HRMAC 21T FTICR Mass Spectrometer First Science Projects: Descriptions and Users

A combined top-down and bottom-up glycoproteome analysis of O-glycoform diversity of the secretome of the lignocellulose degrading fungus Neurospora crassa, PI-Christopher Somerville, University of California at Berkeley.

Enzymatic hydrolysis of (ligno)cellulose by cellulases is key for the production of second generation biofuels, which represent a long-standing leading theme in the field of sustainable energy. Filamentous fungi are long-known and powerful cellulase producers. Filamentous fungi, particularly Trichoderma reesei, are the most efficient sources of large amounts of industrial enzymes. However, the enzymes produced by T. reesei and other industrial fungi are not optimal for lignocellulose depolymerization and the enzymes they produce are rapidly inactivated under industrial depolymerization conditions through a variety of mechanisms, including lignin binding. This inefficiency of the enzymatic destruction of (ligno)cellulose is a big hurdle in making biofuels economically viable. Recent research has suggested that glycosylation of cellulases is a possible key factor. Supported by recent molecular dynamics studies, we hypothesize that glycosylation of these enzymes is pivotal in preventing nonproductive enzyme absorption to lignin, which significantly hinders efficient biofuel production from lignocellulose. Unfortunately, little is known about cellulase glycosylation, and the secreted glycoproteome of filamentous fungi is complex. Deciphering how filamentous fungi glycosylate their proteins and also understanding the influence of glycosylation is hence required to advance our understanding of these processes.

We use the well-characterized filamentous fungus Neurospora crassa, which is closely related to T. reesei, to study glycosylation of cellulases using a combined top-down and bottom-up glycoproteomic approach. N. crassa serves as a model system for filamentous fungi and a powerful genetic tool set is available, including a nearly comprehensive set of gene knockouts. The high mass resolution MS available at the EMSL is perfectly suited to elucidate this complex system.

Building the lignin metabolic map for the production of advanced biofuels, PI-Blake Simmons, Sandia National Laboratory.

Lignin represents a tremendous opportunity for the advanced biofuels community, but is currently viewed as one of its biggest challenges with no short-term solution. As lignin is typically 15-28% of the total dry weight of biomass, it represents a significant unutilized source of carbon for conversion to commercial products. However, commercial biofuel producers currently consider it a waste stream that is typically burned to generate power and waste heat, and fail to take advantage of or maintain the unique properties of lignin in terms of chemical bonds and aromatic backbones that could be used to replace consumer demands within the fuels sector currently met by petroleum. For example, lignin represents the only significant source of renewable aromatics on the planet, but is very difficult to convert to any desired end products due to its heterogeneity and chemical bond complexity that vary as a function of plant species and pretreatment processes. The advent of efficient lignin bioconversion is therefore considered one of the most compelling scientific and technical challenges facing the realization of advanced biofuels at any scale, and is one of the clearest current examples of where fundamental discoveries in biology could have an immediate and significant impact in terms of the role
of biofuels as a robust, reliable, and sustainable component in the transportation energy supply of the nation. The current gaps in lignin conversion arise due to an incomplete understanding of how lignin is broken down in nature, coupled with very limited knowledge of metabolic pathways that are capable of converting lignin intermediates (e.g., monoaryl, biaryl, β-aryl ether, biphenyl, diarylpropane, and phenylpropane) into biofuels.

The overall goal of this project is to develop a metabolic map in four targeted organisms using 13C-labeled versions of the lignin-derived model compounds known to be capable of supporting microbial growth by analyzing a time series of growth using these compounds as the sole carbon source. Time courses of 13C uptake and conversion of model lignin-derived compounds will be measured by metabolomics (HRMAC), proteomics (Orbitrap), and transcriptomics (RNASeq) in four microbes known to be capable of growing on non-labeled versions of the same lignin intermediates to build and compare the lignin metabolic map in each. Maps of lignin degradation metabolism for the four microbes will be built using BNICE framework based on the results. These metabolic maps will be the first step in developing a synthetic biology toolbox for the engineering of organisms capable of producing biofuels from lignin.


In tropical systems, changes in climate will likely affect both the amplitude and periodicity of redox oscillations due to predicted increases in warming and precipitation intensity. Our research will measure how shifts in soil oxygen/redox patterns affect the fate of complex soil C substrates. Redox conditions are a major driver of soil trace gas fluxes, microbial community dynamics and carbon stabilization, but are poorly constrained in most soil biogeochemical process models and underestimated in representations of upland soils. In humid and wet tropical soils, low redox events are particularly common, occurring on daily to weekly timescales and driven by high biological oxygen demand, high moisture (limiting diffusion), warm temperatures and abundant labile carbon. These oscillations prime tropical soils for rapid C, Fe, P and N cycling, and regulate mechanisms of both carbon stabilization (mineral sorption) and loss (dissolved organic matter (DOM) leaching). Although the importance of tropical soils in the global C cycle is clear, we have a surprisingly poor understanding of how soil C cycling in these systems with inherently low climatic variability will respond to climate change; this makes predicting future climate impacts extremely difficult. Better forecasting of soil C cycling in wet tropical soils depends on a mechanistic understanding of organic matter-mineral interactions, and more detailed knowledge of the chemical nature of DOM.

Previous research has shown that while a significant amount of carbon is stabilized via association with Fe hydro (oxide) minerals common to tropical soils, Fe also may be responsible for up to 50% of C oxidation in periodically anoxic soils. Fe-OM complexes are highly vulnerable to variable redox effects, and can rapidly solubilize and re-precipitate in response to local Eh conditions. Losses of dissolved organic carbon (DOC) are thought to be larger in tropical soils than temperate soils because of high rainfall, substantial substrate supplies, and rapid decomposition rates. However, the role of DOM in tropical soil C cycling and its contribution to SOM formation are very poorly understood. This is in part because its very chemical nature is so complex and poorly constrained, although microbial metabolites, EPS and necromass likely comprise a significant portion.

In our proposed EMSL research, we will use isotope tracing and molecular characterization of both mineral retained and dissolved soil C following manipulations of soil redox. DOM pools will be characterized with high mass accuracy using high field FT-ICR mass spectrometry, while mineral sorbed organic matter will be measured with a multi-modal imaging approach, including C60-FTICR and NanoSIMS. We hypothesize that shifts in soil O2 availability and Fe (hydr)oxide mineral crystallinity will have a significant effect on microbial C processing, leading to altered degradation of
complex C compounds and mineral stabilization. Our results will directly benefit attempts to reduce uncertainties in model predictions of tropical soil carbon balance.

**Glycosylation isoforms of heterologous fungal cellobiohydrolases (CBH1) determined by "top-down" high resolution/high accuracy mass spectrometry, PI-Jonathan Walton, Michigan State University.**

A critical step in the lignocellulosic biofuels pipeline is the extraction of fermentable sugars from plant biomass. Extracellular fungal enzymes are currently the most effective way of deconstructing biomass, but their efficiency needs to be improved. One of the most important of the cellulose-degrading enzymes is cellobiohydrolase1 (CBH1, Cel7A). The Walton lab is studying the natural variation among CBH1 enzymes across all sequenced genomes with the ultimate goal of improving its enzymatic properties relevant to biomass conversion. We have expressed 24 genes, reflecting the entire evolutionary space of CBH1, in the fungal host, Trichoderma reesei, and are in the process of characterizing their enzymological properties. The goal of this proposal is to take advantage of the advanced mass spectrometric capacity at EMSL to analyze the glycosylation and other posttranslational modifications (PTMs) that the heterologous enzymes undergo in T. reesei. In particular, the proposed research will use top down proteomics to characterize in detail the proteoforms (isoforms) of heterologous CBH1 enzymes. A major ultimate goal is to understand what effects the observed PTMs have on critical enzymatic properties such as specific activity, pH and temperature optima, and cooperativity with other enzymes. The expected results will not only be relevant to the discovery of superior forms of CBH1 but also more generally to a better understanding of the limitations of expressing heterologous proteins in filamentous fungi.

**High-resolution, parallel measurements of wetland organic carbon and microbial community metabolism under changing redox conditions, PI-Kelly Wrighton, The Ohio State University.**

We aim to molecularly characterize the distribution of dissolved organic carbon (DOC) in wetland sediments via Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS), and to link biodegradation of specific DOC components to the presence and activity of specific microbial genomes, genes, and processes. Temperate wetlands represent the largest source of atmospheric methane, which is a more potent greenhouse gas than carbon dioxide. While little is known about how DOC is distributed along freshwater wetland gradients, we have even less is known about the functional degradation of DOC by microbial communities, which directly impacts greenhouse gas emission. Microbial interactions with DOC in temperate wetlands are likely to be quite different from interactions driving better-studied ecosystems such as peatlands and saline sites. By understanding these interactions at a molecular level, we hope to improve the accuracy of atmospheric methane modeling under predicted climate-change scenarios. This proposal leverages existing methods-development collaborations with EMSL staff, the unique accuracy of the FT-ICR MS instrumentation available at EMSL, and the expertise in microbial community genomics and transcriptomics of the co-PIs to molecularly characterize both DOC distribution and microbial community metabolism in controlled, tractable laboratory mesocosms. We aim to disentangle the distinct processes happening at both shallow and deeper subsurface depths in the lab, with the long-term goal of field application in a well-instrumented, well-described model temperate wetland. This work is directly relevant to EMSL and DOE missions.

**The effect of biogenic-anthropogenic interactions on the physical and chemical properties of atmospheric organic aerosols, PI-Sergey Nizkorodov, University of California at Irvine.**

Understanding the effect of biogenic-anthropogenic interactions on the chemical composition and physical properties of organic aerosols (OA) is important for accurate modeling of the effect of OA on climate. We will examine the effect biogenic-anthropogenic interactions on the chemical composition and physical properties of particles collected during 'Green Ocean Amazon' (GoAmazon) field campaign sponsored by DOE-BER. To achieve this goal, we will use the unique HRMAC capability developed in EMSL to determine the chemical composition of both low molecular weight and heavier oligomeric
compounds present in ambient particles. The samples will be collected by Dr. A. Laskin in collaboration with Prof. S. Martin, Dr. A. Guenther, and the Brazilian research group of Prof. P. Artaxo during both wet and dry seasons. The HRMAC analysis will be performed with help of tools developed by EMSL scientists Dr. A. Laskin and Dr. J. Laskin. To understand the mechanism of biogenic-anthropogenic transformations in isoprene SOA targeted experiments on laboratory model systems will be carried out by Prof. S. Nizkorodov. The HRMAC analysis of both GOAmazon and lab samples will be combined with characterization of particle physical properties using scanning transmission x-ray spectroscopy (STXM) by Dr. M. Gilles from LBNL, and ice nucleation (IN) activity using environmental scanning electron microscopy (ESEM) by Dr. B. Wang from EMSL and by Prof. D. Knopf. High resolution and accuracy of HRMAC will enable for the first time unambiguous identification of the elemental compositions of oligomeric products of isoprene oxidation and aging in the presence of multiple anthropogenic pollutants. Furthermore, the composition will be correlated to viscosity/surface tension and IN abilities of individual particles. This information will facilitate our understanding of the effect of biogenic-anthropogenic interactions on the climate-related physical properties of SOA.