CHEM4710

Honours Project

in

Chemistry or Biochemistry

2017/2018 Project Presentations

April 7th, 2018

201 Armes Building

University of Manitoba

Program:

9:30	**Opening Remarks**	Dr. I. Oresnik (*Associate Dean of Science*) Dr. V. Nemykin (*Head of Chemistry)* Dr. M. Bieringer (*Course Coordinator*)

Time	Student	Title	Supervisor
9:40	Liting Bi	*Towards the Development of an Antibiotic Hybrid Consisting of the Aminoglycoside Tobramycin and the Fluoroquinolone Enoxacin*	Dr. F. Schweizer
10:00	Charles Killeen	*Kinetic and Mechanistic Investigation of Organocatalytic Carbon Dioxide Trapping by Alkynylindoles*	Dr. R. Davis
10:20	Daniel Padeanu	*Solid State NMR of Paramagnetic Metal Organic Frameworks*	Dr. S. Kroeker
10:40	**Coffee Break**	**Foyer of Armes Bldg.**	
11:00	Liam Berry	*Synthesis and Antimicrobial Evaluation of Levofloxacin Derivatives*	Dr. F. Schweizer
11:20	Zhe Xia	*Tentative identification of halogenated polycyclic aromatic compounds in samples from Alberta Oil-Sands region*	Dr. G. Tomy
11:40	Jessy Slota	*Production and Analysis of Truncations made at the C-terminal Quadruplex Unwinding Region of Helicase DDX21*	Dr. S. McKenna
12:00	Shixing Lei	*Incorporation of E. coli Integral Membrane Protein Glycerol Facilitator (GlpF) in a Nanodisc Environment*	Dr. J. O'Neil
12:20	**Lunch Break**	**Pizza lunch in Parker Bldg.**	
1:30	Matthew Stecy	*Regulation of Scleraxis by miRNA*	Dr. M. Czubryt
1:50	Alexandra Burnett	*LC-MS/MS analysis of adjuvants effect on antibiotic accumulation in Pseudomonas aeruginosa*	Dr. G. Tomy
2:10	Leo McKay	*A Retinoic Acid Deficiency Mouse Model of FASD Results in Hypothalamic Oxytocin Deficiency and Maternal Care Deficits*	Dr. G. Hicks
2:30	Oluwadamilola Daramola	*Optimization of GC-MS/MS parameters for the efficient separation of Alkylated Polycyclic Aromatic Hydrocarbons (APAHs)*	Dr. G. Tomy
2:50	**Coffee Break**	**Foyer of Armes Bldg.**	
3:20	Fabian Heide	*Uptake Rates and Relative Binding Affinities between Polycyclic Aromatic Hydrocarbons and RHCC protein nanotube*	Dr. J. Stetefeld
3:40	Lok Tin Hui	*Comparison in enzymatic activity between full length and individual domains of human 2'-5'-oligoadenylate synthetase isoform 2.*	Dr. S. McKenna
4:00	Todd Curtaz	*Development of a High Throughput Method for Flame Retardant Neurotoxicity*	Dr. G. Tomy
4:20	**Closing**	Dr. M. Bieringer	
4:22		**End of Event**	

4:30 **CGSA Sponsored Social at Degrees Restaurant (snacks and drinks)**

Towards the Development of an Antibiotic Hybrid Consisting of the Aminoglycoside Tobramycin and the Fluoroquinolone Enoxacin

Liting Bi (Schweizer group)

9:40 am – 10:00 am

Bacterial infections due to multidrug-resistant (MDR) Pseudomonas aeruginosa are associated with high mortality and morbidity. Treatment options are limited due to the organism's intrinsic resistance to antibiotics, the lack of cell penetration and extensive efflux.1 Aminoglycosides are particularly active against several Gram-negative pathogens and are commonly used to treat severe P. aeruginosa infections.1 However, resistance to this important class of antibiotic as well as other antipseudomonal agents is escalating. The Schweizer group have previously reported heterodimeric agents that are composed of two clinically-used antibiotics that are covalently-linked together, termed antibiotic hybrids.1,2 The successful synthesis of antibiotic hybrids composed of the aminoglycoside tobramycin and either the fluoroquinolones ciprofloxacin or moxifloxacin revealed a potent adjuvant scaffold to synergize with existing antibiotics.1,2 For instance, it was shown that tobramycin-ciprofloxacin hybrids enhance the in vivo efficacy of minocycline, rifampicin and fluoroquinolone antibiotics against MDR P. aeruginosa.1,2 Herein, we present the synthesis and evaluation of a novel type of antibiotic hybrid that is composed of tobramycin and the fluoroquinolone enoxacin. The two clinically-used antibiotics are covalently linked by a 12-carbon long aliphatic tether. We hypothesize that this new antibiotic hybrid enhances, the adjuvant properties observed in our previously reported tobramycin-fluoroquinolone hybrids.

References

(1) Gorityala, B. K.; Guchhait, G.; Fernando, D. M.; Deo, S.; McKenna, S. A.; Zhanel, G. G.; Kumar, A.; Schweizer, F. *Angew. Chem. Int. Ed. Engl.* **2016**, 55 (2), 555.

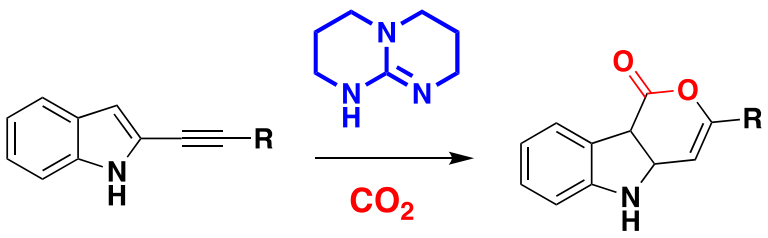
(2) Gorityala, B. K.; Guchhait, G.; Goswami, S.; Fernando, D. M.; Kumar, A.; Zhanel, G. G.; Schweizer, F. *J. Med. Chem.* **2016**, 59 (18), 8441.

Kinetic and Mechanistic Investigation of Organocatalytic Carbon Dioxide Trapping by Alkynylindoles

Charles Killeen (Davis group)

10:00 am – 10:20 am

Carbon dioxide activation and sequestration is a field of ever-increasing importance due to the influence of human activity on Earth's climate. Although consisting of two highly polar C=O bonds, high thermal stability and kinetic barriers make carbon dioxide activation and sequestration a unique challenge. The trapping of CO_2 by alkynylindoles using a guanidine-derived organocatalyst to form a tricyclic lactone-containing species has recently been established. In this project, we have attempted to gain insight into the mechanism of this reaction through kinetic and mechanistic studies, hoping to better understand the role of the catalyst in activating CO_2 and promoting C-C bond formation. The reaction progress was investigated using in-situ infrared (IR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy. Mechanistic studies were also performed to probe the role of specific proposed intermediates in the reaction pathway. Throughout the course of our research, we were able to disprove the role of a CO_2:catalyst adduct as a proposed intermediate in the reaction, and develop a scope of reaction conditions to demonstrate the robustness of the reaction.



Solid State NMR of Paramagnetic Metal Organic Frameworks

Daniel Padeanu (Kroeker group)

10:20 am – 10:40 am

Solid state nuclear magnetic resonance (ssNMR) has had a great impact in materials chemistry but has generally been limited to diamagnetic phases. In the presence of unpaired electrons, the typical diamagnetic chemical shift ranges are overwhelmed by paramagnetic contact shifts, rendering functional group fingerprints useless. Recently, fast magic angle spinning (MAS) ssNMR of paramagnetic compounds coupled with density functional theory (DFT) calculations of their crystal structures has led to a better understanding of these contact shifts, and has proven valuable for peak assignments and insight into bonding. A prominent target for paramagnetic NMR is metal organic frameworks (MOFs). These microporous materials constitute a diverse and rapidly growing field, with applications ranging from catalysis and carbon capture to proton conduction. Furthermore, with many MOFs being poorly crystalline, structural analysis through X-ray diffraction is limited, making MAS ssNMR with DFT calculations an attractive alternative for structural determination. This technique was applied to a series of chromium (III) phosphonate MOFs, allowing for peak assignments and an improved understanding of the bonding. ^{31}P and ^{13}C MAS NMR were collected and the systems with known crystal structures were modelled using DFT. The calculations provide spin density maps which illustrate the spin transfer mechanisms of polarization and delocalization, and helps account for the observed paramagnetic shifts. The paramagnetic chemical shifts and the spin transfer mechanisms are interpreted in terms of the chromium-phosphonate bonding motifs, including the type and number of bonds connecting chromium and phosphorus, the effects of which can be observed experimentally. This work shows the potential of the combined NMR/DFT approach to characterize the structure of MOFs.

Synthesis and Antimicrobial Evaluation of Levofloxacin Derivatives

Liam Berry (Schweizer group)

11:00 am – 11:20 am

As multidrug-resistant bacteria become widespread, and the development of new antibiotics stagnates, we are steadily approaching a “post-antibiotic era” in which common bacterial infections may again result in patient mortality. The highly restrictive outer membrane of Gram-negative bacteria presents a significant challenge for the development of new antimicrobial agents. Existing antibiotics are able to enter the outer membrane by several mechanisms, including nonspecific porin channels as seen in fluoroquinolone (FQ) antibiotics or through a “self-promoted uptake” mechanism seen in some cationic antimicrobial peptides. One of the key structural features of these peptides is their amino acid side chains (such as amines or guanidines) which are protonizable at physiological pH and confer cationic character. These positively charged groups are able to interact with negatively-charged lipopolysaccharides studded on the outer membrane of Gram-negative bacteria, displacing the divalent cations that are responsible for the overall structural integrity of these lipopolysaccharides. This would result in destabilization of the outer membrane that would consequently facilitate a self-promoted uptake of the antimicrobial peptide. We hypothesize that the antibacterial activity of FQ antibiotics may be enhanced by covalently adding these cationic amino acids, thereby allowing the FQ to penetrate the cell through porins (FQ’s native mode of cellular entry) as well as through the self-promoted uptake mechanism that the cationic peptides may bestow. In addition, bacteria develop resistance to FQs by expelling them out of the cell via efflux pumps, and we hypothesize that these new structural modifications may serve to prevent efflux. Overall, we expect an increased membrane penetration and intracellular accumulation of the FQ derivatives. In this talk, we present the preparation of twelve derivatives of levofloxacin, which is a clinically used FQ, by incorporating varying amounts of amino acids via solid-phase peptide synthesis. Moreover, their antibacterial activity against multidrug-resistant pathogens will be discussed.

Tentative identification of halogenated polycyclic aromatic compounds in samples from Alberta Oil-Sands region

Zhe Xia (Tomy group)

11:20 am – 11:40 am

Polycyclic aromatic compounds (PACs) are a complex class of compounds that are present in fossil material, such as petroleum oils. The common PACs include polycyclic aromatic hydrocarbons (PAHs), alkylated PAHs and heterocyclic aromatic compounds. Like the non-halogenated PACs, halogenated PACs (HPACs) are likely to be environmentally persistent and hazardous for biological organisms. To date, there are limited numbers of reports for halogenated PACs in biological organism. This is due in part to the lack of authentic analytical method for identification and quantification of these compounds and because of the lack of analytical standards for halogenated PACs. Historically, the Alberta Oil-Sands was covered by sea water millions year ago, which would have contained high concentrations of chloride and bromide ions. Based on this, the hypothesis of this project is that there are HPACs present in abiotic and biotic samples from this region. The aim of the project is to establish an analytical method for identification and quantification of halogenated PACs by gas chromatography-tandem mass spectrometry (GC-MS/MS). Firstly, the electronic ionization (EI) multiple reaction monitoring (MRM) method was established with sixteen commercially purchased HPACs analytical standards. Sample preparation process and established MRM method were verified by using protocols at Centre for Oil and Gas Research and Development (COGRAD) and Eurachem Guide. The behavior of PAH and HPAC was similar under the same preparation method, which implies the previously processed samples could be used for analysis. Finally, both abiotic and biotic samples from Alberta Oil-Sands were analyzed by GC-MS/MS with the established MRM method to identify these compounds. Samples from three species (snail, fish, and otter), were analyzed; 2,7-dibromofluorene and 9,10-dibromophenanthrene were identified in all three species. In snails (n=3), total concentration of HPACs (ΣHPAC) was determined to be 8.80±1.4 ng/g, which was three times smaller than total concentration of 16 PAHs (Σ_{16}PAH = 23.4±24.0 ng/g). In fish (n=5), 5-bromoacenaphthene and 9,10-dibromoanthracene were also detected; ΣHPAC was determined to be 8.77±4.2 ng/g, which was two times greater than total concentration of 16 PAHs (Σ16PAH = 4.34±5.58 ng/g). In otter liver (n=9), 5-bromoacenaphthene and 9,10-dibromoanthracene were also detected; ΣHPAC was determined to be 10.3±7.0 ng/g, which was smaller than total concentration of 16 PAHs (Σ_{16}PAH = 15.6±36.7 ng/g).

Production and Analysis of Truncations made at the C-terminal Quadruplex Unwinding Region of Helicase DDX21

Jessy Slota (McKenna group)

11:40 am – 12:00 pm

Guanine quadruplexes (G4s) are nucleic acid structures that arise from multiple stacked planar guanine tetrads. These structures form in both DNA and RNA and are thought to participate in several regulatory mechanisms. The formation of quadruplexes is controlled by enzymes which unwind them, known as G4 helicases. One such enzyme is DDX21, an RNA helicase recently identified by our group as possessing RNA G4 unwinding activity. To facilitate the investigation of the interaction between DDX21 and its quadruplex substrates, truncations made at the G4 unwinding region of DDX21 were cloned into a suitable expression vector for production in bacteria. Following overexpression in E. coli, and purification via nickel nitriloacetic acid (Ni-NTA) chromatography, large amounts of each truncation were obtained. Subsequent analysis revealed that the truncations retain the RNA binding and quadruplex unwinding properties of DDX21. Therefore, the production of these truncations in bacteria is a valuable tool which could permit the structural and functional analysis of the G4 unwinding activity possessed by DDX21. Such analysis could reveal important insights into the regulatory mechanisms mediated by G4s.

Incorporation of E. coli Integral Membrane Protein Glycerol Facilitator (GlpF) in a Nanodisc Environment

Shixing Lei (O'Neil group)

12:00 pm – 12:20 pm

Aquaporins are a family of polytopic transmembrane channel proteins that facilitate water and flux across cellular membranes in a large diversity of organisms from prokaryotes to humans.[1] These proteins are exquisitely designed to facilitate the transport of small polar molecules while at the same time preventing ion transport that would alter membrane electrochemical potential difference.[2] Understanding the substrate-specificity and ion selectivity of these channel proteins requires knowledge of the atomic dynamics of the proteins over a wide range of timescales. Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful tool for studying atomic dynamics of proteins but its application to integral membrane proteins is problematic. Membrane proteins cannot be studied in their native bilayer by NMR but removal from the bilayer often leads to destabilization of the protein fold, protein precipitation, loss of activity and degradation of the quality of NMR spectra. Recently, lipid nanodiscs have been used to stabilize membrane proteins yielding high-quality NMR spectra.[3] Nanodiscs are non-covalent assembled discoidal lipid bilayers stabilized by encircling amphipathic helical scaffold proteins termed membrane scaffold proteins (MSP).[4] MSPs were genetically engineered based upon the human serum apolipoprotein A-1 (ApoA1), the primary protein component of high-density lipoprotein (HDL).[5]

The aim of my research project was to prepare GlpF that is amenable to study by highresolution liquid NMR spectroscopy by incorporation into nanodiscs. I obtained the genes for 3 different His-tagged MSP proteins, transformed them into E. coli BL21(DE3), and purified all three proteins using Immobilized Nickel Affinity Chromatography. All of the proteins were analyzed by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and showed the correct molecular weight. Circular Dichroism (CD) spectra were obtained for all 3 proteins and they all showed strong α-helical signatures suggesting that the proteins were correctly folded. Analysis of the GlpF tetramer crystal structure determined that the transmembrane helix surface area is 3920 Å^2. On this basis MSP1E3D1 was chosen from among the three MSPs for further analysis based on its predicted diameter (12.1 nm) that should be able to accommodate the GlpF tetramer and associated lipid. Lipid-filled nanodiscs were formed with MSP1E3D1 and DMPC (Dimyristoylphosphatidyl choline). CD spectra showed that the MSP is helical in the lipid nanodisc. Differential scanning calorimetry (DSC) of the lipid-filled nanodiscs showed the gel-to-liquid crystal phase transition at 27ºC and the unfolding of the MSP at 87ºC. One attempt was made to incorporate GlpF into the lipid-filled nanodisc but no elevation of the GlpF melting point was observed casting doubt on the success of the preparation. In the future, optimization of the GlpF nanodiscs should be possible by optimizing the temperature, time, amount of hydrophobic sorbent, ratios of MSP, lipid, and GlpF. GlpF nanodisc formation will be confirmed by Small Angle X-ray Scattering.

References

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2. Verkman et al. (2013) Curr Biol. 52–55.
3. Denisov et al. (2017) Chem Rev 4669–4713.
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Regulation of Scleraxis by miRNA

Matthew Stecy (Czubryt group)

1:30 pm – 1:50 pm

Heart disease is currently the second leading cause of death in Canada. A significant contributor to this growing issue is a pathological process termed cardiac fibrosis. Characterized by the production of extra-cellular matrix at a rate that exceeds basal turnover, this pathological process ultimately leads to unfavourable stiffening of the heart. Scleraxis has been identified as a key transcriptional regulator for many of the cellular processes leading to cardiac fibrosis. As such, it serves as a potential target for novel anti-fibrotic therapeutics. Through in vitro genetic analysis with NIH-3T3 fibroblast cells, I have identified microRNA 7087-3p as a repressor of Scleraxis translation. Furthermore, through a luciferase reporter system, I have elucidated its mode of action to function through interaction with the Scleraxis 3' untranslated region. These findings illustrate how microRNAs may play a pivotal role in the regulation of Scleraxis, and are the first examination of microRNA-mediated regulation of Scleraxis. Further studies will examine NIH-3T3 cells for endogenous regulatory microRNAs, and whether their levels change upon treatment with the pro-fibrotic agonist Transforming Growth Factor β.

LC-MS/MS analysis of adjuvants effect on antibiotic accumulation in Pseudomonas aeruginosa

Alexandra Burnett (Tomy group)

1:50 pm – 2:10 pm

Last year a woman died from an antibiotic resistant bacterial infection that could not be treated with any of our current antibiotics. Gram negative bacteria are particularly prone to resistance due to their double layered membranes and their efflux pumps, which actively work to pump antibiotics out of bacteria. As a possible solution to antibiotic resistance the Schweitzer group has synthesized adjuvants, which are molecules that work to help increase the concentration of antibiotics in the cell. In order to assess the effectiveness of the adjuvants the concentration of antibiotics in bacterial cells must first be known. Using liquid chromatography tandem mass spectrometry (LC-MS/MS) a method to analyze the concentration of the antibiotic rifampicin was developed. The method was validated by assessing the following performance characteristics: accuracy, precision, linearity, matrix effects and limit of detection. Quantitation was based on isotope-dilution using d4-rifampicin as an internal standard (IS). The accuracy and precision of the method were measured by extracting known amounts of rifampicin intentionally fortified into the physiologically relevant medium Mueller Hinton Broth (n=10). Liquid-liquid extraction was performed using ethyl acetate. The average measured value of rifampicin in the fortified solutions was 64% of the known value and the precision of the overall method was 11% (relative standard deviation). The calibration curve was linear over the concentration range of 10pg/µl to 100pg/µl. The limit of detection was determined to be 6pg/µl. Matrix effects were assessed by fortifying extracted Mueller Hinton Broth medium with varying amounts of rifampicin and comparing the slopes of the matrix calibration curve to the calibration curve of the analytical standards. A small but statistically significant difference between the slopes was observed, however, the use of the mass labeled IS corrected for this. There was a small but insignificant (Student t-test) d-isotope exchange observed between the d4-IS and the native compound. Based on the performance characteristics of the method, the method is fit for its intended purpose.

A Retinoic Acid Deficiency Mouse Model of FASD Results in Hypothalamic Oxytocin Deficiency and Maternal Care Deficits

Leo McKay (Hicks group)

2:10 pm – 2:30 pm

Oxytocin (Oxt) is a neurotransmitter hormone that regulates many social behaviours, including creating the strong bond between a mother and her child. In a retinoic acid (RA) deficiency model of Fetal Alcohol Spectrum Disorder (FASD; Gsc:Cyp26A1 mice) we observed that Gsc:Cyp26A1 mothers have no offspring survive to weaning. Interestingly, human mothers with FASD report problems with maternal care and stress-related disorders, and many of maternal care behaviors are known to be regulated by Oxt in both species. We hypothesized that our Gsc:Cyp26A1 mice have behavioural deficits in maternal care due to an oxytocin deficiency. Here, we report that Gsc:Cyp26A1 mothers have maternal care deficits. In pup survival tests, no offspring survived post-natal day 1 with a Gsc:Cyp26A1 mother, compared to 100% if the mother was WT (n = 11 and 11, respectively). Perinatal death was typically cannibalism, and not the result of failure to thrive, suckle or dependent on the pups genotype. In pup retrieval tests Gsc:Cyp26A1 dams performed poorly when compared to wild-type mice, having much longer retrieval times than the wild-type mothers. We next determined whether Oxt-producing tissues in the hypothalamus of these dams expressed Oxt normally. Oxt-Neurophysin I immunohistochemistry assays on brain sections revealed that Gsc:Cyp26A1 mice are deficient in Oxt expression in both the paraventricular nucleus (PVN) and the supraoptic nucleus (SON), as compared to WT mice. It is well established that Oxt expression is regulated by retinoic acid. To determine whether Oxt deficiency was a result of developmental aberrations or due to the transgenic cassette, we examined Gsc:Cyp26A1 brains for Gsc expression by immunohistochemisitry. No detectable levels of Gsc was observed in either Gsc:Cyp26A1 mice or wild-type littermates; however, aberrant morphology of the PVN in the Gsc:Cyp26A1 hypothalamus does suggest evidence of neurodevelopmental malformations. Taken together, our data demonstrate that RA deficiency during early embryogenesis may result in forebrain malformations and observed maternal care deficit outcomes. This work may reveal new molecular etiologies of FASD and suggests new treatment paradigms that may reverse or reduce behavioural outcomes that can result in significant FASD secondary disabilities

Optimization of GC-MS/MS parameters for the efficient separation of Alkylated Polycyclic Aromatic Hydrocarbons(APAHs)

Oluwadamilola Daramola (Tomy group)

2:30 pm – 2:50 pm

Polycyclic aromatic hydrocarbons (PAHs) are a complex class of organic molecules that consist of two or more fused aromatic rings. These persistent compounds pose a potential health risk to both the aquatic and terrestrial environment. The alkylated homologues of PAHs (APAHs) are less studied chemicals which have been shown to be of higher health risk due to their carcinogenic and mutagenic properties. The separation of APAHs is challenging as there is an exponential increase in the number of theoretical isomers as the alkylation level increases. This produces a chromatogram with large amount s of co-eluting peaks. The traditional methodology used for separation and identification of APAHs is a GC-MS/MS method with He as the carrier gas. The MS/MS method increases the S/N ratio and prevents misidentification of different APAHs that may co-elute due to similar m/z values and structure. The MS/MS method is created for 29 APAH mixture used in this experiment. The confirmation ions are identified based on alkylation level of the APAHs and are achieved with the optimization of the collision energy of the tandem MS. The factors that affect separation of isomers include the type and length of GC-column used, the optimization of the oven gradient temperature, and the type of carrier gas employed. Hydrogen is a smaller and less viscous gas than the He which allows for lower mass exchange in the column leading to a smaller degree of peak widening that occurs in the capillary GC-columns. This could lead to a more efficient separation at higher flow rates. In my study, the separation efficiency of eight GC-columns was first optimized using He as the carrier gas. The oven temperature program was optimized to yield the highest efficiency. The He method proved sufficient for separating closely eluting methylphenanthrene isomers (1 and 9) with an average retention time of 10.3min for 30m columns and 8.4 min for 20m columns. The most efficient column proved to be the Agilent CP-7462 Select PAH (30m x 0.25mm, 0.25um). Once the method was optimized with He as the carrier gas, the method was reoptimized using H_2. The oven temperature program was retained to confirm that the optimal flow rate of H_2 differs to that of He. A series of linear ramps were used as an initial oven program to determine the optimal flow rate. The oven program was further optimized until a baseline resolution was obtained for the 1-methylphenanthrene and 9-methylphenanthrene in half the He runtime. The efficiency and retention times of both the He and H_2 carrier gas methods were analyzed with a statistical testing to confirm that the changes in efficiency were significant. The baseline resolution achieved from the H_2 run proved that a switch from the conventional He carrier gas to H_2 in APAH separation methods will yield a significant increase in efficiency and a decrease in overall method runtime.

Uptake Rates and Relative Binding Affinities between Polycyclic Aromatic Hydrocarbons and RHCC protein nanotube

Fabian Heide (Stetefeld group)

3:20 pm – 3:40 pm

Right-Handed Coiled Coil (RHCC) is a hyperthermo- and pH- stable protein nanotube that has the potential to bind and carry small hydrophobic molecules. This property of RHCC makes it an excellent candidate for the uptake of environmentally significant polycyclic aromatic hydrocarbons (PAHs), and could serve the purpose of measuring PAH concentrations and removing PAHs from the environment. Further examination of the binding of PAHs to the two large, hydrophobic binding sites of RHCC is necessary to establish a purposeful industrial tool. Previous data suggests that the binding affinities of 16 different PAHs would decrease with an increase in molecular size. To examine the relative binding affinities between PAHs and the RHCC binding sites an exposure study was set up to sample the uptake of these 16 different PAHs over a time period of 2 weeks. The PAH concentrations were measured using GC-MS/MS from which the relative binding affinities and uptake of PAHs by RHCC could be observed at various time points. The data showed that RHCC in comparison to control samples has an increased capacity of uptake for all examined PAHs, which confirms storing of the hydrophobic molecules in the binding sites. Also the initial uptake of the small molecules showed that certain PAHs like acenaphthylene, anthracene, benzo(k)fluoranthene, benzo(a)pyrene and benzo(ghi)perylene were taken up in high amounts, while the other PAHs were limited in uptake. However, the results did not establish a general trend but showed that binding to RHCC might be specific for each molecule and not solely depending on molecular size.

Comparison in enzymatic activity between full length and individual domains of human 2'-5'-oligoadenylate synthetase isoform 2

Lok Tin Hui (McKenna group)

3:40 pm – 4:00 pm

Human 2'-5'- oligoadenylate synthetase isoform 2 (OAS2) is an essential innate immune system protein produced upon viral infection. OAS2 can specifically bind viral double-stranded RNA (dsRNA) and polymerize deoxyadenosine triphosphate (ATP) into 2'-5' oligoadenylate chains (2'-5' A). 2'-5'A will bind to an endoribonuclease, RNAse L, which ultimately helps degrade cellular and viral RNA to prevent viral replication (1,2). OAS2 contains two domains, a presumed catalytic domain and a non-catalytic dsRNA-binding domain. Comparison of the structure and catalytic activity of these individual domains has not been investigated, as the eukaryotic expression system used to make full-length OAS2 cannot produce sufficient yield of individual domains. Recombinant OAS2 domains were expressed in bacterial cells as an N-terminal fusion to a solubility/affinity tag (3). Protein was expressed and purified with an affinity chromatography step followed by removal of the N-terminal fusion. Size exclusion chromatography was then used to isolate purified OAS2 domains. OAS2 domains eluted as a single peak with a higher than expected size. Inline UV spectrophotometry confirmed that protein was not bound to nucleic acids after purification. To test the catalytic activity of OAS2 domains and full-length protein, a colorimetric assay was used to detect inorganic pyrophosphate (byproduct of 2'-5'A formation) in the presence of polyinosinic:polycytidylic acid (a dsRNA standard) at different time intervals. The concentration of pyrophosphate can be determined with the addition of molybdate reagent (1-amino-2-naphthol-4sulfonic acid in bisulfite) and 2-mercaptoethanol to induce color formation; the color intensity can be determined by absorption spectroscopy (4). My results demonstrated that while the full-length protein is catalytically active, individual OAS2 domains or both domains added in trans are not sufficient for activity. The results suggested that OAS2 requires both domains play a role in the ability of OAS2 to interact with dsRNA substrates and activate the innate immune response.

References:

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Development of a High Throughput Method for Flame Retardant Neurotoxicity

Todd Curtaz (Tomy group)

4:00 pm – 4:20 pm

As the bioaccumulation of flame retardants becomes of concern a method for determining their neurological effects becomes apparent. A representative model of the aquatic environment The Great Pond Snail (Lymnaea Stagnalis) is selected for a variety of reasons to perform these neurological toxicity tests on. The flame retardant 1, 2-dibromo-4-(1, 2-dibromoethyl)-cyclohexane, also known as TBECH or DBE-DBCH, is chosen for initial testing due to known effects on a mammalian model shown through electrophysiological work. Positive controls are performed on the aquatic model to demonstrate the membrane potential and voltage gated sodium channels can be manipulated, this is done via 15mM potassium chloride and a sodium exclusion experiment, respectively. A concertation of 100uM TBECH is used to determine what effects the flame retardant has when exposed to neurons. When the neurons are exposed to the flame retardant there is a time delay before the effects can be observed, the effects are a depolarization of the cell membrane, a decrease in the latency of the first action potential, a depolarization of the after hyperpolarization potential, and the suppression of subsequent action potentials. These effects are consistent with TBECH interfering with both the voltage gated sodium channels and the voltage gated potassium channels, this can be confirmed via voltage-clamp experiments. The effects of TBECH on the neurons of Lymnaea Stagnalis will require further experimentation to determine a dose-response curve and whether other membrane proteins are subject to the effects of TBECH.