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Supporting information for article:

A GH13 α-glucosidase from *Weissella cibaria* uncommonly acts on short-chain maltooligosaccharides

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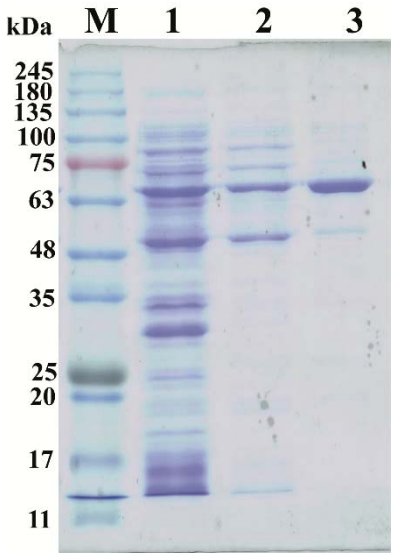

Figure S1 SDS-PAGE analysis of purified *Wc*AG. Lane: M is protein molecular weight marker, lane: 1 is a crude protein from *E. coli* BL21 (DE3) harboring pET*Wc*AG, lane: 2 and lane: 3 are pooled protein fraction with enzyme activity from DEAE and phenyl column, respectively.

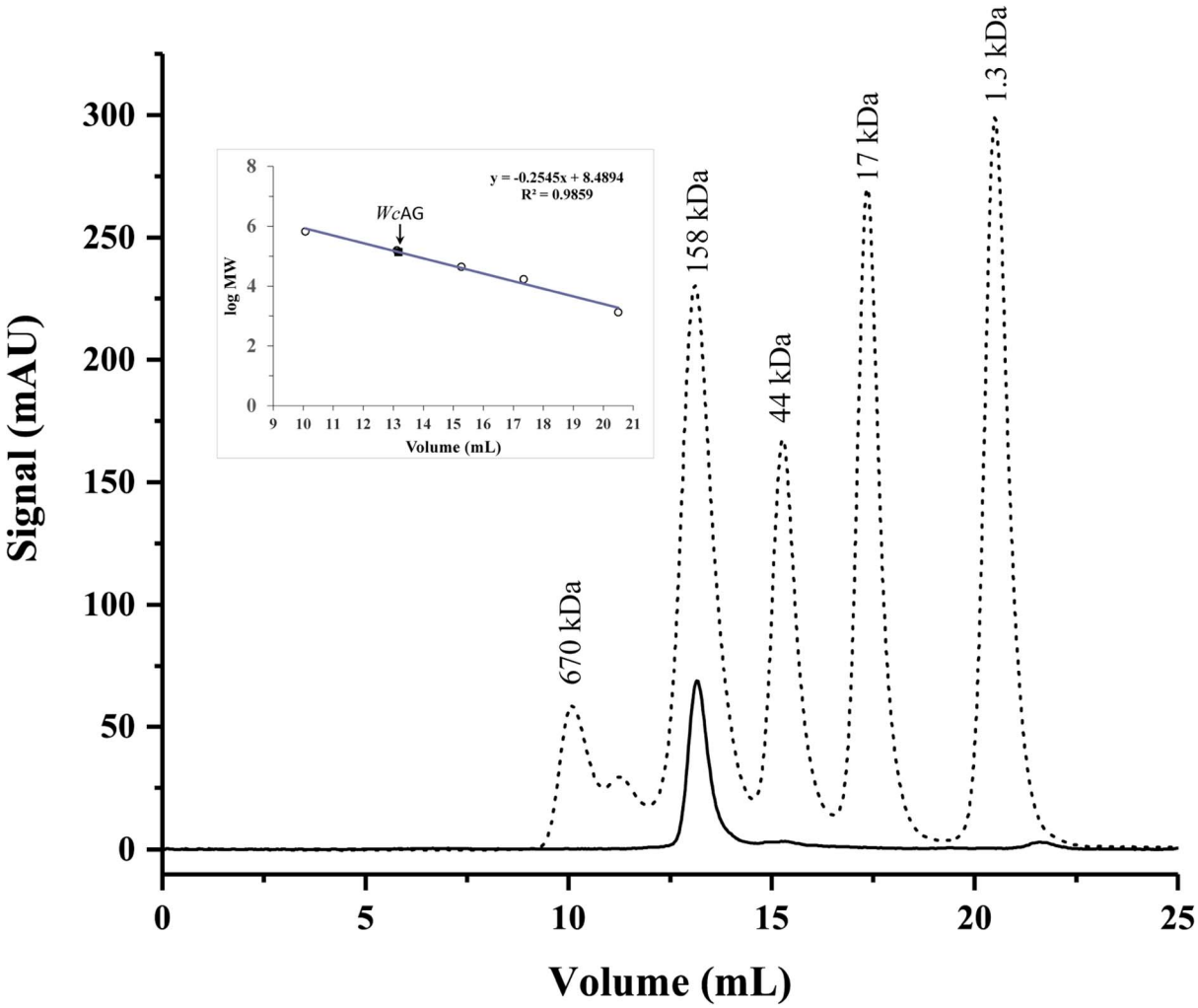

Figure S2 Size-exclusion chromatography. Purified *Wc*AG was analysed by size-exclusion chromatography using Superdex200 column. The system was run in 50 mM phosphate buffer pH 7.4 and 150 mM NaCl at 4 °C with a flowrate 1 mL/min. The solid line represents chromatogram of *Wc*AG, while the dashed line is the elution profile of a protein standard mixture (Bio-Rad).

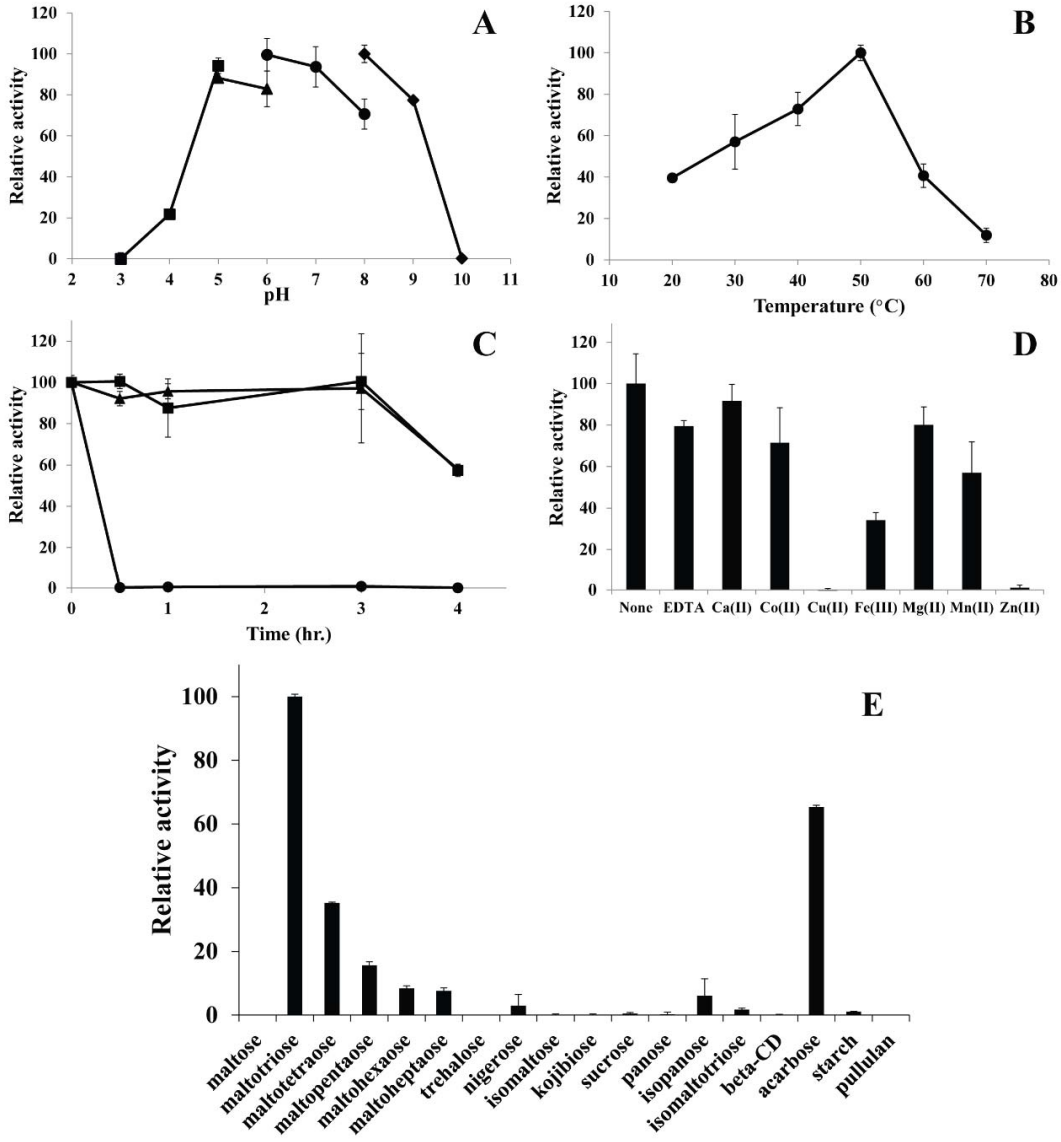

Figure S3 Characterisation of *Wc*AG. (A) Effect of pH on *Wc*AG activity. Buffer used: 50 mM citrate buffer pH 3-5 (■), 50 mM acetate buffer pH 5-6 (▲), 50 mM phosphate buffer pH 6-7 (●) and 50 mM glycine buffer pH 8-10 (ϕ) at 40°C. (B) Effect of temperature on *Wc*AG activity. The reactions were carried out in 50 mM phosphate buffer pH 6 at 20-70°C. (C) Thermostability of *Wc*AG. The enzyme was incubated in 50 mM phosphate pH 7.4 at (ϕ) 30, (■) 40, (●) 50°C and taken at each time point for activity assays in 50 mM phosphate buffer pH 6 at 50 °C. (D) Effect of metal ions on *Wc*AG activity. The reactions were performed in 50 mM acetate buffer pH 6, in the presence of EDTA or metal ions at 10 mM. (E) Substrate specificity of *Wc*AG. The enzyme was incubated with 30 mM of maltose, maltotriose, maltotetraose, maltopentaose, maltohexose, maltoheptaose, trehalose, nigerose, isomaltose, kojibiose, sucrose, panose, isopanose, isomaltotriose, acarbose, β-cyclodextrin (β-CD) 0.6% (w/v) starch and pullulan and was then assayed by glucose oxidase assay.

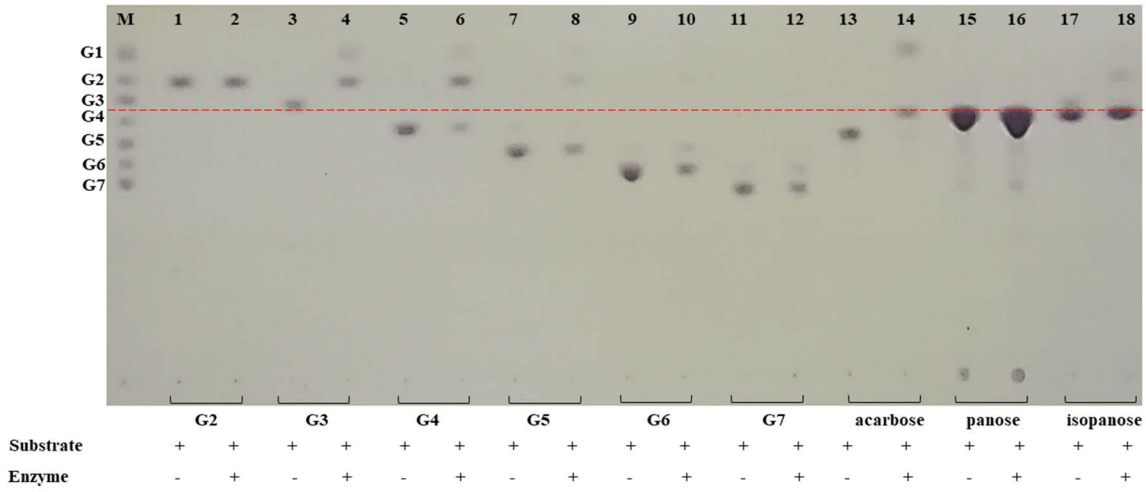

Figure S4 TLC analysis of oligosaccharide digestion. Lane: M is standard malto-oligosaccharides (G1-G7). Lane: 1, 3, 5, 7, 9, 11, 13, 15, and 17 are maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, maltoheptaose, acarbose, panose, and isopanose standards. Lane: 2, 4, 6, 8, 10, 12, 14, 16, and 18 are 30 mM maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, maltoheptaose, acarbose, panose, and isopanose incubated with 10 U/mL *Wc*AG for 30 min at 50 °C in 50 mM phosphate buffer pH 6. The red dash line indicates the positon of maltotriose.

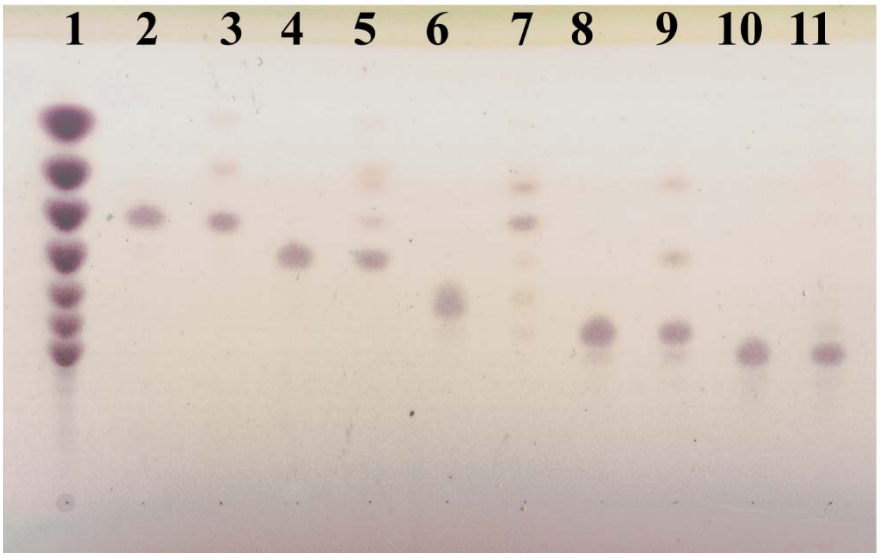

Figure S5 Oligo-pullulan digestion by *Wc*AG on TLC. Lane: 1 is standard malto-oligosaccharides (G1-G7), Lane: 2, 4, 6, 8 and 11 are tri, tetra, penta, hexa and heptasaccharride of pullulan hydrolysate. Lane: 3, 5, 7, 9, 11 are tri, tetra, penta, hexa and heptasaccharride of pullulan hydrolysate, incubated with 10 U/mL *Wc*AG in 50 mM phosphate buffer pH 6 at 50 °C for 30 min.

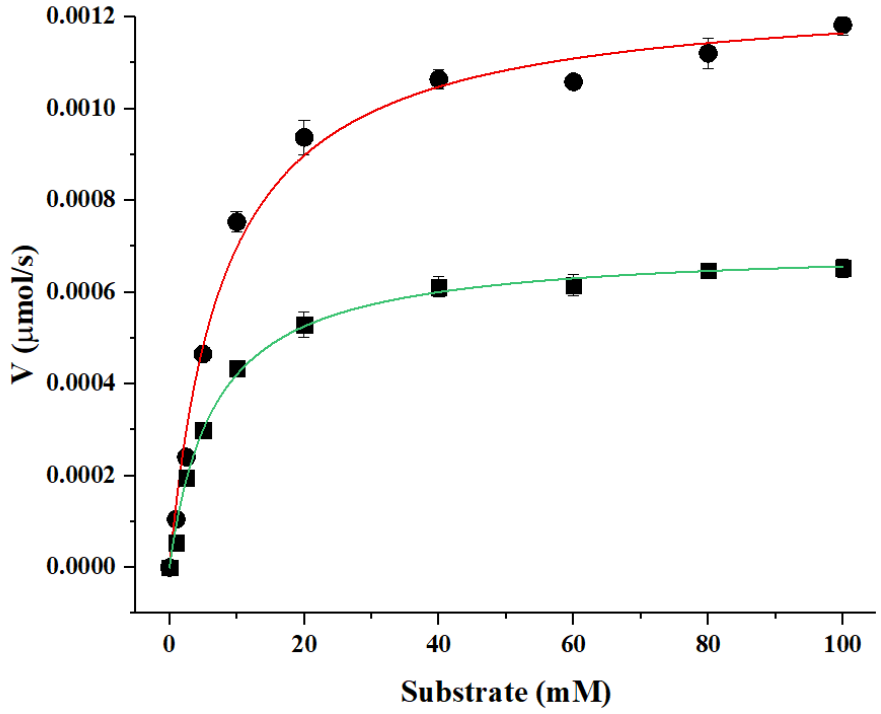

Figure S6 Kinetic study of *Wc*AG. Steady-state reactions were performed as described in Materials and Methods. The red line represents the best fit to Michaelis-Menten equation when *Wc*AG were incubated with maltotriose, giving k_{cat} = 363.4 ± 6 s^{-1} and K_M = 8.0 ± 0.6 mM. The green line was a plot of the observed rates of *Wc*AG against concentration of acarbose, resulting in Michaelis-Menten parameters as k_{cat} = 201.8 ± 3 s^{-1} and K_M = 6.6 ± 0.5 mM.

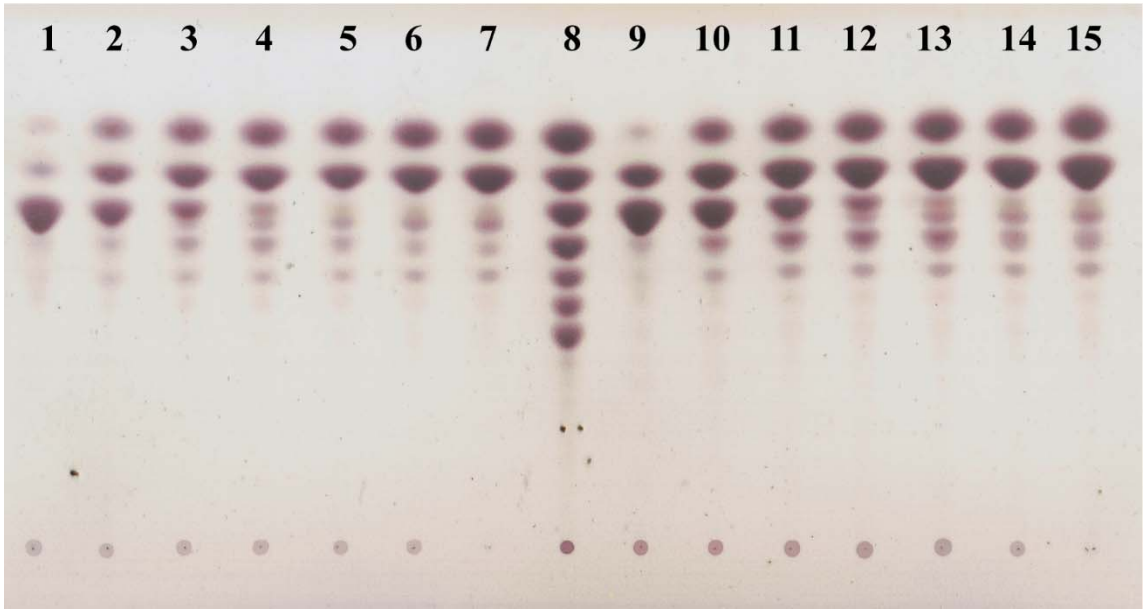

Figure S7 Transglycosylation activity of *Wc*AG on TLC. Lane: 1-7 are the time variation of reaction containing 100 mM maltotriose at 0, 5, 15, 30, 60, 120, 180 min. Lane: 8 is standard malto-oligosaccharides (G1-G7). Lane: 9-15 are the reaction containing 100 mM maltotriose and 50 mM maltose at 0, 5, 15, 30, 60, 120, 180 min. The reactions were performed in 50 mM phosphate buffer pH 6 at 40°C using 10U/mL of *Wc*AG.

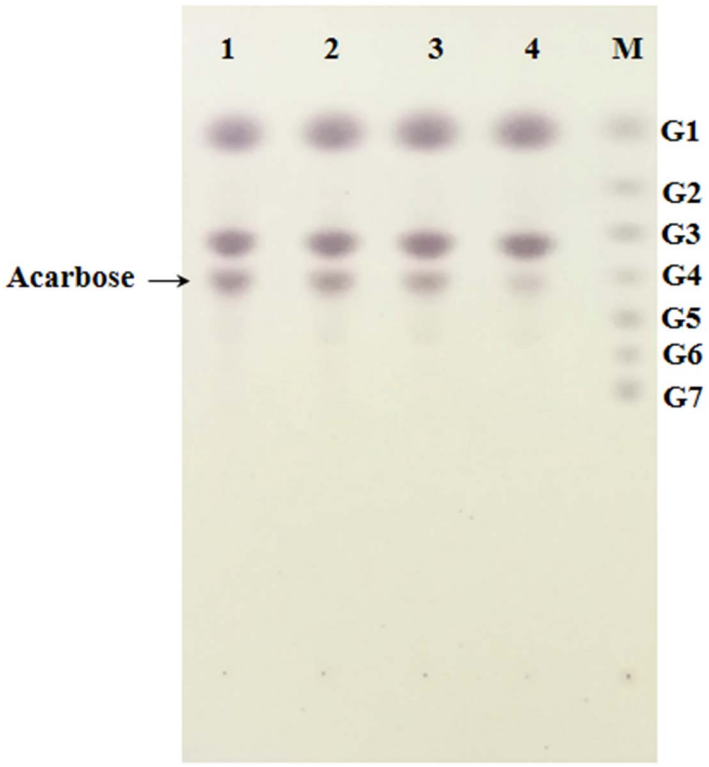

Figure S8 Hydrolysis of acarbose. Lane: 1-4 are the reactions at 30, 50, 75, and 100 min. Lane: M is standard malto-oligosaccharides (G1-G7). The reaction were performed by incubation of 10U/mL of *Wc*AG and 100 mM acarbose in 50 mM phosphate buffer pH 6 at 40°C for 100 min.

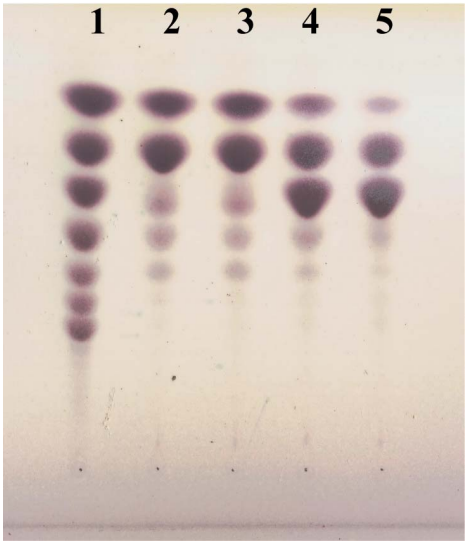

Figure S9 Effect of temperature to tranglycosylation activity containing 10% (v/v). Lane: 1 is standard malto-oligosaccharides (G1-G7), Lane: 2, 3, 4 and 5 are the reaction incubated at 20, 30, 40 and 50°C, respectively. *Wc*AG (10 U/ml) was incubated overnight with 100 mM maltrotriose and 50 mM maltose in 50 mM phosphate buffer pH 6 with the addition of 10 (v/v) acetonitrile.

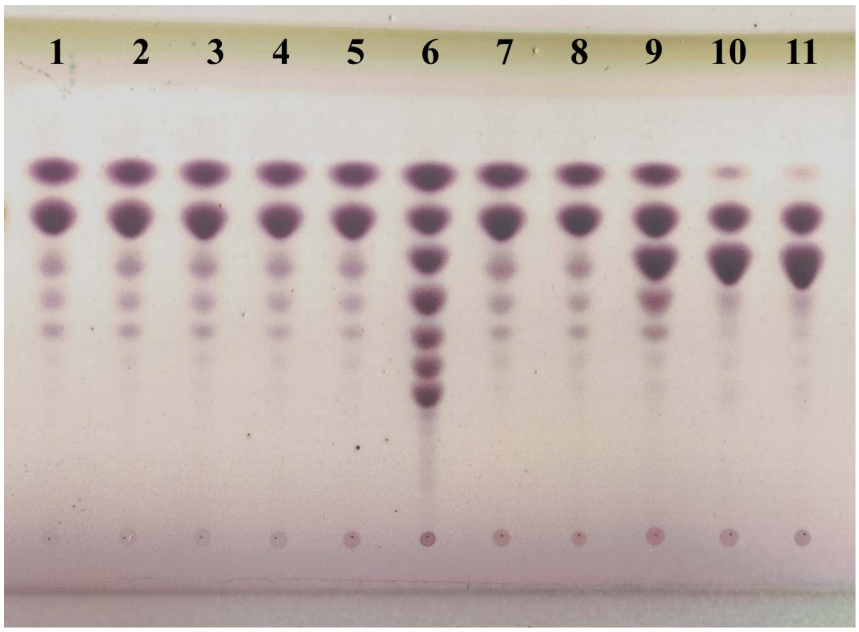

Figure S10Effect of solvent on tranglycosylation activity. Lane: 6 is standard malto-oligosaccharides (G1-G7), Lane: 1, 2, 3, 4 and 5 are the reaction containing 20, 15, 10, 5 and 0 % (v/v) DMSO, respectively. Lane: 7, 8, 9, 10 and 11 are the effect of acetonitrile concentration at 0, 5, 10, 15, 20% (v/v), respectively All reactions consisted of 10 U/ml of *Wc*AG**,** 100 mM maltrotriose and 50 mM maltose in 50 mM phosphate buffer pH 6. The reactions were carried out at 40°C for overnight.

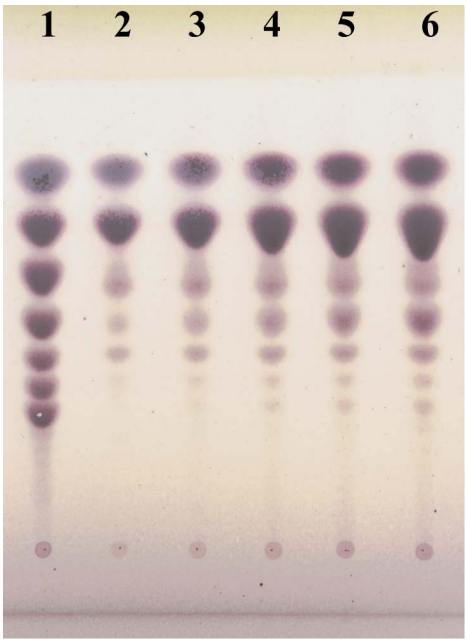

Figure S11 Effect of maltose acceptor on tranglycosylation activity. Lane: 1 is standard malto-oligosaccharides (G1-G7), Lane: 2, 3, 4, 5 and 6 are the reaction consisted of 0, 50, 100, 150, 200 mM maltose and incubated with 100 mM maltotriose in 50 mM phosphate buffer pH 6, using 10 U/ml of *Wc*AG at 30°C for overnight.

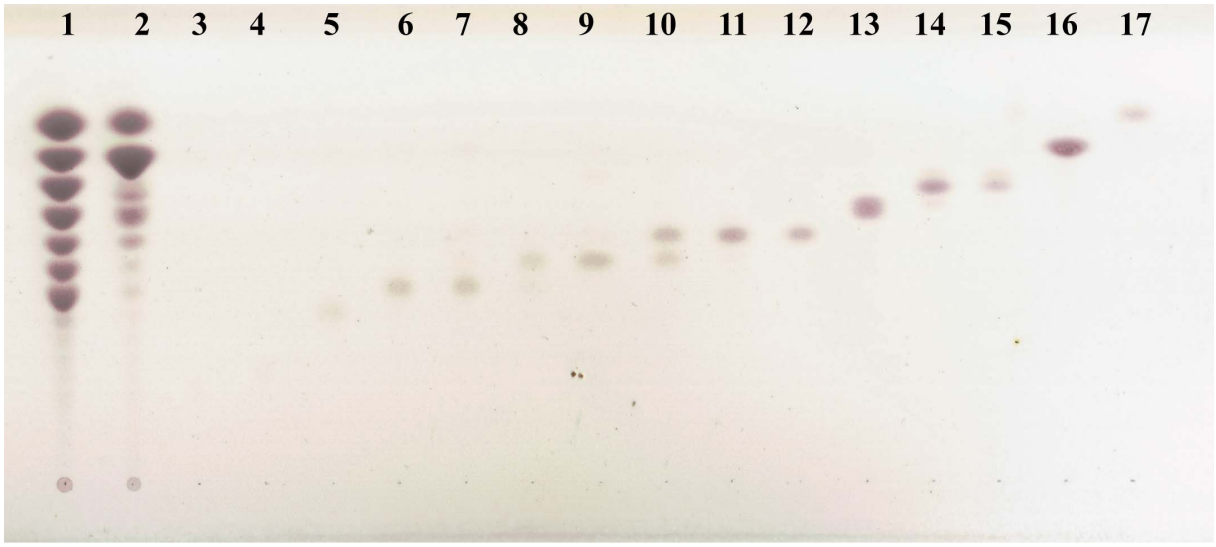

Figure S12 Separation of *Wc*AG transglycosylation products by size-exclusion chromatography. The mixture of *Wc*AG transglycosylation products was separated by Bio-Gel P-2 at 50°C. Lane: 1 is standard malto-oligosaccharides (G1-G7), lane: 2 is a crude mixture of *Wc*AG transglycosylation products, lane: 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 are the fraction No 37, 38, 43, 45, 46, 47, 48, 49, 50, 51, 55, 58, 59, 64, 73, respectively**.**

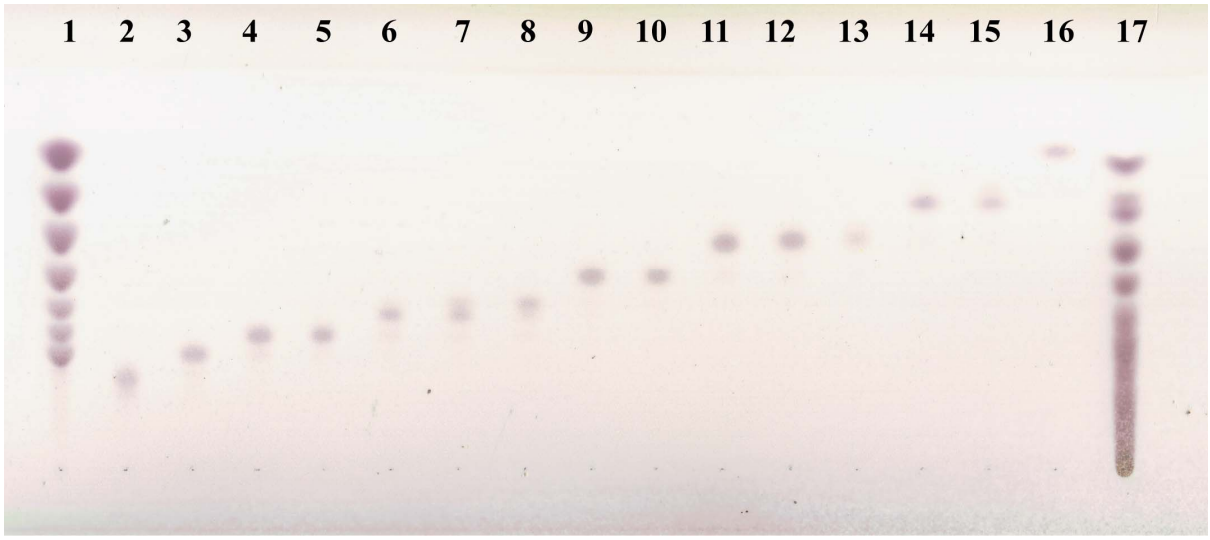

Figure S13 Separation of pullulan hydrolysates by size-exclusion chromatography. The mixture of pullulan hydrolysates were prepared by boiling 20% (w/v) pullulan (Wako, Japan) in 1 M HCl for 40 min and then neutralized by equivalent of NaOH. The pullulan hydrolysates were then separated by Bio-Gel P-2 at 50°C. Lane: 1 is standard malto-oligosaccharides (G1-G7), lane: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 are the fraction No. 43, 46, 48, 49, 51, 52, 53, 56, 57, 60, 61, 62, 65, 66, 73, respectively and lane: 17 represents crude pullulan hydrolysates.

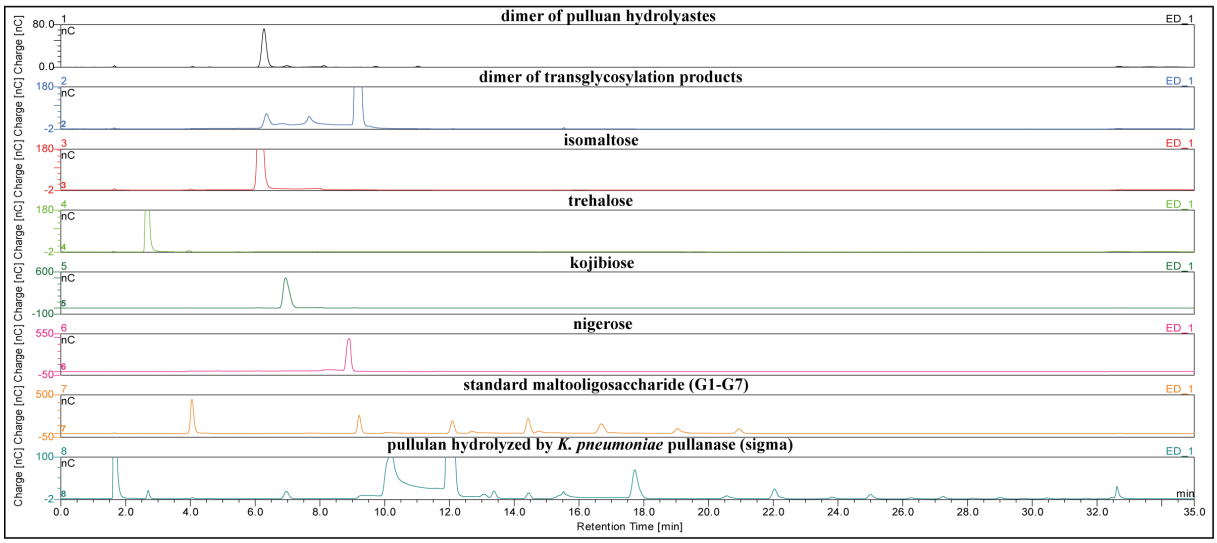

Figure S14 HPAEC-PAD analysis of disaccharides from *Wc*AG transglycosylation products. The dimer products were compared with dimer of pullulan hydrolysates, isomaltose, trehalose, kojibiose, nigerose, standard malto-oligosaccharides (G1-G7) and pullulan hydrolyzed by *K. pneumonia* pullanase (Sigma), respectively.

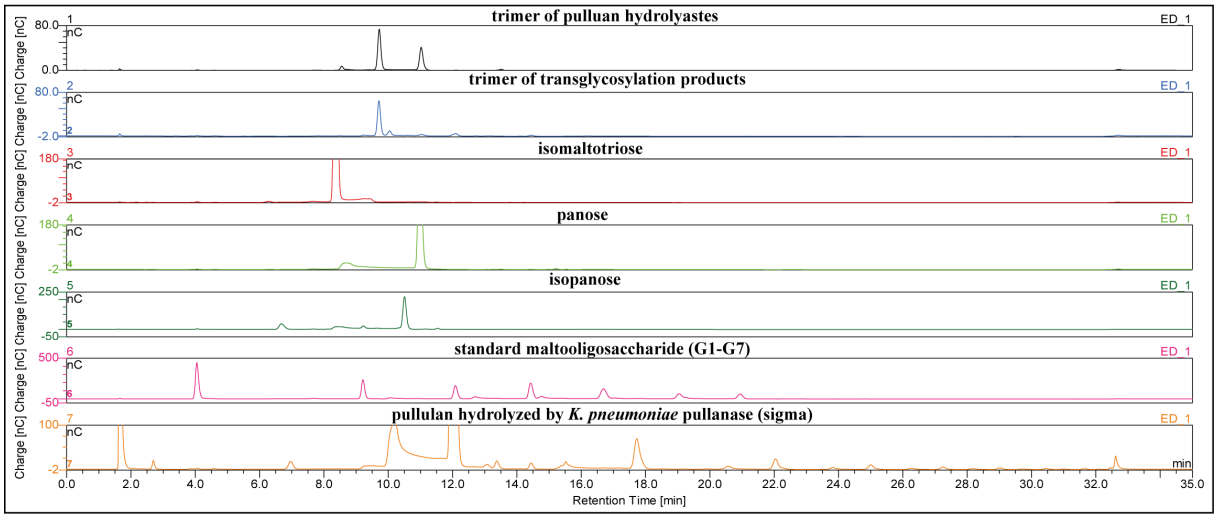

Figure S15 HPAEC-PAD analysis of trisaccharides from *Wc*AG transglycosylation products. The trimer products were compared with trimer of pullulan hydrolysates, isomaltotriose, panose, isopanose, standard malto-oligosaccharides (G1-G7) and pullulan hydrolyzed by *K. pneumonia* pullanase (Sigma), respectively.

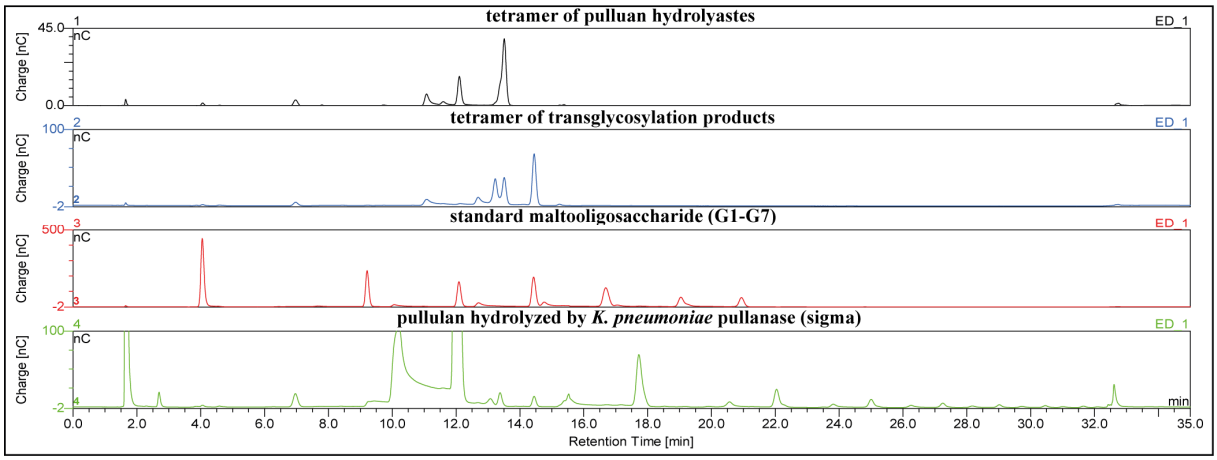

Figure S16 HPAEC-PAD analysis of tetrasaccharides from *Wc*AG transglycosylation products. The tetramer products were compared with tetramer of pullulan hydrolysates, standard malto-oligosaccharides (G1-G7) and pullulan hydrolyzed by *K. pneumonia* pullanase (Sigma), respectively.

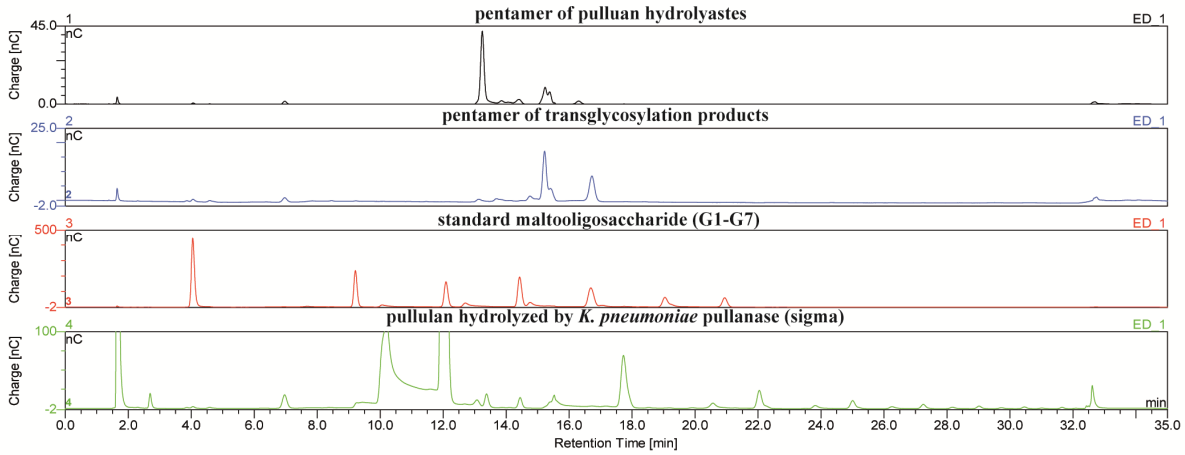

Figure S17 HPAEC-PAD analysis of pentasaccharides from *Wc*AG transglycosylation products. The pentamer products were compared with pentamer of pullulan hydrolysates, standard malto-oligosaccharides (G1-G7) and pullulan hydrolyzed by *K. pneumonia* pullanase (Sigma), respectively.

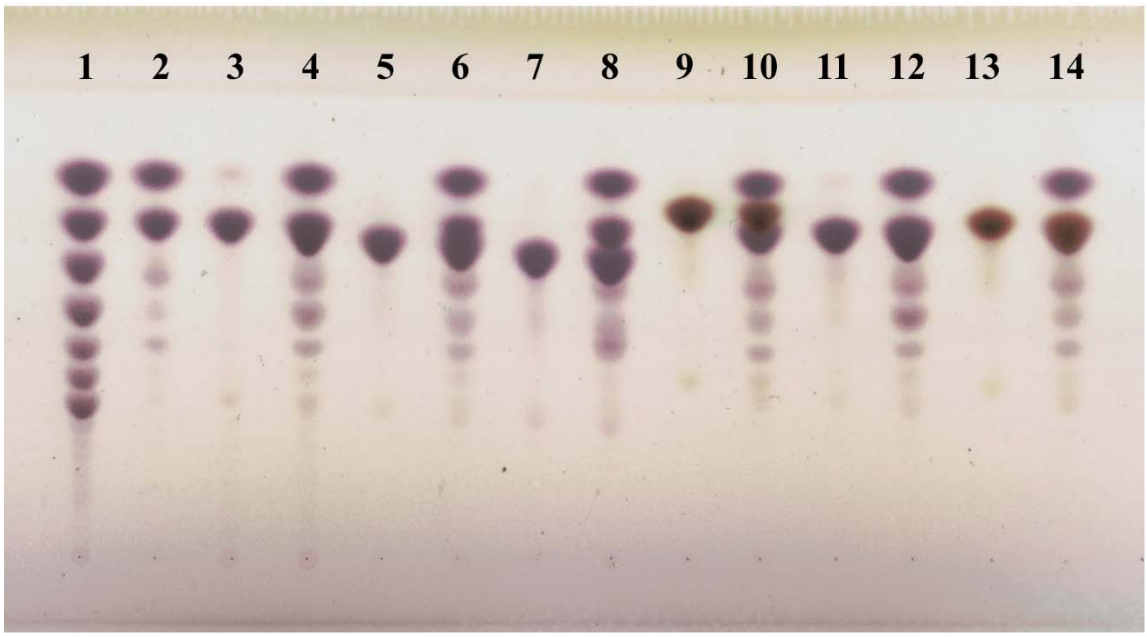

Figure S18 Effect of acceptor on *Wc*AG transglycosylation. Lane: 1 is standard maltooligosaccharide (G1-G7), Lane: 2, 3, 5, 7, 9, 11 and 13 are the reactions incubated with 100 mM of maltrotriose, maltose, isomaltose, melibiose, sucrose, cellobiose and palatinose, respectively. Lane: 4, 6, 8, 10, 12 and 14 are the reactions containing 100 mM maltotriose and 100 mM acceptor molecule, including maltose, isomaltose, melibiose, sucrose, cellobiose and palatinose, respectively. The reactions were carried out overnight in 50 mM phosphate buffer pH 6 with 10% (v/v) acetonitrile at 30°C using 10 U/ml of *Wc*AG.

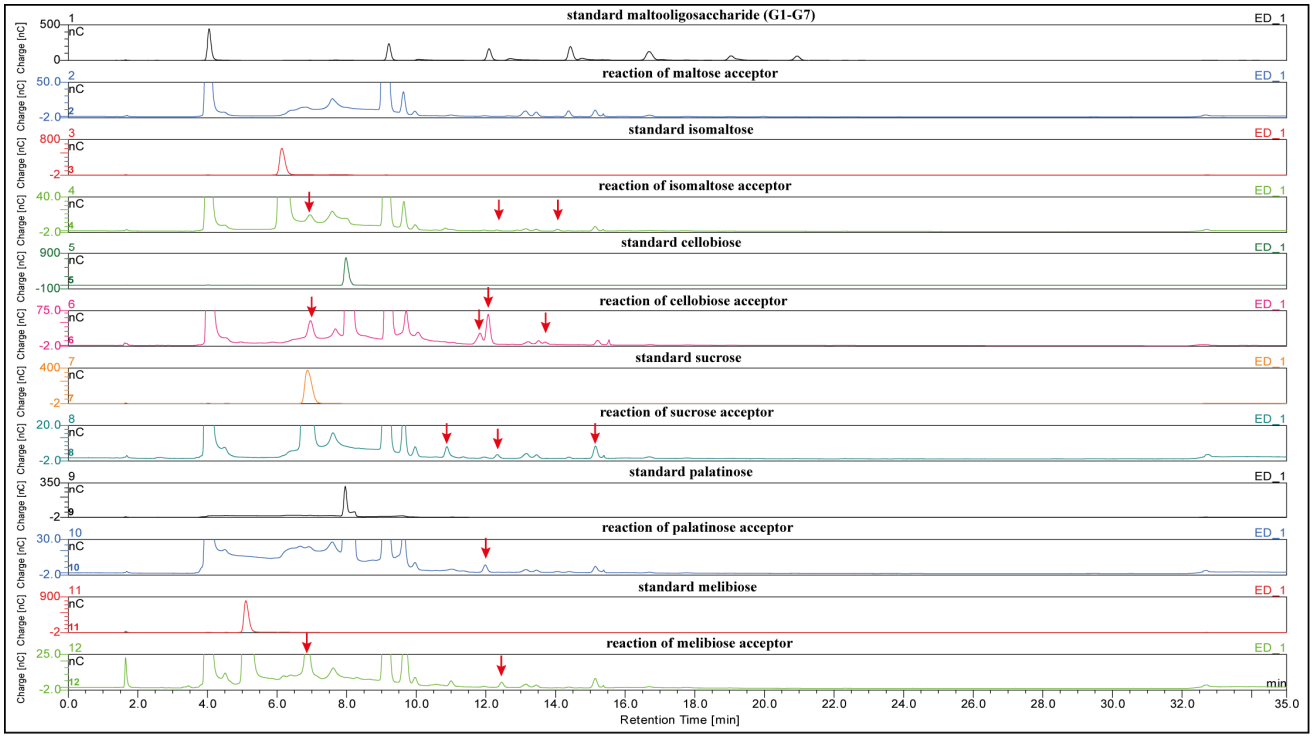

Figure S19 HPAEC-PAD analysis of *Wc*AG transglycosylation products using different acceptors. The products were synthesized using 10 U/ml of *Wc*AG, 100 mM of maltrotriose and 100 mM of various acceptor molecules (maltose, isomaltose, melibiose, sucrose, cellobiose and palatinose). The red arrows indicated different pattern products when other acceptors were added instead of maltose.

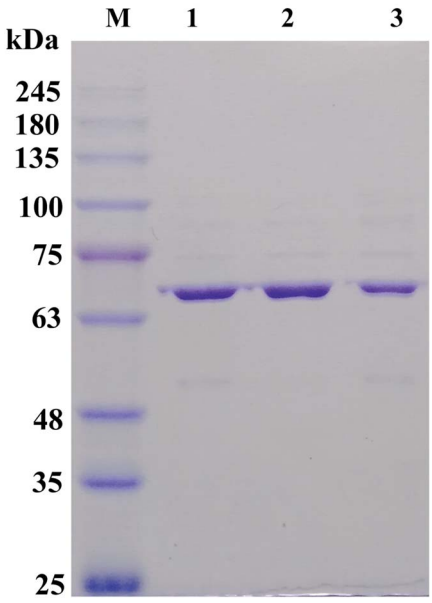

Figure S20 SDS-PAGE analysis of purified wild-type, D345N and E374Q *Wc*AG enzymes. Lane M is protein molecular weight marker, lane1, 2, and 3 are purified wild-type, D345N and E374Q *Wc*AG enzymes, respectively.

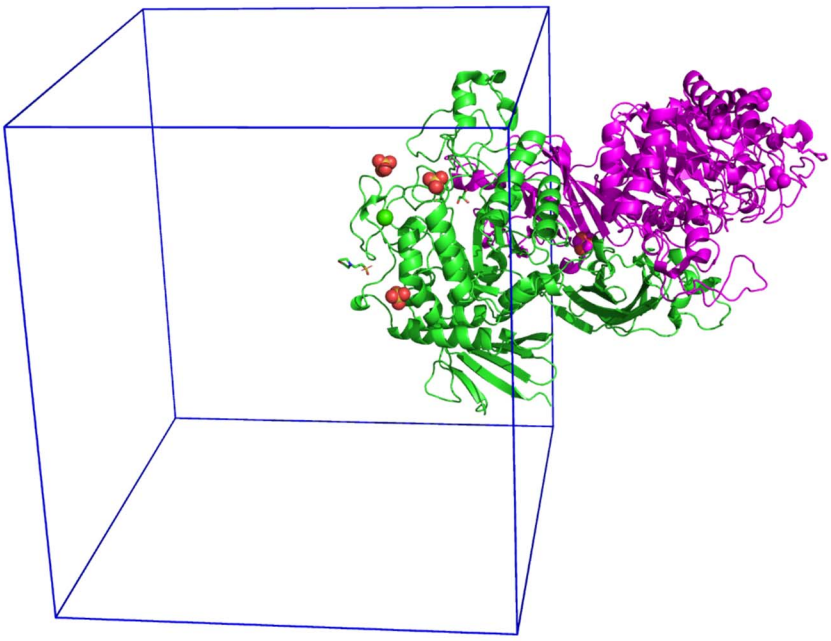

Figure S21 The assembly of *Wc*AG. One molecule of *Wc*AG (green) is presented in an asymetric unit (blue lines), while a 2-fold symmetry-related molecule is in pink.

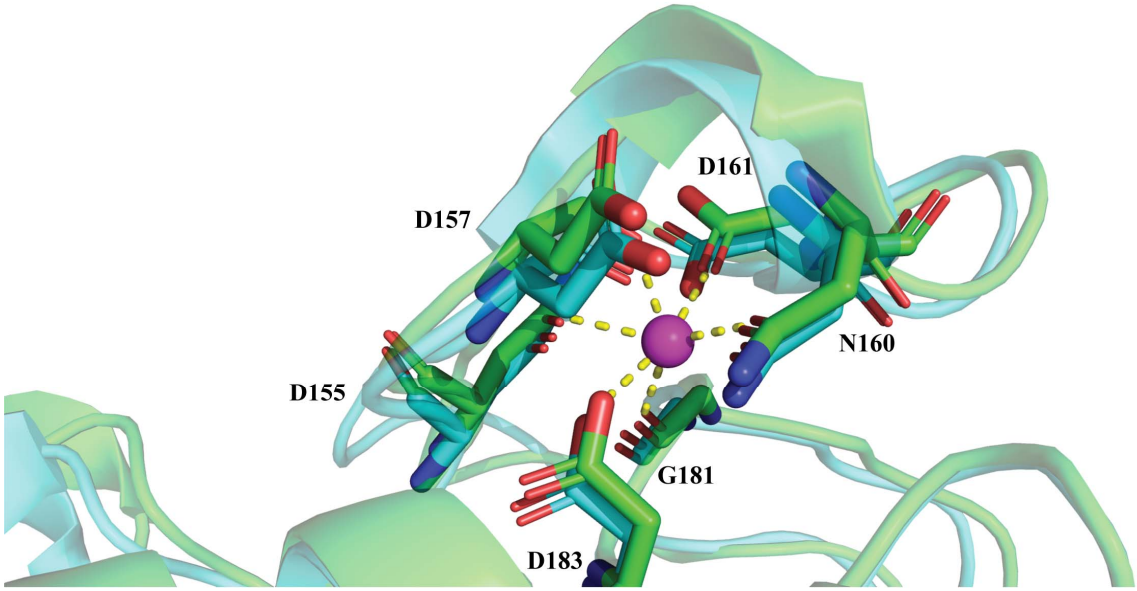

Figure S22 Calcium binding site of *Wc*AG and *Tv*AII. Crystal structure of *Wc*AG (PDB ID: 7D9B) (green) was superimposed with crystal structure of *Tv*AII (cyan). Calcium ion (green sphere) interacts with N155, D157, N160, D161, G181, and D183 of *Wc*AG, corresponding to N143, D145, N148, D149, G169, and D171 of *Tv*AII (PDB ID: 1WZK). The Ca^{2+} ion is presented in magenta.

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TVAI        AANDNNVEWNGLFHDQGPLFDNAPEPTSTQSVTLKLRTFKGDITSANIKYWDTAD-----      55
WcAG        -----MGNLAGIMHRPD---SEMAYVVNEQTVNIRLRTAKDDIVSVELLAGDPYSLRSLP      52
TVAII       ------MLLEAIFHEAK---GSYAYPISETQLRVRLRAKKGDVVRCEVLYADRYASPE--      49
CDase       ------MLKEAIYHRPK---NNYAYAYSKDTLHIRLRTKKNDLTQVELLYADPYNWNE--      49
ThMA        ------MFKEAIYHRPK---DNFAYAYDEQTLHIRLRTKKNDVEHVRLIYGDPYEWEN--      49
NPase       ------MRKEAIYHRPA---DNFAYAYDSETLHLRLRTKKDDIDRVELLHGDPYDWQN--      49
                      .: *      ..     .   : ::**: *.*:   .:   *

TVAI        --NAFHWVPMVWDSNDPTGTFDYWKGTIPASPSIKYYRFQINDGTS-TAWYNGNGPSSTE     112
WcAG        TDEKFYQVPKQMTKIMSDGISDFWQVTVTEPKRRLAYAFLVTDMLGIQKIYSDKGFFKVA     112
TVAII       --EEL--AHALAGKAGSDERFDYFEALLECSTKRVKYVFLLTGPQGEAVYFGETGFSAER     105
CDase       --DGWLYEQKTMRLEASDHLFDYWIIDVTVPYRRLRYGFKLTSEDE-TLYYTEKGFYETA     106
ThMA        --GHWQVSYQSMQKSGTDELFDYWFIALTPPYRRLRYGFELTSNNE-QIVYTEKGFYRTA     106
NPase       --GAWQFQMMPMRKTGSDELFDYWFAEVKPPYRRLRYGFVLYSGEE-KLVYTEKGFYFEV     106
                          *::   :        * * : .       :  .*

TVAI        PN---ADDF-------YIIPNFKTPDWLKNGVMYQIFPDRFYNGDSSNDVQTGSYTYNGT     162
WcAG        DADLMDMNFYFRMPFFQTIDQYNAPEWVTDTVWYQIFPERFANGDVSND-----------     161
TVAII       -----SKAGVFQYAYIHRSEVFTTPEWAKEAVIYQIFPERFANGDPSND-----------     149
CDase       PT--DDTAYYFCFPFINPVDIFQAPEWVKKTVWYQIFPERFANGDSSIN-----------     153
ThMA        PM--DDTAYYFCFPFLNKIDVFQAPEWVKDTIWYQIFPERFANGNEALN-----------     153
NPase       PT--DDTAYYFCFPFLHRVDLFEAPDWVKDTVWYQIFPERFANGNPSIS-----------     153
                          : :*:* .. : *****:** **: : .

TVAI        PTEKKAWGSSVYADPGYDNSLVFFGGDLAGIDQKLGYIKKTLGANILYLNPIFKAPTNHK     222
WcAG        PVGTKPWDSTDHPG-----REDFYGGDLQGILDHLDHLQ-ELGISGIYLNPIFQAPSNHK     215
TVAII       PPGTEQWAKDARPR-----HDSFYGGDLKGVIDRLPYLE-ELGVTALYFTPIFASPSHHK     203
CDase       PASTLPWGST-EAT-----PTNFFGGDFEGILNHLDYLV-DLGINGIYFTPIFKAKSNHK     206
ThMA        PAGTLPWGSA-DPT-----PTSFFGGDFEGIIQKLDHLV-DLGVNGIYFTPIFKASSNHK     206
NPase       PEGSRPWGSE-DPT-----PTSFFGGDLQGIIDHLDYLV-DLGITGIYLTPIFRSPSNHK     206
            *  .  * .             *:***: *: ::* ::   ** . :*:.*** : ::**

TVAI        YDTQDYMAVDPAFGDNSTLQTLINDIHSTANGPKGYLILDGVFNHTGDSHPWFDKYNNFS     282
WcAG        YDTQDYMTVDPHFGDAKLFKQLVQAAHERG----IRVMLDAVFNHIGDKSVQWQDVLKNE     271
TVAII       YDTADYLAIDPQFGDLPTFRRLVDEAHRRG----IKIILDAVFNHAGDQFFAFRDVLQKG     259
CDase       YDTIDYMEIDPQFGDKETFRKLVNACHEKG----IKIMLDAVFNHSGYYFEAFQDVLKHQ     262
ThMA        YDTIDYFEIDPQFGDKQTFKRLVELCHQKA----IRVMLDAVFNHSGYEFPPFQDVLKYG     262
NPase       YDTADYFEVDPHFGDKETLKTLIDRCHEKG----IRVMLDAVFNHCGYEFAPFQDVWKNG     262
            *** **: :** ***   :: *::  *  .      ::**.**** *     : .  :

TVAI        SQGAYESQSSPWYNYYTFYTWPDSYASFLG--------------FNSLPKLNYGNSGSA     327
WcAG        Q-------ASPYADWFHIHQFPATYTPTDNFEFAADATYDTFDY-TPHMPKLNTSNPE--     321
TVAII       E-------QSRYKDWFFIEDFPVSKTSRTN--------YETFAVQVPAMPKLRTENPE--     302
CDase       E-------QSKYKDWFHIRDFPVTPGPKPN--------YDTFGF-VEYMPKLNTENQE--     304
ThMA        E-------NSKYKHWFHIREFPLQTVPRPN--------YDTFAF-TPNMPKLNTENQE--     304
NPase       E-------SSKYKDWFHIHEFPLQTEPRPN--------YDTFRF-VPQMPKLNTANPE--     304
            .        * : .:: :  :*       .                  :***.  *

TVAI        VRGVIYNNSNSVAKTYLNPPYSVDGWRLDAAQYVDANGNNGSDVTNHQIWSEFRNAVKGV     387
WcAG        VVDYLL-----NIATYWVKEFDIDAWRLDVANEI-----------DHHFWRKFHDAMMAL     365
TVAII       VKEYLF-----DVARFWME-QGIDGWRLDVANEV-----------DHAFWREFRRLVKSL     345
CDase       VKDYLL-----KVARYWIEEFNIDGWRLDVANEV-----------DHQFWRDFRREVKTI     348
ThMA        VKNYLL-----DVATYWIREFDIDGWRLDVANEV-----------DHQFWREFRQAVKTI     348
NPase       VKRYLL-----DVATYWIREFDIDGWRLDVANEI-----------DHEFWREFRQEVKAL     348
            *   :          :     .:*.****.*: :           :* :* .*:  :  :

TVAI        NSNAAIIGEYWGNANPWTAQGNQWDAATNFDGFTQPVSEWITGKDYQNNSASISTTQFDS     447
WcAG        KPDFYILGEIWHTSQSWL-VGDEFTAVMNYS-YTGAILQYFL-------ENE-SADALVQ     415
TVAII       NPDALIVGEIWHDASGWL-MGDQFDSVMNYL-FRESVIRFFA-------TGEIHAERFDA     396
CDase       NPDVYILGEIWHDSMPWL-QGDQFDAVMNYP-FTVAALDYIA-------KDKINAEEFAH     399
ThMA        KPDAYILGEIWHDAMPWL-RGDQFDAVMNYP-FTNGTLRFFA-------QHEIRASQFVG     399
NPase       KPDVYILGEIWHDAMPWL-RGDQFDAVMNYP-FTDGVLRFFA-------KEEISARQFAN     399
            : :  *:** *  :  *   *::: :. *:  :      ::          .  :  :
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TVAI        WLRGTRANYPTNVQQSMMNFLSNHDITRFATRSGGDLWKTYLALIFQMTYVGTPTIYYGD     507
WcAG        KMSHQLMLYRDATNRMMFNTVDSHDTPRLMTLAHEDKQLAKSILTFTFMQPGVPSIYYGT     475
TVAII       ELTRARMLYPEQAAQGLWNLLDSHDTERFLTSCGGNEAKFRLAVLFQMTYLGTPLIYYGD     456
CDase       QLTDVLCSYPANIHEVTFNLLGSHDTARVLTVCKDNKEKTKLLYLLLLSSKGSPCIFYGE     459
ThMA        MMTHVLHSYPTNVNEVAFNLLGSHDTPRLLTLCKEDVRKAKLSFLFQLSFTGTPCIYYGD     459
NPase       QMMHVLHSYPNNVNEAAFNLLGSHDTSRILTVCGGDIRKVKLLFLFQLTFTGSPCIYYGD     459
             :      *     .   * :..**  *. * .  :         : :   * * *:**

TVAI        EYGMQGGADPDNRRSFDWSQATPSNSAVALTQKLITIRNQYPAL-RTGSFMTLITDDTNK     566
WcAG        EYGMTGENDPDDRKPMVWQPELQDHDLYDFMQKLVQVRRQVIAKLSDDKIIFDVIG--ER     533
TVAII       EIGMAGATDPDCRRPMIWEEKEQNRGLFEFYKELIRLRHRLASL-TRGNVRSWHADKQAN     515
CDase       EIGMAGENDPGCRDCMIWEEDQQDLEFKAFIKKCIELRKTEPAFSSEASFEIVEANTESN     519
ThMA        EIGITGDQDPGCRKCMIWDEHQQNRELFRHVQKLIALRKAYKAFGNRGNLHFIDANDETN     519
NPase       EIGMTGGNDPECRKCMVWDPMQQNKELHQHVKQLIALRKQYRSL-RRGEISFLHADDEMN     518
            * *: *  **  *  : *.    .       :: : :*.   :     ..     .   .

TVAI        IYSYGRFDNVNRIAVVLNNDSVSHTVNVPVWQLSMPNGSTVTDKITGHSYTVQNGMVTVA     626
WcAG        QIRLTREDNQTRIVGVFNNGTTDLTVAQPT---SI---------------LLKTNQSETQ     575
TVAII       LYAFVRTVQDQHVGVVLNNRGEKQTVLLQV---PESGGKTWLDCLTGEEVHGKQGQLKLT     572
CDase       HLIYARELDGERIYFVINPTEQPITVTLPI---DP-TGQQIKDAWTDKSIEAVDNKVTMD     575
ThMA        HLIYMKTFEEEAIIFLVNNNEQEIEITLPL---SL-KGKLLTNLWTNEQFSAEADTLKST     575
NPase       YLIYKKTDGDETVLVIINRSDQKADIPIPL---DA-RGTWLVNLLTGERFAAEAETLCTS     574
                 :      :  :.*       :

TVAI        VDGHYGAVLAQ----        637
WcAG        LAPNDFMIWTEPVR-        589
TVAII       LRPYQGMILWNGR--        585
CDase       ISATGFGVIKVI---        587
ThMA        LPPYGFFIYKIEDWL        590
NPase       LPPYGFVLYAIEHW-        588
            :      :
```

Figure S23 Sequence alignment of *Wc*AG. *Wc*AG was aligned to TVAI [BAA02471.1], TVAII [BAB40638.1], CDase [O82982], *Th*MA [Q45490], and NPase [P38940.1] by Clustal OMEGA. Three catalytic residues are shown in red and underlined. Yellow highlights represent conserved signature sequence of neopullulanase subfamily ("MPKLN" and "VANE") (Oslancova, A. and Janeček, Š., 2002 and Kuchtová, A. and Janeček, Š., 2016). Blue highlights show the sequence of CBM34 and magenta highlights reveal six conserved aromatic residues of CBM34 (Janeček, Š. *et al.*, 2019).

Figure S24 Phylogenetic tree. *Wc*AG, TVAII, CDase, *Th*MA, and NPase sequences were analysed and phylogenetic tree were generated by Clustal OMEGA.

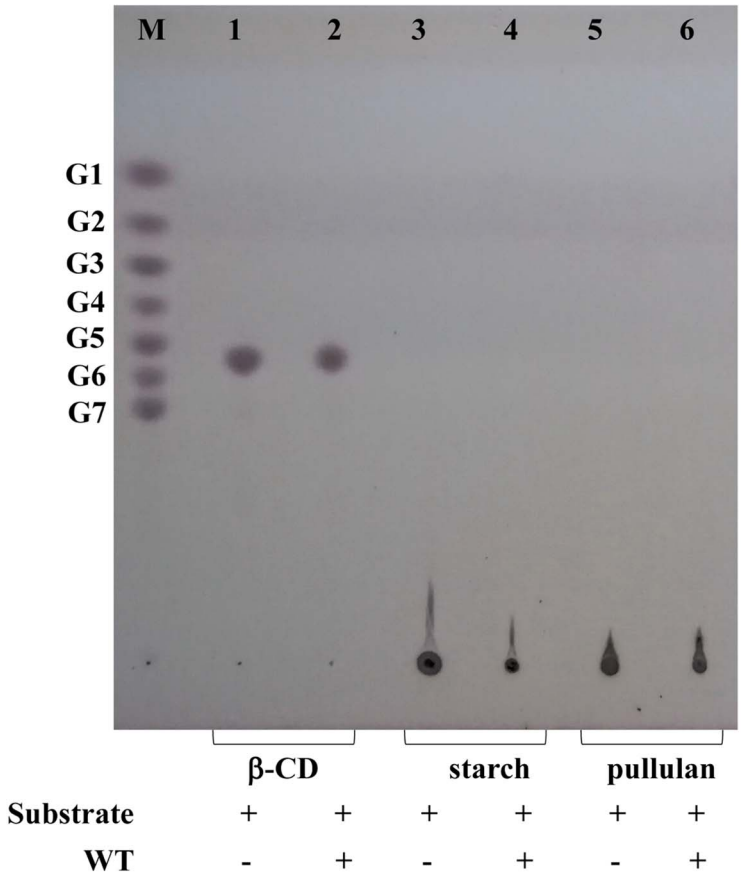

Figure S25 Hydrolysis of cycligosaccharide and polysaccharides by wild-type *Wc*AG by TLC analysis. Wild-type *Wc*AG (1 mg) was incubated with either 5 mg/mL β-CD, 1% (w/v) starch or pullulan in 50 mM phosphate buffer pH 6 at 30 ^{O}C overnight.

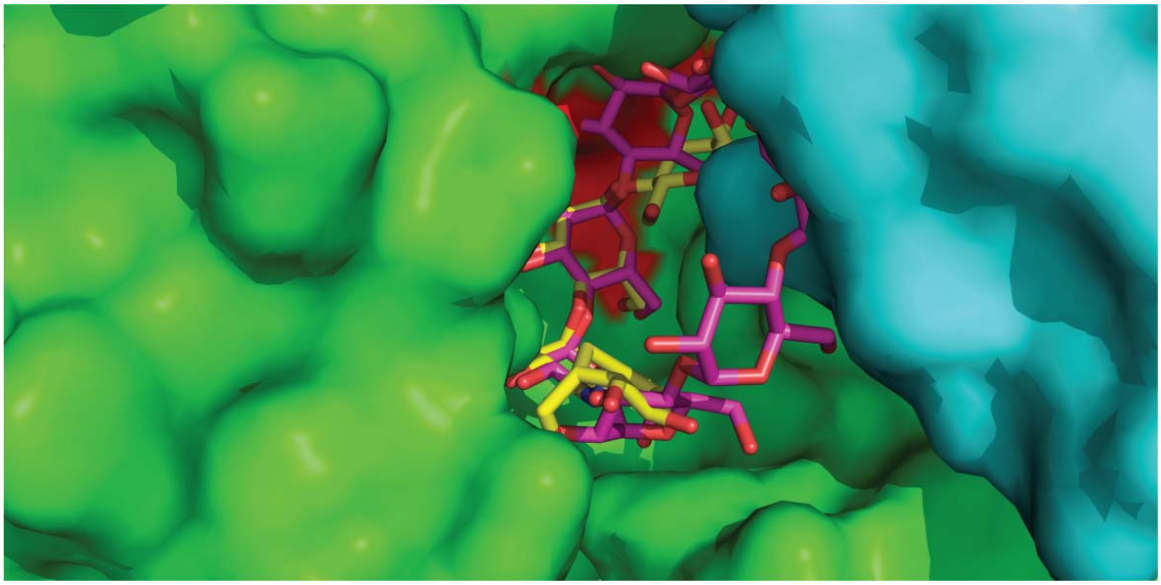

Figure S26 Comparison of the active site of *Wc*AG and *Th*MA. *Th*MA complexed with β-cyclodextrin (PDB ID: 1GVI) is superimposed with E374Q *Wc*AG structure in complex with acarbose (PDB ID: 7DCH). Green represents one subunit of *Wc*AG, and another displays in cyan. Acarbose and β-cyclodextrin are represented in yellow and magenta, respectively.

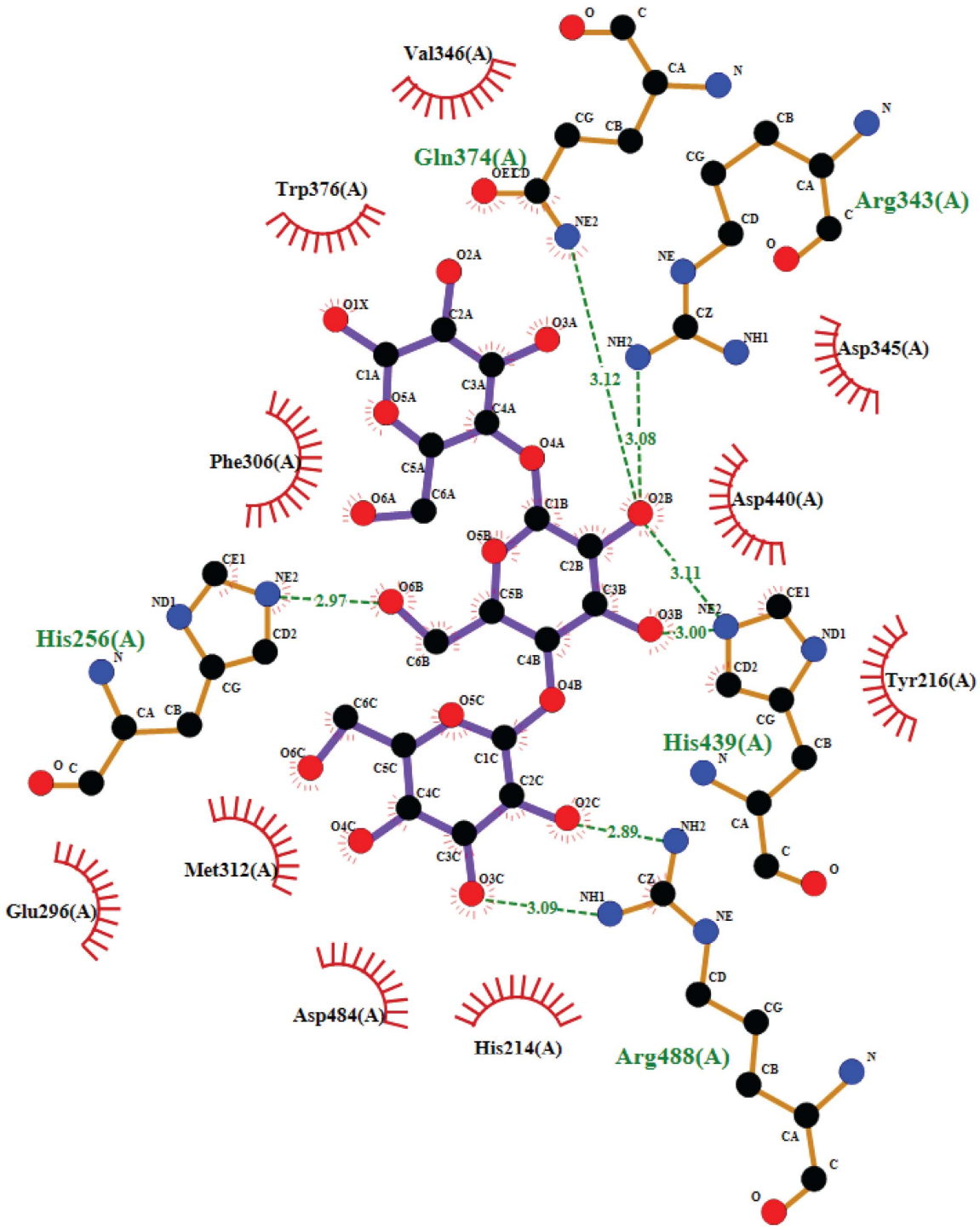

Figure S27 Interactions between maltotriose and E374Q *Wc*AG. The interaction 2D diagram was generated by Ligplot software (https://www.ebi.ac.uk/thornton-srv/software/LIGPLOT/).

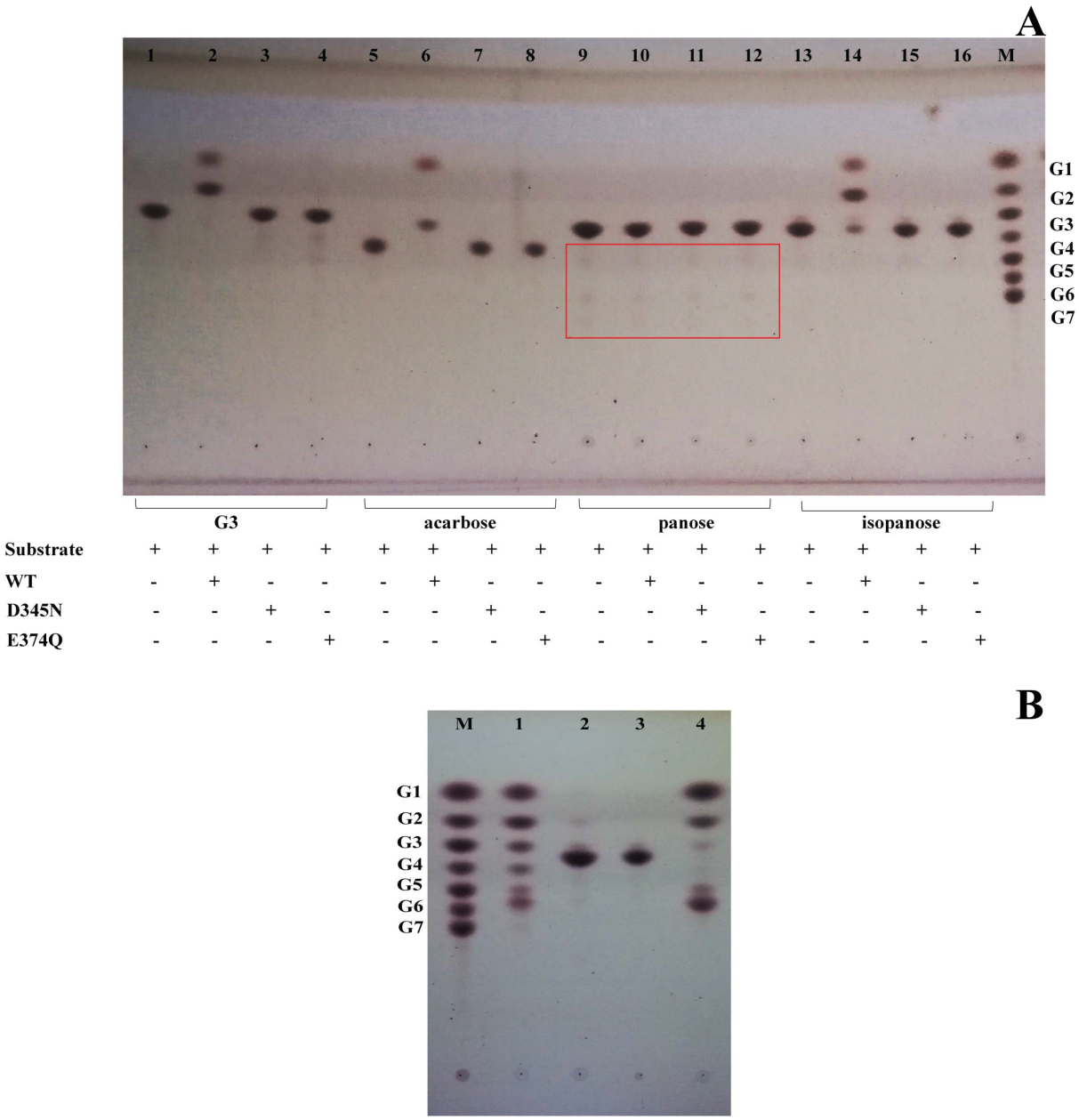

Figure S28 Product analysis of wild-type, D345N, and E374Q *Wc*AG enzymes by TLC.

(A) Wild-type, D345N, and E374Q *Wc*AG (0.02 mg/mL, ~0.15 nM) were incubated with 5 mg/mL maltotriose (G3), acarbose, panose, and isopanose in 50 mM phosphate buffer pH 6 at 30 ^{O}C overnight. Red box indicates impurity of standard panose.

(B) D345N of 5 mg/mL (~36.6 nM) was incubated with 5mg/mL G3 (Lane 1) and isopanose (Lane 2), respectively. E374Q of 5 mg/mL was incubated with 5 mg/mL G3. The reactions were conducted in 50 mM phosphate buffer pH 6 at 30 ^{O}C overnight (Lane 4). Lane M is maltooligosaccharide marker, while standard isopanose is shown in Lane 3.

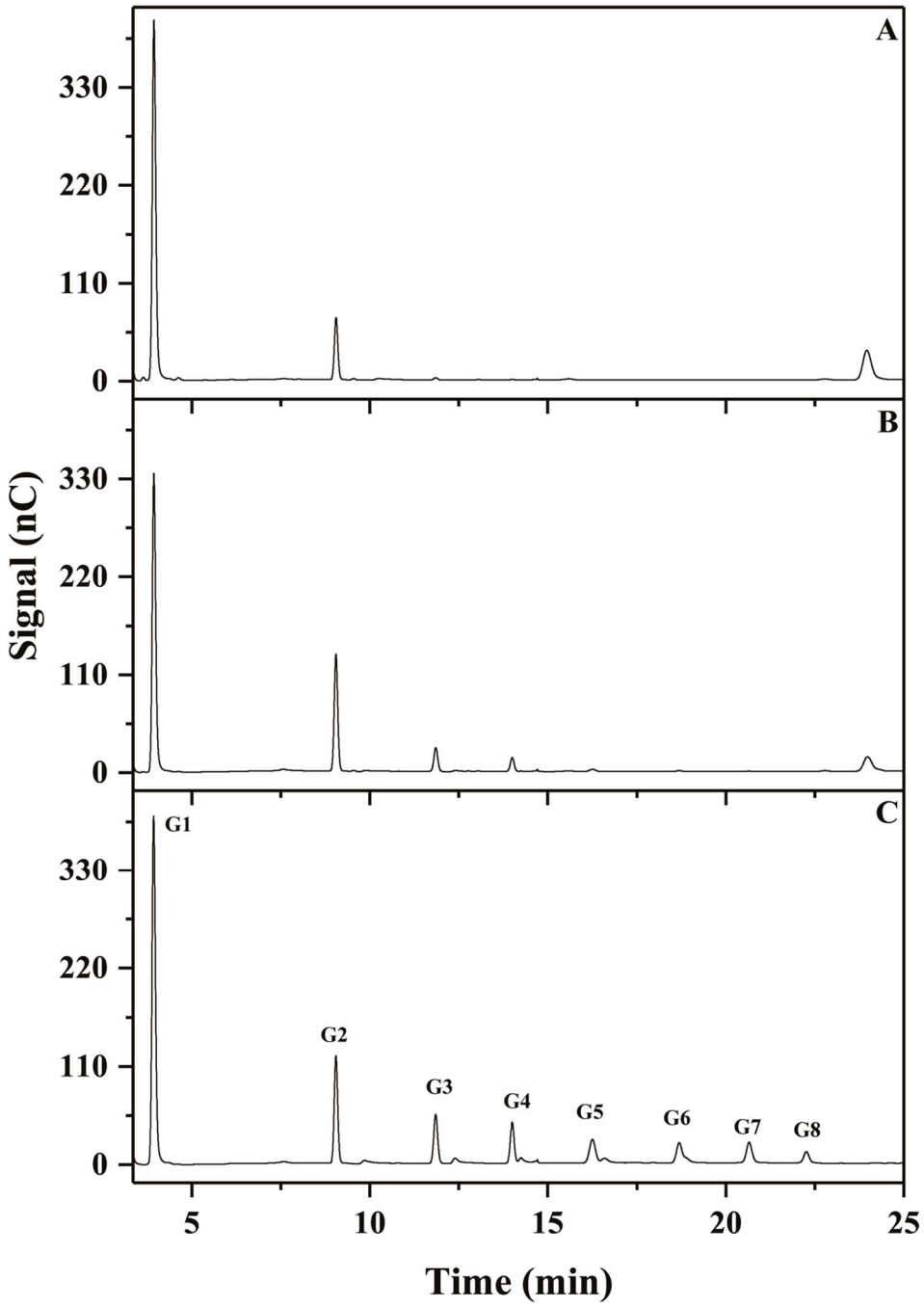

Figure S29 HPAEC-PAD analysis of hydrolysis products of maltotriose by E374Q (A) and D345N (B) *Wc*AG enzymes. Maltotriose (G3) of 5 mg/mL was incubated with either E374Q or D345N *Wc*AG (5 mg/mL) in 50 mM phosphate buffer pH 6 at 30 ^{O}C overnight. Standard malto-oligosaccharides (G1-G8) is shown in Panel C.

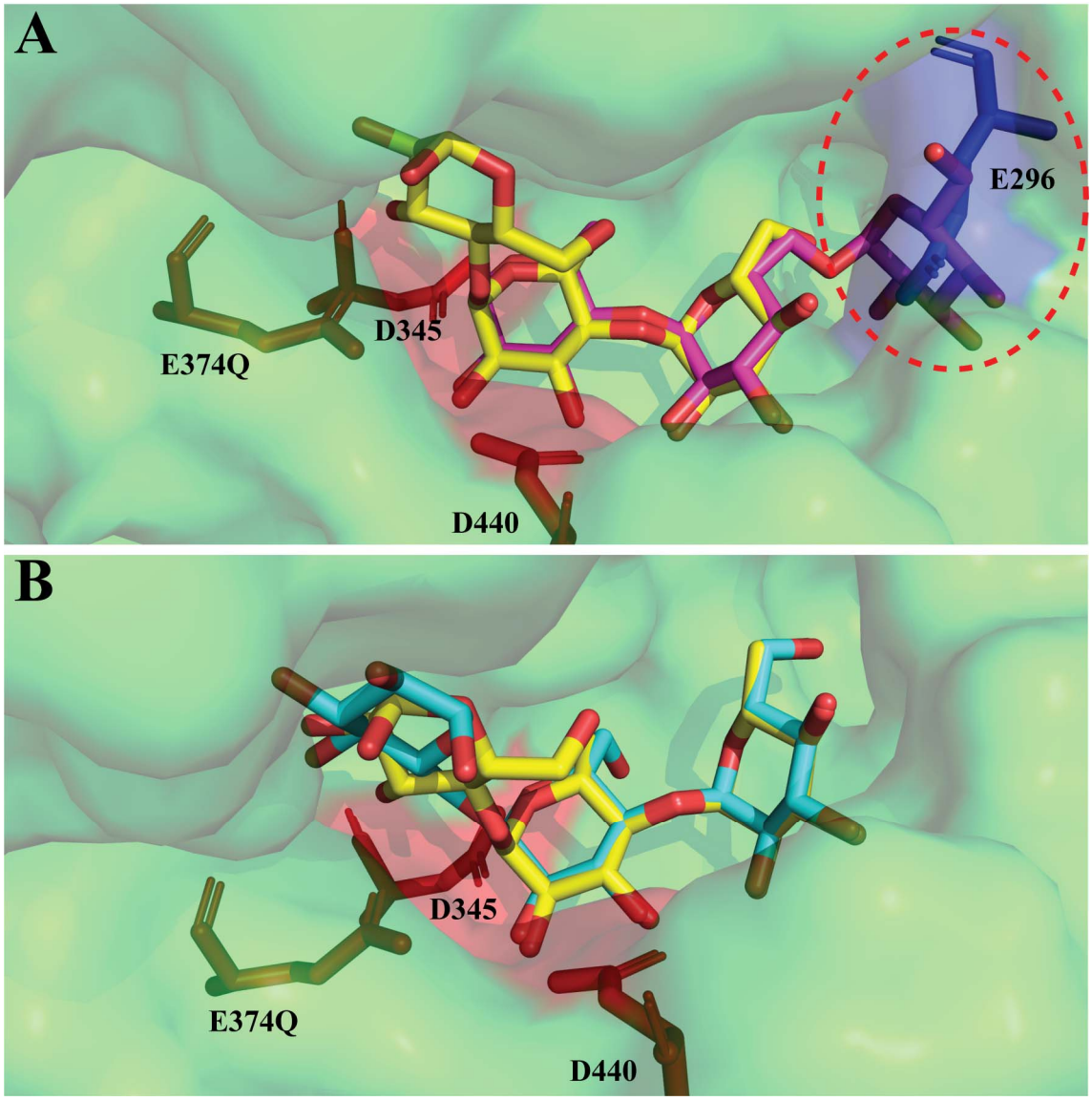

Figure S30 Comparison of the active site of *Wc*AG and NPase. NPase structure complexed with panose (PDB ID: 1J0I) (A) and isopanose (PDB ID: 1J0K) (B) are superimposed with E374Q *Wc*AG structure in complex with maltotriose (PDB ID: 7DCG). The maltotriose, panose and isopanose are in yellow, magenta, and cyan, respectively.

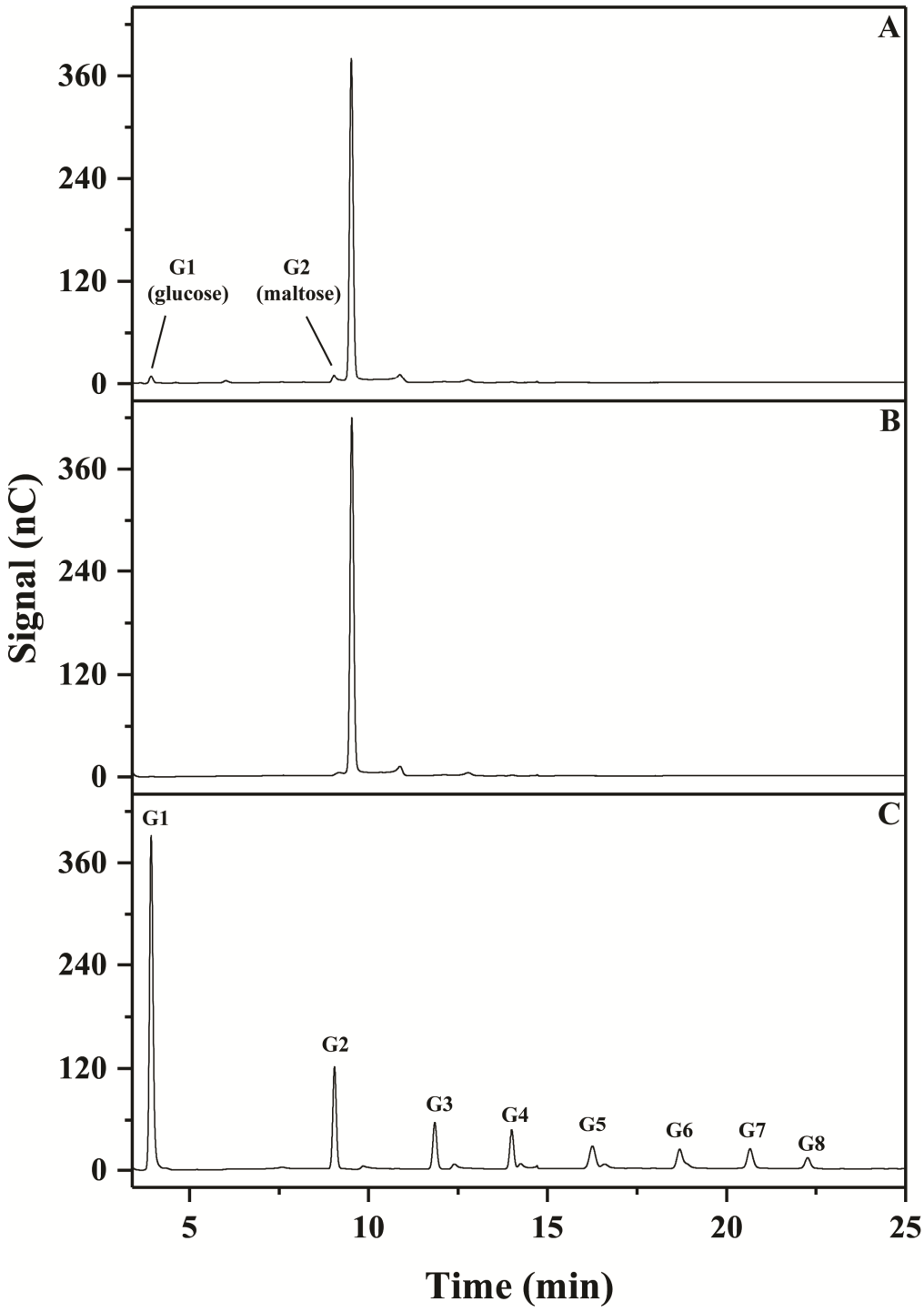

Figure S31 HPAEC-PAD analysis of hydrolysis products of isopanose by D345N mutant.

(A) D345N of 5 mg/mL was incubated with 5 mg/mL isopanose in 50 mM phosphate buffer pH 6 at 30 ^{O}C overnight. (B) and (C) are standard isopanose and malto-oligosaccharides (G1-G8), respectively.

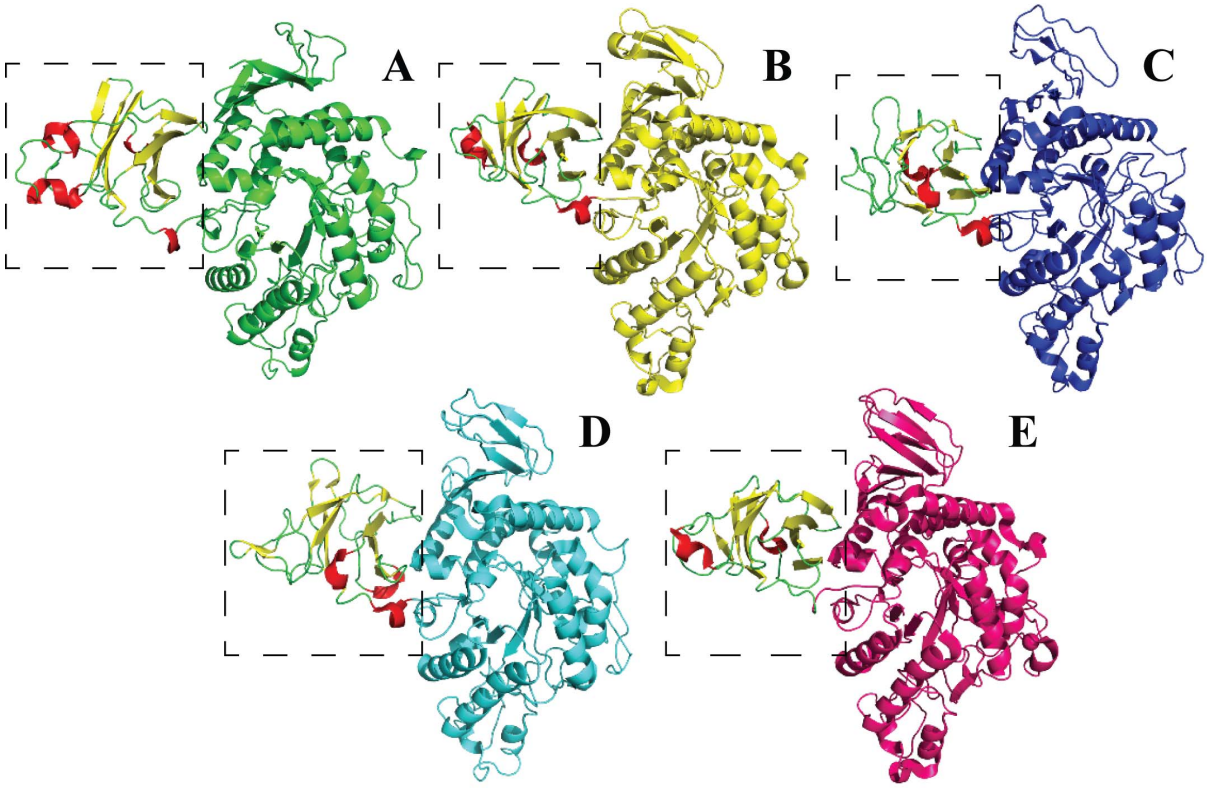

Figure S32 Comparison of the N-terminal domian of monomer structures of (A) *Wc*AG, (B) TVAII, (C) CDase, (D) *Th*MA and (E) NPase. PDB code: *Wc*AG (PDB ID: 7D9B), *Tv*AII (PDB ID: 1WZK), CDase (PDB ID: 1EA9), *Th*MA (PDB ID: 1SMA) and NPase (PDB ID: 1J0H).

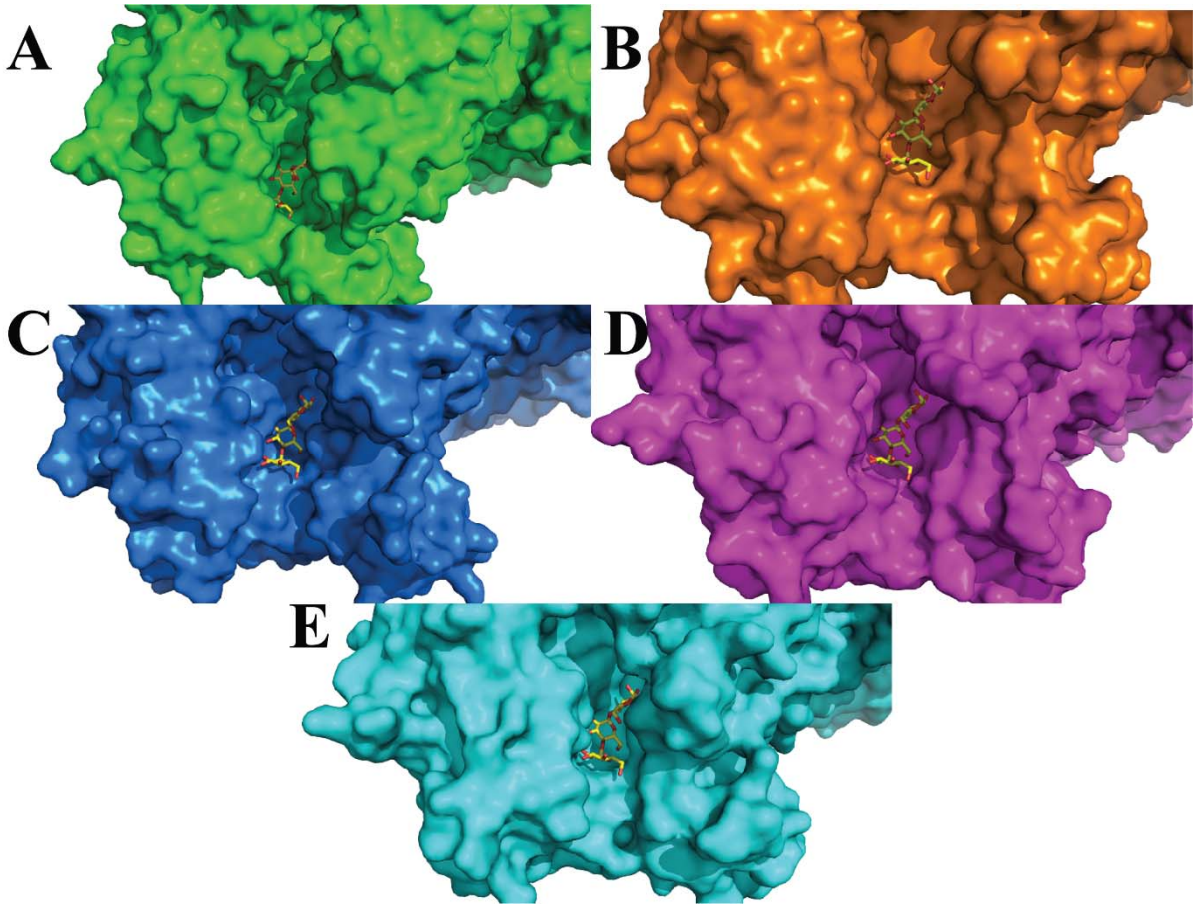

Figure S33 Comparison of the active site of *Wc*AG, *Tv*AII, CDase, *Th*MA and NPase. (A) E374Q *Wc*AG structure in complex with maltotriose (PDB ID: 7DCG, green) is superimposed with (B) *Tv*AII (PDB ID: 1WZK, orange), (C) CDase (PDB ID: 1EA9, blue), (D) *Th*MA (PDB ID: 1SMA, magenta) and (E) NPase (PDB ID: 1J0H, cyan).

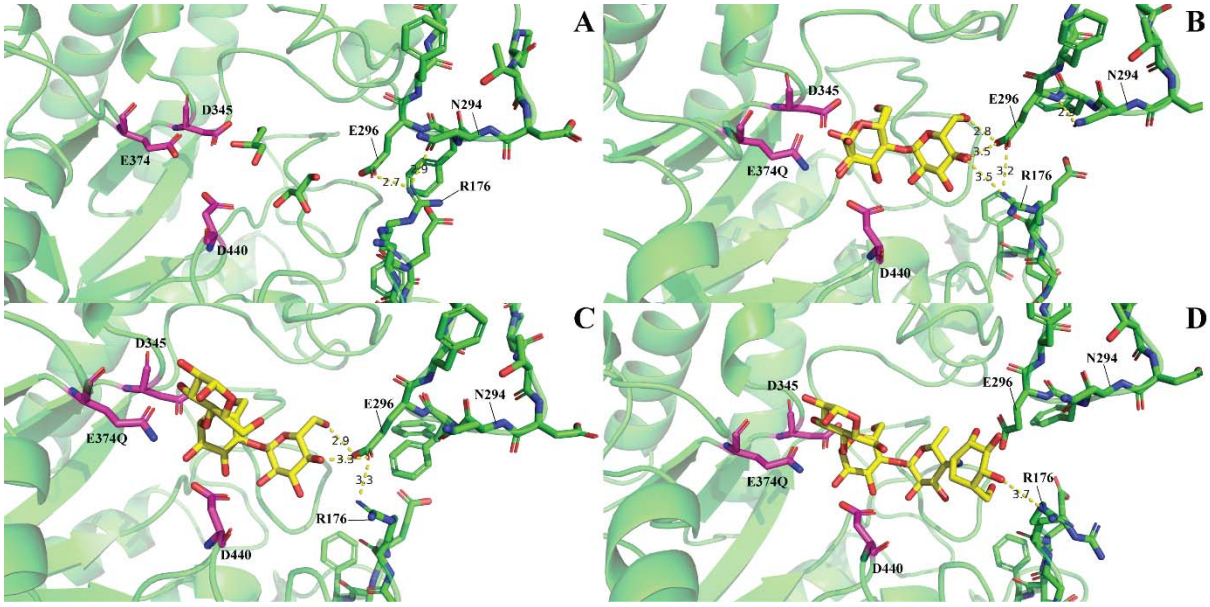

Figure S34 The flexibility of the Arg-Glu gate in front of the *Wc*AG active site. The figure represents the movement of two loops (P174-Y180 and T290-D300) in front of the active site when there is no substrate bound (A), or the active site is occupied by maltose (B), maltotriose (C), and acarbose (D). The ligands are displayed in yellow, while the catalytic residues are shown in magenta.

Table S1 Lists of primer sequences.

Primer name	Primer sequence (5'-3')
F_startNcoI	CCTT**CCATGG**GCAACCTAGCAGGTATTATGC
R_stopXhoI	CCAT**CTCGAG**TTAGCGCACTGGCTCGGTCCAAATC
F_E374Q	GATTTTTACATTCTAGGTCAGATTTGGCACACATCG
R_E374Q	CGATGTGTGCCAAATCTGACCTAGAATGTAAAAATC
F_D345N	GCTTGGCGTCTAAACGTTGCCAATGAAATTG
R_D345N	CAATTTCATTGGCAACGTTTAGACGCCAAGC

The underlines show restriction endonuclease site, *Nco*I and *Xho*I, respectively. Yellow highlights present the positions of mutated condons.

Supplementary Data

Propose of interface interaction of *Wc*AG

From Protein Interactions Calculator (http://pic.mbu.iisc.ernet.in/)

K. G. Tina, R. Bhadra and N. Srinivasan, PIC: Protein Interactions Calculator, Nucleic Acids Research, 2007, Vol. 35, Web Server issue W473–W476.

Protein-Protein Hydrophobic Interactions

  **[help]**

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Hydrophobic Interactions within 5 Angstroms

Position	Residue	Chain	Position	Residue	Chain
4	LEU	A	72	ILE	C
5	ALA	A	31	ALA	C
5	ALA	A	72	ILE	C
31	ALA	A	5	ALA	C
46	TYR	A	306	PHE	C
46	TYR	A	308	TYR	C
51	LEU	A	297	PHE	C
51	LEU	A	308	TYR	C
52	PRO	A	297	PHE	C
72	ILE	A	108	PHE	C
72	ILE	A	4	LEU	C
72	ILE	A	5	ALA	C
108	PHE	A	72	ILE	C
126	PRO	A	382	TRP	C
297	PHE	A	51	LEU	C
297	PHE	A	52	PRO	C

306	PHE	A	46	TYR	C
308	TYR	A	46	TYR	C
308	TYR	A	51	LEU	C
382	TRP	A	126	PRO	C

NO PROTEIN-PROTEIN DISULPHIDE BRIDGES FOUND

Protein-Protein Main Chain-Main Chain Hydrogen Bonds


[help]

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DONOR			**ACCEPTOR**		**PARAMETERS**	

Dd-a = Distance Between Donor and Acceptor
Dh-a = Distance Between Hydrogen and Acceptor
A(d-H-N) = Angle Between Donor-H-N
A(a-O=C) = Angle Between Acceptor-O=C
MO = Multiple Occupancy
Note that angles that are undefined are written as 999.99

Protein-Protein Main Chain-Side Chain Hydrogen Bonds



[help]

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[View the original hbond output]

DONOR				ACCEPTOR				PARAMETERS			
POS	**CHAIN**	**RES**	**ATOM**	**POS**	**CHAIN**	**RES**	**ATOM**	**MO**	**Dd-a**	**Dh-a**	**A(d-H-N)**	**A(a-O=C)**
86	A	ARG	NH1	305	C	THR	O	1	3.10	2.96	87.32	160.87
86	A	ARG	NH1	305	C	THR	O	2	3.10	2.60	110.50	160.87
86	A	ARG	NH1	307	C	ASP	O	1	3.00	3.83	32.23	116.95
86	A	ARG	NH1	307	C	ASP	O	2	3.00	2.06	154.28	116.95
86	A	ARG	NH2	307	C	ASP	O	1	3.27	4.19	24.10	116.95
86	A	ARG	NH2	307	C	ASP	O	2	3.27	2.43	139.80	116.95
129	A	GLN	NE2	349	C	GLU	O	1	2.96	2.63	97.59	116.50
129	A	GLN	NE2	349	C	GLU	O	2	2.96	2.55	103.35	116.50
351	A	ASP	N	129	C	GLN	NE2	-	3.40	3.50	76.35	101.57
352	A	HIS	N	132	C	ASP	OD1	-	3.38	2.88	113.20	82.13
352	A	HIS	N	132	C	ASP	OD2	-	2.78	1.83	163.94	110.14
353	A	HIS	N	132	C	ASP	OD1	-	3.03	2.19	146.03	132.91
378	A	THR	N	105	C	ASP	OD2	-	2.93	2.00	167.34	139.34
86	C	ARG	NH1	305	A	THR	O	1	3.10	2.96	87.34	160.87
86	C	ARG	NH1	305	A	THR	O	2	3.10	2.60	110.50	160.87
86	C	ARG	NH1	307	A	ASP	O	1	3.00	3.83	32.23	116.95
86	C	ARG	NH1	307	A	ASP	O	2	3.00	2.06	154.29	116.95
86	C	ARG	NH2	307	A	ASP	O	1	3.27	4.19	24.10	116.95
86	C	ARG	NH2	307	A	ASP	O	2	3.27	2.43	139.80	116.95
129	C	GLN	NE2	349	A	GLU	O	1	2.96	2.63	97.59	116.50
129	C	GLN	NE2	349	A	GLU	O	2	2.96	2.55	103.35	116.50
351	C	ASP	N	129	A	GLN	NE2	-	3.40	3.50	76.35	101.57
352	C	HIS	N	132	A	ASP	OD1	-	3.38	2.88	113.20	82.13
352	C	HIS	N	132	A	ASP	OD2	-	2.78	1.83	163.94	110.14

353	C	HIS	N	132	A	ASP	OD1	-	3.03	2.19	146.03	132.91
378	C	THR	N	105	A	ASP	OD2	-	2.93	2.00	167.33	139.34

Dd-a = Distance Between Donor and Acceptor
Dh-a = Distance Between Hydrogen and Acceptor
A(d-H-N) = Angle Between Donor-H-N
A(a-O=C) = Angle Between Acceptor-O=C
MO = Multiple Occupancy
Note that angles that are undefined are written as 999.99

Protein-Protein Side Chain-Side Chain Hydrogen Bonds



[help]

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[View the original hbond output]

DONOR				ACCEPTOR				PARAMETERS				
POS	**CHAIN**	**RES**	**ATOM**	**POS**	**CHAIN**	**RES**	**ATOM**	**MO**	**Dd-a**	**Dh-a**	**A(d-H-N)**	**A(a-O=C)**
3	A	ASN	OD1	3	C	ASN	ND2	1	2.87	2.05	131.91	999.99
3	A	ASN	OD1	3	C	ASN	ND2	2	2.87	3.53	44.41	999.99
3	A	ASN	ND2	3	C	ASN	OD1	1	2.87	1.84	167.21	999.99
3	A	ASN	ND2	3	C	ASN	OD1	2	2.87	3.43	49.63	999.99
3	A	ASN	ND2	3	C	ASN	ND2	1	3.37	2.71	120.21	999.99
3	A	ASN	ND2	3	C	ASN	ND2	2	3.37	4.24	28.07	999.99
10	A	ARG	NH1	14	C	GLU	OE1	1	3.16	3.79	46.54	999.99
10	A	ARG	NH1	14	C	GLU	OE1	2	3.16	2.15	168.78	999.99
10	A	ARG	NH1	14	C	GLU	OE2	1	3.48	4.45	19.03	999.99
10	A	ARG	NH1	14	C	GLU	OE2	2	3.48	2.75	128.19	999.99
10	A	ARG	NH2	14	C	GLU	OE2	1	2.84	3.55	40.65	999.99
10	A	ARG	NH2	14	C	GLU	OE2	2	2.84	1.87	159.05	999.99
12	A	ASP	OD2	352	C	HIS	NE2	1	2.80	1.90	140.30	999.99
12	A	ASP	OD2	352	C	HIS	NE2	2	2.80	3.04	66.60	999.99
46	A	TYR	OH	349	C	GLU	OE2	-	2.66	9.99	999.99	999.99
50	A	SER	OG	307	C	ASP	OD1	-	2.61	9.99	999.99	999.99
50	A	SER	OG	307	C	ASP	OD2	-	3.41	9.99	999.99	999.99
85	A	ARG	NH2	281	C	HIS	ND1	1	3.11	3.65	51.90	999.99
85	A	ARG	NH2	281	C	HIS	ND1	2	3.11	2.20	147.89	999.99
85	A	ARG	NH2	304	C	ASP	OD2	1	2.84	1.85	153.92	999.99
85	A	ARG	NH2	304	C	ASP	OD2	2	2.84	3.21	59.72	999.99
308	A	TYR	OH	44	C	ASP	OD2	-	2.62	9.99	999.99	999.99
352	A	HIS	NE2	12	C	ASP	OD2	-	2.80	1.93	159.35	999.99
356	A	ARG	NH1	386	C	ASP	OD2	1	2.68	3.45	36.11	999.99

356	A	ARG	NH1	386	C	ASP	OD2	2	2.68	1.69	161.99	999.99
356	A	ARG	NH2	386	C	ASP	OD2	1	3.26	4.24	17.88	999.99
356	A	ARG	NH2	386	C	ASP	OD2	2	3.26	2.53	127.91	999.99
378	A	THR	OG1	105	C	ASP	OD1	-	2.87	9.99	999.99	999.99
378	A	THR	OG1	105	C	ASP	OD2	-	3.20	9.99	999.99	999.99
381	A	SER	OG	12	C	ASP	OD2	-	2.77	9.99	999.99	999.99
3	C	ASN	OD1	3	A	ASN	ND2	1	2.87	2.05	132.02	999.99
3	C	ASN	OD1	3	A	ASN	ND2	2	2.87	3.53	44.40	999.99
3	C	ASN	ND2	3	A	ASN	OD1	1	2.87	1.84	167.21	999.99
3	C	ASN	ND2	3	A	ASN	OD1	2	2.87	3.43	49.63	999.99
3	C	ASN	ND2	3	A	ASN	ND2	1	3.37	2.71	120.21	999.99
3	C	ASN	ND2	3	A	ASN	ND2	2	3.37	4.24	28.07	999.99
10	C	ARG	NH1	14	A	GLU	OE1	1	3.16	3.79	46.54	999.99
10	C	ARG	NH1	14	A	GLU	OE1	2	3.16	2.15	168.78	999.99
10	C	ARG	NH1	14	A	GLU	OE2	1	3.48	4.45	19.03	999.99
10	C	ARG	NH1	14	A	GLU	OE2	2	3.48	2.75	128.19	999.99
10	C	ARG	NH2	14	A	GLU	OE2	1	2.84	3.55	40.65	999.99
10	C	ARG	NH2	14	A	GLU	OE2	2	2.84	1.87	159.05	999.99
12	C	ASP	OD2	352	A	HIS	NE2	1	2.80	1.90	140.27	999.99
12	C	ASP	OD2	352	A	HIS	NE2	2	2.80	3.04	66.60	999.99
46	C	TYR	OH	349	A	GLU	OE2	-	2.66	9.99	999.99	999.99
50	C	SER	OG	307	A	ASP	OD1	-	2.61	9.99	999.99	999.99
50	C	SER	OG	307	A	ASP	OD2	-	3.41	9.99	999.99	999.99
85	C	ARG	NH2	281	A	HIS	ND1	1	3.11	3.65	51.90	999.99
85	C	ARG	NH2	281	A	HIS	ND1	2	3.11	2.20	147.89	999.99
85	C	ARG	NH2	304	A	ASP	OD2	1	2.84	1.85	153.92	999.99
85	C	ARG	NH2	304	A	ASP	OD2	2	2.84	3.21	59.72	999.99
308	C	TYR	OH	44	A	ASP	OD2	-	2.62	9.99	999.99	999.99
352	C	HIS	NE2	12	A	ASP	OD2	-	2.80	1.93	159.35	999.99
356	C	ARG	NH1	386	A	ASP	OD2	1	2.68	3.45	36.11	999.99
356	C	ARG	NH1	386	A	ASP	OD2	2	2.68	1.69	161.99	999.99
356	C	ARG	NH2	386	A	ASP	OD2	1	3.26	4.24	17.88	999.99
356	C	ARG	NH2	386	A	ASP	OD2	2	3.26	2.53	127.91	999.99
378	C	THR	OG1	105	A	ASP	OD1	-	2.87	9.99	999.99	999.99

378	C	THR	OG1	105	A	ASP	OD2	-	3.20	9.99	999.99	999.99
381	C	SER	OG	12	A	ASP	OD2	-	2.77	9.99	999.99	999.99

Dd-a = Distance Between Donor and Acceptor
Dh-a = Distance Between Hydrogen and Acceptor
A(d-H-N) = Angle Between Donor-H-N
A(a-O=C) = Angle Between Acceptor-O=C
MO = Multiple Occupancy
Note that angles that are undefined are written as 999.99

Protein-Protein Ionic Interactions


[help]

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Ionic Interactions within 6 Angstroms

Position	Residue	Chain	Position	Residue	Chain
10	ARG	A	14	GLU	C
12	ASP	A	352	HIS	C
14	GLU	A	10	ARG	C
84	LYS	A	304	ASP	C
85	ARG	A	304	ASP	C
86	ARG	A	307	ASP	C
86	ARG	A	349	GLU	C
105	ASP	A	419	HIS	C
124	ARG	A	349	GLU	C
132	ASP	A	352	HIS	C
304	ASP	A	84	LYS	C
304	ASP	A	85	ARG	C
307	ASP	A	86	ARG	C
349	GLU	A	124	ARG	C
349	GLU	A	86	ARG	C

352	HIS	A	12	ASP	C
352	HIS	A	132	ASP	C
353	HIS	A	360	ASP	C
356	ARG	A	386	ASP	C
356	ARG	A	387	GLU	C
360	ASP	A	353	HIS	C
386	ASP	A	356	ARG	C
387	GLU	A	356	ARG	C
419	HIS	A	105	ASP	C

Protein-Protein Aromatic-Aromatic Interactions


[help]

Rasmol **Jmol**

Aromatic-Aromatic Interactions within 4.5 and 7 Angstroms

Residue	Position	Chain	Residue	Position	Chain	D(centroid-centroid)	Dihedral Angle
46	TYR	A	306	PHE	C	5.53	103.34
306	PHE	A	46	TYR	C	5.53	103.36

NO PROTEIN-PROTEIN AROMATIC-SULPHUR INTERACTIONS FOUND

Protein-Protein Cation-Pi Interactions


[help]

Rasmol **Jmol**

Cation-Pi Interactions within 6 Angstroms

Position	Residue	Chain	Position	Residue	Chain	D(cation-Pi)	Angle
127	PHE	A	314	LYS	C	4.04	159.40
127	PHE	C	314	LYS	A	4.05	159.24
308	TYR	A	84	LYS	C	4.82	39.54
308	TYR	A	86	ARG	C	5.30	30.49
308	TYR	C	84	LYS	A	4.82	39.81
308	TYR	C	86	ARG	A	5.30	30.18