220. High-Throughput Nanostructure-Initiator Mass Spectrometry (HT-NIMS) Metabolite Screening at JBEI

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Project Goals: The Joint BioEnergy Institute (JBEI) is a United States Department of Energy Bioenergy Research Center dedicated to developing advanced biofuels—liquid fuels derived from the solar energy stored in plant biomass that can replace gasoline, diesel and jet fuels. JBEI's approach to biomass derived biofuels uses a combination of novel feedstock engineering, ionic liquid biomass pretreatment and high performance enzymes to effectively produce low-cost fermentable sugars. In parallel, synthetic biology approaches are being pioneered to enable conversion of these sugars into high-performance drop-in biofuels. In this case, the disconnect between the rate of clone production vs. our ability to measure biofuel yield and composition presents a major technical challenge. Typically, this requires either non-specific assays or radical down selection prior to chemically specific analysis using mass spectrometry. In this project we are pioneering HT-NIMS as a tool to rapidly screen metabolite composition in support of fuels engineering efforts at JBEI.

We have recently developed high throughput enzyme activity analysis capabilities using the novel integration of nanoliter-scale acoustic sample deposition with nanostructure-initiator mass spectrometry (NIMS) analysis platform to rapidly detect and characterize screen large libraries of glucosidases. Central to this approach has been the use of bioconjugate chemistries enabling modification of glycans for high sensitivity and quantitative from complex mixtures. This HT- NIMS technology enables rapid screening of enzyme libraries across relevant process conditions to support the rapid develop enzyme cocktails for a given plant biomass.

Here we describe recent efforts to extend this platform to synthetic biology based fuel engineering efforts at JBEI. In some cases, relevant molecules (e.g. fatty acids) can be detected without modification enabling rapid screening of lipid composition. In other cases, reactive chemical probes are used to improve the mass spectrometry characteristics of target molecules for NIMS analysis. Probes can be added using oxime chemistry and are found to effectively label ketone containing molecules enabling screening of both fatty acid methyl ketones and polyketides. Fatty acid methyl ketones can have high cetane numbers making them highly desirable as diesel fuels. Reduced polyketides provide a largely unexplored approach to producing biofuels and has great potential for producing renewable gasoline and jet fuels.
Overall, HT-NIMS has tremendous potential to enable direct screening that will help optimize the yield and composition for both yield and composition.

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