Title: LigCHIP Technology for Bond-Specific Analysis of Lignocellulose Deconstructing Enzymes

Authors: Jenny R. Onley[1,2]* (jronley@lbl.gov), Kai Deng[1,2], Noel S. Ha[1], Markus de Raad[1], Le Thanh Mai Pham[1,2], Kenneth L. Sale[1,2], Paul D. Adams[1], Trent R. Northen[1], **Jay D. Keasling**[1]

Institutions: [1]Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, Emeryville, CA, USA; [2]Sandia National Laboratories, Livermore, California, USA

Website URL: https://www.jbei.org/

Project Goals: Establish the scientific knowledge and new technologies to transform the maximum amount of carbon available in bioenergy crops into biofuels and bioproducts.

Abstract Text: Lignocellulosic biomass has great potential to provide a sustainable source of fuel, and enzymatic deconstruction of lignocellulose is a key component of biofuel production. Lignocellulose is composed of cellulose, hemicellulose, and lignin. While deconstruction of cellulose and hemicellulose has improved greatly in recent years, further optimization is required to make the process efficient enough for biofuels to compete in the energy market. Lignin is composed of a variety of subunits linked together with a variety of bond types. We have a limited understanding of the enzymatic mechanisms for the breakage of different lignin bonds. Lignin is highly recalcitrant, and breakdown produces a complex, heterogenous mixture of products. Further understanding of the activities of enzymes that degrade cellulose, hemicellulose, and especially lignin is required to lower biofuel production costs and increase efficiency. We utilized the unique mass spectrometry assay (nanostructure-initiated mass spectrometry or "NIMS")[1]. Briefly this platform uses lignin model compounds containing target lignin bonds enabling bond-specific kinetic analysis of enzyme activities. In addition to the lignin linkages, these substrates have perfluorinated and cationic moieties enabling rapid separation from complex mixtures and efficient ionization from small samples. Each substrate has a unique mass enabling them to be analyzed from mixtures. Quantification is achieved using ^{13}C labeled internal standards. NIMS can be scaled up when it is combined with mass spectrometry imaging (MSI) and open-source MSI software[2], providing rapid measurements of multiple samples. Additionally, coupling mass spectrometry methods with microfluidics further increases the high throughput power of these technologies.[3] We constructed a microfluidics device for combinatorial screening of enzyme activities though droplet microfluidics.[4] A commercial droplet generator is used to prepare cocktails of droplets containing different enzymes and substrates. Our system spontaneously merges random pairs of droplets in parallel, replacing deterministic liquid handling operations and providing the potential for testing many more combinations of enzymes and substrates. The microfluidics device was coupled with NIMS, depositing the reaction products onto the NIMS chip for MSI analysis. We demonstrated the use of these technologies with model lignocellulose compounds that represent common linkages in lignin (β-O-4', β-β', and 5-5'), cellulose, and hemicellulose. In the past we observed

cleavage of the β-O-4’ bond, testing lignolytic enzymes alone and in combination with glycoside hydrolases. Through collaboration with the Deconstruction Division at JBEI, we presented detailed studies for the degradation of a phenolic β-O-4 dimeric model compound by lignin peroxidase isozyme H8 as a function of pH.[5] Here we used our microfluidics device to gather performance data for an array of lignocellulolytic enzymes. Interestingly we demonstrated the oxidative cleavage of β-β’-linked lignin by fungal laccases, a phenomenon that is poorly characterized in the literature. Further work with the LigCHIP (our unique technological combination of NIMS, MSI, droplet microfluidics, and lignin substrates) will include scaling up and expanding these technologies to a greater number of enzymes and substrates.

References/Publications

1. Deng, K. *et al.* Rapid characterization of the activities of lignin-modifying enzymes based on nanostructure-initiator mass spectrometry (NIMS). *Biotechnol. Biofuels* **11**, 1–9 (2018).

2. De Raad, M. *et al.* OpenMSI Arrayed Analysis Toolkit: Analyzing Spatially Defined Samples Using Mass Spectrometry Imaging. *Anal. Chem.* **89**, 5818–5823 (2017).

3. Ha, N. S. *et al.* Faster, better, and cheaper: harnessing microfluidics and mass spectrometry for biotechnology. *RSC Chem. Biol.* **2**, 1331–1351 (2021).

4. Ha, N. S., de Raad, M. & Northen, T. R. Picoliter droplet sample processing and deposition for mass spectrometry. 1–21 (2021). Patent 17/062,609.

5. Pham, L. T. M. *et al.* Experimental and theoretical insights into the effects of pH on catalysis of bond-cleavage by the lignin peroxidase isozyme H8 from *Phanerochaete chrysosporium*. *Biotechnol. Biofuels* **14**, 1–11 (2021).

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