Science 16 May 1997:
Vol. 276. no. 5315, pp. 1027 - 1029
DOI: 10.1126/science.276.5315.1027

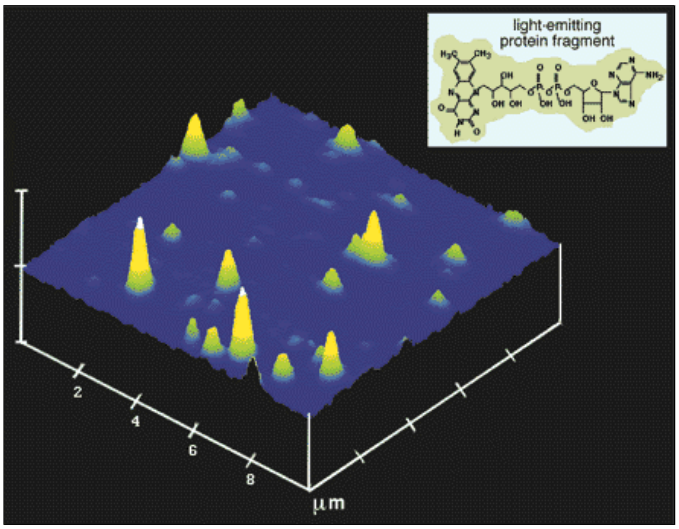
RESEARCH NEWS

Chemistry: Chemists Explore the Power of One

Robert F. Service

By tracking the behavior of individual molecules as they react, chemists are finding answers to long-standing questions ranging from how muscles contract to why light-emitting polymers burn out

SAN FRANCISCO--Look in any chemistry textbook, and you'll see an ideal version of molecular behavior, a far cry from what goes on in most research labs. In books, a basic chemical reaction is a transaction in which two single molecules create a third. In life, most chemists study the to and fro of trillions of molecules at the same time. That's fine for seeing the big picture of a reaction: what compound gets created and how long it usually takes. But chemists know that not all the untold numbers of molecules in a beakerful of solution react the same way, although the reasons behind this have long been shrouded in mystery.



Spotlighting the single life. Bursts of fluorescence of different intensity indicate the location of individual proteins in a thin film.

H. P. LU AND S. XIE/PNNL AND L. XUN/WASHINGTON STATE UNIV.

In recent years, however, a handful of researchers around the globe have begun using lasers and other instruments to lift the veil on how individual molecules behave, making the textbook view of reactions a reality. Initially, such single-molecule sightings--such as spotting the light coming from a single fluorescent molecule or recording the electronic blip when one ion gives off an electron--were little more than a novelty, proof that such sensitive detection could be accomplished. No longer. "The field is really beginning to explode," says Hansgeorg Schindler, a biophysicist at the University of Linz, in Austria. "Its usefulness cuts across all frontiers of science."

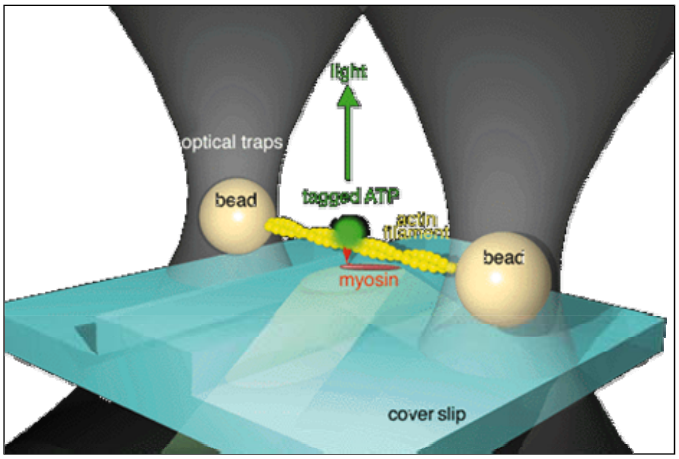
By looking at the behavior of molecules one at a time, the new techniques, which often rely on tiny samples, lasers, and sophisticated light detectors, have an unparalleled ability to reveal the precise timing of molecular events, including how a lone molecule changes its shape during a chemical reaction. At the American Chemical Society meeting held here last month--in a symposium dubbed the "Woodstock" of single-molecule imaging--researchers presented a raft of new results showing how monitoring single molecules can get to the heart of intractable scientific problems ranging from why all molecules of an enzyme don't work at the same pace to what limits the lifetime of new computer-display polymers.

First sight

Simply being able to see single molecules isn't new. Researchers have long been able to detect the presence of massive molecules such as DNA and many polymers using conventional optical and electron microscopes, while the advent of the scanning tunneling microscope in the early 1980s allowed researchers to image individual atoms on surfaces. But spying small molecules buried within solids or in solution--the natural environment for biomolecules--remained exceedingly difficult.

That began to change in 1989, when William E. Moerner and his colleagues at the University of California, San Diego (UCSD), first used a laser to "see" single, small organic molecules trapped inside a transparent host crystal. They scattered molecules sparsely throughout the crystal, shone the laser through it, and measured the light absorbed by the individual molecules.

Today, researchers still use lasers and sensitive photon detectors, but instead of monitoring absorption, they typically trigger and then detect brief light flashes from fluorescent tracer molecules, which are often linked to other molecules like proteins. The flashes reveal more than just a molecule's presence: By tracking the fluorescence over time, researchers can infer changes in the molecule's structure and activity.



Tracking myosin's moves. Optical traps and lasers allow researchers to measure how much force myosin exerts on actin--and how far it moves along the actin filament.

T. YANAGIDA/OSAKA UNIVERSITY

To view only one molecule at a time, experimenters typically confine a dilute solution of light-emitting molecules to an ultrasmall volume of liquid, for example by trapping the solution in a tiny, transparent capillary tube or in the matrix of a clear polymer gel. Then, they fire a laser at the sample, and zoom in with their microscope and photon detectors to capture any light emitted by molecules in the microscope's focal plane.

At the meeting, physical chemists Sunney Xie and H. Peter Lu of the Pacific Northwest National Laboratory (PNNL) in Richland, Washington, used a variation on this experiment to watch--for the first time--as individual enzyme molecules carry out their reactions in real time. Their goal, in part, was to learn why similar enzyme molecules react at different rates.

This puzzle was studied 2 years ago by Ed Yeung and Quifang Xue of Iowa State University in Ames, who used a single-molecule setup to show that identical copies of enzymes can churn out fluorescent products at rates that vary by up to a factor of 4. They suggested that the enzyme molecules--although they all had the same chemical sequence--folded themselves into different three-dimensional conformations, thereby affecting their chemical reactivity.

Meanwhile, other researchers had suggested that shape changes in individual proteins over time could also affect reaction rates. Past measurements had shown that even nonreactive proteins can jump between several stable conformations, implying that these quiescent proteins are continuously shifting their shapes, and leaving chemists wondering whether the proteins are more active in one conformation than another. But large ensemble measurements--in which researchers shine a laser beam into a solution containing a vast number of molecules to trigger a reaction and then probe it with another beam a fraction of a second later--have never been able to witness this level of detail.

To do so, Xie and Lu looked at naturally fluorescent enzymes called flavoenzymes. When a flavoenzyme catalyzes a reaction, it gains an electron, which blocks its ability to emit light, and then later loses the electron again. So, an individual molecule blinks on, off, and on again with each full catalytic cycle--something Xie and Lu were able to watch using their single-molecule setup. They found that the flavoenzymes blinked as often as once a millisecond as the protein carried out reactions one after the other.

Next, they put their flavoenzymes in a solution without any other reagents and shone a laser on single enzymes. Each molecule gave off a steady glow, indicating that it was a healthy enzyme ready to undergo a reaction. But the precise color of that glow continually shifted, likely reflecting subtle changes in the protein's shape, says Xie.

What's more, these colors fluctuated around an average value over the course of a millisecond to a second--roughly the same time scale as the enzymatic cycle. The similar tempos, says Xie, imply that these subtle conformational changes do in fact alter the protein's chemical reactivity. Such results are "very interesting," says University of Illinois protein-folding expert Peter Wolynes, because they may also help researchers track how proteins cycle between different stable conformations.

Motor molecules at work

Other researchers at the meeting disclosed equally compelling results on the dynamics of individual proteins, including the ubiquitous molecules that power muscle contraction. Researchers led by Toshio Yanagida from Osaka University in Japan traced the contortions of myosin, a protein that is found in all eukaryotic cells and is at the heart of muscle contraction. Myosin converts a cell's chemical energy--adenosine triphosphate, or ATP--into mechanical force by lining up with others of its kind in strands that tug on neighboring filaments composed of the protein actin. In relaxed muscle fibers, these filaments overlap by a small amount. But during muscle contraction, myosin proteins bind to actin filaments, forcing the neighboring filaments past one another and increasing their overlap, thereby shortening the muscle.

A long-held model for this process holds that one ATP molecule reacts with one myosin molecule (a reaction known as ATP hydrolysis), causing the myosin to change shape and march down the actin filament by one step. And in 1994, Yanagida and his team became the first to spy on this process. By tracking individual fluorescent-labeled ATP molecules, they saw bursts of light as myosin molecules reacted with ATP and bound to actin. They then watched as these flashes marched down the actin filament, corresponding to steps taken by the myosin.

Meanwhile, the Osaka team and others had developed another laser-based technique to measure the minuscule force--just one piconewton--that an individual myosin molecule exerts on actin. At the ACS meeting, Yanagida reported combining these previous experiments into one--in

the first simultaneous measurements of the chemical and mechanical behavior of a single molecule. And he arrived at some surprising findings.

The team attached plastic beads to the two ends of an actin filament and held them steady with a pair of laser beams (see diagram). Another laser then measured the nanometer-scale movements of one of the beads when a myosin molecule bound to the actin, providing a measure of the mechanical force exerted by the myosin. Meanwhile, the researchers used their fluorescence monitoring setup to confirm that they were seeing single myosin molecules attach and march down the actin filament. When tracked together, the researchers found that sometimes, a single ATP hydrolysis reaction caused myosin to move along the actin by two or three steps, exerting a piconewton of force at each step. "That's basically blasphemy for motor protein experimenters," says Shimon Weiss, a single-molecule expert at the Lawrence Berkeley National Laboratory in California.

Indeed, if the finding holds up, it will likely revolutionize ideas about myosin's behavior. The multiple steps suggest, says Yanagida, that "the chemical energy driven by ATP hydrolysis is stored in the myosin protein and slowly released," rather than being used all at once. Myosin, he says, may act like a spring that releases its energy in a series of small steps, perhaps as the protein moves through a series of conformations. Yanagida quickly acknowledges, however, that "there has been no evidence for that using conventional techniques."

Few myosin specialists heard the talk at the chemistry meeting, and they say that whether it is accurate "is hard to know without seeing the full picture" of the group's as-yet-unpublished results, says Ron Vale, a motor protein specialist at the University of California, San Francisco. "But in theory, this is the definitive experiment," he says, because it allows researchers to directly correlate the chemical and mechanical reactions taking place. Still, says Xie, the hypothesis of slow-acting myosin requires "extreme proof."

Yanagida doesn't have that proof yet, but at the meeting he described still another single-molecule experiment that is at least the first step: He showed that after binding to ATP, myosin can indeed adopt several stable conformations. He and colleagues tagged separate parts of a myosin molecule with two different fluorescent groups, which emit distinct colors of light depending on their proximity to each other. Then, they blasted the protein with laser light and watched the resulting flashes of color. They were able to see that after myosin bound to ATP, the labeled regions ended up in several different positions relative to one another. "That is a suggestion the protein is really changing its [conformation]," says UCSD's Moerner. Next, the Osaka researchers must link these two results, showing that myosin winds its way through several conformations as it marches several steps down the actin filament.

Preventing burnout

Single-molecule experiments are opening a window on the workings of artificial molecules as well. For example, at the meeting, University of Minnesota researchers led by physical chemist Paul Barbara described new work that begins to explain why light-emitting polymers suffer burnout. In recent years, researchers have been struggling to make these polymers into flexible panels for computer displays, but the polymers' fast burnout has hampered commercialization (*Science*, 16 August 1996, p. 878).

Such polymers work by absorbing energy from lasers or an electric current and later reemitting it as photons of light. But the light output of the films can drop by as much as 50% in just a few minutes. Researchers haven't known why--whether 50% of the individual light-emitting molecules turn off completely, or whether all the molecules drop their light output by 50%. "There's no way to find that out except by looking at each molecule independently," says Barbara.

So, his team did just that. They started by adding trace concentrations of a yellow light-emitting polymer known as poly-p-pridylene-vinylene (PPyV) to a film of a nonemitting polymer. Then, they trained a pair of lasers on the film to excite light emission from the scattered PPyV molecules, and zoomed in with a microscope to watch the action unfold.

"What we found really surprised us," says Barbara. The molecules neither immediately winked out nor dropped their emission slowly. Rather, individual polymer molecules blinked on and off thousands of times before going dark for good. Barbara theorizes that the incoming laser light triggers the blinking: By kicking an electron off the polymer, the light creates a defect in the chain that causes excess energy to be released as heat instead of light. If a free electron jumps back onto the polymer, the light switch is turned back on. Eventually, however, another unknown type of defect quashes the light emission. Whether such results will help chemists design longer lasting polymers is "still too early to say," says Barbara. In any case, he adds, "it will give them some new things to think about."

Polymer chemists won't be the only ones with new findings to ponder. Single-molecule experimenters are also exploring a host of other areas, including rapid DNA sequencing by detecting the subtle light-emission differences in the molecule's four bases, imaging proteins in cell membranes, and creating optical data-storage systems. These initial forays into the tiny world of single molecules show that life in the lab is not only catching up to the idealized view in chemistry textbooks; it is rewriting them.

Science. ISSN 0036-8075 (print), 1095-9203 (online)