An Advanced Experimental Approach for Exploring Structure and Function of Redox Intermediates in Metalloenzymes

Start Time: Tuesday, July 13, 2021

End Time:

Exploring Structure and Function of Redox Intermediates in [NiFe]-Hydrogenases by an Advanced Experimental Approach for Solvated, Lyophilized and Crystallized Metalloenzymes

Christian Lorent, Dr. Vladimir Pelmenschikov, Dr. Stefan Frielingsdorf, Janna Schoknecht, Dr. Giorgio Caserta, Dr. Yoshitaka Yoda, Dr. Hongxin Wang, Dr. Kenji Tamasaku, Dr. Oliver Lenz, Prof. Dr. Stephen P. Cramer, Dr. Marius Horch, Dr. Lars Lauterbach and Dr. Ingo Zebger

Transition metals are often involved in chemical and enzymatic catalysis. In nature, metal-containing enzymes catalyze a variety of reactions, especially the conversion of small gaseous molecules like CO_2, N_2, or H_2. This type of chemistry is relevant for establishing alternative strategies for energy conversion and the production of carbon-neutral fuels. Many of these metalloenzymes, including carbon monoxide dehydrogenase, nitrogenase and hydrogenase, are attractive targets for biotechnological application and can serve as blueprints for bioinspired chemistry. However, their rational utilization requires a thorough mechanistic understanding, typically requiring multiple spectroscopic techniques.

In order study metalloenzymes in detail, the research group of Ingo Zebger developed a new experimental setup allowing a controlled preparation of catalytic intermediates for the characterization by various spectroscopic techniques. Together with three further UniSysCat groups of Marius Horch, Oliver Lenz, Vladimir Pelmenschikov/Martin Kaupp and one Einstein visiting fellow, Stephen P. Cramer, this setup was applied for the *in situ* monitoring of redox transitions by infrared spectroscopy in enzyme lyophilizate, crystal and solution during gas exchange in a wide temperature range. Two O_2-tolerant [NiFe]-hydrogenases were here investigated as model systems.

The platform was utilized firstly for the preparation of highly concentrated hydrogenase lyophilizate in a paramagnetic state harboring a bridging hydride in the active site. This procedure proved beneficial for ^{57}Fe nuclear resonance vibrational spectroscopy and revealed, in combination with density functional theory calculations, the vibrational fingerprint of this catalytic intermediate. The same in situ IR setup, combined with resonance Raman spectroscopy, provided detailed insights into the redox chemistry of enzyme crystals, underlining the general necessity to complement X-ray crystallographic data with spectroscopic analyses.

The findings of Lorent et al. are open-access published in *Angewandte Chemie International Edition*: C. Lorent, V. Pelmenschikov, S. Frielingsdorf, J. Schoknecht, G. Caserta, Y. Yoda, H. Wang, K. Tamasaku, O. Lenz, S. P. Cramer, M. Horch, L. Lauterbach, I. Zebger, *Angew. Chem. Int. Ed.* **2021**, 60, 15854, DOI: https://doi.org/10.1002/anie.202100451