Princeton University

Department of Chemistry

Summer Undergraduate Research Program
Poster Session 2016

Thursday, August 4th
10:00 – 11:30 AM
Frick Laboratory

Name	***	University	Advisor	Title
Christine An	*e*	Princeton University	Ando	An Improved Analysis of X-ray Crystallography Data and Its Protein Sources
Rachel Ashmore	*a*	Millersville University	Scholes	Excimer Formation in Pyrene Molecules Interacting in Their Ground State
An Chu	*cgm*	Princeton University	Bocarsly	Probing the Stability of Alkanethiol Monolayers in Common Electrochemical Environments
Emily Cliff	*a*	Ripon College	Muir	Generation of Designer Histones H3K36me1/2/3
Bruce Culbertson	*cn*	Princeton University	Sorensen	Progress Towards a Base Metal Catalyzed, Environmentally Friendly Alternative to Stoichiometric DDQ
Jacqueline Dragon	*b*	Princeton University	Pelczer	NMR Spectroscopy & PCA Differentiate the Metabolomic Components of and Reveal Outliers for Locally-Purchased Paprika Varieties
Blake Feldman	*cn*	Princeton University	Doyle	A Simple, One-Pot, Three-Component Coupling Featuring Nickel Catalyzed Reductive Amination
Michael Gao	*b*	Princeton University	Ando	Characterization of the Structure and Dynamics of Cobalamin-Independent Methionine Synthase
Samuel Garfinkle	*b*	Princeton University	Groves	Enantioselectivity Study of Porphyrin- and Salen-Coordinated Manganese Catalysts for Celestolide Functionalization
Norman Greenberg	*dgi*	Princeton University	Hyster	Directed Evolution of a Ketoreductase to Perform Radical Dehalogenation and Chiral Hydrogen Atom Transfer
Carol Gu	*dhj*	Princeton University	Knowles	Sulfonamidyl-Mediated Remote C-H Fluorination: A Catalytic Protocol Enabled by Proton-Coupled Electron Transfer
Martina Hale	*n*	Princeton University	Knowles	Enantioselective Halogenated Pyrroloindoline Cyclizations Enabled by PCET
Noah Han	*b*	Princeton University	Pelczer	Identifying 4-Methylcyclohexanemethanol (MCHM) in Water Through the Use of ^{1}H NMR
Stephanie Jeong	*cdn*	Princeton University	Rabinowitz	Mechanistic Effects of Inhibiting Serine Hydroxymethyl Transferase in Blood Cancer Cells
Liat Kugelmass	*a*	Vassar College	Ando and Barstow	Electron Uptake in *S. oneidensis*: A Whole Genome High-Throughput Colorimetric Survey
Jared Lockwood	*l*	Princeton University	Hecht	Fusion to a *de novo* Protein Leads to Increased Production of Some Poorly-Expressed Proteins in *E. coli*
Amanuella Mengiste	*b*	Princeton University	Doyle	Development of Enantioselective Nickel-Catalyzed Cross Coupling of Styrenyl Aziridines
Caitlin Miller	*cn*	Princeton University	Nelson	Crosslinked Chitosan Scaffolds for Tissue Engineering Applications
Taylor Myers	*cn*	Princeton University	Hecht	Characterizing Computationally Designed Libraries of *de novo* proteins with Life-Sustaining Properties
Arman Odabas	*dg*	Princeton University	Donia	A Type II Polyketide Biosynthetic Gene Cluster from the Human Microbiome
Yuzki Oey	*b*	Princeton University	Ando	Crystallizing Class 1b Ribonucleotide Reductase
Alejandro Ramirez	*a*	University of California, Berkeley	Bocarsly	Cyanogel Techniques to Create Ni/X (X = Group 13 Metals) Alloys
Orestes Riera	*a*	Florida International University	Doyle	Optimization of Deoxyfluorination Via Cheap Bulky Bases and Inexpensive Fluorinating Reagents.
Paul Rosen	*cikn*	Princeton University	Seyedayamdost	Milking Bacteria for Antibiotics: RiPP Discovery and Biosynthesis in Streptococci
Ellie Sell	*cn*	Princeton University	Groves	Probing the Rebound Mechanism: Insights into Manganese-Catalyzed C-H Bond Halogenations

Grzegorz Skrzypek	*b*	Princeton University	Chirik	Cobalt and Iron Precatalysts with NHC Ligands for Alkene Hydroboration
Spyder-Ryder Sloman	*cdn*	Princeton University	Bocarsly and Cava	Water Splitting Semiconductors: Experimental Realization of New Candidate Materials
Nancy Song	*dfgi*	Princeton University	Yang	Characterizing the Dynamics of DNA Hairpin Hybridization through smFRET and Multi-Trajectory Expectation Maximization
Laura Srivichitranond	*cn*	Princeton University	Cava	Searching for Superconductivity in New Intermetallic Phases
Arjuna Subramanian	*b*	Princeton University	Scholes	Models for Reactive Coordinate Trends under Vibronic Coupling
Uri Tayvah	*g*	Princeton University	Bocarsly and Selloni	A Computational Study of the Stability of $CuRhO_2$ Photoelectrodes for Water Splitting
Ashley Tsue	*cgn*	Princeton University	Scholes	Electronic Spectroscopy of Novel Dehydro[12]annulene: A Spectral Analysis and Time-Dependent Density Functional Theory Study
Miah Turke	*a*	Michigan State University	Yang	The Equilibrium of DNA Hybridization is Different on Surfaces and in Solution
Cecilia Vollbrecht	*a*	Centre College	Carrow	Synthesis of a Sulfur-Functionalized Metal-Organic Framework to Support Oxidase-Type Catalysis
Joshua Wang	*b*	Princeton University	Rabinowitz	Quantitative Analysis of G6PD Inhibition with Michaelis-Menten Kinetics
Monica Wei	*cn*	Princeton University	Rabinowitz	Regulation of the Pentose Phosphate Pathway in Cancer Cells
David Weiner	*n*	Princeton University	Wang and Knowles	Synthesis of Bis-CyHQ-Dopamine, a Novel Doubly Caged Dopamine Analog With Expected Sensitivity to Chemical Two-Photon Excitation
Evelyn Wu	*e*	Princeton University	Groves	Recombinant Production of Novel Heme-Thiolate Protein using the *Pichia pastoris* Expression System
Bufan Zhang	*a*	Vassar College	Carrow	Finding BuPhos: Synthesis and Coordination Chemistry of an Exceptionally Bulky Trialkyl Phosphine
Henry Zheng	*n*	Princeton University	Rabinowitz	Characterizations of Compensatory NADPH Production Pathways in G6PD-Deficient Hypomorphs and Knockouts

***Summer research support provided with many thanks to:

a	Summer Undergraduate Research Program for Diversity in Chemistry, supported by the Princeton University Office of the Provost and Department of Chemistry
b	Leach Summer Scholars Program supported by the Charles Henry Leach, II Endowment Fund for Student Research in the Sciences
c	Anthony B. Evnin ’62 Fund
d	Office of the Dean of the College
e	Office of Undergraduate Research, ReMatch Internship
f	Fred Fox Class of 1939 Fund
g	Princeton Environmental Institute
h	University Center for Human Values
i	Peter N. Curtin, Class of 2008 Memorial Fund
j	Program in Global Health and Health Policy
k	Health Grand Challenge Grant
l	Freshman Scholars Institute
m	Department of Chemistry
n	Faculty Advisor's Research Grants

An Improved Analysis of X-ray Crystallography Data and Its Protein Sources

Christine An
Mentors: Saad Imran and John Bacik
Advisor: Nozomi Ando

X-ray crystallography has been a fundamental method in structural biology. Though it has shown to be a robust technique, conventional crystallography requires extremely well-ordered crystals in order to solve protein structures at high resolution. In addition, it fails to reflect protein dynamics, a feature crucial to the understanding of the protein's function. Diffuse scattering, a weaker signal observed in diffraction images, has been correlated with protein movement and flexibility, yet large crystals are needed to make these measurements. Crystal nucleation and growth were optimized by varying components involved in vapor diffusion methods, including the buffer, buffer pH, salt concentration, precipitant concentration, protein concentration, temperature, and presence of other additives. These methods are extremely versatile and as a result, they were able to be applied to two engineered proteins as well as one protein vital to microbial carbon fixation. Promising results have been observed particularly with the addition of low-melting agarose, where the slowed growth rate is believed to allow the formation of high-ordered crystals.

Excimer Formation in Pyrene Molecules Interacting in Their Ground State

Rachel Ashmore
Mentors: Ryan Pensack and Nhu Tran
Advisor: Gregory Scholes

The efficiency of solar energy conversion, which converts light to other forms of energy, is reduced by loss pathways such as excimer formation. In order to study excimer formation, a procedure was developed to prepare nanoparticles comprising pyrene molecules that form excimers exclusively, and absorption, fluorescence, and transient absorption spectroscopy were performed on the nanoparticles. The chromophores in these nanoparticles are pre-associated in their ground state, and selective excitation of these chromophores results in only the excimer population. It was found that, while excimer formation occurs in 4 ps, a singlet excited state feature of pyrene continues to evolve through 20 ps, indicating an intermediate state currently assigned as a "hot" excimer state is required to fully describe the dynamics. Further study of this intermediate state is necessary to fully understand excimer formation dynamics in these pyrene nanoparticles and potentially other excimer-forming systems.

Probing the Stability of Alkanethiol Monolayers in Common Electrochemical Environments

An Chu
Mentor: James Pander
Advisor: Andrew Bocarsly

Alkanethiol self-assembled monolayers (SAMs) on gold electrodes contain charge transfer characteristics that suggest a possible application as efficient carbon dioxide reduction catalysts. We investigate the stability of alkanethiol SAMs via contact angle measurements in various solvents and electrochemical techniques common to the analysis of heterogeneous catalysts, such as cyclic voltammetry (CV), bulk electrolysis (BE), $NaHCO_3$ and H_2SO_4 supporting electrolyte. Contact angle measurements on the electrode surface before and after CV and BE are shown to be within error of each other. This result indicates the instability of the SAM structure under the negative potential windows investigated in this work.

Generation of Designer Histones H3K36me1/2/3

Emily Cliff
Mentor: Krupa Jani
Advisor: Tom Muir

Methylation of lysine 27 (K27) on histone H3 is associated with gene silencing in chromatin. Polycomb repressive complex 2 (PRC2), the enzyme responsible for methylation of K27 on histone H3, may sense the methylation state of lysine 36 (K36) on the same histone tail via a putative binding pocket. In order to investigate the effect of post translational modifications, specifically methylation of H3K36, on PRC2's ability to methylate H3K27, designer histones H3K36me1/2/3 are produced through solid phase peptide synthesis and three piece native chemical ligation. These designer histones will be incorporated into nucleosomes, which will be used in pulldowns with PCR2 to check binding affinity qualitatively and in enzyme activity assays using wild type PRC2 and various mutants to test this hypothesis. .

Progress Towards a Base Metal Catalyzed, Environmentally Friendly Alternative to Stoichiometric DDQ

Bruce Culbertson
Mentor: Dylan Abrams
Advisor: Erik Sorensen

2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) is a commonly used oxidizing agent in organic synthesis, but its usefulness is limited by its high cost and toxicity when used in stoichiometric quantities. In this study, we attempted to mitigate this problem by using base metal catalysts tetrabutylammonium decatungstate (TBADT) and cobaloxime (COPC) to regenerate DDQ from its reduced form, rendering it catalytic. Reactions were run for five days under argon and ultraviolet light, and reaction mixtures were periodically examined by ^{1}H and ^{13}C NMR. Although we have so far been unsuccessful in incorporating DDQ into the proposed catalytic cycle, we showed that benzoquinone, a closely related oxidant, can be regenerated from its reduced form by TBADT and COPC, suggesting that our catalytic system may be more readily applied to reagents with lower reduction potentials.

NMR Spectroscopy & PCA Differentiate the Metabolomic Components of and Reveal Outliers for Locally-Purchased Paprika Varieties

Jacqueline Dragon
Mentor: Ken Conover
Advisor: István Pelczer

Mass spectroscopy (MS) is a common but intrinsically nonquantitative and destructive process for discovering and measuring product counterfeiting and contamination within industries like the food industry. Here, we use varied nuclear magnetic resonance spectroscopy (NMR) experiments---including ^{1}H, ^{13}C, 1D HR-MAS, 2D HR-MAS TOCSY and HSQC experiments, and SNIF-NMR---as alternatives for characterizing the metabolomic components of and differences between locally-purchased paprika varieties. Using connectivity information from 2D HR-MAS experiments, comparisons with literature, and principle component analysis (PCA), we gave peak assignments, visually and quantitatively determined differences between paprika spectra, and identified the unexpected fermentation of a sample over time. These results both characterize the previously-untested metabolome of paprika samples and demonstrate the efficacy of NMR as a quantitative and nondestructive testing alternative for product quality verification.

A Simple, One-Pot, Three-Component Coupling featuring Nickel Catalyzed Reductive Amination

Blake Feldman
Mentor: Kevin Wu
Advisor: Abigail Doyle

The three-component synthesis of α-substituted amines, which are important pharmaceutical precursors, from the combination of aldehyde, amine, and boronic acid has most recently been demonstrated by aryl-nucleophilic substitution to iminium with palladium, rhodium, and copper catalysts; but the literature has yet to demonstrate a cross-coupling approach which utilizes cheap and abundant nickel. We sought to find the optimal conditions for iminium formation, oxidative addition, transmetallation, and reductive elimination by varying the nickel ligand, solvent, amine, additives, and phenyl organoborate source in a series of assays. Although we were unable to optimize conditions to produce significant yield, we were able to generate small amounts of product, and we found that the reaction has better performance with bulkier phosphine ligands in non-polar solvents. One challenging aspect of this reaction is that iminium formation is favored by acidic conditions, while transmetallation is favored by basic conditions, so optimization of one step may come at the cost of hindering the other. One strategy to improve reactivity might be to replace the in-situ formed iminium with a pre-formed and less sterically-hindered imine species, which removes the possibility of aldehyde arylation and could favor binding to the nickel.

Characterization of the Structure and Dynamics of Cobalamin-Independent Methionine Synthase

Michael Gao
Mentor: Maxwell Watkins
Advisor: Nozomi Ando

Cobalamin-independent methionine synthase (MetE) generates methionine by directly catalyzing a methyl transfer from methyl tetrahydrofolate (MTHF) to homocysteine (Hcy). However, the structural dynamics of MetE, which allow it to catalyze the reaction by bringing MTHF and Hcy in close enough proximity for the methyl transfer, are largely undetermined. We sought to further investigate and understand structural properties of MetE by (1) obtaining crystals for X-ray diffraction and (2) performing limited proteolysis experiments to test the degree of enzyme “closing” upon catalysis. Consistent with our prior small-angle X-ray scattering data, limited proteolysis experiments revealed that the two catalytic domains of MetE adopt a closed conformation upon binding of either both its substrates or MTHF alone, but not with Hcy alone or no substrate. Promising crystallization conditions were further identified; future work will revolve around further optimizing these conditions in the hope of obtaining protein crystals analyzable via X-ray diffraction.

Enantioselectivity Study of Porphyrin- and Salen-Coordinated Manganese Catalysts for Celestolide Functionalization

Samuel Garfinkle
Mentor: Thompson Zhuang
Advisor: John Groves

Recent research on inorganic catalysis has demonstrated the versatility of porphyrin- and salen-coordinated manganese complexes in promoting sp^3 C-H bond activation and hydrocarbon functionalization. This catalytic pathway saw expansion in both ligand variety and substrate scope, but its enantioselectivity, a critical trait of many modern synthetic pathways, remained uncharted territory. Here, we report the enantioselective functionalization of celestolide using chiral, salen-coordinated manganese catalysts, with enantiomeric excess (ee) measured by HPLC traces from the final product. This result opens the door to highly directed syntheses of specific stereoisomeric products, an application with utility in fields such as drug discovery and chemical manufacturing.

Directed Evolution of a Ketoreductase to Perform Radical Dehalogenation and Chiral Hydrogen Atom Transfer

Norman Greenberg
Advisor: Todd Hyster

While photoredox catalysis allows researchers to perform high-energy reactions under mild conditions, the reactions are difficult to render asymmetric. In this project, a nicotinamide-dependent ketoreductase was evolved to perform a radical dehalogenation and chiral hydrogen atom transfer from the nicotinamide cofactor, when irradiated with visible light. Using site-saturated mutagenesis of residues in the active site of the wild type ketoreductase, which is otherwise inactive for the dehalogenation of halolactones, a variant of the enzyme was created to perform the reaction with an er of over 96:4 for the (R)-isomer. The evolution of a ketoreductase to perform such a highly stereoselective hydrogen atom transfer indicates that cofactors within the active site of an enzyme can be utilized to perform chiral photoredox reactions.

Sulfonamidyl-Mediated Remote C-H Fluorination: A Catalytic Protocol Enabled by Proton-Coupled Electron Transfer

Carol Gu
Mentor: Gilbert Choi
Advisor: Robert Knowles

Organofluorine chemistry is valuable in many disciplines, and as such, chemists continue to seek ways to incorporate fluorine substituents into various compounds. In this work, it is proposed that proton-coupled electron transfer (PCET) enables the homolytic activation of N-H bonds in an N-alkyl sulfonamide; the resulting sulfonamidyl radical serves as an intermediate for the fluorination of remote C-H bonds in the substrate. After screening a series of reaction conditions, the fluorinated product was obtained in modest yield. If this protocol can be further optimized, it would constitute an efficient method of selectively introducing fluorine to target molecules, which would benefit medicinal chemistry as well as other fields in which fluorine chemistry plays a large role.

Enantioselective Halogenated Pyrroloindoline Cyclizations Enabled by PCET

Martina Hale
Mentor: Emily Gentry
Advisor: Robert Knowles

Using TEMPO-mediated carbocation generation for polymerization of cyclotryptamine alkaloids offers a promising method for achieving stereoselectivity during synthesis but can be complicated by undesired arene addition to give a mixture of products. 5-chloro-, 5-bromo-, and 5,7-dibromotryptamine were synthesized and tempolated to determine if temporarily blocking the para position of the arene facilitates selective ortho addition during polymerization. 5-chloro- and 5-bromotryptamine exhibited enantioselectivity, both giving 94% ee. Moving forward we seek to use these activated, halogenated pyrroloindolines in the dimerization reaction to determine the regioselectivity and diastereoselectivity of the dimerized product.

Identifying 4-methylcyclohexanemethanol (MCHM) in Water Through the Use of ^{1}H NMR

Noah Han
Mentor: Ken Conover
Advisor: István Pelczer

On January 9, 2014, a chemical holding tank owned by Freedom Industries spilled roughly 10,000 gallons of chemicals, mostly comprised of 4-methylcyclohexanemethanol (4-MCHM), into the Elk River upstream of Charleston, West Virginia from affecting over 300,000 people and leading to the hospitalization of 14 residents. In this experiment, we used NMR and cryopobe technology to detect residual 4-MCHM in water samples collected from 9 different locations around the area Charleston on August 11, 2014, and came to the conclusion that concentration of 4-MCHM was below 200 µg/L, the threshold concentration of contaminant able to be detected without pre-concentration. There were no peaks seen in the resulting spectra that would indicate the presence of 4-MCHM in the samples, however, there were other organic contaminants observed in the samples, revealed by various peaks that showed up on the spectra that could be identified with further experimentation and testing. The result of this experiment is a good example of how NMR could be applied as a quick and effective method of detecting organic contaminants in a H_2O solvent sample with minimal preparation.

Mechanistic Effects of Inhibiting Serine Hydroxymethyl Transferase in Blood Cancer Cells

Stephanie Jeong
Mentor: Gregory Ducker
Advisor: Joshua Rabinowitz

Cancer cells demonstrate particularly high rates of one-carbon (1C) metabolism, which is crucial for cell growth and proliferation. Serine is the main source of 1C units in the cell, and the enzyme serine hydroxymethyl transferase (SHMT) converts serine to glycine by transferring a 1C unit from serine to THF. Lymphomas are of particular interest, as previous work suggests that many of these blood cancer cell lines show sensitivity to SHMT inhibition. The current work utilizes LC-MS (liquid-chromatography mass spectrometry) techniques, cell growth assays, and flow cytometry to determine how blood cancer cells use serine and glycine, and to examine the mechanistic effects of using SHMT inhibitors to block the function of this enzyme. By characterizing the way that SHMT inhibition affects serine and glycine metabolism in blood cancer cells, greater specificity in targeted therapies for lymphomas may be developed.

Electron Uptake in *S. oneidensis*: A Whole Genome High-Throughput Colorimetric Survey

Liat Kugelmass
Advisors: Nozomi Ando and Buz Barstow

Electroactive bacteria, such as *Shewanella oneidensis*, are able to exchange electrons with solid surfaces such as electrodes through Extracellular Electron Transport (EET), creating the possibility of engineering bacteria where flexible carbon fixation and fuel synthesis is driven by renewable electricity. However, despite major advances such as next-generation sequencing, there is still much left to be understood about the genome and the mechanisms for electron uptake and outflow by EET. To further understand EET, we have created the first whole genome knockout collection for *S. oneidensis* by the Knockout Sudoku method. We have used this collection to conduct a genome-wide kinetic high-throughput colorimetric screen of microbial electron uptake using oxidation of the redox dye, anthrahydroquinone-2,6-disulfonate (AHDS), in order to determine which genes are involved in electron uptake for *S. oneidensis*. The assay reveals the role of several genes with previously uncharacterized function which may play an important role in EET. These results give insight into the molecular mechanisms used by autotrophic organisms that do not rely upon sunlight for energy and provide building blocks for synthetic organisms that use the flexibility of biological metabolism to store and retrieve renewable electricity.

Fusion to a *de novo* Protein Leads to Increased Production of Some Poorly-Expressed Proteins in *E. coli*

Jared Lockwood
Mentor: Shlomo Zarzhitsky
Advisor: Michael Hecht

Because of their importance and scope of uses in biomedical fields of study, cost-efficient methods of producing various proteins and peptides plays a key role in biological research. In this study, we outline a system that relies on fusion of a desired protein to a *de novo* tag in order to enhance the expression of the desired protein in vivo. We find that our system allows for the production of some poorly-expressed proteins in greater quantities than an alternative system that is commonly used in labs today. The enhanced efficiency of our system both improves upon a current method of protein expression and indicates the ability of synthetic proteins to be used as effective fusion partners for protein overexpression.

Development of Enantioselective Nickel-Catalyzed Cross Coupling of Styrenyl Aziridines

Amanuella Mengiste
Mentor: Dr. Brian Woods
Advisor: Abigail Doyle

Ring-opening reactions of aziridines via transition-metal catalysis can be particularly valuable to the synthetic community. Currently, the Doyle lab is developing a method for nickel-catalyzed asymmetric reductive cross coupling of styrenyl aziridines. This method yields ß-phenethylamines, important precursors for pharmaceutical and natural products. The desired amines are produced with good enantioselectivity, under relatively mild conditions, and uses an inexpensive Ni(II) source.

Crosslinked Chitosan Scaffolds for Tissue Engineering Applications

Caitlin Miller
Advisor: Celeste Nelson

The rapid advancements in biomaterial research and 3D printing technology for tissue engineering has opened the door for major breakthroughs in biomedical research, such as restoration of 3D anatomic defects, reconstruction of organs with complex 3D microarchitecture, and development of scaffolds for regenerative medicine and tissue development. Here, the hydrogel chitosan is shown to be a viable scaffold material for tissue engineering due to its biocompatibility as well as its potential to introduce nanoarchitecture into 3D printed scaffolds through its porous, yet mechanically robust structure. This is accomplished through immobilizing chitosan onto other biomaterials to increase biocompatibility, or lyophilizing crosslinked chitosan to form a porous structure suitable for cellular proliferation. Furthermore, the study confirmed that as the scaffold stiffness increased, which correlates with an increase in crosslinking density, the bioactivity and cellular proliferation increased as well, stemming from the mechanosensitivity of cells.

Characterizing Computationally Designed Libraries of *de novo* Proteins with Life-Sustaining Properties

Taylor Myers
Mentor: Christina Karas
Advisor: Michael Hecht

Through binary patterning and computational design, the Hecht lab has created over a million DNA sequences unemployed by nature that are expected to form well-folded, four-helix bundle proteins. Some of these sequences demonstrate function in vivo through the ability to “rescue” conditionally lethal auxotrophs in *E. coli*. Life or death assays determine whether the proteins are responsible for auxotroph rescue through site-directed mutagenesis to introduce stop codons and frame shifts. Circular dichroism experiments and multi-dimensional Nuclear Magnetic Resonance are used to characterize the proteins’ structure and stability. Initial spectra suggest that the structures of the synthetic proteins are solvable via NMR, but the rigid and highly stable structures suggest the proteins do not perform the rescue directly.

A Type II Polyketide Biosynthetic Gene Cluster from the Human Microbiome

Arman Odabas
Mentor: Dr. Yuki Sugimoto
Advisor: Mohamed Donia

Many of our most valuable and powerful drugs are bacterial secondary metabolites; however, most of these compounds are isolated from soil bacteria, and when used as drugs in humans, tend to have numerous adverse side effects. Using computational genome-mining techniques, the human microbiome has been identified as a potentially rich source of these secondary metabolites. These are important to study because they are more likely to be active against human pathogens, less likely to have detrimental effects on humans and the human microbiome, and therefore more likely to be better drugs. Here, we report progress toward heterologous expression of a type II polyketide biosynthetic gene cluster found in *Blautia wexlerae*, a human gut bacterium. By growing *B. wexlerae* and heterologously expressing the gene cluster, we hope to isolate and characterize its product and test its bioactivity to assess its value as an antibiotic.

Crystallizing Class 1b Ribonucleotide Reductase

Yuzki Oey
Mentor: William C. Thomas
Advisor: Nozomi Ando

Ribonucleotide reductases (RNRs) catalyze the conversion of ribonucleotides to deoxyribonucleotides and therefore play a crucial role in DNA synthesis and repair. Low resolution data of the *Bacillus subtilis* RNR protein, NrdE, collected in solution using small angle X-ray scattering techniques show evidence that nucleotide binding causes conformational changes, but X-ray diffraction can provide complementary higher resolution information about the mechanisms of regulation. Therefore, we attempted to crystallize NrdE from *B. subtilis* in the presence of substrates (NDPs) and allosteric effectors (dNTPs). Promising crystal conditions were optimized, while large crystals were harvested to be taken to a synchrotron source for diffraction measurements.

Cyanogel Techniques to Create Ni/X (X = Group 13 Metals) Alloys

Alejandro Ramirez
Mentor: Aubrey R. Paris
Advisor: Andrew Bocarsly

Production of Ni-containing alloys and oxides is often time- and energy-intensive, but using techniques involving cyanogels could provide a more efficient pathway. Cyanogels are hydrogel polymeric structures formed by reacting chlorometalates and cyanometalates in aqueous solution, and they can be manipulated to form alloys and oxides. The Ni/X (X = group 13 metal) materials synthesized displayed signs of bridging cyanides yet neither gelled nor formed pure alloys after heating in inert atmosphere, although Ni carbides were observed. These outcomes could result from terminal cyanides existing in the structure and require further investigation.

Optimization of Deoxyfluorination Via Cheap Bulky Bases and Inexpensive Fluorinating Reagents.

Orestes Riera
Mentors: Matthew Nielsen and Derek T. Ahneman
Advisor: Abigail Doyle

Fluorinated compounds are widely used in pharmaceutical drug design as they provide specific transport properties and metabolic stability. Here we report an optimized method for carrying out deoxyfluorination (conversion of an OH to an aliphatic fluoride) affording yields mostly above 80% with inexpensive reagents. Considering commercial availability, price and cleanliness of reaction, we report that *tert*-butyl-TMG and 2-cyanobenzenesulfonyl fluoride are optimal reagents for fluorination of unactivated primary alcohols, whereas PuFlour remains optimal for secondary substrates. These results provide a viable, cost-effective fluorination method which could potentially be used in the pharmaceutical industry for innovative drug design.

Milking Bacteria for Antibiotics: RiPP Discovery and Biosynthesis in Streptococci

Paul Rosen
Mentor: Kelsey Schramma
Advisor: Mohammad Seyedayamdost

Natural products derived from bacteria have traditionally provided an important source of antibiotics, and increasing resistance to these drugs has made the discovery of new drug leads more critical than ever. To this end, we have focused our attention on the enzymology and biosynthesis of a promising new class of natural products: ribosomally synthesized, post-translationally modified peptides (RiPPs). Here, we experimentally probe the binding underpinning RiPP biosynthesis and attempt to discover new RiPPs in Streptococci. We have developed a fluorescence polarization assay to interrogate binding, and we are developing an untargeted mass spectrometric approach to discover new RiPPs.

Probing the Rebound Mechanism: Insights into Manganese-Catalyzed C-H Bond Halogenations

Ellie Sell
Mentor: Gang Li
Advisor: John Groves

Halogenated organic compounds play a crucial role in organic chemistry, constituting important components of a wide variety of biologically and pharmacologically active molecules. To explain the novel halogenating reactivity of manganese porphyrins and salens, radical trapping experiments were used to probe halogen transfer from reactive Mn^{IV} complexes. Using both thermolytic and photolytic radical generation methods, the reactive manganese porphyrin showed no preference for chlorination or fluorination, whereas all three manganese salens tested resulted in increased chlorination. This indicates that axial as well as planar ligands can significantly affect the rebound rate. Probing a wider variety of planar and axial ligands may provide insight into exactly how various ligands impact the rebound, potentially illuminating the details of manganese catalyzed C-H functionalization.

Cobalt and Iron Precatalysts with NHC Ligands for Alkene Hydroboration

Grzegorz Skrzypek
Mentor: Nadia Leonard
Advisor: Paul Chirik

Metal-catalyzed alkene hydroboration is a valuable process in organic synthesis that allows access to organoboronate esters - versatile synthetic intermediates that can be readily transformed into an array of functional groups. However, state-of-the-art catalysts predominantly used utilize expensive and toxic metals such as Rh and Ir. First-row transition metals offer a more inexpensive, earth-abundant, and environmentally-friendly alternative, often with distinct reactivity and selectivity. Here we report the activity of cobalt and iron precatalysts with NHC ligands for the hydroboration of terminal alkenes, which form the branched product as the major product from styrene. Gas chromatography and NMR spectroscopy confirm that hydroboration-isomerization of 1-octene occurs with all the cobalt precatalysts, whereas all the iron precatalysts give the linear product. Experiments also suggest that the two cobalt complexes (*i*Mes)$Co(CH_2SiMe_3)_2$ and (*i*Pr)$Co(CH_2SiMe_3)_2$ are deactivated for hydroboration in toluene and benzene.

Water Splitting Semiconductors: Experimental Realization of New Candidate Materials

Spyder-Ryder Sloman
Mentor: Aubrey Paris
Advisors: Andrew Bocarsly and Robert Cava

The conversion of water into oxygen and hydrogen gas using visible sunlight and semiconductor catalysts is a crucial area of research yet to be resolved; many photo-stable materials have wide band gaps that correspond to high energy UV light. Additionally, it is difficult to predict new candidate semiconductors. Using Pt and NiO co-catalysts, electrolyte solutions of varying pH values, and the hole scavenger triethanolamine, a quick screen was developed, optimized using semiconductors with known band-edge potentials, and employed to predict new possible catalysts. Twenty-six promising materials were identified using this method, seven of which produce hydrogen gas under visible light irradiation. Especially successful materials include semiconductors of the form $AgIn(Lanthanide)_2S_4$ as well as barium cobalt oxides, results that provide a good foundation for further investigation and synthesis of viable photocatalytic semiconductors.

Characterizing the Dynamics of DNA Hairpin Hybridization through smFRET and Multi-Trajectory Expectation Maximization

Nancy Song
Mentor: Hao Li
Advisor: Haw Yang

Though the thermodynamics of DNA hybridization are well understood, little is known about the exact mechanism of DNA hybridization. We investigate the reaction dynamics of a DNA hairpin binding to a surface immobilized target using single-molecular Fluorescence Resonance Energy Transfer (smFRET) and multi-trajectory Gaussian mixture expectation-maximization algorithm (GMM-EM). We found there to be at least two observable intermediates, indicating the presence of a critical intermediate in DNA hybridization. In using these transient states to develop a mechanism for DNA hybridization, we gain a better understanding of a fundamental biological process and can apply this insight to improve biotechnologies such as bio-sensing and guided drug delivery.

Searching for superconductivity in new intermetallic phases

Laura Srivichitranond
Mentor: Elizabeth Seibel
Advisor: Robert Cava

Materials of the antiperovskite crystal structure were tested for potential new superconductors. Samples were synthesized from elemental starting materials by arc-melting under an argon atmosphere, and their magnetic properties were measured. Superconductivity was observed for both rare earth-4d transition metal-carbides and silicides, with critical temperatures of 10 K and 9.5 K. Efforts are made to isolate the phase in which this superconductivity occurs.

Models for reactive coordinate trends under vibronic coupling

Arjuna Subramanian
Mentors: Siwei Wang and Desmond Toa
Advisor: Gregory Scholes

Existing models of energy-transfer processes are crucial for understanding light-harvesting, but do not fully account for the impact of vibronic interactions on these processes at small timescales. A displaced harmonic oscillator framework for reactive coordinates visualizes electronic wavefunction behavior under these interactions. Simulations performed under weak coupling conditions show gradual wavefunction localization at the lowest potential, with the addition of vibrational energy relaxation parameters increasing the convergence rate. These findings are consistent with standard expectations of reaction dynamics, bolstering the validity of the model.

A Computational Study of the Stability of CuRhO2 Photoelectrodes for Water Splitting

Uri Tayvah
Mentor: Xiao Shi
Advisors: Andrew Bocarsly and Annabella Selloni

Photocatalytic water splitting allows solar energy to be stored as H_2, providing a promising source of alternative fuel. However, the materials used as electrodes in this process can still be improved in many respects, including stability. Prior research in our group has suggested that one such photoelectrode material, $AgRhO_2$, offers improved stability toward photoreduction when compared to $CuRhO_2$, another d^{10} delafossite photoelectrode material. This improvement in stability was attributed to different contributions of Ag and Cu to the electronic density of states in the photoactive region of $AgRhO_2$ and $CuRhO_2$ respectively. We obtained insight into these findings by means of DFT and hybrid functional based calculations. Our results show a strong dependence on the choice of the DFT exchange-correlation functional: LDA/GGA functionals largely agreed amongst themselves, but differed from both previously published meta-GGA results and hybrid functional calculations. This suggests that more advanced electronic structure methods and further experimental characterization are needed to conclusively establish the differences between these materials.

Electronic Spectroscopy of Novel Dehydro[12]annulene: A Spectral Analysis and Time-Dependent Density Functional Theory Study

Ashley Tsue
Mentor: Jacob C. Dean
Advisor: Gregory Scholes

Dehydro[12]annulenes have become attractive due to their optoelectronic properties as well as their potential as building blocks for 2D carbon networks and novel carbon materials. 1,2,7,8-tetrakis(((*tert*-butyldimethylsilyl)oxy)methyl)[12]dehydroannulene (TBDMS[12]DA) and its electronic transitions were characterized using steady state and transient absorption spectroscopy coupled with time dependent density functional theory calculations. Both experimental and computational results support TBDMS[12]DA exhibiting three optical accessible states that exhibit complex photoinduced dynamics with time scales ranging from hundreds of femtoseconds to tens of nanoseconds. These excited states follow two independent pathways, including a nonradiative decay path from a higher bright state to a lower dark state and an internal conversion pathway followed by fluorescence from the lowest excited state following Kasha's rule.

The Equilibrium of DNA Hybridization is Different on Surfaces and in Solution

Miah Turke
Mentor: Hao Li
Advisor: Haw Yang

A number of important biochemical processes happen at surfaces, however there are unexplained discrepancies between the binding constants of biomolecules measured by surface-based and solution-based techniques. Surface-based Single-Molecule Spectroscopy and solution-based Fluorescence Spectroscopy techniques were implemented using target ssDNA's and their hybrid Molecular Beacon Probes with the aim of uncovering a relationship behind these differences. It was found that in the system tested, the dissociation constant found using the solution-based technique was greater than the dissociation constant found using the surface-based technique. The quantification of the difference in binding at a surface versus in solution could lead to a new understanding of how the binding process is influenced near a surface.

Synthesis of a Sulfur-Functionalized Metal-Organic Framework to Support Oxidase-Type Catalysis

Cecilia Vollbrecht
Mentor: Long Wang
Advisor: Brad Carrow

Palladium catalysts are important for a variety of oxidative and oxidase-type reactions, yet these methods still frequently require high catalyst loading due to slow rates and catalyst decomposition. Metal-organic frameworks (MOFs) may provide a tunable support to stabilize the catalyst by preventing aggregation of Pd(0) at the slow aerobic oxidation step and controlling the coordination number of the metal. An organic linker was synthesized for a MOF derivative and some initial trial reactions were run. The success of the sulfur-modified MOF in stabilizing palladium catalyst lifetime would be reflected in increased turnover numbers. The selectivity of products formed could also be correlated to confinement effects by modulating the pore aperture.

Synthesis of Bis-CyHQ-Dopamine, a Novel Doubly Caged Dopamine Analog With Expected Sensitivity to Chemical Two-Photon Excitation

David Weiner
Mentor: Brendan Lainhart
Advisors: Samuel Wang and Robert Knowles

Caged neurotransmitters, appended with a photoremovable protecting group that renders them inert until light exposure, allow localized and rapid optical stimulation and modulation of neural tissue. Chemical two-photon uncaging, which attaches two inactivating groups to the biomolecule, increases the spatial resolution and lowers the receptor interference of uncaging systems by requiring two simultaneous photocleavages to produce the bioactive compound. This paper reports progress towards the synthesis of Bis-CyHQ-dopamine, a novel doubly caged dopamine analog with expected chemical two-photon uncaging properties. Studies of the photophysical and photochemical properties will be conducted once the synthesis is complete. This compound will contribute to the currently limited set of optical tools for emulation of short-term dopaminergic transmission, thus enabling the investigation of dopamine's poorly understood biological effects.

Quantitative Analysis of G6PD Inhibition with Michaelis-Menten Kinetics

Joshua Wang
Mentor: Jonathan Ghergurovich
Advisor: Joshua Rabinowitz

Many cancer cells require copious amounts of NADPH, a biological hydride source, for the biosynthesis of cellular building blocks and for defending against redox stress. The primary mammalian NADPH source is the oxidative pentose phosphate pathway, of which glucose 6-phosphate dehydrogenase (G6PD) is the first and rate limiting step, and overexpressed in various cancers. Studies suggest inhibition of G6PD with small molecules may be a means of blocking cancer cell proliferation; however, few useful G6PD tool compounds have been reported to exist. The inhibitor most commonly used, dehydropiandrosterone (DHEA), lacks the potency and selectivity (as a steroid derivative) to be a useful tool compound. To identify novel non-steroidal inhibitors of G6PD, a high-throughput screen of a small molecule compound library was conducted using resazurin-coupled fluorescence assays; hits were confirmed in orthogonal assays (such as absorption) and characterized in Michaelis-Menten experiments to identify binding kinetics. Two classes of inhibitors were discovered with similar potency to DHEA: one that competitively inhibited G6PD with respect to NADP+ and noncompetitively and/or noncompetitively inhibited G6PD with respect to G6P, and a second that noncompetitively and/or uncompetitively inhibited G6PD with respect to NADP+ and G6P. These results will supplement other efforts in lab, including protein crystallography, with the aim of improving these inhibitors in terms of selectivity and potency.

Regulation of the Pentose Phosphate Pathway in Cancer Cells

Monica Wei
Mentor: Lukas Tanner
Advisor: Joshua Rabinowitz

A hallmark of cancer metabolism is the increase in glycolysis known as the Warburg effect. This phenomenon does not occur in isolation, however; the pentose phosphate pathway (PPP) draws from glycolysis to provide reducing power and ribose-5-phosphate that are critical for cancer cell survival. As with glycolysis, PPP activity is increased in tumor cells, but how enzymes within the PPP regulate these changes is still obscure. Better understanding of PPP regulation will help identify therapeutic targets within the PPP, which to date remain elusive. Here we study PPP regulation by overexpressing enzymes within the PPP and glycolysis. This allows us to examine the contribution of individual reactions to global pathway changes and identify reactions with significant control of pathway utilization. We used a combination of metabolomics and isotope-tracer studies to track metabolism. These techniques provide a quantitative and comprehensive analysis of an array of PPP states. We found that the PPP and glycolysis are closely regulated, as shown by broad changes in metabolite concentrations, but that most PPP enzymes do not control glycolytic flux. We investigated a particularly striking change in NADPH and NADP levels using kinetic modeling. We also observed that transaldolase overexpression led to significant changes in PPP flux. In future studies we will utilize radioactive and deuterium-labeled glucose tracers to further estimate PPP flux.

Recombinant Production of Novel Heme-Thiolate Protein Using the *Pichia pastoris* Expression System

Evelyn Wu
Mentor: Christin Monroe
Advisor: John Groves

There has been a recent interest in studying aromatic peroxygenases (APOs), a class of highly stable proteins, because of their unique ability to catalyze oxidations on various unreactive hydrocarbon substrates. Recently, work has been done to produce a novel APO recombinantly using the *Pichia pastoris* expression system. Expression of the DNA sequence and mRNA of the novel protein were confirmed, and it is believed that the protein may be present intracellularly. Once the presence of the APO is confirmed and the location is determined, steps will be taken to isolate the APO for characterization and mechanistic studies.

Finding BuPhos: Synthesis and Coordination Chemistry of an Exceptionally Bulky Trialkyl Phosphine

Bufan Zhang
Mentor: Liye Chen
Advisor: Brad Carrow

Bulky alkyl phosphines have the potential to increase the reactivity of palladium catalysts due to their high polarizability and electron-releasing characters. As a result, industrial syntheses of fine chemicals could be cheaper, and pharmaceuticals safer. Here we report the synthesis and coordination chemistry of BuPhos, a highly sterically hindered trialkyl phosphine. The compound has shown its uniqueness by demonstrating better solubility and different coordinating ability compared to PAd3, a similar but less hindered phosphine.

Characterizations of Compensatory NADPH Production Pathways in G6PD-Deficient Hypomorphs and Knockouts

Henry Zheng
Mentor: Li Chen
Advisor: Joshua Rabinowitz

Although G6PD deficiency affects over 400 million people worldwide, the compensatory mechanisms of alternative NADPH production pathways under the diminished oxidative PPP conditions remain poorly understood. Through CRISPR gene editing, enzyme assays, and Western Blots, we were able to visualize the changes in G6PD, 6PGD, IDH1, and malic enzyme in G6PD knockout and hypomorphic cell lines. There does not seem to be a significant difference among the activities of the enzymes involved in the alternative NADPH production pathways suggesting that a different underlying mechanism is responsible for the compensatory effects. We plan to continue looking for this mechanism through different lenses in the future.