Thermostablility Improvement of a *Streptomyces* Xylanase by Introducing Proline and Glutamic Acid Residues

Kun Wang,[1§] Huiying Luo,[1§*] Jian Tian,[2] Ossi Turunen,[3] Huoqing Huang,[1] Pengjun Shi,[1] Huifang Hua,[1] Caihong Wang,[1] Shuanghe Wang,[1] and Bin Yao[1*]

Key Laboratory for Feed Biotechnology of the Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, People's Republic of China, [1] *Biotechnology Research Institute, Chinese Academy of Agricultural sciences, Beijing 100081, People's Republic of China,* [2] *and Department of Biotechnology and Chemical Technology, Aalto University, Aalto FI-00076, Finland* [3]

Running title: Engineering Xylanase for Improved Thermostablility

[§] K.W. and H.L. contributed equally to this paper.

*Corresponding authors. Mailing address: Key Laboratory for Feed Biotechnology of the Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences, No. 12 Zhongguancun South Street, Beijing 100081, People's Republic of China. Phone: 86-10-82106053. Fax: 86-10-82106054. E-mail: binyao@caas.cn; luohuiying@caas.cn.

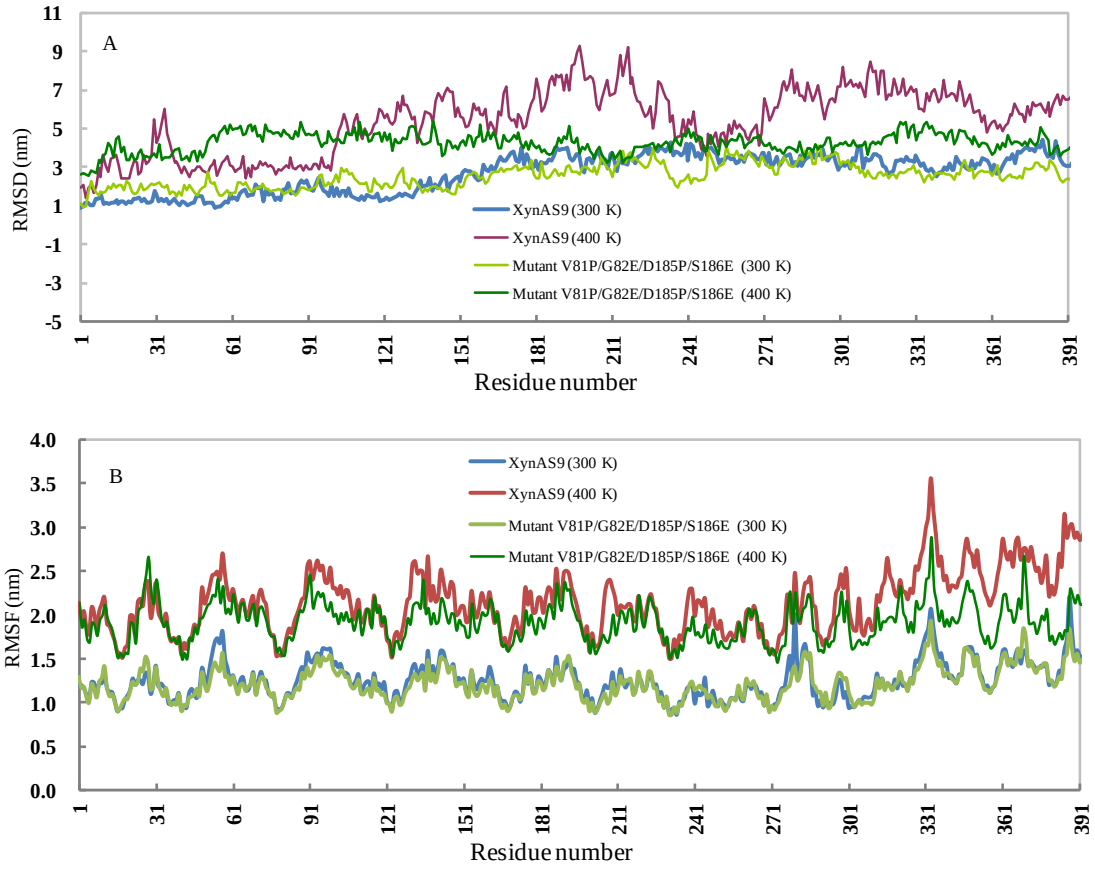

FIG S1 Molecular dynamic simulations (MDS) of XynAS9 and its mutant V81P/G82E/D185P/S186E with NAMD at 300 K and 400 K, respectively. (A) RMSD values during a 20-ns MDS. (B) RMSF values calculated over the last 5 ns.

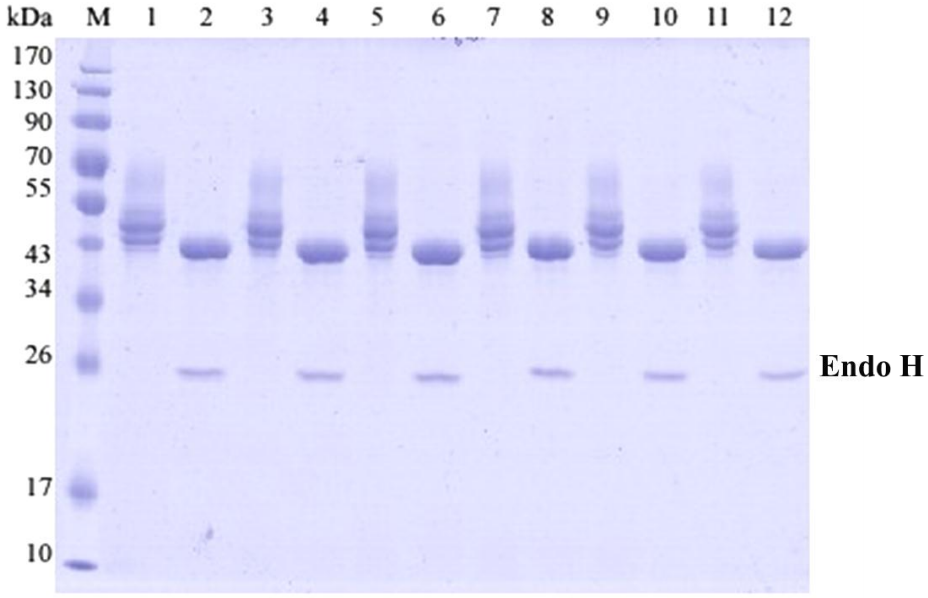

FIG S2 SDS-PAGE analysis of purified and deglycosylated XynAS9 and its mutants. Lanes 1, 3, 5, 7, 9, and 11: purified XynAS9, and mutants V81P, G82E, V81P/G82E, D185P/S186E and V81P/G82E/D185P/S186E, respectively; lanes 2, 4, 6, 8, 10, and 12: deglycosylated XynAS9, and mutants V81P, G82E, V81P/G82E, D185P/S186E and V81P/G82E/D185P/S186E, respectively.

FIG S3 Structural changes between wild-type XynAS9 (A) and mutants V81P/G82E (B) and V81P/G82E/D185P/S186E (C) at 400 K based on MDS analysis with beams of 1 ns, 2 ns, and 5 ns, respectively.

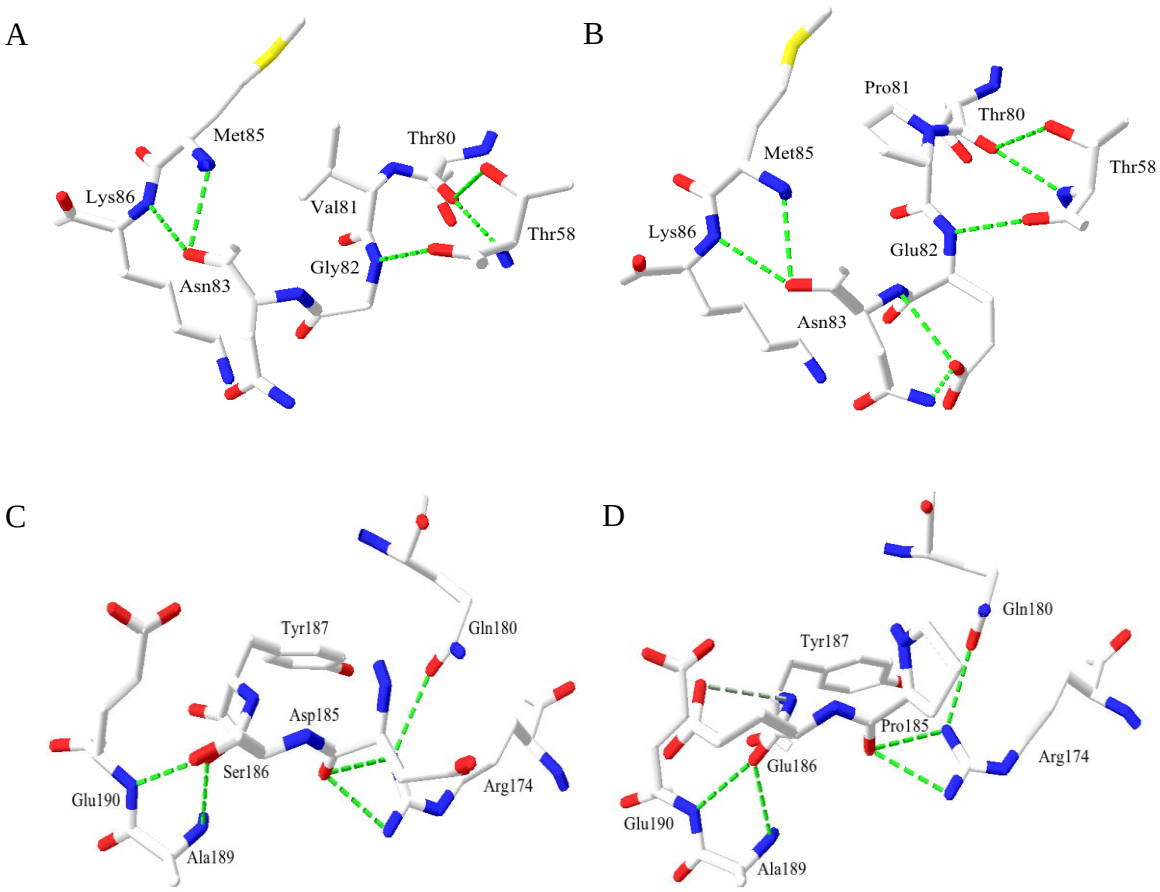

FIG S4 Putative hydrogen bonds in wild-type XynAS9 (A and C) and its mutants V81P/G82E (B) and D185P/S186E (D) predicted using Swiss-PdbViewer 4.0.4. Hydrogen bonds are shown in green dashed lines.

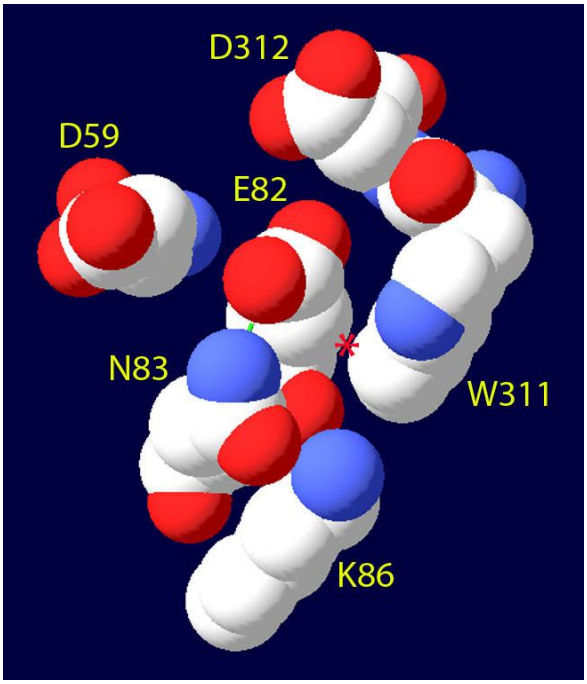

FIG S5 Interactions of mutant G82E with other surrounding residues predicted by Swiss-PdbViewer 4.0.4. The atom oxygen, nitrogen and carbon is colored red, blue, and white, respectively, which may form hydrophobic interactions. The potential hydrogen bond between E82 and N83 is shown in green dashed line. The hydrophobic interaction area between the side chain groups of E82 and W311 is indicated by a star.

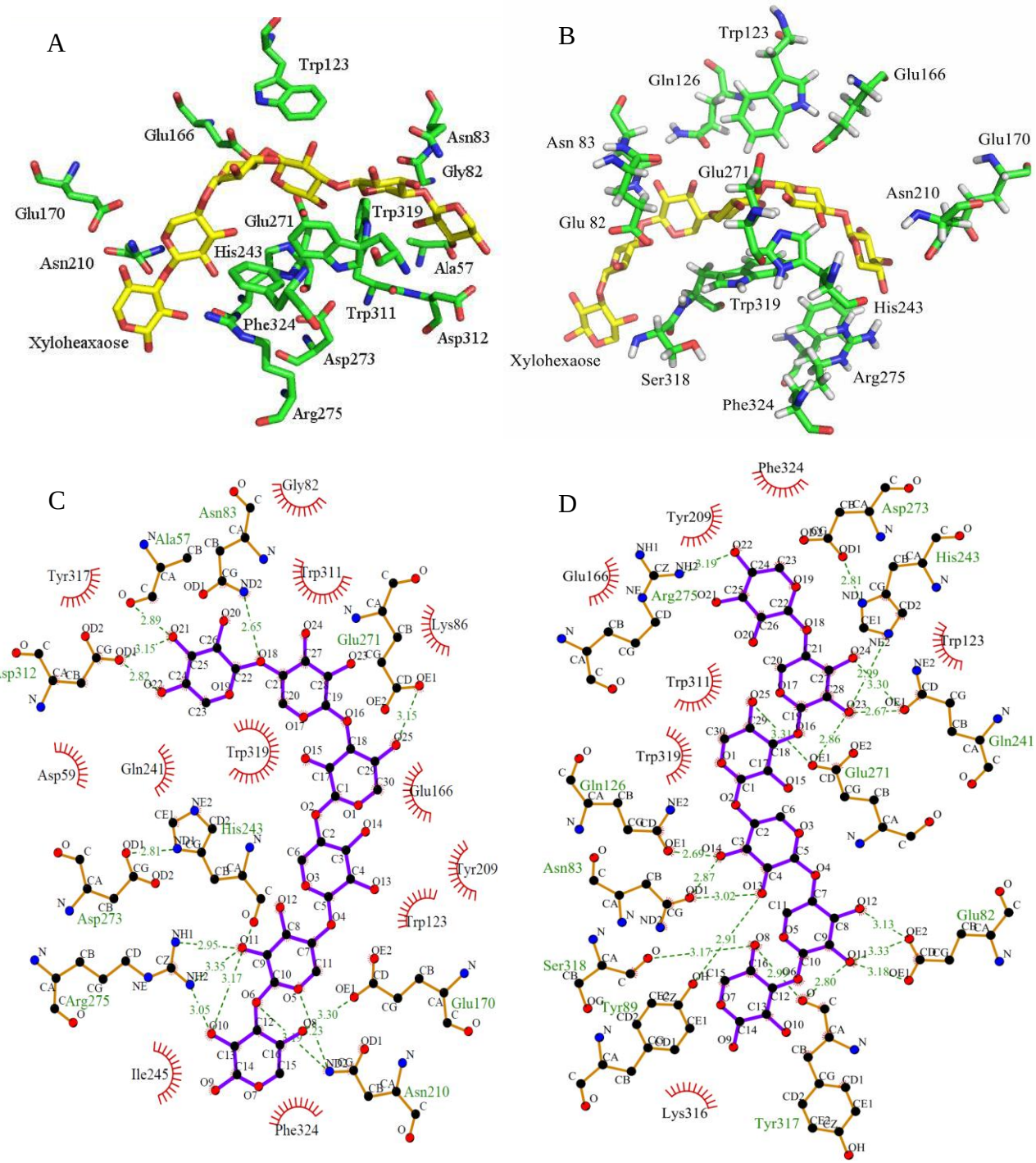

FIG S6 Docking results of xylohexaose with XynAS9 (A and C) and mutant V81P/G82E (B and D). Green dashed lines in Ligplot illustrate the putative hydrogen bonds.

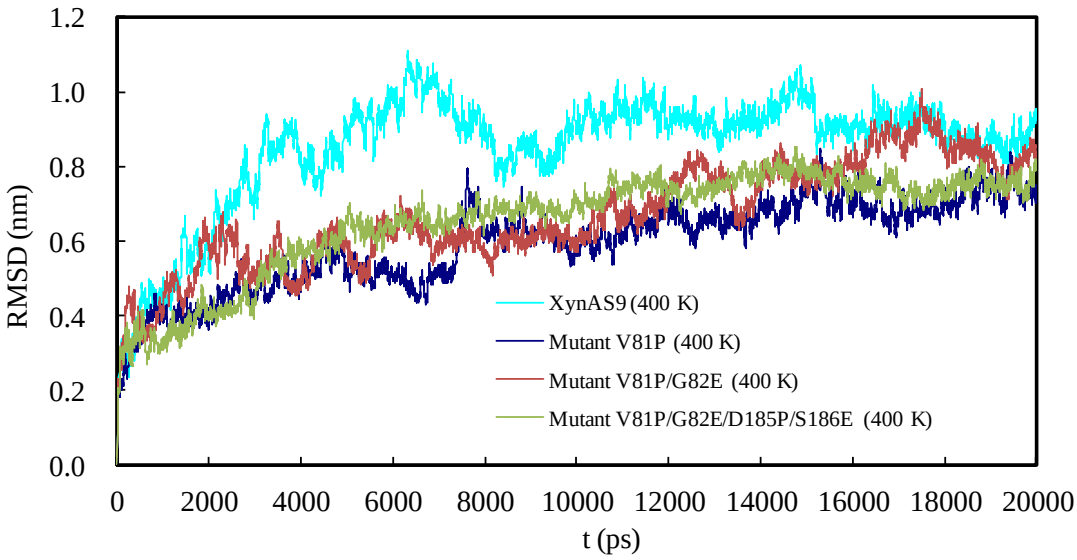

FIG S7 MDS analysis of XynAS9 and its mutants with Gromacs at 400 K.

TABLE S1 Primers used in this study.

Enzyme	Primer sequences (5'→3')[a]
XynAS9	TAGAATTCGACACCGCCACCCTGGGCGAACT
	TATGCGGCCGCCTACGCCGAAGTCCCGGACGGC
Mutant V81P	GCCAGATCACCCCCGGCAACACCATGAAGT
	ACTTCATGGTGTTGCCGGGGGTGATCTGGC
Mutant G82E	GCCAGATCACCGTCGAAAACACCATGAAGT
	ACTTCATGGTGTTTTCGACGGTGATCTGGC
Mutant V81P/G82E	GCCAGATCACCCCCGAAAACACCATGAAGT
	ACTTCATGGTGTTTTCGGGGGTGATCTGGC
Mutant D185P/S186E	AGAAGATCGGCCCCGAGTACATCG
	CGATGTACTCGGGGCCGATCTTCT

[a] The restriction sites *Eco*RI and *Not*I and mutation sites are underlined, respectively.