Design of α-L-transfucosidases for the synthesis of fucosylated HMOs

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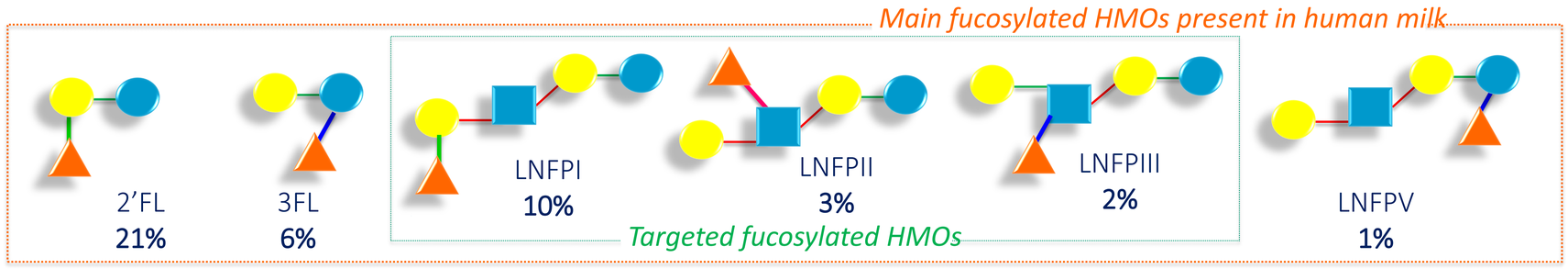
HMOs

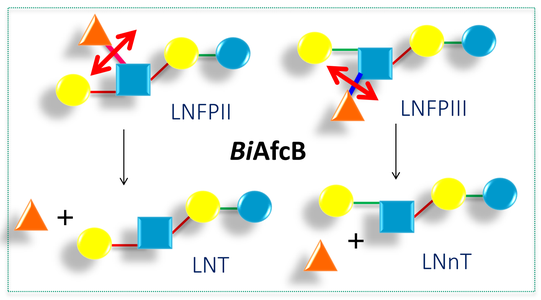
Human Milk Oligosaccharides (HMOs) are known for their prebiotic effects, their anti-adhesive and immunomodulation properties. **But the biggest roadblocks in HMOs research remains the limited availability of HMOs ressources.** Most of these oligosaccharides are fucosylated (50%).



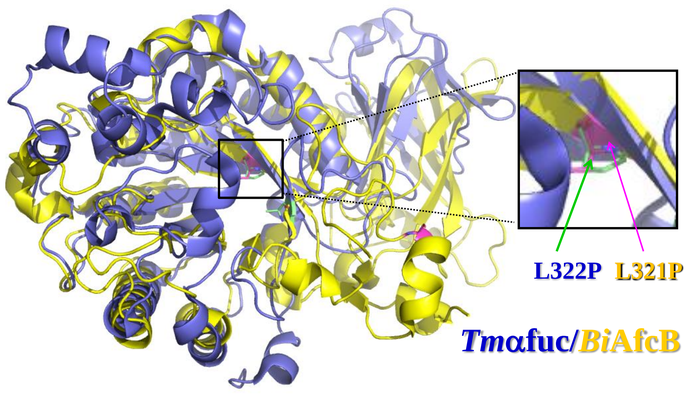
Objective : Design transfucosidases from fucosidases to decorate HMOs with fucosyl residues using a semi-rational approach.

TARGETED ENZYMES

*Bi*AfcB



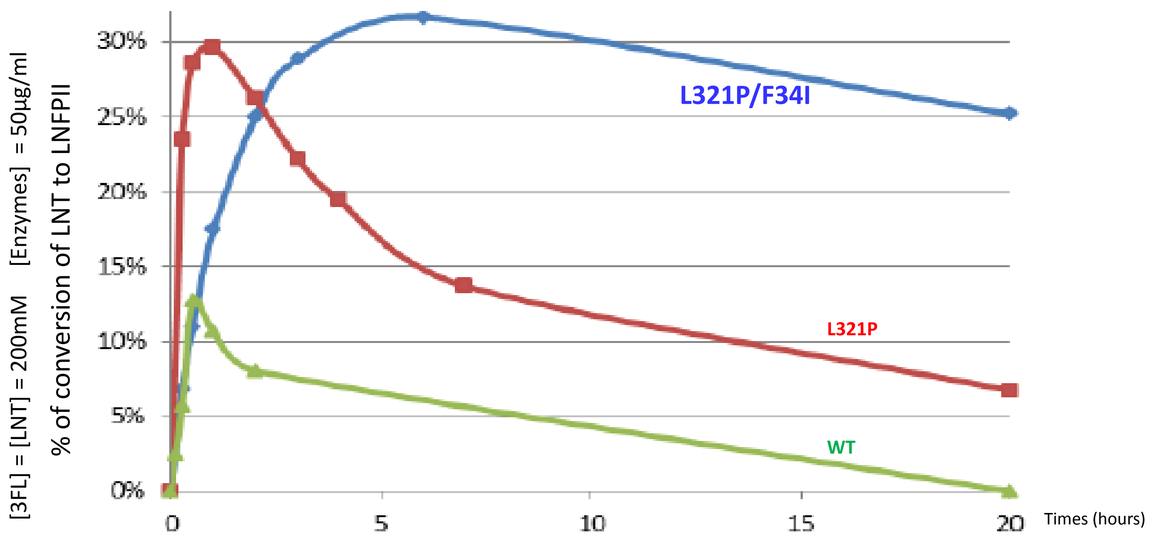
The ***Bi*AfcB** fucosidase from *Bifidobacterium longum* subsp. *Infantis*, cleaves preferentially α(1-3) or α(1-4) linkages such as those present respectively in HMO LNFP-II and LNFP-III, but has low transfucosidase properties.



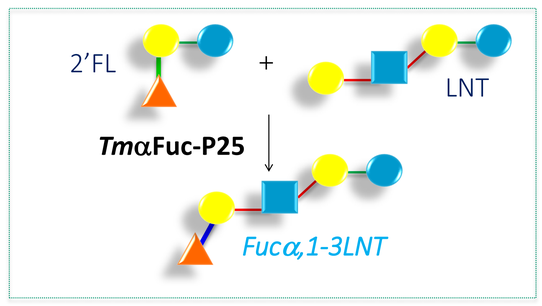
Previous work[1] on the evolution of *Tm*αFuc identified the L322P mutation which **dramatically improves the transglycosidase activity.**

The L321P mutation of *Bi*AfcB is **structurally homologous** with L322P of *Tm*αfuc. Some mutations (e.g. F34I) in the -1 site[2] are performed.

LNFPII production by *Bi*AfcB mutants



P25-*Tm*αFuc



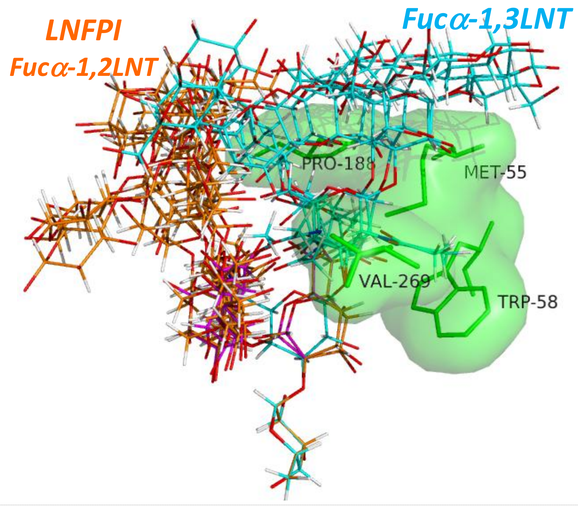
The **P25-*Tm*Fuc**[1] transfucosidase (α-L-fucosidase mutant from *Thermotoga maritima*) transfers a fucosyl residue in position 3 of the LNT terminal galactose with 2'FL as donor, to form Fuc- α(1-3)LNT, which is not an HMO.

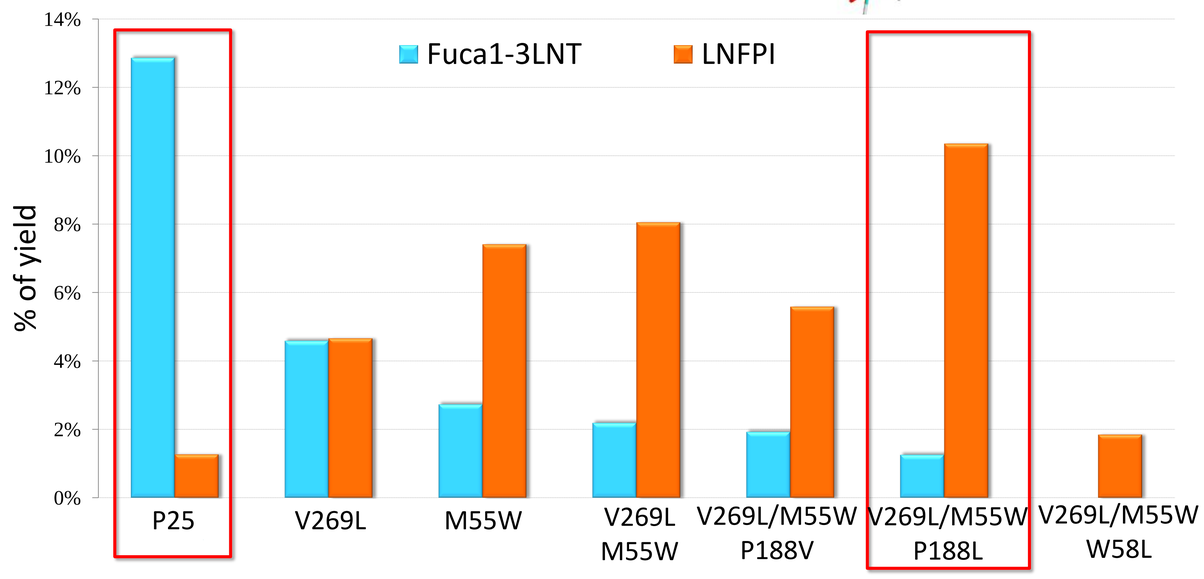
The objective was to change regioselectivity from α(1-3) fucosyl linkage to α(1-2) to synthesize LNFP-I HMO.

All possible conformations of LNFP-I and Fuc-α(1-3)LNT were docked in the P25-*Tm*αFuc active site.

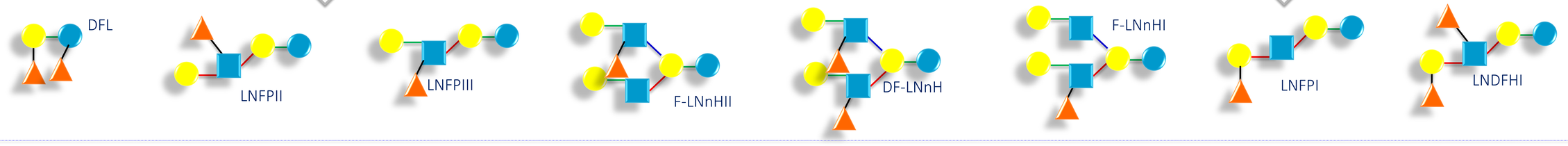
Several positions were targeted since they were close to GlcNAc residue (+2 subsite) of conformers.

Only mutations close to Fuc-1-3LNT +2 subsite induced a modification on regiospecificity, such as V269, M55, P188, W58 positions.





Fucosylated HMOs accessible with these two transfucosidases



Conclusion : *These engineered transfucosidases provide an efficient way to synthesize in vitro 8 fucosylated HMOs.*

[1] Osanjo G[1], Dion M, Drone J, Solleux C, Tran V, Rabiller C, Tellier C., *Biochemistry*, **2007**, 46 (4), pp 1022–1033; **[2]** Teze D, Hendrickx J, Czjzek M, Ropartz D, Sanejouand YH, Tran V, Tellier C, Dion M. *Protein Eng Des Sel.* **2014** Jan;27(**1**):13-9

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