The interaction between carbohydrate active enzymes and polysaccharide substrates includes contributions from extra binding sites situated outside of the active site, which secure efficient contact with macromolecular substrates. Most commonly carbohydrate binding modules (CBMs) carry such extra binding sites, however, an increasing number of reports describe identification and function of so-called surface binding sites (SBSs), which are situated at a certain distance from the active site on the surface of the catalytic domain or an intimately associated domain [1,2]. SBSs have most readily been identified by x-ray crystallography in structures of enzyme carbohydrate ligand complexes. We have recently undertaken development of simple tools to facilitate identification of SBSs. One such tool is affinity gel electrophoresis (AGE) in which migration of a protein in native polyacrylamide gels that contain polysaccharides can potentially be retarded due to binding of the protein to the immobilised polysaccharide [3]. GH62 is a quite small family containing only α-L-arabinofuranosidases (ABFs). It forms GH clan F together with GH43 that is much larger and has a range of specificities including ABFs for which structures have been determined. Very recently the first GH62 structures were published [3]. Modelling of the Aspergillus nidulans AnABF62A-m2,3 enzyme that has about two orders of magnitude higher activity on wheat arabinoxylan (WAX) than other GH62 ABFs and is strongly retarded in AGE by WAX, pointed to two residues, Trp63 and Tyr194, situated outside of the predicted substrate main chain binding cleft and about 30 Å from the catalytic site. While retardation was significantly reduced for W23A and W23AY44A mutants, retardation of Y44A and wild-type was comparable. Noticeably substrates such as WAX and oat spelt xylan, as well as barley β-glucan and birchwood xylan, which are not hydrolysed by AnABF62A-m2,3, caused retardation in AGE. This is in support of the polysaccharides binding to an SBS. The poor substrate L-arabinan did not elicit retardation. The activity was generally similar, but for a DP3 arabinoxylomannosaccharide (AXOS) all mutants only had 3% activity of wild-type, increasing to 10 and 25% for DP4 and DP5 AXOS, respectively. This suggests an allosteric activation of AXOS hydrolysis upon binding of carbohydrate at the SBS. GH13 and GH77 of GH clan H together with xylanases in GH10 and GH11 are the best studied GH families with respect to SBS by containing about half of the reported SBSs [2]. In GH13 SBSs are seen in 15 subfamilies and the few thoroughly characterized examples indicate SBSs with different roles in the same enzyme [4,5]. From GH77 circumstantial evidence is provided for the presence of an SBS in MalQ from Escherichia coli, a relative of DPE2 involved in starch metabolism from Arabidopsis.

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Identifying and improving glycosidases through metagenomics and directed evolution

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Mother Nature has already supplied an enormous diversity of biocatalysts, each of which could serve as a starting point for directed evolution studies. The problem can be in accessing this diversity in a reasonably efficient manner. Here we shall describe our use of activity-based, or functional metagenomics to generate a library of over 200 expressed glycosidases. We shall also describe the high-throughput characterisation of these enzymes for substrate specificity, thermal stability, pH profile and mechanism. Finally we shall describe the use of this library to identify preferred catalysts for cleavage of specific unnaturally modified sugars (e.g. azido sugars) and the generation of "glycosynthesis" versions that can be used to "tag" glycans. Such libraries can be used to select the optimal candidate for further improvements through directed evolution.