ESRF	**Experiment title:** Time-resolved Wide Angle X-ray Scattering Studies of Photosystem II in solution	**Experiment number**: CH-3177
Beamline: ID09B	**Date of experiment**: from: 09/12/2010 to: 14/12/2010	**Date of report**:
Shifts: 16b	**Local contact(s)**: Michael Wulff	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Jan Davidsson*, **Alexandr Nasedkin*, Moreno Marcellini***, **Fikret Mamedov***, Uppsala University, Department of Photochemistry and Molecular Science, Division of Chemical Physics, Box 523, SE - 75120 Uppsala

Richard Neutze, Gergely Katona*, **Linda Johansson***, **Erik Malmerberg***, Göteborg University, Department of Biochemistry and Biophysics, Box 461, SE-40530 Göteborg.

Aim of the experiment

The aim of this experiment was to investigate the global and local changes of the protein complex Photosystem II (PBD accession codes for this protein are 3BZ1 and 3BZ2 for the two monomers, 2AXT, 1S5L, 1W5C, 1ILX, 1FE1, 1IZL) extracted from *Spinacia oleracea*, which is responsible of the water oxidation during the photosynthetic process. This process occurs at a metallic cluster of 4 manganeses atoms and the calcium atom ($CaMn_4$). By light excitation of the harvesting complex (chlorophyll protein complex) and following charge-separation and electron-transfer from the $CaMn_4$ cluster active site, changes in global and local conformation should be observed. The most important and noticeble changes should occur in the $CaMn_4$ cluster. This complex goes through 4 different oxidation states in order to oxidize water before recoil to the original redox state and the time for completing each step is different, Figure 1. The original, dark, stable state is called S_1 state and each next transition-step is light-driven. Previous experimental results agree that the largest change in the $CaMn_4$ occurs between the S_2 and S_3 states. We focused on the time-resolved X-rays scattering measurements in time-scale relevant for this transition.

Experimental

The sample solution was infused in a quartz capillary and was continuously flowed in both directions during one single set of measurements. The light excitation was driven by the *in-situ* nanosecond pulse green laser. The dedicated time was split into two halfs, one where we investigated the local change in the $CaMn_4$ cluster at large q-space measurements and one

half where we focused on low q-ranges were the precence of large scale dynamics in the protein complex would appear.
Two laser pulses were used to excite the Photosystem II complex: the first one induced the S_1 to S_2 transition. The S_2 state is long living so that the second laser flash after 300 μs induced the S_2 to S_3 transition. The S_3 state lives longer than S_2 state and its life time were extended by adding on electrons acceptor. We measured the new state at a time-delay of 3 ms after the second flash (ON). Several X-ray flashes were collected on the MarCCD before dumping the image. Because of the long lasting S_3 state, the X-ray pulse train was extended to 5 μs long. The single differential diffraction pattern $dS_{3\,ms}$ was then computed as usual [1]. The average of such $dS_{3\,ms}$ is sketched in Figure 2. The main humps between 1.5-4.0 $Å^{-1}$ are due to the solvent heating.
Similar experiments were carried out to catch the S_1 to S_2 transition.
In this case, only one laser flash excited the solution and we measured the laser-excited solution after 4 ms ($dS_{4\,ms}$). The average of several single differential scattering is presented in Figure 3. Also here the main humps are due to the solvent heating.
The second half of the time was dedicated to the global change in the Photosystem II complex after charge-separation, i.e. we focused on small angle X-rays scattering (TR-SAXS). We could measure 2 different samples: a similar to the WAXS measurements, plus a so-called PhotosystemII core, a more purified preparation. In this specific case only one time-delay measurements were carried out at a time delay of 4 ms, trying to catch the global change after charge separation. The results are similar to Figure 2 and 3, when watching a $q < 2.5$ $Å^{-1}$. By comparing the two protein complexes (membranes and core complexes) we can also filter out the signal coming from changes in the membranes or catch the global changes in the membranes of the Photosystem II complex.

Technical problems encountered.

The synchronization of the two laser flashes and the X-ray probe pulse was initially a problem since the available software was not developed for such a set-up. The software code had to be altered by the beam line scientists but eventually the timing worked correctly. Also, there were some initial problems with the CCD that had to be solved by the technicians.
During the collection of the data, suddenly, the automatic conversion from CCDraw files to EDF files stopped due to an expectable crash of the main computer which induced the crash in the subsystems. From this point all the CCDraw to EDF files conversions were run manually.
In order to establish the heating component, the solution was heated by a small infrared diode and the usual OFF-ON experiment was carried out. Unfortunately, these experiments were not working properly due to a combination of problems related to the flow rate in the capillary, focus spot of the laser and the delivery of pulses from the software. This problem will be circumvented in the future by using a new IR laser for water heating with a small beam diameter.
Another obstacle was discovered after the beam-time had ended. It was found that the pin-hole used at the beam-line and used to align the laser and the X-ray beams, as well as to determine the focus of the laser, was damaged. The size of the pin- hole was much larger (400 um) then expected (50 um) which means that the overlap between the X-ray and laser

probably was very pore. It is also a delicate experimental balance between the flow rate of sample through the capillary and the delivery of the two laser pulses so that new sample is hit in every experimental cycle. If the focal spot of the laser is large and the overlap is slightly wrong it is likely that the sample is exposed to more than two flashes each time bringing the photo cycle back to ground state again.

Results

Thus, in conclusion, the problems with the heating measurements and the pin-hole unfortunately make it very difficult to extract any useful and trustful data from this experiment.

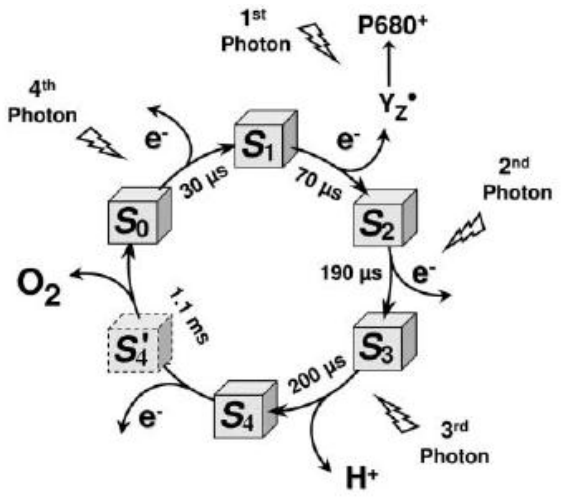

*Figure 1: Sketch of the S state and timing of the transition between different states during water Photosystem II oxidation (from: M. Haumann et al., Science, Vol 310, 1019, **2005**)*

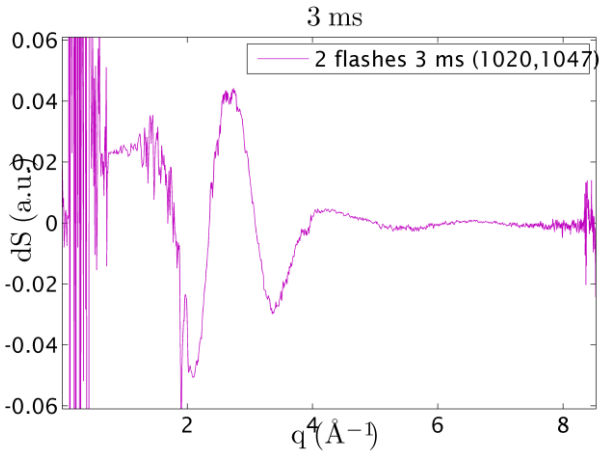

Figure 2: Differential scattering of the Photosystem II complex after 2 laser flashes at the time delay of 3 ms after the second flash.

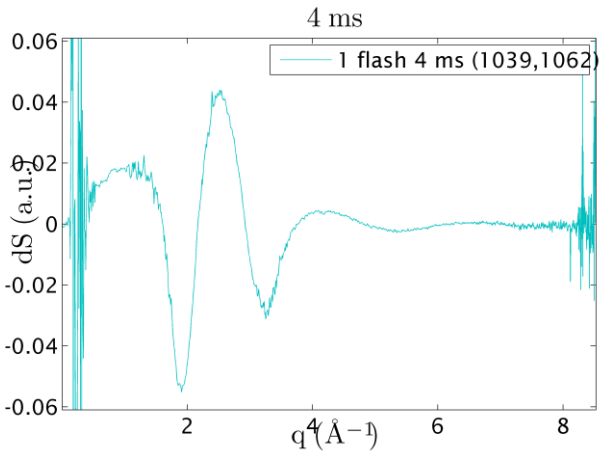

Figure 3: Differential scattering of the Photosystem II complex after 1 laser flash at the time delay of 4 ms. Here the movement from S_1 to S_2 state could be catched

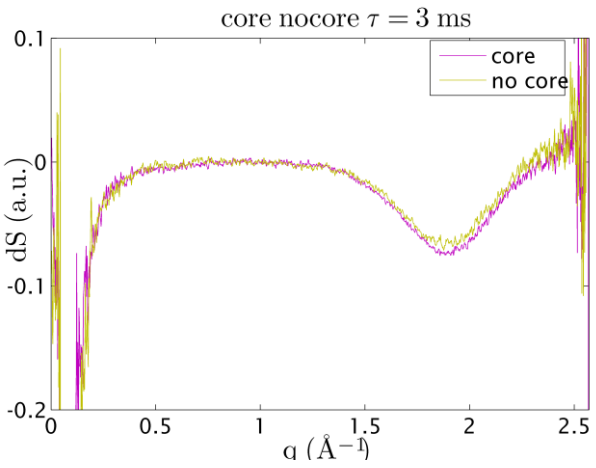

Figure 4: Example of the two protein complexes in TR-SAXS

[1] Marco Cammarata, Matteo Levantino, Friedrich Schotte, Philip A. Anfinrud, Friederike Ewald, Jungkweon Choi, Antonio Cupane, Michael Wulff & Hyotcherl Ihee, *Nature Methods*, **5**, 881 (2008)