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Perspective

Enzymatic Routes to Designer Hemicelluloses for Use in Biobased Materials

Thu V. Vuong,[#] Mohammad Aghajohari,[#] Xuebin Feng, Amanda K. Woodstock, Deepti M. Nambiar, Zeina C. Sleiman, Breeanna R. Urbanowicz,* and Emma R. Master*

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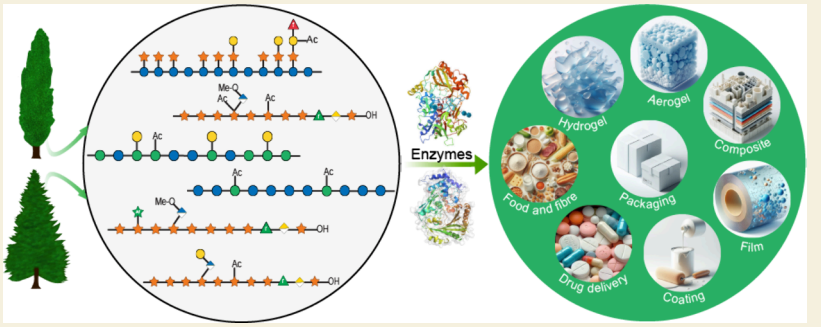

ABSTRACT: Various enzymes can be used to modify the structure of hemicelluloses directly in vivo or following extraction from biomass sources, such as wood and agricultural residues. Generally, these enzymes can contribute to designer hemicelluloses through four main strategies: (1) enzymatic hydrolysis such as selective removal of side groups by glycoside hydrolases (GH) and carbohydrate esterases (CE), (2) enzymatic cross-linking, for instance, the selective addition of side groups by glycosyltransferases (GT) with activated sugars, (3) enzymatic polymerization by glycosynthases (GS) with activated glycosyl donors or transglycosylation, and (4) enzymatic functionalization, particularly via oxidation by carbohydrate oxidoreductases and via amination by amine transaminases. Thus, this Perspective will first highlight enzymes that play a role in regulating the degree of polymerization and side group composition of hemicelluloses, and subsequently, it will explore enzymes that enhance cross-linking capabilities and incorporate novel chemical functionalities into saccharide structures. These enzymatic routes offer a precise way to tailor the properties of hemicelluloses for specific applications in biobased materials, contributing to the development of renewable alternatives to conventional materials derived from fossil fuels.

KEYWORDS: *hemicellulose, lignocellulose, carbohydrate-active enzyme, glycoside hydrolase, glycosyl transferase, carbohydrate oxidoreductase, amine transaminase, biobased material*

1. HEMICELLULOSES: STRUCTURAL DIVERSITY

Hemicelluloses are polysaccharides present in all plant cell walls and are defined by a β-(1 → 4)-linked backbone of glucose, xylose, or mannose, where C1 and C4 of adjacent sugars are in the equatorial configuration.[1] They are structurally diverse and include xyloglucans, xylans, mannans, glucomannans, and mixed linkage β-glucans (Figure 1). Mixed linkage β-(1 → 3,1 → 4)-glucans are characteristic to grasses and cereals[2] and will not be discussed here in detail. Hemicellulose structure and nomenclature are defined by a combination of the backbone sugar and the primary glycosyl substituent. Hardwoods (angiosperms) and softwoods (gymnosperms) vary in the structures and abundance of different classes of hemicellulose. These differences are significant, from a commercial perspective, for the generation of bioderived fuels, chemicals and materials. The predominant hemicellulose in hardwoods is glucuronoxylan, composed of a backbone of β-(1 → 4)-D-xylopyranosyl (Xyl*p*) residues with α-(1 → 2)-linked glucuronic acid (Glc*p*A) groups that are almost always 4-*O*-methylated in woody tissues. These hemicelluloses constitute 20−30% of the secondary cell wall in hardwoods, based on dry weight.[1,3] Glucuronoxylan is highly decorated with *O*-acetyl groups, which can occur as mono- or diacetylated substituents on *O*-2 and/or *O*-3 of xylose backbone residues. Glucuronoxylan (GX) has been reported to have an approximate degree of polymerization (DP) between 100−150.[4] *Eucalyptus dalrympleana* was recently found to contain the more complex side chains, including

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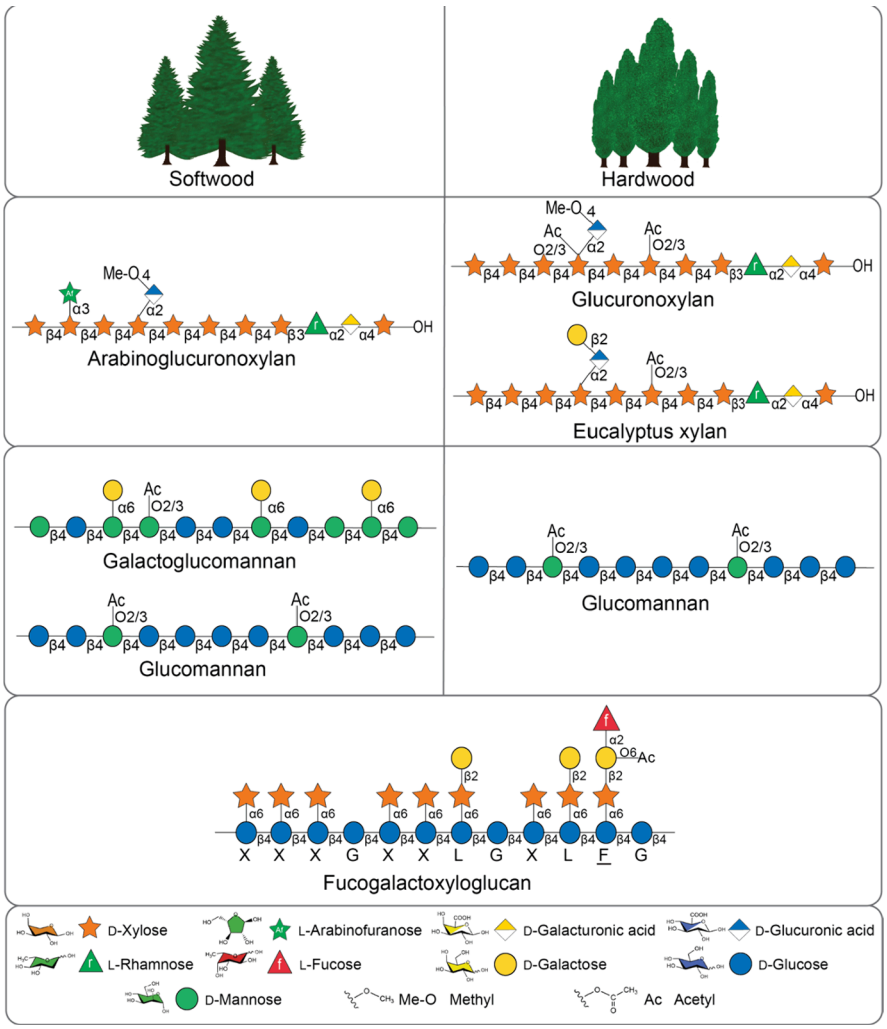

Figure 1. Main tree hemicellulose compositions and structures illustrated using SNFG nomenclature. Depending on the botanical source, 20−30% of dry cell wall materials are composed of various xylans, 10−15% are composed of different mannans, and 10−20% are composed of xyloglucans.

Gal-β(1 → 2)-Glc*p*A-α(1 → 2)- and Ara*p*-α(1 → 2)-Glc*p*A-α(1 → 2)-, that may be more widely distributed in herbaceous dicots.[5] Softwood secondary walls on the other hand, contain glucuronoarabinoxylans which possess α-(1 → 3)-linked arabinofuranose (Ara*f*) substituents,[1] and little to no *O*-acetyl substituents.[6] Studies on spruce, birch, and poplar have shown that hardwood and softwood xylans contain a unique glycosyl sequence at their reducing terminus: β-D-Xyl*p*-(1 → 3)-α-L-Rha*p*-(1 → 2)-α-D-Gal*p*A-(1 → 4)-D-Xyl.[4,7]

Glucomannans are polysaccharides with a backbone of β-(1 → 4)-mannosyl (Man) residues that can be interspersed with β-(1 → 4)-glucosyl (Glc) residues and, like xylan, backbone Man residues are *O*-acetylated at *O*-2 and *O*-3 in hardwoods[8] and softwoods.[9] Glucomannans are a minor component of hardwood secondary cell walls, present at 3−5%, based on the dry weight. Galactoglucomannans are the major component of softwood secondary cell walls, present at 10−30% of the dry weight, and have been reported to have an approximate DP between 100−150.[3] They have a glucomannan backbone that is appended with α-(1 → 2)-linked galactopyranose (Gal*p*) side groups, which can be further substituted with β-(1 → 2)-Gal*p*, forming a disaccharide side chain terminated with Gal*p*.[10,11] They are often structurally defined by their mannose to glucose to galactose (Man:Glc:Gal) ratio, which is 3.5−4.5:1:0.5−1.1 in wood tissue.[12] In softwood, there are two types of acetylated galactoglucomannan. One type is rich in galactose (1:1:3) comprising 5−8% of the dry wood, while the other is galactose poor (0.1:1:3) and makes up to 10−15% of the dry weight.[3] Galactoglucomannans are either absent or present in low quantities (up to 3%) in hardwood secondary cell walls.[1]

The fucogalactoxyloglucans found in the walls of woody dicots and angiosperms consist of a β-(1 → 4)-Glc backbone that is substituted with tandem α-D-Xyl*p* residues attached at *O*-6 (Figure 1). To discuss xyloglucan structure and synthesis, it is important to note that the glycosyl sequence is almost exclusively described using a single-letter code.[13] This method of nomenclature simplifies the representation of complex xyloglucan structures, making it easier to understand and analyze their compositions. The letter G represents an unsubstituted backbone glucose unit. The letter X represents the most basic side chain where α-D-Xyl*p* residues attach at *O*-6, which is often further substituted at *O*-2 by other sugars. Two of the most common side chains include one where the xylose residue is appended with galactose, termed L, which can be further extended by fucose at *O*-2 (F). The arrangement of these side chain structures in xyloglucan is denoted by the order of these letters (Figure 1). The xyloglucan in most dicots

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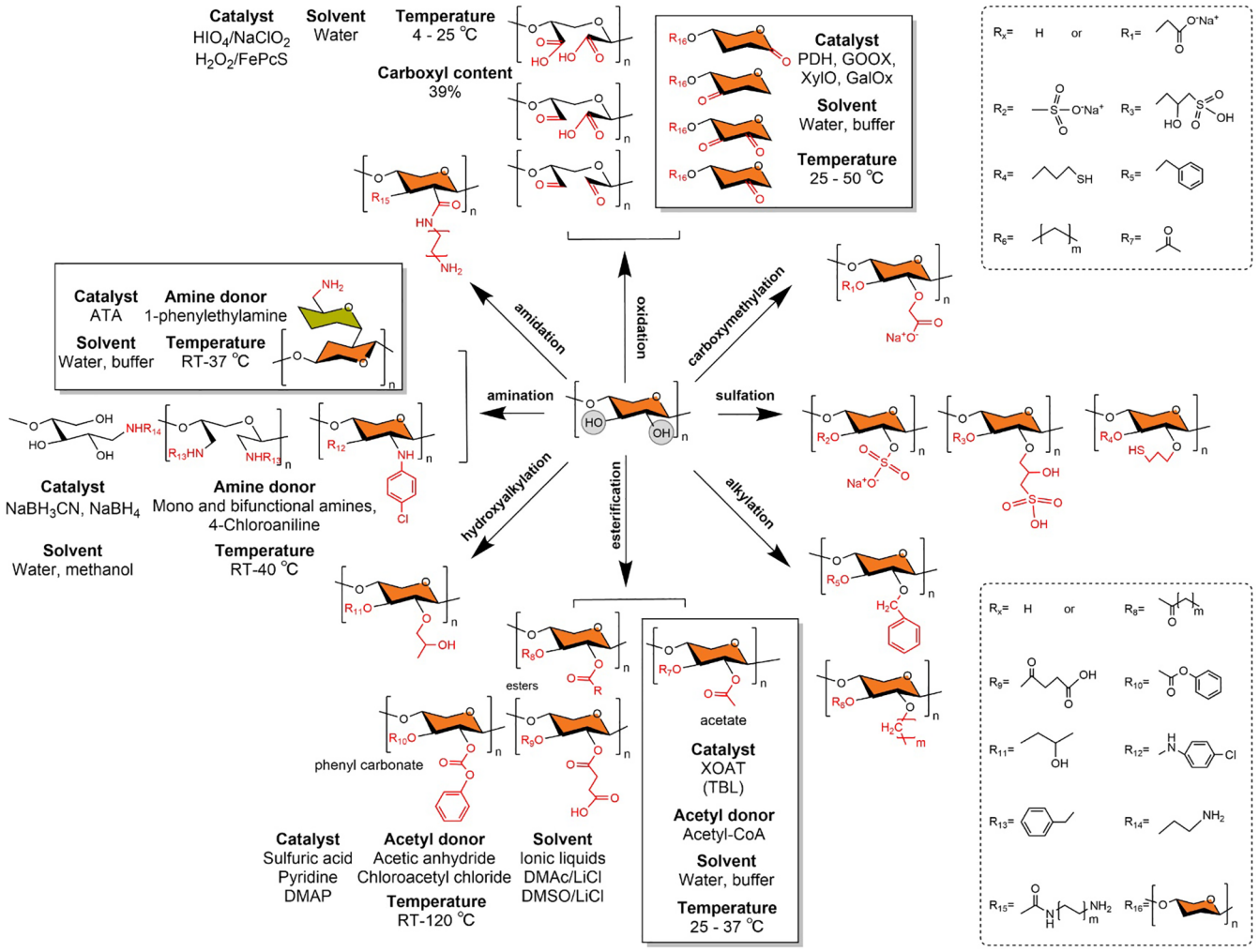

Figure 2. Chemical and enzymatic reactions for hemicellulose modification. The reactions in which both chemical(s) and enzyme(s) can be used are indicated by a bracket, and the reaction conditions and products of the enzymatic reaction are highlighted in shadowed boxes. Newly introduced functional moieties are highlighted within dashed boxes. RT: reaction condition reported as room temperature.

is a typical fucogalactoxyloglucan with an XXXG core structure and L and F side chains. Few comprehensive studies have investigated the composition of xyloglucans in gymnosperm primary walls, unlike extensive research conducted on angiosperms. However, xyloglucan characterized from the two Pinales, *Araucaria angustifolia* and *Cryptomeria japonica*,[14,15] revealed that oligosaccharides released by xyloglucan-specific endoglucanase (XEG) exhibit the typical characteristics of the XXXG core structure fucogalactoxyloglucans described in dicots.[16]

2. PERSPECTIVE—HEMICELLULOSES AS RENEWABLE FEEDSTOCKS FOR CARBON EFFICIENT BIOMANUFACTURING

The high hemicellulose content in agricultural and wood fiber means its broader use will be critical to ensuring the sustainability of emerging biobased economies. Today, the hemicellulose fraction of wood fiber either remains bound to the cellulosic fiber used in paper and packaging or is dissolved during the kraft process and burned. The low heat value of carbohydrates compared to lignin underscores opportunities for value-added applications of hemicelluloses if extracted prior to kraft pulping.[17] Similarly, hemicelluloses from agricultural feedstocks that leave the field largely end up in animal feed, following little to no value-addition. When designing new materials, incorporating enzymatic instead of chemical methods can address many environmental challenges by avoiding toxic chemicals and harsh conditions; moreover, enzymes offer the catalytic selectivity needed to tailor diverse hemicellulose structures for use in new, biobased materials. Already, there are well characterized routes for the enzymatic deconstruction of hemicelluloses to sugars that are fermented to liquid transportation fuels,[18,19] sweeteners,[20] and commodity chemicals.[19] Enzymatic deconstruction relies on the concerted action of multiple glycoside hydrolases (GHs), esterases, and oxidative enzymes, which have been thoroughly reviewed elsewhere.[21−23] Green chemistry metrics, such as the concept of the atom economy, pioneered by Trost, include process assessments that quantify the proportion of atoms from the starting material that ultimately contribute to the desired product.[24] Notably, bioprocessing via fermentation inevitably leads to partial loss of carbon in hemicellulose and other biomass feedstocks to carbon dioxide. By comparison, higher atom economy is achieved when hemicelluloses are first used in their intact form, incorporating all of the atoms from a starting material into the final product. Examples of this would be hemicelluloses used in coatings and films,[25,26] hydrogels for

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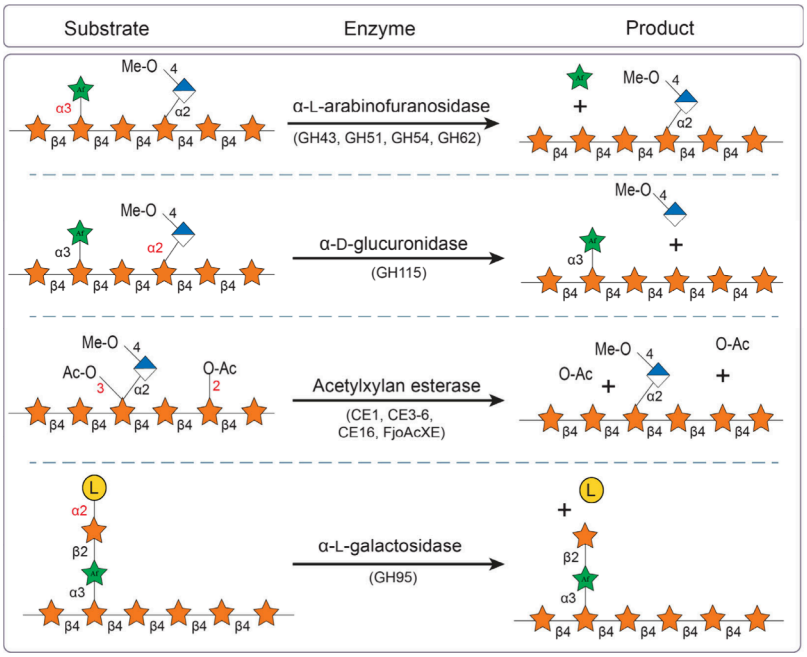

Figure 3. Examples of accessory carbohydrate-active enzymes, including glycoside hydrolases (GH) and carbohydrate esterases (CE), for selective removal of hemicellulose side groups. Target linkages are highlighted in red.

medical and environment applications,[25,27−29] and emulsifiers and prebiotics for food applications.[30,31]

Applications of intact hemicelluloses in value-added materials depend on technologies that extract them from plant biomass without destroying their native structures. Extraction methods must account for intermolecular linkages between hemicelluloses and other plant cell wall components. For example, lignin can form ester linkages with acetyl and MeGlc*p*A substituents in hemicellulose chains, generating so-called Lignin-Carbohydrate Complexes.[32−34] Extensive hydrogen bonding within cellulose microfibrils and hemicelluloses also limits the processability and reactivity of plant cell wall polymers.[35,36] Although extraction strategies will depend on the biomass feedstock, common extraction methods include the application of hot water at alkali pH, organic solvents, and ionic liquids.[37] Table S1 of the Supporting Information (SI) summarizes the most common extraction methods to obtain hemicelluloses and highlights remaining challenges to their use, including molecular heterogeneity (e.g., differing side group composition and DP); absence of chemical functionalities on hemicellulose structures (e.g., carbonyl, amine, or acyl groups) required for specific end applications; and formation of byproducts that can inhibit subsequent processing steps and reduce product purity (e.g., organic acids, hydroxymethylfurfural, and phenolic groups). Whereas chemo-catalytic routes have been developed to address some of these key challenges (Figure 2), efficacy and scale-up of chemo-catalytic approaches for hemicellulose modification are often limited by low catalytic selectivity that curtail product yield, use of organic solvents that impose safety risks, and application of catalysts that are deemed hazardous if not recovered and reused.[38] Alternatively, enzyme technologies permit regiospecific modifications that typically increase product yield, use water as a solvent, and are produced biologically, ensuring their biocompatibility.

This Perspective focuses on untapped opportunities to use enzymes for biomass processing beyond degrading to fermentable sugars and instead for upgrading glycopolymers into value-added materials through tailoring intact hemicellulose structures. After reviewing enzymes relevant to controlling hemicellulose DP and side group composition, enzymes that increase cross-linking functionality and introduce new chemical handles to hemicellulose structures will be described.

3. ENZYMES TO CONTROL STRUCTURAL HETEROGENEITY

3.1. Perspective—Enzymes for All Seasons

Several variables at the level of primary structure, including the DP of the backbone as well as the amount, identity, and patterning of side groups, play a critical role in the physicochemical properties of polymer interactions in the plant cell wall and material properties of extracted polymers. Hemicelluloses can be extracted as intact polymers or as oligomers at an industrial scale and often retain their inherent solubility for downstream transformation. The diversity of hemicellulose structures, which depend on botanical source and extraction method, presents conditions particularly amenable to enzymatic strategies for targeted modifications that reduce the heterogeneity and tailor the functionality of hemicellulose-derived products. Here, we describe a multitude of enzyme classes that are now available for use to develop singular or combinatorial strategies for the generation of defined saccharide structures for desired end uses.

3.2. Accessory Carbohydrate-Active Enzymes for Selective Removal of Hemicellulose Side Groups

The known repertoire of Carbohydrate-Active enZymes (CAZymes) are classified into sequence-derived families based on analysis of amino acid sequence similarities,[39] and are annotated in the CAZy database (http://www.cazy.org).[40,41] Glycosyltransferases (GT), *O*-acetyltransferases, *O*-methyltransferases, and GHs are the four important classes of enzymes that contribute to the hemicellulose structure and

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diversity in planta. These classes of enzymes also present biocatalytic routes for the incorporation of desirable functionalities into hemicellulose structures both in and ex vivo. Plant GTs involved in the synthesis of plant matrix polysaccharides are Golgi localized and play a major role in backbone synthesis and substituent addition. GHs produced by plants are often apoplastic, and play a role in the modification of hemicelluloses after synthesis and deposition in the cell wall, thus contributing to structural diversity. Although largely applied so far for hemicellulose deconstruction, the so-called accessory hemicellulases that include GHs and carbohydrate esterases (CE) selectively remove hemicellulose side groups (Figure 3), and can be used to control physicochemical properties of hemicellulose-based films and hydrogels[42,43] as well as digestibility of food components.[44] Early examples demonstrate that selective removal of Ara*f* from arabinoxylan using an α-L-arabinofuranosidase targeting Ara*f* units on monosubstituted Xyl*p* residues generates xylan films with increased degree of crystallinity and decreased oxygen permeability;[45] α-L-arabinofuranosidases can be found in GH families 43, 51, 54, and 62. Also, the selective release of MeGlc*p*A from glucuronoxylans using a GH115 α-D-glucuronidase was previously shown to increase xylan adsorption to cellulose, thereby increasing water absorbency by the cellulose material.[46] Carbohydrate esterases including acetylxylan esterases (CE families 1, 3−6, and 16), as well as glucuronoyl esterases (CE15) and feruloyl esterases (CE1) that act on polymeric xylan are also used to modify the corresponding xylans.[47] FjoAcXE, an acetylxylan esterase identified from a polysaccharide utilization locus, cleave singly and doubly acetylated Xyl*p*, as well as internal 3-*O*-acetyl-Xyl*p* linkages in (2-*O*-MeGlc*p*A)3-*O*-acetyl-Xyl*p* residues and densely substituted and branched xylooligomers.[48] Reversely, feruloyl esterase (*St*FaeC) can catalyze the transferuloylation from methyl ferulate to L-Ara*f* in low water-content systems, opening a potential pathway for enzymatic feruloylations of oligo- and polysaccharides, yielding biodegradable polymers with modified hydrophobic and rheological characteristics.[49] In complex carbohydrates such as superbranched corn fiber xylan, less-known α-L-galactosidase (GH95) can remove terminal L-galactose units,[50] allowing selective removal of hemicellulose side groups. The precedent for using enzymes to tailor hemicellulose chemistry for material purposes is thus clearly illustrated by the applications of accessory hemicellulases. Just as such applications emerged from a deepened understanding of hemicellulose deconstruction, it is our perspective that the increasing understanding of hemicellulose biosynthesis will likewise, and perhaps more profoundly, reveal innovative opportunities for smarter use of this abundant biomass fraction. The next section thus focuses on the biochemistry and use of enzymes that catalyze the formation of glycosidic bonds, including GTs and glycosynthases (GSs), which are engineered GHs.

3.3. Glycosyltransferases for Selective Synthesis of Hemicellulose

Deciphering the suites of molecular machinery used by plants to synthesize cell wall polysaccharides brings us closer to designing them in vivo or in vitro with defined structures, reduced heterogeneity, and improved functionalities for intended applications. GTs belong to a large superfamily of CAZymes that catalyze the stereospecific transfer of a monosaccharide moiety from an activated donor, generally a nucleoside diphosphate sugar, to a carbohydrate or protein acceptor, forming a specific glycosidic bond.[51] A large repertoire of GTs is involved in synthesis of plant cell wall structures and has been shown to represent more than 2% of the total coding sequences in any given plant genome.[52] GTs are promising candidates for enzyme engineering and the generation of novel transgenic cell wall morphotypes in important woody bioenergy crops such as *Populus*. A prerequisite for exploitation of the GTs is an understanding of the structure−function relationship of the resultant products, the molecular basis of substrate recognition, and their mechanism of action.

GTs are diverse and are categorized based on their sequences in the CAZy database. Plant GTs are mostly localized in the Golgi-ER (endoplasmic reticulum) secretory lumen. Some possess multiple transmembrane domains, such as the members of the cellulase synthase superfamily from CAZy family GT2 that are involved in synthesis of the β-(1 → 4) backbones of cellulose and most hemicelluloses,[53] apart from xylan.[54] Most plant GTs, and almost all known GTs involved in hemicellulose side group addition, are Type II membrane proteins with an N-terminal signal-anchor peptide sequence comprising a single pass transmembrane domain and a C-terminal globular catalytic domain that projects into the lumen.

The structure and catalytic properties of GTs are governed by their regioselectivity and stereoselectivity, specificity to activated donors and acceptors, and the glycosidic bond linkage generated.[55] The core structure of a GT comprises one of three main conserved tertiary fold forms: GT-A, GT-B, or GT-C. The GT-A fold includes a single Rossmann-like fold that includes a nucleotide sugar donor binding domain, constituted by alternating β strands and α helices. The majority of the members of this fold class harbor a characteristic DxD motif that binds divalent cations, often Mn^{2+} or Mg^{2+}, functioning to coordinate carboxyl groups of Asp (or nitrogen atoms of His) and the phosphodiester bond of the activated donor. The GT-B fold forms two Rossmann-like folds. These enzymes lack the DxD motif and do not generally require metal cofactors. GT-B enzymes use conserved Lys or Arg side chains for interactions with the activated donor phosphodiester bond. GT-C proteins are comprised of multiple transmembrane α helices, require lipid-linked sugar donors, and are less significant in the context of plant cell wall synthesis.

The GTs are specific to a range of activated sugar donors. Mechanistically, all GTs synthesize glycosidic linkages either by retention or inversion of the anomeric linkage of the synthesized glycosidic bond relative to the linkage of the donor substrate,[55] and these catalytic mechanisms are characteristic and conserved for a given family. The xylan synthase is an example of a GT47 enzyme, a family that employs an inverting catalytic mechanism; accordingly, a UDP-α-D-xylose donor is used to synthesize a β-(1 → 4)-D-xylan polymer. Thus, knowledge of the respective nucleotide sugar donor will also provide information regarding the anomeric configuration (α/β) of the glycosidic bond formed by each individual enzyme within a GT family. GTs which are involved in transferring Glc, Gal, *N*-acetyl glucosamine (GlcNAc), *N*-acetyl galactosamine (GalNAc), glucuronic acid (GlcA), galacturonic acid (GalA), and Xyl moieties use uridine diphosphate (UDP)-α-D sugar derivatives. GTs transferring Ara, Man, and Fuc use UDP-ß-L, guanosine diphosphate (GDP)-α-D and GDP-α-L

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sugar derivatives, respectively. Recently, broad analysis of diverse GT-A fold enzymes has led to the identification of evolutionary and structural features involved in catalysis and in donor and acceptor interactions.[56]

GT-A fold enzymes were found to share a common core, including catalytic DxD, xED, G-loop, and C-His motifs involved in donor interaction and catalytic functions. In the case of acceptor binding, GT-A proteins employ additional hypervariable regions that are believed to dictate acceptor substrate specificity. The GT-B acceptor binding domain is at a cleft created between the two Rossmann folds. GT-Bs, in general, have not been as well-characterized, and conserved catalytic features have not been identified thus far. This could be partially attributed to the fact that conserved structural motifs in GT-Bs, which clearly define their substrate specificities, remain to be discovered. The modular GT protein architecture brings together different domains, thus facilitating precise relative positioning of chemically different donors and acceptors at the catalytic site, enabling wide substrate specificities. Further, genetic mutations causing semiconserved and nonconservative amino acid substitutions in donor and acceptor binding domains can contribute to altered substrate specificity or affinity. This structural dynamicity offers great potential for enzyme engineering and synthetic biology approaches.

The different mechanisms of action of the GTs also provide a basis for classification, as they are not correlated with the conserved fold structure. They can be broadly classified as inverting and retaining reactions.[55] Inversion reactions involve altered stereochemical configurations in the product. They can be either the SN2 or SN1 type, with the former being more common. Both reactions require a nucleophilic attack by a specific deprotonated hydroxyl group of the acceptor carbohydrate molecule on the anomeric carbon (C1) of the activated sugar donor. SN2 is a bimolecular nucleophilic substitution reaction. This reaction is governed by a single rate-limiting step due to the concerted steps of nucleophilic bond formation and the departure of the leaving group (UDP). It is bimolecular since both the donor and acceptor simultaneously initiate the rate-limiting step. Either of the conserved Glu, Asp, or His residues acts as the enzyme base catalyst for the deprotonation of the acceptor hydroxyl group. SN1 has two rate-limiting steps since the departure of the leaving group and nucleophilic substitution are two separate steps. Both SN1 and SN2 reactions entail a backside attack, wherein the leaving group departs from the side opposite to that of the nucleophilic bond formation. The reception of electrons following nucleophilic attack causes the existing carbon bonds to shift to the opposite side. This results in the reversal of the stereochemical configuration, from the α to the β steric form at C1 in the product. The retention mechanism is less understood. It could either be a double displacement involving two inversion steps and consequentially a retention in the stereochemical configuration at the C1. This type of reaction has not been evidenced in GTs so far. The other proposed mechanism, the SNi type, is theorized to involve a frontside attack by the deprotonated nucleophilic hydroxyl group of the acceptor on the C1 of the activated sugar donor. This results in the retention of the α steric form at C1 in the product. The stereoselective and regioselective nature of GTs ensures chemical sequence and structure preservation in complex cell wall polysaccharides through repeated synthesis cycles without a template.

Plant cell wall synthesis relies on diverse transferases that function together in backbone synthesis and the subsequent addition of glycosyl and nonglycosyl substituents. This joint action thus helps shape the primary sequence of the resultant polysaccharide, which in turn dictates polymer confirmation and the numerous chemical interactions that hold together the complex hierarchical cell wall structure. Thus, GTs are valuable candidates for both transgenic and synthetic biology approaches to obtaining consistent hemicellulose structures, where bioresources making plant cell wall components serve as a living chassis for the development of engineered biomaterials. A case in point is utilization of yeast (*Komagataella phaffii*, previously known as *Pichia pastoris*) as a synthetic biology chassis for the engineering plant cellulose synthase-like A (CSLA) proteins (GT2). This approach showed that yeast can be used as a biofactory to gain mechanistic insights into mannan and glucomannan production and yield insights into modulation of Glc:Man ratios during backbone synthesis.[57] There is increased interest in further adapting polysaccharide synthesis enzymes to perform specific biocatalytic reactions in vitro or in planta. Extensions of this approach for multimembrane spanning enzymes like GT2s have been proposed using the yeast OrthoRep system, which employs a continuous directed evolution approach that facilitates the enhancement of various functional attributes of plant enzymes, and also enables the adaptation of enzymes from nonplant sources to efficiently operate within the plant cellular environment.[58] Another example is cell-free synthesis of xylan polymers using recombinant algal xylan synthase (*Klebsormidium flaccidum*).[59] These enzymes are thus an area of interest for extensive research in the context of generation of novel cell wall-related biomaterials.

3.4. Polysaccharide Assembly Using Glycosynthases and Transglycosylases

The chemical synthesis of hemicelluloses presents significant challenges, including harsh reaction conditions, the need for multiple protection and deprotection steps to limit unwanted reactions, and a notable lack of specificity, often resulting in products that poorly mimic the natural diversity and complexity of these essential polysaccharides.[60,61] In nature, GTs finely orchestrate glycosidic bond formation, assembling complex polysaccharides with remarkable precision. The GTs involved in hemicellulose synthesis use either UDP or GDP sugars as donors. Leveraging sugar-nucleotide-dependent (Leloir) GTs for industrial applications has been hampered by their perceived difficulty to work with and the need for the activated sugars as donors in glycosylation reactions, limiting their practical utility in biotechnological applications.[62] Sugar nucleotides are not widely available as commercial reagents and can be unstable in reactions. However, rapid advances in our knowledge of biosynthetic pathways have led to facile biocatalytic synthesis of activated NDP-sugar donors, largely using a "two-block" biocatalytic strategy where sugars are converted into sugar-1-phosphates in "block one", then to NDP-sugars in "block two", by kinases and pyrophosphorylases, respectively.[63−65] These enabling technologies allow us to assemble multiple enzymes into one-pot multienzyme (OPME) systems for the generation of NDP-sugar substrates and utilization by GTs in a single reaction.[65−67]

In contrast to GTs, GHs facilitate the breakdown of complex carbohydrates into simpler sugars by hydrolyzing the glycosidic bonds. Such reactions, however, can operate in the reverse

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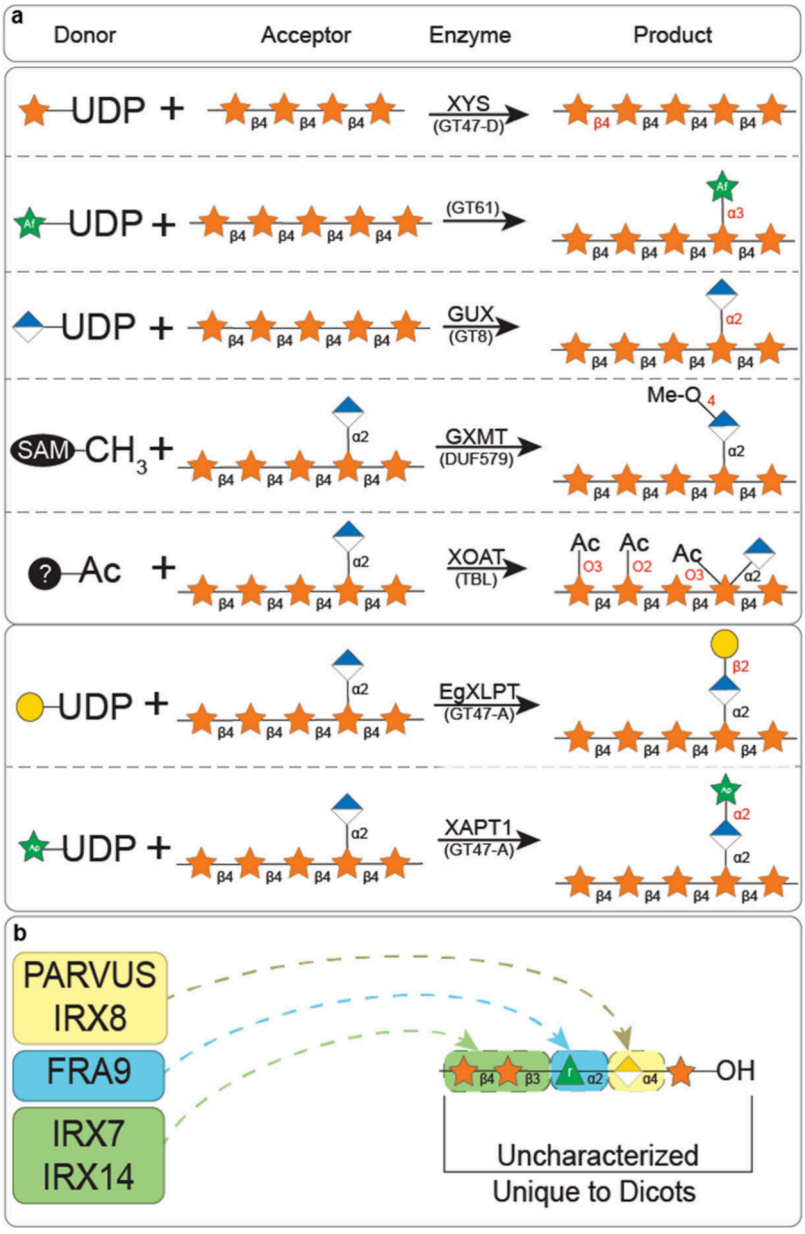

Figure 4. (a) Visual table representing plant enzymes involved in xylan biosynthesis. Xylan synthase (XYS; UDP-Xyl) catalyzes the formation of the main xylan backbone (i.e., GX) while glucuronoxylan xylosyltransferase (GUX; UDP-Glc*p*A), xylan arabinopyranosyltransferase (XAPT1; UDP-Arap), xylan galactopyranosyltransferase (XLPT; UDP-Galp), glucuronoxylan methyltransferase (GXMT; SAM), and xylan *O*-acetyltransferase (XOAT; Unknown) are involved in substituent addition. Enzyme names are denoted by their short form acronyms followed by their CAZyme glycosyltransferase (GT) superfamily. Bonds that are formed by the specified enzyme are highlighted in red. (b) Visual representation of the proposed enzymes involved in formation of the unique reducing-end oligosaccharide. All plant genes putatively involved in synthesis of the reducing end sequence were identified based on mutant analysis in model dicot *A. thaliana*. Each gene name is highlighted to match the proposed sugar and bond formed. Donors utilized by each enzyme are indicated in parentheses together with the abbreviated enzyme name.

direction, catalyzing polysaccharide synthesis. Briefly, GHs can be classified based on their point of action into exoglycosidases that hydrolyze glycosidic bonds from terminal monosaccharides or endoglycosidases that act on internal glycosidic bonds. GHs are also categorized based on their mechanism of action into two main groups. Inverting GHs hydrolyze glycosidic bonds via a single displacement, resulting in the inversion of the anomeric carbon, while the retaining GHs proceed through a double-displacement mechanism, preserving the configuration of the anomeric carbon. Specific GHs, more prominent in retaining enzymes, are capable of acting in reverse. Transglycosylation is a process in which GHs catalyze the transfer of a glycosyl moiety from a donor molecule to an acceptor. On the basis of the concentrations of substrates, the nature of the donor and acceptor, and the reaction conditions, the balance between transglycosylation and hydrolysis can be controlled.

Transglycosylases (GH1, GH3, GH5, GH10, GH16, GH17, etc.) maintain the anomeric configuration of the glycosyl donor and create new glycosidic bonds. These retaining GHs form a covalent glycosyl-enzyme intermediate through a two-step double-displacement mechanism, which can then be intercepted by a glycosyl group, allowing the enzyme to transfer the glycosyl group to the substrate instead of a water

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Table 1. Summary of Select GSs Developed for Hemicellulose Polymer Synthesis: Enzymatic Origins, Mutations, And Product Specifications[a]

Organism	Parent GH	Mechanism	Mutation	Donor	Product	Reference (PDB ID)
Paenibacillus pabuli	Xyloglucanase XG5 (GH5)	Retaining	E323G E323A E323S	XLLG-α-F	Digalactosylated xyloglucan with Mw = 12 kDa	(2JEQ,2JEP)[80]
Humicola insolens	HiCel7B (GH7)	Retaining	E197A E197S	XXXG-α-F	Mw up to 60 kDa	(6YOZ,6YP1)[76]
Bacillus halodurans	Reducing-end exo-oligoxylanase (GH8)	Inverting	Y198F D263N D263C	Xyl-β-(1→4)-Xyl-α-F	X_3	(IWU4)[78]
Aspergillus aculeatus	Endo-(1→4)-β-xylanase (GH10)	Retaining	E265G	X_2F	Mw up to 80 kDa (DP: 606)	(6Q8M)[77]
Talaromyces amestolkiae	Endo-(1→4)-β-xylanase (GH10)	Retaining	E236G	X_2F	Up to X_{10}	[86]
Cellulomonas fimi	Endo-(1→4)-β-xylanase (CFXcd) (GH10)	Retaining	E235G	Xyl-β-(1→4)-Xyl-α-F	Linear xylan chain up to X_7	(1EXP)[81]
Thermotoga maritima	Xylanase XylB (GH10)	Retaining	E259G E259A E259S	Xyl-β-(1→4)-Xyl-α-F	Higher than X_5	(1VBR)[82]
Bacillus halodurans	Xylanase XynA (GH10)	Retaining	E301G E301A E301S	Xyl-β-(1→4)-Xyl-α-F	Higher than X_5	(7D88)[82]
Clostridium stercorarium	Xylanase XynB (GH10)	Retaining	E293G E293A E293S	Xyl-β-(1→4)-Xyl-α-F	Higher than X_5	[82]
Cellulomonas fimi	Xylanase Cex (GH10)	Retaining	E233G E233A E233S	Xyl-β-(1→4)-Xyl-α-F	Higher than X_5	(2EXO)[82]
Geobacillus stearothermophilus	Endo-(1→4)-*b*-xylanase (XT6; GH10)	Retaining	E265G	α-Xyl-F	Up to X_{12}	(4PRW)[85]
Bacillus licheniformis	Xyloglucanase BlXG12 (GH12)	Retaining	E155A	XXXG-α-F XLLG-α-F		(2JEN)[76]
Tropaeolum majus	Xyloglucan endotransferase 1/ xyloglucanase NXG1 (GH16)	Retaining	E94A E94G E94S	XXXG-α-F XLLG-α-F	8 repeating units	(2UWA)[76]
Populus tremula x Populus tremuloides	Xyloglucan endotransglycosylase 16A (PttET16−34) (GH16)	Retaining	E85G E85A E85S	XXXG-α-F XLLG-α-F	6 repeating units	(1UMZ,1UN1)[83]
Cellvibrio japonicus	Mannanase (Man26A) (GH26)	Retaining	E320G E320S	Mannobiosyl-α-F	DP: 3	(4CD5,4CD4)[84]
Geobacillus stearothermophilus	β-Xylosidase XynB2 (GH52)	Retaining	E335G E335S E335A	α-Xyl-F	Self-condensation of donor (X_2) and X_3	(4C1P,4RHH)[79]

[a]X_n: number of xylose residues in the compound; Mw: molecular weight.

molecule. This process occurs simultaneously at the same active site of a GH. Therefore, a high yield of oligosaccharide production is limited due to the competition between the sugar acceptor and the water molecules in the active site. Nevertheless, the efficiency of transglycosylase can be optimized through various strategies, including screening the enzyme source, controlling water accessibility,[68] minimizing steric interference,[69] or improving acceptor molecule binding. Additionally, increasing the concentration of acceptor molecules[70] or adding organic solvents to the reaction mixture[71] can suppress water activity, thereby favoring transglycosylases over the hydrolysis reaction, enabling the synthesis and modification of complex carbohydrates.

Investigations on the LeMAN4 enzyme from ripe tomato fruit belonging to the GH5 family show transglycosylase activity, enabling the enzyme to transfer segments of the donor mannan polysaccharide backbone to galactoglucomannan or mannose-based oligosaccharides, acting as an acceptor. The dual activity of LeMAN4 was confirmed through recombinant expression in *Escherichia coli*, revealing that the dual functionality of the enzyme can be manipulated by salt concentration and pH.[72] Synergistic use of a double mutant of TrMan5A, a β-mannanase belonging to the GH5 family, and Aga27A (α-galactosidase, GH27) creates a novel pathway for allyl glycosides synthesis. Aga27A is capable of removing the galactosyl side groups, exposing more of the β-(1 → 4)-mannan backbone to TrMan5A enzyme. This allows a higher yield of the desired product and diversifies the product range.[73] Cyclomaltodextrin glucanotransferases (CGTases) from the GH13 family can catalyze the conversion of linear starch into cyclodextrins (CDs) through intramolecular transglycosylation.[74] The enzyme cuts a segment from the amylose and cyclizes to form CDs containing six, seven, or eight glucose units. The ability to engineer CGTases to produce specific

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CDs can lead to new material development with applications in drug delivery, food flavoring, and pollutant removal technologies.

In 1998, the first ever report of a mutant retaining enzyme, GH1 β-glucosidase from *Agrobacterium* sp., having a mutation at its nucleophilic residue (Glu358) and catalyzing synthesis of β-glucosides using α-glucosyl fluoride as a donor and different acceptors, opened up a whole new avenue for engineering carbohydrates.[75] GSs are mutated glycosyl hydrolases in which the catalytic nucleophile in the active site is exchanged for a non-nucleophilic residue, thus inactivating the hydrolytic reaction. Therefore, the enzyme catalyzes the formation of new glycosidic bonds by ligation of activated sugar donors, such as glycosyl fluorides, to suitable acceptor substrates in a higher yield than the wild-type glycosyl hydrolases, which form glycosidic bonds in a reversible reaction. When GSs based on endoglycosylases are used, the activated glycosyl donor acts as both the donor and acceptor, resulting in a polymerization reaction. In this way, polysaccharides of different classes with molecular masses up to 60 kDa have been produced.[76] Xylanase-derived GSs have also been reported and used to synthesize homopolymers of xylan.[77]

3.5. Enzymes Involved in Xylan Synthesis and Design

3.5.1. Glycosyltransferases for In Vivo Xylan Synthesis. Xylan has a glycosyl sequence at the reducing end that is hypothesized to initiate synthesis.[88] This "elongation sequence 1" with the unique structure D-Xyl*p*-(1 → 3)-α-L-Rha*p*-(1 → 2)-α-D-Gal*p*A-(1 → 4)-D-Xyl was initially identified by Johanson and Samuelson in 1977,[89] and has only been found in dicots, specifically aspen, spruce and later in the herbaceous model plant *Arabidopsis thaliana*.[90] To date, four genes have been proposed to encode GTs that function in the formation of elongation sequence 1: IRX8 (GT8), FRA8/IRX7 (GT47), PARVUS (GT8), and FRA9 (GT106) (Figure 4).[91]

The β-(1 → 4)-linked Xyl*p* backbone is elongated by xylan synthase complexes (XSCs) that contain GTs from at least two different families: GT43 and GT47.[92,93] The xylan synthase (XYS; EC 2.4.2.-) that directly catalyzes the transfer of Xyl*p* residues from UDP-xylose (UDP-Xyl) to xylo-oligosaccharide acceptors is a GT47 enzyme, capable of forming polymeric xylan in vitro.[54] GX is further modified during synthesis in the Golgi through substitution of Glc*p*A at the α-(1 → 2) position by glucuronoxylan xylosyltransferase (GUX; EC 2.4.1.-) enzymes from GT8. Recently, a member of GT47 family was found in *Eucalyptus* that modifies the Glc*p*A side chain by adding a β-(1 → 2) galactose residue.[5] Furthermore, GX is modified by nonglycosyl substituents like acetylation and methylation. These GX modifications are made from the following metabolites: *S*-adenosyl methionine (SAM) and acetyl-coenzyme A (Ac-CoA). Glucuronoxylan methyltransferase (GXMT; EC 2.1.1.112; PFAM 04669) adds a methyl group from the universal methyl donor SAM to the Glc*p*A at O-4.[94] *O*-Acetylation of GX is catalyzed by xylan *O*-acetyltransferases (XOATs; EC 2.3.1.-; PFAM 13839; PDB: 6CCI),[54] which are members of the Trichome birefringence family. Synthesis of the metabolic precursor SAM is derived from L-methionine, but the donor of Ac-CoA is currently debated (Figure 4).

More recently, the XYS enzyme from the alga *Klebsormidium flaccidum* was demonstrated to work as a biocatalytic tool for the bottom-up synthesis of pure β-(1 → 4) xylan polymers.[95] The ability to carry out the bottom-up synthesis of polysaccharides presents unique opportunities for understanding the design rules for engineering of plant biomass with highly controlled structures using native or engineered enzymes. Previously, we successfully combined XYS with XOAT1 variants, demonstrating a biochemical pathway for the production of xylo-oligomers with highly controlled degrees of acetylation.[59] This work was extended to include the utilization of different primers for synthesis, showing that these fine structural elements have a major impact in polymer−polymer interactions in solution.[95] By combining backbone and side chain synthesis systems, we can envision leveraging OPME systems of increased capacity to more rapidly study processes related to synthesis of finely structured polymers. Taken together, these capabilities allow us to exploit natural plant enzymatic pathways, opening doors for the development of biosynthetic technologies directed toward the tailored biosynthesis of glycopolymers for material by design.

3.5.2. Glycosynthases for In Vitro Xylan Synthesis. The application of GSs in xylan synthesis represents a significant advancement, allowing the production of xylan chains with defined lengths and branching patterns (Table 1).[77,85,87] This enzymatic approach is both specific and efficient, opening new avenues for the exploration of xylan's structural diversity and its application in materials science. Expanding the toolkit for enzymatic xylan synthesis is not limited to engineered, retaining GHs. GSs mutated from an inverting GH, based on a reducing-end specific, xylose-releasing exo-oligoxylanase (Rex, EC 3.2.1.156, GH8) can facilitate formation of xylooligosaccharides (X_3) from α-xylobiosyl fluoride and xylose. Mutating the catalytic base Asp263 through saturation mutagenesis, specifically to either Cys or Asn, facilitates the formation of xylotriose. Enzyme-mediated activation of the glycosyl fluoride donor, followed by the transfer of the glycosyl group to xylose, inverting the configuration at the anomeric center, results the formation of a new glycosidic bond.[78]

Xylanases from GH10 have also been engineered to polymerize xylan chains of up to 22 Xyl*p* units by condensing -X_2F molecules. GH10 xylanases from four different organisms have been modified by exchanging the nucleophilic glutamic acid residues with non-nucleophilic amino acids such as glycine (G), alanine (A), and serine (S) showing that the affinities of the GSs on X_n (n = number of xylose residues in the compound) as acceptor molecules depend on the number of subsites required to fix the acceptor molecule at the proper position. The G mutations (E259G, E293G, E301G, and E233G) demonstrate the highest activity for polymerizing xylooligosaccharides by polymer precipitating out of the solution. When X_n is a poorer acceptor, no reaction change occurs. If X_n is an equal or better acceptor and the subsequent $X_{(n+2)}$ is also a better acceptor, then it immediately extends the chain, increasing the reaction rate due to a higher acceptor concentration. However, if $X_{(n+2)}$ has equal acceptor quality to X_n, its formation does not lead to further polymerization due to higher concentration of X_n.[82]

A conjugated reaction involving the GH52 β-xylosidase mutant XynB2 E335G and GH10 xylanase mutant XT6 E265G results in the synthesis of β-(1 → 4)-xylans with DPs of up to 100 Xyl units. The reaction leverages α-D-xylopyranosyl fluoride (XylF) as the sole substrate, resulting in high molecular weight xylans.[79] Artificial polysaccharides were also synthesized by integrating chemo-enzymatic methods with GS

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technology. To achieve this, chemically synthesized arabinoxylan oligosaccharide fluorides, with a known branching pattern, were used as substrates to elongate xylan and arabinoxylan chains using XynA E265G to obtain chains of up to 9.2 kDa and 80 kDa in molecular weight, respectively.[77] The same enzyme was later used in another chemo-enzymatic synthesis scheme to generate xylan dodecasaccharides. In that work, the synthesis of long-chain saccharides was elucidated through inclusion of modifications, such as 3-*O*-methylation at the reducing-end xylose, to improve water solubility. Furthermore, the synthesized saccharides served as acceptor substrates in enzyme assays, including a glucuronosyltransferase (*At*GUX3) from *A. thaliana*, that can add a glucuronic acid substituent to central Xyl residues.[85]

3.6. Enzymes Involved in Mannan Synthesis and Design

The glucomannans found in the walls of woody dicots and angiosperms have β-(1 → 4)-Man backbones that can be interspersed with β-(1 → 4)-Glc residues that are often *O*-acetylated at *O*-2 and *O*-3 in hardwoods[8] and softwoods.[9] The backbone is synthesized by CSLA enzymes (EC 2.4.1.32), first identified by Dugga et al. (2004),[96] and use GDP-mannose and GDP-glucose as activated donor substrates.[97] CSLAs may be associated with accessory proteins that affect the biosynthesis of mannan, such as mannan-related synthase (MSR).[57] MSR is an uncharacterized glycosyltransferase from GT106 that is expressed at the same levels and locations as CSLA in fenugreek seeds; transgenic mutant lines in *Arabidopsis* show a change in mannose levels,[98] but this protein has not been biochemically characterized. Voiniciuc et al. (2018) coexpressed various combinations of the MSR and CSLA proteins in the yeast *K. phaffii* and found that MSR1 could modulate the content of the product polysaccharide from mannan to glucomannan with CSLA2. Heterologous expression of AkCSLA3 from *Amorphophallus konjac* and AtCSLA2 from *A. thaliana* allowed yeast to produce β-(1 → 4)-glucomannan or mannan, respectively. Thus, these enzymes represent valuable tools for introducing desired alterations in the cell wall composition.[57,99] The mannan backbone can be substituted at *O*-6 with galactose by mannan α-galactosyltransferase (MAGT; EC 2.4.1.-) from GT34, and this first Gal can be further elongated with Gal by mannan β-galactosyltransferase (MBGT; EC 2.4.1.-) from GT47 at *O*-2.[100,101] The *O*-acetylation of mannan is catalyzed by mannan *O*-acetyltransferases (MOATs), which are members of the plant specific trichome birefringence-like (TBL) family.[102] CSLAs, mannan galactosyltransferases, and MOATs are predicted to be localized in the Golgi, like the other hemicellulose synthesis enzymes (Figure 5).

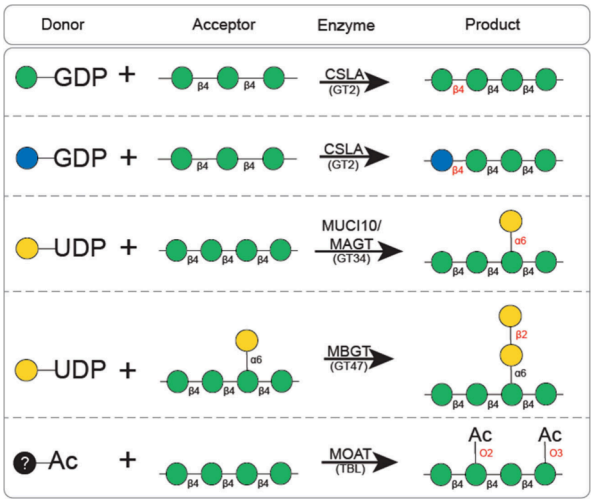

Figure 5. Visual table representing plant enzymes involved in mannan biosynthesis. The backbone is synthesized by cellulose synthase like-A (CSLA; GDP-Glc, GDP-Man) and modified by mucilage related-10 (MUCI10; UDP-Gal), mannan α-galactosyltransferase (MAGT; UDP-Gal), and mannan *O*-acetyltransferase (MOAT; Unknown). Enzyme names are denoted by their short form acronyms followed by their CAZyme glycosyltransferase (GT) superfamily. Bonds that are formed by the specified enzyme are highlighted in red. The donor utilized by each enzyme is indicated in parentheses together with the abbreviated enzyme name.

3.7. Enzymes for Xyloglucan Synthesis and Design

Most of the studies of the enzymes involved in xyloglucan biosynthesis (Figure 6) have been performed by heterologous expression of candidate GTs in the model species *A. thaliana*, which is the appropriate plant model for most angiosperms and even gymnosperms. The β-(1 → 4) glucan backbone is synthesized by members of the GT2 CSLC (EC:2.4.1.-) family,[103,104] and further extended Xyl*p* residues added by xyloglucan xylosyltransferase (XXT; EC 2.4.2.39; PDB: 6BSU) in the GT34 family.[105] Recently, it has been shown that three complementary XXT proteins are required to form XXXG-type xyloglucan.[106] The regiospecific addition of β-(1 → 2) galactoses to the second and third Xyl*p* of an XXXG subunit to form L side chains is conducted by the GT47 enzymes, xyloglucan L-side chain galactosyltransferase position-2 (XLT2; EC:2.4.1.-) and MURUS 3 (MUR3; EC:2.4.1.-), respectively.[107,108] Finally, the F side chain is formed by addition of an α-(1 → 2) fucose residue by GT37 member, fucosyltransferase 1 (FUT1; EC 2.4.1.69; PDB: 5KOE, 5KOP).[109] The GS variant PpXG5 (PDB: 2JEQ, 2JEP) E323G from *Paenibacillus pabuli* shows high catalytic efficiency toward (XLLG) F, making it the first to synthesize digalatosylated xyloglucans with molecular masses up to 60 000 Da. Using *A. thaliana*'s GT37 α(1 → 2)-fucosyltransferase (AtFUT1), it achieves a 3:1 XLFG to XLLG ratio in fucosylated xyloglucans. This, along with HiCel7B E197S glycosynthase, provides a toolset for creating XyG variants with specific branching (Table 1).

4. BIOCATALYTIC STRATEGIES FOR HEMICELLULOSE FUNCTIONALIZATION

4.1. Perspective—Hemicelluloses with Customized Chemical Functionalities

Using enzymes to create hemicelluloses with new chemical functionalities presents an untapped opportunity for green chemistry and biotechnology. As discussed above, the hemicellulose structure varies greatly among different plant species. Section 3.2 explains how accessory hemicellulases can be used to "tame" this diversity by selectively removing specific side groups that are naturally bound to the hemicellulose backbone. Enzymes can also be used to customize the physicochemical properties of hemicelluloses through the targeted introduction of functional groups that are missing from natural hemicellulose structures (e.g., amines and certain carbonyls). So far, most enzymes used to introduce new chemical functionalities into intact polysaccharides generate polysaccharide esters that display higher solubility in organic solvents and improved melt processing.[110] Requisite enzymes

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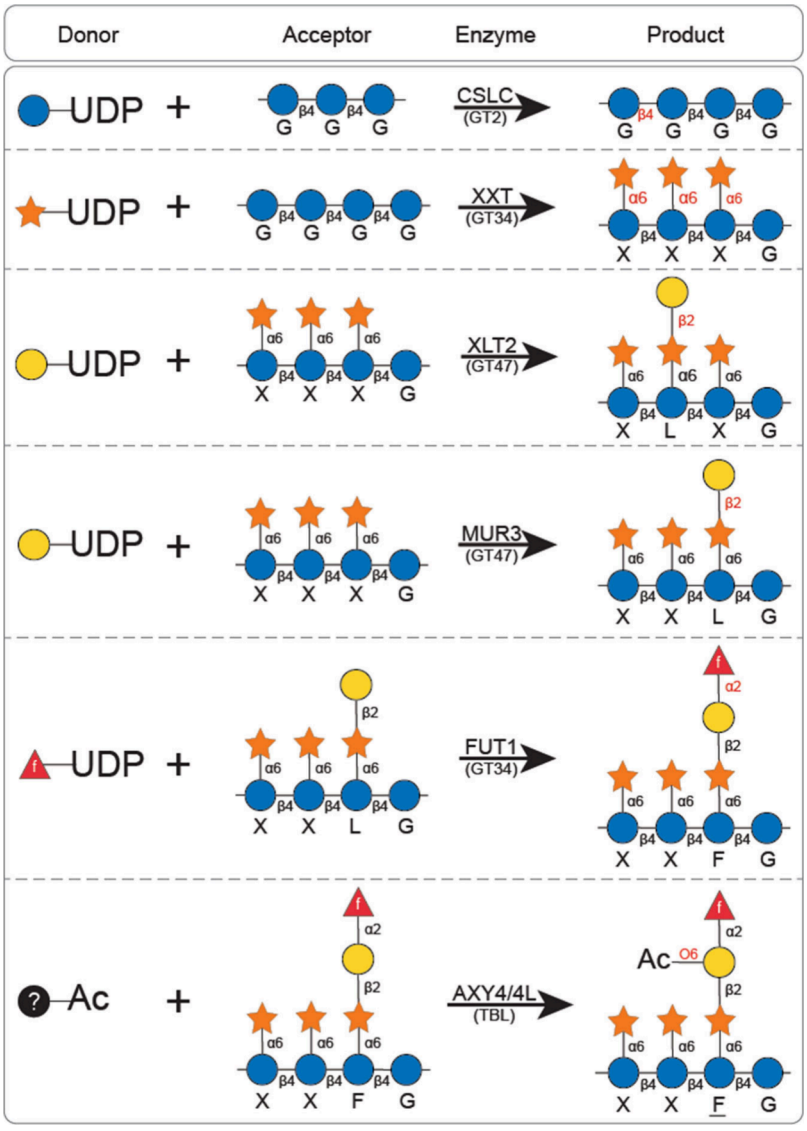

Figure 6. Visual table representing plant enzymes involved in xyloglucan biosynthesis. Cellulose synthase-like C (CSLC; UDP-Glc) synthesizes the main glucose backbone and xyloglucan xylosyltransferase (XXT; UDP-Xyl), xyloglucan L-side chain galactosyltransferase position-2 (XLT2; UDP-Gal), xyloglucan galactosyltransferase (MUR3; UDP-Gal), fucosyltransferase 1 (FUT1; GDP-Fuc), and altered xyloglucan 4/4-like (AXY4/4L; Unknown) catalyze the addition and *O*-acetylation of the side chains. Enzyme names are denoted by their short form acronyms, followed by their CAZyme glycosyltransferase (GT) superfamily when appropriate. Bonds formed by the specified enzyme are highlighted in red. The donor utilized by each enzyme is listed in parentheses together with the abbreviated enzyme name.

include lipases, esterases, and acyltransferase, which catalyze the esterification reaction between polysaccharides and acyl donors (e.g., fatty acids or their derivatives) in nonaqueous solvents. Corresponding enzyme systems have been recently reviewed elsewhere.[110,111] Herein, we will therefore focus on enzymes with potential to functionalize hemicellulose polymers in aqueous systems, including (1) carbohydrate oxidoreductases that introduce new carbonyl functionalities (e.g., aldehyde and keto-groups) into hemicelluloses, which promote hemicellulose cross-linking and further enzymatic modification, and (2) amine transaminases that introduce amine functionality into hemicelluloses, creating plant-derived polyamines relevant to products ranging from personal care to water treatment.

4.2. Enzymes for Hemicellulose Oxidation

Carbohydrates including hemicelluloses can be enzymatically oxidized by carbohydrate oxidoreductases that subtract two electrons from CH−OH positions in carbohydrate substrates and donate them to molecular oxygen (oxidases; EC 1.1.3.x) or other electron acceptors (dehydrogenases; EC 1.1.5.9, EC 1.1.99.18, EC 1.1.99.29, and EC 1.1.99.35). When oxygen is used as the electron acceptor, H_2O_2 is generated along with the corresponding aldehyde, keto-, or acid form of the oxidized carbohydrate. These carbohydrate oxidoreductases all require cofactors including flavin adenine dinucleotide (FAD) that can be noncovalently or (bi)covalently bound to the enzyme,[112] or copper ions that can be required in Cu(I) or Cu(II) oxidation states.[113] To capture the sequence, structural, and functional associations of carbohydrate oxidoreductases, the Auxiliary Activities (AA) class was introduced to the CAZy database in 2013.[41] At present, the CAZy database includes 17 AA families of carbohydrate oxidoreductases. Among these 17 families, the following are of particular interest for hemicellulose oxidation: family AA1 (e.g., laccase and multicopper oxidase), family AA3

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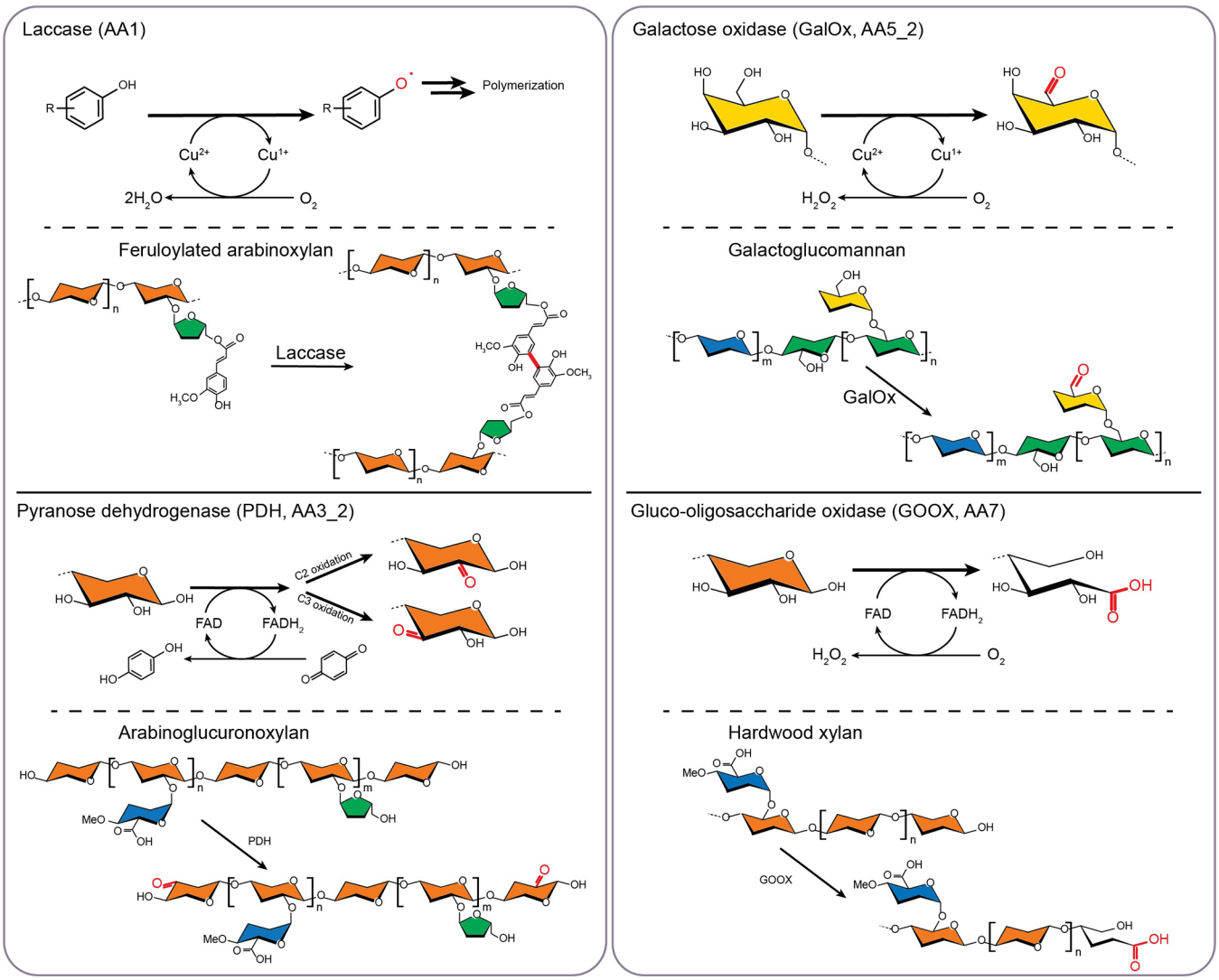

Figure 7. Oxidative enzymes for hemicellulose functionalization from auxiliary activity (AA)family AA1, AA3, AA5 and AA7. For each enzyme family, an overview of the enzymatic mechanism is shown in the upper panel and a substrate example is shown in the lower panel.

especially subfamilies AA3_1 (e.g., xylooligosaccharide dehydrogenase), AA3_2 (e.g., pyranose dehydrogenase or PDH) and AA3_4 (e.g., pyranose 2-oxidase), family AA5 especially subfamily AA5_2 (e.g., galactose oxidase or GalOx),[114] and family AA7 (e.g., gluco-oligosaccharide oxidase or GOOX) (Figure 7). While the cofactor of PDH and GOOX is FAD, that of GalOx and laccase is copper. Several lytic polysaccharide monooxygenases (LPMOs), particularly those in families AA9,[115] AA14,[116] and AA17[117] show oxidation of cellulose-associated glucuronoxylan, cellulose-associated xylan, and pectin correspondingly. Different from the previously mentioned AA families, however, the LPMOs oxidatively cleave the hemicelluloses, facilitating their breakdown into simpler sugars. Given the lytic mode of LPMO action, and because LPMOs have been the subject of numerous recent reviews,[118,119] they are not further discussed in the current perspective.

4.2.1. Enzymes for Hemicellulose Cross-Linking and Polymerization. Laccases (EC 1.10.3.2), versatile enzymes found in fungi and bacteria among other organisms, are recognized for their pivotal role in modifying feruloylated polysaccharides, including feruloylated arabinoxylan (FAX) and feruloylated glucuronoarabinoxylan. Categorized in family AA1, laccases catalyze the oxidation of substrates by transferring electrons from the reduced substrate to molecular oxygen (Figure 7). This process is facilitated by the Cu ions present in the active site of the enzyme. Laccases typically have four copper ions arranged in three sites: Type 1 (T1), Type 2 (T2), and Type 3 (T3).[120] The reduced substrate typically binds to the T1 site. The substrate undergoes oxidation at the T1 copper site, where it donates an electron to the T1 copper ion, which is then transferred to the adjacent Type 2 and Type 3 copper centers. This process results in the reduction of the T1 copper ion from Cu(II) to Cu(I). The reduced oxygen molecule undergoes a series of reactions facilitated by the copper ions in the T2/T3 sites, resulting in the formation of water. The corresponding oxidized product becomes a radical, i.e. the ferulic acid moiety is converted into the semiquinone radical that then initiates nonenzymatic reactions that can induce cross-linking, polymerization, and disproportionation.

Laccases are utilized to modify FAX and feruloylated glucuronoarabinoxylan through an enzymatic process that involves the oxidation of ferulic acid (FA) residues within the arabinoxylan structure. During the enzymatic coupling

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reaction, FA is oxidized and converted to dehydrodimers, including 8−5′-cyclic, 8−5′-noncyclic, 5−5′, 8−8′, and 8-*O*-4′ isomers.[121] The resulting radicals of feruloyl moieties lead to the formation of covalent cross-links between arabinoxylan chains (creating hydrogels) or between arabinoxylan and other molecules present in the system (adding new functionality). The degree of covalent and noncovalent interactions between FAX chains depends on the molecular properties of the source AX, including the degree of substitution (DS), degree of feruloylation, and molecular weight, and will influence the physicochemical properties of the resulting hydrogel. For instance, lower DS of FAX increases elasticity and compact microstructure of FAX hydrogels; it also enhances covalent bonding between FAX chains.[122] The resulting FAX hydrogels exhibit radical scavenging activity relevant to applications in wound healing, cellular repair, and targeted delivery. Notably, peroxidases (EC 1.11.1.-) of family AA2 can also be used to cross-link FAX; for instance, in the presence of H_2O_2 *Amoracia rusticana* horseradish peroxidase (EC 1.11.1.7, PDB: 1ATJ) can cross-link corn-derived FAX to generate hydrogels.[123] A similar peroxidase-H_2O_2 system was able to oxidize and cross-link wheat-derived FAX, and when blending the oxidatively cross-linked FAX with wheat flour, both water absorption and bile acid binding capacity of the flour increased.[124] However, compared to FAX cross-linking by laccases, the rapid cross-linking induced by peroxidase can result in gels with relatively high porosity and heterogeneity.[123]

FAX has also been enzymatically cross-linked to proteins such as casein, caseinate, whey protein, zein, and bovine serum albumin for food applications, particularly to confer semisolid textural attributes to food.[125] However, in protein−polysaccharide cross-linking, free radicals can be formed in both feruloylated polysaccharides and proteins (e.g., tyrosine free radicals). As a result, homogeneous and heterogeneous reactions can occur simultaneously, generating protein−protein, polysaccharide−polysaccharide, and polysaccharide−protein conjugates. Therefore, for amino-containing polysaccharides or protein-associated polysaccharides such as gum arabic[126] or corn fiber gum hydrolysate,[127] transglutaminase (EC 2.3.2.13) can be used to selectively catalyze protein−protein acyl transfer, leading to cross-linkages between the γ-carboxamide group of glutamine residues and primary amines of lysine residues, forming a ε-(γ-glutamyl)lysine isopeptide bond.[128] Protein−polysaccharide cross-linking has been applied to improve protein properties including solubility, emulsifying properties, and filming properties.[128] Furthermore, enzymatic conjugation with polysaccharides can reduce the immunogenicity of food proteins and improve protein therapeutics due to the advantages of enzymatic reactions and biocompatibility of polysaccharides.[128]

4.2.2. AA3 Family for Keto and Acidic Hemicelluloses.

AA3 enzymes belong to the glucose-methanol-choline (GMC) oxidoreductase superfamily, which is a large group of enzymes that catalyze the oxidation of a variety of substrates. There are currently four subfamilies of AA3, of which subfamilies AA3_2, AA3_4, as well as AA3_1 are of interest for modifications of hemicelluloses and their fragments. The catalytic cycle of FAD-carbohydrate oxidoreductases including AA3 enzymes can be divided into two steps: a reductive half reaction followed by an oxidative half reaction (Figure 7). During the reductive half reaction, a hydride transfer to the flavin is assisted by a catalytic base to activate the hydroxyl group located on the substrate. Then, in the oxidative half reaction, the reduced enzyme is oxidized back to FAD by dioxygen for oxidases, which undergoes reduction to H_2O_2 via two distinct single-electron transfer steps or by other electron acceptors for dehydrogenases.

Subfamily AA3_2 includes pyranose dehydrogenases (PDH, EC 1.1.99.29) that can oxidize mono- or disaccharides through monooxidation reactions at either the C1, C2, or C3 position, yielding the corresponding aldonolactones (C1 oxidation) or ketosugars; or double-oxidations at C2/3 or C3/4 yielding the corresponding diketosugars.[129] PDHs display a broader substrate profile with the ability to oxidize many hemicellulose monosaccharides including L-arabinose, D-mannose, D-galactose, and D-xylose, as well as disaccharides including cellobiose and maltose. Moreover, PDHs oxidize different carbon positions depending on the substrates. For instance, *Am*PDH1 of the mushroom *Agaricus meleagris* (aka *Leucoagaricus meleagris*, PDB: 4H7U) oxidizes C2 of arabinose and D-galactose, but it oxidizes C1 of ribose,[130] and when oxidizing oligosaccharides, *Am*PDH1 is able to target both the reducing-end and non reducing-end sugars.[131] *Am*PDH2 and *Am*PDH3 also oxidize D-glucose both at C2 and C3, and upon prolonged reaction C2 and C3 double oxidized glucose is produced.[132] In addition to xylose, *Am*PDH1 was also shown to oxidize xylobiose[130] and oligosaccharides with DP greater than two, such as cellotetraose and maltotriose which are substrates for both *Agaricus bisporus* *Ab*PDH1 and *Am*PDH1.[133,134] More recently, PDHs were shown to oxidize both linear and substituted xylo-oligosaccharides found in hardwood and agricultural resources, by targeting both the reducing and nonreducing ends of the tested substrates.[135] The authors found that activity values decreased with increasing length of the xylooligosaccharide and when using acidic rather than neutral substrates; moreover, the oxidation of linear xylooligosaccharides mostly led to double oxidized products, whereas single oxidized products dominated in reactions containing substituted xylooligosaccharides.[135] This insight was recently applied to enzymatically synthesize telechelic molecules from carbohydrates that can served as biobased alternatives to synthetic cross-linkers.[136]

Pyranose oxidases (pyranose 2-oxidase, POx, EC 1.1.3.10) catalyze the C2 oxidation of D-glucose (both α- and β-D-glucose) and several cellulose- and hemicellulose- derived aldopyranoses to the corresponding 2-keto sugars, concomitant with the reduction of O_2 to H_2O_2. Some POx can additionally oxidize sugars at the C3 position, but this activity is typically much lower than C2 oxidation.[137] C1 oxidation was also found in some POx, for instance, POx from *Trametes multicolor* favors C1 oxidation over C3 oxidation for monosaccharides with axial C4-OH including D-galactose and L-arabinose.[138] Additionally, several POxs can utilize alternative electron acceptors rather than oxygen, some even at catalytic efficiencies higher than for molecular oxygen;[139] therefore, the members of subfamily AA3_4 can include both POxs and PDHs. Recently, a member of subfamily AA3_1 that predominantly consists of cellobiose dehydrogenases, was also found to be a xylooligosaccharide dehydrogenase.[140] This enzyme from *Thermothelomyces myriococcoides* CBS 398.93 preferentially oxidize xylooligosaccharides at the hydroxyl on the reducing anomeric carbon over cellobiose and other cellooligosaccharides, thus it was suggested to use for generating xylan-based aldonic acid and selective removal of xylan fractions from lignocellulosic materials.[140]

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JACS Au 2024, 4, 4044−4065

4.2.3. AA5 Family for Aldehyde and Acidic Hemicelluloses. Family AA5 enzymes are copper radical oxidases (CROs) and currently divided into two subfamilies, where subfamily AA5_2 is particularly relevant to modifying hemicelluloses as it contains galactose oxidases (EC 1.1.3.9) along with the more recently identified raffinose oxidase (EC 1.1.3.-). These enzymes oxidize a primary alcohol of galactose and raffinose to the corresponding aldehyde with concomitant reduction of oxygen to H_2O_2 via a two-step process: the first half-reaction performs the oxidation of the substrate, while the second half-reaction regenerates the oxidation state of the active-site copper with the concurrent reduction of molecular oxygen to H_2O_2 (Figure 7). The process begins by transferring a proton from the hydroxyl group of the substrate to the oxygen atom of the axial tyrosine. Subsequently, a hydrogen atom is transferred from the alkoxy carbon to the tyrosine-cysteine radical. Finally, an electron transfer from the substrate results in the formation of the aldehyde while reducing the copper center to Cu(I). The resulting aldehyde can be oxidized further by the CRO to the corresponding carboxylic acid. The specificity of GalOx toward the C6 primary hydroxyl group of galactose and terminal galactosyl residues in polysaccharides is attributed to hydrogen bonding between an Arg residue and the axial orientation of the C4 hydroxyl in accepted substrates.[141,142]

The extensively researched enzyme within AA5_2 is galactose oxidase from *Fusarium graminearum* (*Fgr*GalOx, PDB: 1GOG), which catalyzes the regioselective oxidation of the C6-hydroxyl group in galactose.[143] *Fgr*GalOx acts on several galactose derivatives including 1-methyl-*β*-galactopyranoside,[144] as well as galactose-containing oligo- and polysaccharides such as lactose, melibiose, raffinose, galactoxyloglucan, galactomannan, and galactoglucomannan.[145,146] In addition, *Fgr*GalOx has been shown to act on guar gum, spruce galactomannan, tamarind galactoxyloglucan, corn arabinoxylan, and larch arabinogalactan.[146] Although the *Fgr*GalOx turnover number is much lower for polysaccharides than for D-galactose, the enzyme shows substantially higher catalytic efficiencies (k_{cat}/K_M) on polysaccharides, due to comparatively low K_M values.[147] The specific pattern of galactose branching and its relative proportion to other side groups within the same carbohydrate play a crucial role in influencing the enzyme's oxidation efficiency.[146] A variety of different mutagenesis and directed evolution experiments have been performed to increase the catalytic activity and to change the substrate specificity of *Fgr*GalOx. Through those engineering attempts, multiple variants with increased activity on D-glucose have been identified such as mutant M3, (aka mutant M-RQW)[148] and mutant Des3−2.[141] Other studies have identified mutant H1 with increased activity toward mannose[149] and mutant F2 with activity toward glucose and *N*-acetylglucosamine.[149] Beside galactose oxidases, subfamily AA5_2 also contains raffinose oxidases. One of these enzymes is raffinose oxidase from *C. graminicola*, which shows negligible activity on galactose, and comparatively high activity on tri- and disaccharides such as raffinose and melibiose, along with the glycolaldehyde dimer.[150]

The AA5_2-mediated oxidation of primary alcohols yields reactive aldehydes, enabling the synthesis of diverse compounds such as uronic acids, indium-mediated allylated and propargylated saccharides, and deoxy sugars.[151−153] Due to the intrinsic affinity of hemicellulose to cellulose, enzymatically oxidized polysaccharides have been applied to functionalize cellulose surfaces, facilitating the generation of biomaterials with new and beneficialproperties. For instance, by employing *Fgr*GalOx, clickable cellulose surfaces,[154] carboxylated polysaccharides for cellulose surface modification,[155] and selectively allylated polysaccharides[153] have been created. Enzymatically oxidized polysaccharides have also been directly applied to create aerogels relevant to food and medical applications.[156] Furthermore, disposable fluorogenic esterase sensors were developed through oxidation of xyloglucan by *Fgr*GalOx followed by click-chemistry and cellulose adsorption.[157]

4.2.4. AA7 Family for Acidic Carbohydrates. The AA7 family was established following the identification of fungal gluco-oligosaccharide oxidase (GOOX, EC 1.1.3.-, PDB: 2AXR) within culture supernatants of *Sarocladium strictum*.[158] Like FAD-containing AA3 enzymes, the catalytic mechanism of AA7 enzymes consists of two half reactions: a reductive half reaction followed by an oxidative half reaction (Figure 7). The majority of the characterized AA7 enzymes exhibit both oxidase and dehydrogenase activities, depending on the presence of electron acceptors; however, some enzymes demonstrate a preference for either oxidase or dehydrogenase activity.[112,159] AA7 oxidases and dehydrogenases are distinguished from AA3_2 and AA3_4 subfamilies by their regioselectivity toward the anomeric position of targeted carbohydrate substrates,[160−162] leading to the release of acidic carbohydrates. Another typical feature of this family is that the majority of AA7 proteins have a bicovalent attachment of the FAD cofactor.[159,163]

AA7 enzymes were found to oxidize carbohydrates with a wide range of DP, from monosaccharides to oligosaccharides to polysaccharides. GOOX is by far the most extensively studied carbohydrate-active AA7 oxidase to date, and has been shown to oxidize a broad palette of carbohydrates, including linear, branched, and acidic xylo-oligosaccharides.[160] GOOX has been used to transform methyl glucuronic acid, released from glucuronoxylan by a GH, into the corresponding glucaric acid for detergent and anticorrosion applications.[164] The regioselectivity of carbohydrate oxidases offers an opportunity to generate diacidic oligosaccharides from a wide range of hemicellulose sources. For example, GOOX has the capability to generate hemicellulosic oligosaccharides containing two carboxylic groups, which could undergo derivatization to incorporate clickable components, thereby serving as building blocks for innovative synthetic biopolymers.[165] Similar to GOOX, the carbohydrate oxidase from *Microdochium nivale* (MnCO, PDB: 3RJA) also acts on monosaccharides (D-glucose, D-maltose, D-xylose, D-lactose, and D-galactose), oligosaccharides[166] as well as polysaccharides, including locust bean gum, guar gum, and polygalacturonic acid.[167] The other members of AA7 family also exhibit a preference for acting on oligosaccharides, including *F. graminearum* chito-oligosaccharide oxidase ChitO (PDB: 6Y0R),[168,169] *Thermothelomyces thermophila* xylo-oligosaccharide oxidase XylO (PDB: 5K8E),[163] and cello-oligosaccharide oxidase from *Sarocladium oryzae*.[170] Although the overall fold of AA7 enzymes is well conserved, the sequences of the corresponding substrate-binding domains are more diverse, as reflected by the distinct substrate profiles of AA7 clades. For instance, UAO from *C. sinensis* (orange) displays selective activity toward uronic acids, including galacturonic acid and glucuronic acid, as well as polygalacturonate;[162] thus this enzyme is able to produce dicarboxylic compounds, which can be used in organic

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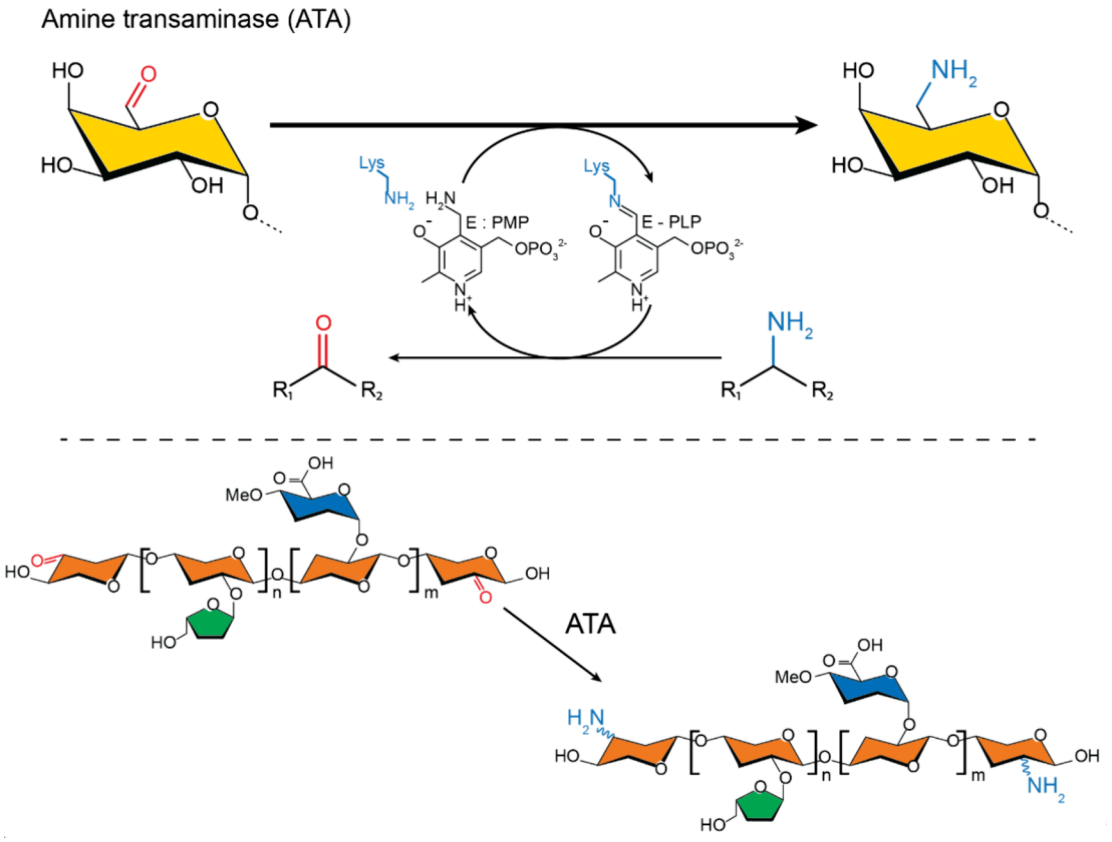

Figure 8. Transaminases for hemicellulose amination. Oxidized hemicelluloses can be further functionalized by adding amino groups to the resulting ketone or aldehyde positions. The top panel shows the mechanism of enzymatic transamination. The bottom panel shows an example of oxidized hemicellulose amination by amine transaminases (ATA).

synthesis and the production of polymers. Several other AA7 members from plants also act on oligogalacturonides.[171,172]

4.3. Enzymes for Hemicellulose Amination

Aminated polysaccharides display polycationic and antimicrobial properties relevant for use as flocculants in wastewater treatment, components of controlled-release drug matrices, beverage stabilizers, and cosmetic ingredients.[173,174] Chitin is the most abundant natural aminated polysaccharide, and it comprises repeating *N*-acetylglucosamine units connected via β-(1 → 4)-linkages and is a major component of arthropod exoskeletons and fungal cell walls.[175] Intensive processing steps are required to isolate chitin from largely animal sources; moreover, the narrow pH range conducive to solubilizing chitosan in water (pH 4.5−5) further restricts its applications as a bioflocculant or component of biomedical material.[176,177] Compared with chitin, hemicelluloses adopt a range of structures with different chemical and physical properties. Although plant polysaccharides, including hemicelluloses, lack amine functionality, chemical routes to their amination include periodate oxidation followed by reductive amination.[177−179] The use of strong oxidizers and toxic metal catalysts, overoxidations, and potential for depolymerization, however, are major drawbacks of such chemical processes and motivate enzymatic alternatives.

Amine transaminases (ATAs, omega-transaminases, EC 2.6.1.-) are pyridoxal 5′-phosphate (PLP) dependent enzymes that catalyze the transfer of primary amino groups from amines to ketones and aldehydes (Figure 8). Compared to other transaminase subgroups, ATAs are more versatile as they do not require a carboxylate group in the amino acceptor and can donate the amino group to α-keto acids as well as other ketones and aldehydes.[180,181] The transamination reaction is carried out via a ping-pong mechanism: the amino group from the donor amine is first transferred to a PLP cofactor, forming a pyridoxamine 5′-phosphate (PMP) intermediate; the PMP then reacts with an acceptor ketone or aldehyde, releasing an aminated product and restoring the PMP to PLP for a new catalytic cycle.[182,183] Active forms of ATAs are homodimers and homotetramers which are held together noncovalently by the PLP cofactor and via layers of interfacial loops.[184] Residues from one chain and a threonine from the other coordinate to form active sites where the PLP is covalently bound via the "catalytic lysine". When the PLP is aminated to PMP, it is released from the catalytic lysine and held in the cofactor binding site by hydrogen bonding and aromatic interactions.[185] This reduced cofactor affinity after half-transamination is believed to promote cofactor dissociation and reduce operational stability of ATAs.[185]

Early studies of the ATA from *Chromobacterium violaceum* (*Cv*ATA, PDB: 4A6D) revealed a broad substrate range and ability to aminate hydroxylated aldehydes such as D-erythrose, glycolaldehyde, and glyceraldehyde.[182] This observation motivated us to investigate the potential of *Cv*ATA to also aminate carbohydrates following their oxidation with galactose oxidase or pyranose dehydrogenase. Proving the concept of an enzymatic cascade to aminated carbohydrates, we demonstrated the production of amino galactose and amino-galactose-containing oligosaccharides through a one-pot cascade that combined *Fgr*GalOx or *Ab*PDH1 with

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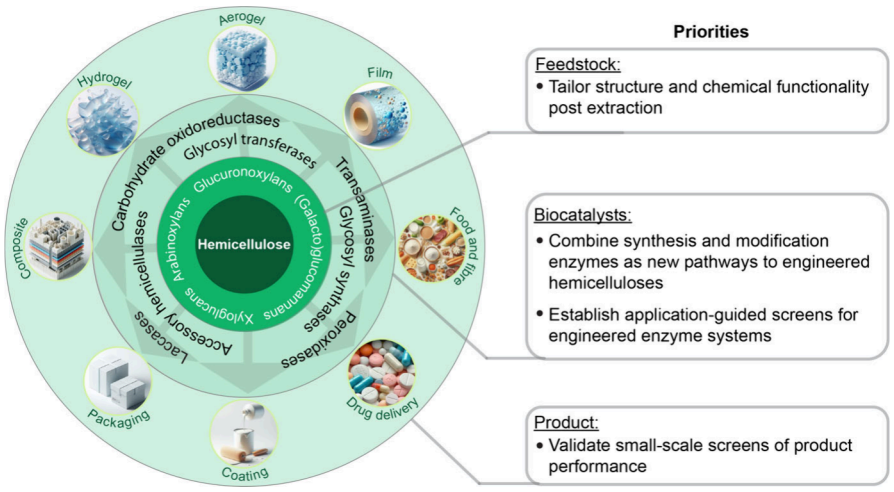

Figure 9. Enzymatic conversion of hemicelluloses to diverse and value-added biomaterials and the key properties to boost enzyme utilization in upgrading hemicellulose.

*Cv*ATA.[186] Using 1-phenylethylamine as the amino donor, nearly 70% of galactose amination could be obtained. More recently, *Cv*ATA and *Sp*ATA (PDB: 3HMU) from *Silicibacter pomeroyi* were compared for ability to produce aminated hemicelluloses.[187] Briefly, *Sp*ATA has been tested on amino acceptors with various sizes and structure and shows high substrate promiscuity.[188] To readily detect the formation of aminated polysaccharides, we established a colorimetric assay using 2-(4-nitrophenyl)ethan-1-amine as the amino donor to enable accurate yield measurements by tracking optical absorbance at 440 nm.[187] Using this assay, the yields of aminated galactomannan and xyloglucan generated by *Sp*ATA used together with *Fgr*GalOx were close to 10%.[187] Despite having much higher activity, *Cv*ATA produced lower product yields under the same reaction conditions which is believed to be the result of its lower operational stability.[187]

Increasing the yield of aminated pokysaccharide is possible by switching to an amino donor that forms a volatile deaminated product. For example, isopropyl amine may be used where the deaminated acetone product is evaporated by depressurization.[189,190] Increasing the operational stability of ATAs is also expected to increase the product yields. In particular, the deactivation of ATA during the catalytic cycle is believed to start with the dissociation of PMP from the active site.[191] The apo form of the ATA dimer is more prone to dissociate to monomers which can irreversibly unfold and precipitate.[191] Recently, we demonstrated the simultaneous deactivation of *Sp*ATA in reactions with variable enzyme loadings and highlighted the dependence of product yield on the enzyme deactivation.[187] So far, efforts to reduce dimer dissociation have focused on engineering amino acids near the dimer interface.[184] Stronger interaction between subunits helps to reduce ATA precipitation; however, high PLP consumption due to PMP release still requires exogenous cofactor addition to maintain ATA activity. Alternatively, engineering amino acids within the cofactor binding pocket to increase the PLP/PMP affinity and reduce PMP release could increase the ATA performance. A recent study proposed the V124N point mutation for *Cv*ATA to stabilize the ionic interaction between the pyridinium nitrogen and the aspartic acid D259.[192] Additional mutations near the pyridinium ring that enhance hydrophilic or aromatic interactions with the PLP/PMP cofactor may further reduce PLP consumption and generate mutants suitable for sustainable and cost-effective routes to hemicellulose amination.

5. PERSPECTIVES ON PRIORITIES TO COMMERCIALIZING ENZYME SYSTEMS FOR HEMICELLULOSE UPGRADING

Hemicelluloses comprise close to 30% of lignocellulose from wood and agricultural feedstocks but remain unused or underused. Processes to recover hemicelluloses from lignocellulose have been established; however, the challenge is how to control the composition and structure of hemicelluloses after extraction. As summarized in the current Perspective, we contend cell-free biocatalysis presents a beneficial approach to transforming complex hemicellulose structures into uniform ones that also comprise chemical functionalities for use in value-added materials (Figure 9). The use of plant cell wall biosynthetic enzymes as biocatalysts to produce designer glycan structures is an attractive approach to broaden the application space of bioderived and biocompatible polymers. The selectivity of enzymes permits reproducible and targeted hemicellulose modifications without requiring laborious protection/deprotection steps that reduce the product yield. Besides the multienzyme systems for hemicellulose engineering described above, we envision that new combinations could include enzymes that first introduce carbohydrates into hemicellulose structures that can be subsequently functionalized using existing carbohydrate oxidoreductases and transaminases. The identification of disaccharide side chains, terminated with Gal, present in both galactoglucomannan and glucuronoxylan in angiosprems presents a new biosynthetic route to insert selective chemical functionalities onto hemicelluloses. We propose that the respective galactosyltransferases can be used to further elaborate native plant structures in vitro or ex vivo through the addition of a terminal galactose unit by either XLPT (Figure 4) or MBGT (Figure 5), for xylan and mannan, respectively. The resulting structures would enable the enzymatic synthesis of aminated xylans using existing enzyme systems.

The commercialization of enzymes and cell-free enzyme systems for hemicellulose upgrading will require identifying expression systems for relevant enzyme families (e.g., GTs) that are amendable to design, build, and test protein

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engineering cycles. Glycosyltransferases are encoded in the genomes of organisms across all domains of life. The GTs discussed herein are of plant origin and localized in the secretory pathways and are thus post-translationally glycosylated. Several advances have been made in making eukaryotic expression systems more accessible. For example, human embryonic kidney (HEK) cell lines that support process scale up, while being capable of carrying out essential post-translational modifications, are currently the preferred platform for heterologous expression of both mammalian and plant GTs.[193,194] The use of mammalian cells can be scaled up for industrial purposes, as this has historically been done for the production of antibodies.[195] More recently, the Expi293F system has been developed to significantly enhance protein production as they can be cultured and transfected at higher densities. This allows protein yields up to six times higher than the original HEK293 cell expression systems, such as the HEK293F/FreeStyle 293-F system, that were first adapted to grow in suspension.[196] Other expression systems that have been used include yeast and insect cells for multimembrane spanning GT2 proteins involved in the synthesis of β-(1 → 4) backbone structures of cellulose[197,198] and mannan.[57] Interestingly, as the production of biopharmaceuticals continues to rise, there has been a concomitant shift toward the use of mammalian-based production systems, with the resulting products representing an almost 80% market share in 2018.[199] High yields (≥100 mg/L) of plant and mammalian GTs, *O*-acetyltransferases, and *O*-methyltransferases are easily achieved using the HEK cell-based expression.[193,194]

Besides large-scale production, challenges to biotechnological applications can include maintaining the solubility and stability of enzymes under desired reaction conditions. Recent advances in computationally guided workflows, such as PROSS 2[200] and ProteinMPNN,[201] to design optimized enzyme variants with improved stability, desired activity, functionality, and even expression are rapidly gaining traction. Together with access to cheap gene synthesis, it is increasingly possible to select a sequence for its catalytic function and application potential and then rapidly optimize using protein sequence design tools targeted for operational stability and high expression in a target biochassis/bioprocessing system. Notably, cell-free enzyme technologies for hemicellulose upgrading typically require the concerted activity of multiple enzymes (e.g., carbohydrate oxidase and transaminase). Accordingly, expanding enzyme engineering platforms that improve single enzymes to also increase the performance of enzyme systems will be important to commercialization. Such platforms for hemicellulose upgrading will often depend on functional screens designed to detect polymeric products that are difficult to measure using conventional high throughput analytical methods (e.g., UV−vis, liquid chromatography, and mass spectrometry). Instead, we envision "screens for enzyme application" rather than "screens for enzyme function", which could measure, for example, change in sample viscosity, light scattering, and analyte chelation. To realize the opportunity to make smarter use of renewable, abundant, and yet underused hemicelluloses, we urge more collaboration between enzymologists and material scientists, who together can establish application-driven screens that are configurable to different biological feedstocks and end-uses, and adopt biophysical measures that predict product performance at scale.

In summary, a decade's worth of careful biochemical studies has delineated the biological role and catalytic mechanism of the many enzymes that evolved to build and deconstruct hemicelluloses from plants and microorganisms, respectively. That knowledge opens possibilities to now tailor hemicellulose structures, so they are applicable for use in diverse and value-added biobased materials. This application of fundamental biochemical studies is gaining importance as we look to renewable bioresources as alternatives to fossil feedstocks and establish biobased economies made resilient through sustainable use and reuse for all renewable components.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacsau.4c00469.

Table summarizing hemicellulose isolation methods, including conditions, efficiency, and potential inhibitory byproducts (PDF)

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Notes

The authors declare no competing financial interest.

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