

## Polymer and structural science behind valorizing lignin using solvents

Marcella C. Berg,<sup>1</sup> Derya Vural,<sup>1</sup> Samarthya Bhagia<sup>1</sup>, Luna Liang<sup>2</sup>, Zhi Yang<sup>1</sup>, Xianzhi Meng<sup>2</sup>, Nidia Gallego<sup>1</sup>, Nathan Bryant,<sup>2</sup> Sai Venkatesh Pingali<sup>1</sup>, Hugh M. O'Neill,<sup>1</sup> Yunqiao Pu,<sup>1</sup> Eugene Mamontov,<sup>1</sup> Arthur J. Ragauskas,<sup>1,2</sup> Jeremy C. Smith,<sup>1</sup> Loukas Petridis<sup>1\*</sup>(petridisl@ornl.gov), **Brian H. Davison<sup>1</sup>**

<sup>1</sup>Oak Ridge National Laboratory, Oak Ridge, Tennessee; <sup>2</sup>University of Tennessee, Knoxville

<http://cmb.ornl.gov/index.php/research/bioenergy/dynamic-visualization-of-lignocellulose>

**Project Goals: The development of renewable biofuels is a key mission of the DOE Genomic Science program. Lignocellulosic biomass has the potential to be an abundant, renewable source material for production of biofuels and other bioproducts. The use of organic solvents to optimize biomass pretreatment has shown considerable promise, but their disruption of microbial membranes is key to toxic effects limiting fermentation titers. The Oak Ridge National Laboratory (ORNL) Scientific Focus Area (SFA) Biofuels Program utilizes multi-length scale imaging with neutron scattering complemented by high performance computer simulations, NMR, biochemistry and targeted deuteration to provide fundamental knowledge about the molecular forces that drive solvent disruption of the critical assemblies of biomolecules that comprise plant cell walls and microbial biomembranes.**

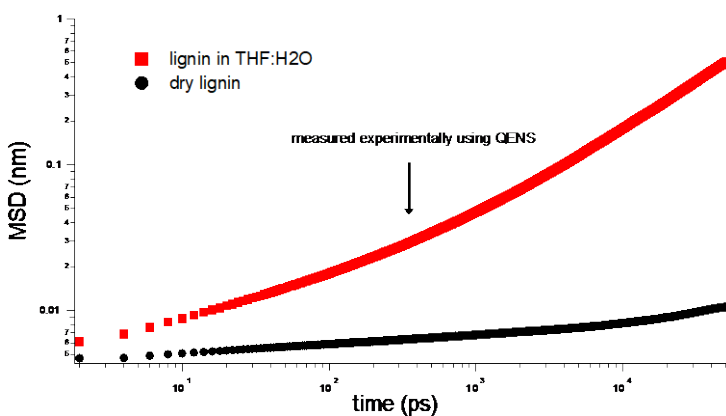
Converting plant biomass to renewable chemicals, fuels and materials can have both economic and environmental impact: sustainable economic growth and reducing the carbon footprint from the industries. The plant polymer lignin is especially well suited as a precursor for valuable renewable biomaterials because of its abundance and high content of carbon. However, the rigidity of lignin makes it the most recalcitrant component of plant biomass. To facilitate deconstruction and conversion of biomass, it is therefore necessary to soften lignin. At an atomic level softening the lignin means increasing lignin atomic fluctuations, making the molecules more dynamic. This is usually done by using high temperatures<sup>1</sup> or pretreatment solvents. Elucidating the motions that give rise to enhanced lignin dynamics may yield fundamental insights that enable rational improvement of biomass pretreatment and processing. However, knowledge about these changes in the dynamics of lignin that arise from the solvent-induced effects is lacking to this day. This study aims to understand the underlying processes that cause the dynamical increase of lignin motion in tetrahydrofuran (THF) solvent, a novel promising multifunctional solvent for biomass pretreatment and fractionation.

Comparative studies of THF and the organic solvents ethanol and gamma-valerolactone (GVL) for extraction of lignin from switchgrass strains found differences in conservation of structural properties dependent on both solvent and lignin characteristics. We studied wild-type Alamo and two transgenic switchgrass strains, one with altered lignin composition (downregulation of caffeic acid 3-O-methyltransferase (COMT) gene) and one with decreased lignin content (overexpression of a lignin biosynthetic repressor MYB transcription factor) that reduce recalcitrance of switchgrass. The two-dimensional <sup>13</sup>C-<sup>1</sup>H heteronuclear single quantum coherence (HSQC) nuclear magnetic resonance (NMR) was carried out on solubilized lignin to obtain structural information on lignin structures. Chemical and physical characterization showed that ethanol pretreatment preferentially cleaved beta aryl ether linkages, while S/G ratio was significantly lower in THF and GVL lignins obtained from COMT and MYB switchgrass, and p-coumarate of THF and GVL lignins was much higher in all three switchgrass samples.

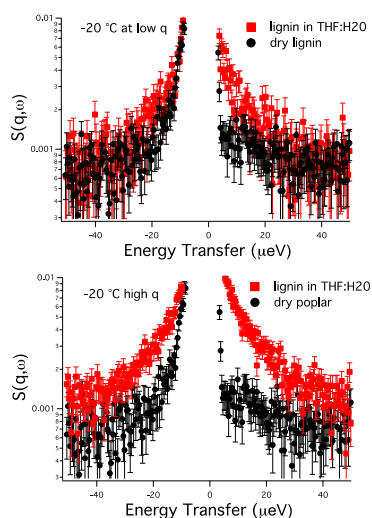
In order to elucidate lignin molecular motions, we employed both experimental and computational methods. Quasi-elastic neutron scattering (QENS) experiments provided direct information on molecular dynamics and geometry of motions in a non-invasive manner that does not unduly perturb the material. The high incoherent scattering cross section of the hydrogen atoms makes the QENS ideal for extracting self-

correlations (dynamics) in materials such as lignin. Molecular Dynamics (MD) simulations access a broad range of time scales (see Figure 1) and provides a full atomistic model of the system. Additionally, MD probes similar length- and time-scales as QENS, so that a theoretical neutron scattering profile can be calculated from the atomic positions in the simulation trajectories, thus results from MD and QENS can be directly compared. For the QENS experiment lignin was extracted and purified from Poplar feedstock corresponding to a molecular weight of 4514 g/mol with composition ratios of S ~ 58%, G~ 30% and H ~ 12 %<sup>2</sup>. The lignin molecules and their solvation concentrations for the MD simulation were modelled to replicate the experimental samples.

Both techniques show that in the presence of the solvent there is a dynamical transition at which lignin purified from polar becomes more dynamic. This dynamical transition is found even at small solvent concentrations and temperatures as low as -20 °C (see Figure 2). We furthermore investigate how THF solvent concentrations affect the atomic fluctuations of the lignin. In conclusion, the study provides a molecular-level understanding of the technologically important motions of lignin that will facilitate the rational improvement of biomass deconstruction and lignin processing.



**Figure 1:** Simulated mean square displacement (MSD) for both dry and solvated lignin at 25 °C. MSD quantifies the amplitude of atomic fluctuations and is a measure of lignin softness. In the simulations, MSD is calculated over a broad time-range. The nano-second timescale, indicated by an arrow, is directly compatible with the experimentally obtained QENS spectra.



**Figure 2:** Experimentally measured QENS signal at -20 °C for dry and solvated lignin. The broadening of the signal indicates mobility and is a measure of lignin softness.

## References

1. Derya Vural, Catalin Gainaru, Hugh O'Neill, Yunqiao Pu, Micholas Dean Smith, Sai Venkatesh Pingali, Eugene Mamontov, Brian H. Davison, Alexei P. Sokolov, Arthur J Ragauskas, Jeremy C. Smith, Loukas Petridis *Green Chem. R. Soc. Chem.* **2018** *20*, 1602-1611
2. Xianzhi Meng, Aakash Parikh, Bhogeswararao Seemala, Rajeev Kumar, Yunqiao Pu, Phillip Christopher, Charles E. Wyman, Charles M. Cai, and Arthur J. Ragauskas *ACS Sustainable Chemistry & Engineering* **2018** *6* (7), 8711-8718

*Oak Ridge National Laboratory is managed by UT-Battelle, LLC for the U.S. Department of Energy under contract no. DE-AC05-00OR22725. This program is supported by the Office of Biological and Environmental Research in the DOE Office of Science.*