



Program for the 2008 RISE Workshop
Acadia University
Wolfville, NS

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Acadia University Faculty of Science

UPEI Faculty of Science

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NSERC Atlantic (Regional Opportunities Fund)

Schedule of Events

Friday, August 22

7:00 – 9:30 Opening mixer, KCIC Garden Room

Saturday, August 23

8:40 - 9:00 Morning Reception and Coffee, KCIC Auditorium

9:00 Welcome and Opening Remarks
Matthew Lukeman

9:20 Daniela Sustac Roman, M. Lukeman
“Progress towards the synthesis of 2-(m-nitrophenyl)acetate protected alcohols”

9:40 Sara Accardi, B. Wagner
“Investigations of a fluorescence-based method for the detection of mastitis in dairy cows”

10:00 Augusto Hernandez, L. Johnston
“Study of lipid rafts in asymmetric bilayers”

10:20 **Nutrition Break** (outside KCIC Auditorium)

10:40 Graeme B. Mulholland, D. Andrews and B. C. Hill
“Investigating a role for free radicals in the sequence of intermediates formed in the reaction of cytochrome oxidase with oxygen”

11:00 Sayuri Freidland, W. Skene
“Comparative study of photophysical and electrochemical properties of simple versus polymeric alkylated fluoreno azomethines”

- 11:20 Amy Murschell, M. Chrétien
"Kinetics of radical addition to acrylate monomers: a laser flash photolysis study"
- 11:40 Margaret Reid, C. Reber
"Application of quantum mechanical models to variable-temperature absorption spectra of nickel(II) complexes"
- 12:00 **Lunch** (KCIC Garden Room)
- 12:45 **Photo of Participants** (KCIC Garden Room)
- 1:00 Katie Thurber, C. Bohne
"The effect of sodium chloride on the aggregation of bile salts"
- 1:20 Lukas Bebjak, P. Karam, W. Mah, and G. Cosa
"Single Molecule Mechanistic Studies on Polymerase Activity"
- 1:40 Chelsy Prince, D. Cramb
"Two-photon Excitation Photobleaching Kinetics of Sensitizers in Optically Trapped Multilamellar Vesicles"
- 2:00 **Nutrition Break**
- 2:20 Ian Duffy, W. Leigh
"Photochemical Synthesis and Characterization of an Electrophilic Germylene by Laser Flash Photolysis of 3,4-dimethyl-1,1-bis-pentafluorophenylgermacyclopent-3-ene"
- 2:40 Alyssa Cruz, G. Loppnow
- 3:00 Robert Godin, P. Kennepohl
"En route to quantifying metal-phosphine bond covalency by XAS"
- 3:20 **Business Meeting** (KCIC Meeting Room)
- 7:00 **Banquet** (Blomidon Inn, 195 Main Street)

Abstracts

Progress towards the synthesis of 2-(m-nitrophenyl)acetate protected alcohols

Daniela Sustac Roman¹ and Matthew Lukeman²

¹University of Toronto, ²Acadia University

Carbanion-mediated photoremovable protecting groups (PPGs) show much promise for the sub-microsecond delivery of bioactive molecules in an aqueous environment. They operate by undergoing photodecarboxylation to give a benzylic carbanion, which can subsequently eliminate a leaving group that is positioned β to it (an E1cb-like mechanism). We have previously demonstrated that nitrophenylacetate PPGs are effective for the rapid and efficient release of halides. The aim of the present project was to develop the synthetic routes for the release of leaving groups with greater biological significance, namely carboxylic acids, alcohols, and amines. Our required starting material was 3-nitrophenyl-2-propionic acid, which we successfully prepared in a 4 step procedure from 3-nitroacetophenone. After protection of the acidic group as either its methyl or t-butyl ester, we successfully hydroxymethylated the benzylic carbon to give us our needed reaction intermediate. A number of routes were attempted to synthesize protected alcohols and amines, both directly protected and through their respective carbonate/carbamates. While many reactions were at least partially successful, compound stability during purification and/or deprotection remained an issue. Successful release of acetate was achieved showing that carboxylic acids, at the very least, can be photoreleased in this manner.

Investigations of a fluorescence-based method for the detection of mastitis in dairy cows

Sara Acardi¹, B. Wagner²

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Mastitis is a bacterial infection that causes inflammation in mammary glands of cows, which is detrimental to milk production. Millions of dollars would be saved annually with a simple test to detect mastitis at early stages. A chemical probe was investigated to determine its ability to detect mastitis at early stages. When the bacterial endotoxin that causes mastitis is first introduced in cows, the amount of active matrix metalloproteinase 2 and 9 (MMP-2 and MMP-9) in the milk is greatly increased. MMP-2 and MMP-9 are two types of gelatinase whose functions are to break down the specific peptide sequence found in gelatin. The chemical probe investigated is a molecular beacon, which shows enhanced fluorescence in the presence of gelatinase. The molecular beacon contains a highly fluorescent tag, 7-methoxy coumarin (7MC), which is bound to a quencher by the same peptide chain that can be broken down by MMP-2 and MMP-9. The presence of the quencher prevents the 7MC from fluorescing. When active MMP is present, it cleaves the peptide sequence, allowing the 7MC to strongly fluoresce. In this project, the use of this spectroscopic probe to detect MMP levels in milk samples was investigated, with the goal of developing an *in vivo* detection method for mastitis in dairy cows.

Study of lipid rafts in asymmetric bilayers

Augusto Hernandez¹, L. Johnston²

¹Universite de Montreal, ²National Research Council, Ottawa

The cell membrane is a selectively permeable barrier separating the intra and extracellular environment. It fulfills many functions in biological processes such as an exchange medium regulating the movement of material into and out of cells, facilitating transport of certain molecules, maintaining cell potential, etc. Moreover, the membrane has a heterogeneous distribution of lipid and proteins. Certain areas have a high concentration of cholesterol and sphingolipids: these regions are termed membrane rafts or microdomains. Rafts are the preferred location of specific proteins, particularly those involved in signaling cascades, they can interact with others raft proteins. In order to understand the interaction in the rafts, we have to use a minimal model membrane; a supported phospholipid bilayers. Previous studies have used symmetric bilayers. However, cell membranes are asymmetric. The outer leaflet is composed primarily of sphingomyelin and phosphatidylcholine while the inner leaflet is composed of phosphatidylserine and phosphatidylethanolamine. For that reason, in this study, asymmetric bilayers will be prepared with the Langmuir-Blodgett and Langmuir-Schaefer technique to compare them to the symmetric ones. To study the organization of supported lipid bilayers, atomic force microscopy and TIRF spectroscopy are used. The combination of topographic and fluorescence contrast leads to a more complete description of the system. Our results suggest that the initial asymmetry of bilayers is difficult to maintain in mixtures with coexisting liquid-ordered and liquid-disordered phases such as DOPC/DEC 221. DEC 221 is a lipid mixture of DOPC (dioleoylphosphatidylcholine), egg sphingomyelin and cholesterol in molar ratios. Finally, an enzyme (sphingomyelinase) will be used to produce ceramide, an important messenger molecule that modulates membrane properties (eg, reorganizes rafts), in the asymmetric bilayers.

Investigating a role for free radicals in the sequence of Intermediates formed in the reaction of cytochrome oxidase with oxygen

Graeme B. Mulholland¹, D. Andrews and B. C. Hill²

¹University of Alberta, ²Queens University

Cytochrome oxidases function to deliver electrons from reduced donor molecules to molecular oxygen. Cytochrome oxidase complexes typically occupy the terminal position within electron transport chains and are conserved among aerobic species. We worked with a specific type of cytochrome oxidase from the aerobic bacterium *Bacillus subtilis* that uses menaquinol as a reductive substrate. The menaquinol oxidase exhibits a radical signal during steady state catalysis in the presence of menaquinol and oxygen. The nature of this radical is not fully characterized and we sought to understand it further by using the radical traps 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), 2-methyl-2-nitrosopropane (MNP) and N-tert-butyl-phenylnitron (BPN). The data indicate one of the radical traps, TEMPO, inhibits cytochrome oxidase. Cytochrome oxidase forms a series of intermediate states in its reaction with oxygen, known as compounds P and F, that can be accessed by reaction with hydrogen peroxide. Addition of TEMPO prevents formation of the second of this series, i.e. compound F. We propose that TEMPO inhibition is due to disruption of the oxygen reaction. Furthermore, reactivity of the oxidase with TEMPO suggests the possibility of a radical state that may be related to the steady state radical observed previously. Further work must be done to characterize the state of the radical in cytochrome oxidase.

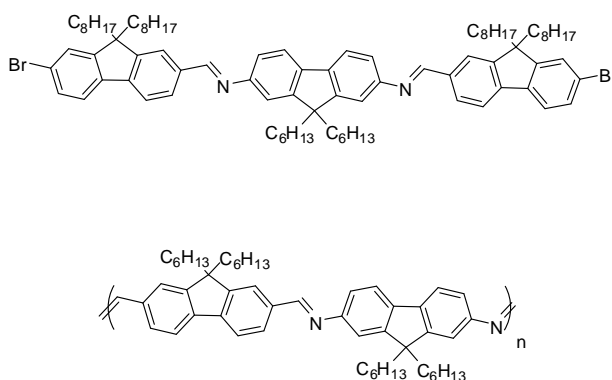
[This work was supported by an operating grant from NSERC to BCH and a USRA to GBM.]

Comparative study of photophysical and electrochemical properties of simple versus polymeric alkylated fluoreno azomethines

Sayuri Freidland¹, W. Skene²

¹University of Victoria, ²Universite de Montreal

Abstract: Compounds consisting of azomethine connections are both hydrolytically and anodically resistant with potential applications in electrochromics and emitting devices. Azomethines consisting of fluorene segments are especially interesting for emitting applications owing to their high degree of fluorescence. Even though azomethine oligomers can be prepared easily and characterized, their polymeric analogues are problematic owing to limited solubility. Dialdehyde- and diamino-substituted fluorene monomers were prepared and condensed to yield a novel pseudo-conjugated soluble fluorene rich polymer. The spectroscopic and electrochemical properties of the prepared polymer are compared to its oligomeric analogues and its thiophene derivatives.



Kinetics of radical addition to acrylate monomers: a laser flash photolysis study

Amy Murschell¹, M. Chrétien²

¹University of Calgary, ²Xerox Research Centre of Canada

Phosphine oxides, α -hydroxy ketones, and α -amino ketones are commonly used as photoinitiators in free radical polymerization. Upon absorption of UV or visible radiation these molecules undergo photolysis to generate a benzoyl and/or a ketyl-like radical. These radicals then react with monomers in the system to initiate polymerization. In this project laser flash photolysis was used to measure the rate constants for addition of nucleophilic ketyl radicals to a series of commercially available monomers. The addition rate constants of α -hydroxy and α -amino radicals were determined by an indirect technique, which employed crystal violet as a probe. The addition rate constant for the radical from Irgacure 819, a phosphine oxide, was determined directly as this phosphinoyl radical has an absorbance at 450 nm. The effects of acrylate chain length, acrylate functional groups, and substitution at the acrylic position will be discussed. The relative rate constants for addition of phosphinoyl, α -hydroxy, and α -amino radicals to a propoxylated neopentyl glycol diacrylate (SR 9003) will also be presented.

Application of quantum mechanical models to variable-temperature absorption spectra of nickel(II) complexes

Margaret Reid¹, C. Reber²

¹McMaster University, ²Universite de Montreal

The use of mathematical quantum models to describe molecular absorption spectra is a relatively unexplored field. When electrons absorb a photon of light, they move between energy states. Absorption spectroscopy is very useful because it accurately shows the differences in energy of possible electronic states. These electronic transitions, under normal circumstances, are governed by selection rules. For example, transitions must take place between orbitals of different energies, and changes in electron spin are forbidden. Allowed absorption bands are theoretically curves with a single maximum, but in reality, there exist forbidden transitions that couple with the allowed bands. These forbidden bands steal intensity from the allowed bands to cause a dip in the absorbance, and a double peak formation in the spectra.

We use a curve-fit equation from the literature¹⁻³ which mathematically describes a two-band coupling system. To test the validity of the equation, different spectra of the same molecule were examined to see if the properties of the curve fit values change with respect to changes in the spectra. Four octahedral nickel(II) complexes were examined. Ni(imidazole)₆(NO₃)₂ and Ni(pyrazole)₆(NO₃)₂ have a forbidden band in between two allowed bands, while Ni(1,10-phenanthroline)₃(NO₃)₂ and Cs[Ni(H₂O)₆](PO₄) have a forbidden band off to the side of two allowed bands. One inadequacy of the model was found to be that the equation only takes into account coupling between one forbidden band and one allowed band. This was determined based on the fact that curve fits using the literature equation did not adequately re-create the spectra, or had a large margin of error when they did.

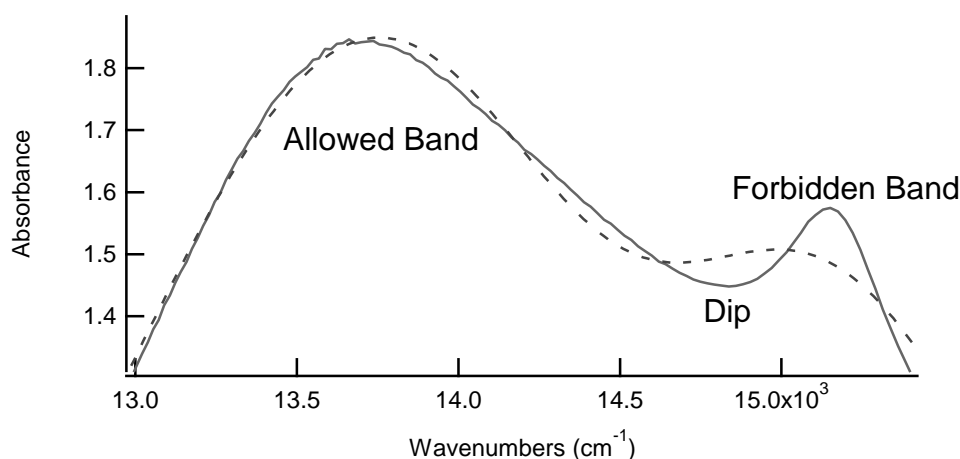


Figure 1: Room temperature absorption spectrum of Cs[Ni(H₂O)₆](PO₄) (solid line), with curve fit (dotted line).

¹ D. Neuhauser, T.-J. Park, J. I. Zink. *Physical Review Letters*, Vol. 85 (2000): 5304-5307.

² M.-C. Nolet, R. Beaulac, A.-M. Boulanger, C. Reber. *Structure and Bonding*, Vol. 107 (2004): 145-158.

³ E. Gonzalez, A. Rodrigue-Witchel, C. Reber. *Coordination Chemistry Reviews*, Vol. 251 (2007): 351-363.

The effect of sodium chloride on the aggregation of bile salts

Katie Thurber¹, C. Bohne²

¹Acadia University, ²University of Victoria

Bile salts are amphiphilic molecules that form complex aggregates in water. At low monomer concentrations primary aggregates are formed and at higher monomer concentrations the primary aggregates form larger structures called secondary aggregates. Previous studies have shown that guest molecules located in the primary aggregate, a hydrophobic environment, are well protected from quenchers that are in the aqueous phase and have long residence times in the aggregate. Guest molecules located in the secondary aggregate, a more hydrophilic environment than the primary aggregate, are well protected from interaction of molecules in the aqueous phase and they have a shorter residence time.

The objective of this project was to determine where the guest molecule, pyrene, was located in the bile salt aggregate when the concentration of salt (NaCl) is increased. Pyrene was used as a guest molecule because the I/III emission intensity ratio of its monomer fluorescence is diagnostic of the polarity of its location. Experiments were carried out looking at singlet excited state fluorescence of pyrene in bile salt aggregates, using steady-state and time-resolved fluorescence quenching experiments. Two different quenchers were used, iodide ions and nitromethane. Results have shown that as the concentration of salt is increased the pyrene is located in a more non-polar environment and the aggregates in which the pyrene resides get smaller.

The second objective was to investigate how the concentration of salt (NaCl) affects the association and dissociation rate constants for the triplet excited state of a guest (1-ethylnaphthalene) located in the bile salt aggregate. These studies were performed using laser flash photolysis experiments. Nitrite ions were used to quench the triplet excited state. Preliminary results indicate that the presence of salt affects the dissociation rate constant of the guest from the aggregate.

Single Molecule Mechanistic Studies on Polymerase Activity

Lukas Bebjak¹, P. Karam², W. Mah², and G. Cosa²

¹McMaster University, ²McGill University

The hepatitis C virus (HCV) is recognized as major human pathogen. Approximately 250000 infected individuals live in Canada. HCV infection is associated with severe liver disease, including cirrhosis and hepatocellular carcinoma (HCC). Unfortunately, the availability of potent inhibitors that block replication of HCV is limited, and by far not everyone benefits from therapy with pegylated interferon-alpha and ribavirin. These drugs are not HCV-specific. Severe toxic side effects, the ability of the virus to evade the host interferon system, and the enormous genetic variability of the virus are recognized as factors associated with treatment failure. A better understanding of specific steps involved in HCV RNA genome replication will likely improve the basis for the development of novel antiviral drugs with improved potency and specificity

Here we describe experiments that are designed to: 1) gain a molecular level understanding of de novo initiation of RNA synthesis catalyzed by HCV and 2) elucidate the functional roles of the GTP binding sites in HCV. We have used Single Molecule and ensemble fluorescence resonance energy transfer, as well as fluorescence polarization studies to address the interactions between HCV and substrate oligonucleotides. We will describe these preliminary results in the presentation.

“Two-photon Excitation Photobleaching Kinetics of Sensitizers in Optically Trapped Multilamellar Vesicles

Chelsy Prince¹, D. Cramb²

¹Queens University, ²University of Calgary

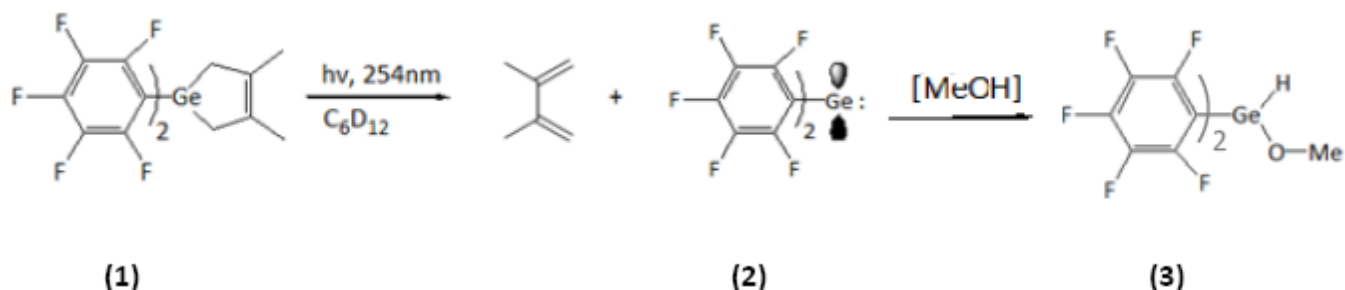
Two-photon excitation photodynamic therapy (TPE-PDT) is being developed as a treatment for age-related macular degeneration, the leading cause of severe vision loss outside of the third world. PDT works by occluding the new blood vessels from coroidal neovascularization in the wet form of the disease, thereby preventing further progress of the disease. Central to the development of this therapy is the determination of an optimized dose of photosensitizer and light to ensure vessel occlusion while limiting collateral damage to the surrounding healthy tissue. The dose can be influenced by many factors one of which is the photobleaching kinetics of the different photosensitizers. In the current study, the dependence of the photobleaching rate constants on the initial concentrations of two photosensitizers, Verteporfin (currently in clinical use) and Lemutoporphin (a new, more water soluble derivative of Verteporfin) was investigated. Optically trapped multilamellar vesicles were used to mimic the photosensitizers' cellular environments. Analysis of fluorescence photobleaching curves revealed that increasing the initial concentration of Verteporfin resulted in a linear increase in both photobleaching rate constants. In contrast, increasing the initial concentration of lemutoorphin did not result in a change in the rate constants over the range tested. This difference in behaviour supports the current hypothesis that the two photosensitizers have different photobleaching mechanisms.

Photochemical Synthesis and Characterization of an Electrophilic Germylene by Laser Flash Photolysis of 3,4-dimethyl-1,1-bis-pentafluorophenylgermacyclopent-3-ene

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¹University of British Columbia, ²McMaster University

The chemistry of 3,4-dimethyl-1,1-bis-pentafluorophenylgermacyclopent-3-ene (**1**) has been characterized by laser flash and steady state photolysis. Trapping products indicate that the corresponding germylene (**2**) is indeed extruded to give the expected O-H insertion product (**3**). While direct detection of (**2**) in hexane solution remains elusive, laser flash photolysis results in coordinative solvents have shown behaviour consistent with that of a very electrophilic germylene. Specifically, coordination complexes have been detected in THF and MeOH solution characteristically blue-shifted from typical diarylgermylene complexes. Interestingly, transient spectroscopy in neat MeOH solution have shown indications of deprotonation of the complex in neat MeOH solution, consistent with that of a very electrophilic germylene – MeOH complex.



En route to quantifying metal-phosphine bond covalency by XAS

Robert Godin¹, P. Kennepohl²

¹University of Ottawa, ²University of British Columbia

Chemical bonds arise from two different types of interactions: direct electrostatic interactions which leads to ionic bonding, and orbital overlap which leads to covalent bonding. In transition metal complexes, metal-ligand bonds generally include a significant covalent component. Therefore, probing the overlap of the atomic orbitals, the bond covalency, yields valuable information about the electronic parameters of the complex. Quantifying the bond covalency between a metal and chlorine or sulphur ligands by X-ray absorption spectroscopy (XAS) has been demonstrated but not for phosphine ligands. Electron paramagnetic resonance (EPR) spectroscopy was used to experimentally determine the covalency of the metal-phosphine bond for a series of copper(II) complexes. XAS studies can then be performed on these standard compounds to develop a method for quantifying the metal-phosphine in other complexes.