	Experiment title: Structural studies on the aconitase family of enzymes	**Experiment number**: LS 1745
Beamline: BM 14	**Date of experiment**: from: 6 December 2000 to: 8 December 2000	**Date of report**: 30-8-2001
Shifts: 6	**Local contact(s)**: Bill Shepard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

These 6 shifts on BM14 had been allocated as a replacement for lost beam time on the same station – see report LS1745-1.

As we had been unsuccessful in obtaining any new seleno-methionine AcnB crystals (see report LS1745-1) data were collected on this trip for two enzymes with unknown structures:

One, imidazole glycerol phosphate dehydratase (IGPD), is an enzyme involved in histidine biosynthesis, which is strongly inhibited by triazole phosphatases and is a potential herbicide target. Preliminary test images on frozen crystals of seleno-methionine IGPD, stored for subsequent data collection at ESRF, indicated that IGPD crystallises in a tetragonal system with cell dimensions a=b=157.3 and c=229.5Å. Mass spectrometry indicated that the selenium incorporation was high, and thus data were collected at the inflection, peak and

high energy remote wavelengths, as determined from a fluorescence scan of the crystal. Due to the long cell dimensions and a mosaicity of 0.6°, the rotation per image used was 0.25°. Preliminary processing and merging of the first few images indicated that the data only merged in a monoclinic system, and thus 711 images were recorded for the inflection and peak energies and 474 for the remote wavelength. Unfortunately, when these data were subsequently processed and analysed in Sheffield, it became apparent that these crystals were perfectly twinned, in space group P4, with a twin fraction of 0.5. Current detwinning algorithms cannot cope with a perfect twin, and thus experiments are underway to alter the crystallisation conditions to hopefully obtain untwinned crystals of this enzyme.

The other protein analysed in this trip is involved in the transacetylation of frontline clinically relevant antibiotics, removing their efficacy, and hence is involved in drug resistance mechanisms. The structure of this enzyme will be important in the design of new antibiotic variants that might avoid this problem. Seleno-methionine crystals of this protein were grown in space group F222, cell dimensions a=181.4, b=183.0, c=184.1 Å and with 6 chains in the asymmetric unit. Data were collected to 2.7Å at the inflection, peak and high energy remote wavelengths of Selenium, as determined by a fluorescence scan of the crystal. FA values were calculated to 3.0Å using a development version of the program XPREP and 46 out of the 48 Se sites were identified using SHELXC. Phases were calculated in MLPHARE and the polypeptide chain was immediately apparent in the initial electron density map. The final model has R = 0.18 and Rfree = 0.23 and a manuscript describing the structure is currently in preparation.