

# The Energy Landscapes and Motions of Proteins

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**Recent experiments, advances in theory, and analogies to other complex systems such as glasses and spin glasses yield insight into protein dynamics. The basis of the understanding is the observation that the energy landscape is complex: Proteins can assume a large number of nearly isoenergetic conformations (conformational substates). The concepts that emerge from studies of the conformational substates and the motions between them permit a quantitative discussion of one simple reaction, the binding of small ligands such as carbon monoxide to myoglobin.**

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PROTEINS ARE DYNAMIC AND NOT STATIC SYSTEMS (1). Indeed, Weber has characterized proteins as “screaming and kicking” (2). Our purpose in this article is not to prove again that proteins move. Excellent reviews of the experimental evidence exist (3), and results from molecular dynamics computations have been elegantly exposed (4). Rather, we want to show that (i) the “screaming and kicking” is not incomprehensible but that the motions can be characterized and classified, (ii) studies from other “complex” systems such as glasses yield information on how to describe the motions, and (iii) the relation between motions and function is beginning to be understood in some simple situations, such as the binding of small ligands to myoglobin (Mb). Studies of biomolecular dynamics today are in some sense where atomic physics was near 1885. A bewildering variety of protein motions has been revealed by fluorescence spectroscopy, nuclear magnetic resonance (NMR), hydrogen exchange, and Raman scattering. Can regularities be found and connected to the structure of proteins, and can the underlying concepts and laws be discovered? We try to show that some progress has been made.

Spectral lines are transitions between energy levels of atoms or molecules, and protein motions can be described as transitions between conformational substates of the protein. Thus, the characterization and classification of these substates is the first task. Different proteins most likely exhibit different sets of conformational substates, but we believe that the general concepts are likely to be universal. We have selected Mb, the protein that reversibly stores O<sub>2</sub> (5), as prototype. Mb is simple enough that dynamic concepts can be studied in detail and yet sufficiently complex that the concepts discovered may be globally valid.

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Our second goal is important because progress is often made by good use of analogies. Proteins and glasses share many properties. Because glasses are simpler, they can serve as guides to the formulation of concepts and theories. Two important features emerge from the comparison of proteins and glasses: (i) Although it is customary to describe the time dependence of protein reactions and motions by simple exponentials and their temperature dependence by the Arrhenius (transition state) expression, neither of these forms is adequate. Glasses suggest what to substitute. (ii) At the theoretical level many properties of the motions of glasses and proteins can be discussed in terms of the features of rugged energy landscapes, which thus provide a unifying language.

The third goal, the exploration of the relation of motions to function, is the most difficult one to reach. We sketch one case, in which a semiquantitative description of the role of motions in function exists, namely, the binding of small ligands to Mb.

## Conformation and Energy Landscape

Even a monomeric protein as small as Mb can execute a large number of motions, and not all will be coupled to function. Functionally important motions can be studied only if they can be selected. In addition, the various motions must be temporally resolved. Originally, experiments at physiological temperatures suggested that the reaction of Mb + O<sub>2</sub> ↔ MbO<sub>2</sub> was a simple one-step process (5). Low-temperature flash photolysis of MbCO and MbO<sub>2</sub> showed that the rebinding of the ligand to the heme active center was nonexponential in time below ~200 K (6). This observation suggested that Mb did not have a single structure but could assume a large number of slightly different structures, each with a different rebinding rate. Because Mb has two globally distinct macrostates, ligand-bound (MbCO or MbO<sub>2</sub>) and unbound (Mb), and there is a spectrum of conformations in either state, these microstates are called “conformational substates (CS)” (7). The cryochemical experiments reveal that a protein in a given state can assume a large number of CS, which form the scaffold for protein motions.

The organization of the CS in MbCO as presently known can be visualized as in Fig. 1 (7, 8). The energy landscape describes the potential energy  $E_c$  of the protein as a function of conformational coordinates; it is a hypersurface in the high-dimensional space of the coordinates of all atoms in Mb. The energy landscape (Fig. 1) has structure on several energy and length scales as illustrated by different one-dimensional cross sections through it. Figure 1 implies that the CS can be roughly classified into a hierarchy (8), where CS<sub>*i*</sub> denotes the substates in the *i*th tier in the organization. The top row of Fig. 1 depicts MbCO in the conventional conception with a unique structure corresponding to a unique energy valley. A hint that MbCO is not as simple comes from infrared (IR) spectra: The bound CO molecules display multiple stretch bands (9). Each band

can be identified with a distinct substate of tier 0. At least three such substates exist,  $A_0$ ,  $A_1$ , and  $A_3$  (10). They have the same primary amino acid sequence but differ, at least, in the geometry of the bound CO as shown in the second row left of Fig. 1 (11). The relative energies, entropies, and volumes for each of these CS0 are known (12, 13). Because the CS0 are distinct and few in number, they can be characterized individually and a taxonomic approach is appropriate.

The CS0 for MbCO are functionally important, as each binds CO with different rates (10).  $A_0$  is the fastest and  $A_3$  the slowest (Fig. 2A). Thus, dynamic biological control may be exerted by switching from one CS0 to another.

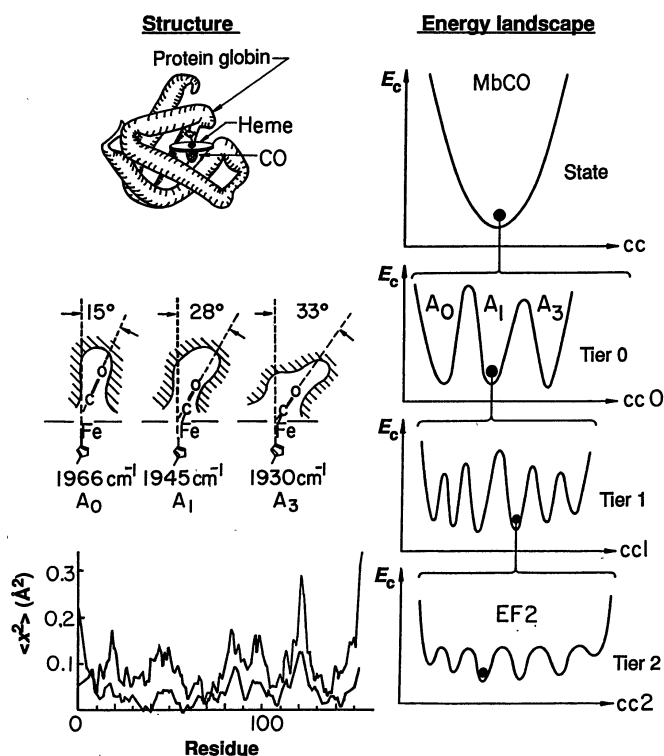
The existence of a second tier of substates, CS1, in each of the preceding CS0 is shown by the inhomogeneous broadening of the individual stretch bands (14) and by the nonexponential time dependence of the CO rebinding (Fig. 2A). This tier of substates may also contribute significantly to the fluctuations in the positions of individual atoms around their averages. These fluctuations are characterized by the mean-square deviations,  $\langle x^2 \rangle$ , derived from the Debye-Waller factor in x-ray diffraction (7, 15). A plot of  $\langle x^2 \rangle$  versus residue number is shown in Fig. 1. In order to interpret Fig. 2A in terms of the substates of tier 1, we note that the rebinding of the CO ligand from the pocket of the protein to the heme iron is determined by an activation barrier of height  $H$  and assume that the rate coefficient  $k(H)$  follows the Arrhenius equation  $k(H) = A \exp(-H/$

$RT)$ , where  $A$  is the preexponential factor,  $R$  is the gas constant, and  $T$  is the temperature. The time dependence of rebinding in a CS with a barrier height  $H$  is given by  $\exp[-k(H)t]$ . Proteins in different CS1 are postulated to have different barrier heights. The probability of finding a protein with a barrier height between  $H$  and  $H + dH$  is denoted by  $g(H)dH$ . The rebinding to an ensemble of proteins in a given CS0 is thus described by

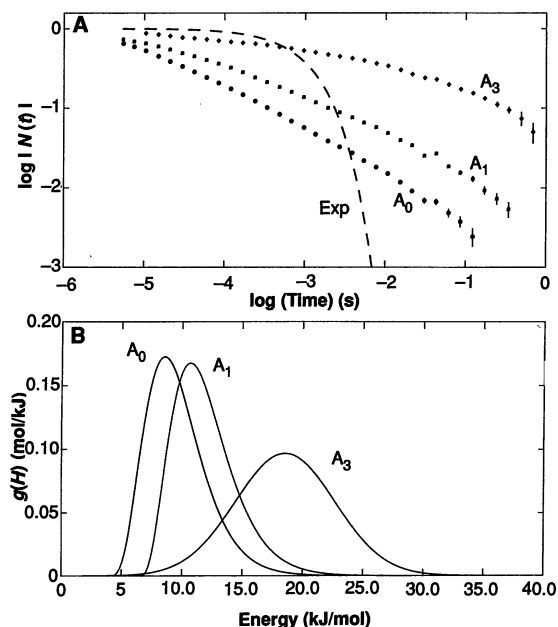
$$N(t) = \int dH g(H) \exp[-k(H)t] \quad (1)$$

where  $N(t)$  is the fraction of proteins that have not rebound a CO ligand at time  $t$  after photodissociation (6). The distribution functions  $g(H)$  derived from the experimental curves in Fig. 2A for substates  $A_0$ ,  $A_1$ , and  $A_3$  are shown in Fig. 2B. The smoothness of the rebinding curves (Fig. 2A) suggests that the number of CS1 for each CS0 is large. Thus a taxonomic approach is impractical and perhaps impossible, in principle. The substates must be described statistically with the use of distributions of the relevant quantities.

Evidence for further branching of the energy landscape comes from "hole burning" experiments (16). Spectral lines in complex systems are inhomogeneously broadened: Proteins in different substates produce lines at slightly different wavelengths. With a laser, a hole can be burned into such a line. At very low temperatures, the holes are persistent and slow refilling can be monitored. Such relaxation experiments provide the evidence for substates of lower tiers. These substates may also be responsible for the linear specific heat of proteins below 1 K (17). The experiments and computer simulations together show that conformational substates with different structural and dynamic properties exist and that they may be organized into a coarse hierarchy. In the top tier (CS0) a taxonomic approach is fruitful, but for lower tiers a statistical viewpoint is needed. The taxonomic attitude is the conventional one of mechanistic chemistry, but the statistical viewpoint requires new concepts. One no longer talks about specific energy levels but about



**Fig. 1.** Structure and conformational energy landscape of MbCO. The top row shows (left) the coarse structure of MbCO and (right) an overall picture of the conformational energy  $E_c$  as a function of a conformational coordinate,  $cc$ . The second row right depicts three substates of tier 0. The three CS0 have different orientation of the bound CO with respect to the heme; possess different enthalpies, entropies, and volumes; and are separated by high barriers. The third row left shows the mean-square deviations for MbCO at 300 K (upper curve) and 80 K (lower curve) (15). The plot of  $E_c$  versus  $cc1$  for tier 1 is vastly oversimplified. In reality,  $E_c$  is a hypersurface in a conformational space of very high dimensions and the number of valleys is extremely large. Valleys and barriers can no longer be characterized individually but must be described by distributions. The same remark applies to lower tiers, such as CS2.



**Fig. 2.** Rebinding of CO to Mb after photodissociation, measured separately for the substates of tier 0 at pH 5.7. (A)  $N(t)$  is the fraction of proteins that have not rebound a CO at the time  $t$  after photodissociation. All three substates ( $A_0$ ,  $A_1$ , and  $A_3$ ) rebound nonexponentially in time. (B) The activation enthalpy spectra, defined through Eq. 1.

the statistics of the energy landscape. This perspective parallels the description of terrestrial landscapes where the higher peaks and deeper valleys are worthy of separate names, but where less specific terms such as “roughness” or “difficulty” characterize the myriads of smaller scale features.

A fruitful source of ideas for analyzing statistically defined energy landscapes may be found in studies of glasses (18) and spin glasses (19). Amorphous systems share features with proteins (20). Unlike nonbiological amorphous systems, however, the energy landscape of a protein can be changed with surgical precision through site-directed mutagenesis (21). Thus, the relation of the energy landscape to function and protein evolution can be elucidated.

Molecular dynamics calculations provide strong support for the concept of conformational substates (22). Unfortunately, the presently accessible time scales of computation do not allow a complete sampling of protein substates and infrequent transitions between substates are a major source of statistical error in current molecular dynamics simulations.

## Conformational Motions

At any given instant, an individual protein molecule is in a specific conformational substate. It usually does not stay there but hops to other substates and explores the energy landscape. This exploration depends critically on temperature. At low temperatures, each protein remains frozen in a particular CS. At room temperature, however, the protein moves through the CS. The motion can be described through analogies with nonbiological systems, such as glasses (18, 23) and spin glasses (18). It is useful to contrast two types of motion, relaxation processes and equilibrium fluctuations. In a relaxation process, the system moves from a nonequilibrium state toward equilibrium. The nonequilibrium state can be created by a chemical reaction or a temperature or pressure jump. The equilibrium fluctuations occur even in quiescent molecules and lead from one CS to another. Both types of motion are related by fluctuation-dissipation theorems (24).

In order to describe the main characteristics of relaxations, we assume that the sample has been brought rapidly to a nonequilibrium state. One can monitor the subsequent relaxation toward equilibrium by measuring an observable  $M(t, T)$  as a function of time  $t$ , and temperature  $T$ .  $M(t, T)$  can, for instance, be the position, width, or intensity of a suitably chosen spectral line. The motion is then characterized by the relaxation function  $\Phi(t, T)$ , defined by

$$\Phi(t, T) = [M(t, T) - M_{\text{eq}}(T)] / [M(0, T) - M_{\text{eq}}(T)] \quad (2)$$

Here  $M(0, T)$  is the value of  $M(t, T)$  immediately after the jump and  $M_{\text{eq}}(T)$  is the value after the new equilibrium has been reached. For simple systems,  $\Phi(t, T)$  is usually exponential in time,  $\Phi(t, T) = \exp[-k(T)t]$ , and the rate coefficient  $k(T)$  satisfies an Arrhenius relation, which indicates the existence of a single relevant energy scale. In glasses and proteins, the complexity of the system is reflected by more complicated time and temperature dependencies. The binding of CO to Mb (Fig. 2A) provides a clear example of a nonexponential time dependence, but  $k(T)$  still satisfies an Arrhenius relation. Conformational motions, however, usually show complex time and temperature behavior. The time dependence can often be approximated by a stretched exponential (25):

$$\Phi(t, T) = \exp\{-[k(T)t]^\beta\} \quad (3)$$

In glasses, the exponent  $\beta$  is often  $\sim 0.3$  and the relaxation function then extends over many orders of magnitude in time.

The temperature dependence of  $k(T)$  provides another signal of complexity. Fits to  $k(T)$  with an Arrhenius relation are good only

locally, and the effective activation energy increases strongly with decreasing temperature. In addition, the preexponential factors, when force-fitted with an Arrhenius law, can become much larger than  $10^{13} \text{ s}^{-1}$  and hence make little sense as the frequencies of atomic motions. Two different empirical relations describe  $k(T)$  in glasses well. One is a relation introduced by Ferry *et al.* (26)

$$k(T) = A \exp[-(E/RT)^2] \quad (4)$$

and the other is the better known Vogel-Tammann-Fulcher expression (23)

$$k(T) = A \exp[-C/(T - T_0)] \quad (5)$$

Either relation can be used to characterize the conformational motions in proteins.

In glasses, different probes reveal two types of motions, denoted as  $\alpha$  and  $\beta$  relaxation (23). The  $\alpha$  relaxation, usually characterized by Eqs. 3 and either 4 or 5, involves large-scale motions. The  $\beta$  relaxation is typically closer to an Arrhenius temperature dependence and is attributed to motions of local regions. In proteins (12, 20), the motions operating in tiers 0 and 1 appear to involve large segments of the protein structure and are similar to the  $\alpha$  relaxation in glasses; motions in lower tiers may involve only local regions.

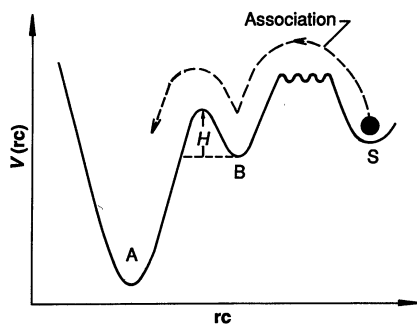
At this point, the reader may ask two questions: Why was the Arrhenius relation used with Eq. 1 whereas Eqs. 4 and 5 are introduced here? And, why was the nonexponential time dependence shown in Fig. 2A characterized by the distribution  $g(H)$  whereas it is now described by a stretched exponential? The answers to both questions follow from the different phenomena that are treated. The binding of CO to the heme iron at low temperatures involves mainly the iron and the CO, and the Arrhenius relation is adequate. Relaxation phenomena usually are collective phenomena involving many atoms, and Eqs. 4 or 5 are appropriate. The nonexponential time dependence shown in Fig. 2A is caused by the fact that different Mb molecules are in different CS, with different activation barriers, as proven by multiple flash experiments (6). Equation 1 is therefore applicable. The nonexponential time dependence in relaxation phenomena is caused by their collective character and occurs presumably even in a single protein.

## Dynamics and Function

Protein motions permit ligands such as  $\text{O}_2$  to enter and leave Mb or hemoglobin, substrates to reach the active center in enzymes, and catalytic groups to come together (27). We are still far from a quantitative treatment of the role of motions in function, and no generally applicable theory exists. Nevertheless, models permit us to describe the main features of some reactions. We treat below the binding of CO to Mb after photodissociation to show that a simple model together with the relations introduced in the previous section describe the observed features over a wide temperature range and that motions are essential in this model (28, 29).

Ligand binding can be described with the reaction surface shown in Fig. 3. Here A denotes the bound state with the CO as a ligand to the heme iron, B the state with CO in the heme pocket, and S the state with CO in the solvent. In photodissociation, the system starts with the ligand bound (Fig. 1) and the incident photon is absorbed by the heme, which results in the rupture of the iron-ligand bond. The ligand moves into the pocket B and then either rebinds ( $B \rightarrow A$ ) or escapes into the solvent ( $B \rightarrow S$ ) (6, 30). The reaction surface  $V(\text{rc})$  is a cartoon of the actual many-dimensional situation in which one reaction coordinate, rc, is singled out. Its barriers are affected by all of the other coordinates of the protein. At low temperatures, where the protein motions are essentially absent, the barriers are

**Fig. 3.** The reaction energy surface for the binding of CO to Mb. Whereas Fig. 1 represents the conformational energy of the protein as a function of conformational coordinates, Fig. 3 shows the energy of the system Mb + CO as a function of the position of the CO for a given and fixed conformational substate. Different CS show the same general  $V(rc)$ , but with different barrier heights.



time- and temperature-independent and the reaction surface is static. At higher temperatures, the conformational energy landscape of Fig. 1 remains temperature-independent, but the motions between substates affect the reaction surface and cause it to be time- and temperature-dependent. Two dynamic effects are seen experimentally and in computer simulations: (i) The effective barrier between the pocket and the heme iron increases with time after dissociation (29, 31, 32), and (ii) the barrier between the pocket and the solvent decreases with increasing temperature (30).

We consider first the reaction at the heme iron. The main steps in the dissociation process are sketched in Fig. 4. Before dissociation the heme is planar with the iron in the plane and the F helix of the protein in its bound-state structure (Fig. 4A). Within 300 fs after photodissociation, the heme domes and twists and is close to the deoxy structure (28, 33). The protein is in a nonequilibrium structure, denoted by Mb\* (Fig. 4B). Below about 160 K, direct rebinding of CO to this metastable state can be observed (6, 10). Rebinding is nonexponential in time and must be described by a distribution in activation enthalpies,  $g(H)$  (Fig. 2). From 40 to 160 K the rebinding data are fit very well by Eq. 1 with a temperature-independent distribution  $g(H)$ .

With increasing temperature, the conformational motions speed up and, above 160 K, Mb\* relaxes toward Mb and the iron moves farther out of plane (Fig. 4C). Rebinding of CO to the relaxed Mb state requires significant structural rearrangements, and  $H$  increases. Such an increase was predicted by Agmon and Hopfield (31). Experimentally, the entire distribution shifts and a barrier of initial height  $H_0$  increases in time according to

$$H(t, T) = H_0 + \Delta H[1 - \Phi^*(t, T)] \quad (6)$$

The relaxation function  $\Phi^*(t, T)$  describes how the barrier  $H(t, T)$  changes as a function of time and temperature, and  $\Delta H$  is the maximum shift. Equations 1 and 6 together permit calculation of the rebinding function  $N(t)$  if  $\Phi^*(t, T)$  is known. If the time dependence of  $\Phi^*(t, T)$  is assumed to be a simple exponential, the data cannot be reproduced. However, a stretched exponential (Eq. 3) with  $\beta = 0.24$  fits the data surprisingly well and gives  $\Delta H \approx 11$  kJ/mol. The relaxation Mb\*  $\rightarrow$  Mb consequently shifts the peak of the distributions shown in Fig. 2 by about 11 kJ/mol, and the shift occurs in a manner reminiscent of glass relaxations. Significantly,  $\Phi^*(t, T)$  and  $\Delta H$  agree with data from spectral shifts measured independently (29).

The maximal shift  $\Delta H \approx 11$  kJ/mol implies that at room temperature CO binding from the heme pocket is about 200 times faster in Mb\* than in Mb. A similar difference is observed in hemoglobin, which can assume two states, R and T. CO binds considerably faster in the R than in the T state. Moreover, extended x-ray absorption fine structure measurements imply that the iron is closer to the heme plane in the R than in the T state (34). Mb\*, where the iron has not yet moved fully out of the heme plane,

consequently is a model for the R state of hemoglobin.

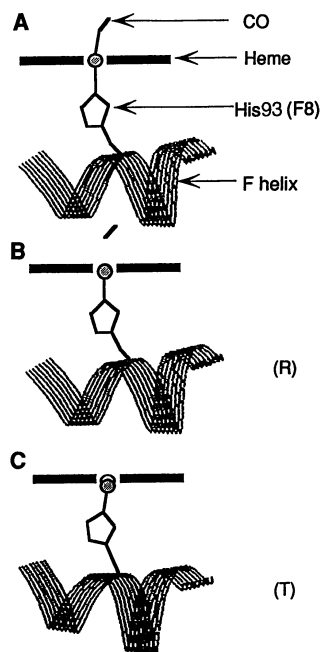
Motions are also critical to the entry and exit step from and to the solvent ( $B \leftrightarrow S$ ). Well below 200 K, all large-scale protein motions are frozen, and the barrier between B and S is so high that ligands in the solvent cannot enter, and any in the pocket cannot escape. Above 200 K, large-scale equilibrium fluctuations open channels to the pocket. Entry is a dynamic dance through a fluctuating gate.

The various motions that are responsible for Mb function occur with different rates and can be tentatively assigned to the different tiers of conformational substates shown in Fig. 1. The relaxation Mb\*  $\rightarrow$  Mb may be due to motions in tier 2. The fluctuations responsible for the opening of pathways through the protein matrix can be assigned to tier 1. The exchange among conformational substates CS0 is involved in the control of ligand binding at the heme and by definition occurs in tier 0 (35).

In this section we have analyzed the effects of motions on the rebinding kinetics of MbCO. Additional information can be obtained from changes of spectral bands that accompany the relaxation phenomena, for instance, from studies of the time dependence of circular dichroism after photodissociation of MbCO (36). Various spectroscopic studies of relaxation after photodissociation have been performed on hemoglobin (37), but much more work will be necessary to explore in depth the connections among structure, function, and spectroscopic markers.

## The Theoretical Underpinning

Equations 3 through 5 are empirical relations that form a consistent frame for analyzing experimental data in protein dynamics. For a deeper understanding, however, it is desirable to appreciate the theoretical justification of these relations and to connect them to the characteristics of the energy landscape. The system most studied theoretically that has a rugged energy landscape is the spin glass (19). Spin glasses are solids with sites that have discrete variables associated with them that can be modeled as spins. An example is a dilute alloy of iron in gold. The interactions between the magnetic moments of the iron atoms favor parallel alignment at some distances and antiparallel alignment at others. Thus, the spins



**Fig. 4.** Sketch of the heme group, the proximal histidine F8, and the CO molecule at various stages. (A) The bound state MbCO. (B) The protein about 300 fs after photodissociation. The iron has moved partially out of the heme plane, but most of the protein has not yet relaxed to the deoxy structure. We hypothesize that this structure, denoted by Mb\*, is a model for the R state of hemoglobin. (C) The protein has fully relaxed to the deoxy structure, and we compare this state to the T state of hemoglobin. The out-of-plane motion of the iron atom and the shift of the F helix are exaggerated.

interact in a pairwise fashion in a random and conflicting way that makes it difficult to determine the lowest energy configuration. The difficulty in satisfying the conflicting interactions leads to a complex energy landscape. The technical term for this phenomenon is "frustration." In folded proteins, the frustrated degrees of freedom are side-chain conformations and the conflicting interactions are thought to arise from local steric and long-range interactions (38). The existence of multiple side chain conformations is well established from x-ray diffraction and NMR studies. Although distributions of barrier heights could be accommodated by a model in which these side chains do not interact with each other, the other relaxation features cannot. Support for this model comes from computer simulations that reveal both local and global displacements on motion between substates (22). Before a protein is completely folded, the backbone degrees of freedom are also relevant and a rugged energy landscape can result from inappropriate associations between side chains. This phenomenon is the basis of spin glass models of protein folding (39-41). In folding it is also crucial to take into account the smooth, correlated minimally frustrated part of the energy landscape that gives quick routes to the folded structure (39).

No matter what the atomic basis, spin glass theory shows that the deeper minima on complex energy landscapes can be grouped into families and that generically there is little in common between members of different families (42). At low resolution the deepest minima thus can be modeled as having uncorrelated random free energies (43). The stretched exponential function arises naturally from the dynamics of hopping between different minima of such an energy landscape (44). Non-Arrhenius temperature dependence also follows from this simplest assumption for a statistical energy landscape both for one-dimensional (1-D) (45) and for many-dimensional energy landscapes (41). A statistical energy landscape leads to the Ferry law, Eq. 4, in the 1-D case (45) and to a modified Ferry law in the  $n$ -D case (41). The increase in apparent activation energy with decreasing temperature arises because at lower temperatures the deeper minima are more likely to be occupied. The energy  $E$  in Eq. 4 is related to the roughness of the energy landscape and in the  $n$ -D case can be further related to the entropy of the "basins of attraction." Although the experimentally observed glass transitions depend on observation time scale, spin glass models exhibit ideal glass transitions in which freezing into low-energy states is thermodynamically obligatory. Dynamics at this point is limited by the search through all of the basins of attraction, just as in Levinthal's famous protein folding paradox where the search through all protein conformations would take cosmological times (46).

The universal nature of the deep parts of the energy landscape is only understood, however, where the range of the interactions is comparable to the size of the system. This condition may be satisfied in proteins, where there are rigid elements such as the  $\alpha$  helices, but for ordinary glasses it is clear that, whatever the conflicting interactions, the effects of the finite range of interactions must be understood. If an ideal glass transition is assumed, Eq. 5 results from a scaling law based on the hypothesis that the underlying glass transition is a random first-order transition that seems to arise generically for spin glasses lacking special symmetries (39, 47). These arguments are modern incarnations of ideas first bruited for polymer transitions (48).

## A Dynamic Future

The history of physics and chemistry shows that a detailed knowledge of the energy levels and the laws governing them is crucial for progress based on a quantitative understanding. Some progress in exploring the energy landscape of Mb has been made

(Fig. 1), but the details and the overall organization are seen only dimly. Many tools are needed to obtain a more complete map of the landscape, and studies must be extended to other biomolecules. The dynamic laws used so far and expressed in Eqs. 3 to 5 are only approximations to the behavior of systems with complex energy landscapes, and more complete expressions are needed.

Although the complete theory of dynamics on complex energy landscapes is still under development, it is important to realize that the analysis embodied by the empirical equations does not imply a more complicated picture of proteins than is absolutely necessary. Indeed, the universality of the glass transition in noncrystalline substances suggests that a complex energy landscape is unavoidable. The complexity of most biological macromolecules demands at least this level of description. The experiments on Mb only provide one clear-cut example. The complexity, expressed empirically through nonexponential time dependences, non-Arrhenius temperature dependences, and multiple relaxation processes, becomes apparent only if proteins are studied over very wide parameter ranges. Experiments performed over narrow time and temperature ranges can be fit to simplified models with simple energy landscapes, but the apparent simplicity reflects merely the lack of information.

Where the dynamics of biomolecules goes far beyond similar problems in physical systems is the connection between dynamics and function and between dynamics and evolution. Evolution occurs through changes in the primary sequence of the proteins, which leads to changes in the structure and the conformational energy landscape. Because of the diversity of the conformational substates, one can see mutations as acting to change some substates more than others. If there is selection pressure, it may be useful to dig a few deeper wells that are more separated than most from the near-continuum of energy states. In this way, allosteric regulation and specific reaction pathways can evolve through energy landscaping. This phenomenon is analogous to the adaptation of neural networks by progressive synaptic modifications (49). The energy landscape perspective may be useful in the planning of genetic engineering approaches because it leads one to think about trying to make many small changes to the energy landscape rather than seeking a few single-site mutations with dramatic effects (50).

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# Perceived Risk, Trust, and the Politics of Nuclear Waste

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The Department of Energy's program for disposing of high-level radioactive wastes has been impeded by overwhelming political opposition fueled by public perceptions of risk. Analysis of these perceptions shows them to be deeply rooted in images of fear and dread that have been present since the discovery of radioactivity. The development and use of nuclear weapons linked these

images to reality and the mishandling of radioactive wastes from the nation's military weapons facilities has contributed toward creating a profound state of distrust that cannot be erased quickly or easily. Postponing the permanent repository and employing dry-cask storage of wastes on site would provide the time necessary for difficult social and political issues to be resolved.

BY THE YEAR 2000, THE UNITED STATES WILL HAVE A projected 40,000 metric tons of spent nuclear fuel stored at some 70 sites and awaiting disposal. By 2035, after all existing nuclear plants have completed 40 years of operation, there will be approximately 85,000 metric tons (1). The U.S. Department of Energy (DOE) has been under intense pressure from Congress and the nuclear industry to dispose of this accumulating volume of high-level waste since the passage of the Nuclear Waste Policy Act in 1982 and its amendment in 1987, by which Yucca Mountain, Nevada, was selected as the only candidate site for the nation's first

nuclear waste repository. The lack of a suitable solution to the waste problem is widely viewed as an obstacle to further development of nuclear power and a threat to the continued operation of existing reactors, besides being a safety hazard in its own right.

Yet, at this time, the DOE program has been brought nearly to a halt by overwhelming political opposition, fueled by perceptions of the public that the risks are immense (2–7). These perceptions stand in stark contrast to the prevailing view of the technical community, which argues that nuclear wastes can be disposed of safely, in deep underground isolation (8–10). Officials from DOE, the nuclear industry, and their technical experts are profoundly puzzled, frustrated, and disturbed by public and political opposition that many of them consider to be based on irrationality and ignorance. Lewis, for example, argued that the risk

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