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High Throughput Mass Spectrometry Based Enzymatic Assays for Biofuels Development

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Mass spectrometry's ability to efficiently generate intact biomolecular ions in the gas phase has led to a wide range of biological applications and is recently being applied for global metabolite profiling ('metabolomics') primarily through liquid chromatography coupled to electrospray mass spectrometry. However the complexity and relatively low throughput of this approach has limited application for high throughput enzymatic assays. To overcome this, we have developed the Nanostructure-Initiator Mass Spectrometry enzymatic (Nimzyme1) assay where enzyme substrates are immobilized on the mass spectrometry surface using fluorophilic phase interactions. This 'soft' immobilization allows efficient desorption/ionization while also allowing surface washing to reduce signal suppression from complex biological samples as a result of the preferential retention of the tagged products and reactants. We have also shown that Nimzyme can detect multiple and competing enzymatic activities and screen for optimal pH, temperature, and enzyme inhibition from crude cell lysates and a hot springs microbial community. This approach is being implemented at the DOE Joint BioEnergy Institute for high throughput functional characterization of both enzyme libraries and environmental samples. Specifically, we are constructing a complete set of glucose polysaccharides (cellobiose to cellihexose) for screening glucohydrolase and glucotransferase activities and a p-coumaryl alcohol substrate for characterization of laccase activity. Together these assays will help to identify and optimize the conversion of lignocellulose into biofuels.

1. Northen Trent R, Lee J-C, Hoang L., Raymond J., Hwang D-R, Yannone S.M., Wong C-H, Siuzdak G. A Nanostructure-Initiator Mass Spectrometry Based Enzyme Activity Assay, PNAS, (2008) 105(10), 3678-3683.

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