Title: A mass spectrometry based high-throughput platform to study lignin modifying enzymes

Kai Deng[1]* (kdeng@lbl.gov), Nicole Ing[1], Le Thanh Mai Pham[1], Carolina A. Barcelos[1], Kenneth L. Sale[1], Paul D. Adams[1], Trent R. Northen[1], **Jay D. Keasling**[1]

[1] Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, Emeryville, CA, USA

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Project Goals: Short statement of goals.

JBEI's mission is to establish the scientific knowledge and new technologies in feedstock development, deconstruction and separation, and conversion needed to transform the maximum amount of carbon available in bioenergy crops into biofuels and bioproducts.

Abstract:

Lignocellulosic biomass has great potential to create sustainable drop-in replacements for conventional petroleum-based fuels. While significant advances have been made in utilizing the cellulose and hemicellulose components of the lignocellulosic biomass, efficient lignin depolymerization remains an enigmatic challenge. Overcoming this challenge to valorize lignin has the potential to dramatically lower the overall cost of biofuel production. However, lignin's complex and variable structure, coupled with our very limited understanding of enzymes that depolymerize lignin, make this a difficult challenge to overcome. As an important first step to rapidly accelerate our understanding of lignin active enzymes we have developed a high throughput analytical assay to assess the bond-specific activities of lignin modifying enzymes/or enzyme cocktails. This has been done by extending the nanostructure-initiator mass spectrometry (NIMS) assays we have developed to study various GH enzymes using model glycan substrates. Recently, we have shown that β-O-4 phenolic and non-phenolic dimeric model compounds are suitable for detailed analysis of laccase enzyme mixtures and peroxidases (e.g. lignin peroxidases,versatile peroxidases, and horse radish peroxidases). Building on these successes we have now synthesizing five other model compounds representing important lignin linkages (β-β, 4-O-5, β-5, 5-5', dibenzodioxocin). Together this collection of lignin dimeric model compounds covers all major lignin linkages. They can be easily integrated into our mass spectrometry-based high throughput assay to study the specificity and activity of various lignin modifying enzymes. These substrates can also be combined with our existing platform of glycan assays to create a high throughput screen for overall lignocellulose decomposition. The information obtained from our assays will help us to develop high performance enzyme cocktails to efficiently break down biomass for bioenergy production.

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