ESRF	**Experiment title:** TDS-XANES of YQR myoglobin	**Experiment number**: SC 1370
Beamline: BM 30B	**Date of experiment**: from: 10 September 2003 to: 18 September 2003	**Date of report**: 30-10-2003
Shifts: 21	**Local contact(s)**: Olivier Proux	*Received at ESRF:*

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Report:

The experiment SC 1370 at BM30B was a continuation of a previous experiment performed at ID 26 (CH 1405). The aim of the experiment is to combine a powerful tool, Temperature Derivative Spectroscopy (TDS), with XANES to study protein dynamics. With TDS, dynamic information is obtained from methods that traditionally require acquisition time of minutes and has mainly been applied to FTIR spectroscopy [1]. In a TDS measurement, the sample is initially perturbed from equilibrium (here we use laser irradiation under different illumination protocols to enhance the formation of particular photoproduct species). The temperature is subsequently increased linearly in time at a slow warming rate (0.3-1.2 K/min) and spectra are collected continuously, integrating over the minimum temperature range to obtain a good signal to noise ratio. Difference spectra are calculated from consecutive spectra and plotted as a function of temperature in a contour plot.

Myoglobin (Mb) is the paradigm for investigating conformational dynamics because it relative simplicity and photosensitivity of the iron–ligand bond, Fe-CO in our case. Upon photodissociation of CO at cryogenic temperatures, a nonequilibrium population of protein is generated with the ligand trapped in one of the possible docking sites inside the protein, or outside in the solvent matrix. We use three main photolysis protocols to select different myoglobin photoproducts: a) CO mainly trapped in the primary docking site (1s illumination at 10 K), b) CO mainly trapped in a secondary docking site (slow cooling under illumination at 3K/min between 140 K and 10K) and c) CO in the solvent matrix (slow cooling under illumination at 3K/min between 180 K and 10 K). The consequent relaxations induced by heating the sample is associated with migration of the ligand to cavities in the protein and with CO rebinding to the iron. CO migration in Mb has

been extensively studied in previous work using FTIR-TDS [2-4 and reference therein]. Our goal with XANES-TDS is to investigate the structural changes that occur in the vicinity of the heme group during ligand migration and rebinding.

To better reproduce the experimental conditions used for FTIR-TDS experiments, we brought with us a four-window cold-finger cryostat from the laboratory in Ulm as well as the Nd:YAG laser for illumination. With the strong help of the beam line staff, we were able to quickly mount our cryostat with temperature controller and start our measurements. Our first aim was to optimize the front window of the sample holder that must be transparent to the x-rays and capable of dissipating the heat induced by x-ray and laser illumination. We tested many different materials and thickness, such as ultra pure Aluminum, Beryllium, Carbon and Copper. We could not use Beryllium or Carbon due to iron contamination that completely swamped the signal, while Copper or Aluminum gave rise to a large diffused signal. We switched back to kapton (already tested in the previous experiments) and aluminated mylar and we were able to reproduce successfully one of the photolysis protocols we wish to investigate in detail (protocol c listed above). The slow cooling protocol was obtained for different samples and in figure 1 and 2 we present the preliminary results from the L29W mutant of Mb. We also collected a first XANES-TDS, using a gradient of 1.2 K/min and an integration time of 600 s (that means that all the spectra collected are integrated over 12 K each). In figure 1, we plotted the CO spectrum (continuous line), the photolyzed spectrum obtained at 10 K (dashed line) and the photoproduct structure generated by cooling under illumination from 160 to 3 K. As expect, the sample illuminated under protocol c showed no significant rebinding between 10 and 170 K. This demonstrates that on the current beam line, BM30B, we were able to eliminate the problem of sample heating due to the x-ray beam we had previously at ID26. In figure 2 we plot the difference spectra between the CO bound species and the photoproduct at 10 K (continuous line) and the difference between the same bound species and the slow-cooling photoproduct after warming again to 100 K. In the same figure (thick line), we show the difference between these two photoproduct spectra. The signal arises from a relaxation process of the heme between 10 K and 100 K in the absence of CO rebinding.

The ultimate goal of the experiment is to investigate the structural changes in the vicinity of the heme during ligand migration and rebinding. Unfortunately, we were unable to obtain a fully photolyzed spectrum at low temperature (protocol a) nor a complete photolysis using protocols b or c, mainly because of heating during illumination. As the sample holder, laser source, cryostat, protein concentration, and the path length are the same used routinely in the laboratory in Ulm, we are confident that the cause of this problem is the window material of the sample holder. For the infrared measurements, we use two CaF_2 windows, each with a thickness of 2 mm. We replaced the rear window with a thin window of sapphire (to avoid the large numbers of Bragg peaks induced by calcium fluoride), but we are now convinced that it is necessary even to have an optical transparent window in front of the x-ray beam. This is necessary for more homogenous illumination of the sample and to confine laser absorption to the sample, avoiding, for example, large absorption of the laser light by the kapton foil at the wavelength of 532 nm. We attribute the difficulties in photolysis at low temperature to the heating of the front window during illumination. We discussed this

problem with the beam line scientists of BM30B and decided to resolve this difficulty by building a sample holder specific for our needs. The sample holder consists of two sapphire windows, the one in front of the x-ray being as thin as possible (5 μm). We will test the sample holder in the laboratory in Ulm, before the start of a next session dedicated to the TDS-XANES.

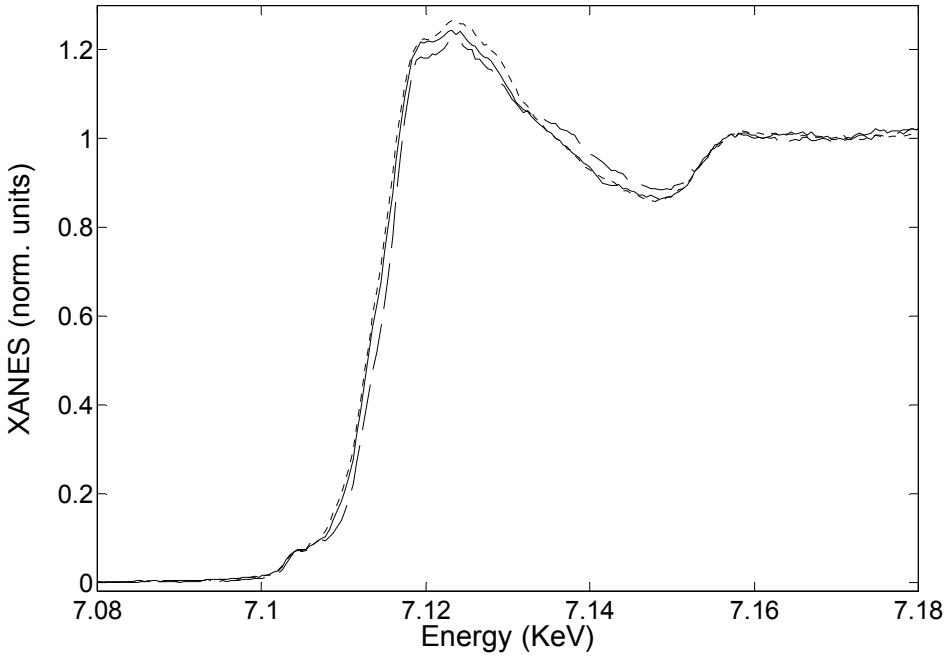

Fig 1: *Dashed line:* Mb-L29W CO bound species at 10 K. *Solid line:* Mb–L29W photolyzed at 10 K. *Dotted line:* Mb–L29W photolyzed species at 10 K after cooling under illumination from 160 K.

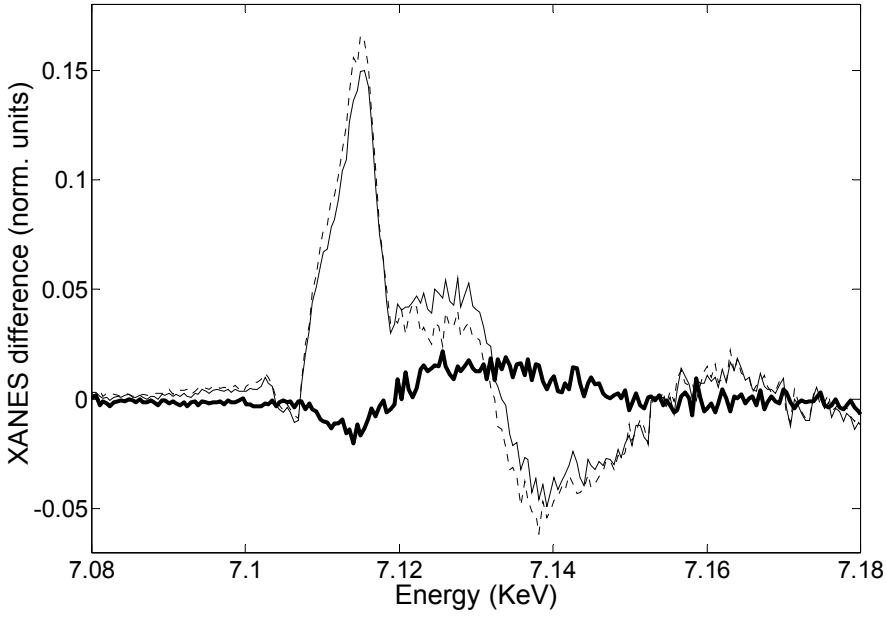

Fig 2: *Thin solid line:* difference spectra between photolyzed species at 10 K and CO bound species. *Dashed line:* difference spectra between photolyzed species under temperature gradient at 100 K and CO bound species. *Thick solid line:* difference spectra between photolyzed species at 10 K and sample photolyzed using protocol c at 100 K.

References

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