Maritimes Biological Chemistry Conference

(MBCC 2023)

Monday, August 28th to Wednesday, August 30th, 2023

KTS Lecture Hall, University of King's College

(2nd floor, New Academic Building, 6350 Coburg Rd, Halifax, NS B3H 2A1)

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Presenters will be considered eligible for student prizes in two categories: PhD and MSc. Winners will be announced at the end of the proceedings.

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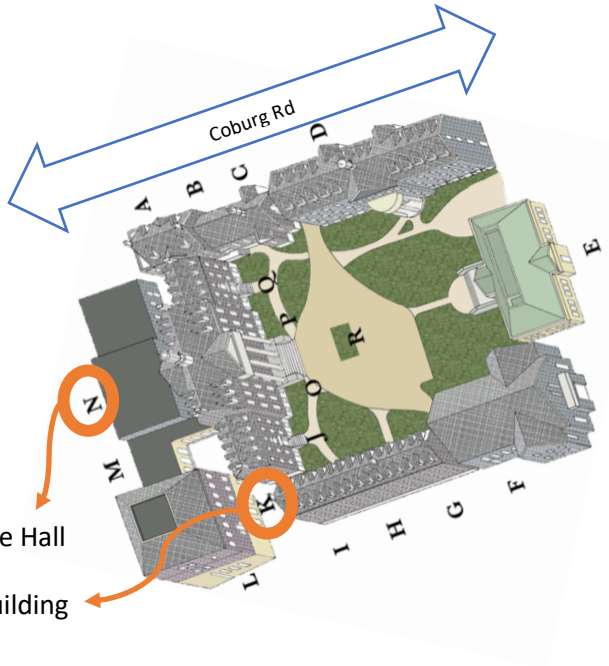
Location of Conference at University of Kings College

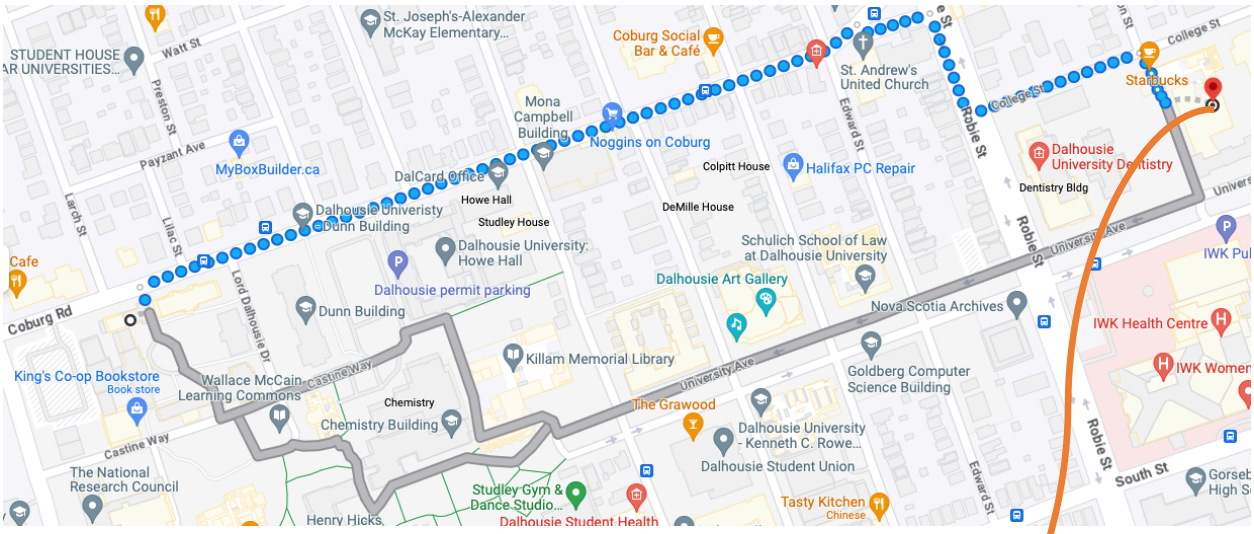


Dining Hall, Prince Hall

KTS Lecture Hall, New Academic Building

Directions from University of Kings College to Tupper Link at Dalhousie University

(Networking Event and Poster Presentation)



DAY 1, Monday, August 28th

9:00 – 9:10 am	**Welcome and Opening Remarks – Prof. Alison Thompson**
9:10 – 10:00 am	**Keynote Address - Dr. Christopher W. Kirby** Associate Director, Agriculture & Agri-Food Canada ***NMR Adventures at Agriculture and Agri-Food Canada***
10:00 - 10:30 am	**Break**
10:30 - 11:20 am	**Keynote Address - Dr. Fabrice Berrué** Research Officer, Team Lead, National Research Council Canada ***Development of NMR Based Methodologies to Ensure the Quality and Safety of Food and Functional Ingredients***
11:20 - 12:00 pm	**Panel Discussion**
12:00 – 13:00 pm	**Lunch (Dining Hall, Prince Hall)**
13:00 - 13:20 pm	**Ruiwen (Raymond) He, MSc Trainee** Biochemistry and Molecular Biology, Dalhousie University ***Characterizing Self-Assembly Features of a Hydrophobin from Wallemia Ichthyophaga***
13:20 - 13:40 pm	**Tran Thanh Tam Pham, PhD Trainee** Biochemistry & Molecular Biology, Dalhousie University ***The Highly Cationic N-Terminal Region of Apelin-17 Drives Its Selective Interaction with Bicelles***
13:40 - 14:00 pm	**Julie Anne Dayrit, MSc Trainee** Chemistry, Saint Mary's University ***Fighting Antimicrobial Resistance (AMR) Using Traditional Philippine Medicinal Plants***
14:00 - 14:20 pm	**Janet Debly, MSc Trainee** Biological Sciences, University of New Brunswick ***Structure Elucidation and Determination of Stereochemistry of Tropicicolide from An Endophyte of The Canadian Medicinal Plant Sorbus Decora***
14:20 – 14:40 pm	**Olivia Roland, MSc Trainee** Chemistry, Saint Mary's University ***Pseudogymnoascus Destructans: the Causative Agent of White-Nose Syndrome in North American Bat Species***
14:40 – 15:00 pm	**Carlie Charron, Assistant Professor** Chemistry, Dalhousie University ***Synthetic Methods for Accessing Heterocycle-containing Peptides***
15:30 – 17:30 pm	**Networking Event and Poster Presentation** **Tupper Link (5850 College Street, Halifax, NS B3H 4H7)** **Dinner and refreshments are provided.**

DAY 2, Tuesday, August 29th

9:00 – 10:00 am	**Keynote Address - Dr. Robert Britton** Chemistry Professor, Simon Fraser University ***Exploiting α-Haloaldehydes in Complex Molecule Synthesis***
10:00 – 10:20 am	**Anupama Ghimire, PhD Trainee** Biochemistry and Molecular Biology, Dalhousie University ***Fabrication and Biophysical-Mechanical Correlation of Recombinant Hybrid Spider Silk Fibers***
10:20 – 10:40 am	**Steve Sequeira, MSc Trainee** Chemistry, Dalhousie University ***Synthesis and Characterisation a Novel Class of Azo Dye; Azo Bispyrroles***
10:40 – 11:00 am	**Break**
11:00 – 11:20 am	**Mostafa Javaheri Moghadam, PhD Trainee** Chemistry, University of New Brunswick ***Quantum Mutual Information: A Novel Insight into Amino Acid Interactions and Chemical Bonding in Biomolecules***
11:20 - 11:40 am	**Allyson Bos, PhD Trainee** Biology, University of New Brunswick ***Discovery of Antimicrobial Natural Products from Fungal Extracts***
11:40 - 12:00 pm	**Meghan Hamilton, MSc Trainee** Biochemistry and Molecular Biology, Dalhousie University ***Characterization of a Novel Racemase for the Production of (S)-Equol: Dihydrodaidzein Racemase***
12:00 – 13:00 pm	**Lunch (Dining Hall, Prince Hall Building)**
13:00 - 13:20 pm	**Nicholas Morehouse**, **PhD Trainee** Biology, University of New Brunswick ***Tolypocaibols: Antibacterial Lipopeptaibols from A Tolypocladium Sp. Endophyte of The Marine Macroalga Spongomorpha Arcta***
13:20 - 13:40 pm	**Mathieu Laprise, MSc Trainee** Biological Sciences, University of New Brunswick ***Determination of The Absolute Configuration of Lignicol and Isolignicol***
13:40 - 14:00 pm	**Sara Evans, PhD Trainee** Biochemistry & Molecular Biology ***Conjugation of Phytoglycogen Nanoparticles to Aciniform Spider Silk Via Disulphide Linkages***
14:00 – 14:20 pm	**Break**
14:20 – 14:40 pm	**Emily Burke, MSc Trainee** Chemistry, Dalhousie University ***Efficient Synthesis and Functionalization of 3-Bromonaphtho[2,3b]thiophene***

DAY 3, Wednesday, August 30th

9:00 – 10:00 am	**Keynote Address - Dr. Jillian L. Rourke** Chemistry & Biochemistry Professor, Mount Allison University ***Using Multiparametric Approaches to Discover Novel Signaling in Health and Disease***
10:00 – 10:20 am	**Peter Oni, MSc Trainee** Chemistry and Biochemistry, Mount Allison University ***Exploring the Molecular Promiscuity of L-Phenylalanine (Phe) Activation of G Protein-Coupled Receptors***
10:20 - 10:40 am	**Karin Reznikov, MSc Trainee** Chemistry, Dalhousie University ***Molecular Characterization and Formation of Novel Reversible Boronic Acid- Diol Complexation***
10:40 - 11:00 am	**Lo Grant, PhD Trainee** Chemistry, Saint Mary's University ***The Terroir of Nova Scotian Natural Wine***
11:00 – 11:20 am	**Break**
11:20 – 11:40 am	**Dr. Alexander Baker, Assistant Professor** Chemistry, Dalhousie University ***Biomaterials for Drug Development and Non-Canonical Residues for Protein Engineering***
11:40 – 12:00 pm	**Dr. James Davey, Assistant Professor** Biochemistry and Molecular Biology, Dalhousie University ***Application of Systems Engineering Principles Toward the Creation of a Rhomboid Protease Activated Repressor of Gene Transcription***
12:00 – 12:20 pm	**Award Ceremony**

Keynote Address

NMR Adventures at Agriculture and Agri-Food Canada

Dr. Christopher W. Kirby

Associate Director
Charlottetown Research and Development Centre
Agriculture and Agri-Food Canada

When pursuing graduate students at an academic institution doing fundamental research, it can be hard to imagine doing research outside the walls of a university. Herein, an NMR spectroscopist adventure will be told which started within the walls of the chemistry department at Dalhousie to the current scene in PEI. Along the way, biopesticide candidate discovery using ICHIP technology and traditional organic solution NMR techniques (1H, 13C 1D and 2D as well as 15N) to help in the control of agriculturally important plant pathogens will get highlighted. An investigation of looking at the oil inside of oilseeds (some of the highest valued crops in Canada) using 1H and 13C high-resolution magic angle spinning (HRMAS) and then being able to plant those seeds will keep your head spinning at 6000 Hz. Lastly using solid-state 31P and 13C NMR to look at metal organic framework (MOF) – pesticide complexes in the aim to improve pesticide remediation will be captivating. While the research stories are unfolding, the career progression from a NMR Chemist (similar to an NMR facility manager) to NMR Research Scientist to the current role of Associate Director in PEI and the technical and professional staff that one interreacts with within AAFC to gain insights into possible careers in chemistry within the Government of Canada.

Keynote Address

Development of NMR Based Methodologies to Ensure the Quality and Safety of Food and Functional Ingredients

Dr. Fabrice Berrué

Research Officer. Team Lead

The agri-food industry faces great challenges in sourcing high-quality raw materials and assuring the quality and specificity of their manufactured products along the supply chain. Moreover, the made-in-Canada brand is generally regarded as having intrinsic value, and thus commands a premium compared to competitors, due to the global perception that it denotes quality and safety. Consequently, it remains important to continuously develop new fit-for-purpose analytical methods to guarantee the quality and authenticity of food products, especially for a global and highly competitive industry.

Foods and functional ingredients are complex, and often contain hundreds of molecules undergoing subtle and complex interactions that dictate organoleptic properties, nutrition value, functional properties, and health benefits. To address this challenge, NRC has been collaborating with industry, academics, and other governmental departments to explore new methods for characterizing the chemical composition of complex food products. In the last few years, NRC researchers have shown that nuclear magnetic resonance (NMR) is an analytical technique of choice for evaluating the composition of complex mixtures in a quantitative and reproducible manner. This talk will discuss a few approaches combining NMR and chemometrics methods which were successfully applied to the study of natural health products, roasted coffee, honey, and more recently pulse-derived products.

Characterizing self-assembly features of a hydrophobin from Wallemia ichthyophaga

Ruiwen (Raymond) He
Biochemistry and Molecular Biology, Dalhousie University

Email: Ruiwen.He@dal.ca
Co-Authors: Calem Kenward, David N. Langelaan
Research Supervisor: Dr. David Langelaan

Hydrophobins are small secreted proteins that play vital roles in the growth and development of filamentous fungi (1–3). Hydrophobins can self-assemble into larger structures called rodlets. These rodlets coat surfaces such as fungal spores, making them extremely water repellent (2). Hydrophobins are functionally separated into two different classes. Class I hydrophobins form durable amyloid-like rodlets, that are heat and acid resistant as well as insoluble in detergent (4). In contrast, class II hydrophobin assemblies are less stable and can be dissociated by detergent solutions. Hydrophobin has been used to modify surfaces to prevent fouling or biofilm formation, as new drug delivery agents, as biosensors, and to make hydrophobic surfaces more biocompatible, which is important for the success of medical implants (5, 6). It is necessary to understand the structure and self-assembly mechanisms of hydrophobins so that their properties can be controlled and applied. This project focuses on WI1, a model hydrophobin from Wallemia ichthyophaga. We first determined the conditions required for self-assembly of WI1 through thioflavin T (ThT) fluorescence assays. Additionally, the surface activity of WI1-treated surfaces was also compared with other hydrophobins and surface-active proteins by measuring water contact angles. We have found that WI1 assembles more slowly than other hydrophobins. WI1 also preferentially assembles at pH 6.5 and in high salt conditions. We are now using atomic force microscopy to characterize the morphology of WI1 assemblies. In the future we will use site-directed mutagenesis to assess the ability of WI1 variants to coat surfaces.

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4. Gandier, J.-A., Langelaan, D. N., Won, A., O'Donnell, K., Grondin, J. L., Spencer, H. L., Wong, P., Tillier, E., Yip, C., Smith, S. P., and Master, E. R. (2017) Characterization of a Basidiomycota hydrophobin reveals the structural basis for a high-similarity Class I subdivision. Sci Rep. 7, 45863

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The highly cationic N-terminal region of apelin-17 drives its selective interaction with bicelles

Tran Thanh Tam Pham, PhD Candidate
Biochemistry & Molecular Biology

Email: Tam.Pham@Dal.ca
Co-Authors: Albarda, J. & Rainey, J. K.
Research Supervisor: Dr. Jan K. Rainey

One important aspect of the peptide-G-protein coupled receptor (GPCR) binding process is the role of the cell membrane as a mediator to facilitate recognition and binding events. Such a role for the cell membrane has been observed for a variety of GPCR-interactive ligands, including neuropeptides and opioid receptor ligands. In the case of the apelin receptor, a class A GPCR involved in many physiological and pathophysiological processes, both of its endogenous peptide ligands - apelin and apela – have been shown to interact with different membrane mimetics. The structural changes observed for apelin isoforms upon membrane interaction are hypothesized to facilitate recognition by the apelin receptor. To test this hypothesis in physiological conditions, we have been developing 19F nuclear magnetic resonance (NMR) approaches by designing fluorinated apelin-17 analogues with 4-trifluorophenylalanine (TFP4) at different positions. We confirmed that there was a conformational change from disordered to α-helical character for apelin-17 and all analogues upon interaction with membrane-mimetic bicellar systems. Using 19F-NMR experiments, we localized the interactions of apelin-17 analogues with bicellar systems. We observed a decrease in diffusion coefficient (Dc) of apelin-17 and fluorinated analogues in the presence of bicelles which suggested binding to bicelles. Localization of binding by STD experiments showed that peptide-membrane interactions exhibit the highest selectivity for the N-terminal region. This is most likely due to cationic residues that bind to anionic headgroups in some bicelles. This study introduces 19F-NMR methodologies as powerful tools to understand the mechanism in apelin-membrane interactions and the hypothesized role of the membrane.

Fighting Antimicrobial Resistance (AMR) using Traditional Philippine Medicinal Plants

Julie Anne Dayrit
Chemistry, Saint Mary's University

Email: julie.anne.dayrit@smu.ca
Co-Authors: Dr. Clarissa Sit
Research Supervisor: Dr. Clarissa Sit

Antimicrobial resistance (AMR) claims at least 700,000 lives per year and are projected to be associated with the deaths of 10 million people per year by 2050 worldwide. Medicinal plant sources have been extensively explored for new chemical entities for therapeutic and drug development purposes and found to have potent antibacterial and antifungal activity. Microbes living in plants, called endophytes, have also been investigated and found to produce beneficial molecules that have therapeutic use. Endophytes are frequently associated with the medicinal properties found in the plant source and some have served as potential candidates for antimicrobial, anti-insect, and anti-cancer drug discovery.

The goal of this project is to extract and identify the active compound responsible for the inhibitory activity against pathogenic bacteria and fungi. The activity of both plant and endophyte extracts will be tested against gram-positive bacteria Bacillus megaterium and Stapylococcus epidermidis; Escherichia coli, a gram-negative bacterium, and Mycobacterium smegmatis, a non-pathogenic proxy for Mycobacterium tuberculosis. As well as pathogenic fungi such as Candida albicans, and Aspergillus brasiliensis. Each endophyte isolates that shows potential to kill other microbes will be grown in bigger scale and will be extracted to characterize the active compound/s responsible for the inhibition.

The potential discovery of new active compound will aid the process selection for active compounds appropriate for clinical drug trials and treatment for infectious diseases caused by microorganisms such as bacteria and fungi. This project will also contribute to the efforts on the exploration and documentation of indigenous medicinal plants and reconnection to Philippine ancestral and indigenous knowledge.

Structure elucidation and determination of stereochemistry of tropicicolide from an endophyte of the Canadian medicinal plant Sorbus decora

Janet Debly
Biological Sciences, University of New Brunswick

Email: jdebly2@unb.ca
Co-Authors: Kirstyn A. Forgrave, John A. Johnson, and Christopher A. Gray
Research Supervisor: Dr. Christopher A. Gray

Natural products produced by endophytic fungi are an excellent source of diverse and complex chemical structures. Mass spectrometry-based prioritization has been proven to be a successful way to isolate novel natural products. In collaboration with Adapsyn BioScience Inc., mass spectrometry data from our fungal extract library was screened in Adapsyn BioScience's dereplication platform. The extract of an endophyte isolated from the Canadian medicinal plant Sorbus decora was highlighted due to the presence of a unique ion peak. Isolation and structure elucidation of the target compound revealed it to be the macrolide, tropicicolide. This presentation will describe the endophyte isolation and fractionation, structure elucidation, and methods used to determine the stereochemistry of tropicicolide.

Pseudogymnoascus Destructans: the Causative Agent of White-Nose Syndrome in North American Bat Species

Olivia Roland
Chemistry, Saint Mary's University

Email: Olivia.Roland@smu.ca
Research Supervisor: Dr. Clarissa Sit

Pseudogymnoascus destructans, previously referred to as Geomyces destructans, is the fungal pathogen that causes a widespread disease known as white-nose syndrome (WNS) in North American bats. An isolate of the fungal pathogen is thought to have been brought to North America from a hibernaculum in Europe or Asia. This travel across the Atlantic, as well as its dispersal in North America, is widely hypothesized to have resulted from human migration, specifically via tourism. P. destructans is a psychrophile, thriving under temperatures of 1 – 20 ℃ which coincidentally is the hibernation temperature of bats. Mortality due to WNS is caused by a disruption to homeostasis, due to the fungal pathogen inflicting severe damage to the wings. Implications of the wing damage include evaporative water loss, increased arousal, energy expenditure, hyperventilation, dehydration, and emaciation as well as electrolyte imbalances and poor circulation. This research project will focus on the potential for compounds collected from the environment (e.g., hibernacula), or the bats' natural microbiome, to assist in inhibiting the growth of P. destructans as a novel method in the treatment of WNS.

Synthetic Methods for Accessing Heterocycle-containing Peptides

Dr. Carlie Charron, Assistant Professor
Chemistry, Dalhousie University

Email: carlie.charron@dal.ca
Co-Authors:

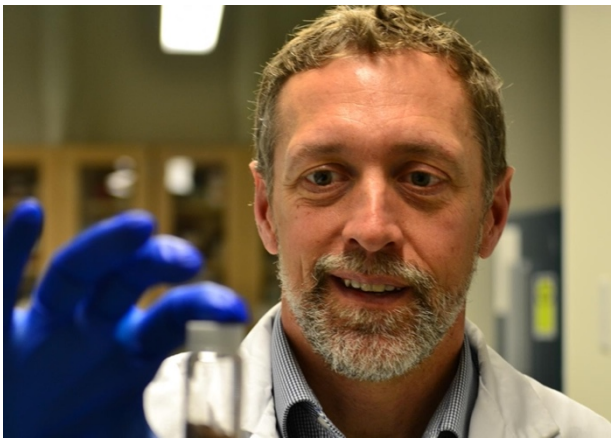
Macrocyclic peptides are peptide structures that contain one or more rings spanning several amino acid residues and have become a wellspring for modern drug discovery. This conformational feature improves bioavailability, lipophilicity, and resistance to proteolytic cleavage when compared to linear peptide counterparts however, their propensity to exist in several stable three-dimensional conformations hampers their ability to associate with a biological target. Heterocyclic rings are a clever modification observed in natural products to improve target association by increasing polar surface area and hydrogen bonding potential while creating a well-defined three-dimensional conformation. Lab synthesis of heterocycle-containing peptides is notoriously challenging using solid phase peptide synthesis and as such, an understudied peptide modification avenue. The Charron lab is currently exploring synthetic methods for incorporating oxazole modifications into peptides using a combination of solution and solid phase peptide synthesis techniques. This presentation will report their progress on obtaining oxazole and methyl oxazole modifications in their pursuit of synthesizing the natural product Wewakazole B using solid phase synthesis.

Keynote Address

Exploiting α-Haloaldehydes in Complex Molecule Synthesis

Dr. Robert Britton

Chemistry Professor
Simon Fraser University



The diastereoselective addition of organometallic reagents to α-chloroaldehydes was first reported in 1959 and is historically significant as the prototypical reaction for Cornforth's model of stereoinduction. Despite clear synthetic potential for these molecules, difficulties associated with producing enantiomerically enriched α-haloaldehydes limited their use in complex molecule synthesis through the latter half of the 20th century. Over the past 20 years, however, a variety of robust, organocatalytic processes have been reported that now provide direct access to optically enriched α-haloaldehydes and have motivated renewed interest in their use as building blocks for complex molecule synthesis. Here, our efforts to produce α-haloaldehydes and exploit these versatile building blocks in complex molecule synthesis will be discussed, including applications in the synthesis of nucleoside analogues, carbohydrate mimics and polyketides.

Fabrication and Biophysical-Mechanical Correlation of Recombinant Hybrid Spider Silk Fibres

Anupama Ghimire
Biochemistry and Molecular Biology, Dalhousie University

Email: an696021@dal.ca
Co-Authors: Lingling Xu, Xiang-Qin Liu, Jan K. Rainey
Research Supervisor: Dr. Jan K. Rainey

Spider silks are natural protein-based biomaterials produced by spiders for purposes such as web construction, locomotion, wrapping of prey and protection of eggs. They are renowned for their mechanical properties and hold great promise for applications ranging from high-performance textiles to the regenerative medicine. Our lab has been focused on two less characterized spider silks: aciniform and pyriform silks. Each silk has distinctive mechanical behaviour and physicochemical properties, with materials produced using combinations of these silks currently unexplored. This study focuses on the development and comparative characterization of hybrid silk fibres spun from fused protein consisting of two repeats of pyriform (Py) followed by two repeats of aciniform (W) silks named as Py2W2. The fused protein was expressed in Escherichia coli and purified by Ni2+-NTA affinity chromatography. Far-UV circular dichroism spectroscopy demonstrated α-helicity for hybrid protein in a fluorinated acid- and alcohol-based solution used to form a dope for wet-spinning. Building on methods previously introduced in our lab, wet-spinning enabled continuous fibre production. Post-spin stretching of the resulting wet-spun fibres (i.e., stretching from the “as-spun” state) in air or ethanol significantly improved the extensibility (~30-fold more extensible) and the tensile strength (~4-fold stronger), respectively. Polarized light microscopy revealed that the anisotropy of hybrid fibres increased upon post-spin stretching, indicating increased molecular alignment along the fibre axis. Fibre-state secondary structuring was evaluated by Fourier transform infrared spectromicroscopy which demonstrated differences in the proportions of α-helix, β-sheet, and other structures as a function of post-stretching conditions. Understanding the properties of hybrid fibres will determine their suitability and tunability for disperate applications.

Synthesis and Characterisation a Novel Class of Azo Dye; Azo Bispyrroles

Steve Sequeira
Chemistry, Dalhousie University

Email: steve.sequeira@dal.ca
Co-Authors: Dr. Alison Thompson
Research Supervisor: Dr. Alison Thompson

Azo dyes are a class of chromophores that can be tuned to access the entire visible range. This characteristic makes azo dyes highly attractive for uses as colouring agents, accounting for the majority of industrial dyes. Although azo benzene is well researched, heteroaryl azo dye remains under-explored. Among all heteroaryl dyes, azo bispyrroles have not been reported. The work herein discusses the isolation of azo bispyrroles, a novel class of azo dyes, and the exploration of their photophysical characteristics.

Quantum Mutual Information: A Novel Insight into Amino Acid Interactions and Chemical Bonding in Biomolecules

Mostafa Javaheri Moghadam
Chemistry, University of New Brunswick

Email: m.javaheri@unb.ca
Co-Authors: S. De Baerdemacker
Research Supervisor: Dr. Stijn De Baerdemacker

Proteins, the essential biomolecules in biological processes, derive their diverse roles from the intricate structure and function of their constituent building blocks, the 20 naturally occurring amino acids. Understanding the interactions between these amino acids is crucial to unveil the secrets of protein functionality. However, accurately describing such interactions demands high-quality ab initio methods, which are often limited to medium-sized systems.

In this study, we present a novel approach to comprehend the chemical interactions of amino acids by introducing the concept of quantum mutual information. Our analysis focuses on the entanglement between pairs of atomic orbitals within the encompassing environment of all other active-space orbitals. By employing von Neumann entropy and a one- or two-particle reduced density matrix [1], we evaluate the orbital entropy and atomic mutual information across a range of dipeptides.

The results of our investigation reveal that atomic mutual information serves as a valuable tool to gauge the strength and character of chemical bonding in biomolecules. This approach provides new insights into potential interactions between amino acids, thereby expanding its applicability to larger systems beyond the scope of high-level ab initio methods. With this quantum mutual information framework, we pave the way for a deeper understanding of the complex interactions that govern protein structures and functions.

Discovery of antimicrobial natural products from fungal extracts

Allyson Bos
Biology, University of New Brunswick

Email: allyson.bos@unb.ca
Co-Authors: John A. Johnson, Christopher A. Gray
Research Supervisor: Dr. Christopher A. Gray

Over the past five decades, antimicrobial resistance has been steadily increasing at a pace that the discovery and development of new antibiotics has not been able to meet. As such, it is becoming increasingly difficult to treat bacterial infections. Natural products are a diverse group of structurally complex and bioactive organic compounds of which fungi are known to be a significant source of therapeutically relevant molecules. Within the fungal kingdom, endophytes (fungi that grow with plant tissues) in particular, provide an under-investigated source of novel chemical diversity although the isolation of previously unknown chemical structures remains a considerable challenge. A growing trend in response to repeated re-isolation of common natural products is the development of prioritization methodologies enabling better navigation of biological extract libraries. Past members of the Natural Products Research Group (NPRG) independently employed bioactivity profiling and mass spectrometry metabolomics strategies to prioritize subsets of the NPRG's library of endophytic extracts. The research that will be discussed in this presentation expands upon that work by utilizing these complementary techniques in tandem to direct the discovery of novel antibiotic natural products from a library of 473 fungal extracts. This modified approach has identified 28 chemical targets in 18 bioactive extracts that are each currently being investigated to determine the putatively novel bioactive compounds.

Characterization of a Novel Racemase for the Production of (S)-Equol: Dihydrodaidzein Racemase

Meghan Hamilton
Biochemistry and Molecular Biology, Dalhousie University

Email: meghan.hamilton@dal.ca
Co-Authors: Dr. Stephen Bearne
Research Supervisor: Dr. Stephen Bearne

Plant-based foods are known to reduce morbidity and mortality of various chronic diseases. More specifically, diets rich in isoflavones from plants coincide with health benefits including diminished risk of cancers, osteoporosis, cardiovascular disease, neurodegenerative diseases, and adverse postmenopausal symptoms. (S)-Equol is a chemoprotective metabolite of the soybean isoflavone daidzein, which is produced by bacteria in the gut of some individuals to induce endocrine effects. (S)-Equol exhibits the greatest antioxidant, the greatest bioavailability, and the slowest clearance rate among all soy-derived isoflavones. Consequently, there is a desire to provide (S)-equol as a health supplement produced either in vitro or via modulation of the gut microbiome. Over the past decade, the pathway for the biotransformation of daidzein to (S)-equol has been investigated. Recently, a novel dihydrodaidzein racemase (DDRC) was discovered that catalyzes the interconversion of (R)-dihydrodaidzein and (S)-dihydrodaidzein required for the biosynthesis of (S)-equol. Thus, the enzymatic activity of DDRC is of therapeutic interest in the production of (S)-equol. We hypothesize that DDRC may be used to conduct a kinetic dynamic resolution in the production of (S)-equol which would allow for 100% yield of (S)-equol from the starting material using a green biosynthetic approach. To exploit the activity of DDRC in the production of (S)-equol the enzymatic activity of DDRC must be further understood. We aim to establish an assay to characterize the enzyme's activity using circular dichroism spectroscopy, to determine the X-ray crystal structure of the enzyme, and to identify the catalytic Brønsted acid-base residues responsible for catalyzing the reaction using site-directed mutagenesis. These studies will afford the first characterization of the mechanism of DDRC which performs an important interconversion in the production of (S)-equol.

Tolypocaibols: Antibacterial lipopeptaibols from a Tolypocladium sp. endophyte of the marine macroalga Spongomorpha arcta

Nicholas Morehouse
Chemistry, University of New Brunswick

Email: nicholas.morehouse@unb.ca
Co-Authors: Andrew J. Flewelling, Dennis Y. Liu, Hannah C. Cavanagh, Roger G. Linington, John A. Johnson, and Christopher A. Gray
Research Supervisor: Dr. Christopher A. Gray

Two new lipopeptaibols, tolypocaibols A (1) and B (2), and the mixed NRPS-polyketide-shikimate natural product maximiscin (3) were isolated from a Tolypocladium sp. fungal endophyte of the marine alga Spongomorpha arcta. Analysis of NMR and mass spectrometry data revealed the amino acid sequences of the lipopeptaibols, which both comprise 11 residues with a valinol C-terminus and a decanoyl acyl chain at the N-terminus. The configuration of the amino acids was determined by Marfey's analysis, and the antibacterial activities of these natural products were assessed against a panel of both Gram-positive and Gram-negative organisms. Tolypocaibols A (1) and B (2) showed moderate, selective inhibition against Gram-positive strains, while maximiscin (3) showed moderate, selective broad-spectrum inhibition.

Determination of the absolute configuration of lignicol and isolignicol

Mathieu Laprise
Biological Sciences, University of New Brunswick

Email: mlaprise@unb.ca
Co-Authors: Nicholas J. Morehouse, Trevor N. Clark, Katie A. Dean, John A. Johnson, Christopher A. Gray
Research Supervisor: Dr. Christopher A. Gray

Determining absolute configuration is important for evaluating the biological effects that a molecule can have on a metabolic system. The focus of my research is to determine the absolute configuration of two natural products (lignicol and isolignicol) isolated from an endophytic Mollisia sp. fungus of the medicinal plant Hypericum perforatum (St. John's wort). While the relative configuration of lignicol has been proposed, its absolute configuration has not been confirmed. To determine the absolute configuration of these molecules, X-ray crystallography will be performed to obtain exact coordinates of each atom in the molecules. On top of this, I will be conducting Mosher's ester analyses and employing Horeau's method to verify that they provide complimentary results to each other and that of the X-ray crystallography. Finally, computational chemistry will be used to calculate the predicted chemical structures using molecular modelling to compare the predicted and the experimental ECD spectra to determine if this method is reliable for these types of molecules. The use of these four methods will provide the utmost confidence in the absolute configuration of both lignicol and isolignicol.

Conjugation of phytoglycogen nanoparticles to aciniform spider silk via disulphide linkages

Sara Evans
Biochemistry & Molecular Biology, Dalhousie University

Email: sara.evans@dal.ca
Co-Authors: Dr. Jan K. Rainey
Research Supervisor: Dr. Jan K. Rainey

Female orb-weaver spiders can produce up to seven kinds of protein-based silks with mechanical properties corresponding to their function. Spiders use aciniform silk to contain prey and line egg sacs, so the silk is both extensible and tough. In this work, engineered cysteine residues are used to form disulphide bonds between the protein and phytoglycogen nanoparticles. Phytoglycogen nanoparticles are nearly monodisperse glucose dendrimers. They have been used to encapsulate molecules to enhance their solubility and bioavailability. We hypothesize that an aciniform/phytoglycogen hybrid material will have the mechanical properties of the silk as well as the encapsulation capabilities of phytoglycogen. Thiol groups are being added to the phytoglycogen via esterification by thioglycolic acid. Thiolated phytoglycogen particles are being characterized by dynamic and static light scattering, Ellman's assay, and zeta potential. The thiolated phytoglycogen nanoparticles will be regiospecifically reacted with cysteine residues in the aciniform protein to form a disulphide bond. Both effectiveness and properties of the resultant materials will be compared when the functionalization of aciniform silk with phytoglycogen is performed in the solution-state before spinning vs in the solid fibrous state. The mechanical properties of the resultant material will be measured using a tensile testing apparatus, the morphology will be evaluated by second and third-harmonic generation microscopy, and the encapsulation efficiency will be determined. $(SR)_{18}$ can be applied to other nanocluster system to investigate the structure-property relationship.

Efficient Synthesis and Functionalization of 3-Bromonaphtho[2,3b]thiophene

Emily Burke
Chemistry, Dalhousie University

Email: em460329@dal.ca
Co Authors: Dr. Alex Speed
Research Supervisor: Dr. Alex Speed

Naphtho[2,3b]thiophene is a sulfur containing aromatic heterocycle. Its synthesis commonly includes a Friedel-Crafts reaction, followed by a Clemmensen or HI/Pred reduction, then redox transformations, and finally a Bradsher cyclization. The substrate for the Bradsher cyclization requires a multistep synthesis involving the use of harsh reagents, multiple oxidation state changes and purification steps. Our work shows a shortened synthesis avoiding the use of harsh reagents by employing a copper-catalyzed cross-coupling reaction to access an acetal-containing Bradsher cyclization substrate. From this technique naphtho[2,3b]thiophene was successfully synthesized. During scale up procedures, a safety concern was raised from the decomposition of a key intermediate due to exothermic ring-alkylation polymerization. Alterations to the thiophene starting material to include a bromine substituent to stabilize the thiophene intermediate and hinder ring-alkylating polymerization tamed the decomposition reaction. Through a similar synthetic route, the 3-bromonaphthothiophene derivative was then synthesized. Further derivatization of the 3 position on 3-bromonaphthothiophene was completed using lithium-halogen exchange, while exploring the importance of the order of addition in this reaction. Quenching the lithium halogen exchange reaction with DMF resulted in the aldehyde containing derivative and quenching with isopropoxy B(pin) afforded the arylpinacolboronate derivative.

Keynote Address

Using Multiparametric Approaches to Discover Novel Signaling in Health and Disease

Dr. Jillian L. Rourke

Chemistry & Biochemistry Professor
Mount Allison University

We recognize that the foods, xenobiotics, and medications that we encounter daily play a critical role in coordinating our metabolic health, where dysfunction increases our risk for obesity, metabolic syndrome, diabetes, heart disease, and cancer. Lower nutrient quality in our foods and increasing use of non-nutritive additives such as sweeteners, indigestible fats, preservatives, and contaminants interact with our cells with unknown functional consequences. The Rourke lab investigates how human cells sense these micro- and macronutrients, and toxins and how imbalances in these sensing activities contribute to the development of disease.

In the Mount Allison Centre for Toxicological and Pharmacological Discovery (CTAP), we use molecular and cell biology techniques to quantify human cell health following nutrient stress, exposure to toxins, and treatment with novel small molecules. The Rourke Lab team seeks to address emergent, high-impact biological questions like: How does cellular nutrient sensing contribute to metabolic diseases like obesity, diabetes, and cancer? And, how can we exploit the power of high throughput experimentation to improve training and new product safety?

To illustrate how we harness high-throughput and multiparametric approaches to empower undergraduate and graduate researchers in biology, biochemistry, and chemistry, this presentation will highlight recent discoveries in the lab including identification of a receptor for the non-nutritive sweetener sucralose, characterization of amino acid signaling across the GPCRome, and profiling the cytotoxic properties of novel nanoparticles and bioactive small molecules.

Exploring the molecular promiscuity of L-phenylalanine (Phe) activation of G protein-coupled receptors

Peter Oni
Chemistry and Biochemistry, Mount Allison University

Email: oponi@mta.ca
Co-Authors: Madeline E. Power and Jillian L. Rourke
Research Supervisor: Dr. Jillian L. Rourke

Introduction: Recent advances in nutrient-sensing mechanisms revealed that amino acids (AA) act as ligands for class C G protein-coupled receptors (GPCRs). The AA Phenylalanine (Phe) is proposed to activate the adhesion GPCRs GPR56 and GPR97 and class A GPCRs GPR142 and GPR139. This study aimed to determine the extent to which Phe activates class A GPCRs.

Methods: We quantified the activation of 277 GPCRs treated with a Phe using a high-throughput B-arrestin2 recruitment assay (PRESTO-Tango). GPCRs were classified as a candidate Phe receptor if the activation magnitude exceeded 2-fold and was statistically significant (GraphPad Prism, two-way ANOVA with multiple comparisons, $p<0.05$).

Results: The treatment of 277 class A GPCRs with Phe revealed that it significantly activates ~53% of the tested receptors and 34 receptors (12.3%) had greater than 5-fold change. The top 5 receptor candidates with the greatest average Z Score fold change were GPR88 (8.36), DRD4 (8.40), NPBW1 (9.23), SSTR4 (9.82), and MTNR1B (11.54). Using in vitro signaling luciferase reporters, significant downstream signalling profiles were identified for some receptors, while others did not impact tested pathways. Further results show that Phe activates multiple established receptor families, and significantly modulate the signalling of Lysophosphatidic acid receptor family.

Conclusions: These data suggest that Phe is a promiscuous endogenous ligand for numerous GPCRs. This contributes to the growing evidence supporting AAs as signalling messengers in nutrient status sensing.

Molecular Characterization and Formation of Novel Reversible Boronic Acid-Diol Complexation

Karin Reznikov
Chemistry, Dalhousie University

Email: Kr826892@dal.ca
Co-Authors: Cyril O'Brien, Ebrahim Soleimani, David Jakeman
Research Supervisor: Dr. David Jakeman

Boronic acids have an impact on a variety of applications in drug discovery and medicinal chemistry. They have even been approved for drug use to treat a variety of conditions, such as multiple myeloma and lymphoma, with the boron atom critical to the drug- target interaction. Therefore, an understanding of their structure and reactivity is essential.

The boranol part of the boronic acid derivative behaves as a lewis acid which can form a tetravalent trihydroxyborate conjugate base, and that could make a sufficient aromatic character which would form a boron oxy conjugate base, and avoid the disruption of ring aromaticity that would occur with a tetravalent boronate anion. In addition, boronic acids are known to bind with compounds containing diol moieties, such as saccharides through reversible ester formation. Such binding allows boronic acids to be used as a recognition moiety when constructing sensors for those saccharides, as nucleotide and carbohydrate transporters and potentially act as antibody mimics targeted on cell- surface carbohydrates.

We have synthesized and investigated the properties of bifunctional molecules containing boronic acids using UV- Visible, NMR and fluorescence spectroscopy to better understand their physicochemical properties including interactions with monosaccharides in aqueous solutions.

Data from these experiments will be presented and discussed.

Biomaterials for Drug Development and Non-Canonical Residues for Protein Engineering

Dr. Alexander Baker, Assistant Professor
Chemistry, Dalhousie University

Email: alexander.baker@dal.ca
Co-Authors: Laura C. Bahlmann, Chang Xue, Yung Hsiang (John) Lu, Allysia A. Chin, Jennifer Cruickshank, David W. Cescon, Molly S. Shoichet

This talk will cover two themes: first, the design of biomaterials for drug development; and secondly, my approach to expanding non-canonical amino acids for protein engineering.

Designing hydrogels to capture the physicochemical attributes of tissues is crucial to achieving the desired cellular phenotype in vitro. Furthermore, the importance of the immune system in response to numerous diseases such as cancer highlights the need for biomaterials compatible with several cell types. Hyaluronan is over-expressed in breast cancer and in several other tumours. We synthesized a biomimetic oxime crosslinked hydrogel composed of hyaluronan and matrix metalloproteinase-cleavable peptide. We identified an optimal extracellular matrix supplement to study the polarization of healthy mammary epithelial cancer cells in vitro. We then extended the platform to study nine different cancer tissue types grown within our engineered hydrogel in vitro. This material was compatible with patient cells isolated from tumor biopsies enabling the establishment of organoids. We observed a differential response to drugs when patient organoids were cultured in Matrigel® versus our engineered hydrogel. We were able to expand sufficient cells in vitro to establish patient-derived xenografts (PDX) using SCID mice. Our hyaluronan-based hydrogel showed superior reproducibility in establishing tumour growth compared to Matrigel®. The immune cell response in vivo was not biased by our delivery hydrogel. In the case of PDX grown in Matrigel® the tumour showed skewed macrophage polarization based on the proportion of alternatively activated resident murine macrophage cells.

Application of systems engineering principles toward the creation of a rhomboid protease activated repressor of gene transcription

Dr. James Davey, Assistant Professor
Biochemistry and Molecular Biology, Dalhousie University

Email: james.davey@dal.ca
Co-Authors: Natalie Goto

Conventional approaches to engineering multidomain protein structures and functions requires that a native protein sequence serve as a template from which constituent protein domains can be modified or replaced to achieve targeted activities. Consequently, design rules are formulated to describe the organization and compatibility of each domain, contributing to an expanding library of modular components. However, this approach cannot be applied to engineer new functions when there exists no related multidomain protein sequence to serve as an engineering template. To circumvent this limitation and capitalize on this database of modular components, we developed a new experimental methodology utilizing a screening strategy to identify functional candidate multidomain protein architectures from a library of unrelated modular components. This function-guided modular design strategy was applied to create a novel membrane-bound repressor that attenuates gene expression from a target promoter upon activation by the proteolytic activity of endogenous bacterial rhomboid protease (GlpG). The architecture of this rhomboid protease activated repressor was identified from a library of candidate multidomain architectures using a three-component genetic circuit to control expression of enhanced green fluorescent protein. This successful application of function-guided modular design suggests a plethora of novel multidomain protein architectures may be readily discovered.