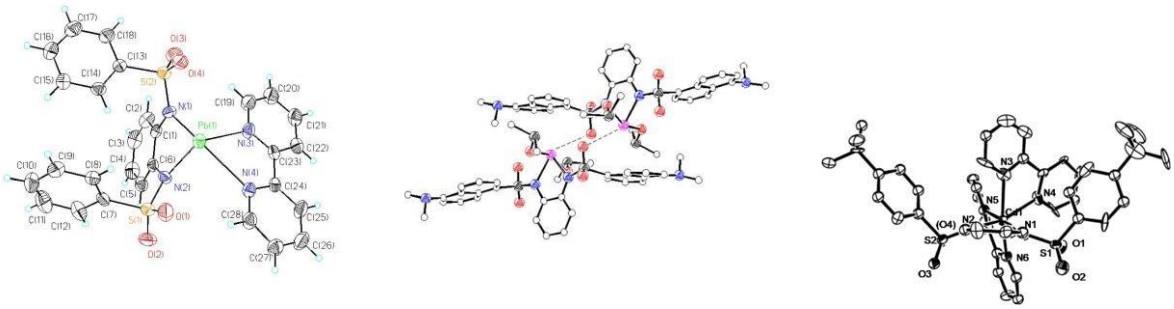
REU Project 1. Coordination Chemistry and Toxic Metal Sensors
Dr. Konstantinos Kavallieratos.

Research in the Kavallieratos group is focused on the design of sensors for toxic metals and ion pairs of biomedical and environmental significance, based on coordination and supramolecular chemistry principles. The REU student will synthesize sensor molecules for Pb(II) based on substituted and conjugated aromatic diamine frameworks, that contain CN and NO_2 electron withdrawing groups, for increased potential for optical and fluorescent sensing. The student will then synthesize and characterize metal complexes with Pb(II). Electrospray Ionization Mass Spectrometry (ESI-MS) will be used for screening potential ligand frameworks for metal binding. The thermodynamic parameters and sensing selectivity in the presence of other metals will be studied by distribution experiments, NMR, and isothermal titration calorimetry. This builds on the knowledge acquired on unsubstituted *o*phenylenediamine derived frameworks, pioneered by previous graduate and undergraduate students in the group.[1-4] The student will acquire skills in synthesis of coordination compounds and spectroscopic (NMR, fluorescence), mass spectrometric, electrochemical, and calorimetric techniques.



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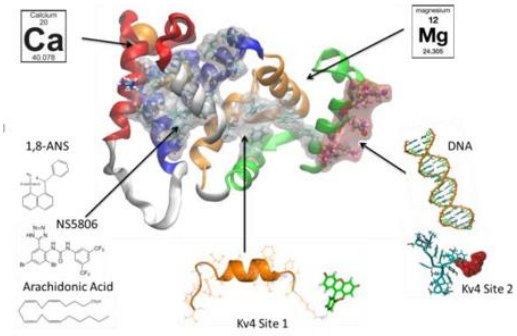
X-ray crystal structures of (a) Pb(II)-disulfonamide-2,2'-bipy (b) Pb(II)-dansylsulfonamide complex both having the stereochemically significant lone pair. Dimer formation in (b) is the probable basis of fluorescence for Pb(II), but not for other metals,[3] and (c) the Cd-sulfonamide-2,2'-bipy complex showing an octahedral coordination pattern.

1. Alvarado R. J.; Rosenberg J. M.; Andreu A; Bryan J. C.; Chen W.-Z.; Ren T.; Kavallieratos K. Structural Insights into the Coordination and Extraction of Pb(II) by Disulfonamide Ligands Derived from oPhenylenediamine. *Inorg. Chem.* (2005) **44**, 7951-7959.
2. Kavallieratos, K.; Sabucedo, A. J.; Pau A. T.; Rodriguez J. M. Identification of Anionic Supramolecular Complexes of Sulfonamide Receptors with Cl^-, NO_3^-, Br^-, and I^- by APCI-MS. *J. Am. Soc. Mass. Spectrom.* (2005) **16**, 1377-1383.
3. Kavallieratos K.; Rosenberg J. M.; Chen W.; Ren T. Fluorescent Sensing and Selective Pb(II) Extraction by a Dansylamide Ion-Exchanger. *J. Am. Chem. Soc.* (2005) **127**, 6514-6515.
4. Kavallieratos K.; Rosenberg J. M.; Bryan J. C. Pb(II) Coordination and Synergistic Ion-Exchange Extraction by Combinations of Sulfonamide Chelates and 2,2'-Bipyridine. *Inorg. Chem*. (2005) **44**, 2573-2575.

REU Project 2. Molecular Aspects of Calcium Signaling by Downstream Regulatory Element Antagonist Modulator

Dr. Jaroslava Miksovska



The research projects in the laboratory of Prof Jaroslava Miksovska are focused on determination of the signaling mechanism of Downstream Regulatory Element Antagonist Modulator (DREAM) protein that belongs to the family of neuronal calcium sensors. (DREAM) is a calcium binding protein that has been shown to directly regulate gene expression as well as regulate K^+ current through interaction with voltage gated potassium channels[1]. In cardiac tissue, a DREAM homolog protein, KChIP2, is necessary for heart pacemaking and decreased expression is associated with cardiac hypertrophy and arrhythmias[2]. In neuronal cells, DREAM regulates expression of pain sensing genes, and the production of Aβ-amyloids through interaction with presenilin[3-6]. These protein-protein interactions are also modulated by small ligands such as fatty acids and NS5806[-8]. Understanding the mechanism of ligand recognition by DREAM is essential to accurately understand its function under physiological and pathological conditions. One of the main objectives is to determine molecular mechanism of DREAM interactions with intracellular partners (DNA, potassium channels, presenilin) and a role of calcium in regulating DREAM specificity for target proteins. REU student participating in this project will receive multi-disciplinary training in biochemical and biophysical techniques and learn how to isolate/purify proteins, carry out fluorescence and ITC measurements, analyze spectroscopic data and model protein structure using molecular dynamic approaches. Results obtaining during this study will be disseminated in form of poster presentation at local/national meetings and publication in peer review journals.

1. Burgoyne, R. D.; and Weiss, J. L. "The neuronal calcium sensor family of Ca^{2+}-binding proteins" Biochem. J. 2001, 353, 1-12.
2. Bahring, R.; Dannenberg, J.; Peters, H. C.; Leicher, T.; Pongs, O.; and Isbrandt, D. "Conserved Kv4 Nterminal domain critical for effects of Kv channel-interacting protein 2.2 on channel expression and gating" J. Biol. Chem. 2001, 276, 23888-23894.
3. Buxbaum, J. D.; Choi, E.; Luo Y.; Lilliehook, C.; Crowley, A. C.; Merriam, D. E.; and Wasco, W. "Calsenilin: A calcium-binding protein that interacts with the presenilins and regulates the levels of a presenilin fragment" Nat. Med. 1998, 4, 1177-1181.
4. Carrion, A. M.; Link, W. A.; Ledo, F.; Mellstrom, B.; and Naranjo, J. R. "DREAM is a Ca2+-regulated transcriptional repressor" Nature. 1999, 398, 80-84.
5. An, W. F.; Bowlby, M. R.; Betty, M.; Cao, J.; Ling, H.; Mendoza, G.; Hinson, J. W.; Mattsson, K. I.; Strassle, B. W.; Trimmer, J. S.; and Rhodes, K. J. "Modulation of A-type potassium channels by a family of calcium sensors" Nature. 2000, 403, 553-556.
6. Ramachandran, P. L.; Craig, T. A.; Atanasova, E. A.; Cui, G.; Owen, B. A.; Bergen, H. R.; Mer, G.; and Kumar, R. "The potassium channel interacting protein 3 (DREAM/KChIP3) heterodimerizes with and regulates calmodulin function" J. Biol. Chem. 2012, 287, 39439-39448.
7. Gonzalez, W. G.; Pham, K.; and Miksovska, J. "Modulation of the voltage-gated potassium channel (Kv4.3) and the auxiliary protein (KChIP3) interactions by the current activator NS5806" J. Biol. Chem. 2014, 289, 32201-32213.

REU Project 3. Characterization of influent and effluent wastewaters by high-resolution mass spectrometry: Figuring out the identity of recalcitrant chemicals released to the environment
Dr. Piero Gardinali

In order to fulfill human "needs" in our rapidly changing society, a great variety of products ranging from pharmaceuticals and personal care products (PPCPs), antibiotics, drugs of abuse (illicit and prescription), artificial sweeteners, nanomaterials, disinfection byproducts, sunscreens, pesticides, to name a few; are being manufactured, consumed, used, and disposed of, on a daily basis in households and industrial settings. This massive collection of compounds (in their unchanged form or as transformation products) is not only able to reach aquatic environments, but in some cases persist and potentially inflict detrimental effects upon it. In South Florida, wastewater treatment plants provide typical secondary treatment, followed by disinfection, and release treated water through, oceanic outfalls, deep well injection, and/or irrigation. In many cases this type of treatment is not sufficient enough to remove all compounds, turning water discharges into a persistent source of contamination. The goal of this studies is to develop comprehensive screening sensing techniques using online-SPE-HPLC ultra high-resolution mass spectrometry to compare influent and effluent wastewater in order to identify and characterize the recalcitrant unknown chemicals or families of chemicals that routinely survive water treatment.

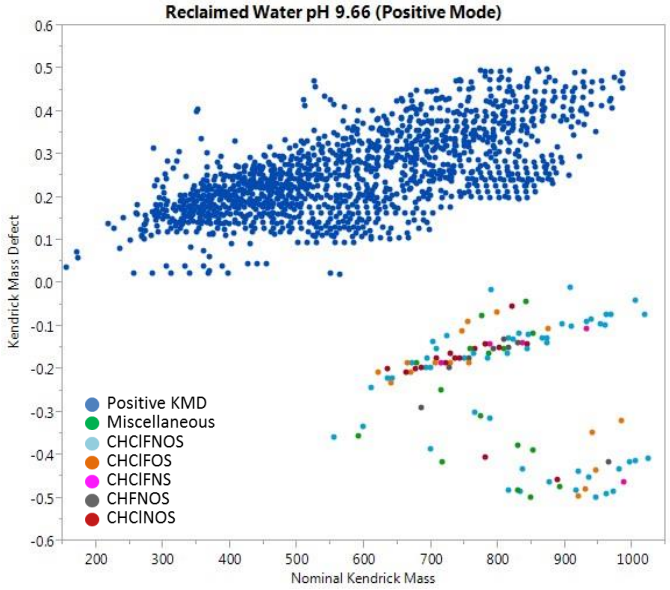
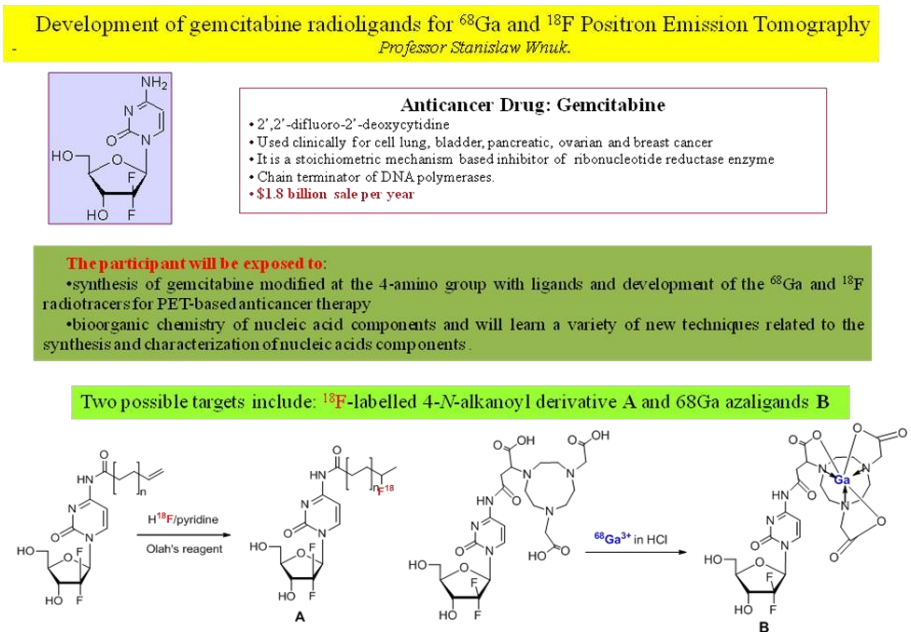


Figure 1 shows and example of the variety of chemical formulas present in a typical treated wastewater effluent collected at the BBC and analyzing using a 7T FT-ICR high resolution mass spectrometer. Each dot is a specific chemical; colored dots show compounds containing heteroatoms. There are about 900 compounds in the figure.

REU Project 4. Development of gemcitabine radioligands for ^{68}Ga and ^{18}F Positron Emission Tomography
Dr. Stanislaw Wnuk.

The REU students working with Dr. Wnuk will pursue projects on bioorganic aspects of sensing. Recognizing the importance of PET technology in nuclear medicine, synthesis of gemcitabine aza-ligands and application of its ^{68}Ga radioligand for a PET-based anticancer therapy is proposed as a training ground for the student. Gemcitabine[10] is a prominent drug, which has been approved for treatment of lung, pancreatic and bladder cancers.[11] Synthesis of gemcitabine modified at the 4-amino group with ligands and development of the ^{68}Ga and ^{18}F radiotracers for PET-based anticancer therapy[12] is proposed. The participant will be exposed to the bioorganic chemistry of nucleic acid components and will learn a variety of new techniques related to the synthesis and characterization of nucleic acids components and radiolabeled bioactive molecules.



1. Shao, J.; Zhou, B.; Chu, B.; Yen, Y. Ribonucleotide reductase inhibitors and future drug design. *Curr. Cancer Drug Targets* (2006) **6**, 409-431.
2. Manegold, C. Gemcitabine (Gemzar) in non-small cell lung cancer. *Exp. Rev. Anticancer Ther.* (2004) **4**, 34560.
3. Zhang, Z-T.; Pitteloud, J.-P. ; Cabrera, L.; Liang, Y.; Toribio, M.; Wnuk, S. F. Arylchlorogermanes/ tetrabutylammonium fluoride. A promosing combination for Pd-catalyzed Germyl-Stille cross-coupling", *Org. Lett.* 2010, 12, 816-819.

REU Project 5: Paper microfluidic sensing for toxicological and forensic applications
Dr. Bruce McCord

Paper microfluidics systems are inexpensive analytical devices based on designs printed using wax-based ink using chromatography paper [1]. The wax creates hydrophilic channels in the paper that

can direct liquid samples toward individual sections of the paper containing colorimetric test reagents. Using these designs, a single device can perform five or more simultaneous analyses on a single analyte by sending portions of the sample into different testing wells. The device costs very little, since the basic design components (paper, wax, and small quantities of reagents) are all inexpensive. Because the reagents can be isolated in a dry, not reactive state, they can be easily stored for long-term performance. Detection occurs using colorimetric, spectroscopic, and chemiluminescent methods. Targeted analytes will include explosive residues, seized drugs, and environmental and chemical toxins.

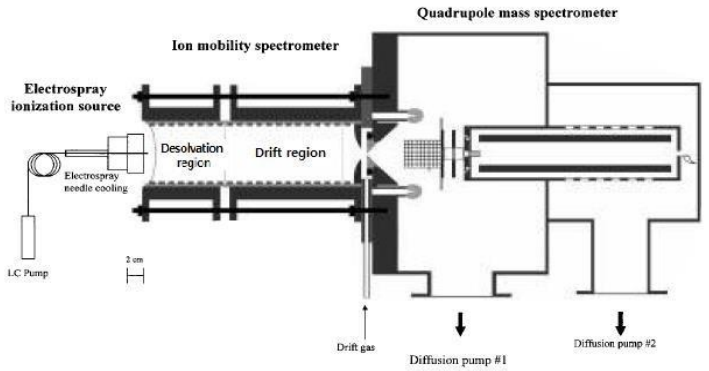
The goal of this project will be to involve the students in various aspects of the developmental validation of these paper microfluidic devices (µPADs) for the on-site, rapid detection of multiple analytes. Two µPADs have already been designed: one for testing inorganic explosives such as flash powders and black powders, while the second tests for seized drugs [2-4]. These devices need to be field tested and refined for use, including proper selection of sensing elements, refinement of chemical and chromatographic processes and testing against potential interferences. Students will develop, test, and optimize the various sensing elements using statistical and experimental design procedures. Results will be published in peer reviewed journals.

1. Phillips ST; Whitesides, GM; **Carrilho E;** Martinez, AW. "Diagnostics for the developing world: microfluidic paper-based analytical devices" *Anal Chem* 2010 Vol 82, 3-10.
2. Musile, G.; Wang, L.; Bottoms, J.; Tagliaro, F.; McCord, B.,"The development of paper microfluidic devices for presumptive drug detection", *Analytical Methods*, in press.
3. Peters, KL.; Corbin,I; Kaufman, LM.; Zreibe,K.;Blanes,L. McCord, B., "Development of Paper Microfluidic Devices for the Rapid, On-Site Detection of Improvised Explosive Compounds," *Analytical methods,* 2015,*7* 63-70.
4. Pesenti, A.; Taudte,RV.; McCord, B.; Doble, P. Roux, C.; Blanes L. Coupling µPAD's and lab on a chip technologies for confirmatory analysis of trinitro aromatic explosives *Anal. Chem.*, 2014, *86* (10), pp 4707– 4714

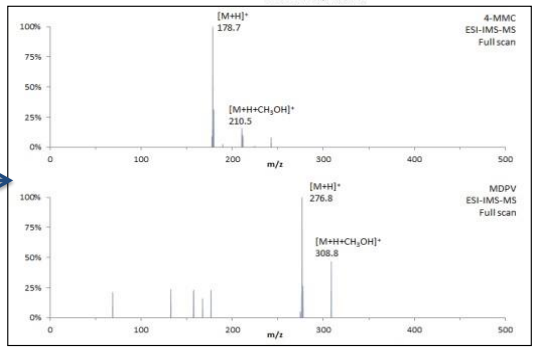
REU Project 6. Forensic Analysis and Identification of Drugs of Abuse Using Chiral Ion Mobility Spectrometry (IMS) coupled to a Mass Spectrometer (IMS-MS)
Dr. José Almirall.

The recent development of the concept of chiral ion mobility spectrometry (CIMS) allows rapid separation and identification of enantiomers and other stereoisomers, within seconds. IMS is a widely accepted analytical method used in a variety of detection scenarios including trace detection of explosives and controlled substances. IMS application in the forensic science laboratory has been limited because of its poor resolution compared to other chromatographic techniques that are coupled to mass spectrometry. An REU student would be trained to operate a *commercial* high-resolution IMS that will enable a CIMS to have *separation performance comparable to that obtained by chromatographic methods*. The CIMS system has the ability to separate stereoisomers of controlled substances for detection using a quadrupole mass spectrometer. The student will use a new analytical tool for identification of chiral drugs. This tool will also be useful for drug analysis in general, as ESI/SESI sample introduction would offer an alternative for the analysis of other drugs that are thermally labile, such as GHB. Such drugs do not survive the temperatures of a GC injector but would be amenable to ESI-IMS-MS analysis. The student will learn to interpret the mass spectral data generated.

High Performance ESI-IMS-MS for Drug Analysis



- Advantages
  - Introduction and detection of non - volatile compounds
  - Fast (< 1 s) analysis time
  - Preservation of the molecular ion with Electron Impact Ionization
  - Reduced false positive by the combination of two spectral approach based on the size and mass of ion



Wu *et al.* (1998)
http://excellims.com/products/ims -ms-systems.html

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REU Project 7. Use of humic acid coated magnetite nanoparticles (HA-MNP) for the removal of radioactive Strontium from groundwater
Dr. Kevin O'Shea.

Groundwater contamination by heavy metals discharge from natural, agricultural and industrial sources have been a growing concern for public health as a large number of peoples around the globe use this water for drinking and cooking purposes [1]. Strontium (Sr) is a heavy metal that has wider industrial application in nuclear power plant, electroceramic and oxide superconductors, medical diagnostics etc. Among different isotopes of Sr, the radioactive isotope ^{90}Sr has smaller half life (28.8 year), greater emission of hazardous beta radiation and higher water solubility which eventually lead to water contamination [2]. This element has lots of structural similarity to calcium and hence, when taken into the body, it can easily deposit in the bone tissue by replacing calcium from there and promote the development of bone sarcomas, leukemia and cancer [3]. The existing methods for the removal of Sr from ground water are mostly suffering from the limitation of poor removal efficiency and/or higher cost [4,5]. Therefore, we are proposing a natural organic matter (humic acid) coated magnetic (magnetite) nanomaterials as an adsorbent of Sr that has been widely applied in environmental [6] and medical sectors but has not been explored yet for the treatment of strontium contaminated water. The nanometer size of the particles provide higher surface area that is ideal for greater adsorption [7] while the magnetic properties of iron oxide [8] (magnetite) helps to separate the particles after treatment using a simple handheld magnet (figure 1). The organic coating of humic acid reduces the auto-oxidation, toxicity and agglomeration of bare magnetite nanoparticles to a significant extent.

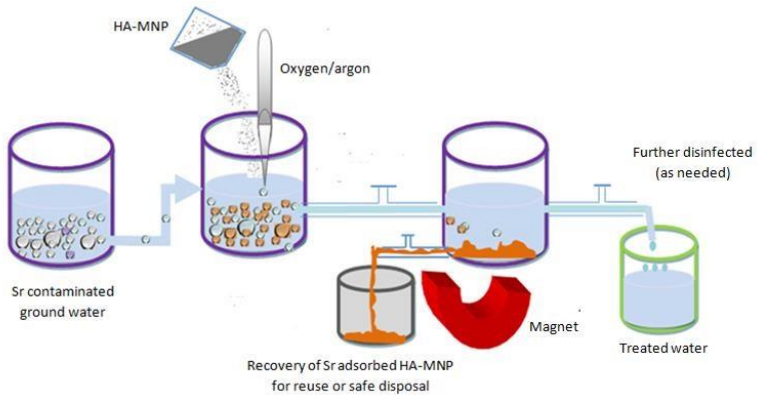


Figure 1: Schematic diagram of Sr contaminated water treatment method

Task 1 (week 1-2): *Syntheses and characterization of humic acid coated magnetite nanoparticles (HAMNPs)*
Synthesis of HA-MNP will be done using the method described in the literature [9]. Fractionated humic acid with different functional groups will be used to synthesize the nanoparticles to see what functional groups generates the best coating and gives higher adsorption of strontium. Characterization of the synthesized materials will be done using FTIR, TEM (or DLS) and TOC analyzer to determine the binding, material size and NOM desorption respectively.

Task 2 (week 3-8): *Investigate the adsorption of Sr on humic acid coated magnetite nanoparticles and characterize the adsorption process*
The adsorption of Sr on HA-MNP will be investigated under different condition to measure the adsorption kinetics, adsorption efficiency as well as the effect of pH and co-existing ions. The findings will be compared with the existing removal methods reported into the literature

Task 3 (Week 9-10): *Writing, reviewing and finalizing the manuscript based on the outcome of the experiment*
A manuscript will be written based on the data obtained from this total set of experiment.

Learning objective: 1) Student will learn the nanomaterials synthesis procedure and get the exposure to sophisticated instruments during the characterization and adsorption experiments
2) Students will acquire hands on experience on writing manuscripts following standard writing style and techniques

Collaboration: Dr. Yong Cai (Dept. of Chemistry, FIU), Dr. Wenzhi Li (Dept. of Physics, FIU), Dr. Dionysios D. Dionysiou (Dept. of Chemistry, University of Cincinnati)

1. Hashim, M. A.; Mukhopadhyay, S.; Sahu, J. N.; Sengupta, B. "Remediation technologies for heavy metal contaminated groundwater" *Journal of Environmental Management.* **2011,** *92,* 2355-2388.
2. Mashkani, S. G.; Ghazvini, P. T. M. "Biotechnological potential of Azolla filiculoides for biosorption of Cs^{+} and Sr^{2+}: Application of micro-PIXE for measurement of biosorption" *Biores.Technol,* **2009**, *100,* 1915-1921.
3. Karasyova, S. N.; Ivanova, L. I.; Lakshtanov, L. Z.; Ovgren, L. L. "Sr^{2+} Sorption on hematite at elevated temperatures" *J. Colloid Interface Sci.,* **1999**, *220,* 419-428.
4. Murthy, Z. V. P.; Parmar, S. "Removal of strontium by electrocoagulation using stainless steel and aluminum electrodes" *Desalination,* **2011**, *282,* 63-67.
5. Kumar, R.; Jain, S. K. "Removal of strontium(II) from aqueous solution using functionalized carbon nanotubes" *International Journal of Nanoscience,* **2012**, *11(2),* 1250019 (6 pages).
6. Jiang, W.; Cai, Q.; Xu, W.; Yang, M.; Cai, Y.; Dionysiou, D. D.; O'Shea, K. E. Cr(VI) Adsorption and Reduction by Humic Acid Coated on Magnetite. *Environ. Sci. Technol.* **2014**, *48*, 8078−8085.
7. Rivera-Reyna, N.*; Hinojosa-Reyes, L; Guzman-Mar, J. L.; Cai, Y.; O'Shea, K; Hernandez-Ramirez, A. "Photocatalytical removal of inorganic and organic arsenic species from aqueous solution using zinc oxide semiconductor", *Photochemical & Photobiological Sciences,* **2013**, *12(4),* 653-659.
8. Jiang, W.;Pelaez, M.; Dionysiou, D. D.; Entezari, M. H.; Tsoutsou, D.; O'Shea, K. "Chromium(VI) removal by maghemite nanoparticles". *Chemical Engineering Journal* **2013**, 527-533
9. Liu, J.-F.; Zhao, Z.-S.; Jiang, G.-B. "Coating Fe_3O_4 magnetic nanoparticles with humic acid for high efficient removal of heavy metals in water" *Environ. Sci. Technol.* **2008**, *42(18)*, 6949−6954.

REU Project 8. Formation pathways of thiolated arsenicals in cells treated with DAR
Dr. Yong Cai.

Darinaparsin (DAR, dimethylarsino glutathione, DMA(GS)) is a novel organic arsenical used as therapeutic agent for hematologic cancers (Mann et al., 2009). However, the mechanisms underlying the clinical anticancer efficacy of DAR are poorly understood, due in part to the lacking of information on the metabolism and transformation of DAR at the molecular level (Matulis et al., 2009). Based on 1) direct detection of dimethylmonothioarsinic acid-glutathione conjugate (DMMTAV-GS) in DAR-treated human cell lines in our previous studies and wide occurrence of thioarsenicals in biological systems (including human) after arsenic exposure, we propose that thioarsenicals are formed during DAR metabolism and are important metabolites possibly involved in mediating the therapeutic efficacy of DAR via interactions with biomolecules, such as proteins. We are planning to perform a number of hypothesis-driven investigations to understand the role of newly identified thioarsenicals, specifically DMMTAV, in the metabolism of DAR.

The REU students will be involved in a study designed to understand how thioarsenicals are formed in biological bodies. Although thioarsenicals have been widely detected in biological systems, little is known about the formation processes of these thioarsenicals. Merely based on the chemical reactions between DMAV (or DMAIII) with hydrogen sulfide that can produce thioarsenicals, hydrogen sulfide has been suspected to be involved in thioarsenical formation in biological systems. However, there is currently no information to support this suspicion. In this research, we hypothesize that it is the living cells that produce hydrogen sulfide that in turn reacts with DAR and/or its metabolites (DMAV and DMAIII) to generate thioarsenicals. Under the supervision of the faculty, and the REU student will perform a set of experiments to test this hypothesis, including chemical reactions and in vitro and in vivo incubation. Incubation experiments will be conducted with cellular hydrogen sulfide production inhibited by adding reagents to inhibit cellular enzymes that are responsible for hydrogen sulfide production. By comparison with regular cell incubation experiments, the student will be able to define the role of hydrogen sulfide in thioarsenical formation.

1. Mann, K.K., Wallner, B., Lossos, I.S., Miller, W.H., 2009. Darinaparsin: a novel organic arsenical with promising anticancer activity. Expert Opinion on Investigational Drugs 18, 1727-1734.
2. Matulis, S.M., Morales, A.A., Yehiayan, L., Croutch, C., Gutman, D., Cai, Y., Lee, K.P., Boise, L.H., 2009. Darinaparsin induces a unique cellular response and is active in an arsenic trioxide-resistant myeloma cell line. Molecular Cancer Therapeutics 8, 1197-1206.

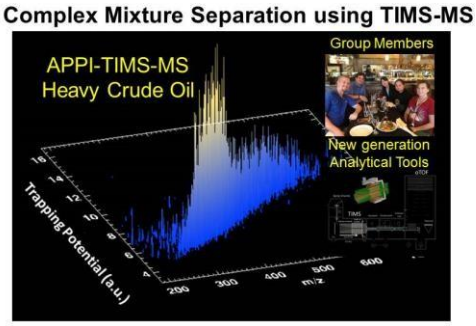
REU Project 9. E-AB sensor development for screening drugs of abuse in seized substance
Dr. Yi Xiao

Single-nucleotide polymorphisms (SNPs) represent genetic variants associated with susceptibility to various common diseases and responses to various drugs.[23] Target-recycling based assays are a novel approach for the direct detection of trace amounts of specific nucleic acids due to the significantly amplified signal triggered by the reused target.[24-26] Recently, we reported an exonuclease III-aided target recycling (EATR) fluorescence DNA sensor which achieved a detection limit as low as 20 aM.[27] As part of this project, the student will use the intercalation of 2-amino-5,6,7-trimethyl-1,8-naphthyridine (ATMND) into abasic sites of duplex DNA for an amplified detection platform. By using signaling probes with different structures (stem-loop, pseudoknot, triple-stem), ATMND, complementary target DNA and exonuclease III, the student will learn the sequence design of DNA probes and will use optimized conditions to develop a method for rapid, room-temperature, PCR-free SNP detections with high sensitivity. Knowledge obtained in this research will be further used for the design and development of ultra-sensitive *in vivo* sensors for various proteins and small molecules.

1. Herne, T. M.; Tarlov, M. J. Characterization of DNA Probes Immobilized on Gold Surfaces. J. Am. Chem. Soc. 1997, 119, 8916–8920.
2. Roncancio, D.; Yu, H.; Xu, X.; Wu, S.; Liu, R.; Debord, J.; Lou, X.; Xiao, Y. A Label-Free AptamerFluorophore Assembly for Rapid and Specific Detection of Cocaine in Biofluids. Anal. Chem. 2014, 86, 11100–11106.

REU Project 10. Characterization of heteroatom hydrocarbons from crude oils using direct infusion Trapped Ion Mobility Spectrometry – Mass Spectrometry
Dr. Francisco Fernandez-Lima

Over the past years, a variety of new types of Ion Mobility Spectrometry (IMS) analyzers have been developed (e.g., periodic focusing DC ion guide, segmented quadrupole drift cell, multistage IMS, field asymmetric IMS and transient wave ion guide). High resolution IMS has been mainly restricted to the use of long IMS cells and low temperature devices. We have recently introduced the Trapped Ion Mobility Spectrometer (TIMS) for the analysis of complex mixtures without the need for pre-fractionation. When coupled to a mass spectrometer (TIMS-MS), this device permits fast, post-ionization, gas-phase separation by taking advantage of the high mobility separation prior to the mass analysis, thus reducing the chemical noise and increasing the dynamic range. In the present project, TIMS-MS separation of complex heteroatom hydrocarbons mixtures (e.g., direct infusion from crude oils) will be performed, which will allow the identification and fingerprint of crude oils with different origins and the structural characterization of the heteroatom classes.



REU Project 11. Detection of Antibacterial Activity Targeting Topoisomerase I
Dr. Yuk-Ching Tse-Dinh

There is an urgent public health need for discovery of novel antibacterial drugs due to the increased prevalence of bacterial pathogens resistant to all current antibiotics. Topoisomerase I enzyme is present in every bacterial pathogen as a potential target for compounds that can kill the bacteria by trapping the reaction intermediate formed by the topoisomerase enzyme and the DNA substrate that can trigger bacterial cell death if accumulated[1,2]. In our laboratory, we conduct high through-put bacterial cell based assays as well as topoisomerase enzyme based assays[2] to identify small molecules and natural products that can target topoisomerase I selectively for antibacterial activity.

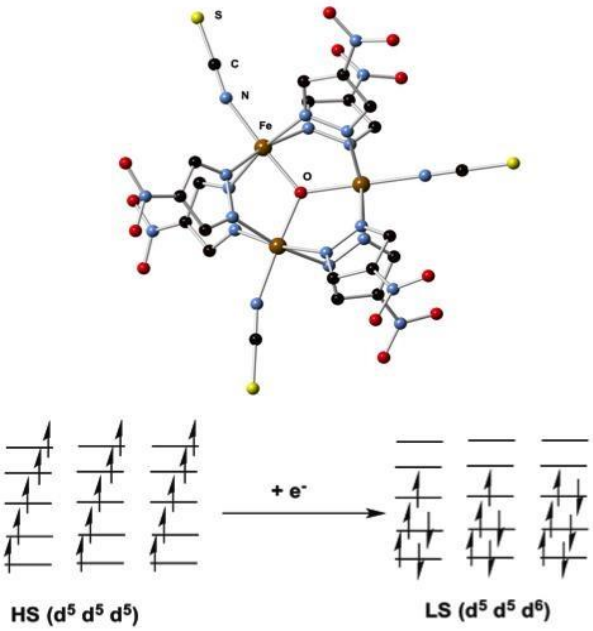
During the 10 week project period, the student will learn to carry out (1) antibacterial assay comparing the Minimal Inhibitory Concentration (MIC) of compounds against isogenic gram negative *Escherichia coli* or gram positive *Mycobacterium smegmatis* strains with different levels of recombinant topoisomerase I expression (2) topoisomerase enzyme activity assay to measure the IC50 for 50% inhibition of relaxation of supercoiled DNA by topoisomerases using agarose gel electrophoresis to separate the reaction substrate and product. The student will monitor the bacterial cell viability by measurement of fluorescence signal from resazurin indicator in the 96-well and 384-well format. Images of supercoiled DNA substrate and relaxed DNA product will be acquired and quantitated. The student will acquire aseptic techniques of monitoring growth of purified microbial cultures. It is expected that the student will complete these tasks for a set of at least 10 compounds. The results of the student project will be presented at scientific conferences and submitted for publication. We are collaborating with faculty in FIU Physics Department to study the topoisomerase-ligand interactions[3]. We also have an ongoing collaboration[4] with Professor Dianqing Sun, a medicinal chemist at the University of

Hawaii at Hilo who has carried out synthesis of natural product and analogs to investigate the associated antibacterial activities.

1. Pommier, Y. "Drugging topoisomerases: lessons and challenges" ACS Chem. Biol. 2013, 8, 82-95.
2. Tse-Dinh, Y. C. "Targeting bacterial topoisomerase I to meet the challenge of finding new antibiotics" Future Med. Chem. 2015, 7, 459-471.
3. Tiwari, P. B.; Annamalai, T.; Cheng, B.; Narula, G.; Wang, X.; Tse-Dinh, Y. C.; He, J.; Darici, Y.; "A surface plasmon resonance study of the intermolecular interaction between Escherichia coli topoisomerase I and pBAD/Thio supercoiled plasmid DNA" Biochem. Biophys. Res. Commun. 2014, 445, 445-450.
4. Lin, H.; Annamalai, T;, Bansod, P.; Tse-Dinh, Y. C.; Sun, D. "Synthesis and antibacterial evaluation of anziaic acid and analogues as topoisomerase I inhibitors" Medchemcomm. 2013, 4, 1613-1618.

REU Project 12. Magnetic Sensors Based on Redox-Sensitive High Spin – Low Spin Crossover.
Dr. Raphael G. Raptis

Sensors based on the detection of magnetization changes are robust and typically quite sensitive. We are now developing the principal components for a magnetic remote sensor of redox environments.
Depending on their coordination environment, transition metals distribute their valence electrons in a way that either maximizes, or minimizes their spin, respectively termed the high-spin (HS) and low-spin (LS) electronic configuration. In multinuclear complexes, the total ground-state spin of the system depends on the sign of interactions – ferromagnetic (positive), or antifferomagnetic (negative) -- among individual spins, while the bulk magnetization of the sample depends on the magnitude of these interactions.



We have discovered a trinuclear complex, $[Fe_3(m_3\text{-}O)(m\text{-}4\text{-}O_2N\text{-}pz)_6X_3]^{2-}$, X = NCS, composed of three HS-Fe^{III} centers antiferromagnetically coupled into a paramagnetic $[Fe_3(m_3\text{-}O)]^{7+}$-unit with an S = 1/2 ground state. Upon one-electron reduction (either chemical, or electrochemical), all-three centers of the formally $Fe^{III}_2Fe^{II}$-cluster convert to LS, resulting in a diamagnetic $[Fe_3(m_3\text{-}O)]^{6+}$-unit with S = 0. New research will be directed at synthesizing related systems with X = CN, NCSe, N_3, OAr and investigating the redox potential and temperature dependence of the spin cross over between HS and LS species. New complexes will be synthesized by solution chemistry methods (4 weeks), will be characterized by single crystal X-ray diffraction analysis and standard spectroscopic techniques (^{1}H-NMR, IR, UV-Vis-NIR,) and electrochemical methods (6 weeks). Further magnetic susceptibility measurements, ^{57}Fe-Mössbauer and EPR spectroscopic analyses and theoretical calculations will be carried out with our international collaborators.

REU Project 13. Construct a type of unique plasmid DNA molecules to study DNA topology and topoisomerases by fluorescence resonance energy transfer (FRET)
Dr. Fenfei Leng

Supercoiling is a fundamental property of the DNA double helix. In most organisms, DNA is typically (-) supercoiled [1]. Free energy constrained in (-) superhelicity greatly promotes a number of essential DNA processes, such as DNA replication, transcription, and recombination [1]. For instance, every stage of DNA replication has topological issues such that (-) supercoiling favors the formation of functional initiation complexes [2-4]. In several cases, DNA replication initiation depends on (-) supercoiling of the DNA templates [4,5]. For replication elongation, as the replication forks proceed along the DNA double helix, (+) supercoils build up ahead of the moving replication forks [6]. Without the help of DNA topoisomerases to remove the (+) supercoils, DNA replication forks would be arrested [7]. For replication termination, the newly replicated, daughter DNA molecules form catenated DNA without the assistance of DNA topoisomerases. Nevertheless, the tools of studying DNA topology and topoisomerases are limited and usually need to use the tedious and time-consuming agarose gel electrophoresis. Therefore, it is important to develop new methods and tools to study DNA topology and topoisomerases.

The objective of the 10-week REU project is to design and construct a type of unique plasmid DNA molecules to study DNA topology and topoisomerases by fluorescence resonance energy transfer (FRET). Task 1 is to make plasmid pUC18_AT42 that carries an (AT)42 sequence between SphI and BamHI sites of pUC18. Task 2 is to ligate an (AT)42 DNA sequence labeled with a fluorophore (fluorescein) and a quencher (dabsyl) to pUC18_AT42. Task 3 is to generate and purify sufficient amount of the double-labeled plasmid DNA template. Task 4 is to study kinetics of *E. coli* DNA topoisomerase I and DNA gyrase using the doubled-labeled DNA template by FRET. The expected outcome is that a new type of plasmid DNA molecules will be available to study DNA topology and topoisomerases. The REU student will gain experimental techniques, such as molecular cloning, DNA purification, and fluorescence.

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REU Project 14. Fabrication of hybrid biomaterials using conjugated polymers and polysaccharides
Dr. Joong-ho Moon

Conjugated polymers (CPs) are attractive photoluminescent materials used for various sensors and optoelectronic devices.[1] When CPs are fabricated into sensory formats such as thin films or particles, aggregation of CPs is an unavoidable issue throughout the formats.[2] Aggregation generally decreases the physical and photophysical properties of CPs, resulting in poor sensing or device efficiency. As part of efforts to reduce or control aggregation of CPs in aqueous media, we previously demonstrated that organic acid treatment of amine-functionalized poly(phenyleneethynylenes) (PPEs) followed by ultrafiltration can control chain-chain interactions in water, resulting in the formation of conjugated polymer nanoparticles (CPNs).[3,4] Although CPNs exhibit necessary photophysical properties for sensing and labeling of biological interests, further fine controlling of conjugation structures would dramatically improve the physical and photophysical properties of CPNs directly associated with the sensing efficiency.

Using negatively charged linear polysaccharides including hyaluronic acid (HA) and chondroitin sulfate (CS), positively charged CPs containing various backbone structures will be complexed with HA or CS to investigate the backbone structure-photophysical property relationship.[5,6] A REU student will conduct 1) polymerization using monomers synthesized by other graduate students; 2) characterization of polymers using gel permeation chromatography, NMR, UV-vis, and fluorescence spectroscopy; and 3) complexation and characterization of CP/HA using dynamic light scattering and optical spectroscopies such as UV-vis, fluorescence, and circular dichroism (CD). Knowledge obtained from the research will be a solid foundation for fabrication of hybrid biomaterials for sensitive labeling and detection of various biological molecules.

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REU Project 15. Ferrocene Derivatizing Reagents for Selective Detection and Isolation of Analytes
Dr. J.M.E. Quirke

Ferrocene-based derivatizing agents are proposed for selective detection of analytes using spectrophotometric or electrochemical methods, which take advantage of Fe(II) oxidation to form the blue-green ferrocenium ion. Nanomolar analyte detection using ESI-MS has been reported.[20,21] The goal of the proposed study is to extend the previous derivatization studies by preparing derivatives of analytes bearing two or more functional groups. As part of the project the REU participant will initially prepare ferrocenoyl azide and derivatize menthol. Then ferrocenoyl ureas of diaminoalkanes will be prepared and characterized by both NMR and ESI-MS. Chain length effects on the ferrocene oxidation will be studied. 2D-TLC-ESI-MS[22] of ferrocenoyl carbamates of menthol will be used as a proof of principle study. The method will yield pure analytes from complex mixtures because polar impurities are removed in the initial elution, and the remaining impurities will be separated from the ferrocenium ion.

Provision of Visual Evidence For Selective Reductions of Functional Groups

INTRODUCTION

My research involves developing experiments to assist students in studyingorganic chemistry.

LONG-TERM AIM AND STATUS

- To provide photographic evidence for the outcome/mechanisms of all the reactions covered in Organic Chemistry I and II.
- My approach is unique in the country. No-one has attempted a systematic study of this kind.
- Develop new reactions laboratory techniques and glassware for the visualization studies.
- Work is complete for about 100 experiments.

IMMEDIATE GOALS

1. Carry out a comprehensive photographic study of reductions of functional groups using a range of reagents includingenzymes,in some cases.
2. To provide visual proof of reaction regioselectivity (or lack thereof).
3. Provide visual proof of enantioselectivity in reductions by enzymes.

EXPECTATIONS/BENEFITS

- You will be trained in the use of NMR and other spectroscopy compounds because all the compounds you make must be fully characterized.
- Your work will be presented at ACS Meetings and will be submitted for publication in the *Journal ofChemical Education*.

Example: Proof that Propanal is Reduced to 1-Propanol.

- Borohydride reduction of propanal to form 1-propanol does not give direct visual evidence of the outcome.
- We confirmed the product by using qualitative tests (shown below) and photographing the boiling point (not shown). **Jones' reagent turns green** for primary and secondary alcohols. **Ceric ammonium nitrate (CAN) turns red brown with alcohols. Reichardt's dye turns violet with primary alcohols and blue with secondary alcohols.**

Distillationof theproductintoa cow containing reagents

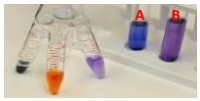
The Initial distillationsetup:

CANtest confirmsalcohol

Strategy

Jonesreagent andReichardt'sdyeconfirmprimaryalcohol

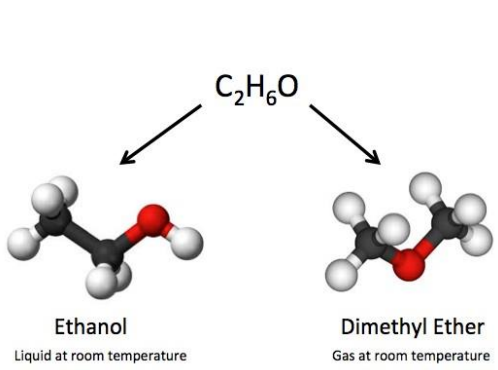
- Ideally, carry out reductions involving color/phase changes.**A** contains pure **2-propanol**.
- If not devise methods to provide visual evidence**B** contains pure **1 – propanol**. of the reaction outcomes, including the use of solvatochromic dyes.



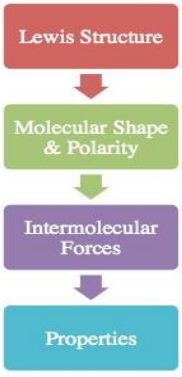
REU Project 16. Investigating introductory college students' understanding of structure-property relationships
Dr. Sonia Underwood

The understanding that a chemical structure can be used to predict how a compound might interact with other species is at the heart of chemistry (Figure 1a). A compound's reactivity is essential in, for example, drug synthesis and signaling processes. Lewis structures are the first type of structural representation introduced to introductory college chemistry students that can provide a pathway to predicting macroscopic properties (Figure 1b). Previous research has shown that college students at all levels have much difficulty making this connection between structure and properties. Typically students are only able to use these structures to predict structural information (e.g. number of bonds or electrons) instead of more implicit, essential, information of reactivity (e.g. acidity/basicity). The long-term research goal is to further explore the ways in which introductory college chemistry students connect structureproperty relationships.

The immediate goal for this summer project is to determine at what point do students engage in the process of predicting structure-property relationships. A series of questions regarding the relationship between structure and properties were administered to general chemistry students (N~100) using *beSocratic* – an online program that allows for free-form student drawing and text responses. The objective for this project is to analyze student responses to determine when students begin to engage in the structure-property relationship. Students engaged in this project will be introduced to the realm of qualitative studies and develop expertise in modern qualitative methods. For example, analysis methods such as open-coding responses to develop themes among the data will be used. In addition, methods to summarize findings for a population such as Sankey diagrams – type of flow diagram – will be explored.



(a)



(b)

Figure 1: (a) an example of how the structure of a compound can lead to different properties and (b) series of information needed to concatenate to predict structure-property relationships for simple molecules. References:

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REU Project 17. Targeted MRI-Contrast Reagents with Redox-Active Metal Centers
Dr. Christopher Dares

MRI contrast reagents can be used to enhance tissue differences necessary for the diagnosis for a variety of health conditions including cancer. One common problem with most reagents is that they do not target cancer cells specifically meaning that a large amount of material must be delivered to the patient, and contrast enhancement may not be profound in the area of interest. By attaching functional groups which include receptors for cancer cells, we will create targeted contrast reagents. This will decrease the amount of material needed to be given to the patient, and, improve contrast where it's required. Work in this area will involve a DTPA framework with four anhydride groups that can be functionalized with molecules for binding to the metal (typically siderophore analogs), while the remaining carboxylic acid group on the DTPA can be derivatized with the cancer receptor.

We will not limit ourselves to the use of Gd(III) which is relatively stable and has 7 unpaired electrons, but, will also develop reagents involving Eu(II) which also has 7 unpaired electrons. While Eu(III) is the typical oxidation state for europium in biological environments, it can be reduced using chemical reductants to Eu(II). We will employ electrochemistry to more cleanly produce Eu(II) complexes, and to aid in our understanding of europium electrochemistry which may facilitate the development of new MRI contrast reagents.