ESRF

**Experiment title:**

**High Resolution Studies and Three-Beam Diffraction of Protein Crystals at Low Temperature**

**Experiment number:**

**Sc 190**

**Beamline:**

D1-SW/NOR

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3

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## Report:

In **previous experiments we have been able to demonstrate, that the experimental determination of triplet phases by a three-beam interference experiments form crystals of small proteins is possible. For example, more than 600 triplet phases have been measured meanwhile from tetragonal lysozyme from which 560 single phases can be deduced. For this kind of experiments the quality of the crystals (mosaic spread) is critical. In general a larger mosaic spread reduces the interference effects. This, however, does not hold for mosaic distributions of large perfect blocks which can** be resolved by a highly collimated synchrotrons beam. As to our experiences with six different proteins of unit cell sizes up to about $10^6 Å^3$ every second or third crystal is suitable for multi-beam diffraction experiments. However, all crystals suffer from radiation damage. Proteins that are considered to be 'stable' can stay for about one day in synchrotrons radiation from an ESRF bending magnet. One possible method to increase the lifetime of protein crystals is the application of cryo temperatures. This method is meanwhile commonly used for the intensity data collections of protein crystals. Two major problems are encountered for multi-beam experiments at low temperatures.

Firstly, from our experience of experiments at room temperature any air flow inside the experimental hutch will give vibrations of the crystal that can be measured as intensity changes due to the small divergence and the sharp rocking curves if crystals are mounted in the traditional way inside a capillary or on top of a glass fiber. Therefore, it is expected that the air flow of the cryo streamer will generate vibrations of the crystal that can influence the interference profile contain-

ing the phases information severely. Using a more rigid mounting technique first experiments with a small molecule tryst al (L-asparagine monohydrate) showed, that it is still possible to measure interference effects and therefore triplet phases at low temperatures using a cryo streamer.

The second problem is the increase of the mosaic spread during cooling of protein crystals due to the crystallization of ice on the surface or inside the crystal. In certain limits this increase of mosaicity can be limited using a suitable cryo protector which suppresses the formation of ice. In recent publications on low temperature experiments with tetragonal lysozyme (Kurinov & Harrison, 1995, Young & Dewan, 1990) a mosaic spread up to one degree was observed. It seems that this is not a severe restriction for an intensity data collection. Crystals like this, however, are not suitable for three-beam interference experiments.

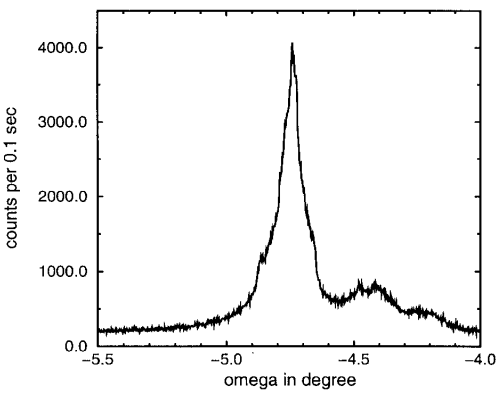


Figure 1: profile of an $\omega$-scan of tetragonal lysozyme at 120K, FWHM = 0.094°

For our tests we used a different recipe published by Petsko (1975) (cryoprotector: lM $NH_4 - At.$, pH 4.7, 40% glycerol). Crystals were mounted in the so called loop technique where they are kept inside a loop of a thin filament and directly frozen to a piece of glass on top of a brass pin. We applied conditions tested already with smaller resolution in our home laboratory. The test experiments were carried out at 120 K. After optimizing the conditions we achieved a mosaic spread measured by high resolution $\omega$-scans that was in the order of 0.1°. This is less than the ones we found from literature but still too large to do reasonable three-beam diffraction experiments. An example for a high resolution $\omega$-scan of tetragonal lysozyme at 120 K is given in Fig. 1. From this results we conclude, that it is not possible to do three-beam interference experiments with crystals of tetragonal lysozyme at cryogenic temperatures with the cryo protectors tested so far. There are other proteins (like Proteinase K and Trypsin) known where there should be less change in mosaicity during freezing. However, this results have been obtained with area detectors experiments, therefore high resolution w-scan still have to be done in order to get more accurate data on the change of the mosaicity. However, due to the limited amount of beam time available for this kind of experiments we had no time to do experiments with Proteinase K and Trypsin.

## References

Kurinov, I. V. & Harrison, R. W. (1995). Acts Cryst. D51, 98-109.

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Young, A. C. M. & Dewan, J. C. (1990). *J. Appl. Cryst.* 23, 215-218.