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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 state. This work also builds upon our recently published results on light-induced structural changes in this photosynthetic reaction centre at low-temperature[4].

To facilitate this goal we pursued Laue diffraction studies on RC crystals grown from the lipidic cubic phase[3]. After approximately one day of beamtime dedicated to optimizing the beam-line, we spent the remainder of the night working on the crystal environment. Many things were tried, but eventually a low-background method which enabled crystals to survive a long time (not drying out) at room temperature was optimized. Much of the second day was then spent working on the ns-laser system and working on the timing between x-ray and laser pulse. We then began to collect laser off/laser on data sets on RC crystals during the evening of the second day. We found that a "good" RC crystal could survive up to 40 Laue diffraction images of 1 ms duration. A representative image is shown in Figure 1. We therefore worked with 2.5^o steps between each image since 90^o should guarantee complete data.

When we began to work with ns laser photo-activated RC crystals we observed that the crystal rapidly died in the x-ray beam and that radiation damage was enhanced by the laser. We therefore worked a lot on trying optimal laser intensity on the crystal, and eventually found a trade-off where the crystal survived about 20 images before becoming unusable. We collected a lot of x-ray diffraction data during the remaining 36 hours of beamtime, and mounted and collected on more than 100 RC crystals in total.

We are working with Dominique Bourgeois in order to process this Laue diffraction data. Since data were collected only 10 days ago we are only at the beginning of this. A preliminary analysis indicates that many of the crystals we collected on were of not sufficient quality to process Laue diffraction data, yet several appear to be processable. We should have a better understanding of the quality of our data and the ease of merging data from several crystals within the next two months. We also have to work on the illumination conditions using

microspectrophotometery in Göteborg and find the optimal trade off between an acceptable photo-excited population (*eg.* 25 %) and the crystal dying too quickly. Nevertheless, since this was our first Laue diffraction study on RC crystals (or any system for that matter) it was an extremely promising start and lays the foundation for a long term programme pursing a 3D movie of structural changes in this light-driven membrane protein complex. This is particularly true since these RC crystals are still consistently improving in quality as we make changes to the crystallization protocol.

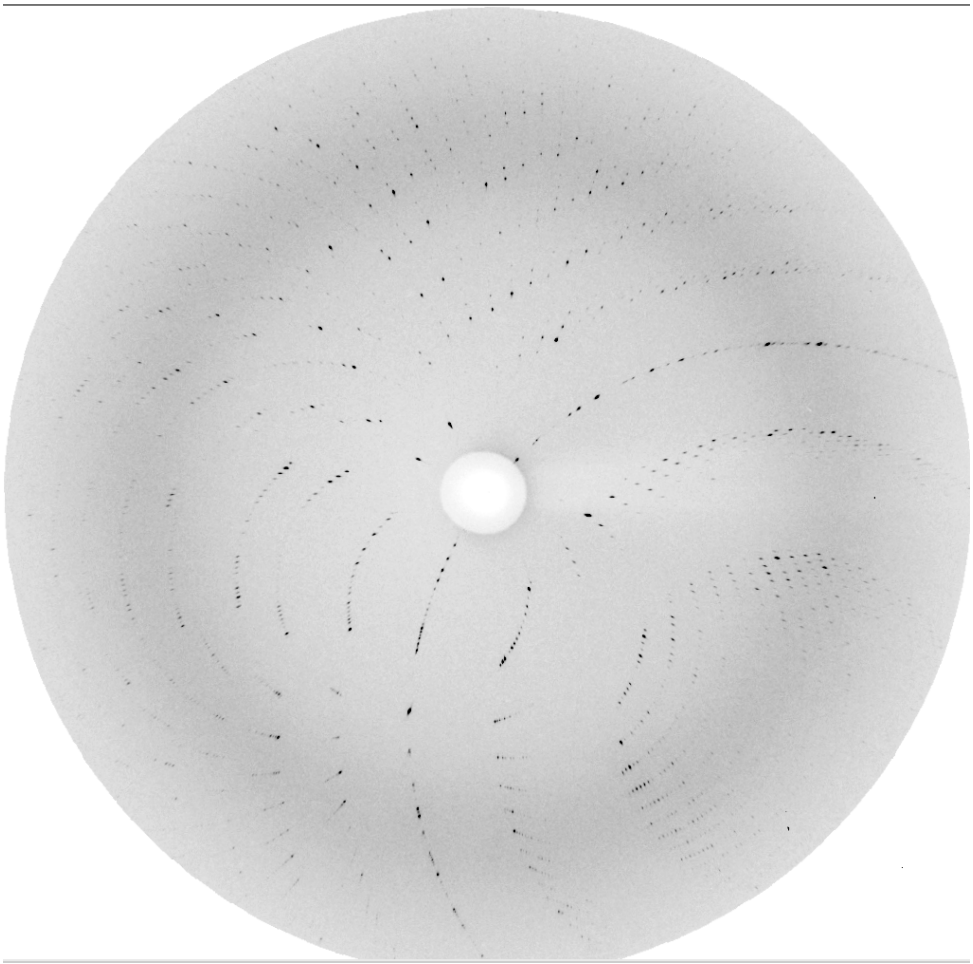

Figure 1: Laue diffraction image from lipidic cubic phase grown crystals of the *R. sphaeroides* reaction centre. Data extends to 2.7 Å resolution.

References.

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