ESRF	**Experiment title:** Physical estimation of triplet phases for solving macromolecular structure. I. Work to define the molecular envelope	**Experiment number:** MI-246
Beamline: ID22	**Date of Experiment:** from: 01.07.98 07:00 to: 07.07.98 07:00	**Date of Report:** 29.08.98
Shifts: 18	**Local contact(s):**	*Received at ESRF :*

Names and affiliations of applicants (*indicates experimentalists):

F. Mo*, R.H. Mathiesen*
Department of Physics, NTNU, N-7034 Trondheim, NORWAY

E. Weckert*
Inst. für Kristallographie, Univ. Karlsruhe, D-76128 Karlsruhe, GERMANY

P.M. Alzari
Institut Pasteur, Immunologie Structurale, 25 rue du Dr. Roux,
F-75724 Paris Cedex 15, FRANCE

Report:

Cellulase (CelA) is a 40 kDa extracellular glycosidase responsible for the hydrolysis of oligo-saccharides and longer cellulose chains. Experiments to explore the suitability of this protein for physical phase estimation were carried out in July, 1997 (ESRF Experiment MI-209). CelA crystallizes in the orthorhombic space group $P2_12_12_1$, $a = 50.05$ Å, $b = 63.50$ Å, $c = 104.75$ Å, $V \sim 333\ 000$ Å^3. Its crystal structure has been determined recently by the MIR technique and refined with data at 1.65 Å resolution [1].

Crystals of the same high quality that were used in the 1997 study could not be produced for the present experiment. Only two crystals of the new sample were acceptable, even though of inferior quality with respect to the cleanness of the intensity profiles, which degraded with increasing X-ray exposure. They had well developed faces and dimensions 1.07 x 0.27 x 0.22 mm^3 (No. 7) and 1.10 x 0.3 1 x 0.25 mm^3 (No. 8). Both specimens had initially narrow single peaks, full-width at half-maximum (FWHM) values from ω rocking curves being in the range 0.006 - 0.012°. During the experiment the mosaicity increased to typical values 0.02 - 0.03°, but in addition the intensity profiles developed shoulders and tails, some of them eventually splitting up in multiple peaks. For crystal No. 8 the maxima of some of the split reflections had a separation of a few 1/100° which made centring difficult both for the calculation of an orientation matrix and in the preparation for ~-scans. One crystal that had been used in the 1997 study (No. 4) was also tried, and surprisingly, after about 25 hrs. exposure to X-rays and subsequent storage for one year this crystal could still be used for a few triplet-phase measurements. Three-beam interference profiles were collected by repeated ψ-scans for pairs of triplets -H/L/H-L and H/-L/-H+L, corresponding to phases $+\Phi_3$ and $-\Phi_3$, respectively.

crystal	Scanned	Unique	Unique estimated
No. 7	*42* tripl.	*39* tripl.	*37* tripl.
No. 8	*39* tripl.	*36* tripl.	*34* tripl.
No. 4	*3* tripl.	1 tripl.	1 tripl.

In total, 84 pairs of three-beam intensity profiles were scanned and estimated in phase. Of the 76 unique triplets 72 could be assigned a phase. The phases were estimated independently by two workers and averaged. Combined with the phases obtained in Experiment MI-209, we have now nearly 110 single-phase values.

Several factors limited the yield of phases in this experiment. Deterioration of the crystal quality with radiation exposure was one, the formation of multiple peaks was in particular a serious obstacle. A second factor, perhaps even more limiting, was weak beam interference implying inferior counting statistics, in some cases not giving an interpretable intensity profile. Our aim is to obtain a structure solution most efficiently from a minimal number of estimated phases via an identification of the Molecular Envelope in the solvent matrix. The strategy involves measurement of a linked sequence of triplets, designed to render single phases in succession, intended for later phase refinement and expansion by direct methods [2]. This implies that some of the triplets that are important as links in a chain of phase development are far from being optimal wrt. experimental feasibility. On this background the percentage of triplets that could be estimated is gratifying. For reasons discussed above the collection of interference profiles was more time-consuming than expected, moreover the poor quality of some of the profiles will probably introduce larger errors than usual in the corresponding phase estimates. In hindsight it would be wise to verify a few derived single phases along the chain of development by short alternative paths of experimental triplets.

About two shifts of the beamtime was used for instrument alignment and checks of the beam with the new mirrors on ID22.

The ψ-scans were made on a six-circle Huber diffractometer (Prof. Hümmer, University of Karlsruhe) presently located on beamline ID22.

[1] P.M. Alzari, H. Souchon & R. Dominguez (1996). *Structure 4,265 - 275.*

[2] F. Mo, R.H. Mathiesen, P.M. Alzari, J. Lescar, B. Rasmussen (1998). ECM-18 Meeting, Praha. Abstracts, p. 484.