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Twitching Proteins Caught in the Act

New research has shown that some enzymes fidget constantly, even as they wait to catalyze a reaction. The study, from Howard Hughes Medical Institute investigator Dorothee Kern, shows that enzymes may not be nearly as passive as scientists once thought.

Biologists have long thought that binding of substrate molecules triggered enzymes to change shape. But Kern has discovered that one key enzyme—and likely many more—snaps shut by itself. This change of shape happens even before the enzyme, called adenylate kinase, binds to its substrates.

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The general notion in enzymology has been that the substrate [on which the enzyme acts] induces the shape change, said Kern, who is also a professor of biochemistry at Brandeis University. We found that this is not true. The enzyme, in fact, changes conformations without the substrate. It's really a shift in the paradigm.

In two papers published November 18, 2007, in advance online publications of the journal *Nature*, Kern and her team detail three years of experiments that prompted the paradigm shift. Employing five different advanced techniques, the team painstakingly constructed a near real-time movie of adenylate kinase in action. Adenylate kinase is a key enzyme that processes adenosine triphosphate, or ATP. ATP is known as cellular gasoline because it acts as cells' primary energy source.

Each method [we used] only gives a partial glimpse of what's happening, said Kern of her multi-faceted approach. It's only when you combine them that you get a more complete picture. The combined data show the clam-shaped enzyme rapidly twitching before snapping shut.

First, the researchers used x-ray crystallography to obtain static snapshots of the protein. In x-ray crystallography, protein crystals are bombarded with x-ray beams. As the x-rays pass through and bounce off of atoms in the crystal, they produce a diffraction pattern, which can then be analyzed to determine the three-dimensional shape of the protein. Kern noticed something strange in the data—various molecules of the enzyme appeared to have quite different shapes in the crystal. Later, Kern realized she had taken three snapshots of the enzyme at three different points as it snapped shut.

We got a little glimpse from our x-ray data that there was something interesting going on, Kern said.

Next, Kern measured the internal motion of the enzyme with a technique called nuclear magnetic resonance (NMR). NMR can detect the motion of individual atoms within a protein. With it, Kern saw that certain parts of the enzyme—the two large flaps she calls lids—oscillated every millisecond or so.

X-ray crystallography gave us detailed structural information but no kinetic information, said Kern. NMR gave us the kinetic information, how fast the enzyme moved, but very limited structural information. These techniques are very complementary.

Kern then moved to a supercomputer, where she ran simulations of the enzyme in motion. We took the starting structure and then calculated how it changes shape over time, said Kern. It's a very powerful technique which can provide both spatial and dynamic information, but there are reservations about it among experimentalists. They say it's always possible to calculate something, to make a movie of the protein, but how do you know it's right?

In this case, the computational results data matched experimental data. The computer-generated movie traced the three states of the enzyme Kern had seen via x-ray crystallography, confirming those experiments. However, there was one big discrepancy. The computer simulations showed the intermediate positions between the open and closed states transitioning in just a few nanoseconds—a very rapid twitching—whereas the motion detected in the NMR experiments took place in a about a millisecond — five orders of magnitude slower.

To unravel the physical origin of this time-scale discrepancy, Kern enlisted the perfect accomplice: her little brother, Christian Huebner, a physicist at Martin Luther-University Halle-Wittenberg in Germany, Kern's country of origin. The pair watched the motion of single molecules using fluorescence resonance energy transfer (FRET)—an analytical method known colloquially as the molecular ruler. This technique detects changes in the relative distances between two fluorescent dyes. In this case, Kern labeled the tips of the two large lids of the enzyme with two different colors. As the lids moved, the laser apparatus built by her brother measured the motion in real time. These data showed the lip snapping shut over a distance of just 10 angstroms, or 10-millionths of a centimeter, in about a millisecond. This experiment suggested that once in a while, the enzyme closes all the way on its own, in

the absence of any target proteins.

Finally, Kern ran a variation on the earlier NMR work, using a technique called paramagnetic relaxation enhancement. In these experiments, Kern labeled one atom of the enzyme with a chemical group with paramagnetisms, and then measured its distance to every other atom in the molecule. It was a home run, said Kern. The closed state of the enzyme looked just like the one during catalysis of the substrates.

A picture emerged in which the enzyme adopts its intermediate — half-open, half-closed - state every few nanoseconds. Only rarely does the enzyme close all the way, snapping its lids shut in milliseconds. That's when the enzyme is getting down to business and can bind with its substrates to perform catalysis, said Kern.

Kern then searched for the physical origin of these large-scale, relatively slow motions. She pinpointed eight segments of the enzyme where she thought the twitching occurred. We superimposed the open and closed states of the enzyme and saw which parts were kinking, said Kern. Strikingly, the atoms in those regions—the hinges—wiggled more than the atoms in other parts of the protein.

By comparing the versions of the adenylate kinase enzyme used by two different organisms - one that lives in an environment where temperatures exceed 200 degrees Fahrenheit and one that thrives in more civilized temperatures — Kern found that thermal energy can cause rapid fluctuations in the positions of individual atoms that, collectively, lead to slower, transient changes in the shape of the protein. Importantly, Kern said, enzymes have evolved to preferentially adopt the shapes they need to catalyze biological reactions.

The novelty here is the combination of the methods to make a near real-time movie of what's going on in the enzyme. None of the methods individually would have solved the problem. Together, Kern says, these techniques can help scientists reveal what she calls the "dynamic personality" of enzymes, and develop a realistic picture of their function.