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ACS Meeting News

### Enzymes' Many Movements

#### Controversy exists over whether motions at various timescales contribute to catalytic efficiency

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**ENZYMES ARE** biology's catalysts, making it possible for chemical reactions to happen on biologically relevant timescales, not to mention at nonlethal temperatures and pressures. Random thermal fluctuations mean that enzymes are always in motion and have not one but many possible conformations that differ ever so slightly. But the question of whether these motions play a role in enzyme function has intrigued experimentalists and theorists for many years—especially in the past decade.

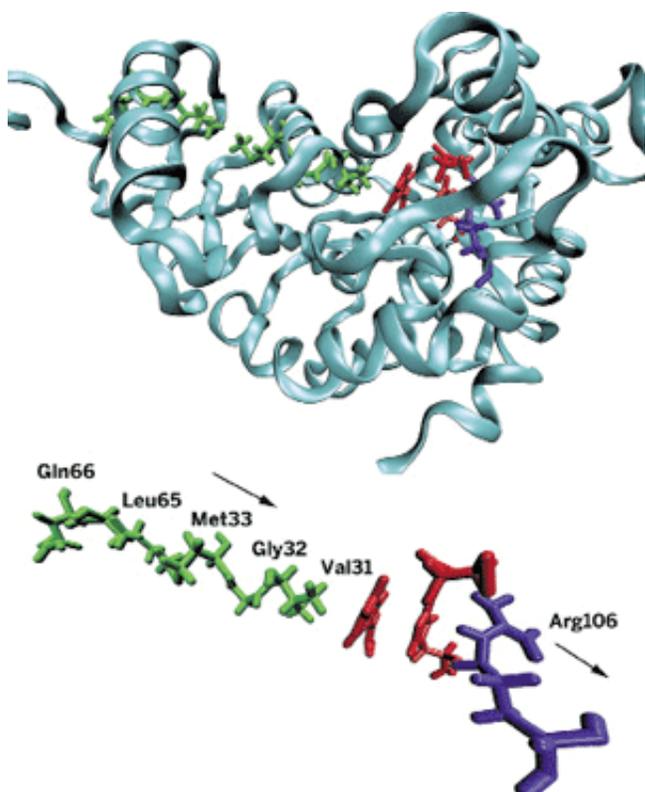
For much of that decade, the functional significance of enzyme motions, or enzyme dynamics as they are often called, has been controversial, particularly the notion that such random motions might contribute to enzymes' catalytic efficiency.

Last month, leading theorists and experimentalists gathered to discuss the current understanding of enzyme dynamics in a four-day symposium cosponsored by the Divisions of Physical Chemistry and Biological Chemistry at the [American Chemical Society](#) national meeting in Salt Lake City. [Amnon Kohen](#) of the University of Iowa and [Arieh Warshel](#) of the University of Southern California (USC) organized the symposium.

"Bringing together experimentalists and theoreticians to present very different experimental approaches and theoretical models addressing the question of enzyme functional motions was important," Kohen told C&EN. "It surely brought the field closer to understanding the agreements, disagreements, and more important, the nature of the phenomena in question." Although disagreements persist, many participants reported a growing sense of converging views in the field.

A vexing challenge to studying the role of protein motions in enzyme function has been the broad range of length and timescales over which those motions occur in portions of the protein both remote from and in the active site. The class of slow motions, which usually involves large regions of the protein, is often called conformational sampling. After the enzyme has found nearly the right conformation for a catalytic action, some people think that faster motions, which [Steven D. Schwartz](#) of Albert Einstein College of Medicine calls "promoting vibrations," fine-tune the active site to push the reaction over the energy barrier.

Because of their varying length and timescales, not all enzyme motions are accessible by a single technique. Nuclear magnetic resonance spectroscopy



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In motion This model of lactate dehydrogenase highlights motions that affect the reaction. Five amino acids (green) compress toward the active site (red), whereas one arginine (blue) relaxes away.

measures slow motions on the order of microseconds or milliseconds, timescales that correspond to the overall turnover rate of many enzymes. But motions also occur at the much faster femtosecond and picosecond timescales of the actual chemical reaction being catalyzed. Theorists' computational methods are best suited to these shorter timescales, but only recently have experimental methods such as ultrafast two-dimensional infrared (2D-IR) spectroscopy become available.

Another, unexpected source of information about protein motions is crystallography. Crystallographic structures are often interpreted as single conformations, but ultra-high-resolution crystal structures (those with resolution better than 1 Å) can represent a range of conformations and indicate the predominant direction of motion of individual atoms.

Structural biologists [Gregory A. Petsko](#) and [Dagmar Ringe](#) of Brandeis University are using crystallography to learn whether a protein's structure constrains the atoms in the protein so that they move predominantly in directions that contribute to the reaction. "There's reasonably good crystallographic evidence that the enzyme structure does restrict the direction of motions," Petsko said. He acknowledged, however, that their work doesn't reveal whether that restriction in motion contributes anything to catalysis.

To provide better starting structures for modelers, Ringe is turning to neutron diffraction. With X-ray diffraction, crystallographers need a resolution of 0.8 Å or better to detect hydrogen atoms. "Because neutrons interact with the nucleus rather than with electrons, we expect that we will be able to see hydrogen," with just 2-Å resolution, Ringe said. The eased resolution requirement means that crystals for neutron diffraction need not be as perfect as for X-ray diffraction.

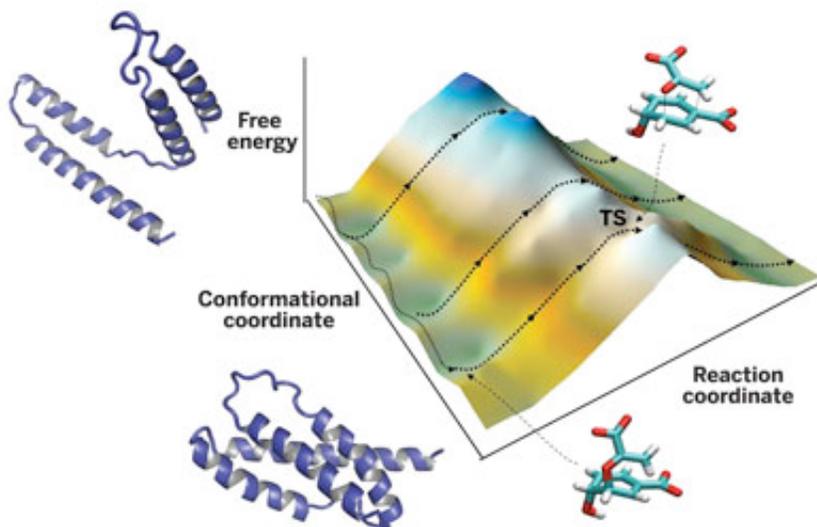
Much of the early work to probe the functional role of enzyme motions focused on hydrogen-transfer reactions. [Judith P. Klinman's](#) group at the University of California, Berkeley, was the first to show that such reactions occur via a quantum mechanical process called tunneling. One of the hallmarks of hydrogen tunneling is that hydrogen and deuterium transfer have very different properties that can lead to larger isotope effects on rates than would be expected given the mass difference between hydrogen and deuterium and to anomalous temperature dependences for those isotope effects.

"Tunneling requires a very close approach between the donor and acceptor to get good wave function overlap," Klinman said. People speculate that this overlap occurs as a result of short, fast motions in the active site that bring the reactants together.

**AT THE ACS MEETING,** Klinman reported the tunneling behavior of a thermophilic enzyme that works best above 30 °C. Below that so-called breakpoint temperature, the tunneling properties change, she said. "When you start to freeze out conformational sampling, you can no longer get precise alignment at the active site," Klinman said. Instead, she continued, deuterium needs more energy to tunnel than does hydrogen, whereas above 30 °C the energies for transfer are about the same. The anomalous tunneling shows that the active site can't achieve its optimized configuration below the breakpoint temperature, Klinman said. These effects are amplified with mutated active sites, she reported.

In addition to the increased enthalpy of activation below the breakpoint temperature, Klinman sees increased entropy as well. This quantity is usually expressed in terms of the Arrhenius prefactor. In the classical picture of enzymes, Klinman notes, this prefactor has an upper limit of  $10^{13}$  reciprocal seconds. "As we go from the wild type through mutants at low temperature, we get violations from that behavior," she said. Klinman and her student Zachary D. Nagel see Arrhenius prefactors many orders of magnitude higher than the classical limit.

Klinman interprets this behavior to mean that the enzyme falls into "deep valleys" in the conformational landscape from which it must escape. The elevated Arrhenius factor "follows from a reduction in the fraction of enzyme that is able to sample the correct conformational space, together with the ability of increased temperature to overcome the low-temperature, mutationally induced defect," she said.



Courtesy of Arieh Warshel

walking uphill As an enzyme (chorismate mutase shown, purple) changes conformation, it moves to a region of the catalytic landscape where its substrate (chorismate, aqua and red) can overcome the energy barrier and reach the transition state (TS).

[Rudolph A. Marcus](#), a theoretical chemist at California Institute of Technology, has modeled some of the odd behavior that Klinman sees in thermophilic enzymes. "That behavior is something like the viscosity of silica above and below the glass point," Marcus said. He suggested that a good theoretical starting point for studying the dynamics of such enzymes would be the concepts employed for glass.

Many of the ideas about the role of enzyme dynamics in hydrogen tunneling have been inferred from the temperature dependence of kinetic isotope effects in studies such as those done by Klinman and others. [Nigel S. Scrutton](#) and coworkers at the University of Manchester, in England, wanted to move beyond such inferences and find direct evidence that enzyme dynamics narrow the energy barrier and permit quantum mechanical tunneling in enzymatic hydride-transfer reactions.

To do that, Scrutton studies the effects of hydrostatic pressure on hydride transfer. Pressure shifts the equilibrium distribution of conformations, increasing the population of conformations with shorter hydrogen-transfer distances. As the pressure increases, the reaction rate should also increase because compressing the protein brings the reactants closer together and makes hydrogen transfer faster and easier. "Through our measurements of reaction rates and isotope effects, we've seen that as you ramp up the pressure, you get kinetic behavior consistent with barrier compression," Scrutton said.

But Scrutton also wants direct spectroscopic evidence for that barrier compression. To obtain it, he made a mimic of an enzyme-substrate complex that gives a spectroscopic signal reporting how the substrate is bound in the active site. He uses the enzyme morphinone reductase because its flavin cofactor changes color when the reaction occurs.

"We're directly accessing the chemical step," Scrutton said. "We've gotten to the point with the experimental data that we're convincingly showing that compressive dynamics has happened."

Scrutton sees pressure as a more subtle conformational tool than site-directed mutagenesis, in which amino acids in the enzymes are changed. Although such pressure effects may be most pronounced in hydrogen tunneling, pressure should be a general tool for studying the role of dynamics in other enzymatic reactions, Scrutton said.

Schwartz and his Einstein College collaborator [Vernon L. Schramm](#) also see evidence of small, fast motions in the active sites of enzymes that catalyze reactions other than hydrogen transfer. For example, in the enzyme purine nucleoside phosphorylase, which breaks the ribosidic bond in purines, they see a rapid motion in the protein that compresses a stack of three oxygen atoms in the phosphate, polarizing the ribosidic bond, Schwartz said. About 100 femtoseconds later, another motion in the protein pushes the ribose ring toward the phosphate.

Although motions within the active site are important, enzymes undergo motions on larger scales, too. [Peter E. Wright](#) of Scripps Research Institute described NMR experiments looking at such larger scale motions in the enzyme dihydrofolate reductase. "We see the enzyme fluctuating in and out of states at rates that correspond precisely to various catalytic events," Wright said. "The rate of product release, which is rate-determining overall, corresponds precisely with the rate at which the enzyme fluctuates into an excited state in which the product binding pocket has changed conformation to look like the empty pocket."

Wright emphasizes that NMR can't capture motions that couple directly to the chemical step. "With NMR, we can't learn about things that affect the height of the barrier for the chemical event," he said. "We can't see the transition state."

But new spectroscopic methods could give scientists direct access to those faster timescales. 2D-IR spectroscopy is allowing scientists to observe picosecond or even femtosecond structural fluctuations. Unlike the slow timescales best suited to NMR, these timescales could help determine what role, if any, enzyme motions play in the actual chemical step.

"Part of the controversy is about whether any of these functional motions related to the chemistry actually have anything to do with the catalytic efficiency of the enzyme. My feeling is that in most cases they don't," said [Christopher M. Cheatum](#), a spectroscopist at the University of Iowa. "As a physical chemist, I'm really interested in understanding how the chemical reaction happens in the context of the enzyme, so I'm interested in the chemical step."

**"Part of the controversy is about whether any of these functional motions have anything to do with the catalytic efficiency of the enzyme."**

To study those key chemical steps, Cheatum works with transition-state analogs. He recently made a transition-state-analog complex of the enzyme formate dehydrogenase, using the azide anion as a spectroscopic reporter in 2D-IR experiments.

Using the transition-state mimic, "we see very different behavior from what's been seen in other systems," Cheatum said. "There are no motions of the enzyme active site that occur on timescales longer than a few picoseconds." From this, Cheatum concluded that the transition-state analog "exhibits an unusually narrow conformational distribution and a very rigid structure."

**IN CONTRAST**, [Michael D. Fayer](#) of Stanford University employs 2D-IR spectroscopy to study motions involved with conformational sampling, using the oxygen transport protein myoglobin (not an enzyme) as an example. With a chemical exchange technique that reports the interconversion between conformations, Fayer observed that myoglobin switches between conformations in approximately 50 ps. Fayer's results suggest that the slow motions of conformational sampling are the sum of many fast motions.

USC's Warshel, a theoretical chemist, has calculated the effects of the various types of protein motions described in the symposium and staunchly maintains that they have no bearing on the catalytic activity of enzymes. For "any motion you claim exists in the protein—if you look at the same process without the protein—you will find that there are similar motions of water molecules that create exactly the same effect," he said. "The barrier height is the only thing that counts."

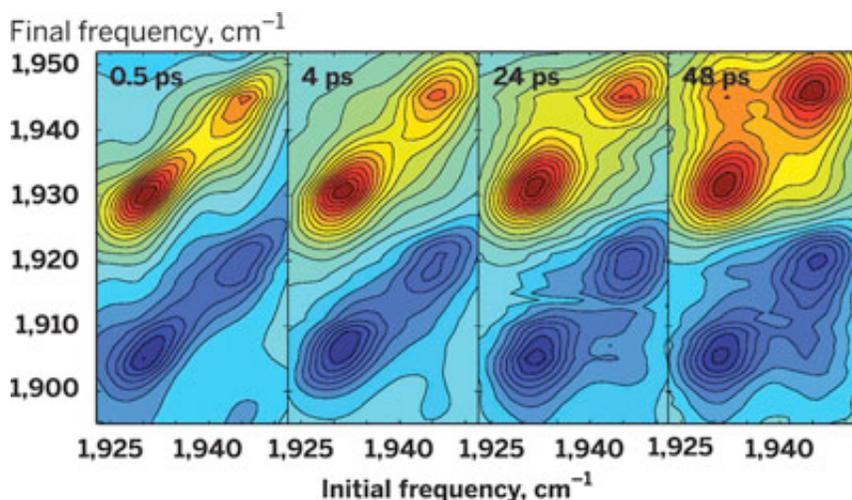
[Sharon Hammes-Schiffer](#), a theorist at Pennsylvania State University, agrees that the free-energy barrier predominantly dictates the overall rate of the reaction, but she believes that protein motions are required to move the reactant up the activation curve to the top of the barrier, the height of which is determined by the relative probability of sampling configurations at the top of the barrier and in the reactant. Fast, thermal, stochastic protein motions lead to slower conformational changes, and she includes these motions in her enzyme models.

Schwartz acknowledged that statistical calculations can reproduce an average reaction rate without invoking rapid dynamics, but such calculations don't address mechanistic issues, he said.

Schwartz believes that protein motions at all timescales contribute to enzyme efficiency. The average energy barrier might be a certain value, but, just as the average speed on a highway says little about the speed of individual cars, individual molecules will experience different reaction rates, he said.

Warshel points out, however, that his calculations, which have reproduced rate constants both in proteins and in water, consider all types of motional effects from femtoseconds to milliseconds and find no major dynamical contribution to catalysis.

Total agreement might be lacking, but "most researchers believe that thermal motions of the protein-reactants complex escort the system to its reacting state and across the barrier," Kohen told C&EN. And big questions remain unanswered: Which motions and timescales are the most important? Do they involve only the active site or the entire protein? Such questions will be answered as experimental and computational tools improve.



Courtesy of Michael Fayer

picosecond by picosecond Time-dependent changes in the peaks (red and blue features) of these 2D-IR spectra provide a direct measure of conformational changes in myoglobin.