# Structural analysis of different substrate affinity in fungal hexosaminidases

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Beta-N-acetylhexosamnidases (HEX) are glycoside hydrolases from the family 20 (EC 3.2.1.52). They cleave terminal glucose/galactose residue from di-/oligosaccharides by a retaining mechanism. Apart of the ability to cleave hexoses specifically, fungal HEX tolerate a variety of substrate modifications [1-3]. This feature together with the ability to hydrolase the transglycosylation reaction makes these enzymes useful in biotransformation to produce modified carbohydrates with defined structures [2-3].

Despite the high primary structure identity (more than 50 %), enzymes from *A. oryzae*, *P.oxalicum* and *T. flavus* have different affinity to modified substrates [4]. To analyze the underpinning structural differences we built homology models of these enzymes, that share the TIM-barrel catalytic domain with identical active site amino acid residues, involved in substrate binding. However long loops close to the active site of the fungal enzymes differ from human and bacterial structures.

Docking of substrates into the active site of HEXs followed by MD revealed some differences in the dynamical behavior of the loops in the vicinity of the active site. Changes in the enzyme structure bound in the  substrate-enzyme complexes let us propose a critical role of some loop residues responsible for different affinity of HEXs to modified substrates. This study demonstrated that substrate affinity of fungal HEX is determined not only by amino acids in direct contact with the substrate, but also regulated by some weaker but still important interactions.

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