Rapid Determination of Enzyme Activities for Lignocellulose Deconstruction and Analysis of Biofuel Molecules Using Nanostructure-Initiator Mass Spectrometry

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Project Goal:
The JBEI mission is to find a viable way to convert lignocellulosic biomass into next generation transportation biofuels.

Abstract:

Our goal is to develop a platform to rapidly characterize the activity of enzymes responsible for the deconstruction of cellulose, hemicellulose and lignin and to screen alcohol and ketone biofuel production. Lignocellulosic biomass is composed of carbohydrate polymers (cellulose, hemicellulose) and an aromatic polymer (lignin). The complexity of the biomass structure requires cost effective enzyme cocktails for its deconstruction. In addition, a robust method to screen biofuel-producing strains for desired products is needed to support development and optimization of strains with high titre productivity. In order to meet these crucial challenges, we are developing mass spectrometry based assays with high-throughput, small sample volume, good sensitivity and importantly, adaptability to automated workstations to facilitate study large enzyme or microbial library strain libraries.

Central to our approach is to use synthetic organic chemistry to prepare chemical probes that enhance nanostructure-initiator mass spectrometry (NIMS) based analysis. This includes model substrates suitable for screening the activities of cellulases, hemicellulases and lignases and a post-reaction products-tagging strategy. Together these enable quantitation of glycan product cascades from biomass deconstruction (to obtain enzyme kinetic parameters so as to help the development of enzyme cocktails), and also methyl ketone and alcohol products (1-butanol, 3-methyl-3-butenol et. al) from biofuel production strains.

For high-throughput analysis of biomass deconstruction, we have standardized a panel of 12 substrates to span the biomass linkages of interest for plant-based biofuel production. To test the value of this standard panel for our high-throughput platform, we characterized the activities of three engineered cellulases CelAcc-CBM3a, CelRcc-

CBM3a, CelEcc-CBM3a and their synergy of combination across a range of reaction conditions and enzyme concentrations. We anticipate that large-scale screening using the standardized platform and substrates will generate critical datasets to enable direct comparison of enzyme activities for cocktail design. Work is underway integrating microfluidics with NIMS and we anticipate this new high-throughput platform will greatly enhance our ability to study biomass deconstruction and biofuel production.

References
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