Supplementary Information:

Table S1. Data collection and refinement statistics.

Wavelength, Å	1.115
Resolution range, Å	29.76 - 3.0 (3.107 -3.0)
Space group	P 6_5 2 2
Unit cell, a, c, Å	153.6, 193.7
Total reflections	327045 (32638)
Unique reflections	27582 (2689)
Multiplicity	11.9 (12.1)
Completeness, %	98 (100)
Mean I/σ(I)	12.4 (0.34)
Wilson B-factor, Å^2	112.21
R-merge	0.204 (8.4)
R-measured	0.213 (8.8)
CC1/2	0.99 (0.11)
CC*	1 (0.44)
Reflections used in refinement	27009 (2197)
Reflections used for R-free	1802 (188)
R-work	0.276 (0.55)
R-free	0.295 (0.50)
CC (work)	0.88 (0.180)
CC (free)	0.93 (0.194)
Number of non-hydrogen atoms	4132
macromolecules	4090
NPG	42
Protein residues	521
RMSD (bond lengths), Å	0.005
RMSD (bond angles), ^{o}	0.73
Ramachandran favored, %	91.8
Ramachandran allowed, %	6.8
Ramachandran outliers, %	1.4
Rotamer outliers, %	12.0
Clash score	5.1
Average B-factor, Å^2	116.4
macromolecules, Å^2	116.3
NPG, Å^2	124.6

Statistics for the highest-resolution shell are shown in parentheses.

Table S2. Hydrogen bonds between $LacY_{ww}$ and Nb9047

$LacY_{ww}$ atom 1, chain A	Nb9047 atom 2, chain B	Distance Å
O (peptide bond), **Lys42**	HD21, **Asn102**, CDR3	2.6
HD22, **Asn245**	O (peptide bond), **Gly103**, CDR3	2.5
HG, **Ser249**	OE1, **Gln99**, CDR3	2.4
O (peptide bond), **Ser249**	HH22, **Arg52**, CDR2	2.3
O (peptide bond), **Phe251**	H (peptide bond), **Tyr106**, CDR3	2.4
O (peptide bond), **Ala252**	H (peptide bond), **Ser107**, CDR3	2.4
NH (peptide bond), **Gly254**	O (peptide bond), **Gly104**, CDR3	2.5
NH (peptide bond), **Gly254**	OE1, **Glu108**, CDR3	2.0
O (peptide bond), **Thr310**	NE, **Arg 57**, CDR2	2.0
OG1, **Ser311**	NH2, **Arg 57**, CDR2	2.9
OE1, **Gln379**	HE1, **Trp53**, CDR2	2.5
H (peptide bond), **Gly380**	OG1, **Thr31**, CDR1	1.9

Table S3. Bond distances in sugar-binding site of $LacY_{WW}$ with bound NPG

Atom 1 Protein sidechain	Atom 2 NPG or protein sidechain	Distance, Å	
		Current structure	Structure without Nb PDB ID 4ZYR, chain A
Arg144 NH1	NPG O6	2.6	3.0
Arg144 NH2	NPG O5	2.8	3.6
Glu126 OE2	Arg144 NH2	3.3	3.2
Glu126 OE1	Tyr 350 OH	2.4	2.4
Glu269 OE2	NPG O4	3.5	3.2
Glu269 OE1	NPG O4	2.9	2.9
Glu269 OE2	NPG O3	3.1	2.8
Glu269 OE1	Asn272 ND2	3.0	3.1
Asn272 ND2	NPG O4	3.1	3.3
His322 NE2	NPG O3	2.8	3.1
His322 NE2	NPG O2	3.9	3.4
His322 ND1	Tyr236 O	2.9	3.3
His322 ND1	Glu325 OE2	3.4	3.2
Trp151 CD2	NPG C5	3.5	3.5
Met23 CE1	NPG C9	3.2	4.7
Cys148 SG	NPG O6	3.6	3.1

Fig. S1. Overall structure of the LacYww/NPG/Nb9047 complex. A. Crystal of LacYww-NPG-Nb9047. B. Side view of the LacYww/NPG/Nb9047 complex. The model is presented as a cartoon rainbow-colored from blue to orange for the backbone of $LacY_{ww}$ (from N- to C-terminus) with red-colored Nb9047 attached to the periplasmic side of $LacY_{ww}$. NPG located in the sugar-binding site is shown as purple sticks. The molecule of detergent n-nonyl-β-D-glucoside is positioned in the vicinity of TM3 and shown as brown sticks. Electron density (2Fo-Fc) map is contoured at 1σ and represented by light orange mesh around the model.

Fig. S2. Effect of Nb binding on the structure of $LacY_{ww}$. Cartoon on the **left** shows comparison of main-chains in the LacYww/NPG/Nb9047 complex and the previously determined outward-open structure of NPG-bound $LacY_{ww}$ (with Nb and NPG removed). The LacYww/NPG/Nb9047 complex is rainbow-colored from blue to orange for TM1 to TM12, and the NPG-bound $LacY_{ww}$ (PDB ID code 4ZYR) is cyan-colored. The plot on the **right** delineates differences in positions of Cα atoms for each amino acid in the aligned structures (RMSD = 0.9Å). Distances between Cα atoms are displayed for transmembrane helices and connecting loops only, including residues 9 - 188 and 221 - 401 in the N- and C-terminal halves of LacYww. Amino acids in loop 6-7 and in N- and C-terminals are omitted. Red and blue dots represent residues in periplasmic and cytoplasmic loops, respectively.

Fig. S3. The crossed-eyes stereo view of the interface in $LacY_{ww}$/Nb9047 complex. Residues in LacYww are shown as sticks (carbon in green, nitrogen in blue, oxygen in red, sulfur in light yellow, and hydrogen in white). Residues in Nb9047 are represented as sticks (carbon in yellow, nitrogen in blue, oxygen in red, sulfur in light yellow, and hydrogen in white). A transparent surface is shown around LacYww. Broken lines present hydrogen bonds. Interactions of LacYww with CDR1, CDR2, and CDR3, are shown on panels A, B, and C, respectively.

Fig. S4. Schematic representation of hydrogen-bond connections between CDR loops of Nb9047 (red) and amino acid residues of $LacY_{ww}$ (green) in the X-ray structure of the $LacY_{ww}$/NPG/Nb9047 complex. Contacts of neighboring residues in CDR3 loop (Asn102 and Gly103) with Lys42 (TM2) and Asn245 (TM7) link N- and C-terminal six-helical halves in LacY (chain of contacts: Lys42 - Asn102 - Gly103 - Asn245). See Table S2 and Fig. S3 for details.

Fig. S5. The NPG binding site in the $LacY_{ww}$/Nb9047/NPG complex. ($2F_o$-F_c) electron density map is contoured at 1σ. NPG is represented by sticks with carbon atoms colored orange, and residues in LacYww shown as sticks with carbon atoms in yellow, nitrogen in blue, oxygen in red, and hydrogen in white. Broken lines represent hydrogen bonds.

Fig. S6. Schematic representation of contacts between NPG and amino acid residues in the $LacY_{WW}$/NPG/Nb9047 complex. Carbon and oxygen atoms in NPG are labeled with blue and cyan marks, respectively. Amino acid residues of $LacY_{WW}$ are shown in green. Bond distances in Å are indicated. See Table S3 for details.

Fig. S7. Accessibility of galactoside-binding site in LacY and effect of Nb9047. NPG binding rates (k_{obs}) were directly measured with $LacY_{WW}$ or $LacY_{WW}$/Nb9047 complex (black or red circles, respectively) and with WT LacY or WT LacY/Nb9047 complex (green or red triangles, respectively) in 50 mM Na-phosphate buffer/0.02% DDM (pH 7.5) as change in Trp fluorescence due to FRET from Trp151 in binding site to NPG, as described (16).The values of association rate constant (k_{on}) for NPG, estimated from linear concentration dependencies of binding rates, are 5.7 or 5.8 μM^{-1} s^{-1} for $LacY_{WW}$ in the absence or presence of Nb9047, respectively, and 0.2 and 6.8 μM^{-1} s^{-1} for WT LacY in the absence or presence of Nb9047, respectively. Dissociation rate constant (k_{off}) is 31 s^{-1} for $LacY_{WW}$ and its complex with Nb9047, 32 s^{-1} for WT LacY/Nb9047 complex, and 41 s^{-1} for WT LacY without Nb (open symbols).

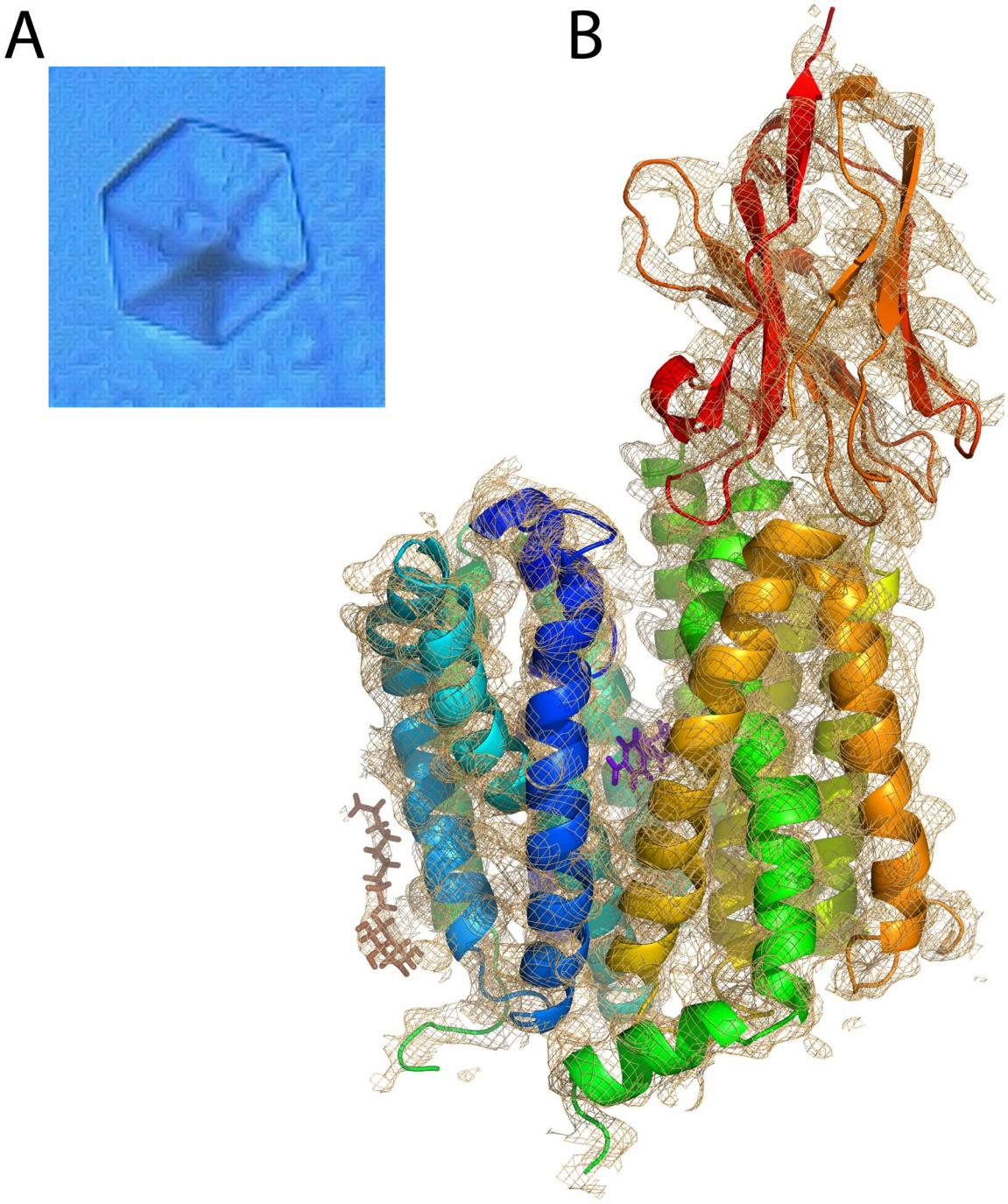


Fig S1

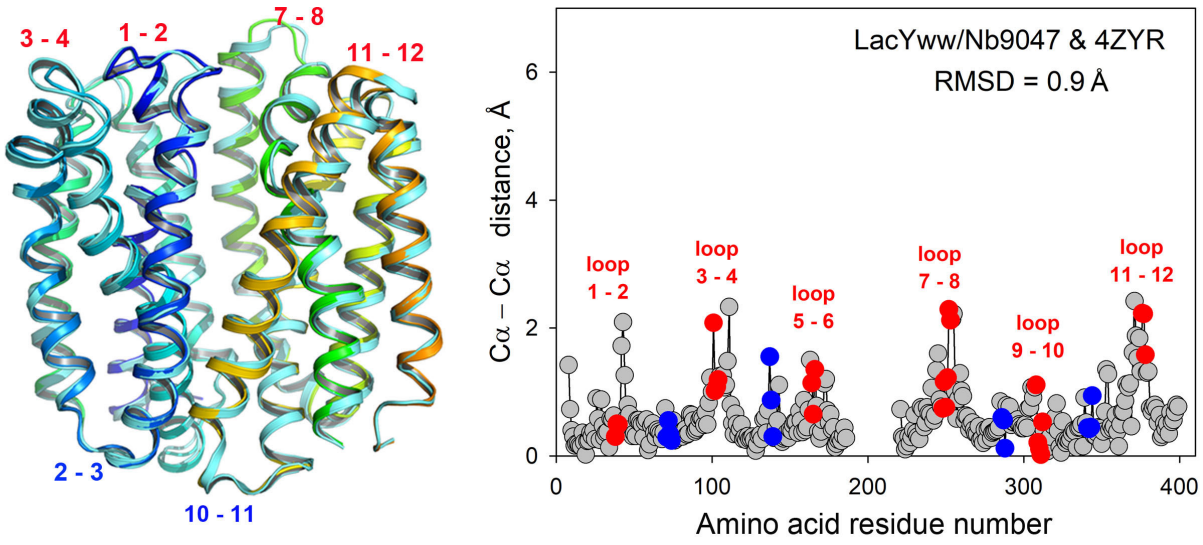
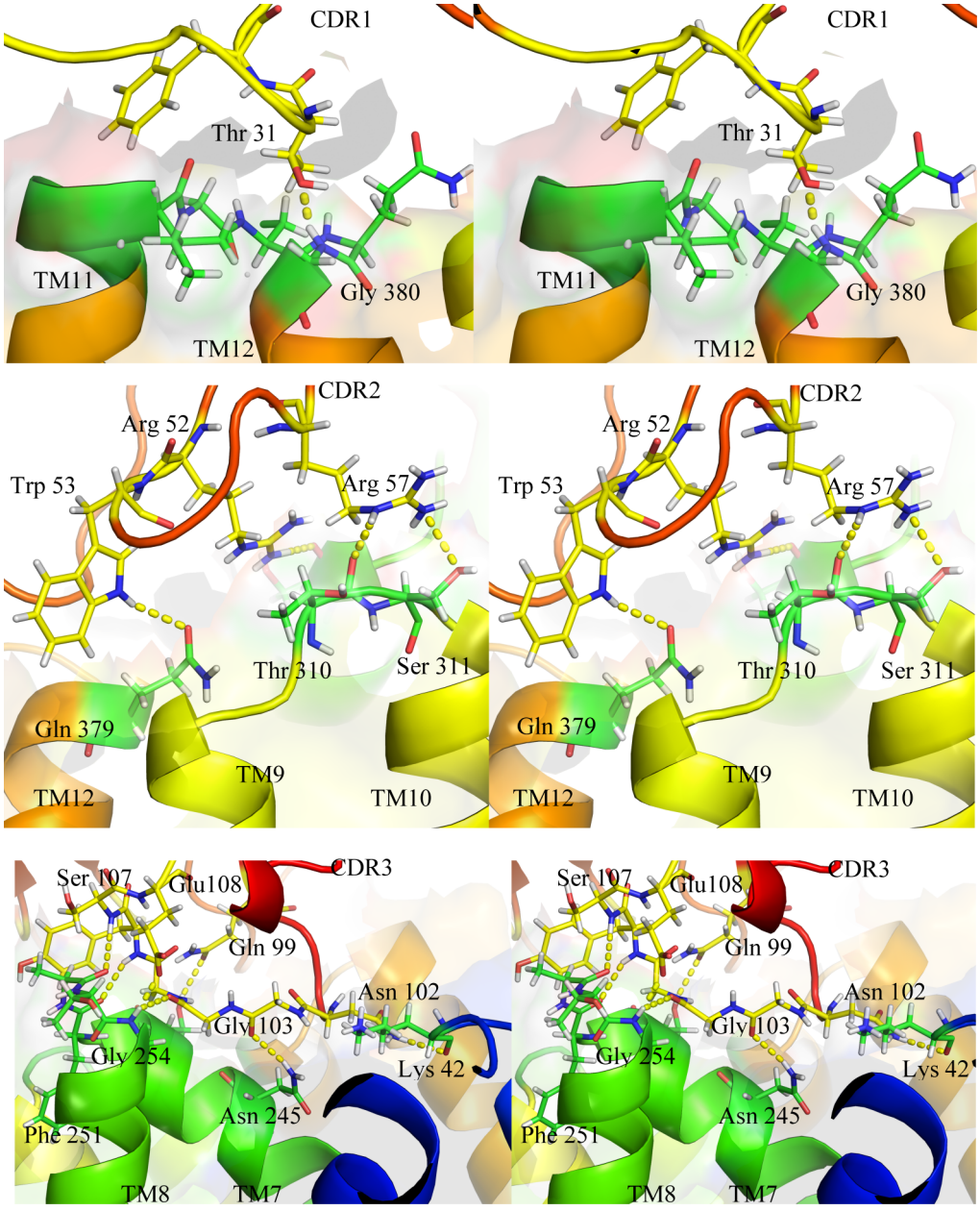

3 - 4
1 - 2
7 - 8
11 - 12
2 - 3
10 - 11
LacYww/Nb9047 & 4ZYR
RMSD = 0.9 Å
Cα – Cα distance, Å
Amino acid residue number
loop 1 - 2
loop 3 - 4
loop 5 - 6
loop 7 - 8
loop 9 - 10
loop 11 - 12
0
2
4
6
0
100
200
300
400

Fig S2


CDR1
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TM11
Gly 380
TM12
CDR1
Thr 31
TM11
Gly 380
TM12
CDR2
Arg 52
Trp 53
Arg 57
Thr 310
Ser 311
Gln 379
TM9
TM12
TM10
CDR2
Arg 52
Trp 53
Arg 57
Thr 310
Ser 311
Gln 379
TM9
TM12
TM10
CDR3
Ser 107
Glu108
Gln 99
Asn 102
Gly 103
Gly 254
Lys 42
Asn 245
Phe 251
TM8
TM7
CDR3
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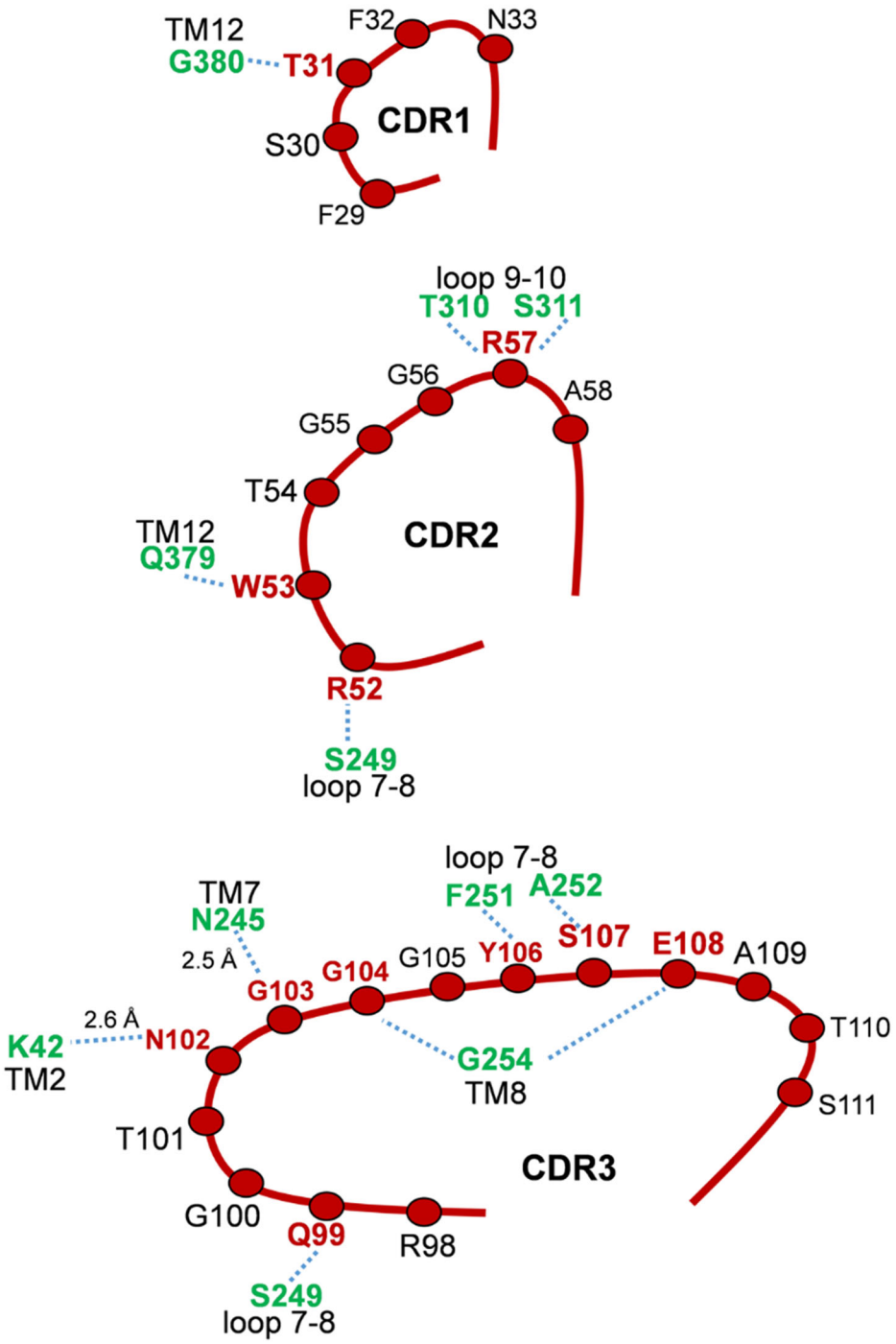
Gly 103
Gly 254
Lys 42
Asn 245
Phe 251
TM8
TM7

Fig S3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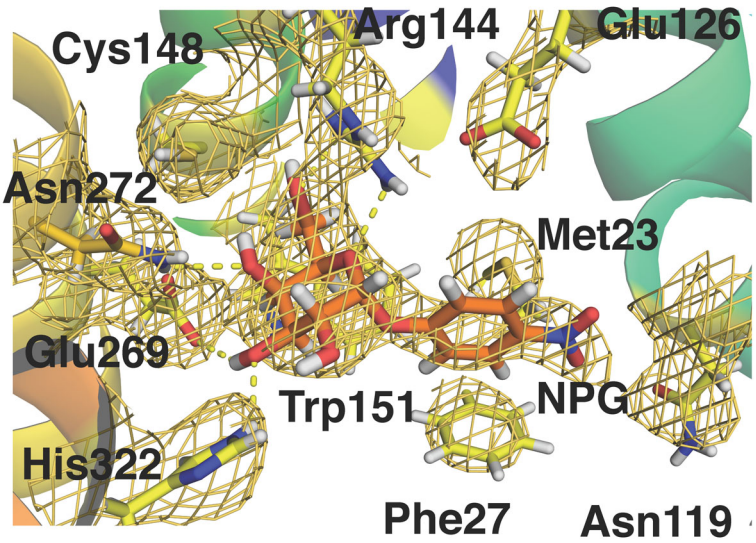
G380
T31
F32
N33
CDR1
S30
F29
loop 9-10
T310
S311
R57
G56
A58
G55
T54
TM12
Q379
W53
CDR2
R52
S249
loop 7-8
loop 7-8
F251
A252
TM7
N245
2.5 Å
S107
E108
A109
G105
Y106
G104
G103
2.6 Å
K42
TM2
N102
T110
G254
TM8
S111
T101
CDR3
G100
Q99
R98
S249
loop 7-8

Fig S4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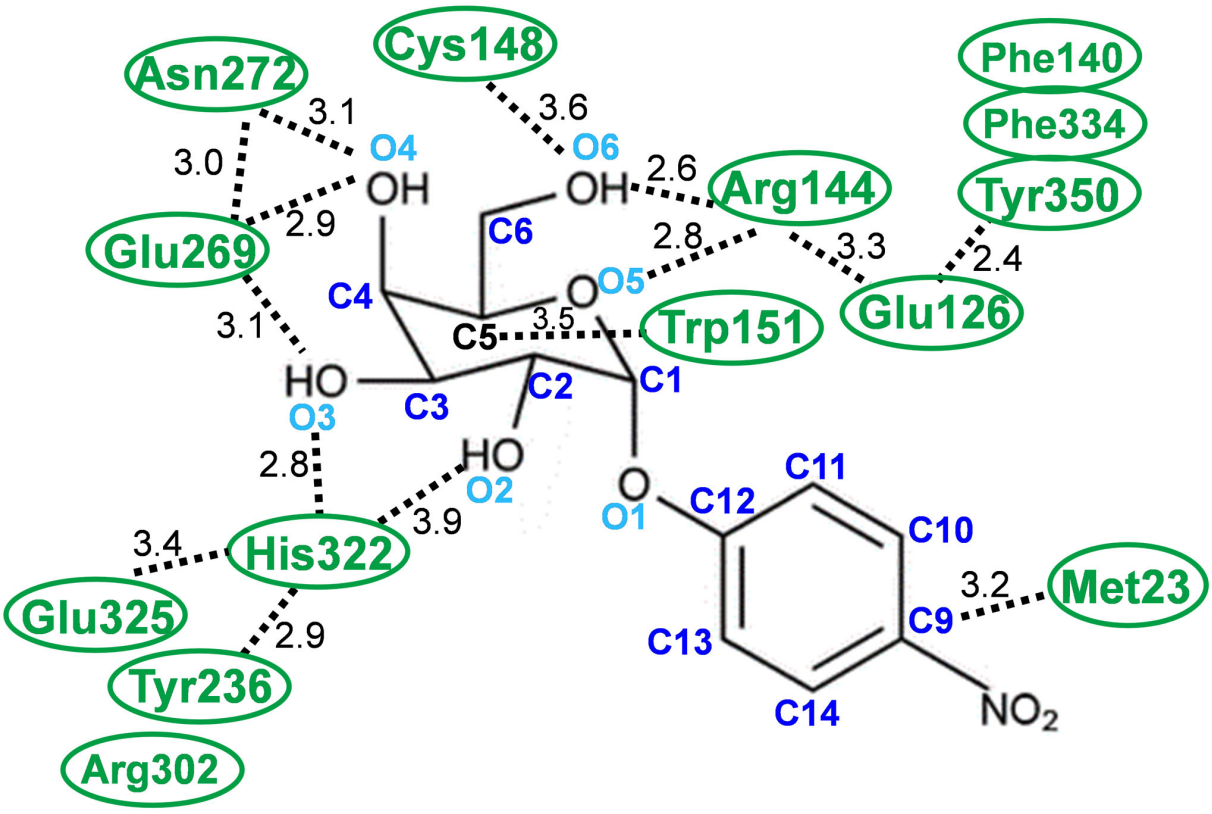
Asn272
Met23
Glu269
Trp151
NPG
His322
Phe27
Asn119

Fig S5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Phe334
Tyr350
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Glu269
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His322
Glu325
Tyr236
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3.6
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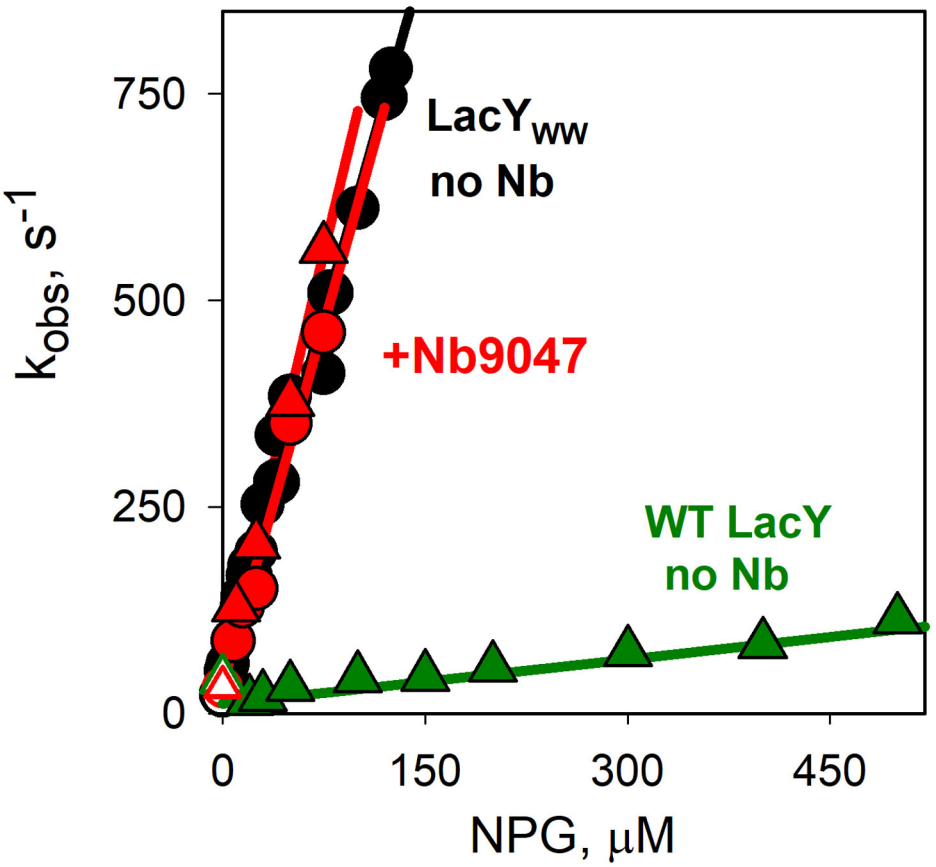
3.2
O4
O6
OH
OH
C6
C4
O
O5
C5
HO
C3
C2
C1
O3
HO
O2
O
O1
C11
C12
C10
C9
C13
C14
NO2

Fig S6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500
250
0
k_{obs}, s^{-1}
$LacY_{ww}$
no Nb
+Nb9047
WT LacY
no Nb
0
150
300
450
NPG, μM

Fig S7