ESRF	**Experiment title:** Glucose 1-phosphate Thymidylyltransferase	**Experiment number:** **LS1803**
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Glucose-1-phosphate thymidylyltransferase (G1P-TT) is the first enzyme in the biosynthesis of dTDP-L-rhamnose, the precursor of L-rhamnose, an essential component of surface antigens, such as the O-lipopolysaccharide, mediating virulence and adhesion to host tissues in many microorganisms. The enzyme catalyses the formation of dTDP-glucose, from dTTP and glucose-1-phosphate, as well as its pyrophosphorolysis. To shed more light on the catalytic properties of glucose-1-phosphate thymidylyltransferase from *Escherichia coli*, specifically distinguishing between ping pong and sequential ordered bi bi reaction mechanisms, the enzyme kinetic properties have been analysed in the presence of different substrates and inhibitors. Moreover, three different complexes of glucose-1-phosphate thymidylyltransferase (co-crystallized with dTDP, with dTMP and glucose-1-phosphate, with d-thymidine and glucose-1-phosphate) have been analysed by X-ray crystallography, in the 1.9 – 2.3 Å resolution range (R-factors of 17.3 – 17.5%). The homotetrameric enzyme shows strongly conserved substrate/inhibitor binding modes in a surface cavity next to the topological switch point of a quasi-Rossmann fold. Inspection of the subunit tertiary structure allows to recognize relationships to other enzymes involved in the biosynthesis of nucleotide-sugars, including distant proteins such as the molybdenum cofactor biosynthesis protein MobA. The precise location of the substrate relative to putative reactive residues in the catalytic center suggests that, in keeping with the results of the kinetic measurements, both catalysed reactions, i.e. dTDP-glucose biosynthesis and pyrophosphorolysis, follow a sequential ordered bi bi catalytic mechanism.

In the course of this period, data collected on G1P-TT complexed with dTDP-glucose and with deoxythimidine and glucose-1-phosphate have been collected. The relevant crystallographic statistics are reported in the table I. The final structures have been refined and deposited within the PDB with the codes 1H5T and 1H5R respectively.

	dTDP-G	Deoxy-thymidine, G1P
λ (Å)	0.933	0.936
Completeness (%)	97.5	85.4
Reflections (total)	421731	586259
Unique reflections	980787	99207
Redundancy	4.3	5.9
I/σ overall Rmerge overall (%)	14.7 4.8	19.3 3.4
Cell constants *a*	72.8 Å	72.8 Å
b	119.5 Å	118.9 Å
c	81.2 Å	80.6 Å
β	112.7°	112.6°
Resolution range (Å) used in refinement	12 – 1.9	12 – 1.9
R-factor (%)	17.4	17.3
R-free (%)	22.4	23.4
Rmsd bond length (Å)	0.019	0.018
Rmsd angles (°)	1.907	1.945
Rmsd planes (Å)	0.019	0.020
Cruickshanks DPI (Å)	0.15	0.17