The limited role of non-native contacts in folding pathways of a lattice protein

Brian C. Gin,1,2,3 Juan P. Garrahan,4 and Phillip L. Geissler∗1,2

1Department of Chemistry, University of California at Berkeley, Berkeley, California 94720
2Chemical Sciences and Physical Biosciences Divisions, Lawrence Berkeley National Lab, Berkeley, California 94720
3School of Medicine, University of California at San Francisco, San Francisco, California 94143
4School of Physics and Astronomy, University of Nottingham, Nottingham, NG7 2RD, U.K.

Models of protein energetics which neglect interactions between amino acids that are not adjacent in the native state, such as the Gō model, encode or underlie many influential ideas on protein folding. Implicit in this simplification is a crucial assumption that has never been critically evaluated in a broad context: Detailed mechanisms of protein folding are not biased by non-native contacts, typically imagined as a consequence of sequence design and/or topology. Here we present, using computer simulations of a well-studied lattice heteropolymer model, the first systematic test of this oft-assumed correspondence over the statistically significant range of hundreds of thousands of amino acid sequences, and a concomitantly diverse set of folding pathways. Enabled by a novel means of fingerprinting folding trajectories, our study reveals a profound insensitivity of the order in which native contacts accumulate to the omission of non-native interactions. Contrary to conventional thinking, this robustness does not arise from topological restrictions and does not depend on folding rate. We find instead that the crucial factor in discriminating among topological pathways is the heterogeneity of native contact energies. Our results challenge conventional thinking on the relationship between sequence design and free energy landscapes for protein folding, and help justify the widespread use of Gō-like models to scrutinize detailed folding mechanisms of real proteins.

Keywords: Gō Model, Non-Native Contacts, Lattice Model, Protein Folding, Principle of Minimum Frustration, Energy Landscape

I. INTRODUCTION

Current understanding of protein folding has been strongly shaped by theoretical and computational studies of simplified models. Such models are typically constructed by discarding fine details of molecular structure or by making simplifying assumptions about the energies of interaction among amino acid residues. A special class of models, based on Gō’s insight, asserts that only a subset of interactions, those between segments of a protein that contact one another in the native state, are crucially important for folding. The Gō model further assumes a unique energy scale for these native contacts. Here, we will focus on elaborated “Gō-like” models that allow for a diversity of native contact energies.

Neglect of non-native contacts offers substantial computational relief to numerical simulations, allowing thorough kinetic and thermodynamic studies to be performed even for detailed molecular representations. It further establishes a basis for theories that focus on gaps in the spectrum of conformational energies and the funnel-like nature of potential energy landscapes. Corroborated by experiment, concepts intrinsic to and inspired by Gō-like models now form a canon of widely accepted ideas about how proteins fold.

The Gō model was originally proposed as a schematic but microscopic perspective on the stability and kinetic accessibility of proteins’ native states. It accordingly provided generic insight into issues of cooperativity, nucleation, and the relationship between sequence and structure. Recent studies have ascribed a much more literal significance to the detailed dynamical pathways defined by Gō-like models. In particular, direct comparisons have been drawn between folding mechanisms predicted by Gō-like models for specific proteins and those suggested by experimental results. However, it is not clear to what extent such a detailed correspondence with Gō-like models should be expected. General theories offer only rough guidance, and few computational studies have compared folding pathways of Gō-like models and their “full” counterparts (in which non-native contact energies are included) in a broad context.

Very favorable interactions between segments of a protein that are not adjacent in the folded state generally impede folding. They might do so by introducing detours or traps on the route to the native state, or simply by stabilizing the ensemble of unfolded conformations. It is often imagined that the former possibility plagues a vast majority of non-natural amino acid sequences, which fold sluggishly if at all. According to this picture, non-native contacts should feature prominently in the convoluted folding pathways of an undesigned sequence. Such kinetic frustration could pose several biological risks in vivo, where aggregation and slow response can be serious liabilities. Indeed, typical proteins taken from living organisms fold reliably and with relative efficiency.

*Corresponding author. E-mail: geissler@berkeley.edu
These notions and observations motivate a “principle of minimum frustration” asserting that natural amino acid sequences have been “designed” by evolution to minimize the disruptive influence of non-native contacts on the dynamics of folding. One might thus apply Gō-like models to these designed sequences with confidence, since, the omitted interactions are precisely the ones whose effects have been mitigated by natural selection. By contrast, one might expect Gō-like models to poorly represent folding mechanisms of slowly folding molecules, whose non-native interactions are presumably responsible for hampering pathways to the native state.

Testing these ideas of sequence design and kinetic frustration is made difficult by several factors. Experimentally, microscopic details of folding kinetics cannot be resolved but only inferred from indirect observables or the effects of mutations. Furthermore, the most concrete hypotheses stemming from the principle of minimum frustration involve Gō-like models, which cannot be realized in the laboratory. Computer simulations of detailed molecular representations can generate, at great cost, dynamical information sufficient to determine a folding mechanism for only the smallest of natural proteins. Although the statistical dynamics of coarse-grained or schematic representations can be readily explored, biology does not provide collections of fast-folding and slow-folding sequences to compare in these artificial contexts. Finally, even when appropriate ensembles of sequences and ensembles of folding trajectories are available, useful comparison of Gō-like models and its full counterpart requires a compact way of characterizing the course of highly chaotic dynamics. A general method for this purpose is not available, though studies of nucleation as a rate-limiting fluctuation provide a useful starting point.

This paper presents the first systematic, large-scale comparison of folding pathways within Gō-like and full models. We focus on a schematic lattice representation of proteins, well-suited for this task in several ways: (a) geometrically, because contacting segments of the chain can be unambiguously identified, (b) statistically, because representative ensembles of folding trajectories can be generated for large numbers of amino acid sequences, and (c) conceptually, because the essential competition between contact energetics and chain connectivity can be isolated from complicating effects of secondary structure, side-chain packing, etc. While these latter effects unquestionably bear in important ways on the folding of real proteins, it is nevertheless imperative to understand the fundamental physical scenarios they enrich and modify. Indeed, much of biologists’ working intuition for protein folding and design was developed in the context of similarly schematic models. Our results challenge some of those notions.

It has been conjectured that well-designed lattice heteropolymers fold through mechanisms that are determined solely by their native structures. Were this hypothesis correct, for both full and Gō-like models, a comparison of fast-folding pathways in the two models would not be especially informative. In that case the sequence of events that advance a molecule toward the native state (which we designate as its folding mechanism) would be exclusively a question of geometry and local mobility. We have found, to the contrary, that a wealth of folding mechanisms are possible even for a single native conformation.

Spanning a range of hundreds of thousands of sequences, with widely varying rates and mechanisms, the work reported in this paper constitutes a thorough test of certain aspects of the principal of minimum frustration and addresses at a new level of kinetic detail the dynamical realism that can be expected from Gō-like models. Our results for the lattice heteropolymer model evidence a remarkably strong mechanistic correspondence between full and Gō-like models. Unexpectedly, this dynamical conformity holds not only for fast-folding sequences but also for the slowest sequences whose folding can be followed in practice. Close correspondence in folding mechanisms holds as long as the Gō-like approximation retains the heterogeneity in native contact energies of the full potential. These findings suggest a profound frustration invariance in the ensemble of trajectories that proceed from deep within the unfolded state all the way to the native structure.

II. METHODS

We focus on lattice heteropolymers, whose folding properties have been studied extensively for specific example sequences, structures, and chain lengths. Here, a protein’s conformation is described by a self-avoiding walk on a three dimensional lattice with spacing a (see for example Fig 1a). Each vertex of this walk represents an amino acid monomer, which possesses no internal structure and interacts only with “contacting” monomers that occupy adjacent vertices. For a chain comprising N monomers the energy of a particular configuration can thus be written

$$E = \sum_{i=1}^{N-1} \sum_{j>i} u_{\text{core}}(r_{ij}) + \sum_{i=1}^{N-3} \sum_{j=i+3}^{N} B_{ij} \Delta(r_{ij} - a),$$

where $r_{ij} = |r_i - r_j|$. The hard-core potential $u_{\text{core}}(r)$, which takes on values of $\infty$ for $r = 0$ and 0 for $r > 0$, imposes the constraint of self-avoidance. Interaction energies $B_{ij}$ are determined by the sequence-dependent identities of monomers $i$ and $j$ according to the model of Miyazawa and Jernigan (MJ), and act only at a spatial separation of one lattice spacing [$\Delta(x) = 1$ if $x = 0$ and vanishes otherwise].

The standard dynamical rules for evolving such a chain molecule proceed from a Metropolis Monte Carlo algorithm. Trial moves, in which one or two randomly selected monomers move in an “edge-flip” or “crankshaft” fashion, are accepted with probabilities that generate a
FIG. 1: (a) 48-mer native structure of the lattice heteropolymer studied in this work. (b) Example of histograms of the order of permanent formation of native contacts (contact appearance order, or CAO) for each of the nine native contacts of a 12-mer lattice structure. Histograms are collected from the set of folding trajectories of a given amino acid sequence. (c) Same as Fig. 1b but shown as a density map. (Right, Top) CAO maps of three fast folding sequences of the 48-mer (Fig. 1a), for both the full potential energy and the Gō-like approximation (which disregards non-native contact energies, but maintains the original heterogeneity in native contact energies). The overlap parameter $q$ quantifies the similarity of CAO maps, and thus topological folding pathways. The overlap of CAO maps between full and Gō-like potentials for each sequence is close to one, $q \approx 0.9$, indicating the similarity of their folding mechanisms. In contrast, the overlap between CAO maps of different sequences is much smaller, $q \lesssim 0.2$. (Right, Bottom) Same as before but now for three slow folding sequences. Again, the CAO distributions of full and Gō-like potentials are very similar, while those between different sequences are not.

Boltzmann distribution at temperature $T = 0.16\epsilon_0/k_B$, where $\epsilon_0$ sets the energy scale of the MJ model. For example, the strongest attractive interaction (between two cysteines) has an energy $\epsilon_{CC} = -1.06\epsilon_0$; for lysine-lysine $\epsilon_{KK} = 0.25\epsilon_0$. Folding trajectories are initiated from swollen configurations drawn from a high-temperature ($k_BT/\epsilon_0 = 100$) equilibrium distribution in which contact energies are negligible compared to typical thermal excitations.

This caricature clearly lacks many of the chemical details underlying the function and secondary structure of real proteins. By capturing an essential interplay between diverse local interactions and constraints of polymer connectivity, it nonetheless recapitulates many nontrivial features of protein statistical mechanics: Even for chains of modest length (say, $N = 27$), the number of possible conformations is sufficiently immense to motivate Levinthal’s paradox, i.e., it is not obvious that they should be able fold at all. Folding occurs in a cooperative fashion, and occurs efficiently only for well-designed sequences. For a given sequence certain residues figure much more prominently in folding kinetics than others; correspondingly, certain residues are more highly conserved than others in computer simulations of evolutionary dynamics.

The Gō-like approximation of the model of Eq. (1) is constructed simply by ignoring the energies of non-native
contacts,
\[ \tilde{E} = \sum_{i=1}^{N-1} \sum_{j>i} N_{ij} B_{ij} \Delta(r_{ij} - a) \]

where \( N_{ij} = 1 \) if the monomers \( i \) and \( j \) are adjacent in the native configuration, and \( N_{ij} = 0 \) otherwise. While disregarding the energy contribution of non-native contacts, the energy function \( \tilde{E} \) of Eq. (2) retains the full heterogeneity in native contacts energies of the original potential, Eq. (1). We will show below that it is a crucial aspect of the Go-like models we study here.

Many studies previously suggested that lattice heteropolymers of modest length fold via a nucleation mechanism\cite{25,26,27}. Formation of a handful of key contacts poises the system at a transition state, from which the chain can rapidly access the folded state or, with equal probability, return to the unfolded state. This set of crucial contacts comprises a “folding nucleus” and serves as a bare synopsis of dynamical pathways that lead to the native state.

A cogent comparison of folding mechanisms requires a means of characterizing dynamical pathways that is both thorough and computationally inexpensive. Identifying the folding nucleus satisfies neither or these necessities well. In particular, locating configurations from which the folded and unfolded states are equally accessible involves propagation of many trajectories and, by itself, does not delineate routes toward and away from the transition state\cite{28,29}. We have devised an alternative measure that is not only succinct and computationally tractable, but also characterizes the entire route from the unfolded to the folded state. Specifically, we record the order in which native contacts form permanently during a protein’s folding mechanism. Our parameters thus chronicle lasting changes in the chain’s “topology”, understood in terms of linkages through the polymer backbone and through non-bonded contacts.

This contact appearance order (CAO) is a highly non-trivial measure of the progress toward folding and provides a detailed characterization of mechanism in the sense we have defined. It is simple to calculate from the time-dependence of a trajectory spanning unfolded and folded states. Like persistence times\cite{30,31} in the context of non-equilibrium systems, such as glasses, it is intrinsically a multi-time quantity; it can neither be computed for a single configuration, nor can it be used to build constrained ensembles whose statistics shed light on the nature of reaction coordinates. But, also like persistence times\cite{32,33} it focuses attention on key dynamical events with unmatched precision. For our purpose of diagnosing the occurrence of lasting topological changes, CAOs serve almost ideally. For some other approaches, e.g., surveying the free energy landscapes on which folding takes place, CAOs would serve poorly.

We have verified that the mechanistic meaning we ascribe to CAOs is consistent with more conventional characterizations of reaction progress. Most importantly, the order of a contact’s appearance correlates strongly with a statistical measure of commitment to folding at the time when that contact forms permanently. We use the parameter \( p_{\text{fold}} \), the probability that a trajectory initiated from a given configuration will reach the folded state before first relaxing to a state with few native contacts\cite{34}, to demonstrate this fact. Fig. 3c shows that the average value of \( p_{\text{fold}} \) rises steadily with CAO, from a value well below \( p_{\text{fold}} = 1/2 \) up to \( p_{\text{fold}} = 1 \).

The point at which \( p_{\text{fold}} \) crosses 1/2 is often considered the transition state for folding. The set of contacts consistently present in such configurations is correspondingly designated as the folding nucleus. We have confirmed that the nucleus identified in this way corresponds closely with the set of contacts that have formed permanently when \( p_{\text{fold}} = 1/2 \). Additionally, we have verified that the CAO-identified nucleus of several sequences from Mirny et al.\cite{35} are consistent with the nucleus identified in that study. While this consistency check reflects favorably on the soundness of exploring folding mechanisms by scrutinizing CAOs, it does not imply that CAO analysis is predicated on putative nucleation mechanisms for folding. Regardless of whether the rate-determining steps in folding are uphill, downhill, or neutral in free energy; regardless of whether folding is kinetically a two-state phenomenon; regardless of whether the progress of folding is plagued by long-lived kinetic traps, CAOs trace a history of conformational change that emphasizes any event with enduring topological consequences.

What CAOs do not resolve is the unproductive development of native structure. Attention is focused solely on segments of time evolution that bridge folded and unfolded basins of attraction. Occasional excursions within the unfolded state amass an atypically large number of native contacts, but due either to topology or to the presence of interfering non-native contacts do not in fact make progress toward folding. CAOs contain no information about these excursions. In comparing full and Go-like models, we therefore make no statements about the character of such non-folding dynamics. By exclusively examining accumulation of native contacts, we also lose direct information regarding the evolution of non-native contacts. If the rupture of a particular non-native contact were a crucial step in folding of a certain sequence, our methods would not detect its occurrence explicitly. We stress, however, that substantial non-native structure is present when the first permanent native contacts are formed. We could therefore indirectly detect the significance of non-native contact dynamics through influences on the pattern of early topological changes.

Compiling the order of permanent contact formation over many folding trajectories of a given sequence, we construct for each native contact a statistical distribution of CAO. Fig. 1b,c illustrate how the set of resulting CAO histograms form a visual fingerprint of a sequence’s folding mechanism. Because the dynamical events it chronicles span a wide range of \( p_{\text{fold}} \), a CAO histogram characterizes not only the transition state for folding, but also...
the dynamics of ascent to and descent from the transition state. The correspondence between an amino acid sequence and its CAO histogram is as subtle as (if not more so) the connection between sequence and native conformation that defines some of the most challenging aspects of the protein folding problem. Most of the results we will present concern a single native structure (that shown in Fig. 1a for $N = 48$), removing a potentially trivial agreement between full and Gō-like potentials. Even for this unique structure, sequences of the full model differing by only a few point mutations can exhibit qualitatively different CAO histograms, reflecting substantial changes in folding pathway. The distribution of contact energies can thus play a critical and complex role in determining folding mechanism, over and above dictating its endpoint. Given this nontrivial relationship it would be surprising if non-native contacts did not generally act to shape or bias CAO statistics.

The primary goal of this paper is to compare the CAO statistics of sequences propagated using full and Gō-like models. In judging their similarities and differences, it is essential to establish for reference how significantly CAO histograms can vary, within either model, for sequences that fold to a common structure. As mentioned above, others have proposed that such variations are weak, i.e., that topology of the folded structure prescribes a nearly unique topological route for folding. Using methods described in the Appendix, we have generated an unprecedentedly diverse set of sequences that fold to the same target structure within the full model. As shown in Fig. 1a variations in CAO statistics within this set are much more substantial than previously thought. Any success of Gō-like models in reproducing folding pathways of the full model cannot be attributed simply to their sharing a common native structure.

We quantify similarity of CAO statistics (for two sequences within the same model, or for full and Gō-like models with the same sequence) using an “overlap” parameter $q$. Inspired by the theory of spin glasses, we define $q$ such that $0 \leq q \leq 1$, with larger $q$ representing greater similarity. The analogy with spin glasses would assign an overlap $q^{(\alpha,\beta)}$ between the CAO distributions for two sequences $\alpha$ and $\beta$ proportional to

$$
\frac{1}{n_{\text{max}}} \sum_{n=1}^{n_{\text{max}}} \sum_{C=1}^{n_{\text{max}}-1} P_n^{(\alpha)}(C)P_n^{(\beta)}(C), \tag{3}
$$

where $P_n^{(\alpha)}(C)$ is the probability that native contact $n$ is
made permanently at order $C$ in a folding trajectory of sequence $\alpha$, and $n_{\text{max}}$ is the total number of native contacts. An accurate numerical estimate of the quantity in Eq. (3), however, is problematic to obtain, requiring the generation of an inordinate number of folding trajectories. As an alternative, we define $q$ using a closely related quantity,

$$q^{(\alpha,\beta)} = \frac{1}{n_{\text{max}}} \sum_{n=1}^{n_{\text{max}}} \left[ \left( \frac{2}{\sigma_n^{(\alpha)} \sigma_n^{(\beta)}} \frac{\sigma_n^{(\alpha)}}{\sigma_n^{(\alpha)}} + \frac{\sigma_n^{(\beta)}}{\sigma_n^{(\beta)}} \right) \right] \times \exp \left( -\left( \frac{\langle C \rangle_n^{(\alpha)} - \langle C \rangle_n^{(\beta)} }{\sigma_n^{(\alpha)} \sigma_n^{(\beta)}} \right)^2 \left( \frac{\sigma_n^{(\alpha)}}{\sigma_n^{(\alpha)}} + \frac{\sigma_n^{(\beta)}}{\sigma_n^{(\beta)}} \right) \right),$$

where $\langle C \rangle_n^{(\alpha)} = \sum_{n=1}^{n_{\text{max}}} p_n^{(\alpha)}(C) C$ is the average CAO of contact $#n$ for sequence $\alpha$ and $(\sigma_n^{(\alpha)})^2 = \sum_{n=1}^{n_{\text{max}}} p_n^{(\alpha)}(C) (C - \langle C \rangle_n^{(\alpha)})^2$ is its variance. Equations (3) and (4) are completely equivalent in the case of Gaussian distributed CAOs. Even for non-Gaussian statistics, $q^{(\alpha,\beta)}$ remains a useful, computationally tractable, and similarly bounded measure of how similarly two sequences fold.

### III. RESULTS AND DISCUSSION

In the ensemble of sequences we generated, the fastest folding sequences access the native state more than 1000 times more rapidly than the slowest. CAO histograms were generated for all sequences, each one evincing a well-defined topological pathway. Typically, the appearance order $C$ of a given native contact varies from one trajectory to another by only a few positions (see below). This regularity belies substantial conformational fluctuations attending each folding event, which exert little influence on the formation of permanent contacts. Sharply peaked CAO histograms do not indicate a lack of complexity, but instead a successful characterization of forward progress along the reaction coordinate for folding.

Figure 1 shows CAO histograms for several sequences folding to this specific 48-mer structure (depicted in Fig. 1a). Results are presented for dynamics propagated according to both full and Gō-like models. Comparing these topological fingerprints across different sequences hints at the broad variety of possible folding pathways. Contacts essential to early stages of folding for one sequence can be irrelevant in the pathway taken by another. This finding contrasts strongly with the “one-structure one-nucleus” hypothesis, bolstering recent reports of dissimilar folding nuclei.

Strong variations in the topological folding pathways chosen from one sequence to another immediately indicate that the original homogeneous Gō model cannot capture the folding behavior of a typical sequence. With a homogeneous set of native contact energies, that model can only discriminate between different native structures, not between different sequences that adopt them. In loose terms folding dynamics of the homogeneous Gō model resemble a superposition of those we determined for diverse sequences of the full model. Whereas in the full model a typical set of contact energies selects a well-defined folding pathway, an egalitarian set of stabilizing energies permits broad sampling of routes to the native state.

Gō-like models that embrace variety in native contact energies, however, capture the topological pathways followed by their full model counterparts with striking accuracy. CAO histograms obtained from full and Gō-like dynamics for any particular sequence can hardly be distinguished, see Fig. 1. Not only are the average CAOs of each contact nearly equivalent, but also fine details of CAO statistics are unaffected by neglect of non-native contact energies. While previous work hypothesized a dynamical correspondence for fast folders, the topological conformity of full and Gō-like mechanisms we observe for slow folders is highly unexpected.

For sequences with folding rates $\lesssim 10^{-9}$, we are unable to harvest folding trajectories in sufficient numbers to construct CAO histograms. According to microscopic reversibility, however, topological routes for folding are identical to time-reversed routes of unfolding. We have therefore extended our analysis of contact appearance order for efficiently folding sequences to one of contact disappearance order (CDO) for very sluggishly folding sequences. The agreement between CDO histograms of full and Gō-like models is no less striking than that of the CAO histograms plotted in Fig. 1, even in cases where the “native” state is grossly unstable. These calculations are somewhat less straightforward: the order of first disappearance (CDO) is equivalent to the order of permanent appearance (CAO), but only for trajectories reaching the unfolded state without revisiting the native state. As such, they require specifying when a molecule has unfolded. For this purpose, we regard a molecule as unfolded when the instantaneous number of native contacts drops to a value consistent with the average number of native contacts in the unfolded state. Additionally, we require that this threshold lie below any value found in equilibrium fluctuations of the native state. We have verified that CAO and CDO histograms indeed match for sequences folding at moderate rates.

Quantitative measures of mechanistic diversity are presented in Fig. 2a. For each pair of sequences generated by our evolutionary simulation we computed the similarity parameter $q$ between CAO histograms for the full model. The resulting distribution of $q$ values is broadly peaked at $q \approx 0.4$, signifying that there is a significant diversity of CAO pathways represented by the sequences in the ensemble. For each individual sequence we also quantified the relationship between CAO histograms generated by full and Gō-like models. These $q$ values are distributed much more narrowly about a considerably higher average, $q \approx 0.9$. Using sequence-to-sequence variation in CAO pathways as a yardstick, the irrelevance of non-
FIG. 3: (a) Number of native contact as a function of time in a folding trajectory, illustrating the “prefolding” (blue) and “folding” (red) phases of the dynamics. The prefolding phase extends from the folding trajectory’s start time until the time the first permanent native contact is formed. The folding phase extends from this time to the time when the native conformation is reached. The full (green) curve shows the $p_{\text{fold}}$, which only departs from zero after the folding phase has started (cf. Fig. 2).
(b, right panels) Distribution of the duration of the prefolding and folding phases, in the full potential and its corresponding Gō-like approximation. For fast-folding sequences (top panel) the distributions for both folding and prefolding durations of the Gō-like model are close to those of the full potential. For slow-folding sequences (middle panel) the Gō-like model reproduces the distribution of folding duration, but underestimates the prefolding times. If the Gō-like potential of slow-folding sequences is supplemented by random non-native contact energies (bottom panel) the prefolding distributions can be made to match, without disrupting the correspondence in the folding phase distributions. (c) Ratio between full and Gō-like models’ folding (red) and prefolding (blue) phase durations, for all sequences ordered according to folding rate; the full lines are the average ratios for each scatter plot. For fast folders, the average times as calculated from the full and Gō-like models are comparable, both for the folding and prefolding phases. For slow folders, the prefolding time in Gō-like model is much smaller than that in the full potential, and this difference increases with decreasing folding rate.

native contacts for the topological folding pathway is beyond doubt. The inset to Fig. 2a emphasizes that this result has little to do with folding efficiency. Typical $q$ values for the full/Gō-like comparison are just as high for the slowest folders examined as for the fastest.

Figure 2b quantifies the variation of CAO between folding trajectories. For each sequence we quantify the root mean-squared fluctuation in the contact order:

$$\delta C = \frac{1}{n_{\text{max}}} \sum_{n=1}^{n_{\text{max}}} \sqrt{(C_n^2) - (C_n)^2}. \quad (5)$$

Fig. 2b shows the distribution of $\delta C$ among the ensemble of Gō-like sequences. It is peaked at a value of $\delta C \approx 7.5$. In contrast, for the homogeneous Gō model $\delta C \approx 12.5$, indicating that CAO values are much more broadly distributed between trajectories (see inset to Fig. 2b). The homogeneous Gō model indeed lacks the pathway specificity exhibited when contact energies are diverse, as in heterogeneous Gō-like models.

The relevance of CAOs for the folding dynamics is illustrated in Fig. 2c. For two sequences and their Gō-like approximations, it plots $p_{\text{fold}}$ as a function of the total number of permanent native contacts formed, averaged over 200 folding trajectories. $p_{\text{fold}}$ gives the probability for trajectories initiated from a particular configuration to fold completely before visiting the unfolded state, and provides a standard basis for defining transition states in complex systems. Fig. 2c shows that $p_{\text{fold}} \ll 1$ when the first permanent contact is formed. Since $p_{\text{fold}} = 1$ by definition when the last permanent contact is formed, CAO histograms chronicle nearly the entire course of folding dynamics, all the way from the unfolded basin of attraction ($p_{\text{fold}} = 0$) to the native state ($p_{\text{fold}} = 1$).

Insensitivity of topological folding pathways to non-native contact energies by no means implies a complete dynamical equivalence of full and Gō-like models. For example, a sequence’s mean first passage time for folding can differ by as many as three orders of magnitude
for full and Gō-like models. This discrepancy is larger for sequences with slower folding rates. Such discrepancies may be due to the presence of off-pathway traps in the unfolded state, and possibly non-native stabilized intermediates along the folding pathway. However, our calculations suggest that such marked distinctions are largely limited to dynamics occurring before the value of the committor function $p_{\text{fold}}$ increases significantly from zero, i.e. before significant progress has been made along the folding reaction coordinate.

As illustrated in Fig. 3a, we can divide each folding trajectory into a period before any permanent contacts are made (the “pre-folding phase”) and the remaining period in which lasting native structure develops (the “folding phase”). Note that this division takes place well before a molecule commits to the folded state ($p_{\text{fold}} > 1/2$); indeed, the number of non-native contacts at the beginning of the folding phase is typically comparable to that of the unfolded state. Fig. 3b shows the distributions of pre-folding and folding phases’ durations for two sequences representative of fast and slow folders. In both cases the influence of non-native contacts on the folding phase dynamics is weak. Non-native contacts mildly extend the time required to complete folding after the first permanent contact is made, by less than an order of magnitude. By contrast, pre-folding dynamics of poorly designed sequences are quite sensitive to non-native contact energies. For the example shown in the middle panel of Fig. 3b, the waiting period prior to formation of a single permanent contact is roughly three orders of magnitude longer in the full model as in the Gō-like model. No such dilation is observed for sequences that fold quickly in the full model.

Because contact appearance order is a sensitive measure of approach to the dynamical bottleneck for folding, our division of pre-folding and folding phases is a kinetically meaningful one. Most importantly, $p_{\text{fold}} \ll 1$ throughout pre-folding dynamics as seen in Fig. 3a, indicating that the system remains well within the unfolded basin of attraction. Only when permanent contacts are made does $p_{\text{fold}}$ rise significantly, so that the folding phase encompasses entirely departure from the unfolded state and transit to the native structure. It is remarkable that non-native contacts, which can substantially prolong dwell times in the unfolded state, exert no discernible influence on the topological folding order, and only a small effect on the duration of folding phase dynamics.
Our simulations suggest that progress toward the native state is essentially orthogonal to the formation and rupture of non-native contacts. A number of such contacts are certainly present over much of the course of folding, but they do little to decide what conformational rearrangements bring a chain closer to its transition state for folding. To further test this idea, we studied folding dynamics governed by potential energy functions that combine aspects of full and Gō-like models. Specifically, we selected a set of non-native contact energies at random from a Gaussian distribution, see Fig. 3b. The “frustrating” influence of these random energies match precisely the behavior we have reported for the full model: CAO histograms are completely insensitive to the average strength and variance of non-native attractions, while overall folding rates decrease with increasing non-native attraction strength.

The observation of correspondence between dynamics of the full lattice model and that of a heterogeneous Gō-like approximation does not noticeably depend upon chain length or on details of native structure. We have generated sequences with a range of folding rates for several native conformations of chains with lengths 8, 12, 48, and 64. For the two shortest chains, we used each maximally compact lattice structure as a folded state. For the two longest chains, we studied several native structures varying significantly in compactness and in contact order. Typical results shown in Fig. 4 highlight that the fidelity of Gō-like folding mechanisms is a very general feature of these lattice heteropolymers.

IV. CONCLUSIONS

Several arguments have been presented in the literature to justify the use of Gō models in studying the folding mechanisms of real proteins. Most commonly asserted (based on the principle of minimum frustration) is that evolutionary optimization of real sequences removes kinetic barriers and renders the energy landscape smoothly funneled and therefore Gō-like. Biases due to topological features of the native state, unchanged in a protein’s Gō-like representation, have also been invoked to justify mechanistic fidelity. Our results demonstrate, however, that neither of these assumptions need hold for a Gō-like model to reproduce in fine detail the topological ordering of folding events of a lattice heteropolymer.

Robustness of the detailed mechanism for folding to omission of non-native contacts is not a consequence of sequence design within the schematic lattice models we have studied. It is a fundamental emergent feature of their statistical dynamics, independent of folding efficiency over the entire range accessible to our numerical simulations. Rather than introducing kinetic roadblocks that reshape transition states for folding, energetic diversions due to non-native contacts appear to strongly affect only physical properties of the unfolded state. Even the duration of trajectory segments that span folded and unfolded states is essentially determined by native energies alone, despite the fact that substantial non-native structure must be disrupted en route.

Lattice heteropolymers are perhaps the crudest representation of protein mechanics to which our analysis could be meaningfully applied. The correspondence between full and Gō-like folding mechanisms we have revealed might break down in more detailed models. For example, it has been reported that lattice heteropolymers do not exhibit glassy folding dynamics even at very low temperatures, while non-Arrhenius temperature dependence naturally arises in slightly elaborated models that describe side chain packing in addition to backbone conformation. Gō-like energetics could alter folding pathways by abating the frustration underlying such glassy relaxation. This possibility, which merits further investigation, does not however negate the significance of our findings. Our primary purpose is not to justify the use of Gō-like models for detailed study of real proteins’ folding mechanisms. It is instead to establish the influence of non-native interactions on dynamics intrinsic to the fundamental interplay between chain connectivity and heterogeneous contact interactions. That interplay, whose understanding is central to any instructive physical picture of protein folding, is not just present in simple lattice models – it is the exclusive source of their complexity. The results we have presented therefore establish an important point: Mechanistic aspects of protein folding that arise from the basic physics of heteropolymer freezing are remarkably insensitive to non-native structure.

Acknowledgments

We wish to thank D. Chandler, J. Chodera, K. DuBay, R. Jack, and S. Whitelam for useful discussions, and W. Eaton, E. Shakhnovich, and A. Szabo for critical readings of the manuscript.

This research used resources of the National Energy Research Scientific Computing Center, which is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231. This work was supported by the Director, Office of Science, Office of Basic Energy Sciences, Chemical Sciences and Physical Biosciences Divisions, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231. In carrying out this work JPG was supported by EPSRC grant GR/S54074/01.

V. APPENDIX

Our method of sequence generation, which effects a biased random walk in the space of all possible sequences, is an extension of the method of Mirny et al. To generate ensembles of sequences folding to a specific native
structure, we introduce random point mutations and accept them with a Metropolis probability

$$P_{\text{acc}} = \min \left[ 1, \exp \left( -\frac{\Delta F^{(\beta)} - \Delta F^{(\alpha)}}{k_B T} \right) \right]$$  \hspace{1cm} (6)

that generates a Boltzmann-like distribution. Here, $\Delta F^{(\alpha)}$ is an estimated activation free energy for folding of sequence $\alpha$, $k^{(\alpha)} = k_0 \exp(-\Delta F^{(\alpha)}/k_B T)$. We estimate the folding rate constant $k^{(\alpha)}$ for sequence $\alpha$, relative to the rate of basic microscopic motions $k_0$, by computing the fraction of trajectories $\langle h_{\text{fold}} \rangle \tau \approx 1 - \exp(-k^{(\alpha)} \tau)$ that fold within a fixed amount of time $\tau$ (with $k^{(\alpha)} \ll \tau^{-1} \ll k_0$). This strategy offers two distinct advantages: (1) the evolutionary temperature $T_{\text{ev}}$, which governs the stringency of selection for efficient folding, can be controlled systematically; and (2) estimates of folding efficiency via $\langle h_{\text{fold}} \rangle \tau$ can converge much more rapidly than mean first passage time calculations employed in Mirny et al.\textsuperscript{23}

Our evolutionary simulations, conducted at moderate “temperature” $T_{\text{ev}} = 0.05/k_B$, demonstrate that in fact many folding pathways can provide efficient access to a single native state. It is therefore not at all self-evident that a particular, well-designed amino acid sequence should arrive at its native structure via similar routes in full and Gō-like versions of the lattice heteropolymer model.

Using this method, we have generated hundreds of thousands of sequences which fold to given structures (for example that of Fig. 1a) through a variety of folding mechanisms. This is the ensemble of sequences we use in this paper. Further details of the evolutionary dynamics used to generate these large ensembles of sequences will be given in a forthcoming publication.\textsuperscript{10}

\begin{thebibliography}{99}
  \bibitem{1} Shakhnovich, E., 2006. Protein folding thermodynamics and dynamics: where physics, chemistry, and biology meet. \textit{Chem Rev} 106:1559–1588.
  \bibitem{2} Go, N., 1983. Theoretical studies of protein folding. \textit{Annu Rev Biophys Bioeng} 12:183–210.
  \bibitem{3} Shimada, J., A. V. Ishchenko, and E. I. Shakhnovich, 2000. Analysis of knowledge-based protein-ligand potentials using a self-consistent method. \textit{Protein Sci} 9:765–775.
  \bibitem{4} Shimada, J., E. L. Kussell, and E. I. Shakhnovich, 2001. The folding thermodynamics and kinetics of crambin using an all-atom Monte Carlo simulation. \textit{J Mol Biol} 308:79–95.
  \bibitem{5} Takada, S., 1999. Go-ing for the prediction of protein folding mechanisms. \textit{Proc Natl Acad Sci U S A} 96:11698–11700.
  \bibitem{6} Shoemaker, B. A., and P. G. Wolynes, 1999. Exploring structures in protein folding funnels with free energy functionals: the denatured ensemble. \textit{J Mol Biol} 287:657–674.
  \bibitem{7} Shakhnovich, E. I., and A. M. Gutin, 1990. Implications of thermodynamics of protein folding for evolution of primary sequences. \textit{Nature} 346:773–775.
  \bibitem{8} Sali, A., E. Shakhnovich, and M. Karplus, 1994. Kinetics of protein folding. A lattice model study of the requirements for folding to the native state. \textit{J Mol Biol} 235:1614–1636.
  \bibitem{9} Bryngelson, J. D., and P. G. Wolynes, 1987. Spin glasses and the statistical mechanics of protein folding. \textit{Proc Natl Acad Sci U S A} 84:7524–7528.
  \bibitem{10} Onuchic, J. N., P. G. Wolynes, Z. Luthey-Schulten, and N. D. Socci, 1995. Toward an outline of the topography of a realistic protein-folding funnel. \textit{Proc Natl Acad Sci U S A} 92:3626–3630.
  \bibitem{11} Onuchic, J. N., N. D. Socci, Z. Luthey-Schulten, and P. G. Wolynes, 1995. Protein folding funnels: the nature of the transition state ensemble. \textit{Fold Des} 1:441–450.
  \bibitem{12} Onuchic, J. N., Z. Luthey-Schulten, and P. G. Wolynes, 1997. Theory of protein folding: the energy landscape perspective. \textit{Annu Rev Phys Chem} 48:545–600.
  \bibitem{13} Socci, N. D., J. N. Onuchic, and P. G. Wolynes, 1998. Protein folding mechanisms and the multidimensional folding funnel. \textit{Proteins} 32:136–158.
  \bibitem{14} Pande, V. S., A. Grosberg, T. Tanaka, and D. S. Rokhsar, 1998. Pathways for protein folding: is a new view needed? \textit{Curr Opin Struct Biol} 8:68–79.
  \bibitem{15} Onuchic, J. N., and P. G. Wolynes, 2004. Theory of protein folding. \textit{Curr Opin Struct Biol} 14:70–75.
  \bibitem{16} Karanicolas, J., and C. L. Brooks, 2003. Improved Gō-like models demonstrate the robustness of protein folding mechanisms towards non-native interactions. \textit{J Mol Biol} 334:309–325.
  \bibitem{17} Levy, Y., and J. N. Onuchic, 2006. Mechanisms of protein assembly: lessons from minimalist models. \textit{Acc Chem Res} 39:135–142.
  \bibitem{18} Simler, B. R., Y. Levy, J. N. Onuchic, and C. R. Matthews, 2006. The folding energy landscape of the dimerization domain of Escherichia coli Trp repressor: a joint experimental and theoretical investigation. \textit{J Mol Biol} 363:262–278.
  \bibitem{19} Clementi, C., and S. S. Plotkin, 2004. The effects of non-native interactions on protein folding rates: theory and simulation. \textit{Protein Sci} 13:1750–1766.
  \bibitem{20} Sali, A., E. Shakhnovich, and M. Karplus, 1994. How does a protein fold? \textit{Nature} 369:248–251.
  \bibitem{21} Paci, E., M. Vendruscolo, and M. Karplus, 2002. Validity of Gō models: comparison with a solvent-shielded empirical energy decomposition. \textit{Biophys J} 83:3032–3038.
  \bibitem{22} Paci, E., M. Vendruscolo, and M. Karplus, 2002. Native and non-native interactions along protein folding and unfolding pathways. \textit{Proteins} 47:379–392.
  \bibitem{23} Shakhnovich, E. I., and A. M. Gutin, 1993. Engineering of stable and fast-folding sequences of model proteins. \textit{Proc Natl Acad Sci U S A} 90:7195–7199.
  \bibitem{24} Gutin, A., A. Sali, V. Abkevich, M. Karplus, and E. I. Shakhnovich, 1998. Temperature dependence of the folding rate in a simple protein model: Search for a “glass” transition. \textit{Journal of Chemical Physics} 108:6466–6483.
  \bibitem{25} Mirny, L. A., V. I. Abkevich, and E. I. Shakhnovich, 1998. How evolution makes proteins fold quickly. \textit{Proc Natl Acad Sci U S A} 95:4976–4981.
  \bibitem{26} Schaeffer, R. D., A. Fersht, and V. Daggett, 2008. Combining experiment and simulation in protein folding: closing the gap for small model systems. \textit{Curr Opin Struct Biol} 18:4–9.
\end{thebibliography}
27. Pande, V. S., and D. S. Rokhsar, 1999. Folding pathway of a lattice model for proteins. *Proc Natl Acad Sci U S A* 96:1273–1278.

28. Abkevich, V. I., A. M. Guti, and E. I. Shakhnovich, 1994. Specific nucleus as the transition state for protein folding: evidence from the lattice model. *Biochemistry* 33:10026–10036.

29. Sutto, L., G. Tiana, and R. A. Broglia, 2006. Sequence of events in folding mechanism: beyond the Go model. *Proteins Sci* 15:1638–1652.

30. Shakhnovich, 1994. Proteins with selected sequences fold into unique native conformation. *Phys Rev Lett* 72:3907–3910.

31. Miyazawa, S., and R. L. Jernigan, 1985. Estimation of Effective Interresidue Contact Energies from Protein Crystal Structures: Quasi-Chemical Approximation. *Macromolecules* 18:534–552.

32. Dokholyan, N. V., S. V. Buldyrev, H. E. Stanley, and E. I. Shakhnovich, 2000. Identifying the protein folding nucleus using molecular dynamics. *J Mol Biol* 296:1183–1188.

33. Faisca, P. F. N., R. D. M. Travasso, R. C. Ball, and E. I. Shakhnovich, 2008. Identifying critical residues in protein folding: Insights from phi-value and Pfold analysis. *The Journal of Chemical Physics* 129:095108.

34. Ritort, F., and P. Sollich, 2003. Glassy dynamics of kinetically constrained models. *Adv. Phys.* 52:219–342.

35. Fischer, K., and J. Hertz, 1993. *Spin Glasses*. Cambridge University Press.

36. Du, R., V. S. Pande, A. Y. Grosberg, T. Tanaka, and E. I. Shakhnovich, 1998. On the Transition Coordinate for Protein Folding. *Journal of Chemical Physics* 108:334–350.

37. Weikl, T. R., and K. A. Dill, 2003. Folding rates and low-entropy-loss routes of two-state proteins. *J Mol Biol* 329:585–598.

38. Oliveira, L. C., A. Schug, and J. N. Onuchic, 2008. Geometrical features of the protein folding mechanism are a robust property of the energy landscape: a detailed investigation of several reduced models. *J Phys Chem B* 112:6131–6136.

39. Hills, R. D., and C. L. Brooks, 2008. Coevolution of function and the folding landscape: correlation with density of native contacts. *Biophys J* 95:L57–L59.

40. Gin, B. C., J. P. Garrahan, and P. L. Geissler. in preparation.