EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"),*
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

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.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number 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:** Study of distribution and structure of copper and nickel metal centers in microbial protein wires with extraordinary electrical conductivity	**Experiment number**: LS-3138
Beamline:	**Date of experiment**: from: 30/09/2022 to: 03/10/2022	**Date of report**: 20/02/2023
Shifts:	**Local contact(s)**: Madeleine Han	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Bent Smets*

Silvia Hidalgo Martinez*

Eric Boschker

Filip Meysman

Gert Nuyts

Report:

Conclusion:

Experiment LS-3138 successfully investigated the electrically conductive network of cable bacteria with XANES. The conductive protein wires in the cell envelope were examined at the Ni K-edge to study a novel nickel cofactor, while the cell junctions were probed at the Cu K-edge to analyze an unknown copper metalloprotein (see LS3032). Ni XANES spectra show that the cofactor's nickel center in the conductive protein wires likely has a +II oxidation state and exhibits a square planar geometry. The copper center in metalloprotein the cell junction is likely in the +I oxidation state. Further investigation of the nickel cofactor is required since more information about the structure has been collected in other experiments. In future synchrotron experiments, EXAFS could be employed to investigate the coordinating ligands of the nickel cofactor and to determine the Ni-Ni distance between neighboring metal centers.

Introduction:

Experiment LS-3032 confirmed the hypothesis that conductive protein wires in the cell envelope of cable bacteria are enriched in nickel. This finding was rather controversial because biological electron transport is generally thought to be only mediated by Cu- and Fe-containing metalloproteins and indicates that cable bacteria evolved a highly efficient, nickel-based mechanism to transport electrons over centimeter-scale distances. This mechanism relies on a novel nickel cofactor with unknown molecular structure. Additionally, it was found that cell junctions contain a distinct structure made up of a copper metalloprotein. Previous studies proved that this structure is also conductive and plays an important role in electron transport. Both the nickel and copper metalloprotein were prime candidates to examine with Ni K-edge and Cu K-edge XANES respectively in an attempt to collect chemical information (oxidation state, coordination) about their metal centers. The small size of cable bacterium filaments (ca. 4 μm) and their cellular structures (junction, ±400 nm, conductive wires ±50 nm) required the use of the high spatial resolution offered by the ID16B beamline. In order to limit the radiation damage observed in previous experiments, a He cryostat was used to cool samples to ≈10 K.

Results:

Prior to XANES measurements, low-resolution (200 nm) XRF scans were done to image cable bacterium filaments. The beam energy was kept at 9300 eV to excite both copper and nickel. The resulting XRF maps

were used to identify regions with cell junctions (Cu-rich) and the conductive protein wires (Ni-rich) for XANES.

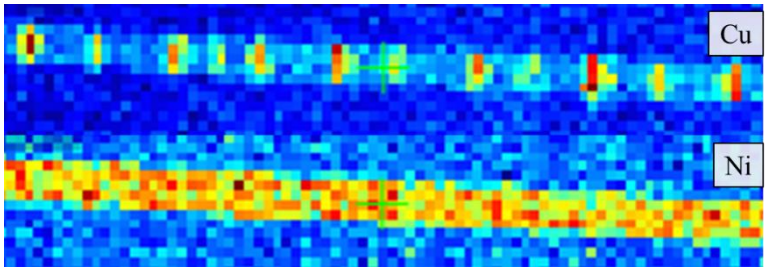

Fig. 1 – Low-resolution XRF maps of a cable bacterium filament. *The top XRF maps depict the regions of interest for Cu K-edge XANES (hotspots) since cell junctions are rich in copper. The conductive protein wires span the whole length of the filament but were only examined at the Ni K-edge in the middle of the cell to avoid radiation damage caused by measurements in the cell junction.*

Native cable bacteria and fiber sheaths (conductive protein wires supported by a basal sheath) were studied at room temperature and in cryogenic conditions (±10 K). Ni K-edge XANES spectra were collected in the middle of cable bacterium cells, while Cu K-edge spectra were measured exclusively in the cell junctions (Figure 1). Additionally, nickel reference compounds ($Ni(OH)_2$, Ni_2O_3, NiO) and copper reference compounds (Cu_2O, CuO, CuS) were examined. Per measurement point, 3 spectra were collected to inspect the effect of repeated radiation exposure on the XANES spectrum.
Measurements performed in cryogenic conditions generally resulted in lower signal intensities with higher noise. Moreover, the shape of the XANES spectra collected at room temperature and cryogenic temperatures were slightly different.

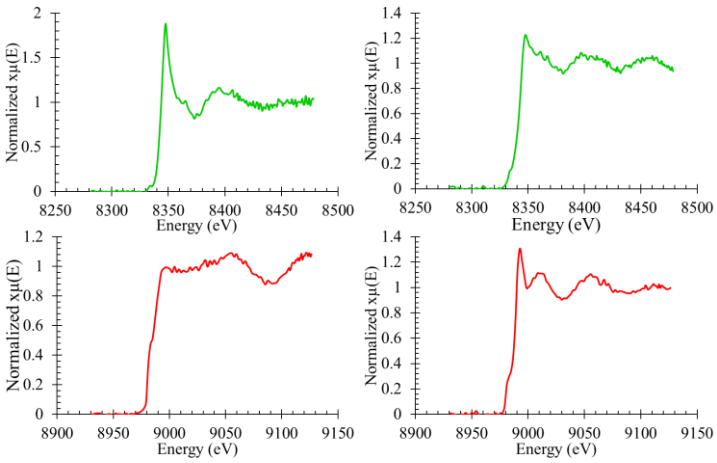

Fig. 2 – Ni XANES *(green) and* ***Cu XANES*** *spectra (red) of intact cable bacteria recorded at room temperature (left) and 10 K (right).*

The Ni K-edge was found to be positioned at 8343 eV in the nickel cofactor, indicating that the nickel center has an oxidation state of +II. The presence of a pre-edge around 8331 eV suggests a square planar geometry.
The Cu K-edge in the copper metalloprotein is located at 8987 eV and displays a clear pre-edge structure. Based on the reference compounds' spectra, we assume copper is in the +I oxidation state.

Discussion:

We observe clear differences in XANES spectra obtained in cryogenic conditions and at room temperature. These differences are likely caused by local ionization and heating of the cable bacterium filaments at room temperature compared to filaments kept in vacuum at cryogenic temperatures. Measurements performed with the cryostat generally yielded lower signal intensities potentially because of partial absorption of the secondary X-rays by the cryostat's windows. However, it is likely that these spectra represent the native state best because radiation damage is avoided. At instances, the signal intensity was also low due to certain detector elements malfunctioning, but overall, spectra were collected successfully.
Recent experiments have revealed that the nickel cofactor found in the conductive fibers of cable bacteria is most likely coordinated by 4 sulfur atoms of 2 dithiolene ligands. Because of these dithiolene ligands, the nickel cofactor has a square planar geometry. Additionally, electron paramagnetic resonance spectroscopy and modeling of the cofactor predict that nickel is in the +II oxidation state. Both findings were confirmed by the experimental Ni K-edge data gathered in LS-3138. In the past months, potential structural analogs of the nickel cofactor have been found. These structural analogs should be compared to the nickel cofactor in future Ni K-edge XANES measurements. Moreover, the EXAFS region should be explored in more detail in order to investigate the ligands of the nickel center. An additional benefit of performing EXAFS would be the possibility to determine the distance between adjacent nickel centers since this is crucial for long-distance electron transport in the conductive wires.