ESRF	**Experiment title:** Structural elucidation of the membrane protein complex photosystem II of water-oxidizing photosynthesis by X-ray crystallographic methods.	**Experiment number**: LS-1503
Beamline: ID02B	**Date of experiment**: from: 20 November 1999 to: 22 November 1999	**Date of report**: 31 August 2000
Shifts: 6	**Local contact(s)**: Dr. Bjarne Rasmussen	*Received at ESRF:*

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Report:

Crystals of photosystem II (PS II) isolated from the thermophilic cyanobacterium *Synechococcus elongatus* are fully active in water oxidation (Zouni et al., 2000a). This opens the opportunity to investigate the three-dimensional structure of this complex, which is essential for oxygenic photosynthesis, by X-ray crystallographic methods.

When experiment LS-1503 was carried out, crystals of PS II were available which typically diffracted X-rays to resolution limits between 4 and 5 Å, if synchrotron sources were used. Although a number of native and potential heavy atom derivative data sets had already been collected at this time, non-isomorphism between the crystals turned out to be a major problem which prevented solving the structure by MIRAS methods. As even different native crystals were observed to be non-isomorphous, differences between proteins obtained from different purification runs were considered to be responsible for non-isomorphism. Especially for a lead and two mercury derivatives, high concentrations of the respective heavy atom compounds used in soaking of the crystals, ranging from 4 mM to 10 mM, obviously induced large shifts of the unit cell parameters. As diffraction experiments at our in-house rotating anode X-ray generator with crystals soaked with the same compounds at concentrations of 2 mM demonstrated that the unit cell parameters were not affected significantly under these conditions, data collections were carried out for a native crystal and the three potential heavy atom derivatives at beamline ID02B, using crystals originating from the same purification batch.

The native, the lead and one mercury derivative data sets were suitable to 4.5 Å, the second mercury derivative data set to 4.8 Å. Upon processing, scaling of the derivative data to the native set indicated isomorphism up to ~ 5 Å resolution between each of the derivatives and the native crystal. Anomalous Patterson syntheses calculated using the heavy atom derivative data sets showed major peaks in the Harker sections of space group $P2_12_12_1$ at positions, which agreed well with the maxima observed in the corresponding isomorphous difference Patterson maps, indicating the usefulness of the anomalous scattering data.

MIRAS phases calculated using these data to 5 Å resolution were employed in the localisation of heavy atom sites in derivative crystals, of which data were collected in the subsequent experiment LS-1663 at ESRF. These improved data finally led to a first structural model of PS II at 3.8 Å resolution (Zouni et al., 2000b)

References:

Zouni, A., Jordan, R., Schlodder, E., Fromme, P. and Witt, H.T. (2000a) First Photosystem II Crystals Capable of Water Oxidation. *Biochim. Biophys. Acta* **1457**, 103-105.

Zouni, A., Witt, H.T., Kern, J., Fromme, P., Krauß, N., Saenger, W., and Orth, P. (2000b), Crystal Structure of Oxygen Evolving Photosystem II from *Synechococcus elongatus* at 3.8 Å Resolution (submitted).