19TH ANNUAL

GRADUATE STUDENT SYMPOSIUM 2024

April 30, 2024

Program & Abstract Booklet

Sponsors

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Human Health & Nutritional Sciences

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Research and Graduate Officer, CBS

Interactive Table of Contents

Schedule of Events

10:00 – 11:00 am ***Concurrent Sessions***

Session 1: Biochemistry [SSC 3317]
Session 2: Knowledge Translation [SSC 1304]
Session 3: Pathogens/Disease [SSC 1306]

11:00 – 12:00 pm ***Concurrent Sessions***

Session 4: Physiology [SSC 1304]
Session 5: Genetics/Genomics [SSC 3317]
Session 6: Ecology [SSC 1306]

12:00 – 1:00 pm ***Lunch***

Complimentary lunch served in Waasamawin (formerly the SSC Atrium)

12:30—1:00 pm ***Plenary Speaker***

Dr. Emma Allen-Vercoe (MCB)

1:00 – 2:15 pm ***Keynote Speakers*** [Waasamawin, formerly the SSC Atrium]

Dr. Mike Tymko (HHNS)
Dr. Angela Scott (MCB)
Dr. Andy Turko (IB)

2:30 – 3:30 pm ***Concurrent Sessions***

Session 7: Cell Biology [SSC 1306]
Session 8: Nutrition/Metabolism [SSC 2315]
Session 9: Neuroscience [SSC 3317]
Session 10: Proteomics [SSC 1511]

3:30 – 5:00 pm ***Poster session*** [Waasamawin, formerly the SSC Atrium]

Odd numbered posters – Session A 3:30 – 4:15 pm
Even numbered posters – Session B 4:15 – 5:00 pm
Appetizers will be served
Bartender will serve domestic beer ($6), premium beer ($7), coolers ($7), wine ($6), soft drinks ($2), and other non-alcoholic drinks ($2).

Plenary Speaker

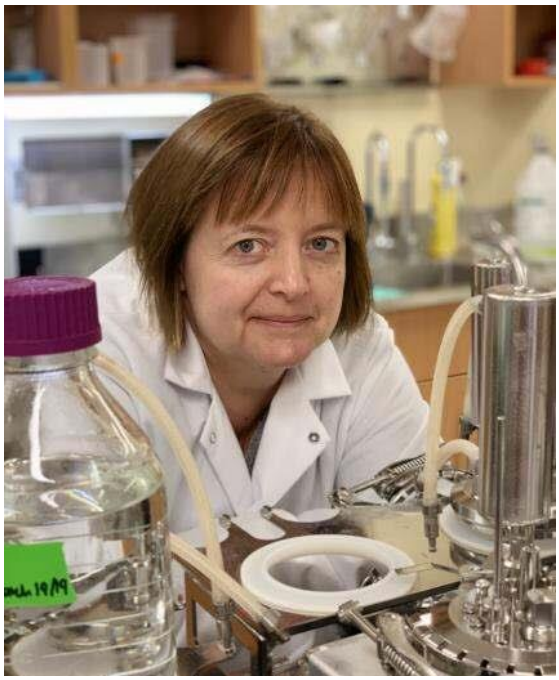
Dr. Emma Allen-Vercoe

Molecular and Cellular Biology

I began my research career with undergraduate and graduate studies at the Central Veterinary Laboratories (now Veterinary Laboratories Agency) and the Centre for Applied and Microbiological Research (CAMR, now the Health Protection Agency), UK, under the direction of Prof. Martin Woodward. There, I studied the enteric pathogen Salmonella enterica serovar Enteritidis, and developed a sound appreciation of the many obstacles that a enteric pathogen must overcome in the gut in order to cause disease. I became fascinated by the huge arsenal of virulence factors required by enteric pathogens in order to survive and proliferate in the gut environment.

I spent a brief postdoctoral period at CAMR, learning to work with technically challenging pathogens such as *Mycobacterium tuberculosis* and *Campylobacter jejuni*, before I relocated to Canada in 2001 to start a postdoctoral position at the University of Calgary, under the joint direction of Drs. Rebekah DeVinney and Mike Surette. Here I worked on Enteropathogenic and Enterohemorrhagic *E. coli* (EPEC and EHEC), using cell and molecular biology techniques to probe the fascinating interactions of their type III secretion systems with host cells.

I had always been interested in learning more about the normal microbial population inside the human gut, and in 2004 I was fortunate enough to win a Fellow-to-Faculty Transition award through the Canadian

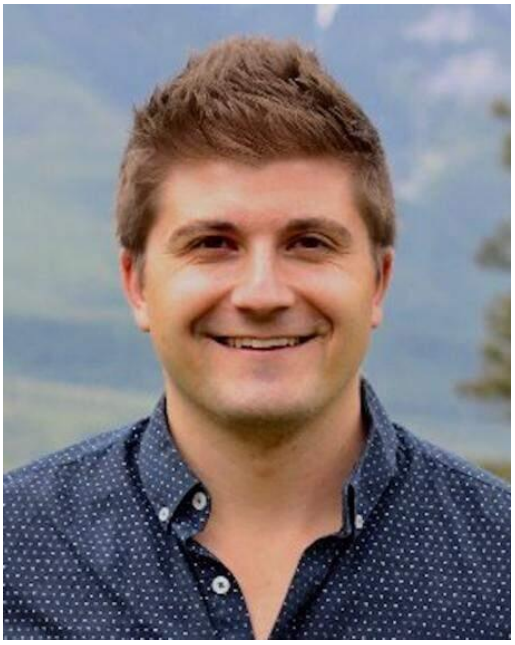


Association of Gastroenterology. This award allowed me to develop an independent research program aimed at the study of the normal human microbiota and its influence on human health and disease, a program that I brought with me to Guelph in December 2007.

Since I've been here, with thanks to my very talented staff and students I have built a world-class anaerobic microbiology facility and directed it both towards answering fundamental research questions, and to create translational opportunities to move the science into the clinic. In 2013, I co-founded NuBiyota, a company whose mission is to develop "Microbial Ecosystem Therapeutics" to treat disorders that have gut microbial dysbiosis as a root cause.

Keynote Speakers

This year's GSS 2023 theme for the keynote addresses is "Journey Through Academia". We have a professor from each of our three departments who will be discussing their own unique stories from their research training up until becoming a professor.



Dr. Mike Tymko

Human Health and Nutritional Sciences

My research interests in environmental and cerebrovascular physiology began when I was an undergraduate student at Mount Royal University under the supervision of Dr. Trevor Day. As an undergraduate, I participated on a research expedition to the Nepal Himalaya where our team conducted a series of human physiology experiments. This experience led me to completing my MSc under Dr. Glenjamin Foster where I studied the effects of changes in oxygen and arterial blood tension on respiratory function, and my PhD with Dr. Philip Ainslie where I studied the impact of high altitude on peripheral and cerebrovascular function.

After the completion of my graduate training, I completed a postdoctoral fellowship in Dr. Craig Steinback's laboratory at the University of Alberta exploring the relationship between autonomic nervous activity and cerebrovascular function, and then a second postdoctoral fellowship with Dr. Mypinder Sekhon at the University of British Columbia where I continued this line of work in mild brain injury (i.e., concussion). My graduate and postdoctoral training shaped the theme of my current research program at the University of Guelph. In my laboratory, we use various methods to evoke alterations in autonomic nervous activity (e.g., hypoxia, carbon dioxide, orthostatic stress, thermal stress) to explore the link between these autonomic stressors and the regulation of brain blood flow in health and disease.

Dr. Angela Scott

Molecular and Cellular Biology

Neural communication and the intricate choreography of signals required for the formation and preservation of neural function is heavily dependent on the interactions between neurons and glial cells (myelinating cells, astrocytes, microglia). While neurons provide the essential wiring within the nervous system, glial cells are responsible for a myriad of functions that determine the quality, maintenance, and re-growth of these connections. Given the cellular diversity of the nervous system, uncovering the roles of glial cells is integral to understanding basic neurobiological function and the pathology of many neurological disorders. In the lab, we take an integrative and comparative approach to study glial-neuronal interactions in relation to early development, physical injury, and neurological disease that spans across multiple levels of biological organization (molecular and cellular physiology > systems biology) and multiple model species (zebrafish and mice).

Dr. Andy Turko

Integrative Biology

Turko Comparative Conservation Physiology Lab opened its doors at the University of Guelph in 2024. Prior to this date, I was an NSERC Postdoctoral Fellow at Wilfrid Laurier University, where I worked with Jonathan Wilson. Before that, I was an Eastburn Postdoctoral Fellow at McMaster University where I worked with Graham Scott and Sigal Balshine, and a ReNewZoo Postdoctoral Fellow at the University of Windsor where I worked with Trevor Pitcher. I completed my PhD at the University of Guelph under the supervision of Patricia Wright.

Instructions for Presenters

All oral presentations are **10-12 minutes** long with **3-5 minutes of questions**.

In-person oral presentations

- Please email your presentation to the chair of your session by Monday April 29th.
- Please arrive at least 5 minutes before the start of the session.

Poster presentations

- See page 22 for your poster number
- Please hang up your poster before 1:00 pm
- For odd numbered posters: Please stand by your poster from 3:30 pm – 4:15 pm
- For even numbered posters: Please stand by your poster from 4:15 pm – 5:00 pm

Concurrent Session Schedule

Concurrent Sessions 1-3
10:00 – 11:00 am
[in-person]

	Session 1	Session 2	Session 3
Session Theme	Biochemistry	Knowledge Translation	Pathogens/ Disease
Room	SSC 3317	SSC 1304	SSC 1306
Chair	Isaac Sullivan isulliva@uoguelph.ca	Liz Manchester emanches@uoguelph.ca	Nicole Notaro nnotaro@uoguelph.ca
Presenters			
10:00	Bradley Fitzgerald	Nida Ansari	Noah Keuhfuss
10:15	Allison Leonard	Elizabeth Porter	Hayley Smith
10:30	Victoria Butler	Sidney Evans	Jennifer Holborn
10:45	Amelia Doerksen	-	Andrey Petropavlovskiy

Concurrent Sessions 4-6
11:00 am – 12:00 pm
[in-person]

	Session 4	Session 5	Session 6
Session Theme	Physiology	Genetics/Genomics	Ecology
Room	SSC 1304	SSC 3317	SSC 1306
Chair	Alexa King aking17@uoguelph.ca	Isaac Sullivan isulliva@uoguelph.ca	Victoria Butler vbutler@uoguelph.ca
Presenters			
11:00	Liz Manchester	Charles Sanchez	Kendra Gardner
11:15	Nicole Fletcher	Laura Thompson	Chris Bender
11:30	Barbara Hyde-Lay	Marissa Sim	Derin Calik
11:45	Lily Hopkinson	Ashley Fisher	-

Concurrent Sessions 7-10
2:30 – 3:30 pm
[in-person]

	Session 7	Session 8	Session 9	Session 10
Session Theme	Cell Biology	Nutrition/ Metabolism	Neuroscience	Proteomics
Room	SSC 1306	SSC 2315	SSC 3317	SCC 1511
Chair	Rachel Handy rhandy @uoguelph.ca	Brittany MacIntyre bmacin02 @uoguelph.ca	Sidney Evans sevans05 @uoguelph.ca	Ella Parkinson eparki02 @uoguelph.ca
Presenters				
2:30	Thomas Kunjumon	Joshua Budd	Aimee Dawe	Barret Foster
2:45	Puja Puspa Ghosh	Patrick McTavish	Amanda Wiseman	Stevan Cucic
3:00	Kirsten Berry	Nadine Abraham	Coleman Olenick	Boyan Liu
3:15	-	Anna Kupraty	Jayson Capistrano	Chelsea Reitzel

Concurrent Session Summaries

Session 1: Biochemistry

10:00 am – Bradley Fitzgerald

The Regulated Production of Medium-chain Fatty Acids by Butyrate- fermenting Lachnospiraceae

Pathways for the microbiota's medium-chain fatty acid (MCFA) production have been enigmatic. Here, we demonstrate that Lachnospiraceae produce valerate and hexanoate through their butyrate-fermentation pathway and that MCFA production is regulated by exogenous short-chain fatty acids. Furthermore, encoding two variants of the Acetyl-CoA acetyltransferase enzyme correlates with hexanoate production.

10:15 am – Allison Leonard

Unsaturated Fatty Acids Inhibit Staphylococcus aureus Adhesion to Host Ligands

Staphylococcus aureus colonizes ~30% of the population. Unfortunately, current decolonization strategies are no longer effective. To tackle this problem, I have identified a compound exhibiting broad-spectrum anti-adhesive activity. This compound attenuates *S. aureus*' adhesion to numerous host ligands by preventing the expression and surface presentation of cell-wall anchored adhesins.

10:30 am – Victoria Butler

Glucan-linked phosphate reduces the affinity of glycogen branching enzymes for polyglucans in vitro

Living organisms store energy as complex polyglucans, which are branched by branching enzymes (BEs). Glucan-bound phosphate, found in starch and glycogen, has been found to alter BE substrate affinity. Our research aims to understand how phosphate impacts BE affinity, influencing polyglucan chain length and branching degree.

10:45 am – Amelia Doerksen

Regulation of axonal transport in neuron by protein palmitoylation of p150glued

One mechanism to regulate neuronal protein trafficking is through the covalent addition of fatty acids to cysteine residues, a process known as palmitoylation. We recently demonstrated palmitoylation of the dynein activating complex dynactin subunit p150Glued. Although the functional role of p150Glued palmitoylation is unknown, it may regulate fast axonal transport.

Session 2: Knowledge Translation

10:00 am – Nida Ansari

Contextualizing the learning environment of undergraduate engineering students

To ensure that we are training engineering students to be creative designers, it is important to first understand what their current educational climate looks like. This presentation discusses the Contextual Engineering (CEng) framework and how we may incorporate it into engineering education today to train the innovative engineers of tomorrow.

10:15 am – Elizabeth Porter

(Re)imagining a Manufactured Ecosystem

Climate change necessitates solutions for the degradation of ecosystem services. Technocentric manufactured ecosystems are reminiscent of science fiction, while an approach that supports existing natural systems is more sustainable. The Manufactured Ecosystems project provides an opportunity to explore exciting possibilities for innovation and uncomfortable truths associated with addressing climate change.

10:30 am – Sidney Evans

Glucan-linked phosphate reduces the affinity of glycogen branching enzymes for polyglucans in vitro

Living organisms store energy as complex polyglucans, which are branched by branching enzymes (BEs). Glucan-bound phosphate, found in starch and glycogen, has been found to alter BE substrate affinity. Our research aims to understand how phosphate impacts BE affinity, influencing polyglucan chain length and branching degree.

Session 3: Pathogens/Disease

10:00 am – Noah Kuehfuss

Overcoming intrinsic antibiotic resistance mechanisms in *Escherichia coli*

The intrinsic resistance mechanisms of *Escherichia coli* render many of the clinically used antibiotics ineffective. I discovered the novel adjuvant activity of the natural product, kanosamine. Studies I have completed demonstrates that kanosamine potentiates rifampicin against pathogenic *E. coli* in a more unconventional method.

10:15 am – Hayley Smith

ShcD adaptor protein modulates EGFR signalling and invasion in breast cancer cells

Triple-negative breast cancers lack effective therapies due to high metastasis rates. Adaptor protein ShcD is upregulated in these tumours, correlating with poor patient survival. ShcD enhances EGFR phosphorylation, promoting invasion in traditional in vitro and novel cerebral organoid models. Targeting ShcD-EGFR interactions with indomethacin reduces invasion, suggesting a therapeutic strategy.

10:30 am – Jennifer Holborn

Investigating the Anticancer Potential of Cannflavin A and B in Glioblastoma Multiforme

Cannflavin A and Cannflavin B, flavonoid derivatives from Cannabis Sativa with known anticancer properties, demonstrate potential against glioblastoma multiforme (GBM). Increasing doses of Cannflavins reduce cell viability, while low doses of both compounds hinder migration. Notably, Cannflavin B at low dose demonstrates significant anti-migratory and anti-invasive effects in GBM cells.

10:45 am – Andrey Petropavlovskiy

S-acylation as a regulator of localization and function of endoplasmic reticulum chaperone GRP78/BiP in glioblastoma

Glioblastoma cells can survive and proliferate under unfavourable microenvironment conditions that cause protein misfolding. This is due to upregulation of the ER-luminal chaperone GRP78 (BiP). We have found that GRP78 is modified by long-chain fatty acids and that this process is regulated by ER stress and could modify GRP78 function.

Session 4: Physiology

11:00 am – Liz Manchester

The functional and structural response of the zebrafish (*Danio rerio*) cardiovascular system to chronic hypoxia exposure

The current study focuses on how the cardiovascular system of zebrafish responds to exposure to seven weeks of chronic environmental hypoxia. We demonstrate that zebrafish remodel the cardiovascular system in response to hypoxia, as indicated by: improved performance in a test of cardiorespiratory capacity, reduced ventilation rates in hypoxic water, maintained heart function (measured using cardiac ultrasound), and upregulated hematocrit.

11:15 am – Nicole Fletcher

Sex Differences are not Present in Arteriolar Reactivity in Skeletal Muscle at the Microvascular Level

We investigated sexual dimorphisms in arteriolar reactivity to better understand how female microvascular networks coordinate blood flow responses to muscle contraction. We demonstrated that arteriolar reactivity to muscle contraction and the mechanisms involved in driving the vascular response to contraction were similar between males and females.

11:30 am – Barbara Hyde-Lay

The morphology and network architecture of pericytes in the skeletal muscle

Pericytes have been observed to be associated with the microvasculature in a variety of tissues. In the skeletal muscle, the network architecture and morphology of pericytes remains unknown. Our aim is to characterize both the types of pericytes present and their network structure in a variety of skeletal muscles.

11:45 am – Lily Hopkinson

Sex-differences in the cardiac response to splenectomy

Newly identified functions of the spleen (e.g., blood volume regulation and inflammatory modulation) may support optimal cardiovascular health, yet no studies exist characterizing the effect of splenectomy on the heart in healthy animals. Using echocardiography, invasive hemodynamics, and histology, we identified sex-specific consequences of splenectomy for cardiac structure and function.

Session 5: Genetics/Genomics

11:00 am – Charles Sánchez

Genome-wide association study (GWAS) for salmon rickettsial syndrome (SRS) resistance in rainbow trout using whole-genome sequence level imputed genotypes

Imputation consists of completing missing genotypes and correcting genotyping errors. Performing it from WGS reduces genotyping costs and mapping of disease-resistance trains. GWAS for using imputed WGS genotypes identified genes associated with resistance against *Piscirickettsia salmonis* in rainbow trout.

11:15 am – Laura Thompson

The next generation of the Efflux Platform: A genetic toolkit to study compound transport across the Escherichia coli cell envelope

Efflux pumps are intrinsic resistance elements that reduce the influx of antimicrobial agents. Escherichia coli harbours 35 putative efflux-encoding genes, and the collective efflux network habours overlapping activities and expression profiles. To overcome this, we generated an Efflux Platform to study efflux pumps in a highly deficient genetic background with normalized expression.

11:30 am – Marissa Sim

Genetic Manipulation of Non-Model *Blautia luti* Reveals a Novel Mechanism of Succinate Production

The mechanism by which *Blautia* produce succinate is currently uncharacterized. Until recently, this genus has been genetically intractable. Here, we implemented a genetic manipulation pipeline to knockout a gene in a putative succinate production pathway. To our knowledge, these represent some of the first reported mutants of *Blautia luti*.

11:45 am – Ashley Fisher

The roles Dbf4-dependent kinase, Chromatin assembly factor 1, and Topoisomerase interacting factor 1 in epigenetic regulation in *Saccharomyces cerevisiae*

DDK is a serine/threonine kinase with an essential role in DNA replication initiation. It also targets non-essential substrates, but these functions are largely uncharacterized. Using *Saccharomyces cerevisiae* as a model organism, this project will provide novel insight into DDK and its regulation of two chromatin-associated factors, CAF1 and Tof1.

Session 6: Ecology

11:00 am – Kendra Gardner

Changes in Lumpfish Diet with Size Inside Atlantic Salmon Sea Cages in Southern Newfoundland

Parasite management in Atlantic salmon aquaculture is important because they have become genetically resistant to chemical and nutritional controls, shifting focus to mutualistic biological controls such as lumpfish. My study assess the relationship between diet and cleaning efficacy in relation to lumpfish size using morphological species identification and DNA metabarcoding.

11:15 am – Chris Bender

No Stomach, No Problem: an Integrated Morpho-Molecular Approach to Assessing the Diets of the Cunner Wrasse, among Coastal, Nearshore Regions of Atlantic Canada

Cunner wrasse are a potential cleaner fish solution to problems in the aquaculture industry posed by pest organisms, yet minimal information on natural dietary variation exists. Using traditional morphological and modern dDNA metabarcoding analysis, we illuminate general dietary composition and delineate trends in sample similarity across different grouping factors.

11:30 am – Derin Calik

Identifying developmental windows of sensitivity to diluted bitumen exposure in sockeye salmon (*Oncorhynchus nerka*)

Early life stages of fish are sensitive to diluted bitumen; however, little is known about how the timing of exposure influences the magnitude and scope of toxicity. This study compares key fitness metrics in sockeye embryos exposed to low, realistic concentrations of water-soluble fractions of dilbit during discrete developmental windows.

Session 7: Cell Biology

2:30 pm – Thomas Kunjumon

Chloroplast-ER Membrane Contact Sites have functional implications for plastid morphology and behaviour

Membrane contact sites between the ER and chloroplasts may facilitate lipid transfer. A putative lipase, BnCLIP1 localizes at ER-chloroplast junctions, potentially mediating MCS formation. My ongoing research demonstrates the role of BnCLIP1 as a chloroplast-ER MCS marker and the physical consequences of plastid-ER connectivity in plastid movement and stromule formation.

2:45 pm – Puja Puspa Ghosh

Investigating the Role of Endoplasmic Reticulum (ER) in Plastid Division

Plastids multiply via binary division, facilitated by a protein Accumulation and Replication of Chloroplast 5 (ARC5). However, mechanisms of ARC5 recruitment to the plastid division site and separation of daughter plastids after division remain unknown. Time-lapse imaging-based observations strongly support the involvement of endoplasmic reticulum (ER) in both processes.

3:00 pm – Kirsten Berry

The *Staphylococcus aureus* esterase FmtA is required for proper wall teichoic acid D-alanylation and host ligand adhesion

The teichoic acid D-alanylation pathway is important for regulating *S. aureus* cell surface charge and enabling proper host-ligand adhesion. Here, we have demonstrated that the esterase, FmtA, is an essential component of this pathway, facilitating the removal of D-alanine from lipoteichoic acids for the transfer to wall teichoic acids.

Session 8: Nutrition/Metabolism

2:30 pm – Joshua Budd

A ketogenic diet with or without omega-3 long chain polyunsaturated fatty acids does not affect glucose homeostasis or skeletal muscle insulin response in rats

The ketogenic diet (KD) is high in fat and low in carbohydrates. The effects of the KD on skeletal muscle glucose homeostasis are controversial. KDs also lack omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs), which may benefit glucose homeostasis. When animals were pair fed, a KD with or without n-3 LC-PUFAs did not affect skeletal muscle glucose homeostasis.

2:45 pm – Patrick McTavish

Investigating the Role of Omega-3 Polyunsaturated Fatty Acids on Lipid Uptake in White Adipose Tissue: Current Knowledge and Future Directions

Omega-3 polyunsaturated fatty acids (n-3 PUFA) can influence whole body lipid metabolism through various mechanisms. Peroxisome proliferator-activated receptor-gamma (PPARγ) in particular, and its downstream genes, represent a major point of regulation . Our research aims to advance understanding of the molecular mechanisms underlying the benefits associated with n-3 PUFA.

3:00 pm – Nadine Abraham

Detoxification of the mycotoxin patulin by evolutionarily divergent reductases

Biodetoxification of the mycotoxin patulin by reductases isolated from G. oxydans ATCC 621 expands upon current approaches to mitigate levels in finished apple juice products. Building upon previous work, structural and functional analysis was conducted on the two reductases GOX0716 and GOX1462 to establish optimal reaction conditions for each enzyme.

3:15 pm – Anna Kupraty

Metabolic adaptations to exercise in adipose tissue

Exercise and metabolism study in rodents on adipocyte phenotypes utilizing functional measurements to evaluate training status and define metabolic adaptations to exercise.

Session 9: Neuroscience

2:30 pm – Aimee Dawe

The Effect of Myocardial Infarction on Behaviour and the Brain: A Pilot Study in Mice

Development of neurological disease is associated with cardiovascular dysfunction. To investigate this relationship, we used a surgical model of myocardial infarction in mice, examining changes in behaviour and brain volume. Preliminary findings show differences in behaviour as well as total brain volume between early and late stages of heart failure.

2:45 pm – Amanda Wiseman

Investigating the Behavioural Consequences of Stress-Induced Inhibition to Forebrain Neurogenesis in Adult Zebrafish (*Danio rerio*)

Zebrafish (*Danio rerio*) have a great capacity for adult neurogenesis in the telencephalon, which can be inhibited by chronic stressors, including social subordination. This decrease will be indicated by fewer BrdU/NeuN co-labelled neurons. The functional outcomes of these changes will be assessed through novel tank tests and mirror (aggression) tests.

3:00 pm – Coleman Olenick

Is Luminance Contrast the Same as Attentional Weight?

We distinguish attentional weight and target selection of objects competing for selection by varying the luminance of a target and competing distractor. Eye movement trajectory deviations were used as a dynamic measure of attentional weighting and compared against task performance which is classically used to infer attentional weight.

3:15 pm – Jayson Capistrano

Understanding the role of gut-derived metabolites in brain development and in the etiology of autism spectrum disorder

The gut microbiota produces small neuroactive molecules called metabolites that can impact the brain. However, the contributions of gut-derived metabolites influence the brain, or vice-versa, remains poorly understood. We hypothesize that distinct metabolites derived from different diets and/or mental health profiles result in unique phenotypic effects in the developing brain.

Session 10: Proteomics

2:30 pm – Barret Foster

Label-free quantitative proteomic analysis of Norwegian kveik during a prolonged thermal stress implicates multiple mechanisms responsible for superior thermotolerance

In order to elucidate the thermotolerant adaptations of Norwegian kveik, a group of stress tolerant ale yeast from Western Norway, we performed label-free quantitative proteomics with these strains undergoing a prolonged thermal stress and identified significant differences to multiple pathways such as ergosterol biosynthesis, antioxidant enzyme abundance, and mitochondrial function.

2:45 pm – Stevan Cucic

Multi-omic Characterization of the Lytic Listeria Phage, CKA15

We report multi-omic characterization a lytic bacteriophage infecting Listeria monocytogenes. We could thus identify the promoter and terminator elements controlling phage gene expression during infection, the time-resolved progression of phage infection (phage and bacterial host transcript abundance) and the complement of phage-encoded proteins that form the mature phage particle.

3:00 pm – Boyan Liu

Defining remodelling of the global temporal proteome responses of Fusarium head blight-resistant and -susceptible wheat cultivars

Fusarium graminearum, a fungal pathogen, causes Fusarium head blight in cereals, threatening food security. The severity is expected to increase with climate change. Our research uses proteomics to improve crop resistance and combat the disease, identifying key defence responses in wheat. This could significantly enhance food security.

3:15 pm – Chelsea Reitzel

Iron Availability Influences Lon Protease, which Plays a Role in Cell Growth, Biofilm Formation and Virulence, as evidenced by the Phosphoproteome of *Klebsiella pneumoniae*

This study is the first investigation into proteome-wide phospho-signaling for *K. pneumoniae* in response to iron and zinc-depleted conditions. Seven proteins (gene names: wzb, tolQ, mgtE, gntR, sbmA, osmC and lon) were selected for further characterization to determine the role they had in growth, virulence, and biofilm formation to identify candidate proteins for disruption as an antimicrobial strategy.

Poster Presentations

1. Panuya Athithan
2. Alyssa Banaag
3. Natalina Becke
4. Abigail Buckle
5. Mariel Burnside
6. Cassandra Clausen
7. Alyssa Clews
8. Ethel Closa
9. Jason Cousineau
10. Myah Crosby
11. Bradley Davis
12. Andrew Dolson
13. Isaac Firth
14. Meea Fogal
15. Gwendolyn Freeze
16. Nick Gervais
17. Connor Gianetto-Hill
18. Monica Goncalves
19. David Good
20. Neil Greenwood
21. Colin Guth
22. Davier Gutierrez Gongora
23. Loudon Herold
24. Dipendra Karki
25. Alexa King
26. Chloe King
27. Jordan Ko
28. Joyce Kuipers
29. Patrick Lameront
30. Sean Lee
31. Sarah Milinkovich
32. Elma Misini
33. Morgan Mizzoni
34. Benjamin Muselius
35. Liam Noseworthy
36. Una Pantic
37. Evan Perehiniak
38. Nathaniel Petersen
39. Alicia Plourde
40. Noah Presley
41. Nicolas Rolfe
42. Erin Rudolph
43. Neethu Shaji Saji
44. Erka Shata
45. Margaret Smith
46. Isaac Sullivan
47. Charlotte Townsend
48. Madison Turner
49. Brandon Ulch
50. Jordan Willis
51. Michael Woods

Session Abstracts

Session 1 – Biochemistry

Bradley Fitzgerald

The Regulated Production of Medium-chain Fatty Acids by Butyrate- fermenting Lachnospiraceae

Fitzgerald B, Moore A, Sorbara M

Bacteria inhabiting the human gut microbiota produce fatty acids, which can be divided into either short-chain fatty acids (SCFAs) such as acetate (2C), propionate (3C) and butyrate (4C) or medium-chain fatty acids (MCFAs) such as valerate (5C) and hexanoate (6C). The benefits of these MCFAs are beginning to be uncovered, however, the species which produce them in the gut are currently unknown. In industrial settings, the microbe Megasphaera is known to produce MCFAs through their butyrate-fermentation pathway but is rarely present in the gut. Fortunately, genera in the Lachnospiraceae (Lachno) family possess a similar pathway and are abundantly present in the gut. Therefore, this study aims to determine if Lachno produces MCFAs in the human gut.

Metabolomic analysis revealed that all tested Lachno isolates produce valerate with propionate supplementation, whereas only isolates from the Coprococcus produce hexanoate, which increases significantly with butyrate supplementation. Following this, an investigation of the butyrate-fermentation pathway showed that hexanoate-producers possess a second variant of the acetyl-CoA acetyltransferase (thlA) enzyme which forms the initial C-C bond in a Claisen condensation reaction. Protein alignment further revealed that the second variant was distinct from the first. AlphaFold analysis revealed that both thlA variants have a similar structural configuration, however, they had different ligand binding patterns when multiple CoA molecules were docked using AutoDock Vina. These findings show that the hexanoate-producing Coprococcus isolates have a second distinct thlA variant which may enable their ability to produce hexanoate.

Finally, to confirm that Lachno are utilizing their butyrate-fermentation pathway to produce MCFAs, 13C-labelled acetate utilization was traced in the presence of propionate and butyrate. Labelled carbons were observed in butyrate (2 or 4), valerate (2) and hexanoate (2). Overall, the findings of this study demonstrate the ability of Lachno to produce MCFAs and identifies a possible mechanism which controls hexanoate production.

Allison Leonard

Unsaturated Fatty Acids Inhibit Staphylococcus aureus Adhesion to Host Ligands

Leonard AC, Bao R, Myers M, Menjivar C, Berry KA, Bayles KW, Cox G

Staphylococcus aureus frequently colonizes the human nasal cavity, by tightly adhering to the skin's keratinized stratified squamous epithelium. These endogenous isolates are the predominant cause of hospital-acquired infections, highlighting the necessity of decolonizing

strategies. I have screened a chemical library composed of compounds with confirmed biological activity to identify inhibitors of *S. aureus* keratin adhesion, a major host ligand enabling skin and nasal colonization. I identified a naturally occurring unsaturated fatty acid, Geranylgeranoic acid (GGA), exhibiting dose-dependent inhibition of *S. aureus* keratin adhesion at concentrations subinhibitory for bacterial growth. GGA exhibited broad-spectrum anti-adhesive properties, inhibiting *S. aureus* adhesion to keratin, fibronectin, fibrinogen and immunoglobulins. Structure-activity relationship studies indicated that the carboxylic acid moiety and degree of unsaturation were important determinants of activity. As such, a wide array of unsaturated fatty acids, including host-derived fatty acids, were also shown to be potent anti-adhesive agents. Mechanistic studies indicated that GGA reduced the surface abundance of S. aureus cell wall-anchored proteins, including the fibronectin-binding proteins (FnBPs), iron surface determinant A (IsdA), and protein A (Spa), as well as, the cellular abundance of FnBPs and Spa. For the FnBPs, this was correlated with unsaturated fatty acid-mediated inhibition of the SaeRS two-component system. In summary, I have identified a potent naturally occurring anti-adhesive compound that inhibits *S. aureus* host adhesion and could represent an efficacious alternative decolonization strategy to overcome the rapid emergence of antibiotic resistance.

Victoria Butler

Glucan-linked phosphate reduces the affinity of glycogen branching enzymes for polyglucans in vitro

Butler V, Shabaan H, Tetlow L, Whiting M, Nitschke F, Tetlow IJ

All living organisms form complex polyglucans which provides short- or long-term energy storage. In plants, energy is stored as insoluble starch granules in the plastid, while bacteria, archaea, and heterotrophic eukaryotes store water-soluble glycogen in the cytosol. Starch and glycogen are synthesized in their respective cellular compartments by key enzymes such as glucosyltransferases, branching enzymes, debranching enzymes, and α-glucan phosphorylases. Branching enzymes (BEs) introduce branch points to complex polyglucans by removing short α-glucan chains by α-1,4 bond hydrolysis from an existing α-glucan chain, and reintroducing that short chain with an α-1,6 glycosidic linkage. BEs are critical determinants of starch and glycogen branching degree, each BE possesses a unique substrate preference and chain length transfer preference which ultimately influences overall polyglucan structure. Certain forms of starch and glycogen are known to possess covalently-linked phosphate monoesters (or glucan-bound phosphate) which can occupy the third or sixth carbon of a glucosyl unit within an α-glucan chain. The small negative charge of glucan-bound phosphate groups is thought to contribute to the dissociation of glucan double helices from their semi-crystalline structure, increasing hydration of glucan chains, ultimately preparing the polyglucan for eventual degradation. Recent evidence has shown that across multiple kingdoms of life, glucan-bound phosphate reduces the affinity of BEs for their substrate. Our research aims to further understand whether the presence of glucan bound phosphate on amylopectin and glycogen is capable of modifying BE affinity and activity for their glucan substrates, ultimately modifying glucan chain lengths that are transferred by Bes.

Amelia Doerksen

Regulation of axonal transport in neuron by protein palmitoylation of p150glued

Doerksen A, Leekha A, Sanders S

Neurons are large, complex cells requiring efficient trafficking and delivery of proteins and organelles to specific subcellular locations. Fast, continuous, anterograde and retrograde transport of cargo along axonal microtubules by dynein and kinesin motors is critical for neuronal function. The activity of motor proteins is tightly regulated, and aberrant activity can result in neurodegeneration or neurodevelopmental deficits. One important mechanism to regulate neuronal protein trafficking is the covalent addition of fatty acids to cysteine residues, a process known as palmitoylation. Several kinesin and dynein motor subunits and their activators have been identified in high throughput palmitoyl-proteomic studies as being potentially palmitoylated. Indeed, we recently demonstrated palmitoylation of the dynein activating complex dynactin subunit p150Glued. Dynactin is critical for dynein activation and processivity. P150Glued is palmitoylated predominantly in nervous system tissues on cysteines 617 and 1252 by the ZDHHC12 palmitoyl acyltransferase. p150Glued is the largest dynactin subunit that mediates dynein-dynactin microtubule binding and processive motility. The functional role of p150Glued palmitoylation is unknown, but due to the importance of p150Glued in dynein-mediated fast axonal transport, p150Glued palmitoylation likely regulates transport. Interestingly, when palmitoylation-resistant (C617/1252A; CCAA) p150Glued-GFP is expressed in neurons, less GFP signal is present in distal axons and in the vesicular fraction compared to wild type expressing neurons. This suggests that palmitoylation may regulate association of p150Glued with vesicular cargos. This study will be the first to investigate the function of p150Glued palmitoylation. Our findings will provide novel insights into how palmitoylation can regulate neuronal transport, contributing to the foundational knowledge within the field of palmitoylation with potential for understanding how trafficking can be altered in various neuropathies.

Session 2 – Knowledge Translation

Nida Ansari

Contextualizing the learning environment of undergraduate engineering students

Ansari N, Hird M, Jacobs S

Engineering is a large and growing umbrella of disciplines responsible for the many scientific and technological innovations of today's rapidly changing world. With a) increasing complexity in global challenges, b) a fourth industrial revolution that values transferable skills alongside disciplinary skills, and c) a diversification of engineering career pathways, we are concerned about whether post-secondary programs prepare graduates for our present context as the gap grows between what is taught and what skills are required for success after graduation (e.g., Burke et al., 2020; Ingram et al., 2013; Nelson & Brennan, 2018; OECD, 2018). Specifically, the conventional models of disciplinary-focused education

designed to achieve high grades interfere with the societal need to train creative innovators in science (Nelson & Brennan, 2018).

To increase the sustainability of engineering solutions, Dr. Witmer (2018; 2019; 2022) established the importance of contextualizing our environment (e.g., considering the socio-cultural and environmental factors) before trying to solve a problem. This is because the context is a dominant driver of a sustainable and meaningful solution. Thus, Dr. Witmer's Contextual Engineering (CEng) theory emphasizes community context as critical to the successful design, implementation, and functioning of an engineering project.

To ensure that all engineering designs are contextualized, it is important to first explore the education and training of engineering students to understand the current climate and knowledge of CEng. Therefore, our project seeks to understand the current attitudes and perceptions of engineering students and their instructors about the value of contextual engineering approaches to curriculum and program design. In this presentation, we will describe the overall CEng project and our research objectives, the methodology of the project, and the planned analytical approach. This project seeks to contribute valuable insights about the current engineering education climate to better inform, introduce and adapt contextualized education approaches into the engineering curriculum.

Elizabeth Porter

(Re)imagining a Manufactured Ecosystem

Dowhaniuk D, Porter E, Lipton M, Jacobs S

Climate change moves us toward a world where naturally functioning ecosystem services are irreparably degraded, or lost entirely. Biodiversity, primary production, pollination, soil formation, and climate regulation are just a few of the many benefits that humans obtain from ecosystems. For continued survival, humans will need to engineer environments to mimic the necessary structure, function, and services to support human life. This will lead us toward a manufactured ecosystem that imitates natural systems and processes to address human needs and challenges as a result of climate change. To change the climate crisis narrative from despair and denial to collaborative action and solution-oriented transdisciplinary approaches, a team of international collaborators came together to create the Manufactured Ecosystems project (https://www.manufacturedecosystems.com). Manufactured Ecosystems explores the potential for nature-based knowledge, techno-knowledge, and imagined knowledge to forecast the future of climate adaptation. It invites people to imagine and learn from complete adaptation strategies that replace ecosystems for survival in a post-climate world. Manufactured Ecosystems provides an opportunity to explore both the exciting opportunities and the uncomfortable truths associated with addressing and mitigating the impacts of climate change. While science can provide us with choices and possible outcomes, it is ultimately culture, society, and community that choose and shape our reality, the steps we take forward, and our future. Our presentation will explore the perspectives and transdisciplinary research that informs the Manufactured Ecosystems

project, and the critical intersections of understanding that come from science and art to support interconnecting ecosystems where we find the resources for well-being and survival.

Sidney Evans

Continuously improving biology education: An analysis of current biology program learning outcomes

Dawson J, Evans S

As our economy continuously evolves, the adaptation of higher education curricula becomes imperative, especially in the discipline of biology. The lack of a standard accreditation body to set program standards has resulted in diverse curricula, resulting in uncertainties regarding the preparedness of graduates for the workforce. Program Learning Outcomes (PLOs) provide a structured framework for developing and revising educational content, delineating the knowledge, skills, and values that students should acquire by graduation. However, the onus of PLO development and revision lies on already burdened institutions. Moreover, a disconnect persists between higher education and the labour market, which is critical for informing curricular advancements. Notable research in the United States, particularly the Vision and Change Report and the BioSkills Guide, has laid a strong foundation for improving biology education. It is critical to contextualize and apply these findings within the Canadian educational landscape, adapting and refining these strategies to fit our unique curricular needs and labour market demands. Networking offers a unique opportunity for feedback and guidance, providing insights that are vital for the progression of biology education. To facilitate this continuous improvement, it is essential to understand the existing state of biology curricula. This study compares the PLOs from Canadian BSc biology programs with internationally validated recommendations to enhance biology programs. We invite you to learn about the current state of Canadian biology education and contribute your vision for its future. Together, we can shape an educational curriculum where students are thoroughly prepared to become successful, contributing members of society.

Session 3 – Pathogens/Disease

Noah Kuehfuss

Overcoming intrinsic antibiotic resistance mechanisms in *Escherichia coli*

Kuehfuss NM, Cox G

Antimicrobial resistance is a major global health concern. With bacteria consistently developing new resistance mechanisms, and intrinsic resistance impeding the discovery of novel antibiotics, it makes it very clear that antibiotic alternatives are necessary. Gram-negative bacteria harness a complex intrinsic resistome which provides high levels of antimicrobial resistance and renders many of the clinically used antibiotics ineffective. This is primarily due to their highly impermeable outer membrane and drug efflux network. Antibiotic adjuvants are compounds that potentiate antibiotics by inhibiting resistance

mechanisms. In the search for natural product antibiotic adjuvants that target the intrinsic resistome of the bacterial pathogen *Escherichia coli*, I discovered the novel adjuvant activity of the compound kanosamine. Kanosamine is a monosaccharide amine sugar with structural resemblance to that of glucose and glucosamine. I have shown that when used in combination with rifampicin, kanosamine highly potentiates rifampicin against *E. coli* increasing susceptibility to the antibiotic by 16-fold. Further studies I have completed demonstrate that kanosamine does not potentiate rifampicin by increasing intracellular accumulation and that the synergy that exists appears to be due to a more unconventional method of antibiotic potentiation. My research describes a potentially novel mechanism of action for antibiotic potentiation that may allow for the revival of previously ineffective antibiotics and help in the discovery of novel antibiotics that target pathogenic *E. coli*.

Hayley Smith

ShcD adaptor protein modulates EGFR signalling and invasion in breast cancer cells

Lau H, Alural B, Tilak M, Smith H, Staples B, New L, Jacquet K, Martin CE, Gingras AC, Bisson N, Lalonde J, Jones N

Triple-negative breast cancers are highly metastatic and present clinical challenges as there are currently no effective therapies. While metastasis is the leading cause of breast cancer mortality, the underlying molecular mechanisms are unclear, and identification of new regulators is crucial. The ShcD phosphotyrosine adaptor protein bridges signalling complexes to classes of receptor tyrosine kinases implicated in metastatic signalling pathways. ShcD shares similar structure with paralog ShcA, which has an established role in mammary tumorigenesis and progression. Here we have identified ShcD upregulation in triple-negative tumours which correlates with overall reduced patient survival. We show that in human breast cancer cells, ShcD expression significantly enhances ligand-stimulated EGFR phosphorylation, reduces cell adhesion, and heightens cell invasion in vitro, with opposing effects upon ShcD knockdown. Furthermore, in a three-dimensional system, we report that ShcD expression enhances the infiltration of spheroids derived from a brain metastatic breast cancer cell line into human cerebral organoids. In each event, effects are mitigated with a ShcD mutant that can no longer engage surface receptors like EGFR or signal to downstream pathways involving Gab1 and Akt. Lastly, we show that treatment of breast cancer cells expressing ShcD with anti-inflammatory drug indomethacin decreases associations between ShcD and EGFR and reduces EGFR phosphorylation, which correlates with reduced cell invasion. Our results link ShcD-induced EGFR hyperphosphorylation to the modulation of metastatic properties and position ShcD as a putative contributor to breast cancer progression. Moreover, we provide a molecular basis for clinical targeting of adaptor-RTK interactions in breast cancers.

Jennifer Holborn

Investigating the Anticancer Potential of Cannflavin A and B in Glioblastoma Multiforme

Holborn J, Gluscevic T, Borenstein A, Carter A, Lalonde J

Glioblastoma multiforme (GBM) is a highly aggressive and invasive brain tumor associated with poor prognosis and limited treatment options. Flavonoids, such as Cannflavin A and Cannflavin B derived from Cannabis Sativa, have previously shown promising anticancer properties in various cancer models. In this study, we explored the therapeutic potential of Cannflavins against GBM utilizing a comprehensive approach involving cell viability, migration, and invasion assays to delineate the impact of these compounds on key aspects of GBM progression.

Our investigation revealed a dose-dependent decrease in GBM cell viability following exposure to increasing concentrations of Cannflavin B (0.5 to 20 µM), with no discernible cytotoxic effects observed. Remarkably, low doses of both Cannflavin A and Cannflavin B exhibited significant inhibition of GBM cell migration in timelapse scratch assays, indicative of their potential to impede tumor dissemination.

Of particular interest, Cannflavin B demonstrated robust anti-migratory and anti-invasive properties at 5 µM concentration, as evidenced by transwell migration and spheroid invasion assays. This concentration of Cannflavin B not only suppressed the migration of GBM cells but also attenuated their ability to invade surrounding tissues, highlighting its multifaceted therapeutic effects.

These findings underscore the promising anticancer attributes of Cannflavin A and Cannflavin B in the context of GBM. Their ability to modulate critical cellular processes implicated in tumor progression suggests a potential role in GBM therapy. Further studies elucidating the underlying molecular mechanisms and in vivo efficacy of Cannflavin A and Cannflavin B are warranted to validate their utility as novel therapeutic agents for GBM treatment.

Andrey Petropavlovskiy

S-acylation as a regulator of localization and function of endoplasmic reticulum chaperone GRP78/BiP in glioblastoma

Petropavlovskiy AA, Al Ramadan M, Sellar È, Sanders SS.

Glioblastoma is the most common adult malignant brain tumor with poor prognosis and limited treatment options. Glioblastoma cells can survive and proliferate under unfavourable microenvironment conditions that cause protein misfolding. Their proliferation is thus reliant on the activation of the unfolded protein response (UPR), which reduces the accumulation of misfolded proteins in the endoplasmic reticulum (ER), and upregulation of various chaperone proteins. The Hsp70 chaperone GRP78 (BiP) facilitates protein folding within the ER lumen and is the central modulator of the UPR. Several proteomics studies indicate that GRP78 could be post-translationally modified by a long-chain fatty acid in a process known as S-acylation (palmitoylation), which regulates localization and function of several glioblastoma-linked proteins. As such, we hypothesize that GRP78 S-acylation modifies its localization and chaperone activity, therefore promoting glioblastoma proliferation. Using metabolic labeling coupled with click chemistry, we confirmed that GRP78 is S-acylated in U-87 MG glioblastoma cells, and that ER stress dramatically upregulates GRP78 S-acylation. Furthermore, we found that GRP78 is S-acylated in several glioblastoma cell lines, and that S-acylation predominantly occurs at Cys420, located in a functionally important interdomain linker. We have also established that, surprisingly, S-acylation does not regulate trafficking of GRP78 outside of the ER. Future work on this project includes identifying the acyltransferase enzymes that S-acylate GRP78, determining how S-acylation regulates the functions of GRP78, and investigating how it contributes to glioblastoma cell proliferation both in 2D and 3D cell culture models. This project is significant as it is, to our knowledge, the first study to investigate how S-acylation modifies functions of ER luminal proteins. Furthermore, given the importance of GRP78 in maintaining the UPR in cancer cells, this project could implicate S-acylation as a new therapeutic target for glioblastoma.

Session 4 – Physiology

Liz Manchester

The functional and structural response of the zebrafish (*Danio rerio*) cardiovascular system to chronic hypoxia exposure

Manchester EM, Gillis TE

The functional capacity of the cardiorespiratory system depends on oxygen availability to the myocardium. However, there are fish species, including the zebrafish (*Danio rerio*), that maintain aerobic function in their natural habitats where exposure to hypoxia is a common occurrence. Previous work in our lab has focused on the ability of fish to modify the structure and function of the heart in response to a sustained environmental challenge, for example, cold acclimation. In the current study, we investigate the response of zebrafish to chronic hypoxia, and the adaptations they use to compensate for the physiological challenges

caused by hypoxia. We exposed zebrafish to 30% dissolved oxygen (DO) for 7 weeks, measured cardiorespiratory capacity via loss of equilibrium (LOE) trials, and then characterized heart function using high frequency cardiac ultrasound. We also investigated heart morphology and composition using histological approaches and explored the expression of a variety of gene transcripts related to the hypoxia response, anaerobic metabolism, and angiogenesis. Our results suggest that exposure to chronic hypoxia enhances cardiorespiratory function, as indicated by improved performance in LOE trials, and a lack of a bradycardic response when subjected to both acute hypoxia and acute cold exposure. These functional responses could be explained by the observed increase in hematocrit, and/or by the predicted modifications to myocardial structure. These results provide novel insight into the physiological response of fish to hypoxia, which is becoming a challenge in aquatic ecosystems worldwide. This work was supported by an NSERC-CGSM scholarship.

Nicole Fletcher

Sex Differences are not Present in Arteriolar Reactivity in Skeletal Muscle at the Microvascular Level

Fletcher NM, Murrant CL

We investigated sex differences in microvascular reactivity to better understand how female microvascular networks match blood flow to skeletal muscle metabolism. We characterized the arteriolar vascular response to muscle contraction in an in situ, blood perfused, anaesthetized, male and female hamster model (8-13 weeks) using intravital microscopy. We contracted 3-5 skeletal muscle fibres overlying an arteriole and quantified the resultant vasodilation. Skeletal muscle fibres were contracted for 2 minutes using a range of twitch and tetanic stimulation parameters: 6, 15 and 60 contractions per minute (cpm) at 20Hz, or 4, 20 and 70Hz at 15cpm (250msec train duration). We did not observe any significant differences in the magnitude of the vasodilation between males and females under any contractile conditions. In vitro control experiments were included to determine whether sexual dimorphisms in force production, and therefore differences in the vascular stimulus in the in-situ experiments existed. We stimulated isolated retractor muscle from male and female hamsters using the same stimulation parameters used in situ and found no sex differences in force production or fatigue, which confirmed that stimulation of the vasculature in the in-situ experiments was similar between the sexes. To determine if the mechanisms driving the microvascular response to muscle contraction differed between males and females, we stimulated arterioles via micropipette application of vasodilators associated with muscle contraction: nitric oxide (NO,10-5M), adenosine (ADO, 10-6M) and potassium (K^+, 20mM) and quantified the resultant vasodilation. Arteriolar reactivity to NO, ADO and K^+ did not significantly differ between males and females during the 2 min agonist application. Therefore, sexual dimorphisms are not apparent in arteriolar reactivity to muscle contraction under varying metabolic demands, or in the mechanisms that are involved in driving the observed vasodilatory response to muscle contraction in muscles where specific force was constant between the sexes.

Barbara Hyde-Lay

The morphology and network architecture of pericytes in the skeletal muscle

Hyde-Lay BM, Charter MC, Murrant CM

Pericytes are associated with the microvasculature in many different tissues. In the brain, it has been documented that ensheathing, mesh-type, and thin strand (TS) pericytes are associated with the microvasculature including arterioles, capillaries, and venules. However, in skeletal muscle, the architecture of the pericytic network and the types of pericytes present remain unknown. We sought to determine morphological and structural characteristics of the pericytic network and varieties associated with the microvasculature in skeletal muscle. We visualized the pericytes using male and female Tg(Cspg4-DsRed.T1)1Akik/J reporter mice, where NG2 membrane proteins were labeled with red fluorescent protein (RFP). We viewed pericyte architecture compared to the microvascular network by perfusing the RFP mice with fluorescent dyes that label the vascular endothelial cells. Gluteus Maximus, Diaphragm, and Cremaster muscles were then excised and whole mount viewed using fluorescent microscopy. TS pericytes are associated with capillaries in all muscles observed. No pericytes were observed to be associated with venules. The architecture of the pericyte network and how they are distributed differed between muscles and capillaries appeared to be largely covered by TS pericytes. Pericyte cross projections appear to extend freely between capillaries. No sex differences appear to exist between pericyte morphology or network architecture in any of the muscles studied. In conclusion, TS pericytes are associated with capillaries but consistently not associated with venules in each of the muscles observed. Free cross projections between capillaries implies that not all pericyte projections must be anchored to the capillary basement membrane. Whether this is specific to skeletal muscle or occurs in other tissues has yet to be determined.

Lily Hopkinson

Sex-differences in the cardiac response to splenectomy

Hopkinson LD, Brunt KR, Simpson JA

Our understanding of the physiological functions of the spleen, long thought to be a vestigial organ, has advanced significantly in the past decade. The spleen's primary role is to filter the blood of senescent erythrocytes; importantly, recent findings support the existence of a cardio-splenic axis. The spleen may support optimal cardiac function through its regulation of blood volume, breakdown of cardiotoxic lipoproteins, and capacity to modulate inflammatory immune responses following cardiac insult. Intriguingly, splenectomy (spx) increases one's long-term risk of cardiovascular events. Yet, no research has evaluated heart structure and function following splenectomy in otherwise healthy animals. Thus, the aim of this work is to characterize the role of the spleen in maintaining left ventricle (LV) structure and function. The vast majority of existing spleen research has been conducted exclusively in males; thus, we will perform our investigation in both sexes to determine whether there are sex differences in the cardio-splenic relationship.

Male and female wistar rats underwent sham or spx surgery, and LV structure and function were evaluated by echocardiography and invasive hemodynamics, respectively, at 9-weeks. Histological assessment of cardiomyocyte cross-sectional area and interstitial fibrosis were conducted to investigate LV hypertrophy and fibrosis - drivers of cardiac dysfunction. At 9-weeks post-surgery, splenectomy increased LV wall thickness in both sexes, in the absence of cardiomyocyte hypertrophy or reductions in LV dimensions. Despite LV remodelling, systolic function was maintained in both sexes.There was a sex-specific effect of splenectomy on fibrotic remodelling and diastolic function; namely, females exhibited increased quantity of interstitial fibrosis, and elevated end-diastolic pressure indicative of increased LV stiffness. This study identifies for the first time, a sex-specific role for the spleen in maintaining healthy cardiac structure and function, paving way for future work on the consequences of these changes for cardiac function long-term.

Charles Sánchez

Genome-wide association study (GWAS) for salmon rickettsial syndrome (SRS) resistance in rainbow trout using whole-genome sequence level imputed genotypes

Sánchez-Roncancio C, Garcia B, Gallardo-Hidalgo J, Yañez J

Rainbow trout production is greatly affected by salmonid rickettsial syndrome (SRS) caused by the bacteria, *Piscirickettsia salmonis*. In Chile, the mortality associated with SRS was 49.4% of the total mortality in production for the year 2022. Vaccines against *P. salmonis* infection and administration of antibiotics during disease outbreaks have not been effective in controlling SRS. Fortunately, resistance to *P. salmonis* has a moderate heritability (0.34-0.44) in rainbow trout. Therefore, an alternative and non-harmful strategy is genetic selection of trout that are resistant to *P. salmonis*. Genome-wide association studies (GWAS) with Single Nucleotide Polymorphisms (SNPs) have been used to map quantitative trait loci (QTL) and to identify some genes associated with pathogen resistance. A major challenge in GWAS is the cost of genotyping. An important approach to reduce costs is genotype imputation. Imputation consists of predicting unknown genotypes in offspring genotyped using a low-density panel of SNPs, ("Validation set") using their parent's genotypes ("Reference set") from either with a high-density panel of SNPs or from low-coverage whole genome sequencing, WGS, this allows completion of the missing offspring genotypes and correction of genotyping errors by taking advantage of the information from inherited haplotypes. Imputation is much more cost-effective then WGS of the offspring because offspring sequences will be very similar to those of their parents. In whole genome sequencing data, the accuracy of genomic prediction is no longer restricted by the degree of linkage disequilibrium (LD) between the SNP markers and the causative mutations affecting the SRS-resistance trait. Some potential genes in genome regions associated with resistance against SRS under controlled challenges were discovered.

Laura Thompson

The next generation of the Efflux Platform: A genetic toolkit to study compound transport across the *Escherichia coli* cell envelope

Thompson LK, O'Neill LC, Walia E, Kuehfuss NM, Cox G

Overcoming intrinsic antimicrobial resistance requires an in-depth understanding of compound permeation and transport across the bacterial cell envelope. The outer membrane (OM) is a formidable barrier for entry, which synergizes with the activities of drug efflux pump systems. To facilitate the study of drug efflux pump function, we previously reported the generation of EKO-35 (Efflux Knockout-35), a highly susceptible *Escherichia coli* K-12 (BW25113) mutant devoid of 35 known or putative drug efflux pumps. Here, we report the next-generation of this mutant, EKO-352.0. Efflux pump-encoding genes were disrupted using CRISPR-Cas9 mediated counterselection, producing a pan efflux-deficient mutant that harbours only one secondary nonsynonymous mutation compared to the

parental strain. Additionally, *E. coli* K-12 laboratory-associated cell envelope mutations were repaired including restoration of the wbbL locus, enabling production of O-antigen, and the BW25113-associated fabR missense mutation. Next, we expanded the Efflux Platform to encompass the entire *E. coli* efflux network, creating 35 efflux pump-encoding markerless single-copy genomic integrations under the control of the constitutive PLacI promoter. To enable the study of compound permeation, we also integrated an open and nonselective variant of the OM siderophore transporter FhuA, referred to as a 'pore', in each of these strains. To summarize the physicochemical properties and functional groups of compounds impacting the substrate specificity of each efflux pump, we profiled the Efflux Platform against a curated and diverse compound collection. Overall, we demonstrate the Efflux Platform is an essential resource to explore the contribution of efflux pumps and the OM to intrinsic antimicrobial resistance. Efflux pumps have also been suggested to extrude physiological substrates, and we also profiled the Efflux Platform under a variety of different environmental conditions to assess the role of drug efflux pumps in *E. coli* physiology. Overall, the Efflux Platform is an invaluable tool to define exogenous and endogenous substrates of drug efflux pumps, which will advance current strategies to overcome antibiotic resistance.

Marissa Sim

Genetic Manipulation of Non-Model *Blautia luti* Reveals a Novel Mechanism of Succinate Production

Sim M, Fitzgerald B, Moore A, Sorbara MT

The gut microbiota is a diverse community of microorganisms that engages in several regulatory processes such as colonization resistance (CR). CR involves numerous safeguarding functions which prevent the expansion of pathogens. For example, an acidic extracellular environment coupled with abundant short chain fatty acids (SCFA) promotes intracellular acidification (IA) and prevents pathogen replication. CR is lost when perturbations such as antibiotics disrupt the established equilibrium. This creates a favourable environment for pathogen expansion and increases host susceptibility to infections. Therefore, strategies to mediate pathogen inhibition and restore CR are critical. The Lachnospiraceae family are commensal gut anaerobes which produce SCFA and engage in CR. Here, we investigate the genus *Blautia* for their ability to produce succinate, a mechanism which is currently uncharacterized. Succinate is a cross-feeding metabolite commonly used to produce the SCFA, propionate. Interestingly, however, *Blautia* are seemingly devoid of the terminal components of this metabolic pathway. Succinate is a dianion with one pKa value lower than those of abundant SCFA. Therefore, we hypothesize that succinate aids in establishing an acidic environment that drives IA. Through genomic analysis and metabolomic screening, we identified an 11-gene cluster with homology to a flavin based electron bifurcation (FBEB) complex. We predict this mechanism is responsible for the reduction of fumarate to succinate. While *Blautia* have traditionally been genetically intractable, we implemented a recently established pipeline to knockout a gene in the putative FBEB complex. To our knowledge, these represent some of the first knockouts in *Blautia luti*. We confirmed the validity of these mutants and observed prominent phenotypes,

including succinate elimination, a shift in central metabolism, the inability to lower media pH, and a reduced capacity to inhibit the multi-drug resistant pathogen, Klebsiella pneumoniae. Together, this research will help potentiate the candidacy of succinate producing *Blautia* as biotherapeutic agents to restore CR.

Ashley Fisher

The roles Dbf4-dependent kinase, Chromatin assembly factor 1, and Topoisomerase interacting factor 1 in epigenetic regulation in *Saccharomyces cerevisiae*

Fisher AK, Yankulov K

DDK is a serine/threonine kinase with an essential role in DNA replication initiation. It also targets non-essential substrates, though these functions are largely uncharacterized. In vitro, DDK phosphorylates the Cac1 subunit of CAF1, a histone chaperone that reassembles nucleosomes behind replication forks to ensure the preservation of chromatin structures after DNA replication. The mutation of a putative DDK target site on Cac1 results in the loss of gene silencing at a repressed model locus, suggesting that DDK-directed phosphorylation is crucial to CAF1 activity. Intriguingly, the double deletion of CAC1 and TOF1, a member of the Fork Protection Complex that is required for replication fork pausing, causes aberrant gene silencing at a model locus. A separate line of evidence suggests that the phospho-regulation of Tof1 is required for its activity, and that DDK may be the catalyzing kinase. Thus, I hypothesize that DDK, Tof1, and CAF1 possess uncharacterized roles in chromatin reassembly. Using Saccharomyces cerevisiae as a model organism, I aim to examine the roles of DDK in epigenetic regulation, and establish a connection between DDK, CAF1, and Tof1 in vivo. To accomplish this, I will first confirm that DDK targets Cac1 and Tof1 in vivo using mass spectrometry methods. Next, I will introduce GFP reporter genes into two repressed model loci, and examine how mutations in putative DDK target sites on Cac1 and Tof1 affect GFP expression via flow cytometry. Separately, I will compare the phosphoproteomes of strains with wildtype and defective DDK to determine if other epigenetic regulators are targeted by DDK in vivo. Overall, this will provide novel insight into the role of DDK in epigenetic maintenance. It will also clarify the phospho-regulation and activities of CAF1 and Tof1 in vivo, and contribute to a model that links Tof1 to epigenetic regulation.

Session 6 – Ecology

Kendra Gardner

Changes in Lumpfish Diet with Size Inside Atlantic Salmon Sea Cages in Southern Newfoundland

Gardner KL, Boulding EG

Atlantic salmon (*Salmo salar*) held in sea cages may become infested with ectoparasitic copepods called “sea lice”. The salmon louse (*Lepeophtheirus salmonis*) is the prominent species in Canadian Atlantic salmon farms in Southern Newfoundland and can significantly reduce production and cause salmon welfare issues. Chemical and nutritional controls have been used as a way to manage the infestations; however, the sea lice have become genetically resistant over time. The use of mutualistic biological controls to manage parasite infestations is important because it is a more sustainable and environmentally friendly solution. Lumpfish (*Cyclopterus lumpus*) are commonly used as a biological control agent for

sea lice in cold-water environments. However, lumpfish are highly opportunistic generalist feeders that forage on prey other than lice. My study aims to assess the relationship between lumpfish diet composition and cleaning efficacy as a function of their size within Atlantic salmon sea cages using morphological prey identification and DNA metabarcoding. My hypothesis is that smaller lumpfish will eat more sea lice because their smaller mouth prevents them from eating large non-target prey items - such as krill and pellets formulated for salmon. Initial morphological stomach analysis showed that there is a wide range of prey items being consumed by lumpfish. The prey categories with the highest frequency of occurrence were "Unidentifiable" (41%)", "Salmon Pellets" (21%)", and "Amphipod/Caprellid" (19%). My DNA barcoding results will provide a better estimate of the delousing efficacy of lumpfish of different sizes when held with Atlantic salmon in sea cages.

Chris Bender

No Stomach, No Problem: an Integrated Morpho-Molecular Approach to Assessing the Diets of the Cunner Wrasse, among Coastal, Nearshore Regions of Atlantic Canada

Bender CJD, Moir CD, Hajibabaei M

Recent expansion and intensification of marine aquaculture along the Northwest Atlantic coast have resulted in the growth of non-target biological organisms that parasitize farmed species or opportunistically colonize equipment. The cunner wrasse (*Tautogolabrus adspersus*) is a potential cleaner fish species that has been experimentally used to control the salmon louse that infest Atlantic salmon inside sea cages. Little is known about the natural feeding ecology of the cunner wrasse across its wide geographic area found from Northern Newfoundland to Chesapeake Bay. We predicted dietary composition to vary amongst regions because of differences in prey species distributions. Gastrointestinal tract samples were collected across two sampling years (2019 and 2022) and 14 locations spread across 5 separate regions. Samples underwent traditional morphological identification of gut contents, prior to sample homogenization, DNA extraction and PCR amplification using three COI primers for dDNA metabarcoding analysis. Samples were assigned to operational taxonomic units (OTUs) using the MetaWorks 1.12 bioinformatics pipeline, and data was visualized and analyzed through the vegan package in R. Permanova results suggest that sampling region, location and year all have influence on diet composition, with region having the largest proportional effect on variation. Primary constituents of diet across all samples are dominated by sessile/benthic organisms, such as mussels, invasive ascidians and bryozoans. DNA metabarcoding enabled identification of many prey taxa to the species level. Next steps include further analysis of correlations between environmental conditions (temperature) and dietary composition across the full breadth of this species' range to delineate potential mechanisms behind these observations.

Derin Calik

Identifying developmental windows of sensitivity to diluted bitumen exposure in sockeye salmon (*Oncorhynchus nerka*)

Calik DM, Reside AM, Su G, Kennedy CJ, Alderman SL, Gillis TE

Early life stages of fish are sensitive to diluted bitumen (dilbit), a Canadian crude oil product that is transported at high volumes across North America and can enter aquatic environments by pipeline failure. However, little is known about how the timing of exposure relative to embryonic developmental stage influences the magnitude and scope of negative outcomes. The goal of this study was to compare key fitness metrics in sockeye salmon (*Oncorhynchus nerka*) embryos exposed to low, realistic concentrations of water-soluble fractions of dilbit during discrete developmental windows: fertilization to eyed stage, eyed to hatch, and hatch to swim-up. The fitness metrics included cardiorespiratory performance at the swim-up stage, relative growth (mass, body length), and cumulative mortality. We found that cardiorespiratory performance was compromised in sockeye salmon exposed to dilbit (6 µg/L total initial PAC), however the extent of this impairment was dependent on the developmental window in which they were exposed. Specifically, sockeye exposed from fertilization to eyed stage and subsequently reared in clean water to swim-up showed no difference in performance relative to unexposed controls. However, those exposed from eyed to hatch or from hatch to swim-up experienced a 14% and 30% reduction in cardiorespiratory performance, respectively, when measured at swim-up stage. Data analyses for relative growth and cumulative mortality are ongoing. These findings suggest that sockeye salmon may be more vulnerable to dilbit exposure during the period from hatching to the swim-up stage, and that a sufficient period of depuration may mitigate the negative effects of dilbit on cardiorespiratory performance. This data is valuable for informing evidence-based decision-making in the event of oil spills and for enhancing the accuracy of risk assessments regarding the impact of such incidents on salmon populations.

Session 7 – Cell Biology

Thomas Kunjumon

Chloroplast- ER Membrane Contact Sites have functional implications for plastid morphology and behaviour

Kunjumon TK, Mathur J

Direct communication as well as inter-organelle exchanges are facilitated by membrane contact sites (MCS) representing areas of close apposition of organelle membranes. While MCSs have been extensively studied in mammalian and yeast cells, our understanding of MCS in plants is still limited. MCS have been identified for a few major plant organelles such as Golgi bodies, plasma membrane, and mitochondria. Notably, the ER serves as the common apposing organelle in the formation of these MCSs.

The major defining organelle of green plants, the chloroplast has a major role in photosynthesis, and the synthesis of fatty acids and higher lipids. Chloroplasts maintain a close association with the ER. We hypothesized that potential MCS between chloroplasts and the ER reside within PLAMs (Plastid associated membranes). Indeed, based on its presence at chloroplast-ER junctions a Brassica napus Chloroplast Lipase Protein1 (BnCLIP1), has been suggested as a MCS marker. Given the extensive lipid flux that occurs

at PLAMs lipases with major roles in lipid modifications likely aid in the lipid exchange at the plastid-ER connection sites. My ongoing research employing live cell imaging has been supportive in establishing BnCLIP1 role as a chloroplast-ER MCS marker and demonstrates the physical consequences of plastid-ER connectivity in facilitating plastid movement and stromule formation.

Puja Puspa Ghosh

Investigating the Role of Endoplasmic Reticulum (ER) in Plastid Division

Ghosh PP, Mathur J

Plastids, postulated endosymbiotic organelles, generally multiply by binary division across the mid-plane of a pre-existing plastid yielding two daughter plastids of equal size. A crucial player in this process is a cytosolic Dynamin-related Protein 5B (DRP5B) or Accumulation and Replication of Chloroplast 5 (ARC5), which constricts and severs the plastid membrane. While ARC5 localizes to the plastid division site through its interaction with plastid envelope membrane proteins, the precise mechanism of its recruitment to this site remains unknown. Moreover, the separation of daughter plastids following membrane fission is poorly understood. Based on findings described for the division of other organelles, this study investigated if ER participates in plastid division. Time-lapse imaging-based observations of stable transgenics of Arabidopsis thaliana expressing ER and ARC5-targeted fluorescent proteins strongly support ER involvement in facilitating ARC5 recruitment to the plastid division site and daughter plastid separation.

Kirsten Berry

The *Staphylococcus aureus* esterase FmtA is required for proper wall teichoic acid D-alanylation and host ligand adhesion

Berry K, Verhoef M, Al-Abdul-Wahid S, Cox, G

Staphylococcus aureus, a highly prevalent pathobiont, relies on its cell envelope for pathogenesis. Teichoic acids (TAs), including lipoteichoic acid (LTA), affixed to the membrane, and wall teichoic acid (WTA), linked to the cell wall, are important components of the cell envelope. The TA D-alanylation pathway allows *S. aureus* to regulate its cell surface charge by decorating TAs with positively charged D-alanine (D-Ala) residues, counteracting the negatively charged TA backbone. Recently, the Cox Lab demonstrated that the TA D-Ala esterase, FmtA, is important for host ligand adhesion. Here, we sought to explore the basis of this association.
To begin, we confirmed that disruption of fmtA, negatively impacted adhesion to host ligands. Additionally, we found that disrupting the GraRS two-component system, which decreases the expression of the dlt operon responsible for incorporating D-Ala residues into LTAs, resulted in similar adhesion defects. This observation was unexpected, as fmtA and graRS mutants were anticipated to possess higher and lower levels of TA D-alanylation, respectively. To investigate further, we assessed surface charge in fmtA and graS mutants using a cytochrome c binding assay. Surprisingly, both mutants demonstrated increased surface

electronegativity, despite FmtA's role in the removal of D-Ala groups that reduce the intrinsic negative charge of the TA backbone. Therefore, we proposed that in a fmtA mutant, D-Ala cannot be released from LTA for incorporation into the surface-exposed WTA. To confirm this, WTA purified from wildtype, ΔfmtA, and graS::Tn was analyzed using H-NMR. Results confirmed that WTA from fmtA and graS mutants both exhibited reduced D-alanylation.
In conclusion, our findings support the theory that FmtA releases D-Ala from LTAs, enabling incorporation into WTAs. Furthermore, disruptions to WTA D-alanylation impacts the affinity of *S. aureus* for host ligands, which we propose is due to repulsive electrostatic charges.

Session 8 – Nutrition/Metabolism

Joshua Budd

A ketogenic diet with or without omega-3 long chain polyunsaturated fatty acids does not affect glucose homeostasis or skeletal muscle insulin response in rats

Budd JM, Notaro NM, Macleod B, Mutch DM, Dyck DJ

The ketogenic diet (KD) is extremely low in carbohydrates and high in fat. Despite evidence suggesting that KDs can promote weight loss and improve glucose metabolism in humans, inconsistent findings in rodents have left uncertainties regarding their true effect on glucose homeostasis. Additionally, while skeletal muscle is the primary location of insulin-stimulated glucose disposal, the effect of KD feeding on this process is poorly studied. Most KDs are predominantly composed of saturated and monounsaturated fatty acids, with almost no omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs). Evidence supports a beneficial role for the n-3 LC-PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in insulin-stimulated glucose disposal in skeletal muscle in the context of a metabolic challenge; however, it is unknown if their inclusion in a KD has beneficial metabolic effects. The objective of this study was to examine the impact of a KD on whole-body glucose tolerance and skeletal muscle insulin-stimulated glucose uptake, and to determine if altering the fatty acid composition of a KD with n-3 LC-PUFAs could improve metabolic parameters. Male Sprague Dawley rats were pair-fed either a low-fat diet, high-fat diet, KD, or a KD supplemented with n-3 LC-PUFAs (KDn-3) for 8 weeks. No significant differences in whole-body glucose tolerance, skeletal muscle insulin signaling, or skeletal muscle insulin-stimulated glucose uptake were detected between the dietary groups. In conclusion, our findings suggest that KD feeding, with or without supplementation of n-3 LC-PUFAs, does not affect whole-body glucose homeostasis or the skeletal muscle response under pair-feeding conditions.

Patrick McTavish

Investigating the Role of Omega-3 Polyunsaturated Fatty Acids on Lipid Uptake in White Adipose Tissue: Current Knowledge and Future Directions

McTavish PV, Mutch DM

After a meal, lipids are cleared from the circulation through uptake by various tissues. White adipose tissue (WAT) serves as the body's largest depot for storing excess fats as triacylglycerols (TAGs). Fatty acid uptake into adipocytes is facilitated by lipoprotein lipase (LPL), a membrane protein that hydrolyzes circulating TAGs to release fatty acids from the glycerol backbone. LPL activity is inhibited post-translationally by angiopoietin-like protein 4 (ANGPTL4), a secreted protein involved in lipid homeostasis. Angptl4 is negatively regulated by insulin signaling via a transcriptional connection with forkhead box-O1 (FOXO1), which promotes LPL activity post prandially. We recently found that the omega-3 polyunsaturated fatty acids (n-3 PUFA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) increase murine Angptl4 gene expression both in-vitro and in-vivo. We hypothesize that this

is mediated by peroxisome proliferator-activated receptor-gamma (PPARγ) due to 1) the demonstrated PPAR agonist activity of n-3 PUFAs, and 2) the ability of PPARγ to bind to the Angptl4 gene. The implications of these findings are contrasted by the positive metabolic outcomes observed in adipose-specific Angptl4 -/- mice, which show higher LPL activity and lower plasma TAG. Taken together, this presents an intriguing paradox, where the absence of Angptl4 and dietary n-3 PUFA both lower plasma TAG, despite having seemingly opposite effects on Angptl4 expression in white adipose tissue. This novel finding warrants future investigation with experiments specifically designed to understand the impact of Angptl4 expression on lipid uptake. To study this, we will first measure Angptl4 gene expression, TAG content, and LPL activity in a murine in-vitro adipocyte model across multiple treatments including n-3 PUFAs, PPAR agonists, and siRNA-based gene inhibition of Angptl4 and Pparγ. Collectively, this research aims to advance our understanding of the mechanisms by which n-3 PUFA improve metabolic health.

Nadine Abraham

Detoxification of the mycotoxin patulin by evolutionarily divergent reductases

Abraham N, Chan ETS, Li XZ, Mats L, Zhou T, Seah S

Penicillium expansum is a destructive post-harvest pathogen that causes blue mold rot in pome fruits such as apples. The fungus also produces the mycotoxin patulin, which has genotoxic, neurotoxic, and gastrointestinal effects. Patulin contamination poses a serious health hazard and an economic burden to the fresh-fruit industry and fruit-processing industry since levels can exceed the 50 µg/kg (ppb) limit in finished products.
Biodetoxification, involving the application of patulin detoxifying microbes or their associated enzymes presents a promising means to curtail patulin levels. One prospective microbe is the acetic acid bacteria, Gluconobacter oxydans ATCC 621 which possesses a broad repertoire of evolutionarily divergent reductases that detoxify patulin to the less toxic metabolite, ascladiol. Previously, four NADPH-dependent reductases with this requisite activity were isolated and two of these candidates, GOX0525 and GOX1899 were characterized. This study builds upon previous work by continuing the functional characterization of the other two candidate enzymes, GOX0716 and GOX1462.
Bioinformatic analysis revealed that GOX0525, GOX1899, and GOX0716 cluster into evolutionarily divergent short-chain dehydrogenase/reductase (SDR) families. Conversely, GOX1462 was classified as an aldo-keto reductase (AKR) family member. Functional characterization of GOX0716 and GOX1462 was conducted and their optimal activity was determined to occur at pH 6 and 7 respectively. GOX0716 was more thermostable than GOX1462, with a half-life of approximately 5 hours at 55 degrees celsius. Additionally, both enzymes also demonstrated broad substrate specificity towards other endogenous and xenobiotic aldehydes and ketones. Overall, among all four reductases, GOX0525 displayed the highest catalytic efficiency towards patulin.

Anna Kupraty

Metabolic adaptations to exercise in adipose tissue

Brunetta H, DesOrmeaux G, Holloway GP, Kupraty A

Evidence has accumulated to identify that brown adipose tissue (BAT) could be a potential therapeutic target to aid in the manifestation of obesity. This has been demonstrated as it is highly oxidative and is considered an extremely metabolic organ due to its elevated expression of uncoupler protein 1 (UCP-1). Within previous literature utilizing rodent models, when examining the effects of exercise on adipocytes, there are several factors that are included, such as thermogenic stress (i.e. cold exposure) and injections of beta 3 agonists to induce 'beiging' in white adipose tissue. Separately, there is additionally a lack of functional measurements within previous literature that could provide mechanistic and conclusive evidence when exploring the topic of exercise adaptations on adipose tissue. These factors thereby limit the translatability for human trials. By utilizing the preliminary data collected, following an exercise protocol of 3x a week for 4 weeks, in addition to voluntary wheel running (average of approx. 10 000 rpms per day), female mice demonstrate a significant increase in their distance for a standardized exhaustion trial when compared to their untrained counterparts. This has allowed us to confirm that the training protocol was successful. Given that subcutaneous white adipose tissue (iWAT) has been established to respond well to exercise, we will evaluate its differences from BAT, by measuring functional adaptations following the training protocol. By implementing a functional measurement such as testing mitochondrial respiratory capacity within the tissues, we have been able to show differences in coupled respiration following exercise in BAT (via GDP inhibition % and maximal ADP respiration). This is a functional indication of 'beiging' as the futile uncoupling cycle resulting from increased UCP-1 content becomes constrained. Results will be discussed based on two separate respiration protocols that were completed using the Oroborus Oxygraph 2K. We hypothesize that the 'beiging' will not be as significant in thermoneutrality when compared to previous literature, however, we do expect the exercised mice to exhibit metabolic adaptations from exercise. That said, to substantiate our findings, we are awaiting results from the male cohort of mice, in addition to confirmation from western blots and histology slides. Combining these methods with our respiration and in vivo data we will be able to confidently confirm or deny 'beiging' in mice kept in thermoneutrality and how this may be impactful for further research on adipocytes and exercise.

Session 9 – Neuroscience

Aimee Dawe

The Effect of Myocardial Infarction on Behaviour and the Brain: A Pilot Study in Mice

Dawe AM, King AN, Gupta BR, Simpson JA, Alpaugh MJ

The development of neurological disease is associated with a variety of risk factors, including cardiovascular dysfunction. To investigate this relationship, we used a surgical model of myocardial infarction (MI) in CD-1 wild-type mice. Specifically, after undergoing either the MI or sham surgical procedure, mice were followed post-surgically at 3,7 and 14 days to gain a comprehensive understanding of how the brain and behaviour changed across the beginning stages of heart failure. General differences in behaviour and cognition were measured using

the Open Field test, and alterations in brain volume were observed through structural analysis of sectioned tissue. Preliminary results show motor impairment and increased anxiety-like behaviour in the MI mice at 3- and 7-days post-surgery, with the effects most notably observed at 3 days. Interestingly, cognition was reduced only at the 14-day timepoint in the MI mice when compared to sham and non-surgery control mice. These behavioural differences at the early versus late time points suggest a variety of underlying mechanisms; currently, we hypothesize there will be an increase in inflammation at early time points and reduced cerebral blood flow at later time points, which will be investigated moving forward. Initial analysis of brain structure yielded an overall decrease in total and lateral ventricle volume between 3- and 7-days post-surgery; further analysis is necessary to parse out specific differences between the MI mice and controls.

Amanda Wiseman

Investigating the Behavioural Consequences of Stress-Induced Inhibition to Forebrain Neurogenesis in Adult Zebrafish (*Danio rerio*)

Wiseman A, Young F, Alderman S

Zebrafish (*Danio rerio*) have the greatest capacity for adult neurogenesis yet described in vertebrates, particularly in the telencephalon. Adult neurogenesis is inhibited by chronic stress, such as social subordination, as indicated by fewer BrdU-labelled cells in the telencephalon, but the functional outcomes of stress-induced changes to neurogenesis are understudied. We tested the hypothesis that decreased neurogenetic rates in the telencephalon associated with the chronic elevation in cortisol will promote the altered behavioural phenotype of anxiety-like behaviour. To test this hypothesis, baseline behaviours of zebrafish were assessed using a novel tank test and a mirror test. Zebrafish were then size-matched and placed in a 96-hour social subordination, where towards the end, fish were injected with BrdU. Once done, all fish were returned to their home tank intermittently undergoing a novel tank test and a mirror test directly after social subordination was finished, and 5, 10, or 14 days after that. In the novel tank test, time spent in the lower half of the tank, frequency of transitions between halves, and time frozen, were observed to be similar to baseline behaviours when compared to the time points post-social subordination. Subsequent analysis will analyze the mirror test, looking at the frequency and times of attacks on the mirror and will quantify neurogenesis by counting the amount of BrdU and NeuN co-labelled neurons. This research will help to understand the functional significance of the stress-induced decrease in neurogenesis and may be further used as a model for the neurobehavioral effects of social stress.

Coleman Olenick

Is Luminance Contrast the Same as Attentional Weight?

Olenick CE, Rosen N, Jordan H, Fallah M

Models of attention assume a relationship between attentional selection and luminance. Brighter objects are thought to have a greater attentional weight relative to dimmer objects. We investigated this assumption using a saccadic delayed match-to-sample task in which the trajectories of saccades provided a sensitive measure of attentional weights of target and distractor. Previous studies have shown that actively competing distractors have an attractive effect on saccade trajectories, but once competition is resolved the distractors repel the saccade. To examine the hypothesis that luminance is functionally equivalent to attentional weighting, we varied the relative luminance of the target and distractor, ensuring it was irrelevant to the task. Salience-based models of attention would predict saccade trajectory deviations towards the distractor that scale with the relative luminance of the distractor during active competition. Once the competition is resolved, distractors with lower relative luminance should elicit reduced trajectory deviations away from the distractor. Fast reflexive saccades tended to be executed to the brighter object, independent of their target/distractor identity. Reflexive saccades had trajectory deviations towards the distractor

that depended on the relative luminance of both the target and distractor. Once the target was discriminated from the distractor, saccades to the target deviated away from the distractor as predicted. Surprisingly, only the luminance of the target altered the magnitude of deviation after the completion of decision making. Taken together, salience drives the execution of reflexive saccades, but once visual discrimination has completed, the amount of distractor suppression does not depend on the salience of the distractor. Therefore, luminance is not synonymous with attentional weighting in saccade target selection throughout the selection process. This runs counter to attentional models that suggest attention works via increasing perceived contrast.

Jayson Capistrano

Understanding the role of gut-derived metabolites in brain development and in the etiology of autism spectrum disorder

Capistrano JDR, Rea V, Tran PNG, Ball T, Van Raay T

Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders caused by genetic and environmental factors. Due to its complex and often pleiotropic nature, its etiology has been difficult to elucidate—ultimately, hampering the identification of definitive diagnostic markers and development of potential therapies/treatments. Interestingly, most individuals with ASD suffer from gastrointestinal problems, which underscores a potential connection between ASD and the gut. Metabolites produced from the gut have been reported to impact the brain; however, the mechanisms of how these metabolites influence the brain or vice-versa remain poorly understood. Here, we hypothesize that distinct metabolites derived from different diets and/or mental health profiles result in unique phenotypic effects in the developing brain. In the first study, metabolites from naïve fecal samples that were grown in a robogut in the presence of different diets (high fiber Western, low fiber Western, Mediterranean, and Yanomami) were isolated. In the second study, age- and gender-matched fecal samples from neurotypical (NT) and children with ASD were used to isolate metabolites. We then evaluated the effects of these gut-derived metabolites on neurodevelopment by looking into changes in gene and protein expression, sensory organ development, and behavioral responses using germ-free zebrafish as our model. Thus far, our results suggest that the zebrafish model may not be sensitive enough to detect the effects of metabolites derived from different diets. However, we found that zebrafish neurodevelopment seems to be sensitive enough to detect the different effects of ASD and NT metabolites, where we see unique behaviors and distinct alterations in sensory organ development and gene expression profiles. Our goal is to eventually uncover the molecular mechanisms underlying the contributions of gut-derived metabolites on the development of the brain, which has implications for ASD and other relevant diseases and disorders.

Session 10 – Proteomics

Barret Foster

Label-free quantitative proteomic analysis of Norwegian kveik during a prolonged thermal stress implicates multiple mechanisms responsible for superior thermotolerance

Foster B, Oszahin E, Chalk B, Rowlands H, Tyrawa C, McAllister J, Geddes-McAllister J, Van der Merwe G

Norwegian kveik are a group of fast fermenting *Saccharomyces cerevisiae* ale yeast from Western Norway regarded for their thermo- and ethano-tolerance. Their capacity to survive and ferment rapidly at temperatures up to 42°C are attractive for further commercial applications. While previous work identified significant trehalose accumulation as a likely factor in their high-temperature survivability, the broader, cell-wide impact of their thermal stress response remains uncharacterized. This study utilized mass spectrometry-based label-free quantitative proteomics to analyze the total proteome of three Norwegian kveik strains and two control strains following a thermal stress of 40°C over six hours. We aimed to elucidate the complex mechanisms of high temperature adaptation by comparing protein abundance both within and between these strains, thereby identifying potential pathways responsible for enhanced thermotolerance. This proteomic analysis revealed significant differences in protein abundance related to the heat shock response, mitochondrial function, carbon metabolism, oxidative stress response, and sterol metabolism between kveik and the control strains. Notably, kveik strains exhibited an enrichment of proteins associated with ATP synthesis, elevated concentrations of the antioxidant catalase Ctt1, and a significantly higher abundance of enzymes associated with ergosterol biosynthesis. This study not only advances our understanding of the unique stress tolerant adaptations of kveik, but also sets the stage for future research into their stress resistance and potential for industrial applications.

Stevan Cucic

Multi-omic Characterization of the Lytic Listeria Phage, CKA15

Cucic S, Putzeys L, Boon M, Lepp D, Lavigne R, Anany H, Khursigara C

Listeria monocytogenes is a foodborne pathogenic bacterium that can undergo a physiological switch from saprophytism to parasitism and persist in food processing environments. Strictly lytic Listeria phages have shown promise as biosanitation and biocontrol agents. Phage CKA15 is a linear dsDNA phage with 3,134bp long terminal repeats and a genome length of 137,077bp. It belongs to the genus Pecentumvirus, of which P100 is the type species. In this research, we used a proteogenomics-based approach to identify virion-associated proteins, Illlumina-based RNAseq to analyze time-resolved host and phage transcript abundance during infection, and ONT-cappable-seq to experimentally determine the operon structure of the phage genome. We detected 29 phage-encoded putative particle-associated proteins, of which 19 are in the structure and assembly module. During infection, there was a progressive decrease in host transcript abundance and an increase in phage transcript abundance, which accounted for approximately 70% of transcripts by 25-minutes post-infection. We identified three highly transcribed putative phage ncRNA regions. The progression of phage gene expression showed a switch in functions from hypothetical at 5 minutes, nucleic acid metabolism at 15-, structural proteins at 25-, and DNA packaging,

tail assembly and lysis at 40 minutes post-infection. The timing of expression of predicted phage-encoded sigma and anti-sigma factors we observed is consistent with a switch in promoter recognition occurring after 15 minutes post-infection and being mediated by these two proteins. Using ONT-cappable-seq, we identified 81 phage transcription start sites (TSS) and 66 transcription termination sites (TTS). We grouped the TSS according to the timing of maximal expression of genes downstream of these TSSs and performed motif analysis, identifying two classes of promoters. The early promoter motif resembles a σA consensus sequence. Of the 66 TTS, 11 sites were predicted to be rho-independent terminators. There were profound changes in the host transcriptome evident after 5 minutes post-infection. GO enrichment and KEGG pathway analysis indicated a downregulation of host transcription factor expression and an upregulation of translation, cobalamin biosynthesis and propanediol metabolism. This research contributes to a systems-level understanding of the infection process of a strictly lytic phage infecting an important foodborne pathogen.

Boyan Liu

Defining remodelling of the global temporal proteome responses of Fusarium head blight-resistant and -susceptible wheat cultivars

Liu B, Reid B, Mitra S, Gedded-McAllister J

The fungal pathogen, *Fusarium graminearum*, is the primary causative agent of Fusarium head blight (FHB) in cereal crops around the world, posing a significant threat to food security. FHB reduces the crop yield and contaminates food products with mycotoxins, such as deoxynivalenol (DON). Moreover, the destruction caused by FHB is expected to increase in severity in the coming years due to its association with climate change, leading to a broader geographic impact. Consequently, mitigation of FHB requires innovative strategies to combat the disease. Our research, which utilizes mass spectrometry-based proteomics, has the potential to significantly impact food security by improving cultivar resistance and preventing fungal diseases in cereal crops. We have identified time- and cultivar-dependent defence responses in the wheat proteome and pinpointed Gene Ontology terms that contribute to resistance at the early stage of infection. These findings are crucial in our collective efforts to ensure a stable and secure food supply.

Chelsea Reitzel

Iron Availability Influences Lon Protease, which Plays a Role in Cell Growth, Biofilm Formation and Virulence, as evidenced by the Phosphoproteome of *Klebsiella pneumoniae*

Reitzel C, Geddes-McAlister J

Bacterial pathogens, such as *Klebsiella pneumoniae*, pose a significant health risk to immunocompromised individuals by causing pneumoniae, septicemia and meningitis. Alarmingly, due to antibiotic overuse and misuse, *K. pneumoniae* has increasingly become multi-drug resistant, making infection more difficult to treat. Attempts to discover new antimicrobials have yielded diminishing results; thus, novel targets are needed. Proteins involved in adaptation to nutrient-limited conditions could provide new antimicrobial targets

since these proteins are important for cellular processes within bacteria, such as growth, biofilm formation and virulence. This study is the first investigation into proteome-wide phospho-signaling for *K. pneumoniae* in response to iron and zinc-depleted conditions and provides comprehensive phosphoproteome profiling of *K. pneumoniae* under these regulated conditions. Optimization of the phosphopeptide enrichment strategy combined with improved bioinformatics allowed for the identification of 640 phosphopeptides from 313 phosphoproteins. This dataset identified several proteins with significant changes in the abundance of phosphorylation events in metal-limited versus iron- or zinc-replete conditions. Seven proteins (gene names: wzb, tolQ, mgtE, gntR, sbmA, osmC and lon) were selected for further characterization to determine the role they had in growth, virulence, and biofilm formation. Growth curves showed that deletion of the lon gene resulted in an increase in growth rate and it was observed that there was an increase in biofilm formation via crystal violet assay and biofilm colony forming units. Additionally, Δlon showed an increase in virulence compared to wildtype *K. pneumoniae* based on colony forming units of phagocytosed bacteria following BALB/c macrophage co-culture. The remaining six proteins will be characterized to find a protein target for disruption, causing a reduction in growth, biofilm formation or virulence as an antimicrobial strategy. This data provides new insights into cell signaling in *K. pneumoniae* in response to nutrient-limited conditions and illustrates the power of proteomics – specifically, phosphoproteomics – as a platform for understanding the mechanisms of bacterial adaptation to nutrient-limited conditions.

Poster Session Abstracts

1. Panuya Athithan

The impact of human milk oligosaccharides on the gut symbiont, *Ruminococcus gnavus*, in relation to gastrointestinal and metabolic diseases

Athithan P, Sorbara MT, Allen-Vercoe E

Breastmilk contains high concentrations of structurally diverse oligosaccharides that constitute the third most abundant component in human milk which are known as human milk oligosaccharides (HMOs). HMOs are best known for their prebiotic effects on the infant gut microbiome and confer several beneficial functions to infant development such as aiding in commensal microbe colonization and host immune cell modulation. Due to these benefits, there has been considerable interest in the impact of HMOs on the adult gut microbiome as recent studies have shown that HMOs have the potential to restore health-associated microbial communities in individuals with gut diseases. Dominant species of gut microbes capable of HMO utilization have a growth advantage over those lacking this ability. Recent findings have shown growth advantages employed by a wide diversity of resident gut bacteria isolated from infant fecal samples in the presence of HMOs. However, several strains of bacteria across a variety of species and genera experienced HMO-induced growth inhibition. Due to this novel antimicrobial property, there is considerable interest in investigating the range of HMO-induced inhibition across pathobiont species, including *Ruminococcus gnavus* (*R. gnavus*). *R. gnavus* is widely distributed at low abundance in the gut microbiomes of healthy individuals. However, it is disproportionately overrepresented in gut microbiomes of individuals with chronic metabolic diseases such as inflammatory bowel disease and type 1 diabetes. Thus, the aim of this study is to investigate the impact of HMOs on *R. gnavus* strains, the nature of HMO inhibition, and discern intra-species variation within *R. gnavus*. HMO utilization was assessed by treating *R. gnavus* strains with pooled HMOs (pHMOs) and measuring optical density (OD600). Strain specificity detected amongst *R. gnavus* through their different growth properties when exposed to pHMOs. While many of the strains demonstrated enhanced growth, some strains experienced a spectrum of pHMO-induced growth inhibition. These findings advance our understanding of HMO interactions with the gut microbiota at the strain level and opens new avenues for exploring the therapeutic potential of HMOs in modulating microbial composition.

2. Alyssa Banaag

Proteomics of *P. aeruginosa* strains using data dependent analysis and data independent analysis

Banaag A, Garnier N, Allan B, Park AJ, Brewer D, Gauthier J, Kukavica-Ibrulj I, Quang Henri Nguyen G, Mohammadi S, Potvin M, Renaud V, Levesque RC, Khursigara CM

Proteomics in *P. aeruginosa* research has been a valuable method to identify the differences in the proteome between strains, different modes of growth, resistance profiles after antibiotic treatment, and identification of potential systems involved in response to environmental stressors. With the popularity of discovery-based quantitative mass spectrometry methods in bottom-up proteomics, there have been two commonly used methodologies in the literature. In bottom-up proteomics, two standard acquisition modes are used: data-dependent acquisition (DDA) and data-independent acquisition (DIA). When comparing the two modes of acquisition and their pros and cons, one can ask which technique is most helpful in analyzing proteomic datasets from single-species strains. This project aims to tease apart the differences between DDA and DIA, to show how DIA offers greater quantitative depth, and how DIA can prove to be a better option when looking at proteomic analyses for single-species strains. To analyze the differences between these two methods, I first looked at protein identification with *P. aeruginosa* PAO1. Using these two methods, this analysis did not show strong differences between DDA and DIA. However, when analyzing the label free quantification (LFQ) between a pairwise comparison between *P. aeruginosa* strains PAO1 and LESB58, there are drastic differences. In this comparison, DIA shows more reproducible data than DDA according to sample correlation plots and principal component analyses. DIA quantified more proteins than DDA. Furthermore, DIA provided more descriptive information on biological processes involved through a 1D annotations and phenotypic assays showing that DIA gives greater quantitative depth. With this reasoning, DIA was selected as a method to conduct LFQ of a selected five strains of *P. aeruginosa*. The analysis on the five strains demonstrated unique differences in strain comparisons. This research aims to provide a rationale as to why DIA can be a method to use for single-species large scale proteomic analysis.

3. Natalina Becke

CREB is activated by store-operated calcium entry via calmodulin-dependent signalling pathways in iPSC-derived human neural progenitor cells

Becke N, Hewitt T, Proud E, Anderson E, Sheridan SD, Perlis RH, Brind'Amour J, Lalonde J

cAMP Response Element-Binding Protein (CREB) is a calcium (Ca2+)-sensitive transcription factor that has been implicated in multiple neuronal processes. CREB becomes active when it is phosphorylated at serine 133 by signaling pathway kinases, many of which are Ca2+-dependent. In neural progenitor cells (NPCs), intracellular Ca2+ is regulated by storage-operated Ca2+ entry (SOCE)—a mechanism that promotes Ca2+ influx through ORAI channels when Ca2+ stores are empty. Evidence shows that SOCE in mature neurons influences synaptic growth and plasticity while in NPCs, this pathway contributes to the proliferation and differentiation of NPCs. Interestingly, no direct connection has been made

between SOCE and CREB activity in NPCs to date. Therefore, we tested whether CREB phosphorylation is influenced by SOCE-facilitated Ca2+ influx and seek to understand how this interaction could affect downstream gene expression using human induced pluripotent stem cell (iPSC)-derived NPCs. Indeed, our efforts suggest that SOCE activation promotes the phosphorylation of CREB and significant changes in neurodevelopment-related gene expression. Further testing with a pharmacological inhibitor suggests that CREB phosphorylation appears to be dependent on calmodulin signaling. Downstream effectors of calmodulin include Ca2+ /calmodulin-dependent protein kinase II (CaMKII) and Ca2+ /calmodulin-dependent protein kinase IV (CaMKIV), both of which can directly interact with and phosphorylate CREB. However, protein expression tends to vary at different stages of neuron development, as shown with a completely diminished expression of CaMKII in NPCs when compared to neurons who were differentiated for 8-weeks or primary mouse cortical neurons. In contrast, CaMKIV is expressed at a similar level to the 8-week neurons, making it our kinase of interest for the activation of CREB following SOCE events. Taken together, these findings begin to piece together the signaling mechanism that connects SOCE to its effect on the early stages of neuron growth and differentiation.

4. Abigail Buckle

Circadian rhythm and nutrition

Buckle A, Ma DWL

Circadian rhythm is the twenty-four-hour regulation of the body and plays an important role in the maintenance of health. Circadian rhythm is regulated centrally from the brain and peripherally with regulation in major tissues including the liver, heart, adipose tissue, and muscle. Environmental cues such as light and dark, feeding and fasting, and stress influence circadian regulation. Circadian regulation occurs when clock genes Clock and Bmal1 create a complex and a negative feedback loop with Cryptochrome and Period, dependent on the light cycle. A second negative feedback loop is created when Rev-erb and ROR compete for binding on Bmal1 to influence Bmal1 expression. These circadian rhythm genes interact with various nutrients including vitamin D, vitamin A, saturated fatty acids, and polyunsaturated fatty acids (PUFA). Vitamin D is important for brain development and health, specifically in regions with circadian function. There is evidence showing that low levels of vitamin D can lead to disrupted sleep and wake cycles resulting in disruptions to circadian rhythm. Vitamin A is important for vision and cellular function. Low levels of vitamin A have been associated with disruptions to circadian rhythm genes. There is evidence demonstrating that vitamin A interacts with the Clock and Bmal1 complex and Period expression has shown influence from vitamin A. Saturated fatty acids and PUFAs are important lipids for cellular function. High levels of saturated fatty acids negatively impact circadian gene expression. There is also evidence to support that increased levels of saturated fatty acids lead to circadian rhythm

and disruptions to circadian rhythm increase levels of saturated fatty acids. PUFA levels in the blood follow a diurnal pattern, suggesting an association with circadian rhythm. Overall, the current body of evidence suggests circadian rhythm genes and nutrients interact across various pathways.

5. Mariel Burnside

Investigating the effects of plastidial oligogalactolipid-synthesizing SFR2 on stromule formation and behaviour

Burnside MI, Lobbezoo M, Mathur N, Mathur J

The plastid, a characteristic organelle of green plants, is the site of numerous important metabolic activities including photosynthesis and de novo fatty acid synthesis. While generally ellipsoid in shape, plastids spontaneously extend and retract stroma-filled tubules (stromules) through mechanisms and for functions that are not yet understood. The Mathur laboratory's investigations on stromule formation have led to considering the involvement of plastid acyl-lipid synthesis. Unlike most phospholipid-based membranes in eukaryotic cells, plastid membranes are predominantly composed of galactolipids. An OEM-localized protein named SENSITIVE TO FREEZING 2 (SFR2; At3g06510) uses monogalactosyldiacylglycerol, a galactolipid prevalent in plastidial membranes, as a substrate in processive galactosyltransferase activity to produce oligogalactolipids, bilayer-stabilizing glycolipids consisting of three or more chained galactoses bound to a DAG moiety. It is hypothesized that SFR2 activity has an effect on stromule formation and behaviour. Investigations of this hypothesis have been based upon the transgenic overexpression of fluorescently-tagged SFR2 in model plant Arabidopsis thaliana. To characterize the overexpression phenotype, the stromule formation frequency and polar lipid profile of a representative transgenic line have been analysed using confocal laser scanning microscopy and gas chromatography, respectively. The results of this research provide some insight into the morphology and behaviour of this essential plant organelle.

6. Cassandra Clausen

Investigating the role of adaptor protein ShcD in the oligodendrocyte lineage

Alural B, Clausen C, Jones N

Within the central nervous system, cells can be divided into two major classes: neurons and glia. Oligodendrocytes are members of the glial cell family and are crucial to the proper functioning of neurons. Oligodendrocytes produce myelin that wraps around the axons of neurons, facilitating rapid action potential propagation and providing metabolic support to energy demanding neurons. Myelinating oligodendrocytes are derived from their precursors, oligodendrocyte progenitor cells (OPCs), through a complex differentiation process that is regulated by various growth factors and neurotrophins. Crosstalk between intracellular signaling cascades are at the helm of coordinating this differentiation process and the function of the cells at each stage of differentiation.

The Shc family of adaptor proteins are well established modulators of many intracellular signaling pathways and have been noted to be expressed within the central nervous system.

ShcD, the most recently identified and least well characterized Shc, has been found to be uniquely expressed within the oligodendrocyte lineage, particularly in OPCs; however, an understanding of the role ShcD plays within these cells has yet to be elucidated.
We aim to investigate ShcD in the oligodendrocyte lineage with the use of ShcD knockout and ShcD wildtype mouse derived primary OPCs. Probing differences in OPC proliferation and migration, as well as comparing differences in their differentiation capacity, will allow for a better understanding of the importance of ShcD within these cells. In parallel, we aim to investigate differences between ShcD KO and WT mouse myelination profiles in vivo using immunofluorescence, to identify the significance of this protein in oligodendrocytes within the neural environment. The culmination of these efforts allows for a more comprehensive understanding of ShcD and the role it plays within the oligodendrocyte lineage, while also allowing insight into various intracellular pathways that may be involved in regulating oligodendrocyte dynamics and function.

7. Alyssa Clews

Insights into a protein complex putatively occupying membrane contact sites across chloroplast-endoplasmic reticulum membranes in plants

Clews AC, Jesionowska M, Bowering N, Mullen RT, Xu Y

Plant-derived lipids represent a multibillion-dollar global industry because of their plethora of applications, ranging from food/feed/nutraceuticals, cosmetics to biofuels. The commercial demand for plant lipids is predicted to greatly increase in upcoming years due to a combination of driving-forces such as population growth, and bioengineering of lipid metabolism represents a cost-effective means to enhance production efficiency. As such, there has been a surge of research tailored towards elucidating cellular/biochemical mechanisms underlying plant lipid metabolism. This includes the transfer of lipids and/or their precursors across organelles, especially between chloroplasts and endoplasmic reticulum (ER), which are responsible for lipid precursor biosynthesis and assembly, respectively. Although, our understanding of membrane contact sites (MCS) that facilitate lipid trafficking across ER and chloroplasts remains limited.

Brassica napus Chloroplast-Lipase-Protein (CLIP) is one of a few proteins known to reside at an ER-chloroplast MCS, however its conserved function and participation in a potential oligomeric complex across planta remains unclear. To expand our understanding of MCS proteins in plants and inform future bioengineering strategies, the presented project utilizes BnCLIP as a molecular ‘bait’ to identify novel ER-chloroplast bridging protein complexes in the model plant Arabidopsis thaliana. More specifically, BnCLIP fused to TurboID, a mutated biotin ligase (i.e., a ‘hook), was stably expressed in Arabidopsis and the resultant biotinylated proteins (i.e., ‘prey’) were screen as candidate components of MCS protein complexes. Showcased in this poster are key findings to date, such as the confirmation of BnCLIP-

TurboID localization to MCS' via microscopy and western blot analysis, as well as the identification of putative interactor candidates following biotinylated protein isolation and mass-spectrometry. Initial assessments of a representative candidate interactor (Apoptotic Death-1-Like-Lipase 3; DALL3), including subcellular co-localization and bimolecular fluorescence complementation assays as well as protein structural predictions will also be discussed.

8. Ethel Closa

Bacterial growth stages control NOD2 activation of diverse gut commensal family Lachnospiraceae

Closa E, Wallworth H, Sweeney A, Sorbara MT

A central pillar of the homeostatic crosstalk between the mucosal immune system and gut microbiota is the activation of intracellular NOD1/2 pattern recognition receptors (PRRs) that trigger pro-inflammatory signaling. These PRRs recognize conserved fragments of bacterial peptidoglycan that can be taken up by epithelial cells. Notably, a loss-of-function allele in nod2 is strongly associated with Crohn's Disease (CD), a chronic inflammatory condition of the gastrointestinal tract. This suggests that a tonic level of NOD2 activation is necessary to maintain barrier functions of a healthy gut environment. However, the extent to which anaerobic members of the gut microbiota provide NOD2 signaling is largely uncharacterized. One such taxon is the highly abundant and diverse family of Gram-positive anaerobes, Lachnospiraceae, which produce multiple beneficial metabolites and are often reduced in CD patients. Here, we determine if Lachnospiraceae can activate tonic levels of NOD2 signalling.

The ability of diverse Lachnospiraceae to induce NOD2 activation was assessed by stimulating reporter cells expressing human NOD2 receptors with culture supernatants and pellet suspensions. These Lachnospiraceae isolates originated from healthy human donors and were grown in a rich media culture to either 8 or 24 hours prior to the stimulation assay. Bacterial viability was confirmed using plate counts and continuous growth curves were generated for each isolate tested. We demonstrate that members of the Lachnospiraceae family are differentially able to activate NOD2. Some species, including *Ruminococcus gnavus* and *Coprococcus comes*, do not activate NOD2 signaling, while others like *Anaerostipes hadrus* trigger strong NOD2 activation with significant strain level variation. We further demonstrate that changes in NOD2 stimulatory capacity coincide with changes in the cell wall of some Lachnospiraceae upon entry into stationary phase. Our study reveals that the regulation of NOD2 signalling is linked to the species-dependent growth stage in Lachnospiraceae.

9. Jason Cousineau

Assessing the relationship between hypertension, blood-brain barrier breakdown, and neurological dysfunction

Cousineau J, Marquez P, Sellar P, Alpaugh M

Hypertension is highly associated with physiological changes to the body, such as altered blood flow to the brain and damage to the blood-brain barrier (BBB). These changes are known to influence the development and progression of dementia in general and Alzheimer's

disease in particular. However, little is known about how hypertension may interact with other neurological disorders. Therefore, we aim to assess the effect of hypertension on the blood vessels of the cortex and the glial cells of the surrounding area, within the context of Huntington's disease (HD) and Schizophrenia. HD, a dominantly inherited protein-misfolding neurodegenerative disease, and Schizophrenia, a neuropsychiatric disorder with strong genetic ties to cardiovascular health, have both been found to have decreased tight-junction (TJ) expression and have been linked to earlier onset of disease when comorbid with hypertension. Hypothesis. Hypertension will reduce BBB integrity and enhance neuropathology in a disease-specific manner within cellular models of HD and Schizophrenia. Methods. A 3D microfluidic cellular model of the BBB will be used to examine the effects of inducing hypertensive-like pathology (HLP) in vessels produced using human control, HD, or Schizophrenia induced pluripotent stem cells. Specifically, BBB integrity, TJ protein expression, and disease-specific indicators will be measured after the induction of HLP. This will be accomplished by way of BBB integrity assays, western blots, and immunofluorescence respectively. We anticipate variations between disease groups in vessel integrity, TJ protein expression, endothelial cell size, and glial behavior, showing an exacerbated effect when HLP is present. Results. Preliminary data shows that there is increased permeability of the BBB after the induction of HLP (high salt diet) in control vessels, but this effect is modulated in disease conditions. Conclusion. Our current results suggest that disease status has a modulatory effect on the response of the BBB to hypertension-related stressors.

10. Myah Crosby

Regulation of starch phosphorylases under cellular redox stress

Crosby M, Tetlow I

Starch metabolism is a metabolic process necessary for plant fitness and survival and serves as a critical source of stored energy vital for developing responses to stressors. Plastidal starch phosphorylases (Pho1) are key regulators of plant starch metabolism and may also play a crucial role for mitigating abiotic stress. Although extensive research is dedicated to the role of Pho1 during abiotic stress at the whole plant level, knowledge of Pho1 in relation to regulation during cellular redox stress is comparatively limited. Within our lab, two unpublished phenomena have been discovered in Pho1. Firstly, under simulated oxidative stress induced by a glutathione-specific reagent (diamide), Pho1 increases in catalytic activity and is shown to be glutathionylated. Secondly, Pho1 separates into two catalytically active forms under simulated reducing conditions (DTT).

My research aims to elucidate the changes in activity and to identify key cysteine modifications during cellular redox stress. Additionally, my research aims to understand how these phenomena relate to mitigating abiotic and cellular stress. This project addresses the

impacts of environmental stressors on starch metabolism in plants relating to ongoing climate change concerns at a cellular level. This may be accomplished through identifying potential targets for manipulation in major crops to improve plant health and stress tolerance.

11. Bradley Davis

Fluorinated ethylamines as electrospray-compatible neutral pH buffers for native mass spectrometry

Davis BTV, Velyvis A, Vahidi S

Native electrospray ionization mass spectrometry (ESI-MS) has emerged as a potent tool for examining the native-like structures of macromolecular complexes. Despite its utility, the predominant “buffer” used, ammonium acetate (AmAc) with pKa values of 4.75 for acetic acid and 9.25 for ammonium, provides very little buffering capacity within the physiological pH range of 7.0-7.4. ESI-induced redox reactions alter the pH of the liquid within the ESI capillary. This can result in protein unfolding or weakening of pH-sensitive interactions. Consequently, the discovery of volatile, ESI-compatible buffers, capable of effectively maintaining pH within a physiological range, is of high importance. Here we demonstrate that 2,2-difluoroethylamine (DFEA) and 2,2,2-trifluoroethylamine (TFEA) offer buffering capacity at physiological pH where AmAc falls short, with pKa values of 7.2 and 5.5 for the conjugate acids of DFEA for TFEA, respectively. Native ESI-MS experiments on model proteins cytochrome c and myoglobin electrosprayed with DFEA and TFEA demonstrated the preservation of non-covalent protein-ligand complexes in the gas phase. Protein stability assays and collision-induced unfolding experiments further showed that neither DFEA nor TFEA destabilized model proteins in solution or in the gas phase. Lastly, we demonstrate that multi-subunit protein complexes such as alcohol dehydrogenase and concanavalin A can be studied in the presence of DFEA or TFEA using native ESI-MS. Our findings establish DFEA and TFEA as new ESI-compatible neutral pH buffers that promise to bolster the use of native ESI-MS for the analysis of macromolecular complexes, particularly those sensitive to pH fluctuations.

12. Andrew Dolson

Analysis of Rif1 and Spt16 to understand their genetic interactions at the replication fork

Yankulov K, Dolson A

Chromatin structure plays a critical role in the regulation of gene expression in eukaryotes. The preservation of chromatin state throughout rounds of cell divisions is vital for maintaining gene expression programs and maintaining cell stability. The disassembly and reassembly of nucleosomes during DNA replication is central to these processes. Epigenetic markers on histones are partially responsible for encoding chromatin state and the retention of encoded histones during DNA replication ensures the copying of that information to nascent strands of DNA, resulting in faithful propagation of chromatin state. The histone chaperone FACT is known to ferry disassembled histones behind the replication fork during its passage and participate in their reassembly into new nucleosomes. Recent evidence has shown that the

Spt16p subunit of FACT physically and genetically interacts with other replication fork factors, suggesting a role in the regulation of fork stability and propagation of chromatin state. Rif1p is a telomere associated factor involved In regulating timing of origin firing during S-phase. It is also suggested that Rif1p acts during elongation in regulating the pausing and the rescuing of forks at sites where blockage of elongation occurs, effecting the ability of encoded histones to be ferried to newly replicated DNA. The goal of my study is to analyse the genetic interactions SPT16 and RIF1 genes have with other replication factors and histone chaperones, as well as describe the effects of these interaction have on the stability of heterochromatic loci in the model organism *Saccharomyces cerevisiae*.

13. Isaac Firth

The role of urease in gut commensal Lachnospiraceae

Firth IJ, Fitzgerald B, Hess S, Sweeney A, Sim M, Sorbara MT

The human gut microbiota is a complex group of microbes that are associated with various aspects of host health. Approximately twenty percent of host-derived urea is released into the gut where it is metabolized by gut microbes. Microbial urease cleaves urea into ammonia and carbon dioxide, however the roles of urea utilization in the physiology of commensal gut anaerobes are largely unknown. Health-associated microbiota communities produce high levels of short-chain fatty acids (SCFA) and decrease the pH of the proximal colon triggering inhibition of enteric pathogens. The Lachnospiraceae are a family of anaerobes that are associated with SCFA production, show high levels of genomic diversity, and vary in their capacity to acidify culture media. Here, we investigate the ability of Lachnospiraceae to tolerate pH and SCFA stress, and test the hypothesis that Lachnospiraceae-encoded urease alters SCFA production and acidification. Using a random forest model to predict Lachnospiraceae pH and acetate production based on gene presence/absence identified multiple subunits of urease as important predictors of model outcomes. Here, we demonstrate that urease-encoding Lachnospiraceae show urea-dependent changes in SCFA production, acidification, and growth. Encoding urease increases the tolerance of ureolytic Lachnospiraceae to acid stress and increases SCFA production under acidic conditions. Furthermore, urease-positive Lachnospiraceae directly incorporate the carbon from urea into produced SCFAs particularly under acidic conditions, demonstrating that urease activity directly supports SCFA production. This research provides mechanistic insight into the role of commensal-encoded urease in supporting healthy gut conditions that can inhibit the expansion of pathogens in the gut.

14. Meea Fogal

Optimizing and scaling up CRISPR interference for pooled genome-wide functional genomics in *Candida albicans*

Fogal M, Wensing L, Gervais NC, Shapiro RS

Candida albicans is an opportunistic pathogen and a commensal member of the human microbiota. Infections caused by *C. albicans* can be severe and life-threatening and often originate from the gut, via translocation into the bloodstream. While its pathogenicity has been extensively studied, comparatively less is known about the fundamental molecular mechanisms underpinning *C. albicans*' commensalism. Genetic manipulation is critical to understand such molecular mechanisms. The Shapiro lab has validated the use of a transcriptional repression system, known as CRISPR interference (CRISPRi), to probe gene function in *C. albicans*. In optimizing our CRISPRi system, we have identified an optimal region of target for our CRISPRi system relative to the transcriptional start site (-200 bp to +50 bp). We have also validated that the system can be regulated via tetracycline and is able to repress the transcription of even essential genes. We have scaled the system up to create a pooled CRISPRi functional genomic library targeting (96%) ORFs in the *C. albicans* genome. Screening this pooled genome-wide library in a murine model of gastrointestinal colonization will allow us to identify genes that, when repressed, influence commensalism. This library will help to deepen our understanding of fungal biology and serve as a tool for researchers in the field to rapidly study *C. albicans* functional genomics on a genome-wide scale.

15. Gwendolyn Freeze

Characterization of *Parvimonas micra* pathogenic potential, host cell interactions, and strain variation among six distinctly sourced isolates in the context of colorectal cancer

Freeze G, Daisley B, Vancuren S, Allen-Vercoe, E

The human gut microbiome is composed of trillions of different microorganisms, including bacteria, archaea, viruses, and fungi. A balanced gut microbial community is often associated with overall host health, whereas disruptions to the gut microbial composition can lead to the progression of diseases. In the case of colorectal cancer (CRC), an imbalanced gut microbial community and the enrichment of specific oncomicrobes (i.e., microbes associated with the development of cancer) are often implicated in CRC pathogenesis. One such oncomicrobe, *Parvimonas micra*, is newly emerging in its striking association with CRC. Recent work has revealed the overabundance of *P. micra* in CRC patients compared to controls, although few studies have investigated the mechanistic role of *P. micra* in the disease. As such, the objectives of this study are two-fold: 1) to better characterize the genomic and physiological attributes of *P. micra* that may be relevant to pathogenesis, and 2) to determine the interactions of *P. micra* with host cells in vitro. Furthermore, by utilizing

six distinctly sourced *P. micra* isolates, this work also focuses on characterizing strain-variation within the species. Overall, we found that *P. micra* possesses an abundance of toxin genes and virulence factors, a strong ability to co-aggregate with other known oncomicrobes, a remarkable tolerance to air, as well as a sensitivity to certain gut-related stressors such as pH and short chain fatty acids, with the extent of these effects varying among strains. Furthermore, strains of *P. micra* were found to differentially stimulate host cell proliferation and inflammatory responses in vitro, as well as adhere to and invade into host cells to varying extents. Overall, this work provides insight into the potential role of *P. micra* in colorectal tumorigenesis, as well as highlights interesting strain-variation within the species that may be relevant to its pathogenesis in CRC.

16. Nick Gervais

Exploring aneuploidy and drug susceptibility in *Candida albicans* via CRISPR activation screening

Gervais NC, Wensing LF, Fogal M, Rogers R, Bukari ARA, Sethi P, Maciel EI, Ene IV, Gerstein AC, Shapiro RS

Candida albicans is an opportunistic human fungal pathogen that lives as a commensal in most healthy adults, though can lead to invasive infections in vulnerable populations. The emergence of *Candida* strains with decreased susceptibility to antifungal drugs is a critical threat to human health globally. One mechanism of acquiring increased drug resistance or drug tolerance is *C. albicans*' ability to readily undergo chromosomal rearrangements and alter ploidy from its typical diploid state. However, the genetic pathways underpinning many of these aneuploidies remain uncharacterized. It has recently been shown that a trisomy of chromosome R (ChrR) in *C. albicans* results in a decreased susceptibility to azole antifungal drugs. This suggests that the overexpression of one or more genes on ChrR is associated with antifungal drug response phenotypes. Better characterization of the genes found on ChrR through genetic manipulation will therefore promote our understanding of the molecular mechanisms mediating the evolution of resistance and tolerance to antifungal drugs. Here, we present the optimization of a new CRISPR activation (CRISPRa) system in *C. albicans* that allows for targeted genetic overexpression and demonstrate its application towards characterizing fungal genes involved in antifungal drug resistance and virulence. Presently, we are exploiting our CRISPRa system to construct a novel CRISPR-based overexpression library that targets genes on ChrR (~1000 genes) in *C. albicans*. Scaling this technology up to target an entire chromosome has allowed us to mimic the gene dosage conditions present when there is an extra copy of ChrR in *C. albicans* cells, and we are screening this library in the presence of antifungal drugs to identify key genetic factors involved in ChrR trisomy-mediated drug resistance. Our resulting data will therefore help to uncover fundamental mechanisms that influence antifungal drug responses, as well as demonstrate functionality for an important new CRISPR screening platform in *C. albicans*.

17. Connor Gianetto-Hill

Assessing gut microbiota differences in adolescents at risk of type 2 diabetes using a colonic bioreactor model

Gianetto-Hill C, Granato A, Hamilton J, Danska J, Allen-Vercoe E

The global incidence of Type 2 Diabetes (T2D) is on the rise, posing an escalating concern, particularly among adolescents. Addressing this surge necessitates a deeper understanding of the risk factors contributing to the disease, including the involvement of the gut microbiome.

Existing literature on the gut microbiome's association with T2D predominantly focuses on populations over 30 already diagnosed with the condition. To bridge this gap, we utilized stool samples obtained from adolescents at risk of developing T2D to inoculate bioreactors designed to model the colonic environment. In parallel, stool from healthy young participants were inoculated in bioreactors to discern differences between the healthy and the at-risk of T2D gut microbiomes. Given the established influence of dietary nutrients on gut microbiome composition, bioreactors were initially supplied with nutrient-rich media, transitioning to a nutrient-poor medium after 28 days to investigate dietary impacts on community composition and function. Shotgun metagenomic sequencing of stool samples revealed some differences in bacterial composition and predicted functions between healthy and T2D risk groups. Furthermore, bacterial community composition in bioreactors was assessed using 16S rRNA marker gene sequencing, while metabolites were identified and quantified using proton nuclear magnetic resonance (1H-NMR), facilitating a comparative analysis between healthy and at-risk adolescents. This approach aims to identify absent bacterial taxa or functions in at-risk adolescent microbiomes, with the ultimate goal of designing potential gut therapeutics to restore these microbial communities.

18. Monica Goncalves

Inhibition of mitochondrial ClpP protease via orthosteric and allosteric compounds

Goncalves MM, Watson I, Uday AB, Forrester T, Jitkova Y, Currie SQ, Kim A, Joshi D, Mohammed M, Velyvis A, Yudin A, Al-war R, Harkness R, Kimber M, Zeytuni N, Schimmer A, Vahidi S

The human mitochondrial ClpP protease is a validated drug target for the treatment of acute myeloid leukemia (AML), a type of blood cancer with poor prognosis. It remains critical to investigate novel ways to target AML using strategies that are rooted in a mechanistic understanding of the disease and the infrastructure AML cells use to survive. ClpP is a tetradecameric serine protease that degrades damaged or misfolded respiratory chain proteins. Unfortunately, currently available inhibitors of ClpP are non-specific. Past studies of several bacterial ClpPs show that these systems are highly dynamic, and their function is tightly regulated via allosteric conformational changes. Here we describe the mechanism of action of two groups of ClpP inhibitors: orthosteric boron-based inhibitors that directly target the ClpP active site; and allosteric inhibitors that bind away from the active site and exploit the inherent conformational dynamics of ClpP to dysregulate its function. My poster highlights a strategy that exploits structure-guided iterative improvements to develop novel compounds as selective and potent ClpP inhibitors. We combine structural biology approaches such as methyl-TROSY NMR, HDX-MS, and cryoEM to quantify and probe the ClpP-inhibitor interactions. The integration of these sensitive and complementary structural biology approaches improves our understanding the ClpP biology. Given that ClpP is

overexpressed in a broad range of cancers, this project has the potential to have translational value beyond AML.

19. David Good

Dietary influence on gut microbiota diversity: introducing Yanomami bacteria to Western cultures

Good D, Allen-Vercoe E, Vancuren S

Recent research highlights the significantly increased microbial diversity in the gut microbiota of the Yanomami, an indigenous group that inhabits the Amazon rainforest. This diversity may be attributed to their diet rich in fibrous plant foods providing an array of substrates for microbial fermentation. Studies have emphasized the functional importance of specific bacterial genera prevalent in these populations, such as *Prevotella* and *Treponema*, demonstrating an enhanced ability to degrade dietary fibers and produce short-chain fatty acids due to adaptation. In comparison, Western and industrialized societies exhibit significantly lower diversity and abundance of these beneficial gut-associated bacteria. This study aims to study the impact of introducing Yanomami gut-derived bacterial taxa to Western-based microbial culture. Single-stage, continuous-flow bioreactors will be used to simulate the distal colon's physiological conditions to recapitulate the gut microbial community derived from stool samples. Using this model, we will challenge steady-state microbial cultures derived from Yanomami and Western stool samples by swapping their respective diet conditions and measuring their effect on microbial composition and functional profile. These changes will be assessed using 16s rRNA gene profiling, shotgun metagenomic analysis, and metabolic profiling techniques such as proton nuclear magnetic resonance (1H-NMR) and mass spectrometry.

To investigate if bacterial strains derived from the Yanomami gut could persist in a Western gut community, we will inoculate a Western microbial culture with a suite of Yanomami bacterial strains under varying diet conditions. We will observe whether any introduced bacterial strains persist and how they alter the overall microbial ecology. Provided the inoculum receives the appropriate and sufficient substrates, we hypothesize that introducing the Yanomami-derived bacterial strains into a Western-based microbial culture will shift the gut microbial profile towards increased diversity and production of health-associated metabolites. These experiments will offer valuable insights into the intricate interplay between diet and microbial ecology.

20. Neil Greenwood

Structural modelling and oligomeric state of cell division protein FtsK using co-variance analysis, SECMALS, and immunofluorescence

Greenwood N, Seidel L, Warywoda K, Kimber M, Khursigara C

Bacterial cell division is a highly coordinated process involving the duplication and segregation of genetic material, cell elongation, and septation of the cell envelope. Septation

is mediated by a complex of essential and non-essential proteins known as the divisome. The focus of our research is the integral inner-membrane protein FtsK. FtsK is an essential divisome protein, which has been implicated to act as a critical checkpoint regulator during divisome assembly. The method by which FtsK accomplishes this has yet to be described. The only essential domain of FtsK is the membrane segment (FtsKN) for which there is no structural model. Several programs exist for ab initio protein folding, the premier program being Deep Mind's AlphaFold. For FtsKN, the general AlphaFold model is generally confident, biophysically plausible, and aligns well with the experimentally derived topology. However, the confidence in the model in the periplasmic regions is low. The periplasmic exposed region is particularly important, as it is believed to be the site of important protein-protein interactions. Using a simple cell-length based assay, we have tested over 100 amino acid residue pairs and have confirmed a series of mutations which break and then restore septation, suggesting that these residues likely interact within the structure. We have used these experimental interactions to generate a hybrid model that refines the AlphaFold model. We have also demonstrated that this method can be extended beyond intra-protein contacts and be used for protein oligomerization studies, and likely protein-protein interaction studies. Using SECMALS analysis, we have demonstrated that FtsK forms both hexamers and monomers in in solution, and that specific mutations identified through co-variance analysis can impair and restore FtsKN's ability to oligomerize. Immunofluorescence analysis demonstrates that there are no significant changes to FtsK localisation when the oligomeric state is impaired, suggesting that oligomerization is not necessary for protein localisation. Overall, this research furthers our understanding of bacterial cell division, while demonstrating additional tools for easier investigation into the structure of recalcitrant membrane proteins

21. Colin Guth

The mast cell receptor, MRGPRB2, promotes infected wound healing through the neuroimmune axis

Guth CR, Pundir P

Mast cells, as primary reservoirs of bioactive and immunomodulatory molecules in the skin, influence processes such as pathogen clearance, tissue remodeling, and vascular regulation. In the context of skin repair, mast cells play a pivotal role during the early stages of wound healing, with emerging data underscoring their significance in recovering from infected wounds. Our research in mouse models of bacterial infection and neurogenic inflammation has uncovered a mast cell-specific receptor, Mrgprb2, and its human homolog, MRGPRX2, which contribute to bacterial clearance and neuropeptide-mediated inflammation, potentially impacting wound healing—an area yet to be explored. We hypothesize that Mrgprb2/X2 are

critical for promoting infected wound healing through communication with immunomodulatory neuropeptides.

Aims: 1) Evaluate whether Mrgprb2 controls skin wound infection and promotes wound healing. 2) Characterize the effect of cutaneous neuropeptides on Mrgprb2/MRGPRX2 activation to stimulate mast cell mediator release and wound healing.
Results: We demonstrate that Mrgprb2 is essential for normal healing of infected, excisional full-thickness skin wounds. In wildtype mice, wound size reduces rapidly upon injury with wounds closing by day 10. Conversely, Mrgprb2-deficient mice exhibit increased wound size, with wounds remaining at 30% of their original size even after 10 days, signifying impaired closure. Additionally, we show that catestatin, a neuroendocrine peptide secreted by skin cells upon injury, activates MRGPRX2-expressing cells with an EC50 of 4.1± 0.26μM. In vitro functional assays further demonstrate that catestatin stimulates mast cells to release histamine, prostaglandins, and the cytokine CCL2, important for monocyte recruitment and wound healing. However, this activation is significantly reduced in mast cells lacking MRGPRX2.
Conclusions: Our findings reveal that the neuropeptide catestatin is an agonist for Mrgprb2/X2, potentially capable of inducing Mrgprb2-mediated infected wound healing. Our work advances knowledge of key cellular and molecular players in infected wound repair, suggesting new therapeutic targets for this debilitating condition. Funding: This research was supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC RGPIN-2022-03453), the J.P. Bickell Foundation, the Canada Foundation for Innovation, and the College of Biological Science Team Building Grant.

22. Davier Gutierrez Gongora

Discovery of peptidase inhibitors from mollusks against the human fungal pathogen, *Cryptococcus neoformans*

Gutierrez-Gongora D, Bohra M, Woods M, Chan N, Prosser R, Geddes-McAlister J

Cryptococcus neoformans is an environmental fungal pathogen responsible for 19% of AIDS-related deaths worldwide. Treatment of this pathogen relies on three drug classes: azoles, polyenes, and pyrimidine analogs. However, azole drugs are also used to treat plant fungal pathogens and are accelerating the antifungal resistance of human environmental pathogens even outside the host. A recent approach consists of identifying new compounds that inhibit fungal virulence factors rather than killing the pathogen, leading to less selective pressure and resistance. Invertebrates (e.g., mollusks) stand out for their high diversity, chemical defences, and similitude with animal cells (e.g., humans), which would produce new drugs with less off-target or cytotoxic effects. In this investigation, we evaluated the efficacy of five mollusk species extracts against the production of well-defined virulence determinants (i.e., thermotolerance, melanin, capsule, and biofilm) and fluconazole

resistance in *C. neoformans*. We demonstrated the extracts' influence on virulence factor production, biofilm formation, and susceptibility to fluconazole. Additionally, we measured the inhibitory activity of extracts against different peptidases (family representatives of cryptococcal orthologs) related to fungal virulence determinants and fluconazole resistance. We integrated these phenotypic findings with quantitative proteomics profiling to define distinct signatures of each treatment followed by validation of a new mechanism of anti-virulence action. Further purification steps and mass spectrometry analysis revealed the presence of small peptidase inhibitors in active fractions against virulence-related cryptococcal enzymes. Similarly, using cryptococcal peptidases as baits in a pull-down assay, we isolated and identified specific inhibitors against these targets. We are now expressing, purifying, and assessing these putative novel peptidase inhibitors using in vitro and in vivo models for antifungal activity. Furthermore, this work highlights the potential of compounds derived from natural sources to inhibit virulence factors in *C. neoformans* and other clinically significant fungal pathogens like *Aspergillus fumigatus* and *Candida* spp.

23. Loudon Herold

Examining carbohydrate utilization within Gram-positive anaerobic bacterium from the Lachnospiraceae family

Herold L, Fitzgerald BG, Sorbara MT

The composition of the human gut microbiota is shaped by the host's diet. A microbe's ability to replicate in the dense gut environment depends on their success in competing for limited available nutrients compared to their competitors. Understanding how specific microbes access a suite of nutrient niches can provide insight into their contributions to microbial dynamics and the host's physiological state. Genomic regions have been characterized that express enzymes to transport, cleave, and metabolize carbohydrates. This area has been primarily investigated in the Gram-negative Bacteroidota phylum, but remains relatively unknown in the Gram-positive Bacillota phylum. This project aims to investigate intra- and inter-species variation in the utilization of simple and complex carbohydrates by members of the Lachnospiraceae family and the impacts of different carbon sources on metabolite production.

Strains of *Anaerostipes hadrus* and *Blautia wexlerae* were profiled for their carbohydrate utilization, which revealed extensive intra-species variability. Using this dataset, strains of *A. hadrus* and *B. wexlerae* can be clustered solely based on carbon source utilization. Tanglegrams were generated from genomic analyses, which revealed a significant correlation between the clustered observed growth phenotypes and whole genome sequence annotation data for all genes and relevant carbohydrate utilization genes. Furthermore, putative utilization operons were identified for carbohydrates that showed

strain-dependent growth. Gas-chromatography mass-spectrometry analysis revealed that the SCFAs produced by Lachnospiraceae are both carbohydrate- and species-dependent with differences in the ratio of SCFA production at a strain level.
Lachnospiraceae demonstrate intra- and inter-species variability in carbon source use. Genomic analyses demonstrate that these differences can be strongly linked to the strain-level variations in the genomic repertoire. Additionally, the strain and particular carbon source are both determinants in resulting metabolite production. Understanding the impacts of strain-level variation on nutrient niche access and metabolite production will aid in the development of future microbiome-directed therapeutics.

24. Dipendra Karki

Elucidation of the Function of the Novel AlkB domain of grapevine viruses

Karki D, Meng B

Alkylation B (AlkB) proteins are ubiquitous in diverse cellular organisms except for archaea, which are involved in reversing methylation damage, such as 1-methyladenine and 3-methylcytosine in DNA and RNA. An AlkB domain was also discovered as part of replicase (poly)protein in a subset of single-stranded positive-sense RNA viruses primarily infecting woody perennial fruit crops, including a few grapevines viruses belonging to the families Betaflexiviridae and Closteroviridae. As a newly identified protein domain in RNA viruses, the experimental evidence regarding the functional size of the viral AlkB for the most active demethylation was not determined. Furthermore, the role of viral AlkB in virus replication and virus-host interactions in the context of an intact viral genome infection in its natural host was still unknown. We used phage reactivation assays to reactivate the methylated RNA MS2 bacteriophages by using four different-sized viral AlkB constructs each for four grapevine viruses to determine the functional size of viral AlkB. In addition, we generated wildtype and alkB mutants full-length infectious cDNA clones of the Grapevine Pinot gris virus, one of the economically important emerging grapevine viruses with worldwide distribution to examine the role of viral AlkB in virus replication and long-term survivability in its natural host Vitis vinifera. The phage reactivation assay using viral AlkB showed viral AlkB with size 170 aa showed the most AlkB-mediated demethylation and further showed the impairment of the methylated phage reversal with the mutation of conserved residues. The mutant infectious clone of GPGV with mutation of conserved residues of viral AlkB exhibited reduced systemic infection, reduced pathogenicity, and low virus titer compared to the wildtype GPGV clone. Our results demonstrated the biological relevance of viral AlkB-mediated repair of deleterious methylation damage to maintain the long-term survivability of these RNA viruses in the woody perennial hosts.

25. Alexa King

Myocardial infarction causes transient pulmonary inflammation and edema in mice

King AN, Cochrane K, Brunt KR, Simpson JA

Following a myocardial infarction (MI), many patients suffer from respiratory complications, such as pulmonary edema, which decreases quality of life and survival. Pulmonary edema can develop as a consequence of pulmonary hypertension (a common pathological sequalae of heart failure), or from increased permeability of the alveolar-capillary barrier (from pulmonary inflammation). Following an MI, pulmonary edema is commonly attributed to pulmonary hypertension, due to the impaired cardiac function. However, patients also develop pulmonary edema in the absence of pulmonary hypertension, emphasizing that the response of the lung following an MI is relatively unknown. Therefore, the purpose of this study was to investigate the mechanism(s) driving fluid accumulation in the lungs post-MI. We hypothesized that pulmonary inflammation would be apparent post-MI but transient and contribute to the development of pulmonary edema. To model an MI, the left anterior descending coronary artery was permanently ligated in male CD-1 mice. At 3, 7, or 14 days following surgery, mice underwent echocardiography and cardiac catheterization of the left and right ventricles to evaluate structure and function. Mice underwent bronchoalveolar lavage for visualization of alveolar cells, and lungs were frozen for molecular analysis. Mice developed sustained impairments in left ventricle systolic and diastolic function by 3 days post-MI. Pulmonary edema (i.e., increased lung weight) was present at 7 days post-MI, but was transient, and could not be explained by pulmonary hypertension (i.e., right ventricle pressure). However, pulmonary inflammation preceded edema and was transient post-MI, shown by increased leukocytes and red blood cells in the bronchoalveolar fluid. Finally, RNA sequencing of whole lungs 3 days post-MI showed upregulation of inflammatory cytokines and genes involved in leukocyte migration and chemotaxis. Overall, we show that pulmonary inflammation is evident, precedes pulmonary edema, and is likely a major contributor to impairments in respiratory function following an MI.

26. Chloe King

Unveiling the mechanism of disease-causing cardiac actin mutations

King C, Dawson J

Cardiomyopathies are a type of heart disease associated with dysfunction in the ventricular myocardium, leading to reduced cardiac output and progressive heart failure. The main forms are dilated cardiomyopathy (DCM), characterized by atrophy of the ventricular myocardia, and hypertrophic cardiomyopathy (HCM), marked by hypertrophy of the ventricular myocardia. Mutations in cardiac sarcomere proteins are a common cause of cardiomyopathies. Understanding these variants helps uncover molecular mechanisms

driving DCM and HCM, enabling personalized treatments. The Sf9-baculovirus expression system utilizes insect cells to express wildtype and variant human recombinant actin, which can be used in a variety of assays such as the in vitro motility assay, ATPase assay, and tropomyosin binding assay, to determine the properties and disease mechanism of each variant cardiac actin. This research aims to delineate activity differences between variant ACTC1 and wildtype proteins to advance individualized treatment strategies, a promising approach to mitigate the global impact of cardiomyopathies.

27. Jordan Ko

Probing the role of extracellular DNA in Borrelia burgdorferi aggregates

Ko J, McCullough T, Wills M, Khursigara C

Extracellular DNA (eDNA) is a common feature of many bacterial biofilms across multiple species. eDNA is largely thought to serve as a stabilizing factor and a structural scaffold for the extracellular matrix that houses cells within biofilms. However, the degree to which it contributes to the overall biofilm biomass, and subsequent function, appears to vary. Therefore, we sought to investigate the potential role of eDNA in aggregates formed by Borrelia burgdorferi, the Lyme disease spirochete. By using a combination of confocal microscopy and enzymatic treatments, we sought to determine if eDNA is present in B. burgdorferi aggregates and if exposure to DNAse1 would impair aggregate development or disrupt pre-existing aggregates. Fluorescence microscopy of eDNA using a cell-impermeable nucleic acid stain, propidium iodide, was confounded by the presence of spirochetes with compromised membranes within the aggregate structure. Continuous exposure to DNAse1 did not significantly impair the development of aggregates in either abundance or individual volumes. Developed aggregates exposed to DNase1 did not undergo significant changes in volume or abundance. All together, the evidence does not indicate that eDNA is integral to the structure and formation of B. burgdorferi aggregates.

28. Joyce Kuipers

Neisseria gonorrhoeae and AMR: biofilms show limited attenuation in the presence of antimicrobials

Kuipers J, Khursigara CM

Neisseria gonorrhoeae is the etiological agent of the sexually transmitted infection gonorrhea. Studies have demonstrated there is strong evidence to suggest that biofilms play a role in the virulence of this organism. However, this role in natural infection remains unknown. Another growing concern is the development of antimicrobial resistance (AMR), and how biofilms contribute to this issue is not well studied. In this project, AMR profiles of gonococcal biofilms are described to the clinically relevant antimicrobials azithromycin, ceftriaxone,

gentamicin, and ciprofloxacin for the strains 49226 and FA1090. Minimum inhibitory concentrations (MIC) were determined using an adapted agar dilution method. Minimum biofilm inhibitory concentrations (MBIC) were determined using crystal violet staining to assess biofilm biomass. Challenged against these antimicrobials, the MIC values of both strains were <1 ug/mL. The corresponding MBIC values ranged from 1024 ug/mL to >8192 ug/mL. These results demonstrate gonococcal biofilms display high levels of resistance, showing limited growth attenuation in the presence of antimicrobials. Biofilms in context of antimicrobial resistance for *N. gonorrhoeae* has not been extensively studied, so these results provide insight that antimicrobials have limited impact on the maturation of biofilms. Overall, this project demonstrates that gonococcal biofilms may play a significant role in AMR and further study is needed into the mechanisms behind these interactions.

29. Patrick Lameront

Establishment of experimental systems to elucidate the functions and structural properties of unique open reading frames of Grapevine leafroll-associated virus 3

Lameront P, Meng B

The wine and grape industry plays a significant economic and cultural role and is impacted by over 80 viruses. Of those, Grapevine leafroll-associated virus 3 (GLRaV-3), the putative agent of grapevine leafroll disease, is the most widespread and devastating. Infected vines show impaired source-to-sink photosynthate and phloem transport, leading to characteristic downward rolling and darkening or chlorotic leaves. Disease symptoms increase year over year, reducing berry sugar accumulation and causing a decrease in juice quality. On a cellular level, the disease impacts the mitochondria and photosynthate trafficking from source tissue. GLRaV-3 contains the largest genome of its viral family *Closteroviridae* and encodes thirteen open reading frames (ORF). Several of these ORFs, including ORF8, ORF9 and ORF10, are unique to the *Ampelovirus* genus and little is known about their role in the viral replication cycle. The immediate goal of the proposed research project is to characterize these ORFs and elucidate their role in the cycle of infection of GLRaV-3. To this end ORF8, ORF9 and ORF10 have been individually cloned, tagged with fluorescent proteins, and ectopically expressed with *Nicotiana benthamiana* leaves to observe their transient subcellular localizations in vivo. Results suggest that when ORF8, ORF9 and ORF10 localize to the cytosol, cortical microtubules, and the nucleus, respectively. Further co-infiltrations with organelle markers and site directed mutagenesis have been done to verify these findings. In addition to microscopy, a variety of in-silico analysis using Colabfold, APBS, DALI, Plant PLoc and INSP has been conducted to support the initial findings. These approaches will be followed up with protein crystallography and structural determination of the three gene products. Ultimately, the aim of this project is to elucidate underlying GLRaV-3 infection

cellular mechanism which, in turn, may represent novel targets for grapevine biodefense strategies.

30. Sean Lee

Investigating temperature effects on the gene expression and activity of urease in *Sporosarcina pasteurii* for bio-cementation

Lee S, Khursigara MC, Monfared AK

The Gram-positive bacteria *Sporosarcina pasteurii*, is capable of performing microbiologically induced calcite precipitation (MICP) by catalyzing the hydrolysis of urea by utilizing the urease enzyme encoded within it. MICP is a natural biomineralization process and research in this field has gained traction in recent years for its applicative potential as a sustainable biological cement for environmental applications such as soil stabilization. While MICP via ureolysis shows strong potential under optimal laboratory conditions, environmental applications are limited by uncontrollable variables such as temperature. We aimed to investigate the effect that temperature has on *S. pasteurii* and its MICP capability by assessing urease activity and gene expression. The urease activity assay revealed the enzymatic activity at 20°C, 10°C, and 4°C negatively impacted urea hydrolysis from 1.97mM/min at 30°C to 1.11mM/min, 0.28mM/min, and 0.266 mM/min respectively. From this result, the gene expression of the urease operon (ureABCDEFG) was conducted and relative to control at 30°C, revealed a fold change difference of at least 0.5 across all genes and temperatures. However, comparisons across treatment samples revealed no significant changes in expression. Considering the relative differences between enzyme activity and relative gene expression, the disparity in urease activity do not correlate to the difference in fold change observed. The results indicate that the decrease in activity is not influenced solely by expression of ureABCDEFG but other additional factors may be contributing to the reduction in enzymatic activity.

31. Sarah Milinkovich

Identification of a small molecule inhibitor of group 2 capsule production in *Escherichia coli*

Milinkovich S, Mainprize I, Davis B, Vahidi S, Whitfield C

Capsular polysaccharides (CPSs) form capsule structures surrounding many bacteria. Group 2 glycolipid CPSs facilitate evasion of host immune responses and are critical virulence factors in extracellular pathogenic *E. coli* (ExPEC) isolates, which are prominent in neonatal meningitis, and bloodstream and urinary tract infections. ExPEC are increasingly resistant to carbapenems and extended β-lactams, so new therapeutics are needed. A high-throughput screen of a compound library including FDA-approved drugs, revealed group 2 CPS synthesis was inhibited by glitazones, a class of compounds developed to combat insulin resistance. A sub-fragment (5-(4-Hydroxybenzyl)thiazolidine-2,4-dione; hereafter INH7B) was responsible for the activity. In western immunoblots, CPS was barely detectable in the presence of 5 μM inhibitor but bacterial growth was unaffected by concentrations up to 500 μM. The group 2 CPS biosynthesis gene locus is arranged in two convergent transcriptional units and q-PCR experiments showed INH7B decreased transcription from both promoters by more than 100-fold. The compound structure, and an understanding of capsule transcriptional regulators, led to the hypothesis that the target is MprA, a member of the MarR superfamily. Differential scanning fluorimetry of purified MprA in the presence of 2 mM INH7B results in a 10°C increase in Tm compared to the ligand-free protein, suggesting a strong interaction, and providing evidence MprA is the cellular target of INH7B. These studies provide a foundation for systematic investigations to elucidate the mechanism of action of INH7B and describe an interesting antivirulence candidate active against pathogens on the WHO critical priority list.

32. Elma Misini

Modeling cardiovascular disease: investigating cardiac actin mutations in zebrafish for insights into cardiomyopathy and musculoskeletal abnormalities

Misini E, Prill K, Dawson J

Actin is a highly conserved protein found in all eukaryotic cells. It plays crucial roles in various cellular processes, notably cellular contractility. Within the sarcomere, interactions between actin filaments, myosin motor proteins, along with regulatory thin filaments troponin and tropomyosin orchestrate the generation of contractile forces. Mutations in genes encoding sarcomere proteins, such as the Alpha cardiac actin 1 (ACTC1) gene have been implicated in cardiovascular diseases such as cardiomyopathy in human patients. Patients with congenital heart disease may also manifest non-cardiac related congenital abnormalities, with musculoskeletal abnormalities being the most prevalent. To investigate the effects of

mutations in the ACTC1 gene, a transgenic CRISPR/Cas9 zebrafish line can be used as a model to study cardiac disease progression during key stages of development. The transparent appearance of larvae allow for clear visualization of both the pericardial cavity as well as the tail muscle, displaying phenotypes of cardiomyopathy and musculoskeletal myopathy. Birefringent light analysis on tail muscles show disorganized tail muscle in variant larvae when compared to the wildtype counterparts throughout early development. Brightfield imaging of the pericardial sac with subsequent heart rate analysis show a difference in heart rate between variant and wildtype larvae. Phenotype analysis provides a crucial platform for correlating observable traits with underlying genetic and protein expression patterns, aiding in the comprehensive understanding of biological mechanisms and disease processes. Overall, zebrafish provide a powerful model system for elucidating fundamental aspects of heart development, function, and disease, offering valuable insights into muscle disorders in humans.

33. Morgan Mizzoni

Autophagy-dependent increase in a ribosomal protein S24 splice variant aids in hypoxic cancer cell survival

Mizzoni M, Specker E, Kerry J, Uniacke J

In recent years, the view of the ribosome has shifted from a uniform, indiscriminate machine, to a dynamic protein complex with specific roles in gene regulation. The ribosome can vary in ribosomal protein composition in different cell types or in response to environmental stimuli such as hypoxia. Hypoxia, or low oxygen availability, is a feature of solid tumours that has been associated with malignant progression and reduced therapeutic response. Recently, a hypoxia-induced alternative splicing event in ribosomal protein S24 (RPS24) has been shown to produce distinct long and short RPS24 protein isoforms that can incorporate into ribosomes. Our group has found that the RPS24 long (RPS24L) to RPS24 short (RPS24S) transcript ratio is consistently increased in hypoxia, and to a greater degree in spheroids (in vitro tumour models), in several cancer cell lines. This hypoxic increase in the RPS24L transcript variant is dependent on autophagy induction, a process in which cytoplasmic components are degraded within the lysosome. Here, we show that overexpressing the RPS24L isoform in glioblastoma cells significantly increased cell proliferation and viability during hypoxic stress. Furthermore, the overexpression of RPS24L increased spheroid growth and the expression of hypoxia response genes. As translational machinery can be modified in response to stress, the RPS24L isoform may be incorporated into specialized ribosomes that aid in malignant progression. Additionally, the RPS24L protein isoform exhibited increased stability in hypoxia compared to the RPS24S isoform, which may have implications on the stability of the ribosome during times of stress. Future directions will involve examining nascent protein synthesis levels in RPS24L and RPS24S overexpressing

cell lines to determine if the stability difference between these two isoforms influences the rate of protein synthesis in times of stress. Further investigation may be of benefit in the treatment of cancers where tumour hypoxia contributes to therapeutic resistance.

34. Benjamin Muselius

Harnessing tandem mass spectrometry-based proteomics to construct a *C. neoformans* organ atlas

Authors: Muselius B, Droit A, Roux-Dalva F, Geddes-McAlister J

The ability to profile changes in both the host and pathogen proteomes during in vivo infection provides a unique opportunity to identify novel interactions driving infection for improved understanding of a diseased state and strategies for treatment. My PhD Thesis profiles the interplay between the human fungal pathogen, *Cryptococcus neoformans*, and the mammalian host using high-resolution mass spectrometry-based proteomics to define novel diagnostic and prognostic markers of disease. Here, I established an in vivo murine model of cryptococcal infection and collected 13 clinically relevant tissues and fluids across a temporal continuum of disease. Tandem mass tags combined with high pH fractionation were used to improve protein quantification and to increase proteome coverage, respectively, across the 780 samples. By profiling protein level alternations of *C. neoformans* in vivo I identified novel factors regulating important pathogenic events, such as pathogen infiltration of the blood brain barrier, identifying important fungal targets for novel therapeutics. Conversely from the host perspective, we define known host responses to fungal infection (e.g., cytokine production, complement cascade activation), as well as temporal distinctions across innate and adaptive immune system activation. While the proteomic profiles of each organ are distinct, common trends across the dataset provide promising avenues for discovery of novel biomarkers of disease. The goal of this research is to unveil a comprehensive cryptococcal organ atlas as a resource for the scientific community to identify weaknesses in the fungal arsenal for the development of targeted therapeutics and opportunities to empower the host immune system for clearance of infection. In this presentation I will present as yet unpublished findings from the data collected as well as the organ atlas resource database.

35. Liam Noseworthy

A structural and functional investigation into pyruvyltransferase protein WbbZ from *Klebsiella pneumoniae*

Noseworthy L, Kelly S, Kimber M, Ailas M, Lowary T

Pyruvyltransferases are a class of enzymes, found across diverse bacterial taxa, that link a pyruvyl group from a phosphoenolpyruvate donor to two adjacent hydroxyl groups (i.e. a ketal linkage) of a specific terminal monosaccharide within a polysaccharide. Pyruvylation alters the net charge, hydrophobicity and behaviour of the resulting sugar. Pyruvyltransferases are increasingly clinically relevant in light of the discovery of the O1b glycoform of the O1 lipopolysaccharide O antigen from *Klebsiella pneumoniae*, which is

partially modified by WbbZ through the addition of a terminal pyruvyl group. Lipopolysaccharide O antigen polysaccharides offer potential targets for the development of new immunotherapeutic treatments for carbapenem-resistant *Klebsiella pneumoniae*, a pathogen currently listed as a critical threat by the World Health Organization. This project aims to elucidate the detailed structural mechanistic features of the pyruvyltransferase WbbZ from *K. pneumoniae* as understanding the mechanism and role of this enzyme is important for the future development of immunotherapeutics. Currently, pyruvyltransferases are poorly biochemically characterized, with no structures known for any enzyme-substrate complexes, and even the order of ketal-pyruvate bond formation remaining uncharacterized. This investigation has thus far yielded a high-resolution structure of the enzyme WbbZ with phosphoenolpyruvate; this is the first such structure for a ketal-pyruvyltransferase and shows that the active site rearranges upon substrate binding. Notable changes include a reorganization and partial ordering of the extended loop between β6 and α8, as well as significant shifts in several key polar residues that either directly coordinate PEP, or which appear to become positioned to better bind the incoming acceptor. A synthetic acceptor has been produced in collaboration with the Lowry Lab and preliminary kinetic data has been collected for point mutations of six proposed catalytically relevant residues.

36. Una Pantic

Dok1/2 adaptor proteins regulate adhesion in kidney podocytes

Dutta NT, Martin CE, Phippen NJ, Pantic U, Lu P, New LA, Aoudjit L, Takano T, Gingras AC, Jones N

Podocyte adhesion to the underlying glomerular basement membrane is required for proper glomerular filtration, and this is mediated by dynamic signalling events within focal adhesions. Integrins are a major component of focal adhesions, and their activity and matrix binding is negatively regulated by adaptor proteins Dok1 and Dok2. Using proteomic techniques we identified specific binding partners for Nck1/2 in podocytes and one of the identified interactors was Dok1 thus we proceeded to investigate the role of Dok1/2 in podocytes. We evaluated the renal phenotype of Dok1/2 total body knockout (KO) mice and tested their response to podocyte injury using the nephrotoxic serum (NTS) model. We found that the loss of Dok1/2 does not impact glomerular function with aging. However, Dok1/2 KO mice produce an attenuated response to podocyte injury. We used BioID to determine Dok proximity interactors and we show that they associate with adhesome components, specifically cell-cell adhesion and actin filaments-based processes. Accordingly, we further show that glomeruli from Dok1/2 KO animals display higher surface levels of integrin β1 relative to WT controls with a concomitant increase in active integrin β1, and that phosphorylation of the focal adhesion protein paxillin is significantly increased. In primary podocytes, we observe enhanced paxillin levels in focal adhesions in the KO cells. Similarly,

in Dok1/2 KO podocytes generated by CRISPR-Cas9 genome editing, we observe that the KO's display higher surface levels of integrin β1 relative to the control and that there is a significant increase in adhesion. Finally, we demonstrate that Dok1 levels are increased following injury both in vitro and in glomeruli of PAN-treated rats. In conclusion, Dok1/2 regulate integrin signalling in podocytes, which may have implications in adhesion and maintenance of glomerular filtration.

37. Evan Perehiniak

Phosphorylation as a regulatory mechanism in SNARE-mediated ECM degradation

Perehiniak E, Coppolino M

SNAREs (Soluble N-ethylmaleimide-sensitive factor Attachment Protein Receptors) are proteins that mediate membrane fusion during vesicle trafficking pathways in cells. SNAREs have well-defined roles in neurotransmission and hormone secretion, and have recently been identified to play important roles in cell-extracellular matrix (ECM) interactions, including cell adhesion, cell migration, and cell invasion. SNARE-mediated trafficking is responsible for the transport of cellular components to and from ECM interaction sites. Components such as MT1-MMP, a key proteolytic enzyme that facilitates ECM degradation in invasive tumour cells, has been shown to be trafficked in a SNARE-dependent manner. This suggests that the regulation of SNAREs could play an important role in the intracellular trafficking that contributes to tumour cell invasion. Phosphorylation is a key post-translational modification, but its role in SNARE-mediated cell-ECM interactions and metastasis are largely unknown. Preliminary work suggests phosphorylation of SNAREs is a key mechanism that can regulate cell-ECM interactions such as adhesion and migration. A SNARE complex containing Stx4-SNAP23-VAMP7 that plays a previously characterized role in cell-ECM interactions appears to be regulated by phosphorylation. Particularly, Stx4 phosphorylation disrupts SNARE complex formation, correlating with reduced ECM degradation in tumor cells, while SNAP23 phosphorylation appears to promote SNARE complex formation, correlating with enhanced ECM degradation in tumour cells. This preliminary data highlights the complexity of the specific phosphorylation profiles of SNAREs and its impact on SNARE complex formation. These findings will be further explored by employing site-directed mutagenesis to investigate the impact of phosphorylation at specific amino acid residues in SNAREs. Current and future research aims to elucidate the role of SNARE regulation by phosphorylation in the trafficking of cellular components that are essential to cell-ECM interactions.

38. Nathaniel Petersen

Modulation of receptor tyrosine kinases using Ubiquitin Variant Induced Proximity

Petersen N, Liu Q, Jones N, Zhang W

Targeted protein degradation is increasingly being shown as a promising tool for disease treatment and protein research. One prominent method of targeted protein degradation is through the use of Proteolysis Targeting Chimeras (PROTACs). PROTACs function by hijacking the cell's ubiquitination system. This is done through the PROTAC's E3 ligase binding domain and target protein binding domain, which facilitates E3 activity to cause the target's ubiquitination and subsequent degradation by the 26S proteasome. PROTACs have been successful in protein degradation, however they are limited in target range by the few

recruitable E3 ligases. In the human proteome, over 600 E3 ligases exist, however only 4 are used commonly among PROTACs. To address this, the Zhang lab developed Ubiquitin Variant Induced Proximity (UbVIP) molecules, which involve a similar, but protein-based structure. UbVIP uses ubiquitin variants (specifically mutated ubiquitin moieties) as the E3 recruiting domain, allowing for unique E3s to be recruited. The protein-based structure of UbVIP may also allow for more easily developed target binders by incorporating endogenous protein binders into the UbVIP system. In my work, I have integrated SH2 domains, as well as some recently developed SH2 superbinding domains into UbVIP molecules to target several receptor tyrosine kinases (RTKs) as a novel method for their degradation. To further show the versatility of UbVIP, I have assisted in developing proof-of-concept stabilizing UbVIP molecules; by swapping the UbVIP E3 ligase binder for a deubiquitinase binder, the mechanism changes from the addition of ubiquitin to its removal. This allows for proteins that may be excessive targets of degradation by the ubiquitin proteasome system to be stabilized – such as some tumor suppressors. My research shows that there is potential for UbVIP molecules to modulate levels of RTKs, and in ways that may be unique to PROTACs.

39. Alicia Plourde

Understanding the role of conformational dynamics in the substrate promiscuity of the Pup-proteasome system in mycobacteria

Plourde A, Vahidi S

Mycobacterium tuberculosis uses the Pup proteasome system (PPS) to resist the human immune response. Therefore, components of the PPS are potential drug targets against TB. PafA is the sole ligase that links prokaryotic ubiquitin-like protein (Pup) to exposed lysine residues of target proteins in an ATP-dependent manner. Pupylated substrates are recognized and degraded by the proteasome machinery. The literature largely treats PafA as a monomer in solution, with only subtle indications that it may exist as a dimer with modulated activity and affinity towards pupylation targets. This aspect of PafA structure: function relationship remains largely unexplored. Here, we use HDX-MS together with biochemical and biophysical tools to examine the structure and dynamics of PafA in different oligomeric and nucleotide states.

SEC revealed that PafA elutes as a pair of monomeric and dimeric peaks, with bias towards the monomer. This distribution shifts over time, further favouring the monomeric state. Interestingly, the dissociation of the dimer is slowed in the presence of nucleotide, whereas monomeric PafA remains monomeric in the presence or absence of nucleotide. Moreover, none of our explored conditions promoted the dimerization of PafA, further supporting the notion that monomeric PafA predominates in solution.

We used the data above to guide our HDX-MS experiments to capture the differences in the conformational dynamics of the dimeric and monomeric fractions of PafA. Interestingly, several PafA peptides exhibit EX1 kinetics, suggesting the presence of cooperative unfolding events. These data suggest PafA may interconvert slowly between various conformational or oligomeric states. Moreover, these data revealed a decrease in deuterium uptake in the predicted dimerization interface when comparing nucleotide-free and -bound states of monomeric PafA. Intriguingly, this finding is in contrast with our SEC data that showed nucleotide binding does not promote dimerization of PafA.

40. Noah Presley

Development of a high-throughput in-vitro motility assay

Presley N, Dawson J

Cardiovascular diseases are a leading cause of death among the global population. Within cardiovascular diseases, cardiomyopathies including hypertrophic cardiomyopathy (HCM), and dilated cardiomyopathy (DCM) are significant diseases contributing to large financial strain on the economy. Furthermore, treatments for HCM focus on the treatment of symptoms not the underlying cause of disease and fail to provide comprehensive relief for a significant portion of patients. Currently there are no commercially available treatments for DCM and treatments also focus on reliving symptoms however are insufficient and result in the need for heart transplantation frequently. Current assays at use to screen novel compounds for treatment are limited by their throughput and detail into the causative mechanisms relating protein function to disease. This project focuses on developing a high-throughput in vitro motility assay to meet the needs of drug discovery for disease treatment.

41. Nicolas Rolfe

Exploring substrate specificity of Sad, the only class-3 aldehyde dehydrogenase that prefers steroid substrates

Rolfe N, Seah SYK

Aldehyde dehydrogenases (ALDHs) constitute an enzyme superfamily encompassing more than 10 discrete classes. Within the bile acid side chain degradation pathway, an aldehyde dehydrogenase, Sad (side chain aldehyde dehydrogenase) is the only class-3 member (ALDH-3) with the unique ability to catalyze reduction of steroid substrates. To investigate the mechanisms underlying steroid binding, Sad from the mesophile, *Comamonas testosteroni* (Sad C. test) and the thermophile, *Aquabacterium tepidiphilium* (Sad A. tepid) were purified and shown to have broad specificity towards steroids containing different substituents and low specificity towards non-steroidal aldehydes. Sad (A. tepid) was also crystallized and its 3D-structure solved to 1.8 Å resolution using X-ray crystallography and molecular replacement. Sad and its homolog benzaldehyde dehydrogenase (BADH) share conserved structures and catalytic residues but BADH prefers significantly smaller substrates, containing a single aromatic ring. Several residues in the BADH active site were found to correspond to smaller residues in Sad (A. tepid). These residues in BADH were replaced to the corresponding residues in Sad (A. tepid) by site-specific mutagenesis. While BADH showed negligible activity towards steroid aldehydes, the F400A and L125T variants have significantly increased specificity towards steroids. Insights gained from this study can inform future protein engineering efforts to create a biocatalyst for the synthesis of novel

steroid-based drug precursors with improved pharmacological properties, including highly reactive steroids with 3C and 5C side chains aldehydes.

42. Erin Rudolph

Inhibition of MAPK signaling by *Pseudomonas aeruginosa* quorum sensing molecules leads to attenuation of mast cell immune functions

Rudolph E, Guth C, Andersen A, Devereaux A, Clark AJ, Pundir P

Mast cells are innate immune cells abundant in the tissues adjacent to the external surfaces such as skin, lung, or intestine and play a pivotal role in host defense. Given the potent antimicrobial and immunoregulatory functions of mast cells, if bacteria could employ strategies to suppress mast cell function is paramount of their survival and dominance. Multi-drug resistant *Pseudomonas aeruginosa* is notoriously hard to treat due to its capacity for quorum sensing, a process that allows Pseudomonas to form antibiotic-resistant biofilms and evade immune responses. We recently identified that mast cells can detect bacterial quorum sensing molecules (QSMs) through cell surface receptors. The interaction between *P. aeruginosa* QSMs and mast cells remains unknown. We hypothesize that *P. aeruginosa* QSMs alter immune functions of mast cells by interfering with receptor-mediated downstream signalling pathways.

Aims: 1. To determine whether *P. aeruginosa* QSMs affect FceRI- and MRGPRX2-mediated human mast cell activation and mediator release. 2. To dissect the underlying signaling mechanism impacted by *P. aeruginosa* QSMs on FceRI- and MRGPRX2-activated human mast cells

Results: We demonstrate that *P. aeruginosa* QSMs inhibit human mast cell degranulation and histamine release through impairment of FceRI- and MRGPRX2-induced intracellular calcium mobilization. Moreover, these QSMs attenuate the release of early- and late-phase do novo synthesized proinflammatory mediators following FceRI- and MRGPRX2-mediated human mast cell activation. Treatment with *P. aeruginosa* QSMs inhibits PI3K and PLCg phosphorylation and downstream MAPK signaling in both FceRI- and MRGPRX2-activated human mast cells.

Conclusions: Our findings show that *P. aeruginosa* QSMs are inhibitors of FceR1and MRGPRX2 G protein-coupled receptor pathways of mast cell activation, providing insight into how *P. aeruginosa* can maintain its dominance and evading the immune system. This work advances our knowledge in crucial areas of host-pathogen interactions and opens new avenues for targeting antimicrobial resistant pathogens. Funding: This research was supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC RGPIN-2022-03453), the Canada Foundation for Innovation, and the College of Biological Science Team Building Grant.

43. Neethu Shaji Saji

Investigating the oligomeric state of FtsK(N) in *Escherichia coli* cell division

Shaji Saji N, Khursigara CM

Escherichia coli is a model organism extensively used to study bacterial cell division. It divides by forming a multiprotein complex called 'divisome' at the division site. Filamentous Temperature Sensitive Protein K (FtsK) is an essential protein for divisome formation. The N-terminal of FtsK (FtsKN) is the only region necessary for cell division, and it is proposed to oligomerize into a hexamer upon recruitment to the midcell. Despite being essential in cell division, neither its structure nor FtsK(N) function is fully understood. The principle of co-variance analysis states that amino acids that interact with each other in a tertiary structure are likely to co-evolve to maintain the structure. Previous studies in our laboratory showed that a specific single mutation (L32F) in FtsKN (220) generated using covariance analysis produces cells with elongated phenotypes and voids within the cell. The typical phenotype is recovered when another mutation (C169F) is introduced along with the first mutation. According to the current model of FtsKN (220) and AlphaFold models, these residues are not in proximity to each other to co-evolve. Another potential explanation is that these residues might contact each other during oligomerization. The single mutation, L32F might disturb the oligomerization of FtsK (N220) and produce elongated cells. I will use Size Exclusion Chromatography to compare the oligomeric state of L32F and L32F-C169F to wild-type FtsK(N220). This may help identify whether the impaired cell division producing elongated phenotype with voids is due to the inability of FtsK (N220) to oligomerize properly. In this project, I will address central questions concerning the structure, function, and interactions of FtsK) that are highly conserved across bacterial species, including pathogens. My proposed research aims to help elucidate the complex role of divisome in cell division.

44. Erka Shata

Nck adaptor proteins regulate breast cancer cell metastasis and invasion

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The adaptor proteins Nck1 and Nck2 are well established signaling nodes in pathways regulating actin cytoskeleton remodeling. Work from multiple laboratories suggests Nck may be involved in regulating processes correlated with invasion and metastasis of cancer, and although these proteins were first identified as oncogenes nearly 30 years ago, there is scarce in vivo evidence supporting their ability to induce tumour development or metastasis. We have now determined that Nck1 and Nck2 are central regulators of breast cancer progression. We have systematically profiled Nck across TCGA-BRCA and related datasets and identified upregulation of Nck in breast cancer which correlates with negative outcomes. We confirmed these findings in patient tumours, and further showed that overexpression of Nck1 and Nck2 in breast cancer cells results in enhanced invasion and gelatin degradation.

Next, using an in vivo loss of function strategy in mice which allows simultaneous expression of activated oncogene HER2/ErbB2 and Cre recombinase in mammary epithelial cells, we have shown that deletion of both Nck1 and Nck2 (Nck-DKO) significantly extends survival by delaying tumour onset and also reduces incidence of metastasis. Protein analysis of tumours lacking Nck1 and Nck2 shows significant alterations to focal adhesion signaling dynamics. To identify key Nck-dependent regulators, we have used CRISPR to generate matching WT and Nck-DKO cell lines and performed RNA-seq. Our findings provide new physiological insights verifying the role of Nck as an oncogene, and they reveal its potential as a target to inhibit breast cancer.

45. Margaret Smith

Exploring the impact of positive charge distribution on the ability of dehydrins to interact with proteins in vivo

Smith M

Dehydrins are intrinsically disordered proteins that improve plant desiccation tolerance. The defining feature of a dehydrin is the K-segment: a semi-conserved sequence motif, rich in lysine. One way in which dehydrins are thought to improve desiccation tolerance is through the stabilization of proteins. However, it is not clear how this is achieved. There is evidence of a sequence-order-independent effect acting on lactate dehydrogenase. Incongruently, a sequence-order-dependent effect has been found to increase structure in yeast frataxin homologue 1. This appears to be related to the positive charge distribution of the dehydrin. I propose that the positive charge distribution found in the K-segments may improve the ability of dehydrins to interact with and stabilize a wide array of proteins. To test this, I will express a YSK2 -type dehydrin in tobacco BY-2 cells and *E. coli*. In-cell cross-linking will be performed followed by mass spectroscopy to identify the interaction partners of the dehydrin. This will be repeated for dehydrin constructs with a rearranged sequence. Finally, volume exchange for convective transfer will be used to deliver dehydrin into BY-2 cells in the hopes that in-cell NMR can be performed. This could help to improve our understanding of the ways in which intrinsically disordered proteins can function.

46. Isaac Sullivan

Purinergic regulation of the zebrafish spinal-cord-injury response

Sullivan I, Stefanova E, Scott A

In contrast to mammals, adult zebrafish (*Danio rerio*) undergo successful neural regeneration following spinal cord injury (SCI). Radial glia lining the zebrafish central canal function as neural progenitors that undergo a massive injury-induced proliferative response before differentiating into both neurons and glial cells. However, the molecular mechanisms that underlie these processes remain elusive. Among the signaling pathways that are dysregulated following mammalian SCI is the purinergic signaling system. While purines such as ATP and its metabolites mediate diverse cellular processes within the mammalian central nervous system (CNS), their roles have not been explored within the zebrafish CNS. Given that the purinergic system is evolutionarily conserved among vertebrates, we sought to characterize potential roles for P2X7 and P2Y2 receptor signaling in neurogenesis following SCI in adult zebrafish. Our findings demonstrated that expression of P2X7 and P2Y2 receptors were both upregulated following injury, and activation of P2X7 signaling in particular enhanced injury-induced neurogenesis in this species. Further work will elucidate the roles of both receptors in these natural regenerators following SCI.

47. Charlotte Townsend

Unravelling the dynamic regulation of Kv1 channel localization at the axon initial segment

Townsend C, Sanders S, Becke N

The axon initial segment (AIS) serves as a pivotal site for action potential initiation within neurons, housing a diverse array of ion channels, notably the voltage-gated potassium ion channels (Kv1). Disruption of Kv1 channel trafficking has been implicated in various channelopathies, including episodic ataxia type-I and epilepsy, emphasizing their fundamental role in neuronal function. Still, the molecular mechanisms governing the precise AIS distribution of Kv1 channels remain poorly understood. The AIS is dynamic and plastic, such that there are alterations in morphology and ion channel composition in response to neuronal activity. The post translational lipid modification, palmitoylation is critical for the clustering of Kv1 channels at the AIS. Palmitoylation involves the covalent attachment of long-chain fatty acids to cysteine residues via a thioester linkage. Importantly, palmitoylation is critical for the clustering of Kv1 channels at the AIS and the reversible nature of palmitoylation makes it well suited to dynamically regulate Kv1 channel localization in response to changes in neuronal activity. Preliminary data following cLTD treatment reveal alterations in the AIS localization of Kv1 channel subtypes and changes in Kv1 channel palmitoylation. These findings could suggest interplay of dynamic palmitoylation and Kv1 channel localization, shedding light on the molecular mechanisms underlying synaptic plasticity and neuronal excitability. Furthermore, this research holds promise for uncovering novel therapeutic targets for Kv1-related channelopathies.

48. Madison Turner

Allosteric regulation of proteasome function as established by H/D exchange mass spectrometry, cryo-EM, and molecular dynamics simulations

Turner M, Hoff SE, Uday AB, Velyvis A, Zeytuni N, Bonomi M, Vahidi S

Proteasome systems are key regulators of cellular homeostasis via the degradation of redundant or damaged proteins. Central to these pathways is a self-compartmentalized protease, known as the 20S core particle (CP). This barrel-shaped oligomer is formed through the stacking of four heptameric rings in an α7-β7-β7-α7 configuration, resulting in the catalytic chamber being sequestered at the center of the complex. To protect against spurious degradation, the α-rings cap each end of the proteasome, with the N termini of each subunit spanning the axial pore to bar substrate entry. This necessitates interaction with regulatory particles (RPs) to initiate proteolysis. RP binding to the α-ring induces a conformational change resulting in an opening of the axial pore. Recently, RP binding was also shown to allosterically activate proteolytic function in the eukaryotic and archaeal 20S

CPs. However, allosteric relationships have yet to be described in the less common, bacterial proteasomes. Of interest is the proteasome system of *Mycobacterium tuberculosis* (Mtb), which is a critical virulence factor during tuberculosis infection. Kinetic findings suggest that the Mtb 20S CP displays long-range (~80 Å) allosteric modulation across the α- and β-subunits, yet these structural changes have been largely indiscernible in the available high-resolution structures. This implies that subtle conformational shifts may regulate Mtb 20S CP activity. This project has therefore explored regions of conformational plasticity within the Mtb proteasome to investigate allosteric pathways that modulate its function. Three 20S CP variants have been functionally and structurally characterized using kinetic assays and hydrogen/deuterium-exchange mass spectrometry, respectively. My findings support an allosteric network across the α- and β-rings that extend from the RP binding sites to the catalytic residues in the Mtb proteasome. By understanding regions of communication, this research can uncover dynamic points of regulation within this molecular machine that may have implications for future tuberculosis therapeutics.

49. Brandon Ulch

The role of phosphatidylcholine:diacylglycerol cholinephosphotransferase (PDCT) in enhancing unsaturated oil production in soybean seeds

Ulch B, Xu Y

Soybean is a widely cultivated crop known for its abundant production of valuable oils and proteins. It is of particular interest because of its high abundance of unsaturated fatty acids. However, the mechanisms responsible for this phenotype require further research. To address this knowledge gap, this study aims to investigate the role of an understudied enzyme called phosphatidylcholine:diacylglycerol cholinephosphotransferase (PDCT) in soybean seed oil biosynthesis.

PDCT has been demonstrated to catalyze the interconversion of phosphatidylcholine (PC) and diacylglycerol (DAG), two crucial molecules involved in triacylglycerol (TAG) biosynthesis. Fatty acids attached to DAG are often incorporated directly into TAG, while those attached to PC are instead recognized as substrates for acyl-modifying enzymes such as desaturases or hydroxylases. Therefore, PDCT likely plays an important role in determining oil quality in plant species by shuttling fatty acids between DAG and PC, enabling modification of their acyl-chains.

The aim of this project is to uncover the molecular mechanisms by which the two isoforms of Glycine max PDCT contribute to the accumulation of unsaturated fatty acids in Glycine max. We will be characterizing these proteins by determining where they are most expressed, performing in-vivo enzymatic assays in *Saccharomyces cerevisiae* and complementation assays in *Arabidopsis thaliana* knock-out lines to determine their activity. Findings from this

work will bolster our understand of how TAG is synthesized in plants and may lead to the development of novel soybean varieties with improved nutritional and industrial applications.

50. Jordan Willis

GID1 and GID5 of the GID E3 ubiquitin ligase have a broad impact on gene expression in response to changing carbon conditions in *Saccharomyces cerevisiae*

Willis J, Ozashin E, Rentz J, van der Merwe G

The yeast *Saccharomyces cerevisiae* has evolved to utilize a wide range of carbon sources but typically preferentially ferments glucose, resulting in the inhibition of gluconeogenic processes through carbon catabolite repression. Fermentative metabolism requires a concert of synergistic mechanisms involved in carbon sensing, transcriptional inhibition, and protein degradation, thereby enabling the cell to rapidly respond to changes in environmental and intracellular sugar availability. The GID protein complex (GIDc), also known as the Vid30 Complex (Vid30c), is an E3 ubiquitin ligase required for the degradation of metabolic enzymes, including fructose-1,6-bisphosphatase (FBPase), to inhibit gluconeogenic processes. While best understood in the context of carbon metabolism, recent studies have uncovered new recognition subunits and posttranslational targets for GIDc ligase activity under a variety of cellular conditions. While these discoveries have greatly enhanced our knowledge of its potential role in overall metabolic regulation, the impact of GID gene deletion upon the transcriptional regulation of metabolism is still poorly understood. Here, we use a combination of qRT-PCR, RNA-Seq, Western blotting, and bioinformatics tools to delineate the genes and pathways affected in yeast cells by GID gene deletion. Our results show GIDc involvement in the gene expression of multiple biological processes including carbon, amino acid, and acetyl-CoA metabolism. Furthermore, we show that these GID subunits are required for the carbon-sensitive posttranslational regulation of the well-studied transcription factor Mig1, a key participant in carbon catabolite repression. In combination, these results expand upon our understanding of GIDc involvement in the transcriptional regulation of metabolism.

51. Michael Woods

Unraveling the mechanism behind ClpX disruption in overcoming antifungal resistance in *C. neoformans*

Woods M, Geddes-McAlister J

Of the known invasive fungal species, *Cryptococcus neoformans*, an opportunistic mammalian fungal pathogen found ubiquitously in the environment, is the major causative agent of cryptococcosis in immunocompromised individuals (e.g., HIV/AIDS). Optimal treatment of cryptococcosis is not readily available in many regions of the world, leaving fluconazole monotherapy as the primary prescribed treatment. Importantly, the exposure of the fungus to azole fungicides used in agriculture that are functionally similar to fluconazole can lead to cross-resistance with environmentally obtained strains showing reduced

susceptibility to fluconazole in the clinic. With the lack of diversity in current antifungal treatments, it is imperative to understand the mechanisms behind resistance to find methods to overcome resistance, allowing available treatment to work as intended. Recent works in the JGM lab have characterized the disruption of ClpX, an ATP-dependent unfoldase involved in protein homeostasis in the mitochondria of eukaryotic cells, to overcome fluconazole resistance in fluconazole-resistant *C. neoformans* strains. Our goal here was to determine how the disruption of ClpX leads to this phenomenon. Fluconazole targets lanesterol-14**α** demethylase, a critical enzyme in the ergosterol biosynthesis pathway, altering sterol composition and ultimately leading to inhibition of fungal growth. Here, we found a decrease in ergosterol content of clpXΔ deletion fluconazole-resistant *C. neoformans* strains after fluconazole treatment. Additionally, using state of the art mass spectrometry based quantitative proteomics, we compared the proteome of fluconazole-resistant *C. neoformans* to the clpXΔ deletion mutant and found significant differences in proteins involved in ergosterol biosynthesis, iron regulation and heme biosynthesis. Lastly, clpXΔ deletion strains treated with iron and heme showed a reduction in sensitivity to fluconazole, suggesting there is a connection between these pathways and fluconazole susceptibility that is influenced by ClpX. In all, this study highlights the relationships between iron, heme and ergosterol content during fluconazole resistance, with ClpX providing an essential role.