Discovery of Glycosyltransferases Using Carbohydrate Arrays and Mass Spectrometry

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Abstract

Glycosyltransferases (GTs) catalyze the reaction between an activated sugar donor and an acceptor to form a new glycosidic linkage. GTs are responsible for the assembly of oligosaccharides *in vivo* and are also important for the *in vitro* synthesis of these biomolecules. However, the functional identification and characterization of new GTs are both difficult and tedious. This paper describes an approach that combines arrays of reactions on an immobilized array of acceptors with analysis by mass spectrometry to screen putative GTs. A total of 14,280 combinations of GT, acceptor and donor in four buffer conditions were screened and led to the identification and characterization of four new GTs. This work is significant because it provides a label-free method for the rapid functional annotation of putative enzymes.

Glycosyltransferases (GTs) are among the most abundant enzymes in nature and are important for the biosynthesis of glycans [1]. *In vivo*, GTs display high specificity in transferring a sugar from a nucleotide donor to an acceptor substrate to form glycosidic

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linkages. However, for GT-mediated reactions that are performed *in vitro*, the enzymes typically tolerate a broader set of substrates and therefore have been useful catalysts in the synthesis of oligosaccharides and derivatives [2–4]. The use of GTs to synthesize complex oligosaccharides offers the benefits that the enzymes create defined glycosidic linkages, avoid the need to employ tedious protection and deprotection steps that are required in organic synthesis, and are efficient catalysts at neutral pH [5]. Genomic analysis can be used to identify putative GTs, but functional assays are ultimately required to validate the transferase activities of the enzymes [6–7]. Furthermore, the discovery of GTs that have novel specificities for the donor and acceptor substrates they accept remains an important, but difficult, goal. For example, among the 60,000 putative GTs organized in the CAZy database (http://www.cazy.org/GlycosylTransferases.html) only a small fraction have been functionally characterized, reflecting a lack of efficient tools and systematic strategies to functionally characterize GTs [8–9].

To address this challenge, we developed a label-free assay that is based on a combination of self-assembled monolayers (SAMs) on gold that present carbohydrate substrates and mass spectrometry to identify products of the glycosylation reactions (Fig. 1a). The monolayers are well-suited to solid-phase assays of biochemical activities because the substrates are presented in a regular environment and the tri(ethylene glycol) groups that surround the substrate are highly effective at preventing nonspecific interactions with proteins and thereby ensure that the interactions of soluble proteins occur only by way of the immobilized ligands [10]. More importantly, the monolayers are compatible with matrix-assisted laser desorption-ionization mass spectrometry (in a technique referred to as "SAMDI") [11–14]. When the monolayer is irradiated with the laser, the alkanethiolates are desorbed from the gold substrate but undergo little fragmentation, providing spectra that directly reveal the masses of the ligand-substituted alkanethiols (or alkyl disulfides). In this way, the spectra show clear peaks for both the substrate and products of an enzyme-mediated conversion and are valuable in efficiently identifying enzyme activities.

We used the SAMDI assay to evaluate nearly 60,000 reactions comprising unique combinations of a putative GT, nucleotide donor, immobilized acceptor and buffer composition. Forty-four reactions showed new peaks that correspond to individual glycosylation products and gave a functional validation of four new GTs, including an enzyme that displayed a novel specificity in forming a glycosidic linkage.

RESULTS

Reaction screening

We analyzed the genomes of several microorganisms to identify putative glycosyltransferases to include in the screen. In this way, we emphasize that the SAMDI method can be used in a non-biased screen to identify unanticipated transferase activities, though we note that this approach can also be used to analyze GTs that are taken from a single species or directed at a mechanistic question. Some fraction of these enzymes may not catalyze glycosylation reactions—they could, for example, have hydrolase activity or act on acceptors that use other functional groups as the nucleophile—and would not be active in the present screen. To speed the analysis, we used a high throughput screening platform

based on metal plates having an array of 384 gold-coated islands in the standard microtiter plate geometry [15]. We prepared 24 oligosaccharide acceptors (Supplementary Methods, Supplementary Table 1 and Supplementary Fig. 1–4) and immobilized individual acceptors on the gold features by either forming monolayers from carbohydrate-terminated alkanethiol reagents, or performing immobilization reactions of a thiol-functionalized carbohydrate with a maleimide-terminated monolayer, or an azido-functionalized carbohydrate with an alkyne-terminated monolayer (Supplementary Fig. 5). Our use of multiple strategies for immobilizing the acceptors owes to the availability of, or efficient access to, the carbohydrate reagents. The choice of chemistries used to link the acceptor to the monolayer should not have a significant influence on the glycosylation reaction since GTs generally display selectivity for the terminal carbohydrate unit of the acceptor. We therefore expect the activities that are discovered in the screen to not be compromised by the choice of the immobilization reaction; in support of this idea, we discuss later the use of a homogeneous format to verify the activities of GTs found in this work.

An example of the screening assay is shown in Figure 1 and starts with a monolayer to which the acceptor lactose is immobilized. A SAMDI spectrum of the monolayer showed a peak at m/z 1296 that corresponds to the mixed disulfide with a single lactose group. The peak at m/z 693 represented the tri(ethylene glycol) disulfide. The monolayer was treated with bovine α1,3-galactosyltransferase (GGTA1) and analyzed by SAMDI to reveal a peak at m/z 1458 that corresponds to the mixed disulfide containing the trisaccharide that resulted from enzymatic galactosylation of lactose [16]. The absence of a peak at m/z 1296 demonstrated that the enzymatic reaction was essentially complete. We applied this assay to screen 85 GTs (Supplementary Results, Supplementary Table 4)—including 76 putative bacterial enzymes that had not been previously characterized—with the goal of identifying new GT activities.

Because traditional methods for protein expression and purification are tedious, time-consuming, and can compromise protein activity, we used an *in vitro* expression system to rapidly prepare the GTs, which were then assayed in unpurified form (Supplementary Fig. 7). Each individual protein was first mixed with one of the 7 sugar donors dissolved in one of four buffers—which were selected to vary the divalent metal ion and pH—and then applied to individual gold islands presenting the sugar acceptor (Fig. 1). The four buffers were selected because of their common use in enzyme assays and include different divalent ions and values of pH (see Methods).

From the 57,120 reactions tested in the screen, forty-four showed new glycosylation products (Supplementary Table 5). Included in these hits were glycosylation activities for four previously uncharacterized enzymes (Fig. 2). Two of these, BF0009 (GT80) and BF0614 (GT84), are from *Bacteroides fragilis*, and catalyze the GalNAcylation of β-glucose and cellobiose, and the galactosylation of N-acetylglucosamine (GlcNAc), respectively (Fig. 2a–c). The two others, HD0466 (GT24) and AAF28363.1 (GT09), are from *Haemophilus ducreyi* and transfer GlcNAc from UDP-GlcNAc to lactose and to GlcNAc, respectively (Fig. 2d–e). We also found that two known galactosyltransferases could utilize donors that had not been reported previously. The GTs GGTA1 (GT02) and LgtC (GT06), in addition to utilizing the UDP-Gal donor were also found to glycosylate their substrates with UDP-Glc

for the former and with UDP-Glc and GDP-Man for the latter (Fig. 2f and Supplementary Fig. 8).

To elaborate on one example, the protein expressed from the gene BF0009 (GT80) was mixed with the donor UDP-GalNAc and applied to a spot presenting β-glucose. The SAMDI spectrum revealed a new peak at m/z 1337 (Fig. 2a), which was 203 Dalton greater than the peak for the acceptor-terminated alkyl disulfide (m/z 1134) and is consistent with the addition of GalNAc to the acceptor. To estimate the yield with which the GTs were expressed in active form, we included nine known GTs in the screen and found that six were active (Supplementary Table 5). We believe that this fraction provides a fair estimate of the yield for the expression of the other putative enzymes examined in this work, though we have not determined whether the inactivity arises from improper folding of the enzymes, requirement for cofactors or regulatory domains, or an inaccessibility of the immobilized substrates.

Identification of the glycosidic linkages in the products

The SAMDI screen is effective at identifying combinations of GT, donor and acceptor substrates that generate new glycosidic linkages, but the use of mass spectrometry does not provide information on the regio- and stereo-chemical structure of the linkage. To characterize the products, we expressed the four new enzymes in *E. coli* BL21(DE3) cells and used them in preparative glycosylation reactions to generate milligram quantities of the products, which we then characterized using 1D and 2D NMR (Fig. 2, Supplementary Table 2 and Supplementary Results). We found that: BF0009 (GT80) catalyzes the formation of aβ1,3 linkage between GalNAc and glucose; BF0614 (GT84) joins galactose and GlcNAc through a β1,4 bond; HD0466 (GT24) joins GlcNAc and lactose through a β1,3 linkage; and AAF28363.1 (GT09) creates aβ1,4 linkage between two GlcNAc residues.

Biochemical characterizations of the new GTs

We selected three GTs that gave new activities in the screen and characterized their kinetic parameters. These experiments used a 'pull-down' format wherein reactions were performed in solution, quenched and then applied to a monolayer to allow the substrate and product to undergo immobilization prior to analysis by SAMDI [17]. This method avoids perturbations that may arise from presentation of the ligands at the surface. We used an azido-modified oligosaccharide as the acceptor and a monolayer presenting terminal alkyne group to selectively immobilize the substrate and product of the reaction (Supplementary Table 3 and Supplementary Fig. 9). Previous work has shown that the SAMDI method provides quantitative information for the rates of enzyme-catalyzed reactions, including reactions of GTs [17–18]. In one example, we characterized the BF0009-mediated transfer of UDP-GalNAc to an azido-glucose substrate by performing a series of reactions with the glucose azide and UDP-GalNAc present at several concentrations and for several reaction times (Supplementary Fig. 9c). The yields were determined by integrating the areas of the mass peaks for the product and acceptor substrate (Supplementary Fig. 9b and Supplementary Fig. 10). Kinetic parameters were determined from double reciprocal plots (Supplementary Fig. 11) [19–20] and are summarized in Table 1. We also performed this experiment using a radiolabeled assay and found that those results agreed with our determination of kinetic

parameters using the SAMDI pull-down assay (Supplementary Table 6). These experiments revealed several features of the GTs. The value of K_b for the UDP-Gal donor is an order magnitude lower with the human enzyme, reflecting a stronger interaction between the human homologue and the donor. In another example, HD0466 and the closely related β1,3-N-acetylglucosaminyltransferase from *Neisseria meningitidis* (LgtA) [3] each interact with the donor with similar affinities and perform the glycosylation reaction with similar values of V_{max}.

Many GTs display conserved folds and structural motifs that bind, and require, divalent metal ions for activity. We therefore also investigated the metal-dependent activities of the GTs that were found in this work (Supplementary Fig. 6 and Supplementary Table 7) [22]. The addition of EDTA resulted in a loss of activity for each of the three enzymes described above, consistent with a requirement for metal ions. The three GTs were most active in the presence of Mn^{2+} and Mg^{2+}, but were also active with other divalent metal ions. For example, BF0009 displayed the highest activity with Mg^{2+}, retained activity when Mn^{2+}, Co^{2+} or Mo^{2+} ions were present in the buffer, had only minor activity when Cu^{2+} or Ni^{2+} were present, while Ca^{2+}, Fe^{2+} or Zn^{2+} elicit no activity. Comparison of HD0466 and LgtA surprisingly showed differences in metal dependence, with HD0466 having higher activity in the presence of Mg^{2+} relative to Mn^{2+} whereas LgtA showed the reversed trend.

DISCUSSION

While GTs represent one of the most abundant protein families, they are also among the least characterized. This discrepancy owes to the limitations inherent to traditional assays of GT activity. For example, *in vitro* studies of substrate specificities require significant quantities of protein, donors and acceptors, all of which are expensive. The assays commonly use radioactive donors and remain tedious and difficult to adapt to high throughput formats. Moreover, the *in vitro* characterization of a GT is typically pursued only after a relevant glycosylation function has been identified by other means (i.e., genetic experiments), reflecting the difficulty in discovering these activities based on activity profiling. Over the past dozen years, several groups have contributed to the development and application of carbohydrate arrays that allow a higher throughput and comprehensive characterization of GTs [23–29]. Much of this work has relied on fluorescense as a detection method by using fluorescently-labeled lectins [24], biotinylated sugar nucleotide donors [25] or labeled polysaccharide acceptors [26]. These impressive examples demonstrate the utility of glycan arrays in profiling enzymes that modify carbohydrate substrates and motivated the development of 'label-free' strategies that could find even broader use in glycobiology.

Our reports that mass spectrometry could be used to analyze self-assembled monolayers provided early examples of a label-free assay of GT activity [11, 13]. A related approach was used to assess the specificity of a polypeptide GalNAc transferase using an array of peptides immobilized on SAMs [27]. Other work used MALDI-TOF mass spectrometry to identify reaction products of glycan arrays on an aluminum oxide-coated glass slide [28] and of alkyl-glycans that were immobilized onto monolayers using hydrophobic interactions [29]. The example we describe in the present work is significant because it integrates laboratory automation protocols with mass spectrometry to efficiently perform tens of thousands of

reactions for the purpose of discovering novel GTs. The requirement for minimal amounts of enzyme also enabled the use of an *in vitro* expression system, which offers the potential of generating hundreds of proteins quickly, though only at pmol levels. Finally, the label-free assay has the advantage that it avoids the possible interference of labels with enzymatic activities and it enables the discovery of unanticipated events [14].

The present example resulted in the discovery of four bacterial proteins that have specific GT activities. Although the biological roles of the enzymes remain unknown, they provide new catalysts that can be used to generate oligosaccharides with novel structures. For example, the protein BF0009 from *B. fragilis* is particularly significant because the enzyme catalyzes a reaction that yields a linkage that has not previously been observed in bacterial systems. Further, this enzyme only recognizes UDP-GalNAc as the donor substrate among the seven donors screened, making it more selective than related enzymes, including bovine milk galactosyltransferase, which accepts UDP-Gal, UDP-Glc and UDP-GalNAc [30]. We also note that carbohydrate associated antigens are related to the pathogenesis of *B. fragilis* [31] and future work may reveal whether the novel GalNAcβ1,3-Glc linkage exists in the bacterium and whether it is relevant to the pathogenesis.

In summary, this work demonstrates an effective strategy for combining carbohydrate arrays and mass spectrometry for the functional annotation of enzyme families. The method is significant because it combines the immobilized arrays with a true label-free detection method that allows direct read-out of the biochemical activities on the array. This method for the high throuput characterization of GT activity offers a new opportunity for the identification of novel and interesting GTs from both bacterial and eukaryotic sources. We believe it will also be important for the functional annotation of other enzyme families.

METHODS

In vitro expression of putative glycosyltransferases

The sources of materials including bacteria strains, genomic DNAs, plasmids, and growth conditions can be found in Supplementary Methods. The *in vitro* expression of the putative GTs was performed using the Expressway Cell-Free *E. coli* expression system as described by the vendor. In brief, 2 μg of plasmid was used for each 100 μL volume of the reaction volume, and the reactions were incubated at 37 °C for 4 hours. Insoluble particulates were removed by centrifugation and the supernatant containing soluble protein was used immediately in glycosylation reactions. A vector provided by the manufacturer (harboring a non-GT gene) was used as a positive control for protein expression, and the empty-vector pMCSG7 was used as a negative control and gave no detectable endogenous GT activity. The expression of the target enzymes were confirmed by western blot using mouse anti-His antibody and anti-mouse IgG horseradish peroxidase secondary antibody (Supplementary Fig. 7). The films were developed using the ECL with western blot detection reagent.

Reaction screening

Reactions were performed on metal plates having a 24 by 16 array of gold islands modified with monolayers. The preparation of the oligosaccharide-terminated monolayers is discussed

in detail in the Supplementary Methods. Each sugar nucleotide donor was dissolved in one of the four buffer systems (0.75 mM, 4 μL) and combined with protein (2 μL) in a 384 well plate. The buffers used in this work were the following: Tris-HCl (50 mM, pH 8.0) and MnCl$_2$ (10 mM); sodium cacodylate (100 mM, pH 6.0) and MnCl$_2$ (10 mM); Tris-HCl (50 mM, pH 7.5) and CaCl$_2$ (10 mM); HEPES (50 mM, pH 7.5). The resulting reaction mixtures were then transferred to individual gold islands on a 384 metal plate presenting one of the 24 sugar acceptors. Liquid handling was performed by a Tecan Freedom Evo 200 robot. We estimate that enzymes were present at concentrations in the low nM range. The plates were kept in humidified chambers at 37 °C for 2 hours, and then rinsed with water followed by ethanol. The plates were dried under nitrogen, treated with a solution of 2,4,6-trihydroxyacetophenone matrix (THAP, 5 mg mL^{-1}, 0.5 mL per plate) and analyzed by SAMDI mass spectrometry. A 355 nm Nd:YAG laser was used as the desorption/ionization source with an accelerating voltage of 20 kV and extraction delay time of 50 ns. All spectra were acquired automatically using positive reflector mode. The combinations of GT, donor and acceptor substrate that gave a glycosylation reaction are summarized in Supplementary Table 5. The new enzymes were expressed again in *E. coli*, and were used to synthesize oligosaccharide products at preparative scale. See Supplementary Methods for the details of enzyme expression, oligosaccharide preparation and structure characterization. LgtC and GGTA1 were also expressed in *E. coli* and their activities with UDP-Glc and GDP-Man (for LgtC), and UDP-Glc (for GGTA1) were confirmed in a SAMDI assay.

Determining kinetic parameters of GTs using SAMDI

To obtain kinetic constants for the new GTs, reactions were performed in solution and then the substrate and product were immobilized to a monolayer by way of a Click reaction [18]. Reactions were initiated in 384 well plates and quenched through the addition of EDTA and then applied to monolayer and analyzed by SAMDI, according to the following protocols. Each reaction contained one of the purified enzymes, the sugar donor, the acceptor, Tris-HCl (50 mM, pH 8.0) and MnCl$_2$ (10 mM) in a total volume of 10 μL. The enzymes were used at the following concentrations: BF0009, 0.14 mg mL^{-1}; BF0614, 0.3 mg mL^{-1}; HD0466, 0.3 mg mL^{-1}. The concentrations of donors ranged from 100 μM to 5 mM and those of the acceptors ranged from 250 μM to 5 mM. For each set of concentrations, the reactions were carried out for times ranging from 2 to 30 min at intervals of 2 to 3 min and terminated by adding a mixture of cold ethanol and EDTA (10 mM, 20 μL). Each reaction mixture was then applied to an individual gold circle of the array (in a volume of 2 μL) that was modified with the alkyne-terminated monolayer. An aqueous solution (1μL per reaction) containing CuBr (2 mM) and triethylamine (0.5 mM) was applied to each circle and the reactions were incubated at room temperature from 30 min to 6 hours, depending on the concentrations of the azido sugars. The completion of the reactions was monitored by SAMDI. The slide was then rinsed with water, followed by ethanol, and dried under nitrogen. For quantification, the extent of glycosylation (R) was determined from the peak intensities for product (I$_p$) and acceptor substrate (I$_s$) on the SAMDI spectra using the relation: $R = I_p/(I_p+I_s)$. We confirmed that the measured ratio reflects the actual ratio of the two azido sugars in solution by performing a calibration experiment, which is discussed in detail in the **Supplementary Methods** and Supplementary Fig. 10. The yield of the glycosylation, calculated from the equation in Supplementary Fig. 9b, was plotted against

the reaction time. The linear region of the plot was fitted to obtain the slope which represented the initial velocity (v_0). Double reciprocal plots of initial velocities are plotted in Supplementary Fig. 11. For these plots, the donors were the variable substrates and the acceptors were the constant substrates. The data were fit to equation (1), which has been used to describe bisubstrate enzyme kinetics [19–20].

$$\frac{1}{v_0} = \frac{K_{ia} \times K_b + K_a \times [B] + K_b \times [A] + [A] \times [B]}{V_{max} \times [A] \times [B]} \quad (1)$$

In this equation, [A] is the concentration of the acceptor, [B] is the concentration of the donor, V_{max} is the maximum velocity, K_a and K_b represent the cognate Michaelis constants for substrates A and B, respectively and K_{ia} is the dissociation constant of the substrate A. The metal activity studies were carried out in a similar fashion to obtain initial velocities and the details can be found in Supplementary Methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Ohtsubo K, Marth JD. Glycosylation in cellular mechanisms of health and disease. Cell. 2006; 126:855–867. [PubMed: 16959566]

2. Koeller KM, Wong CH. Enzymes for chemical synthesis. Nature. 2001; 409:232–240. [PubMed: 11196651]

3. Blixt O, van Die I, Norberg T, van den Eijnde DH. High-level expression of the *Neisseria meningitidis* lgtA gene in *Escherichia coli* and characterization of the encoded *N*-acetylglucosaminyltransferase as a useful catalyst in the synthesis of GlcNAcβ1→3Gal and GalNAcβ1→3Gal linkages. Glycobiology. 1999; 9:1061–1071. [PubMed: 10521543]

4. Yu H, et al. A multifunctional *pasteurella multocida* sialyltransferase: a powerful tool for the synthesis of sialoside libraries. J Am Chem Soc. 2005; 127:17618–17619. [PubMed: 16351087]

5. Breton C, et al. Structures and mechanisms of glycosyltransferases. Glycobiology. 2006; 16:29R–37R. [PubMed: 16049187]

6. Yi W, et al. Bacterial homologue of human blood group A transferase. J Am Chem Soc. 2008; 130:14420. [PubMed: 18842049]

7. Li M, et al. Characterization of a novel alpha 1,2-fucosyltransferase of *Escherichia coli* O128: B12 and functional investigation of its common motif. Biochemistry. 2008; 47:378–387. [PubMed: 18078329]

8. Aharoni A, et al. High-throughput screening methodology for the directed evolution of glycosyltransferases. Nature Methods. 2006; 3:609–614. [PubMed: 16862135]

9. Cantarel BL, et al. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for glycogenomics. Nucleic Acids Res. 2009; 37:D233–238. [PubMed: 18838391]

10. Houseman BT, Mrksich M. Carbohydrate arrays for the evaluation of protein binding and enzymatic modification. Chem Biol. 2002; 9:443–454. [PubMed: 11983333]

11. Su J, Mrksich M. Using mass spectrometry to characterize self-assembled monolayers presenting peptides, proteins, and carbohydrates. Angew Chem Int Ed. 2002; 41:4715–4718.

12. Min DH, Su J, Mrksich M. Profiling kinase activities by using a peptide chip and mass spectrometry. Angew Chem Int Ed Engl. 2004; 43:5973–5977. [PubMed: 15547909]

13. Ban L, Mrksich M. On-chip synthesis and label-free assays of oligosaccharide arrays. Angew Chem Int Ed Engl. 2008; 47:3396–3399. [PubMed: 18383458]

14. Mrksich M. Mass spectrometry of self-assembled monolayers: a new tool for molecular surface science. ACS Nano. 2008; 2:7–18. [PubMed: 19206542]

15. Gurard-Levin ZA, Scholle MD, Eisenberg AH, Mrksich M. High-throughput screening of small molecule libraries using SAMDI mass spectrometry. ACS Comb Sci. 2011; 13:347–350. [PubMed: 21639106]

16. Fang JW, et al. Highly efficient chemoenzymatic synthesis of alpha-galactosyl epitopes with a recombinant alpha(1→3)galactosyltransferase. J Am Chem Soc. 1998; 120:6635–6638.

17. Min DH, Yeo WS, Mrksich M. A method for connecting solution-phase enzyme activity assays with immobilized format analysis by mass spectrometry. Anal Chem. 2004; 76:3923–3929. [PubMed: 15253625]

18. Guan W, et al. Combining carbochips and mass spectrometry to study the donor specificity for the *Neisseria meningitidis* beta 1,3-*N*-acetylglucosaminyltransferase LgtA. Bioor Med Chem Lett. 2011; 21:5025–5028.

19. Leskovac, V., et al. Comprehensive enzyme kinetics. 1. Springer; New York: 2003.

20. Cleland WW. Statistical analysis of enzyme kinetic data. Methods Enzymol. 1979; 63:103–138. [PubMed: 502857]

21. Bella A, Whitehead JS, Kim YS. Human plasma uridine diphosphate galactose-glycoprotein galactosyltransferase: purification, properties and kinetics of the enzyme-catalyzed reaction. Biochem J. 1977; 167:621–628. [PubMed: 414742]

22. Breton C, Mucha J, Jeanneau C. Structural and functional features of glycosyltransferases. Biochimie. 2000; 83:713–718. [PubMed: 11530202]

23. Rillahan CD, Paulson JC. Glycan microarrays for decoding the glycome. Annu Rev Biochem. 2011; 80:797–782. [PubMed: 21469953]

24. Park S, Shin I. Carbohydrate microarrays for assaying galactosyltransferase activity. Org Lett. 2007; 9:1675–1678. [PubMed: 17394347]

25. Blixt O, et al. Glycan microarrays for screening sialyltransferase specificities. Glycoconj J. 2008; 25:59–68. [PubMed: 17914671]

26. Kosik O, et al. Polysaccharide microarrays for high-throughput screening of transglycosylase activities in plant extracts. Glycoconj J. 2010; 27:79–87. [PubMed: 19953317]

27. Laurent N, et al. Enzymatic glycosylation of peptide arrays on gold surfaces. ChemBioChem. 2008; 9:883–887. [PubMed: 18330850]

28. Chang S-H, et al. Glycan array on aluminum oxide-coated glass slides through phosphonate chemistry. J Am Chem Soc. 2010; 132:13371–13380. [PubMed: 20822102]

29. Sanchez-Ruiz A, Serna S, Ruiz N, Martin-Lomas M, Reichardt N-C. MALDI-TOF mass spectrometric analysis of enzyme activity and lectin trapping on an array of N-glycans. Angew Chem Int Ed Engl. 2011; 50:1801–1804. [PubMed: 21328643]

30. Palcic MM, Hindsgaul O. Flexibility in the donor substrate specificity of β 1,4-galactosyltransferase: application in the synthesis of complex carbohydrates. Glycobiology. 1991; 1:205–209. [PubMed: 1823163]

31. Cerdeno-Tarraga AM, et al. Extensive DNA inversions in the *B. fragilis* genome control variable gene expression. Science. 2005; 307:1463–1465. [PubMed: 15746427]
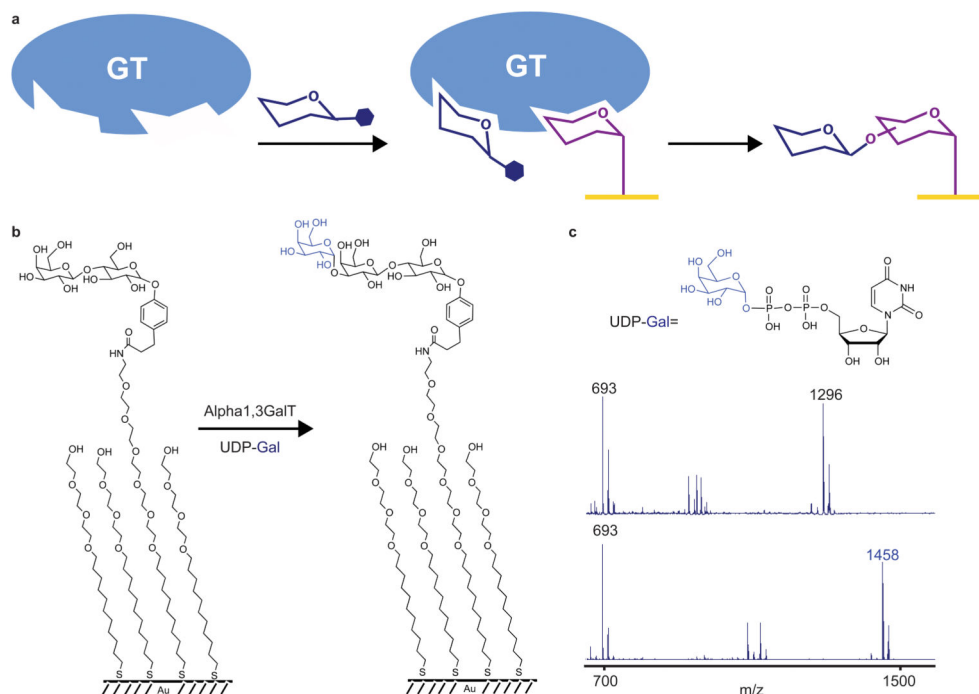
Figure 1.
Screens of putative GTs were performed on arrays of sugar acceptors. (a) Glycosyltransferase (GT) assays were performed by applying solutions containing a GT and a sugar donor (blue) to regions of a self-assembled monolayer presenting carbohydrate acceptors (purple). SAMDI mass spectrometry was then used to analyze the monolayers to identify those combinations of GT, donor and acceptor that give a glycosylation reaction. (b) In one example, GGTA1 and the sugar donor UDP-Gal were applied to a lactose-terminated monolayer. (c) SAMDI spectra revealed that the lactose-substituted alkyl disulfide (at m/z 1296) was glycosylated to give the trisaccharide (at m/z 1458).
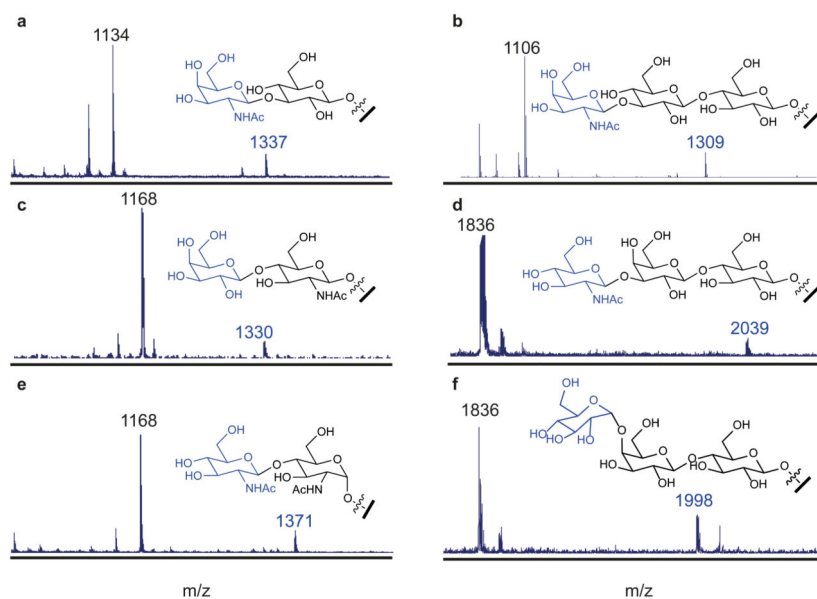
Figure 2.
The screen of putative GTs resulted in the discovery of several enzyme activities. Mass spectra of the monolayers that revealed new activities are shown for the following combinations of GT, donor and acceptor: (**a**) BF0009 from *B. fragilis*. donor: UDP-GalNAc; acceptor: β-glucose. (**b**) BF0009 from *B. fragilis*. donor: UDP-GalNAc; acceptor: cellobiose (Glcβ1,4-Glc). (**c**) BF0614 from *B. fragilis*. donor: UDP-Gal; acceptor: GlcNAc. (**d**) HD0466 from *H. ducreyi*. donor: UDP-GlcNAc; acceptor: βlactose. (**e**) AAF28363.1 from *H. ducreyi*. donor: UDP-GlcNAc; acceptor: GlcNAc. (**f**) LgtC. donor: UDP-Glc; acceptor: β-lactose. Additional activities are shown in Supplementary Fig. 8.

Table 1

Kinetic Parameters for Glycosylation Reactions

SAMDI was used to obtain kinetic parameters for glycosylation reactions mediated by three GTs. K_a and K_{ia} are the cognate Michaelis constant and dissociation constant of the constant substrate (acceptor), respectively. K_b represents the cognate Michaelis constant for the variable substrate (donor). The values are reported with the standard errors in parenthesis of three parallel experiments.

Parameters	BF0009	BF0614	HD0466
K_a (mM)	4.51 (0.071)	4.92 (0.075)	2.89 (0.032)
K_{ia} (mM)	6.16 (0.047)	5.62 (0.068)	3.52 (0.041)
K_b (mM)	2.88 (0.039)	0.446 (0.023)	0.366 (0.044)
V_{max} (nmol min^{-1})	0.25 (0.013)	0.22 (0.011)	0.19 (0.0095)