Report on MX655: ID09B September 11th to 13th 2007

Photosynthetic organisms thrive by generating chemiosmotic potential across their biological membranes. One such bacterium is *Rhodopseudomonas viridis* in which the light-driven reaction takes place in a membrane-bound protein complex called the reaction centre (RC). When a photon is absorbed by the special pair (P_{870}) 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.

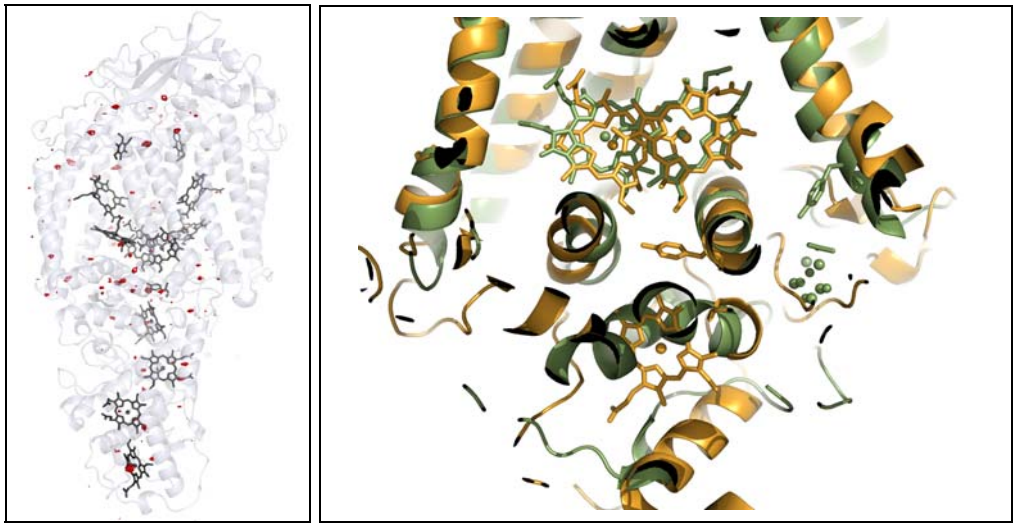
In reaction centres light induced structural changes were first predicted for the $P^+Q_B^-$ state based on kinetic evidence. For example the electron transfer from static ubiquinone (Q_A) to Q_B was much faster in frozen samples when the reaction centres were illuminated prior freezing[1]. Stowell *et 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[2]. However, no change in the protein matrix could be detected in the charge-separated structure. 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[3].

To facilitate this goal we pursued Laue diffraction studies on RC crystals grown from the sponge phase[4]. Since our working group had another experiment just before MX655 beam time we could take advantage of the beamline setup. The first shift of day one was used for switching from polychromatic to monochromatic mode. Already in the afternoon, the beamline setup was optimized and the first crystals could be mounted and tested for diffraction. The following two shifts have been used for laser on/off monochromatic data collection. At the same time data collection strategies have been tried out in order to get highest completeness. The crystals diffracted to about 2.2Å resolution, but it turned out that the crystal is dying already after a few frames. Due to this problem it was not possible to collect a complete dataset from one crystal and therefore we decided to switch to polychromatic mode. In the beginning of day two a few hours were spend on switching to polychromatic X-ray mode. There were initial problems with the lauecollect software and the sample alignment script. The experiment could proceed around 15:00 on day two as planed. The next shift was used to get data on oxidised crystals, which did not diffract to high resolution. During the remaining shifts about 15 datasets on reduced reaction centre crystals were collected. The final shift was

used for two electron transport reactions, but due to laser problems just laser off data was collected.

Since the CH2271 beam time laser on and laser off datasets have been processed using the program precognition (Renz Research). The data could be processed to 2.4 Å resolution with a completeness of about 60%. Data has been collected on different positions on the same crystal using 6° oscillation range of each image spanning 0° to 180°. This data collection strategy turned out to be better compared to experiment CH2271, where data was collected from 0°- 360° in steps of 12° oscillation range. Using this strategy the completeness was improved to about 15%.

After experiment CH2271 it turned out that the joule meter of ID09b has lost the right calibration and it returns an energy per pulse that is half the real one, so the energy has been to low and the photo cycle was not induced. In this experiment we repeated all the relevant experiments using the correct laser power in order to initiate the photo cycle. Until now tree datasets have been processed to 2.4Å and difference density maps have been calculated (see figure 1A). All the tree datasets show difference density on residue Tyr162, which is located between the high potential heme3 and the special pair P_{860} of reaction centre. This tyrosine molecule is supposed to play a role in the electron transfer reaction and resembles TyrZ of photosystem II in plants (see figure 1B). This interesting finding of the experiment and the prospects of this project motivates us to further investigate reaction centre pursuing a 3D movie of structural changes in this light-driven membrane protein complex.



(A) (B)

Figure 1: (A) overall difference diffraction map (2.4Å) of reaction centre from *Rhodopseudomonas viridis.* (B) Overlay of the special pair (P_{860}) of reaction centre from *Rhodopseudomonas viridis* (orange structure) with plant photosytem II (green structure).

References.

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