ESRF

ESRF	**Experiment title:** Structural studies of ion pumps	**Experiment number**: LS-2167
Beamline: ID14-1	**Date of experiment**: from: 18th May to: 20th May	**Date of report**: 02/09/2002
Shifts: 6	**Local contact(s)**: Dr. Andrew McCarthy	*Received at ESRF:*

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Report:

The bacterial reaction center from *Rhodobacter spheroides* belong to type II reaction centers where the final electron acceptor is a ubiquinon molecule. Photosynthetic reaction centres are the basic functional protein complexes that can perform the primary photochemical event in photosynthesis: light driven charge separation. Following photoexcitation an electron flows from the special pair of chlorophylls to the dissociable ubiquinon (Q_B), which is consequently reduced to semiquinol. The semiquinol is further reduced to ubiquinol in a second light driven electron transfer step.

At Chalmers in Gothenburg we have grown crystals of bacterial reaction centre from *Rhodobacter spheroides* using lipidic phase crystallisation (1). This crystal form, which forms stacked two dimensional crystals, enables us to study the structure of an integral membrane protein in a “native like” environment. Crystals of photosynthetic reaction center from were grown in the presence of 60 % monoolein and 40 % protein solution. Plate like crystals of about 0.05 mm x 0.05 mm x 0.02 mm were grown in three days at room temperature. These crystals were screened for diffraction at

MAX-LAB prior to travelling to the ESRF, and the best crystals diffracted to 3.3 Å resolution in Lund. These crystals were saved at liquid nitrogen temperatures, and data were collected from the same crystals at ID14 EH1 of the ESRF. Due to the higher brilliance at the ESRF the structure could be refined to 2.35 Å resolution. A manuscript describing this novel crystal form of the bacterial reaction centre is currently in preparation and should be submitted within the next few months. We believe that this work will be of interest since this will be the first lipidic cubic phase structure to be reported from a membrane protein not belonging to the archeal rhodopsin family. There are also some interesting new insights regarding the plane of the membrane, crystal contacts, and a minor conformational change relative to the highest resolution detergent structures (2).

During this experiment we also tried the first intermediate trapping experiments, using illumination with IR light at low temperature. The initial difference Fourier Maps calculated from this data established the viability of the trapping protocol, and appeared to offer a reasonable mechanistic interpretation. However, before structural results from these experiments can be published, the resolution must be improved and the trapping experiment optimised. For this reason it is necessary to have access to further beamtime at the ESRF as the structural changes associated with charge separation are characterised.

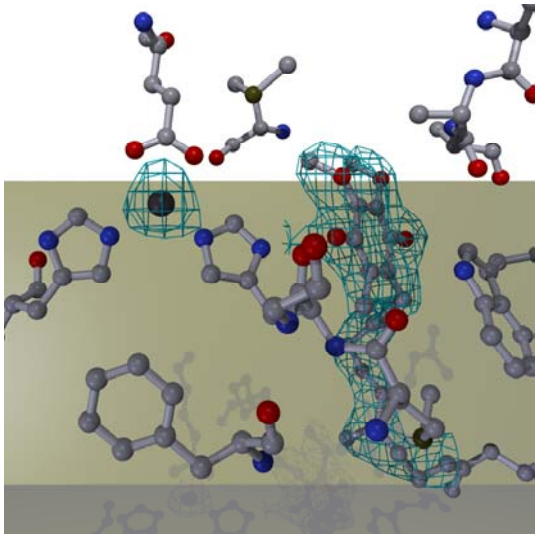

Figure 1: electron density for the tightly bound quinone, Q_A, of the bacterial reaction centre at 2.35 Å resolution.

References:

1. Landau, E. M., and Rosenbusch, J. P. (1996) *Proc Natl Acad Sci U S A* **93**, 14532-14535
2. Stowell, M. H., McPhillips, T. M., Rees, D. C., Soltis, S. M., Abresch, E., and Feher, G. (1997) *Science* **276**, 812-816