EUROPEAN **S**YNCHROTRON **R**ADIATION **F**ACILITY

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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:	**Experiment number**:
	The structure of Photosystem II with emphasis on the water splitting reaction mechanism	MX426
Beamline: ID14EH2/ID29	**Date of experiment**: 15July2005-16July2005 (ID14EH2) 23July2005-24July2005(ID29)	**Date of report**: 10 August 2005
Shifts: 6	**Local contact(s)**: Celia Romao (ID14EH2) and Laurent Terradot (ID29)	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Prof. James Barber FRS

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Dr. James Duncan*

Mr Karim Maghlaoui*

Dr. Joanna Kargul*

Report:

This report covers 6 shifts of beam time 3 on ID14-2 and 3 on ID29. For both sets of shifts a few hours were lost to technical difficulties with the beam line or machine.

Studies on photosystem II (PSII) - a 600KDa membrane protein complex.

We scanned 161 PSII crystals for diffraction quality, some up to 500μm in length. Unfortunately, none were of sufficient quality to significantly improve on our existing 3.5Å crystal structure. PSII crystals where the Ca ion in the catalytic centre had been replaced a Sr ion (Sr-PSII) were of lower quality than the native Ca-PSII crystals, and we were unable to obtain diffraction beyond about 6Å. These crystals were from new preparations of material and from different crystallization conditions. These results will enable us to perform further optimisation of our biochemical procedures and crystallization conditions in order to obtain better diffracting crystals.

We were able to conduct a fluorescence scan over the strontium K-edge on the Sr-PSII crystals. This confirmed the presence of strontium in the crystals. The determination of a Sr-PSII structure is a key aim for us, as it will enable the position of the calcium site within the water-slitting site to be unambiguously determined.

We used the ESRF Cryobench facility to investigate the effect of X-ray radiation on the spectral properties of the crystals. Fig. 1 shows a crystal that has been irradiated. The path of the beam in the mother liquor is clear. This purple colour (which disappeared over time), probably represents the hydrated electron.

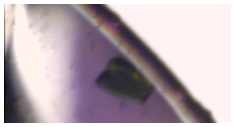

Fig 1. *A PSII crystal at 100K that has been exposed to X-rays. The path of the beam through the loop is visible as a purple line.*

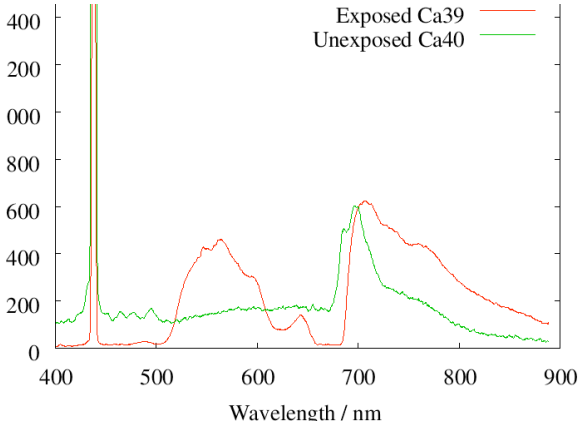

Fig 2. *The effect of X-ray irradiaton on the absorption spectrum of a photosystem II crystal. An absorption maximum at 541nm (attributed to pheophytin), disappears and is replaced by and the appearance of two new absorption maxima at 545nm and 553nm.*

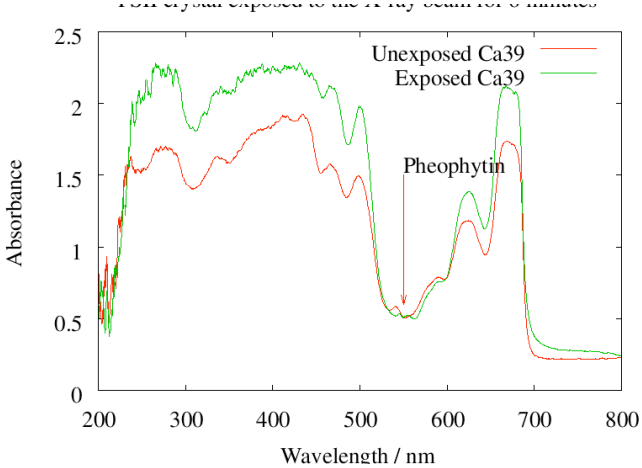

Fig 3. *The effect of X-ray irradiation on the fluorescence spectrum of a photosystem II crystal. The unexposed crystal showed two fluorescence peaks at 685nm and 697nm, corresponding to chlorophyll a contained within the CP43 and CP47 subunits respectively. An X-ray exposed crystal showed a new peak at 707nm, corresponding to a red-shift in the chlorophyll spectrum and also new unassigned peaks at 642nm and 546nm.*

Despite the very high visible absorption of the crystals, it was possible to show the likely photoreduction of pheophytin (Fig 2.) the primary physiological electron acceptor from the excited P680* donor. There is therefore a correlation between physiological function and chemical behaviour in the beam.

We measured the fluorescence response to excitation by a 440nm laser of a native PSII crystal and an X-ray exposed crystal (Fig. 3). The irradiation seemed to completely convert one fluorescing chlorophyll species to another. In addition several new fluorescence peaks were created, at 642nm and 546nm. These peaks are as yet unassigned, although it is likely that they are from some of the chromophores within PSII. The results need to be repeated but seem to provide an assay for damage/modifications occurring within PSII crystals on exposure to X-ray radiation. This interesting discovery will require a more rigorous analysis.

In summary, we scanned many crystals from different crystallization conditions for diffraction and obtained data which provides the basis for further optimisation experiments. We were also able to obtain novel spectroscopic results on these crystals. When combined with better diffraction data we will be able to provide chemical and structural pictures of PSII at different states of radiation damage and of the physiological reaction cycle.