Supporting Information

Engineering the substrate preference of glucose oxidase for the enzymatic oxidation of xylose

Yue Wang,‡[a] Xueting Cao,‡[b] Shanshan Jiang,[a] Liwei Gao,[c] Xiaolong Han,[d] Jingyao Qu,[a] Xukai Jiang,[b] Guodong Liu,*[a,b] and Yinbo Qu[a,b]

[a] *State Key Laboratory of Microbial Technology, Shandong University, Qingdao 266237, China*
[b] *National Glycoengineering Research Center, Shandong Key Laboratory of Carbohydrate Chemistry and Glycobiology, NMPA Key Laboratory for Quality Research and Evaluation of Carbohydrate-Based Medicine, Shandong University, Qingdao 266237, China*
[c] *Tobacco Research Institute of Chinese Academy of Agricultural Sciences, Qingdao 266101, China*
[d] *School of Life Science and Bioengineering, Jining University, Jining 273155, China*

‡Equal contributors. These authors contributed equally.
*Corresponding Author. Email: gdliu@sdu.edu.cn (G. Liu)

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Supplemental data

Coding sequence of wild-type *Aspergillus niger* glucose oxidase used in this study (mature protein, codon optimized for *Pichia pastoris*):

TCTAATGGTATTGAAGCTTCTTTGTTGACTGATCCAAAAGATGTTTCTGGTAGAACTGTT
GATTATATTATTGCTGGTGGAGGTTTGACTGGTTTGACTACTGCTGCTAGATTGACTGAA
AATCCAAATATTTCTGTTTTGGTTATTGAATCTGGTTCTTATGAATCTGATAGAGGTCCA
ATTATTGAAGATTTGAATGCTTATGGTGATATTTTTGGTTCTTCTGTTGATCATGCTTATG
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TCTTGGGAAACTGTTTTTGGTAATGAAGGTTGGAATTGGGATAATGTTGCTGCTTATTCT
TTGCAAGCTGAAAGAGCTAGAGCTCCAAATGCTAAACAAATTGCTGCTGGTCATTATTT
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TGATTATTCTCCAATTGTTAAAGCTTTGATGTCTGCTGTTGAAGATAGAGGTGTTCCAAC
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ATGTTATGACTGTTTTTTATGCTATGGCTTTGAAAATTTCTGATGCTATTTTGGAAGATTA
TGCTTCTATGCAATAA

Table S1. Primers used in this study.

Primer	Sequence (5'-3')	Note
Sc Alpha-m+GOx-Pp F	TCTAGAACTAGTGGATCCCCCATGAGATTTCCTTCAATTTTTAC	Amplification of *Angox* sequence upstream of T110 codon
T110-R	ACCACCATTAACCAAAGTAGAACCACCCAAACCATTACCAG	
T110-F	CTACTTTGGTTAATGGTGGTNNKTGGACTAGACCACATAAAGC	Amplification of *Angox* sequence downstream of T110 codon
Sc GOx-Pp+Alpha-m R	ACTAATTACATGAGGGCCCCCCCTTATTGCATAGAAGCATAATC	
F414-R	CAATTCAGAATAAGCAACATTGTGATTGACGATCCAATCTCTA	Amplification of *Angox* together with Sc Alpha-m+GOx-Pp F
F414-F	CAATGTTGCTTATTCTGAATTGNNKTTGGATACTGCTGGTGTTG	Amplification of *Angox* together with Sc GOx-Pp+Alpha-m R
Pp-GOx-Pp F	AGGCTGAAGCTTACGTAGAATTCTCTAATGGTATTGAAGCTTCTTTG	Amplification of *Angox* sequence upstream of T110V codon
T110V R	CTTTATGTGGTCTAGTCCAAACACCACCATTAACCAAAGTAG	
T110V F	CTACTTTGGTTAATGGTGGTGTTTGGACTAGACCACATAAAG	Amplification of *Angox* sequence downstream of T110V codon
Pp-GOx-Pp R	TTAATTCGCGGCCGCCCTAGGGTTATTGCATAGAAGCATAATC	
F414L R	GCAACACCAGCAGTATCCAACAACAATTCAGAATAAGCAAC	Amplification of *Angox* together with Pp-GOx-Pp F
F414L F	GTTGCTTATTCTGAATTGTTGTTGGATACTGCTGGTGTTGC	Amplification of *Angox* together withPp-GOx-Pp R

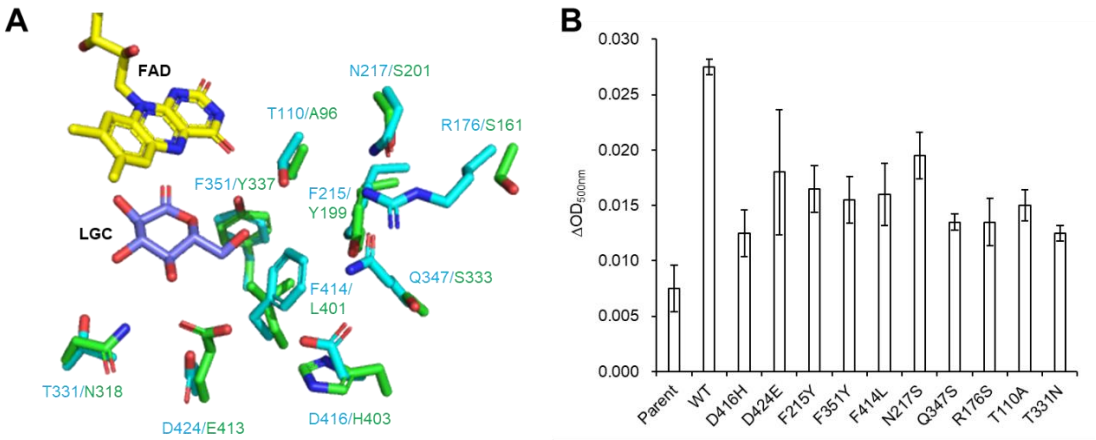

Fig. S1. Mutagenesis of AnGOx based on the comparison with AfGDH. (A) Amino acid variations between AnGOX (cyan) and AfGDH (green) around the substrate-binding pocket. (B) $\Delta OD_{500\ nm}$ values in the D-xylose oxidase assay of the culture supernatants of *Saccharomyces cerevisiae* strains expressing AnGOx mutants. Data represent the mean ± SD from duplicate cultivations.

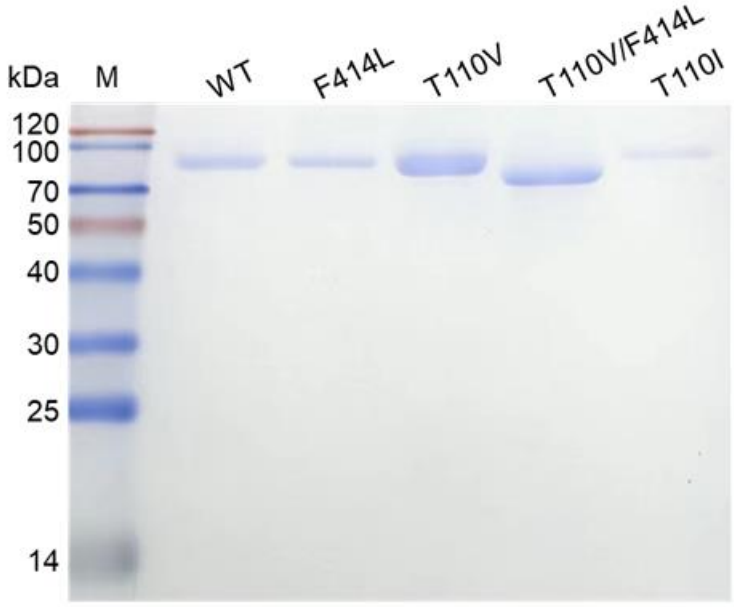

Fig. S2. SDS-PAGE analysis of purified enzymes.

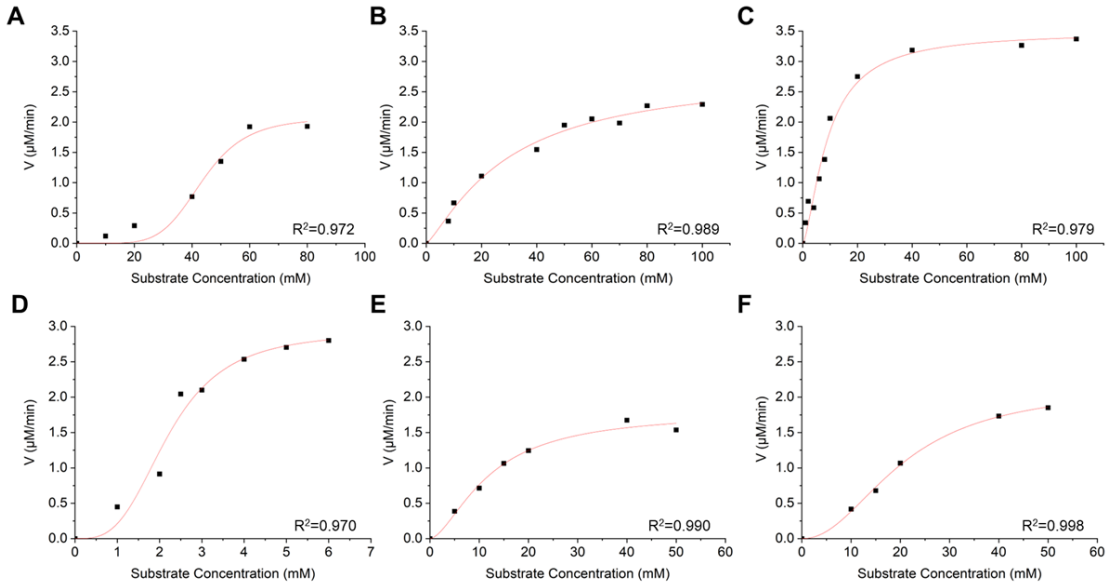

Fig. S3. Plots for the determination of kinetic parameters of enzymes. The Hill equation was used to fit the plots. (A–C) WT, T110V and T110V/F414L on D-xylose, respectively. (D–F) WT, T110V and T110V/F414L on D-glucose, respectively.

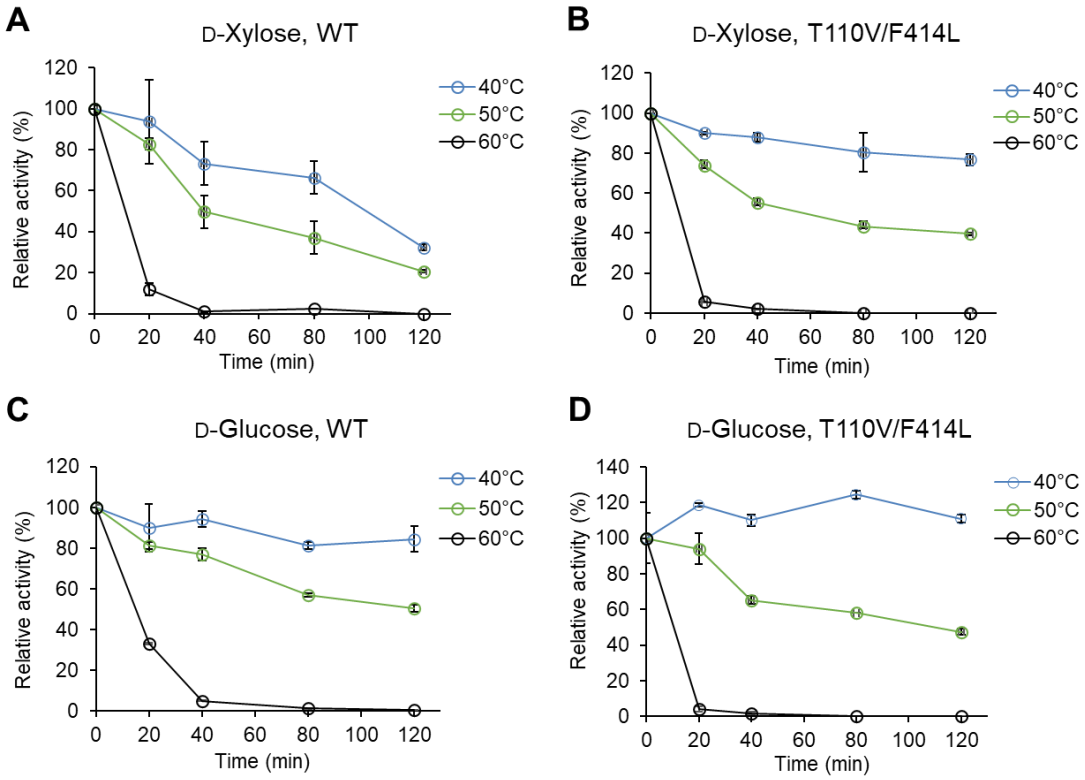

Fig. S4. Thermostabilities of AnGOx (WT) and the T110V/F414L mutant. The purified proteins were incubated at different temperatures for the indicated time, and the activities towards D-xylose (A, B) and D-glucose (C, D) were measured at pH 6.0 and 35 °C. Data represent the mean ± SD from duplicate measurements.

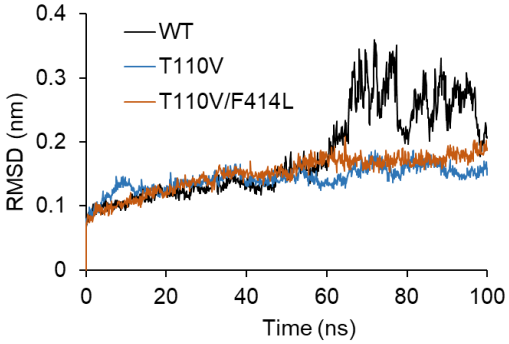

Fig. S5. Time evolution of RMSD for AnGOx, T110V, and T110V/F414L with D-xylose.

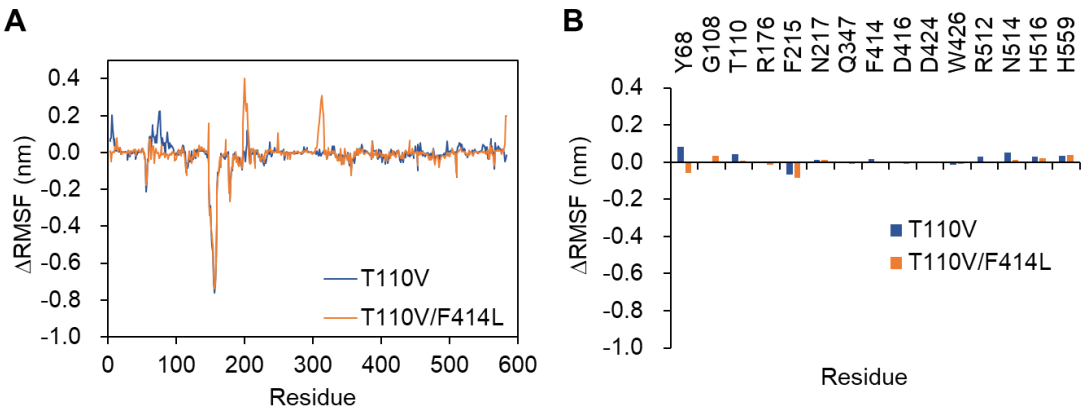

Fig. S6. Changes in the RMSF values of all residues (A) and active-site residues (B) of T110V and T110V/F414L relative to the wild-type AnGOx. The data were analyzed using the simulations with D-xylose.

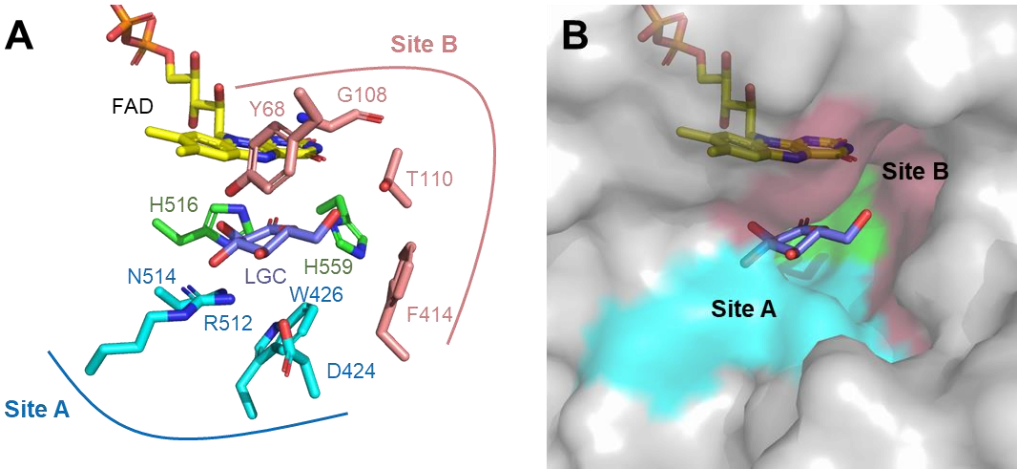

Fig. S7. Front view of the substrate pocket of AnGOX. The D-glucose oxidation product LGC was placed in the model by superimposing the structure of AnGOx (PDB: 1CF3) onto that of the AfGDH-LGC complex (PDB: 4YNU). Sites A (cyan) and B (pink) defined in the text are shown. (A) Stick representation. (B) Surface representation.

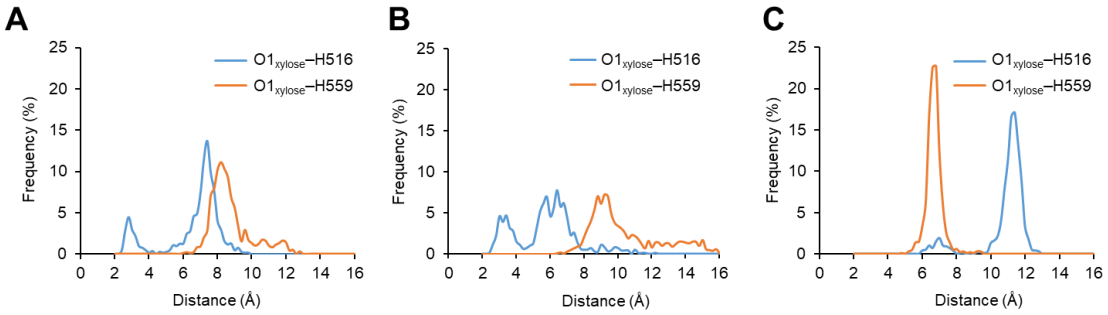

Fig. S8. Distribution of distances between the O1 atom of D-glucose and catalytic histidine residues of AnGOx (A), T110V (B), and T110V/F414L (C), during MD simulations.

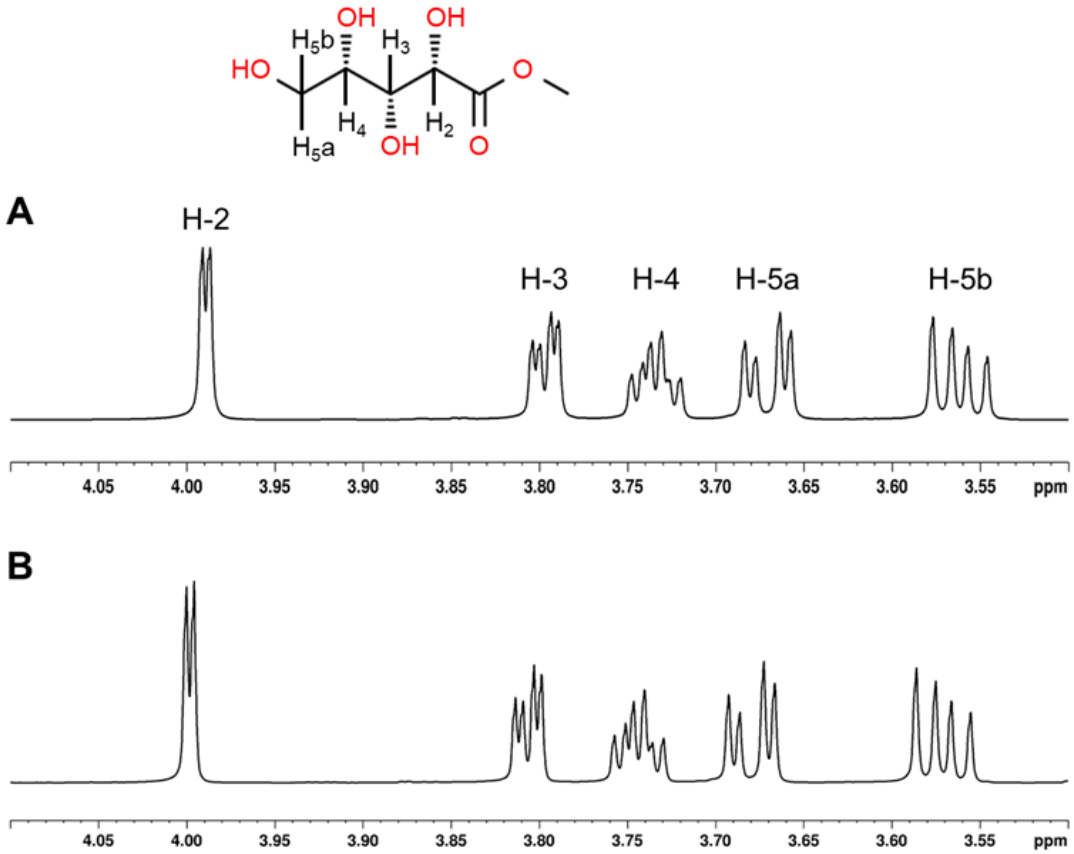

Fig. S9. ^{1}H NMR spectra of commercial lithium D-xylonate as a standard (A) and purified D-xylonate product (B).