Press Release

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Enzyme Structural Dynamics Captured in Real Time During Catalysis

Researchers use cutting-edge X-ray crystallography to reveal how proteins move during chemical reactions, offering valuable insights into enzyme function.

In a groundbreaking study, researchers have captured real-time "molecular movies" showing how an enzyme changes shape during catalysis. Using an advanced technique called mix-and-inject serial crystallography at Japan's SACLA X-ray free-electron laser facility, the team observed domain movements and structural changes in the enzyme, copper amine oxidase enzyme over millisecond timescales, revealing dynamics that are nearly impossible to observe by other methods.

Enzymes are nature's catalysts, that speed up biochemical reactions essential for life. Scientists have long known that enzymes change their shape, called conformational changes, during catalysis. Such protein motions can occur over a wide range of timescales, from picoseconds to milliseconds. However, due to these extremely short timescales, capturing these movements has remained a significant challenge. Traditional crystallography techniques can provide static snapshots of enzyme structures, but observing enzymes in action as they bind substrates, transform them into products and release them, requires specialized techniques capable of tracking changes over extremely short timescales.

In a breakthrough, a research team led by Dr. Takeshi Murakawa from the Department of Biochemistry at Osaka Medical and Pharmaceutical University in Japan, has successfully captured time-resolved structures of an enzyme during its catalytic cycle. This was carried out using the mix-and-inject serial crystallography (MISC) at Japan's SPring-8 Angstrom Compact free electron LAser (SACLA) facility.

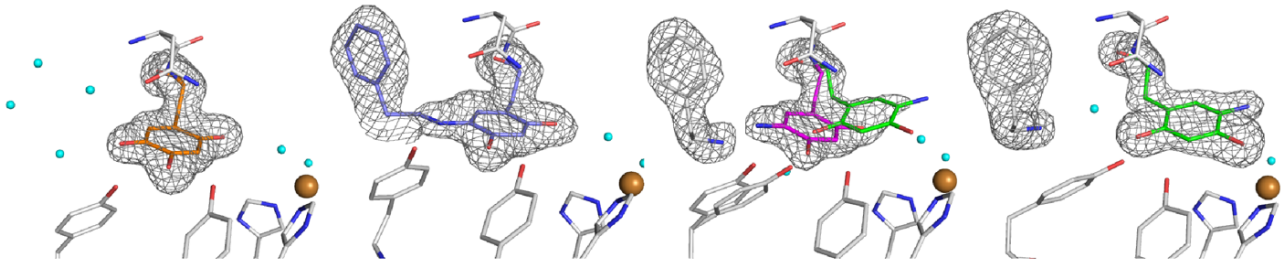
"*Through MISC we have illuminated, at the atomic level, a part of the sophisticated and rapid movement that enzymes undergo during catalysis,*" explains Dr. Murakawa. "*This is extremely valuable for molecular design of novel enzymes with highly specific functions.*" The team also included Associate Professor Toshihide Okajima from the University of Osaka, and Professor Eriko Nango from Tohoku University. Their study was published in Nature Communications on December 18, 2025.

The researchers focused on copper amine oxidase, derived from the bacterium *Arthrobacter globiformis* (AGAO), which they have studied extensively for over two decades. Copper amine oxidases are widespread in nature, occurring in organisms ranging from bacteria to humans. These enzymes contain a unique protein-derived cofactor called topaquinone (TPQ), which plays the central role in oxidizing primary amines through a complex multi-step mechanism.

To observe the enzyme in action, they prepared uniform AGAO microcrystals and mixed them with an amine substrate under precisely controlled conditions. A key piece of technology enabling this experiment was a two-liquid mixing device, developed and provided by SACLA. Utilizing MISC, they then captured 12 distinct structural snapshots at nine distinct time-points, spanning from 22 milliseconds (ms) to 1 second during the enzyme's reductive-half reaction.

Structural changes in the cofactor TPQ during catalytic process

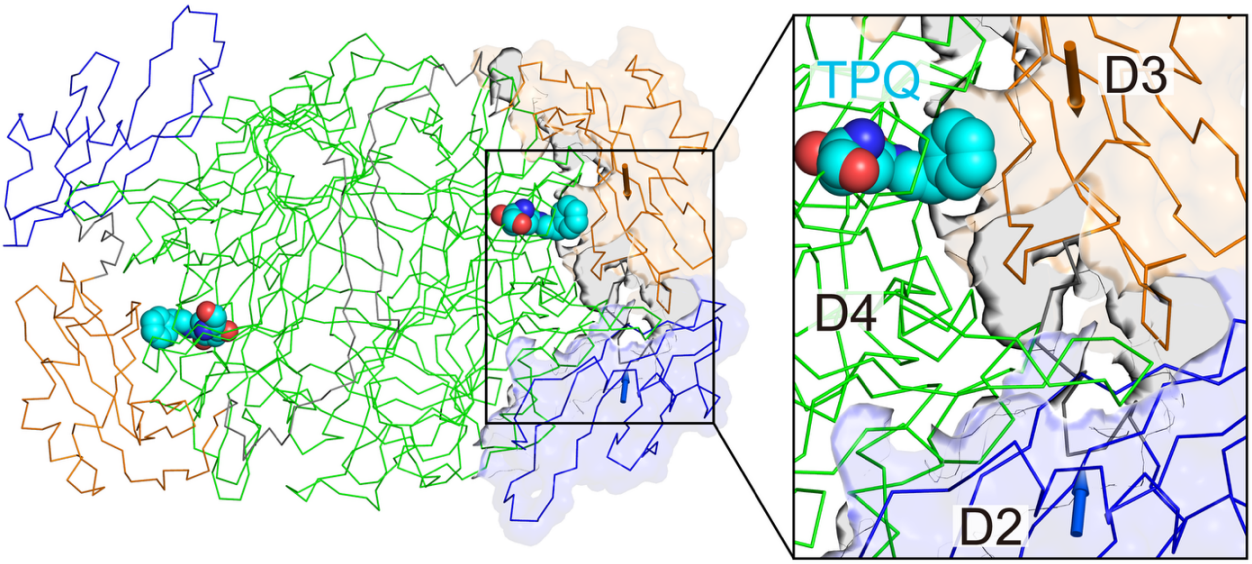
The snapshots reveal key reactions steps.



At 0 ms, water molecules occupy the substrate-binding site.
At 50 ms, a substrate-cofactor intermediate forms.
At 100 ms, the product is released, and the next cycle's substrate enters.
At 1000 ms, the cofactor flips and slides in preparation for the next half-reaction.

By putting together these snapshots into a molecular “movie”, the researchers were able to observe the domain movements associated with substrate binding and the formation of catalytic intermediates in real-time. One unexpected observation was the unidirectional protein shrinkage associated with substrate binding. When the substrate enters the enzyme's active site, specific protein domains move closer together, compressing the crystal lattice by nearly one angstrom.

Overall structural changes in copper amine oxidase during catalysis



Upon substrate binding, Domain 2 (D2) and Domain 3 (D3) contract in the direction indicated by the arrows.
This enzyme has a homodimeric structure, and similar movements are observed in both D2 and D3.

The team also showed that crystal packing and substrate diffusion can significantly slow certain catalytic steps. This actually provides an important benefit. As Dr. Okajima explains, “*These slowed-down steps in crystal-based reactions allow us, through MISC, to capture the key intermediates that would be too short-lived to observe in solution.*”
The implications of this research extend far beyond understanding copper amine oxidase. “*The quest to visualize catalysis in action is a long-standing shared dream among many enzyme scientists worldwide,”* remarks Dr. Nango. *“MISC represents a versatile approach for this purpose and is also applicable to a wide range of enzymes.*"

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About Osaka Medical and Pharmaceutical University

Osaka Medical and Pharmaceutical University is a comprehensive institution that offers education and research in the departments of medicine, pharmacy, and nursing. The university has been providing thousands of clinical professionals by its unique curriculum. Not only basic scientific research but also interdisciplinary research activities are conducted to solve unmet medical needs. Of note, various medical devices have been invented there by active industry-academia-government collaborations. The University Hospital provides various advanced medical treatments, such as the state-of-the-art cancer therapies including boron neutron capture therapy, robot surgery, and genomics medicine.
Website: https://www.ompu.ac.jp/

About Dr. Takeshi Murakawa

Dr. Takeshi Murakawa is currently an Associate Professor at the Department of Chemistry, Faculty of Medicine, in Osaka Medical and Pharmaceutical University, Japan. His research focuses on the structural and mechanistic studies of enzymes using advanced crystallographic techniques, including serial femtosecond X-ray crystallography and neutron crystal structure analysis. He has published over 40 articles to date, that have received numerous citations.

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