| ESRF | **Experiment title:** Temperature dependent local structure conformation in heme proteins studied by EXAFS | **Experiment number:** LS 860 |
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| **Beamline:** | **Date of experiment:** from: 4/2/98 to: 9/2/98 | **Date of report:** |
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**Report:**

This proposal was aimed to study the protein fluctuations by EXAFS and XANES. The beam time has been allocated togheter with proposal LS-871 therefore we present a joint report for the two proposals.

We have developed a new experimental approach, based on TDS (Temperature Derivative Spectroscopy), to probe the conformational landscape of horse heart carbonmonoxymyoglobin (MbCO), it allows us to study the behaviour of different conformational coordinates of the local iron active site structure, as function of the temperature. From the analysis of several single energy values of Fe-K edge X-ray absorption in the temperature range 13- 120 K, we have determined the distribution function g(H) of activation enthalpy barriers H relative to the photodissociation event, and related to relaxation of the Fe-Np distances and CO recombination. We have named this experimental procedure Temperature Derivative X-ray Absorption Spectroscopy (TDXAS). This study has been performed on MbCO in : a) water solution; b) a trehalose glass; c) a sucrose glass, and we found a variation in the dynamics of the recombination process in those three different environment

The X-ray absorption spectra of myoglobin have been measured at the ESRF synchrotron radiation facility in Grenoble on the beam line BM29. The 6 GeV storage ring was operating in hybrid mode with a typical current of 160 mA. The synchrotron radiation emitted was monochromatized by a fixed-exit double crystal Si(311) and focused on the sample, positioned at 45" with respect to the incident beam. Data were acquired with a 13 elements Canberra ultra pure Ge array detector, positioned perpendicularly to the X-ray beam. The energy resolution of 170 eV was detected at the Fe Ka fluorescence peak. The samples were kept in a 2 mm thick pure aluminium cell with mylar windows and covered with steal scotch to prevent iron contamination arising from the sampleholder. Data have been recorded at fixed energy values ( $E_1$=7.123 KeV and E2= 7.147 KeV) in the temperature range 13-120 K, being the sample mounted in a closed- cycle two stage cryostat with He exchange gas. Temperature was monitored using a PID controller with an accuracy of ±0.5 K. Photodissociation was performed at this temperature by illuminating the sample for l hour using two fiber optic lamps FORT GLI 156 P. For statical measurements, in order to obtain high signal to noise ratio, MbCO XANES spectra were acquired at 13 K, 5 s/point integration time each, in the energy range of 6980 - 7200 eV, being the iron edge positioned at 7120 eV. The zero of the energy scale ($E_0$) is fixed at the Fe metal K-edge defined as the first maximum of the derivative spectrum: $E_0$=7112.5 eV. For dynamical measurements the single-energy x-ray absorption detection, performed while heating the sample in a arbitrarly wide T range, have been recordered using 5 s/point integration time, requiring about 3 hours each spectrum and the sample temperature was ramped linearly.

The landscape of potential barriers for the CO recombination have been determined by this method. We show that it is possible to follow different conformational parameters of the local Fe site, such as the Fe position , the Fe-CO bond angle and the doming of the heme. For each conformational parameter we have found slightly different landscapes of potential barriers.These results shade new light on this fundamental problem of protein fluctuations that was studied before using infrared spectroscopy and are complementary results to time resolved x-ray diffraction. These results has been object of a comunication at XAFS X Conference and a longer paper has been written.

1) TDXAS study of the conformational landscape of MbCO in water solution, in a treahalose and a sucrose glass at different degree of hydration.Francesca Natali, Maria Lucia Alosi, Sveva Grande, Luca Maragliano, Fabio Librizzi, Alessandra Lanzara Antonio Bianconi, *J. of Synchrotron radiation to be published*