ESRF	**Experiment title:** X-RAY ABSORPTION STUDY OF THE PURPLE MEMBRANE CATION BINDING SITES	**Experiment number:** LS-726
Beamline:	**Date of experiment:** from: July 5,1997 to: July 8, 1997	**Date of report:** 27-08-1997
Shifts: 9	**Local contact(s):** Adriano Filipponi	*Received at ESRF:* 1 SEP. 1997

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Report:

Bacteriorhodopsin (BR) is the unique protein of the purple membrane (PM) of Halobacterium halobium. It drives a proton transport from the inside to the outside of the cell, which operates under illumination. The proton pumping activity proceeds through a photochemical cycle which comprises several intermediates. In particular, proton release at the external surface occurs before the M412 intermediate, and proton binding at the cytoplasmatic surface occurs after the M412. The characterization of the M412 intermediate constitutes one of the goals in the study of the proton pumping activity. Native purple membrane contains one bound Ca2+ and four Mg2+ per BR molecule. Removal of these cations from the purple membrane induces a reversible transition of BR to a blue form which no longer pumps protons . The purple colour can be regenerated by the addition of a variety of cations, as for example Mn. Our previous studies have shown the presence of one binding site of high affinity and four sites of medium affinity for Mn2+ per BR. In particular, we have shown by using EXAFS, that Mn2+ in the high-affinity site is coordinated with six atoms of oxygen, in an octahedral disposition, and that this binding site is located in the protein. The aim of this study is the characterization, by means of EXAFS, of the Mn environment at the different

affinity sites, both when M412 is and is not present, to shed light on the structural changes .that the BR undergoes to allow or to inhibite the proton pumping activity.

Preliminary measurements have been carried out at BL29, recording, in the fluorescence mode at 70K, the EXAFS spectra at the Mn K-edge, on purple membrane samples regenerated with 1, 3 and 5 Mn^{2+}/BR, in such a way to compare high, medium and low affinity sites respectvely. A reference sample of water Mn solution, with a similar Mn concentration (about 1mMol.), has also been measured. The EXAFS spectra and FT transforms of the Mn solution (b) and of the 3 Mn^{2+}/BR sample are shown in the figure.

Two main results can be drawn. First, the Mn-O bondlength in the BR samples (2.17Å), is different from the Mn-O bondlength observed in the solution (2.20 A), indicating that the Mn atoms are bonded to the protein complexe. Second, the absence of further shells contribution, related to the presence of S or P as next nearest neighbors, in the BR spectra, indicates that Mn binding site is located in the protein and not in the lipidic region of the membrane. In addition, slight differences seem to appear, comparing between the medium and low affinity Mn site environment, but a higher data quality is needed for a reliable modeling of the system. These preliminary results are consistent with a previous study performed study on the high affinity site and can be considered as a good base to pursue the investigation on the M412 intermediate, i.e. on the changes of the Mn environment during the photocycle determining the proton pumping activity. Having the possibility of performing further measurements on samples with a lower Mn concentration (10^{-4}-10^{-5} Mol.) at a beamline dedicated to the study of very diluted samples, is crucial for the success of the experiment.