

STRANGE ACTION AT THE ACTIVE SITE

Quantum simulation of an enzyme reaction gives initially baffling results that point toward a surprising new insight

STRUCTURE
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For the sake of discussion, let's say that enzyme biochemistry is like football. You can represent an enzyme's reaction mechanism with diagrams that resemble the Xs and Os of a football play. For both football and enzymes, there's a series of steps that have to happen for things to work. A ball carrier has to get the handoff just in time to hit the hole, for instance, and the hole has to open when he gets there.

Within a living cell, the atoms of an enzyme are like superfast, super-small players. Enzyme reactions often occur in timeframes approaching a nanosecond (a billionth of a second) and distances of an angstrom (a hundred-millionth of a centimeter) or less. And if something isn't working as it should, the result can be disease or death.

Researchers can't see enzyme reactions within a living cell, but with help from powerful supercomputing systems they can simulate them and see what happens in minute detail. They can make animations from the simulation and look at the reactions as if watching a movie. Using these sophisticated and powerful tools, PSC scientist Troy Wymore and University of Pittsburgh biochemist John Hempel have simulated reactions in an important enzyme family called ALDH (aldehyde dehydrogenase).

Genetic malfunctions in human ALDH lead to a variety of debilitating disorders. ALDHs also affect the cancer-fighting activity of one of the most-used chemotherapy drugs. In 2002, with better understanding of ALDH reactions as the goal, Wymore, Hempel and their PSC colleagues David Deerfield and Hugh Nicholas focused on a particular ALDH in humans, ALDH3. Their detailed quantum-mechanical simulations identified a

novel mechanism for a step in the enzyme reaction that had been generally disregarded but which their work showed to be crucial.

The mechanism they found — a proton transfer from the "backbone" of the enzyme's structure — may be a key to understanding ALDH-related disease. "We think these diseases occur because something interrupts this proton transfer," says Wymore. "What we think we've done is give an *atomic* rationale for ALDH-related metabolic diseases. It opens the door to possible intervention strategies."

To further explore their hypothesis, in 2006 they used PSC's newest system, the Cray XT3, for more simulations. This time they focused on another ALDH, ALDH2, and the simulations showed an unexpected intermediate reaction step, one never seen experimentally and completely unexpected — to such an extent that the researchers had to pause and think about the validity of the ALDH2 model they used. It seemed like a back-to-the-drawing-board moment.

Not long after, however, Hempel learned of a laboratory study that showed the same never-before-observed reaction step. This not only confirmed their surprising simulation result, it also suggested they had flipped a page in ALDH chemistry to uncover a more complex and more complete description of ALDH's reaction mechanism.

"This shows," says Hempel, "that simulations of enzyme chemistry have to be taken seriously. They reveal details you can't otherwise see, and as a result we find that now we have to look closely at things nobody thought needed to be considered."

A DEEPER LOOK

At least 18 different versions of ALDH are known in plants and animals, and they all do essentially the same thing. They take a toxic molecule called an aldehyde, produced during metabolism, and change it (oxidize it) into a form (carboxylic acid) that can pass safely out of the cell and into the bloodstream. In humans, malfunctioning ALDH is involved in two known diseases, one of which, an inherited disorder called Sjögren-Larsson syndrome, leads to skin scaling and mental retardation.

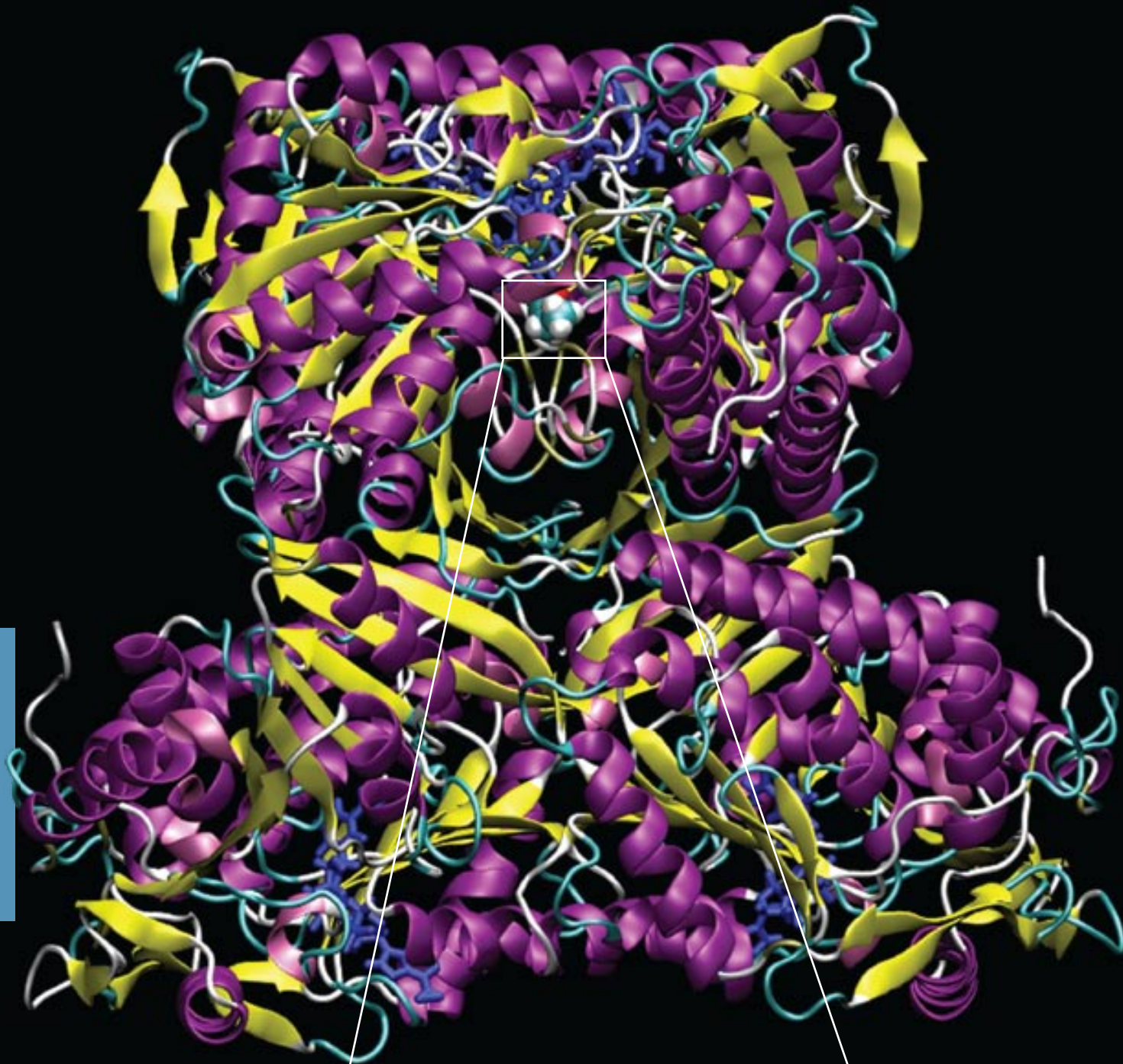
ALDHs are also involved in cancer therapy. A widely used chemotherapy drug breaks down in the body to an aldehyde, and the normal action of ALDHs interferes with the drug's ability to destroy cancer cells, requiring higher doses with hard-to-tolerate side effects. In this context, researchers would like to be able to create drugs that reduce ALDH's ability to react with aldehydes, at least in the local area of a tumor under treatment.

In both cases, what's needed is deeper understanding of how ALDH works. With the promising clue of their work on ALDH3, a complicated proton-relay mechanism in the enzyme's "active site" — where the reactions occur, Wymore decided to look at ALDH2 for similar results. Again, as with ALDH3, he employed a powerful approach called quantum mechanics/molecular mechanics (QM/MM) — a hybrid approach that tracks the movement of electrons and protons with quantum theory in the active site and uses a less computationally demanding method to keep track of the atoms in neighboring parts of the enzyme.

Wymore used a precise structure of ALDH2 obtained recently with x-ray crystallography. He worked closely

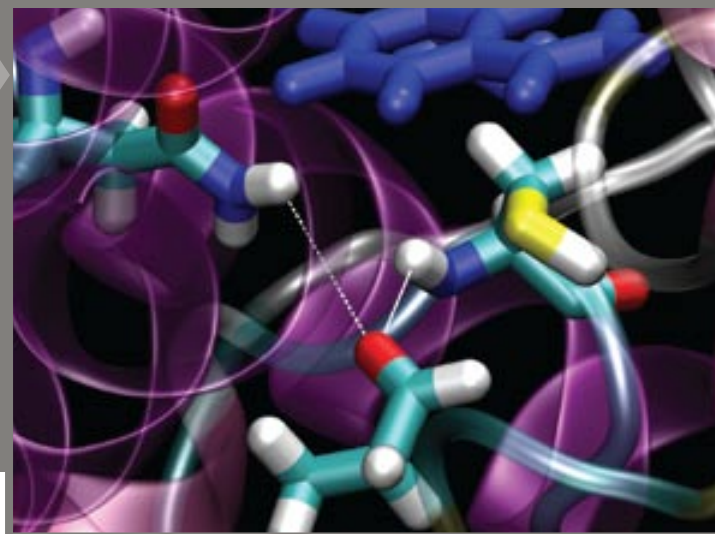


Shawn Brown, Troy Wymore, John Hempel



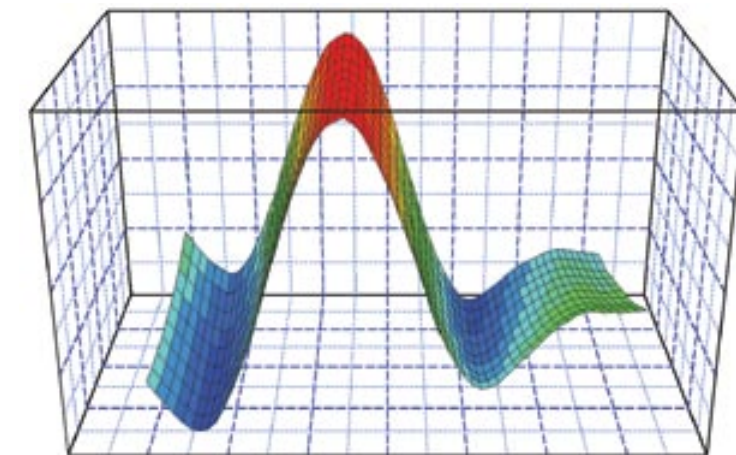
Zooming-In on the Active Site

This closeup of the active site of human ALDH2, shows the region of Wymore's QM/MM simulation in which the enzyme reaction was simulated quantum-mechanically, to capture the breaking and making of bonds as the reaction proceeds from reactants to products. The stick-figure closeup shows the NAD co-enzyme (upper right) and the aldehyde substrate (center), held in the active site by a carbon-sulfur bond and with hydrogen bonds (dotted lines) before this stage of the reaction begins. Colors represent atoms: N (dark blue), S (yellow), H (white), C (light blue), O (red).



Free Energy

This graphic is a "free-energy profile" that represents by vertical height the amount of energy required for a reaction to occur. (Colors correspond to energy, increasing blue-to-green-yellow-orange-red.) This profile shows results of Wymore's simulations of the proton transfer from ALDH's backbone (right side of plot) to an oxygen atom on the aldehyde substrate (left side of the plot), an intermediate step in the overall ALDH reaction mechanism. The front-to-back dimension corresponds to distance between a carbon atom in the aldehyde substrate and the active-site sulfur (in a cysteine amino-acid residue) to which it binds. The profile shows that the C-S bond becomes stronger (increased slope in the low-energy valley) as the proton moves from the nitrogen to the oxygen atom.



A NEW PICTURE EMERGES

The calculation ran for 9,450 XT3 processor hours. The surprising result showed a sulfur atom from ALDH2 reacting with NAD to form NAD-S, a reaction that, as far as Wymore and Hempel knew, had never been seen experimentally. Their first thought was that the ALDH2 structure from x-ray crystallography may have been flawed for their purposes, slightly shifted from its living-cell configuration in which the reaction would proceed as expected.

It was at this point, however, that Hempel saw a manuscript about a related ALDH by Sergey Krupenko's group at the University of South Carolina Medical School. These scientists reported a laboratory study of an ALDH reaction that also produced NAD-S, as the XT3 simulations predicted.

Although this laboratory work showed that NAD-S forms only in small amounts, it confirmed what Hempel and Wymore had seen in simulations. And it forced new thinking about ALDH's enzyme mechanism. In what cases did NAD-S form instead of NADH?

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Using PSC's Jonas system, Wymore mounted further simulations with the aim of answering this question. The researchers turned to Jonas because of its large memory. "We ran on Jonas," says Wymore says, "because we need 40-60 gigabytes of memory." They used 64 Jonas processors, and with data from many of these runs, four to five years of processor time, a new picture of the ALDH mechanism emerged.

"It looks like a more ordered mechanism," says Wymore, meaning that it requires a greater number of sequenced steps. "Until our work on this, the accepted thinking was that the initial reaction always happens with NAD in position to receive the hydride." Based on their new simulations, their working hypothesis is that a staged process must occur, starting with the proton transfer they found previously, for the reaction to form NADH.

NAD is located slightly away from the active site most of the time and when it sweeps in at the correct instant — after the proton transfer from the backbone stabilizes the aldehyde (which then binds with the sulfur atom) — it is converted to NADH. When this sequence of events plays out, the average ALDH enzyme does its job and converts a molecule of aldehyde into carboxylic acid.

Occasional missteps in the mechanism, however, block the proton transfer, and this accounts for formation of small amounts of NAD-S, as both the simulation and experiment show. "Basically," says Wymore, "it comes down to whether or not a proton moves half an angstrom." In rare cases, mutations in ALDHs block the proton transfer and render the enzyme useless. People with Sjögren-Larsson syndrome have structural changes (mutations) in their ALDH that disrupt this transfer entirely.

Along with the therapeutic gains that can result from deepened knowledge of the enzyme mechanism, the work by Wymore, Hempel and Brown contributes to enzyme modeling and simulation science. A goal of this research, supported by NIH's National Center for Research Resources, is to develop tools that other researchers can use to advance from simpler simulations to QM/MM studies that uncover the transient, hard-to-observe details of enzyme reactions. "It's really hard to set up these systems," says Wymore. "We're trying to develop tools to make these simulations more accurate and easier for other researchers to use. We're a long way from thorough understanding of how enzymes function." (TP)

MORE INFORMATION:
www.psc.edu/science/2007/aldh.html