ESRF	**Experiment title:** The rhamnose pathway, a target for therapeutic intervention	**Experiment number**: WT-12
Beamline: BM14	**Date of experiment**: from: 01.11.00 to: 02.11.00	**Date of report**: 04.12.00
Shifts: 3	**Local contact(s)**: Germaine Sainz	*Received at ESRF:*

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Report:

L-rhamnose is a key residue of the cell wall of many pathogenic bacteria. It is generated from glucose-1-phosphate in a synthesis involving four enzymes (termed RmlA to RmlD), yielding dTDP-L-rhamnose as the precursor for cell wall generation. As L-rhamnose is not found in humans and as the enzymes of the rhamnose pathway show a high degree of conservation amongst microorganisms the pathway is an attractive target for the development of novel antibiotics. With the generous allocation of beam time at the ESRF in Grenoble it has been possible to solve the structures of all four enzymes over the last two years. RmlA has a novel tetrameric structure with two binding sites for thymidine containing compounds and is likely the point of control for the whole pathway. RmlB and RmlD as NAD(P)-dependent enzymes possess a Rossman fold.

The structure of the third enzyme in the pathway, RmlC (6-deoxy-D-xylo-4-hexulose 3,5-epimerase; E.C. 5.1.3.13), has been described in the literature earlier this year for the first time (see previous report). It is a dimeric, mainly ß-sheet jelly-roll like protein that catalyses the epimerisation of the glucose scaffold at positions 3 and 5 and is therefore of prime importance in determining the stereochemistry of L-rhamnose biosynthesis. The active center of the published RmlC structure from *Salmonella enterica* suggests an important role for two histidine residues that are almost completely conserved. In order to fully understand catalytic mechanism of RmlC we set out to determine the structure of an RmlC protein that is a more distant relative to the elucidated structure and in which one of the histidine residues is replaced by another amino acid. RmlC from *Streptococcus suis* fulfills these requirements with a sequence identity of approx. 20 % to RmlC from *Salmonella*. After many tedious attempts it was finally possible to obtain a crystal form of *S. suis* RmlC that was suitable for X-ray data collection. Structure solution with molecular replacement was not successful so that we decided to collect anomalous data of seleno-methionine labeled protein. Since the crystals belong to space group $P2_1$ and a structural template existed in *Salmonella* RmlC a SAD experiment performed at a wavelength giving maximum f'' contribution seemed appropriate to accumulate enough phase information for solving the *S. suis* RmlC structure.

Data collection statistics are given in Table I. Even if the crystal was relatively mosaic (> 1º) the anomalous signal was strong enough to find four of six possible heavy atom sites with SOLVE1.18. Density modification in RESOLVE1.04 leads to a map with clear solvent boundaries and features of protein secondary structure (Figure 1). This makes a positioning of the structure template possible (Figure 2) and will allow a rough modification of the trace in places where both proteins differ markedly. The refinement will proceed with multi-crystal averaging to transfer the phase information to higher resolution data sets collected at other ESRF beamlines, followed by the application of automatic structure building procedures.

Table I

		Resolution Shell (Å)	**Anom. R_{merge}**[a]	**R_{merge}**[b]
Wavelength (Å)	0.9788	30.0 – 5.42	5.9	8.1
Resolution (Highest Shell, Å)	30.0 – 2.37 (2.44 – 2.37)	5.42 – 4.30	4.4	6.1
Space group	$P2_1$	4.30 – 3.76	4.3	6.0
Cell constants (Å; º)	46.3, 82.6, 53.0; ß=107.7	3.76 – 3.42	4.4	6.1
V_M	1.68	3.42 – 3.17	4.6	6.2
Total measurements[a]	66450	3.17 – 2.99	5.8	7.9
Unique reflections[a]	27438	2.99 – 2.84	6.3	8.2
Average redundancy[a]	2.4 (2.6)	2.84 – 2.71	7.2	9.1
I/s	23.0 (14.4)	2.71 – 2.61	6.9	8.7
Completeness (%)[b]	92 (56)	2.61 – 2.52	7.5	9.1
Anom. completeness (%)[a]	87 (17)	2.52 – 2.44	9.7	11.4
Anom. R_{merge}[a]	5.2	2.47 – 2.37	10.7	12.3

[a] Data collection statistics follow SCALEPACK, treating F+ and F- as separate observations
[b] These values are for non-anomalous treatment in SCALEPACK

Figure 1

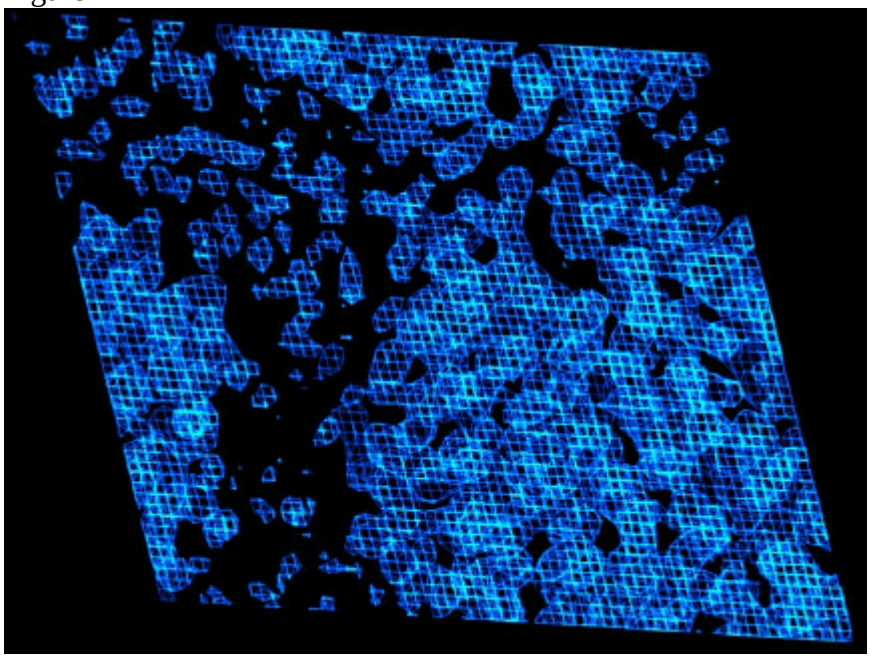

Figure 2