Table S1. *E. coli* plasmids

Plasmid	Relevant features	Source
pDG680	*T. reesei* XynII AA 2-190 with C-terminal His_6 tag optimized for *E. coli* expression in pJexpress401	Wan *et al.* (in press)
pSBN44D	pDG680 modified with primers TrXE-N44D and TrXE-N44D-anti	Wan *et al.* (in press)
pSBN44H	pDG680 modified with primers TrXE-N44H and TrXE-N44H-anti	Wan *et al.* (in press)
pSBN44V	pDG680 modified with primers TrXE-N44V and TrXE-N44V-anti	This study
pSBV46L	pDG680 modified with primers TrXE-V46L and TrXE-V46L-anti	This study
pSBW18N/D20N	pDG680 modified with primers TrXE-W18N_D20N and TrXE-W18N_D20N-anti	This study
pSBA175L	pDG680 modified with primers TrXE-A175L and TrXE-A175L-anti	This study
pSBA175S	pDG680 modified with primers TrXE-A175S and TrXE-A175S-anti	This study
pSBA175V	pDG680 modified with primers TrXE-A175V and TrXE-A175V-anti	This study

Table S2. Oligonucleotide primers used for site-directed mutagenesis

Primer name	Sequence	Function
TrXE-W18N_D20N	ACGGCTACTTCTACTCCTACAATAATAACGGTCACGGTGGTGTCACC	W18N & D20N sense primer
TrXE-W18N_D20N-anti	GGTGACACCACCGTGACCGTTATTATTGTAGGAGTAGAAGTAGCCGT	W18N & D20N antisense primer
TrXE-N44D	ATTGGTCTAACAGCGGCGACTTTGTGGGTGGTAAG	N44D sense primer
TrXE-N44D-anti	CTTACCACCCACAAAGTCGCCGCTGTTAGACCAAT	N44D antisense primer
TrXE-N44H	ATTGGTCTAACAGCGGCCACTTTGTGGGTGGTAAG	N44H sense primer
TrXE-N44H-anti	CTTACCACCCACAAAGTGGCCGCTGTTAGACCAAT	N44H antisense primer
TrXE-N44V	AATTGGTCTAACAGCGGCGTCTTTGTGGGTGGTAAGGG	N44V sense primer
TrXE-N44V-anti	CCCTTACCACCCACAAAGACGCCGCTGTTAGACCAATT	N44V antisense
TrXE-V46L	TGGTCTAACAGCGGCAACTTTTTGGGTGGTAAGG	V46L sense primer
TrXE-V46L-anti	CCTTACCACCCAAAAAGTTGCCGCTGTTAGACCA	V46L antisense primer
TrXE-A175L	GTACTATGGATTACCAGATTGTGCTAGTTGAAGGTTATTTCTCTAGCGG	A175L sense primer
TrXE-A175L-anti	CCGCTAGAGAAATAACCTTCAACTAGCACAATCTGGTAATCCATAGTAC	A175L antisense primer
TrXE-A175S	TACTATGGATTACCAGATTGTGAGCGTTGAAGGTTATTTCTCTAGC	A175S sense primer
TrXE-A175S-anti	GCTAGAGAAATAACCTTCAACGCTCACAATCTGGTAATCCATAGTA	A175S antisense primer
TrXE-A175V	CTATGGATTACCAGATTGTGGTCGTTGAAGGTTATTTCTCTAG	A175V sense primer
TrXE-A175V-anti	CTAGAGAAATAACCTTCAACGACCACAATCTGGTAATCCATAG	A175V antisense primer
pTF	CTCGAAAATAATAAAGGGAAAATCAG	5’ sequencing primer
pTR	TGGTAGTGTGGGGACTC	3’ sequencing primer
5TrXynIIN	GGAGATAAAACATATGACGATC	5’ XynII primer
3TrXynIIN	CCGCGGATCCTTAGCTCACGGTAATGCTTGCGC	3’ XynII primer

Table S3. Data collection and refinement statistics.

Crystals	E177Q-X6	N44H-X3	*apo*-N44H	XynII-TrisX2-X3	*apo*-E177Q
Data Collection					
space group	$P2_122_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_1$	$P2_12_12_1$
unit cell dimensions(Å)	a=38.5 b=45.5 c=111.4	a=42.4 b=59.6 c=62.1	a=48.3 b=59.0 c=69.7	a=41.88 b=38.43 c=56.32	a=48.2 b=59.2 c=69.7
	α=β=γ=90º	α=β=γ=90º	α=β=γ=90º	β=105.9º	α=β=γ=90º
molecules per asu	1	1	1	1	1
wavelength (Å)	0.9793	0.9793	0.9793	1.5417	1.5417
resolution (Å)	20-1.15 (1.17-1.15)	20-1.55 (1.58-1.55)	20-1.10 (1.12-1.10)	40-1.65 (1.71-1.65)	20-1.5 (1.53-1.50)
unique reflections	63877 (2967)	23195 (1141)	81313 (3947)	19614 (1087)	32599 (2066)
redundancy	6.5 (6.9)[a]	3.8 (3.6)[a]	4.3(3.7)[a]	3.8 (2.1)[a]	3.7 (3.4)[a]
Completeness (%)	90.8 (84.2)[a]	98.5(99.7)[a]	99.8 (98.6)[a]	93.6 (52.2)[a]	99.6 (97.5)[a]
R_{sym}[b]	0.070 (0.622)[a]	0.099(0.521)[a]	0.051(0.629)[a]	0.062 (0.211)[a]	0.069 (0.408)[a]
$\langle I/\sigma(I)\rangle$	27.6 (3.4)[a]	13.8(2.7)[a]	26.7(1.9)[a]	14.9 (3.3)[a]	15.2 (2.8)[a]
Refinement					
R_{work}/R_{free} (%)[c]	11.1 (12.7)[a]	14.9 (17.9)[a]	11.9 (12.9)[a]	15.5 (21.9)[a]	17.1 (18.6)[a]
RMSD bond length/angle	0.012Å/1.485º	0.006Å/1.179º	0.007Å/1.315º	0.008Å/1.150º	0.006Å/1.160º
No. of atoms					
Protein	2882	1466	2959	1480	1478
water	202	256	260	248	222
xylooligomer	54	27	-	55	-
glycerol	29	-	-	-	-
citrate	12	-	-	-	-
iodide	-	-	3	-	6
calcium	-	-	-	1	-
Mean B value ($Å^2$)					
main chain/ side chain	7.7/11.7	15.4/16.8	10.4/13.0	12.6/15.1	8.9/10.2
water/xylose	25.4/11.7	27.4/15.8	19.2/-	28.5/17.9	20.8/-
Ramachandran Statistics (%)					
most favorable	97.9	97.3	98.5	89.6	97.9
allowed	2.1	2.7	1.5	10.4	2.1
disallowed	0	0	0	0	0
PDB ID	4HK8	4HK9	4HKL	4HKW	4HKO

[a] Numbers in parenthesies represent values in the highest resolution shell.

[b] $Rsym=\mathbf{\Sigma}(|Ii - \langle I\rangle|)/\mathbf{\Sigma}(Ii)$, where Ii is the measured intensities and $\langle I\rangle$ is the mean intensity of all measured observations equivalent to reflection Ii.

[c] $Rwork=\mathbf{\Sigma}||Fobs| - |Fcalc||/\mathbf{\Sigma}|Fobs|$, where |Fobs| are the observed diffraction amplitude, |Fcalc| is the corresponding calculated structure factor amplitude. Rfree is defined by Rwork, but involved 5% of the measured reflections not used in refinement and set aside for cross-validation purpose.

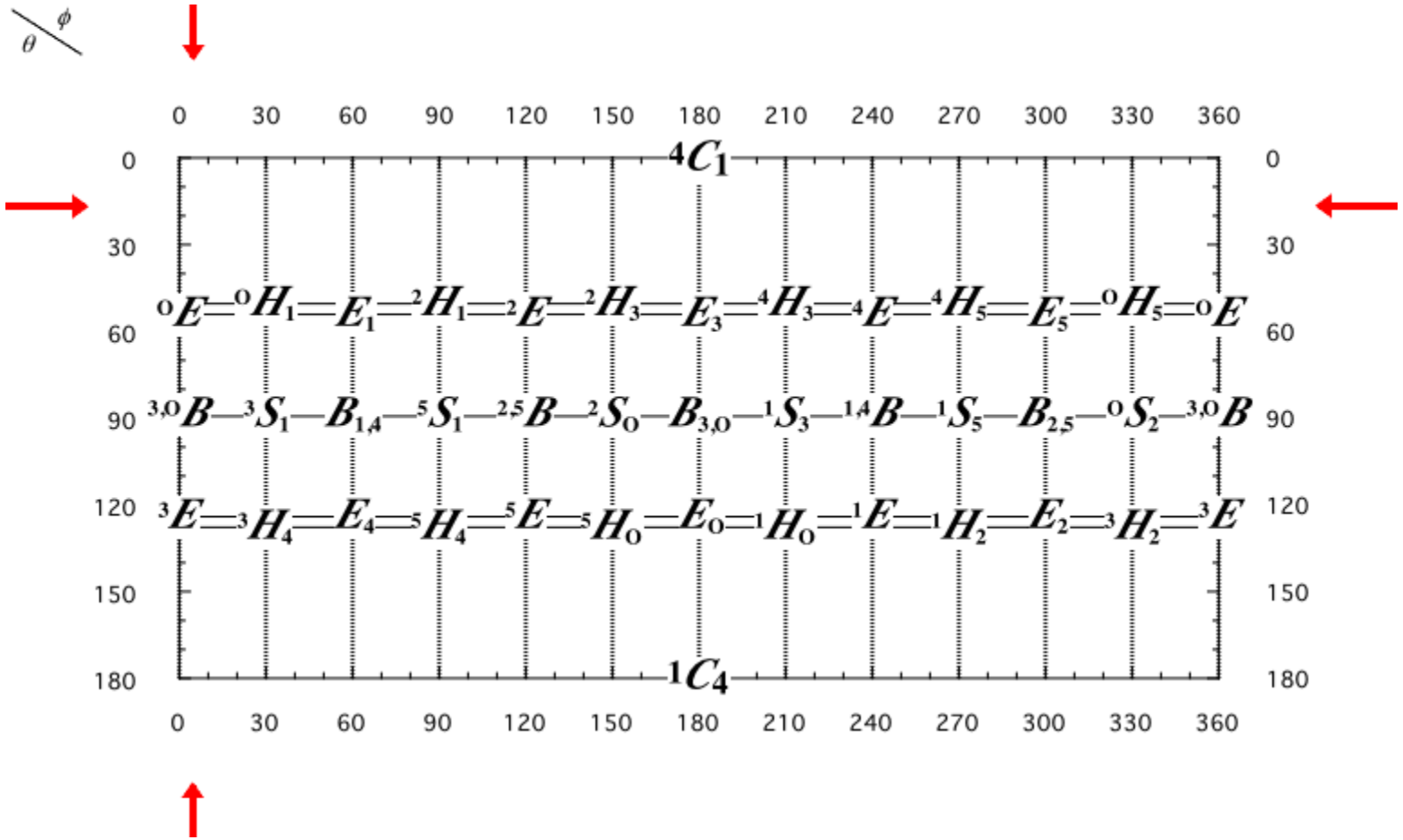

Figure S1. Mercator plot of the xylose unit -1 conformation in Q177N-X6 Michaelis complex showing the departure from $^{4}C_{1}$ conformation towards ^{O}E conformation.

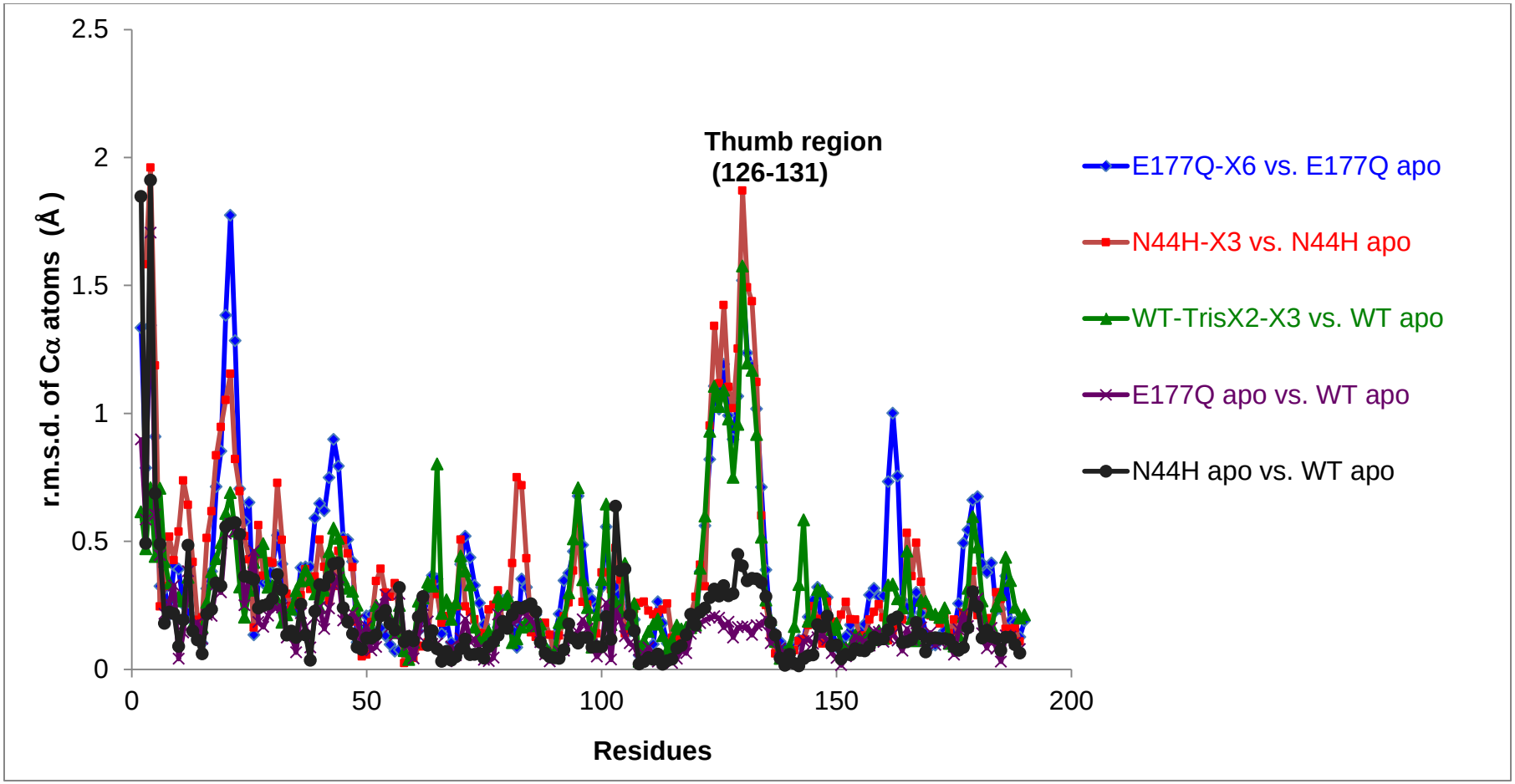

Figure S2. The root mean square deviation (RMSD) of the main-chain atoms between different structures studied.

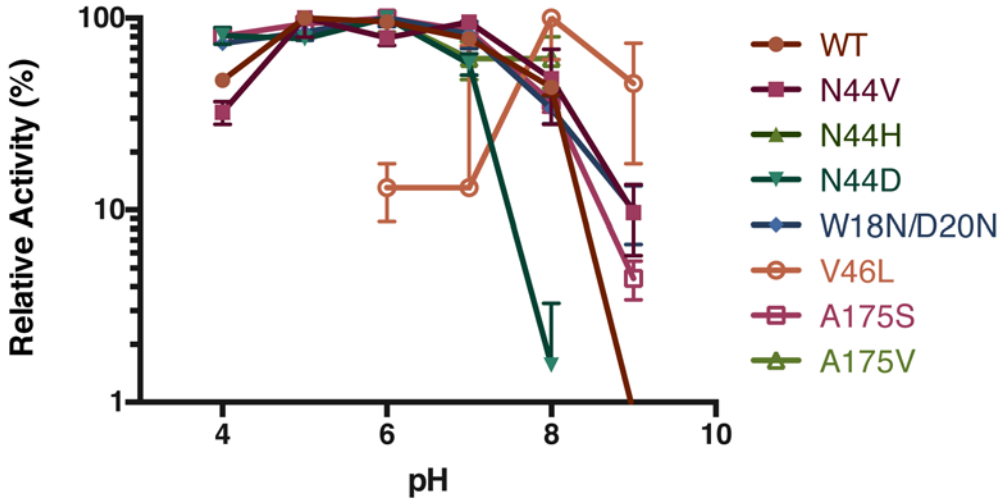

Figure S3. **(a)** Specific xylan hydrolytic activity of WT and variant XynII proteins at the indicated pH values are shown relative to maximum activities at acidic pH.

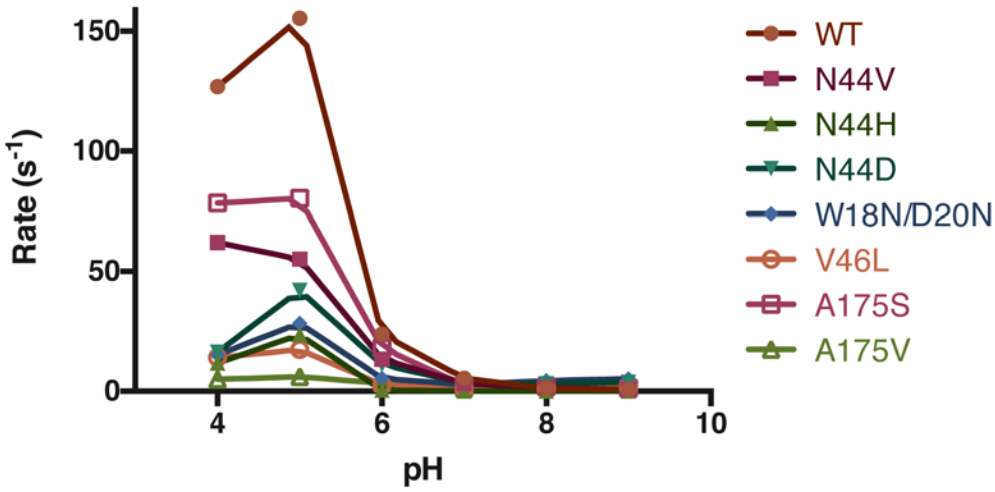

Figure S3. **(b)** Rates of p-nitrophenylxylobioside (PNPX2) hydrolytic activity were measured in continuous assays at the indicated pH values using WT and variant XynII proteins.

Figure S4. Structural comparison between ligand-free E177Q (green) and E177Q-X6 (red) complexes. The most significantly different region is highlighted in a dashed circle.

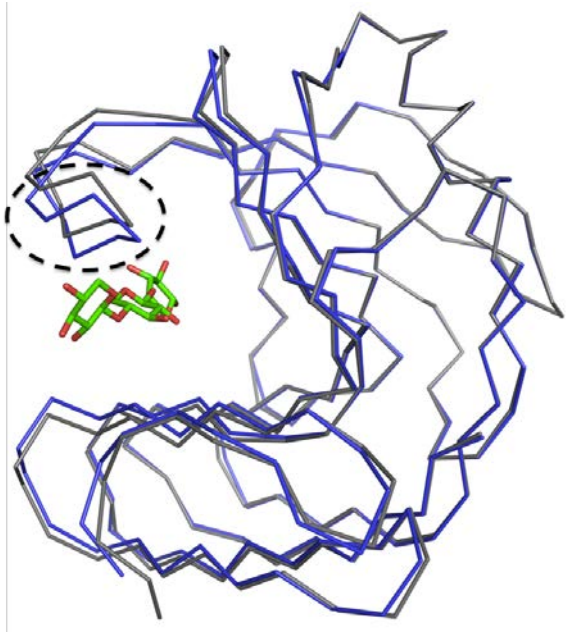

Figure S5. Structural comparison between ligand-free N44H (gray) and N44H-X3 (blue) complexes. The most significantly different region is highlighted in a dashed circle.

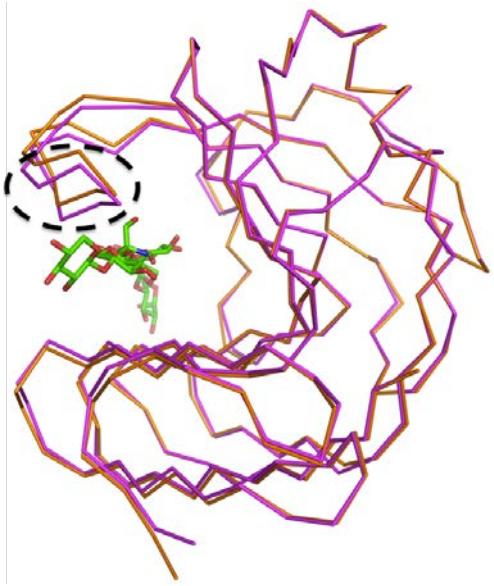

Figure S6. Structural comparison between ligand-free WT (orange) and WT-TrisX2-X3 (magenta) complexes. The most significantly different region is highlighted in a dashed circle.

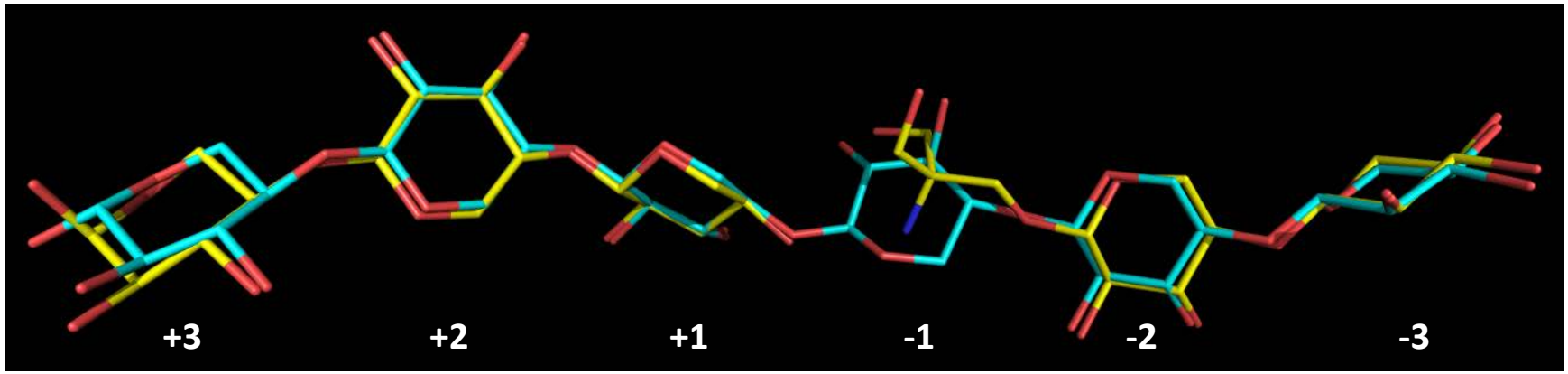

Figure S7. Superposition of sugar units of X6 and TrisX2-X3. Ligands are colored by atom type, X6 with cyan carbon atoms and Tris-X2-X3 with yellow carbon atoms.

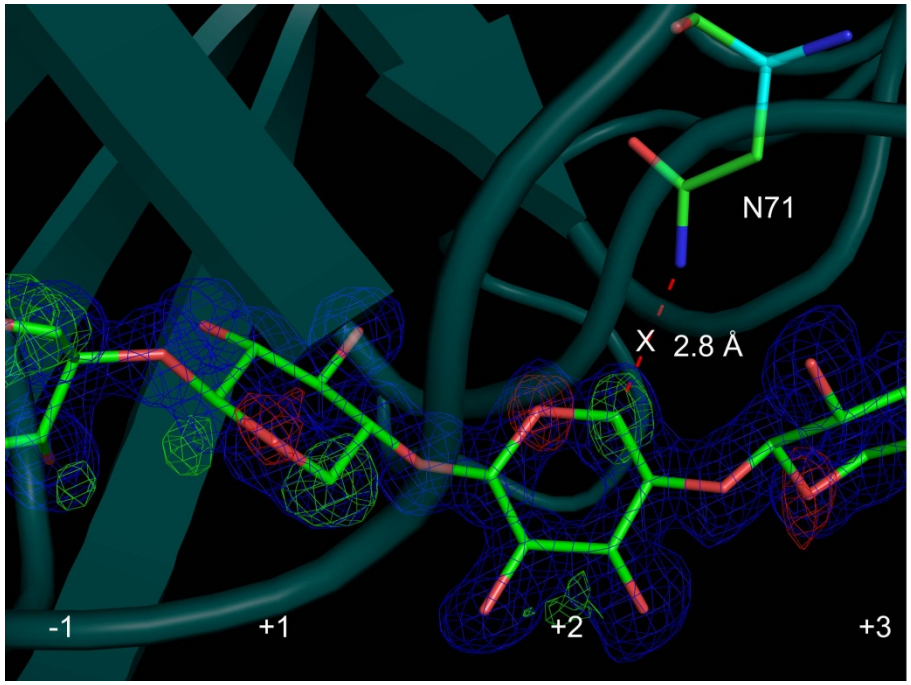

Figure S8. $F_O - F_C$ difference electron density map (contoured at the 3σ level) shows that the reversed orientation of X6 is not correct. The positive density is green, while the negative density is red. The distance between C5 of the -2 xylose and ND2 atom of N71 would be only 2.8 Å in the incorrect oligosaccharide orientation; such an interaction would be repulsive and should not occur, as shown by the X.