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

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, supersmall players. Enzyme reactions often occur in timeframes approaching a nanosecond (a billionth of a second) and distances of an angstrom (a hundred millionths 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 how ALDH works. With the promising clue of their recent work on ALDH1, a complicated proton-relay mechanism in the enzyme’s “active site” — where the reactions occur — Wymore decided to look at ALDHs 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.

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, malfunctions of ALDH are 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 when ALDH works. With the promising clue of their recent work on ALDH1, a complicated proton-relay mechanism in the enzyme’s “active site” — where the reactions occur — Wymore decided to look at ALDHs 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 ALDH1 obtained recently with x-ray crystallography. He worked closely with PSC scientist Shawn Brown, who modified software called DYNAMO to run QM/MM efficiently (with linear scaling) on the XT3. To take advantage of the XT3’s parallelism, Brown devised the simulation — a total of 36,711 atoms — among 900 XT3 processors.

The new simulations included a “substrate” molecule — an aldehyde — and also included a co-enzyme, a molecule that sits in the active site and must be present for the reaction to occur. The co-enzyme, NAD (nicotinamide adenine dinucleotide), was expected to react with a hydrogen ion (hydride) to form NADH.

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STRUCTURE AT THE ACTIVE SITE

Strange Action

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

This closeup of the active site of human ALDH2, Zooming-In on the Active Site A NEW PICTURE EMERGES

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 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. This 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.

As the proton moves from the nitrogen to the oxygen atom, the C-S bond becomes increasingly stronger (increased slope in the low-energy valley). This profile shows results of Wymore’s simulations of the proton transfer from the backbone of ALDH to an oxygen atom on the aldehyde substrate (center), held in closeup shows the NAD co-enzyme (upper right) from reactants to products. The stick-figure and making of bonds as the reaction proceeds quantum-mechanically, to capture the breaking in which the enzyme reaction was simulated shows the region of Wymore’s QM/MM simulation.

A NEW PICTURE EMERGES

The calculation ran for 9,450 XT3 processor hours. The surprising result showed a sulfur atom from ALDH 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 ALDH 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.

A NEW PICTURE EMERGES

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?

IT COMES DOWN TO WHETHER OR NOT A PROTON MOVES HALF AN ANGSTROM.

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, “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.

“One 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.

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NAD is located slightly away from the active site most of the time and when it sweps 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.

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.”

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