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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 using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now 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.

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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;
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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:** SoxR from *Vibrio salmonicida*. *Vibrio salmonicida*, a halophilic (salt loving) and psychrophilic (cold loving) curved gram-negative bacteria.	**Experiment number**: MX-687
Beamline: ID23-1	**Date of experiment**: from: 20.04.07 to: 21.04.07	**Date of report**: 10.05.07
Shifts: 2	**Local contact(s)**: Dr Didier NURIZZO	*Received at ESRF:*

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REPORT

Background and summary

Two shifts of beam time were granted in order to solve the crystal structure of the transcriptional regulator SoxR from *Vibrio salmonicida* (VsSoxR, 148 AA). VsSoxR is a member of the MerR family of bacterial transcriptional activators involved in superoxide and nitric oxide stress protection, by regulating oxidative stress genes (Hidalgo and Demple, 1997; Watanabe *et al.*, 2006). Reactive oxygen species (ROS) generated from both internal metabolism and external sources are highly toxic to bacterial cells. Superoxide dismutase (SOD), which is regulated by SoxR, and other peroxidases form the first line of defense against ROS. Oxidative stress in *V. salmonicida* has been a research topic in Tromsø from which two crystal structures have been solved, the catalase (Riise *et al.*, 2007) and the iron superoxide dismutase (VsFe-SOD, 194 AA, unpublished).

Data collected in Tromsø on a protein crystal co-crystallized with $KAu(CN)_2$ using the rotating anode with wavelength of 1.54 Å allowed the identification of anomalous signals which were suggested by XPREP to correspond to three heavy atom sites. The theoretical anomalous signal (f'') for Au is 7.3 electrons at this wavelength. The data were unfortunately not good enough to phase the structure, therfore beam time at the ESRF was applied for.

The structure was solved using a three wavelength MAD data set at (i) the peak and (ii) inflection point of the gold L-III edge and (iii) one high energy remote dataset. The initial model determined by MAD could further be used as starting model for molecular replacement on a dataset to 1.1 Å collected previously. After the structure was solved, we realized to our horror and despair that the structure was not that of *Vibrio salmonicida* VsSoxR but that of Fe-superoxide dismutase (VsFe-SOD), a protein which has been solved previously.

RESULTS

The majority of the crystals had been mounted in standard SPINE sample holders and vials, and the sample changer was used with great success. Several fluorescence scans of crystals co-crystallized with Au were easily performed, and the MXCuBE software was programmed to collect data in SAD or MAD mode. Our

typical crystals were about 400x300x300 μm^3, they were exposed for 1-3 second with 0.2-0.5 degree oscillation and 10% transmission of the beam. These settings were used through out the data set. The beam size used was either 100x100 μm^2 or 50x50 μm^2. Some of the crystals were translated between data collection scans.

In total 22 data sets were collected on two different crystal forms of the protein. The first crystal form is the one containing Au and belonged to space group $P6_521$ with cell axes of a=b=99.5 Å and c=231.8 Å. Two high multiplicity (~20) SAD experiments, 5 MAD experiments (three with 3 wavelengths and two with 3 wavelengths) were recorded in this space group. In addition, other data sets were collected after translating the crystals and shooting at a second place in order to try to keep the second data set isomorphous with the first data collected. One higher resolution data set to 2.15 Å was obtained with an overall R-merge of 8.0%, mean I/σ_I of 8 and 99.7% complete data. This was the best data every from this crystal form, and a great improvement from previous data collected to 2.5 Å resolution.

Three data sets we recorded on a new crystal form in a cubic space group (I23), where the best diffracted to 1.9 Å resolution, had an overall R-merge of 7.5%, mean I/σ_I of 16 and 100% complete data. The cell of these data were a=b=c=175.1 Å.

Phasing – One of the three wavelength MAD dataset at the peak (at 11.9153 keV), the inflection point (at 11.9102 keV) and a high energy remote (at 12.20 keV) was put through the program SOLVE which found three sites interpreted as Au atoms. The coordinates for these sites were put into SHARP, which further refined the positions, the occupancies (to1.4, 1.3, 1,1) and B-factors (to 69, 59 and 74 $Å^2$) for the three sites, respectively. The phasing statistics using all data from 25-2.4 Å were FOM of 0.502 / 0.296 (Acent./Cent.), anomalous phasing power of 1.8, 1.4 and 1.7 and anomalous R-cullis of 0.68, 0.78,0.71 for peak, inflection and remote, respectively. The refined contributions from f' and f'' (e^-) are shown in Table 1.

Table 1 *Refined values of f' and f'' (e^-) after running SHARP.*

	f' / f''
peak	-22.9 / 10.3
inflection	-24.0 / 7.2
remote	-19.0 / 9.0

The phases from SHARP were put into the density modification program DM, which with 61 % solvent and phase extension with the 2.15 Å data set, improved the phases sufficiently for ARP/wARP to built 290 residues. These residues were found to belong to two molecules. One of the molecules was used as a molecular replacement model for the 1.1 Å data set collected earlier. MolRep found two molecules in the asymmetric unit which allowed ARP/wARP to build 360 residues using data from 25-1.6 Å. However, the chains could not be docked into sequence. Manual inspection of the 1.6 Å electron density map allowed the identification of the amino acid sequence and revealed that the structure of a wrong protein had been solved, namely VsFe-SOD. After feeding ARP/wARP with the VsFe-SOD sequence the model, including the side chains, could be built. Analysis of all data sets obtained so far on this project, verified that unfortunately all were of VsFe-SOD.

CONCLUSIONS

Due to some unfortunate human mistake at an earlier stage of this project clones must have been interchanged so that what we believed to be VsSoxR, in this experiment was found to be VsFe-SOD. Both VsSoxR and VsFe-SOD had been verified by DNA sequencing, and interchanged afterwards. It was very unfortunate that the incorrect protein structure was solved by experimental phasing during this experiment and not the new target. Improved routines have now been established in the biochemical lab, by doing mass spec analysis of the purified protein and not only DNA sequencing, to prevent similar mistakes to happen in the future. We were very sorry about this protein being mixed up with another target, and that the use of beam time did not give the intended scientific results.

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