EUROPEAN SYNCHROTRON RADIATION FACILITY

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ESRF

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://wwws.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.

ESRF	**Experiment title:** HERFD XAS and XES Studies of Biological Birch Reduction^	**Experiment number:** **LS-2674**
Beamline: ID26	**Date of experiment:** from: 17.05.2017 to: 23.05.2017	**Date of report:** 17.08.2017
Shifts: 18	**Local contact(s):** Lucia Amidani	*Received at ESRF:*

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Report:

Bacteria containing benzoyl-coenzyme A reductases (BCR) play a crucial role in the degradation of aromatic compounds. The mild conditions may serve as inspiration for an environmentally-friendly alternative to the harsh Birch reduction regularly used in synthetic inorganic chemistry. Recently a subunit of a class II BCR from Deltaproteinbacterium *Geobacter metallireducens* was shown to contain a W-bispyranopterin monophosphate active site (Figure 1, left)[1]. Our aim was to understand the electronic structure of the active site and identify an unknown ligand as well as disclose the oxidation state of tungsten.

During the assigned beam time, we collected W $L\beta_3$ high-energy resolution fluorescence detected X-ray absorption (HERFD) spectra from the subunit of a benzoyl-coenzyme A reductase (BamBC) (Fig.1, left) and several model complexes with different oxidation states (a: W(0), b: W(II), c: W(IV). d: W(VI), Figure 1, right). The samples were kept at ~10 K by using a LHe-cooled cryostat. W $L\beta_3$ HERFD were collected by scanning the L_1-edge XAS, while monitoring the W $L\beta_3$ emission line (9819 eV) using Si(660) crystals at 80.55°. Radiation damage tests were performed to assess the maximum dose per spot.

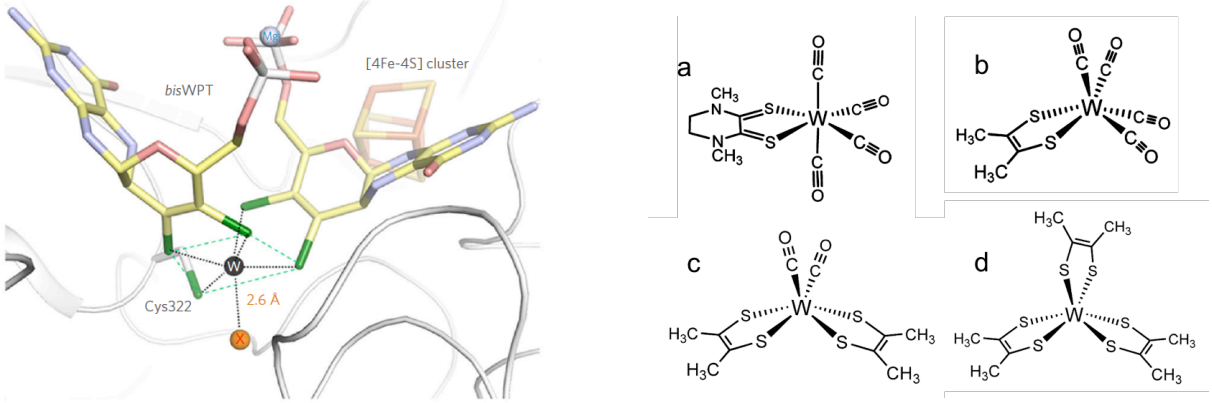

Figure 1: left: X-ray structure of the BisWPT cofactor in the BamBC subunit; right: Different model compounds, a: *[W(Me$_2$pidpt(CO)$_4$]; b: [W(mdt)(CO)$_4$], c: [W(mdt)$_2$(CO)$_2$], d: [W(mdt)$_3$].*

Comparing the spectra for the protein with and without dienoyl-CoA as substrate it is possible to see a small shift in the rising edge to lower energy for the sample with dienoyl-CoA (Fig. 2). This shift can be assigned to a lowering in the W oxidation state, which is consistent with literature[1].

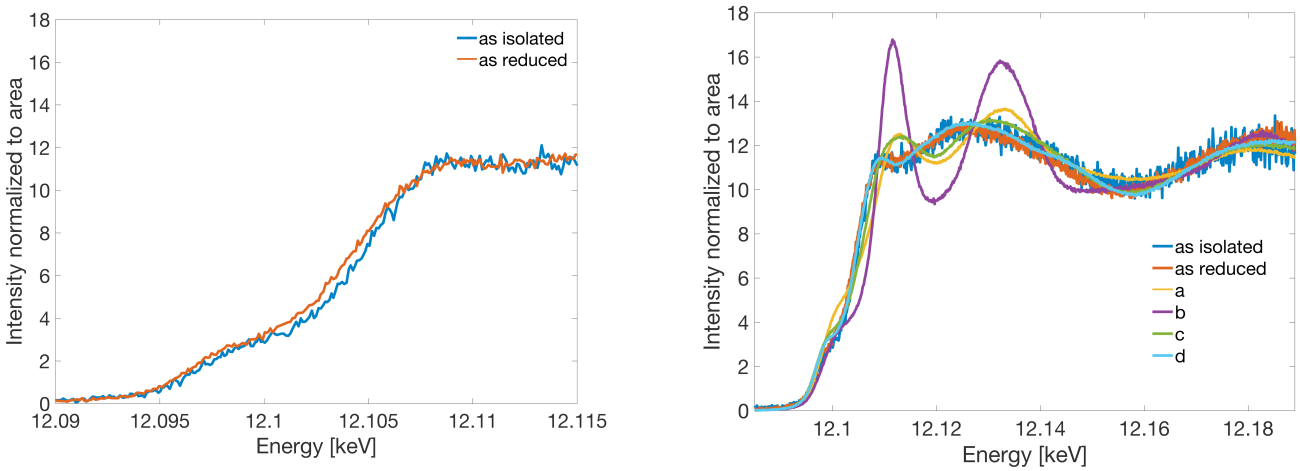

Figure 2: left: Zoomed in W L_1-edge from BamBC as isolated and BamBC as reduced (with dienoyl-CoA); right: Comparison of four model compounds with both protein preparations.

The spectra on the right side of figure 2 show a comparison between the two different protein preparations and four selected model compounds. It is nicely visible that the compounds with two or more CO ligands show distinct spectra compared to the protein ones. Only the fourth compound (d) with three dithiolene ligands and therefore six sulfurs bound to the W overlaps really nicely with both protein spectra. This is consistent with the crystal structure showing two dithiolenes and a cysteine ligand (Fig.1). In addition, the W L_1-edge seems to be sensitive to changes in the local site symmetry around the tungsten atom showing tremendous differences in the edge intensities for compound a and b.

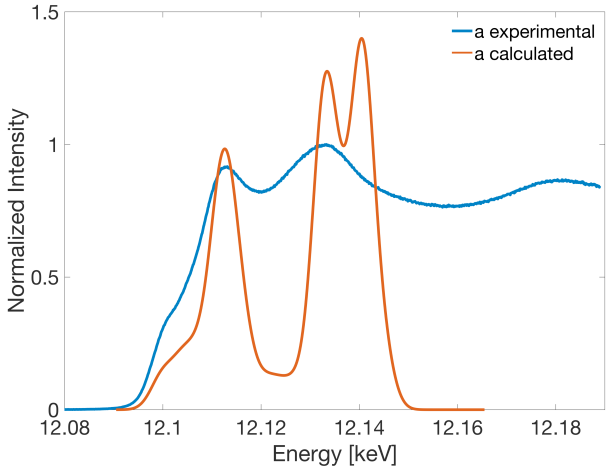

Figure 3:XAS spectra for compound a: Experimental data compared with a spectrum from a TDDFT

Figure 3 shows the comparison between an experimentally measured spectrum and a spectrum calculated using time-dependent density functional theory (TDDFT). It can be ascertained that the TDDFT calculations can really nicely reproduce the experimental spectra especially for the edge and the pre-edge region and will therefore help to understand the electronic structure for the different tungsten compounds and the protein.

Overall it can be summarized that the L_1-edge shows sensitivity for changes in the oxidation state of tungsten as well as for changes in the local site symmetry around tungsten. Compared to the featureless L_3-edge, the L_1-edge seems to be a powerful tool for analyzing tungsten compounds and tungsten containing proteins using x-ray absorption spectroscopy[2]. With support from TDDFT calculations it should be possible to increase the information we can get from the W L_1-edge and build up a good basis for a calibration study on tungsten compounds using XAS spectroscopy in combination with TDDFT calculations. The results of these studies are presently being prepared for publication.

References

1. Weinert, T. *et al.* Structural basis of enzymatic benzene ring reduction. *Nat. Chem. Biol.* **11,** 586–591 (2015).
2. Musgrave, K. B. *et al.* X-ray spectroscopy of enzyme active site analogues and related molecules: bis(dithiolene)molybdenum(IV) and -tungsten(IV, VI) complexes with variant terminal ligands. *Inorg Chem* **39,** 5238–5247 (2000).