ESRF	**Experiment title:** Physical Estimation of Triplet Phases for Solving Macromolecular Structure. I. Exploratory Experiments	**Experiment number:** MI-209
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Report:

Cellulase (CelA): This is a 40 kDa extracellular glycosidase responsible for the hydrolysis of oligosaccharides and longer cellulose chains. CelA is part of a high molecular weight (> 2 MDa) thermostable complex - the cellulosome - produced by the Gram-positive, anaerobic bacterium C. *thermocellum.* The cellulosome, which comprises at least 14 different polypeptides, is one of the most active cellulase systems known to date. Endoglucanase CelA crystallizes in the orthorhombic space group $P2_12_12_1$, $a = 50.05$ Å, $b = 63.50$ Å, $c = 104.75$ Å, $V \sim 33\ 3\ 000$ Å^3. Its crystal structure has been determined recently by the MIR technique and refined with data at 1.65 Å resolution [1].

Two prismatic crystals with well developed faces and physical dimensions 1.2 x 0.24 x 0.21 mm^3 (No. 4) and 1.1 x 0.22 x 0.14 mm^3 (No. 5) were used for the triplet-phase measurements. Both crystals had small mosaicity, full-width at half-maximum (FWHM) values from ω rocking curves were in the range 0.006 - 0.015° for both specimens. Three-beam interference profiles were collected by repeated ~-scans for 49 pairs of triplets -H/L/H-L and H/-L/-H+L, corresponding to phases $+\Phi_3$ and $-\Phi_3$, respectively.

Crystal	Scanned	Not estimated	Remeasured
No. 4	24 tripl.	3 tripl.	2 tripl. with No. 4, 3 tripl. with No. 5
No. 5	25 tripl.	1 tripl.	3 tripl. also with No. 4

In total, 45 pairs of intensity profiles were scanned and estimated in phase, five of these were

estimated twice, thus rendering 40 unique triplet-phase values. The number of scanned intensity profiles was limited, both due to initial problems with crystals of inferior quality and an instability in the mounting of crystal No. 4. Three examples of experimental profile pairs are shown in Fig. 1, the upper profile in each pair corresponds to the triplet -H/L/H-L.

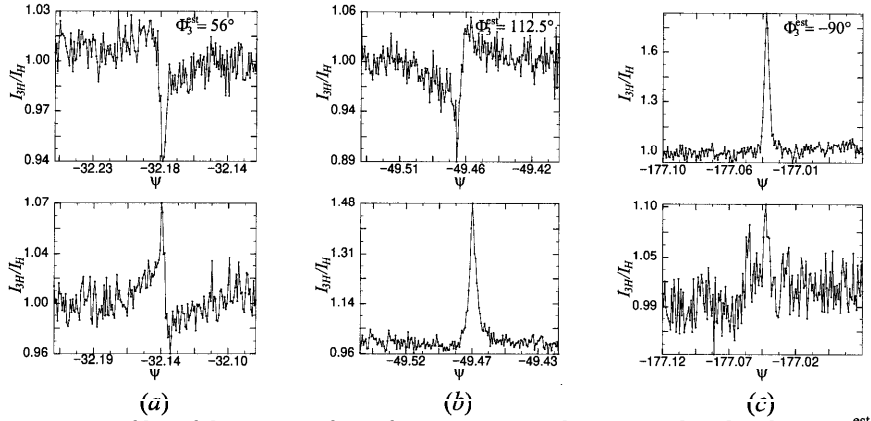

Fig. 1. Y-scan profiles of three pairs of interference maxima, the estimated triplet phase, Φ_3^{est}, is given in the upper profile of each pair. (a) Triplet 8 9 26/ -7 -5 -2O/ -1 -4 -6, $\Phi_3^{calc} = 43^o$. (b) Triplet 14 1 8/ -14 0 -10/ 0 -1 2, $\Phi_3^{calc} = 117^o$. (c) Triplet 5 5 20/ -7 -5 -2O/ 2 0 0, $\Phi_3^{calc} = -61^o$.

Phases were estimated independently by two workers and averaged. The general precision attempted was 22.5^o, except near $\pm 90^o$ where a more accurate estimate is feasible. Of the 45 independent estimates, 32 were identical, four differed by about 10^o, and nine by 22.5^o. No estimate differed by more than 22.5^o. The average difference, $<\Delta\Phi_3>$, between the 40 estimated unique triplet phases and those calculated from the crystallographic refinement is 15.9^o. The set includes four special-valued $(0/180^o)$ triplets. Most triplets contain one very strong reflection with indices 10 1, 0 11, 111, 0 12 or 200, i.e. low-resolution data containing information on the coarse structure. With a suitable strategy for the triplet-phase measurements and their subsequent application in a method for phase expansion, it is envisaged that this technique can be used to define the molecular envelope in the solvent matrix.

Complex α*-D-glucose · NaCl · H₂O (2:1:1):* This complex has been subjected to physical phase estimation in previous experiments at SNBL to distinguish between two different but very similar crystal structures [2, 3]. In the present run about 25 reflections with 28 in the range $18 - 76^o$, of which 70% had $2\theta > 45^o$, were collected for two crystals, one of each form (λ = 0.99998 Å). Cell parameters calculated with esd's including a $\sigma(\lambda) = 10^{-4}$ Å showed that the two sets of parameters are equal within 1 σ.

The ψ-scans were made on a six-circle Huber diffractometer (Prof. Hummer, University of Karlsruhe) located on the Swiss-Norwegian Beamlines, ESRF.

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

[2] Mo, F. *et al.* (1997). ESRF Project Reports, Experiments MI-l 17 and HS-37.

[3] Mathiesen, R.H., Mo, F., Eikenes, A., Nyborg, T. & Larsen, H.B. (1998). *Acta Cryst.* **A54.** In press.