# MANA 2024 Conference Scientific Program

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| **Monday (Oct. 21st)** | | |
| 3:00-6:00 PM | Vendor Setup in Atrium | |
| 4:30-6:00 PM | Interactive Workshops (p. 3) | |
| 6:00-8:30 PM | Reception in Atrium | |
| **Tuesday (Oct. 22nd)** | | |
| 8:45-9 AM | **Welcome:** Elsa Flores, PhD **Orientation:** John Koomen & Tim Garrett | |
| 9-9:45 AM | **Plenary Presentation 1: Gina DeNicola, PhD** in Couch Auditorium (p. 3) | |
| 9:45-10:00 AM | Break in Atrium (Coffee, Tea, & Snacks) | |
| 10:00-11:55 AM | **Parallel Session 1: Biomarkers** (Murphey) Tim Garrett (p. 4) | **Parallel Session 2: Lipidomics** (Couch) Jace Jones and Jeffrey McDonald (p. 5) |
| 12:00-1:00 PM | Lunch & Corporate Presentations (Thermo Interactive Workshop in Couch Auditorium (p. 6), Panome Bio in Trustees Boardroom, Waters in Murphey Conference Room, Sciex in Ferman Conference Room) | |
| 1-2:30 PM | Poster Presentations in Atrium (All Posters) (p. 6-8) | |
| 2:30-3:00 PM | **Early Career Rising Star Award: Ipsita Mohanty, PhD** in Couch Auditorium (p. 9) | |
| 3:00-3:45 PM | **Plenary Presentation 2: Tao Huan, PhD** in Couch Auditorium (p. 9) | |
| 3:45-4:00 PM | Break in Atrium (Beverages & Snacks) | |
| 4:00-5:55 PM | **Parallel Session 3: Environment & Ecology** (Murphey) Valerie Copie (p. 10) | **Parallel Session 4: Multi-omics** (Couch) Xiuxia Du (p. 11) |
| 6:00-7:00 PM | Interactive Workshops (p. 12)/Independent Poster Viewing in Atrium | |
| 7:00-9:00 PM | ECM Reception | |
| **Wednesday (Oct. 23rd)** | | |
| 8:00-8:55 AM | Corporate Breakfasts (Leco in Murphey Conference Room, IROA in Ferman Conference Room) | |
| 9-9:45 AM | **Womix Mentorship Award: Rachel Kelly, PhD** in Couch Auditorium (p. 12) | |
| 9:45-10:00 AM | Break in Atrium (Coffee, Tea, & Snacks) | |
| 10:00-11:55 AM | **Parallel Session 5: AI/ML** (Murphey) Paul Stewart (p. 13) | **Parallel Session 6: Health & Disease** (Couch) Teklab Gebregiworgis (p. 14) |
| 12:00-1:00 PM | Lunch & Corporate Presentations (Agilent in Ferman Conference Room, Bruker in Couch Auditorium, Thermo in Murphey Conference Room, Beckman Coulter in Trustee's Boardroom) | |
| 1-2:30 PM | Poster Presentations in Atrium (All Posters) (p. 15-17) | |
| 2:30-3:00 PM | **Lightning Talks** (6*5 min) in Couch Auditorium (p. 18) | |
| 3:00-3:45 PM | **Plenary 3: Julia Laskin, PhD** in Couch Auditorium (p. 18) | |
| 3:45-4:00 PM | Break in Atrium (Beverages & Snacks) | |
| 4:00-5:55 PM | **Parallel Session 7: Single Cell Organisms & Microbiomes** (Murphey) Ipsita Mohanty (p. 19) | **Parallel Session 8: New Technologies & Methodologies** (Couch) Lloyd Sumner (p. 20) |
| 6:00-7:00 PM | Interactive Workshops (p. 21)/Independent Poster Viewing in Atrium | |
| **Thursday (Oct. 24th)** | | |
| 9-9:45 AM | **Plenary Presentation 4: Patricia Scaraffia, PhD** in Couch Auditorium (p. 21) | |
| 9:45-10:00 AM | Break in Atrium (Coffee, Tea, & Snacks) | |
| 10:00-11:55 AM | **Parallel Session 9: Cancer Metabolism** (Murphey) Vanessa Rubio (p. 22) | **Parallel Session 10: Metabolite ID** (Ferman) Emily Gentry (p. 23) |
| 12:00-1:00 PM | Lunch & MANA Members Meeting in Couch Auditorium | |
| 1-2:30 PM | Poster Presentations in Atrium (All Posters) (p. 24-26) | |
| 2:30-3:00 PM | **Mark P. Styczynski Early Career Award in Computational Metabolomics: Joshua Mitchell, PhD** in Couch Auditorium (p. 27) | |
| 3:00-3:45 PM | **Plenary Presentation 5: Oliver Fiehn, PhD** in Couch Auditorium (p. 27) | |
| 3:45-4:00 PM | Break in Atrium (Beverages & Snacks) | |
| 4:00-5:55 PM | **Parallel Session 11: Food & Nutrition** (Murphey) Waylon Hastings (p. 28-29) | **Parallel Session 12: Flux** (Couch) Stacy Sherrod (p. 30) |
| 6:00-8:00 PM | Awards in Couch Auditorium & Closing Dinner in Atrium | |

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# Thanks to the MANA Corporate Members!

# Monday

## MANA 2024 Monday Afternoon Interactive Workshops (4:30-6 PM)

**Spatial Isotopic Tracing in Mammalian Tissues**
Organizer(s): Matthew Merritt; Ramon Sun
Location: Ferman Conference Room

**Unveiling the mQACC Living Guidance for QA/QC Best Practices in LC-MS-Based Untargeted Metabolomics**
Organizer(s): Jonathan Mosley; Dajana Vuckovic
Location: Couch Auditorium

**Hands-on Workshop on using benchmarking dataset to evaluate software tools for preprocessing mass spectrometry-based metabolomics data**
Organizer(s): Xiuxia Du; Gary Patti
Location: Murphey Conference Room

**Engaging with MANA and Increasing the Impact of Metabolomics through MANA Interest Groups**
Organizer(s): Ewy Mathé; Arpana Vaniya
Location: Trustees Boardroom

# Tuesday

## MANA 2024 Plenary Lecture 1

Gina M. DeNicola, PhD
H. Lee Moffitt Cancer Center
**NRF2 and cysteine metabolism in cancer**
Tuesday, October 22nd, 9:00-9:45 AM
Location: Couch Auditorium

## Session 1: Biomarkers

Session Chair: Tim Garrett
Location: Murphey Conference Room
Time: Tuesday, October 22nd, 10:00 AM - 12:00 PM

**10:00** **Applications of NMR metabolomics to wildlife management: monitoring of the nutritional states of wild bighorn sheep using 1H NMR spectroscopy**.
Copie, Valerie; O'Shea-Stone, Galen; Tripet, Brian, Garrott, Robert, and Thomson, Jennifer.
Montana State University-Bozeman.

**10:20** **High Throughput Plasma Profiling of Human Liver Disease Samples using Rapid Chromatography and a Multi-Reflecting Time-of-Flight Mass spectrometer**.
Martin, LeRoy; King, Adam M; Sanchez-Lorenzo, Ana, Marsden-Edwards, Emma, Want, Elizabeth.
Waters Corp, Marblehead, MA; Waters Corp, Wilmslow, Cheshire, UK; Department of Metabolism, Digestion and Reproduction, Imperial College, London, UK; Waters Wilmslow; Imperial College.

**10:40** **Evaluation of Biomarkers of Reproductive Success in Atlantic Salmon Biofluids using NMR-based Metabolomics and Machine Learning**.
Casu, Fabio; Schock, Tracey; Bayless, Amanda; Mahynski, Nathan; Boggs, Ashley.
National institute of Standards and Technology (NIST), Chemical Sciences Division.

**11:00** **Plasma Metabolome as a Biomarker for Immunotherapy in Recurrent or Metastatic Head and Neck Cancer**.
Eldridge, Ronald C.; Anderson, Allyson; Magliocca, Kelly; Shi, Qiuying; Patel, Mihir R.; Bates, James E.; Schmitt, Nicole C.; Steuer, Conor E.; Shin, Dong M.; Liu, Yuan; Teng, Yong; Chung, Christine H.; Saba, Nabil F.
Emory University and Moffitt Cancer Center.

**11:20** **The Human Metabolome Atlas unveils metabolic heterogeneity across cell types and stratifies cancer subtypes**. J. Rafael Montenegro Burke, Jeremy K. Chan, William D. Gwynne, Nicholas Ly, Olivia Taverniti, Brandon Y. Lieng, Mathula Muhundan, Alexandra Denhart, Andrew T. Quaile.
Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, ON.

**11:40** **Metabolic Changes in Response to Hormonal Therapy in Advanced Prostate Cancer**.
Mohammad Alyamani, Nima Sharifi.
University of Miami.

## Session 2: Lipids & Lipidomics with the International Lipidomics Society

Session Chairs: Jace Jones and Jeffrey McDonald
Location: Couch Auditorium
Time: Tuesday, October 22nd, 10:00 AM - 12:00 PM

**10:00 Deciphering Spatial Variation in Lipid Profiles of X-Ray Irradiated Cells using nLC and Orbitrap 240 MS.**
Susan S Bird-1; Rahul Ravi Deshpande-1; Kyle D.G. Saunders-2; Johanna Gerichten-2; Bashar Amer-1; Melanie Bailey-2.
1-Thermo Fisher Scientific, San Jose, CA; 2-Department of Chemistry, University of Surrey, Guildford, United Kingdom.

**10:20 Measurement of cholesterol and bile acids synthesis with deuterated water and mass spectrometry.**
Xiaorong Fu, Adrianna Maurer, Stanislaw Deja, Justin A. Fletcher, Sean Slater, Jeffrey G. McDonald, Jay D. Horton, John P. Thyfault, Matthew A. Mitsche and Shawn C. Burgess
Center for Human Nutrition, The University of Texas Southwestern Medical Center, Dallas, TX 75390

**10:40 Ozonolysis kinetics for the study of cis/trans isomerism.**
Troy R Scoggins IV, Boone M. Prentice.
Department of Chemistry, University of Florida, Gainesville, FL, 32603; USA.

**11:00 Artificial Intelligent Agents for Automating Deep Lipidomics Workflows to Investigate Alzheimer's Disease and Aging-Related Lipid Droplets.**
Randolph C 1 , Muhoberac M 1 , Beveridge C 1 , Manchanda P 1 , Iyer S 1 , Poad B 3 , Blanksby S 3 , Tichy S 4, Chopra G 1,2
1 Department of Chemistry, Purdue University, West Lafayette Indiana, United States of America
2 Department of Computer Science, Purdue University, West Lafayette Indiana, United States of America
3 Queensland University of Technology, Brisbane Queensland, Australia
4 Agilent Technologies, Santa Clara California, United States of America

**11:20 Bridging Gaps in Honey Bee Pheromone Analysis Using State-of-the-Art Lipidomics Analysis**.
Alcazar, Armando; McAfee, Alison; Hoover, Shelley E.; Foster, Leonard J.
1. Michael Smith Laboratories, Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada 2. Life Sciences Institute, Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada 3. Department of Applied Ecology, North Carolina State University, North Carolina, NC, USA 4. Department of Biological Sciences, University of Lethbridge, Lethbridge, AB, Canada

**11:40 ILS Americas and an Interactive Checklist for Lipidomic Researchers. Partnering with MANA to Improve the Quality of Lipidomic Measurements.**
Jace W. Jones and Jeffrey G. McDonald.
University of Maryland School of Pharmacy and UT Southwestern Medical Center

## MANA 2024 Tuesday Lunch Workshop

**Metabolic Flux Analysis and Stable Isotope Resolved Metabolomics**

Organizer(s): Rahul Deshpande; Bashar Amer; Susan Bird

Location: Couch Auditorium

Time: Tuesday, October 22nd, 12:00 PM - 1:00 PM

## Tuesday Posters

**Poster #1:** **A role for Untargeted Metabolomics in cultured meat production through optimizing media composition and growth conditions** Cohen Tom; Stancliffe, Ethan; Richardson, Adam; Mehta, Ashima; Gandhi, Monil; Guzior, Doug V; Cho, Kevin; Patti, Gary. Panome Bio, Washington University in Saint Louis

**Poster #2:** **Biochemical phenotyping of null alleles in human induced pluripotent stem cell-derived cell lineages: integrating genetics with metabolomics** Thapa, Maheshwor; Zheng, Shujian; Mitchell, Joshua; Diniz, Juliana; Oliveira, Nelio; Tagger, Arti; Chi, Yuanye; Siddiqa, Amnah; Gong, Minghao; McDonough, Justin; Skarnes, William; Robson, Paul; Li, Shuzhao. The Jackson Laboratory for Genomic Medicine, 10 Discovery Dr, Farmington, CT 06032

**Poster #3:** **Genetic Mechanisms Controlling Development: Insights from Metabolomics** Jaiyesimi, Olakunle; Extavour, Cassandra 1. Department of Organismic and Evolutionary Biology, Harvard University, 2. Department of Molecular and Cellular Biology, Harvard University 3. Howard Hughes Medical Institute, Chevy Chase, Maryland, United States of America

**Poster #4:** **Multi-omics approaches for the identification of HCC biomarkers in patients with liver cirrhosis** Rashid, Md Mamunur; Varghese, Rency; Ressom, Habtom. Georgetown University

**Poster #5:** **RaMP-DB 3.0: A Relational Database for Multi-Omic Data Interpretation** Mehta, Khyati Y.; Patt, Andrew; Sheils, Timothy; Tisch, Adam; Sayer, Jaden; Braisted, John; Kelleher, Keith J.; Mathé, Ewy A. National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD

**Poster #6:** **A quantitative assay for measuring 1200+ metabolites in biofluids** Mandal, Rupasri; Zheng, Jiamin ; Zhang, Lun; Johnson, Mathew; Wishart, David S. University of Alberta

**Poster #7:** **Activity guided fractionation to identify the quorum molecule in Neurospora crassa** Molina, Alexis; Esselman, Christopher; Edison, Art. University of Georgia

**Poster #8:** **Application of NMR-based metabolomics to identify potential cerebrospinal biomarkers of disease progression in patients with multiple sclerosis.** Nadia Ashrafi, Ahmet Tarik Baykal, PhD; Boran Aksakal, Ceyda Buyuker, Eda Tahir Turanli, PhD, Stewart F. Graham, PhD and Ali Yilmaz, PhD.

**Poster #9:** **Combination of Low Glucose and SCD1 Inhibition Impairs Cancer Metabolic Plasticity and Growth: A Comprehensive Metabolomic and Lipidomic Analysis.** Zhu Wentao; Raftery Daniel. University of Washington

**Poster #10:** **Dual MSTUS sample-to-sample Normalization as a Batch-to-Batch normalization correction.** Beecher C.(2), Ghosh D.(1), de Jong F.(2), Shulaev V.(1) 1Department of Biological Sciences, College of Science, University of North Texas, Denton, Texas; 2 IROA Technologies LLC, Chapel Hill, North Carolina

**Poster #11:** **ECIDbase.org : Exposome Correlation and Interpretation Database (ECID)** Dinesh Barupal. Icahn School of Medicine at Mount Sinai

**Poster #12:** **Effects of light intensity on Phaeodactylum tricornutum primary carbon metabolism and TAG synthesis** Zheng, Amy; Wang, Bo; Ruiz-Marquez, Kevin; Cheah, Yi Ern; Paton, Andrew; Kassaw,Tessema; Peers, Graham;Young, Jamey. Vanderbilt University (1-4,8), Colorado State University(5-7)

**Poster #13:** **EnrichMet: an R package for quick and easy pathway enrichment** Dhake, Neha : Stewart, Paul* 1 Department of Bioinformatics & Computational Biology, Morsani College of Medicine, University of

South Florida, Tampa, FL 2 Department of Biostatistics and Bioinformatics, Moffitt Cancer Center, Tampa, FL

**Poster #14:** **Estimating the Biological Potency of 2,3-Benzofluorene via Untargeted Metabolomics Analysis of Plasma from a Short-Term in vivo Study** Crizer, David; Rice, Julie; Auerbach, Scott Division of Translational Toxicology/National Institute of Environmental Health Sciences

**Poster #15:** **Exploring Acyl Carnitines in the Human Metabolome Using Reverse Metabolomics** Emily C. Gentry, Sara Pacini, Lindsay E. Sandusky Virginia Tech, Department of Chemistry, Blacksburg, VA, USA

**Poster #16:** **High throughput metabolite quantification in biological samples with Pyxis, a matrix-agnostic AI/ML tool.** Ana S. H. Costa, Craig Knisley, Devesh Shah, Timothy Kassis, Mimoun Cadosch Delmar, Jennifer M. Campbell, Jack Geremia. Matterworks, Inc, Somerville, MA

**Poster #17:** **Identifying Feature Recurrence via All-by-All Alignments.** Hitchcock, Daniel; Krejci, Jesse; Jeanfavre, Sarah; Avila-Pacheco, Julian; Clish, Clary. Metabolomics Platform, The Broad Institute

**Poster #18:** **Investigating Metabolic Phenotypes For the Diagnosis of Sarcoidosis and Explore Immunometabolic Phenotypes and Unraveling Disease Mechanisms**. Mohammad Mehdi Banoei, Abdulrazagh Hashemi Shahraki, Kayo Santos, Gerg Holt, Mehdi Mirsaeidi. University of Calgary

**Poster #19:** **Large scale combinatorial synthesis to create a MS/MS reference library for the discovery of disease associated molecules.** Patan, Abubaker 1,2; Charron-Lamoureux, Vincent 1,2; Deleray, Victoria 1,2; Vittali, Kyle 1,2; Lee,Carlynda 1,2; Leanos, Daniel1,2; Mohanty, Ipsita1,2; Mannochio-Russo, Helena 1,2; Xing, Shipei 1,2; El-Abiad, Yasin 1,2; Siegel, Dionicio 1; Dorrestein, Pieter C. 1,2* 1Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, CA, United States. 2Collaborative Mass Spectrometry Innovation Center, University of California San Diego, La Jolla, CA, United States.

**Poster #20:** **METABOLOMIC AND LIPIDOMIC ANALYSIS FROM FORMALIN-FIXED PARAFFIN-EMBEDDED GASTRIC BIOPSIES SAMPLES.** Ramm, Maximiliano; Martinez, David, Delgado, Carolina; Muñoz, Esteban; Barrera, María Paz & Bustamante, Luis. 1 Departamento Análisis Instrumental, Facultad de Farmacia, Universidad de Concepción, Concepción, Región del Biobio, Chile. 2 Departamento de Especialidades, Facultad de Medicina, Universidad de Concepción, Concepción, Región del Biobio, Chile.

**Poster #21:** **Metabolomic Changes in Biofilm of Pseudomonas Aeruginosa Under Tobramycin Treatment Identified by COLMARq.** Munki Choo,1 Da-Wei Li,2 Devin Sindeldecker,3 Paul Stoodley,3 Lei Bruschweiler-Li,2 and Rafael Brüschweiler1,2,4* 1Department of Chemistry and Biochemistry, 2Campus Chemical Instrument Center, 3Department of Microbial Infection and Immunity, 4Department of Biological Chemistry and Pharmacology, The Ohio State University, Columbus, Ohio 43210, U.S.A.

**Poster #22:** **NIST SRM 1950 Beyond the Certificate of Analysis: mQACC Results of Community-Driven Qualitative and Quantitative Data.** Bayless, Amanda*; Davis, W. Clay*; Aristizabal-Henao, Juan; Artati, Anna; Barsch, Aiko; Beecher, Chris; Beger, Richard; Bowden, John; Broeckling, Corey; Cho, Joo-Youn; Dunn, Rick; Flores, Roberto; Fradin, Manon; Franchina, Flavio A.; Gouveia, Goncalo J.; Harms, Amy; Hartung, Thomas; Haznadar, Majda; Henriques da Costa, Sofia Ana; Jones, Christina; Kang, Kyo Bin; Lewis, Matthew; Lippa, Katrice; Malinowska, Julia; Nair, Sindhu; Ngere, Judith; Ntai, Ioanna; Percy, Andrew; Plumb, Robert; Raftery, Dan; Rahman, Anas Abdel; Schock, Tracey*; Sun, Jinchun; Tayyari, Fariba; Theodoridis, Georgios; Torta, Federico; Ulmer Holland, Candice Z.; Velagapudi, Vidya; Wilson, Ian; Zhang, Bo; Cumeras, Raquel. *National Institute of Standards and Technology, Charleston, SC; and members of Metabolomics Quality Assurance and Quality Control Consortium in the Reference and Test Materials Working Group

**Poster #23:** **Precision Targeting of Ferroptosis in Colorectal Cancer: Sex and KRAS Mutation-Driven Metabolic Vulnerabilities and Drug Repurposing.** Shen, Xinyi; Yan, Hong; Yao, Yisha; Khan, Sajid; Ma, Shuangge; Johnson, Caroline. Yale School of Public Health

**Poster #24:** **Progesterone Metabolism and Breast Cancer Risk using Simultaneous Quantitation and Discovery (SQUAD) Liquid Chromatography Mass Spectrometry.** Kenney, Katherine; German, Rana; Moore, Samuel; Gaul, David A.; Kim, Jaeyeon; Fernandez, Facundo M. School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332 2) Petit Institute of Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332 3) Department of Biochemistry and

Molecular Biology, Indiana University School of Medicine, Indiana University, Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN 46202 4); The Komen Tissue Bank, Indiana University School of Medicine, Indiana University, Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN 46202

**Poster #25:** **Quantification of underivatized acylcarnitines and carnitine intermediates using RP chromatography and ion funnel triple quadrupole.** Silva, Bianca; Cuthbertson, Daniel. Agilent Technologies

**Poster #26:** **Quantitative analysis and structural characterization of bile acids using the ZenoTOF 7600 system.** Colquhoun David(1);Baker, Paul RS(1); Proos, Robert(1); Seferovic ,Maxim D(2); and Horvath, Thomas D(3,4) 1) SCIEX, USA; 2) Dept of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX; 3) Dept of Pathology, Texas Children's Hospital, Houston, TX; 4) Dept of Pathology & Immunology, Baylor College of Medicine, Houston, TX

**Poster #27:** **Reducing Complexity in Polar Feature Detection in Non-Targeted LC-MS Experiments from the Presence of Salt in Sample Preparation.** David A. Gaul, Ying Liu, Samuel Moore 1School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332

**Poster #28:** **Sample Preparation Optimization for Metabolomics Analysis of Cell Culture Media to reduce Maillard reaction products.** Nguyen, Vyncent; Asik, Didar; Campbell, Andy; Goldfuss, Jaime; Tu, Chengjian. Thermo Fisher Scientific

**Poster #29:** **Semi-automated extraction of cell and tissue samples for multi-omic analysis using the Biomek i7 workstation.** Smith, Zachary; Jones, Martin; Viant, Mark. Beckman Coulter Life Sciences; Phenome Center Birmingham University of Birmingham

**Poster #30:** **The depth of the annotatable metabolome is a function of the mass spectrometer and software versions.** Barnes, Stephen (1); Berryhill, Taylor F (1); Wilson, Landon (1); Youngmee Kim (2). University of Alabama at Birmingham (1); University of Miami (2)

**Poster #31:** **Untargeted analysis of lipid biomarker to inform carbon storage potential in soils.** Mioko Tamura, Tyler McIntosh, Vidya Suseela, and Nishanth Tharayil. Clemson University, South Carolina, USA

**Poster #32:** **Untargeted Metabolomics of Blood Plasma from Short-Term Repeat Dose in vivo Studies to Estimate Biological Potency of PFAS Compounds.** Rice, Julie; Auerbach, Scott; Crizer, David. Division of Translational Toxicology/National Institute of Environmental Health Sciences

**MANA 2024 Early Career Rising Star Award**

Ipsita Mohanty, PhD

University of California, San Diego

**Diving deeper into the bileome: MS2 fragmentation-based filtering identifies bile acid regio- and stereoisomers to reveal unique patterns in biology**

Tuesday, October 22nd, 2:30-3:00 PM

Location: Couch Auditorium

**MANA 2024 Plenary Lecture 2**

Tao Huan, PhD

University of British Columbia

**A deep dive into sample normalization for improved quantitative performance in untargeted metabolomics**

Tuesday, October 22nd, 3:00-3:45 PM

Location: Couch Auditorium

## Session 3: Environment & Ecology

Session Chair: Valerie Copie
Location: Murphey Conference Room
Time: Tuesday, October 22nd, 4:00 PM - 6:00 PM

**4:00 Development of metabolomics toolbox for identification and discovery of non-protein amino acids in plants.**
Sandhu, Pawanjit K; Murch, Susan J
University of British Columbia Okanagan

**4:20 Mass Spectrometry-Based Metabolomics to Elucidate Spider Mite-Host Interactions for the Development of RNAi-Based Biopesticides.**
Sharma, Chetan; Abiskaroon, Brendan; HA, Ricardo; Singh, Vinayak; Zhurov, Vladimir; Maglov, Jorden; Harrison, Alexander; Antonacci, Michele; Chruszcz, Maksymilian; Grbic, Vojislava.
Western University, London, Ontario, Canada

**4:40 Metabolomic Profiling of Coral Microhabitats: Understanding the Metabolic Response of Coral Tissue, Mucus, and Skeleton.**
Garcia, Brianna M.; Kido Soule, Melissa C.; Brandt, Marilyn; Apprill, Amy; Kujawinski, Elizabeth B.
Woods Hole Oceanographic Institution, Woods Hole, MA 02543; Woods Hole Oceanographic Institution, Woods Hole, MA 02543; University of the Virgin Islands, St Thomas, Charlotte Amalie West, St Thomas 00802, U.S. Virgin Islands; Woods Hole Oceanographic Institution, Woods Hole, MA 02543; Woods Hole Oceanographic Institution, Woods Hole, MA 02543

**5:00 Leveraging untargeted and targeted approaches to characterize molecular complexity and conservation of compounds released from the degradation of plant substrates.**
Xia, Mengxue; Suseela, Vidya; Tharayil, Nishanth
Clemson University

**5:20 Comprehensive profiling of Nepeta Cataria using multidimensional gas chromatography, and high-performance mass spectrometry.**
David E. Alonso, Joseph Binkley, and John Hayes
LECO Corporation

**5:40 Innovative application of Weighted Quantile Sum in Mediation Analysis of Metabolomics, PFAS Exposure, and SARS-CoV-2 IgG Levels in Pregnancy.**
Haibin, Guan; Shelley, Liu; Georgia, Dolios; Jia, Chen; Lauren, Petrick
Department of Environmental Medicine and Public Health at the Icahn School of Medicine at Mount Sinai

## Session 4: Multi-Omics

Session Chair: Xiuxia Du
Location: Couch Auditorium
Time: Tuesday, October 22nd, 4:00 PM - 6:00 PM

**4:00 Mega Metabolomics: A Big Data Challenge and Opportunity with 13,700 human plasma samples for the TEDDY cohort.**
Keshet, Uri; Wohlgemuth, Gert; Li, Yuanyue; Fiehn, Oliver
West Coast Metabolomics Center, UC Davis

**4:20 Leveraging Scale with Pan-Repository Scale Analysis: Tracing the Evolutionary Chemistry of Life through Mining of Public Metabolomics Data.**
El Abiead, Yasin; Strobel, Michael; Hagey, Lee; Mohanty, Ipsita; Gentry, Emily; Mannochio-Russo, Helena; Gomes, Paulo Wender P; Zuffa, Simone; Wang, Mingxun; Dorrestein, Pieter C.
Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA, USA; Department of Computer Science and Engineering; University of California Riverside, 900 University Ave, Riverside, CA, 92521, USA; Department of Chemistry, Virginia Tech, Blacksburg, VA, USA; Collaborative Mass Spectrometry Innovation Center, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA, USA

**4:40 Dysregulation of immunometabolomics in ME/CFS patients is linked to xenobiotics and predictive of clinical symptoms.**
Gong, Minghao; Gunter, Courtney; Karabacak, Fatih; Kozhaya, Lina; Xiong, Ruoyun; Oh, Julia; Vernon, Suzanne; Bateman, Lucinda; Unutmaz, Derya; Li, Shuzhao
1. The Jackson Laboratory for Genomic Medicine, 10 Discovery Drive, Farmington, CT 06032, USA 2. Bateman Horne Center, Salt Lake City, Utah, USA. 3. University of Connecticut School of Medicine, Farmington, CT 06032, USA.

**5:00 Comprehensive multi-omic profiling of lung squamous cell carcinomas.**
Paul Stewart1#, Isis Narvaez-Bandera1, Ashley Lui1, Vanessa Rubio1, Min Liu1, Eric Welsh1, Dalia Ercan1, Hayley Ackerman1, Guohui Li2, Lancia Darville1, Bin Fang1, Steven Eschrich1, John Koomen1, Brooke Fridley3, Eric Haura1, Gina DeNicola1, Elsa Flores1
1Moffitt Cancer Center, 2Thermo Fisher Scientific, 3Children's Mercy Hospital, #Presenting Author

**5:20 Analysis of Metabolic Consequences Associated with Variants in ALDH18A1 Uncovers Perturbations in Multiple Amino Acid and Antioxidant Pathways.**
Colonna, Maxwell B.; Lyons, Michael J.; Flanagan-Steet, Heather; Steet, Richard
Greenwood Genetic Center

**5:40 Multi-omics Analysis of Staphylococcal Enterotoxin B Exposure in a Soldier-on-a-Chip: An Advanced Micro Physiology System.**
Raquel L. Shortt, Conor C. Jenkins, Gabrielle M. Rizzo, Allison E. Clay, Tyler D. Goralski, Elizabeth S. Dhummakupt.
DEVCOM CBC

## Tuesday Evening Interactive Workshops (5:45-7 PM)

### Career Development Q&A/Building and Leveraging your Personal Brand to Accomplish your Professional Goals

Organizer(s): John Koomen; Tim Garrett; Arpana Vaniya; Maryam Goudarzi; Stephanie Bishop; Prasanna Kumaar; Khayti Pathak; Nicole Prince; Goncalo Gouveia; Erica Forsberg

Location: Murphey Conference Room

### AI in NMR Metabolomics

Organizer(s): Hamid Eghbalnia; Leo Cheng; Art Edison; Valerie Copie

Location: Ferman Conference Room

### Round Robin Study Design for Inter-Lab Reproducibility Analyses in Metabolomics

Organizer(s): Haley Chatelaine; Chris Beecher

Location: Couch Auditorium

# Wednesday

## MANA 2024 WOMIX Mentorship Award Lecture

Rachel Kelly, PhD

Brigham and Women's Hospital, Harvard University

Location: Couch Auditorium

Wednesday, October 23rd, 9:00-9:45 AM

## Session 5: Artificial Intelligence & Machine Learning

Session Chair: Paul Stewart
Location: Murphey Conference Room
Time: Wednesday, October 23rd, 10:00 AM - 12:00 PM

**10:00 Convolutional Neural Network-Based Metabolite Annotation**
Chau, Katharine; Zhang, Xinran; Varghese, Rency; Ressom, Habtom
Georgetown University

**10:20 What do data on environmental exposures in the NHANES dataset tell us?**
Annie Cen, Xiuxia Du
UNC Charlotte

**10:40 Spectrum-guided de novo molecular generation from molecular scaffolds**
Wang, Yinkai; Chen, Xiaohui; Liu, Liping; Hassoun, Soha
Tufts University

**11:00 MassID: A Cloud-Based Untargeted Metabolomics Pipeline for Enhanced Biochemical Discovery.**
Stancliffe, Ethan; Richardson, Adam; Gandhi, Monil; Mehta, Ashima; Guzior, Douglas V.; Cho, Kevin; Cohen, Tom; Patti, Gary
Panome Bio, Inc.; Washington University in St. Louis

**11:20 Optimizing Neural Networks for Real-Time Quantification of Metabolites in Complex NMR Spectra**
Tipirneni-Sajja, Aaryani; Johnson, Hayden
University of Houston

**11:40 Mapping The Landscape of Metabolomics Research**
Bifarin, Olatomiwa; Yelluru, Varun; Simhadri, Aditya; Fernández, Facundo
Georgia Institute of Technology, Atlanta, Georgia

## Session 6: Health & Disease

Session Chair: Teklab Gebregiworgis
Location: Couch Auditorium
Time: Wednesday, October 23rd, 10:00 AM - 12:00 PM

**10:00 Precision Targeting of Ferroptosis in Colorectal Cancer: Sex and KRAS Mutation-Driven Metabolic Vulnerabilities and Drug Repurposing**
Shen, Xinyi; Yan, Hong; Yao, Yisha; Khan, Sajid; Ma, Shuangge; Johnson, Caroline
Yale School of Public Health

**10:20 Multi-Omic Analysis Links Neighborhood Disadvantage to Elevated Stress Hormones and Dysregulated Immune Response in ER+ Breast Cancer Patients**
Hannah Heath(1), Farizi Fazli(1), Oana C. Danciu(2), Garth Rauscher(3), Natalie Pulliam(4), Elona Liko-Hazizi(4), Sarah Friedewald(5), Seema Khan(5), Julie Kim(5), William Gradishar(5), Jonna Frasor(2), Kent F. Hoskins(2), Zeynep Madak-Erdogan(1,6)
1)Department of Food Science and Human Nutrition, University of Illinois, Urbana-Champaign, Urbana, IL USA, 2) Division of Hematology/Oncology, University of Illinois at Chicago, Chicago, IL USA, 3) School of Public Health, University of Illinois at Chicago, Chicago, IL, USA, 4) Northwestern Memorial Hospital, Chicago, IL, USA, 5) Northwestern University Feinberg School of Medicine, Chicago, IL, USA, 6) Cancer Center at Illinois, University of Illinois, Urbana-Champaign, Urbana, IL USA

**10:40 Untargeted metabolomics reveals PFOA-induced immune modulation in a SARS-CoV-2 infection model**
Lanier, Deanna; Uchimiya, Mario; Rowe, Dawne; Tompkin, Stephen; DeWitt, Jamie; Woodlief, Tracey; Edison, Arthur
University of Georgia Institute of Bioinformatics; Complex Carbohydrate Research Center

**11:00 Serum Metabolomics for Capturing Dynamic Metabolic Responses to Burn Injuries and Risk Assessment for Sepsis in Critical Care**
Ana Ruxandra Stanciu(1); Marc Jeschke(2); Diana Tedesco(2); Fadi Khalaf(2);Philip Britz-McKibbin(1)
1. Department of Chemistry and Chemical Biology, McMaster University, Hamilton, Ontario; 2. David Braley Research Institute, Hamilton Health Sciences, Hamilton, Ontario.

**11:20 Medication Exposure Read-out in Untargeted Metabolomics Using a MS/MS Library of Drugs and Metabolites Propagated from Repository-scale Analog Search**
Zhao, Haoqi; Kvitne, Kine E.; Mannochio-Russo, Helena; Lamichhane, Santosh; Lamoureux, Vincent; Dorrestein, Pieter C.
Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego

**11:40 Comprehensive discovery lipidomic workflow which utilizes a novel, multi-reflecting ToF with integrated informatics, providing highly confident lipid characterization and quantification**
Kass, Ignatius J 1;Munjoma, Nyasha C.2; Kirk Jayne 2; Gethings, Lee A 2; Tiberi, Paolo 3; Goracci, Laura 4; Lock, Richard 2
1. Waters Corporation, Milford, MA 2. Waters Corporation, Wilmslow, United Kingdom; 3. Mass Analytica Ltd, Sant Cugat del Vallés, Spain; 4. University of Perugia, Perugia, Italy

## Wednesday Posters

**Poster #1:** **A comprehensive untargeted fecal metabolomics workflow on the Orbitrap Astral MS to achieve deep metabolome coverage and confident compound annotation.** Hermanson, Daniel1; Amer, Bashar1; El Abiead, Yasin2; Dorrestein, Pieter2; Bird, Susan S.1 1 Thermo Fisher Scientific, San Jose, California, USA; 2 University of California - San Diego, California, USA

**Poster #2:** **Metabolomics as a complementary conduit to elucidate the mechanisms of priming-mediated stress memory in plants.** Kaur Gagandeep; Kumar Rohit; Leonard Elizabeth; Tharayil Nishanth. Department of Plant and Environmental Sciences, Clemson University, Clemson, South Carolina 29634, United States

**Poster #3:** **Optimization of sample preparation and LC-MS analysis for high throughput untargeted lipidomics and metabolomics.** Bennouna, Djawed ; Chatelaine, Haley ; Mehta, Khyati; Tisch, Adam; Beecher, Chris; LeClair, Christopher; Mathé Ewy. All authors except Beecher Chris affiliation: National Center for Advancing Translational Sciences (NCATS), Rockville, Maryland, USA. Beecher, Chris affiliation: IROA Technologies, Chapel Hill, NC, USA

**Poster #4:** **Simultaneous quantitation and discovery (SQUAD) metabolomics workflow for the analysis of fecal bile acids.** Stewart, Allison K.1; Amer, Bashar1; Midha, Ayush2; Jain, Isha2; Percy, Andrew J.3; Backiel, Krista3; Deshpande, Rahul R.1; Kline, Joshua P.1; Bills , Brandon1; Bird, Susan 1 1 Thermo Fisher Scientific, San Jose, California, USA; 2 Gladstone Institute, UCSF, California, USA; 3 Cambridge Isotope Laboratories, Inc., Tewksbury, MA

**Poster #5:** **User-Friendly In-Silico Bioprocess Optimization Tool Integrating Flux Balance Analysis with Genetic Algorithms.** Oğuzcan Ünver, Benjamin Gerber, Fırat Kahya, Boran Saruhan. Metastate Bio Inc

**Poster #6:** **A Flexible Cloud Framework for Untargeted Metabolomics Streamlining High-Throughput Analysis.** Gandhi, Monil; Stancliffe, Ethan; Richardson, Adam; Mehta, Ashima; Guzior, Douglas V.; Cho, Kevin; Cohen, Tom; Patti, Gary. Panome Bio, Inc.; Washington University in St. Louis

**Poster #7:** **A High-throughput Mass Spectrometry Platform (Rapidfire-IM-QTOF) to Support Biomarker Discovery.** Xiang Tian, Cristina Di Poto, Sonja Hess, Erik L. Allman. AstraZeneca

**Poster #8:** **An Accelerated Workflow to Extract Biological Pathway Information from Untargeted Metabolomic Datasets.** Montefusco, David1; Xie, Longsheng2,3; Saligrama, Siri1; Yue, Yang1; Liu,Jinze2. 1.Virginia Commonwealth University Department of Biochemistry. 2.Virginia Commonwealth University Department of Biostatistics. 3.George Mason University Department of Biostatistics.

**Poster #9:** **Analyzing the Impact of In-source Fragmentation on Phosphors Derivative Metabolites to Improve Untargeted Metabolomics Analysis.** Yue, Yang*; Cowart, L. Ashley; Allegood, Jeremy; Scalzo, Megan. Lipidomics and Metabolomics Shared Resource, Massey Cancer Center, Virginia Commonwealth University

**Poster #10:** **Annotation of nontargeted LC-MS features in the Jackson Heart Study.** Avila-Pacheco, Julian; Chen, Zsu-Zsu; Tahir, Usman; Katz, Dan; Yan, Gao; Shuilang, Deng; Wilson, James; Gerszten, Rob; Clish, Clary. Broad Institute of Harvard and MIT, Cambridge, MA, US.

**Poster #11:** **Assessing redundancy in untargeted metabolomics feature picking to produce high-fidelity quantitative datasets.** Bonitatibus, Sarah; Henke, Matt. Department of Pharmaceutical Sciences, University of Illinois at Chicago.

**Poster #12:** **Comparison of Sample Preparation methods for At-home Feces Collection in Global Metabolomics.** Lan, Renny; Assress, Hailemariam; Malaviarachchi, Priyangi; Kay, Colin. Metabolomics Core, Arkansas Children's Nutrition Center, Arkansas Children's Hospital, USA

**Poster #13:** **Comprehensive Non-Targeted Characterization of Persistent Organic Pollutants (POPs) in Human Plasma Using GC and GCxGC with High-Performance TOFMS.** John Hayes, David E. Alonso, and Joeseph E. Binkley. LECO Corporation

**Poster #14:** **Cough Breath: A Method to Detect Airway Pathogens of Cystic Fibrosis Patients in the Age of Highly Effective Modulator Therapy.** Karunarathne, Hansani1; Bridges, Christopher1; Remisoski, Lacy1; Crane, Maddey1; Casanova, Claudia1; Kinne, Samantha2; Castillo Bahena, Alicia2; Gil, Marissa3; Padillo, Lienwil3; Querido, Gabriel3; Mielke, Jenna3; McClelland, Marc2; Conrad, Doug3; and

Quinn, Robert1. 1Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, USA. 2 Corewell Health, Grand Rapids, MI, USA. 3 Department of Medicine, University of California San Diego, La Jolla, CA, USA.

**Poster #15:** **Deep Matter annotation using 4D-Metabolomics in Glycyrrhiza uralensis used for Traditional Chinese Medicine.** Forsberg, Erica M.; Nakabayashi, Ryo. Bruker Daltonics

**Poster #16:** **Integrative LC-MS and GC-MS Metabolic Profiling Unveils Dynamic Changes during Barley Malting.** Whitcomb, Sarah; Rani, Heena; Standish, Andy; Walling, Jason. USDA-ARS Cereal Crops Research Unit

**Poster #17:** **Dietary Vitamin B6 Deficiency Impairs Liver and Fecal Metabolites in a Mouse Model of Non-Alcoholic Fatty Liver Disease.** Mayengbam, Shyamchand; Amarasena, Sathya; Hossain, K Shaharina; Rasauli, Ava. Department of Biochemistry, Memorial University of Newfoundland, St. John's, Canada

**Poster #18:** **Elucidating exercise-responsive features in human plasma using multidimensional chromatography and a custom compound annotation workflow.** Abraham Raskind, Charles R. Evans, Phd, Christopher Patsalis, M.S, Gayatri Iyer, PhD, Alexander Raskind, PhD, Alla Karnovsky, PhD, Charles F. Burant, M.D, PhD. University of Michigan

**Poster #19:** **Emerging Opportunities and Obstacles in Metabolomic Epidemiology: A ChatGPT Study**. Waylon J. Hastings. Texas A&M University Department of Nutrition

**Poster #20:** **Fast scanning MRM quantitative lipidomics analysis.** Paul RS Baker (1), Rebekah Sayers (2), David Calquhoun (1), and Ian Moore (3). 1. SCIEX, US; 2. SCIEX, UK; 3. SCIEX, CA

**Poster #21:** **FraGNNet: A Deep Probabilistic Model for Mass Spectrum Prediction.** Fei Wang, Adamo Young, Bo Wang, Hannes Röst, Russ Greiner, David S Wishart. University of Alberta, University of Toronto

**Poster #22:** **Glucose and Glutamine Metabolism in Hypertrophic Cardiomyopathy and Its Association with Inflammatory Pathway Activation.** Venturini, Gabriela; Padilha, Kallyandra; Pereira, Alexandre, Toepfer, Christopher; Seidman, Jon; Seidman, Christine. Department of Genetics, Harvard Medical School; Howard Hughes Medical Institute; Heart Institute, University of Sao Paulo; Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford

**Poster #23:** **High-plex metabolomic profiling on the Orbitrap Astral mass spectrometer.** Tan, Lin; Myer, Ciara; Xiong, Yun; Wei, Bo; Ding, Jibin; Tautenhahn, Ralf; Lorenzi, L Philip. 1. Metabolomics Core Facility, MD Anderson Cancer Center, Houston, TX, United States 2, ThermoFisher Scientific

**Poster #24:** **iModMix: Integrative Modules for Multi-omics Data.** Narvaez-Bandera, Isis; Lui, Ashley; Sulman, Noah; Wilson, Christopher; Welsh, Eric; Ercan, Dalia; Rubio, Vanessa; Ackerman, Hayley; Li, Guohui; Darville, Lancia; Fang, Bin; Eschrich, Steven; Koomen, John; Haura, Eric; DeNicola, Gina; Li, Qian; Chen, Ann; Flores, Elsa; Fridley, Brooke; Stewart, Paul. Moffitt Cancer Center

**Poster #25:** **Integrating untargeted metabolomics and lipidomics with drug adherence monitoring in heart failure patients.** Cajka, Tomas; Hricko, Jiri; Hola, Veronika; Rudl Kulhava, Lucie; Novakova, Michaela; Paucova, Michaela; Rakusanova, Stanislava. Institute of Physiology of the Czech Academy of Sciences, Videnska 1083, 14200 Prague, Czech Republic

**Poster #26:** **Investigating systemic consequences following the depletion of native murine intestinal flora**. Guzior, Douglas V; Stancliffe, Ethan; Rowles, Joe L; Gandhi, Monil; Cho, Kevin; Mehta, Ashima; Richardson, Adam; Cohen, Tom; Patti, Gary J. Panome Bio, Inc.; Washington University in St. Louis

**Poster #27:** **Reverse Metabolomics in the Discovery of 3-Hydroxy N-Acyl Amides.** Victoria Deleray1,2, Helena Mannochio-Russo1,2, Vincent Charron-Lamoureux1,2, Abubaker Patan1,2, Ipsita Mohanty1,2, Pieter C. Dorrestein1,2 1Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, CA, United States. 2Collaborative Mass Spectrometry Innovation Center, University of California San Diego, La Jolla, CA, United States.

**Poster #28:** **Root exudate chemodiversity in sorghum: implications for crop-mycorrhizal symbiosis.** Garg, Chahat; Tharayil, Nishanth; Suseela, Vidya. Clemson University

**Poster #29:** **SLC45A4 encodes a mitochondrial transporter that promotes GABA synthesis from**

**ornithine**. Xiaoyang Su, Cecilia Colson, Yujue Wang, James Atherton. Rutgers University

**Poster #30:** **Targeted metabolomics analysis of key pathways to optimize cell culture media and feeds for Chinese Hamster Ovary cells.** Asik, Didar; Nguyen, Vyncent; Ciganda, Martin; Tu, Chengjian; Goldfuss, Jaime S; Jacobia, Scott ; Dodson, Elizabeth; Campbell, Andy M. Thermo Fisher Scientific, Grand Island, NY; Thermo Fisher Scientific Hunt Valley, MD

**Poster #31:** **The propensity for non-obese diabetic mice to develop Type 1 diabetes is modulated by antibiotic treatment and cecal material transplant: a lipidomics study.** Diaz-Rubio, M. Elena; Zhang, Xuesong; Su, Xiaoyang; Blaser, Martin. Cancer Institute of New Jersey, Rutgers University, New Brunswick, NJ, United States; Center for Advanced Biotechnology and Medicine, Rutgers University, New Brunswick, NJ, United States; Department of Medicine, Rutgers Robert Wood Johnson Med Sch, New Brunswick, NJ.

**Poster #32:** **The role of SNAT2 in the metabolic switch upon post-treatment glioblastoma recurrence**. Bozelli, Jr Jose C1; Puri, A2,3; Venugopal, C3,4; Singh, SK2,3,4; Britz-McKibbin, P1 1Department of Chemistry and Chemical Biology, McMaster University, Hamilton, ON, Canada; 2Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON, Canada; 3Centre for Discovery in Cancer Research (CDCR), McMaster University, Hamilton, ON, Canada; 4Department of Surgery, Faculty of Health Sciences, McMaster University, Hamilton, ON, Canada

**Poster #33:** **Untargeted profiling of submetabolomes using chemical derivatization.** Shujian Zheng; Joshua Mitchell; Shuzhao Li. The Jackson Laboratory For Genomic Medicine.

## MANA 2024 Lightning Talks (5 minutes each)

| | |
|---|---|
| Waylon Hastings | **Emerging Opportunities and Obstacles in Metabolomic Epidemiology: A ChatGPT Study** |
| Rohit Kumar | **Metabolomics for deciphering cellular responses of gene dosage variation** |
| Renny Lan | **Comparison of Sample Preparation methods for At-home Feces Collection in Global Metabolomics** |
| Jose C Bozelli, Jr | **The role of SNAT2 in the metabolic switch upon post-treatment glioblastoma recurrence** |
| Hansani Karunarathne | **Cough Breath: A Method to Detect Airway Pathogens of Cystic Fibrosis Patients in the Age of Highly Effective Modulator Therapy** |
| Gabriela Venturini da Silva | **Glucose and Glutamine Metabolism in Hypertrophic Cardiomyopathy and Its Association with Inflammatory Pathway Activation** |

Location: Couch Auditorium
Time: Wednesday, October 23rd, 2:30-3:00 PM

## MANA 2024 Plenary Lecture 3

Julia Laskin, PhD
Purdue University

**Advances in Nanospray Desorption Electrospray Ionization (nano-DESI) Mass Spectrometry Imaging**

Location: Couch Auditorium
Time: Wednesday, October 23rd, 3:00-3:45 PM

## Session 7: Single Celled Organisms & Microbiomes

Session Chair: Ipsita Mohanty
Location: Murphey Conference Room
Time: Wednesday, October 23rd, 4:00 PM - 6:00 PM

**4:00 Effects of Crabtree-induced metabolic rewiring on essential cellular processes in model yeast species**
Harini Sridharan, April Miguez, Mark P. Styczynski
Georgia Institute of Technology

**4:20 Deciphering bacterial-fungal chemical interaction between Burkholderia cenocepacia and Aspergillus fumigatus using Metabolomics**
Jin, Jiangpeiyun; Garg, Neha
Georgia Institute of Technology

**4:40 Lyso-Lipid Dynamics Reflect Thermal Tolerance in Reef Building Corals**
Cline, Thomas; Samuel, Darnilla; Rosset, Sabrina; Roach, Ty; Tortorelli, Giada; Drury, Crawford; Quinn, Robert
Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, USA; Duke Marine Lab, Duke University, Beaufort, NC, USA; Hawaii Institute of Marine Biology, University of Hawaii and Manoa, Kaneohe Bay, HI

**5:00 Investigating how abiotic stressors alter Cladocopium metabolism and impact coral holobiont dysbiosis**
Chiles, Eric N (1,2); Su, Xiaoyang (2,3)
1. Graduate Program in Microbial Biology, Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ 08901, USA, 2. Metabolomics Shared Resource, Rutgers Cancer Institute of New Jersey, Rutgers University, New Brunswick, NJ 08901, USA, 3. Department of Medicine, Division of Endocrinology, Robert Wood Johnson Medical School, Rutgers University, New Brunswick, NJ 08901, USA

**5:20 High throughput targeted metabolomics library generation on a novel mass spectrometer applied to microbiome analysis**
Amer, Bashar1; Jacob, Cristina1; Remes, Philip M.1; Deshpande, Rahul Ravi1; Jafari, Reza2; Moehring, Thomas1; Bird, Susan S.1
1 Thermo Fisher Scientific, San Jose, California, USA. 2 MetaSci, Canada

**5:40 Forward and Reverse Cosine Similarity Scoring During Real-Time Library Search for Triggering Additional Experiments on Indole Compounds**
Bills, Brandon; Christopher, Michael W.; Yedla, Sunandini; Amer, Bashar; Bird, Susan; Deshpande, Rahul; Barshop, William; Prentice, Boone M.; Garrett, Timothy J.; Zabrouskov, Vlad
Author Affiliations
BB, SY, BA, SB, RD, WB, and VZ: Thermo Fisher Scientific, San Jose, California, USA; MC, BP, and TG: University of Florida, Department of Chemistry

## Session 8: New Technologies & Methodologies

Session Chair: Lloyd Sumner
Location: Couch Auditorium
Time: Wednesday, October 23rd, 4:00 PM - 6:00 PM

**4:00 Serial metabolite extractions reveal de novo formation of metabolite-protein interactions that mask analytes from detection**
Ryan D. Sheldon, Molly T Soper-Hoper, Sydney Shenk, Rae J. House, Megan Gendjar
Van Andel Institute

**4:20 Standardization and Software Enabling Automated Quantitative Metabolomics using Microchip CE-MS**
Thompson, J. Will; Stewart, Sam; Redman, Erin; Mellors, Scott; Guerrette, Josh
908 Devices Inc

**4:40 Rapid profiling of the human skin metabolome with heat pulse desorption mass spectrometry (HPD-MS)**
Rankin-Turner, Stephanie; Hiraoka, Kenzo; Shimada, Haruo; Kinoshita, Kazumasa; Ninomiya, Satoshi
University of Pittsburgh, University of Yamanashi

**5:00 Divergent Metabolic Fates of Aromatic Amino Acid-Derived Isomers: Insights from Metabolomics and HDX-HRMS/MS-Based Resolution of Tautomers**
Christopher, Michael [1]; Ericson, Aiden [1]; Klug, Alexander [1]; Prentice, Boone [1]; Garrett, Timothy [1,2]
[1] Department of Chemistry, University of Florida, [2] Department of Pathology, Immunology, and Laboratory Medicine, University of Florida

**5:20 Customizable Polymeric Nanocarriers for Interrogating Cellular Metabolism with Hydrophobic Tracers and Drugs**
Vincent, Michael; Ellis, Abigail; Sheldon, Ryan
Van Andel Institute

**5:40 Overcoming Speed and Sensitivity Barriers in Lipidomics with the Orbitrap Astral Mass Spectrometer**
Ciara Myer, Rahul Ravi Deshpande, Bashar Amer, Susan S Bird
Thermo Fisher Scientific

## Wednesday Evening Interactive Workshops (5:45-7 PM)

**Working like a data scientist on Jupyter**
Organizer(s): Shuzhao Li
Location: Ferman Conference Room

**Quantitation in untargeted high resolution- mass spectrometry assays: innovations and challenges**
Organizer(s): Lauren Petrick
Location: Couch Auditorium

**The role and career path of early-career staff in core facility across North America**
Organizer(s): Maryam Goudarzi, Uri Keshet, Prassana Kumaar, Hanan Alwaseem
Location: Murphey Conference Room

**Overcoming challenges in ISO 15189 for an effective precision and translational medicine implementation.**
Organizer(s): Yamilé López Hernández and Sindhu Nair
Location: Trustees Boardroom

# Thursday

## MANA 2024 Plenary Lecture 4

Patricia Y. Scaraffia, PhD
Tulane University

**Unlocking the metabolic secrets of Aedes aegypti mosquitoes using reverse genetics and mass spectrometry-based metabolomics**.

Thursday, October 24th, 9:00-9:45 AM
Location: Couch Auditorium

## Session 9: Cancer Metabolism

Session Chair: Vanessa Rubio
Location: Murphey Conference Room
Time: Thursday, October 24th, 10:00 AM - 12:00 PM

**10:00 Discovering Distinct Metabolic Alterations for Colorectal Cancer Diagnosis and Monitoring**
Jiangjiang (Chris) Zhu
The Ohio State University

**10:20 Using Metabolites and Metabolomics to Identify Regulatory Allosteric Sites for Drug Discovery**
Thomas Roddy, Gordon Murray, Joseph LaPointe, Anil Padyana, Marion Dorsch, Maria Jesus-Blanco, Shomit Sengupta
Atavistik Bio

**10:40 Charting the metabolic biogeography of the colorectum in cancer: challenging the right sided versus left sided classification**
Abhishek Jain 1, Montana T. Morris 2, Domenica Berardi 1, Trisha Arora 3, Xavier Domingo-Almenara 3, Philip B. Paty 4, Nicholas J. W. Rattray 5, Daniel Kerekes 2, Lingeng Lu 6, Sajid A. Khan 2, Caroline H. Johnson 1
1 Department of Environmental Health Sciences, Yale School of Public Health, 60 College Street, New Haven, CT, 06510, USA .2 Department of Surgery/Surgical Oncology, Yale School of Medicine, 333 Cedar Street, New Haven, CT, 06510, USA .3 EURECAT - Technology Centre of Catalonia, Omics Sciences Unit, Avda. Universitat 1, 43204 Reus, Catalonia, Spain. 4 Department of Surgery, Memorial Sloan Kettering Cancer Center, 1275 York Ave, New York, NY, 10065, USA. 5 Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, UK .6 Department of Chronic Disease Epidemiology, Yale School of Public Health, 60 College Street, New Haven, CT06510, USA

**11:00 NMR Metabolomics Biomarkers of Human Prostate Cancer from Biopsies After More Than 5-Year Follow-Up**
Leo Cheng, Ella Zhang, Jiaqi Lu, Chin-lee Wu, Adam S. Feldman
Harvard Medical School

**11:20 Metabolic Reprogramming of Tumor Microenvironment by Nitric Oxide**
Erika M Palmieri1, Jonathan M. Weiss1, David A Wink1 and Daniel W McVicar1
1 National Cancer Institute, NIH, USA

**11:40 Triboelectric Nanogenerator-Powered Laser Ablation/Nanoelectrospray Ionization (TENG LAESI) Mass Spectrometry Imaging Ion Source to Study Renal Cell Carcinomas**
Ma, Xin; Leontyev, Dmitry; Asef, Carter K; Arnold, Rebecca S.; Petros, John A.; Fernández, Facundo M.
Department of Chemistry and Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology; Emory School of Medicine and Winship Cancer Institute, Emory University

## Session 10: Metabolite Identification

Session Chair: Emily Gentry
Location: Couch Auditorium
Time: Thursday, October 24th, 10:00 AM - 12:00 PM

**10:00 COLMAR1d: A web server for automated, quantitative 1D NMR-based metabolomics at arbitrary magnetic fields**
Da-Wei Li, Rodrigo Cabrera, Munki Choo, Lei Bruschweiler-Li, Alexandar L. Hansen, and Rafael Brüschweiler
The Ohio State University, Columbus, Ohio 43210, USA

**10:20 Development of a Simultaneous Quantitation and Untargeted Discovery (SQUAD) workflow for coeluting sugar-phosphates using orthogonal fragmentation techniques**
Yedla, Sunandini; Bills, Brandon; Deshpande, R. Rahul; Amer, Bashar; Bird, S. Susan; Zabrouskov, Vlad
Thermo Fisher Scientific

**10:40 Metabolomic Analysis of Papanicolaou Tests for the Discovery of Ovarian Cancer Biomarkers**
Schwiebert, Elisabeth(1); Sah, Samyukta(1,2); Moore, Samuel(1,2); Gaul, David (1,2); Boylan, Kristin (3); Skubitz, Amy (3); Fernández, Facundo (1,2)
School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332 (1), Petit Institute of Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332 (2), Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN 55455 (3)

**11:00 Assessing the utility of organ-on-a-chip technology for human relevant diagnostic biomarker identification**
Clay, Allison; Goralski, Tyler; Fudge, Dylan; Jenkins, Conor; Lee, Priscilla; Dhummakupt, Elizabeth; Rizzo, Gabrielle
US Army DEVCOM Chemical Biological Center; Excet, A Precise Systems Company

**11:20 How deep is the metabolome? Modified chromatography, multidimensional separations and derivatization enhance sensitivity and identification in untargeted metabolomics**
Evans, Charles; Raskind, Abraham; Anderson, Brady
University of Michigan, Department of Internal Medicine

**11:40 A consensus serum metabolome by large-scale data mining reveals major gaps in metabolomic measurements and modeling**
Li, Shuzhao; Chi, Yuanye; Mitchell, Joshua; Thapa, Maheshwor; Zheng, Shujian; Smirnov, Aleksandr; Du, Xiuxia
The Jackson Laboratory for Genomic Medicine

# Thursday Posters

**Poster #1:** **Comprehensive Metabolite Profiling in Formalin-Fixed and Paraffin-embedded Tissue.** Mehta, Ashima; Stancliffe Ethan; Richardson, Adam; Gandhi, Monil; Guzior, Douglas V.; Cho, Kevin; Cohen, Tom; Patti, Gary. Panome Bio Inc.; Washington University in St. Louis

**Poster #2:** **Molecular formulae from fine structures of ultra-high-resolution LC-MS isotope patterns.** Goncalo J. Gouveia (1), Delia Qu (2), Aaron M. Ferber (2), Maximilian J. Helf (3), Carla P. Gomes (2), Frank C. Schroeder (1) 1 - Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY, 14853, USA; 2- Department of Computer Science, Cornell University, Ithaca, NY 14853; 3 - Novartis Fabrikstrasse 2, 4056 Basel, Switzerland

**Poster #3:** **Reproducibility of metabolomic profiles generated using the TruQuant platform in a multi-lab "round-robin" study design.** Haley Chatelaine (1), Chris Beecher (2), Ewy A. Mathé (1) (1) National Center for Advancing Translational Sciences - National Institutes of Health, Rockville, MD; (2) IROA Technologies, Chapel Hill, NC

**Poster #4:** **A pipeline for domain detection and annotation of spatial metabolomics data using hierarchical clustering and Shiny app integration.** Oscar E. Ospina1, Eric Welsh2, Vanessa Rubio3, Lancia Darville4, Min Liu4, Joseph O. Johnson5, John Koomen3, Elsa R. Flores3, Brooke L. Fridley6, Paul Stewart1* 1 Department of Biostatistics and Bioinformatics, Moffitt Cancer Center, Tampa, FL; 2 Biostatistics and Bioinformatics Shared Resource, Moffitt Cancer Center, Tampa, FL; 3 Department of Molecular Oncology, Moffitt Cancer Center, Tampa, FL; 4 Proteomics and Metabolomics Core, Moffitt Cancer Center, Tampa, FL; 5 Analytic Microscopy Core, Moffitt Cancer Center, Tampa, FL; 6 Division of Health Services and Outcomes Research, Children's Mercy Hospital, Kansas City, MO

**Poster #5:** **A potential end-to-end workflow for analysing time-series NMR.** Hossain, Zarif; Delaglio, Frank; Arnold, Jonathan; Edison, Arthur S. Institute of Bioinformatics, University of Georgia

**Poster #6:** **ADAP informatics for analyzing untargeted LC-MS/MS metabolomics data for the NIH Common Fund's Nutrition for Precision Health, powered by the All of Us Research Program.** Xiuxia Du, Aleksandr Smirnov, Toan Nguyen, Radha Krishna Balaji Ponnuru, Blake Rushing, Susan McRitchie, Wimal Pathmasiri, Susan Sumner. UNC Charlotte, UNC Chapel Hill

**Poster #7:** **Application of the sulfo-phospho-vanillin assay for total lipid pre-quantitation and correlation to proteins for sample normalization in untargeted lipidomic LC-MS/MS.** Bailey, Laura S.; Basso, Karri B. University of Florida.

**Poster #8:** **Automated and Efficient In-House Standards Database Development using R.** Tisch, Adam; Bennouna, Djawed; Chatelaine, Haley; Mathé, Ewy. National Center for Advancing Translational Sciences (NCATS), Rockville, Maryland, USA.

**Poster #9:** **Comparative study of NMR based metabolomics platforms for blood analysis from various species.** Miki Watanabe-Chailland and Lindsey Romick-Rosendale. Cincinnati Children's Hospital Medical Center

**Poster #10:** **Development of a Synthetic Fecal Metabolite Calibration Solution.** Da Silva, Sandra M.; Urbas, Aaron A.; Schock, Tracey; Davis, Clay and Sade, Youssef B. National Institute of Standards and Technology (NIST)

**Poster #11:** **Differential Microbial and Metabolites Abundances in Rotten Fruits Drives Metabolic Variation Across Drosophila Genotypes.** Fijabi Oluwatobi; Laura K. Reed. The University of Alabama, Tuscaloosa

**Poster #12:** **Elucidating A Metabolic Trigger Leading To The Stoppage Of Transcription-Translation In Cell-Free Expression Systems.** Vora, Soor; Styczynski, Mark. Georgia Tech Department of Chemical and Biomolecular Engineering

**Poster #13:** **Evaluating the concordance of untargeted and quantitative metabolomics for characterizing metabolic dysregulation in colorectal cancer.** Richardson, Adam D; Cho, Kevin; Stancliffe, Ethan; Mehta, Ashima; Guzior, Douglas V; Gandhi, Monil; Cohen, Tom; Patti, Gary J. Panome Bio, Inc.; Washington University in St. Louis

**Poster #14:** **Investigation of diagnosis potential of Salivary metabolites for early detection of Vascular Cognitive impairment.** Ali Yilmaz, PhD; Nadia Ashrafi, PhD; Delanie Goniwiecha; Stewart F. Graham, PhD.

**Poster #15:** **iPRM-PASEF - a novel workflow for the analysis and interpretation of spatial on-tissue tandem mass spectrometry.** Ramachandran Sumankalai(1), DelaCourt Andrew(1), Tao Nannan(1), Heijs Bram(2), Boskamp Tobias(2), Deininger Sören-Oliver(2), Kessler Nikolas(2), Fütterer Arne(2), Behrens Arne(2), Henkel Corinna(2), T. Smit Nadine(2), Stumpo Kate(1) (1)Bruker Scientific Inc; (2)Bruker Daltonics GmbH

**Poster #16:** **LC-HRMS/MS analysis of phase II metabolites of common mycotoxins.** Myriam Mireault, Elissa Mariani, Irina Slobodchikova, Calin Zainea and Dajana Vuckovic. Concordia University, Chemistry and Biochemistry department, Montreal, QC, Canada

**Poster #17:** **Macrophage Infiltration in KRAS-Driven Lung Adenocarcinoma is driven by Lipid Metabolism and TAp73 Status.** Vanessa Y. Rubio1, Hayley D. Ackerman1,2, Nicole Hackel1,2, Christina L. Carr1,2, Jaden Baldwin1,2, John M. Koomen1, Elsa R. Flores1,2 1Department of Molecular Oncology, 2Cancer Biology and Evolution Program, Moffitt Cancer Center, Tampa, Florida, USA, 33612.

**Poster #18:** **MANA SODA: The Software and Data Exchange.** Hitchcock, Daniel; Wu, Yue. The Broad Institute; Stanford University

**Poster #19:** **Metabolomics and Exposome Laboratory at the UNC Nutrition Research Institute.** Blake Rushing. University of North Carolina-Chapel Hill, Nutrition Research Institute, Department of Nutrition, Kannapolis, NC 28081

**Poster #20:** **Metabolomics for deciphering cellular responses of gene dosage variation.** Kumar, Rohit; Tharayil, Nishanth. Clemson University, SC, USA

**Poster #21:** **Metabolomics uncovered the reason for differential rooting capacity within Eucalyptus hybrid species, highlighting potential biomarkers for mother plant selection.** Salinas, Ignacio; Medina, Alex; Emhart, VerÃ³nica; Pérez, Andy J. Department of Instrumental Analysis, University of Concepcion, Chile.

**Poster #22:** **Method for simplified simultaneous quantitation of the constituents of a chemically complex mixture and establishing quantitative linearity.** de Jong F.(2), Ghosh D.(1), Beecher C.(2), Shulaev V.(1) 1Department of Biological Sciences, College of Science, University of North Texas, Denton, Texas; 2 IROA Technologies LLC, Chapel Hill, North Carolina

**Poster #23:** **Organoid Analysis using Ultra-High Lateral Resolution AP-SMALDI Mass Spectrometry Imaging.** Zhou, Mandy; Ghezellou, Parviz ; Elisa Badin, Max A. MÃ¼ller,1,3 Svenja Pauer,4 Jasmin Ballout,4 Martin Diener,4 Kerstin Strupat,5 Bernhard Spengler1,3 1. Institute of Inorganic and Analytical Chemistry, Justus Liebig University Giessen, Giessen, Germany; 2. Department of Chemical Sciences, University of Padova, Padova, Italy; 3. TransMIT GmbH, Giessen, Germany; 4. Institute for Veterinary Physiology and Biochemistry, Justus Liebig University Giessen, Giessen, Germany; 5. Thermo Fisher Scientific (San Jose, San Jose, California

**Poster #24:** **Plant Metabolomics Applied to Establishing the Foundation for Building Resistance Against Gonipterus platensis Defoliation in Eucalyptus Inter-Specific Hybrids.** Pérez, Andy J.; Campos, Jasna V.; Salinas, Ignacio; Mardones, Claudia. Laboratorio de MetabolÃ³mica de Plantas e ImagenologÃ-a Espectral de Masas (MetPlant & MSI Lab), Departamento de AnÃ¡lisis Instrumental, Facultad de Farmacia, Universidad de ConcepciÃ³n, ConcepciÃ³n, Chile

**Poster #25:** **Plasma metabolomics of inhaled corticosteroid response in asthma patients.** Dung T. Tran, MS, PhD; Yulu Chen PhD; Lourdes Ramirez, MD; Jessica Lasky-Su, M.S., ScD; Ann C. Wu, MD; Kelan G. Tantisira, MD, MPH; Michael McGeachie PhD; Scott T. Weiss, MD, MS; Amber Dahlin, PhD, MMSc. Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA USA

**Poster #26:** **Quantitation and structural characterization of lipid mediators by high-resolution mass spectrometry.** Sayers, Rebekah(1); Proos, Robert(2); Baker, Paul RS(2); Norris, Paul(2); Zheng, Yi(3); Leyen, Klaus van(3); and Holm, Ted(4). 1) SCIEX, UK; 2) SCIEX, USA; 3) Neuroprotection Research

Laboratories, Massachusetts General Hospital, Charlestown, MA; 4) Dept of Chemistry and Biochemistry, University of California at Santa Cuz, Santa Cruz, CA

**Poster #27:** **Semi-quantification of triglycerides with resolved fatty acid composition using a targeted MS3 approach on a novel hybrid nominal mass instrument.** Charles Maxey(1), Hector Gallart-Ayala(2), Julijana Ivanisevic(2), Rahul Ravi Deshpande(1), Bashar Amer(1), Susan S. Bird(1), Philip Remes(1), and Cristina C. Jacob(1) (1)Thermo Fisher Scientific, San Jose, CA; (2)Metabolomics Platform, Faculty of Biology and Medicine, University of Lausanne, Switzerland

**Poster #28:** **SERCA activation shifts cardiac substrate utilization without impairing cardiac function in obese mice.** Banerjee, Deveena; Hasenour, Clinton; Rahim, Mohsin; Bednarski, Tomasz; Young, Jamey. Vanderbilt University

**Poster #29:** **Simultaneous quantitation and discovery (SQUAD) metabolomics workflow for the analysis of flavonoids and their conjugates in blood, urine and food samples.** Carvajal-Miranda, Yendry; Amer, Bashar; Deshpande, Rahul; Bird, Susan S.; Bills, Brandon; Bennette, Brian; Newman, John; Fiehn, Oliver. Thermo Fisher Scientific Intern; PhD Student, Fiehn Lab, UC Davis

**Poster #30:** **Simultaneous Quantitation and Discovery Analysis (SQUAD) of Lipids in Commercial Vegetable Oils using LC-HRAM-Tribrid platforms.** Mattos, Thiago; Zhu, Mengyuan; Moy, Edmund; Bernal III, Claude. Thermo Fisher Scientific, West Palm Beach, FL, USA

**Poster #31:** **Unraveling Brain Aging: Lipidomic and Metabolomic Insights from the CSF and Choroid Plexus.** Prasanna Vadhana Ashok Kumaar. Buck Institute for Research on Aging

**Poster #32:** **Untargeted Metabolomics Reveal Antioxidant and Anti-inflammatory Effects of Exercise in Drosophila.** Kolapo-Mabayoje, R. ,Tolulope; Cross, Hogland; Gabriella, Bicanovsky; Jarmacus, Monroe; Laura, K. Reed. Department of Biological Sciences. The University of Alabama, Tuscaloosa

**MANA 2024 Mark P. Styczynski Early Career Award in Computational Metabolomics**

Joshua Mitchell, PhD

The Jackson Laboratory for Genomic Medicine

**High-performance metabolomics data processing using the Asari suite of tools**

Tuesday, October 22nd, 2:30-3:00 PM

Location: Couch Auditorium

**MANA 2024 Plenary Lecture 5**

Oliver Fiehn, PhD

University of California-Davis

**Standardizing nontargeted metabolomics and exposomics: The LC-BinBase environment**

Time: Thursday, October 24th, 2:50-3:35 AM

Location: Couch Auditorium

## Session 11: Food, Nutrition, & Natural Products

Session Chair: Waylon Hastings
Location: Murphey Conference Room
Time: Thursday, October 24th, 4:00 PM - 6:00 PM

**4:00 Improved Soil-Health and Pasture Phytochemical Richness Underlies Improved Cattle Health and Meat Nutritional Composition in Southern US Grass-Finished Beef Systems**
Muhammad Ahsin; Jennifer Cloward; Robert E. Ward; Joseph V. Varre; Matt H. Poore; Johnny Rogers; Alan Franzluebbers; Sierra N. Young; Scott L. Kronberg; Frederick D. Provenza; James R. Bain; and Stephan van Vliet
Department of Nutrition, Dietetics, and Food Sciences, Utah State University, Logan, UT, USA 84322; Department of Animal Science, North Carolina State University, Raleigh, NC, USA, 27695; USDA-Agricultural Research Service, Raleigh, NC, USA, 27695; Department of Civil and Environmental Engineering, Utah State University, Logan, Utah, USA; USDA-Agricultural Research Service, Mandan, ND, USA 58554; Department of Wildland Resources, Utah State University, Logan, UT, USA 84322; Duke Molecular Physiology Institute, Duke University Medical Center, Durham, NC, USA, 27701

**4:20 Exploring Metabolic Diversity in American Elderberry Fruits and Flowers**
Sumner, Lloyd W.1; Kranawetter, Clayton 1; Ho, Khank-Van 1; Moore, Sydney2; O'Neal, Caleb3; Thomas, Andrew L.3
1Division of Biochemistry, Bond Life Sciences Center, Interdisciplinary Plant Group, University of Missouri, Columbia, MO. 2Division of Plant Science and Technology, University of Missouri, Columbia, MO. 3Division of Plant Science and Technology, Southwest Research, Extension and Education Center, University of Missouri, Mt. Vernon, MO

**4:40 The impact of grass vs. grain-finishing practices on plant-secondary derived metabolites in beef: a nation-wide profiling study**
Ali, Lamis; Ahsin, Muhammad; V Varre, Joseph; Cloward, Jennifer; Ward, Robert; Carbonell Herrera, Mittendorf, Camille; Statham, Travis; Marble, Shawna; Pinelli, Amanda; Kittredge, Dan; Van Vliet, Stephan
Department of Nutrition, Dietetics and Food Science, Utah State University, Logan, Utah; 2The Bionutrient Institute, MA

**5:00 Juicy Insights: A Standardized, Nontargeted Metabolomics Approach to Facilitate a Global Comparison of Apples to Apples**
Odenkirk, Melanie1; Vaniya, Arpana2; Read, Margaret1; Michel, Cole3; Doenges, Katrina3; Chaparro, Jacqueline1,4; Mitchell, Susan1; Montgomery, Nathan4; Broeckling, Corey4; Brinkley, Sarah5; Leaptrot, Katrina6; Sherrod, Stacy6; May, Jody6; Chaura, Juliana7; Velez Meija, Gabriel7; de Vos, Ric8; Fiehn, Oliver2; Reisdorph, Richard3; Reisdorph, Nichole3; McLean, John6; Jaramillo-Botero, Andres7,9; Hall, Robert8; Chien, Chi-Ming10; Shafizadeh, Tracy10; Prenni, Jessica10; Watkins, Steve10
1Department of Horticulture and Landscape Architecture, Colorado State University, Fort Collins, CO, United States; 2West Coast Metabolomics Center, University of California Davis, Davis, CA, United States; 3Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado, Denver, CO, United States; 4Analytical Resources Facility, Colorado State University, Fort Collins, CO, United States; 5CIAT Bioversity Alliance, Texas A&M University, College Station, TX, United States; 6Department of Chemistry and Center for Innovative Technology, Vanderbilt University, Nashville, TN, United States; 6iÃ"MICAS Institute, Pontificia Universidad Javeriana, Cali, Valle del Cauca, Colombia; 8Bioscience, Wageningen University and Research Centre (Wageningen-UR), PO Box 16, 6700 AA Wageningen, The Netherlands; 9Chemistry and Chemical Engineering Division, California Institute of Technology, Pasadena, CA 91125, United States; 10Verso Biosciences, Inc., Davis, CA, United States

**5:20 Food for Thought: Characterizing 500 Commonly Consumed Foods through Standardized Metabolomics for The Periodic Table of Food Initiative**
Arpana Vaniya; Jessica Prenni; Jacqueline M. Chaparro; Melanie Odenkirk; Margaret Read; Susan B. Mitchell; Corey Broeckling; Nathan Montgomery; Nichole Reisdorph; Richard Reisdorph; Cole Michel; Katrina A.

Doenges; Oliver Fiehn; Stacy D. Sherrod; Katrina L. Leaptrot; Jody C. May; John A. McLean; Chi-Ming Chien; Tracy Shafizadeh; Steve Watkins
University of California Davis West Coast Metabolomics Center

**5:40 Urinary Metabolite Profiling to Non-Invasively Monitor the Omega-3 Index**

Kroezen, Zachary;1 Shanmuganathan, Meera;1; McIntyre, Britanny;2 Mutch, David;2 Britz-Mckibbin, Philip1
1. McMaster University, Dept. Chemistry and Chemical Biology, Hamilton, ON, Canada; 2. University of Guelph, Dept. Human Health and Nutritional Sciences, Guelph, ON, Canada

## Session 12: Flux & Isotope Tracer Experiments

Session Chair: Stacy Sherrod
Location: Couch Auditorium
Time: Thursday, October 24th, 4:00 PM - 6:00 PM

**4:00 Acidic Methanol Treatment Facilitates MALDI-Mass Spectrometry Imaging of Energy Metabolism**
Lu, Wenyun; Park, Noel R; TeSlaa, Tara; Jankowski, Connor SR; Rabinowitz, Joshua D; Davidson, Shawn M
Princeton University

**4:20 Metabolic reprogramming during mammalian sperm activation**
Melanie Balbach
Michigan State University

**4:40 Carbon and Nitrogen Positional Isotopomer Determination in Metabolites using Stellar MS - a novel hybrid nominal mass instrument**
Rahul Ravi Deshpande-1; Ayush Midha-2; Bashar Amer-1; Isha Jain-2; Cristina C. Jacob-1; Susan Bird-1
1-Thermo Fisher Scientific, San Jose, CA; 2-Gladstone Institutes, San Francisco, CA;

**5:00 E. coli-based cell-free systems used in synthetic biology have complex metabolic dynamics**
Mark P. Styczynski, April M. Miguez, Yan Zhang, Fernanda Piorino
Georgia Institute of Technology

**5:20 Simultaneous in vivo multi-organ fluxomics reveals divergent metabolic adaptations in liver, heart, and skeletal muscle during obesity**
Young, Jamey D.; Rahim, Mohsin; Bednarski, Tomasz K.; Hasenour, Clinton M.; Banerjee, Deveena R.; Trenary, Irina
Vanderbilt University

**5:40 Serum biomarkers of Multiple Sclerosis are associated with metabolic flux rearrangement in brain cells: combining 1H metabolomics and 13C fluxomics.**
Probert, Fay
Department of Chemistry, University of Oxford, Oxford, United Kingdom Department of Pharmacology, University of Oxford, Oxford, United Kingdom

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## MANA 2024 Plenary Lecture Abstracts

**Tuesday, October 22nd, 9:00-9:45 AM**
Gina M. DeNicola, PhD
H. Lee Moffitt Cancer Center
**NRF2 and cysteine metabolism in cancer**
Introduction: Redox regulators are emerging as critical mediators of lung tumorigenesis. Notably, NRF2 and its negative regulator KEAP1 are commonly mutated in human lung cancers. These mutations lead to NRF2 accumulation and constitutive expression of NRF2 target genes, many of which are at the interface between antioxidant function and anabolic processes that support cellular proliferation. One such metabolic process is the uptake and metabolism of the amino acid cysteine, which is required for maintaining cellular redox homeostasis in both normal and transformed cells. However, how tissues and tumors acquire cysteine in vivo is not well understood.
Methods: We generated genetically engineered mouse models of lung cancer with activating mutations in NRF2 and a liver cancer model driven by Myc overexpression. We infused healthy and tumor bearing mice with $^{13}$C-serine and $^{13}$C-cystine to track the transsulfuration pathway (de novo cysteine synthesis) and cystine uptake, respectively. Moreover, we deleted the enzymes required for cystine reduction in the lung cancer mouse model to examine the effect on lung tumor formation.
Preliminary Data: Our studies revealed that the liver and liver tumors were metabolically flexible with the ability to both synthesize cysteine and uptake it from the circulation. In contrast, healthy lung and lung tumors lacked the ability to synthesize cysteine and derived their cysteine from circulating cystine, consistent with their overexpression of the cystine transporter xCT. Interestingly, perturbation of cystine reduction only modestly impaired lung tumor formation, suggesting the presence of an alternative metabolic mechanisms of cysteine acquisition.
Novel Component: We performed the first in vivo analysis of the source of cysteine in cancer models.

**Tuesday, October 22nd, 3:00-3:45 PM**
Tao Huan, PhD
University of British Columbia
**A deep dive into sample normalization for improved quantitative performance in untargeted metabolomics**
Introduction: Variations in starting material leads to a big challenge in the quantitative comparison of metabolites in biological samples, such as urine and saliva. For fair quantitative comparisons, sample normalization is essential during the metabolomics pipeline. Over the years, many post-acquisition sample normalization methods have been proposed. Yet, how to select the right one remains under explored. This work benchmarked six commonly used post-acquisition sample normalization methods using data simulations: sum, median, quantile, class-specific quantile, probabilistic quotient normalization (PQN), and maximal density fold change (MDFC). Using data simulations and experimental results, our work provides a mechanistic understanding of the discrepancy between sample normalization algorithms. Based on the discoveries, we proposed an evidence-based normalization workflow to facilitate accurate quantitative comparisons.
Methods: To simulate LC-MS-based metabolomics data, intensities from real experimental data were used to generate a reference sample. Next, additional samples were simulated using Gaussian noise. Each sample were then scaled by a dilution factor to represent concentration differences. To represent metabolite dysregulation from biological variation (e.g., diet and lifestyle), a set of features were dysregulated using uniform distributions. Normalization methods were benchmarked by comparing the estimated normalization factors with the true dilution factors. To examine the impact of data quality on normalization performance, we analyzed urine samples with known dilution factors. Notably, we examined how the following issues impact normalization performance: 1) non-biological features; 2) poor quantitative response; 3) fold-change bias; 4) missing values.
Preliminary Data: In our simulations, we found that most normalization methods perform poorly with unbalanced metabolite dysregulation. This is the common phenomenon where the percentage of up- and downregulated features are unequal. In these cases, normalization methods are unable to accurately estimate true dilution factors. The implications are severe as improper normalization will result in misleading quantitative comparisons. The only normalization method that was able to consistently perform well across a diverse range of data structures was MDFC. Beyond the choice of normalization method, we examined four aspects of data quality on normalization performance. Our results demonstrated that the using non-biological features and features with poor quantitative response resulted in inaccurate normalization factors. Interestingly, we observed that correcting

fold-change compression resulted in the greatest improvement in normalization performance. We also show that normalization should be performed prior to missing value imputation. The reasoning is that imputation strategies can introduce artifacts that can bias normalization factors. To facilitate accurate quantitative comparisons, we propose an evidence-based normalization workflow that captures both data quality and normalization method. In this approach, a metabolomics dataset is first cleaned up by addressing the data quality issues described previously. We then introduced the idea of symmetry factors (SFs) to estimate the percentage of samples with unbalanced data. Based on this information, the normalization method can be chosen accordingly. We demonstrate this workflow using public metabolomics datasets.
Novel Component: This work deepens the understanding of sample normalization and proposes an innovative normalization workflow to achieve more reliable quantitative results.

**Wednesday, October 23rd, 3:00-3:45 PM**
Julia Laskin, PhD
Purdue University
**Advances in Nanospray Desorption Electrospray Ionization (nano-DESI) Mass Spectrometry Imaging**
Mass spectrometry imaging (MSI) is a powerful technique for molecular mapping of biological samples with high sensitivity and molecular specificity. Ambient ionization techniques enable imaging of biological samples with minimal sample pretreatment. We have developed an ambient MSI technique based on nanospray desorption electrospray ionization (nano-DESI). Nano-DESI is a liquid extraction-based technique, in which molecules are extracted from the sample into a dynamic liquid bridge formed between the nano-DESI probe and sample surface. The extracted analytes are transferred to a mass spectrometer inlet and ionized by electrospray ionization. The high sensitivity of nano-DESI enables imaging with high spatial resolution of 6-10 microns, which opens new directions for molecular mapping of individual cells in biological tissues. Furthermore, we have developed approaches for correlative imaging of lipids, metabolites, peptide, proteins, and glycans in biological tissues and used immunofluorescence microscopy of the same or adjacent tissue sections to extract cell-specific molecular signatures. We have also examined the effect of solvent composition on both the extraction and ionization efficiency, which provided significant enhancements in sensitivity and molecular coverage of nano-DESI MSI. These developments have established nano-DESI MSI as a powerful technique for studying biological systems.

**Thursday, October 24th, 9:00-9:45 AM**
Patricia Y. Scaraffia, PhD
Tulane University
**Unlocking the metabolic secrets of Aedes aegypti mosquitoes using reverse genetics and mass spectrometry-based metabolomics.**
Introduction: Despite our best efforts to control Aedes aegypti mosquitoes, these vectors of arboviruses causing infectious diseases represent a serious public health burden worldwide. The blood-sucking female mosquito must deal with a potentially life-threatening overload of nitrogen that makes up a disproportionate amount of the nutrients in a blood meal. This metabolic challenge involves most of the amino acids derived from a blood meal being oxidized, leading to a massive deamination. The application of a multidisciplinary approach including RNA interference (RNAi) and stable-label isotope tracing based metabolomics, has allowed us to discover that Ae. aegypti mosquitoes overcome the lack of a urea cycle by using unique metabolic pathways and mechanisms of regulation for ammonia detoxification and nitrogen disposal.
Methods: Currently, we are investigating how ammonia pathways closely crosstalk with the polyamine biosynthetic pathway to maintain nitrogen homeostasis in blood-fed Ae. aegypti females. To achieve this goal, we are using classical and modern techniques, including qPCR, western blots, reverse genetics and mass spectrometry-based metabolomics. Furthermore, a targeted, quantitative LC-MS/MS method - with a new mixed-mode chromatographic separation – is under development to simultaneously measure metabolites involved in both ammonia and polyamine pathways.
Preliminary Data: We identified genes encoding S-adenosylmethionine decarboxylase (SamDC, AAEL001176), spermidine synthase (SdS, AAEL010071), and spermine synthase (SmS, AAEL001378) within the Ae. aegypti genome. These proteins catalyze different steps in the polyamine biosynthetic pathway. We analyzed SamDC, SdS and SmS gene and protein expression in tissues dissected from sugar- and blood-fed females during the first gonotrophic cycle by qPCR and western blots. We found that the three genes are constitutively expressed, whereas the proteins SamDC, SdS and SmS each have a distinct profile in certain tissues. Our preliminary data also indicate an increase in polyamine levels measured in the fat body of blood-

fed females dissected at 24 h post blood meal (PBM) when compared to the fat body of sugar-fed females. We have also begun to knockdown SdS and SmS by RNAi to determine the impact of their deficiencies on protein expression and uric acid (UA) excretion. Notably, we observed that RNAi-driven SdS deficiency in fat body causes a pronounced decrease in SdS protein level as expected, but also a significant reduction of SmS protein level and UA excretion at 24 h PBM. Interestingly, RNAi-mediated knockdown of SmS in fat body results in a significant decrease in SmS and SdS protein expression and a significant reduction of UA excretion at 24 h PBM. Altogether, our data not only uncover crosstalk regulations within the polyamine biosynthetic pathway but also provide strong evidence of unique crosstalk regulations between the polyamine and UA pathways. A better understanding of these metabolic processes during blood meal digestion could lead to the discovery of metabolic targets or their regulators, and ultimately provide a foundation for the development of novel metabolism-based mosquito control strategies.
Novel Component: Unique crosstalk regulations between polyamine and UA pathways maintain nitrogen homeostasis in blood-fed Ae. aegypti females.

**Thursday, October 24th, 3:00-3:45 PM**
Oliver Fiehn, PhD
University of California-Davis
**Standardizing nontargeted metabolomics and exposomics: The LC-BinBase environment**
Introduction: Current nontargeted MS/MS data do not give standardized reports and are difficult to compare. Data processing including naming and annotating metabolites is not harmonized or under quality-control. Reported compound identifications are difficult to be scrutinized by the scientific community.
Methods: We here showcase the computational infrastructure to make nontargeted analyses standardized, validated, and useful for comparisons comparable across multiple organs and studies. The LC-BinBase environment defines sample metadata via a standardizer that employs automatic user input curation through vocabularies and ontologies in MeSH, NCBI, Cellosaurus, NCIT and FDA databases. Raw data acquisition is continually monitored in the LC-BinBase control panel, including QC chart violations of upper and lower intervention limits and missed internal standards.
LC-BinBase standardizes retention times into retention index values by normalizing to 42 internal standards for the BEH-amide (HILIC) assay, and 76 internal standards for the BEH-C18 lipidomics assay. LC-BinBase then generates 'accurate mass_MS/MS_retention time' triplets (Bins) that are mapped to unique Spectral Hash Keys.
Preliminary Data: From over 20,000 samples that have been acquired so far, LC-BinBase has generated between 10,000-20,000 Bins per assay. MS/MS spectra are cleaned via LibGen 2.0 denoising. MS/MS spectra are automatically analyzed via Flash Entropy similarity search in MassWiki, including identity-, hybrid-, neutral loss- and open search similarity queries. The public MassWiki sites include open libraries such as MassBank.us and GNPS, while licensed libraries such as NIST23 are kept in-house. Normalized retention times are matched against machine learning predictions in Retip 2.0 software. From human clinical cohort data, plasma metabolome data with more than 1,200 unique annotated compounds per study are reported, including more than 500 exposome compounds. Chemical metadata are enriched by adduct information, InChI keys and SMILES chemical identifiers. Through links to MassIVE/GNPS and WCMC metadata, all spectra are linked to biological information such as the frequency at which they were detected in diverse species and organs. Results are presented for several studies, from plasma-based exposomics to clinical trials in small intestinal diseases.
Novel Component: A standardized, open-access database that will be developed into a community resource.

## MANA 2024 Early Career Member Awards Abstracts

**Tuesday, October 22nd, 2:30-3:00 PM Early Career Rising Star Award**

Mohanty, Ipsita; Xing, Shipei; El Abiead, Yasin; Hagey, L. R.; Dorrestein, P. C. Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego; 3Department of Pharmacology, University of California San Diego; 6Center for Microbiome Innovation, University of California San Diego; 5Collaborative Mass Spectrometry Innovation Center, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego

**Diving deeper into the bileome: MS2 fragmentation-based filtering identifies bile acid regio- and stereoisomers to reveal unique patterns in biology**

Introduction: Bile acids are critical signaling molecules that regulate host metabolism, immunity, and energy uptake. They are synthesized in the liver from cholesterol and released into the small intestine after a meal to facilitate fat digestion. Gut bacteria modify the bile acids into various forms, such as sulfated, oxidized, and amidated bile acids. We have recently revealed the underappreciated diversity of microbially modified candidate bile acids using mass spectrometry-based advanced computational tools. We created an MS2 spectral library of candidate bile acid modifications by mining billions of spectra from public LC-MS/MS untargeted metabolomics datasets. However, the MS2 fragmentation spectra in the library lacked isomer assignment, with the position and stereochemistry of the hydroxyl groups on the steroid core remaining undefined.

Methods: To address this, we developed a mass spectrometry query language-based filtering approach to identify isomeric bile acids from public LC-MS/MS untargeted metabolomics data. Careful manual inspection of MS2 fragmentation spectra of taurine-conjugated bile acids acquired in positive ionization mode showed differences in the ratio of relative peak intensities of specific ion pairs which were used to design filters for unique isomers of mono-, di-, and trihydroxylated bile acids. The filters were tested for specificity using the bile acid-specific spectral libraries on Global Natural Products Social Molecular Networking (GNPS) containing more than 5000 bile acid MS2 spectra. A false discovery rate (FDR) of <5% for most of the filters was obtained. After their specificity was validated, the filters were used to bin the 21,549 MS2 spectra in the candidate bile acid modifications library into unique regio- and stereoisomers.

Preliminary Data: The filtering approach to bin spectra into unique isomer groups allowed us to further refine the MS2 spectral library of candidate bile acid modifications. User metadata for these MS2 spectra deposited in the ReDU platform was analyzed to provide deeper insights into tissue distribution, disease associations, and matches to microbial reference databases for unique bile acid isomers. We provide novel biological insights and associations for previously understudied and potentially undetected bile acid isomers. This is a first-of-its-kind approach to get the stereochemical assignment of bile acid steroid cores from just the MS2 fragmentation spectra. The filtering workflow is also easy to adapt to large repository-scale mining or for analysis of individual metabolomics datasets, as we have highlighted with two human studies to reveal unique distribution patterns of bile acid isomers. One use case we highlight is the differential relative abundances of previously unknown conjugated bile acid isomers, including the microbially modified steroid cores, such as deoxycholic acid and hyodeoxycholic acid, between intestinal fluid and feces in 14 healthy subjects. The filters were also used to discover a previously unknown polyamine conjugated to deoxycholic acid in lion feces. The preliminary data highlights the potential impact of the filters in revealing detailed biological patterns for bile acids if the unique isomers can be identified using MS2 spectra, and circumventing the need to synthesize hundreds of possible candidates.

Novel Component: Human-interpretable MS2 fragmentation-based filtering workflow to identify bile acid isomers and reveal unique biological patterns at the repository scale.

**Thursday, October 24th, 2:30-3:00 PM Mark P. Styczynski Early Career Award in Computational Metabolomics**

Mitchell, Joshua M; Chi, Yuanye; Thapa, Maheshwor; Li, Shuzhao The Jackson Laboratory for Genomic Medicine, 10 Discovery Drive, Farmington, CT 06032, USA (all authors); University of Connecticut School of Medicine, Farmington, CT 06032, USA

**High-performance metabolomics data processing using the Asari suite of tools**

Introduction: Significant challenges remain in metabolomics data processing including issues of provenance, reproducibility, and performance. The existing computational metabolomics ecosystem is predominantly R-based with limited interoperability and inconsistent terminology between software tools. As metabolomics becomes an increasingly common component of biomedical studies, there is an urgent need to standardize

terminology, define interoperable data structures, and create reproducible workflows to facilitate the adoption of metabolomics data processing tools by the broader bioinformatics community.
Methods: We previously developed Asari, an LC-MS pre-processing tool that uses transparent and interoperable data structures and new approaches to spectral processing to yield high-quality feature tables with best-in-class performance. Here we describe a fully featured pipeline that integrates Asari into an end-to-end data processing pipeline. The pipeline can pre-process LC-MS datasets, quality control the resulting feature tables, and generate annotations for empirical compounds detected in the dataset using MS1 and MS2-based methods. Support for GC-MS is in development. We also introduce the MetDataModel, a set of programming-language agnostic data structures representing key concepts in computational metabolomics. These data structures enable the integration of our tools and are presented to the community for feedback and reuse.
Preliminary Data: The Python Centric Pipeline for Metabolomics (PCPFM) was evaluated on multiple metabolomics and lipidomics datasets. All tests were performed using a variation on a reusable workflow to exemplify the generalizability and reusability of PCPFM-based workflows. The ability of the in-built quality control metrics to correct common data quality issues including batch effects, failed injections, and outlier samples is demonstrated on multiple datasets. Feature detection was evaluated by comparing the PCPFM to a state-of-the-art MetaboAnalystR 4.0 workflow where both tools detected similar sets of high-confidence metabolites in an exemplar dataset. At the same time, the PCPFM offered superior computational performance in all test cases. MS2 annotation accuracy was estimated by comparing PCPFM-generated annotations against those produced by CompoundDiscoverer on the same in-house dataset. Although similar annotations were generated by both tools, the closed-source nature of CompoundDiscoverer prevented an in-depth evaluation of the differences in the results, highlighting the need for open-source and transparent software for computational metabolomics. Finally, the ability of the PCPFM to produce biologically meaningful results was demonstrated by re-analyzing two public datasets: Bowen et al. 2023 which sought to characterize sunitinib metabolites in cardiomyocyte samples and Ansone et al. 2021 which compared COVID-infected patients to recovered and healthy controls. Our generic workflow identified all but one of the sunitinib metabolites in the Bowen dataset without the need for a specialized xenobiotic focused workflow. In Ansone 2021, our pipeline recapitulated the main finding of a COVID-specific metabolic phenotype while also detecting that the most impacted metabolite from COVID exposure was a component of respiratory surfactant, a finding not reported in the original analysis. While Asari requires little to no modification for GC-MS data processing, an EI-MS aware version of Khipu, our pre-annotation tool, is under development to cluster features from the same EI-MS spectrum for annotation.
Novel Component: The PCPFM and MetDataModel fill a critical need for reusable, transparent, and interoperable metabolomics data processing.

## MANA 2024 Monday Afternoon Interactive Workshops (4:30-6 PM)

**Spatial Isotopic Tracing in Mammalian Tissues**

Organizer(s): Matthew Merritt; Ramon Sun

Objectives: Detail the workflow for obtaining high quality spatial maps of tracer uptake and metabolism; Data interpretation.

Outcomes: Learners enabled to design appropriate tracer based experiments for MALDI Mass Spectrometry Imaging (MSI).

**Unveiling the mQACC Living Guidance for QA/QC Best Practices in LC-MS-Based Untargeted Metabolomics**

Organizer(s): Jonathan Mosley; Dajana Vuckovic

Objectives: 1. To disseminate findings from the mQACC Best Practices Working Group's extensive community engagement efforts to establish best practices for LC-MS data collection in untargeted metabolomics. 2. To solicit further feedback from the international metabolomics community on the open-access best practices living guidance document that will be freely accessible to researchers.

Outcomes: 1. Attendees will learn about minimum and best practices for untargeted LC-MS-based metabolomics as proposed in Living Guidance by mQACC Best Practices Working Group. The guidance covered will include pooled QC, internal standards, and design of analytical batch. 2. Attendees will be able to identify how to participate in mQACC, including mechanisms to contribute to the best practices community engagement efforts and Living Guidance document.

**Hands-on Workshop on using benchmarking dataset to evaluate software tools for preprocessing mass spectrometry-based metabolomics data**

Organizer(s): Xiuxia Du; Gary Patti

Objectives: To demonstrate to and teach researchers and software developers on how to use a carefully generated benchmarking dataset for evaluating the performance of software tools for preprocessing mass spectrometry-based untargeted metabolomics data.

Outcomes: Participants will understand the built-in structure of the benchmarking data, how where to obtain the data, and how to use the data to evaluate software tools.

**Engaging with MANA and Increasing the Impact of Metabolomics through MANA Interest Groups**

Organizer(s): Ewy Mathé, Arpana Vaniya

Objectives: The mission of MANA is to promote the growth and development of the field of metabolomics, particularly on the continent of North America. The MANA Interest Groups (IGs) play a key role in fulfilling this mission through impactful publications that articulate perspectives and best practices in metabolomics, and by providing opportunities for networking and collaborations through virtual meetings and workshops. The current MANA IGs are: 1) Early Career Member; 2) Metabolomics Cores; 3) Microbiome; 4) NMR Metabolomics; 5) Precision Medicine; 6) SODA; 7) Womix. The first objective of this workshop is to provide updates on the latest activities offered by the active MANA IGs and to allow discussions on future plans and how new members can get involved. The second objective is to provide an overview of the guidelines that outline how to start up and lead an IG to encourage the formation of new IGs. As part of this second objective, participants will be asked to share interests in relevant and state-of-the-art topics in the metabolomics field. Overall, this workshop aims to solicit new ideas and engagement from all our members in our IG activities.

Outcomes: Participants of the workshop will have clarity on current IG activities and how to get involved. They will be encouraged to share their thoughts on new activities and standing up new IGs. This workshop will thus be interactive and will provide networking opportunities. Discussions and input from participants will help shape IG activities for the following year and bring cohesion and alignment between IG activities.

## MANA 2024 Tuesday Lunch Workshop (12-1 PM)

**Metabolic Flux Analysis and Stable Isotope Resolved Metabolomics**

Organizer(s): Rahul Deshpande; Bashar Amer; Susan Bird

Objectives: This workshop is designed to educate participants about metabolic flux analysis and metabolomics using stable isotopes. It will cover the distinctions between these two experiments and outline the fundamental workflows for each approach. For metabolic flux analysis, attendees will start by learning about steady-state fluxes and constructing basic stoichiometric models for their calculation using material balances. They will then explore the use of stable isotope tracers to decipher and quantify metabolic fluxes through crucial metabolic pathways. The users will also learn about metabolomic studies using stable isotopes which aim to look at metabolic differences by quantifying the incorporation of the isotope in key metabolites. The use of an appropriate stable isotope labelled compound for deciphering key biochemical pathways and key analytical strategies for both the workflows will be discussed. They will also be introduced to free and commercially available software used for this analysis.

Metabolic flux analysis is computationally intense compared to stable isotope labeling studies. Outcomes: The attendees after the workshop should be able to understand the key differences in the two approaches to study metabolism. They should understand the experimental design of these workflows and appropriate controls to use. They will be familiarized with some key concepts in labeling studies such as isotopomers, isotopologues, natural abundance correction etc. They will also have knowledge on the analytical techniques used for these workflows with the advantages and disadvantages of these. If an attendee wishes to incorporate this workflow in her/his scientific study, this workshop should give her/him the knowledge and tools to start.

## Tuesday Evening Interactive Workshops (5:45-7 PM)

**Career Development Q&A/Building and Leveraging your Personal Brand to Accomplish your Professional Goals**

Organizer(s): John Koomen; Tim Garrett; Arpana Vaniya; Maryam Goudarzi; Stephanie Bishop; Prasanna Kumaar; Khayti Pathak; Nicole Prince; Goncalo Gouveia; Erica Forsberg

Objectives: 1. Discuss Advantages and Disadvantages of Different Career Paths; Provide Alternatives to Academics. 2. Forum Between Corporate Members and Early Career Members. 3. Empower MANA members within the metabolomics community by enhancing their understanding of the significance of personal branding using various platforms, such as social media, professional networks, and industry publications. Develop a personalized action plan to achieve professional goals in the field of metabolomics.

Outcomes: 1. Early Career Members and Trainees generate different ideas and strategies for their career paths. 2. Learn about the importance and benefits of industry collaboration in advancing metabolomic research and enhancing career opportunities. They will also be equipped with skills in identifying potential industry partners, assessing compatibility, and understanding the specific needs and priorities of corporate members in metabolomics. 3. Participants will achieve enhanced awareness of the importance and impact of personal branding on their professional lives. They will develop improved communication skills to effectively convey their personal brand through various channels, thereby increasing their visibility and influence within the metabolomics community. Attendees will also gain access to increased professional opportunities, such as leadership roles, collaborative projects, and speaking engagements, by leveraging their personal brand. Additionally, the workshop will facilitate networking and community engagement, allowing participants to build stronger connections within the MANA community and the metabolomics field. Ultimately, attendees will experience boosted confidence and professional growth, empowering them to pursue and achieve their career aspirations more effectively.

**AI in NMR Metabolomics**

Organizer(s): Hamid Eghbalnia; Leo Cheng; Art Edison; Valerie Copie

Objectives: NMR spectroscopy is governed by quantum mechanics (QM). Over half a century of research has resulted in a deep understanding of the behavior of NMR-active nuclei in a magnetic field. Until the past decade or so, the computation of accurate NMR spectra required a deep knowledge of QM, which largely

excluded non-experts from the powerful tools. Fortunately, much of this situation has changed. Many methods are available to compute NMR chemical shifts and coupling constants, using QM calculations, empirical approximations, or—more recently—artificial intelligence (AI). Complex NMR spectra can now be computed directly using full QM rules or modeled using reasonably accurate approximations. This "computability" of NMR enables unique opportunities in many areas of machine learning (ML). Recent AI/ML approaches have greatly improved spectral analysis with tools to perform jobs such as peak-picking. Computability of NMR, and the ability to compute accurate NMR spectra from QM rules means that the calculation of huge numbers of realistic training sets for ML with known ground truth can be produced with ease. An expanding resource of available experimental NMR data can be used to augment training data, improve ML performance, as well as validate models.

Outcomes: Provide an overview of what practitioners need for creating a good ML model Expose metabolomics scientists to the inherent computability of NMR spectra. Introduce accessible tools that can be used to compute NMR data. These will largely be through NMRbox but might also use additional computational resources. Demonstrate new AI/ML tools that can be used today. Demonstrate some approaches that could be used in training future AI/ML metabolomics NMR models.

**Round Robin Study Design for Inter-Lab Reproducibility Analyses in Metabolomics**

Organizer(s): Haley Chatelaine; Chris Beecher

Objectives: Participants in this workshop will learn about previous round robin studies to gain an understanding of strengths and weaknesses in study designs. They will then, in breakout groups, identify an area of interest in evaluating metabolomics reproducibility, create a tentative study design, and then evaluate potential pitfalls and strengths.

Outcomes: Participants will come away from the workshop with an understanding of the necessity for round robin study designs in evaluating metabolomics data reproducibility on an inter-lab scale. Participants may also take their ideas further to establish collaborative efforts in round robin studies to address current gaps in understanding of inter-lab metabolomics data reproducibility.

## Wednesday Evening Interactive Workshops (5:45-7 PM)

**Working like a data scientist on Jupyter**

Organizer(s): Shuzhao Li

Objectives: To train non-programmers to use online Jupyter notebooks (Colab) for data processing, annotation, visualization and analysis. With new tools asari, khipu (regular and isotope data), JMS and pcpfm pipeline.

Outcomes: Understanding of data processing and analysis; new toolkit enabled by interactive notebooks.

**Quantitation in untargeted high resolution- mass spectrometry assays: innovations and challenges**

Organizer(s): Lauren Petrick

Objectives: Introduce hybrid assays for quantitation and discovery at the same time- examples of different workflows used in metabolomics Discuss theoretical and practical approaches for quantitation of environmental chemicals in non-targeted analysis Provide a step-by-step example of building a PFAS hybrid assay and discuss challenges and technical considerations Discuss QA/QC and communicating analyte measurement assurance from assays that include a range of concentration confidences.

Outcomes: A fundamental understanding of different hybrid assays and workflows Highlighting the pros and cons so that investigators will be able to determine whether building a hybrid assay is right for their research objectives Knowledge to critically evaluate the confidence of measurements from a hybrid assay.

**The role and career path of early-career staff in core facility across North America**

Organizer(s): Maryam Goudarzi, Uri Keshet, Prassana Kumaar, Hanan Alwaseem

Objectives: To have a discussion on viable career paths for early-career staff in service cores, best ways to build a publication record and maintain a competitive edge in the scientific community.

Outcomes: Attendees will learn about the essential skills and competencies needed to excel in as a core facility member, including technical expertise, data analysis, project management, and soft skills. The workshop will provide solutions to early career members on maintaining healthy careers in service cores and identifying opportunities to reach their career goals. The event will also facilitate networking and mentorship connections, helping early career professionals build relationships with experienced leaders and peers, and offering personalized advice to support their career development in academic, government, and industry settings across North America.

**Overcoming challenges in ISO 15189 for an effective precision and translational medicine implementation.**

Organizer(s): Yamilé López Hernández and Sindhu Nair

Objectives: This workshop aims to tackle the challenges faced by research laboratories in implementing the ISO 15189 standard, particularly those involved in discoveries that lead to medical innovation. By demystifying the complexities of specific ISO 15189 clauses, the workshop will facilitate a smoother transition for research laboratories towards accreditation, ultimately benefiting the broader healthcare community. This instructional workshop will be divided in two parts. A single presenter will present the challenges in academic research laboratories that analyze clinical samples collected in health institutions. During the second part, the presenter will introduce ISO 15189:2022 and how to implement it in clinical research laboratories. The intent of this workshop is to highlight bottlenecks in ISO 15189:2022 to the metabolomics community and to teach participants practical solutions to clear these bottlenecks for moving metabolomics towards precision medicine. Objectives Part 1: Objective1: Understand the challenges that research laboratories face through practical examples (metabolomics studies conducted with clinical samples) Part 2: Objective 2: Understand why quality management is required in clinical labs Objective 3: Learn on how to implement the clauses of ISO15189 in a clinical research lab environment Objective 4: Learn the major challenges and expert ideas to resolve it Objective 5: Understand about the process flow of how a precision discovery can transition to medical health outcome

Outcomes: For research laboratories that transition into diagnostic applications, achieving ISO 15189 accreditation becomes a critical step. ISO 15189 is a comprehensive standard designed to enhance the quality management systems of medical and clinical laboratories, ensuring they meet the rigorous requirements necessary for high-quality medical care. Despite its importance, many research laboratories face significant challenges in implementing ISO 15189, including but not limited to documentation, workload, lack of laboratory safety measures and management support, poor staff motivation, and deficiencies in maintenance and calibration of equipment. Through this workshop, research laboratories will gain valuable insights into the specific challenges of ISO 15189 implementation and acquire practical strategies to overcome them. This will help them in effectively navigating the accreditation process, ultimately improving their operational standards and contributing positively to the healthcare community.

Full Abstracts for the Artificial Intelligence & Machine Learning Session (5)

**Author(s)** (Presenting author should be listed first)
Chau, Katharine; Zhang, Xinran; Varghese, Rency; Ressom, Habtom
**Author Affiliations**
Georgetown University
**Title**
Convolutional Neural Network-Based Metabolite Annotation
**Introduction**
Spectral matching of tandem mass spectrometry (MS/MS) against those in public spectral libraries is one of the most widely used methods for metabolite annotation. However, the application of this method in a typical untargeted metabolomics study is limited by the lack of comprehensive spectral libraries. Publicly available spectral libraries represent a very small fraction of known compounds. Deep learning methods provide an opportunity to break through this challenge and annotate unknown compounds based on relationships they learn between spectral data and compound features.
**Methods**
In this study, we trained a convolutional neural network (CNN) to predict molecular fingerprints based on over one million MS/MS spectra obtained from MoNA, NIST 23, and HMDB. Prior to training, we performed selection of the most relevant features from the peaks of the training MS/MS spectra and binary fingerprints of the reference compounds from which the spectra data were derived. For unknown metabolites, we used fingerprints predicted by CNN along with formulae predicted by SIRIUS based on MS/MS and MS spectra, respectively, of the unknown metabolite to rank candidates retrieved from a compound database according to the precursor m/z values.
**Preliminary Data**
The deep learning-based method introduced in this study is evaluated via CASMI 2016, CASMI 2017 and CASMI 2022 benchmark datasets in terms of its ability to rank putative metabolite IDs obtained based on precursor m/z values. The performance of the CNN model is compared against CSI:FingerID. The results show the method has a good ability to perform fingerprint prediction and assist metabolite candidate annotation. We would like to emphasize that MS/MS spectra that correspond to the compounds in the benchmark datasets were removed from the training data. Thus, the performance evaluation is based on previously unseen or blinded data. We observed the use of MS1 data along with MS/MS data aids in improving metabolite fingerprint prediction by providing insight into the isotopic pattern of an unknown compound. Our future work will focus on including MS1 in the training data to improve fingerprint prediction and seeking other deep learning models to further improve the top-k ranking performance.
**Novel Component**
A deep learning-based method for metabolite annotation is introduced by learning the relationships between spectral data and compound features.

**Author(s)** (Presenting author should be listed first)
Annie Cen, Xiuxia Du
**Author Affiliations**
UNC Charlotte
**Title**
What do data on environmental exposures in the NHANES dataset tell us?
**Introduction**
The National Health and Nutrition Examination Survey (NHANES) is a major program of the National Center for Health Statistics (NCHS). NCHS is part of the Centers for Disease Control and Prevention (CDC) and has the responsibility for producing health statistics for the Nation. NHANES is designed to assess the health and nutritional status of adults and children in the United States. It examines a nationally representative sample of about 5,000 persons each year. The examination component consists of medical, dental, physiological measurements and laboratory tests administered by highly trained medical personnel. NHANES has produced a rich dataset. The aim of this project is to discover associations between environmental factors and human health and provide pointers for in-depth metabolomics studies.
**Methods**

Data mining has been conducted on the NHANES laboratory data, demographics data, dietary data, and questionnaire data that spans more than two decades from 1999 to 2023. In particular, we have examined the laboratory measurement data on various environmental compounds including PFAS, arsenic, chromium, flame retardants, lead, cadmium, selenium, manganese, mercury, cholesterol, nickel, organophosphate insecticides, perchlorate, phthalates and plasticizers metabolites, volatile organic compounds, and trihalomethanes.

**Preliminary Data**

We have discovered strong and weak associations between environmental compounds and other entities, including: (1) between compounds within a category of compounds, (2) between compounds across different categories of compounds, (3) between compounds and demographics, (4) between compounds and dietary information, and (5) between compounds and medical conditions. Both the analysis scripts in python and analysis results are presented in HTML format for users to easily interact with the results. With this analysis and presentation of the analysis results, the NHANES data is now alive, rather than being static data tables hosted on the CDC website anymore. We hope that this will maximize the utility of the NHANES data and facilitate researchers to use the data for various research purposes. In particular, we anticipate that the results will motivate researchers to design and conduct mechanistic studies for better understanding how environmental chemicals affect human health using metabolomics methods.

**Novel Component**

Extensive data mining of the NHANES data and a resulting resource for maximizing the data's utility for metabolomics research

Wang, Yinkai; Chen, Xiaohui; Liu, Liping; Hassoun, Soha

**Author Affiliations**

Tufts University

**Title**

Spectrum-guided de novo molecular generation from molecular scaffolds

**Introduction**

The discovery of small molecules through mass spectrometry is vital for drug development, biomarker discovery, and other biotechnology applications. Two common approaches to annotation are using spectral similarity against reference spectral libraries or exploring putative molecular candidates from large databases. However, both approaches are limited to measured and/or known compounds. De novo molecular generation provides an alternative approach for the annotation of novel compounds not yet annotated in reference or compound libraries. Prior de novo approaches (MSNovelist, MS2Mol, and Spec2Mol) utilized encode-decoder models that generate a SMILES string from a query spectrum. We explore in this work a novel molecular generation approach that assumes knowledge of the underlying molecular scaffold. Generation is guided via the query spectra.

**Methods**

We propose a spectrum-guided graph generation model for de novo molecular generation. Our model utilizes an initial scaffold graph and atoms from the chemical formula to generate new structures. The atoms and edges within the scaffold are fixed. Our model learns a distribution in the discrete space between the scaffolds and target molecules. We employ Classifier-Free Guidance (CFG) for improved generation quality and normalize mass spectra (m/z, intensity) pairs to enhance learning. Cross-attention mechanisms integrate mass spectra with the molecular graph, while self-attention identifies important peaks. Molecules are represented as graphs, and mass spectra as (m/z, intensity) pairs, with a spectra encoder embedding each peak.

**Preliminary Data**

We evaluate the effectiveness of our de novo generation approach from a target scaffold on the CANOPUS. This dataset was released with the prior published CANOPUS tool and has been used prior as a benchmark evaluations set for annotation. The evaluation metrics are the accuracy, similarity, and Maximum Common Edge Substructure (MCES) at rank 1 and at rank 10. The top-k accuracy indicates the portion of the test set within the top-k molecules where the target molecule was generated. The similarity and MCES at k reports the highest similarity and smallest MCES within the top-k set. The baseline model omits spectra guidance, thus generating de novo molecules based on the target molecular formula and the distribution of the training dataset.

Our model achieved a top-1 accuracy of 6.7% and a top-10 accuracy of 16.6%. The similarity scores for top-1 and top-10 predictions were 0.207 and 0.564, respectively. The MCES values were 73.80 for top-1 predictions and 29.53 for top-10 predictions. Without the spectral guidance, we obtain the following results. In contrast, the

baseline model achieved a top-1 accuracy of 2.2% and a top-10 accuracy of 3%. The similarity scores are 0.046 as top-1 and 0.151 as top-10. As for MCES, the baseline model has 93.84 for top-1, 78.82 for top-10. Our preliminary results demonstrate that our model performs well in generating molecules from their scaffolds when guided with the spectra. We caution that the generated molecules are based on their scaffolds, and hence the results assume known scaffolds. We are currently assessing the performance when considering scaffolds from the candidate sets and exploring scaffold prediction and generation models. Our work highlights the potential for de novo scaffold-based generation approaches guided by the query spectrum.

**Novel Component**

Novel formulation and competitive results for spectra-guided de novo molecular generation from molecular scaffolds under a chemical formula constraint

**Author(s)** (Presenting author should be listed first)
Stancliffe, Ethan; Richardson, Adam; Gandhi, Monil; Mehta, Ashima; Guzior, Douglas V.; Cho, Kevin; Cohen, Tom; Patti, Gary

**Author Affiliations**

Panome Bio, Inc.; Washington University in St. Louis

**Title**

MassID: A Cloud-Based Untargeted Metabolomics Pipeline for Enhanced Biochemical Discovery.

**Introduction**

Metabolomics holds immense potential for understanding human disease. However, current metabolomics technologies face limitations in comprehensively analyzing metabolomics data in a robust and comprehensive manner. This presentation introduces MassID, a novel, cloud-based untargeted metabolomics data analysis pipeline designed to overcome the limitations of existing technologies. This innovative pipeline leverages advanced bioinformatics and machine learning to enhance metabolite detection, curation, and identification. MassID integrates multiple existing software programs (e.g., PeakDetective, mz.unity, and more) as well as two novel software modules: PeakDefrag, which annotates and removes noise signals arising from unintentional metabolite fragmentation, and DecoID2, which employs probabilistic modeling to assign quantitative confidence scores to metabolite identifications. These functionalities address the critical challenges of untargeted analysis in metabolomics.

**Methods**

The MassID processing pipeline begins with raw LC/MS data being uploaded to the Google Cloud Platform (GCP). PeakDetective, enhanced with PeakDefrag, performs unbiased peak detection, alignment, grouping, and filtering of fragments, contaminants, and other degeneracies (via mz.unity). PeakDefrag removes fragment ions by mapping unidentified metabolite signals to a database of predicted low-energy fragment ions generated by CFM-ID 4.0. Next, DecoID2 identifies the remaining metabolites by comparing their MS/MS spectra and retention times to a comprehensive metabolite library, assigning a probability score to each identification through a Bayesian integration of the data. This enables the inclusion of previously unusable ambiguous identifications in downstream analyses. Finally, users can interact with the processed data through a web application and perform downstream analysis.

**Preliminary Data**

To showcase MassID's usefulness, serum metabolomics data from healthy individuals and stage IV CRC patients were acquired and analyzed. MassID detected 48,549 metabolite signals from these data.~38,000 of these signals were discarded for not providing unique biological information through the application of blank subtraction and mz.unity, leaving ~10,500 presumed unique biologically relevant features being detected. Of the remaining features, 1,509 could be structurally identified, leaving 8,973 signals that could not be annotated as noise nor identified. Additionally, 518 of these unannotated signals showed a significant association with CRC status, suggesting many potential novel compounds of relevance to CRC. However, when applying PeakDefrag to these unidentified signals, 8,093 of the 8,976 unannotated features were classified as an unintentional fragment of another identified metabolite, which reduced the number of statistically significant unannotated signals from 518 to just three. This filtration not only leads to a >98% annotation of the dataset but enables accurate prioritization of hits suitable for de novo structure elucidation.

When considering the identified metabolites, 123 compounds reached statistical significance. However, only 28 of these compounds met the requirements for a L1 or L2 identification by the metabolomics standards initiative (MSI) guidelines. Traditionally, only results from these identification levels are considered in biological data interpretation. To enable contextualization of all metabolites, we applied DecoID2 to calculate the identification

confidence of all identified metabolites, resulting in 343 metabolites being identified at >95% confidence. Of note, 30 statistically significant metabolites that did not meet the MSI Level 1 or Level 2 requirements, achieved a >95% identification confidence, exemplifying the enhanced discovery power made possible by MassID. Biological interpretation of the results of this study, highlighted changes in multiple metabolic pathways including, ascorbic acid, homocysteine, and glucose metabolism that have been corroborated in the literature.

**Novel Component**

A novel platform for untargeted interrogation of metabolomics data that achieves near complete data annotation and quantitative identification confidence scores.

**Author(s)** (Presenting author should be listed first)
Tipirneni-Sajja, Aaryani; Johnson, Hayden
**Author Affiliations**
University of Houston

**Title**

Optimizing Neural Networks for Real-Time Quantification of Metabolites in Complex NMR Spectra

**Introduction**

Neural networks can be applied to quantify metabolites in thousands of NMR spectra in real time; however, the diversity of neural network algorithms available and the many nuances of dataset preparation and model training make preparing the optimal model a non-trivial task. This study aims to identify and optimize the most important aspects of designing a robust, accurate neural network for metabolite quantification. We use simulated spectra of common aqueous metabolites to generate training and validation datasets of varying spectral complexity and magnetic field-strengths, and we explore architectural variations and learning parameters using multi-layered perceptrons (MLP), convolutional neural networks (CNN), and transformers.

**Methods**

An MLP, CNN, and transformer network are developed and applied to representative simulated NMR spectra, and models are compared using mean absolute percent error. To develop the optimal training dataset, each model is trained using simulated 400-MHz spectra datasets with up to 44 metabolites and with varying concentration distributions and modifications mimicking realistic experimental variations. Learning parameters (loss function, learning rate, ect.) and model architecture (# of layers, kernel sizes, ect.) for each model are then optimized by Bayesian hyperparameter optimization using the most promising dataset. The best model architectures and parameter sets are further trained and compared on spectra containing up to 8, 21, 44, and 87 metabolites generated at 100, 400, and 700-MHz.

**Preliminary Data**

In dataset optimization, datasets using a combination of uniformly distributed, low-concentration mimicking the distributions measured in real metabolomics datasets, and high-dynamic range concentration distributions with concentration magnitudes extending slightly beyond the range of intended use resulted in the most accurate models. Increasing the training/testing dataset size from 20,000 to 50,000 improved the performance of the transformer and CNN but not MLP. In loss function optimization, relative absolute error loss achieved the most accurate metrics. The MLP and transformer were the most accurate for representative input spectra with a high dynamic range of concentrations. The transformer had the most robust performance in cases of interfering signals in the form of non-analyte singlets added at random, followed by the CNN and then the MLP. The transformer was the most effective model in handling spectral complexity with increasing number of metabolites in spectra. All three models performed similarly on 100-MHz and 700-MHz spectra as they did on 400-MHz spectra. In summation, all three models were accurate across a broad range of possible input spectra; however, in this study, the transformer model achieved the best combination of accuracy and robustness.

**Novel Component**

First application and optimization of neural networks, especially transformers, for quantification of aqueous metabolites in highly complex, realistic NMR spectra.

**Author(s)** (Presenting author should be listed first)
Bifarin, Olatomiwa; Yelluru, Varun; Simhadri, Aditya; FernÃ¡ndez, Facundo
**Author Affiliations**

Georgia Institute of Technology, Atlanta, Georgia

**Title**

Mapping The Landscape of Metabolomics Research

**Introduction**

The field of metabolomics has experienced exponential growth since its emergence in the late 1990s, as evidenced by the thousands of publications. However, this rapid expansion makes it challenging to track the field's evolution. While databases like PubMed facilitate the identification of studies through keywords and **Title**s, and the tracking of citations, they lack the capacity to provide a comprehensive overview of the field. This limitation hinders the identification of evolving research trends such as research questions, and techniques used. Thus, there is a pressing need for a comprehensive, high-angled analysis of the metabolomics literature to understand its trajectory.

**Methods**

Utilizing the Biopython library and Entrez API, we queried PubMed for abstracts mentioning "metabolomics" or "metabonomics." Abstracts were then classified into broader fields (e.g., oncology, plant biology, microbiology) based on the journals in which they were published. For the embeddings generation, we tokenized and processed each abstract using PubMedBERT, a variant of the Bidirectional Encoder Representations from Transformers (BERT) model trained on PubMed abstracts and articles. This yielded 768-dimensional vectors for each abstract. For visualization, we employed t-distributed Stochastic Neighbor Embedding (t-SNE) to reduce dimensionality to two, enabling the identification of trends and patterns within the metabolomics literature.

**Preliminary Data**

We compiled a dataset of over 82,000 abstracts from 1998 to January 2024. Our analysis confirmed the expected annual increase in publications since the late 1990s, with the most significant growth occurring between 2020 and 2021. As anticipated, a consistent slower growth rate was observed in the field's early years. The modal abstract length was found to be 200 words. The top three journals for metabolomics publications were Scientific Reports, Metabolites, and PLoS ONE. Frequently used terms in abstracts include "mass spectrometry," "found," "associated," "level," "P" (indicating P-value), amongst others. Our embeddings revealed distinct clusters for fields like plant sciences and more dispersed clusters for analytical chemistry. We observed overlap between fields such as toxicology and environmental sciences, and cancer biology and immunology. Our methodology also enables keyword searches to uncover trends, such as clustering of COVID-19 metabolomics publications, and the tracking of groundbreaking publications mentioning phrases like "for the first time." Analysis of embeddings by publication year did not reveal clear trends, but the embeddings were used to investigate trends in P-value mentions, reported sample sizes, journal title length, abstract length, and number of authors. We have developed a web application to allow scientists to explore these embeddings and facilitate the discovery of trends in research topics and techniques in their respective domain within metabolomics. Future directions include topic modeling with open-source models like Llama 3 for the complete labeling of embeddings.

**Novel Component**

This work provides a unique mapping of the evolution of metabolomics within the life sciences and biomedical fields.

Full Abstracts for the Biomarkers Session (1)

**Author(s) (Presenting author should be listed first)**
Copie, Valerie; O'Shea-Stone, Galen; Tripet, Brian, Garrott, Robert, and Thomson, Jennifer
**Author Affiliations**
Montana State University-Bozeman
**Title**
Applications of NMR metabolomics to wildlife management: monitoring of the nutritional states of wild bighorn sheep using 1H NMR spectroscopy
**Introduction**
Rocky Mountain bighorn sheep are iconic symbols of the American West and have adapted to survive in some of the most rugged and remote landscapes of the region. Their nutritional status is a critical determinant of their survival, reproduction, and population dynamics. Conservation efforts have had limited success, partially due to a lack of knowledge about these animals' metabolic characteristics. During winter months, when forage is scarce and of lower nutritional value, bighorn sheep rely heavily on their body reserves and experience severe nutritional deficits. Metabolic adaptations are thus critical for the animals' health and fitness yet are poorly understood. This study employed 1H NMR metabolomics to identify essential metabolic pathways underlying wild bighorn sheep nutritional stress responses.
**Methods**
Serum samples from 388 wild bighorn sheep were collected between 2014 and 2017 across Wyoming and Montana. Animal captures and sample collections took place from December to March during three consecutive winters. Due to seasonal changes, wild bighorn sheep were sustained on senescent native forages resulting in sub-maintenance diets. Animals captured in December months were designated as the least impacted by seasonal changes and classified as 'early submaintenance' (Early-SM), while animal captured in January months as 'moderate submaintenance' (Mod-SM) and February through March as 'severe submaintenance' (Severe-SM). Following metabolite extraction and recording of NMR spectra, univariate & multivariate statistics and machine learning methods were employed to identify distinct metabolic interactions and discriminate between early and severe sub-maintenance nutritional states.
**Preliminary Data**
Significant differences were observed in key metabolites including formate, thymine, glucose, choline, among others, pointing to distinct and important alterations in one-carbon, amino acid, and central carbon metabolic pathways. Results from PLS-DA modeling revealed clear separation between early and severe sub-maintenance groups underscoring the distinct metabolic states associated with the different degrees of nutritional stress. Most noteworthy were alterations in metabolites associated with one carbon and amino acid metabolism. The discovery of alterations in one-carbon (1C) metabolism in bighorn sheep (to be further detailed in our presentation) provided valuable insight into one of the fundamental metabolic pathways which is essential for survival and therefore adaptation to nutritional stress. Findings from our study emphasize the intricate interplay between metabolic responses and seasonal, environmental, and nutritional changes. Our metabolomics results underscore the value of examining polar serum metabolite profiles to strengthen our understanding of the metabolic shifts accompanying varying degrees of nutritional stress in wild bighorn sheep. The metabolites and metabolite level changes identified in this work offer potentially robust biological indicators for early detection and differentiation of nutritional states, paving the way for further research into their roles and implications in animal health and disease. This study provides valuable insights into biochemical mechanisms of metabolic resilience of bighorn sheep, emphasizing the potential for targeted nutritional interventions to support restoration and conservation efforts. This research enhances the understanding of metabolic responses to environmental stressors in wild ruminants, offering a foundation to develop more effective management practices to restore and maintain healthy bighorn sheep population.
**Novel Component**
Monitoring wild bighorn sheep nutritional status via metabolomics; Environmental metabolomics; Application of NMR metabolomic methods to guide conservation efforts of wild ungulates; one-carbon and amino acid metabolism in wild ruminants; Application of machine learning tools to NMR metabolomics data analysis

**Author(s) (Presenting author should be listed first)**
Martin, LeRoy; King, Adam M; Sanchez-Lorenzo, Ana, Marsden-Edwards, Emma, Want, Elizabeth
**Author Affiliations**

Waters Corp, Marblehead, MA; Waters Corp, Wilmslow, Cheshire, UK; Department of Metabolism, Digestion and Reproduction, Imperial College, London, UK; Waters Wilmslow; Imperial Colleg

**Title**

HIGH THROUGHPUT PLASMA PROFILING OF HUMAN LIVER DISEASE SAMPLES USING RAPID CHROMATOGRAPHY AND A MULTI-REFLECTING TIME-OF-FLIGHT MASS SPECTROMETER

**Introduction**

Acute-on-chronic liver failure (ACLF) is a serious condition which develops based on acute decompensation (AD) of cirrhosis and is characterized by intense systemic inflammation, multiple organ failure, and high short-term mortality. However, there are still no validated biomarkers for the diagnosis of ACLF. Understanding the role of metabolites and lipids in the pathogenesis of ACLF would help the development of new diagnostic and therapeutic strategies. Investigating biomarkers for large scale studies requires robust, high-throughput analytical methods. Rapid microbore metabolic profiling (RAMMP) methods and conventional high-resolution mass spectrometry have previously been shown to considerably reduce analysis time. Here we compare these methods with vacuum jacketed technology (VJC) and ultra high-resolution mass spectrometry for the analysis of plasma from liver disease patients.

**Methods**

Human plasma samples from four patient groups, healthy control (n=25), cirrhosis (n=25), ACLF (n=25), and acute liver failure (ALF) (n=25), underwent extraction for lipids and small molecules. Quality-control (QC) sample was prepared by combining aliquots of each study sample and phenotypic pools were created by combining aliquots within the study groups. Chromatographic separation was performed using the RAMMP methodology with small molecules analysed using the HILIC ACQUITY BEH amide (1.0 x 50 mm) and lipids profiled using a ACQUITY BEH C8 (1.0 x 50 mm) column. Mass spectrometry data was acquired on the SELECT SERIES MRT using the data independent acquisition (DIA) mode MSE in both positive and negative ESI mode. All data were processed using MARS and Lipostar software.

**Preliminary Data**

Conventional LC-MS analysis using chromatographic methods between 10-30 min per sample provides detailed characterisation of biological matrices. However, analysing large collections of samples using these analysis regimes places a burden on costs, resourcing and can lead to batch response variation, impacting data quality. The need for fast, high throughput methods has increased in recent years to tackle these burdens. Deploying RAMMP and VJC chromatographic methodologies affords much shorter analysis times but increases the need for faster scanning MS acquisitions to maintain peak fidelity. While traditional Tof-based mass analysers enable fast acquisition speeds, generally their mass resolution capabilities are limited (10-90K FWHM). In comparison ion trap-based analysers offer higher mass resolution (>100K FWHM) but require longer scan times to achieve this elevated resolution making them less compatible with rapid analysis. The SELECT SERIES MRT system overcomes the limitations of orthogonal Tof and trapping mass analysers. Using Multi Reflecting Time-of-flight it achieves mass resolution of >200K FWHM at scan rates compatible with RAMMP and VJC methodologies.

Data analysis of the entire batch of 112 injections was performed in a single polarity using both a RAMMP method and with VJC technology with the same LC parameters. VJC produced narrower chromatographic peaks (typical peak width 0.6s). The fast MS scanning for this acquisition enabled collection of >20 data points across these peaks, fully defining the analytical peak.

Interrogation of the HILIC small molecule data by OPLS-DA determined significant differences in amino acids and biogenic amines, in particular depletion of branched chain amino acids and an increase in aromatic amino acids. For the lipid data, much lipid dysregulation was seen in the diseased patients with both increases and decreases seen particularly for LPA, LPC and PC lipid species.

**Novel Component**

Plasma Biomarker profiling using high-throughput chromatography and very high accuracy mass spectrometry.

**Author(s) (Presenting author should be listed first)**

Casu, Fabio; Schock, Tracey; Bayless, Amanda; Mahynski, Nathan; Boggs, Ashley

**Author Affiliations**

National institute of Standards and Technology (NIST), Chemical Sciences Division

**Title**

Evaluation of Biomarkers of Reproductive Success in Atlantic Salmon Biofluids using NMR-based Metabolomics and Machine Learning

**Introduction**
Atlantic salmon, a key species in marine aquaculture, has experienced a production drop of over 35% since 2000, partly due to decreased embryo survival rates. Currently, selective breeding programs use metrics that are mostly based on growth related traits, but these traits do not correlate well with reproductive performance. We are developing measurement techniques to assess broodstock quality through the analysis of biofluids (plasma, ovarian fluid, and skin mucus) collected from Atlantic salmon female broodstock with the objective of identifying a suite of metabolic markers that correlate with reproductive success by comparing metabolite profiles in high performers (>70% eye-up rate eggs) and low performers (<70% eye-up rate eggs) using NMR metabolomics in combination with machine learning.
**Methods**
Plasma, ovarian fluid and skin mucus were collected in collaboration with USDA and the University of Maine. Two cohorts were sampled during spawning in 2021 and 2022. Plasma and ovarian fluid were filtered using centrifugal filters (3 kDa MWCO). Mucus samples collected on filter paper, were extracted with 70% methanol. NMR spectra were acquired at 298K on a Bruker Avance II 700 MHz spectrometer using both 1D and 2D NMR experiments (1H NOESY, and 1H,13C-HSQC). Spectra were binned, normalized to total spectral area, and scaled (Pareto) prior to multivariate analysis (PCA and PLS-DA). Metabolite identification was performed by comparison of experimental chemical shifts to reference values in available metabolomics databases (Chenomx, HMDB and BMRB), and an in-house library.
**Preliminary Data**
Our preliminary results from metabolomics analysis of 2022 data show that among the three different biofluids analyzed in this study, ovarian fluid NMR metabolite profiles showed better clustering according to egg eye-up rates. Since ovarian fluid is the obvious reproductive matrix, machine learning approaches will be used to identify correlating metabolic features in other biofluids like plasma and mucus to evaluate the fish metabolome for reproductive fitness. Skin mucus is of particular interest since it constitutes a more readily accessible and non-invasive matrix. Results from this study will guide the development of robust molecular tools for efficient broodstock selection, with the potential for early culling of low-yield broodstock, thereby enhancing reproductive success, and improving environmental sustainability.
**Novel Component**
Development of predictive biomarkers of reproductive performance in Atlantic salmon broodstock.

**Author(s) (Presenting author should be listed first)**
Eldridge, Ronald C.; Anderson, Allyson; Magliocca, Kelly; Shi, Qiuying; Patel, Mihir R.; Bates, James E.; Schmitt, Nicole C.; Steuer, Conor E.; Shin, Dong M.; Liu, Yuan; Teng, Yong; Chung, Christine H.; Saba, Nabil F.
**Author Affiliations**
Emory University; Emory University; Emory University; Emory University; Emory University; Emory University; Emory University; Emory University; Emory University; Emory University; Emory University; Moffit Cancer Center; Emory University
**Title**
Plasma Metabolome as a Biomarker for Immunotherapy in Recurrent or Metastatic Head and Neck Cancer
**Introduction**
Plasma metabolomics has emerged as a valuable prognostic biomarker for cancer patient outcomes. Our research has demonstrated the association of pretreatment plasma metabolic profiles with weight loss, symptoms, quality of life, and overall survival in head and neck cancer (HNC) patients undergoing standard therapy. A significant advancement in this field would be the ability to use metabolomics to predict which patients will respond to novel immunotherapies. The combination of pembrolizumab (a PD-1 inhibitor) and cabozantinib (a VEGF inhibitor) has shown promising disease control in HNC patients with recurrent or metastatic disease (RMHNC). However, effective biomarkers for predicting response remain elusive. Our study aims to identify pre- and post-treatment plasma metabolites as potential indicators of therapeutic response to this combination immunotherapy.
**Methods**
In our phase II single-arm trial of immunotherapy in RMHNC patients, plasma was collected before treatment and 9 weeks after the first cycle. Using HILIC-positive and C18-negative high-resolution LC-MS, we extracted and identified 171 metabolites, matched within 10 ppm m/z accuracy to laboratory authenticated compounds. These included 4 amines, 50 amino acid derivatives, 11 carbohydrates, 21 cholines/betaines/carnitines, 28

lipids, 7 nucleotides, 34 simple organic acids, 6 vitamins/cofactors, and 10 others. Treatment outcomes (complete or partial tumor response) were determined using the Response Evaluation Criteria in Solid Tumors v1.1. We z-score standardized the metabolites and examined significant ($P<0.05$) changes over time and across treatment response using repeated measures ANOVA. KEGG pathway enrichment analysis determined the metabolic profile associated with treatment response.

**Preliminary Data**

Of the 14 treated patients, 11 were male and 9 were white, with a median age of 59 years. Half had human papillomavirus (HPV)-positive RMHNC and six were fully active with no performance restrictions. Six patients responded to the combination immunotherapy, with response rates evenly distributed across all patient characteristics, including HPV status. One notable finding suggests that the plasma metabolome is relatively stable after undergoing immunotherapy, as lysine ($P=0.006$) was the only metabolite that significantly differed pre- and post-treatment. We observed higher levels of lysine after treatment in both responders and non-responders. Another intriguing discovery, twenty-two metabolites differed significantly between responders and non-responders with 14 being amino acids or their derivatives (Î²-Alanine, Î³-Aminobutyric acid, creatinine, proline, guanidoacetic acid, threonine, leucine, ureidopropionic acid, methionine, aminoadipic acid, phenylalanine, tyrosine, histidine, carnosine). The elevated levels of all these amino acids observed in responders strongly suggests that amino acid metabolism may be a critical determinant of susceptibility to anti-PD-1 immunotherapy. This aligns with the well-established role of amino acid catabolism in immune suppression within the tumor microenvironment. Our enrichment analysis further underscored the intricate metabolic dysregulation of amino acids pathways: five of the 22 metabolites were linked to Î²-alanine metabolism ($P<0.00001$), two to phenylalanine/tyrosine metabolism ($P<0.001$), two to valine/leucine biosynthesis ($P=0.002$), and three to arginine/proline metabolism ($P=0.004$). Importantly, none of the patient characteristics (e.g., age, sex, HPV status) meaningfully confounded the associations between metabolites and response. However, our data did suggest that the effectiveness of amino acids as biomarkers for immunotherapy response may be particularly pronounced in HPV-negative patients compared to HPV-positive patients. This novel study paves the way for utilizing plasma metabolomics to enhance patient stratification and optimize immunotherapy outcomes in RMHNC.

**Novel Component**

Despite a small sample size, this is the first study to report a metabolomic blood biomarker for RMHNC immunotherapy.

**Author(s) (Presenting author should be listed first)**

J. Rafael Montenegro Burke, Jeremy K. Chan, William D. Gwynne, Nicholas Ly, Olivia Taverniti, Brandon Y. Lieng, Mathula Muhundan, Alexandra Denhart, Andrew T. Quaile

**Author Affiliations**

Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, ON

**Title**

The Human Metabolome Atlas unveils metabolic heterogeneity across cell types and stratifies cancer subtypes

**Introduction**

Science has exerted significant efforts to understanding the metabolic complexity and heterogeneity of human cells and tissues to discern context-specific biology amenable to diagnostic stratification and treatment of disease. However, compared to transcriptome and proteome data, which have been deeply profiled across different tissues and cells, the coverage of metabolomics data is lacking. Our rudimentary understanding of the human metabolome represents a substantial bottleneck towards discovery. To better appreciate the complexity of metabolic heterogeneity, we mapped the metabolomes of 69 human cell lines from various normal and malignant tissues. The Human Metabolome Atlas (HMA) provides a reference map of key biological processes, and we demonstrate how it can identify unique metabolic signatures and potential metabolic vulnerabilities within cancer.

**Methods**

Metabolome and lipidome profiles of 69 human cell lines that are commonly used in biomedical research and that represent 18 diverse tissues were generated using standardized methodology. Cells were cultured in replicates (n=5), spiked with internal standards (Avanti UltimateSplash ONE & IROA TruQuant Yeast Extract) for data normalization, and subjected to a biphasic methyl tert-butyl ether-based protocol for metabolome and lipidome extraction. Data were acquired using a Vanquish Horizon UHPLC system coupled to an Orbitrap IQ-X Tribrid (Thermo Scientific). Separation of metabolites and lipids was achieved using a ZIC-pHILIC (2.1 x 150

mm, 5 mm) (Millipore Corp.), an Acquity BEH Amide (2.1 x 100 mm, 1.7 mm) and an Acquity CSH C18 (2.1 x 100 mm, 1.7 mm) column (Waters).

**Preliminary Data**

The deep metabolomic and lipidomic profiling of our cell line biobank identified (MSI level 1 and 2) and quantified of over ~300 endogenous metabolites and ~1,300 lipids across multiple metabolic pathways. Long-term reproducibility and variation were evaluated through the recovery of spiked internal standards within each sample. These same internal standards were also used for evaluating ion suppression effects; despite the numerous matrices present within the HMA, suppression levels were consistent across metabolites and lipids. Overall, their inclusion enabled comparisons across cell types and improved data fidelity. Given the breadth of the HMA, we were able to confirm the broad applicability of our metabolomic and lipidomic cell line profiles for determining cell-type specific biology by recapitulating expected phenotypes from previous studies (e.g., TG abundance in liver cells, long and unsaturated PEs in retinal cells, etc.). These results were leveraged to identify novel metabolic signatures specific to individual cell types and tissue. For example, we observe a separation in the clusters of cells derived from hematological from other malignancies. Surprisingly, this separation in PCA is driven by levels of saturation within glycerolipids and the abundance of purine nucleotides. It is noteworthy that most cell lines within the HMA are of malignant origin, providing a reference and the necessary context to determine the specificity of metabolomic signatures in different cancer types. For instance, the lipidomes of 12 breast tumor cell lines can be used to separate them into their respective molecular subtypes (i.e. Luminal and Basal); driven by lipid classes including complex sphingolipids and phospholipids. These signatures may prove to be useful as potential prognostic markers for breast cancer. Together, the HMA combines both analytical breadth and depth to accelerate research towards novel, context-specific metabolic vulnerabilities and diagnostic biomarkers within defined cancer lineages.

**Novel Component**

The HMA unveils unique metabolomic signatures of 69 diverse cell-types unlocking deeper understanding of human metabolism and cancer-type specific biology.

**Author(s) (Presenting author should be listed first)**

Mohammad Alyamani, Nima Sharifi

**Author Affiliations**

University of Miami

**Title**

Metabolic Changes in Response to Hormonal Therapy in Advanced Prostate Cancer

**Introduction**

Prostate cancer (PCa) is a major health problem in the United States, being the most frequently diagnosed cancer and the second leading cause of cancer-related death in men. It is well known that advanced prostate cancer is driven by the androgen axis. Therefore, androgen deprivation therapy (ADT), by either medical or surgical castration, is the front-line treatment for advanced (metastatic) disease. Patients initially respond to ADT but then resistance occurs. Apalutamide, a potent next-generation androgen receptor antagonist, is FDA-approved to treat advanced PCa. Apalutamide confers a survival benefit. However, drug resistance still eventually occurs, and is the cause of nearly all PCa deaths.

**Methods**

Selecting an optimal treatment is challenging in PCa because there are no effective predictive biomarkers for personalized medicine. In our effort to identify biomarkers and explore the metabolic changes caused by hormonal therapy, we analyzed serum samples from 42 patients with advanced PCa treated with apalutamide plus ADT. Samples were collected at baseline (just before the start of the treatment) and after 28 days. Our initial analysis targeted steroid changes with respect to the treatment. These in-house analyses were performed on an ABSciex 5500Qtrap. We then send samples to Metabolon to perform an untargeted analysis on a subset of patients (n=18) who had serum available.

**Preliminary Data**

ADT suppresses testosterone levels. In addition to this known observation, our targeted analysis revealed an alteration in systemic glucocorticoids. The major active glucocorticoid, cortisol, and its downstream metabolites (via C5 reduction) were elevated after treatment.

In addition to alterations in steroidogenesis, our untargeted analysis revealed alterations in major physiological pathways. A total of 142 metabolites were altered after ADT and apalutamide, of which 60 were elevated and 82 were suppressed. These metabolites cover most biochemical classes and pathways, including amino acids,

bile acids, and fatty acids. Mechanistic studies on the effects of these metabolites on drug resistance in advanced PCa are ongoing.

**Novel Component**

This is the first study to investigate metabolic alterations in patients with PCa in response to apalutamide plus ADT treatment.

Full Abstracts for the Cancer Metabolism Session (9)

**Author(s)** (Presenting author should be listed first)
Jiangjiang (Chris) Zhu
**Author Affiliations**
The Ohio State University
**Title**
Discovering Distinct Metabolic Alterations for Colorectal Cancer Diagnosis and Monitoring
**Introduction**
Colorectal cancer (CRC) stands as the third most frequently diagnosed cancer and ranks second among the leading causes of cancer-related mortality within the United States. Hence, there is an urgent clinical demand for CRC screening **Methods** that is less invasive, easily accessible, and more convenient comparing to established **Methods** such as colonoscopy. In this study, we utilized metabolomics and transcriptomic data to study the metabolic abnormalities of CRC patients at different cancer stages. A new data analysis pipeline integrating the methodologies of both partial least squares discriminant analysis (PLS-DA) and advanced artificial neural network (ANN), was also established to enhance CRC diagnosis and progression monitoring.
**Methods**
Multiple cohorts of CRC patients and age/gender matched healthy controls were utilized for the study. Blood samples were collected from various clinical sets in the State of Ohio and respective IRB approvals were obtained. Metabolomics analyses were performed on a Thermo Vanquish UPLC system coupled with a Q-Exactive Orbitrap mass spectrometer equipped with a heated electro-spray ionization probe as well as a XBridge BEH Amide XP column. Raw mass spec data for biological samples and targeted list PQCs were converted for spectral peak extraction using the XCMS R package. Then PQCs were annotated by comparing with our commercial standards. The normalized colon adenocarcinoma mRNAseq data and clinical data were obtained from the Broad Institute (https://gdac.broadinstitute.org).
**Preliminary Data**
Three distinct cohorts of samples were obtained for metabolomics analyses, while an additional cohort of transcriptomic data was acquired from TCGA databases to serve as validation of findings from metabolomics results and facilitate multiomics integration analyses. Following metabolomic data collection, a rigorous preprocessing protocol was implemented. Overall, 240 metabolites were confidently annotated via standard references and high-resolution databases. Subsequently, both PLS-DA and ANN models were deployed to address key research inquiries, encompassing the identification of diagnostic and prognostic biomarkers, as well as delineating mutation-associated dysfunctional pathways. We demonstrate the application of benchmark classification methods, including the separate use of PLS-DA and ANN, and the development of the advanced in-tandem pipeline of these methods, PANDA, in model classification. Simultaneously, we utilize cross-validation to ensure the reliability and generalizability of our classification results. In order to discover the metabolic disparities between CRC and HC cohorts, we employed both PLS-DA and ANN modeling techniques on plasma metabolite data. To corroborate the discriminative capacity of the metabolite dataset between the two groups, we also performed transcriptomics analyses of TCGA data. It was observed that both the PLS-DA (accuracy=0.8721, AUC=0.8879) and the ANN-based model (accuracy=0.9412, AUC=0.9727) have a good separation prediction between HC and CRC cases. Meanwhile, in ANN models, both the metabolomic data (accuracy=0.9412, AUC=0.9727, loss=0.1780) and the transcriptomic data (accuracy=0.9767, AUC=1, loss=0.0555) exhibited superior performance on the classification. To delve into CRC progression monitoring, we also studied a subset of CRC patients in our analyses which we have detailed staging information to investigate the alterations in metabolic profiles across varying tumor and lymph node (LN) stages. In total, 23 and 72 compounds were selected and visualized in heatmaps for T stage and N stage, respectively. Overall, there is an observed downward trend in compound levels as stages advanced, coupled with the stronger association of N stage with metabolic profile alterations, underscores the relevance of LN stage progression in CRC.
**Novel Component**
We identified the metabolic profiles changes in CRC diagnosis and progression in a large cohort utilized a novel bioinformatics pipeline.

**Author(s)** (Presenting author should be listed first)

Thomas Roddy, Gordon Murray, Joseph LaPointe, Anil Padyana, Marion Dorsch, Maria Jesus-Blanco, Shomit Sengupta
**Author Affiliations**
Atavistik Bio
**Title**
Using Metabolites and Metabolomics to Identify Regulatory Allosteric Sites for Drug Discovery
**Introduction**
Metabolites are known to control many biological processes of normal and disease physiology through direct interactions with proteins. These interactions include acting as substrates and products of enzymes, and are also the result of allosteric regulation of many classes of proteins, such as enzymes, transcription factors, and others. We have developed technology that uses metabolomics and our custom endogenous metabolite library to rapidly and systematically discover functional allosteric sites on therapeutic targets of interest. Next, we apply our AI-enabled structure-based drug discovery engine to design small molecules for these sites, enabling the discovery of novel precision allosteric therapeutics. This process is demonstrated by our lead precision allosteric oncology drug discovery program.
**Methods**
Our **Methods**, based on technology developed at University of Utah, use equilibrium dialysis to allow endogenous metabolites from our library to interact with proteins and, if binding, to enrich with the protein in a 2-sided 96-well equilibrium dialysis plate. Once equilibrium is complete, we extract metabolites from both the protein and buffer side of the equilibrium dialysis plate. Next, LC-HRMS techniques using multiple LC and positive and negative MS conditions allow detection of this variety of metabolites from the protein and the buffer sides of the device. This results in the identification and relative quantitation of the metabolites on each side, indicating which metabolites are enriched with the protein and, therefore, bound bind to the protein if interest.
**Preliminary Data**
We have screened over 80 protein targets so far and have found metabolite binders in >95% of the cases. In the 15 cases that we selected and characterized the effect of the metabolites on protein function, we identified functional binders in 100% of the cases. Out of those cases, we identified 7 novel allosteric metabolite protein binding sites by crystallography, allowing the rapid development of cell or in-vivo active compounds with medicinal-like properties in at least 5 cases to date. Two examples of screening and follow-ups will be presented, including data for MCAD (Medium chain acyl desaturase), a key enzyme in fatty acid oxidation, and BCR-ABL, an oncologic transcription factor target.
**Novel Component**
Metabolomics and a metabolite library are used to discover novel allosteric sites and drugging mechanisms, enabling AI/ML-enhanced structure-based drug design.

**Author(s)** (Presenting author should be listed first)
Abhishek Jain 1, Montana T. Morris 2, Domenica Berardi 1, Trisha Arora 3, Xavier Domingo-Almenara 3, Philip B. Paty 4, Nicholas J. W. Rattray 5, Daniel Kerekes 2, Lingeng Lu 6, Sajid A. Khan 2, Caroline H. Johnson 1
**Author Affiliations**
1 Department of Environmental Health Sciences, Yale School of Public Health, 60 College Street, New Haven, CT, 06510, USA .2 Department of Surgery/Surgical Oncology, Yale School of Medicine, 333 Cedar Street, New Haven, CT, 06510, USA .3 EURECAT - Technology Centre of Catalonia, Omics Sciences Unit, Avda. Universitat 1, 43204 Reus, Catalonia, Spain. 4 Department of Surgery, Memorial Sloan Kettering Cancer Center, 1275 York Ave, New York, NY, 10065, USA. 5 Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, UK .6 Department of Chronic Disease Epidemiology, Yale School of Public Health, 60 College Street, New Haven, CT06510, USA
**Title**
Charting the metabolic biogeography of the colorectum in cancer: challenging the right sided versus left sided classification
**Introduction**
Colorectal cancer (CRC) is usually classified as proximal, distal and rectal cancer. Clinicopathological, molecular features and risk factors do not change abruptly at splenic flexure and variations exist even among the refined subsites. Moreover, extensive evidence indicates that distinct anatomic sublocations in the colorectum harbor significantly different gut microbiota, contributing to disease heterogeneity. If not addressed,

subsite-associated variability may contribute to the failure of mechanism-targeted therapies for CRC as well as poor accuracy of potential CRC biomarkers. We generated the first comprehensive CRC metabolome map, integrating gut microbial culturomics and bioinformatics tools, to describe etiological and survival heterogeneity in cancers of different subsites of the colorectum.

**Methods**

Colon tissues were acquired from surgery for colectomy and prospectively collected on 736 stage Iâ€“IV CRC patients in the period 1991â€“2001 at Memorial Sloan-Kettering Cancer Center, USA. Utilizing 372 tumor tissues with matched normal mucosa liquid chromatography-mass spectrometry (LC-MS) was applied to examine metabolomics profiles along 7 subsites of the colorectum: cecum (n=63), ascending colon (n=44), transverse colon (n=32), descending colon (n=28), sigmoid colon (n=75), rectosigmoid colon (n=38) and rectum (n=92). Cox proportional hazard regression analysis was used for survival analysis. Linear distribution of metabolites across the colorectum was tested by Linear regression. Untargeted metabolomics data was obtained from 310 in-vitro cultures of gut bacterial species and integrated with CRC metabolome to identify the microbial contribution to disease heterogeneity.

**Preliminary Data**

A comparative analysis of tumors with matched normal mucosa identified exclusive alterations in metabolite markers within specific CRC subsites. Notable metabolites included alanyl-leucine, alanine, valyl-methionine in the cecum; maltotriose, succinate, valerylcarnitine, lysoPE(P18:0) in the ascending colon; decanoylcarnitine in the transverse colon; hexanoylcarnitine, octanoylcarnitine, epsilon-(gamma-glutamyl)lysine in the descending colon; estradiol-17B-glucuronide, glycocholic acid, glycodeoxycholic acid, vitamin A,arachidonic acid in the sigmoid colon; lysoPE(18:0) in the rectosigmoid; and cysteinylglycine, cytidine, cytidine monophosphate, phenylalanine in the rectum. Integration of metabolic features from 310 gut bacteria, along with significantly altered metabolic markers in each CRC subsite, identified bacterial species predominantly influencing metabolic changes. Utilizing an R script, the top 30 bacteria with the most substantial impact on metabolome changes in each subsite were identified. Further investigation revealed co-occurring triplet bacterial markers within the top 30 bacteria, influencing subsite-specific changes: Helicobacter pullorum, Campylobacter coli,Campylobacter upsaliensis in the cecum; Anaerococcus lactolyticus, Oxalobacter formigenes, Gordonibacter pamelaeae in the ascending colon; Collinsella stercoris, Allobaculum stercoricanus, Paraprevotella clara in the transverse colon; Bacteroides finegoldii, Bacteroides caccae, Bacteroides dorai in the descending colon; Butyricoccus faecihominis, Allobaculum stercoricanus,Collinsella stercoris in the sigmoid colon; Oxalobacter formigenes, Gordonibacter pamelaeae,Varibaculum cambriense in the rectosigmoid;Helicobacter pullorum, Campylobacter coli,Campylobacter upsaliensis in the rectum. Next, we compared inter-subsite differences.40 and 76 significantly altered metabolites (including bile acids, lysoPCs and LysPEs) between tumors and normal mucosa showing metabolic heterogeneity existed between CRC subsites. Gradual changes in metabolites abundance with a significantly linear trend from cecum to rectum: 23 metabolites in tumor, 30 metabolites in normal mucosa and 15 metabolites in both tumor and normal mucosa, confirmed a concentration gradient exists across colorectum and it depends on the disease status. Cox regression analysis revealed metabolites associated with survival were different and unique to each subsite. Gut bacterial metabolites contributed to intertumor and survival heterogeneity across colorectum.Finally, an interactive and publicly accessible CRC metabolome database was designed to enable access and utilization of this rich data resource (https://colorectal-cancer-metabolome.com/yale-university).

**Novel Component**

The first comprehensive CRC metabolome map challenges the conventional classification of CRC in proximal and distal cancer

**Author(s)** (Presenting author should be listed first)
Leo Cheng, Ella Zhang, Jiaqi Lu, Chin-lee Wu, Adam S. Feldman
**Author Affiliations**
Harvard Medical School

**Title**

NMR Metabolomics Biomarkers of Human Prostate Cancer from Biopsies After More Than 5-Year Follow-Up

**Introduction**

Prostate cancer (PCa) is one of the most prevalent and fatal malignancies for men worldwide. The combination of multiparametric magnetic resonance imaging (mpMRI) and ultrasound allows for improved visualization of anatomical structures, assessment of tumor aggressiveness, and probe-tracking capabilities;

however, such biopsies are still prone to false-positive and false-negative results due to the presence of various factors. Metabolomics, as a promising field for cancer biomarker discovery, can provide new perspectives for evaluating PCa prognosis, aggressiveness, clinical significance, and promote developments of in vivo metabolomic imaging. In this >15years study, we present the capacity for metabolomic analyses to not only distinguish between PCa and benign tissues, but also differentiate patients who are diagnosed with PCa years after initial negative biopsy.

**Methods**

Biopsy cores. Over a period of 15 years and with 10 years of follow-up, 432 biopsy cores from 332 patients with suspicious for PCa underwent biopsies. One or two cores underwent NMR analysis. Spectroscopy. All cores were analyzed by high-resolution magic angle spinning (HRMAS) NMR on a Bruker 600MHz spectrometer at 4ÂºC with a rotor-synchronized Min(A,B) protocol with spinning at 600 and 700Hz. Data analysis. Spectra were curve-fit using a MATLAB-based program. Metabolic spectral regions of interest (ROIs) (n=48) with >60% of samples presenting detectable values were analyzed with principal component analysis (PCA) and other statistical tools. Following the NMR analysis, all cores returned to pathology for quantitative pathology evaluation and recorded in patient records.

**Preliminary Data**

Prediction of PCa through patient follow-ups. We classified all 432 cores according to their diagnosis at three separate time points: Class 1 reflects patient diagnosis at the time of biopsy (t=0, 1a: our analyzed cores were benign and the entire biopsy was benign; 1b: our analyzed cores were benign, but the entire biopsy was cancer; and 1c: our analyzed cores were cancer), Class 2 at 5 years post-biopsy (t=5, 2a: the case was still benign after 5 years follow-up; and 2b: the case changed from benign to cancer), and Class 3 at 10 years post-biopsy (t=10), where cancer cases were assigned their scores of the prostate cancer prognostic grading group (PGG). HRMAS NMR could differentiate between cancer and benign cores at the time of biopsy; however, benign cores obtained from PCa positive patients presented their different associations with cancer or benign for different ROIs, with principal components presented the most significant difference between Class 1a and 1b. Based on measurement biopsy core at time t=0, metabolomic differentiation between Class 2a and 2b (PCa discovered within five years after biopsies) patients was possible, with their trends moving from 1a to 1b on the PC2 plot. Finally, we randomly divided all cases in training and testing cohorts, and observed metabolomic profiles that can predict case PGG after prostatectomies from biopsy core PGG.

Here, we demonstrate the capability of measuring PCa metabolomics non-destructively from human biopsy cores using ex vivo HRMAS MRS, and present PCa metabolomic predictive potential measured from biopsy cores through patient follow-ups 5~15 years. Most notably, predictions of negative biopsy patients to be discovered with PCa within 5 years may be achieved with MRs-based metabolomics, while longer follow-ups are needed and continued in our laboratory.

**Novel Component**

Human prostate cancer metabolomics analysis of biopsy cores with >5 year patient follow-up

**Author(s)** (Presenting author should be listed first)
Erika M Palmieri1, Jonathan M. Weiss1, David A Wink1 and Daniel W McVicar1
**Author Affiliations**
1 National Cancer Institute, NIH, USA

**Title**

Metabolic Reprogramming of Tumor Microenvironment by Nitric Oxide

**Introduction**

Expression of Nitric oxide synthase-2 (NOS2) in breast cancer predicts reduced patient survival. We have shown that endogenous nitric oxide (NO) reprograms macrophage mitochondrial metabolism by limiting aconitase (ACO)-2 and pyruvate dehydrogenase (PDH) activities. NO controls indeed the levels of immunomodulatory metabolites and uses the PDH-cofactor lipoate to generate nitroxyl, a radical that forms irreversible modifications on proteins. Despite this knowledge, the actual concentration of NO within the tumor microenvironment (TME) remains unclear; it is unknown if the levels of NO present are sufficient to induce metabolic reprogramming. Therefore, we want to understand the impact of NO on tumor growth utilizing EO-771 breast tumors.

**Methods**

EO-771 murine cell line was propagated in vitro and CRISPR-Cas9 system was used to generate knockout line for Nos2. Tumors were formed by injecting 1x10^5 EO771 cells in 4th inguinal mammary fat pad in a 50%PBS-50%matrigel suspension.
Tumor interstitial fluid (TIF) and tumor-associated macrophages (TAMs), specifically F4/80 positive cells, were isolated from both wild type and Nos2 knockout tumors at day 14 from inoculation. Metabolomic analyses were performed on TIF and TAMs employing Agilent 6546 LC-QTOF and MS-DIAL for data alignment. Further statistical and pathway analyses were carried out through Gene Set Enrichment Analysis (GSEA) and mummichog algorithms within MetaboAnalyst.
TMRE staining was used for assessment membrane potential and Seahorse assays were conducted to measure energy phenotype of TAMs through flux analysis.

**Preliminary Data**

Mice bearing Nos2 knockout tumors exhibited slower tumor growth and resistance to necrosis. Metabolomic analysis identified a clear NO imprint within the TIF. This is characterized by dysregulated energy metabolism, lipoate disturbances, and regulation of oxygen tension. These metabolic shifts highlight the extensive influence of NO on the tumor microenvironment. Moreover, TIF analysis shows dysregulation of metabolites belonging to pathways related to proline metabolism and extracellular matrix remodeling, hinting at a role for NO in invasion and aggressiveness.
Metabolomic profile of TAMs highlighted the significant metabolic differences attributed to NO exposure. TAMs from wild type tumors demonstrated profound dysregulation in oxidative metabolism, with clear arginine-pathway related signatures. This was coupled with a rewiring of metabolic pathways mediated by the presence of reactive oxygen species and subsequent changes in compounds associated with endoplasmic reticulum (ER) stress. Corroborating this data, we observed that TAMs from knockout tumors possess higher mitochondrial membrane potential and additionally, these exhibited a pronounced aerobic and energetic phenotype through flux analysis. This indicates a profound metabolic reprogramming in response to NO.
Our study reveals that the metabolic traits observed in murine macrophages producing large quantities of NO are recapitulated in tumor settings where tumor-derived NO crucially shapes the microenvironment. This interaction significantly affects the metabolism and functionality of macrophages surrounding the lesion, thus promoting tumor growth.

**Novel Component**

Our insights into the metabolic dynamics within the tumor microenvironment suggest potential avenues for targeted metabolic interventions in cancer therapy.

**Author(s)** (Presenting author should be listed first)
Ma, Xin; Leontyev, Dmitry; Asef, Carter K; Arnold, Rebecca S.; Petros, John A.; FernÃ¡ndez, Facundo M.
**Author Affiliations**
Department of Chemistry and Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology; Emory School of Medicine and Winship Cancer Institute, Emory University

**Title**

Triboelectric Nanogenerator-Powered Laser Ablation/Nanoelectrospray Ionization (TENG LAESI) Mass Spectrometry Imaging Ion Source to Study Renal Cell Carcinomas

**Introduction**

Triboelectric nanogenerators (TENG) have been proven to be a highly efficient power supply for pulsed ionization in mass spectrometry (MS). Nanoelectrospray ionization (nESI) powered by â€œlarge areaâ€ TENG devices follow a mixed ESI/APCI-like ionization mechanism that enables C=C bond epoxidation in lipids, which can be coupled with collision induced dissociation for lipid structural elucidation. Herein, we introduce a new TENG-powered MS imaging (MSI) ion source for in-depth spatial lipidomics. We optimize and quantify its performance using renal cell carcinoma (RCC) tissues and compare against MALDI and DESI MSI platforms.

**Methods**

An OPOTEK IR laser operated at 2,940 nm wavelength with a repetition rate of 20 Hz was used for desorbing lipids from the tissue surface. The laser was mounted on a Thermo Q Exactive Orbitrap mass spectrometer, the laser beam guided by a gold-coated right-angle prism mirror. Lenses with focal lengths of 20 to 30 mm were used to optimize the laser spot size on the tissue. A TENG device was connected to a nESI emitter via a platinum wire for ionization and epoxidation of desorbed lipids. nESI solution was 50% methanol. Tissues were

mounted on a slide attached to an XY stage controlled by an Arduino microcontroller. The stage was placed in front of the MS extended ion inlet.

**Preliminary Data**

Proof-of-concept experiments were conducted using a Leu-enkephalin (MW = 555.62 g mol-1) standard solution (200 pg Î¼L-1), several lipid standard solutions (LPC 18:1, MW = 521.35 g mol-1 and PC 18:1, MW = 785.59 g mol-1, 200 pg Î¼L-1) purchased from Avanti Polar Lipids and banked human RCC tissue sections. The Orbitrap mass spectrometer was operated in positive ion mode in the 100 â€“ 1,200 m/z range and the mass resolution was set to 17,500 (at m/z 200). No sheath or auxiliary gas flow was utilized. The temperature of the modified ion inlet was set to 320 oC. Standard solutions (10 Î¼L) deposited on glass slides, or 10 Î¼m-thick RCC tissue sections were placed onto the XY stage prior to MSI experiments. The vertical distance between the extended inlet and the tissue was 5 â€“ 10 mm. The speed of the TENG sliding electrode was set to 80%, and the pulse time between each half TENG cycle was kept at 550 ms for optimal lipid desorption. Further geometry optimization including additional laser focusing and stage positioning is being conducted. Leu-enkephalin and lipid standard solutions were successfully desorbed and protonated, resulting in 10 â€“ 12 TENG pulses. Ion intensities of protonated molecules exhibited excellent pulse-to-pulse stability with relative standard deviations (RSD%) ranging from 6% to 11%. Protonated, sodiated and potassiated lipid molecules in the 200 â€“ 1,200 m/z range were readily detected in RCC tissue sections. For comparison purposes, DESI and MALDI MSI experiments were conducted on sections collected from the same kidney on a Waters Select series cyclic ion mobility mass spectrometer and a Bruker solariX 12T mass spectrometer. Multi-pass (up to 20 passes) ion mobility separation was attempted for putative annotation of certain key lipids (e.g. PC 34:1). Determination of C=C positions is currently being attempted using TENG LAESI-MSI, followed by MS/MS.

**Novel Component**

A new triboelectric nanogenerator-powered MSI ion source for spatial lipidomics with in-depth lipid structural information.

Full Abstracts for Environment & Ecology (3)

**Author(s)** (Presenting author should be listed first)
Sandhu, Pawanjit K; Murch, Susan J
**Author Affiliations**
University of British Columbia Okanagan
**Title**
Development of metabolomics toolbox for identification and discovery of non-protein amino acids in plants
**Introduction**
In addition to twenty amino acids that are required for protein synthesis, plants are known to produce hundreds of natural non-protein amino acids (npAAs). The npAAs have functions in anti-herbivory, stress signaling, nitrogen storage and environmental toxins. It has been predicted that more than 800 npAAs occur across plant kingdom, however the exact scope of diversity and distribution of the npAAs is not known. We are developing a liquid-chromatography tandem mass spectrometry (LC-MS/MS)-based metabolomics toolbox for identification, quantification and discovery of npAAs in plants.
**Methods**
We created a method training set of 42 compounds containing both -N[H] and -COOH functional groups for optimization of chromatographic separations, determination of derivatization suitability and tuning of MS parameters. The training set contains npAAs that are not commonly included in traditional amino acid analyses. Derivatization protocols for -N[H] derivatization reagent (Waters AccQ-Tag (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC))) and a -COOH derivatization reagent (4-bromo-N-methylbenzylamine (4-BNMA)) were optimized for unusual amino acids. Conditions for gradient elution from reverse phase chromatography and DDA analysis in positive ionization mode were developed. A set of plants spanning the tree of life were collected and extracted to investigate the scope of npAA metabolism. npAAs are detected in complex extractions by common fragment ion produced of the derivatization reagent and deconvolution of the parent ion mass. Matching of parent ion masses with our in-house library of >8000 amino acids and retention time with the ReTip algorithm is used for confidence in identification of novel npAAs in plants.
**Preliminary Data**
Based on multiple -N[H] and -COOH derivatization sites, derivatization resulted in 88 structures for -N[H] and 46 structures for -COOH derivatization for 42 compound training set. The optimized LC method consists of 30-minute gradient elution of 0.1% formic acid (solvent A) and 0.1% formic acid in acetonitrile (solvent B) on a Cortecs C18 column (Waters) with parent ion monitoring of m/z 171.06, the cleavage product of AQC. The MS/MS condition optimization for 4-BNMA derivatization is underway. The preliminary analysis of derivatized Arabidopsis thaliana extracts shows 100+ potential amino acid peaks highlighting the need and validity of the developed toolbox.
**Novel Component**
This study combines multiple derivatization chemistries with targeted analysis and parent ion fragmentation to identify and discover novel npAAs in plants.

**Author(s)** (Presenting author should be listed first)
Sharma, Chetan; Abiskaroon, Brendan; HA, Ricardo; Singh, Vinayak; Zhurov, Vladimir; Maglov, Jorden; Harrison, Alexander; Antonacci, Michele; Chruszcz, Maksymilian; Grbic, Vojislava.
**Author Affiliations**
Western University, London, Ontario, Canada
**Title**
Mass Spectrometry-Based Metabolomics to Elucidate Spider Mite-Host Interactions for the Development of RNAi-Based Biopesticides
**Introduction**
Tetranychus urticae, the two-spotted spider mite, is a notorious pest known for rapid reproduction, adaptability, and pesticide resistance. As a generalist herbivore, it infests over 150 crops and adapts quickly to unfavorable hosts, overcoming plant defense mechanisms. This leads to reduced yields and significant financial losses due to the extensive use of chemical pesticides, which are costly, unsafe, and prone to resistance. Arabidopsis thaliana, a model plant, contains amino acid-derived secondary metabolites like glucosinolates and phenylpropanoids, which serve as major anti-herbivory defenses. This study aims to develop an untargeted

metabolomics workflow to understand the host adaptation strategies of spider mites and identify phytochemicals and enzymatic defense mechanisms involved.

**Methods**

Transcriptome data analysis was conducted to observe gene expression for detoxification enzymes such as Glutathione S-transferases, Cytochrome P450s, and Intra-diol dioxygenases. Mass spectrometry-driven untargeted and targeted metabolomics were utilized to elucidate the dynamic interactions between spider mites and Arabidopsis thaliana. These analyses provided insights into the evolutionary aspects as well as new and informed sustainable plant protection strategies.

**Preliminary Data**

The transcriptome data analysis of the spider mite reveals a high expression or upregulation of genes for enzymes linked with detoxification and host adaptation, including Glutathione S-transferases, Cytochrome P450s, and Intra-diol dioxygenases. Mass spectrometry-based metabolomics elucidates the dynamic interactions between the spider mite's detoxification enzymes and plant defense molecules, particularly glucosinolates and phenylpropanoids. Untargeted metabolomic analysis of spider mite-infested A. thaliana Col-0 (wild) versus its different mutants revealed a high accumulation of phenylpropanoids, indole glucosinolates, and their breakdown products. Toxicological assessment of Indole-3-acetonitrile and sinapoyl malate demonstrated their toxicity against spider mites, showing excellent potential in reducing fecundity and increasing mortality. Furthermore, the role of TuGSTm12 in the detoxification of glucobrassicin (indole-3-methyl glucosinolate) has been confirmed through in-vitro reactions with the recombinant protein of TuGSTm12. We also described the putative mechanism of this detoxification reaction through targeted metabolomic experiments with LC-HRMS. These findings suggest that targeting the detoxification pathways in spider mites using RNAi could be an effective strategy for developing RNAi-based biopesticides, potentially offering a sustainable and environmentally friendly alternative to conventional chemical pesticides. The integration of transcriptomics and metabolomics provides a comprehensive understanding of the molecular interactions between spider mites and their host plants, paving the way for innovative pest management.

**Novel Component**

Indole glucosinolates and phenylpropanoids like sinapoyl malate are major plant defense metabolite against spider mite which uses its enzymatic detoxification machinery to survive.

**Author(s)** (Presenting author should be listed first)
Garcia, Brianna M.; Kido Soule, Melissa C.; Brandt, Marilyn; Apprill, Amy; Kujawinski, Elizabeth B.

**Author Affiliations**

Woods Hole Oceanographic Institution, Woods Hole, MA 02543; Woods Hole Oceanographic Institution, Woods Hole, MA 02543; University of the Virgin Islands, St Thomas, Charlotte Amalie West, St Thomas 00802, U.S. Virgin Islands; Woods Hole Oceanographic Institution, Woods Hole, MA 02543; Woods Hole Oceanographic Institution, Woods Hole, MA 02543

**Title**

Metabolomic Profiling of Coral Microhabitats: Understanding the Metabolic Response of Coral Tissue, Mucus, and Skeleton

**Introduction**

Corals are complex colonial organisms comprising genetically identical individual polyps, photosynthetic dinoflagellate symbionts, and diverse microorganisms within a coral host. Each polyp features distinct layersâ€”surface mucus, tissue, and skeletonâ€”termed microhabitats. These microhabitats support unique microbial communities, serve distinct physiological functions, and are hypothesized to exhibit varying responses to local stressors. However, current metabolomic studies largely focus on bulk coral homogenates, overlooking potential metabolic and stress response differences among microhabitats. Understanding the chemical compositions and roles of these physiological components, and their associated microbial communities, is crucial for comprehending coral health. In the face of increasing environmental and anthropogenic pressures, there is a need to advance current strategies to mitigate these impacts on coral reefs and enhance their resilience.

**Methods**

Orbicella faveolata coral fragments were separated into mucus, tissue, and skeleton and their individual metabolomes were compared to the bulk coral homogenate metabolome and exometabolome (metabolites released into seawater). Fragments were swabbed to remove the bulk of their surface mucus layer, wrapped in combusted aluminum foil, and flash frozen. A piece of each coral was removed to serve as the homogenate.

Flash frozen samples were lyophilized for 48-hours. Dried tissue was separated from the skeleton using a firm bristle brush. Homogenate and skeleton were pulverized under liquid nitrogen. Metabolites from mucus, tissue, skeleton, and homogenate were extracted with organic solvents and sonication; seawater was extracted using PPL-SPE. Samples were analyzed via UHPLC-MS/MS on a ThermoFisher Fusion Lumos Tribrid Mass Spectrometer.

**Preliminary Data**

Untargeted metabolomics data was collected in positive and negative mode using a ThermoFisher Fusion Lumos Tribrid Mass Spectrometer and data dependent acquisition. Data was processed using mzMine4.0 on a high-performance computing cluster. Focusing on positive mode, a total of 44,739 features were detected. Features were blank filtered to remove potential processing contaminants and non-biologically related features. Principal components analysis (PCA) of all five investigated sample types (tissue, mucus, skeleton, homogenate, and exometabolome) showed strong separation along PC1 (28.5%) and PC2 (26.3%). Coral mucus and exometabolome samples largely contributed to this variation, having drastically different profiles than the coral tissue, skeleton and homogenate. Exclusion of the coral mucus and exometabolome samples from the PCA showed clear separation of the coral tissue/homogenate from the skeleton. The coral tissue and homogenate overlapped with one another (within the 95% CI), however, the coral tissue showed tighter clustering and less variability than the coral homogenate. Preliminary analyses with PLS-DA showed interesting metabolite differences between the coral tissue, skeleton, and homogenate with many signals present in the separated tissue and skeleton samples being absent or largely decreased in abundance in the homogenate, confirming our hypothesis that bulk metabolomic analyses may be limiting our understanding of the complexity and dynamic nature of coral metabolites across it's various microhabitats. GNPS feature based molecular networking and database matching resulted in 276 unique putative compound annotations with a cosine score > 0.7. Metabolites previously observed in coral metabolomics studies, such as betaine lipids, amino acids, nucleotides and various osmolytes were among the annotations observed. Analyses are ongoing to further investigate metabolite trends across sample groups. Outcomes of this work will highlight the implications of coral reef sampling strategies and demonstrate the importance of considering microhabitat differences when asking questions related to coral reef metabolism, ecology, and immune response dynamics.

**Novel Component**

This new method provides the first metabolomic analysis and comparison of separated coral tissue, mucus, and skeleton.

**Author(s)** (Presenting author should be listed first)
Xia, Mengxue; Suseela, Vidya; Tharayil, Nishanth

**Author Affiliations**

Clemson University

**Title**

Leveraging untargeted and targeted approaches to characterize molecular complexity and conservation of compounds released from the degradation of plant substrates

**Introduction**

Plants produce a large number and highly diversified array of phytochemical compounds. Through abiotic/microbial degradation, these compounds are released into the environment, representing significant carbon fluxes from plants to soils. These plant-substrate-released compounds attract attention in environmental/ecological studies for their potential to form stable soil organic carbon by associating with soil minerals. The transformation/stabilization of these compounds in soils are regulated by their molecular properties. However, molecular-level chemistry of compounds released from plant substrate degradation remains poorly understood. Here, we ask whether plant species and tissue type (leaf vs. root) impact the consortia of compounds released, resulting in distinct chemical phenotypes, and whether these substrate-specific chemical phenotypes persist or converge, losing much of their initial distinction as degradation progresses.

**Methods**

This study addresses knowledge gap in the molecular-level chemistry of compounds released from plant substrate degradation by characterizing molecular composition of leachate collected from 18 plant substrates in a 16-month incubation with soil microbial inoculums. Ultra-high-resolution Orbitrap MS with nano-electrospray ionization was utilized to characterize chemistry at molecular formula levels, with formulae assigned to chemical classes using a multidimensional stoichiometric approach. Because a number of

common formulae were observed from all plant substrates in later biodegradation stages, we conducted tandem MS to determine if these universal formulae across substrates share similar molecular structures. An in-house R program addressed chimeric spectra and assigned fragments to precursors by matching fragments resulting from common neutral losses and their combinations to a corresponding precursor.

**Preliminary Data**

The 18 plant substrates in our 16-month incubations produced leachate with highly variable molecular compositions, and this variability can be largely attributed to plant identity, tissue type, and biodegradation stage. The initial substrates imposed a strong influence on the molecular composition of leachate, which exhibited distinct chemical phenotypes not only among plant species, but also between leaves and rootsâ€”compounds produced from root decomposition typically had a higher weighted means of molecular mass and a greater degree of unsaturation and aromaticity than those from leaves, and these differences were relatively consistent across six phylogenetically-diverse species. Whether the molecular characteristics of leachate remain distinct among plant substrates or converge as degradation progresses is context-dependent and depends on specific chemical aspects. Several key molecular properties that have been associated with reactivity of compounds in soils (e.g., molecular mass, aromaticity, and nominal oxidation state of carbon) remained divergent by species and tissue type even after extensive degradation, suggesting that plant species and tissue type have persistent influences on the compounds released, shaping the molecular fingerprints of leachate from both fresh and highly decomposed plant residues. On the other hand, regardless of initial substrates, leachate from later degradation stages converged to exhibit a large number of unique molecules in low concentration (i.e., high Shannon diversity). We also identified an increasing pool of common molecular structures, frequently containing carboxyl substitutions, across all plant substrates as degradation progressed. This supports the existence of conservative, substrate-independent biochemical pathways mediating the formation of compounds from the degradation of plant substrates. Our findings of the persistent influences of initial plant substrates, generalizable differences between compounds release form leaves and roots, and the emerging pool of common molecular structures offer a novel molecular basis for understanding the chemical variability and conservation of compounds resulting from the degradation of plant substrates.

**Novel Component**

Persistent influences of plant species and tissue types; emerging pool of common molecular structures in the later stages of degradation

**Author(s)** (Presenting author should be listed first)
David E. Alonso, Joseph Binkley, and John Hayes
**Author Affiliations**
LECO Corporation

**Title**

): Comprehensive profiling of Nepeta Cataria using multidimensional gas chromatography, and high-performance mass spectrometry

**Introduction**

Nepeta Cataria (Catnip) is well-known for its intoxicating effects on felines. This euphoric effect is characterized by playful, sometimes aggressive cat behavior. Catnip essential oil is rich in bioactive compounds that have led to its use in the past as a traditional medication. It has recently been used as an antioxidant, anti-inflammatory, anti-cancer, and antimicrobial material. In addition, the oil has shown promise as a natural insect repellent. New analysis **Methods** should be implemented to characterize this valuable plant material. Unfortunately, the plant is very complex due to its large number of constituents with different physiochemical properties that are present in a wide range of concentrations. In this study, we developed a method that combines enhanced chromatographic separation with high-performance time-of-flight mass spectrometry for the comprehensive profiling of catnip.

**Methods**

The analytical methodology included effective sample extraction techniques, automated data acquisition, and processing to maximize the total number of characterized metabolites. Samples were prepared using solid-liquid extraction **Methods**, Solid Phase Microextraction (SPME), and thermal desorption to extend the detectability of catnip components. The extracted materials were analyzed using GC- and GCxGC with high-performance time-of-flight mass spectrometry. Data processing for confident metabolite annotation included peak finding with deconvolution, spectral database matching, retention index filtering, and mass accuracy

calculations for molecular, fragment, and adduct ions. Downstream statistical processing was performed to differentiate the catnip samples.

**Preliminary Data**

The analytical methodology resulted in comprehensive data with increased S/N and high-quality spectra for superior database comparisons to large, well-established libraries (i.e., NIST 23, Wiley 12). The combination of enhanced chromatographic resolution with high-performance time-of-flight mass spectrometry resulted in an increased total number of confidently annotated metabolites. Five times more compounds were annotated using GCxGC-TOFMS as compared to traditional GC-TOFMS analysis. Retention index filtering and mass delta calculations increased confidence in the metabolite annotations. Classes of compounds present in peak tables after automated data processing included alkanes, alkenes, alcohols, amines, aromatics, indoles, terpenes, terpenoids, aldehydes, ketones, and esters. In addition, several different iridoids (e.g., structural and stereoisomers of nepetalactone) were detected and annotated. Unknown compounds were characterized using a combination of multimode ion source (EI, PCI, and NCI) with GCxGC-HRTOFMS. In these cases, metabolite annotation was achieved through formula determinations for fragment, molecular, and adduct ions using high-resolution accurate mass data. Downstream statistical processing using Fisher ratios and principal component analysis plots facilitated the identification of markers that differentiated the samples.

**Novel Component**

Comprehensive characterization of catnip using a combination of enhanced chromatography, and high-performance time-of-flight mass spectrometry.

**Author(s)** (Presenting author should be listed first)

Haibin, Guan; Shelley, Liu; Georgia, Dolios; Jia, Chen; Lauren, Petrick

**Author Affiliations**

Department of Environmental Medicine and Public Health at the Icahn School of Medicine at Mount Sinai

**Title**

Innovative application of Weighted Quantile Sum in Mediation Analysis of Metabolomics, PFAS Exposure, and SARS-CoV-2 IgG Levels in Pregnancy

**Introduction**

We previously reported a negative association between PFAS mixture exposures and SARS-CoV-2 IgG levels in pregnant individuals. However, the underlying mechanism remains unclear. To gain mechanistic insights, we conducted a novel mediation analysis using Weighted Quantile Sum on metabolomics and PFAS exposures to identify metabolites or pathways that may mediate the relationship between PFAS exposure and SARS-CoV-2 IgG antibody levels.

**Methods**

The study included 59 pregnant participants from the Generation C Study in NYC. PFAS levels and metabolomics profiles in maternal plasma were measured using LC-MS/MS, and SARS-CoV-2 anti-spike IgG antibody levels were measured using ELISA. We applied weighted quantile sum (WQS) regression to assess the effects of PFAS exposures on IgG levels, followed by random-subset WQS to construct a metabolites-index conditioned on the PFAS-index. Two mediation analyses were performed: one with the metabolites-index as a single mediator, and another using a parallel design to control mutual influence between metabolites indexes. Analyses adjusted for maternal demographics and health factors, with enrichment analyses identifying key metabolic pathways.

**Preliminary Data**

The outcome-related PFAS-index constructed by WQS was negatively associated with the SARS-CoV-2 IgG antibody levels (beta = -0.273, SE=0.08, p=0.002). Two metabolite mixture indexes were constructed, one positively (beta = 0.296 [95% CI=0.191, 0.407] ) and one negatively (beta = -0.277 [95% CI=-0.362, -0.198]), associated with PFAS-index. For PFAS-index the most significant individual PFASs contributing to SARS-CoV-2 IgG antibody levels were PFHPS and PFHXS, with assigned weights 0.355 and 0.249, respectively. For metabolite-index, the key metabolites contributing to the positive association were Xanthine and Sphingosine-1-phosphate (d18:1) while the key metabolites contributing to the negative association were Adipate, Ketoleucine, Guanidinoacetic acid and Glycine. In mediation analyses. We observed a significant indirect effect by negative metabolite-index where 60.8% of the PFAS joint mixture effect on SARS-CoV-2 IgG antibody levels were partially explained (mediated) by metabolite-index in the negative direction. Enrichment analysis reveals that Alpha Linolenic Acid and Linoleic Acid Metabolism, Arginine and Proline Metabolism, Glycine and Serine Metabolism and Ammonia Recycling were the underlying key pathways.

**Novel Component**
Using Weighted Quantile Sum in mediation analysis to assess the mediation effect of metabolites-index on PFAS exposure and IgG levels in pregnant individuals.

## Full Abstracts for Flux & Isotope Tracer Experiments (12)

**Author(s)** (Presenting author should be listed first)
Lu, Wenyun; Park, Noel R; TeSlaa, Tara; Jankowski, Connor SR; Rabinowitz, Joshua D; Davidson, Shawn M
**Author Affiliations**
Princeton University
**Title**
Acidic Methanol Treatment Facilitates MALDI-Mass Spectrometry Imaging of Energy Metabolism
**Introduction**
Detection of small molecule metabolites (SMM), particularly those involved in energy metabolism using MALDI-mass spectrometry imaging (MSI), is challenging due to factors including ion suppression from other analytes present (e.g., proteins and lipids). Ion suppression during MALDI occurs from a combination of other analytes, including proteins, matrix compounds, salts, and analytes with high ionization efficiency (IE) or are present in high abundance. One potential solution to enhance SMM detection is to remove analytes that cause ion suppression from tissue sections before matrix deposition through solvent washes. Here, we systematically investigated solvent treatment conditions to improve SMM signal and preserve metabolite localization.
**Methods**
Serial sections of mouse tissues at a thickness of 10 Î¼m were acquired using a cryostat and thaw-mounted on indium tin oxide (ITO)-coated glass slides. Tissue slides were desiccated under vacuum for 10 min and underwent solvent wash before matrix application, which involves placing the tissue slide on an incline and pipetting solvents (5 ml) over the tissue surface. The slide was desiccated again for 5 min before matrix application. MALDI image runs were performed on a solariX XR FT-ICR mass spectrometer with a 9.4 T magnet. Data were analyzed using IsoScope. The metabolites were identified using high-resolution accurate mass with a ppm window of 10 ppm compared to an in-house metabolite list established on liquid chromatography-mass spectrometry (LCâ€“MS) using authenticated standards.
**Preliminary Data**
Washing with acidic methanol significantly enhances the detection of phosphate-containing metabolites involved in energy metabolism. The improved detection is due to removing lipids and highly polar metabolites that cause ion suppression and denaturing proteins that release bound phosphate-containing metabolites. Stable isotope infusions of [13C6]nicotinamide coupled to MALDI-MSI (‘Iso-imaging’) in the kidney reveal patterns that indicate blood vessels, medulla, outer stripe, and cortex. We also observed different ATP:ADP raw signals across mouse kidney regions, consistent with regional differences in glucose metabolism favoring either gluconeogenesis or glycolysis. In mouse muscle, Iso-imaging using [13C6]glucose shows high glycolytic flux from infused circulating glucose in type 1 and 2a fibers (soleus) and relatively lower glycolytic flux in type 2b fiber type (gastrocnemius). Thus, improved detection of phosphate-containing metabolites due to acidic methanol treatment combined with isotope tracing provides an improved way to probe energy metabolism with spatial resolution in vivo.
**Novel Component**
We developed a washing strategy to significantly improve the detection of phosphate metabolite on MALDI-MSI .

**Author(s)** (Presenting author should be listed first)
Melanie Balbach
**Author Affiliations**
Michigan State University
**Title**
Metabolic reprogramming during mammalian sperm activation
**Introduction**
Mammalian sperm are stored in the epididymis in a dormant state. Upon ejaculation, they must immediately start producing sufficient energy to maintain motility and support maturation in the female genital tract (capacitation). While this increased energy demand during capacitation is well-established, it remains unclear how mammalian sperm modify their metabolism to meet this need. Capacitation is regulated by soluble adenylate cyclase (sAC)-mediated cAMP increase and protein phosphorylation via PKA. However, the interplay between these signaling pathways regulating sperm capacitation and sperm metabolism remains mostly unexplored.
**Methods**

Using an extracellular flux analyzer, metabolomics and metabolic flux analysis we compare the central carbon, amino acid and fatty acid metabolism of non-activated and activated mouse and human sperm. Additionally, we include sperm treated with sAC inhibitors and sperm from sAC knockout mice, where the signaling pathway regulating sperm activation is hampered.

**Preliminary Data**

By combining these techniques we show that the flux through glycolysis and oxidative phosphorylation increases during capacitation. We find that in the presence of glucose, sperm utilize the Warburg effect and simultaneously suppress the pentose phosphate pathway while in the presence of pyruvate, sperm similarly generate lactate and TCA cycle metabolites. Hence, we confirm a functional link between glycolysis and oxidative phosphorylation in mouse and human sperm despite a potential compartmentalization of glycolysis and oxphos in different areas of the flagellum. We identify phosphofructokinase and lactate dehydrogenase as rate-limiting enzymes and reveal that a subset of glycolytic steps are regulated by sAC. Moreover, we find that mammalian sperm also utilize endogenous substrates like fatty acids and amino acids for energy production.

**Novel Component**

Our study provides novel insights into sperm metabolic reprogramming and reveals a functional link between sAC/PKA-regulated signaling pathways and mammalian sperm metabolism.

**Author(s)** (Presenting author should be listed first)

Rahul Ravi Deshpande-1; Ayush Midha-2; Bashar Amer-1; Isha Jain-2; Cristina C. Jacob-1; Susan Bird-1

**Author Affiliations**

1-Thermo Fisher Scientific, San Jose, CA; 2-Gladstone Institutes, San Francisco, CA;

**Title**

Carbon and Nitrogen Positional Isotopomer Determination in Metabolites using Stellar MS - a novel hybrid nominal mass instrument

**Introduction**

Fluxomics or flux analysis aims to quantify metabolism by computing the flow of carbon or nitrogen atoms through various metabolic pathways. Use of stable isotopes for flux analysis in biology has contributed greatly to the understanding of cellular metabolism and regulation. However there remains a challenge to determine accurate flux rates for metabolites which are formed from multiple precursors utilizing different enzymatic pathways. Positional isotopomer distribution of metabolites obtained by utilizing multiple tracers are invaluable pieces of information for the computation of these fluxes. Classically done by GC-MS and NMR, here we show the application of liquid chromatography-based separation of metabolites with the use of hybrid dissociation strategies using HCD and CID MSn fragmentation for positional isotopomer determination.

**Methods**

HEK cells were grown under different oxygen tensions (50%, 21% and 1.5%). Cells were unlabeled or labeled with 13C6 Glucose, 15N2 Glutamine or both 13C6 Glucose and 15N2 Glutamine. Initial data were acquired on a Thermo Scientificâ„¢ Orbitrap IQ-Xâ„¢ Tribrid Mass Spectrometer. The use of a tribrid allows the use of hybrid strategy utilizing higher-energy collisional dissociation (HCD) and collisionally induced dissociation (CID) for fragmentation of metabolites up to MSn level. Thermo Scientificâ„¢ Mass Frontierâ„¢ 8.0 and Compound Discovererâ„¢ 3.3 software were used for the metabolite annotation and data processing. A targeted MSn based assay utilizing the above data to get the structural information of the metabolites was developed on a novel hybrid nominal mass instrument.

**Preliminary Data**

GC-MS and NMR have been used extensively for positional isotopomer determination in fluxomics. However, NMR lacks the sensitivity to detect low abundant metabolites and GC-MS cannot give all the positional isotopomers of a metabolite. Moreover, GC-MS lacks the resolution for dual tracer experiments (for e.g., using 13C and 15N). Use of high-resolution accurate mass instruments with MSn capability can help overcome the challenges of using GC-MS.

Initial experiments were done using 12C and U-13C labeled E.Coli cell extracts. Metabolites were separated on HILIC-AEX column. Based on biological importance and complexity ATP molecule was chosen for the analysis. HCD and CID MS/MS spectra of the metabolites was annotated and curated using Mass Frontier 8.0 which is a spectral interpretation software. Based on the structural information of the fragments obtained from Mass Frontier 8.0, MS3 experiments were designed to target fragments which could potentially give us all the positional isotopomers. HCD and CID MS3 spectra from these fragments were again annotated and curated using Mass Frontier 8.0 for determining the optimum secondary ions to fragment.

An initial HCD/CID MS2 profiling experiment was done on the HEK cell extracts. A targeted MS3 experiment was designed, from the data obtained, for all the detected isotopologues and the optimized labeled MS2 fragments of the metabolites. The targeted assay was run on Orbitrap IQ-X Tribrid Mass Spectrometer and a novel hybrid nominal mass spectrometer. Differences in the nitrogen and carbon labeling of the metabolites in three different oxygen tension were quantified and correlated to metabolic pathways.

**Novel Component**

Workflow and application of positional isotopomer determination of metabolites using LC-MS with a hybrid dissociation strategy.

**Author(s)** (Presenting author should be listed first)
Mark P. Styczynski, April M. Miguez, Yan Zhang, Fernanda Piorino
**Author Affiliations**
Georgia Institute of Technology

**Title**

E. coli-based cell-free systems used in synthetic biology have complex metabolic dynamics

**Introduction**

Cell-free systems represent an exciting new application space in synthetic biology based on replacing the use of whole cells with processed cellular lysates devoid of the cell's genome and membrane but capable of executing transcription and translation. These systems have great potential for synthetic biology applications ranging from biosensors to manufacturing of therapeutic proteins. While these systems are best known for their protein expression potential, our group recently identified that metabolic reactions appear to play a critical role in their total expression capacity. However, the details and dynamics of this role remained poorly understood, suggesting that metabolic studies could enable improved cell-free bioengineering applications.

**Methods**

We used two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GCxGC-MS) to perform a detailed metabolic analysis of E. coli cell-free systems. We measured the small molecules in the different components of cell-free systems and of components in combination at multiple time points to track metabolic dynamics. We also measured differences in metabolic profiles after incubation at different temperatures, between lysates made using different processing parameters, and after supplementation with purified metabolic enzymes.

**Preliminary Data**

Consistent with our previous results, we found that the metabolic profile of a cell-free reaction changed significantly as a function of protein expression reaction time, with the greatest changes in metabolite profiles at the beginning and end of the reaction. Some of the most significant metabolic changes occurred in the beta-alanine biosynthesis, DHAP metabolism, and TCA cycle pathways. Unsurprisingly, for the defined chemical mixture of small molecules provided as substrates for a cell-free reaction (sometimes referred to as "energy mix") we saw little variation in concentrations with incubation time, suggesting that the molecules are stable over the timescale of the reaction in the absence of endogenous metabolic activity. Lysate metabolite concentrations, on the other hand, changed significantly when incubated without the chemical mixture, even though the metabolic content of the lysate was expected to be comparatively trivial. We found that while changing the total assembled reaction starting state via lysate preincubation impacts protein production, it has a comparatively small impact on metabolic state. We also demonstrated that changes to lysate preparation have a larger effect on protein yield and temporal metabolic profiles, though general metabolic trends are conserved. Finally, we used our metabolic results to pose hypotheses about the potential importance of different reactions in the endogenous metabolism of cell-free systems, and then sought to test those hypotheses via targeted supplementation of metabolic enzymes to the cell-free reaction. We found that while supplementation of some enzymes improves protein production, the endogenous metabolic activity is fairly resilient to these enzymatic perturbations. Overall, this work highlights the robust nature of CFE reaction metabolism as well as the importance of understanding the complex interdependence of metabolites and proteins in CFE systems to guide optimization efforts.

**Novel Component**

The metabolism of cell-free systems in synthetic biology is poorly understood and understudied, yet critically important.

**Author(s)** (Presenting author should be listed first)
Young, Jamey D.; Rahim, Mohsin; Bednarski, Tomasz K.; Hasenour, Clinton M.; Banerjee, Deveena R.; Trenary, Irina
**Author Affiliations**
Vanderbilt University
**Title**
Simultaneous in vivo multi-organ fluxomics reveals divergent metabolic adaptations in liver, heart, and skeletal muscle during obesity
**Introduction**
Control of nutrient metabolism is distributed across a network of interacting tissues and organs and is best studied in an in vivo model system. Isotope-based metabolic flux analysis (MFA) has enabled quantification of obesity-induced flux alterations within individual organs, but prior MFA studies have not attempted to simultaneously examine flux dysregulation within multiple organs that together control whole-body nutrient metabolism. As a result, it has been challenging to examine the hypothesized connections between different tissues and their functional states in vivo.
**Methods**
Metabolic fluxes were estimated by fitting a comprehensive atom-mapping model to isotope labeling measurements obtained from infusion of a combination of stable and radioactive tracers. This platform was scaled to examine metabolic flux adaptations in age-matched cohorts of mice exhibiting varying levels of obesity. Mice were fully conscious and unrestrained throughout the entire period of isotope infusion, which minimizes the potential effects of stress and anesthesia on the experimental measurements. The generality of our modeling approach allows for future expansion to include additional organs and has the potential for broad usage in metabolism research.
**Preliminary Data**
As a physiologically relevant test case, the multi-tissue fluxomics platform was applied to assess metabolic flux alterations within the liver, heart, and skeletal muscle of mice during progression of obesity. In the most severe case, chronic obesity caused an increase in hepatic gluconeogenesis and endogenous glucose production that was accompanied by elevations in glycolytic and mitochondrial cardiac fluxes but opposite changes in skeletal muscle. Skeletal and cardiac muscle have conventionally been presumed to undergo parallel metabolic responses to obesity at the transcriptional level. However, our study unveils discordant metabolic flux adaptations within these two tissues, shedding light on a striking dichotomy in fuel utilization between cardiac and skeletal muscle during the progression of cardiometabolic disease. In summation, our findings underscore the divergent impacts of obesity on metabolic fluxes across multiple organs that together control whole-body nutrient metabolism. This multi-tissue MFA technology can be extended to address important questions about in vivo regulation of metabolism (and its dysregulation under disease conditions) that cannot be answered through studies of single organs or isolated cells/tissues.
**Novel Component**
This is the first in vivo study to simultaneously quantify intermediary fluxes in multiple tissues of individual, conscious mice.

**Author(s)** (Presenting author should be listed first)
Probert, Fay
**Author Affiliations**
Department of Chemistry, University of Oxford, Oxford, United Kingdom Department of Pharmacology, University of Oxford, Oxford, United Kingdom
**Title**
Serum biomarkers of Multiple Sclerosis are associated with metabolic flux rearrangement in brain cells: combining 1H metabolomics and 13C fluxomics.
**Introduction**
Multiple sclerosis (MS) is a demyelinating autoimmune disease of the central nervous system which affects 2.8 million people worldwide. While chronic neuroinflammation is a hallmark of MS, the mechanisms by which inflammation contributes to cell death are not well understood and there remains no biofluid diagnostic test. Consequently, diagnosing MS can be highly challenging. Here we used a combination of 1H metabolomics (of human serum) and 13C fluxomics (of model rodent and cell culture systems) to identify novel diagnostic biomarkers of MS for use in the clinic, map the metabolic fluxes the identified biomarkers in brain cells, and determine whether the identified blood-borne biomarkers are directly associated with MS-like lesions in brain.

**Methods**

Spectra were acquired using a 700-MHz (16.4 T) Bruker AVII spectrometer with 1H (13C/15N) TCI cryoprobe. 1H NMR spectra used either a 1D NOESY 2 s presaturation scheme or Carr-Purcell-Meiboom-Gill (CPMG) sequence (Ï„ 400 Î¼s, 80 loops, 32 data collections, 1.5 s acquisition time, 2 s relaxation delay). Proton-decoupled 13C NMR spectra used a standard 30Â° excitation pulse and pore-gated 1H decoupling with 2048 scans and 2 s relaxation decay. Orthogonal partial least squares discriminant analysis with 10-fold external cross-validation and 100 repetitions with permutation testing was applied to identify robust biomarkers in human blood samples. For fluxomics analysis, Wistar rates were infused 1.1 mM [1,2-13C]-glucose, or [2-13C]-acetate, or [3-13C]-lactate. Dissected brain tissue was extracted in 50% v/v acetonitrile.

**Preliminary Data**

We have demonstrated that NMR metabolomics of serum samples collected from people with MS at each stage of the diagnostic pathway can 1) distinguish between people with MS and healthy controls (100% accuracy), 2) identify MS patients at the earliest signs of disease (83% accuracy), 3) distinguish MS from other neurological diseases which mimic the clinical and radiological signs of MS (81% accuracy), 4) determine the stage of disease progression (91% accuracy), and 5) identify clinical relapse and, thus, treatment response. The biomarkers identified by these human studies reveal dysregulation of cholesterol, glutamate/glutamine, and glucose chemical processes in MS patients which we then confirmed are associated with active neuroinflammation using ex vivo 13C NMR fluxomics analysis of brain extracts from a rodent model of neuroinflammation. Significant increases in [4,5-13C]-glutamine and [2,3-13C]-lactate coupled with a significant decrease in [4,5-13C]-glutamate were observed in brain extracts of [1,2-13C]-glucose infused animals following an inflammatory challenge. As glutamine is produced from glutamate by astrocyte-specific glutamine synthetase, the increase in [4,5-13C]-glutamine reflects increased production of glutamine by astrocytes. This was confirmed by infusion with astrocyte substrate [2-13C]-acetate and, independently, using astrocyte cell culture. As lactate is metabolized in the brain to produce glutamate, the simultaneous increase in [2,3-13C]-lactate and decrease in [4,5-13C]-glutamate suggests decreased lactate utilization, which was confirmed using [3-13C]-lactate as a metabolic precursor. Finally, we investigated the relationship between brain and blood metabolite levels and confirmed that the identified biomarkers correlate with brain lesion size in a rodent model of MS.

Taken together these results demonstrate that neuroinflammation results in rearrangement of the glutamine/glutamate metabolic network in the brain which, in turn, results in profound and detectable metabolite changes in blood that correlate with MS lesion burden suggesting that NMR-metabolomics of blood could be useful in the clinic to aid MS diagnosis.

**Novel Component**

The first study to identify NMR-detectable blood-borne biomarkers to stage MS progression and validate those biomarkers in 13C fluxomics studies.

Full Abstracts for Food, Nutrition, & Natural Products (11)

**Author(s)** (Presenting author should be listed first)
Muhammad Ahsin; Jennifer Cloward; Robert E. Ward; Joseph V. Varre; Matt H. Poore; Johnny Rogers; Alan Franzluebbers; Sierra N. Young; Scott L. Kronberg; Frederick D. Provenza; James R. Bain; and Stephan van Vliet
**Author Affiliations**
Department of Nutrition, Dietetics, and Food Sciences, Utah State University, Logan, UT, USA 84322; Department of Animal Science, North Carolina State University, Raleigh, NC, USA, 27695; USDA-Agricultural Research Service, Raleigh, NC, USA, 27695; Department of Civil and Environmental Engineering, Utah State University, Logan, Utah, USA; USDA-Agricultural Research Service, Mandan, ND, USA 58554; Department of Wildland Resources, Utah State University, Logan, UT, USA 84322; Duke Molecular Physiology Institute, Duke University Medical Center, Durham, NC, USA, 27701
**Title**
Improved Soil-Health and Pasture Phytochemical Richness Underlies Improved Cattle Health and Meat Nutritional Composition in Southern US Grass-Finished Beef Systems
**Introduction**
As concerns about beef production's impact on health grow, interest in improving the soil-plant-animal-human health continuum increases. We compared soil, forages, and meat samples from grass-fed and grain-fed beef across three farms and paired cropland fields in the Southeastern US. This project aimed to identify differences in metabolite profiles of grass-fed versus grain-fed beef using untargeted metabolomics analysis, linking these differences to soil health and forage nutrient content. Additionally, the study examined health biomarkers in animals, exploring how finishing diets in cattle affects health and meat composition using the same untargeted metabolomics **Methods** on plant samples.
**Methods**
All forage/feed (n=25) and meat (n=16) samples were protein precipitated with methanol after vigorous shaking followed by centrifugation. Methanolic extracts were analyzed using ultra-high performance liquid chromatography coupled with a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer. This system was interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer. MS analysis alternated between MS and data-dependent MSn scans using dynamic exclusion, covering m/z 70â€“1000 at a resolving power of R=35,000, optimized at fifty percent of the maximum peak height. Metabolites were identified by automated comparison of ion features in the samples to a reference library of chemical standard entries. Soil samples were submitted for total organic matter, and macro and micro mineral analysis.
**Preliminary Data**
We observed that 165 out of 784 profiled compounds in grass-fed (n=8) and grain-fed (n=8) ribeye steaks were different (all, $P < 0.05$), with phenolic metabolites, co-factors/vitamins, and lipid metabolites emerging as the most discriminatory classes. On average, phenolic/xenobiotics were ~3-fold higher in grass-fed beef compared to grain-fed beef ($P < 0.05$), which was directly traceable to the increased presence of plant-secondary compounds in forage samples, which were combined 38-fold higher than the total mixed rations (TMR) samples ($P < 0.05$). We also found 4.9-fold and 4.2-fold higher concentrations of Vitamins A (carotene) and E (alpha-tocopherol) in the grass-fed vs grain-fed beef samples, respectively, similarly reflecting the greater amounts of these in the forage vs TMR samples. Carotene and alpha-tocopherol were 2.14-fold and 5.13-fold higher, respectively, in the forage samples compared to the TMR samples. The higher antioxidant/phytochemical richness of the meat appeared to have a beneficial effect on animal health, which was reflected in meat biomarkers. Urate, a major intracellular antioxidant, was elevated 2-fold in grass-fed samples while 4-hydroxynonenal glutathione, an advanced lipoxidation end-product associated with worsened metabolic health, was 37% elevated in grain-fed samples (all, $P < 0.05$). Similarly, homocysteine, a common inflammatory marker, was 35% higher in the grain-fed beef samples (all, $P < 0.05$). We also observed generally healthier soils in pasturelands compared to cropland, where corn constituted 50â€“80% of total mixed rations. Soil organic matter content was higher in the pastureland soil samples (6.36%) than in feed crop soil samples (4.49%) ($P < 0.05$). Additionally, various minerals, including potassium, phosphorus, calcium, selenium, and zinc, exhibited levels 20% to 300% higher in the pasture soil samples (all, $P < 0.05$). In contrast, cropland soils contained 19% to 180% higher amounts of manganese, cobalt, and aluminum (all, $P < 0.05$).
**Novel Component**

Improved grassland soil health contributes to forage phytochemical richness, resulting in improved animal health and nutrient density in grass-fed beef.

**Author(s)** (Presenting author should be listed first)
Sumner, Lloyd W.1; Kranawetter, Clayton 1; Ho, Khank-Van 1; Moore, Sydney2; O'Neal, Caleb3; Thomas, Andrew L.3
**Author Affiliations**
1Division of Biochemistry, Bond Life Sciences Center, Interdisciplinary Plant Group, University of Missouri, Columbia, MO. 2Division of Plant Science and Technology, University of Missouri, Columbia, MO. 3Division of Plant Science and Technology, Southwest Research, Extension and Education Center, University of Missouri, Mt. Vernon, MO

**Title**
Exploring Metabolic Diversity in American Elderberry Fruits and Flowers

**Introduction**
American elderberry (Sambucus nigra L. subsp. canadensis (L.) Bolli) is a growing specialty crop and posited superfood indigenous to the United States. Elderberry fruits, leaves, and flowers possess health-benefitting compounds such as chlorogenic acid, anthocyanins, flavonoids, and their glycoconjugates. In plants, these compounds possess antimicrobial and antioxidant properties, thereby mediating plant-microbe and plant-stress responses. These compounds also possess antioxidant, anti-diabetic, cardioprotective, anticancer, and neuroprotective properties in humans. However, substantial metabolic and genetic diversity exist across the various cultivated varieties that can affect plant resilience, as well as the quality and value of derived nutraceutical products. Thus, we are using a metabolomics approach to examine the metabolic diversity in multiple cultivars and in a sizable genome by environment (GxE) study.

**Methods**
American elderberry cultivars utilized included Bob Gordon, Wyldewood, Pocahontas, Ozark, and Kelly 7-14. Tissues were harvested from plants cultivated in the same orchard and on the same day to minimize outside effects on metabolism. Ten milligrams (10Â±0.05 mg) of lyophilized tissues were extracted with 1 mL of 80% methanol:20% water containing 18 Âµg/mL umbelliferone as the internal standard. UHPLC-QTOF-MS, UHPLC-QTOF-MS/MS, and UHPLC-TIMS-PASEF-MS were performed on a Bruker TIMS-TOF mass spectrometer coupled to a Waters ACQUITY UHPLC system. Separations were achieved using a linear gradient and mass spectra were acquired in the negative electrospray ionization mode. Raw data were examined using Bruker DataAnalysis and Metaboscape 2022. Processed data were exported to csv files and uploaded into MetaboAnalyst 5.0 for statistical analyses.

**Preliminary Data**
Clear metabolic differences were observed amongst the various elderberry cultivars examined. More specifically, fruits of Bob Gordon, Wyldewood, Pocahontas, Ozark, and Kelly 7-14 cultivars exhibit readily apparent differential metabolite composition. Considerable variance is seen in Kelly 7-14 and Pocahontas cultivars, while Bob Gordon, Wyldewood, and Ozark cultivars are more similar.
A HCA heatmap was generated to visualize all mass features and revealed subsets found only in specific elderberry cultivars, hinting at metabolite fingerprints characteristic of individual cultivars. Further HCA heatmap visualization of identified metabolites revealed individual and groups of specific metabolites that vary depending on the cultivar. Key metabolite classes demonstrating these patterns included glycosylated flavones/flavonols, triterpenoids, and phenylpropanoic acids. An example of this includes maslinic acid (a triterpenoid, KEGG: C16939) and isorhamnetin 3-O-glucoside (a glycosylated flavonol, KEGG: C22589) differentially accumulating in the Ozark cultivar but not in Pocahontas. These data will ultimately guide breeding and cultivar selection to maximize bioactive compounds important to plant health and productivity along with enhancement of nutraceutical content for human disease mitigation or management. Our data indicate distinct, cultivar-specific metabolite accumulation patterns that will support development of metabolite profile-based phylogeny identification tools.
A sizable number of the metabolic features could be chemically identified, which is necessary to understand the biological implications and context. However, many metabolic features could not be identified even though they were clearly differentiated. Advanced technologies are being developed and applied to the identification of differentially accumulated metabolites. These include UHPLC-trapped ion mobility and UHPLC-MS-SPE-NMR.

Metabolic analyses of American elderberry flowers from the GxE experiment are currently underway, and these data will also be reported.

**Novel Component**

American elderberry cultivars demonstrate cultivar-specific metabolite profiles containing bioactive compounds relevant to plant health/productivity and human nutraceuticals.

**Author(s)** (Presenting author should be listed first)

Ali, Lamis; Ahsin, Muhammad; V Varre, Joseph; Cloward, Jennifer; Ward, Robert; Carbonell Herrera, Mittendorf, Camille; Statham, Travis; Marble, Shawna; Pinelli, Amanda; Kittredge, Dan; Van Vliet, Stephan

**Author Affiliations**

Department of Nutrition, Dietetics and Food Science, Utah State University, Logan, Utah; 2The Bionutrient Institute, MA

**Title**

The impact of grass vs. grain-finishing practices on plant-secondary derived metabolites in beef: a nation-wide profiling study

**Introduction**

Beef is an important source of vitamins, minerals, fatty acids and other metabolites in the human diet. These components can be influenced by cattle diet, especially during the finishing phase. Cattle finished on forages, grass, and/or legumes (grass-fed), are typically exposed to higher phenolic levels compared to feedlot-finished cattle (grain-fed), which typically includes rations of corn and soy, which have been found to be lower in phenolics. There is currently a growing consumer interest in potential nutritional differences between grass-fed and grain-fed; however, few studies have profiled how cattle finishing practices impact phenolic-derived metabolite profiles in beef. Thus, the goal of this work was to perform a survey of commercially available grass-fed and grain-fed beef samples to understand potential differences in phytochemical richness

**Methods**

Commercial beef producers across North America submitted three ribeye samples, which were ground and homogenized using liquid nitrogen. Samples (100 mg each) were mixed with 1250 Âµl of pre-cooled (-20 Â°C) extraction solvent containing methyl tert-butyl ether: methanol (2:1, v:v) followed by 5-minute lysis at 30 oscillations/second, 30-minutes of sonication, and 4-hour protein precipitation at -80Â°C. Samples were mixed with 750 Âµl of water and centrifuged at 18,000 rcf for 10 min. The aqueous: methanolic phase was evaporated and reconstituted in 100 Âµl of methanol:water (1:1, v:v) with 0.1% formic acid prior to injection. Metabolites were detected using multiple reaction monitoring (MRM) on a SCIEX Hybrid Triple Quad 7500 system with chromatographic separation on a Kinetex F5 reverse phase column.

**Preliminary Data**

The analysis revealed that grass-fed beef (n=243) has significantly higher levels of 8 out 29 measured phenolic-derived metabolites compared to grain-fed beef (n=72), while 1 compound (trans-ferulic acid) was higher in grain-fed beef (all $p < 0.05$). Notable compounds included hippuric acid (62.1 Î¼g/100g) with a 2.3-fold increase ($p < 0.01$), p-cresol sulfate (19.4 Î¼g/100g) with a 1.3-fold increase ($p < 0.05$), and 4-hydroxybenzoic acid (1.30 Î¼g/100g) with a 2.8 foldincrease ($p < 0.01$), compared to grain-fed beef. Additionally, significant increases were observed in caffeic acid (7.6 Î¼g/100g) with a 7.6-fold increase ($p < 0.001$), and catechol sulfate (11.6 Î¼g/100g) with a 4.1- fold - increase ($p < 0.01$). Other significant differences, with higher concentrations in grass-fed beef, were found in 3,4-dihydroxybenzoic acid, salicylic acid, and enterolactone (all, $p <0.05$). These differences may arise from benzoic acid, a common phenol in forages, being metabolized into compounds like 4-hydroxybenzoic acid and salicylic acid via enzymatic hydroxylation by gut microbiota and into hippuric acid through glycine conjugation in the liver. Conversely, trans ferulic acid (0.55 Î¼g/100g) was 27.5 times higher in grain-fed beef compared to grass-fed beef ($p < 0.001$). This compound is known to be present in high levels in grains, likely explaining its higher presence in tissue of grain-fed cattle.

**Novel Component**

Grass-fed beef has higher levels of phenolic-derived metabolites, likely attributed to higher phenolic levels in forages compared to grain-based rations.

**Author(s)** (Presenting author should be listed first)
Arpana Vaniya; Jessica Prenni; Jacqueline M. Chaparro; Melanie Odenkirk; Margaret Read; Susan B. Mitchell; Corey Broeckling; Nathan Montgomery; Nichole Reisdorph; Richard Reisdorph; Cole Michel; Katrina A. Doenges; Oliver Fiehn; Stacy D. Sherrod; Katrina L. Leaptrot; Jody C. May; John A. McLean; Chi-Ming Chien; Tracy Shafizadeh; Steve Watkins
**Author Affiliations**
University of California Davis West Coast Metabolomics Center
**Title**
Food for Thought: Characterizing 500 Commonly Consumed Foods through Standardized Metabolomics for The Periodic Table of Food Initiative
**Introduction**
Conventional food databases routinely track and measure at most 160 food components. However, technological advances in untargeted metabolomics enable comprehensive characterization of the chemical composition of food. The Periodic Table of Food Initiative (PTFI) is a global, non-profit effort to catalog the biomolecular composition of the world's food supply to improve human and planetary health. The PTFI aims to create a public database of food composition using liquid chromatography-mass spectrometry (LC-MS) and other analytical techniques. Here we explore 500 commonly consumed foods using the PTFI standardized untargeted metabolomics method. We will discuss how standardized metabolomics can be used to generate comprehensive molecular profiles of foods, and how this information can be leveraged to improve human health and well-being.
**Methods**
500 food samples were lyophilized, homogenized, and extracted with the PTFI internal retention time standard (IRTS) reagent. Lipids were removed using Agilent Captiva EMR-Lipid Cartridges. Using high-resolution mass spectrometry, each sample was analyzed using the Agilent ZORBAX Stable Bond Aqueous (SB-Aq) column in both positive and negative ESI modes. Raw data files were uploaded to the PTFI Lab Portal, a cloud-based data processing pipeline. The pipeline performs data conversion, feature finding, peak grouping, normalization, and retention time alignment. Metabolite annotations were based on accurate mass and retention index matching to the PTFI consensus compound library which contains over 2,800 compounds. This standardized workflow was used in two academic laboratories each analyzing the same 500 food samples demonstrating agreement across laboratories.
**Preliminary Data**
Previously, we acquired data for 10 foods to establish the standardized extraction **Methods**, reverse phase LC-MS method, and data processing pipeline. We have now applied the optimized standardized **Methods** to the analysis of 500 foods. The diversity of these 500 foods spans 56 unique food ontologies from Actinidiaceae (i.e., kiwi) to Zingiberaceae (i.e., ginger). These 56 unique ontologies can be grouped into six FoodOn categories, a globally harmonized food ontology system used to describe food in various stages (i.e., farm to fork). Specifically, the 500 foods include 46 plants (10 types of vegetables and 37 types of fruits), 19 animal species (domesticated and wild), algae, fungi, bacteria, and prepared (complex) meals. These 500 foods were sourced from 11 distinct origin locations such as Austria, the United States, Jamaica, and Thailand. Lastly, these foods also include various production processes (e.g., organic vs. conventional) and preparation (e.g. raw vs. cooked). The PTFI consensus compound library was first tested with 40 standards sent to three laboratories that acquired data in both positive and negative ESI modes. Among the 40 standards, 31 were consistently identified across all three laboratories in positive mode. Using IRTS for retention time alignment, data were aligned to the retention index (RI). The average RI standard deviation for a compound was Â± 1.58 seconds and the median standard deviation was Â± 1.47 seconds. Preliminary analysis of 10 foods spanning 7 unique ontologies using the standardized reverse phase PTFI method demonstrated the accurate annotation of authentic standards spiked into each food sample. After alignment using the IRTS, the absolute average RI difference was 0.85 seconds, with a standard deviation of Â±1.02 seconds. Collectively, these results demonstrate the application of standardized nontargeted metabolomics for the analysis of a broad range of food matrices.
**Novel Component**
Characterizing the biomolecular composition of 500 commonly consumed foods using standardized untargeted metabolomics for PTFI.

**Author(s)** (Presenting author should be listed first)
Odenkirk, Melanie1; Vaniya, Arpana2; Read, Margaret1; Michel, Cole3; Doenges, Katrina3; Chaparro, Jacqueline1,4; Mitchell, Susan1; Montgomery, Nathan4; Broeckling, Corey4; Brinkley, Sarah5; Leaptrot, Katrina6; Sherrod, Stacy6; May, Jody6; Chaura, Juliana7; Velez Meija, Gabriel7; de Vos, Ric8; Fiehn, Oliver2; Reisdorph, Richard3; Reisdorph, Nichole3; McLean, John6; Jaramillo-Botero, Andres7,9; Hall, Robert8; Chien, Chi-Ming10; Shafizadeh, Tracy10; Prenni, Jessica10; Watkins, Steve10
**Author Affiliations**
1Department of Horticulture and Landscape Architecture, Colorado State University, Fort Collins, CO, United States; 2West Coast Metabolomics Center, University of California Davis, Davis, CA, United States; 3Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado, Denver, CO, United States; 4Analytical Resources Facility, Colorado State University, Fort Collins, CO, United States; 5CIAT Bioversity Alliance, Texas A&M University, College Station, TX, United States; 6Department of Chemistry and Center for Innovative Technology, Vanderbilt University, Nashville, TN, United States; 6iÃ“MICAS Institute, Pontificia Universidad Javeriana, Cali, Valle del Cauca, Colombia; 8Bioscience, Wageningen University and Research Centre (Wageningen-UR), PO Box 16, 6700 AA Wageningen, The Netherlands; 9Chemistry and Chemical Engineering Division, California Institute of Technology, Pasadena, CA 91125, United States; 10Verso Biosciences, Inc., Davis, CA, United States

**Title**
Juicy Insights: A Standardized, Nontargeted Metabolomics Approach to Facilitate a Global Comparison of Apples to Apples

**Introduction**
Small molecules are essential contributors to both the nutritional content and flavor profiles of food. The advancement of our understanding of food composition heavily relies on high-throughput methods and techniques for characterizing these molecules. However, despite individual investigations into how variable practices of extracting, measuring, and annotating compounds bias metabolic results, niche metabolomics methods remain incredibly common across laboratories. In this context, we explore the ramifications of this prevailing landscape of metabolomics for characterizing food within and across three established laboratories. This work is juxtaposed by a standardized, nontargeted metabolomics workflow and custom internal retention time standard (IRTS) mix developed by the Periodic Table of Food Initiative (PTFI) to reproducibly survey the small molecule composition of food across laboratories.

**Methods**
Two separate experiments were conducted to (1) ascertain the comparability of results from variable nontargeted metabolomics workflows, and (2) to illustrate how standardized methodology and reagents enhance result agreement. The first experiment was completed by three laboratories (two academic and one commercial) following in-house methodologies for extraction, reversed-phase LC-MS data acquisition, and data processing. This was completed with nine foods (in duplicate) to assess intra- and inter-result reproducibility. Conversely, validation of the standardized, nontargeted metabolomics method developed by the PTFI was completed on six identical foods by five laboratories across three continents. In addition to global representation, a wide range of instrumentation (four LC-QTOF instruments from three vendors (Waters, Shimadzu, and Agilent) and one LC-Orbitrap FTMS system (Thermo)) was included.

**Preliminary Data**
Our landscape assessment aimed to evaluate both intra- and inter-laboratory agreement of the measured small molecule composition of food using variable, in-house metabolomics methods. Initially, within labs we observed average correlation values of 98%, indicating that each lab was capable of reproducibly characterizing the small molecule composition of nine unique foods. However, across labs, our assessment of overlapping coverage across foods was minimal, with an average of only 3 formula being observed across all three labs. To mitigate methodological and data processing biases, we next performed an experiment where six foods in duplicate were analyzed by five laboratories across three continents using a standardized extraction, acquisition, and data processing protocol. From standardization alone, we observed several hundred unique features reproducibly annotated across labs. However, inconsistencies in retention times as large as +/-25 seconds across laboratories precluded further alignment of data. To circumvent this issue, a standardized methodology was adopted with IRTS containing 34 non-endogenous compounds with elution times spanning the entire chromatographic separation. This resulted in a reduction of cross-laboratory retention time drift to less than 5 seconds. Following the retention time alignment of data, qualitative coverage of small molecule composition across each method was compared. The overlapping formula assignments across labs improved significantly relative to the overlap of results from variable, in-house methodologies.

Unique formula assignments across each lab were also common, likely due to instrumentation biases such as in-source fragmentation. Collectively, the application of a standardized, nontargeted metabolomics method, and the incorporation of data alignment with IRTS allowed for significantly improved agreement of data and demonstrates the potential of the PTFI's standardized, methodological approach for the generation of comparable food composition data by multiple laboratories and instruments.

**Novel Component**

We present a standardized, nontargeted metabolomics strategy for food characterization that enables the generation of comparable data across laboratories.

**Author(s)** (Presenting author should be listed first)
Kroezen, Zachary;1 Shanmuganathan, Meera;1; McIntyre, Britanny;2 Mutch, David;2 Britz-Mckibbin, Philip1
**Author Affiliations**
1. McMaster University, Dept. Chemistry and Chemical Biology, Hamilton, ON, Canada; 2. University of Guelph, Dept. Human Health and Nutritional Sciences, Guelph, ON, Canada

**Title**

Urinary Metabolite Profiling to Non-Invasively Monitor the Omega-3 Index

**Introduction**

Optimal dietary intake of omega-3 long-chain polyunsaturated fatty acids (n3-LCPUFA) is critical to human health across the lifespan with a high prevalence of deficiency in the global population. The Omega-3 Index (O3I) reflects eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) content in erythrocytes. However, omega-3 index (O3I) determination is not routinely assessed due to complicated procedures for n3-LCPUFA analysis from the phospholipid (PL) fraction of erythrocytes. We investigated whether urinary metabolites could be used to non-invasively monitor the O3I in an exploratory analysis of a previous placebo-controlled, parallel arm randomized clinical trial.

**Methods**

Fasted blood and first-void urine samples were collected at baseline and following supplementation, and they were analyzed via gas chromatography and multisegment injectionâ€“capillary electrophoresisâ€“mass spectrometry (MSI-CE-MS), respectively. Eighty-eight participants completed the 12-week double-blind, parallel arm, randomized trial in which the effects of ~3 g/day olive oil (OO; control), EPA, or DHA supplementation were compared. Briefly, 20 ÂµL of urine was diluted five-fold with 60 ÂµL deionized water, and a 20 ÂµL mixture of internal standards for a final volume of 100 ÂµL. A targeted and nontargeted data workflow was applied for characterizing urinary metabolites when using temporal signal pattern recognition with multiplexed separations in MSI-CE-MS under positive and negative ion mode in full-scan data acquisition.

**Preliminary Data**

Overall, the technical variance for repeated analysis of 125 authenticated metabolites (including creatinine) in a pooled urine sample was acceptable with a median CV = 13.4% for QCs (n = 33) as compared to their greater between-subject biological variance with a median CV = 60.5% (n = 178). We first examined changes in creatinine-normalized urinary metabolites in response to supplementation and time. For normally distributed metabolites, significant interactions (Pint < 0.05) were observed for nine urinary metabolites, of which four remained significant after applying a Bonferroni adjustment: S-carboxypropylcysteamine (CPCA; $p < 0.001$), tiglylglycine ($p < 0.001$), an unknown cation (300.215:0.841:p, $p = 0.004$), and glucuronic acid ($p = 0.014$). We tentatively identified CPCA as a novel urinary biomarker reflecting O3I status, which increased following both EPA and DHA ($p < 0.001$), but not OO supplementation, and was positively correlated to the O3I ($R = 0.30$, $p < 0.001$). In ROC curve analyses, CPCA outperformed all other urinary metabolites in distinguishing both between OO and EPA or DHA supplementation groups (AUC > 80.0%), whereas the unknown dianion performed best in discriminating OO from DHA alone (AUC = 93.6%). Stability studies were also performed on lead candidate biomarkers of the O3I that tolerated delays to freezing and repeat freeze-thaw cycles and we explored the potential for screening using dried urine spots on filter paper cards.

**Novel Component**

Candidate urinary biomarkers of the O3I were identified that lay the foundation for a non-invasive assessment of omega-3 status.

## Full Abstracts for Health & Disease (6)

**Author(s)** (Presenting author should be listed first)
Shen, Xinyi; Yan, Hong; Yao, Yisha; Khan, Sajid; Ma, Shuangge; Johnson, Caroline
**Author Affiliations**
Yale School of Public Health

**Title**
Precision Targeting of Ferroptosis in Colorectal Cancer: Sex and KRAS Mutation-Driven Metabolic Vulnerabilities and Drug Repurposing

**Introduction**
The metabolic landscape of sex differences in Colorectal Cancer (CRC) remains poorly characterized, particularly in relation to oncogenes like KRAS. Our previous work revealed decreased ferroptosis, an iron-dependent cell death mechanism, in KRAS-mutant tumors from male patients. This study examines the metabolic landscape of ferroptosis in the context of KRAS mutations and sex differences. We aim to identify metabolic vulnerabilities in KRAS-mutant CRC and explore drug repurposing opportunities. By integrating multi-omics data and applying advanced analytical approaches, we seek to uncover sex- and KRAS-specific differences in ferroptosis regulation at both transcriptional and metabolic levels. This research aligns with precision oncology efforts and addresses critical gaps in understanding CRC metabolism.

**Methods**
We analyzed three datasets: GSE39582 gene expression microarray, TCGA-COADREAD RNA-seq, and metabolomics data from an in-house MSKCC cohort. Variable Importance (VIMP) analysis and Gaussian mixture model were applied to identify genes and metabolites predictive of KRAS status by sex. A Random Survival Forest with Backward Elimination (RSF-BE) algorithm, using 1000 bootstraps, determined ferroptosis-related predictive molecular features stratified by sex and KRAS status. Partial dependence plots visualized non-linear relationships between predictors and outcomes. Gene Ontology (GO) enrichment and joint-pathway analyses revealed over-represented biological themes. Drug response analysis utilized data from the Genomics of Drug Sensitivity in Cancer (GDSC) resource. This integrated approach comprehensively explored metabolic vulnerabilities and their potential therapeutic implications in CRC.

**Preliminary Data**
Our analysis revealed significant sex- and KRAS-specific differences in ferroptosis-related gene expression and metabolite levels in CRC tumors. We identified differential expression of ferroptosis suppressors and drivers (e.g., SLC1A5 and SLC7A11) between KRAS mutant and wild-type tumors in male patients, but not in females. Metabolomics analysis detected 20 significantly altered metabolites in male KRAS mutant tumors, while only stearic acid differed in female patients.

RSF-BE models demonstrated high efficacy in identifying prognostic ferroptosis-related genes and metabolites. Distinct features were found to predict 5-year overall survival when stratified by sex and KRAS status. Notably, genes and metabolites involved in arginine synthesis and glutathione metabolism were uniquely associated with prognosis in tumors from males with KRAS mutations.

Joint-pathway analysis of prognostic ferroptosis genes and metabolites confirmed the enrichment of arginine biosynthesis and glutathione metabolism pathways in males with KRAS mutations. This finding was further supported by Kaplan-Meier analysis of the GSH/GSSG ratio from our MSKCC cohort, which showed that a lower ratio was associated with worse overall survival only in males with KRAS mutations.

Drug response analysis using CRC cell line data uncovered sex- and KRAS-specific associations between ferroptosis gene expression and drug sensitivity. Notably, expression of ferroptosis suppressor genes like DHODH, GCH1, and AIFM2 in KRAS mutant CRC cell lines correlated with resistance to cisplatin and paclitaxel. This finding provides insight into why these drugs may be ineffective for patients with KRAS mutant CRC.

These results collectively unveil novel metabolic vulnerabilities in CRC and offer promising avenues for drug repurposing strategies tailored to specific patient subgroups based on sex and KRAS mutation status. Our comprehensive metabolic map provides valuable biological insights for future investigations in CRC metabolism and ferroptosis, with significant implications for precision oncology.

**Novel Component**
Metabolic landscape of ferroptosis in CRC regarding KRAS mutations, sex differences, and drug repurposing, reveals new targets for precision oncology.

**Author(s)** (Presenting author should be listed first)
Hannah Heath(1), Farizi Fazli(1), Oana C. Danciu(2), Garth Rauscher(3), Natalie Pulliam(4), Elona Liko-Hazizi(4), Sarah Friedewald(5), Seema Khan(5), Julie Kim(5), William Gradishar(5), Jonna Frasor(2), Kent F. Hoskins(2), Zeynep Madak-Erdogan(1,6)
**Author Affiliations**
1)Department of Food Science and Human Nutrition, University of Illinois, Urbana-Champaign, Urbana, IL USA, 2) Division of Hematology/Oncology, University of Illinois at Chicago, Chicago, IL USA, 3) School of Public Health, University of Illinois at Chicago, Chicago, IL, USA, 4) Northwestern Memorial Hospital, Chicago, IL, USA, 5) Northwestern University Feinberg School of Medicine, Chicago, IL, USA, 6) Cancer Center at Illinois, University of Illinois, Urbana-Champaign, Urbana, IL USA
**Title**
Multi-Omic Analysis Links Neighborhood Disadvantage to Elevated Stress Hormones and Dysregulated Immune Response in ER+ Breast Cancer Patients
**Introduction**
Estrogen receptor positive (ER+) BC (BC) patients living in disadvantaged neighborhoods experience worse survival compared to patients living in advantaged neighborhoods. Recent studies have implicated chronic stress as a mediator of this disparity, as chronic stress is linked to dysregulated immune responses that may drive the development and progression of BC. The chronic stress hormone cortisol is known to regulate immune response, yet the role of cortisol in ER+ BC remains understudied.
We conducted targeted plasma metabolomics and proteomic analyses to investigate the association of pretreatment cortisol and immune response protein levels with cancer diagnosis and neighborhood disadvantage in racially diverse women with primary ER+ BC.
**Methods**
Pretreatment plasma samples were collected from women with primary ER+ BC (n=75) and cancer-free controls (n=75). Samples came from three Chicago, IL hospitals serving either disadvantaged or advantaged neighborhoods. Cases and controls were matched on race and hospital. Plasma samples were analyzed via UHPLC-MS/MS using a targeted steroid hormone assay and via Olink Proximity Extension Assay using a targeted inflammatory, immune response, and cancer-related proteins panel. Metabolites were quantified using 13-point calibration curves adjusted for labeled internal standards. Mixed models were fitted, including random effects for individual patients and adjusting for race, to compare: 1) cases versus controls, 2) samples from hospitals serving advantaged (n=54) versus disadvantaged (n=96) neighborhoods, 3) cases from hospitals serving advantaged (n=27) versus disadvantaged (n=48) neighborhoods.
**Preliminary Data**
A total of 92 proteins and 13 steroid hormones were detected. Proteomic analysis comparing all samples from hospitals serving advantaged versus disadvantaged neighborhoods found that those from disadvantaged neighborhoods had higher levels of twenty different cytokines, interleukins, immune response, and cancer-associated proteins, including PD-L1 and VEGFA ($p<0.05$). Among BC cases only, this elevation in immune response and cancer-associated proteins was also observed in samples from hospitals serving disadvantaged neighborhoods compared to those serving advantaged neighborhoods ($p<0.05$). No significant differences in steroid hormones were found in these comparisons, but a comparison of cases compared to controls revealed an elevation of the cortisol/cortisone ratio, 11-deoxycortisol, and sum_21-deoxycortisol_corticosterone in cases compared with controls ($p<0.05$). The elevated cortisol/cortisone ratio in cases versus controls was driven by an increase in cortisol, suggesting elevated chronic stress.
Correlation analysis of detected proteins with the cortisol/cortisone ratio was performed in all samples. A weak positive correlation was found between the cortisol/cortisone ratio and PD-L1 ($p<0.05$, Pearson correlation coefficient=0.18), a protein involved in cancer immune evasion known to be upregulated by cortisol. A similar weak positive correlation was found between the cortisol/cortisone ratio and VEGFA ($p<0.05$, Pearson correlation coefficient=0.18). VEGFA is a known marker of cancer aggressiveness that is upregulated in pro-inflammatory conditions.
These findings suggest that, at baseline, individuals living in disadvantaged neighborhoods have a dysregulated immune and inflammatory response. A BC diagnosis appears to raise cortisol levels, potentially worsening this dysregulation and contributing to the poorer outcomes seen in these patients. This may occur through cortisol's influence on immune response and cancer aggressiveness proteins such as PD-L1 and VEGFA. Future directions include in vitro experimentation to validate the regulation of PD-L1 and VEGFA by cortisol in ER+ breast cancer cell lines.
**Novel Component**

Multi-omic approaches using pretreatment plasma samples links neighborhood disadvantage to elevated stress hormones and immune dysregulation in breast cancer patients.

**Author(s)** (Presenting author should be listed first)
Lanier, Deanna; Uchimiya, Mario; Rowe, Dawne; Tompkin, Stephen; DeWitt, Jamie; Woodlief, Tracey; Edison, Arthur
**Author Affiliations**
University of Georgia Institute of Bioinformatics; Complex Carbohydrate Research Center
**Title**
Untargeted metabolomics reveals PFOA-induced immune modulation in a SARS-CoV-2 infection model
**Introduction**
Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic chemicals known for their persistent, bioaccumulative, and toxic properties. These substances are linked to negative health outcomes like immunotoxicity and immune system suppression in rodents and humans. Perfluorooctanoic acid (PFOA), a well-studied PFAS found in human and wildlife serum, has been shown to suppress T-cell-dependent antibody response and reduce vaccine efficacy in humans. As such, environmental agents like PFOA may exacerbate the severity COVID-19. We conducted untargeted metabolomics studies on serum to elucidate how PFOA exposure affects COVID-19 infection severity. We hypothesized that PFOA exposure would alter the metabolome and impair the host immune response, leading to a more severe COVID-19 outcome.
**Methods**
In the pilot dosing phase, ferrets (n=12) were divided into three groups and administered PFOA at 0, 2, or 10 mg/kg/day. For the loading phase, ferrets (n=16) were divided into two groups and given 0 or 10 mg/kg/day in feed for seven days prior to SARS-CoV-2 infection. All animals were challenged with SARS-CoV-2, with blood samples collected every other day. Clinical observations, including weight, temperature, and viral load, were recorded bi-daily. Serum IgG was detected using ELISA, and lung tissue histopathology was collected. Untargeted NMR metabolomics data were collected to identify metabolites driving differential immune responses. Statistical analyses included O-PLS-DA, t-tests, and mixed-effects models. Metabolites were identified using the 2D spectral data and database-matching software like COLMARm.
**Preliminary Data**
NMR metabolomics and statistical analysis identified eight metabolites â€” succinic acid, citric acid, hydroxybutyrate, proline, choline, glucuronate, glycine, and threonine â€” differentiating PFOA and control ferrets before the infection challenge. O-PLS-DA and T-tests significant differences in these metabolites after one week of daily dosing. Mixed-effects models were used to evaluate changes over time, considering repeated measures as random effects and time and dosing as fixed effects. Two metabolitesâ€”choline and lactic acidâ€” were associated with time, peaking one day after SARS-CoV-2 inoculation, irrespective of PFOA dosage. Four metabolitesâ€”threonine, proline, glycine, and glucuronateâ€” significantly differentiated control versus PFOA-exposed ferrets over time post- SARS-CoV-2 challenge, indicating an altered metabolome due to the exposure. Three metabolitesâ€”histidine, citric acid, and leucineâ€” showed significant interaction effects between time and dosing, indicating their role in the differential immune response. Viral quantification showed PFOA-exposed ferrets reached peak infectivity on day three, while control ferrets peaked on day five with a lower viral load. Four metabolites correlated with these data. Serology revealed higher serum IgG absorbance in control ferrets at day four post-challenge compared to PFOA-exposed ferrets, and histology indicated viral infection in the lung tissue of both groups. This study demonstrates the use of ferrets as a model to study PFOA-related immune response changes, supporting our hypothesis that PFOA exacerbates COVID-19 severity, with changes detectable via metabolomics.
**Novel Component**
Utilizing ferrets to model PFOA-induced immune changes with the use of untargeted NMR metabolomics
**Methods** evaluating these effects.

**Author(s)** (Presenting author should be listed first)
Ana Ruxandra Stanciu(1); Marc Jeschke(2); Diana Tedesco(2); Fadi Khalaf(2);Philip Britz-McKibbin(1)
**Author Affiliations**
1. Department of Chemistry and Chemical Biology, McMaster University, Hamilton, Ontario; 2. David Braley Research Institute, Hamilton Health Sciences, Hamilton, Ontario.

**Title**
Serum Metabolomics for Capturing Dynamic Metabolic Responses to Burn Injuries and Risk Assessment for Sepsis in Critical Care
**Introduction**
Burn injuries are under-appreciated injuries that result in physical trauma and extreme metabolic changes that may be life-threatening. Burns can vary in severity and body surface area that can result in severe infection and a dysregulated immune response with a high risk for sepsis and mortality for susceptible individuals. The challenge in a critical care setting is knowing when clinicians should intervene with more aggressive treatment to prevent the onset of an aberrant hypermetabolic/hyperinflammatory state. By performing metabolomic analysis on repeat serum samples from burn patients, we aim to identify circulating biomarkers for early detection of sepsis during hospitalization, to understand the pathophysiology of metabolic distress, and to explore therapeutic interventions to improve survivorship of vulnerable patients, such as metformin.
**Methods**
In this pilot study, untargeted metabolite profiling was performed on 144 repeat serum samples acquired from 57 patients, admitted to the critical care unit for burn injuries due to various causes (e.g. flame, chemical, etc.). Patients were classified into four sub-groups; burn patients who developed sepsis (n=14), burn patients without sepsis (n=20), burn patients treated with metformin (n=12), and those who have passed away (n=11). Repeat serum samples were collected over four time intervals during hospitalization for up to 30 days since admission. Capillary electrophoresis-mass spectrometry was used to characterize dynamic changes in the serum metabolome of burn patient sub-groups relative to non-burn controls (n=10). Lipidomic analysis from methyl-tert-butyl ether serum extracts will be conducted using reversed-phase liquid chromatography-mass spectrometry.
**Preliminary Data**
A rigorous data workflow was performed to authenticate reliably measured polar/ionic metabolites from serum filtrate samples when using capillary electrophoresis-mass spectrometry under positive and negative ion mode, which was performed in both a high throughput and high sensitivity configuration. Metabolites were selected for subsequent data analysis if they were measured with adequate precision (CV of QC < 30%) with adequate frequency in most patient samples (> 70%). We first quantified serum creatinine and lactic acid concentrations as common clinical biomarkers in critical care for sepsis risk assessment, which were not significantly associated with sepsis or mortality of burn patients. Complementary multivariate and univariate statistical **Methods** were then used to identify serum metabolite trajectories that differentiated patient sub-groups relative to non-burn controls at early stages of hospitalization. Preliminary analysis indicated that patients who developed sepsis had increasingly higher levels for two short-chain acylcarnitines over time, including O-propionylcarnitine and O-butyrylcarnitine after adjustment for age and sex ($p < 0.05$) in contrast to non-sepsis burn patients and non-burn controls. Additionally, in the sepsis sub-group, plasma isoleucine increased significantly only at early period of hospitalization unlike controls, which may reflect greater risk for insulin resistance reflecting metabolic distress in vulnerable burn patients. Work is currently underway to complete data processing of distinct temporal changes in the plasma metabolome of burn patients using a multi-platform analytical approach.
**Novel Component**
Metabolic responses to burn injuries in critical care; early detection of sepsis; treatment responses to metformin

**Author(s)** (Presenting author should be listed first)
Zhao, Haoqi; Kvitne, Kine E.; Mannochio-Russo, Helena; Lamichhane, Santosh; Lamoureux, Vincent; Dorrestein, Pieter C.
**Author Affiliations**
Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego
**Title**
Medication Exposure Read-out in Untargeted Metabolomics Using a MS/MS Library of Drugs and Metabolites Propagated from Repository-scale Analog Search
**Introduction**
Drugs represent one of the most important human chemical exposures. To study the relationships between drug exposure and health outcomes, clinical research relies on medical records or self-reported medication usage. However, such information is costly to acquire and often inaccurate, and does not reflect drug exposure

from non-medication routes. Untargeted metabolomics offers the opportunity to provide objective drug read-out, but it suffers from the limited number of reference spectra of drugs and even lower coverage for drug metabolites. Moreover, interpretation of the annotations requires extensive literature searches to understand the therapeutic indications of the drugs. There is currently no resource to provide objective, systematic, and efficient read-out of drugs in untargeted metabolomics.

**Methods**

We developed a MS/MS spectral library for drugs, metabolites, and analogs connected with drug metadata. From GNPS Public Spectral Library, we collected reference spectra of drugs and metabolites through partial name search against four drug knowledge bases. We then created a propagated â€œanalog libraryâ€ by searching against 1.2 billion MS/MS spectra in public metabolomics datasets to retain those with high similarity to drug reference spectra. An ontology-based metadata table was created and hand-curated to connect drug detections to the therapeutic indications. We tested the performance of the library on 322 fecal samples from people with the human immunodeficiency virus (HIV). We disentangled the effects of disease status and drug usage on an important family of circulating lipid signaling molecules, the N-acyl lipids.

**Preliminary Data**

As of July 2024, the GNPS Drug Library contains 99,122 reference spectra of drugs (4,723 unique drug structures), 4,087 reference spectra of drug metabolites (belonging to 470 drugs), 3,232 unique reference spectra of drug analogs (belonging to 577 drugs) derived from mining public metabolomics data, and hand-curated drug metadata based on an ontology of the pharmacologic class, therapeutic area, therapeutic indication, and mechanism of action. In fecal samples from the HIV cohort, we observed 169 drugs, with HIV medications, cardiovascular disease medications, and antidepressants being the most frequently detected categories. People with HIV have a higher chance to develop depression and dementia at higher ages, which agrees well with the observation of depression and cardiovascular drugs in this cohort (age of this cohort: 55 Â± 12 years). Interestingly, 33% of the drugs were annotated together with their metabolites or analogs, and the occurrences of the drug metabolites and analogs highly agree with those of the corresponding drugs. To demonstrate the metabolic sources of the drug analogs, we cultured 10 drugs frequently observed in this cohort with a synthetic microbial community mimicking human gut microbiota, and we confirmed that analogs of five drugs could be sourced from microbial metabolism. We observed that some N-acyl lipids, an important family of circulating lipid signaling molecules, showed significant changes with the HIV status of the people. Using the GNPS Drug Library, we further revealed that these changes could be caused by the HIV drug exposure. The changes of N-acyl lipids were not observed using reported HIV drug consumption information (which is developed by expert clinicians but still contains a high degree of inconsistent information), highlighting the importance of performing metabolomics-based drug read-out in clinical studies. Parallel metagenomics analysis suggested gut microbiome changes as a possible mechanism for the different N-acyl lipids.

**Novel Component**

A resource for systematic and efficient drug read-out was developed for the first time in untargeted metabolomics.

**Author(s)** (Presenting author should be listed first)

Kass, Ignatius J 1;Munjoma, Nyasha C.2; Kirk Jayne 2; Gethings, Lee A 2; Tiberi, Paolo 3; Goracci, Laura 4; Lock, Richard 2

**Author Affiliations**

1. Waters Corporation, Milford, MA 2Waters Corporation, Wilmslow, United Kingdom; 3Mass Analytica Ltd, Sant Cugat del Vallés, Spain; 4University of Perugia, Perugia, Italy

**Title**

Comprehensive discovery lipidomic workflow which utilizes a novel, multi-reflecting ToF with integrated informatics, providing highly confident lipid characterization and quantification

**Introduction**

Lipidomics allows researchers to probe changes in the lipidome as a result of disease, treatment, lifestyle, etc. Analysis of these lipids in a discovery mode is normally performed by a combination of liquid chromatography (LC) and accurate mass spectrometry (MS). Despite developments in analytical technology the detection and identification of lipids remains a significant challenge. Here we show the key benefits of a novel, benchtop MS and the features it provides to help overcome some of the drawbacks outlined for lipid analysis. Combining this novel MS data with third-party informatic solutions, demonstrates a powerful lipidomic workflow. The benefits of

this approach are demonstrated using plasma samples from colon and rectum cancer and healthy control plasma

**Methods**

Lipid standard mixes containing a range of lipid classes and the NIST SRM 1950 plasma were used to assess instrument performance. As a proof-of-concept (POC), colon and rectum cancer and healthy control plasma were used to demonstrate the workflow. Study samples were prepared using protein precipitation with IPA solvent containing premixed deuterated standards. The resulting supernatant was chromatographically separated using a high throughput lipid profiling method which consisted of a 12-minute gradient. The eluent from the chromatography system was coupled to a novel, benchtop multi-reflecting ToF mass spectrometer (Xevo MRT). The subsequent data were then processed using Lipostar software to provide lipid identifications and relative quantification. Statistical analysis involving multivariate analysis (MVA) was used to establish group differences

**Preliminary Data**

Lipid extracts originating from the standard mixes was first assessed to establish instrument performance. Data were acquired using either data dependent (DDA) or data independent analysis (DIA). Resolution and mass accuracy were initially evaluated, achieving 100,000 (FWHM) and 200 ppb respectively for each lipid component. Based on previous literature, the most commonly identified lipids from human plasma (based on the NIST standard) highlighted the reduction in false positive identifications following database searching due to the high mass accuracy provided. A dilution series of lipid standards (0 to 1000ng/mL) spiked into a matrix of human plasma also benchmarked the level of sensitivity achieved from the MS platform. Data showed that sensitivity levels equivalent to tandem quadrupole measurements were readily achieved, whilst an in-solution dynamic range of > 4 orders was also demonstrated. Data acquisition rates up to 50-100Hz were utilized for the MS methods, providing the ability to run with faster gradient profiles. Cancer-based study samples were prepared using the same protocol described for the lipid standards. Data processing via Lipostar software was used for peak picking, data normalisation and lipid identification. Statistical analysis involving a range of MVA tools showed a clear differentiation between the cancer types and healthy controls. Identification of the differential markers responsible for the group separation, was conducted using a database comprised of LIPIDMAPS and in-house libraries. The primary lipid classes identified included ceramide (Cer), phosphocholine (PC) and sphingomyelin (SM). Integrated pathway analysis revealed that these lipid species were implicated in the signalling and cell proliferation pathways.

**Novel Component**

Enhanced lipidomic workflow using a novel, benchtop multi-reflectron ToF for comprehensive detection and identification of key biological lipids

## Full Abstracts for the Lipids & Lipidomics Session (2)

**Author(s)** (Presenting author should be listed first)
Susan S Bird-1; Rahul Ravi Deshpande-1; Kyle D.G. Saunders-2; Johanna Gerichten-2; Bashar Amer-1; Melanie Bailey-2;
**Author Affiliations**
1-Thermo Fisher Scientific, San Jose, CA; 2-Department of Chemistry, University of Surrey, Guildford, United Kingdom;
**Title**
Deciphering Spatial Variation in Lipid Profiles of X-Ray Irradiated Cells using nLC and Orbitrap 240 MS
**Introduction**
Lipidomics is of significant interest in cell biology because cells contain lipid droplets, which play an important role in managing cell stress in response to infection, irradiation and drug treatment. Herein, we describe an optimized workflow for single cell lipidomics utilizing HRAM and ddMS2 fragmentation of lipids from a single cell in both positive and negative mode on the Orbitrap Exploris 240 using nano-LC based seperation.
It is known that cells irradiated by photons can instigate bystander effects in neighboring cells leading to DNA damage, apoptosis and even secondary cancers. After live spatial sampling of irradiated cells and their unirradiated neighbors, we apply this workflow to characterize the lipidomic impact of X-ray radiation and its bystander effect.
**Methods**
Adherent PANC-1 cells were irradiated with X-Ray (6 Gy) prior to sampling using an irradiation cabinet (Xstrahl), select areas of the dish were shielded with lead. Following radiation, live cell imaging and spatial sampling of single cells was performed on a Single Cellomeâ„¢ SS2000. Lipid droplets and nuclei were visualized with BODIPY 493/503 and Hoerst respectively (Invitrogen). For nanoLC-MS, lipids were separated on a Thermo Scientific PepMapâ„¢ - column connected to a Thermo Scientific Vanquishâ„¢ Neo UHPLC system. Data were acquired on a Thermo Scientific Orbitrap Explorisâ„¢ 240 Mass Spectrometer, both in positive as well as negative mode ionization using polarity switching. Thermo Scientific LipidSearchâ„¢ 5.0 and Compound Discovererâ„¢ 3.3 software was used for lipid annotation and data processing.
**Preliminary Data**
Lipid standards and bovine liver lipid extracts were analyzed using nano-LC-MS. The flow rates and run time for the nLC were optimized for greater separation and resolution of the eluted lipid species. Dilution series of cells were run to benchmark the reproducibility, robustness and the sensitivity of the workflow.
For sampling single cells, the Single Cellomeâ„¢ System SS2000 (Yogokawa) was used. The system utilizes high-resolution images captured with a confocal microscope to automatically and accurately collect single cells. For cells irradiated with X-Ray, the system was used to collect single cells spatially, based on their distance from irradiated areas. The cells collected were immediately transferred to dry ice, before transfer to LC-MS vials using the starting mobile phase spiked with internal standard (EquiSPLASHÂ©, 16 ng/mL, Avanti). Samples were then freeze-dried and shipped to San Jose for nLC-MS analysis. The SS2000 can be used to isolate single adherent cells under incubation, preserving metabolic activity and reducing the need to flow cells prior to sampling. In addition, the behavior of lipid droplets within the cell populations was also profiled as lipid droplets pertain to treatment resistance and cellular stress.
Single cells lipid extracts were run using the optimized nLC-MS workflow run with polarity switching to yield comprehensive lipid species coverage. The data obtained was analyzed using LipidSearch and Compound Discoverer. More than 500 lipid species were annotated using MS/MS fragmentation data. Differences in cellular lipid profiles were observed based on the spatial location of the cells. Major differences were seen between cells on the periphery and cells in the middle of the plate.
**Novel Component**
nLC-MS based method for lipidomics was used for spatially resolved single cell application.

**Author(s)** (Presenting author should be listed first)
Xiaorong Fu, Adrianna Maurer, Stanislaw Deja, Justin A. Fletcher, Sean Slater, Jeffrey G. McDonald, Jay D. Horton, John P. Thyfault, Matthew A. Mitsche and Shawn C. Burgess
**Author Affiliations**
Center for Human Nutrition, The University of Texas Southwestern Medical Center, Dallas, TX 75390
**Title**

Measurement of cholesterol and bile acids synthesis with deuterated water and mass spectrometry

**Introduction**

High cholesterol causes atherosclerosis and has emerging roles in other diseases. The majority of cholesterol is eliminated from the body by its conversion to bile acids (BAs), molecules that aid dietary lipid absorption and activate receptors that modify metabolism. Here, we evaluated high-resolution orbitrap mass spectrometry method for measuring cholesterol synthesis by measuring the incorporation of a 2H tracer using two different cholesterol fragments on GCMS and LCMS platforms. The method resolved 2H enrichment from natural abundance 13C and permitted quantification of cholesterol synthesis in cultured cells and mice. BA synthesis was determined as 2H-BAs in mouse liver using conventional LC-MS/MS and was validated by its response to a BA sequestrant.

**Methods**

High resolution Orbitrap gas chromatography mass spectrometry (HR-Orbitrap-GCMS) and high-resolution Orbitrap liquid chromatography mass spectrometry (HR-Orbitrap-LCMS) were used for the quantitative assessment of the synthesis of cholesterol by analyzing the 2H incorporation into cholesterol after administration of 2H2O tracer. HR-Orbitrap-GCMS operated in electron ionization at 240,000 resolution was applied to measure the isotopic enrichment of cholesterol acetyl ester fragment ion 247 (m/z C18H31). Also, HR-Orbitrap-LCMS at 500,000 resolution was used to measure the cholesterol labeling by analyzing the pseudo-molecular ion 369 (m/z C27H45).

Synthesis of taurine-conjugated BAs, specifically tauro-Î±-muricholic acid (TÎ±MCA), tauro-Î²-MCA (TÎ²MCA), taurocholic acid (TCA), tauro-chenodeoxycholic acid (TCDCA) and tauro-deoxycholic acid (TDCA) were determined as 2H-BAs in mouse liver using conventional LC-MS/MS.

**Preliminary Data**

We aimed to provide a mass spectrometry-based method of how 2H2O tracer allowed the synthesis of BAs and cholesterol to be measured in the same experiment, which provides a view into how the metabolism of these related sterols is coordinated. Here, we evaluated high-resolution orbitrap mass spectrometry method for measuring cholesterol synthesis by measuring the incorporation of a 2H tracer using two different cholesterol fragments on GCMS and LCMS platforms. The method resolved 2H enrichment from natural abundance 13C and permitted quantification of cholesterol synthesis in cultured cells and mice. Regardless of the platform, the capability to resolve 2H and 13C allowed cholesterol synthesis to be measured using 2H2O doses 20 times lower than typically used in mouse experiments. This sensitivity advantage may be leveraged in tissues with inherently low cholesterol synthesis rates or to measure 2H incorporation after a very short tracer exposure. In addition to improved sensitivity, the combined analysis of these fragments revealed the near complete labeling of sites originating from NADPH/H+, but incomplete and variable labeling of sites originating from acetyl-CoA. Taurine-conjugated BAs, specifically TÎ±MCA, TÎ²MCA, TCA, TCDCA and TDCA constitute the largest proportion in the liver. Their synthesis were determined as 2H-BAs in mouse liver using conventional LC-MS/MS and was validated by its response to a BA sequestrant. After one month of BA sequestrants treatment, mice were given a 20 ÂµL/g IP injection of 99% 2H2O/saline. BA synthesis strongly correlated with cholesterol synthesis, despite modest effects on the concentrations of both. Notably, cholesterol synthesis and its disposal into BAs could be measured simultaneously, revealing a strong correlation between these fluxes that reflect the coordination more deeply than measuring their concentrations alone. These new tools will provide deeper insight into in vivo cholesterol and BA metabolism.

**Novel Component**

We demonstrate a novel 2H tracer approach to detect cholesterol and bile acids synthesis simultaneously.

**Author(s)** (Presenting author should be listed first)

Troy R Scoggins IV, Boone M. Prentice

**Author Affiliations**

Department of Chemistry, University of Florida, Gainesville, FL, 32603; USA.

**Title**

Ozonolysis kinetics for the study of cis/trans isomerism

**Introduction**

Cis/trans isomerization significantly impacts the functions of many biomolecules and has implications in health and disease. Traditional methods for distinguishing these isomers require chromatographic techniques, which may not be successful with all analytes. This study presents an innovative approach leveraging ozonolysis kinetics combined with mass spectrometric analysis to differentiate and quantify cis and trans lipid isomers. By

enhancing the ozonolysis reaction efficiency through the use of metal-cationized ion types, we aim to provide a method for lipid geometrical isomer analysis. Our preliminary findings suggest that certain alkali metal ions, particularly lithium, significantly increase the reaction rate, thereby facilitating more precise kinetic studies and improving sample.

**Methods**

Ozonolysis reactions were conducted on lyso PC 18:1 and dioleoylphosphatidylcholine (DOPC) lipid standards using a Bruker HCT 3D ion trap mass spectrometer (Bruker Daltonics, Billerica, MA), modified for ozone **Introduction**. Samples were introduced via electrospray ionization (ESI) and subsequently subjected to ozonolysis within the instrument. Different ion types, including alkali metal ions (lithium, sodium, potassium), were studied for their impact on reaction efficiencies. The reaction progress was monitored at various time points to establish kinetic rates. Comparative studies were performed on cis and trans DOPC to assess differences in reaction rates. The resulting reaction rates were analyzed and utilized for the relative quantification of a DOPC cis/trans mixture, validating the method's effectiveness.

**Preliminary Data**

A Bruker HCT 3D ion trap has been modified to allow for the introduction of ozone. Briefly, ozone generated using an Atlas 30 ozone generator is admitted into the vacuum manifold of the mass spectrometer via a metering valve connected to the trap gas supply. Our Preliminary Data demonstrate significant differences in ozonolysis reaction efficiency with varying ion types. Ozonolysis reaction time points were acquired from 0.5 to 5 seconds, at 0.5 second intervals. The natural log of normalized parent lipid ion decomposition is then plotted versus reaction time to generate a kinetic plot. The slope of this plot gives the kinetic rate of parent ion decomposition in the presence of ozone. Lithiated lipids exhibited reaction rates approximately 1.8 times faster than sodiated lipids, 2.3 times faster than potassiated lipids, and 3.3 times faster than protonated lipids. The kinetic studies also revealed distinct reaction rates for cis and trans isomers of DOPC. For example, analysis of sodiated DOPC lipids revealed that trans isomers reacted approximately twice as fast as cis isomers, allowing for clear differentiation between the isomers. Lithiated DOPC samples exhibited an even greater disparity, with trans isomers reacting approximately three times faster than cis isomers. These differences in reaction kinetics underscore the crucial role of ion type in enhancing ozonolysis efficiency. To validate our approach, we applied the kinetic data to quantify a 50:50 mixture of cis and trans DOPC isomers. The observed reaction rates accurately predicted the isomer ratio, demonstrating the method's precision in determining isomeric composition. This accurate prediction confirms the potential of ozonolysis kinetics for reliable lipid isomer analysis without the need for chromatographic separation. This methodology can potentially be used to increase throughput in lipidomic studies. This chemistry can also be used to study small molecule and pharmaceutical isomerism.

**Novel Component**

Utilizing ozonolysis kinetics to differentiate cis and trans lipid isomers in the gas phase.

**Authors** (Presenting author should be listed first)
Randolph C 1 , Muhoberac M 1 , Beveridge C 1 , Manchanda P 1 , Iyer S 1 , Poad B 3 , Blanksby S 3 , Tichy S 4, Chopra G 1,2

**Author Affiliations**

1 Department of Chemistry, Purdue University, West Lafayette Indiana, United States of America
2 Department of Computer Science, Purdue University, West Lafayette Indiana, United States of America
3 Queensland University of Technology, Brisbane Queensland, Australia
4 Agilent Technologies, Santa Clara California, United States of America

**Title**

Artificial Intelligent Agents for Automating Deep Lipidomics Workflows to Investigate Alzheimer's Disease and Aging-Related Lipid Droplets

**Introduction**

Lipid droplets (LDs), or cellular "fat," have been recently demonstrated by our research to hold significance in Alzheimer's disease (AD). However, detailed composition of LDs remains unknown to date. Conducting large-scale lipidomics experiments is time-intensive and yields extensive data, complicating annotation and interpretation. Presently, there are no **Methods** to automate workflows, including experiment method generation and bioinformatic analysis. To address this, we've developed artificial intelligent (AI) agents. These agents enable users to interact in a chatbot-style manner, facilitating detailed lipid annotation and identification via multiple reaction monitoring (MRM)-profiling and liquid chromatography ozone electrospray ionization MRM

(LC-OzESI-MRM) experiments. The AI-agents decipher the intricate tissue- and region-specific lipidomic profiles of LDs from age-matched AD and aged mouse brains.

**Methods**

LDs were extracted from diverse brain regions of 13-17 month-old AD (5xFAD) and age-matched WT mice, spanning cortex, hippocampus, diencephalon, and cerebellum. Tailored AI agents were developed, utilizing large language models (LLMs), to comprehend user inputs for experiment planning, execution, and analysis. Guided by an AI manager, these agents employed instrument-specific tools, gleaned insights from scientific literature, and consulted instrument documentation for troubleshooting, thereby streamlining lipidomics profiling and data analysis, interpretation and visualization. Optimized worklists compatible with Agilent MassHunter were generated, facilitating MRM-profiling and LC-OzESI-MRM deep lipidomics profiling with “ozone on” and “ozone off” experiments. The AI agents meticulously processed and annotated lipids, identifying acyl chain composition and unsaturation sites, and conducted comparative bioinformatics/pathway analyses to elucidate lipidomic patterns.

**Preliminary Data**

Users interact with AI-agents in a chatbot-manner to do several tasks: (a) generate worklists for the Agilent MassHunter software, (b) identifies lipid species present in samples (e.g. brain and liver) specifying number of replicates for differential analysis including fatty acyl (FA) chains and double bond locations, (c) recommended initial LC parameters and cleaning frequency for the experiments, (d) assisted in generating detailed plots to elucidate the experimental results, and (e) provides insights from mass spectrometry lipidomic literature. The AI-agents were used to schedule experiments for MRM-profiling for LDs from different brain regions in 5xFAD and WT mice. AI agents annotated and identified 3000 lipids from 10 lipid classes in LDs in a region- and tissue-specific manner with triacylglycerols (TGs) and cholesterol esters but also identified a variety of previously unknown lipid species spanning acyl carnitine, sphingomyelin, phosphatidylethanolamine, and ceramide subclasses. Deep profiling of TGs with LC-OzESI-MRM resolved isomeric lipid structures with varying carbon-carbon double bond (C=C) position(s). Forty-seven unsaturated TGs were identified and quantified in liver tissue, with 26 and 22 TGs with specific C=C locations in LDs from AD and wild-type mice were compared. The agent also utilized OzESI tools to identify mono and polyunsaturated fatty acyl (PUFAs) isomer double bond isomers in LDs from four distinct brain regions of AD mice. Similar to monounsaturated fatty acyls, AI agents compiled a list of PUFAs double bond locations using “ozone off” and “ozone on” mode. Finally, the AI agent iteratively learnt from errors to write custom code for data analysis and visualization. AI-agents answered specific questions about the results and troubleshooted runtime errors, like LC leaks or poor MS signal, providing specific information and citations from the user manuals of the Agilent 6495C QQQ LC-MS, thereby streamlining rapid and detailed characterization of LDs to elucidate its functional role in AD.

Novel Aspect

AI agents offer expert guidance to plan, execute, analyze and troubleshoot MRM- and LC-OzESI-MRM experiments to profile LDs in AD.

**Author(s)** (Presenting author should be listed first)

Alcazar, Armando; McAfee, Alison; Hoover, Shelley E.; Foster, Leonard J.

**Author Affiliations**

1. Michael Smith Laboratories, Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada 2. Life Sciences Institute, Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada 3. Department of Applied Ecology, North Carolina State University, North Carolina, NC, USA 4. Department of Biological Sciences, University of Lethbridge, Lethbridge, AB, Canada

**Title**

Bridging Gaps in Honey Bee Pheromone Analysis Using State-of-the-Art Lipidomics Analysis

**Introduction**

Queen honey bee (Apis mellifera) pheromones, crucial for colony dynamics, include polar lipids such as hydroxylated short chain fatty acids and fatty acid esters. Traditionally analyzed via gas chromatography-mass spectrometry (GC-MS), this field has seen no new studies in over two decades. However, recent advancements in lipidomic methodologies, characterized by integrating state-of-the-art high-resolution UHPLC columns and enhanced sensitivity of high-resolution mass spectrometers, present a better alternative to conventional methods.

**Methods**

In both targeted and untargeted approaches, queen bee extracts from mated and unmated bees were analyzed using liquid chromatography-high-resolution mass spectrometry (adapted from Cajka et al., 2017). Samples were supplemented with labeled internal standards before extraction. The dried pellets were resuspended in 70:30 acetonitrile before LCâ€“HRMS/MS analysis. A multistep elution gradient was optimized to resolve isomeric pheromones (hydroxylated short-chain fatty acids) and prevent the coelution and peak suppression of key pheromones eluting in the void volume in traditional lipidomics methods. The mass spectrometer (Bruker Impact-II) operated in high-resolution data-dependent acquisition mode. The mobile phase consisted of A: water with 10 mM ammonium formate and 0.1% formic acid, and B: 10:90 acetonitrile with 10 mM ammonium formate and 0.1% formic acid.

**Preliminary Data**

We established a lipidomics method using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to compare the pheromonal and lipidomic profiles of mated and unmated queen bees. This method allowed us to measure the honey bee queen retinue pheromone (QRP) in connection to age, egg-laying status, and queen acceptance, while also conducting concurrent metabolomic and lipidomic analyses.

Through this method, we identified new isomers of known mandibular pheromone components as well as various important hydroxylated short-chain fatty acids and fatty acid esters. We were able to annotate over 250 lipid species in the pheromone/lipidomic extract, revealing significant differences between mated and unmated queens. In addition, we found disparities in prostaglandins and thromboxanes between mated and unmated queens, indicating their potential role in egg development. We noticed that older queens produced higher levels of QRP components such as 9(R)-HDA, LEA, and HVA compared to younger queens, with HVA being correlated with ovary mass.

Metabolomic and lipidomic analyses revealed that samples grouped by queen age and mating status, rather than ovary mass, suggesting other physiological changes in the queen's early life. This study provides valuable insights into the biochemical landscape of queen bees and the signals influencing their acceptance and reproductive success. Furthermore, this approach emphasizes the importance of using LC-HRMS/MS alongside gas chromatography-mass spectrometry (GC-MS) for studying bee pheromones and lipidomic pathways.

Finally, the spectral data and experimental design are openly accessible for further analysis or improvements in annotation as new pathways are uncovered and new standards become accessible. This comprehensive spectral data will serve as a legacy dataset for the ongoing exploration of the queen honey bee (Apis mellifera) pheromones and colony dynamics (URLs: www.metabolomicsworkbench.org; http://dx.doi.org/10.21228/M81B11).

**Novel Component**

Exploring Bee Pheromones and Colony Dynamics Using State-of-the-Art Lipidomics Analysis

**Author(s)** (Presenting author should be listed first)
Jace W. Jones and Jeffrey G. McDonald
**Author Affiliations**
University of Maryland School of Pharmacy and UT Southwestern Medical Center

**Title**

ILS Americas and an Interactive Checklist for Lipidomic Researchers. Partnering with MANA to Improve the Quality of Lipidomic Measurements.

**Introduction**

The International Lipidomics Society (ILS) is a scientific non-profit organization representing and promoting lipidomics through worldwide cooperation. ILS aims to foster the development of new technologies, techniques, resources, skills and training through international collaborations. ILS strives to foster global collaboration and transparent communication in lipidomics projects by gathering leading international laboratories from a wide range of disciplines. ILS organizes annual international meetings, workshops and training courses to foster the translation of lipidomics data to biological meaning. ILS North America (NA) is an education-focused branch of ILS.

**Methods**

The rapid increase in lipidomic data generation has led to a collaborative effort within the community to establish standards and criteria for producing, documenting, and disseminating lipidomic data. Creating a dynamic checklist that condenses key information about lipidomic experiments into common terminology will

enhance the field's consistency, comparability, and repeatability. We will describe the structure and rationale of established Lipidomics Minimal Reporting Checklist to increase transparency in lipidomics research.

**Preliminary Data**

The Lipidomics Minimal Reporting Checklist has been established and is continuously curated by the Lipidomics Standard Initiative (LSI) an interest group affiliated with ILS. The checklist is based on consensus-driven guidelines for lipidomics implemented in a publicly available web-based questionnaire. Its main purpose is to describe all essential steps of lipidomic experiments in a standardized way. The checklist output, a PDF document, is intended to assist editors and referees in reviewing research studies containing lipidomic data. The checklist can be viewed as guideline on â€˜good lipidomics practice' for both new and experienced lipidomic investigators. The checklist covers preanalytics, lipid extraction, analytical platform, lipid identification and quantitation, quality control, method validation, and reporting summary. In addition to reviewing the ILS checklist, we will also provide examples of lipidomic reporting in literature highlighting the need for increased consensus and oversight for lipidomic research.

**Novel Component**

ILS NA seeks to promote lipidomic research based on best practices for the benefit of trainees, investigators, reviewers and editors.

## Full Abstracts for the Metabolite Identification Session (10)

**Author(s)** (Presenting author should be listed first)
Da-Wei Li, Rodrigo Cabrera, Munki Choo, Lei Bruschweiler-Li, Alexandar L. Hansen, and Rafael Brüschweiler
**Author Affiliations**
The Ohio State University, Columbus, Ohio 43210, USA
**Title**
COLMAR1d: A web server for automated, quantitative 1D NMR-based metabolomics at arbitrary magnetic fields
**Introduction**
The field of metabolomics, which is quintessential in today's omics research, involves the large-scale detection, identification, and quantification of small-molecule metabolites in a wide range of biological samples. Nuclear magnetic resonance spectroscopy (NMR) has emerged as a powerful tool for metabolomics due to its high resolution, reproducibility, and exceptional quantitative nature. One of the key bottlenecks of metabolomics studies, however, remains the accurate and automated analysis of the resulting NMR spectra with good accuracy and minimal human intervention measured at arbitrary magnetic B0-field strengths.
**Methods**
Here, we present the COLMAR1d platform, consisting of a user friendly, interactive web server and an optimized database, for automatic 1D NMR-based metabolomics analysis to address these challenges. The COLMAR1d database comprises over 480 metabolites from GISSMO enabling database query of spectra measured at arbitrary magnetic field strength. To deal with 1D spectra with significant peak overlapping, the COLMAR1d query algorithm runs an exhaustive search and ranks all possible assignments to find the most accurate spectral interpretation considering chemical shifts, J-coupling multiplet patterns, and peak height variations, as well as the possibility that some peaks might fall below the detection limit or are obscured by larger peaks from other compounds.
**Preliminary Data**
COLMAR1d is demonstrated for different metabolomics samples, including mouse serum, DMEM cell growth medium, and wine measured on NMR spectrometers ranging from 80 MHz benchtop to 1.2 GHz ultrahigh field NMRs showing excellent capabilities for quantification.
**Novel Component**
COLMAR1d combines the GISSMO database with tools for automated processing, deconvolution, database query, and quantification for improved accuracy and efficiency.

**Author(s)** (Presenting author should be listed first)
Yedla, Sunandini; Bills, Brandon; Deshpande, R. Rahul; Amer, Bashar; Bird, S. Susan; Zabrouskov, Vlad
**Author Affiliations**
Thermo Fisher Scientific
**Title**
Development of a Simultaneous Quantitation and Untargeted Discovery (SQUAD) workflow for coeluting sugar-phosphates using orthogonal fragmentation techniques
**Introduction**
Sugar-phosphates are key primary metabolites in carbohydrate metabolism. Identifying these compounds is challenging because they are structurally similar and often not resolved chromatographically. Also, they commonly don't produce unique fragments with a single fragmentation type. Employing multiple fragmentation techniques to generate unique fragment ions can improve the chance of unambiguous identification when multiple isomers are present.
Here we deployed several fragmentation techniques, including Higher-energy Collision Dissociation (HCD), Collision Induced Dissociation (CID), and Ultraviolet Photodissociation (UVPD) on a Thermo Scientificâ„¢ Orbitrapâ„¢ IQ-Xâ„¢ Tribridâ„¢ MS to investigate the potential to quantify isomeric sugar-phosphates using unique MS/MS fragments.
**Methods**
Samples of six sugar-phosphate standards (Sigma-Aldrich, MO) were prepared at different concentrations using 60% acetonitrile.
HPLC solvents: 10mM ammonium acetate with 0.1% acetic acid in acetonitrile and water (Fisher Scientific, MA).

Analysis was carried out using a SeQuantÂ® ZICÂ®-pHILIC column connected to a Thermo Scientificâ„¢ Vanquishâ„¢ Horizon LC and Orbitrap IQ-X with HCD, CID, and UVPD fragmentation options to generate and quantify diagnostic fragments.
Individual standards for three coeluting sugar-phosphates and their adducts were evaluated using UVPD, HCD, and CID with targeted analysis performed using a dilution series (30ÂµM to 1.25mM).
Spectra were evaluated for unique fragments, quantified using Thermo Scientificâ„¢ FreeStyleâ„¢ v. 1.8 and annotated using Thermo Scientificâ„¢ Mass Frontierâ„¢ 8.0. Calibration curves were generated using Thermo Scientificâ„¢ TraceFinderâ„¢.

**Preliminary Data**

Fragmentation spectra of six isomeric sugar phosphates were evaluated for unique ions. The fragments were treated as uniquely diagnostic if they were only observed for that sugar phosphate. Several such unique diagnostic ions were observed for UVPD, CID, and HCD fragmentations, providing enough evidence for the differentiation of three coeluting sugar phosphates. The instrument response for the most intense fragment and the most intense unique fragment of galactose-1-phosphate was found to be linear down to 16.3ng and 65ng respectively. For glucose-1-phosphate the instrument response for most intense ion and most intense unique ion is found linear down to 16.3ng and 32.5 ng respectively. The water loss of fructose-6-phosphate parent showed an MS1 signal (m/z 243.0264) that was an order of magnitude higher than the other coeluting isomers at similar concentrations. In addition, it showed a UVPD fragment at 25X higher relative intensity than the other isomers, enabling quantification within the concentration range even in the presence of the other sugar-phosphates.
It is planned to evaluate additional sugar-phosphates including the impact of variable concentrations of multiple coeluting isomers on quantitative linear dynamic range of this approach. SQUAD analysis will then be used to quantify up to six isomeric sugar phosphates in plant extract.
For Research Use/Purposes Only â€“ Not for Diagnostic Procedures

**Novel Component**

Here we used HCD, CID, MSn, and UVPD MS/MS experiments to differentiate and quantify co-eluting sugar phosphate isomers.

**Author(s)** (Presenting author should be listed first)
Schwiebert, Elisabeth(1); Sah, Samyukta(1,2); Moore, Samuel(1,2); Gaul, David (1,2); Boylan, Kristin (3); Skubitz, Amy (3); Fernández, Facundo (1,2)

**Author Affiliations**

School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332 (1), Petit Institute of Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332 (2), Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN 55455 (3)

**Title**

Metabolomic Analysis of Papanicolaou Tests for the Discovery of Ovarian Cancer Biomarkers

**Introduction**

Ovarian cancer (OC) remains one of the most lethal cancers among women due to most cases going undiagnosed until later stages. Early detection and treatment of this malignancy provides the best prognosis, but the lack of an accurate and sensitive screening tool combined with ambiguous symptoms hinders these diagnoses. In contrast, screening for cervical cancer via Papanicolaou (Pap) tests is a widespread practice that greatly reduces the cancer's mortality rates. Interestingly, previous studies show evidence of OC cells in Pap tests, suggesting that proteins, and potentially lipids, shed from ovarian tumors end up in the cervix. The goal of this study is to evaluate the practicality of using Pap tests as biospecimens for OC screening-related metabolomics.

**Methods**

To evaluate the effectiveness of using residual Pap test samples as biospecimens for potential metabolomic work, 29 Pap test samples, collected from women over the age of 50 with normal cytology and no visible blood contamination, were first obtained from the University of Minnesota with IRB approval. These samples were centrifuged to recover the cell pellets from the supernatants. The cell pellets underwent a biphasic extraction followed by RP-LC-MS analysis, while the supernatants underwent two separate extractions and analyses, including RP-LC-MS and HILIC-LC-MS. Non-targeted features were detected in the range of 220-1000 m/z to determine the sensitivity and scope of the various extraction and analytical workflows, as well as evaluating residual Pap test samples as viable metabolomic biospecimens.

**Preliminary Data**
The biphasic extraction and subsequent RP-LC-MS analysis of the isolated cell pellets from all 29 samples yielded informative, exploratory data, highlighting the potential of using residual Pap test samples as biospecimens for metabolomics, specifically lipidomics, studies. Each sample was analyzed in both positive and negative ion mode, and in total, 11,051 features were detected with 7,318 in positive ion mode and 3,733 in negative ion mode. In contrast, the RP-LC-MS and HILIC-LC-MS analyses of the supernatants yielded significantly fewer features. The RP-LC-MS analysis yielded 56 and 46 features, while the HILIC-LC-MS analysis yielded 58 and 75 in positive and negative ion mode respectively. Since the cell pellets yielded more features, only the annotations of these features were pursued. Using both Thermo Scientific™ mzCloud library as well as an in-house mzVault library, 22.85% and 36.19% of features were annotated in positive and negative ion mode respectively. Among these detected features, 453 unique lipids, representative of 20 different lipid subclasses, were annotated in all 29 samples. Of the various lipid subclasses represented from the detected lipids, ceramides, triacylglycerols, hexosylceramides, and phosphatidylcholines contributed to over half (53.3%) of the detected lipids at 16.2%, 13.0%, 12.8%, and 11.3% respectively. The detection of these common lipids across all patients establishes a relative lipidome baseline for women over the age of 50 with normal cervical cytology. This exploratory study is the first investigation to utilize residual Pap test samples as biospecimens in a metabolomics/lipidomics workflow.
**Novel Component**
RP-LC-MS detection and annotation of 453 unique lipid species, classified within 20 lipid subclasses, from residual Pap test samples.

**Author(s)** (Presenting author should be listed first)
Clay, Allison; Goralski, Tyler; Fudge, Dylan; Jenkins, Conor; Lee, Priscilla; Dhummakupt, Elizabeth; Rizzo, Gabrielle
**Author Affiliations**
US Army DEVCOM Chemical Biological Center; Excet, A Precise Systems Company
**Title**
Assessing the utility of organ-on-a-chip technology for human relevant diagnostic biomarker identification
**Introduction**
While microphysiological system (MPS) technologies (also known as organ-on-a-chip) are gaining traction as a viable alternative model for toxicity studies, further characterization is necessary to explore the full translational potential of MPS to human physiology, as well as serving as a diagnostic. One question that remains is how well does bioinformatic data collected from organ chips match the bioinformatic data collected from human samples. Correlations between published human omics and MPS technology omics data will inform on the potential of organ chips to accurately represent human responses and provide an alternative approach for improved biomarker discovery for toxicity assessment and exposure identification. To interrogate these potential overlaps, multi-organ chips were exposed to acetaminophen and subjected to proteomic and metabolomic analysis.
**Methods**
TissUse Chip3 multi-organ chips (MOCs) were seeded with kidney organoids, liver organoids, and full thickness lung tissue. Each chip was exposed to acetaminophen (APAP) solutions at varying doses which included a low dose, therapeutic dose, and toxic dose of drug. After 24 hours, all chip effluent, tissues, and organoids were collected from the MOCs. All samples were then homogenized using a high-speed homogenizer and methanol. Protein pellets were removed for proteomic sample preparation. Methanol supernatant was dried down for metabolomic sample preparation. Proteomic samples underwent clarification, protein normalization, digestion, and peptide normalization before being analyzed on a Orbitrap mass spectrometer. Metabolomic samples underwent extraction and normalization before being analyzed on a Hybrid Quadrupole-Orbitrap mass spectrometer.
**Preliminary Data**
Thermo Fisher Scientific Proteome Discover and Compound Discoverer along with BioTransformer 3.0. MS1 features were used to preform untargeted proteomics and metabolomics data analysis. At 24 h post APAP exposure, 661 significantly dysregulated proteins were identified from lung tissues; 2052 from the kidney organoids; and 697 from liver organoids. Several of the dysregulated proteins identified by proteomic analysis correlated to published human multi-omics data that described proteins and biological processes shown to be

dysregulated after APAP administration. Also identified were hallmark protein biomarkers confirmed to be associated with APAP toxicity.
APAP was detected in all three metabolomic tissue samples, indicating that the drug distributed throughout the chip despite direct exposure occurring on the liver organoids alone. The presence of APAP in the tissues was observed in a dose dependent manner, where the highest concentration of APAP was detected in chips that received the toxic high dose, and the lowest concentration was observed in chips that received the low dose. Acetaminophen sulfate and acetaminophen glucuronide, two known primary APAP metabolites that are formed predominately by the human liver, were identified in all three tissues in a dose dependent manner. Both metabolites were detected in a previous study in human blood and urine via mass spectrometry analysis. NAPQI, the toxic intermediate that forms during APAP metabolism, was most abundant in liver tissues, followed by kidney. Cell death that occurs in the liver and kidney after toxic doses of APAP is attributed to oxidative stress that results from the formation of NAPQI.
The data from our organ chips largely match biomarkers and dysregulations identified in published human omics data, to include the identification of several known acetaminophen metabolites and biotransformation products. This suggests that organ chips may be a suitable surrogate for human biomarker identification and drug or agent exposure diagnosis.

**Novel Component**

Organ-on-a-chip technologies are a suitable surrogate for human biomarker identification and drug or agent exposure diagnosis.

**Author(s)** (Presenting author should be listed first)
Evans, Charles; Raskind, Abraham; Anderson, Brady
**Author Affiliations**
University of Michigan, Department of Internal Medicine

**Title**

How deep is the metabolome? Modified chromatography, multidimensional separations and derivatization enhance sensitivity and identification in untargeted metabolomics

**Introduction**

In recent years it has been convincingly demonstrated that most features in untargeted LC-MS metabolomics data do not represent unique chemical compounds but rather reflect contaminants, secondary adducts, in-source fragments and other degenerate signals. These observations have led to the conclusion that the observable metabolome may be smaller than previously estimated and even to speculation that the human metabolome may be close to fully characterized. However, these conclusions are based in part on limitations of typical methods and instrumentation. In this presentation, we describe and compare three experimental strategies to more deeply characterize the metabolome, all of which can be implemented using commercial instrumentation accessible in most metabolomics labs.

**Methods**

First, we describe multi-modal LC-MS/MS of human plasma and assess the potential of altered chromatographic gradients, increased column loading and altered MS/MS acquisition parameters to increase compound identification performance. Next, we report further improvements in sensitivity and compound identification that can be achieved using offline multidimensional chromatography, using RPLC x RPLC and RPLC x HILIC to characterize the fecal metabolome. Finally, we assess the potential of chemical derivatization reagents, including 3-nitrophenylhydrazine and dansyl chloride, to enhance analysis of compound classes that are difficult to detect or identify under non-derivatized conditions.

**Preliminary Data**

Simple modifications to chromatographic conditions including increasing column loading and gradient length, when combined with iterative MS/MS acquisition, allowed identification of over 2000 unique metabolites in human plasma based on defined spectral scores and retention time alignment windows. This represents an over 5-fold improvement over conventional chromatographic conditions. Next, we investigated the fecal metabolome using offline multidimensional liquid chromatography (LC x LC). Over 50 fractions were collected from a semi-preparative separation of a fecal metabolite extract were analyzed with an orthogonal LC-MS/MS method. The resulting spectra were searched against commercial, public, and local spectral libraries with validation using retention time prediction, resulting in identification of 3,414 unique compounds, an over 2.25-fold improvement over the compound identifications that could be achieved by a multi-modal combination of LC-MS/MS runs. LC x LC successfully identified several rare and novel compounds, including previously

unreported conjugated bile acid species. Many features identified by LC x LC were detectable but not identifiable in the original single-dimensional LC-MS data. Derivatization allowed identification of additional metabolite classes not typically detected in untargeted metabolomics including labile and volatile compounds in plasma and other matrices. We contrast metabolome coverage against derivatization-free methods and assess challenges of all methods, including false discovery and incomplete derivatization. We also describe strategies to automate these approaches and incorporate them into a practical workflow.

**Novel Component**

Enhanced chromatographic conditions, multidimensional separations and derivatization identify more compounds than conventional LC-MS and reveal new depths of the metabolome

**Author(s)** (Presenting author should be listed first)
Li, Shuzhao; Chi, Yuanye; Mitchell, Joshua; Thapa, Maheshwor; Zheng, Shujian; Smirnov, Aleksandr; Du, Xiuxia

**Author Affiliations**

The Jackson Laboratory for Genomic Medicine

**Title**

A consensus serum metabolome by large-scale data mining reveals major gaps in metabolomic measurements and modeling

**Introduction**

Blood analysis is the most common in biomedical applications and a reference metabolome will be critical for effective annotation and for guiding scientific investigations. However, compiling such a reference is hindered by many technical challenges, despite the availability of large amount of metabolomics data today. We have designed a series of data structures and tools, including metDataModel (PLoS Computational Biology 20 (6): e1011912), asari (Nature Communications 14, 4113) and khipu (Analytical Chemistry 95, 6212), which enabled a first draft of assembling a consensus serum metabolome from large-scale public data.

**Methods**

We have collected LC-MS metabolomics data from human serum or plasma samples from two major repositories, Metabolomics Workbench and MetaboLights. The collection included 77,097 acquisition files (140 million spectra) from 110 studies using the Orbitrap mass spectrometers. They led to 813 datasets as most studies employed more than one method, and we partitioned supersized studies to avoid technical complications. In addition, 26,253 acquisition files from the TOF mass spectrometers are analyzed to compare to the Orbitrap based results. These datasets are processed with the asari software, and consensus mass tracks are determined by the most frequently observed m/z values using kernel density estimation. Pre-annotation by khipu is performed on each dataset then combined cross studies.

**Preliminary Data**

The KDE approach led to 25,868 consensus mass tracks are identified in the positive ionization mode, and 23,316 in the negative mode. Compared to the 1,251 metabolites reported in the serum metabolome by Bar et al (Nature 2020), 95% of them are matched in our consensus mass tracks. After pre-annotation, they map to 42,499 neutral mass values, which are linked to annotation models utilizing consensus features per method, chemical descriptors and previous literature records. About 25% of this consensus serum metabolome is covered by HMDB v5, 50% by PubchemLite and 5% by the current human genome scale metabolic models, in a frequency dependent manner. The results indicate significant gaps in the current databases and metabolic models. The CSM can significantly improve cross-study comparison and feature annotation. Additional GC-MS and MS/MS data reintegrated into CSM from ADAP-KDB. We will report both the tool development and scientific findings, and the resource will be freely available via a web service.

**Novel Component**

Assembly of reference serum metabolome by largest data collection. This provides a platform to cumulate and share annotation.

Full Abstracts for the Multi-Omics Session (4)

**Author(s)** (Presenting author should be listed first)
Keshet, Uri; Wohlgemuth, Gert; Li, Yuanyue; Fiehn, Oliver
**Author Affiliations**
West Coast Metabolomics Center, UC Davis
**Title**
Mega Metabolomics: A Big Data Challenge and Opportunity with 13,700 human plasma samples for the TEDDY cohort.
**Introduction**
Large metabolomics studies can provide insights into the metabolic status and health of patients, as well as their interactions with the environment. However, metabolomics also poses significant challenges for data acquisition, processing, and analysis, especially when dealing with large and complex cohorts. Here, we present our metabolomics analysis of 13,700 human plasma samples for the TEDDY consortium, a multinational, large-scale longitudinal study that aims to identify The Environmental Determinants of type 1 Diabetes in the Young (TEDDY). We describe how we designed the analytical parts of the study to measure and compare the metabolite profiles of TEDDY participants, and how we addressed the big data challenges and opportunities arising from this endeavor.
**Methods**
Samples were extracted for HILIC- and C18-based LC-Orbitrap mass spectrometry using biphasic MTBE/Methanol/Water liquid-liquid extractions in 96 well plates. Method-blanks, pools, and NIST SRM1950 standard plasma were used as QC samples. Internal standards (46 for HILIC and 73 for C18-based lipidomics) were added to each sample for quality control and as markers to generate a retention index. Data was converted to mzml and uploaded to Amazon Web Services. LC-BinBase performs online QC monitoring of data files by the abundance and retention times of the internal standards and validates every detected LC-MS/MS feature, converts retention times to retention indexes, and processes individual files, on the database level, without the need for retention time alignment.
**Preliminary Data**
All samples were acquired by four LC-MS/MS **Methods**: the polar extraction phases were used for BEH Amide HILIC-MS/MS, and the MTBE phases were used for BEH -C18 chromatography-MS/MS for lipidomics, both acquired in negative and positive ESI modes. Three ThermoFisher Scientific Orbitrap instruments acquired data simultaneously. Over 60,000 data files were acquired, including 20% quality control samples. A subset of 2,300 TEDDY samples was used to generate sufficient QC pool samples to last throughout the study. Raw retention time drifts were found at <3s shifts across 1 year of data acquisition. A range of Quality Control parameters were used,  from automatic QC controls that alerted technical staff on potential issues in detecting internal standards or on instrument sensitivity drifts, to SOPs, training protocols, and variance scale analysis to detect outlier samples before and after data normalization by SERFF (systematic error removal by random forest). Data files were automatically converted from .RAW file format into formats used by the MS-DIAL software that we have re-written for use in Amazon Web Services for cloud-based data processing. Files were flagged â€œpassedâ€□ or â€œfailedâ€□ within minutes, even when many instruments are acquiring data at the same time. Reports were available to the operators in a simple web page, using a unique study ID. Detected peaks with MS/MS spectra were automatically added to our LC-Binbase database which is currently populated with 5,000-8,000 compounds per assay and ESI mode. MS/MS entries were annotated against MassBank.us, GNPS and NIST23 spectral libraries using a flash entropy similarity searches in MassWiki. Compound identifications were compared to results from Compound Discoverer software (ThermoFisher). Our QA/QC measures resulted in increased throughput and improved confidence levels of annotations. We will present and discuss our results in terms of instrument stability, method robustness, and general quality management performance.
**Novel Component**
We demonstrate a full analytical design, SOPs and quality control measures for a large-cohort metabolomics study.

**Author(s)** (Presenting author should be listed first)
El Abiead, Yasin; Strobel, Michael; Hagey, Lee; Mohanty, Ipsita; Gentry, Emily; Mannochio-Russo, Helena; Gomes, Paulo Wender P; Zuffa, Simone; Wang, Mingxun; Dorrestein, Pieter C

**Author Affiliations**
Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA, USA; Department of Computer Science and Engineering; University of California Riverside, 900 University Ave, Riverside, CA, 92521, USA; Department of Chemistry, Virginia Tech, Blacksburg, VA, USA; Collaborative Mass Spectrometry Innovation Center, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA, USA

**Title**
Leveraging Scale with  Pan-Repository Scale Analysis: Tracing the Evolutionary Chemistry of Life through Mining of Public Metabolomics Data

**Introduction**
The evolutionary trajectory of life's chemistry, from unicellular to complex multicellular organisms, is a vast unexplored domain. Our research leverages public metabolomics data repositories, including Metabolights, MetabolomicsWorkbench, and GNPS/MassIVE, to chart this chemical evolution. Utilizing untargeted LC-MS/MS-based metabolomics, we index and harmonize metadata across these platforms, enhancing the searchability of known and undefined MS/MS spectra. This approach integrates molecular data with taxonomic information, offering a unique perspective on the metamorphosis of life's chemistry across evolutionary timelines. Our study bridges evolutionary biology with advanced metabolomics, aiming to illuminate the molecular developments that have shaped the diversity of life.

**Methods**
We integrated raw data from key public metabolomics repositories, including Metabolights (ML), MetabolomicsWorkbench (MWB), and GNPS/MassIVE (GNPS). To ensure consistency across these diverse datasets, we automated the harmonization of  metadata. This harmonization methology and automation was designed to employ standardized terminology based on NCBI taxonomic identifiers and UBERON body parts. A critical part of our process involved downloading and indexing the mass spectra from these repositories, enabling their searchability through the FASST MS/MS search tool. Additionally, we annotated the raw data from all repositories using the GNPS MS/MS library, ensuring comprehensive data analysis.

**Preliminary Data**
We have aggregated a substantial collection of taxonomic identifiers from the Global Natural Products Social Molecular Networking (GNPS) database, comprising more than 600K LC-MS files from over 1,800 microbes, 3,500 plants, and 1,400 animals, including several extinct species such as neanderthals, ground sloth (extinct 7,000 years ago), Japanese otter (extinct 2012), white rhino (extinct 2018) and moa (extinct in 1445). This collection is set to expand with the ongoing integration of metadata from the additional aforementioned repositories. The scale of this dataset requires the efficient indexing and searching of these spectra, which was achieved with the FASST search tool.

A crucial innovation of our study lies in the integration of NCBI taxonomic identifiers into a phylogenetic tree with mass spectral/molecular data. This data integration allows for a more nuanced interpretation of both annotated and unannotated MS/MS spectra, facilitating analyses within an evolutionary context. Our approach thus enables a unique perspective for the analysis of metabolomics data, leveraging the combined power of taxonomy and untargeted metabolomics.

As a prime example, we illustrate how various steroidal molecules, including newly discovered bile amidates, exhibit distinct evolutionary patterns. For instance, unconjugated trihydroxy bile acids display different presence/absence patterns among animalsâ€”horses and deer, for example, are among the few mammals lacking them. In contrast, birds almost entirely lack unconjugated trihydroxy bile acids, with exceptions found only in certain seabirds and birds of prey. Furthermore, we demonstrate how analyzing fragmentation patterns can distinguish the stereochemistry of these trihydroxy bile acids. Finally, we show how integrating phylogeny with large-scale metabolomics data can help establish cross-species relationships across the tree of life.

**Novel Component**
This represents the first effort to merge large-scale untargeted metabolomics data with evolutionary biology using advanced data mining techniques.

**Author(s)** (Presenting author should be listed first)
Gong, Minghao; Gunter, Courtney; Karabacak, Fatih; Kozhaya, Lina; Xiong, Ruoyun; Oh, Julia; Vernon, Suzanne; Bateman, Lucinda; Unutmaz, Derya; Li, Shuzhao
**Author Affiliations**

1. The Jackson Laboratory for Genomic Medicine, 10 Discovery Drive, Farmington, CT 06032, USA 2. Bateman Horne Center, Salt Lake City, Utah, USA. 3. University of Connecticut School of Medicine, Farmington, CT 06032, USA.

**Title**

Dysregulation of immunometabolomics in ME/CFS patients is linked to xenobiotics and predictive of clinical symptoms

**Introduction**

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a debilitating condition with symptoms like enduring fatigue, cognitive impairment, chemical sensitivities and post-exertional malaise (PEM), closely resembled symptoms manifested in long COVID. Specific molecular biomarkers and established biological mechanisms are missing in both diseases, hindering diagnosis and treatments. While immune and metabolic disturbances are noted, the influence of diet, medication, and environmental factors on ME/CFS symptomatology is underexplored. We systematically profiling 312 immune cell populations and over 1,300 metabolites from blood samples of 153 ME/CFS patients (75 under 4 years and 78 over 10 years of diagnosis) and 75 healthy controls. We not only scored 100+ symptom-related variables, but also curated dietary and prescription records to approximate xenobiotic components.

**Methods**

- We used multi-omics factor analysis (MOFA) to integrate immune and metabolic modalities, reducing variables into latent factors (LFs). Statistical association analyses were then performed to understand relationships between LFs and metadata.
- Linear regression identified metabolites that significantly stratify patients of short or long durations with healthy controls.
- We used ordinary logistic regression to decode dietary questionnaire items-associated metabolic features.
- We expanded similar analyses to symptom variables, exploring associations of metabolites/metabolic ratios predictive of symptoms.
- Correlation analyses between immune cells and metabolites were performed in different cohort subsets.
- Subset-dependent correlation patterns led to expanded MOFA frameworks to identify LFs explaining variances in immune and metabolic modalities.
- A web component was established for visualizing complex analysis results for scientists and patients.

**Preliminary Data**

- MOFA explains variances across different data types in the ME/CFS cohort. The first two LFs driving immune data exhibit strong positive associations with chronological age and female sex, respectively, while the top metabolomics-driven LFs exhibit positive association with older age and female gender, confirming the significant influences of age and gender on the overall immune and metabolic status.
- Self-reported dietary intakes strongly associated with the second major metabolomics-driven LF, indicating the critical components of xenobiotics in blood metabolomics.

The metabolomic signatures are associated with disease duration, with elevated lipid levels in long-term patients, including cholesterols, carnitines and sphingomyelins.

- Symptomatology association analyses demonstrated multiple metabolites (e.g., lactate, kynurenine) significantly associated with symptom severity scores. Interestingly, metabolite ratios between pyruvate and multiple closed related metabolites aligned with severity of PEM. In contrast, exogenous metabolites (e.g., ergothioneine, thioproline) negatively associated with the scores.
- Strikingly, the correlations between metabolites and immune cells show vastly different patterns among the patients and among the controls. Such unique correlations are even more pronounced in subset of patients.
- Consistent with results obtained from differential correlation analyses, extensive MOFA analyses on subsets of cohorts (e.g., fibromyalgia patients) uncovers LFs that has coordinated variances explaining both immune and metabolic modalities that otherwise fail to uncover from the previous MOFA using the full cohort data. And the top-LF-weighted metabolites (e.g., 2,4-di-tert-butylphenol, mannonate) and their ratios with the immune cell counterparts uniquely associated with symptom severity.
- This study demonstrates the strong connections between metabolites, xenobiotics and the immune system, and their interactions are predictive of clinical symptoms. It reveals â€œchemical sensitivesâ€□, and many other symptoms are of clear molecular basis. Finally, it paves ways not only for metabolic biomarker discovery and but endorse precision nutrition as an indispensable component in personalized medicine.

**Novel Component**

Sophisticated immunometabolism study in ME/CFS cohorts; developed algorithms for bridging multiple MOFA models, metabolic network analysis, and web visualization.

**Author(s)** (Presenting author should be listed first)
Paul Stewart1#, Isis Narvaez-Bandera1, Ashley Lui1, Vanessa Rubio1, Min Liu1, Eric Welsh1, Dalia Ercan1, Hayley Ackerman1, Guohui Li2, Lancia Darville1, Bin Fang1, Steven Eschrich1, John Koomen1, Brooke Fridley3, Eric Haura1, Gina DeNicola1, Elsa Flores1
**Author Affiliations**
1Moffitt Cancer Center, 2Thermo Fisher Scientific, 3Children's Mercy Hospital, #Presenting Author
**Title**
Comprehensive multi-omic profiling of lung squamous cell carcinomas
**Introduction**
A lack of targetable genomic drivers and poor response rates to immune checkpoint blockade means patients with lung squamous cell carcinoma (LUSC) need new drug targets and better biomarkers, respectively. To this end, we previously integrated DNA copy number, somatic mutations, RNA-sequencing, and expression proteomics in a cohort of 108 LUSC patients. A major finding was identification of three proteomic subtypes, two of which made up the majority (87%) of tumors: the â€œInflamedâ€□ subtype was enriched for B-cell rich tertiary lymphoid structures, and the â€œRedoxâ€□ subtype was enriched for NFE2L2/KEAP1 alterations and increased redox pathways but had significantly less immune infiltration. We hypothesized that these subtypes would give rise to distinct metabolomic profiles.
**Methods**
We performed untargeted metabolomics on 87 tumors from the same LUSC proteogenomics cohort using ultra-high performance liquid chromatographic separation on a HILIC column, followed by analysis on a Q Exactive HF high-resolution mass spectrometer. This analysis yielded 7,392 features, and we identified 446 metabolites using m/z and retention time matching to an in-house reference library.
**Preliminary Data**
We found that the metabolomics data recapitulated the proteomic subtypes using a random forest classifier (AUC 0.73). Glutathione, a key redox metabolite, was elevated in the Redox proteomic subtype compared to the Inflamed subtype (0.58 log2 ratio, p = 1.14E-05), and pathway enrichment using MetaboAnalyst and the KEGG database revealed Glutathione metabolism was significantly enriched in Redox (p = 1.26E-06). To further integrate the metabolomics and proteomics data, we developed a novel, network-based method dubbed iModMix (â€œintegrative Modules for Multi-omicsâ€□). This approach takes large, empirical interaction networks created from omics data and distills them down into small, biologically-relevant modules. Importantly, iModMix can accommodate abundances from unidentified metabolites, so metabolomics modules can consist of both identified and unidentified metabolites. Our approach captures interactions that are not observable with individual omics approaches, and our approach can capture novel biology related to LUSC because modules are empirically derived and are not constrained to pre-existing pathway databases such as KEGG or GO. Applied to our LUSC data, iModMix identified 287 metabolite modules, 73 protein modules, and 133 gene modules, and in line with the Inflamed and Redox phenotypes, it identified 35 pairs of highly correlated modules ($|R| > 0.5$, $p < 0.05$) with enrichment in oxidative stress and immune pathways. Interestingly, one of the oxidative stress modules contained unidentified metabolites that were strongly differentially expressed and elevated in the Redox subtype. Analysis of the m/z patterns suggested that these are adducts of the same parent metabolite, and we hypothesize that this molecule is an as-of-yet uncharacterized redox-related metabolite. Ongoing experiments seek to validate this finding.
**Novel Component**
The first large scale multi-omic characterization of lung squamous cell carcinomas for molecular classification to develop novel therapies.

**Author(s)** (Presenting author should be listed first)
Colonna, Maxwell B.; Lyons, Michael J.; Flanagan-Steet, Heather; Steet, Richard
**Author Affiliations**
Greenwood Genetic Center
**Title**
Analysis of Metabolic Consequences Associated with Variants in ALDH18A1 Uncovers Perturbations in Multiple Amino Acid and Antioxidant Pathways
**Introduction**

ALDH18A1 encodes the enzyme, pyrroline-5-carboxylate synthetase (P5CS), which plays a pivotal role in the biosynthesis of proline. Proline, while not an essential amino acid, is a crucial component of collagens, plays a role in osmoregulation, and has been shown to serve as an antioxidant capable of scavenging reactive oxygen species. Variants in ALDH18A1 have been linked to a diverse array of neurocutaneous conditions, encompassing spastic paraplegia and cutis laxa. Our work identified a novel homozygous ALDH18A1 variant of uncertain significance (p.Thr331Pro) in four patients from two unrelated families who display clinical features consistent with P5CS deficiency. While possibly pathogenic, these patients did not display abnormal metabolite levels in plasma or urine typical of disease-causing ALDH18A1 variants.

**Methods**

To assess the effect of the variant on the metabolome and indicate a potential pathogenic effect, NMR metabolomics studies were conducted on patient-derived fibroblasts. Adherent primary fibroblasts from a patient and an unaffected control were cultured in replicate, washed with cold PBS, and flash-frozen with liquid nitrogen. Metabolites were extracted from cells with 80% methanol. 1H NMR spectra were acquired on a 600 MHz spectrometer. Additional two-dimensional spectra were acquired on an internal pooled sample for metabolite annotation. RNAseq was later performed on fibroblasts from the same control, patient, and the patient's family members. Western blots were performed with protein extracts to assess levels of P5CS protein as well as agreement of protein expression with gene expression results.

**Preliminary Data**

Patient fibroblast cells carrying the p.Thr331Pro variant showed significant changes in several amino acids related to proline metabolism, including decreased glutamate, glutathione, glycine, and increased arginine. Proline levels in patient cells were also decreased but were not statistically significant. Analysis of RNAseq data revealed that carrier parents exhibit an intermediate phenotype between patients and controls, supporting a partial loss-of-function effect of the p.Thr331Pro variant. Pathway analysis highlights altered expression of extracellular matrix (ECM) proteins, including several collagen protein subunits, implicating altered TGFÎ² signaling. Western blots of SDS-PAGE confirmed the enrichment and depletion of the most up and downregulated ECM proteins, namely MMP3 and COMP, respectively. Native PAGE also showed that the p.Thr331Pro variant resulted in reduced oligomerization of P5CS complexes, previously shown to be essential for function. Together, these data suggest expression of the p.Thr331Pro variant results in a decrease in P5CS enzyme activity, leading to decreased steady state proline levels and altered ECM protein expression in fibroblasts. The decrease in reduced intracellular glutathione and upregulation of glutathione peroxidase transcripts suggest oxidative stress in variant expressing cells, possibly contributing to the observed transcriptional reprogramming. Forthcoming work will employ semi-targeted LC-MS metabolomics studies of the family set of donor fibroblasts to measure a broader set of metabolites and lipids and assess the ability of drug treatments to restore a normal metabolic phenotype in cells.

**Novel Component**

Leveraged metabolomics study to help determine pathogenicity of a novel genetic variant in a metabolic enzyme

**Author(s)** (Presenting author should be listed first)
Raquel L. Shortt, Conor C. Jenkins, Gabrielle M. Rizzo, Allison E. Clay, Tyler D. Goralski, Elizabeth S. Dhummakupt

**Author Affiliations**

DEVCOM CBC

**Title**

Multi-omics Analysis of Staphylococcal Enterotoxin B Exposure in a Soldier-on-a-Chip: An Advanced Micro Physiology System

**Introduction**

Micro physiology systems (MPS) are used to emulate human organ systems, to collect more physiologically relevant data. This is especially important in understanding the human host’s response to exposures of lethal chemicals (i.e. sarin, VX) and biological organisms (i.e. Bacillus anthracis, Western equine encephalitis). In this project, a multi-organ system situated on a single chip was exposed to Staphylococcal enterotoxin B (SEB); the organ systems exposed were lung tissue, cardiac organoids, and skin tissue. These tissue systems are linked on-chip and share flow through micro-pumps, which is meant to mimic blood flow through a human organ system.

**Methods**

The three tissue types were harvested 24 hours after exposure to SEB and prepared for (1) proteomic and (2) metabolomic analyses via (1) Thermo Scientific Eclipse Tribrid Orbitrap mass spectrometer and (2) Thermo Scientific Q Exactive Plus Orbitrap mass spectrometer. Data was then analyzed via Proteome Discoverer 2.5 and Compound Discoverer 3.2. For proteomic analysis, each tissue type dataset was processed with its respective Human Proteome Atlas database along with the Housekeeping Protein database and the Transcription Regulation database.

**Preliminary Data**

Within the three tissue types, between 239 and 340 proteins were found to be significantly changing. In the skin tissue, SUMO-conjugating enzyme UBC9 was significantly downregulated from the PBS-exposed control sample. This enzyme plays a role in sumoylation, a process in which SUMO proteins are covalently attached to specific lysine residues in target proteins and used to regulate various aspects of protein function, like transcription, subcellular localization, DNA repair and cell cycle. Also in skin, the mitochondrial fission 1 protein (FIS1) was significantly upregulated. This protein is part of maintaining functional mitochondria when cells experience metabolic or environmental stresses. Disruptions in this process have been implicated in neurodegenerative diseases.

**Novel Component**

This study uniquely integrates multi-omics strategies to identify potential therapeutic targets for and taxological effects of SEB on an advanced microfluidics model

Full Abstracts for the New Technologies & Methodologies Session (8)

**Author(s)** (Presenting author should be listed first)
Ryan D. Sheldon, Molly T Soper-Hoper, Sydney Shenk, Rae J. House, Megan Gendjar
**Author Affiliations**
Van Andel Institute
**Title**
Serial metabolite extractions reveal de novo formation of metabolite-protein interactions that mask analytes from detection
**Introduction**
Contrary to prevailing dogma, we have recently demonstrated that metabolite extraction with organic solvent does not fully precipitate and remove proteins. Moreover, a subset of these proteins retain catalytic activity even after drying and resuspension. Stemming from these observations, we hypothesized that preserved protein structure across the proteome could lead to protein-metabolite interactions that would render portions of the metabolome inaccessible. To test this, we performed a series of sequential and orthogonal metabolite extractions to determine the extent of this protein-masked metabolome.
**Methods**
Experiments were conducted on pulverized and pooled frozen mouse liver as a model system. Primary metabolite extraction using 40% acetonitrile, 40% methanol, and 20% water (AMW20). The supernatant was collected, and insoluble pellets were subjected to a battery of secondary extractions including Bligh-Dyer (BD; chloroform, methanol, water), a second AMW20, or AMW with 50% water (AMW50). In a separate experiment, four serial AMW20 extractions were performed. In some experiments, 13C15N ATP was added during primary extraction. Metabolomics data were collected using three orthogonal LCMS **Methods**: (1) tributylamine ion-paired chromatography in ESI- on an Orbitrap Exploris 240, (2) low-pH BEH amide chromatography in ESI+ on an Orbitrap ID-X, and (3) low pH T3 reversed phase chromatography in ESI+ on an Orbitrap ID-X.
**Preliminary Data**
We tested the hypothesis that a portion of the metabolome remains trapped in the insoluble, protein-containing pellet during metabolite extraction. Performing a secondary BD extraction on the pellet revealed that only one third (49 of 160 detected metabolites) were recovered (as a percentage of the total recovery defined as the summed peak area of the primary and secondary extraction for each metabolite) greater than 90% in liver primary AMW20 extracts. Alarmingly, eleven metabolites were recovered at less than 25% in the initial AMW20 extraction, including ATP (21.8%), NADPH (10.7%), pyridoxal-5-phosphate (4.8%), and fructose 1,6 bisphosphate (1.7%). The balance of these metabolite pools was recovered in the secondary BD extraction on the pellet. These data reveal a complex, analyte specific interaction with the sample matrix during extraction. We next asked whether this could be due to incomplete homogenization of the sample. To test this, we added 13C15N labeled ATP to the primary AMW20 extraction solvent and repeated the BD secondary extraction on the pellet. Remarkably, labeled ATP correlated strongly ($r=0.99$, $p<0.0001$) to endogenous sample arising ATP, indicating de novo in-extract formation of ATP-matrix interactions. This experiment was repeated using four serial AMW20 pellet extractions and, again, labeled ATP behaved identically to endogenous ATP. Moreover, even four AMW20 extractions failed to recover ATP to the level achieved by primary BD extraction alone. This suggests that extraction solvent influences which metabolites bind the matrix and to what extent in metabolite extracts. Finally, we extended this paradigm to broad-coverage metabolomics profiling. These efforts revealed a sweeping, complex interaction between sample-type (tissue, plasma, cells), extraction modality, and specific analytes, including fatty acids, acyl-carnitines, amino acids, nucleotides, vitamins, and more.
**Novel Component**
Metabolite-protein interactions form during metabolite extractions that mask analytes from detection and may skew biological phenotype detection.

**Author(s)** (Presenting author should be listed first)
Thompson, J. Will; Stewart, Sam; Redman, Erin; Mellors, Scott; Guerrette, Josh
**Author Affiliations**
908 Devices Inc
**Title**
Standardization and Software Enabling Automated Quantitative Metabolomics using Microchip CE-MS

**Introduction**
Capillary Electrophoresis has long held promise as a powerful separation technique for metabolites, and yet has had poor adoption in the metabolomics community. We suggest that the limitations to adoption have stemmed from several factors, including difficulty of use and lack of available methods for automated migration time correction and quantification. Leveraging the power of Skyline's capabilities for small molecule data processing, we propose novel automated workflows for suitability testing (SST), raw data quality checks/control, and metabolite quantification in Microchip CE-MS.
**Methods**
Amino acid standards (Promega) were diluted in Peptides Diluent (908 Devices) and utilized as a system suitability standard (SST) for ZipChip CE-MS analysis (908 Devices) on an Exploris 240 (Thermo). Custom stable isotope standards were developed with Cambridge Isotope Laboratories. SST Pass/Fail criteria were developed using historical criteria from hundreds of analyses, and included migration time, peak shape (resolution), and migration index. Samples including plasma, serum, and urine (BioIVT) were extracted using a simple methanolic protein precipitation including internal standards and ammonium acetate. Open source application was developed in Python with standard data science toolset (e.g. pandas, numpy). The application utilizes Skyline as a backend for data extraction and for steps of the quantitation pipeline.
**Preliminary Data**
When evaluating whether an analytical system is suitable for analysis, the SST should be robust enough to detect failed analyses, but not so strict that false-positive failures occur often. We determined that a set of 5 metabolites was sufficient to detect system failures nearly perfectly in CE-MS. Using a set of several hundred analyses across multiple chips, we established pass/fail criteria for the following metrics in SST: 1) migration time of lysine (highest mobility), 2) migration time of aspartic acid (lowest mobility), 3) separation resolution of isoleucine and leucine, and 4) migration of valine compared to Ile/Leu. These metrics were then incorporated into a GUI which provides users immediate feedback at system startup, with guided procedures for next step if the SST fails.
It is equally important to quicky detect failures in data quality or missed injection during an analytical batch; we screened 36 internal standards and identified 4 measurements that could detect in an automated manner whether the sample was injected properly, if the sample was prepared correctly, and whether the separation quality was acceptable. Using several hundred runs to build acceptance criteria from a variety of sample types (serum, plasma, urine) these four internal standards could determine key failure modes with nearly 100% accuracy, allowing automated resubmission or exclusion from data analysis as required.
Finally, most metabolomics software often has difficulty correctly selecting and integrating low abundance target peaks in the presence of high abundance, isobaric interference. Examples include alloisoleucine in the presence of isoleucine or leucine, or beta-alanine and sarcosine in the presence of alanine. We demonstrate a widely-generalized solution to these challenges using the Hungarian algorithm which take situations where Skyline selects the correct analyte peak 0% of the time (i.e. never), to making the assignment with nearly 100% accuracy. We plan to open-source this solution on GitHub.
**Novel Component**
Automated, High-Throughput Quantitative Metabolomics using MicroChip CE-MS

**Author(s)** (Presenting author should be listed first)
Rankin-Turner, Stephanie; Hiraoka, Kenzo; Shimada, Haruo; Kinoshita, Kazumasa; Ninomiya, Satoshi
**Author Affiliations**
University of Pittsburgh, University of Yamanashi
**Title**
Rapid profiling of the human skin metabolome with heat pulse desorption mass spectrometry (HPD-MS)
**Introduction**
The human skin releases a complex blend of metabolites through sweat, sebaceous glands, and the diverse microbes inhabiting its surface. The study of the skin metabolome has garnered interest in various fields of research, being utilized to identify disease biomarkers, study skin conditions and treatments, develop chemical tools for the location of trapped humans in disasters, and understand the role of human scent in mosquito attraction to humans. As such, the development of new methods to facilitate the chemical characterization of skin metabolites is of great interest. In particular, ambient ionization mass spectrometry, which enables the

rapid analysis of samples in their native state without pretreatment, offers an exciting approach to study the skin metabolome almost in real time.

**Methods**

A heat pulse desorption mass spectrometry (HPD-MS) with proximity corona discharge method was developed for the rapid analysis of human skin metabolites. In HPD-MS, a brief (~50ms) pulse of heated NÂ¬2Â¬ gas is applied to the sample in front of an open mass spectrometer inlet. This induces the rapid desorption of analytes for subsequent ionization and detection by mass spectrometry. In this work, regions of the human body were sampled with cotton swabs to collect skin secretions from various body sites, which were subsequently analyzed by HPD coupled with an Orbitrap mass spectrometer. Samples were collected from different participants in addition to being collected from the same individual over multiple days, demonstrating the inter- and intra-individual variation in skin metabolites.

**Preliminary Data**

Heat pulse desorption mass spectrometry with proximity corona discharge ionization enabled the detection of diverse polar and non-polar metabolites from the skin, ranging from volatile compounds such as acetone and geranylacetone, to lipids such as triacylglycerides and wax esters. Participants across a range of ages were sampled (aged 22 to 79). Many metabolites were consistently detected across all participants, though notable variation in the relative abundance of metabolites was observed. Whether this variation is a result of natural variation amongst humans or if there are age-specific differences requires a larger scale study to confirm. The composition of the skin metabolome is furthermore likely to vary across sampled body sites due to the variation in the skin microbiome across different regions and the distribution of sweat and sebaceous glands. In this work, numerous skin sites were sampled, including the forehead, nose, axilla, back, and feet. As expected, there was extensive variation in the presence and abundance of identified analytes. Finally, samples were collected from a single participant over multiple days to evaluate the day-by-day stability of the skin metabolome. Notable variation in the presence and relative abundance of metabolites was observed across multiple days, highlighting a key challenge in developing robust assays for using skin metabolomics in biomedical research. This pilot study demonstrates that HPD-MS is a promising new tool for the rapid characterization of diverse human skin metabolites without the need for complex sample pretreatment, though various challenges must be explored and tackled in future work.

**Novel Component**

Application of a novel ambient ionization MS technique to the characterization of skin metabolites in <10 seconds.

**Author(s)** (Presenting author should be listed first)

Christopher, Michael[1]; Ericson, Aiden[1]; Klug, Alexander[1]; Prentice, Boone[1]; Garrett, Timothy [1,2]

**Author Affiliations**

[1] Department of Chemistry, University of Florida, [2] Department of Pathology, Immunology, and Laboratory Medicine, University of Florida

**Title**

Divergent Metabolic Fates of Aromatic Amino Acid-Derived Isomers: Insights from Metabolomics and HDX-HRMS/MS-Based Resolution of Tautomers

**Introduction**

Tautomers are one of the many types of isomers. Following metabolism of aromatic amino acids into aromatic pyruvic acids, structures with a tautomeric equilibrium arise. The corresponding enol and keto tautomer occur at the keto carbon alpha to the carboxylic acid. The different electron configuration of the compounds is hypothesized to lead to different chemical and biological outcomes. An ex vivo and computational approach was used to study how the tautomers differentially metabolize. One of the metabolites discovered was tautomeric, but too massive for HDX to assign tautomer chromatogram peaks. HDX-HRMS/MS was developed to access the high resolution and low m/z range conditions needed to resolve a deuterated keto from the [13]C M+1 isotope EIC of the keto and enol.

**Methods**

Whole blood was collected via venous puncture and used for ex vivo metabolomics. The addition of indole-pyruvate (IPyA) and IPyA-d5, to a concentration of 25 ÂµM, was used to discover new metabolic fates. Untargeted metabolomics was used to find feature pairs which differed by exactly 4 or 5 deuterium atoms and had retention times no greater than 0.8 min of each other. Two unknown metabolites were discovered and

characterized with HCD and UVPD MS/MS. An HDX labeling method, in combination with HRMS/MS, was used to differentially label and resolve the keto and enol tautomers of the new metabolite.

**Preliminary Data**

Two novel compounds were found to be involved in the metabolism of IPyA. The first, named 3-(1H-indol-3-yl)-2,3-dioxopropanoic acid (i.e., indole-3-oxopyruvic acid), was structurally characterized using HCD. This compound results from the loss of two hydrogen atoms and the addition of an oxygen atom to the alpha carbon of the carbonyl carbon, relative to IPyA. The second compound resulted from glutathionylation of IPyA, and required both HCD and UVPD for full structural characterization to identify the conjugation site. The conjugation site sparked a computational investigation into the relative reactivity of the carbon in its enol versus keto states, which showed drastically higher methyl anion affinity and reactivity towards GSH in the enol configuration. Structural characterization of GSHIPyA revealed the potential for two tautomeric states. The deuterated and [13]C M+1 isotopes at nominal m/z 510 would not be resolved via an MS1 measurement alone (i.e., using a resolving power of 140,000 defined at m/z 200). However, high resolution tandem mass spectrometry (HRMS/MS) of the tautomeric product ions allowed for baseline peak resolution and accurate assignment of the chromatographic peaks by aligning EICs of the product ions with the parent ions.

**Novel Component**

The development of HDX-HRMS/MS for resolution of differentially deuterated tautomeric product ions from otherwise large, unresolvable ions.

**Author(s)** (Presenting author should be listed first)
Vincent, Michael; Ellis, Abigail; Sheldon, Ryan

**Author Affiliations**

Van Andel Institute

**Title**

Customizable Polymeric Nanocarriers for Interrogating Cellular Metabolism with Hydrophobic Tracers and Drugs

**Introduction**

The field of metabolomics seeks to characterize dynamic networks of small molecules and lipids that shape physiology in health and disease. Stable isotopes and metabolism-perturbing drugs provide basic tools for this purpose yet issues with specificity and/or bioavailability often limit their ability to probe metabolic phenotypes in specific cell types in vivo. Here, we developed polymeric nanocarriers to address key issues with the systemic metabolic effects of tracers and drugs, as well as problems with compound solubility, stability, and accumulation within the target cell type(s). Our initial proof-of-concept studies evaluated three types of polymeric nanocarriers as vehicles for delivering U-13C-palmitate tracer in vitro. Labeling was benchmarked against the traditional approach of complexing 13C-palmitate with bovine serum albumin (BSA).

**Methods**

Polymeric nanocarriers encapsulating hydrophobic dye or U-13C-palmitate tracer were self-assembled from amphiphilic block copolymers by method of nanoprecipitation. Physical and chemical properties were characterized by dynamic light scattering, electrophoretic light scattering, and transmission electron microscopy (TEM). The cellular uptake kinetics of nanocarriers encapsulating a fluorescent dye was assessed by flow cytometry. Metabolomics and lipidomics tracing studies examined labeling in cells treated with nanocarriers encapsulating U-13C-palmitate or U-13C-palmitate-BSA complexes at multiple timepoints over a 48 h period. Cell extracts were analyzed using liquid chromatography coupled to high resolution tandem mass spectrometry (LC-MS/MS). Data were analyzed using Skyline software (peak picking) and custom python-based software (statistical analysis).

**Preliminary Data**

Nanocarriers were monodisperse with diameter between 30-50 nm and a surface charge between -10 mV and -15.5 mV. Subsequent characterization by TEM confirmed the formulations consisted of monodisperse spherical nanostructures with physical properties consistent with the expected micelle morphology. Flow cytometry studies demonstrated nanocarrier internalization was detectable by 2 h post-administration. Our metabolomics studies found rapid decreases in the labeled palmitate (FA 16:0) pool that coincided with an increase in stearic acid (FA 18:0) labeling, indicative of successful nanocarrier uptake, payload release, and entry into cellular metabolic networks. Label enrichment was negligible in tricarboxylic acid cycle intermediates compared to the greater labeling observed in complex lipids, suggesting cells primarily used the tracer payload for lipid biosynthesis rather than beta-oxidation under the conditions examined. Finally, labeling patterns

correlated between groups receiving nanocarrier-encapsulated U-13C-palmitate versus U-13C-palmitate-BSA complexes.

**Novel Component**

Polymeric nanocarriers are suitable vehicles for delivering hydrophobic tracers and can be customized to probe metabolism in specific cell types.

**Author(s)** (Presenting author should be listed first)

Ciara Myer, Rahul Ravi Deshpande, Bashar Amer, Susan S Bird

**Author Affiliations**

Thermo Fisher Scientific

**Title**

Overcoming Speed and Sensitivity Barriers in Lipidomics with the Orbitrap Astral Mass Spectrometer

**Introduction**

Untargeted lipidomics offers a powerful tool for detailed characterization of a sample's lipid profile. However, limitations in sensitivity and scan speed can hamper in-depth and confident analysis. This study investigates the potential of a mass spectrometer with MS2 scan speeds of up to 200 Hz to improve depth of coverage, confident annotation, and analysis throughput for high-resolution lipidomics studies.

**Methods**

Lipids were extracted from diverse sources including liver, plasma, cells and plants using the Bligh and Dyer method. Separations were achieved on a C30 column using a Thermo Scientificâ„¢ Vanquishâ„¢ Horizon UHPLC system and analyzed on a Orbitrapâ„¢ Astralâ„¢ mass spectrometer. MS1 scans were acquired in the Orbitrap analyzer at 120,000 resolution and fast, sensitive MS2 was acquired in the Astral analyzer at 80,000 resolution to facilitate in-depth fragmentation and confident annotation of lipid species. Data analysis was performed using various software platforms.

**Preliminary Data**

Several parameters were optimized to ensure efficient transmission and detection in the Astral analyzer. This included radio frequency (RF) lens voltage, injection time, automatic gain control (AGC) target and collision energy. Notably, low AGC targets allowed the instrument to operate at its maximum speed of 200 Hz while maintaining spectral quality. This resulted in a significant number of MS2 spectra (over 250,000) within a 30-minute run. Further analysis with various sample types confirmed the instrument's reliability and robustness, indicating its potential for advancing lipidomics research.

**Novel Component**

This study presents a high-throughput lipidomics workflow that achieves deep coverage and confident annotation in a single injection.

Full Abstracts for the Single Celled Organisms to Microbiomes Session

**Author(s)** (Presenting author should be listed first)
Harini Sridharan, April Miguez, Mark P. Styczynski
**Author Affiliations**
Georgia Institute of Technology
**Title**
Effects of Crabtree-induced metabolic rewiring on essential cellular processes in model yeast species
**Introduction**
The impacts of a phenotype can be felt across a range of cellular regulatory levels â€“ from genomic expression to metabolic pathways. One such extensively studied phenotype in select yeast species is the Crabtree effect: the production of ethanol through fermentation despite the presence of oxygen, which typically supports respiration over fermentation. This phenotype has facilitated the production of biofuels and various biochemicals, and it holds significant potential for biotechnological and biomedical applications. The Crabtree effect has been extensively studied at the genomic, transcriptomic, and proteomic levels. However, a comprehensive systems-scale analysis of the metabolic rewiring associated with the Crabtree effect has yet to be conducted, despite the metabolome offering the most direct insight into an organism's phenotype.
**Methods**
We moved towards this goal by selectively deleting genes of importance in the central carbon metabolism in two model yeast species: Saccharomyces cerevisiae, which exhibits the Crabtree effect, and Kluyveromyces lactis, which does not. The overall metabolic profiles of the deletion strains were compared with the control (wild-type strain) at two different growth stages â€“ exponential and stationary. The time points were chosen to reflect two different physiological states of the cell. GCXGCâ€“TOF MS was used to profile the metabolome of the yeast samples. The dataset was analyzed using multivariate and univariate statistical tools such as Principal Component Analysis (PCA) and Analysis of Variance (ANOVA) respectively.
**Preliminary Data**
Multivariate analysis suggested changes in the metabolic profile of the deletion strains in both S. cerevisiae and K. lactis, when compared to their respective control strains. Univariate analysis highlighted metabolites with notable differences in abundance among the deletion strains and between the species, aiding in the generation of biologically interpretable hypotheses. Several of these metabolites, such as sterols and phospholipids, were identified as part of pathways that contribute to yeast cell membrane stability. The levels of these metabolites show differences in their relative levels in K. lactis deletion strains. However, similar trends are not observed in the orthologous S. cerevisiae deletion strains. This may indicate differences in the metabolic regulations associated with cell membrane stability in S. cerevisiae and K. lactis. Experiments specifically probing the cell membrane stability of the variants will be conducted to confirm the relative variances in cell membrane stability.
Another such metabolism that showed consistently notable changes in S. cerevisiae and K. lactis is the biosynthesis of specific amino acids such as lysine and leucine. These amino acids have been known to play significant roles in cellular stress response to oxidative and ethanol stress. The dataset suggested a relationship between redox balance and fermentative capacities of S. cerevisiae strains. On the other hand, the levels of metabolites belonging to the leucine biosynthesis pathway in K. lactis show differences in specific strains associated with the respiratory metabolism, which is localized in the mitochondria, where leucine biosynthesis begins.
**Novel Component**
This study is the first to explore the Crabtree effect on the metabolome, providing the most direct downstream readout of the phenotype.

**Author(s)** (Presenting author should be listed first)
Jin, Jiangpeiyun; Garg, Neha
**Author Affiliations**
Georgia Institute of Technology
**Title**
Deciphering bacterial-fungal chemical interaction between Burkholderia cenocepacia and Aspergillus fumigatus using Metabolomics
**Introduction**

Cystic fibrosis patients face a lifelong threat with chronic airway infections and inflammation. CF airway is a vulnerable host to a complex community of microorganisms, allowing polymicrobial interaction. Such interaction contributes to shift the pathogen virulence, antimicrobial susceptibility and driving poorer clinical outcomes. The interplay between bacterial and fungal CF pathogens has not been well characterized. We utilized metabolomics to investigate the chemical interaction between the most feared bacterial pathogen, Burkholderia cenocepacia, and the dominant fungal pathogen, Aspergillus fumigatus, and to characterize the outcome of microbial interaction on their pathogen physiology. This study demonstrates the impact of environmental factors on shaping microbial metabolomic profiles and will improve our understanding on the complexity of CF lung microbiomes.

**Methods**

Burkholderia cenocepacia was cocultured with Aspergillus fumigatus, and trimethoprim was added to the cultures at sublethal concentrations to examine the effect of sublethal antibiotics on the chemical interaction between the two species. Metabolites were extracted from B. cenocepacia and A. fumigatus mono- and cocultures, and then subjected to UPLC-MS/MS-based untargeted metabolomics data acquisition. MALDI-TOF IMS was employed to directly visualize the spatial distribution of the metabolites involved in the bacterial-fungal crosstalk. Various multivariate statistical analyses were performed to characterize metabolomic shifts between different culture conditions and to identify important metabolite features for further analysis. Additionally, tools including GNPS molecular networking and in silico structure predictions were utilized to aid in metabolite annotation.

**Preliminary Data**

Significant inhibition of Aspergillus fumigatus growth by Burkholderia cenocepacia was observed on ISP2 agar supplemented with sublethal concentrations of trimethoprim. Additionally, increasing the trimethoprim concentration resulted in enhanced inhibition. Principal component analysis and hierarchical clustering analysis revealed that cocultivation and antibiotic exposure induced variations in the global metabolomic profiles of both species. An UpSet plot was generated to visualize the intersection of metabolite features detected across different samples, demonstrating that cocultivation induced metabolomic responses from one or both strains, with 155 features detected exclusively under coculture conditions. Annotations of these differentially detected metabolite features included several bacterial and fungal secondary metabolites with significant roles in the virulence of these pathogens. Our approach not only revealed global shifts in the metabolomic profile due to the interaction between the two species but also demonstrated the emerging role of sublethal antibiotics as signaling molecules to modulate biosynthetic gene cluster expression, thereby altering the bacterial-fungal chemical interaction. The workflow employed in our study highlights the powerful application of untargeted metabolomics in identifying microbial chemical communication from both microorganisms and underscores the importance of considering environmental factors when studying microbial pathogenicity.

**Novel Component**

We show novel insights into the interaction between B. cenocepacia and A. fumigatus and how sublethal antibiotic exposure influenced their interaction. Preferred Format of Presentation

**Author(s)** (Presenting author should be listed first)
Cline, Thomas; Samuel, Darnilla; Rosset, Sabrina; Roach, Ty; Tortorelli, Giada; Drury, Crawford; Quinn, Robert

**Author Affiliations**

Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, USA; Duke Marine Lab, Duke University, Beufort, NC, USA; Hawaii Institute of Marine Biology, University of Hawaii and Manoa, Kaneohe Bay, HI

**Title**

Lyso-Lipid Dynamics Reflect Thermal Tolerance in Reef Building Corals

**Introduction**

Coral reefs are fundamental ocean ecosystems threatened by climate change. Scleractinian corals are a symbiotic association of a Cnidarian host and symbiotic dinoflagellate algae and the principal biological architect of reefs. Under heat stress, corals bleach, expelling their algal symbionts and turning white, threatening the organism’s survival. Previous work has shown bleaching resistance is strongly correlated with the lipidome of the symbionts. Members of the algal clade Durusdinium are more resistant to bleaching and have higher proportions of lyso-lipids than the thermally sensitive Cladocopium. The strongest predictor of thermal tolerance among these symbionts were lyso-betaine lipids, especially the DGCC form unique to marine

algae. However, how these lipids and their dynamics provide resistance to bleaching remains poorly understood.

**Methods**

We tested the lipid dynamics and lipase activity of Galaxea fascicularis corals hosting either Durusdinium or Cladocopium algae in aquaria. Samples were collected by cutting tentacles at the base and transferring to a collection tube on ice. Tentacles were incubated at room temperature (25Â°C) in 100% methanol for 5 minutes, 15 minutes, 30 minutes, 1 hour, 4 hours, 8 hours, and 24 hours before being placed in a -80Â°C freezer. Lipid extractions were performed in 3:1 MTBE:methanol and dried with N2 gas before reconstitution in 2:1:1 IPA:ACN:water and 65:35:1 methanol:water:formic acid for lipidomics. Extracts were analyzed on a Thermo Q-Exactive mass spectrometer coupled to a Vanquish UHPLC system. Resultant mass spectra were processed using MZmine 3 and analyzed on GNPS.

**Preliminary Data**

Results showed differential responses between G. fascicularis corals hosting either Durusdinium or Cladocopium symbionts. Concurrent with previous findings on live reefs in Hawaii, Durusdinium hosting corals showed higher lyso-DGCC abundance at time zero and this did not change during the incubation. The Cladocopium hosting corals however, increased their lyso-DGCC (16:0) abundance over time in a linear fashion. Diacyl forms of these lipids including DGCC (34:4) and PC (36:4) did not change in either holobiont type. Remarkably, similar dynamics were observed in corals in Kaneohe Bay, HI under heat stress, where only Cladocopium hosting corals increased lyso-DGCC abundance under high temperature conditions. The increase in Cladocopium lyso-lipid abundance during incubation points to heightened lipase activity natively present in these algae that are also active during heat stress. The Durusdinium-hosting corals in aquaria and on live reefs have a static lipidome that primes their resistance to elevated water temperatures. These results suggest lyso-lipids and lipase activity may be a major driver of thermal resistance in Scleractinians and points to a future where Durusdinium hosting corals with higher lyso-lipids dominate reefs in a changing climate.

**Novel Component**

Stress-induced lipase activity causes a dynamic change of the coral lipidome trending towards the profile associated with heat resistance.

**Author(s)** (Presenting author should be listed first)
Chiles, Eric N (1,2); Su, Xiaoyang (2,3)

**Author Affiliations**

1. Graduate Program in Microbial Biology, Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ 08901, USA, 2. Metabolomics Shared Resource, Rutgers Cancer Institute of New Jersey, Rutgers University, New Brunswick, NJ 08901, USA, 3. Department of Medicine, Division of Endocrinology, Robert Wood Johnson Medical School, Rutgers University, New Brunswick, NJ 08901, USA

**Title**

Investigating how abiotic stressors alter Cladocopium metabolism and impact coral holobiont dysbiosis

**Introduction**

Coral reef systems are threatened globally due to anthropogenic climate change. Understanding the metabolic relationship between the coral holobiont, comprised of the cnidarian animal host and its algal and bacterial symbionts, is essential for conservation efforts. Inhabiting oligotrophic waters, scleractinian corals rely on a mutualistic symbiosis with dinoflagellate Symbiodiniaceae to survive. Algal symbionts efficiently assimilate both dissolved organic and inorganic precursors from the water and the animal host to fix them into photosynthate metabolite products. Our central question is whether LC-MS based metabolomic approaches can be used to characterize nutrient assimilation in algal symbionts and assess how it is impacted by abiotic stress, providing a mechanistic understanding of bleaching and giving new insights to improve coral health and resilience.

**Methods**

We designed a controlled experiment using cultures of Cladocopium (Strain 2488 obtained from Bigelow Lab) Symbiodiniaceae, endemic to the Hawaiian Reef system (our study site was Kaneohe Bay, Oahu). Permutations of combined abiotic factors including heat and light were exposed to the cultures spanning either ambient or high temperatures, i.e., 26Â°C or 31Â°C, and low and high light for 7 days. Cultures were then subjected to stable isotopic enrichment using 100ÂµM 15NH3 for 18 hours before being collected for subsequent targeted and untargeted LC-MS analysis under both positive and negative polarities. The 15N enrichment was determined using AccuCor natural abundance corrector.

**Preliminary Data**

Previous studies considering the holobiont showed considerable nitrogen assimilation into several metabolite classes of primary and secondary metabolism including purines and pyrimidine nucleotides and derivatives, amino acids, metabolic cofactors and their precursors after an 18-hour incubation with the tracer. Statistically significant isotopic enrichment between treatment groups was not observed. Isotopic tracer incorporation was also observed in dipeptides, a secondary metabolite class that is positively correlated to heat stress. This was consistent among multiple coral species, however, dipeptide production could not be localized to any one constituent of the holobiont. Here, by separating the algal symbionts and examining their metabolic capacity individually, we show that they are predominantly produced by the alga. This supports the canonical role of the symbiont as the driver of holobiont metabolism.
Prolonged alterations to the diurnal light cycle can also induce a stress response. Light deprivation leads to a cessation of photosynthesis in the alga and subsequent reduction of photosynthates being translocated to the animal host. This starves the host similarly to heat induced dysbiosis. During low light isotopic tracer incorporation significantly reduced in dipeptides, as well as other primary and secondary metabolite classes. Contrastingly, high irradiance can also saturate algal photosynthesis. Here we have a comparative analysis on both abiotic factors in tandem and assess each's overall contribution to the algal stress response observed in the holobiont. We observe that altered light conditions do not significantly alter nitrogen assimilation or labeling in heat stress correlated dipeptides. These preliminary results show that high temperature is the predominant driver of the coral stress response among tested conditions. Additionally, nitrogen assimilation into the coral holobiont is compartmentalized, with rapid assimilation and quick dipeptide turnover occurring within the algal symbiont and a slow turnover of other nitrogen containing metabolites in another region. Work exploring nitrogen assimilation compartmentalization is ongoing.

**Novel Component**

To investigate where nitrogen assimilation is compartmentalization in scleractinian corals and assess its impact by abiotic stress.

**Author(s)** (Presenting author should be listed first)
Amer, Bashar1; Jacob, Cristina1; Remes, Philip M.1; Deshpande, Rahul Ravi1; Jafari, Reza2; Moehring, Thomas1; Bird, Susan S.1

**Author Affiliations**

1 Thermo Fisher Scientific, San Jose, California, USA. 2 MetaSci, Canada

**Title**

High throughput targeted metabolomics library generation on a novel mass spectrometer applied to microbiome analysis

**Introduction**

Recent studies underline the crucial role of the human gut microbiome in maintaining a healthy gastrointestinal tract and influencing pathological processes. The fecal metabolome, increasingly studied for clinical diagnosis potential, reveals insights into the microbiome's impact on health. Targeted MS-metabolomics identifies and quantifies fecal metabolites, but limitations like instrument speed and selectivity can reduce target numbers. A new mass spectrometer (Thermo Scientificâ„¢ Stellarâ„¢ MS) with fast scan speed, high selectivity, and detection sensitivity was employed to build a comprehensive fecal library for analyzing polar and non-polar metabolites.

**Methods**

A Fecal Metabolites library, containing over 500 compounds sourced from MetaSci, was prepared using various solvent mixes according to vendor instructions. Pure methanol was used for non-polar compounds, while methanol-water mixtures were used for medium and high-polar compounds. Calibration curves were created by combining aliquots of each standard. Fecal samples were collected from 8-week-old mice subjected to different diets with varying fat sources for 29 days. Metabolites were extracted using 80% methanol before analysis. Thermo Scientificâ„¢ reversed-phase, mixed mode, and HILIC columns connected to a Thermo Scientificâ„¢ Vanquishâ„¢ Horizon system separated metabolites. Thermo Scientificâ„¢ Stellarâ„¢ MS provided fast scanning MS/MS and MSn levels for precise annotation and extended quantitation, with Thermo Scientificâ„¢ TraceFinderâ„¢ 5.2 used for data processing and quantitation.

**Preliminary Data**

The Stellar mass analyzer's high scan speed reduced LC acquisition time, crucial for high-throughput analyses with sample stability challenges. MSn-based quantitation provided the selectivity necessary for detecting and accurately quantifying co-eluting isomers and isobars, improving discrimination between analyte signals and

those from matrix interferences. The established library allowed reliable quantitation over a broad dynamic range (5 orders of magnitude) in fecal components, with an improved sensitivity reflected in a low limit of quantification of 10 femtomoles and a low limit of detection of 0.25 femtomoles for most targets. Assessment of isotopically labeled internal standards demonstrated high data quality, reliability, and robust measurement, evidenced by minimal chromatographic shift and consistent signal responses, as indicated by low % CV for quality control and sample replicates. The developed methods were used to measure metabolites in fecal extracts from mice subjected to different dietary interventions.

**Novel Component**

A comprehensive fecal library using a fast, sensitive, and selective novel mass spectrometer enabling high throughput and accurate microbiome metabolomics.

**Author(s)** (Presenting author should be listed first)
Bills, Brandon; Christopher, Michael W.; Yedla, Sunandini; Amer, Bashar; Bird, Susan; Deshpande, Rahul; Barshop, William; Prentice, Boone M.; Garrett, Timothy J.; Zabrouskov, Vlad

**Author Affiliations**

BB, SY, BA, SB, RD, WB, and VZ: Thermo Fisher Scientific, San Jose, California, USA; MC, BP, and TG: University of Florida, Department of Chemistry

**Title**

Forward and Reverse Cosine Similarity Scoring During Real-Time Library Search for Triggering Additional Experiments on Indole Compounds

**Introduction**

Studying unknown compounds of a specific class in biological systems can be challenging. Relevant compounds must be identified in complex matrices amongst an abundance of background compounds. Even if fragmentation data is collected on a relevant compound, it may not be enough to annotate. Alternate fragmentation techniques can provide additional information but take additional time and reduces the number of features that can be investigated in a single run. Real-Time Library Search (RTLS) allows the instrument to check spectra against a library and trigger additional data acquisition for similar compounds. In this work we look at the efficacy of forward versus reverse cosine similarity searching for triggering additional data acquisition on indole compounds in a plant matrix.

**Methods**

Library data was generated by direct infusion of 25 indolic small molecules using a Thermo Scientificâ„¢ Orbitrapâ„¢ IQ-Xâ„¢ Tribridâ„¢ mass spectrometer. The [M+H]+ ions were fragmented using a range of normalized collision energy from 10 to 70 and the spectra were curated using Thermo Scientificâ„¢ mzVaultâ„¢. Plant extracts were run via reverse phase liquid chromatography both without RTLS and with RTLS using a cosine score threshold of 20 and either forward or reverse cosine scoring. Precursors that passed the threshold triggered a follow up UVPD MS2 scan and three ddMS3 scans. Results were evaluated for the number of each type of scans that were collected as well as whether potential indole containing compounds were successfully targeted for additional data collection.

**Preliminary Data**

Real-Time Library Searching acts as a filter that prevents the instrument from running additional experiments unless it passes a score threshold. This allows for more compounds to be interrogated with ddMS2 while still collecting additional information when relevant. In experiments with RTLS, 3-4x more ddMS2 spectra were collected relative to running without RTLS.

Forward cosine similarity scoring compares experimental spectra and library spectra against each other with the presence/absence and relative intensity of the fragments in both used to generate a score. In reverse cosine scoring the fragments in the library are compared to the experimental spectra (not vice versa) without penalty for additional fragments being present in the experimental spectra.

Initial results from studying plant extract show that out of the collected ddMS2 scans scored against a library of indole compounds, approximately 5% of spectra had a forward similarity score of 20 or higher with only 1.3% having scores above 30. The same sample analyzed using reverse cosine scoring shows 10% of scans score 20 or higher and they show a more even distribution of scores between 20 to 90. Because forward scoring only gives a high score when the experimental and library spectra closely match, it is better suited for identity matching. Reverse scoring doesn’t penalize additional fragments in the experimental spectra making it better suited for similarity searching. To show this, results were evaluated for features that had MS2 fragments commonly found in indole containing compounds and whether these potential indole containing features had

additional data collected on them. Due to forward scoring giving lower scores when there are additional fragments in the experimental spectra, only about one third of the potential indole containing features with MS2 data had additional data collected. In contrast, with reverse scoring over 90% of these features had additional data collected.

**Novel Component**

Reverse cosine similarity scoring during RTLS for more effective triggering of additional experiments on compounds similar to indole compounds.

## Full Abstracts for Tuesday Posters

**----------Poster Board #1----------**

**Title**

**A role for Untargeted Metabolomics in cultured meat production through optimizing media composition and growth conditions**

**Author(s) (Presenting author should be listed first)**

Cohen Tom; Stancliffe, Ethan; Richardson, Adam; Mehta, Ashima; Gandhi, Monil; Guzior, Doug V; Cho, Kevin; Patti, Gary

**Author Affiliations**

Panome Bio, Washington University in Saint Louis

**Introduction**

Cultured meat production, the cultivation of animal cells to create meat products without livestock, offers a sustainable and ethical alternative to traditional meat. However, high costs and scaling challenges have hindered its commercialization. To address these limitations, we applied untargeted metabolomics to optimize media formulation and bioreactor conditions in a cultured meat production process. By analyzing media and cells from a leading cultured seafood manufacturer using Panome Bio's metabolomics workflow, we identified critical media modification strategies and a need to modify oxygen levels that when implemented improved cell growth efficiency.

**Methods**

Panome Bio used untargeted metabolomics to identify metabolites consumed by a cellular population, metabolites secreted into media, and pathway-level metabolic changes during growth. First, cell pellets and supernatant media were collected from seven timepoints (n=4). Then both cells and media were subjected to separate polar and lipid sample preparation methods. Data was then acquired through reversed-phase and hydrophilic interaction liquid chromatography coupled to QTOF a mass spectrometer. Raw mass spec files were processed through the Panome bioinformatic analysis pipeline which includes unbiased deep learning-based signal detection, robust quality control and filtering, and MS/MS and retention time-based metabolite identification. Hits were then identified, followed by pathway analysis that fed into our biological interpretations of how the systems changed over time.

**Preliminary Data**

1691 metabolites were detected in cell pellets while 1301 metabolites were detected in supernatant media. 73 of these metabolites in cell pellets, and 71 in supernatant media were found to be significantly altered over time with high-confidence. The time-course nature of this experiment allowed us to determine which of the significantly altered metabolites were being excreted by the cells into the media, which were consumed from the media by the cells, and which were significantly altered in both cell and supernatant media samples. Pathway analysis on these hits identified four significantly affected metabolic pathways in the cultured cells while changes in the supernatant media sample metabolite composition resulted in one significantly affected metabolic pathway. This data together was used to generate three main hypotheses 1) the media contains an abundance of a specific nutrient, 2) The feed media contains excess of a specific amino acid, and 3) The bioreactor appears to be oxygen deficient. With this information, the process parameters were adjusted to increase growth efficiency of the cell population.

**Novel Component**

Successful and interesting application of untargeted metabolomics (improving cultured meat production)

**----------Poster Board #2----------**

**Title**

**Biochemical phenotyping of null alleles in human induced pluripotent stem cell-derived cell lineages: integrating genetics with metabolomics**

**Author(s) (Presenting author should be listed first)**

Thapa, Maheshwor; Zheng, Shujian; Mitchell, Joshua; Diniz, Juliana; Oliveira, Nelio; Tagger, Arti; Chi, Yuanye; Siddiqa, Amnah; Gong, Minghao; McDonough, Justin; Skarnes, William; Robson, Paul; Li, Shuzhao.

**Author Affiliations**
The Jackson Laboratory for Genomic Medicine, 10 Discovery Dr, Farmington, CT 06032

**Introduction**
Induced pluripotent stem cells (iPSCs) are a key tool for biomedical research and drug discovery. Null alleles can be created in iPSCs using CRISPR-Cas9 based gene editing and the cells subsequently differentiated into many cell types. The NIH/NHGRI recently initiated the Molecular Phenotypes of Null Alleles in Cells (MorPhiC) consortium which aims to create a comprehensive catalog of null alleles for every human gene with associated cellular differentiation schemes and subsequent phenotyping. Here, we employ multiple metabolomics methods to demonstrate a robust, unbiased tool for phenotyping biochemical pathways in iPSC-derived cell types. Comparative metabolomics between wild-type and null alleles enables the genetic dissection of these biochemical pathways toward comprehensive characterization of gene function.

**Methods**
Comprehensive metabolic profiling methods were developed utilizing liquid chromatography coupled with ultrahigh-resolution mass spectrometers. This included two-phase and one-phase extraction methods for cell pellets and supernatant and reverse phase and HILIC chromatography methods for both ionization modes. Chemoselective derivatization methods using reagents such as dansyl hydrazine, dansyl chloride, and dimethylaminophenacyl bromide were developed to enhance the detection of poorly ionizable metabolites. Furthermore, C13 isotope tracing protocols utilizing multiple tracers were developed. Combinations of these methods were applied to samples from cultured iPSCs with null mutations for alleles of interest, along with corresponding wild type and suitable controls. Furthermore, we have compiled the detected metabolites into a metabolic atlas with a user-friendly front interface to assist researchers utilizing iPSCs in their research.

**Preliminary Data**
Biochemical phenotyping and the resulting compendium of data is a powerful tool to enable the discovery of novel biology. To date, we have generated 20 datasets including chemoselectively-derivatized, isotopically-labeled metabolomics, and lipidomics datasets for 4 null allele mutations. These datasets have been analyzed using our Python-centric pipeline for metabolomics and the detected metabolites, both known and unknown, are summarized in a public-facing, searchable, and navigable, iPSC metabolic atlas (https://ipsc-atlas.web.app/).

Metabolomics and lipidomics datasets of iPSCs differentiated into primitive syncytium (PrSyn) illustrate the application of biochemical phenotyping to understand gene function on a molecular level. Through the comparison of KOs and controls, the effects of mutations and the culture microenvironment can be dissected. For example, media and supernatant samples of PRLR KO-containing PrSyn clearly cluster with respect to their genotype, but also in accordance with the media on which they were cultured, illustrating the substantial impact of the culture microenvironment on observed metabolic phenotypes. However, many metabolites of interest are not observable using traditional LC-MS techniques, including the steroids implicated in the differentiation and function of PrSyn, necessitating the need for chemoselective derivatization to enable their detection. Using dansyl chloride and dansyl hydrazine derivatization of cell pellets and cell extracts, key steroids, including progesterone and estrogen, are detectable. Using these techniques the role of key genes in the steroidogenesis pathway in PrSyn including: CYP19A1, CYP11A1, HSB17B1, ABCG2, and SLC22A11. Cells for each KO were cultured in media containing DHEA-S, a signaling molecule that stimulates steroidogenesis, and without DHEA-S for these comparisons along with wild-type controls.

**Novel Component**
The data compendium and atlas provide a powerful tool to support the discovery of novel biology.

**----------Poster Board #3----------**

**Title**
**Genetic Mechanisms Controlling Development: Insights from Metabolomics**

**Author(s) (Presenting author should be listed first)**
Jaiyesimi, Olakunle; Extavour, Cassandra

**Author Affiliations**
1. Department of Organismic and Evolutionary Biology, Harvard University, 2. Department of Molecular and

Cellular Biology, Harvard University 3. Howard Hughes Medical Institute, Chevy Chase, Maryland, United States of America

**Introduction**

Ripe Morinda citrifolia, also called noni, fruit increases egg-laying and improves developmental traits in Drosophila sechellia relative to standard laboratory diet. However, the ripe noni fruit is toxic to its generalist sister, D. melanogaster. The fruit inhibits generalists' egg-laying traits, and all adult flies die within a few days of exposure relative to standard laboratory diet. The molecular mechanism underlying these differential traits relative to environment is unknown. Many phenotypes are multifactorial, polygenic, and are influenced by environmental factors. In this study, we aim to use metabolomics to identify candidate genes and pathways with potential roles in specifying the differential reproductive and developmental traits in D. melanogaster and D. sechellia when exposed to ripe noni fruits.

**Methods**

Age-matched adult virgin female laboratory populations of D. melanogaster and D. sechellia were mated and exposed to different proportions of ripe noni fruits in standard laboratory diet for 15 days. The number of eggs laid by both species exposed to the different diets were counted daily throughout the experimental period. The rate of development of the F1 progenies was also determined. The ovaries of the F0 females were dissected and subjected to LCMS-based untargeted metabolomics data acquisition in both positive and negative ionization modes using the Zic pHILIC column 150x2.1mm 5micron. Feature-selection was performed on curated data, and the top 250 annotated and harmonized LCMS features were subjected to pathway analysis using the Drosophila melanogaster KEGG pathway library.

**Preliminary Data**

We report that D. melanogaster flies laid no eggs and all flies died in 100% ripe noni fruit. Although D. sechellia laid more eggs in 100% ripe noni fruit relative to standard laboratory diet, the development of the F1 generation was halted at pupation. When exposed to a specific mix of ripe noni fruit and standard laboratory diet, both D. melanogaster and D. sechellia laid more eggs relative to standard diet, but only D. sechellia displayed increased rate of development to adulthood.

Our pathway analyses revealed pathways with intermediates whose abundance distribution across the treatment groups strongly correlated with the observed phenotypic responses of D. melanogaster and D. sechellia to different diets. We report pathways with potential contributions to differential reproductive and developmental traits between adult female D. melanogaster and D. sechellia exposed to different ripe noni fruit proportions in standard laboratory diet. These include tryptophan metabolism via the kynurenine pathway, methionine metabolism and urate biosynthesis amongst others.

**Novel Component**

The use of untargeted metabolomics to highlight genes and pathways with potential contributions to phenotypes of interest.

**----------Poster Board #4----------**

**Title**

**Multi-omics approaches for the identification of HCC biomarkers in patients with liver cirrhosis**

**Author(s) (Presenting author should be listed first)**

Rashid, Md Mamunur; Varghese, Rency; Ressom, Habtom

**Author Affiliations**

Georgetown University

**Introduction**

Hepatocellular carcinoma (HCC), the most common form of liver cancer, is the third highest cause of death worldwide. Patients diagnosed with HCC have a poor prognosis mostly because symptoms typically appear during advanced stages of the disease. Furthermore, traditional biomarkers exhibit inadequate performance in early-stage HCC detection, hence emphasizing the necessity for the discovery of more potent biomarkers. This study aims to discover and confirm a panel of serum biomarkers for the detection of HCC in patients with liver cirrhosis (CIRR) through multi-omics approaches including metabolomics and lipidomics.

**Methods**
Serum samples from 20 HCC cases and 20 patients with CIRR were analyzed using ultra-high-performance liquid chromatography-Q-Exactive mass spectrometry (UHPLC-Q-Exactive-MS). Both univariate and multivariate analyses including t-test, principal component analysis (PCA), and partial least square discriminant analysis (PLS-DA) were performed to compare the data from the two groups and to identify metabolites and lipids significantly altered in HCC vs. CIRR.
**Preliminary Data**
Metabolites and lipids that are significantly altered in HCC vs. CIRR have been identified. These consist of organic acids, amino acids, TCA cycle intermediates, fatty acids, bile acids, glycerophospholipids, sphingolipids, and glycerolipids. Most of these identified putative metabolites were further verified against the commercially available standards by comparing their MS/MS fragments and retention time (RT) values. The concentrations of fatty acids, glycerophospholipids, and bile acids displayed the most notable variation. Whereas phosphatidylethanolamine (PE) and triglycerides (TG) levels were increased significantly in HCC cases, fatty acid (FA) and phosphatidylcholine (PC) levels were significantly decreased. Furthermore, the selected metabolites were found to have a better area under the receiver operating characteristic (ROC) curve compared to alpha-fetoprotein (AFP). Pathway analysis of these metabolites revealed fatty acid, lipid, and energy metabolism as the most impacted pathways. Future work will focus on validating biomarker candidates identified in this study by targeted quantitation using a large cohort of independent samples.
**Novel Component**
This study led to the identification of metabolites and lipids as biomarkers for HCC in patients with liver cirrhosis.

**----------Poster Board #5----------**
**Title**
**RaMP-DB 3.0: A Relational Database for Multi-Omic Data Interpretation**
**Author(s) (Presenting author should be listed first)**
Mehta, Khyati Y.; Patt, Andrew; Sheils, Timothy; Tisch, Adam; Sayer, Jaden; Braisted, John; Kelleher, Keith J.; Mathé, Ewy A.
**Author Affiliations**
National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD
**Introduction**
RaMP-DB is a public, up-to-date and comprehensive resource for biological, chemical, ontology and reaction annotations and for metabolomic and multi-omic data interpretation. We present here our version 3.0 which includes some key new features: updates to currently included knowledge sources, conversion to an SQLite database, the addition of Rhea reactions, interactive visualizations, and additional enrichment functions. RaMP-DB has multiple access points through the R package, SQLite database, APIs, and an interactive web application. This version also supports batch queries on annotations. Of note, RaMP-DB has a transparent and reproducible process which enables frequent updates. The database itself is also useful for improving and developing new pathway analysis methods.
**Methods**
RaMP-DB parses information from HMDB, KEGG (through HMDB), Reactome, WikiPathways, LIPIDMAPS, Rhea, and ChEBI. Analyte mappings across databases is semi-automated by cross-referencing IDs from each source database. Potential errors in mapping are identified by calculated molecular weight differences which are then manually curated and fixed. Another major upgrade is that RaMP-DB 3.0 is available as an SQLite file, which replaces MySQL, allowing for significant ease in integration with other tools, such as MetaboAnalyst and the Bioconductor Computational Metabolomics Ecosystem. The R package installation automatically downloads the SQLite Database file and stores it in a local file cache. This has simplified the previous 8-step MySQL installation process into 3 simple steps of download, load, and run the package.
**Preliminary Data**
The current version of RaMP-DB includes 254,860 chemical structures, of which 43,338 are lipids, 15,389 genes, 53,745 pathways, 15,849 curated reactions and an additional 791,513 predicted reaction, and 699

ontologies. In addition to the expansion of content from the source databases, there has been an increased focus on lipid annotations and an incorporation of curated reactions from Rhea. For streamlined mapping of analytes (metabolites, proteins/genes) to the resource, RaMP-DB handles mixed IDs for input analytes (e.g. LIPIDMAPS, PubChem, HMDB for metabolites and UniProt, HMDB, and Ensembl IDs for genes/proteins). A complete list of supported IDs is easily retrievable through a simple lookup function available within the package.
Two major components of RaMP-DB analyses are lookups and enrichment analyses. First, RaMP-DB can perform simple queries to â€˜lookup' information such as what chemical class or ontological information about the analytes. These lookups enable users to globally evaluate how much is known about analytes of interest. Second, RaMP-DB can facilitate multi-omic pathway enrichment analysis and chemical class enrichment for metabolites. Clustering of enriched pathways is performed to group together similar or redundant pathways thereby easing interpreting of results. Functions to generate interactive visualizations, such as sunburst and upset plots, have also been added. These plotting functions take from outputs of existing functions to streamline data exploration.
The RaMP-DB R package includes vignettes, example data, documented functions, and all the code is publicly available at https://github.com/ncats/RaMP-DB. The website UI https://rampdb.nih.gov/ has been redesigned to condense similar query pages and expose query options supported by the RaMP R package. Programmatic access is also available via our API and includes Swagger documentation. Overall, RaMP-DB is a multi-use tool that integrates widely used knowledge sources for facilitating multi-omics data interpretation through various access portals.
**Novel Component**
User-friendly and programmatic access to comprehensive multi-omic annotations and enrichment analyses.

**----------Poster Board #6----------**
**Title**
**A quantitative assay for measuring 1200+ metabolites in biofluids**
**Author(s) (Presenting author should be listed first)**
Mandal, Rupasri; Zheng, Jiamin ; Zhang, Lun; Johnson, Mathew; Wishart, David S.
**Author Affiliations**
University of Alberta
**Introduction**
Metabolomics involves the comprehensive characterization of small molecule metabolites in specific biological specimens, such as blood, urine, feces and saliva. Metabolomics has traditionally been divided into two complementary but somewhat different approaches: untargeted and targeted metabolomics. Untargeted metabolomics is a discovery-based approach that aims to â€œqualitativelyâ€ measure all detectable spectral features over a large number of biospecimens. Targeted metabolomics studies are aimed at quantitatively measuring a small number of targeted compounds that can be readily used in clinical chemistry, medical diagnoses, and disease biomarker discovery studies. While untargeted methods can generate data on up to 1000 compounds, targeted methods usually generate data only on 100-200 compounds. In an effort to allow targeted metabolomics to achieve the same coverage as untargeted metabolomics, we have developed a custom made, comprehensive, quantitative LC-MS/MS-based assay for targeted metabolomics analysis of biospeimens
**Methods**
Our method uses a combination of direct injection (DI) mass spectrometry with a reverse-phase LC-MS/MS. It combines the derivatization and extraction of analytes, and the selective mass-spectrometric detection using multiple reaction monitoring (MRM) pairs. Two separate panels involving two different precolumn derivatization reactions were developed for this assay: Panel A - Phenylisothiocyanate (PITC) derivatization targeting amine-containing compounds and Panel B - 3-nitrophenylhydrazine (3-NPH) derivatization targeting keto- and carboxyl-containing compounds. Isotopically-labeled internal standards are used for metabolite quantification. Calibration of metabolite concentration ranges in both panels was adjusted for different biofluid types. Mass spectrometric analysis was performed on Sciex 5500 QTrapÂ® tandem MS instrument equipped with an Agilent 1290 series UHPLC system

**Preliminary Data**
This custom assay can be used for the targeted identification and quantification of up to 1000 metabolites across 21 chemical classes including amino acids and derivatives, biogenic amines, organic acids, nucleotides/nucleosides, ketone and keto acids, indole derivatives, vitamins and derivatives, sulfates, dipeptides and 11 different classes of lipids. In addition, 400 biomedically related metabolite sums and ratios can be calculated from concentrations of these 1000 targeted metabolites. In total, this custom assay can measure up to 1400 metabolites and ratios/sums. The accuracy of QC standards with 3 different concentration levels are in the range of 80% to 120% with satisfactory precision values of less than 20%. The recovery rates of spiked serum, urine and fecal extract samples with three different concentration levels are in the range of 80% to 120% with satisfactory precision values of less than 20%. More than 1500 biological samples such as serum, urine and fecal ex
tracts have been analyzed in our laboratory variations of this assay since 2021. The analysis time is approximately 1 sample/hour on a single LC-MS/MS instrument or about 100 samples over 4 days.
**Novel Component**
This assay allows for the identification of 1200+ metabolites along with another 400 metabolite sums and ratios.

**----------Poster Board #7----------**
**Title**
**Activity guided fractionation to identify the quorum molecule in Neurospora crassa**
**Author(s) (Presenting author should be listed first)**
Molina, Alexis; Esselman, Christopher; Edison, Art
**Author Affiliations**
University of Georgia
**Introduction**
Quorum sensing serves to benefit a fungus by allowing spores to exhibit homing and create Conidial Anastomosis Tubes (CATs). The reduced branching of CATs creates a more compact structure, providing higher chances of colonization in environments which do not have enough glucose for the number of cells present. Consistent with this theory, we have noticed CAT formation in high density cultures, and a lack of homing in low-density cultures of the filamentous fungus, Neurospora crassa. While much is known about the CAT formation, the quorum molecule(s) have not been identified.
**Methods**
I will first spike my low density cultures with unfractionated, spent high density media. This will induce a high-density phenotype if the quorum molecule is in the high density media. I will use HPLC to separate metabolites within the spent media from high density cultures. I will use the fractions to â€œspikeâ€ my low density cultures with high density metabolites. I expect one or more of these fractions to induce a high density phenotype in a low density culture. I will use NMR and MS to further validate and identify the signaling molecule(s) once a fraction induces a high density phenotype.
**Preliminary Data**
Current data has revealed that the quorum molecule is in the media and can induce a high density phenotype from a low density inoculation. When low density cultures were spiked with some level of high density media, there was a clear and visible CAT formation in the cultures. From here, we began to separate the metabolites by HPLC. By using a Hilic HPLC column we were able to successfully extract metabolites from our spent high density media. By using HPLC I can create fractions which separate metabolites present in the high density media. I can spike low-density cultures with these fractionated metabolites to deduce which fraction contains the quorum sensing molecule(s). I can then use NMR to determine the structure of the quorum sensing compound.
**Novel Component**
Activity guided fractionation

**----------Poster Board #8----------**
**Title**

**Application of NMR-based metabolomics to identify potential cerebrospinal biomarkers of disease progression in patients with multiple sclerosis.**

**Author(s) (Presenting author should be listed first)**
Nadia Ashrafi, Ahmet Tarik Baykal, PhD; Boran Aksakal, Ceyda Buyuker, Eda Tahir Turanli, PhD, Stewart F. Graham, PhD and Ali Yilmaz, PhD.
**Author Affiliations**
Nadia Ashrafi, Ahmet Tarik Baykal, PhD; Boran Aksakal, Ceyda Buyuker, Eda Tahir Turanli, PhD, Stewart F. Graham, PhD and Ali Yilmaz, PhD.

**Introduction**
Multiple sclerosis (MS) is a chronic and progressive neurological disorder, characterized by neuroinflammation and demyelination within the central nervous system (CNS). Despite ongoing research, the underlying causes and mechanisms of MS remain elusive. Currently, there are no effective treatments, reliable diagnostic tools, or accurate prognostic indicators for MS. To address this knowledge gap, our study focused on identifying metabolic changes in cerebrospinal fluid (CSF) obtained longitudinally followed patients with primary and secondary progressive types of MS to explore differences across various MS subtypes.

**Methods**
Utilizing proton Nuclear Magnetic Resonance (1H NMR) spectroscopy we quantitatively profiled CSF from patients with primary MS (n = 40) and those who are diagnosed with secondary MS (n = 38). Further, we systematically evaluated the utility of several machine learning techniques to identify significant differences between the metabolite profiles of those MS groups.

**Preliminary Data**
1H NMR spectroscopy-based metabolomics, in combination with bioinformatic analyses, provided useful information highlighting previously unreported biochemical pathways and CSF-based biomarkers associated with both primary and secondary MS. Results of this metabolomics study indicated that metabolite panel consists of adenosine triphosphate (ATP), tryptophan, formate, glutathione, inosine, pantothenate, and nicotinamide adenine dinucleotide (NAD) exhibited highest classification accuracy between MS patients.

**Novel Component**
This study highlights the potential of 1H NMR-based metabolomics in comprehensively profile the metabolome of cerebrospinal fluid (CSF), which serves as a proxy for brain metabolism. To predict MS risk, future studies should explore the practical application of these CSF-derived biomarkers in more easily accessible biological samples. Moreover, it is crucial to verify whether the identified biochemical pathways are mirrored in the brains of MS patients, with the ultimate goal of uncovering potential targets for therapeutic intervention.

**----------Poster Board #9----------**

**Title**
**Combination of Low Glucose and SCD1 Inhibition Impairs Cancer Metabolic Plasticity and Growth: A Comprehensive Metabolomic and Lipidomic Analysis.**

**Author(s) (Presenting author should be listed first)**
Zhu Wentao;  Raftery Daniel
**Author Affiliations**
University of Washington

**Introduction**
Cancer cells exhibit remarkable metabolic plasticity, enabling them to adapt to fluctuating nutrient conditions. This study investigates the impact of a combination of low glucose levels and inhibition of stearoyl-CoA desaturase 1 (SCD1) using A939572 on cancer metabolic plasticity and growth.

**Methods**
A comprehensive metabolomic and lipidomic analysis was conducted to unravel the intricate changes in cellular metabolites and lipids. MCF-7 cells were subjected to low glucose conditions, and SCD1 was inhibited using A939572. The resulting alterations in metabolic pathways and lipid profiles were explored to elucidate the synergistic effects on cancer cell physiology.

**Preliminary Data**

The combination of low glucose and A939572-induced SCD1 inhibition significantly impaired cancer cell metabolic plasticity. Metabolomic analysis highlighted shifts in key glycolytic and amino acid pathways, indicating the cells' struggle to adapt to restricted glucose availability. Lipidomic profiling revealed alterations in lipid composition, implying disruptions in membrane integrity and signaling cascades.

**Novel Component**

Our findings underscore the critical roles of glucose availability and SCD1 activity in sustaining cancer metabolic plasticity and growth

**----------Poster Board #10----------**

**Title**

**Dual MSTUS sample-to-sample Normalization as a Batch-to-Batch normalization correction**

**Author(s) (Presenting author should be listed first)**

Beecher C.(2), Ghosh D.(1), de Jong F.(2), Shulaev V.(1)

**Author Affiliations**

1Department of Biological Sciences, College of Science, University of North Texas, Denton, Texas; 2 IROA Technologies LLC, Chapel Hill, North Carolina

**Introduction**

We had previously shown that the Dual-MSTUS normalization was useful for batch-to-batch error correction. Here we demonstrate an improvement to this normalization that reduces sample variance further. In any MSTUS algorithm it is assumed that the more compounds that are included the better. We now show that variances are further reduced if we use a subset of all compounds seen (the current algorithm) namely, only those compounds found in all samples (Core compounds). The current belief is to include all validated compounds that may appear only in a small portion of samples as their presence in the subset of samples will alter their normalization. The rationale and consequences of this will be discussed.

**Methods**

In this study Saccharomyces cerevisiae were treated with 4 different oxidants known to be involved in oxidative stress. An Internal Standard (IROA-IS) was included in every sample. Samples from the entire experiment were processed and analyzed in batches on several different days across several weeks. After the fourth batch, the electrospray ionization source was removed, cleaned, and re-tuned to articulate very strong batch effects before processing further batches. Data obtained from mass-spectrometer was used to optimize our Dual-MSTUS normalization method. A Dual-MSTUS normalization corrected for the batch effect errors easily, but sample-to-sample (STS) variances could be reduced further by using only the â€œCoreâ€ peaks (seen in all samples) rather than the current standard of â€œAllâ€ peaks seen in each sample.

**Preliminary Data**

In this study Saccharomyces cerevisiae were treated with 4 different oxidants known to be involved in oxidative stress. An Internal Standard (IROA-IS) was included in every sample. Samples from the entire experiment were processed and analyzed in batches on several different days across several weeks. After the fourth batch, the electrospray ionization source was removed, cleaned, and re-tuned to articulate very strong batch effects before processing further batches. Data obtained from mass-spectrometer was used to optimize our Dual-MSTUS normalization method. A Dual-MSTUS normalization corrected for the batch effect errors easily, but sample-to-sample (STS) variances could be reduced further by using only the â€œCoreâ€ peaks (seen in all samples) rather than the current standard of â€œAllâ€ peaks seen in each sample.

**Novel Component**

Enhanced MSTUS Normalization procedure, IROA, Internal Standard, workflow

**----------Poster Board #11----------**

**Title**

**ECIDbase.org : Exposome Correlation and Interpretation Database (ECID)**

**Author(s) (Presenting author should be listed first)**

Dinesh Barupal

**Author Affiliations**

Icahn School of Medicine at Mount Sinai

**Introduction**

Inter-chemical correlations are ubiquitously observed in metabolomics and exposomics datasets. ECID is a new NIEHS-funded Biomedical Knowledgebase (U24ES035386) to catalog inter-chemical correlations observed in the targeted and untargeted biomonitoring datasets for human specimens (blood, urine, feces, saliva, tissues, cell-lines). These correlations can indicate biochemical and chemical relationships among metabolites and exposome chemicals.

**Methods**

We have included 315 datasets of human specimens covering ~ 260,000 samples, > 5,000 annotated compounds and thousands of not yet identified compounds. Targeted datasets (sample count=171,000) were sourced from NHANES, HHEAR and ECHO databases. Untargeted data (sample count=89,000) were sourced from EBI MetaboLights, Metabolomics WorkBench and GNPS data repositories and supplementary tables of selected metabolomics publications. Inter-chemical correlations were computed using the Spearman coefficient.

**Preliminary Data**

We have identified several thousands strong correlations (Spearman coefficient > 0.50) in these datasets. An interpretation resource was compiled using NCBI biochemical, chemical and literature databases. These correlations were interpreted using nine different levels covering reactions (enzymatic, transformations), pathways, GO metabolic processes, MeSH terms, chemical classes, literature co-occurrences, chemical similarity and sources, yielding a curated set of ~ 15,000 unique correlations. Inclusion of formula annotation of unknown metabolites have annotated several closely related metabolites that are potentially new reactants in known biochemical reactions. In a test study, we find distinct changes in inter-chemical correlations in a cardiovascular metabolomics dataset, indicating how the exposome-metabolome relationships differ by CVD groups. The database is being actively updated. We have made this resource available at ecidbase.org and all the underlying data will be made available at Zenodo.org.

**Novel Component**

ECIDbase will serve as a new discovery-driving engine to gain biomedically useful insights from targeted and untargeted metabolomics and exposomics datasets for human specimens.

**----------Poster Board #12----------**

**Title**

**Effects of light intensity on Phaeodactylum tricornutum primary carbon metabolism and TAG synthesis**

**Author(s) (Presenting author should be listed first)**

Zheng, Amy; Wang, Bo; Ruiz-Marquez, Kevin; Cheah, Yi Ern; Paton, Andrew; Kassaw,Tessema; Peers, Graham;Young, Jamey

**Author Affiliations**

Vanderbilt University (1-4,8), Colorado State University(5-7)

**Introduction**

The model microalgae, Phaeodactylum tricornutum (Pt), has received significant attention as a sustainable feedstock for biodiesel production due to their ability to accumulate lipids. Optimizing the carbon flux within microalgal metabolism is crucial for enhancing lipid yields while maintaining biomass productivity. Recent advances in metabolic flux analysis (MFA), particularly 13C isotopic nonstationary metabolic flux analysis (INST-MFA), have enabled measurement of intracellular fluxes to systematically develop gene targets. This research focuses on leveraging INST-MFA to dissect the metabolic responses of Pt under varying light intensities, with the goal of improving lipid productivity for biodiesel applications. This study investigates the role light intensities effects primary metabolism and carbon allocation.

**Methods**

To explore the metabolic shifts in Pt under varying light conditions, cultures were grown under low (60Î¼E) and high light intensities (250Î¼E). We employed isotope tracer studies where cultures are labeled with a 13C tracer. Samples were taken at multiple time points to capture tracer incorporation into metabolites. The isotopic labeling of key metabolites was quantified using gas chromatography-mass spectrometry (GC-MS) and tandem liquid chromatography-mass spectrometry (LC-MS/MS). The time-resolved labeling data was fitted to a

computation model of the metabolic network using INST-MFA. The resulting flux map quantifies the metabolic fluxes with defined confidence intervals.

**Preliminary Data**

Our isotope labeling studies in Pt revealed that varying light intensities have a profound effect on the partitioning of carbon between biomass and lipid production. Under low light conditions, carbon primarily flows toward biomass synthesis, with minimal accumulation of lipids. However, when cultures are exposed to high light intensities, a notable shift occurs, where carbon is directed not only toward biomass but also toward the accumulation of triacylglycerols (TAGs), the major precursor for biofuels. This shift is observed without substantial changes in the fluxes of primary metabolic pathways, indicating that light intensity specifically affects the carbon endpoint allocation rather than the overall metabolic throughput.

At low light, carbon fixation leads primarily to cellular growth and maintenance, with TAG formation being minimal. However, high light encourages more carbon fixation, leading to biomass production and significant TAG accumulation. Despite this shift in carbon fate, primary metabolic fluxes remain relatively unchanged across both light conditions, suggesting a robustness in core metabolic functions. The redirection of carbon toward TAG at high light occurs downstream of primary metabolism, likely influenced by regulatory mechanisms responding to the energy surplus generated under these conditions. These findings demonstrate that light intensity is a key factor in controlling the metabolic fate of carbon.

**Novel Component**

Light intensity shifts carbon partitioning toward TAG without significant alteration to primary cycle fluxes.

**----------Poster Board #13----------**

**Title**

**EnrichMet: an R package for quick and easy pathway enrichment**

**Author(s) (Presenting author should be listed first)**

Dhake, Neha : Stewart, Paul*

**Author Affiliations**

1 Department of Bioinformatics & Computational Biology, Morsani College of Medicine, University of South Florida, Tampa, FL 33612 2 Department of Biostatistics and Bioinformatics, Moffitt Cancer Center, Tampa, FL 33612 * Corresponding author

**Introduction**

Untargeted mass spectrometry-based metabolomics studies can identify hundreds of metabolites in a single experiment, posing a challenge for biological interpretation. Pathway enrichment approaches consolidate numerous metabolites into pathways, offering insights into how these metabolites relate to underlying biological processes. However, most modern metabolomics-based pathway enrichment tools face challenges, including reliance on web interfaces that yield non-reproducible results, lack of appropriate background correction, and slow external database dependencies. Our R package, EnrichMet, addresses these issues by offering a fast, local solution for pathway enrichment. Its main functionality requires only a single line of code in R and incorporates background correction to enhance accuracy.

**Methods**

EnrichMet takes a list of interesting metabolites (e.g., differentially expressed) and a list of background metabolites as input. It performs pathway enrichment analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and Fisher’s Exact Tests, which compares the frequency of metabolites in predefined pathways to a background set to assess statistical significance. P-values can be adjusted using common methods such as Benjamini-Hochberg, Storey's Q-values, and Bonferroni. Results can be exported as a table in CSV format or as figures in PNG, TIFF, or PDF format. Importantly, our package has minimal external dependencies, ensuring that it can be quickly installed and that it will work on nearly all workstations.

**Preliminary Data**

To facilitate pathway enrichment analysis, we used the keggrest R package to parse all of KEGG and compile a comprehensive table of metabolites and their associated pathways. This table, along with other resources, will be included in the final package. We obtained a list of example differentially expressed metabolites from MetaboAnalyst, tested our package functionality on this data, and received comparable results to

MetaboAnalyst's own pathway enrichment functionality. Resulting p-values were computed and transformed to -log10(p-values) for visualization, and the results were represented using a bar plot to highlight the most significant pathways. Ongoing work will incorporate additional resources, including the Human Metabolome Database (HMDB), to further enhance pathway enrichment capabilities. Additionally, a Shiny app is planned to provide a web interface for users without R experience to access our tool.

**Novel Component**

Quick and easy pathway enrichment with a single R command.

**----------Poster Board #14----------**

**Title**

**Estimating the Biological Potency of 2,3-Benzofluorene via Untargeted Metabolomics Analysis of Plasma from a Short-Term in vivo Study**

**Author(s) (Presenting author should be listed first)**

Crizer, David; Rice, Julie; Auerbach, Scott

**Author Affiliations**

Division of Translational Toxicology/National Institute of Environmental Health Sciences

**Introduction**

Polycyclic aromatic hydrocarbons (PAHs) are a class of compounds to which humans are widely exposed. 2,3-benzofluorene is a member of this class of compounds which has been associated with numerous toxicological effects. Currently, there exists a lack of in vivo toxicological data on 2,3-benzofluorene. Previously, short-term in vivo gavage studies in rats coupled with transcriptomics of liver and kidney tissue has been shown to be a good estimate of biological potency when compared to apical endpoint from longer-term toxicity studies. In this work, we used untargeted metabolomics of rat plasma samples from a short-term repeat dose in vivo study to estimate the biological potency of 2,3-benzofluorene.

**Methods**

A short-term in vivo study on 2,3-benzofluorene in adult male and female Sprague Dawley rats was conducted by administering a daily oral gavage for 5 days. 2,3-benzofluorene was administered at 10 doses ranging from 0 to 1,0000 mg/kg body weight. After final dose, animals were euthanized, and plasma samples were collected. Plasma samples were extracted and then analyzed via C18 (nonpolar) and HILIC (polar) ultra-high pressure liquid chromatography-mass spectrometry (UHPLC-MS) methods using a Thermo Vanquish LC system in conjunction with a Thermo Q Exactive Plus. Modeling was conducted to identify benchmark doses (BMDs) associated with metabolic changes in blood plasma.

**Preliminary Data**

Short-term in vivo gavage studies with transcriptomics on liver and kidney tissue have recently been used to estimate biological potencies that provide a good approximation of toxicological potency in long-term guideline toxicity studies. These short-term in vivo studies can be used to generate estimates of biological potency more rapidly for chemicals with little, if any, in vivo toxicological information. However, a potential drawback of this approach is a reliance on select target organ tissues for assessment. The analysis of blood plasma samples from these short-term in vivo gavage studies using metabolomics offers an alternative solution to this reliance on sentinel organ tissues. Here, we analyze blood plasma samples from Sprague Dawley rats dosed once daily with 2,3-benzofluorene using untargeted metabolomics analysis in conjunction with benchmark dose (BMD)analysis to estimate biological potency and better understand biological changes elicited by test chemicals. The untargeted metabolomics analysis used consists of four UHPLC-MS assays: polar (positive and negative mode) and nonpolar (positive and negative mode). XCMS was used to deconvolute UHPLC-MS data to detect individual features. These features were then filtered for quality and imported in BMDExpress for benchmark dose analysis. BMDs were derived for each of the four UHPLC-MS assays and then combined to determine an overall estimate of biological potency for 2,3-benzofluorene. This analysis resulted in finding 148 metabolite features that fit a dose-response relationship. The most sensitive metabolic feature (m/z =271) was found to have a BMD value of 0.14 mg/kg. Work is ongoing to annotate those metabolite features that exhibited a dose-response fit.

**Novel Component**

Using untargeted metabolomics and benchmark dose analysis to estimate biological potency of a data poor polycyclic aromatic hydrocarbon (PAH), 2,3-benzofluorene.

**----------Poster Board #15----------**

**Title**

**Exploring Acyl Carnitines in the Human Metabolome Using Reverse Metabolomics**

**Author(s) (Presenting author should be listed first)**

Emily C. Gentry, Sara Pacini, Lindsay E. Sandusky

**Author Affiliations**

Virginia Tech, Department of Chemistry, Blacksburg, VA, USA

**Introduction**

Structure identification is essential for untargeted metabolomics data interpretation yet remains a major bottleneck in analysis. Typically in untargeted metabolomics experiments, compounds are detected first, prioritized based on biological significance, and then structurally identified. In reverse metabolomics, the process is flipped. Compound classes of interest are first identified and synthesized combinatorially, then their MS/MS spectra are searched for in public metabolomics data. This strategy enables high-throughput discovery and phenotypic profiling of metabolites in humans. Here, reverse metabolomics was used to explore where acyl carnitines are detected in humans and other vertebrates.

**Methods**

Over 100 acyl carnitines with unique molecular formulas were synthesized and product structures were confirmed by LC-MS/MS. The MS/MS spectra obtained were then searched against public metabolomics data available on the Global Natural Products Social (GNPS) Molecular Networking database using a mass spectrometry search tool called MASST. The outputs of these searches were analyzed to identify where each metabolite was found (e.g. sample type, species, disease state).

**Preliminary Data**

Analysis will be presented for reverse metabolomics searches of acyl carnitines derived from saturated, unsaturated, hydroxylated and dicarboxylic fatty acids. Overall, acyl carnitines were found to be completely ubiquitous in humans with some structures detected in nearly every system of the body. A variety of acyl carnitine structures were present in the GI tract while urine samples contained only those derived from short-chain fatty acids. Several metabolite-disease associations, some of which are novel, were discovered and will be presented here.

**Novel Component**

Using organic synthesis in combination with mass spectrometry and public data mining for repository-scale annotation and biomarker discovery

**----------Poster Board #16----------**

**Title**

**High throughput metabolite quantification in biological samples with Pyxis, a matrix-agnostic AI/ML tool**

**Author(s) (Presenting author should be listed first)**

Ana S. H. Costa, Craig Knisley, Devesh Shah, Timothy Kassis, Mimoun Cadosch Delmar, Jennifer M. Campbell, Jack Geremia

**Author Affiliations**

Matterworks, Inc, Somerville, MA

**Introduction**

The technological advances in mass spectrometry have enabled the field of metabolomics to evolve and provide better and deeper understanding of cell metabolism. As data acquisition became faster and more expansive, the conventional approaches for absolute quantification of metabolites, and our ability to rapidly process those data, became the bottlenecks in high throughput experimentation. Fast conversion of MS files into biologically relevant and actionable data is, therefore, highly desirable for biomedical research and bioprocessing. Pyxis

overcomes the limitations of conventional quantitative methods and allows for the inference of metabolite concentrations directly from raw MS data. Herein, we demonstrate Pyxis capabilities in the analysis of a variety of matrices compared to stable isotope-based methods.

**Methods**

Metabolites were isolated from eight different matrices by precipitating proteins with an organic solution spiked with stable isotope labeled standards and StandardCandles. Sample extracts were analyzed using a Transcend LX-2 multichannel system fitted with an Atlantis Premier BEH Z-HILIC column (Waters) and coupled to an Orbitrap Exploris 120 mass spectrometer (Thermo Fisher Scientific). High resolution spectra were acquired for 6.7 minutes in full scan and polarity switching mode. Calibration curves were prepared with pure standards and analyzed under the same conditions. TraceFinder software was used for stable isotope-based absolute quantification of metabolites. In parallel, the raw MS files were converted into an open format and analyzed with Pyxis.

**Preliminary Data**

Pyxis uses the signal of a small number of matrix-independent calibrators, StandardCandles, to infer the absolute concentration of metabolites, thus eliminating the need to invest in analyte-matched stable isotope labeled standards, as well as to prepare and analyze calibration curves. We developed a deep learning-based model that spans a concentration range of up to four orders of magnitude, and covers a wide range of metabolic pathways. To generate representative samples, metabolites from mammalian cells, cell culture media, and human biofluids (cerebrospinal fluid, amniotic fluid, urine, saliva, dried blood spots and blood plasma) were extracted with organic solvents with different sample:solvent ratios. We analyzed sample extracts from different matrices using Pyxis, and benchmarked it against the traditional analytical chemistry approach for absolute quantification of metabolites. Pyxis was able to identify and infer absolute concentrations of up to 89 metabolites in the various matrices, including matrices the model had not previously been benchmarked on. Results from the comparison to the current gold standard approach for targeted quantitative metabolomics showcases the immense potential of Pyxis in routine metabolomics analyses. The median R-squared between the two methods ranged between 0.62 (dried blood spots) and 0.88 (cell extracts). Furthermore, preliminary data indicates the metabolic profile of the different sample types was correctly characterized using Pyxis. This AI tool requires no method development by the end user, and analyte identification and concentration prediction takes only a few seconds per sample, it therefore, has the potential to become indispensable for both experienced researchers and beginner users for a variety of applications.

**Novel Component**

An AI/ML-based tool provides absolute metabolite quantification in trained and untrained biological matrices with comparable results to traditional methods.

**----------Poster Board #17----------**

**Title**

## Identifying Feature Recurrence via All-by-All Alignments

**Author(s) (Presenting author should be listed first)**

Hitchcock, Daniel; Krejci, Jesse; Jeanfavre, Sarah; Avila-Pacheco, Julian; Clish, Clary

**Author Affiliations**

Metabolomics Platform, The Broad Institute

**Introduction**

Determining which features are noise and which metabolites were missed in an LC-MS dataset is challenging. In fact, it is not even known how many metabolites an LC-MS method can measure, as every acquisition reveals its own unique set of features. However, given many datasets, we expect patterns to emergeâ€”specifically, that real features recur in subsequent acquisitions while noise does not. While the premise is simple, identifying feature recurrence in datasets with tens of thousands of features, most of which are unknown, is not trivial.

**Methods**

To address this, we demonstrate a novel approach to identify and measure feature recurrence using our Python module, Eclipse. We performed All-by-All alignments of 105 mixed matrix HILIC-Pos (HP-Mixed) datasets and 21 plasma (HP-Plasma) datasets using retention time and m/z as matching criteria. We then validated

random features at various recurrence thresholds to verify the correlation between recurrence rate and feature â€œequality.â€ We intend to use this information for noise removal, feature prioritization for analysis, identifying missed features that require targeted extraction, discovering novel metabolites, and gaining a better understanding of the metabolome as described by an LC-MS method.

**Preliminary Data**

In HP-Plasma, 387 features were observed in all 21 datasets, and 4,867 in more than 10 datasets. In HP-Mixed, 130 features were found in all 105 datasets, and 4,351 in more than 53 datasets.

**Novel Component**

All-by-All LCMS Dataset Alignment, Leveraging Feature Recurrence as a QC Metric

**----------Poster Board #18----------**

**Title**

**Investigating Metabolic Phenotypes For the Diagnosis of Sarcoidosis and Explore Immunometabolic Phenotypes and Unraveling Disease Mechanisms**

**Author(s) (Presenting author should be listed first)**

Mohammad Mehdi Banoei, Abdulrazagh Hashemi Shahraki, Kayo Santos, Gerg Holt, Mehdi Mirsaeidi

**Author Affiliations**

University of Calgary

**Introduction**

Sarcoidosis, a granulomatous disease affecting multiple organ systems, presents a diagnostic challenge due to its varied clinical presentation and lack of specific diagnostic tests. Blood biomarkers such as ACE, sIL-2R, CD163, CCL18, serum amyloid A, and CRP are currently used for the diagnosis and monitoring of sarcoidosis. Promising applications of metabolomics include identifying highly sensitive and specific biomarkers. This study aimed to use metabolomics to diagnose sarcoidosis at an early stage and identify metabolic phenotypes associated with disease progression.

**Methods**

Serum samples from sarcoidosis patients (n=40, including stage 1 to stage 4), were analyzed for small molecule metabolites by semi-untargeted liquid chromatography-mass spectrometry (LC-MS). Metabolomics data from sarcoidosis patients were compared with those from COVID-19 patients and healthy controls to select differentiating metabolic biosignatures. Univariate and multivariate data analysis was applied to obtaine diagnostic and prognostic metabolic phenotypes.

**Preliminary Data**

Significant changes in metabolic profiles distinguished stage 1 sarcoidosis from healthy controls, and potential biomarkers include azelaic acid, itaconate and glutarate. Different metabolic phenotypes were observed across the stages of sarcoidosis, with stage 2 showing heterogeneity compared to stages 1, 3, and 4. We investigated immunometabolic phenotypes related to inflammation in sarcoidosis patients particulary stages 3, 4 and 1 using compared it to patients with COVID-19 and healthy controls, showing metabolic pathways were upregulated in different stages of sarcoidosis.

**Novel Component**

The study identified potential biomarkers for each stage of sarcoidosis and validated findings with previous research

**----------Poster Board #19----------**

**Title**

**Large scale combinatorial synthesis to create a MS/MS reference library for the discovery of disease associated molecules**

**Author(s) (Presenting author should be listed first)**

Patan, Abubaker 1,2; Charron-Lamoureux, Vincent 1,2; Deleray, Victoria 1,2; Vittali, Kyle 1,2; Lee,Carlynda 1,2; Leanos, Daniel1,2; Mohanty, Ipsita1,2; Mannochio-Russo, Helena 1,2; Xing, Shipei 1,2; El-Abiad, Yasin 1,2; Siegel, Dionicio 1; Dorrestein, Pieter C. 1,2*

**Author Affiliations**

1Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, CA, United States. 2Collaborative Mass Spectrometry Innovation Center, University of California San Diego, La Jolla, CA, United States.

**Introduction**

Discovering new molecules and biomarkers in untargeted metabolomics remains challenging. In this study, we used reverse metabolomics as a novel data science strategy to link phenotypic information to newly synthesized compounds. This approach starts by acquiring MS/MS spectra of the synthesized compounds using liquid-chromatography tandem mass spectrometry (LC-MS/MS). A fast version of MASST (mass spectrometry search tool) is used to query all newly acquired MS/MS spectra against metabolomics repositories. Then, metadata information is used to link any phenotypic information to each MS/MS spectrum retrieved from the public domain. To validate this strategy, we are creating a reference library of 5,000,000 compounds and exploring various classes of metabolites.

**Methods**

To discover metabolites and attach phenotypic information via reverse metabolomics, we are synthesizing, over the upcoming 5 years, up to 5,000,000 molecules using combinatorial synthesis. This number seems large, but coupling of 2,000 molecules with 2,500 other host or diet-derived molecules would reach the goal. For instance, a bile acid; cholic acid can be combined with the 20 amino acids to synthesize all amino acids conjugated to cholic acid in a single reaction. This strategy can be applied to other molecular species such as amino acids, fatty acids, saccharides, bile acids, sterols, flavonoids, carnitines, and other metabolite classes. Given that mass spectrometry is a sensitive analytical technique, the reaction mixture can be analyzed directly by LC-MS/MS. This approach accelerates the creation of a large-scale reference MS/MS spectrum library for untargeted metabolomics. Reverse metabolomics -using a repository scale mass spectrometry search tool- allows us to connect MS/MS spectra from the combinatorial synthesis to understand if the new metabolites we synthesized are found to be disease associated.

**Preliminary Data**

To demonstrate the effectiveness of reverse metabolomics, we first employed combinatorial synthesis to generate complex mixtures of amino acids, fatty acids, saccharides, bile acids, sterols, flavonoids, carnitines, and other potential metabolites classes and perform the possible diversifications of the products such as (de)methylation, (de)sulfation, (de)phosphorylation, (de)glycosylation, and oxidation, including multi-step reactions. We then acquired MS/MS data of these synthetic mixtures and have obtained 182,516 MS/MS spectra from 14,000 newly synthesized compounds.

Then, we aimed to attach any biological information by recovering similar MS/MS spectra from metabolomics repositories and link metadata information to uncover phenotypic associations. We leverage the GNPS-based framework to explore their associations with health conditions in humans and rodents. We discovered that the newly synthesized molecules of the N-acyl amides bile acids classes were found in specific human diseases such as Crohn's disease, Alzheimer's disease, Kawasaki disease, and inflammatory bowel disease. reverse metabolomics can be used for structurally known and unknown molecules and attach any biological information by leveraging public metabolomics data and metadata information. The reverse metabolomics approach resulted in an expansion of metabolic knowledge using simple chemical transformations. This method can be expanded to any synthetically accessible compound class that can ionize and fragment well in a mass spectrometer.

References

1. Gentry, E. C. et al. Reverse metabolomics for the discovery of chemical structures from humans. Nature 626, 419â€“426 (2024).

2. Mohanty, I. et al. The underappreciated diversity of bile acid modifications. Cell 187, 1801-1818.e20 (2024).

**Novel Component**

Creating a vast reference library of 500,000 compounds and linking it to their respective phenotype associations.

**----------Poster Board #20----------**

**Title**

**METABOLOMIC AND LIPIDOMIC ANALYSIS FROM FORMALIN-FIXED PARAFFIN-**

## EMBEDDED GASTRIC BIOPSIES SAMPLES

**Author(s) (Presenting author should be listed first)**
Ramm, Maximiliano; Martinez, David, Delgado, Carolina; Muñoz, Esteban; Barrera, María Paz & Bustamante, Luis.
**Author Affiliations**
1 Departamento Análisis Instrumental, Facultad de Farmacia, Universidad de Concepción, Concepción, Región del Biobio, Chile. 2 Departamento de Especialidades, Facultad de Medicina, Universidad de Concepción, Concepción, Región del Biobio, Chile.

**Introduction**
In Chile, gastric cancer is the top in mortality in men and the third in women. Metabolomics and lipidomics studies on this disease could allow a better understanding on the physiopathological alterations related to tumor progression and differentiation. In metabolomics, samples should ideally be rapidly collected and frozen with liquid nitrogen; however, it can be difficult to collect a large cohort of biopsies. In this regard, formalin-fixed and paraffin-embedded (FFPE) samples could be an approach, as they are regularly collected, studied and stored in clinical services. Some disadvantages can be expected from FFPE samples, such as loss of several metabolites and lower recovery compared to fresh frozen (FF) samples.

**Methods**
Gastric mucosa biopsies for a patient, who went under total gastrectomy, were collected as fresh frozen samples (with liquid nitrogen) and also processed as FFPE blocks. Metabolome and lipidome methods consisted in monophasic secuential liquid extraction with 80% methanol and later 90% isopropanol including solid-phase liquid extraction for phospholipids removal; also deparaffinization with xylene was evaluated. Extracts were preconcentred and analyzed through reverse phase liquid chromatography coupled to high ressolution mass spectrometry by an UHPLC-QTOF instrument (ESI+/ESI-).
Chromatograms were transformed to format and processed with MSDial and Metaboanalyst.

**Preliminary Data**
Results shows better method performance at the highest amount of FFPE sample and increased repeatability when applying xylene for paraffin removal. Some of the annotated metabolites could be related to the xylene treatment. Furthermore, more than 18% of the features related to midpolar metabolites are preserved compared to FF samples, which could be related to FFPE sample conservation techniques. Unexpectedly, an important amount of lipids were recovered from the FFPE sample, such as glycerolipids, sphingomyelines, ceramide derivatives, phospholipids and steroids, among others; which represents up to 78% of the FF detected lipids. Following steps in this study will include the comparation between cancerous and non-cancerous tissues in both FF and FFPE samples, as it will validate that metabolomic alterations are conserved in file samples.

**Novel Component**
File pathology samples as an input for metabolomics

**----------Poster Board #21----------**

**Title**

## Metabolomic Changes in Biofilm of Pseudomonas Aeruginosa Under Tobramycin Treatment Identified by COLMARq

**Author(s) (Presenting author should be listed first)**
Munki Choo,1 Da-Wei Li,2 Devin Sindeldecker,3 Paul Stoodley,3 Lei Bruschweiler-Li,2 and Rafael Brüschweiler1,2,4*
**Author Affiliations**
1Department of Chemistry and Biochemistry, 2Campus Chemical Instrument Center, 3Department of Microbial Infection and Immunity, 4Department of Biological Chemistry and Pharmacology, The Ohio State University, Columbus, Ohio 43210, U.S.A.

**Introduction**
Biofilms are linked to 80% of persistent microbial infections within the body and are responsible for 25% of patient deaths in hospitals. They are formed by pathogens, such as Pseudomonas aeruginosa, as a gel-like structure composed of extracellular polysaccharides, DNA, and proteins that surround and protect these

bacteria. Biofilm exhibits much higher resistance to antibiotics than that of their planktonic growth form. Biofilms are difficult to eradicate and thereby significantly contribute to the ongoing surge of infectious diseases.

**Methods**

To better understand the biochemical response of biofilm to antibiotic treatment, we treated biofilms by tobramycin, a widely used antibiotic, to identify and quantify metabolic changes. To identify metabolomic markers, we used the COLMARq method (Complex Mixture Analysis by NMR for quantification), which is available as a publicly accessible web server for the quantitative untargeted metabolomics analysis of cohorts of 2D NMR spectra enabling their streamlined semi-automated metabolite identification, quantification, and statistical analysis.

**Preliminary Data**

Using COLMARq, we could identify statistically significant differences for more than 10 metabolites in dose dependent manner providing direct pharmacological insights into the behavior of biofilms when treated by antibiotics.

**Novel Component**

Dose-dependent metabolomic analysis of biofilms' response to antibiotics by COLMARq

**----------Poster Board #22----------**

**Title**

**NIST SRM 1950 Beyond the Certificate of Analysis: mQACC Results of Community-Driven Qualitative and Quantitative Data**

**Author(s) (Presenting author should be listed first)**

Bayless, Amanda*; Davis, W. Clay*; Aristizabal-Henao, Juan; Artati, Anna; Barsch, Aiko; Beecher, Chris; Beger, Richard; Bowden, John; Broeckling, Corey; Cho, Joo-Youn; Dunn, Rick; Flores, Roberto; Fradin, Manon; Franchina, Flavio A.; Gouveia, Goncalo J.; Harms, Amy; Hartung, Thomas; Haznadar, Majda; Henriques da Costa, Sofia Ana; Jones, Christina; Kang, Kyo Bin; Lewis, Matthew; Lippa, Katrice; Malinowska, Julia; Nair, Sindhu; Ngere, Judith; Ntai, Ioanna; Percy, Andrew; Plumb, Robert; Raftery, Dan; Rahman, Anas Abdel; Schock, Tracey*; Sun, Jinchun; Tayyari, Fariba; Theodoridis, Georgios; Torta, Federico; Ulmer Holland, Candice Z.; Velagapudi, Vidya; Wilson, Ian; Zhang, Bo; Cumeras, Raquel

**Author Affiliations**

*National Institute of Standards and Technology, Charleston, SC; and members of Metabolomics Quality Assurance and Quality Control Consortium in the Reference and Test Materials Working Group

**Introduction**

Certified reference material (CRM) values provide a known and standardized reference point against which the results of a metabolomic study can be compared. However, the breadth of metabolomics hinders the feasibility of certifying concentration values for all chemical constituents of interest. The Standard Reference Material (SRM) 1950, Metabolites in Frozen Human Plasma, is by far the most used reference material by the metabolomics community. NIST SRM 1950 provides certified and/or reference values for select metabolites and lipids such as fatty acids, electrolytes, vitamins, hormones, and amino acids. The metabolomics community would greatly benefit from consensus values and identification of metabolites and lipids associated with multiple analytical platforms in SRM 1950 to increase harmonization and meaningful comparisons.

**Methods**

The Reference and Test Materials Working Group (RTMWG) of mQACC collected data from multiple instrument platforms (LC-MS, GC-MS, NMR) including qualitative identifications and quantitative values of metabolites and lipids in NIST SRM 1950 beyond those listed on the NIST Certificate of Analysis. Data was collected via google doc submissions and by gathering publicly available published data. These were combined to produce a publicly available database of community-generated 1) consensus concentration values for quantified metabolites and lipids of critical interest within the community and 2) compounds identified but not quantified in SRM 1950.

**Preliminary Data**

As expected, the data received was disparate and required additional formatting and conversions. For the

quantitative data, nomenclature was standardized, lipid species were converted to their summed composition notation, and concentrations were converted to ÂµM to obtain an average, standard deviation, median, min, and max around each analyte. From the 12 quantitative sources, between one and seven values were obtained per compound for calculating the consensus concentration. The standard deviation and range in concentrations for the 1,030 analytes was compound dependent and further demonstrated the need for consensus values. Having more values and information available for SRM 1950 metabolites and lipids will allow researchers to confidently investigate a broader range of analytes in their studies, which in turn could lead to a better understanding of the underlying biological mechanisms. The mQACC RTMWG continues to accept data submissions and will iteratively build upon this data set as submissions are received (https://www.mqacc.org/srm1950). This data will soon be publicly available for end users.

**Novel Component**

The first compilation of publicly available and user contributed metabolite and lipid qualitative and quantitative data for SRM 1950

**----------Poster Board #23----------**

**Title**

**Precision Targeting of Ferroptosis in Colorectal Cancer: Sex and KRAS Mutation-Driven Metabolic Vulnerabilities and Drug Repurposing**

**Author(s) (Presenting author should be listed first)**

Shen, Xinyi; Yan, Hong; Yao, Yisha; Khan, Sajid; Ma, Shuangge; Johnson, Caroline

**Author Affiliations**

Yale School of Public Health

**Introduction**

The metabolic landscape of sex differences in Colorectal Cancer (CRC) remains poorly characterized, particularly in relation to oncogenes like KRAS. Our previous work revealed decreased ferroptosis, an iron-dependent cell death mechanism, in KRAS-mutant tumors from male patients. This study examines the metabolic landscape of ferroptosis in the context of KRAS mutations and sex differences. We aim to identify metabolic vulnerabilities in KRAS-mutant CRC and explore drug repurposing opportunities. By integrating multi-omics data and applying advanced analytical approaches, we seek to uncover sex- and KRAS-specific differences in ferroptosis regulation at both transcriptional and metabolic levels. This research aligns with precision oncology efforts and addresses critical gaps in understanding CRC metabolism.

**Methods**

We analyzed three datasets: GSE39582 gene expression microarray, TCGA-COADREAD RNA-seq, and metabolomics data from an in-house MSKCC cohort. Variable Importance (VIMP) analysis and Gaussian mixture model were applied to identify genes and metabolites predictive of KRAS status by sex. A Random Survival Forest with Backward Elimination (RSF-BE) algorithm, using 1000 bootstraps, determined ferroptosis-related predictive molecular features stratified by sex and KRAS status. Partial dependence plots visualized non-linear relationships between predictors and outcomes. Gene Ontology (GO) enrichment and joint-pathway analyses revealed over-represented biological themes. Drug response analysis utilized data from the Genomics of Drug Sensitivity in Cancer (GDSC) resource. This integrated approach comprehensively explored metabolic vulnerabilities and their potential therapeutic implications in CRC.

**Preliminary Data**

Our analysis revealed significant sex- and KRAS-specific differences in ferroptosis-related gene expression and metabolite levels in CRC tumors. We identified differential expression of ferroptosis suppressors and drivers (e.g., SLC1A5 and SLC7A11) between KRAS mutant and wild-type tumors in male patients, but not in females. Metabolomics analysis detected 20 significantly altered metabolites in male KRAS mutant tumors, while only stearic acid differed in female patients.

RSF-BE models demonstrated high efficacy in identifying prognostic ferroptosis-related genes and metabolites. Distinct features were found to predict 5-year overall survival when stratified by sex and KRAS status. Notably, genes and metabolites involved in arginine synthesis and glutathione metabolism were uniquely associated with prognosis in tumors from males with KRAS mutations.

Joint-pathway analysis of prognostic ferroptosis genes and metabolites confirmed the enrichment of arginine biosynthesis and glutathione metabolism pathways in males with KRAS mutations. This finding was further supported by Kaplan-Meier analysis of the GSH/GSSG ratio from our MSKCC cohort, which showed that a lower ratio was associated with worse overall survival only in males with KRAS mutations.
Drug response analysis using CRC cell line data uncovered sex- and KRAS-specific associations between ferroptosis gene expression and drug sensitivity. Notably, expression of ferroptosis suppressor genes like DHODH, GCH1, and AIFM2 in KRAS mutant CRC cell lines correlated with resistance to cisplatin and paclitaxel. This finding provides insight into why these drugs may be ineffective for patients with KRAS mutant CRC.
These results collectively unveil novel metabolic vulnerabilities in CRC and offer promising avenues for drug repurposing strategies tailored to specific patient subgroups based on sex and KRAS mutation status. Our comprehensive metabolic map provides valuable biological insights for future investigations in CRC metabolism and ferroptosis, with significant implications for precision oncology.

**Novel Component**

Metabolic landscape of ferroptosis in CRC regarding KRAS mutations, sex differences, and drug repurposing, reveals new targets for precision oncology.

**----------Poster Board #24----------**

**Title**

**Progesterone Metabolism and Breast Cancer Risk using Simultaneous Quantitation and Discovery (SQUAD) Liquid Chromatography Mass Spectrometry**

**Author(s) (Presenting author should be listed first)**

Kenney, Katherine; German, Rana; Moore, Samuel; Gaul, David A.; Kim, Jaeyeon; Fernandez, Facundo M.

**Author Affiliations**

School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332 2) ; Petit Institute of Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332 3) ; Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indiana University, Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN 46202 4); The Komen Tissue Bank, Indiana University School of Medicine, Indiana University, Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN 46202

**Introduction**

Breast cancer is the most prevalent cancer globally, accounting for 12.5% of new annual cases worldwide. Women with BRCA1/2 mutations face a significantly increased risk, with up to 80% higher likelihood of developing breast cancer. Emerging evidence suggests that the steroid hormone progesterone may be a critical intrinsic factor for breast cancer risk in BRCA1/2 carriers. Using metabolomics, we are examining the association between metabolomic alterations and progesterone levels in both BRCA1/2 carriers and noncarriers as well as breast cancer patients. We aim to identify metabolite changes linked to progesterone levels and signaling and to determine whether these steroidomic alterations are associated with breast cancer risk and development in BRCA1/2 carriers.

**Methods**

We studied serum samples from 450 breast cancer and control patients from the Komen Tissue Bank at Indiana University. Fifty of these were BRCA positive. Using a SQUAD liquid chromatography mass spectrometry (LC-MS) approach we collected both targeted and non-targeted metabolomics data in a single experiment. SQUAD is an innovative acquisition scheme that interleaves targeted and non-targeted scans in tribrid mass spectrometry platforms. Non-targeted experiments enable the generation of hypothesis associated with progesterone and breast cancer based on BRCA status. Targeted experiments included progesterone, beta-estradiol, estrone, cortisone, hydrocortisone, corticosterone, testosterone, androstenedione, and 17alpha-hyroprogesterone, producing data that can be compared across batches and over time. These targets were selected to cover a variety of enzymatic steps along the progesterone pathway.

**Preliminary Data**

Both targeted and non-targeted SQUAD LC-MS experiments yielded informative preliminary data, highlighting

the potential differences among steroid pathways. Targeted data focused on species of interest that contribute to the steroid metabolic pathway such as progesterone, cortisone, beta-estradiol, hydrocortisone, corticosterone, estrone, testosterone, androstenedione, and 17alpha-hydroxyprogesterone. All steroids except beta-estradiol were analyzed in the positive ion mode, while beta-estradiol analysis was conducted in the negative ion mode. Additionally, MS/MS was collected for the untargeted data, to help identify several of the steroids of interest when confirmation was needed or to annotate unknowns with significant fold changes. MS2 data was compared against an in-house mzVault database along with accurate mass measurements. Annotation of known and unknown metabolites presents both an opportunity to better understand breast cancer biology but also a significant challenge. The annotation process is widely recognized as the major bottleneck of current metabolomics workflows because of the large chemical space that the metabolome and lipidome span. The SQUAD LC-MS approach ensures that both qualitative and quantitative differences in the steroid and metabolome profiles are thoroughly investigated, providing deeper insights into breast cancer biology.

**Novel Component**

SQUAD LC-MS detection and quantification of specific steroids and their metabolites related to breast cancer risk and development in BRCA-mutation carriers.

**----------Poster Board #25----------**

**Title**

**Quantification of underivatized acylcarnitines and carnitine intermediates using RP chromatography and ion funnel triple quadrupole**

**Author(s) (Presenting author should be listed first)**

Silva, Bianca; Cuthbertson, Daniel

**Author Affiliations**

Agilent Technologies

**Introduction**

Acylcarnitine profile analysis is a commonly requested analysis in genetic disease research laboratories due to their wide metabolic involvement. Acylcarnitines detection can be important markers for numerous disorders as they are intermediates of fatty acid oxidation and amino acids metabolism in tissues and body fluids. Currently, acylcarnitines are often tested using flow injection analysis, which compromises their quantification because various species occur as isomers and/or have very low concentrations. To address many issues inherent to such analysis, a new comprehensive LC-MS/MS method was developed to quantify 32 acylcarnitine species, with acyl-chain lengths from C0 to C20 in plasma samples without derivatization step.

**Methods**

Acylcarnitines were extracted using acidified isopropanol (0.3% formic acid) to precipitate proteins. After mixing and centrifuging, samples were diluted and analyzed on an ion funnel triple quadrupole mass spectrometer coupled to a biocompatible LC system, which is coated with an iron free alloy. Optimal source conditions and MRM transitions for all compounds were determined individually using commercial optimization software. Robust chromatography separation for the main isomers compounds was achieved without ion pairing reagents. A gradient elution was obtained using a solution of methanol:water (95:5) (B) and water (A) both phases containing 15mM of Ammonium acetate and 0.3% formic acid. Absolute quantification was obtained using commercial labeled compounds spiked into samples. Relative response calibration curves were made for quantitative analysis of each analyte.

**Preliminary Data**

The method achieved the desired separations of the main isomers, such as: (C5) isovaleryl, valeryl and 2-methylbutyryl-L-carnitine; (C4) butytryl and isobutytryl-L-carnitine and (C6DC) 3-methylglutaryl and Adipoyl-L-carnitine without ion pairing additives, which can cause ion suppression. This separation used a standardized configuration intended for discovery analysis and workflow to allow flexibility for different studies. The chromatography was reproducible, and the figures of merit evaluated for each analyte were linearity, detection and quantification limits, recovery, and precision. Simple sample preparation was obtained avoiding any derivatization. Protein precipitation is well known for its efficiency in extracting metabolites from plasma and reduces processing time. Method reproducibility showed RSD < 10%. Limits of quantification ranged from 0.01

to 1 ng/L for all metabolites. All the compounds showed a great linearity (R2 > 0.994) and RSD below 12%. This data shows the method is capable of quantifying acylcarnitines and carnitines intermediates at levels relevant to plasma for monitoring metabolic alterations. Compared with literature and previous data, the ion funnel LC/TQ contributes to the analyte sensitivity and reproducibility. The method developed on the standardized system can be easily transferred and applied to perform different experiments from pathway discovery metabolomics to absolute quantitation.

**Novel Component**

New sensitive method developed to quantify acylcarnitines using a wide acyl-chain length without derivatization in a standardized metabolomics system.

**----------Poster Board #26----------**

**Title**

**Quantitative analysis and structural characterization of bile acids using the ZenoTOF 7600 system**

**Author(s) (Presenting author should be listed first)**

Colquhoun David(1);Baker, Paul RS(1); Proos, Robert(1); Seferovic ,Maxim D(2); and Horvath, Thomas D(3,4)

**Author Affiliations**

1) SCIEX, USA; 2) Dept of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX; 3) Dept of Pathology, Texas Children’s Hospital, Houston, TX; 4) Dept of Pathology & Immunology, Baylor College of Medicine, Houston, TX

**Introduction**

Primary bile acids are essential for emulsifying and absorbing dietary fats and can be converted to secondary bile acids by the gut microbiota. Bile acid analytical methods often use triple quadrupole mass spectrometersâ€”the quantitative performance of which can suffer from chemical interference. High-resolution mass spectrometers with sufficient sensitivity can reduce background interferences and improve the quality of quantitative data. Bile acids are comprised of multiple groups of isomers that cannot be distinguished using CID-based fragmentation alone. However, electron-based fragmentation such as EAD, provides diagnostic fragments that distinguish these isomers. Here, the speed and sensitivity of the ZenoTOF 7600 system was leveraged to quantify and structurally characterize bile acids in human plasma samples.

**Methods**

Plasma was isolated from human blood by centrifugation, extracted, mixed with an internal standard solution, and split for injection on the QTRAP 7500 and ZenoTOF 7600 systems. Chromatographic separations were performed using a Phenomenex Kinetex XB-C18 column for the ZenoTOF 7600 system and a Restek Raptor C18 column for the QTRAP 7500 system using mobile phases of 10 mM ammonium formate in water (A) and pure acetonitrile (B). Samples were analyzed on both systems using a scheduled multiple reaction monitoring (sMRM) scan mode. EAD-based product ion spectra were acquired in positive ion mode for structural characterization. LOD and LOQ were calculated from calibration curves, and Pearson correlation calculations were performed using Prism software.

**Preliminary Data**

A bile acid standard curve was generated and analyzed on both the QTRAP 7500 and ZenoTOF 7600 systems. Optimized parameter settings for each instrument allowed for the separation of isomeric bile acids with baseline resolution. The internal standard curve ranged from 0.01 to 1000 nM, but the QTRAP 7500 experienced detector saturation at 1000 nM, unlike the ZenoTOF 7600. To match conditions, the dynamic range was truncated to 0.1 to 100 nM for final calculations, sufficient for measuring bile acids in human plasma. Rat plasma extracts were analyzed, and data were acquired using a high-resolution multiple reaction monitoring (sMRMHR) scan mode. The ZenoTOF 7600 uniquely identified specific lipid molecular species using both CID- and EAD-based fragmentation. The system's high-resolution capabilities excluded contaminating peaks, increasing measurement accuracy. Standard curves were created for each analyte to calculate LOD and LOQ. Both instruments showed comparable sensitivity, with the QTRAP 7500 being slightly more sensitive for some compounds. Both instruments effectively measured bile acids in human plasma, showing linear correlation in concentration data with r values >0.99 and p values <0.0001. The ZenoTOF 7600's EAD-based fragmentation

provided detailed structural information, distinguishing bile acid isomers and potentially identifying novel conjugated bile acids. This capability is valuable for bile acid metabolism research, highlighting the system's quantitative sensitivity and qualitative capabilities

**Novel Component**

Simultaneous quantitation and structural characterization of bile acids in human plasma

**----------Poster Board #27----------**

**Title**

**Reducing Complexity in Polar Feature Detection in Non-Targeted LC-MS Experiments from the Presence of Salt in Sample Preparation**

**Author(s) (Presenting author should be listed first)**

David A. Gaul, Ying Liu, Samuel Moore

**Author Affiliations**

1School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332

**Introduction**

Non-targeted metabolomic experimental design has sought to reduce as many technical variables as possible. When working with cells and tissues, frequently prior to extraction, residual buffered solution is difficult to remove completely resulting in non-consistent salt content. Effects from differences in salt concentrations are further complicated when cell numbers are not consistent. Generally, salt is expected to suppress analyte ion signal due to possibly competition for charge or by decreasing vapor pressure during the electrospray ionization process.

**Methods**

One excised rat liver was subdivided for one portion of this study. Bead homogenization with a TissueLyser II extracted metabolites into methanol:aqueous solvent (80:20), where the aqueous solvent was water, PBS, or concentrated PBS. In addition, both liver and Vero cells were prepared by exchanging PBS salts for Mass Spectrometry friendly ammonium salts (ammonium formate, ammonium acetate, and ammonium bicarbonate). Cell viability was assessed following salt exchange. Serial dilutions were prepared for each set of samples. Triplicate extracts for each study condition were separated using ultra performance liquid chromatography with a Waters BEH Amide column and analyzed with an Orbitrap IDX mass spectrometer. Data processing was with Compound Discoverer v3.3 software.

**Preliminary Data**

Based on a non-targeted profiling experiment that had relatively large differences in cell numbers, we observed variable results, which were not controlled by targeting a consistent extraction ratio of cells to extraction solvent. We hypothesized the variability of residue PBS may be responsible, so we designed this experiment to investigate the role of salt in sample preparation for an LC-MS experiment analyzing polar metabolites. The sodium chloride, 0.137M in PBS, interacts with the mobile phase additive ammonium formate to create salt clusters (NaOCH2 and NH4Cl) which elute over 4-6 min of the 12 min gradient. Of the 3331 detected features, 24% of these features had a 2x fold change with the use of PBS in the extraction. The salt addition was observed to enhance or to suppresses ion detection dependent on the analyte. The observed signal differences were unlikely related to solubility or extraction efficacy, but instead related to increased stabilization of ionic complexes during sample preparation and LC separation. Salt removal with solid phase extraction led to increase complexity in data analysis. While PBS replacement with mass spectrometry friendly additives was shown to reduce the suppression and the enhancement effects observed with PBS present. These results suggest the importance in controlling the salt content of the samples during sample preparation to minimize the addition of unwanted technical variation which could lead to misinterpretation of the data.

**Novel Component**

Sample preparation method to avoid PBS effects on polar analyte detection that complicate interpretation of non-targeted profiling experiment.

**----------Poster Board #28----------**

**Title**

## Sample Preparation Optimization for Metabolomics Analysis of Cell Culture Media to reduce Maillard reaction products

**Author(s) (Presenting author should be listed first)**
Nguyen, Vyncent; Asik, Didar; Campbell, Andy; Goldfuss, Jaime; Tu, Chengjian
**Author Affiliations**
Thermo Fisher Scientific

**Introduction**
The presence of Maillard reaction products (MRP) can affect culture media stability and performance by depleting essential nutrients such as peptides and amino acids via reaction to carbonyl groups on reducing sugars. Artificial MRP from sample preparation, specifically the drying steps, have been misidentified as endogenous metabolites from bioproduction processes. This has led to misinterpretation of the dataset and prolonged data validation analysis time. While methods to control MRP have been evaluated in the food industry, to best of our knowledge, none have been reported in sample preparation in metabolomics analysis of cell culture media. Here, we compared different sample preparation methods for their effectiveness in reduction and control of MRP using nontargeted and targeted high throughput LC-MS/MS metabolomics analysis.

**Methods**
We selected Gibcoâ„¢ Dynamisâ„¢ Medium and spent media from a 10-day fed-batch cultivation as test samples. The LC/MS sample preparation protocol was adapted from Kofeler et al., and the drying steps were carried out with an Organomation Microvap Evaporator or a Thermo Scientificâ„¢ SpeedVac Concentrator. The samples were resuspended in 5% acetonitrile and 0.1% formic acid, and further diluted with water and 0.1% formic acid. The UHPLC-MS data acquisition was carried out with a Vanquishâ„¢ HPLC coupled with either a Thermo Scientificâ„¢ Orbitrap Fusionâ„¢ Tribridâ„¢ ID-X or a TSQ Altisâ„¢ Triple Quadrupole Mass Spectrometer for nontargeted and targeted analysis, respectively. Differential analysis and metabolite identification were processed by Compound Discovererâ„¢ 3.3 (CD3.3), and quantification was performed with MacCoss Skyline software.

**Preliminary Data**
We utilized CD3.3 SP2 for differential analysis and metabolite identification using FISh coverage scores of ms2 spectral matching greater than 60 against spectral library mzCloud and ChemSpider databases. A total of 14 MR metabolites were identified between samples dried with SpeedVac concentrator and the nitrogen evaporator. Among these, Glucosyl-Glutamate, 310.11348 m/z (Gluc-Glu), is misidentified as sialic acid (SA), which plays a significant role in glycosylation. In evaluating MRP expression, Gluc-Glu and MRP Fructosyl-lysine intensities were 8,300% and 20,000% higher in samples dried with SpeedVac than those of the nitrogen evaporator. By controlling temperature and processing time, we were able to reduce these differences significantly. Furthermore, Gluc-Glu and SA cannot be separated by chromatography in spent media analysis. By applying MS/MS unique fragment selection method in targeted analysis, we were able to evaluate SA expression in spent media and Gluc-Glu levels. Overall, this investigation provides a comprehensive comparison between different sample preparations for metabolomics analysis of cell culture media, and highlights some cautionary steps to avoid, or work around, based on each lab's settings.

**Novel Component**
Evaluation of Maillard reaction products in metabolomics analysis in cell culture media

**----------Poster Board #29----------**

**Title**

## Semi-automated extraction of cell and tissue samples for multi-omic analysis using the Biomek i7 workstation

**Author(s) (Presenting author should be listed first)**
Smith, Zachary; Jones, Martin; Viant, Mark
**Author Affiliations**
Beckman Coulter Life Sciences; Phenome Center Birmingham University of Birmingham

**Introduction**
â€˜PrecisionTox', a â‚¬20M EU Horizon 2020-funded research project, aims to develop new methods for

chemical hazard assessment based on a fusion of: 1) transcriptomics and metabolomics analyses, 2) machine learning, and 3) chemical exposure experiments in phylogenetically disparate model organisms (Daphnia magna, Danio rerio, Drosophila melanogaster, Caenorhabditis elegans and Xenopus laevis) and the HepG2 human cell line. With >10,000 samples being generated across 6 different laboratories in Europe and the US, there is a considerable need to automate the extraction of polar and lipophilic compounds from cells and tissues. We report progress in the development of a high-throughput semi-automated extraction method using a Biomek i7 Hybrid automated workstation from Beckman Coulter Life Sciences.

**Methods**

Each of the >10,000 PrecisionTox samples will undergo in-depth molecular characterisation using transcriptomics and liquid-chromatography-mass-spectrometry-based metabolomics. Extraction workflow, established on a Biomek i7 workstation equipped with cryogenic positions for preserving samples underpinning these vital analyses. Each extraction batch consists of up to 96 samples (including quality control samples), wherein samples are homogenized by bead beating before transfer to a 96-well plate. A fraction of each homogenate is removed and quenched for RNAseq analysis, with the remainder undergoing a modified Bligh and Dyer biphasic extraction (2:2:1.8 v/v/v chloroform:methanol:water) to yield polar and lipid metabolomic fractions for drying and subsequent metabolomic analysis.

**Preliminary Data**

Our semi-automated extraction workflow facilitates the extraction of up to 192 samples per day for downstream multi-omic analyses (polar metabolomics, lipidomics and RNAseq) â€“ an approximate 5-fold increase in throughput versus manual extractions. Intra- and inter-day variability are found to be excellent, with no clear trends observed in terms of the day of sample extraction, nor position of samples on the well plate (edge vs inner wells). Moreover, diverse sample matrices including crustacea, flies, fish larvae, embryos and human cells lines, relevant to both toxicological and biological research, are shown to process adequately.
Metabolomic analyses of PrecisionTox samples revealed clear shifts in metabolism related to growth and development of, e.g. Daphnia magna and Danio rerio, control (i.e. non-exposed) samples throughout the exposure period, demonstrating the ability of the combined extraction and analysis workflow to reveal (expected) molecular perturbations. With regards to chemical-exposed samples, our extraction and analysis workflow revealed stark metabolic shifts (relative to time-matched control samples) across all PrecisionTox organisms. Here, we present observations for both Caenorhabditis elegans and Drosophila melanogaster, including a summary of perturbed metabolites throughout exposure time.

**Novel Component**

A semi-automated extraction workflow supporting multi-omic analyses, without which a world-leading, â‚¬20M omics-based project would be undeliverable.

**----------Poster Board #30----------**

**Title**

**The depth of the annotatable metabolome is a function of the mass spectrometer and software versions**

**Author(s) (Presenting author should be listed first)**

Barnes, Stephen (1); Berryhill, Taylor F (1); Wilson, Landon (1); Youngmee Kim (2)

**Author Affiliations**

University of Alabama at Birmingham (1); University of Miami (2)

**Introduction**

LC-MS instruments have improved remarkably over the past decade and have allowed investigators to acquire very large datasets containing retention times, MS and MSMS and for some ion mobility data on the observable compounds in biological samples. Nevertheless, annotation of the reproducible ion features remains limited and error-prone. The goal of this study was to compare the acceptable annotations for samples after (1) a transition from collecting MSMS data at 16 Hz to 133 Hz, (2) under two collision conditions (20 and 30 eV) and (3) using different versions of MS-DIAL software in combination with public, commercial and in-house metabolite databases.

**Methods**

Extracted samples (fecal and charcoal-stripped serum) were analyzed on a SCIEX ZenoTOF 7600, collecting

data in a duty cycle consisting of 100 msec hi-res TOF-MS data followed by the top 30, five msec, hi-res TOF MSMS spectra. Metabolites were separated by reverse-phase UPLC on a Luna Omega column using a 6-minute linear gradient of acetonitrile in 0.1% formic acid at a flow rate of 400 microL/min. Collected data were processed using different versions of MS-DIAL (4.90, 4.92 and versions 5). Databases used for annotation were version 17 in the MS-DIAL suite and an IROA Technologies metabolite standards library.

**Preliminary Data**

Use of the Zenotrap on the ZenoTOF 7600 increased the intensities of ions 5-fold compared to it off. A single LC-MS run consisted of 1,440 MS and up to 43,200 MSMS hi-res TOF spectra. The in-house positive ion IROA metabolite standards library consisted of 373 metabolites and was supplemented with 140 bile acid-amino conjugates (provided by BileOmix). It was used as a home truth since it also provided LC retention times [RT] and the same MSMS collision conditions as the test samples. Using MS-DIAL version 4.90, a total of 21 bile acid-amino acid conjugates were annotated in fecal extracts with acceptable MSMS spectra. However, for all subsequent versions of MS-DIAL (4.92 and those up to 5.3.240704) only the conventional, hepatically-derived glycine and taurine-conjugated bile acids were observed. The post 4.90 versions of MS-DIAL coincided with achange to parsing the raw data from profile to centroid data. Reducing the MSMS criteria within MS-DIAL for versions 4.92 and above allowed recovery of up to 18 bile acid-amino acid conjugates, however, it was at the cost of increased low-score annotations of many other metabolites. Further, peak areas of annotated metabolites in version 4.90 were five times larger than in all subsequent versions of MS-DIAL. LC-MS analysis of charcoal-extracted serum with the ZenoTOF 7600 yielded 29,042 positive ion features of which 761 (2.6%) were annotated at level 2 (MS and MSMS criteria) using the public version 17 library. With the in-house IROA library, 51 ions (13.7%) were annotated without using RT for scoring but only 22 (5.9%) with it. MSMS data collected at 20 and 30 eV revealed that the higher CE resulted in unacceptable loss of most molecular ions as well as their higher mass product ions.

**Novel Component**

Acceptable high quality metabolite annotations are functions of both the instrument, the software version and the data library.

**----------Poster Board #31----------**

**Title**

**Untargeted analysis of lipid biomarker to inform carbon storage potential in soils**

**Author(s) (Presenting author should be listed first)**

Mioko Tamura, Tyler McIntosh, Vidya Suseela, and Nishanth Tharayil

**Author Affiliations**

Clemson University, South Carolina, USA

**Introduction**

Soil lipids serve as molecular biomarkers indicative of the origins and degradation stages of soil organic carbon (SOC) and could complement characterization of SOC. Biomarker retention occurs through chemical and physical interaction during plant degradation, a/biotic catabolism and resynthesis, and sequestration in soil particles. Because of the chemical complexity and polydispersity of the SOC, targeted extractions, coupled with an untargeted analysis, are better suited to elucidate their molecular identity. The current approaches require laborious extraction, derivatization with hazardous chemicals and less-standardized interpretation of complex mass spectra, which could result in a lack of widespread adoption despite its potential utility in defining extractable SOC. The current study applied modified procedures to improve extraction efficiency, identification, and source assignment across four soil types under different vegetation and management practices.

**Methods**

Soils were collected from deciduous and pine forests, forage fields, and legume-incorporated organic farms. The samples were freeze-dried and subjected to sequential extraction first with non-polar solvents to release free lipids, and then subjected to base- and acid-hydrolysis for transesterification and methylation of compound lipids. Extraction procedures were modified for free lipid recovery, and esterification reaction workflow. The lipids were converted to trimethylsilyl derivatives and analyzed using GC-MS. The compounds were positively

identified by comparing mass fragmentation patterns to the NIST libraries, external standards, and literature. A spectral library for biomarkers was created to enhance workflow efficiency. Metabolic pathways of plants and microbes were examined to clarify the source assignment of the biomarkers.

**Preliminary Data**

Traditionally, extracted free lipid fractions are directly silylated, resulting in an underestimation of lipid abundance as compound lipids in the solvent extract remain unaccounted for. To overcome this, the extracts were subjected to a base-hydrolysis followed by acid hydrolysis to generate methyl esters from all lipid fractions and offered better chromatographic separation of complex lipids compared to the traditional silyl derivatives. Further, by switching solvents, we were able to limit the use of water in the liquid-liquid partitioning of the trans-esterified lipids that would have otherwise resulted in ester hydrolysis, thus avoiding the need for re-methylation with diazomethane. The new approach not only excluded the utilization of water, but also introduced consecutive hydrolysis by base, followed by acid to ensure the methylation of the fatty acid in the extract. The one-step hydrolysis is beneficial in terms of reducing time and hazardous solvents and facilitates a more sensitive and robust instrumental analysis.

**Novel Component**

Our method accurately characterizes the soil lipid, is less cumbersome and could lead to broader adoption of biomarker to characterize the extractable SOC

**----------Poster Board #32----------**

**Title**

## Untargeted Metabolomics of Blood Plasma from Short-Term Repeat Dose in vivo Studies to Estimate Biological Potency of PFAS Compounds

**Author(s) (Presenting author should be listed first)**

Rice, Julie; Auerbach, Scott; Crizer, David

**Author Affiliations**

Division of Translational Toxicology/National Institute of Environmental Health Sciences

**Introduction**

PFAS, or per- and polyfluoroalkyl substances, are used in many products, such as stain repellant and nonstick cookware. These manufactured substances are ubiquitous in the environment and have been linked to adverse health effects like liver damage and cancer. Due to their persistence in the environment and the human body they have been nicknamed â€˜forever chemicals' and there is concern for public health and environmental safety. Analysis of blood plasma samples from Sprague Dawley rats dosed once daily with a PFAS compound was done using untargeted metabolomics analysis in conjunction with benchmark dose (BMD) analysis to estimate biological potency and better understand biological changes elicited by test chemicals.

**Methods**

Male and female Sprague Dawley rats were exposed once daily for five consecutive days by oral gavage with 1 of 3 PFAS compounds: 6:1 Fluorotelomer Alcohol (6:1-FTOH),10:2 Fluorotelomer Alcohol (10:2-FTOH), and Perfluorohexanesulfonamide (PFHxSA). Doses ranged from 0 to 1000 mg/kg body weight. Animals were then sacrificed, and samples were collected. Plasma samples were extracted and analyzed via HILIC (polar metabolites) and C18 (lipids) liquid chromatography mass spectrometry (LCMS) methods in both positive and negative mode using a Thermo Q Exactive Plus in conjunction with a Thermo Vanquish LC system.

**Preliminary Data**

Previous work has investigated the utility of transcriptomics analysis of liver and kidney tissues from 5-day repeat dose studies in rats for estimating benchmark doses (BMDs). When compared to apical (histopathological) BMDs from chronic or sub-chronic toxicity studies, the transcriptional BMDs were found to be consistently within a 10-fold cut-off of the apical values. Recently, this approach has been used to estimate BMDs for a set of perfluoroalkyl substances (PFAS) where there is minimal (if any) data available from longer time toxicity studies. One potential drawback of this approach is the reliance on specific sentinel tissues and the issue that poses when investigating chemicals that don't affect the liver or kidney. Here we analyze blood plasma samples from Male Sprague Dawley rats exposed once daily for five consecutive days by oral gavage to a PFAS compound. Metabolomics analysis of plasma samples consisted of four assays in total: polar (positive

and negative mode) and nonpolar (positive and negative mode). LCMS data was deconvoluted using XCMS to detect individual features. Features were then filtered for quality. Data were then imported into BMDExpress to derive BMDs for each of the four LCMS assays. The derived BMDs for each of the LCMS assays are then combined to determine an overall metabolomics point of departure for each chemical. For example, the analysis of plasms samples from rats dosed with 10:2 Fluorotelomer alcohol resulted in the identification of 325 potential metabolite features that fit a dose-response relationship. The most sensitive metabolic feature (m/z = 1044) was found to have a BMD value of 1.48 mg/kg. Work is ongoing to annotate those metabolite features that exhibited a dose-response fit.

**Novel Component**

Estimating biological potencies of PFAS chemicals using untargeted metabolomics and benchmark dose analysis.

## Full Abstracts for Wednesday Posters

**----------Poster Board #1----------**

**Title**

**A comprehensive untargeted fecal metabolomics workflow on the Orbitrap Astral MS to achieve deep metabolome coverage and confident compound annotation**

**Author(s) (Presenting author should be listed first)**

Hermanson, Daniel1; Amer, Bashar1; El Abiead, Yasin2; Dorrestein, Pieter2; Bird, Susan S.1

**Author Affiliations**

1 Thermo Fisher Scientific, San Jose, California, USA; 2 University of California - San Diego, California, USA

**Introduction**

Discovery fecal metabolomics using mass spectrometry aims to gather comprehensive insights into the metabolic diversity of samples. This approach enables the identification of a broad spectrum of molecules, facilitating the potential discovery of novel, previously unrecognized compounds through matches in mass spectra (MS1 and MS2) and compound databases. This can empower clinical diagnosis to highlight the valuable link between the microbiome and health.

Nevertheless, challenges like mass spectrometer speed, sensitivity, and accuracy may result in suboptimal production of high-quality MS2 spectra, leading to reduced annotation percentage and confidence. Here, an untargeted fecal metabolomics approach was established using Thermo Scientificâ„¢ Orbitrapâ„¢ Astralâ„¢ high-resolution mass spectrometer, known for its capability of faster MS2 scanning together with high-resolution accurate mass orbitrap detection.

**Methods**

Human Whole Stool reference material and isotope-labeled compounds (MSK-QReSSâ„¢ Kit; used as QC internal standards (IS)) were obtained from NIST and CIL, respectively. The fecal sample was extracted with 50% methanol, and the supernatant was evaporated and then reconstituted in either water or 50% methanol solutions containing IS. Metabolites underwent separation, first on a reversed-phase column and then on a HILIC column connected to a Thermo Scientificâ„¢ Vanquishâ„¢ Horizon system. Data were acquired in positive (RP) and negative (HILIC) polarities on an Orbitrap Astral mass spectrometer, using Orbitrap full MS1 scans and Astral analyzer MS2 scans. Thermo Scientificâ„¢ Compound Discovererâ„¢ 3.4 software was used for data processing, statistical analysis, and unknown annotation.

**Preliminary Data**

In this study, the standard NIST stool sample extracted with excess methanol was used as a proof of concept to build an LC-MS thorough untargeted discovery workflow to characterize the fecal metabolome using the Orbitrap Astral mass spectrometer, which can simultaneously run a full-scan Orbitrap HRAM MS1 analysis and a DDA fast and sensitive Astral HRAM MS2.

A high percentage of detected compounds had MS2 fragmentation using the developed workflow. In particular, the fragmentation of lower-abundance compounds in the very complex human stool matrix was higher. For the reversed-phase positive mode, 92% of compounds had MS2 fragmentation data. For the HILIC negative mode, 86% of compounds had MS2 fragmentation data. The large percentage of compounds with MS2 fragmentation is a result of the faster scanning rates and higher sensitivity of the Astral analyzer, which enables the collection of more fragmentation data for the confident annotation of unknown compounds.

High data quality, reliability, and robustness of measurement were observed by evaluating the performance of isotopically labeled compounds (from CIL's MSK-QReSSâ„¢ mixes) over time through metric tracking (e.g., retention time, mass accuracy, signal response). In brief, the QCs demonstrated minimal chromatographic shift and consistent signal responses, as evidenced by a low % CVs across analytical and technical replicates. Furthermore, sub-ppm mass accuracy was detected for all targets over the entire acquisition period. The stable metrics provided assurances of the quality of data rendered from the untargeted MS-based workflow.

**Novel Component**

A thorough untargeted LC-MS metabolomics workflow to facilitate deeper coverage and confident annotation of fecal metabolites.

**----------Poster Board #2----------**

**Title**

**Metabolomics as a complementary conduit to elucidate the mechanisms of priming-mediated stress memory in plants**

**Author(s) (Presenting author should be listed first)**

Kaur Gagandeep; Kumar Rohit; Leonard Elizabeth; Tharayil Nishanth

**Author Affiliations**

Department of Plant and Environmental Sciences, Clemson University, Clemson, South Carolina 29634, United States

**Introduction**

Environmental conditions that deviate from the growth optimum alter the rate of critical physiological processes in plants, thereby imposing stress. Plants have evolved various biochemical strategies to circumvent many of these stresses. Plants could â€˜memorize' the stress response, where biochemical responses to sub-lethal doses of stress could outlive the initial stress event and could equip (â€˜prime') the resilient plants for a more efficient and less disruptive response to future stress that is otherwise lethal. At the molecular level, stress memory is regulated by epigenetic modifications that include chromatin remodeling and DNA methylation. However, stress memory-mediated resilience in plants remains less known at a biochemical level. We studied the metabolic landscape associated with stress memory in Amaranthus palmeri, an economically destructive weed that causes considerable damage to the agricultural production systems of the Southern United States. By employing nontargeted metabolomic approaches, we try to elucidate the metabolic pathways involved in stress memory of amaranth when exposed to a lethal dose of herbicide, after exposure to a non-lethal dose of the same herbicide.

**Methods**

Two biotypes of Amaranthus plants were grown from seeds and subjected to various herbicide treatments. For priming, four-week-old plants were sprayed with six sub-lethal doses of glyphosate (0x, 0.005x, 0.02x, 0.1x, 0.2x, and 0.5x, where 1x represents the field recommended dose of the herbicide). Plants were allowed to recoup for 72 hrs., after which half the plants from each sublethal herbicide dose were sprayed with 0.5x of herbicide (trigger dose). The top whorl of young leaves, along with the meristem, was harvested from all plants after 48 hrs., and the pool of metabolites was measured using global metabolomic platforms and data processing pipelines.

**Preliminary Data**

Our preliminary data revealed a significant increase in shikimate concentration in non-primed amaranth plants, wherein the primed plants (plants subjected to non-lethal glyphosate dose) showed a minimal increase in shikimate accumulation after the trigger dose of glyphosate. Using an untargeted metabolomics approach, we captured the variation in approximately 4500 mass features between primed and non-primed plants, which were separated into distinct groups in principal component analysis (PCA). Overall, the result suggests that, following the trigger treatment, glyphosate-induced metabolic perturbations were profoundly lower in primed plants. Time-course analysis of shikimate abundance post-priming indicated an increase in shikimate levels until 48 hours after herbicide application. The shikimate levels stabilized by 72 hours and subsequently returned to a new baseline at 98 hours, slightly above the native level, suggesting a time-dependent regulation of shikimate in response to priming. These findings support our hypothesis that a sub-lethal dose of herbicide could confer resilience to a higher dose of herbicidal stress in some agricultural weeds.

**Novel Component**

Our study highlights the potential of metabolomics to elucidate stress resilience in plants and paves a path for focusing on specific epigenetic mechanisms regulating these metabolic changes.

**----------Poster Board #3----------**

**Title**

**Optimization of sample preparation and LC-MS analysis for high throughput untargeted lipidomics and metabolomics**

**Author(s) (Presenting author should be listed first)**

Bennouna, Djawed ; Chatelaine, Haley ;  Mehta, Khyati; Tisch, Adam; Beecher, Chris; LeClair, Christopher; Mathé Ewy.
**Author Affiliations**
All authors except Beecher Chris affiliation: National Center for Advancing Translational Sciences (NCATS), Rockville, Maryland, USA. Beecher, Chris affiliation: IROA Technologies, Chapel Hill, NC, USA

**Introduction**
Developing high-throughput metabolomics and lipidomics workflow is essential to reduce analytical errors and provide high data quality with greater consistency and reliability. By automating sample extraction and optimizing chromatographic conditions and mass spectrometry parameters, we aim to reduce the extraction time and human error with parallel pipetting and reduce the chromatography time range used to separate metabolites. Our overall goal is to increase signal intensities while reducing the overall time of sample analysis. To this end, we developed a biphasic extraction protocol on 384 well plates using a robotic arm and optimized LC-MS methods using short columns.

**Methods**
The biphasic extraction protocol was optimized manually on 30 uL of plasma before its automation on 384 well plates, starting with only 9 wells. The automatic extraction protocol was performed with the Bravo Automated Liquid Handling Platform (Agilent), equipped with 384ST head. The main steps of the extraction included protein precipitation, lipid extraction, lipid extract collection, solvent polarity switching, and polar phase collection. LC-MS methods for metabolomics and lipidomics were developed and optimized on a 50 mm C8 column with a 4 min run, and 50 mm HILIC column with a 5 min run, respectively, using native and labeled lipids and polar standards. The detection of the separated compounds was performed on Agilent QTOF in both positive and negative modes.

**Preliminary Data**
The LC-MS gradient applied with the C8 column showed clear separation of the main lipid classes, including omega 3,6 and 9 fatty acids, Lysophospholipids, sterols, phospholipids, ceramides, sphingomyelins, and triglycerides. By comparing the separation profile with our classical lipidomics method of 22 minutes, the new short method allowed a significant reduction of the separation window from 13 minutes to 2 minutes while maintaining the same elution order for the lipid compounds. For example, omega-3 fatty acids, omega-6 fatty acids and omega-9 fatty acids kept the same elution order and their separation window reduced from 7 min to 0.5 min. The separation window of phospholipids was also reduced to under one minute, while keeping the same separation order compared to the other lipid classes in the chromatogram. The separation window of triglycerides was also reduced while keeping their position in the last segment of the chromatogram. Similarly, the optimized metabolomics LC-MS method using HILIC column showed good separation of the polar compounds involved in major metabolic pathways such as purines, indoles, amino acids and vitamins. Reproducibility was assessed by comparing repeated consecutive injections several days and months apart and results show a perfect retention time overlapping. We further tested our automated 384 well plate method in 9 plasma samples and again showed strong overlap between the TICs of the lipid and polar extracts and in the extracted ion chromatograms of known polar and non-polar compounds. Notably, the automated biphasic extraction protocol on 384-well plates was performed in under an hour.
Overall, our automated biphasic extraction protocol provides a robust platoform for advancing translational science efforts that evaluate large numbers of samples (hundreds to thousands).  These efforts include investigation of metabolic dysregulations that underlie disease etiology, identification of novel treatments, and identificiation of biomarkers to monitor treatment responses and disease progression.

**Novel Component**
Reducing analysis time of 384 injections in positive and negative mode from a month to 4 days with high-quality data

**----------Poster Board #4----------**

**Title**
**Simultaneous quantitation and discovery (SQUAD) metabolomics workflow for the analysis of fecal bile acids**

**Author(s) (Presenting author should be listed first)**
Stewart, Allison K.1; Amer, Bashar1; Midha, Ayush2; Jain, Isha2; Percy, Andrew J.3; Backiel, Krista3; Deshpande, Rahul R.1; Kline, Joshua P.1; Bills , Brandon1; Bird, Susan 1
**Author Affiliations**
1 Thermo Fisher Scientific, San Jose, California, USA; 2 Gladstone Institute, UCSF, California, USA; 3 Cambridge Isotope Laboratories, Inc., Tewksbury, MA

**Introduction**
Bile acids (BA), crucial for lipid digestion, are synthesized from cholesterol in the liver and act as biomarkers and signaling molecules, influencing disease states through complex interactions with gut microbiota. A metabolomics LC-MS workflow: Simultaneous Quantitation and Discovery (SQUAD), was implemented for fecal BA and BA conjugates analysis. The workflow incorporates Real-Time Library Search (RTLS), for enhanced identification confidence of relevant unknowns through spectral similarity measures during method execution.

**Methods**
Human stool material and BA standards from NIST and CIL were utilized to build the library needed for SQUAD. The feces were spiked with labeled standards and extracted with 80% methanol. Fresh fecal samples were also obtained from a dietary intervention involving mice fed one of three diets, each comprising 15% of kcal from fat. The diets varied in fat source: standard (soybean oil), SFA-rich (cocoa butter and soybean oil), and MUFA-rich (olive and soybean oils). Following 29 days under each dietary condition, fecal samples were collected, and metabolites were extracted using 80% methanol. Subsequently, metabolites were separated using reversed-phase column. The tribrid mass spectrometer enabled sensitive PRM-quantitation on the linear ion trap and HRAM Orbitrap MS1 scanning for higher annotation rates.

**Preliminary Data**
The labeled and unlabeled BA standards were used to construct calibration curves for absolute quantitation. The quantitation provided a wide dynamic range with metrics of 10 fmol lower limit of quantification and a 0.25 fmol lower limit of detection for most targets. MSn-based quantitation enabled selective detection of co-eluting isomers and isobars, enhancing discrimination between analyte signals and matrix interferences.

This study enables the assessment of bile acids and other metabolic profile differences in the mice fecal samples across various groups due to improved annotation capabilities of the HRAM data. Finally, confidence in the targeted and untargeted MS-based workflow's quality was obtained by regularly monitoring the spiked IS.

**Novel Component**
SQUAD metabolomics workflow for parallel targeted quantitation, deep coverage, and confident annotation of fecal bile acids using IQ-X Tribrid.

**----------Poster Board #5----------**

**Title**
## User-Friendly In-Silico Bioprocess Optimization Tool Integrating Flux Balance Analysis with Genetic Algorithms

**Author(s) (Presenting author should be listed first)**
Oğuzcan Ünver, Benjamin Gerber, Fırat Kahya, Boran Saruhan
**Author Affiliations**
Metastate Bio Inc

**Introduction**
Metastate Bio has developed a user-friendly software tool that integrates Flux Balance Analysis (FBA) with Genetic Algorithms (GA) to refine metabolic models and optimize bioprocesses. This tool serves as a cost-effective replacement for traditional wet-lab experiments, allowing researchers to simulate and optimize processes such as fermentation and cultivation within minutes. The intuitive user interface (UI) ensures accessibility for researchers and scientists in biotechnology, pharmaceuticals, and synthetic biology.

**Methods**
Our tool utilizes publicly available metabolic models and proprietary genome-scale models. Through a user-

friendly interface, researchers can input data and perform hundreds of thousands of tests to match experimental data by turning off non-essential reactions and adding new ones from databases like HMDB, KEGG, Reactome, and MetaCyc. The GA optimizes the metabolic pathways to fit historical data, providing high-fidelity, reproducible models. This process significantly accelerates research and development by enabling rapid iteration and optimization.

**Preliminary Data**

Feasibility studies estimate that the tool can reduce experimental time by 90% and costs by 70% compared to traditional methods. The tool is designed to evaluate over 100,000 combinations for processes such as recombinant production, medium optimization, and parameter optimization, with a 1000-fold increase in the number of combinations evaluated. These estimates highlight the potential efficiency and usability for researchers.

**Novel Component**

Genetic Algorithm-Enhanced FBA Tool with User Interface

**----------Poster Board #6----------**

**Title**

**A Flexible Cloud Framework for Untargeted Metabolomics Streamlining High-Throughput Analysis**

**Author(s) (Presenting author should be listed first)**

Gandhi, Monil; Stancliffe, Ethan; Richardson, Adam; Mehta, Ashima; Guzior, Douglas V.; Cho, Kevin; Cohen, Tom; Patti, Gary

**Author Affiliations**

Panome Bio, Inc.; Washington University in St. Louis

**Introduction**

Next-generation metabolomics, driven by advances in analytical techniques like liquid chromatography-mass spectrometry (LC-MS), generates massive, complex datasets requiring extensive processing. Conventional workflows, often reliant on local computational resources, struggle to efficiently handle the scale and complexity of modern metabolomics studies. To address this challenge, we have developed a scalable, cloud-based metabolomics workflow leveraging Google Cloud Platform (GCP). Our solution provides a robust, flexible environment for high-throughput metabolomics data processing, enabling seamless peak detection, metabolite identification, and downstream analysis. By facilitating efficient data handling and analysis, our infrastructure empowers researchers to accelerate the pace of discovery in metabolomics, paving the way for new insights into biological systems and disease mechanisms.

**Methods**

We transitioned our ML/TensorFlow-based metabolomics pipeline from an 8-core local setup to a scalable cloud-based infrastructure on Google Cloud Platform (GCP). The pipeline is executed on VMs, initially provisioned as n1-standard-32 instances, and then scaled up or down (to 64-core VMs) based on the specific computational requirements of each analysis. This flexibility allows for efficient resource utilization and optimal performance across a wide range of metabolomics studies. Leveraging Docker containers ensures consistent environments and reproducibility across different computational resources. Google Buckets provide scalable storage for both raw LC-MS data and processed results.

Additionally, a dedicated 32-core VM hosts JupyterHub, enabling collaborative data analysis and visualization. This separate environment allows team members to securely access and interact with processed data

**Preliminary Data**

Our cloud-based infrastructure has been validated across multiple projects, demonstrating its efficiency and robustness in handling metabolomics datasets of varying sizes. The ability to utilize VMs running Docker images, each tailored to specific client requirements, has proven crucial in enabling the simultaneous processing of multiple client projects, showcasing the platform's capacity for parallel execution. We successfully processed a large-scale COVID-19 study comprising 707 samples, further demonstrating the platform's ability to handle

demanding real-world applications.

In addition to the scalable pipeline execution environment, our infrastructure includes a dedicated VM running JupyterHub, enabling seamless collaborative analysis and visualization. JupyterHub is accessed securely through a DNS and username/password authentication, with each team member having their own allocated storage space. Centralized administration ensures robust security management for both the JupyterHub and pipeline VMs.

The use of Docker containers and GCP VMs further allows us to scale both horizontally (by adding more containers or VMs) and vertically (by increasing the resources allocated to individual VMs) to meet varying computational demands, ensuring optimal performance and resource utilization across a diverse range of metabolomics studies.

**Novel Component**

A validated cloud-native solution for scalable and efficient metabolomics, surpassing on-premises capabilities

**----------Poster Board #7----------**

**Title**

**A High-throughput Mass Spectrometry Platform (Rapidfire-IM-QTOF) to Support Biomarker Discovery**

**Author(s) (Presenting author should be listed first)**

Xiang Tian, Cristina Di Poto, Sonja Hess, Erik L. Allman

**Author Affiliations**

AstraZeneca

**Introduction**

High-throughput mass spectrometry is essential for large-scale discovery omics studies. Conventional LC-MS methods require minute-level separation times, which hinder the application to large cohort projects (e.g., >1000 samples). To address this challenge, we are developing a novel Rapidfire-IM-QTOF approach, which leverages on-line SPE cartridges (graphitic carbon, HILIC, C18, and mixed mode), to select for a wide range of molecules and enable biofluid sampling at a remarkable speed (~10s/sample). Public and in-house CCS (cross collision section) libraries will assist in the annotation of detected molecules (e.g., metabolites, lipids). This method will significantly increase the scale of our data acquisition and open the door to integrating with other large cohort omics initiatives, such as genomics, transcriptomics, and proteomics

**Methods**

A Rapidifre 400 and Agilent IM-QTOF 6560 mass spectrometer were coupled to build a fast data acquisition platform. Various extraction and loading/eluting solvent combinations were tested for each SPE cartridge to optimize the experimental conditions. Subsequently, the platform was used to acquire omics data on different sample types (e.g., human urine, plasma, and serum from healthy volunteers) in both positive and negative ion modes, to test analyte coverage and reproducibility. For data processing, PNNL-preprocessor (v4.0), IM-MS browser (v10.0), and MS-Dial (v4.8) were used to perform demultiplexing, CCS calibration, and peak extraction, respectively. Public (CCS compendium) and in-house CCS libraries were used to annotate the extracted features.

**Preliminary Data**

Human urine, plasma, and serum were collected from healthy volunteers recruited by the research specimen collection program at AstraZeneca in Gaithersburg, MD, USA. Data were acquired as described above. First, several optimized cartridge conditions were identified; for Graphitic Carbon metabolomics this was - loading solvent: 100% ACN with 0.1% HCOOH, eluting solvent: ACN/ACE/Water (3:3:4) with 0.1% HCOOH. Under these conditions and based on the intensity/coverage results from a mixture of isotopic labeled amino acids - 13 of 17 amino acids were detected with low relative standard deviations (RSD) from 3.5% to 11.6%. Second, in human urine, different extraction ratios (1:10, 1:50, and 1:100) were tested. In the 1:10 dilution, approximately 1268 MSI Level 3 features were found while 1081 and 1068 were found in the1:50 and 1:100 dilutions, respectively. After referencing the CCS libraries and filtering for signal quality (Signal/Blank>3 and

RSD<30%), ~90 total metabolites were annotated at MSI Level 2 in the 1:10 dilution, with 54 in the 1:50 dilution and 48 in the 1:100 dilution. Known urine metabolites like N,N-dimethylarginine, citrulline, ornithine, uric acid, and lysine, key components of arginine metabolism, were detected, further validating the method. Third, during the human serum/plasma optimization, around 500 features were detected. In serum, ~20 metabolites (e.g., 1-methylhistidine, phenylalanine, and cystine) and 60 lipids (PC, PE, LPC, LPE, and TG) were filtered and confidently annotated, which indicated both lipidomics and metabolomics were achievable in this platform. Attempts to remove polar lipids during preparations are ongoing, in the hopes of expanding our metabolite coverage. Additionally, we will continue to implement other SPE cartridges and optimize preparation methods for lipid-rich samples like serum/plasma and tissue. This high-throughput data acquisition method will significantly benefit biomarker discovery and drug development efforts by enabling large cohort metabolomics or lipidomics projects

**Novel Component**

A novel high-throughput discovery metabolomics data acquisition platform.

**----------Poster Board #8----------**

**Title**

**An Accelerated Workflow to Extract Biological Pathway Information from Untargeted Metabolomic Datasets**

**Author(s) (Presenting author should be listed first)**

Montefusco, David1; Xie, Longsheng2,3; Saligrama, Siri1; Yue, Yang1; Liu,Jinze2

**Author Affiliations**

1.Virginia Commonwealth University Department of Biochemistry. 2.Virginia Commonwealth University Department of Biostatistics. 3.George Mason University Department of Biostatistics.

**Introduction**

There is an ever-growing library of tools that facilitate identification biological, disease and drug pathways from high throughput data sets including metabolomics. These include Metabolanalyst, KEGG toolkits, Ingenuity Pathway Analysis (IPA) and others. Despite the vast array of options provided by these tools, their design does impose limitations on choices provided to the user. More importantly, the process of going from raw metabolomics data to a list of usable identifiers best suited to each application imposes a series of bottlenecks that limits the number of analyzable targets. Our enhanced Python-based workflow translates compound names into KEGG and HMBD IDs with substantially greater effectiveness than other readily available tools. Additionally, it performs comparison statistics based on user-provided variables including multifactorial analysis, followed by enrichment analysis using a novel HMDB-based enrichment analysis tool which distinguishes between metabolic, disease and drug-targeted pathways. Additionally, outputs are formatted for rapid input into Metaboanalyst, KEGG and IPA toolkits.

**Methods**

Our workflow is Python based. The key scripts perform the translation and enrichment analysis. The translation script queries compound name inputs against a carefully curated database of compound identifiers. These compound names are largely drawn from the HMDB database, but have been thoroughly filtered to remove redundant, ambiguous or duplicate identifiers. The finished translator greatly outperforms readily available web-based translators such as that available through Metaboanalyst in terms of name coverage and the rate of misidentifications. The enrichment analysis performs overrepresentation analysis on significant metabolites selected automatically based on the Tukey-HSD P-value. The enrichment analysis draws from all of the HMDB categories including Disease, Drug Action, Drug Metabolism, Metabolic, Physiological, Protein, Signaling, and a category called Pathway. Among these categories, the three richest in metabolites, that is Disease, Metabolic and Drug Action are selected for enrichment from within each category.

**Preliminary Data**

We applied our workflow to the challenging problem of identifying a role for SPTLC3 in metabolic adaptation to acute alcohol exposure. SPTLC3 is an inducible subunit of the SPT complex which carries out the initial reactions of the sphingolipid metabolic pathway. Knocking out SPTLC3 in hepatocytes and cardiomyocytes revealed to have a mitochondrial defect, and in a parallel study we are determining if the altered mitochondrial

function of hepatocyte-specific SPTLC3 could be protected from alcoholic liver disease. Bridging in vivo and in vitro models of alcoholic liver disease is challenging and requires precise dissection of cell pathways to pinpoint the direct effects of alcohol in the in vitro model. Our preliminary analysis of wild type and SPTLC3 knockout (SPTLC3ko) hepatocytes indicates that with ethanol treatment SPTLC3hko hepatocytes show higher levels of metabolites associated with oxidative stress. A parallel mouse feeding study implies some level protection in STPLC3hko mice from liver damage with alcohol feeding. These data imply that protection from oxidative stress may be a part of this protective effect.

**Novel Component**

The workflow provides two novel components. First is the dramatic improvement in translation of compound names to useful identifiers. Second is the enrichment analysis based on categories in the HMDB.

**----------Poster Board #9----------**

**Title**

**Analyzing the Impact of In-source Fragmentation on Phosphors Derivative Metabolites to Improve Untargeted Metabolomics Analysis**

**Author(s) (Presenting author should be listed first)**

Yue, Yang*; Cowart, L. Ashley; Allegood, Jeremy; Scalzo, Megan

**Author Affiliations**

Lipidomics and Metabolomics Shared Resource, Massey Cancer Center, Virginia Commonwealth University

**Introduction**

Electrospray ionization (ESI) is widely used for the targeted and untargeted metabolomics analysis. However, as an atmospheric ionization mode, in-source fragmentation or collision induced dissociation (CID) is an inevitable occurrence when charged and non-polar metabolites transfer from the ion source to the vacuum chamber. Phos-nucleotides and Phos-sugars indicate obvious in-source fragmentation that partial phosphor group releases from parent metabolites and exhibits fragments signals at MRM or MS full scan. As a result, it increases the risk of mis annotation of fragment products or underestimation of the abundance of parent metabolites. Our study quantitatively analyzed the in-source fragmentation products of 21 common phosphor derivatized metabolites and provided a correction approach to avoid the misannotated fragmentation products and improve the reliability of the analysis of the abundance of phosphor derivates. Overall, this approach should increase the identification of phosphor metabolites and analysis of their abundances with untargeted metabolomics

**Methods**

The in-source fragmentation products of 21 common Phos-nucleotides and Phos-sugars metabolites were quantitively analyzed and compared to their parents respectively with AB SCIEX QTRAP 6500 system. A correction method was set up to estimate the real abundance of parent metabolites based on the fragmentation ration at different injected concentrations. Combining the retention time of Phos-nucleotides and Phos-sugars metabolites on Vanquish ultra-high performance liquid chromatography (UHPLC) system, the annotations of fragment products of above phospho-derivate compounds were firstly validated. The setup algorithm was then used to correct the abundance and the fragments products on a Q-Exactive HF mass spectrometer with electrospray ionization source (HESI-II) operating in positive (+ESI) and negative (-ESI) modes.

**Preliminary Data**

A total of 21 common Phos-nucleotides and Phos-sugars metabolites on glycolysis, pentose phosphate pathway and nucleotides metabolic pathways were used to estimate the impact of in-source fragmentation effect on the abundance of parent metabolites under ESI mode. The retention time ranged from 1.2 min to 5.8 min with clear separation of fragments products and parent metabolites. Multiple algorithms were investigated to correlate and fit the abundances of fragment products with parent metabolites at different injected concentration of standard compounds with R program. For Phos-nucleotides, such as ATP and ADP, a liner regression indicated best fitting with more than 90% confidence and consistent with the mechanism of phosphorylation breakage on pyrophosphate group.

Above modeling was optimized and applied for untargeted analysis. Matrix effect was also estimated in this study with mouse liver samples. To maximize the annotation coverage from above 21 metabolites, automatic

iterative exclusion with data-dependent fragmentation (ddMS2) of the top 5 most abundant peaks from a full MS scan were acquired in both positive and negative modes. The detected fragment products were validated after removing false positives generated from in-source fragmentation based on the retention time from our in-house library. A total of 2968 features were detected with 524 annotations, among which 29 annotations contained single or multiple phosphor groups. The abundances of detected parent phosphor derivate metabolites from treatment and control samples were respectively corrected by adding a proportion of abundances of those removed false positive fragments. This correction did not impact the distribution on PCA plot but could exhibit significant difference on fold change of some phosphor compounds, and improvement the quantitative enrichment analysis with the pathways of glycolysis and pentose phosphate pathway. Although the annotation could not cover all 21 investigated metabolites, this study provides a feasible approach to improve the confidence of annotation and reduce the impact of in-source fragmentation for untargeted metabolomics analysis

**Novel Component**

Frist time to correct the annotation and variation caused by in-source fragmentation on analyzing phosphor derivates with untargeted metabolomics

**----------Poster Board #10----------**

**Title**

**Annotation of nontargeted LC-MS features in the Jackson Heart Study**

**Author(s) (Presenting author should be listed first)**

Avila-Pacheco, Julian; Chen, Zsu-Zsu; Tahir, Usman; Katz, Dan; Yan, Gao; Shuilang, Deng; Wilson, James; Gerszten, Rob; Clish, Clary

**Author Affiliations**

Broad Institute of Harvard and MIT, Cambridge, MA, US.

**Introduction**

The annotation of nontargeted liquid chromatography - mass spectrometry (LC-MS) features is a major challenge, given the large number of unknown features that are often detected, and the complexities associated with predicting correct compound matches. In this study, we used a variety of approaches to identify fasting plasma metabolites that were associated with incident diabetes and whole genome allelic variants in samples from 2,750 participants in the Jackson Heart Study (JHS), a community cohort of self-identified African Americans.

**Methods**

Plasma polar metabolites were analyzed using hydrophilic liquid chromatography (HILIC) coupled to high resolution mass spectrometry (HRMS). Feature abundances for annotated compounds and unknown features were used to compute associations with diabetes and whole genome data. Unknowns of interest were then targeted for product ion mass spectra (MS/MS) generation. The resulting spectra were used to produce Molecular Networks (Global Natural Product Social Molecular Networking) and machine learning-based structure predictions to rank structural matches for unknown features of interest.

**Preliminary Data**

We identified a novel isomer of 1-deoxyceramide Cer(m18:1/24:0) that was associated with incident diabetes (mean 10.2 years of follow-up) and the genomic variants in the MEIS2 and CPS1 loci. Moreover, we found 37 high confidence annotations for unknowns with whole genome associations, five of which (all-trans-retinol, zeaxanthin, 5,6 dihydrouridine, AICA-Riboside, and cholestanone) had not been previously reported.

**Novel Component**

We incorporated genomic data, chemical phenotyping, and MS/MS to elucidate novel metabolite-locus associations in a population that is often underrepresented in omics studies.

**----------Poster Board #11----------**

**Title**

**Assessing redundancy in untargeted metabolomics feature picking to produce high-fidelity quantitative datasets**

**Author(s) (Presenting author should be listed first)**
Bonitatibus, Sarah; Henke, Matt
**Author Affiliations**
Department of Pharmaceutical Sciences, University of Illinois at Chicago

**Introduction**
While untargeted quantitative metabolomics techniques are employed in a variety of applications, these experiments require accurate quantitation of all metabolites present within a sample to produce robust representations of the metabolomic phenotypes under study. However, careful consideration must be given to raw data pre-processing approaches, as these procedures contribute greatly to variability in metabolite feature quantitation. As a result, downstream statistical tests (such as differential abundance analysis), have lower integrity and may misrepresent biological phenomena in the form of false positives and false negatives. This study addresses this concern by introducing a redundancy metric to evaluate the fidelity of feature quantitation in untargeted metabolomics feature tables.

**Methods**
The redundancy metric was developed using feature tables generated from the raw untargeted metabolomics data collected as a part of the Integrative Human Microbiome Project's (HMP2) investigation of Inflammatory Bowel Disease (IBD). This simple calculation is defined as the proportion of features in a table that have at least one redundant match within the specified m/z similarity and retention time difference cutoff ranges. The published feature tables from HMP2 were compared in terms of feature redundancy and metabolome coverage to a series of feature tables generated using a multivariate parameter sweep method in ASARI, an open-source software tool for metabolomics data feature picking. The redundancy metric and all downstream statistical analyses were implemented using R and RStudio.

**Preliminary Data**
The redundancy metric can quickly assess the fidelity of feature quantitation in untargeted metabolomics data. Thus, it is a useful tool for critically evaluating the integrity of a single feature table when raw data are unavailable, or for comparing groups of feature tables produced from the same raw data under different conditions (software tools, algorithmic strategies, parameter selections, etc.). Using a multivariate sweeping approach, feature tables were generated using ASARI with combinations of values selected for the min_peak_height, min_intensity_threshold, wlen, gaussian_shape, min_timepoints, and snr parameters. Then, the results of this parameter sweep were used to construct a multivariate regression model describing the impacts of feature quantitation and parameter selection on the redundancy metric and metabolome coverage. A feature binning approach preliminarily shows potential to minimize redundancy while optimizing metabolome coverage without sacrificing quantitation integrity. Finally, differential abundance analyses were performed to assess the impact of variances in quantitation on the associations of features with health and disease. To establish a ground truth dataset containing the most accurate peak areas of metabolites detected in HMP2, manual integration was performed on a subset of metabolites to enable comparisons in quantitation across feature tables. It was found that redundancy serves as a marker of poor quantitation, as deviations in differential abundance analysis results were detected more often among redundant features. Under suboptimal feature picking conditions, poorly quantitated features resulted in greater rates of false positives and false negatives in downstream statistical analyses. The redundancy metric can be used to gauge the extent to which this issue is affecting a feature table, as a method of assessing the fidelity of data processing methods. This work moves towards understanding how data processing can lead to misrepresentations of biological phenotypes, and preliminarily shows potential paths to remediation of suboptimal feature picking.

**Novel Component**
The redundancy metric reflects feature quantitation integrity, supporting critical evaluation of biological hypotheses generated from untargeted metabolomics experimental data.

**----------Poster Board #12----------**

**Title**
**Comparison of Sample Preparation methods for At-home Feces Collection in Global Metabolomics**

**Author(s) (Presenting author should be listed first)**

Lan, Renny; Assress, Hailemariam; Malaviarachchi, Priyangi; Kay, Colin
**Author Affiliations**
Metabolomics Core, Arkansas Children's Nutrition Center, Arkansas Children's Hospital, USA

**Introduction**
Feces is a matrix of choice to study gut metabolomics, the study of metabolites produced by the gut microbiome, which has offered critical insights into the interactions between diet, gut microbiota, and the host's health. However, feces is difficult to work with due to its matrix complexity and heterogeneity between and within samples. Variation can also arise during fecal sample collection and storage that may introduce microbial interference or affect the stability and recovery of the metabolites. In this study, OMNImet GUT (ME-200), a novel sampling device that provides an all-in-one solution for ambient temperature collection and transport of human fecal samples for metabolomics were examined to identify optimal methodology for extraction and metabolomics analysis.

**Methods**
Samples were collected from one healthy donor into three OMNImet GUT tubes using the OMNImet tube collection protocol and tubes were left at room temperature for 48 hours before fecal slurry was transferred and aliquot to screw cap microtubes. Flash-frozen feces were collected, weighed, put on dry ice, and used as controls. The flash-frozen feces samples were prepared using an in-house feces extraction method, while the OMNImet feces samples were prepared using five separate extraction methods for metabolomics analysis. Pooled quality control (QC) samples and four spiked internal standards were used to ensure data integrity. Reverse-phase chromatography was used to analyze the extracted samples using LC-HRMS. Data was processed using Compound Discoverer v3.3.

**Preliminary Data**
In this study, five sample preparation methods for feces samples collected using OMNImet GUT and one existing extraction method for flash-frozen feces samples were tested for untargeted metabolomics. The extraction methods used for the OMNImet included 1) Spin and inject: take aliquot equivalent to 100 mg, centrifuge and inject, 2) Reconstitution solvent exchange: take aliquot equivalent to 100 mg, dry, reconstitute in 5% methanol , 3) Bead beating homogenization: take aliquot equivalent to 100 mg, homogenize, spin, dry and reconstitute in 5% methanol, 4) Extraction solvent exchange: take aliquot equivalent to 100 mg, dry, add extraction solvent (90% ACN: MeOH (1:1)), homogenize, spin, dry and reconstitute in 5% methanol, 5) Extraction solvent exchange: same as method 4 but starting with 50 mg feces. The flash-frozen sample extraction involved taking 100 mg of feces, homogenization with beads, spinning, drying, and reconstitution in 5% methanol. Overall, Low variation in the wet weight of feces collected from the three OMNImet GUT tubes was observed among three replicates (CV = 5.06%). The number of total features and annotated metabolites shared a similar trend across all methods in which Method 3 and Method 4 were comparable to the method used to prepare the flash-frozen feces. Exchanging the extraction solvent and homogenization step improved the number of level 1 & level 2 annotated metabolites. In addition, lowering the sample weight by half to 50 mg (Method 5) improved the number of metabolites annotated at level 1 & level 2, which could be attributed to a reduced matrix effect. Variations in the sample preparation methods also presented differences in chemical classes of metabolites, highlighting the importance of study design to address study-specific research questions to identify relevant metabolic pathways.

**Novel Component**
Feces is a matrix of choice to study gut metabolomics, the study of metabolites produced by the gut microbiome, which has offered critical insights into the interactions between diet, gut microbiota, and the host's health. However, feces is difficult to work with due to its matrix complexity and heterogeneity between and within samples. Variation can also arise during fecal sample collection and storage that may introduce microbial interference or affect the stability and recovery of the metabolites. In this study, OMNImet GUT (ME-200), a novel sampling device that provides an all-in-one solution for ambient temperature collection and transport of human fecal samples for metabolomics were examined to identify optimal methodology for extraction and metabolomics analysis.

**----------Poster Board #13----------**

**Title**

## Comprehensive Non-Targeted Characterization of Persistent Organic Pollutants (POPs) in Human Plasma Using GC and GCxGC with High-Performance TOFMS

**Author(s) (Presenting author should be listed first)**
John Hayes, David E. Alonso, and Joeseph E. Binkley
**Author Affiliations**
LECO Corporation

**Introduction**
Persistent organic pollutants (POPs) can be present in varying concentrations in human bodily fluids. They are often measured individually or in classes despite being present with other hazardous compounds in human plasma. These materials are typically introduced through diet and/or inhalation and have the potential to accumulate over time. Many of these substances are harmful even at low concentrations. Therefore there is a need to identify these foreign compounds collectively to avoid health complications associated with these toxins. A comprehensive analytical approach is ideal for the qualitative analysis of plasma. The objective of this study was to utilize gas chromatography and comprehensive two-dimensional gas chromatography â€“ high-resolution time-of-flight mass spectrometry (GC- and GCxGC-HRTOFMS) for profiling human plasma.

**Methods**
Method development was conducted using NIST SRM 1950 and 1957 plasma standards. The developed methodology was applied to the analysis of thirty human plasma samples. The plasma constituents were extracted, dried, and treated with derivatization reagents for liquid injections. Data was collected using GC-, and GCxGC-TOFMS and processed using automated Peak Find (Non-targeted). The compounds were annotated using spectral similarity searches (NIST & Wiley Databases), mass accuracy calculations for formula determinations, and retention index filtering. Characterization of unknowns was accomplished using a multi-mode source (EI, PCI, NCI), and high-resolution time of flight mass spectrometry (HRT). This not only increased the total number of compounds annotated but also added confidence to compound characterization. Differentiation of diseased and control samples was accomplished using novel statistical processing software based on Fisher ratios.

**Preliminary Data**
The comprehensive analysis of the plasma samples resulted in the annotation of metabolites such as amino acids, diacids, fatty acids, sterols, phosphates, and a wide variety of xenobiotics. The list of foreign compounds found in plasma included halogenated phenols, pesticides, polyaromatic hydrocarbons, dibenzothiophenes, phthalates, bisphenols as well as illicit, over-the-counter, and prescription drugs, and their metabolites. Downstream statistical processing facilitated rapid differentiation of samples and annotation of pollutants.

Targeted and semi-targeted methods have been used in the past to screen biological samples for contaminants. This type of analysis is often required due to the complexity of these samples and the low concentration of the pollutants. The developed methodology has the advantage of both enhanced chromatography and mass spectral resolution which increases the total number of annotated compounds.

**Novel Component**
New methodology for the comprehensive characterization of complex samples.

**----------Poster Board #14----------**

**Title**

## Cough Breath: A Method to Detect Airway Pathogens of Cystic Fibrosis Patients in the Age of Highly Effective Modulator Therapy

**Author(s) (Presenting author should be listed first)**
Karunarathne, Hansani1; Bridges, Christopher1; Remisoski, Lacy1; Crane, Maddey1; Casanova, Claudia1; Kinne, Samantha2; Castillo Bahena, Alicia2; Gil, Marissa3; Padillo, Lienwil3; Querido, Gabriel3; Mielke, Jenna3; McClelland, Marc2; Conrad, Doug3; and Quinn, Robert1
**Author Affiliations**
1Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, USA. 2

Corewell Health, Grand Rapids, MI, USA. 3 Department of Medicine, University of California San Diego, La Jolla, CA, USA.

**Introduction**

Obtaining sputum samples from people with CF (pwCF) for microbial analysis has become challenging due to the positive clinical effects of the CFTR modulator triple therapy, Elexacaftor-Tezacaftor-Ivacaftor (ETI). As an alternative, breath analysis has gained interest in the scientific community as a non-invasive technique that can detect airway pathogens in CF research. Our study employed a novel dual sampling approach called â€˜Cough Breath' (CB), which combined cough-based detection and volatilomics in a single non-invasive procedure. We aimed to identify unique volatile organic compounds (VOCs) signatures linked to CF respiratory tract infections in the Exhaled Breath Condensate (EBC) of pwCF, providing insights into the volatile compounds associated with CF infections.

**Methods**

We used the CB method, where participants coughed into a microbial filter and intermittently breathed through it into an EBC collection device. This technique enabled the detection of common CF pathogens via culture-based analysis of the CF filters. The CF pathogens isolated from CB or sputum and the EBC of pwCF were analyzed using purge and trap gas chromatography-mass spectrometry (GC-MS). To identify unique VOC signatures, VOCs in EBC (n = 98) from pwCF (n = 71) were matched with those produced by clinically relevant CF pathogens, such as Pseudomonas aeruginosa (PA) and Staphylococcus aureus (SA), creating a comprehensive map of the pathogens' volatilome.

**Preliminary Data**

Thirty VOCs identified in the EBC were exclusively associated with PA isolates (n = 21), and 23 VOCs originated solely from SA isolates (n = 24). Among these VOCs, methyltartronic acid and 2-methyl-1-propene, exclusively produced by SA and PA, respectively, were identified as top VOCs in EBC and showed potential for distinguishing between pwCF positive for these specific pathogens. Additionally, twelve VOCs produced only by Achromobacter xylosoxidans (AX; n = 10) were detected in the EBC of pwCF, while eight VOCs were linked exclusively to Streptococcus sp. (n = 7). Some key VOCs produced by CF pathogens frequently detected in EBC include propyl pyruvate and 2,3-pentanedione. Among the VOCs identified in EBC and produced by CF isolates, several compounds have been previously reported as volatiles of clinical isolates. These include methyl isobutyl ketone, 1-chloro-3-methyl-butane, 2-pentanone, 2-butanone, 3-methyl-2-butanone, and methyl vinyl ketone produced by PA; propanal, acetoin, 2-methyl-1-propene, butane, and 2-butene produced by SA; and 2,3-butanedione produced by Streptococcus sp. Additionally, we observed that while some VOCs produced by CF pathogens matched with the EBC volatiles of CF patients, their presence was inconsistent. Therefore, a preliminary analysis was conducted to explore the variation in the volatilome produced by these isolates at different growth phases. The results showed that the production of VOCs by CF isolates varies based on both strain and growth phase, which could potentially impact the EBC volatilome of CF patients.

**Novel Component**

The novel CB approach offers valuable insights into CF infections by providing comprehensive metabolomic data.

**----------Poster Board #15----------**

**Title**

**Deep Matter annotation using 4D-Metabolomics in Glycyrrhiza uralensis used for Traditional Chinese Medicine**

**Author(s) (Presenting author should be listed first)**

Forsberg, Erica M.; Nakabayashi, Ryo

**Author Affiliations**

Bruker Daltonics

**Introduction**

Plant derived natural products (phytochemicals) have been used as traditional medicines for centuries. They contain chemically diverse secondary/specialized metabolites that often possess bioactivity that may be beneficial as therapeutics or identifying lead compounds for drug development. Characterization of natural

products requires in-depth spectral characterization using mass spectrometry (MS). In addition to accurate mass, isotope pattern and fragmentation pattern, ion mobility adds an orthogonal measurement, collisional cross section (CCS) that is unique to the size and shape of a molecule. Trapped ion mobility spectrometry (TIMS) is a highly accurate technique for measuring CCS and when coupled with MS-derived measurements, provides more confident annotations. Here we demonstrate characterization of Glycyrrhiza species extracts using TIMS-MS and the untargeted metabolomics software MetaboScape.

**Methods**

Three root samples (Glycyrrhiza uralensis, G. grabra, and roasted mixture of the two species) were purchased from Uchida Wakanyaku Ltd. and roughly milled. Samples were extracted with 80% methanol (300 ÂµL/mg dried weight of the samples) using a BeatBox (PreOmics). Samples were centrifuged at 13,000 rpm for 5 minutes at 4Â°C . Supernatants were analyzed in triplicate using a timsTOF fleX with a VIP-HESI source and an Agilent 1290 liquid chromatograph with a C18 column on a reverse phase gradient from 95% water to 95% acetonitrile both with 0.1% formic acid over 15 min. Data was acquired with default 4D-Metabolomics parameters which employ DDA-PASEF. Experiments were performed in both positive and negative polarity. Data analysis was performed in MetaboScape 2023b.

**Preliminary Data**

Positive and negative mode DDA-PASEF data were processed in MetaboScape2024 using default T-ReX 4D parameters for feature detection, retention time alignment, and deisotoping. Detected MS features were 10797 and 11535, and MS/MS features were 7607 and 8199 in positive and negative ion mode, respectively. Finally, 2439 features were annotated in merging data of both ion modes. Principal component analysis showed a clear separation between æ□±åŒ—ç”˜è□‰ and è¥¿åŒ—ç”˜è□‰, and ç‚™ç”˜è□‰, suggesting the change of metabolites depended on roasting. Features were annotated with open-source natural product libraries that contain structural information, either InChI or SMILES, in order to generate predictive CCS values with the machine learning algorithm CCS-Predict Pro, and in silico fragmentation patterns for matching with experimental data. These include KNApSAcK (http://www.knapsackfamily.com/KNApSAcK/) and MS/MS libraries (Bruker MetaboBase, NIST, and HMDB). All features that were annotated were inspected manually to determine accuracy of the annotation. Trapped ion mobility spectrometry plays a key role in separating isomeric and isobaric metabolites, which increases the number of secondary metabolites that are detected.

**Novel Component**

LC-TIMS-MS separation and annotation workflow for isomeric secondary metabolites from natural products

**----------Poster Board #16----------**

**Title**

**Integrative LC-MS and GC-MS Metabolic Profiling Unveils Dynamic Changes during Barley Malting**

**Author(s) (Presenting author should be listed first)**

Whitcomb, Sarah; Rani, Heena; Standish, Andy; Walling, Jason

**Author Affiliations**

USDA-ARS Cereal Crops Research Unit

**Introduction**

Malt is required for beer production and is considered the backbone of beer quality and taste. Malting starts from dry grain, typically barley, and comprises three steps: steeping, germination, and kilning. As the barley transforms during malting from a quiescent seed to germinating grain to kilned and dehydrated malt, a myriad of molecular changes occurs in the seed. Shotgun proteomics and transcriptomic studies have revealed the dynamic protein composition and breadth of gene expression changes across malting stages. However, these approaches only provide partial insights into the complex biochemical changes taking place during malting. The lack of a detailed metabolic understanding of this process limits our ability to assess and enhance malt quality.

**Methods**

This study employed untargeted GC-MS and LC-MS metabolite profiling across six malting timepoints: dry seed (malting input), post-steep (DOG0), on alternating days of germination (DOG1, DOG3, and DOG5), and at the end of kilning (malting output).

**Preliminary Data**

We identified 4,980 known metabolites, 82% of which exhibited significant changes during the malting process. Here we identified stage-dependent metabolic shifts and dynamic chemical classes and pathways between each studied stage. These results can guide the fine-tuning of malting conditions to improve malt quality for beer production and other malt-based applications. Additionally, metabolites with antimicrobial properties were identified, underscoring the interplay between barley and microbial metabolic processes during malting. Further research into these microbial metabolites and cognate microbes may lead to novel malting assessment traits for high-quality and safe malted barley.

**Novel Component**

Kilning increased heterocyclic compounds, known for their flavor-active properties. Antimicrobial compound classes with dynamic levels during malting were identified.

**----------Poster Board #17----------**

**Title**

**Dietary Vitamin B6 Deficiency Impairs Liver and Fecal Metabolites in a Mouse Model of Non-Alcoholic Fatty Liver Disease**

**Author(s) (Presenting author should be listed first)**

Mayengbam, Shyamchand; Amarasena, Sathya; Hossain, K Shaharina; Rasauli, Ava

**Author Affiliations**

Department of Biochemistry, Memorial University of Newfoundland, St.John's, Canada

**Introduction**

Non-alcoholic fatty liver disease (NAFLD) is a complex liver disorder affecting a significant number of people worldwide. Research has indicated that dietary micronutrients are associated with the onset of NAFLD. Specifically, a deficiency in vitamin B6 can disrupt one-carbon (1C) metabolism, leading to alteration in key metabolic pathways which exacerbate oxidative stress and liver fat accumulation. This study aimed to investigate how dietary vitamin B6 influences the development of NAFLD, with a particular focus on its effects on 1C metabolism.

**Methods**

A total of thirty-two male C57BL/6J mice were fed one of four diets for eight weeks: a control diet (n=8), a high-fat, high-sugar (HFHS) diet (n=8), an HFHS diet high in vitamin B6 (HFHS-HB6, n=8), or an HFHS diet low in vitamin B6 (HFHS-LB6, n=8). Body weights were measured weekly, and urine and fecal samples were collected at three time points. At the end of the study, blood and tissue samples were collected for biochemical analysis. We performed targeted LC-MS/MS-based analysis of serum 1C metabolites, as well as 1H-NMR-based untargeted metabolomics for liver and fecal samples.

**Preliminary Data**

The body weights of HFHS-LB6 mice were significantly lower compared to those of the control and HFHS mice ($P<0.001$). However, the percentage of liver weight was significantly higher in the HFHS-LB6 group ($P<0.001$) compared to the other groups . Interestingly, liver triglyceride concentration was also significantly higher in the HFHS-LB6 group ($P=0.007$) compared to the other three groups. Targeted analysis of 1C metabolites revealed significant elevations in serum homocysteine, glycine, and serine, while the concentrations of betaine and glutamic acid were reduced in HFHS-LB6 animals compared to the other treatment groups. This impairment in 1C metabolism was also observed in liver metabolites through 1H-NMR-based metabolomics analysis, where betaine was lower and glycine was elevated. Furthermore, fecal 1H-NMR-based metabolomics revealed significant differences in fecal metabolites in HFHS-LB6 animals compared to the other groups, with tryptophan identified as the key metabolite responsible for the treatment effect. Our study highlights the critical role of vitamin B6 in the pathophysiology of NAFLD and suggests impairments in both gut and host metabolites.

**Novel Component**

Vitamin B6 deficiency exacerbates metabolite perturbations in NAFLD.

**----------Poster Board #18----------**

**Title**

## Elucidating exercise-responsive features in human plasma using multidimensional chromatography and a custom compound annotation workflow

**Author(s) (Presenting author should be listed first)**
Abraham Raskind, Charles R. Evans, Phd, Christopher Patsalis, M.S, Gayatri Iyer, PhD, Alexander Raskind, PhD, Alla Karnovsky, PhD, Charles F. Burant, M.D, PhD
**Author Affiliations**
University of Michigan

**Introduction**
The health benefits of physical activity, such as improved cardiovascular health, enhanced immune response, and increased longevity, are well documented. However, the mechanisms that underlie these benefits are incompletely understood. Metabolomics offers a useful to explore how multiple complex metabolic processes may contribute to these health benefits, but not all exercise-responsive compounds that can be detected in untargeted metabolomics data can be identified. By combining techniques including chromatographic fractionation and tandem mass spectrometry with custom software, we can obtain higher quality data which facilitates identification of unknowns of interest. This poster will describe a workflow that uses these techniques, alongside additional publicly available software tools, to better characterize both known and unknown exercise responsive metabolites.

**Methods**
First, data from two distinct metabolomics studies measuring exercise response in blood plasma were aligned to generate a consensus set of exercise responsive molecules, including unknowns. Then, pooled human plasma was extracted and fractionated using a semi-preparative LC column. Reversed-phase LC-MS/MS data was acquired from the resulting fractions and from three different sources of unfractionated plasma. Using our custom software tool, MetIDTracker, and the aligned feature set, potential matches to exercise-responsive unknowns were flagged based on m/z and RT proximity to features of interest. Identity and hybrid MS/MS search results were evaluated and annotated when possible. GNPS and SIRIUS were used to construct spectral networks and to aid in structural characterization of unidentified molecules of interest.

**Preliminary Data**
Using the aligned dataset, 860 features, including both knowns and unknowns, were determined to be differentially abundant in exercise-conditioned plasma. Most of these features lacked MS/MS data from the original analysis in which they were first detected. Of these, 459 potential matches with MS/MS data were found in fractionated and unfractionated pooled plasma. 284 of the resulting features had the highest intensity and MS/MS quality in fractionated pooled plasma, 90 were found in one of the three unfractionated plasma samples, and 4 were excluded due to being observed excess abundance in a solvent blank. 81 potential matches were found only after pooled plasma was analyzed using targeted MS/MS to follow-up on features not detected during the first round of data-directed LC-MS/MS of the fractions. When multiple distinct features matched the m/z and RT of a differentially abundant molecule, the one with the closest proximity was assigned as the primary â€œmatchâ€□, but all possible candidates were reviewed. Of metabolites that were observed with MS/MS in both fractionated and unfractionated plasma, signal intensity, MS/MS spectrum quality, and spectral match scores (when available) were generally higher in fractionated samples. The three pooled plasma samples also showed some differences in feature abundance and MS/MS quality. Once the features had been annotated to the extent possible in MetIDTracker, a GNPS network was constructed out of all generated MS2 data. Several features of interest were located in multi-feature subnetworks, revealing potential structural motifs that are currently under investigation. MS2 data processed using SIRIUS for in-silico annotation revealed additional putative structural information, serving another possible aid in unknown identification. Preliminary results indicate previously unidentified exercise-responsive include carnitine-related compounds, bile acids, multiple dipeptides, steroid derivatives and other compound classes.

**Novel Component**
A combination of multi-dimensional chromatography, MS/MS annotation with custom software, and additional tools helped characterize exercise-responsive known and unknown metabolites.

**----------Poster Board #19----------**

**Title**

**Emerging Opportunities and Obstacles in Metabolomic Epidemiology: A ChatGPT Study**

**Author(s) (Presenting author should be listed first)**

Waylon J. Hastings

**Author Affiliations**

Texas A&M University Department of Nutrition

**Introduction**

Metabolomics is an increasingly powerful tool to study diverse interactions in the etiology of human disease, leading to the emergence of metabolomic epidemiology as a research field. In 2023 an expert consensus of early career scientists within the Consortium of Metabolomics Studies (COMETS) identified a set of eight challenges and future directions facing the field of metabolomic epidemiology: (i) metabolite identification, (ii) metabolite harmonization, (iii) metabolite stability and reproducibility, (iv) metabolomics and diet, (v) tissue-specific metabolomics, (vi) single cell metabolomics, (vii) biobank metabolomics, and (viii) metabolomic studies in diverse populations. This investigation analyzes how these opportunities and obstacles have evolved over time by reviewing research published across the intervening period since this expert consensus.

**Methods**

Relevant publications were identified using a PubMed search for articles with a post-date following that of the early career perspective that had â€œmetabolomicsâ€ AND â€œepidemiologyâ€ MeSH terms OR those having â€œmetabolomicsâ€ AND â€œepidemiologyâ€ in the title/abstract. Following initial screening, the discussion, conclusion, strengths/limitations, and/or future directions text of qualifying articles was extracted. Following preliminary training on the expert consensus, relevant text of each article was input into the ChatGPT 4Î¿ AI learning module with a directive to evaluate how opportunities and obstacles in metabolomic epidemiology have developed over time.

**Preliminary Data**

66 articles were identified in the initial literature search. A total of 13 articles were removed during primary screening due to being duplicate records (n=2), descriptive cohort profiles (n=2), or articles with a disease or methodological focus outside metabolomic epidemiology (n=9), resulting in a final sample of 53 articles for evaluation. ChatGPT 4Î¿ AI identified a set of five opportunities and obstacles in initial training that reflect a synthesis of the expert consensus: (i) metabolite identification and harmonization across platforms, (ii) advancement of single-cell and spatial metabolomics, (iii) reproducibility and longitudinal stability of metabolomic measurements, (iv) integration of disease-relevant tissues, and (v) addressing population diversity and health disparities. These opportunities and obstacles were evaluated iteratively after subsequent training on 10, 27, and 53 articles to develop a final set of opportunities and obstacles currently facing the field of metabolomic epidemiology: (i) standardized protocols, methodologies, and statistics, (ii) integration of multi-omics approaches, (iii) longitudinal and diverse population-based studies, (iv) exploration of environmental and lifestyle exposures, and (v) clinical translation and regulation. Changing themes reflect the emergence of new research questions, such as using metabolomics to investigate exposure to environmental toxins and pollutants, as well as new approaches to address existing challenges, such as determining the effect of pre-analytical factors on scalar differences in metabolomics measurements in different tissues. Finally, these opportunities and obstacles were used to generate a collection of high-priority skills that will position researchers for success in the emerging field of metabolomic epidemiology.

**Novel Component**

Using AI to synthesize literature, identify research priorities, and generate relevant skills to facilitate future success

**----------Poster Board #20----------**

**Title**

**Fast scanning MRM quantitative lipidomics analysis**

**Author(s) (Presenting author should be listed first)**

Paul RS Baker (1), Rebekah Sayers (2), David Calquhoun (1), and Ian Moore (3)

**Author Affiliations**

1. SCIEX, US; 2. SCIEX, UK; 3. SCIEX, CA

**Introduction**

Herein, a method is presented and tested to sensitively detect and quantify lipids using a comprehensive lipidomics panel using the multiple reaction monitoring (MRM) scan mode with an ultrafast mass spectrometer. The panel consists of ~2000 lipid molecular species identified at the fatty acid level of structural specificity and depends on chromatography to separate lipids by class to mitigate isobaric interference. Due to lipids eluting as a class, the speed at which individual MRM transitions are measured is crucial to ensure good coverage and quantitative accuracy. A balance must be struck between the extent of the target list and the resulting data quality, especially during periods of high MRM concurrency. However, the SCIEX 7500+ system with fast MRM technology addresses this problem. It is ideal for large panel quantitative screening to provide more data points across individual peaks to improve peak shapes and the calculated %CVs for quantitative measurements.

**Methods**

Experiments were designed to compare the quantitative performance of lipid analysis using different dwell and pause times. Our findings reveal that a combined scan time of 3 ms (1.5 ms dwell and 1.5 ms pause times) on the SCIEX 7500+ system resulted in data with superior quantitative precision compared to the classic SCIEX 7500 system running at its fastest recommended combined scan rate of 5 ms. This advantage was most evident during periods of high MRM concurrency, with 59% more molecular species quantified with a %CV < 20 compared to previously established methods. The faster data acquisition yielded more data points across analyte peaks, which generated better peak shapes and improved lipid isomer resolution compared to the SCIEX 7500 system.

**Preliminary Data**

There appears to be a movement within small molecule omics from the classic untargeted experimental approach to biomarker discovery to a targeted method that utilizes a broad panel of metabolites. This is partly due to the challenges associated with untargeted data analysis and the growing need to measure biomolecules quantitatively. Consequently, metabolomic analyte panels are expanding from single-class panels to more extensive lists incorporating multiple classes. A faster scanning speed is required to maintain quantitative performance to accommodate the increasing lists of biomolecule targets.

The SCIEX 7500+ system can measure analytes at a combined speed of 1.2 ms per MRM transition (0.5 ms dwell and 0.7 ms pause times), enabling an 800 MRM/s scan speed. This fast scan rate was used to analyze samples in an unscheduled fashion to establish retention times, which resulted in 7-9 data points acquired across the analyte peak, facilitating RT determination. Faster data acquisition also improved the analyte peak shape, which resulted in better resolution of closely eluting isomers and a significant improvement in quantitative precision overall for the assay. During a period of the experiment with the highest MRM concurrency (~925 concurrent MRMs), there was a 60% increase in the number of molecular species measured with a %CV <20% using the SCIEX 7500+ system at a scan speed of 3 ms. Overall, the faster scan speed improved quantitative precision across all lipid classes in a global, broadly targeted panel of lipids. These results establish this platform as an ideal instrument for any large panel of analytes.

**Novel Component**

An ultra-fast MRM scanning experiment improves the quantitative precision of large panel analyte measurement

**----------Poster Board #21----------**

**Title**

**FraGNNet: A Deep Probabilistic Model for Mass Spectrum Prediction**

**Author(s) (Presenting author should be listed first)**

Fei Wang, Adamo Young, Bo Wang, Hannes Röst, Russ Greiner, David S Wishart

**Author Affiliations**

University of Alberta, University of Toronto

**Introduction**

Tandem mass spectrometry (MS/MS) plays an important role in untargeted metabolomics analysis. MS/MS workflows attempt molecular structure inference from mass spectral data: this is a challenging problem, and

reliable automated solutions remain elusive. Existing identification strategies often rely on retrieval from spectral libraries, but these approaches are limited by poor library compound coverage and incomplete instrumentation information about reference spectra.In this work we propose a new computational method for high resolution and interpretable MS/MS prediction, FraGNNet. Our model can be used to rapidly generate in-silico spectral libraries for compounds that lack ground truth spectra, increasing the effectiveness of retrieval-based approaches for spectrum identification.

**Methods**

Our method combines combinatorial fragmentation with deep learning to predict the mass spectrum. First, a bond-breaking algorithm generates a large set of plausible fragment structures from the input molecule. Then, a graph neural network predicts a distribution over the fragments, identifying which ones are most likely to appear during fragmentation. This fragment distribution is subsequently mapped to a distribution over chemical formulae, whose masses are used to determine precise peak m/z values in the spectrum.

**Preliminary Data**

FraGNNet achieves over 0.70 cosine similarity (using a 0.01 Da binning resolution) when evaluated on held-out data, outperforming other spectrum predictors. In terms of retrieval-based spectrum identification, FraGNNet performs well as an in silico library generation tool. FraGNNet is also highly interpretable, providing mass formula and fragment annotations for each predicted peak in the spectrum and an estimate of the total peak intensity that the model cannot explain. Through ensembling, unreliable peak intensities and annotations can be identified to increase user confidence in the model's predictions.

**Novel Component**

Novel machine learning model for mass spectra prediction

**----------Poster Board #22----------**

**Title**

**Glucose and Glutamine Metabolism in Hypertrophic Cardiomyopathy and Its Association with Inflammatory Pathway Activation**

**Author(s) (Presenting author should be listed first)**

Venturini, Gabriela; Padilha, Kallyandra; Pereira, Alexandre, Toepfer, Christopher; Seidman, Jon; Seidman, Christine.

**Author Affiliations**

Department of Genetics, Harvard Medical School; Howard Hughes Medical Institute; Heart Institute, University of Sao Paulo; Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford

**Introduction**

Cardiomyopathies are a significant cause of morbidity and mortality, affecting millions annually. Hypertrophic Cardiomyopathies (HCM) can be caused by mutations in sarcomere proteins that disrupt cardiac muscle fibers, leading to altered metabolism and disease progression. These metabolic changes, including impaired mitochondrial function, exacerbate cardiac dysfunction and are linked to immune responses, worsening inflammation and injury. Despite advances, the precise metabolic pathways and their role in disease development remain underexplored. Comprehensive studies integrating metabolomics with genetic and molecular analyses, along with new IPS-derived cell models, are essential to uncover novel therapeutic targets, improve patient outcomes, and reduce the global burden of these diseases. Here, we describe the main metabolic pathways altered in HCM using mass spectrometry, 13C-labeled fuel, and drug inhibition.

**Methods**

In this study, we developed cardiomyocytes derived from IPS cells edited via CRISPR to carry HCM-associated mutations in MYH7, TNNT2, TNNI3, MYBPC3 and WT treated with endothelin to induce hypertrophy. Contraction function was measured under different fuel conditions. Targeted polar LC-MS metabolomics was performed on all cell models, with altered metabolites validated in MYH7-mutant mouse hearts and explanted human hearts containing MYH7 mutation. The 13C glucose and glutamine flux was measured in HCM cell models using LC/MS, and cell function was assessed after inhibiting glycolysis and glutamine uptake with 2DG and BPTES. The association between metabolic alterations and immune response was evaluated using western blot and transcriptional alteration after glycolysis inhibition was measures by RNAseq.

**Preliminary Data**
Previous data showed IPS-cardiomyocytes carrying HCM-associated mutations in MYH7, TNNT2 and TNNI3 genes, and WT treated with endothelin, exhibited increased contractility and altered mitochondrial function. Here, we demonstrated that HCM cell models cultivated in media with minimal glutamine, glucose, and fatty acid content reduced contraction function within 12hr, whereas WT maintained normal contraction up to 48hr. All HCM models showed altered metabolites compared to WT, with phenotypes like WT cells treated with endothelin. These models exhibited increased ATP production, higher ATP/ADP ratios, and low AMP levels. Phosphocreatine/ATP and creatine levels were also very low. Similar findings were observed in animal models. The TCA cycle was altered in all HCM models, with downregulation of citrate, isocitrate, and aconitate. Glycolysis was upregulated at different points in the cycle, with MYH7 showing increased fructose 1,6-bisphosphate, DHAP, and glyceraldehyde 3-phosphate, while 1,3-bisphosphoglycerate was increased in TNNI3 and TNNT2. Both animal and human HCM hearts with MYH7 mutations showed increased glycolysis-associated metabolites. Using 13C-glucose, we identified increased glucose uptake in HCM models, primarily used for the pentose phosphate pathway and the oxidative step of glycolysis. Additionally, glucose use for protein glycosylation was decreased, and glucose partially supported the TCA cycle in HCM models. Interestingly, glucose use in the TCA cycle ceased when glutamine entered the cycle.
Inhibiting glycolysis with 2DG did not impair contractility in HCM but did impair the upregulation of inflammatory pathways, as shown by RNAseq. All inflammasome machinery was downregulated after glycolysis inhibition. Despite glycolysis's importance for signaling in HCM cardiomyocytes, it was not associated with contraction alterations. Inhibiting glutamine uptake in HCM cells resulted in complete contraction cessation within 30 minutes. Treating cells with 13C-glutamine showed higher glutamine uptake in HCM compared to WT, with glutamine used for alpha-ketoglutarate, succinate, and fumarate production, and incorporation into the redox system.
**Novel Component**
First work highlighted common metabolic alterations in cardiomyopathy mutations, the roles of glucose and glutamine in cardiomyocyte contraction, and inflammation.

**----------Poster Board #23----------**
**Title**
**High-plex metabolomic profiling on the Orbitrap Astral mass spectrometer**
**Author(s) (Presenting author should be listed first)**
Tan, Lin; Myer, Ciara; Xiong, Yun; Wei, Bo; Ding, Jibin; Tautenhahn, Ralf; Lorenzi, L Philip
**Author Affiliations**
1. Metabolomics Core Facility, MD Anderson Cancer Center, Houston, TX, United States 2, ThermoFisher Scientific
**Introduction**
The Orbitrap Astral mass spectrometer (Astral MS) is a high-resolution accurate mass (HRAM) analyzer that includes Orbitrap and an innovative HRAM Asymmetric Track Lossless (Astral) analyzer. The system can perform an ultra-high resolution full-scan MS1 analysis on the OT followed by a fast DDA MS2 scan (up to 200 HZ) on the Astral analyzer, successfully combining high-confidence annotation and accurate quantitation for non-targeted and targeted analyses within the same injection. Here we compared the performance of a HILIC chromatographic system coupled with Astral MS or Orbitrap-Exploris 240 (OE240 MS) systems, The Astral MS yielded a 2-fold increase in high-confidence metabolite annotations and a much shorter run time. We tested the method for metabolomic profiling of tissue and plasma samples.
**Methods**
NIST SRM1950 plasma and MCF7 cell samples were extracted with 0.1% formic acid in 90/10 acetonitrile/water, and extracts were injected on a Vanquish Neo LC-Astral MS system and a Vanquish Horizon LC-OE240 MS system. Normal phase chromatographic conditions included a Waters Acquity BEH Amide 1x100 mm column with 1.7 Âµm particle size and mobile phases consisting of acetonitrile/0.1% formic acid (MPA) and water/0.1% formic acid (MPB) were used for metabolites separation. Data were acquired in full-scan MS1 and DDA MS2 for both ESI positive and negative modes. Raw files were analyzed using Thermo

Scientific Compound Discoverer 3.3 and Trace Finder 5.1 for non-targeted and targeted data processing, respectively.

**Preliminary Data**

The Astral MS generated ~2-fold greater high-quality MS2 spectra than the OE240 MS system. After data processing in Compound Discoverer, the NIST SRM1950 plasma standard yielded 1715 compounds and 153 high-confidence mzCloud-based annotations on the OE240 MS system. The Astral MS system yielded a comparable number of compounds (1799) but 314 high-confidence annotations. To assess dynamic range, we injected dilutions of a metabolite extract equivalent to 10, 20, 50, 100, 200, and 1,000 MCF7 cells on both systems , The Astral MS system exhibited up to 91% MS2 coverage, whereas the OE240 exhibited up to 58%. Compound Discoverer detected 149 high-confidence metabolites with mzCloud database search from the 1,000 cell injection on the Astral MS, whereas the OE240 yielded only 64 high-confidence metabolite annotations. Strikingly, the Astral MS system accurately measured ~80 annotated metabolites in 10 cells. We will also present results from application of the method for deep profiling of tissue and plasma samples.

**Novel Component**

We demonstrated a fast and deep metabolite profiling method using the Orbitrap Astral MS system.

**----------Poster Board #24----------**

**Title**

**iModMix: Integrative Modules for Multi-omics Data**

**Author(s) (Presenting author should be listed first)**

Narvaez-Bandera, Isis; Lui, Ashley; Sulman, Noah; Wilson, Christopher; Welsh, Eric; Ercan, Dalia; Rubio, Vanessa; Ackerman, Hayley; Li, Guohui; Darville, Lancia; Fang, Bin; Eschrich, Steven; Koomen, John; Haura, Eric; DeNicola, Gina; Li, Qian; Chen, Ann; Flores, Elsa; Fridley, Brooke; Stewart, Paul.

**Author Affiliations**

Moffitt Cancer Center

**Introduction**

Multi-omic co-expression networks for the integrative analysis of data from high-throughput genomic technologies hold immense potential for advancing our understanding of diseases and improving therapeutic strategies. This study presents iModMix, a novel integrative analysis framework designed to navigate the methodological intricacies of network-based analyses, with a specific focus on elucidating the complex relationships within biological systems.

**Methods**

Weighted Gene Co-expression Network Analysis (WGCNA) serves as the foundation, providing a comprehensive understanding of each omic dataset. In iModMix the construction of network-based modules entails using sparse Gaussian graphical models (GGMs with lasso penalty) that focus only on the direct associations between genes, proteins, and metabolites. In contrast, WGCNA uses both the direct and indirect associations between features in the network used in module construction. Subsequently, hierarchical clustering based on the topographical overlap matrix and the sparse partial correlation matrix generates modules comprising related features. The interrelation between omic modules is assessed through the correlations of the first eigenvector of each module, facilitating quantitative comparisons across data types and capturing intricate biological interactions.

**Preliminary Data**

Applying iModMix to integrate genomics, proteomics, and metabolomics datasets from an 81-patient cohort with lung squamous cell carcinoma (LSCC) produced noteworthy insights. The metabolomic dataset started with an initial dataset of 6,042 features, of which 446 metabolites were identified using m/z and retention time matching to an internal reference library. iModMix constructed 287 modules with an average size of 21 features. Similarly, within the protein data, originating from an initial dataset of 5,115 features, 73 modules were constructed with an average size of 70 features. Concurrently, the RNA-seq dataset, initially consisting of 14,411 features, constructed 442 modules with an average size of 33 features. A correlation threshold of â‰¥ |0.5| revealed 35 pairs of protein-metabolite modules and 207 pairs of gene-metabolite modules. Analysis of the top 5 correlations in both protein-metabolite and gene-metabolite studies unveiled that most metabolites within

highly correlated modules were unidentified. Notably, the identified metabolite, tryptophan (Trp), emerged in the second pair with a correlation of 0.6 (51 proteins and 28 metabolites). Again, tryptophan was identified in the fifth pair with a correlation of 0.7 (41 genes and 28 metabolites). Given the current focus on Trp metabolism modulation for lung cancer diagnosis, prognosis, and therapies, these findings hold significant clinical relevance. Furthermore, the identified 3-(2-Hydroxyphenyl) propanoic acid and glutamic acid emerged in the fifth pair with a negative correlation of -0.59 (98 proteins and 26 metabolites). Glutamic acid has been associated with cancer, and its negative correlation in this context warrants further investigation. Further analysis revealed that ten genes (COL1A2, COL5A2, FBLN2, COL3A1, SPARC, VCAN, COL1A1, COL5A1, PCOLCE, FKBP7) were shared between the pair of groups where D-Tryptophan emerged. These genes demonstrated high enrichment in critical pathways, including protein digestion and absorption, and ECM-receptor interaction pathways.

**Novel Component**

iModMix: A multi-omic analysis platform integrating genomics, proteomics, and metabolomics to better understand the relationships between different omics layers.

**----------Poster Board #25----------**

**Title**

**Integrating untargeted metabolomics and lipidomics with drug adherence monitoring in heart failure patients**

**Author(s) (Presenting author should be listed first)**

Cajka, Tomas; Hricko, Jiri; Hola, Veronika; Rudl Kulhava, Lucie; Novakova, Michaela; Paucova, Michaela; Rakusanova, Stanislava

**Author Affiliations**

Institute of Physiology of the Czech Academy of Sciences, Videnska 1083, 14200 Prague, Czech Republic

**Introduction**

Liquid chromatography-mass spectrometry (LC-MS) is the preferred technique for analyzing small molecules such as polar metabolites, complex lipids, and drugs in biofluids like plasma and serum. However, capturing the full range of these compounds requires more than a single extraction method or instrumental platform. Therefore, the primary goal is to cover polar metabolites, complex lipids, and drugs using as few platforms as possible while maintaining the necessary precision and accuracy. Additionally, fast LC-MS methods (<5 minutes) for high-throughput applications are advantageous for large-scale studies, enabling the analysis of over a hundred samples daily.

**Methods**

Polar metabolites and complex lipids were isolated using an â€˜all-in-one' extraction with a methanol/methyl tert-butyl ether mixture and water, while drugs were extracted with a methanol/ethanol mixture. Complex lipids were separated using RPLC (C18 column) with ESI(+) and ESI(â€“) analysis, while polar metabolites were separated using HILIC (amide-based column) in ESI(+) and RPLC (C18 column) in ESI(â€“). Drug analysis utilized RPLC (C8 column) in MRM-ESI(+)/(â€“) mode. For untargeted methods, simultaneous acquisition of MS1 and MS/MS spectra was performed on each platform. The raw instrumental files were processed with MS-DIAL 4 software. MRM mode was used for drug analysis, followed by targeted data processing.

**Preliminary Data**

We developed an LC-MS workflow for extracting polar metabolites, complex lipids, and drugs from human plasma, followed by rapid, high-throughput LC-MS methods. Extraction was performed using methanol, methyl tert-butyl ether, and water, with separate analyses of organic (complex lipids) and aqueous (polar metabolites) layers for untargeted metabolomics and lipidomics. Due to the wide range of polarities of drugs, a separate extraction with a methanol/ethanol mixture was employed. The optimized LC-MS methods achieved <5 minutes of injection-to-injection times, enabling fast analysis. Using MS-DIAL software and combined MS/MS spectral libraries, we annotated over 400 complex lipids and 100 polar metabolites in more than 560 plasma samples from heart failure patients. Targeted drug analysis allowed quantifying 84 drugs and some metabolites at 1-10 ng/mL, sufficient for monitoring drug adherence. (Supported by the Czech Health Research Council, NU22-02-00161.)

**Novel Component**
Fast and robust LC-MS platforms for comprehensive metabolome, lipidome, and drug adherence analysis in plasma samples of heart failure patients

**----------Poster Board #26----------**
**Title**
**Investigating systemic consequences following the depletion of native murine intestinal flora**
**Author(s) (Presenting author should be listed first)**
Guzior, Douglas V; Stancliffe, Ethan; Rowles, Joe L; Gandhi, Monil; Cho, Kevin; Mehta, Ashima; Richardson, Adam; Cohen, Tom; Patti, Gary J
**Author Affiliations**
Panome Bio, Inc.; Washington University in St. Louis
**Introduction**
The composition of our bile, a mixture of water, salts, and bile acids (BAs), has been known to play a significant role in health for hundreds of years. BAs themselves are the cornerstone of this mixture; they function as key detergents in fat absorption, signaling molecules, and antimicrobial compounds shaping the composition of our gut microbiome. Members of our gut microbiome modify BAs through various metabolic pathways, altering their biochemical properties and subsequent physiological effects. Recently uncovered forms of BA metabolism, particularly conjugation, expanded the potential diversity of BAs with impacts of these novel bile species remain largely unknown. Here, we leveraged a microbiome-deplete mouse model to uncover systemic metabolic consequences following antibiotic-induced depletion of native gut flora.
**Methods**
7-week-old C57BL6/J mice were randomly assigned to control or antimicrobial-induced microbiota depletion (AIMD) groups. Microbiome depletion was performed by twice-daily oral gavage of an antibiotic cocktail for three days followed by ad lib availability in drinking water. Mice were euthanized 14 days after the initial gavage. Serum and fecal samples were subjected to untargeted polar and lipid analyses in addition to targeted analyses of bile acid and short-chain fatty acid profiles. Unbiased peak detection and spectral alignment was performed using PeakDetective. DecoID, and mz.unity. In-house and publicly available libraries were used for annotation. Statistical analysis was performed in R and Python.
**Preliminary Data**
Microbiota depletion resulted in marked shifts in host metabolism and the circulating metabolome, particularly the host bile acid pool. Notable shifts in secondary BAs were observed and mechanistically connected to the loss of species responsible for producing these compounds. Our methods allow for the detection of nearly 200 unique bile acids and bile acid derivatives including multiple forms of noncanonical amino acid conjugates, products of microbial bile acid metabolism referred to as microbially conjugated bile acids (MCBAs) or bacterial bile acid amidates (BBAAs). Similar shifts were observed in SCFA abundances. Gastrointestinal microorganisms are responsible for producing SCFAs, such as butyrate and propionate, through fermenting dietary fibers. We observed significant shifts in fecal SCFA profiles for AIMD mice, reflecting the loss of microbial production.

Integrating results across all metabolomic assays results in a comprehensive view of system-wide metabolic shifts occurring within microbiota-deplete mice. Our observations reinforce our gut microbiota's role in supporting host health through production of probiotic metabolites, as well as its role in regulating overall gut health through modification of host-derived compounds. Investigating correlations between fecal and serum metabolites provided further insights into compounds capable of crossing the intestinal barrier, circulating throughout the body.
**Novel Component**
This work captures metabolic consequences of microbiome-depletion in addition to characterizing microbial metabolites taken up and circulated throughout the host.

**----------Poster Board #27----------**

**Title**

**Reverse Metabolomics in the Discovery of 3-Hydroxy N-Acyl Amides**

**Author(s) (Presenting author should be listed first)**

Victoria Deleray1,2, Helena Mannochio-Russo1,2, Vincent Charron-Lamoureux1,2, Abubaker Patan1,2, Ipsita Mohanty1,2, Pieter C. Dorrestein1,2

**Author Affiliations**

1Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, CA, United States. 2Collaborative Mass Spectrometry Innovation Center, University of California San Diego, La Jolla, CA, United States.

**Introduction**

N-acyl amide structure consists of two components, an amine and lipid chain which can be conjugated by gut microbial enzymes. While N-acyl amides have been previously reported, their chemical diversity and functions in humans have only just been discovered. Our study aims to explore a modified N-acyl amide class, with a hydroxyl group on the carbon 3 of the fatty acid, a previously unknown structural motif. This large class of compounds demonstrated many signaling functions including regulating immunity, memory, stress response, and glucose levels and also found in diseased states including Type 1 Diabetes.

**Methods**

To enhance our knowledge of N-acyl amides diversity, we used a novel data science strategy called Reverse Metabolomics. Through this, we performed large scale repository searches against the 1.8 billion spectra on GNPS/MassIVE using the MS/MS spectra of synthetic standards. We did combinatorial reactions to obtain an inventory of standards and create an MS/MS library of the 3-OH acyl amides by acquiring LC-MS/MS data. Next, we used the MASST search tool to query MS2 spectra and provide phenotypic association against public untargeted metabolomic datasets using comprehensive metadata. Spectral matches between the queried and reference MS/MS spectrum were filtered using a minimum cosine similarity score of 0.7. Associations with body part distribution and disease conditions were investigated using visualization techniques.

**Preliminary Data**

We used 8 saturated, 3-OH fatty acids and combinatorically reacting those with 41 amines. These amines included proteinogenic amino acids, polyamines, and other human relevant metabolites such as the neurotransmitter serotonin and citrulline. Due to their high prevalence of these compounds in the human gut, we hypothesized that these small molecules are conjugated to the fatty acid. The synthesis of these 3-OH acyl amides led to the creation of a library containing 273 spectra. Upon the MASST search, 321,622 matching spectra were derived from the public domain, and integrating ReDU metadata with controlled vocabulary allowed insights into potential origin and distribution of the synthesized N-acyl amides across different body parts. To improve confidence in these biological discoveries, we further filter and perform manual inspection of the collected spectra from the public domain. 20.8% of organ-associated spectral matches come from fecal samples and 10.3% come from blood plasma samples. Five different 3- OH acyl amides were found in a type 1 diabetes dataset, in which glutamic acid-3-OH lauric acid and glutamic acid-3-OH octanoic acid were found statistically higher in the healthy group
compared to diabetic individuals. This study serves as an enhancement to the understanding of the diverse structures and roles that N-acyl amides take using a methodology that allows efficiency and high-throughput analysis of biological samples.

**Novel Component**

Innovative workflow to discover previously understudied class of compounds.

**----------Poster Board #28----------**

**Title**

**Root exudate chemodiversity in sorghum: implications for crop-mycorrhizal symbiosis**

**Author(s) (Presenting author should be listed first)**

Garg, Chahat; Tharayil, Nishanth; Suseela, Vidya

**Author Affiliations**

Clemson University

**Introduction**
The limited availability of phosphorus (P) in soils for plant uptake poses a significant challenge to agriculture. Arbuscular mycorrhizal fungi (AMF) could offer a widely applicable and sustainable solution to this problem by enhancing plant P uptake via the mycorrhizal pathway. Plants produce diverse root exudates that help initiate, establish, and maintain a functional AMF symbiosis. The limiting soil P conditions trigger P starvation responses in plants, resulting in the production of signaling molecules such as strigolactones. The strigolactones are apocarotenoid compounds that help in AMF spore germination and hyphal branching, necessary for initiating the plant-AMF symbiotic association. Our study aims to elucidate the role of root exudate chemical diversity in facilitating the assemblage of AMF in diverse sorghum accessions.
**Methods**
In this study, we selected 100 genetically diverse sorghum accessions, 82 accessions were chosen using the Partitioning-Around-Medoids (PAM) algorithm based on variant data from the Sorghum Bioenergy Accession Panel and the remaining accessions include those from carbon-partitioning nested association mapping (CP-NAM) parents, reference genomes, and accessions likely to be used for biofuel production. In an ongoing experiment, we are collecting root exudates of sorghum accessions in a hydroponics system and using a novel mass spectrometric approach to document the chemodiversity.
**Preliminary Data**
Our results demonstrate that different sorghum accessions exude varying compositions and concentrations of variety of compounds, such as strigolactones and sorgoleones, under P deficiency.
**Novel Component**
The effect of root exudates chemodiversity on the hyphal branching of different AMF species will be further investigated.

**----------Poster Board #29----------**
**Title**
**SLC45A4 encodes a mitochondrial transporter that promotes GABA synthesis from ornithine**
**Author(s) (Presenting author should be listed first)**
Xiaoyang Su, Cecilia Colson, Yujue Wang, James Atherton
**Author Affiliations**
Rutgers University
**Introduction**
Solute carrier (SLC) proteins are membrane transporters that govern the cross-membrane exchanges of nutrients, inorganic ions, and other small molecule metabolites. Many SLC genes have been shown to be causes of Mendelian diseases in humans, and a number of SLC transporters are important drug targets. However, due to myriad technical difficulties, a large fraction of SLC family members are still orphan transporters without known substrates, which represents both a significant knowledge gap and a huge opportunity for new drug development. Our goal is to investigate the biochemical functions of uncharacterized SLC transporters using mass spectrometry.
**Methods**
We developed a workflow for transcriptomic-metabolomic association analysis. Using this approach, we identified an uncharacterized gene, SLC45A4, that is the single greatest determinant of Î³-aminobutyric acid (GABA) levels in human cancer cells. Using stable isotope tracing and high-resolution mass spectrometry, we identified the dominant GABA biosynthetic pathway, and we studied the role of SLC45A4 in the regulation thereof.
**Preliminary Data**
Since the expression of SLC45A4 strongly correlates with cellular GABA level, we investigated the potential role of SLC45A4 as a GABA transporter. When the cells were fed with 13C4-GABA, SLC45A4 depletion did not decrease the level of 13C4-GABA in the cells but decreased the unlabeled GABA. This result suggests SLC45A4 does not contribute to GABA uptake as a GABA transporter but it regulates GABA de novo synthesis. Using 13C5-glutamine as the tracer, we observed little labeling in GABA, suggesting that glutamate decarboxylases (GADs) are not involved in GABA synthesis in A549 and H1299 cells. Using 2H6-ornithine as

the tracer, we observed a significant fraction of GABA being 2H6- or 2H4-labeled, suggesting that ornithine is the most preferred substrate for GABA synthesis. Using diamine oxidase inhibitors (DAOIs) and monoamine oxidase inhibitors (MAOIs), we showed that GABA production depends on DAO activities. We also generated SLC45A4 KO cells in the A549 and H1299 background. The SLC45A4 KO cells show a significantly decreased GABA production from ornithine, but not from putrescine. Our immunofluorescence microscopy results show that SLC45A4 is a mitochondrial transporter, which may regulate the intracellular distribution of putrescine to promote GABA synthesis. Moreover, the isolated mitochondria fraction is capable of converting 13C4-Putrescine to 13C4-GABA, suggesting a compartmentalized GABA synthesis.

**Novel Component**

We have discovered an SLC transporter regulating cellular GABA de novo synthesis.

**----------Poster Board #30----------**

**Title**

**Targeted metabolomics analysis of key pathways to optimize cell culture media and feeds for Chinese Hamster Ovary cells**

**Author(s) (Presenting author should be listed first)**

Asik, Didar; Nguyen, Vyncent; Ciganda, Martin; Tu, Chengjian; Goldfuss, Jaime S; Jacobia, Scott ; Dodson, Elizabeth; Campbell, Andy M

**Author Affiliations**

Thermo Fisher Scientific, Grand Island, NY; Thermo Fisher Scientific Hunt Valley, MD

**Introduction**

The biopharmaceutical industry places significant emphasis on the development and optimization of cell culture media specifically designed for Chinese Hamster Ovary (CHO) cells. We have developed a targeted metabolomics approach to study ~140 metabolites covering 15 specific metabolic pathways in four commonly used CHO cell lines using different medium and feed combinations. High resolution targeted quantitative metabolomics analyses of media and cellular samples can provide a quick and better evaluation of selected metabolites for optimization of cell culture media to improve growth, titer, and protein quality.

**Methods**

The selected cell culture metabolite list consists of subgroups such as amino acids, organic acids, vitamins, nucleosides, fatty acids, and sugars. Acquisition of small-molecule mass spectra was performed on Thermo ScientificTM OrbitrapTM ID-XTM and TribidTM Mass Spectrometry (MS). The raw files were analyzed by Compound DiscovererTM to create a CHO cell line panel metabolite library and a list containing KEGG, HMDB, and CAS IDs. Selected metabolites were further optimized on a TSQ AltisTM MS via direct injection and QED/SRM method was developed. Additionally, LOD and LOQ values of the CHO basal medium metabolites in GibcoTM DynamisTM Production were determined based on their standard deviation of the response and slope method.

**Preliminary Data**

43 metabolites of 4 CHO basal media and 2 feed media (GibcoTM DynamisTM Production, GibcoTM Efficient-ProTM, GibcoTM CD-CHO and GibcoTM CD OptiCHOTM, Efficient-Pro Feeds 1 and 2) were quantified. The LOD and LOQ values of CHO basal media metabolites were calculated. Quantitative targeted metabolomics methods on TraceFinderTM and Skyline (23.1) software were created and used.

Our Targeted Metabolomics approach demonstrates excellent sensitivity and is capable of accurately quantifying metabolites specific to certain pathways, thereby offering valuable insights into CHO metabolism. The utilization of targeted proteomics will greatly enhance the understanding of changes in cellular metabolism during cell culture, allowing us to identify bottlenecks in greater detail and thus optimize cell culture media and feeds for CHO cells.

**Novel Component**

Targeted metabolomics gains a comprehensive understanding of the therapeutic protein production process in CHO cells by closely monitoring key pathways.

**----------Poster Board #31----------**

**Title**

**The propensity for non-obese diabetic mice to develop Type 1 diabetes is modulated by antibiotic treatment and cecal material transplant: a lipidomics study.**

**Author(s) (Presenting author should be listed first)**

Diaz-Rubio, M.Elena; Zhang, Xuesong; Su, Xiaoyang; Blaser, Martin

**Author Affiliations**

Cancer Institute of New Jersey, Rutgers University, New Brunswick, NJ, United States; Center for Advanced Biotechnology and Medicine, Rutgers University, New Brunswick, NJ, United States; Department of Medicine, Rutgers Robert Wood Johnson Med Sch, New Brunswick, NJ.

**Introduction**

Type 1 diabetes (T1D), is characterized by the destruction of pancreatic insulin-producing beta cells by immune effectors. The gut microbiota affecting host immunity plays a role in the early stages of T1D pathogenesis in the non-obese diabetic (NOD) mouse model. Gut microbiota produce diverse lipid molecules, which affect host responses and metabolism. Maternal cecal microbiota transplant (CMT) to NOD mice after early-life antibiotic exposure mitigated the induced T1D enhancement by partially restoring microbial diversity, and depleted metabolic pathways. The objective of this work was to use non-targeted lipidomic approaches to investigate the profiles of microbially-produced lipid molecules in NOD mice, evaluating the effects of early-life antibiotic perturbation and CMT on these lipid profiles and the corresponding host innate immune responses.

**Methods**

To establish a lipidomic profile of the murine gut microbiome, a lipidomic analysis was conducted on cecal contents of 6-week-old germ-free (n=5) and conventional mice (n=5) as well as on their diets. The effects of antibiotic treatment (1P) and restoration (CMT) compared to control on lipid profiles of NOD mouse cecal contents also were investigated and lipid analysis performed with samples from postnatal day 23 (P23) in the three treatment groups (n=6/group)]. The untargeted lipidomic analysis was performed on a Vanquish Horizon UHPLC system with a Poroshell 120 EC-C18 column coupled to a Thermo Q Exactive PLUS Mass Spectrometer. To further confirm the identity of key lipid species, targeted data acquisition using PRM was performed.

**Preliminary Data**

Based on the antibiotic-induced metagenomic differences involving lipogenesis we used non-targeted lipidomics to characterize gut microbiota lipid profiles in conventional and germ-free mice and identified a group of specific microbiota-produced lipid compounds.

Once the lipids of microbial origin were established, in a second experiment comparing cecal contents from male NOD mice at postnatal day 23 (P23) in three treatment groups [(C, Control; 1P, 1PAT; CMT, 1PAT followed by maternal cecal transplant, we identified a group of gut lumen lipids that had been reduced by antibiotic treatment and restored by CMT. We then used the targeted lipidomic methods to elucidate the structure of several of those key lipids, that were tested as suppressors of macrophage-induced inflammation through the NFÎºB pathway

**Novel Component**

These findings indicate potential therapeutic roles of specific microbially-produced lipid compounds in mitigating risk for T1D and other inflammatory disorders involving the distal intestinal tract.

**----------Poster Board #32----------**

**Title**

**The role of SNAT2 in the metabolic switch upon post-treatment glioblastoma recurrence**

**Author(s) (Presenting author should be listed first)**

Bozelli, Jr Jose C1; Puri, A2,3; Venugopal, C3,4; Singh, SK2,3,4; Britz-McKibbin, P1

**Author Affiliations**

1Department of Chemistry and Chemical Biology, McMaster University, Hamilton, ON, Canada; 2Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON, Canada; 3Centre for Discovery in Cancer Research (CDCR), McMaster University, Hamilton, ON, Canada; 4Department of Surgery, Faculty of Health Sciences, McMaster University, Hamilton, ON, Canada

**Introduction**
Glioblastoma (GBM), the most aggressive brain tumor in adults, is known for its resistance to treatment and frequent recurrence. While it is widely accepted that metabolic reprogramming of cancer cells is vital for cell survival, invasion, and sensitivity to genotoxic therapies in GBM models, little is known about the metabolic alterations upon GBM recurrence. This knowledge gap hinders our understanding of treatment failure in most patients. Some of us have recently investigated the functional drivers of post-treatment recurrent GBM. This led to identifying sodium-coupled neutral amino acid transporter 2 (SNAT2) as a selective vulnerability in recurrent, but not primary, GBM. Here, the role of SNAT2 in the metabolism of recurrent GBM using in vitro GBM models is investigated.
**Methods**
SNAT2 was knocked out using CRISPR in a recurrent GBM specimen obtained at first disease recurrence after standard of care. SNAT2 KO metabolome was compared to its empty vector (EV) control and a patient-matched primary GBM specimen (obtained at the initial diagnosis before chemotherapy) to gain insight into the role of SNAT2 in GBM recurrence. Cell pellets were extracted following a modified methyl-tert-butyl ether protocol. Both aqueous (polar/charged metabolites) and organic (lipids) fractions were separated, and full-scan data under positive (cations and zwitterions) and negative (anions) modes was acquired for comprehensive metabolomic analysis. Polar metabolite species were resolved and quantified via multisegment injection-capillary electrophoresis-mass spectrometry (MSI-CE-MS). Lipid species were separated and quantified via liquid chromatography (LC)-MS.
**Preliminary Data**
A comprehensive metabolome analysis was performed using MSI-CE-MS, a multiplex separation platform for quantifying polar/charged metabolites. MSI-CE-MS uses a serial injection of thirteen samples/run, allowing higher sample throughput, side-by-side sample comparison, and signal pattern recognition. The precision of the method was validated by analyzing internal standards in pooled quality control (QC) samples (mean CV = 10.93%, no outliers exceeding Â± 2*SD) and observing lower technical variance (CV = 3.72%) in QC compared to biological variance (CV = 7.71%) in cellular extracts. The method's accuracy was excellent, with a recovery rate of 104Â±14%. Metabolites in cell extracts were rigorously selected, filtered, and annotated according to MSI signal pattern recognition (to reject spurious/background signals), signal absence in blank (processed similarly to samples), accurate m/z, isotopic pattern, m/z error (< 10 ppm), and unique relative migration time (RMT). Forty-one metabolites were measured (31 in positive mode, 20 in negative mode, 11 overlapping). A volcano plot (FC = 1.5, FDR-adjusted p-value = 0.05) identified 11 significantly decreased metabolites upon SNAT2 knockout. Notably, intracellular glutamine decreased ca. 3-fold (p-value = 1.19x10-5, unpaired t-test, n = 3 biological replicates), supporting SNAT2's role as a glutamine transporter. The most significant decrease (ca.7-fold, p-value = 2.0x10-6, unpaired t-test, n = 3 biological replicates) was observed for an unknown metabolite (198.0868:0.769, m/z:RMT). Pathway enrichment analysis revealed significant changes in the metabolism of alanine, aspartate, glutamate, nitrogen, glyoxylate/dicarboxylic acid, and arginine biosynthesis. All data has been acquired; however, preliminary analysis has solely been done on the polar metabolome of SNAT2 KO and its EV counterpart. It is proposed that final data analysis and unknown identification will yield significant findings that have the potential to impact our understanding of GBM recurrence and, ultimately, pave the way for novel therapeutic strategies.
**Novel Component**
SNAT2's role in the metabolism of GBM cells goes beyond glutamine transport, highlighting its importance in GBM recurrence.

**----------Poster Board #33----------**
**Title**
**Untargeted profiling of submetabolomes using chemical derivatization**
**Author(s) (Presenting author should be listed first)**
Shujian Zheng; Joshua Mitchell; Shuzhao Li
**Author Affiliations**
The Jackson Laboratory For Genomic Medicine

**Introduction**

Metabolomics aims to comprehensively detect, identify, and quantify small molecules in an organism. Liquid chromatography coupled with high-resolution mass spectrometry (LC-MS) is the most popular technique due to its high sensitivity and selectivity. A challenge in metabolomics is achieving consistent detection across diverse metabolites and complex biological samples. Chemoselective derivatization (CSD) enhances detection by introducing ionizable structures but faces issues like reagent purity and reaction consistency. This work presents a novel approach to CSD in untargeted metabolomics, where isotope-labeled and unlabeled reagents are mixed prior to reaction. This method allows for robust detection and mapping of derivatives to study samples with appropriate controls for accuracy.

**Methods**

The multiplexing of derivatization results in complex mass spectral patterns that encode the number of reactions that have occurred for a given reagent isotopologue mixture as follows. Using the unlabeled derivatized samples, the underlying metabolite intensity distribution is calculated by removing the natural abundance contribution introduced by the reagent. This distribution is then combined with the known ratio of the reagent isotopologues, assuming one too many reactions have occurred, to yield many theoretical spectral patterns. The similarity between a theoretical pattern and one observed in the pooled labeled derivatized samples can be evaluated statistically and thus, the correct number of derivatization reactions inferred. From the number of reactions, the back calculation of the metabolite's underivatized m/z becomes trivial.

**Preliminary Data**

Our multiplexing approach was first evaluated on authentic standards mixtures derivatized using a roughly 50:50 mixture of m+13C2 and monoisotopic reagents. First, we evaluated the approach on TCA authentic standards derivatized with DmPA. All TCA metabolites, with the exception of Succinyl-CoA, were observed and detected with both the targeted and untargeted versions of the algorithms. This includes multiple derivatives of several metabolites as well as unexpected derivatives such as a 3*DmPA derivative of fumarate which was examined using MS2. Similar results were observed in steroidogenesis standards derivatized with DnHz and DnCl. Second, we evaluated the improvements in metabolite coverage provided by untargeted chemoselective derivatization in multiple datasets including CHO wild type and Kolf2.2J cells with null mutations.

**Novel Component**

mixed isotope-labeled and unlabeled reagents prior to reaction

## Full Abstracts for Thursday Posters

**----------Poster Board #1----------**

**Title**

**Comprehensive Metabolite Profiling in Formalin-Fixed and Paraffin-embedded Tissue**

**Author(s) (Presenting author should be listed first)**

Mehta, Ashima; Stancliffe Ethan; Richardson, Adam; Gandhi, Monil; Guzior, Douglas V.; Cho, Kevin; Cohen, Tom; Patti, Gary

**Author Affiliations**

Panome Bio Inc.; Washington University in St. Louis

**Introduction**

With the progression of disease, the metabolic programs of cells and tissues change, making metabolomics an imperative tool for identifying key diagnostic biomarkers. While formalin-fixed and paraffin-embedded (FFPE) tissue preservation presents challenges such as metabolite degradation, it also provides the benefit of long-term storage and the opportunity for retrospective analysis. Untargeted MS-based metabolomics was utilized to compare frozen and FFPE tissue samples and to evaluate the composition of metabolites retained after fixation and paraffin embedding. In order to optimize metabolomics in FFPE tissues, we obtained matched frozen and FFPE liver and kidney mouse tissue samples. The results obtained highlight the feasibility of performing metabolomics studies with FFPE material, and hence its significance for clinical research.

**Methods**

Untargeted metabolomics profiling of polar and lipid metabolites was accomplished through HILIC/MS and RPLC/MS on an Agilent 6546 mass spectrometer. Resulting data were processed with a custom pipeline that includes the PeakDetective, DecoID, and mz.unity software. A QC sample was also prepared by pooling metabolite extracts from all the samples, which showed the clear separation in the Principal Component Analysis, representing a chemical average of the study.

**Preliminary Data**

Global metabolomics analyses detected a total of 17,093 peaks. More than 90% of these signals were attributed to contaminants, artifacts, and redundant metabolite signals. Across all the assays, the coefficient of variation (CV) values was less than 10%. The polar fraction of the samples exhibited a diverse range of metabolites, including nucleosides, and smaller peptide
groups (single amino acids, dipeptides, etc.). The lipid data revealed the presence of various glycerophospholipids, glycerolipids, and other non-polar compounds.

We observed a total of 946 unique metabolites and identified 878 at MSI Levels 1-3. Among these identified compounds of known identity, 125 were also observed in matched FFPE tissues, each with a corrected p-value of less than 0.05 and an absolute log fold change greater than 3. Several metabolite classes proved particularly amenable to FFPE-based analysis and were not lost during the fixation process including nucleotides, energy metabolites, amino acids, lipids, and small peptides. The detection of metabolites in FFPE samples seems to be significantly influenced by specific chemical substituents. This is demonstrated by the markedly higher levels of phosphatidylethanolamines in comparison to phosphatidylcholines. Additionally, we observed varied behavior within the lipid class. Some metabolites, like fatty acids, showed reliable detectability whereas others, like fatty acid esters, were less detectable. While the methods presented here capture and measure a significant number of biologically relevant metabolites and pathways, we are currently exploring additional sample preparation methods to expand FFPE metabolomic coverage. FFPE methods continue to be effective, especially for lipid metabolism studies, as lipids are among the metabolites that are relatively well-preserved in FFPE samples from various tissue types.

**Novel Component**

This study reveals that metabolite profiling in FFPE tissue sections can effectively elucidate biologically significant compounds and pathways, offering new insights into disease mechanism

**----------Poster Board #2----------**

**Title**

## Molecular formulae from fine structures of ultra-high-resolution LC-MS isotope patterns

**Author(s) (Presenting author should be listed first)**
Goncalo J. Gouveia (1), Delia Qu (2), Aaron M. Ferber (2), Maximilian J. Helf (3), Carla P. Gomes (2), Frank C. Schroeder (1)
**Author Affiliations**
1 - Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY, 14853, USA; 2- Department of Computer Science, Cornell University, Ithaca, NY 14853; 3 - Novartis Fabrikstrasse 2, 4056 Basel, Switzerland

**Introduction**
Living organisms produce millions of unidentified metabolites, but methods for their LC-MS-based identification at-scale are lacking. Towards this goal, assignment of molecular formulae (MFs) is an important step. However, even at high mass accuracy, MS1 data alone are insufficient to infer a single correct MF above m/z ~300, and therefore, additional data is need. While analysis of MS2 data can be used to constrain the number of MFs compatible with the MS1 data, we here show that at ultra-high mass resolution, the fine structure of isotope patterns become sufficiently resolved to distinguish nominal isobars, i.e. the 13C and 15N-derived M+1 peaks. The resulting isotopic fine structures (IFS) can be predicted for all candidate MFs and compared to the experimental data.

**Methods**
Here we introduce new capabilities of Metaboseek (Helf), an open-source platform for LC-MS data analysis, that enables assignment of MFs of unknowns based on comparison of experimental and predicted IFS patterns. Using ultra-high-resolution data, predicted MFs are ranked by considering IFS pattern match and mass accuracy. Ranking employs a stratified cosine similarity metric that weighs each isotope cluster individually plus global pattern fitting to identify most likely candidates. This module leverages a modified HR2 (Kind, Fiehn) brute force formula generator and the R-package â€˜envipat’ (Loos) to generate theoretical IFS patterns for every candidate MF. In addition, it allows for the simultaneous visualization of experimental data (averaged and individual scans), top best fitting predicted IFS patterns, and MS/MS data.

**Preliminary Data**
Development of the IFS analysis module used test data sets of whole-metabolome samples of the model organism C. elegans, containing several 10,000 unknown metabolites. MS data were collected using an Orbitrap IQ-X in both positive and negative mode, using a resolution of 1,067,105 (FWHM/m/z) at 200 Da. To reduce incidence of co-elution or overlap of different metabolites, LC-MS data were acquired a using a 2h-long LC method. Profile-mode MS1 data was converted using mzmine without any processing for subsequent IFS analysis in Metaboseek. Profile mode data sets are extremely large, pushing the boundaries of non-high-performance workstations capabilities. Therefore, we additionally developed the â€˜rIFSTools’ R package integrating both custom and existing functionalities to make data processing and handling feasible. Using the IFS module enabled assignment of correct MFs for all members of a test set of known compounds with m/z = 300-800, whereby the correct MF in many cases did not correspond to the MF which matched best when considering mass accuracy alone. Moreover, IPFS enabled high confidence assignment of MFs for a large number of yet unidentified metabolites. Plausibility of the MFs assigned to unknowns was further supported by MS2 analysis of select examples.
The flexibility of the Metaboseek front-end allows the user to easily import data from different data processing engines or use the integrated XCMS data processing module. Special emphasis is put on data visualization for effective, multi-layered interaction with MS1 and MS2 data, which paired with statistical methods facilitates filtering and prioritization of differentially regulated molecular features, in particular when the analysis is discovery-oriented and focuses on the identification of unknowns. The IFS module hypercharges the metabolomics workflow, greatly simplifying MF assignments as the first step in the structural elucidation process.

Kind, Fiehn, doi.org/10.1186/1471-2105-8-105; Loos, et al, doi.org/10.1021/acs.analchem.5b00941; Helf, et al, doi.org/10.1038/s41467-022-28391-9

**Novel Component**

Computational tools to assign molecular formulas from ultra-high resolution experimental data and calculate isotopic fine structures

**----------Poster Board #3----------**

**Title**

**Reproducibility of metabolomic profiles generated using the TruQuant platform in a multi-lab "round-robin" study design**

**Author(s) (Presenting author should be listed first)**

Haley Chatelaine (1), Chris Beecher (2), Ewy A. Mathé (1)

**Author Affiliations**

(1) National Center for Advancing Translational Sciences - National Institutes of Health, Rockville, MD; (2) IROA Technologies, Chapel Hill, NC

**Introduction**

Establishing reproducibility in metabolomics results is required to ensure reliable clinical conclusions, both by accurately identifying metabolites and reporting reproducible relative abundances. The TruQuant platform is a potential solution to address this challenge. The approach uses 95%/5% 13C/12C isotope ratio standards, derived from yeast, to generate mass spectral peaks that directly correspond to biological metabolites, rather than lab-idiosyncratic artifacts. These standards are then used to identify metabolites and to reproducibly estimate their relative abundances in samples, ensuring results are directly related to biological variability, not technical error. The goal of this study is to assess the reproducibility of the TruQuant approach when multiple labs, using their own LC-MS parameters, collect TruQuant data on the same set of samples.

**Methods**

We evaluated the reproducibility of the TruQuant approach in 8 labs in the US and UK. Each lab received identical aliquots from 32 samples, containing multiple known mixtures of beef, pork, and chicken extracts, each mixture analyzed in duplicate, from which they collected metabolomics data using their own methods. Anonymized, processed data was analyzed for: 1) data quality; 2) reproducibility across labs, including qualitative assessment of metabolite identities detected and quantitative assessment of relative abundances of shared metabolites; and 3) Prediction of sample types from metabolite profiles. While the first two assessments evaluate intra- and inter-lab reproducibility, the third provides preliminary data for the relevance of metabolomics in clinical settings to differentiate tissues.

**Preliminary Data**

The study design includes varying purity levels of extract mixtures (i.e., pure beef, chicken, and pork; half of each; and one-third of each), serving as a case study for differentiating metabolically heterogeneous tissues (e.g., tissues with both diseased and healthy cells). Each sample mixture was also varied in concentration to assess normalization capabilities of the TruQuant approach, and each sample type (based on purity level and concentration) was analyzed in duplicate. Data from all labs was first analyzed for quality, using the duplicate samples. Metabolite abundances between duplicate samples within each lab had high correlation values (all but one with $r > 0.9$) and low total drift in metabolite abundance over run time, ensuring high quality data. Instrumentation differences resulted in a range of total features detected by each lab (minimum = 397, maximum = 1334 features). Despite differences in numbers of features detected, metabolomic profiles from each of the 8 labs differentiated sample mixture types via unsupervised clustering analyses (principal component analysis and unsupervised random forest). Notably, cluster distributions reflected sample mixture composition, with pure beef, pure pork, and pure chicken samples clustering furthest apart, and samples of increasing mixture complexity clustering toward the middle. Clusters were also primarily driven by sample composition, rather than relative concentration of extracts, as expected after applying TruQuant normalization. Pairwise Pearson correlations of relative abundances for shared metabolites between labs also showed strong positive associations (mean = 0.48 +/- 0.44), indicating that, regardless of LC-MS data collection platform, the TruQuant workflow enabled consistent measurements of metabolite profiles across samples. Machine learning prediction models of data mixtures from each lab are ongoing. Overall, our â€œround-robinâ€□ study design and our data show that the TruQuant approach enables reproducible measurements across different labs and platforms.

**Novel Component**
The rigorous study design and isotope ratio standards enable evaluation of reproducibility across multiple labs and platforms and potential clinical utility.

**----------Poster Board #4----------**
**Title**
**A pipeline for domain detection and annotation of spatial metabolomics data using hierarchical clustering and Shiny app integration**
**Author(s) (Presenting author should be listed first)**
Oscar E. Ospina1, Eric Welsh2, Vanessa Rubio3, Lancia Darville4, Min Liu4, Joseph O. Johnson5, John Koomen3, Elsa R. Flores3, Brooke L. Fridley6, Paul Stewart1*
**Author Affiliations**
1 Department of Biostatistics and Bioinformatics, Moffitt Cancer Center, Tampa, FL; 2 Biostatistics and Bioinformatics Shared Resource, Moffitt Cancer Center, Tampa, FL; 3 Department of Molecular Oncology, Moffitt Cancer Center, Tampa, FL; 4 Proteomics and Metabolomics Core, Moffitt Cancer Center, Tampa, FL; 5 Analytic Microscopy Core, Moffitt Cancer Center, Tampa, FL; 6 Division of Health Services and Outcomes Research, Children's Mercy Hospital, Kansas City, MO
**Introduction**
Spatially-resolved metabolomics (SM) is advancing the spatial omics revolution, enhancing our understanding of tissue function and disease progression. A key SM method, Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI), profiles tissues at high resolution across thousands of molecules. Due to data complexity, advanced computational tools are essential. While open-source solutions exist, user-friendly interfaces are scarce. We introduce spatialMET, a pipeline for detecting and annotating spatial domains via hierarchical clustering of MALDI-MSI pixels. The pipeline includes a Shiny app for manual annotation and differential abundance analysis, aiding data exploration and hypothesis generation.
**Methods**
The spatialMET pipeline integrates the popular R package Cardinal for processing of .imzML and .ibd files generated by the mass spectrometer. Text files containing the peak intensities and pixel 2D coordinates are then passed to a multi-threaded C executable that performs noise thresholding, peak filtering, normalization, and hierarchical clustering of the pixels and metabolites. The result is a classification of each MALDI-MSI pixel into a spatial domain based on metabolomic similarity. Finally, users can take the pixel classifications and peak intensities and upload them to our Shiny app for visualization, manual annotation of spatial domains, and differential abundance analysis.
**Preliminary Data**
The spatialMET pipeline builds upon the Cardinal package, combining the strengths of both C and R for computational efficiency and user-friendly visualization. The Shiny app's interactive interface allows users to annotate clusters manually and identify regions of interest (ROIs), for which the application can test for statistical differences in intensity. Users can also opt to test for differentially abundant molecules among the hierarchical clusters predicted by our pipeline. To demonstrate the utility of the tool, we have predicted spatial domains on lung cancer samples profiled at ~20Âµm lateral resolution with the Bruker timsTOF flex equipment at the Proteomics and Metabolomics Core at Moffitt Cancer Center.
**Novel Component**
Developed a spatial metabolomics pipeline integrating Cardinal, multi-threaded C, and Shiny app, enabling spatial domain detection, annotation, and differential analysis

**----------Poster Board #5----------**
**Title**
**A potential end-to-end workflow for analysing time-series NMR**
**Author(s) (Presenting author should be listed first)**
Hossain, Zarif; Delaglio, Frank; Arnold, Jonathan; Edison, Arthur S.
**Author Affiliations**

Institute of Bioinformatics, University of Georgia

**Introduction**

The large-scale analysis of metabolites in a living organism establishes the foundation for studying biochemical pathways in an integrated way. We present a novel end-to-end workflow to model and analyze time-series NMR data on Neurospora crassa. The spectrum from each time point was modeled into a peak table using Spectral Automated NMR Decomposition (SAND). Then, the peak tables were combined to quantify metabolite concentration over time. This quantification facilitated the comparison of metabolites in aerobic and anaerobic conditions. The method demonstrated here showed us the difference in the contribution of periods between these groups. Furthermore, dynamic Flux Balance Analysis (DFBA) was performed to compare fluxes between conditions. This has a potential application for studying network-level flux differences in quorum-sensing and biofuel synthesis networks.

**Methods**

Spectral Automated Time Domain (SAND) was implemented to model the peak parameters f, A, and Î» for each peak. As a result, each spectrum was decomposed into a peak table. Tables from different time points were combined to quantify the metabolite peaks over time. Each peak was mapped across different time points using the minimum pairwise distance of chemical shifts, and the amplitude of the mapped peaks was plotted together. Furthermore, as a proof of concept, ridge data from a previous study was used to combine experiments. T-tests were performed on mapped peaks to see the difference in time points for annotated compounds using in-house MATLAB codes. Lastly, Dynamic Flux Balance Analysis was implemented on the COBRA Toolbox, choosing biomass maximization as the objective function. Cytoscape was used to observe network-level differences in fluxes.

**Preliminary Data**

One aerobic time-series experiment was considered to optimize the SAND process. SAND modeled the individual spectrum well and resolved the overlaps by modeling them as underlying peaks. This was evident when we compared the FT and the SAND modeled spectra together, and it especially resolved the baseline distortion well. The SAND-modeled time-series NMR surface was at least as good as one of the FTs. The resolution of the moving peaks was at least as good as the FT. To extract the ridge from the tabular domain, the algorithm mentioned in the method was applied. The results from the computer vision-based algorithm previously developed in the lab were compared with the one currently being developed to exemplify how it did. It produced identical results for the metabolites in the time series. Although SAND might seem to be another trivial algorithm for peak modeling, its real power lies in its ability to model and quantify double the amount of time-series features than the previously published protocol.

Besides the peak mapping for quantification, combining time-series experiments also needed peak mapping of the first time point spectra based on pairwise minimum distance. The peak mapping performed well, especially in the aerobic and anaerobic data datasets. When t-tests were performed for annotated compounds at every time point, it generated interesting time signatures and metabolites, which might have completely different dynamics but had the same pattern, leading to clustering. The goal is to extend the technique to unannotated compounds, too.

The results of the dFBA simulations are still under development. The genome-scale metabolic model iJDZ836 is being used to simulate aerobic and anaerobic conditions. The network level change for the TCA cycle is being studied. By the time of the presentation, the comparison of the aerobic and anaerobic fluxes by Cytoscape is intended.

**Novel Component**

Time-series metabolite quantification from tabular domain by SAND is intended to be novel, automatic, and based on single unix command line. Using t-tests to understanding the importance of time-points can be a new addition to time-series data analysis.

**----------Poster Board #6----------**

**Title**

## ADAP informatics for analyzing untargeted LC-MS/MS metabolomics data for the NIH Common Fund's Nutrition for Precision Health, powered by the All of Us Research Program

**Author(s) (Presenting author should be listed first)**
Xiuxia Du, Aleksandr Smirnov, Toan Nguyen, Radha Krishna Balaji Ponnuru, Blake Rushing, Susan McRitchie, Wimal Pathmasiri, Susan Sumner
**Author Affiliations**
UNC Charlotte, UNC Chapel Hill

**Introduction**
Untargeted metabolomics is an integral part of the NIH Common Fund's Nutrition for Precision Health (NPH), powered by the All of Us Research Program. The program aims to develop algorithms that predict individual responses to food and dietary patterns. Toward that end, researchers need to examine individual differences observed in response to different diets by studying the interactions between diet, genes, proteins, microbiome, metabolism and other individual contextual factors. Metabolomics plays an important role for this examination and informatics tools are essential for making sense of the large-scale untargeted LC-MS/MS metabolomics data that are generated for this examination. Additional informatics capabilities are also needed for rigorous quality control of the data and making the data available for the NPH program.

**Methods**
The suite of ADAP informatics tools for the NPH program establishes a computational and informatics workflow for handling untargeted LC-MS/MS metabolomics data. The suite consists of ADAP-BIG, ADAP-KDB, ADAP-Analytics, and ancillary computer scripts. ADAP-BIG is a desktop software tool for preprocessing raw LC-MS/MS metabolomics data and extracting compound-relevant signals. ADAP-KDB is a cloud platform that identifies and annotates compounds by matching these resulting signals against compound signals in the physical standards library and public databases. ADAP-Analytics is designed to work in the All of Us Researcher Workbench for researchers to analyze and interrogate the ADAP-BIG and ADAP-KDB results. The ancillary computer scripts have been developed to package ADAP-BIG and ADAP-KDB results and make it available on Metabolomics Workbench.

**Preliminary Data**
ADAP-BIG is written in Java. It is specifically designed and developed for processing large-scale untargeted metabolomics data that involve thousands of samples. This capability was made possible through a highly modular software design and by utilizing as many computing cores as possible and adopting memory-efficient data models. Through ADAP-BIG, chromatographic peaks and the associated MS/MS spectra can be sent to the cloud resource ADAP-KDB for compound identification and annotation. The ADAP-KDB results are then sent back to ADAP-BIG to be visually examined. ADAP-Analytics is a Python program that takes the ADAP-BIG semi-quantitation results, make the data machine learning ready, and perform machine learning and statistical analysis. Ancillary computer scripts written in Python are to perform quality control of the ADAP results and prepare data packages to be uploaded to Metabolomics Workbench and to be made available in the All of Us Researcher Workbench.

**Novel Component**
Integrated informatics pipeline designed for handling untargeted metabolomics data for NIH programs

**----------Poster Board #7----------**

**Title**

## Application of the sulfo-phospho-vanillin assay for total lipid pre-quantitation and correlation to proteins for sample normalization in untargeted lipidomic LC-MS/MS

**Author(s) (Presenting author should be listed first)**
Bailey, Laura S.; Basso, Karri B.
**Author Affiliations**
University of Flordia

**Introduction**
Sample normalization is vital for quantitation. Lipidomic LC-MS/MS has been normalized using mass/counts, DNA, or protein; however, there is no widely accepted method nor have lipid-based normalizations been

applied. Unlike many lipid quantitation methods where entire classes may be neglected, the sulfo-phospho-vanillin assay (SPVA) quantifies total lipid concentrations via a single lipid C=C.
As the first published SPVA pre-quantitation method to lipidomic LC-MS/MS, similar to a Bradford protein quantitation, biologically sourced standards yielded smaller standard deviations and larger relative concentration differences compared to gravimetric or protein sample normalization methods. We continue investigating the SPVA on true biological lipid extracts, showing the relative lipid concentration effects when lipid or protein concentrations are artificially altered.

**Methods**

Tissue extracts, with and without purified lipid and/or protein spiking (0-100ÂµL), were prepared using chloroform:methanol. Lipid content was measured using an optimized SPVA methodology in a 96-well glass-coated plate [1]. The absorbance was measured at 530nm, and the lipid concentration interpolated from Avanti lipid standards. Protein content was measured via Qubit.
LC-MS/MS measurements were conducted on Acquity UPLC column (Waters) and TIMStof Pro2 (Bruker) mass spectrometer. Separation was conducted using a 20-minute gradient of 60/40% acetonitrile/water and 90/8/2% isopropanol/acetonitrile/water, both containing ammonium formate and formic acid. Analyses were conducted in positive mode, employing PASEF. Gravimetric-normalized injections (3ÂµL) were compared to 2-3Âµg lipid-normalized content based on SPVA and protein measurements. Metaboscape was used for bioinformatics.

**Preliminary Data**

A proof-of-concept investigation used biologically sourced Avanti standards of E.coli and porcine brain to evaluate lipid sample normalization (gravimetric (MQ) vs. lipid (SPVA)). A micro-scaled SPVA showed total lipid concentration linearity (R2=0.99, both simple and complex mixtures), high accuracy (14% E.coli concentration error and 9% brain) and precision (7% E.coli and 10% brain), and smaller relative lipid standard deviations (30-90% better than MQ).
Further investigations on true biological lipid extracts of plasma, E.coli lysate, and mouse kidney, brain, and heart evaluated MQ, protein (PQ), and SPVA sample normalization. Total protein concentrations estimated lipid content using the accepted rule: [lipids]â‰ˆ4*[proteins]. Extracted total lipid showed high deviation from the 4-time lipid estimate, least to most deviation:  heart (4.5-times), E.coli (5.6-times), kidney (7.0-times), brain (11.6-times), and plasma (0.7-times). LC-MS/MS MQ, PQ, and SPVA sample normalization showed greater reproducibility (8%, 26%, 8% RSD, respectfully) and tighter statistical clustering (PCA) using SPVA. While MQ and SPVA showed similar lipid relative relationships, PQ showed some inverse lipid concentrations. To investigate these results, pure, non-native lipids and/or proteins were spiked into tissue homogenate. Lipid-spiked-kidney showed unchanged total protein (Â±0.02Âµg/ÂµL) and linearly increasing total lipid (slope=0.0234Âµg/ÂµL2). Inversely, protein-spiked-kidney showed unchanging total lipid (Â±0.2Âµg/ÂµL) and linearly increasing total protein (slope=0.003Âµg/ÂµL2). LC-MS/MS sample normalization was evaluated using significant ions (spike vs non-spike), where non-native lipids should up-regulate and down-regulation should be minimal. As lipid-spike concentration increased, MQ and PQ up-regulated features increased quadratically, and PQ showed logarithmically increasing artificial â€œdown-regulatedâ€ native-lipids. In protein-spiking experiments, PQ also showed more abundant artificial â€œdown-regulatedâ€ native-lipids. SPVA lipid-spiking showed linearly increasing up-regulated non-native lipids while protein-spiking experiments showed negligible lipid significance. Combined with previous SPVA sample normalization experiments, SPVA better accounts for sample lipid concentrations, yielding more biologically significant results with less artificial significance.
[1] Bailey. Analy.Chem. 2022, 94.

**Novel Component**

Evaluating artificial lipid or protein enhancements on tissue extracted lipid pre-quantitation using the sulfo-phospho-vanillin-assay for LC-MS/MS global lipidomics.

**----------Poster Board #8----------**

**Title**

**Automated and Efficient In-House Standards Database Development using R**

**Author(s) (Presenting author should be listed first)**
Tisch, Adam; Bennouna, Djawed; Chatelaine, Haley; Mathé, Ewy
**Author Affiliations**
National Center for Advancing Translational Sciences (NCATS), Rockville, Maryland, USA.

**Introduction**
Untargeted metabolomics is a powerful analytical technique used to detect and measure metabolites in biological samples, providing insights into biomarkers and mechanisms of action. One significant challenge in metabolomics is identifying compounds from their mass spectrometry (MS) signal, a time-consuming process that is necessary for biological data interpretation. An in-house standards library is often necessary to overcome this major bottleneck. However, manual inspection and annotation of each standard with its adducts is also a lengthy procedure. To streamline this process, we developed a computational workflow to accelerate the integration of compounds, thereby improving the speed and efficiency of library development.
**Methods**
603 compounds from the IROA Technologies Mass Spectrometry Library of Standards (MSMLS) were analyzed with LC-MS and converted to mzML files before being processed with R (4.4.0) and mzR (2.38.0). First, an expected mass to charge ratio (m/z) was calculated for all expected adducts of each compound. The dplyr::filter function identified peaks within a 30-ppm error window around each adduct's m/z value, and retention time (RT) was estimated at the maximum signal intensity for each compound. Extracted ion chromatograms (EICs) were visualized using ggplot, highlighting assigned RT values to aid accuracy assessment. Finally, a library matrix was generated, detailing compound identities, m/z values, adducts, and RTs for annotating unknown data. This workflow was validated by manual inspection for EIC shape, intensity, and quality.
**Preliminary Data**
Our workflow showed similar results to manual integration and assessment using both proprietary and open-source software. RT assignment was accurate even for low-abundance compounds, though challenging for noisy peaks or poorly defined peaks with multiple apices, which were removed from the final library by visual inspection for both manual and automated methods. The comparison of 54 compounds processed by both manual integration using MassHunter and our automated approach exhibited an RMSD of 0.0095 min between their assigned RT values, indicating high precision.

Our automated approach is designed as an efficient, specialized tool for library data processing. While visual assessment remains essential for verification of peak quality and RT value assignment, our tool significantly accelerates in-house library development, reducing the integration time per compound from 5 minutes to just 20 seconds. This improvement is particularly advantageous when dealing with thousands of standards, where manual peak integration would be overly time-consuming and inefficient. Additionally, our tool's reproducibility suggests that it will be of interest to users seeking to build their own libraries, regardless of the LC-MS instruments used for analyzing their standards. We also plan to enhance our tool by adding functionalities such as the integration of data files containing multiple compounds in a single run, reflecting the common practice of analyzing several compounds simultaneously. Our open-source code will be made publicly available on GitHub for broader access by the metabolomics community.
**Novel Component**
Rapid, efficient, and automated processing of metabolite standards for building standard libraries.

**----------Poster Board #9----------**
**Title**
**Comparative study of NMR based metabolomics platforms for blood analysis from various species.**
**Author(s) (Presenting author should be listed first)**
Miki Watanabe-Chailland and Lindsey Romick-Rosendale
**Author Affiliations**
Cincinnati Children's Hospital Medical Center
**Introduction**

The conventional workflow of NMR-based metabolomics includes sample collection and processing, NMR data acquisition and processing, metabolite quantification, statistical analysis, and data interpretation. Over the past decade, tremendous effort has been made to optimize, standardize and automate each step of the workflow. The Bruker AVANCE-IVDr (In Vitro Diagnostics research) platform provides automated NMR data acquisition and processing to metabolite quantifications on human biofluids. In this study, the metabolite quantification method using the conventional (Basic) method and the IVDr platform were compared in blood samples. Additionally, the effect of sample sources, collection tubes and NMR sample sizes on data reproducibility and accuracy were investigated.

**Methods**

Blood samples were obtained from five species (human, rat, mouse, horse, and sheep) in serum tube and three types of anticoagulant (Heparin, EDTA and Citrate) tubes for Basic and IVDr platforms. Our standard NMR-based metabolomics (Basic) platform involves plasma/serum filtration, 1D-NOESY data acquisition and processing, metabolite assignments, and quantification using ChenomxÂ® NMR Suite profiling. In the IVDr platform, samples were prepared by an automated Bruker-SamplePro Tube system and NMR data acquisitions were performed according to the Bruker-IVDr protocol. The IVDr-BioBank-QC (IVDr-QC) method evaluates the sample integrity and spectral quality based on human plasma/serum samples. The IVDr-Quantification-Plasma (IVDr-Quant) method reports quantification of 41 pre-set metabolites. Over 90 samples were assessed by both methods including five in-house Heparin-plasma control material (PCM) samples.

**Preliminary Data**

All PCM samples passed the IVDr-QC evaluation parameters. However, in human Citrate- and EDTA-plasma , the effect of anticoagulant was observed in the citrate chemical shift which was below the range (2.672 - 2.680ppm). Each non-human species tested had a unique matrix which resulted in failing of various parameters. For example, concentrations of acetic acid above the maximum limit (0.01 mmol/L) were detected in horse (ave. 0.61mmol/L), sheep (ave. 0.21mmol/L), and mouse (ave. 0.17mmol/L) samples. Due to the limited sample volume, all mouse samples were prepared in 3mm NMR tubes which consistently failed to meet 90-degrees pulse within the target range (9-15ÂµsecÂ).

The initial comparisons between the platforms were conducted with the metabolites reported from PCM samples. In the Basic method, 42 polar metabolites were manually assessed and quantified from each spectrum with <20% RSD. The IVDr-Quant method only reported 24 metabolite concentrations, >20% RSD were found for 2-Aminobutyric acid, proline, glycerol, and dimethylsulfone. The concentrations of lactate, citrate and glucose reported by the basic method agreed with those in the IVDr method; however, acetate and formate concentrations were close to 2-fold higher in the basic method.

In all human test samples, the Basic and IVDr-Quant platforms commonly reported 23 metabolites. The correlation analysis revealed the presence of large discrepancy in quantification in some of the metabolites e.g. lysine and histidine ($R^2<0.02$). However, high correlations ($R^2>0.9$) were observed in acetone, formate, glycine, lactate and pyruvate. Significantly higher levels of acetone in Citrate-plasma and formate in EDTA-plasma compared to other samples indicate anticoagulant interference.

The preliminary analysis suggests that the sample origin, anticoagulants, NMR tube size, and analysis platforms influence each metabolite quantification differently; therefore, independent assessments are necessary. Automated NMR-based metabolomics platforms increase throughput and limit human error; however, reproducibility and accuracy need to be evaluated prior to each study.

**Novel Component**

Reproducibility and accuracy of automated NMR-based metabolomics platforms need to be evaluated on a study-by-study basis.

**----------Poster Board #10----------**

**Title**

**Development of a Synthetic Fecal Metabolite Calibration Solution**

**Author(s) (Presenting author should be listed first)**

Da Silva, Sandra M.; Urbas, Aaron A.; Schock, Tracey; Davis, Clay and Sade, Youssef B.

**Author Affiliations**

National Institute of Standards and Technology (NIST)

**Introduction**

The gut microbiome plays a pivotal role in maintaining host health by influencing metabolic processes, immune function, and disease resistance or susceptibility. Research into the human gut microbiome is rapidly evolving, and metabolomics, the study of small molecules metabolites within cells, tissues, or organisms, has emerged as a powerful tool to assess the functional state of the gut microbiome. Analysis of stool samples, including identification and quantification of metabolites produced by microbial activity, helps reveal the functional metabolic interactions between the host and microbiota, offering insights into the overall health, including dysbiosis, of the gut ecosystem.

**Methods**

However, there is a lack of comparability within and across studies arising from the inherent complexity of stool samples, the variety of analytical workflows, and the variability in instrumentation responses. NIST is developing a synthetic fecal metabolite calibration solution to help improve measurement confidence in this field. [1-3] NMR and LC-MS are being utilized in the characterization of this material.

**Preliminary Data**

The goal of this presentation is to give an update to the community on the status of this effort. We currently have 200 vials of a pilot batch of a mixture of 70 metabolites at biologically relevant relative concentrations found in human stool (RGTM 10212). The details of the preparation, challenges, and future work will be discussed.

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**Novel Component**

Synthetic Fecal Metabolite Calibration Solution

**----------Poster Board #11----------**

**Title**

## Differential Microbial and Metabolites Abundances in Rotten Fruits Drives Metabolic Variation Across Drosophila Genotypes

**Author(s) (Presenting author should be listed first)**

Fijabi Oluwatobi; Laura K. Reed

**Author Affiliations**

The University of Alabama, Tuscaloosa

**Introduction**

Diet is a major modulator of host phenotype. The nutritional profile of Drosophila lab diet differs from what they encounter in the wild. Therefore, our study seeks to uncover the environmental influence on Drosophila metabolism in the lab using fermented peach and strawberry diets. We hypothesize that differences in natural diets may inform different metabolic states across Drosophila genotypes

**Methods**

To answer this question, we did a 6-day fermentation and homogenization of organic peach (P) and strawberry (S). These were fed to sterilized 1st instar larvae of three DGRP genotypes. The homogenized samples were subjected to ITS and 16S microbial analysis while metabolites were characterized using untargeted GC/MS in diet and 3rd instar larvae samples. We characterized survival and development time to 3rd instar (L3) fed P and S

**Preliminary Data**

Our ITS data showed that non-Saccharomyces yeast of the Ascomycota phyla were the core fungi in P and S with S higher in beta-diversity ($p<0.01$). The 16S result showed that phyla Proteobacteria, Actinobacteriota,

Verrucomicrobiota and Acidobacteriota were core bacteria with S higher in beta-diversity ($p<0.05$). Sugar oxidizing bacteria (Gluconobacter oxydan) is more abundant in P compared to S. Metabolomics analysis of diet showed elevated level of trehalose, TCA, essential amino acid metabolites in S, while sucrose, fructo-oligosaccharide, raffinose and stigmasterol were elevated in P. The metabolite profile of P fed larvae may suggest shunting pentose sugar pathway to compensate for lowered bioavailability of nutrients while S fed larvae use the conventional glycolysis pathway. TCA intermediates were elevated in S fed larvae except succinic acid compared to P. Genotypes showed differential levels for ribitol metabolite. Genotype-by-diet interaction influenced survival of larvae to L3($p<0.01$). Survival was higher in S (62%) compared to P (53%) ($p=0.0076$). Larvae on S developed faster (5.7 days) compared to P (7 days, $p<0.001$). Together, our results show differential response of Drosophila to P and S due to variation in nutritional and microbial composition with S fed larvae reflecting healthier phenotypes. Genetic variation also strongly impacts metabolic profile

**Novel Component**

Our study shows the importance of nutrition, microbiome, and genotype in driving metabolic fate in Drosophila

**----------Poster Board #12----------**

**Title**

**Elucidating A Metabolic Trigger Leading To The Stoppage Of Transcription-Translation In Cell-Free Expression Systems**

**Author(s) (Presenting author should be listed first)**

Vora, Soor; Styczynski, Mark

**Author Affiliations**

Georgia Tech Department of Chemical and Biomolecular Engineering

**Introduction**

Cell-free expression systems (CFES) use cellular extracts to express proteins in vitro. Because of their robustness, tunability, and focused metabolism toward desired products, they are a popular chassis for point-of-care diagnostics and are being increasingly explored as a means for biomanufacturing.

However, the limited productivity and lifetime of batch CFES serve as bottlenecks for their biomanufacturing applicability, as their tendency toward halted transcription-translation (TX-TL) activity caps their protein yields.

While standard CFES reporters indicate timings of protein production cessation, the metabolic changes leading to this may not occur simultaneously. We aim to use metabolomics to identify timings, key metabolites, and pathways defining the metabolic trigger behind TX-TL stoppage, informing process changes to improve the productivity and lifespan of batch CFES.

**Methods**

To gain insights generalizable to all E. Coli-based CFES, we employ simple plasmid systems with expression coming from only a fluorescent reporter and an antibiotic resistance marker. To assess the timing of inflecting protein production and its sensitivity to process parameters, we conducted continuous fluorescent reads of CFES reactions in microplates.

Our metabolomics experimentation starts with incubating cell-free reactions for durations of interestâ€”revealed by fluorescence experimentsâ€”to examine metabolome-level and pathway-level changes. From there, samples undergo protein precipitation and derivatization to ensure suitability for metabolite identification and quantification via GC-MS. We then perform a series of data processing steps on the raw spectra and chromatograms, followed by statistical analysis and pathway enrichment to provide mechanistic understanding.

**Preliminary Data**

GC-MS protocols were optimized for analyzing metabolite profiles in cell-free reactions using an Agilent 5977/7890b GCMS. Experimental sensitivity studies and data analysis initiatives led to an ideal parameter set and a pipeline for peak deconvolution, alignment, and statistical analysis, providing an avenue toward mechanistic takeaways.

Our analyses of E. coli cell-free reaction datasets previously collected by our group reveal that the time reporter production starts decelerating â€“ essentially, the inflection in a protein production curve â€“ varies with plasmid concentration, promoter strength, and operator. This biologically reasonable but important finding highlighted the need to use continuous fluorescence reads with our own cellular extracts to determine the timing for metabolomics sample collection. In addition, the differences between operators highlighted the importance of internal consistency within our extracts, as consistent experimental timescales are important for properly executing metabolomics experimentation.

We have conducted and analyzed continuous reporter-only cell-free experiments expressing sfGFP to understand the inflection time and lifetime for overall protein synthesis. We also expressed a fluorescent RNA aptamer to glean transcription-only timescales. These experiments have provided valuable information about the inflection times and productivity of our lysates and revealed that changes in various process conditions can manipulate the lifetime and productivity of CFES.

Our cell-free metabolomics studies to date have reproduced previously published trends and revealed that conditions with similar initial and final metabolomes can lead to very different levels of protein expression. Negative and positive controls whose metabolomes took different paths to the same metabolic endpoint showed us that the path taken by a CFES reaction's metabolome could be critical for its productivity. They also revealed a mid-process metabolic shift that could point to a metabolic trigger that stops TX-TL machinery. Ongoing experimentation is attempting to build on this by focusing sample timings on hypothesized inflection points to find a specific metabolic trigger.

**Novel Component**

This is the first study to identify connections between specific timings of the cell-free metabolome and the stoppage of expression.

**----------Poster Board #13----------**

**Title**

**Evaluating the concordance of untargeted and quantitative metabolomics for characterizing metabolic dysregulation in colorectal cancer.**

**Author(s) (Presenting author should be listed first)**

Richardson, Adam D; Cho, Kevin; Stancliffe, Ethan; Mehta, Ashima; Guzior, Douglas V; Gandhi, Monil; Cohen, Tom; Patti, Gary J.

**Author Affiliations**

Panome Bio, Inc.; Washington University in St. Louis

**Introduction**

Colorectal cancer (CRC) presents a significant global health concern, underscoring the pressing need for sensitive and robust methods for early detection, characterization, and therapeutic monitoring. Metabolomics, the study of small molecules within biological systems, is rapidly emerging as a powerful approach for investigating the complex metabolic reprogramming that underpins cancer development and progression. Targeted and untargeted metabolomics offer distinct yet complementary analytical strategies for comprehensively profiling the metabolome. Here, we investigated the concordance of untargeted and quantitative metabolomics analyses of the same serum sample set from individuals with stage IV CRC and matched healthy controls to assess the strengths of each approach for investigating metabolic dysregulation in CRC.

**Methods**

Serum samples from individuals with stage IV CRC and matched healthy controls were subjected to both untargeted and quantitative metabolomics analyses. Untargeted metabolomics profiling was performed using a combination of liquid chromatography and high-resolution mass spectrometry (LC/MS) encompassing both polar and lipid fractions. Targeted metabolomics analysis was conducted using the Biocrates MxPÂ®Quant 500 XL kit, employing a combination of liquid chromatography (LC) and flow injection analysis (FIA) coupled with tandem mass spectrometry (MS/MS) for absolute quantification of a targeted panel of metabolites. Following

data acquisition, both datasets were processed for feature detection, noise removal, signal drift correction, and missing value imputation. Statistical analyses, including principal component analysis (PCA), hierarchical clustering analysis (HCA), and pathway enrichment analysis, were performed to identify differentially abundant metabolites and dysregulated metabolic pathways.

**Preliminary Data**

Untargeted metabolomics analysis revealed 1,616 metabolite features, with 1,115 confidently identified, while the targeted approach yielded measurements for 1,019 metabolites, with 454 reliably quantified. Unsupervised analyses of both datasets showed clear separation between healthy and CRC samples. Specifically, PCA revealed distinct clustering patterns, while HCA highlighted the segregation of metabolic profiles, indicating distinct metabolic profiles associated with CRC.

Differential analysis of the untargeted dataset revealed 20 statistically significant metabolites, while the targeted analysis yielded 9 significant metabolites. Notably, both approaches identified alterations in amino acid metabolism, fatty acid metabolism, and bile acid biosynthesis, underscoring the involvement of these pathways in CRC development.

Pathway enrichment analysis of the untargeted dataset revealed upregulation of pathways related to unsaturated fatty acids, prostaglandins, and octadecanoids, indicating increased inflammation in the CRC group. The targeted approach identified similar trends, with alterations in glycerophosphoethanolamines, triacylglycerols, and bile acid biosynthesis. Moreover, cystine levels were significantly elevated in the targeted dataset, aligning with the untargeted analysis's observation of dysregulated cysteine catabolism. This suggests a potential role of oxidative stress and altered redox balance in CRC.

**Novel Component**

This study aims to compare the results obtained from untargeted and quantitative metabolomics to assess the strengths and limitations of each approach for studying CRC.

**----------Poster Board #14----------**

**Title**

**Investigation of diagnosis potential of Salivary metabolites for early detection of Vascular Cognitive impairment**

**Author(s) (Presenting author should be listed first)**

Ali Yilmaz, PhD; Nadia Ashrafi, PhD; Delanie Goniwiecha; Stewart F. Graham, PhD

**Author Affiliations**

Assistant Professor

**Introduction**

Vascular Cognitive Impairment (VCI) is the second most common cause of dementia in the elderly. VCI is not a homogeneous condition but encompasses various pathomorphological and pathogenetic syndromes that are united by the association of cognitive impairments with cerebrovascular pathology. VCI often develops subtly and gradually, making it difficult to detect until noticeable symptoms emerge. As a result, VCI frequently goes undiagnosed until it has progressed significantly. Therefore, finding effective prevention strategies and developing efficient treatments for VCI symptoms remains a pressing challenge in the medical field. Saliva, a readily accessible biological fluid, offers valuable source of biological information and has great potential for diagnostic purposes.

**Methods**

In this novel study combining two advanced analytical techniques, namely one-dimensional proton Nuclear Magnetic Resonance (1D 1H NMR) Spectroscopy and High-Resolution Liquid Chromatography coupled Mass Spectrometry (LC-MS), the metabolic profiles of saliva from patients with VCI (n=37) and age and sex-matched cognitively healthy controls (HC, n=40) were studied. Advanced machine learning and bioinformatic analyses were applied to identify metabolic changes associated with VCI and evaluate the potential of salivary metabolites as diagnostic biomarkers to differentiate VCI patients from healthy controls.

**Preliminary Data**

A random forest (RF) model discriminated VCI from HC subjects with an accuracy of 0.883 and an area under

the receiver operating characteristic curve (AUROC) of 0.931. A support vector machine (SVM) model had an accuracy of 0.857 and an AUROC of 0.946. Bioinformatics analysis uncovered novel metabolic pathways that had not been previously associated with VCI, suggesting a significant role in its development. This discovery highlights the immense potential of saliva as a diagnostic tool for understanding the underlying mechanisms of VCI, paving the way for new insights into the disease's pathology.

**Novel Component**

Saliva metabolomics using both 1H NMR and LC-MS provided noninvasive identification of perturbations in several metabolomics pathways in patients with VCI. Biomarker panel reported here serve as a non-invasive, clinically feasible tool to reliably support the early diagnosis of VCI.

**----------Poster Board #15----------**

**Title**

**iPRM-PASEF - a novel workflow for the analysis and interpretation of spatial on-tissue tandem mass spectrometry**

**Author(s) (Presenting author should be listed first)**

Ramachandran Sumankalai(1), DelaCourt Andrew(1), Tao Nannan(1), Heijs Bram(2), Boskamp Tobias(2), Deininger Sören-Oliver(2), Kessler Nikolas(2), Fütterer Arne(2), Behrens Arne(2), Henkel Corinna(2), T. Smit Nadine(2), Stumpo Kate(1)

**Author Affiliations**

(1)Bruker Scientific Inc; (2)Bruker Daltonics GmbH

**Introduction**

Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI Imaging) is a powerful analytical technique that provides detailed spatial characterization of tissue samples and allows for the analysis of a wide range of molecules such as proteins, lipids, metabolites, and other biomolecules, at high spatial resolution. However, accurately identifying these species based on specific mass-to-charge ratios (m/z) or combinations of m/z and collisional cross section (CCS) alone is not possible and often the integration of other analytical techniques is necessary to confidently annotate a compound. The iprm-PASEF method developed here offers a fully integrated solution for CCS-enabled MALDI Imaging (MS1 data) of lipids with iprm-PASEF (MS2 data), streamlining the entire workflow from precursor selection to data acquisition and data interrogation.

**Methods**

A 10 Âµm-thick cryosection from a fresh-frozen rat brain was sprayed with N-(1-naphthyl) ethylenediamine dihydrochloride (NEDC) matrix and imaged on a timsTOF fleX MALDI-2 instrument with microGRID. Following data acquisition, the MS1 data was imported into SCiLS Lab 2025a for advanced processing, including T-ReX3 feature finding, and automated CCS feature extraction. A list of features was selected based on their abundance in key morphological areas, exported as a precursor list and imported into the timsControl 6.0 software to set up the iprm-PASEF acquisition. The MS2 data was also imported into SCiLS Lab 2025a. Using the MetaboScape-powered Molecular Annotation workflow, lipid MS/MS spectra, generated by T-ReX3 feature finding in the iprm-PASEF dataset, were annotated using the rule-based Lipid Species annotation tool.

**Preliminary Data**

CCS-aware spatial lipidomics data were obtained by MALDI Imaging on a timsTOF flex MALDI-2 instrument with microGRID. The data was imported into SCiLS Lab, and T-ReX feature finding was used to find the top 200 most intense mass-mobility features. Based on a PCA analysis, the white matter region of the brain was selected as a region of interest. Several highly abundant features in the white matter region were selected based on their PCA scores. The selected features were exported as a precursor list required to set up the iprm-PASEF acquisition. Additionally, the white matter region was exported, to only focus the MS2 analysis on the cerebral white matter areas. Note that the MS2 analysis was performed from the same tissue area as the MS1 analysis. The iprm-PASEF MS2 dataset was loaded in SCiLS Lab 2025a, and T-ReX feature finding was used to generate MS/MS spectra for each of the selected precursors. These were subsequently annotated using the built-in MetaboScape-powered Molecular Annotation workflow. The rule-based lipid species annotation tool in MetaboScape interpreted the MS/MS spectra and was able to confirm the identity of the precursors. Using the

computational tools in SCiLS the correlations between different fragment ions were calculated, and fractional contribution images were generated to assess the spatial correlation between the different fragments and the precursor ions. This workflow streamlines the study of lipid distributions in key tissue structures, improving the focus and accuracy of downstream lipidomic investigations.

**Novel Component**

New workflow that streamlines the accuracy of lipid identification and enables high-resolution molecular imaging of tissue samples.

**----------Poster Board #16----------**

**Title**

**LC-HRMS/MS analysis of phase II metabolites of common mycotoxins**

**Author(s) (Presenting author should be listed first)**

Myriam Mireault, Elissa Mariani, Irina Slobodchikova, Calin Zainea and Dajana Vuckovic

**Author Affiliations**

Concordia University, Chemistry and Biochemistry department, Montreal, QC, Canada

**Introduction**

Mycotoxins are toxic secondary metabolites produced by filamentous fungi and found in a wide range of foods, including pasta, nuts, milk and meat products. Their structural diversity can lead to carcinogenic, teratogenic, genotoxic, cytotoxic or hepatotoxic effects. Food monitoring ensures that mycotoxins in food do not exceed regulatory guidelines set by different countries. However, dietary preferences can influence an individual's exposure to mycotoxins. Human biomonitoring of mycotoxins measures mycotoxin levels in human biofluids to ensure that established guidelines provide adequate safety. However, analyses generally focus on the parent compounds which can lead to an underestimation of exposure. Here, we characterize the phase II metabolites of mycotoxins found in Canadian foods, focusing on sulfate and glutathione metabolites.

**Methods**

In vitro incubations were performed using human liver S9 fractions on 34 mycotoxin standards in the presence of the cofactors PAPS, GSH, and/or NADPH. Agilent HPLC 1260 coupled to either an LTQ-Orbitrap Velos HRMS/MS or an Agilent 6545 QTOF system was used in positive and negative mode. A Phenomenex PFP column and a Waters CORTECS T3 Column were used for chromatographic separation, with a gradient of water and methanol, containing 0.1% or 0.02% acetic acid (v/v), respectively, for ESI (+) and ESI (-) mode. Data was processed using XCalibur software with an extraction window of 5 ppm window or, alternatively, using Agilent MassHunter Qualitative Analysis with an extraction window of 20 ppm.

**Preliminary Data**

Through in vitro microsomal incubations using human liver S9 fractions, a total of 24 sulfates and 14 glutathione conjugates were reported for the first time. These included novel sulfates of HT-2 toxin, 15-acetyldeoxynivalenol (15-AcDON) and citrinin (CIT), as well as a series of trichothecene glutathione metabolites. The primary metabolites of CIT and 15-AcDON were identified as 8-SULF-CIT and 15-AcDON-7-SULF, respectively. These metabolites were the result of a rearrangement including reduction (8-SULF-CIT) or from a rearrangement of the acetyl group at a 6-member ring (15-AcDON-7-SULF). These results demonstrate the value of direct monitoring of metabolites instead of studying only parent mycotoxin levels after enzymatic deconjugation. In addition, known sulphate and glutathione were generated to build an in-depth spectral library using a combination of two chromatographic methods. Altogether, these results provide better understanding of possible mycotoxin metabolism in humans and have enabled the building of a comprehensive spectral library of mycotoxin metabolites, to support exposomics studies. This library can also be used in biomonitoring and for toxicological studies of mycotoxins.

**Novel Component**

Better understanding of mycotoxin metabolites for biomonitoring and toxicological studies.

**----------Poster Board #17----------**

**Title**

**Macrophage Infiltration in KRAS-Driven Lung Adenocarcinoma is driven by Lipid Metabolism and**

## TAp73 Status

**Author(s) (Presenting author should be listed first)**
Vanessa Y. Rubio1, Hayley D. Ackerman1,2, Nicole Hackel1,2, Christina L. Carr1,2, Jaden Baldwin1,2, John M. Koomen1, Elsa R. Flores1,2
**Author Affiliations**
1Department of Molecular Oncology, 2Cancer Biology and Evolution Program, Moffitt Cancer Center, Tampa, Florida, USA, 33612.

**Introduction**
To understand the tumor suppressive role of TAp73 in the context of Kras-induced lung adenocarcinoma (LuAD), we have induced tumor formation with different adenoviral vectors expressing Cre recombinase. The ubiquitous promoter in the Ad-CMV-Cre virus allows for recombination to occur in multiple cell types, including some immune cells, while the cell-type specific promoter in the Ad-SPC-Cre virus only transduces alveolar type II cells. To characterize the role of TAp73 in the tumor microenvironment, we have applied an integrated â€˜omics approach to tumors derived from the KrasG12D-driven murine LuAD model with and without the loss of TAp73 after infection with either Ad-CMV-Cre or Ad-SPC-Cre.

**Methods**
Murine LuAD tumors with and without the loss of TAp73 after infection with either Ad-CMV-Cre (n = 3 per genotype) or Ad-SPC-Cre (n = 3 per genotype) were pulverized and underwent extraction for metabolomics, lipidomics, and proteomics using the same tissue using aqueous 80% methanol, isopropanol alcohol, and tandem mass tag, respectively. Metabolomic and lipidomic data were acquired using ultra high-performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS) on a Vanquish UHPLC interfaced with a Q Exactive HF mass spectrometer. Additionally, spatial analysis was performed on frozen murine LuAD lung lobes cryosectioned and sprayed with N-(1-naphthyl) ethylenediamine dihydrochloride matrix before acquisition of matrix-assisted laser desorption/ionization (MALDI) imaging with ion mobility (IM). MALDI-IM-MS was performed using 20 um spatial resolution on a Bruker timsTOF Flex with MALDI-2.

**Preliminary Data**
The effect of TAp73 loss on tumor development is much more dramatic with Ad-CMV-Cre versus Ad-SPC-Cre and is associated with an increase in tumor associated macrophages. This result suggests that losing TAp73 in the tumor microenvironment, especially in alveolar macrophages, has a much stronger effect on tumor development than losing TAp73 in the tumor cells themself. With Ad-CMV-Cre, there was a significant effect on several pathways involved in phospholipid regulation. Tumors with TAp73 were enriched for pathways giving rise to arachidonic acid-derived prostaglandins and thromboxanes while tumors without TAp73 favored omega-6 lipids via linoleic acid. Conversely, the models induced by Ad-SPC-Cre do not show the same changes in lipid metabolism and, instead, show a diminished lipidomic profile overall. Changes in prostaglandin E2 (PGE2) levels are known to induce insulin-like growth factor 1 (IGF-1), and we see increased secretion of IGF-1 from macrophages lacking TAp73. To determine the spatial distribution of these lipid molecules, matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) was performed on intact lung lobes containing tumors from all four models. Notably, ion signals matching the expected m/z, CCS, and fragment ions for arachidonic acid and its precursor lipids were seen increased in areas of concentrated macrophages near tumor-bearing regions for the Ad-CMV-Cre models when TAp73 is present but not when it is lost. Altogether, the data suggest that the loss of TAp73 in macrophages that occurs with Ad-CMV-Cre infection results in changes in lipid metabolism coupled with induction of IGF-1 that support tumor development and progression.

**Novel Component**
Integrative 'omics for elucidation of lung cancer tumor microenvironment

**----------Poster Board #18----------**

**Title**

## MANA SODA: The Software and Data Exchange

**Author(s) (Presenting author should be listed first)**
Hitchcock, Daniel; Wu, Yue

**Author Affiliations**
The Broad Institute; Stanford University
**Introduction**
The landscape of metabolomics tools and data is vast. Metabolomics encompasses many technologies (NMR, LCMS, GCMS, etcâ€¦), and even the application of a single technology is not standardized. To process and analyze data, researchers create discrete tools to handle the ever-growing list of metabolomics formats and technologies. However, navigating this arena is tedious and identifying the best tool for the job, if it even exists, is not trivial. At best, this slows down research; at worst, this results in the wrong tool being used and producing inferior data.
**Methods**
The goal for the MANA SODA initiative is to develop a community-driven effort that provides information on current software, datasets, and data analysis results. SODA will provide a community driven medium to communicate between tool creators and data generators. It is our hope that through open community interaction, the available tools for a job would be represented, and perhaps consensus can be reached on what would be the best for a given scenario.
**Preliminary Data**
Our current initiatives include SODA Meetups and the Curated Software List.
**Novel Component**
MANA Interest Group

**----------Poster Board #19----------**
**Title**
**Metabolomics and Exposome Laboratory at the UNC Nutrition Research Institute**
**Author(s) (Presenting author should be listed first)**
Blake Rushing
**Author Affiliations**
University of North Carolina-Chapel Hill, Nutrition Research Institute, Department of Nutrition, Kannapolis, NC 28081
**Introduction**
A major role of the MEL has been to provide support to the NIEHS funded Human Health Exposure Analysis Resource (HHEAR) program, the Environmental Influences on Child Outcomes (ECHO) program, and the NIH Common Fund Nutrition for Precision Health (NPH) study (U2CES030857, MPIs Sumner, Du, Fennell; U24CA268153, PI Sumner). In the past several years, the MEL has analyzed over 10,000 human biospecimens using high resolution mass spectrometry (HRMS) untargeted metabolomics analysis of the internal exposome. These investigations are creating resources for investigators to access through the HHEAR/ECHO and NPH programs. The MEL also supports studies with model systems. Our applications span areas of cancer, cardiovascular disease, pregnancy complications, maternal and child health, osteoarthritis, drug addiction, environmental exposures, and more.
**Methods**
Biospecimen extracts (spiked with 13C standards) are randomized and interspersed with 10% quality control study pools (QCSPs), blanks, and reference materials (e.g., NIST). Data is acquired on a UHPLC Q-Exactive HFx Mass Spectrometry (UHPLC-HR-MS) and preprocessed using blanks for background removal, 13C standards for alignment, QCSPs for within batch filtering, and reference materials for inter-batch corrections. Biospecimens and QCs are processed identically. A system suitability check is regularly performed to assess the retention time stability, signal intensity, and mass accuracy of a mixture of standards. Members of the HHEAR team developed an Automated Data Analysis Preprocessing (ADAP) software that includes algorithms for rapid matching to the MEL inhouse library of over 5,000 compounds, and over 1.7M spectra in public databases.
**Preliminary Data**
The untargeted UHPLC-HR-MS metabolomics platform typically detects 10,000-40,000 features in biospecimens (e.g., urine, plasma, serum, red blood cells, seminal plasma, stool). Using rapid algorithms, 1,000 to1,500 peaks are typically matched to the MEL in-house physical standards library, and 10,000 to 20,000 peaks

are matched to public databases. The MEL in-house retention time (RT), exact mass (MS) and fragmentation (MS/MS) reference library was built by acquiring data for reference standards under the same instrument conditions that are used to analyze biospecimens. Currently, the in-house library contains > 5,000 compounds, including over 1,000 host and microbial metabolites (i.e. metabolic pathways associated with amino acids, carboxylic acids, biogenic amines, polyamines, nucleosides, nucleotides, carnitines, sugars, saturated/unsaturated fatty acids, steroids, bile acids, vitamins, omega-3/omega-6 fatty acids, neurotransmitter metabolites, and one-carbon metabolites), over 100 drugs/medications, over 500 metabolites from foods (including various phytochemicals), and 1,000s of environmental and lifestyle-related compounds, including those commonly detected in the National Health and Nutritional Examination study (NHANES), as well as the Environmental Protection Agency (EPA) ToxCast library. These include metabolites of pesticides, herbicides, insecticides, parabens, volatile organic compounds, phthalates, tobacco related analytes, polyaromatic hydrocarbons, and more. This extensive and growing library enhances our ability to identify and annotate metabolites accurately, with the potential for re-mining existing data as new databases and libraries become available.

The MEL uses an ontology system to clearly communicate the evidence that supports the identification of annotation of each peak in the untargeted metabolomics datasets. The ontology system labels each peak match based on combinations of MS, RT, MS/MS, and isotope pattern data, and whether the match evolved from the MEL in-house library or a public database. This system enables investigators to rapidly understand the evidence behind each match. Pathway analyses, univariate/multivariate statistical approaches, and other modeling techniques are used to understand differences between study phenotypes.

**Novel Component**

The MEL platform simultaneously analyses host and microbial metabolism with lifestyle and environmental exposures to inform biomarkers and intervention strategies.

**----------Poster Board #20----------**

**Title**

**Metabolomics for deciphering cellular responses of gene dosage variation**

**Author(s) (Presenting author should be listed first)**

Kumar, Rohit; Tharayil, Nishanth

**Author Affiliations**

Clemson University, SC, USA

**Introduction**

Gene dosage variations (GDV) refer to the variation in the number of copies of a particular DNA sequence in an organism's genome. The GCV produces morphophysiological alterations from cellular to the whole organism level, some of which have detrimental consequences (cancer, neurodegenerative disorders and Down, Edwards syndromes). A spectrum of GDV in a population also provides a unique platform to elucidate the dosage effect of the varying genes on the phenotype. Metabolomics, by capturing a comprehensive snapshot of biochemical activities, could reveal the dynamic response of an organism to GDV. One of the most detrimental effects of GDV plaguing the present-day agricultural production system is yield loss due to weedy species (estimated loss of $43 billion annually). This is further aggravated by the development of herbicide resistance in weeds, especially glyphosate resistance in Palmer amaranth (Amaranthus palmeri) due to multiple copies of the target gene EPSPS. By analyzing 310 plants varying in EPSPS copies from thirty populations, we attempt to elucidate the physiological manifestation of the EPSPS gene dosage effect using metabolomics.

**Methods**

Plants were grown in the greenhouse. The meristematic leaves from glyphosate and control treatments were harvested and stored at -80Â°C until analysis.

Copies of the EPSPS gene were determined through qPCR (QuantStudioâ„¢ 3 Real-Time PCR). The EPSPS gene copy number was determined with two technical replicates using the 2Î”CT method relative to the Î²-tubulin gene.

The metabolites were extracted using 70% methanol containing 0.5 ppm diosmetin-d3. Untargeted primary

metabolome analysis was performed after chloroform partitioning and derivatization using Gas chromatography connected to a mass spectrometer. The secondary metabolome was analyzed on ultra-high performance liquid chromatography (UHPLC) coupled to an Orbitrap Fusion Tribrid mass spectrometer (Thermo Scientific, Waltham, MA). Pooled samples were also run for QC. Statistical analyses were performed using R packages.

**Preliminary Data**

An ample amount of variation was observed in gene dosage, ranging from a minimum of one copy in susceptible biotypes to a maximum of 85 copies in resistant biotypes. Three hundred ten amaranth plants with diverse copy numbers were selected for subsequent untargeted metabolomic analysis. For these plants, 254 primary and 6354 secondary mass features were detected (identification with exact mass, retention time, and fragmentation spectrum matching). Principal component analysis (PCA) showed a clear distinction of metabolomic profiles before (CTRL) and after glyphosate (GLY) spray. Our hypothesis of distinguishing GS from GR biotypes, based on native metabolic profile, supported by distinct clustering, driven by high abundances of nonaromatic amino acids, glucose and sugar acids, D-glucaric acid and hexaric acid in the GR-biotypes and ascorbic acid, galactinol, and glucose in the GS-biotypes. After GLY treatment, different gene dosage groups (0-5, 6-20, 21-40, 41-60 and 61-90) have distinct associated metabolic profiles with partial to complete overlap for primary and secondary metabolome. Further, using gene additive model, we predicted 21 and 185 primary mass features in CTRL and GLY treatment respectively, out of 254, significantly ($P < 0.05$) associated with GDV. For the secondary metabolome, there were 785 and 3117 mass features out of 6354 significantly associated with GDV. We also predicted copy numbers needed to achieve half maximum concentration of a metabolite. Finaly, we investigated the overall perturbation in the primary and secondary metabolome in GLY compared to CTRL in different copy number ranges including 0-5, 6-20, 21-40, 41-60 and 61-90. Our results indicated an interesting pattern, with perturbation in metabolome decreasing (p-value of significance and Log2 fold change) with increasing GDV. The effects of GDV percolates through different pathways as shown by the pathway enrichment analysis. At low copy number (0-5) major enriched pathways were amino acid metabolism, glyoxylate and dicarboxylate metabolism and TCA cycle. Conversely, plants with high copy number displayed a notable enrichment of pathways involved in fatty acid biosynthesis upon glyphosate exposure.

**Novel Component**

To our knowledge, this is the first study where metabolomics is utilized to study the gene dosage effect across all organisms. Our approach captures the threshold differences in GDV that influence primary vs secondary metabolites.

**----------Poster Board #21----------**

**Title**

**Metabolomics uncovered the reason for differential rooting capacity within Eucalyptus hybrid species, highlighting potential biomarkers for mother plant selection.**

**Author(s) (Presenting author should be listed first)**

Salinas, Ignacio; Medina, Alex; Emhart, VerÃ³nica; Pérez, Andy J.

**Author Affiliations**

Department of Instrumental Analysis, University of Concepcion, Chile

**Introduction**

Plantations of the Eucalyptus genus are widely used for paper production, with E. globulus and E. nitens being the most planted species in Chile. However, due to the low adaptability of E. globulus to cold climates, hybridization between these two species has been chosen, obtaining the E. nitens x E. globulus that better tolerates the frost, maintaining an acceptable wood quality. Unfortunately, hybrids present, in addition, undesired variability in their rooting capacity for vegetative propagation. Therefore, we applied metabolomics and spatial omics to reveal the metabolome changes during rhizogenesis to understand the underlying biochemical mechanisms that differentially affect such physiological processes among hybrid genotypes.

**Methods**

Cuttings from mother plants (MP) of E. nitens x E. globulus and E. nitens genotypes were propagated and collected at different times after planting. According to historical rotting records provided by the Chilean forest

company CMPC, two groups of ten genotypes each, including high (>70%) and low (<30%) rooting capacity, were considered. Plant materials were divided into aerial and underground parts and processed for LC-MS-based metabolomics analysis. Localization of differentially expressed metabolites within the root tissues was then visualized by mass spectral imaging through MALDI-Imaging.

**Preliminary Data**

Our results clearly showed differentiation between the constitutive metabolic profiles of the E. nitens species concerning the E. nitens x E. globulus genotypes. At the same time, an intraspecific metabolome homogeneity was observed in each genotype, indicating that rooting deficiency cannot be attributed to its constitutive specialized metabolite accumulation. In addition, hydrolyzable tannins such as 1,2,6-trigalloyl-glucose and 1,2,3,6-tetragalloyl-glucose were identified to be differentially accumulated in the aerial parts of the low-capacity clones, presumably affecting rooting. Their mapping within underground tissues using MALDI-Imaging finally confirmed their intense accumulation surrounding the place of cutting, which presumably avoids auxin movement leading to rizogenesys inhibition.

**Novel Component**

LC-MS-based metabolomics and spatial omics reveals the most probable cause of rooting deficiency in Eucalyptus hybrids vegetatively propagated.

**----------Poster Board #22----------**

**Title**

**Method for simplified simultaneous quantitation of the constituents of a chemically complex mixture and establishing quantitative linearity.**

**Author(s) (Presenting author should be listed first)**

de Jong F.(2), Ghosh D.(1), Beecher C.(2), Shulaev V.(1)

**Author Affiliations**

1Department of Biological Sciences, College of Science, University of North Texas, Denton, Texas; 2 IROA Technologies LLC, Chapel Hill, North Carolina

**Introduction**

Stable-isotope-labeled Internal Standards (IS) are key for accurate metabolomics quantitation. When analyzing a few compounds in a targeted protocol, it is more accurate to quantify compounds individually, but when the Standard is complex, it is neither efficient nor economical. We developed a workflow to efficiently quantify hundreds of metabolites in an IS that is suitable for both isotopic and natural abundance (NA) mixtures and sufficiently accurate for most purposes. A NA mixture is made, in which the concentration of every compound is known, and used to establish the concentration of the same compounds in the IS by serial dilution and comparison. In addition to quantitating compounds, it gives insights into the range of quantitative accuracy achievable.

**Methods**

In this method we used a library of 600 authentic (unlabeled, IROA-LSMLS) metabolites that was derived from accurately prepared mother liquors which were formatted in seven 96-well plates, each well containing 1 mg of compound dissolved in 90 ÂµL of MeOH:H2O. For each plate 30 ÂµL per well was pooled to create a solution that was serially diluted, aliquoted and dried. A fixed concentration of isotopically-labeled IS was added to all dried aliquots. Samples were analyzed using an Orbitrap Velos Pro mass spectrometer interfaced with Accela UHPLC system (Thermo Scientific). Metabolites were separated on an Ace Excel 2 C18-PFP column. Area ratios were used to calculate equivalent measurements for each compound.

**Preliminary Data**

Each compound in the library had been previously analyzed to determine its RT, and other physical parameters including MS/MS spectra. These identification parameters were loaded into a database to establish compound identity for subsequent analysis. The pooled serially diluted solutions containing IS were analyzed using our standard LC methodology. Using the database library peaks were identified and examined for their NA and IS isotopolog clusters using ClusterFinderâ„¢ software run in calibration mode and the resulting dataset exported as a .tsv file. An R program was written to perform the subsequent analysis. The cluster apical ratio areas (12C/13C) were calculated and graphed to establish their point of equality. Since the concentrations of the 12C

side were known (according to their aliquot size) therefore, the point at which the ratio was equal to 1 was used to the quantity the compounds on the IS (13C) side. In order to balance the equation all of the isotopes for each of the NA and IS clusters had to be considered and summed. The analysis was performed in both positive and negative ionization modes because some compounds showed up in only one mode. This served as a secondary validation for some compounds.
The resultant quantification of the IS makes it possible to measure hundreds of metabolites more accurately than simple relative peak comparison. This will not be as accurate as absolute quantitation of each individual compound, but it is a step forward and well within the error of any measurement in a semi-targeted metabolomics analysis.
Analysis of the serially diluted compounds over many orders of magnitude has provided the opportunity to measure relative balance between the ratio and its systemic error at the extreme ends of its range.
**Novel Component**
A workflow for simultaneous quantification of compounds in an isotopic complex mixture, IROA-LSMLS, calibration

**----------Poster Board #23----------**
**Title**
**Organoid Analysis using Ultra-High Lateral Resolution AP-SMALDI Mass Spectrometry Imaging**
**Author(s) (Presenting author should be listed first)**
Zhou, Mandy; Ghezellou, Parviz ; Elisa Badin, Max A. MÃ¼ller,1,3 Svenja Pauer,4 Jasmin Ballout,4 Martin Diener,4 Kerstin Strupat,5 Bernhard Spengler1,3
**Author Affiliations**
1. Institute of Inorganic and Analytical Chemistry, Justus Liebig University Giessen, Giessen, Germany; 2. Department of Chemical Sciences, University of Padova, Padova, Italy; 3. TransMIT GmbH, Giessen, Germany; 4. Institute for Veterinary Physiology and Biochemistry, Justus Liebig University Giessen, Giessen, Germany; 5. Thermo Fisher Scientific (San Jose, San Jose, California
**Introduction**
Organoids are laboratory-developed 3D organ models, originating from stem cells and are gaining importance as research tools for, e.g., disease modeling, drug discovery, and personalized medicine.â€‹ Here, we present a methodology and results that provide the metabolite spatial distributions in targeted organoids using an atmospheric-pressure scanning microprobe MALDI (AP-SMALDI) MSI setup with a lateral resolution of 1.5 Î¼m at a mass resolution of more than 100,000.
**Methods**
The organoids were treated with 100 ng/ml proinflammatory cytokine tumor necrosis factor-Î± (TNFÎ±) over three days and compared with untreated ones. Various sample preparation techniques were employed to optimize the cryosectioning process of organoids. These included embedding the samples in 10% gelatine after freezing them in N2-cooled isopentane, ensuring the preservation of the samples' morphological integrity.

The sections were thawed in a desiccator and subsequently covered with DHB (for positive-ion mode) or 9AA (for negative-ion mode) matrix using a SMALDIPrep pneumatic sprayer system (TransMIT GmbH, Giessen, Germany).

Mass spectrometry imaging measurements were performed using an ultra-high-resolution prototype AP-SMALDI ion source (TransMIT GmbH, Giessen, Germany), coupled with a Thermo Scientific Q Exactive mass spectrometer (Thermo Fisher Scientific (Bremen) GmbH, Bremen, Germany) at pixel sizes and laser spot sizes down to 1.5 Âµm in positive- and 2 Âµm in negative-ion mode.
**Preliminary Data**
Using ultra-high lateral resolution AP-SMALDI MSI, we observed alterations of ceramide (Cer) and cholesterol sulfate (CS) distributions in the treated samples, suggesting that TNF-Î± triggers a metabolic pathway that increases endogenous Cer- and CS-lipid levels, potentially activating the intrinsic cell death pathway. Cer lipids, belonging to the sphingolipids category, are known for their role in mediating fundamental cellular

processes such as apoptosis, mitophagy, cell cycle arrest, and senescence.3 The elevation of CS has been associated with both, protective and apoptotic effects on cells. The dual role of CS lipids in cellular processes underscores its context-dependent impact on apoptosis, influenced by specific cell types and the cellular microenvironment.

Our study showcases the remarkable potential of the AP-SMALDI setup for label-free molecular imaging of microscopic biological samples, particularly 3D organoids, with an high spatial resolution of 1.5 Î¼m.

**Novel Component**

Highlighting high spatial resolution of 1.5 Î¼m we successfully conducted lipid profiling of distinct organoids, revealing valuable insights into the localization of cell-type-specific metabolites and their functional roles within organoid environments.

**----------Poster Board #24----------**

**Title**

**Plant Metabolomics Applied to Establishing the Foundation for Building Resistance Against Gonipterus platensis Defoliation in Eucalyptus Inter-Specific Hybrids**

**Author(s) (Presenting author should be listed first)**

Pérez, Andy J.; Campos, Jasna V.; Salinas, Ignacio; Mardones, Claudia

**Author Affiliations**

Laboratorio de MetabolÃ³mica de Plantas e ImagenologÃ-a Espectral de Masas (MetPlant & MSI Lab), Departamento de AnÃ¡lisis Instrumental, Facultad de Farmacia, Universidad de ConcepciÃ³n, ConcepciÃ³n, Chile

**Introduction**

Eucalyptus plantations in temperate regions often face productivity challenges due to frost damage. One solution has been creating inter-specific hybrids by crossing the highly valuable but frost-sensitive E. globulus with cold-hardy E. nitens, giving rise to the E. nitens Ã— E. globulus hybrids. Besides, hybrids also inherit resistance/susceptibility to pests, such as the specialist defoliator Gonipterus platensis (eucalyptus weevil). Its control is challenging due to both the prohibition of using pesticides by the Forest Stewardship Council International standards and the exclusion of genetic engineering by international certification bodies endorsing sustainable forest management. Therefore, there is a need to identify measurable biomarkers helping to distinguish differential resistance among hybrid genotypes, which may lead to an alternative breeding strategy for building resistance.

**Methods**

In this study, we used LC-MS-based untargeted metabolomics to identify the metabolic resistance traits transferred to E. nitens Ã— E. globulus hybrids by their parents. We focused on the constitutive and the inducible defenses against G. platensis herbivory based on non-volatile specialized metabolites from trees grown in a field trial. In this way, two sampling times were implemented for the same individuals. The first was at a time without any signs of defoliation, and the second was after an intense weevil attack occurring in the same season. Finally, mass spectral imaging through MALDI-Imaging was used to map the localization of differential metabolites within Eucalyptus leaf tissues to attribute their physiological functionality.

**Preliminary Data**

Findings revealed that, unlike the susceptible E. globulus, hybrids have the necessary enzymes to synthesize stilbenes constitutively, a trait that provides resistance to E. nitens. However, both the constitutive and the inducible stilbene levels in hybrids were significantly lower than in E. nitens, making them less resistant than this parent species. However, results suggested that differential resistance among hybrid genotypes is based on the downregulation fold-change of Roseoside upon herbivory, a glucoside of the monoterpene Vomifoliol. Those genotypes that downregulated this metabolite at a rate higher than 2.0-fold showed better protection against herbivory, suffering only moderate defoliation. Accordingly, the resistance parent, E. nitens, downregulated Roseoside by 3.34-fold, providing it with the highest protection and suffering minimal defoliation. Due to Vomifoliol's lipophilic nature, it was first presumed that it could be sequestered after Roseoside cleavage within the secretory cavities (SC), where the essential oil is accumulated in the leaves. This

hypothesis was then proved by MALDI-Imaging, which indicated that localization of such metabolite was exclusively insight the SC throughout the leaf. During weevil feeding, such SC may be disrupted, releasing the essential oil containing Vomifoliol and allowing it to exert a deterrent effect. These findings mark a critical initial milestone in developing resistance for E. nitens Ã— E. globulus hybrids. They offer unprecedented measurable biomarkers that can assist in genotype selection for large-scale propagation, aiming to prevent future extensive defoliation caused by G. platensis and the resulting economic losses.

**Novel Component**

Metabolomics application uncovered the inducible defense in Eucalyptus hybrids upon defoliation, laying groundworks for building resistance.

**----------Poster Board #25----------**

**Title**

**Plasma metabolomics of inhaled corticosteroid response in asthma patients**

**Author(s) (Presenting author should be listed first)**

Dung T. Tran, MS, PhD; Yulu Chen PhD; Lourdes Ramirez, MD; Jessica Lasky-Su, M.S., ScD; Ann C. Wu, MD; Kelan G. Tantisira, MD, MPH; Michael McGeachie PhD; Scott T. Weiss, MD, MS; Amber Dahlin, PhD, MMSc

**Author Affiliations**

Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA USA

**Introduction**

The development of asthma is due in part to the interaction of genetics and environmental factors, and response to inhaled (ICS) and oral (OCS) corticosteroids shows significant variability among patients. Poor symptom control of asthma can result in exacerbations, leading to increased mortality and worse therapeutic outcomes for patients. Both pharmacogenomics and metabolomics studies have greatly improved our understanding of the differences in ICS responses between individuals. The goal of this study was to investigate whether plasma metabolomic profiles are associated with the risk of asthma exacerbations in patients undergoing ICS treatment.

**Methods**

A total of 810 plasma metabolites from 447 asthma patients on ICS treatment from the Mass General Brigham (MGB) Biobank were profiled. Association between metabolites and asthma exacerbation on ICS was investigated using Cox-regression, followed by validation in an independent cohort of 315 asthma subjects. The primary outcome was the time to first OCS use after plasma collection. We also examined the effect of medication on the metabolite-exacerbation associations by analyzing ICS prescriptions using stratified analysis. Pathway and network analyses, incorporating data-driven and knowledge-driven approaches, were conducted to discover altered pathways. Then, we extracted a list of related genes involved in these pathways using KEGG pathway database and performed gene-based tests to identify significant genes associated with the altered pathway.

**Preliminary Data**

Of the 810 endogenous metabolites tested for association with risk of asthma exacerbation, four were validated by replication in an independent asthma cohort. Two of these metabolites, taurine and stearoylcholine, had hazard ratios (HR) less than 1 (HR = 0.63 (95% CI: 0.45 â€“ 0.88) and HR = 0.84 (95% CI: 0.71 â€“ 0.99) respectively), suggesting that higher levels of these metabolites were statistically associated with reduced risk of asthma exacerbation. Conversely, the other two metabolites, lactate and oleoyl-linoleoyl-glycerol (18:1/18:2) [2] , had hazard ratios higher than 1 (HR = 1.33 (95% CI: 1.01 â€“ 1.75) and HR = 1.27 (95% CI: 1.01 â€“ 1.59) respectively) indicating that higher levels were statistically associated with an increased risk. Additionally, we detected potential effect modification by the number of ICS prescriptions in nine out of the top ten significant metabolites associated with exacerbation risk in the discovery cohort including the validated metabolite, taurine. Pathway and network analyses revealed 26 altered biochemical pathways. Next, we formed a list of 559 candidates genes associated with these 26 alerted pathways from the KEGG database. Forty-six genes had an empirical p-value less than 0.05 and these genes were linked to 18 altered pathways. Of the 18 pathways, Glycerophospholipid metabolism had 11 genes associated with exacerbations, followed by Fatty

Acid Metabolism with nine genes and Methionine, Cysteine, SAM, and Taurine metabolism with seven genes.

**Novel Component**

Our findings show specific metabolites and genes affect asthma exacerbation risk. In addition, we identified unique pathways for ICS response.

**----------Poster Board #26----------**

**Title**

**Quantitation and structural characterization of lipid mediators by high-resolution mass spectrometry**

**Author(s) (Presenting author should be listed first)**

Sayers, Rebekah(1); Proos, Robert(2); Baker, Paul RS(2); Norris, Paul(2); Zheng, Yi(3); Leyen, Klaus van(3); and Holm, Ted(4)

**Author Affiliations**

1) SCIEX, UK; 2) SCIEX, USA; 3) Neuroprotection Research Laboratories, Massachusetts General Hospital, Charlestown, MA; 4) Dept of Chemistry and Biochemistry, University of California at Santa Cuz, Santa Cruz, CA

**Introduction**

Lipid mediators are crucial for the initiation, propagation, and resolution of inflammation, making them key targets for anti-inflammatory drug development. These molecules, present in pM-nM concentrations, are challenging to analyze and typically require sensitive triple quadrupole mass spectrometers. However, a new ultra-sensitive high-resolution mass spectrometer now provides precise in vivo measurements of lipid mediators while simultaneously providing for their structural characterization.

The ZenoTOF 7600 system, a high resolution mass spectrometer, measures endogenous lipid mediators with the sensitivity comparable to that of a high-end triple quadrupole instrument. It uses traditional collision-induced dissociation (CID) and electron-activated dissociation (EAD) for detailed structural characterization. The system's tunable electron beam produces EAD-based fragments to distinguish lipid mediator isomers.

**Methods**

Rats underwent ischemic stroke (MCAO model), and plasma samples were taken after 24 hours. Rat plasma serum was extracted using a solid phase extraction (SPE) column, eluted with methanol (MeOH), reconstituted in 100 Î¼L MeOH, and stored at -20Â°C. For HPLC MS/MS analysis, 10 Î¼L of the sample was injected on a ZenoTOF 7600 system with an OptiFlow Turbo V ion source and an Exion UHPLC. The mobile phases were (A) 0.1% acetic acid in water and (B) 0.1% acetic acid in 84:16 ACN/MeOH, at 0.4 mL/min over 21 minutes. Data were acquired using a high-resolution multiple reaction monitoring (sMRMHR) scan mode and processed with SCIEX OS software.

**Preliminary Data**

The data presented demonstrate the use of the ZenoTOF 7600 system to quantitate and fully characterize lipid mediators using both CID- and EAD-based fragmentation. The results presented will demonstrate that this level of molecular characterization can be achieved on a liquid chromatography (LC) time scale, which enables high throughput data acquisition in samples of diverse origins. The data presented will show that the ZenoTOF 7600 system with EAD is uniquely capable of the specific structural identification of lipid molecular species in simple and complex matrices.

**Novel Component**

Simultaneous quantitative and qualitative analysis of endogenous lipid mediators

**----------Poster Board #27----------**

**Title**

**Semi-quantification of triglycerides with resolved fatty acid composition using a targeted MS3 approach on a novel hybrid nominal mass instrument**

**Author(s) (Presenting author should be listed first)**

Charles Maxey(1), Hector Gallart-Ayala(2), Julijana Ivanisevic(2), Rahul Ravi Deshpande(1), Bashar Amer(1), Susan S. Bird(1), Philip Remes(1), and Cristina C. Jacob(1)

**Author Affiliations**

(1)Thermo Fisher Scientific, San Jose, CA; (2)Metabolomics Platform, Faculty of Biology and Medicine, University of Lausanne, Switzerland

**Introduction**

Triglycerides (TAGs) are the most abundant lipids in human plasma made up of a glycerol backbone and three fatty acids which are connected via ester linkages. The measure of their total circulating level in plasma has been a key clinical biomarker of metabolic and cardiovascular diseases, however, this lipid class comprises a wide variety of chemically diverse TAG species. Their individual measurement with higher selectivity would lead to a better understanding of their role in health and disease.

In this work we used the fragmentation data acquired in an untargeted LC-HRMS lipidomics experiment to generate the list of transitions for a targeted MS3 assay aiming at quantification of individual TAGs in human plasma using the new hybrid nominal mass instrument.

**Methods**

Plasma lipid extracts were separated on a reverse-phase C30 column and analyzed using an untargeted lipidomics approach on Thermo Scientific Orbitrap IQ-X Tribrid mass spectrometer. In this method, TAGs were automatically targeted by specific product ion or neutral losses which enabled further CID MS2/MS3 characterization in the same ddMS2 acquisition. TAGs were annotated with LipidSearch 5.1 software. TAGs with the highest confidence level were selected to develop a targeted MS3 assay on a new hybrid nominal mass instrument for their estimated quantification in human plasma. Estimated concentrations are measured via single-point calibration or spike with multiple internal standards with varying fatty acid chain composition. Method performance was evaluated using isotopically labeled triglyceride standards spiked in NIST plasma at different concentrations.

**Preliminary Data**

TAGs profiling is typically performed by LC-HRMS using HRAM mass spectrometers while their quantification is commonly performed using a Selected Reaction Monitoring (SRM)-based approach. In SRM-based methods, TAG precursor masses are the corresponding ammonium adducts and the product ions are the neutral losses of one of the three fatty acids residues and the ammonium ion. Further fragmentation of these product ions allows the determination of the complete fatty acid composition of TAGs. Thus, a targeted MS3 method will enable the quantification of individual TAG species with high selectivity resolving their specific fatty acid composition.

In this work, LipidSearch software was used to match the experimentally acquired MS2 and MS3 spectra of potential TAG species detected in NIST plasma against its in silico predicted spectral library (More than 100 TAGs annotated with high confidence (â€œgrade Aâ€□) were the fed into a targeted MS3 assay on the new hybrid nominal mass instrument for their routine quantification in human plasma. This method allowed for the measurement of more than 100 individual TAG species with fully resolved fatty acid chain composition and high precision (CV<20%).

**Novel Component**

Targeted-MS3 approach for the quantification of TAG species with resolved fatty acid composition using the new hybrid nominal mass instrument.

**----------Poster Board #28----------**

**Title**

**SERCA activation shifts cardiac substrate utilization without impairing cardiac function in obese mice**

**Author(s) (Presenting author should be listed first)**

Banerjee, Deveena; Hasenour, Clinton; Rahim, Mohsin; Bednarski, Tomasz; Young, Jamey

**Author Affiliations**

Vanderbilt University

**Introduction**

Prior studies suggest that restoring calcium homeostasis by activating SERCA improves liver health and metabolism in a mouse model of metabolic syndrome. We were interested to know if these benefits extended to the heart. Obese mice show increased rates of cardiac glucose and fat oxidation. Here, we test the impact of pharmacological SERCA activation by CDN1163 on heart metabolism. We modeled data from in vivo 13C

tracer experiments to determine cardiac metabolic fluxes in obese mice at the end of an 8-week CDN1163 treatment period. Flux measurements were combined with echocardiography data, gene expression, and enzymatic assays to assess the effects of SERCA activation on heart function and metabolism.

**Methods**

All mice in the study were hyperphagic Mc4r-/- mice transitioned to Western diet (WD) at eight weeks of age. Half of the mice received intraperitoneal injections of CDN1163 thrice weekly, while other animals received vehicle injections. Stable and radio-labeled isotopes were infused and blood was sampled from unrestrained, conscious mice through jugular vein and carotid artery catheters, respectively. Fluxes were estimated by fitting a mathematical model of liver and cardiac metabolism to 13C metabolite enrichment measurements of plasma and tissue metabolites taken at the end of the infusion. A separate cohort of mice was used for echocardiography, gene expression, and protein analysis.

**Preliminary Data**

We found that CDN1163 treatment decreased glucose uptake ($p<0.05$), and that the fractional contribution of plasma glucose to citric acid cycle (CAC) metabolites citrate, glutamate, succinate, and malate were also decreased ($p<0.05$). However, the fractional contribution of plasma glucose to the glycolytic metabolite phosphoenolpyruvate was unchanged between vehicle- and CDN1163-treated mice. These metabolite and labeling differences indicated that cardiac metabolism was different between CDN1163- and vehicle-treated Mc4r-/- mice on WD. Modeling of fluxes from the metabolite enrichment revealed that glycolytic flux downstream of glucose uptake, including pyruvate kinase (PK) and pyruvate dehydrogenase (PDH) were also decreased ($p<0.05$). However, the flux of citrate synthase (CS) relative to glucose uptake was increased (~2.5-fold, $p<0.05$), suggesting that non-pyruvate sources were used for carbon flow through the CAC. Indeed, Î²-oxidation flux (Î²ox) flux was increased (~5-fold, $p<0.05$). Furthermore, the flux of Î²ox relative to PDH was increased (~2.5-fold, $p<0.05$), indicating a substrate switch induced by CDN1163 treatment from glucose to fatty acids in the hearts of obese mice. Additionally, expression of genes of fatty acid oxidation were upregulated ($p<0.05$), consistent with flux results and an increase in mitochondrial mass ($p<0.05$). These metabolic shifts occurred without changes in cardiac function or compensatory structural remodeling of the left ventricle. Overall, our findings support that the oxidation of glucose-alternative substrates fuel the heart under CDN1163 treatment in obese mice.

**Novel Component**

We use 13C metabolic flux analysis to study the effects of pharmacological SERCA activation on cardiac metabolism

**----------Poster Board #29----------**

**Title**

**Simultaneous quantitation and discovery (SQUAD) metabolomics workflow for the analysis of flavonoids and their conjugates in blood, urine and food samples**

**Author(s) (Presenting author should be listed first)**

Carvajal-Miranda, Yendry; Amer, Bashar; Deshpande, Rahul; Bird, Susan S.; Bills, Brandon; Bennette, Brian; Newman, John; Fiehn, Oliver

**Author Affiliations**

Thermo Fisher Scientific Intern; PhD Student, Fiehn Lab, UC Davis

**Introduction**

Consumption of dietary pulses (legumes) such as beans, chickpeas, and lentils are potentially beneficial to human health, mainly due to their content of flavonoids (FV) and isoflavonoids (IFV). These polyphenols and their conjugates could serve as potential biomarkers of pulse intake, adding a layer of objective accuracy to self-directed food questionnaires. A metabolomics LC-MS workflow - Simultaneous Quantitation and Discovery (SQUAD) - was developed to analyze FV, IFV and conjugates in legumes. This workflow incorporates Real-Time Library Search (RTLS) to enhance the identification confidence of relevant unknown compounds through spectral similarity measures during method execution.

**Methods**

A total of 40 standard compounds, including flavonoids, isoflavonoids, and few conjugates, were used to create

the library required for RTLS discovery and targeted SQUAD analysis. Urine, plasma, and food samples were collected from a cross-sectional dietary study involving 18 individuals fed randomly with three diets (high in pulse, low in pulse, and control) over seven days. The samples were extracted using 80% methanol, and metabolites were separated using an RP-column with (0.1% FA) and acetonitrile as the mobile phase. Quantification was done using an IQ-X tribrid mass spectrometer, enabling sensitive PRM-quantitation on the linear ion trap and HRAM Orbitrap MS1 scanning for higher annotation rates. LOD and LOQ for the 40 standards were determined.

**Preliminary Data**

Flavonoid standards were used to create calibration curves for accurate quantification. The MSn-based quantification allowed us to selectively detect co-eluting isomers and isobars, improving our ability to distinguish between analyte signals and matrix interferences. The development of a SQUAD method revealed differences in the metabolic profiles of the different groups, provided sensitive monitoring of known potential biomarkers and allowed for new biomarkers to be identified.

**Novel Component**

Metabolomics, Mass spectrometry, SQUAD, Food Compounds, Flavonoids, Isoflavones,

**----------Poster Board #30----------**

**Title**

**Simultaneous Quantitation and Discovery Analysis (SQUAD) of Lipids in Commercial Vegetable Oils using LC-HRAM-Tribrid platforms**

**Author(s) (Presenting author should be listed first)**

Mattos, Thiago; Zhu, Mengyuan; Moy, Edmund; Bernal III, Claude.

**Author Affiliations**

Thermo Fisher Scientific, West Palm Beach, FL, USA

**Introduction**

Vegetable oil is primarily comprised of triglycerides (TAG) and diglycerides (DAG). The analyses of these species are traditionally done by chemical hydrolysis followed by an evaluation of the fatty acid profile by GC-MS. This approach provides an indirect analysis of the TAG and DAG species, and it is not able to provide details into their composition in the vegetable oils. In this study, we applied simultaneous Quantitation and Discovery (SQUAD) method that combines targeted and untargeted workflows for the analyses of lipids in commercial vegetable oils samples using a Thermo Scientificâ„¢ Orbitrapâ„¢ LC-HRAM-Tribrid mass spectrometer platform. The instrument acquisition versatility allowed simultaneous targeted lipid quantification and unknown lipid characterization with a single HPLC-MS run.

**Methods**

Six different types of commercial vegetable oil samples were analyzed using reversed phase chromatography on a Thermo Scientificâ„¢ Vanquishâ„¢ Horizon UHPLC system coupled to a Thermo Scientificâ„¢ Orbitrap Ascendâ„¢ Mass Spectrometer. A Tribrid-based mass spectrometer equipped with a high-resolution Orbitrap and sensitive linear ion trap mass analyzers which allows for high confidence in untargeted analysis using the orbitrap as well as a high dynamic range tSIM quantitation utilizing the linear ion trap. FullScan-data dependent MSn approach with the combination of HCD MS2 and CID MSn, and UVPD MSn was employed for structural determination of triglyceride species in the vegetable oils. Thermo Scientificâ„¢ TraceFinderâ„¢ and Compound Discovererâ„¢ software were used for data processing, compound annotation, structural elucidation, and statistically analysis.

**Preliminary Data**

The results presented herein serve as a proof of principle of using Simultaneous Quantitation and Analysis (SQUAD) method for lipidomic analysis in any biological matrix. In a single LC-MS run, both targeted ion trap data (for targeted quantitation) and high resolution Orbitrap data (for unknown annotation) were collected. The FullScan and MSn data generated by Orbitrap Tribid mass spectrometer with high resolution and sub-ppm mass accuracy enables confident lipid annotation and structural characterization. Data was analyzed using Compound Discovererâ„¢ for unknown characterization and TraceFinderâ„¢ for quantitation. Compound Discovererâ„¢ software offers a comprehensive approach for comparative analyses, annotation, and data visualization,

allowing for the identification of dozens of lipid species in this study. The ion trap quantitative results showed remarkable sensitivity and a great dynamic range for the lipid standard compounds, with limit of quantitation (LOQ) reaching low ppb levels.

**Novel Component**

A streamlined and robust method for the analysis of triglycerides and diglycerides will become available to the community.

**----------Poster Board #31----------**

**Title**

**Unraveling Brain Aging: Lipidomic and Metabolomic Insights from the CSF and Choroid Plexus**

**Author(s) (Presenting author should be listed first)**

Prasanna Vadhana Ashok Kumaar

**Author Affiliations**

Buck Institute for Research on Aging

**Introduction**

Understanding the mechanisms of brain aging is crucial for developing interventions against age-related neurodegenerative diseases. We focused on capturing the true metabolic state in the choroid plexus and cerebrospinal fluid (CSF) which are critical in their central roles in brain homeostasis, nutrient transport, and waste clearance. However, these tissues are underexplored in mouse brain aging research due to the challenges they presentâ€"limited CSF volumes with compromised purity, and small and structurally complex choroid plexus. Despite these difficulties, we developed a workflow for metabolomic and lipidomic analyses of the choroid plexus and CSF with the aim of uncovering aging-related metabolic changes and identifying biomarkers.

**Methods**

We optimized extraction methods for CSF and choroid plexus samples for comprehensive metabolomic and lipidomic analyses using the IQ-X Tribrid mass spectrometer. For CSF, a biphasic extraction with methanol and methyl tert-butyl ether efficiently partitioned metabolites from 10 ÂµL volumes, with a SPLASH Lipidomix kit as the internal standard in QC samples. Choroid plexus samples were extracted with 80% methanol for small molecule analysis, with extracts dried using a speed vacuum.

For lipidomics, dried extracts were reconstituted and analyzed via reversed-phase chromatography coupled with high-resolution mass spectrometry (HRMS) in positive and negative ionization modes. Polar metabolites were analyzed using HILIC coupled with HRMS. ESI source ionization parameters were optimized, employing AQUIREX deep scanning for enhanced data quality.

**Preliminary Data**

The results from our metabolomics analysis reveal significant lipid alterations in the cerebrospinal fluid (CSF) of aging mice, shedding light on the molecular processes underlying brain aging. By comparing lipid profiles between 6-month-old and 18-month-old mice, representing early adulthood and the onset of aging, we successfully annotated 250 distinct lipids in the CSF, including sphingomyelins, fatty acids, and phosphatidylcholines. This detailed lipid map provides crucial insights into the metabolic shifts occurring as the brain ages. Two key findings emerged: an increase in cardiolipin and a decrease in sphingomyelin 42:2 in the CSF of 18-month-old mice. Cardiolipin, essential for mitochondrial function, showed elevated levels, possibly reflecting an adaptive response to age-related mitochondrial dysfunction. This suggests that the aging brain may attempt to maintain mitochondrial efficiency and counteract declining cellular energy metabolism through increased cardiolipin production. Conversely, the decrease in sphingomyelin 42:2, a lipid crucial for cell membrane integrity and signal transduction, suggests reduced membrane stability in the aging brain. This reduction could impair neuronal communication and increase susceptibility to neurodegenerative processes by weakening membrane structure and function. The changes in cardiolipin and sphingomyelin 42:2 not only serve as potential biomarkers of aging but also highlight key pathways that could be targeted for therapeutic intervention.

Ongoing metabolomic analysis of the choroid plexus in wild-type mice will further illuminate the metabolic

changes associated with aging. As the choroid plexus regulates CSF composition, its analysis may reveal additional biomarkers and pathways influencing brain aging. Overall, these results emphasize the critical role of lipid metabolism in brain aging and demonstrate the power of CSF and choroid plexus metabolomics in advancing our understanding of aging and neurodegeneration.

**Novel Component**

The novel component is the untargeted and targeted metabolomic analysis of CSF and choroid plexus, uncovering region-specific aging biomarkers previously underexplored.

**----------Poster Board #32----------**

**Title**

**Untargeted Metabolomics Reveal Antioxidant and Anti-inflammatory Effects of Exercise in Drosophila**

**Author(s) (Presenting author should be listed first)**

Kolapo-Mabayoje, R. ,Tolulope; Cross, Hogland; Gabriella, Bicanovsky; Jarmacus, Monroe; Laura, K. Reed

**Author Affiliations**

Department of Biological Sciences. The University of Alabama, Tuscaloosa

**Introduction**

Obesity, type II diabetes, cardiovascular diseases are compendium of disorders associated with metabolic syndrome. Physical activity is known to be a non-invasive treatment not only for obese individuals but also for other diseases like Alzheimer's, Parkinson, and cancer. Drosophila is used to further understand the molecular mechanism behind the multifaceted benefits of exercise. Drosophila is suitable because most of the pathways that trigger pathophysiological responses are conserved in fruit flies and humans. Metabolomics is a useful approach to understand both the genetic and environmental contributions to end products of metabolism in biological systems. Since exercise is an external/environmental force that impacts biological systems, our study seeks to identify clusters of metabolites that are associated with molecular pathways that are triggered during exercise.

**Methods**

We conducted untargeted metabolomics using the GC/MS approach. We used a strain from the Drosophila Genetic Reference Panel (DGRP) and we collected their eggs. Four days post eclosion, they were separated into male and female cohorts that proceeded into three treatments; Exercise(E), Control on the device (C), and No Exercise (NE). This were further randomly assigned to either Power Tower (PT) device or the TreadWheel (TW) device. These flies exercised for 30 minutes on the devices and were immediately frozen in liquid Nitrogen. Three replicates from each treatment-device combination were used for further analysis.

**Preliminary Data**

Following the untargeted metabolomics analysis of the samples, the raw data was log transformed, normalized and statistical analysis were conducted using Metaboanalyst and JMP. The result reveals that device impacts the abundance of metabolites, we identified 17 metabolites that were more abundant in flies trained on the Power Tower compared to those trained on the TreadWheel and the No Exercise cohort. These metabolites include vanillic acid, malonic acid, threitol, aconitic acid, and pipecolinic acid, most of which are known for their antioxidant and anti-inflammatory properties. The abundance of xylose and threitol suggests the upregulation of Glucuronate pathway that converts xylose to threitol during energy metabolism. These metabolites further reveal that oxidative stress and anti-inflammatory pathways that includes NRF2/Keap1 pathway are triggered. Our study also reveals that sex impacts the abundance of 86 other metabolites, of these metabolites most females had increased relative abundance of amino acids and sugars while most male flies had more long chain fatty acids in abundance regardless of exercise. We further see exercise interact with sex to impact the abundance of eight metabolites that include alanine, stigmasterol, adipic acid, indole-3-lactate (known to be involved in tryptophan metabolism, immune cell differentiation and synthesis of cytokines). With this study we have been able to identify some pathways linked to exercise in Drosophila, and our study shows baseline metabolite distribution in male and female Drosophila prior to exercise. We also see that both sex and exercise interact to induce a change in the metabolome of Drosophila.

**Novel Component**

This study to our knowledge is the first exercise metabolome research conducted with Drosophila.