a place of mind

Irving K. Barber School of Arts and Sciences

The University of British Columbia Okanagan

Undergraduate Research Conference

April 7, 2011

ABSTRACTS

POSTER PRESENTATIONS

UBC Okanagan Undergraduate Research Conference
Poster Presentations
April 7, 2011 – FIPKE Centre Foyer, Ground Floor
11:00 am – 1:00 pm

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6	Meghan Dutot	Mathematical Modelling of a Biological Control Agent
7	Michael Cowper	The Synthesis of Iron- and Ruthenium-Containing Terpyridine-Based Polymers
7	Patrick Bobyn	Tissue expression patterns of the copper transporter *atp7* in larval *Aedes aegypti* following acute exposure to environmental copper
8	Ryan Lohre	An NMR-based Method for the Metabolomic Analysis of the American Cranberry
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9	Suud Nahdi	Cellular trafficking of B_{12}

Rapid Synthesis of Ligand-Based Radicals from Chromium(II) Compounds

Student: Addison Desnoyer
Supervisor: Dr. Kevin Smith

Radicals are compounds that contain an unpaired electron. Although radicals are generally highly reactive, they play crucial roles in almost all fields of chemistry, ranging from the chemistry of metals to the chemistry of biological systems. Diimines and quinones are known to form stable radical species when bound to first-row transition metals. Although these "redox non-innocent" ligands have been extensively studied by inorganic spectroscopists, the reactivity consequences of ligand-based radicals on metal catalyzed reactions have only recently been actively investigated. Octahedral chromium(III) complexes provide a particularly useful framework for studying ligand-based radicals, as these complexes have strong metal-ligand bonds, slow ligand dissociation rates and effective antiferromagnetic coupling between the single unpaired electron on the ligand and the three non-bonding chromium-based electrons. Better routes are required to a) prepare these compounds cleanly in non-polar solvents at room temperature and b) obtain structure-activity relationships. The chromium(III) complex $Cr[(OC^tBu)_2CH]_2$(bipyridine) was selected as an initial target due to its expected solubility in Et_2O. Reaction of the square planar chromium(II) complex $Cr[(OC^tBu)_2CH]_2$ with bipyridine in Et_2O provided a clean and rapid route to the complex. This route was extended to other diimines, pyridyl imines, pyridyl ketones and 1,2-diketones in order to compare a related series of radical ligand complexes. Sequential treatment of Cp_2Cr or $Cr[N(SiMe_3)_2]_2(THF)_2$ with bipyridine and 1,3-diketones afforded the corresponding series of $Cr[(OCR)_2CH]_2$(bipyridine) complexes via protonolysis. The UV-visible and ^1H NMR spectra of these complexes and their electrochemical behaviour will be discussed.

Energy and Conformation of Glycinate ($NH_2CH_2COO^-$) on a Copper Surface

Student: Ahmad Abd-El-Aziz
Supervisor: Dr. David Jack

Computational calculations of the electronic structure and conformation of the amino acid glycine on the (110) surface of solid copper are presented. The energy of the molecule was calculated for several positions on the copper surface using the GAMESS set of computer programs on the WestGrid computing facility. The calculations used are based on Schrodinger's equation for quantum mechanics. The solid surface was simulated using a patch of 10 copper atoms fixed in position. The glycine molecule was then placed on top of the surface and the computer program determined the lowest energy conformation. The glycine molecule was assumed to be in its anionic form (glycinate). By incrementally moving the molecule and performing additional energy calculations, an estimate of the energy barrier for the movement of the molecule across the surface was obtained. The barrier height for diffusion is estimated to be ~8-10 kcal/mol (~4000-5000 K). The binding energy to the copper surface was found to be 44.2 kcal/mol in agreement with previous calculations [1] and consistent with experimental measurements [1].

The electronic structure and surface chemistry of glycine adsorbed on Cu.110, Nyberg, M., Hasselström, J. Karis, O., Wassdahl, N., Weinelt, M., Nilsson, A., Pettersson, L. G. M. Journal of Chemical Physics 112(12): 5420-5427, (2000)

Martian meteorites: Geochemical inferences on the mineralogy and composition of the martian mantle

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Student: Avee Ya'acoby
Supervisor: Dr. John Greenough

Geochemical data for 13 basaltic Martian meteorites from the NASA "Meteorites From Mars" website are used to compare the mantles of Mars and Earth. Incompatible transition metals have concentrations that are comparable to or higher than those in Earth basalts with Cr particularly anomalous. Nevertheless, they, along with similar Mg# values (Mg# = Mg/(Mg+0.9*Fe)) suggest formation in a peridotite mantle similar to Earth. A plot of SiO_2 (pressure indicator) versus La/Yb or Nb/Y (melting percentage proxies on Earth) shows that, unlike on Earth, Martian basalts form over a narrow range of melting percentages that are high and that increase slightly with depth. The range of pressures (based on SiO_2) appear similar to or slightly lower than those for oceanic island basalts (OIB) on Earth. The narrow range of melting percentages and positive relationship with depth resembles preliminary information for the Moon (based on KREEP) and may reflect restricted mantle convection in the interior of small planetary bodies. The relative incompatibility of ~30 incompatible "trace" elements is well established for the Earth. When the behaviour of ~160 incompatible element ratios (more-incompatible element in the numerator, based on Earth) in the Martian meteorites are compared, most ratios bearing elements with dramatically different bulk distribution coefficients (i.e. different incompatibility) show the same behaviour as in Earth basalts. This suggests similar mantle mineralogy for Mars and Earth. Comparison of OIB and Martian basalts using element ratios calculated from elements with similar incompatibility (i.e. ratios that reveal source region variability on Earth) shows that Martian basalts come from materials most-similar to depleted mantle on Earth. Absolute element concentrations lead to the same conclusion. However, in detail these similarly incompatible element ratios organize Martian basaltic source regions into three categories that have been described as Enriched, Depleted and Intermediate by geochemists studying the martian interior with isotopes. Thus crust formation on Mars apparently led to a variably depleted mantle interior but there is no evidence for recycled lithospheric materials such as those that yield the mantle component sources of OIB on Earth.

A Rotationally Averaged Interaction Potential for H_2 molecules Based on Quantum Mechanics

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Student: Brandon Kootnekoff
Supervisor: David Jack

The development of intermolecular H_2-H_2 and H_2-MgO surface potentials for rotating molecules are presented. These rotationally averaged potentials include quantum mechanical effects and are characterized by the distance between molecules as well as the orientation of the molecule. The interaction energy as a function of separation distance between pairs of molecules for a variety of orientations are presented and compared with previous calculations [1] that ignored the rotational averaging. The interaction potentials were subsequently modified for surface interactions and used in a Monte Carlo computer simulation of a film of H_2 molecules adsorbed on the (001) face of an MgO crystal. These results were compared with previous simulations based on classical interactions [2].

1. P. Diep and J. K. Johnson, J. Chem. Phys. **112**, 4465 (2000).
2. J. N. Dawoud and D. B. Jack, Appl. Surf. Sci. **256**, 1443 (2009).

Cobalamin: An Important part of the Processes and Functioning of Life.
A look into Methylmalonyl Mutase and its molecular chaperone MMAA.

Student: Brayden Gourdeau
Supervisor: Dr. Kirsten Wolthers

Vitamins, cofactors and enzymes are important in every aspect of life. One such vitamin is cobalamin, more commonly known as vitamin B-12, which is important in the metabolic processes of both mammals and bacteria alike. It is a complex molecule that takes over 25 metabolic steps to synthesize and is most disguisable for its cobalt center which can have up to 6 ligands. Cobalamin by itself has no known biological function however when paired with a cofactor it assumes an active role. Methyl malonyl mutase (MCM) requires one such cofactor called Adensylcobalamin (AdoCbl) to catalyze the isomerization of methyl malonyl-CoA to succinyl-CoA using radical based chemistry. MMAA functions as a molecular chaperone to MCM and is a member of the G3E subfamily of P-loop GTPases. Although its function is not known it is believed to provide a gate keeping role for MCM. Our research looks into the molecular interactions of these two proteins through stop flow spectroscopy and florescence experiments. In our results we provide evidence for MMAA function as a chaperone to MCM as well as its role in protection.

Secrets and Lies: Involuntary Leakage in Deceptive Facial Expressions As a Function Emotional Intensity

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Student: Brendan Wallace
Supervisor: Dr. Stephen Porter

Darwin (1872) posited that aspects of facial communication are uncontrollable and can betray a deceiver's true emotion, particularly when emotional arousal is high. We examined the presence of inconsistent emotional expressions in genuine and deceptive facial expressions. Participants viewed high and low intensity disgusting, sad, frightening, and happy images, responding to each with a genuine or deceptive (masked or neutralized) expression. Each 1/30-s frame (256,650 frames in 1,711 expressions) was analyzed for the presence and duration of universal emotions and microexpressions (1/25th to 1/5th-second expressions; Ekman, 1992). Relative to genuine emotions, masked emotions were associated with more inconsistent expressions, particularly when participants were attempting to mask a high intensity emotion. Further, neutralizing a high intensity emotion lead to greater emotional leakage than low intensity emotional neutralization. Emotional leakage often lasted up to one second, much longer than Ekman microexpressions, which occurred rarely. In general, negative emotions were more difficult to falsify than happiness. Results replicate and extend previous findings, provide support for Darwin's (1872) inhibition hypothesis and contribute to our understanding of the communicative properties of the human face more generally.

Chromium benzyl complexes for selective radical reactions

Student: Caitlyn Liberto
Supervisor: Dr. Kevin Smith

Metals are useful in catalyzing carbon-carbon bond forming reactions. Newer, first row catalysts have pathways often involving carbon based radicals. The Smith group research has synthesized well-defined organometallic chromium complexes capable of reversibly generating highly reactive neopentyl or cyclohexyl radicals for organometallic-mediated radical polymerization of vinyl acetate, toluene carbon-hydrogen bond activation, and radical cyclization of halo acetals. The purpose of this study was to investigate the potential of chromium benzyl complexes for selective radical carbon-hydrogen bond functionalization and carbon-carbon bond formation reactions. The chromium benzyl complex was considered because although the chromium-carbon homolysis rate of the chromium benzyl complex is close to that of a chromium neopentyl complex, the benzyl complex is easier to handle due to the increased stability of the carbon based benzyl radical. Due to the stability of the benzyl radical, the radical has reduced reactivity, which should result in increased selectivity. Cyclized tryptophan derviatives were evaluated as substrates for carbon-hydrogen bond activation with benzyl radicals. This functionalization could be followed by either radical dimerization or coupling with a second benzyl radical. Furthermore, chromium catalysts were used in cross-coupling reactions of benzyl chloride and benzyl magnesium chloride. Organic radical coupling products were explored and identified using ^{1}H NMR while in the presence of paramagnetic organochromium by products.

Meso-Scale Variations in Snow Accumulation in Relation to Forest Stand Age

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Student: Chris Jaeggle
Supervisor: Dr. David Scott

In this study, the purpose was to better understand the relationship between snow accumulation and the age of the surrounding forest canopies. The aim was to make other effects on snow accumulation, such as temperature, aspect, slope, and elevation negligible. Similar studies have been done, but many fail to exclude the above effects. This study was done on a logging site near the James Lake reservoir, outside of Kelowna, B.C. The site was chosen because it had forest stands of three different ages, all within a square kilometer of each other. One stand was an old growth stand, one was a secondary growth stand that had been previously logged and replanted, and one was a clearcut stand. In order to quantify the relationship between stand age and accumulation, forest characteristics such as tree stem density and crown closure, indicative of canopy interception, were measured. These parameters generally increase with stand age, so it is expected that the older stands will intercept more snow and yield smaller accumulations. Snow accumulation measurements were made in each of the stands for the months of December, January, February, and March, using a Standard Federal Snow Tube. This instrument took a core sample of the snowpacks, and gave values of snow water equivalent (SWE). The SWE for each stand, on each month, was then compared to the associated density and crown closure measurements for each stand. As hypothesized, the old growth stand displayed the smallest accumulations. The secondary growth stand showed less accumulation than the clearcut, however not by a large margin. These results are useful as they can be used to predict snowmelt rates and spring runoff, which in turn are useful for predicting soil erosion, flooding, and future water supply problems.

The effect of sexual competition on the Drosophila virilis immune response

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Student: Claire Vincent
Supervisor: Dr. Bob Lalonde

Immune function has been shown to be influenced by sexual selection, and decreases in males with increased sexual activity. Sexual selection is a strong factor influencing evolutionary processes associated with immune response. In this study, the effect of increasing sexual competition on immunocompetence of offspring of *Drosophila virilis* was explored. Adults were placed in one of three treatments; monogamy (one male and one female), female competition (one male and five females), or male competition (five males and one female). Offspring were then inoculated with tetracycline resistant *Escherichia coli* (XL-1 Blue) and were plated onto tetracycline plates after 4 days to see how many cells remained in the individuals. There was no difference found in immune response of offspring among the treatments. However, there was a significant difference between males and females independent of the treatment. Daughters were found to clear bacteria less well than sons independently of the amount of sexual competition faced by their parents, suggesting that females are limited by another factor causing fewer resources to be allocated to immunocompetence. The results suggest that the amount of sexual competition experienced by parents does not affect the immunocompetence of offspring after one generation. It has been found that the Drosophila immune system can evolve after 60 generations, thus our findings demonstrate that the evolutionary processes associated with immune response operate at a longer temporal scale than was explored in this study.

Determining if *Alloclavaria purpurea* is an Ectomycorrhizal Fungi or a Saprotrophic Fungi

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Student: Courtney Paterson
Supervisor: Dr. Melanie Jones

Ectomycorrhizal fungi (EMF) are dominant players in forest microbial activity because of their symbiotic relationship with roots of trees; they exchange organic nitrogen for carbon that the tree fixed from the atmosphere. Saprotrophic fungi (SAF) do not form this relationship; instead they acquire nutrients from dead organic matter on forest floors. SAF are the primary decomposers of forest ecosystems. This study was conducted to determine if the fungus *Alloclavaria purpurea* is an ectomycorrhizal fungus or a saprotrophic fungus. With recent changes in the phylogenetic placement of *A. purpurea,* as well as frequent detection in fungal DNA isolated from ectomycorrhizal roots, we have hypothesized that *A. purpurea* are playing similar roles to EMF rather than SAF which is what they were assumed to be previously. SAF have a higher ratios of ^{13}C to ^{12}C ($\delta^{13}C$) than EMF, and EMF have a higher ratio of ^{15}N to ^{14}N ($\delta^{15}N$) than SAF in most cases. I will be comparing isotopic ratios of *A. purpurea* to those of known EMF and SAF in hopes of better understanding its role in forests communities. Sporocarps (mushrooms) of several well-known EMF and SAFs were collect from spruce-fir forests at the Sicamous Creek Silvicultural Site on September 19, 2010. Sicamous Creek lies seven kilometres east-southeast of Sicamous and approximately two hours from Kelowna B.C. No samples of *A. purpurea* were found on our trip; however, samples from Sicamous Creek collected in September 2009 were available. Fifty samples were used in my experiment as well as 12 dried samples that were collected by Jen Walker in 2009. The sporocarps were dried in an oven, ground in a ball mill, weighed with a 5 point balance into tin capsules, and sent to New Hampshire for isotope analysis. DNA sequencing was performed to confirm the identities of each sporocarp. Preliminary analysis of the isotope data suggests that *A. purpurea* has isotopic ratios more similar to EMF than to SAF. These findings with help better understand the trophic status of *A. purpurea* and its significance in its ecosystem.

Geochemical Analysis of Trace Elements in Calcite Shells of *Panopea generosa*, Geoduck Clams.

Student: Devon Jollimore
Supervisor: Dr. Karen Perry

Geoduck clams, a marine bivalve mollusc, form shells of calcite, which are formed in concentric rings similar to those of tree growth rings. These rings potentially hold records of paleo-climate, water composition, sea surface temperatures (SSTs), salinity, and many other environmental conditions. Sample shells were collected from Poole inlet, Haida Gwaii, British Columbia, Canada. The shells were aged 94 and 108 years old, and cut along the axis of maximal growth, where each growth ring was found to be the largest. Beginning with the youngest rings, the first 20 years of growth of two shells were analyzed using a laser ablation inductively coupled plasma mass spectrometer (LA-ICP-MS) run in low resolution. Samples were externally standardized using a doped NIST 610 glass using calcium as an internal standard. The concentrations of 42 metals including alkali earths, some transition metals, as well as some rare-earth elements (REEs) were measured from each annual ring by selectively sampling the lighter calcite centres. Elemental trends between two different shells appeared to be quite similar. The elements Mg, Cu, Si, and U were very high in the first growth ring followed by a sharp decrease to relatively stable concentrations for both shell samples. It appears that these elements are enriched during the early stages of shell deposition.

Characterisation of Ornithine Amino Mutase: Radical Intermediate Stabilization

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Student: Francois Miros
Supervisor: Dr. Kirsten Wolthers

D-ornithine 4,5 aminomutase (OAM) is an anaerobic enzyme naturally found in *Clostridium Stricklandii* involved in a Stickland reaction: a metabolic pathway in which the reduction of one amino acid is coupled to the oxidation of another. OAM interconverts D-ornithine to 2,4 diaminopentanoic acid using an uncommom radical based isomerisation mechanism. Homolysis of an adenosylcobalamin (AdoCbl) cofactor generates a carbon-centered substrate intermediate that can undergo intramolecular isomerisation to form product. Pyridoxal 5' phosphate (PLP), along with active site residues hold the d-ornithine in position as well as stabilize the radical intermediate during the isomerisation. An understabilized substrate can form an unfavoured product and prevent AdoCbl reformation. A challenge for OAM, like all radical-based enzymes, is to control radical trajectories, thereby minimize unwanted side reactions that would lead to enzyme inactivation.

The purpose of this study was to examine how the radical intermediate of d-ornithine 4,5 aminomutase (OAM) is stabilized during enzyme turnover. A similar enzyme lysine aminomutase (LAM) performs similar radical chemistry but undergoes suicide inactivation much more readily than OAM. This has been suggested to be due to the lack of a histidine residue (H225) which is found in OAM. As such, two mutants were generated to see how critical this residue is in OAM activity. UV-vis spectroscopic techniques were used to identify the activities of substrates, d-ornithine and DABA, within these mutants by tracking OHCbl formation. Results showed that alteration of H225 resulted in a 10-fold increase in enzyme O_2-inactivation with D-ornithine, but decreased O_2-inactivation with D-2, 4 diaminobutyric acid.

Disruption of Coenzyme Binding in Methionine Synthase Reductase

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Student: Frida Gustafsson
Supervisor: Dr. Kirsten Wolthers

Cobalamin-dependent methionine synthase is an important house-keeping enzyme as it recycles homocysteine (a toxic metabolite) to methionine and converts methyltetrahydrofolate to tetrahydrofolate, an important precursor for protein and DNA synthesis. Methionine synthase reductase (MSR) maintains MS function by reductively remethylating inactive MS-cob(II)alamin, to form active MS-methylcob(III)alamin. MSR is a 78 kDa diflavin dehydrogenase that transfers reducing equivalents from NADPH via its protein-bound FAD and FMN redox centres to MS-cob(II)alamin. Previous studies show that MSR has a weak binding affinity for NADPH, which may restrict MS reactivation and folate metabolism. The aim of this study is to determine structural features of the NADPH active site that affect coenzyme binding. In the initial steps of hydride transfer from NADPH to FAD, the nicotinamide ring must stack so that the C4 atom of NADPH is in close proximity of the N5 atom of the FAD. The indole ring of Trp^{697}, the second to last residue of MSR, is positioned over the isoalloxazine ring of FAD and the π-π interactions are thermodynamically stable, obstructing the electron transfer and affecting substrate specificity. For this reason, the MSR mutants W697H and S698Δdel were created, and subsequently expressed and purified from *E. coli* by affinity and ion exchange chromatography. Steady state kinetic assays show that the turnover rate for W697H and S698Δdel increased by 5.75 and 6.93 fold respectively. The mechanistic implications and increased efficiency of the enzyme on MS reactivation, according to these results, are analyzed and discussed.

The effects of disturbance on arbuscular mycorrhizal fungi communities

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Student: Jessica DeMarinis
Supervisor: Dr. Miranda Hart

Regarding life-history strategies in plants and animals, the facts are well-known and clearly defined. Among other characteristics, these strategies dictate the species' response to disturbance in their habitat. However, little is known about how AMF communites respond to disturbance. . We performed a metanalysis of recent literature to answer the question, do disturbed sites select for distinct communities of arbuscular mycorrhizal fungi? In both natural and disturbed systems we looked for broad changes in AMF community structure, including differences in species richness, phylogenetic diversity, presence/absence of 'generalists' and cosmopolitan genotyopes. For the majority of the variables, each system did not show significant differences. However, the results indicated that the cosmopolitan genotype *Glomus mosseae* was present in the disturbed sites significantly more than the undisturbed.

"Social, Environmental and Cultural Impacts of Flooding: Two Case Studies"

Students: Joanne Taylor and Erica Sure
Supervisor: Dr. John Wagner

Flooding has always been a major issue throughout the world. In recent history the negative impacts of flooding have been exacerbated by climate change and modern technologies and interventions, such as those associated with dam construction. Much of the flooding and human suffering that coincides with the effects of flooding and damming can be mitigated. This project seeks to examine how flooding in various parts of the world affects local and especially marginalized people socially, culturally, and environmentally. Two case studies have been conducted in two different areas of the world based on a review of available literature. The first case study examines the Three Gorges Dam and the second looks at the annual flooding in Bangladesh. Our poster will be a composite representation of both case studies*.

The Role of Neurosteroids on Neurogenesis and Neuronal Repair in Adult *Carassius auratus*

Student: Jonathan Mitchell
Supervisor: Dr. Bruce Mathieson

Neurogenesis, which is the production of new neurons, occurs in the brain of an adult mammal to a very limited extent. Interestingly, neurogenesis is confined to only a few specific regions in the mammalian brain. In contrast, teleost fish are able to undergo a large amount of neurogenesis throughout their lifetime, in many more regions of the brain than mammals (Pellegrini et al., 2007). In addition, fish can repair and regenerate damaged neurons, whereas mammalian species mostly lack this ability (Zupanc, 2008). One of the most interesting areas of research concerning neurogenesis involves the role of neurosteroid hormones in the control of neurogenesis. For example, the neurosteroid allopregnanolone has been shown to promote neurogenesis and survival of new neurons in the mammalian brain (Romer et al., 2010). This raises the central question of our current study: are neurosteroids responsible for the robust neurogenic and repair capacity of fish? To test this hypothesis, we are measuring the rate of neurogenesis in the brains of adult goldfish (*Carassius auratus*), using a microscopy marker or neuronal cell division called BrdU incorporation. Neurogenesis in the optic lobe and cerebellum will be measured in normal brains and in brains that receive a small surgical lesion to stimulate repair processes. In addition, the effects of depleting allopregnanolone levels on neurogenesis will be measured. Reductions in neurogenesis following neurosteroid depletion could help explain why the brains of higher vertebrates have such limited capacity for repair of neuron damage in adult animals

Source Region Comparison of Sub Continental Lithospheric Mantle Using Continental Flood Basalts and Implication for the Generation of Economic Layered Mafic Intrusions.

Student: Jordan McDivitt
Supervisor: Dr. John Greenough

Studies of oceanic basalts have been used to shed light on mantle heterogeneity in terms of the source region signatures present in isotopes, trace elements and primitive transition metal concentrations within mid ocean ridge basalts (MORB) and ocean Island basalts (OIB). Isotopic studies of OIB's have shown enriched source regions are present in the sub crustal oceanic lithosphere (EM1, EM2). It has been hypothesized that EM1 represents delaminated subducted Archean SCLM (sub-continental lithospheric mantle) and EM2 is representative of younger (Proterozoic-Paleozoic) delaminated SCLM. This study involves various continental flood basalt provinces from around the world that have been extruded through continental crust of various ages. Continental flood basalts (CFB's) are thought to form in similar ways to OIB's, by hotspot generated melting of the SCLM. Evidence exists that suggests the same source regions signatures defined in OIB's exists under the continental crust but exhibit a much stronger signature. This hypothesis can be tested by examining the source region characteristics of CFB's which are thought to be generated by melting of the continental SCLM. Statistical comparison (multi dimensional scaling) of OIB's and CFB's shows distinctly different source region characteristics in terms of similar incompatible element ratios (SIER). CFB's extruded through Archean crust separate from those extruded through younger crust based on SIER as well. This indicates different SCLM characteristics between the Archean and Proterozoic. Economic layered mafic intrusions (LMI's) generally form in Archean crust, display a stong EM1 signature and exhibit a high percentage melting. Comparison of source region characteristics in Archean SCLM and younger SCLM has shown distinctly different properties between the two that suggests the formation of economic LMI's as a result of high percentages of melting may reflect the susceptibility of the Archean SCLM to form higher percentage melts in standard plume environments.

Expression of the ORF23/24 Protein in the pET28a Vector

Student: Julianne Beam
Supervisor: Dr. Mary Forrest

Asukamycin is a novel secondary metabolite produced by the Gram-positive bacterium, *Streptomyces nodosus. spp. asukaensis*. Asukamycin is related to the manumycin group metabolites which show antimicrobial, antifungal, antitumor, and possible anticancer and anti-inflammatory activities. Asukamycin is structurally very similar to other manumycins with one difference. This difference is at asukamycin's upper polyketide chain which ends with a cyclohexane carboxylic acid moiety (CHCA), not found in other manumycins. One of the genes responsible for CHCA production is the open reading frame (ORF) 23-24, which codes for the enzyme(s), EPSP synthase/CHC-CoA ligase. The speculated function of the enzyme(s) is that it places the CHCA moiety onto the upper polyketide chain during biosynthesis of asukamycin. My aim is to express and study the ORF23/24 protein in the pET28a vector. The protein has been previously extracted in an insoluble form which has proven inaccessible. In order to express the protein in a soluble form, two methods were used. The first method was to express the protein *in vitro* using a protein expression system. The second method was to express the protein *in vivo* using Isopropyl-β-D-thio-galactoside (IPTG) induction. Using both SDS and native polyacrylamide gel electrophoresis (PAGE), the proteins produced were studied. In both methods, the conditions were altered to increase the chance of protein product solubility.

Inhibition of postharvest fungal pathogens in apples with mixtures of antagonistic soilborne bacteria

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Student: Kaela Loffler
Supervisor: Dr. Louise Nelson

Postharvest fungal pathogens infect and destroy 20-25% of harvested fruits and vegetables annually in North America. The use of naturally-antagonistic bacteria as biocontrols to combat these postharvest pathogens is under study as a means to reduce fungicide use. However, there have been few studies of the application of mixtures of biocontrol agents to control postharvest decay. *Penicillium expansum*, *Botrytis cinerea*, and *Mucor piriformis*, major postharvest pathogens of pome fruit, were selected. Three antagonistic bacterial strains, *Pseudomonas fluorescens* strains 1-112 and 4-6 and *Serratia plymuthica* strain 6-25 were applied alone and in combination in both *in vitro* inhibition plate assays and *in vivo* apple inoculations against these pathogens. All of the strains alone and in combination significantly inhibited the growth of the pathogens in the *in vitro* inhibition plate assays. A combination of antagonistic strains 1-112 and 4-6 was most effective in suppressing growth of *P. expansum in vivo* and greater inhibition was seen when the two strains were combined as opposed to alone. Strain 6-25 and the combinations of 1-112 and 4-6, and 1-112 and 6-25, were most effective in the suppression of *B. cinerea* growth *in vivo* after 90 days incubation at 4 ºC. This suggests that the combination of antagonistic strains may have synergistic effects on inhibition of the two pathogens. Based on the above data, the effect of varying the proportions of the *P. fluorescens* strains 1-112 and 4-6 were tested on apples inoculated with *P. expansum* at 24 ºC. Ratios of both strains from 25:75 to 75:25 were not significantly different from the infection severity of 4-6 alone but were significantly different from 1-112 alone after 16 days. Thus, different combinations of antagonistic strains may enhance efficacy in controlling growth of postharvest fungal pathogens. The mechanisms of action for these effects may require further study.

A Survey of Ability of Rhizosphere Bacteria to Suppress Fungal Pathogens Associated with Apple Replant Disease

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Student: J. Kyle MacPherson
Supervisor: Louise Nelson

Plant growth promoting rhizobacteria (PGPR) have been shown to form a mutualistic relationship with the roots of plants. PGPRs may promote an increase in root size yield by producing indole acetic acid, cytokinins and in some cases dissolving organic and inorganic phosphate in soil making it more available to the plant roots. PGPRs may also enhance plant growth indirectly by suppressing the growth of root-associated fungal pathogens. Replant disorder (RD) is a complex problem usually associated with several fungal pathogens and can occur when one crop is planted at a site previously devoted to the same crop. In orchards RD can lead to small or decayed root systems, lower or less frequent fruit yield and possibly death of the plant. Finding a PGPR strain that can suppress the fungal pathogens associated with replant disorder may provide an important and much needed method which we can use to control or prevent RD.

In this study I screened 18 promising strains of growth promoting rhizobacteria from a collection in Dr. Nelson's laboratory for their ability to inhibit the growth of three fungal pathogens commonly associated with replant disorder, including Rhizoctonia, Fusarium and Cylindrocarpon, using *in vitro* plate inhibition assays. The 5 most promising strains were found to be *Serratia plymuthica* 5-6 and 6-25, *Enterobacter agglomerans* 6-20 and 6-117 and *Enterobacter intermedius* 6-76. These 5 strains are now being tested in an *in vivo* assay with apple seedlings in growth pouches in the presence and absence of pathogens to see if they will promote growth and suppress disease. These 5 strains are also being tested in pots containing potting soils inoculated with the fungal pathogens to assess their potential to enhance seedling growth. This research could be useful in identifying a PGPR that might be used in the future in larger scale apple orchards in order to promote a greater crop yield and reduce deleterious effects of replant disorder.

The Evidence of Cardiomyocyte damage in high n-6 PUFA fed mice via the MCP-1 mediated ER stress apoptotic pathway

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Student: Laura Bakker
Supervisor: Dr. Sanjoy Ghosh

In Canada, more than 60% of all adults and a quarter of all children are either overweight or obese. This soaring increase in obesity and obesity-related cardiovascular disease (CVD) in the western world has lead to substitution of saturated fats with polyunsaturated fatty acids (PUFA's). Indiscriminate substitution has led to a current intake of around 7gms of PUFA daily. Although advocated, such high doses go against evolutionary dietary patterns. Further, previous studies have shown increased heart inflammation with such diets but the exact mechanisms remain inconclusive. In this study, I have investigated the role of omega-6 PUFA high fat fed mice on heart inflammation. High fat diets (40% energy from fats) included blending of different oils like corn oil (high in omega-6 PUFA) and canola oil (low in omega-6 PUFA). Methodologies I have used include looking at the endoplasmic reticulum (ER) stress pathways and cardiovascular damage in heart sections of mice. In

order to study the effects of these different diets on the heart, I have used SDS-PAGE techniques for western blotting to obtain data for protein levels of various ER stress markers, and then used chemiluminescent horseradish peroxidase substrate with analogue detection. In order to evaluate the influence of inflammatory pathways on such omega-6 PUFA induced ER stress, I have also analyzed data from mice lacking monocyte chemoattractant protein 1 (MCP-1), which were fed the same diets. I predict that with the lack of MCP-1, ER stress biomarkers will be attenuated even with omega-6 PUFA, thus confirming the activation of ER stress pathways in the omega-6 PUFA fed hearts via an inflammatory pathway.

Saccharomyces cerevisiae strain composition during an inoculated Pinot Noir fermentation

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Student: Liz Halvorsen
Supervisor: Dr. Daniel Durall

Wine fermentations are typically carried out using one of two techniques: spontaneous or inoculated. During a spontaneous fermentation, yeast indigenous to the winery and vineyard environment facilitate the conversion of glucose to ethanol. In contrast, inoculated (guided) fermentations inoculate grape must with a commercial Active Dry Yeast (ADY) strain of *Saccharomyces cerevisiae*, which is thought to allow a more rapid and even rate of fermentation with a product of consistent quality. In association with Quails Gate Estate Winery, Kelowna, BC, this study analyzed the strain composition of *Saccharomyces cerevisiae* during two stages of a Pinot Noir ferment inoculated with the ADY strain Lalvin® RC212. The aim of this experiment was to determine which strains of *S.cerevisiae* were present in the coldsoak and end stages of fermentation. Results from this experiment were compared to a previously conducted study at Quails Gate Estate Winery, to observe any variability from year to year. Wine samples from three Quails Gate Estate Winery Pinot Noir vessels – R3, R4 and R18 – were collected for the coldsoak and end stage of fermentation and isolated on agar. DNA from colonies displaying *S.cerevisiae* morphology (Pallmann et al. 2001) was extracted and subsequently amplified using six hypervariable microsatellite loci. Each genetic fingerprint was then compared to an ADY database compiled by Hall (2010) to establish strain identity. Four different *S.cerevisiae* strains were isolated in end stage fermentation in vessels R4 and R18, including Lalvin® RC212 (the inoculant), Lalvin® ICV D254, Red Star® Premier Cuvee, and an unknown strain. Results for tank R3 are still being processed. All samples isolated from coldsoak expressed non-*Saccharomyces* morphology. Sequencing and NCBI Basic Local Alignment Search Tool (BLAST) results indicated that all isolates from the coldsoak were *Hanseniaspora uvarum,* a species indigenous to the vineyard.

Synthesis of Sulfonamide Pyrazole Derivatives as Potential Antibiotic Compounds

Student: Matt Taron
Supervisor: Dr. Ed Neeland

Our lab has created a parent pyrazole compound **1** and a number of derivatives which have been shown to possess potent medicinal properties. These pyrazole compounds are highly effective anti-inflammatory and neuroprotective drugs. Building on these results, we envisioned linking the pyrazole template to a sulfonamide unit to create a new drug which was both an antibiotic and anti-inflammatory. Using conditions previously successful in our lab, the parent **1** was reacted directly with sulfanilamide under a variety of pH, temperature, and solvent conditions. Surprisingly, the desired product would not form. We found a clue to this mystery with the isolation of a salt which formed from an unexpected reaction between sulfanilamide and parent **1**. The basic amine groups of sulfanilamide were abstracting the amido hydrogen atoms and forming a chelated salt compound. This structural feature was confirmed using X-ray crystallography on an isopropyl derivative. To circumvent this salt formation, the N-H moieties in **1** were reacted with methyl iodide to exchange the troublesome amido hydrogen atoms with methyl groups and so eliminate the unwanted chelating reaction. These reactions resulted in partial methylation, and in some cases were seen to affect the diamagnetic anisotropy present in **1.** This was determined by ^{1}H NMR spectroscopy in which the phenyl multiplet of the starting material was changed into a phenyl singlet. The reluctance of sulfanilamide to react with **1** necessitated a change in strategy whereby the pyrazole sulfonamide was instead synthesised by first reacting sulfanilamide with oxalyl chloride to followed by subsequent nucleophilic attack of the pyrazole precursor. This successful reaction appears to contain 2 isomeric products which formed by nucleophilic attack of both sulfanilamide amine groups.

Mathematical Modelling of a Biological Control Agent

Student: Meghan Dutot
Supervisor: Dr. Rebecca Tyson and Dr. Louise Nelson

Postharvest diseases of apples, especially those caused by *Penicillium expansum* and *Botrytis cinerea*, cause considerable damage to apples in controlled atmosphere (CA) storage, resulting in economically significant losses. Annually, up to twenty percent of the crop stored in CA rooms may be lost to disease, despite the use of fungicides. Non-pathogenic bacteria antagonistic to fungi are a potentially more effective and ecologically friendly alternative to fungicides. These biological control agents have been shown to reduce the growth of the fungal pathogens. The population dynamics between the fungi and bacteria are complex, and difficult to elucidate solely with experimental work. This study focuses on the development of a mathematical model for biological control agents on apple fruit in long-term storage. It is based on a system of three differential equations modelling the interactions between fungi, bacteria, and the concentration of available nutrients. Parameter values were determined through fitting with experimentally determined growth curves. Results from simulations can be used to predict the severity of disease and the level of inhibition provided by biocontrols. In addition, the application of this model would reduce both the time and cost of future experimentation.

The Synthesis of Iron- and Ruthenium-Containing Terpyridine-Based Polymers

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Student: Michael Cowper
Supervisor: Dr. Alaa Abd-El-Aziz

Polymers are ubiquitous in modern society, possessing a vast array of properties and applications. The goal of this project was to synthesize novel organometallic and coordination polymers based on the molecule terpyridine. These types of polymers involve the incorporation of metal atoms into the polymer, yielding interesting properties such as magnetic susceptibility, increased solubility, and electrical conductivity. Terpyridine was chosen as a basis for this project as it strongly coordinates metal atoms, with two terpyridine molecules coordinating to one metal atom to form a linkage between monomers. With these metal linkages, when the monomers have the potential to conduct electricity, the newly synthesized polymer will have conductive properties. For this purpose, terpyridine was attached to thiobisbenzenethiol, a molecule containing alternating aromatic rings and sulfur atoms, allowing for the transfer of electrons. Alternatively, when terpyridine is contained in a side chain attached to the backbone of a polymer, its strong ability to coordinate metal atoms produces polymers with potential filtration applications. Polymers based on substituted valeric acid were prepared with side chains containing terpyridine. The side chains of these polymers were then coordinated to iron or ruthenium, producing ladder-shaped polymers with the backbones connected through metal-coordinated terpyridine. The prepared polymers were characterized using NMR spectroscopy, as well as differential scanning calorimetry, gel permeation chromatography, and thermogravimetric analysis.

Tissue expression patterns of the copper transporter *atp7* in larval *Aedes aegypti* following acute exposure to environmental copper.

Student: Patrick Bobyn
Supervisor: Dr. Mark Rheault

The transition metal copper is an essential trace element that is critical to the metabolic function of all organisms. Copper is found primarily as a divalent cation ($Cu2^{+}$) in the environment. Due to its redox potential, it is used as a critical cofactor by numerous enzymes, such as cytochrome c oxidase. Cu^{2+} is tightly regulated and is rarely found in its unbound form within cells. A number of copper transporting proteins have been identified in vertebrate and invertebrate species, which are responsible for copper regulation. The Cu-ATPase family in vertebrates consists of two representative proteins; CuATP7A and CuATP7B, which are responsible for Menke's and Wilson's disease respectively. In invertebrates there is only a single *atp7* gene, which is orthologous to the vertebrate genes. In this study we examined the mRNA expression pattern of the putative copper transporter *atp7* from the yellow fever mosquito *Aedes aegypti*. Fourth instar larvae were acutely exposed to 0.5 and 2.0 mmol l^{-1} Cu^{2+} and the expression of *atp7* relative to unexposed controls was measured in the whole body, midgut, hindgut, Malpighian tubules, and anal papillae using quantitative reverse transcription polymerase chain reaction (qRT-PCR). Results from this study will be used to develop the *Aeaatp7* gene as a molecular biomarker of copper toxicity. Additionally, this study supports the development of *Aedes aegypti* as an invertebrate indicator species for aquatic environmental metals toxicology.

An NMR-based Method for the Metabolomic Analysis of the American Cranberry

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Student: Ryan Lohre
Supervisor: Dr. Paul Shipley

The American cranberry (*Vaccinium macrocarpon*) has been used in the treatment and prevention of urinary tract infections (UTIs) since the early 19th century. Clinical evidence supports the traditional use of cranberries in decreasing the number of symptomatic cases of UTIs in women, however the mechanism of action remains inconclusive. Around 500 million tons of cranberries are produced each year in North America, of which British Columbia represents 12% of total fruit.

A reproducible method for the analysis of *V. macrocarpon* that considers the global phytochemical profile is required. This will allow for the differentiation of cranberries contributing to an annual harvest based on the variability of metabolites and illustrate the need for optimized growing conditions to produce the best possible commercial products. To do this, we have investigated the metabolome of 22 cranberry fruit samples randomly chosen from 22 locations in the Lower Mainland of British Columbia, representing the diversity of farms, growing conditions, water shed and environmental conditions present in an annual cranberry fruit harvest. We chose to use nuclear magnetic resonance (NMR)-based metabolomic profiling to provide a picture of the entire cranberry metabolome, and determine the variability of cranberries at the time of harvest. A method for aqueous cytosolic fluid extraction as well as direct deuterated acidified methanol and acetone extraction of lyophilized berry tissue was optimized. NMR experiment parameters and processing of data sets were developed, utilizing a 2D J-Resolved (2D JRES) pulse sequence followed by application of window functions, baseline correction, binning and normalization. Principle Component Analysis

(PCA) reduces the dimensionality of the data, and when supplemented by Partial Least Squares Discriminant Analysis (PLS-DA) provides a visualization of the variability present in a cranberry harvest, as well as the metabolites responsible for differentiating them.

Hydraulic Properties of Burned and Unburned Forest Soils in Interior British Columbia

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Student: Stuart MacKinnon
Supervisor: Dr. David Scott

Water repellent (hard-to-wet) soils exist naturally in many conifer forests in British Columbia (BC), but the degree of repellency can be enhanced by forest fires. With the loss of vegetation and ground cover that results from forest fires, the water repellent soil has lower infiltration rates and an increased risk of erosion. When adequate rainfall occurs, the probability of overland flow over the surface increases, resulting in rill and gully formation. These post-fire effects are well known elsewhere in western North America, particularly in California, where the "fire-flood-erosion" sequence is known to pose serious economic risks.

This study assessed the effect of fire on soil water repellency, and the recovery of wettability in the soils. Soils were examined at four major forest fires that burned in BC's southern interior over the last eight years. For every burned soil, a comparable soil from a nearby unburned location was collected as an indication of the natural, or background, water repellency.

Water repellency was tested using water drop penetration time (WDPT), critical surface tension (CST) tests, and inferred through infiltration rates determined using mini-disk infiltrometers (MDI). These tests were conducted in the field and in the laboratory on dry, sieved soils. Capillary rise and soil water characteristic curves (SWCCs) were also developed for the soils.

Severely burned soil had higher levels of repellency and altered hydraulic properties relative to the unburned soils. This effect diminished with time after the fire, becoming less important. The exact recovery time varies. Soils from the most recent fire showed highly repellent soils, but minimal repellency in the unburned soils. At each of the other three fire sites there was more variability in wettability between and within burned and unburned soils, and a minimum of one unburned soil at each site displayed water repellency, indicating background water repellency.

Cellular trafficking of B_{12}

Student: Suud Nahdi
Supervisor: Dr. Kirsten Wolthers

Since its first total synthesis, vitamin B12 (cobalamin) has been a central focus of study. Its role in human body is of particular interest especially due to its critical role in metabolism. There are two mammalian enzymes that are cobalamin dependent: methionine synthase (MS) and methylmalonyl CoA mutase (MCM). Humans cannot synthesize B12 *de novo*, therefore the body has a complex mechanism consisting of a network of proteins that transport and processes the vitamin for metabolic use by MCM and MS. Cobalamin has a cobalt metal that is coordinated to four nitrogen ligands from the tetrapyrrole corrin ring and a fifth lower axial ligand composed of 5,6-dimethylbenzimidazole base. The sixth upper axial position is variable depending on the form of the VitaminB12. This variable region can be composed of four different groups; cyano (CN), Methyl (CH_3), 5'-deoxyadenosyl (Ado) and hydroxide (OH) which will determine the form of Vitamin B12. Methylmalonic aciduria and homocystinuria type C protein (MMACHC) is a cytosolic cobalamin trafficking chaperone that is implicated with the transport and conversion of different forms of vitamin B12 to active cofactor that can be used by the enzymes. MMACHC is a 29kDa protein found in the cytosol that aids in the transport of vitaminB12 to MS in the cytosol and MCM in the mitochondria. MMACHC protein converts cyanocobalamin and other VitaminB12 forms to methylcobalamin for MS and adenosylcobalamin for MCM. MMACHC does this by converting the cobalamin into cob(II)alamin oxidized state by removing the base 5,6-dimethylimidazole, referred to as "base-off", hence facilitating the conversion of the cofactor needed. Deficiency in MMACHC protein causes reduced activity for both MS and MCM enzymes, which signifies its importance in conversion and transport of cobalamin.