

**Experiment title:**

Crystallographic studies of the Src-kinase domain.

Experiment number:

LS452

Beamline: Date, of Experiment:

BL4/ID2 from: 16.2.96 (07.00) to: 18.2.96 (07.00)

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Report:

The major goal of our visit to beamline BL4/ID2 at the ESRF (16.2.96-18.2.96) was to collect data from Src crystals. Altogether data was collected for two different projects:

- 1) Chicken Src Kinase-SH2-SH3 construct.
- 2) Rat mitochondrial enoylCoA-isomerase.

1) Crystals of chicken Src were approximately $0.16 \times 0.08 \times 0.04$ mm. One crystal, mounted in a normal glass capillary, diffracted to approximately 2.6 \AA resolution. Crystal quality appeared to be reasonable but in the course of data collection, severe radiation damage rendered only 15 of the 40 images collected useful. Initial processing of this data indicates that the crystal is orthorhombic (P2,2,2,) with cell dimensions of $a = 55.6 \text{ \AA}$, $b = 90.2 \text{ \AA}$, $c = 110.4 \text{ \AA}$ and angles of $\alpha, \beta, \gamma = 90$. The mosaicity is approximately 0.95° . At room temperature, the half life of the crystal due to the radiation damage is approximately 30 minutes as judged by the scaling factors of sequential frames.

Subsequently, much time was devoted to find a protocol for data collection at 100K. The use of small hoops ($d=0.15\text{mm}$) and no cryoprotectant apparently works best for these crystals (grown in approximately 25% PEG2000 or 25% PEG4000). After many trials, two datasets were collected at 100K with a resolution of approximately 4.0\AA and 3.0\AA . Processing of the 3.0\AA data set confirms that the crystal has $P2_12_12_1$ symmetry with cell dimensions of 54.3\AA , 89.1\AA , 9.9\AA and the mosaicity is 0.95° . The initial images of this crystal showed diffraction beyond 3\AA . Processing of the second data set gives the same space group, similar cell dimensions and a mosaicity of 1.4° . Molecular replacement calculations of both sets are in progress.

2) Enoyl CoA isomerase has been crystallised in the presence of 20% PEG6000. A few cryo experiments had been done on the rotating anode at EMBL-Heidelberg just before the ERSF visit. Diffraction spots were observed at a maximal resolution of 4\AA , at 100K. At the ERSF data was collected from a small loop (0.15mm), and, once again, without cryoprotectant. A complete dataset (3\AA resolution) was collected. The cell dimensions are $a=95$, $b=128.8\text{\AA}$, $c=134.2\text{\AA}$, α , $\beta, \gamma=90$ with spacegroup $P2_12_12_1$. For 2 trimers per asymmetric unit the $V = 2.4\text{\AA}^3/\text{D}$. Mosaicity is 1.5° .

Further optimisations of the crystallisation conditions and the cryocooling protocol will probably allow for a data set with a resolution better than 3\AA for both proteins.