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

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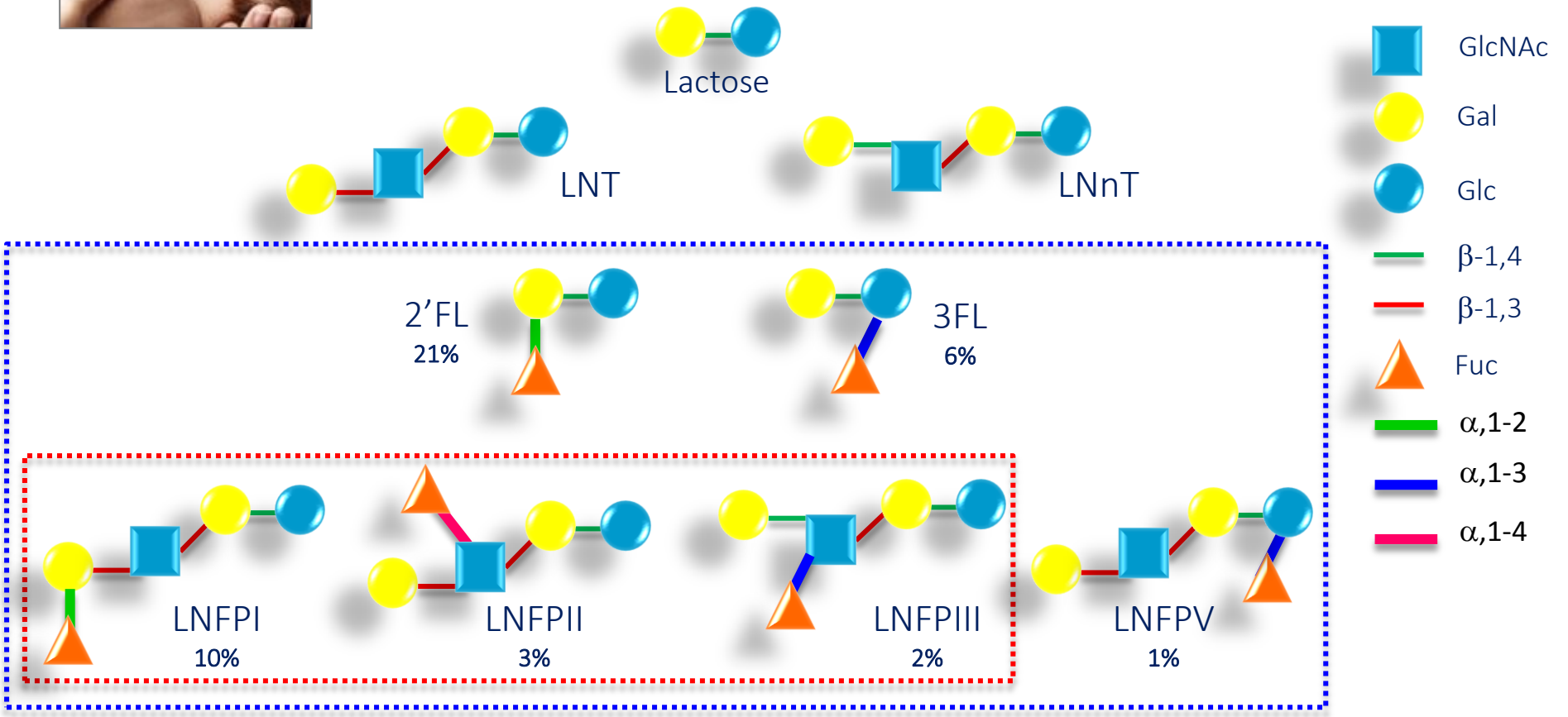
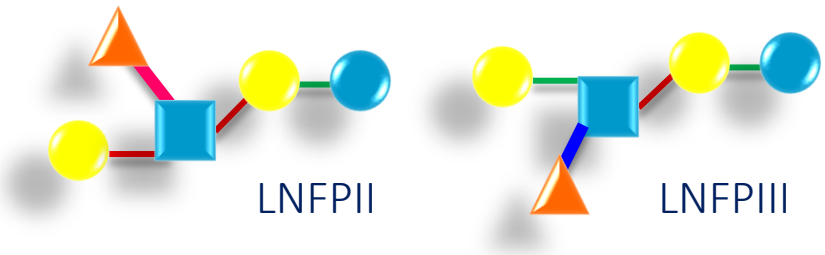
Human Milk Oligosaccharides are major components of breast milk and provide benefits in the short and long-term in infants.



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

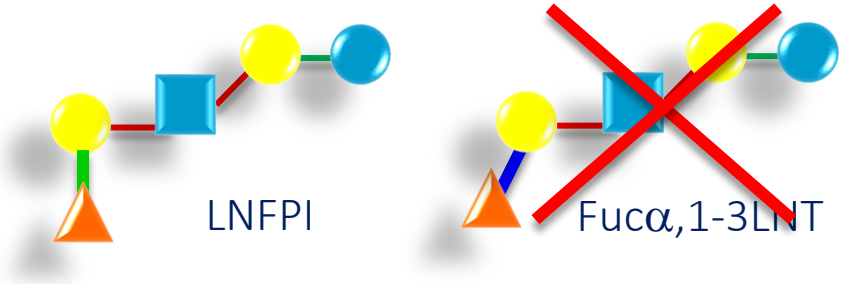
Objective : Obtain fucosylated tetrasaccharides (LNT or LNnT)

*Bi*AfcB



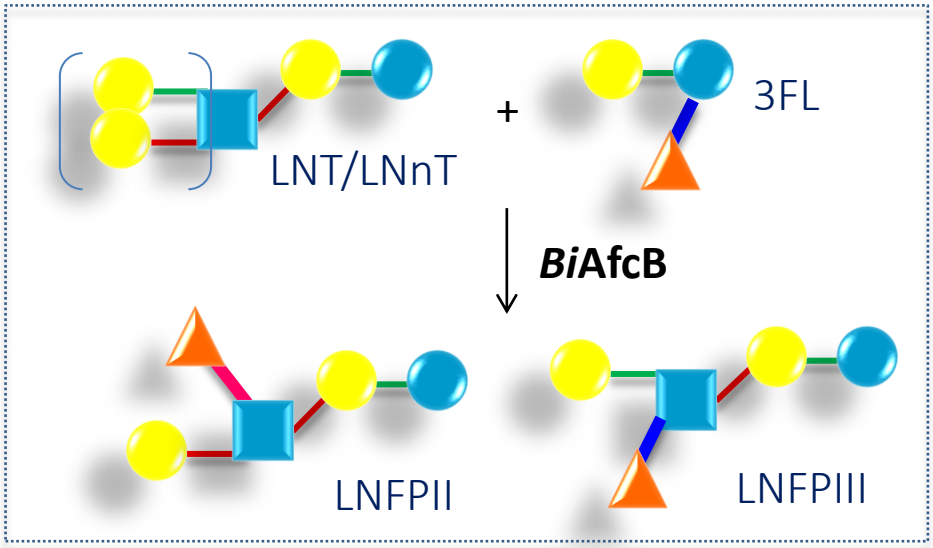
***Bi*AfcB→** Evolve fucosidase from *Bifidobacterium longum* subsp *infantis* to have an α1-3 transfucosidase and synthesize **LNFPII** and **LNFPIII.**

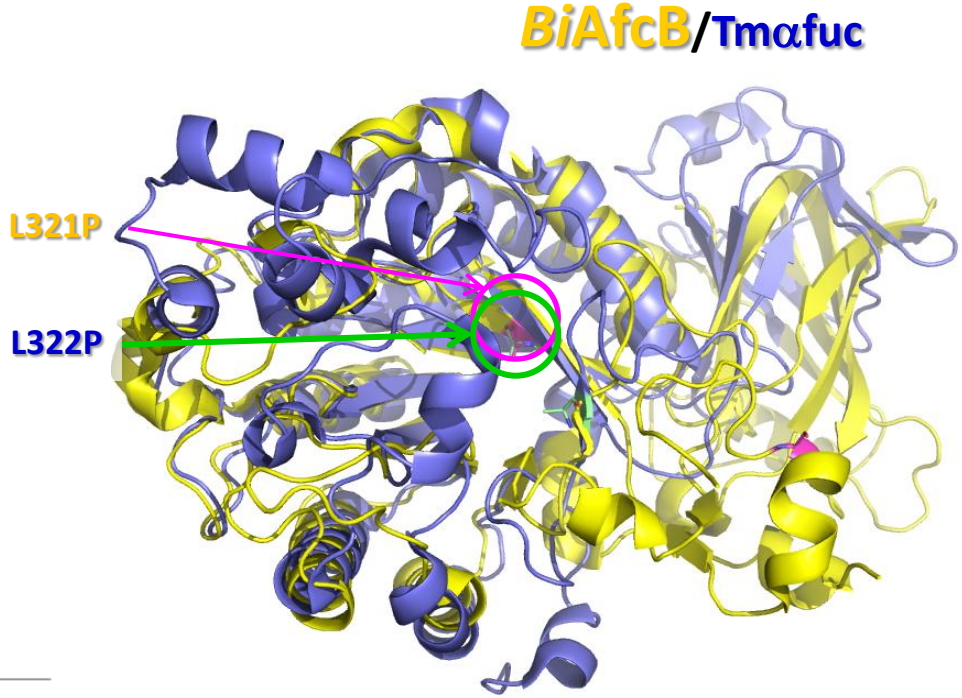
*Tm*αFuc-P25



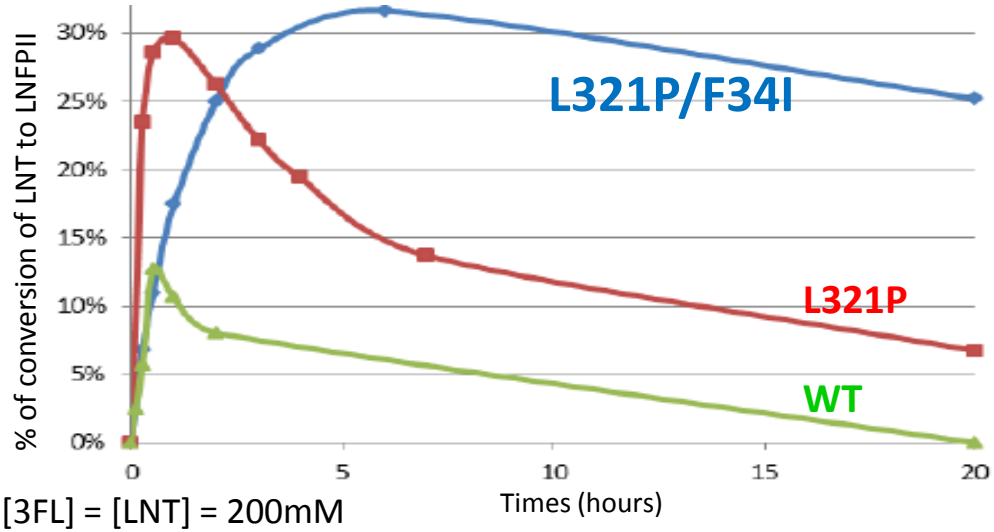
***Tm*αFuc-P25→** Modify the regioselectivity of P25-*Thermotoga maritima* transfucosidase mutant to prevent Fucα-1,3LNT formation and favor **LNFPI** synthesis.

*Bi*AfcB evolve in α1-3 transfucosidase to synthesize LNFPII and LNFPIII





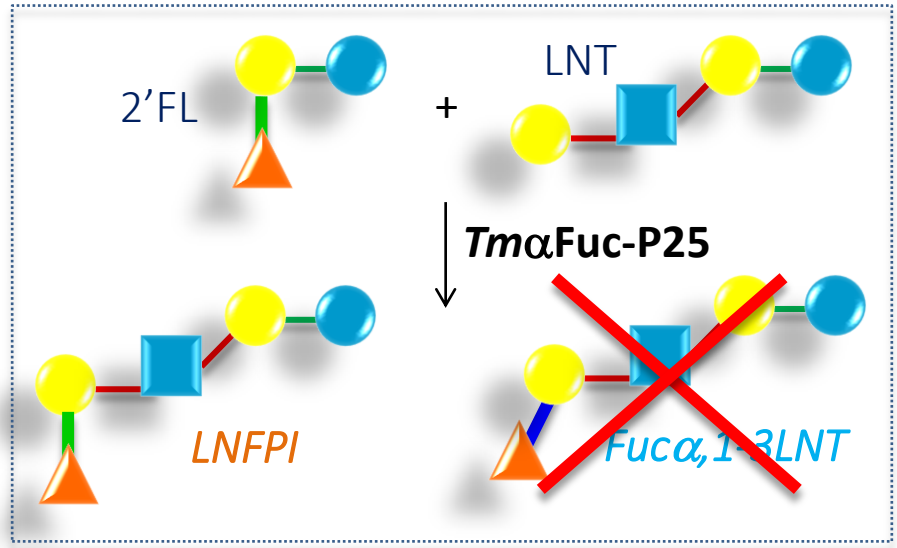
LNFPII production by *Bi*AfcB mutants

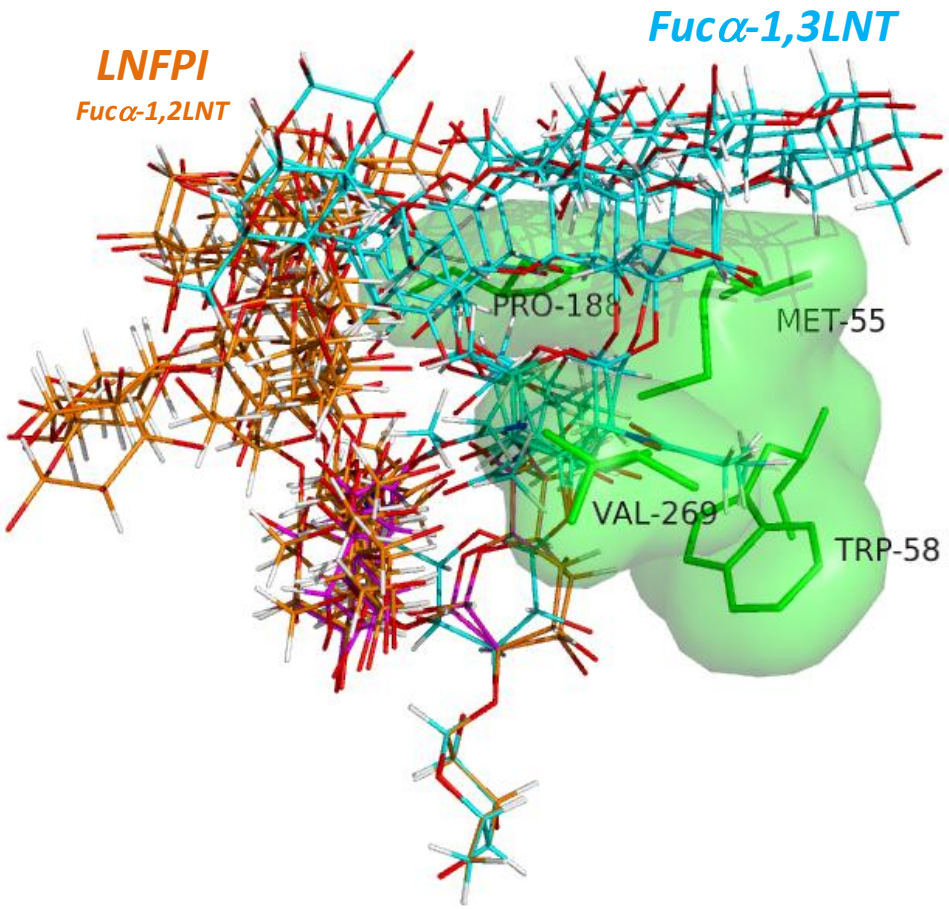


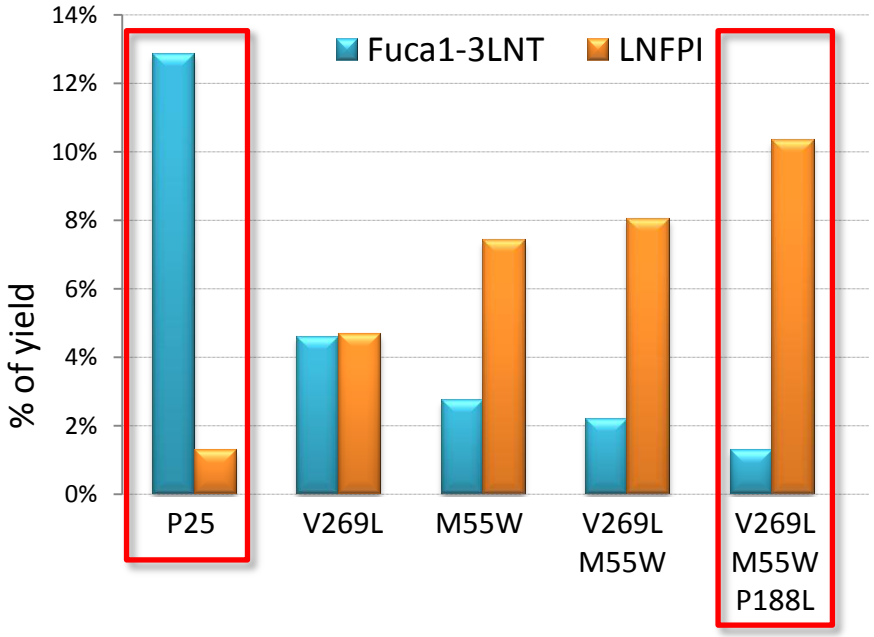
[3FL] = [LNT] = 200mM
[Enzymes] = 50µg/ml

→ ***Bi*AfcB/L321P-F34I is able to recognize LNT and LNnT to produce LNFPII and LNFPIII respectively with a transfucosylation yield of 32%.**

*Tm*αFuc-P25, *regioselectivity modification to synthesize LNFPI*







→ P25-V269L/M55W/P188L is able to produce LNFPI with a similar yield to P25 to produce Fucα,1-3LNT.

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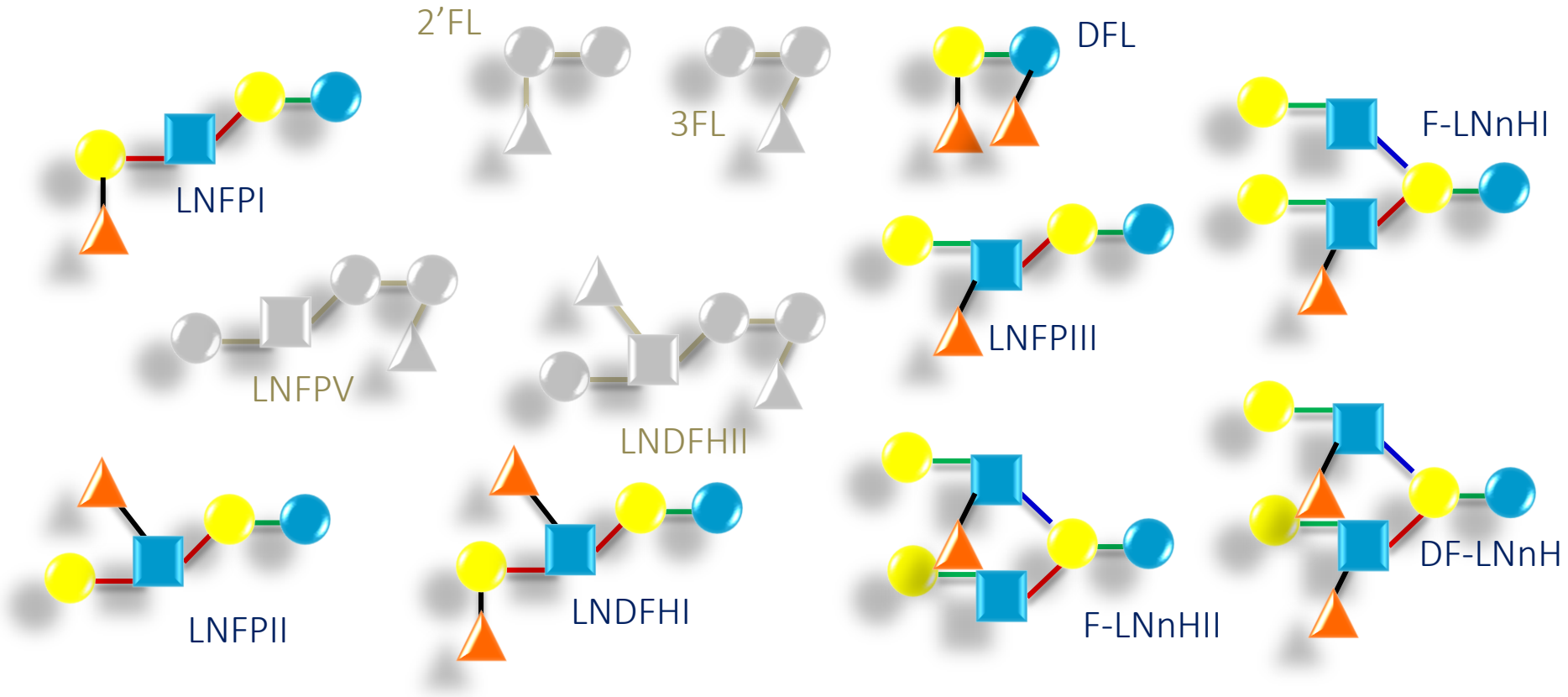
To summarize



To conclude

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

Thank you for your attention and
thank you to everyone involved in this project

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