	Experiment title: Essential bacillus subtilis gene products	**Experiment number**: 14-U-740
Beamline: BM14U	**Date of experiment**: from: 17 December 2004 to: 28 December 2004	**Date of report**: 3-11-05
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Essential Bacterial gene products.

Bacterial pathogens are becoming of increasing importance in health care due to the spread of multi-antibiotic resistant strains. In order to develop new treatment regimes for infectious diseases it is crucial to identify novel therapeutic targets. Essential gene products are ideal, as they are required for cellular viability. In *Bacillus subtilis*, around 30 novel putative essential genes have been identified via functional genomics. Many of these are present in a wide range of bacterial pathogens. We are analysing these essential 30 genes from Bacillus and have initiated cloning, overexpression and purification of each, and have determined the structures of a number of these proteins, mostly by three-wavelength MAD data collected at the ESRF. One such protein is the gene product ykuR, which 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 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 have tried to determine the structure of this enzyme by soaking a crystal in mercury and collecting 3 wavelength MAD data and also by soaking a separate

crystal in a platinum solution and collecting single wavelength SAD data (MX148, 8-12-03). Neither of these approaches were successful, however fluorescence scans on the crystals at ID29 showed that they contained Fe and Mn. In a further attempt to determine the crystal structure we proprosed to collect Iron and Sulfur SAD data using the remaining diffracting crystals. A trip to ID23.1 to undertake this experiment proved fruitless, due to problems with the fluorescence detector on this station (MX 216). Therefore, collecting these SAD data was the purpose of this visit to BM14.

Fluorescence scans on a crystal of ykuR confirmed the presence of Fe in the crystal and test images showed diffraction to 3.5 Å. A total of 1440 images (1 degree per image) were collected at the iron edge (7.129 KeV) and a further 90 images at the inflection and remote wavelengths. There is a small, but hopefully detectable, anomalous signal from sulfur at the iron edge, and so it was hoped that these data would give both the Fe and S positions in the asymmetric unit. Analysis of the anomalous signal to noise in XPREP, whilst the data were being collected, showed a small, but improving, signal as more data were accumulated.

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. Attempts to determine the anomalous substructure using SHELXD only gave one clear site with a number of less significant sites. Extensive analysis of the data using both MAD and SAD techniques with SHARP and the SHELX suite has unfortunately, yet to yield interpretable electron density maps.