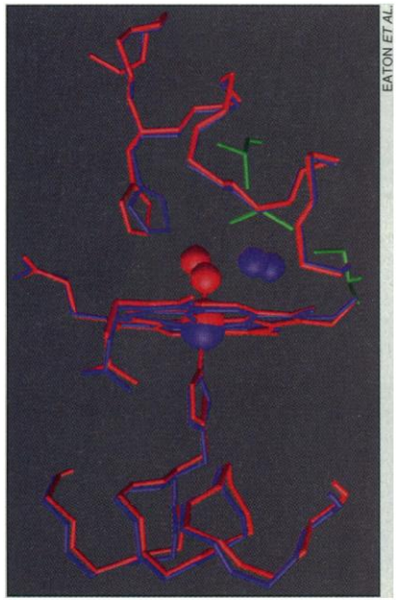
MOLECULAR MOVIES

Fast-Action Flicks Draw Chemists' Rave Reviews

Looking for the fastest paced action-adventure film of the summer? Forget Spielberg and Lucas. Keith Moffat and Michael Wulff have a couple of new, action-packed thrillers that are knocking the socks off viewers worldwide. They're leaving audiences "breathless," proclaims one reviewer. But don't bother with the popcorn. The movies are over so fast, you won't even have a chance to reach for a kernel. Still, what they lack in duration, they make up for in splendid closeup shots: They reveal—in exquisite, three-dimensional, atomic detail—how the show's stars, protein molecules, change shape as they undergo simple reactions.



EATON *ET AL.*

Double take. Superimposed models of part of myoglobin before (red) and after (blue) dissociation of carbon monoxide (double spheres). Residues in green contact dissociated CO.

These two new action flicks aren't the only ones on release. Scientists around the globe are turning out molecular movies of proteins, semiconductor crystals, and even simple molecules reacting in a gas, using short pulses of x-rays and electrons to freeze the action of molecules in motion. "We've dreamed of doing these kinds of things for decades," says Moffat, a biochemist at the University of Chicago. "Now they're finally happening."

The new movies are by no means the first efforts to detect high-speed changes in molecules. Researchers have used laser-based techniques for years to track the knitting and breaking of chemical bonds, events that occur in just a few quadrillionths of a second. Those techniques essentially just detect when the events occur, however. By contrast, the new x-ray and electron-beam schemes take repeated frames of the position of each atom in the spotlighted molecule, giving scientists successive snapshots of its complete atomic structure and a direct look at exactly how a host of chemical reactions unfold.

Just as film grew out of still photography, the new movies are an outgrowth of a molecular snapshot-taking technique known as diffraction, which is widely used to produce still lifes of molecules. Researchers begin by firing a beam of x-rays or electrons at a sample of aligned molecules—such as innumerable copies of a protein lined up in a crystal. By recording and analyzing how the waves or particles ricochet off the sample, the investigators can determine the precise location of each atom in a molecule.

Diffraction is traditionally done with a continuous beam of x-rays or electrons. But by pulsing their beam instead of leaving it on, researchers can create an effect like that of a strobe light, freezing the action of molecules in motion. And in recent years, by using shorter and shorter strobe pulses, researchers have been able to capture faster and faster action sequences. One problem, however, is that molecular moviemakers need roughly the same number of x-rays or electrons to make each diffraction image. So, as the pulses get shorter—and that's no easy feat in itself—researchers have had to find ways to boost the flux of their x-ray and electron beams.

For x-ray movies—at this point the most popular of the genre—that has meant turning to high-powered synchrotrons, which produce ultrabright flashes of x-rays. Most current synchrotrons can produce bright enough x-ray pulses that last about 10 milliseconds. But even that isn't short enough to capture the fastest action of proteins in real time, which occurs in just billionths of a second, or nanoseconds. So molecular moviemakers have had to come up with a variety of tricks to slow down the action, such as cooling their protein crystals to ultralow temperatures, which can drop the speed of a reaction 10 billion–fold (*Science*, 21 October 1994, p. 364).

Researchers worry, however, that the frigid temperatures may affect the behavior of their molecular actors. "You may not merely slow down the normal reaction," says Moffat. "You may get abnormal reactions at that temperature." But x-ray moviemakers have begun to solve that problem using a trio of new synchrotrons, such as the European Synchrotron Radiation Facility (ESRF) in Grenoble, France, which are so bright that they can pack enough energy into pulses lasting just 150 picoseconds (1000 picoseconds equal 1 nanosecond). In just the past 6 months, for example, Moffat and Wulff, an ESRF physicist, along with other colleagues, have used the 150-picosecond x-ray pulses at ESRF to produce the first pair of real-time movies of proteins in action at room temperature.

In the group's first such movie, the researchers unveiled the most detailed picture yet of how the iron-containing protein myoglobin—a common muscle protein—carries out its task of storing and releasing small molecules such as oxygen. In this case, the film tracked the release and rebinding of a single carbon monoxide molecule—a stand-in for oxygen—from myoglobin's central iron atom.

For each frame the Chicago-ESRF team shot, the action was triggered by blasting a crystal of myoglobin molecules in a vacuum chamber with a short burst of laser light. The light was precisely tuned to be absorbed by the molecule's iron-containing heme group, breaking the iron atom's bond to CO. A fraction of a second later, the researchers opened a high-speed shutter, steering a 150-picosecond x-ray pulse into the chamber to produce a diffraction image of the crystal. By taking multiple snapshots, varying the length of time between the trigger pulse and the probing x-rays for each one, the researchers assembled a movie that shows how CO drifts away from the iron and later rebinds. It revealed such details of the action as how the iron atom and others close by in the protein withdraw slightly from the protein's center after the CO–iron bond breaks, allowing the CO to drift away (*Science*, 6 December 1996, p. 1726).

The team's second feature shows the gyrations of a bacterial photoreceptor called photoactive yellow protein, as a small organic group in the protein absorbs a photon and drastically changes its shape in response. Together, these two releases show that high-speed x-ray movies have come of age for tracking real-time atomic changes in proteins, but there's still plenty of action that even these fast cameras can't capture. For that reason, other teams are working to come up with still faster x-ray pulses for tracking even speedier events.

At last month's Quantum Electronics Laser Sciences Conference in Baltimore, for instance, researchers led by physicist Chris Barty at the University of California, San Diego, reported creating subpicosecond x-ray pulses by firing ultrafast laser pulses at a moving copper wire, which then sheds the excess energy as x-rays. They used those pulses to produce x-ray–diffraction movies that track the initial atomic motions involved in the melting of laser-heated, gallium arsenide semiconductor crystals. And last fall, researchers led by

Charles Shank at the Lawrence Berkeley National Laboratory (LBNL) in California reported producing x-ray pulses lasting just 300 femtoseconds (or 3/10 of a picosecond) by firing near-infrared laser light across an accelerated beam of electrons; the energetic electrons essentially give the infrared photons a kick, boosting them to x-rays (*Science*, 11 October 1996, p. 236). The LBNL team has yet to produce images with its ultrashort, but as yet relatively low-flux, pulses. But "ultimately, we want to be able to look at chemical reactions as they occur," says Shank.

No matter how short, x-ray pulses will still miss plenty of action, such as reactions in gases rather than in solids like protein crystals. That's because x-rays interact only weakly with atoms: Generally, researchers need huge numbers of atoms lined up in crystals to deflect enough x-rays to provide high-quality images, explains Ahmed Zewail, a physicist at the California Institute of Technology (Caltech) in Pasadena. So Zewail and other moviemakers interested in tracking chemical reactions in gases have turned to short pulses of electrons, which interact with atoms more readily than x-rays do.

While electron-diffraction experts have also been making movies for years—in this case of gas-phase chemical reactions—techniques here, too, have been advancing rapidly. In the 13 March issue of *Nature*, for example, Zewail and his Caltech colleagues reported making the fastest paced electron-diffraction movies to date by shortening their electron pulses about 1000-fold, from several nanoseconds to about 10 picoseconds, using a femtosecond light pulse to create short bursts of electrons that were then focused on their target.

The Caltech team shot movies of molecules as they are torn apart by laser light. To do so, the researchers first used a laser to fire a pulse of photons into a vacuum chamber filled with a methane derivative containing two iodine atoms. The photons began breaking apart some of the methane molecules—essentially starting a reaction stopwatch. A second pulse, fired a fraction of a second later, hit a metal-coated cathode ray tube, stripping away the electrons and creating an ultrashort electron pulse, which was channeled into the vacuum chamber to produce a diffraction image of the dissociating methanes. In this case, those images don't reveal the exact position of each atom, because molecules in a gas are oriented randomly and are not lined up like those in a crystal. Nevertheless, because all the molecules have the same constituent atoms, the diffraction images are able to reveal the precise distance between atomic neighbors within the molecules.

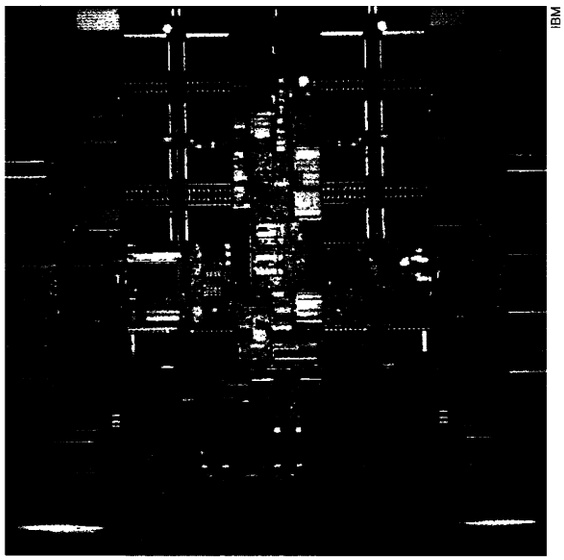
Buoyed by this success, the Caltech researchers hope to do even better. At this point, the movie only shows the methanes just before and after they break apart. To capture the bonds in the process of breaking will require shortening the pulses another 1000-fold, says Zewail's postdoc Jianming Cao. Nevertheless, says Carl Lineberger, a chemist at the University of Colorado, Boulder, "it's very exciting to see people taking steps toward seeing electron diffraction in real time." That excitement will undoubtedly grow if, as expected, the number of new movie releases begins to take off.

–Robert F. Service

MICROELECTRONICS

Catching Speeding Electrons in a Circuit City

Take a long-exposure aerial photograph of a city at night, and you will see traffic patterns traced in the bright streams and dense clusters of car headlights. The image (right), obtained by a team of researchers at the IBM Thomas J. Watson Research Center in Yorktown Heights, New York, is the equivalent shot of a functioning microprocessor, the 1997 S/390 used in the current generation of IBM mainframes. The "traffic" consists of electrons emitting light as they pass through the transistors, or crossroads, of this silicon city. By directly viewing such traffic patterns, circuit designers can look for weak spots and bottlenecks in the millions of components on a chip.



IBM

"Researchers have known since the 1980s that electrons emit light as they pass through the field-effect transistors [FETs] at the heart of most modern microchips," says Jeffrey Kash, of the IBM team that made the images. The light, which is in the near infrared and is extremely weak, can be detected only with cooled charge-coupled devices or special photomultiplier imaging tubes. Kash and his colleague James Tsang investigated this particular microprocessor because it consumed two orders of magnitude more current than it should have when it was not performing any operations. Kash and his colleagues obtained images of the chip in this "quiescent" state that showed a series of spots indicating that this excessive current was confined to a small portion of the chip. They couldn't tell which of the 7.8 million transistors were at fault, however, because they couldn't identify individual transistors in their images. "The issue was, how do you know where you are, how do you navigate," says Kash.

The team solved the navigation problem by spying on the individual FETs as they shuttled electrons around the chip when it was operating normally. "The only time a current is flowing is when you have a change of logic state," says Kash. The FETs produce picosecond light pulses as they switch on and off, so by photographing the chip in normal operation, Kash and his colleagues obtained a "road map" of the positions of the FETs. When the researchers superimposed this image on the photo showing the excess leak currents, they pinpointed exactly where the leaks occur.

The technique is useful for more than troubleshooting. "We can look at hundreds of thousands of FETs on a chip," says Kash, "and that is very helpful" in improving the design of subsequent chips. Indeed, Ingrid De Wolf of IMEC, Belgium's Interuniversity Microelectronics Center in Leuven, says optical-emission diagnosis of chips is beginning to spread throughout the microelectronics industry. Researchers are going beyond simple imaging, she adds: "We are now also trying to get more information from the spectrum of the emitted light, so we can measure the energy of the electrons."

–Alexander Hellemans

Alexander Hellemans is a science writer in Paris.