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

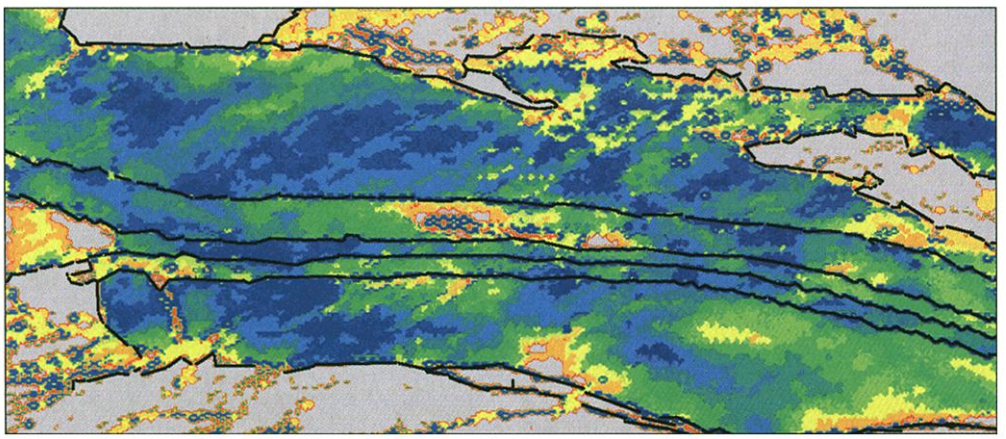
Florian Heidelbach

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,

The author is at the Bayerisches Geoinstitut, Universität Bayreuth, 95440 Bayreuth, Germany. E-mail: Florian.Heidelbach@uni-bayreuth.de



Complex orientation pattern. Orientation gradients (shades of green) inside a single copper grain (outlined by thick black lines) are induced by rotation and bending of the crystal lattice as a result of dislocation movement and pile-up during deformation. The process leading to this complex structure can now be followed directly with the method of Margulies *et al.* (*1*). The orientation map was produced by electron backscattering diffraction in a scanning electron microscope with a spatial resolution of 1 μm. The width of the map is 175 μm, and the spread of orientations is about 15°. [Adapted from (*8*)]

but these conditions are rarely fulfilled in real materials. More recent theories such as the self-consistent model (*5*) may yield more realistic predictions for texture development but are hampered by the problem that very similar final textures may be produced under wide ranges of model parameters. Tracking orientation changes of single grains inside a polycrystal directly during deformation therefore represents an important step toward understanding the deformation behavior of polycrystals and developing new and better models.

To date, orientation changes on the scale of a single grain have mostly been studied with electron microscopy, operating either in transmission (*6*) or in scanning mode (*7*). In both cases, analyses are restricted to two-dimensional sections bound by at least one free surface. Hence, orientation development of single grains in the bulk material can only be measured after the deformation process. A typical example is shown in the figure, which displays the curvature and distortion of the crystal lattice in a single copper grain after deformation (*8*). The continuous wavelike orientation gradient is caused by the accumulation of dislocations in the crystal lattice, illustrating the complex interactions between external stresses and grain deformation.

The orientation before deformation of the grain shown in the figure is not known, and, hence, its rotation path cannot be reconstructed by electron microscopy. The synchrotron-based approach of Margulies *et al.* (*1*) allows this rotation path to be tracked starting from the undeformed material. The method uses the penetration depth of a focused high-energy x-ray beam to monitor the orientation changes of lattice reflections from single grains within a deforming polycrystal. By analyzing the width of the reflections, it furthermore allows the spread of orientations resulting from the deformation within one grain to be determined. The rotation path can be compared directly with predictions from polycrystal plasticity theory, enabling the deformation behavior to be understood in detail.

The method demonstrated by Margulies *et al.* makes it possible to map orientations and their dynamic change in three dimensions, opening the opportunity to comprehend the deformation process in space as well as in time. With the rapidly increasing number of synchrotron facilities around the world, this pioneering approach should become widely applied in the future, leading to a better understanding and visualization of the complex processes in polycrystal plasticity.

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PERSPECTIVES: CHEMISTRY

Dynamic Combinatorial Chemistry

Jean-Marie Lehn and Alexey V. Eliseev

Combinatorial chemistry is now widely used to generate vast libraries of molecules that can be screened for biologically active substances and new materials. In a parallel development, self-assembly processes directed by molecular recognition are investigated in supramolecular chemistry. By merging features of both areas, dynamic combinatorial chemistry (DCC) offers access to a wide range of substances assembled from relatively small libraries, without the need to synthesize each substance individually. Although formulated only recently, this approach is already showing success in the search for compounds binding to specific biological and nonbiological targets.

DCC uses mixtures (libraries) of constituents that interconvert in dynamic equilibrium. The constituents are assemblies of components connected through reversible reactions or interactions; the whole set of possible self-assembled constituents forms a virtual combinatorial library. When a molecular target such as a biopolymer or a small molecule is added, some library constituents bind to it selectively and are removed from the pool of interconverting compounds. The equilibrium then shifts, amplifying the good binders and minimizing the concentration of poor binders in the library. The method thus enables the discovery of individual compounds or noncovalent assemblies that recognize small molecules or biopolymers.

DCC was conceptualized and implemented only recently in various chemical systems [see recent reviews (*1–4*)], although some characteristic features may have been implicitly present in earlier studies. Initial investigations focused on proofs of principle and on methodological procedures that established, for example, which reversible chemical transformations

J.-M. Lehn is at ISIS, University Louis Pasteur, 67000 Strasbourg, France. E-mail: lehn@chimie.u-strasbg.fr
A. V. Eliseev is at Therascope AG, Hans-Bunte-Strasse 20, 69123 Heidelberg, Germany.