

Nanostructure-initiator mass spectrometry (NIMS): high throughput enzyme activity assays for biofuel development

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Project Goals:

This project meets an urgent need for a highly specific activity screening approach and offers tremendous potential for the high-throughput identification and optimization of industrial enzymes and enabling application of biological approaches utilizing large libraries.

The efficient deconstruction of lignocellulosic biomass into biofuels represents a critical and formidable challenge. JBEI is addressing this challenge using a multifaceted approach that is highly dependent on enzyme discovery, optimization and synthetic biology. The optimization of deconstruction processes requires technologies for the high throughput screening and identification of glycoside hydrolase activities. The high sensitivity, specificity, and resolution of mass spectrometry make it well suited for the analysis of sugar molecules. However, the low throughput of conventional GC/MS and LC/MS precludes implementation for screening purposes. Here we present a multiplexed approach based on nanostructure-initiator mass spectrometry (NIMS) that allows for the rapid analysis of several glycolytic activities in parallel under diverse assay conditions. By forming colloids, it was possible to perform aqueous reactions in microwell plates despite the substrate analogs' hydrophobic perfluorinated tags. Our assay can be used both for the characterization of known enzymes (pH and temperature profiles, kinetic studies, ionic liquid tolerance), and the identification of yet unknown activities, even from complex biological samples (environmental and enrichment cultures). We are now integrating this assay with acoustic printing resulting in a 100-fold increase in throughput.

This work conducted by the Joint BioEnergy Institute was supported by the Office of Science, Office of Biological and Environmental Research, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.