

**Welcome to the 2nd Annual
Interdisciplinary Biological Sciences
Symposium**

From Code to Cure: Bridging the Gap in
Biological Sciences

April 3-4, 2024

Keynote Speakers
Wednesday April 3, 2024
Memorial Union – South Ballroom

9:00 – 10:00 - Dr. Akhila Rajan - Associate Professor - [Fred Hutch Cancer Center](#)
"Lard of the flies: Investigating how fat cells communicate with the brain"
-Introduction by Anurag Das

Dr. Akhila Rajan studies how bodies sense how much energy they have available, and how this information then influences factors such as activity and hunger. Our bodies store energy as fat, to be used later when external sources of energy run low. How much energy is stored is an important piece of information that influences behavior. For example, as our bodies sense that our energy stores are dropping, hunger, and the drive to find more food, will increase. But a dysfunctional energy-sensing system may underlie obesity, in which our bodies may not properly sense that we have enough energy stored already. Dr. Rajan uses fruit flies to understand how fat signals the brain, and how chronic nutrient surplus disrupts this communication. She hopes to reveal fundamental insights into this nutrient-sensing network that could point the way toward strategies to tackle obesity.



2:30 – 3:30 - Jacob Hoyle - Lead Research Scientist - [Living Carbon](#)
"Biotech Trees for Carbon Storage"
-Introduction by Sabrena Rutledge



Jacob Hoyle is a plant molecular biologist and geneticist working to benefit growers and improve the climate. To be adopted, climate benefiting nature-based technologies need to be profitable, so whether that means row crops or forests, they need to grow well. Jacob has taken this philosophy to the biotech startup sector, first with soy-cheese startup Nobell Foods, and in climate and forestry with Living Carbon. Jacob leads construct design and gene editing teams for Living Carbon PBC, a climate biotech company with a mission to rebalance the carbon cycle with the power of plants. Living Carbon has planted thousands of trees enhanced with a respiration shunt pathway to increase the carbon drawdown potential of photosynthesis, generating high-quality carbon removal projects. Continuing research interests include increased permanence, disease resistance, and drought tolerance.

Keynote Speakers
Thursday, April 4, 2024
Hach Hall – Thiel Atrium

10:00 – 11:00 - Dr. Joan Lunney - Senior Research Scientist –
USDA Animal Parasitic Diseases Laboratory
"Combining immunology and genomics research to improve pig health and biomedical research"
- Introduction by Vishesh Bhatia



Dr. Joan Lunney is a Distinguished Senior Research Scientist working in the [Animal Parasitic Diseases Laboratory](#), Beltsville Agricultural Research Center, Agricultural Research Service (ARS), US Department of Agriculture, Beltsville MD USA. Dr. Lunney designed the US PRRS (porcine reproductive and respiratory syndrome) Host Genomics Consortium which assesses the role of genetics in determining pig resistance and susceptibility to PRRS virus infection, pathology, and associated growth effects. In collaboration with Canadian scientists, she has probed mechanisms controlling fetal resistance to congenital PRRS virus infection. She is an active member of the US NC229 multi-station research consortium which addresses stakeholder-driven needs to combat swine infectious diseases and identify scientific solutions to improve animal health.

Dr. Lunney coleads the US Swine Immune Toolkit efforts aimed at developing new monoclonal antibodies and immune assays for assessment of pig health and vaccine responses and for use in biomedical models of human health and disease. She is actively involved in mentoring younger scientists, particularly women scientists. She was selected as a Fellow of the American Association for the Advancement of Science (1998), the International Society for Animal Genetics (2017), and the Conference of Research Workers in Animal Disease (2022). She received the ARS national Outreach Diversity, and Equal Opportunity Award (2014), and was inducted into the ARS Hall of Fame (2019). In 2022 she was announced as a U.S. Presidential Rank Awardee as a Meritorious Senior Professional and in 2023 was honored as the Distinguished Veterinary Immunologist at 13th International Veterinary Immunology Symposium (IVIS2023) in Kruger National Park, South Africa Nov. www.ivis2023.org. Dr. Lunney has served on numerous grant panels, journal editorial boards and in leadership positions for animal genetic and veterinary immunology societies.

2:00 – 3:00 - Dr. Ming "Tommy" Tang - Director of Computational Biology at [Immunitas Therapeutics](#)
"Bridging the Gap: how to learn computational biology the hard right way"
- Introduction by Mehak Kapoor

With over a decade of experience in computational biology, Dr. Ming Tommy Tang specializes in genomics, epigenomics, and (single-cell) transcriptomics data analysis. He has taken on pivotal roles in various cancer research projects, notably contributing to the NCI's Cancer Moonshot initiative at the Dana-Farber Cancer Institute.



As the Director of Computational Biology at Immunitas Therapeutics, he and his team employ machine-learning techniques to investigate immune cells in human tumors by analyzing single-cell RNAseq, single-cell TCRseq, and spatial transcriptome data. Their goal is to develop novel therapeutics for cancer patients.

He was a self-trained computational biologist. Beyond his professional work, Tommy is passionate about promoting open science and improving bioinformatics education to equip biologists with computational skills. More about him can be found at his website <https://divingintogeneticsandgenomics.com/>.

Wednesday, April 3, 2024 - Memorial Union

Morning Events in South Ballroom

8:00 - 8:45 Breakfast, Networking, and Registration

8:45 - 9:00 Introduction and announcements - **Anurag Das, Mehak Kapoor, and Brian Zebosi** - Directors of the Symposium

Opening remarks - **Dean William Graves**, Dean of the Graduate College

9:00 - 10:00 Keynote speaker - Dr. Akhila Rajan - Associate Professor - Fred Hutch Cancer Center
"Lard of the flies: Investigating how fat cells communicate with the brain"
-Introduction by **Anurag Das**

10:00 – 11:30 - 1st Session - Student Oral Talks (South Ballroom)

10:00 - 10:15 **Alyssa Hohman** - Genetics and Genomics

"Human conserved drosophila miR-6 inhibition has profound impact on lipid accumulation in the heart while mitigating age-related cardiac dysfunction"

- Advisor: Elizabeth McNeill, FSHN

10:15 - 10:30 **Ankur Kumar** - Genetics and Genomics

"The Interplay of peroxisome and mitochondrial dynamics during aging in Drosophila melanogaster"

-Advisor: Hua Bai, GDCB

10:30 - 10:45 **Solomon Eshun** - Applied Mathematics

"Estimating Gender Differences in Glioblastoma Outcomes using Propensity Score Matching"

-Advisor: Claus Kadela, Math

10:45 - 11:00 **Astha Tuladhar** - MCDB

"E3 ubiquitin ligase CUL3-KLHL41 regulates ER homeostasis in skeletal muscle"

-Advisors: Jeff Essner, GDCB and Jinhoh Kim, BMS

11:00 - 11:15 **Anurag Das** - Neuroscience

"Peroxisomal Gene Expression and protein import are regulated by circadian oscillations in Drosophila Glial cells: Title - Peroxisomal Gene Expression and protein import are regulated by circadian oscillations in Drosophila Glial cells"

-Advisor: Hua Bai, GDCB

11:15 - 12:30 - 1st Poster Presentation (Great Hall)

Poster No. Presenters name, graduate/undergraduate program, poster title, and advisor

1 **Sage Becker** - Immunobiology

"Impact of butyrate on short-chain fatty acid receptor expression in porcine monocytes"

-Advisor: Crystal Loving, USDA-ARS

3 **Molly Kroeger** - Immunobiology

"First detection of PCV4 in the United States and its association with PCV2 and OCV3 coinfection"

-Advisor: Pablo Pineyro, VDPAM

5 **Claudia Carrillo** - Nutritional Sciences

"Low to moderate doses of dietary resistant starch and a renin-angiotensin system inhibitor exhibit nephroprotective effects through similar mechanisms in a preclinical model of diabetes"

-Advisor: Matthew Rowling, FSHN

7 **Kelby Kies** - Bioinformatics and Computational Biology

"Identifying key regulators of uterine function during pregnancy"

-Advisor: Geetu Tuteja, GDCB

- 9 **Fazhir Kayondo** - Genetics and Genomics
"A genome-wide association study of stress hormone levels in hair of young healthy pigs"
 -Advisor: Jack Dekkers
- 11 **Leonora James** - Genetics and Genomics
"Fatty acids inferred from milk spectral are predictive of feed intake in lactating Holstein cows"
 -Advisor: James Koltes, Animal Science
- 13 **Claudia Lorena Arriaga** - Nutritional Sciences
"Effects of corn-resistant starch consumption along with intake of ACE inhibitor, captopril, on metabolic syndrome biomarkers in a type-2 Diabetes rodent-model"
 -Advisor:
- 15 **Manuela Chaves** - Genetics and Genomics
"Characterizing the Activating Transcription Factor 1 (ATF1)"
 -Advisor: Julien Roche
- 17 **David Hall** - Genetics and Genomics
"Mosquito immune cells enhance dengue and Zika virus dissemination in Aedes aegypti"
 -Advisor: Ryan Smith, PPEM
- 19 **Henri Chung** - Bioinformatics and Computational Biology
"EpicTope: narrating protein sequence features to identify non-disruptive epitope tagging sites"
 -Advisor: Iddo Friedberg, VMPPM
- 21 **Catherine Fonder** - Molecular, Cellular, and Developmental Biology
"Electrical Stimulation Effects on Adult Rat Hippocampal Progenitor Cell Behavior"
 -Advisor: Don Sakaguchi, GDCB
- 23 **Mubashrah Mahomood** - Animal Science
"Cross-Species Sperm Proteomic Analysis for Human Male Fertility Model Establishment"
 -Advisor: Karl Kerns, Animal Science
- 25 **Marissa Roghair Stroud** - Microbiology
"Design and optimization of a robust CRISPR interference system to identify Pseudomonas putida genes essential for rhizosphere microbiome formation"
 -Advisor: Larry Halverson, PPEM
- 27 **Katherine Gisi** - Electrical and Computer Engineering
"Biomedical Imaging Datasets using PA as the Ground Truth"
 -Advisor: Pramanik Manojit

12:00 - 1:00 - Lunch (South Ballroom) ISU Dining - Classic Mediterranean

1:00 – 2:30 - 2nd Session - Student Oral Talks (South Ballroom)

- 1:00 - 1:15 **Megan DeTemple** - Plant Biology
"Novel use of CWDEs as a tool to elucidate cell wall-mediated signaling during pathogenesis"
 -Advisor: Olga Zabolina, BBMB
- 1:15 - 1:30 **Brian Zebosi** - Genetics and Genomics
"An effective and inexpensive maize seed chipping protocol using chipping piers: Its applications in small-scale genotyping laboratories and marker-assisted breeding"
 -Advisor: Erik Vollbrecht, GDCB
- 1:30 - 1:45 **Elizabeth Glynne** - Ecology and Evolutionary Biology
"The effect of miniaturization on the evolution of sexual size dimorphism in geckos"
 -Advisor: Dean Adams, EEOB
- 1:45 - 2:00 **Panchali Chakraborty** - Plant Biology
"Investigating the dynamic regulation of plasmodesmata during bacterial infection"
 -Advisor: Kyaw Aung, GDCB

- 2:00 - 2:15 **Kaitlin Higgins** - Genetics and Genomics
"Time point analysis in maize reveals dynamic expression of imprinted genes and three imprinted zeins"
 -Advisor: Sarah Anderson, GDCB
- 2:15 - 2:30 Break

2:30 - 3:30 Keynote speaker - **Jacob Hoyle** - Lead Research Scientist - Living Carbon
"Biotech Trees for Carbon Storage"
Introduction by Sabrena Rutledge

3:30 - 5:00 - 2nd Poster Presentation (Great Hall)

Poster No.	Presenters name, graduate/undergraduate program, poster title, and advisor
2	Prathyusha Cheguri - Genetics and Genomics "Photothermal Effects on Inflorescence Development in <i>Poa bulbosa</i>" -Advisor: Shui-Zhang Fei, Horticulture
4	Joseph DeTemple - Genetics and Genomics "Incorporating gene network information into the prediction of maize flowering time " -Advisor: Jianming Yu, Agronomy
6	Ashley Paulsen - Genetics and Genomics "The MARS community: a synthetic community to identify rules governing maize rhizosphere microbiome assembly" -Advisor: Larry Halverson, PPEM
8	Allison Treibe - Genetics and Genomics "The Role of GAUT Proteins in Arabidopsis thaliana Root Development" -Advisor: Dior Kelley, GDCB
10	Laura Zinnel - Mathematics "Detection of Brain Midline Shift using 2D and 3D Convolutional Neural Networks" -Advisor: Sarah Bentil
12	Anna Maria Luciano - Postdoc "Characterization of a zebrafish model of MYC-driven acute myeloid leukemia"
14	Matthew Wendt - Genetics and Genomics "Multi-environment multivariate genome-wide association studies identify candidate loci underlying cuticular wax accumulation and composition on maize silks" -Advisor: Marna Yandea-Nelson
16	Kassidy Sullivan - Biology and Statistics "Arabidopsis as a model for spontaneous haploid genome doubling in maize" -Advisor: Sarah Pfeffer
18	Claudia Carrillo - Nutritional Sciences "Disruption of Ovarian One-Carbon Metabolism in the Agouti Mouse" -Advisor: Kevin Schalinske, FSHN
20	Beatriz Pereira - Neuroscience "Single-nuclei RNA-Sequencing: heterogeneity of striatal astrocytes and the effect of stress" -Advisor: Elizabeth McNeill, FSHN
22	Anthony Sillman - Molecular, Cellular, and Developmental Biology "Identification of Hemogenic Endothelial Cells Through a Multi-omics Approach" -Advisor: Raquel Espin-Palazon, GDCB
24	Alexandra Keller - Genetics and Genomics "Identification of distinct proteome differences between fresh and cryopreserved bull spermatozoa" -Advisor: Karl Kerns, Animal Science
26	Xiaohan Jiang - Bioinformatics and Computational Biology "Anomaly detection for breed purity analysis" -Advisor: Juan Steibel, Animal Science

Thursday, April 4, 2024 - Hach Hall - Thiel Atrium

- The Creative Arts Showcase will be located in the south side of the Thiel Atrium

8:00 - 8:45 - breakfast (Panera) and networking

8:45 - 9:00 - Welcome and opening remarks - **Anurag Das, Mehak Kapoor,** and **Brian Zebosi** - Directors of the Symposium

9:00 – 10:00 - 3rd Session - Student Oral Talks - Hach Hall - Thiel Atrium

9:00 - 9:15 **Xiaoyi "Chelsea" Cheng** - Molecular, Cellular, and Developmental Biology
"Nod1-dependent NF-kB activation initiates hematopoietic stem cell specification in response to small Rho GTPases"
- Advisor: Raquel Espin-Palazon, GDCB

9:15 - 9:30 **Pengxin Yang** - Biochemistry
"Definition of regulatory elements and transcription factors controlling immune cell gene expression at single cell resolution using single nucleus ATAC-seq"
-Advisor: Chris Tuggle, Animal Science

9:30 - 9:45 **Chinmayi Gudi** - Chemical Engineering
"An Engineered Variant of E. coli Nissle Unveils Enhanced Transformation Efficiency and Versatility in Probiotic Engineering"

9:45 - 10:00 **Break**

10:00 - 11:00 **Keynote speaker - Dr. Joan Lunney** - Senior Research Scientist - USDA Animal Parasitic Diseases Laboratory
"Combining immunology and genomics research to improve pig health and biomedical research"
- Introduction by Vishesh Bhatia

11:00 – 12:00 - 4th Session - Student Oral Talks - Hach Hall - Thiel Atrium

11:00 - 11:15 **An Phan** - Bioinformatics and Computational Biology
"A Longitudinal Analysis of Biases in Function Annotation and Publication Mentions in the Human Proteome"
-Advisor: Claus Kadelka, Math

11:15 - 11:30 **Abbigail McCune** - Genetics and Genomics
"An investigation of Stat3 in Grna-driven myeloid differentiation"
-Advisor: Raquel Espin-Palazon

11:30 - 11:45 **Sabrina Rutledge** - Bioinformatics and Computational Biology
"Improving analysis and interpretation of single-cell sequencing approaches"
-Advisor: Geetu Tuteja, GDCB

11:45 - 12:00 **Nicole Kling** - Nutritional Sciences
"Trust in nutritional science is moderately high but depends on political and religious beliefs"
-Advisor: Lorraine Lanningham-Foster, FSHN

12:00 - 1:00 Lunch - [Schonert's Corporate Catering](#) (boxed lunches)

1:00 – 2:00 - 5th Session - Student Oral Talks - Hach Hall - Thiel Atrium

1:00 - 1:15 **Parnal Joshi** - Bioinformatics and Computational Biology
"A comparison of protein function annotations of model species to orthologous proteins in humans"
-Advisor: Iddo Friedberg, VMPPM

1:15 - 1:30 Yuwei Zhang - Molecular, Cellular, and Developmental Biology
"Development and Quantitative Analysis of a 3D Scaffold-Based Model for Simulating Tumor Microenvironment Using Light Sheet Microscopy"
-Advisor: Jing Wang, Chemical and Biological Engineering

1:30 - 1:45 **Yunhui Qi** - Statistics
"Detection of Gene Expression Mid-parent Heterosis with Single Biological Replicate"
-Advisor: Peng Liu, Statistics

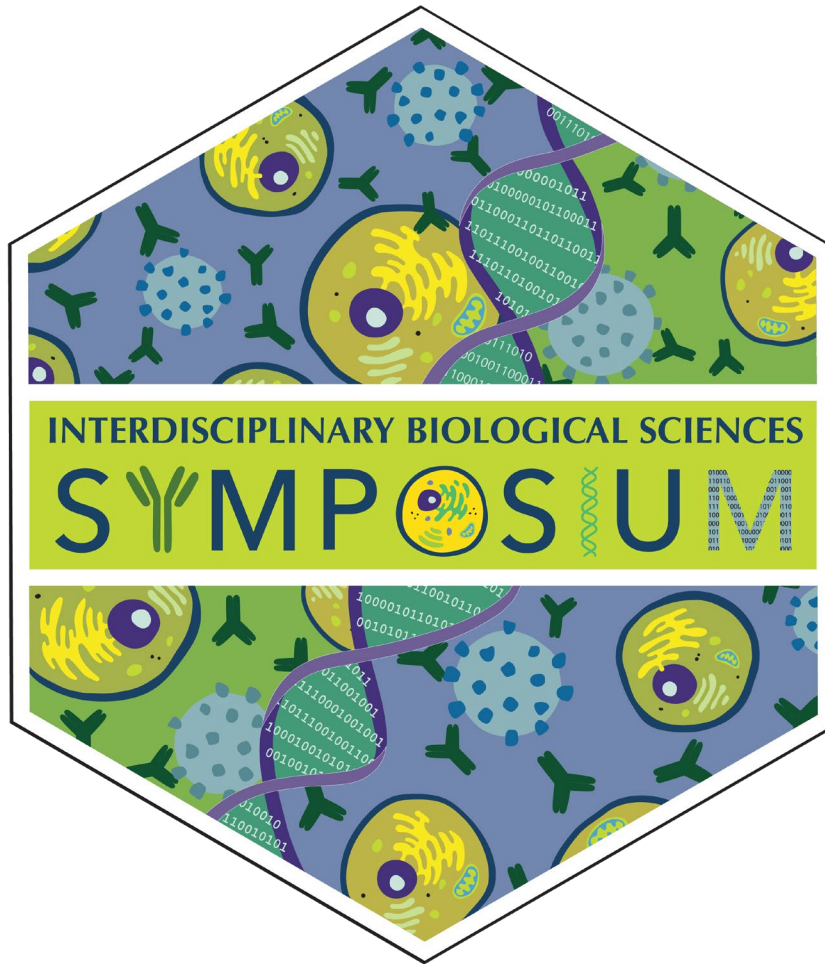
1:45 - 2:00 Break

2:00 - 3:00 Keynote Speaker - **Dr. Ming "Tommy" Tang** - Director of Computational Biology at Immunita Therapeutics
"Bridging the Gap: how to learn computational biology the ~~hard~~ right way"
- Introduction by Mehak Kapoor

3:00 - 4:00 Closing remarks by Associate Dean Heather Greenlee, Graduate College

Awards Ceremony

- Student Oral Presentation - 1st place, 2nd place, 3rd place, people's choice
- Student Poster Presentation - 1st place, 2nd place, 3rd place, people's choice
- Creative Component - 1st place, 2nd place, 3rd place, people's choice
- Lauter Award for Graduate Student Creativity and Research Excellence



Student Oral Presentation Abstracts

Student Oral Abstracts

Human conserved drosophila miR-6 inhibition has profound impact on lipid accumulation in the heart while mitigating age-related cardiac dysfunction.

Alyssa M. Hohman, Jackson Komp, Elizabeth M. McNeill

MicroRNAs (miRNA), short non-coding RNAs that post-transcriptionally regulate gene expression have emerged as ideal candidates for regulation of genes in the heart. Circulating miRNAs have been implicated in various diseases, including cardiovascular disease, yet there are large gaps in our knowledge about their function in these diseased states. One of these microRNAs, mammalian miR-27, is highly conserved with the *Drosophila melanogaster* microRNA-6 (miR-6). Here, we use *Drosophila* as a model to investigate the role of this conserved regulatory mechanism. Utilizing the miRNA sponge technology, we characterized the function of miR-6 loss on heart function, morphology, and lifespan. Functional and morphological changes to the heart were evaluated using SOHA (semi-automatic optical heartbeat analysis) software and immunohistochemistry. Utilizing in silico target analysis, we identified the conserved miRNAs share 149 predicted gene targets. We show heart-specific miR-6 inhibition results in reduced heart diameter, mitigates age related increases in arrhythmia Index, while increasing lipid accumulation within the cardiomyocytes, and significantly extends lifespan. Through qPCR we show an increase to conserved predicted target LRP1 in the hearts of flies with miR-6 inhibition. We have also shown that in the hearts of mice fed a high-fat- high-sugar diet there is a significant reduction of miR-27b expression. Our results establish a new role of miR-6 as a regulator of lipid metabolism, heart health, and longevity in *Drosophila*, with implications for the improved understanding of heart health. These results have the potential to inform therapeutics to address heart disease and ease the age-related decline of human heart function.

The Interplay of peroxisome and mitochondrial dynamics during aging in *Drosophila melanogaster*

Ankur Kumar, Hua Bai

Genetics, Development, and Cell Biology, Iowa State University, Ames, IA, USA 50011

Healthy mitochondria play an essential role in maintaining cellular homeostasis. It is known that mitochondrial structure and function are impaired during aging, likely due to dysregulated mitochondrial fission and fusion processes. Through TEM and confocal imaging analysis, we find that mitochondrial size increases in aged oenocytes, the hepatocyte-like cells in *Drosophila*. Interestingly, we find that mitochondria from aged oenocytes do not exhibit paraquat-induced mitochondrial fission in contrast to young flies. Additionally, aged flies have decreased numbers of mitochondria but increased size as compared to young flies. Our preliminary studies suggest impairment in peroxisomal function in adult oenocytes results in mitochondrial morphology alterations similar to the old flies. However, peroxisomal impairment in indirect flight muscle did not show mitochondrial morphology alteration. Further imaging analysis showed that muscles have fewer peroxisomes than the oenocytes, which again supports the involvement of peroxisome in regulating mitochondrial morphology. Therefore, we screened peroxisomal genes; among those, only *Gnpat* showed similar mitochondrial morphology. Further, we find that the knockdown of plasmalogen biosynthesis enzymes (e.g., *Gnpat* and *Kua/PEDS1* Glyceronephosphate O-acyltransferase) blocks paraquat-induced mitochondrial fission and the recruitment of fission factor *Drp1* to mitochondria, which was also confirmed by utilizing our newly developed live cell imaging technique. We discovered the decrease of mitochondrial plasmalogen C18:0/C18:1-PE aged flies using Mass spectrometry. To discover the involvement of plasmalogen in mitochondrial fission, we performed the lipid pull-down assay using synthetic biotinylated C18:0 plasmalogen PE (pPE) and identified interacting mitochondrial fission proteins such as *Drp1* and *Mff*. To further validate the interacting partner, we pulled down the crude mitochondrial extract, which showed that the *Drp1* and *Mff* interact with biotinylated pPE. Together, our findings suggest that the peroxisomal plasmalogen synthesis pathway plays an important role in maintaining normal mitochondrial health during animal aging.

Estimating Gender Differences in Glioblastoma Outcomes using Propensity Score Matching

Solomon Eshun

Department of Mathematics, Iowa State University
Carver Hall, Morill Rd, Ames, IA, 50014

Gender disparities in health outcomes have garnered significant attention, prompting investigations into their underlying causes. Glioblastoma (GBM), a devastating and highly aggressive form of brain tumor, serves as a case for such inquiries. Despite the mounting evidence on gender disparities in GBM outcomes, investigations specific at the molecular level remain scarce and often limited by confounding biases in observational studies.

Methods: In this study, I aimed to investigate the gender-related differences in GBM outcomes using propensity score matching (PSM) to control for potential confounding variables. The data used was accessed from the Cancer Genome Atlas (TCGA), encompassing factors such as gender, age, molecular characteristics and different glioma grades. Propensity scores were calculated for each patient using logistic regression, representing the likelihood of being male based on the baseline characteristics. Subsequently, patients were matched using the nearest-neighbor (with a restricted caliper) matching to create a balanced male-female group.

Results: After PSM, 303 male-female pairs were identified, with similar baseline characteristics in terms of age and molecular features. The analysis revealed a higher incidence of GBM in males compared to females, after adjusting for potential confounding factors.

Conclusions: This study contributes to the discourse on gender equity in health, paving the way for targeted interventions and improved outcomes, and may guide efforts to improve gender-specific treatment strategies for GBM patients. However, further investigations and prospective studies are warranted to validate these findings and explore additional factors that might contribute to the observed gender-based differences in GBM outcomes aside from the molecular characteristics.

E3 ubiquitin ligase CUL3-KLHL41 regulates ER homeostasis in skeletal muscle.

Astha Tuladhar, Tamara Moretti, Yotesawee Srisomboon, Scott O'Grady, Jeffrey Essner and Jinoh Kim

Cullin3 (CUL3) is a scaffold protein of CUL3 RING ubiquitin ligases (CRL3s) for the RING protein RBX1 and one of BTB domain proteins. The BTB domain proteins serve as substrate adaptors. One hundred eighty-three genes have been identified to encode a BTB domain in the human genome. *KLHL41* is a BTB domain protein in skeletal muscles and the heart. Mutations of the *KLHL41* gene cause nemaline myopathy. A pool of *KLHL41* proteins is associated with the sarcoplasmic reticulum (SR). We observed that depletion of *KLHL41* expression increased the key SR calcium pump SERCA1 protein levels, whereas overexpression of *KLHL41* led to a reduction in *KLHL41* protein levels in C2C12 myotubes. Our qPCR data suggests a post-transcriptional regulation of SERCA1 by *KLHL41*. Furthermore, elevated SERCA1 levels in *KLHL41*-depleted myotubes led to altered calcium dynamics. Recombinant *KLHL41* did not alter recombinant SERCA1 levels in a non-muscle cell line. Interestingly, in undifferentiated myoblast cells, recombinant *KLHL41* appeared to stabilize recombinant SERCA1. To determine the significance of our findings *in vivo*, we generated *klhl41* knockout mutants in zebrafish. The zebrafish knockout mutants showed disruptions in sarcomere organization. Consistent to our findings in C2C12 myotubes, we also observed a post-transcriptional increase of SERCA1 protein levels in the mutants. In addition, the EM analysis of the zebrafish mutants showed a significant dilation of the SR. Our data reveals a new role of CRL3KLHL41 that it maintains SR homeostasis and SERCA1 levels, thus securing proper Ca²⁺ flows in skeletal muscle.

Peroxisomal Gene Expression and protein import are regulated by circadian oscillations in *Drosophila* Glial cells.

Anurag Das, Hua Bai

Genetics, Development, and Cell Biology, Iowa State University, Ames, IA

Interdepartmental Neuroscience PhD Program, Iowa State University, Ames, IA

Peroxisomes are responsible for metabolic activities through the regulation of antioxidant enzymes and oxidases. An important process highlighted during cellular aging is when increased oxidative stress leads to a reduced import efficiency of peroxisomal matrix proteins containing a C-terminal peroxisomal targeting signal type 1 (PTS1). One of the key import factors that is predicted to enable cargo proteins holding PTS1 and its delivery to the peroxisomal matrix is the PEX5 (peroxin-5) protein (Huang et al., 2020). Even though there is emerging evidence of peroxisomal distribution in and its dependency on glia cells (Chung et al., 2020) but an overarching question remains – “which adult glial subtype among *Drosophila* CNS contains peroxisomes?” We have made a novel identification that the cortex glia has a predominance of peroxisomes in the adult *Drosophila* brain. Further, we aim to establish a closely intertwined connection between peroxisome biology and glial of the adult *Drosophila* during the process of cellular aging. We have seen the dynamic changes in transport of the peroxisomal import machinery from the cytosol to the peroxisome marked with eYFP carrying the peroxisomal targeting signal - PTS1. We demonstrate that eYFP.PTS1 reporter driven by Repo-GAL4, a pan- glial marker, can be used to understand the decline of peroxisomal transport in the glial cells of the adult *Drosophila* brain. Our preliminary data suggest that peroxisome import declines in aged glial cells. We have further uncovered how peroxisomal genes and proteins are oscillating at different zeitgeber time points of the diurnal day in the unique glial cell clusters. This was possible through mining & extracting of publicly available *Drosophila* sleep single cell data deposited through the works of Joana Dopp et al., 2024 Nature Neuroscience. I have further validated this through my own experimental testing. Among future directions, I am currently testing the working mechanism to understand how these peroxisomes enriched in the cortex glia which are in very close proximity with the neuronal soma control diurnal metabolism via lipid enrichment in the cortex glia resulting in a unique wake-promoting (sleep) phenotype in my cortex-glia Gal4>PEX5 mutant flies to understand how circadian/sleep disruption due to PEX5 knockdown in glial cells affect human health & disease.

Novel use of CWDEs as a tool to elucidate cell wall-mediated signaling during pathogenesis

Megan DeTemple, Sivakumar Swaminathan, and Olga Zobotina

The cell wall is the plant’s first line of defense against microbial pathogens. During infection, microbial pathogens produce a notable quantity and variety of cell wall degrading enzymes (CWDEs) to invade the plant cell. The plant has evolved plasma membrane receptors to specifically sense these cell wall modifications which activate signaling pathways, triggering the host immune system. Here we present a novel approach to study CWDEs and their role in pathogenesis. Our lab has transformed the pathogen enzyme genes one at a time into the plant genome tagged with a signal peptide which will secrete the CWDEs into the plant apoplast where it is normally found during infection. We can then evaluate their resistance to infection and measure the changes in plant defense gene transcript levels before, and after infection. Our results show that not only are plant defense genes in transgenic plants upregulated before infection, but also that some of these plants are more resistant to infection. We hypothesize that the resistant plants experience only a minor change in their cell wall that causes plant immunity to be elevated without compromising its normal growth and development. Our lab has previously published on a few CWDEs that confer resistance in *Arabidopsis*. Here I present an evaluation of an additional 17 more genes in *Arabidopsis thaliana* against the fungal pathogen *Botrytis cinerea*. Additionally, we have transformed a few of these genes into maize to see if we observe the same effects.

An effective and inexpensive maize seed chipping protocol using chipping piers: Its applications in small-scale genotyping laboratories and marker-assisted breeding.

Brian Zebosi

Department of Genetics and Developmental Biology

In marker-assisted breeding and positional cloning, leaf sampling and plant tracking are vital steps in the genotyping pipeline. They enable the identification of desirable seedlings, saving time and reducing the cost and space required for high plant vigor, especially for greenhouses and winter nurseries. Small-scale marker-assisted selection laboratories rely heavily on leaf-based genotyping, which involves planting segregation populations, leaf sampling, genotyping, and backtracking, which is costly and laborious. Thus, there is a need to adopt seed-based genotyping to reduce costs and save time. Therefore, we improvised and optimized a safe and cheap seed-chipping prototype using chipping piers to chip seed for genotyping before planting. To identify a cost-effective DNA extraction method, we assessed the quality of seed-based DNA extracted across four extraction methods (CTAB, SDS, Urea, and Guanidine-HCl) and compared it to leaf-based DNA. Except for the Urea-based method, the quality of seed-based DNA was comparable to leaf-based DNA across the other methods. We also compared seed-based and leaf DNA for the same individuals from a segregating population and found zero genotyping disparities between seed-chip and leaf genotypes that would be associated with pericarp contaminations. Seed chipping did not affect germination rates, as we observed 100% and 95.3% between chipped and unchipped seeds in B73 and Mo17, respectively. However, chipped seeds grew slower within 7-10 days after planting. Despite this shortcoming, seed sampling using chipping piers provides a safe and high-throughput alternative to selecting specific genotypes before planting, which is paramount for marker-assisted selection and mutant screening.

The effect of miniaturization on the evolution of sexual size dimorphism in geckos

Elizabeth Glynne, Dean Adams

The evolution of miniaturization can result in dramatic alterations of morphology, physiology and behavior; however, the effects of miniaturization on sexual dimorphism remain largely unknown. Here we investigate how miniaturization influences patterns of sexual size dimorphism (SSD) in geckos. Measuring 1,875 individuals from 131 species, we characterized patterns of SSD relative to body size across two families. We found that miniaturized species were more female-biased than non-miniaturized species. Additionally, one family, Phyllodactylidae, contained few miniaturized species and displayed an isometric trend in SSD with body size, where changes in female body size were accompanied by similar changes in male body size. By contrast, Sphaerodactylidae, which contained many miniaturized species, displayed strongly allometric patterns, where larger species were male-biased and smaller species were more female biased. Smaller species in this lineage also produced proportionally larger eggs. Together, these observations are consistent with the hypothesis that selection for increased reproductive success in small species drives the positive SSD allometry observed in the lineage, and that selection for increased miniaturization in the clade may be offset by selection on maintaining a female size in smaller taxa that ensures reproductive success.

Investigating the dynamic regulation of plasmodesmata during bacterial infection

Panchali Chakraborty*, Su-Ling Liu*, Zhongpeng Li, Christian Montes-Serey, Justin Walley, and Kyaw Aung

*These authors contributed equally to this work.

Department of Genetics, Development, and Cell Biology, Iowa State University, Ames, Iowa 50011

Plasmodesmata (PD) provide physical connections between adjoining plant cells, serving as communication channels. Given their fundamental roles, PD are important for plant survival and defense. It has been reported that PD-located proteins (PDLs) play important roles in plant immunity. The expression of PDL5 is upregulated by bacterial infection, whereas *Pseudomonas syringae* (Pst) effector HopO1-1 targets Arabidopsis PDL5 and PDL7. We recently implemented enzyme-catalyzed proximity labeling (PL) to identify functional partners of PDL5. We further utilized the PL assay to capture the dynamic changes in PDL5-containing protein complexes upon bacterial infection. We identified over 200 proteins that might function with PDL5 during bacterial infection. GO enrichment analysis shows that proteins involved in response to stress (GO:000695), vesical-mediated transport (GO:0016192), and localization (GO:0051179) are significantly enriched by bacterial infection. We first selected over 20 candidates to examine their PD association. We detected 9 proteins to be associated with PD. We then focus on functional, characterizing cysteine-rich receptor-like kinases (CRKs). CRK2 has been reported to function at PD and phosphorylates NADPH/respiratory burst oxidase protein D (RBOHD). Using the protein complex modeling program AlphaFold-Multimer (AF-M), we predicted CRKs-PDL5, CRKs-RBOHD, and CRKs-RBOHF heterocomplexes. Our preliminary data suggest the role of the CRK13 and CRK15 as negative regulators plant immunity against bacterial infection. We hypothesize that CRKs regulate reactive oxygen species production during bacterial infection to regulate the PD function and plant immunity. Our study demonstrates the power of the PL assay and AF-M in studying the dynamic regulation of PD during plant immunity.

Time point analysis in maize reveals dynamic expression of imprinted genes and three imprinted zeins.

Kaitlin Higgins, Vital Nyabashi, Sarah Anderson

Department of Genetics, Development, and Cellular Biology. Iowa State University, Ames, IA 50011.

Genomic imprinting is a phenomenon where alleles are expressed in a parent-of-origin dependent manner, and has been found to occur in flowering plants, mammals, and some insects. Though imprinting was discovered over 50 years ago, only 5 imprinted genes were known in flowering plants as recently as 2011. Since 2011 advances in transcriptome sequencing have enabled us to identify hundreds more imprinted genes in Maize, Rice, and Arabidopsis, however most of these studies have evaluated imprinted expression at just one time point in development. In this study, we evaluated imprinted expression at four timepoints throughout endosperm development to identify transiently and consistently imprinted alleles as well as identify patterns of imprinting based on whether the allele is ever expressed when inherited in the opposite parental genome. In total, we find 725 maternally expressed genes and 323 paternally expressed genes that are imprinted at one or more timepoints. To better understand dynamics of imprinting, we split our genes into three patterns based on imprinting call changes across time. Some genes lose imprinting due increased expression from the opposite parent, some genes gain imprinting due to decreasing expression, and many genes maintain uni-parental expression across time points. We next compared these patterns of imprinted expression to genes identified as imprinted in nine additional maize lines and found a link between the pattern of imprinted expression across development and the conservation of imprinted expression throughout the maize lines assessed. Finally, this study allowed us to evaluate imprinting for zeins, which are starch accumulation proteins that are expressed at very high levels in late endosperm development. We found three zeins that are imprinted, suggesting that imprinted expression may shape expression of agronomically important genes.

Nod1-dependent NF- κ B activation initiates hematopoietic stem cell specification in response to small Rho GTPases

Xiaoyi Cheng

Molecular, Cellular, and Developmental Biology

Uncovering the mechanisms regulating hematopoietic specification not only would overcome current limitations related to hematopoietic stem and progenitor cell (HSPC) transplantation, but also advance cellular immunotherapies. However, generating functional human induced pluripotent stem cell (hiPSC)-derived HSPCs and their derivatives has been elusive, necessitating a better understanding of the developmental mechanisms that trigger HSPC specification. Here, we reveal that early activation of the Nod1-Ripk2-NF- κ B inflammatory pathway in endothelial cells (ECs) primes them to switch fate towards definitive hemogenic endothelium, a pre-requisite to specify HSPCs. Our genetic and chemical embryonic models show that HSPCs fail to specify in the absence of Nod1 and its downstream kinase Ripk2 due to a failure on hemogenic endothelial (HE) programming, and that small Rho GTPases coordinate the activation of this pathway. Manipulation of NOD1 in a human system of definitive hematopoietic differentiation indicates functional conservation. This work establishes the RAC1-NOD1-RIPK2-NF- κ B axis as a critical intrinsic inductor that primes ECs prior to HE fate switch and HSPC specification. Manipulation of this pathway could help derive a competent HE amenable to specify functional patient specific HSPCs and their derivatives for the treatment of blood disorders.

Definition of regulatory elements and transcription factors controlling immune cell gene expression at single cell resolution using single nucleus ATAC-seq

Pengxin Yang, Ryan Corbett, Lance Daharsh, Juber Herrera Uribe, Kristen A. Byrne, Crystal L. Loving, Christopher Tuggle

Department of Animal Science, Iowa State University, 2255 Kildee Hall, Ames, IA, 50011, USA.

USDA-ARS, NADC, 1920 Dayton Ave, Ames, IA, 50010, USA.

Correspondence: cktuggle@iastate.edu

The transcriptome of porcine peripheral blood mononuclear cells (PBMC) at single cell (sc) resolution is well described, but little is understood about the cis-regulatory mechanism behind scPBMC gene expression. Here, we profiled the open chromatin landscape of porcine PBMC using single nucleus ATAC sequencing (snATAC-seq). Using clustering based on open chromatin pattern similarity, we demonstrate that cell type annotations using snATAC-seq are highly concordant to that reported for sc RNA sequencing. The differentially accessible peaks (DAPs) for each cell type were characterized and the pattern of accessibility of the DAPs near cell type markers across cell types was similar to that of the average gene expression level of corresponding marker genes. Additionally, we found that peaks identified in snATAC-seq have the potential power to predict the cell type specific transcription starting site. We identified both transcription factors (TFs) whose binding motif were enriched in cell type DAPs of multiple cell types and cell type specific TFs by conducting transcription factor binding motif (TFBM) analysis. Furthermore, we identified the putative enhancer or promoter regions bound by TFs for each differentially expressed gene (DEG) having a DAP that overlapped with its transcription start sites by generating cis-co-accessibility networks (CCAN). The regulator TF-target DEG pairs predicted through TFBM analysis for each CCAN were largely consistent with the results reported in the ENCODE Transcription Factor Targets Dataset. This snATAC-seq approach provides insights into the chromatin accessibility landscape of porcine PBMCs and enables discovery of TFs predicted to control DEG through binding regulatory elements.

An Engineered Variant of *E. coli* Nissle Unveils Enhanced Transformation Efficiency and Versatility in Probiotic Engineering

Chinmayi R Gudi, William J Neilson, Thomas J Mansell

In recent years, the spotlight has increasingly focused on the gastrointestinal microflora, driving scientific, veterinary, and medical research interest. Consequently, probiotics, as live biotherapeutic agents, have garnered substantial attention. Among these, *E. coli* Nissle (EcN), a non-pathogenic gut isolate bacterium, has gained significant popularity. However, a formidable bottleneck in harnessing EcN's potential as a probiotic has been its poor transformation efficiency, relative to other bacterial strains. In this study, we present a novel engineered strain of EcN, developed through adaptive laboratory evolution, showcasing a remarkable enhancement in transformation efficiency. This new strain (provisionally patented) has been comprehensively characterized in comparison to the wildtype EcN, through assessments of fitness, growth under gut-mimicking duress conditions, and membrane study assays. Since EcN is known to compete with pathogenic strains in the gut for iron, the competition dynamics and iron consumption of the strain were also significant factors which were evaluated. Furthermore, we conducted genome sequencing and comparative analysis, which along with gene ontology enrichment studies confirm the engineering of a most robust EcN strain with tremendous potential as a probiotic candidate. This development heralds a groundbreaking frontier in probiotic engineering, endowing EcN with superlative potential while preserving its biological functionality.

A Longitudinal Analysis of Biases in Function Annotation and Publication Mentions in the Human Proteome

An Phan, Parnal Joshi, Karin Dorman, Claus Kadelka, Iddo Friedberg

Gene Ontology (GO) annotation databases contain the knowledge of protein function, but they are biased towards the well-studied proteins that are of biomedical interest to researchers and funding agencies. Experiments annotating protein functions focus on these well-studied proteins, leaving many others under-annotated. To better understand these biases and how they change over time, we focused on GO annotations of human proteins, emphasizing on annotations coming from experiments. We examined the information content of annotations assigned to each protein, and analyzed the inequality in the knowledge distribution of proteins in the past decade. We also used the number of full publication equivalents (FPEs) of these proteins to capture their interest to researchers. We show that the inequality of knowledge among human proteins is high and does not decrease over time. Interestingly, we show that the gain in knowledge of proteins is negatively correlated with their initial knowledge quantified ten years ago, whereas the gain in interest is positively correlated with their initial interest. In other words, researchers kept on studying well-known proteins while results suggested that the knowledge of these proteins may have reached saturation. To mitigate the research bias towards well-studied proteins, we should shift our resources to studying and annotating moderately-annotated and less-annotated proteins. We hope our work shall ameliorate the "rich-get-richer" phenomenon that has persisted in human proteome studies for decades, especially among the studies of protein functions.

An investigation of Stat3 in Grna-driven myeloid differentiation

Abigail McCune

Genetics and Genomics

In studying blood development (hematopoiesis) and hematopoietic diseases, many researchers have focused on how genes contribute to malignant phenotypes rather than how these genes contribute to normal hematopoietic function. Thereby, leaving gaps in the understanding of healthy hematopoiesis and how it compares to malignant hematopoiesis. Here, we demonstrate how one gene studied in acute myeloid leukemia (AML), *signal transducer and activator of transcription (stat3)*, functions in normal myelopoiesis in the zebrafish model. We utilized morpholino knockdown, chemical inhibition, and mutant knockout of *stat3* to study the effect of Stat3 on myeloid populations. Loss of Stat3 reduced both primitive and erythroid-myeloid progenitor (EMP)-derived neutrophil and macrophage populations. However, the loss of Stat3 also resulted in an increased immature EMP population. Using motif predictive software and CUT&RUN, we also found that Stat3 binds to the *granulin a (grna)* gene, previously identified as necessary for neutrophil and macrophage differentiation. A connection between Stat3 and Grna is also supported by the knockdown and knockout of *grna*, which produces a phenocopy of the Stat3 myeloid phenotypes (Campbell et al., 2021). Cumulatively, our results indicate that Stat3 drives Grna expression, which is necessary for 1) the development of the primitive neutrophil and macrophage populations and 2) the differentiation of the EMP population into terminal neutrophils and macrophages. Our findings provide new possibilities for utilizing GRN to treat AML by forcing the differentiation of immature myeloblasts into terminal identities.

Improving analysis and interpretation of single-cell sequencing approaches.

Sabrina Rutledge^{1,2}, Arun Seetharam^{1,3}, Geetu Tuteja^{1,2}

1: Genetics, Development, Cell Biology Department, Iowa State University, Ames, IA

2: Bioinformatics and Computational Biology Program, Iowa State University, Ames, IA

3: Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, IA

Single-cell RNA sequencing (scRNA-seq) has revolutionized the field of genetics, recovering nuanced transcriptional profiles that can be lost with whole-tissue RNA sequencing. Despite the advantages, challenges remain in the analysis, interpretation, and reproducibility of data generated by scRNA-seq. For example, the lack of data reporting standards impedes analysis reproducibility. While these factors are often ignored, they can impact both the number and composition of cell clusters and thus potentially the conclusions drawn. Additionally, the cluster identities are often labelled based on the expression of only a few genes, which is a time-consuming and biased approach. Our goals were to: (1) perform a parameter sweep with the most commonly used scRNA-seq pipeline to identify parameters that impact cell cluster composition; (2) develop an automated tool for labeling cluster identities; and (3) establish recommendations for reporting scRNA-seq data that improve reproducibility. Of 20 parameters tested, three impacted overall results, increasing the number of clearly-defined trophoblast clusters. Ten parameters had a minor impact in data labeling without impacting the overall conclusions. Six parameters only impacted data visualization. We developed a tool, *celltypeEnrich*, to annotate cell clusters. Given a set of input genes, *celltypeEnrich* calculates enrichment of cell-specific genes for cell-types across 24 fetal and adult human tissues. We found that *celltypeEnrich* outperforms other related tools, especially when annotating single-cell data generated from the placenta. In summary, considering parameter choice in data analysis, developing user-friendly tools, and establishing clear criteria for data reporting will make results from scRNA-seq approaches more robust and reproducible.

Trust in nutritional science is moderately high but depends on political and religious beliefs

Nicole Kling MS, Katie Dentzman PhD, Lorraine Lanningham-Foster PhD

To describe how trust in nutritional science is moderated by demographics such as political and religious beliefs and to compare trust in nutritional science to other scientific fields that study our food system.

Methods: We surveyed 400 Americans. Respondents answered 5-point Likert-items (1 = completely distrust, 5 = completely trust) about trust in different fields of sciences (i.e., nutrition, food, agriculture, and environment). Besides trust, we also measured potentially moderating sociodemographic factors and beliefs (i.e., politics, religion) that previous research on trust in science have shown to be relevant. Preliminary analysis includes descriptive statistics (means (+SD)) and tests of the trust scales' internal consistency with Cronbach's alpha.

Results: Trust in nutritional science (3.83 (+0.86)) was comparable to trust in food (3.85 (+0.84)), agricultural (3.98 (+0.84)), and environmental (4.02 (+0.94)) sciences. However, the most politically liberal and non-religious individuals reported higher levels of trust in all sciences (range from 3.96 (+0.84) to 4.55 (+0.43)) compared to the most politically conservative and highly religious individuals (range from 2.57 (+0.84) to 3.58 (+0.98)). Our scales of trust in science have good internal consistency based on Cronbach's alpha scores which ranged from 0.85 to 0.92.

Conclusions: Trust in the field of nutritional science is moderately high and consistent with other agri-food-related scientific fields. Religious and political beliefs are important predictors of trust, where more politically liberal and non-religious individuals report higher trust in science. Future research should explore differences in trust depending on the practitioner of nutritional science (i.e., scientist, nutritionist, dietitian, etc.)

A comparison of protein function annotations of model species to orthologous proteins in humans

Parnal Joshi^{1,2}, **Qi Li**³, **Ido Friedberg**^{1,2}

1. Program in Bioinformatics and Computational Biology
2. Department of Veterinary Microbiology and Preventive Medicine, Iowa State University
3. Department of Computer Science

The accurate annotation of the biological function of proteins is an open problem in life science. While improvements in sequencing techniques have facilitated novel data generation, translating sequence data into biologically meaningful functions remains a challenge. Comprehensive knowledge of protein function is crucial to understanding life's mechanisms and their evolution and also for practical applications such as characterizing disease genotypes and phenotypes, identifying antimicrobial resistance, and selecting targets for drug design. Although the best means, experimental assays are resource-intensive and limited to certain species, leading us to rely on model organisms to infer protein function in humans. However, the contribution of model organisms to function annotations has not yet been systematically quantified. In our study, we developed a framework for comparing Gene Ontology annotations between species using information content to indicate the specificity of an annotation to any given species. Our analysis shows the species-specificity of experimentally determined annotations in humans surpasses the sum of contributions from model species. Next, we measured the functional similarity between annotations of orthologous proteins across species. Consistent with previous studies, we observed no correlation between function similarity of experimental annotations and sequence divergence across model organisms, highlighting the limitations of individual biological systems. Individual model systems contribute only a partial understanding of protein function. We conclude that experimental annotations across species are complementary rather than redundant, and that a state-of-the-art assessment of the annotation landscape will allow us to identify avenues for experimental characterization in model organisms and provide suggestions for biocuration.

Development and Quantitative Analysis of a 3D Scaffold-Based Model for Simulating Tumor Microenvironment Using Light Sheet Microscopy

Yuwei Zhang, **Sriram Vijendran**, **Alina Kirillova**, **Oliver Eulenstein**, **Jing Wang**

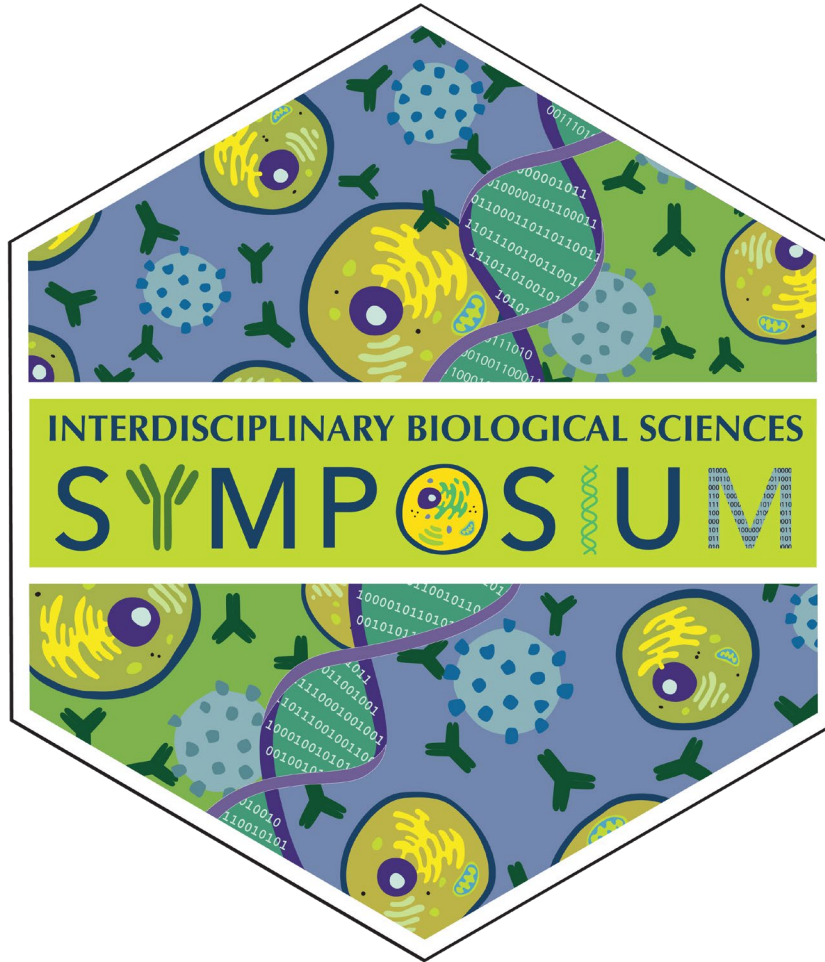
2D and 3D imaging techniques have been utilized to assess the efficacy of in vitro biological models. Among various 3D imaging methods, light sheet microscopy stands out for its depth penetration, making it suitable for examining larger and more complex samples. Despite these advances, quantitatively evaluating the effectiveness of biological models, particularly those simulating tumor angiogenesis, remains challenging. In this study, we introduce a transparent, biocompatible 3D scaffold designed to model tumor angiogenesis and extravasation. Initially, human umbilical vein endothelial cells (HUVECs) are cultured on the scaffold to monitor growth kinetics. Once the HUVEC layer becomes confluent, collagen and collagen infused with Lewis lung carcinoma cells (LL2) are introduced atop the HUVEC monolayer, respectively. This is intended to mimic the process by which tumor cells intravasate through the extracellular matrix into blood vessels. After that, all samples are fixed for imaging with light sheet microscopy. Through the application of Arivis4D for quantitative analysis, we observed that the growth kinetics of HUVECs on the 3D scaffold are consistent with those seen in 2D cultures. Moreover, the presence of LL2 in the conditional medium promotes the movement of LL2 towards the HUVEC layer, indicating interaction between tumor cells and endothelial cells. In summary, our study successfully establishes a model that simulating tumor angiogenesis and cancer cell intravasation. This model, combined with light sheet microscopy, offers a new approach for studying tumor dynamics in a controlled environment.

Detection of Gene Expression Mid-parent Heterosis with Single Biological Replicate

Yunhui Qi, Peng Liu

Department of Statistics, Iowa State University

Mid-parent heterosis (MPH) is a phenomenon in which the traits of hybrid offspring significantly diverge from the mean traits of their parents. A critical aspect of this divergence is seen in gene expression levels, making the detection of genes that exhibit MPH essential for uncovering the genetic mechanisms underlying heterosis of phenotypic traits. Traditional Bayesian methods for detecting MPH genes rely on multiple biological replicates per genotype and unrealistic hyper-parameter assumptions, which are particularly problematic in the context of multi-family RNA-seq experiments, where typically hundreds of genotypes but only a single biological replicate per genotype is available. Addressing these challenges, our study introduces a novel two-stage likelihood ratio test (2sLRT) leveraging a negative binomial distribution, conditional on precisely estimated dispersion parameters, to identify MPH genes in multi-family RNA-seq experiment with single biological replicate. Our method employs Poisson likelihood ratio test to detect null families with equal genotype means, followed by hierarchical clustering and local polynomial regression for robust dispersion parameter estimation. Validation through rigorous simulation studies and the application to an extensive maize dataset of 599 families highlights the 2sLRT's superior capability in maintaining a low false discovery rate and significantly improving the accuracy of MPH gene detection. This performance notably surpasses that of traditional methods for MPH detection based on the Poisson distribution. Our method offers a practical solution for the genetic research on heterosis, enabling accurate identification of MPH genes under the constraint of limited biological samples, thus providing valuable insights into the genetic basis of mid-parent heterosis.



Student Poster Presentation Abstracts

Morning Poster Session

Impact of butyrate on short-chain fatty acid receptor expression in porcine monocytes

Sage R. Becker and Crystal Loving

The short-chain fatty acid (SCFA) butyrate is a microbial-produced metabolite associated with enhanced mucosal immunity and increased barrier integrity. Despite many studies investigating the impact of SCFAs on pig health and production, the function of SCFA receptors and their role in the immunomodulatory activities of butyrate is minimally characterized in pigs. In particular, blood monocytes likely migrate into the intestine and differentiate in response to local cues, including butyrate and LPS. To identify whether SCFA receptors are present on porcine monocytes and the impact of butyrate on SCFA receptor expression by monocytes, porcine blood-derived monocytes were cultured with LPS +/- butyrate for 4h. Under basal conditions, SCFA receptors FFAR2, HCAR2, and FFAR3 were all expressed by peripheral monocytes. HCAR2 had the highest expression when compared to FFAR2 and FFAR3. Butyrate exposure did not alter FFAR3 expression but did increase expression of HCAR2 and FFAR2. Butyrate alone (1 mM) increased FFAR2 expression over monocytes exposed to LPS only. Additionally, compared to LPS alone, co-stimulation of monocytes with LPS and butyrate (0.25 mM) slightly decreased HCAR2 expression; whereas LPS with 1mM butyrate increased HCAR2 expression. Co-stimulation of low and high doses of butyrate with LPS increased FFAR2 expression compared to LPS alone. Overall, our data suggests butyrate can modulate peripheral blood monocyte SCFA receptor expression, and expression is further modulated by LPS exposure. Understanding how SCFA receptors are expressed and how butyrate affects SCFA receptor responses in peripheral blood monocytes is important for understanding how SCFAs modulate intestinal health in pigs.

Keywords: porcine, butyrate, monocytes, SCFA receptors

First detection of PCV4 in the United States and its association with PCV2 and PCV3 coinfection

Molly Kroeger, Diana S. Vargas-Bermudeza, Jairo Jaime, Julian Parada, Phil Gauger, Pablo Piñeyro

Porcine circovirus 4 (PCV4) was first reported in Chinese pigs in 2019 displaying respiratory and enteric disease. Since, PCV4 has been reported in South Korea, Thailand, and Spain. Currently, no studies report the presence of PCV4 in the US. Therefore, the objectives were to determine the detection rate of PCV4 in the US and its association with PCV2 and PCV3 codetection.

512 clinical samples were obtained from the ISU Veterinary Diagnostic Laboratory from June-September 2023 from lung ($n=100$), feces ($n=100$), spleen ($n=100$), serum ($n=100$), lymphoid tissue (lymph node and tonsil, $n=64$), and fetus ($n=48$) samples. Nucleic acid extracts were tested by PCV4 qPCR and PCV2/3 qPCR.

PCV4 was detected in 8.6% of the total samples with detection occurring in lung (9%), feces (5%), spleen (9%), serum (10%), and lymphoid tissue (17.2%). The overall average PCV4 Ct was 33 and the average Ct between different sample types was not significantly different. Of the PCV4 positive samples, most were only positive for PCV4 (52.3%). However, some PCV4 positive samples had codetection of PCV2 (31.8%), PCV3 (6.8%), and PCV2/3 (9.1%). Reported clinical signs in cases where PCV4 was detected most commonly included enteric and respiratory disease in 2-17-week-old pigs. Coinfections were reported in 90% of the PCV4 positive cases.

PCV4 was detected for the first time in the United States. Given the relatively high detection rate in cases with PCV2, PCV3, and other coinfections, future studies should address the role of PCV4 in a coinfection model to determine the effect on clinical disease.

Low to moderate doses of dietary resistant starch and a renin-angiotensin system inhibitor exhibit nephroprotective effects through similar mechanisms in a preclinical model of diabetes

Claudia Carrillo, Claudia Arriaga, Rachel O'Brien, Annie Balk, Frances Loeffelholz, Grace Wood, Matthew Rowling

The investigative goal of our study was to explore the mechanisms of nephroprotection of a drug-diet intervention for type II diabetes (T2D) in a pre-clinical rat model. Our working hypothesis proposed that a combination of low-to-moderate doses of dietary resistant starch (RS) and renin-angiotensin system (RAS) inhibitor would decrease renal biomarkers associated with the progression of T2D through the modulation of the renal RAS. Zucker Diabetic Fatty (ZDF) rats (n=48) were randomly assigned to one of three diets: AIN-93G diet with 0% RS (control), AIN-93G with 5% RS or 10% RS, which were adjusted at the expense of cornstarch. They were also randomly assigned to daily intraperitoneal (IP) injections of either captopril (cap) (0.25 mg/kg of body weight) in a saline and ascorbic vehicle or placebo solution, which consisted of just the vehicle. Zucker lean rats (n=7) were assigned to the control diet and daily placebo injections, for eight weeks. ZDF 10% RS cap group had a decrease of 62.5% in urinary protein creatinine ratio, 29% of urinary albumin, and 18% of 25-hydroxy vitamin D (25D) levels, compared to the diabetic control group, while also exhibiting an increase of 62% in serum 25D levels. The 5% RS placebo group exhibited a significant decrease in relative abundance of kidney injury molecule-1. Our results suggest that a combination of dietary RS and cap similarly help protect kidney integrity in T2D, evidenced by a decrease of biomarkers in the urine and increased expression of key proteins of the RAS and kidney structure.

Identifying key regulators of uterine function during pregnancy

Kelby Kies, Geetu Tuteja

During early pregnancy, the tissue lining the inside of the uterus must transform into a new tissue called the decidua to support embryonic growth and formation of the placenta. If the development of the decidua is compromised, complications can occur often resulting in pregnancy loss. Although individual genes underlying decidua development have been identified, their interactions are not clearly established. Therefore, it is vital to define the molecular mechanisms regulating decidua development to prevent early pregnancy complications.

The goal of this project is to identify genes, regulatory networks and transcription factors that regulate the two processes that are essential for development of the decidua: decidualization and angiogenesis. To achieve this goal, we generated transcriptomic and epigenetic data from the mouse decidua at two time points in early pregnancy.

Using the transcriptomic data, we first performed a differential expression analysis between the two timepoints and built protein-protein interaction networks (PPI). Next, we used these networks to predict novel genes that could have a role in each process. Lastly, we integrated the epigenetic data into each PPI to identify novel transcription factors that are regulating genes previously known in each process. Future experiments will be conducted to validate these genes' roles in decidualization and angiogenesis. The findings of this project will help researchers understand the complex biological processes necessary for decidua development and contribute to the overall goal of preventing pregnancy complications.

A genome-wide association study of stress hormone levels in hair of young healthy pigs

Fazhir Kayondo, Hayder Al-Shanoon, Yolande M. Seddon, David Janz, Dylan Cayette, Carmen Cole, Frederic Fortin, John C. S. Harding, Michael K. Dyck, Graham Plastow, Pig Gen Canada, and Jack C. M. Dekkers

Levels of cortisol, cortisone, DHEA, and DHEA-S in the hair from the backs of 863 young and healthy Landrace x Yorkshire F1 barrows are being evaluated for their potential as genetic indicators of resilience to infectious and non-infectious stress in pigs. Estimates of heritability for the concentrations ranged from 0 to 0.33. Genetic correlation between hormone concentrations and responses to a 30 s back-test ranged from low to high. Cortisol was highly genetically correlated with the number of struggles and with the intensity of vocalizations. A genome-wide association study for the hormone concentrations was performed, and a 1 Mb window that explained > 1% of the genetic variance was considered a QTL. This was expanded 2 Mb up- and downstream to constitute a 5 Mb QTL region (QR). Candidate genes in the QRs were identified with BioMart using the Sscrofa11.1 genome build. No QTL was identified for cortisone. A QR on SSC2 harboring the NR3C1, SRA1, and HDAC3 genes explained 45.3% of the genetic variance for cortisol and 1.4% for the CL/DS ratio. A QR on SSC3 harboring the NCOA1 and FKBP1B genes explained 3.0% of the genetic variance for DHEA-S and 6.7% for the CL/DS ratio. A QR on SSC5 explained 1.4% of genetic variance for the CL/DS ratio and harbors the ENSSSCG00000056452 and EMP1 genes. These results highlight the genetic control of response to non-infectious stress in young healthy pigs. More work will look at the relationship between these stress hormones levels and resilience to a polymicrobial challenge.

Fatty acids inferred from milk spectral are predictive of feed intake in lactating Holstein cows

Leonora M. James, Mary S. Mayes, Cori J. Siberski-Cooper, Juan P. Steibel, Francisco Peñagaricano, James E. Koltes

Feed production contributes greatly to dairy cattle sustainability. A solution is to breed for more feed efficient animals. Feed intake data from individual animals is required to determine feed efficiency, but unfortunately data collection is cost prohibitive on large scale. This limits genetic improvement since less data means lower prediction accuracy compared to breeding values for other traits. Thus, cheaper, and scalable indicator traits for feed intake are needed. Millions of dairy cows are routinely milk tested but the spectral data associated with these tests are underutilized. The goal of this study was to evaluate the use of milk data to predict feed intake. Fatty acids inferred by Fourier-Transformed Infrared Spectrometry from weekly milk tests were collected on 357 cows. Multiple statistical methods were evaluated to predict feed intake, including linear models, random forest, and partial least squares. Predictive performance was evaluated in a 4-fold cross-validation. The best prediction model was the linear model that included parity, contemporary group, days in milk, and body and milk energy sinks, resulting in an R^2 of 0.85 and a concordance correlation coefficient of 0.92. Alternative models were further evaluated to determine the feasibility of predicting feed intake without the use of variables not regularly collected on farm, such as body weight and body condition score. Integration of genomic relationships was also explored. Those models performed similarly to the full models. Results indicate, milk spectra data could be used to predict feed intake phenotypes or as an indicator trait to genetically improve feed efficiency.

Effects of corn-resistant starch consumption along with intake of ACE inhibitor, captopril, on metabolic syndrome biomarkers in a type-2 Diabetes rodent-model.

Claudia Lorena Arriaga

Objectives: Evaluate the effects of a nutritional intervention, with resistant starch (RS) accompanied by injections of captopril, on metabolic syndrome biomarkers in a type-2 Diabetes rodent-model.

Methods: Six-week-old, Zucker diabetic fatty rats-ZDF and lean rats (n=56), assigned randomly to experimental and control groups, were kept in individual plastic cages under a 12-h-light/-dark cycle for 8 wks, consuming feed and water ad libitum. Three ZDF-groups were fed with 0%, 5%, and 10% RS, all receiving captopril injections; three ZDF-groups with the same RS interventions, receiving placebo injections; one group of lean rats, fed with 0% RS, receiving placebo injections. Animals were put in metabolic cages 12 h. before euthanized; whole blood was obtained by cardiac puncture; biomarkers were analyzed in serum using commercial or analytical essays. Mann-Whitney tests and descriptive statistics were conducted.

Results: RS groups, regardless of captopril or placebo injections, showed attenuated FBG average values in comparison to the 0%RS + placebo group that showed the highest average value, 594 ± 26.3 mg/dl, indicating an effect of RS on this biomarker. Data showed that groups receiving captopril injections showed TG's values that were 60-70% lower than those of their counterparts with placebo injections.

Conclusions: RS seems to have attenuated FBG levels in all groups with RS. Captopril showed positive effects on decreasing TG's levels when compared with their counterparts that didn't receive captopril injections. Insulin resistance indicators were not conclusive on whether RS or captopril are modulating insulin levels.

Characterizing the Activating Transcription Factor 1 (ATF1)

Manuela Chaves Mejia, Tracie Hennen-Bierwagen, and Julien Roche

The Activating Transcription Factor 1 (ATF1) is a member of the CREB/ATF family of bZip transcription factors. The CREB/ATF family is involved in the expression of stress-related genes that regulate cell growth, metabolism, and survival. Despite their association with several types of cancer (such as lung cancer, clear sarcoma, and colorectal cancer, among others), the molecular mechanisms underlying CREB/ATF activation and regulation are not well understood. The CREB/ATF members share a common mechanism of activation through phosphorylation of a conserved serine residue and a mechanism of gradual inactivation by multiple-phosphorylation of a serine cassette. Remarkably, ATF1 uniquely possesses an additional phosphorylation site, implicated in the recruitment of Pin1, a prolyl isomerase that has been targeted for cancer therapies due to its high expression levels in cancer cells. Despite its crucial functional importance, the molecular basis of Pin1 recruitment by ATF1 remains yet elusive. Through biochemical techniques, such as Nuclear Magnetic Resonance (NMR), Fluorescence Polarization (FP), and Intact Mass Spectrometry (IMS), along with Molecular Dynamic (MD) simulations, this project aims to elucidate the molecular mechanisms of activation and regulation of ATF1, its interaction with DNA, and protein dynamics, data that will allow to understand ATF1 as a unique target for further cancer therapies.

Mosquito immune cells enhance dengue and Zika virus dissemination in *Aedes aegypti*

David R. Hall, Rebecca M. Johnson, Hyeogsun Kwon, Zannatul Ferdous, S. Viridiana Laredo-Tiscareño, Bradley J. Blitvich, Doug E. Brackney, Ryan C. Smith

Mosquito-borne viruses are a rapidly increasing threat to public health, causing more than 400 million annual infections and placing over half of the world's population at risk. Despite this importance, the mechanisms by which arboviruses infect the mosquito host and disseminate to tissues of importance are not well understood. Herein, we provide evidence that mosquito immune cells, known as hemocytes, play a critical role in the dissemination of dengue virus (DENV) and Zika virus (ZIKV) in the mosquito *Aedes aegypti*. Utilizing a chemical approach to deplete phagocytic hemocytes, we demonstrate that these immune cell populations have little influence on midgut virus infection and instead facilitate virus dissemination to the ovaries and salivary glands, essential tissues required for virus transmission. Immunofluorescence experiments show that phagocytic hemocytes are a focal point for virus infection and are capable of attaching to ovary and salivary gland tissues, suggesting a role in virus dissemination in the mosquito host. Through additional transfer experiments, we show that virus-infected hemocytes can transmit virus to non-infected mosquitoes, demonstrating that hemocytes are an important location for virus infection and enhance dissemination. Taken together, our data support a model of virus dissemination in the mosquito host where infected hemocytes transport virus through the hemolymph to tissues required for arbovirus transmission. This study significantly advances our understanding of virus infection dynamics in mosquitoes and the integral role of hemocytes in mosquito vector competence for DENV and ZIKV.

EpicTope: narrating protein sequence features to identify non-disruptive epitope tagging sites

Joseph Zinski, **Henri Chung**, Parnal Joshi, Finn Warrick, Brian D. Berg, Greg Glova, Maura McGrail, Darius Balciunas, Iddo Friedberg, Mary Mullins

Epitope tagging is an invaluable technique enabling the identification, tracking, and purification of proteins *in vivo*. We developed EpicTope, a tool that integrates predictions of protein structure, solvent accessibility, disordered binding regions and sequence conservation to predict optimal sites for epitope tagging, without disrupting function. We performed a computational assessment of EpicTope predictions on a set of pathogenic and benign non-frame insertion human gene mutants. Our results show EpicTope identifies benign mutants with 0.76% accuracy, comparable to other pathogenic indel mutation predictors. Notably, unlike other methods, EpicTope identifies multiple locations in a protein where potential insertions are non-disruptive to function. We then tested the efficacy of EpicTope empirically and predict suited tagging sites for Smad5 in Zebrafish. We constructed zebrafish Smad5 variants with V5-tags at two internal sites, along with N- and C-terminal V5-tags for comparison. We show that internally tagged Smad5 can rescue zebrafish smad mutant embryos, while the N- and C-terminal tagged Smad5 cannot. We also show that the internally tagged smad5 is accessible to antibodies for immunohistochemistry. We then that internally tagged Smad5 proteins are nuclearly localized in the presumptive ventral region of the zebrafish gastrula. We have released EpicTope as an R package. Our work provides an accessible tool for predicting optimal epitope-tag sites and validates its predictions both in computational and empirical systems.

Electrical Stimulation Effects on Adult Rat Hippocampal Progenitor Cell Behavior

Catherine Fonder, John Swanson, Gabrielle Sawin, Metin Uz, Surya K. Mallapragada, Donald S. Sakaguchi

Neurodegenerative diseases and brain injury often result in significant neuronal loss and cognitive deficits. Current treatment strategies show limited success and often lead to life-long disabilities, reduced quality of life, and heavy socio-economic burdens. Developing methods to replace lost and compromised neural cells relies on new advances in tissue and cellular engineering strategies. Electrical stimulation (ES) approaches applied *in vitro* to direct the differentiation of neural stem cells (NSCs) have potential applications for nervous system rescue and repair. In this study, adult rat hippocampal progenitor cells (AHPCs) were cultured on 3D printed, biodegradable, electrically conductive PLA/graphene scaffolds to determine the effects of electric field stimulation on the viability, proliferation, and differentiation of the AHPCs. These multipotential AHPCs can differentiate *in vitro* into neurons, oligodendrocytes, and astrocytes, the three primary central nervous system cell types, making them ideal for use in NSC differentiation experiments. The circuit design within the scaffolds was composed of an interdigitated pattern, which allowed for the application of ES within spatially defined areas of the scaffold. Varying stimulation voltage and duration showed parameter-dependent stimulation effects on AHPC differentiation without affecting cell viability. Stimulation at 125 mV for 10 min. each day for 7 days resulted in significant increases in the differentiation of AHPCs into immature neurons and oligodendrocytes compared to no or lower stimulation voltages. Stimulation for 15 min. a day resulted in significantly higher oligodendrocyte differentiation compared to controls. These results demonstrate the potential use of ES for cost-effective and efficient selective NSC differentiation.

Cross-Species Sperm Proteomic Analysis for Human Male Fertility Model Establishment

Mubashrah Mahmood and Karl Kerns

Male fertility is a complex process regulated by thousands of protein-encoding genes. As transcriptionally silent cells, spermatozoa are reliant on post-translational modifications occurring upon leaving the male. A proteome is a constellation of all the proteins present in the cell and its subcellular structures. Owing to ethical limitations for human use as a research subject, the mouse has served as a model for human fertility for decades, though the genomic sequences coding for proteins (1.5% of the complete genome) in mice are only approximately 70% the same as a human. A cross-species sperm proteomic analysis was conducted to determine a relevant animal model for human fertility studies. For this, published sperm proteome data of boar (n=2), bull (n=5; *bos taurus* = 4, *bos indicus* = 1), buffalo (n=1), mouse (n=3), and human (n=4) were used for the comparison. Combining datasets and removing duplicates within species yielded 4092 boar, 2133 bull, 2147 buffalo, 4641 mouse, and 7554 human sperm proteins. NCBI protein BLAST function was then used to compare the proteome of each species against the human sperm proteome. Of all the proteins analyzed, each species had the following mean percent identity to the human sperm proteome (median in parenthesis): boar 85.1% (89.9%), bull 83.7% (90.2%), buffalo 84.5% (90.6%), and mouse 76.4% (84.4%). The results indicate that livestock species are a more suitable model for human sperm/male infertility research than mouse at the proteome level. Supported by the National Institute of Food and Agriculture, U.S Department of Agriculture, grant 2022-67015-36298 (KK).

The Role of GAUT Proteins in *Arabidopsis thaliana* Root Development

Allison R. Triebe, Zach Steinmetz, Damilola Olatunji, Dior R. Kelley

Cell wall dynamics are regulated during root development through the activity of cell wall modifying enzymes. However, how cell wall composition is modulated in root stem cell populations to influence organogenesis is not well understood. The pectin modifying enzyme GALACTURONOSYLTRANSFERASE 10 (GAUT10) has been shown to be involved in both primary root elongation and cell division, as *GAUT10* mutants have a short root phenotype that is dependent on sucrose. GAUT proteins can form complexes and may work in concert with one another. Gene expression mining indicates that *GAUT3*, *GAUT8*, *GAUT10* and *GAUT11* are all enriched in root stem cell populations. Through bimolecular fluorescence complementation (BiFC) assays, it appears that GAUT10 can interact with GAUT3, GAUT8, and GAUT11 in the Golgi apparatus. To test the overlapping function of these GAUT proteins during root development, mutant combinations of these four *GAUT* genes have been made. Preliminary phenotyping done so far has shown that these genes have non-redundant and epistatic interactions. Specifically, *gaut11-3* roots are longer compared to wild-type (WT) and loss of *gaut3* and *gaut11* can suppress the short root phenotype of *gaut10*. These results provide fresh insight into how closely related *GAUT* genes influence root development. Continued investigations will examine the impact of *GAUT8* and cell wall composition changes in the absence of these *GAUT* genes in a combinatorial fashion.

Biomedical Imaging Datasets using PA as the Ground Truth

Katherine Gisi, Paige Sparber, Manojit Pramanik

Background - Deep learning (DL) for biomedical imaging is a dynamic research field encompassing countless clinical practices. However, there remains ubiquitous systematic challenges. One of which is the tediousness of dataset annotation for multitudinous systems and procedures. One such DL-aided procedure involves ultrasonic (US) needle-tracking. This minimally invasive technique is used for peripheral nerve blocks, tumor biopsies, and fetal blood sampling. Better technology could even increase the range of biological and medical applications.

Methods - Our approach pairs US with photoacoustic imaging (PAI). PAI is an emerging imaging modality which is readily used in tandem with US. PAI has the capability to identify surgical tools such as needles and offers a higher spatial resolution. Some research pairs the two methods for final clinical translation, but we are exploring a different route: the use of a neural network trained on PAI images to identify needles on US equipment.

Results - Our outcomes are twofold. First, we propose a dataset preparation pipeline. This automates the process of transforming recorded US signal to a robust dataset. A 24 second US/PAI data acquisition produces up to 1280 images and their corresponding masks. Second, preliminary results from networks based on DeepLabV3 and a UIU-Net (using the automatically annotated datasets), exhibit sufficient accuracy for segmentation of needles.

Conclusion - Developing a translatable dataset creation pipeline can remove the need for resource-consuming annotation and system characterization. In future applications, enhanced US imaging could be performed apart from PAI equipment on any given system and multiple different regions of interest.

Afternoon Poster Session

Photothermal Effects on Inflorescence Development in *Poa bulbosa*

Prathyusha Cheguri, Shui-Zhang Fei

The transition from prairies to annual crops with a short growing season renders the land barren during the fallow period. This practice leads to sustainability challenges with the loss of many ecosystem services. Planting annual cover crops can eliminate the fallow period, but they result in complications in planting and harvesting time of the cash crop and require significant labor and seed costs. Planting perennial groundcover (PGC) with cash crops provides continuous soil cover and supports intensive agricultural production with less management compared to annual cover crops. However, this interplanting introduces a unique challenge as PGCs can compete with cash crops for critical resources during the growing season, potentially reducing grain yields. Two methods can manage this competition: chemical suppression of PGCs or using summer dormant PGCs to eliminate the need for chemicals. *Poa bulbosa*, with its summer dormancy trait, could be an ideal PGC that can eliminate competition with cash crops. Summer dormancy in *P. bulbosa* is mostly regulated by the internal biochemical signals and is genetically controlled. It reproduces vegetatively as bulbils in the seedhead and bulbs at the base of the plant, and sexually through seed. This species is native to Eurasia. When introduced to this country, the reproductive pathway is altered, and it mostly reproduces vegetatively. Understanding the environmental cues for true flower and seed formation is crucial for controlled hybridization in breeding *P. bulbosa*. This study explores the impact of photothermal conditions on inflorescence development across six unimproved USDA *P. bulbosa* accessions- PI314172, PI317470, PI314301, PI314162, PI254907, PI226526. All the accessions are allowed to grow in the growth chamber set up at 15°C/11 hr. Simulating the natural photothermal conditions of average of September and October 2022. After there is sufficient vegetative growth, the first set of the plants were moved to 21°C/8 Hr., 21°C/16Hr., 24°C/8 Hr., 24°C/16 Hr., 27°C/8 Hr., 27°C/16 Hr. growth chamber treatments, (without vernalization treatment) to check for seed heads formation. Subsequent sets of plants were vernalized at 5°C/8h for 2, 4 and 6 weeks. Six-week vernalization treatment and 24°C/16h flower initiation treatment is ideal for inflorescence development, true sexual flower and true seed formation. The future aim is to elucidate the pollination behavior of *P. bulbosa*, whether it is predominantly self-pollinated or cross-pollinated. To understand the impact of pollination methods on seed production by analyzing under different conditions (bagged and unbagged seedheads). These results can further be utilized for future breeding purposes to establish a good PGC- based cropping system.

Incorporating gene network information into the prediction of maize flowering time

DeTemple, Joseph; Li, Dongdong; Muszynski, Michael; Li, Xianran; Yu, Jianming

The flowering time in crops is a critical trait for consistent agricultural performance and developing lines for specific environments. Due to the complex quantitative genetic architecture of flowering time, prediction of diverse lines in new environments is difficult. Much of this complexity arises from the large network of genes and regulatory elements that interact to hasten or slow the transition from vegetative to reproductive growth in the developing maize plants. These interactions, detected through either functional genetics studies with knockout mutants or quantitative genetics studies with natural genetic diversity, contain information critical to understanding flowering time differences between different maize lines. Using gene network information in a predictive context will shift prediction methods towards a systems biology approach, where individual alleles cannot be considered separately from the gene network and genetic background. Although including expression data from over 50 genes in a single model, for example a static mixed-effect model or a dynamic gene regulatory network model, may not be feasible, it is important to consider that in individual maize lines, a unique subset of genes within the network may be able to capture a majority of the trait variation and be sufficient for accurate prediction of flowering time. Simplified gene networks, based only on genes segregating in the genetic background of interest, can incorporate gene network information into modeling approaches without requiring extremely complicated, comprehensive models. Incorporating gene network information within prediction models will also increase the interpretability of results by connecting to functional processes at the cellular level. Genes involved in the flowering time network have diverse functions, including light receptors, transcription factors, and enhancer elements, and the significance of each of these categories in the prediction step can give insight into the biological consequences of gene interactions. The tropical inbred lines CML277 and Tzi8 are given as examples of how network information can be used in predicting the flowering time of maize lines significantly different than the standard reference line B73.

The MARS community: a synthetic community to identify rules governing maize rhizosphere microbiome assembly

Ashley Paulsen, Marissa Roghair Stroud, Dua Vang, Hannah Burkhardt, Larry J Halverson

Abstract:

The maize rhizosphere microbiome is a complex network of interactions between plants, microbes, and soil that can influence plant health. We developed the MAize Rhizosphere Synthetic community (MARSc) to facilitate identifying the rules governing microbiome assembly. MARSc, is comprised of 31 bacterial strains, representing 4 pPhyla and 20 families, was isolated from roots of maize grown in Iowa soils with low inorganic nitrogen (N) inputs, and includes the model rhizosphere colonist *Pseudomonas putida* KT24420. MARSc composition is based on the observed phylogenetic diversity of the maize rhizosphere and on members' interactions with KT2440. MARSc-inoculated maize has more ramified root systems than uninoculated maize in both sterile and non-sterile systems, with the effect being greatest in low-N fertilized conditions. Compared to untreated plants, 28-day old MARSc-treated maize grown in low-N soil also had significantly greater biomass than untreated. Interaction networks are being developed to identify relationships governing MARSc members behaviour and function. One pairwise network identifies bacteria whose growth or behavior is modified by interactions with another species, illustrating context-dependent stimulatory, inhibitory, and neutral interactions. We are exploring how root metabolites influence inter-species and plant-microbe interactions on biofilm formation are being investigated to assess how they may affect root colonization. Moreover, de novo genome assemblies of MARSc members are being mined for traits that could contribute to plant growth promotion, stimulatory or inhibitory interactions, and rhizosphere colonization. These results provide the foundation for using MARSc to identify rules of microbiome assembly.

Design and optimization of a robust CRISPR interference system to identify *Pseudomonas putida* genes essential for rhizosphere microbiome formation

Marissa Roghair Stroud, Dua Vang, Ashley Paulsen, and Larry J. Halverson

The rhizosphere microbiome is essential to plant growth and health, but relatively little is known about the mechanisms by which these microbiomes form or the interactions that occur within. We are interested in identifying the genes used for rhizosphere microbiome assembly and maintenance by the model rhizosphere colonist *Pseudomonas putida* KT2442. We designed a CRISPR interference (CRISPRi) expression system optimized for KT2442 using the native promoter system XylS/PM, which has tunable expression with low levels of background. We have experimentally validated our CRISPRi expression system using the genes *ftsZ* (cell division, essential) and *pvdH* (siderophore synthesis, non-essential). We have shown that CRISPRi of *ftsZ* is functional in the maize rhizosphere each during the onset of *P. putida* root colonization and after *P. putida* has colonized the root, both leading to decreased root colonization. We have created the MAize Rhizosphere Synthetic Community (MARS-C), a community of bacteria representative of roots of maize grown in Iowa soils, and we identified organisms within this community who are able to stimulate/inhibit the growth of *P. putida* or vice versa. When the gene *pvdH* is repressed and *P. putida* is unable to produce pyoverdine, it loses its ability to inhibit other microbes *in vitro*, indicating that pyoverdine production is a key contributor to *P. putida*'s inhibitory interactions. These results provide the foundation on which to use CRISPRi-seq to identify *P. putida* genes that contribute to microbiome assembly and maintenance in the maize rhizosphere.

Identification of distinct proteome differences between fresh and cryopreserved bull spermatozoa

Alexandra Keller, Emma Keller, George Perry, and Karl Kerns

Keywords: sperm, bull, proteomics, cryopreservation

Abstract:

Semen cryopreservation of high-quality semen from high genetic indexing sires has become standard practice in the livestock industry. Allowing for accelerated genetic improvement around the world and the ability to cover more females per ejaculate. One drawback to these procedures is that they could compromise the fertilization competency of sperm cells. We used a bottom-up proteomic approach to monitor proteomic changes before and after cryopreservation and identify differentially abundant protein candidates for biomarkers to better understand sperm cryopreservation factors and the effects on spermatozoa maturation through capacitation. Semen from five bulls was used for protein extraction before and after cryopreservation. Proteins from sperm pellets were extracted and submitted for MS analysis at the Iowa State University Proteomics Core. A total of 2411 proteins were identified between both treatments. Of those, 402 were unique to the fresh replicates, and 57 were unique to cryopreserved replicates. Principle component and cluster analysis revealed 154 proteins with differential abundances ($p < 0.05$). We found 106 proteins less abundant and 48 proteins more abundant in cryopreserved samples. PANTHER molecular and biological function analysis revealed the following functions as the most prominent: catalytic activity, binding, cellular processes, metabolic processes, and response to stimulus. This data emphasizes the effects that cryopreservation has on semen quality by showing that cryopreserved sperm have a distinctly different proteome than fresh sperm and that the processes that are affected change metabolic and cellular processes that could affect spermatozoa ability to undergo capacitation, bind the oviductal sperm reservoir, and ultimately its ability to fertilize.

Supported by the National Institute of Food and Agriculture, U.S Department of Agriculture under grant number 2022-67015-36298 (KK) and Multistate Hatch Project 9835.

Single-nuclei RNA-Sequencing: heterogeneity of striatal astrocytes and the effect of stress

Beatriz B. Pereira, Trevor J. Buhr, Meghan G. Connolly, Lynna Chu, Justin S. Rhodes, Zachary V. Johnson, Peter J. Clark, Elizabeth M. McNeill

Our group has shown that exposing rats to a single episode of unpredictable tail shocks can result in a persistent reduction in physical activity, outlasting well-characterized depression- and anxiety-like behaviors by at least a month. To that end, young adult male SD rats were housed in cages with locked running wheels for one week. Half of the rats were exposed to a single episode of 100 uncontrollable tail shocks (stress), and the remaining half were left undisturbed in home cages (no stress). Forty-eight hours later, the running wheels were unlocked for half of the rats in the acute stress (stress running, $n=6$) and no stress conditions (no stress running, $n=6$), which received free access to running wheels for 42 days. Running wheels remained locked for the other half of the rats in the acute stress (stress sedentary, $n=6$) and no stress conditions (no stress sedentary, $n=6$). On the final day of wheel access, rats were sampled. Single-nuclei RNA-sequencing (snRNA-seq) was performed in rat striatum to identify cell-specific molecule events. Here, we conducted a detailed analysis of changes to the expression of genes within the astrocyte cell population, as astrocytes can reflect the overall physiological conditions of the striatum in response to stress. Results suggest that five genetically disparate populations of astrocytes can be derived in the striatal area. Within these populations, 68 differentially expressed genes (DEGS) were found due to stress exposure, involved in processes related to mitochondrial function, sleep regulation and circadian rhythm, angiogenesis, and oxidative phosphorylation.

Identification of Hemogenic Endothelial Cells Through a Multi-omics Approach

Anthony Sillman, Xiaoyi Cheng, Rodolfo Calderón, Abigail McCune, Masuma Khatun Usha, Radwa Barakat, Clyde Campbell, and Raquel Espín-Palazón

The processes contributing to the development of hematopoietic stem and progenitor cells (HSPCs) are still not fully established. A comprehensive understanding of the genes and components necessary for this process would provide the groundwork for developing HSPCs from human induced pluripotent stem cells. This could overcome current barriers to safe and effective HSPC transplantation. Here, we utilize a multi-omics approach to identify novel functions for genes not previously implicated in HSPC development. Single-cell RNA sequencing along with bulk RNA sequencing and publicly available human protein and RNA expression databases are used to identify candidate genes. The most promising candidate genes include *cxc5b*, *mef2cb*, *slc9a3r1a*, and *cdh6*. Prior research has shown these genes to be involved in non-hematopoietic roles such as osteoblast differentiation, cardiomyocyte differentiation, cancer cell autophagy, and kidney development. To confirm whether the candidate genes have a substantial effect on the differentiation of endothelial cells into hemogenic endothelial cells and eventually into HSPCs, we utilized whole mount *in situ* hybridization and confocal imaging of transgenic lines of zebrafish.

Detection of Brain Midline Shift using 2D and 3D Convolutional Neural Networks

Laura Zinnel and Sarah A. Bentil

Traumatic brain injury (TBI) is a prevalent neurological disorder that can have life-long impacts, and quick diagnosis of TBI can play an important role in the effectiveness of treatment. A common sign of TBI is midline shift. The brain midline, which divides the hemispheres of the brain, is normally positioned down the center of the skull. However, in cases of injury or build-up of intracranial pressure, the midline can become shifted from this center position. This is called midline shift (MLS). MLS can be used as an indicator of the severity of TBI, and it can be used as a predictor of the outcome of injury. Computerized Tomography (CT) scans are normally used by radiologists to check for MLS in a patient's brain. However, this process is time consuming, so it is desirable to develop a method to automatically detect MLS from CT scans.

In this study, both a 2D and 3D convolutional neural network (CNN) were trained to detect MLS of more than 5 mm from head CT scans. The results of the models were compared to help understand how to develop a deep learning model for the detection of MLS, which will speed up the process of diagnosis of patients with TBI and lead to faster treatment.

Characterization of a zebrafish model of MYC-driven acute myeloid leukemia

Anna M. Lucianò, Juan-Francisco Rodríguez-Vidal, Miriam Fernández-Lajarín, María L. Cayuela, Diana García-Moreno, Victoriano Mulero

Background: Acute myeloid leukemia (AML) is a clonal malignancy of the stem cell precursors of the myeloid lineage associate with uncontrolled proliferation and impaired differentiation of hematopoietic stem and progenitor cells. The main causes are genetic and epigenetic variations that led to neoplastic changes and clonal proliferation. Among the different genes found amplified in AML, the oncogene MYC is one of the prognostic factors with an impact of AML and several type of cancer. Albeit MYC is a well know factor associated with the uncontrolled proliferation of AML, the mechanism that led to the oncogenesis established by MYC are not well known. In the last years zebrafish has emerged as an attractive model organism for studying cancer development because of its genetic accessibility.

Methods: After the generation of a transgenic zebrafish line expressing the human MYC under the control of the neutrophils-specific promoter lysozyme (*lyz:hMYC*), we proceeded with flow cytometry analysis of adult kidney marrow of *lyz:hMYC* fish. Immunohistochemical analysis of whole zebrafish has been made in order to check the infiltration of cells and possible metastasis. May-Grunwald stain was made to check the kidney marrow population. Gene expression profile of the whole kidney marrow has been performed to check the expression of human MYC and the endogenous *myc* as well as other genes associated with the MYC pathway and the ability of the cells to proliferate. Metagenomic analysis has been conducted to search for possible variations in the intestinal microbiota that may be associated with the disease causing its progression.

Results: Flow cytometry analysis of *lyz:hMYC* zebrafish revealed the expansion of myeloid cells with a significant reduction of erythroid cells, which may be associated with anemia, a characteristic trait of patients with AML. The immunohistochemical analysis of whole *lyz:hMYC* zebrafish showed the infiltration of myeloid cells into different organs confirming that those cells are able not only to proliferate unconditionally but also to metastasize. May-Grunwald analysis showed a higher number of blast cells that were unable to differentiate towards mature granulocytes in *lyz:hMYC* zebrafish than in their wild type siblings. The gene expression profile of whole kidney marrow demonstrated not only the overexpression of exogenous MYC, but also of endogenous *myc*, suggesting an autoregulatory positive regulation of MYC. Metagenomic analysis revealed that the gut microbes of AML fish were significantly altered with the possibility to be associated the progression of AML.

Conclusion: The model developed here is an excellent tool to further understand the mechanisms involved in MYC-induced AML and the relevance of the gut microbiota on this disease.

Multi-environment multivariate genome wide association studies identify candidate loci underlying cuticular wax accumulation and composition on maize silks.

Wendt, Matthew, Hattery, Travis J; Hirsch, Candice; Yandea-Nelson, Marna

As global temperatures rise, precautions must be taken to mitigate the adverse effects of drought on water retention rates in crops. The cuticle is a hydrophobic layer that coats all aerial plant tissues. It consists of a matrix of cutin that is coated by and intercalated with very long chain cuticular waxes to form a barrier that limits rates of non-stomatal water loss and protects against other environmental stresses. We recorded the concentrations of forty-seven very long chain cuticular wax metabolites (hydrocarbons, fatty acids, fatty alcohols) from the silks of 448 inbreds of the Wisconsin Diversity panel, each grown in replicate across three year-by-location environments (Iowa and Minnesota, 2016-2017). Total accumulation varied 17-fold, with hydrocarbon, fatty acid, and fatty alcohol constituents displaying 18-, 23-, and 99-fold variation, respectively. To better understand the genetic mechanisms underlying cuticular wax variation, genome wide association studies (GWAS) were conducted for each trait individually. Further, we conducted multivariate GWAS using multiple wax traits or traits from multiple environments to uncover loci responsible for pleiotropic control of the cuticular wax profile. Combined, GWAS and multivariate GWAS yielded two-hundred unique, high-quality candidates, accounting for a high level of genetic variance across all measured traits; among these candidates are known stress responsive transcription factors and hormone receptors, as well as enzymes involved in wax biosynthesis. These candidate genes could be implemented directly into breeding programs or targeted engineering to ameliorate drought stress in silks, thereby improving pollen reception of the silks.

Arabidopsis as a model for spontaneous haploid genome doubling in maize

Kassidy Sullivan

Arabidopsis Thaliana is a common weed with a unique property of spontaneous haploid doubling. As the demand for maize increases in global markets, efficient production is critical. Traditionally having a laborious breeding process, the need for fertile and rapid generations is the goal of haploid induction and genomic doubling. Genome doubling leads to a 100% homozygous generation after one breeding. The methods used are haploid inducers are used to create double haploids. After identifying fertile siliques, the mutant genes and mutant seeds are processed to identify how to trigger and select for the mutations and increase the seed yield. Then, crossing the mutants allows for a more significant haploid evaluation.

By identifying haploid fertility genes, arabidopsis, and maize can move further in the pipeline for double haploid technology, reducing costs and labor and increasing the efficiency of double haploid technology.

Anomaly detection for breed purity analysis

Xiaohan Jiang

Recent advancements in genotyping technologies have revolutionized our ability to estimate breed composition in pigs. The classic compositional regression used requires a multibreed panel to detect crossbreeding and its application is limited to breeds in the panel. In this study, we presented a protocol based on classic anomaly detection algorithms that use single breed panel to detect crossbreeding. Our dataset consisted of 44616 SNP genotypes recorded in purebred Large White, Landrace, Duroc, Pietrain (N=2000 from each breed) and 1010 crossbred pigs (50% Pietrain, 25% Large White, 25% Landrace). 1500 Large White pigs were randomly selected as reference set. The remaining 500 Large White pigs, other purebred pigs, crossbred pigs, and simulated crossbred animals of known proportion of Large White segments were test sets. We applied three anomaly detection methods: 1) Principal Component Analysis (PCA). 2) deep learning autoencoders (AE) 3) Phasing and imputation (Imputation). For PCA the top 600 eigen vectors capturing 85% cumulative proportion of variance in the reference set were selected. Test set genotypes were projected onto the principal components and then they were reconstructed to the full genotype set. For AE, we utilized the model trained in the reference set to reconstruct test sets. For imputation, SHAPEIT 5 and IMPUTE 4 were applied to reconstruct test sets. Accuracy of reconstruction of genotypes was computed using correlation and mean square error. The proportion of rejected genotypes was used to assess the ability of anomaly detection algorithms to detect crossbreeding. Results showed Imputation provided better properties to detect genomic outliers compared to PCA and AE.

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1st Annual Biological Sciences Oral and Poster Winners

April 2023

Oral Presentation

1st place - David Hall

2nd place - Marissa Roghair Stroud

3rd place - Ha. T.H. Vu

Poster Presentation

1st place - Sahiba Siddiqui

2nd place - Samuel Shobade

3rd place - Anurag Das & Colleen Yanarella

People's Choice

Oral Presentation - Jaspreet Rishi

Poster Presentation - Mehak Kapoor

Creative Arts Showcase - Prathyushi Cheguri

Special Recognition

Joan Meis and Anna Schwake

Lauter Award for Graduate Student Creativity and Research Excellence

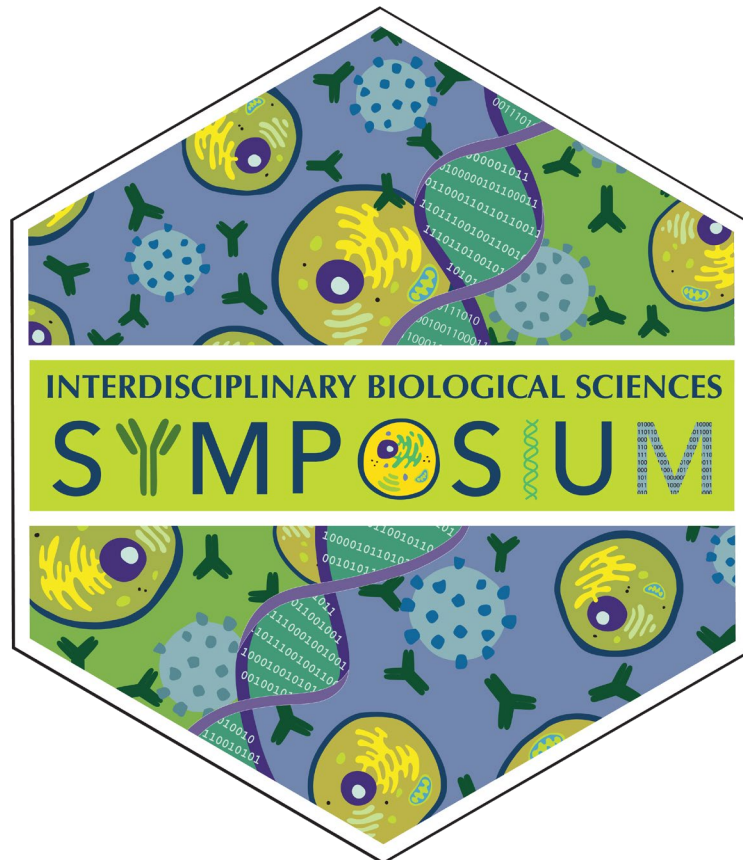
Clayton Carley and Swathy Krishna

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Looking forward to next year and hope to see you there.

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Plant Pathology, Entomology and Microbiology (PPEM)
Raymond F. Baker Center for Plant Breeding
Statistics
Veterinary Microbiology and Preventative Medicine (VMPPM)
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