The donor subsite of trehalose-6-phosphate synthase: binary complexes with UDP-glucose and UDP-2-deoxy-2-fluoro-glucose at 2Å resolution*.

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The atomic coordinates and structure factors (codes 1uqt and 1uqu) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rscb.org.).

Running Title: OtsA donor-site complexes

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Abstract
Trehalose is an unusual non-reducing disaccharide which plays a variety of biological roles from food storage to cellular protection from environmental stresses such as desiccation, pressure, heat-shock, extreme cold, and oxygen radicals. It is also an integral component of the cell-wall glycolipids of Mycobacteria. The primary enzymatic route to trehalose first involves the transfer of glucose, from a UDP-Glc donor, to glucose-6-phosphate to form α,α-1,1 trehalose-6-phosphate. This reaction, in which the configuration of two glycosidic bonds are set simultaneously, is catalysed by the glycosyltransferase OtsA which acts with retention of the anomeric configuration of the UDP-sugar donor. The classification of activated-sugar dependent glycosyltransferases, into approximately seventy distinct families based upon amino-acid sequence similarities, places OtsA in family GT-20. The recent 2.4 Å structure of *Escherichia coli* OtsA revealed a two-domain enzyme with catalysis occurring at the interface of the twin β/α/β domains. Here we present the 2.0 Å structures of the *E. coli* OtsA in complex with either UDP-Glc or the non-transferable analogue UDP-2FGlc. Both complexes unveil the donor subsite interactions, confirming a strong similarity to glycogen phosphorylases, and reveal substantial conformational differences to the previously-reported complex with UDP and Glc-6-P. Both the relative orientation of the two domains and substantial, up to 10 Å, movements of an N-terminal loop (residues 9-22) characterise the more open “relaxed” conformation of the binary UDP-sugar complexes reported here.

1 The abbreviations used are: GT glycosyltransferase; 2FGlc 2-deoxy-2-fluoroglucone; GT-20, glycosyltransferase from family 20

2 See afmb.cnrs-mrs.fr/CAZY/
Trehalose (α-D-glucopyranosyl α-D-glucopyranoside) is a widely distributed non-reducing disaccharide in which the two glucosyl moieties are linked together in an α,α−1,1 linkage, Fig 1. Initially, trehalose was viewed “merely” as an unusual energy source, substituting for glycogen, indeed it is still regarded as highly important in these contexts in certain insect tissues and in fungal spores. More recently, however, the critical roles of trehalose in the protection of organisms from environmental stresses and its role in the formation of the trehalated glycolipids of Mycobacteria have become apparent (1). The biosynthetic pathways to trehalose synthesis have thus become important areas of research both for their exploitation potential in biotechnological applications (2) and their inhibition for the treatment of mycobacterial and fungal infections.

Trehalose is undoubtedly one of the key players in the protection of living organisms against extremes of environmental stress (reviewed in 2,3). It is perhaps most well characterised under conditions of water-stress. “Resurrection plants”, nematodes, brine shrimps and yeast, amongst others, all survive under extreme desiccation where up to 99% of their water has been removed. Under these conditions cell viability is maintained through the synthesis of trehalose which can reach levels up to 10-20% of the dry weight of these organisms. Stabilisation reflects two major factors: the substitution for solvent water in hydrogen-bonding to proteins and the prevention of lipid-phase transitions at biological membranes (for example 4). Even in “normal” organisms, trehalose synthesis is elevated under stress conditions (most recently reviewed in 1), including dehydration, stationary phase culture, salt-stress (5), heat-shock (2), high pressure (6), extreme cold (7), exposure to oxygen radicals (8,9) and anoxia (10).

The stabilising effects of trehalose have also been harnessed for biotechnological applications (2). Trehalose increases the tolerance of protein samples to higher concentrations of chemical denaturant and acidity in vitro (11). The addition of exogenous trehalose at concentrations from 25mM to 350mM dramatically increases the viability of *E. coli* cells after 5 days of desiccated storage(12). For these reasons, there is now concerted effort to over-express trehalose-synthesis pathway genes in organisms...
where stress is a major problem. Recent success with salt, drought, and low-temperature stress-resistant rice suggests that this is a powerful approach for the future (13).

Trehalose plays numerous other roles. It is the main component of the trehalated glycolipids of Mycobacteria, including the immunogenic “cord-factor”, indeed trehalose metabolism is a validated therapeutic target in *M. tuberculosis* (14-16). Recent investigations also reveal the importance of the sugar and its mono-phosphorylated derivative trehalose-6-phosphate, for embryonic growth for *Arabidopsis thaliana* through the control of carbohydrate utilisation (17, reviewed in 18). Metabolic studies into microbial pathogenesis of *Magnaprtthe grisea*, a rice blast fungus, point to trehalose biosynthesis as having widespread significance during the initial infection (19) whilst infection by the human pathogenic fungus *Candida albicans* is also impaired if the trehalose-6-phosphate synthase gene is deleted (20).

This emerging spectrum of biological roles all highlight the importance of trehalose synthesis both for its inhibition and exploitation. The primary biological route to trehalose, in most organisms (1), involves the initial formation of trehalose-6-phosphate followed by dephosphorylation to yield trehalose, Fig 1. In *E. coli*, the enzyme responsible for the formation of α,α-1,1 trehalose-6-phosphate is the trehalose-6-phosphate synthase, OtsA, which uses UDP-Glc as the donor with the α-anomer of Glc-6-P as acceptor. Those species for which trehalose is extremely important under non-stress conditions, such as Mycobacteria and Corynebacteria, also find alternative pathways to trehalose including the transglycosylation of maltose and starch-derived oligosaccharides (16,21). Indeed, the three alternative pathways of these organisms may all substitute for each other and only the triple knock-out is lethal (22).

At the chemical level, activated sugar dependent glycosyltransferases show two distinct stereochemical outcomes. The configuration of the anomeric carbon of the donor sugar may either be retained or inverted following catalysis, Fig 2a, leading to the common terms “retaining” and “inverting” glycosyltransferases. For most reactions (and assuming D-sugars) the donor will be an α-linked species and thus inversion leads to a β-D linkage
whilst retention gives an $\alpha$-D linked product. Whilst the mechanism for inverting enzymes is comparatively clear (reviewed, for example, in 23), the mechanism leading to retention of anomeric configuration is poorly understood (see, for example, 24). Trehalose itself is an unusual non-reducing disaccharide in which the anomeric carbons of both glucosyl moieties are linked to each other in a “double” $\alpha-\alpha$ glycosidic linkage (Fig 1, inset and Fig 2b). The reaction catalysed by OtsA thus involves the formation of two glycosidic linkages and the enzyme must both select the $\alpha$-anomer of the acceptor and act with net retention of configuration of the ($\alpha$-linked) UDP-Glc donor; OtsA is thus a “retaining glycosyltransferase”.

At the sequence level, over 8700 sequences (as of 15/09/03) for activated-sugar dependent glycosyltransferases have been reported (25). The carbohydrate-active enzymes server “CAZY” currently sorts these into sixty-seven distinct families on the basis of amino-acid sequence similarities. 3-D structures are available only for twelve of these families GT-1, 2, 6-8, 13, 20, 28, 35, 43, 63 and 64 (reviewed in 25). OtsA is classified into family GT-20 which currently has 86 related members from all branches of the taxonomic tree. The *E. coli* OtsA structure is the only reported structure from this family (26).

Thus far, only two protein topologies have been associated with the twelve families of activated-sugar dependent glycosyltransferases with a known 3-D structural representative. These have been termed fold “GT-A” and “GT-B” (25,27) as well as “SpsA-like” (reflecting its first observation in the structure of the putative glycosyltransferase from family GT-2 (28) and “phage T4 $\beta$-glucosyltransferase-like” (again reflecting its first description in that structure (29)), Figure 3. The GT-A fold reveals two tightly associated and abutting $\beta/\alpha/\beta$ domains which tend to form a continuous central sheet of at least 8 $\beta$-strands (hence some authors describe this as a single domain fold (30)), Fig 3a. The second fold observed thus far, GT-B, again displays two Rossmann-like $\beta/\alpha/\beta$ domains, but these are much more independent, lying “opposite” each other, Fig 3b, and conformational changes in the relative-orientation of these domains are expected to accompany ligand binding (for example 31). Thus far, five
GT-B fold UDP-dependent glycosyltransferases have been reported. Four of these are “inverting” glycosyltransferases: GtfB (32) and GtfA (33) from family GT-1, the *E. coli* MurG from family GT-28 (34) and the original phage T4 β-glucosyltransferase, now classified into family GT-63. In addition, one retaining UDP-sugar glycosyltransferase has been solved which displays this fold, the family GT-20 OtsA (35), described further here.

We recently described the structure of *E. coli* OtsA in a “non-competent” complex with both UDP and Glc-6-P (35). This revealed a “bi-domain” β/α/β domain topology, Fig 3c, unveiling OtsA as the first retaining UDP-sugar dependent enzyme to display the GT-B fold. Whilst the complex, with UDP and Glc-6-P, allowed mapping of the acceptor subsite interactions, firm identification of the donor subsite was not possible. Furthermore, the UDP/Glc-6-P complex revealed a “closed” structure demanding that conformational change accompanied ligand binding from the native state. Here we present the structures of OtsA in binary donor complex; both those with UDP-Glc and the non-transferable analogue UDP-2FGlc at 2.0 Å resolution. The two binary structures (which are essentially identical) reveal the donor subsite interactions, confirming a striking similarity with glycogen and maltodextrin phosphorylases and highlight conformational changes, compared to the original UDP/Glc-6-P complex (35), as a result of ligand-binding. These latter changes feature both domain reorientation and large-scale movement of active centre loops with differences of up to 10 Å between binary and UDP/Glc-6-P complexes in some active-centre loops.

**EXPERIMENTAL PROCEDURES**

*Synthesis of UDP-2FGlc*

UDP-2FGlc was synthesized essentially as described previously (36). Briefly, treatment of 3,4,6-tri-*O*-acetyl glucal with Selectfluor (37) gave, in a 1:1 mixture, 3,4,6-tri-*O*-acetyl 2-deoxy-2-fluoro glucose and 3,4,6-tri-*O*-acetyl 2-deoxy-2-fluoro mannose. Acetylation of the anomic position afforded the per-*O*-acetylated sugars which could be readily separated by column chromatography. 1,3,4,6-Tetra-*O*-acetyl 2-deoxy-2-fluoro glucose
was isolated as a mixture of anomers. This mixture was treated with HBr/AcOH to yield the alpha-bromide and conversion to the desired beta-acetate was accomplished with silver acetate. MacDonald phosphorylation of the beta-acetate followed by lithium hydroxide mediated hydrolysis gave the fully deprotected 2-deoxy-2-fluorogluco- alpha-phosphate as the dilithium salt (as described in Ref. (38). Conversion of the lithium salt to the tri-n-octylamine salt, as required for the morpholidate coupling, was achieved using a cation exchange resin. Coupling of this alpha-glucosyl phosphate analog with uridine monophosphate was accomplished using the morpholidate coupling procedure described by Wong (39). Reaction progress was monitored by $^{31}$P-NMR until no further reaction was observed. The desired 2-deoxy-2-fluorogluco uridine diphosphate product was isolated by ion-exchange and size-exclusion chromatography.

_Crystallisation and X-ray data collection_

Gene over-expression and enzyme purification was carried out as previously described (26). The purified enzyme was concentrated in a buffer of 20mM TrisHCl pH 8 and 200 mM NaCl to 10 mgml$^{-1}$. Initial crystallisation screening was performed in 100nL sitting droplets using a Mosquito robotic system (TTP Labtech Ltd. Royston U.K.) a strategy which was essential to obtain preliminary conditions with small quantities of ligand. Optimisation of initial conditions was achieved using the hanging drop method with an equal amount of mother liquor to enzyme/ligand solution. The enzyme was co-crystallised either with 25 mM UDP-glucose and 50 mM 1-deoxy-Glc-6-P or 25 mM UDP-2FGlc with 20 mM Glc-6-P. These pairs were chosen so as to try to provide a ternary complex with either a non-transferable donor (UDP-2FGlc) or a non-viable acceptor (1-deoxy Glc-6-P). Crystals appeared, with both ligand combinations, after 36 hours in 30% (w/v) PEG4000 (Fluka), 200 mM ammonium acetate and 100 mM TrisHCl pH 8. Crystal evaluation and subsequent data collection required that 25% ethylene glycol be added to the growth liquor to produce a suitable cryo-protectant.

Data were collected, at a single wavelength, on the ID14 beam-lines of the European Synchrotron radiation Facility, Grenoble, France. Crystals for both forms are in space group $P_{2_1}2_12$ with approximate cell-dimensions $a=88$ Å, $b=102$ Å, $c=118$ Å. Data were
indexed and subsequently processed with MOSFLM/SCALA/TRUNCATE from the CCP4 suite (40).

**Structure solution and Refinement**

The structures were solved by molecular replacement using AMORE (41) using the “A” molecule of the UDP/Glc-6-P non-competent ternary complex (PDB code 1GZ5; (35)) as the search model with data between 20 and 4 Å and an outer radius of Patterson integration of 25 Å. The asymmetric unit contains a dimer of OtsA with the tetramer (35) being completed by the crystallographic 2-fold axis. Initial electron density maps suggested both local and “rigid-body” movements. 5% of the observations were set aside for cross-validation analysis and used to guide maximum-likelihood strategies. The structure was first treated as 4 independent bodies corresponding to the N- and C-terminal domains of the A and B molecules of the asymmetric unit. Further refinement involved maximum-likelihood positional and temperature-factor refinement using REFMAC (42). Manual rebuilding was performed with the X-Fit routines of QUANTA (Accelrys, San Diego, USA). Following convergence, UDP-sugar ligands were incorporated with refinement dictionaries calculated from ideal structures provided by QUANTA. Structural overlaps were performed with LSQMAN (43). Coordinates have been deposited with the protein data bank (http://www.rcsb.org).

**RESULTS**

*The structure of OtsA*

OtsA displays the twin-domain structure associated with fold “GT-B” glycosyltransferases with two β/α/β Rossmann-folds and a catalytic center at their interface. The N-terminal domain, residues 1-225, has a core of nine β-strands together with six associated helices, Fig 3c. The C-terminal domain (226-456) is of similar topology with a central parallel β-sheet of 6 strands flanked by seven α-helices. A common feature to GT-B fold enzymes is a kink in the C-terminal helix (in this case at residue 438) causing the C-terminal residues to cross-over and interact with the N-terminal domain.
In order to obtain donor-complexes co-crystallisation, harnessing extensive screening using high-throughput robotics, afforded two closely related crystal forms for OtsA from solutions containing either UDP-Glc and 1-deoxy Glc-6-P or UDP-2F-Glc and Glc-6-P, respectively. Both crystal forms diffract to around 2.0Å resolution, Table I, a significant improvement over the 2.45 Å of the original UDP / Glc-6-P complex (35) and both allow, for the first time, analysis of the UDP-sugar donor site for this class of retaining glycosyltransferase. Oddly, however, neither complex reveals electron-density for Glc-6-P in the acceptor site which is, instead, compromised as a result of conformational changes (or their absence), described below. Both structures are therefore binary complexes with intact UDP-sugar donor species only.

Ligand-induced conformational change

The 3-D structures of OtsA in binary complex with either UDP-Glc or UDP-2F-Glc reveal essentially identical structures in which electron density for the whole of the intact UDP-sugar ligand is unambiguous. There is absolutely no evidence for production or accumulation of a covalent β-2-F-glycosyl-enzyme intermediate as has been claimed for the bovine α-Gal transferase (44) in complex with the equivalent, but galacto-configured, ligand (although these results have been questioned by others (45)) The overall twin-domain structure of OtsA is slightly more “open” in the binary complex compared to the UDP/Glc-6-P complex described previously with the apex of the N-terminal domain approximately 1-1.5Å displaced relative to the UDP/Glc-6-P, Figure 4,5. Significantly larger conformational changes are observed in the acceptor-binding region, in particular the loop from residues 9-22, and these are described further below.

The overall fold of the enzyme is unperturbed by the binding of ligands. Within the catalytic center, however, there are significant conformational changes. In the UDP/Glc-6-P complex described previously the loop region from Arg9 to Gly22 made extensive interactions with the acceptor site, especially Arg9 which interacted with the phosphate moiety of Glc-6-P. We proposed that this loop thus provided “cross-talk” between the domains through the interaction of Gly22 on the acceptor domain with the distal
phosphate of UDP-Glc on the C-terminal donor sugar domain (26). This loop is unusual in that although catalytically important it contains a number of insertions, of up to eight residues, in other GT-20 sequences with the *E. coli* enzyme displaying the shortest loop (see http://afmb.cnrs-mrs.fr/CAZY/). In the absence of the Glc-6-P acceptor, there is a significant change in the conformation of this loop, Figures 4,5. In the binary complex with UDP-Glc in which the acceptor sugar site is vacant, there is no Arg9-Glc-6-P interaction and the loop folds back. This “open” conformation is stabilized through a new interaction between the carbonyl of Ala20 and the terminal nitrogen of Arg341. This conformational change involves main-chain differences, in excess of 10Å for some residues, Figure 5. Arg9 no longer lies in a position to interact with an appropriately placed acceptor sugar and the interaction of Gly22 with the distal phosphate of UDP-Glc is lost. The new position of the loop subsequently affects the flanking helix and loop region (residues Leu23-Gly36 and Val55-Thr61) setting them back within the domain, all contributing to an overall open, more 'relaxed' conformation than was observed previously for the UDP/Glc-6-P complex.

*The donor subsite of OtsA*

Observation of unambiguous electron density for UDP-Glc (and UDP-2FGLc), Figure 6, now permits description, Figure 7, of the donor subsite interactions of OtsA and by inference, this whole class of retaining glycosyltransferases. The O6 hydroxyl of the glucosyl moiety interacts with the side-chain of His154, mirroring the interaction seen in glycogen and maltodextrin phosphorylases. O6 also interacts with the side-chain amide of Gln185. The structurally equivalent residue to Gln185 of OtsA is Val455 in glycogen phosphorylase. Phosphorylase, however, achieves a chemically similar interaction with O6 through a hydrogen bond to Asn484, which is instead derived from a different part of the structure. The glucoside O4 makes a single side-chain interaction with Asp361 structurally and chemically equivalent to a glutamate-derived O4 interaction in phosphorylases. The majority of the O3 and O4 interactions are, however, with main-chain amide groups. O3 interacts primarily with the NH of Met363 (2.8Å) and O4 with the backbone amide of Asn364 (2.8 Å). The extensive use of main-chain interactions is consistent with the lack of plasticity reported for the donor sugar. The interactions of the
UDP-moiety are essentially identical to those reported previously for the UDP / Glc-6-P complex with the exception of the main-chain amide interaction from Gln22 of the acceptor domain which characterizes the closed ternary complex.

The phosphates of UDP-Glc fold back “underneath” the sugar moiety. In both UDP-Glc and UDP-2FGlc complexes this allows a 2.7 Å interaction between one of the phosphate oxygen atoms and the O2 (or F2) of the donor sugar. This close interaction is very reminiscent of the interaction of the carbonyl of the carboxylate nucleophile with the O2 hydroxyl of retaining β-glycoside hydrolases which is believed to contribute in excess of 40 kJ mol\(^{-1}\) to transition-state stabilization in those enzymes (46,47). It is also very similar to the conformation seen for the 2-fluoro-glucose-1-phosphate complex of glycogen phosphorylase (48). The close interaction of O2 (F2) with the phosphate, in this “curled under” conformation is consistent with the inhibition of glycogen phosphorylases by glucose-1,2 cyclic phosphates (\(K_i\) values from 250 – 900µM; (49)). Phosphorylase inhibitors may be able to act as lead compounds for the development of trehalose-6-phosphate synthase inhibitors.

**DISCUSSION**

The binary UDP-Glc (and UDP-2FGlc) complexes of OtsA clearly reveal significant conformational changes compared to the previous UDP/Glc-6-P pseudo-ternary complex. Compared to the binary UDP-Glc complex, the non-competent ternary complex is characterised both by a slight closing of the domains, perhaps triggered by the interaction of Gly22 on the N-terminal acceptor domain with the distal phosphate of UDP-Glc on the C-terminal donor domain, and by a significant conformational change of the “acceptor loop”, from residues 9-22, which encloses the acceptor site. It is unclear however, why the crystallised complexes, described here, do not display any occupancy of the acceptor site. One possibility is that the lattice-contacts somehow favour trapping of the UDP-Glc binary complex, although it is not evident how this might be so, or it might be that initial formation of the UDP-sugar complex somehow precludes binding of the acceptor although this would seem unlikely.
In this context, the striking similarities and differences between retaining UDP-sugar glycosyltransferases, OtsA, and glycogen phosphorylases are fascinating. Of the retaining UDP-GTs perhaps only LgtC has received an extensive analysis of its kinetic mechanism. LgtC displays an ordered bi-bi mechanism in which UDP-Gal binds first followed by the acceptor lactoside (50). Following transfer, the oligosaccharide product is released prior to UDP; these results being consistent with the structural rearrangements necessary for the formation of the acceptor binding-site following binding of the UDP-sugar. Glycogen phosphorylases, however, instead display a rapid equilibrium random kinetic mechanism in which both donor and acceptor substrates must be bound prior to the chemical event, but without constraint on their binding order (reviewed in 51). The observed 3-D structures of binary and abortive ternary complexes of OtsA do not favour one mechanism over any other. There does not appear to be pre-formation of the acceptor site as a result of UDP-Glc binding, in contrast to (52,53), indeed the UDP-Glc complex leaves some acceptor-binding residues over 10Å from the appropriate location.

It is clear that in terms both of kinetic and catalytic mechanism, retaining glycosyltransferases are still a challenging area. At the mechanistic level, whilst most authors agree that glycoside hydrolysis with net retention of anomeric configuration occurs via the formation and subsequent breakdown of a covalent intermediate either enzyme-derived (see for example (54) or substrate-derived (for example 55) there has been little credible evidence for a similar mechanism involving glycosyl transfer from activated sugars with anionic leaving groups. It is the absence of strong experimental data (such as mass-spectrometry, kinetic isotope effects, or accumulation of intermediates), for a covalent intermediate that leads to the widespread citation of an “S_N1-like” mechanism involving concerted but asynchronous front-face attack and departure as the likely mechanism for some retaining glycosyltransferases (for example, Refs (45,50,52,56)). Whilst such reactions are not without chemical precedent (57,58) they are exceedingly difficult to demonstrate experimentally. Here we have unveiled, for the first time, the donor subsite interactions of the GH-B class of retaining UDP-sugar glycosyltransferase. Whilst this confirms the similarity with glycogen and maltodextrin
phosphorylases the nature of the transition-state and reaction mechanism for this class of enzyme remains unclear.

ACKNOWLEDGEMENTS
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Legends to Figures.

**Figure 1 The biosynthesis of trehalose.**
The most common route to trehalose is via the formation of trehalose-6-phosphate from UDP-Glc and Glc-6-P, catalysed by the “retaining” glycosyltransferase OtsA.

**Figure 2. The stereochemical outcomes of enzyme-catalysed glycosyltransfer.**
(a) The use of $\alpha$-(D) linker activated sugar donors may give rise to products in which the anomeric configuration is retained or inverted. Trehalose (See Fig 1) is an unusual non-reducing disaccharide in which the two anomeric centers are linked together through the glycosidic oxygen; it thus has two glycosidic linkages as shown in simplified form in (b).

**Figure 3. Two folds, thus far, for NDP-sugar glycosyltransferases.**
To date, the 3-D structures of glycosyltransferases have revealed just two protein topologies termed the (a) GT-A and (b) GT-B fold (reviewed in 25), first revealed for NDP-sugar dependent enzymes by the structure determinations of SpsA (28) and the phage T4 $\beta$-glucosyltransferase (29). OtsA displays the GT-B fold (c). In this figure the N-terminal domains are shown in red and the C-terminal domains in blue. Ligands (UDP in (a) and (b) and UDP plus Glc-6-P in (c) are shown in “ball-and-stick” representation.

**Figure 4**
3-D structural overlap of OtsA in binary complex with UDP-2F\text{Glc} (gold with red ligand, this work) and in abortive complex with UDP/Glc-6-P (green with ligands in blue from (26). This figure was drawn with BOBSCRIPT (59).

**Figure 5**
Residue-by-residue comparison of OtsA in complex with UDP/Glc-6-P and OtsA in complex with UDP-Glc alone (main-chain positions only).

**Figure 6**
Observed electron density for the UDP-2F\text{Glc} complex of OtsA. The map is a maximum likelihood(42) / $\sigma_A$(60) weighted 2$F_{\text{obs}}$-F$_{\text{calc}}$ synthesis at 0.4 electrons / Å$^2$ and is shown in divergent wall-eyed) stereo. This figure was drawn with BOBSCRIPT (59).

**Figure 7**
Schematic diagram of the interactions of OtsA with UDP-2F\text{Glc} (identical interactions are observed for the UDP-Glc complex).
Table I X-ray data and structure quality for OtsA in complexes with UDP-Glc and UDP-2FGlc.

<table>
<thead>
<tr>
<th>Data(^a)</th>
<th>UDP-Glc</th>
<th>UDP-2FGlc</th>
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<tbody>
<tr>
<td>Resolution</td>
<td>20 – 2.0</td>
<td>20 – 2.0</td>
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<td>(R_{\text{merge}})</td>
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<td>0.061 (0.455)</td>
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<tr>
<td>Completeness (%)</td>
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<tr>
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<td>3.72 (2.66)</td>
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<tr>
<td>Mean (I/\sigma I)</td>
<td>12.4 (1.79)</td>
<td>18.7 (2.33)</td>
</tr>
</tbody>
</table>

Structure

| Number of non-H atoms | 7086 | 7393 |
| Number Reflections used /free | 65900 / 3507 | 67542 / 3594 |
| \(R_{\text{cryst}}\) | 0.232 | 0.217 |
| \(R_{\text{free}}\) | 0.269 | 0.254 |
| r.m.s.d (bonds/ Å) | 0.014 | 0.018 |
| r.m.s.d (angles / °) | 1.49 | 1.57 |
| Mean B value main-chain (Å\(^2\)) | 20.5(A) 22.6(B) 21.6(A) 23.7(B) |
| Mean B value ligand (Å\(^2\)) | 27.3(A) 26.7(A) 48.0 (B) |

\(^a\) outer resolution bin statistics are given in parentheses
References

UDPGlc + \( \alpha \)-Glc-6-phosphate \rightarrow \text{trehalose-6-phosphate} \rightarrow \text{Trehalose}

Gibson et al., Fig 1
Gibson et al., Fig 2
Gibson et al., Figure 4
Gibson et al., Fig 5
Gibson et al., Fig.6
The donor subsite of trehalose-6-phosphate synthase: binary complexes with
UDP-glucose and UDP-2-deoxy-2-fluoro-glucose at 2A resolution
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