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 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.

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: **Structural analysis of halophilic proteins from *Halobacterium salinarum***	**Experiment number**: LS1660
Beamline: ID14-3	**Date of experiment**: From: 6.5.00 to: 14.7.00	**Date of report**: 15-3-01
Shifts:	**Local contact(s)**: S. Arzt	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Lars-Oliver Essen*
Stefanie Offermann*
Boris Bieger
Dieter Oesterhelt

Report:

The kingdom of archaea excels through a remarkable amount of extremophilic organisms. One example are the halophilic archaea which live in extremely high salt environments. A unique feature of proteins from these halobacteria is their stability under nearly saturated salt conditions. The structural properties of halophilic proteins are only partially understood as compared to their mesophilic homologs. However, a broader structural repertoire for halophilic proteins would not only advance the understanding of this extremophily on an molecular level, but would be highly beneficial for adapting other mesophilic proteins to high-salt conditions by protein engineering als well. We investigate the structures of two halophilic proteins which comprise as hollow spheres inner nanocompartments.

The first example is the DNA-protective protein dps from *Halobacterium salinarum* which was crystallized in two different crystal forms (hsod, hprot). Two native datasets have been collected earlier for the crystal form hsod but the method of molecular replacement failed to yield any consistent results until now. For both crystal forms heavy atom trials have been made, for the second crystal form a native dataset has been collected at 2.8 resolution using the beam-line EH3.

The table, below, summarizes the collection statistics of the dps datasets.

Dataset Name	Cell dimensions	Resolution	Completness	spg.	Rmerge	Remark
hsod3	**92.52, 92.52, 224.79**	**3.2**	**99.1 %**	**p321**	**8.4%**	**native**
hsod6	**91.67, 91.67, 222.61**	**3.2**	**95.6 %**	**p321**	**5.4%**	**native**
hgsod1	**92.37, 92.37, 224.17**	**3.0**	**91.2 %**	**p321**	**7.4 %**	**mercury deriv.**
hsodgad	**92.13 92.13 223.82**	**3.5**	**90.6%**	**p321**	**6.5%**	**Gd^{3+}-deriv.**
hsodpb	**91.80 91.80 223.17**	**2.7**	**93.1%**	**p321**	**3.4%**	**lead-deriv.**
hprot1	**91.01 91.01 150.23**	**2.8**	**99.5%**	**p321**	**8.3%**	**native**
hprot2	**90.99 90.99 150.82**	**2.7**	**99.6**	**p321**	**8.5%**	**mercury deriv.**
hprotpb1	**91.17 91.17 152.29**	**3.3**	**94.8%**	**p321**	**7.2%**	**lead deriv.**

The structure determination is now in progress for the second crystal form. Analysis of the self-rotation functions demonstrated the presence of two dps dodecamers in the hprot crystal form where the second dodecamer is rotated by 90¡ along its twofold relative to the first dodecamer. Consequently, the lattice symmetry was found to be P3 instead of the observed crystal symmetry P321 and a first packing model (24/3 monomers per a. s. u.) was derived for the halophilic dps protein which is now subjected to refinement.

The second example of a halophilic protein that was crystallized by us is the lumichrome-binding protein from *H. salinarum*. We collected at ID14-3 two native datasets to higher resolution than before of which one is now used for the further refinement of this remarkable protein. The lumichrome-binding protein assembles like the dps protein to hollow-sphere like dodecamers. Along the twofolds of this 23-symmetric particle a dimer of lumichromes is bound between two symmetry-related tryptophanes. The biological function of the lumichrome-binding protein which is present in several pathogenic eubacterial species is currently a matter of investigation.

Dataset Name	Cell dimensions	Resolution	Completness	spg.	Rmerge	Remark
lumbb1a5	**142.79**	**1.7**	**99.0 %**	**F4132**	**8.7%**	**native**
lumbb1a6	**141.97**	**1.7**	**99.7 %**	**F4132**	**6.0 %**	**native**