ESRF	**Experiment title:** The Structure Elucidation of the Membrane Intrinsic Protein Complex Photosystem I by X-Ray Crystallographic Methods	**Experiment number**: LS-1367
Beamline: ID02B	**Date of experiment**: from: 06 February 1999 to: 09 February 1999	**Date of report**:
Shifts: 9	**Local contact(s)**: Dr. Bjarne Rasmussen	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Katrin Geßler*, Norbert Krauß*, Patrick Jordan and Wolfram Saenger

Institut für Chemie/Kristallographie, Freie Universität Berlin, Takustr. 6, D-14195 Berlin

Athina Zouni*, Petra Fromme* and Horst Tobias Witt

Max-Volmer-Institut für Biophysikalische Chemie und Biochemie, Technische Universität Berlin, Straße des 17. Juni 135, D-10623 Berlin

Report:

For many years our attempts to elucidate the three-dimensional structural of photosystem I (PS I) isolated from the thermophilic cyanobacterium *Synechococcus elongatus* had only led to electron density maps at relatively low resolutions. The most recent structural model determined at 4 Å resolution (Klukas et al., 1999a, b) was incomplete and not suitable for crystallographic refinement. The limitations in the available maximum resolution was caused by the large unit cell constants, the relatively high mosaicity and radiation damage of the crystals at the temperature (T = 277 K) where the X-ray diffraction data were collected.

The development of a protocol for cryocooling the PS I crystals and improvements of the crystal quality (Fromme and Witt, 1998) led to the first complete native data set suitable to 3 Å resolution collected under cryogenic conditions (T = 100 K). The preceding experiments LS-932 and LS-1133 at beamline ID02B had been successful as they resulted in MIRAS phases to 3.5 Å resolution, from which after density modification an interpretable electron density map could be calculated, and further improved native data to 2.5 Å resolution.

At the time experiment LS-1367 was carried out it was not clear if the initial model would be suitable for crystallographic refinement (but see report for experiment LS-1133), and we planned to improve the experimental basis for the MIRAS phases by collecting data from two additional heavy atom derivative data sets. In analogy to our experiments at 277 K, which resulted in significantly different occupancies of common heavy atom sites if derivatives were prepared using the same mercury and platinum compounds at different

concentrations, we soaked PS I crystals with solutions of these heavy atom compounds at concentrations which were reduced with respect to the last successful experiment (LS-932). The data sets were of reasonable quality: mercury derivative data were 82.7 % complete from 22 to 2.9 Å resolution with R_{sym} = 0.07 and $\langle I/\sigma(I)\rangle$ = 18.1 (R_{sym} = 0.15, $\langle I/\sigma(I)\rangle$ = 2.9 and completeness only 41.0% from 3.0 to 2.9 Å because of 0.6° mosaicity set with DENZO). Platinum derivative data were collected to 96.9 % completeness from 30 to 3.0 Å resolution with R_{sym} = 0.08 and $\langle I/\sigma(I)\rangle$ = 16.1 (R_{sym} = 0.29, $\langle I/\sigma(I)\rangle$ = 3.1 and completeness 95.8% from 3.12 to 3.0 Å, mosaicity 0.3°). Isomorphous difference and anomalous difference Patterson maps contain maxima at identical positions, confirming isomorphism with the native data and a reasonable anomalous scattering signal.

From a crystal of a complex of trimeric PS I with its soluble electron acceptor ferredoxin a 65 % complete data set was collected at 100 K, which was only suitable to 8.5 Å resolution with R_{sym} = 0.09 and $\langle I/\sigma(I)\rangle$ = 10.2 (R_{sym} = 0.24, $\langle I/\sigma(I)\rangle$ = 5.0 and completeness 63 % from 9.2 to 8.5 Å, mosaicity 1.0°). Obviously, these crystals have to be improved in order to obtain diffraction data suitable for solving the structure by molecular replacement, even at low resolution. An explanation for the poor quality of these crystals could be incomplete binding of ferredoxin to PS I.

References

Fromme, P. and Witt, H.T. (1998) Improved Isolation and Crystallization of Photosystem I for Structural Analysis. *Biochim. Biophys, Acta* **1365**, 175-184.

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**, 7360-7367.