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 biofuels using CBP with cotreatment at high rates, titers and yield in combination with catalytic upgrading into drop-in hydrocarbon fuel blendstocks.

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 employ solid state Nuclear Magnetic Resonance (ssNMR) techniques, including methods developed in house [1], to probe polymer-polymer interactions within the secondary cell wall of ^{13}C-enriched poplar wood in great detail. The major ssNMR observables that informed the construction and validation of the SCW atomistic molecular models of poplar include the following: 1) cellulose microfibrils are on the order of 3 nm in diameter with tightly bound water and possibly some acetylated xylan trapped between; 2) spin-diffusion rate constants and loose inter-polymer distance estimates confirm that xylan is “in the middle” between lignin and cellulose and that xylan interacts with cellulose in a 2-fold arrangement with its decorations pointing away from the cellulose surface; 3) the inter-polymer distance between xylan acetyl groups and the lignin surface is ~0.3 – 0.5 nm; 4) ~80% of all lignin and ~40% of cellulose chains are within ~1 nm of xylan acetyl carbons; 5) ~60% of all xylan and ~20% of all cellulose carbons are within ~1 nm of lignin ring carbons; and 6) lignin and xylan are most likely of linear morphology, not globular, and lignin/xylan heterogeneities are generally not larger than ~1 nm in size.

Even for a known polymer composition (*e.g.,* 50% cellulose, 25% lignin, 25% hemicelluloses), 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. Our approach of building atomistic models with varied spatial arrangements of constituent biopolymers, benchmarked with ssNMR data presents a robust protocol for the development of realistic and holistic plant cell wall models. These ssNMR-informed and experimentally-validated atomistic models lay the foundations for myriad future *insilico* explorations to gain insights into the molecular level determinants of the emergent properties of naturally occurring, treated and engineered biomass.

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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