

## Session F – Chemistry (Alphabetical)

### Adapting CLUSTALW for RNA

Kathryn M. Brennan

Mentors: John SantaLucia, Jr. and Robert McKeown

To create automatic sequence alignments of RNA and accurate phylogenetic trees this project focused on adapting CLUSTALW, an open source multiple sequence alignment program for proteins, to be compatible with RNA. The adapted program has scoring matrices corresponding to ribosomal RNA (further research could develop scoring matrices for other RNA types) and uses a Structure Based Sequence Alignment algorithm for pairwise and multiple sequence alignment. The SBSA algorithm uses RNA secondary structure as alignment criteria, increasing accuracy of the alignments and phylogenetic trees produced by the program. This version of CLUSTALW for RNA has been interfaced with RNA123, a program developed by the SantaLucia lab.

### Multi-Step Electron Tunneling Across Outer Membrane Protein A: A Study of Ruthenium-Labeled OmpA Folding

Eric Y. Chang

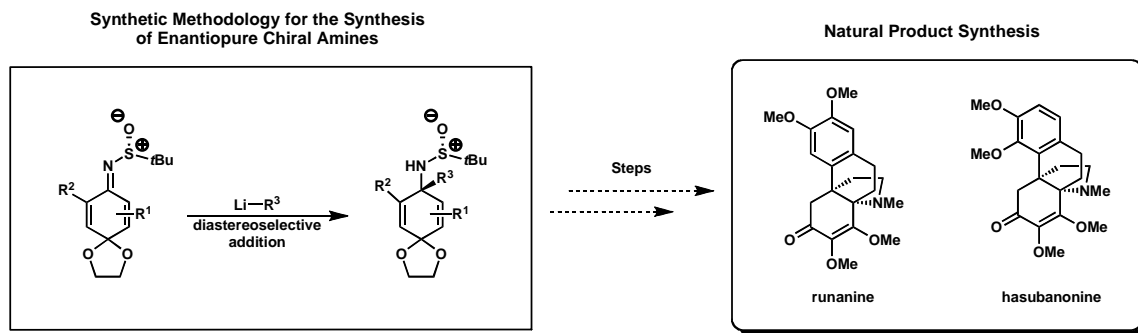
Mentors: Harry Gray and Bert Lai

Multi-step electron tunneling has been demonstrated experimentally using a ruthenium-labeled metalloprotein in solution. However, in natural redox systems, such as those involved in photosynthesis and respiration, multi-step electron tunneling must occur across a membrane. The research here involves studying electron tunneling through a transmembrane protein, namely outer membrane protein A (OmpA). A ruthenium sensitizer complex will be covalently bound to a cysteine residue folded inside the cavity of a small unilamellar vesicle. It will be studied spectroscopically to determine whether OmpA folds correctly when singly labeled with ruthenium.

### An Auxiliary-Based Approach to Chiral Quinone-Derived Amines

Kangway Chuang

Mentor: Sarah Reisman



Natural products have played a major role in the discovery of new drugs for the treatment of disease. The hasubanan alkaloids, such as runanine and hasubanone, contain a structural resemblance to morphine, offering potential analgesic and anti-inflammatory properties. As a result of their interesting biological activity and challenging structures, alkaloids such as runanine and hasubanone serve as excellent target molecules for synthetic chemists. Their complex tetracyclic structures, embedded with vicinal quaternary centers, represent significant synthetic challenges.

The synthesis of these complex structures necessitates reliable, stereoselective methodology for the production of chiral quinone-derived amines. As a step towards the total synthesis of these molecules, we are currently developing a diastereoselective nucleophilic addition of organolithium reagents to quinone *tert*-butyl sulfonamides through the use of chiral auxiliaries. Preliminary results on simple quinone sulfonamides have shown excellent diastereoselectivity, demonstrating the viability of the class of reactions. In order to establish our reaction as an effective synthetic tool, we are currently working towards optimizing reaction conditions and expanding our substrate scope, which would ultimately facilitate the enantioselective total synthesis of the hasubanan alkaloids.

### ***A Structural Study of the Pathway for Membrane Integration of Tail-anchored Proteins by Get3***

Alan Deng

*Mentors: William Clemons and Christian Suloway*

Although numerous proteins have been identified which insert into cellular membranes by way of a tail-anchor segment in their C-termini, the process by which they are integrated into these membranes is poorly understood. Stefanovic, et al. and Schuldiner, et al. have previously identified TRC40 (or Get3 in yeast) as an essential component of this pathway. In this project, our goal is to determine the effect of Get3 on TA protein translocation and targeting by way of several experiments. First, we are using a localization assay on GFP-tagged TA substrate to monitor its movement and integration into the membrane. Second, we are constructing a Get3 fusion dimer to observe how the interactions between the two monomers affect their function in binding substrate. Third, we are screening for dominant negative mutations that inhibit the Get3 pathway, from a list of deleterious mutations observed in the knockout. By doing these experiments, we hope to gain some insight into understanding the nature of the interaction between Get3 and its TA substrates.

### **The Folding Mechanism of Cytochrome c-b562 Detected by Time-Resolved Fluorescence Energy Transfer**

Yuehan Huang

*Mentors: Harry B. Gray and Nicole Bouley*

Cytochrome c-b562 is a modified mutant of the four-helix bundle cytochrome b562. The mutation enables covalent bonds between the heme and to the protein helix, allowing for more detailed measurements of the folding of cytochrome c-b562. Studies from other groups have suggested that the protein apo-cytochrome b562 has a folding intermediate state, in which helix 1 of the protein remains unfolded. To study if such an intermediate state exists in cytochrome c-b562, time-resolved fluorescence energy transfer (FET) data of the folding process of cytochrome c-b562 are measured to provide information on the distribution of distances between helix 1 and the heme during different stages of the folding process.

### **Experimental and Theoretical Studies of Interfacial Chemical Reactions using Field Induced Droplet Ionization**

Madiha Hussain

*Mentor: J.L. Beauchamp*

Interfacial chemistry involves the study of chemical processes that occur at phase boundaries such as gas-liquid, solid-liquid, and gas-solid interfaces. These interfaces present a unique environment for studies of chemical reactions, the mechanism and energetics of which can differ significantly from reactions in either phase alone. However, the experimental methods appropriate for examining chemical reactions at phase interfaces are limited, since ideally they must be able to analyze molecular species localized within the interfacial region. A new and promising methodology for these studies, developed in the laboratory of Professor J. L. Beauchamp at Caltech, is field induced droplet ionization (FIDI), which provides mass spectrometric analysis of molecular species at gas-liquid interfaces. We used this methodology to study a range of chemical reactions occurring at the surface of a small suspended droplet.

### **Detecting the Effects of Cholesterol and Other Sterols on Aggregation of Lipids in the Pulmonary Surfactant Layer Using Field-Induced Droplet Ionization Mass Spectrometry**

Tony Z. Jia

*Mentors: J. L. Beauchamp, Evan Neidholdt, and Chang Ho Sohn*

Field-induced droplet ionization (FIDI) mass spectrometry is a useful application for studying the chemical reactions at the gas-liquid interface by preferentially sampling ions from the surface of small droplets. FIDI subjects a droplet to a strong electric field which causes the droplet to elongate and emit jets of opposite charge, generating desolvated gas-phase ions; these are sampled by an ion trap mass spectrometer for analysis. Recent research has pointed to cell membranes as being composed of complex lipid domains including lipid rafts, as opposed to a homogeneous fluid. Segregation of membrane lipids is affected by sterols, most notably cholesterol. This study utilizes FIDI-MS to examine the structure of mixed pulmonary surfactant lipid layers with varying amounts of sterols including cholesterol and sphingomyelin. More than 90% of the pulmonary surfactant layer is made of lipids, and thus by examining these interactions, we may gain more insight into lung function. This study also includes basic studies of the mechanism of FIDI-MS by comparing FIDI spectra to electrospray ionization mass spectra of samples containing species with different surface activities. The data obtained suggest that FIDI does indeed sample preferentially from the surface.

## **Tail-Anchored Protein Expression Systems for Structural and Biochemical Studies**

Yea-ra Jo

*Mentors: William M. Clemons and Christian Suloway*

Tail-anchored (TA) proteins are proteins that are anchored to the lipid bilayer by a single transmembrane domain (TMD) near their C-terminal. There are many examples of TA proteins with broad functional importance in various membrane systems of the cells of diverse eukaryotic species. Nevertheless, the proper machinery by which TA proteins are targeted and inserted into the endoplasmic reticulum (ER) membrane remains poorly understood and characterized, except that Get3 constitutes a major component in the nonspontaneous posttranslational targeting and insertion pathway of most TA proteins with recruitment of ATP hydrolysis in the yeasts. Therefore, in order to study this mechanistic basis underlying TA insertion pathway structurally and biochemically, we have worked to characterize the interaction of Get3 with TA protein substrates by structure determination using X-ray crystallography. TA proteins using spontaneous targeting and insertion pathways, and TA proteins localized to intracellular membranes other than ER membranes were chosen as controls.

## **An Electrochemical Study of Nitric Oxide Synthase and The W70H Mutation**

Katherine Lavoie

*Mentors: Harry Gray and Charlotte Whited*

We are working to electrically "wire" nitric oxide synthase (NOS) to an electrode surface in an attempt to initiate enzymatic activity electrochemically. Wiring NOS to gold electrodes through variable chain-length linkers allows us to control the rate of electron transfer into the enzyme. Toward this end, we have synthesized three azide-terminated thiols of different lengths to connect the electrode to the protein. In addition we have expressed both wild-type and a W70H mutant of *Geobacillus stearothermophilus* NOS (gsNOS) that feature a surface cysteine. Our efforts are now directed toward preparing an ethynyl/maleimide linker to conjugate the gsNOS to gold electrodes *via* CLICK chemistry to the azide-terminated thiol monolayers.

## **Synthesis and Characterization of a Redox Active Metal-Ligand Framework for Oxygen Activation**

Eva Nichols

*Mentors: Theodor Agapie and Sibö Lin*

The activation of molecular oxygen is very important to the field of clean energy research. In an attempt to mimic biological systems known to catalyze the reduction of oxygen, the synthesis of a series of complexes containing nitrogen and oxygen donors and redox-active transition metals has been attempted. The target ligands consist of a rigid terphenyl backbone with pendant pyridine and alcohol groups. Synthetic procedures for two similar organic frameworks and their subsequent metalations are described. Reactions with oxygen sources ( $O_2$ ,  $H_2O$ ) are also investigated.

## **Fundamental Studies of Ozonolysis at Gas-Liquid Interfaces**

Keshav Sapatnekar

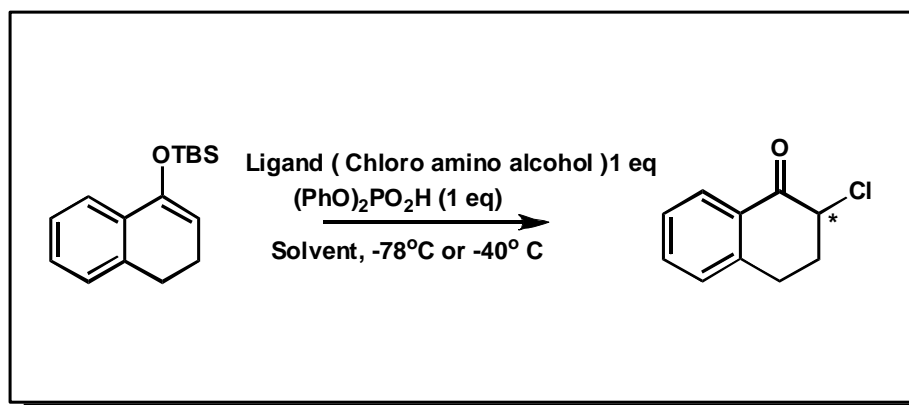
*Mentor: J. L. Beauchamp*

Reactions at the interfaces of gases and liquids display unique properties. While these reactions have been widely studied in terms of the liquid phase, the gaseous is not as well characterized. Ozonolysis of fatty acids proves to be an ideal reaction for study because of the amphiphilicity of these compounds. In particular, oleic and linoleic acids were studied. The expected products of their ozonolysis included nonanal for oleic acid, and nonenal and hexanal for linoleic acid. To perform these reactions, low concentration aqueous solutions of the fatty acids were run in a bubble column reactor. Compressed air was run during the first period, and UV light was used to generate ozone during the second period. Then, two periods with no ozone determined the effects of bulk-dissolved ozone. The principal gaseous phase compound produced appears to be nonanal, although it may be necessary to lower concentrations to 1 micromolar or lower before this is definitive. It is also notable that nonenal only appears in solutions of both acids after ozone generation has ended, indicating that the hydrophobic end of the linoleic acid molecule only abuts into the air at some point before its ninth carbon.

## Asymmetric $\alpha$ -Chlorination of Silyl Enol Ethers

Vincentius (Jeremy) Suhardi

Mentors: Sarah E. Reisman and Raul Navarro



$\alpha$ -Chloro ketones are important intermediates for the synthesis of natural products. Whereas the conversion of silyl enol ethers into racemic  $\alpha$ -chloro ketones are well known reactions that can be found in general organic chemistry textbooks, method to promote this transformation asymmetrically have not yet been fully developed. This project is focused on the utilization of N-Chloro amino alcohols or N-chloro diamines in conjunction with phosphoric acid activators as reagents for asymmetric  $\alpha$ -chlorination of silyl enol ethers. In the long term, it is envisioned that this methodology could give rise to a general strategy for catalytic, enantioselective electrophilic chlorination.