ESRF	**Experiment title:** Structure solution of UDP-glactose mutase	**Experiment number:** LS-1166
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Report:

The aim of the experiment was to determine the structure of the *E. coli* protein UDP-galactose mutase. The protein is responsible for the inter-conversion of UDP-galactose in the six membered ring form (pyranose) to the five membered ring form (furanose). A homologue of the protein is found in the pathogen mycobacteria tuberculosis. In this pathogen galactofuranose plays a key role in cell viability by anchoring the cell wall and peptidoglycan. As glactofuranose is not found in man, it presents a good target for therapeutic intervention.

With Dr Leonard we had collected a native data set on protein to 2.9Å on BM14. We prepared the SeMet protein. The presence of the selenium was confirmed by mass spectroscopy. As stated in the proposal, mutase crystals are very small and require multiple seeding to reach 0.05mm in thickness. The selenometionine crystals produced smaller crystals (0.01mm) which were difficult to grow bigger. By continued re-seeding we were able to grow crystals to approximately 0.04mm. These crystals did not diffract on the lab home

source and could not be characterised before visiting the synchrotron. Once cryo protected the crystals diffracted to beyond 3.0Å. The EXAFS scan showed a clear signal for selenium. Unfortunately the crystal was highly mosaic (> 2.5°). The quality of the diffraction varied enormously from crystal to crystal and we exposed around 15 different crystals. All the larger crystals (> 0.2mm) were highly mosaic. The small crystals (0.01mm) did not diffract beyond 4-6Å on BM14. However, Drs. Leonard & McSweeney have shown that these diffract to 3.0Å on ID14. On the basis of a 1 second exposure they conclude the crystals are not mosaic and should yield a structure. We then switched to putative platinum derivatives. The EXAFS scan showed the presence of platinum and scaling of native to derivative data suggested an isomorphous signal. We collected a full MAD data set on these crystals despite the high mosaic spread (2.0°). Although automated methods (SOLVE) found heavy atom sites these did not refine sufficiently well to phase the structure. We concluded that either the mosaic spread confounded the experiment or that the platinum was bound at many low occupancy sites.

We used the remaining time to good effect. We diffracted native crystals of three other protein crystals. Three good native data sets on frozen crystals were obtained. The data sets RmlB (dTDP-D-glucose 4,6-dehydratase EC4.2.1.46), 2.9Å, RmlC (dTDP-6-deoxy-D-xylo-4-hexulose 3,5-epimerase EC5.1.3.13) 2.1Å and RmlD (dTDP-6-deoxy-L-lyxo-4-hexulose reductase EC 1.1.1.133) 2.8Å were all considerably higher resolution than we had obtained in the laboratory. A paper describing the native data collection on RmlC is in press. We are wiring the other two papers. These proteins are in the rhamnose biosynthetic pathway in *Salmonella typhimurium*. This pathway is responsible for the conversion of glucose to rhamnose (figure 1). The pathway is found only in bacteria and is a good therapeutic target in *M. tuberculosis*, where rhamnose plays a similar role to galactofuranose. As a result of this trip and a further collaboration with Dr Leonard, BM14 has produced two new MAD structures RmlC and RmlD based on selenomethionine multiwavelength diffraction experiments. We have also solved by molecular replacement RmlB. We are currently refining RmlC and RmlB and Dr Leonard is tracing RmlD. We will publish these papers when this has been completed.

Figure 1 The rhamnose pathway