

Freeze-Framing a Fidgety Molecule

A sister-brother team conspires to pin down the ultrafast movements of enzymes in action.



Dorothee Kern is changing the way scientists think about enzymes—proteins that speed up chemical reactions.

Leah Fasten

DESPITE THEIR HEATED SCIENTIFIC DEBATES AS KIDS IN GERMANY, DOROTHEE

Kern never imagined turning to her little brother to collaborate on her research. But Christian Huebner, three years her junior, is now a physicist and offered just the know-how Kern needed to resolve a puzzle about the behavior of a restless protein. ¶ With his help, Kern, who has previously collaborated with her parents—also scientists in Germany—

made sense of her experimental observations that the protein could adopt three distinct conformations in one crystal. In the process, she turned a long-standing biochemistry assumption on its head.

Kern, an HHMI investigator at Brandeis University, studies the dynamics of enzymes—proteins that speed up chemical reactions by clamping onto one or more substrates and efficiently converting them into products. In this case, she was studying adenylate kinase, an enzyme that processes ATP, ADP, and AMP—molecules that give cells energy and are building blocks of DNA. Adenylate kinase exists in every organism, from bacteria to humans. Kern wanted to know how the enzyme adapted to one of its most extreme environments—inside bacteria that thrive at 220 degrees Fahrenheit in deep ocean vents. Most proteins unravel at such high temperatures.

She already knew the molecular structure of adenylate kinase at more moderate temperatures but not what the heat-loving version looked like. “We really needed a high-resolution structure to see subtle differences,” says Kern.

She and her colleagues turned to x-ray crystallography—they bombarded crystallized protein with x-rays and used the resulting diffraction pattern to determine the protein’s three-dimensional arrangement. “We thought it would be easy,” says Kern.

Not quite. The data gave a jumbled picture of atoms that seemingly existed in three places at once. Kern’s take on this puzzling result: three different structures were present in a single crystal.

“We had one crystal of one unique protein, but three conformations of the protein,” says Kern. “These are just snapshots though—static and frozen in the crystal.”

What Kern needed was a way to measure whether the protein, when it wasn’t stuck in a crystal, actively alternated between these structures. Nuclear magnetic resonance (NMR), which can detect the movement of atoms, provided just such an approach. Using NMR, Kern’s team calculated that the protein switched conformation about every millisecond or so, but they were not able to see exactly which conformations.

To bridge the information obtained by crystallography and NMR, Kern’s team performed a third experiment—a computer simulation that calculated how fast the molecule could move between the structures seen in the crystal. The simulation only added confusion. It indicated that the enzyme could move between the open and partially closed states seen in the crystal in nanoseconds—that is, thousands of times faster than the milliseconds suggested by NMR.

Kern had an idea of what was going on but needed the help of her brother, a physicist at Germany’s Martin Luther University Halle-Wittenberg, to experimentally reveal the inner workings of adenylate kinase. “It was a real collaboration where we were flying back and forth across the ocean,” says Kern, who credits her light-hearted brother for making the partnership lots of fun.

They performed single molecule fluorescence resonance energy transfer (FRET) experiments—tacking fluorescent molecules onto the upper and lower lids of

the clam-shaped enzyme and tracking its opening and closing by measuring changes in fluorescence. Huebner had designed and built a unique ultrasensitive laser that allowed precise measurements and time resolution in microseconds.

The siblings found that every few nanoseconds—as the computer simulation had shown—the enzyme twitches partially shut. But every few milliseconds—as suggested by NMR—it closes all the way.

The traditional view had been that an enzyme snaps shut only when it makes contact with its substrate. But Kern and Huebner showed that an enzyme can constantly fidget and take on a new structure without that contact. And it’s not just in this one instance—Kern has detected twitching in the handful of other enzymes she’s tested so far using NMR. “This is really a paradigm shift,” she says. “We want to encourage scientists to consider that this happens with their own systems. So far, it seems that these short-lived, higher energy states quite often are the biologically active states.”

Returning to her original question on heat-loving adenylate kinase, Kern performed new experiments showing that the hinges between the lids of the enzyme are more rigid in heat-loving bacteria, which slows the enzyme’s movements, presumably keeping it from unraveling at high temperatures. The team’s findings appeared in two papers online in *Nature* on November 18, 2007. Next, Kern wants to find out just how the protein manages to switch states without losing its structure.

“The risk of a protein being flexible is that it can fall apart,” she says. “The fascinating part to me is how nature keeps that from happening. These proteins are really living on the edge.”

As for the sibling team, the collaboration continues. “He’s simply the best,” says Kern of Huebner. Calling the teamwork between the labs “electrifying,” Kern says, “The combination of these different biophysical techniques provided us with a much deeper understanding of the fundamental principles of protein function. We’re already planning new projects together.” ■

—SARAH C.P. WILLIAMS

“The risk of a protein being flexible is that it can fall apart. The fascinating part to me is how nature keeps that from happening. ”

DOROTHEE KERN