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Developing Fluorescent Probes to Shed Light on Brain Signalling

Student: Sydney Acton
Supervisor: Frederic Menard

Ca2+ signaling in astrocytes is essential to neuronal activity, yet the molecular mechanisms underlying this process are poorly understood. This project involves the design and synthesis of a receptor-specific molecular probe based on domoic acid (a naturally-occurring red algae neurotoxin) to monitor and control Ca2+ signaling between neurons and astrocytes. Domoic acid is an agonist for ionotropic glutamate receptors (iGluR) and its binding site has been established by X-ray co-crystallization. Using this information, we synthesized a derivative of domoic acid that contains a reporter tag (fluorescent dye) to conduct imaging studies in live cells. This molecular probe is composed of three parts: a domoic acid analog, a linker chain, and a fluorescent dye. The modular strategy enables variation of the linker and the fluorophore to alter parameters such as solubility. Our first-generation molecular probe will be used in HEK cells heterologously expressing iGluRs, and will provide the foundation for characterization of Ca2+ signalling in astrocytes using imaging and electrophysiology techniques.

Accurate Detection and Quantification of beta-methylamino-L-alanine in Food and Environmental Samples

Student: Teesha Baker
Supervisor: Susan Murch

β-N-methylamino-L-alanine (BMAA) is a non-protein amino acid, produced by symbiotic cyanobacteria in cycad (Cycas micronesica Hill) plants. It has been found that BMAA is a neurotoxin associated with neurodegenerative disease in humans. In 2002, it was hypothesized that BMAA accumulates in some food webs but there has been significant controversy in the literature with respect to the analytical methods for detection and quantification. More than 59 methods papers have been published with 11 describing a failure to detect BMAA in either cyanobacteria or human samples. Two of these analytical methods were compared for analysis of BMAA by liquid chromatograph tandem mass spectrometry using 2 different derivatizing agents, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) and propylchloroformate (EZ:faast) in 5 sample matrices. While both AQC and EZ:faast derivatization detected BMAA at relatively high levels in blue crab, only the AQC method detected BMAA in brain and neither detected BMAA in the negative control matrices. The AQC method quantified BMAA and the isomers N-(2-aminoethyl) glycine (AEG) and 2,4-diaminobutyric acid (DAB) in different Nostoc 29150 cultures with the characteristic fragments of 258.1 m/z, 214 m/z, and 188 m/z, respectively. The different Nostoc cultures included a standard BG11 medium, a BG11 medium supplemented with 1.0 mM glutamine, and a nitrate-free BG11 medium to determine the role of environmental nitrogen on BMAA, AEG, and DAB production. The BMAA content of Nostoc was significantly decreased by the addition of glutamine to the media and significantly increased in the cultures grown in the absence of nitrate in the medium. In contrast, AEG was increased by addition of glutamine and both AEG and DAB were decreased in cultures grown on nitrate-free media. Together these data provide optimized methods for detection and quantification of BMAA in cyanobacteria and evidence demonstrating that the biosynthesis of BMAA and its isomers is regulated by environmental nitrogen.

Wolbachia parasitism in Diplolepis wasps throughout the Okanagan Valley

Student: Danielle Bartkiewicz
Supervisor: Bob Lalonde

Wasps of the genus Diplolepis form galls on wood roses (Rosa gymnocarpa) throughout the Okanagan valley, ranging from Vernon to the Canadian-American border. The wasps are often parasitized by the bacteria Wolbachia, which feminizes males and thus can alter the sex ratios and population structure of Diplolepis wasps. It has been demonstrated that Wolbachia is sensitive to temperature, and the range of climate conditions across the valley may influence which wasp populations have been infected. This study involved sampling galls from three wasp species (Diplolepis variabilis, Diplolepis rosafolii and Diplolepis spinosa) along a

north-south gradient in the Okanagan valley. Half of the collected galls were dissected to extract larvae, and a PCR assay was conducted to amplify the CO1 mitochondrial locus to confirm species identity, and the Wsp locus to test for the presence of Wolbachia. The remaining galls were left intact to allow adult emergence in order to estimate the sex ratio of the wasps at each sampling location.

Synthetic Pyrethroid Insecticides Inhibit the Electron Transport Chain in Mammalian Mitochondria

Hallway

Student: Kiranjot Birk, Manjot Birk
Supervisor: Anthony Stea

Pyrethrins are a naturally occurring insecticides produced by several Chrysanthemum species. However, the instability of these natural compounds make them inadequate for residential and agricultural use. Several generations of synthetic variants known as pyrethroids have since been produced, with each generation having increased potency and photostability. These pyrethroid insecticides can be classified as type I or type II based on the symptoms they induce and the presence of an alpha cyano group. Although significant research has explored mammalian toxicity of pyrethroids on ion function, very few studies have investigated possible adverse effects on mammalian mitochondrial electron transport chain. In this study, we develop an assay system to measure electron transport chain activity via oxygen consumption. One main objective of this study was to compare the effects of type I pyrethroid permethrin and type II pyrethroid cypermethrin on electron transport chain activity. Another objective was to determine the effects of three different generations of pyrethroid: allethrin, tetramethrin, and fenvalerate. Using succinate as an electron donor, 100 mM permethrin and cypermethrin blocked oxygen consumption on average 50.99% +/- 8.96% (n=10) and 50.63% +/- 4.23% (n=8) respectively. The first and second generation pyrethroids, allethrin and tetramethrin showed significantly greater (p<0.01) block of O2 consumption (100 mM: 87.67% +/- 2.22%, n=9; 89.00% +/- 2.31%, n=8, respectively). While the third generation pyrethroid showed a block of 67.05% +/- 3.69%, n=8. These results suggest that a change in chemical structure of the pyrethroids, making them more stable and effective as pesticides appears to lower the toxic effects of these compounds in mammalian mitochondria.

The Relationship of ADHD Symptomology with Bidirectional and Unidirectional Intimate Partner Violence

ART 219

Student: Sarah Brennan
Supervisor: Zach Walsh

Attention-deficit/hyperactivity disorder (ADHD) has been associated with perpetration of intimate partner violence (Fang, Grosse, Mercy, Massetti, & Ouyang, 2010; Wymbs, et al., 2012) as well as other forms of maladaptive behaviour in adolescence and adulthood. However, this association is usually studied in the context of physical perpetration of IPV and among those who have a previous diagnosis of ADHD. The tumultuous relationships often experienced by those with ADHD symptoms (Biederman, Faraone, Spencer, Mick, Monuteaux, & Aleardi, 2006) may put them at greater risk of both victimization and bidirectional patterns of abuse in intimate relationships. This study seeks to examine the relationship between ADHD symptomology and victimization and perpetration of IPV. It is anticipated that when controlling for conduct disorder and psychopathy, those reporting high ADHD symptoms will show greater instance of perpetration and bidirectional violence.

Spatial Data Analysis applied to Environmental Pollution and Geochemical Mapping

Hallway

Student: JoonHwan Cho
Supervisor: Sylvia Esterby

The purpose of spatial data analysis is to explain the spatial variation of a property in terms of other attributes taking the spatial positions of the sampling points into account. Methods of spatial statistics incorporate both the distribution of the sample data

and the spatial correlation among sampling points which are positioned in some reference coordinate system. Spatial analysis is widely applied in fields where a key feature is the assumption that close locations are more similar than locations far apart. In geostatistics, researchers spatially interpolate and make predictions at un-sampled locations to evaluate mineral and petroleum resources, and in hydrology and environmental assessment, as examples. It is also applied in economics to study the variation in economic growth rates across regional economies. In this study, we explore the application of spatial analysis through two separate data sets. Near-shore sediment samples from the Gulf of Mexico after the Deepwater Horizon oil spill in April 2010, collected by United States Environmental Protection Agency (EPA), are used to investigate the impact of the oil spill on the shores through exploratory spatial data analysis. Additionally, chemical data collected for the Kola Ecogeochemistry Project are used to evaluate multiple approaches for spatial modeling. Various descriptive modeling approaches including trend surface models and semi-variogram models (kriging) are compared relative to their representation of the spatial variation in data.

The effects of blood feeding on multi drug resistance transporter expression in the tissues of the yellow fever mosquito Aedes aegypti | Hallway

Student: Nicholas Chow
Supervisor: Mark Rheault

Multidrug resistance has been increasingly problematic in both the medical and agricultural field. Many pests, illnesses, and cancers that were once simply treated are resistant to various management strategies. This resistance has been attributed to an ABC (ATP-binding Cassette) superfamily of transport proteins found in both prokaryotic and eukaryotic organisms. The study of the superfamily has led to the identification of multidrug resistant genes: P-gp, mdr, or ABC-B (Rheault, 2005). These genes encode for ATP dependent transporters that aid in the excretion of higher molecular weight polyvalent organic cations and anions. In this study, we examined the transcript expression of the single putative mdr transporter found in the genome of the yellow fever mosquito (Aedes aegypti). Previous studies have examined the expression of various transporters in mosquitoes after a blood meal, however none have examined the mdr expression in Aedes aegypti. We used quantitative real-time polymerase chain reaction (qRT-PCR) to determine whether the mdr transcript expression levels in midgut and Malpighian tubules of A. aegypti were altered after a blood meal at 3, 12 and 24-hour post feed. Our results showed that mdr transcripts were initially higher in the Malpighian tubules compared to the midguts of adult female A. aegypti. This trend was also similar with the A. aegypti larval samples. Furthermore, mRNA transcripts in the midgut demonstrated a 3-fold increase in the 12-hour post blood fed females in comparison to the 3-hour post blood samples. However, transcript levels returned to preliminary levels where 24-hour post blood females were equal 3-hour post blood fed females. In addition, there were no significant changes in the level of mdr expression in the Malpighian tubules post blood meal. Our findings suggest that within the first 12-hours post blood meal, the mdr gene is highly up-regulated in the midgut while Malpighian tubules displayed no observable change. This indicates that the midgut is an important tissue dedicated to the excretion of toxic exogenous organic cation metabolites during blood feeding.

Development of a Ligand-Directed Chemical Ligation of Lysines | Hallway

Student: Jocelyn Creelman
Supervisor: Frederic Menard

An important challenge in chemical biology is the ability to label and monitor proteins in their native environment without genetic modifications. A general strategy was developed to label proteins with a fluorescent probe via a site-directed, tag-release mechanism. The molecular probe was composed of a ligand, a tag-release linker, and a fluorescent probe. For this method to be utilized in live cells, the protein must retain its functionality. Upon chemoselective reaction with residues on the surface of a protein, the ligand is cleaved to restore a functional protein. A tag-release linker was synthesized to demonstrate this strategy. The linker was constructed with an aromatic ester to act as an electrophile towards the amine of a lysine residue on the surface of a protein. The rate of the trans-acylation was tested in vitro with a simple lysine molecule in buffered water and monitored with 1H NMR spectroscopy. The selectivity profile of the chemical ligation was tested with other biomolecules, including small peptides.

Frizzled-independent septate junction protein, Akap200, in the wing epithelia of Drosophila melanogaster

Student: Laura De La Giroday
Supervisor: Dennis Venema

It is the frizzled-signaling pathway that determines the distal-proximal polarity of the epithelial cells of Drosophila wings; however, there is another mechanism, independent of frizzled, for establishing the local polarity of the cells. This frizzled-independent signaling pathway requires the proper localization of various components, septate junction proteins, which include zipper and nervana2. Akap200, A-kinase anchoring protein, is molecularly involved with protein localization and planar cell polarity, as well as regulation of the Ras signaling pathway and autophagic cell death. Drosophila melanogaster GFP-tagged alleles were utilized to visualize the target proteins, and screened for proper expression; the wings were collected at various points of pupal development—38 hours and 42 hours after pupal formation. After collection, the pupal wings were immunostained, to visualize the proteins as well as F-actin. The pupal wings were imaged via fluorescence confocal microscopy, and the images were edited using ImageJ64 and Photoshop CC. The preliminary results suggest that the septate junction proteins are localizing as expected, however data is still in the process of being collected and analyzed.

Expression of sesquiterpene synthase genes in response to fungal attack in flax (Linum usitatissimum)

Student: Katie Del Buono

Supervisor: Soheil Mahmoud

Flax (linum usitatissimum) is commonly grown for its oil, which is widely used in the medical and food industries. One of the main diseases of flax, which is a setback for many farmers, is an infection caused by the fungus Fusarium oxysporum. There is evidence that sesquiterpenes, a class of plant specialized metabolites, have potent antimicrobial properties, and the genome of flax encodes several sesquiterpene genes. I hypothesize that some of these genes are involved in defense against fungal attack. In order to test this hypothesis, I will study the changes in the expression of ten sesquiterpene genes by Polymerase Chain Reaction (PCR). Briefly, flax plants will be infected by Fusarium oxysporum. Next, RNA extracted from the root tissue, hypocotyl tissue, and leaves of infected and control (uninfected) plants will be reverse transcribed to produce cDNA. The sesquiterpene synthase genes will then be quantitatively amplified by standard and quantitative PCR to show any changes in their expression in infected plants as compared to the controls. The outcomes of this research effort are to improve resistance to Fusarium oxysporum through biotechnology.

Using Autonomous, Infrared Drone Technology for Cattle Tracking

Student: Christopher Foster

Supervisor: Kevin O'Neil, John Church

Cattle ranching in Western Canada is on the cusp of a technological revolution. The old approach of turning the cattle out on the range for the summer feeding season and then collecting them back in the fall via cowboys on horseback is a model dating back 200 years in the Kamloops region. Over the past 25 years some technology has been applied, with the cowboy on a horse in some instances being replaced by a cowboy on a Quad off-road vehicle, for example. Some large ranches even use a helicopter to direct the search for the cattle. Technology has the ability to revolutionize ranching over the next decade with several different technologies being used to track cattle. Drones equipped with infrared imaging equipment show great potential for being a very low cost alternative to the current approaches to searching for cattle. We are prototyping a hardware and software solution to integrate an infrared camera with a dedicated on-board computer to process the video stream in real time. We've assessed some available drones, infrared cameras, and durable embedded computers and are now in the process of mounting the camera and on-board computer on the drone. We have software capable of running real time analysis on an infrared video stream to identify the hotspots left by cattle, and match those identifications with the GPS location they were discovered at. We are currently

scheduling test flights to tweak the threshold values of the algorithm and expect the software to be fully optimized by the end of the project. We aim to provide a low-cost, autonomous alternative to manually searching for cattle.

Fish Oil Supplementation Has a Differential Effect During Murine Colitis Dependent on Background Fatty Acids | Hallway

Student: Sandeep Gill

Supervisor: Deanna Gibson

Inflammatory bowel diseases (IBD), like ulcerative colitis, are a major health burden in the West and there is currently no known cure or definite cause. Finding methods for preventing IBD should be a priority in order to reduce the disease burden in Canada. Emerging evidence has identified dietary lipid intake to be an important factor in contributing to the etiology of IBD. However, the effects of specific fatty acids on intestinal inflammation remain unknown. Research suggests that high ω-6 polyunsaturated fatty acid (PUFA) diet has been shown to exacerbate intestinal damage during murine colitis, monounsaturated fatty acids (MUFA) tend to be anti-inflammatory and have anti-oxidant effects, and while saturated fatty acids (SFA) have contradictory results. Furthermore, it is unknown if the effectiveness of fish oil supplementation is dependent on the predominant type of dietary fat consumed. The aim of this study was to investigate the effects of fatty acids, with or without fish oil supplementation, on dextran sodium-sulfate (DSS)-induced colitis. Post weaned mice were fed lipid diets rich in ω-6 PUFA (corn oil), MUFA (olive oil) or SFA (anhydrous milk fat) alone or supplemented with ω-3 PUFA (fish oil) for 5 weeks. The mice were then challenged with 3% DSS for 7 days to induce colitis. Before induction of colitis, short chain fatty acids were analyzed to determine the most protective lipid diet. After induction of colitis, factors such as colonic damage, immune cell infiltration and cytokine expression were examined. ω-3 PUFA supplementation was shown to have the most beneficial effect on a background diet rich in SFA, both before and during colitis. This study shows that fish oil supplementation has a differential effect depending on the background diet that is being consumed. These results have significant implications when considering the contradicting evidence for dietary fish oil supplementation.

Gender, Objectification, and Intimate Partner Violence | ART 219

Student: Melissa Jonnson

Supervisor: Zach Walsh

Intimate partner violence (IPV) is a substantial health concern, especially among university students (Straus, 2004). Therefore, identifying factors that predispose certain people to engage in IPV is a research priority. Although both males and females perpetrate and are victimized by IPV at high rates, women are overrepresented as primary victims and underrepresented as primary perpetrators (Henning, Renauer, & Holdford, 2006; Walsh et al., 2010). In addition, women often sustain more severe injuries from IPV (Straus, 2004). These findings have evoked a call for continued research into the gendered aspects of IPV (Ansara & Hindin, 2009; Johnson, 2006; Straus, 2006). The objectification of women is an important factor that has been linked to IPV perpetration (Tully, 2012). In this context, objectification refers to treating someone as an object, rather than a person (Fredrickson & Roberts, 1997). Research has discovered that women are objectified to a greater extent than men are and women self-objectify more than men self-objectify (Strelan & Hargreaves, 2005). These gendered objectification trends may help to explain the gender asymmetry found in IPV perpetration and victimization. This study builds upon prior research by examining the relationship between gender differences in objectification and gender differences in IPV. A sample of 954 university students completed a series of online questionnaires designed to assess levels of objectification and personal experience with IPV. Male perpetrators demonstrated the highest levels of objectification of the opposite sex, while female victims demonstrated the highest levels of self-objectification. What makes these differences in objectification interesting is that they cast doubt on the notion that male and female perpetrators and victims are equivalent. In other words, the reasons that perpetrators use violence and the effects that violence has on victims may be different across genders. In sum, this study explores a relatively new avenue of investigation in IPV research, provides insight into potential similarities and differences between male and female perpetrators and victims, and ultimately adds to the conversation about how to reduce intimate partner violence.

Calcium channel imaging tag for use in fluorescence microscopy

Student: Jeff Kerkovius

Supervisor: Frederic Menard

One of the key factors in understanding the mechanisms of heart and neurodegenerative disease is to learn the role of sarco/endoplasmic reticulum calcium - ATPase (SERCA) pump proteins in these illnesses. Such studies require a labelling probe that can enable real time visualization of these proteins in action. The ideal labelling probe will attach a fluorescent tag to only the protein of interest. To meet this need, we have synthesized a fluorescent probe joined to a reactive linker that contains a selective SERCA pump ligand. After optimization the molecule will be used to investigate SERCA pumps in live cells using super resolution fluorescence microscopy.

A comparative analysis of Acorus americanus Morphotypes and their Application in Traditional Aboriginal Medicine

Student: Nicole Labine

Supervisor: Paul Shipley

Plants from the genus Acorus have historically been used as a traditional herbal remedy to treat throat and lung infections. This project focused on identifying the chemical differences between two morphotypes of Acorus calamus var. americanus, also known as Ratroot by the Cree people, collected in the Northwest Territories. Aboriginal traditional usage has identified one morphotype for medicinal usage and the other has been identified as poisonous. Under the guidance of a traditional knowledge holder we have collected and prepared these two morphotypes using Aboriginal traditional methods. The rhizomes were extracted with an appropriate solvent, then nuclear magnetic resonance spectrometry was used to investigate the metabolome, or total small molecule chemical profile, of each morphotype. Multivariate statistical analysis allows us to identify specific chemical differences between the morphotypes. Recent results have shown that a significant difference between the morphotypes can be attributed to the stigmastane family of sesquiterpenes, which are known for their antiinflammatory, antimicrobial and antiulcer properties. It is our hypothesis that differences in chemistry between the morphotpes are responsible for the traditionally reported difference in medicinal properties, making the stigmastanes candidate molecules for further study.

Investigation of Matrix Assisted Laser Desorption/Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF-MS) for detection of Clostridium difficile toxin A and B from stool samples

Student: Cindy Lam

Supervisor: Naowarat Cheeptham

Objective: This study investigates the use of MALDI to detect C. difficile toxins A and B from stool samples. Methods: C. difficile toxin positive stool samples sent to Royal Inland Hospital were diluted 10-fold in deionized water or phosphate buffered saline (PBS) and vortexed to create a relatively homogeneous suspension. Samples were then centrifuged and the pellet removed. Proteins in the supernatant were precipitated with acetonitrile or ammonium sulfate and the solution was centrifuged again. The pellet was resuspended in deionized water or TA30 and spotted on a MALDI plate with a sinnapinic acid (SA) or SDHB matrix co-crystalized. Results: MALDI analysis showed no difference between samples diluted in deionized water and those diluted in PBS. Protein precipitation with acetonitrile produced higher quality spectra than protein precipitation with ammonium sulfate. Sample co-crystalization with SA provided higher quality spectra than SDHB. MALDI analysis showed a peak at 56 kDa in four samples. No peaks were seen in the 63 kDa range in any of the samples. Autocleavage of a commercially known toxin A also failed to show the expected peak at 63 kDA Conclusion: We were unable to use MALDI to detect C. difficile toxin A and B from crude stool protein extracts. Further studies would be required to ascertain the possibility of using this technological tool to detect C. difficile toxin as an alternative method of diagnosis to the tests currently available. Although inconclusive, this study is a starting point for the use of MALDI as a diagnostic tool in a clinical setting.

Substitution of active site aspartic acid 634 reveals its role in regulating NADPH binding in cytochrome P450 reductase.

Student: Alex Louder

Supervisor: Kirsten Wolthers

Cytochrome P450 reductase (CPR) is an enzyme that catalyzes electron transfer from the obligate two electron donor, NADPH, to a variety of physiological redox partners through two bound flavin cofactors, FAD and FMN. In humans, the enzyme is vital for supporting the function of microsomal cytochrome P450s involved in drug degradation and steroid biosynthesis. Crystal structures of NADP+-free and NADP+- bound CPR show that a conserved aspartic acid residue (D634) undergoes a conformational change upon NADPH binding. Judging from the disulfide-cross linked substrate-free structure, the side chain of D634 appears to block NADPH binding through steric clashes with the pyrophosphate moiety of the coenzyme. To evaluate the role of D634 in binding and catalysis, the residue was substituted with alanine (D634A) and asparagine (D634N). Steady state kinetic assays and stopped flow experiments were employed to study these variants. Steady state kinetic assays reveal that the variants bind NADPH more tightly than wild type CPR, severely attenuating the catalytic turnover rate of the enzyme. Stopped-flow experiments suggest that this decrease in catalytic turnover is the result of impaired electron transfer from FAD to FMN. Analysis of these data in conjunction with structural information contributes to our understanding of CPR catalysis.

The impact of climate change on predator-prey systems and their risk of extinction

Student: Jessa Marley

Supervisor: Rebecca Tyson

The relationship between predator and prey is fundamental to natural ecosystems. Anticipating how predator-prey systems will respond to climate change is critical for the management of species in danger of extinction. Populations suffering from extremely low numbers can struggle to reproduce, resulting in a faster population decline. This effect coupled with changing climatic patterns could be enough to endanger more species than is currently expected. I have developed a mathematical model that incorporates low density extinction into a cyclic predation-prey system and introduce simple perturbations that simulate a variable environment in order to quantify extinction risk as a function of changing climatic conditions.

Synthesis of Water-Soluble Cobalt Catalysts for the Green Oxidation of the Lignin Model Compound Veratryl Alcohol.

Student: Philip Martens

Supervisor: Stephen McNeil

The binding agent of wood is a complex polymeric material called lignin, which is usually wasted and burned by the paper and pulp industry, an environmentally detrimental process. This project sought to develop new methods to render lignin into a potentially renewable chemical feedstock. The ultimate goal regarding the usage of lignin is to find a chemical catalyst that is environmentally friendly, suitable for use under mild conditions, and operates without harsh reagents or harmful solvents. This would mean finding a catalyst that is environmentally friendly, suitable for use under mild conditions, used in water, and does not require any additives except oxygen to function properly. Due to the high complexity and irregularity of lignin, this has proven to be a challenging task. To identify a catalyst for the systematic breakdown of lignin, the general properties of a potentially successful catalyst have to be determined. This project focused on finding synthetic routes to very specific catalysts, and testing their ability to catalyze reactions with simple compounds that model key lignin functional groups. The catalysts studied contain cobalt, and follow a promising pattern found in previous research. By undertaking a few key changes, it was possible to test some conclusions drawn from previous research in the hope of finding key changes needed to be made in order to drive this field of research forward. Since lignin is so complex and no catalyst has been found yet which can come close to performing the desired task, all catalysts are tested on some model compounds which in one way or another, represent parts of lignin. In the case of this project, the synthesized catalysts were tested for their ability to oxidize veratryl alcohol. By obtaining catalytic data at very

specific conditions such as catalyst loading, pH and temperature, it was possible to make comparisons to previously synthesized catalysts to draw conclusions on what underlying principles need to be followed to obtain an efficient catalyst in future research.

What is on your plate? Health attributes of meat, poultry, and fish fatty acids | ART 219

Student: Lucas Martins

Supervisor: John Church

Lucas E. Martins Supervisor(s): J.S. Church, D. Prema, T.D. Turner Meat, poultry and fish are recognized as excellent sources of protein, but also provide our diet with omega-6 (n-6) and omega-3 (n-3) essential fatty acids (FA) (i.e. 18:2n-6, 18:3n-3) and their more biofunctional long-chain (LC) derivatives (i.e.20:4n-6, 20:5n-3, 22:5n-3, 22:6n-3). Increased incidences of human metabolic disorders/inflammatory response are thought to be influenced by LC-FA intake and high dietary n-6/n-3 ratio. In this study, the polar and neutral lipid FA profiles of muscle tissues were compared by feeding system within monogastric, ruminant and fish. Neutral FA were rich in SFA, c-MUFA and in the case of ruminants, t-MUFA, whereas PUFA were more abundant in membrane-bound polar lipids. Intensive production systems utilizing grains and oilseeds tend to decrease the proportion of desirable FA, raising the n-6/n-3 ratio. Fish are an excellent source in desirable LCn-3 FA, largely related to being poikilotherms and diets rich in phytoplankton derived FA. Monogastrics had a high proportion of PUFA, however, this was mostly 18:2n-6, leading to an elevated n-6/n-3 ratio. Grain-fed ruminant profiles were more similar to monogastrics, whereas, grass-fed ruminants had higher proportions of desirable n-3 FA. In conclusion, grass-fed ruminants have a desirable n-6/n-3 ratio below 4:1 and are the best alternative to fish to increase LCn-3 intake.

Quantum Tunnelling in a Radical Enzyme | Hallway

Student: Michael McGregor

Supervisor: Kirsten Wolthers

Enzymes that employ radical chemistry are able to break and re-form C—C, C—N, and C—H bonds in catalytically inert substrates. The ability to catalyze these energetically challenging reactions with exquisite control makes radical-based enzymes attractive as industrial biocatalysts. Adenosylcobalamin (AdoCbl)- and pyridoxal 5'-phosphate (PLP)-dependent ornithine 4,5-aminomutase (OAM) serves as a model system for advancing our understanding of radical-based enzymatic catalysis. OAM, from Clostridium sticklandii, catalyzes the isomerization of D-ornithine to (2R,4S)-2,4-diaminopentanoate. Radical chemistry initiates with homolytic rupture of the Co—C bond in AdoCbl, which generates a reactive deoxyadenosyl radical (Ado•). Ado• rapidly abstracts a hydrogen from the substrate, generating a substrate radical intermediate that is able to undergo intramolecular isomerization to form product. In native OAM, hydrogen abstraction exhibited a deuterium kinetic isotope effect (KIE, kH/kD) of 8, which slightly exceeds the semi-classical limit of ~7. Given that the magnitude of the KIE is suggestive of quantum tunnelling, we investigated the temperature-dependence of the KIE. Arrhenius analysis indicated that the KIE was temperature-independent for both native and a variant of OAM (Y187F), which elicited an elevated KIE of 16.7. These data indicate that over the temperature regime studied, hydrogen abstraction occurs via full quantum tunnelling, whereby the hydrogen penetrates the activation energy barrier, rather than surmounting it. In addition, we generated and studied a second OAM variant (N226D) in an effort to stabilize and characterize radical intermediates that form along the reaction pathway. The results of these experiments provide insight into how the enzyme is able to catalyze reactions with high activation energy barriers, ultimately contributing to a greater understanding of AdoCbl-dependent radical-based catalysis.

Electronic Structure and Imidyl Radical Reactivity in Chromium Phenylenediamido Complexes | Hallway

Student: Andre Mottier

Supervisor: Kevin Smith, Gino DiLabio

Chromium compounds isolated by the Smith research group will remove hydrogen atoms from some substrates to form new N-H bonds in a reaction like: R''-H + [Cr]-N*R' à R''* + [Cr]-NHR ([Cr] = CpCr[C6H4(NR)2]) where R is a substituent on [Cr], N*R is a substituted nitrogen group on [Cr] with an unpaired electron centered on the nitrogen atom, R''-H is the substrate, and R*'' is the substrate minus a hydrogen atom plus an unpaired electron. It has been shown independently that the reactivity of the "N*R'" portion of analogous complexes may depend on the presence of an unpaired electron on "N" and that interactions between R and R' may influence the electronic structure and reactivity "N*R'" by relocating an unpaired electron on "N" in a reaction like: [M]=NR' [M]*-N*R'. Recent literature reports and our own efforts have suggested that density functional computational models (in particular the BP86//6-311+G(2d,2p)/Cr-CRENBL method) are capable of accurately calculating the energies associated with reactions like those given above. In order to understand how R and R' influence the electronic structure and reactivity of [Cr] we have designed a set of model reactions for which the calculated energies are representative of the influence of R and R' on the observed hydrogen atom abstraction reactivity and from which the particular nature of the interactions between R and R' can be deduced. We have set a goal of locating a set of R and R' substituents which 1) are not so large as to prevent binding of the substrate to [Cr]-N*R', 2) drive the equilibrium reaction [M]=NR' [M]*-N*R' towards more [M]*-N*R' than [M]=NR', and 3) destabilize [Cr]-N*R' and stabilize [Cr]-NHR' enough for reactions like R''-H + [Cr]-N*R' à R''* + [Cr]-NHR to be spontaneous for a wide range of substrates. Results from the computational study will influence future design of [Cr] type complexes and improve our understanding of this and related metal mediated hydrogen abstraction systems.

Annotation and Analysis of Contig72 of Chromosome 3L of D. biarmipes   ART 219

Student: Kenneth Muir

Supervisor: James Bedard

Comparative genomics has applications in discovering evolutionary and phylogenetic processes. The Genomics Education Partnership (GEP) is an organization which annotates the genomes of Drosophila species in order to conduct comparative genomic research. In this study, contig72, a 40 kb genomic DNA sequence of from Drosophila biarmipes chromosome 3L, was examined. Databases and web-based tools of FlyBase, GEP UCSC Genome Browser, NCBI BLAST, Gene Record Finder, and Small Exon Finder were used for gene annotation of this region. The genomic DNA sequence was compared with the Drosophila melanogaster genome and seven putative orthologs were annotated: Taf6, ash1, CG9368, CG9330, CG9231, Shal, and CG14100. These genes showed conservation with D. melanogaster genes, ranging between 83-100% identity conservation. Genes known to have specific and biologically crucial functions, such as ash1 (%ID=90.4) and Shal (%ID=100.0), were highly conserved; genes suspected to have crucial functions, such as Taf6 (%ID=94.1) and CG9330 (%ID=98.0), were also highly conserved. Hidden Markov Model gene predictor programs suggested the presence of other genes on contig72; these were investigated but ruled out. Potential transcription start sites (TSSs) for these seven genes were also annotated. By studying the 5'untranslated regions (UTRs) using GEP UCSC Genome Browser Mirror, the approximate location of some TSSs were discovered. Using NCBI BLAST and FlyBase, the 5' UTRs were searched for DNA motifs with known distances to the TSS using GEP UCSC Genome Browser Mirror. These annotations will be submitted to the GEP to be used in further comparative genomics research.

UNC-53 Functions In Innate Immunity Through Expression in the Excretory Cell   Hallway

Student: Nathaniel Noblett

Supervisor: Eve Stringham

UNC-53 in Caenorhabditis elegans is homologous to the human NAV-2 navigator protein, an essential part of development that has modified expression in many types of cancer. Previous work has characterized the protein to be essential in neuron, muscle and vulva formation in the worm, with null mutations leading to a phenotype consisting of uncoordinated movement and egg laying defects. Recently, studies have suggested a role for UNC-53 in resistance to Pseudomonas aeruginosa through a number of signalling pathways like the DAF-2 and Map kinase signalling pathways. Despite this previous work, data about what tissues express UNC-53 in its immune role has not been found. UNC-53 is required for immunity but its presence in neural or epithelial tissue, alone or in combination, is insufficient to produce a rescued immune response phenotype. The hypothesis of this study is that UNC-53 is required in the excretory cell in order to rescue innate immunity of C. elegans to P. aeruginosa. This study took five

strains of C. elegans with wild type (N2), decreased lifespan (pmk-1), increase lifespan (daf-16), immunocompromised (unc-53 null allele N166) and excretory cell rescue (unc-53::ppgp-12;unc-53(n166)::GFP) phenotypes. The worms were synchronized to the L4 stage of development, rendered sterile and then picked to either pathogenic P. aeruginosa or non-pathogenic E. coli OP50. Lifespan assays were then conducted at 25oC, taking note of the decrease in population over time. These statistics can be analyzed using a Kaplan-Meier plot to evaluate trends. While current data is not able to support or deny the hypothesis, it is expected that the rescued excretory cell population will have a longevity greater than that of the unc-53 mutant.

Evaluation of the role of UNC-53 and Wnt signalling in the outgrowth of the excretory cell in C. elegans	**Hallway**

Student: Vanessa Porter

Supervisor: Eve Stringham

The Caenorhabditis elegans protein UNC-53 is homologous to the three neuronal navigator proteins in vertebrates that are differentially expressed in many cancer types. Within the worm, UNC-53 has an important role of regulating the cytoskeletal rearrangements that facilitate the outgrowth of sex muscles, neurons, and the excretory cell. It facilitates the movement cell-autonomously along with UNC-71/ADAM and is mediated by the Rho-specific guanine nucleotide exchange factor (GEF) domain in UNC-73E/TRIO. The underlying signal that triggers the UNC-53 pathway has yet to be determined, although cross talk between other developmental signal pathways and UNC-53 suggested that Wnt signaling might be involved. To observe if the two pathways have a functional commonality, the excretory cell (EC) morphology was analyzed in null alleles of unc-53 and the Wnt ligands and their receptors. All Wnt ligands, five out of six receptors, and unc-53 null alleles all had truncated phenotypes of the EC, which suggested a common outgrowth function between the two pathways. Furthermore, the EC was analyzed in double and triple mutants of four Wnt ligands, two Frizzled receptors, and one negative regulating receptor with unc-53. So far, the double and triple mutants showed statistically different truncation patterns than unc-53 alone, which suggested they were not in the same pathway. Each Wnt ligand has a specific expression pattern and is in cooperation with several different pathways. It still may be possible that Wnt signaling controls the EC outgrowth by mediating several pathways including the one controlling UNC-53.

HABIT Change: A Brief Implementation Intention Intervention to Decrease Alcohol Consumption in Undergraduates	**ART 219**

Student: Gillian Russell

Supervisor: Marvin Krank

Having a goal does not guarantee success; often there is a gap between our intentions and our actions. Implementation intentions support goal achievement by requiring the individual to determine where, when, and how to perform a specific action. The advantage of developing this linkage is that it allows action control to switch from conscious and effortful to being efficient and automatic, without the need for conscious intent. The aim of the present study is to investigate the effects of a brief implementation intention intervention on the alcohol consumption of undergraduates. Participants were randomly divided into three conditions: control, goal formation (GF), and implementation intention intervention (III). Measurements of cognitive variables and substance use were assessed through self-report questionnaires at the beginning of the study (time 1) and at one-month follow-up (time 2). At time 1, participants that reported consuming excessive amounts of alcohol were given feedback indicating that they were consuming alcohol at levels that may be hazardous to their health and a number of the consequences that they may face. Also at time 1, all participants were provided with information about the negative cognitive and academic impacts associated with alcohol use, and then given examples of goals and implementation intentions that could be formed in response to the article. Participants in both the GF and III groups were asked to write down a goal regarding their own alcohol use, and those participants in the III group then formed implementation intentions for those situations where they did not feel 100% confident that they would achieve their goal. We propose that those in the two experimental conditions will exhibit reduced alcohol consumption when compared to control participants, with those who formed implementation intentions exhibiting a greater reduction in consumption. Furthermore, we propose that levels of alcohol-related cognitions will be reduced in the group who formed implementation intentions. Data collection and analysis are currently underway and a multivariate analysis of variance (MANOVA) will be performed with one between-subjects factor (3 levels: control, GF, III) and one within-

subjects factor (2 levels: Time 1, Time 2). The results of this study may have implications for the treatment and prevention of substance use disorders.

Individual-based modelling of bear foraging in Whistler, BC to assess the effectiveness of management strategies in reducing human-bear interactions

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Hallway

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Student: Joe Salkeld

Supervisor: Lael Parrott, Rebecca Tyson

The removal of known conflict animals through extermination is not a sustainable solution to existing wildlife management issues. Instead, pro-active methods should be considered for situations where black bears are enabled to become conflict animals. It is important to identify what proactive measures are most effective and of highest priority for an area based on animal response and resources. We developed an individual-based model using NetLogo to simulate the seasonal movement of a black bear on a natural landscape that includes urban environment, namely the Resort Municipality of Whistler (RMOW) and the surrounding areas. The model simulates the movement of individual bears as they forage on the landscape over a single growing season (early spring to late summer as determined by ripening of local berries). The bear movement in the model depends on the current and remembered food quality (in terms of bear preference) of the landscape, which was validated using data from collared bears in the RMOW. The forage food quality values are spatially generated from relative rankings of bear habitat types that are based on provincial Terrestrial Ecosystem Mapping (TEM) Data, in combination with forage plant phenology. The modelled landscape also includes natural and human food sources within urban areas that attract bears, and thus leads to bear habituation towards humans. The model is used to predict the location and frequency of human-bear interactions undergoing proactive measures versus landscapes without any methods to reduce these interactions from happening. The simulation results give us insight into the relative effectiveness of different management strategies, such as selective elimination of urban access points or spatial configurations of human education to reduce bear attractants and increase aversive conditioning.

Evidence-based manual gene annotation of 45 kb of Drosophila biarmipes 3L chromosome through comparative genomics

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Student: Ashandeep Sandhu

Supervisor: James Bedard

Since the Human Genome Project began in 1990, the field of genomics has expanded tremendously. Genomes of numerous species have now been sequenced. The breadth of knowledge that gene annotation provides has led to significant advances in various branches of biology, including medicine and evolution. This is primarily due to the essential information gene annotation provides for the field of comparative genomics. By comparing the species of interest in this project, Drosophila biarmipes, with the closely-related, reference species, Drosophila melanogaster, we can begin to understand the evolutionary mechanisms and processes taking place in this particular species. To compare these two species, gene models were created for a 45 kilobase portion of the D. biarmipes 3L chromosome through evidence-based annotation. Numerous programs and bioinformatic tools such as gene predictor tracks, RNA expression data, and BLASTx alignments with D. melanogaster, were used to provide evidence for the correct gene models. Based on the results collected from these gene models, we determined that there are six orthologs present within this region of the 3L chromosome: CG7442, CG14562, DNApol-η, CS-2, CG7448, and CG7458. The biological function and significance of many of these genes are widely unknown, serving as a subject for future investigations. The results from this study will contribute to a larger research project organized by the Genomics Education Partnership.

Climatic influences on snowpack accumulation in southern British Columbia

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ART 219

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Student: Kristi Silk

Supervisor: Ian Saunders

The Intergovernmental Panel on Climate Change reported in 2013 that snow extent and depth in North America was generally declining, driven by climate change. The present study looked at the influences of synoptic-scale climate on snowpack accumulation in southern British Columbia, based on the premise that local climate variability typically depends on processes occurring at much larger scales. Time-series analysis showed strong short-term variability in springtime snowpack superimposed on an overall decreasing trend. Springtime snowpack accumulation recorded at ten stations with long periods of record was correlated with synoptic-scale climate indices including El Nino/Southern Oscillation (ENSO, using the Southern Oscillation Index), Pacific Decadal Oscillation (PDO), North Pacific Index (NP), and Pacific North American pattern (PNA). A t-test analysis generally showed significant differences in snow accumulation between positive and negative phases of climate indices, which suggested that large-scale climate variability affects snow accumulation in southern BC. Correlation analysis showed that snow accumulation was negatively correlated with PDO and PNA and weaker positive correlations existed between SOI and NP. These results demonstrated teleconnections between surface climate and synoptic-scale climatic patterns.

Comparison of Nitrogen Response of Camelina and Canola in the Brown Soil Zone — ART 219

Student: Emma Soeten

Supervisor: David Clements

Camelina (Camelina sativa L. Crantz) and canola (Brassica napus L.) are important oil-seed crops belonging to the Brassicaceae, or mustard, family. Compared to canola, camelina has moderate to low nutrient requirements, is regarded as drought resistant and frost tolerant, and is affected by fewer pests and diseases. Camelina is therefore seen as a more cost-effective and environmentally sustainable oil-seed crop. There is great potential for camelina to be used as a biodiesel, as previous studies have proven the beneficial and cost-effective nature of using camelina oil in this manner. While the optimal applied nitrogen rate for camelina has been determined in several studies around the world, it differs greatly with soil type and climate. Specifically, there has been very little research done on the nitrogen requirement of camelina in the marginal land of the brown soil zone in Southwest Saskatchewan, Canada. To this extent, the effect of applied nitrogen fertilizer on seed and straw yield as well as the nitrogen fertilizer use efficiency (NFUE) of camelina in comparison to canola in the brown soil zone was investigated. A randomized complete block design (RCBD) was implemented using applied nitrogen rates as the plot and one cultivar of Camelina sativa and one cultivar of Brassica napus as the sub-plot. Seven different applied nitrogen rates were used, including rates of 0, 40, 80, 120, 160, 200, and 240 kg/ha. Results showed comparatively higher yields in the camelina than the canola, with camelina showing a quadratic response to the nitrogen levels. It was evident that camelina was able to grow at lower fertilization levels than canola. The introduction of camelina, and its numerous benefits, to the marginal land of the brown soil zone would be cost-effective while providing many environmental advantages over the use of conventional crops.

Creation and validation of the Composite Cannabis Assessment Scale — ART 219

Student: Michelle Thiessen

Supervisor: Zach Walsh

An increasing number of Canadians use cannabis across diverse contexts, and adapting our appreciation of cannabis use to the evolving socio-medical landscape is a research priority. Although only a small proportion of cannabis use is problematic, the majority of cannabis use assessment tools focus exclusively on problematic use, and thus fail to provide a comprehensive assessment of cannabis use. The Composite Cannabis Assessment Scale (CCAS) was developed to better identify and differentiate the diverse motivations and consequences associated with problematic, recreational and therapeutic cannabis users. The aggregation of these diverse aspects of cannabis use in a single measure has the potential to elucidate the complex motives that underlie cannabis, and pending adoption also has the potential to further continuity and reliability across diverse research settings. We have adapted items from previously validated measures: the Cannabis Use Disorders Identification Test (CUDIT) and the Marijuana Motives Measure, and have also added novel items where extant measures were lacking. We will validate the Composite Cannabis Assessment Scale in a clinical, therapeutic, and undergraduate sample of cannabis users with factor analysis and item response theory.