ESRF	**Experiment title:** Development of new crystallographic methods applying phase-sensitive multiple-beam diffraction (**Summary**)	**Experiment number:** MI-244
Beamline: ID22	**Date of experiment:** from: 21.1.1998 to: 8.12.1999	**Date of report:** February 29, 2000
Shifts: 110	**Local contact(s):** M. Drakopoulos	*Received at ESRF:* 02 MAR. 2000

Names and affiliations of applicants (* indicates experimentalists):
Edgar Weckert*, Ralf Müller*, Johannes Zellner* and Kurt Hümmer
Institut für Kristallographie
Universität Karlsruhe (TH)
D-76128 Karlsruhe
Germany

Report:
This report is intended to summarize the activities and results during our long term proposal **MI-244**. A more detailed report has been submitted for each individual beam time period.

1. The first question we were interested in was the advantage of an **undulator beamline compared to a bending magnet** for the 'standard' measurements of three-beam interference profiles of protein crystals. Since these experiments require a very low mosaicity cryo techniques can not be applied for protein crystals. This limits the maximum flux that can be used in order to keep radiation damage at a reasonable rate. Another limitation is the driving speed of the diffractometer. With good quality test crystals we achieved a measurement rate about twice as fast as at a bending magnet ($\approx$ 10 triplet phases per hour). The stability of the beamline proved to be excellent for three-beam interference experiments (which need very high stability on a time scale of one ψ-scan ca. 40 s) if there were no technical problems with e.g. the monochromator.

2. The determination of the **absolute structure** (chirality, conformation or polarity) is normally achieved by exploiting anomalous dispersion effects. For light atom structures, however, with a very low oxygen content or with no oxygen atoms the method is not very reliable. Since three-beam interferences do not depend on anomalous dispersion effects we could determine the absolute structure of three light atom compounds:
a: $C_{14}H_{12}O$ (Methylbenzophenon): for this compound the correlation between the optical activity and the chirality of the molecule is studied.

b: $C_{23}H_{25}N_2$: this is a chiral drug pre product, it is one of the first determination of the absolute structure of an oxygen free compound.
c: $CHO_2Li \cdot H_2O$ (Li-formiate monohydrate): for this compound the polarity of the atomic arrangement will be correlated to the polar morphology of the crystals.

3. The availability of an extremely bright undulator source made some **diffraction physical** investigations possible that could not have been carried out otherwise:
a: We could show for the first time that it is possible to measure three-beam interference effects in layered structures. The results of these experiments could be verified by theoretical calculations based on the Tagaki-Taupin theory for the three-beam case. In future applications these method might provide additional informations that can not be obtained by standard two-beam rocking curve or reciprocal space mapping techniques. This experiments also showed that three-beam interferences can take place in crystalline regions as thin as 100nm.
b: In case of mosaic crystals the three-beam interference profiles of all mosaic blocks that fulfill the diffraction condition will be superimposed. In general this will lead to interference profiles that can not be interpreted. The availability of high resolution two dimensional CCD cameras at ID22 made diffraction topographic experiments possible in which the interference effect of single mosaic blocks or homogeneous regions of a crystal could be recorded. This has been demonstrated with a less perfect quasicrystal for the first time. In order to evaluate this information properly the theoretical intensity distribution in a reflection during a three-beam interaction has to be known. In order to check the validity of the developed computer programs experiments using perfect crystals were also carried out. The quantitative analysis is just now under investigation. In general this method is very promising since it would open the experimental phase determination by three-beam interference experiments to a wide range of less perfect crystals. However, the practical limitations are the very low quantum efficiencies of high resolution CCD cameras which require extreme radiation doses on the crystals. Even with the full undulator beam and strongly scattering crystals these experiments were quite time consuming compared to a standard measurements (hours versus minutes).
c: Crystals showing well separated large mosaic blocks in their rocking curves are still suitable for three-beam interference experiments since by the very parallel synchrotron beam often a single block can be 'selected'. It would be of great advantage for planing the experiments if the 3D orientation of each mosaic block could be determined. We developed a method which uses tomographic algorithms to reconstruct the scattering distribution around a single reciprocal lattice point from rocking curves at different azimuthal angles. A procedure to determine the orientation distribution of all mosaic blocks from a number of these scattering distributions is under development at present. As a test case we selected a 6H-SiC crystal which shows only a small number of large mosaic blocks.

4: Another problem for the determination of triplet phases of protein crystals is the very low rate compared to an intensity data collection besides crystal quality and radiation damage. Recently in literature a method to use an area detector has been proposed. From the so far published results a more critical investigation seems to be

will be successful the measurement of triplet phases might provide another possibility for the solution of quasicrystalline structures.

7: One of the main goal of this long term proposal was the attempt to solve the **unknown crystals structure of a small protein** (nettle lectin, 89 amino acids, SG: $P\ 2_1\ 2_1\ 2_1$, a=37.56Å, b=49.03Å, c=57.30Å) that could not be solved by other methods up to now. What seemed to be an achievable goal, since we had already 220 triplet phases (about half the number that will be needed) measured from two crystals from our first beam time with this compound, worked out not to be successful up to now. The crystals grew either not to a size that is large enough for three-beam interferences or if large enough their mosaicity was too high for this kind of experiments. In total more than 60 crystals were investigated. Some batches of these crystals also showed another metric (a=38.67Å, b=45.83Å, c=57.32Å and a=37.58Å, b=47.52Å, c=61.75Å). Some of the crystals of the two other modifications were good enough for three-beam interference experiments. We were able to collect from both modifications a small number ($\approx 50+10$) of triplet phases, but the differences of these modifications compared to the first one are too large in order be able to use this additional phase information. Since to our present knowledge only two further crystals of the quality, that was already available once, are necessary to complete this project our collaborator (I. Zegers, University of Brussels) will try to systematically improve crystal quality and to correlate various parameters with the measured mosaicity. First experiment in this direction have already been carried out (MI-422) which showed that the transport of the crystals does very likely not increase their mosaicity.
In addition crystals of two other proteins were investigated. One was a test experiment with a medium size protein of known crystal structure ('hisf', SG: C 2, a=81.11Å, b=45.21Å, c=64.33Å, $\beta = 111.43°$, crystals made a available by D. Lang, EMBL Hamburg). The rate for measured triplet phases was about three per hour due to their slightly increased mosaicity ($0.01 - 0.025°$). The other protein, which has not been solved up to now by the standard methods, had a rather large cell for this kind of experiments (nickel superoxide dismutase, SG: $P\ 2_1\ 2_1\ 2_1$, a=113.38Å, b=115.47Å, c=131.09Å, crystals made available by Y. Modis, EMBL Heidelberg) and showed a quite small mosaicity ($0.008 - 0.01°$). However, since the crystals were small ($< 100 - 200 \mu m$) the intensity of individual reflections is too weak in order to accumulate enough statistics for the interpretation of three-beam interference profiles within the life time of a crystal. From this we conclude that proteins of that size can only be investigated by three-beam interferences if large crystals ($> 400\ \mu m$) are available. In any case the measured phase information for structures of that size will be restricted to low resolution reflections.

necessary. In a first approach we were able to simulate this technique using a point detector. This results, however, indicated that the requirements on crystal quality will be even more strict than the standard diffractometer method. Since it would be highly desirable to measure a large number of triplet phases in a much shorter time we considered it to be worth to make own tries using a CCD camera. The first approach with the Medoptic camera from the ESRF detector pool was rather unsuccessful due to the limited dynamic range and the intensity reproducibility of this camera. For our next try we borrowed a Siemens AXS SMART System from HASYLAB/Hamburg. However, even this system showed a reproducibility for the intensity worse than 2% even for reflections measured with a nominal statistics of 0.1%. Since the expected interference signal is smaller than 2% no interference profiles could be observed except large *Umweganregung* effects containing practically no phase information. The same crystals showed reasonable interference effects in the point detector control experiments afterwards. In order to check the feasibility of this sort of experiments further experiments with better quality CCD-cameras are necessary.

5: Radiation decay is one of the main problems in protein crystallography at high brightness sources. Most of the investigations so far have been carried out using area detectors. Our investigation using a high resolution diffractometer aimed to obtain complementary information. In this first step we were using crystals of l-asparagine monohydrate that should have an absorption similar to a protein crystal as a model system. Our first question was whether there is any beam heating by a 10^{12} ph/s undulator beam, which should be measured by a slight change of the lattice parameter that can easily be detected by small changes in the Bragg angle. We did not find any beam heating effect at 100K (nitrogen gas flow cooling) but a linear and irreversible increase of the d-spacing. We assumed that this irreversible change does directly correlate to the radiation damage. Further investigations showed (i) the change of lattice parameter (radiation damage) is linear with time (ii) it is nearly independent form the radiation energy between 15 and 28 keV (iii) the rate of lattice parameter change can be described by an Arrhenius type expression with an activation energy of about 100*k (k: Boltzmann constant). From this we conclude that (i) there is no advantage to use extremely high energy radiation for light atom structure (ii) radiation damage can be significantly reduced by measuring at e.g. 40 K. These experiments were thought as a sort of benchmark for protein crystals. Whether these results can be extrapolated to protein crystals at cryogenic temperatures has still to be investigated.

6: Various experiments were carried out mainly with **quasi crystals** from the decagonal system AlNiCo (crystals made available by M. Estermann and T. Haibach, ETH Zürich). In some preliminary studies we tried to resolve a literature dispute on whether this crystals show deviation from centrosymmetry. We could not find significant deviations from centrosymmetry even for very weak reflections. Since we observed many additional three-beam interference effects not indicated by our intensity data set a new more complete data set had to be measured. This proved to be very valuable for our final attempt to measure a larger number of triplet phases recently (more than 220 triplet phases, MI-422). With this triplet phase data set we will try to derive an ab initio structural model for comparison with the known structure. If this development

Publications and Conference Proceedings related to this project:

- K. Hölzer, K. Hümmer, K. Schroer, E. Weckert, J. Zellner, Strukturlösung mit gemessenen Triplettphasen von kleinen Proteinstrukturen, (1998) *Z. Kristallogr.*, Suppl. 15, 78.
- F. Mo, B.C. Hauback, R.H. Mathiessen, Å. Kvick, E. Weckert, Physical Estimation of Triplet Phases - Effect of Different Radiation Sources and Modes for Profile Scans, (1998), *J. Synchrotron Rad.* **5**, 1369-1374.
- F.-J. Volk, G. Mattern, E. Weckert, A.W. Frahm, The Absolute Configuration of *cis*-(αS,1S,2R)- 2-Methyl-1-(α-methylbenzylamino) cyclohexanecarboxamide, (1998) *Acta Cryst.* C**54**, 387-389.
- E. Weckert, K. Hümmer, On the Influence of the Diffraction Geometry on Three-Beam Interference Profiles, (1998), *Cryst. Res. Technol.*, **33**, 651-674.
- E. Weckert, Einbeziehung experimenteller Triplettphasen in ein statistisches Verfahren zur Strukturlösung, (1998), *Z. Kristallogr.*, Suppl. 15, 204.
- E. Weckert, K. Hölzer, R. Müller, K.Schroer, J. Zellner, Using Maximum-Entropy Methods to Exploit Measured Triplet Phases for the Structure Determination of Small Proteins, *Bulletin of the Czech and Slovak Crystallographic Association* (1998), **5b**, 483.
- J. Zellner, K. Hölzer, R. Müller, K. Schroer, E. Weckert, Using Synchrotron Radiation to Determine the Orientation Distribution of Mosaic Blocks, (1998) *Bulletin of the Czech and Slovak Crystallographic Association* **5B**, 462.
- K. Schroer, K. Hölzer, E. Weckert, K. Hümmer, Dreistrahlinterferenz-Experimente an Schichstsystemen, (1998), *Z. Kristallogr.* Suppl. 15, 129.
- R. Müller, E. Weckert, K. Schroer, K. Hölzer, J. Zellner, K. Hümmer, Anwendung eines Analysatorkristalls für Dreistrahlinterferenzexperimente, (1998) *Z. Kristallogr.* Suppl. 15, 160.
- J. Zellner, K. Hölzer, R. Müller K. Schroer, E. Weckert, Bestimmung der Orientierungsverteilung von Mosaikblöcken durch Tomographie, (1998) Suppl. 15, 207.
- J. Stahn, A. Pucher, U. Pietsch, J. Zellner, E. Weckert, Experimental Determination of Electric Field Induced Differences in Structure Factor Phases in the Order of 2%, (1999) *Acta Cryst.* A**55**, 1034–1037.
- E. Weckert, K. Hölzer, K. Schroer, J. Zellner, K. Hümmer, Feasibility Study for the Measurement of a Large Set of Triplet Phases from a Small Protein, (1999) *Acta Cryst.* D**55**, 1320-1328.

- E. Weckert, R. Müller, Optimal Selection of Measured Triplet Phases: Maximum-Entropy and Density Modification Methods, (1999) *Acta Cryst.* A**55**, C-185.
- J. Zellner, R. Müller, E. Weckert, M. Drakopoulos, A. Snigirev, C. Raven, Ortsaufgelöste Messung von Dreistrahlinterferenzen an perfekten und nichtperfekten Kristallen, (1999) *Z. Kristallogr.*, Suppl. 16. 138.
- R. Müller, E. Weckert, M. Drakopoulos, On the Advantages Using an Area Detector for the Measurement of Triplet Phases from Protein Crystals, (1999) *Acta Cryst.* A**55**, C-234.
- K. Hölzer, E. Weckert, K. Schroer, K. Hümmer, Experimentally Determined Triplet Phases Used for Structure Solution of Lysozyme, (1999) *Acta Cryst.* A**55**, C-185.
- J. Zellner, E. Weckert, R. Müller, M. Drakopoulos, On the Feasibility of Triplet Phase Determination from Non-Perfect Crystals, (1999) *Acta Cryst.* A**55**, C-527.
- K. Schroer, K. Hölzer, E. Weckert, K. Hümmer, Phase Sensitive Diffraction for one Dimensionally Distorted Crystals, (1999) *Acta Cryst.* A**55**, C-529.
- K. Hümmer, T. Jouffre, R. Müller, E. Weckert, J. Zellner, The Determination of the Absolute Structure of Low Z<=7 Compound by Three-Beam Diffraction, (1999) *Acta Cryst.* A**55**, C-530.
- K. Hölzer, E. Weckert, K. Schroer, Properties of an Electron-Density Map Derived from a Limited Number of Experimentally Determined Triplet Phases, (2000) *Acta Cryst.*, D**56**, 322–327.