ESRF	**Experiment title:** **Experimental Phase Determination by Three-Beam Diffraction of an Unknown Protein Struktur**	**Experiment number:** MI-167
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Report:

The experimental determination of triplet phases from macromolecules by three-beam diffraction experiments was developed using crystals of various small proteins (Weckert & Hümmer, 1997). In case of tetragonal lysozyme about 850 triplet phases have been measured from which more than 750 single phases can be deduced by a suitable maximum entropy approach. An electron density map using these phases is traceable and shows the lysozyme molecule almost complete (Holzer, Weckert, Schroer & Hümmer, 1997).

The intention of this proposal was to apply the expertise developed with proteins of known structure to unknown ones. For this purpose 'deacetoxycephalosporin C synthase' (space group R3, a=b=107 Å, c=72 Å, MW=38000) was first selected since this structure resisted solution by standard methods for several years. Screening several samples crystals with quite low mosaicity (FWHM=0.019°) could be found. However, after one hour of radiation exposure the width of the same reflection has already increased by a factor of three (FWHM=0.058°). After this first fast decay the crystals were stable for several hours, but only two out of the first five three-beam interference profiles could be interpreted (about 98% were interpretable for tetragonal/triclinic lysozyme). Other experiments carried out later revealed that the crystals of this compound are probably merohedral twins (Hajdu, 1997).

As a second compound in collaboration with I. Zegers (Vrije Universiteit Brussel) 'nettle lectin' (a=37.56 Å, b=49.03 Å, c=57.30 Å, SG: $P2_12_12$) was chosen. For this compound it was not possible to find any heavy atom derivatives since several years. Some crystals of sufficient quality for three-beam interference experiments could be found that were reasonably stable in the beam (life

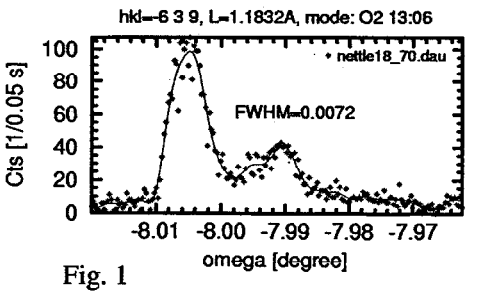

Fig. 1

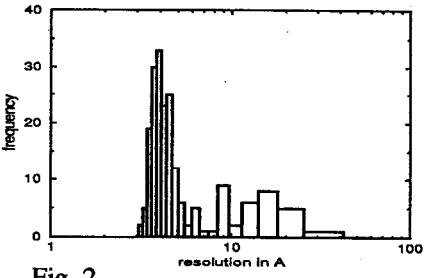

Fig. 2

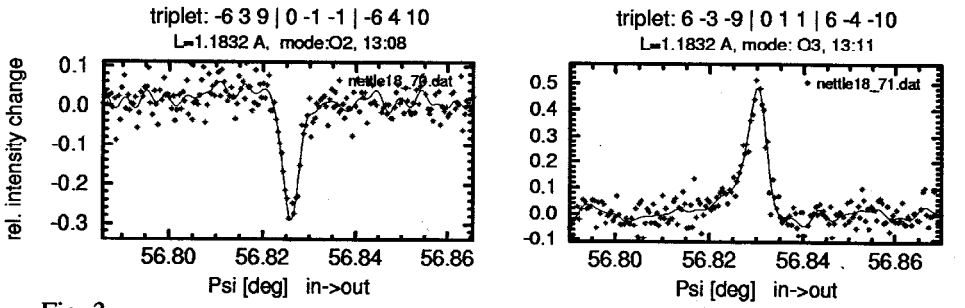

Fig. 3

Fig. **1:** Typical rocking curve of the investigated **nettle lectin crystals. Fig. 2: Distribution of the** resolution of measured triplet reflections. Fig. 3: Example of the three-beam interference profiles of a centrosymmetric related three-beam case of nettle lectin.

time more than 24 h). Since the three-beam interference method requires a complete data set also to low resolution a data collection up to 7Å resolution was carried out (R_{sym} = 2.8%). In the remaining nine shifts from two crystals about 220 triplet phases could be determined despite the somewhat small crystal volume ($\approx$ 70 x 80 x 500μm) for this kind of experiments at an unfocussed bending magnet. A typical rocking curve giving an idea on the crystals quality is shown in Fig. 1. Even this crystals that were far from perfect can give rise to interference effects of more than 30% intensity changes due to the very good collimation of the incident beam (Fig. 3). On average the interference effects were in the range of 4-15% intensity change. The distribution versus resolution of the reflections involved in the measured triplets is given in Fig. 2.

Due to our experience from previous experiments and taking into consideration the size of the nettle lectin molecule the measured number of triplet phases is almost half of what is needed to solve the structure directly from the measured triplet phases alone. Investigations on how far the already measured triplet phases lead in conjunction with other methods are under way.

References

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