

## High-Throughput Screening of Lignocellulosic Biomass Degrading Enzymes Utilizing Mass Spectrometry

Nicole Ing<sup>1,2</sup>, Noel Ha<sup>1,3</sup> \* ([noelha@lbl.gov](mailto:noelha@lbl.gov)), Kai Deng<sup>1,2</sup>, , Markus de Raad<sup>1,3</sup>, Fangchao Song<sup>3</sup>, Jose Henrique Pereira<sup>1,3</sup>, Martina Aulitto<sup>1,3</sup>, Yan Chen<sup>1,3</sup>, Jennifer Gin<sup>1,3</sup>, Carolina, Barcelos<sup>1,2</sup>, Kenneth Sale<sup>1,2</sup>, Blake Simmons<sup>1,3</sup> Chris Petzold<sup>1,3</sup> Steve Singer<sup>1,3</sup>, Paul Adams<sup>1,3</sup>, Anup Singh<sup>1,2</sup>, and **Trent Northen**<sup>1,3</sup>

<sup>1</sup>Joint BioEnergy Institute, Technology Division, Emeryville, CA <sup>2</sup>Sandia National Laboratories, Livermore, CA <sup>3</sup>Lawrence Berkeley National Laboratory, Berkeley, CA

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**Project Goal: Establish the scientific knowledge and new technologies to transform the maximum amount of carbon available in bioenergy crops into biofuels and bioproducts. This work aims to develop an analytical platform for high-throughput analysis of lignocellulosic biomass deconstruction reactions. This platform combines surface-based mass spectrometry with bioconjugation chemistry to screen enzymatic activity toward lignin, cellulose, and hemicellulose degradation.**

Lignocellulosic biomass is the most abundant raw material on the planet and is composed primarily of the cellulose, hemicellulose and lignin. Understanding how to deconstruct this complex material is critical to achieving a cost-competitive replacement for petrochemicals. Enzymes and microbes are capable of degrading this biomass in nature; however, the species, mechanisms, and pathways involved in deconstructing this material are vast and complex. To this end, we are developing highly sensitive nanostructure-initiator mass spectrometry (NIMS)-based assays to screen for novel enzyme activity and gain mechanistic insight into biomass degradation to support our efforts to develop optimal enzyme cocktails at the Joint BioEnergy Institute (JBEI).

Here we describe the development of a library of NIMS substrates representing major lignin linkages, the development of a multiplexed assay to screen for both ligninase and cellulase activity, and the development of a high throughput microfluidic device integrating droplet mixing with NIMS analysis. We have now extended our perfluorinated tagging technique targets for analysis of hemicellulose and cellulases to characterize lignin-modifying enzymes and synthesized a variety of lignin model probes representing the most common lignin linkages. Importantly, unlike existing colorimetric assays, this NIMS-based lignin assay provides information about specific bond-cleavage reactions.

We have combined our lignin and glycan probes to create a multiplexed assay to screen for enzyme activities on mixed. The vast combinations of lignin modifying enzymes and glycoside hydrolases necessitates increasing the throughput and reducing the volumes of our current assays. Hence, we have developed a droplet-based microfluidic device, which combines droplet sample loading and processing onto a NIMS surface. We have now demonstrated array generation, droplet merging, and NIMS analysis and will next extend this to screen the activities of single and mixed enzymes.

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