THE 3RD ANNUAL

Ohio Mass Spectrometry and Metabolomics Symposium

Abstracts

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Contents

Invited Abstracts

In alphabetical order

Mass spectrometry imaging applications to support clinical decision making

Nathalie Y.R. Agar, PhD
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Mass spectrometry provides multiple options for the direct characterization of tissue to support surgical decision-making, and provides significant insight in the development of drugs targeting tumors of the central nervous system (CNS). Using an array of mass spectrometry (MS) applications, we rapidly analyze specific tumor markers ranging from small metabolites to proteins from surgical tissue for rapid diagnosis and surgical guidance. Using similar clinical protocols, we visualize drug and metabolites penetration in brain tumor tissue and correlate with tumor heterogeneity and response to support drug development.

Connecting the world's mass spectrometry data to understand the chemistry of life – a Big Data strategy

Pieter Dorrestein, PhD
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Imagine if we could figure out the chemical composition of any sample and understand their relationships of the molecules to all other samples in seconds – akin to a Google search but instead of using a text we use mass spectrometry information. This is now beginning to be possible. The foundation for such infrastructure is being created through community wide knowledge capture and analysis tool development. In this lecture I will discuss the steps we have taken with building such community tools. This infrastructure is now used by 78,000 people worldwide. I will showcase how world-wide users are applying these tools in understanding the role of the microbiome in health and disease via reuse of public data, including non-clinical data. We have processed 100,000s of datasets and from this I will show representative examples associated with ecological questions, early therapeutic discovery, disease, sleep deprivation, personal care, malnutrition but also several single patient case studies, including a single patient phage therapy treatment but also how useful it is to enable rapid turn around of the analysis in a clinical setting.

Harnessing metabolomics to combat infectious diseases

Ian Lewis, PhD
Dept. of Biological Sciences, University of Calgary

The global rise in the prevalence of antibiotic resistant organisms is an imminent threat to global health and is projected to lower North American life expectancies by over a decade. Diagnostic technology could play a pivotal role in fighting antimicrobial resistance by enabling clinicians to make timely, evidence-based, decisions regarding the type antibiotics to prescribe to each patient. Unfortunately, it currently takes 2-5 days to identify microbial pathogens and measure their susceptibility to antimicrobials – a timeline that directly contributes to preventable deaths. To address this, the Lewis Research Group has recently developed a suite of metabolomics-based

tools that can identify bloodstream and urinary tract infections in a fraction of the time required by more traditional approaches. The key to this transformative technology is a new metabolomics strategy that tracks the metabolic preferences of microbes. The presentation will discuss this new strategy and the potential role metabolomics could play in shaping global health.

The exposome meets precision nutrition

Susan Sumner, PhD
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The use of metabolomics in precision medicine often involves the development of biomarkers to detect, stage, and monitor disease, as well as to determine individuals' responses to drug treatments. Similarly, in the field of precision nutrition, individuals' have different nutrient requirements, and individuals' respond differently to nutrient intake. Lifestyle factors (e.g., stress, smoking, physical activity), use of medications or illicit drugs, dietary intake, and exposure to environmentally relevant chemicals are all part of our Exposome, and can lead to perturbations in endogenous metabolism to create new endogenous Exposomes. Genetics, polymorphisms, and metabolic individuality all contribute to the adverse or positive responses of an individual to the exposures they encounter. The intersection between genomics, metabolomics, and the environment (e.g., foods, nutrients, chemicals, drugs, lifestyle factors) are important to inform the development of nutritional intervention at the individual level. This section will cover examples of the challenges of Exposome Research to inform Precision Nutrition [U2CES026544; 1U24DK097193].

Lunch and Learn Abstracts

In alphabetical order

Spatial visualization of metabolite and lipid markers using MS imaging

Anthony Midey, PhD
Biomedical Research Group, Waters Corporation

Mass spectrometry imaging (MSI) extends the discovery process of identifying key markers in lipidomics and metabolomics to include spatial distribution information, increasing the understanding of how different mechanisms proceed. Understanding where metabolites are located and how their distributions evolve offers keen insights into disease pathology, drug targets, drug treatments, and other processes. Desorption electrospray ionization (DESI) for source sampling and ionization provides a label-free method for MSI under ambient conditions with no sample preparation for direct analysis. Adding ion mobility complementary structure/shape pre-separation prior to MS analysis in MSI resolves isobaric mass peaks, isomers, and extends peak capacity. An overview of how DESI, ion mobility, and mass spectrometry imaging work and what tools are available will provide the basis for a review of applications in lipidomics, metabolomics, and drug metabolism and kinetics (DMK), including direct monitoring of metabolism in cell cultures.

Integrated metabolomics analyses to uncover metabolic vulnerabilities in cancer

Shawn McGuirk[1], Yibo Xue[1], Mathieu Vernier[1], Kaiqiong Zhao[3,4], Geneviève Morin[1], Celia MT Greenwood[3,4,5,6], Vincent Giguère[1], Sidong Huang[1], Julie St-Pierre[1,2]

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Development of chemotherapy resistance is a critical barrier in cancer treatment. Increased reliance on mitochondrial metabolism has been described as a distinctive characteristic of resistant cancers, however it is unknown whether enhanced oxidative metabolism is an intrinsic property or whether the metabolic signature of resistant cancers is dependent on the therapeutics. Here we show that two anthracyclines, doxorubicin and epirubicin, elicit distinct primary metabolic vulnerabilities in human breast cancer cells. Doxorubicin-resistant cells rely on glutamine to drive oxidative phosphorylation and *de novo* glutathione synthesis, while epirubicin-resistant cells display markedly increased bioenergetic capacity and mitochondrial ATP production. Targeting the global metabolic regulator PGC-1α abrogated growth and survival of doxorubicin- and epirubicin-resistant cells, as it controls both drug-specific metabolic vulnerabilities. Overall, our work reveals that targeting global regulators of metabolism like PGC-1α may provide an effective strategy to overcome resistance to multiple therapeutic agents.

Using metabolomics in freshwater mussel conservation

Ieva Roznere, PhD

Department of Evolution, Ecology, and Organismal Biology, The Ohio State University

Freshwater mussels are the most endangered group of animals in North America. As numbers of freshwater mussels continue to decline, conservation efforts are more critical than ever in order to protect existing populations. Freshwater mussels are often translocated between habitats or into captivity for purposes such as propagation, basic research, or as temporary refugia. Although translocation is an important tool that is increasingly used in wildlife conservation and management, it often results in increased mortality and reduced growth. Despite the necessity, there is limited knowledge of mussel health and the effects of environmental stressors on mussel physiology. I leverage the power of metabolomics to better understand the physiology of these animals and how they respond to environmental stress, such as translocation. Adult *Amblema plicata* were translocated from the Muskingum River to the Columbus Zoo and Aquarium Freshwater Mussel Conservation and Research Center and to Big Darby Creek. Hemolymph samples were taken from wild and translocated mussels every few months for one year. Samples were analyzed by Metabolon, Inc. using GS-MS and LC-MS and approximately 100 metabolites were identified. Glucose and lipid metabolism remained similar among groups but differences were observed in altered amino acid and nucleotide metabolism. The results are indicative of a general stress response, which is evident after a year post-translocation. The mussels in our research center were also subjected to a separate food limitation study. Because metabolite production is closely

associated with environmental conditions, studying changes in these biological molecules is especially helpful in understanding how animals react to environmental stressors. In this presentation I will discuss the insights gained from using metabolomics to study freshwater mussel physiology and how this research helps to improve ongoing and future conservation efforts.

Novel approaches to quantitative metabolomics

Baljit Ubhi, PhD
Global Metabolomics & Lipidomics Applications, SCIEX

The major challenge in the field of metabolomics is to accurately identify and quantify hundreds of metabolites within a single analytical run. The ability to measure biologically relevant metabolites and quantitate them in complex matrices has always been a major objective in any Omics lab. Therefore the flexibility of an analytical platform to offer the flexibility of quantitative and qualitative workflows is deemed critical. These workflows can be delivered these objectives. Recently variable window SWATH acquisition has shown to identify a higher number of metabolites compared to the traditional Data Dependent Acquisition (DDA) approach, thus enabling broader metabolome coverage. Using MS/MS fragments for metabolite quantitation provides better selectivity, and ultimately increased sensitivity. Variable window SWATH Acquisition provides quality quantitative data for metabolites in complex matrix. Targeted workflows on QTRAP technology have shown increased metabolite coverage (by upto 50%) by utilizing low flow rates, through the M5 microflow platform. Quantitation of large panels of metabolites (n=310) and lipid molecular species (n=1500) is possible given the speed of the QTRAP platform in a single analytical run. Given the overlap of MRMs in the small molecule space, the TRAP allows a user to run added confirmatory experiments for qualitative data review through library matching workflows. All of these workflows will be demonstrated during this presentation.

OSU Core Facility Abstracts

In alphabetical order

The OSU CCIC Mass Spectrometry and Proteomics Facility

Árpád Somogyi[1], Liwen Zhang[1], Andrew Reed[1], Florian Busch[1,4], Nan Kleinholz[1], Sophie Harvey[1,4], Matt Bernier[1], Michael Freitas[2], Vicki H. Wysocki[3,4]
[1]OSU CCIC MSP Facility, [2] SBS-Cancer Biology and Genetics, OSU, [3] Department of Chemistry and Biochemistry, OSU, [4]Resource for Native MS-Guided Structural Biology, OSU

The Mass Spectrometry and Proteomics Facility (MSP) is part of OSU's Campus Chemical Instrument Center (CCIC) and serves a wide variety of research groups from OSU, other universities and industry nationwide. MSP provides considerable expertise in bottom up and top down proteomics, quantitative proteomics analysis, untargeted (qualitative) and targeted (quantitative) metabolomics analyses (including lipid analyses), and the analysis of complex organic and inorganic matter and synthetic polymers. The MSP houses state-of-the-art mass spectrometry instrumentation to support research needs of all investigators at the State of Ohio Consortium. Modern high performance instruments that are house in the core include: i) a Thermo Orbitrap Fusion tandem HPLC MS/ MS system, ii) a Thermo Quantiva QQQ HPLC MS/MS, iii) a Bruker 15 T

SolariX XR FT-ICR ultrahigh resolution instrument, iv) a Thermo QE Plus HPLC MS/MS system, and v) an Agilent 4650 HPLC MS Q-TOF, and a vi) Bruker timsTOF Pro nanospray ion mobility MS/MS system. Representative examples for collaborative projects and services will be shown in the presentation.

Shared CCIC-NMR Facility at The Ohio State University

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Campus Chemical Instrument Center, NMR Facility, The Ohio State University, Columbus, OH

The shared NMR facility at the Ohio State University (OSU) houses 9 state-of-the-art high-field NMRs from Bruker Biospin with 5 instruments at 800 MHz and above. The Campus Chemical Instrument Center (CCIC) NMR facility has unique capabilities in solution NMR, with 5 instruments equipped with cryoprobes (including TCI, TXO, and QCI probes) and automated, temperature-controlled sample changers (SampleJet and SampleCase), fast-MAS solid-state NMR, Dynamic Nuclear Polarization (DNP), and micro-imaging. CCIC NMR also develops the public suite of web servers (COLMAR) for the automated analysis of multidimensional NMR spectra for the accurate identification and quantification of metabolites in complex biological mixtures. CCIC NMR instruments and staff support a wide range of research interests at OSU, in the state of Ohio, and beyond. Here we present an overview of current capabilities highlighting several ongoing projects.

Resource for Native Mass Spectrometry Guided Structural Biology

Árpád Somogyi[1], Sophie Harvey[1,3], Florian Busch[1,3], Dalton Snyder[3], Vicki H. Wysocki[1,2,3]

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The Resource for Native Mass Spectrometry Guided Structural Biology (nMS➔SB) was established in 2018 with a 6.8M P41 grant from the NIH National Institute of General Medical Sciences. As a national Biomedical Technology Research Resource, our mission is to develop improved native MS as a routine tool and disseminate the technology to the biomedical research community through vendor partnerships and training. We are building an integrated MS-based workflow for intact, native complexes, i.e. "complex-down" characterization, with state-of-the-art instrumentation and software that can answer structural biology questions. An integrated workflow for native MS will define the m/z of the intact complex, m/z of subcomplexes, stoichiometry, heterogeneity, connectivity of partners, topology, conformational diversity, collision cross sections, and relative subunit binding strengths of intact, native protein complexes with high sensitivity and throughput. The Resource works with investigators across the nation and globe on challenging biomedical projects that have both biomedical significance and substantial technical structural characterization challenges. These Driving Biomedical Projects, ranging from viral hemorrhagic fevers and HIV to cataract formation and neurological disorders, are integrated with Technology Research and Development projects and serve as the drivers and testbeds of developing technologies. Collaboration and Service Projects show investigators how developed technologies can begin to answer their questions for structural biology. Technology Research and Development is fostered by collaborations with Software Consultants, Technology Partners, and National Lab Partners.

Nutrient and Phytochemical Analytics Shared Resource (NPASR)

Ken Riedl, PhD, Acting Director
Department of Food Science & Technology; OSU Comprehensive Cancer Center

NPASR brings world class expertise in applying LCMS technologies to measure key bioactives and biomarkers in foods and biologics. We excel at targeted and untargeted metabolomics for biomarker identification, quantification, and metabolite discovery. Metabolomics experiments (targeted and untargeted) provide broad metabolite coverage to aid in identifying new relationships between metabolites and associations with clinical metadata. We have particular experience in targeted and untargeted metabolomics supporting a 'Crops to Clinic' approach in which dietary interventions are used for cancer prevention and provide analytical support for the OSU Discovery Themes - Foods for Health. NPASR service goals: provide investigators with bioanalytical method development and quantitative analyses of nutrients and phytochemicals in foods and their metabolites in biological samples, enhance understanding of the role of dietary compounds in cancer prevention and control, metabolite and biomarker discovery through untargeted LCMS metabolomics techniques, and offer lipidomics delivering >1,000 lipid species over 13 classes with our Lipidyzer platform and smaller focused LCMS panels to recover less abundant classes like eicosanoids.

OARDC Metabolite Analysis Cluster (OMAC)

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The OARDC Metabolite Analysis Cluster (OMAC) is a metabolomics facility located in the Ohio Agricultural Research and Development Center (OARDC), at The Ohio State University. The facility is focused on using the tools of metabolomics to dissect biochemical and physiological responses and metabolic partitioning. One area of expertise is simultaneously using multiple analytical platforms (LC-MS/MS, GC-MS, HPLC, FPLC, TLC, and zonal capillary electrophoresis) to generate "metabolic fingerprints" of compounds and pathways involved in plant cells/tissues/organs, microbial cells or cellular exudates, food and beverage samples, and/or animal tissue or fluid samples. In sample analyses, we focus on using multiple analytical approaches in parallel to allow the breadth of a broad spectrum metabolomic approach, while still retaining the sensitivity and low limit of detection of a targeted metabolomic approach. To enable these assays, we have developed tools and techniques allowing the micro- extraction and quantification of low-volume or low-mass samples. OMAC also assists researchers in the development of procedures for the extraction and analysis of their compounds of interest. Compounds in which the facility specializes include: terpenoids, phytohormones, organic acids, sugars/polysaccharides, lipid signaling molecules and membrane components, plant secondary metabolites, neurotransmitters, nucleotides. protein purification and enzyme characterization, plant secondary/specialized metabolism. OMAC also provides services in the areas of metabolic engineering of plant, microbial, and cellular systems; detection of herbicide and pesticides; protein purification and enzymatic assay development; kinase/phosphatase assay development; and the biochemical dissection of cellular signaling and organismal stress responses.

Oral Abstracts

In alphabetical order

Identification of consumer liking markers in whole wheat bread using flavoromics

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Despite the numerous health benefits attributed to whole grain intake, consumers fail to meet the recommended amount of whole grains, mainly due to associated negative sensory properties. The overall objective of this study was to apply an untargeted chemical profiling approach (flavoromics) to identify flavor active compounds that drive consumer acceptance of whole wheat bread. A total of 30 bread samples, made with fresh and aged whole wheat flours, were subjected to UPLC-ToF-MS chemical profiling and consumer acceptance testing. Multivariate data analyses were then performed to correlate non-volatile chemical features with liking scores to select chemical markers driving consumer acceptance of whole wheat bread. Two OPLS models were generated to model consumer liking against chemical profiles of fresh samples and aged samples, separately. All OPLS models showed good fit ($R^2Y > 0.97$) and strong predictive power ($Q^2 > 0.94$). Six putative markers driving consumer liking of whole wheat bread that highly correlated to liking were subsequently isolated by multi-dimensional preparative LC/MS and further identified by MS and NMR (consisted of lipid oxidation products). The sensory relevance of the chemical markers will be established by recombination testing. This work provides a basis to improve the consumer liking of whole wheat bread to promote consumption and health impact.

Dietary inulin decreases circulating ceramides by suppression of neutral sphingomyelinase expression and activity

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Elevated circulating levels of ceramides are associated with increased risk of cardiometabolic diseases. Although the mechanisms involved are likely complex these observations suggest that lowering circulating ceramide levels be protective against these diseases. Dietary fiber such as inulin have been reported to promote cardiovascular and metabolic health. However, the mechanisms involved are not well understood. We studied effects of inulin on lipid metabolism in Ldlr deficient atherosclerosis mouse model using lipidomics and transcriptomics. Plasma and tissues were collected at 10 days and/or 12 weeks after feeding an atherogenic diet supplemented with inulin or cellulose (control). Compared with controls, Inulin-fed mice displayed 39-51% lower levels of plasma ceramides which were associated with VLDL and LDL. Liver transcriptomic analysis revealed that Smpd3, a gene that codes for neutral sphingomyelinase (NSMase), was downregulated by 2-fold in inulin-fed mice. Hepatic NSMase activity

was 3-fold lower in inulin-fed mice than controls. Furthermore, liver redox status and compositions of phosphatidylserine and free fatty acid species, the major factors that determine NSMase activity were also modified by inulin. Taken together, inulin can decrease plasma ceramide levels through reductions in neutral sphingomyelinase expression/activity, suggesting a mechanism by which fiber could reduce cardiometabolic disease risk.

Application of omics and native mass spectrometry approaches to understand *Salmonella* pathogenesis

Angela Di Capua[1,2], Jikang Wu[1], Mikayla Borton[3], Anindita Sengupata[1], Anice Sabag-Daigle[4], Brian Ahmer[4], Venkat Gopalan[1], Kelly Wrighton[3,5], and Vicki Wysocki[1,2]

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Salmonella is a foodborne pathogen that causes illness and death each year. There are no drugs that specifically target *Salmonella*, and the use of broad-spectrum antibiotics is disfavored as they prolong shedding and cause antibiotic resistance. We are using untargeted metabolomics and proteomics to characterize *Salmonella* infection in mice to identify *Salmonella*-specific processes that could be targeted therapeutically. One exemplar in this regard is the pathway for utilization of fructose-asparagine (F-Asn). Inhibition of the *Salmonella* FraB deglycase, which is essential for F-Asn catabolism, causes accumulation of a metabolic intermediate that in turn retards growth of the organism *in vitro* and *in vivo*. Thus, FraB is a potential target to inhibit *Salmonella*.We are using specific applications of mass spectrometry (MS) to better understand the *Salmonella* infection. The FraB deglycase is believed to employ an initial isomerization followed by hydrolysis of a Schiff base to convert 6-phosphofructose-asparate to glucose-6-phosphate and aspartate. When we tested the activity of FraB at different pH values ranging from 4.7 to 9.8, we observed a bell-shaped pH-rate profile with optimal activity at pH ~8. Because FraB is characterised by two active sites/homodimer we used native MS to investigate if pH affects homodimer formation and the protein conformation, as well as the binding interaction with the 6-P-F-Asp. In our omics studies, we demonstrated how *Salmonella* infection affects the proliferation of specific members of the murine microbiota. Short chain fatty acids are often implicated as regulators of intestinal inflammation. We also reported that the cumulative concentrations of acetate, butyrate, and propionate in the highly-inflamed *Salmonella* gut were significantly depleted relative to the non-inflamed control (value below the limit of detection).

Exploring natural variation in tomato steroidal glycoalkaloids: Using small tomatoes to answer big questions

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Tomatoes (*Solanum lycopersicum*) are an important crop for nutritional and health reasons that biosynthesize many phytochemicals including carotenoids, vitamin C, and steroidal glycoalkaloids (tSGAs). Tomatoes uniquely produce tSGAs which provide protection against biotic stress due to their fungicidal and insecticidal properties. Pre-clinical studies suggest that tSGAs may contribute to health benefits observed in mice that consumed tomato-rich diets. However, little is known about the chemical diversity, structure, and concentrations of most tomato tSGAs. We hypothesized that genetic variation would influence the type and concentration of tomato fruit tSGAs. To test this hypothesis, we developed

a panel of 108 genetically diverse tomato accessions including 25 accessions of *Solanum pimpinellifolium* and 32 accessions of *Solanum lycopersicum* var. *cerasiforme*. In addition, we included commercial processing and fresh market germplasm. Our diversity panel was grown in three environments and red ripe fruits were characterized for 22 tSGAs using an ultra-high performance liquid chromatography tandem mass spectrometry method. We found that tSGA concentrations and profiles varied considerably in tomatoes primarily due to genetic background. Wild tomato accessions exhibited higher total tSGA concentrations than processing tomatoes commonly used for human consumption. Data from populations constructed to determine the inheritance of α-tomatine, a prominent tSGA, indicate that high levels are recessive. This observation suggests loss of function; either loss of degradation, modification, or sequestration. Genetic mapping experiments have revealed QTL associated with high levels of α-tomatine in ripe fruits and candidate gene identification is underway. Our data indicate that there is substantial natural variation present in tomato tSGAs that may be exploited to develop new germplasm potentially with improved biotic stress resistance and human health benefits.

A data science perspective on transparent and reproducible analysis of complex omic data

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Big data is a term often used to describe data sets that are too large to be analyzed with commodity computer hardware running end-user software. Until recently, high complexity big data sets from genomics and proteomics were obtained by domain experts and analyzed by dedicated bioinformatics staff. Rapid advances in next-generation sequencing and mass spectrometry have driving down the cost of genomic and proteomic experiments making the acquisition of these complex data sets available to a much broader research community. More and more investigators are seeking answers from next gen sequencing and proteomics in lieu of traditional bench-top molecular biology. The adoption of omics in the lab has been further aided by a fervent community of scientists creating user friendly data analysis software. Investigators now have a dizzying array of options for analyzing short read sequencing or tandem mass spectrometry data. Given these trends in omic science, the call for transparent and reproducible data analysis pipelines is gaining momentum. This talk will explore examples of integrative genomic and proteomic workflows from the perspective a data scientist attempting to address the ongoing challenges for developing transparent and reproducible scientific workflows. A strategy for how data analysis pipelines can be efficiently disseminated and reproduced across research labs will be presented.

Structure-guided targeting of psilocybin gene homolog metabolites: a novel autonomous approach

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The Center for Disease Control has declared antimicrobial resistance a global health crisis. Natural products (NP) and their synthetic derivatives are the primary source of antimicrobials and make-up over 50% of all pharmaceuticals. However, microbes quickly develop resistance to drugs. To combat antimicrobial resistant bacteria and fungi, it is important to continue mining microbes' repertoires of compounds. NP mining efforts often employ untargeted metabolomics and largely focus on specific groups of bacteria and Fungi in the phylum Ascomycota. Yet searching understudied groups of microbes and targeting derivatives of structures with broad activity may elucidate novel NP. Here, we present a genomic-guided tandem mass spectrometry (MSMS) methodology for targeting small molecules. We developed a pipeline to autonomously detect conserved fragments of structurally similar

compounds, cluster peaks from precursor ion scans (PIS) of these fragments, and extract overlapping clusters from multiple PIS to provide a target list with layered support. In parallel, we developed an automated pipeline to cluster high resolution peak data and calculate molecular formulas to support PIS targets. We applied these pipelines for tryptamine-derived compounds produced by homologs of psilocybin genes in *Fibularhizoctonia* sp. (Fibsp), a fungus in the phylum Basidiomycota that is symbiotic with termites. We targeted four compounds from methanol-water extract of Fibsp that appeared on three or more PIS of conserved tryptamine fragments. These targets are supported by molecular formulas derived from their high resolution molecular ions and are currently undergoing isolation and structure elucidation. This methodology is broadly applicable for autonomous structure-guided peak targeting via MSMS.

An ultra-sensitive paper-based diagnostic platform of detecting colorectal cancer via mass spectrometry

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Colorectal cancer (CRC) is ranked as 3RD most malignant cancer. Early detection technique is needed due to no specific symptom that can be found in early stage of CRC. Current CRC detection methods include colonoscopy, which give high accuracy but is associated with high-cost that limits its usage. Stool-based tests have been proposed that are simpler, however low selectivity and sensitivity leave room for improvement. We propose a cost-effective paper-based blood test that utilizes exosomes as more specific biomarkers for CRC diagnosis. We have designed novel pH-sensitive cleavable amine probes to enable ultra-sensitive analysis of the paper-based immunoassay platform via mass spectrometry. The selected biomarker, CRC exosomes, is isolated from HCT 116 via ultracentrifugation and its characterization are performed. Prior to detection, sandwich immunoassay approach is adopted on the paper substrate using anti-A33 and -CD63 antibodies. The presence of CRC exosomes is determined by mass spectrometry (MS) detection of amine probes that are coupled to the detection antibody. The synthesized amine probes have unique characteristics of being cleavable depending on pH and having small mass to enable analysis on portable mass spectrometers. Therefore, after exosome capture via immunoassay in paper, a solvent optimum pH is added to cleave the amine mass tag, which is subsequently detected by paper spray MS. The exosome biomarker will be analyzed from both PBS buffer and serum on the paper substrate. Stability studies will also be performed, and results compared to the traditional enzyme-linked immunoassay platform. The expectation is that the amine-based immunoassay performed on paper could be stored under ambient conditions compared to enzyme-based assays which require cold temperature storage. This approach will open doors in medical healthcare fields to reach under-served population from remote locations.

Investigating the metabolic impact of green tea extract and its major constitutes on gut microbial metabolism in obese mice model

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Green tea extract (GTE) may alleviate obesity by modulating gut microbial metabolism, but direct evidence for its specific bioactive components responsible for these benefits remain limited. We hypothesized that there will be significant differences in the metabolic regulation by GTE, EGCG, and CAT on gut microbial metabolism in an obese mouse model. To test this, we utilized a targeted metabolomics approach to assess shifts in metabolites from feces obtained from male C57BL6/J mice

fed a low-fat diet, a high-fat (HF) diet, or the high-fat diet containing GTE, epigallocatechin gallate (EGCG) or catechin (CAT). After the 8-wk intervention, HF mice had higher ($P<0.05$) body mass, adipose mass, and insulin resistance compared to LF mice. Without affecting dietary energy intake, GTE fully protected against HF-induced increases in these parameters. However, EGCG and CAT partly protected obesity and only EGCG alleviated insulin resistance to that of GTE. PLS-DA of 119 metabolites comparing GTE, CAT, and EGCG groups indicated that the metabolic profiles of these groups were significantly different. 11 metabolites (variance importance in the projection score > 2) explained these metabolic differences. Five metabolic pathways (citrate and glyoxylate cycles, metabolism of dicarboxylate, amino acids, and biotin) were altered between GTE and CAT, and four between GTE and EGCG ($-\log(p)>5$ and pathway impact>0.2). Several phenolic metabolites were also well-associated with CAT or EGCG supplementation, and fecal EGCG and ECG detected from EGCG-treated mice and fecal 3,4-dihydroxybenzoic acid from CAT-treated mice may help to explain lowered insulin resistance and the observed metabolic impacts to the gut microbes.

Untargeted spatial lipidomics of colon carcinoma spheroids using a LC-QTOF platform

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Multicellular tumor spheroids are valuable *in vitro* tumor models which are frequently used to evaluate drug penetration and efficacy. Spheroids can accurately recapitulate many aspects of *in vivo* tumors, such as the chemical microenvironment, that 2D cell cultures fail to achieve. To better characterize the spatial lipidome of spheroids, we subjected colon carcinoma HCT116 spheroids to serial trypsinization to obtain cellular populations from the outer proliferating, middle quiescent, and core layers and compare them with the 2D cell culture. Using Untargeted lipidomics was performed using an ultra-high-performance liquid chromatography quadruple/time-of flight mass spectrometry (UHPLC-QTOF MS) instrument operated in both negative and positive ion mode and utilizing C30 reversed-phase chromatography. Through statistical analyses and normalization by using quality control pooled samples, we obtained lipid profiles that revealed statistically relevant alterations in the levels of fatty acyls (FA), phosphatidylcholines (PC), lysophosphatidylcholines (LPC), phosphatidylethanolamines (PE), ceramides (Cer), sphingomyelin (SM), and triacylglycerols (TAG) between spheroids and 2D cultures. We utilized the MS-DIAL software platform which contains an *in-silico* lipid database to identify lipids at their “molecular lipid” identification level. We found specific alterations between the spheroid layers and 2D cultures suggesting different lipid metabolism occurring in each layer of the spheroid. Additionally, we examined the retention time behavior to improve the identification of lipids including certain TAG species which elute according to their carbon chain length and degree of unsaturation of the acyl chains. This untargeted lipidomics study of tumor spheroids shows similar lipid level alterations from previous studies done in cancer tissues. Using spheroids as an *in vitro* platform, we can improve our understanding of the complex molecular environment of these tumor models and the lipid signatures found can be used to monitor changes during preclinical evaluation of drug therapeutics.

Poster Abstracts

In alphabetical order

(1) A deeper dive into HILIC chromatography

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Analyzing DNA and RNA oligonucleotides has become increasingly important as more is learned about the transcriptome and more oligonucleotide drugs are being produced in an attempt combat various diseases. Traditionally, oligonucleotides are analyzed using ion pairing reverse phase liquid chromatography (IP-RP-LC). IP-RP-LC mobile phase additives that aid the chromatographic retention or gas-phase ionization of oligonucleotides. While this method has been very effective, ion pairing reagents are difficult to work with and eliminate from HPLC systems. Thus, most oligonucleotide liquid chromatography – mass spectrometry (LC-MS) assays require dedicated instruments. Recently, hydrophilic interaction liquid chromatography (HILIC) for oligonucleotide separation during LC-MS analysis has been shown to provide competitive chromatographic and mass spectrometric figures of merit. The focus of this project is to further understand the retention mechanism as well as the limitations of HILIC chromatography for oligonucleotide LC-MS assays. The sample types being investigated include nucleosides, nucleotides, and short oligonucleotide. For these sample types, we are seeking to compare the relative performance and utility of silica-based dihydroxyl (DIOL) functionalized columns and polymer-based (poly-vinyl alcohol) columns. Through these experiments, we expect to gain a clearer understanding of nucleoside, nucleotide and oligonucleotide retention mechanisms in HILIC, which should help advance the LC-MS detection of DNA and RNA oligonucleotides without the use of ion pairing reagents.

(2) Optimization of sample preparation method for proteomics analysis of gut microbiota

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Salmonella infection is one of the common causes of gastroenteritis that could affect and alter the microbial composition of the gastrointestinal (GI) tract.[1] In order to study the effect of *Salmonella* infection on the gut microbiota, we look at the potential of proteomics to provide a flexible, dynamic platform for the identification and quantification of proteins that directly represent cellular biochemistry.[2] However, protein extraction from gut microbiota in fecal samples is challenging, mainly due to the resistant bacterial cell walls. Therefore, protein extraction methods used for proteomics sample preparation could dramatically affect the proteomics results. Currently, there is a lack of consistency in protein extraction methods in published proteomics studies of gut microbiota. To address this issue and ensure appropriate recovery of bacterial proteins, we have optimized cell lysis procedures for application to our proteomics study of the gut microbiome. During our method optimization we first focused on evaluating the effects of different lysis buffers (sodium dodecyl sulfate (SDS)-based, SDS/urea-based, and Tris-HCl/SDS/urea-based lysis buffer) and mechanical disruption methods (probe sonication and advanced bath sonication using Bioruptor®) on the metaproteome characterization of gut microbiota. To further enrich bacteria from fecal samples and facilitate extraction of microbial proteins, a differential centrifugation (DC) method was applied along with the selected lysis conditions.[3] Our results have revealed that the DC method along in combination with the Tris-HCl/SDS/urea lysis buffer and a trap-based filter (S-trap) increases the ratio of identified bacterial proteins to the host

proteins and the total number of identified proteins. Moreover, our optimized method provides a high degree of reproducibility compared to traditional methods for protein extraction during bottom-up proteomics methods.

(3) The effects of doxorubicin-based chemotherapy and omega-3 supplementation on the mouse brain lipidome

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Breast cancer chemotherapy agents negatively affect long-term brain functioning, known as chemotherapy-induced cognitive impairment (CICI), in some breast cancer survivors. We hypothesized that neuroinflammattory damage may change brain structure or alter signaling, resulting in CICI. Our goal was to determine which brain lipids are modified following doxorubicin-based chemotherapy (DOX), and how omega-3 (n3-FA) supplementation may confer protection. Ovariectomized mice received a diet with supplemental EPA+DHA (0% or 2% kcal) for 4 weeks, followed by two injections of either DOX (9 mg/kg) + cyclophosphamide (90 mg/kg), or vehicle. In study 1 animals were sacrificed at 4, 7, and 14 days after the last injection (n = 120, whole brain collected) and in study 2 at 10 days after last injection (n = 40, hippocampus collected). Brain samples from study 1 were analyzed via targeted UHPLC-MS/MS methods to quantify specialized pro-resolving mediators. UHPLC-QTof lipidomics analyses were performed on extracts from study 2 to determine changes in the hippocampal lipidome. Results from study 1 revealed that resolvinD1 was present in all samples, but no significant differences in concentration were observed, regardless of treatment or dietary group. In addition, resolvin D3, protectin D1 and maresin 1 were detected in a small subset of samples. Study 2 results revealed that chemotherapy was positively correlated with concentrations of hippocampal lipids involved in the omega-9 fatty acid synthesis pathway, also shown to be impacted by other cognitive diseases (e.g. Alzheimer's). N-3 FA supplementation maintained levels of plasmalogens, lipids negatively correlated with Alzheimer's disease. No statistically significant treatment*diet effects were observed. Results demonstrate that DOX increases lipids associated with diseases of cognitive decline, and2% EPA+DHA supplementation favorably alters lipids associated with cognitive function. Future investigations will determine if the same biomarkers of chemotherapy and n-3 FA consumption are observed in human breast cancer survivors.

(4) Linking genomics and metabolomics for nutrition-driven germplasm improvement of apples

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Apples are one of the most commonly consumed fruits in America, and "an apple a day keeps the doctor away" is a well-known adage. The commercial and nutritional importance of apples has prompted interest in varietal improvement. A major factor limiting progress in apple improvement is the long juvenile period (5-10 years before fruiting), which delays fruit evaluation for quality traits such as phytochemical composition. Additionally, there is a disparity between the genomic breeding of most

crops and current apple-breeding initiatives. Apple breeders have begun implementing genotype-driven early-seedling selection for some traits, but strategies to enable breeding for specific fruit phytochemicals have yet to be developed. In response, we are developing an integrated genomic-metabolomic platform to better understand gene-metabolite associations in apples. This platform is being built with high-throughput genomic and metabolomic assessment of ~250 unique apple selections. These include progenies from crosses of several Midwest-derived commercial varieties, members of their pedigrees, wild accessions, and additional varieties to capture the diversity of apple germplasm. Genotypic data generated by single nucleotide polymorphism arrays are being analyzed for estimation of resemblance among genotypes. Polar apple extracts are being characterized with untargeted metabolomics via UHPLC-QTOF-ESI-MS and ^{1}H NMR. Raw MS data will be processed in Profinder (Agilent). TopSpin and AMIX (Bruker) will be used for NMR spectral processing and post-processing. Multivariate and univariate analyses of deconvoluted data will be performed to evaluate sample grouping and relative differences in metabolite concentrations between selections. Compounds will be identified with combinations of MS/MS, authentic standards, as well as 1D and 2D NMR. Genomic and metabolomic data will be integrated using a pedigree-based analysis for quantitative trait loci mapping. Determining gene-metabolite relationships in apple will inform breeding and facilitate future marker-assisted selection for improved nutrition or other attributes including flavor and disease resistance etiology.

(5) Metabolome-based genome wide association profiling of innate immunity in rice

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Rice is a staple food grain for about half of the world's population. Rice blast, caused by the fungus *Magnaporthe oryzae*, routinely causes 10-30% yield losses worldwide. Rice blast is most effectively controlled by host resistance, which also eliminates fungicide contamination on rice grains, thereby protecting human health. While the genetic foundation of host immunity is well-studied, the role of specific metabolites in this process is unclear. Here we present a metabolomic study focused on elucidating biochemical pathways responsible for host immunity to rice blast. We metabolomically profiled infected vs. non-infected leaves in a rice variety resistant to rice blast, as well as a cultivar sensitive to this pathogen, and have identified clusters of features linked to rice pathogen responses.

(6) Development and validation of a sensitive LC-MS/MS method for quantifying dynorphin B

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Dynorphins are endogenous opioid peptides that have been implicated as initiators of immune and inflammatory response through upregulation of inflammatory cytokine and chemokine production, as well having a role in glutamate-induced neuro-inflammation and neurotoxicity. Being extremely potent peptides, the physiologic concentrations of dynorphins are very low ranging from 0.16 pg/mL during the absence of a stimulus to 23.5 pg/mL when stimulated in disease condition. Previously published HPLC-mass spectrometry techniques have insufficient detection capabilities for quantification and detection of dynorphins. As a result, immunoassay quantification has been the most utilized technique

for analysis of dynorphins in physiologic samples. A sensitive LC-MS/MS technique has been developed and validated in the present work which can quantify dynorphin B by mass spectrometry below their low physiologic concentrations in mouse serum. To achieve this level of sensitivity, the intact peptide was digested using a novel metalloendopeptidase called Lys-N. This digestion process produced fragments which are extremely sensitive to detection by mass spectrometer and very specific to dynorphin B. Sensitivity achieved by this method is 4000 times more than previously published HPLC-mass spectrometry techniques.

(7) Untargeted metabolomic profiling of early diet energy intake in C57BL/6N mice indicates tentative differences correlated with diet and colon region

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A high-fat (H) diet leads to obesity, a known risk-factor of colorectal cancer (CRC) development. Animal models of calorie restriction (E) demonstrate reduced CRC development. Although these diets are believed to alter the colon metabolome to favor/reduce CRC risk, a comparison between the colon metabolome resulting from H and

E diets, relative to a normocaloric control diet (C), has not been made. The different influences of these diets on the proximal (PC), medial (MC), and distal (DC) colon metabolome has also not been studied. Thus, the objectives were to compare the metabolome of H, E, and C diets in each colon region and to correlate these differences with abberant crypt foci (ACF) number, a marker of CRC risk. Female C57BL/6N mice were fed a C, E, or H diet for 21 weeks, injected with azoxymethane between 16-21 weeks, and either maintained or changed diets (for 9 groups: CC, CH, CE, HH, HC, HE, EE, EH, EC) for an additional 39 weeks. Monophasic extracts of PC, MC, and DC were analyzed using HILIC LC-HRMS in positive and negative modes, as well as via ^{1}H NMR. A total of 451 compounds were detected by LC-HRMS in positive ionization mode and 407 compounds in negative ionization mode. Principle components analysis (PCA) of these samples reveals clear differences between colon regions, and additional univariate fold-change comparisons are being used to determine differences based on diet groups. Once discriminant analytes are clearly defined, identification of metabolites that differ between groups, and correlation with ACF number and microbial species abundances, will identify which analytes are correlated with increased and decreased risk of CRC development.

(8) Characterization and differentiation of bispecific antibody chain pairing variants by native and ion mobility mass spectrometry

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Bispecific antibodies (BsAbs) are composed of two unique pairs of heavy and light chains linked by disulfide bonds. They can bind with two distinct antigens simultaneously, showing a potential application in antibody-based therapeutics.1 However, the qualification and quantification of BsAbs is challenging due to the formation of unwanted mispaired species, specifically when a mispaired

molecule is isobaric with the target molecule of interest. Yin et al. have developed an ion exchange mass spectrometry method to separate target and off-target BsAbs, but it relies on the difference between isoelectric points (pI) of BsAbs.2 Here we take an alternative characterization approach by focusing on antibody-antigen binding, tandem mass spectrometry, and unfolding behaviors of BsAbs using ion mobility, in order to develop a more universal characterization method for BsAbs. Antigen-antibody binding is observed only for correctly paired BsAbs by high-resolution nMS, indicating antigen-antibody binding as a promising method to differentiate correctly paired BsAbs from double mispaired isomers. In general, tandem mass spectrometry is a powerful strategy to identify isomers but the direct dissociation of antibodies is difficult due to the high energy barriers required for the cleavage of disulfide bonds between chains in antibodies. BsAbs were subjected to a "gentle" disulfide bond reduction and dissociated in the gas-phase. Dissociation by collision-induced dissociation (CID) yields mostly light chains while surface-induced dissociation (SID) can generate two characteristic half antibodies that can be used to distinguish isomeric BsAbs. Additionally, unfolding behaviors of antibodies can be used to recognize the position and number of disulfide bonds.3 Compared to collision-induced unfolding (CIU), surface-induced unfolding (SIU) can provide additional insight into the characterization of antibodies.

(9) Modification mapping of large subunit ribosomal RNA through overlapping digestion products

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Ribosome is a dedicated molecular machine for intracellular protein synthesis. The prokaryotic ribosome is composed of 50S (large) and 30S (small) subunits. The 23S ribosomal RNA (rRNA) of large subunit harbors the peptidyl transferase center (PTC), which makes peptide bonds between amino acids[1]. The rRNA contains 2904 nucleotides, of which 25 are post-transcriptionally modified (either methylations of the base or sugar and isomerization of uridine). These modifications are known to be important in maintaining the conformation of ribosome, enhancing the subunit association, and providing antibiotic resistance among other functions[2,3]. Therefore, location-specific changes in modification levels due to stress responses or metabolic changes is expected to influence the ribosome structure and function, and potentially, the cell survival. The location-specific information is generally obtained through RNA modification mapping involving liquid chromatography coupled with mass spectrometry. In the current project, we have explored the utility of C-and U-specific ribonucleases in combination with G-specific ribonuclease to obtain modification location information with high accuracy. Usage of enzymes with complementary nucleobase specificity improved the sequence coverage through generation of overlapping digestion products. The observed benefits and opportunities for further improvement of this analytical technology will be discussed in the presentation.

(10) Discrimination of differently pasteurized orange juices and identification of marker compounds using untargeted UHPLC-IMS-qToF-MS

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Orange juice belongs to the most popular fruit beverages, accounting for 60% of juice consumption in Western Europe and North America. To extend the shelf life of fruit juices and purees, pasteurization is necessary to inactivate microorganisms and enzymes and to ensure stable and safe products. However, conventional thermal pasteurization has been reported to adversely affect the texture, flavor, nutritional value, color, and the bioactivity of fruit compounds. Therefore, there is a growing interest in more gentle

preservation methods such as pulsed electric fields (PEF) and high pressure processing (HPP). So far, there is no analytical marker to differentiate between conventional thermal and alternative pasteurization techniques, which may be used to detect food fraud.
In this study, untargeted UHPLC-IMS-qToF-MS approaches were utilized to reveal differences in the profile of non-volatile secondary plant metabolites, especially polyphenols and carotenoids, between untreated, low, and high thermal pasteurized, PEF, and HPP treated orange juices. The objective of the study was to identify marker compounds for these pasteurization techniques and to elucidate their formation. The different modes of action of these techniques like heat, pressure, electric pulses, and possible formation of free radicals probably result in different isomerization, oxidation, and cleavage pathways of plant metabolites. Moreover, structure-dependent stabilities were shown for several compounds such as carotenoids. Violaxanthin esters seem to be sensitive toward heat and might undergo further isomerization to 5,8-epoxids and cleavage.

(11) Modulating the chemical micro-environment of dried blood spheroids for long-term storage of labile organic molecules and biomarkers

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Dried Blood Spots (DBS) are a simple minimally invasive sample collection, transportation, storage, and processing medium well-suited for direct-to-consumer healthcare. However, to preserve the sample, DBS must be stored under cold conditions (e.g -20°C) and stowed away from sunlight, heat, and high moisture content. Blood samples applied on hydrophobic paper beads up on top of the paper surface, forming a spheroid. This spheroid has a smaller surface area-to volume ratio compared to DBS and is less exposed to oxidative stresses. It has been shown experimentally that the spheroid contains an outer protective layer, which traps labile analytes within the bulk and prevents rapid degradation. The chemical composition of these dried blood spheroids can be altered to prolong the signal of small labile organic molecules (e.g., cocaine) and biomarkers (e.g., carnitines) by the addition of polymer and surfactants. A small amount of polymer solution can be added on top of a dried blood spheroid to artificially coat the sample, forming an artificial thin protective film. This is crucial for preventing and fixing cracking that occurs naturally within the spheroids, which can lead to oxidative degradation. To investigate the viability of polymer-coating as a method of preserving the sample, cocaine-spiked plasma was coated with polymer and the signal was monitored for a two-week period. The MS/MS signal for cocaine persisted much longer for polymer-infused and polymer-coated samples relative to the control sample. Previous work in our group has shown that enzymes retain some activity in dried blood spheroids, which can lead to reduced ion signal. To inhibit enzyme activity, surfactin is spiked into blood and the MS/MS signal of carnitines are monitored over a two-week period. It is hypothesized that surfactin deactivates the enzymes responsible for carnitine metabolism, preventing a decrease in signal due to enzyme activity.

(12) Identification of compounds that contribute to the bitterness perception of coffee brew

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Coffee consumption habits have been reported to be impacted by the bitterness attributes of the beverage. Bitter compounds in coffee have also been linked to gastroesophageal reflux disease. Consequently, understanding the molecular basis of coffee bitterness is of interest. Bitter compounds in coffee have been identified using taste-guided analytical approach; however, the sensory relevance of these compounds and their contribution to overall bitterness in coffee are not well established. The

overall objective of the current project was to apply an untargeted chemical profiling approach (flavoromics) to identify chemical drivers of coffee bitterness. A total of 14 commercial roasted coffee beans were selected from the market. Their respective coffee brews were chemically profiled using UPLC/MS-QTOF. Sensory analysis by 8 trained panelists was carried out to evaluate the bitter intensity of same brews. The chemical profiles and the corresponding bitter intensity ratings were modeled by orthogonal partial least squares (OPLS) with good fit ($R^2Y > 0.9$) and predictive ability ($Q^2 > 0.9$). Thirteen chemical markers that highly correlated to bitter intensity were subsequently isolated by multi-dimensional preparative LC/MS and further identified by MS and NMR. The sensory relevance of the chemical markers will be established by recombination testing. This work will provide new understanding of the chemistry driving overall coffee bitterness and allow the industry to tailor coffee products to different consumer preferences.

(13) A multi-omics approach to investigate the role of EZH2 in chromatin remodeling, cell proliferation and tumorigenesis

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Diffuse large b-cell lymphoma (DLBCL) is the most common form of Non-Hodgkin's Lymphoma, accounting for about 40% of new cases. The process of B cell maturation occurs in the lymph nodes, involving the precise timed suppression of cellular checkpoint genes by EZH2. Mutations in the catalytic domain of EZH2 have been linked to increased proliferation, tumorigenesis and adverse patient outcome, yielding increased or decreased levels in the epigenetic silencing mark, H3K27me3.

To understand this dual oncogenic and tumor-suppression mechanism behind EZH2, we created gain of function (GOF) and loss of function (LOF) cell lines relative to the activity on H3K27. Data-independent mass spectrometry analysis of histones extracted from these isogenic mutant cell lines revealed no significant changes in histone H4 post-translational modifications. The F667I/H689A LOF cell line resulted in significant enrichment in H3K9me3 and depletion of H3K27me3 (*p-value* = 0.05, *p-value* = 0.0004). RNA sequencing identified 26 potential targets primarily related to tumor progression via differentiation, migration, proliferation and signaling pathways including MMP14, TCIM, ADAMTS5, EPIPL, MAGEA and CCND1 (*q-value* < 0.05). Further, H2B ubiquitin ligase, UBE2E1, that results in increased H3K9me3 was up-regulated and WIZ, a protein that links H3K9 methyltransferases EHMT1 and EHMT2 to the CTBP co-repressor machinery was down-regulated, suggesting this catalytically inactive form of EZH2 inhibits PRC2 and other reader/writer complexes by physically blocking their association with chromatin. In comparison, the Y641F and A677G GOF cell lines revealed 85 down-regulated genes in response to increased H3K27me3 (fold-change > 2). These involve transcription factors, kinases, phosphatases, cell cycle checkpoint and tumor suppressors that include PGR, RAB3C, PDGFD, FGFBP1 and CD1D. While there is commonality among the gene targets in the GOF mutants, the DIA-MS/MS results suggests the GOF mutants operate distinctly to increase H3K27me3 perhaps in a histone-variant specific manner.

(14) Tomato steroidal glycoalkaloids are absorbed, metabolized, and stored in pigs

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Tomatoes account for 22% of vegetable intake for Americans, and their consumption has been associated with various health benefits. Often, these benefits are attributed to lycopene, a red-colored carotenoid. However, tomatoes contain thousands of metabolites, and research shows carotenoids are not the only bioactives. Recently tomato steroidal glycoalkaloids (tSGAs), an understudied class of secondary plant compounds, have been shown to be absorbed from the diet and deposited in skin in mice, and have *in vitro* antiproliferative properties. These discoveries have led to a growing interest in these compounds and their function in the body. Tomatoes uniquely produce tSGAs and these compounds undergo various glycosylations, acetylations, and hydroxylations during fruit ripening. There is little information regarding what happens to these compounds once they are consumed from tomatoes. In order to understand the potential bioactivity of tSGAs, it is important to know where they deposit and how they are metabolized. To investigate how tSGAs are processed in the human GI tract, we conducted a parallel study in weaned piglets (aged 3 weeks) as a physiologically relevant model for humans, fed a control diet (n=10) or a diet containing 10% tomato powder (n=10) for 2 weeks. Pigs were then sacrificed, and the following samples were collected: blood, prostate, liver, skin, muscle, adipose, lung, pancreas, spleen, stomach, colon, small intestine, and digesta and mucosal scrapings from both colon and small intestine. Samples were analyzed for tSGAs via UHPLC-QTOF-MS. Preliminary results show that tSGAs are absorbed and deposited in various bodily tissues. Additionally, it was found that sugar moieties are cleaved *in vivo*, and the remaining aglycones undergo phase I and phase II metabolism. This information provides a basis for further research on understanding the bioactivity of tSGAs in humans and their potential for health benefit.

(15) Improved high-throughput targeted lipidomic analysis with sMRM Pro Builder

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The quantitation of lipids via global lipid profiling is challenging. The sMRM Pro Builder tool enables acquisition of large numbers of compounds based on multiple reaction monitoring (MRM) of compound's MS/MS transitions and retention times when creating the optimized MRM acquisition method. Time scheduling MRMs reduces the requirement for multi-period experiments to cover large number of compounds. This algorithm maximizes points across the chromatographic peak to give better peak detection and improved reproducibility. The sMRM Pro Builder simplifies the retention time scheduling of over 1150 lipid analytes. The tool also aids in the optimization of dwell weight and dynamic window extension to maximize sensitivity and capture of detected analytes.

In order to confirm separation efficiency, lipids standards (one standard per lipid class) were injected individually to confirm that there was no isomer crosstalk among different lipid classes.

Preliminary data is collected with no MRM scheduling on a representative matrix (with or without internal standards) for any biological study. Results from this unscheduled MRM datasets are entered into the sMRM Pro Builder and an initial rough approximation of the retention times is determined. Next replicate injections are performed on the matrix using a preliminary retention time scheduled MRM method, ideally with a pooled sample from all the biological samples. Data analysis here provides information

on peak width, RT variance, lipid signal, then the sMRM Pro Builder computes a final optimized time scheduled MRM method. Excellent reproducibility was observed with majority of lipid species showing retention time standard deviations below 0.05 minutes.
Lipid standards from 19 different classes, which are either heavy isotopic labeled lipids or odd chain lipids, were used as internal standard. This method provided extensive lipid class coverages including, CE, CER, DCER, HCER, LCER, TAG, DAG, MAG, LPC, PC, LPE, PE, LPG, PG, LPI, PI, LPS and PS.

(16) Are steroidal glycoalkaloids imparting bitterness in tomato?

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Like other solanaceous crops, tomatoes (*Solanum lycopersicum,* L.) make unique steroidal glycoalkaloids (tSGAs) which are cholesterol derivatives that impact plant and, potentially, human health. α-Tomatine, a tSGA, is abundant in vegetative parts of the plant and serves as a phytoanticipin, discouraging herbivory and helping prevent microbial infection. α-Tomatine also occurs in immature green fruit, and as ripening progresses enzymes decorate the molecule with chemical groups and rearrangements, resulting in dozens of other downstream tSGAs. A recent survey of diverse tomato germplasm revealed higher tSGA concentrations and unique tSGA profiles in wild relatives of cultivated varieties. Selected studies suggest a group of Peruvian tomatoes with an abundance of α-tomatine differ in perceived bitterness. α-Tomatine and other tSGAs may have human health implications, but before introducing this trait to test nutritional hypotheses, we should understand if it impacts flavor or other quality attributes. We hypothesize that bitterness in tomato is not definitively linked to the presence of α-tomatine. To evaluate this hypothesis, 36 accessions representing three tomato species (*S. lycopersicum*, *S. pimpinellifolium*, and *S. peruvianum*) and several types (currant-, cherry-, pear-, plum-, and round-type) were planted in triplicate (n=108) in a greenhouse arranged in a Latin square design. Accessions were selected based on tSGA profile, geographic location, and varying bitterness. Twenty-two tSGAs will be quantified via ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). Additionally, fruits will be comprehensively profiled using untargeted, high resolution MS. Ripe fruits will also be evaluated via a trained panel to characterize associations between tSGAs, other fruit metabolites, and bitterness. These data will allow insight into the chemical basis of bitterness, and the improvement of tomatoes for heath and flavor outcomes.

(17) Benzo(a)pyrene and its interactions with modified RNA

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A number of studies have linked benzo(a)pyrene (B[a]P) exposure to tumorigenesis in specific cancers.[1,2] These studies have revealed genomic DNA damage through DNA adduct formation.[3] However, the influence of B[a]P on cellular RNA and their post-transcriptional modifications remains largely unclear. In the absence of extensive repair systems, unlike DNA, the damaged RNA can impair biochemical activities, if it is not turned over inside the cell. RNA plays critical roles in protein synthesis; therefore, it remains to be seen whether the adverse effects of B[a]P on RNA are connected to cellular transformation and tumorigenesis. Thus, the goal of research is to understand whether exposure to B[a]P elicit qualitative and quantitative changes to ribonucleotides and their posttranscriptional modifications through formation of covalent adducts. Using *Saccharomyces cerevisiae* RNA as a model system, we are documenting the effects on tRNA nucleotides, canonical and modified alike, through in

vitro and intracellular studies coupled with liquid chromatography tandem mass spectrometry. These studies are expected to provide insight into the effects of B[a]P in influencing the tRNA structure-function relationships during protein synthesis and any potential link to cancer development.

(18) Novel standardized metabolomics/lipidomics analysis tool for comprehensive targeted profiling

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Mass spectrometry (MS) is the key analytical technique for comprehensive metabolomics/lipidomics analysis. Reliable analytical results and improved inter-laboratory comparability, automation, and standardization of the metabolomics workflow is of utmost importance. Here, we present the newly developed standardized, quantitative MxP® Quant 500 kit-based assay for multiplexed MS/MS analysis of 630 metabolites/lipids from 26 metabolite/lipid classes in only 10 µL sample volume. The assay is designed for 80-sample batches and allows the broadest coverage of metabolite/lipid standardized analysis in kit format in a variety of biological sample matrices (e.g. blood, feces, tissue) and species. This assay is also the worldwide first tool for comprehensive microbiome-host interaction analysis covering host and gut bacteria-derived metabolites and lipids. The MxP® Quant 500 kit is designed to cover a broad range of biological matrices (e.g. blood, feces, tissue) and species, and requires only 10 µL of sample volume. The ready-to-use assay combines UHPLC-MS/MS and FIA-MS/MS into a single workflow. Automated and fast data analysis of >60K MRM chromatograms from analytes and internal standards analyzed in 96 tests (blanks, QC, standards, and samples) was performed with Biocrates' Met*IDQ*™ software, which automatically controls the entire workflow, from sample registration to data processing and result reporting. For beta-testing 14 samples (NIST SRM 1950, 6 human male/female plasma samples, 2 human male/female serum samples, lipemic human plasma pool, mouse plasma pool, rat plasma pool, mouse liver pool, human feces pool) were evaluated.

(19) Enzymatic and chemical hydrolysis in the identification of sugars in citrus pectin using ^{1}H and ^{13}C NMR

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Prebiotics are non-digestible compounds used to promote the growth of beneficial bacteria that exist in the digestive system. The consumption of prebiotics has been shown to exert a plethora of health-promoting effects and can be protective against several chronic diseases although the mechanisms of action for these health-promoting affects have not been fully elucidated. Pectin and pectic oligosaccharides (POS) have been shown to have significant prebiotic activity and can be utilized by certain beneficial microorganisms as a carbon source. The goal of this research is to gain a better understanding of the structure-function relationships of pectin and POS, as related to their prebiotic activity. A combined enzymatic and chemical hydrolysis was performed on citrus pectin in order to identify its sugar composition. The enzymatic hydrolysis of pectin was performed at 50°C using Peclyve CP, a pectin lyase, polygalacturonase, and pectin-methyl-esterase derived from *Aspergillus niger*. Samples were taken throughout to ensure the enzymatic hydrolysis was complete before beginning the chemical hydrolysis. Chemical hydrolysis followed using .2M trifluoroacetic acid (TFA) at 80°C. Samples taken during both hydrolyses were cooled, centrifuged, and trimethylsilylpropanoic acid (TSP) was added as an internal standard. ^{1}H and ^{13}C NMR spectra were obtained in order to identify sugars present in the samples; in-house ^{1}H and ^{13}C sugar libraries were used in the identification of sugars. We

are currently producing POS from pectin using enzymatic hydrolysis and we will evaluate the impact of specific carbohydrates on the metabolism and growth of probiotic bacteria. We will integrate NMR, liquid chromatography (LC) and mass spectrometry for the analysis of pectin and the accurate identification of POS. We will conduct an NMR-based metabolomics study and a detailed structural analysis to obtain structure-function information and determine the importance of factors such as monosaccharide composition, glycosidic linkage and molecular size on the prebiotic activity.

(20) Detection and discovery of ribonucleosides by higher-energy collisional dissociation mass spectrometry (HCD MS)

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Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the gold-standard technique for the discovery, detection and structural characterization of post transcriptional ribonucleoside modifications. Through collision-induced dissociation (CID), the RNA modification is assigned based on the diagnostic nucleobase ion (BH_2^+) observed following cleavage of *N*-glycosidic bond of the protonated ribonucleoside molecular ion (MH^+). Various laboratories have exploited this characteristic fragmentation to create powerful analytical platforms for the characterization of known and unknown modifications. Orbitrap-based mass spectrometers offer higher-energy collisional dissociation (HCD) as an alternate tool for fragmentation of molecular ion precursors. Here, we show the utility of HCD to generate nucleoside-specific MS/MS spectra (*i.e.*, fingerprints) for the characterization of modified ribonucleosides. Our studies demonstrate the capabilities of HCD fingerprints for: (a) accurate identification of positional isomers of ribonucleosides (*e.g.*, m^3C, m^4C, m^5C) exclusively based on their mass spectrometric features; (b) a rapid software-based retention time-independent detection or ribonucleosides through spectral matching; and (c) discovery and structural characterization of new RNA modifications through fingerprint comparisons.

(21) A pilot study of untargeted urine metabolomics in sarcoma patients treated with HD-AIM

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Soft-tissue sarcomas are an uncommon, diverse set of mesenchymal malignanices which affect 12,000 adults in the United States with a median survival of less than 15 months. Unfortunately, the current gold standard treatment, high dose adriamycin with ifosfamide (HD-AIM), has significant life-threatenting toxicities. Urinary metabolites may give early indication of treatment-induced toxicity. Urine samples from 7 sarcoma patients treated with HD-AIM therapy were collected over the course of 5 days and two treatment cycles. Liquid chromatography/mass spectrometry of these samples and pooled QC samples was performed on a Sciex TripleTOF 5600 and data preprocessing was performed with XCMS. Because pooled QC samples were purchased commercially, and thus did not include metabolites associated with treatment, the following two criteria were used to filter features: 1) missing in <=1 QC sample and CV <30% in QC samples or 2) present in >10% of patient samples and mean CV in sample duplicates <30% yielding a total of 4,047 features. Time course plots of known drug metabolites showed an expected ramp up to steady state at the beginning of each cycle in most patients and metabolites.

In unsupervised PCA, samples mainly clustered by patient and one patient (TTR-164) had a very distinct metabolomic profile. Patient TTR-164 was also an outlier in time course plots for adriamycin (low) and carboxyphosphamide (high). This patient suffered from acute toxicity leading to their withdrawal from the trial and passed away shortly thereafter. Despite the small number of patients evaluated, this pilot study indicates that urine metabolomics may uncover putative metabolite biomarkers of HD-AIM toxicity. Indeed, we find urinary excretion of unmetabolized adriamycin and carboxyphosphamide were significantly disturbed in one of two patients who withdrew from the study due to toxicity. Larger numbers of patients and further analysis would be required to evaluate the predictive capacity of putative biomarkers.

(22) Putting together the pieces of archaeal RNase P

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RNase P is an essential housekeeping enzyme that catalyzes the Mg^{2+}-dependent 5′-maturation of precursor (pre)-tRNAs in all three domains of life. Defective tRNA processing in animals has been associated with a variety of diseases, including neurodegeneration, underscoring the need for a mechanistic understanding of RNase P assembly and catalysis. The canonical form of RNase P is a ribonucleoprotein (RNP) complex that is exemplified by archaeal RNase P, which consists of one catalytic RNA (RNase P RNA; RPR) and up to five protein subunits (RNase P proteins; RPPs): POP5, RPP21, RPP29, RPP30, and L7Ae. Four of these RPPs function as binary complexes, with POP5•RPP30 enhancing the enzyme's cleavage rate and RPP21•RPP29 improving substrate binding. However, the role of L7Ae, which binds RNA structural modules called kink-turns (K-turns) to induce axial bending, is unclear. We recently reported that L7Ae binds to three K-turns in the *Pyrococcus furiosus* (*Pfu*) RPR, including a 'double K-turn' that likely facilitates foldback of the P12 region. Thus, L7Ae may play a structural role in aiding RPR folding and RPP binding. To further characterize how different suites of protein cofactors modulate the structure, function, and dynamics of a catalytic RPR, we use native mass spectrometry (nMS) to study the composition, stoichiometry, and subunit interactions of *Pfu* RNase P assembled with wild-type or K-turn-mutant RPRs and varying suites of RPPs. In parallel biochemical experiments, which include site-directed hydroxyl radical-mediated footprinting and rapid quench-flow-based cleavage assays, we seek to establish a functional context for the nMS results. Thus far, we have used nMS to determine the stoichiometry of (1) RPR + POP5•RPP30, (2) RPR + RPP21•RPP29, (3) RPR + L7Ae, and (4) RPR + POP5•RPP30 + RPP21•RPP29, and have preliminary results for (5) RPR + POP5•RPP30 + RPP21•RPP29 + L7Ae. Together, our complementary analyses provide valuable insights into protein-aided catalysis in this ancient, multi-subunit RNP.

(23) Using multiple ionization techniques to understand dissolved organic matter (DOM) oxidation by permanganate

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Dissolved organic matter (DOM) is a complex heterogeneous mixture of biotically and abiotically degraded biomolecules. DOM is ubiquitous in aqueous systems and is problematic in drinking water treatment operations because DOM can react with disinfectants to form carcinogens. Therefore, DOM must be effectively removed or its reactivity reduced. One strategy to reduce DOM reactivity is

permanganate oxidation. However, it is unknown what DOM components are oxidized by permanganate. We characterized a commonly studied DOM standard, Suwannee River Fulvic Acid (SRFA), before and after permanganate oxidation to gain insight on this process. Fourier transform-ion cyclotron resonance-mass spectrometry (FT-ICR-MS; Bruker SolariXR 15T) was used to identify chemical formulae in DOM in the *m/z* range of 200 to 700. Traditionally, DOM is ionized using only negative mode electrospray ionization (ESI), which ionizes only a fraction of compounds present in DOM. To address this limitation, four ionization techniques were used to target different fractions of DOM: negative mode ESI and LDI to observe CHO formulae, such as polyphenols, and positive mode ESI and LDI to observe nitrogen- and sulfur-containing compounds. We anticipated that compounds with high permanganate reaction rate constants, such as alkenes and phenols, would preferentially be oxidized by permanganate. Instead, we found that condensed aromatic-like and tannin-like components (likely containing alkyl benzene moieties) and nitrogen-containing aromatic components (possibly benzylamines or phenylamines) were primarily oxidized by permanganate. These compounds were oxidized to benzoic acid-containing moieties and nitrogen-containing aliphatic carboxylic acids. If we had only used negative mode ESI, as is traditional, we would not have observed the oxidation products that were observed by the positive ion modes and the reactants that were observed by negative mode LDI. This study, therefore, highlights the value of using multiple ionization techniques to comprehensively characterize DOM and its oxidation products by permanganate.

(24) Evaluation of sample processing parameters for optimized 2D NMR metabolomics experiments

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Two-dimensional nuclear magnetic resonance (2D NMR) exhibits excellent quantitative features for metabolite measurement in complex mixtures such as urine and plasma, especially when coupled to advanced data analysis methods (Gronwald et al. 2008; Klein, Oefner, and Gronwald 2013). Additionally, 2D NMR is recognized as the most reliable method for identification of metabolites, outperforming mass spectrometric methods (Sumner et al. 2014). Still, the inherent low sensitivity of NMR leaves many metabolic processes “invisible”, which has led to a slow adoption of NMR based techniques in the field of metabolomics. While these limitations are in part driven by the laws of physics and therefore unavoidable, we hypothesized that improved sample preparation protocols could at least lessen the impact of these limitations. We carefully optimized sample preparation procedures and tested metabolite quantification performance using ^{1}H-^{13}C HSQC experiments in order to determine optimal preparation parameters. Our results indicate that optimized sample processing of urine and plasma/serum can improve quantification performance for 2D NMR metabolomics experiments.

(25) Metabolomic analysis of an endophytic *Streptomyces sp.* from the liverwort *Bazzania trilobata*

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Microbial-derived natural products have played an important role in drug discovery and represent a major component of modern therapeutics. In particular, many clinically approved antibiotic and anticancer agents have been developed from the rich pool of bioactive compounds produced by *Actinomycetes*. While the demand for potent bioactive molecules remains, it is important to search in unique settings, thereby increasing the probability of discovering novel chemical scaffolds and valuable

leads for future development. This philosophy has fueled the recent interest in searching the underexplored chemistry of endophytic microorganisms. All plants harbor microorganisms that live asymptomatically within the cells and tissues of the plant, these endophytes play an important role in host protection from microbial pathogens. Recent reports have also identified plant endophytes as the producers of clinically important molecules (e.g., paclitaxel and vincristine) and unique chemical skeletons. As part of an ongoing search for bioactive compounds from microbial associates of liverworts, our lab has identified an endophytic *Streptomyces* sp. from *Bazzania trilobata* (Lepidoziaceae) that produces a crude extract active against the human breast (MCF-7) and colorectal (HT-29) adenocarcinoma cell lines with IC_{50} values of 8.6 µg/mL and 16.5 µg/mL, respectively. Isolation of the major constituents of the extract using high-performance liquid chromatography with diode array detection (HPLC-DAD) led to the discovery of several known compounds belonging to the structurally unique spirotetronate polyketide family of abyssomicins. Untargeted metabolomics using the Global Natural Products Social Molecular Networking (GNPS) platform was then used to identify putative abyssomicin derivatives and determine which compounds may be responsible for the observed biological activity. The metabolomics analysis, isolation, structure identification, and biological data will be presented.

(26) Identifying urinary biomarkers of tomato consumption using untargeted metabolomics

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Tomatoes are one of the most consumed vegetables and their intake has been associated with reduced risk for certain diseases. Understanding the relationship between tomato intake and health benefits requires an accurate measurement of tomato consumption. Using plasma lycopene as an indicator of tomato intake is problematic due to variation in lycopene absorption from different foods and between individuals. Alternatively, urine sampling is non-invasive and typically more representative of dietary intake, resulting in a more robust assessment of consumption. Untargeted metabolomics allows for detection of differences in small molecules between groups without preconceived bias, allowing for several classes of compounds to be evaluated as potential biomarkers. The objective of this study is to identify urinary biomarkers of tomato consumption using untargeted metabolomics. Healthy subjects (n=35) consumed 360 mL/day of high-β-carotene tomato juice, high-lycopene tomato juice, or a macronutrient matched control juice (derived from cucumber) for four weeks. Two varieties of tomato juices were included in order to understand how variation in tomato source contributes to intake biomarkers observed. Urine was collected at baseline, 2 and 4 weeks after beginning the intervention. UHPLC-QTOF-MS will used to compare osmolality-normalized urine samples at each time point. Data will be collected in both positive and negative modes for comprehensive coverage of metabolites. Raw data will be processed using ProFinder (Agilent) and unsupervised and supervised analyses will discern differences due to diet. Differentiating metabolites will be identified using online databases, MS/MS and comparison to authentic standards. Based on previous literature, we expect to see glucuronidated and sulfonated derivatives of tomato fruit secondary metabolites, specifically steroidal glycoalkaloids and phenolic acids. This study will act as a pilot in identifying potential urinary biomarkers for future studies evaluating tomato consumption.

(27) Subunit interaction of glutathionylated human hemoglobin probed by surface-induced dissociation/ion mobility mass spectrometry

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Glutathionylation is a reversible post-translation modification of cysteine residues via thiol-disulfide exchange with oxidized glutathione. Under elevated oxidative stress conditions, βCys93 of adult hemoglobin (HbA_0) undergoes S-glutathionylation. Glutathionylated hemoglobin (GSHb) is a biomarker of oxidative stress associated with several clinical conditions such as diabetes mellitus, chronic renal failure, iron deficiency anemia, and atherosclerosis. Apart from protecting proteins from oxidative modifications, glutathionylation regulates and modulates protein activity that is critical to redox signaling, rendering this modification essential to explore. Therefore, as a biomedical model, a structure-function correlation of GSHb can serve as a valuable reference system for other proteins undergoing glutathionylation. We investigated the structural integrity and overall architecture of GSHb using native mass spectrometry and surface-induced dissociation. Glutathionylation of hemoglobin leads to structural perturbation by the dissociation of important inter-subunit and intra-subunit interactions across globin subunits in the tetrameric hemoglobin. The weakening of subunit interactions leads to increased dissociation equilibrium constants of both tetramer/dimer (K_{d1}), and dimer/monomer (K_{d2}) of GSHb by 1.91 fold and 3.64 fold, respectively. The functional abnormality of tighter oxygen binding of GSHb may be attributed to the increased dissociation of the tetramer and its transition towards an oxy-hemoglobin-like conformation. MD simulations showed the average radius of gyration for deoxy-HbA_0, oxy-HbA_0, deoxy-GSHb, oxy-GSHb were 24.04 (±0.11) Å, 24.26 (±0.10) Å, 24.01 (±0.09) Å, 23.60 (±0.06) Å, respectively. The difference of radii of gyration by ≤1 Å indicated that the overall structures of the molecules do not change significantly upon binding of two glutathione molecules per tetramer and the overall size and shape of the molecules remain unchanged. We observed a relatively small difference in the interfacial dissociation of HbA_0 and GSHb in the energy-resolved mass spectrometry (ERMS) plots obtained from surface-induced dissociation which cleaves the weakest interfaces and is reflective of the protein structure.

(28) Comparison of strategies for the enrichment of cross-linked peptides

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The mass spectrometric analysis of cross-linked peptides from proteins and protein complexes can provide information on intra- and inter-molecular residue-residue distances, which can then be used as constraints to model protein tertiary and quaternary structures. A fundamental and challenging aspect of this technique is to detect and identify the cross-linked peptides in the presence of an excess of non-cross-linked peptides. In this work, we examined strategies used to enrich for cross-linked peptides prior to LC-MS and found that employing multiple strategies can help to retain more information on cross-links present within a protein/protein complex. We cross-linked homo-dimeric enolase from *Saccharomyces cerevisiae* and proteins from the tryptophan synthesis pathway with disuccinimidyl sulfoxide (DSSO) and either enriched for inter-molecular cross-links by separating oligomers via SDS-PAGE prior to protein digestion, or enriched for inter- and intra-molecular cross-links after in-solution digestion. For the latter, we used size-exclusion chromatography (SEC), mixed-mode strong cation-exchange (MCX), and offgel electrophoresis prior to LC-MS. All data were collected on an

Orbitrap Fusion mass spectrometer and analyzed with XLinkx. From the five enrichment strategies preformed on enolase, over two hundred cross-links were identified containing over one hundred unique cross-links with less than twenty cross-links common across all strategies. The results indicate that there is a variability in cross-links identified depending on the enrichment strategy and demonstrate the utility of combining enrichment strategies to allow for a high coverage of cross-links across the protein complex.

(29) AcquireX… A deep Ddve into the sea of metabolic features

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Compound identification remains a challenge in untargeted metabolomics. In LC/MS based untargeted metabolomics experiments, the detection of thousands of features in a single sample is routinely accomplished. However, this should not be equated to "global" metabolome coverage, as only a small percentage of those metabolites are of biological origin. Data-dependent acquisition (DDA) often provides information for the most abundant ions. Recently developed AcquireX acquisition software can determine on-the-fly features corresponding to background contaminants and compound degeneracy, such as isotopes, adducts, and dimers, enabling more efficient MS/MS and MS^n sampling of unique biologically relevant metabolites. Unlike traditional DDA, during which the fragmentation of background ions dominates the duty cycle, the AcquireX workflow selects precursors intelligently by excluding background ions and targeting unique metabolites of biological relevance for fragmentation. Here, we used samples of varying matrix and complexity, to demonstrate the utility of AcquireX acquisition across several sample types. By excluding background and degenerate signals, the total number of fragmentation targets was reduced without compromising metabolite coverage. By focusing acquisition on biologically relevant compounds, more time could be spent collecting multistage (MS^n) fragmentation data, without affecting experiment length. MS^n provided additional structural information and confidence for compound annotations and, in the case of flavonoids, isomeric compound annotation candidates could be differentiated without the need for additional experiments. Ultimately, AcquireX intelligent acquisition enabled annotation of non-biological and redundant features on-the-fly, resulting in comprehensive MS^n coverage regardless of sample type, complexity, and concentration. For Research Use Only. Not for use in diagnostic procedures.

(30) Modifying a 15T FT-ICR platform for native mass spectrometry applications

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The understanding of protein complex structure is integral for determining how changes in architecture affect function and possibly malfunction. Native mass spectrometry coupled with ion mobility and surface-induced dissociation (SID) offers a powerful analytical tool capable of probing the gas-phase structure of protein complexes in a single analysis. Fourier Transform- Ion Cyclotron Resonance (FT-ICR) mass spectrometers offer ultrahigh resolution capable of measuring small mass differences such as post translational modifications or ligand binding to complex biological molecules. However, most

commercially available FT-ICR mass spectrometers are geared toward small molecule analysis, requiring multiple instrument modifications for native mass spectrometry experiments.
The standard entrance funnel of the FT-ICR has been replaced with a trapped ion mobility spectrometry (TIMS) analyzer, capable of separating ions based on their mass, charge, and shape. TIMS has been used to separate isobaric and isomeric ions, ranging from peptides such as bradykinin to native proteins like ubiquitin, that have different conformations in the gas-phase. The original Bruker collision cell has been replaced with a surface-induced dissociation (SID) device to probe the connectivity and stoichiometry of proteins and protein complexes. The SID device also allows for collision-induced dissociation (CID), allowing for multiple activation experiments with one instrumental set up. Finally, the original selection quadrupole had an upper selection limit of 6000 *m/z*. A newly designed RF driver has been installed to allow isolation of large protein complexes up to *m/z* 20,000.
TIMS-SID studies with quad isolation have been performed for various protein complexes, including streptavidin (53 kDa homotetramer) and C-reactive protein (115 kDa homopentamer) for proof of concept experiments. Different charge states can be mobility selected in the TIMS region and then dissociated with SID. Additionally, the low frequency RF driver allows for the selection of charge states of large protein complexes, including glutamate dehydrogenase, a 330 kDa protein complex.

(31) MDM2 copy number aberrations alter ceramide glycosylation in liposarcoma tumors, impacting drug response

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Dedifferentiated liposarcoma (DDLPS) is an aggressive mesenchymal cancer. Patients have dismal prognosis, with a five-year survival rate of less than 50%, partially due to the high toxicity and low efficacy of current treatments. DDLPS tumors are characterized by amplified levels of MDM2, an inhibitor of the tumor suppressor TP53. Patients with high MDM2 amplification have worse prognosis compared to patients with low MDM2 amplification. We hypothesize that MDM2 levels impact lipid synthesis pathways in tumor cells, leading to differences in drug response. We leveraged metabolomic and lipidomic profiling of patient-derived DDLPS cell lines to test this hypothesis.
Four MDM2 high (Lipo224, Lipo141, Lipo224B, Lipo246) and two MDM2 low (Lipo815, Lipo863) patient-derived DDLPS cell lines were treated with atorvastatin. Metabolomic and lipidomic profiles were obtained for the six treated and untreated cell lines (Metabolon platform and SCIEX 5600 TripleTOF, respectively). Our metabolomics data are comprised of 481 metabolites, 12 of which were significantly higher in MDM2 high cells (FDR-adjusted t-test pval < 0.05, |FC| > 2) and 1 of which were significantly higher in MDM2 low. Of note, two glycosylated ceramides were significantly upregulated in MDM2 high cells.

Our lipidomics data are comprised of 517 identified lipids, 46 of which were significantly higher in MDM2 low cells, and 13 of which were significantly higher in MDM2 high cells (FDR-adjusted $p < 0.05$, $|FC| > 2$). One of the 13 significantly upregulated lipids in MDM2 high was another glycosylated ceramide, GlcCer_NS(d18:1/25:0). Additionally, we identified three pairs of glycosylated/nonglycosylated forms of the same ceramide in which the ratio of glycosylation to nonglycosylation was higher in MDM2 high cells. Together, our results suggest that reduced levels of nonglycosylated ceramides in MDM2 high tumor cells compromise the ability of the cells to undergo apoptosis, thereby driving drug resistance.

(32) Development of gradient chromatofocusing -mass spectrometry in the determination of protein isoforms

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The gradient chromatofocusing (GCF) technique generates a linear pH gradient externally in time prior to entering the column by mixing an elution buffer mobile phase in successively greater proportions with an application buffer at a different pH. This smooth linear decreasing/increasing pH gradient is then employed on to the anion/cation exchange column. The gradient mixing of mobile phase buffers with multiple buffer components that have closely spaced pKa values provides buffering capacity throughout the pH gradient. The gradient mixing allows a gradual change in pH instead of an instantaneous change in pH and gradual introduction of elution buffer generates a smooth pH gradient without any disturbance or irregularities in pH gradient. GCF also has an ability to manipulate the pH gradient profile by changing the slope of the external pH gradient and the flexibility in employing a wide range of buffer concentrations. Particularly relevant is that this technique can be directly interfaced to the mass spectrometer in the determination of proteins for accurate mass determination by using volatile low molecular weight buffers.

The present proposal seeks to develop a gradient chromatofocusing technique interfaced to a mass spectrometry for two model proteins that have multiple isoforms in which the isoelectric point (pl) distribution and molecular weight distributions are narrow. The model proteins with multiple isoforms are basic myelin protein which has been reported to have 4-6 isoforms with pls ranging from 11.1-11.8 in mice and 5 isoforms of rat pituitary prolactin with pls varying 5.14-5.34. Results of experiments will be compared for buffer systems having closely spaced pKa components versus less closely spaced pKa components and with different gradient slopes (change of pH with time). Increased resolution of the isoform peaks is anticipated for lower pH gradient slopes and for buffer systems having closely spaced pKa components.

(33) Mass spectrometry-based detection of genetically variable peptides: An alternative to DNA typing

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DNA typing is one of the most prominent methods for identifying individuals. However, DNA can degrade and may be difficult to recover from samples such as skin and hair. Thus, proteins are an attractive alternative, as they are more robust than DNA and may contain unique genetic variations in the form of single amino acid polymorphisms (SAPs). Here we evaluate different sample preparation and liquid chromatography tandem mass spectrometry (LC-MS/MS) methods to develop a bottom-up proteomics approach to identifying SAP-containing genetically variable peptides (GVPs). Proteins were extracted from individual skin samples by sonicating in solutions containing varying concentrations of

Rapigest SF or ProteaseMAX surfactant. Extracted proteins were digested with trypsin at varying temperatures and times prior to LC-MS/MS analysis using a Thermo Scientific Q Exactive Plus (QE). Peptide and protein IDs were generated using Proteome Discoverer, Mascot, and OpenMS for identification of human proteins and unique GVPs. Our results suggest that sample digestion is less efficient at higher temperatures, as more human proteins and GVPs were observed at 37°C than at 50°C. Meanwhile, digestion at 37°C for either 3 h or 16 h yielded a comparable number of human proteins and GVPs. The good overlap between the samples suggests that neither the surfactant nor the incubation time considerably changed the proteomic outcome. However, fewer missed tryptic cleavages were observed with RapiGest (7.8%) than ProteaseMAX (12.3%). Using the optimized sample preparation protocol and a 1D 5hr LC gradient, an average of 280 proteins were identified in each sample, with 655 GVPs detected across all 25 samples. In addition, analysis of the samples on a Bruker timsTOF Pro resulted in a 70-80% increase in the number of identified human proteins as well as a significant increase in protein sequence coverage, despite the shorter analysis time and lower sample injection amount.

(34) Assessment of the effects of black raspberry phytochemicals on the mechanisms of allergic contact dermatitis

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Allergic contact dermatitis (ACD) is a common occupational disease with significant public health impact. It affects about 20% of the population, causing erythema, dryness, edema, or blistering of the skin. Immune cells present in the skin tissue including macrophages and T cells play critical roles in the development of allergic contact dermatitis. Black raspberry phytochemicals have been shown to possess potent anti-inflammatory and immunomodulatory properties. We therefore examined the effect of black raspberry phytochemicals and metabolites on mechanisms of ACD in vitro and in vivo. Diets containing black raspberry powder, and black raspberry extracts were prepared for use in a murine model of contact hypersensitivity. Using untargeted metabolomics, we profiled small molecular weight compounds present in black raspberry and black raspberry extract diets. Our results demonstrated that black raspberry extract and black raspberry metabolites protocatechuic acid and ellagic acid suppressed macrophage activation in vitro as determined by their ability to produce nitric oxide. In our murine model of contact hypersensitivity, mice fed black raspberry supplemented diets showed a 49% reduction in ear swelling. Our data suggests a correlation between reduced swelling associated with experimental contact hypersensitivity and suppression of macrophage activity by black raspberry phytochemicals. We conclude that bioactive phytochemicals present in black raspberries can mitigate the pathogenesis of ACD.

(35) Assessment of dietary compliance and catechin/catechin-derived microbial metabolite pharmacokinetic responses in obese and healthy individuals following consumption of a novel green tea extract-rich confection

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Green tea extract (GTE) limits endotoxemia-mediated inflammation in obese mice by alleviating gut barrier dysfunction and microbial dysbiosis. However, translational evidence in obese humans is equivocal, partly due to lack of dietary control, objective compliance measures, and limited understanding of catechin/catechin-derived microbial metabolite bioavailability. Thus, we hypothesized that obese persons will have greater bioavailability of parent catechins due to increased gut permeability, but reduced abundance of catechin-derived microbial metabolites likely due to gut dysbiosis. We conducted a 12-h pharmacokinetics trial prior to a 4-wk randomized, double-blinded, placebo-controlled intervention in obese and age- and gender-matched healthy persons. For the pharmacokinetics trial, participants consumed a 0.5 g GTE confection (447 mg total catechins) and blood and urine were collected over 12-h and 24-h, respectively. After a 3-d washout, participants were randomized to either a GTE-rich (1 g/day) or placebo confection while maintaining a low-polyphenol diet during the intervention. Blood and 3-d diet records were collected at day 0, 14, and 28 to assess endotoxemia and dietary compliance. Plasma and urinary catechin/catechin-derived microbial metabolites were assessed by LC-MS. Confection-derived catechins and associated microbial metabolite, 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone, were bioavailable regardless of obesity status, but with high within- and between-group variability in pharmacokinetic responses. During the intervention, total polyphenol intakes were reduced in both treatment groups to a similar extent. Intervention compliance was corroborated by detecting little to no circulating catechins in participants receiving placebo confections regardless of health status. Total plasma catechins and 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone were significantly increased in participants receiving the GTE confection with no reported adverse events. In-progress findings demonstrate feasibility to implement a low-polyphenol diet while delivering a GTE-rich confection for a 4-wk intervention. Studies are underway examining gut-level improvements by GTE that limit endotoxemia in support of establishing GTE as an effective dietary strategy to mitigate endotoxemia-induced inflammation in obesity.

(36) A second-generation device for surface-induced dissociation of protein complexes in a 15 T FT-ICR mass spectrometer

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A second-generation (“Gen 2”) cell for surface-induced dissociation (SID) and collision-induced dissociation (CID) of protein complexes in a 15 T FT-ICR has been experimentally compared to a first-generation device (“Gen 1”). In the second-generation design we focused on increasing sensitivity and mass range for large multimeric protein complexes. Compared to Gen 1, Gen 2 exhibits an approximate 10x increase in sensitivity in CID and SID modes and an approximate doubling of the useful mass range (from *m/z* 8,000 to *m/z* 15,000) using a rectilinear ion trap with smaller inscribed radius or tripling (to *m/z*

25,000) using a hexapole collision cell. We demonstrate the increased mass range and sensitivity on a variety of model protein complexes including hexameric glutamate dehydrogenase (330 kDa) and 14mer GroEL (801 kDa).

(37) The effect of diet on the metabolome and microbiome of the colon in a lifetime obesity murine model

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Obesity is a known risk factor for developing colorectal cancer. To better understand how lifetime obesity could affect the development of colon cancer, we evaluated the metabolome of colon biospecimens excised from a colon cancer model fed a specific diet to model lifetime obesity based on C57BL/6N mice. Specifically, the mice were fed either a control diet (C), a calorie-restricted diet (E), or a high-fat diet (H), which mimics a Western diet, from 3-21 weeks of life. At 21 weeks, the mice either switched diets or stayed on the same diet to produce 9 total groups (CC: n = 35, CE: n = 34, CH: n = 35, EC: n = 30, EE: n = 38, EH: n = 24, HC: n = 24, HE: n = 24, HH: n = 24), and were fed this diet until euthanasia at week 60. From week 16 to week 21, the mice received weekly intra-peritoneal (IP) injections of azoxymethane at a dose of 10mg/kg of body weight to promote development of colon cancer. Microbiome data (16S profiling) have previously been collected in these same samples. To complement these microbiome data, we performed metabolomics profiling using liquid chromatography coupled with mass spectroscopy (LC-MS), yielding 248 metabolites in ESI+ mode and 194 metabolites in ESI- mode. Metabolite abundances were normalized by tissue weight and altered metabolites were defined by One-way ANOVA (FDR-adjusted p-values < 0.1) based on the diet, followed by Tukey testing (adj p-value < 0.1 and $\log_2$ fold change > 1.5). IntLIM was then applied to the metabolome and microbiome profiles to identify microbe-metabolite pairs with diet-type dependent relationships (e.g. positive correlation in one diet group but no correlation in another). Shifts in metabolomic profiles and gene-metabolite relationships due to diet and ACF formation will be presented.

(38) Using metabolomics to classify the underlying effects of multi-nutrient supplementation in ADHD Youth

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Attention-deficit/hyperactivity disorder (ADHD) is a neuropsychiatric disorder with increasing global prevalence and high heritability commonly diagnosed in childhood. Pharmaceutical treatment options provide a poor long-term risk:benefit ratio. A broad-spectrum multi-nutrient formula for ADHD has shown promise in children, but its effects on nutrient status and the underlying nutrient-metabolome interactions have not been characterized. We propose to use targeted measures of vitamin status as well as LC-MS metabolomics to develop new hypotheses as to the influence of nutrients on pathways

related to the disorder. Blood samples were collected at baseline and post-intervention from medication-free children with ADHD enrolled in a double–blind randomized placebo-controlled 8-week multinutrient supplement trial. ELISA Assays and targeted LC-MS/MS analyses will be performed to assess the blood nutrient status of nutrients whose levels are predicted to negatively correlate with ADHD symptom severity (i.e. tyrosine, phenylalanine, magnesium, zinc, long-chain omega-3 fatty acids, and iron). An untargeted LC-MS metabolomics screening approach using 3 separation methods (HILIC, C18, and Normal Phase analyses) will be used to assess very polar, polar, and nonpolar analytes in blood plasma extracts using an Agilent 1290 UHPLC interfaced with an Agilent QTof 6545. Metabolite levels will be compared pre-and post- supplement intervention, and correlated with symptom severity. We hypothesize that distinct differences in nutrient status and the metabolome will be observed in children randomized to the placebo vs. children randomized to the multinutrient supplement. Furthermore, we hypothesize that a subset of metabolites (e.g. those involved in neurotransmitter synthesis or branched chain amino acid metabolism) will be correlated with improvements in ADHD symptoms. This proposed results will address a significant gap in the nutritional psychiatry literature and provide new hypotheses as to how multi-nutrients may benefit a pediatric ADHD population.

(39) Metabolite profiling and authentication of pomegranate juice using high-resolution NMR spectroscopy and chemometrics.

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NMR spectroscopy is an emerging technology in food science and offers significant advantages compared to traditional methods of analysis. NMR allows the rapid and reproducible determination of many compounds in complex mixtures, such as foods, without using purification and separation steps. Here we applied high-resolution NMR for the compositional analysis and authentication of pomegranate juice (PJ). PJ produced from fresh pomegranate fruits of three varieties and commercial PJ of twenty brands were examined. Compound identification was performed using multidimensional NMR experiments, databases, literature and spiking with model compounds. The main compounds identified in PJ were sugars, organic acids and amino acids. Various 1D and 2D pulse sequences were investigated as quantification tools using Potassium hydrogen phthalate (PHP) as an internal standard. A good linearity and reproducibility were observed overall, which is a notable result for the development of NMR as a quantitative tool, in general. In addition, untargeted metabolomics were implemented using NMR coupled with multivariate statistical analysis. It was able to discriminate among varieties, geographical origins of PJ and detect PJ adulteration with apple juice, showing its strong potential for PJ traceability.

(40) Probing the mechanism of inhibition for inhibitors of metallo-β-lactamase VIM-2

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Resistance to β-lactam antibiotics has become increasingly prevalent since the introduction of antibiotics. Each year in the United States approximately two million people acquire bacterial infections that are resistant to antibiotics. A common mechanism for β-lactam resistance is the production of β-lactamases that hydrolyze the β-lactam ring, thus rendering the drugs inactive. Today there are more than 2000 β-lactamases, but this study focuses on the B1 subclass known as metallo-β-lactamases (MBLs) specifically New Delhi Metallo-β-lactamase (NDM-) and Verona Integrin-encoded Metallo-β-

lactamase (VIM-). MBLs are capable of inactivating all β-lactam antibiotics, except monobactams, and do not have any known clinical inhibitors, thus the development of these inhibitors is crucial. In an ongoing study the mechanisms of inhibition of the following inhibitors are being studied (using spectroscopic techniques) with VIM-2: captopril; ethylenediaminetetraacetic acid (EDTA); dipicolinic acid (DPA); dithiol; thiorphan; tiopronin; AC10-4; ANT-431; compound 36; and with NDM-1 ANT-431. The native-state ESI-MS results showed the formation of a ternary complex between VIM-2 and the following inhibitors: captopril; AC10-4; ANT-431; compound 36; dithiol; thiorphan; and tiopronin; while a metal stripping mechanism was shown with EDTA and DPA. The native-state ESI-MS results showed the formation of a ternary complex between NDM-1 and ANT-431. The data collected using ICP and UV-Vis supported the results shown by native-state ESI-MS for both VIM-2 and NDM-1 and their respective inhibitors. In conclusion, native MS is a powerful tool to probe for the formation of non-covalent enzyme-inhibitor complexes and is being used to guide inhibitor redesign efforts.

(41) In vitro evaluation of apple pomace as a source of prebiotics: an NMR metabolomics approach

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Prebiotics are defined as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of probiotic bacteria in the colon, which may lead to improved health of the host" (Gibson and Roberfroid 1995). Commonly, prebiotics on the market are produced from limited and/or high-cost sources, such as chicory root and Jerusalem artichoke. Previous studies show that pectin can have prebiotic activity, thus there is an increased interest for the production of prebiotics from alternative, low-cost sources such as pectin from food waste. Evidence shows that the structure of prebiotic oligosaccharides can affect prebiotic activity in the gut. However, the mechanisms of action and the factors that determine prebiotic efficacy have not been fully elucidated. The objective of this work is to extract and characterize pectin from apple pomace, an agricultural waste product, and conduct an *in vitro* study to understand the prebiotic effect of apple pomace pectin using a Nuclear Magnetic Resonance (NMR)-based metabolomics approach. Pectin was extracted using HCl and citric acid and characterized using NMR and IR. Overall, the HCl method produced the highest yield for pectin extraction and the citric acid extraction required an additional washing step for NMR analysis. Media will be created to simulate gut conditions (Gullon et al. 2008), and pure cultures (*Lactobacillus amylovorus, Lactobacillus acidophilus*) will be inoculated *in vitro* with pectin as the carbohydrate source, compared to inulin as a positive control. The supernatant will be filtered and analyzed using NMR following 24-hour incubation at 37°C. Untargeted, multivariate statistical analysis will be employed to elucidate the differences among carbohydrate sources to better understand the change in gut metabolites following prebiotic consumption.

(42) Iron chlorophyllin metabolites in simulated digestion and Caco-2 human small intestinal cell model

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Iron deficiency anemia (IDA) affects ~5.6% of the US population. It causes fatigue, dizziness, and weakness, and can be life-threatening when left untreated, especially in the developing world. Currently, inorganic iron supplementation and iron fortified food products are used for IDA treatment and prevention. However, inorganic iron has low bioavailability (5-20%) compared to the type found in

red meat, heme iron (20-35%), and also causes adverse gastrointestinal effects, which may increase subject noncompliance. Iron chlorophyllin (IC), a chlorophyll derivative with the central Mg^{2+} exchanged for Fe^{2+}, is a structural analogue to heme. A previous study showed that IC increases ferritin concentrations in Caco-2 human small intestinal cells, demonstrating its ability to serve as a substitute for heme iron. However, IC biotransformation during digestion and small intestinal cell absorption is unknown. This study aims to identify the metabolites of IC generated in simulated (*in vitro*) digestion and Caco-2 cell absorption, for further understanding of the gastrointestinal metabolism of this potential iron supplement. An IC supplement was digested under gastric and small intestinal conditions, and the filtered micellular fraction used to treat differentiated Caco-2 cells (13 days post-confluency) for 4 h before harvest. Chlorophyll derivatives and their metabolites in the IC supplement, digested chyme, the micellular fraction, and the Caco-2 cells, were extracted. Extracts were separated and detected using UHPLC-DAD-QTOF with an ESI ionization probe in positive mode, and peaks at 400 nm monitored. Results demonstrated that the primary iron chlorophyll product in the supplement (major peak m/z 606.1929, Fe-isochlorin e4) was partially taken up and metabolized by the Caco-2 cells, and novel metabolites absorbing in the chlorophyll region were apparent in the cell extracts. Analyses are underway to identify these novel metabolites, and results-to-date will be presented.