

Engineering and evolution of old enzymes for new tasks : glycoside assembly

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Traditional routes to the synthesis of oligosaccharides have either involved the use of Nature's own biosynthetic enzymes, the glycosyl transferases, or glycosidases run in transglycosylation mode. Each approach has its drawbacks. Glycosynthases are mutant glycosidases in which the catalytic nucleophile has been removed. When used in conjunction with glycosyl fluorides of the *opposite* anomeric configuration to that of the substrate, these enzymes function as highly efficient transferases, frequently giving stoichiometric yields. Thioglycosylases are a new class of mutant glycosidases in which the acid/base catalyst has been mutated. These enzymes synthesise sulfur-linked oligosaccharides when an activated donor is used in conjunction with a thiosugar acceptor. Recent results in the engineering of these two classes of mutant enzymes, as well as of "classical" glycosyl transferases, will be discussed. Particular attention will be paid to their application to oligosaccharide, glycolipid and glycoprotein synthesis. Emphasis will be placed upon approaches towards the directed evolution of these enzymes using a variety of screening methodologies including FACS sorting and phage display.