UNIVERSITY OF SASKATCHEWAN

Proteomics Research in Interactions and Structure of Macromolecules

UNIVERSITY OF SASKATCHEWAN
College of Medicine
MEDICINE.USASK.CA

10th Annual Protein Structure, Function and Malfunction (PSFaM) meeting

June 15-16, 2023

Saskatoon, Saskatchewan

Human Health, RNA Biology and Function, and Cross-disciplinary Methodologies

Albert M. Berghuis
McGill University

Lisa Craig
Simon Fraser University

Justyna Fert-Bober
Cedars-Sinai Heart Institute

GB03, Health Sciences Building, University of Saskatchewan

PRISM Center: medicine.usask.ca/prism/

Contacts:
Dr. Adelaine Leung: adelaine.leung@usask.ca; Dr. Jenny-Lee Thomassin: jet198@mail.usask.ca;
Dr. Erique Lukong: kiven.lukong@usask.ca

Proteomics Research in Interactions and Structure of Macromolecules

PSFaM Protein Structure, Function and Malfunction

Bringing Western Provinces Together for Scientific Exchange

June 15-16, 2023
Saskatoon, Saskatchewan

Sub-Themes and Methodologies:
Dive into three exciting research areas with wide-ranging applications in structural biology, bioinformatics, and mass spectroscopy:

- Human Health: Investigate infectious and cardiovascular diseases, and explore the development of plant-based technologies to improve our environment
- RNA Biology and Function: Unravel the complexity and versatility of RNA molecules
- Cross-disciplinary Methodologies: Discover novel insights, therapeutic targets, and cutting-edge techniques that span across diverse research areas

Connect with experts and learn about cutting-edge methodologies, techniques, and tools that transcend specific research areas.

Keynote Speakers:
- Albert Berghuis – McGill University
- Lisa Craig – Simon Fraser University

Rising Star Speaker:
- Justyna Fert-Bober – Cedars-Sinai Heart Institute

Confirmed Speakers from Western Provinces:
- Gerd Prehna – University of Manitoba
- Ute Kothe – University of Manitoba
- Andrew MacMillan – University of Alberta
- Howard Young – University of Alberta
- Douglas Muench – University of Calgary
- Nicole Hansmeier – University of Regina

University of Saskatchewan Western College of Veterinary Medicine WCVM.USASK.CA

VIDO

University of Saskatchewan College of Medicine MEDICINE.USASK.CA

University of Saskatchewan College of Medicine Department of Biochemistry, Microbiology and Immunology MEDICINE.USASK.CA

University of Saskatchewan College of Medicine Department of Anatomy, Physiology and Pharmacology MEDICINE.USASK.CA

BRUKER

SHRF Saskatchewan Health Research Foundation

Agilent

PacBio

HORIBA

ThermoFisher Scientific

GIFS Global Institute for Food Security

Growing science for life

Nutrien - a Founding Partner

Register Here: https://medicine.usask.ca/prism/events.php

June 15, 2023

Dear Delegates,

Welcome to the 10th Annual Protein Structure, Function and Malfunction (PSFaM) Meeting taking place on June 15 and 16, 2023, in the Health Sciences Building at the University of Saskatchewan. The PSFaM Meeting, inaugurated in 2012, was conceived by the Proteomics Research in Interactions and Structure of Macromolecules (PRISM) members at the University of Saskatchewan. The meeting aims to bring together leading academic scientists, researchers, research scholars and trainees from universities and institutes in Western Canadian provinces to exchange and share their experiences and research results on all aspects of protein structure and function, engage in discussions about their research, and meet their peers. The PSFaM community, therefore, fosters an inclusive and collaborative environment and encourages knowledge sharing and the advancement of research and innovation in protein topics.

The 10th PSFaM Meeting focuses on three exciting research areas with wide-ranging applications in structural biology, bioinformatics, and mass spectroscopy. These areas include human health, RNA biology and function, and cross-disciplinary methodologies. We have outstanding invited speakers for this milestone meeting, including two excellent keynote speakers, Dr. Albert Berghuis (McGill University) and Dr. Lisa Craig (Simon Fraser University). Our Rising Star speaker (a new PSFaM program feature) is Dr. Justyna Fert-Bober from the Cedars-Sinai Medical Center in Los Angeles. We also have a slate of confirmed speakers from the University of Manitoba, the University of Alberta, the University of Calgary, the University of Regina, and the University of Saskatchewan. It is important to stress that the PSFaM meetings also provide a platform for trainees/students and future rising stars to give oral and poster presentations, networking opportunities, exposure to interdisciplinary methodologies, and access to valuable resources for career development.

On behalf of the organizing committee and delegates, we thank all our sponsors for their invaluable and generous support. It is through their contributions that we have been able to uphold our commitment to making this symposium free and accessible to all participants. We are also thankful to all the student volunteers, session chairs, oral and poster presentation judges and all those working behind the scenes to make this conference successful.

PSFaM 2023 Organizing Committee:

Dr. Adelaine Leung (Chair)
Dr. Jenny-Lee Thomassin (Co-Chair)
Robyn Claypool (Administrative Coordinator)
Dr. Maruti Chandra Uppalapati
Dr. Kristen Conn
Dr. Poonam Dhindwal
Dr. Tony Ruzzini
Dr. Kiven Erique Lukong (Director, PRISM)

Notes

10th Annual PSFaM Program

Day 1 - June 15, 2023

8:15	**Registration and Light Breakfast**
	Atrium Health Sciences Building
8:45	**Welcome and Opening Remarks**
	GB03 Health Sciences Building
8.45 - 8.55	Opening Remarks: Adelaine Leung (2023 PSFaM chair) and Erique Lukong (PRISM Director)
8:55 - 9:00	Welcome address: Marek Radomski, Vice-Dean Research, Medicine, University of Saskatchewan
9:00	**Session 1 - Part 1**
	GB03 Health Sciences Building Chairs: Jenny-Lee Thomassin and Hannah Braun
9:00 - 9:45	**Keynote Lecture: Albert M. Berghuis (McGill University)** Antibiotic Resistance up close: a structural biology perspective on a global health threat
9:45 - 10:00	**Trainee Talk: Dinesh Wellawa (University of Saskatchewan)** Bioluminescent Salmonella Enteritidis to study the role of ferrous and ferric iron uptake in the colonization and infection of *Gallus gallus domesticus*
10:00 - 10:30	**Invited Speaker: Gerd Prehna (University of Manitoba)** Molecular mechanism of type VI secretion system membrane protein chaperones
10:30	**Coffee Break**
	Atrium Health Sciences Building
11:00	**Session 1 - Part 2**
	GB03 Health Sciences Building
11:00 - 11:15	**Trainee Talk: Maria Yousefi (University of Saskatchewan)** Stability of APOBEC3F in presence of APOBEC3 Antagonist HIV-1 Vif Increases at the Expense of co-expressed APOBEC3H Haplotype I
11:15- 11:45	**Invited Speaker: Nicole Hansmeier (University of Regina)** Making themselves at “home”- remodeling of cell surfaces and phagosomes by a food pathogen
11:45	**Lunch Break**
	Pick-up at Room 1B21 Health Sciences Building, please bring ticket
13:00	**Session 2**
	GB03 Health Sciences Building Chair: Adelaine Leung
13:00 - 13:30	**Invited Speaker: Howard Young (University of Alberta)** Functional and structural re-classification of variants of uncertain significance (VUS) in cardiomyopathies
13:30- 14:15	**Keynote Lecture & Rising Star: Justyna Fert-Bober (Cedars-Sinai Heart Institute)** Challenges and strategies for targeted citrullination site identification and quantification using mass spectrometry
14:15	**Networking Break** **Optional Tour of the Canadian Light Source (CLS) and/or Western College of Veterinary Medicine (WCVM)**
	Tour leaves from GB03
15:30	**Poster Session**
	Louis' lower level
19:00	**Dinner Buffet**
	Louis' lower level, please bring ticket

Notes

Day 2 - June 16, 2023

8:15	**Registration and Light Breakfast**
	Atrium Health Sciences Building
9:00	**Session 3 – Part 1**
	GB03 Health Sciences Building
	Chairs: Adelaine Leung and Maruti Uppalapati
9:00 - 9:30	**Invited Speaker: Michael Levin (University of Saskatchewan)**
	Peptide-derived small molecules that target the RNA binding protein hnRNP A1 inhibit neurodegeneration – implications for the pathogenesis and treatment of multiple sclerosis
9:30 - 9:45	**Trainee Talk: Madison Adolph (Vanderbilt University)**
	RADX controls RAD51 filament dynamics to regulate replication fork stability
9:45 - 10:15	**Invited Speaker: Douglas Muench (University of Calgary)**
	Sequence-specific RNA-binding proteins: structural and functional characteristics and potential for engineering RNA target specificity
10:15	**Coffee Break**
	Atrium Health Sciences Building
11:00	**Session 3 - Part 2**
	GB03 Health Sciences Building
11:00 - 11:15	**Invited Speaker: Ute Kothe (University of Manitoba)**
	Modifications in the T arm of tRNA globally determine tRNA function and cellular fitness
11:15- 11:45	**Invited Speaker: Andrew MacMillan (University of Alberta)**
	Regulation of gene expression by the protein heart of the spliceosome
11:45	**Lunch Break**
	Pick-up at Room 1B21 Health Sciences Building, please bring ticket
13:00	**Session 4**
	GB03 Health Sciences Building
	Chairs: Jenny-Lee Thomassin and Hannah Braun
13:00 - 13:30	**Keynote Lecture: Lisa Craig (Simon Fraser University)**
	Understanding Type IV pilus-mediated uptake and secretion
13:30- 14:15	**Trainee Talk: Ahmed Oraby (University of Alberta)**
	Unlocking the secrets: unleashing allosteric inhibitors for viral protein targeting through computational structure-based drug design
14:15	**Networking Break - Optional Tour Protein Crystallization and Characterization Facility (PCCF), Museum of Natural Sciences**
	Tour leaves from GB03
15:00	**Session 5**
	GB03 Health Sciences Building
	Chair: Maruti Uppalapati
15:00 - 15:30	**Invited Speaker: Franco Vizeacoumar (University of Saskatchewan)**
	Targeting the CINful genome
15:30 - 15:45	**Trainee Talk: Vincent Maranda (University of Saskatchewan)**
	Developing combination therapies for telomerase-overexpressing clear cell carcinoma of the ovaries
15:45 -16:00	**Selected talk: Prem Prakash Das (National Research Council)**
	Label-free parallel reaction monitoring (PRM) for targeted protein quantification: a case study in foodomics
16:00	**Awards Ceremony and Closing Remarks**
	GB03 Health Sciences Building
	Awards presented by Erique Lukong, PRISM Director

Notes

Table of Contents

Notes

Links

PSFaM 2023 Website Link

https://medicine.usask.ca/prism/events.php

Online Poll to vote for Keynote Speakers for 2024

https://PollEv.com/discourses/doOO6G0UsVrzjfjVVGDga/respond

ORAL PRESENTATIONS

Keynote Speakers

Albert Berghuis (McGill University)

Antibiotic Resistance up close: a structural biology perspective on a global health threat

The dangers antibiotic resistance poses to human health needs little introduction. Statistics, such as that more than one million deaths annually are directly attributable to resistant bacteria, have been well publicized. The WHO has advocated a multi-prongeds approach to address this global health threat, which includes developing new medicines. We have used structural biological approaches to examine various mechanisms of antibiotic resistance, with the objective of informing the development of new therapeutic options. Notably, we have examined enzyme-mediated resistance to aminoglycoside and macrolide antibiotics. Our findings have underscored the difficulties of drug development in the context of widespread multi-drug resistance but have also revealed viable avenues to combat resistance.

Lisa Craig (Simon Fraser University)

Understanding Type IV pilus-mediated uptake and secretion

Type IV pili are long thin protein polymers present on many bacterial pathogens, including *Vibrio cholerae*, enterotoxigenic *E. coli* and *Neisseria gonorrhoeae*. These filaments are critical virulence factors and attractive targets for vaccines and therapeutics. Type IV pili are both dynamic and adhesive, features that are key to their functions in host cell adherence, twitching motility, DNA and bacteriophage uptake, and secretion. Type IV pili rapidly extend across the cell wall, adhere to cell or abiotic surfaces or to substrates such as DNA or phage, then just as rapidly retract, pulling the bacteria close to or along the bound surface or pulling substrates into the cell. We have used X-ray crystallography and cryo-electron microscopy to decipher the molecular structure of the pilus and pilin subunits and to understand pilus assembly and retraction. We are now focused on the molecules located at the pilus tip, which is becoming recognized as the “business end” of the pilus. For this seminar, I will describe our structural, biochemical and genetic data, which have provided a mechanistic understanding of Type IV pilus dynamics and functions with insights into how they might be exploited for antibacterial therapies.

Justyna Fert-Bober (Cedars-Sinai Heart Institute)

Challenges and strategies for targeted citrullination site identification and quantification using mass spectrometry

By altering amino acids after protein synthesis, posttranslational modifications (PTMs) increase the structural and functional diversity of the proteome. Therefore, PTMs control key biological processes, ranging from cellular signaling to functions in homeostasis and pathology. Citrullination is the posttranslational conversion of a gene-encoded arginine into the non-encoded amino acid citrulline [3], mediated by peptidyl arginine deiminases (PADs) in a calcium-dependent manner. The introduction of

citrulline dramatically changes the structure and function of proteins. It has been implicated in several physiological and pathological processes. Physiological processes include epithelial terminal differentiation, gene expression regulation, and apoptosis. Rheumatoid arthritis, multiple sclerosis, Alzheimer's disease, and carcinogenesis are examples of human diseases in which protein citrullination involvement has been demonstrated. Despite its importance, the 'ultimate' method to identify and quantify site-specific protein citrullination using mass spectrometry (MS) has not yet been fully established. This is as much a function of the dynamic range of instrumentation as it is the complexity surrounding the isolation and behavior of citrullinated peptides. Citrullinoprotoeome analysis using MS can be quite challenging when analyzing and quickly becomes a daunting task when attempting to perform proteome-wide measurements since citrullination only results in an increase in the protein mass with less than one mass unit and the loss of one positive charge. Data-dependent tandem MS-based methods that are useful for the discovery and characterization of novel citrullination sites often lack the dynamic range and quantitative aspect required for studying the citrullinoproteome. While targeted methods such as multiple reaction monitoring do provide a highly specific and quantitative methodology, they are not suited for the initial discovery of previously unreported sites of citrullination. Data-independent acquisition represents a relatively new approach for simultaneous qualitative and quantitative sample analysis that holds promise for filling this technological gap. Better detection and quantification methods for citrullination on a protein will help to further elucidate the biology of this PTM.

Invited Speakers from the Western Provinces

Gerd Prehna (University of Manitoba)

Molecular mechanism of type VI secretion system membrane protein chaperones

Type VI secretion systems (T6SSs) are dynamic nanomachines that Gram-negative bacteria use for numerous biological functions, including killing competing bacteria and pathogenesis. Bacteria use the T6SS to deliver protein effectors into adjacent cells to kill rivals and establish niche dominance. In the evolutionary arms race of potent toxic effectors, the T6SS has been adapted to both secrete effectors directly into the periplasm of a prey-cell, and to secrete membrane proteins that target a toxin to the prey-cell cytoplasm. Using the Salmonella T6SS as a model system, our lab is studying the versatility of both T6SS effector secretion and function. Current research is focused on revealing the molecular mechanism of chaperone-mediated membrane protein secretion by the T6SS and characterizing the biochemistry of the unique effectors that allow Salmonella serovars to outcompete the host microbiome.

Ute Kothe (University of Manitoba)

Modifications in the T arm of tRNA globally determine tRNA function and cellular fitness

All tRNAs are highly modified and harbor 5-methyluridine at position 54 and pseudouridine at position 55 in the T arm, which are generated by the enzymes TrmA and TruB, respectively. Escherichia coli TrmA and TruB have both been shown to act as tRNA chaperones, and strains lacking trmA or truB are outcompeted by wildtype. Here, we investigate how TrmA and TruB contribute to cellular fitness. Deletion of trmA and

truB in E. coli causes a global decrease in aminoacylation and alters other tRNA modification such as acp3U47 and 4-thiouridine. Whereas global protein synthesis is not significantly changed in DtrmA and DtruB, the expression of many specific proteins is altered at the translational level. In conclusion, we demonstrate that universal modifications of the tRNA T arm are critical for global tRNA function by enhancing other tRNA modifications, tRNA folding, tRNA aminoacylation, and translation of specific genes thereby improving cellular fitness and explaining their conservation.

Andrew MacMillan (University of Alberta)

Regulation of Gene Expression by the Protein Heart of the Spliceosome

Splicing of precursor-messenger RNAs (pre-mRNAs) is an essential part of the maturation of protein-coding RNAs and is a key gene regulatory mechanism in all eukaryotic cells. Removal of pre-mRNA introns and ligation of exons is a two-step process carried out by a complex RNA-protein assembly: the spliceosome. While the essential chemistry of splicing is RNA-catalyzed, proteins regulate the fidelity and efficiency of splicing through the orchestration of assembly and transitions within the spliceosome. We are interested in the role of a large protein, PRP8, the most highly conserved nuclear protein in eukaryotes, found at the heart of the spliceosomal machinery. We have identified a regulatory switch found within PRP8 and are working to understand its role in the regulation of the spliceosomal mechanism.

Franco Vizeacoumar (University of Saskatchewan)

Targeting the CINful genome

Tumor heterogeneity represents a major therapeutic challenge that underlies treatment resistance. The polo-like kinase 1 (PLK1) is overexpressed across all cancers, inducing chromosomal instability (CIN). As CIN promotes intra-tumor heterogeneity, and direct PLK1 inhibition has not yielded clinical advances, we aimed to target its genetic interactions by using the synthetic dosage lethality (SDL) approach to overcome the challenges associated with heterogeneity and ensure the selective killing of PLK1 overexpressing tumor cells. We performed a genome-wide shRNA screen and selected 105 most likely SDL candidates using unbiased computational strategies. We evaluated them by an in vivo pooled CRISPR screen and a matching in vitro arrayed CRISPR screen in a patient-derived xenograft model of breast cancer. This identified 65 top SDL hits. We next used direct capture Perturb-seq to characterize individual SDL hits at the single cell level and assess their potential in overcoming tumor heterogeneity. Our unbiased strategy identified IGF2BP2/IMP2 as a top SDL hit, whose loss-of-function effectively eliminates PLK1 overexpressing cells. We also found that IGF2BP2 suppression, either genetically or pharmacologically, downregulates PLK1 and, ultimately, reduces tumor growth. Taken together our observations strongly suggest that targeting genetic interactions of PLK1 should be considered as a promising therapeutic avenue. As PLK1 is overexpressed in multiple cancers, our work is likely to trigger broad therapeutic implications.

Howard Young (University of Alberta)

Functional and Structural Re-Classification of Variants of Uncertain Significance (VUS) in Cardiomyopathies

As the genetic landscape of cardiomyopathies continues to expand, the identification of missense variants in disease-associated genes frequently leads to a classification of variant of uncertain significance (VUS). For the proper reclassification of such variants, functional characterization is an important contributor to the proper assessment of pathogenic potential. Several missense variants in the calcium transport regulatory protein phospholamban have been associated with dilated cardiomyopathy. However, more than 40 missense variants in this transmembrane peptide are currently known and most remain classified as VUS with little clinical information. Similarly, missense variants in cardiac myosin binding protein have been associated with hypertrophic cardiomyopathy. However, hundreds of variants are known and many have low penetrance and are often found in control populations. Herein, we focused on novel missense variants in phospholamban, an Ala15-Thr variant found in a 4-year-old female and a Pro21-Thr variant found in a 60-year-old female, both with a family history and clinical diagnosis of dilated cardiomyopathy. The patients also harbored a Val896-Met variant in cardiac myosin binding protein. The phospholamban variants caused defects in the function, phosphorylation, and dephosphorylation of this calcium transport regulatory peptide, and we classified these variants as potentially pathogenic. The variant in cardiac myosin binding protein alters the structure of the protein. While this variant has been classified as benign, it has the potential to be a low-risk susceptibility variant because of the structural change in cardiac myosin binding protein. Our studies provide new evidence for missense variants previously classified as benign or VUS.

Douglas Muench (University of Calgary)

Sequence-specific RNA-binding proteins: structural and functional characteristics and potential for engineering RNA target specificity

RNA-binding proteins (RBPs) have important roles in regulating the physiology of their RNA targets throughout the lifetime of the RNA, from processing in the nucleus to translation in the cytosol. Several classical RBP types exist, each having different mechanisms of binding to their RNA target. RBPs typically bind to a wide range of RNA targets, often recognizing RNA structure and sequence as well as interactions with regulatory proteins. However, some RBP types bind to their RNA targets with nucleotide sequence specificity. The PUF family of RBPs is widespread in eukaryotes and typically consists of eight tandem Puf repeats that each binds a single nucleotide in a one-repeat:one base manner. We identified an RNA target sequence of an Arabidopsis nucleolar PUF that is ten nucleotides in length, thereby increasing the sequence specificity of its binding to mRNA across the transcriptome. This RNA-binding domain represents a novel backbone that can be engineered and appended to functional domains to regulate specific mRNA targets in the cell. Recent RNA-interactome studies have also identified new types of RBPs, including those that 'moonlight' as RBPs on top of their primary role in the cell. We have identified a metabolic enzyme that possesses sequence-specific RNA-binding activity that may serve to regulate the activity of the enzyme or its synthesis through translational autoregulation. Although these two RBP types demonstrate sequence-specific binding, their mechanisms of binding are distinct.

Nicole Hansmeier (University of Regina)

Making themselves at "home"- remodeling of cell surfaces and phagosomes by a food pathogen

Successful infections require intricate interplays between pathogens and their respective hosts. These start on the surface of host cells and include adhesion and continue with clever manipulations of host mechanisms, which ultimately result in a pathogenic niche for the pathogen to live and reproduce. In this

talk, I will present a number of approaches to investigate these interactions on the model pathogen *Salmonella enterica* serovar Typhimurium. Specifically, I will discuss a method to express and investigate the adhesiome, pathways leading to cytoskeletal and phagosomal remodeling and present some insights on intracellular replication niche structures on the intracellular life of the pathogen.

Michael Levin (University of Saskatchewan)

Peptide-derived small molecules that target the RNA binding protein hnRNP A1 inhibit neurodegeneration – implications for the pathogenesis and treatment of multiple sclerosis

Introduction: Neurodegeneration, the death and damage to neurons and axons, underlies permanent disability in multiple sclerosis (MS). Yet, regardless of their marginal effect on neurodegeneration, immunotherapies are still the primary treatment for MS. We discovered that dysfunction of the RNA binding protein heterogeneous nuclear ribonucleoprotein A1 (A1), including its cytoplasmic mislocalization and aggregation in neurons, drives neurodegeneration in MS and its models. We hypothesize that correcting A1 dysfunction will rescue neurodegeneration in relevant MS models.

Methods: Two model systems were used to assess A1 dysfunction: stress-induced A1 dysfunction in primary mouse neurons and optogenetics induced A1 aggregation in a differentiated neuronal cell line. An A1-specific peptide was used to inhibit A1 dysfunction. In silico modeling and thermal shift binding assays were used to model peptide-A1 interactions, which identified small molecules with the potential to inhibit A1 dysfunction. Small molecules were examined for their ability to inhibit A1 dysfunction and neurodegeneration in vitro as well as for toxicity and efficacy in mice.

Results: A1 nucleocytoplasmic mislocalization and cytoplasmic A1 aggregation caused decreased neurite length, a marker of neurodegeneration. The A1-specific peptide reduced A1 mislocalization ($p<0.001$) and A1 aggregation ($p<0.0001$) and rescued neurite length ($p<0.001$) in both model systems. In-silico modeling identified three peptidomimetic small molecules that bound A1 and reduced A1 mislocalization ($p<0.001$) and aggregation ($p<0.001$) and rescued neurites ($p<0.01$) like the A1-specific peptide. The small molecule with the greatest in vitro effects was non-toxic and efficacious in mice with experimental autoimmune encephalomyelitis (EAE) ($p<0.01$), the most common preclinical model of MS.

Conclusion: A1 dysfunction contributes to the pathogenesis of MS and A1 specific small molecules inhibited A1 dysfunction and ameliorated neurodegeneration, the root cause of disability in MS, thus fulfilling a major therapeutic gap in the treatment of MS.

Selected Speakers

Madison Adolph (Vanderbilt University)

RADX controls RAD51 filament dynamics to regulate replication fork stability

The RAD51 recombinase forms nucleoprotein filaments to promote double-strand break repair, replication fork reversal, and fork stabilization. The stability of these filaments is highly regulated, as both too little and too much RAD51 activity can cause genome instability. RADX is a single-strand DNA (ssDNA)

binding protein that regulates DNA replication. Here, we define its mechanism of action. RADX directly and selectively interacts with ATP- bound RAD51, stimulates ATP-hydrolysis, and destabilizes RAD51 nucleofilaments. The RADX interaction with RAD51, in addition to its ssDNA binding capability, is required to maintain replication fork elongation rates and fork stability. Furthermore, BRCA2 can overcome the RADX-dependent RAD51 inhibition. Thus, RADX functions in opposition to BRCA2 in regulating RAD51 nucleofilament stability to ensure the right level of RAD51 function during DNA replication.

Maria Yousefi (University of Saskatchewan)

Stability of APOBEC3F in presence of APOBEC3 Antagonist HIV-1 Vif Increases at the Expense of co-expressed APOBEC3H Haplotype I

The seven human APOBEC3 enzymes (APOBEC3A through H, excluding E) are host restriction factors. Most of the APOBEC3 enzymes can restrict HIV-1 replication with different efficiencies. The HIV-1 Vif protein combats APOBEC3-mediated restriction by inducing ubiquitination and degradation in the proteasome. APOBEC3F and APOBEC3G can hetero-oligomerize, which increases their restriction capacity and resistance to Vif. Here we determined if APOBEC3C, APOBEC3F, or APOBEC3G could hetero-oligomerize with APOBEC3H haplotype I. APOBEC3H haplotype I has a short half-life in cells due to ubiquitination and degradation by host proteins, but is also resistant to Vif. We hypothesized that hetero-oligomerization with APOBEC3H haplotype I may result in less Vif-mediated degradation of the interacting APOBEC3 and stabilize APOBEC3H haplotype I, resulting in more efficient HIV-1 restriction. Although we found that all three APOBEC3s could interact with APOBEC3H haplotype I, only APOBEC3F affected APOBEC3H haplotype I by surprisingly accelerating its proteasomal degradation. However, this increased APOBEC3F levels in cells and virions in the absence or presence of Vif and enabled APOBEC3F-mediated restriction of HIV-1 in the presence of Vif. Altogether, the data suggest that APOBEC3 enzymes can co-regulate each other at the protein level and that they cooperate to ensure HIV-1 inactivation rather than evolution.

Vincent Maranda (University of Saskatchewan)

Developing combination therapies for telomerase-overexpressing clear cell carcinoma of the ovaries

Epithelial ovarian cancer (EOC) is the most common cause of gynecological cancer death. Each year, an estimated 230,000 women are diagnosed with EOC globally, and 150,000 women die of the disease. Ovarian cancer ranks fifth in cancer deaths among women, accounting for more deaths than any other cancer of the female reproductive system. Ovarian clear cell carcinoma (OCCC), a subtype of ovarian cancer, is the second most common type of ovarian cancer affecting approximately 10-13% of women with ovarian tumors. OCCC is also the predominant histologic type of gynecological malignancy that harbors activating mutations within the telomerase reverse transcriptase (hTERT) promoter, causing its increased expression. The telomerase (hTERT) gene is hyperactive in many cancers and has been identified as a potential target for treatment. Although this led to multiple telomerase-targeting approaches, disappointingly, none have been successful in clinics. To circumvent this concern, our team has applied a genetic approach called synthetic dosage lethality (SDL), to exploit hTERT overexpression to identify potential targets to treat cancer. SDL is a genetic concept, where a normally non-lethal gene inactivation kills cells only in the context of overexpression of another gene like hTERT. My project aims to apply this concept to treat hTERT-overexpressing cancers and specifically, ovarian cancer. Our laboratories have used lentiviral-based, pooled CRISPR/Cas9 and pooled shRNA-screening platforms to systematically query the entire genome and recently identified several SDL partners of hTERT. These potential partners will be

validated using a novel CRISPR-based strategy in an in vivo pooled screen, which is already in progress. We also plan to apply these therapies in combination with existing therapies to amplify the efficiency of treatment against cancers. The results will identify new targets exploiting hTERT overexpression and provide preclinical evidence to support the development of novel OCCC therapies.

Dinesh Wellawa (University of Saskatchewan)

Bioluminescent Salmonella Enteritidis to study the role of ferrous and ferric iron uptake in the colonization and infection of Gallus gallus domesticus

Salmonella enterica subspecies *enterica* Serovar Enteritidis (SEn) is a major bacterial cause of gastroenteritis in humans. Consumption of contaminated chicken meat, eggs, and other poultry products has been driving outbreaks of salmonellosis. We investigated the kinetics of early events after oral gavage of day-old chickens, using the bioluminescent imaging (BLI) technique. SEn was genetically engineered to continuously produce light (490nm) using the light-emitting module called lux operon. We were able to accurately define the onset of primary colonization as early as 30 hours post-infection (p.i.) in the cecum with the aid of photonic signal strength detected by ex-vivo imaging and colony count values. For the first time, utilizing BLI, we identified a potential virulence factor which can significantly impact yolk sac colonization/infection: iron homeostasis may be critical to pathogenesis in a chicken model. Lack of information in a chicken model encouraged us to investigate the role of high-affinity iron uptake systems namely siderophore (enterobactin and salmochelin) mediated ferric iron uptake and FeoABC-mediated ferrous iron uptake. Our approach was to use mutant strains devoid of each of the uptake systems in a co-infection chicken model together with the wild-type strain. One strain in each mutant versus wildtype combination was chromosomally marked with the lux operon to enable differential detection and counting in the co-infection experiment. There was a significant colonization defect in the cecum and extraintestinal sites associated with a strain devoid of both siderophore synthesis genes and feoBC. The two uptake systems were functionally redundant by contributing to the virulence of SEn and the data suggested that chicken mount an iron starvation response towards SEn. Our findings indicated that the interaction of Salmonella with the avian host started in an environment close to the equilibrium regarding adequate iron supply to SEn but facing a mounting iron starvation during early colonization. This underlines the importance of having alternative routes for iron import and explains a functional redundancy between the two high-affinity iron acquisition systems.

Ahmed Oraby (University of Alberta)

Unlocking the Secrets: Unleashing Allosteric Inhibitors for Viral Protein Targeting through Computational Structure-Based Drug Design

Respiratory viruses are the most common cause of disease in humans causing high rates of morbidity and mortality worldwide. Among these, respiratory syncytial virus (RSV) infection is the biggest cause of severe lower respiratory infections in children in North America and one of the leading causes of infant death worldwide. There is no vaccine, limited drug treatment options, and so there is a great need for effective drugs. The development of antiviral agents became a top priority due to the emergence of SARS-CoV-2 in late 2019, which is causing a global pandemic of acute respiratory disease. The viral RNA-dependent RNA polymerase complex (RdRp) represents an attractive therapeutic target for inhibition of virus infection because it makes the RNA genomes that are packaged into progeny virus particles. If this process is halted or slowed then virus infection is stopped. This has been a highly successful approach in the antiviral drugs

developed for HIV and hepatitis C virus (HCV) infections. Broad-spectrum antiviral agents are desirable because they could protect against the emergence of new viruses, like pandemic SARS-CoV-2. We have discovered a new class of compounds that inhibit the infectivity of RSV and SARS-CoV2. In this project, we are determining the mechanism of viral inhibition caused by this class of compounds. RNA-dependent RNA polymerase assays showed that the compounds act by targeting the RdRp replication complex. With no solved co-crystallized ligands with our viral RdRp polymerase targets, we have employed computational studies, including docking, molecular dynamics, and binding free energy calculations, to model the interactions of our compounds with RSV and SARS-CoV-2 RdRp polymerases. These efforts have led to the discovery of two compounds with substantial viral polymerase inhibitory activity for both viruses.
In summary, this project may lead to the development of a new class of broad-spectrum, non-nucleoside antiviral compounds against RSV and SARS-CoV-2 with high selectivity, effectiveness, and low toxicity.

Prem Prakash Das (National Research Council)

Label-free Parallel Reaction Monitoring (PRM) for targeted protein quantification: A case study in foodomics

PRM represents a targeted quantitative proteomics technique that enables the simultaneous quantification of multiple target proteins. The procedure involves acquiring prior knowledge of the target proteins and developing an assay, followed by analyzing the samples using mass spectrometry (MS) in PRM mode. This approach offers exceptional selectivity, sensitivity, and high-throughput capabilities for quantifying specific proteins within complex matrices compared to other targeted quantitative methods such as selected reaction monitoring (SRM) or multiple reaction monitoring (MRM). When employing a label-free targeted PRM strategy, external standards (e.g., BSA peptide) are utilized in defined quantities for PRM MS runs, and the most suitable peptide is subsequently chosen for generating a calibration curve. The PRM method serves the purpose of quantitating and validating target proteins (for example biomarkers and allergenic proteins) that have been identified through discovery-based proteomics investigations. Moreover, it is an efficient technique for quantifying proteins present in low concentrations within a given sample.

Pea (*Pisum sativum*) serves as a cost-effective and high abundant alternative source of protein, cultivated for its fresh and dried seeds. The protein content of peas varies from 23% to 31% based on factors such as environmental conditions and pea crop varieties. Pea protein can be obtained in different forms, including flours, air-classified protein-enriched flours (containing approximately 40-60% protein), or wet fractionated isolates (containing roughly 90-95% protein). Among legumes, pea proteins have great nutritional value and high protein content. However, the presence of potentially allergenic proteins, such as non-specific lipid transfer proteins (nsLTP) and convicilin (Pis s 2), is a matter of growing concern as consumption of plant-based proteins increase in North America. Label-free-targeted quantification using PRM can be utilized to accurately determine the amount of allergen present in variety of sample types. In this case study, pea flour, concentrate and isolate samples were analyzed by PRM and the amounts of potential allergen targets were compared. The results obtained through PRM revealed variations in allergen levels across the different samples. Therefore, PRM is one of the most advanced proteomics tools for the simultaneous quantification of multiple proteins in complex samples with a femtomole-level detection sensitivity.

POSTER PRESENTATIONS

1. **Mohammad-Amin Abdollahifar** (University of Saskatchewan)

Vimentin as a contributing factor in SARS-CoV-2-induced orchitis: a study on postmortem testicular autopsy of COVID-19 patients

Introduction: Coronavirus disease 2019 (COVID-19) emerged in late December 2019, leading to a global pandemic with significant morbidity and mortality. The causative agent, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), utilizes distinct receptors and co-receptors for cell entry. Vimentin has recently been identified as a potential co-receptor for SARS-CoV-2. Objective: The present study aimed to investigate the relationship between vimentin expression and SARS-CoV-2-induced orchitis, given the high expression of vimentin in testis tissue. Methods: Testes autopsy samples were collected from COVID-19 patients and a control group who died due to accidental causes. Gene expression, immunohistochemical, and stereology studies were conducted to assess vimentin expression level, leukocyte infiltration, and testicular damage. Results: Our findings revealed a significant overexpression of vimentin in COVID-19 patients compared to controls ($P < 0.001$). Additionally, COVID-19 patients exhibited a substantially higher level of leukocyte infiltration than the control group ($P < 0.001$). Severe testicular damage was observed in COVID-19 patients, with a significant reduction in spermatogenic, Sertoli, and Leydig cells. Conclusion: These results suggest a strong association between vimentin expression and COVID-19-induced orchitis. Further investigations are warranted to elucidate the precise mechanism by which vimentin contributes to SARS-CoV-2 entry into testicular cells.

2. **Shabnam Abdi** (University of Saskatchewan)

Expression and function of the EphA2 receptor in human and canine melanoma

Melanoma is the most lethal type of malignant skin cancer in humans and dogs since it spreads rapidly throughout the body. Despite significant advances in treatment, cancer at an advanced stage has a poor prognosis. Hence, more effective treatments are needed to enhance outcomes with fewer side effects. Erythropoietin-producing hepatocellular receptors are the largest family of receptor tyrosine kinases and are divided into two subfamilies, EphA and EphB, both of which play a significant role in disease especially cancer. Due to their association with proliferation and invasion in many aggressive types of cancer, Eph receptor tyrosine kinases (Eph RTKs) are promising cancer therapy molecules. Because these receptors have not been studied in canine melanoma, we investigated how EphA2 influences survival and tumorigenicity of melanoma cells.

Expression of EphA2 protein in canine melanoma cell lines (Parks and Jones) and human melanoma cell line (A375) was evaluated by Western blot. Melanoma cells were transduced with lentiviral particles encoding Eph-targeting shRNAs or non-silencing shRNAs (control) for silencing the expression of an Eph receptor and silencing was confirmed by Western blotting and immunofluorescence. The effect of siRNA treatment on cellular proliferation, colony formation, invasion was analyzed by Resazurin assay, Matrigel invasion assay and wound healing respectively.

Expression of EphA2 was detected in canine and human melanoma cell lines. Moreover, stably silencing EphA2 by specific shRNAs significantly and consistently decreased the expression of EphA2 protein in both human and canine melanoma cells. Proliferation, colony formation, invasion and migration of melanoma cells were significantly decreased in EphA2 siRNA-treated cells compared to the control.

Our data provide the first functional evidence that the EphA2 receptor plays a critical role in the malignant cellular behavior of melanoma.

3. **Nayoung Kim** (University of Saskatchewan)

Defining the Proximity Interacting Partners of the Tyrosine Kinase SRMS

Tyrosine kinases play an important role in the intracellular signaling cascade and are involved in regulating various key biological processes including growth, metabolism, differentiation, and apoptosis in response to both external and internal stimuli. SRMS (Src-Related kinase lacking C-terminal regulatory tyrosine and N-terminal Myristylation Sites) is one of three members of the non-receptor type tyrosine kinase family known as the BRK family kinases. The other two members of the family include BRK and FRK. Like BRK and FRK, SRMS possesses three relatively conserved, structured domains, namely the SH3, SH2, and kinase domain. While both BRK and FRK possess a C-terminal regulatory tyrosine residue known to regulate their enzymatic activity, SRMS lacks such a residue. Therefore, its mode of autoregulation has been found to involve its unique 50 amino acids-long N-terminal region. Previous research has found BRK to display oncogenic properties including promoting cellular growth and proliferation, and FRK playing an opposing role in its involvement in tumour suppression. Unfortunately, the role of SRMS to date is currently limited. However, recent publications show promising insight of SRMS in promoting tumour growth through phosphorylating the scaffolding protein FKBP51, as well as its promise as a biomarker for colorectal and gastric cancers. Additionally, SRMS was also found to play a potential role in platinum resistance in ovarian cancer. SRMS displays an interesting, primarily cytoplasmic, punctate localization within the cell, known as the SRMS Cytoplasmic Punctae (SCP). Currently, it is unknown what proteins SRMS may be interacting with to form these structures. Therefore, we aim to identify the proximity interacting partners of SRMS using a technique known as Biotin Identification (BioID) in the hopes to better understand the potential cellular functions of the kinase. Recently, we have conducted biotin affinity purification and mass spectrometry analyses in collaboration with the Gingras Lab at the Lunenfeld-Tanenbaum Research Institute (LTRI) in Toronto for the identification of SRMS proximity partners. We have narrowed down and chosen proteins to validate via co-immunoprecipitation experiments. We predict that proteins involved in the upstream regulation of the PI3K-AKT pathway interact with SRMS and we hope to conduct functional assays for these pathways. Defining the proximity partners of SRMS may result in a clearer understanding of the regulation and function SRMS and the role this kinase plays in diseases such as cancer.

4. **Andrew Poholka** (University of Alberta)

Regulation of pre-mRNA Splicing During Starvation in Yeast

Splicing of precursor-messenger RNAs (pre-mRNAs) through the removal of introns is an essential part of the maturation of protein-coding RNAs in eukaryotic cells. Recently, it has been shown that introns mediate the starvation response in yeast, downstream of the TORC1 and PKA pathways. Both pre-mRNA and intron accumulation under starvation conditions result in a downregulation of splicing of RNAs coding

for ribosomal proteins with a resulting decrease in ribosome synthesis and enhanced cell survival. Intron deletion has been shown to negatively affect cell survival in stationary phase; cell viability can be restored by reintroduction of the deleted introns. Here, we show that significant enhancement of splicing by mutation of an essential splicing factor also negatively affects cell survival, further demonstrating the importance of the splicing pathway in response to changing growth conditions in yeast.

5. **Iris Yuxiu Chen** (University of Saskatchewan)

Content and immunogenicity of gluten-like proteins in Canadian oats

Glutens in cereals such as wheat are ethanol-soluble prolamins comprising two distinct groups gliadins and glutenins. These proteins contain repetitive sequences of six-to-eight amino acids rich in proline and glutamine, and some of them are highly immunogenic which can cause an enteropathy disorder (Celiac disease) in the small intestine leading to chronic diarrhea and malnutrition. Oat is a cereal crop with increasing popularity due to the presence of unique healthy compounds. However, it also contains a small amount of avenins, a group of prolamins similar to wheat gliadins. The purpose of this study is to determine content and immunogenic activity of gluten-like proteins in Canadian oats.

Twenty-four oat cultivars from East and West Canada were used to extract albumins, globulins, avenins (gliadin-like) and aveninin (glutenin-like) sequentially. Quantitative assays showed avenins were in range of 6 to 17%, and aveninins were in a range from 5 to 11% of the total proteins in Canadian oats. Sandwich ELISA assays with two different types of monoclonal antibodies against immunogenic epitopes in wheat α-gliadin were used to measure the levels of immunogenic gluten-like proteins in Canadian oats. Results showed that almost all Canadian cultivars possessed the immunogenic gluten-like proteins less than or close to 20 ppm (or 20 mg/kg, the gluten-free standard), except for one cultivar Reid from East Canada that was substantially higher than the standard (about 150 ppm). This result indicates most Canadian cultivars are safe and suitable for consumption for all consumers including Celiac disease individuals.

6. **Ananaya Charaya** (University of Saskatchewan)

Identification of DDX41 protein interactome under stress conditions by BioID and Affinity Purification.

DDX41 (DEAD-box 41 protein) is a member of superfamily 2 family of RNA helicases. It is involved in crucial processes, including genome stability, mRNA splicing, ribosome biogenesis, translation, cell differentiation, and innate immunity. Mutations in DDX41 cause myeloid malignancies, especially myelodysplastic syndrome and acute myeloid leukemia (MDS/AML). Previous studies in Dr. Wu's lab have showed that DDX41 protein level experienced a dynamic change when the cells are exposed to distinct stress conditions like oxidative stress (sodium arsenite), microbial infection (HSV-1), and radiation exposure (Infrared radiations). The dynamic expression is accompanied by formation of P-bodies (marker EDC4) and shifting in the sub-cellular localization of DDX41 from the nucleus to cytoplasm. However, the molecular mechanisms regulating DDX41's expression and sub-cellular localization are unknown. We hypothesize that DDX41-interaction proteins may regulate the dynamic expression and cellular localization of DDX41. Although DDX41 has been found in the spliceosomal complex, its interactome under stress conditions has not been reported.
To identify the interacting proteins of DDX41, we utilized two approaches: BioID and affinity purification. For BioID, we constructed DDX41 fusion proteins (WT and a MDS/AML patient mutant R525H) tagged with

FLAG at N-terminus and TurboID at C-terminus. We have successfully constructed the vectors; however, the N-terminal FLAG tagged DDX41 is localized in the cytoplasm, which is different from endogenous DDX41 that is localized in the nucleus. Alternatively, we used affinity purification, where DDX41 is tagged with FLAG at its C-terminus, and immunofluorescence showed the protein is localized in the nucleus. Now we are optimizing the stress treatment conditions and scaling up samples for mass spectrometry analysis. Identification and validation of DDX41 interacting proteins will lead to better understanding of the regulatory pathways and potential drug targeting in treatment of DDX41 related blood cancer.

7. **Taniya Saha** (University of Saskatchewan)

Deciphering the role of oncogenic lemur tyrosine kinase 3 (LMTK3) in breast cancer stem cells.

Deciphering the role of oncogenic kinase Lemur tyrosine kinase 3 (LMTK3) in breast cancer stem cells
Breast cancer (BC) is the most frequent type of malignancy in women worldwide. Breast cancer stem-like cells or bCSCs (CD44+CD24-/low) are tumor-initiating cells believed to be responsible for therapeutic resistance and cancer relapse in BC. Lemur tyrosine kinase 3 (LMTK3), a member of LMTKs family of serine–threonine kinases, has been implicated in breast, lung, gastric carcinoma for its contribution to cellular proliferation, migration, invasion, actin cytoskeleton remodeling, and intrinsic & acquired endocrine resistance. LMTK3, identified as an estrogen receptor α (ERα) regulator, is over-expressed in most aggressive forms of BC and confers both chemo- and endocrine resistance. Hence, molecular targeting of LMTK3 should be a promising therapeutic approach to target bCSCs. Elucidating LMTK3-driven signaling in bCSCs and targeting LMTK3 kinase activity using novel small molecule inhibitors will provide an innovative therapeutic approach to the treatment of drug-resistant BC.

8. **Andrew Gierys** (University of Manitoba)

Point mutations in the chaperone EagT6 reveals residues critical for the interaction with the second transmembrane domain of the effector Tse6 in Pseudomonas aeruginosa

The type-6 secretion system is used by Gram-negative bacteria to deliver toxic effector-proteins directly into the periplasm of neighbouring bacteria. However, many effectors have cytoplasmic targets. To overcome this, these effectors contain TMDs (TransMembrane Domains) that are hypothesized to penetrate membranes to help deliver the effector into the cytoplasm. The effectors are co-translated with chaperone proteins that bind the TMDs to prevent erroneous membrane insertion. T6SS membrane effectors can have one TMD (class 1) or two TMDs (class 2). Past work by our lab revealed the structure of the chaperone protein EagT6 bound to the first TMD, or TMD1, of the toxin Tse6. This structure revealed that EagT6 distorts the structure of TMD1 to prevent proper folding to inhibit membrane penetration. How the chaperone binds to the second TMD of Tse6 is currently unknown. Given this, the aim of this work is to characterize both the structure and binding mechanism of EagT6 to the second transmembrane domain (TMD2) of the effector Tse6. To achieve this goal, we have taken a structural and biochemical approach to probe the interaction of EagT6 and TMD2. Currently I have purified an EagT6:TMD2 complex and characterized its stability using nano-differential scanning fluorimetry. Both thermal denaturation experiments and chemical denaturation challenges demonstrate that the EagT6:TMD2 complex is significantly less stable than the EagT6:TMD1 complex. These results are supported several crystal structures where organic precipitants such as PEG in the crystallization condition displaced TMD2. Finally, I have assayed the ability of various EagT6 point variants destabilize the EagT6:TMD2 complex highlighting

key residues critical for binding TMD2. Our results show that the same point variants in EagT6 also stabilize the EagT6:TMD1 complex, suggesting that EagT6 binds a conserved α-helix motif found in both TMD1 and TMD2 of Tse6.

9. **Yuping Lu** (National Research Council of Canada)

Improvement of pea protein quality using solid-state fermentation: A quantitative proteomics approach to understand molecular changes during processing

Pea (*Pisum sativum*) is an inexpensive and highly abundant alternative source of protein which grown for both fresh and dried seeds. Pea protein components are available as flours (protein 23 to 31%), air-classified protein enriched flours (protein ~40-60%), and as isolates (protein ~90-95%). Pea protein fractionation process increases the protein content and hence offers many applications for producing plant-based protein products. These plant-based products are healthy in comparison to meat-based products. However, pea proteins have a limited supply of some essential tryptophan and sulfur-containing amino acids (methionine, cysteine). Additionally, pea proteins offer poor techno-functional properties which limits their use in many food product applications. There are bioprocessing techniques like fermentation and enzymatic digestion that have been utilized to improve nutritional and functional aspects of pea proteins. However, there is a lack of information about molecular and compositional changes occurring in food matrix during fermentation processes of the pea substrate. In this research, we used solid-state fermentation (SSF) of pea protein isolate with Aspergillus oryzae to explore proteome changes at different time-points using label-free quantitative proteomics. It enabled us to identify underlying functional pathways of fungus that is required for establishment of the organism on the pea protein substrate and understand its role in potential improvement of nutritional and functional value of pea proteins. We identified a significantly upregulated enzyme, methionine synthase, from L-methionine biosynthesis pathway, which catalyzes methionine production. This might suggest how fermentation processes could be used to improve the presence of sulfur containing amino acids in pea ingredients.

10. **Lacey Winstone** (University of Saskatchewan)

DDX41 Helicase in P-bodies Formation and Myeloid Malignancies

DDX41 is a member of the DEAD-box helicase family. Mutations in DDX41 cause myeloid malignancies, including myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). MDS is characterized by unsuccessful hematopoiesis and peripheral blood cytopenia, and AML is characterized by the spread of immature myeloid cells. One-third of MDS will progress to AML. DDX41 has been implicated in innate immunity and mRNA splicing. The predominant mutation in DDX41 is R525H; however, its molecular pathogenesis in MDS/AML is still unknown.
Processing bodies (P-bodies) are cytoplasmic ribonucleoprotein granules comprised primarily of decapped mRNAs and proteins involved in mRNA decay and translationally repression. They follow a dynamic assembly and disassembly according to their local cellular condition. In normal conditions, P-bodies recruit mRNA decay-promoting components alongside translational factors. Under stress conditions, P-bodes increase in size and frequency. P-body dysregulation has been associated with various diseases and cancers, including MDS/AML. Three proteins, EDC4 (decapping component), LSM14A (mRNA decay factor), and 4E-T (translational repression factor), are considered vital for P-bodies dynamic continuation. Comparing DDX41 knockout (KO) and wild-type (WT) cells, we found that DDX41 is required for P-bodies

formation (EDC4 as a marker) but not stress granules (G3BP1) under stress conditions. To understand whether DDX41 affects P-bodies proteins' expression, we measured EDC4, 4E-T, and LSM14A protein expressions by Western blotting. EDC4 and 4E-T expression increased till 4 h post sodium arsenite treatment, then decreased in DDX41-KO HeLa cells. Whereas in WT cells, their expression peaked at 2 h and then decreased; overall, their expression was higher than DDX41-KO. In contrast, the absence of DDX41 did not influence LSM14A levels. We performed immunofluorescence to understand the change of expression at a subcellular level and to observe if DDX41 expression alters P-bodies dynamics. We found that EDC4 foci increased till 2 h post-treatment in both cell types, but DDX41-KO cells had reduced foci compared to WT cells. To test if DDX41 has a role in the splicing of P-bodies proteins, we investigated endogenous and exogenous of 4E-T and DCP1A, another P-bodies component. The foci of endogenous EDC4 and DCP1A increased until 2 h and then decreased in WT cells, whereas DDX41-KO cells showed reduced foci. Exogenous GFP tagged DCP1A also increased till 2 h, then decreased.; however, there is no significant difference between DDX41-KO and WT cells. Lastly, to understand the R525H mutation on P-bodies dynamic, we re-constituted DDX41 KO cells with DDX41-R525H and DDX41-WT genes, respectively and found that DDX41 mutation caused an increase in the P-bodies formation. Currently, we are examining the expression of endogenous DCP1A protein by Western blot and GFP-4E-T foci formation by immunofluorescence.
In conclusion, DDX41 is required for P-bodies formation but not stress granules. DDX41 influences the expression of P-bodies proteins EDC4 and 4E-T but not LSM14A. Endogenous P-bodies proteins differ between DDX41-KO and WT cells, whereas exogenous P-bodies proteins expression remained similar, indicating DDX41's role in mRNA splicing. The DDX41 R525H mutation causes an increase in P-bodies amount, which might be the molecular pathogenesis leading to MDS/AML.

11. Joy Rameille Santos (University of Alberta)

SEQSIM: A novel software to conduct full genome analysis of sequence similarity in upstream gene regions"

The upstream regions of genes (UGRs), sometimes known as promoter regions, contain binding sites for proteins that play a crucial role in gene regulation. The presence of similar promoter sequences suggests co-regulation and functional relatedness between genes. By studying these sequences, researchers gain insights into gene regulation, cellular processes, disease mechanisms, and evolutionary relationships. Analyzing these UGRs and their sequence similarities provides valuable information for predicting gene co-expression. Additionally, DNA sequence similarity may also be associated with three-dimensional (3D) structure and physical proximity of UGRs. The 3D folding of the genome within the nucleus may bring distant DNA segments, including UGRs, into closer proximity which allows for regulatory interactions. A genome-wide analysis of these UGRs has yet to be conducted.
We developed an algorithm and scoring system to compare the 2000 nucleotides upstream of every human gene. Our algorithm was validated against a known sequence comparison software, Clustal Omega. We used tools such as NCBI's Multiple Sequence Alignment Viewer and Gephi to further visualize the matches. Additionally, we created heatmaps with genes arranged sequentially along both axes for each chromosome utilizing both Clustal Omega's comparisons and our derived similarities. The algorithm resulted in a matrix 57064 by 57064 large that comprehensively compared every UGR in the Genome Reference Consortium Human Build 38 and showed percent similarity between each sequence. When visualized in a heat map, our algorithm similarities resulted in similar patterns to that of Clustal Omega. Analysis of the heat map for each chromosome elucidated unique patterns of similarity in adjacent genes. Patterns are highly suggestive of a 3D chromatin arrangement previously

unidentified. We developed 3D models of the chromatin and plan to test these predictions using structural biology techniques.
The patterns that emerge from the similarity heatmaps of each chromosome may give some insight on the regulation of genes on the sequence level. We proposed that these patterns may also be related to the folding and overall structure of chromatin. Our findings suggest that the folding of chromatin may play a role in the spatial architecture of regulatory domains which has implications for our understanding of gene regulation.

12. **Narasimha Pujari** (University of Saskatchewan)

Flirty Flies to Funky Physiology- Feeding Response in Mated Drosophila Females is Regulated by NUCB1 in the Nervous System

In the common fruit fly, the sex peptide is an accessory protein that is transferred from the male to the female during copulation. Sex peptide binding to its receptor in the female initiates post-mating response (PMR), which includes increased food intake and other physiological changes to support the increased metabolic demand of reproduction. Recently, our lab discovered that the highly conserved Drosophila nucleobindin-1 (dNUCB1) has anti-hunger properties, as observed in several mammalian systems. We hypothesize that dNUCB1 is involved in post-mating food intake increase. First, we confirmed that mating changes dNUCB1 mRNA level within the first 2 hours of mating and the change is dependent on sex peptide signaling. Then, we evaluated the effect of pan-neuronal knockdown of dNUCB1 on food intake in virgin and mated females using a dye-based assay. We observed that pan-neuronal knockdown of dNUCB1 increased food intake only in mated females. Our immediate future goal is to identify the specific dNUCB1-expressing neurons that are responsible for this mating-induced feeding response.

13. **Harsha Benny** (University of Saskatchewan)

Identification of ICP4-interacting proteins to characterize the mechanism of ICP4 to regulate transcription and histone dynamics.

Herpes simplex virus 1 (HSV1) is a double-stranded DNA virus that causes lytic or latent infections. During lytic infection viral genes are expressed and infectious viral progeny are produced whereas in latent infection most viral genes are not expressed, and no progeny virions are produced. Latent infectious are life long and it cause lytic infection. During lytic infection, HSV1 has a temporal cascade of gene expression with genes categorized as Immediate Early (IE), Early (E), or Late (L) based on their expression kinetics. Infected cell polypeptide 4 (ICP4) is one of the five IE proteins. It is an essential viral transcription activator that interacts with cellular RNA polymerase II and general cellular transcription machinery to regulate viral gene expression. Gene expression requires access to DNA. Access to the DNA is regulated by chromatin. Chromatin is composed of regularly spaced nucleosomes that consist of two molecules each of core histone (H2A, H2B, H3, and H4), wrapped in 147bp of DNA. The linker histone H1 binds to DNA entry and exit sites of the nucleosome to stabilize and compact chromatin. DNA assembled in stable, compacted chromatin is generally not accessible to factors that require a DNA template, whereas DNA assembled in unstable “open” chromatin is accessible to such factors. Consequently, transcription requires that DNA is assembled in accessible, “open” chromatin. Chromatin compaction is affected by the assembly of histone variants into chromatin, post-translational modifications (PTMs) of

histones, and the association of other chromatin proteins that bind to histone-DNA or histone-histone interactions. Such factors also influence the chromatin exchange of histones (histone dynamics). HSV1 genome enters the nucleus devoid of histones. During lytic infection, HSV1 genome is assembled in highly unstable nucleosomes. The unstable HSV1 chromatin is consistent with the observation that histone dynamics increased in HSV1-infected cells. The histone dynamics are higher in replication compartments where HSV1 genomes and ICP4 localize than in the cellular chromatin, consistently ICP4 is sufficient to enhance histone dynamics. Based on these data I propose a model in which ICP4-mediated histone exchange regulates HSV1 chromatin accessibility to regulate transcription. To test this model, I will first identify ICP4-interacting partners to characterize the mechanisms of ICP4 to regulate histone dynamics and test how this activity relates to HSV1 transcription and chromatin stability.
Previous studies used harsh conditions to purify ICP4 interacting proteins that only identify very strong and direct protein interactions. However, these proteins are likely not involved in histone dynamics based on their known functions. Therefore, I hypothesis ICP4 interacts with more cellular proteins.
I will use BioID (Proximity-dependent labeling) to identify ICP4-interacting partners, including those interacting partners with transient, temporal, weak, and indirect protein-protein interactions. PCR sequence of ICP4 amplified from HSV1 genome. Subcloned PCR amplified ICP4 expression vector is labeled with BirA biotin ligase. BiraA biotin ligase catalyzes the generation of reactive biotinyl-AMP from biotin and ATP. Biotin will be added to the cells to biotinylate the ICP4 interacting proteins within a 10 nm radius. Mass spectrometry will be used to identify the isolated proteins.
I expect to identify weak, transient, temporal, and indirect ICP4-interacting proteins. Previous studies used harsh protein purification conditions that only detect very strong direct protein-protein interactions. In my study, I will use the BioID method, which is never used for ICP4, to make possible the identification of transient, temporal, and indirect protein-protein interactions.

14. Himen Salimizand (VIDO)

Characterize and test the immunogenicity of peptides representing the hypervariable regions (HVRs) of outer membrane proteins, LamB, OmpF, OmpA, and FhuA of Avian Pathogenic Escherichia coli (APEC)

The intestinal tract of poultry species is a habitat for Avian pathogenic *Escherichia coli* (APEC).
In the poultry industry, however, APEC in broilers; extraintestinal sites can result in a variety of diseases that can result in economic losses. Flock vaccination is considered one of the strategies for controlling APEC. Only two are commercially available, however, the efficacy of these vaccines is questionable since there is a lack of heterogenous APEC protection.
Recently, comparative genomics showed that beta-barrel outer membrane proteins are the most proteins under positive selection which leads them to be very diverse and make hypervariable regions (HVRs). However, this diversity was restricted to the outer membrane loops that faced out the cell, and the beta-barrel segment of these proteins in the outer membrane layer
remained constant. Despite the well-established fact that hypervariable regions in proteins are responsible for strain diversity, new research indicates that HVR regions in *E. coli* have a limited range of possibilities.
HVRs are known to be potent immunogens, and the four selected OMPs, FhuA, LamB, OmpF, and OmpA, play a vital role in the interactions between *E. coli* and the host immune system. Hence, these HVRs are attractive candidates to test as vaccine antigens in chickens. Based on these basic data, from Saskatchewan poultry farms, 245 *E. coli* isolates were collected and the whole genome was sequenced. *E. coli* isolates were collected from three sources, diseased (n=91), healthy (n=61), and environmental (n=93) samples. The sequence of these proteins was analyzed and the frequency of HVRs was detected.

A construct of each HVR of each protein was designed. In order to find out how protective they are, they will be challenged by APEC to prove their immunogenicity in chickens.

15.Liliia Kyrylenko (University of Saskatchewan)

Mapping Synthetic Lethal Interactions Amongst DNA Repair Genes

Cancer is a leading cause of death, and a key challenge in cancer research is to identify novel drug targets that, when inhibited, should affect only the cancer cells but not the normal cells. Over the past two decades, the concept of synthetic lethality that queries novel genetic relationships between gene pairs has slowly emerged as one of the best strategies to selectively eliminate cancer cells. By definition, two genes are said to exhibit a synthetic lethal interaction (SLI) if a single knockout (SKO) of individual genes has no effect on cell survival, but their simultaneous double knockout (DKO) results in cell death. Thus, if one of the genes is cancer-specific, then it provides a "tumour-specific context" or acts as a "biomarker", in which the second gene becomes a "vulnerable target" to eliminate cancer cells. Cyclin-dependent kinase inhibitor 1A (CDKN1A) functions as a cell cycle regulator with leading roles in DNA damage and response. Uncorrected DNA lesions activate tumour suppressor gene p53 that can inhibit the cyclin-dependent kinases CDK2 and CDK4 through an induced expression of p21 (the protein form of CDKN1A), leading to G1 cell cycle arrest and preventing potential replication of damaged DNA. Therefore, the cell cycle regulatory role of CDKN1A and its impact on growth regulation led it to be regarded primarily as a tumour suppressor, with a loss-of-function (LOF) mutation indicated across several cancers. Furthermore, BRG1/BRM associated factor (BAF) complex functions as a tumour suppressor in many cancers, and it is crucial for the regulation of gene expression and differentiation. Mutations in the genes encoding mSWI/SNF subunits are found in more than 20% of cancers across all types.
Our hypothesis is that the SLI between members of the BAF complex and CDKN1A can be exploited for cancer therapeutics. We propose to confirm the robustness of the SLIs between CDKN1A and members of the BAF complex to exploit them for therapeutics.

16. Alain Morejon Morales (University of Saskatchewan)

Analysis of the EphB6-EGFR crosstalk in cancer cells

Cancer is the leading cause of death in developed countries with increasing figures of incidence and mortality. EphB6 is a cell surface receptor protein that controls multiple aspects of cell behaviour in malignant tumors. According to our recent findings, EphB6 acts to enhance cancer cell proliferation when this response is simultaneously driven by another pro-oncogenic protein, the Epidermal Growth Factor Receptor (EGFR). My research project aims to determine how these two proteins interact to control tumor development, and to perform screening of large drug libraries to identify compounds that effectively eliminate cancer cells and tumors with high EphB6 levels. To achieve this, I am studying the effect EphB6 on cancer cells and tumor growth driven by EGFR upon stimulation with its natural ligand, the Epidermal Growth Factor (EGF). I am also examining if EphB6 stimulation with its own ligand, ephrin-B2, affects EphB6-controlled modulation of EGFR activity in tumor cells and the effect of EphB6 on the growth of tumors that express high levels of EGFR. In addition, I am screening libraries of thousands of clinically approved or novel small molecule compounds to select reagents that specifically kill EphB6/EGFR overexpressing cancer cells without producing negative effects on normal tissues. The

completion of my analyses of the EphB6-EGFR crosstalk should support the development of novel effective therapeutic strategies co-targeting EphB6 and EGFR, while my screening work is expected to identify drugs that can be used based on high EphB6/EGFR levels in tumors without causing toxic side effects detrimental for patients. Ultimately, my findings are expected to assist in establishing new clinical protocols improving survival of cancer patients.

17. Radwa Asar (University of Saskatchewan)

Arthrobacter citrus biotransformation of bile acids

Bile acids and their bacterial biotransformation products play an important role in both the health and disease of humans and animals. Bile acid modifying bacteria participate in diversifying the bile acids pool in the gut through the formation of new bile acid metabolites. By screening hundreds of bacterial colonies, *Arthrobacter spp*. (a common member of bison gut microbiome) was chosen as a model for bile acid modifying bacteria. We hypothesize that the modified bile acids produced by *Arthrobacter spp*. can affect the host immune system. Using a multidisciplinary research design, 12 distinct bile acids biotransformation products from the primary and secondary bile acids cholic and deoxycholic acid, respectively were isolated, and their structures were assigned. The isolated biotransformation products are intermediaries relate to the early 2,3-Seco degradation pathway of bile acids (an understudied pathway that has been reported since the 1950s). The impact of these molecules on the health of animals remains unknown. By shifting our attention to the genes and corresponding enzymes that are involved in bile acids metabolism by *Arthrobacter spp*., we were able to characterize and purify the bile salt hydrolase (BSH) enzyme. BSH is considered to be the gateway to bile acid bacterial metabolism through the deconjugation process liberating free bile acids that can be further modified in the gut. The crystal structure of the enzyme has been solved to 2 Å resolution. This poster will summarize our findings, including the isolation of the biotransformation intermediaries and the characterization of the bile salt hydrolase enzyme.

18. Dinesh Wellawa (University of Saskatchewan)

The role of histamine in iron acquisition by multidrug-resistant Acinetobacter baumannii

Histamine is a potent immunomodulator that plays a key role in the innate immune response. Mast cells and basophils release histamine upon exposure to an antigen. The biological effects of histamine are diverse and include increasing vascular permeability and enhancing mucus production. Increased blood vessel permeability functions to allow escape of immune cells into the damaged tissue, allowing them to directly engage the antigen. Interestingly, the emerging opportunistic and extensively drug-resistant pathogen *Acinetobacter baumannii* also produces histamine. *A. baumannii* causes an array of infections, including bacteremia and pneumonia, where mortality rates can exceed 35%, but little is known about the factors that contribute to its pathogenesis. In *A. baumannii*, histamine appears to be produced as a key precursor molecule to production of the siderophore acinetobactin, an iron-binding molecule that facilitates bacterial capture of this essential nutrient. Here, using wild-type (WT) *A. baumannii* and a mutant impaired for the decarboxylation of histidine to histamine (ΔbasG), we sought to assess the contribution of endogenous and exogenous histamine to iron acquisition and siderophore production by *A. baumannii*. We find that disruption to histamine biosynthesis inhibits bacterial growth under iron restriction and on human serum, transferrin, and lactoferrin as sole sources of the essential metal. In the absence of endogenously synthesized histamine, exogenous addition of the amine restores the iron-

dependent growth of and siderophore production by *A. baumannii*. These results suggest that the bacterium may be able to sense and utilize host-derived histamine to facilitate its iron-dependent growth in vivo. Together, it appears that histamine plays a complex and multifactorial role in the survival and pathogenesis of A. *baumannii.*

19. **Kathyana Deeyagahage** (University of Saskatchewan)

An Antimicrobial peptide that assembles into a supramolecular pore

Short cationic peptides have been studied extensively as antimicrobials and host-defense peptides with a range of plausible mechanisms of action proposed. Pores that span bacterial membranes are among the most frequently described antimicrobial mechanisms, though there is a dearth of structural information available. We therefore completed a structural and functional investigation of an AMP based on the delta-toxin of S. auricularis, reporting a 1.5 Å resolution model of supramolecular pore assembled from the 17 amino acid peptide. Importantly, this staphylococcal toxin-inspired peptide, named STIP3-1, demonstrated an ability to form pores in vitro. The quaternary structure represents a new scaffold for AMP structure-activity relationship studies and has the potential to serve biotechnological needs.

20. **Jamsad Mannuthodikayil** (University of Saskatchewan)

Advancing Precision Cancer Therapies through Direct Protein-Drug Conjugate Discovery

Our study utilizes an innovative platform specifically engineered to directly discover protein-drug conjugates for precision cancer therapies. Our discovery approach focuses on identifying potent combinations of short affinity proteins and drug conjugates that target relevant biomarkers with great specificity, expediting the development of short protein-based therapeutics for precision cancer diagnosis and treatment. By leveraging unique but large libraries of protein-drug conjugates and employing advanced screening techniques, our platform enables the direct discovery of highly efficient protein-drug conjugates targeting cancer. This direct discovery approach eliminates the challenges associated with post-protein discovery (drug) conjugation, by effectively addressing concerns related loss of specificity or reductions in potency during the conjugation of toxins/markers/chelators with the discovered protein affinity reagents.

Our primary objective is to apply this innovative platform to discover short protein-drug conjugates against cancer biomarkers, with a specific emphasis on targeting the Nectin-4 (PVRL4). Nectin-4, a transmembrane protein from the nectin family, exhibits frequent overexpression in various human malignancies and has been associated with a poor prognosis in triple-negative breast cancer. Moreover, it serves as an independent biomarker for overall survival in multiple cancer types, making it a highly promising target for the development of precision anticancer therapeutics.

Our study aims to develop short protein-based precision therapeutics that specifically target the Nectin-4 prognostic biomarker, capitalizing on the favorable properties of short protein drug conjugates, including their rapid extravasation from blood vessels and high tissue penetration. These properties facilitate precise and effective targeting of tumor sites, surpassing the capabilities of larger-sized antibodies-based drug conjugates. Additionally, our work underscores the limitations of traditional approaches in discovering short proteins and subsequently conjugating them with

toxins/markers/chelators for precision diagnosis and therapies while emphasizing the significant advantages offered by our platform's direct discovery of protein conjugates.

21. **Hannah Braun** (University of Saskatchewan)

Identification of Exoproteins Secreted by the Klebsiella pneumoniae Type II Secretion System

Klebsiella pneumoniae is one of the top causative pathogens for nosocomial infections worldwide and is increasingly difficult to treat due to emerging multi-drug resistance in both community and hospital isolates. This bacterium uses a nanomachine called a type II secretion system to secrete proteins out of the cell. These secreted proteins, called exoproteins, are usually involved in host colonization and nutrient acquisition, thus enabling different bacteria to sustain infections within a host. Type II secretion systems in most bacteria recognize 7-25 exoproteins. To date, the only identified *K. pneumoniae* exoprotein is an enzyme called pullulanase. We hypothesize that like other bacteria possessing a type II secretion system, *K. pneumoniae* secretes more than one exoprotein. Mass spectrometry was previously used to identify putative *K. pneumoniae* exoproteins. My project currently involves validating the mass spectrometry findings by cloning a select subset of exoprotein genes into low copy vectors. These constructs were transformed into *Escherichia coli* model strains which express either a functional or a non-functional reconstituted *K. pneumoniae* type II section system. Secretion status of putative exoproteins was assessed through in vitro assays, with exoproteins visualized on Coomassie blue gels. In addition to the aforementioned assay, putative exoproteins had a FLAG tag fused to the C-terminus and secretion status of exoproteins was assessed via Western blot. Future work will focus on characterizing the activity of confirmed exoproteins through in vitro assays. This study will be the first to identify additional proteins secreted by the *K. pneumoniae* type II secretion system. The identification of additional exoproteins may provide novel therapeutic targets to treat multi-drug resistant *K. pneumoniae* infections.

22. **Janvier Anleu Algeria** (University of Saskatchewan)

Prediction of Breast Tumour Kinase (BRK) substrates derived from alignment-free similarity analysis of proteins in dysregulated pathways of drug-resistant MCF7 cell lines

Breast tumour kinase (BRK) is a non-receptor tyrosine kinase that is overexpressed in most breast cancers and has been linked to a poor prognosis. Its role and mechanisms of action are not yet fully understood and elucidating some of the kinase's targets could help understand its functions. Classical biochemical techniques to study protein-protein interactions are time-consuming and expensive, but recent advances in proteomics and bioinformatics have helped narrow down and predict potential targets, which can then be experimentally validated. We have used a protein mass spectrometry dataset of parental, tamoxifen-resistant, and fulvestrant-resistant MCF7 breast cancer cell lines to predict BRK substrates. We analyzed differentially expressed proteins and dysregulated pathways between the three cell lines through pairwise comparisons using the MSqRobSum and pDis algorithms, respectively. This was followed by subcellular localization filtration and alignment-free k-mer similarity analyses of the proteins in the dysregulated pathways. The differential expression analysis showed that the proteome of the tamoxifen-resistant cell line diverged significantly from those of the parental and fulvestrant-resistant cell lines, while the latter two were more alike. The pathway analysis identified 129 distinct dysregulated pathways that included pathways linked to cancer hallmarks like MAPK, apoptosis, protein processing in the endoplasmic reticulum, and estrogen signalling pathways. We also identified 14 BRK substrate candidates based on

subcellular localization filtration and alignment-free similarity analysis from the pathway analysis dataset of almost 7,000 proteins. Three candidates, IRS2, AGAP2, and KMT2A have known associations with cancer, including breast cancer, and are ideal for experimental validation as BRK substrates. Overall, our workflow holds vast potential to efficiently predict protein kinase substrates, streamlining the study of protein-protein interactions and cellular mechanisms.

23. Kayla Abrametz (University of Saskatchewan)

Rational design of a hyperactive telomere resolvase

Species in the *Borrelia* and *Agrobacterium* genera possess linear chromosomes. The ends of these linear chromosomes are protected by covalently closed hairpin loops, called hairpin telomeres. Hairpin telomeres are resolved from a replication intermediate containing replicated telomere junctions in a process called telomere resolution. Telomere resolvases use a DNA breakage and rejoining mechanism related to that used by type IB topoisomerases and tyrosine recombinases (like Cre). The agrobacterial telomere resolvase, TelA, is also a ssDNA annealing enzyme. The N-terminal domain of TelA is required for annealing activity but is dispensable for telomere resolution. We describe here the reaction properties of TelA in telomere resolution. We show that telomere resolution is dramatically stimulated by divalent metal ions and that telomere resolution in an N-terminal deletion mutant of TelA becomes independent of divalent metal ions. We also show that the N-terminal domain autoinhibits TelA, rendering the reaction metal responsive. Autoinhibition also suppresses reverse and side reactions that telomere resolvases can promote. We modeled TelA with AlphaFold2 to predict the structure of TelA as a monomer and discovered two potential autoinhibitory interfaces that may control the DNA cleavage and rejoining activity of TelA. Mutation of these interfaces is shown to hyperactivate telomere resolution.

24. Matthew Van Schepdael (University of Manitoba)

Point mutants in the chaperone EagT6 reveals residues critical for the interaction with the second transmembrane domain of the effector Tse6 in Pseudomonas aeruginosa

The ability to secrete proteins is an essential component of bacterial communication and virulence. The type VI secretion system (T6SS) is a dynamic protein nanomachine that forcibly secretes toxic effectors into prey-cells in a contact-dependent manner. Many T6SS effectors possess transmembrane domains (TMDs) in their N-termini which are hypothesized to catalyze the transition of the toxin across the inner-membrane of a prey-cell. For proper loading onto the T6SS prior to secretion, TMD-containing effectors require assistance from Effector Associated Gene (Eag) chaperones. Eag chaperones bind directly to the TMD regions of the toxin effectors and hold the TMDs in a biologically inactive conformation. This allows the Eag bound T6SS effector to be properly loaded onto the tip of the T6SS. For the TMDs to adopt their biologically active conformations, the Eag chaperone is removed during the secretion process by a currently unknown mechanism. To elucidate the mechanism of Eag chaperone removal, we have taken a biophysical approach to characterize the energetics and molecular features that contribute to the stability of Eag-TMD complexes. Nano differential scanning fluorimetry (nDSF) and differential scanning calorimetry (DSC) experiments have shown that Eag-TMD complexes are highly-stable and are resistant to chemical denaturation. Further mutational analysis reveals certain residues are critical in complex stability and when mutated can lead to destabilization and disruption of the Eag-TMD

complex. Co-crystal structure of one of these Eag-TMD mutants shows certain molecular features are required for complex stability and may suggest a possible mechanism of Eag chaperone removal. Together, our work sheds light on the bioenergetics and the molecular mechanism of Eag chaperone removal from effector transmembrane domains.

25. **Jared Price** (University of Saskatchewan)

Exploring synthetic lethal dosage interactions of telomerase

The enzyme telomerase is frequently overexpressed in most cancer types which is generally thought to be related to tumorigenesis. This has raised the hypothesis that telomerase's inhibition will lead to a profound antitumor strategy. Unfortunately, despite many clinical efforts, a direct telomerase-targeting approach has yet to produce success. Here I propose a novel strategy called synthetic dosage lethality (SDL) to overcome the deficiencies of previous efforts and apply it to models of telomerase over expressing cancers. The identification of SDL interactions is of therapeutic interest because if a particular gene exhibits SDL with a tumor-driving molecular alteration, then targeting this SDL gene will cause lethality selectively in cancer cells harboring this alteration. The Vizeacoumar lab has utilized a pooled lentiviral CRISPR and shRNA screen against the entirety of the human genome to identify gene knockouts which was cross-referenced with cell line specific gene essentiality data from DepMap to create a short list of potential targets. These targets were then stratified according to canonical function, drugability, loss-of-fitness specificity, and essentiality. Here we validate the top SDL interactions through isogenic telomerase overexpression lines and further evaluate in cell lines with intrinsic differences in telomerase expression. One such gene, PPAN, demonstrates decreased proliferation in telomerase overexpressing cells and reduced tumor growth in xenograft models. Interestingly, a potential interference with the noncanonical functions of PPAN and telomerase interaction may be identified by abnormalities in mitochondrial structure from PPAN loss-of-function in telomerase overexpressing cells. As telomerase is overexpressed in most cancers, our research outcomes have pan-cancer applicability.

26. **Neil Lorente Cobo** (University of Manitoba)

Molecular characterization of effector/immunity pairs in the Salmonella Typhimurium type VI secretion system

In most microenvironments, bacteria have evolved survival mechanisms to allow them to outcompete other microorganisms and harvest the limited resources available to them. One such mechanism is the type VI secretion system (T6SS). The T6SS is an efficient nanomachine responsible for contact-dependent toxin translocation into neighboring prey cells. *Salmonella Typhimurium* contains a single T6SS present in *Salmonella* pathogenicity island 6 (SPI-6) that is utilized for interbacterial competition in the host gut microbiota. In addition to the core T6SS genes, there are 4 clusters of noncore genes which are predicted to be effector/immunity pairs. Two effector-immunity pairs of interest are Tlde1a/Tldi1a and Rhs2/SciX, which have been shown to play roles in pathogenicity and improve bacterial survival in vivo. To date, I have elucidated the structure of Tlde1a by X-ray crystallography and characterized its ability to modify the peptidoglycan in vitro. This work showed how Tlde1a has been modified structurally by combining various molecular features to create a unique toxin in the T6SS competition arms-race. A partially complete structural model of Rhs2 predicts the protein to be similar to a tripartite complex found in insect pathogens. Toxicity assays in *E. coli* show significant cell death when Rhs2 is

induced. Currently, I have solved a structure of the immunity protein SciX by X-ray crystallography and assigned its NMR spectrum. This structural data will be used to probe the dynamics of the immunity protein and map the residues necessary for effector/immunity binding. These studies will provide unique insight into the arsenal of weaponry that *Salmonella* species possess, as well as develop our understanding on bacterial specificity towards toxin immunity.

27. Sohaumn Mondal (University of Saskatchewan)

Identifying Synthetic Lethality Targets in DDX41-mutated MDS/AML

DDX41 is a DEAD-box helicase. Mutations in DDX41 cause myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) and the missense mutation R525H is found in over 70% of affected patients. MDS/AML typically occurs in the ageing population but can also occur in younger individuals due to inherited mutations, including DDX41. Direct targeting DDX41 is challenging because DDX41 is one of the 37 DEAD-box helicases in humans that share a similar structure. Synthetic lethality (SL) represents an effective strategy instead of direct targeting. Two genes are said to exhibit SL interaction, if the loss of function of both these genes affects cellular viability, while neither has any effect on their own. Collaborating with Dr. Franco Vizeacoumar's group, we have used bioinformatic analyses and identified several potential SL interactions of DDX41. Using DDX41 knockout cell lines that we established (Singh et al., 2022), we validated some potential SL candidates by shRNA knockdown and drug inhibitors. In this project, I will continue to use cell-based assays to validate the SL hits generated by computational methods. Second, I will use a pooled CRISPR/Cas9 screen to identify SL interactions of DDX41. Third, combining computational analysis and CRISPR screening, I will choose and validate some hits in cell-based and mouse models of DDX41-mutated MDS/AML. Our success in this proposal will significantly improve the treatment and survival of MDS/AML patients by identifying novel targets for DDX41 mutated blood cancers.

28. Zhihong Du (University of Saskatchewan)

Molecular analysis of front-end desaturases in biosynthesis of polyunsaturated fatty acids in microalga Prorocentrum micans

The marine dinoflagellate Prorocentrum micans produces a high amount of omega-3 polyunsaturated fatty acids (PUFAs), including stearidonic acid (SDA, 18:4n-3), octadecapentaenoic acid (OPA, 18:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). The mechanisms of how DHA and OPA are synthesized in P. micans remain unknown. Sequence analysis of ribonucleic acid (RNA)-seq data of P. micans indicated that the aerobic pathway, which involves multiple elongases and desaturases, is probably responsible for the biosynthesis of PUFAs. This study mainly focused on the front-end desaturases, which can introduce carbon-carbon double bonds between the carboxyl end and the pre-existing double bond in a fatty acid chain. Four endoplasmic reticulum (ER)-based candidate genes, designated Fed-C1, Fed-C2, Fed-C3 and Fed-C6, were cloned out from complementary deoxyribonucleic acid (cDNA), and their function was characterized by heterologous expression in yeast. The feeding experiments of transgenic yeast with exogenously fatty acid substrates showed that only Fed-C6 encodes a functional D6 fatty acid desaturase, which can convert linoleic acid (LA, 18:2n-6) to gamma-linolenic acid (GLA, 18:3n-6), and alpha-linolenic acid (ALA, 18:3n-3) to stearidonic acid (SDA, 18:4n-3), respectively. Fed-C6 showed a strong substrate preference toward ALA with a conversion efficiency of 28% compared with LA of 2%.

29. Iryna Myziuk (University of Saskatchewan)

A new family of serine-dependent macrolide esterases

Antimicrobial resistance genes (ARGs) that are shared between disparate biological niches pose significant risks to the practice of human and veterinary medicine. Moreover, AMR surveillance systems are incomplete: unreported ARGs that have been disseminated between environmental and animal, including human, reservoirs exist. The discovery of these known unknowns remains essential to better inform AMR surveillance, stewardship programs and clinical practice. As part of our recent AMR surveillance efforts at beef cattle feedlots in western Canada, we isolated a multidrug-resistant bacterium and identified that it carried a plasmid-borne cluster of known ARGs (*tetX*, *floR* and *erm35*) that included an uncharacterized α/β-hydrolase. This uncharacterized enzyme was determined to encode for a macrolide esterase, which we named EstT, and had the capacity to hydrolyze the macrocyclic lactone rings of tylosin and related antibiotics. Our discovery of EstT and our work to re-identify, characterize, and compare it to a second member of the α/β-hydrolase superfamily that has been overlooked as an ARG for decades will be presented.

30. Nabeela Kanwal (University of Saskatchewan)

Characterization of the Type II Secretion System in Citrobacter rodentium

Citrobacter rodentium, a Gram-negative bacterium, is a murine pathogen that is accepted as a surrogate model for a human pathogen enterohemorrhagic *Escherichia coli* (EHEC). Both of these pathogens share many virulence factors and cause similar infections. These bacteria have specialized secretion systems that they use to secrete virulence factors that are involved in pathogenicity. One secretion system that is found in EHEC and might also be used by *C. rodentium* is the type II secretion system (T2SS). The overall aim of this research was to characterize T2SS from *C. rodentium*, and to identify the *in vitro* conditions that promote T2SS-activation. First, a bioinformatic analysis of the T2SS was performed. All genes required for a functional T2SS machinery were identified. In addition, *in silico* analysis predicted the presence of two putative promoters, one at the beginning of each operon encoding T2SS gene, and two terminators, one at the end of each operon. Next, promoter activity was monitored using transcriptional *lacZ* fusion assays. The data indicates low level constitutive activity of the T2SS promoter under nearly all growth conditions tested. In addition, secretion assays were performed under various growth conditions to identify T2S proteins. These assays revealed the presence of a major protein species in the supernatant of wild-type *C. rodentium* that was absent from that of a T2SS deletion strain. For the identification of the novel protein species the samples have been sent for mass spectrometry. Future work will focus on validating the putative hits.

Notes

Notes

Sponsors

We are grateful to:

UNIVERSITY OF SASKATCHEWAN
Western College of Veterinary Medicine
WCVM.USASK.CA

UNIVERSITY OF SASKATCHEWAN
College of Medicine
DEPARTMENT OF BIOCHEMISTRY, MICROBIOLOGY AND IMMUNOLOGY
MEDICINE.USASK.CA

GIFS | GLOBAL INSTITUTE FOR FOOD SECURITY
Growing science for life
Nutrien - a Founding Partner

Office of the Vice-President Research
UNIVERSITY OF SASKATCHEWAN

UNIVERSITY OF SASKATCHEWAN
College of Medicine
DEPARTMENT OF ANATOMY, PHYSIOLOGY AND PHARMACOLOGY
MEDICINE.USASK.CA

UNIVERSITY OF SASKATCHEWAN
College of Medicine
MEDICINE.USASK.CA

UNIVERSITY OF SASKATCHEWAN

UNIVERSITY OF SASKATCHEWAN
College of Medicine
MEDICINE.USASK.CA

Annual Protein Structure, Function and Malfunction (PSFaM) meetings

Proteomics Research in Interactions

PRISM

and Structure of Macromolecules

PRISM Center: medicine.usask.ca/prism/