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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

	Experiment title: Structural Studies of photosynthetic complexes	**Experiment number**: MX471
Beamline: ID14EH4 /ID29	**Date of experiment**: 3 March 2006 – 6 March 2006	**Date of report**: 25 Jan 2007
Shifts: 9	**Local contact(s)**: John McGeehan	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Prof. James Barber FRS (Imperial College)

Dr. Martin Weik (IBS)

Dr. James W. Murray (Imperial College)*

Mr Karim Maghlaoui (Imperial College)*

Dr. Joanna Kargul (Imperial College)*

Report:

This report covers 9 shifts of beamtime on ID14-4, including a few hours on ID29 that were made available to us by the local contact. For ID14-4 shifts a few hours were lost to technical difficulties with the beamline or machine.

Studies on photosystem II (PSII) - a 700KDa membrane protein complex (Ferreira et al, 2004).

We scanned about 150 PSII crystals for diffraction quality, some up to 500µm in length. Unfortunately, none were of sufficient quality to improve on our existing 3.5Å crystal structure. The Sr-PSII crystals were of lower quality than the native crystals, and we were unable to obtain diffraction beyond about 5Å. These crystals were from new preparations of material and from different crystallization conditions. These results have enabled us to perform the further optimisation of our crystallization conditions that will be necessary to obtain better diffracting crystals.

We used the ESRF online microspectrophotometer facility to investigate the effect of radiation on the spectral properties of the crystals.

Fig. 1 shows the appearance of two absorption maxima at 533nm and 554 nm on a PSII crystal exposed to X-rays. These peaks correspond to the alpha and beta bands respectively of the reduced low potential form of cytochrom b_{559} (Kruk and Strzalka, 2001). We were also able to observe the appearance of the hydrated electron in the samples and slow decay of the 673nm chlorophyll red peak with X-ray dose.

We collected time-series of absorption spectra from concentrated vitrified PSII solutions at 100K before, during and after exposure to X-rays. These experiments were conducted in the presence of

the potential free-radical scavengers DTT, glutathione, ascorbate and acetate. A full analysis of these datasets will allow the effect of these scavengers on X-ray induced changes to be determined.

On ID29 we collected some composite datasets using the meethod of Berglund *et al* (2002). These preliminary results show that the technique works for this system, and can be used in the future to generate structures that vary by dose. This is particularly important for the 5 metal water oxidation complex, which is known to be very prone to radioreduction in the X-ray beam.

In summary, we scanned many crystals from different crystallization conditions for diffraction, the basis for for further optimisation experiments. We were also able to obtain novel spectroscopic results on these crystals. We also evaluated data collection techniques. When combined with better diffraction data we will be able to provide a 4-dimensional picture of PSII and the structural and chemical changes that occur in the X-ray beam.

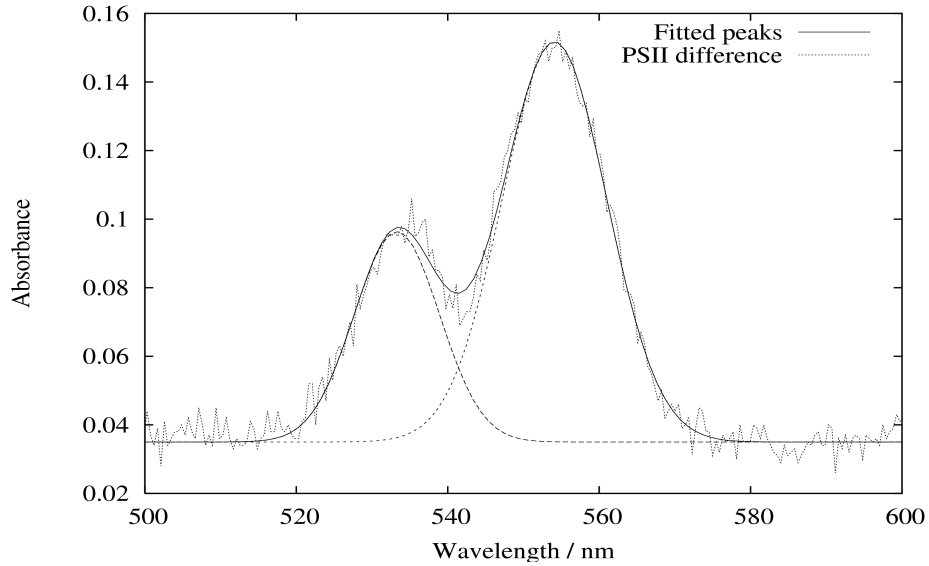

Figure 1: The change in absorption of a PSII crystal on exposure to X-rays.

References

Ferreira, K. N., Iverson, T. M., Maghlaoui, K., Barber, J., and Iwata, S. (2004). Architecture of the photosynthetic oxygen-evolving center. Science, 303(5665):1831–1838.

Kruk, J. and Strzalka, K. (2001). Redox changes of cytochrome b(559) in the presence of plastoquinones. J Biol Chem, 276(1):86–91.

Berglund, G. I., Carlsson, G. H., Smith, A. T., Szőke, H., Henriksen, A., and Hajdu, J. (2002). The catalytic pathway of horseradish peroxidase at high resolution. Nature, 417:463–468.