In Fig. 2(c) and (d) we report the *energy maps* of targets T_1 and T_5 respectively. Each square represents a native contact whose mean energy, averaged over 100 sequences, falls in a range indicated by the

colour. Since there is no correspondence between the colour patterns of figures 2(a) and 2(c) and between those of figures 2(b) and 2(d) we conclude that the difference is driven by geometrical constraints. A quantitative analysis of the correlation between the contact's frequencies and energies yields modest correlation coefficients, $r = 0.63$ and $r = 0.65$ for targets T_1 and T_5 respectively.

Let the contact time, t_0 , be the mean FPT of a native contact averaged over 100 MC runs (the FPT of a native contact is number of MC steps up to the first time the contact is formed). The contact time, averaged over the contacts in each interval of backbone distance, are shown in Table II, and plotted in Fig. 4: In both targets the set up of local contacts occurs largely before the LR contacts are established and, for LR contacts, there is no correlation between the contact time and the backbone distance. In view of these results one may be tempted to conclude that the higher folding time of T_5 is due to the fact that it has more LR contacts. However, the folding time is non-additive and a simple calculation shows that the higher number of LR contacts cannot justify the observed folding time of T_5 . Indeed the longest contact time ($\ln t_0 = 12.24$) is two orders of magnitude shorter than the folding time of T_5 and the sum of contact times is $\ln(\sum_{i=1}^{57} t_0^i) = 15.51$ much lower than the observed folding time $\ln t = 17.59$.

From the results of Fig. 4 we infer that the average contact times, over a given range, are similar for both targets. Thus the differences in the observed frequencies reported in Fig. 3 distinguish different cooperative behaviours.

Results obtained so far suggest that two broad classes of folding mechanisms exist for the MJ lattice-polymer model. What distinguishes these two classes is the presence, or absence, of a monotonic decrease of contact frequency with increasing contact range that may be related to different cooperative behaviour. The monotonic decrease of contact frequency with increasing contact range appears to be specific of the folding to low- CO targets. In this case the folding is also less cooperative and seems to be driven by the backbone distance: Local contacts form first while LR contacts form progressively later as the contact range increases.

At this point one may ask if the different folding mechanisms identified in the previous discussion are not a consequence of analysing only two different structures, i.e. there could be intermediate mechanisms for intermediate native structures. In order to clarify this point, we have analysed the folding of Shakhnovich and Gutin sequences designed to three target

geometries with intermediate contact order (0.163, 0.173 and 0.189) and the results are reported in Fig. 3(c). The folding times associated with these 3 structures are 15.67 ± 0.09 , 16.46 ± 0.09 and 16.04 ± 0.12 and we found that the contact order and the folding times, for the 9-element target set, correlate well ($r = 0.82$). The average sequence energy is in the same range as that of the targets reported in Table I. However, it is clear from the figure that intermediate and high-*CO* proteins fold via the same type of cooperative mechanism.

Contact order and the exploration of the conformational space

In this section we analyse the time evolution of the 57 native contacts of targets T_1 and T_5 to obtain a picture of the ‘global’ structural changes that occur during folding.

In the folding process a chain explores conformations that may be characterised by the fraction of native contacts, Q . Different native contacts will contribute to conformations with the same Q . In a MC run the probability of occurrence of a certain native contact is equal to the number of times that the contact occurs over the number of times that conformations with a given fraction of native contacts, Q , are sampled.

Since in a given run some native contacts are more probable (or more frequent) than others one may consider different probability intervals and ask, from the total number of native contacts, how many occur within a given probability interval, at fixed Q . The result gives the dependence of the number of contacts, C , on P , the probability of a contact being formed, and on Q , the fraction of native contacts [23]. Results, averaged over 100 simulation runs, are reported in Fig. 5(a) for T_1 and in Fig. 5(b) for T_5 where the coordinate Q may be interpreted as a monotonic ‘time’ coordinate along the folding process. Accordingly, early folding corresponds to low- Q while late folding occurs at high- Q .

A first look at the figures suggests smooth dynamics for the time evolution of T_1 ’s 57 native contacts by comparison with T_5 that exhibits a considerably more ‘rugged’ behaviour. Indeed, for a fixed probability interval, the variation, as a function Q , of the number of native contacts C which are present with that probability is clearly more pronounced for T_5 than for T_1 . This suggests that T_5 does not keep a considerable number of its native contacts as it evolves from a conformation $\Gamma(Q)$ to another conformation $\Gamma'(Q')$ during its exploration of the conformational space towards the native fold. A closer look shows other important differences. In the early folding ($Q < 0.35$) of T_1 there are a few near permanent contacts,

that is highly probable contacts ($P \geq 0.80$), by contrast with T_5 where highly probable contacts occur only later ($Q \geq 0.50$). Indeed, in the late folding of T_5 , there are still a few contacts with rather low probability $P \approx 0.25$. Moreover, as Q increases, the number of contacts in the two highest probability intervals increases smoothly for T_1 , while for T_5 the number of high-probability contacts shows a sudden increase only at $Q \approx 0.7$. These dynamical features are consistent with a folding scenario according to which T_1 explores more correlated native-like conformations as time evolves. For T_5 , however, even though the chain is getting more compact as it evolves towards the native fold it still explores many uncorrelated conformations up to the late folding stage.

Contact order and non-native contacts

To investigate the effects of non-native contacts in the folding dynamics to geometrically different native structures we have computed the dependence of the averaged number of non-native contacts, $\langle N_{nat} \rangle$, with Q . The average is taken over 100 MC runs. Results reported in Fig. 6 show that it is possible to identify two distinct dynamical regimes: For $Q > 0.5$ the number of non-native contacts decreases monotonically with Q independent of target geometry. However, for lower Q , the dynamics is target sensitive with the high- CO target displaying a larger number of total contacts. This data is consistent with a folding scenario where, in the early folding of the high- CO target, conformational sampling is geometrically restricted due to pre-existing compact structures.

Within the context of the energy landscape theory significant energy barriers, or kinetic traps, are known to exist between compact denaturated structures slowing down the folding process. Can the observed compact structures act as kinetic traps in the folding of T_5 ?

In order to answer this question we have computed the transition probability curves for both targets where the presence of plateaus indicates the presence of kinetic traps. In a transition probability curve the folding probability, $P_{fold}(t)$, is plotted against t . Results for targets T_1 and T_5 are shown in Fig. 7 where no plateaus are visible. Thus, based on these results, one cannot claim that the longer folding time of T_5 is due to the presence of kinetic traps.

Why do high- CO structures form compact denaturated states? We associate these conformers with the existence of high-frequency, LR native contacts. Indeed, native contacts

with a backbone separation in the range $35 \leq |i - j| \leq 39$ and frequency in the range $0.40 \leq \omega_{ij} \leq 0.57$ correspond to conformers characterized by $Q \approx 0.18$ and a total number of contacts close to 30 (of which ≈ 20 are non-native). Figure 6 confirms that for $Q = 0.18$ target T_5 is considerably more compact than target T_1 that has only (≈ 12) non-native contacts.

These findings suggest the following interpretation of the behaviour observed in Fig. 5 for the dynamics of the high- CO target’s ensemble of native contacts: The promiscuous formation of LR contacts takes the chain through low conformational entropy states from where it reorganises in a time consuming process towards the native fold. This major reorganization explains why even in the late stages of folding the chain is still exploring sets of unrelated conformations.

CONCLUSIONS AND FINAL REMARKS

In the present work we have carried out a thorough statistical analysis of the folding dynamics of 48 mers, within the MJ lattice-polymer model, designed to high, intermediate and low- CO target structures, in order to investigate the folding mechanisms associated with different target geometries, and the corresponding folding rates.

We found two broad classes of folding mechanisms for the MJ lattice-polymer model. The main feature of the first class, that describes the folding of low- CO targets, is a monotonic decrease of contact frequency with increasing contact range; indeed, such dependence seems to be a specific trait of the dynamics associated with low- CO targets. The building up of native structure is driven by backbone distance with local contacts forming first and non-local contacts forming progressively later as contact range increases. Moreover, the analysis of the time evolution of the 57 native contacts shows a progressive cumulative construction of the native fold with the chain exploring more correlated native-like conformations as time evolves. Folding to low- CO native structures is therefore gradual rather than abrupt (or cooperative). Folding to intermediate and high- CO targets belongs to a different class, where the dependence of contact frequency on contact range is non-monotonic. The folding is markedly more cooperative with many high-probability contacts forming suddenly only in the late stages of folding. Our results suggest that the higher cooperativity of the high- CO folding dynamics is due to the presence of LR contacts. A similar conclusion on the role of

LR contacts in the folding dynamics was obtained by Abkevich *et al.* in Ref. [18].

A common feature of the two folding classes is that the dynamics is dominated by local contacts in the sense that they are the most frequent during the folding process. This feature results, in part, from the local nature of the move set used in the simulations which favours the formation of local contacts.

At this stage a word on the correlation between CO and folding times is in order. Although the correlation coefficient between CO and T_f for the 6 targets of Table I is high ($r = 0.82$) the difference in folding times is relatively modest and this correlation should be taken with caution. Indeed, if one includes the 9 targets studied in this work the correlation coefficient decreases, a clear indication that these numbers are not conclusive. However, the geometry driven cooperativity appears to be rather robust and this implies an increase in folding times as the cooperativity increases.

Related studies, have investigated the physical mechanisms behind the (empirical) geometry-dependent kinetics exhibited by two-state folders. Work on the ‘topomer search model’ (TSM), concludes that the topology-dependence of real two-state folders is ‘a direct consequence of the extraordinary cooperative equilibrium folding of simple proteins’ [24]. In agreement with the TSM results Jewett *et al.* [12] showed that modified $G\bar{o}$ type polymers, exhibiting enhanced thermodynamic cooperativity, display a larger dispersion of the folding rates and a stronger topology-dependent kinetics than traditional, non cooperative $G\bar{o}$ polymers. In a very recent study, Kaya and Chan suggested that the way thermodynamic cooperativity is achieved may be as important as thermodynamic cooperativity *per se* in topology-dependent kinetics [5]. By studying a modified $G\bar{o}$ model, with many-body interactions, the authors found folding rates, well correlated ($r = 0.914$) with CO , spanning a range two orders of magnitude larger than that of $G\bar{o}$ models with additive contact energies.

The results for the modified $G\bar{o}$ models and our current results for the MJ model shed light on our previous finding [11] of a particularly strong correlation ($r \approx 0.80$) between higher- CO structures and longer logarithmic folding rates; These structures have a larger number of LR contacts that enhance the cooperativity of the folding transition. This cooperativity appears to be the essential ingredient of topology-dependent kinetics.

TABLE I: Summary of target properties with targets organized with increasing contact order parameter, CO . Targets T_0 , T_1 and T_2 constitute the low- CO target set while targets T_3 , T_4 and T_5 make the high- CO target set. $\langle E \rangle$ is the averaged trained energy and σ its standard deviation, $\ln_e t$ is the logarithmic folding time, Q is the fraction of native contacts with mean frequency $\langle \omega \rangle$ (we specify the number of long-range (LR) native contacts) and N_{nnat} is the number of non-native contacts with a marginal lifetime.

Target	CO	$\langle E \rangle \pm \sigma$	$\ln t$	$Q_{\langle \omega \rangle \geq 0.5}$	$Q_{0.4 \leq \langle \omega \rangle < 0.5}$	$N_{nnat}(\langle \omega \rangle \geq 0.1)$
T_0	0.126	-25.80 ± 0.03	16.44 ± 0.11	0.25(0LR)	0.19(0LR)	22
T_1	0.127	-26.27 ± 0.03	14.99 ± 0.13	0.28(1LR)	0.13(1LR)	16
T_2	0.135	-25.78 ± 0.04	16.09 ± 0.12	0.19(3LR)	0.13(1LR)	25
T_3	0.241	-25.77 ± 0.03	16.83 ± 0.16	0.05(0LR)	0.19(5LR)	40
T_4	0.254	-25.11 ± 0.03	17.35 ± 0.12	0.11(1LR)	0.16(4LR)	38
T_5	0.259	-26.16 ± 0.02	17.59 ± 0.12	0.11(2LR)	0.12(4LR)	52

TABLE II: Fraction of native contacts, Q , at consecutive intervals of backbone distance for targets T_1 and T_5 .

Target	backbone distance							
	[3, 8[[8, 13[[13, 18[[18, 23[[23, 28[[28, 33[[33, 38[[38, 43[
T_1	0.49	0.18	0.19	0.07	0.05	-	0.02	-
T_5	0.23	0.04	0.09	0.19	0.14	0.05	0.14	0.13

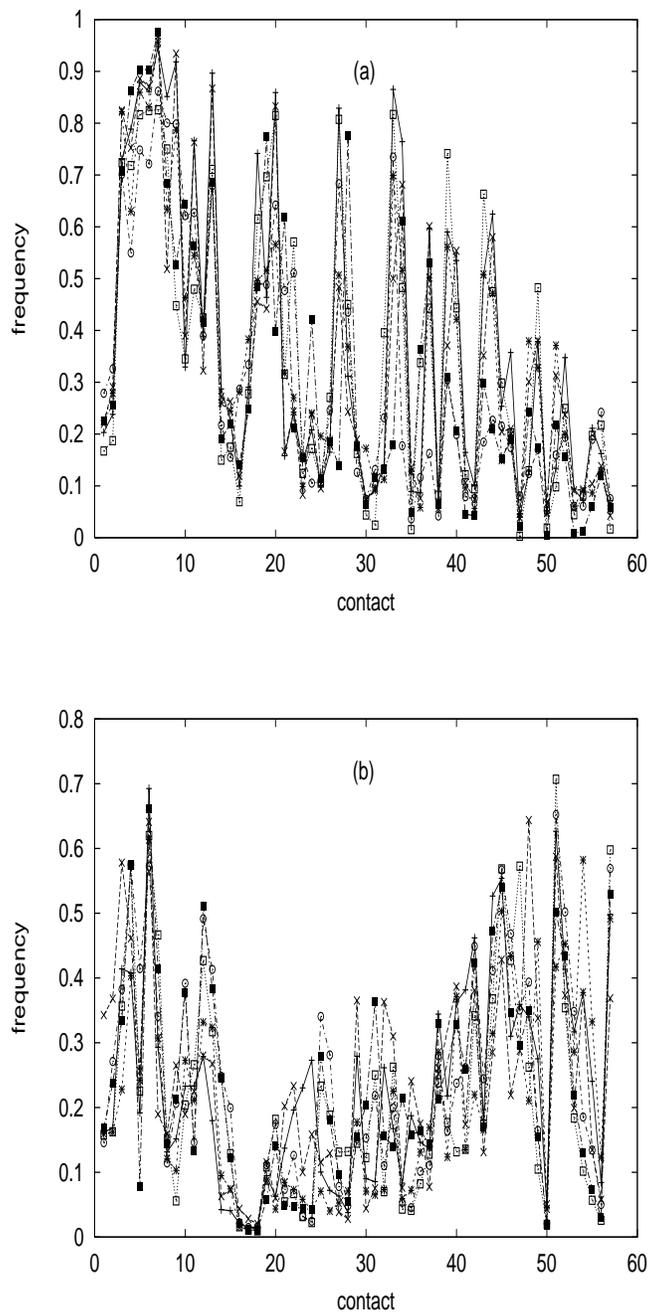
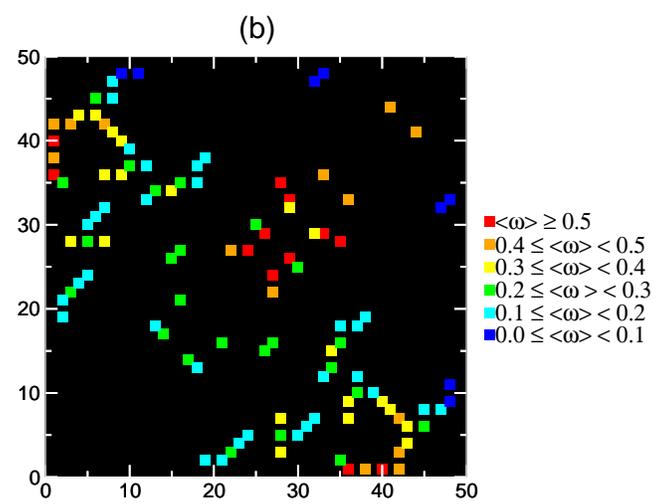
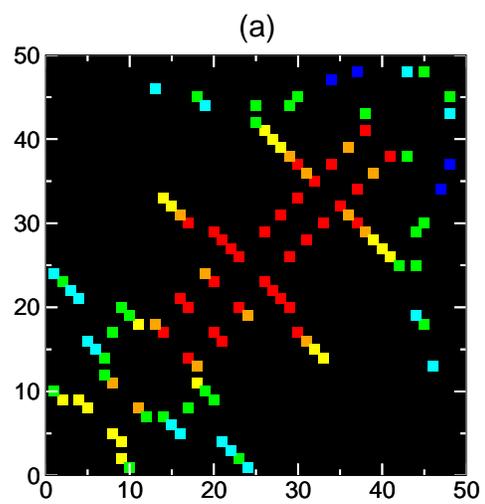


FIG. 1: Frequency with which a native contact (numbered from 1 to 57) occurs in the folding simulations of six randomly chosen sequences trained for targets T_1 (a) and T_5 (b). The contact frequency is the ratio of the number of times a native contact occurs in a MC simulation to the folding time. Note how frequency of occurrence of particular contacts has strong correlation between different trained sequences; that is a clear dependence on conformation alone.



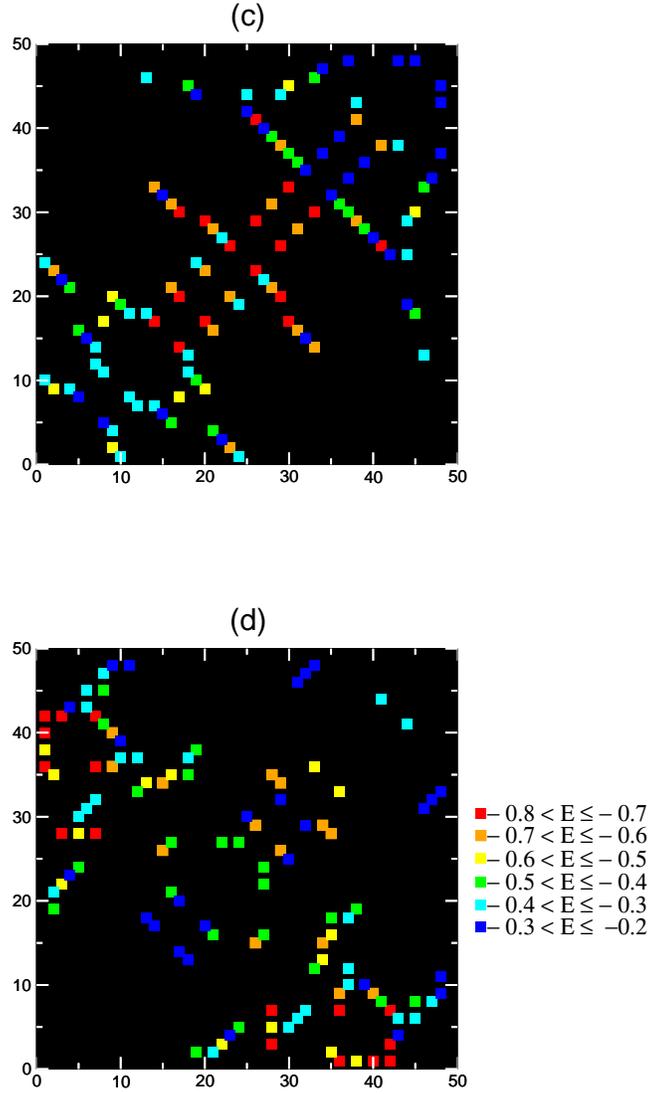
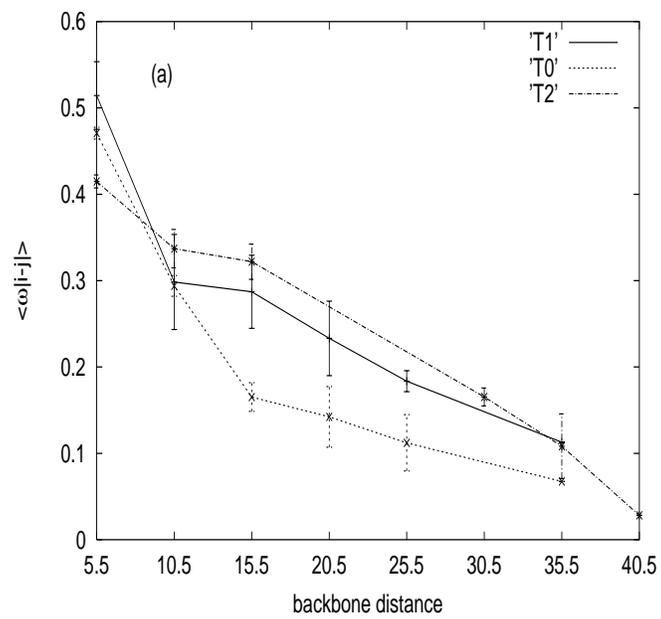


FIG. 2: (Color online). Frequency maps of targets T_1 (a) and T_5 (b) and energy maps of targets T_1 (c) and T_5 (d). A coloured square represents a native contact with an averaged mean frequency $\langle \omega \rangle$ or an averaged mean energy E . Averages are taken over 100 MC runs.



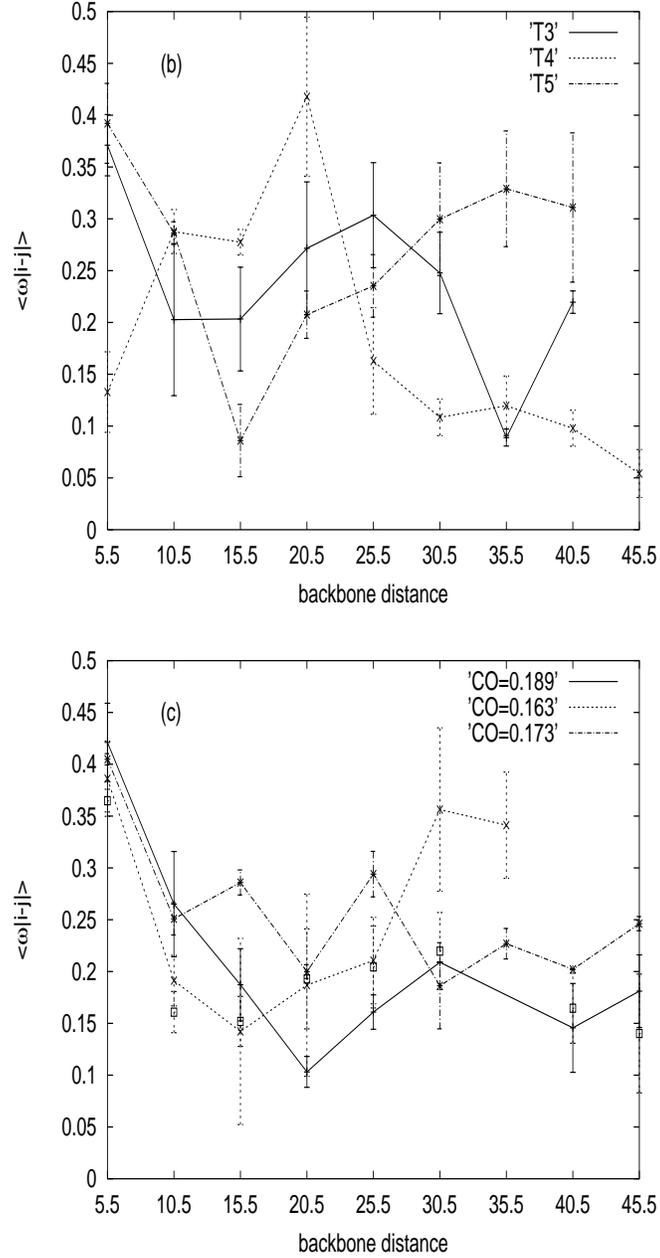


FIG. 3: The backbone frequency, $\langle \omega_{|i-j|} \rangle$, as a function of the backbone separation for the low- CO (a), high- CO (b) and intermediate- CO targets (c). The backbone frequency is the mean value of $\langle \omega \rangle$ averaged over the number of contacts in each interval of backbone separation as shown in Table II.

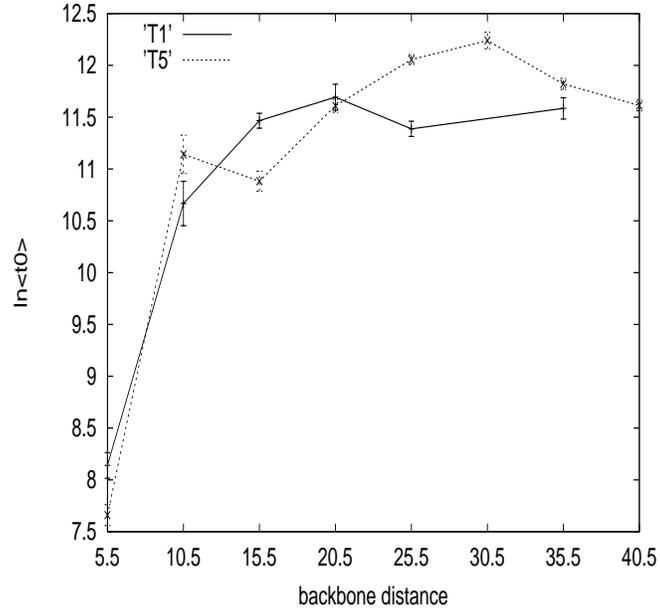


FIG. 4: The averaged contact time, $\ln_e \langle t_0 \rangle$, as a function of the backbone distance. t_0 was averaged over the number of contacts in each interval of backbone separation as shown in Table II.

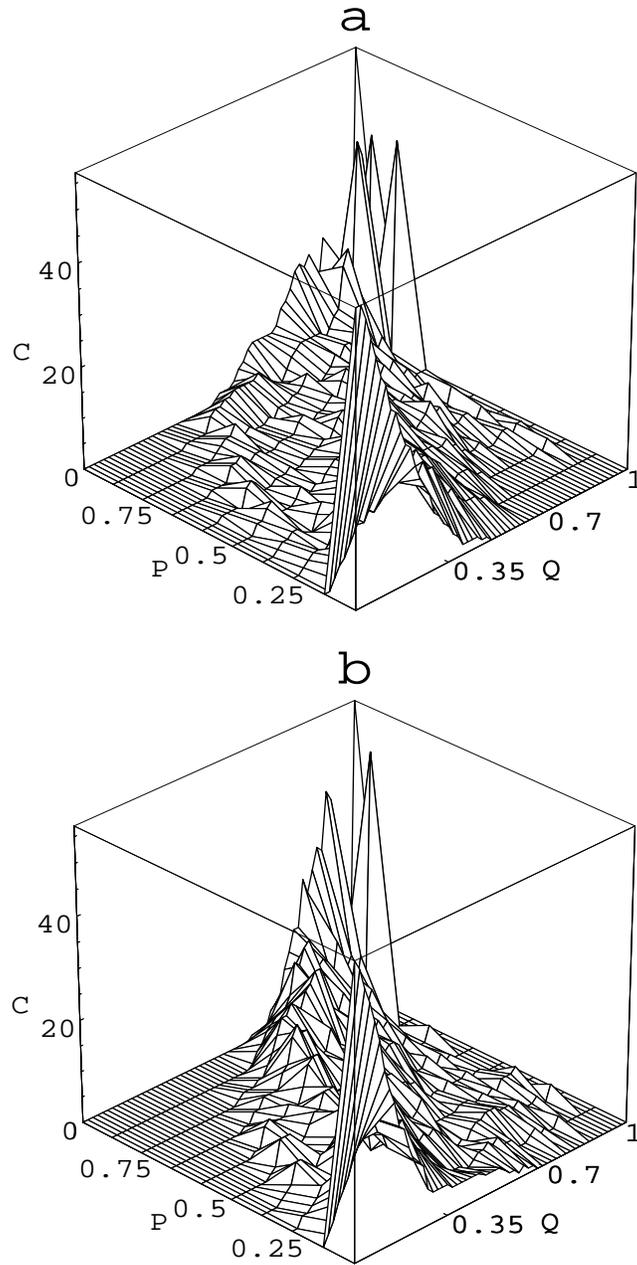


FIG. 5: Number of contacts, C , with a given probability of being formed, P , as a function of Q , the fraction of native contacts for targets T_1 (a) and T_5 (b). These are results averaged over 100 MC folding runs.

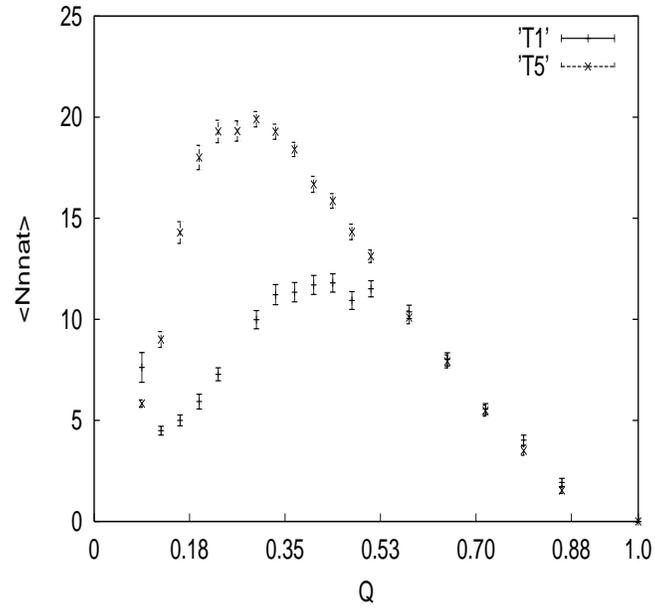


FIG. 6: Mean number of non-native contacts, $\langle N_{nnat} \rangle$, averaged over 100 MC runs, as a function of the fraction of native contacts.

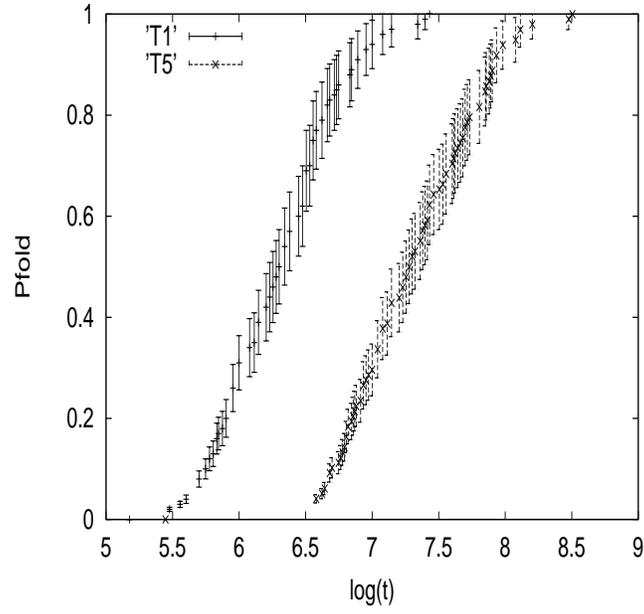


FIG. 7: Dependence of the folding probability, P_{fold} , on $\log_{10}(t)$. P_{fold} was calculated as the number of folding simulations which ended up to time t normalized to the total number of runs.

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