

Trapping distinct states to characterize the MurJ-Lipid II complex

Kylin M. Elliott

Mentors: William M. Clemons and Grace F. Baron

MurJ is the essential lipid II flippase involved in peptidoglycan biosynthesis, the pathway responsible for cell wall formation that is conserved across bacteria. When bacteria cannot form a cell wall, cells will lyse from osmotic pressure, making MurJ an emerging antibacterial target. Due to conformational changes and subsequent substrate release, it has been impossible to capture the substrate-bound structure of MurJ or to fully understand its transport mechanism. To solve this problem, constructs with point mutation pairs were created, specifically engineered to form disulfide bonds that trap an inward-facing conformation of MurJ. We hypothesize that trapping this distinct state will allow for substrate binding in vitro and allow structural characterization of the complex. The primary methodology includes construct design and multi-step membrane protein purification, and evaluation of disulfide formation using assays with trypsin digestion and oxidative treatments. Disulfide bond formation in the constructs has been confirmed, and CryoEM analysis will be performed to evaluate the state of the proteins before moving forward with structural characterization of the lipid II-bound state.

Removal of C55-P-P-GlcNAc-ManNAcA from MjAglH for improved structural analysis

Grace H. Otos

Mentors: William M. Clemons and Jacob Kirsh

N-glycosylation is a post-translational modification where glycans are added to asparagine residues on proteins. In humans these modifications are important for cell adhesion, migration, and signaling. In archaea they are essential for forming a protective proteinaceous layer on the membrane, enabling survival in extreme environments. The initiating proteins for this process in humans and archaea are homologs DPAGT1 and AgIH. Structural analysis of AgIH in the Clemons lab thus far has found it to be structurally similar to DPAGT1, making it of interest for comparative analysis. However, the existing structural data for AgIH shows the binding pocket to be occupied by the non-native lipid, C55-P-P-GlcNAc-ManNAcA; a result of the E.Coli expression system. This project explores techniques to obtain an apo AgIH sample to enable further study of AgIH. In order to obtain an apo sample, we applied three strategies: catalytically ablated mutants, a catalytically active mutant, and a lipid stripped wild type sample. Through mass spectrometry and lipidomics, we were able to identify the successful removal of C55-P-P-GlcNAc-ManNAcA from both catalytically ablated mutants and continue to work to purify and analyze our catalytically active and lipid-stripped samples for the removal of the lipid.

Identifying cellular targets of phage-encoded single gene lysis proteins in *Escherichia coli*

Nihira Padidam

Mentors: William M. Clemons and Roujon Nowzari

Antimicrobial resistance poses a global health threat that demands investigation of alternative target discovery. Single-gene lysis (Sgl) proteins encoded by lytic bacteriophages induce lysis in host bacterial cells. Due to their rapid evolution and diversity, the study of Sgl lysis mechanisms offers abundant opportunities to identify new druggable targets. While the number of newly discovered Sgls has increased, most Sgl targets continue to remain unknown. Here, we aim to elucidate the mechanism of lysis in *E. coli* of three newly discovered Sgls ESO 003, ESE 027, and EMM 000. We seek to develop a genetic screen to identify resistance mutations mapping to Sgl-specific targets and apply it to our Sgls of interest. Additionally, we aim to assess bacterial cellular morphology during Sgl-induced lysis to determine whether these Sgls target cell wall biosynthesis. By defining the targets of Sgl proteins, our work aims to identify bacterial vulnerabilities that can be leveraged to develop new drugs and combat the prevalence of drug-resistant pathogens.

Circulating microbial and vesicle-associated signatures as potential biomarkers of atherosclerotic plaque states

Emily Tu

Mentors: Rustem F. Ismagilov and Roey Lazarovits

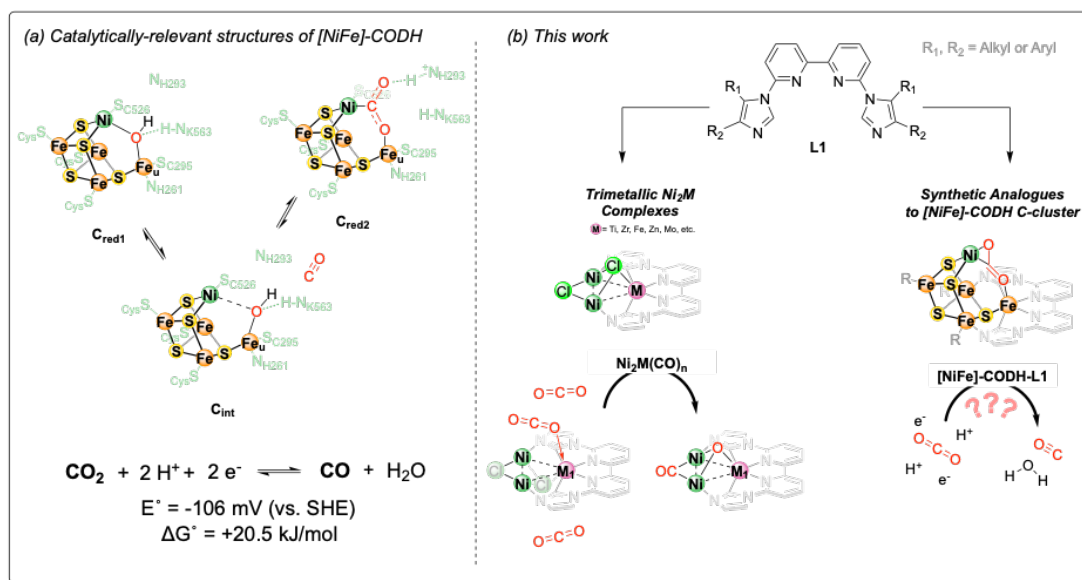
Atherosclerotic plaques vary in their composition and behavior as some remain stable, whereas others become inflamed, calcified, rupture-prone, or otherwise high-risk. The underlying mechanisms that drive different plaque states remain incompletely characterized. One possibility is that microbial and vesicle-associated biomarkers contribute to plaque heterogeneity, particularly if circulating material can be traced to the oral microbiome. Periodontal disease and gingival disruption have been associated with cardiovascular disease and allow microbial products to enter the bloodstream. Once in circulation, microbial cell-free nucleic acids, extracellular vesicles, and bacterial vesicles could link oral microbial communities and vascular disease. This project aims to investigate whether oral microbial biomarkers are detectable in blood and whether they connect to plaque inflammation, calcification, and necrotic core formation. The current approach focuses on establishing a paired oral and blood sampling framework and optimizing methods for detecting cell-free nucleic acids and vesicle-associated material.

Trimetallic nickel-based CO₂ reduction catalysts and progress towards a synthetic analogue to the [NiFe]-carbon monoxide dehydrogenase C-cluster

Vassilios P. Dresios

Mentors: Theodor Agapie and Leo A. Morag

Multimetallic catalytic centers are useful for converting inert molecules, such as CO₂, into value-added products. An enzyme expressed natively in some anaerobic microorganisms, carbon monoxide dehydrogenase ([NiFe]-CODH), contains a NiFe₄S₄ cluster active site at which CO₂-CO conversion at inoffensive temperatures and pressures. The catalytic mechanism of the cluster can be better understood *via* a synthetic analogue of this metal cluster, which would facilitate study of the electronic structure of molecules similar to the cluster at different points during catalysis. The binding of a 6,6'-di(N-imidazolyl)-2,2'-bipyridyl ligand **L1** to an iron-sulfur cubane cluster (Fe₄S₄R₄) may open a promising avenue towards assembling a synthetic model for [NiFe]-CODH. The possible nickel-iron cooperativity in this metallic active site moreover inspires other potential multimetallic catalysts for CO₂ reduction. Towards this goal, the proposed dianionic bis(carbene)bipyridyl ligand may also host trimetallic Ni₂M (M = transition metal) complexes for cleaving C=O bonds. The impact of the identity of M on the ability of this trimetallic complex to catalyze CO₂ reduction is investigated.



Developing a charged palladium catalyst to synthesize photodegradable polyethylene

Chloe I. Hu Pesh

Mentors: Theodor Agapie and Jocelyn Y. Zhang

Polyethylene persists in the environment long after its disposal because its inert carbon-carbon bonds are not readily hydrolyzed and resist photodegradation. When the polymer does eventually degrade, this degradation begins at sites of defects including unsaturated C=C bonds and carbonyls, making the intentional incorporation of these functionalities to the polymer an area of interest. Ethylene/carbon monoxide (E/CO) copolymers which include ketones have been found to be photodegradable while maintaining desirable commercial properties. Although researchers have only achieved single CO insertion in polyethylene, back-to-back CO insertion, which would create an even more photodegradable polymer has not been observed. This project works towards the synthesis of a charged palladium catalyst for the back-to-back insertion of CO into E/CO copolymers. The synthesized anionic catalyst will carry cationic counterions, which we hypothesize will form a thermodynamically favored 5-membered chelate with the diketone functionality formed after the second CO insertion, making back-to-back CO insertion more favorable.

Towards a convergent fragment-coupling approach of grayanane diterpenoids via selective lithium-halogen exchange of a vinyl dibromide

Zhehao Zhang

Mentors: Sarah E. Reisman and Joshua Belfield

Grayananes are a class of natural products (diterpenoids) with a fused 5/7/6/5 carbon skeletal structure, with the central 7-membered ring being of great synthetic challenge. Herein, we report our progress towards a fragment coupling strategy to forge the central 7-membered ring, via selective lithium-halogen exchange into a vinyl dibromide fragment. We report the successful synthesis and lithiation of the proposed vinyl dibromide fragment, as well as progress continuing from last SURF on developing the bicyclo[3.2.1]octane fragment as a viable coupling partner. We envision that with both fragments in hand, two selective couplings will enable access to the Grayanane skeleton, which can be elaborated to targets such as Pieristoxin S.

Identifying and steering with interpretable features in protein models using sparse autoencoders

Ryan D. Yu

Mentors: Grant Rotskoff, Kara D. Fong, Jason Liu, and Soojung Yang

Generative protein models have recently become one of the most important advances in modern biology. In particular, AlphaFold has become incredibly important for predicting protein structures without lengthy spectroscopy experiments. Other models like BioEmu add additional functionality like generating protein samples along a Boltzmann distribution. However, these models are still mostly black boxes. To improve general understanding of these models, we train sparse autoencoders on these generative protein models to identify features corresponding to structural and thermodynamic properties, as well as at what point in the model these features appear. From this, we show that these features can be used to steer generation towards desired properties.

Engineering cpSRP43 for enhanced chaperone activity against amyloid aggregation through directed evolution

Fanhao Kong

Mentors: Shu-ou Shan and Bryce Yeazell

Chloroplast signal recognition particle 43 (cpSRP43) is evolved to enhance its chaperone activity toward amyloidogenic aggregation-prone proteins, including α -synuclein and amyloid- β (A β), associated with neurodegenerative disease. The L189R mutation—a key allosteric residue predicted to stabilize the closed conformation of cpSRP43—was introduced into four previously evolved tailor-made molecular chaperones (TMMCs), and circular dichroism showed that L189R destabilizes the closed conformation of cpSRP43 at physiological temperature, and seeded α -synuclein fibrillation assays showed that L189R broadly impairs anti-fibrillation activity. In parallel, a single-region saturation-

mutagenesis library targeting seven substrate-binding regions of cpSRP43 was constructed and is being screened against A β 42 by HigB/HigA Toxin-Antitoxin Chaperone Selection (HITACS). Variants beginning to emerge from this screen will inform combinatorial library design to probe epistasis and will serve as starting points for continuous directed evolution via the Orthogonal Replication System (OrthoRep).

Determining the chaperone activity and interaction interface of *Chlamydomonas reinhardtii* chloroplast signal recognition particle to its client proteins

Muping Wang

Mentors: Shu-ou Shan and Yelim Yi

The 43-kDa subunit of the chloroplast signal recognition particle (cpSRP43) is a chaperone protein that protects proteins relevant to the assembly of the light-harvesting complex (LHC) in chloroplasts. Past research in *Arabidopsis thaliana* (*A. thaliana*), a model plant, has demonstrated that *A. thaliana* cpSRP43 is a dual-function chaperone that both protects light-harvesting chlorophyll *a/b*-binding proteins (LHCPs) in transit to the thylakoid membrane and protects GENOMES UNCOUPLED 4 (GUN4) in the tetrapyrrole biosynthesis (TBS) pathway from heat stress. However, the chaperone activity of cpSRP43 is not well-understood in algae. Here, we demonstrate that cpSRP43 in *Chlamydomonas reinhardtii* (*C. reinhardtii*), a model alga, has GUN4-specific chaperone activity. Via light scattering assays, we show that GUN4 is disaggregated by *C. reinhardtii* cpSRP43 in a concentration dependent manner. We also show *C. reinhardtii* cpSRP43 has weak chaperone activity against LHCB5, a representative LHCP and weak binding to the L11 peptide, a conserved cpSRP43 recognition sequence in LHCPs. Our cross-linking coupled with mass spectrometric studies further identified the binding interface of *C. reinhardtii* cpSRP43 to GUN4. This information can be used to engineer *C. reinhardtii* cpSRP43 variants with alternate chaperone activity to modify the assembly of the LHC, and in turn, improve the photosynthetic efficiency of *C. reinhardtii*.

[4+2] Cycloaddition on β -ketoester with tethered alkene and siloxane tether

Sarah E. Madden

Mentors: Brian M. Stoltz and Sara Siddiqui

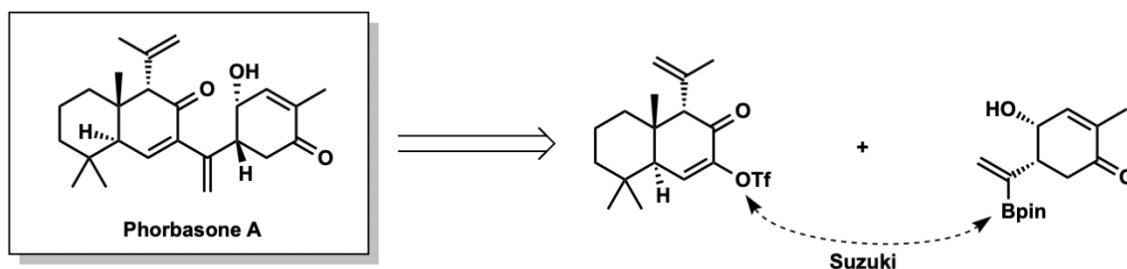
Metal-catalyzed allylic alkylation is a well-explored process that allows for the synthesis of organic molecules. In particular, variations of decarboxylative asymmetric allylic alkylation have been studied extensively on β -ketoesters over the last several years. Protonation, conjugate addition, and the aldol reaction have been performed on variations of a chiral palladium enolate already, as well as a [4+2] cycloaddition. In order to access a more diverse variety of products and scaffolds with a palladium enolate undergoing a [4+2] cycloaddition, we are modifying the β -ketoester substrate commonly used by the Stoltz lab. We are appending an alkyne tethered to the enone through a siloxane linker, which will serve as a synthetic handle for further product manipulation. The siloxane will likely break the five-membered carbocycle that has formed on past attempts of a [4+2] cycloaddition on similar substrates within the lab. Then, we will test and refine the conditions of the [4+2] cycloaddition on this substrate.

Approaching the total synthesis of Phorbasone A

William R. Putzer, Jr.

Mentors: Brian M. Stoltz and Sean Corman

In this paper, we aim to conduct a total synthesis of Phorbasone A, a complex natural product isolated from marine sponges. We explore a synthetic pathway that is currently proposed to start with cheap, commercially-available materials (R)-carvone and dimethylcyclohexanone. A series of chemical reactions transform the starting materials until the resultant fragments are at last joined together via a Suzuki coupling. Synthesis of the western fragment (originating from dimethylcyclohexanone) has been demonstrated, while synthesis of the eastern fragment (originating from (R)-carvone) is still under speculation and experimentation.

**Extending temperature control capabilities for Laser-driven Ultrafast Impedance Spectroscopy**

Jack S. Yu

Mentors: Scott K. Cushing, Amy Lin, and Natan Spear

Solid-state electrolytes are promising materials for next-generation batteries due to their potential to improve safety and energy density compared to conventional liquid electrolytes. However, the microscopic mechanisms that enable rapid ion transport through these materials remain incompletely understood. Recent studies suggest that lattice vibrations, or phonons, influence how ions move through a crystal structure, but directly determining how these vibrations affect ionic transport remains experimentally challenging. This project develops a temperature-controlled sample stage for Laser-driven Ultrafast Impedance Spectroscopy (LUIS), a technique that uses laser excitation to probe changes in ion hopping on ultrafast timescales. The stage uses thermoelectric cooling to control the temperature of solid-state electrolyte samples, allowing the equilibrium population of thermal phonons to be systematically varied. Temperature-dependent LUIS measurements will be demonstrated using systematic selenium substitutions in sodium thiophosphate ($\text{Na}_3\text{PS}_{4-x}\text{Se}_x$), a highly conductive solid-state electrolyte, to examine how changes in the initial phonon population influence phonon-ion coupling. Ultimately, the integration of temperature control into LUIS provides an additional experimental parameter to systematically compare ion transport across different temperatures and materials, enabling a better understanding of the factors governing ionic conduction.