Engineering Plant Cell Wall Polysaccharide *O*-Acetyltransferases with Altered Specificity

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The Center for Bioenergy Innovation (CBI) vision is *to accelerate domestication of bioenergy-relevant, non-model plants and microbes to enable high-impact innovations at multiple points in the bioenergy supply chain.* CBI has identified key barriers for the current bioeconomy in (1) high-yielding, robust feedstocks, (2) lower capital and processing costs via consolidated bioprocessing (CBP) to specialty biofuels, and (3) methods to create valuable byproducts from the lignin residues. CBI will identify and utilize key plant genes for growth, yield, composition and sustainability traits as a means of achieving lower feedstock costs, focusing on the perennial feedstocks - poplar and switchgrass. We will convert these feedstocks to specialty biofuels (C4 alcohols and esters) using CBP at high rates, titers and yield. CBP will be combined with cotreatment or pretreatment. CBI will maximize process and product value by *in planta* modifications of lignin and biological funneling of lignin to value-added chemicals. Techniques for rapid domestication of non-model microbes and plants will be improved.

We aim to modify lignocellulosic feedstocks with adjusted acetylation levels towards reducing cell wall recalcitrance via the understanding and control of key acetylation enzymes. *O*-Acetylation is a universal modification of non-cellulosic polysaccharides in most bioenergy feedstocks, which is an obstacle for saccharification and fermentation in the process of biomass conversion into bioproducts. Our goal in this project is to understand the molecular basis of the catalytic activity of *O*-acetyltransferases from plants that are used as pilot organisms in CBI, such as *Populus* and *Arabidopsis*.

The level of acetylation of the hemicelluloses that constitute plant cell walls can have significant impacts on lignocellulosic biomass-based biofuel production. *O*-Acetyl groups play a major role in polymer-polymer interactions in the cell wall, such as those formed between xylan-lignin and xylan-cellulose, and also sterically hinder hydrolases from accessing their polysaccharide substrates. In addition, *O*-acetyl groups are one of the most abundant substituents of the plant cell wall components, particularly xylans and mannans; thus, the acetate released during both pretreatment and deconstruction also inhibits downstream microbial fermentation processes. The enzymes involved in *O*-acetylation of hemicelluloses belong to a plant-specific Trichome Birefringence-Like (TBL) protein family. Understanding the interactions between TBL proteins

and their substrates could drive the development of engineered enzymes and modified feedstocks for improved conversion into fuels and products. In our research, we used molecular docking and molecular dynamics simulations to analyze enzyme-substrate interactions of the *Arabidopsis thaliana* TBL protein xylan *O*-acetyltransferase 1 (XOAT1/TBL29) with xylan. The results point out the key amino acid residues involved in substrate-binding at the active site, and kinetic analysis of the resulting XOAT1 mutant variants substantiates their crucial role, providing us an opportunity to potentially tune acetylation levels in biomass through genetic engineering (see figure). Additionally, we employed high-throughput computational modeling of the TBL family in *Populus* to identify the molecular determinants of substrate specificity. This computational and experimental workflow suggests a new strategy of using predicted models to fine-tune the biochemical properties of the plant cell wall.

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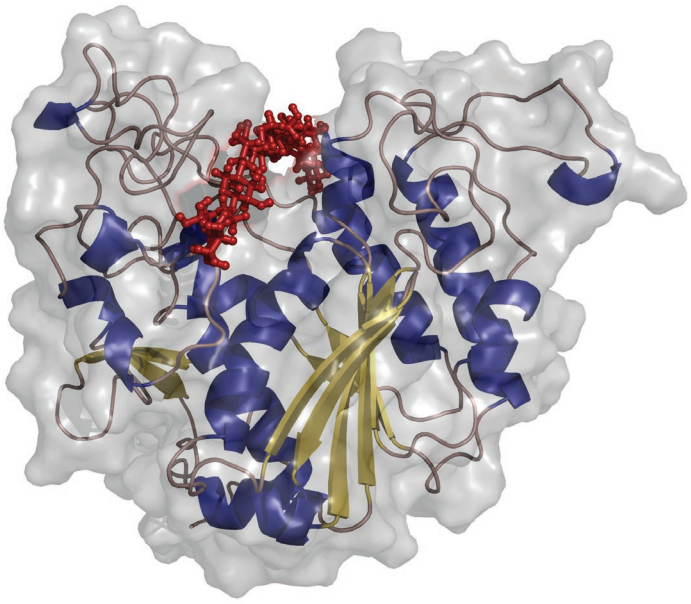

XOAT1 is at the heart of xylan synthesis. The structure of XOAT1 reveals a putative substrate binding groove shown here with a xylodecaose (red stick representation) bound in MD simulations.

Lunin, Vladimir V., et al. "Molecular mechanism of polysaccharide acetylation by the Arabidopsis xylan O-acetyltransferase XOAT1." *The Plant Cell* 32.7 (2020): 2367-2382.