## **Supplementary Information**

## Molecular structure of a metal-independent bacterial glycosyltransferase that catalyzes the synthesis of histo-blood group A antigen

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## **Supplementary Figures**

	D1 D2
α3GT	ESKLKLSDWFNPFKRPEVVTMTKWKAPVVWEGTYNRAVLDNYYAKQKITVGLTV
GTA	LPRMVYPQPKVLTPCRK-DVLVVTPWLAPIVWEGTFNIDILNEQFRLQNTTIGLTVFAIK
GTB	LPRMVYPQPKVLTPCRK-DVLVVTPWLAPIVWEGTFNIDILNEQFRLQNTTIGLTVFAIK
B0GT6a	MRIGILYICTG
Parach	MLFSHSLYAANVGLLVMATG
	D3 L3
α3GT	RYIEHYLEEFLTSANKHFMVGHPVIFYIMVDDVSRMPLIELGPLRSFKVFKIKPEKRW
GTA	KYVA-FLKLFLETAEKHFMVGHRVHYYVFTDQPAAVPRVTLGTGRQLSVLEVRAYKRW
GTB	KYVA-FLKLFLETAEKHFMVGHRVHYYVFTDQPAAVPRVTLGTGRQLSVLEVGAYKRW
BoGT6a	KYDI-FWKDFYLSAERYFMQDQSFIIEYYVFTDSPKLYDEENNKHIHRIKQKNLGW
Parach	KYVS-FVPPLVKSADHFFCKNHKVTYFVFTDG-YLEPMPNVVPIFHAKMGW
	▲ D4 D5 D5,7 A1 A2
α3GT	QDISMMRMKTIGEHIVAHIQHEVDFLFCMDVDDVFQDKFGVETLGESVAQLQAWM
GTA	QDVSMRRMEMISDFCERRFLSEVDYLVCVDVDMEFRDHVGVEILTPLFGTLHPGF
GTB	QDVSMRRMEMISDFCERRFLSEVDYLVCVDVDMEFRDHVGVEILTPLFGTLHPSF
BOGT6a	PDNTLKRFH-IFLRIKEQLERETDYLFFFNANLLFTSPIGKEILPPSDSNGLLGTMHPGF
Parach	PYDTMRYH-VYDMHRDAFAGQ-DYLYACDADMLFVGEVGDEILGNRVATRHPGF
	A3 A4 L1 D8,9 L2
α3GT	YKADPNDFTYERRKESAAYIPFGEGDFYYHAAIFGGTPTQVLNITQECFKGILKDKKNDI
GTA	YGSSREAFTYERRPQSQAYIPKDEGDFYYLGGFFGGSVQEVQRLTRACHQAMMVDQANGI
GTB	YGSSREAFTYERRPQSQAYIPKDEGDFYYMGAFFGGSVQEVQRLTRACHQAMMVDQANGI
BoGT6a	YNKPNSEFTYERRDASTAYIPEGEGRYYYAGGLSGGCTKAYLKLCTTICSWVDRDATNHI
Parach	INRPKSSYTYERNPLSTAYIPQGEGNDYFAGGFYGGTKDEFLNIVHTNAVNIDQDMQNGI
	D10,11,12
α3GT	EAQWHDESHLNKYFLLNKPTKILSPEYCWDYH-IGLPADIKLVKMSWDTKEYNVVRNNV
GTA	EAVWHIESHLNKYLLRHKPTKVLSPEYLWDQQLLGWPAVLRKLRFTAVPKNHQAVRNP-
GTB	EAVWHDESHLNKYLLRHKPTKVLSPEYLWDQQLLGWPAVLRKLRFTAVPKNHQAVRNP-
BoGT6a	IPIWHDESLINKYFLDNPPAITLSPAYLYPEG-WLLPFEPIILIRDKNKPQY-GG-
Parach	IAVWHDESHWNRFCINNPPTVILSPSYCYPQG-LRIPFLPKLIALDKNHEEMRKG-
	A5 A6 D13 D14

Figure S1. A multiple sequence alignment (using Clustal X2) of GT6 family members used in this study. Loop1, Loop2, Loop3 and C-term are secondary structural regions of BoGT6a that exhibit significant conformational changes upon ligand binding. A1-A7 are residues that contribute towards acceptor binding and are boxed in blue colour. D1-D14 are donor binding residues and are boxed in green colour. The residues in the box coloured in gold are the manganese ion binding D-*X*-D motif of GTA/B and/or  $\alpha$ 3GT. In BoGT6a this is replaced by N-*X*-N motif.



Figure S2. C-terminal conformational flexibility as observed in X-ray crystallographic structures of BoGT6a and  $\alpha$ 3GT. (A) BoGT6a (yellow) and BoGT6a-FAL (green); (B)  $\alpha$ 3GT (pea colour) and  $\alpha$ 3GT in complex with UDP and GalNAc (PDB id: 1GX4) (light purple).



**Figure S3. Isothermal titration of BoGT6a with ligands.** Titrations were with **(A)** UDP-GalNAc **(B)** FAL and **(C)** FAL in the presence of 3 mM UDP **(D)**. Both the ligand and sample were in 20mM HEPES buffer pH 7.5, containing 1 mM DTT and 0.2M NaCl and the reaction cell was maintained at 25 °C. The first injection in each titration was 5  $\mu$ l of ligand and subsequent injections were 10

 $\mu$ l. The upper section of each panel shows the exotherms of binding for each injection of ligand and the lower, the integrated binding isotherm with the experimental points (filled circles) and fitted line that gave the values for thermodynamic parameters give in **Table 3**.