ESRF	**Experiment title:** A systematic triple-axis diffractometry study of the influence of the flash-cooling conditions and the annealing history on mosaicity and strain in protein crystals	**Experiment number**: LS-1408
Beamline: BM5	**Date of experiment**: from: 01.12.99 to: 05.12.99	**Date of report**: 17.02.00
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REPORT: EXPERIMENTAL TECHNIQUE AND SETUP

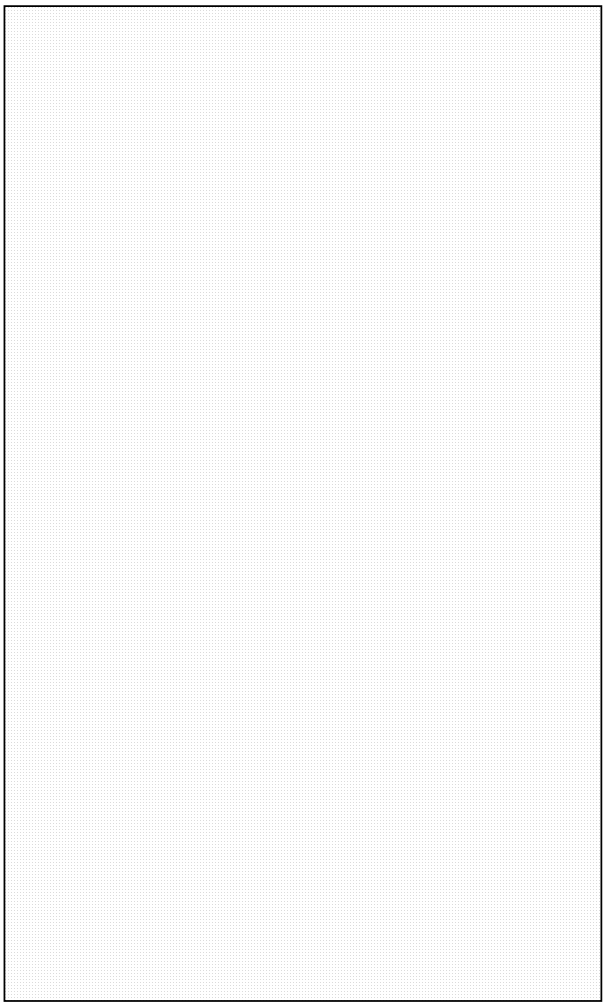
Triple-axis diffractometry allows mosaicity to be distinguished from lattice strain. The three crystals are arranged non-dispersively, with the first and third crystal acting as monochromator and analyser, respectively and the sample being mounted on the second axis. The reciprocal space map of a reflection is obtained by carrying out 2D scans of sample (ω_2) and analyser (ω_3), which can be transformed in reciprocal space coordinates according to $\mathbf{q} = (q_y, q_z) = |\mathbf{k}|[\cos\Theta_B\ \omega_3, \sin\Theta_B\ (\omega_3 - 2\omega_2)]$, where $\mathbf{k}$ is the wavevector and Θ_B is the Bragg angle.

The triple-axis diffractometer at the ESRF optics beamline BM5 was used for the experiments. The beam was unfocussed, i.e. its divergence was below 20 µrad. All measurements were carried out at 1.05 Å wavelength using Si(111) crystals. An Eulerian cradle was mounted on the sample stage with an Oxford cryostream system being mounted perpendicular to its ϕ-axis. The temperature of the nitrogen gas was 100 K during the experiments.

SAMPLE PREPARATION

Hen-egg white lysozyme crystals were grown using the hanging drop method (solution: 50 or 25 mg/ml of lysozyme (Fluka Nr. 62970) in 50 mM Na-Acetate pH 4.5; reservoir: 500 µl of 1.4 M NaCl in 50 mM Na-Acetate pH 4.5; drop: 5 µl of solution and reservoir, crystals appear after 1 day at 20°). OmpF porin was crystallised using the microdialysis technique (8.5 – 10.5 % PEG 2000, 700mM $MgCl_2$, 50 mM Tris·HCl (pH 9.8), with 0.6% n-octyl-2-hydroxethylsulfoxide, 0.1% octylPOE as detergents) [1]. The optimal cryo-protectant was found to be paraffin oil (Fluka Nr. 76235) for the lysozyme crystals and 15% glycerol for Porin resulting in mosaicities of 0.45 and 0.8 °, respectively. Crystals were either mounted in capillaries or cryo-loops (Hampton Research).

RESULTS



Room temperature measurements of lysozyme resulted in rocking curve widths down to 0.002 ° at 4 Å resolution (sample size: 500 µm). Crystals exposed directly to the N_2 gas stream exhibited no sign of strain but an increased mosaicity of 0.05 °. The upper plot in Figure 1 shows the reciprocal space map of a flash-frozen crystal (sample size: 700 µm). It exhibits a clear splitting of the reflection associated with a high degree of mosaicity and strain. This sample was annealed by exposing it for 3 min to the paraffin oil and gas frozen afterwards. The treatment removed the splitting of the peak and all strain. The rocking curve width decreased from 0.08 to 0.04 ° and diffuse scattering was significantly reduced (see Figure).

Porin was measured at room temperature only. The reciprocal space map revealed that the sample consists of several small subdomains. The rocking curve width of the single reflection was found to be as small as 0.04 ° vs. typical values of 0.3 ° for the whole crystal as determined with MOSFLM.

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Figure 1: Reciprocal space map of lysozyme after flash freezing in LN_2. The lower figure shows the same sample after 3 min annealing and subsequent freezing in gaseous N_2

CONLUSIONS AND OUTLOOK

It was demonstrated that triple-axis diffractometry can reveal the deterioration mechanism of cryo-cooled samples. Due to the low flux available from an unfocussed bending magnet, only a small number of samples could be studied which is statistically not representative. At present stage it can only be stated that cryo-cooled samples exhibit primarily mosaicity and only to a smaller extend strain.

More experiments are required on a regular protein crystallography beamline, which provides sufficient flux to study more samples and allows the orientation matrix to be determined thereby permitting to measure always the same reflection.

The narrow rocking curve width of room temperature and cryo-cooled samples is remarkable. While similar results were reported at room temperature [2], typical values at cryogenic temperatures are one order of magnitude larger. This seems to indicate that the rocking curve width of a single reflection is significantly smaller than the overall mosaicity of the crystal. The result could have important consequences for data acquisition, since it would require taking very fine ϕ–slices even from frozen crystals.

REFERENCES

[1] R.A. Pauptit et al, J. Mol. Biol. (1991), **218**, 505-507

[2] R. Fourme et al, J. Synchr. Rad., (1995), **2**, 136-142