Enzymatic glycosynthesis GeTs better

Benjamin G Davis

The use of biocatalysts for glycoside bond formation is an attractive strategy in chemical synthesis, but the tight specificity of enzymes can be a significant limitation. An ingenious screening strategy has led to the discovery of a particularly plastic glycosyltransferase.

Despite a long-standing desire to use enzymes for the synthesis of carbohydrate-containing molecules, the field has long been plagued by the thorny issue of tight enzyme specificity. In this issue, Williams *et al.* have taken a subfamily of glycosyltransferases, the group of enzymes that nature uses to assemble sugars, and developed a pragmatic method that has allowed them to quickly discover new, unnatural catalysts with a wonderful potential breadth of applications[1]. The importance of this paper lies not only in the inherent biocatalytic function of these enzymes, but also in its illustration that sequence data and structural hypotheses alone will not successfully inform functional understanding. This work elegantly demonstrates that only iterative cycles of combined analysis of sequence, structure and function will succeed.

For a number of years, researchers in biocatalysis have been exploring methods for generating or improving synthetically useful functions from protein catalysts. The starting point for this type of work is often enzymes that have evolved to perform tasks that are not necessarily ideally suited to chemical synthesis. The challenge has therefore been to maintain the beauty and advantages of enzymes as catalysts, such as efficiency and selectivity that outstrip those of most purely chemical catalysts, while at the same time removing some of their disadvantages. One of the primary limitations of enzymes is lack of substrate tolerance: many enzymatic systems are highly specific for only a limited range of substrates. The frustrating result may be that enzymes can be found that are brilliant at a given transformation, but the transformation can only be performed on one or two compounds rather than the many hundreds to which synthetic chemists might want to apply it.

One of the long-standing strategies for enhancing the synthetic utility of enzymes has been to re-engineer them through various methods, including logical choice of mutagenesis sites, chemical modification and 'forced/directed evolution'. The latter provides a means to allow near-random mutagenesis at a variety of positions in the catalyst followed by the screening of, or selection for, mutated catalysts with enhancement in the function of interest.

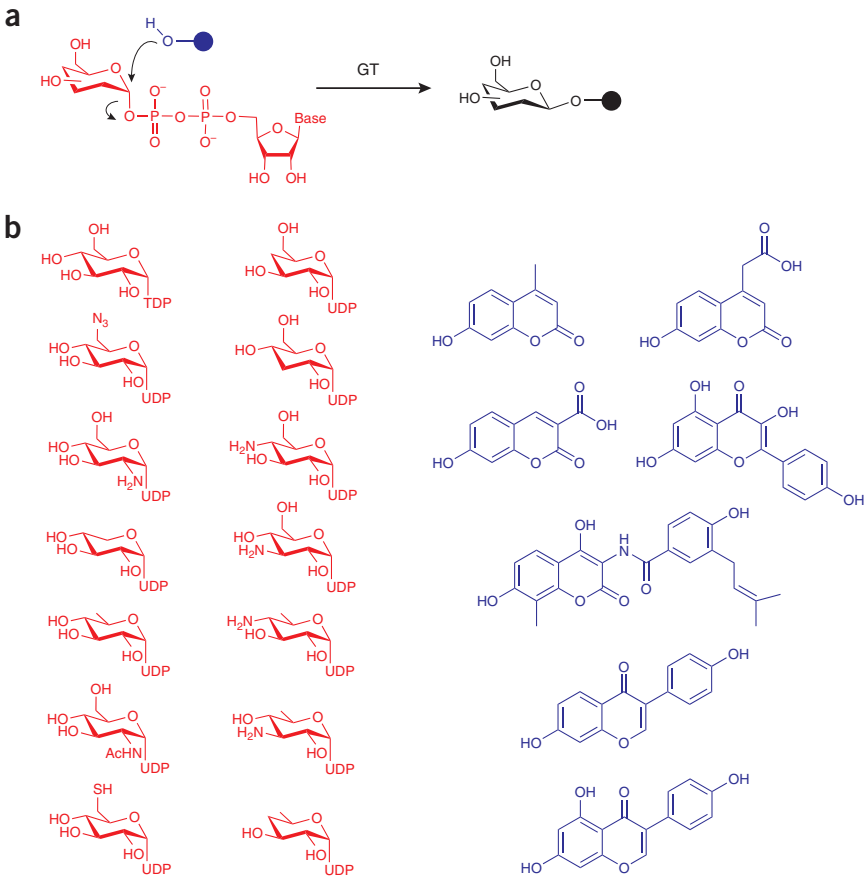

Figure 1 It's GeTting better all the time. (**a**) General mechanism for inverting Leloir-type glycosyltransferases. (**b**) The breadth of substrates (both donor and acceptor) that the evolved, plastic variant of OleD will process. Donors are shown in red and acceptors in blue.

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At the same time, one of the most difficult chemical transformations to achieve synthetically with good overall efficiency and selectivity has been the construction of glycosidic linkages, the bonds to the anomeric centers of sugars. It has therefore long been clear that glycosyltranferases, the enzymes that nature uses to make such bonds, could be powerful synthetic tools (**Fig. 1a**). Yet these enzymes are particularly capricious, mechanistically still quite poorly understood and greatly underexploited. Methods for creating more tolerant, more plastic glycosyltransferases would be invaluable. To date, despite its great success in simpler enzyme systems, applying forced evolution to glycosyltransferases has been difficult, in part due to the lack of screening methods that would allow the high throughput needed, and there have been relatively few successful examples[2].

In this context, the work of Williams *et al.*[1] critically demonstrates the first example, to my mind, of the evolution of the GT-B subfamily, a group of proteins that is characterized structurally by the presence of two Rossmann-like βαβ domains. A semi-tolerant enzyme—OleD from *Streptomyces antibioticus*, which catalyzes glucosyl transfer with inversion of anomeric configuration from UDP-glucose to the antibiotic oleandomycin—has been broadened wonderfully in an unexpected manner. Through the ingenious use of a previously known[3] acceptor substrate, a simple yet powerful fluorescence assay system was established that allowed the authors to determine the effect of key rounds of mutation and evolution. The results of the screen were exciting examples of unexpected, but useful, broadening of substrate tolerance—benefits in plasticity were observed not only for acceptor substrates, as one might have expected, but also for donor (sugar) substrates (**Fig. 1b**). Therefore, this paper shows that as we progress in the field of enzyme engineering, the much quoted-adage of forced evolution "you get what you screen for" is becoming less and less solid.

Intriguingly, this paper also contains a real hidden gem: the coalescence of a common mutation site (P67T) that emerged previously[4] through logical redesign work on the related GT enzymes UrdGT1b/c and that emerges here in OleD through near-chance evolution. This is a rare 'hotspot' event in enzyme engineering, and an exciting one, because it is strikingly rare based on statistics alone and highlights the value of the 'middle ground' between design and evolution. Thus, in addition to the successful account of the creation of a useful, synthetic catalyst, the truly powerful, underlying message of this paper lies in the implications for the ways in which we should strategically approach enzyme engineering as we start to grapple with more subtle (but more interesting) enzyme classes: we must seek to guide this future work by the judicious combination of design, mechanistic understanding and elegant strategies for functional analysis.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

1. Williams, G. J., Zhang, C. & Thorson, J.S. *Nat. Chem. Biol.* **3**, 657–662 (2007).
2. Aharoni, A. *et al. Nat. Methods* **3**, 609–614 (2006).
3. Yang, M. *et al. J. Am. Chem. Soc.* **127**, 9336–9337 (2005).
4. Hoffmeister, D. *et al. Chem. Biol.* **9**, 287–295 (2002).

A carbohydrate vaccine exceeds the sum of its parts

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A fully synthetic three-component vaccine has been shown to induce high titers of antibodies against the mucin Tn antigen of human cancer cells. The vaccine's superior properties are a result of the covalent incorporation of a ligand for Toll-like receptors and the presentation of the vaccine in a liposome format.

For two decades, researchers have been intensively working toward the development of therapeutic vaccines against tumor-associated carbohydrate antigens. To date there has been some success. For instance, antibody production specific for certain cell-surface glycoproteins and glycolipids in patients treated with cancer vaccines is associated with a more favorable prognosis. Although these initial observations suggest that a cancer vaccine is feasible, intrinsic problems abound: carbohydrates are not immunogenic, they often remain poor immunogens even when conjugated to highly immunogenic proteins and, despite considerable advances in methodology, the challenges of oligosaccharide synthesis continue to be a major hurdle. In a paper in this issue, Ingale *et al.* address several aspects of these problems[1]. The authors describe the design, synthesis and initial evaluation of a fully synthetic vaccine that incorporates a B-cell epitope, a T-cell epitope and an adjuvant in a single, chemically defined molecule.

Carbohydrate-based vaccines can be either preventative (prophylactic) or therapeutic. Prophylactic vaccines against polysaccharide-encapsulated pathogens, such as *Streptococcus pneumoniae*, date back to the pre-antibiotic era. Although high-molecular-weight polysaccharides and blood-group glycoproteins (mucins) can induce antibodies in adults, antibacterial vaccines that use purified polysaccharides are often poorly immunogenic, especially in infants. As a means to increase their immunogenicity, polysaccharides can be covalently conjugated to highly immunogenic proteins such as tetanus toxoid or a fragment of diphtheria toxin. These so-called 'conjugate vaccines' have shown efficacy against bacterial meningitis and streptococcal pneumonia in North America and Europe[2]. However, not all carbohydrates are readily converted to effective conjugate vaccines. The group B *Neisseria meningitides* capsule—a homopolymer of sialic acid (*N*-acetylneuraminic acid), which is also present on NCAM, a neural cell adhesion glycoprotein—has failed to provide protective antibodies even when conjugated to a potent immunogenic protein. Developing vaccines that target tumor-associated carbohydrate antigens requires raising antibodies to what are essentially 'self-antigens', and therefore these vaccines have encountered similar challenges to those encountered by the group B meningococcal conjugate vaccine.

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