PERSPECTIVES: STRUCTURAL BIOLOGY

Flipping a Switch

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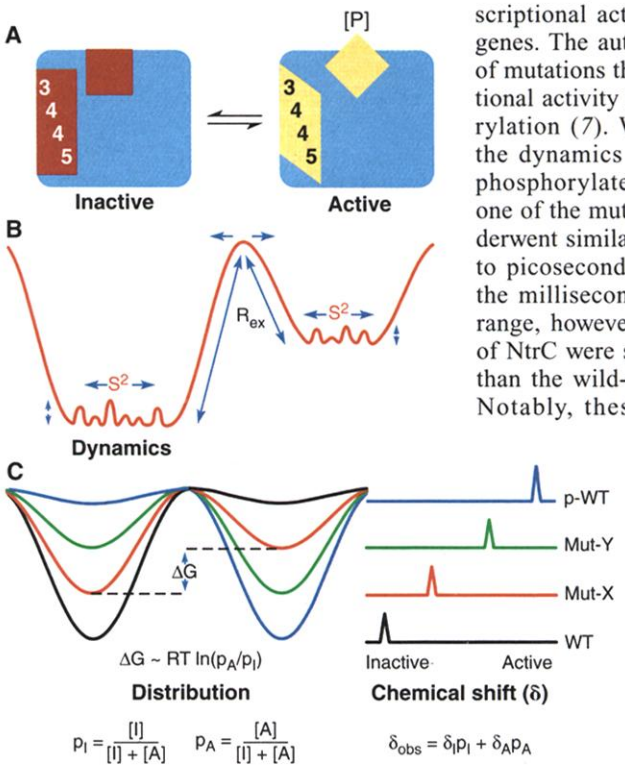
Structures of biological macromolecules are often presented as static images, yet biophysical studies have established that proteins and nucleic acids are in constant motion. They move with frequencies ranging from several fluctuations per picosecond to a few every hour or even one every few weeks. Corresponding to these time scales, the amplitude of motions ranges from tiny vibrations of covalent bonds to large-scale reorientations of domains and global unfolding. Macromolecular dynamics are essential for the binding and release of ligands by receptors, as well as for the catalytic activity of enzymes (*1, 2*). The motions of a protein, like its biological activity, are determined by its structure. But assessing the interrelationships among a protein's motions, activity, and structure has proven problematic because, experimentally, it is often difficult to observe all three characteristics simultaneously.

Nuclear magnetic resonance (NMR) spectroscopy is an extremely powerful tool for characterizing the structures of proteins and their motions (transitions between conformational states), over a range of time scales and at atomic resolution (*3, 4*) (see the figure). However, the dynamic data measured by NMR can be difficult to interpret, in part because particular structures (and their biochemical activities) often cannot be ascribed to different interconverting states. Furthermore, a protein's rate of interconversion between different states is difficult to manipulate experimentally in a manner that does not affect its activity. Such complexities mean that only correlations can be drawn between the dynamics and activity of a protein. On page 2429 of this issue, Volkman *et al.* (*5*) use NMR to correlate the structural states of a protein and its interconversion dynamics directly with biochemical activity. With their results, they develop a model for the regulation of nitrogen regulatory protein C (NtrC) of bacteria, an important signaling molecule. Their study, along with those of other groups, demonstrates that NMR measurements of protein structure and dynamics must be integrated with protein activity data to unlock the details of functional motion.

The response regulator NtrC is a member of the bacterial two-component family of signaling molecules that control gene expression, chemotaxis, and many other processes. A two-component signaling pathway consists of a highly conserved histidine kinase and a response regulator, a molecular switch that alternates between an inactive unphosphorylated and an active phosphorylated state. The comparison of NMR solution structures of NtrC in both states shows that phosphorylation causes displacement of several structural elements resulting in exposure of a hydrophobic surface on one side of the molecule, the so-called "3445" face (*6*). These changes are thought to enable NtrC dimers to bind to each other, forming oligomers with transcriptional activity that switch on target genes. The authors also describe a series of mutations that lead to partial transcriptional activity in the absence of phosphorylation (*7*). With NMR they measured the dynamics of unphosphorylated and phosphorylated wild-type NtrC, and of one of the mutants, and found that all underwent similar motions on a nanosecond to picosecond (ns-ps) time scale (*5*). In the millisecond to microsecond (ms-μs) range, however, unphosphorylated forms of NtrC were substantially more dynamic than the wild-type phosphorylated form. Notably, these ms-μs movements occurred in the regions of the protein that also underwent the greatest conformational changes upon phosphorylation. These observations establish a correlation between motion and the structural changes accompanying activation, but do not define the conformational states involved or how the motions are related to the level of activity observed.



NMR analysis of protein conformation and dynamics. (**A**) The bacterial response regulator NtrC is depicted in its inactive (I) and active (A) conformations. The regions with major structural differences between the two states are shown in red and yellow. (**B**) NMR analyses have enabled motions of inactive and active NtrC of various time scales and magnitudes to be measured. The order parameter, S^2 (red), reflects the magnitude of dynamics that occur on the ns-ps time scale, which have low activation barriers and typically involve smaller motions of relatively few coupled atoms. R_{ex} (blue) reflects the frequency of motions on the ms-μs time scale, which have larger activation barriers and typically involve larger structural changes and greater numbers of atoms. Vertical arrows indicate activation energies associated with transitions; horizontal arrows indicate transitions between different conformational states (energy minima). NMR measurements of NtrC dynamics yield information about the transition frequencies and barrier heights between conformational states (*3, 4*). (**C**) Different distributions of the two conformational states (A and I) in the mutants X and Y are revealed by differences in chemical shift (that is, differences in the position of the NMR signal of a given atom nucleus in the protein). The distribution of conformational states and their thermodynamic stabilities are measured with respect to reference distributions, such as 100% of the phosphorylated state.

To associate functionally distinct structures and ms-μs dynamics, Volkman *et al.* used a property of NMR called the chemical shift. With chemical shift data, they examined the environment of individual atoms at the phosphorylation site and at the 3445 face for unphosphorylated NtrC, the series of partially active mutants, and phosphorylated versions of each. Chemical shifts in all of the phosphorylated proteins were nearly identical and, because they also had the same

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maximal level of activity, they could be correlated with a common active conformation. Moreover, chemical shifts of each mutant were located in between those of unphosphorylated and phosphorylated wild-type NtrC and could be correlated with the mutant's activity. The correspondence between chemical shift and activity data in the wild-type and mutant proteins strongly suggests that all forms of NtrC adopt the same two-state equilibrium, and that the ms-μs dynamics include transitions between an inactive conformation (having the structure of the unphosphorylated wild-type protein) and an active conformation (having the structure of the phosphorylated wild-type protein). So, the different chemical shifts result from different equilibrium ratios of the active and inactive conformations, rather than from unique active conformations (which would almost certainly have uncorrelated chemical shifts). The changes in chemical shifts observed support a switch mechanism for the activation of NtrC that is rapid on the NMR time scale, involving a direct coupling between the structural transitions at the phosphorylation site and those at the 3445 face. Phosphorylation strongly shifts the equilibrium to the active conformation, where ms-μs dynamics are quenched because the inactive conformation is no longer adopted. Thus, by combining chemical shift changes and transcriptional activity analyses, Volkman *et al.* have developed a unified structural and dynamic model for the activation of NtrC.

How general is this model? NMR dynamics data from other bacterial two-component response regulators suggest that, like NtrC, they are activated through a shift in equilibrium between two conformations (*8, 9*). Similarly, the eukaryotic protein calmodulin shifts between Ca^{2+}-free (inactive) and Ca^{2+}-bound (active) states (*10*). In such systems, the inactive or unphosphorylated protein regularly "samples" the active state even in the absence of ligand or covalent modification. This behavior, however, cannot provide the large change in activity and sharp switching needed by many signaling systems. In the case of NtrC, this problem is solved through superimposing a second repressive mechanism, the requirement of oligomerization for activity, over the phosphorylation switch (*5*). Because conformational transitions in strongly biased equilibria are likely to be slow, the coupling of multiple weak regulatory steps may be an advantage for proteins that must be highly repressed in their inactive states but simultaneously capable of rapid activation in response to appropriate signals. An analogous argument, based on the rapid dissociation kinetics of multivalent binding interactions, explains the prevalence of multivalent systems throughout biology (*11*). Such multilevel regulation may also provide the tight specificity required in signal transduction, given that multiple independent signaling inputs must be applied simultaneously to achieve activation. A multilevel system would be well suited for evolutionary change, as the effect of any individual input could be modified with only a modest effect on overall activity.

Many motions of proteins do not directly affect their activity. For example, dynamics at the protein surface may arise simply because of a lack of packing restraints. But even general features of protein structures such as these have been exploited in biology (*12*). In several systems, changes in the extent of ns-ps motions detected by NMR after ligand binding have been interpreted in thermodynamic terms (*3, 4*). Motions in the ms-μs range typically involve several amino acids (if not larger structural units) in collective transitions and could be important kinetically, given that their time scale is similar to that of many biochemical processes. During information transfer between macromolecules, for example, signaling proteins often undergo large conformational changes or unfolding events that coincide with their binding to other proteins (*13*). Here, intrinsic ms-μs dynamics may limit the rate at which activation can occur. Recently developed methods to measure ms-μs conformational exchange directly, when coupled with biochemical measurements such as those conducted by Volkman *et al.*, hold great promise for deciphering how motions influence the function of proteins.

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PERSPECTIVES: MATERIALS SCIENCE

Watching Grains Deform

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Most crystalline solids—from aluminum sheets to rock masses—are not perfect crystals, but rather agglomerates of crystalline grains. Theoretical attempts to understand the mechanisms by which such polycrystals deform have been hampered by the lack of experimental tools for the in situ monitoring of the material during deformation. Such a tool is now reported by Margulies *et al.* on page 2392 of this issue (*1*). The authors have developed a novel method for following individual grain rotations during plastic deformation, thereby enabling a direct view into the mechanisms and dynamics of texture development in a bulk material.

Plastic deformation of polycrystalline materials is governed by the interplay between deformation within individual grains through dislocation movement and the external applied stress. The movement of crystal dislocations (such as missing or additional rows or planes of atoms) allows grains to deform in certain directions when stresses far below the theoretical strength of the perfect crystal are applied. Simultaneously, the deforming grain is forced to rotate in response to the external stresses exerted upon it by its neighbors. The resulting preferential alignment of crystalline grains (texture) reflects the deformation conditions as well as the deformation mechanisms of the material. It causes the polycrystal to develop anisotropic (direction-dependent) physical properties such as elasticity or electrical conductivity.

The development of texture and anisotropy in a polycrystal has important consequences for processes as diverse as solid-state convection in the deep Earth and the production of metal cans or polymer fibers. Despite a vast amount of experimental data on postdeformation textures (*2*), theoretical understanding of texture development during deformation remains incomplete. Early theories for polycrystal plasticity were derived for conditions of stress equilibrium (*3*) or homogeneous strain (*4*) in the deforming material,

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