**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 NO₂ 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.

X-ray crystal structures of (a) Pb(II)-disulfonamide-2,2’-bipy¹ (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,⁵ and (c) the Cd-sulfonamide-2,2’-bipy complex showing an octahedral coordination pattern.


**REU Project 2. Singlet oxygen production within zeolite-like metal-organic frameworks (ZMOF) – Dr. Jaroslava Mikovska**

Porous metal-organic frameworks (MOFs), also known as coordination networks or coordination polymers, have attracted significant interest over the past few years largely due to their potential applications in gas storage, host-guest exchange, sensing, and catalysis.⁵⁻⁸ The REU participant will explore the application of the ZMOF as support materials for photosensitizer molecules. The participant will synthesize rho-ZMOF frameworks with negatively charged and neutral internal cavities and functionalize them with common photosensitizers using cation exchange or the “ship-in-the-bottle” approach.⁹ Photo-physical properties of functionalized frameworks will be evaluated using steady-state and time-resolved absorption and fluorescence spectroscopies. The functionalized material will then be probed for singlet oxygen production. The proposed research will provide a unique opportunity for the
student to become familiar with the synthesis of metal-organic materials as well as with steady-state and time-resolved fluorescence techniques and data analysis.


**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.

Two possible targets include: $^{18}$F-labelled 4-alkanoyl derivative A and 68Ga azaligands B


REU Project 5: Microfluidic Systems for DNA typing: Dr. Bruce McCord.

Professor McCord’s current research interests encompass the fields of analytical forensic chemistry, nanochemistry, and mass spectrometry. One current research project is the development of microfluidic systems for the analysis of trace levels of DNA utilized in forensic human identification. In this project the student will develop improved multiplex short tandem repeat markers for application to microchip electrophoresis. These systems will permit rapid and precise genotyping of DNA from convicted offenders and crime scenes. Key intellectual challenges include primer design and computer modeling of
electrophoretic injections. The REU participant will develop expertise in DNA extraction, real time PCR, and entangled polymer synthesis for DNA separation on microfluidic devices.

REU Project 6: Trace detection of explosive residues – Dr. Bruce McCord.
A second project is focused on the development of methods for the extraction of trace levels of compounds of forensic interest on surfaces. The students will prepare and extract inorganic explosive simulants and other components on surfaces and develop methods for extraction and analysis using monolithic capillary electrochromatography. The key to the project is the development of appropriate non-aqueous extraction and analysis techniques to maximize formation of ion pairs. Separation will be performed using novel laboratory-synthesized monolithic stationary phases embedded in capillary columns. These columns will be synthesized based on the photo-initiated polymerization of acrylate polymers and utilized to concentrate and separate the isolates. Students involved in this project will develop skills in polymer synthesis, reaction kinetics, microfluidic electrophoresis, and mass spectrometry (Time-of flight and ESI-MS).

This project focuses on novel ‘bar bell’ shaped molecules having redox functionality at both ends. This architecture provides a unique topological basis for producing redox active molecules capable of bridging narrow gaps, spanning membranes and thin films, and modifying surfaces where electron transfer occurs. Redox reactions are among the most fundamental of sensor mechanisms and can provide exquisite sensitivity and selectivity in the quantitative measurement of a wide range of analytes. Redox-active molecules based on xanthophylls will be synthesized and characterized, and their flexibility will be investigated with computer models. Dr. Landrum will guide the REU student in the synthesis and characterization of symmetric, ligand-modified carotenoids capable of complexing metal ions, using fundamental and well-recognized reaction chemistry. In addition the student will use computational methods to model the conformational mobility of these species under the direction of Dr. Chatfield. The synthesis and computational studies will proceed simultaneously. Calculations on conformational energetics of carotenoids, focused primarily on the single bonds of the polyene chain, will be performed. These calculations will provide insight into the geometric ‘plasticity’ of these and related species and the probability of end-to-end self-exchange redox via folding versus through-bond electron transfer.
REU Project 8. 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/SES1 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


The REU student will test the working hypothesis that superoxide anion radical, singlet oxygen and possibly hydrogen peroxide (from superoxide disproportionation) are the primary reactive oxygen species (ROS) in VLA TiO₂ photocatalysis and that surface effects also play an important role. It has been shown that irradiation of VLA TiO₂ yields both superoxide anion radical and singlet oxygen. In addition, substitution of D₂O for H₂O does not increase the lifetime of singlet oxygen formed under irradiation, suggesting that its reactivity is confined to the catalyst surface. In order to meet the project goals the student will learn to use physical, analytical and chemical methods to investigate the formation, fate, and reactivity of different ROS generated during visible (or UV) light irradiation of VLA TiO₂ in the presence of microcystins (MCs), domoic acid (DA), nodularin (NOD) and cylindrospermopsin (CYN).
Spin trapping using nitrones can provide strong evidence for the formation of ROS. Therefore, the student will learn to use commercially available Electron Paramagnetic Resonance (EPR) spin-traps, such as DEPMPO (5-Diethoxyphosphoryl-5-methyl-1-pyrroline-N-oxide) for superoxide detection. Fluorescein-based deprotection probes, which are highly selective for singlet oxygen, hydrogen peroxide or superoxide will also be employed to corroborate the results of spin trapping studies. The results will be analyzed in order to develop a detailed mechanistic understanding of the photochemistry, which will lay the foundation for VLA-TiO₂ photocatalysis optimization strategies.

**Solar Powered Decontamination**

![Diagram of TiO₂ photocatalysis]

### REU Project 10. Thiol-Arsenic Interactions. Dr. Yong Cai.

The overall objective of this study is to investigate the interactions of thiols and arsenic, and the formation of As complexes with biomolecules, which will provide insight into cellular defense mechanisms and toxicological effects of As. The REU student will synthesize Arsenic-GSH complexes by incubating aqueous solutions buffered at different pH and with different ratios of As(III), MMA(III), DMA(III) and GSH. Analysis of the resulting complexes will be carried out by HPLC-ICP/MS and LC/MS. Kinetic and thermodynamic parameters will be calculated. Effects of pH and temperature, and GSH concentration on complex formation and stability will be identified. Comparison of the results with previously reported values at high concentrations will determine whether the proposed mechanisms are viable at biological conditions. Since this research is a collaborative study between Emory Medical School and FIU, the REU student will have an exposure to an interdisciplinary research environment.


**REU Project 11. 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 studying organic 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 including enzymes, in some cases.
2. To provide visual proof of reaction regioselectivity (or lack thereof).
3. Provide visual proof of enantioselectivity in reductions by enzymes.

Strategy
• Ideally, carry out reductions involving color/phase changes.
• If not devise methods to provide visual evidence of the reaction outcomes, including the use of solvatochromic dyes.

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 of Chemical 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.

Distillation of the product into a cow containing reagents

Jones reagent and Reichardt’s dye confirm primary alcohol

A contains pure 2-propanol.
B contains pure 1-propanol.

REFERENCES

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.


**REU Project 13. 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 14. Bacterial Topoisomerase I as a novel target for discovery of new antibiotics  
– Dr. Yuk-Ching Tse-Dinh
Topoisomerase I is present in all common bacterial pathogens as potential target for bactericidal poison inhibitors. Gene function is essential for viability of Mycobacterium tuberculosis and Helicobacter pylori. Type IA topoisomerases present a novel target for drug resistant bacterial pathogens.28

Structure of the E. coli topoisomerase I N-terminal domain and ssDNA (in green) covalent complex with four domains shown in different colors. The catalytic Y319 is in domain D3. D1 and D4 provide the binding groove for DNA. D2 connects D3 and D4 in forming the central cavity.


REU Project 15. Iron-hyaluronic acid conjugates as ovarian and breast cancer-specific MRI contrast agents. - Dr. Raphael G. Raptis
Advances in the application of MRI in cancer screening, staging, and treatment monitoring have been hampered by a poor tumor-to-background ratio. To improve this ratio, one approach is the development of contrast agents (CA) with greatly enhanced sensitivity and targeting. Molecularly targeted MRI-CAs offer a further dimension of molecular specificity to the anatomical and functional data that MRI and other imaging modalities provide. Targeted CAs typically have both a reporter group(s) and a high-affinity targeting moiety that binds to specific cell membrane receptors/biomarkers, providing the
pharmacodynamic effect of increasing CA relaxivity, and therefore the MR signal. Due to inadequate MRI sensitivity and the low expression level of most cellular targets, strategies based on multiple reporter groups bound to a single targeting moiety to amplify the signal are being developed, as they circumvent both limitations. Some typical biomarkers of breast and ovarian cancers include folate receptors, CD20 and CD44 (the focus in this proposal), which are overexpressed on both histiotypes.

We are synthesizing novel well-defined molecular octanuclear Fe-cluster (Fe₈)-based CAs, with potential application to molecularly targeted MR imaging of ovarian, breast and other cancers. This is achieved by conjugating the paramagnetic Fe₈-MRI module to hyaluronic acid (HA), the natural ligand for cell-surface CD44 proteoglycans, over-expressed on many cancer types as well as on cancer stem cells. We will optimize the composition of the Fe₈-HA conjugates via iterative synthesis-evaluation cycles. The evaluation of the targeted Fe₈-HA conjugates consists of determination of their contrast enhancing properties in vitro and tumor vs. normal targeting, contrast enhancing and physiological properties in vivo (Figure 1).

![Figure 1. T₂-weighted scans of MDA-MB-468-bearing nude mouse before (left) and 45 min after (right) i.v. injection of 7 mg of an HA-Fe₈ conjugate.](image)