Session E Abstracts

Molecular Mechanism of Nucleocytoplasmic Transport of p38α and cGAS
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Mentors: André Hoelz and Chia Yu Chien

Nested between the inner and outer lipid layers of the nuclear envelope, nuclear pore complexes (NPCs) are the sole gateways that mediate the bidirectional macromolecule transport between the cytoplasm and nucleus. One of the macromolecules that relies on the NPC for intracellular transport is mitogen-activated protein kinase 14 (MAPK14), also known as p38α. MAPK14 is critical for directing cell growth, differentiation, and apoptosis in addition to stimulating immune responses against external stressors. Thus, the hyperactivity of MAPK14 in the nucleus carries detrimental health implications ranging from mild inflammatory disorders to autoimmune diseases and cancers. By researching the route taken by MAPK14 into the nucleus, it becomes possible to explore the nuclear transport inhibition as a solution to the negative effects of dysregulated MAPK14. Alongside the research being conducted on MAPK14, the transport of the primary immune sensor of cytosolic DNA, cyclic GMP-AMP synthase (cGAS), is also under investigation. Progress on recombinant protein purifications and pull-down technique has allowed for further pathway analysis with the hopes of identifying the critical transport factors for p38α and cGAS.

Development of a Deep Sea Thermophilic Model Organism for the Study of Large Scale Protein Assemblies
Filipe Andreas Melo
Mentors: Andre Hoelz and George Mobbs

Alvinella pompejana is a polychaete worm identified at hydrothermal vents near the sea floor, a habitat exhibiting temperatures as high as 80°C. This extreme environment has led to an unusually high degree of thermostability within the *A. pompejana proteome*, making the expression of recombinant proteins an attractive option for structural biologists aiming to determine structures that have proven intractable from alternative higher eukaryotic model systems, such as humans due to their relative lack of stability. We aim to demonstrate *A. pompejana* as a useful model organism by showing the structural homology of proteins within its nuclear pore complex (NPC), compared to their human and fungal counterparts, which have recently been characterized. The NPC is an ~110MDa protein complex that makes up the only pathway through the nuclear envelope and is central to the export of mRNA from the nucleus to cytoplasm. I aimed to recapitulate crystal structures of the NPC protein Nup214, the proteins Nup214 and Ddx19 in complex, and Ddx19 in complex to Gle1 to compare them to their human homologs. I did this by expressing these proteins in *E. coli*, purifying them to a high degree of homogeneity by affinity, ion exchange, and gel filtration chromatography, then screening for crystallization conditions. This study demonstrates the suitability of this structural model organism with ample yields and promising crystallization hits obtained.

Structural Characterization of Human Nuclear Pore Membrane Protein GP210
Philip Spyrou
Mentors: Andre Hoelz and Sema Edjer

The human nuclear pore is an anchored macromolecular assembly forming the only bidirectional gateway regulating the exchange of macromolecules across the nuclear membranes. One of the 34 unique proteins which assemble the nuclear pore, GP210 is an integral membrane protein composed of a singular helical transmembrane domain and 17 repeated domains of an Ig like fold. GP210 is one component of the inner ring of the nuclear pore complex, and is theoretically responsible for anchoring the nuclear pore, providing flexibility, and stabilizing the nuclear envelope curvature. Due to its high flexibility, GP210 was subdivided into individual Ig-like domains. Seventeen Ig-like domain have been identified, which were each individually tested for expression and solubility. Soluble Ig-like domains were tagged with a histidine-SUMO tag for Nickel affinity purification by and then ion exchange chromatography based on the pI of the purified domain. Finally, a size exclusion chromatography allowed researchers to obtain highly pure protein sample suited for crystallization. Commercially available crystallization screens were used to increase the probability to obtain protein crystals. Crystals for Ig17 have been obtained and been optimized. Due to poor yields of other soluble Ig domains, new expression constructs are being generated to create longer chains of Ig domains with potentially better expression and solubility. Structural characterization at high resolution of these Ig domains will allow researchers to understand the flexibility of GP210 which acts like a belt allowing the NPC to open and close for nucleocytoplasmic transport.

Biochemical Reconstitution of Nuclear Pore Complex Cytoplasmic Filaments Using Thermophilic Eukaryotic Model Organisms
Wentao Zhang
Mentors: André Hoelz and George Mobbs

Within eukaryotic cells, massive ~110-MDa transport complexes called nuclear pore complexes (NPCs) facilitate the selective transport of macromolecules across the nuclear envelope. Protein constituents of the NPC, called

nucleoporins (nups), are essential to biology's central dogma, facilitating directional transport of RNA from nucleus to cytoplasm. We utilize *Alvinella pompejana*, a thermophilic extremophile worm living near deep-sea vents, as a model organism to investigate the structure and biochemical interfaces of the cytoplasmic filament nup205. Using cryo-EM, X-ray crystallography, and affinity assays, we will elucidate the structure and interaction interfaces of nup205 to near-atomic resolution. Our findings will facilitate a more complete understanding of NPC structure and assembly mechanics and will lead to development of targeted therapies targeting erroneous NPC homologies.

Structural and Biochemical Characterization of *Alvinella pompejana* Nucleoporins
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Mentors: Andre Hoelz and George Mobbs

Challenges in expressing and purifying requisite amounts of relevant human proteins have significantly hindered contemporary investigations of post-synaptic biochemistry, limiting our understanding of the fundamental biochemical processes that underlie neural function. In order to overcome these challenges, the Hoelz lab has identified deep-sea worm *Alvinella pompejana* as a promising model organism candidate for sourcing proteins with superior biochemical stability, high amenability for large-scale expression, and significant structural homology to their human counterparts. With the broad goal of validating *A. pompejana* as a novel model organism for the study higher-order biochemical processes, we seek to structurally characterized several protein constituents—termed nucleoporins—of the *A. pompejana* nuclear pore complex (NPC), the structure of which is well-characterized and conserved across a wide range of phyla. We focus our efforts on several nucleoporin constituents of the cytoplasmic filament nucleoporin complex—a key-sub-complex of the NPC which plays several roles in not only nucleocytoplasmic transport but also the structural integrity of the NPC—the solenoid region of nucleoporin 93, the R2 region of nucleoporin 35, and nucleoporin Gle1. We seek to express, purify, and crystallize the nucleoporins in order to verify structural similarity to their human homologues, thus establishing *A. pompejana* as a suitable model organism for the study of neural biochemistry.

Stabilization of High Oxidation State Complexes for Nickel-Catalyzed Aryl Fluoride Cross-Coupling
Eric Ramos
Mentors: Theodor Agapie, Meaghan Bruening, and Matthew Espinosa

In catalysis, high oxidation state compounds can significantly lower the activation energy to perform difficult transformations. One such process is the formation of aryl-fluoride bonds, which are a common motif in pharmaceuticals, agrochemicals, and materials. Nickel has been shown to facilitate this coupling through some high-valent nickel species, and that these states can be supported by pyridine-containing ligands. The isolation of these species is necessary to enable reactivity studies. Fluorinated silicates developed in the Agapie Group could be a solution to this problem. By appending these silicates to a pyridine-based ligand core, we hypothesize that high oxidation state metal complexes can be isolated. To synthesize the target ligand, a Negishi Coupling approach was taken. We report spectral data that indicates the product is formed, but it remains a challenge to isolate and purify the material before metalation.

PdPtOx Thin Film Catalyst Synthesis for Olefin Epoxidation
Yamilet M. Rivera Cintrón
Mentors: Karthish Manthiram and Chenyu Jiang

Production of propylene oxide with hydrogen peroxide requires the direct burning of fossil fuels to reach elevated reaction temperatures. Electricity can serve as a greener alternative to fossil fuels, sustainably functionalizing olefins with the aid of efficient and selective electrocatalysts. However, understanding mechanisms of electrochemical reactions remains untrivial due to dynamic changes of the catalysts under applied potential. Olefin epoxidation using water as the oxygen atom source has been previously demonstrated in ambient conditions with PdPtOx/C nanoparticles as the catalyst drop-cast onto carbon paper (CP) electrodes (Chung et. al. 2023, *Science, manuscript under review*), achieving a Faradaic efficiency (FE) higher than 90% for cyclooctene oxide. Due to its porous nature, CP is regarded as an unsuitable substrate for *in situ* spectroscopies that require an ultra-high vacuum environment, such as soft X-ray absorption spectroscopy. By changing the substrate to a nonporous material and depositing the catalyst without sacrificing its high catalytic performance, the mechanism of this oxidation reaction can be investigated to reveal the reason why PdPtOx excels as a novel catalyst for olefin epoxidation. This study provides insight on how deposition methods of PdPtOx affect its structure and catalytic properties by analyzing the FE obtained from the use of CP, indium tin oxide, and glassy carbon as substrates. PdPtOx thin films were prepared by drop-casting, sputtering, and spin coating, and chronopotentiometry was carried out at 40 mA/cm^2 until a total charge of 20C was passed. A series of characterization methods were performed to obtain information on the oxidation states, morphology, and thickness of PdPtOx thin films.

Progress in the Electrochemical Carboxylation of Aryl Aldehydes
Nuren Lara
Mentors: Karthish Manthiram and Thu Ton

Electrochemical carboxylation has emerged as a promising method for converting CO_2 into value-added organic compounds. The electrochemical carboxylation of organic compounds has several advantages, such as using a non-toxic and abundant carbon source to perform C-C bond formation. Additionally, the electrochemical carboxylation of aldehydes results in the synthesis of alpha hydroxy acids (AHAs), a commercially significant compound. A sacrificial-anode free method for the electrochemical carboxylation of benzaldehyde to mandelic acid was studied. Efforts focused on increasing the faradaic efficiency and yield by attempting to reduce the hydrogenation side reaction. Preparing electrolytes in water-free environments suggest that reducing the water content of salts and solvents increases the faradaic efficiency of the reaction. Future work will focus on the development of a high performance liquid chromatography method for the simultaneous quantification of mandelic acid, benzaldehyde, and other side products in the reaction mixture.

Accessing Medium-sized Rings *via* Vinyl Carbocation Intermediates
Naiara Lebrón Acosta
Mentors: Hosea Nelson and Zhenqi Zhao

Cyclic structural motifs are commonly found in natural products, pharmaceuticals, and advanced materials. Among cyclic compounds, 5- to 6-membered rings are the most prevalent due to their stability and ease of preparation. Synthetic methods for small-ring cyclic structures (5–7 membered rings) have been well-established and developed. However, these methods encounter challenges when attempting to achieve the synthesis of medium-sized rings (8–11 membered cycles). Medium-sized rings possess torsional and transannular strains, rendering them less stable and consequently more difficult to synthesize. The Nelson Lab has recently laid the foundation for generating vinyl carbocation intermediates through Lewis acid-weakly coordinating anion catalysis. In this study, we present a catalytic method for synthesizing medium-sized ring systems via intramolecular Friedel-Crafts reactions of vinyl carbocations.

Towards an Enantioselective Total Synthesis of Pedrolide
Jessica Wang
Mentors: Sarah E. Reisman and Cedric Lozano

Complex natural product synthesis is of great interest to organic chemists from both applied and intellectual standpoints. The Reisman Group has had a long standing interest in total synthesis, with particular attention to synthesizing natural products via convergent coupling approaches. Pedrolide, a novel tigliane-type diterpenoid that is heptacyclic and features an embedded bicycle[2.2.1]heptane system, is a compelling synthetic target based on its complex structure alone. Pedrolide also has interesting biological activity and medicinal potential. Our convergent synthetic strategy towards pedrolide relies on a radical-based cyclopropane fragmentation to construct the bicycle[2.2.1]heptane, and an enantioselective homo-Diels—Alder—developed in collaboration with the Sigman Group at the University of Utah—to construct the cyclopropane. Herein we report efforts regarding two enantioselective synthetic routes towards pedrolide, focused on answering synthetic questions regarding functionalization and our key synthetic steps. These efforts will ultimately contribute towards an enantioselective total synthesis of pedrolide.

Using Supervised Machine Learning to Predict the Regioselectivity of C–H Oxidation Sites in Complex Molecules
Gina Lee
Mentors: Sarah Reisman, Jules Schleinitz, and Alba Carretero

Targeted C–H oxidations, which split C–H bonds into C–O bonds, can derive complex molecules from other pre-existing molecules rather than synthesizing them *de novo*. As a result, they allow for greater efficiency in the synthesis of complex molecules. However, due to the multitude of existing sites where oxidation may occur, it is extremely difficult to manually pinpoint the most effective site to create the product. Thus, this project aims to utilize supervised machine learning to predict the most reactive sites within a complex molecule, where the desired product will be created. A Random Forest model, which utilizes a "wisdom of the crowd" method by generating numerous decision trees, is used, and a set of electronic descriptors are generated through quantum chemical methods for each carbon atom in the data set. The model is then tested and run through the data set of 288 unique reactions using their descriptors, with the reactions split between the testing and training sets. The accuracies are then compared to a baseline established by a method based on the atoms' Gasteiger charges, and the model is improved based on its performance with numerous proportions of testing and training data splits.