**Numerical Simulation of Atmospheric Photochemistry in a Laminar-Flow Tube Reactor**

Michael Bauer  
**Mentors:** John H. Seinfeld and Matthew Coggon

The climatological impact and chemical characteristics of atmospheric aerosols remain very uncertain. The latest step in this field is the experimental use of UV-lit gas-phase tube reactors to simulate days to weeks of atmospheric photochemistry. These reactors have typically been modeled as plug-flow reactors. We have constructed a more detailed model to describe the new laminar-flow tube reactor constructed by the Seinfeld Group. This model assumes radial symmetry and takes into account the non-uniform velocity distribution in the tube’s flow. However, it ignores gas-phase diffusion since preliminary simulations indicated that it may not have a large effect. A numerical simulation of this model was constructed to consider the steady-state addition of an arbitrary number of reactants. Starting with a list of reactions and reaction rate constants, this simulation can generate an estimate of species concentrations for each species everywhere in the reactor. It does this by repeatedly solving an N-dimensional ODE for different reactor streamlines using backward differentiation formulae and Newton iteration. This allows for the simulation of very large and very stiff kinetic systems within the reactor. Experimental evaluation of the model’s accuracy is necessary. Diffusion may also be considered in the future.

**Gas Vesicles as Functional Ultrasonic Reporters**

Suchita Nety  
**Mentors:** Mikhail Shapiro and Anupama Lakshmanan

Ultrasound is a widely used non-invasive imaging modality in biomedicine, yet its high spatiotemporal resolution has not yet been fully exploited for molecular imaging due to the lack of suitable contrast agents. Gas vesicles (GVs), hollow protein-shelled nanostructures from buoyant microorganisms, have recently been identified as a new class of ultrasonic reporters, and our present goal is to engineer GVs as functional imaging agents. We aimed to enable covalent attachment of any protein of interest to the GV surface by employing the genetically-encoded SpyTag-SpyCatcher protein-tagging system. SpyTag-coated GVs were prepared and reacted with fluorescent SpyCatcher proteins in vitro. Collapse pressure assays and ultrasound imaging indicated that covalent attachment of fluorescent proteins to the GV surface did not perturb GV mechanical and acoustic properties. GV fluorescence was confirmed by fluorescence microscopy, and the degree of labeling was quantified by SDS-PAGE. Ongoing efforts are focused on co-labeling GVs with multiple proteins (i.e. targeting peptide and fluorescent protein), thus establishing GVs as functional multimodal contrast agents.

**Preventing Environmental Release of Genetically-Modified Gut Bacteria With a Thermally-Activated Kill Switch**

Gloria Ha  
**Mentors:** Mikhail Shapiro and Mohamad Abedi

The human body carries about 100 trillion microorganisms in its intestines, a number ten times greater than the total number of human cells in the body. These organisms play a major role in maintaining our health and mood, and are targeted by engineered bacteria and medicines. Unfortunately, therapeutic bacteria engineered to modify the gut microbiota are eventually excreted into the environment, where they continue to propagate. In order to design engineered gut bacteria that are incapable of spreading into the environment, we used TlpA, a coiled-coil protein found in *Salmonella typhimurium*, to establish heat-modulated bacteria that can survive at gut temperatures (37°C) but not outside (25°C). To this end, we targeted the production of D-alanine, an amino acid that is essential for cell membrane formation, and thus for bacterial growth. *Alr-100*, an essential gene for D-alanine production, was made dependent on TlpA binding, and auxotrophic bacteria incapable of D-alanine production were transformed with these plasmids. Future work will use the optimized constructs to design bacteria that are activated at fever temperatures (40°C) but not at body temperature (37°C).
Encapsulins as Molecular Reporters for Spatiotemporal Control of Biological Systems
Vasant Iyer
Mentors: Mikhail G. Shapiro and Pradeep Ramesh

Continued advancement in molecular medicine and our understanding of biological systems depends on developing techniques to modulate gene expression, cell behavior, and tissue properties in a spatiotemporally specific and noninvasive manner. We aim to develop such a technique using proteins of a new class of bacterial nanoparticles called encapsulins, which natively contain guest proteins that enable bacteria to sequester iron in times of oxidative stress. We have targeted proteins to the interior of encapsulins to create a redox-controlled environment in which magnetite particles may be nucleated upon iron supplementation, yielding T2 contrast under magnetic resonance imaging (MRI). Further work involves developing encapsulins as noninvasive agents for cellular control based on hyperthermia induced by incident radio frequency waves.

Biological Imaging Using Genetically Encoded Magnetic Contrast Agents
Jiemin Sheng
Mentors: Mikhail G. Shapiro and Pradeep Ramesh

Though optical microscopy is a powerful modality of imaging, its applications are limited by the penetration depth of light in tissue. To circumvent this limitation, we wish to exploit the robust, non-invasive nature of magnetic resonance imaging (MRI) in order to observe interesting systems biology, in vivo. We are working to create genetically encoded magnetic nanoparticles in order to enhance T2 (spin dephasing) contrast in MRI. This is done by borrowing the genome of the magnetotactic bacteria (MTB), a bacteria that creates magnetic particles, called magnetosomes, in order to help it align along the Earth's magnetic field. By engineering, transplanting, and targeting a minimal set of the MTB genome, along with other helper proteins, into mammalian cells, we hope to create a robust contrast agent which will allow us to image tissues in vivo and investigate processes such as tumor metastasis, which has been very hard to do thus far.

Development of a Three Wavelength Cavity Ring-Down Instrument
Karim Lakhani
Mentors: Geoffrey Smith, Al Fischer, Austen Scruggs, and Mitchio Okumura

Ambient aerosols are one of the largest contributors to air pollution, and determining their optical properties allows scientists to create better models to study air pollution. However, because of their low extinctions ($10^{-7} \text{ m}^{-1}$), special techniques besides UV-Vis spectroscopy are necessary to detect them. We attempted to build a three wavelength (red, green, blue) cavity ring-down spectrometer to measure extinction by these particles. Cavity ring-down uses ultra-high reflectivity mirrors to create a large path length to detect molecules with small absorbance cross sections. This project focused on coupling the three lasers into the same cavity and interweaving them to obtain information for each wavelength at high repetition rates. I worked on setting up the spectrometer, writing the Labview program that modulated the lasers and calculated the exponential fits in real time, and calibrating the spectrometer to known gases. Future studies involve using the cavity ring-down spectrometer outside of the laboratory setting to detect ambient aerosols.

Immobilization and Study of Fluorinated Molecular Catalysts on Graphitic Surfaces
Ayush Gupta
Mentors: Harry B. Gray and James D. Blakemore

Interfacing well-defined molecular catalysts with electrode surfaces is a key step toward constructing devices for selective solar-fuel production. Noncovalent interactions between fluorinated aromatic cycles and other aromatic cycles have been observed in a variety of compounds. Most notably of these is the interaction between hexafluorobenzene and benzene. We now report synthesis of a new bipyridine ligand appended with two perfluorobiphenyl groups. The ligand was synthesized by reacting 4,4′-Dimethyl-2,2′-bipyrindine with lithium disopropylamide to afford the reduced bipyridine. This was then reacted with decafluorobiphenyl to form the final compound. The ligand was then reacted with a rhodium cyclopentadiene dimer to form the proton reduction catalyst that was studied. To better study the surface-attached catalyst. A high surface-area carbon material was prepared on highly oriented pyrolytic graphite using Ketjen black and a conductive polymer. Preliminary studies with the carbon material show a wide range of stable potentials that can be applied. Further data about the attachment and stability of the catalyst will be provided once the experiments are completed.
**New Manganese and Rhenium Complexes for CO₂ Reduction Catalysis**
Thomas Sheridan  
*Mentors: Harry Barkus Gray and James Blakemore*

Manganese and rhenium carbonyl complexes with bipyridine ligands have been shown in prior work to function as electrocatalysts for CO₂ reduction. In our new work, related compounds bearing the analogous nitrogen-rich bipyrimidine (bpm) or bipyrazine (bpz) ligands were synthesized, characterized, and tested for their ability to catalyze CO₂ reduction. Our new compounds include [Mn(CO)₃(bpm)Br], [Mn(CO)₃Br(bpm)Mn(CO)₃Br], [Mn(CO)₃Br(bpm)Re(CO)₃Br], and [Mn(CO)₃(bpz)Br]. These new compounds were characterized by ¹H NMR spectroscopy, mass spectrometry, and single-crystal X-ray diffraction. Cyclic voltammetry showed promising catalytic activity for a number of complexes, with the most robust catalysis found with the Mn-bpm monomer and Mn-bpm-Re dimer. Results from on-going experimental work will be presented, including characterization of the products of CO₂ reduction.

**Mechanistic Investigations of Cobalt Glyoximes**
Lucille Wells  
*Mentors: Harry Gray and Sarah Del Ciello*

Using the sun’s energy to split water and store fuels has been proposed as an alternative to the fossil fuels used today. Earth-abundant catalysts are needed to facilitate the reduction of protons to form hydrogen gas in the water-splitting process. Cobalt glyoximes are known to be catalysts for hydrogen evolution; however, the mechanisms require further investigation. This study aimed to address whether the mechanism of the cobalt glyoxime was homolytic or heterolytic through the principle of microscopic reversibility. In addition, the investigation of cobalt glyoximes led to a focus on finding a protonated intermediate in hydrogen evolution by reducing the cobalt glyoxime and protonating it with a weak acid.

**Structural and Functional Characterization of the Interaction of mRNA Export Factor Gle1 With Cytoplasmic Filament Protein yNup42/hNup12 at the NPC**
Sarah Cai  
*Mentors: André Hoelz and Daniel Lin*

The mRNA export factor Gle1 is an essential nucleoporin that functions in the export of poly(A)+ mRNA through the nuclear pore complex (NPC). Mutations in Gle1 can result in defects in mRNA export—amyotrophic lateral sclerosis (ALS) is linked to a mutation removing the Nup12 binding domain on Gle1. Gle1 has two known binding partners, one of which is the cytoplasmic filament protein hNup12 (yNup42 in *S. cerevisiae*). A minimal binding construct was determined, expressed in *E. coli*, and purified. We solved the atomic structure of the yGle1-yNup42 complex, refined to 1.75 Å resolution. The structure reveals several key residues that mediate the interaction, and experiments are being done in vivo with *S. cerevisiae* using mutants to validate the structure and to study NPC localization patterns of yGle1 and yNup42. The homologous human complex (hGle1-hNup12) has been purified and is in the process of screening for crystallization conditions. Understanding the atomic structure of these interactions can be applied toward the study of diseases such as ALS that result from mRNA export defects.

**Investigating a Potential Role for Karyopherin-α in the Assembly of the Nuclear Pore Complex**
Nina Butkovich  
*Mentors: André Hoelz and George Mobbs*

The nuclear pore complex (NPC) is an essential, massive macromolecular structure composed of approximately 30 different proteins known as nucleoporins, providing the sole gateway between the nucleus and cytoplasm in eukaryotic cells. While significant regions of its architecture have recently been resolved, the NPCs assembly is not well characterized. Karyopherin-α, a nuclear transport factor, has been shown to interact with several nucleoporins and is hypothesized to play a role in the assembly and oligomerization of the NPCs distinctive ring like structures. To further study its role in assembly, we have investigated how Karyopherin-α interacts with Nup53, a flexible linker which interacts with a wide array of nucleoporins, and Nup133, the base of the coat nucleoporin complex. We have successfully expressed, purified and crystallized Karyopherin-α with a short peptide corresponding to the minimal binding region of Nup53. We have also identified a minimal Karyopherin-α binding region in Nup133 through testing various truncated constructs using size-exclusion chromatography. This work shows that Karyopherin-α forms physiologically significant interactions with two crucial nucleoporins, which opens the door for a larger role in NPC assembly.
Evaluation of the Optical Properties of NiOx Protective Coating Under Working Conditions Using Operando Optical Spectroscopy
William Schmidt
Mentors: Nathan S. Lewis and Ke Sun

The process of artificial photosynthesis uses solar energy to split water into oxygen and hydrogen in acidic or basic conditions. Traditional photovoltaic materials with small energy band gaps are unstable under such conditions and thus require additional protective coating to increase durability for photoelectrochemical solar fuel conversion. A nickel oxide coating has been shown to extend the life of the device from a few hours to over 1000 in 1.0 M KOH, but its optical properties under the conditions are less explored.

In this SURF project, we are using Operando optical spectroscopy, particularly ellipsometry, to study the optical properties of the protective film. This would allow us to directly reconstruct the optical parameters of the films under water oxidation conditions, which will help to understand the degradation of protective coatings and design stable photoelectrodes for solar fuel conversion.

Experiments on samples from different deposition conditions in pH 9.5 solution reveals that under bias near water oxidation voltage the amplitude ratio of s and p waves increases at higher energy wavelengths, while the peak phase difference decreases. This change is more pronounced on samples prepared at low temperature and high deposition rate, while less significant on high temperature and slow deposition.

Mechanistic Studies on QUINAP and Its Triflate Precursor
Ashay Makarand Gore
Mentor: Scott Virgil

A novel method of the asymmetric synthesis of the chiral ligand QUINAP via dynamic kinetic resolution was recently developed. This synthesis involves an isomerization process of the arylpalladium intermediate in which the isoquinoline piece can adopt two positions in relation to the naphthalene ring. Mechanistic studies to deduce the mode of isomerization of the arylpalladium intermediate and the triflate precursors via deuterium labeling and kinetic analysis were performed. Work on isolating the arylpalladium intermediate was also conducted.

Engineering Prolyl tRNA Synthetase for Accommodation of Non-Canonical Amino Acids
Mary Boyajian
Mentors: David Tirrell, Katherine Yan Fang, and Seth Lieblich

Current methods of engineering recombinant proteins primarily involve the replacement of one canonical amino acid with another; however, for certain proteins of heavy research and commercial interest, all reasonable canonical mutations have been extensively explored. Diabetes is a disease that affects millions in the world; it is the result of an inability to produce or respond to insulin. As a result, insulin has been the subject of intensive engineering efforts for decades. Few, if any further, engineering opportunities remain undiscovered using canonical amino acids; however, the replacement of canonical amino acids (cAAs) with non-canonical amino acids (ncAAs) is relatively new and opens new unexplored chemical space wherein beneficial engineering via mutations is possible. Previous research in the field has demonstrated that hundreds of ncAAs can be incorporated into newly synthesized proteins by bacterial and mammalian cells. These ncAAs have unique side chains and can introduce novel chemical, physical, and biological changes that cannot be achieved with the natural cAAs. Aminoacyl-tRNA synthetases (aaRSs) are responsible for catalyzing the specific ligation of amino acids to their cognate tRNAs; in most cells, there are at least 20 aaRSs, one for each natural amino acid. While some ncAAs are promiscuously charged to tRNA by aaRSs, most ncAAs require a mutant aaRS to enable protein translation. Insulin has a single proline in its mature form: B28; this position is known to be critical for controlling insulin oligomer states and has served as the most important site of alteration and modification in new biologic drugs. In this project, certain mutations will be made to the prolyl tRNA synthetase (ProRS) to accommodate the different side chains on ncAAs in order to explore new protein sequence space for the engineering of improved insulin variants.