	Experiment title: Feasibility of a MAD experiment at the uranium M_V absorption edge (3.5 keV) with an uranyl derivative of lysozyme crystal	**Experiment number**: MI481
Beamline: ID01	**Date of experiment**: from: 4th April 2001 to: 8th April 2001	**Date of report**: 4th October 2001
Shifts: 9	**Local contact(s)**: P. Boesecke	*Received at ESRF:*

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Report:

Diffraction experiments with soft X-rays face several challenges. Among these there are:

- the optimisation of transmission,
- the increase of the solid angle of diffraction.

These two points were attacked in the project MI481. Tetragonal lysozyme was chosen as sample.

The set up was similar to that which had been used successfully in September 2000 (MI384): the protein crystal was cooled by cold helium gas, using the set up developed at EMBL. The modifications concern the windows:

- the Kapton window at the entrance of the helium box has been replaced by a beryllium window of 25 µm thickness,
- the 20 µm Mylar window in front of the IP has been removed, i.e. the IP was exposed directly to the scattered radiation,
- the angular range has been extended from 75° (in September 2000) to 106° with a newly constructed (home made) helium box, which schematically is shown in Fig 1.

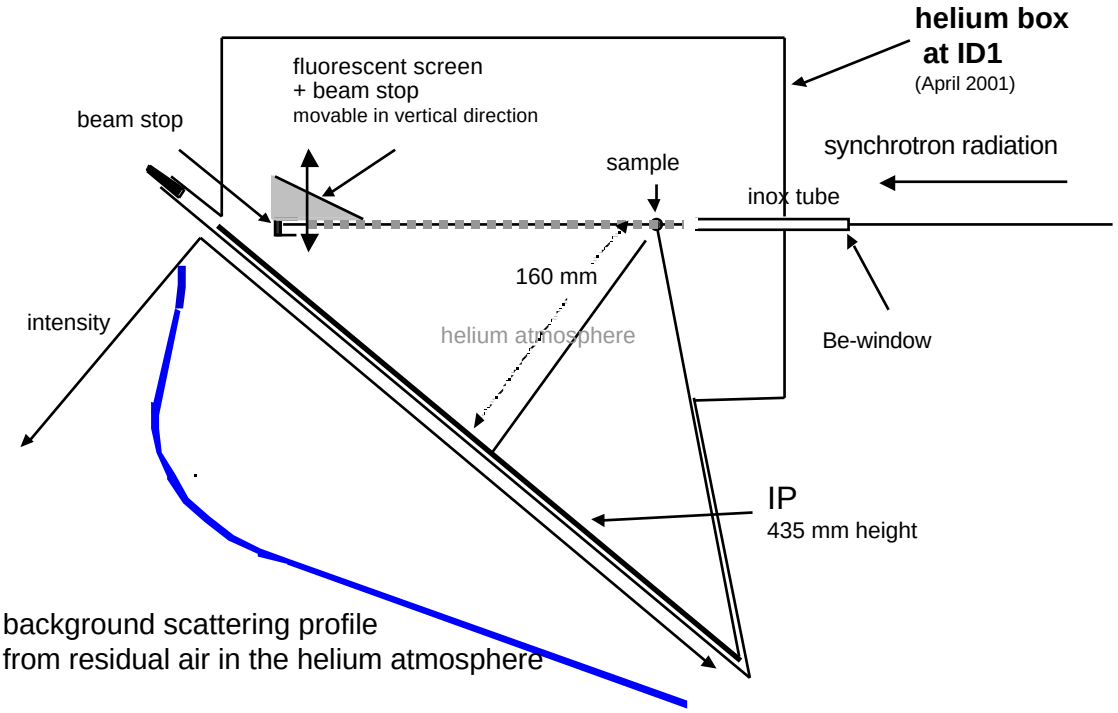

Figure 1. Schematic drawing of the helium box (in black) and background intensity profile (in blue).

The experiment demonstrated quite clearly that the crystal of the lysozyme doped with uranyl $(UO_2)^{2+}$ ions diffracts 3.5 Å photons up to the maximum scattering angle of 106° covered by the IP, i.e. to 2.2 Å resolution. 12 scans of 2° at three energies (3520 eV, 3552 eV and 3558 eV) close to the M_V-edge of uranium were measured. The experiment had to be terminated because of ice formation close to the sample crystal. The cold helium gas cooling system of EMBL worked very reliably due to further improvements.

The new box was not as helium-tight as it had been expected. Each time the image plate was taken out of the box through a narrow slit at the upper end of the box, air entered the helium box and replaced about one quarter of the helium inside the box, as could be monitored by the oxygen content of the outlet gas. Humidity was introduced in this way. Ice crystals near the sample were growing and finally obscuring the beam.

The residual amount of air in the helium box remained unexpectedly high (a few percent). This explains the strong, large background peak, the profile of which is shown in Fig 1. The air along the beam path between the outlet of the stainless steel tube and the beam stop (dotted line in the figure) acted as a line source of strong diffuse X-ray scattering. These observations are now well understood.

Most of the beam time was spent to find the origin of background scattering and to attempt reducing the leaks of the helium box. There were also some problems with the vacuum of the last section of the beam line ID1 at the very beginning of the beam time. The ESRF staff of ID1 was extremely helpful in fixing all technical problems and helping to overcome these as far as possible.

The results of this experiment are of technical importance, as they provide a guideline for a future construction of a helium box for a cylindrically bent image plate.