STRUCTURAL BIOLOGY

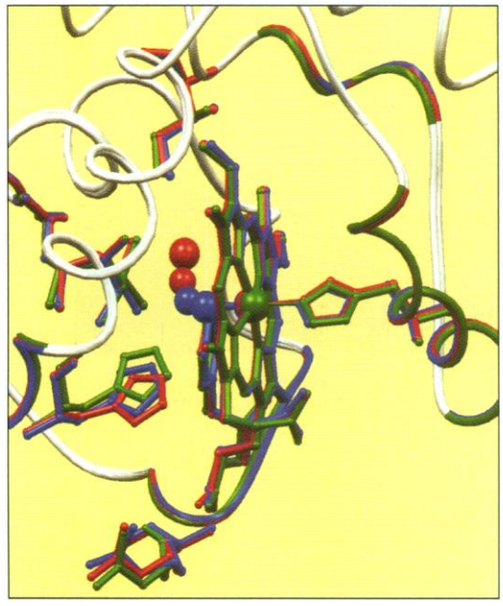
X-ray Movies Start to Capture Enzyme Molecules in Action

The project would defeat even the boldest Hollywood director. The script hadn't been written, the action all took place in a fraction of a second, and the film had to be shot with x-rays and in 3D. Yet this past summer, two research groups put their work on general release. Their films consist of only a few frames, and they had to trick the main actor into slowing down by chilling the set to 40 degrees above absolute zero. But the movies are drawing rave reviews. They show the iron-containing protein myoglobin—a common muscle protein that has been called the hydrogen atom of biology because of its simplicity—going about its vital task: storing and releasing small molecules, such as oxygen.

The groups—one led by biophysicist Joel Berendzen of the Los Alamos National Laboratory and the other by biochemist Keith Moffat of the University of Chicago—aren't the first to make x-ray movies of a protein in action. But theirs are the first to capture molecular events that normally take place in a few nanoseconds, the blinding pace typical of many biochemical reactions. First reported in July at a meeting of the American Crystallographic Association in Atlanta, the work is another step toward the ultimate goal: to get "as close as you can imagine to a moving picture or video of molecules and enzymes changing conformation" in real time, as Joel Sussman, director of the Protein Data Bank at the Brookhaven National Laboratory, puts it. And the tools needed to make a real-time molecular movie may now be in hand: new synchrotron facilities around the world that provide the intense x-rays needed for action-stopping snapshots, and new schemes for getting arrays of molecules to begin their moves precisely on cue.

These molecular movies are actually an outgrowth of x-ray crystallography, a technique for imaging the 3D structure of a molecule by passing a beam of x-rays through a crystal containing countless copies of it. By recording and analyzing the scattered x-rays, researchers can pinpoint the locations of the molecule's hundreds or thousands of atoms. In x-ray movie-making, however, the goal is not a still life but a series of frames showing the molecule in action as it responds to a chemical trigger or a flash of light. "If you could look at these biological molecules actually doing their stuff," says Moffat, "you'd get a better idea of how they work."

As every photographer knows, action shots require fast exposures—in this case, many orders of magnitude faster than the exposures typical in traditional crystallography. As a result, crystallographers have looked for ways to put the brakes on the reactions they want to study. Last year, for instance, a collaboration led by Bauke Dijkstra of the University of Groningen in the Netherlands combined low temperatures and a change in pH to slow down the catalytic cycle of the enzyme haloalkane dehalogenase, which breaks down organic compounds in bacteria. These manipulations, says Moffat, transformed the enzyme's fleeting transitions into "molecular structures that are designed to be stable and to resemble in some way authentic intermediates." Dijkstra and collaborators were then able to make x-ray images of these intermediates.



JOEL BERENDZEN *ET AL.*

Protein in motion. X-ray images reveal the shape of myoglobin's active site with a carbon monoxide bound to the protein's iron *(blue)*, while the carbon monoxide is released *(red)*, and with the site unoccupied *(green)*. The carbon, oxygen, and iron atoms are spheres.

The problem with chemically arresting a catalytic process, explains Moffat, is that doing so may affect the authenticity of the reaction. Very low temperatures alone—much lower than Dijkstra used—are an alternative, as Moffat and Berendzen have shown. Temperatures below that of liquid nitrogen, 77 kelvins, can slow a fast reaction by a factor of 10 billion, stretching to hundreds of seconds the binding and release processes, which normally take less than a microsecond. The challenge is preventing any unevenness in the cooling process from turning an ordered protein crystal into a "popsicle," as Berendzen calls it—something too disordered to yield structural information.

Berendzen says his group—which also included George Phillips of Rice University, Ilme Schlichting of the Max Planck Institute for Medical Research, and Robert Sweet of Brookhaven National Laboratory—"spent a long time learning how to cool the crystals while preserving their order." Moffat and his colleagues Tsu-Yi Teng and Vukica Srajer credit their success to a similar combination of trial and error and painstaking attention to detail. Both groups managed to cool their myoglobin crystals to below 40 K without disrupting their internal order. They then captured multiple frames of the protein as it held a molecule of carbon monoxide—a stand-in for the oxygen that is its usual target—released it in response to a flash of light, and then recaptured it.

The images reveal something never seen before: how the target molecule extricates itself from the myoglobin after the bond holding it is broken and how the protein rearranges itself afterward. And these glimpses of myoglobin in action, which Moffat and his colleagues have now published in this month's issue of *Nature Structural Biology*, may hold insights into the operation of more complicated iron-containing proteins, such as hemoglobin.

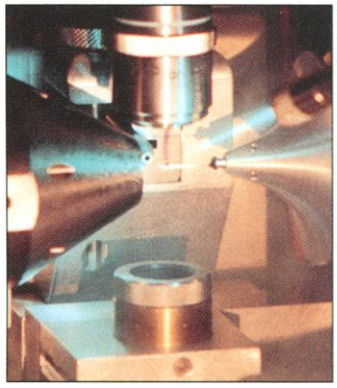
Letting it rip. Moffat says he isn't satisfied, however. "By having to slow the reactions down," he says, "you may in fact be trapping the system in an abnormal state. You don't know; you hope not, but you've always got to worry about it." The dream, he says, is to "let the reaction rip at physiological temperatures on normal proteins."

To make the short exposures required, crystallographers will need much brighter light sources. Synchrotrons, a trillion times brighter than laboratory x-ray sources, are the obvious choice. Synchrotrons have already sped up some x-ray crystallography work, but to simplify the analysis of the data, researchers have tended to make use of just one wavelength in the synchrotron beam, in effect throwing away the rest of the energy. At even the brightest existing synchrotrons, the resulting exposures are too slow for real-time studies.

A technique that harnesses the full spectrum of x-rays, known as Laue diffraction, has long been available, and by the early 1980s, desktop workstations and new computer algorithms had made its computational complexities "a great deal easier to handle," says Moffat. But using Laue diffraction to make real-time molecular movies has turned

out to be surprisingly difficult. The main stumbling block has been finding a way to prompt the 10^{13} or so molecules in a typical protein crystal to initiate their moves in concert, so that the snapshot isn't blurred by molecules that are responding out of step.

The problem is that the two standard reaction triggers—a chemical diffused into the crystal or a flash of light from a laser—can both produce an uneven spatial gradient in the crystal, says Moffat. The result is that instead of taking place at the same instant throughout the crystal, the reaction sweeps through it like a "roll of thunder," as he puts it, blurring the individual snapshots of enzyme structure. And while a very bright laser pulse can initiate the reaction simultaneously all through the crystal, it does so at the risk of damaging the crystal structure.



KEITH MOFFAT/UNIVERSITY OF CHICAGO

Movie set. A protein crystal is bombarded with blue laser light to initiate a reaction, white light to monitor the reaction's progress, and invisible x-rays to capture images.

As a result, the Laue technique so far has only succeeded on reactions that are slow to begin with. In 1987, Louise Johnson and Janos Hajdu at Oxford University managed to take time-resolved x-ray crystallography frames of phosphorylase, a huge molecule that takes tens of minutes to hours to convert a stubborn substrate into a product. And in 1991, Sweet and his colleagues at Brookhaven studied the enzyme trypsin, a digestive enzyme that breaks down proteins by hydrolyzing them—encouraging them to react with water.

But Moffat thinks the technology for moving on to faster reactions may now be in hand. The key may be a device called a microspectrophotometer, which measures a crystal's optical absorption spectrum at the same time as it is being hit by x-rays. By doing so, the device can reveal when and where the reaction is under way. That should enable researchers to deliver a jolt of laser light just strong enough to start the reaction uniformly, without running the risk of damage. "We can measure just how much energy is put in, and can put in just enough to trigger the reaction but not so much as to damage the crystal," Moffat explains.

In studies of a light-sensitive protein known as photoactive yellow protein (PYP), which acts as a bacterial photoreceptor, he and Elizabeth Getzoff of the Scripps Research Institute are testing that promise. They've taken multiple, time-resolved frames of PYP as it responds to light. The work is in progress, and none of the researchers will say much about it. But as Louise Johnson says, "if the PYP work can be pulled off, it would be very exciting." She adds that it might be "the breakthrough" the field has been waiting for.

If that's the case, the field will have a lot to look forward to in the next few years. Three powerful new synchrotron sources are coming on line: the European Synchrotron Radiation Facility in Grenoble, France, which has just begun producing light, and the Advanced Photon Source at Argonne National Laboratory and SPring-8 in Tsukuba, Japan, both scheduled to turn on within 3 years. These machines will provide x-ray crystallographers with brighter, tighter beams, says Moffat, and "enable us to study even faster reactions." Crystallographers may be about to discover what Hollywood discovered long ago—that there's nothing like action to draw the crowds.

–Gary Taubes

BIOLOGY AWARDS

Ernst Mayr Wins the Japan Prize

When the Nobel committee makes its annual wake-up calls to scientists to tell them they have won science's top prize, researchers in most areas of biology don't lose any sleep. There are no Nobel Prizes for biology other than medicine and physiology. As a result, some of the greatest biologists of the 20th century have no chance of making the trip to Stockholm. One of the most renowned, however, this week garnered an award designed to make up for this oversight: Evolutionary biologist Ernst Mayr was given the prestigious Japan Prize by the Committee on the International Prize for Biology.

Mayr, age 90, is the Alexander Agassiz Professor of Zoology, Emeritus, at Harvard University, and was recognized for his groundbreaking work in systematics: defining the evolutionary relationships among organisms. Other scientists are applauding his selection. Evolutionary biologist John Maynard Smith of the University of Sussex says that "Ernst is one of the great shining figures in evolutionary biology." Then he asked: "Did he win a lot of money? I like to see my friends get a lovely nest egg."

In fact, Mayr will walk away with $100,000 and a medal at an awards ceremony with the Japanese emperor in Tokyo on 28 November. Reached at his home in Cambridge, Massachusetts, Mayr obviously relishes the recognition: "Some years ago, I was picked as the world's best evolutionary biologist for the Balzan Prize. Now, I've been picked as the world's best systematist. It's rather gratifying."

ANNETTE COLTRELL

Prize in hand. Biologist Ernst Mayr's work on classifying birds was the foundation for a brilliant career.

Indeed, the award was given to Mayr because he was "without a doubt, the outstanding systematist in the world," says Columbia University systematist Walter Bock, who was a member of the 17-member committee that selected Mayr. Ever since Mayr left his native Germany at the age of 23, sailing to the Southwest Pacific to study the wild birds of New Guinea and the Solomon Islands, he has been devising methods of classifying species and subspecies of organisms. His observations of birds in the wild, and his work as a curator in charge of bird collections at the American Museum of Natural History for 21 years, led to the publication of the leading textbook on systematics, *Systematics and the Origin of Species* (1942).

The prize committee also took note of Mayr's other scientific contributions. He is best known as one of the architects of the so-called "modern synthesis" of evolutionary biology, which showed in the 1930s to 1950s that Darwin's notion of natural selection could be used to explain all evolution—not only the way plants and animals change over time, but why genes evolve at the molecular level. In particular, Mayr cleared up an area that had confused Darwin—how new species arise. Says Smith: "His chief observation is that species arise when the members of a species are separated in space and time"—either by mountains, the sea (particularly if they live on islands), dense forests, or other geographical barriers. Over time, separate populations of the same species evolve different traits—so-called "isolating mechanisms"—that discourage them from interbreeding, and eventually become so different genetically that they form different species. For this insight and others, Mayr too, as his peers and prize committees agree, has shown himself to be a breed apart.

–Ann Gibbons