Engineering stereospecificity in glycoside hydrolases through mutagenesis

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Enzymes are biocatalysts which use specific substrates to carry out chemical reactions inside the cell. The interaction between the active site of an enzyme and the substrate is refined by evolution such a way that enzymes can specifically target unique molecules and even distinguish their stereoisomers. A great example of stereospecificity came from glycoside hydrolases which can distinguish between the alfa and beta substrate conformation to catalyze the hydrolysis of O-glycoside bond from glycoconjugates (**Figure 1a**).[1] Here, I used a well characterized beta stereospecific glycosidase scaffold[2] to design a variant able to use alfa-substrate. The software FuncLib[3] was used to insert mutations using the input scaffold to expand the substrate repertoire in the active site without modifying the catalytic residues. The designed glycosidase has 47 mutations compared to the scaffold wild type (**Figure 1b**) and the prediction of the variant's structure with AlphaFold suggests the mutated residue W143F at the active site (**Figure 1c**) as a key factor to allow the newly alfa activity. The designed variant was experimentally purified and is active to both, alfa (*p*–nf α–glucoside: 62.5 $M^{-1}s^{-1}$) and beta (*p*–nf β–glucoside: 1.1 $M^{-1}s^{-1}$) substrates. Further structural investigations with experimentally determined crystal structures should shed light on the molecular basis of stereospecificity in glycosidases which may lead to biotechnology innovations, specially towards biomass valorization.

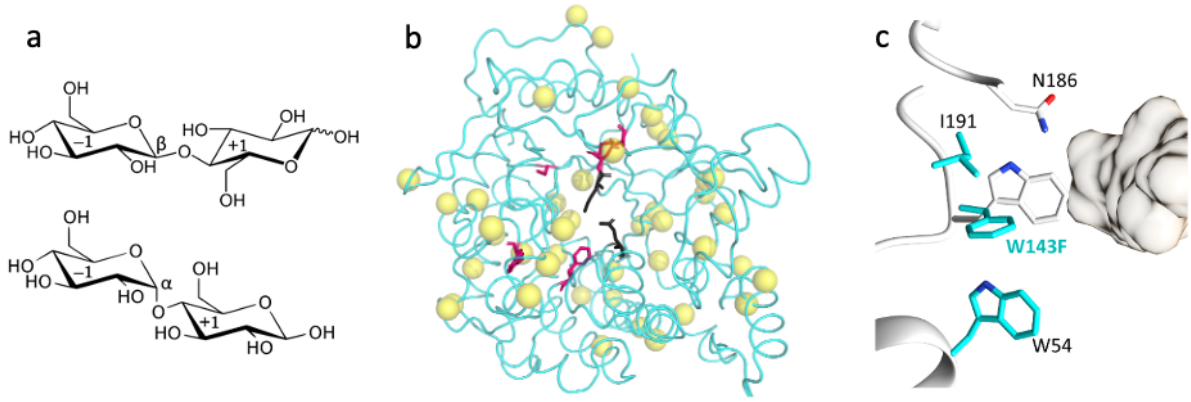

Figure 1. **a**) Example of beta and alfa glycoconjugate used as substrate for glycosidases. **b**) Designed alfa and beta glycosidase variant depicting mutations through its AlphaFold predicted structure. **c**) Active site of the superimposed scaffold (white) and variant (cyan) with an alfa-substrate depicted as Van der Waal's-surface.

References

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