ESRF	**Experiment title:** **Structural Studies on Thymidylyltransferase (RmlA) from *Pseudomonas aeruginosa*** 12 JUN 2000	**Experiment number:** WT12
Beamline: ID14EH1	**Date of experiment:** from: 27.02.00 to: 03.03.00	**Date of report:** 9th June 2000
Shifts:	**Local contact(s):** Dr. G. Leonard	*Received at ESRF:*

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Report:

RmlA (glucose-1-phosphate thymidylyltransfase, E.C. 2.7.7.24) catalyses the first step in the conversion of D-glucose-1-phosphate (G1P) to deoxy-thymidylyl-diphospho-L-rhamnose (dTDP-rhamose), a key component for the synthesis of cell walls in many pathogenic bacteria. As L-rhamnose is not found in mammals any enzyme involved in its generation is of high interest as potential target for the development of new antibiotics.

The protein chain of RmlA consists of approx. 300 amino acids. It shows no sequence relationship to any of the proteins deposited in the PDB, making experimental phase determination a crucial step its structure elucidation. Six methionine residues per chain render MAD-phasing of seleno-methionine labeled protein the method of choice this purpose.

Recombinant RmlA of *Pseudomonas aeruginosa* was obtained by overexpression in *E. coli* and optimal crystallisation conditions were determined. A first rotating anode dataset showed these crystals to be triclinic with a fairly large unit cell (a = 71.5 Å, b = 73.1 Å, c = 134.7 Å; α = 89.9 °, β = 80.9° and γ = 81.1°), further necessitating the utilisation of a high quality tuneable beamline to collect multiwavelength MAD data in an acceptable amount of time. We were collected a three-wavelength MAD dataset on ESRF's BM14 consisting of three times 760 images of 0.5° oscillations. Using SOLVE these data allowed us to locate 24 selenium sites showing that the triclinic unit cell contains two RmlA tetramers. Initial

electron density maps based on the MAD data are of excellent quality and will allow us to unambiguously build a structure model of RmlA.

Data collection statistics are given in table 1

Table 1: Data collection statistics for MAD experiment on BM14

Position	**Peak**	**Inflection**	**Remote**
Wavelength (Å)	0.9790	0.9791	0.8855
Resolution (Å)	30.0 – 2.8 (2.87 – 2.80)		
Unit cell (Å; °)	a=71.6, b=73.9, c=133.8; α=89.8, β=80.3, γ=80.2		
V_m (Å^3/Da)	2.54		
Total measurements	221219	220585	224654
Unique reflections	121843	121982	121112
I/σ	31.7 (9.0)	31.6 (8.8)	29.7 (9.8)
Average redundancy	1.8 (1.0)	1.8 (1.0)	1.9 (1.7)
Completeness (%)	93.0 (63.2)	93.0 (63.2)	92.7 (69.4)
R_{merge} (%) †	2.2 (8.9)	2.3 (9.5)	2.4 (8.9)

† $R_{-merge} = \Sigma\Sigma I(h)_j - \langle I(h) \rangle / \Sigma\Sigma I(h)_j$ *where I(h) is the measured diffraction intensity and the summation includes all observations*

A manuscript describing these experiments has been accepted for publication:

Blankenfeldt, W.; Giraud, M.F.; Leonard, G.; Rahim, R.; Creuzenet, C.; Lam, J.S. & Naismith, J.H. (2000). The purification, crystallisation and preliminary structural characterisation of glucose-1-phosphate thymidylyltransferase (RmlA), the first enzyme of the dTDP-L-rhamnose synthesis pathway from *Pseudomonas aeruginosa*. *Acta Cryst.* D**56**, in press