Rational Enzyme Design for Controlled Functionalization of Acetylated Xylan

U.S. DEPARTMENT OF ENERGY | Office of Science

Background

- Xylan O-acetyltransferase 1 (XOAT1) is involved in *O*-acetylating the backbone of hemicellulose xylan.
- *O*-acetylation influences polymer-polymer interactions, biorefinery operations, and valorization processes.
- **Our goal** is to develop feedstocks with adjusted xylan acetylation levels.

Approach

- Key residues involved in enzyme-acceptor substrate interactions were investigated through a combination of computational modeling followed by site-directed mutagenesis, kinetic analysis, and *in vitro* synthesis of *O*-acetylated xylan which was confirmed via MALDI-TOF MS.

Outcome

- The major lobe of XOAT1 is important for xylan binding.
- An *in vitro* cell-free platform was developed to study xylan acetyltransferases and produced xylo-oligomers with highly controlled degrees of acetylation.

Significance

- Molecular insights into the enzyme-substrate interactions that modulate xylan binding in XOATs lays the foundation for rational enzyme engineering strategies that can precisely modulate acetylation levels in plant secondary cell walls and produce, using natural plant pathways, biomass with tuned properties.

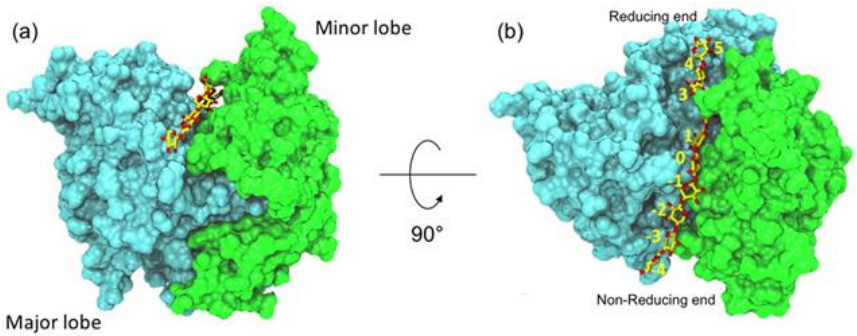

Fig. 1. Residues of the two lobes interact with different sections of xylan; the major lobe is in blue.

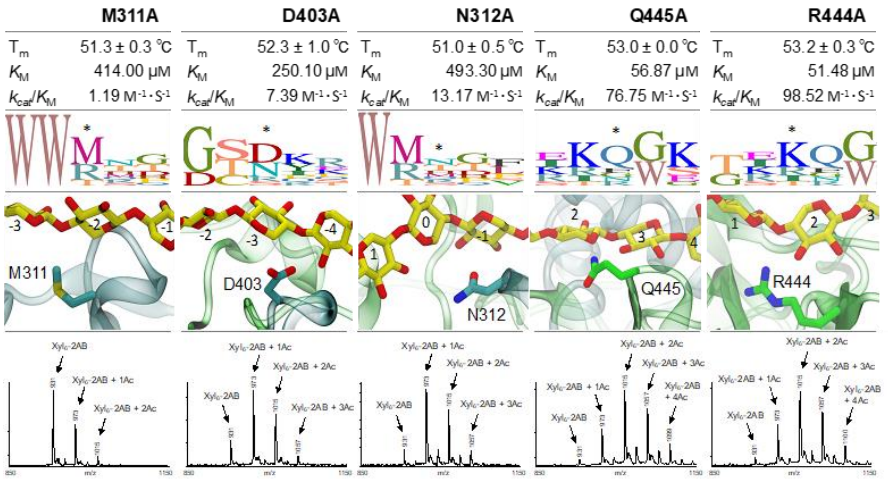

Fig. 2. Single AA substitutions in the substrate binding lobes used to tune catalytic efficiency. In each case the mutated residue is marked *. The bottom row is the MALDI-TOF MS spectra showing the different acetylation patterns.

Wang H.T., Bharadwaj, V. S., Jeong Yeh Yang, Thomas M. Curry, Kelley W. Moremen, Yannick J. Bomble, and Breeanna R. Urbanowicz (2021) Rational enzyme design for controlled functionalization of acetylated xylan for cell-free polymer biosynthesis. *Carbohydrate Polymers* https://doi.org/10.1016/j.carbpol.2021.118564.

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