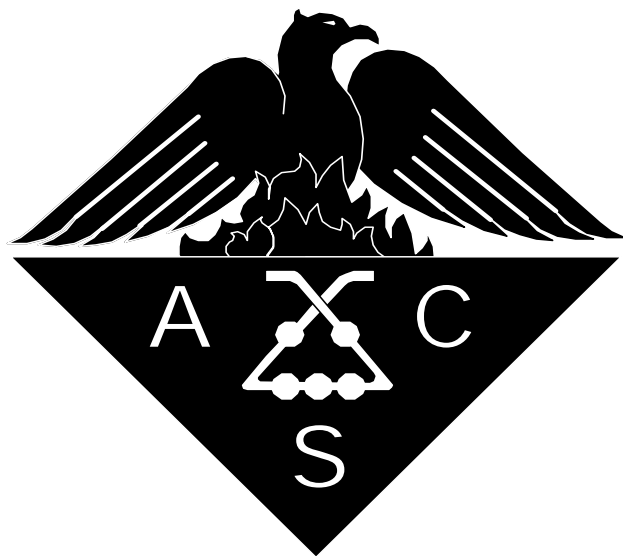


2011 Cleveland ACS Meeting-In-Miniature Program

Ursuline College
Pepper Pike, Ohio

March 16, 2011



BIOLOGICAL CHEMISTRY SESSION

Pilla Center 101

2:30 p.m.

Preventing the Aggregation of Islet Amyloid Polypeptide (IAPP) in Rat Insulinomas Using Novel Beta-Sheet Breakers

Melissa Barranger-Mathys, Ursuline College, Department of Chemistry

Islet amyloid polypeptide (IAPP), or amylin, is a 37-residue polypeptide that is co-secreted with insulin by pancreatic β -cells. IAPP self-associates, forms cytotoxic fibrils, and aggregates into a β -sheet conformation even at nanomolar concentrations. This aggregation is the main constituent of pancreatic amyloid, which is observed in patients with Type II Diabetes Mellitus. Because the aggregation of IAPP causes the death of β -cells, we are interested in inhibiting fibrillogenesis. Research suggests that a class of compounds known as β -sheet breakers have the ability to alter the secondary structure of amyloid plaques, making them more soluble and unable to self associate and aggregate. Using insulinomas in rat pancreatic β -cells, we tested the ability of our novel five amino acid β -sheet breakers to decrease the aggregation of IAPP. Immunofluorescent FITC tags allowed the visualization of IAPP in the cells with and without β -sheet breaker peptide treatment.

2:50 p.m.

Expression and purification of recombinant Peptidyl Arginine Deiminase from *Danio rerio*.

Jessica McClarren,* Baldwin-Wallace College

Multiple sclerosis is a demyelinating disease of the central nervous system (CNS). The etiology of the disease seems to result from a variety of influences including genetic, environmental, and immunological factors (Kim, 2003). Myelin basic protein (MBP) is a major component in the composition of myelin, a material composed of lipids and proteins responsible for increasing the propagation of an action potential down an axon. After numerous essential post-translational modifications to this protein (phosphorylation, deamidation, deimination, arginine methylation, and N-terminal acylation), healthy myelin is stable, compact, and wound tightly around an axon (Kim, 2003). Peptidyl arginine deiminase (PAD) is an enzyme responsible for converting positively charged arginine residues to neutral citrulline residues in brain tissue (Moscarello, 2007; Wegner, 2008). This conversion from arginine to citrulline by PAD is irreversible (Kim, 2003). In a recent study, 20% of the MBP isolated from normal human adult cells was citrullinated, in contrast to 45% in chronic MS patients and 90% in fulminating MS patients (Kim, 2003). This increase in citrullination strongly suggests that an increase in the PAD enzyme activity is responsible for the pathogenesis of multiple sclerosis.

The objective of this project is to express and purify the recombinant peptidyl arginine deiminase (PAD2) enzyme from *Danio rerio* (zebrafish). The PAD2 enzyme has been found to be correlated with an increase in citrullinated myelin basic protein (MBP) (Moscarello et al., 2002). Citrullinated MBP contributes to demyelination which is one of the hallmarks of multiple sclerosis (MS) (Moscarello et al., 2007). Therefore, this project will ultimately contribute to the determination of the role of PAD2 in the pathogenesis of MS.

- Kim, J.K., Mastronardi, F.G., Wood, D.D., Lubman, D.M., Zand, R., & Moscarello, M.A. An important role for post-translational modifications of myelin basic protein in pathogenesis. (2003). *Molecular & Cellular Proteomics*, 2(7), 453-462.
- Moscarello, M.A., Mastronardi, F.G., Wood, D.D. The role of citrullinated proteins suggests a novel mechanism in the pathogenesis of multiple sclerosis. (2007). *Neurochem Res*, 32, 251-256.
- Moscarello, M.A., Pritzker, L., Mastronardi, F.G., Wood, D.D. Peptidyl arginine deminase: a candidate factor in demyelinating disease. (2002). *J Neurochem*, 81, 335-343.
- Wegner, N., Wait, R., Venables, P.J. Evolutionarily conserved antigens in autoimmune disease: implications for an effective etiology. (2009). *The International Journal of Biochemistry and Cell Biology*, 41, 390-397.

3:10 p.m.

A Modular Isotopomer Synthesis of γ -Hydroxybutyrate (GHB) Reveals Catabolism via Alpha Oxidation

Sushabhan Sadhukhan,^{†**} Guo-Fang Zhang,[‡] Henri Brunengraber,[‡] Gregory P Tochtrop[†],
Department of Chemistry[†] and Department of Nutrition[‡], Case Western Reserve University,
10900 Euclid Avenue, Cleveland, OH 44106, USA

γ -Hydroxybutyric acid (GHB), the smallest 4-hydroxy acid primarily derived from γ -Aminobutyric acid (GABA) in brain, possesses a number of physiological and pharmacological properties. GHB has been clinically used as an anesthetic drug as well as for the treatment of alcohol and opiate dependence and narcolepsy-associated cataplexy. On the other hand, due to its ability to induce short-term amnesia, GHB is being used illicitly as a drug to facilitate acquaintance sexual assault and got a reputation of a *date rape drug*. GHB gets accumulated at supraphysiological concentrations in succinic semialdehyde dehydrogenase (SSADH) deficiency, which is a rare inborn error of GABA metabolism caused by the deficit of GABA degradative enzyme SSADH. Despite being such an important small fatty acid and expanding clinical and illicit consumption, the catabolism of GHB remains elusive. In the past, we reported the catabolism of 4-hydroxy acids in perfused rat livers using a combination of metabolomics and mass isotopomer analysis. A key finding of that work was that 4-hydroxy acid catabolism can proceed via two parallel pathways that involve either a phosphorylation and isomerization of the C-4 hydroxyl group or a β -oxidation/ α -oxidation sequence. Surprisingly, GHB being a 4-hydroxy acid does not follow the same pathway that others do. Instead, we figured out that GHB produces least amount of 4-phospho acyl CoA in the series which lead us to further investigate that particular fatty acid. In the study of GHB metabolism, we found α -oxidation and β -oxidation playing a key role. Transient stability of formyl CoA, product of α -oxidation makes its detection difficult to perform. We then decided to incorporate heavy stable isotopes (¹³C) at specific positions of GHB to track formate. But, the observation of M1 formate is not sufficient to conclude the α -oxidation because of the anaplerotic incorporation of ¹³C labeled intermediates. Hence, we need an efficient analytical method to confirm α -oxidation. We synthesized a number of ¹³C labeled GHB and defined a precursor to product relationship as a new index of α -oxidation. The mass-isotopomer analysis revealed key features including the direct evidence for the existence of α -oxidation and β -oxidation in the course of its catabolism. Together these

findings delineate pathways for GHB metabolism and will enable a better understanding in its therapeutic uses and drug overdose.

3:30 p.m.

Analytical Approaches to Ovarian Cancer: Development of an ELISA for a Biomarker Mimic Peptide

Kevin Hu,* Rebecca Whelan, Department of Chemistry and Biochemistry, Oberlin College
Clinical successes in monitoring ovarian cancer patients by quantification of serum biomarker CA125 have motivated further investigations into its structure and function. CA125 genetic sequencing has enabled the characterization of specific structural motifs. Specifically, a 21-mer peptide containing the epitope for anti-CA125 monoclonal antibodies has been synthesized, and characterized using FT-IR. To understand the immunoreactivity and structure-activity relationships of this 21-mer epitope “mimic” peptide, an ELISA is under development to quantify this antigen. Sandwich and indirect protocols are being studied and optimized. Preliminary results suggest that the mimic peptide does not retain completely equivalent immunoreactivity as native CA125, and that a sandwich assay will prove more sensitive than an indirect assay.

3:50 p.m.

Identification of non-peptidic, substrate competitive Abl kinase inhibitors

Amelia Zessin*, Sonali Kurup², Mike E. Steffey², Matthew B. Soellner², John Carroll University
Departments of Biology and Chemistry, University Heights, OH 44118; ²University of Michigan, Department of Medicinal Chemistry, Ann Arbor, MI 48109.

Protein tyrosine kinases (PTKs) catalyze the transfer of the γ -phosphate on ATP to the tyrosine residues on proteins. These enzymes have been implicated in diseases including cancer, inflammation, diabetes, hypertension, angiogenesis, and immune disease. Specifically, the PTK c-Abl plays a role in cell growth and development. The increased kinase activity of c-Abl has been shown to play a role in several cancers including chronic myelogenous leukemia (CML). Current approved therapeutics targeting inhibition of c-Abl are predominantly ATP-competitive inhibitors. We believe that protein substrate-competitive inhibition of c-Abl offers several important benefits over ATP-competitive inhibition, including selectivity for c-Abl and a lesser probability of resistant mutations. We have utilized a fragment based screening approach based on suitable peptide scaffolds to specifically orient inhibitor fragments in the substrate pocket of kinases. Removal of the peptide backbone has allowed for the identification of novel inhibitory fragments. A library of inhibitory fragments has been developed and evaluated as inhibitors of Abl kinase. The K_i values against c-Abl have been determined using a fluorescence based assay.

4:10 p.m.

Molecular Mechanism Underlying the Flavonoid-Induced Inhibition of A β (1-42) Fibrillation

Lijun Zhu**, Michael Zagorski, Chemistry Department, Case Western Reserve University

Background: Alzheimer's disease (AD) is characterized by the abundance of intraneuronal neurofibrillary tangles and the extracellular deposition of the A β peptide into amyloid plaques.

The A β becomes neurotoxic to cortical cell cultures when aggregated as amyloid-like β -sheet structures, A β protofibrils, or other soluble aggregates. Previously we showed that certain flavonoids inhibit A β (1-42) aggregation into amyloid fibrils. The flavonoids selected for our studies are polyphenols found in wines and blueberries, and the flavonoid myricetin showed the greatest inhibition. My research is focused on unraveling the molecular mechanism associated with this inhibition.

Methods: The analytical techniques utilized are ESI-MS, CD, NMR, and Th-T.

Results: The molecular mechanism underlying the myricetin-induced inhibition involves noncovalent binding to the A β (1-42) monomer and oxidation of the M35 side chain. This prevented aggregation and the random coil \rightarrow β -sheet conversion that occurs during amyloid fibril formation.

Conclusion: My work has established that several factors rather than a single one contribute to the inhibition of WT A β (1-42) fibrillation induced by myricetin. The myricetin inhibiting A β (1-42) fibrillation may serve as a model for the development of therapeutic drugs in combating Alzheimer's Disease.

4:30 p.m.

Cytoskeletal Connection Between Integrin Binding Partners and the Effects on Endothelial Cell Response to Shear Stress

Sidney Jones^{1,2},** Kasia Bialowska², Edward Plow²

¹Cleveland State University, Department of Chemistry, Cleveland, OH 44115, United States

²Department of Molecular Cardiology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH 44195, United States

Atherosclerosis is prominently located in bifurcated regions of vasculature. These regions have been associated with disturbances in hemodynamic flow patterns which induce shear stress experienced as a mechanical stimulus by endothelial cells lining the vessel wall. In response, endothelial cells are unable to respond to disturbed flow and develop a sustained inflammatory state which primes the vascular region for atherogenesis. Numerous investigations which have used shear flow assays have shown that integrins transduce mechanical forces applied by fluid shear stress into biochemical events through cytoskeletal structure distortion-dependent changes either locally at the site of integrin-extracellular matrix protein binding or distally, such as the cell surface in contact to flow. In this study, we provide insight into the role of the kindlin-2 and kindlin-3 in integrin mediated mechanotransduction by applying low shear stress to human umbilical vein endothelial cells (HUVECs). HUVECs express β 1 and β 3 integrins and can interact with various substrates. The kindlins, via their F3 subdomain of the FERM domain, have been shown to bind the conserved NITY⁷⁵⁹ motif of the C-terminal region of integrin β cytoplasmic tails and consequently participate in bidirectional signaling events attributable to integrin activation. Kindlin-2 has been shown to bind to other cytoskeletal proteins, such as migfilin, and may facilitate the localization of proteins required for mechanotransduction to the cytoplasmic tail of integrins. Our data indicates that under low shear stress conditions, kindlin-3, but not kindlin-2, experiences changes in its distribution which may implicate kindlin-3 to have a role in mechanotransduction. Low shear stress has been reported to

induce changes in vesicle displacement, gene regulation, and secretion of proteins from endothelial cells. We suggest that the changes in kindlin-3 distribution may involve interactions with vesicles or secreted proteins resulting in its redistribution under shear. In summary, the cytoskeletal connection between the β 3 subunit of integrins and kindlins may be involved in mechanotransduction in endothelial cells.

4:50 p.m.

The Influences of Polyphenols on the Stability of A β Fibrils

Fang Han**, Michael Zagorski, Case Western Reserve University

Background: Alzheimer's disease (AD) is characterized by the abundance of intraneuronal neurofibrillary tangles and the extracellular deposition of the A β peptide into amyloid plaques. The A β peptide is the major component of the amyloid plaques. A β peptide is an extremely sensitive system that undergoes environment dependent aggregation or oligomerization. The Met-35 side chain of A β is thought to play some critical roles because the oxidation of methionine makes the peptide less prone to aggregation.

Methods: The disruption of A β (1-42) fibrils by polyphenols will be monitored by electron microscopy (EM), thioflavin T (Th-T) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The data will be carefully analyzed to find out the most effective polyphenol to disaggregate the insoluble aggregates.

Results: My data demonstrate that Myricetin can disassemble the A β aggregates. The reason is that hydrogen peroxide is generated during the so-called autoxidation of polyphenols and then induces the methionine oxidation, and thus the disaggregation.

Conclusions: Disassembling pre-existing amyloid plaques constitutes a therapeutic approach for possible treatment of AD.

ORGANIC/PHARMACEUTICAL CHEMISTRY SESSION

Pilla Center 217

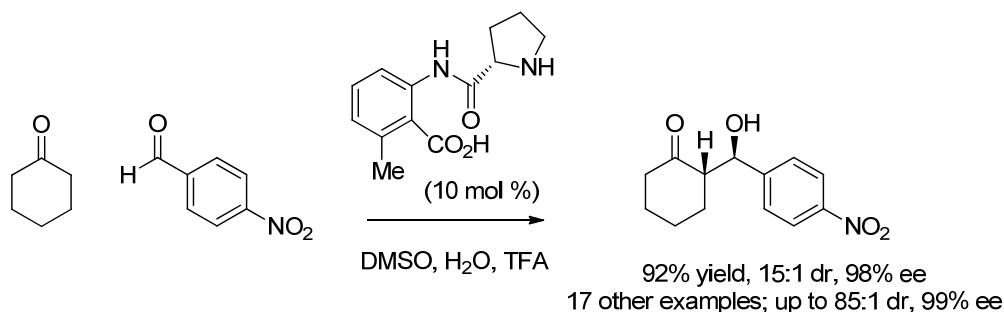
2:30 p.m.

N-Prolinylanthranilic Acid Derivatives as Bifunctional Organocatalysts for Asymmetric Aldol Reactions.

Santanu Panda** and Anthony J. Pearson, Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106

Organocatalysis is currently one of the most rapidly growing areas of organic synthesis research, and one entire recent issue of Chemical Reviews was devoted to this topic.¹ The aldol reaction is one of the most commonly applied C-C bond forming reactions to form β -hydroxy ketone compounds. Its great usefulness for building up natural products has promoted the rapid evolution of efficient chiral catalysts. The earliest example of the use of L-proline to promote asymmetric aldol reactions is the intramolecular

version published by Eder et al.² in 1971, and by Hajos and Parrish in 1974. More recently, List and co-workers introduced the use of proline as a catalyst for asymmetric intermolecular aldol reactions.³ One of the disadvantages is that it is not very soluble in many organic solvents, limiting its compatibility with many aldol substrates. Also, while it does catalyze aldol reactions in water, it affords racemic products under those conditions. Consequently, modifications of this system have been of considerable recent interest. Our own approach is to employ the secondary amine of L-proline as the functional catalytic unit and use of an anthranilic acid unit for a strong hydrogen bonding interaction. The reacting anthranilamide has two important hydrogen bonding units, one is the NH of the amide and another is the OH of the acid unit. These two hydrogen bonding units participate in hydrogen bonding in the TS resulting high diastereo and enantioselectivity. The catalyst was shown to promote enantioselective direct aldol reactions between a wide variety of aldehyde with various ketones with an excellent diastereoselectivity (85:1) and enantioselectivity up to 99%.



1. List, B., Ed. *Chem. Rev.* **2007**, *107*(12), 5413-5883.
2. Hajos, Z. G.; Parrish, D. R. *J. Org. Chem.* **1974**, *39*, 1615-1621.
3. List, B.; Lerner, R. A.; Barbas, C. F. *J. Am. Chem. Soc.* **2000**, *122*, 2395-2396.

2:50 p.m.

Eumelanin Analog Synthesis

Nathaniel Kadunce,* Jason M. Belitsky, Department of Chemistry and Biochemistry, Oberlin College, Oberlin, OH 44074

While “melanin” is a well-known biochemical entity among the general public, scientists know surprisingly little about the fundamental chemistry of melanins. Much remains to be learned about the structures of melanins, including eumelanin, the black to brown pigment in humans. Eumelanin is thought to be composed of organic nanoparticles derived from oligomers of dihydroxyindoles. We are using a variety of metal-mediated reactions to functionalize simple indole monomers and construct well-defined indole oligomers. This talk will describe our recent progress toward an indole trimer and iterative routes to indole and dihydroxyindole oligomers relevant to eumelanin structure.

3:10 p.m.

Analysis of Hydrogen Peroxide Fe(II) Mediated Linoleic Acid Peroxidation Products by GC-MS

Danielle Maholtz* and Michael A. Nichols, Department of Chemistry, John Carroll University

Currently, there are few published undergraduate biochemical experiments addressing the important issue of lipid peroxidation. Lipid peroxidation can occur through the reaction of a lipid with a reactive oxygen species such as the hydroxyl radical, which can be formed by a reaction of Fe^{2+} ions with hydrogen peroxide. When linoleic acid, an essential ω -6 fatty acid, is treated with ascorbic acid, Fe^{2+} ions and H_2O_2 , several bifunctional electrophiles can be formed as products. These include 2,4-decadienal, 4-hydroxy-2(E)-nonenal (HNE), and 4-oxo-2(E)-nonenal (ONE). The concentrations of these oxidation products can be determined using Gas Chromatograph-Mass Spectrometry (GC-MS). Currently, an analysis of the efficiency of the method using direct extraction from GC vial by ether is being performed. This method can then be used to study the effects of antioxidants such as ascorbic acid or α -tocopheryl acetate (vitamin E acetate) on the peroxidation reaction of linoleic acid in an undergraduate biochemistry course.

3:30 p.m.

Synthesis, Characterization and Use of the Ligand PQAM in Copper Analysis

Meaghan Brown* and Michael A. Nichols, Department of Chemistry, John Carroll University

The ligand PQAM (2-Pyridyl-N,N-bis[(8-quinolyl)amino]methane) has been shown to react with copper chloride to yield a new paramagnetic copper complex, CuCl_2 -PMAQ (8-[(2-Pyridine)methylideneamino]quinoline). The research has five main goals: (1) to optimize the synthesis and purification of PQAM; (2) fully structurally characterize PQAM using infrared (IR) and nuclear magnetic resonance (NMR) spectroscopies; (3) to optimize the synthesis of and fully characterize the structure (using ultraviolet-visible (UV-Vis) and fluorescence spectroscopies) and measure the magnetic susceptibility (in solution using NMR and in the solid state) of the CuCl_2 -PMAQ complex; (4) investigate whether the CuCl_2 -PMAQ complex can be prepared by a direct metal-assisted condensation of CuCl_2 and the reactants that form PQAM, 8-aminoquinoline and 2-pyridine carboxaldehyde; and (5) to develop a spectrofluorimetric assay to measure the concentration of copper in aqueous solutions using the synthesized PQAM.

3:50 p.m.

Reducing Sugar Reactions with Oxidants: Stoichiometric Evidence that is Inconsistent with Aldehyde Oxidation

Melanie Malinas*, Simone Koster, Naomi Onsongo, Inyang Udo-Inyang, and William Fuchsman, Dept. of Chemistry & Biochemistry, Oberlin College, Oberlin OH 44074

A long-established hypothesis about the nature of reducing sugars is that during reducing sugar assays, the aldehyde forms of reducing sugars oxidize to carboxylate compounds. Using the quantitative dinitrosalicylate (DNS) assay, we have found that (1) effects of reducing sugar structures on the assay's sensitivity appear inconsistent with aldehyde oxidation, (2) there are aldehydes that do not behave like reducing sugars, and (3) there is at least one ketone that does behave like a reducing sugar even though it cannot rearrange into an aldehyde. In order to examine the possibility that structural effects upon reduction potentials could account for most of our findings, we have used two methods to determine the optimum reducing sugar to DNS ratios for different reducing sugars. The ratios, which likely represent approximate stoichiometries for very complicated reactions, are inconsistent with the aldehyde oxidation hypothesis. We offer an alternative hypothesis for reducing sugar oxidations that is consistent with our evidence thus far.

4:10 p.m.

Biomimetic Functionalization of Magnetic Nanoparticles

Pratima Vabbilisetty** and Xue-Long Sun, Department of Chemistry, Cleveland State University

Nanoparticles such as magnetic nanoparticles have been showing great potential to revolutionize biological imaging and drug delivery, but their use has been limited by difficulties in obtaining nanoparticles that are biocompatible. To address this problem, surface modification of nanoparticles has been widely explored. Among them, lipid coating, micelle encapsulation and liposome encapsulation have been demonstrated to enhance their compatibility with the biological milieu *in vitro* and *in vivo*. In this report, we describe a biomimetic functionalization of magnetic nanoparticle, namely, carbohydrate/lipid-coated magnetic nanoparticle hybrid systems for improved targeted drug delivery and imaging application. Briefly, magnetic nanoparticles were synthesized by the thermal-decomposition of iron oleates in high-boiling solvents containing surfactants in order to obtain surfactant-coated monodisperse nanoparticles. Lipid-coated magnetic nanoparticles were prepared by standard thin-film hydration method and sonication process. Chemically selective glyco-functionalization of lipid-coated magnetic nanoparticles was conducted *via* Staudinger ligation. The structural characteristics of the nanoparticles were confirmed by IR, TEM and DLS techniques.

4:30 p.m.

Fabrication of Molecularly Controlled Glyco-Macroligand Array

Satya Nandana Narla** and Xue-Long Sun, Department of chemistry, Cleveland State University, Cleveland, OH 44115

An oriented and density controlled glyco-macroligand array formation was demonstrated by immobilizing O-cyanate chain-end functionalized glycopolymer imprinted with boronic acid ligands of different sizes. Phenylboronic acid ligands of different sizes such as phenylboronic acid-polyacrylamide, lysozyme and BSA conjugates were synthesized, with which the O-cyanate chain-end functionalized glycopolymer was pre-modified and then imprinted onto amine functionalized glass slide *via* isourea bond formation followed by releasing of phenylboronic acid ligands. The protein (lectin) binding capacities of the non-imprinted and imprinted glycopolymer were evaluated by glycoarray and SPR techniques, where both the techniques showed same trend of affinity to lectin. This glycoarray platform presents sugar chains in defined orientation and density configurations. It is, thus, uniquely useful tool for exploration of glycan-binding protein interactions.

PHYSICAL/MATERIALS CHEMISTRY

Pilla Center 239

2:30 p.m.

Determination of Chemical Shift Anisotropy Orientation in Tripeptides Using Rotational Echo Double Resonance NMR

Hannah A. Fuson,^{1,*} Anil K. Mehta,² and Manish A. Mehta¹

1. Oberlin College, Department of Chemistry and Biochemistry, Oberlin, OH 44074

2. Emory University, Department of Chemistry, Atlanta, GA 30322

Valuable information about the secondary structure of biological molecules is contained in the chemical shift anisotropy (CSA) and its orientation in the molecular frame. Studying molecules in the solid state makes this CSA information accessible. Rotational-echo double-resonance (REDOR) solid-state NMR experiments are common for measuring distances between heteronuclei through measurement of the internuclear dipolar coupling. However, further information about the orientation of the internuclear dipole vector can be extracted from these experiments by examining the dephasing across the span of the CSA. We report a study of a series of tripeptides, spanning a range of secondary geometries, where we determine the orientation of carbonyl ¹³C shift anisotropy in the molecular frame using REDOR. In conjunction with known crystal structures, we can use the CSA to learn more about the hydrogen-bonding patterns in these molecules.

2:50 p.m.

Selection of Metallic Single-Walled Carbon Nanotube with Specific Chirality by Using PPE-PPV Copolymer

Yusheng Chen,^{**} Wenhui Yi, Yi Pang, Department of Chemistry, University of Akron, OH 44325

Single-walled carbon nanotubes (SWNT) are graphite sheets rolled along a certain vector defined by the chiral indices (n,m). The superior electronic and mechanical properties of SWNTs have led to their various application, including flexible electronics, biosensors, and transistor. The electronic structure and optical properties of individual SWNT are uniquely dependent on the chiral indices (n,m) which separates the tubes into metallic and semiconducting forms.

Conjugated polymers are consisting of a sequence of sp² – hybridized carbon. Their structural similarity to SWNT and capability to form an ordered assembly offers an attractive option to achieve selective interaction with SWNTs.

Highly pure metallic (12,6) SWNT can be obtained by sequential treatment of HiPco sample with nitric acid and a conjugated copolymer PPE-PPV. The polymer with alternating phenyleneethynylene (PE) and phenylenevinylene (PV) segments can not only effectively dispersed carbon nanotubes, but also exhibited a remarkable selectivity to differentiate metallic (12,6) and (8,8) tubes.

3:10 p.m.

Ultrafast Dynamics in Nitro- and (Organophosphine)gold(I) Polycyclic Aromatic Hydrocarbons

R. Aaron Vogt,** Christian Reichardt, Carlos E. Crespo-Hernandez, Thomas G. Gray, Department of Chemistry and Center for Chemical Dynamics, Case Western Reserve University, Cleveland, Ohio 44106, USA

Femtosecond UV/Vis broadband transient absorption was used to investigate the excited-state dynamics in nitronaphthalenes and (organophosphine)gold(I) naphthalenes. Both groups of compounds display similar dynamics. Following excitation with UV light the compounds undergo fast and efficient intersystem crossing to the triplet manifold with a lifetime between 0.1 and 0.3 ps. Once in the triplet manifold, internal conversion occurs from the receiver triplet state to the lowest-energy triplet state (T_1). Vibrational cooling in the T_1 state then proceeds with a lifetime between 3 and 11 ps, which depends on the substituent on the naphthalene moiety. Quantum chemical calculations including solvent effects are used to substantiate the interpretation of the experimental results. Comparisons between the naphthalene derivatives and the previously studied pyrene-containing derivatives^{1,2} will be discussed.

(1) Crespo-Hernández, C. E.; Burdzinski, G.; Arce, R. *J. Phys. Chem. A* **2008**, *112*, 6313.

(2) Vogt, R. A.; Peay, M. A.; Gray, T. G.; Crespo-Hernández, C. E. *J. Phys. Chem. Lett.* **2010**, *1*, 1205.

3:30 p.m.

Infrared Spectroscopy of Trapped Hydrogen in Metal-Organic Frameworks

J. Schloss,* C. Pierce, B. Thompson, and S. FitzGerald, Oberlin College Department of Physics and Astronomy; J. Rowsell, Oberlin College Department of Chemistry and Bio-Chemistry

Hydrogen gas shows promise as an alternative fuel, although its storage for use in vehicles presents challenges. A new storage method involves trapping hydrogen in highly porous materials called metal-organic frameworks (MOFs). Unfortunately, MOFs bind hydrogen too weakly to be practical hosts at room temperature; thus there is much research aimed at enhancing the hydrogen-binding mechanism within MOFs. We have been using infrared spectroscopy to investigate the dynamics of trapped hydrogen. Specifically, we have studied the spectra of H_2 in an isostructural series of materials called M-MOF-74, with $M=Co, Mg, Mn, Ni,$ or Zn , at a range of temperatures and pressures. The intensities and redshifts of the H_2 vibrational peaks tell us about the nature and strength of binding to the MOF, which may help in the construction of hydrogen-storage materials that are practical for use at room temperature.

3:50 p.m.

Excited-State Dynamics in 6-Thioguanosine from Femtosecond to Microsecond Time Scale

Cao Guo,** Christian Reichardt and Carlos E. Crespo-Hernández, Department of Chemistry and the Center for Chemical Dynamics, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, Ohio 44106

6-Thioguanine is a widely used pro-drug¹ in which the oxygen atom in the carbonyl group of guanine is replaced by a sulfur atom. Previous studies have shown that patients treated with 6-thioguanine can metabolize and incorporate it in DNA as 6-thioguanosine (6tGuo). These patients show a high incidence of skin cancer when they are exposed to extended periods of sunlight irradiation. In this work, the photodynamics of 6tGuo is investigated by broad band time resolved transient spectroscopy.² Similar to previously studied 4-thiothymidine,^{3,4} our results show that excitation of 6tGuo with UVA light at 340 nm results in efficient ultrafast intersystem crossing to the triplet manifold ($\tau = 0.31 \pm 0.05$ ps) and a high triplet quantum yield ($\phi = 0.8 \pm 0.2$). The triplet state has a lifetime of 720 ± 10 ns in N₂-saturated vs. 460 ± 10 ns in air-saturated aqueous solution. In addition, a minor picosecond deactivation channel (80 ± 15 ps) is observed, which is tentatively assigned to internal conversion from the lowest-energy excited singlet state to the ground state. Quantum chemical calculations support the proposed kinetic model. Based on the high triplet quantum yield measured, it is proposed that the phototoxicity of 6tGuo is due to its ability to photosensitized singlet oxygen, which can result in oxidative damage to DNA.

¹ P. O'Donovan, C. M. Perrett, X. Zhang, B. Montaner, Y.-Z. Xu, C. A. Harwood, J. M. McGregor, S. L. Walker, F. Hanaoka, P. Karran, *Science* **2005**, 309, 1871.

² C. Reichardt, C. Guo, C. E. Crespo-Hernández, *J. Phys. Chem. B.* **2011**, *in press*.

³ C. Reichardt, C. E. Crespo-Hernández, *J. Phys. Chem. Lett.* **2010**, 1, 2239;

Reichardt, C. E. Crespo-Hernández, *Chem. Comm.* **2010**, 46, 5963.

⁴ C.

4:10 p.m.

Excited State Dynamics in 2-Aminopurine Ribonucleoside: From Femtosecond to Microsecond Time Scale

Chengwei Wen**, Christian Reichardt, Carlos E. Crespo-Hernández, Department of Chemistry and Center for Chemical Dynamics, Case Western Reserve University, 10900 Euclid Ave., Cleveland, Ohio 44106

2-Aminopurine is a structural isomer of adenine that is widely used as fluorescence probe in DNA because its fluorescence lifetime and quantum yield respond sensitively to local DNA duplex conformation and neighboring microenvironment. Despite its broad utilization in biochemical and biophysical studies, there is ongoing debate about the mechanism by which fluorescence is quenched in 2-aminopurine in different solvents. Establishing the excited-state relaxation pathways that lead to fluorescence quenching is essential for a meaningful interpretation of the experimental data in terms of local structure and dynamics. In this contribution, the excited state dynamics in 2-aminopurine ribonucleoside is investigated by using broad-band transient absorption spectroscopy in different solvents. Excitation is performed at 320 nm and the spectral evolution is monitored in the spectral region from 330 nm to 700 nm. Three exponential functions are required to fit globally the transient absorption signals from femtosecond to microsecond time scale. The first lifetime is assigned to solvent relaxation dynamics in the excited singlet state. The singlet state population then bifurcates to two different decay channels. The first channel is the well-documented fluorescence emission while the second channel is assigned to intersystem crossing to the triplet manifold. The extent of participation of this nonradiative decay channel depends sensitively on the polarity and hydrogen bonding ability of the solvent. The triplet state population decays back to the ground state in

hundreds of nanoseconds and is quenched by molecular oxygen. Our results unequivocally show that the excited-state dynamics in 2-aminopurine is more complex than previously thought.

4:30 p.m.

Functionalization of Cellulose Whiskers with Thermo-responsive Polymers via Reversible Addition Fragmentation Chain Transfer (RAFT) Polymerization

Amanda E. Way,** Lorraine Hsu, Christoph Weder, Stuart J. Rowan

Department of Macromolecular Science & Engineering, Case Western Reserve University

The use of cellulose whiskers as bio-inspired nanocomposite fillers has proved to be of great interest over the last several years. As fillers, the cellulose whiskers have been used to dynamically alter the modulus of nanocomposites, utilizing the reversible on/off whisker self-interactions triggered by addition or removal of a hydrogen-bonding solvent. In this work, utilizing thermo-responsive whiskers to alter the modulus of nanocomposites was investigated. Cellulose whiskers were harvested from the mantles of tunicates and subjected to oxidation and peptide coupling reactions, followed by RAFT polymerization to obtain thermo-responsive poly(ethylene glycol)monomethyl ether acrylate grafted whiskers. Kinetic studies indicate that the PEGA polymers grow with control from the surface of the whiskers. The solution properties of these thermo-responsive surface whiskers were investigated, as well as the thermal switching behavior of the whisker fillers in PVAC nanocomposites.

INORGANIC/MATERIALS CHEMISTRY

Pilla Center 228

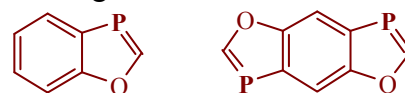
2:30 p.m.

Novel Organophosphorus Electroluminescent Materials

Shanshan Wu,** John D. Protasiewicz, Department of Chemistry, Case Western Reserve University

Low-coordinated organophosphorus compounds featuring P=C (phosphaalkene) and P=P (diphosphene) units have got intensive study in recent years due to their unique optoelectronic properties. These π -conjugated materials have great potential application in electronic device, OLEDs (organic light emitting diodes) ^[1].

Benzoxaphospholes (BOPs) is a class of heterocycles which a benzene ring fused to a phosphorus and oxygen containing five membered ring. And its extended materials benzobisoxaphospholes (BBOPs) are developed successfully by our group (Figure 1). Recent paper reported that benzoxaphospholes and benzobisoxaphospholes shown remarkable luminescent properties ^[2]. In order to optimize their luminescent properties for potential application in OLED, we are interested in preparing soluble polymers containing BOP or BBOP units.



Benzoxaphosphole Benzobisoxaphosphole

Figure 1. Structure of BOP and BBOP.

References:

[1] Baumgartner, T.; Regis, R. *Chem. Rev.* 2006, *106*, 4681-4727. Wright, V. A.; Gates, D. P., *Angew. Chem. Int. Ed.* 2002, *41*, 2389-2392. Smith, R. C.; Chen, X.; Protasiewicz, J. D. *Inorg. Chem.* 2003, *42*, 5468-5470. Smith, R. C.; Protasiewicz, J. D. *J. Am. Chem. Soc.* 2004, *126*, 2268-2269.

[2] Heinicke, J.; Tzschach, A. *Phosphorus Sulfur Relat. Elem.* 1985, *25*, 345. Washington, M. P.; Gudimetla, V. B.; Laughlin, F. L.; Deligonul, N.; He, S.; Payton, J. L.; Simpson, M. C.; Protasiewicz, J. D. *J. Am. Chem. Soc.* 2010, *132*, 4566-4567.

2:50 p.m.

Conjugated Materials with Phosphorus

Feng L. Laughlin,** Marlena P. Washington, Vittal B. Gudimetla, John D. Protasiewicz,
Chemistry Department, Case Western Reserve University

A series of new conjugated materials with phosphorus-benzoxaphospholes (BOP), benzobisoxaphospholes (BBOP) and naphthoxaphospholes (NOP) were synthesized. Compared to diphosphene and phosphalkene, these new materials retain the P=C bond(s) and fluorescence. They are further more stable and planar thus they have better conjugation ability.

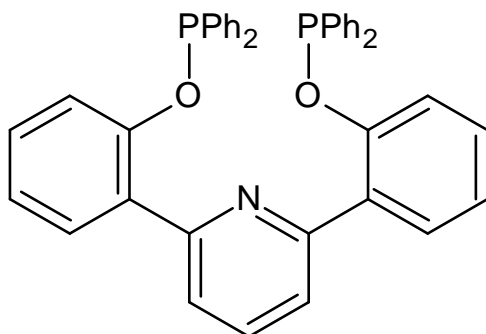
This presentation will discuss the synthesis and characterization of BBOP and NOP including their: NMR spectra, X-ray crystal structures, photoluminescence, and cyclic voltammetry.

3:10 p.m.

Synthesis Toward A Novel Diphosphinite 2,6-Diphenylpyridine Analog of *m*-Terphenyl Pincer Ligands.

Erin Childers,* Ursuline College; Dr. Sarah Preston, Ursuline College; and Dr. John Protasiewicz, Case Western Reserve University

A novel PNP pincer ligand (see below) will be synthesized beginning with a Suzuki Coupling resulting in 2,6-bis-*o*-methoxyphenylpyridine, followed by demethylation promoted by pyridine HCl, then, reaction with chlorodiphenylphosphine will give the final diphosphinite ligand. The ligand will be coordinated to a variety of late transition metals and screened for catalytic activity. Ligands are a class of compounds used to stabilize metals in low oxidation states and come in a variety of sizes, shapes and charges. The pincer ligand class has garnered much attention due to its high thermal stability and bulky substituents. Unlike the more studied pincer ligand, *m*-xylyl, meta-terphenyls are neutral donors, allowing for metals to retain lower oxidation states which are necessary for catalysis. The proposed ligand will replace the central binding carbon atom with a nitrogen atom which also creates a neutral ligand. The determination of catalytic activity, thermal stability, and the geometry around the metal will be determined and compared to the meta-terphenyl class of ligands as well as the *m*-xylyl class. This new pincer ligand shows promise as an active catalyst for use in organic synthesis.



3:30 p.m.

Synthesis and Reversible Dehydration of Basic Lead Carboxylates

Clay C. Easterday*, Gabrielle White-Dzuro, Catherine M. Oertel, Department of Chemistry and Biochemistry, 119 Woodland St., Oberlin College, Oberlin, OH 44074

Basic lead carboxylates are produced during the corrosion of lead and lead alloys in historical objects. These compounds can also be viewed as metal organic frameworks (MOFs), which have been heavily researched for their high porosity and catalytic activity. Previous work in our laboratory has elucidated the structures of two of these corrosion products, basic lead acetate $[\text{Pb}_3\text{O}_2(\text{CH}_3\text{COO})_2 \cdot 0.5\text{H}_2\text{O}]$ and basic lead formate $[\text{Pb}_2\text{O}(\text{CHOO})_2]$. Our current interest is in basic lead acetate (BLA), which is able to lose and regain its water of hydration without loss of crystallinity. By contrast, many MOFs show collapse of the open pore network upon exposure to moisture. We are studying this reversible exchange of water with the environment using thermogravimetric analysis, variable-temperature powder X-ray diffraction, and polarized light microscopy. Further, we are synthesizing and characterizing new basic lead carboxylates for an expanded study of hydration within this largely unstudied class of compounds.

3:50 p.m.

Guest-Dependent Optical Properties of Cobalt MOF-74 and its Solid Solutions

Matt Chaves* and Jesse Rowsell, Oberlin College Department of Chemistry and Biochemistry

Metal-organic frameworks are currently receiving an enormous amount of attention for their potential applications in the fields of renewable energy and greenhouse gas sequestration. Highly porous structures are readily synthesized, some displaying record-breaking pore volumes that have been confirmed experimentally through gas adsorption measurements. It has been proposed that such materials could also be employed in small molecule sensors, but such applications are complicated by a lack of materials demonstrating electronic, optical or magnetic responses to guest molecules.

We examine evidence for semiconductivity in a cobalt(II) carboxylate framework (MOF-74) that is sensitive to adsorbed water. This material is one of a family of isostructural frameworks, in which none of the other members display this sensitivity. We hypothesize that synthesizing solid solutions (i.e. mixed metal frameworks) will allow for tunable band gaps, allowing control of the material's response. Solvothermal methods for preparing Mg-Co, Zn-Co, and Ni-Co frameworks are tested, and resulting products are desolvated under vacuum. The as-

synthesized and solvent-free materials are characterized by a battery of techniques, including powder X-ray diffraction, infrared and visible spectroscopy, thermogravimetric analysis, and atomic absorption spectroscopy. UV-Vis-NIR diffuse reflectance spectrophotometry is used to investigate the materials' optical response to varying guest molecules.

4:10 p.m.

Hybrid Lithium Oxalate-Phosphinate Salts

Andrew R. Shaffer, Nihal Deligonul, Daniel A. Scherson, and John D. Protasiewicz
Case Western Reserve University, Cleveland, OH

Safety concerns have limited the widespread implementation of lithium ion batteries into the automotive industry. Among the major hurdles to be overcome is the danger associated with the flammability of the organic solvents. As such, the development of flame-retardant additives, aimed at preventing catastrophic device failure, has been at the forefront of many researchers.

In hopes to combine the electrolyte functionality and flame retardant properties, rationally designed Flame Retardant Ions (FR Ions) were targeted as a possible solution. Recently, several novel organophosphorus containing lithium salts, $\text{Li}[(\text{C}_2\text{O}_4)\text{B}(\text{O}_2\text{PAr}_2)_2]$ were synthesized and characterized using a variety of spectroscopic techniques as a initial leads. These compounds display high air and water stability and are also thermally robust, properties needed of electrolytes for possible use as electrolytes and/or additives in Li ion battery applications.

4:30 p.m.

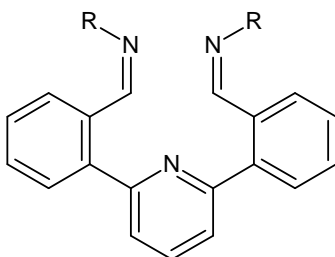
Synthesis Toward A Novel 2,6-Diphenylpyridine Analog of *m*-Terphenyl Pincer Ligands

Elizabeth Gadowski*, Sarah Preston, Ursuline College; John Protasiewicz, Case Western Reserve University

A novel NNN diimine pincer ligand will be synthesized by the Suzuki coupling of 2-formylphenylboronic acid and 2,6-dibromopyridine followed by reaction with an amine. The ligand will be coordinated to a variety of late transition metals, such as Ru, and screened for catalytic activity.

Ligands are a class of compounds used to stabilize metals in low oxidation states and come in a variety of sizes, shapes and charges. The pincer ligand class has garnered much attention due to its high thermal stability and bulky substituents. Unlike the most studied pincer ligand, *m*-xylyl, meta-terphenyls are neutral donors, allowing for metals to retain lower oxidation states which are necessary for catalysis. The proposed ligand will replace the central binding carbon atom with a nitrogen atom which also creates a neutral ligand.

The catalytic activity, thermal stability, and geometry around the metal will be determined and compared to the meta-terphenyl class of ligands as well as the *m*-xylyl class. The use of chiral amines allows for the possibility of asymmetric catalysis. This new pincer ligand shows promise as an active catalyst in pharmaceutical synthesis.



4:50 p.m.

Synthesis, Characterization, and Antimicrobial Application of Trinuclear Copper (II) Complexes of Oxalic Dihydrazone Schiff-base

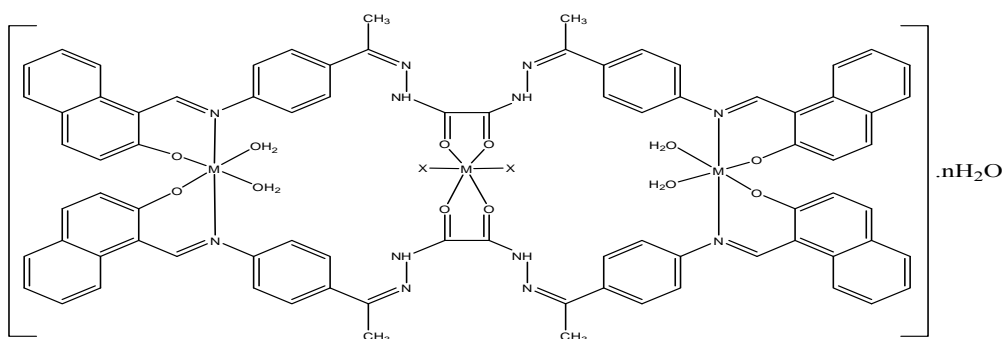
Haitham F. Kalil,^{1,*}, Shehab A. Sallam,¹ Adel S. Orabi,¹ Mekki Bayachou²

¹Department of Chemistry, Faculty of Science, Suez Canal University, Ismailia, Egypt

²Department of Chemistry, Science & Research Center, Cleveland State University, Ohio, USA

Schiff-base was synthesized by the reaction of oxalic dihydrazone with 4-aminoacetophenone; the reaction product was condensed with naphthaldehyde in EtOH. The purified ligand was characterized by elemental analysis, differential scanning calorimetry (DSC), gas chromatography coupled to mass spectrometry (GC-MS), proton NMR, as well as UV and IR spectroscopy.

Complexes of various copper–II salts and the ligand synthesized were prepared. The reaction between the different copper (II) salts and the synthesized ligand was in 3:2 molar ratio to form the complexes of general composition $[\text{Cu}_3(\text{H}_2\text{L})_2\text{X}_2(\text{H}_2\text{O})_4] \cdot n\text{H}_2\text{O}$ where $\text{X} = \text{NO}_3^-$, Cl^- or CH_3COO^- while $n = 2, 6$ and 7 respectively, Scheme 1.



Scheme 1. Proposed structure of the Cu-H₄L Schiff-base complexes

The Cu(II)-complexes were air-stable solid powders, and soluble in DMF and DMSO. We characterized the complexes by elemental analysis, conductivity measurements, IR, UV-Vis., electron spin resonance (ESR) spectroscopy, and magnetic moment measurements. The ligand showed deprotonation of its phenolic protons during complexation. Absorbance and ESR spectroscopy confirmed that the complexes adopt octahedral structures. Magnetic moment data indicate anti-ferromagnetic interaction between metallic centers. Thermogravimetric and differential thermal analyses (TGA/DTA) confirmed the proposed chemical formulation of the complexes and their thermal decomposition and thermodynamic parameters were evaluated. The molar conductance values of these complexes indicated that the non-electrolytic nature of the complexes.

The complexes illustrated similarity in structure, color, spectroscopic analyses and thermal behavior, which rules out a major role of the effect of the nature of the anion in the coordination sphere of the metal ion. However, the nature of the anion was crucial in the antimicrobial activity; in fact, only the copper nitrate complex showed high activity against pathogenic fungi and also a slight efficacy against both gram positive and negative bacteria.

ANALYTICAL/PHARMACEUTICAL CHEMISTRY

Pilla Center 215 (until 3:50; then Pilla Center 238)

2:30 p.m.

Nitric Oxide Synthase (NOS) immobilized in electrospun fibers: towards novel nitric oxide (NO) release membranes

Bhagya Gunasekera**, Mekki Bayachou, Chemistry Department, Cleveland State University, Cleveland, OH 44115

Background

Nitric oxide is a molecule known to counteract platelet aggregation, and thus can stop the thrombosis cascade on the surface of blood-contacting medical implants. Nitric oxide synthases are enzymes (NOSs) responsible for catalytic conversion of the substrates L-arginine to NO and L-citrulline. By using NO releasing biomaterial in their closest native characteristics to mammalian tissue, one may be able to solve the issue of thrombosis and restenosis on the surface of foreign devices implanted or used as part of cardiovascular procedures. In the past we have tested the use of NOS enzymes in layer-by-layer thin films as source of *in-situ* NO synthesis and release. Our objective in the current work is to use NOS enzymes trapped in electrospun fiber matrices as biocompatible platform for NO release.

Methods

In this project, we investigate embedding of nitric oxide synthase (NOS) as a functional component contained in aqueous pockets of electrospun biopolymer matrices; namely, polycaprolactone (PCL) and Polyurethane (PU). A guided stream of polymer solution containing suspended aqueous pockets of enzyme solution is directed towards a collector drum in strong electric field. In its path of acceleration towards the target, the solvent evaporates and the charged jet thins-out leaving a fibrous membrane, devoid of solvent and containing 'nodes' of aqueous pockets with entrapped NOS enzymes.

Surface characterizations such as Transmission Electron Microscopic (TEM) and Atomic Force Microscopic (AFM) imaging are carried out on the newly formed NOS-containing electrospun fibers. Further, the NOS-modified membranes are tested electrochemically using a characteristic electrocatalytic reaction mediated by entrapped NOS enzymes. Finally, the NOS-containing electrospun membranes are subject to assays under various conditions to determine the structural integrity of NOS enzymes and their enzymatic activity.

Major results

Morphology of the NOS containing nodes in individual microfibers imaged at different stages of electrospinning shows evidence of success of spin-trap process. Griess assay and hemoglobin deoxy assay shows quantitative release of NO from the NOS-modified fibers under physiologic conditions. This confirms electrochemical characterization using the NOS-mediated catalytic reduction reaction of exogenous NO of the same fibers.

Conclusion:

Together, these results show that the native structure of the entrapped NOS in the aqueous pockets is conserved and is functional under physiologic conditions.

2:50 p.m.

Development and Optimization of an HPLC-UV Method for the Measurement of Triapine

Ye Feng,** Yan Xu, Department of Chemistry, Cleveland State University, 2121 Euclid Avenue, Cleveland, Ohio 44115

Triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone, or 3-AP) is a novel anticancer drug which belongs to a family of drugs known as ribonucleotide reductase (RNR) inhibitor. Due to its chelating effect, triapine can remove iron (III) from the catalytic di-iron-tyrosyl radical center of RNR. Preclinical study showed that triapine significantly decreased RNR activity and enhanced radiation-mediated cytotoxicity in cervical and colon cancer cells. These preliminary findings warrant further clinical study of this compound. Therefore, a sensitive analytical method is critically needed to support pharmacological study of triapine.

The major challenge in the development of a quantitative method for the measurement of triapine has been the nature of triapine as metal chelator. In this work, we have studied the complexation reactions of triapine with Fe (III) under various conditions. Our data showed that the reaction pH, buffer composition, temperature, and other coexisting chelators have effects on the complexation reactions. Based on the optimization of these influencing factors, we have developed a quantitative HPLC-UV method for the determination of triapine. In this method, hydrazinecarbothioamide {2-[(3-methoxy-2-pyridinyl)]-methylene} was used as internal standard. Triapine and the internal standard are separated on a Waters Xterra RP18 column (2.1 x 150 mm, 5 μ m particle size), and detected at the wavelength of 365 nm by photodiode array detector. The mobile phase for chromatographic separation consists of 18.0% acetonitrile and 82.0% EDTA-ammonium bicarbonate buffer [5 mM EDTA and 25 mM NH_4HCO_3 , pH 8.5] (v/v). This method has a linear calibration range of 15.6-5200 nM for triapine with correlation coefficient of 1.00. The lower limit of quantification (LLOQ) of the method was 15.6 nM. Our work shows that triapine can be measured accurately and reliably if the influencing factors are under controls. This method may be useful for preclinical and clinical studies of triapine.

3:10 p.m.

Glyco-Engineering of Recombinant Thrombomodulin

Valentinas Gruzdyś* and Xue-Long Sun, Department of Chemistry, Cleveland State University

Thrombomodulin (TM) is an endothelial membrane protein and plays a critical role in local haemostasis by binding thrombin and subsequently converting protein C to its active form (APC), which is an anticoagulant protease that selectively inactivates coagulation factors Va and VIIIa. We propose a recombinant TM-glycan conjugate as potential antithrombotic agent. Glycoengineering aimed adding glycans to proteins to alter their pharmacokinetic properties such as increasing *in vivo* activity and prolonging the duration of action. Particularly, covalent attachment of synthetic macromolecules is an effective way to improve protein stability with reduced immunogenicity and extended plasma half-lives. In addition, secondary property can be introduced, such as protein targeted delivery. In this presentation, expression and purification of

recombinant thrombomodulin, and synthesis of glycopolymer, a glycan-containing polymer and its protein modification will be described. Specifically, a chain end-functionalized glycopolymer for well-defined protein-glycopolymer conjugation is reported.

3:30 p.m.

Quantitative Analysis of Benzyl Isothiocyanate, a Dietary Anticancer Agent, in Mouse Plasma by LC-MS/MS

Simuli L. Wabuyele,** Yan Xu, Department of Chemistry, Cleveland University, 2121 Euclid Avenue, Cleveland, Ohio 44115

Benzyl isothiocyanate (BITC) is a naturally occurring compound in a variety of cruciferous vegetables as glucotropaeolin (benzyl glucosinolate), which is enzymatically released upon dietary intake/consumption. BITC has been shown to have chemopreventative properties against various types of cancers, including pancreatic, lung and breast. To determine the bioavailability and pharmacokinetic properties of this compound, a sensitive and selective analytical method is critically needed. In this work, a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for quantitative measurement of BITC in mouse plasma has been developed. BITC was extracted from plasma using hexane as the organic solvent and the derivatization reaction with ammonia to benzylthiourea was optimized. Isocratic baseline separation of BITC and internal standard (methoxy benzylthiourea) was achieved on a Waters Phenyl C18 column. Positive electrospray ionization (ESI⁺) and multiple-reaction-monitoring (MRM) modes were used for quantification with mass transitions m/z 167 → 91 and m/z 197 → 121 for benzylthiourea and the internal standard, respectively. The linear calibration range and lower limit of quantification (LLOQ) for BITC have been established. The developed method will be validated in mouse plasma for pharmacological studies of the compound.

****Move to Pilla Center 238****

3:50 p.m.

Measurement of Intracellular Nitric Oxide in Human Sperm Using Flow Cytometry

Saleem Bani Hani ^{**1}, Ashok Agarwal², Rakesh Sharma², Mekki Bayachou^{1*}

1. Department of Chemistry, Cleveland State University, 2399 Euclid Ave, Cleveland, OH 44115

2. Center for Reproductive Medicine, Cleveland Clinic, 9500 Euclid Ave, Cleveland, Ohio 44195

Nitric oxide is a biological molecule synthesized by a family of enzymes called Nitric Oxide Synthases (NOSs). It is involved in a myriad of biological processes such as vasodilation, neurotransmission, and host defense, as well as in an increasing list of pathological states. Studies showed that nitric oxide (NO) has a major contribution in the function of male reproduction system. NO's mechanisms of action in sperm remain unclear; one important reason is that methods for direct NO-single cell measurements are complex, insensitive, and non-specific. Here we demonstrated a method to measure intracellular NO in human sperm by means of flow cytometry using NO-specific probe 4,5-diaminofluorescein-2-diacetate (DAF-2DA). Freshly motile sperm were prepared using a modified direct swim-up procedure. Both sperm counts and DAF-2DA concentrations were standardized using flow cytometry. Optimum

fluorescence was reached when sperm were incubated with DAF-2DA for 2 hours at 37°C. Sperm samples incubated with L-Nitro arginine methyl ester (L-NAME), a Nitric oxide synthase inhibitor, showed lower fluorescence in a dose-dependent manner. Sperm samples supplemented with Diethylamine NONOate (DEA NONOate), a nitric oxide donor, showed higher fluorescence compared to the control. Application of this method to the determination of NO in sperm sample from patients showing low sperm motility will allow us to shed light on the role of NOS activity and NO release in sperm function.

4:10 p.m.

Post-Mortem Analysis of Illicit Drugs BZP and TFMPP by LC-MS/MS

Kerri M. Smith ^{**}, Szabolcs Sofalvi ^b, Eric S. Lavins ^b, Frank P. Miller III ^b, Yan Xu ^{a,*}

^a Department of Chemistry, Cleveland State University, 2121 Euclid Avenue, Cleveland, Ohio 44115

^b Cuyahoga County Coroner's Office, 11001 Cedar Avenue, Cleveland, Ohio 44106

1-Benzylpiperazine (BZP) and 1-(3-trifluoromethylphenylpiperazine) (TFMPP) are designer drugs of abuse that synergistically mimic the effects of 3,4-methylenedioxymethamphetamine (MDMA or Ecstasy) in human. Distributed through the internet as powder-filled capsules or pressed tablets, these drugs are often marketed as an alternative to Ecstasy. In 2002, the US Drug Enforcement Administration placed BZP on emergency Schedule I status, making its possession illegal. BZP has a high adverse effect profile and can be lethal when ingested together with MDMA. Due to a rising prevalence of BZP and TFMPP, a sensitive method for their detection and quantification in biological matrices is necessary. This work describes for the first time an isocratic liquid chromatography tandem mass spectrometric method for the quantitative determination of BZP and TFMPP in hemolyzed post-mortem human blood using a simple sample preparation procedure with internal standard. The method has linear calibration ranges from 0.500 to 250 ng/mL for BZP and 0.500 to 500 ng/mL for TFMPP with recoveries greater than 87%. This method is adoptable for the analysis of phenylpiperazine derivatives.

4:30 p.m.

Site-Specific Modification of Recombinant Thrombomodulin

Rui Jiang and Xue-Long Sun, Department of Chemistry, Cleveland State University, Cleveland, OH 44115

Thrombomodulin (TM) is an endothelial cell membrane protein and is a critical regulator of the protein C pathway and represents a major anticoagulant mechanism that is operative under physiological conditions. The extracellular region of human TM consists of three tentative domains, an *N*-terminal domain, a domain involving six continuous epidermal growth factor (EGF1-6)-like structures, and an *O*-glycosylation-rich domain. Previous studies have shown a TM fragment containing last three EGF-like structures (TM456) is the minimum functional domain for protein C activating cofactor activity and recombinant TM456 provides as potential antithrombotic agents. In order to modify protein in a controlled way, new functionalities need to be introduced in a defined manner. In this report, we presented a strategy for enzymatic site-specific modification of a recombinant TM456 derivative at the *C*-terminus, which contains EGF-like domains 4-6 and a conserved pentapeptide motif (Leu-Pro-X-Thr-Gly, X represents any residue) as a *C*-terminal linker for *Staphylococcus aureus* Sortase A catalyzed ligation with

N-terminal glycine-containing molecules. The recombinant TM456 fusion protein was synthesized in *Escherichia coli*. Two kinds of molecular probes were conjugated to the *C*-terminal of TM456 *via* Sortase-catalyzed ligation, respectively. The recombinant TM and its conjugates were confirmed with SDS-PAGE and fluorescent imaging techniques. This protein engineering technique permits the unique attachment site on the protein for numerous applications such as immobilization and polymer conjugation.

The Cleveland Section of the American Chemical Society would like to thank the following for generously contributing funds for the undergraduate and graduate awards:

The Amalgamated Tuna Company
Dr. Dwight Chasar
Cleveland State University Department of Chemistry
Energizer Corporation
Lubrizol Corporation
Dr. Lily Ng

The Cleveland Section ACS thanks the following for volunteering their time to the 2011 Meeting-in-Miniature:

Dr. Sarah Preston, Ursuline College
Dr. Melissa Barranger, Ursuline College
Dr. Daniel Landfried, Ursuline College
Dr. Tony Pearson, Case Western Reserve University