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| | **Experiment title:**<br>Structural Studies of Novel proteins with potential for antibiotic targets. | **Experiment number**:<br>MX 216 |
| **Beamline**:<br>ID 23.1 | **Date of experiment**:<br>from: 30 April 2004 to: 1 May 2004 | **Date of report**:<br>3-11-05 |
| **Shifts:**<br>3 | **Local contact(s)**<br>Dr Didier NURIZZO | *Received at ESRF:* |

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## (1) Glutamate Racemase

# Substrate-Induced Conformational Changes in *Bacillus subtilis* Glutamate Racemase and Their Implications for Drug Discovery

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**Structure, Vol. 13, 1–7, November, 2005**

Summary
D-glutamate is an essential building block of the peptidoglycan layer in bacterial cell walls and can be synthesized from L-glutamate by glutamate racemase (RacE). The structure of a complex of *B. subtilis* RacE with D-glutamate reveals that the glutamate is buried in a deep pocket, whose formation at the interface of the enzyme's two domains involves a large scale conformational rearrangement. These domains are related by pseudo-2-fold symmetry, which superimposes the two catalytic cysteine residues, which are located at equivalent positions on either side of the *a* carbon of the substrate. The structural similarity of these two domains suggests that the racemase activity of RacE arose as a result of gene duplication. The structure of the complex is dramatically different from that proposed previously and provides new insights into the RacE mechanism and an explanation for the potency of a family of RacE inhibitors, which have been developed as novel antibiotics.

**(2) 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. 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. 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. Using this single drop of crystals we had previously tried to determine the structure of this enzyme at ID29 of the ESRF by soaking crystals in mercury and collecting 3 wavelength MAD data, which proved unsuccessful (MX148). Fluorescence scans on a crystal of ykuR on ID29, showed that they contained Fe and Mn. In an attempt to determine the crystal structure we wished to collect Fe MAD data for these crystals. However, there were problems with the fluorescence detector on ID23.1, and we were limited to collecting SAD data, at an energy (8KeV) estimated to produce some Fe anomalous signal. Despite collecting a total of 360 degrees of data, very little anomalous signal was present in the data, and it has proved impossible to determine a heavy metal substructure.

(3**) RusA resolvase**
RusA endonuclease is involved in resolution of Holliday junctions in the process of homologous recombination. Data for a mutant of this enzyme was collected to a resolution of 1.2 Å in space group $C222_1$ with cell dimensions of a = 60, b = 85, c = 57 Å. The structure of this mutant has provided important insights into Holliday junction recognition and resolution during recombination (Macmaster *et al.* in preparation).