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Structural Analysis of *Thermus thermophilus* HB27 Mannosyl-3-Phosphoglycerate Synthase Susana Gonçalves, Nuno Borges, Ana M. Esteves, Bruno Victor, Cláudio M. Soares, Helena Santos, Pedro M. Matias. *Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal.*
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Glycosyltransferases (GTs) catalyze the synthesis of an immense diversity of oligosaccharides, polysaccharides, and glycoconjugates. Many of these nucleotide-sugar dependent transferases are grouped within two well-characterized structural folds, GT-A and GT-B [1]. GTs are classified as either retaining or inverting according to the stereochemical conservation of their sugar substrates in the reaction products. The enzymatic mechanisms proposed for GTs are based on those established for their glycoside hydrolase counterparts [1]. However, due to the lack of a consensual nucleophilic residue in the retaining GTs together with difficulties in the trapping of a covalently bound glycosyl-enzyme intermediate, the mechanistic picture for this enzyme class is still incomplete.
Mannosyl-3-phosphoglycerate synthase (MpgS; EC 2.4.1.217) belongs to the retaining GT55 family (www.cazy.org), and is involved in the synthesis of mannosylglycerate, a compatible solute that accumulates in response to salt and/or heat stresses and is widespread in hyper/thermophilic bacteria and archaea from marine environments [2-4]. The crystallographic structure of MpgS from *Thermus thermophilus* HB27 in its binary complex form, with GDP-α-D-mannose and Mg^{2+}, shows a second metal binding site about 6 Å away from the mannose moiety. Kinetic and mutagenesis studies provided evidence for the first time that this metal site plays a role in catalysis, and is most likely to be relevant for enzymatic activity in all MpgSs in the GT55 family [5]. Additionally, Asp167 in the DXD motif is found within van der Waals contact distance of the anomeric C1' atom in the mannopyranose ring, suggesting its action as a catalytic nucleophile during the glycosyl-transfer mechanism, either in the formation of a glycosyl-enzyme intermediate in light of a double-displacement S_N2-like reaction mechanism, or in the stabilization of the oxocarbenium ion-like intermediate according to the $D_N{*}A_{Nss}$ (S_Ni-like) reaction mechanism. We propose that either mechanism may occur in the retaining GTs with a GT-A fold, and based on the gathered structural information we identified an extended structural signature towards a common scaffold between the inverting and retaining GTs**.**

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Structural basis for a new mode of glycosyltransferase inhibition. Rene Jørgensen[a], Thomas Pesnot[b], Monica M Palcic[a], Gerd K Wagner[b]. [a]*Carlsberg Laboratory, Copenhagen, Denmark.* [b]*School of Pharmacy, University of East Anglia, Norwich, UK.*
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In all domains of life, the biosynthesis of complex glycoconjugates requires the concerted action of a multitude of glycosyltransferases (GTs)-enzymes that catalyze the transfer of a mono- or oligosaccharide from a glycosyl donor (for example, a sugar-nucleotide) to a suitable acceptor (for example, a glycan, peptide or lipid)[1]. GTs play a key role in many fundamental biological processes underpinning human health and disease, such as cell signaling, cellular adhesion, carcinogenesis and cell wall biosynthesis in human pathogens. The development of small-molecule GT inhibitors is therefore of considerable scientific interest in chemical glycobiology and drug discovery. We have developed several new, base-modified UDP-Gal derivatives with an aromatic or heteroaromatic substituent in position 5 of the uracil base as chemical tools for the investigation of glycosyltransferases and other UDP-Gal dependent glycoprocessing enzymes. The most potent of these new derivatives act toward five different GTs, as a inhibitors of glycosyl transfer, with K_i values in the low micromolar to nanomolar range. To understand the molecular basis for the enzymological behavior of these inhibitors we chose a mutant of the ABO(H) blood group A and B glycosyltransferases which catalyze the final step in the synthesis of the A and B antigens. This mutant is a cis-AB mutant capable of transferring both Gal and GalNAc to the H-antigen with equal efficiency. We have solved high-resolution crystal structures of several of these inhibitors bound to the cis-AB mutant. Surprisingly, the inhibitors block the closure of a flexible loop in the active site by preventing the stacking of two amino acid residues where one is placed in the flexible loop and one in the C-terminus. This is a new mode of inhibition for GTs that, given the strong mechanistic similarities between many GTs, will probably also be applicable to other enzymes in this class.

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Chemokine Binding Protein from Orf Virus Modulates Immune Function- a new twist on an old motif Kurt L. Krause[a], Rafael Counago[a] Stephen Fleming[b], Andy Mercer[b], [a]*Biochemistry, University of Otago, Dunedin. New Zealand,* [b]*Microbiology and Immunology, University of Otago, Dunedin, New Zealand*
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Chemokine binding proteins (CBPs) are viral proteins that modulate inflammation by interfering with host chemokine signaling. CBPs bind to their cognate partner with picomolar affinity via an extended beta sandwich structure. Here we