	Experiment title: Structural studies on Aconitase A	**Experiment number**: MX 148
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(1) **Aconitase A** (AcnA) is the first aconitase of *E.coli* and has 30% identity to the porcine mitochondrial aconitase (whose structure is known) and 53% identity to human Iron Regulatory Protein 1 (IRP1) which binds an RNA stem-loop known as an iron response element (IRE) and acts as an iron-sensor to control expression of various iron-containing proteins. Very small crystals (~20 micron) of native AcnA and a potential mercury derivative were taken to ESRF, but failed to diffract.

(2) Having previously solved the structure of **Aconitase B** containing an iron-sulphur cluster (AcnB; Williams et al (2002) *Nature Struct. Biol.* 9: 447-452), we are also interested in the apo-AcnB as is the RNA-binding form of this dual-function protein. Small (~50 micron) hexagonal crystals of apo-AcnB were grown. Data were collected at ESRF station ID29 on the native crystals and a potential mercurial derivative, both to 3.5 Å resolution. Cell dimensions were a=b=121Å, c= 258Å, $\alpha=\beta=90°$, $\gamma=120°$ and the space group initially appeared to be P622. However, merging statistics were poor, and reprocessing of the data in P3 shows that the crystals are merohedrally twinned with a twinning fraction close to 0.5. Attempts at molecular replacement have not been successful.

(3) A high resolution data set (1.6 Å) was collected on crystals of the E. coli toxin **hemolysin E / cytolysin A** (HlyE). This has enabled high resolution refinement of the structure, which has revealed exceedingly interesting water clusters around hydrophobic surface residues that may become buried on assembly of the toxin into its membrane-bound pore form (Stillman *et al*, in preparation).

(4) **ykuR**. During this trip data were also collected on the *Bacillus subtilis* essential gene product *ykuR.* This protein is a member of the M20 hydrolase superfamily. A knockout of this gene is bacteriocidal in *B.subtilis*, indicating that this enzyme is a good target for novel antimicrobial therapies. We have cloned, expressed and purified ykuR, however, diffraction quality crystals have proved very difficult to obtain. It is possible to routinely grow crystals that are large (>1mm), yet only diffract X-rays to 12Å at best. However, one batch of protein gave one crystallization drop that contained 150 micron crystals that diffracted to 3.5Å on a rotating anode source, these crystals have proved impossible to reproduce. Using this single drop of crystals we tried to determine the structure of this enzyme by soaking crystals in heavy atoms and collecting 3 wavelength MAD data. A fluorescence scan on a back soaked, mercury soaked crystal showed the presence of mercury and data were collected for the peak, inflection and remote wavelengths. The crystals are in space group R32, cell dimensions a=b=213.2 Å c=180.1 Å, and Vm analyses indicate that there are probably 2 or 3 copies of ykuR in the asymmetric unit. However, despite extensive analysis of the data using both MAD and SAD techniques with SHARP and the SHELX suite, interpretable electron density maps could not be obtained. A second crystal was soaked in a solution containing platinum, this crystal was not back soaked as any bound platinum would presumably soak out. A fluorescence scan showed, unsurprisingly, that Pt was present, however data collected at the peak wavelength had no anomalous signal, indicating that Pt had not bound to the protein. A further fluorescence spectrum was obtained, which suggested that both Fe and Mn may be present in the crystal. However, time constraints precluded data collection at these wavelengths.