

#### Molecular Biology, Genetic Engineering and Recombinant Protein production Genetic engineering of nucleobase-specific ribonucleases

Primary goal of this project is to develop ribonucleases that recognize each of the four nitrogenous bases to cleave the phosphodiester bond in RNA. It includes either improving the specificity of existing ribonucleases, for example, Rnase U2 for adenosine, or engineering novel enzymes to cleave RNA at either uridine (Rnase MC1) or cytidine. The research project employs molecular biology techniques including site-directed mutagenesis, molecular cloning and heterologous expression of recombinant genes in bacteria. The expressed proteins will be purified by affinity chromatography for investigating their RNA cleavage patterns by liquid chromatography and mass spectrometry (LC-MS). Although no prior research experience is required, the student will benefit from having basic background in biochemistry and biomolecules. The project also requires a minimum of a 2-semester commitment by the student.

### **Biochemistry**

## RNA modifications and their relation to stress responses

The goal of this project is to understand the impact of stress (oxidative stress, radiation) on RNA integrity and posttranscriptional modifications in RNA. The RNA following exposure to a defined stress will be analyzed for changes in modification profiles and location of damage. The student will use model organisms to understand these effects. The student will learn cell culture, RNA isolation, radiation treatment and LC-MS analysis techniques. A minimum of 2 semester commitment is required to undertake these studies. Although prior experience is not required, the student has to have interest in biochemistry, radiation biology and nucleic acid analysis techniques.

### **Bioanalytical Chemistry and Method Development**

### Sample preparation method development for LC-MS analysis of modified ribonucleosides

One of the key factors that determine the success of LC-MS analysis is sample preparation and dynamic range levels of targeted compounds for a given sample source (also termed as matrix). The goal of this project is enrichment of modified nucleosides through optimization of binding and elution conditions of RNA hydrolysate mixtures (composed of unmodified and modified ribonucleosides). The student will test the various stationary phases made from different metal oxides for their efficacy to retain and elute cis-diol containing ribonucleosides and 2'-O-modified ribonucleosides. The protocols will initially be optimized with commercially available RNA samples. These optimized conditions will be used to detect ribose methylations of mammalian tRNA. The student is required to have interest and/or background in analytical chemistry of biological molecules.

### Identification of optimal conditions for RNA modification mapping by LC-MS

The goal of this project is identification of optimal mass spectrometry conditions for mapping chemical modifications in RNA, referred to as RNA modification mapping. The student will evaluate the efficiency of different lab protocols and instrument conditions for qualitative and quantitative acquisition of mass spectral data with a high mass accuracy mass spectrometer.

The conditions so identified with the commercially available RNA samples will be utilized to map tRNA modifications in human RNA samples. The student will get familiar with the sample preparation, electrospray ionization, parameters involved in liquid chromatography coupled with mass spectral acquisition and data analysis in a semester or two semester time frame. The student is required to have interest and/or background in analytical chemistry of biological molecules.

Office: 305 Crosley Telephone: 513-556-9370 Email: Noe.Alvarez@uc.edu

The *Alvarez Lab* research is focused on carbon nanomaterials synthesis and assembly into macroscopic materials for sensor applications. We synthesize and assemble carbon nanotubes (*Fig 1*) into fibers and films and use them for physiological and electrochemical sensors, as well as energy storage devices. Besides of the fundamental chemistry such as synthesis and electrochemistry, students in the Lab are exposed to engineering aspects of nanomaterials development.

*Physiological Sensors:* We are developing microelectrodes suitable for physiological applications such as recording extracellular activity for neuroscience research and the ability to stimulate neurons in a targeted fashion. Brain related treatments like epilepsy and Parkinson's disease,



Fig. 1: Single a and 4-walled carbon nanotubes

require microelectrodes implants that are flexible, biocompatible and reliable electrodes. This research is focused on a bottom-up approach that allows us to combine carbon nanotubes (CNTs) into macroscopic flexible electrodes that can be adjusted to application-specific requirements. Electron transfer rates are currently under study using Electroretinogram (ERG) for signal recording and electrical stimulation. Biocompatible polymer coatings and control over their porosity and stiffness are topics of interest in neuroscience as implants to prevent damaging brain tissue.

*Electrochemical Sensors:* Detection of heavy metals in our drinking water has become high priority for our societies, particularly for people living in cities where water infrastructure was built more than 50 years ago. This research intents to develop an electrochemical sensor based on CNTs to detect toxic metals such as Pb, Cd and Hg that have been detected in drinking waters. The sensor will quantify trace levels of multiple heavy metal ions simultaneously and should operate autonomously. Current electrochemical approach employs anodic stripping voltammetry and can detect nanomolar concentrations, however the sensitivity heavily depends on the material characteristics. Besides of the material, miniaturization of the electrodes allows the fabrication of small sensors that can potentially assist human wellbeing.

*Energy Storage Devices:* Miniaturized, flexible and wearable electronics devices are topics of growing interest. Powering these devices needs for compatible energy storage units that can exhibit similar mechanical strength and flexibility. Fiber-based energy storage devices that are light-weight, and flexible can be easily integrated into textiles. The large surface area, electrical conductivity, and mechanical strength of CNTs makes their fiber assemblies an ideal material for fiber supercapacitors and batteries. This research is oriented to the development of microns thick energy storage devices based on CNTs and thin films in combination with polymers.

Research in the Ayres Lab is focused on synthetic polymer chemistry for applications in biomaterials. We have ongoing projects investigating polymer biomimics for blood-contacting biomaterials, reversible gels with self-healing properties, and shape memory polymers. The experiments we perform are rooted in organic synthesis, but no prior knowledge of polymer science is required, and we welcome undergraduate student participation, especially from transfer students.

### Synthesis of copolymers for biomaterials used to repair bone

In this project we are preparing a polymer called poly(propylene fumarate) and copolymerizing it with different monomers to impart new value-added properties. Poly(propylene fumarate) is

biodegradable and has been used for a variety of tissue engineering and regenerative medicine strategies, in particular those strategies focused on repairing bone. However, poly(propylene fumarate) suffers from low strength and hydrophobicity. We believe we can solve these continuing problems with



our synthesis routes. We prepare a range of sulfur-containing polymers where we control the material properties such as brittleness, hydrophilicity, surface charge, or presence of functional groups and then cross-link the poly(polypropylene fumarate) with these sulfur-containing polymers using a photochemical thiol-ene addition reaction.

### Synthesis of shape memory polymers



Shape memory polymers are polymer materials that can be fixed into some temporary dimensions, but upon experiencing

(From L-to-R) Picture of fused salt template; SEM image of fused salt template; picture of polyurea/urethane foam; SEM image of open cell foam.

some stimuli the material can change dimensions to a previously determined permanent shape. We have taken advantage of this phenomenon to prepare shape memory foams that are blood compatible, i.e. prevent the formation of blood clots. The foams we prepare are every easy to make and use a salt template. By changing the properties of the salt template we can change the properties of the resulting foams, including their strength, flexibility, and how much they can expand or contract. These changes in physical properties will affect their biocompatibility. Undergraduate researchers in the Ayres group can contribute to these projects in many different ways. To learn more about our research please contact Dr. Ayres at neil.ayres@uc.edu or visit our website at ayreslab.squarespace.com.

# **Undergraduate Research Opportunities in the Baldwin Group**

Our research group is interested in designing new transition metal complexes that may have useful applications, and are inspired by bioinorganic systems, but are not limited to biologically available components. For example, one project in our group uses Ni(II), which is generally unreactive with O<sub>2</sub>, to catalyze oxidation of various organic compounds using O<sub>2</sub> as the oxidant. Catalysis of aerobic substrate oxidations like this is considered to be environmentally friendly "green chemistry". This chemistry is accomplished by choosing ligand donor groups, oximates, which form a remarkable metal-organic redox hybrid with the nickel. This kind of redox hybrid is used in nature by various metalloenzymes, including the copper-containing galactose oxidase and amine oxidases, which catalyze the same kind of chemistry as our nickel complex. Another project in our group involves the development of bio-inspired, light-activated metal transport agents. These complexes bind Fe(III) very tightly, but release it as Fe(II) upon photolysis by an appropriate wavelength of light. Among the potential applications of these "siderophore mimics" is site-specific delivery of an activating metal to a metal dependent pharmaceutical, such as a chemotherapy agent that would be activated by the light-triggered release of an appropriate metal only at the tumor site.

A typical undergraduate project in any of these areas would involve synthesis of a new ligand designed for the particular application, characterization of its metal complex (with nickel, iron, or other appropriate metal), and screening the new complex for the desired chemistry. This will provide the student with experience in organic and inorganic synthesis, a variety of spectroscopic and analytical methods, and the evaluation of useful new chemistry. Several undergraduates working on these projects have become co-authors on published papers based on their research.

Computer simulation, quantum chemistry, water, biophysics, energy science, batteries, supercapacitors. We use computer modeling to study complex systems involving liquids, interfaces, and proteins. The research has wide applications in modern chemistry.

### **Bioinformatics methods for targeted drug-design:**

Our group is developing and applying database mining approaches and other bioinformatics methods to the determination of binding motifs at interfaces between various biological molecules; the goal of this research is to build a repository of specific and non-specific interactions between macromolecules which can be used for targeted drug-design.

## Computer modeling of biological macromolecules dynamics and function:

We are studying the conformational space in proteins using simplified methods that encode specific characteristics of the polypeptide chain; an example of a project is to target the metastable states that represent obligatory intermediates on the pathway of folding of a protein from a fully unfolded state to its native functional form. Knowledge of all the relevant intermediates for the reaction from the unfolded to the folded form of a molecule can be used to gain insight into the details of its function.

The research in the Guan group lies at the interface between inorganic and organic chemistry focusing on the development of homogeneous catalysts based on first-row transition metals such as nickel, cobalt, iron and copper. Such efforts are motivated by the fact that precious metals, which are widely used today in catalysis for synthesizing commodity and specialty chemicals, are expensive, limited in supply, and sometimes difficult to remove from organic products. The challenge of using first-row transition metals for catalysis starts with the difficulty in identifying ligands that can not only bind tightly to the metals but also promote precious metal-like reactivity or de-emphasize metal's role. Our investigation of pincer-ligated metal hydrides has led to the discovery of nickel and iron based catalysts for the hydroboration of CO<sub>2</sub> to methanol derivatives and the hydrogenation of fatty acid methyl esters to fatty alcohols. Our ongoing projects build on these initial successes and focus more on the improvement of catalytic efficiencies through further modification of the catalyst structures. Students involved in these projects will learn various synthetic techniques including the handling of air- and moisture-sensitive compounds. They will also be trained to conduct mechanistic studies using NMR spectroscopy, X-ray crystallography, and chemical kinetics. Furthermore, the research projects will teach students the concept of increasing energy efficiency by performing catalytic reactions and the notion of sustainability by using renewable feedstock and readily available materials.

### **Photorelease of Fragrances**

We are interested in the release of alcohols since they are used as fragrances in applications such as body care and household cleaning goods. One of the drawbacks of using volatile alcohols in fragrances is that the desired aroma is detected for only a relatively short time in applications. Thus by forming a phenyl butyric ester (1) of a volatile alcohol it is possible to release the fragrance in a controlled manner over an extended time period by exposure to light.

An undergraduate project would focus on preparing various derivatives of ester **1** and investigate if they release alcohol upon exposure to sunlight. The undergraduate student working on this project will gain experience in carrying out simple synthesis and



how to purify the starting material by column chromatography. The student will also learn to use 1H-NMR, IR and MS spectroscopy to characterize the starting materials.

## Solid State Photoreaction: Green Chemistry

One of the advances of studying chemical reactions in the solid state is that it reduces the use and disposal of potentially hazardous solvents, an important consideration in this era of

increased environmental awareness. Furthermore due to the restricted motions of molecules in crystals, solid state reactions are generally more selective than their counterparts in solutions. Crystal lattices can therefore pose as an effective technique for controlling chemical reactivity. We are interested in study the reactivity of azidoarylketones in the solid state, since they can be used to make interesting heterocyclic compounds.



An undergraduate working on this project would focus on synthesizing and characterizing various derivatives of the azidoarylketones. The photoproducts from solution and solid state photolysis of these compounds can then be isolated and characterized. Obtaining X-ray structure analysis of the staring material will allow us to connect the solid state reactivity with the structure of the starting material. By studying a series of closely related compounds we can attempt to correlate the molecular structure and the molecular packing arrangements in the crystals, a concept known as "crystal engineering". The ultimate goal of this research is to

control the regioselectivity of photochemical reactions by slight changes in the molecular structure of the substrates to obtain specific crystal lattices.

The student working on this project will gain experience in carrying out synthesis in solutions and in the solid state. The student will have contributed to the development of using crystals as a reaction media for synthesis.

## **Statement of Research in the Jiang Group**

The interdisciplinary research in the Jiang group involves expertise in a wide variety of areas (materials science, renewable energy, molecular engineering, and spectroscopy). Undergraduate students in our group will have options to work on various project(s) based on their expertise and research interests.

Our research interests center on the development of advanced materials with novel properties to address the main question in the renewable energy field. Leveraging synthetic and molecular engineering techniques, we will design and synthesize a series of model materials to understand the molecular interactions underpinning renewable energy storage. Our research is focused in two areas: catalysis for renewable fuel production and rechargeable battery for energy storage, as briefly described below.

Photo- and electrocatalysis for renewable fuel production. Photocatalytic nanoreactors have

the advantage that substrates and catalysts are confined in a nanosized compartment for improved compatibility and catalytic performance. Our group will design and construct self-assembled, hybrid nanoreactors as a platform for photocatalysis of various substrates, with special interest in  $CO_2$  photocatalytic reduction to renewable fuels. Each of the photosynthetic components (photosensitizers and catalysts) is positioned at specific sites within the nanoreactor to allow efficient energy transfer from the chromophores to the catalytic centers. The modular hybrid

architecture facilitates the molecular engineering for overall efficiency improvement. Metrics of success include product selectivity, catalyst stability, and electrochemical (over)potentials.

Next-generation rechargeable batteries for stationary and mobile energy storage. Redox

flow batteries (RFBs) have received increasing attention as a largescale stationary energy storage technology due to their safety, cost efficiency and scalability. Enhancing energy density is one of the most pressing issues in the development of RFBs. Our group focuses on the development of highly soluble, multielectron-active, polymeric metal complexes with tunable redox properties for improved overall battery performance. Synthetic tools will be used to prepare a series of metal complexes with variations on metal centers, peripheral ligands, and polymer backbones and side chains. Electrochemical and mechanistic studies will then be performed to elucidate the structure-function relationships to provide information to design, synthesize and characterize the next generation of polymeric metal complexes with



combining desirable properties. The identified candidates will be tested in flow batteries, and the feedback obtained will facilitate iterative development of polymeric metal complexes with improved performance.

Fast-growing demand for high power-density batteries for electronic devices and electric cars has motivated the exploration of energy storage devices with high charge capacity and stable cyclability. The lithium-sulfur battery has a high theoretical specific capacity yet has been retarded by the loss of active sulfur species during charge/discharge cycles. Our group will design and synthesize a saccharidebased polymer cathode material to (1) entrap the sulfur material in the cathode region by covalent



cathode material. Sulfur redox reactions and confinement mechanisms will also be studied at the molecular level, the results of which will in turn guide iterative material designs for better performance.

<u>Poly(ADP-ribosy)lation (PARylation) is a reversible post-translational modification that regulates</u> DNA repair, gene expression, and cell fate. Targeting poly(ADP-ribose) (PAR) metabolism and signaling emerges as a promising strategy for tumor-specific therapy and precision cancer medicine. Research projects in our lab are focused on understanding the structure, mechanism, and function of a PAR turnover enzyme PARG, a PAR-dependent tumor suppressor CHFR, and PAR signaling proteins. We are particularly interested in developing small-molecule modulators

of those proteins as novel anticancer therapeutics. Students will use multidisciplinary research tools, including X-ray crystallography, timeresolved fluorescence resonance energy transfer (TR-FRET), small-angle X-ray scattering (SAXS), highthroughput screening, various biochemical assays, and chemical synthesis, to investigate 1) how proteins and domains communicate for their specialized functions, and 2)



how they specifically recognize small-molecule ligands and substrates.

**Project 1. Structural biology of PAR turnover and signaling proteins:** Poly(ADP-ribose) metabolism is essential for maintenance of genomic integrity and downstream DNA damage response. PAR glycohydrolase (PARG) is a primary enzyme that turns over PARylation and thereby counteracts the PAR synthesis by PARP1. Using X-ray crystallography and SAXS, we are investigating the structure and protein-substrate/inhibitor interactions of PARG and downstream PAR signaling & DNA repair enzymes. This structural work (*project 1*), along with a high-throughput assay (*project 2*), will be a framework to develop novel chemical modulators/probes (*project 3*) for these bio-medically important enzymes.

**Project 2. Development of a high-throughput TR-FRET assay for PAR turnover and signaling proteins:** We are using a <u>Time-Resolved Fluorescent Resonance Energy Transfer (TR-FRET) as a</u> primary assay system. TR-FRET assay can be developed in a 384-well format that is suitable for high-throughput screening, and is a convenient mix-and-read type of assay with very low background signal. We are currently using or developing a TR-FRET assay for PARG, CHFR, DTX3L, and DNA ligase III for biochemical analysis of their enzymatic activities and for highthroughput screening.

**Project 3. Cancer drug discovery through a high-throughput screening and a structure-based lead optimization:** We aim to discover and develop new cancer therapeutics by targeting PAR metabolism and signaling pathways. Once we establish a TR-FRET assay (**project 2**), we actually run a pilot screening or medium- to high-throughput screening *in house* to identify new chemical scaffolds. We will optimize our lead compounds through a structure-activity relationship and a high-resolution crystal structure of protein-ligand complex.

### Aquaponics

Our research group is interested in the water chemistry of aquaponics systems. Aquaponics is the integration of fish farming and hydroponic vegetable growth. The waste produced by the fish and the uneaten feed is treated with the help of nitrifying bacteria to transform ammonia into nitrates, as the preferred vegetable nitrogen source. The water is pumped from the fish tank into a bio-filter, then by gravity if flows to a floating raft hydroponic grow bed, and then returns to the fish tank, creating a closed re-circulating system. The advantages of this emerging food production system are the dramatic reduction of water needed, the elimination of polluted water discharges and the addition of a secondary crop in the form of vegetables. Aquaponics is a very promising food production method, but many technical aspects need to be refined before it can be widely applied. The nutrient balance and a strong bacteria population are the key for success. We explored the use of legumes as means of nitrogen recovery alternative with very good results. The accumulation of toxicants within the systems is our biggest concern, specifically toxic elements like As, Cd, Pb, Hg, and nutrients that in high levels are toxic like Se. The closed-loop design with constant addition of feed and water makes accumulation of these toxicants a possible drawback.

In order to understand the mobility and accumulation of toxic elements in the aquaponics systems a comprehensive water chemistry description is necessary. Water parameters like pH, dissolved organic matter, oxygen content and temperature are expected to alter the mobility and chemical speciation of the elements and therefore its toxicity. We currently have a complete analysis on the distribution of Se and Hg in aquaponics systems. Se accumulates in fish and solids as Se<sup>0</sup> which for a long term system presents a problem. Mercury gets accumulated in roots, while we are exploring the possibility of the bacteria reducing and volatilizing it as adaptive protection mechanism.

The systematic study of the effect of water parameters in the mobilization and chemical transformations of Se and Hg are of high interest for improved system reliability. The accumulation of organic toxicants is another research venue for undergrad research work.

## Role of Zn in immunity

The integration of biomedical research with analytical chemistry is a necessity to advance in the life sciences. Our group has a strong collaboration with the UC infectious diseases department, where we study the effect of Zn and its chemical distribution on the immune response against intracellular pathogens. The versatility of the immune cells allows macrophages to act on different scenarios, where sometimes wound healing is needed, versus when an infection is combated. By using atomic and molecular mass spectrometry coupled to liquid

chromatography we can elucidate changes in low molecular Zn and the Zn-proteome in response to different white blood cells activators. By combining molecular biology methods like gene silencing and transcription analysis, we have unveiled the role of Zn in the modulation of macrophages to accommodate to its different functions. The effects of Zn on the immune system are just recently being explored in detail, and we have a very broad spectrum of effects to be studied in detail. The cell metabolism, is altered depending on the levels of free Zn and this affects the way the cells interact and function.

This great collaboration is a good opportunity to learn the resources that an analytical chemistry lab has to offer to the biomedical research. Several parts of this project can be performed at an undergraduate research level.

My research interests can be divided into two areas of research

- 1. Host-Guest Interactions of Fullerene Fragments
- 2. Green Chemistry

### Host-Guest Interactions of Fullerene Fragments.

Fullerenes and nanotubes have unique electronic properties which potentially will play a large role in the field of nanotechnology. Since these molecules have exhibited such potential, interest has expanded to incorporate the area of fullerene fragments. Fullerene fragments, also known as bowl-shaped polyarenes, are molecules which map onto the surface of fullerenes. One such molecule is corannulene,  $C_{20}H_{10}$ , which represents 33% of a

fullerene[60] molecule. Similar to fullerenes, fullerene fragments possess an electron-poor convex shell and an electron rich concave surface. The electron-rich concave surface may exhibit strong sites for cationic binding. We are interested in synthesizing molecules which covalently link two fullerene fragments for the study of their interactions with various guests. One such target molecule in this class is a [6,6] 1,8-corannulene cyclophane with enediyne bridges. Using the enediyne bridges to connect the two fragments may allow the molecule to accommodate large guest such as fullerenes as well as small guest such as ammonium ion.



### Green Chemistry.

The chemical community has recently been concerned with green chemistry. These concerns have led to an increasing interest in chemical waste minimization. One of the primary sources of chemical waste is volatile organic compounds (VOCs). Many VOCs have been targets of waste minimization since the Clean Air Act Amendments of 1990. In research

laboratories organic solvents generally comprise most of the waste involved in a reaction. Common practice has been to use milligram quantities of reagents and gram quantities of solvents. At the conclusion of such reactions the small amounts of reagents are recovered and the large volumes of solvent discarded. VOCs such as carbon tetrachloride and benzene both appear on several of the EPA's minimization priority lists. Carbon tetrachloride has been shown to be an ozone depletion chemical, while benzene is a known carcinogen. Although both of these chemicals have a history of environmental disdain they are continually used in the research laboratory as well as in industrial processes, especially in the area of radical chemistry. Benzene has been cited in more than 1,400 publications as the solvent for various reactions in 2002. Likewise carbon tetrachloride, given its tag as an ozone depletion chemical and its mark up in price over the past several years has been used as a solvent in more than 400 publications in 2002. We are interested in exploring solvent-free reactions utilizing high-speed vibrational milling. HSVM is a procedure in which solid reactants (crystals or powders) are placed inside a steel vessel along with ball bearings. The vessel is sealed and placed inside the milling apparatus whereby it is vigorously agitated. The high speed agitation (60 Hz) forces the ball bearings to pulverize the reagents, causing them to react. Reactions under HSVM conditions potentially will have large impact in organic synthesis with little to no solvent waste.

*Reactive Oxygen Responsive Bioactivation*. Though the term excessive reactive oxygen species (ROS) and oxidative stress are widely used their biochemical basis and methods to utilize this unique environment remain limited. The most useful definition put forward has been that excessive ROS is a state with a disruption in ROS signaling and control. Importantly, ROS molecules tend to be transient, hard to detect, and location-specific causing a lack of a chemical understanding of this key cellular process. Current detection and activation strategies are lacking. There are several issues with exsiting methods including (1) high rates of reactivity with ROS, (2) limited substrate scope and (3) a limited ability to tune the reaction rate. To overcome these issues we have designed Reactive Oxygen Responsive Bio-activation. We covalently attach an intact bio-active molecule to a particular scaffold. This attachment leads to a loss of activity. In high ROS environments the bio-active molecule is released. Chemically inclined undergraduate researchers will either synthesize the agents and more biologically inclined students will determine the activation rates (Km and Vmax) in the presence of biologically relevant oxidases and within cells.

## The Conversion of an Epoxide to a Cyclic Carbonate

Several years ago, we discovered the very easy and synthetically useful conversion of an aziridine (a three membered ring compound with nitrogen in the ring) to an oxazolidinone (a five membered heterocyclic system) using carbon dioxide (CO<sub>2</sub>). This stereo- and regiospecific reaction may be done using THF or water as the solvent, and it also may be done in the absence of any solvent. This reaction uses common salts, such as LiI, KBr, and NH<sub>4</sub>I, as the catalyst.

Our goal is to extend this chemistry to the conversion of an epoxide (1) to a cyclic carbonate (2) using similar reaction conditions.



An undergraduate student will investigate various substitution patterns on the epoxide, different solvents, and different salts as potential catalysts. In addition, our goal will be to determine the regio- and stereochemistry of the conversion of compound **1** to compound **2**.

## Generation of Carbon-Carbon Bonds with Water as the Solvent

Fenton's Reagent (FeSO<sub>4</sub> and  $H_2O_2$  in water) is well known for its ability to remove a hydrogen atom from DNA and cause the double helix to unravel. It also causes the degradation of other biological systems, such as proteins and lipids. In contrast, we have studied the use of Fenton chemistry not to destroy a molecule, but rather to create new carbon-carbon bonds. Specifically, we have investigated the radical coupling of acetonitrile, acetone, and acetophenone using water as the solvent.

Two molecules of acetonitrile (**3**) couple to give succinonitrile (**4**) in good yield. However, the coupling of two molecules of acetone (**5**) only occurs in very low yield. Surprisingly, when acetone and acetonitrile are mixed, the acetone coupling product **6** predominates.



An undergraduate student will investigate ways to improve the yield of the coupling of ketones and also will investigate the coupling of various other functional groups.

#### **Dr. Ashley Ross**

Office: 201 Crosley Telephone: 513-556-9314 Email: <u>Ashley.Ross@uc.edu</u>

Research in the Ross lab focuses on developing methods to probe signaling within and between the brain and the immune system. Our lab primarily uses a combination of electrochemistry, microfluidics, and fluorescence microscopy to reveal the intricate signaling network across these organs. We are specifically interested in understanding the mechanism of how neurotransmitters are regulating important "danger signals" within the brain and how this communication is extended into the immune system during autoimmunity. Undergraduates are required to commit to a minimum of 2 semesters in the Ross lab so that adequate learning of the analytical methods and contribution to the overall goals of the lab (discussed below) are achieved. Students will have the opportunity to aid in the following projects:

#### Characterizing guanosine signaling using fast-scan cyclic voltammetry:

Intricate chemical signaling within and between the brain and the immune system governs much of health and disease. Neurotransmitters and neuromodulators are key components of this signaling network, and their signaling pathways often go awry during neuroinflammation and disease. Guanosine, a purine nucleoside, is an important extracellular signaling molecule and neuromodulator in the brain and is often used as a therapeutic for neuroinflammation. Despite its therapeutic uses, real-time detection methods for quantitative monitoring in both the brain and immune system have not been fully developed. Guanosine is electroactive and easily detected with electrochemical techniques. Fast-scan cyclic voltammetry at carbon-fiber microelectrodes is an electrochemical method often used to detect real-time neurotransmitter release, like dopamine (Fig.1), in the brain. Guanosine oxidation at carbon-fiber microelectrodes has not been fully explored with fast-scan cyclic voltammetry. Students on this project will help characterize guanosine detection at carbon-fiber microelectrodes by fabricating, designing, and testing electrodes.



**Figure 1:** Dopamine detection using fast-scan cyclic voltammetry at carbon-fiber microelectrodes. Dopamine undergoes a quasireversible 2-electron oxidation reaction to dopamine-ortho-quinone and is detected at a 7-µm in diameter carbon-fiber microelectrode.

#### Electrochemical detection of neurotransmitters and proteins in the lymph node:

The lymph node is a tiny organ important for regulating appropriate immune responses during both health and disease. Evidence suggests that neurotransmitters, like dopamine and serotonin, can be synthesized and released from immune cells; however, the mechanisms of release and clearance within the immune system is unknown. Dopamine and serotonin signaling is believed to be vital for modulating specific immune responses but real-time detection of these analytes in the immune system has not been done. Additionally, cytokine signaling (important proteins) in the lymph node is thought to be regulated by dopamine and serotonin under specific inflammatory conditions; however, real-time electrochemical detection of protein secretion in lymph node tissue has not been explored. Students on this project will aid in developing electrochemical tools to probe both neurotransmitter and protein signaling within the lymph node in real-time.

#### Local stimulation and modulation of tissue samples on-chip:

Microfluidics is a powerful platform which utilizes micron-size features to manipulate tiny volumes with precise spatial and temporal control. Undergraduate students will aid in design and fabrication of microfluidic platforms for studying local stroke within the brain and cross-communication between the brain and the immune system.

The Sagle group has two main interests: (1) the development of biocompatible surface enhanced Raman spectroscopy substrates, so that one can measure *single* biological molecules and (2) the development of new bionanomaterials, mainly for biosensing. There is ample opportunity for undergraduate students to take part in both projects.

**Single Molecule Biophysical Measurements Using SERS.** Single molecule SERS is an emerging new field that has not yet been applied towards biophysics effectively and offers a lot of advantages over other techniques in its ability to probe protein flexibility, carry out site-selective folding measurements, and measure specific interactions. Because proteins are associated with plasmonic nanoparticles, this technique also offers the ability to carry out the first nanoparticle-assisted temperature-jump folding studies. Some of the first proteins used in this new technique will be horse heart Cytochrome c, Hemoglobin A, and the SH3 Domain. Proteins of interest will be trapped inside a liposome in which metallic structures are attached to or grown on the outside. As an undergraduate researcher in the group, some of the first measurements may involve (1) comparing the spectroscopic properties of different hollow nanoshell or liposome-based materials or (2) comparing the folding properties of proteins in solution versus proteins encapsulated in our substrates.

Biosensing With Protein-Nanoparticle Biomaterials. Currently, localized surface plasmon resonance (LSPR) based biosensors are more sensitive than other types of biosensors, but specificity and biofouling (the binding of unwanted substances) plague many applications. By linking plasmonic nanoparticles together with proteins to make 2-D protein-nanoparticle arrays, these problems can be circumvented. These 'smart' proteinnanoparticle arrays yield a response that is not driven by something binding to the surface, but rather a protein binding a specific ligand and changing conformation, thus changing the spacing between the plasmonic particles. These type of biosensors are expected to be much more sensitive because changes in protein conformation will give rise to differences in plasmonic coupling, which should shift the LSPR peak maximum (or color) by hundreds of nanometers instead of only 5-10 nm that is typically observed by LSPR-based biosensors currently being used. In addition, these biosensors should have increased sensitivity and resistance to biofouling since the response comes from a specific ligand binding to the protein and not a change in local index of refraction. These protein-nanoparticle arrays will be built from proteins known to form discrete large-scale structures both in solution and on surfaces, such as collagen microtubules and fibrinogen. Other proteins of interest can be attached to these structure-forming proteins via genetically engineered fusion proteins and nanoparticles will be attached via covalent and noncovalent linkages. These biosensing arrays will eventually be incorporated into microfluidic, on-chip devices for sensing of markers associated with various diseases such as cancer and Alzheimer's.

**Biosensing With Flexible Nanoparticle Arrays.** Drastic improvements in biosensing sensitivity and versatility can come from interfacing patterned nanoparticle arrays with optical fibers and cavities. This combination can pave the way for not only next generation biosensing devices, but also improved electro-optical devices. These projects will involve using nanolithography to pattern nanoparticles onto various flexible plastic substrates, and then wrap

these substrates around commercially available optical fibers. The substrates will be characterized using microscopy and the optical effects characterized using UV-Vis spectroscopy.

The Smithrud Research Group uses mimetic chemistry to investigate the intricate interplay that exist between functional groups at protein binding domains in order to determine the underlying forces that give proteins their unique ability to recognize molecules and to develop novel synthetic devices. This exploration involves using various synthetic and analytical techniques, such as 1D- and 2D-NMR spectroscopy, fluorescence and UV-vis spectroscopy, and fluorescence microscopy. Molecular modeling calculations are performed to provide insights into the binding mechanisms and to guide in the development of the next agents. Our research has produced novel binding agents, cell transport agents, and compounds that bind the major groove of DNA. Current projects are to develop smart contrast agents to advance MRI diagnosis, construct and investigate novel aptamers that selectively target cancer cells, and develop anticancer agents that deliver Ca<sup>2+</sup> into cells.

**Rotaxane binding agents** Novel artificial receptors and antibodies, based on the rotaxane architecture, have been constructed and shown to ineract strongly with a variety of guests in a variety of solvents, including water. Host-rotaxanes are readily constructed in a few synthetic steps. A simple switch of the recognition pocket results in binding agents that show a dramatic difference in guests from aromatic compounds to cations.



**Cellular transport agents** Host rotaxanes (HRs) bind and transport fluoresceinated peptides into cells through a passive mechanism. The rotaxanes operate as molecular machines, relying on the wheel to adjust its position to counteract the inhospitable environments encountered with

cellular delivery. The next step in to make switchable rotaxanes, whose operation is controlled by external stimuli. With such smart devices in hand, we will be able to control the delivery a variety of compounds into cells and construct a wide range of sensors for industrial and environmental



devices.

New metal cation delivery devices for novel therapeutics. Crown ether rotaxanes, such as 15C5, selectively bind Ca<sup>2+</sup> ions and deliver them into cells, resulting in cell death via apoptosis. The next steps for drug development include derivatizing the CEHR's to enhance toxicities, link them to a RNA-aptamer to provide cancer cell selectivity, and further investigate the mechanism of cell death.



Research in Stan's group is focused on computational modeling of biological nanomachines involved in essential protein quality control mechanisms of protein folding assistance and degradation.

We develop and apply computational molecular modeling tools, such as the widely used program CHARMM, in combination with extensive data mining of protein databases. This is an opportunity to acquire a diverse set of computational skills and apply them to problems of biomedical interest. In addition, our supercomputer cluster provides a chance to learn about designing and maintaining high-performance computers for data intensive applications.

#### Molecular modeling of chaperonin-assisted protein folding

Chaperonins are biological nanomachines that employ a spectacular mechanism to assist protein

folding. During the chaperonin cycle, concerted, large scale, rigid body conformational changes, ultimately driven by ATP hydrolysis, result in a dramatically expanded chaperonin cavity serving as folding chamber. Currently, very little is known about the annealing action of eukaryotic chaperonins. Questions that we are trying to address are what are the chaperonin binding sites for substrate proteins, how does protein folding assistance take place in the absence

of a change in chemical environment and how does the sequential opening of the eukaryotic chaperonin promote protein folding.

#### Computational models of protein degradation by bacterial proteases

Protein quality control such as degradation mechanisms prevent deleterious off-pathway reactions of misfolding or aggregation. In the degradation pathway, AAA+ (ATPases associated with various cellular activities) nanomachines, such as bacterial Caseinolytic protease (Clp) ATPases, unfold and translocate SPs through narrow central pores as a prerequisite for the ultimate destruction of the polypeptide chain within the peptidase chamber. We focus on probing the dependence of the Clp ATPase unfoldase function on the direction of application of mechanical force. Multiscale modeling of Clp-mediated unfolding of SPs with discernible mechanical anisotropy will yield detailed information of these properties. We propose to probe mechanisms of unfolding and translocation along the restricted direction of the N-C termini. The substrate proteins to be studied will involve wild-type and variants of proteins probed in single-molecule experiments. Unfolding and translocation patwhays obtained in these studies will be contrasted with those in multidirectional pulling geometries, which mimic the cellular environment.

Office: 430 Rieveschl

Telephone: 513-556-0227

Email: yujie.sun@uc.edu

Undergraduate students are encouraged to join our group to participate in a variety of multidisciplinary research projects. The overall theme of our laboratory is to use our expertise in coordination chemistry, materials science, electrochemistry, and photochemistry to address two frontier challenges: energy and health. Students will be trained in a broad set of skills and inspired to become critical-thinking scientists with a passion on solving urgent problems from a fundamental science perspective.

Currently, we are interested in the following directions:

(1) Inexpensive solid-state materials for renewable energy catalysis and organic transformation, including water splitting,  $H_2$  oxidation,  $N_2$  reduction, and biomass valorization.

(2) Bioinspired molecular catalysts for small molecule activation, such as O<sub>2</sub> evolution.

(3) Photoactivable complexes and materials for luminescence sensing and phototherapy.



Undergraduate research in the Tsang laboratory involves introduction and analysis of the structure and function of important biochemical molecules such as proteins and nucleic acids. The research is focused upon understanding how essential proteins involved in protein biosynthesis recognize and interact with their cognate RNA molecules using techniques such as gel electrophoresis, spectroscopy and fluorescence.

#### The White Group @ UC

#### Overview

Our research lies at the intersection of nanoscience, electrochemistry and the biological interface. Research interests in our group focus on the development of new (bio)analytical methods to probe chemical and biological systems with unprecedented spatial and temporal resolutions afforded by working at the nanoscale. The scope of this research is quite broad, ranging from studies of fundamental chemical and biological phenomena to the development of applied sensor technologies. As such, students in the group can expect to gain valuable interdisciplinary laboratory experience.

### **Electrochemical Biosensors and Bioanalytical Chemistry**

The White Group @ UC develops electrochemical biosensors to tackle a wide variety of bioanalytical applications ranging from single cell analysis to biomedical diagnostic devices. As such, undergraduate students can become involved in different aspects of biosensor developing. The projects include fundamental studies of the electrochemical behavior of the sensor readout, biomolecular engineering and design of nucleic acid-based recognition elements, and the development and use of protein channels for single molecule sensors. Sensor development is always rooted in the application of the sensor and students will have opportunities to then apply their developed sensors to different biological systems.

### **Nanoelectrodes and Sensors**

In line with our bioanalytical applications, we aim to develop small-scale sensors that are amenable to single cell analysis and electrochemical imaging. Undergraduates working in this area can become involved in new ways to make small electrodes, electrode characterization, sensor fabrication, and ultimately electrochemical imaging using scanning ion conductance or electrochemical microscopy. Nanoscience is at the unexplored frontiers of science and engineering, and it offers one of the most exciting opportunities for innovation in technology. One of the hopes for nanoscience and technology is that the combination of a number of areas - from physics and chemistry to material science and biology - will create a new area and lead to major advances both in understanding of science and in their applications in technology. Key to this new era is research across many disciplinary interfaces. As illustrated in the following diagram, the central theme of this research program is metal-enhanced spectroscopies and their applications, typically biorelated, based on various types of nanomaterials.

### Development of upconversion nanomaterials for sensing

At present, luminescence-based assays generally provide high sensitivity, large dynamic range, and the simultaneous use of multiple fluorophores with different spectral characteristics (multiplexing). Nevertheless, greater sensitivity, improved multiplexing, and performance under extreme test conditions is continuously demanded. On the other hand, upconversion emission, i.e., the emission of light at shorter wavelength than the excitation, has been observed and studied in many lanthanide-doped bulk materials. In this effort, we intend to synthesize lanthanide-doped photon-upconverting nanoparticles. Once such upconverting nanoparticles are prepared, their surfaces can be easily modified to conjugate biomolecules of interest. Because most non-target materials under study do not possess upconversion properties, an enhanced signal-to-noise ratio is expected when these phosphor nanoparticles are used for sensing, imaging and photodynamic therapy. The ultimate goal of this project is to develop biocompatible nanomaterials for biologically related applications.

#### Development of nanoparticle-based photosensitizers for photodynamic antibacterial therapy

We are developing nanoparticles as photosensitizers to be used in photodynamic antimicrobial therapy. The particle sizes range from <10 to 100 nm. The versatility of such nanoparticle-based photosensitizers lies in the fact that the surface of these nanoparticles can be modified to have either positive or negative charges so as to be specific to a class of bacteria, or be coated with antibodies specific to a certain type of bacterium. Experiments are underway to test the efficacy of these photosensitizers towards several bacteria, such as P. fluorescens, E. coli., S. epi., and MRSA.