	Experiment title: **Time-resolved WAXS studies bacterial rhodopsins**	**Experiment number**: SC2546
Beamline: ID09B	**Date of experiment**: from: 27/11/2008 to: 02/12/2008	**Date of report**: 12/02/2009
Shifts: 15	**Local contact(s)**: Dr. Marco CAMMARATA	*Received at ESRF:*

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Report:

Retinal proteins are involved in both energy transduction and sensory perception. Thus the family of bacterial rhodopsins exploit both the energy and information content of sunlight. Of all membrane proteins, bacteriorhodopsin has the best structurally characterised mechanism of action and a structural model for vectorial proton pumping has emerged from several intermediate trapping experiments performed in 3D crystals at low temperature [1]. However, as with all low-temperature trapping experiments, there is a need to validate the major conclusions using other room-temperature methodologies.

This experiment aimed to take time-resolved wide-angle X-ray scattering (WAXS) methods developed in collaboration with members of staff of ID09B of the ESRF for studying small molecules in the liquid phase [2,3,4] over to the study of membrane proteins. Members of the staff of ID09 have recently pioneered the use of time-resolved WAXS to study soluble proteins [5].

Our first time-resolved WAXS studies, one year earlier, on the retinal proteins bacteriorhodopsin and proteiorhodopsin were successful, and a manuscript describing that work had been prepard and submitted to a a high impact journal. Following the reviewers reports, it was apparent that there was a need to improve the time-resolution in this data, in particular for proteorhodopsin. Thus we pushed the temporal resolution down from 2 μs to 350 ns in this study, and improved he signal to noise for the early data points. The data has since been successfully merged with the earlier experimental data, and a paper describing the light-driven structural dynamics of both proteorhodopsin and bacteriorhodopsin (Fig. 1), probed by time-resolved WAXS, has been submitted [6].

The second goal of this experiment was to extend the studies of bacteriorhodopsin and proteorhodopsin, two light-driven proton pumps, to sensory rhodopsin II, a light-driven sensory receptor. This aspect of the project was also extremely successful, with high-quality data recorded from sensory rhodopsin II. The analysis and modelling of this data is ongoing.

A third goal of this experiment was to repeat a study of proteorhodopsin with the retinal modified chemically, by replacing the C_{20} methyl group with an iodine atom. This idea builds upon our suggestion as to how time-resolved WAXS could simultaneously probe both local and global protein structural dynamics [7]. As with the data from sensory rhodopsin II, its too early to be certain that the data we recovered is of sufficient quality for publication, but the initial analysis seemed very promising.

Despite these very encouraging results, there were some technical difficulties. In particular we were one of the first users of the new Image-intensified CCD detector developed at the ESRF, and there were quite a few teething-issues. A shadow of earlier images remaind on this detector, and this is a major problem when we are trying to accurately measure changes of 0.1 % or less in the X-ray scattering intensities. Also there were software issues, such that some images were corrupted due to the detector being exposed to X-rays during readout. We have been informed that the ESRF is aware fo these probems and they will be sorted out for future experiments.

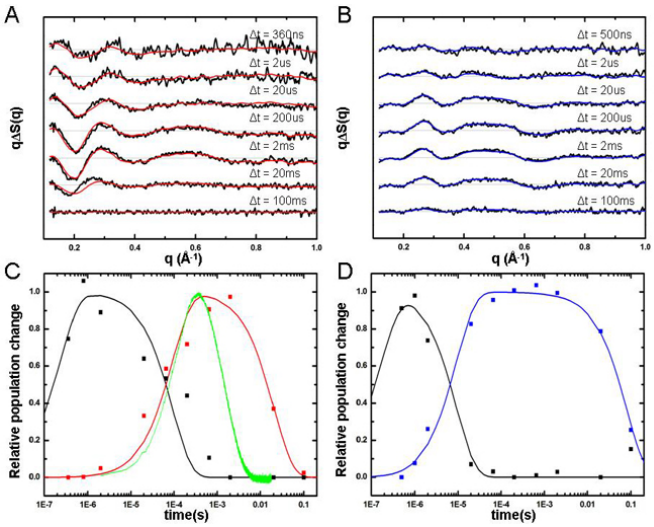

Figure 1: Time-resolved wide angle X-ray scattering data recorded from bacteriorhodopsin (left) and proteorhodopsin (right) and its decomposition into fast (black) and slow (red, bacteriorhodopsin; blue proteorhodopsin) components. Data were recorded at ID09B of the ESRF. A manuscript describing these data and their analysis has recently been submitted for publication [].

References

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