	Experiment title: **Time-resolved WAXS studies bacterial rhodopsins**	**Experiment number**: SC2812
Beamline: ID09B	**Date of experiment**: from: 16/12/2009 to: 21/12/2009	**Date of report**: 01/03/2010
Shifts: 15	**Local contact(s)**: Dr. Michael WULFF	*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]. Time-resolved wide angle X-ray scattering (WAXS) was developed by us in collaboration with members of staff of ID09B of the ESRF for studying small molecules in the liquid phase [2,3,4]. Members of the staff of ID09 have recently pioneered the use of time-resolved WAXS to study soluble proteins [5].

We recently pioneered the application of time-resolved WAXS to study membrane protein structural dynamics at this beamline, with the experiments performed during two previous beamtimes in 2007/2008 [6]. We were able to demonstrate that the difference data reflects the helical rearrangements of the proton pump bacteriorhodopsin, and structural refinement showed that two major movements dominate the photocycle. These are outward movements of the extracellular part of helices E/F and an inward movement of the cytoplasmic part of helix C. In the same paper, we also used time-resolved WAXS to demonstrate that the same underlying structural dynamics occur in proteorhodopsin from marine bacteria [6].

A **first goal** of this experiment was to record time-resolved WAXS data for visual rhodopsin, the photoreceptor in vision. The photoreaction is irreversible, which poses a major challenge for the data acquisition strategy, since each sample volume can only be used once: Can sufficiently high signal-to-noise be obtained for a sample where only few milligrams of protein are available? We addressed this challenge by constructing a carefully designed pumping scheme, where a fresh part of the liquid column in a glass capillary is exposed with each laser and X-ray pulse. Using approximately 25 mg of highly concentrated

protein solution (10 mg/ml) we recorded data as shown in the Figure. Due to the limited amount of protein available, only two time-points (20 ms and 20 µs) were acquired, and there is a definite need to return to ID09b to acquire a more complete timing history and to improve the data quality.

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 successful, with high-quality data recorded from sensory rhodopsin II. The analysis and modelling of this data is ongoing. Even for this experiment, we replaced the sample by pumping it with a syringe pump (at 10Hz), which proved much more robust than the previously employed strategy, which was to scan the capillary perpendicularly to the x-ray beam using a fast linear stage.

The **third goal** of the experiment was to record time-resolved WAXS data of bacterial cytochrome c oxidase, which is the last enzyme in the respiratory electron transport chain. In this case the enzyme was reduced and carbon monoxide bound to the active side. Thus the study was similar in concept to light-driven structural studies of hemeglobin [5], but there was an unknown scientific question as to whether or not the protein relaxed structurally in response to CO being dislodged from its active site. We recovered some preliminary data from this study, but it absolutely has to be repeated more thoroughly before the question of publication arises.

The beamtime, and especially the use of the pump system, delivered some very encouraging results. In particular, the pumping scheme allowed us to record data on an irreversible system and it yielded much more stable data than scanning the capillary. However, it was difficult to synchronize the pumps with the acquisition system and it would be beneficial if such a scheme would be permanently available at the beamline. With this in place, more complex acquisition strategies will be realistic considering the limited amount of beamtime available.

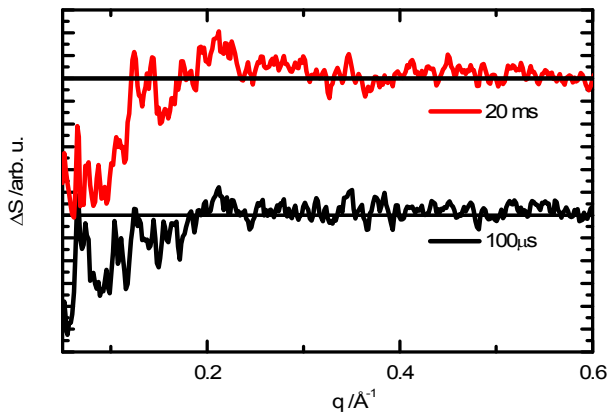

Figure 1: Time-resolved wide angle X-ray scattering data recorded from visual rhodopsin at delay time 20 ms and 100 µs.

References

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