Caltech
Student-Faculty Programs

Session D Abstracts

Refinement of cpSRP43 chaperone variants via secondary selection in a yeast model of Parkinson's disease
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Mentors: Shu-ou Shan and Arpit Gupta

Parkinson's disease is characterised by the pathological aggregation of alpha-synuclein into amyloid fibrils, contributing to degeneration of dopaminergic neurons. Molecular chaperones offer a promising therapeutic avenue by preventing and reversing such misfolding events. One such chaperone, the chloroplast signal recognition particle 43, has been shown to inhibit alpha-synuclein aggregation with sub-stoichiometric efficiency. Nonetheless, its activity is improvable through directed evolution in cellular models of Parkinson's disease. The Shan lab has accomplished this using a novel toxin-antitoxin model in E. coli, though this yielded an excessive number of evolved chaperone variants. To refine this, we conducted secondary selection on a library of successful variants using a different, yeast-based model of PD. In doing so, we selected successful variants with greater stringency, allowing more precise biochemical characterisation of increasingly promising chaperone variants.

Elucidation of cpSRP43-GUN4 protein dynamics through proximity-based biophysical techniques
Dennis Rui
Mentors: Shu-ou Shan, Alex R. Siegel, and Yelim Yi

Photosynthetic organisms rely on properly functioning light harvesting complexes (LHCPs) which serve as antennas for photosynthetic complexes to mediate carbon fixation for 99% of the biological energy expenditure on Earth. The proper biogenesis of this pathway is in part mediated by cpSRP43, a conserved molecular chaperone in chloroplasts. It has been shown that cpSRP43 harbors two distinct conformational states: a *closed*, structured state dedicated to the *de novo* biogenesis of LHCPs, and an *open*, disordered conformation, which protects tetrapyrrole biosynthesis (TBS) enzymes that mediate chlorophyll synthesis from heat-induced destabilization. However, the mechanism behind the client recognition of cpSRP43 is not well understood. A combination of biophysical approaches, namely XL-MS, NMR, and fluorescence (specifically FRET) spectroscopies, will be used to provide the first molecular model of how the conformation-dependent chaperone activities of cpSRP43 can recognize and remodel mature TBS enzymes to reshape their aggregation behavior during heat stress. Specifically, we aim to identify the molecular interactions between the disordered *open* conformation of cpSRP43 and GUN4, a well-characterized TBS client that serves as an accessible model system. Elucidating these molecular mechanisms will provide insight into the design of engineered proteins with switchable functions and therapeutic strategies targeting protein control pathways.

Enzymatic in-situ generation of silylium ions via protodesilylation of allylsilanes
Shreya Sivaramakrishnan
Mentors: Hosea Nelson and Kunal Jha

Protodesilylation of allylsilanes is the cleavage of Silicon – Carbon (σ) bond through the protonation at the allylic γ – carbon centre. However, silicon containing molecules are not native substrates to enzymes due to its low abundance in living organisms, which adds complexity to the presented problem. This project explores a biocatalytic route to generate silylium ions within the enzyme pocket as enzymes are known for its high selectivity and mild reaction conditions. Oleic Acid Hydratase (OAhyd) are flavin dependent enzymes known to catalyse the hydration of non-activated CC double bonds in olefins; and it has been reported to have hydrophobic active site. This makes OAhyd enzyme library ideal for this study as the generated silylium ions are highly electrophilic, which can be stabilised within a hydrophobic active site with relative ease. We have taken a dual approach for this study 1) Enzymatic screening of 12 substrates against the OAhyd enzyme library and 2) Design and synthesis of a "target molecule" mimicking the native substrate of the enzyme and subsequently

screen it against the library. Despite the enzyme having a hydrophobic active site, at present, we expect silanol formation due to the possibility of nucleophilic attack by hydroxide (from the buffer environment) on the silylium ion. Thus, we identified Silanol using gas chromatography–mass spectrometry (GC-MS), to verify the activity of the enzymes and find the most potent enzyme for this reaction. Further exploration of silylium ion stabilization within the enzyme's active site, prior to nucleophilic attack, can open multiple possibilities in organic transformations since silylium ions are known to act as potential reactive lewis acid catalysts.

A data-driven approach towards the electrochemical nickel-catalyzed reductive cross-coupling of piperidines
Ruchira S. Hariharan
Mentor: Sarah E. Reisman

Piperidines are a functional group ubiquitous in medicinal motifs, present in anticancer agents, Alzheimer's medications, antibiotics, analgesics, antipsychotics, and antioxidants. Thus, piperidine functionalization remains desirable, and an electrochemical Nickel-catalyzed reductive cross-coupling offers a mild, user-friendly platform to this transformation. Our optimization takes a data-driven approach to chiral ligand design: in employing multi-variate linear regression models, we elucidate the ligand features integral to the system's success. Our model provides a metric for exploring chemical space undiscovered via traditional empiricism, in addition to guiding our team on the best ligands for synthesis. Via high-throughput electrochemistry, we then screen these ligands and update our model with the results in an active learning fashion.

Designing mechanophore-containing polymers for targeted release of molecular cargoes
Zoe R. Grogan
Mentors: Maxwell J. Robb and Liam A. Ordner

While typical methods to induce chemical reactions include exposure to heat and light, mechanochemistry provides an alternative pathway by employing mechanical force (primarily introduced via ultrasound) to aid chemical reactions by lowering their activation energy barriers. Molecules known as mechanophores are specifically designed to enable productive covalent transformations in response to the stimulus of mechanical force when incorporated into polymers. This project is dedicated to constructing a mechanophore whose productive chemical response is the release of a small-molecule chemical cargo. Previous studies into these molecules revealed a relationship between cargo release kinetics and mechanophore substitution caused by stabilizing influences of substituent groups. We aim to expand upon these studies by synthesizing a mechanophore containing a methoxy electron dontating group and a methyl substituent. This mechanophore will allow for further investigation of the structure-activity relationship for molecular release, as well as provide insight regarding the release mechanism.

Successful synthesis of this molecule has been achieved, and future work includes the incorporation of the cargo-loaded mechanophore into a polymer and subsequent sonication to collect data regarding the release kinetics of this mechanophore.

Mechanism of DNA2-dependent replication fork degradation
Anusha K. Chatha
Mentors: Daniel R. Semlow, Maria Altshuller, and Victoria MacKrell

During interstrand crosslink (ICL) repair, replication forks must be restored after fork reversal and lesion unhooking. The nuclease-helicase DNA2 has been implicated in the restoration step, but its exact activity at the replication fork remains unclear. Hyperactivation of DNA2 leads to degradation of 3′ ends of fork structures generated during ICL repair, although it is not yet known whether this affects both the reversed fork and the fork abutting the ICL. This raises the hypothesis that DNA2 can resect a nascent leading strand at a replication fork. This project aims to test whether DNA2 can degrade a base-paired 3′ end at a replication fork and whether this activity is aided by RPA, a single-stranded DNA-binding protein known to influence DNA2 resection. We will express and purify DNA2 and RPA from insect cells, validate their activity using known flap substrates, and assemble a

synthetic Y-shaped DNA substrate that mimics a stalled fork. Using gel electrophoresis and phosphorimaging, we will assess whether DNA2 resects the labeled leading strand in the presence of RPA. These studies will provide insight into the mechanism by which DNA2 processes replication fork structures during ICL repair.

Understanding the mutagenic bypass of HMCES peptide adduct
Yushan Li
Mentor: Daniel R. Semlow

AP-interstrand cross-links (AP-ICLs) are cytotoxic as they covalently link the two strands of the DNA duplex, preventing strand separation and blocking essential cellular processes such as DNA replication and transcription. The repair mechanism for AP-ICLs is coupled to DNA replication and involves generation of an AP site on single-stranded (ss)DNA that will be cross-linked by the 5-hydroxymethylcytosine binding, embryonic stem-cell-specific (HMCES), forming a DNA protein crosslink (DPC). The HMCES-DPC stabilizes AP sites and suppresses double strand breaks (DSBs) during AP-ICL repair. The AP site stabilized by HMCES will later be bypassed by translesion synthesis (TLS) polymerase and the sequencing of AP-ICL repair products indicates that HMCES-DPC also influences the mutagenicity of TLS past an AP site, favoring deoxyguanosine (dG) insertion opposite the AP site. However, the basis of this mutagenic signature remains unclear. To understand what drives this mutagenicity, we will be studying 1) whether the bypass of the AP site by TLS polymerases happens during or after the proteolysis of HMCES-DPC and 2) the specific TLS polymerases that bypass the AP site during the AP-ICL repair. To address these questions, this project involves purifying the TLS polymerase Pol κ and generating a point mutation on the SRAP domain of HMCES to make it self-reversal deficient. We have successfully cloned SRAP E129A and are currently optimizing the purification procedure for Pol κ. Once Pol κ is purified, it will be utilized to observe the replication intermediates in AP-ICL repair reactions involving the HMCES-DPC.

Investigating the non-canonical binding interface between NEIL3 glycosylase and PCNA
Sophia R. Martinez-Whitman
Mentors: Daniel R. Semlow and Richa Nigam

DNA interstrand cross-links (ICLs) are highly toxic lesions that stall DNA replication and must be resolved to preserve genome integrity. NEIL3, a DNA glycosylase, initiates the repair of certain ICLs by cleaving glycosidic bond without disrupting the DNA backbone. This summer, I am investigating how NEIL3 interacts with the replication processivity factor PCNA, which is known to bind at a non-canonical interface within NEIL3's glycosylase domain. Using pre-constructed NEIL3 plasmids containing alanine substitutions in this domain, I am expressing and purifying wild-type and mutant NEIL3 proteins in *E. coli*. After optimizing induction and purification conditions, I successfully purified wild-type NEIL3 and several individual mutants using nickel affinity chromatography. *In vitro* pull-down assays will compare the ability of wild-type and mutant NEIL3 to bind PCNA. Loss of binding in specific mutants will help identify residues critical for this interaction. These studies aim to define the non-canonical NEIL3-PCNA binding interface and provide insight into how NEIL3 is regulated during ICL repair.

Investigating the substrate specificity of O-GlcNAc transferase (OGT)
Jesus E. Mendoza
Mentors: Linda C. Hsieh-Wilson and Maia Helterbrand

The addition of O-linked β-*N*-acetylglucosamine (O-GlcNAcylation) to serine or threonine residue in proteins is involved in a variety of cellular processes, with its dysregulation being linked to the onset of various neurodegenerative diseases. The addition of this sugar is catalyzed solely by the enzyme, O-GlcNAc transferase (OGT), which poses the question of how OGT can selectively glycosylate many different substrates. The adaptor protein hypothesis proposes that OGT can interact with specific interactors or "adaptor proteins" which guide OGT to specific locations and substrates. We focused on two putative adaptor protein candidates, DDX6 and ATXN2, which were identified through our lab's previously generated NOTISE proteomics networks. We then generated DDX6 knockout (KO) and ATXN2 KO HEK293T cell lines through CRISPR/Cas9 and fluorescence-activated cell sorting (FACS).

These cell lines were then validated via western blotting and lysed for downstream chemoenzymatic labeling and enrichment for mass spectrometry-based proteomic analyses. If DDX6 or ATXN2 are adaptor proteins, we expect to observe changes to O-GlcNAcylation on specific substrates in the KO lines compared to the control. Deeper investigation into this mechanism can guide new interventions that selectively modify O-GlcNAcylation events by targeting OGT to specific substrates, especially in pathways that are implicated in neurodegenerative diseases.

O-GlcNAc transferase activity and protein-protein interactions at synapses
Sigriður B. Karlsdóttir
Mentors: Linda C. Hsieh-Wilson and Jamison Takashima

O-linked glycosylation of nuclear and cytoplasmic proteins is a covalent post-translational modification catalyzed by *O*-GlcNAc transferase (OGT). Abberrant OGT activity has been shown to be correlated with cancer, diabetes and Alzheimer's disease (AD) and although OGT is known to localize to synapses, its role in synaptic function is not fully understood. We chemoenzymatically labeled AD brain samples with biotin and blotted the samples with a streptavidin conjugated dye to examine *O*-GlcNAcylation levels. We observed a decrease in *O*-GlcNAcylation in the crude synaptosome, compared to age matched control samples, as well as an increase of *O*-GlcNacase and decrease in OGT which was in line with exprectations and previous data. We then performed co-immunoprecipitation to confirm the interaction of OGT and PSD-95 among other synaptic proteins such as Synapsin 1 and Homer1. Unlike what was expected, the only interaction observed was between OGT and the inhibitory protein gephyrin, contrary to previously acquiered results which shown that OGT and PSD-95 interact. Therefore it is likely that these results stem from problems in experimental setup and we are currently optimizing an in vitro pull-down experiment to determine the site on OGT where PSD-95 interacts.