BIOMOLECULAR FOLDING

Moments of excitement

Direct measurements of protein folding paths agree with theoretical predictions

By **Peter Wolynes**

At the atomic level, biomolecular dynamics, like war to its soldiers, consists of long periods of boredom interspersed by brief, intense moments of excitement. During the vast majority of its existence, a biomolecule flails about randomly, with large-amplitude motions when unfolded and with smaller-amplitude motions when folded. Rare transitions between these two states of often apparently aimless activity occur through a fleeting series of steps: a set of transition paths guided by the biomolecular energy landscape. On page 239 of this issue, Neupane *et al.* use single-molecule force spectroscopy to study the transition paths for folding a nucleic acid and for misfolding a prion protein (*1*). They are able to confirm some very basic aspects of biomolecular energy landscape theory.

Much of our knowledge of how molecules fold has come from painstaking measurements of the typical length of time that biomolecules spend in their states of boredom under many thermodynamic conditions, and then using our imagination (disciplined by theory and computation) to fill in the gap of how the molecule actually moves between its folded and unfolded ensembles (*2*). Recently, however, dynamical fluorescence spectroscopy has made the exciting moments of transition between folded and unfolded states directly accessible to experimental observation (*3*). These studies have provided estimates for the typical time involved in a folding transition.

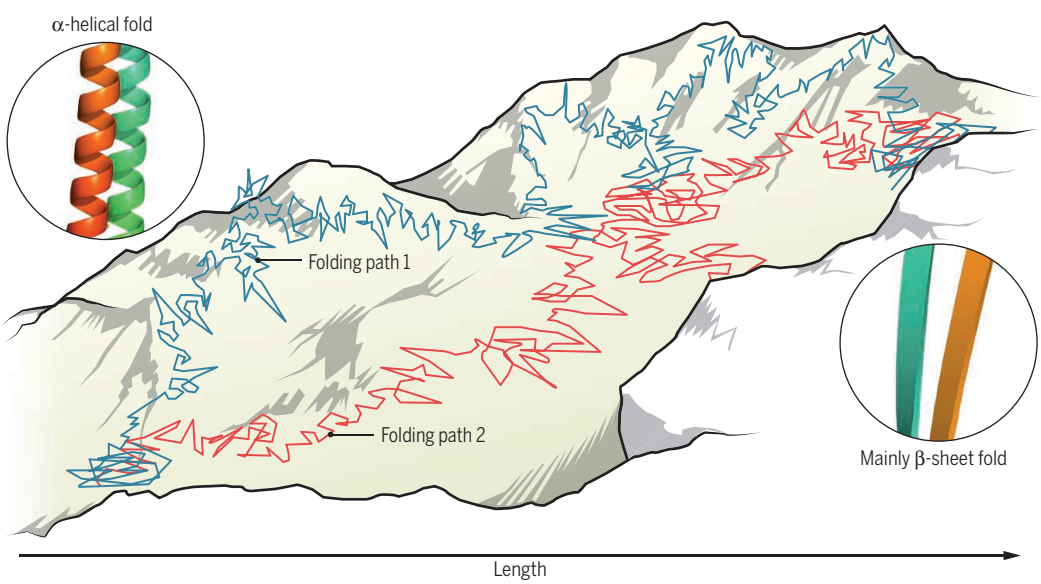
Neupane *et al.* use a different technique, single-molecule force spectroscopy, in which a force is exerted on a single biomolecule while simultaneously monitoring its length with subnanometer precision as it repeatedly unfolds and refolds. The extraordinary stability of the apparatus allows tens of thousands of transitions to be monitored over a period of hours. Each folding or unfolding transition takes place in only a few tens of microseconds; the molecule spends vastly more time simply ambling about. By monitoring the length during these transitions as a function of time and then analyzing the specific time histories, the authors build up a one-dimensional view of how each molecule folds. Even glancing at a few such histories settles a long-standing question: Is there a unique folding pathway? The authors find that not all histories are the same, and there is thus no evidence for an obligate single folding pathway. Instead, the authors find a multiplicity of paths, as has come to be expected from modern energy landscape theory.

A more quantitative test of energy landscape theory is provided by measuring the statistics of the transitions. Biomolecular folding involves random diffusion on an energy landscape with many dimensions. Nevertheless, this motion can often be projected onto a single coordinate such as the length, especially when the energy landscape is funneled. For funneled landscapes, the fraction of native structures acts as a good reaction coordinate, monitoring the reaction's progress. After projection, the motion is no longer completely random, but rather is guided by free energy gradients. The diffusion coefficient for this motion measures how easily and how often the molecule can escape from local minima on the full many-dimensional energy landscape.

The projected free energy surface typically has a barrier, because the entropic cost of organizing the molecule is not immediately repaid by increased stability of conformations with partially formed correct native structure. The free energy barrier explains why the waiting times are much longer than the time to traverse the barrier. By pulling continuously and measuring the work needed to unfold the molecule, the thermodynamic free energy profile can be measured. According to theory, the transition time scales inversely with diffusion rate, and measuring the typical transit time therefore gives a good estimate for diffusion rates. The overall waiting time, however, not only depends on diffusion but also scales exponentially with the thermodynamic barrier, which makes the waiting time so long.

Neupane *et al.* find that their single-molecule measurements of the barrier, waiting times, and transition times agree in the main with the simplest one-dimensional theory. The kinetic barrier for folding, however, turns out to be smaller than the thermodynamically determined one. The authors trace this discrepancy to an excess of short transit times relative to the expected simple exponential form for a strictly one-dimensional problem (*4*).

Department of Chemistry, Rice University, Houston, TX 77251, USA. E-mail: pwolynes@rice.edu



More than one path. This schematic two-dimensional free energy plot shows that the free energy of a protein under force depends on the fraction of ß sheet–like secondary structure as well as on the molecule's length. The latter is the only coordinate monitored by Neupane *et al*. The protein can follow different paths during folding; two possible paths are superimposed on the plot.

ILLUSTRATION: ADAPTED FROM MINGCHEN CHEN/RICE UNIVERSITY BY P. HUEY/*SCIENCE*

This apparent excess could come from unwanted influence of the Brownian motion of the tethers that pull on the molecule, or it might indicate that more coordinates of the biomolecule in addition to the length are needed to describe the transition. Such an explanation would not be surprising, because the length coordinate is not expected to be perfectly correlated with the folding or misfolding reaction coordinate (see the figure). The protein system studied by Neupane *et al.*—a prion—also probably does not have a well-funneled energy landscape, given that its configurational diffusion is extremely slow. For rugged landscapes, theory suggests that the logarithms of the escape times nearly follow a normal Gaussian distribution. This distribution gives a wider range of transit times than the prediction for strictly one-dimensional diffusion (*5*).

Now that Neupane *et al.* have directly confirmed some of the most basic notions of energy landscape theory by observing transition paths, we can expect future refinements to give more structural details about the transitions. To access these details, one can simultaneously measure fluorescence and length (*6*). However, existing measurements of this sort must be extended in time range and stability to uncover the multidimensional aspects of folding transition paths. Even without such enhancements, combining the capabilities of protein engineering with transition path measurement will give direct access to the structural aspects of the transition path ensemble. These structural factors have been predicted by theory and simulation for many proteins (*2*). Leaving its days of controversy, biomolecular folding is now on its way to becoming one of the best-understood processes in biochemistry. ■

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CLIMATE CHANGE

A heated mirror for future climate

Climatic changes 55.9 million years ago resemble those expected in the future

By **Richard B. Alley**

Climate has always changed naturally, and this is not good news when contemplating a human-forced future. The natural responses have been as large as, or larger than, those simulated by leading models for shorter time scales, with major biological and physical impacts. The possible effects of rapid carbon dioxide (CO_2) release may be clearest from the Paleocene-Eocene Thermal Maximum (PETM) about 55.9 million years ago, when a large, natural CO_2 release drove strong warming that caused amplifying feedbacks, dwarfing of large animals, ecosystem disruptions, soil degradation, water-cycle shifts, and other major changes (see the figure). The climatic changes during the PETM occurred over longer time scales than those of anthropogenic climate change. The impacts of the latter may thus be even more severe.

The source of the initial CO_2 release that drove the PETM remains debated (*1*). Increasing evidence points to a concentrated igneous outpouring during the opening of the North Atlantic, which intruded oil-bearing and otherwise carbon-rich rocks (*2*). The PETM was amplified and extended by sustained CO_2 release, probably at least in part because the warming released organic carbon stored in soils, seafloor sediments, or elsewhere (*1*).

Most estimates of the total CO_2 added to the atmosphere during the PETM are similar to, or somewhat lower than, the total CO_2 that would arise from burning all fossil-fuel resources estimated to exist on Earth—especially if, as suggested by the PETM and by current understanding, warming releases additional carbon from reservoirs such as tundra soils and seafloor hydrates (*1*). However, the initial CO_2 rise during the PETM took place over the course of a few millennia, about a factor of 10 slower than if humans burned the remaining fossil-fuel resources under a business-as-usual scenario (*3*). PETM CO_2 remained elevated for more than 150,000 years, confirming the long persistence expected for human-released CO_2 (*1*).

The strong PETM warming suggests that climate is highly sensitive to rising CO_2. This implies a higher climate sensitivity than the lower end adopted for somewhat shorter times by the Intergovernmental Panel on Climate Change (IPCC), and perhaps larger than the higher end (*4*). Thus, temperatures may rise more than currently projected.

During the PETM, the rise in CO_2 and resulting climate shifts caused further changes propagating across the Earth system. On land, enhanced erosion and sediment transport to the sea (*5*, *6*) are consistent with the expected increase in hydrological variability from warming; larger or more intense storms separated by longer and drier intervals likely contributed to regional loss of vegetation, soil carbon, and soil fertility (*5*).

Over the course of the PETM, terrestrial species migrated long distances poleward or upward and crossed land bridges between continents. Some types became extinct, whereas others spread. Ecosystems during the event were notably different from those before or afterward. Wing and Currano have shown that of a sample of 91 common plant taxa known from fossils during a million-year-long interval starting 200,000 years before the PETM in Wyoming's Bighorn Basin, only 7 persisted before, during, and after the PETM. Another 12 experienced at least local extinction at the onset, 20 were confined to the event, 40 were locally absent during the event but present before and after, and 12 first appeared after the event (*5*).

PETM plant leaf fossils from the Bighorn Basin are almost twice as likely to show insect damage as the average from before and after; one PETM leaf shows 10 different types of damage. Possible reasons include increased insect feeding as higher CO_2 reduced nutritional value of plants, invasion by new insects, and disruption of established ecological balances (*7*). Heat and water stress and loss of soil fertility likely also challenged plants (*5*). Large mammals became notably dwarfed, perhaps because of heat stress or the lower nutritional value of their food (*8*).

In the ocean, the high CO_2 levels during the PETM raised acidity while ocean warming

Department of Geosciences and Earth and Environmental Systems Institute, Pennsylvania State University, University Park, PA, USA. E-mail: rba6@psu.edu

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