Caltech
Student-Faculty Programs

Session E Abstracts

Developing a molecular replacement method for phasing low-resolution MicroED data of paramagnetic transition metal complexes
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Mentors: Hosea Nelson and Samuel J. Mussetter

Existing spectroscopic techniques struggle to characterize catalytically relevant paramagnetic transition metal complexes, and crystallography remains the gold standard. Microcrystal electron diffraction (MicroED) extends this to submicron crystals, but dynamical scattering, poor crystallinity, low completeness, preferred orientation, and short-lived intermediates frequently complicate or preclude ab initio phasing. Here we develop a fragment-based molecular replacement (MR) method for small-molecule transition metal complexes, enabling phasing of otherwise intractable MicroED data. MR searches rotational and translational space for the fragment placement that best reproduces the experimental diffraction data, and its ability to phase beyond the resolution regime accessible to direct methods has made it routine in macromolecular crystallography. To adapt MR to small molecules, we assemble a library of metal-ligand fragment search models, prioritized using the known synthetic route and complementary characterization data, and we have built a Phaser pipeline suitable for transition metal complexes. We demonstrate the approach on iron bipyridine complexes, including structures in which Fe(II) and Fe(III) species co-crystallize within a single asymmetric unit. Ultimately we aim to deliver an automated, user-directed workflow for routine structural characterization, with broad applications in catalysis and high-throughput structure determination.

Proteomic analysis of NAC interactions
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Mentors: Shu-ou Shan, Radoslaw J. Gora, and Sunny Huang

The nascent polypeptide-associated complex (NAC) coordinates co-translational protein biogenesis at the ribosomal exit tunnel, yet the domain-specific interactomes of its flexible α-N, α-C, and β-C termini remain incompletely defined. Here, we employ TurboID-based proximity labeling coupled with quantitative LC-MS/MS proteomics to systematically map terminal-specific NAC interactions in HEK293T cells. In preliminary single-run data, known co-translational factors (including Naa acetyltransferases, NMT1/2, METAP1/2, SRP, and HYPK1) showed enrichment at NAC termini relative to TurboID-only controls, validating that our proximity labeling assay captures specific interactions. Several uncharacterized candidates, including taxilin family members, prefoldin subunits, and annexins, also met our preliminary enrichment cutoff and await statistical validation from ongoing triplicate proteomic analysis. In parallel, we are optimizing an orthogonal RNA Affinity Purification with Poly-Lysine (RAPPL) assay to validate candidate interactions under conditions that preserve ribosome-associated interactions. Together, this work establishes a robust pipeline for quantitative interactome analysis and provides a foundation for defining how individual NAC termini coordinate the recruitment of protein biogenesis factors at the ribosomal exit tunnel.

Molecular basis of CK1δ nuclear import in circadian rhythm modulation
Emma J. Berg
Mentors: André Hoelz and Chia-Yu Chien

The circadian clock is an internal biological timing system that aligns physiological processes with external light-dark cues. At the molecular level, it is regulated by a transcription-translation feedback loop involving the clock proteins PER and CRY, whose activities are modulated by Casein Kinase 1δ (CK1δ). CK1δ is reported to be localized both in cytosolic and nuclear compartments, and its transport between the two is mediated by the karyopherin (KAP) family. However, the detailed molecular mechanisms governing the shuttling of CK1δ and how it participates in distinct stages of signaling remain elusive. This project aims to validate the predicted nuclear localization signal (NLS) of CK1δ and determine how its interactions affect its binding to α-KAPs and how it enters the nucleus.

To accomplish this, we tested the interaction between CK1δ and mKapα2 through Size-Exclusion Chromatography coupled with Multi-Angle Light Scattering (SEC-MALS). The results suggested that CK1δ binds α-KAPs with low affinity, and the C-terminal extension might be essential for the interaction. Additionally, we conducted subcellular localization and Western blot analysis to visualize CK1δ's localization within mammalian cells. Aligned with our biochemical findings, we found that CK1δ localizes to both the nucleus and cytosol, which demonstrates a property of low-affinity NLS-tagged cargoes.

Developing chemical tools for the inhibition of chondroitin sulfate glycosaminoglycan sulfotransferases to enhance neuronal regrowth

Martin B. Panicacci
Mentors: Linda C. Hsieh-Wilson, Cassandra N. Cancemi, and Sophia Gonzalez

Chondroitin sulfate proteoglycans (CSPGs), ubiquitous in the extracellular matrix of the central nervous system, consist of core proteins modified with chondroitin sulfate (CS) glycosaminoglycan (GAG) chains. CSPGs are diversified through the patterned sulfation of their GAG chains by sulfotransferases, which is known to influence interactions with cytokines, receptors, and growth factors. Notably, the CS-E sulfation motif has been associated with potent inhibition of neuronal recovery, thus the disruption of CSPG expression may yield insights towards the development of therapeutics. However, tools to interrogate the role of specific sulfation motifs are not widely accessible. Previously, oxindole and cyanoacrylamide scaffolds have been investigated as inhibitors of Chst15, the sulfotransferase responsible for the CS-E motif. Preliminary screening studies have identified Rottlerin as a potent inhibitor of Chst15. Towards the definition of a structure-activity relationship and tuning its activity, we have synthesized a fragment of Rottlerin and derivatives exhibiting substitutions known to increase potency against Chst15 in established scaffolds. In the future, these will be evaluated by a fluorescence-based enzyme-coupled assay and disaccharide analysis of treated Neu7 astrocyte cell cultures. Together, this work aims to further understand Rottlerin as a Chst15 inhibitor, expanding our library of GAG sulfotransferase inhibitors.

Mechanistic studies of β-oxidation in saturated dietary fatty acids

Wiktor Sobczynski
Mentors: Johannes Morstein and Binyou Wang

Mitochondrial β-oxidation is one of the fundamental metabolic processes in the cell, converting fatty acids into shorter acyl-CoA units. Even though the mechanism has been thoroughly studied, there are still unanswered questions regarding the β-oxidation of saturated fatty acids and which ones seem to undergo the greatest extent of oxidation. Here, we utilized lipidomic analysis to understand which fatty acids need the carnitine shuttle to enter the mitochondria and which ones get oxidized the most. We treated HeLa cells with deuterated saturated fatty acids and observed their incorporation into acylcarnitines (CARs), phosphatidylcholines (PCs), and triacylglycerols (TAGs). After fatty acid treatment, PCs and TAGs constituted around 80% of the total lipids in the cell. By investigating them, we optimized the work needed to determine plausible results. We observed the 10 most abundant lipid species of PCs and TAGs. We saw that, out of all the fatty acids, arachidic acid was oxidized the most. These findings suggest that shorter fatty acids are incorporated into multiple lipid species in their native form or undergo elongation, whereas longer fatty acids undergo β-oxidation to a greater extent.

Mechanistic investigation of lanthanide reactivity and imine substrate scope in a photodriven Sm-catalyzed asymmetric ketyl–olefin coupling

Elizabeth Y. Bank
Mentors: Sarah E. Reisman, Nathan C. Friede, and Will Chen

Samarium(II) iodide (SmI_2) is a versatile single-electron reductant widely employed in organic synthesis. Although SmI_2-mediated transformations have traditionally required (super)stoichiometric quantities of reagent, recent advances have enabled catalytic Sm-mediated reactivity. Specifically, development of a photodriven Sm-catalyzed asymmetric ketyl–olefin coupling by the Reisman group has established a new strategy for catalytic samarium chemistry, providing a platform for investigating

the fundamental factors governing catalytic reactivity and selectivity. This work combines mechanistic investigations with electrochemical studies, including cyclic voltammetry, to examine two complementary objectives within the Sm–PyBOX catalytic cycle: elucidating the influence of lanthanide identity on catalyst reactivity and extending the methodology to enantioselective imine–olefin cross-coupling. Screening reactivity of lanthanide–PyBOX complexes investigates how ionic radius, Lewis acidity, 4f electronic structure, and redox properties influence catalytic activity and stability. In parallel, mechanistic studies of imine substrates seek to identify the factors governing substrate activation, catalyst coordination, and suppression of competing background reactivity. Collectively, this research aims to advance the mechanistic understanding of catalytic samarium chemistry and guide the development of new enantioselective lanthanide-catalyzed reductive cross-coupling reactions.

Investigating the interactions of the NEIL3 beta hairpin and KY motif with MCM2–7 in DNA interstrand cross-link repair
Hannah L. Bowman
Mentors: Daniel R. Semlow and Gabriel Ashton-Rickardt

DNA interstrand cross-links (ICLs) covalently link the two strands of DNA, stalling replication forks and threatening genome stability. One class of ICLs, abasic site-induced ICLs (AP-ICLs), is repaired by the NEIL3 glycosylase. Although several domains within the NEIL3 C-terminal domain have established roles in replication-coupled ICL repair, the functions of two highly conserved motifs – the KY motif and the β-hairpin – remain poorly understood. Our lab's AlphaFold modeling suggests that the β-hairpin interacts with a cleft in the MCM2–7 helicase, potentially positioning the glycosylase domain of NEIL3 to unhook ICLs at stalled replication forks. To investigate this model, we generated a panel of NEIL3 mutants containing deletions, alanine substitutions, and targeted point mutations within the KY motif and β-hairpin to test the contribution of residues predicted to mediate interactions with MCM2–7. Following DNA cloning, baculovirus-mediated expression in Sf9 insect cells, and protein purification, mutant proteins will be evaluated in *Xenopus* egg extract using biochemical rescue-depletion assays to determine whether these motifs are required for NEIL3-dependent ICL repair. These studies will clarify how the KY motif and β-hairpin support replication-coupled ICL repair and provide a foundation for future studies examining how these motifs mediate interactions with the MCM2–7 helicase.

Converting carbon dioxide into useful chemicals and fuels
Daniel C. Darahdgian
Mentors: Harry B. Gray and Jay R. Winkler

Lowering carbon dioxide emissions while also reducing demand for fossil fuels is made possible through the catalytic hydrogenation of carbon dioxide into hydrocarbons. However, few catalysts can hydrogenate CO_2 while not reducing the carbon-carbon double bonds characteristic of olefins or unsaturated hydrocarbons. Therefore, we studied a carbon nanotube supported iron-potassium catalyst, which has previously been reported to have high CO_2 conversion while maintaining great olefin selectivity. The catalyst was prepared by sequential loadings of iron and then potassium on multi-walled carbon nanotubes and catalytic tests were carried out on a fixed reactor bed setup. In our experiments, we found that catalyst loading had a significant impact on the product distribution. Lower catalyst loading resulted in primarily oxygenated products while higher catalyst loading resulted in mostly hydrocarbon products.

Towards precise simulation of molecules with memory-efficient analytical gradients from auxiliary-field quantum Monte Carlo
Daniel S. Lai
Mentor: Sandeep Sharma

Molecules can be simulated to near chemical precision using electronic structure theory in quantum mechanics. For example, computing the gradients of the total molecular energy with respect to certain variables enables the prediction of many useful properties, such as the geometric structure and dipole moments of a molecule. Auxiliary-field quantum Monte Carlo (AFQMC) stands out as a powerful and scalable approach that is a rising alternative to traditional quantum chemistry methods. The current

approach to reliably compute energy gradients from AFQMC relies on standard reverse-mode automatic differentiation, which is analytical and time-efficient but incurs a significant memory overhead. We explore a new analytical strategy for energy gradients with low memory cost comparable to the evaluation of the energy itself, which could extend the current capability of high-precision simulations of larger molecules.

Synthesis of complex ring systems via a [2+2] cycloaddition followed by a base-induced rearrangement
Elise R. Hastie
Mentors: Brian M. Stoltz and Chloe Cerione

Dimeric complex ring systems are observed in sesquiterpenoid dimers that exhibit potent anticancer, anti-inflammatory, and neuroprotective properties; however, synthetic access to these molecules remains limited due to their structural complexity and lengthy synthetic routes. Building upon previous research in the Stoltz group, we test the viability of a [2+2] cycloaddition and base-induced cascade approach for rapid access to various complex ring systems. To assess the scope of this approach, we have dimerized multiple simple butenolide substrates via [2+2] cycloaddition and subjected the resulting dimers to sodium methoxide to observe the resulting rearrangements. Additionally, we are working toward the synthesis of chloranthalactone A, which we expect can be dimerized and subjected to base treatment to yield chloranthalactone F. All chemical structures were confirmed by nuclear magnetic resonance (NMR) and/or single-crystal X-ray diffraction (SCXRD). Ongoing work will optimize yield and expand upon the substrate scope established by this work and previous studies conducted by the Stoltz group.

Catalytic asymmetric semipinacol-type rearrangement for the construction of all-carbon quaternary stereocenters
Junrui Zheng
Mentors: Brian M. Stoltz and Weicheng Xu

All-carbon quaternary stereocenters are common in structurally complex molecules, yet their catalytic enantioselective construction remains challenging. This project aims to develop an asymmetric semipinacol-type rearrangement in which a cyclobutanol-containing α,β-unsaturated 1,4-dicarbonyl undergoes strain-releasing 1,2-carbon migration to form a cyclopentanone bearing an all-carbon quaternary stereocenter. A modular furan-based route will provide electronically and sterically varied substrates, while reaction development will examine how chiral Brønsted-acid ion can organize the migration of two enantiotopic cyclobutane bonds. By separating productive ring expansion from competing fragmentation and establishing relationships among catalyst structure, reaction rate, and enantioselectivity, this work seeks to define a general strategy for stereocontrolled skeletal rearrangement.