Caltech
Student-Faculty Programs

# Session C Abstracts

**Grand canonical quantum mechanics study for $CO_2$RR on PcFe MOF catalyst**
Samhitha Venkat
*Mentors: William A. Goddard III and Sejun Kim*

As reducing $CO_2$ levels becomes increasingly important to curbing greenhouse gas emissions, the electrochemical reduction of $CO_2$ to form hydrocarbon products offers a promising route to achieving this goal. While Cu-based catalysts are able to produce significant quantities of methane and ethylene, catalysts that operate at lower overpotentials and achieve greater product selectivity are required for electrochemical reduction to become industrially viable. This study investigates the effectiveness of a phthalocyanine based two-dimensional metal-organic framework with Fe active sites in catalyzing methane and ethylene products using the grand canonical DFT method. We have thus far obtained intermediates for the proposed reaction pathway for methane formation. Further calculations are required to obtain a complete energy profile for the methane and ethylene formation pathways.

**Expanding the scope of C(sp2)–F bond activation processes facilitated by light-absorbing Ni–based complexes**
Annalissa Valdez
*Mentors: Ryan G. Hadt, Jacob O. Rothbaum, and Maria Blankemeyer*

Photocatalysis has gained significant attention as a useful tool for aiding the development of new reactivities in organic synthesis. Moreover, merging thermal catalysis with photochemistry has had a profound impact in facilitating challenging sp2–sp2 and sp3–sp3 C–C or C–X coupling reactions. The Hadt group synthesized (t-Bubpy)Ni(II)(p-tetrafluoropyridyl)2 and characterized the complex using single-crystal X-ray diffraction, NMR, and mass spectrometry. Preliminary mechanistic studies suggest its formation via multimolecular, para-selective C(sp2)–F activation of pentafluoropyridine. The complex is air- and moisture-stable under ambient conditions and undergoes a clean C–C bond-forming transformation upon visible light irradiation at room temperature. Furthermore, Ni(p-PyF4)2(t-Bubpy) activates stronger C(sp2)–F bonds, thus broadening the substrate scope of Ni-mediated C(sp2)-F activation. Fluorimeter and UV-Vis spectroscopy was utilized to analyze photophysical properties. Absorbance spectra show charge transfer bands and a λmax at 370nm, consistent with a metal-to-ligand charge transfer (MLCT) transition. Emissions observed between 450-550 nm indicate the formation of an emissive species.

**Structural characterization of PER2 nuclear import via the PER2-NLS2–mmKap-α2 Complex**
Philip-David Medows
*Mentors: André Hoelz and Chia-Yu Chen*

Period Circadian Regulator 2 (PER2) is a key transcriptional regulator in the mammalian circadian rhythm, acting as a transcriptional inhibitor in the nucleus following nuclear import. This nuclear import occurs after the formation of a trimeric complex consisting of PER2, an α-karyopherin protein that binds to one PER2's nuclear localization signals (NLS), and a β-karyopherin protein. This project aims to characterize PER2's nuclear import mechanism by crystallizing one of its two NLS sites in complex with mmKap-α2, a mouse α-karyopherin. We will use x-ray crystallography to determine the structure of this NLS–mmKap-α2 complex, thereby enabling the identification of key molecular interactions essential for PER2 nuclear import. These findings will inform future studies of circadian regulation at the molecular level.

**Nucleocytoplasmic transport of circadian clock proteins**
Talaysha S. Simonds
*Mentors: André Hoelz and Sabrina Doerrich*

The molecular circadian clock is an important regulator of diurnal physiology and behavior with a rhythm of 24-hours. At the molecular level, it consists of a transcriptional-translational feedback loop (TTFL) in which positive elements CLOCK and BMAL drive the transcription of negative regulators PER and CRY that inhibit their own expression. To block the transcriptional activation of CLOCK-BMAL, the negative regulators PER and CRY have to be translocated into the nucleus. This shuttling of cargos through the nuclear pore complex mediated by transport factors called Karyopherins is known as nucleocytoplasmic transport. While transport of circadian clock proteins is essential to regulate the circadian oscillations, it has not been studied systematically. Our research project aims to investigate the interactions between circadian clock proteins and Karyopherins. To accomplish this, we recombinantly express and purify the required proteins and ultimately test their interactions through size exclusion chromatography coupled with multi-angle light screening (SEC-MALS).

**Engineering single-chain AMP-activated protein kinase complexes to investigate metabolic regulation and hearing loss**
Isabella H. Yang
*Mentors: André Hoelz and Michael S. Gruhne*

In cells, chemical energy is consumed and produced to propel key metabolic processes. Cells must quickly sense the energy needs of the organism, utilizing or producing ATP in response. Chiefly, AMP-activated protein kinase (AMPK), a cellular energy sensor, recognizes and maintains the ratio of ATP to AMP. In the cochlea, metabolic activity of inner hair cells HCs) is necessary for the conversion of auditory stimuli into neural signals. Loss of IHC activity, caused by noise overexposure, aging, or ototoxic drug treatments, results in permanent hearing loss. AMPK inactivation has been heavily implicated in IHC death and resulting ear damage, suggesting that overexpression of certain AMPK isoforms may protect against auditory impairment. However, overexpression of individual AMPK subunit isoforms can broadly alter the expression of other subunits, preventing high-confidence overexpression of specific complexes. To remedy this, individual human AMPK subunit isoforms were linked together to produce single-chain AMPK (scAMPK) complexes. scAMPK complexes were expressed in *E. coli*, purified via column chromatography, and compared to their respective heterotrimeric forms through folding pattern and activity. Such development of scAMPK proteins allows complexes with designated isoforms to be expressed with increased reliability, optimizing AMPK overexpression for widespread clinical applications, particularly therapies for hearing loss.

**Expanding in-situ capabilities of a table-top soft X-Ray absorption spectrometer for probing electrocatalyst electronic structure**
Jacob A. Cho
*Mentors: Scott K. Cushing and Alejandro Arellano*

Soft X-ray absorption spectroscopy (SXAS) is an element-specific technique for probing the local electronic and geometric structure of materials, making it ideal for studying catalytic systems under operating conditions. *In-situ* and *operando* SXAS further improves the versatility of the technique by revealing changes in oxidation state, bonding environment, and active-site structure during electrochemical reactions, providing critical insight into catalytic mechanisms. This project aims to expand the *in-situ* capabilities of the Cushing Lab's table-top SXAS system through the design and implementation of a vacuum-compatible adapter that integrates both a commercial liquid electrochemical TEM holder and a static TEM holder with the beamline. In parallel, static SXAS measurements of nitrogen-substituted pyridinium (NsP) films electrodeposited on silver electrodes were conducted to establish baseline spectra of the C K-edge, N K-edge, and Ag $M_{4,5}$-edges. These systems are of interest because NsP films have been shown to suppress the hydrogen evolution reaction during $CO_2$ reduction, enabling highly selective CO production, and is a viable candidate for *in-situ* SXAS on the table-top. The adapter will ultimately enable *in-situ and operando* gas- and liquid-phase SXAS measurements, allowing real-time tracking of electronic structure changes during

catalysis. By improving the flexibility and accessibility of *in-situ* SXAS in a lab-scale setting, this work aims to accelerate the study of catalytic materials and inform the design of more efficient systems for $CO_2$ conversion and other energy-relevant reactions.

**Multimetallic complexes for small molecule reactivity: Expanding dinuclear nickel systems through third-metal variation**
Sherlyn Cazares
*Mentors: Theodor Agapie and Matt R. Espinosa*

Multimetallic complexes represent a new direction in inorganic chemistry, offering novel approaches to small molecule activation and catalytic transformations by mimicking nature's metalloenzymes.. Bimetallic systems, particularly those involving nickel and cobalt centers, have demonstrated enhanced reactivity and selectivity compared to their monometallic analogues by stabilizing reactive intermediates. Recent advances suggest that introducing a third metal can further expand the scope of reactivity and modulate electronic environments.

Trimetallic systems are yet to be fully explored in current literature. However, those that do appear in literature have shown unique capabilities in redox chemistry and C-H bond activation, highlighting the potential of such complexes to surpass the limits of mono- and bimetallic systems.

This project proposes the synthesis of novel bimetallic nickel complexes with a tetrapyridine ligand designed to support the strategic placement of a third metal (scheme 1). The objective of this project is to systematically evaluate how the identity of a third metal influences small molecule activation. This work aims to develop new structure-function relationships in multimetallic catalysis by leveraging geometric adaptability and electronic modulation, untimely advancing the design of catalysts capable of complex and selective chemical transformation.

**The role of DNA2 in the cGAS-STING signalling pathway**
Anya J. Peedle
*Mentors: Judith L. Campbell and Eunny Bae*

DNA2 is a structure specific 5'-3' nuclease/helicase that displays ATPase activity. It has roles in cell cycle regulation, telomere maintenance and mitochondrial replication. DNA2 aids in DNA replication, specifically Okazaki fragment maturation and 5' long flap cleavage. It also repairs DSBs by initiating homologous recombination and re-starting stalled replication forks with WRN or BLM. DNA2 is overexpressed in tumours including breast cancer, ovarian cancer and pancreatic cancer as it mitigates replication stress providing a survival advantage. Therefore DNA2 is a good therapeutic target, where inhibition limits DNA repair and sensitises cells to chemotherapeutics. Here, we are looking at the role of DNA2 in cGAS-STING signalling. This is an innate immune pathway that detects cytosolic DNA and triggers inflammatory responses. DNA2 can also be targeted for degradation by CDK12 phosphorylation, which triggers ubiquitin-proteasomal degradation. We hope to overexpress DNA2 using CDK12 inhibitors, hypothesised to increase cGAS-STING signalling. We also used tamoxifen induced knockout of DNA2 to demonstrate decreased levels of cGAS-STING signalling. Chronic cGAS-STING activation leads to multiple inflammatory diseases including heart failure and liver disease, which can be potentiated by chemotherapies such as doxorubicin. Therefore understanding the role of DNA2 could yield therapeutic benefits for aberrant cGAS-STING signalling.

**Engineering native N-Terminal access to a membrane enzyme: Dual strategies for structural isolation of HyMraY**
Camilla Fezzi
*Mentors: William M. Clemons, Jr., and Beebee Yusrah Kaudeer*

The enzyme MraY catalyzes the first membrane-anchored step in bacterial peptidoglycan biosynthesis by attaching UDP-MurNAc-pentapeptide to the C55 lipid carrier to form Lipid I. Despite its crucial role and importance as an antibiotic target, understanding the structure of MraY remains challenging due to difficulties in obtaining the protein with a truly native N-terminus in a detergent-solubilized, folded state. In this project, we explore two complementary strategies to isolate HyMraY with an unmodified

N-terminus from E. coli: (1) an affinity-based approach using a His-tagged nanobody Nb7 that specifically binds to folded HyMraY, and (2) an intein-mediated trans-splicing system designed to automatically remove purification tags after expression. We engineered two plasmids: one expressing InteinC fused to HyMraY, and another expressing InteinN with a His-tag, allowing the conditional reassembly of the full intein in trans. At the same time, we expressed and purified Nb7 and performed membrane extraction, Ni-NTA pull-down, SDS-PAGE, western blotting, and size-exclusion chromatography (SEC) to assess complex formation with HyMraY. While initial SEC and western blot data indicate some nanobody binding to MraY, further optimization of binding and detergent conditions is in progress. These two approaches aim to create a powerful platform for high-resolution structural and mechanistic studies of transmembrane enzymes with their native terminal features, with potential broader applications in drug discovery and membrane protein biophysics.

**Towards a mechanistic characterization of type II single gene lysis proteins via *in vivo* and *in silico* approaches**

Diego Antonio Velazquez Vargas
*Mentors: William M. Clemons, Jr., and Roujon Nowzari*

Single stranded RNA (ssRNA) bacteriophages are viruses that exert bacterial autolysis by the expression of a single protein, referred to as Single Gene Lysis proteins (SGLs). These proteins can be categorized based on their mode of action: while type I SGL disrupt the synthesis of bacterial cell wall, type II induce host lysis without affecting the net production of peptidoglycan. Although Type II SGLs have been extensively studied since the discovery of the lysis protein L from ssRNA phage MS2 (almost 50 years ago), their lytic mechanism remains a long-standing unresolved question, whether it involves enzyme inhibition, pore-like structure formation, or entirely novel interactions. To clarify these possibilities, we aim to apply a dual *in vivo* and *in silico* approach. The first approach involves fusing the lysis proteins to MiniTurboID, an enzyme that labels proteins in a ~10 nm radius, which may identify possible targets of type II SGLs. In parallel, we conducted molecular dynamics simulations using GROMACS to model L oligomerization within a lipid bilayer, aiming to show its impact on membrane stability. These experiments may help clarify the lytic mechanism of L, contribute to the characterization of other type II SGLs, and ultimately support combating multidrug-resistant bacteria.