ARTICLE
PUBLISHED ONLINE: 22 JULY 2012 | DOI: 10.1038/NCHEMBIO.1022

Discovery of glycosyltransferases using carbohydrate arrays and mass spectrometry

Lan Ban[1,2,10], Nicholas Pettit[3,4,10], Lei Li[5–7,10], Andreea D Stuparu[1,2], Li Cai[3,4], Wenlan Chen[3,4], Wanyi Guan[3–6], Weiqing Han[7,8], Peng George Wang[7,8]* & Milan Mrksich[1,2,9]*

Glycosyltransferases catalyze the reaction between an activated sugar donor and an acceptor to form a new glycosidic linkage. Glycosyltransferases 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 glycosyltransferases is difficult and tedious. This paper describes an approach that combines arrays of reactions on an immobilized array of acceptors with an analysis by mass spectrometry to screen putative glycosyltransferases. A total of 14,280 combinations of a glycosyltransferase, an acceptor and a donor in four buffer conditions were screened, leading to the identification and characterization of four new glycosyltransferases. This work is notable because it provides a label-free method for the rapid functional annotation of putative enzymes.

© 2012 Nature America, Inc. All rights reserved.

Glycosyltransferases are among the most abundant enzymes in nature and are important for the biosynthesis of glycans[1]. *In vivo*, glycosyltransferases have high specificity in transferring a sugar from a nucleotide donor to an acceptor substrate to form glycosidic linkages. However, for glycosyltransferase-mediated reactions that are performed *in vitro*, the enzymes typically tolerate a broader set of substrates and have therefore been useful catalysts in the synthesis of oligosaccharides and derivatives[2–4]. The use of glycosyltransferases to synthesize complex oligosaccharides offers the benefits that the enzymes create defined glycosidic linkages, they avoid the need to use tedious protection and deprotection steps that are required in organic synthesis, and they are efficient catalysts at neutral pH[5]. A genomic analysis can be used to identify putative glycosyltransferases, but functional assays are ultimately required to validate the transferase activities of the enzymes[6,7]. Furthermore, the discovery of glycosyltransferases that have previously unidentified specificities for the donor and acceptor substrates they accept is an important, but difficult, goal. For example, among the 60,000 putative glycosyltransferases 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 glycosyltransferases[8,9].

To address this challenge, we developed a label-free assay that is based on a combination of mass spectrometry and self-assembled monolayers (SAMs) on gold that present carbohydrate substrates 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 the products of an enzyme-mediated conversion and are valuable for efficiently identifying enzyme activities.

We used the SAMDI assay to evaluate nearly 60,000 reactions comprising unique combinations of a putative glycosyltransferase, a nucleotide donor, an immobilized acceptor and a buffer composition. Forty-four reactions showed new peaks that corresponded to individual glycosylation products and gave a functional validation of four new glycosyltransferases, including an enzyme with a previously unknown 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 nonbiased screen to identify unanticipated transferase activities, although we note that this approach can also be used to analyze glycosyltransferases that are taken from a single species or can be 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 thus would not be active in the present screen. To speed up the analysis, we used a high-throughput screening platform based on metal plates that have 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 Figs. 1–4**) and immobilized individual acceptors on the gold features by either forming

[1]Howard Hughes Medical Institute, Northwestern University, Evanston, Illinois, USA. [2]Department of Chemistry, Northwestern University, Evanston, Illinois, USA. [3]Department of Chemistry, Ohio State University, Columbus, Ohio, USA. [4]Department of Biochemistry, Ohio State University, Columbus, Ohio, USA. [5]National Glycoengineering Research Center, Shandong University, Shandong, China. [6]State Key Laboratory of Microbial Technology, Shandong University, Shandong, China. [7]State Key Laboratory of Medicinal Chemical Biology, Nankai University, Tianjin, China. [8]College of Pharmacy, Nankai University, Tianjin, China. [9]Department of Biomedical Engineering, Northwestern University, Evanston, Illinois, USA. [10]These authors contributed equally to this work. *e-mail: pwang@nankai.edu or milan.mrksich@northwestern.edu

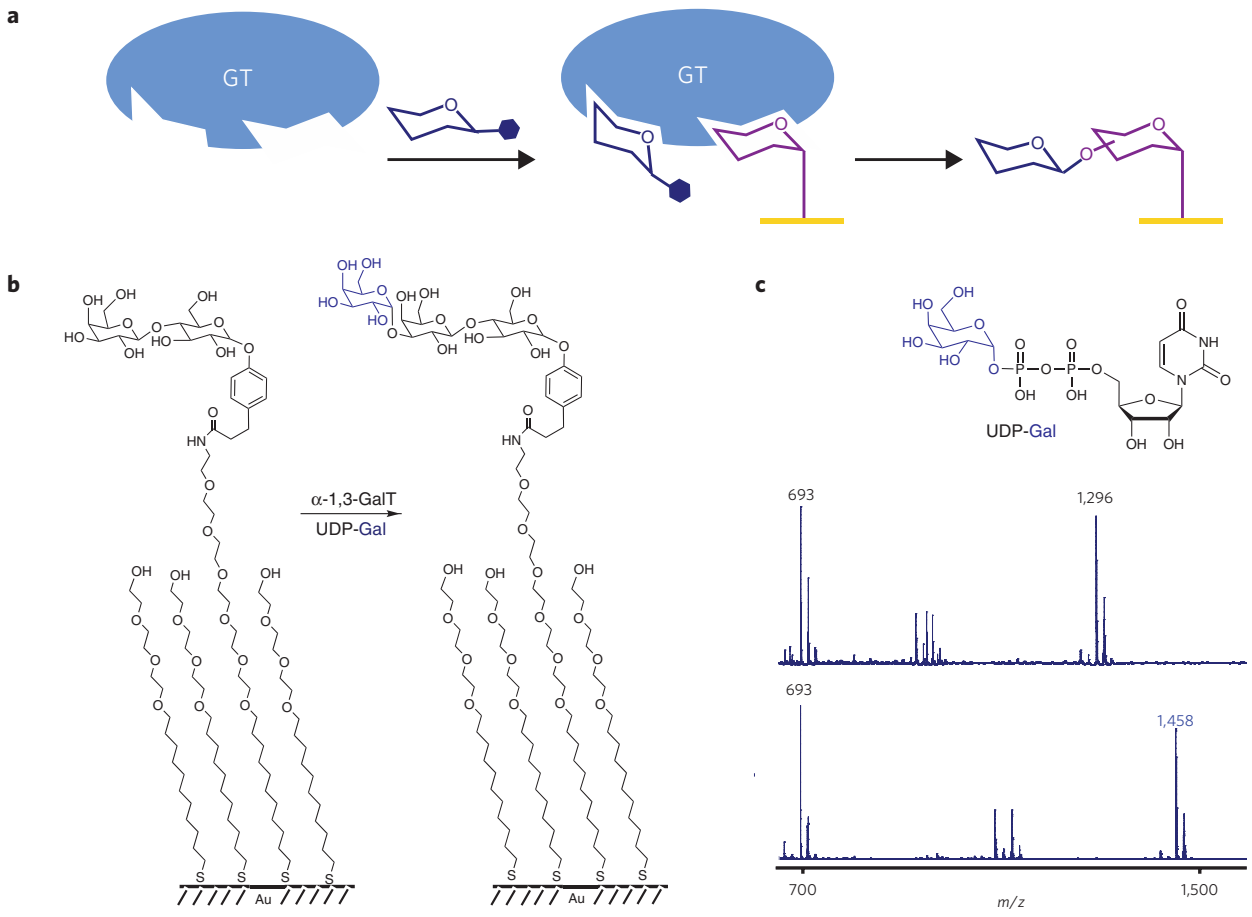

Figure 1 | Screens of putative glycosyltransferases performed on arrays of sugar acceptors. (**a**) Glycosyltransferase (GT) assays were performed by applying solutions containing a glycosyltransferase and a sugar donor (blue) to regions of a SAM presenting carbohydrate acceptors (purple). SAMDI mass spectrometry was then used to analyze the monolayers to identify those combinations of glycosyltransferase, 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. GalT, galactosyltransferase. (**c**) The SAMDI spectra revealed that the lactose-substituted alkyl disulfide (at *m/z* 1,296) was glycosylated to give the trisaccharide (at *m/z* 1,458).

© 2012 Nature America, Inc. All rights reserved.

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 major influence on the glycosylation reaction, as glycosyltransferases generally have selectivity for the terminal carbohydrate unit of the acceptor. We therefore expect that the activities that are discovered in the screen will not be compromised by the choice of immobilization reaction; in support of this idea, we discuss later the use of a homogeneous format to verify the activities of the glycosyltransferases 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 a mass to charge ratio (*m/z*) of 1,296, which corresponds to the mixed disulfide with a single lactose group. The peak at *m/z* 693 represents the tri(ethylene glycol) disulfide. The monolayer was treated with bovine α1,3galactosyltransferase (GGTA1) and analyzed by SAMDI to reveal a peak at *m/z* 1,458, which corresponds to the mixed disulfide containing the trisaccharide that resulted from the enzymatic galactosylation of lactose[16]. The absence of a peak at *m/z* 1,296 indicated that the enzymatic reaction was essentially complete. We then applied this assay to screen 85 glycosyltransferases (**Supplementary Results** and **Supplementary Table 4**), including 76 putative bacterial enzymes that had not been previously characterized, with the goal of identifying new glycosyltransferase activities.

Because traditional methods for protein expression and purification are tedious and time consuming and can compromise protein activity, we used an *in vitro* expression system to rapidly prepare the glycosyltransferases, which we then assayed in an unpurified form (**Supplementary Fig. 7**). Each individual protein was first mixed with one of the seven sugar donors dissolved in one of four buffers 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 included different divalent ions and pH values (Methods).

From the 57,120 reactions tested in the screen, 44 had new glycosylation products (**Supplementary Table 5**). Included in these hits were glycosylation activities for four previously uncharacterized enzymes (**Fig. 2**). Two of these enzymes, BF0009 (GT80; GTs are numbered as listed in **Supplementary Table 4**) 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 other

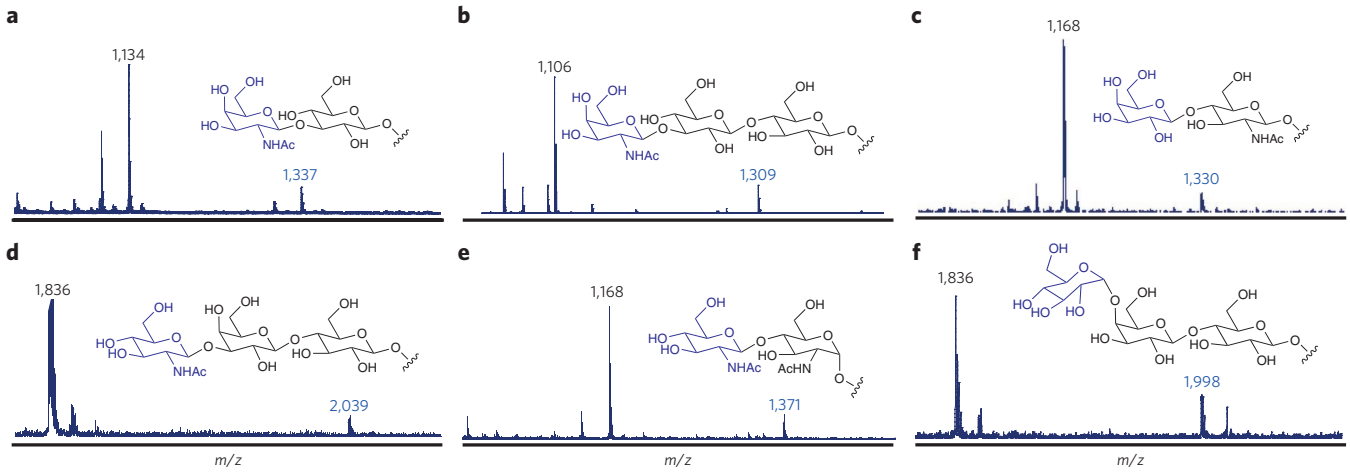

Figure 2 | The screen of putative glycosyltransferases resulted in the discovery of several enzyme activities. Shown are the mass spectra of the monolayers that revealed new activities for various combinations of glycosyltransferase, donor and acceptor. (**a**) BF0009 from *B. fragilis*, with UDP-GalNAc as the donor and β-glucose as the acceptor. (**b**) BF0009 from *B. fragilis*, with UDP-GalNAc as the donor and cellobiose (Glc-β1,4Glc) as the acceptor. (**c**) BF0614 from *B. fragilis*, with UDP-Gal as the donor and GlcNAc as the acceptor. (**d**) HD0466 from *H. ducreyi*, with UDP-GlcNAc as the donor and β-lactose as the acceptor. (**e**) AAF28363.1 from *H. ducreyi*, with UDP-GlcNAc as the donor and GlcNAc as the acceptor. (**f**) LgtC, with UDP-Glc as the donor and β-lactose as the acceptor. Additional activities are shown in **Supplementary Figure 8**.

© 2012 Nature America, Inc. All rights reserved.

two enzymes, 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 use donors that have not been reported previously. The glycosyltransferases GGTA1 (GT02) and LgtC (GT06), in addition to using the UDP-galactose (UDP-Gal) donor, were also found to glycosylate their substrates with UDP-glucose (UDP-Glc) (for GGTA1) or with UDP-Glc and guanosine diphosphate mannose (GDP-Man) (for LgtC) (**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* 1,337 (**Fig. 2a**), which is 203 Da greater than the peak for the acceptor-terminated alkyl disulfide (*m*/*z* 1,134) and is consistent with the addition of GalNAc to the acceptor. To estimate the yield with which the glycosyltransferases were expressed in the active form, we included nine known glycosyltransferases 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, although we did not determine whether the inactivity arose from improper folding of the enzymes, a 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 glycosyltransferases, donors and acceptor substrates that generate new glycosidic linkages, but the use of mass spectrometry does not provide information on the regiochemical and stereochemical structure of the linkage. To characterize the products, we expressed the four newly identified enzymes in *Escherichia coli* BL21(DE3) cells and used them in preparative glycosylation reactions to generate milligram quantities of the products, which we then characterized using one-dimensional and two-dimensional NMR (**Fig. 2**, **Supplementary Table 2** and **Supplementary Results**). We found that BF0009 (GT80) catalyzes the formation of a β1,3 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 a β1,4 linkage between two GlcNAc residues.

Biochemical characterization of the new enzymes

We selected three glycosyltransferases that gave new activities in the screen and characterized their kinetic parameters. These experiments used a pulldown format wherein reactions were performed in solution, quenched and then applied to a monolayer to allow the substrate and product to undergo immobilization before 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 a 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 glycosyltransferases[17,18]. In one example, we characterized the BF0009-mediated transfer of UDP-GalNAc to an azidoglucose substrate by performing a series of reactions, with the glucose azide and UDP-GalNAc being 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 the 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 the results agreed with our determination of kinetic parameters using the SAMDI pulldown assay (**Supplementary Table 6**). These experiments revealed several features of the glycosyltransferases. The value of the Michaelis constant (K_b) for the UDP-Gal donor is an order of magnitude lower with the human enzyme, reflecting a stronger interaction between the human homologue and the donor[21]. In another example, HD0466 from *H. ducreyi* and the closely related β1,3N-acetylglucosaminyltransferase from *Neisseria meningitidis* (LgtA)[3] each interact with the donor with similar affinities and perform the glycosylation reaction with similar maximum velocities, V_{max}.

Many glycosyltransferases have conserved folds and structural motifs that bind, and require, divalent metal ions for activity. Therefore, we also investigated the metal-dependent activities of the

Table 1 | Kinetic parameters for glycosylation reactions

Parameter	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)

SAMDI was used to obtain the kinetic parameters. 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.

glycosyltransferases that we identified 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, which is consistent with a requirement for metal ions. The three glycosyltransferases were the most active in the presence of Mn^{2+} and Mg^{2+} but were also active in the presence of other divalent metal ions. For example, BF0009 had the highest activity when Mg^{2+} was present in the buffer, retained some activity when Mn^{2+}, Co^{2+} or Mo^{2+} was present, had only minor activity when Cu^{2+} or Ni^{2+} was present and had no activity when Ca^{2+}, Fe^{2+} or Zn^{2+} was present. A comparison of HD0466 and LgtA showed notable differences in metal dependence, with HD0466 having higher activity in the presence of Mg^{2+} relative to Mn^{2+}, whereas LgtA showed the reverse trend.

DISCUSSION

Although glycosyltransferases comprise one of the most abundant protein families, they are also among the least characterized. This discrepancy owes to the limitations inherent in traditional assays of glycosyltransferase activity. For example, *in vitro* studies of substrate specificities require substantial quantities of protein, donors and acceptors, all of which are expensive. The assays commonly use radioactive donors and are tedious and difficult to adapt to high-throughput formats. Moreover, the *in vitro* characterization of a glycosyltransferase is typically pursued only after a relevant glycosylation function has been identified by other means (for example, 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 for higher throughput and a comprehensive characterization of glycosyltransferases[23–29]. Much of this work has relied on fluorescence as a detection method by using fluorescently labeled lectins[24], biotinylated sugar nucleotide donors[25] or labeled polysaccharide acceptors[26]. These examples show the utility of glycan arrays in profiling enzymes that modify carbohydrate substrates, and they motivated the development of label-free strategies that could have even broader use in glycobiology.

Our previous reports that mass spectrometry can be used to analyze SAMs provided early examples of a label-free assay of glycosyltransferase 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 alkylglycans that were immobilized onto monolayers using hydrophobic interactions[29]. The example we describe here is notable because it integrates laboratory automation protocols with mass spectrometry to efficiently perform tens of thousands of reactions for the purpose of discovering new glycosyltransferases. The requirement of 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 picomole levels. The label-free assay also has the advantage that it avoids the possible interference of labels with enzymatic activities, and it enables the discovery of unanticipated events[14].

The example we report here resulted in the discovery of four bacterial proteins that have specific glycosyltransferase activities. Although the biological roles of these enzymes are unknown, they provide new catalysts that can be used to generate oligosaccharides with new structures. For example, the protein BF0009 from *B. fragilis* is particularly interesting 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 newly identified GalNAc–β1,3Glc linkage exists in the bacterium and whether it is relevant to pathogenesis.

In summary, this work describes an effective strategy for combining carbohydrate arrays and mass spectrometry for the functional annotation of enzyme families. The method is noteworthy because it combines the immobilized arrays with a true label-free detection method that allows for direct readout of the biochemical activities on the array. This method for the high-throughput characterization of glycosyltransferase activity offers a new opportunity for the identification of new and interesting glycosyltransferases from both bacterial and eukaryotic sources. We believe it will also be key for the functional annotation of other enzyme families.

METHODS

***In vitro* expression of putative glycosyltransferases.** The sources of the materials used, including bacteria strains, genomic DNAs and plasmids, as well as the growth conditions used, can be found in **Supplementary Methods**. The *in vitro* expression of the putative glycosyltransferases was performed using the Expressway Cell-Free *E. coli* Expression System (Invitrogen), as described by the vendor. In brief, 2 μg of plasmid was used for each 100 μl volume of the reaction, and the reactions were incubated at 37 °C for 4 h. Insoluble particulates were removed by centrifugation, and the supernatant containing the soluble protein was used immediately in glycosylation reactions. A vector provided by the manufacturer (harboring a nonglycosyltransferase 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 glycosyltransferase activity. The expression of the target enzymes was confirmed by western blot using mouse histidine-specific antibody and mouse IgG-specific conjugated horseradish peroxidase secondary antibody (**Supplementary Fig. 7**). The films were developed using the enhanced chemiluminescence with the western blot detection reagent.

Reaction screening. Reactions were performed on metal plates that had a 24 × 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 as follows: 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); or HEPES (50 mM, pH 7.5). The resulting reaction mixtures were then transferred to individual gold islands on a 384-well metal plate presenting one of the 24 sugar acceptors. Liquid handling was performed by a Tecan Freedom EVO 200 robot. We estimate that the enzymes were present at concentrations in the low nanomolar range. The plates were kept in humidified chambers at 37 °C for 2 h and then rinsed with water followed by ethanol. The plates were dried under nitrogen, treated with a solution of 2,4,6-trihydroxyacetophenone (THAP) matrix (5 mg ml^{-1}, 0.5 ml per plate) and analyzed by SAMDI mass spectrometry. A 355-nm neodymium-doped yttrium aluminum garnet (Nd:YAG) laser was used as the desorption/ionization source with an accelerating voltage of 20 kV and an extraction delay time of 50 ns. All spectra were acquired automatically using the positive reflector mode. The combinations of glycosyltransferase, 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 a preparative scale. See the **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) or with UDP-Glc (for GGTA1) were confirmed in a SAMDI assay.

Determining the kinetic parameters of glycosyltransferases using SAMDI. To obtain kinetic constants for the new glycosyltransferases, reactions were performed in solution, and then the substrate and product were immobilized to a monolayer

© 2012 Nature America, Inc. All rights reserved.

by way of a Click reaction[18]. Reactions were initiated in 384-well plates and quenched by the addition of EDTA and then applied to a monolayer and analyzed by SAMDI, according to the protocols listed here. 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}; and HD0466, 0.3 mg ml^{-1}. The concentrations of the donors ranged from 100 µM to 5 mM, and the concentrations of the acceptors ranged from 250 µM to 5 mM. For each set of concentrations, the reactions were carried out for durations of 2–30 min at intervals of 2–3 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 (1 µl per reaction) containing CuBr (2 mM) and triethylamine (0.5 mM) was applied to each circle, and the reactions were incubated at 25 °C for between 30 min and 6 h, depending on the concentration of the azido sugars. The completion of the reactions was monitored by SAMDI. The slide was then rinsed with water and then ethanol and then was dried under nitrogen. For quantification, the extent of glycosylation (R) was determined from the peak intensities for the product (I_p) and acceptor substrate (I_s) on the SAMDI spectra using the formula $R = I_p/(I_p + I_s)$. We confirmed that the measured ratio reflected 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 Figure 10**. The yield of the glycosylation, calculated from the equation given in **Supplementary Figure 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 the initial velocities are shown in **Supplementary Figure 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 previously been used to describe bisubstrate enzyme kinetics[19,20].

$$\frac{1}{v0} = \frac{K_{ia} \times K_b + K_a \times [B] + K_b \times [A] + [A] \times [B]}{V_{max} \times [A] \times [B]} \quad (1)$$

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 manner to obtain the initial velocities, and the details can be found in the **Supplementary Methods**.

Received 28 December 2010; accepted 8 June 2012; published online 22 July 2012

References

1. Ohtsubo, K. & Marth, J.D. Glycosylation in cellular mechanisms of health and disease. *Cell* **126**, 855–867 (2006).
2. Koeller, K.M. & Wong, C.H. Enzymes for chemical synthesis. *Nature* **409**, 232–240 (2001).
3. Blixt, O., van Die, I., Norberg, T. & van den Eijnde, D.H. 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* **9**, 1061–1071 (1999).
4. Yu, H. *et al.* A multifunctional *Pasteurella multocida* sialyltransferase: a powerful tool for the synthesis of sialoside libraries. *J. Am. Chem. Soc.* **127**, 17618–17619 (2005).
5. Breton, C. *et al.* Structures and mechanisms of glycosyltransferases. *Glycobiology* **16**, 29R–37R (2006).
6. Yi, W. *et al.* Bacterial homologue of human blood group A transferase. *J. Am. Chem. Soc.* **130**, 14420–14421 (2008).
7. Li, M. *et al.* Characterization of a novel α 1,2-fucosyltransferase of *Escherichia coli* O128: B12 and functional investigation of its common motif. *Biochemistry* **47**, 378–387 (2008).
8. Aharoni, A. *et al.* High-throughput screening methodology for the directed evolution of glycosyltransferases. *Nat. Methods* **3**, 609–614 (2006).
9. Cantarel, B.L. *et al.* The Carbohydrate-Active EnZymes database (CAZy): an expert resource for glycogenomics. *Nucleic Acids Res.* **37**, D233–D238 (2009).
10. Houseman, B.T. & Mrksich, M. Carbohydrate arrays for the evaluation of protein binding and enzymatic modification. *Chem. Biol.* **9**, 443–454 (2002).
11. Su, J. & Mrksich, M. Using mass spectrometry to characterize self-assembled monolayers presenting peptides, proteins, and carbohydrates. *Angew. Chem. Int. Ed. Engl.* **41**, 4715–4718 (2002).
12. Min, D.H., Su, J. & Mrksich, M. Profiling kinase activities by using a peptide chip and mass spectrometry. *Angew. Chem. Int. Edn Engl.* **43**, 5973–5977 (2004).
13. Ban, L. & Mrksich, M. On-chip synthesis and label-free assays of oligosaccharide arrays. *Angew. Chem. Int. Edn Engl.* **47**, 3396–3399 (2008).
14. Mrksich, M. Mass spectrometry of self-assembled monolayers: a new tool for molecular surface science. *ACS Nano* **2**, 7–18 (2008).
15. Gurard-Levin, Z.A., Scholle, M.D., Eisenberg, A.H. & Mrksich, M. High- throughput screening of small molecule libraries using SAMDI mass spectrometry. *ACS Comb. Sci* **13**, 347–350 (2011).
16. Fang, J.W. *et al.* Highly efficient chemoenzymatic synthesis of α-galactosyl epitopes with a recombinant α(1→3)galactosyltransferase. *J. Am. Chem. Soc.* **120**, 6635–6638 (1998).
17. Min, D.H., Yeo, W.S. & Mrksich, M. A method for connecting solution-phase enzyme activity assays with immobilized format analysis by mass spectrometry. *Anal. Chem.* **76**, 3923–3929 (2004).
18. Guan, W. *et al.* Combining carbochips and mass spectrometry to study the donor specificity for the *Neisseria meningitidis* β 1,3-*N*-acetylglucosaminyltransferase LgtA. *Bioorg. Med. Chem. Lett.* **21**, 5025–5028 (2011).
19. Leskovac, V. *Comprehensive Enzyme Kinetics* 1st edn (Springer, New York, 2003).
20. Cleland, W.W. Statistical analysis of enzyme kinetic data. *Methods Enzymol.* **63**, 103–138 (1979).
21. Bella, A., Whitehead, J.S. & Kim, Y.S. Human plasma uridine diphosphate galactose-glycoprotein galactosyltransferase: purification, properties and kinetics of the enzyme-catalyzed reaction. *Biochem. J.* **167**, 621–628 (1977).
22. Breton, C., Mucha, J. & Jeanneau, C. Structural and functional features of glycosyltransferases. *Biochimie* **83**, 713–718 (2001).
23. Rillahan, C.D. & Paulson, J.C. Glycan microarrays for decoding the glycome. *Annu. Rev. Biochem.* **80**, 797–783 (2011).
24. Park, S. & Shin, I. Carbohydrate microarrays for assaying galactosyltransferase activity. *Org. Lett.* **9**, 1675–1678 (2007).
25. Blixt, O. *et al.* Glycan microarrays for screening sialyltransferase specificities. *Glycoconj. J.* **25**, 59–68 (2008).
26. Kosík, O. *et al.* Polysaccharide microarrays for high-throughput screening of transglycosylase activities in plant extracts. *Glycoconj. J.* **27**, 79–87 (2010).
27. Laurent, N. *et al.* Enzymatic glycosylation of peptide arrays on gold surfaces. *ChemBioChem* **9**, 883–887 (2008).
28. Chang, S.-H. *et al.* Glycan array on aluminum oxide–coated glass slides through phosphonate chemistry. *J. Am. Chem. Soc.* **132**, 13371–13380 (2010).
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. Edn Engl.* **50**, 1801–1804 (2011).
30. Palcic, M.M. & Hindsgaul, O. Flexibility in the donor substrate specificity of β 1,4-galactosyltransferase: application in the synthesis of complex carbohydrates. *Glycobiology* **1**, 205–209 (1991).
31. Cerdeño-Tárraga, A.M. *et al.* Extensive DNA inversions in the *B. fragilis* genome control variable gene expression. *Science* **307**, 1463–1465 (2005).

Acknowledgments

M.M. acknowledges the National Science Foundation (EEC-01180205), US National Institutes of Health (P50 GM086145) and Defense Advanced Research Projects Agency (FA 9550-08-1-0221), and P.G.W. acknowledges the National Basic Research Program of China (973 Program, no. 2012CB822100 and no. 2012CB910300) for financial support. L.L. acknowledges support from the China Scholarship Council (2007102057). We thank J. Modica for assistance with synthesis. We thank Z.-J. Liu (Institute of Biophysics of the Chinese Academy of Sciences) for providing the vector pMCSG7.

Author contributions

L.B., A.D.S. and L.C. performed the synthesis of sugar acceptors. N.P., L.L., W.C., W.G. and W.H. constructed the plasmids and expressed the proteins. L.B. and A.D.S. performed the screening reactions. P.G.W. and M.M. provided project management. L.B., A.D.S., P.G.W. and M.M. prepared the manuscript.

Competing financial interests

The authors declare no competing financial interests.

Additional information

Supplementary information and chemical compound information is available in the online version of the paper. Reprints and permissions information is available online at http://www.nature.com/reprints/index.html. Correspondence and requests for materials should be addressed to P.G.W. or M.M.

© 2012 Nature America, Inc. All rights reserved.