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Zhiyong Guo, Lei Wang, Lingqia Su, Sheng Chen, Wei Xia, et al.. A Single Hydrogen Bond Controls the Selectivity of Transglycosylation vs Hydrolysis in Family 13 Glycoside Hydrolases. *Journal of Physical Chemistry Letters*, 2022, 13 (24), pp.5626-5632. 10.1021/acs.jpcclett.2c01136 . hal-03758367

HAL Id: hal-03758367

<https://hal.science/hal-03758367>

Submitted on 25 Oct 2022

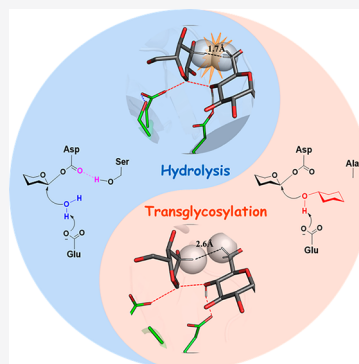
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A Single Hydrogen Bond Controls the Selectivity of Transglycosylation vs Hydrolysis in Family 13 Glycoside Hydrolases

Zhiyong Guo, Lei Wang, Lingqia Su, Sheng Chen, Wei Xia, Isabelle André, Carme Rovira,*
Binju Wang,* and Jing Wu*

ABSTRACT: Converting glycoside hydrolases (GHs) from hydrolytic to synthetic enzymes via transglycosylation is a long-standing goal for the biosynthesis of complex carbohydrates. However, the molecular determinants for the selectivity of transglycosylation (T) vs hydrolysis (H) are still not fully unraveled. Herein, we show experimentally that mutation of one active site residue can switch the enzyme activity between hydrolysis and transglycosylation in two highly homologous GHs. Further QM/MM simulations reveal that the mutation modulates the T vs H reaction barriers via the presence/absence of a single H-bond with the nucleophile Asp. Such a H-bond controls the product selectivity via a dual effect: on one hand, it facilitates the breaking of the glycosyl-enzyme intermediate. On the other, it displaces the sugar acceptor, resulting in a reduced affinity and significant steric repulsion for transglycosylation. These findings expand our understanding of the molecular mechanisms that modulate the T/H balance in GHs.

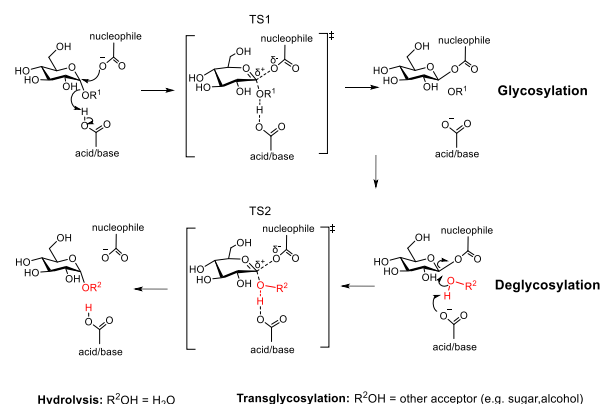


Biosynthesis of carbohydrates or glycosides is mainly catalyzed by glycosyltransferases (GTs).^{1–3} These enzymes require activated sugar phosphates as their donor substrates. However, sugar phosphates are usually difficult to obtain.⁴ In addition, heterologous expression of GTs is challenging in most cases. These two factors have greatly limited the *in vitro* use of GTs as catalysts for glycoside biosynthesis in the bioindustry.⁵ As an alternative avenue, the retaining glycoside hydrolases (GHs) can be exploited for carbohydrates synthesis via the transglycosylation or the glycosynthase reactions.^{6,7} GHs can recognize a broad range of substrates, which are normally not activated sugar phosphates and thus readily available.

The reaction mechanism of retaining GHs involves two key catalytic steps: glycosylation and deglycosylation (Scheme 1). In the glycosylation step, a catalytic nucleophile residue attacks the sugar anomeric center, while a catalytic acid/base residue (acting as a general acid) transfers a proton to the glycoside oxygen atom, leading to the formation of a glycosyl-enzyme covalent intermediate (GEI).^{8,9} In the subsequent deglycosylation step, an acceptor molecule (water or sugar) attacks the anomeric carbon and transfers a proton to the acid/base residue (now acting as a general base).

If the acceptor is a water molecule, the deglycosylation step leads to hydrolysis. Alternatively, if the acceptor is a sugar molecule, the process results in the synthesis of a new carbohydrate, and it is known as transglycosylation (Scheme 1). Although more than 160 GH families have been characterized to date,^{10–12} most of them primarily catalyze the hydrolysis of carbohydrates and therefore are classified as

Scheme 1. Double-Displacement Mechanisms of Retaining GHs



hydrolytic GHs. Only a few of them^{13–18} display transglycosylation activities.

In recent years, many practical strategies have been developed to convert specific GHs into TGs,^{5,19–25} such as the use of an activated glycosyl donor,¹⁹ the inactivation of the

nucleophile,²⁶ or enzyme engineering at both donor and acceptor sites.²⁷ Concerning enzyme engineering at acceptor sites, it was found that the transglycosylation vs hydrolysis activity ratio (T/H) can be improved by either enhancing the hydrophobicity of the active site or increasing the binding strength of sugar acceptors.^{20–24} Additionally, alteration of the water dynamics could be beneficial to the transglycosylation activity.²⁵ Concerning enzyme engineering at donor sites, it was speculated that mutation of conserved residues at the –1 subsite may destabilize the transition state of hydrolysis compared to that of transglycosylation, leading to increased T/H ratios.^{28–30} However, the molecular determinants for the selectivity of T/H, especially regarding how TGs can suppress hydrolysis in favor of transglycosylation, have not been fully unraveled yet.³¹

In order to address the aforementioned long-standing issues, we herein investigate two similar enzymes that strongly differ in their T/H ratio by a combination of experimental and computational methods. Specifically, we focus on amylases (ASs, EC 2.4.1.4) from *Neisseria polysaccharea* (NpAS)^{32–36} and *Deinococcus geothermalis* (DgAS)³⁷ as well as their hydrolase counterparts, sucrose hydrolases (SHs, EC 3.2.1.-) from *Xanthomonas axonopodis* (XaSH, 57% identical to NpAS)³⁸ and *Caulobacter crescentus* (CcSH). Both enzymes are members of the GH family 13. ASs convert sucrose into amylose-like α -(1,4)-glucans (Supporting Information, Figure S1), while SHs display almost exclusive preferences for hydrolysis, converting sucrose into equal amounts of D-glucose and D-fructose. Our experimental results show that a single mutation (serine to alanine) at the –1 subsite in SHs convert hydrolytic enzymes into TGs and, conversely, introducing the reverse mutation (alanine to serine) in ASs convert TGs into hydrolytic enzymes. Using a combination of molecular dynamics (MD) simulations and quantum mechanics/molecular mechanics-based metadynamics (QM/MM MetD), we unravel the molecular determinants of this conversion, revealing the specific role of a single hydrogen bond in modulating the balance between transglycosylation and hydrolysis.

As a first step in our investigation, we performed a multiple sequence alignment to identify residues that could be responsible for the T/H preferences among ASs and SHs (Figures S2–S5). The conserved residues Ala287/Ser281 (in NpAS/XaSH), which forms hydrogen bond interactions with the nucleophile, were identified as being key for the T/H selectivity. Therefore, site-directed mutations were focused on the swapping of Ala/Ser in ASs and SHs.

To determine the effect of the Ala/Ser single mutation on the hydrolysis and transglycosylation activities, the T/H ratios of wild-type and mutants at a single donor concentration (D-sucrose) were determined without an acceptor. In such a case, hydrolysis and transglycosylation compete to reach an equilibrium. The product of hydrolysis is D-glucose, whereas the main product of transglycosylation can be either an α -1,4 glucan with a high degree of polymerization for ASs or an oligosaccharide with a low degree of polymerization for SHs (Figure S6). The measured T/H ratio reflects the preference for transglycosylation vs hydrolysis. As shown in Table 1, NpAS_{A287S} reduces the T/H ratio by 59-fold (from 8.80 to 0.15) with respect to the wild-type NpAS. The opposite trend happens in XaSH_{S281A}, which increases the T/H ratio by 28-fold (from 0.05 to 1.40). Therefore, the single mutation can completely shift the selectivity from specific hydrolysis to

Table 1. Transglycosylation and Hydrolysis Ratio Measured for all GH13 Enzymes Investigated and Their Corresponding Ala/Ser Mutants^a

enzyme	transglycosylation (%)	hydrolysis (%)	T/H
NpAS	89.8 ± 1.3	10.2 ± 0.4	8.80
NpAS _{A287S}	12.9 ± 1.2	87.1 ± 1.2	0.15
XaSH	5.1 ± 1.8	94.9 ± 1.8	0.05
XaSH _{S281A}	58.4 ± 6.0	41.6 ± 1.1	1.40
DgAS	87.7 ± 1.9	12.3 ± 0.8	7.13
DgAS _{A285S}	16.5 ± 2.8	83.5 ± 2.9	0.20
CcSH	6.4 ± 0.3	93.6 ± 0.3	0.07
CcSH _{S271A}	52.7 ± 5.4	47.3 ± 0.4	1.11

^aThe T/H ratio was measured as a percentage of the reaction products once the reaction reached final equilibrium (24 h) using the natural donor sucrose as the donor. All reactions were performed for 24 h at 30 °C in a 10 mL reaction system (pH = 7.0) containing 0.1 mg/mL enzyme (DgAS, XaSH, CcSH, XaSH_{S281A}, and CcSH_{S271A}), 0.2 mg/mL enzyme (NpAS) or 0.4 mg/mL enzyme (NpAS_{A287S} and DgAS_{A285S}).

transglycosylation in XaSH and *vice versa* for NpAS (Figure S7).

Subsequently, kinetic parameter analyses were performed (Table S1). Sucrose was used as the donor substrate for both hydrolysis and transglycosylation, while D-glucose was used as the acceptor for transglycosylation only.³⁹ In these conditions, the transglycosylation product is D-maltose. Wild-type NpAS has a high preference for transglycosylation, with a $k_{\text{cat}}^{\text{T}}/k_{\text{cat}}^{\text{H}}$ value of 13.43. However, upon A287S mutation, the transglycosylation activity ($k_{\text{cat}}^{\text{T}}$) decreased significantly (0.7 s^{–1} in NpAS_{A287S} versus 9.4 s^{–1} in NpAS). Meanwhile, the hydrolysis activity ($k_{\text{cat}}^{\text{H}}$) is enhanced (1.9 s^{–1} in NpAS_{A287S} versus 0.7 s^{–1} in NpAS). Likewise, XaSH exhibits high preference for hydrolysis, with a $k_{\text{cat}}^{\text{T}}/k_{\text{cat}}^{\text{H}}$ value of 0.02, while the Ser281 → Ala281 mutation in XaSH switches the hydrolysis and transglycosylation activities, with the transglycosylation being preferred over hydrolysis ($k_{\text{cat}}^{\text{T}}/k_{\text{cat}}^{\text{H}} \sim 1.7$). Similar results were obtained for another pair of enzymes of the same family (DgAS, a transglycosylase, and CcSH, a hydrolase) (Figure S8).

Overall, our experimental results for both single and double concentration models demonstrate that the Ala → Ser mutation convert ASs into hydrolytic enzymes, while the corresponding reverse mutation, Ser → Ala, convert SHs into transglycosylases. Therefore, only one amino acid mutation (Ala/Ser) can remarkably modulate the product selectivity (T/H) in ASs and SHs.

To get further insight into the effect of a single mutation on the T/H ratio, we performed QM/MM-based metadynamics simulations of the hydrolysis and transglycosylation reactions in NpAS and XaSH. As both reactions share the first step of the reaction (glycosylation, Scheme 1), we focused on the second step (deglycosylation), which determines the T/H balance. The glucose molecule (the natural acceptor) was docked into the active site to model the transglycosylation reaction. The chemical reaction was driven by three collective variables (Figure S29): the first one (CV1) measures the degree of GEI bond cleavage, the second one (CV2) quantifies the degree of nucleophilic attack by the acceptor (water or glucose), and the third one (CV3) considers proton transfer from the acceptor to the acid/base residue.

For NpAS, the hydrolysis reaction starts with the nucleophilic attack of the catalytic water on the anomeric

carbon atom C1 (Figure 1A, Table S2, Movie S1). As the catalytic water molecule approaches the C1 atom, its covalent

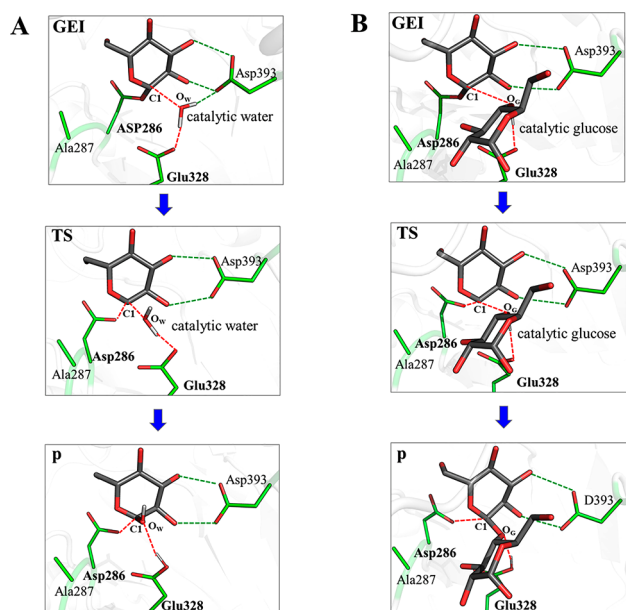


Figure 1. Representative snapshots from QM/MM modeling of hydrolysis (A) and transglycosylation (B) in *NpAS*. TS, transition state; P, product.

bond distance with Asp286 ($C1-O_{\delta 1}$) gradually increases. In line with previous studies, both hydrolysis and transglycosylation reactions transpire via a highly asynchronous and dissociative D_NA_N mechanism^{4041–43} (see Movie S2, Figure 1B, Figure S9 and Table S3). The reaction free energy barrier for transglycosylation is 8.2 kcal/mol (Table 2), which

Table 2. Computed Reaction Free Energy Barriers (in kcal/mol) for Hydrolysis and Transglycosylation in *XaSH* and *XaSH_{S281A}*^a

enzyme	ΔG_H^\ddagger	ΔG_T^\ddagger
<i>NpAS</i>	8.9	8.2
<i>NpAS_{A287S}</i>	7.1	9.6
<i>XaSH</i>	10.3	20.3
<i>XaSH_{S281A}</i>	13.5	9.6

^a ΔG_H^\ddagger , free energy barrier for the hydrolysis reaction; ΔG_T^\ddagger , free energy barrier for the transglycosylation reaction.

is 0.7 kcal/mol lower than that of hydrolysis, indicating that transglycosylation is favored over hydrolysis. Such a finding is in line with the experimental value (Table S1). Noteworthy, the computed structure of the enzyme complex with the transglycosylation product is in good agreement with the crystal structure of the enzyme complex with the natural substrate, which occupies the same −1 and +1 subsites (Figure S10).

For *NpAS_{A287S}*, both the hydrolysis and transglycosylation mechanisms turned to be similar to those in the wild-type enzyme (see Movies S3 and S4, Figures S11 and S12 and Tables S4 and S5). The computed free energy barrier of hydrolysis in *NpAS_{A287S}* is 7.1 kcal/mol (Table 2). For transglycosylation, the calculated barrier is 9.6 kcal/mol, which is 2.5 kcal/mol higher than the hydrolysis reaction. These results show that the balance between hydrolysis and

transglycosylation has been reversed in *NpAS_{A287S}* compared to the wild-type enzyme, i.e., *NpAS* changes its activity preference from transglycosylation to hydrolysis upon the A287S mutation. These findings are consistent with our experimental data, which show that the T/H ratio has been fully reversed in *NpAS_{A287S}* compared to wild-type *NpAS* (Table S1).

To verify the reverse effects of Ser → Ala mutation in *XaSH*, we conducted similar MD and QM/MM-MD simulations in *XaSH* and *XaSH_{S281A}*. The catalytic mechanism of *XaSH* turned out to be very similar to *NpAS* (see Figures S13–S16, Tables S6–S9, and Movies S5–S8). In WT *XaSH*, hydrolysis and transglycosylation show energy barriers of 10.3 and 20.3 kcal/mol, respectively (Table 2). This is in line with our experimental results that WT *XaSH* is dominated by hydrolysis (Table S1), while the transglycosylation activity is negligible.⁴⁴ However, the energy barrier of hydrolysis increases to 13.5 kcal/mol in *XaSH_{S281A}* upon Ser → Ala mutation, while the barrier of transglycosylation is significantly reduced to 9.6 kcal/mol. Therefore, the activity preference has shifted to transglycosylation. All these findings are basically in line with our experiments.

To identify the root cause of Ala → Ser mutation on the T/H balance, we superimposed the MD-equilibrated structures of the glycosyl-enzyme intermediate (GEI) for the WT *NpAS* and *NpAS_{A287S}*, as shown in Figure 2A. This revealed that, in *NpAS_{A287S}*, the hydroxyl group of Ser287 forms a tight hydrogen bond (present over 95% time of the MD simulation) with the catalytic nucleophile (Asp286) (see also Figure S17 and Table S10). The hydroxyl O_S atom of Ser287 maintains an average distance of around 2.7 Å with the $O_{\delta 2}$ atom of Asp286, while its backbone −NH group forms a hydrogen bond with the catalytic acid/base residue (Glu328). Compared to the WT *NpAS*, the new hydrogen bond causes the movement and rotation of Glu328 and significantly displaces the sugar acceptor from its position in the wild-type enzyme, weakening the interaction between the sugar and Arg446 (Table S11).

Indeed, a linear interaction energy analysis (Figure 2B) shows that nonbonding interactions between the sugar acceptor and the protein are significantly reduced in *NpAS_{A287S}*, compared with those in wild-type *NpAS*. Altogether, the simulations predict a decreased binding affinity of the sugar acceptor upon mutation. This is consistent with our kinetic results showing that the K_m value for the sugar acceptor increases in *NpAS_{A287S}* (Table S1). A similar trend was observed in chitinases of GH family 18.²⁴ Noteworthy, the displacement of the sugar also creates a large cavity around Arg446, which allows solvent molecules to penetrate into the active site, facilitating hydrolysis (W1 and W2 in A). We observed a similar phenomenon in the hydrolase *XaSH* (WI in Figure S19a).

Analysis of the transition state structures for transglycosylation, evidence that the presence of the Ser... nucleophile H-bond introduces significant steric repulsion, as shown in Figure 3A. Due to the H-bond induced rotation of the sugar acceptor (Figure S18), the pyranose ring at the −1 subsite and the acceptor glucose suffer from significant steric repulsion at the TS of transglycosylation, which results in a high barrier of transglycosylation in *XaSH*. By contrast, the S → A mutation in *XaSH_{S281A}* remarkably reduces the steric repulsion (Figure 3B), resulting in a low barrier of transglycosylation (Table 2). A similar scenario was observed in *NpAS* (Figure S19b,c).

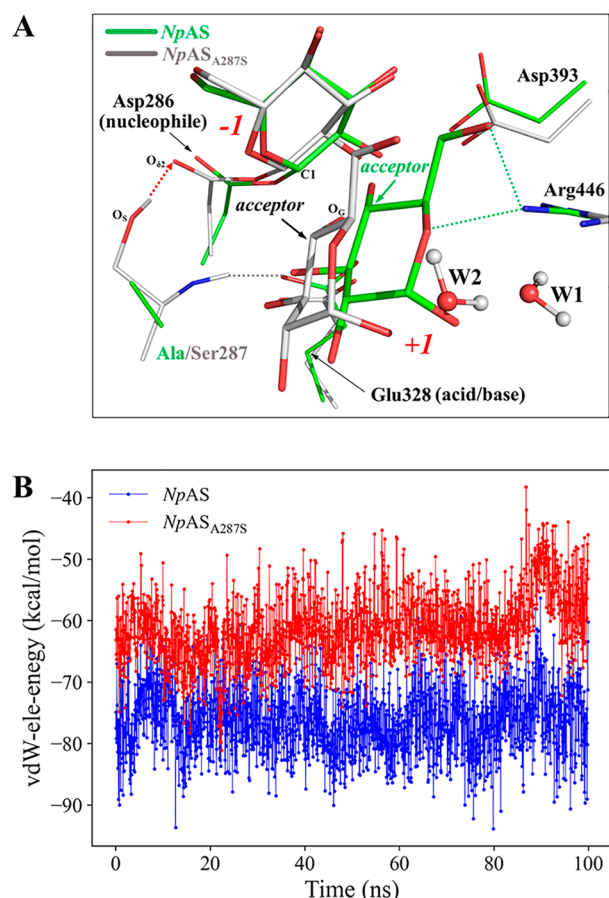


Figure 2. (A) Superposition of the GEI of *NpAS* (green carbon atoms) and *NpAS*_{A287S} (gray carbon atoms) bound to glucose. The hydrogen bond between Ser287 and the nucleophile is shown as a red dashed line. The hydrogen bond between the backbone -NH group of Ser287 and the oxygen atom of Glu328 in the wild-type enzyme is shown as a gray dotted line. The hydrogen bond between the glucose and Arg446 in the wild-type enzyme is shown as green dotted lines. For the sake of clarity, only H atoms bound to O and N of Ser287, as well as those of water molecules, are shown. (B) Calculated electrostatic (ele) and van der Waals (vdW) interaction energies between glucose and protein along the MD trajectory in *NpAS* and *NpAS*_{A287S} using linear interaction energy analysis.

In summary, our simulations clearly reveal the specific role of a single H-bond in modulating the balance between hydrolysis and transglycosylation in GH13 enzymes by dual effects. On one hand, the Ser...nucleophile hydrogen bond facilitates the glycosyl-enzyme covalent bond (C1-O_{δ1}) cleavage during both hydrolysis and transglycosylation (Table S12). On the other hand, it disrupts the binding and positioning of the sugar acceptor, resulting in a reduced enzyme affinity for the sugar acceptor and significant steric repulsion between it and the pyranose ring of the -1 subsite during the transglycosylation reaction. These dual effects increase hydrolysis but reduce transglycosylation activity, decreasing the T/H ratio. These findings could be extended to other members of GH families for which active site interactions have been suggested to play a role in the T/H ratio (Figure S20, Table S13).^{39,45–48} The insights on the key roles of hydrogen bonds in controlling T/H preferences provided here could also be useful in the engineering of other GHs toward the synthesis of new carbohydrates.

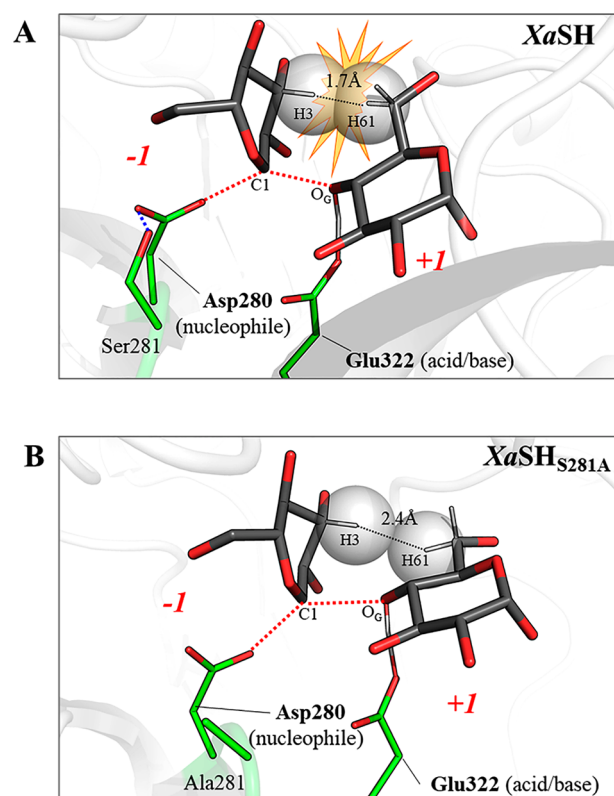


Figure 3. Comparison of transition state structures in *XaSH* and *XaSH*_{S281A}. The two closest H atoms between the pyranose ring at the -1 subsite and the acceptor glucose are represented as van der Waals spheres.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jpclett.2c01136>.

Bacterial strains and materials; cloning and mutagenesis; protein production and purification; enzyme assay and measurement of kinetic parameters; biosynthesis and HPLC analysis; high-performance anion exchange chromatography analysis; NMR spectroscopy analysis; classical MD simulations; QM/MM MD simulations; QM/MM metadynamics simulations; pK_a calculations; and Cartesian coordinates (PDF)

Movie S1, reaction details of hydrolysis reaction catalyzed by *NpAS*; Movie S2, reaction details of the transglycosylation reaction catalyzed by *NpAS*; Movie S3, reaction details of the hydrolysis reaction catalyzed by *NpAS*_{A287S}; Movie S4, reaction details of the transglycosylation reaction catalyzed by *NpAS*_{A287S}; Movie S5, reaction details of the hydrolysis reaction catalyzed by *XaSH*; Movie S6, reaction details of the transglycosylation reaction catalyzed by *XaSH*; Movie S7, reaction details of the hydrolysis reaction catalyzed by *XaSH*_{S281A} mutant; and Movie S8, reaction details of the transglycosylation reaction catalyzed by *XaSH*_{S281A} mutant (ZIP)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This study was financially supported by the National Natural Science Foundation of China (NSFC; Grants 31730067 and 21933009), the National Science Fund for Distinguished

Young Scholars (Grant 31425020), the NSFC (Grants 22073077, 32001637, and 31972032), the Spanish Ministry of Science and Innovation (Grants PID2020-118893GB-I00 and MDM-2017-0767), the European Research Council (Grant ERC-2020-SyG-95123), the National First-Class Discipline Program of Light Industry Technology and Engineering (Grant LITE2018-03), and the Postgraduate Research & Practice Innovation Program of Jiangsu Province (Grant KYCX19_1839).

REFERENCES

- (1) Breton, C.; Fournel-Gigleux, S.; Palcic, M. M. Recent Structures, Evolution and Mechanisms of Glycosyltransferases. *Curr. Opin. Struct. Biol.* **2012**, *22* (5), 540–549.
- (2) Nidetzky, B.; Gutmann, A.; Zhong, C. Leloir Glycosyltransferases as Biocatalysts for Chemical Production. *ACS Catal.* **2018**, *8* (7), 6283–6300.
- (3) Weijers, C. A. G. M.; Franssen, M. C. R.; Visser, G. M. Glycosyltransferase-Catalyzed Synthesis of Bioactive Oligosaccharides. *Biotechnol. Adv.* **2008**, *26* (5), 436–456.
- (4) Gantt, R. W.; Peltier-Pain, P.; Cournoyer, W. J.; Thorson, J. S. Using Simple Donors to Drive the Equilibria of Glycosyltransferase-Catalyzed Reactions. *Nat. Chem. Biol.* **2011**, *7* (10), 685–691.
- (5) Bissaro, B.; Monsan, P.; Faure, R.; O'Donohue, M. J. Glycosynthesis in a Waterworld: New Insight into the Molecular Basis of Transglycosylation in Retaining Glycoside Hydrolases. *Biochem. J.* **2015**, *467* (1), 17–35.
- (6) Malet, C.; Planas, A. From B-Glucanase to B-Glucansynthase: Glycosyl Transfer to A-Glycosyl Fluorides Catalyzed by a Mutant Endoglucanase Lacking Its Catalytic Nucleophile. *FEBS Lett.* **1998**, *440* (1), 208–212.
- (7) Mackenzie, L. F.; Wang, Q.; Warren, R. A. J.; Withers, S. G. Glycosynthases: Mutant Glycosidases for Oligosaccharide Synthesis. *J. Am. Chem. Soc.* **1998**, *120* (22), 5583–5584.
- (8) Vocadlo, D. J.; Davies, G. J.; Laine, R.; Withers, S. G. Catalysis by Hen Egg-White Lysozyme Proceeds Via a Covalent Intermediate. *Nature* **2001**, *412* (6849), 835–838.
- (9) Ardevol, A.; Rovira, C. Reaction Mechanisms in Carbohydrate-Active Enzymes: Glycoside Hydrolases and Glycosyltransferases. Insights from Ab Initio Quantum Mechanics/Molecular Mechanics Dynamic Simulations. *J. Am. Chem. Soc.* **2015**, *137* (24), 7528–7547.
- (10) Henrissat, B. A Classification of Glycosyl Hydrolases Based on Amino Acid Sequence Similarities. *Biochem. J.* **1991**, *280* (2), 309–316.
- (11) Lombard, V.; Golaconda Ramulu, H.; Drula, E.; Coutinho, P. M.; Henrissat, B. The Carbohydrate-Active Enzymes Database (CAZy) in 2013. *Nucleic Acids Res.* **2014**, *42* (D1), D490–D495.
- (12) Drula, E.; Garron, M.-L.; Dogan, S.; Lombard, V.; Henrissat, B.; Terrapon, N. The Carbohydrate-Active Enzyme Database: Functions and Literature. *Nucleic Acids Res.* **2022**, *50* (D1), D571–D577.
- (13) Monsan, P.; Remaud-Simeon, M.; Andre, I. Transglucosidases as Efficient Tools for Oligosaccharide and Glucoconjugate Synthesis. *Curr. Opin. Microbiol.* **2010**, *13* (3), 293–300.
- (14) Gangoiiti, J.; Pijning, T.; Dijkhuizen, L. Biotechnological Potential of Novel Glycoside Hydrolase Family 70 Enzymes Synthesizing Alpha-Glucans from Starch and Sucrose. *Biotechnol. Adv.* **2018**, *36* (1), 196–207.
- (15) Uitdehaag, J. C. M.; Mosi, R.; Kalk, K. H.; van der Veen, B. A.; Dijkhuizen, L.; Withers, S. G.; Dijkstra, B. W. X-Ray Structures Along the Reaction Pathway of Cyclodextrin Glycosyltransferase Elucidate Catalysis in the A-Amylase Family. *Nat. Struct. Biol.* **1999**, *6* (5), 432–436.
- (16) Montagna, G.; Cremona, M. L.; Paris, G.; Amaya, M. F.; Buschiazzi, A.; Alzari, P. M.; Frasch, A. C. The Trans-Sialidase from the African Trypanosome *Trypanosoma Brucei*. *Eur. J. Biochem.* **2002**, *269* (12), 2941–2950.
- (17) Baumann, M. J.; Eklöf, J. M.; Michel, G.; Kallas, A. S. M.; Teeri, T. T.; Czjzek, M.; Brumer, H., III Structural Evidence for the

Evolution of Xyloglucanase Activity from Xyloglucan Endo-Transglycosylases: Biological Implications for Cell Wall Metabolism. *Plant Cell* **2007**, *19* (6), 1947–1963.

(18) Aguilera, B.; Ghauharali-van der Vlugt, K.; Helmond, M. T. J.; Out, J. M. M.; Donker-Koopman, W. E.; Groener, J. E. M.; Boot, R. G.; Renkema, G. H.; van der Marel, G. A.; van Boom, J. H.; et al. Transglycosidase Activity of Chitotriosidase: Improved Enzymatic Assay for the Human Macrophage Chitinase *. *J. Biol. Chem.* **2003**, *278* (42), 40911–40916.

(19) Williams, S. J.; Withers, S. G. Glycosyl Fluorides in Enzymatic Reactions. *Carbohydr. Res.* **2000**, *327* (1), 27–46.

(20) Madhuprakash, J.; Dalhus, B.; Rani, T. S.; Podile, A. R.; Eijssink, V. G. H.; Sorlie, M. Key Residues Affecting Transglycosylation Activity in Family 18 Chitinases: Insights into Donor and Acceptor Subsites. *Biochemistry* **2018**, *57* (29), 4325–4337.

(21) Ortiz-Soto, M. E.; Possiel, C.; Görl, J.; Vogel, A.; Schmiedel, R.; Seibel, J. Impaired Coordination of Nucleophile and Increased Hydrophobicity in the + 1 Subsite Shift Levansucrase Activity Towards Transfructosylation. *Glycobiology* **2017**, *27* (8), 755–765.

(22) Durand, J.; Biarnés, X.; Watterlot, L.; Bonzom, C.; Borsenberger, V.; Planas, A.; Bozonnet, S.; O'Donohue, M. J.; Fauré, R. A Single Point Mutation Alters the Transglycosylation/Hydrolysis Partition, Significantly Enhancing the Synthetic Capability of an Endo-Glycoceramidase. *ACS Catal.* **2016**, *6* (12), 8264–8275.

(23) Rosengren, A.; Reddy, S. K.; Sjöberg, J. S.; Aurelius, O.; Logan, D. T.; Kolenová, K.; Ståhlbrand, H. An *Aspergillus* *Nidulans* B-Mannanase with High Transglycosylation Capacity Revealed through Comparative Studies within Glycosidase Family 5. *Appl. Microbiol. Biotechnol.* **2014**, *98* (24), 10091–10104.

(24) Zakariassen, H.; Hansen, M. C.; Jøranli, M.; Eijssink, V. G. H.; Sorlie, M. Mutational Effects on Transglycosylating Activity of Family 18 Chitinases and Construction of a Hypertransglycosylating Mutant. *Biochemistry* **2011**, *50* (25), 5693–5703.

(25) David, B.; Irague, R.; Jouanneau, D.; Daligault, F.; Czjzek, M.; Sanejouand, Y.-H.; Tellier, C. Internal Water Dynamics Control the Transglycosylation/Hydrolysis Balance in the Agarase (AgaD) of *Zobellia* *Galactanivorans*. *ACS Catal.* **2017**, *7* (5), 3357–3367.

(26) Faijes, M.; Pérez, X.; Pérez, O.; Planas, A. Glycosynthase Activity of *Bacillus* *Licheniformis* 1,3–1,4-B-Glucanase Mutants: Specificity, Kinetics, and Mechanism. *Biochemistry* **2003**, *42* (45), 13304–13318.

(27) Abdul Manas, N. H.; Md. Illias, R.; Mahadi, N. M. Strategy in Manipulating Transglycosylation Activity of Glycosyl Hydrolase for Oligosaccharide Production. *Crit. Rev. Biotechnol.* **2018**, *38* (2), 272–293.

(28) Teze, D.; Zhao, J.; Wiemann, M.; Kazi, Z. G. A.; Lupo, R.; Zeuner, B.; Vuillemin, M.; Rønne, M. E.; Carlström, G.; Duus, J. Ø.; et al. Rational Enzyme Design without Structural Knowledge: A Sequence-Based Approach for Efficient Generation of Transglycosylases. *Chem.—Eur. J.* **2021**, *27* (40), 10323–10334.

(29) Zhao, J.; Tandrup, T.; Bissaro, B.; Barbe, S.; Poulsen, J.-C. N.; André, I.; Dumon, C.; Lo Leggio, L.; O'Donohue, M. J.; Fauré, R. Probing the Determinants of the Transglycosylation/Hydrolysis Partition in a Retaining A-L-Arabinofuranosidase. *N. Biotechnol.* **2021**, *62*, 68–78.

(30) Teze, D.; Daligault, F.; Ferrières, V.; Sanejouand, Y.-H.; Tellier, C. Semi-Rational Approach for Converting a GH36 A-Glycosidase into an A-Transglycosidase. *Glycobiology* **2015**, *25* (4), 420–427.

(31) Raich, L.; Borodkin, V.; Fang, W.; Castro-López, J.; van Aalten, D. M. F.; Hurtado-Guerrero, R.; Rovira, C. A Trapped Covalent Intermediate of a Glycoside Hydrolase on the Pathway to Transglycosylation. Insights from Experiments and Quantum Mechanics/Molecular Mechanics Simulations. *J. Am. Chem. Soc.* **2016**, *138* (10), 3325–3332.

(32) Daudé, D.; Vergès, A.; Cambon, E.; Emond, S.; Tranier, S.; André, I.; Remaud-Siméon, M. Neutral Genetic Drift-Based Engineering of a Sucrose-Utilizing Enzyme toward Glycodiversification. *ACS Catal.* **2019**, *9* (2), 1241–1252.

(33) Tian, Y.; Xu, W.; Zhang, W.; Zhang, T.; Guang, C.; Mu, W. Amylosucrase as a Transglucosylation Tool: From Molecular Features to Bioengineering Applications. *Biotechnol. Adv.* **2018**, *36* (5), 1540–1552.

(34) Champion, E.; Guérin, F.; Moulis, C.; Barbe, S.; Tran, T. H.; Morel, S.; Descroix, K.; Monsan, P.; Mourey, L.; Mulard, L. A.; et al. Applying Pairwise Combinations of Amino Acid Mutations for Sorting out Highly Efficient Glucosylation Tools for Chemo-Enzymatic Synthesis of Bacterial Oligosaccharides. *J. Am. Chem. Soc.* **2012**, *134* (45), 18677–18688.

(35) Skov, L. K.; Mirza, O.; Henriksen, A.; De Montalk, G. P.; Remaud-Simeon, M.; Sarcabal, P.; Willemot, R. M.; Monsan, P.; Gajhede, M. Amylosucrase, a Glucan-Synthesizing Enzyme from the Alpha-Amylase Family. *J. Biol. Chem.* **2001**, *276* (27), 25273–25278.

(36) Champion, E.; André, I.; Moulis, C.; Boutet, J.; Descroix, K.; Morel, S.; Monsan, P.; Mulard, L. A.; Remaud-Siméon, M. Design of A-Transglucosidases of Controlled Specificity for Programmed Chemoenzymatic Synthesis of Antigenic Oligosaccharides. *J. Am. Chem. Soc.* **2009**, *131* (21), 7379–7389.

(37) Guérin, F.; Barbe, S.; Pizzut-Serin, S.; Potocki-Véronèse, G.; Guieysse, D.; Guillet, V.; Monsan, P.; Mourey, L.; Remaud-Siméon, M.; André, I.; et al. Structural Investigation of the Thermostability and Product Specificity of Amylosucrase from the Bacterium *Deinococcus Geothermalis*. *J. Biol. Chem.* **2012**, *287* (9), 6642–6654.

(38) Kim, H.-S.; Park, H.-J.; Heu, S.; Jung, J. Molecular and Functional Characterization of a Unique Sucrose Hydrolase from *Xanthomonas Axonopodis* Pv. *Glycines*. *J. Bacteriol.* **2004**, *186* (2), 411–418.

(39) Bissaro, B.; Durand, J.; Biarnés, X.; Planas, A.; Monsan, P.; O'Donohue, M. J.; Fauré, R. Molecular Design of Non-Leloir Furanose-Transferring Enzymes from an α -L-Arabinofuranosidase: A Rationale for the Engineering of Evolved Transglycosylases. *ACS Catal.* **2015**, *5* (8), 4598–4611.

(40) Alonso-Gil, S.; Coines, J.; Andre, I.; Rovira, C. Conformational Itinerary of Sucrose During Hydrolysis by Retaining Amylosucrase. *Front. Chem.* **2019**, *7*, 269.

(41) Raich, L.; Borodkin, V.; Fang, W.; Castro-Lopez, J.; van Aalten, D. M.; Hurtado-Guerrero, R.; Rovira, C. A Trapped Covalent Intermediate of a Glycoside Hydrolase on the Pathway to Transglycosylation. Insights from Experiments and Quantum Mechanics/Molecular Mechanics Simulations. *J. Am. Chem. Soc.* **2016**, *138* (10), 3325–3332.

(42) Romero-Tellez, S.; Lluch, J. M.; Gonzalez-Lafont, A.; Masgrau, L. Comparing Hydrolysis and Transglycosylation Reactions Catalyzed by *Thermophilus Beta-Glycosidase*. A Combined Md and QM/MM Study. *Front. Chem.* **2019**, *7* (200), 200.

(43) Jitonnorn, J.; Ketudat-Cairns, J. R.; Hannongbua, S. QM/MM Modeling of the Hydrolysis and Transfructosylation Reactions of Fructosyltransferase from *Aspergillus Japonicas*, an Enzyme That Produces Prebiotic Fructooligosaccharide. *J. Mol. Graphics Model.* **2018**, *79*, 175–184.

(44) Kim, M. I.; Kim, H. S.; Jung, J.; Rhee, S. Crystal Structures and Mutagenesis of Sucrose Hydrolase from *Xanthomonas Axonopodis* Pv. *Glycines*: Insight into the Exclusively Hydrolytic Amylosucrase Fold. *J. Mol. Biol.* **2008**, *380* (4), 636–647.

(45) Teze, D.; Hendrickx, J.; Czjzek, M.; Ropartz, D.; Sanejouand, Y. H.; Tran, V.; Tellier, C.; Dion, M. Semi-Rational Approach for Converting a GH1 Beta-Glycosidase into a Beta-Transglycosidase. *Protein Eng. Des. Sel.* **2014**, *27* (1), 13–19.

(46) Ortiz-Soto, M. E.; Possiel, C.; Görl, J.; Vogel, A.; Schmiedel, R.; Seibel, J. Impaired Coordination of Nucleophile and Increased Hydrophobicity in the + 1 Subsite Shift Levansucrase Activity Towards Transfructosylation. *Glycobiology* **2017**, *27* (8), 755–765.

(47) Schroeven, L.; Lammens, W.; Van Laere, A.; Van den Ende, W. Transforming Wheat Vacuolar Invertase into a High Affinity Sucrose:Sucrose 1-Fructosyltransferase. *New Phytol.* **2008**, *180* (4), 822–831.

(48) Ritsema, T.; Hernández, L.; Verhaar, A.; Altenbach, D.; Boller, T.; Wiemken, A.; Smeekens, S. Developing Fructan-Synthesizing

Capability in a Plant Invertase Via Mutations in the Sucrose-Binding
Box. *Plant J.* **2006**, 48 (2), 228–237.