Scientists uncover structure of light-driven enzyme with potential biofuel applications

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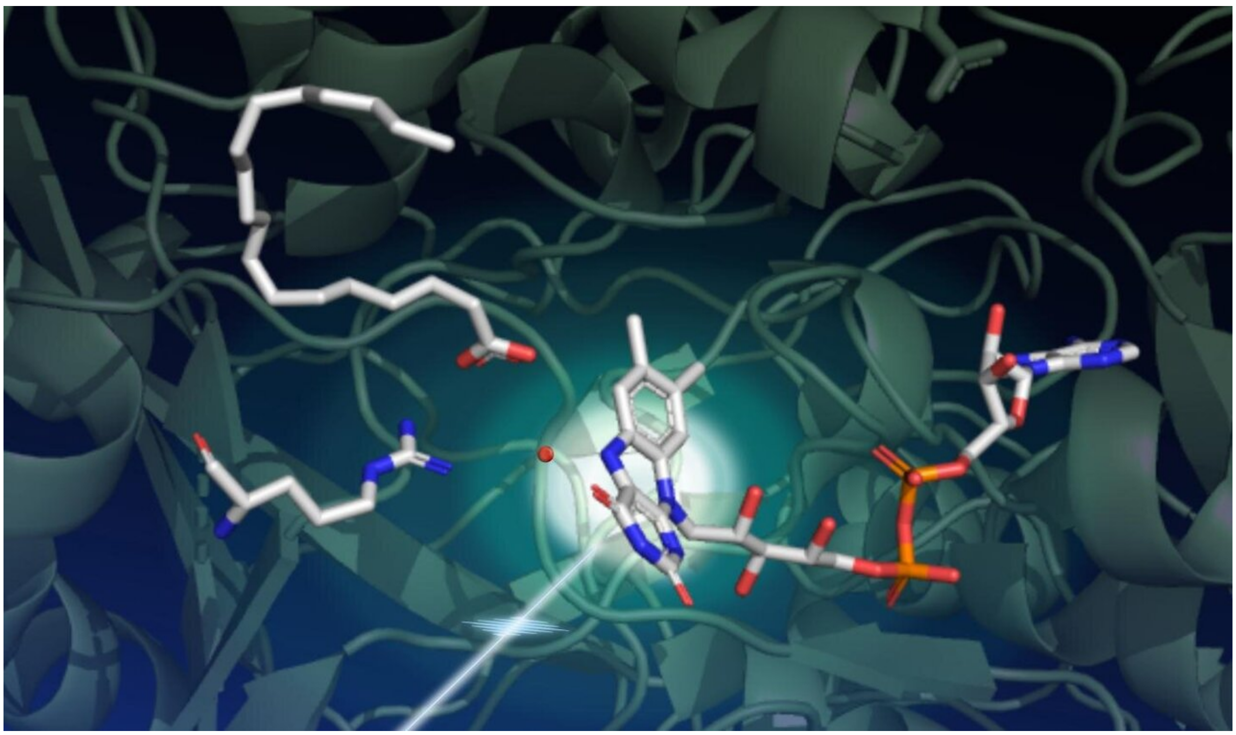

A study using SLAC's LCLS X-ray laser captured how light drives a series of complex structural changes in an enzyme called FAP, which catalyzes the transformation of fatty acids into starting ingredients for solvents and fuels. This drawing captures the starting state of the catalytic reaction. The dark green background represents the protein scaffold. The enzyme's light-sensing part, called the FAD cofactor, is shown at center right with its three rings absorbing a photon coming from bottom left. A fatty acid at upper left awaits transformation. The amino acid shown at middle left plays an important role in the catalytic cycle, and the red dot near the center is a water molecule. Credit: Damien Sorigue/Universite Aix-Marseille

Although many organisms capture and respond to sunlight, enzymes—proteins that catalyze biochemical reactions—are rarely driven by light. Scientists have identified only three types of natural photoenzymes so far. The newest one, discovered in 2017, is fatty acid photodecarboxylase (FAP). Derived from microscopic algae, it uses blue light to catalyze the conversion of fatty acids, found in fats and oils, into alkanes and alkenes.

"A growing number of labs envision using FAPs for green chemistry applications, because alkanes and alkenes are important components of solvents and fuels, including gasoline and jet fuels. And the transformation of fatty acids into alkanes or alkenes happens in a single step within the enzyme," says Martin Weik, the leader of a research group at the Institute of Biologie Structurale at the Universite Grenoble Alpes.

Weik is a primary investigator of a new study that has captured the complex sequence of structural changes FAP undergoes in response to light, called a photocycle, which drives this fatty acid transformation. Although researchers previously proposed a FAP photocycle, the fundamental mechanism was not understood. The scientists didn't know how long it took a fatty acid to lose its carboxylate, the chemical group attached to the end of its long chain of hydrocarbons, a critical step in forming alkenes or alkanes.

In collaboration with SLAC scientists, experiments at the Linac Coherent Light Source (LCLS) at the Department of Energy's SLAC National Accelerator Laboratory helped answer many of these outstanding questions. The researchers describe their results in *Science*.

All the tools in a toolbox

To understand a light-sensitive enzyme like FAP, scientists use many different techniques to study processes that take place over a broad range of time scales—because photon absorption happens in femtoseconds, or millionths of a billionth of a second, while biological responses on the molecular level often happen in thousandths of a second.

"Our international, interdisciplinary consortium, led by Frederic Beisson at the Universite Aix-Marseille, used a wealth of techniques, including spectroscopy, crystallography and computational approaches," Weik says. "It's the sum of these different results that enabled us to get a first glimpse of how this unique enzyme works as a function of time and in space."

The consortium first studied the complex steps of the catalytic process at their home labs using optical spectroscopy methods, which investigate the electronic and geometric structure of atoms in the samples, including chemical bonding and charge. Spectroscopic experiments identified the enzyme's intermediate states accompanying each step, measured their lifetimes and provided information on their chemical nature. These results motivated the need for the ultrafast capabilities of the LCLS.

Next, a structural view of the catalytic process was provided by serial femtosecond crystallography (SFX) with the LCLS X-ray free-electron laser (XFEL). During these experiments, a jet of tiny FAP microcrystals was hit with optical laser pulses to kick off the catalytic reaction, followed by extremely short, ultrabright X-ray pulses to measure the resulting changes in the enzyme's structure.

By integrating thousands of these measurements—acquired using various time delays between the optical and X-ray pulses—the researchers were able to follow structural changes in the enzyme over time. They also determined the structure of the enzyme's resting state by probing without the optical laser.

Surprisingly, the researchers found that in the resting state, the enzyme's light-sensing part, called the FAD cofactor, has a bent shape. "This cofactor acts like an antenna to capture photons. It absorbs blue light and initiates the catalytic process," Weik says. "We thought the starting point of the FAD cofactor was planar, so this bent configuration was unexpected."

The bent shape of the FAD cofactor was actually first discovered by X-ray crystallography at the European Synchrotron Radiation Facility, but the scientists suspected this bend was an artifact of radiation damage, a common problem for crystallographic data collected at synchrotron light sources. Only SFX experiments could confirm this unusual configuration because of their unique ability to capture structural information before damaging the sample, Weik says.

"These experiments were complemented by computations," he adds, "Without the high-level quantum calculations performed by Tatiana Domratcheva of Moscow State University, we wouldn't have understood our experimental results."

Next steps

Despite the improved understanding of FAP's photocycle, unanswered questions remain. For example, researchers know carbon dioxide is formed during a certain step of the catalytic process at a specific time and location, but they don't know its state as it leaves the enzyme.

"In future XFEL work, we want to identify the nature of the products and to take pictures of the process with a much smaller step size so as to resolve the process in much finer detail," says Weik. "This is important for fundamental research, but it can also help scientists modify the enzyme to do a task for a specific application."

More information: D. Sorigué et al, Mechanism and dynamics of fatty acid photodecarboxylase, *Science* (2021). DOI: 10.1126/science.abd5687

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