	Experiment title: Online monitoring of spectral to unravel the structure of a key covalent intermediate in Baeyer-Villiger monooxygenases	**Experiment number**: MX-1083
Beamline:	**Date of experiment**: from: 20 feb 2010 to 21 feb 2010 to	**Date of report**: Aug 27th 2010
Shifts: 3	**Local contact(s)**: Martin Weik	*Received at ESRF:*

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We have performed a first round of microspectrophotometry analysis and X-ray data collection on phenylacetone monooxygenase (PAMO), a flavin-dependent Baeyer-Villiger monooxygenase. These experiments have been extremely insightful and provide the ground for another set of experiments aimed at the elucidation of the crystal structure of key intermediates in catalysis as described in the newly submitted beam-time application.

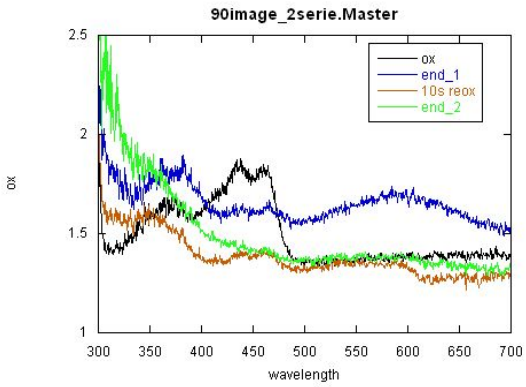
The main results we obtained are summarized below:
1) PAMO crystals give excellent spectra fully consistent with those measured in solution and typical for an oxidized flavoprotein. In the figure below, one can clearly see the characteristic peak at 458 nm followed by a shoulder (black spectrum).

2) As soon as the crystals are exposed to X-rays, quite "some chemistry" starts to take place. In particularly, there is the formation of a broad 500-600 nm peak (blue spectrum) which is due to the accumulation of hydrated electrons whose formation is favored by the high PEG concentrations present in the mother liquor. Consistently, the crystals acquire the typical "bluish" color that is a typical feature of hydrated-electrons generation. Interestingly, the 500-600 nm peak is transient in that after continuation of the exposure its intensity decreases (green spectrum). We also have found that addition to the mother liquor of radical scavengers (tocopherol, ascorbic acid) effectively protects from formation of hydrated electrons.

3) In parallel to the 500-600 peak formation, there is also formation of a transient peak at around 380 nm (see blue spectrum). The cause if this peak is probably the formation of some radical species (such as di-chloro radicals).

4) During X-ray exposure there is progressive bleaching of the 458 nm peak of the flavin (green spectrum). This clearly and unambiguously indicates that the crystalline protein is photo-reduced by the X-rays.

5) We could confirm that soaking in dithionite-containing solutions reduces the crystalline protein very effectively.



6) Both dithionite- and X-ray-reduced crystals are quickly re-oxidized upon exposure to air.

7) The crystals are remarkably stable under X-rays and do not suffer freeze-and-thaw cycles. For instance, we could measure four data sets on the same crystal. At the end of each data collection experiment, the crystal was warmed-up to expose to air and subsequently cryo-cooled.

8) Refinement of the structures obtained after photo-reduction, dithionite-reduction, and re-oxidation indicate the presence of conformational changes in a critical Arg residue and in the binding (only to the re-oxidized enzyme) of a negative anion such as chlorine and/or sulphite. This is information is very insightful in terms of understanding the structural elements and conformations that underlie the stabilization of catalytic intermediates. A further round of experiments is needed to further validate these observations and to attempt the elucidation of the critical flavin-peroxide intermediate as explained in the newly submitted application.