Imaging the smallest atoms provides insights into an enzyme's unusual biochemistry

A team led by researchers at Osaka University uses neutron crystallography to image all of the atoms in an enzyme's biochemical reaction intermediates, which might help in the design of enzymes for the chemical industry

Osaka, Japan – When your wounds heal and your liver detoxifies a poison such as histamine you ingested, you can thank the class of enzymes known as copper amine oxidases for their assistance. Identifying the exact positions of the smallest hydrogen atoms in these enzymes is challenging with commonly used technologies, but is critical to engineering improved enzymes that exhibit unusual yet useful biochemical reactivity.

Now, in a study recently published in *ACS Catalysis*, a team led by researchers at Osaka Medical and Pharmaceutical University and Osaka University has used neutron crystallography to image the atom-by-atom structure of a copper amine oxidase enzyme. This study provides unprecedented structural insights into the enzyme's biochemistry.

Some copper amine oxidase enzymes exhibit unusual biochemistry, such as quantum tunneling, which enables otherwise inexplicably fast reaction rates. Although it is often challenging to determine the exact position of each hydrogen atom in the enzyme, such knowledge is important for designing corresponding artificial enzymes. Researchers commonly obtain the atom-by-atom structure of enzymes by X-ray crystallography. However, this technique obtains structural information by diffraction from electrons in the enzyme. Thus, it is insufficient for imaging hydrogen atoms, which generally contain only one electron. Neutron crystallography, which analyzes diffraction from atomic nuclei in the enzyme (all atoms have an atomic nucleus), is an alternative imaging technique that the researchers chose for their work.

"There are pH-dependence, conformational change, and radical intermediate stabilization questions of our enzyme that X-ray crystallography in itself cannot fully explain," explains Takeshi Murakawa, lead author of the study. "Neutron crystallography is well-suited for answering these questions."

The researchers obtained numerous insights. For example, they imaged the protonation/deprotonation state (related to the pH) of sites within the enzyme that are important for stabilizing radical species (i.e., especially reactive atoms that contain an unpaired electron). They also characterized the motions of the enzyme's topaquinone cofactor—sliding, upward tilting, and half-rotation—that facilitate single-electron transfer within the enzyme.

"We disclose binding of a second molecule of high-affinity amine substrate during the enzymatic reaction, a previously unknown event in the enzyme active site," says Toshihide Okajima, senior author. "X-ray crystallography misses such insights."

This work has provided previously undisclosed structural details in a copper amine oxidase enzyme that has many functions in biochemical metabolism. Revealing the exact position of the hydrogen atoms in the enzyme helps to explain its efficiency at physiological temperatures and pressures. In the future, researchers might apply these insights to designing artificial enzymes that function used in the chemical industry.

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The article, “Neutron crystallography of a semiquinone radical intermediate of copper amine oxidase reveals a substrate-assisted conformational change of the peptidyl quinone cofactor,” was published in *ACS Catalysis* at DOI: https://doi.org/10.1021/acscatal.3c02629

Summary: A team led by researchers at SANKEN (The Institute of Scientific and Industrial Research), at Osaka University has used neutron crystallography to image all of the atoms in a radical intermediate of a copper amine oxidase enzyme. They disclosed previously unknown details, such as precise conformational changes, that help to explain the enzyme's biochemistry. This work might help researchers engineer enzymes that facilitate unusual chemistry or are highly efficient at room temperature that are useful in chemical industry.

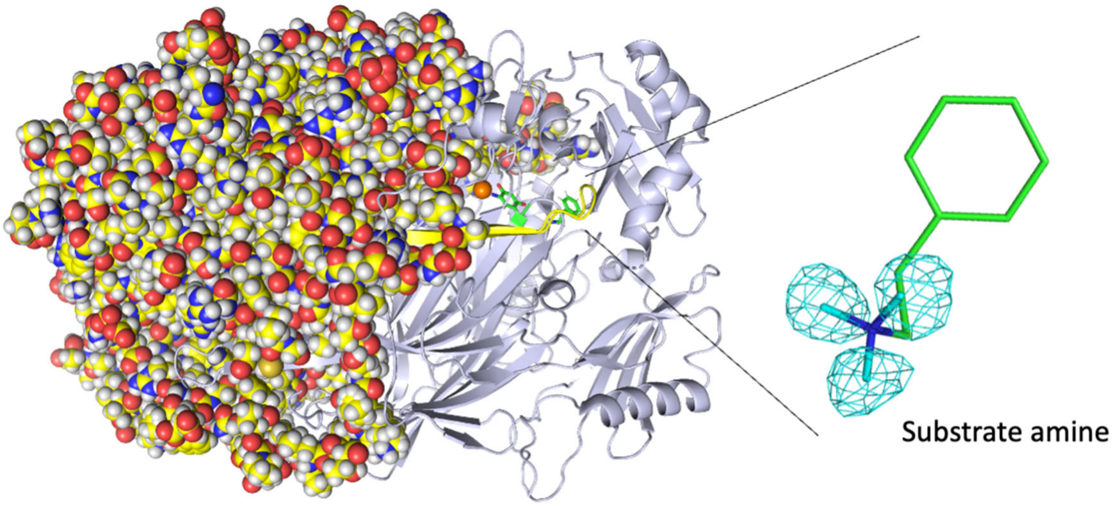

Fig. 1
Entire neutron crystal structure of copper amine oxidase. (Left) Neutron crystallography determined the entire protein structure, including the hydrogen atoms. (Right) The precise positions of the hydrogen atoms indicate that the detected molecule is not a product aldehyde but a substrate amine. Maps for the hydrogen atoms (more precisely, deuterium) are presented by using a cyan mesh.

Credit： Takeshi Murakawa, Toshihide Okajima

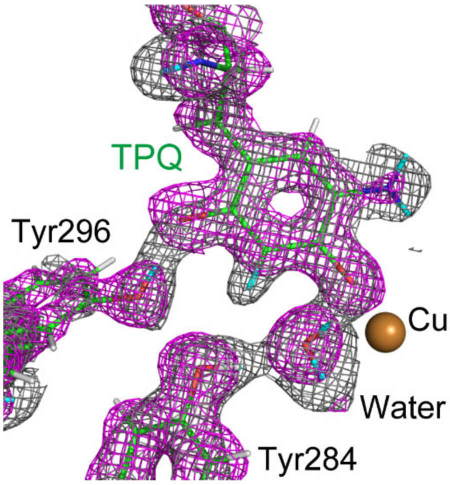

Fig. 2
Structure of semiquinone radical intermediate. Maps from X-ray and neutron crystallography are presented by using red–purple and grey mesh, respectively.

Credit：Takeshi Murakawa, Toshihide Okajima

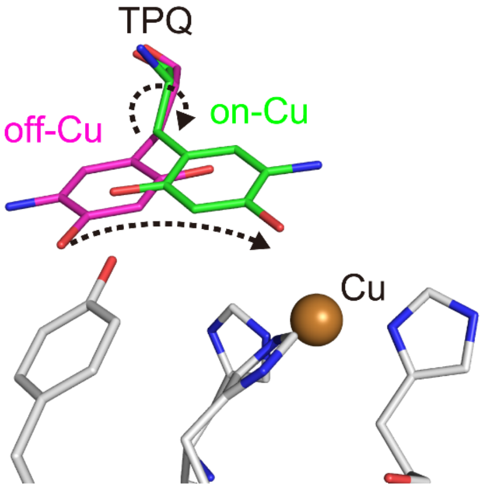

Fig. 3
Conformational change of cofactor TPQ during the catalysis. TPQ undertakes a conformational change from off-Cu to on-Cu in a manner that generates a semiquinone radical intermediate.

Credit： Takeshi Murakawa, Toshihide Okajima

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Authors: Takeshi Murakawa, Kazuo Kurihara, Mitsuo Shoji, Naomine Yano, Katsuhiro Kusaka, Yoshiaki Kawano, Mamoru Suzuki, Yasuteru Shigeta, Takato Yano, Motoyasu Adachi, Katsuyuki Tanizawa, and Toshihide Okajima

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About Osaka University

Osaka University was founded in 1931 as one of the seven imperial universities of Japan and is now one of Japan's leading comprehensive universities with a broad disciplinary spectrum. This strength is coupled with a singular drive for innovation that extends throughout the scientific process, from fundamental research to the creation of applied technology with positive economic impacts. Its commitment to innovation has been recognized in Japan and around the world, being named Japan's most innovative university in 2015 (Reuters 2015 Top 100) and one of the most innovative institutions in the world in 2017 (Innovative Universities and the Nature Index Innovation 2017). Now, Osaka University is leveraging its role as a Designated National University Corporation selected by the Ministry of Education, Culture, Sports, Science and Technology to contribute to innovation for human welfare, sustainable development of society, and social transformation.

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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 department 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.

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