ESRF	**Experiment title: .** Study of the high frequency collective dynamics in hydrated protein: myoglobin in water solution	**Experiment number:** LS-558
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Report:

This experiment aimed to investigate the existence of high frequency collective excitations in hydrated proteins, and their dependence on the thermodynamic state and the hydration level. The chosen system was myoglobin, prepared in the weight ratio of 0.34 g of water per gram of dry protein. This corresponds to a value just above the activation threshold of the protein functions. The experiment was performed in the temperature range 180-300 K, and we excluded radiation damage by studying different parts of the sample at different times.
As shown in Fig. 1, at $T=240\ K$, it is possible to observe a weak inelastic scattered intensity, which has a Q dependence consistent with a linear dispersion. The speed of sound is $3000 \pm 300\ m/s$. The absolute intensity of this inelastic scattered signal is comparable to our previous measurements on liquid water [l], although its intensity ratio with respect to the elastic/quasi-elastic line is much more unfavorable. This is due to the presence of a very intense feature in the static structure factor S(Q) at Q $\approx 6\ nm^{-1}$, which is responsible for the strong elastic signal, and it limits to $1.5 \div 3\ nm^{-1}$ Q values the region where we have sufficient contrast to observe the inelastic signal (this, inspite of the fact that we used the best energy resolution available at the beamline: Si(l1 11 11) reflection , 1.5 meV energy resolution).
We investigated whether one can observe a temperature dependence of the inelastic signal across the protein glass transition, expected to be around 190 K. The mode coupling theory (MCT) [2], in fact, predicts the existence of a critical temperature T_c marking the idealized glass-liquid transition. At T_c, the Debye-Waller factor, which is related to the intensity ratio of the elastic to inelastic scattered intensity and to the high frequency sound velocity, has a square root behavior with a cusp at T_c. Therefore one can obtain relevant informations on the glass transition directly from the dynamic structure factor. A selection of spectra taken at $Q=1.8\ nm^{-1}$ at different temperatures is reported in Fig. 2.

The statistical accuracy of the available data, unfortunately, is not sufficient, at present, to derive any conclusion on the existence of a temperature dependence of either the sound velocity, $v_s = \omega/Q$, or of the elastic to inelastic intensity ratio. Further data analysis is in progress, The data are being fitted by the convolution of the experimentally determined resolution function (essentially represented by the deconvoluted central line contribution to the spectra in the figures) with a lorentzian for the central line and a damped oscillator model for the inelastic signal. Few preliminary conclusions can already be extracted from the present results: i) We confirm the presence of collective excitations in hydrated proteins [3], which have a Q-dependence consistent with the linear dispersion of a sound mode. ***ii)*** The speed of sound of these excitations is similar to that of liquid water in the same considered high Q-region. iii) The signal quality, at present, does not allow to get information on a possible temperature dependence of the dynamics, that could be analysed within the formalism of liquid-glass transition theories. ***iv)*** The inelastic x-ray scattering instrument is capable to detect these very weak excitations inspite'of the very unfavorable situation where an intense peak in the S(Q) is present in the Q-region of interest. ***v)*** The available beamtime was not sufficient to investigate a possible dependence of the collective dynamics on the hydration level, and to study the dry protein.
This study needs extra beamtime to continue the work on myoglobin, and to investigate another protein, that, in solution, gives a reduced structural elastic contribution to the scattered intensity.

References:

[l] J. Teixeira et al., Phis. Rev. Lett. 54, 2681 (1985) F. Sette et al., Phis. Rev. Lett. 75, 850 (1995)
[2] W.Gotze, in "Liquids, Freezing and the Glass Transition", edited by J.P. Hansen, D. Levesque and J. Zinn-Justin (North-Holland, Amsterdam, 1991).
[3] M.G. Bellisent-Funel et al., Biophys. J., 56, 713 (1989).

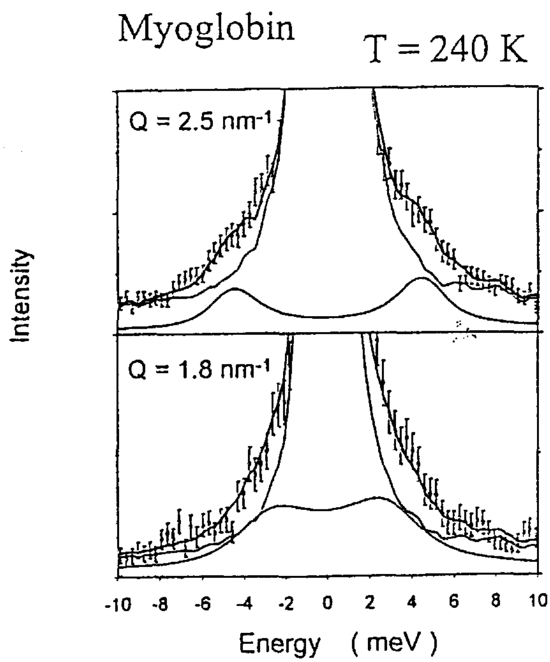

Fig. 1

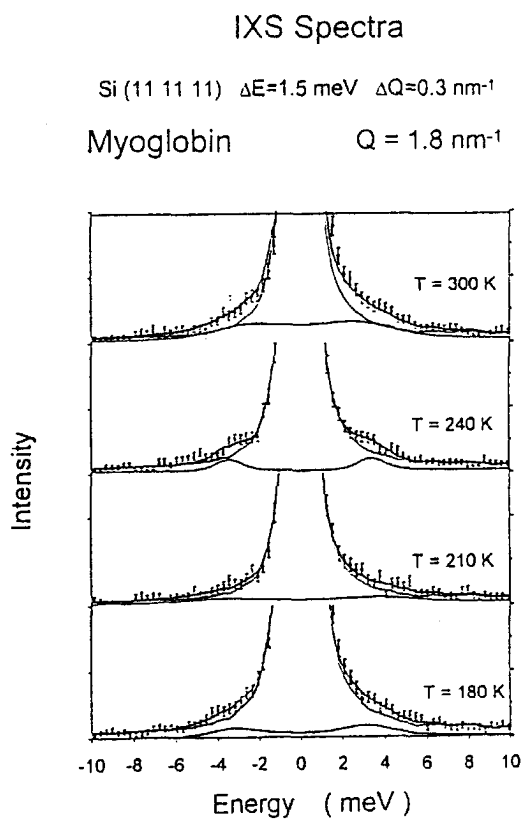

Fig. 2