

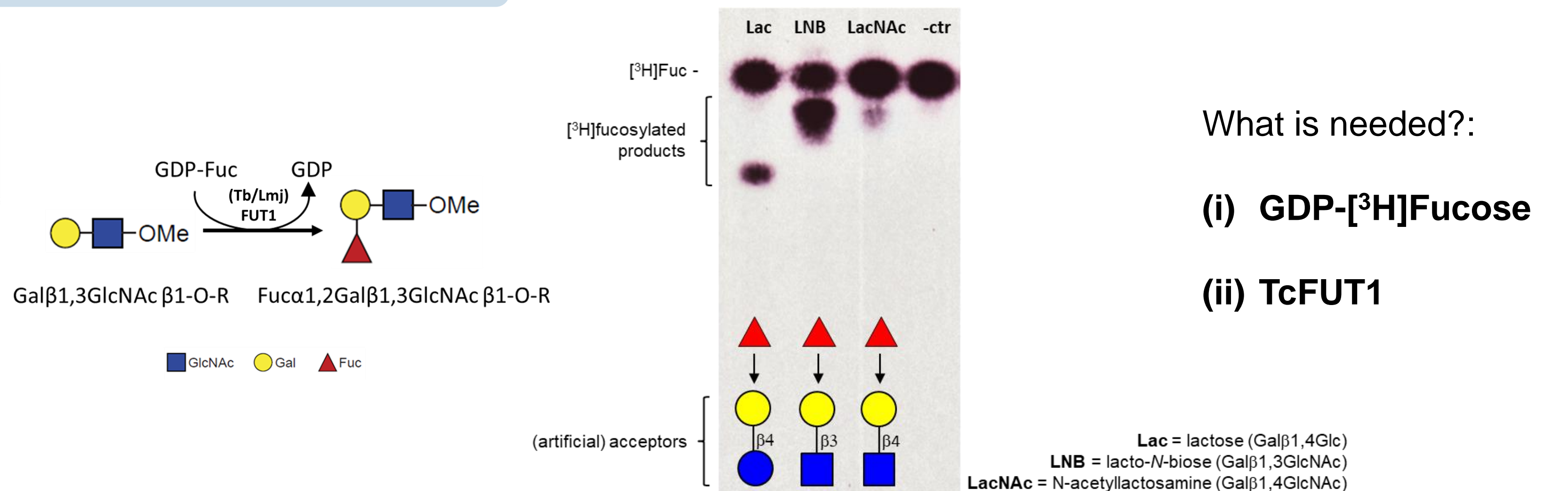
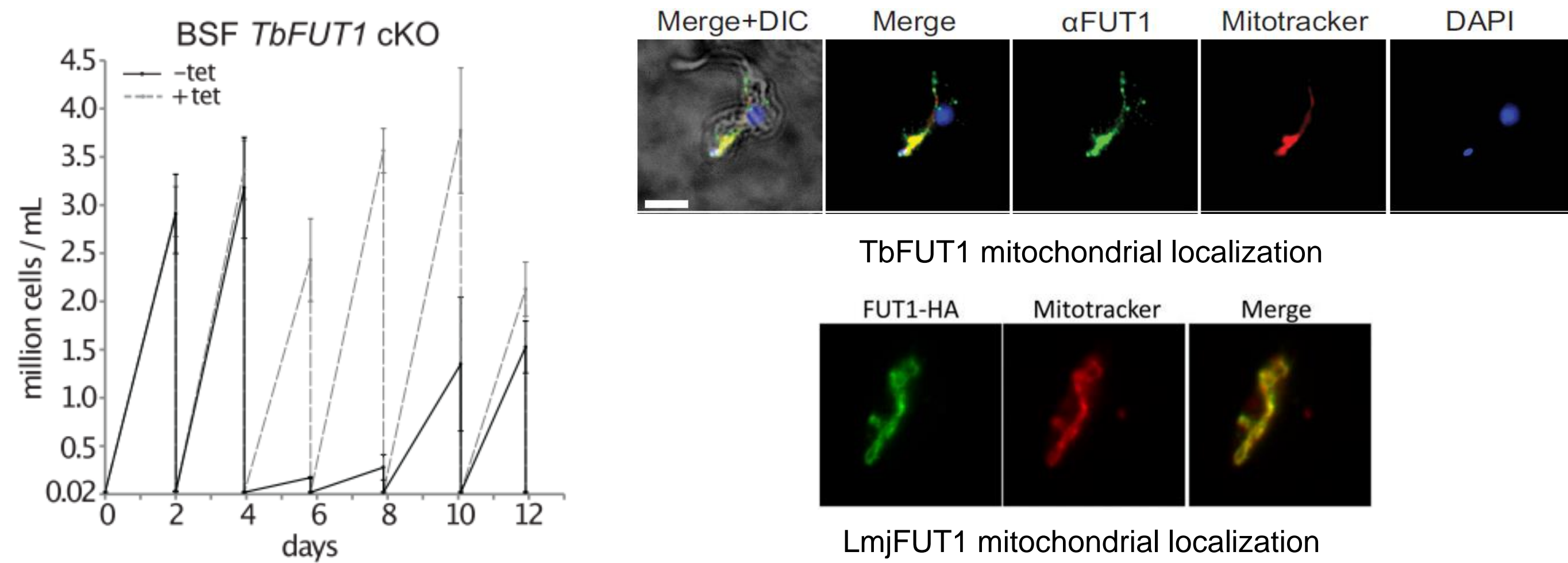
# Expression and characterization of a mitochondrial fucosyltransferase from *Trypanosoma cruzi* and use of monoxenous parasite *Crithidia fasciculata* as an enzymatic source for synthesis of radioactive GDP-Fucose.

Jose Carlos Paredes-Franco<sup>1</sup> and Michael A. J. Ferguson<sup>1</sup>

<sup>1</sup>Wellcome Centre for Anti-Infectives Research, Division of Biological Chemistry and Drug Discovery, University of Dundee, Dundee, UK.

- Trypanosomatids include insect-vector-transmitted parasites that cause sleeping sickness (*Trypanosoma brucei*), Chagas' disease (*Trypanosoma cruzi*), and leishmaniasis in humans, and are included in the WHO portfolio of priority Neglected Tropical Diseases. Recently, an essential fucosyltransferase (GDP-Fuc:β-D-galactose α-1,2-fucosyltransferase) has been identified in the single mitochondrion of *Trypanosoma brucei* (TbFUT1) and of *Leishmania major* (LmjFUT1), which could be a potential drug target.
- We aim to investigate the *T. cruzi* orthologue (TcFUT1) by expressing a recombinant version that can be used in bioluminescence and radioactivity (<sup>3</sup>H) – based activity assays in order to confirm its activity, and obtain <sup>3</sup>H-labelled glycosylated products which can be further characterized.
- We have developed a method to synthesize GDP-<sup>3</sup>H]Fucose, the sugar donor needed in TcFUT1 activity assays, using the endogenous nucleotide sugar biosynthesis machinery of the monoxenous trypanosomatid *Crithidia fasciculata*.

## Current knowledge of FUT1<sup>a,b</sup>.

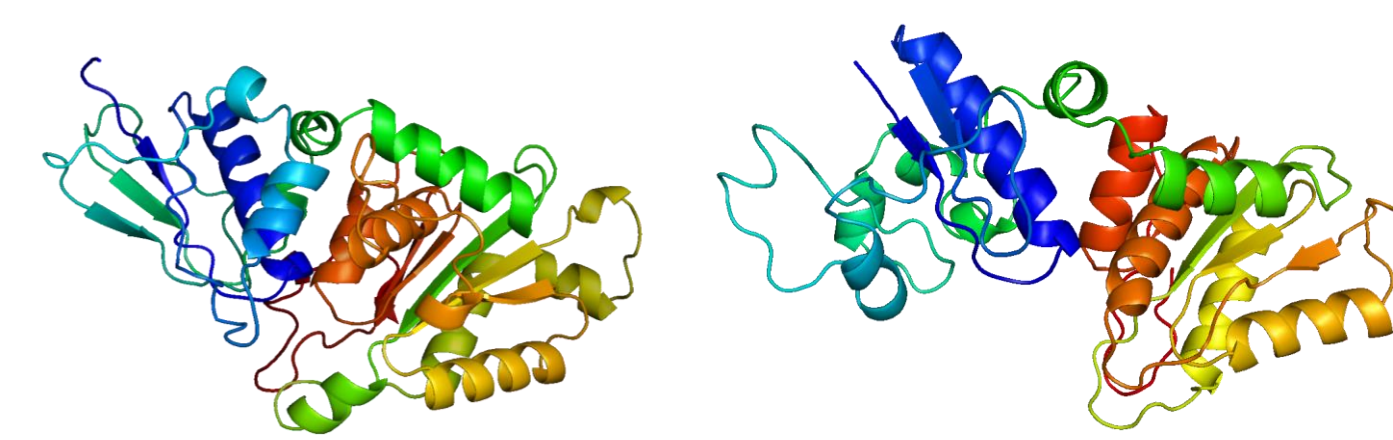


What is needed?:

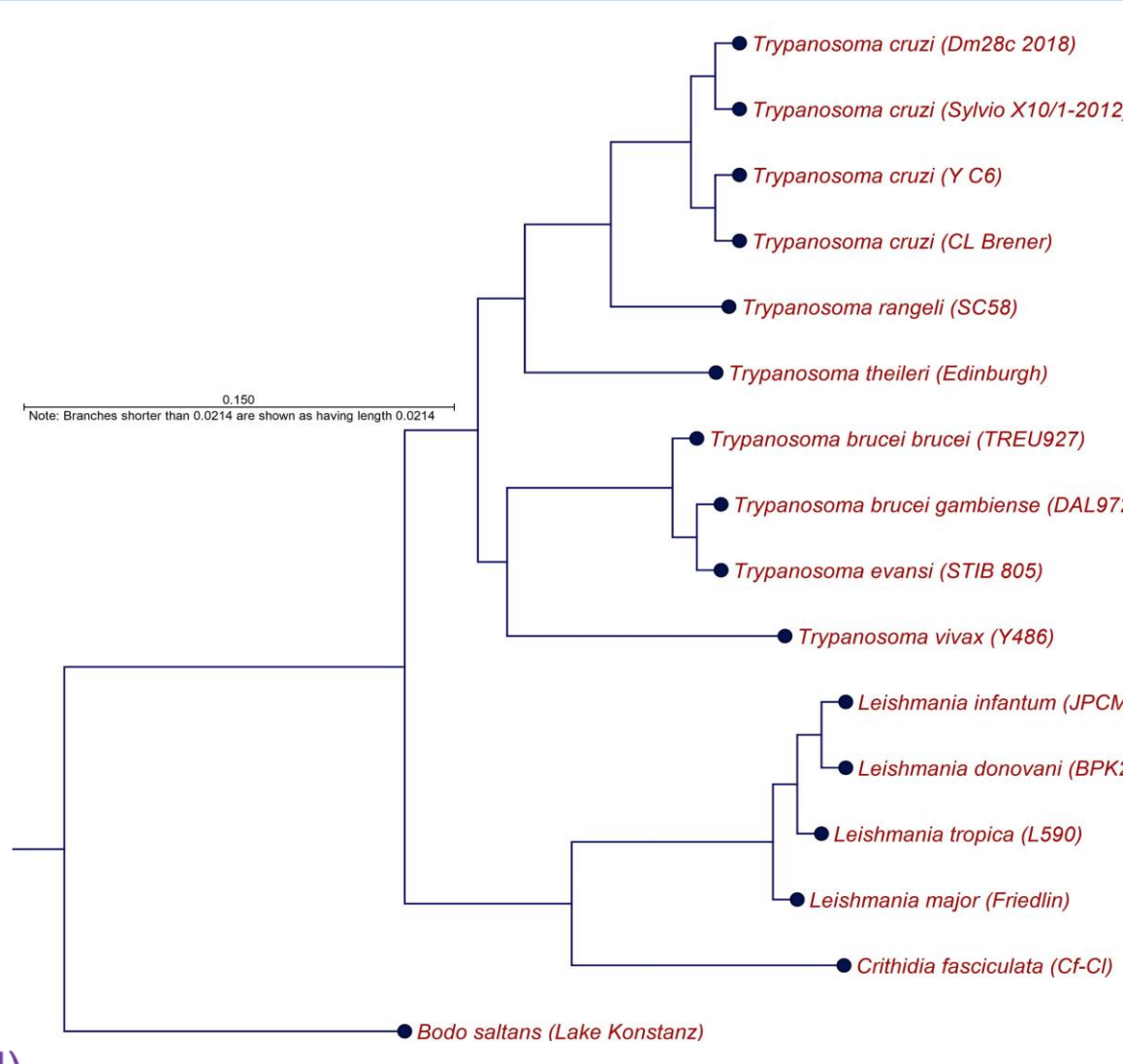
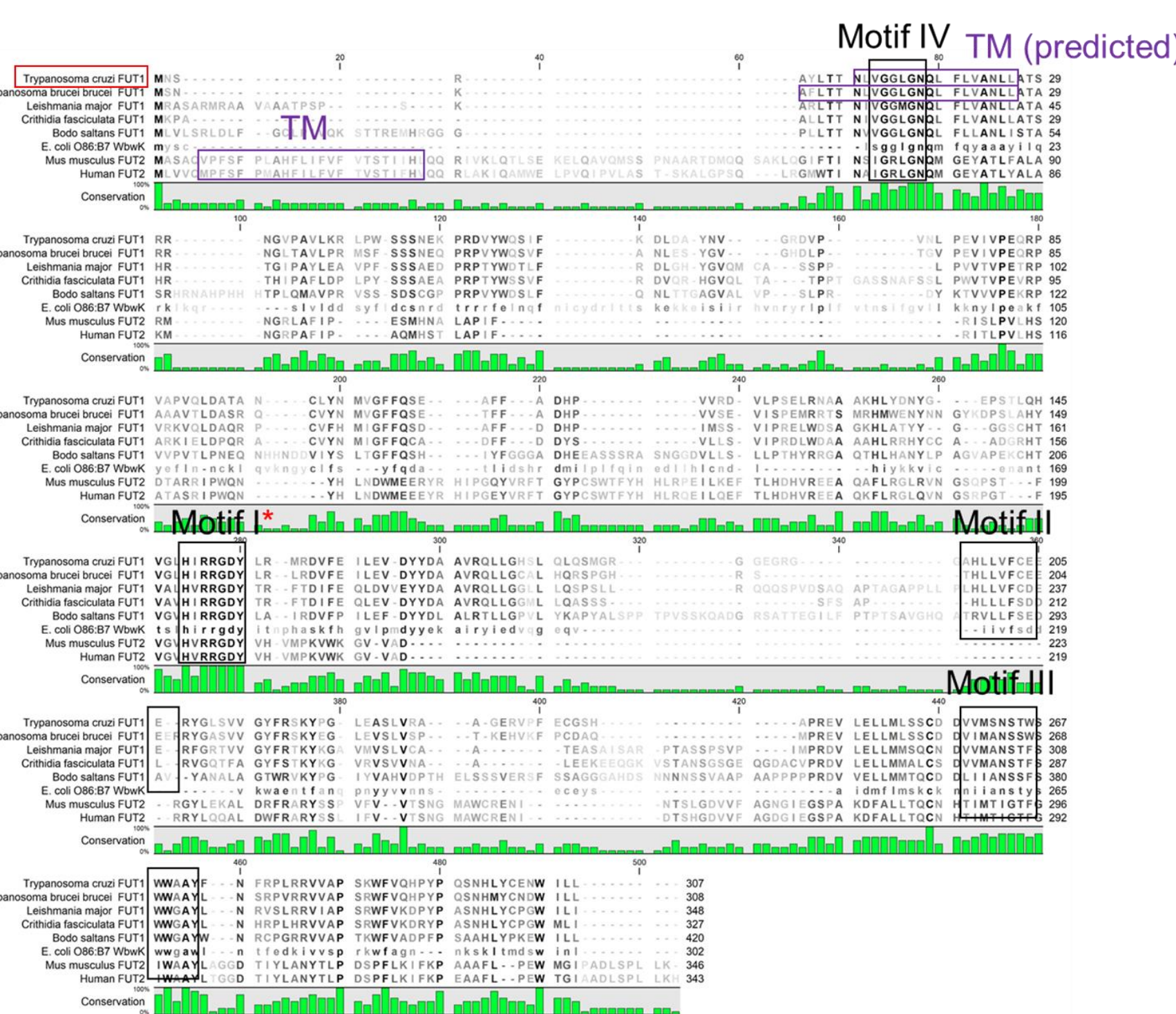
- GDP-<sup>3</sup>H]Fucose
- TcFUT1

## The GT11 family FUT1 is encoded in all kinetoplastids.

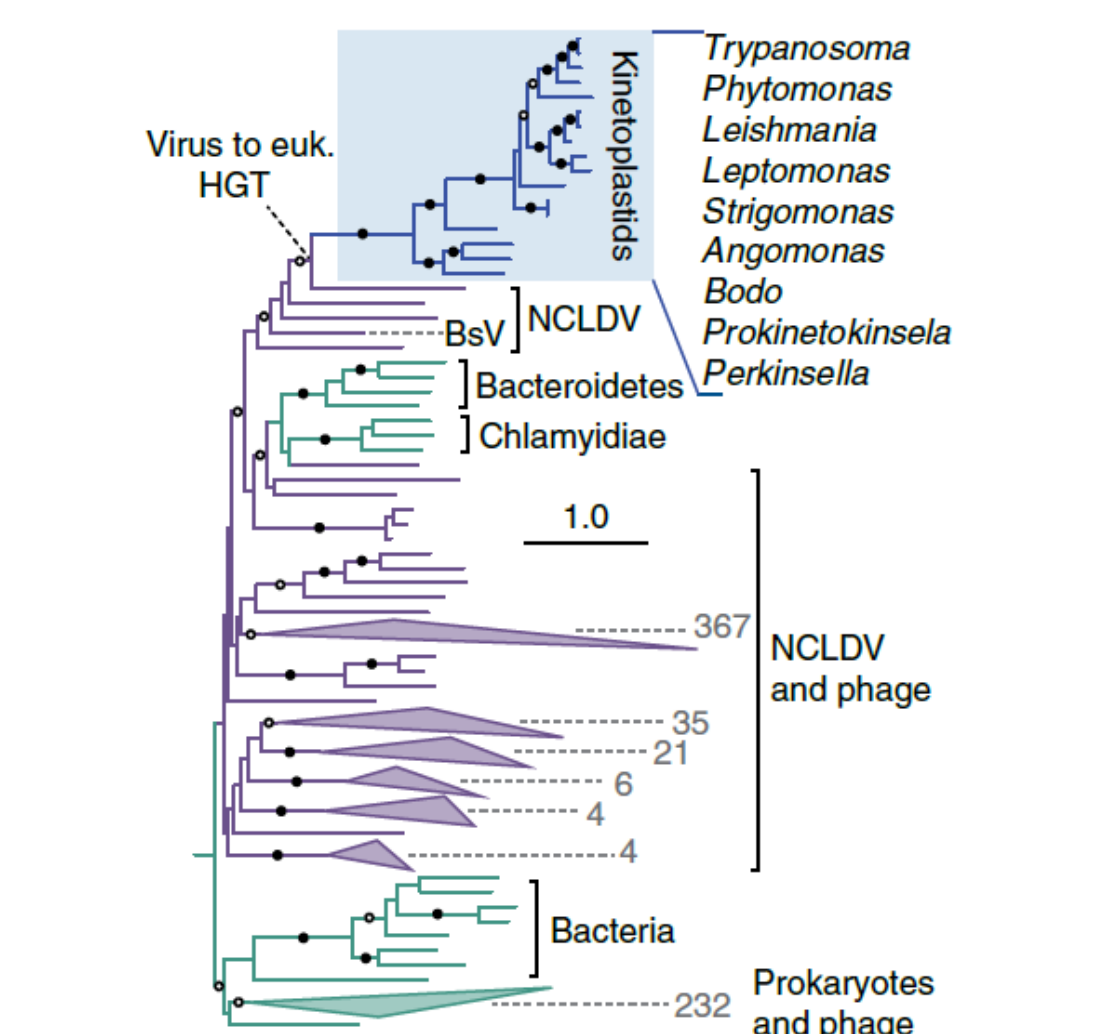
Predicted TcFUT1 structure (Robetta, left; Phyre2, right)



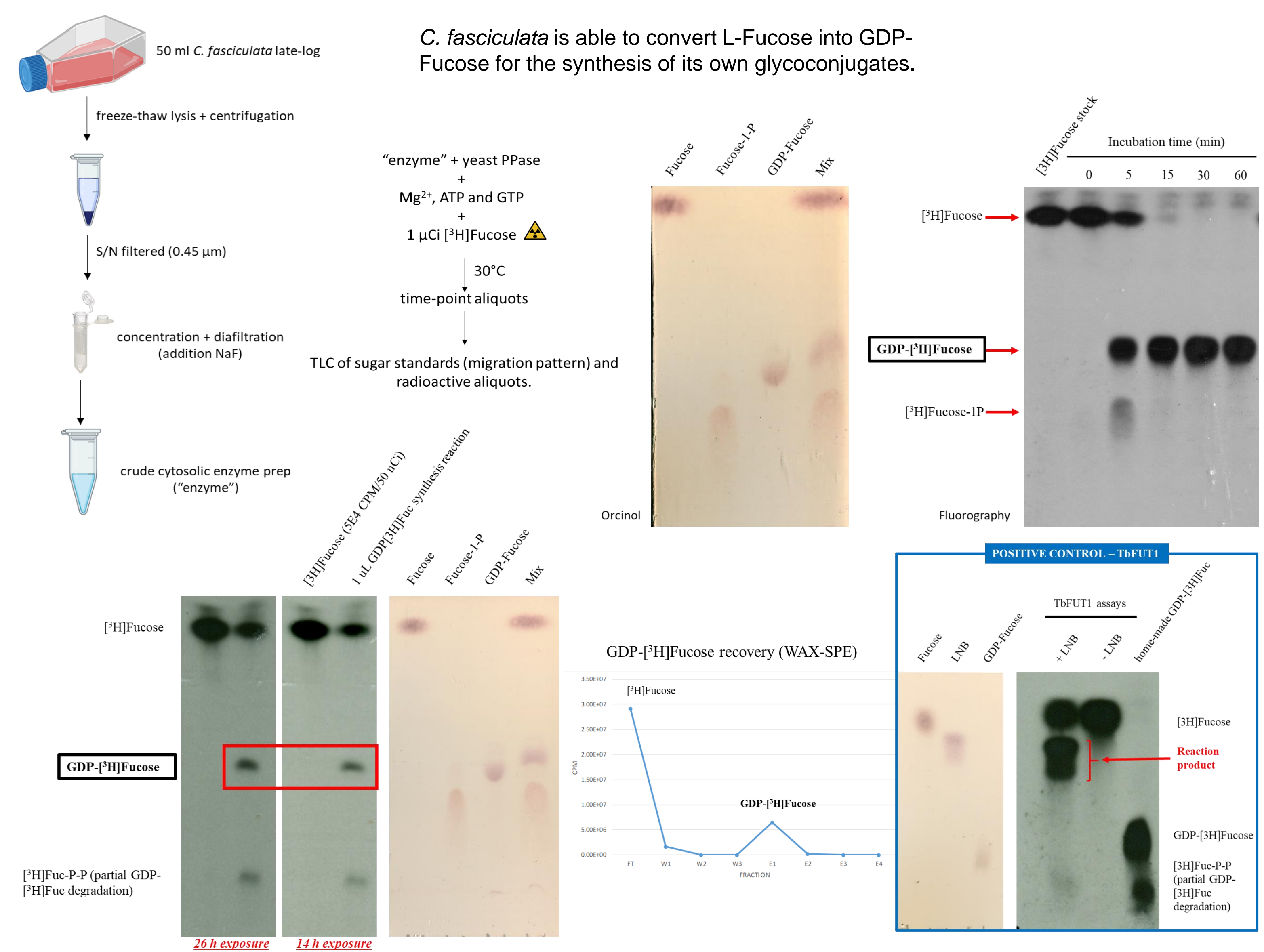
Closest crystal structures are fucosyltransferases (non-GT11) from human, *A. thaliana* and bacteria.



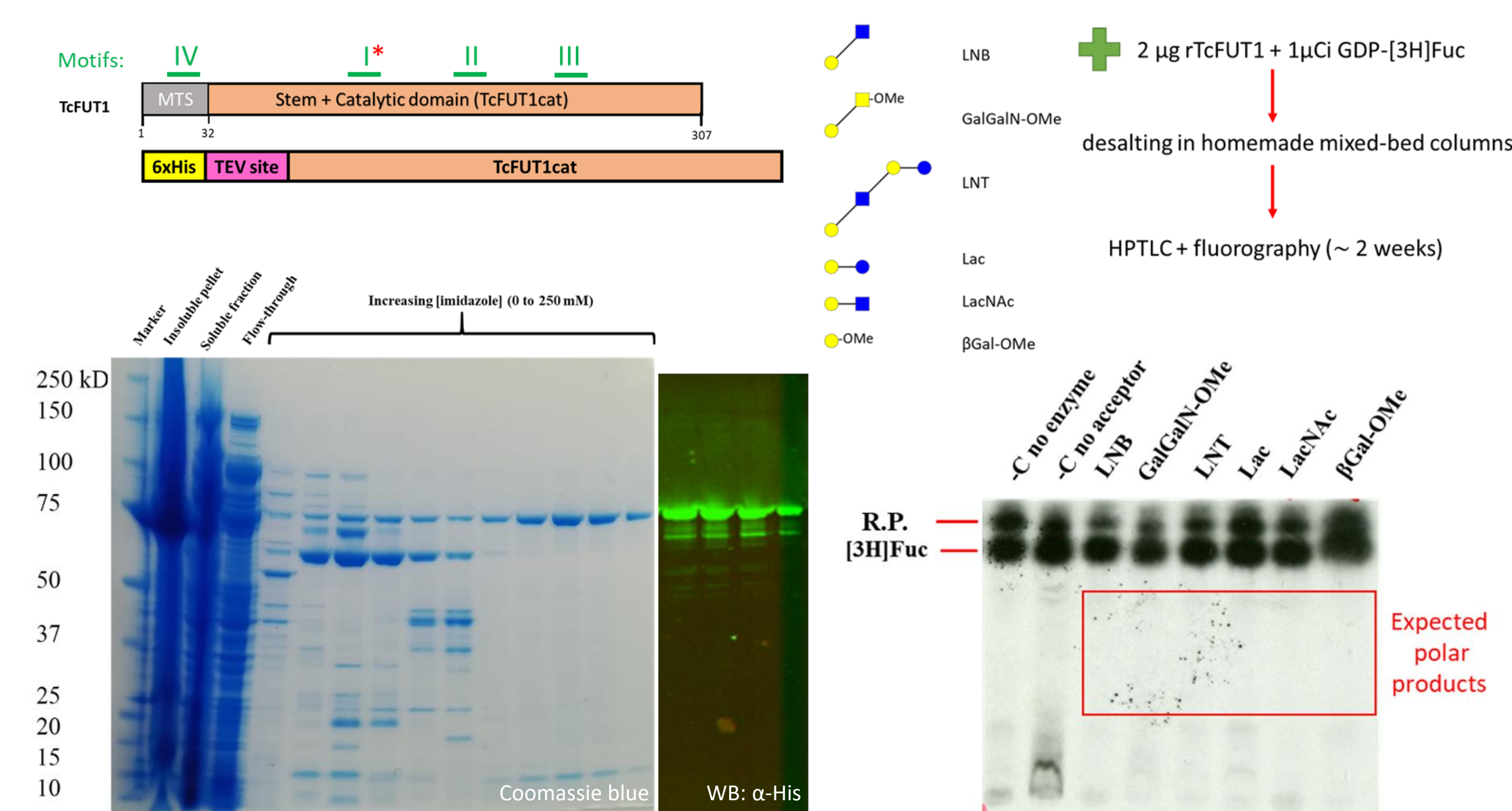
The kinetoplastid FUT1 is a bacterial-derived gene obtained by horizontal gene transfer<sup>c</sup>.



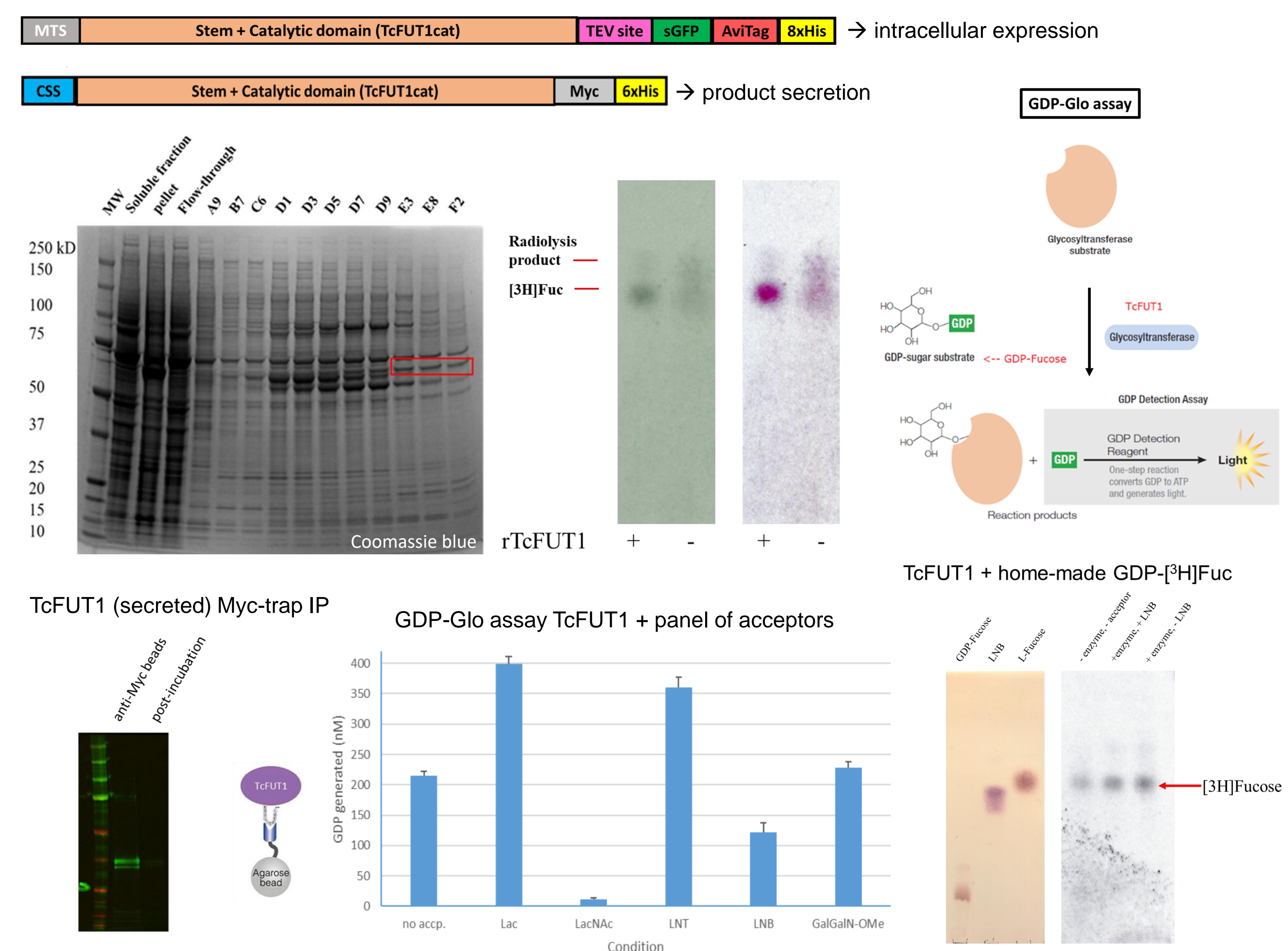
## Synthesis of GDP-<sup>3</sup>H]Fucose using *Crithidia fasciculata* lysate as enzymatic source<sup>d,e</sup>.



## Expression of TcFUT1 in bacteria and GDP-<sup>3</sup>H]Fuc activity assays.

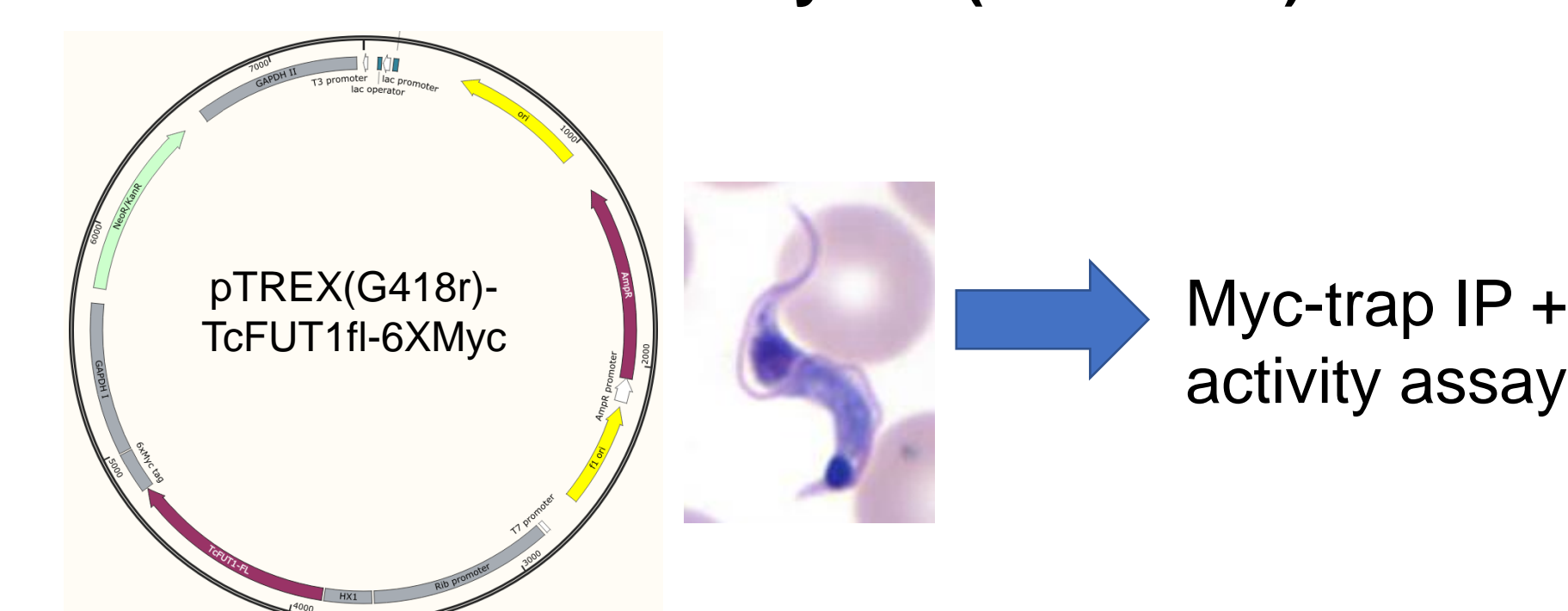


## Eukaryotic expression of TcFUT1: clues of GDP-<sup>3</sup>H]Fuc hydrolysis and fucosyltransferase activity.



## Ongoing work

- [sup3H]-labelling in *C. fasciculata* to identify endogenous substrates.
- TcFUT1 overexpression in *T. cruzi*.
- TcFUT1 localisation by IFA (α-TbFUT1).



**References:**

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b. Guo, H., et al. A broadly active fucosyltransferase LmjFUT1 whose mitochondrial localization and activity are essential in parasitic *Leishmania*. *PNAS*, 118 (33) e2108963118 (2021).

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d. Schneider, P., et al. The biosynthesis of GDP-D-arabinopyranose in *Crithidia fasciculata*: characterization of a D-arabino-1-kinase activity and its use in the synthesis of GDP-[sup3H]-D-arabinopyranose. *Biochemical Journal*, 311, 307-315 (1995).

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