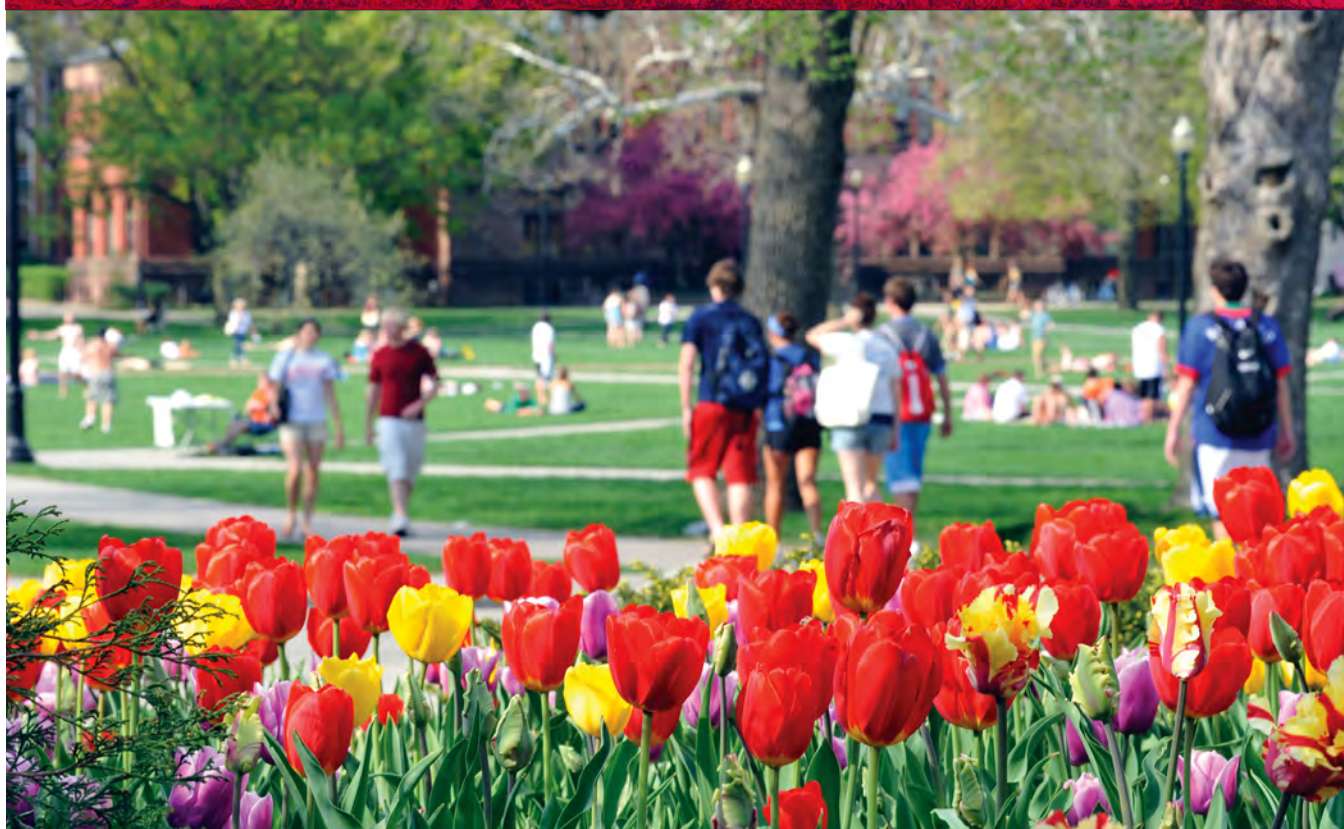


JOINT MEETING OF

The Inaugural Conference on Food and Nutritional Metabolomics for Health

and

The 14th Annual Ohio Mass Spectrometry Symposium



THE OHIO STATE UNIVERSITY

MAY 17 – 18, 2017
OHIO UNION
THE OHIO STATE UNIVERSITY


Agenda Overview

Wednesday, May 17

8-9 a.m.	Registration and Continental Breakfast for Tutorial Participants		
9-12:00 p.m.	Introduction to Metabolomics Tutorial Interfaith Room, 3rd Floor Ohio Union		
12-1 p.m.	Lunch for Tutorial Participants		
12-1 p.m.	Conference Registration		
1-1:10 p.m.	Conference Welcome: Dr. Caroline Whitacre 🍷 Performance Hall		
1:10-1:55 p.m.	Keynote Speaker: Dr. Gary Patti 🍷 Performance Hall		
1:55-2:40 p.m.	Plenary Speaker: Dr. Rafael Brüschweiler 🍷 Performance Hall		
2:40-3 p.m.	BREAK		
3-5 p.m.	Metabolomics Oral Presentations 🍷 Great Hall 1&2	Mass Spectrometry Oral Presentations ✳ Great Hall 3	
5-7 p.m.	Reception and Poster Session 🍷 Performance Hall		

Thursday, May 18

8-8:30 a.m.	Registration and Continental Breakfast		
8:30-9:15 a.m.	Keynote Speaker: Dr. Robert Gerszten 🍷 Performance Hall		
9:15-9:30 a.m.	BREAK		
9:30-11:30 a.m.	Oral Presentations 🍷 Performance Hall		
11:30-11:50 a.m.	Pick Up Lunch		
11:50-12:30 p.m.	Lunch & Learn Session 1A Aiko Barsch 🍷 Performance Hall	Lunch & Learn Session 1B Leah Shriver 🍷 Great Hall 1&2	Lunch & Learn Session 1C Amanda Souza ✳ Great Hall 3
12:30-12:40 p.m.	BREAK		
12:40-1:20 p.m.	Lunch & Learn Session IIA Michel Aliani 🍷 Great Hall 1&2	Lunch & Learn Session IIB Paul Baker ✳ Great Hall 3	
1:20-1:30 p.m.	BREAK		
1:30-2:15 p.m.	Plenary Speaker: Dr. Devin Peterson 🍷 Performance Hall		
2:15-3 p.m.	Plenary Speaker: Dr. Ewy Mathé 🍷 Performance Hall		
3-4 p.m.	Poster Session & Networking		

 } See map of Ohio Union on page 4

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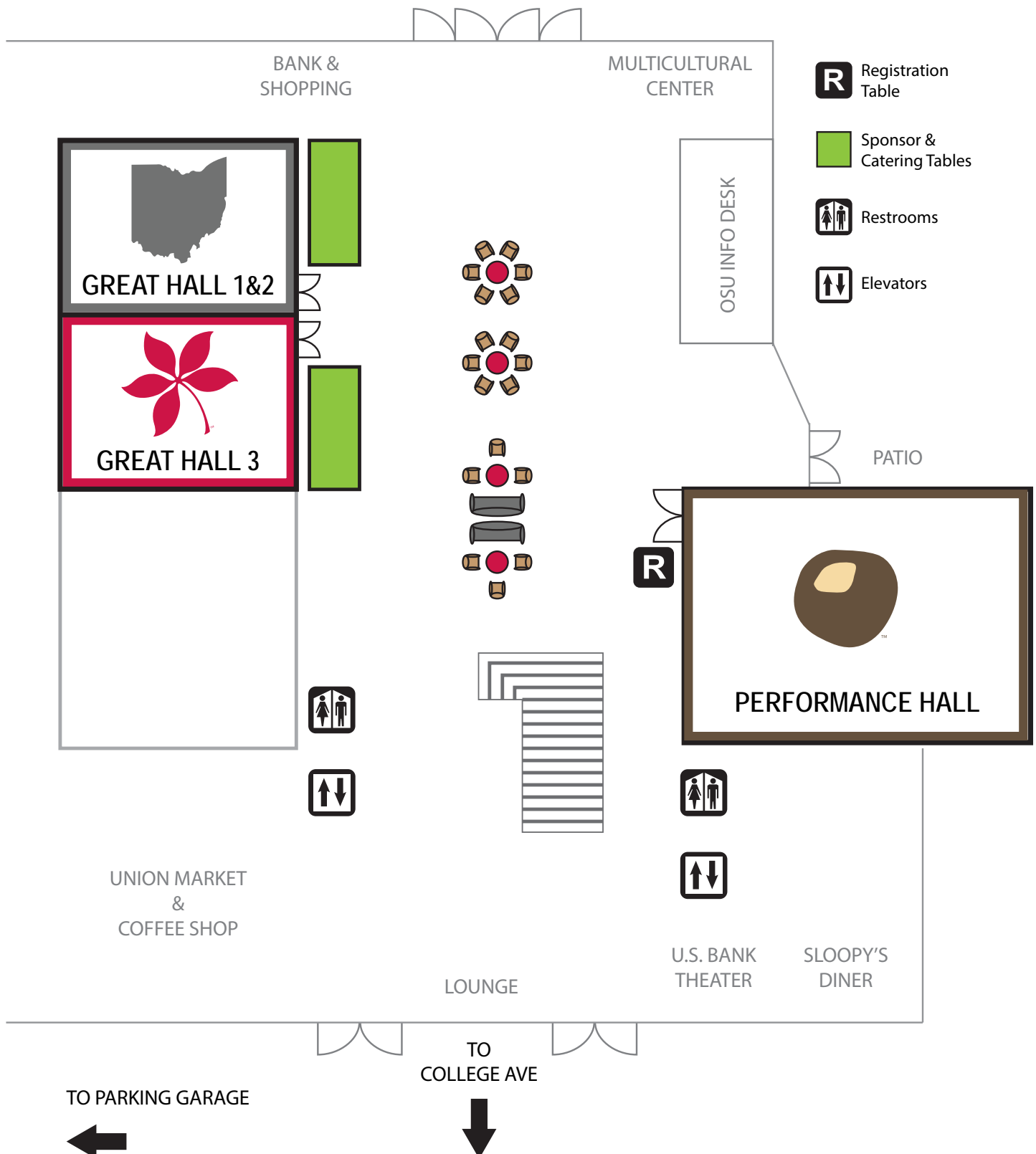
TO PARKING GARAGE



TO HIGH STREET

Ohio Union Map

First Floor



WELCOME FROM THE PLANNING COMMITTEE FOR:

The Inaugural Conference on Foods and Nutritional Metabolomics and The 14th Annual Ohio Mass Spectrometry Symposium

Dear 2017 Conference Participant,

It is our pleasure to welcome you to The Ohio State University campus for the Inaugural Conference on Food and Nutritional Metabolomics and the 14th Annual Ohio Mass Spectrometry Symposium! Our two programs are pleased to partner this year to provide all attendees with a high-quality and intellectually stimulating conference that is attracting researchers from the Ohio Valley region and beyond. This joint conference will serve as a wonderful opportunity to expand scholarly interactions and foster new collaborations among researchers in the academic and corporate sectors.

The conference kicks off with an informative 'Introduction to Metabolomics Tutorial' that will be beneficial for both beginners and serve as a refresher for more seasoned investigators. The program also will include Keynote and Plenary lectures by several well-established scholars, and oral and poster presentations that address diverse topics focused on metabolomics, proteomics, mass spectrometry and NMR to showcase highlights of recent research.

We sincerely thank all of our generous sponsors as well as our media partners, The Metabolomics Society and the journal Metabolites, for their support. Funds contributed by the Metabolomics Society and matched by the Foods for Health program will provide ten travel awards to offset the expenses of graduate students and postdoctoral fellows who will be traveling from outside the greater Columbus area to present their discoveries. We would also like to thank the Ohio State University Discovery Theme Initiative for their targeted investment in Food and Nutritional Metabolomics for Health (learn more at discovery.osu.edu/ffh).

We look forward to a great conference!

Sincerely,

- **Besma Abbaoui**, Program Manager, OSU Foods for Health Discovery Theme
- **Kamal Aboshamaa**, Executive Director, OSU Foods for Health Discovery Theme
- **Mark Failla**, Professor Emeritus and Interim Faculty Lead, OSU Foods for Health Discovery Theme
- **Alyssa High**, Business Assistant, OSU Campus Chemical Instrument Center
- **Brittany Poast**, Business Administrator, OSU Campus Chemical Instrument Center
- **Arpad Somogyi**, Associate Director, OSU CCIC Mass Spectrometry & Proteomics Facility
- **Laura VanArsdale**, Project Coordinator, OSU Foods for Health Discovery Theme
- **Vicki Wysocki**, Ohio Eminent Scholar, Professor, Director of OSU Campus Chemical Instrument Center



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Keynote Speakers



GARY PATTI

Gary Patti, PhD, is an associate professor at Washington University in St. Louis in the departments of chemistry and medicine. He trained as a graduate student in the laboratory of Jacob Schaefer, inventor of cross-polarization magic-angle spinning NMR. There, he integrated solid-state NMR and LC/MS technologies to examine alterations in bacterial metabolism related to antibiotic resistance. He joined the laboratory of Gary Siuzdak as a postdoctoral fellow, where he helped develop mass spectrometry-based resources for the field of metabolomics (such as the XCMS software and the METLIN metabolite database). His laboratory is interested in understanding basic mechanisms of metabolic regulation. To this end, his group focuses on both developing and applying metabolomic technologies. His work is supported by the NIH and the Pew Charitable Trust, as well as the Camille Dreyfus, the Sloan, and the Mallinckrodt foundations.



ROBERT GERSZTEN

Robert Gerszten, MD, serves as chief of cardiology at Beth Israel Deaconess Medical Center, professor of medicine at Harvard Medical School, and a senior associate member of the Broad Institute. Dr. Gerszten received his medical education at Johns Hopkins University School of Medicine and performed his residency at the University of Pennsylvania. From 2006-2015, he served as director of clinical and translational research in the MGH Heart Center. Dr. Gerszten's investigative career focuses on the nexus of cardiac and metabolic diseases. His translational research program is a national leader in the use of metabolomics and proteomic technologies for the discovery of new biomarkers and pathways contributing to atherogenesis and its complications. His group has identified novel biomarkers that identify—a decade before disease onset—patients destined to develop diabetes. His work is funded by the NIH and the American Heart Association, from whom he received an Established Investigator Award. In addition to his investigative focus, Dr. Gerszten has been an active clinician in the coronary care Unit and the consultation services at MGH, and serves on the executive committee of the MGH Cardiac Unit Associates. Dr. Gerszten is a member of the American Society for Clinical Investigation, the Association of American Physicians, and the Association of University Cardiologists.

Plenary Speakers



RAFAEL BRÜSCHWEILER

Rafael Brüsweiler received his PhD in 1991 in physical chemistry from ETH Zurich, Switzerland, before doing a postdoc at the Department of Molecular Biology at the Scripps Research Institute in La Jolla, Calif. In 2004, he joined Florida State University in Tallahassee as full professor and the National High Magnetic Field Laboratory as associate director for biophysics. He joined The Ohio State University in August 2013 with joint appointments in the Department of Chemistry & Biochemistry in the College of Arts and Sciences, and in the Department of Biological Chemistry & Pharmacology in the College of Medicine. He is an Ohio Research Scholar (endowed) and NMR executive director for the Ohio State Campus Chemical Instrument Center. His research interests include metabolomics, protein structural dynamics, interactions and function, computer simulations of proteins, and new NMR methods for better resolution and sensitivity.



DEVIN PETERSON

Devin Peterson, PhD, is a Professor in the Department of Food Science and Technology at The Ohio State University. He earned a doctoral degree in flavor chemistry (2001) at the University of Minnesota. In 2001, he joined the faculty in the Department of Food Science at Penn State University. In 2004, during his tenure at Penn State, he received the Presidential Early Career Award for Scientists and Engineers (PECASE) from the White House Office of Science and Technology Policy. In 2009, he returned to the University of Minnesota as an associate professor and was promoted to professor in 2014. He received the Excellence in Flavor Science Award from the Flavor and Extract Manufacturers Association in 2015. In 2016, he relocated his research program to Ohio State as part of the Discovery Themes Initiative. His research focuses on characterizing chemical stimuli that impart flavor to foodstuffs (taste, aroma, chemesthetic, mouthfeel), investigating multi-flavor interactions on perception, mapping pathways of flavor generation/stability and mechanisms of flavor delivery. His program is based on a utility inspired fundamental research platform with an overarching goal to get more from nature, guided by science, to support the development of sustainable, nutritious, and healthy foods with higher product acceptability. He initiated and directs the Flavor Research and Education Center (<http://frec.osu.edu/>), a partnership between academic researchers and industry leaders focused on the creation, exchange and application of knowledge to drive innovation in the food marketplace.



EWY MATHÉ

Ewy Mathé, PhD, is an assistant professor in the Department of Biomedical Informatics at The Ohio State University. Her position is funded in part by the Translational Data Analytics program, a foundational piece of the Discovery Themes Initiative. Her primary research interests are to leverage epigenomics, genomics, nucleotide variants and metabolic patterns to 1) understand how the genetic and epigenetic landscape affects disease phenotypes, particularly cancer; 2) define cell-type and disease-type specific molecular characteristics to uncover novel biomarkers and guide the search of novel therapeutic targets. Prior to joining the department, she was a bioinformatics staff scientist in the Laboratory of Immunogenomics at NIAMS/NIH in Bethesda, Md. Before that, she delved into molecular epidemiology during her postdoctoral training in the Laboratory of Carcinogenesis at the National Cancer Institute, where she discovered putative esophageal and lung cancer biomarkers using miRNA microarrays and metabolomics. She received her BS in biochemistry from Mount Saint Mary's University in Maryland in 2000 and her PhD in bioinformatics from George Mason University in Virginia in 2006.

Lunch and Learn Speakers



MICHEL ALIANI

Dr. Michel Aliani is a professor in the Department of Human Nutritional Sciences at the University of Manitoba and a member of the Canadian Centre for Agri-Food Research in Health and Medicine in Winnipeg, Manitoba. He was educated in France (B.Sc. and engineering degree in Agri-Food Biochemistry) and in Northern Ireland (PhD, and post-doctoral at Queen's University Belfast) prior to his move to University of Manitoba in 2007. His area of scientific expertise includes food science, mass spectrometry and metabolomics. His research interests include studying the molecular basis for the development and successful marketing of functional foods targeted to patients as well as health populations in the world, and investigating the effect of active compounds on metabolic pathways in animal and human models.

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PAUL BAKER

Dr. Paul Baker received his PhD in Biochemistry from Wake Forest University School of Medicine, where he began his training in lipids with the study of the metabolism and signaling actions of ether-linked lipid mediators. He did his post-doctoral work with Bruce Freeman at the University of Alabama at Birmingham, where he led the discovery of a novel class of anti-inflammatory lipid mediators—nitrated lipids. He continued his work on nitrated lipids as an assistant professor at the University of Pittsburgh School of Medicine until June 2011, when he joined AB SCIEX, where he is the Global Applications Lead in Lipidomics. At AB SCIEX, Paul has pioneered the use of differential mobility spectrometry in the study of lipids.

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AIKO BARSCH

Dr. Aiko Barsch is a qualified biologist and has been involved in metabolomics research since 2002. During his PhD studies in Professor Karsten Niehaus' lab at Bielefeld University in Germany, he established GC-MS and LC-MS-based techniques enabling the study of molecular interaction between *Medicago sativa* plants and root-nodule-forming *Sinorhizobium meliloti* bacteria. These studies were co-supervised by Professor Joachim Kopka from the MPI in Golm, Germany. Aiko joined Bruker Daltonics in Bremen in January 2007 as an application scientist for ESI-(Q)-TOF systems with a focus on small molecule analyses. Since the beginning of 2010 he has been fully committed, once again, to metabolomics research, in his position as Global Metabolomics Market Manager. Collaborating with cutting-edge research partners across the globe provides Aiko with profound insights into the current state of metabolomics. In addition to his continuing work as the global Market Manager for Metabolomics, in October 2014 he became Director of Life Sciences, and manages a team of proteomics and metabolomics specialists. This provides important insights to facilitate integrated OMICS research.

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LEAH SHRIVER

Dr. Leah Shriver received her B.A. in Biology at Mount Holyoke College and completed PhD training at the Medical College of Wisconsin. She then joined the laboratory of Dr. Marianne Manchester at the Scripps Research Institute and the University of California, San Diego, as a postdoctoral fellow where she developed new therapeutics for multiple sclerosis using untargeted metabolomics and a novel imaging technology, nanostructure-initiator mass spectrometry. In addition, she developed the plant virus, Cowpea mosaic virus, as a novel nanoparticle platform for the delivery of drugs and imaging agents to the central nervous system. Shriver joined the faculty at The University of Akron as an Assistant Professor in autumn 2012 and continues her research using these novel technologies to identify and develop new neuroprotective agents for the treatment of CNS disease.

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AMANDA SOUZA

Amanda Souza is currently the Metabolomics Program Manager at Thermo Fisher Scientific in the Chromatography and Mass Spectrometry Division. Prior to this recent transition to the marketing team, Amanda was a member of the Center of Excellence, where she supported customers directly involving metabolomics and lipidomics applications. Before joining Thermo Fisher Scientific, she worked under Dr. Clary Clish at the Broad Institute in the Metabolite Profiling Platform where she was involved in all aspects of experimental design and execution of profiling experiments, both pilot and large clinical studies.

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Conference Agenda

WEDNESDAY, MAY 17

INTRODUCTION TO METABOLOMICS TUTORIAL

- 8-9 a.m. **Registration & Continental Breakfast for Tutorial Participants**
- 9-12 p.m. **Introduction to Metabolomics Tutorial**
Interfaith Room, 3rd Floor Ohio Union
- Morgan Cichon, Ph.D., The Ohio State University**
Research Scientist, Foods for Health Discovery Theme
- Ken Riedl, Ph.D., The Ohio State University**
Acting Director, Nutrient and Phytochemical Analytics Shared Resource
- Rachel Kopec, Ph.D., The Ohio State University**
Assistant Professor, Department of Human Sciences, Foods for Health Discovery Theme
- Matt Teegarden, M.S., The Ohio State University**
Graduate Fellow, PhD Candidate, Department of Food Science & Technology
- 12-1 p.m. **Lunch for Tutorial Participants**

CONFERENCE

- 12-1 p.m. **Conference Registration**
- 1-1:10 p.m. **Conference Welcome**
🍷 Performance Hall
- Dr. Caroline Whitacre, The Ohio State University**
Senior Vice President for Research, Professor, Department of Microbial Infection and Immunity
- 1:10-1:55 p.m. **Keynote Speaker**
🍷 Performance Hall
- Dr. Gary Patti, Washington University in St. Louis**
Associate Professor, Departments of Chemistry & Medicine
Profiling Metabolites at the Global Level: A Last Resort or the Next Frontier?
- 1:55-2:40 p.m. **Plenary Speaker**
🍷 Performance Hall
- Dr. Rafael Brüschweiler, The Ohio State University**
Professor, Department of Chemistry & Biochemistry,
NMR Executive Director of Campus Chemical Instrument Center
NMR and NMR/HS Hybrid Approaches for Targeted and Untargeted Metabolomics
- 2:40-3 p.m. **BREAK**
- 3-5 p.m. **Parallel Oral Presentation Sessions**
- Session IA: Metabolomics Oral Presentations**
Session Moderator: Dr. Emmanuel Hatzakis The Ohio State University
🍷 Great Hall 1&2
- **Jiangjiang (Chris) Zhu, Miami University**
Enhanced Inhibitory Function and Metabolic Dysregulation in E. coli induced by L. acidophilus Fermented Tea Extracts
 - **Geoffrey A. Dubrow, The Ohio State University**
Improving Sugar-Free Fruit Spread Flavor Using Metabolomics
 - **Jikang Wu, The Ohio State University**
Quantification of Fructose-Asparagine in Human Foods, Animal Foods and Mouse Ceca
 - **Matt Teegarden, The Ohio State University**
Elucidating Urinary Markers of Strawberry Consumption in Smokers and Nonsmokers
 - **Enkhtuul Tsogtbaatar, The Ohio State University**
Application of ¹³C-labeling Approach to Reveal Biochemical Mechanism of Oil Synthesis in Pennycress (Thlaspi arvense L.) Embryos

Session IB: Mass Spectrometry Oral Presentations

Session Moderator: Dr. Chrys Wesdemiotis, The University of Akron

★ Great Hall 3

- **Manasses Jora, University of Cincinnati**
Induction-Based Fluidics: An Alternative Ionization Source for LC-MS/MS Analysis of RNA Nucleosides
- **Joshua Gilbert, The Ohio State University**
Implementation of an Ion Carpet Array for Facile and Compact Surface Induced Dissociation
- **Kevin Endres, The University of Akron**
First Application of SL-MALDI-MS Imaging to a Synthetic Material Towards Understanding Interfacial Characteristics of Polymer Films
- **Stacey Nash, The Ohio State University**
Investigation of Protein-Protein Interactions Using Surface Induced Dissociation (SID) MS
- **Krishani Rajanayake, The University of Toledo**
Determination of the Origin of Doubly-Cationized Monosialylated Fragments in MS/MS Spectra of Singly-Cationized LSTb and GM1 using Ion Mobility Mass Spectrometry

5-7 p.m.

Reception and Poster Session

● Performance Hall

THURSDAY, MAY 18

8-8:30 a.m.

Registration and Continental Breakfast

8:30-9:15 a.m.

Keynote Speaker

● Performance Hall

Dr. Robert Gerszten, Harvard Medical School

Chief of Cardiology, Beth Israel Deaconess Medical Center, Professor of Medicine,
Harvard Medical School

Mining the Blood for New Cardiometabolic Hormones

9:15-9:30 a.m.

BREAK

9:30-11:30 a.m.

Metabolomics/Mass Spectrometry Oral Presentations

Session Moderator: Dr. Rachel Kopec, The Ohio State University

● Performance Hall

- **Michael Petriello, University of Kentucky**
Positive Associations Between Serum Levels of Dioxin-like Pollutants and the Circulating Cardiometabolic Disease Risk Biomarker Trimethylamine-N-oxide
- **Peter Lobue, University of Cincinnati**
Data-Independent Mass Spectrometry of Modified RNA
- **Jessica Cooperstone, The Ohio State University**
Metabolomics Aids in Discovery of Putative Bioactive Compounds from Tomatoes in a Model of Keratinocyte Carcinoma
- **Alyssa Stiving, The Ohio State University**
Use of Surface Induced Dissociation (SID) as a Top-Down Fragmentation Technique
- **Joshua Blakeslee, The Ohio State University**
Metabolome-based Genome Wide Association Profiling of Innate Immunity in Rice
- **Deidre Damon, The Ohio State University**
Investigation of Surface Energy in Paper Spray Ionization for the Direct Analysis of Dried Biofluids Using Mass Spectrometry

11:30-11:50 a.m.

Pick Up Boxed Lunch

continued on next page...


Conference Agenda

THURSDAY, MAY 18 *continued...*

11:50-12:30 p.m.

Lunch & Learn Sessions – Session I


IA: Aiko Barsch, Bruker Daltonics

 Performance Hall

Deeper Insights with Metabolomics: Studying the War between Plants and Insects

Sponsored by Bruker Daltonics


IB: Leah Shriver, The University of Akron

 Great Hall 1&2

Cuprizone Toxicity: The Intersection of Nutrition and Disease

Sponsored by Waters

IC: Amanda Souza, Thermo Fisher Scientific

 Great Hall 3

Removing the Data Processing Bottleneck: Move Efficiently from Data to Structure to Pathway with Thermo Scientific™ Compound Discoverer™ 2.0

Sponsored by Thermo Fisher Scientific


12:30-12:40 p.m.

BREAK

12:40-1:20 p.m.

Lunch & Learn Sessions - Session II

IIA: Michel Aliani, University of Manitoba


 Great Hall 1&2

Blood pressure-lowering effects in lentil-fed spontaneously hypertensive rats (SHR):

A nutritional metabolomics approach

Sponsored by Agilent

IIB: Paul Baker, SCIEX

 Great Hall 3

Differential Ion Mobility Spectrometry Dramatically Improves the Specificity of Untargeted Lipidomics Analysis by 'Shotgun' Analytical Approaches


Sponsored by SCIEX

1:20-1:30 p.m.

BREAK

1:30-2:15 p.m.

Plenary Speaker

 Performance Hall


Dr. Devin Peterson, The Ohio State University

Professor, Food Science & Technology, Director, Flavor Research & Education Center

Decoding Food Flavor for Health Promotion: Targeted and Untargeted Approaches

2:15-3 p.m.

Plenary Speaker

 Performance Hall




Dr. Ewy Mathé, The Ohio State University

Assistant Professor, Department of Biomedical Informatics

Integrating Gene Expression and Metabolomics Data Unravels Cancer-Specific Molecular Signatures

3-4 p.m.

Poster Session & Networking



 } See map of
Ohio Union
on page 4

Abstracts

KEYNOTE & PLENARY SPEAKER ABSTRACTS

Listed in order of presentation

Profiling Metabolites at the Global Level: A Last Resort or the Next Frontier?

Gary J. Patti

Washington University in St. Louis, Departments of Chemistry and Medicine

During the last ten to fifteen years, advances in mass spectrometry-based technologies have greatly simplified the acquisition and processing of metabolomic data. Whether one acquires the data in their own laboratories or uses a service core, it is now dangerously simple to obtain untargeted metabolomic results. The question has become, how should one interpret these complex datasets? Most commonly, untargeted metabolomic datasets contain tens of thousands of unnamed signals that cannot be interpreted intuitively. This talk will highlight recent technologies from our lab to help guide data analysis. Some do's and don'ts of interpreting untargeted metabolomic data will be discussed. Evidence will be presented that challenges some of the common assumptions made about untargeted metabolomic datasets. The pros and cons of several metabolomic technology platforms that we have developed will be evaluated, and applications to cancer metabolism will be illustrated.

NMR and NMR/HS Hybrid Approaches for Targeted and Untargeted Metabolomics

Rafael Brüschweiler

Department of Chemistry and Biochemistry, Department of Biological Chemistry and Pharmacology Campus Chemical Instrument Center, The Ohio State University

A key challenge in metabolomics concerns the comprehensive, rapid and reliable identification of large numbers of metabolites in complex mixtures without the need for extensive purification. I will present new multidimensional NMR methods that significantly help accomplish this task, some of which are implemented on our public COLMAR suite of web servers and databases (<http://spin.ccic.ohio-state.edu/index.php/colmar>). A large number of metabolites are present in many biological systems whose identity is unknown, i.e. they are not yet contained in metabolomics databases. A general approach is described that synergistically combines NMR with modern mass spectrometry and combinatorial cheminformatics, which is directly applicable to complex mixtures without the need of any spectroscopic database information. It is further shown how the addition of nanoparticles to the NMR sample further simplifies this task by either quenching the NMR signals of metabolites with certain physical-chemical properties or by eliminating proteins as is the case for human serum. The interaction strengths between nanoparticles and metabolites can be quantitatively monitored by ¹³C transverse NMR spin relaxation experiments. In turn, the interaction propensities of individual amino acids can then be used to accurately predict the sequence-specific interaction strengths of intrinsically disordered proteins with nanoparticles. Such information should prove useful for the better understanding of nanotoxicity and for the design of nanomaterials with improved interaction properties.

Mining the Blood for New Cardiometabolic Hormones

Robert Gerszten

Beth Israel Deaconess Medical Center and Harvard Medical School

Our laboratory focuses on the nexus of cardiac and metabolic diseases. To expand the novelty and clinical impact of our studies, we have developed and incorporated emerging mass-spectrometry and aptamer technologies towards the discovery of new biomarkers and pathways. We make observations in humans and then turn to cell and animal based systems to test for causal relationships. Because metabolites and proteins are downstream of genetic variation and transcriptional changes, they serve as “proximal reporters” of physiology and may be highly relevant biomarkers for human diseases. A key question is whether proteins and metabolites identified in our discovery work participate in a functional manner in disease pathogenesis. In addition to cell and animal studies, we have turned to human genetics to understand the genetic architecture of circulating proteins and metabolites to identify causal pathways. Our research incorporates basic molecular and cell biology, genetics, chemistry, mass spectrometry and bioinformatics, all with a foundation in clinical medicine.

Decoding Food Flavor for Health Promotion: Targeted and Untargeted Approaches

Devin Peterson

Department of Food Science and Technology; Flavor Research and Education Center, The Ohio State University

Despite improvements in the food supply over the past century, consumer choice still plays an important role in the global burden of disease. One of the main factors that influence food choice is the product acceptability/ flavor quality. Hence flavor is key to implementing dietary consumption patterns that promote a healthy lifestyle. Advancements in horticulture and crop science have largely focused on disease/pest resistance and yield, and have overlooked the drivers that influence food flavor. This practice has challenged the consumption of healthier food options which are typically inferior in flavor quality. Flavor is a multimodal sensation arising from the integration of the gustation, olfaction, and somatosensory systems. This presentation will discuss analytical strategies to characterize flavor compounds in foodstuffs (i.e. whole grain, dairy, fruits) by targeted and untargeted chromatographic/mass spectrometry-based approaches. The application of flavor discovery to get more from nature, guided by science, to support the development of sustainable, nutritious, and healthy foods with higher product acceptability will be presented.

Integration of gene expression and metabolomics data unravels cell-type specific molecular signatures

Ewy Mathé

Department of Biomedical Informatics, The Ohio State University

While the value of metabolomics in translational research is undeniable, functional interpretation of disease-associated metabolites is difficult, and the biological mechanisms that underlie cell type or disease-specific metabolomics profiles are oftentimes unknown. To help fully exploit metabolomics data and to aid in its interpretation, we propose a global approach that integrates gene expression and metabolite measurements. Such integration will expand our understanding of the molecular mechanisms that underlie cancer and other diseases, which in turn will help guide the identification of novel therapeutic targets and clinically useful diagnostic and prognostic biomarkers. To accomplish this integration, we have developed a novel linear modeling approach that directly evaluates the relationship between gene and metabolites that are specific to a phenotype (e.g. cell type). Our approach was applied to publicly available data, including the NCI-60 cell lines, to test the hypothesis that gene:metabolite correlations differ between cell types, for example cells originating from different cancers or cells that respond differently to treatment. Subsequent pathway analysis on relevant gene:metabolite pairs reveals molecular functions that are dysregulated in specific cell types. Our proposed approach specifically uncovers gene and metabolite relationships affected by a cell or disease phenotypes, and will soon be available through a user-friendly framework.

LUNCH & LEARN ABSTRACTS

Listed in order of presentation

Deeper Insights with Metabolomics: Studying the War between Plants and Insects

Sven Heiling¹, Emmanuel Gaquerel², Aiko Barsch³, Heiko Neuweiger³ and Ian T. Baldwin¹

¹Max Planck Institute for Chemical Ecology, Department for Molecular Ecology, Jena, Germany; ²Centre for Organismal Studies Heidelberg, University of Heidelberg, Germany; ³Bruker Daltonics, Bremen, Germany

Exploring the diversity of natural products in plants requires efficient methods to gain sufficient structural information to rapidly discriminate known compounds from novel or closely related ones. This process can be rendered extremely challenging when analyzing profiles of genetically-manipulated plants in which natural product biosynthetic pathways are manipulated. Especially on further trophic levels, like herbivores feeding on those plants, the consequences of these manipulations are difficult to grasp. Efficient workflows which combine statistical data mining and automatic compound identification routines are therefore needed. Here, we present a software solution for a quick and efficient metabolite profile screening to unravel the function of wild tobacco plants in which the expression of several glycosyltransferase genes has been manipulated. These genes are part of the biosynthetic pathway leading to defensive 17-hydroxygeranyllinalool diterpene glycosides (HGL-DTGs). Furthermore we analyze the next trophic level, by profiling the metabolites and their changes within the tobacco hornworm *Manduca sexta*. We illustrate the power of this software based workflow for the discovery of gene-mediated glycosylations in the HGL-DTG pathway in tobacco and their consequences in the herbivore *M. sexta*.

Seminar Sponsored by Bruker Daltonics

Cuprizone Toxicity: The Intersection of Nutrition and Disease

Leah Shriver

The University of Akron

Multiple sclerosis (MS) is an immune-mediated neurological disorder that results from the destruction of the myelin-producing oligodendrocytes in the brain and spinal cord. Cuprizone, a copper chelator, is used to induce demyelinating lesions in mice and this serves as a preclinical model to test new regenerative therapies for MS. While the histological characteristics of the demyelinating lesions have been extensively explored, the biochemical mechanisms of toxicity are not well-characterized. We have used transcriptomics and metabolomics to explore cellular pathways involved in cuprizone-induced cell death. In addition, we investigated the dynamics of copper chelation by using mass spectrometry to characterize copper binding in the presence of small molecule mimics of protein copper sites. We find that in cells and animals, cuprizone treatment induces alterations in pathways associated with amino acid metabolism. Furthermore, cuprizone cannot chelate copper away from active site mimics suggesting that the *in vivo* effects of cuprizone on copper metabolism are more complex than copper depletion. Instead, cuprizone has the capacity to disrupt transaminase activity by forming a monohydrolyzed species with the vitamin cofactor pyridoxal 5' phosphate (P5P) bound through a Schiff base linkage. Our studies highlight the power of a systems approach to explore the *in vivo* effects of cuprizone and indicate that toxicity in the central nervous may be due to non-copper interactions and could be linked to the inhibition of P5P-containing enzymes.

Seminar Sponsored by Waters

Removing the Data Processing Bottleneck: Move Efficiently from Data to Structure to Pathway with Thermo Scientific™ Compound Discoverer™ 2.0

Amanda Souza

Thermo Fisher Scientific

Tackling the entire metabolome requires the right tools. Compound Discoverer 2.0 streamlines discovery metabolomics in one complete package. CD 2.0 offers robust algorithms for retention time alignment and unknown peak detection of high resolution accurate mass data. The program is fully integrated with mzCloud™, a comprehensive spectral library for confident identifications, and KEGG Pathway database for functional interpretation. CD also has built in statistical tools for data mining that reduce large datasets to a subset of meaningful metabolites.

Seminar Sponsored by Thermo Fisher Scientific

Blood pressure lowering effects in lentil-fed spontaneously hypertensive rats (SHR): A nutritional metabolomics approach

Michel Aliani^{1,2,3}, Matthew Hanson^{2,3}, Carla G. Taylor^{1,2,3}, Peter Zahradka^{1,2,3}

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Hypertension is a multifactorial disorder originates due to a complex combination of genetic, environmental and other factors such as diet, salt intake, age, race and gender as well as smoking, lack of exercise, stress and excessive alcohol intake. Pulse crops are nutrient dense foods containing high concentrations of fiber, protein, and various phytochemicals. In a previous study, we showed that lentils could attenuate the rise in blood pressure that occurs in SHR rats as they age, while other pulses such as beans, peas and chickpeas were ineffective. The objectives of this study were twofold: 1) to compare the metabolic profile of the urine samples from control and pulse-fed SHR in relation to the compounds present in lentils but not in other pulses, and 2) to identify the biochemical pathway(s) and the compound(s) in lentils responsible for the observed effect on blood pressure. Urine samples were collected from 17 week old male SHR rats (n=8/ group) after 4 weeks on a pulse-fortified diet (30% w/w) of either lentils (mixture of green and red), beans (mixture of pinto, red kidney, white navy and black beans), peas (mixture of yellow and red) or chickpeas, or a pulse-free control diet. All urine samples, pulse powders and control diets were analyzed by high performance liquid chromatography/quadrupole time-of flight mass spectrometry (LC-QTOF-MS) in both positive and negative electrospray ionization modes. Blood pressure was measured before the onset of experimental feeding and during the final week of the study (week 4) using tail-cuff plethysmography. Among the 27 significant metabolites found in urine from lentil-fed SHR compared to SHR fed control diet, 7 metabolites were not detected in the urine of SHR fed with other pulses. Citrulline was the only metabolite that has a known connection to blood pressure regulation *via* production nitric oxide (NO), a vasodilator involved in blood pressure regulation. Several arginine-related compounds that function as NO synthase substrates or inhibitors were also detected in lentils but not the control diet or in other pulse powders. In conclusion, consumption of lentils may increase the availability of arginine and several related compounds that could potentially contribute to the blood pressure-lowering effects of lentil-rich diets by elevating the production of NO.

Seminar Sponsored by Agilent

Differential Ion Mobility Spectrometry Dramatically Improves the Specificity of Untargeted Lipidomics Analysis by “Shotgun” Analytical Approaches

Paul RS Baker

SCIEX, Framingham, MA

The study of lipids in food has evolved in recent years from a basic composition analysis to the need for detailed lipid molecular species analysis. This change is driven in part by the recognition that the relationship between diet and health must be evaluated at the molecular level, and lipid analysis must provide specific structural details to fully understand the metabolism. Lipid identification starts with global lipid profiling strategies using electrospray ionization mass spectrometry (ESI-MS/MS). The complex data arrays generated during sample analysis can then be processed by lipid identification software and principal component analysis to generate candidate lipid biomarkers, which can then be validated by targeted quantitative analysis. Despite this seemingly straight-forward means to lipid biomarker discovery, the actual process is quite challenging due to the high number of lipid isobars and isomers that interfere with qualitative and quantitative analysis. Recently, Differential Ion Mobility Spectrometry (DMS) coupled to mass spectrometry has been shown to be very effective at resolving complex mixtures of lipid isomers without the need for extensive chromatography. In this report, DMS was used in tandem with a TripleTOF[®] instrument to generate untargeted lipidomics data via infusion in the MSMS^{ALL} scan mode (a universal “shotgun lipidomics” approach). The DMS enabled class isolation during analysis that removed isobaric interference during MS/MS experiments and provided clear identification and quantitation of the molecular species within each lipid class. Additionally, it was observed that different adducts within the same lipid class (e.g., chlorine Vs. acetate adducts of PC) can also be resolved, effectively solving a particularly challenging problem in infusion-based lipidomics. In summary, the combination of DMS with the MS/MS^{ALL} workflow provides a powerful means to improve the specificity of untargeted lipidomics analysis and increase confidence in lipid identification and quantification.

Seminar Sponsored by SCIEX

ORAL PRESENTATION ABSTRACTS

Wednesday, May 17th; Session 1A: Metabolomics Oral Presentations

Listed in order of presentation

Enhanced inhibitory function and metabolic dysregulation in *E. coli* induced by *L. acidophilus* fermented tea extracts

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Functional food such as tea drinks have been known for long time to have beneficial health effect. Meanwhile, the gut bacteria population was also known to modulate the utilization of food components, but the synergistic effect between these two factors were still unclear, their underline mechanism in disease prevention is not fully studied. This study examined the ability of *Lactobacillus acidophilus* (LA) to modify tea phenolic compounds, and therefore to enhance their cellular uptake when the pre-fermented black tea extract (BTE) was co-incubated with *E. coli*. The inhibitory effect of BTE with and w/o pre-fermentation were compared, several intracellular phenolic compounds and metabolic profiles of *E. coli* with and w/o treatments were also analyzed using HPLC-MS/MS-based approach. Our results showed statistically significant decrease of *E. coli* survival when treated with 25 mg/mL BTE, but when treated with LA pre-fermented BTE, 5, 10 and 25 mg/mL BTE all induced decreased *E. coli* growth. Intracellular concentration of (+)-catechin-3-gallate/(-)-epicatechin-3-gallate and (+)-catechin/(-)-epicatechin were significantly higher when the *E. coli* were treated with pre-fermented BTE in comparison to BTE treatment w/o LA pre-fermentation. Metabolic profiles of *E. coli* were also investigated to understand their metabolic response when treated with BTE, and much significant metabolic changes of *E. coli* were observed when treated with pre-fermented BTE. Clear trend of BTE concentration dependent effect to metabolites, such as creatinine, phenylalanine, valine, lactose, inosine 5'-triphosphate and NADP was noticed. Metabolic profiles were also used in partial least square-discriminant analysis (PLS-DA) to distinguish the pre-fermented BTE treatment groups from the control group and the BTE treatment groups, which indicated a large-scale *E. coli* metabolic activities dysregulation induced by the pre-fermented BTE. Our findings showed that LA fermentation may be an efficient approach to enhance phenolic bioavailability and to inhibit bacterial cells by dysregulating their metabolic activities.

Improving Sugar-Free Fruit Spread Flavor using Metabolomics

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Fruit spreads are a broadly consumed category of foods, with over one billion dollars in annual sales within the United States. However, due to the high sugar content of traditional jams, consumers with calorie-restrictions are unable to consume these products within the constraints of their diets, leading to stagnant sales. Although sugar-free and low-sugar spreads offer an alternative to traditional spreads for consumers, current products are plagued by flavor defects not present in traditional spreads. These flavor defects lead to low consumer acceptance of sugar-free spreads, making it more likely that calorie-restricted consumers may choose to avoid fruit spreads rather than consume unpalatable product. In order to improve the consumer acceptance of sugar-free spreads, a fusion of sensory science and small-molecule metabolomics, termed Flavoromics, has been used to understand chemical differences between traditional and sugar-free products which may contribute to these flavor defects. Twenty-three spreads from seven different fruits have been profiled using UPLC/ToF-MS, and models predictive of spread type have been developed and validated. Compounds significantly differing between product types have been isolated, recombined with jams, and analyzed using sensory panels to determine if they have a causal relationship with flavor, and the potential to influence acceptance. Using these techniques, compounds which modulate acidity and contribute to a sense of "rounded" flavor in fruit jams have been discovered. Knowledge of these compounds will allow for producers to tweak raw ingredient selection, fruit breeding strategies, and processing parameters, in order to naturally produce better tasting sugar-free fruit spreads.

Quantification of fructose-asparagine in human foods, animal foods and mouse ceca

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Salmonella, a worldwide foodborne disease, is estimated to cause more than 1.2 million illnesses with 450 deaths in the U.S. every year. It was recently discovered that fructose-asparagine (F-Asn), an Amadori product, can be utilized as a novel nutrient by *Salmonella enterica*. Knowing the concentration of F-Asn in human foods, animal foods, and mouse ceca will facilitate the understanding of *Salmonella* growth as well as benefit the development of therapeutics. The goal of this study is to build a liquid chromatography-mass spectrometry (LC-MS) method for F-Asn quantification and to quantify F-Asn from human foods, animal foods and mouse ceca. The F-Asn concentrations (nmol/mg of dry weight) were measured for these human foods: raw, fried and boiled Russet potatoes, raw, fried and boiled Yukon gold potatoes, raw, fried and boiled Red potatoes, French fries and potato chips, raw, fried and boiled rice, bread crust and inside, raw and boiled corn, fresh, dried and canned apricots, raw and canned peaches, raw and boiled asparagus, raw lettuce. All food samples except for raw dried rice had detectable amounts of F-Asn. Dried apricots had the highest F-Asn concentration, 8.4 ± 1.0 nmol/mg. Fried potatoes had much higher level of F-Asn than raw potatoes, consistent with previous studies measuring acrylamide. The measured concentrations (nmol/mg of dry weight) of F-Asn from animal foods were 0.007 ± 0.002 , 0.07 ± 0.02 and 0.40 ± 0.01 for hog feeds, poultry feeds and mouse chow, respectively. Germ free mouse cecal contents contained 2.7 ± 0.2 nmol/mg of F-Asn while germ free mice colonized with *Salmonella* had 0.008 ± 0.003 nmol/mg. Germ free mice had more than 300 times greater concentration of F-Asn accumulated in the ceca compared with the *Salmonella* infected group, which suggested that mice cannot utilize F-Asn but *Salmonella* does.

Elucidating urinary markers of strawberry consumption in smokers and nonsmokers

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Promising results from clinical and pre-clinical research support a potential role for berries and their associated phytochemicals in the prevention of oral cancer. Strawberries are a commonly consumed source of berry phytochemicals. Key phytochemicals, such as anthocyanins and flavonoids, are hypothesized to be the bioactive agents in berries, but these compounds tend to be poorly absorbed or metabolized prior to absorption. Little work has been done to understand major metabolites associated with strawberry consumption. Our group recently conducted a randomized, cross-over, placebo-controlled clinical study, in which cigarette smokers and non-smokers consumed a novel confection containing lyophilized strawberry powder (24 g/day) and placebo confections, each for 7 days. The objective of this work was to investigate key markers of strawberry exposure in the urine of healthy individuals and individuals at high risk for oral cancer using a metabolomics approach. Urine samples from 9 smokers and 10 non-smokers following placebo and strawberry interventions were volumetrically normalized according to osmolality and profiled using UHPLC-QTOF-MS (ESI+/-). Data were modeled using multilevel PLS-DA to capitalize on the paired design of the study. Significant markers of strawberry exposure were selected based on VIP score (>1) and *P* value following a paired t-test with Benjamini-Hochberg multiple testing correction ($P < 0.05$). A total of 46 markers were found, 31 of which were elevated following the strawberry treatment and 15 following the placebo. Example marker identities include urolithin A, as well as phase II metabolites of 4-hydroxy-2,5-dimethyl-3(2H)-furanone, an important aroma compound found in strawberries. The urinary metabolomic fingerprint found in this work will inform future studies on the potential health benefits of strawberries and will also provide insight for new urinary markers for compliance measurements in future clinical trials.

Application of ^{13}C -labeling approach to reveal biochemical mechanism of oil synthesis in pennycress (*Thlaspi arvense* L.) embryos

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Pennycress (*Thlaspi arvense* L.) produces oil up to 35% of the total seed biomass, and its overall fatty acid composition has shown to be suitable for industrial products and biodiesel. However, for this plant to become an economically viable, its oil production needs to be improved. In pennycress embryos, fatty acid synthesis (FAS) requires carbon skeletons, energy, and reducing power; all of these are provided by central metabolism. We hypothesize that one or more steps is/are limiting FAS. To test this hypothesis, we have previously conducted a metabolomics study that identified the main carbon and nitrogen sources, and the active biochemical pathways during FAS in pennycress embryos. We are now performing ^{13}C -Metabolic Flux Analysis to quantify *in vivo* carbon fluxes through each metabolic pathway, which will pinpoint potential bottleneck(s). First, this approach requires establishing *in vivo* culture conditions that mimic the development of pennycress embryos *in planta*. Given that the endosperm provides embryos for necessary substrates, the endosperm composition was analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) to design the culture growth medium. Second, the efficiency with which pennycress embryos convert carbon into biomass was measured to be 93.6%, which is higher than other Brassicaceae, indicating the importance of revealing biochemical mechanisms oil synthesis in these embryos. Third, pennycress embryos were incubated with ^{13}C -labeled substrates until metabolic and isotopic steady states are attained. The labeling abundances in intracellular metabolites were then quantified by LC-MS/MS. The main findings from the ^{13}C -glucose labeling experiment show the higher activity of oxidative pentose phosphate pathway in cytosol than in plastid, and reversibility of glycolysis due to aldolase activity. In addition, the ^{13}C -glutamine labeling experiment results indicate no occurrence of gluconeogenesis, but reversibility of isocitrate dehydrogenase in Krebs cycle and active NADP-dependent malic enzyme. Future plans include the incorporation of all the labeling information into a mathematical model to generate a flux map, which will identify the bottleneck(s) in FAS. Understanding the biochemical basis of oil synthesis in pennycress embryos is fundamental to advance future breeding and/or metabolic engineering efforts aiming at increasing FAS.

Induction-Based Fluidics: An Alternative Ionization Source for LC-MS/MS Analysis of RNA Nucleosides

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Ribonucleic acids (RNA) play important roles in an organism. RNA can be post-transcriptionally modified, and these modifications may affect RNA stability, structure, function, and translation. Liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) has been the gold standard for the analysis of modifications in ribosomal (rRNA) and transfer (tRNA) RNAs for many years. Routine analysis of RNA modifications by LC-ESI-MS/MS typically requires 5-50 µg of sample. Since rRNA and tRNA together account for 95-99% of the total RNA content of an organism, sample limitation has not generally been an issue. However, recent discoveries have pushed the field more towards the detection of modifications in other kinds of RNA (e.g. messenger RNA or mRNA). In this case, sample consumption becomes more critical. For example, current protocols for mRNA purification yield tens to hundreds of nanograms of purified mRNA. One way to reduce sample requirements would be to make use of capillary chromatography and nanoelectrospray (nESI). Unfortunately, due to the matrix complexity of biological samples, nESI capillary clogging is a recurring problem. Recently, Sauter and collaborators have shown that induction based fluidics (IBF) can generate femto- to nano-liter drops with high reproducibility. Additionally, they have shown that IBF can be used as an ionization source with the potential to both increase ionization and transmission efficiencies in MS. The work presented here is a proof of principle for LC-IBF-MS/MS. The study was carried out using a standard RNA nucleosides mixture, employing an UPLC (nanoAcquity, Waters) and a linear ion trap mass spectrometer (LTQ-XL, Thermo Fisher). Though it is still in the early stages of research, initial results appear promising.

Implementation of an Ion Carpet Array for Facile and Compact Surface Induced Dissociation

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Surface-induced dissociation (SID) has emerged as an effective tool for probing the topology of protein complexes. Current SID devices are installed in-line with existing ion paths. In such a design, angled electrode voltages are tuned to direct ions into a surface within the device and subsequently pull product ions back onto the original trajectory axis. However, these devices require ten additional electrodes and occupy 3 cm of the ion path. Herein an ion carpet, a set of in-plane, resistively-linked concentric electrodes, is implemented to collect SID products in a novel SID device designed to reduce the number of electrodes and space required for SID, thereby simplifying the experiment. The new SID device was installed preceding a truncated transfer cell in a Synapt G2 system. The ion carpet consists of 14 concentric electrodes, with the innermost electrode (5 mm in diameter) serving as the aperture through which ions pass after the SID collision. Preceding the ion carpet is a cone electrode (5 mm tall with a 4 mm base). In MS experiments, this cone holds a slightly repulsive voltage to direct ions around itself as they pass through the device, similar to jet disruptors in source interface regions. The ion carpet axially focuses ions as they exit the device, allowing for analysis of intact analyte. When ions are sufficiently accelerated into the device, they collide with the cone electrode, and subsequent fragments are funneled through the back end of the device. This was demonstrated with streptavidin, C-reactive protein, and serum amyloid P component. These previously-studied systems are known to undergo specific fragmentation when subjected to SID, cleaving protein complexes to yield subunits or sub-complexes that retain charge that is proportional to their fraction of the intact complex mass. With these reliable indicators, SID of varying energies was achieved with minimal tuning.

First Application of SL-MALDI-MS Imaging to a Synthetic Material Towards Understanding Interfacial Characteristics of Polymer Films

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A material interacts with its environment through its interfaces; therefore, advancing analytical techniques that provide information specific to material surfaces is an important objective for materials scientists. There is a limited number of surface analysis methods, hampering elucidation of the fundamental interactions occurring when materials are applied in practical situations, as in tissue engineering, coatings, and adhesives. Amongst the currently available surface-specific techniques (for example, XPS and SIMS), surface-layer matrix assisted laser desorption ionization mass spectrometry (SL-MALDI-MS) is an emerging surface characterization method utilizing a surface specific sample preparation procedure and the advantages of MS, especially rapid data acquisition and molecular-level information. Here, we combine the imaging capabilities of MALDI with SL-MALDI-MS to image defects and segregation in polymer films. The polymer films were prepared by either spin casting or solution casting methods onto a metal substrate. DCTB and DHB matrices, mixed with silver trifluoroacetate cationization agent, were sublimated onto the casted films so that the representative film surface (top 2 nm) remained unperturbed. Different surface characteristics and deformations have been investigated and imaged, such as mechanical defects, microphase separations, material transfer, and lateral heterogeneity. More importantly, oligomer ion intensity variations in areas with a compression defect were observed in the image of a similar 6,000 Da PS film. The observed variations in intensity suggest that this technique would be suitable for surface defect analysis. Our results indicate that film regions perturbed by compression acquired higher surface roughness, thus providing a larger surface area in contact with matrix. Based on these findings, material transfer caused by mechanical contact or adhesive/interfacial failure would be analyzable with SL-MALDI-MS imaging.

Investigation of Protein-Protein Interactions using Surface Induced Dissociation (SID) MS

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Proteins have well-defined tertiary structures and form quaternary assemblies via non-covalent interactions. Structural characterization of protein structures is necessary to understand how proteins accomplish their physiological functions. One important tool for structural analysis is native mass spectrometry (MS), which is commonly employed to determine the stoichiometry and topology of protein complexes. Currently, it is not evident whether native MS in combination with gas phase disruption techniques is suitable to obtain quantitative information on the number and nature of non-covalent interactions between constituting subunits of a protein complex. To answer this question, a set of homologous protein complexes with distinct non-covalent interactions between constituting subunits was used. Complexes were analyzed by a modified Synapt G2 mass spectrometer (Waters) with a surface induced dissociation (SID) device installed between a truncated Trap cell and the ion mobility cell. SID data were obtained for six homologous tryptophan synthase hetero-tetramers. We observed an energy dependent dissociation into sub-complexes by collision of the corresponding complexes with a surface (SID). Interestingly, homolog complexes differ in the energy necessary for their dissociation. This indicates that similar protein-protein interactions with distinct inter-subunit connections can be differentiated by this gas-phase disruption technique. Surface induced dissociation (SID) is suitable to distinguish between protein-protein interactions of homologous complexes.

Determination of the Origin of Doubly-Cationized Monosialylated Fragments in MS/MS spectra of Singly-Cationized LSTb and GM1 using Ion Mobility Mass Spectrometry

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Sialic acid (NeuAc)-containing fragments $[\text{NeuAc-H}+2\text{Na}]^+$ were surprisingly detected in MALDI-LID-MS/MS spectra of $[\text{M}+\text{Na}]^+$ ganglioside ions in a recent study (*Carbohydr. Res.* 2016, 431, 6-14). The origin of $[\text{NeuAc-H}+2\text{Na}]^+$ fragments in MS/MS spectra of $[\text{M}+\text{Na}]^+$ ions were further investigated by analyzing $[\text{M}+\text{A}]^+$ (where A is Li^+ , Na^+ , K^+ , or Cs^+) ions of a monosialylated glycan (LSTb) and a monosialylated ganglioside (GM1) using a variety of MS/MS techniques. $[\text{NeuAc-H}+2\text{A}]^+$ fragments were observed during MALDI-LID-MS/MS as well as ESI-CID-, ion mobility spectrometry (IMS)-CID-, and HCD-MS/MS of LSTb and GM1 precursor ions whose m/z values corresponded to $[\text{M}+\text{A}]^+$ ions. Additionally, $[\text{NeuAc-H}+\text{A}_1+\text{A}_2]^+$ (A_1 is Li^+ , K^+ , or Cs^+ and A_2 is Na^+) B_1 fragments were detected in MALDI-LID-MS/MS spectra of $[\text{M}+\text{A}_1]^+$ ions. Based on ESI-IMS-CID-MS/MS performed after IMS separation, the $[\text{NeuAc-H}+2\text{Na}]^+$ B_1 ions were detected predominantly due to fragmentation of multiply charged multimer ions $[(n\text{M}+n\text{Na})^+]$, $n = 2-4$ that were isobaric with $[\text{M}+\text{Na}]^+$ ions of LSTb and GM1, but were separated at different IMS drift times. However, $[\text{NeuAc-H}+2\text{Na}]^+$ B_1 ions were also detected with low intensities in ESI-IMS-CID-MS/MS spectra of $[\text{M}+\text{A}]^+$ ions of LSTb and GM1 at higher collision energies. Furthermore, $[\text{NeuAc-H}+2\text{A}]^+$ and $[\text{NeuAc-H}+\text{A}_1+\text{A}_2]^+$ B_1 ions observed during MALDI-LID-MS/MS of LSTb and GM1 may also originate from multiply charged multimers of $[\text{M}+\text{A}]^+$ or $[\text{M}+\text{A}_1+\text{A}_2]^+$ ions. Overall, this study demonstrates that the formation of $[\text{NeuAc-H}+2\text{A}]^+$, originating from sialylated glycan and glycosphingolipid ions and their multimers can be elucidated by combination of IMS separation of precursor ions and MS/MS.

Thursday, May 18th; Oral Presentations

Listed in order of presentation

Positive associations between serum levels of dioxin-like pollutants and the circulating cardiometabolic disease risk biomarker Trimethylamine-N-oxide

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Cardiovascular disorders are largely caused by genetic and environmental factors. Understanding how these factors intersect to determine individual disease risk is a critical challenge. Well-studied “lifestyle dependent” determinants of increased cardiovascular disease risk include smoking, physical inactivity, and poor nutrition, but emerging data now implicate exposures to persistent environmental pollutants as an important contributor to inter-individual variability in cardiovascular disease risk. It is also critical to identify novel biomarkers that link nutrition, toxicant exposure, and cardiometabolic disease risk. Interestingly, emerging diet-derived biomarkers such as trimethylamine-N-oxide (TMAO), carnitine, certain fatty acids, and choline have strong positive relationships with heart disease risk, whereas plasma levels of other nutrients, for example plant-derived carotenoids, sterols and polyphenols are correlated with reduced risk. Quantitating levels of these nutrient biomarkers in individuals with well-defined environmental exposures and well documented metabolic disease histories may shed light on why certain people are more or less prone to environmentally induced diseases. For example, recently, we published that in preclinical models, exposure to dioxin-like pollutants can increase circulating levels of TMAO. In our preclinical studies, dioxin-like PCBs strongly increase the enzyme responsible for TMAO production, FMO3, resulting in amplified increases in TMAO levels. We have now begun to investigate if these associations between pollutant exposure and TMAO are evident in the highly exposed Anniston, Alabama population. We have used mass spectrometry methods to quantitate TMAO in archived plasma samples, and have determined that higher body burden of dioxin-like pollutants is significantly associated with increased circulating TMAO levels in humans.

Data-Independent Mass Spectrometry of Modified RNA

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To continue to push our understanding of the identity, location and role of RNA modifications in human disease, new analytical tools capable of complex sample analysis are needed. The common approach for RNA modification mapping (i.e., enzymatic digestion of an RNA with subsequent analysis by LC-MS/MS) has gone essentially unchanged for many years. In a typical mass spectrometric experiment, the product ion spectra needed for sequence confirmation are generated through data-dependent acquisition (DDA). The limitations of DDA - including bias towards the most abundant ions, lack of reproducibility, and low quality MS1 chromatograms - have been well documented. One approach to overcome the limitations of DDA is the use of data-independent acquisition (DIA) mass spectrometry. MS^E, a type of DIA method, is performed by collecting alternating low and high energy scans over the entire mass range. The low energy scans contain precursor ion information and the high energy scans contain multiplexed product ion spectra where all ions above the detection limit have been fragmented. In our lab we are focused on applying MS^E methodology to RNA modification mapping by LC-MS. A preliminary comparison of DDA and MS^E strategies for RNA modification mapping of *E. coli* total tRNA samples on a Waters Synapt G2-S will be presented. Method development considerations, data processing hurdles, and ion-mobility enhanced MS^E will also be discussed.

Metabolomics aids in discovery of putative bioactive compounds from tomatoes in a model of keratinocyte carcinoma

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Adding tomatoes to the diet has been shown to lessen erythema and inflammation after exposure to ultraviolet light in humans. Carotenoids, ubiquitous plant pigments responsible for the red color of tomatoes, have received the most attention for this photoprotective effect. However, single carotenoids appear less effective than whole foods suggesting other secondary plant metabolites may play a role. Our group has observed that tomato supplementation can decrease tumor number in a murine model of ultraviolet light-induced keratinocyte carcinoma of the skin, suggesting modulation of the skin metabolome by tomato bioactives. Outside of carotenoids however, other tomato phytochemicals have not been well studied in this context. The objective of this study was to use untargeted metabolomics to profile and compare the skin metabolomes of mice fed control and tomato-containing diets to elucidate additional putative bioactive compounds responsible for the observed reduction in tumor number. Male hairless and immunocompetent SKH-1 mice were fed an AIN-93G diet, an AIN-93G diet + 10% freeze dried red tomato powder (high in all-*trans*-lycopene) or 10% *tangerine* tomato (a unique tomato variety high in *cis*-lycopene and lycopene precursors) powder for 35 weeks. Methanolic extracts of skin were analyzed to compare differences in metabolite profiles between the control and tomato fed mice, focusing on compounds derived from tomatoes and their *in vivo* metabolites. By employing UHPLC-QTOF-MS-based untargeted metabolomics, we were able to detect over 6,000 compounds in skin, and distinguish metabolite profiles based on diet type using multivariate statistics. Tomato glycoalkaloid metabolites were significant discriminators of animals fed tomato diets and are plausible bioactive compounds from tomatoes. An understanding of the metabolomic changes in skin with tomato supplementation can generate testable hypotheses to better understand ways in which this fruit may be exerting protective effects.

Use of surface induced dissociation (SID) as a top-down fragmentation technique

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Top-down mass spectrometry has emerged as a useful tool in native mass spectrometry and proteomics due to its ability to analyze intact proteins and identify sites of post-translational modifications, providing increased information about biologically-relevant proteoforms. Currently used dissociation techniques each have their own advantages, but further development of new dissociation methods that can provide sufficient sequence information with multiple ion types and limited instrument modifications is still desired. Work has been done to characterize SID as an alternative dissociation technique for top-down analysis using ubiquitin, cytochrome c, myoglobin, and carbonic anhydrase (8.5 to 29.1 kDa) on both ion mobility and Fourier transform MS instruments. In these experiments, SID has yielded a larger number of backbone cleavages with greater variety in that SID appears to produce *c/z* and *a/x* ion pairs alongside the *b/y* ions of CID. Additionally, SID has shown little charge state dependence, an advantage over charge-dependent fragmentation techniques such as ECD. Traveling-wave ion mobility was employed on the Waters Synapt G2 mass spectrometer to extract out the differently charged fragment ions to deconvolute each spectrum for simpler analysis. While ubiquitin has provided the greatest % sequence coverage thus far, all other proteins have shown sequence coverage comparable to or better than CID on the same instrument as well as varying ion pair types that aid in confidence of fragment assignment. Current SID experiments on the Q-TOF platform require acquisition times greater than other top-down dissociation methods to obtain the signal-to-noise required for thorough sequence coverage, partially because of the larger numbers of fragment ions produced (spreading signal over more fragments). Optimization of product ion collection will be an important next step to perform top-down SID on a chromatographic timescale. Overall, results with SID have shown good sequence coverage, a variety of ion types, and little charge state dependence.

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. Unfortunately, rice is affected by many pathogens that substantially decrease yield worldwide. Reductions in rice production resulting from pathogen attack result in millions of dollars in economic losses to growers and decrease global food security. Among these diseases, rice blast, which is caused by the fungus *Magnaporthe oryzae*, is particularly devastating, routinely causing 10-30% yield losses. Pathogens such as rice blast are most effectively controlled by host resistance, which also eliminates fungicide contamination on rice grains, and thereby protects human health. While the genetic foundation of host immunity is well-studied, the role of specific metabolites and biochemical pathways in this process is unclear. Some insight into the potential biochemical mechanisms underlying host immunity has been provided using genome wide association studies (GWAS), which use a genome-wide set of genetic markers in different individuals or a population to determine whether any variant is associated with a trait. Previous and ongoing work in the Wang lab has generated a GWAS database from rice lines resistant and susceptible to the rice blast fungus. We are combining this data with metabolomic analyses of resistant and susceptible rice during pathogen infection (metabolome GWAS, or mGWAS). To allow profiling of a broad range of compounds, metabolomic analyses are being conducted across a range of metabolomic platforms, including: LC-MS/MS (broad-spectrum metabolomic profiling, phenolics/phenylpropanoids, phytohormones, lipids and sterols); GC-MS (phenolics, terpenoids); and zonal capillary electrophoresis (rapid profiling of sugars and organic acids). Identification of specific metabolite shifts in resistant vs. susceptible rice lines will, when combined with GWAS data, allow the identification of specific QTLs or genes for use in breeding programs designed to increase the resistance of this crop to rice blast.

Investigation of Surface Energy in Paper Spray Ionization for the Direct Analysis of Dried Biofluids Using Mass Spectrometry

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Direct mass spectrometric analysis of both dried and wet blood spots is possible from hydrophobic paper substrates. When filter paper is exposed to silane vapor, the paper's surface energy is decreased to approximately 44 mN/m, and aqueous-based samples (surface tension ~72 mN/m) are unable to wet the treated paper, instead resting on the surface as a drop. After drying, these sample drops are confined to a reproducible area determined by the silane treatment time (e.g., sample area is 0.014 ± 0.003 cm² when paper is treated for 30 minutes). This is compared to the area 0.13 ± 0.05 cm² produced on untreated paper with >2X the relative standard deviation. Because sample is focused on one side of the hydrophobic paper and surface energy of the paper is lowered, target small molecules are less likely to form strong interactions with the paper surface, allowing the molecules to be eluted and ionized more easily when compared to untreated hydrophilic paper substrate. In the detection of drugs of abuse from dried blood spots, this modification has resulted in sub-ng/mL limits of detection, which is an order of magnitude lower than traditional paper spray mass spectrometry. Additionally, by modifying the relative surface energies of the solvent and paper surface, the ionization mechanism can be manipulated, and either electrospray or electrostatic spray may be performed. Investigation of these parameters has been performed with the use of hydrophobic rectangles and triangles. Preliminary data suggests manipulation of (i) surface energy of the paper; (ii) surface tension of the solvent; (iii) distance between the paper surface and the inlet; and (iv) paper geometry determines the type of ionization (e.g., electrostatic spray or electrospray) that occurs which also influences the ion yield.

POSTER PRESENTATION ABSTRACTS

(1) Stressor-induced immunomodulation is attenuated by prebiotics and milk oligosaccharides: role of the colonic metabolome

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Prebiotics and milk oligosaccharides have been shown to impact the gut microbiota and reduce many of the effects of stressor exposure. We tested whether the social disruption (SDR) stressor results in metabolomic changes in the colon, and whether prebiotics (blend of galactooligosaccharides (GOS) and polydextrose (PDX)) and/or milk oligosaccharide sialyllactose (Lacprodan SAL-10®, SL) support a normal metabolome in the presence of stress. Male mice were placed on one of the experimental diets for 14d: a) SL [2.2g/kg], b) GOS+PDX [15g/kg each] + SL [2.2g/kg] or c) Control [cellulose as fiber source]. Mice were then either exposed to the SDR stressor, which entailing repeated social defeat for 2 hrs per day for 6 days, or left undisturbed as controls. Metabolites in the colonic contents were assessed using LC/MS. Stressor-exposed mice fed the control diet showed significant differences in 116 out of 529 colonic metabolites, compared to controls. This was partly due to significant reductions in dipeptides and amino acids, as well as significant increases in nucleotides and sphingolipids. Stressor-exposed mice fed diets enriched with prebiotics or milk oligosaccharides showed similar changes in dipeptides, nucleotides, and sphingolipids, but also showed increases in polyunsaturated fatty acids and endocannabinoids, compared with the non-stress groups. Serum IL-6 and IL-1 β were significantly increased in stressor-exposed mice, but this effect tended to be attenuated by prebiotics or milk oligosaccharides (diet x stress interactions, $p=0.081$ and 0.056 , respectively). This study demonstrates that dietary prebiotics and milk oligosaccharides can impact the colonic metabolome to potentially attenuate stressor-induced immunomodulation.

(2) Extraction of MC-LR from Spiked Serum and its Quantification using LC-ESI-orbitrap-MS

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Microcystins (MCs) are toxins produced by cyanobacteria which appear in fresh water during harmful algal blooms. They are cyclic heptapeptides that contain unusual amino acid ADDA. MCs were responsible for the water crisis of 2014 in Toledo, Ohio, as well as deaths of several patients in Brazil in 1996. Their major toxic effects include hepatotoxicity and neurotoxicity, and it is therefore important to detect MCs in biofluids. The most common microcystin is MC-LR. Two different SPE cartridges were tested for purification and preconcentration of MC-LR from MC-spiked human serum. An optimized SPE procedure led to MC-LR recovery between 95 and 101%. A parallel LC-ESI-SIM-MS and MS/MS method was optimized for the separation and quantification of MC-LR using an orbitrap mass spectrometer (Thermo). HPLC (Shimadzu) was used for separation of MC-LR with a C8 column and its quantification was achieved by ESI-MS in positive ion mode using SIM scans and an Orbitrap Fusion mass analyzer. The peak area of the singly-charged protonated ion of MC-LR (m/z 995.56) was used to calculate the recoveries and quantify MC-LR in serum. The percent recovery was determined when peak area of MC-LR ion after preconcentration was divided by the average extracted ion chromatogram peak area of this ion in a control sample and multiplied by 100. For MS/MS, MC-LR ions were fragmented by higher-energy collisional dissociation (HCD) and its fragments were detected using ion trap mass analyzer. The fragment ion at m/z 135.08 was used to confirm the structure of MC-LR. It was possible to extract and detect MC-LR spiked in serum at concentration level of ~ 1 ppb. Current experiments aim to improve the extraction of MC-LR from serum as well as its detection and quantification by LC-ESI-MS.

(3) Untargeted Metabolomics of Probiotic Yogurt Consumption via LC-MS and NMR Methods

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Probiotic foods are often used to modulate intestinal microbiota to improve colon health. The mechanistic underpinnings and accurate quantification of their impact on metabolism and the composition of gut microbiota remains poorly understood. Combining metabolome and microbiome analyses will allow for the generation of hypotheses that address these topics at the molecular level and may inform nutritional approaches to promote human health. Groups of metabolites may be biomarkers for consumption of specific foods, enabling objective dietary intake measures. We hypothesize that consumption of probiotic yogurt with live and active cultures (*L. acidophilus*, *B. bifidum*, and *L. paracasei*) can generate an identifiable metabolic fingerprint. We will inoculate germ-free mice with a humanized microbiome as a model to determine if the fingerprint is differentiable between probiotic yogurt, inactivated yogurt (yogurt control), milk (dairy control), or water (negative control) (n=12/group). Mice will be randomized (n=5/cage) and fed an AIN-93G diet for 3 weeks before baseline sacrifice (n=1/cage). Remaining mice (n=4/cage) will receive dietary treatment for 3 weeks before sacrifice and tissue collection. Polar and non-polar extracts of colon and serum samples will be analyzed using LC-MS (QToF) and NMR (¹H and ¹³C) approaches. Multivariate statistical analyses—PCA and OPLS-DA—are expected to yield a profile of metabolites that differentiate the four dietary treatments. We anticipate alterations in metabolites associated with anaerobic respiration and the metabolism of branched-chain amino acids, short-chain fatty acids, amino acids, and bile acids in the probiotic yogurt treatment group. Furthermore, we hypothesize that host-microbiome interactions influence metabolic changes. We will attempt to integrate metabolomic and metagenomic microbiome data (Pathway Architect, Agilent) to provide new insight into how the microbiome mediates metabolism. Application of these results may provide more objective assessment measures of yogurt intake and aid in hypothesis generation pertaining to the role of probiotic yogurt in colon health.

(4) Cultivar Specific Changes in Primary and Secondary Metabolites in Pak Choi (*Brassica rapa*, *Chinensis*. Group) by Methyl Jasmonate

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Pak choi is a popularly consumed vegetable in China and is showing an increase in consumption in Europe and North America, primarily due to its comparatively mild flavor. As a *Brassica* vegetable, pak choi provides a number of phytonutrients; in particular, glucosinolates. Although its popularity is increasing, the nutritional quality of pak choi has not been thoroughly investigated, and only a few studies have reported glucosinolate profiles from pak choi. Glucosinolates, their hydrolysis products, and primary metabolites were analyzed in five pak choi cultivars to determine the effect of 500 μ M methyl jasmonate (MeJA) on metabolite flux from primary metabolites to glucosinolates. Among detected glucosinolates, indole glucosinolate concentrations (153-229%) and their hydrolysis products increased with MeJA treatment. Total isothiocyanates concentration changes by MeJA were associated with epithiospecifier protein activity. Total nitrile formation (%) out of total hydrolysis products was significantly increased by MeJA treatment. Goitrin concentration was significantly decreased by MeJA treatment in all cultivar. However, changes in glucosinolates and hydrolysis products differed among cultivars. Primary metabolites including amino acids, organic acids, and sugars also changed with MeJA treatment in a cultivar-specific manner. Decreased sugar level suggests that they might be a carbon source for secondary metabolite biosynthesis in MeJA-treated pak choi. The present study suggests that MeJA can be an effective agent to elevate indole glucosinolates and reduce goitrogenic effect in pak choi.

(5) High-throughput quantification of the levels and labeling abundance of free amino acids by liquid chromatography tandem mass spectrometry

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Given that amino acids (AAs) are extensively involved in numerous biochemical reactions, they play a key role in carbon flow throughout central metabolism. The common and powerful approach for quantifying *in vivo* carbon distribution is ¹³C-based metabolic flux analysis which involves providing a system of interest with ¹³C-labeled substrates and follow their incorporation into biochemical pathways. Accurate assessment of mass isotopomer distributions (MIDs) of intracellular metabolites, such as free AAs, is crucial for quantifying *in vivo* fluxes. To date, the majority of studies that measured AA MIDs have relied on the analysis of proteinogenic rather than free AAs by: i) GC-MS, which involved cumbersome process of derivatization, or ii) NMR, which requires large quantities of biological sample. In this work, the development and validation of a high-throughput LC-MS/MS method allowing the quantification of the levels and labeling of free AAs is described. Sensitivity in the order of the femtomol was achieved using multiple reaction monitoring mode (MRM). The MIDs of all free AAs were assessed without the need of derivatization, and were validated (except for Trp) on a mix of unlabeled AA standards. Finally, this method was applied to determine the ¹³C-labeling abundance in free AAs extracted from maize embryos cultured with ¹³C-glutamine or ¹³C-glucose. Although Cys was below the limit of detection in these biological samples, the MIDs of a total 18 free AAs were successfully determined. Due to the increased application of tandem mass spectrometry for ¹³C-Metabolic Flux Analysis, this novel method will enable the assessment of more complete and accurate labeling information of intracellular AAs, and therefore a better definition of the fluxes.

(6) Alleles of *tangerine* Differentially Alter Carotenoid Profiles in Tomato Fruit

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Carotenoids are naturally occurring pigments that give tomato (*Solanum lycopersicum*) fruits their color. Many carotenoids have nutritional benefits as vitamin A precursors, and their consumption is associated with decreased risk for many diseases. Structural and regulatory genes affecting biosynthesis, development and ripening alter the accumulation of specific carotenoids. Alleles of *tangerine* interrupt the carotenoid pathway by disabling carotenoid isomerase (CRTISO) resulting in the accumulation of bioavailable tetra-*cis*-lycopene. The *tangerine* allele *t* is a deletion spanning the first exon of CRTISO while *tangerine virescent* (*t'*) is a single nucleotide insertion generating a premature stop codon after the second exon. Alleles of the transcription factor RIN regulate the onset of ripening and therefore flux through the carotenoid pathway. To test hypotheses about the effect of allelic variation for *tangerine* with and without the *rin* mutation, a segregating population was created, selfed to F5, and analyzed using high performance liquid chromatography. Fruits with *t'* accumulated ~1.5x more carotenoids than those with *t*, with the differential effect detected in both wild-type and *rin* backgrounds. The *rin* mutation reduced fruit carotenoid concentrations by ~10x. We hypothesize that alleles of *tangerine* differentially affect carotenoid pathway flux as a result of a transcript regulated mechanism. Characterization of other metabolic pathways affected by *tangerine* alleles due to metabolic crosstalk is underway. Studies investigating the transcriptome differences that occur due to allelic variation in *tangerine* will also help elucidate mechanisms underlying changes in carotenoid biosynthesis and related pathways.

(7) Understanding the role of metabolism in the interaction between *Pantoea stewartii* subsp. *stewartii* and Maize

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Stewart's Wilt Disease of maize poses a serious risk to farmers who plant susceptible varieties. Young plants are particularly prone and crop loss can reach 100%, whereas older plants are more likely to survive but suffer poor seed yield and quality. The ability of *Pantoea stewartii* subsp. *stewartii* to proliferate and cause this devastating disease in maize is dependent on WtsE, an AvrE-family type three effector protein translocated from the bacteria into maize cells via type III secretion. In the lab, we examine maize responses to wild-type and *wtsE* mutant strains of *P. stewartii*. We previously determined that WtsE causes induction of maize phenylpropanoid gene expression and resulting accumulation of secondary metabolites, including salicylic acid and coumaroyl tyramine. More recent studies indicate that WtsE also perturbs plant primary metabolism. Because *P. stewartii* can proliferate in the apoplast of maize leaves, we have utilized Liquid Chromatography Tandem Mass Spectrometry to quantify several classes of metabolites accumulating in this intercellular space following infection with wild-type and *wtsE*-mutant strains of *P. stewartii*. We observe WtsE-dependent increase in several metabolites within the apoplast. The apoplast/cellular distribution of other metabolites are stable whether or not the bacteria deliver WtsE, indicating targeted perturbation of the apoplast environment rather than cellular disruption. Additional experiments suggest that *P. stewartii* can utilize several of the apoplast-accumulating compounds as nitrogen sources to facilitate its growth *in planta*. Future studies aim to further elucidate how WtsE-dependent perturbations of the maize apoplast contribute to *P. stewartii* virulence.

(8) Quantitative lipidomic analysis of hepatic steatosis by sequential precursor ion fragmentation on a hybrid quadrupole time-of-flight mass spectrometer

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Mass spectrometry based lipidomics can determine the lipid molecular species composition of biological samples. Because of the central role of lipid metabolism in cellular structure and energy homeostasis and the interplay between lipid metabolism genetics, diet and environment lipidomics can be valuable for diagnosis and prognosis of human disease. Accumulation and retention of lipids in the liver as a result of metabolic disease, exposure to drugs and toxins and alcohols results in hepatic steatosis. The purpose of this study is to examine the impact of steatosis on the liver lipidome. To do this we used a bias-free global lipid profiling acquisition technique of sequential precursor ion fragmentation called MS/MS^{ALL} developed by ABSciex (1). In brief, this method carries a high resolution TOF MS scan followed by a series of high resolution MS/MS experiments of each precursor at 1 amu difference in both positive and negative polarity modes. The data generated using this approach were validated by large scale targeted quantitation of identified lipid species using LC MS based multiple ion monitoring methods on an ABSciex 6500 Q-Trap instrument. Validation of these methods was accomplished using a well characterized mouse model (methionine/choline deficient diet) and our approach was then used to study how environmental toxins and genetic manipulation of signaling pathways that control hepatic lipoprotein secretion impact on pathways of lipid metabolism and signaling. In brief, lipids were extracted using acidified chloroform-methanol extraction process and data acquired using the MS/MS^{ALL} method in the TripleTOF 5600 (ABSciex). The data was then processed using the Lipidview software (version 1.3 beta, ABSciex). Quantification was done using internal standards for each lipid class and these measurements were validated by targeted quantitation of identified lipid species by selected ion monitoring LC MS/MS methods. A comprehensive characterization of the major abundant lipid classes was performed leading to the identification of over 1000 lipid species distributed among 11 lipid classes providing insights into the mechanisms underlying dysregulated hepatic lipid metabolism during liver steatosis.

(9) Probing the mechanism of homotropic allostery in TRAP using native mass spectrometry

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Homotropic allostery describes the cooperative binding of ligands to a macromolecule with multiple binding sites for the same ligand. In the case of positive cooperativity, binding of one ligand increases the affinity of the macromolecule for additional ligands; oxygen binding by hemoglobin is a classic example. To understand the mechanism of such allostery, one must first quantify the thermodynamic coupling between ligand binding sites. This is generally difficult because experimental measurements of affinity yield an *apparent* macroscopic parameter that is composed of many microscopic parameters, including the site-site coupling terms of interest. We are developing an approach to provide access to those microscopic interaction terms by studying allostery in *trp* RNA binding attenuation protein (TRAP). TRAP is an oligomeric protein with 11 identical binding sites for the ligand tryptophan, Trp. TRAP becomes activated upon Trp binding and binds to the 5' leader region of the *trp* mRNA, resulting in both transcription attenuation and translation inhibition, presenting a regulated feedback loop that controls tryptophan biosynthesis in *Bacillus* cells. A key difficulty with accurately obtaining microscopic parameters describing cooperativity is that in typical binding measurements, where binding isotherms are constructed from ligand-dependent changes in fluorescence, NMR chemical shift, or enthalpy, allostery may distort the proportion between the measurable and the number of the bound ligands. However, the mass of a protein with *n* ligands bound is not biased by allosteric effects and reports directly the number of bound ligands. MS can be used to measure populations of bound species, thereby increasing the accuracy with which microscopic parameters can be obtained. By using native mass spectrometry, we measured concentration-dependent binding of Trp to TRAP and use a nearest-neighbor thermodynamic model to quantify the microscopic thermodynamics of the cooperativity in TRAP. In addition, we used solution measurements (ITC) in MS-compatible buffers to test and validate native MS as a tool to measure microscopic thermodynamic parameters.

(10) Using lipidomics to understand the human adipocyte as a metabolic and immune cell

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Currently 35% of the US population is obese. As the major storage depot for excess calories and containing multiple immune cells, adipose tissue is uniquely positioned at the crossroads of immunity and metabolism. Adipocytes are determinants of adipose resident T cell (ART) and adipose tissue macrophage (ATM) subtype and dialogue with immune cells to regulate the production of pro- and anti-inflammatory adipo/cytokines that critically influence systemic metabolic pathways such as insulin action. In human and mouse obesity, adipocytes expand their antigen-presenting capacity to activate CD4⁺ pro-inflammatory Th1 cells, promote pro-inflammatory M1 macrophage polarization, and increase production of leptin and other pro-inflammatory cytokines. Genetic knockout of adipocyte-specific antigen presenting capability decreases adipose expression of Th1 markers and increases immunosuppressive regulatory T cells (Tregs), leading to enhanced systemic insulin sensitivity despite obesity. However, obese adipocytes decrease mitochondrial function and fatty acid oxidation/synthesis, and in humans genes that mediate these pathways correlate with adipose Tregs and inversely associate with antigen-presenting genes. Determining what lipid moieties accumulate in the adipocyte that could regulate its newly defined functions as an immune cell will be important in developing a strategy to curb adipocyte-derived inflammation. Quantitative lipidomics analyses are being performed (ThermoFisher triple quadrupole (QQQ) Quantiva HPLC-MS system) comparing adipocytes from obese vs. lean human samples obtained at elective surgery and from mice fed chow vs. high fat diet to identify novel lipids or lipoproteins that potentially contribute to regulation of adipocyte pro- or anti-inflammatory responses and its dialogue with immune cells.

(11) Direct Detection of Capsaicinoids Residues from a Single Thread: Applications in Forensics and Food Analysis

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Secondary metabolites produced by *Solanaceae* plants such as peppers, tomatoes, and potatoes are natural defenses against many predators. The high pungency of these secondary metabolites, such as capsaicinoids, produced by peppers, has been utilized in spicy food preparation, medicinal creams, and self-defense products. Unfortunately, some adverse effects have been detected following the use of capsaicin-based products. Therefore, accurate determination of pungency level has become critical. One method that is widely accepted is the Scoville's method, based on a tasting committee. Though subjective, the Scoville's method is preferred by the food industry because current analytical techniques are either time consuming or subject to interference. This study explores a simple mass spectrometric method that is capable of using fabric threads for rapid in-situ determination of capsaicinoids in pepper-based products. This novel ionization method utilizes a single thread as a medium for sampling and ionization under ambient conditions. Thread spray ambient ionization is demonstrated through the detection of various capsaicinoids from the placental tissue of pepper fruits. Pepper residues present on the thread were analyzed by the application of DC voltage and solvent to cause field-induced charged droplet generation. Capsaicinoids extracted from the sample are contained in the electrosprayed droplets and transported to the mass spectrometer for characterization. In a second experiment, pepper spray residues present on fabrics were directly detected by pulling a single thread from the fabric. The thread spray mass spectrometry was optimized using commercially available materials such as, 100% cotton, cotton/polyester (35:65), 100% polyester, and nylon fabric. These threads were subsequently used for in-situ analysis of several different pepper fruits and pepper spray residues on fabrics. This exploration indicated that the special physico-chemical characteristic of threads allowed a rapid and convenient sampling and ionization of pepper products for analysis by mass spectrometry.

(12) Effects of Dietary Sphingomyelin on Neonatal Piglet Intestinal Health and Membrane Composition

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Dietary nutrients are essential for gastrointestinal (GI) growth and function, and nutritional support is a significant component of neonatal development. The composition of mother's milk affects GI, mucosal immune system, and neurological development. Therefore, further understanding of nutrient interactions with the mucosa is necessary to define nutritional requirements of the developing GI tract to minimize intestinal complications and neonatal morbidity. Therefore, the purpose of this proposal is to investigate the effects of dietary sphingomyelin from the milk fat globule membrane (MFGM) compared to a soy based diet on piglet's intestinal and plasma lipid chemistry. By using an *in vivo* neonatal piglet model, we will identify how bioactive nutrients modulate gut and blood lipid transport in piglet model. The first stage of the project is focused on preparing a dairy-based formulation enriched with MFGM which is naturally high in sphingomyelin (30% approximately). Buttermilk, a byproduct obtained from butter manufacturing, contains MFGM and residual triglycerides from the fat globules. We are currently evaluating the effect of supercritical CO₂ (ScCO₂) treatment on sprayed-dried powder buttermilk by determining the supercritical parameters (temperature and pressure) to extract the maximum amount of residual triglycerides and therefore, increase the concentration of MFGM and sphingomyelin. We used an extraction system purchased from Waters (Waters, USA) which is composed of a 1 L extraction vessel, a P-200 CO₂ high pressure pump, an automated temperature controller and back pressure regulator. The optimal process found experimentally so far consisted on 125g of powder subjected to an extraction pressure of 350 bar and 77 °C. The CO₂ flow was set at 50 g/min with 3 extraction cycles of 100, 30 and 10 minutes respectively. The collection temperature was set at 55 °C. In conclusion, the methods development for increasing concentration of MFGM and sphingomyelin for utilization in milk formulas has been successful.

(13) Electrospray-based Photo-Catalytic Screening Provides Rational Synthetic Pathways to Aerobic Oxidation of N-Heterocycle Compounds under Ambient Conditions

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Photocatalytic reactions have many advantages over thermal reactions including the ability to overcome large activation barriers, formation of thermodynamically disfavored products and eco-friendliness. Therefore, photo-catalytic screening platforms are essential in discovering new reaction pathways; the ability to achieve on-line optimization, in order to gain efficient product generation, will be an added advantage. Catalytic oxidative dehydrogenations of N-heterocycles are important as they are structural components of natural and pharmacologically active substances. Current methods of oxidative dehydrogenations require sophisticated/expensive catalysts, high O₂ pressures. Aerobic oxidations of 1,2,3,4-tetrahydroisoquinoline (THiQ) terminates with the formation of imine product. Herein, we describe a new synthetic pathway (involving three simple steps) to produce isoquinolines from THiQ by manipulating the electron density. We used a new electrospray-based photo-catalytic platform to study the influence of electronic effects (e.g., inductive and pi-conjugation effects) on the rate of oxidative dehydrogenation of N-derivatives of THiQ using unmodified Ruthenium catalyst in ambient air. Experiments were performed on a newly developed picomole-scale photoreaction screening platform, which involves the coupling of portable laser source with nano-electrospray ionization (nESI) emitter. The THiQ derivative reactant and the Ru(bpy)₃Cl₂ (bpy= 2,2'-bipyridine) photo-catalyst were contained in a single barrel nESI glass capillary; application of ~1.2 kV DC voltage to reaction mixture produced charged droplets, containing the reactants, which were transported to mass spectrometer (MS). In-situ exposure of these charged droplets to the blue coherent laser light (wavelength 452 nm, power 5 mW) initiated photochemical reaction under ambient conditions, and products were promptly characterized by MS in real time. The droplet reaction condition was transferred to bulk-phase for large-scale chemical synthesis. Unlike THiQ, the N-phenyl derivatives undergo complete dehydrogenation to produce derivatized isoquinolines (99% yield for N-(4-methoxyphenyl)THiQ, through the abstraction of four hydrogen atoms. Results from other derivatives that induce conjugation and/or induction will be presented, and supported with computer simulations.

(14) Probing The Charge Distribution on the Gas-phase Dissociation Products of Protein Dimers

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The ability to preserve non-covalent interactions in protein complexes using nano- electrospray ionization(nano-ESI) has enabled native mass spectrometry to emerge as a powerful bioanalytical tool for protein analysis. Gas-phase dissociation methods provide insights into the stoichiometry and geometry of protein complexes. Protein complexes activated by collision induced dissociation(CID) fragment to highly charged monomers and their complementary (n-1)-mer products. In contrast, surface induced dissociation(SID) produces more symmetrical charge state distributions and dissociation products typically provide protein topology and connectivity information. As the charge state in the gas phase is reflective of protein structure, probing the effects of charge manipulation on the dissociation behaviors is of great importance. In agreement with Williams' results, CID of dimers with intramolecular disulfide bonds such as β -lactoglobulin leads to the generation of almost equally charged monomers while CID of the dimers after reduction of the disulfide bonds results in an asymmetric charge distribution. We further found that the precursor charge states also play a role on the symmetry of the charge distribution of the CID products. In charge reduced condition, the disulfide bond reduced β -lactoglobulin shows a symmetric charge distribution. By contrast, SID products show more symmetric charge distribution under all conditions. ZCON-39 is a hetero-dimer with a 12.5kDa chain A and a 13.4kDa chain B. The CID products of ZCON-39 with precursor charge states larger than +8 show asymmetric distribution with greater charge on chain B. For the precursor with reduced charge, the charge distribution of the CID products is more symmetric. In comparison, SID shows symmetric charge partitioning under all conditions. In conclusion, the charge distribution of CID products depends on the precursor charge states, the subunit size and flexibility while SID produces symmetric charge partitioning on the generated sub-complexes.

(15) Determining the translational impact of varying metabolic resources on melanized raditrophic fungi through nucleoside and oligo analysis

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Melanin has been found to play a role in the ability of radiotrophic fungi to convert gamma radiation into a metabolic resource. *Cryptococcus neoformans* and *Cladosporium sphaerospermum* are able to survive extreme conditions including oxidative stress and radiation exposure. The production of melanin further advances their ability to survive cellular stress. Various effects of metabolic resources have been investigated for their role in melanin production and survivability. Through total RNA and transferRNA (tRNA) modifications it will be possible to evaluate effects of resources on cellular function. Reversed phase chromatography was used for nucleoside detection. For oligo nucleotide mapping of transferRNA ion-pair reverse phase LC-MS was used.

(16) The Impact of Metabolic Syndrome on Fat Soluble Vitamin Uptake

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Obesity has been correlated with reduced circulating levels of the essential fat-soluble vitamins (A, E, D, and K). The mechanism(s) involved are not known, but metabolic syndrome (MetS), characterized by obesity and inflammation, has been shown to decrease the absorption and trafficking of vitamin E. We hypothesized that metabolic dysregulation in MetS subjects would also reduce the capacity to absorb vitamins A, D, and K. MetS adults (n=10 per group) and age- and gender-matched healthy adults, ingested α -tocopherol (15 mg) with a soymilk beverage (delivering 120 μ g vitamin A, 120 IU vitamin D2, and 3.4 μ g vitamin K1) prior to isolating plasma and the chylomicron-containing triglyceride-rich lipoprotein fraction (TRL) of blood 12 hours post-prandially. An LC-MS/MS method, using an MTBE/methanol gradient and a C30 column for separation, with the LC interfaced with the MS using an APCI probe operated in positive ion mode, was previously validated for retinyl palmitate, carotenoids, and vitamins K1 and E. The method was adapted to incorporate vitamins D2 and D3 to use for the quantitation of the suite of fat soluble vitamins in the TRL fraction (using stable isotope internal standards). Area-under-the-time-concentration-curve (AUC) values for each micronutrient over 12 h will be assessed via pairwise comparisons to determine the difference in fat-soluble nutrient absorption between MetS and healthy adults. MetS status reduced chylomicron and plasma AUC of α -tocopherol, and is expected to similarly impair absorption of retinyl esters (vitamin A), vitamin D3, and vitamin K1. Metabolomics analyses of non-polar plasma extracts will be performed using LC-HRMS (QToF), in combination with a multivariate statistical approach, to generate new hypotheses as to which stage(s) of fat-soluble nutrient absorption are impaired by MetS status. Metabolomics analyses are expected to reveal clear systematic differences in the mechanisms of intestinal absorption that differentially and/or similarly affect a variety of fat-soluble compounds.

(17) Pico-Litter Based On-line Protein Modification using Accelerated Droplet Chemistry

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Nano-electrospray ionization (nESI) is an efficient process for introducing small volumes of liquid samples for MS characterization. However, nESI has been always operated in a single mode (sample treatment is in bulk solution using large sample volumes). Herein, we describe a novel contained-nESI apparatus to enable *in-situ* treatment and analysis of small sample volumes contained in nESI emitter. Instead of treating the sample before analysis, contained-nESI uses charged droplets as reactions vessel; analytes are treated after charge droplet generation, providing an efficient means to conduct ion chemistry under ambient conditions. This process was applied for unfolding of different proteins. MS analysis of water solutions of myoglobin, ubiquitin, cytochrome C, and carbonic anhydrase II (CA-2) was accomplished by using conventional and contained-nESI using headspace HCl vapor. Protein charge state distribution was compared by employing the intensity weighted average charge state (q_{ave}). Contained-nESI provided significant shift ($\Delta q_{ave} \approx 2$ units) to higher charge states for analyzed proteins when compared to conventional nESI, performed in the absence of HCl. This included the analysis of the acid resistant ubiquitin, which yielded $\Delta q_{ave} = 2.1$, with simultaneous appearance +11 and +12 charge states that could not be detected in the absence of acid. The contained-nESI ion source also allowed a dramatic increase in ion intensity for CA-2. Due to its high molecular weight and low pI, the analysis of native CA-2 in water is often a challenge. With contained-nESI, about three orders of magnitude increase in ion yield was obtained simply by reducing the pH of the resultant droplets. These results demonstrate the ability of the contained-nESI to improve, accelerate and simplify protein analysis. The modification occurred during the lifetime of the droplets and yet substantial charge shifts were recorded (denaturation of the acid-sensitive myoglobin protein occurred before the heme cofactor could leave the unfolding protein).

(18) Absolute Minimal Sampling in High Dimensional NMR Spectroscopy

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Standard three-dimensional Fourier transform (FT) NMR experiments of molecular systems often involve prolonged measurement times due to extensive sampling required along the indirect time domains to obtain adequate spectral resolution. In recent years, a wealth of alternative sampling methods has been proposed to ease this bottleneck. However, due to their algorithmic complexity, for a given sample and experiment it is often hard to determine the minimal sampling requirement, and hence the maximal achievable experimental speed up. Here we introduce an absolute minimal sampling (AMS) method that can be applied to common 3D NMR experiments. We show for the proteins ubiquitin and arginine kinase that for widely used experiments, such as 3D HNCO, accurate carbon frequencies can be obtained with a single time increment, while for others, such as 3D HN(CA)CO, all relevant information is obtained with as few as 6 increments amounting to a speed up of a factor 7 – 50.

(19) Exploring isoflavone metabolic phenotypes and evaluating lipid and immune biomarkers in HIV infected men and women on antiretroviral therapy after soy pretzel intervention

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HIV-infected (HIV+) patients treated via antiretroviral therapy (ART) are at risk for many conditions including altered lipid profiles, hypercholesterolemia, and chronic inflammation as a result of pro-inflammatory cytokines and lymphocyte activation, which can lead to the development of cardiovascular disease (CVD). In order to ameliorate the metabolic disturbance caused by ART and reduce the risk of a cardiac event in HIV+ patients, a novel functional food has been developed to deliver bioactive soy isoflavones to ART-treated HIV+ patients. Soy pretzels rich in bioactive soy isoflavones and soy proteins have been shown to work synergistically to improve dyslipidemia and reduce pro-inflammatory cytokines; however, soy metabolism and absorption is relatively unexplored regarding metabolic disturbances due to ART in HIV+ men and women. Soy isoflavones exist predominantly in their glycoside forms in food but can be hydrolyzed by β -glucosidase in the intestinal brush border to produce aglycones (e.g. daidzein, genistein, glycitein). Daidzein can then be metabolized to various bioactive metabolites including equol and ODMA (O-desmethylangolensin). Isoflavone metabolites can play a significant role in inflammation reduction by altering cell signaling pathways responsible for inflammatory cytokine activation (e.g. IL-6). We hypothesize that daily consumption of soy pretzels (70mg isoflavones and ~21g of soy protein/day) in HIV+ ART-treated patients will significantly reduce low-density lipoproteins (LDL) and triglycerides as well as decrease pro-inflammatory cytokines in those who exhibit novel isoflavone metabolites such as equol or ODMA. A 6-week single arm study will be conducted to evaluate the impact of soy pretzel intervention on isoflavone metabolism in 20 HIV+ ART-treated men and women. The following objectives will be met: To quantify soy isoflavone metabolites in urine and blood after soy pretzel intervention using HPLC-MS/MS and determine individual isoflavone phenotypes. Subsequently, these phenotypes will be correlated with fasting blood lipids (LDL and triglycerides) and pro-inflammatory biomarkers.

(20) MDM2 Alterations Reprogram the Metabolic Functioning of Liposarcoma Cell Lines

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Patients with high-grade dedifferentiated liposarcoma (DDLPS) have a five-year survival rate of less than 50%, and current treatments are unpredictable in their efficacy and toxic effects. There is thus an urgent need to identify alternative treatments. DDLPS is mainly driven by amplification of chromosome 12, which harbors MDM2, the principal cellular antagonist of p53. Unpublished in vitro results from our group show that growth of patient derived DDLPS tumor cells with low MDM2 levels can be reversed with Lipitor treatments. Cells with high MDM2 are unaffected by Lipitor. This difference could be caused by differences in fatty acid or cholesterol metabolism pathways. We hypothesize that MDM2 expression reprograms metabolomic profiles of DDLPS cells, which affects their treatment response. We performed metabolomic (Metabolon) profiling on four MDM2 high and two MDM2 low patient-derived DDLPS cell lines. We identified 30 metabolites differentially abundant between MDM2 high and low cells. Among those identified were 3-hydroxy-3-methylglutarate (FDR adjusted $p = 9.52e-03$), a metabolite used in the mevalonate pathway. This pathway is used in the cell to convert acetate into cholesterol precursors. Previous studies have shown that inhibition of the mevalonate pathway can slow growth in tumor cells with mutant p53. Specific lipids disrupted by MDM2 expression will be further identified through targeted lipidomics (Lipidizer). To validate our in vitro findings, we leveraged public transcriptomic data from human DDLPS patients using the Cancer Genome Atlas. We divided the set into halves based on MDM2 expression, and identified several differentially expressed transcripts involved in lipid pathways, including PPARG, which codes for a nuclear receptor commonly found in adipose tissue that regulates fatty acid storage. By integrating gene and metabolite profiles, we aim to identify dysregulated biological pathways that are responsible for the differential response to cholesterol inhibition treatment between MDM2 high and low cells.

(21) Detection of tRNA modification changes during growth phase transitions in *Bacillus subtilis* by LC-MS/MS

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Transfer Ribonucleic Acids (tRNAs) are most post-transcriptionally modified than the other species of RNA. It is known that these modifications can be dynamic in nature, and yet the causes of these dynamic modifications is still not completely understood. Commonly used modification databases provide static modification profiles, not taking into consideration the effect of the culturing conditions used to obtain those modification profiles. By using liquid chromatography tandem mass spectrometry (LC-MS/MS), one can detect and characterize modifications from total tRNA samples grown under different conditions. Abundance on nucleosides from *Bacillus subtilis* is being analyzed to learn the effects of culturing conditions on the tRNA modification profile.

(22) Green tea treatment in obese mice with nonalcoholic steatohepatitis restores the hepatic global metabolome

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Green tea extract (GTE) limits NFκB-mediated inflammation during nonalcoholic steatohepatitis (NASH). We hypothesized that the antiinflammatory activities of GTE would be attributed to its polyphenolic catechins and related metabolites that promote a shift in the hepatic metabolome. Male C57BL/6J mice (6 wk old) were fed a low-fat (LF) or high-fat (HF) diet for 12 wk to induce NASH. They were then randomized to continue on these diets supplemented with 0 or 2% GTE ($n = 10/\text{group}$) for an additional 8 wk. Profiling of the GTE by LC-PDA-QTOF-MS showed high abundance of the four major catechins (epigallocatechin gallate, epigallocatechin, epicatechin gallate, epicatechin), along with less abundant organic acids (gallic and quinic acid), catechin derivatives (EGC-methyl gallate), and other flavonoids (rutin, kaempferol derivatives). Untargeted metabolomics of the polar extract from livers was performed by UHPLC-QTOF-MS in both positive and negative ionization modes. More than 5,000 metabolites were detected, with >1,000 metabolites showing statistically between-treatment differences. GTE-mediated improvements in histological evidence of NASH occurred in association with changes in liver metabolites, such as derivatives of amino acids and purines, lysophospholipids, and glycerolipids. These findings support that dietary treatment with catechin-rich GTE functions to restore several key biochemical pathways that are otherwise dysregulated in NASH.

(23) Regulation of glucose metabolism by hypoxia in colorectal carcinoma

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Background/Objective: Glucose metabolism is an important pathway to provide energy and building materials to growing cells. Cancer cells favor glycolysis with enhanced lactate production and decreased mitochondrial function (Warburg effect). Pyruvate is the endproduct of glycolysis and it can be metabolized to either lactate, or oxidized in the mitochondria. Pyruvate dehydrogenase (PDH) is a mitochondrial enzyme and a major regulator of pyruvate fate. Inhibition of PDH by the hypoxia-responsive PDH kinases decreases PDH activity and mitochondrial function. Therefore, we investigated the role of the hypoxia-responsive PDH kinases PDHK1 and PDHK3 on metabolism of pyruvate in colorectal carcinoma (CRC). **Methods/Results:** We used two genetic models to investigate PDHK function in CRC: knockout cells *in vitro* and a conditional mouse knockout *in vivo*. RKO CRC cells were engineered to delete either PDHK1 or PDHK3 using CRISPR/Cas9 gene editing technology. The modified cells were tested for hypoxic changes in the three inhibitory phosphorylations (serines 232, 293, and 300) of PDH E1 α and mitochondrial function by Seahorse flux analysis *in vitro*. We find that PDHK1 KO causes reduced hypoxic phosphorylation at Ser232, whereas PDHK3 KO cells show reduced phosphorylation at Ser232, Ser293 and 300. The conditional mouse PDHK1 deletion was combined with Villin-Cre to delete fl/fl PDHK1 in colonocytes followed by treatment of the mice with AOM/DSS to generate colitis associated colon carcinoma. **Conclusion:** We interpret these results to indicate that PDHK3 is more important in the inhibition of PDH activity in hypoxia in CRC cells *in vitro*. Our data also show that PDHK1 KO mice developed tumors, but the statistical analysis to measure the frequency and severity of cancer is underway. Lastly, there is differential hypoxic regulation of PDH by the different PDHK family members, and PDHK induction by hypoxia represents an adaptive metabolic response.

(24) Novel linear modeling approach allows analysis of metabolomics data in context of gene expression and phenotype-specific data

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Metabolomics is playing an increasing role in clinical and translational research and has greatly assisted in identifying novel, putative biomarkers for cancers. However analyzing untargeted metabolite data poses different challenges. One of the challenges is to interpret the metabolomics profiles and understand how they affect, and are affected by genes and the proteins they produce. Toward this end, we can study associations between gene expressions and metabolite abundances. In particular, our aim is to find relationships between gene and metabolite levels that are associated with a phenotype (e.g. cancer vs. non-cancer). This is especially important as the relationship between genes and metabolites will differ with respect to particular phenotypes. To understand the relationship between metabolite levels, gene expression, and phenotypes, we propose a novel linear modeling approach: $m = g + t + g:t$ where m is the metabolite abundance, g is the gene expression levels, t is the phenotype, and $g:t$ describes the interaction between phenotype and gene expression. A statistically significant p-value of the $g:t$ (interaction) coefficient indicates that the gene-metabolite relationship is phenotype-specific. We have applied our linear model to the NCI-60 data set and to previously published data from a breast cancer study. In each of these data sets, we have identified changes in gene-metabolite associations resulting from differing phenotypes. The next steps involve extending the model to analyzing other data sets as well as developing an R package and Shiny App of the novel linear model that will facilitate future studies.

(25) A flavoromics approach in determining key chemical markers in green coffee beans that have significant impact on coffee brew flavor quality

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Coffee is one of the most traded commodities in the world and is predominately sold as raw or green coffee beans. Screening methods utilizing physical properties of green coffee beans and sensory properties of the resulting coffee brew, after roasting, are commonly used to determine flavor quality and greatly influence green coffee bean selling price. These methods, however, can be time consuming and/or unreliable, thus there is a need for a more accurate approach which allows predicting coffee brew quality based on green coffee bean chemistry. A non-targeted flavoromics approach coupled with multivariate statistics analysis was utilized to identify chemical markers in green coffee bean that directly correlate with coffee brew flavor quality. Green coffee bean samples varying in origin, source, processing methods, and flavor quality were chosen in order to include a wide range of inherent chemistry (n=18). Brew quality was assessed by five certified coffee graders based on an objective and standardized scoring method for coffee flavor profile. Molecular fingerprints were collected for both green coffee bean extracts and their resulting coffee brews by Liquid Chromatography/Mass Spectrometry (LC/MS) in positive and negative electrospray ionization (ESI). Data preprocessing and generation of Orthogonal Projection to Latent Structures models which correlate flavor quality scores with chemical features were completed. Signal threshold optimization was performed and models with good fit and predictive scores ($Q^2 > 0.8$) have been established for green coffee beans and work is ongoing to select high correlating markers for further characterization, purification and evaluation to confirm sensory relevance. Future work will correlate the chemistry of the green bean and coffee brew markers to gain better understanding of the origins and formation pathways of relevant compounds. This work aims to facilitate the development of screening methods for price negotiation and allow the improvement of current breeding and processing strategies for flavor optimization.

(26) Hydrophobic Thread Spray Mass Spectrometry for Direct Analysis of Illicit Drugs in Complex Mixtures

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Current needs in forensics and drug discovery require methods that are rapid and sensitive, whilst also minimizing sample preparation steps. Paper Spray mass spectrometry (PS-MS) has become a staple ambient ionization method for biological samples analysis. Recently, we have shown that hydrophobic PS-MS offers increased sensitivity by (i) suppressing endogenous matrix during ionization, (ii) providing prolonged ion signal that enables effective ensemble averaging, and (iii) using electrostatic spray ionization through vigorous droplet vibration that facilitates liquid-liquid extraction. We reasoned that a more reliable and efficient extraction/ionization could be achieved by relying on the natural sharp tips present in threads. In this case, a single thread is placed in a glass capillary and a DC voltage applied to the wet thread in an experiment similar to nano-electrospray ionization (nESI). Unlike nESI utilizing metal emitters, the use of porous threads allow direct bio-fluid analysis from the glass capillary through in-situ selective extraction and ionization. This objective is achieved by using hydrophobic thread, prepared by gas-phase silane functionalization. The analytical capability of this novel method is demonstrated via the direct analysis of cocaine, amphetamine, benzoylecgonine, and methamphetamine from undiluted human blood. Data obtained from traditional hydrophilic cotton threads will be compared to results collected from different hydrophobic cotton threads; thread hydrophobicity was varied by changing their exposure time to the silane vapor. The mechanism governing the enhanced detection and selective extraction will be discussed, including physicochemical data such as surface roughness, peak curvature, and surface texture as it relates to the ionization efficiency of the different thread types. Limit of detection and quantification will be presented as a function of spray solvent type and voltage.

(27) Development of a Cleavable Peptide Probe Unit for Mass Spectrometry-Based Immunoassays

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A paper-based mass spectrometry diagnostic platform is currently being developed that could enable portable mass spectrometers to be used for the detection of protein disease biomarkers. The core of this approach is the use of readily available cleavable ionic probes as mass reporter in immunoassays, which make possible sensitive on-demand detection. The development of platform involves the following steps: (i) optimize conditions for optimum peptide ion generation and fragmentation in order to obtain a highly sensitive mass reporter with characteristic fragment ions, (ii) incorporate the selected mass reporter peptide into a cleavable probe unit based on available starting materials, and (iii) use the synthesized probe in paper-based immunoassays. This objective was evaluated using nano-electrospray ionization mass spectrometry (nESI-MS) and paper-spray ionization. Three peptides were designed, synthesized and characterized: AKRRG, RRGKA, and GARKK, all having molecular weight of 586 Da. Fragment ions from the three isobaric peptides produced in MS/MS are different, providing a simple way to differentiate them as mass tags for different diseases. Also, we observed that fragmentation efficiencies of the $[M+2H]^{2+}$ ions from the selected peptides were different. Ion mobility experiments, however, revealed that the three $[M+2H]^{2+}$ peptide ions have comparable cross sections. This result suggests the ability to derive unique fragment ions from isobaric peptides with different efficiencies may be related to the mobile proton mechanism where restrictions in the movement of the charge affect ease of fragmentation. Moreover, a novel synthetic route for the development of the cleavable peptide probe is proposed for the first time, which involves two steps: (i) protection of the primary peptide amine and (ii) the incorporation of the peptide into a cleavable probe unit by steglich-esterification reaction. Successful incorporation of the peptide unit will be discussed. Results from peptide-based immunoassay test performed on a paper-based microfluidic device will also be presented.

(28) Facilitating Accurate Identification and Quantitation of Metabolites by Non-Uniformly Sampled (NUS) Multi-dimensional NMR

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Accurate identification and quantitation of metabolites play an essential role for monitoring biomarkers and metabolic pathways of certain diseases. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy are the two most powerful analytical methods in metabolomics research. Multi-dimensional NMR experiments provide powerful connectivity and structural information of all detectable metabolites based on the presence of cross-peaks and their chemical shifts. However, conventionally measured multidimensional NMR spectra require significantly longer NMR times compared to 1D NMR. Here, we discuss the implementation of non-uniformly sampled (NUS) multi-dimensional NMR to speed up these measurements for the accurate identification and quantitation of metabolites in model mixtures and mouse urine. The identification and quantitation accuracy of metabolites was analyzed using our lab's COLMAR NMR web server suite and metabolomics database (<http://spin.ccic.ohio-state.edu/index.php/colmar>). The data show that identification and quantitation of metabolites by NUS NMR achieved good performance compared to conventional multi-dimensional NMR methods while the experimental time was reduced by a factor 4. We expect that for relatively high-concentration metabolite samples these NUS NMR methods will become a standard tool for the more rapid acquisition of multi-dimensional NMR spectra in the context of metabolic profiling and quantitative analysis of complex mixtures.

(29) Analysis and assessment of the lipid fraction of green and roasted *Coffea Arabica* beans from various geographical origins under different processing and storage conditions - An NMR approach.

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Approximately 15% of the mass of an Arabica coffee bean, *Coffea Arabica*, consists of lipids. This lipid fraction contains several compounds with the potential to act as biomarkers for classification and evaluation of *Coffea Arabica*. The objective of this study is to employ multinuclear and multidimensional NMR spectroscopy as a rapid and reliable tool for the quantitative analysis and evaluation of the non-polar, including unsaponifiable, part of *Coffea Arabica*. Green and roasted coffee beans from various geographical origins, as well as brewed coffee and spent coffee grounds were analyzed for their lipid components. A number of gradient-selected two-dimensional NMR techniques were applied for a systematic two-dimensional analysis of the various components in coffee oil. Quantification was achieved by integration of the appropriate diagnostic signals in the NMR spectra using 2,6-Di-tert-butyl-4-methylphenol (BHT) as an internal standard, as well as the ERETIC method. Overall, it was found that the major fatty acids in coffee oil are linoleic, oleic and saturated fatty acids. Our preliminary data indicate that only minor changes occur in the fatty acid profile during roasting, however the oxidation status of coffee oil is dependent on external factors such as temperature and storage. In addition, small amounts of partially esterified lipids, such as diacylglycerides appear in roasted and green coffee. NMR allows for the determination of several compounds of the unsaponifiable fraction of coffee oil, such as sterols and terpenes. The ³¹P NMR spectra indicate the presence of phospholipids and may be a reliable tool for their quantification. Our results suggest that multinuclear (¹H, ¹³C, ³¹P) NMR spectroscopy can be a valuable tool for the determination of several bioactive compounds in coffee oil and can be used for quantifying the impact of typical coffee processes.

(30) Nanoparticle-Assisted Metabolomics: from Protein Removal to Specific Metabolite Identification

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Metabolomics aims at a complete characterization of all metabolites in biological samples, which reflect the physiological processes and cellular functions that have profound impacts in areas such as nutrition, pathology, biomarker discovery, and disease diagnosis. Mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR) are two primary and highly-complementary techniques in metabolomics. In practice, metabolite samples with high protein content, such as serum, pose substantial challenge to both methods, and the removal of protein is a typical prerequisite for subsequent analysis. Existing protein removal methods include ultrafiltration and organic-solvent-induced protein precipitation, and currently there is no standard operating procedure. Here we introduce a novel, highly efficient, and environmentally friendly protein-removal method using nanoparticles. In brief, serum proteins are absorbed on the surface of silica nanoparticles (SNPs) forming protein-nanoparticle complexes and can be later removed by centrifugation, leaving the native metabolome unperturbed. As a result, the SNP-treated sample leads to remarkably clean NMR spectra that compare favorably to samples treated with other methods. This nanoparticle-assisted protein removal method is very promising for the routine processing of serum samples for MS and NMR analysis on large scale. For samples containing both proteins and metabolites, SNPs interact primarily with proteins. Interestingly however, for metabolite samples in the absence of proteins, SNPs can weakly yet specifically interact with certain metabolites. For example, positively charged metabolites tend to bind negatively charged SNPs and vice versa. Such binding events, even when taking place on transiently occur, can considerably broaden and usually suppress the corresponding resonances on NMR spectra, which makes NMR amenable to differentiate metabolites based on their electric charges. This has been demonstrated both for artificial model mixture and human urine. In addition, this method also simplifies the NMR spectra of complex samples, and hence greatly improves the accuracy of metabolite identification. In conclusion, we have successfully applied nanoparticles in metabolomics study for protein removal and specific metabolite identification. With the booming of nanotechnology, we expect to further find niches for nanomaterial in MS- and NMR-based metabolomics for better metabolite identification, quantitation and beyond.

(31) Probing protein structure via surface induced dissociation, gas phase hydrogen-deuterium exchange, and electron induced dissociation in a hybrid FT-ICR

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In structural biology studies, mass spectrometry has emerged as a powerful tool to obtain topology and quaternary structural information on protein complexes. Gas phase hydrogen-deuterium exchange (HDX) and electron induced dissociation (EID) are capable of probing structure of proteins. Surface induced dissociation (SID), which can provide quaternary structure information on non-covalent protein complexes, has been successfully applied in a FT-ICR (Fourier transform ion cyclotron resonance) platform, providing high resolution and mass accuracy. In this study, structures of protein complexes were studied by SID, gas-phase HDX and EID in a modified Bruker SolariX XR 15 T FT-ICR mass spectrometer. The experiments were performed on a modified Bruker SolariX XR 15 T FT-ICR mass spectrometer with an SID device replacing the original collision induced dissociation (CID) cell. The gas connected to the ICR cell was ND₃ instead of Ar. The instrument was modified so that the ions trapped in the ICR cell can interact with ND₃ before mass detection. Both the protein complex ions and their SID fragments were further studied in the HDX experiment in the ICR cell. The EID experiment were also performed in the ICR cell. SID is known to dissociate protein complexes into fragments that correspond well to their crystal structures. The streptavidin tetramer, a dimer of dimers, has been shown to dissociate into abundant dimers in SID but not CID. These dimers (5+, 6+ and 7+) generated in the SID of the charge reduced streptavidin tetramer (12+) were allowed to undergo HDX. The similar low mass shift of the 5+, 6+, and 7+ dimers upon HDX indicates that dimers with similar folded structures were generated from SID. Covalent fragments from EID also provide insight on the structure of streptavidin and C-reactive protein.

(32) Molecular characterization of the *Salmonella Typhimurium* infection in mice gallbladder by NMR/MS-based metabolic profiling of bile

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Bile is a rarely studied, but important body fluid, which contains numerous bile acids that play pivotal roles in the maintenance of cholesterol homeostasis through facilitating lipid absorption. Recently, bile acids were also attributed to certain regulatory function as signaling molecules in metabolic pathways within the host metabolome. The host metabolome is however constantly interacted by microbiota formed by a variety of microbes colonized in the host which can lead to numerous diseases associated with the host's nutrient absorption, energy metabolism, pathogenic immune response, etc. *Salmonella Typhimurium* is a type of pathogenic Gram-negative bacteria that has been found in the gallbladder with the potential to influence the host's health through metabolic cross talks. Therefore, it is important to comprehensively characterize the metabolites in bile to uncover the molecular changes associated with such infections. In this study, a series of 2D/3D NMR techniques were applied to establish atomistic structural information of the metabolites obtained from bile samples of mice infected with *S. Typhimurium*. Such spectral information was used for metabolite identification and quantification using our COLMARm database. More than 30 metabolites involved in glycolysis, TCA cycle, urea cycle, and other metabolic pathways were identified as well as primary and secondary bile acids. Novel metabolic structures that are not available in databases, were subjected to analysis by NMR and mass spectrometry with our SUMMIT MS/NMR method. As one of the first comprehensive studies of metabolites in bile, this study may pave the way to fully understand the molecular mechanism of the pathogen-host interactions in gallbladder.

(33) In-tube extraction (ITEX) for green tea flavor analysis

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Green tea is a widely consumed beverage around the world for its mild flavors and health benefits, unfortunately, there are many people who prefer decaffeinated green tea due to caffeine's adverse effects in humans. Most of the conventional decaffeination techniques applied in food use organic solvents. The decaffeination process technique not only reduced caffeine but also reduced some important green tea flavors. However, these important volatile compounds of green tea have not intensively characterized yet. Hence, the objectives of this study is to compare profiles of volatile compounds between regular and decaffeinated green teas to provide biomarkers between regular green tea and decaffeinated green tea. Different extraction techniques such as solid-phase extraction (SPE), head-space solid-phase microextraction (SPME) and stir bar sorptive extraction (SBSE) were developed and applied in flavor analysis. Among them, thermal desorption offers a cost-saving and high sensitivity which make it good alternative to conventional sample preparation methods for the volatile organic chemicals. In-tube extraction (ITEX) is newly developed tool as a thermal desorption technique. In this study, we profiled flavor compounds from both regular green tea and decaffeinated green tea using ITEX tool with Tenex TA absorbent coupled with gas chromatography-massspectrometer. As result of partial least squares-discriminant analysis (PLS-DA), we found that regular green tea showed significantly higher levels of butanoic acid ethyl ester, 3-ethyl-1H-pyrrole, 2-methyl butanoic acid ethyl ester, 2-pentyl furan, 1-methyl-4-(1-methylethyl)-1,3-cyclohexadiene, and o-cymene than decaffeinated green teas, while hexanal, 2-methyl butanal, 1-penten-3-ol, (Z)-4-heptenal and 3-methyl butanal were significantly lower than decaffeinated green tea. These biomarkers can be utilized to improve quality of decaffeinated green tea. Therefore, ITEX tool can be useful to study flavor in green teas, and it also can be potentially used for volatile compounds analysis of samples from drinking water, plant materials, foods, and other environment research.

(34) Detection and Mapping of Post-Transcriptional tRNA Modifications in the Radioresistant Bacterium

Deinococcus radiodurans

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The bacterium *Deinococcus radiodurans* is one of the most ionizing radiation (IR) resistant bacteria discovered to date. It also has extraordinary resistance to all reactive oxygen species (ROS)-generating agents including desiccation, UV radiation, mitomycin C. To obtain a better understanding of the ionizing radiation resistance of *D. radiodurans*, modified nucleosides in transfer RNAs (tRNA) were analyzed by using HPLC tandemed with Orbitrap mass spectrometer when *D. radiodurans* was exposed to different levels of IR. Preliminary data shows *D. radiodurans* has a rich diversity of modified nucleosides in tRNA. The modifications pools almost have no changes even under high IR. However it was found that upon higher IR exposures, levels of some modifications increased as compared to the control. In contrast, some other modifications decreased upon increasing IR exposure. On-going studies are now directed at RNA modification mapping to identify sequence locations whose modifications changed upon IR exposure and by using of Triple quadrupole mass spectrometer to measure the absolute concentrations of modifications. The goal of this project is to determine whether post-transcriptional modifications in tRNA provide any selective advantage for *D. radiodurans* during exposure to high levels of ionizing radiation and to use findings from this model prokaryotic organism to inform other on-going studies with IR-resistant eukaryotes.

(35) Targeted HPLC-MS/MS Metabolomics Differentiates Metabolic Syndrome from Obesity

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Both obesity and the metabolic syndrome (MetS) are risk factors for type 2 diabetes and cardiovascular disease. Identification of novel biomarkers are needed to distinguish MetS from equally obese individuals in order to direct them to early interventions that reduce their risk of developing further health problems. It is important to enhance the prognostic value of established MetS criteria (i.e., waist circumference, blood pressure, and blood glucose and lipid levels) by identifying novel metabolic markers to differentiate MetS from obese non-MetS individuals and to stratify the risk of MetS status based on the incremental number of clinical criteria fulfilled. In this study, targeted metabolic profiling by HPLC-MS/MS was conducted to detect metabolic profile differences between obese individuals with MetS and equally obese individuals not meeting the MetS criteria (obese non-MetS group). Obese (body mass index (BMI) ≥ 30 kg/m²) men and pre-menopausal women were screened for the presence of ≥ 3 of established risk factors for MetS: waist circumference, fasting triglycerides, fasting glucose, resting systolic and diastolic blood pressure, and HDL-cholesterol. Of the 69 participants screened, 26 were classified as MetS and 43 as obese non-MetS. 221 metabolites were selected according to our published work and consistent with previous studies, which represent key metabolites of interest from relevant metabolic pathways. Compared to obese individuals without MetS, univariate statistical analysis and partial least squares discriminant analysis (PLS-DA) showed that a specific group of metabolites from multiple metabolic pathways (i.e., purine metabolism, valine, leucine and isoleucine degradation, and tryptophan metabolism) were associated with the presence of MetS. Receiver operating characteristic curves generated based on the PLS-DA models showed excellent areas under the curve (0.85 and 0.96, for metabolites only model and enhanced metabolites model, respectively), high specificities (0.86 and 0.93), and good sensitivities (0.71 and 0.91).

CORE FACILITY POSTER ABSTRACTS

(36) The New State-of-the-Art CCIC-NMR Facility at the Ohio State University

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

Over the past few years, the shared NMR facility at the Ohio State University (OSU) has seen an expansion with new instruments and update of existing instruments housing now 9 high-field NMRs from Bruker Biospin with 5 instruments at 800 MHz and above. The new 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. These instruments 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.

(37) Targeted Metabolomics Laboratory

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¹*Center for Applied Plant Sciences, The Ohio State University, Columbus, OH;*

²*Department of Molecular Genetics, The Ohio State University, Columbus, OH*

Each cell or tissue features a collection of metabolites (small molecules) which are the end product of cellular processes. Metabolomics is a study of these metabolites, providing a unique opportunity to fingerprint physiological activity. The Targeted Metabolomics Laboratory (TML) serves researchers at The Ohio State University through the detection and quantification of small molecules from a biological source. The TML provides access to state-of-the art chromatographic and mass spectrometric instrumentation including an ultra high performance liquid chromatographer tandem mass spectrometer (UHPLC-DAD-MS/MS), a high performance liquid chromatographer photodiode array detector (HPLC-PDA) and a gas chromatographer mass spectrometer (GC-MS). The list of metabolites detected and quantified contains, but is not limited to: amino acids, fatty acids, flavonoids, organic acids, phosphorylated compounds, polyamines, sterols, sugar alcohols, and sugars. The TML also offers expertise in experimental design, extraction methods, and data interpretation. This poster features key instrumentation and their application to metabolomics studies.

(38) OARDC Metabolite Analysis Cluster (OMAC)

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¹*Department of Horticulture and Crop Science, Ohio Agricultural Research and Development Center (OARDC), The Ohio State University;* ²*OARDC Metabolite Analysis Cluster, Department of Horticulture and Crop Science, OARDC The Ohio State University*

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 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, food and beverage samples, microbial cells or cellular exudates, 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. Areas of specialization of the facility include: phytohormone and membrane biochemistry, protein purification and enzyme characterization, plant secondary/specialized metabolism, metabolic engineering, metabolic profiling, detection of herbicide and pesticides, biochemical dissection of cellular signaling and organismal stress responses.

(39) Nutrient and Phytochemical Analytics Shared Resource

Ken Riedl

Department of Food Science & Technology; OSU Comprehensive Cancer Center

Our core brings unique expertise and world class LCMS instrumentation to analytical exploration of foods and biologics. We excel at targeted and untargeted metabolomics for biomarker identification and metabolite discovery. Metabolomics is an experimental capability providing unrivaled depth of metabolite coverage to enhance scientific rigor of investigations and competitiveness of grants. We have particular experience in metabolomics as applied to dietary interventions and support the OSU Discovery Themes in Personalized Food and Nutritional Metabolomics for Health. NPASR service goals are to provide investigators with bioanalytical method development and quantitative analysis of nutrients and phytochemicals in foods and their metabolites in biological samples. Key outputs of untargeted LCMS metabolomics include metabolite and biomarker discovery. Through these deliverables we aim to enhance understanding of the role of dietary compounds in cancer prevention and control.

(40) The OSU CCIC Mass Spectrometry and Proteomics Facility

Árpád Somogyi¹, Liwen Zhang¹, Cindy James¹, Nan Kleinholtz¹, Sophie Harvey¹, Matt Bernier¹, Michael Freitas², Vicki H. Wysocki³

¹ OSU CCIC MSP Facility, ² SBS-Cancer Biology and Genetics, OSU, ³ Department of Chemistry and Biochemistry, OSU

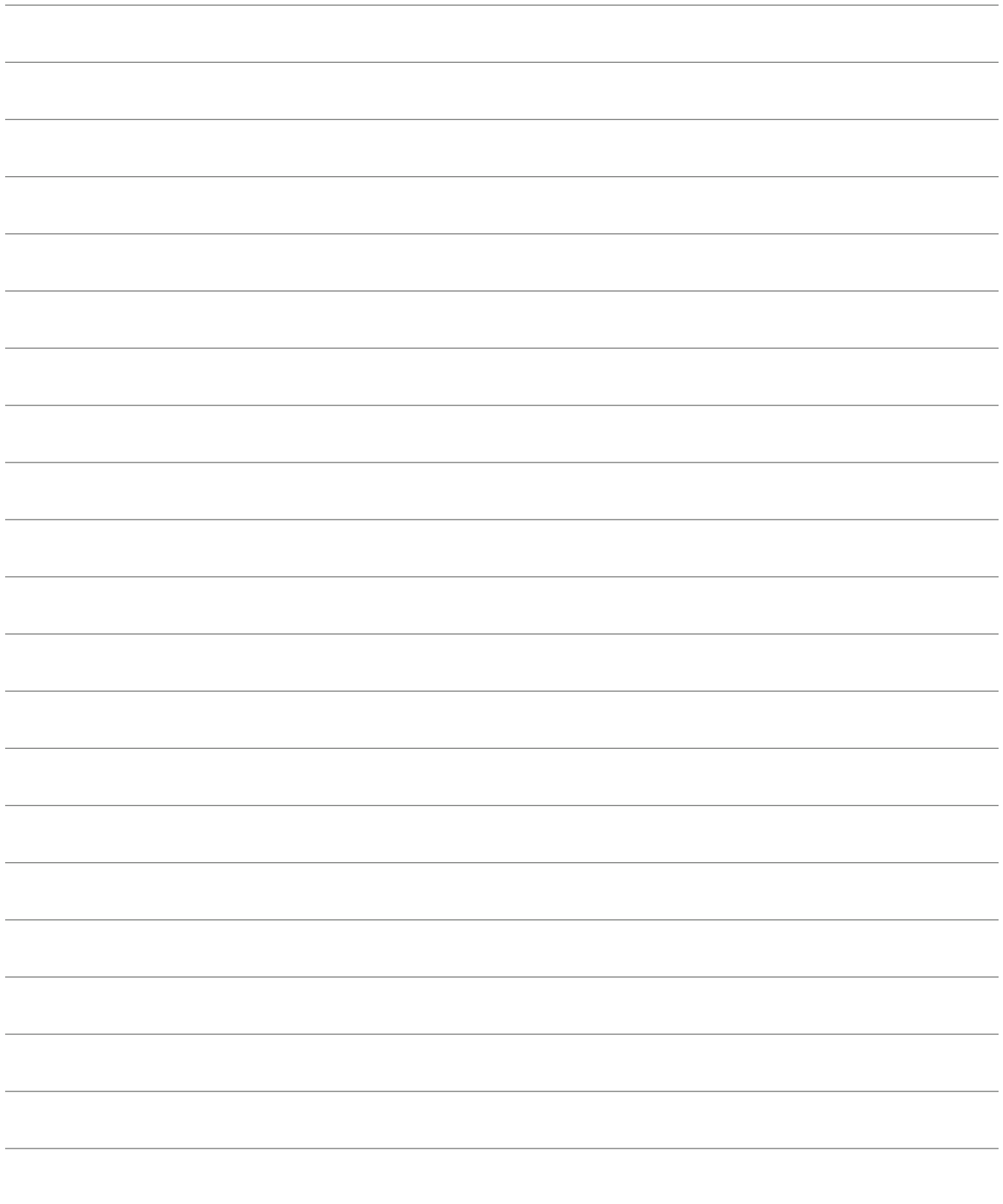
The Mass Spectrometry and Proteomics Facility (MSP) is part of OSU's Campus Chemical Instrument Center (CCIC) and serves a wide variety of research groups from OSU, other universities and industry nationwide. MSP provides considerable expertise in bottom up and top down proteomics, quantitative proteomics 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 ThermoFisher Orbitrap Fusion tandem HPLC MS/MS system, ii) a ThermoFisher Quantiva QQQ HPLC MS/MS, and iii) a Bruker 15 T Solarix XR FT-ICR ultrahigh resolution instrument, and a iv) Thermo QE Plus HPLC MS/MS system. Representative examples for collaborative projects and services will be shown in the presentation.

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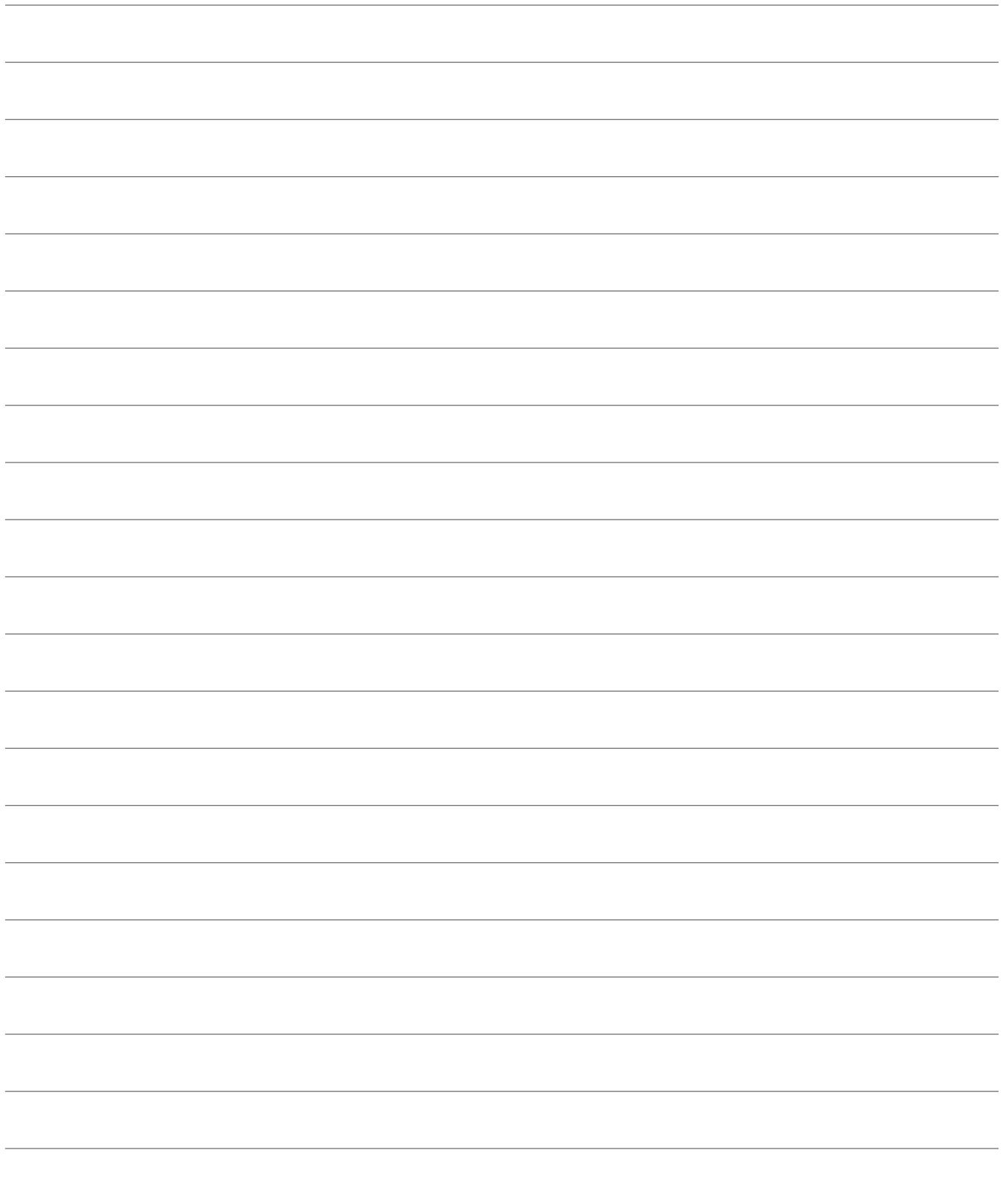
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DISCOVERY AT OHIO STATE

The Inaugural Conference on Food and
Nutritional Metabolomics for Health
and
The 14th Annual Ohio Mass
Spectrometry Symposium

Foods for Health Discovery Theme
discovery.osu.edu/ffh

Campus Chemical Instrument Center
ccic.ohio-state.edu



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