



Professor Keith Moffat makes the first real-time movies of processes that drive – and might help save – life.

Keith Moffat's movies are screened in the quiet back rooms of a facility unknown to almost anyone but scientists. They won't win any Academy Awards. But they explain some fundamental facts of life itself – and may spin off important advances in medicine and pharmacy.

"If there's one thing that drives us," said Moffat, the University of Chicago's Louis Block Professor of Biochemistry and Molecular Biology and newly appointed deputy provost for research, "it's the realization that many of the fundamental principles of the biological process are shared, from the lowest organisms through humans.

by Paul Karr

Molecular Movies



Since 1996, Keith Moffat's pioneering molecular "movies" have been changing the way scientists study the most basic processes of life.
Photo by Peter Kiar

These movies may give us a glimpse into ourselves," he said. "But they're also more profound, illustrating the basic workings of life."

Moffat's stage for these films is a long way from Hollywood: a racetrack-shaped building at Argonne National Laboratory, about an hour west of Chicago. (See related story on page 23.)

His camera is a far cry from Technicolor, too. It consists of laser beams, X-rays and a sealed, thin glass tube in which a protein crystal plays the starring role.

A typical Moffat flick begins with a powerful pulsed laser beam fired directly and briefly at a tiny crystal of proteins, which are known to be sensitive to light, or photoreceptors. The brief flash of light sets off a complex chemical and structural reaction in the protein molecules — a fundamental biological process about which surprisingly little is known.

At almost the same instant the laser is firing and triggering the reaction, a super-bright and narrowly concentrated X-ray beam also is fired directly at the protein crystal. As the beam bombards the crystal, its X-ray photons scatter; the distinctive pattern of scattering forms the picture that Moffat and his team will analyze to learn how the proteins in the crystal changed, instant by tiny instant, after the laser struck it.

Computers analyze data from the scattered photons for weeks. The final product is a series of images of the different stages of the protein's structural change that, until now, had eluded scientific inquiry. Strung together they form the first "movies" of the changes occurring during the most basic molecular processes of life: responses to light signals, photosynthesis, oxygen uptake and the like.

"Keith's pioneering study of myoglobin [seven years ago] showed the international research community that the idea of watching a protein as it functions is no longer an empty pipe dream," said Philip Anfinrud, a senior biomedical research scientist at the National Institutes of Health's Laboratory of Chemical Physics in Bethesda, Md. "It is now reality."

Peeking at proteins

Proteins — long strings of amino acids — are the main building blocks of life and essential to all biological processes. In order to design drugs that affect very specific processes, pharmaceutical researchers must understand both the static structure of proteins and how those proteins move and change.



Photo by George Jochim/ANL

Donning laser safety glasses, scientist Spencer Anderson secures a glass capillary tube that contains a PYP protein crystal to the "molecular camera" in an APS research lab at Argonne.

Compared with ordinary chemical compounds, protein molecules are large and complex, with many "moving parts" to their machinery. These parts shift, wiggle and jiggle between various stages as they undergo the reactions that drive life.

But scientists have not known exactly what stages are present in a reaction nor how proteins get from one stage to another. For instance, what are the intermediate stages between the beginning and end of a reaction? How are they arranged? What are their structures and properties? The stages in processes (such as vision) or reactions (such as photosynthesis) are simply too small and quick to view with either the naked eye or conventional tools.

"Many years ago I had an idea about how to do it," Moffat said.

He began thinking about the idea of taking extremely fast-exposure images of protein reactions during the late 1960s as a Cambridge research student investigating conventional macromolecular crystallography at England's prestigious MRC Laboratory of Molecular Biology.

"I was — and still am — interested in how proteins work, not just in what their structure is," Moffat said. "But investigating proteins while they were working clearly wasn't feasible then, for a whole variety of practical reasons."

So he put the idea on a back burner. As lasers and X-ray production slowly became more advanced during the following two decades, the tools also became available for the experiments he had envisioned. In 1983, Moffat began redeveloping the concept in earnest.

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us, it's the realization that many biological processes are shared through humans.

— Keith Moffat, Professor of Biochemistry and Molecular Biology

To view such fast reactions, scientists need a powerful laser, a very bright X-ray source and an extremely fast “shutter” with which to capture an image of the process. The X-ray sources have become much more brilliant since the mid-1980s, when the first of a series of large, ring-shaped facilities called synchrotrons were built to accelerate and magnetically “tease” particles until they produced super-bright X-rays.

During the 1980s Moffat had used an early synchrotron at Cornell University, where he then was a faculty member. When he joined the University of Chicago in 1990, his team began using the Brookhaven National Laboratory on Long Island and the European Synchrotron Radiation Facility in Grenoble, France — at the foot of the Alps — for weeks at a time to conduct their experiments at more advanced X-ray facilities.

“We’ve been scientific travelers,” Moffat chuckled through his native Scottish accent. “We’ve always gone wherever the best synchrotron has been.”

X-ray tunnel in as little as two microseconds, during which a single X-ray pulse — lasting just 100 picoseconds (that’s one-ten-thousand-millionth of a second) — can pass through the slot in the shutter and fall on the protein crystal.

Fellow lab researcher Zhong Ren developed software to analyze the unusual diffraction patterns of the scattered X-rays; team members Claude Pradervand, Vukica Srajer and Reinhard Pahl devised fast-timing circuitry to tie the opening of the X-ray shutter with both the laser pulses and the X-ray pulses in a precise, controllable way.

“For the new experiments to work, you need the overall experimental design, the hardware, the electronics, the software and the crystal to be in place,” Moffat said. “This demands a team with very diverse skills.”

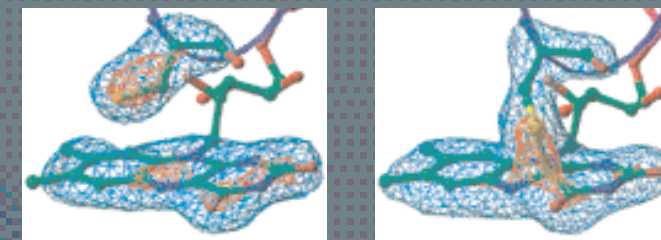
It all comes together when the software draws stop-action images of color-enhanced blobs, shrinking and growing, moving together and disassociating on a tiny scale — the “blobs” are related to the protein atoms whose positions are

control our human clock. Now we have absolutely no idea how that works at the molecular level — we don’t know if it works the same way as the light sensors in plants. But we do know that some of the molecules involved in light sensing in plants and bacteria have relatives in humans. So there’s a good chance of a relationship.”

Plant life depends on sunlight, and plants have evolved a remarkable ability to sense it, to follow the sun around in the sky, to germinate or to flower in response to changes in light. Darwin first studied light-driven motion in plants, a process called phototropism, more than a century ago.

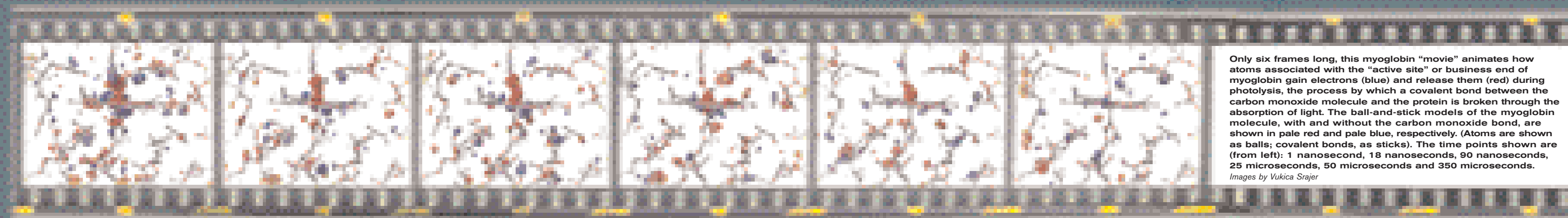
So far, Moffat and his collaborators have successfully captured images of three light-sensitive proteins — usually known as photoreceptors — in “slow motion” using the movies.

LOV2 When this plant protein absorbs the blue spectrum of light, it generates a structural signal that sets in motion a series of events that ultimately causes the plant to turn toward the sun. This response seems to have evolved very early, because it is



Scientists have captured images of the “light-driven molecular switch” of LOV2, which shows one way that plants are able to sense light. The chemical and structural response shown in these figures ultimately leads the plant to bend toward the light, which maximizes the efficiency of photosynthesis. The figure at left shows the protein in the dark state; the right one reveals its photo-excited state. Researchers doubt there are any well-structured stages in between. Images by Sean Crosson

Analyzing it requires almost unfathomable speed. “Absorbing the light occurs in femtoseconds (thousand-million-millionths of a second),” Moffat said. “That alters the electronic structure of FMN in nanoseconds, making it responsive to forming the new, covalent bond; and then the whole process fully reverses within minutes.” This forces the scientists to use different



Only six frames long, this myoglobin “movie” animates how atoms associated with the “active site” or business end of myoglobin gain electrons (blue) and release them (red) during photolysis, the process by which a covalent bond between the carbon monoxide molecule and the protein is broken through the absorption of light. The ball-and-stick models of the myoglobin molecule, with and without the carbon monoxide bond, are shown in pale red and pale blue, respectively. (Atoms are shown as balls; covalent bonds, as sticks). The time points shown are (from left): 1 nanosecond, 18 nanoseconds, 90 nanoseconds, 25 microseconds, 50 microseconds and 350 microseconds. Images by Vukica Srajer

In 1996, one of the world’s best synchrotrons, known as the Advanced Photon Source (APS), opened almost in Moffat’s backyard at the Argonne National Laboratory in DuPage County, just off I-55. Now he can drive there in less than an hour from his University of Chicago offices (though the food, wine and scenery are better in Grenoble, he pointed out wistfully).

At the APS, in a section of the big circle called Sector 14, Moffat carefully orients the “X-ray shutter,” a suspended, slotted titanium triangle that rotates very quickly. The shutter was adapted by an engineering group in Germany from an earlier design by Moffat’s team. It can open and close a small

changing as the reaction proceeds — then assembles them into jerky but fascinating “movies” of reactions.

These movies tell stories that hold the rapt attention of the scientists in the audience because many of the chemical principles involved in bacterial or plant functions being studied by Moffat and his colleagues have human analogs. Take jet lag, for instance.

“If you fly to Japan, you experience jet lag for several days. How does the body adapt and reset its biological clock? The answer is, it senses the light environment in Japan,” Moffat said. “The body has light sensors, which ultimately

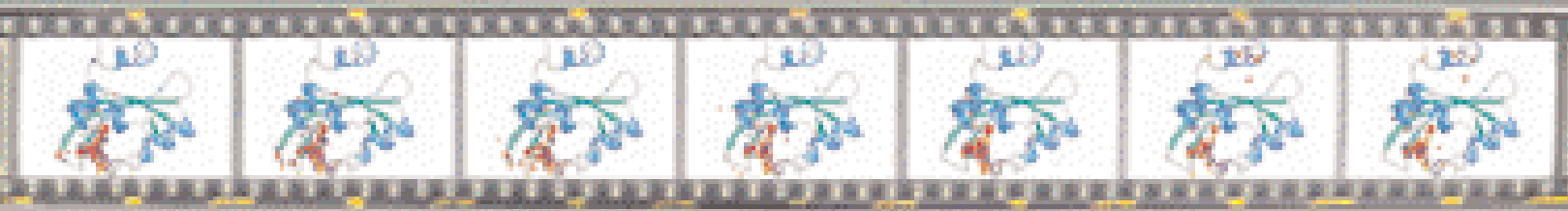
shared among lower forms of bacteria as well as higher plants. So using light as a signaling device may have originated early in Earth’s biological time and may even underlie the control of human circadian rhythms, according to Moffat.

Sean Crosson, while a graduate student with Moffat’s lab, showed that the initial structural signal of LOV2 is produced when a stronger, covalent — but temporary — chemical bond is formed between the protein cofactor flavin mononucleotide (FMN) and the protein itself. That bond forms in microseconds, but weakens during the next few minutes until it breaks completely. Somehow, during the process, a signal has been sent throughout the plant that causes it to bend towards the sun.

“shutter speeds” and repeat experiments to view all the different time scales of change within the protein.

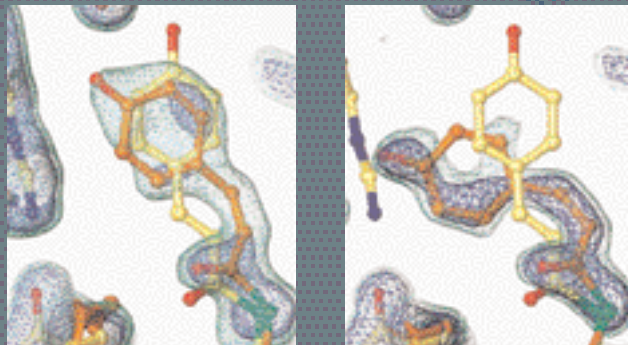
Myoglobin As the body’s chief oxygen-storage protein present in all muscles, myoglobin is studied a great deal. Movies made by Vukica Srajer and Tsu-yi Teng, senior scientists in Moffat’s lab, show that when a laser pulse strikes myoglobin, an iron atom at the center of it breaks its bond with a gas molecule, freeing the gas molecule to move around — either to rebind to the iron promptly or to escape the molecule entirely into the surrounding solution, re-binding more slowly later. This entire process takes just a millisecond, but during that time the protein has changed its structure — “relaxed,” in scientific parlance.

The motions that a molecule goes through to get from one state to the next are essentially invisible because there are so few particles in the molecule. Scientists have yet to determine if these motions are continuous or discrete, or a mixture of both. Within seven frames of the PYP protein illustrated below, several distinct states of the whole protein are revealed. The chromophore region – the part of the protein that actually absorbs the light – lies in the bottom left of the figures. Red represents loss of electrons, and blue represents the gain of electrons. *Images by Spencer Anderson*



The kinetics and pathways of these diffusion, relaxation and rebinding processes are interesting for what they reveal about oxygen and myoglobin and also — now that they are better understood — provide a kind of benchmark for the experiment and for structural changes in proteins.

Photoactive yellow protein (PYP) Bacteria can get overheated and even die if light levels are too high. PYP monitors and responds to blue light to help keep the bacteria alive much as LOV2 does for plants. (LOV2 actually resembles PYP in structure.) In this case, the bacteria's PYP light sensors are coupled with its swimming motion: The PYP absorbs light and generates a signal that causes the bacteria to swim away from the source of blue light whenever that light becomes dangerously strong.



Careful analysis of the chromophore region – the part of the protein that actually absorbs the light – shows two structures, or arrangements, of the chromophore atoms (shown in orange in both illustrations). The structure in the ground or dark state, prior to absorbing light, is shown in yellow. *Images by Spencer Anderson*

Postdoctoral researcher Spencer Anderson and graduate student Sudarshan Rajagopal are trying to find out how the bacterial PYP generates the signal. When struck by light, a double bond in the protein undergoes a process known as isomerization, in which certain atoms move from opposite sides of the double bond to the same side. In response, other atoms in PYP move and push each other around in a kind of molecular chain reaction that ends within about 100 milliseconds, and the bacteria begin moving away from the light. This process is a simpler form of the one used by the retina that human eyes use to react to light.

The movies of PYP in flux, Moffat said, provide a framework that might one day enable us to understand the molecular processes of light sensitivity that underlie such basic biological properties as vision and jet lag.

Brighter future

Moffat is looking forward to even better movies — sequels, if you will. He said the next generation of X-ray sources based on linear accelerators, once built, could reduce the time exposures of his molecular images to as low as 100 or even 10 femtoseconds — that is, to “shutter speeds” a thousand to 10,000 times faster than they are now, with a corresponding boost in the quality of the movies.

“We’re collaborating closely with scientists at Stanford University who have proposed to build the first of these new types of X-ray source,” Moffat said.

For researchers, it will mean critical new information on the ways proteins react to light and how their internal bonds are reorganized, broken or renewed — all of which feeds the slow but important work of drug research, design and discovery. Using movies like Moffat’s, drug researchers soon may be able to better predict — or, perhaps, actually witness — the unfolding reactions of drugs to the molecules they are targeting and acting upon.

“While others tried to imagine how proteins move in real time, a team led by Keith [Moffat] went out and made the first movie,” said NIH researcher Anfinrud. “By watching proteins writhe in real time, we will hopefully be able to make sense out of the molecular choreography that gives rise to a protein’s function.”

The field is still young, Anfinrud said, with much more to be done. “But if the past is a useful predictor of the future,” he said, “Keith will be in the midst of it all.”

For more information about molecular movies, access <http://moffat.bsd.uchicago.edu>.

FastTrack

Argonne National Laboratory’s particle accelerator – best in the business

From above, it doesn’t look like much — a big concrete doughnut, perhaps, or a baseball park without an infield in the quiet of DuPage County about 25 miles west of the University of Chicago. Though it could fit Wrigley Field inside it with room to spare, it’s too homely and far from downtown to do much good as a sports stadium.

But that ring produces some of the fastest, brightest, most powerful X-rays in the world — and scientists are beating a path to its door.

Researchers have used these super-powerful beams to examine such mysteries as the changes that plant proteins undergo during photosynthesis; the ancient Turkish metallurgy used to fashion and repair 5,000-year-old figurines; the detailed composition of the Earth’s crust and moon rocks; and the oxygen uptake mechanisms of insects. (It turns out that they do actually “breathe” oxygen into their tracheas, much like humans. See story on page 9.)

The Advanced Photon Source (APS) facility, part of Argonne National Laboratory — a U.S. Department of Energy lab managed and operated by the University of Chicago — was built in the early 1990s at a cost of \$1 billion to become one of the preeminent X-ray facilities in the scientific world.

There are about 50 synchrotrons in the world, but the APS, the European Synchrotron Radiation Facility and Japan’s Spring8 are the only high-energy machines. They work by firing

pulsed beams of electrons out of a “gun,” speeding them up many times as they traverse a circular track to boost their energy, then releasing them into a larger, almost-circular track about three-fifths of a mile around whose straightaways are lined with large magnets.

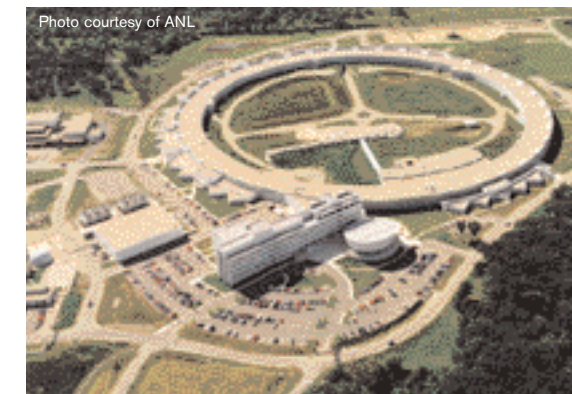
When the fast-moving electrons whiz past the strong magnetic fields, radiation is produced; the resulting X-ray beams are one trillion times brighter than those produced by scientists in the laboratory, yet can be narrowed to as fine a size as one micron — one-one-hundredth the width of a hair. About 70 different experimental stations lining the larger circle harvest X-ray beams with specific individual characteristics (known as “beamlines”) and direct the beams at

That ring produces some of the fastest, brightest, most powerful X-rays in the world — and scientists are beating a path to its door.

various materials, chemicals or other experimental samples. Such experiments might enable researchers to learn about the three-dimensional structure and the electronic properties of matter.

Keith Moffat, for seven years director of the university’s Consortium for Advanced Radiation Sources, uses APS for his research into the changing structures of light-excited proteins. (See cover story.)

Argonne National Laboratory’s Advanced Photon Source



He was closely involved in the original design of the beamlines to harness the X-rays and deliver them to their targets.

“We knew what we wanted, and we built it,” Moffat said. “These beamlines are the very best in the world for our purposes.”

Thousands of researchers from other institutions — assembled into “collaborative access teams” — have used the APS to conduct research in areas as diverse as materials sciences, chemistry, X-ray instrumentation, geophysics, environmental science and biology.

Moffat said he is excited about proposed new accelerators, which could supplement the APS ring and its 100-picosecond bursts of X-rays with even shorter, more intense bursts — and more precise still frames of the reactions he studies.

“There are fundamental reasons why you can’t make the X-ray pulses from the APS much shorter,” Moffat said. “But there are other ways to generate X-rays.”

In fact, accelerator physicists have already drawn up plans for a so-called linear accelerator: a differently shaped, differently constructed accelerator with a new, much longer sort of undulating magnet that will create still more intense X-ray beams than those at APS. Argonne and Stanford have tested both a miniature version of this accelerator and a so-called “bunch compressor” that can concentrate electrons into a very narrow beam. Initial experiments will be underway this year.

— P.K.