Title

High Throughput Mass Spectrometry Based Enzymatic Assays for Biofuels Development

Authors

Northern, Trent R.
Lee, Jinq-Chyi
Petzold, Christopher J.
et al.

Publication Date

2009-12-18

jbei
Joint BioEnergy Institute

High Throughput Mass Spectrometry Based Enzymatic Assays for Biofuels Development

Trent R. Northen*[,], Jinq-Chyi Lee[], Chris Petzold[], Blake Simmons[], Paul Adams[], and Anup Singh[,]Life Sciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, CA 94720, USA. [Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, 5885 Hollis st, Emeryville, CA 94608; [Division of Biotechnology and Pharmaceutical Research, National Health Sciences Institute, Taipei, Taiwan.
* Presenter. Email: trnorthen@lbl.gov

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 though 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 fluorous 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 glucohydralase 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.

Work was done with supported from the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

U.S. DEPARTMENT OF ENERGY | Office of Science

DISCLAIMER