research is the possible role of protein dynamics in aiding the reacting species in crossing the transition-state barrier to the reaction. As originally formulated, the structure of the enzyme was proposed to favor atomic vibrations along the reaction coordinate while disfavoring those that would not lead to productive bond-making or bond-breaking steps (*18*). Recent evidence from different enzyme systems suggests that this factor may indeed contribute to catalytic efficiency (*19*, *20*).

Given that we now have a good understanding of the principles underlying enzyme catalytic proficiency and specificity, it seems appropriate to ask where the field is likely to go next. Practical applications, such as the creation of enzymes catalyzing novel reactions, are under way. Further investigations into the role of protein dynamics in enzymatic catalysis are still needed. But we believe that a crucial next step will be to go beyond the milieu of dilute aqueous solution and individual purified enzymes that has defined enzymology for the past 100 years. Most enzymes function in the interior of the cell, where the substrate concentration is typically very low and the protein concentration may exceed 100 mM. How do enzymes function in a crowded medium of low water activity, where there may be no such thing as a freely diffusing, isolated protein molecule? In vivo enzymology is the logical next step along the road that Phillips, Koshland, and their predecessors and successors have traveled so brilliantly so far.

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21. We dedicate this paper to the memory of our good friend and long-time collaborator Jeremy R. Knowles.

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BIOCHEMISTRY

How Do Proteins Interact?

New results provide support for the hypothesis that interactions between proteins involve selection from an ensemble of different conformations.

David D. Boehr and Peter E. Wright

Interactions between proteins are central to biology and are becoming increasingly important targets for drug design. Upon forming complexes, protein conformations usually change substantially compared to the unbound protein. Two main hypotheses have been advanced to explain these changes (see the figure). According to the "induced fit" hypothesis, the initial interaction between a protein and a binding partner induces a conformational change in the protein through a stepwise process (*1*). In the "conformational selection" model, it is assumed that, prior to the binding interaction, the unliganded protein exists as an ensemble of conformations in dynamic equilibrium. The binding partner interacts preferentially with a weakly populated, higher-energy conformation-causing the equilibrium to shift in favor of the selected conformation. This conformation then becomes the major conformation in the complex (*2*). Although biochemistry textbooks have championed the induced fit mechanism for more than 50 years, there is now growing support for the additional binding mechanism, including the seminal work by Lange, Lakomek, and co-workers on page 1471 of this issue (*3*).

A major stumbling block for the conformational selection hypothesis has been the inability to characterize the structures of the predicted multiple conformations (or conformational substates) of a protein. The structural models resulting from x-ray crystallography tend to identify only a single dominant conformation, although different crystal forms of the same protein can provide insights into the range of conformations accessible to the protein (*4*). Help comes from nuclear magnetic resonance (NMR), a powerful method for characterizing protein dynamics and the protein conformational ensemble at the atomic level. Various NMR observables (*5*, *6*) give structural information about lowly populated, higher-energy conformations that are invisible to other techniques.

In a previous report, Vendruscolo and co-workers (*7*) combined data from NMR relaxation experiments with molecular dynamics simulations to characterize a structural ensemble of the protein ubiquitin. However, the experimental data only covered nanosecond time-scale dynamics and thus failed to capture the slower time scales that are important for molecular recognition.

Lange *et al.* have now extended the methodology to slower time scales by using residual dipolar couplings (RDCs) (*3*), which serve as restraints for structural determination by NMR and also provide dynamic information over a wide range of time scales (*8*). By analyzing RDCs measured for a large range of solution conditions, Lange *et al*. construct a structural ensemble for ubiquitin that describes its dynamic behavior up to the microsecond time scale.

The most striking feature of the ensemble is the presence of conformations that are nearly identical to the 46 known bound forms of ubiquitin observed in x-ray crystal structures. The results provide very strong evidence that complex formation by ubiquitin involves conformational selection processes. Gsponer *et al.* recently reported a similar result for calmodulin. Using the methodology of Vendruscolo and co-workers, they showed that the nanosecond ensemble for apocalmodulin contains conformations similar to calmodulin bound to myosin light chain kinase (*9*).

The structural ensemble reported by Lange *et al.* is consistent with the energy landscape theory of protein folding and function (*2*, *10*, *11*). This theory posits that there are multiple protein conformations in dynamic equilib-

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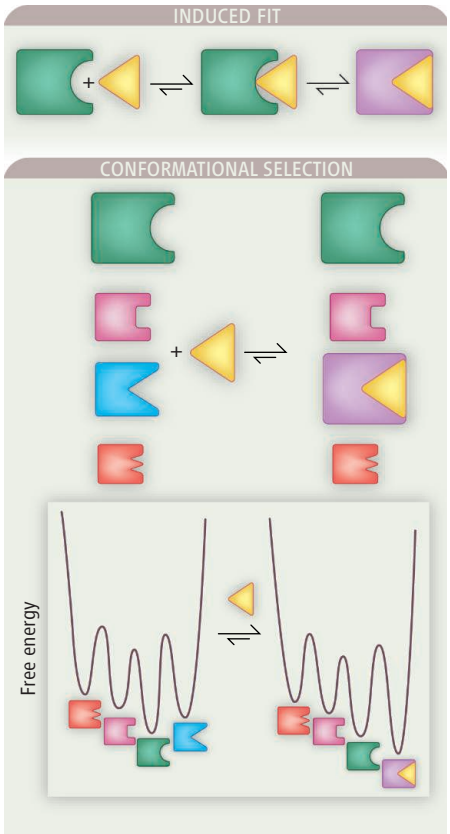

Molecular recognition mechanisms in proteins. Induced fit (**top**) assumes an initial interaction between a protein and its binding partner, followed by conformational changes that act to optimize the interaction. In conformational selection (**bottom**), a weakly populated, higher-energy conformation interacts with the binding partner, stabilizing the complex. Relative populations of conformations are indicated by size. In the structural ensemble presented by Lange *et al*., different conformations may interact with distinct protein-binding partners. The energy diagram depicted is the simplest case; binding partners may have affinity for a number of protein substates that would further modify the structural energy landscape.

rium, with populations that depend on their relative free energies. Changes in the protein environment—such as a binding event—will alter the relative populations of the substates in the conformational ensemble (see the figure). In this context, induced fit and conformational selection are two extremes of a spectrum of possible protein binding mechanisms that can be categorized based on the initial binding interaction and the resulting structural changes in the energy landscape.

Indeed, a large body of structural work supports induced fit mechanisms (*12*), and kinetic signatures for both induced fit and conformational selection have been observed, sometimes in the same system (*13*–*15*). In a model that combines both mechanisms, the interaction proceeds through three steps: a diffusional encounter, recognition of complementary structures contained within the conformational ensembles of the free proteins, and conformational relaxation into the final bound state (*16*). As noted by Lange *et al.*, their results only characterize protein backbone structure and dynamics, and it is possible that minor backbone conformational changes or rotameric rearrangements of side chains may be induced after the initial interaction with a protein binding partner.

The analysis by Lange *et al*. provides much structural insight into the conformational ensemble of ubiquitin, but a more complete picture of the energy landscape would require more detailed kinetic and thermodynamic information. What are the relative populations of the individual structures and the rate constants of exchange among the substates in the conformational ensemble? What is the nature of the thermodynamic barriers between conformations? The information gained about the conformational ensemble can be compared with a careful kinetic analysis of ubiquitin binding interactions to provide us with a richer understanding of the diversity of protein-protein binding mechanisms.

The findings by Lange *et al.* (*3*) also pose intriguing questions about the role of dynamics in protein evolution (*17*). Either the structural fluctuations of ubiquitin evolved to interact with various protein binding partners, or new binding interactions took advantage of the intrinsic protein dynamics. The second case would help facilitate new binding interactions without compromising the structural integrity and original function of the protein. Analysis of structural ensembles populated on time scales slower than molecular tumbling, as begun by Lange *et al.*, will lead to a better understanding of evolution at the molecular level and may provide new approaches to protein engineering and drug design.

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DEVELOPMENTAL BIOLOGY

Sex and Poison in the Dark

Reinhard Fischer

A protein complex moves in and out of the nucleus in response to light, associating with proteins that control fungal development and metabolism.

Filamentous fungi are very successful organisms on our planet because of their metabolic versatility and potential to adapt to and survive extreme conditions. In this context, one important feature is their ability to produce different types of spores, for their dissemination in the environment and for resisting harsh conditions (*1*, *2*). Another factor is their success in chemical warfare—fungi produce molecules that help them to compete with other microorganisms (*2*). The best-known of these compounds are antibiotics, which can benefit one microorganism by inhibiting the growth of others. On the other hand, several other fungal metabolites, such as mycotoxins, cause millions of dollars in losses every year due to contaminated food and animal feed. If ingested by humans, mycotoxins, such as aflatoxin, may cause cancer or even death. Most interestingly, the phenomena of spore development and secondary metabolism are genetically linked (*3*). On page 1504 of this issue, Bayram *et al*. (*4*) unravel this association at a molecular level in the model fungus *Aspergillus nidulans* and show how this connection is controlled by light.

Most research with the filamentous fungus *A. nidulans* involves a strain in which the

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