Report on MX-505: ID09B September 1st to 4th 2006

Local contact: Dr. Qingyu KONG

Photosynthetic organisms thrive by generating chemiosmotic potential across their biological membranes. One such bacterium is *Rhodobacter sphaeroides* 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.

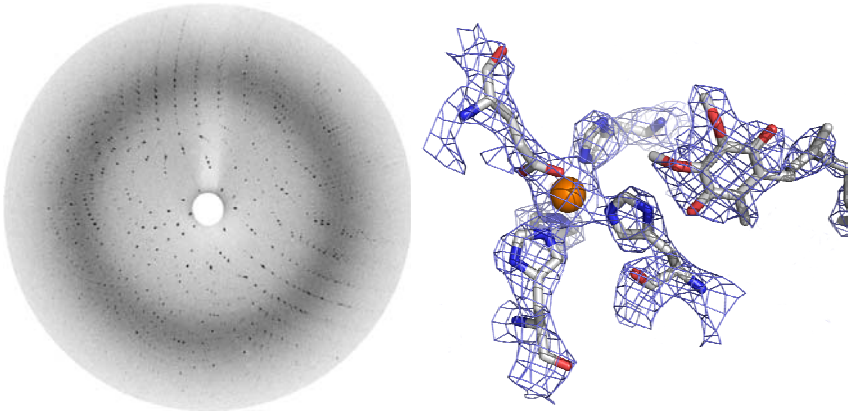
In reaction centres light induced structural changes were first predicted for the $P^+Q_B^-$ state based on kinetic evidence. For example the electron transfer from static ubiquinone (Q_A) to Q_B was much faster in frozen samples when the reaction centres were illuminated prior freezing[1]. Stowell *et 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]. Unfortunately this experiment was not without problems. The first day of this beam time started at 18:00 due to the fact that the previous detector had been seriously damaged a few days before we arrived. Thus detector exchange and accompanying software issues took quite some time. The first shift was used for optimizing the mounting protocol for the crystals. This included background corrections and work out of a fast fishing protocol since it was discovered that speed reflects the ultimate resolution of the crystals. As compared to the previous beam time in February (MX-477) the crystal quality seemed to be good, but not quite as good. From this point we started to set up the ns-laser system. Due to an upgrade of the fs-laser just prior to our experiment, the ns-laser software was gone and needed to be reinstalled on a new computer. Further delays were then incurred due to all optics, previously at the beamline, having gone “missing” when the fs laser was installed. The local contact spent considerable tracking down essential optics, and managed to find most things which were previously at the station. We then ran into serious difficulties with x-ray beam alignment since it turned out that one shutter was miss-placed. It is quite possible that the misplacement of this x-ray optics element was a user-error, but due to the extremely hard work of the local contact up until this point, Dr. Qingyu KONG, we were reluctant to

wake him, and the problem was therefore not solved until the following day. With all these technical problems only a fraction of the last day of beam time was used for collecting about 20 laser on/off datasets using a time delay of 3 ms.

Nevertheless, since this experiment datasets have been processed using the program precognition (Renz Research). The data could be processed to 2.6 Å resolution with a completeness of about 60% (Fig. 1). The reason for the low completeness is probably due to the data collection strategy of 2.5° oscillation range of each image and the 9 mm gap of the undulator 17. A better option would be to increase the oscillation range and use the 6 mm gap of the undulator 17. These changes we plan to make in the future. Processing of all data is continuing and will soon be complete. It is a major step that we can achieve good electron density maps at this resolution from these integral membrane proteins using 1 ms exposures.

The illumination conditions have also been optimized now using a microspectrophotometer and a ns-laser in Göteborg. The optimal power of the laser, relaxation time and occupancy has now been investigated. This has put us in a better position for conducting a successful time resolved light induced experiment of reaction centre.



(a) (b)

Figure 1: (a) Laue diffraction image from sponge phase grown crystals of the *R. sphaeroides* reaction centre. Data extends to 2.6 Å resolution. (b) Refined electron density for QA binding pocket (2Fo – Fc map contoured at 1σ).

References.

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