208. Simulation and Structure of Cel7A during Binding and Hydrolysis of Cellulose

Sai Venkatesh Pingali,[1] Junhong He,[1] Hugh M. O'Neill,[1] Volker S. Urban,[1] Loukas Petridis,[1] William T. Heller,[1] Marcus Foston,[2] Arthur Ragauskas,[2] Barbara R. Evans,[1] Jeremy C. Smith,[1] Paul Langan[1*] (langanp@ornl.gov), and **Brian H. Davison[1] (PI)**

[1]Oak Ridge National Laboratory, Oak Ridge, Tennessee; [2]Institute of Paper Science and Technology, Georgia Institute of Technology, Atlanta, Georgia

Project Goals: Lignocellulosic biomass comprises the vast majority of biomass on Earth and has the potential to play a major role in generation of renewable biofuels if cost- effective conversion can be achieved. Largely composed of plant cell walls, it is a complex biological composite material that is recalcitrant to the structural deconstruction and enzymatic hydrolysis into sugars that is necessary for fermentation to bioethanol. The Scientific Focus Area in Biofuels is developing "Dynamic Visualization of Lignocellulose Degradation by Integration of Neutron Scattering Imaging and Computer Simulation" for multiple-length scale, real-time imaging of biomass during pretreatment and enzymatic hydrolysis. This is providing fundamental information about the structure and deconstruction of plant cell walls that is needed to drive improvements in the conversion of renewable lignocellulosic biomass to biofuels.

Investigation into the mode of action of cellulases on the surface of cellulose is of importance for understanding and optimizing the production of cellulo-oligosaccharides for biofuel production. A significant technical challenge has been the inability to probe the structure of the cellulose- cellulase system at a molecular level while the enzyme is digesting cellulose. Neutron scattering has the potential to overcome this challenge because of its unique ability to obtain scattering contrast between cellulose and enzyme by substituting hydrogen atoms of cellulose by deuterium. We have probed the structure of *Trichoderma reesei* Cel7A, a processive exocellulase enzyme, while it binds to and digests partially deuterated cellulose. The results provide insights into the pH dependent structural properties of cellulases when bound to the cellulose substrate.

Complementary molecular dynamics simulations validate the scattering data which show that *T. reesei* Cel7A assumes a compact conformation during cellulose hydrolysis (pH 4 to 5) and a more extended conformation when it has lower catalytic activity (pH 7). This observation lends support to a 'caterpillar mechanism' for Cel7A's action on cellulose which predicts that the energy required for processive action of Cel7A on cellulose is provided by stretching and compression of the linker region allowing the enzyme to move along a cellulose chain. This study provides important experimental insights into the mode-of-action of cellulases on cellulose surfaces.

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.