	Experiment title: Feasibility test of long wavelengths X-ray diffraction in protein crystallography at ID1	**MI-384**
Beamline: ID01	**Date of experiment**: from: 13-09-2000 to: 18-09-2000	**28.Febr.'01**
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Report:

Using a cold Helium atmosphere for cooling the protein crystal and an image plate as detector diffraction data with fairly high intensity could be taken up to wavelengths of 5 Å (K-absorption edge of sulfur). The intensity of the peaks decreased by a factor 6 on passing from λ = 2.7 Å to λ = 5.0 Å after correction for absorption by windows (28 µm Mylar foil +12 µm Kapton foil) This trend (see Tab. 1 and Fig. 1) arises from the diffraction power of the protein crystal, the sensitivity of the image plate, and to a smaller extent to a change of the incident beam intensity.

At a wavelength of 5.7 Å (K-absorption edge of phosphorus) the diffraction intensity and the background scattering were lower. One reason for this is that the intensity of synchrotron radiation from the wiggler which is required at 5.7 Å is about three times weaker than that from the undulator at λ = 5 Å. Clearly the increased absorption plays also its part.

Diffraction peaks were observed all over the image plate at each wavelength. At the long wavelength side, in particular with λ = 5.7 Å, diffraction peaks at larger scattering angles were more intense than those at smaller angles. The number of peaks observed with a 2° rotation of the crystal decreased as expected with $1/\lambda^3$. Four scans of 2° were taken at each wavelength. Fig. 2 shows the variation of the mean intensity recorded on the image plate in lysozyme diffraction peaks as well as in the background as a function of the wavelength.

In a further step, a preliminary test of a MAD experiment was tried with a uranium derivative of lysozyme at three wavelengths near the M_V edge of uranium (3.5 Å, 3.5 keV). 64 scans of 2° were taken at E = 3520 eV, 3552 eV and 3558 eV and 28 scans of 2° at E = 3552 eV, 3554.5 eV (f”max), and 3558 eV. The diffraction data at three energies were superimposed and for a better separation of the peaks the plate was rotated around the vertical axis by 0.6° after each energy change. Indexation has been done by MOSFLM. The peak integration is in progress.

The cooling device from EMBL providing a cold helium gas stream of T = 40K worked continuously. The liquid helium consumption was 1.2 $l.hour^{-1}$. No significant radiation damage of the crystal of the uranium derivative crystal of lysozyme was observed during the first 64 scans of the experiment described above.

Table 1. Some physical properties of soft X-ray synchrotron radiation relevant to ID1.

λ (Å)	Transmission Be 25 µm	Transmission Mylar 28 µm	Penetration depth in water (µm)	Diffraction from lysozyme (*)/pixel/min	Scattering from Kapton /pixel/min
1.5	0.99	0.96	1000		
2.0	0.98	0.93	460		
2.7	0.96	0.87	178	88330	27122
3.5	0.92	0.75	86	62103	24357
4.4	0.85	0.57	44	43381	16388
5.0	0.78	0.40	30	13337	6672
5.7	0.70	0.29	20	522	403

(*) average intensity of diffraction on peak area

The background scattering was mainly due to the Kapton foil separating the vacuum section from the helium atmosphere.

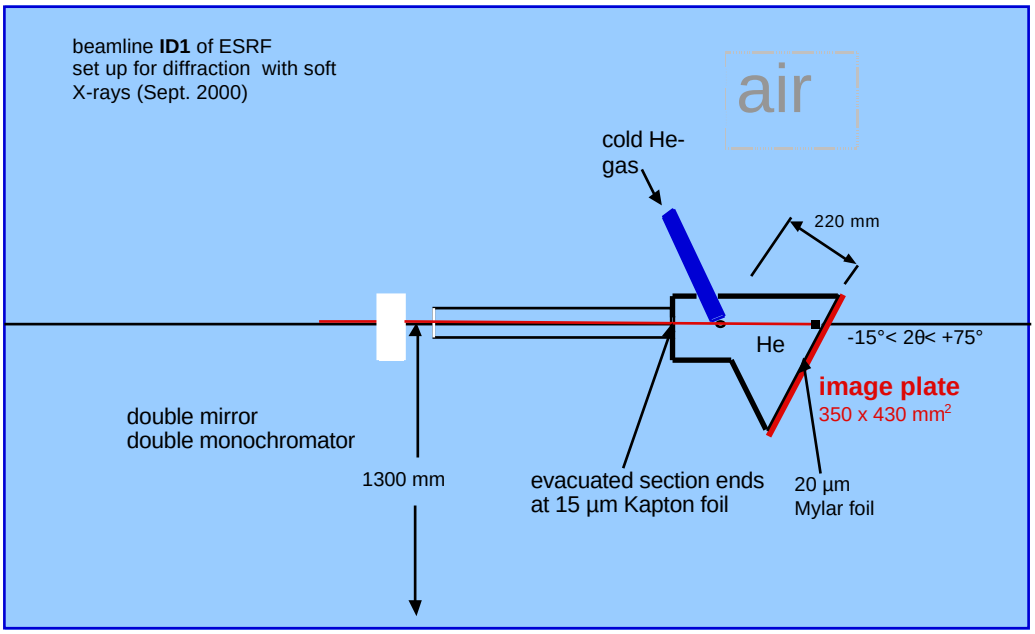

Figure 1. Setup for soft X-ray diffraction at ID1. The protein crystal was kept in a cold helium atmosphere. The image plate is read off-line.

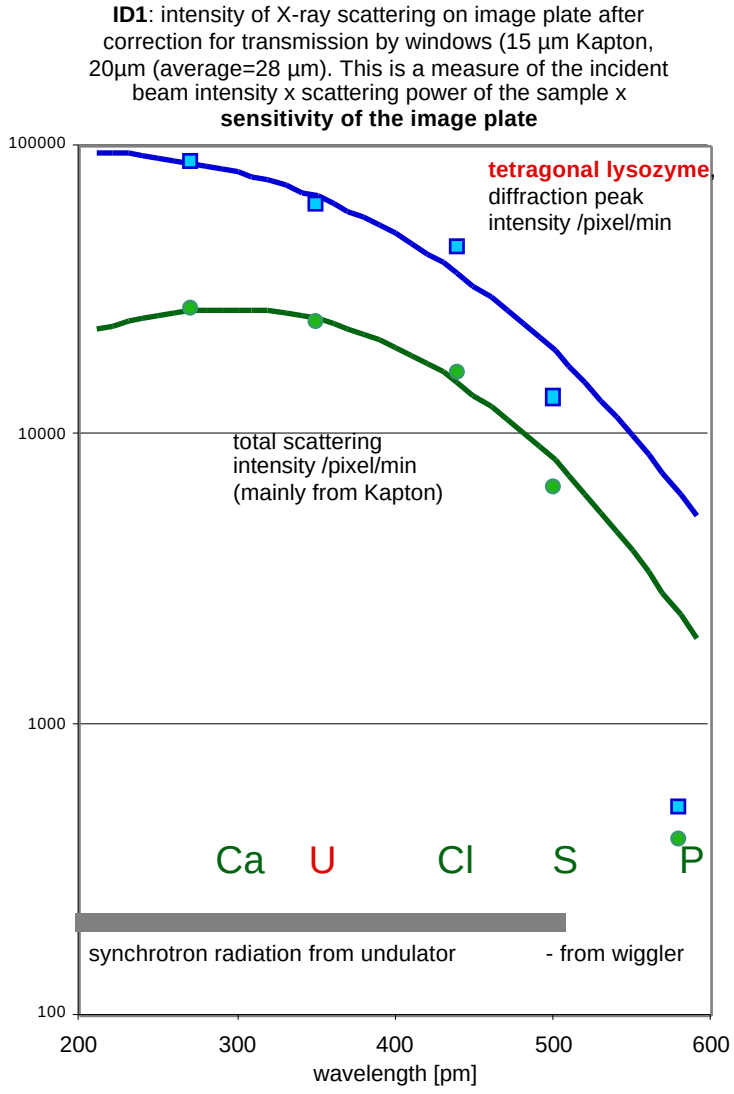

Figure 2. Soft X-ray diffraction from lysozyme at ID1