Recent observations of fast steps in protein folding (1–3) address the following question: what is the shortest time that would be required for folding? The paper by Hagen et al. (4) in this issue of the Proceedings provides strong experimental support for a speed limit that is governed by diffusion. Diffusion is known to limit the rates of many of the activities of proteins, including enzymatic activity, electron transfer, and binding to other macromolecules (5). The new evidence suggests that folding can be added to this list.

The implications of the work by Hagen et al. and other recent data on fast steps in protein folding can best be understood in the context of the energy landscape picture of protein folding (6). This picture can be used quantitatively to describe the thermodynamic and related kinetic issues that are associated with the transformation of an ensemble of initially unfolded molecules to the native, folded structure. Although a wide variety of folding behaviors emerge from the energy landscape picture, depending on the energy parameters, temperature, and other variables, folding from random-coil conformations always begins with the collapse of the polypeptide chains to more compact structures. In the fastest-folding proteins, these compact structures continue “downhill” in free energy to the native state. In other cases, bottlenecks arising from the decreasing chain entropy or other factors result in slower progression to the native state.

About 10 years ago, Bryngelson and Wolynes (7) presented the basis for a diffusion equation (8) that describes the time evolution of an ensemble of chains that start at any given degree of progress toward the folded state. The diffusion coefficient in this equation depends on the energy landscape topography and on the temperature; increasing the temperature increases the diffusion coefficient, in part by stimulating escape of the chain from local minima on the energy surface. For the initial collapse phase of folding, it has been possible to obtain estimates of this diffusion coefficient and the collapse time by analyzing computer simulations of lattice models that have some of the essential features of proteins (8). The work by Hagen et al. now provides experimental data of a similar kind.

Hagen et al. used nanosecond-resolved spectroscopy to determine the rate of collision of sequentially distant segments of unfolded cytochrome c. The system was maintained under strongly denaturing conditions, so the measurements provide estimates of the time required for large loops to form, starting from random-coil conformations. For loops of 50–60 residues, times of the order of 40 \( \mu \)s are found. To make the connection to a collapse time, Hagen et al. argue that collapse cannot occur faster than the formation of the shortest loops that are seen in folded proteins, i.e., 6–10 residues. They then apply theories for the diffusion-controlled contact formation in a polymer to scale the measured times for larger loops to obtain estimated times for the smaller loops. The resulting time, about 1 \( \mu \)s, provides an estimate of the lower limit for the time required for a random-coil protein to collapse to a compact structure. Because the landscape analyses imply that the complete folding process will not be faster than this initial collapse (8), the 1-\( \mu \)s interval represents an estimate of the shortest time required to fold a protein.

The (1 \( \mu \)s)^{-1} speed limit for protein folding proposed by Hagen et al. is in general accord with data from a number of other recent experiments (1–3), although some of these are clearly probing incomplete folding processes. Hagen et al. argue that rapid collapse is of biological importance in preventing the aggregation of newly synthesized, unchaperoned proteins. The fast-folding substructures of larger proteins (6) may also serve this function.

Finally, it is worth noting that Brownian dynamics simulations (9) of the diffusion of model peptide chains have now been extended from the nanosecond time scales of early studies of \( \alpha \)-helix growth (10) to microsecond time scale studies of tertiary structure changes (11, 12). It may soon be possible to characterize the initial collapse of small proteins using sets of such Brownian dynamics simulations. As was mentioned at the start of this commentary, diffusion is known to set the speed limits for the fastest enzymes. Brownian dynamics simulations have deepened our understanding of these speed limits, which may be raised or lowered by electrostatic and other interactions between the enzyme and substrate (5, 13, 14). In fact, such simulations have been used successfully to raise these speed limits in some cases, by guiding mutagenesis to produce enzymes that steer substrate diffusion more effectively to their active sites (15, 16). The simulations in such applications are of relatively simple bimolecular encounter processes, although coupling of the association to internal motions of the enzyme may lead to interesting linkages to the folding problem (14). Diffusional encounters of discrete structures have also been suggested to be involved in protein folding, e.g., in the association of partly folded elements (11, 17–19). Brownian dynamics simulations have proven useful in model studies of the association of preformed helices on the microsecond time scale (11), but such simulations of the diffusional collapse of chains from representative samples of fully unfolded conformations remain to be done. Hagen et al. argue that successful sequences must be ones that collapse rapidly. Theory, simulations, and experiment are all likely to contribute to the study of such issues in the near future.


