Modeling and NMR Methods to Probe Spatial Arrangements in Biomolecules: Towards predictive models of plant cell wall structure

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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 specialty biofuels (C4 alcohols, C6 esters and hydrocarbons) using CBP at high rates, titers and yield in combination with cotreatment, pretreatment or catalytic upgrading. CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

The lignified plant secondary cell wall is comprised of a complex and heterogeneous framework of three major biopolymers; cellulose, hemicelluloses (xylan and glucomannan), and polyaromatic lignin. The intrinsic physical properties of these polymers and the complicated, yet poorly understood, interplay between them, gives rise to a lignocellulosic material that is not only structurally and mechanically sound but also frustratingly recalcitrant to enzymatic and chemical deconstruction methods. Due to inherent limitations of many common analytical techniques applied to heterogeneous materials, detailed molecular-level information on secondary cell wall (SCW) structure and architecture, especially regarding interactions between the constituent polymers, remains scarce. As a result, even the best available SCW models in literature are limited to being mere conceptual renderings rather than acting as frameworks for enhancing our scientific understanding of the role played by molecular-level actors in contributing to emergent properties. A combined experimental and computational approach is being developed to help bridge this gap. Experimentally, we have developed a new solid state Nuclear Magnetic Resonance (ssNMR) technique to probe polymer-polymer interactions within the secondary cell wall of ^{13}C-enriched poplar wood in great detail [1]. The major ssNMR observables that informed the construction and validation of the SCW atomistic molecular models of poplar are as follows: 1) Roughly 70% of all lignin shares surface contact with xylan; 2) Roughly 70% of acetylated xylan is bound to cellulose in a linearized 2-fold structure; 3) the inter-polymer distance between xylan acetyl groups and the lignin surface is ~0.4 – 0.5 nm; 4) little to no direct lignin/cellulose contact is observed; and 5) there is evidence for preferential lignin/hemicellulose surface interaction in the order

Methoxy > S-Lignin > G-Lignin. Even for a known polymer composition, the construction of molecular models of these complex systems entails the consideration of a number of variable factors such as the relative locations of xylan, lignin, and water with respect to cellulose. Quantitative observables from molecular dynamics (MD) simulations (*e.g.*: radial distribution functions, polymer-polymer distances and conformational analyses) of models built with varying arrangements of these components enables the corroboration of ssNMR inferences and lays the foundations for the development of realistic plant cell wall architectures with atomistic details. Here we demonstrate how ssNMR data has enabled the iterative development of these atomistic SCW models leading to the most detailed molecular picture of the plant cell wall architecture to date. Moving forward, we will investigate plant SCW superstructures using a combined ssNMR and MD approach for consideration of molecular-level factors that may be associated with cell wall recalcitrance, sustainability metrics, and other plant phenotypes.

References/Publications

1. Addison, B., et al., *Selective One-Dimensional 13C-13C Spin-Diffusion Solid-State Nuclear Magnetic Resonance Methods to Probe Spatial Arrangements in Biopolymers Including Plant Cell Walls, Peptides, and Spider Silk.* The Journal of Physical Chemistry B, 2020: p. acs.jpcb.0c07759.

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