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

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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.

ESRF	**Experiment title:** The Oxygen-Evolving Photosystem II Manganese Complex - Time-Resolved Analysis of S-state Formation and Decay at Room Temperature	**Experiment number**: LS 1428
Beamline: ID26	**Date of experiment**: from: 17.11.99 to: 22.11.99	**Date of report**: August 18, 2000
Shifts: 15	**Local contact(s)**: Dr. Armando V. Solé	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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The experiments at the ESRF were carried out at ID 26 by:
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Armando Solé (ESRF)

Report:

Photosystem II (PS II) samples were exposed to X-ray irradiation and simultaneously illuminated by single-turnover flashes of intense light (at room temperature). For illumination three synchronized flash-lamps were used (µs flashes, 500-800 nm).

The results of this study prove that a combination of flash illumination and XAS can facilitate investigations on intermediate states of the catalytic S-state cycle of PS II. For the first time, evidence has been provided by means of X-ray absorption spectroscopy at physiologically relevant temperatures that the S_1-S_2 transition of the oxygen-evolving complex is coupled to manganese oxidation and that the oxygen-evolving S_3-S_0 transition is coupled to manganese reduction. We feel that these experiments pave the road to a new type of XAS experiments.

Due to the use of Xenon flash lamps, pronounced S-state mixing occurred (flashes too weak and too long, inappropriate spectral characteristics). Therefore, publication of the results of this (successful) feasibility study would be premature. A satisfactory S-state population requires the use of ns LASER flashes (green light; e.g. 532 nm). We intend to pursue further this promising line of experiments using LASER illumination.

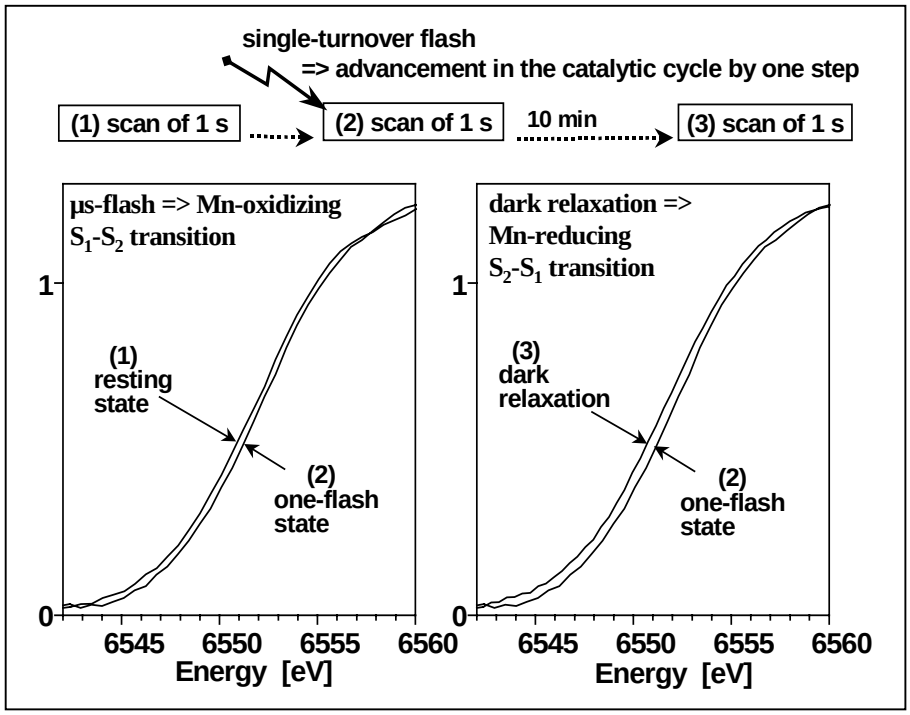

Figure 1. The S_1-S_2 transition was initiated by application of a single µs-flash of intense light; both sides of the optically thick sample were illuminated simultaneously. Within a few milliseconds after the µs-flash, a **rapid XAS scan** was started. Even though the S_1-S_2 transition proceeds in less than 1 ms, the S-state decay requires several minutes. Therefore the S-state decay during the scanning period of only 1 s is negligible. The observed edge shift is indicative of manganese oxidation upon the S_1-S_2 transition.

Figure2. Five µs-flashes of intense light were applied to samples exposed to the X-ray beam (flash frequency of 3.3 Hz). The **timecourse** of the X-ray fluorescence intensity was monitored (fixed X-ray energy). The shown data points represent the fluorescence intensity averaged for 300 ms (between flashes). For energies close to the inflection point of the edge, a decrease (increase) in the fluorescence-detected X-ray absorption points towards manganese oxidation (reduction). The results prove that a combination of flash illumination and XAS can facilitate investigations on intermediate states of the catalytic S-state cycle. Due to the use of inappropriate Xenon flash-lamps pronounced S-state mixing has occurred (estimated miss parameter of 40-50%). Excitation by green-light LASER pulses (of ns duration) should result in significantly reduced S-state mixing.