GLYCOSYLTRANSFERASES GT-B DOMAIN SWAPPING: HOW TO ENHANCE VERSATILITY FOR SUGARS DONORS AND ACCEPTORS

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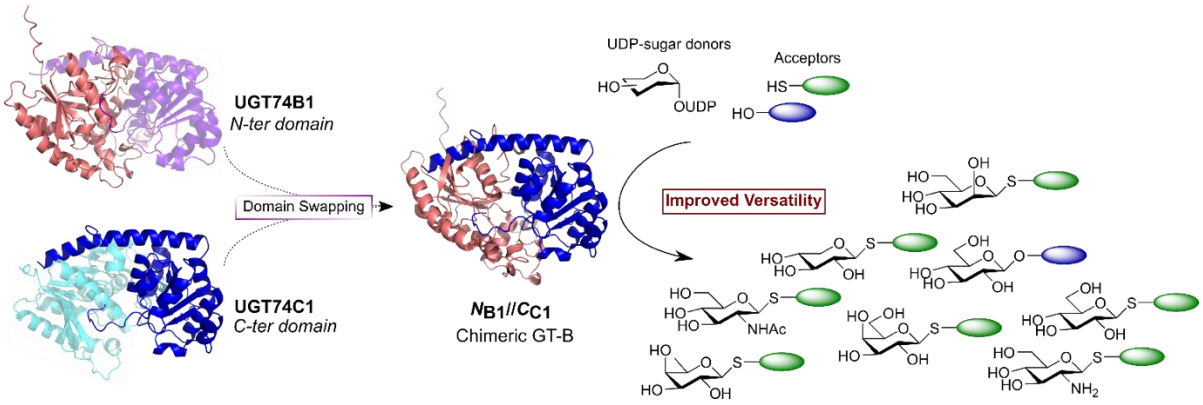
In glycochemistry, a wide range of sugar-metabolising enzymes such as **glycosyltransferases** (GTs) have been developed and used for the chemo-enzymatic synthesis of glycosides, providing an alternative to the chemical synthesis of glycosides. GTs generally catalyse glycosidic bond formation using **sugar donors containing a phosphate-leaving group** to specific acceptors [1]. However, GTs commonly **lack the modularity** required to tailor the nature of the transferred sugar or the acceptor. As a result, the identification of a new GT, followed by its genetic engineering, is required to graft a new sugar onto a given acceptor. In this context, we have **recently developed an engineering approach based on the chimerization of GT domains**, belonging to GT-B structural GT family [2]. This family is constituted of GTs with two domains facing each other: the acceptor recognition domain (*N*-terminus) and the donor binding domain (*C*-terminus), linked by an unstructured loop [3].



We have demonstrated the possibility to **exchange and assemble domains** originating from two GT-B enzymes to **generate a dimeric enzyme** [2]. This resulting chimera was found to exhibit **higher acceptor promiscuity** compared to parent GT-B enzymes, able to generate both *O*- and *S*-glucosides with diverse structures, which was correlated to higher flexibility of domains interface. In addition, we recently investigated the ability of this chimera to transfer a wider range of sugars than the native enzyme. As we observed for acceptor promiscuity, the chimeric enzyme allowed the efficient **transfer of a much broader range of sugars** compared to the native GT-B from UDP-sugars. This approach makes the GT-B domain swapping strategy particularly attractive for the development of a versatile biocatalytic tool for glycoside synthesis.

References:

1. Lairson, L. L *et al. Annual Review of Biochemistry* **2008**, *77*, 521–555.
2. Bretagne, D *et al. J. Biol. Chem.* **2024**, *300 (3)*, 105747.
3. Park, S.-H *et al.* B.-G *Biotechnology and Bioengineering* **2009**, *102*, 988-994.