

## Scaling of Folding Properties in Simple Models of Proteins

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(Received 1 December 1998)

Scaling of folding properties of proteins is studied in a toy system—the lattice Go model with various two- and three-dimensional geometries of the maximally compact native states. Characteristic folding times grow as power laws with the system size. The corresponding exponents are not universal. Scaling of the thermodynamic stability also indicates size-related deterioration of the folding properties.

PACS numbers: 87.14.Ee, 71.28.+d, 71.27.+a

Recent advances in understanding of protein folding have been made, to a large extent, through studies of lattice heteropolymers with a small number of beads,  $N$ , [1,2]. In these toy models of proteins, the beads represent amino acids. Lattice models allow for an exact determination of the native state, i.e., of the ground state of the system, and are endowed with a simplified dynamics. An  $N$  of order 125 is considered to be large in such studies and then special sequences are considered [3]. There are real life proteins [4] with  $N$  as small as of order 30, but most of them are built of several hundreds of amino acids. Apparently there is no protein with  $N$  exceeding 5000 which is orders of magnitude smaller than the number of base pairs in a DNA. The question we ask in this Letter is: How do folding properties of proteins scale with  $N$  and can they lead to a deterioration in stability and kinetic accessibility of the native state that exceed bounds of functionality?

A previous numerical analysis of the scaling has been done by Gutin, Abkevich, and Shakhnovich [5] who studied three-dimensional (3D) lattice sequences with  $N$  up to 175. For each  $N$ , they considered five sequences and selected one that folded the fastest under its optimal temperature  $T_{\min}$ . The corresponding folding time,  $t_{01}$ , was the quantity that was used in studies of scaling. They discovered that  $t_{01}$  grows as a power law with the system size:

$$t_{01} \sim N^\lambda. \quad (1)$$

The exponent  $\lambda$  was found to be nonuniversal—it depended on the kind of distribution of the contact energies  $B_{ij}$  in the Hamiltonian

$$H = \sum_{i < j} B_{ij} \Delta_{ij}, \quad (2)$$

which pointed to the existence of a variety of kinds of the energy landscapes [6]. In Eq. (2),  $\Delta_{ij}$  is either 1 or 0 depending on whether the monomers  $i$  and  $j$  face each other, but not along the chain, or not. For random and designed sequences, with the  $B_{ij}$ 's generated from the database of Ref. [7],  $\lambda \approx 6$  and 4, respectively [5]. Finally, for the Go model [8], in which  $B_{ij} = -1$  for native contacts and 0 for non-native contacts,  $\lambda \approx 2.7$ . There were also phenomenological arguments [9] which suggested that the folding times scale with  $N$  exponentially for all temperatures. Thus the nature of the scaling laws for the folding

times remains puzzling. Perhaps more importantly, Gutin *et al.* [5] did not study scaling of any of the characteristic temperatures that are relevant for folding nor the effects of the dimensionality were explored.

In this Letter, we report on studies of the 2D and 3D Go model, with  $N$  up to 56 and 100, respectively. In the 2D and  $N = 16$  case, we consider all 37 maximally compact conformations (there are 69 such conformations but only 38 of them are distinct due to the end-to-end symmetry of the Go model; furthermore, one conformation cannot be accessed kinetically). In the remaining cases, we study 15 conformations, except for  $N = 80$  and 100 when only 10 and 5, respectively, are considered. Note that each of these structures is equally designable within the model because each is a nondegenerate ground state to one Go sequence. We demonstrate that in this case,  $t_{01}$  is indeed given by Eq. (1). In 2D,  $\lambda$  is  $5.9 \pm 0.2$ . Thus the constraint for the heteropolymer to lie in a plane increases  $\lambda$  compared to the 3D Go model. Our larger statistics also allows us to study median values, not just minimal, of the folding times. The median values also follow the power law with an effective  $\lambda$  of  $6.3 \pm 0.2$  and  $3.1 \pm 0.1$  in 2D and 3D, respectively. Actually, the effective  $\lambda$  depends on whether the folding is studied at  $T_{\min}$  or at the folding temperature  $T_f$ .  $T_f$  is defined operationally as a temperature at which the equilibrium probability of finding the native state is  $\frac{1}{2}$ . We find that in 2D and at  $T_f$ ,  $\lambda = 6.6 \pm 0.1$  (the exponent for the minimal time at  $T_f$  is  $6.3 \pm 0.3$ ) which means that by moving away from conditions which are optimal for the folding kinetics one generates a somewhat increased exponent in the power law.

Notice that good folding takes place for sequences for which  $T_f$  is comparable to or bigger than  $T_{\min}$ . Otherwise the folding is poor. An important novel aspect of our research is that we determine the scaling properties of  $T_{\min}$  and those of the folding temperature,  $T_f$ . We conclude that, both in 2D and 3D, there are indications that there could be a size related limit to good foldicity. We find that  $T_{\min}$  grows linearly with  $N$  whereas  $T_f$  first grows like  $T_{\min}$  but then it falls off and possibly saturates asymptotically. This makes the gap between  $T_{\min}$  and  $T_f$  increase linearly with  $N$  asymptotically which would change the folding kinetics from excellent to bad.

One stumbling block in studies of scaling of random systems is the necessity to compare quantities which are averaged statistically and to have some control of the statistical ensemble used. The advantage of the Go model is that there is no randomness in the values of the contact energies and the ensemble is generated by the set of possible maximally compact conformations that can act as native states—i.e., the variety is due only to the geometry of the native states. The advantage of studying 2D models is that, for  $N = 16$ , it is feasible to determine the full distribution of  $T_f$ ,  $T_{\min}$ , and of the folding times among all of the 37 targets and then to realize that the *median* folding time probes vicinities of the peak in the distribution. Thus, on going to larger  $N$  and taking, as we usually do, 15 targets, it is reasonable to expect that the corresponding median time still probes the peaks of good foldicity. Median quantities are, in addition, more stable statistically, in general, whenever one deals with wide distributions.

As to the selection of the 15 native maximally compact targets: in 2D 10 were obtained by a random construction and 5 were obtained by a multiple quenching of randomly shaped homopolymers until a maximally compact conformation was obtained. The homopolymers had identical attraction in each possible contact. In both methods, we generate targets to which there is a path of kinetic access. In 3D, all targets were obtained by the random construction.

Figure 1 illustrates definitions of quantities that will be studied here. It shows the dependence of the median folding time,  $t_{\text{fold}}$ , on temperature for one target. The target

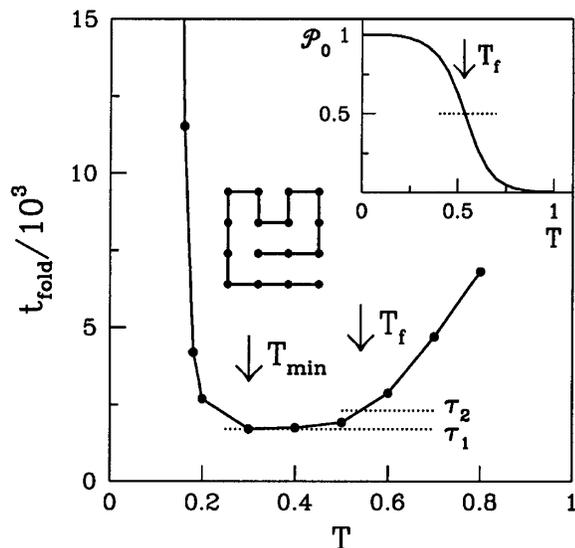


FIG. 1. The dependence of the median folding time,  $t_{\text{fold}}$ , on  $T$  for the 2D Go conformation shown in the center of the figure. The results are averaged over 1000 Monte Carlo trajectories. The inset shows the  $T$  dependence of probability for the sequence to be in the native state as obtained through an exact evaluation of the partition function which involves 802075 conformations.

has  $N$  of 16 and is shown in the center of the figure. The optimal temperature,  $T_{\min}$ , is where  $t_{\text{fold}}$  is the shortest.  $T_{\min}$  signifies the onset of glassy kinetics. This quantity is better suited to study scaling than the glass transition temperature  $T_g$  [10] because the latter involves a cutoff time which necessarily must be  $N$  dependent.  $t_{\text{fold}}$  at  $T_{\min}$  will be denoted by  $\tau_1$ .  $\tau_2$  is defined to be  $t_{\text{fold}}$  at  $T_f$  ( $T_f$  is larger than  $T_{\min}$  for the target shown in Fig. 1). In the statistical ensemble,  $t_1$  is defined to be the median value of  $\tau_1$  and  $t_2$ —the median value of  $\tau_2$ . We also study  $t_{01}$  and  $t_{02}$  which are the minimal values of  $\tau_1$  and  $\tau_2$  among the targets considered.

The folding times were obtained through a Monte Carlo procedure that satisfies the detailed balance condition [11] and was motivated by studies presented in Ref. [12]. For each conformation of the polymer, one first determines the number of possible single and double-monomer (crankshaft) moves—these numbers will be denoted here by  $A_1$  and  $A_2$ , respectively. The maximum value of  $A_1 + A_2$ , among all conformations, is equal to  $A_{\max} = N + 2$ . The probability to attempt a single monomer move is taken to be  $rA_1/A_{\max}$  ( $r = 0.2$ ). For a double monomer move it is  $(1 - r)A_2/A_{\max}$ . The attempts are rejected or accepted as in the standard Metropolis method. The folding time is defined as the first passage time and is measured by the number of Monte Carlo attempts divided by  $A_{\max}$ . For  $N > 16$ , it is determined based on 50 to 200 trajectories. It should be noted that Ref. [5] does not specify whether the detailed balance condition was enforced.

Figure 2 shows the distribution of  $\tau_1$  and  $\tau_2$  for all targets with  $N = 16$ . There is a substantial scatter in the

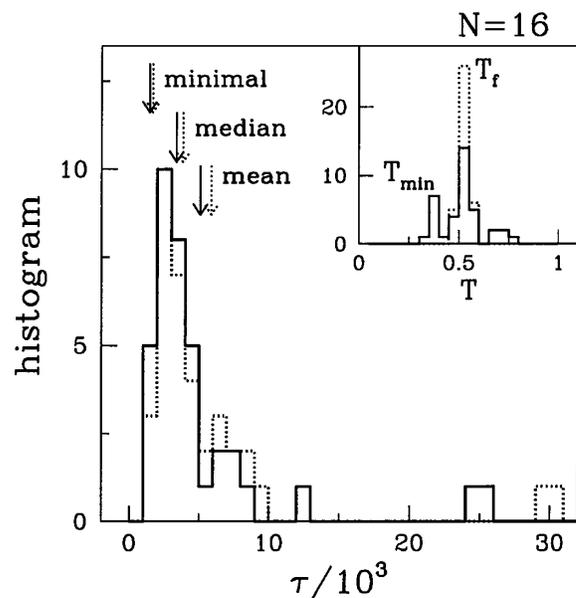


FIG. 2. The distribution of folding times at  $T_{\min}$  (solid lines) and at  $T_f$  (dotted lines) for the 2D  $N = 16$  case. The arrows indicate their median, mean, and minimal values. The inset shows the distributions of  $T_{\min}$  and  $T_f$ .

values of  $\tau_i$  so the usage of the median  $t_i$  appears to be justified. The inset shows the corresponding distributions of  $T_f$  and  $T_{\min}$ . Both are centered and the median and mean values almost coincide. Note that there is very little variation in  $T_f$ : all Go targets with  $N = 16$  have almost identical stability properties:  $T_f$  varies between 0.489 and 0.565. On going to larger  $N$ 's, the distributions of  $\tau_1$  remain clustered around  $t_1$  but the long time tail appears to extend towards longer and longer times. This results in an overall flattening of the distributions on the scale set by  $t_1$ . For  $N = 16$ , the exact distribution of  $\tau_1/t_1$  ends at about 8 whereas our sampling of  $N = 20$  and 42 yields tails in  $\tau_1/t_1$  which are located at around 16 and 10, respectively. Within our statistics, we have not spotted any relatively long lasting folding processes for other values of  $N$ . However, their very existence for  $N = 20$  and 42 suggests an emergence of the tails in distributions if those could be sampled fully.

Figure 3 summarizes our results on the scaling of folding times. It demonstrates the validity of the power laws both for the median and for the minimal folding times. The effective exponents  $\lambda$  depend on  $T$ , i.e., they depend on whether the kinetics was monitored at  $T_{\min}$  or  $T_f$ . This dependence is not substantial but it indicates variations of the free energy landscape with  $T$  and underscores a more general lack of universality.

The generic power laws obtained by Gutin *et al.* [5] and by us contradict the exponential laws derived in the random

energy model [6,9]. They support a generally accepted view that the folding process is a finite volume version of the first order transition [5,13]. In this picture one may visualize the transition stage as an inhomogeneous mixture of the "new" phase in the sea of an "old" phase [14]. The random energy model does not capture such inhomogeneities.

Figure 4 shows the  $N$  dependence of the characteristic temperatures. For both 2D and 3D  $T_{\min}$  grows linearly with  $N$ , whereas  $T_f$  shows a more complex behavior. For  $N > 16$ ,  $T_f$  is determined from the Monte Carlo simulations: (a) we vary the  $T$  in steps of 0.05 or smaller, (b) at each  $T$  we start from the native state and monitor the probability of occupying it, and (c) in most cases the results are averaged over 50 different trajectories. The number of Monte Carlo steps for each  $T$  depends on  $N$  and it ranges from  $10^6$  to  $7 \times 10^6$ . We checked that doubling the selected cutoff times had a negligible effect on  $T_f$ . The procedure yields results which agree with those obtained by the exact enumeration for  $N = 16$ . In 2D, the dependence of  $\langle T_f \rangle$  on  $N$  initially follows that of  $T_{\min}$ . However, on crossing  $N_c$  of 36,  $T_f$  falls off and it may saturate which is suggested by the declining rate of growth. Thus 2D Go conformations appear to have intrinsic limits to their thermodynamic stability. Beyond  $N_c$ , the foldicity becomes gradually poorer and poorer. The same scenario appears to be present also in the 3D case except that the small  $N$  value of  $T_f$  is substantially larger than  $T_{\min}$ .  $T_f$  starts showing signs of the saturation around  $N = 80$ . We

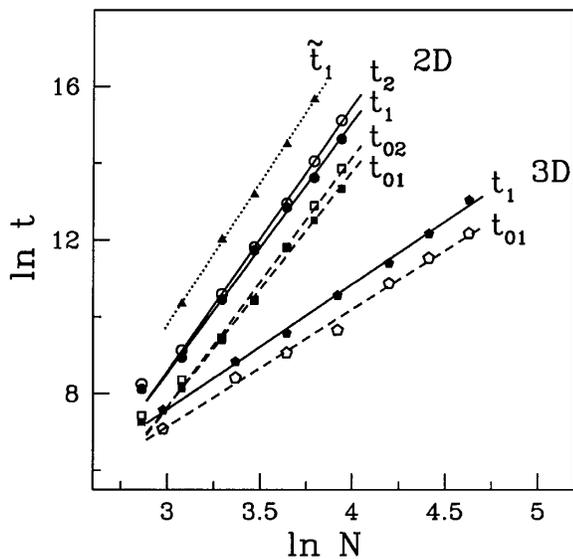


FIG. 3. The dependence of folding times on  $N$ .  $t_1$  and  $t_2$  are the median folding times at  $T_{\min}$  and  $T_f$ , respectively.  $t_{01}$  and  $t_{02}$  are the corresponding minimal folding times found.  $\tilde{t}_1$  is the median folding time at  $T_{\min}$  as obtained by a straightforward Monte Carlo procedure which does not enforce the detailed balance condition. The values of the effective exponent  $\lambda$  for the 2D case are  $7.1 \pm 0.1$ ,  $6.6 \pm 0.1$ ,  $6.3 \pm 0.2$ ,  $6.3 \pm 0.3$ , and  $5.9 \pm 0.2$  when counting clockwise. For 3D, the slopes are  $3.1 \pm 0.1$  and  $2.9 \pm 0.1$ .

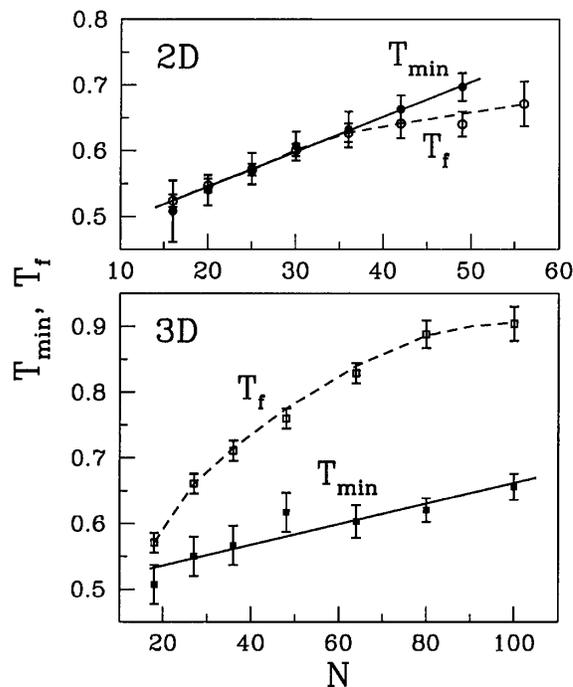


FIG. 4. The dependence of  $\langle T_{\min} \rangle$  and  $\langle T_f \rangle$  on  $N$ .  $\langle T_{\min} \rangle$  is fitted by a linear function:  $0.44 + 0.0053N$  and  $0.505 + 0.0015(7)N$  for 2D and 3D, respectively. The results are averaged over the conformations that were used in the studies of dynamics.

were unable to explore values of  $N$  that were larger than 100 but a saturation of  $T_f$  is expected on general grounds due to the existence of the (first order) phase transition to the folded phase in the thermodynamic limit.  $T_{\min}$ , on the other hand, is expected to grow indefinitely due to the growth of kinetic barriers to cross. In 3D,  $T_f$  and  $T_{\min}$  appear to cross somewhere around  $N_c = 300$ .

In conclusion, we have studied the scaling properties not only of the fastest sequences, as in Ref. [5], but also of those with typical folding rates. The exponents in the resulting power laws for the folding times depend on  $D$ , values of the  $B_{ij}$ 's, and on  $T$ . In addition to the deterioration of the folding kinetics with  $N$ , as described by the growth of  $T_{\min}$  and of the folding times, a relative deterioration of the thermodynamic stability also appears to set in. Thus there will be no rapidly folding heteropolymers of a large size. It would be interesting to determine the scaling properties for more realistic models of proteins.

This work was supported by KBN (Grant No. 2P03B-025-13). Fruitful discussions with Jayanth R. Banavar and Dorota Cichocka are gratefully acknowledged.

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