	Experiment title: **Time-resolved diffuse x-ray scattering studies of a photosynthetic reaction centre and bacterial rhodopsins**	**Experiment number**: CH2271
Beamline: ID09B	**Date of experiment**: from: 7th July 2007 to: 9th July 2007	**Date of report**: 25/08/2007
Shifts: 9	**Local contact(s)**: Dr. Marco CAMMARATA	*Received at ESRF:*

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Report:

This experiment was carried forward from the previous allocation period, since technical problems at ID09 of the ESRF meant that it was not possible to schedule this experiment during the last run. Although at the time we foresaw a first experiment on the diffuse x-ray method for membrane proteins, which we had developed for simple photochemical systems [1,2], the emergence of a new crystal form of the *R. viridis* reaction centre made Laue diffraction studies a higher priority. We therefore made the decision, after consultation with Dr. Michael Wulff (beamline scientist for ID09), to prioritise a continuation of Laue diffraction studies of the reaction centre. This turned out to be a very good decision since the project made a tremendous jump forward. We now have scheduled time for the first liquid phase experiment in September 2007 (CH-2521).

Background

Photosynthetic organisms thrive by generating chemiosmotic potential across their biological membranes. One such bacterium is *Rhodopseudomonas viridis* in which light-driven reactions take place in a membrane-bound protein complex called the reaction centre (RC). When a photon is absorbed by the special pair (P) in the RC an electron transfer reaction is initiated across the membrane which ultimately leads to the reduction of a ubiquinone molecule (Q_B). The ubiquinone molecule diffuses in the membrane to the cytochrome bc_1 complex which oxidises it in two steps on the other side of the membrane. This so called redox loop is responsible for the active translocation of protons across the membrane. As a consequence a proton gradient is maintained and the energy stored in this gradient is harvested to propel vitally important reactions. One such example is the synthesis of ATP, the universal energy currency of the cell, which is primarily synthetised by the ATPsynthase utilising the chemiosmotic potential of H^+ ions.

In reaction centres light induced structural changes were first predicted for the $P^+Q_B^-$ state based on kinetic evidence. For example the electron transfer from static ubiquinone (Q_A) to Q_B was much faster in frozen samples when the reaction centres were illuminated prior freezing [3]. Stowell *et al*. in 1997 compared the illuminated and dark-adapted structure of the reaction centres. The crystals were illuminated before freezing and during data collection with a wide bandpath tungsten light source. In the illuminated crystals, trapped in the $P^+Q_B^-$ state, the head group of the secondary quinone has moved ~5 Å and undergone a 180° rotation compared to the dark-adapted structure [4]. To extend our knowledge about the reaction centre catalytic mechanism, we aimed to observe structural changes associated with this charge separated state. This work also builds upon our recently published results on light-induced structural changes in this photosynthetic reaction centre at low-temperature [5].

Experimental Details

To facilitate this goal we pursued Laue diffraction studies on *R. viridis* RC crystals grown from the sponge phase [6]. During the first shift of day one we established the X-ray and laser beam and the first crystals were mounted and tested for diffraction. The following two shifts were used for ground-state datacollection on *R. viridis* RC crystals. This resulted in reproducible Laue diffraction to 2.5 Å resolution, and this was extremely exciting since it was the first attempt with this new crystal form. However a setback immediately came up since the tunable ns- laser failed, and did not recover throughout the entire experiment. Thus the first night we could not perform a time-resolved experiment since we could not photo-excite.

In the beginning of day two a few hours were thus spent with setting up another ns- laser system (527 nm), which normally is used to pump the fs laser. It was fortutious that the *R. viridis* RC had an absorption at this wavelength, and the experiment could continue despite the failure of the tunable ns laser. When compared to our previous experiment with Laue diffraction in September 2006 (MX-505) using *R. sphaeroides* RC crystals, we changed to a 6 mm gap rather than a 9mm gap for the undulator U17, which gave a higher flux per single shot and a broader x-ray spectrum. The latter we learnt was valuable so as to make it easier to align Laue diffraction images. The *R. viridis* RC crystals were much bigger and of more consistent quality than the *R. sphaeroides* crystals. Their size also enabled a skimming algorithm to be used such that data was collected from a surface-laminnar, and this appeared to work extremely well. Furthermore, by translating these crystals several complementary laser-on/laser-off datasets were collected from the same crystal. Throughout the beamtime which remained we collected 26 laser on/off datasets using a time delay of 3 ms, and a number of variations (*eg*. varying the laser intensity, using two-pulse excitation; and using both reduced and oxidised crystals). This was a massive increase in the data collection over our previous experience primarily because of the significant improvement in the crystal quality, reproduciblity and robustness derived from developing a new purification and crystallisation protocol for the *R. viridis* RC.

Since the CH2271 beam time one laser-on/laser-off datasets has been processed using the program precognition (Renz Research). This data could be processed to 2.5 Å resolution and we recovered a completeness of about 50 % (figure 1). The next step is to process more data sets and merge data from several data-sets together, and thus we expect the completeness to improve significantly. We also need to refine the low-temperature monochromatic structure of this RC to 1.8 Å resolution so as to recover high-quality crystallographic phases for the analysis of this Laue data. Nevertheless, if things go well, we hope to have the first difference electron density maps by the end of September and a complete analysis by the end of this year.

One disappointment is that illumination conditions were optimized in advance of this experiment in Göteborg, using a microspectrophotometer and a ns-laser. After this experiment we were informed that the joule meter of ID09b had an incorrect calibration and returned an energy per pulse that is twice the real one. We do not yet know if this technical error will significantly influence the structural result recovered from this experiment.

In summary, an extremely encouraging outcome resulted from this experiment and the prospects of this project motivates us to further investigate time-resolved Laud-diffraction studies of this reaction centre, aiming at a 3D movie of structural changes in this light-driven membrane protein complex.

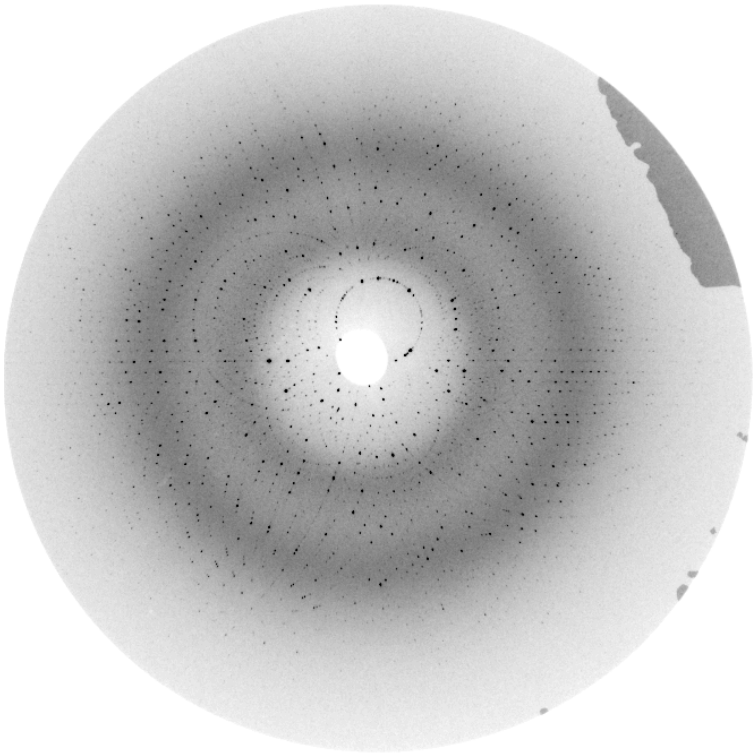

Figure 1: Laue diffraction image from sponge phase grown crystals of the *Rhodopseudomonas viridis* reaction centre. Data extends to 2.5 Å resolution.

References.

[1] R. Neutze, R. Wouts, S. Techert, J. Davidsson, M. Kocsis, A. Kirrander, F. Schotte & M. Wulff, *Visualizing photochemical dynamics in solution through picosecond X-ray scattering*, Physical Review Letters **87**, 195508-1-195508-4 (2001)

[2] J. Davidsson, J. Poulsen, M. Cammarata, P. Georgiou, G. Katona, F. Jacobson, R. Wouts, A. Plech, M. Wulff, G. Nyman, R. Neutze. *Structural determination of a transient isomer of CH_2I_2 through picosecond X-ray scattering.* Physical Review Letters **94**, 245503-1 - 245503-4 (2005)

[3] Kleinfeld, D., Okamura, M. Y. & Feher, G. Electron-transfer kinetics in photosynthetic reaction centers cooled to cryogenic temperatures in the charge-separated state: evidence for light-induced structural changes. *Biochemistry* **23**, 5780-6 (1984).

[4] Stowell, M. H. et al. Light-induced structural changes in photosynthetic reaction center: implications for mechanism of electron-proton transfer. *Science* **276**, 812-6 (1997).

[5] Katona, G. et al. Conformational regulation of charge recombination reactions in a photosynthetic bacterial reaction center. *Nat Struct Mol Biol* **12**, 630-1 (2005).

[6] Wadsten, P., Wöhri, A.B., Snijder, A., Katona, G., Gardiner, A.T., Cogdell, R.J., Neutze, R. & Engström, S. (2006). Lipidic sponge phase crystallization of membrane proteins. *J. Mol. Biol.* **364**, 44-53.