Reveal the Functional Structure of Complex Carbohydrates in Plants and Fungi Using DNP Solid-State NMR

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Complex carbohydrates play crucial roles in energy storage, cell recognition and structural building. Their functional structure is often elusive due to the technical difficulty in characterizing these molecules, which are typically polymorphic and disordered in structure. Here we present two solid-state NMR and DNP studies of carbohydrate-rich biosystems: the disease-relevant, pathogenic fungi and the energy-rich plant biomass. High-resolution of such complex biomaterials is accomplished by systematically investigating the composition, sub-nanometer packing, site-specific hydration and ns-μs motion of polysaccharides and other biomolecules in the near-native cells through a series of 2D ^{13}C-^{13}C/^{15}N experiments. DNP are often needed to overcome sensitivity limitation as well as specifically probe the interaction interface between biomolecules. The fungal cell walls of a major pathogen *Aspergillus fumigatus* is found to contain a hydrophobic scaffold of chitin and α-1,3-glucan, which is surrounded by a hydrated matrix of diversely linked β-glucans and capped by a dynamic, outer layer rich in glycoproteins[1] (**Fig. 1a, b**) This study provides the first high-resolution model of fungal cell walls and enables in-cell, high-resolution characterization of the drug effect to promote the development of wall-targeted antifungals. In the intact stems of multiple energy crops, such as maize and switchgrass, lignin is found to self-aggregate to form hydrophobic nanodomains, which are bridged to cellulose microfibrils by xylan via extensive interface[2] **(Fig. 1c, d)**. The flat conformers of xylan are coating the even surface of cellulose microfibrils and the non-flat conformers bind the intrinsically disordered aromatics of lignin through electrostatic interactions. This study has substantially revised our contemporary views of lignocellulose and has the great potential to facilitate the development of crops with higher digestibility for improving biomass deconstruction and conversion to biofuels. These studies provide invaluable insight into the functional structure of carbohydrates, their interaction with other polymers such as lignin and proteins, and the evolutional structure of cell walls. In addition, the development of DNP methods for structural elucidation and statistical analysis of polysaccharide structure in unlabeled whole-cells[3], as well as the efforts for developing a carbohydrate solid-state NMR database, will also be discussed.

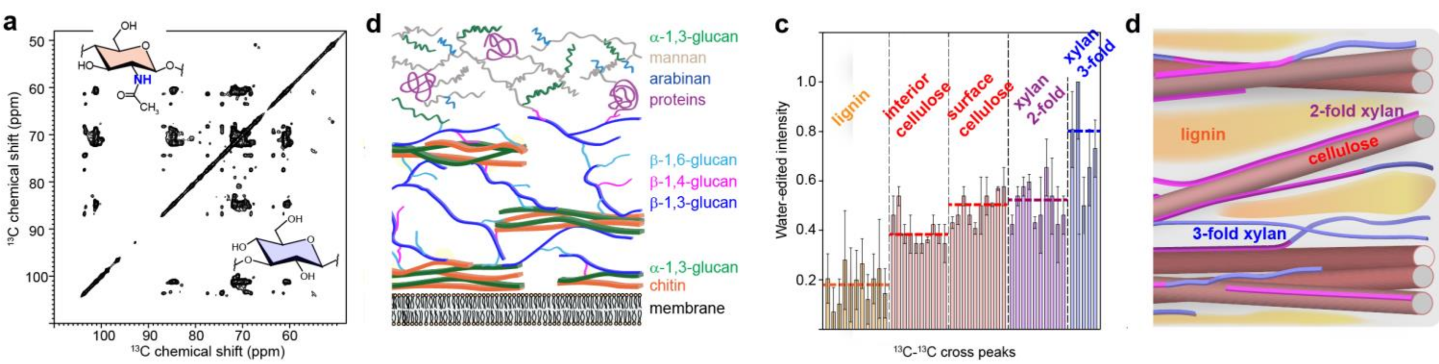

Figure 1. Structural characterization of fungal and plant cell walls. **a**, Representative 2D ^{13}C-^{13}C CORD spectrum of fungal pathogen *A. fumigatus* measured on an 800 MHz spectrometer. **b**, NMR-derived structural model of fungal cell walls. **c**, The site-specific hydration profile of carbohydrates and lignin in intact maize stems. **d**, The supramolecular architecture of secondary plant cell walls.

References

1. X Kang, A Kirui, A Muszyński, MC Dickwella Widanage, A Chen, P Azadi, P Wang, F Mentink-Vigier, T Wang*, "Molecular Architecture of Fungal Cell Walls Revealed by Solid-State NMR", ***Nat. Commun.*** 9, 2747 (2018).

2. X Kang, A Kirui, MC Dickwella Widanage, F Mentink-Vigier, DJ Cosgrove, T Wang*, "Lignin-Polysaccharide Interactions in Plant Secondary Cell Walls Revealed by Solid-State NMR", ***Nat. Commun.***, accepted.

3. A Kirui, Z Ling, X Kang, MC Dickwella Widanage, AD French, T Wang*, "Atomic Resolution of Cotton Cellulose Structure Enabled by Dynamic Nuclear Polarization Solid-State NMR", ***Cellulose***, in press.

Acknowledgement: This research is primarily supported by National Science Foundation NSF/OIA-1833040. The National High Magnetic Field Laboratory is supported by National Science Foundation through NSF/DMR-1644779 and the State of Florida.