Crystal Structure of $\alpha$-Xylosidase from *Aspergillus niger* in Complex with a Hydrolyzed Xyloglucan Product and New Insights in Accurately Predicting Substrate Specificities of GH31 Family Glycosidases

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**ABSTRACT:** Glycoside hydrolase family 31 (GH31) enzymes show both highly conserved folds and catalytic residues. Yet different members of GH31 show very different substrate specificities, and it is not obvious how these specificities arise from the protein sequences. The fungal $\alpha$-xylanase, AxlA, was originally isolated from a commercial enzyme mixture secreted by *Aspergillus niger* and was reported to have potential as a catalytic component in biomass deconstruction in the biofuel industry. We report here the crystal structure of AxlA in complex with its catalytic product, a hydrolyzed xyloglucan oligosaccharide. On the basis of our new structure, we provide the structural basis for AxlA’s role in xyloglucan utilization and, more importantly, a new procedure to predict and differentiate C5 vs C6 sugar specific activities based on protein sequences of the functionally diverse GH31 family enzymes.

**KEYWORDS:** $\alpha$-Xylosidase, Crystal structure, Aspergillus niger, Biomass deconstruction, GH31, Specificity

**INTRODUCTION**

The past two decades have witnessed a boost in lignocellulose-based bioenergy research led by US DOE Bioenergy Research Centers, which proposes to take advantage of the use of both renewable waste biomass (such as corn stover and paper waste) and energy crops grown on marginal lands (such as switchgrass).1–3 These missions are aimed at cost-effective production of renewable bioenergy as a sustainable solution to the world’s unmet energy needs rather than sole reliance on fossil fuels. These “green” efforts owe thanks to the rising awareness of the worldwide environmental impact of fossil fuels as well as economic concerns about energy security. In addition to the bioengineering of both plant feedstock and microbial workhorses, enzyme discovery for biomass deconstruction also remains a major research focus.4,5 Enzyme based biomass deconstruction represents a “green” approach to the alternative harsh chemical pretreatment of biomass as an initial step before conversion into renewable fuels, such as bioethanol and biodiesel, and other value-added products.5–6

In the current study, we present a crystal structure of a recombinant *Aspergillus niger* $\alpha$-xylosidase AxlA in complex with its catalytic product, a hydrolyzed xyloglucan oligosaccharide. AxlA was originally isolated from a commercial enzyme mixture secreted by *Aspergillus niger*.7 AxlA can effectively release terminal xylose from xyloglucan, a major plant hemicellulose.7,8 It belongs to the GH31 family of glycoside hydrolases and catalyzes the hydrolysis of an $\alpha$1,6-linked xylene via a two-step retaining mechanism (classification according to CAZy database, www.cazy.org).9 Optimized ratios in the combinations of AxlA and several other glycosidases have led to significant improvements in yield and efficiency for the total deconstruction of xyloglucan to C6 and C5 fermentable sugars.8

Either native or engineered microbial GH31 family enzymes have also proved useful for oligosaccharide synthesis due to their transglycosylation activity.12,13 Some newly characterized GH31 enzymes were shown to act on uncommon substrates like sulfoquinovosyl diacylglyceride sulfolipids as for *E. coli* YihQ14 or produce cyclic oligosaccharides such as cycloalternan by *Listeria monocytogenes* Lmo2446.15 The human genome also encodes multiple GH31 enzymes with varied physiological localizations and functions, such as lysosomal $\alpha$-
**Materials and Reagents.** Xyloglucan oligosaccharide, XFG heptasaccharide with purity >80%, was purchased from Elcitol (Crolles, France). XFG (see Abbreviations for nomenclature details) was chosen for subsequent crystal soaking experiments for the following reasons: First, it represents the complex sugar components of natural xyloglucan to help reveal different sugar binding sites in the XFG bound enzyme structure. Second, its relatively low degree of polymerization (DP7) may allow successful diffusion into the enzyme active site in the crystalline state to form a complex. Xylose was purchased from Sigma (U.S.A.). Polyethylene glycol 20000 as a 30% (w/v) stock solution as well as crystallization screens Index HT, PEGRx HT, Crystal Screen HT, and SaltRx HT were obtained from Hampton Research (Aliso Viejo, CA, U.S.A.). Morpheus and MIDAS screens were from Molecular Dimensions (Altamonte Springs, FL, U.S.A.). All other chemicals and reagents used for crystal growth were purchased from Sigma (U.S.A.) or Fisher Scientific (U.S.A.) and were used without further purification.

**Cloning, Expression, and Purification of AxlA.** AxlA (UniProt ID: G3XMN9) was expressed and purified according to previous procedures. 

**EXPERIMENTAL PROCEDURES**

Crystallization, Diffraction Data Collection, and Structure Determination. Because proteins expressed in *P. pastoris* can possess excessive N-glycosylation with high-mannose saccharides, we first treated AxlA with various glycosidases prior to crystallization trials. These included Endo H (50000 units/ml), peptide N-glycosidase F (5000 units/ml), and α-xylosidase (3000 units/ml) all from New England Biolabs, Ipswich, MA, U.S.A. with manufacture listed specific activity in parentheses. Deglycosylation reactions contained 10 volumes of AxlA, 1 volume of glycosidases, and 1 volume of 10X reaction buffer provided by the manufacturer. The reactions were incubated at room temperature (22 ± 2 °C) for 24–48 h. Initial crystallization trials were set up by a Mosquito Crystal nanoliter dispenser robot (TTP Labtech, Melbourne, UK) on MRC 2 Well crystallization plates (Hampton Research; Aliso Viejo, CA, U.S.A.) using sitting drop vapor diffusion method testing against all 6 commercial high-throughput screens listed in the Materials and Reagents section each with 96 conditions. Briefly, 200 nl of reservoir solution was laid on top of 200 nl of AxlA stock solution (15 mg/mL−1 supplemented with 10 mM D-xylose) and the mixture was equilibrated against the reservoir solution at 20 °C. Neither the native nor the treated AxlA crystallized under these conditions. However, Endo H pretreatment led to precipitation in the crystallization experiments and in the protein stock solution indicating decreased solubility due to potential deglycosylation of AxlA. Subsequent optimization of the Endo H pretreatment at various pH and temperature values was performed for crystallizations. In particular, Endo H pretreatment at 50 mM in Tris pH 7 and 4 °C overnight did not show precipitation of protein stock solution and led to initial crystal hit in the PEGRx HT screen at the H9 condition (5% v/v 2-propanol, 0.1 M citric acid pH 3.5, 6% w/v polyethylene glycol 20000). The crystal appeared as near cubic shape with the longest dimension of ~70 μm after growing for 2 days. Further optimization of crystallization conditions was performed at varied pH and precipitant concentrations. Prior to harvesting, a subset of crystals was soaked with xyloglucan oligosaccharide XFG for 1–5 min. Crystals were either directly flash-frozen in liquid nitrogen or cryoprotected by transferring into MiTeGen’s LV CryoOil (MiTeGen, Ithaca, NY) before being frozen.

The best diffracting crystal showed diffraction to 2.7 Å resolution and was collected at the Argonne National Laboratory on the LS-CAT 21-ID-G beamline from a single crystal (grown at the modified condition 2% v/v 2-propanol, 0.1 M citric acid pH 3.5, 3% w/v polyethylene glycol 20000, soaked with xyloglucan derived oligosaccharide XFG for 2 min, cryoprotected with oil) using a X-ray wavelength of 0.97856 Å. The data were indexed, integrated, and scaled using XDS. The initial phase problem was solved by molecular replacement using AutoMR (Phase) using *Cellulibrio japonicus* GH31 α-glycosidase as the search model (PDB code 4BY9). Autobuild programs of the PHENIX suite were used to partially complete the polypeptide coordinates without including ligands or water. Two copies of the protein were found in the asymmetric unit displaying an overall noncrystallographic pseudo-C2 symmetry. The phases were improved, and the structure was completed with alternating rounds of manual model building with COOT and refinement with PHENIX using default noncrystallographic symmetry restraints. Water molecules were added and updated during refinement. For relatively poor electron density regions, the loops were built based on traceable albeit weak backbone electron density and by relying on favorable side-chain rotamers and favorable geometry statistics after real-space refinement in COOT. N-Glycosylation GlcNAc and mannose residues as well as solvent molecules were also built in based on clear residual electron density in the 2mFo–dFc omit maps. Prior to final rounds of refinement, enzyme ligands were built into the structure based on clear difference density, revealing the hydrolysis product of an XFG molecule at each active site of the dimer and also one putative xyloglucan oligosaccharide fragment per asymmetric unit sandwiched between two aromatic residues mediating a crystallographic contact.

The final structures were refined to the same resolution limit as in data collection with favorable Rcryst and Rfree values (Table 1). Model
Regarding the mechanism of action and the specificity of this enzyme are revealed by the presence of a hydrolyzed oligosaccharide in the active site of the enzyme.

**Oligomeric State of AxlA.** AxlA comprises two chains in the asymmetric unit of the unit cell of the crystal with pseudo-C2 noncrystallographic symmetry (Figure 1A). This 2-fold interaction was predicted to be stable by the PDBePISA server based on calculations of interface area of $\sim2358 \AA^2$ per dimer (Figure 1B) and a positive dissociation free energy of 21.1 kcal/mol.

EPPIC is a biological assembly analysis program recommended by PDB to distinguish biological interfaces from artificial crystal contacts. It enumerates all possible symmetric assemblies with a prediction of the most likely assembly based on probabilistic scores from pairwise evolutionary scoring (sequence entropy signals). The EPPIC server predicted AxlA to be a homotetramer with 0.99 probability based on four additional interfaces between crystallographic symmetry-related dimers of either $\sim1255$ (Figure 1C) or $\sim1082 \AA^2$ (Figure 1D) forming a total interfacial area of $\sim9390 \AA^2$ in the tetrameric assembly. Thus, we conclude that the physiological state of AxlA is most likely a tetramer based on both bioinformatic analysis and physical analysis of favorable interfacial interactions.

**Post-Translational modifications of AxlA.** Seven out of the nine N-glycosylation sites of AxlA as predicted by the NetNGlyc 1.0 Server displayed glycosylation in the structure (Figure 1A). These include asparaginino amino acids at positions 6, 21, 314, 358, 453, 637, and 683 but not 369 and 658 (residue numbers are based on the secreted form of AxlA with the N-terminal signal peptide sequence removed). The N-glycans on Asn358 and 637 both retained a chitobiose core and high mannose type substitutions, possibly protected from Endo H due to limited solvent exposure. At residues 6, 314, 453, and 683, only one GlcNAc is present, indicating a result of successful deglycosylation by Endo H treatment prior to setup of crystallization. Asn261 shows high mannose glycan in one chain but not the other, which possesses only a GlcNAc. The N-linked glycan on Asn637 contains a complex branched structure of GlcNAcβ1-4GlcNAcβ1-3(Man1-3(Man1-2Man1-6)Man1-6)Man1-3Man1-2Man1-2Man (Figure 2A). We have previously reported a similar effect of Endo H treatment on a glycosylated GH29 family $\alpha$-fucosidase secreted by the same Pichia eukaryotic expression system. Asn639 is not glycosylated and is relatively more buried with its side-chain amide group forming favorable interactions with two nearby main-chain amide groups. This is consistent with a warning by the NetNGlyc 1.0 server on the accuracy of the prediction of Asn639 glycosylation because of a preceding Pro residue in the sequence. There is very little electron density in our experimental maps to convincingly suggest N-glycosylation on Asn658 despite bioinformatics predictions.

It should be noticed that the partially deglycosylated recombinant AxlA in the crystal structure has an average molecular weight per monomer ($\sim85.4$ kDa considering both protein and N-glycan contents) very similar to that reported for its native form from A. niger ($\sim85$ kDa). This suggests that the crystallographically observed N-glycan modifications and tetrameric assembly of AxlA may also be preserved in the native form. However, in the absence of deglycosylation treatment, recombinant AxlA heterologously expressed in P. pastoris was reported to have a much higher average molecular weight per monomer ($\sim110$ kDa) than the native form from A.
**niger** despite their very similar kinetic properties in catalyzing the hydrolysis of small α-xyloside substrates. This suggests a much higher extent of glycosylation of AxlA expressed in *P. pastoris* than its native host *A. niger*.

While the function of glycans are not known, several scenarios are possible such as the enhancement of solubility or stability of the protein. A support of solubility function comes from the observation of decreased solubility of the AxlA protein upon deglycosylation treatment as described in the Experimental Procedures section. One argument in support of stability function is that the Asn637 linked glycan locates at the dimer interface and possibly stabilizes dimerization via hydrogen-bonding and van der Waals interactions because of its proximity and its shape complementarity between the glycan and the dimer crevice (Figure 2B). This observation suggests that glycan modifications can be a useful strategy for protein design and engineering.

**Structure Determinants of Substrate Specificity.** We observed a reaction product at the active sites of both AxlA subunits in the crystal asymmetric unit from the hydrolysis of an added xyloglucan oligosaccharide substrate XFG (Figure 3A–C). In particular, the α1–6 glycosyl bond between the terminal xylose at −1 site and glucose at +1 site is cleaved to yield a free D-xylose. We were able to trace the experimental electron density for all the sugar residues of the XFG except for the +2 site branched fucose (Fuc) residue (Figure 3A–C) possibly due to disorder of the fucose in exposure to the bulk solvent. AxlA forms multiple polar and van der Waals interactions with the reaction product. All the hydroxyl groups of the −1 site xylose and nearly all the +1 site glucose interact with one or more hydrogen-bonding partners from the protein active site (Figure 3A). The protein–ligand interactions at +2 site, +3 site and +2 branch sites appear to be dominated by van der Waals interactions and shape complementarity. The current product bound complex supports the specific role of...
AxlA in xyloglucan biomass deconstruction and provides a structural basis for further optimization of its enzyme activity in the renewable biofuel industry.

The conserved nucleophile Asp395 (D395) and general acid Asp487 (D487) residues are located at the opposite sides of the catalytic labile C1 position of xylose. The averaged distances between the carboxylic oxygen atoms of D395 and D487 are 6.38 and 6.55 Å for each active site of the dimer, respectively. These values are consistent with a retaining catalytic mechanism involving double displacement steps (Figure 3D), as seen in other GH31 enzymes.

GH31 family α-glycosidases comprise a variety of enzyme activities with highly conserved catalytic residues conferring catalytic activity in the structurally conserved core (β/α)_{8}-barrel domain. In addition, the sequence identity ranges from 21%–33% between AxlA and other GH31 members (α-xylosidases, a majority of α-glucosidases, α-quinovosidases, and α-galactosidases, etc.) with no clear identity cutoff value to specify a particular type of substrate specificity. Thus, the overall protein structure and pairwise identity are relatively poor predictors of α-xylosidase activity. However, we differentiated α-xylosidases from other GH31 enzymes based on sequence phylogenetic analysis of all the structurally characterized GH31 enzymes in the CAZy databases (Figure 4A). The only exception is an E. coli α-xylosidase (PDB code 2F2H) that appears to be clustered with the α-glucosidases, α-glucosyltransferase, and α-quinovosidase GH31 family members.

We further identify a key molecular determinant of substrate specificity at the −1 site corresponding to Tyr286 of AxlA (PDB code 6DRU) despite the poor sequence conservation at this position among GH31 family enzymes. Almost all the structurally available GH31 α-xylosidases possess a bulky aromatic residue at the spatially equivalent position to Tyr286 again except for the E. coli α-xylosidase with Cys307 (PDB code 2F2H) compared to relatively smaller residues at this site in α-glucosidase, α-quinovosidase, and α-galactosidase (Figure 4B). These bulky aromatic side chains necessarily form steric clash with C6 hydroxyl groups of glucose, galactose and the C6 sulfo group of sulfoquinovose but not xylose. This is because xylose is a “C5 sugar” which lacks C6 and hence the corresponding O6/S6 groups that are present in the “C6 sugars”. In addition, the exact spatial orientation and proximity of the side chain to −1 site sugar also appear to play a role in the discrimination of substrates. For example, the relatively large sulfur atom of Cys307 is located closer to the O6 equiv position at the −1 site to cause potential steric clash, although perhaps to a lesser extent. Consistent with this molecular basis, the E. coli α-xylosidase (PDB code 2F2H) was reported to show minimal but not completely abolished α-glucosidase activity.

Figure 3. Reaction product complex structure of AxlA and proposed catalytic mechanism. (A) The hydrolyzed XFG heptasaccharide catalytic product is shown as sticks (carbon in white) with the corresponding difference omit map contoured at 3.5 σ. The active site residue side chains within 4 Å of the hydrolyzed oligosaccharide are shown as sticks (carbon in cyan) with corresponding 2mFo-dFc map contoured at 2 σ. The hydrogen-bonding interactions between the ligand and active site residues are indicated as black dashes. The conserved nucleophile D395 and general acid D487 aspartate residues and catalytic labile C1 of xylose at −1 site are also labeled. The branched xyloglucan oligosaccharide binding site is connected to a surface pocket of the adjacent subunit (pink), although with no apparent direct interactions with the ligand. (B) shown the same way as part A but in the other active site of the dimer in the asymmetric unit. (C) chemical structures of XFG and α-xylose (atom number labeled). XFG is named according to an existing nomenclature for xyloglucan-derived oligosaccharide (see Abbreviations). (D) Proposed two-step double displacement catalytic mechanism of AxlA leads to conformational retention at the catalytic labile C1 position between the substrate and product.
activity compared to its much higher α-xylosidase activity.31 Interestingly, among the limited number of structurally characterized GH31 enzymes, both Bacteroides ovatus and Cellvibrio japonicus GH31 α-xylosidases (PDB codes 5JOU and 2XVK) were also reported to act on xyloglucan derived substrates.32,33 The functional diversity of the GH31 enzymes suggests evolutionary robustness of their conserved fold to be tailored for different carbohydrate chemistry to gain specific fitness advantages.

■ CONCLUSIONS

In summary, we determined the structure of a GH31 family α-xylosidase AxlA from an industrially relevant fungus, Aspergillus niger, in a form with its bound catalytic product. Both the active site shape complementarity and polar interactions with the ligand support the role of AxlA in xyloglucan utilization with application potential in the renewable biofuel industry. We identified useful functional predictors on the basis of phylogenetic and active site residue analyses, and also suggested that the overall protein fold and pairwise sequence identity are poor functional predictors of substrate specificity of GH31 family enzymes.

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Notes
The authors declare no competing financial interest.

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■ ABBREVIATIONS

AdxA, α-xylosidase of Aspergillus niger; PDB, Protein Data Bank; P. pastoris, Pichia pastoris; GH, glycoside hydrolase; Xyl, α-D-xylose; Glc, α-D-glucose; Gal, α-D-galactose; Fuc, L-fucose; α1-6Glc, L is for Gal 1,4-linked D-glucoses modified by additional branched sugar units, where X is for Xylor1-6Glc, L is for Gal1-2Xylor1-6Glc, F is for Fuc1r-2Gal1-2Xylor1-6Glc, and G is for Glc, according to the above nomenclature.14

■ REFERENCES

(1) Gilna, P.; Lynd, L. R.; Mohnen, D.; Davis, M. F.; Davison, B. H. Progress in understanding and overcoming biomass recalcitrance: a BioEnergy Science Center (BESC) perspective. Biotechnol. Biofuels 2017, 10, 285.
(2) U.S. DOE. Lignocellulose Biomass for Advanced Biofuels and Bioproducts: Workshop Report, DOE/SC-0170. U.S. Department of Energy Office of Science. 2015, https://genomics.energy.gov/biofuels/lignocellulose/.
(3) Dale, B. E.; Holtzapple, M. The Need for Biofuels. Biomass recalcitrance: a BioEnergy Science Center (BESC) perspective. 2017, 60, 125–132.
(4) Meng, X.; Sun, Q.; Kosa, M.; Huang, F.; Pu, Y.; Ragauskas, A. J. Physiochemical Structural Changes of Poplar and Switchgrass during Pretreatment and Enzymatic Hydrolysis. ACS Sustainable Chem. Eng. 2016, 4, 4563–4572.
(5) Lombard, V.; Ramulu, G.; Drula, E.; Coutinho, P. M.; Henrissat, B. The Carbohydrate-active enzymes database (CAZY) in 2013. Nucleic Acids Res. 2014, 42, D490–D495.
(6) Rao, L. V.; Goli, J. K.; Gentela, J.; Koti, S. Bioconversion of lignocellulosic biomass to xyitol: An overview. Bioresour. Technol. 2016, 213, 299–310.
(7) Deloche, C.; Minondo, A. M.; Bernard, B. A.; Bernerd, F.; Salas, F.; Garnier, J.; Tancrede, E. Effect of C-xylose on morphogenesis of the dermal epidermal junction in aged female skin. An ultrastuctural pilot study. Eur. J. Dermatol. 2011, 21, 191–196.
(8) Brummer, H.; YicI, 1,4-linked D-glucoses modified by additional branched sugar units, where X is for Xylor1-6Glc, L is for Gal1-2Xylor1-6Glc, F is for Fuc1r-2Gal1-2Xylor1-6Glc, and G is for Glc, according to the above nomenclature.14

(9) Light, S. H.; Cahoon, L. A.; Halavaty, A. S.; Freitag, N. E.; Anderson, W. F. Structure to function of an α-glucan metabolic pathway that promotes Listeria monocytogenes pathogenesis. Nat. Microbiol. 2017, 2, 16202.
(10) Petersen, T. N.; Brunak, S.; von Heijne, G.; Nielsen, H. SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat. Methods 2011, 8, 785–786.
(11) Gupta, R.; Jung, E.; Brunak, S. Prediction of N-glycosylation sites in human proteins. 2004 http://www.cbs.dtu.dk/services/NetNGlyc/.
(12) Krissinel, E.; Henrick, K. Inference of macromolecular assemblies from crystalline state. J. Mol. Biol. 2007, 372, 774–797.
(13) Bliven, S.; Lafita, A.; Parker, A.; Capitani, G.; Duarte, J. M. Automated evaluation of quaternary structures from protein crystals. PLoS Comput. Biol. 2018, 14, e1006104.
(14) Papadopoulos, J. S.; Agarwala, R. COBALT: constraint-based alignment tool for multiple protein sequences. Bioinformatics 2007, 23, 1073–1079.
(15) Wildt, S.; Gerngross, T. U. The humanization of N-glycosylation pathways in yeast. Nat. Rev. Microbiol. 2005, 3, 119–128.
(16) Kabsch, W. XDS. Acta Crystallogr., Sect. D: Biol. Crystallogr. 2010, 66, 125–132.
(17) McCoy, A. J.; Grosse-Kunstleve, R. W.; Adams, P. D.; Winn, M. D.; Storoni, L. C.; Read, R. J. Phaser crystallographic software. J. Appl. Crystallogr. 2007, 40, 658–674.
(18) Adams, P. D.; Afonine, P. V.; Bunkoczi, G.; Chen, V. B.; Davis, I. W.; Echols, N.; Headd, J. J.; Hung, L. W.; Kapral, G. J.; Grosse-Kunstleve, R. W.; McCoy, A. J.; Moriarty, N. W.; Oeffner, R.; Read, R. J.; Richardson, D. C.; Richardson, J. S.; Terwilliger, T. C.; Zwart, P. H. PHENIX: a comprehensive Python-based system for macromolecular structure solution. Acta Crystallogr., Sect. D: Biol. Crystallogr. 2010, 66, 213–221.
(19) Emsley, P.; Cowtan, K. Coot: model-building tools for molecular graphics. Acta Crystallogr., Sect. D: Biol. Crystallogr. 2004, 60, 2126–2132.
(20) Chen, V. B.; Arendall, W. B.; Headd, J. J.; Keedy, D. A.; Immormino, R. M.; Kapral, G. J.; Murray, L. W.; Richardson, J. S.; Richardson, D. C. MolProbity: All-atom structure validation for macromolecular crystallography. Acta Crystallogr., Sect. D: Biol. Crystallogr. 2010, 66, 12–21.
(21) Karplus, P. A.; Diederichs, K. Linking crystalllographic model and data quality. Science 2012, 336, 1030–1033.
(22) Cao, H.; Walton, J. D.; Drumm, P.; Phillips, G. N., Jr. Structure and substrate specificity of a eukaryotic fucosidase from Fusarium graminearum. J. Biol. Chem. 2014, 289, 25624–25638.
(23) McCarter, J. D.; Withers, S. G. Mechanisms of enzymatic glycoside hydrolysis. Curr. Opin. Struct. Biol. 1994, 4, 885–892.
(24) Okuyama, M.; Kaneko, A.; Morii, H.; Chiba, S.; Kimura, A. Structural elements to convert Escherichia coli alpha-xylosidase (YicI) into alpha-glucosidase. FEBS Lett. 2006, 580, 2707–2711.
(25) Hemsworth, G. R.; Thompson, A. J.; Stepper, J.; Sobala, L. F.; Coyle, T.; Larsbrink, J.; Spaduti, O.; Goddard-Borer, E. D.; Stubbs, K. A.; Brumer, H.; Davies, G. J. Structural dissection of a complex Bacteroides ovatus gene locus conferring xyloglucan metabolism in the human gut. Open Biol. 2016, 6, 160142.
(26) Larsbrink, J.; Izumi, A.; Ibatullin, F. M.; Nakhai, A.; Gilbert, H. J.; Davies, G. J.; Brumer, H. Structural and enzymatic characterization of a glycoside hydrolase family 31 α-glucosidase from Cellvibrio japonicus involved in xyloglucan saccharification. Biochem. J. 2011, 436, 567–568.
(27) Fry, S. C.; York, W. S.; Albersheim, P.; Darvill, A.; Hayashi, T.; Immormino, R. M.; Kapral, G. J.; White, A. R. An unambiguous nomenclature for xyloglucan-derived oligosaccharides. Physiol. Plant. 1993, 89, 1–3.
(28) Lepore, R.; Kryshtafovych, A.; Alahunta, M.; Verasuto, H. A.; Bomble, Y. J.; Bufton, J. C.; Bullock, A. N.; Caba, C.; Cao, H.; Davies,
O. R.; Des fosses, A.; Dunne, M.; Fidelis, K.; Goulding, C. W.; Gurusaran, M.; Gutsche, I.; Harding, C. J.; Hartmann, M. D.; Hayes, C. S.; Joachimiak, A.; Leiman, P. G.; Loppnau, P.; Lovering, A. L.; Lunin, V. V.; Michalska, K.; Mir-Sanchis, I.; Mitra, A.; Moul t, J.; Phillips Jr, G. N.; Pinkas, D. M.; Rice, P. A.; Tong, Y.; Topf, M.; Walton, J. D.; Schwede, T.; et al. Target highlights in CASP13: Experimental target structures through the eyes of their authors. *Proteins: Struct., Funct., Genet.* 2019, 87, 1037–1057.