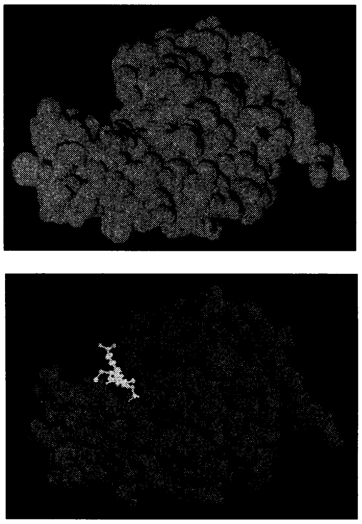
MICROSCOPY

A New Way of Seeing Proteins in Motion

It's easy enough to time a heartbeat or the speed of a nerve impulse. But some of the most basic biological movements occur on a much smaller—and faster—scale, when a protein changes shape. These minute contortions speed up reactions, control gates across cell membranes, and make muscles contract. Yet directly detecting these conformational changes of enzymes or other types of proteins has been beyond the reach of any instrument, and researchers had to content themselves with "before" and "after" snapshots. Now, however, scientists have put their finger—or, more precisely, a microscopic probe—on an enzyme in the midst of chewing up its target molecule or substrate. And as they did so, they felt its jaws move.



RADMACHER AND FRITZ/UC SANTA BARBARA

Reaction shot. These models of lysozyme show that after a reaction *(bottom)*, the chain on the right side of the molecule extends out farther, increasing the lysozyme's diameter.

On page 1577, Manfred Radmacher, Monika Fritz, Helen Hansma, and Paul Hansma, all of the University of California, Santa Barbara (UCSB), report lowering the tip of an atomic force microscope (AFM) onto an egg-shaped enzyme called lysozyme while it was reacting with another molecule. During one fraction of a second, the lysozyme appeared to change its shape, growing in height as its binding cleft grabbed the molecule.

Such reactions have previously been seen only indirectly, as researchers detected changes in shape by looking at a protein before and after a reaction with x-ray crystallography techniques or measured the reaction dynamics of many protein molecules in solution by spectroscopy or other means. The UCSB team's experiment "is the first attempt to try to see a signal of a dynamic process at a molecular level. So in that sense, this is a first, and it's very exciting," says Carlos Bustamante, a biophysicist at the University of Oregon. Scientists like Bustamante are excited by the promise of being able to measure real-time shape changes that are too slight or too quick to detect in other ways.

The AFM was able to perform this observational feat because it measures attractive and repulsive forces on an atomic scale. The scope has a sensitive tip that is usually dragged over a surface; as the tip encounters bumps and dips, the sample surface is raised or lowered to keep the contact force constant. These changes are used to plot molecular or atomic contours.

The UCSB group homed in on yet another level by using the AFM to monitor a process within a single molecule. They placed the tip on mica coated with a one-molecule-thick layer of lysozyme, a common enzyme whose function is to split molecules called polysaccharides in bacterial cell walls by adding a molecule of water to a bond. Crystallographic data indicate that, as the lysozyme grasps the substrate in its wedge-shaped binding cleft, the enzyme becomes slightly thicker.

The researchers set the AFM tip to tap lightly on the lysozyme about every 50 microseconds for a 32-second period, because leaving it in constant contact with a single spot would damage the molecule. The height measurements were constant when the lysozyme was covered by plain buffer. But when an oligoglycoside, a polysaccharide fragment, was present in the buffer, spikes indicating an increase in height appeared in the AFM signal. The spikes disappeared when the investigators added an enzyme inhibitor, indicating the spikes were the result of the enzyme's reaction.

But the group "is cautious with [its] interpretation" of whether or not the height changes corresponded directly to a change in the diameter of the enzyme, Radmacher says. That's because the experiment produced some anomalous results: The height measured for the lysozyme and the magnitude of the subsequent shape change were about twice what the scientists expected. The researchers are trying to determine whether elastic or other forces might exaggerate their measurements.

The greater value of the group's experiment, however, is not what it showed about lysozyme, but that it demonstrated a potential new use for the AFM, Radmacher says. Scientists "have learned that we have the capability," Bustamante adds. "Now people will think of applications." Iowa State University molecular biologist Eric Henderson, for one, can see setting the AFM's tip on ribosomes, the cellular machinery for translating RNA into proteins. During RNA translation, ribosomes are "like a car factory with arms and robots swinging around, but we don't know how the arms are interacting," he says. The AFM may give scientists a view that takes them right down on the factory floor where the proteins are working.

–Jocelyn Kaiser

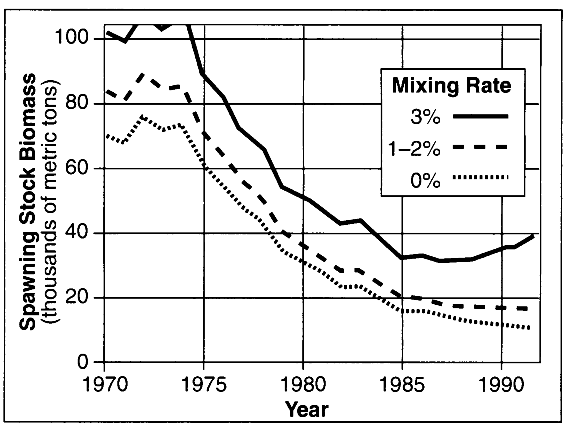
FISHERIES MANAGEMENT

Tuna Stocks: East Meets West

In the western Atlantic, bluefin tuna are a hot commodity: Many end up in the seafood markets of Japan, where a big one can fetch as much as $10,000. That demand has been linked to a reported sharp decline among the big fish in the Western Atlantic—a population plunge of perhaps 85% since 1975. Environmentalists have used these reports, based on catch data from fishers, to campaign to have bluefin listed as an endangered species.

But a new report from the National Research Council (NRC) says these reports of tuna decline have been exaggerated, and stocks have actually been stable since 1988. The estimates have been revised largely because of evidence that there is a "significant" mixing of tuna from the eastern and western parts of the ocean, with eastern fish replenishing western fisheries.

The notion of separate tuna stocks was embraced in 1982 when declining catches led to the formation of the International Commission for the Conservation of Atlantic Tunas (ICCAT), a coalition of 22 nations including the United States. The ICCAT divided the Atlantic down the 45 degree longitude line into eastern and western regions, each with its own spawning ground. And it began setting catch quotas in the west.



Fish lines. NRC projections show decline in western Atlantic tuna stocks would vary depending on the degree of trans-Atlantic tuna exchange.

Last year, worries about a continuing tuna decline, based on ICCAT statistics, led the National Oceanic and Atmospheric Administration to ask the NRC to do its own assessment for the benefit of this year's ICCAT meeting in November. The report, "An Assessment of Atlantic Bluefin Tuna," concluded that the situation in the west is not that bad. While ICCAT estimated that the 1993 catch was only 78% of the 1988 catch, NRC found "no evidence that abundance of western Atlantic bluefin tuna has changed significantly" in that period.

The committee, chaired by zoologist John Magnuson, director of the Center for Limnology at the University of Wisconsin, reported that one of the main factors in the revised assessment was the rejection of the "two-stock hypothesis." The panel pulled together all available tagging data for Atlantic bluefin and concluded ICCAT was coming up with "skewed" estimates by treating the two sides of the Atlantic separately. The committee estimated that about 2% of eastern tuna head west each year, and 1% of the westerners go east. An annual transfer rate of 2% may seem small, but it's far from trivial, as it means that over 10 years, some 20% of the fish on one side of the Atlantic may have moved to the other.

Whether ICCAT will revise its policies to accommodate NRC findings and recommendations remains to be seen. Said one NRC committee member, Terrance J. Quinn of the University of Alaska in Juneau: "Our job as scientists, assessors, and managers gets a lot more difficult under the mixing hypothesis," because now data from both sides of the Atlantic have to be integrated. Fish movements and changes in distribution are little understood, Quinn says, and there's not enough genetic data to be able to track subgroups. Getting a handle on Atlantic tuna, it seems, is a slippery business.

–Constance Holden

MEETING BRIEFS

Astronomers Gossip About The (Cosmic) Neighborhood

The Hague, Netherlands, last month welcomed 2000 astronomers from around the world for the 22nd General Assembly of the International Astronomical Union (IAU). From 15 to 27 August, they participated in symposia and discussions on topics ranging from the down-to-Earth issue of light and radio-frequency pollution to the creation of elements at the farthest reaches of time and space, in the big bang. Some of the most striking news, however, came in new findings from our galaxy and its immediate surroundings.

New Galaxy on the Block

Much of the glory in astronomy comes from probing the farthest reaches of the universe with the largest telescopes. But poking around closer to home with more modest instruments has its own rewards—such as finding a sizable galaxy right in the Milky Way's own backyard. That's what happened recently, when radio astronomers turned the 25-meter Dwingeloo radio telescope in the Netherlands—a puny thing next to the 100-meter dishes and miles-long arrays common in radio astronomy—on a neglected patch of sky.

The new galaxy, designated Dwingeloo-1, is massive enough to be influencing the motion of our own galaxy and others nearby, its discoverers reported at the IAU meeting. Yet earlier searches with optical telescopes overlooked it because they tended to bypass the region of sky behind the Milky Way; it was too difficult to distinguish anything behind the dust and stars in the Milky Way's disk. To penetrate that dusty cloud, Renee Kraan-Korteweg of the University of Groningen and collaborators from the Netherlands, Britain, and the United States organized the Dwingeloo Obscured Galaxy Survey last year. By searching not for visible light but for the 21-centimeter radio waves emitted by atomic hydrogen gas in unidentified galaxies, the researchers hoped to spot them through the haze of the Milky Way.

The first hints that they had succeeded came on 4 August, when the Dwingeloo telescope picked up radio emissions with a spectral signature indicating they came from a massive, rotating collection of stars and gas—presumably a spiral galaxy. Soon afterward, the much larger Westerbork Synthesis Radio Telescope confirmed that the signals were emitted by a galaxy spinning at about 100 kilometers a second. Within days, the astronomers contacted colleagues who photographed the galaxy at infrared wavelengths with the United Kingdom Infra-Red Telescope on Mauna Kea, Hawaii. The images revealed starlight and a hint of a spiral pattern.

The Dwingeloo group estimates that the new galaxy lies just 10 million light-years away, about five times farther than Andromeda, the nearest large galaxy. From its size and rotation speed, the researchers estimate the galaxy's total mass to be about a quarter that of the Milky Way. That makes Dwingeloo-1 far too big to be ignored any longer; it's big enough, they say, to be tugging on the Milky Way and dozens of its neighbors, altering their wanderings through space.

Cosmic Lithium Factories

For an element, lithium has a delicate constitution. Originally forged in the big bang, astronomers believe, lithium is quickly destroyed in the hot interiors of stars, leaving only faint traces visible in the light from a star's surface. So it came as quite a surprise 2 years ago when a group of astronomers announced that they had spotted a normal, sunlike star in which lithium was 10,000 times more abundant than it is in ordinary stars. What made the finding even more surprising was that the suspect star belongs to an x-ray–emitting binary system known as V404 Cygni, in which the other member is thought to be a black hole. A black hole's violent surroundings seemed an even less hospitable environment for lithium than normal stars are.

But it now seems that lithium and x-ray binaries are natural companions. At the IAU meeting, the same research team, Eduardo Martin and Rafael Rebolo of the Instituto de Astrofísica de Canarias in Tenerife and Jorge Casares and Philip Charles of Oxford University, announced that they have detected signs of abundant lithium in two other x-ray binaries. One, A0620-00, is also thought to contain a black hole; the other, Centaurus X-4, harbors a different kind of powerhouse, a neutron star. Now the researchers are trying to understand how these violent objects might step out of character to create rather than destroy lithium.

One possibility, they say, is that lithium is created near the neutron star or black hole, where material sucked in by the object's potent gravity is accelerated to very high speeds. High-speed helium nuclei might collide with each other and combine to form lithium through a process known as spallation. The blast of radiation emitted by the superheated matter in the black hole's accretion disk—the last way station for infalling material before it slips into the black hole itself—might then blow some of the lithium-rich material outward onto the sunlike companion star.

An alternative scenario comes from Rashid Sunyaev of the Space Research Institute in Moscow, who believes the lithium might be formed where it is detected—in the companion star's atmosphere. He thinks some high-energy helium nuclei might escape from the black hole or neutron star and plunge into the atmosphere of the sunlike