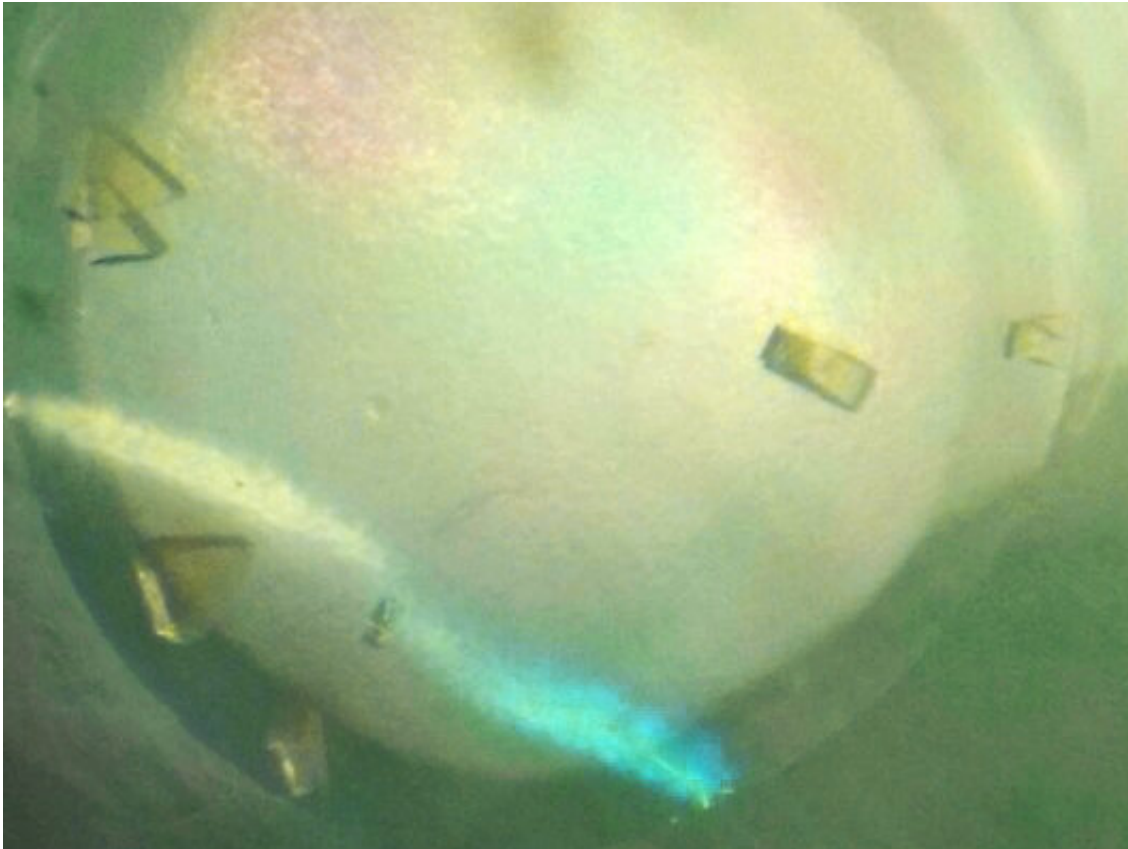


# Understanding oxygen-reducing enzymes

September 28 2020

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Pictures of F420H<sub>2</sub>-oxidase crystals obtained aerobically with a typical size of 0.1 mm. With oxygen the Fe and flavin inside the enzyme give the natural yellow color to the crystals. Credit: Max Planck Institute for Marine Microbiology/T. Wagner

Methane is a powerful greenhouse gas that plays a central role in the global carbon cycle. At the same time, it is an important energy source for us humans. About half of its annual production is made by

microorganisms known as methanogens that decompose organic material such as dead plants. This normally takes place in a habitat without oxygen as this gas is lethal to methanogens. But even in actually oxygen-free habitats, oxygen molecules occasionally appear. To render these intruders harmless, methanogens possess a special enzyme that is able to convert oxygen into water.

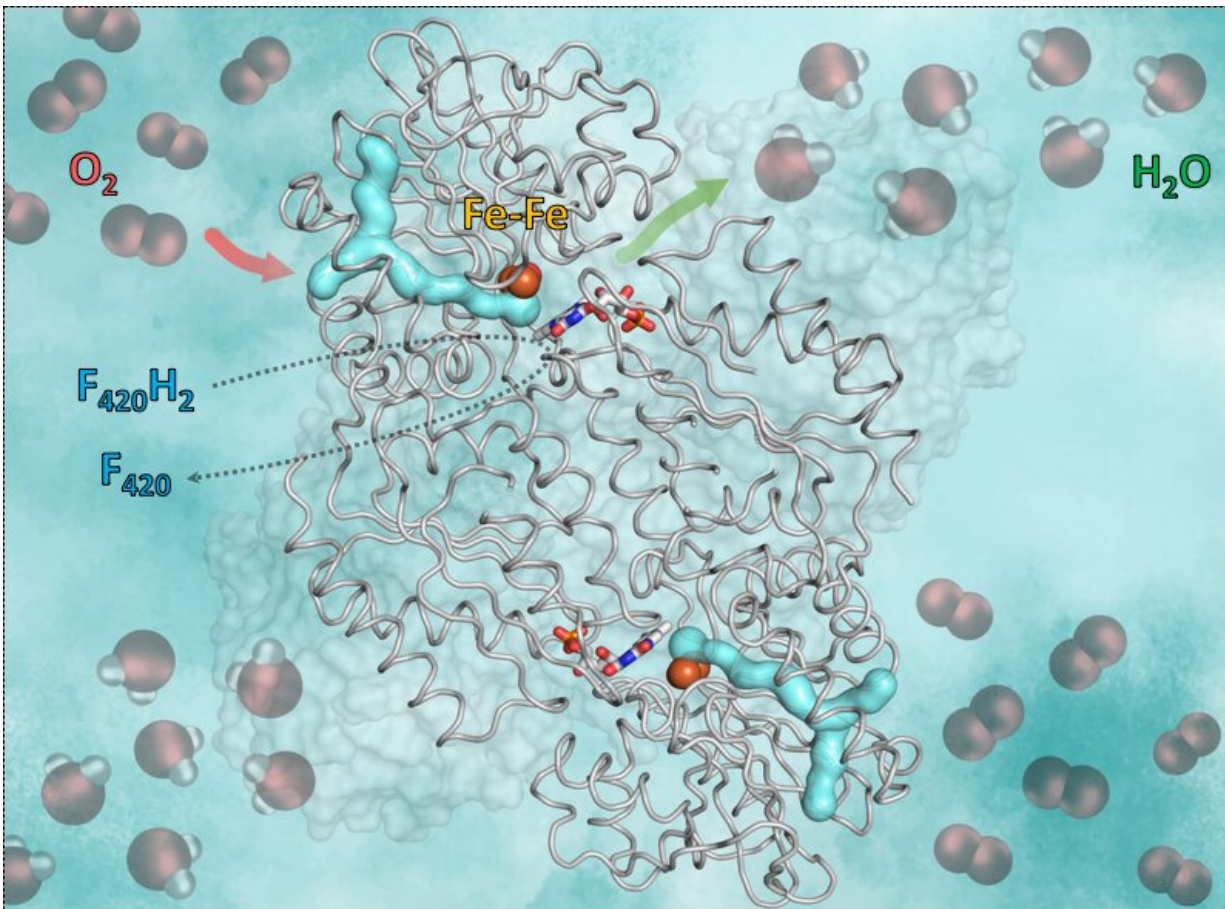
"Enzymes are vital components of the metabolism of all living organisms and the goal of our laboratory is to understand how these nanomachines are working at the molecular level," says Tristan Wagner from the Max Planck Institute for Marine Microbiology and first author of the study, published in the scientific journal *Chemical Communication* in September 2020. For the study, Wagner cultivated anaerobic microorganism called *Methanothermococcus thermolithotrophicus*, which originated from the sediment of the Gulf of Naples. He purified the [enzyme](#) F420-oxidase, a flavodiiron protein, and crystallized it, a common method to study the functioning of enzymes.

"It was already known that F420-oxidase can convert oxygen into water," says Wagner. "But we succeeded to decrypt the mechanism." The study is a cooperation of scientists from the Max Planck Institute for Marine Microbiology, the Max Planck Institute for Terrestrial Microbiology, the Paul Scherrer Institute, the Interdisciplinary Research Institute of Grenoble and the European Synchrotron Radiation Facility.

## **Oxygen is locked in**

The mechanism, the researchers revealed, has an important requirement: Oxygen is very reactive, so it is crucial that the reaction is controlled correctly by the enzyme and no solvents are floating around. Otherwise the oxygen could accidentally be transformed in superoxide and kill the anaerobe. The trick of the enzyme F420-oxidase is to use a gas channel and a gating system. The oxygen molecule is first funneled in the

specific channel to an appropriate anhydrous catalytic cavity containing iron. Then iron transforms the oxygen in water that will be released by a gating mechanism. For that the cavity begins to move and opens a small "door." Thanks to the movement, the newly generated water is transported outside. The empty cavity closes again and is available for the next oxygen molecule.



This graphic shows the enzyme F420H2-Oxidase and the way it works. The cyan y-formed part is the gas-channel. The red arrow shows the way in of the oxygen to the catalytic cavity containing iron. The green arrow symbolizes the way out of the water. Yet, the blue-red sticks in the middle shows the flavin (FMN) accepting electrons from the reduced coenzyme F420, which brings the hydrogen necessary to convert the oxygen into water. Credit: S. Engilberge and

T. Wagner

To gain insights into this mechanism the scientists used X-ray crystallography. They first obtained the [crystal structure](#) without oxygen, where they could see the anhydrous catalytic cavity isolated from the solvent. Then, they gassed the enzyme crystals with the inert gas krypton, which, unlike oxygen, can be made visible by X-rays. Afterwards they irradiated the enzyme crystals and were able to detect krypton atoms showing the gas channel leading to the catalytic cavity. The flavodiiron protein and its channel is conserved not only in methanogens, but also in other microorganisms like clostridia (who live mainly in soil or in the digestive tract), in the sulfur bacteria *Desulfovibrio gigas* or even in the intestinal parasite *Giardia intestinalis*.

## **The faster the better**

"This reaction is really fast," says Sylvain Engilberge from the Paul Scherrer Institute and first author of the study next to Tristan Wagner. "This velocity is also the high importance of our investigation." Similar enzymes like laccase are much slower. "For future application of bio-inspired electrochemical processes, we need to learn more from the chemical reaction, structure and function of different groups of oxygen-reducing enzymes," says Engilberge. It would also pave the way of protein engineering to convert a high-rate O<sub>2</sub>-detoxifier into an electron sink for industrial processes.

"Our next step would be to understand the diversity of flavodiiron protein," says Tristan Wagner. Some homologues are not targeting oxygen but the poisonous nitric oxide, their enzymes can discriminate between both gases with high specificity. But what is the selective filter? The gas channel? The environment of the catalytic cavity? "More studies

have to be carried out to understand how the protein discriminates oxygen and nitric oxide," adds Wagner. With such knowledge, it would be for instance possible to predict from genomic information if a flavodiiron protein would be an [oxygen](#) or a nitric oxide scavenger.

**More information:** Sylvain Engilberge et al, Krypton-derivatization highlights O<sub>2</sub>-channeling in a four-electron reducing oxidase, *Chemical Communications* (2020). [DOI: 10.1039/d0cc04557h](https://doi.org/10.1039/d0cc04557h)

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