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BIOPHYSICS

Rough passage across a barrier

The dynamics of chemical reactions in solution are described by Kramers' theory, but the parameters involved have eluded direct measurement. A study of protein folding reveals how this problem can be overcome.

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Proteins fluctuate between different conformations to perform their sophisticated tasks. The random motion of water molecules around proteins provides an inexhaustible reservoir of thermal 'kicks', which act as the molecular driving forces of such conformational dynamics. Counterintuitively, the same thermal motions of the solvent also limit the speed of biomolecular motion, an effect known as solvent friction. But it has become increasingly clear that, in some important cases, friction within a protein molecule might be the dominant impediment to its molecular dynamics. In a paper published on *Nature*'s website today, Chung and Eaton[1] report one of the most impressive studies so far in which the contribution of such internal friction to dynamics is quantified for protein molecules caught in the act of folding. Remarkably, the results have implications far beyond protein folding.

The rate at which chemical reactions proceed is most commonly conceptualized in terms of a barrier-crossing process. In the simplest case considered in most chemistry textbooks, this barrier might correspond to the energy required to break a single chemical bond in a molecule in the gas phase. The generalized concept of barrier crossing, developed by the Dutch physicist Hans Kramers and published[2] in 1940, can also be applied to much more complex processes[3], including reactions in solution, and even protein folding[4].

The formulation of such a simplified description of reaction kinetics requires two key ingredients: the shape of a suitable 'free-energy surface' that describes the energetic and entropic properties of a system at equilibrium, and the magnitude of the frictional forces that determine how fast the system can move around on its free-energy surface. Any molecule will spend almost all of its time in free-energy valleys (minima of the free-energy surface). In the case of protein folding, these valleys correspond to the folded and the unfolded states (Fig. 1).

The probability of a molecule passing from one valley to another — that is, how frequently the reaction takes place — is dominated by the height of the barrier between the valleys. The most interesting event, however, which contains essentially all of the information about the sequence of molecular steps in the reaction, is the actual crossing of the barrier. The molecules spend only a tiny fraction of their time in this transition-state region, and information about their passage is correspondingly hard to get hold of.

Chung, Eaton and colleagues last year succeeded[5] in measuring these microsecond transition-path times by recording the fluorescence from individual protein molecules and analysing the signal photon by photon, using a clever, previously reported method[6]. They have now taken these investigations a crucial step further by probing the dynamics of a small helical protein in the transition-state area in unprecedented detail.

For protein folding, information about the structural properties of molecules at the top of the barrier has previously been inferred from investigations of how individual changes in a protein's amino-acid sequence affect its folding kinetics[7]. The timescales of barrier crossing have been studied in laser-induced temperature-jump experiments[8] (in which ensembles of molecules are heated by irradiation with a laser) for very small barriers and, more recently, by single-molecule measurements[5,9].

What has remained a daunting challenge, however, is quantifying the key ingredients for a Kramers-like description of protein-folding reactions, especially the role of internal friction and how it changes as folding proceeds. Earlier work has shown that internal friction can be a critical factor in folding kinetics[10,11]; that it can be highly localized to specific regions of the free-energy surface[12]; and that its contribution tends to increase as unfolded proteins become more compact[13]. Now, Chung and Eaton have investigated the nature of the barrier for the folding of individual molecules directly.

The authors measured the transition-path times for a protein (called α_3D) as a function of temperature and solvent viscosity to reveal characteristic signatures of both the solvent and internal friction. The presence of a multitude of simultaneous inter- and intramolecular

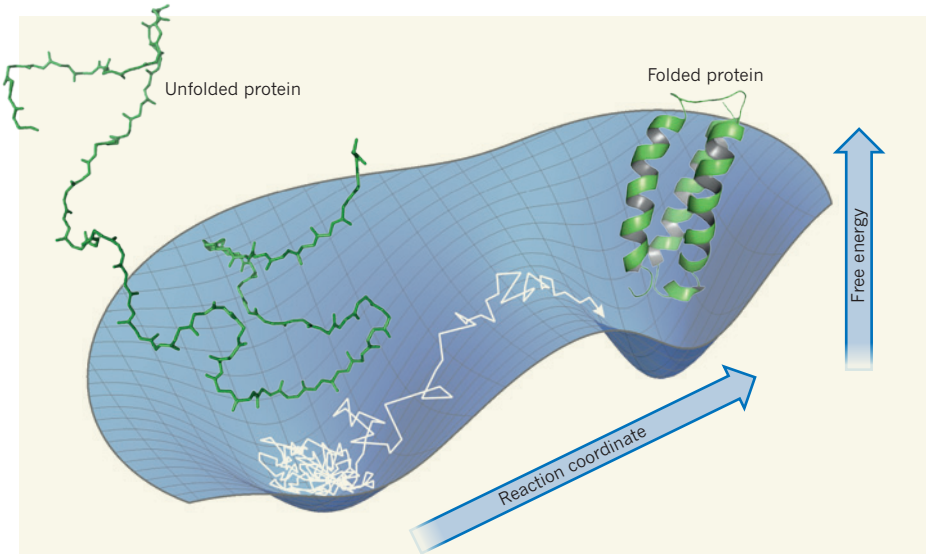

Figure 1 | Barrier crossing in protein folding. Many molecular processes can be described in terms of the diffusion of a particle on a free-energy surface, which depicts how the combined effects of energy and entropy change along a suitably chosen coordinate that represents the progress of a reaction. Here, a protein in its unfolded state corresponds to a basin on a free-energy surface; the protein must cross a free-energy barrier to reach its folded state, which constitutes another basin. The white arrow indicates the diffusive passage of the protein across the surface. Chung and Eaton[1] have used optical single-molecule experiments to probe the dynamics of the process at the top of the barrier.

interactions that slow down barrier crossing might explain why Kramers' theory applies in this case. By contrast, for some reactions of small molecules, barrier crossing can be so rapid that the solvent cannot keep up, and the simple theory fails[3].

Chung and Eaton's results allowed them to estimate the height of the barrier directly — a difficult task in general, because of the large entropic contributions to the folding process, but a fundamental one, because the barrier height is a key determinant of kinetics. To model the shape of the free-energy surface, the authors took advantage of improvements in computational methods that allow simulations of protein folding in atomic detail[14] and that agree remarkably well with experimental folding rates and transition-path times.

Two goals, however, have yet to be achieved: resolving the sequence of events that occur on the top of the barrier directly from single-molecule experiments, rather than from simulations; and understanding the molecular origin of internal friction. It is still unclear whether internal friction is dominated by steric hindrance (clashes of chemical groups) during rotations about the bonds in the polypeptide chain, by the transient formation of intramolecular hydrogen bonds or of clusters of hydrophobic groups, or by other short-lived interactions that must be broken to allow correct folding to proceed[10,12]. However, the convergence of results from sophisticated experiments such as those reported by Chung and Eaton and results from simulations is a promising development, because it will increase our understanding of the detailed mechanisms of biological dynamics at the molecular scale. That will allow us to identify the requirements for applying Kramers' theory, which is widely used for describing dynamic processes in physics and chemistry[3]. ■

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