Integrating Synthetic Biology and Polysaccharide Synthesis for Designer Polymers with Tunable Properties

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Project Goals: 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 addresses strategic barriers to the current bioeconomy in the areas of 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. CBI will identify and utilize key plant genes for growth, composition, and sustainability phenotypes as a means of achieving lower feedstock costs, focusing on poplar and switchgrass. We will convert these feedstocks to biofuels using CBP with cotreatment at high rates, titers and yield in combination with catalytic upgrading into drop-in hydrocarbon fuel blendstocks.

Plant cell wall represent one of the most abundant sources of renewable material available for the production of biobased fuels and products. Plant cell walls are comprised primarily of a complex matrix of cellulose, lignin and a diverse group of polysaccharides known as hemicelluloses. Hemicelluloses such as xylan and xyloglucan are essential to plant growth and fitness and can affect outcomes of biomass deconstruction and fermentation. For these reasons engineering hemicellulose structure is an attractive approach to improving biomass quality for bioprocessing. We are seeking to apply principles of synthetic biology to plant hemicellulose engineering to create novel or well-defined polysaccharides that may serve to improve the properties of biomass for deconstruction and valorization. Using recombinantly expressed plant cell wall glycosyltransferases as biocatalysts, we are building and modifying plant cell wall polymers *in vitro.* This approach allows for the determination of structure-function relationships of polysaccharides and other wall components which are difficult to obtain due to the complexity of structures often exhibited from polysaccharides isolated from plant feedstocks.

As an example of this approach, we are using a Xyloglucan Xylosyl Transferase (XXT1) to modify mixed-linkage glucans (polymers typically devoid of glycosyl branches), with α 1,6 xylosyl or α 1,6 glucosyl residues *in vitro*. By controlling the amount of substitutions on the mixed linkage glucan backbone, we have demonstrated the ability to fine tune the interactions of mixed-linkage glucans with nanocellulose surfaces as determined by a Quartz Crystal Microbalance based assay. In addition, we have developed a platform for the bottom-up synthesis of acetylated xylan polymers using a recombinant Xylan Synthase (XYS1) and Xylan *O*-acetyl Transferase (XOAT1) *in vitro*[1]. Insights gained by computationally modeling enzyme-substrate interactions were used for the rational design of XOAT1 mutants capable of creating xylan polymers with well-defined acetylation patterning. In summary, we are bringing synthetic biology to plant polysaccharide synthesis by using plant biosynthetic enzymes as biocatalysts to build structurally defined

polymers. This work will help to advance our understanding of polysaccharide structure-function relationships and can serve as a basis for the engineering of plant polysaccharides to create biomass better suited for processing to fuels and materials.

References/Publications

1. Wang, H. T., Bharadwaj, V. S., Yang, J. Y., Curry, T. M., Moremen, K. W., Bomble, Y. J., & Urbanowicz, B. R. Rational enzyme design for controlled functionalization of acetylated xylan for cell-free polymer biosynthesis. *Carbohydrate polymers*, *2021 Dec 1; 273*, 118564.

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