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An increasing number of transglycosylase-based processes provide access to oligosaccharides or glycoconjugates, some of them reaching performance levels compatible with industrial developments. Nevertheless, the full potential of transglycosylases has not been explored because of the challenges in transforming a glycoside hydrolase into an efficient transglycosylase. Advances in studying enzyme structure/function relationships, screening enzyme activity and generating synthetic libraries guided by computational protein design or machine learning methods should considerably accelerate the development of these catalysts. The time has now come for researchers to uncover their possibilities and learn how to design and precisely refine their activity to respond more rapidly to the growing demand for well-defined glycosidic structures.

1. Introduction

Glycosylation is a major reaction in numerous biological processes, including cell communication, signaling and protection, and in energy storage. The *in cellulo* transfer of glycosyl from a donor to an acceptor substrate is almost exclusively catalyzed by Leloir glycosyltransferases (GTs) [1]. The demand for oligosaccharides and glycoconjugates has steadily increased over the last decades. Well-defined structures are needed to investigate the role of glycosylation in biological phenomena. In addition, there is growing interest in the application of bioactive oligosaccharides and glycoconjugates in the food, health, and cosmetic industries. GTs are not ideal candidates for the *in vitro* and large-scale synthesis of glycoproducts, since they use expensive donors that are in limited supply. Reverse phosphorolysis catalyzed by glycoside phosphorylases (GPs) is another option. Sucrose phosphorylases for example were proven to be efficient for glycosylglycerol, resveratrol glucoside, kojibiose or nigerose synthesis [2]. However, such reactions may also have limitations: unfavorable thermodynamic equilibrium frequently occurs and sugar-1-phosphates are also rather expensive [3]. The third enzyme-based alternative is the use of glycoside-hydrolases (GHs), especially retaining GHs that cleave the osidic bond and retain the anomeric configuration [4]. These enzymes use abundant substrates, such as disaccharides, oligosaccharides, and polysaccharides, which is advantageous in the context of bio-economic development. Some GH families are naturally rich in very effective transglycosylases (TGs). This characteristic indicates evolutionary pathways between GHs and TGs, which can help researchers to create optimal synthetic tools. In this review, wild-type TGs have been targeted as prime examples to demonstrate the potential of these enzymes. Then, we focus on the strategies and methods used to accelerate the discovery of TGs and optimize their performance in synthesizing glycoproducts (Figure 1).

2. Native TGs for oligosaccharides

The TGs from GH families are enzymes that adopt a double-displacement as originally described by Koshland [5, 6] (Fig. 2a) or a substrate-assisted mechanism [7, 8] (Fig. 2b). In standard reaction conditions, the competition between transglycosylation and hydrolysis (T/H ratio) is under kinetic control.

2.1 Synthesis using naturally highly effective transglycosylases

These transglycosylases are involved in the synthesis or rearrangement of oligo or polysaccharide structures and can be found in the GH 13, 16, 68, 70 and 77 families. Their transglycosylation capacity is exceptional and mainly governed by the architecture of the active site that promotes interactions with the sugar acceptor while protecting the glycosyl-enzyme from water attack. These catalysts are some of the best tools to produce oligosaccharides, in particular as functional ingredients [9–12]. For example, inulooligosaccharides were obtained from sucrose using the GH68 inulosucrase InuO from *Bacillus agaradhaerens* [11]. They are very similar to plant derived inulin with a degree of polymerization (DP) ranging from 2 to 24. Raffinose and lactose were also glucosylated, from sucrose, using the novel GH70 glucansucrase URE13-300 from *Leuconostoc mesenteroides* URE13, with conversion of 47% and 59%, respectively. The resulting oligosaccharides were efficiently metabolized by various probiotic strains [11]. Other promising GH70 enzymes are the branching sucrases that graft isomaltooligosaccharides with α-1,3 linked glucosyl units, resulting in new and non-digestible functional ingredients [9, 13, 14]. In addition, up to three GH70 and GH13 glucansucrases that synthesize different types of α-glucans were used in cascade to produce glucooligosaccharides with mixed but controlled types of linkages from sucrose only [15]. Extending this principle to other transglycosylases would certainly give rise to unusual and unique oligosaccharide structures.

2.2 Synthesis using weak to moderate transglycosylases. Enzymes with weak synthetic ability are found in many GH families (e.g., GH1, 2, 16, 18, 20, 31, 35, 42A, 68 and 85). They display low T/H ratios and the transglucosylation products are often degraded by secondary hydrolysis. However, reaction engineering (i.e., high substrate concentrations; reduced water activity; modulated acceptor/donor ratios, pH, and temperature) can favorably impact transglycosylation [7, 16, 17]. For example, β-galactosidases produce prebiotic β-galactooligosaccharides (GOS) at lactose concentrations higher than 200 g/L with a yield of more 50% [17]. These enzymes can also catalyze chitosan galactosylation to produce oligosaccharides inhibiting the adhesion of enterotoxigenic bacteria [18]. Finally, the use of transglycosylases gains in interest to produce beneficial and non-digestible human milk oligosaccharides (HMO). Of the > 200 HMOs found in human milk, 29 structures have been produced enzymatically, as reviewed by Faijes et al. [19]. The latest developments mainly concern fucosylated compounds, which are involved in systemic immunity of breastfed babies [20]. Recently, Shi et al. [21] reported the production of 2' and 3' fucosyllactose with conversion of up to 85% using a novel α-L-fucosidase from *Pedobacter* sp., with *p*NP-Fuc as the donor and lactose as the acceptor. Obtaining sialylated oligosaccharides is another challenge and a novel exo-α-sialidase from *Bacteroides fragilis* NCTC9343 was shown to produce 6'-sialyllactose, a dominant sialylated HMOs, from 40 mM sialic acid dimer and 1M lactose, with a conversion above 20% [22].

3. Potential of wild-type TGs for glycoconjugate synthesis

Enzymatic decoration of secondary metabolites (polyphenol, terpenoids), alcohol or peptides with sugars is a robust method to access valuable and sought-after compounds with modified physico-chemical and biological properties compared with their aglycone counterparts [23]. A broad variety of glycoconjugates with glucosyl, galactosyl, fucosyl, fructosyl or oligosaccharidyl moieties can be obtained by using TGs (Fig. 3, Table 1).

3.1. Glycosylation of plant secondary metabolites

Over the past two years, many different polyphenol structures (mainly flavonoids) have been glycosylated with GH13, GH70 glucansucrases and GH68 levansucrases, highlighting the broad acceptor promiscuity of these catalysts. Transglycosylation modulates the hydrophilic/hydrophobic balance. For example, daidzin-mono and di-glucosides are 127 and 1686 times more soluble than daidzin, respectively [24]. The conversion, regioselectivity and degree of glycosylation are substrate and enzyme dependent (Table 1). By taking advantage of the distinct product specificity of two levansucrases from *Gluconacetobacter diazotrophicus* and *Bacillus subtilis*, a one-pot bi-enzymatic cascade was successfully implemented to produce puerarin-polyfructosides [25]. Glucansucrases (GH70) and cyclodextrin glucanotransferases have also been shown to glucosylate a large diversity of terpenoids, including ginsenoside, steviol glycosides and stilbenes. The new-to-nature α-glycosylated ginsenoside F1 (G1-F1) outperforms ginsenoside in terms of solubility, cytotoxicity and inhibiting effect toward tyrosinase for dermocosmetic applications [26]. Similarly, α-redaubioside C glucosylation has improved sweetness and solubility, but reduced bitterness compared with redaubioside C [27]. Hence, promising results have been obtained on generating novel glycoproducts from plant secondary metabolites to modulate their bioactivity.

3.2. Glycosylation of other hydroxylated compounds

There has been increasing interest in using glycopolymers (synthetic polymers with carbohydrate pendants) to study protein-sugar interactions and for biomaterials for gene therapy, drug delivery, disease inhibition, and biosensors [28]. Glycopolymers can be obtained by chemical polymerization of glyco-monomers. A variety of transglycosylases have been used to enzymatically produce β-mannosyl acrylates and β-glucosyl acrylamide [29, 30]. Hoffmann et al. [31] reported the gram scale synthesis of 2-(β-galactosyl)-ethyl methacrylate using the hyperthermostable β-glycosidase (GH1) from *Pyrococcus woesei* and a *p*NP-Gal/methacrylate ratio of 1/140 to achieve a yield of 88%. Different glycoside hydrolases from the GH5, GH13, GH39, GH52 families were also tested for their ability to synthesize alkyl glycosides and to produce a new variety of biosurfactants. GH20 β-N-acetylhexosaminidases can also be used for glycoconjugate synthesis. The enzyme from *Penicillium oxalicum* was recently found capable of transferring GalNac from *p*NP-GalNAc using a broad range of acceptors, including secondary and tertiary alcohols, coniferyl alcohol, inositol, and pyridine-3-aldoxim [32]. A recently discovered endo-1,3-fucanase from the marine bacterium *Wenyingzhuangia fucanilytica* (GH168 family) was reported to transfer sulfated fucose to glycerol and methanol from sulfated fucan, revealing a promising synthetic activity for the synthesis of sulfated glycoconjugates [33].

Increasing the repertoire of usable TGs for developing new pathways for naturally occurring glycosylated compounds or for synthesizing novel molecules is of great interest but not without challenges. However, recent advances give cause for optimism.

Table1: Glycoconjugate synthesis catalyzed by transglucosylases reported over the two last years

GH Family	Enzymes	Species	Donor substrate	Acceptor substrate (mM)	[a] Yield [b] Conversion	Product	Ref
GH1	β-galactosidase	*Pyrococcus woesei*	*p*NP-Gal	Ethylmethacrylate (up to 7200 mM)	88%[a]	O-galactoside	[31]
GH5	β-mannanase	*Trichoderma reesei*	β-mannotetraose	Methanol, Hexanol	-	O-mannoside	[34]
		Trichoderma reesei	β-mannotetraose	2 hydroxyethyl metacrylate	-		[30]
		Aspergillus niger		2 hydroxyethyl metacrylate Allyl-alcohol	-		
GH11	Xylanases	*Thermobacillus xylanilyticus*	Xylans	Catechol, Resorcinol, Hydroquinone,Pyrogallol (500 mM)	-	O-xylosides	[35]
GH13	Amylosucrase	*Cellumonas carboniz T 26*	Sucrose	Hydroquinone (5 mM)	40%-44,7%[a]	O-glucoside	[36]
		Deinococcus geothermalis	Sucrose	Apigenin (7.4 mM)	19.6% ± 5%[b]	O-glucoside	[37]
				Chrysin (7.9 mM)	-		
				6,7-dihydroxyflavone (7.9 mM)	56% ± 1.5%[b]		
				Homoorientin (4.4 mM)	57% ± 1.4%[b]		
				7-hydroxyflavone (8.4 mM)	-		
				Isorhoifolin (3.5 mM)	1.8% ± 0.7%[b]		
				Luteolin-3', 7-diglucoside (3.3 mM)	-		
				Orientin (4.5 mM)	-		
				Luteolin (7 mM)	86%[b]		
		D. geothermalis		α–isoquercitin (2.15 mM)	97.6%[b]	O-glucoside	[38]
		D. geothermalis DSM 11300	Sucrose	Hydroquinone (400 mM)	81%[a]	O-glucoside	[39]
				Resorcinol (200 mM)	65%[a]		
				Catechol (300 mM)	95%[a]		
		D. geothermalis	Sucrose	Daidzin (0.6 mM)	89%[b]	O-glucoside	[24]
		D. geothermalis	Sucrose	Hydroxyflavones (0.05mM)	-	O-glucoside	[40]
		D. geothermalis	Sucrose	8-hydroxydaidzein (0.4 mM)	-	O-glucoside	[41]
		D. wulumuqiensis	Sucrose	Isovitexin (5.8 mM)	-	O-glucoside	[42]
		Neisseria polysaccharea	Sucrose	Aesculetin, Aesculin (1 mM)	-	O-Glucoside	[43]
		*Xanthomonas campestris pv. Campestris str.*ATCC 33913	Sucrose	Hydroquinone (125 mM)	95.5%[a]	O-glucoside	[27]
		X. campestris pv. Campestris	Sucrose	Hydroquinone (fed batch)	-	O-glucoside (α-arbutin)	[44]
	Amylomaltase	*Thermus sp*	Starch	α, β-arbutin (20 mM)	83%[b]	O-glucoside	[45]
	Cyclodextrin-glucanotransferase	*Bacillus macerans*	β-cyclodextrin	α, β-arbutin (20 mM)	70%[b]	O-glucoside	[45]
		Thermoanaerobacter sp.	β-cyclodextrin	Resveratrol (3.9 mM)	35%[a]	O-glucoside	[46]
		Thermoanaerobacter sp.	β-cyclodextrin	Rebaudioside A (20 mM)	-	O-glucoside	[47]
		Thermoanaerobacter sp.	Dextrin	Ginsenoside F1 (1.6 mM)	-	O-glucoside	[26]
		Thermoanaerobacter sp.	Starch	Epigallocatechin gallate (20 mM)	58%[b]	O-glucoside	[48,
				Hesperetin (49 mM)	4%[b]		49]

		Thermoanaerobacter sp.	Starch	Pterostilbene (19 mM)	1.5%[a]	O-glucoside	[50]
		Thermoanaerobacter sp.	Starch	*Tert*-butanol	-	O-glucoside	[51]
GH32	β–fructosidase	*Xanthophyllomyces dendrorhous*	Sucrose	Hydroxytyrosol (162 mM)	45.6%[a]	O-fructoside	[52]
GH39	β– xylosidase	*Sphingomonas sp. JB13*	*p*NP-Xyl	Methanol, Ethanol, Propanol, Butanol, *iso*pentanol, glycerol	-	O-xyloside	[53]
GH52	β-xylosidase	*Anoxybacillus sp. 3M*	*p*NP-Xyl	Methanol, Ethanol, n-Propanol	-	O-xyloside	[54]
GH68	Levansucrase	*Gluconoacetobacter diazotrophicus*	Sucrose	Puerarin (25mM)	93.8%[b]	O-fructoside	[55]
				Ferulic acid (25mM)	6.4%[b]		
				Caffeic acid (25mM)	9%[b]		
				Rosmarinic acid (25mM)	15.2%[b]		
				Methyl-gallate (25mM)	5.9%[b]		
				Resveratrol (25mM)	7 %[b]		
				Mangiferin (25mM)	4 %[b]		
				Catechin (25mM)	11 %[b]		
				Neohesperidin (25mM)	2.5 %[b]		
				Coniferyl alcohol (25mM)	25 %[b]		
				Vanillin (25mM)	5%[b]		
		G. diazotrophicus	Sucrose	Puerarin (25mM)	88.7 %[b]	O-fructoside	[25]
		Bacillus subtilis			24.8%[b]		
		Leuconostoc mesenteroides			7.3%[b]		
		Zymomonas mobilis			9 %[b]		
GH70	Alternansucrase	*L. citreum*	Sucrose	Rebaudioside C (9.46 mM)	-	O-glucoside	[56]
	Dextransucrase	*L. mesenteroides* B-512 F/KM	Sucrose	Stevioside (62 mM)	98%[b]	O-glucoside	[57]
	Glucansucrase Gtf180-ΔN	*Lactobacillus reuteri*	Sucrose	Rebaudioside A (50 mM)	55%[b]	O-glucoside	[58]
	Glucansucrase	*L. citreum* DSM 5577	Sucrose	Protocatechuic acid (10 mM)	54%[a]	O-glucoside	[59]
				Caffeic acid (10 mM)	66%[a]		
		L. pseudomesenteroides DSM 20193	Sucrose	Nordihydroguaiaretic acid (30 mM)	95.5%[b]	O-glucoside	[60]
		Weissella beninensis DSM 22752	Sucrose	Protocatechuic acid (10 mM)	34%[a]	O-glucoside	[59]
				Caffeic acid (10 mM)	32%[a]		
				Umbellic acid (10 mM)	5%[a]		

4. Future directions to increase the panel of usable TGs

4.1. How to tap into biodiversity in an intelligent way?

Bioinformatic tools are used to explore sequence databases and extract sequences that encode efficient transglycosylases, but this is not without limitations. Phylogenetic analyzes based on Multiple Sequence Alignment (MSA) or sequence similarity networks (SSNs) generate robust data to categorize GH subfamilies. However, GH transglycosylation capability and enzyme substrate promiscuity toward donor or acceptor substrates are not clearly predicted, as recently illustrated by the SSN analysis of 23 000 sequences from the GH16 family [61]. This large family contains both hydrolases and transglycosylases that cleave the β-1,4- or β-1,3-glucosidic bonds of several glucans and galactans *e.g.* endo-β-1,3-glucanases, endo-β-1,3/β1,4-glucanases, β-1,3 glucanosyl transferases, hyaluronidase, elongating β-1,3-transglucosylase, etc. [62]. The SSN approach rapidly distinguished 23 different subfamilies; results were similar to those obtained from classical phylogenetic approaches using a more restricted set of sequences. However, it failed to segregate hydrolases and transglycosylases [62] found in four fungal subfamilies ((GH16_1, GH16_2, GH16_18, and GH16_19)) and one plant subfamily (GH16_20) because the sequence determinants of transglycosylation were too diluted. In this context, coupling the acquisition of biochemical and structural information with machine learning approaches is a promising emerging alternative as exemplified by Yang et al. [63] who predicted the transferase activity of GT1 glycosyltransferases from *Arabidopsis thaliana*. First, a functional dataset was generated by screening the library of 107 GT1s against 13 potential electrophilic donors and 91 nucleophilic acceptors using high-throughput MS based assays. The results obtained with 54 active GT1s were then used to train decision tree models incorporating physico-chemical information. The models successfully validated uncharacterized glycosyltransferase annotations and predicted reactivity of novel substrates. Such strategies could be extended to predict the functional profile of transglycosylases that also adopt a retaining mechanism working on an electrophilic donor and nucleophilic acceptor.

4.2. Expanding the scope of glycoside synthesis using enzyme engineering

Random technologies

Protein engineering technologies offer many options to improve TG performance with the objectives of increasing the T/H ratio and catalytic efficiency, and improving thermostability and/or substrate specificity. Directed evolution involving iterative cycles of random gene diversification coupled with selective screening is the most powerful method in the absence of structural or mechanistic information on the enzyme. However, this approach is rarely applied to TG engineering due to the lack of screening tests for the direct detection of transglycosylation products at very high throughputs (>10^6 mutants per day) without relying on surrogate or labeled substrates. To by-pass this technical limitation, Daudé et al. [64] applied the "neutral genetic drift" laboratory-driven method to evolve the amylosucrase from *Neisseria polysaccharea*. The method generated iteratively small-sized libraries that accumulated random amino-acid changes and did not modify the parental enzyme function. After four rounds, a library of 500 variants was screened using conventional liquid chromatography and variants were identified with enhanced activity toward an alternative donor and several acceptors of which one was not recognized by the parental enzyme. This demonstrates the efficiency of the method for multi-parameter engineering of TGs at low throughput. Moreover, for ultra-high throughput screening (excellently reviewed in Markel et al. [65]), emerging versatile technologies, such as MALDI-MS detection of analytes on colonies or label-free ESI-MS droplet screening, which should be extendable to many enzyme families, clearly open interesting perspectives for screening fully randomized libraries of TGs [66]. Such advances will also considerably accelerate biochemical data acquisition. We can expect a massive increase of QSAR methods and machine learning approaches to train algorithms and extract valuable information in order to track TG sequences in databases, further understand the mechanisms sustaining transglycosylation, and better guide the rational engineering of TGs.

Rational approaches

To date, rational and/or semi-rational methods have clearly taken center stage in TG engineering. They incorporate mechanistic and structural data, increasingly rely on protein/ligand interactions obtained from X-ray structures or molecular modeling approaches, and also start to integrate data from computational protein design algorithms [4]. The generation of glycosynthases and thioligases is certainly one the best illustrations of mechanism-based engineering that have made synthetic oligosaccharides and glycoconjugates widely available today. In glycosynthases, the catalytic nucleophile is mutated and the enzyme is fed with an activated donor substrate showing an anomeric configuration opposite to that of the natural substrate. After transfer to a suitable acceptor, no further hydrolysis can occur [67]. Numerous α- or β-glycosynthases from different GH families have been obtained to produce a broad range of oligosaccharides and glycoconjugates at the laboratory scale. A new pipeline for the generation of custom glycosynthases with new activities has recently been reported [68, 69]. A library of 175 GHs from the GH1 family, produced from synthetic genes, was first screened for hydrolysis of 6-amino- and azido-glucoside donors. The most active hits (8 out of 106) were subsequently tested against 83 different acceptors. Seven new glycosynthases synthesizing di- and tri-saccharides labeled with azido or amino groups were generated, thus validating a strategy, which could be extended to other GH families. The concept has been extended to enzymes from the GH18, 20 and 85 families mainly for HMO [7] and glycoprotein synthesis [8]. Notably, a chemoenzymatic process for lacto-N-triose II (LNT II) production, which used D746E glycosynthase derived from GH20 β-D-hexosaminidase LnbB from *Bifidobacterium*, converted 90% of GlcNac-1,2 oxazoline and lactose (both at 600mM) to obtain 500 mM (280 g/L) of LNT II [70]. The same enzyme was immobilized on agarose beads and used in a packed-bed reactor to produce LNT II with quantitative yields in continuous flow [71]. These processes outperform the current alternatives and highlight that both the enzyme and reaction need to be considered to optimize transglycosylation reactions. Another type of incompetent hydrolase, namely the thioglycoligases, is obtained by mutating GHs on their acid/base catalysts. They work with a donor exhibiting a good leaving group and a nucleophile with a low pKa. The range of glycosides prepared with these engineered enzymes has significantly increased during the two last years [72, 73]. Hence, thioligases have been designed to react with acids and produce arabino- and galacto-furanosyl esters [72].

The redesign of the glycone and aglycone subsites is certainly the most appealing option to modulate T/H ratio without altering the retaining mechanism. To this purpose, mutations causing destabilization of hydrolysis transition state, disruption of catalytic water binding, together with a better recognition of acceptors are often attempted [6, 15] but it must be underlined that understanding at the molecular level their impact on T/H ratio can be challenging [74]. A sequence-based approach using iterative MSA was recently applied to point the most conserved residues (6 to 12) in GHs from several families (*e.g.* Family GH 2, 10, 20, 29, 51). Replacing them with structural analogs increased by 2 to 9 fold the transglycosylation yield for more than 50% of the mutants indicating the method is worth to be tested with other retaining GHs (IMN Teze et al., bioRxiv doi: 10.26434/chemrxiv.11538708.v1). Several studies also suggested that mutations affecting the acid-base catalyst flexibility enhance transglycosylation [75, 76]. Loop engineering has been very effective in shifting the reaction toward acceptor glycosylation and generating efficient TGs from a GH20 hexosaminidase [7]. Acceptor subsites have also been successfully reshaped on several occasions to adapt sucrose active-enzymes from the GH70 and GH13 families to bulky flavonoids [77] or chemically protected saccharides [78], and generate TGs from the GH68 levansucrase [79] and GH70 dextransucrase [80] synthesizing size-defined oligosaccharides and polymers. Finally, the engineering, deletion or addition of domains, which are far from the catalytic sites, but are known to mediate binding with transglycosylation products, offer interesting perspectives to control glycan or oligosaccharide size. Two examples can be given. First, one mutation in the sugar binding pocket of domain V of several GH70 glucansucrases and/or the deletion of this domain produced downsized polymers [81–83]. Second, the inverse addition of a family 3 carbohydrate-binding module to the glycosynthase derived from the GH5 cellulase CelE from *Clostridium thermocellum* favored the formation of oligosaccharides up to DP5 [84].

5. Conclusion

Transglycosylases are effective tools to synthesize a wide variety of oligosides. A large number of proteins are available in nature that are currently not being exploited. Advances in gene sequencing and synthesis, combined with the development of ultrafast screening assays possibly coupled with the generic detection of glycosylation products, should considerably accelerate the acquisition of biochemical data and the discovery of novel catalysts. Therefore, transglycosylase profiling on a broader set of donor and acceptor substrates should be conducted in a much more systematic manner in order to generate high quality data that will complement computer-based protein design and machine learning algorithms to improve rational protein design approaches. Using transglycosylases of different specificities in one-pot synthesis or in cascade reactions can further diversify the panel of molecules that can be synthesized using TGs. Researchers should also consider using chemical steps in the synthesis process since they can increase yields and comply with the rules of green chemistry, especially if the toxic compounds can be easily recycled. More generally, an eco-design approach based on life cycle analysis needs to be followed as early as possible in the synthetic process, to ensure the eco-responsible synthesis of glycoproducts [85].

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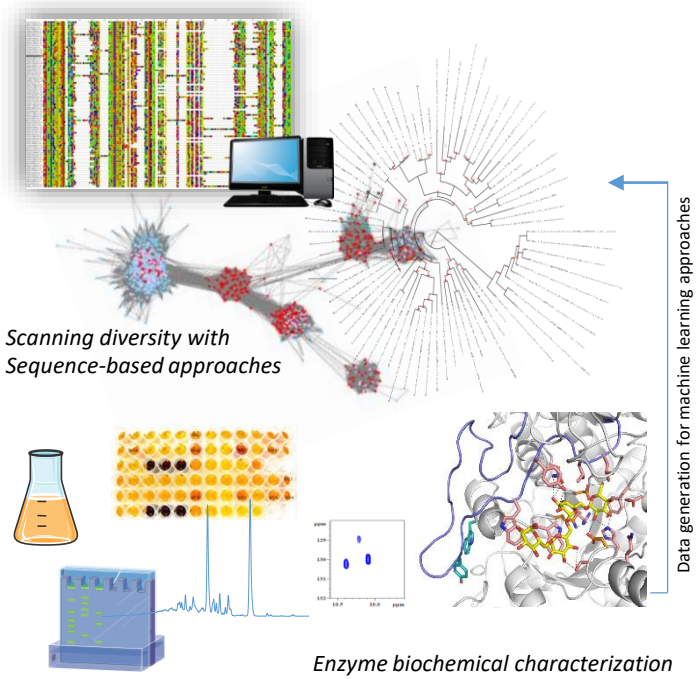
EXPLORING THE NATURAL DIVERSITY



Scanning diversity with Sequence-based approaches

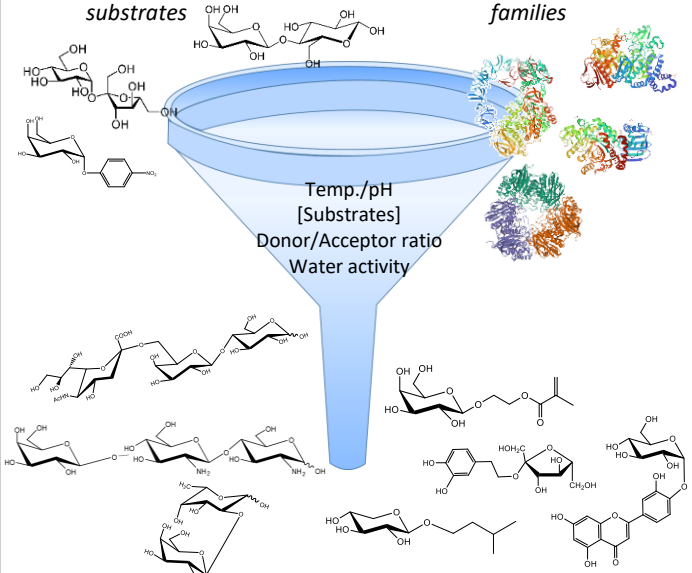
Data generation for machine learning approaches

Enzyme biochemical characterization

TRANSGLYCOSYLASES & GLYCOPRODUCTS

Cheap & available substrates

TGs from various GH families

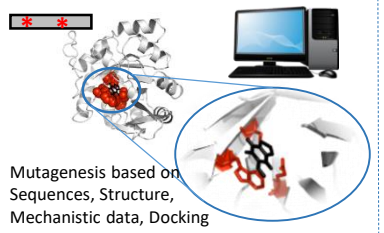


Temp./pH
[Substrates]
Donor/Acceptor ratio
Water activity

Broad diversity of oligosaccharides & glycoconjugates

EXPANDING THE DIVERSITY USING ENGINEERING

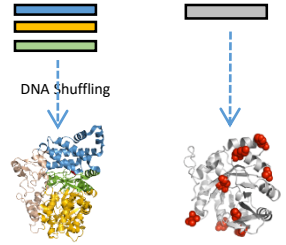
Rational / Semi-rational



Mutagenesis based on Sequences, Structure, Mechanistic data, Docking experiments, Protein Design

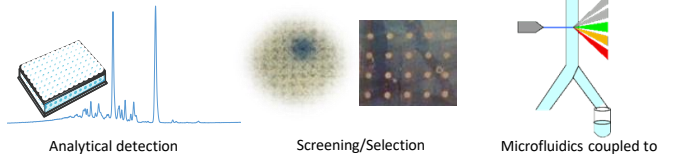
Small focused libraries (10-10^3)

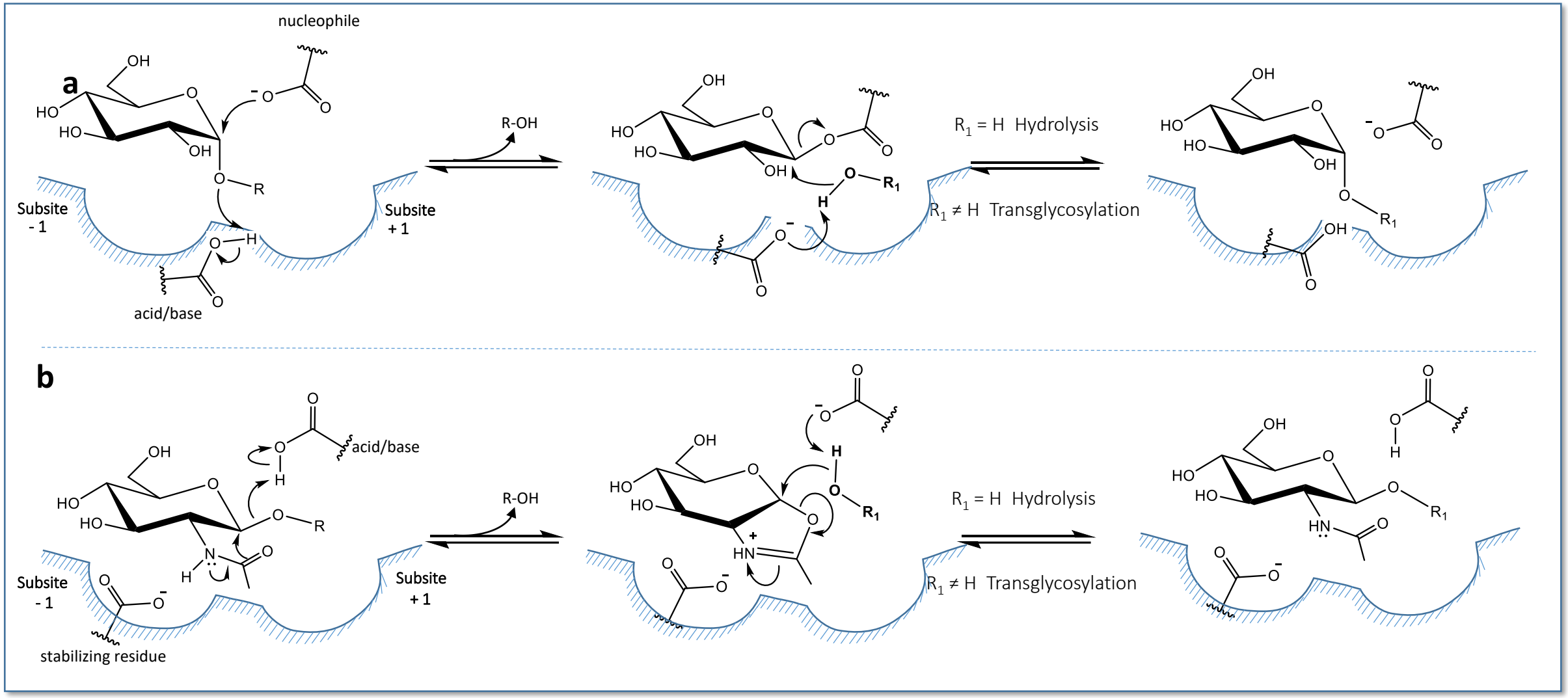
Random approaches

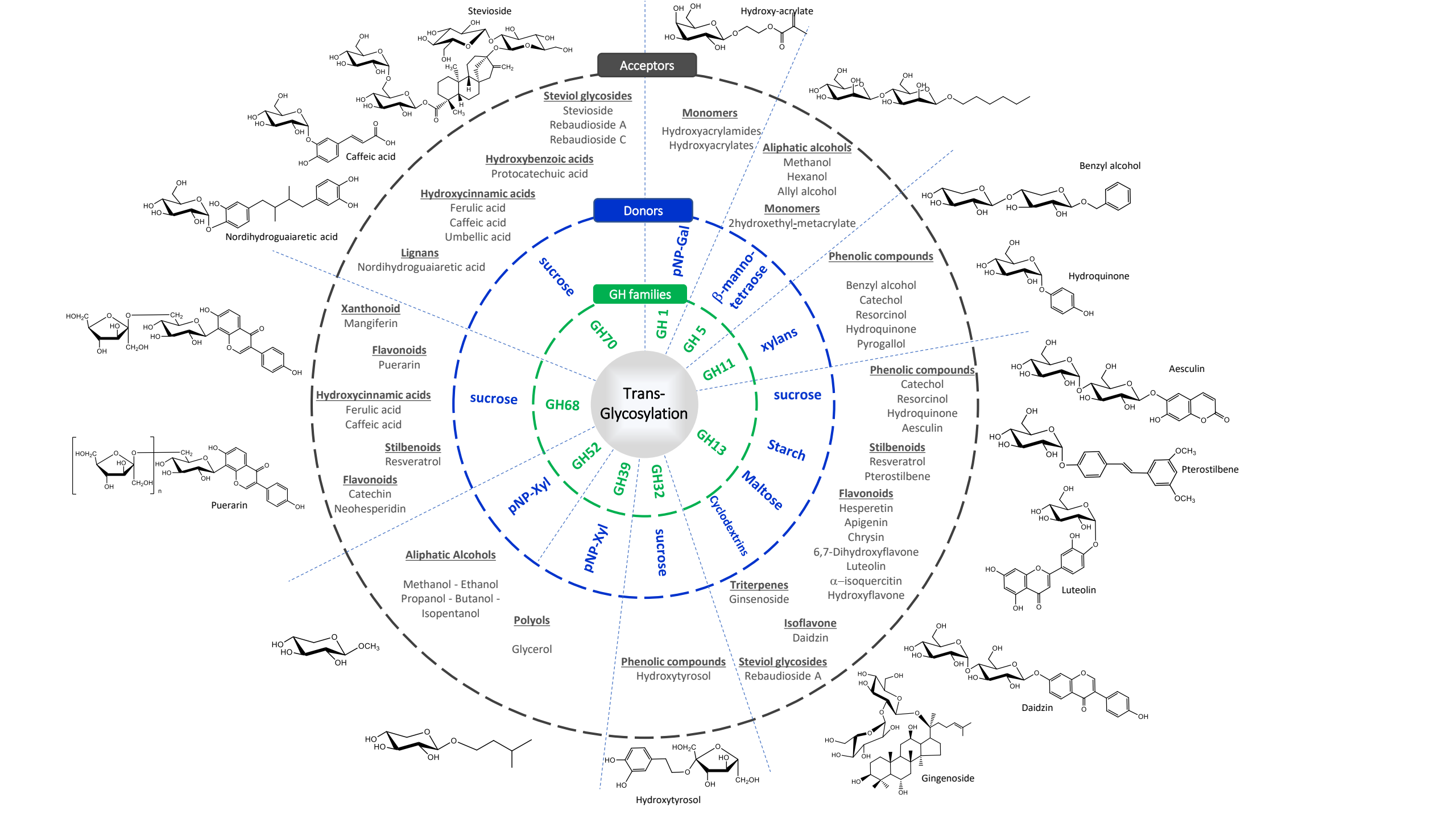


Large enzyme libraries (10^3-10^9)

Screening methods




a
nucleophile
Subsite
- 1
Subsite
+ 1
acid/base
R-OH
R1 = H Hydrolysis
R1 ≠ H Transglycosylation
b
acid/base
Subsite
- 1
Subsite
+ 1
stabilizing residue
R-OH
R1 = H Hydrolysis
R1 ≠ H Transglycosylation


Trans-Glycosylation
Acceptors
Donors
GH families
Steviol glycosides
Stevioside
Rebaudioside A
Rebaudioside C
Hydroxybenzoic acids
Protocatechuic acid
Hydroxycinnamic acids
Ferulic acid
Caffeic acid
Umbellic acid
Lignans
Nordihydroguaiaretic acid
Xanthonoid
Mangiferin
Flavonoids
Puerarin
Hydroxycinnamic acids
Ferulic acid
Caffeic acid
Stilbenoids
Resveratrol
Flavonoids
Catechin
Neohesperidin
Aliphatic Alcohols
Methanol - Ethanol
Propanol - Butanol -
Isopentanol
Polyols
Glycerol
Phenolic compounds
Hydroxytyrosol
Triterpenes
Ginsenoside
Isoflavone
Daidzin
Steviol glycosides
Rebaudioside A
Flavonoids
Hesperetin
Apigenin
Chrysin
6,7-Dihydroxyflavone
Luteolin
α–isoquercitin
Hydroxyflavone
Stilbenoids
Resveratrol
Pterostilbene
Phenolic compounds
Catechol
Resorcinol
Hydroquinone
Aesculin
Phenolic compounds
Benzyl alcohol
Catechol
Resorcinol
Hydroquinone
Pyrogallol
Aliphatic alcohols
Methanol
Hexanol
Allyl alcohol
Monomers
2hydroxethyl-metacrylate
Monomers
Hydroxyacrylamides
Hydroxyacrylates
sucrose
pNP-Gal
β-manno-tetraose
xylans
sucrose
Starch
Maltose
Cyclodextrins
sucrose
pNP-Xyl
pNP-Xyl
GH70
GH 1
GH 5
GH11
GH68
GH13
GH52
GH39
GH32
Stevioside
Hydroxy-acrylate
Caffeic acid
Nordihydroguaiaretic acid
Benzyl alcohol
Hydroquinone
Aesculin
Pterostilbene
Luteolin
Daidzin
Gingenoside
Hydroxytyrosol
Puerarin