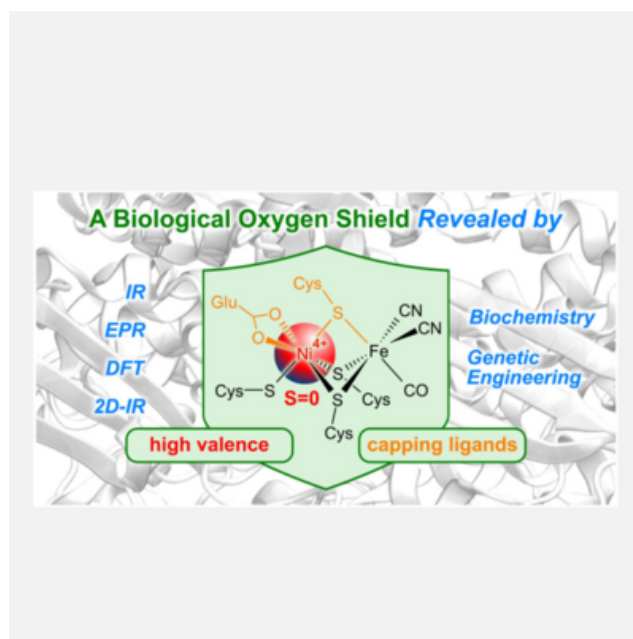


How a hydrogenase protects itself from oxygen

Start Time: Monday, January 23, 2023

End Time:



The use of hydrogen (H_2) as an energy storage medium is currently being treated as a key technology in the energy transition. In the search for new and improved ways to produce and activate H_2 , researchers in UniSysCat are also taking nature as a model. For example, they want to employ hydrogenases, enzymes that can catalyze the reversible splitting of H_2 into two protons and two electrons. These hydrogenases could be used, e.g., as building blocks for the lightdriven production of H_2 through water splitting. For this and other applications, however, they have to be tolerant to oxygen (O_2), which represents a notable challenge.

All hydrogenases contain metal centers made of nickel (Ni) and/or iron (Fe) as their catalytic sites and can be classified accordingly as [FeFe] hydrogenases, [NiFe] hydrogenases, and [Fe] hydrogenases. Most hydrogenases are known to be deactivated by O_2 and elevated temperatures. However, a few specific types of hydrogenases resist such conditions, such as the soluble NAD^+ -reducing [NiFe] hydrogenase from *Hydrogenophilus thermoluteolus* (HtSH). Thus, HtSH represents a promising candidate for biotechnological applications, such as the regeneration of nucleotide cofactors and the light-driven production of hydrogen. How exactly the O_2 tolerance in HtSH works and which structural features lead to it is not yet fully understood, but a deeper understanding of these aspects would be desirable for future applications.

A current study involving the UniSysCat groups of [Christian Teutloff](#), [Oliver Lenz](#), [Ingo Zebger](#) and [Marius Horch](#) examines the catalytic activity and active-site structure of HtSH and variants in which a glutamate residue in the active-site cavity was replaced by other amino acids. Using biochemical, spectroscopic and theoretical approaches, the authors gain deeper insights into the mechanism of O₂ tolerance in HtSH and structural features of the enzyme that are relevant for this special property.

The spectroscopic and theoretical results suggest that HtSH features an unusual glutamate coordination at its oxidized active site. This structural motif is found to represent a biologically relevant feature of the hydrogenase that is also formed under physiologically representative conditions. Moreover, the study shows that this motif is selectively and reversibly formed under oxidic conditions, especially upon rapid exposure to high O₂ levels. This process is accompanied by the formation of formal Ni(IV) oxidation state of the active site that is unprecedented in biology. Thus, formation of this high-valent glutamate-coordinated state represents a response toward O₂ that could protect the enzyme from oxidative damage.

The authors conclude that the unusual glutamate coordination represents the key element in the O₂ protection mechanism of HtSH. Finally, this protective mechanism is very similar to that of other metalloenzymes. Reversible binding of glutamate or other suitable ligands, triggered by an off-site redox stimulus, may represent a general mechanism for preventing undesired reactions of catalytic metal sites with O₂.

This study has been published in the *Journal of the American Chemical Society*: C. J. Kulka-Peschke, A.-C. Schulz, C. Lorent, Y. Rippers, S. Wahlefeld, J. Preissler, C. Schulz, C. Wiemann, C. C. M. Bernitzky, C. Karafoulidi-Retsou, S. L. D. Wrathall, B. Procacci, H. Matsuura, G. M. Greetham, C. Teutloff, L. Lauterbach, Y. Higuchi, M. Ishii, N. T. Hunt, O. Lenz, I. Zebger and M. Horch, Reversible Glutamate Coordination to High-Valent Nickel Protects the Active Site of a [NiFe] Hydrogenase from Oxygen, *J. Am. Chem. Soc.* **2022**, 144, 37, 17022–17032, <https://doi.org/10.1021/jacs.2c06400>