Caltech
Student-Faculty Programs

Session C Abstracts

Decompression of the bacteriophage MS2's genome utilizing cell-free systems
Grace R. Tuhabonye
Mentors: William M. Clemons, Jr., and Yan Zhang

The motivation of this project comes from the rise of multidrug-resistant bacteria, which can be dangerous when trying to fight bacterial diseases. Bacteriophages, bacterial viruses, can serve as an alternative to antibiotics because they evolve with bacteria and are highly specific. However, most phages have compressed genomes, where many genes overlap, making them difficult to engineer. The objective of this project is to decompress the MS2 genome to enable more efficient genome editing. Decompressed MS2 is synthesized using cell-free TX-TL systems because these reactions maximize product formation without relying on living cells. The synthesized phages are analyzed with plaque assays to visually confirm bacterial lysis and with reverse transcriptase–polymerase chain reaction (RT-PCR) to amplify and sequence MS2 DNA to verify the decompressed genomic structure. Functional phages were observed once in plaque assays using various concentrations of Mg, but no DNA product matched the expected MS2 RNA length. Future steps are to synthesize phages with 4 mM Mg to confirm plaque formation and optimize RT-PCR protocols to successfully amplify MS2 at the correct length.

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.

Structural analysis of AglH as a comparative model for DPAGT1
Grace H. Otos
Mentors: William M. Clemons, Jr., 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, bacteria, and archaea are homologs DPAGT1, MraY, and AglH respectively. Through preliminary AlphaFold analysis,

we find AglH is more structurally similar to DPAGT1 than MraY. This analysis motivates us to further investigate AglH as a model system to understand DPAGT1. This project compares the sequence and structure of DPAGT1 and AglH to identify key similarities and differences. To enable an in-depth comparison we employed a combined computational and experimental approach: AlphaFold to predict structures and investigate features of interest, and CryoEM to obtain high-resolution structural data for both proteins. A structure for DPAGT1 has already been solved by the Clemons lab. Building on this, we focused on analyzing DPAGT1 bound to a truncated muraymycin analog, a potential cancer drug, to characterize its binding interactions. We are continuing to process CryoEM data to determine the structures of both AglH and DPAGT1 in complex with the drug.

Thin or monolayer 2D conducting metal-organic frameworks by exfoliation
Katie M. Dao
Mentors: John Anderson and Jonas C. Peters

Metal-organic frameworks offer great potential for various electronic and physical applications due to their modular nature and tunable structures. However, and despite these known benefits, observing unusual physical phenomena in MOFs is often limited by their poor crystallinity. Trimming down 2D systems in other materials has shown unusual behavior and moving to thin or monolayer 2D MOFs would similarly avoid issues with crystallinity. However, such similar studies in potentially interesting MOFs have been quite limited. 2D nanosheets of MOFs might enable different properties compared to their bulk. This allows for the ideal properties of the materials to be utilized and studied. In this work, we synthesized our base MOF linker, SnTHT, from which we make the MOF NiTHT. With this material, we have used a range of techniques; sonication, exfoliation, and Scotch tape method; to find the best resulting nanosheets.

Terahertz time-domain investigation of molecular quantum bits
Alan Song
Mentors: Geoffrey A. Blake and Jax Dallas

Molecular quantum bits (qubits) that store quantum information on an S=½ or S=1 metal center are tunable, precise, and customizable (Bayliss et. al. 2020). Extending the spin coherence time (Tm) would therefore increase the utility of the qubit and make applications such as quantum computing more accessible. Near room temperature, Tm is limited by the vibration-mediated spin relaxation time (T1), a timescale the Hadt group hypothesizes is controlled by the thermal population of totally symmetric vibrational modes (Kazmierczak et. al. 2024).

Terahertz (THz) time-domain spectroscopy provides a direct probe into the dynamics of these vibrational energy states. A qubit's response to a broadband femtosecond pulse in the 0-8THz range can be tracked with an 800nm probe pulse in conjunction with a gallium phosphide (GaP) detector crystal. Cyclic olefin copolymer (TOPAS) and high-density polyethylene (HDPE) have good transparency in this frequency domain and can be used as sample media, either as solid solutions of qubit in polymer or pressed pellet mixes.

With THz scans of the qubit targets Cu(acac)2, Cu(TMHD)2, and CuTPP, we hope to demonstrate the population of thermally accessible vibrational modes in these molecules. Computational density functional theory (DFT) calculations indicate which of these vibrational modes are totally symmetric. Correlating the spectroscopic data with the known Tm of these qubits over this temperature range will confirm or deny the Hadt group's hypothesis, allowing for more informed and targeted molecular qubit synthesis in the future.

References

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Modeling the chemistry of the RNO 90 and AS 209 protoplanetary disks: A computational approach revealing radial profiles using CO and H_2O spectroscopic data
Alana C. Nisperos
Mentors: Geoffrey A. Blake and Emma Dahl

Protoplanetary disks are differentially rotating structures of gas, dust, and ice that surround young stars. Their inner regions are sites of planetary system formation, as the materials present within are thought to be the building blocks that constitute any resulting planets. However, our understanding of this innermost material is greatly limited. This project combines ground based high-resolution near-infrared spectra acquired with the Keck II telescope with mid-infrared spectroscopy from the James Webb Space Telescope (JWST). Together, these observations are used to determine the physical conditions that arise within two disks: AS 209 and RNO 90. To do this, we ran retrieval models that used Bayesian inference to initially constrain the probable temperature, column density, and emitting area parameters for the CO molecule, via high dispersion rovibrational spectra covering 4.6-5.3 microns. We ultimately mapped the radial column density profiles of the CO disk emission and compared the results to H_2O retrievals generated by JWST. This provides estimates of the C/O ratios in the warm gas near the inner disk surface of AS 209 and RNO 90. Long term, comparing the chemistry of disks to that of exoplanetary atmospheres can provide deeper insight into disk structure and resulting planetary formation.

The electronic structure of Holm clusters
Aadarsh Balaji
Mentors: Garnet K. Chan and Chenghan Li

It is only within the last two decades that the molecular structure of the nitrogenase enzyme has become well understood, and only in the last decade that progress has been made in describing its electronic structure via *ab initio* methods. Because of the complexity of nitrogenase's electronic structure, and due to the presence of dense, low-lying excited states that can contribute to room-temperature chemistry, a rigorous understanding of the dinitrogen reduction mechanism has not yet been developed. Currently, our goal is to describe the FeMoco active site of nitrogenase, but describing large systems that have many open-shell metal atoms is challenging due to the multi-reference nature of the ground state wavefunction. For qualitative insight, we begin by focusing on the so-called Holm clusters, which are synthetic "cubane" metal-iron-sulfur clusters that we use as model systems. We study the ground states of these clusters using several post-Hartree Fock methods and additionally describe the theory and computational implementations of some of these methods as well.

Stereospecific synthesis of vicinally brominated polycycooctene (PCOE) and downstream chemistries
Haleigh J. Gardner
Mentors: E. Bryan Coughlin and Gregory C. Fu

Vicinal 1,2-substitution is an underexplored structure in commodity polymers and is thus an interesting route for the chemical up-cycling of polyolefins. A pathway for the postpolymerization modification of double bonds in a polycycloocctene (PCOE) backbone is used to generate a brominated polymer (Br_2-PCOE). Every internal C=C bond is stereospecifically transformed into vicinal dibromide, mapping the 84% trans content of the parent backbone to an 86% erythro product. Thermogravimetric analysis confirms bromination: a 60% mass loss at 383 °C corresponds to dehydrobromination of a high conversion backbone, and negligible char remains above 700 °C. NMR evidence demonstrates the same with a complete disappearance of the olefin C=C peak. Differential scanning calorimetry reveals an amorphous material with a glass transition temperature (Tg) between −35 and −25 °C and no crystallinity from −70 to 70 °C, showing the possibly disruptive effect of stereoregular vic-dibromides. Nucleophilic substitution of the bromides with NaN_3 introduces vicinal diazides, evidenced by an azide stretch at 2100 cm^{-1}; solubility limits prompt a protected-azide route (Me_3SiN_3/TBAF) now in progress to enable full spectroscopic verification and downstream click

reactions. Future directions include: thiol-bromo click, alkyne-azide click, and Staudinger reduction. These parallel pathways—stereoselective dihalogenation followed by displacement—open access to a family of regio- and stereodefined vicinal polymers, setting the stage for PolyUP's broader objective of converting mixed polyolefin waste into high-value, functionally diverse materials.

Investigating the role of angiotensinogen (AGT) in oxidative stress and inflammation in retinal pigment epithelium (RPE) cells with high-risk CFH (Y402H)
Isabella G. Kedikian
Mentors: Deborah Ferrington, Dennis A. Dougherty, Johnson Hoang, and Peng Shang

Age-related macular degeneration (AMD), a leading cause of blindness in the elderly, is strongly linked to both cigarette smoke exposure and the Y402H polymorphism in the Complement Factor H (CFH) gene. This project investigates the role of angiotensinogen (AGT) and the renin-angiotensin system (RAS) in driving oxidative stress and inflammation in retinal pigment epithelium (RPE) cells carrying this high-risk (HR) CFH variant. Previous research from induced pluripotent stem cell-derived (IPSC) RPE cells from human donors had shown elevated levels of AGT expression in HR cells, and this was confirmed through ELISA. Experiments exposed cells to varying concentrations of cigarette smoke extract (CSE), angiotensinogen converting enzyme (ACE), and renin to assess RAS activation via AGT and angiotensin II levels, lipid droplet formation, reactive oxygen species (ROS) production, and NFkB signaling. Results revealed increased lipid droplets and mitochondrial ROS in response to CSE, though inconsistencies between time points and limitations in ELISA and Western Blot sensitivity posed as challenges. Future experiments aim to refine treatment timing and explore RAS and CSE interactions more precisely. This work contributes to understanding how the CFH Y402H polymorphism and CSE interact through RAS signaling and converge to drive AMD pathology, offering potential therapeutic targets.

Mechanistic study of N_2 activation on intermetallic surfaces
Chrystal Duan
Mentors: William A. Goddard III and Sejun Kim

The development of an efficient, environmentally friendly method for ammonia synthesis has long been a subject of interest. NH_3 plays a crucial role in fertilizers, food production, and holds promise as a long-term energy carrier and carbon-neutral fuel. Industrially, NH_3 is synthesized through the Haber-Bosch process, which requires extreme temperatures and pressures, resulting in significant energy consumption and environmental impact. A major challenge in developing alternative catalytic routes is the activation of chemically inert nitrogen gas (N_2). In this work, we employ computational methods, such as Density Functional Theory (DFT), to investigate the catalytic behavior of LaCoSi and related rare-earth intermetallic compounds. Through a stepwise analysis of the reaction mechanism on the catalyst surface, we demonstrate how the distinct electronic and structural characteristics of these materials facilitate N_2 activation and reduce the energy barrier for NH_3 formation.