

<b>Experiment title</b>	<b>Crystal structure of L-ala ligases implicated in cell wall synthesis</b>
<b>Experiment number</b>	<b>30-01-695</b>
<b>Dates of experiment</b>	<b>20 / 22 May 2004</b>

The femABX protein family is constituted by enzymes involved in the synthesis of the peptidoglycan, the major component of the bacterial cell wall. These proteins catalyze the addition of an amino acid on the peptidoglycan precursor by using aminoacylated tRNA as a substrate. We recently solved the structure at 1.7 Å resolution of the FemX ligase of *Weissella viridescens* (Biarrotte-Sorin et al, 2003, 2004). FemX consists of two structurally equivalent domains, separated by a cleft containing the binding site of the substrate, and arranged in a three-dimensional fold similar to the fold of a large N-acetyltransferase superfamily, the GCN5-related N-acetyltransferase (GNAT) superfamily, which catalyzes the transfer of the acetyl group from acetyl coenzyme A to a primary amine. This led us to investigate the structural studies, and especially the thermostability of the GNAT fold.

Beside all the technical problems we encountered during the 6 shifts of the 30-01-695 experiment (NFS crash of the DEC computer, secondary X-ray source...), we collected a native data set to 2.8 Å resolution of a putative N-acetyltransferase of *Pyrococcus furiosus*. Determination of this structure will provide new insights into the substrate specificity of this acetyltransferase and the thermal stability of the N-acetyltransferase domain.. We are trying by now to solve this structure by molecular replacement, but no solution could be obtained. Statistics of the data collection to 3 Å resolution are summarized in Table I. We are currently trying to optimize the crystals in order to collect a higher quality data set.

	pfGNAT
Number of images	180
Oscillation (°)	1
Space group	P622
Unit-cell parameters (Å)	a = b = 82.60 c = 105.92
Data-collection statistics	
Wavelength (Å)	0.9795
Resolution range (Å)	27-3
No. Observations	60747
No. Unique reflections	46010
Completeness (%)	98.6 (99)
Redundancy	12.9 (13.1)
R <sub>sym</sub> (%)	4.7 (16.8)
I/σ(I)	21.7 (6.12)

**Table I.** Statistics of data collection. The values in parenthesis are for the highest resolution shell. Data have been processed with *XDS*.

## **Publications**

- S. Biarrotte-Sorin, A.P. Maillard, J. Delettré, W. Sougakoff, D. Blanot, K. Blondeau, J.-E. Hugonnet, C. Mayer & M. Arthur (2003). Crystallization and preliminary X-ray analysis of *Weissella viridescens* FemX UDP-MurNAc-pentapeptide:L-alanine ligase. *Acta Cryst.* **D59**, 1055-1057.
- S. Biarrotte-Sorin, A.P. Maillard, J. Delettré, W. Sougakoff, M. Arthur & C. Mayer. (2004) Crystal structure of *Weissella viridescens* FemX transferase and its complex with UDP-MurNAc-pentapeptide: Insights into FemABX family substrate recognition. *Structure*, 12, 57-67.
- A.P. Maillard, S. Biarrotte-Sorin, R. Villet, S. Mesnages, A. Bouhss, W. Sougakoff, C. Mayer & M. Arthur. Structure-Based Site-Directed Mutagenesis of the UDP-MurNAc-pentapeptide-binding Cavity of the FemX Alanyl Transferase from *Weissella viridescens*. *J. Bacteriology*. Submitted.