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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.

ESRF	**Experiment title:** EXAFS Study of the M412 intermediate of bacteriorhodopsin	**Experimen number**: LS1265
Beamline: ID26	**Date of experiment**: from: 09 June 1999 to: 14 June 1999	**Date of repo** 01/03/2003
Shifts: **18**	**Local contact(s)**: Dr. V. Armando Sole	*Received at ESI*

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Report:

Bacteriorhodopsin (BR) is the photoreceptor protein in the purple membrane of *Halobacterium salinarium*. It acts as a proton pump, allowing the transformation of light into chemical energy. Purple membrane (PM) binds 5 mols of Ca^{2+} per mol of protein, that play important structural and functional roles. The extraction of these cations leads to a deionised form of the membrane that possesses characteristic features. The purple form can be regenerate by the addition of mono-, di- or trivalent cations. The research concerning the cation binding to the purple membrane has mainly been directed towards the using of several indirect techniques as for example NMR, Scatchard plots, DSC (refs), but these techniques are unable to give structural and geometrical information about the coordination of the metals. In this sense, X-ray absorption spectroscopy is a direct technique to obtain information about the local structure around a metal. It allows to study purple membrane in solution, i.e. in an environment which is much closer to its physiological state. Also has the advantage of being element-specific and does not require a crystalline sample. The nature and location of the cation binding site is currently in dispute and is the subject of intense investigations by a number of research groups. Our previous studies have shown: i) the presence of one site of high affinity and four sites of medium affinity and five sites of low affinity for Mn^{2+}, ii) that the low-affinity sites are located on the BR C-terminal segment, and iii) by using EXAFS, that the binding sites are located in the protein and not in the lipidic region. (See references of our proposal Ref. N. 5377).

The main goal of our research was the study of the Ca^{2+}-high affinity binding site in the bacteriorhodopsin. In this experiment we have performed a detailed study of the absorption spectrum close to the absorption edge (XANES) that is very sensitive to the site geometry. We performed ab initio calculations of the X-ray absorption cross section based on a full multiple scattering approach

(Benfatto and Della Longa, J. Synchrotron Rad. 8, 1087-1094). A best fit of the experimental data was also performed by changing the cluster geometry (Fig 1).
We have characterized the Ca^{2+}-high affinity binding site in the bacteriorhodopsin. The Ca^{2+}-PM environment is compared with a Ca^{2+} water solution, showing differences both in the oxygen coordination numbers and cluster geometry.
The results of this experiment shows that the Ca^{2+} is hexa coordinated with the two atoms of the Asp-212, with one of the oxygen atoms of the Asp-85 and with three water molecules (Fig. 2 and Table I). This cluster shows a very distorted octahedral geometry with the 6 first neighbours located at an average Ca-O distance of 2.30 Å.
The great biochemical importance of this work is that Ca^{2+} is one of the native cations that have been found bound to the purple membrane in physiological conditions. Therefore the results of this experiment are of fundamental interest in order to know the structure-function relationship of bacteriorhodopsin, and also are a strong evidence for the specific binding site of the metal cation to bacteriorhodopsin.

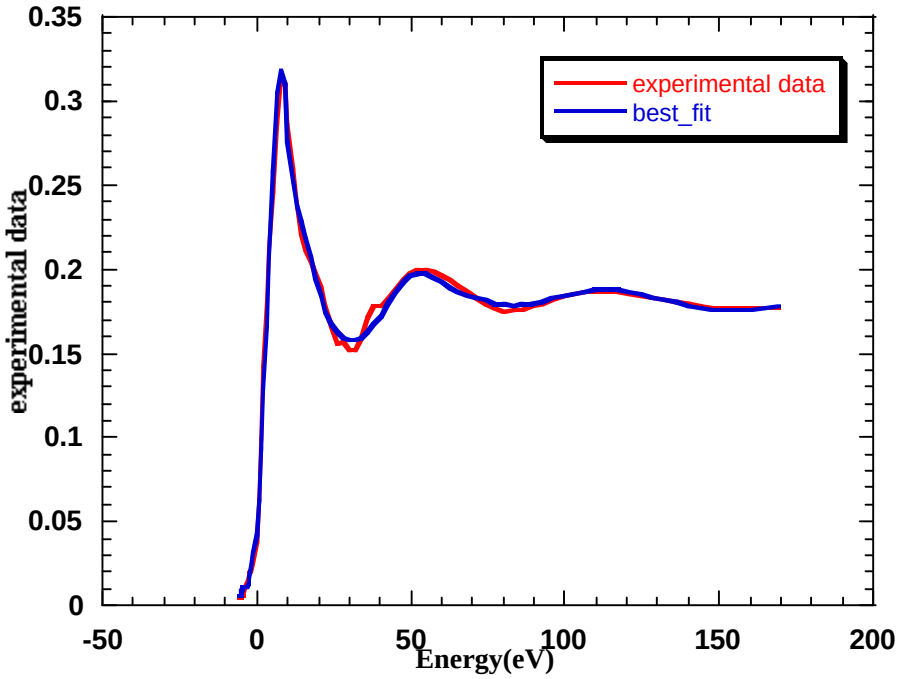

Figure 1. Theory-*vs*-experiment best fit results obtained for the cation binding site of bacteriorhodopsin

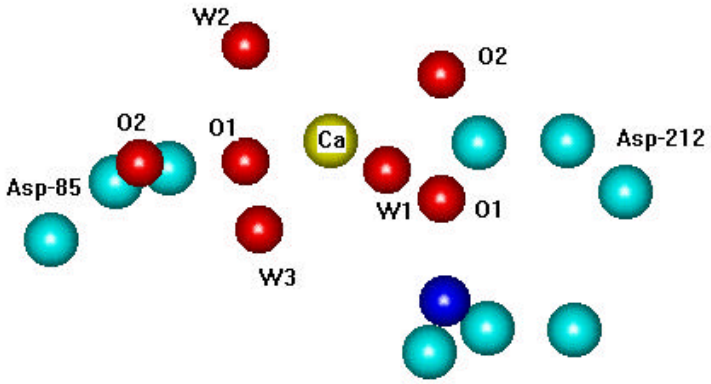

Figure 2. Optimized geometry of the cation binding site of bacteriorhodopsin.

Atom	O_1-Asp85	O_2-Asp85	O_1-Asp212	O_2-Asp212	W1	W2	W3	Mean
Ca	2.31	3.36	2.22	2.37	2.25	2.36	2.29	2.30 (excluding Ca-O_2-Asp85 distance)

Table 1. Selected distances of the optimized molecular cluster. All distances are in Angstroms.