ESRF	**Experiment title:** Three-Beam Interference Experiments for Experimental Phase Determination at Low Temperatures with Macromolecular Crystals	**Experiment number:** MI-123
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Names and affiliations of applicants (*indicates experimentalists):

Edgar Weckert*, Kerstin Hölzer*, Klaus Schroer*, Ralf Müller*, Johannes Zellner*, Kurt Hümmer
Institut für Kristallographie, Universität Karlsruhe (TH), Kaiserstr. 12, D-76128 Karlsruhe

Report:

In previous experiments we have shown that it is possible to determine a larger number (850) of triplet phases by three-beam interferences also from crystals of small proteins like tetragonal lysozyme (Weckert & Hümmer, 1997, Weckert, 1997). For this kind of experiments crystals of low mosaic spread are required. One of the main problems is radiation decay, e.g. the maximum number of triplet phases that can be obtained from one crystal of tetragonal lysozyme is about 150 (= more than 24h).

For intensity data collections cryo-techniques are meanwhile successfully applied. In a first three shift test experiment (SC190) it was tried to apply the same technique to crystals of tetragonal lysozyme. We did, however, not succeed in preserving the low mosaic spread to cryo temperatures. The original intention of this proposal was to test further compounds (trypsin and proteinase K) which can easily be frozen for intensity data collections. First a test experiment at room temperature was carried out. The investigated sample of proteinase K (a=b=68.289 Å, c=108.326 Å, SG: $P4_32_12_1$) was about 100 x 100 x 200 μm^3 in size. The FWHMs were in the range of 0.0025-0.014". At room temperature three-beam interference measurements were possible at a speed of about three triplet phases per hour (Fig. 1). Direct freezing experiments in liquid nitrogen with proteinase K using 10 - 20% glycerol as cryo-protector revealed mosaic widths with FWHMs in the range of 0.3 - 0.7". The crystals size in all case was approximately 150 x 150 x 50 μm^3. These values are probably still suitable for a intensity data collection but they can not be used for three-beam interference experiments.

The investigated crystals of trypsin (a=54.8Å, b=58.7Å, c=67.8Å, SG: $P\ 2_1\ 2_1\ 2_1$) showed at ambient temperatures a FWHM of 0.005 - 0.02". All crystals were of approximate size 100 x 100 x 600 μm^3. Three-beam interference effects could be observed at ambient temperature as well. As it was the case for proteinase K using an appropriate cryo protector (8g phytohistol, lml ethylenglycol and added reservoir solution to give 10ml) the mosaicity also increased considerably during

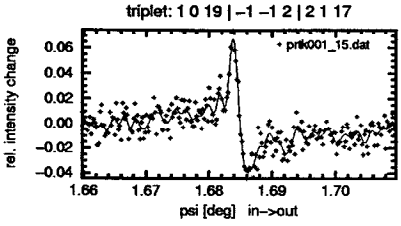

Fig. 1

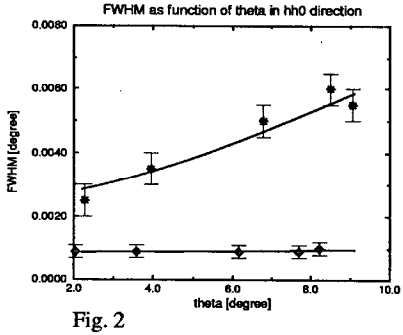

Fig. 2

Fig. 1: Example of a three-beam interference profile of proteinase K at 300K. Fig. 2: Reflection width obtained during $\omega/2\theta$ scans using an analyzer crystal; upper curve: frozen crystal, lower curve: room temperature comparison.

freezing in liquid nitrogen. From about 10 crystals FWHM in the range from 0.24 - 0.85° were measured, which are again too wide for three-beam experiments.

From these results it has to be concluded that proteinase K and trypsin can not be frozen any better than tetragonal lysozyme. In order to investigate the influence of different freezing methods on the final mosaic spread a further series of experiments were carried out using either the cryo stream alone at about 100 K (for less rapid cooling) or liquid propane (for extreme fast cooling). In addition freezing experiments without cryo-protector or with the crystals covered by a thin film of parafine oil were tried. As a result of these investigations no significant differences in the FWHMs between various methods / sizes / cryo-protectors were observed.

In order to obtain a deeper insight into the nature of the mosaic spread of frozen protein crystals reciprocal space maps around the reciprocal lattice points were carried out. The crystals showed typical FWHM of about 0.25" for an omega scan. By an $\omega/2\theta$ scan using a perfect Si (111) crystal as analyzer in front of the detector information about the mosaic spread in direction of the reciprocal lattice vectors can be measured. The development of this profile width as a function of the scattering angle in (h h 0) direction of a frozen lysozyme crystal is shown in Fig. 2. The increase of the FWHM with θ in comparison to the non frozen crystal can be explained by the assumption that there exists about 0.066% strain in the crystal which might be due to different cooling rates inside the sample.

Cooling close to 0" C or even to temperatures slightly above the freezing point of the mother liquid may slow down radiation damage in some cases. In a last experiment the FWHM of two reflections of a tetr. lysozyme crystal (original FWHM: 0.0018 - 0.002°) during slow cooling ≈ 1 ***K/minute*** was monitored. No change of the FWHM was observed down to 273 K as long as the crystals temperature was homogeneous. Below 273K the FWHM did also not change dramatically, however the 'tails' of the reflection profile became more pronounced. Ice formation set in at about 240 K and destroyed the crystal. The last measured FWHM at 243K was 0.0036°. This experiment shows that cooling a protein crystal to temperatures just above the ice formation nearly maintains its original quality. Further experiments to investigate to what extend radiation damage is already reduced for this conditions are planned for different proteins.

References

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