ESRF	**Experiment title:** Crystal structures of complexes of the bifunctional enzyme GlmU	**Experiment number:** LS1657
Beamline: ID14-2+3	**Date of experiment:** from: 10-4-00 to: 11-4-00	**Date of report:** Aug00
Shifts: 3	**Local contact(s):** Mark van RAAIJ/ Julian LESCAR	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Gerlind Sulzenbacher*, Laurent Gal and Yves Bourne

Report:

The crystal structures of a truncated form of GlmU (GlmU-Tr) from *E. coli* and of the enzyme in complex with UDP-GlcNAc have been solved at 2.25 Å resolution in our laboratory (1). The 49 kDa protein is organized in two domains: (i) a pyrophosphorylase (PPase) N-terminal domain that shares homology with other pyrophosphorylase (PPase) enzymes over residues Met1-Ala120 and (ii) an acetyltransferase domain that possesses 23 times the hexapeptide repeat '(LIV)-(GAED)-X2-(STAV)-X', typically found in other bacterial acetyltransferases.

Recently we have solved the structure of the full-length enzyme from *Streptococcus pneumoniae* by Se-Met MAD at beamline BM14. In order to elucidate the structural basis required for both the uridyltransferase and acetyltransferase activity we have performed soaking and co-crystallization experiments with substrates, reaction intermediate and the final product. Data were collected for a number of the complexes obtained (see Table 1). As for the truncated form, the full-length enzyme assembles into a trimeric arrangement, with the LβH acetyltransferase domains packed tightly against each other in a parallel fashion, and the PPase domains being projected away from the threefold axis by a long α-helical arm. The last 25 C-terminal residues stretch over the a neighbouring LβH domain and end in an α-helix, which coils back towards the N-terminal and packs tightly against two neighbouring subunits, thereby forming a narrow tunnel where acetyl-CoA can bind. However, the C-terminal becomes stabilized only upon binding of acetyl-CoA. Clear difference density density could be observed for bound acetyl-CoA, UDP-GlcNAc, UTP and Mg^{2+}, giving insight both into substrate recognition and reaction mechanism. The exact mode of binding for GlcN-1-P remains unknown, as no electron density for this substrate could be observed. However, a narrow pocket opening in the proximity of the acetyl-CoA binding tunnel and surrounded by hydrophilic residues strongly suggests this being the binding site for GlcN-1-P.

Table 1 Data statistics for GlmU complexes (Values in parenthesis refer to the highest resolution bin)						
Complex with	UDP-GlcNAc 1	UDP-GlcNAc 2	Acetyl-CoA	Acetyl-CoA + GlcN-1-P + UDP-GlcNAc	Acetyl-CoA + GlcNAc-1-P + UDP-GlcNAc	Acetyl-CoA + GlcNAc-1-P + UTP + Mg^{2+}
Beamline	ID14-EH2	ID14-EH2	ID14-EH2	ID14-EH3	ID14-EH3	ID14-EH3
Resolution (Å)	40-2.5 (2.56-2.50)	15-2.4 (2.47-2.40)	50-2.5 (2.56-2.50)	50-1.9 (1.95-1.90)	50-1.75 (1.80-1.75)	50-2.2 (2.26-2.20)
R_{sym} (%)	4.7 (25.3)	5.5 (32.5)	2.4 (6.2)	3.3 (24.5)	4.7 (37.7)	5.1 (24.8)
I/σ (I)	9.1 (2.7)	5.8(2.3)	23.1 (10.8)	18.1 (2.9)	12.3 (1.9)	5.3 (2.4)
Completeness (%)	98.4 (100)	97.8 (99.0)	94.7 (70.9)	92.2 (83.1)	97.9 (96.4)	92.9 (82.9)
Redundancy	2.5	2.7	2.5	2.8	2.3	2.3

References: 1) Brown, K., Pompeo, F., Dixon, S., Mengin-Lecreulx, D., Cambillau C. & Bourne Y. (1999), *EMBO J.* **18**, 4096-4107.

Publications: Sulzenbacher, G., Gal, L. & Bourne, Y. Crystal structures of native and complexed forms of the *Streptococcus pneumoniae* N-acetylglucosamine-1-phosphate uridyltransferase, GlmU. Article in preparation.