

# Mechanism of Fucosylation in Xyloglucan Biosynthesis revealed in *Arabidopsis thaliana*

## Background

- Understanding glycosyltransferases at the molecular level is paramount for gaining fundamental insights into key steps in plant biomass formation.
- FUT1 is a plant fucosyltransferase that carries out the final step in the synthesis of the hemicellulose xyloglucan, which is a major component of the plant cell wall and is involved in cell growth and expansion, energy metabolism, and signaling.
- Crystal structures of eukaryotic glycosyltransferases, especially from plants, are highly underrepresented in structural databases, despite their biological importance, due to difficulties in expressing and purifying plant proteins.

## Approach

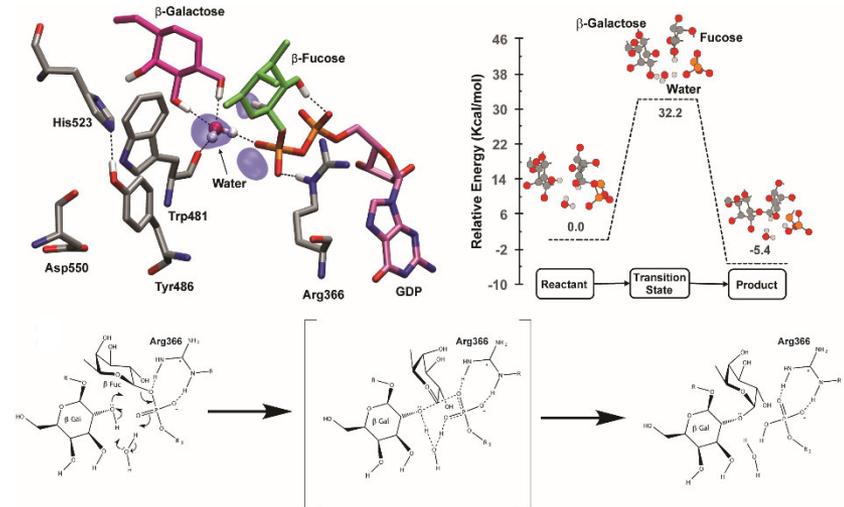
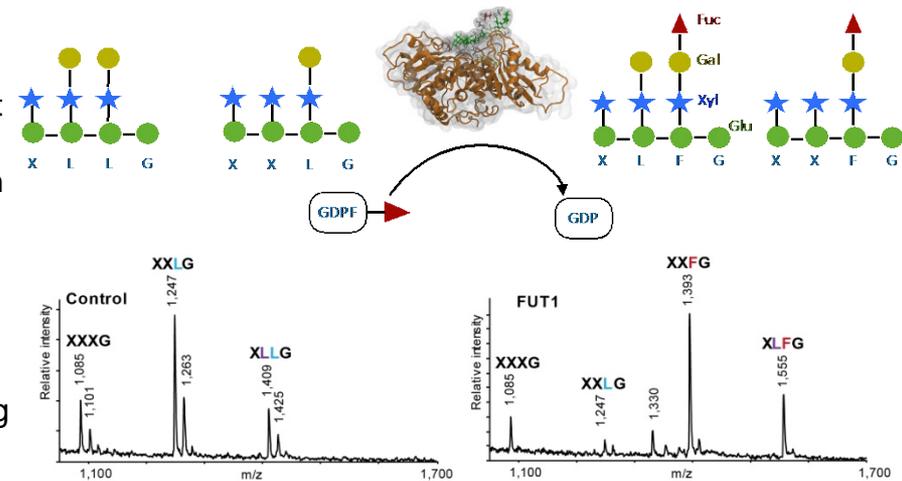
- Mammalian cell culture-based expression enabled recombinant production of significant amounts of enzyme (100 mg/L).
- X-ray crystallography revealed the structural architecture of FUT1 in complex with bound donor and acceptor substrate analogs, forming the basis for *in silico* studies that unraveled the mechanistic basis for fucosylation in GT37 enzymes.

## Outcome

- Structural and mutagenic analyses determined that there was no active site amino acid residue that could act as a base for catalyzing the reaction.
- In silico* studies revealed the presence of a crucial water molecule that could conduct catalysis by shuttling the proton to enable a  $S_N1$ -like mechanism.

## Significance

- This work presents one of the first instances of the structural, biochemical, and mechanistic elucidation of an important glycosyltransferase involved in the biosynthesis of a key polymer in plant biomass.



# BESC glycosyl transferase work featured on cover of



Front cover: The cover image represents a rendition of the *Arabidopsis thaliana* fucosyltransferase (AtFUT1) enzyme in action on a xyloglucan chain. The galactose residues on the acceptor xyloglucan chain are represented in magenta, while the fucose residue on the donor is represented in green. The inset shows a close up of the active site with the donor fucose, acceptor galactose along with the reaction mediating water molecule. The background is set on an confocal microscopy image of the plant cell wall. For details, see article by Urbanowicz et al. (pp.931–949).