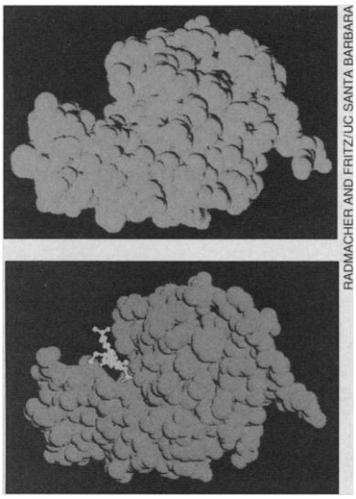
MICROSCOPY

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

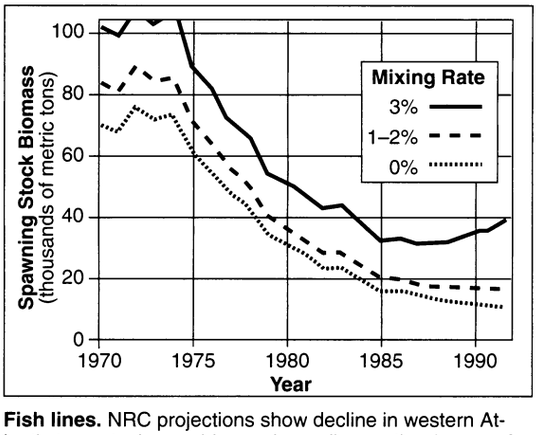
FISHERIES MANAGEMENT

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.