230. Rapid Kinetic Characterization of Glycosyl Hydrolases (GHs) Based on Oxime Derivatization and Nanostructure-Initiator Mass Spectrometry (NIMS)

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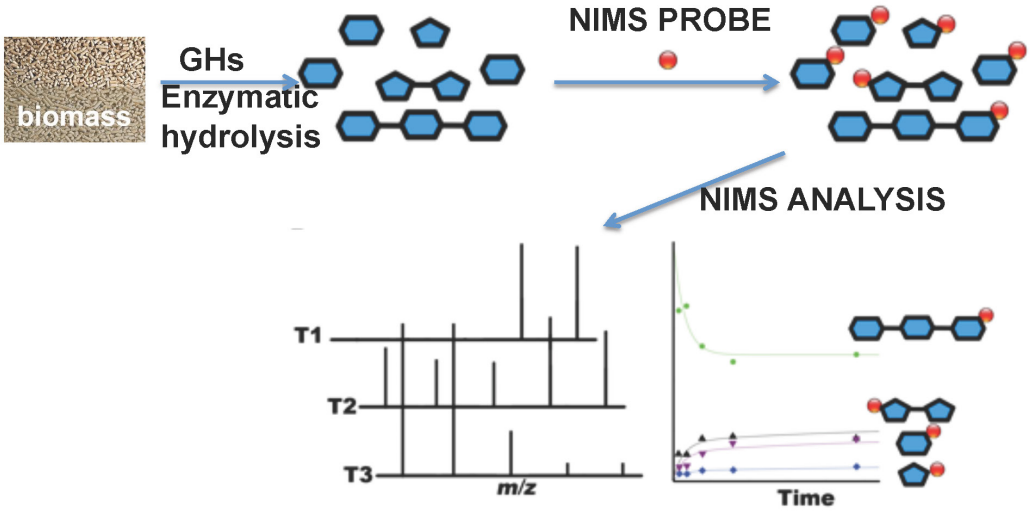
Project Goals: Glycoside hydrolases (GHs) are ubiquitous enzymes in nature and play significant roles in many biological and industrial processes. Unfortunately only a small fraction of the hundreds of thousands of putative GHs have been functionally characterized. Thus methods are urgently needed to rapidly provide direct biochemical evidence. Here we describe an oxime chemistry for nanostructure-initiator mass spectrometry characterization of in situ expressed mono and multifunctional GHs relevant to biomass conversion to biofuels. This approach enabled simultaneous detection of hexose and pentose product cascades from the biomass hydrolysis to understand enzyme kinetics. Overall, this integrated platform of robotic cell-free translation with mass spectrometry analysis can rapidly provide kinetic information for GHs to improve our functional understanding of this important class of enzymes.

Abstract: Glycoside hydrolases (GHs) are critical to cycling of plant biomass in the environment, digestion of complex polysaccharides by the human gut microbiome, and industrial activities such as deployment of cellulosic biofuels. High throughput sequencing methods show tremendous sequence diversity among GHs, yet relatively few examples from the over 150,000 unique domain arrangements containing GHs have been functionally characterized. Here, we show how cell-free expression, bio-conjugate chemistry, and surface-based mass spectrometry allows detection of new glycoside hydrolase specificities and enables kinetic analysis of their activities with plant biomass. Detection of soluble products is achieved by coupling a unique chemical probe to the reducing end of oligosaccharides in a stable oxime linkage, while the use of ^{13}C-labeled monosaccharide standards (xylose and glucose) allows quantitation of the derivatized glycans. We apply this oxime-based nanostructure-initiator mass spectrometry (NIMS) method to characterize the functional diversity of GHs secreted by *Clostridium thermocellum*, a model cellulolytic organism. New reactions are identified for previously unassigned enzymes, and differences in rates and yields of individual enzymes are demonstrated in reactions with biomass substrates. Numerical analyses of time series data suggests that synergistic combinations of mono- and multi-functional GHs can decrease the complexity of enzymes needed for the hydrolysis of plant biomass during the production of biofuels.



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