

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

<https://www.esrf.fr/misapps/SMISWebClient/protected/welcome.do>

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.



	Experiment title: Defining the Intermediates in Biological Hydrogen Conversion	Experiment number: CH - 4046
Beamline:	Date of experiment: from: 16.04.2014 to: 22.04.2014	Date of report:
Shifts: 18	Local contact(s): Pieter Glatzel	<i>Received at ESRF:</i>

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Hydrogenases catalyze the reversible oxidation of molecular hydrogen. These enzymes have attracted significant attention since they catalyze an environmentally clean reaction with very high efficiency. Understanding the catalytic cycles of the active sites is crucial for exploring how hydrogen could be used as an energy carrier. Starting in 2005^[1] and 2009^[2] the first models were investigated that mimic not only the heterobimetallic thiolate-bridged active site of the enzyme, but also the short metal-metal distance of 2.45-2.6Å that was reported for the Ni-R and the Ni-Sia states^[3]. Nowadays even more models of the EPR silent [Ni-Fe] Hydrogenase states were synthesized^[4].

While the active site of this enzyme contains both one Fe and one Ni center, the whole enzyme also consists of 3 Fe-S clusters. Hence, within the large hydrogenase heterodimer, there are 12 total Fe atoms. That makes Ni much more accessible for element specific spectroscopy methods like XAS/XES.

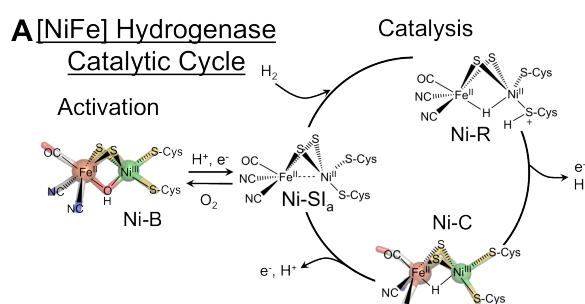


Figure 1 – Catalytic Cycle of [Ni-Fe] Hydrogenase

The experiments undertaken during the previous beamtime were aimed at understanding the electronic and the geometrical structure that underlies the intermediate states of the [Ni-Fe] Hydrogenase – in particular the Ni-R state and multiple model systems (Rauchfuss, Ogo, DuBois complexes). To obtain this information Ni K β HERFD XAS, and K β XES data, in both the mainline and the valence to core (VtC) region were measured.

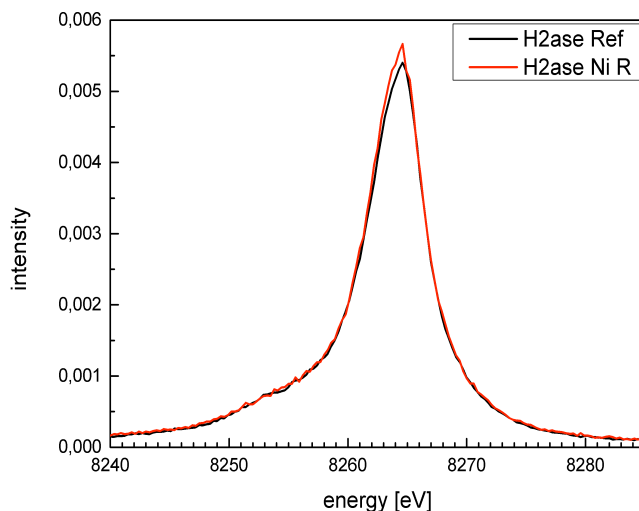


Figure 2 – Mainlines of [NiFe] Hydrogenase

Figure 2 shows the K β mainlines of two states of [Ni-Fe] Hydrogenase. The shape, intensity and features of these mainlines give information about the spin-states. Via comparison with other Ni complexes we were able to obtain insight to the Ni spin state, which has been a subject of intense debate. We are currently completing a detailed restricted active space configuration interaction analysis (RASCI) of these data in order to assess if the spectra can only be described as low spin, or if covalent contributions may be complicating the picture.

In addition to the the spin states, the existence of a bridging hydride vs. a covalent bound proton is a question of major interest. Therefore valence to core XES data were obtained for both the hydrogenase protein samples and a series of models. These models vary in the formal oxidation state of the Ni (Ni⁰, Ni^I, Ni^{II}) and in the presence or absence of a hydrogen. Dramatic differences are observed (Figure 3), which indicate that in certain cases, this method can be used to directly detect the presence of a covalently bound hydride. We are currently completing DFT calculations on this series, and a publication describing these results is in preparation.

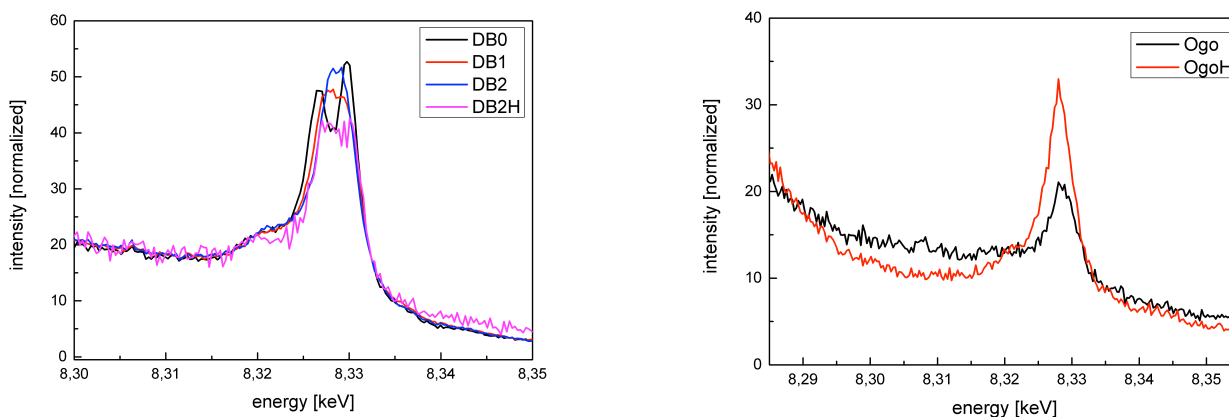


Figure 3 - V2C of various model systems to examine the effect of a proton bound to the Ni center

- [1] W. Zhu, M. Schröder et al., *PNAS* (2005), **102**, 18280-18285
- [2] B. E. Barton, T. B. Rauchfuss et al., *JACS* (2009), **131**, 6942-6943
- [3] G. Davidson, M. J. Maroney et al., *Biochemistry* (2000), **39**, 7468-7479

[4] S. Ogo, T. Ohhara et al., *Science* (2013), **339**, 682-684