- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.
- bear in mind that the report will be reduced to 71% of its original size. A type-face such as "Times", 14 points, with a 1.5 line spacing between lines for the text, produces a report which can be read easily.

ESRF	**Experiment title:** St. Andrews Dundee BAG	**Experiment number:** LS-1683
Beamline: ID14-1/2	**Date of experiment:** from: 27th FEB 2000 to: 29th FEB 2000	**Date of report:** 15th March 2000
Shifts: 3+3	**Local contact(s):**	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

St. Andrews Structural Biology Group

James H. Naismith Principal Investigator *

David A.R. Sanders*

Louise V. Buchanan*

Wulf Blankenfeldt*

Report:

UDP-galactomutase catalyses an extremely unusual reaction in biology, the conversion of a six membered sugar ring into a five membered ring. This contraction of the ring occurs by an unknown mechanism. The enzyme requires flavin to function however it appears it does not function by a redox process. The enzyme is a target in many pathogens including *M. tuberculosis*.

We have determined the structure of the enzyme using a variety of data sets obtained at the ESRF. We obtained a MAD data set to 3.2Å and native crystal to 2.7 Å on $P2_1$ crystals (Cell a= 69.5 b=56.9 c=97.3 Å β=96.4^{O}) at the ESRF on ID14-4 on a previous award to Naismith (report submitted). The crystals diffract very poorly, even with unattenuated beam several seconds an exposure is required to see diffraction beyond 4.0 Å for SeMet crystals. The

crystals have a mosaic spread in excess of 2.0° and unsurprisingly in light of radiation damage the data were not of high quality R-merge 15.0%, completeness 76% for the final wavelength.

We used the time on ID14-1 and ID14-2 to screen very small new fresh crystals of mutase grown under a slightly different conditions. These crystals measured a maximum 0.05mm in each dimension. We estimate approximately 40% of these crystals do not diffract, a further 40% or unusuably mosaic (>5°). However, by screening many crystals we found one which diffracted to 2.4 Å. The crystal indexed and merged in $P2_12_12_1$. The cell dimensions were a = 134, b = 59, c = 98 Å. We collected a complete data set on this crystal (R-merge 5%, completeness 100%). We also screened $P2_1$ SeMet crystals and collected a single wavelength experiment on a very small crystal which was significantly non-isomorphous with all other $P2_1$ crystals (cell a=65.0 b=53.9 c=95.6Å β=98.4°). The crystal was very small and was unlikely to survive a MAD experiment, however, as it was non-isomorphous we collected a data set.

We used the phases we obtained from the ID14-4 MAD data set to calculate a map. A volume of this map corresponding to our 'best guess' of the protein was cut out and molecular replaced into the $P2_12_12_1$ native crystal form, the non-isomorphous $P2_1$ SeMet (mosaic spread 2.5∞ resolution 3.5 Å) and the 2.7 Å native. A clear solution was obtained in each case.

We then cross crystal averaged, using only the phases to 4.5 Å from the MAD experiment. The resulting map at 2.4 Å is interpretable and so far we have traced 320 out of 380 residues and located the flavin molecule. Preliminary post-mortem examination suggests that to work all four data sets are required, as the phases are so poor (function of the poor crystals).

The structure solution required synchrotron radiation. The crystals diffract too poorly to be examined in house, multiwavelength methods were key to the problem. The facility of BAG time allowed us flexibility in scheduling the experiment. The crystals require to be examined when fresh and do not grow at a predictable rate. The BAG also allowed us to screen for crystals at the synchrotron over a period of time and to select the best crystal. It is this approach that ultimately solved the structure. Without BAG time, the solution would have taken longer.