ESRF	**Experiment title:** The Structure Elucidation of the Membrane Intrinsic Protein Complex Photosystem I by X-Ray Crystallographic Methods	**Experiment number**: LS-1133
Beamline: ID02B	**Date of experiment**: from: 30 October 1998 to: 02 November 1998	**Date of report**: 26 August 1999
Shifts: 9	**Local contact(s)**: Dr. Bjarne Rasmussen	*Received at ESRF:*

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Report:

The present model of the structure of photosystem I (PS I) from the thermophilic cyanobacterium *Synechococcus elongatus* at 4 Å resolution (Schubert et al., 1997, Klukas et al., 1998a, b) lacks many important details, as the amino acid side chains and the exact orientation of the numerous cofactors. The aim of the last years experiments was to obtain a first structural model of PS I containing more or less complete polypeptide backbones, an assigment of the amino acid sequences of the 11 protein subunits and the complete set of cofactors bound to the complex: 3 [Fe_4S_4] clusters, ca. 95 chlorophyll *a*, 2 phylloquinone and ca. 25 β-carotene molecules.

The preceding experiment LS-932 at beamline ID02B was the first which led to a set of complete isomorphous data from a native and two heavy atom derivative crystals including reasonable anomalous scattering data collected under cryogenic conditions. At the time experiment LS-1133 was carried out it was not yet clear if the MIRAS phases derived from these data could be of sufficient quality to calculate an interpretable electron density map.

Diffraction experiments at 277 K had already shown that the procedure of cocrystallizing the mercury compound used to produce a heavy atom derivative with PS I led to derivative crystals with the heavy atom sites occupied to a degree different from the derivative crystals obtained by conventional soaking techniques. Therefore a data set of a cocrystallized mercury derivative was collected at $\lambda = 0.99$ Å and T = 100 K to 2.9 Å resolution. The data

set was almost complete only in the low resolution shells, while the completeness was smaller than 50 % already at 3.5 Å resolution. On the other hand, native data collected from two crystals were significantly improved with respect to the data previously collected. The data were 97.1 % complete from 50 to 2.5 Å (88.9 % from 2.59 to 2.50 Å) with R_{sym} = 0.07 (0.24 from 2.59 to 2.50 Å). The large completeness even in the highest resolution shells could be achieved because for the new crystals the mosaicity was reduced to 0.3º, compared to values larger than 0.7º for the crystals which had been available previously.

In the meantime, MIRAS phases could be calculated to 3.5 Å and an electron density map calculated after density modification, from which a first incomplete structural model of PS I (with 1572 out of 2293 amino acid side chains being assigned) was derived and subjected to crystallographic refinement against the new native data to 2.5 Å resolution. At present the R-value of the refinement has reached 0.358 (R_{free} = 0.379) and a further progress of the model building and refinement can be expected. The final structural model should explain many details of protein-pigment, pigment-pigment and protein-protein interactions and form the basis for simulations on the energy and electron transfer in PS I.

References

Schubert, W.-D., Klukas, O., Krauß, N., Saenger, W., Fromme, P. & Witt, H.T. (1997) Photosystem I of *Synechococcus elongatus* at 4 Å Resolution: Comprehensive Structure Analysis. *J. Mol. Biol.* **272**, 741-769.

Klukas, O., Schubert, W.-D., Jordan, P., Krauß, N., Fromme, P., Witt, H.T. and Saenger, W. (1999a) Photosystem I, an Improved Model of the Stromal Subunits PsaC, PsaD and PsaE. *J. Biol. Chem.* **274**, 7351-7360.

Klukas, O., Schubert, W.-D., Jordan, P., Krauß, N., Fromme, P., Witt, H.T. and Saenger, W. (1999b) Localization of Two Phylloquinones, Q_K and Q_K', in an Improved Electron Density Map of Photosystem I at 4 Å Resolution. *J. Biol. Chem.* **274**, 7361-7367.