# 2022 CHEMICAL BIOLOGY PROGRAM RETREAT PROGRAM

THURSDAY, APRIL 14 8:30 AM TO 4 PM UCD STUDENT COMMUNITY CENTER

# 2022 CHEMICAL BIOLOGY PROGRAM RETREAT AGENDA

8:30 - 9:00 AM:

### Breakfast and Coffee Check-In

9:00 AM - 9:10 AM:

Welcome and Introduction\* https://ucdavis.zoom.us/j/93647029642 CBP Director Peter Beal

### 9:10 - 10:30 AM:

Student Presentations\* https://ucdavis.zoom.us/j/93647029642

-David Villarreal (Britt lab/CBP Trainee) -Elizabeth Lotsof (David lab) -Nathaniel Harder (Heffern lab)

### 10:30 AM - 10:50 AM

### **Community Coffee Break**

(brief activity built in)

10:50 AM-12:10 PM

Keynote Speaker Presentation\* *Translational Science* <u>https://ucdavis.zoom.us/j/94557324097</u> Dr. Paul Wender

Stanford University

# 12:10 - 1 PM:

**BBQ Luncheon** 

by Olive & Vine Catering

(gluten-free/vegetarian/vegan options available)

1:00 - 2 PM:

# **CBP Faculty Trainer**

### Presentation

Dr. James Letts

2:10-3:10 PM:

### **Conflict Resolution Workshop**

Dr. Angelique Louie & Dr. David Segal (CBP Trainers & Executive Committee Members)

3:10-3:15 PM:

## Group photo/break

3:15 - 4 PM:

### **Poster Session**

\*Notes hybrid sessions.

# 2022 CHEMICAL BIOLOGY PROGRAM RETREAT FEATURED SPEAKERS



# KEYNOTE SPEAKER Dr. Paul Wender / Stanford University

Dr. Wender holds appointments in the Chemistry and Chemical and Systems Biology Departments, as well as on the science advisory board of the Stanford Imaging Center and is a fellow of the ChEM-H Institute. He served as co-founder of the Chemical Biology Program, and serve as a member of the Stanford Cancer Institute, the NIH Biotechnology Training Program, the Stanford Molecular Imaging Program, the Stanford Molecular Pharmacology Training Program, and the Center for Molecular Analysis and Design. Additionally he also founded a course, Science Communication and Innovation (SCI), and undergraduate club (https://faSCInatepublication.org ) at Stanford focused on sharing the "fun, excitement and value of science" with undergraduates and high school students. The Wender group is involved in >30 collaborations involving academia, not-for-profit organizations and biotech companies. Over the last 3 decades, he has consulted with companies on medicinal and process chemistry, served on advisory boards for several universities and institutes and has research that has been licensed by or led to the founding of several biotech companies and advancement of leads into clinical trials.



# FEATURED TRAINER PRESENTATION Dr. James Letts / University of California, Davis

The Letts group focuses on how organisms use electron transport membrane proteins to convert energy from the food that we eat into a form that can be used by cells across many essential processes, as well as in cellular defense and signaling. The Letts lab seeks to characterize the structures and functions of these important membrane protein complexes in order to learn about how they work and how their dysfunction results in disease.

# 2022 CHEMICAL BIOLOGY PROGRAM RETREAT FEATURED SPEAKERS



# WORKSHOP CO-FACILITATOR Dr. Angelique Louie / University of California, Davis

Dr. Louie aims to enhance the diagnostic value and quality of noninvasive imaging through the use of advanced, targeted and activatable contrast agents. The Louie group focuses on the synthesis and development of small molecule, polymer and nanoparticulate agents for contrast-enhanced imaging in living systems. Current projects involve multimodal imaging of inflammation in atherosclerosis and the brain (MRI/PET), theranostic nanoparticles for atrial fibrillation, macrophage behavior in cancer progression and regression and small molecule sensors for MRI. In addition to Dr. Louie's research and other accolades she is also currently part of the inaugural UC Davis Faculty Academy of Graduate Student Well-Being.



# WORKSHOP CO-FACILITATOR Dr. David Segal - University of California, Davis

Almost every disease has a genetic component. Often this information is used only to determine how condemned a person is to develop disease. The Segal group would like to use the genetic information to fix the disease. A guiding principle for their work has been to study how nature does what it does, then attempt to use that knowledge to make useful tools to improve public health, either through increased knowledge or therapeutic intervention. Specific research foci in the Segal lab revolve around engineering zinc finger, TALE, and CRISPR/Cas nucleases and transcription factors. We also continue to make methodological improvements, many of which have been widely adapted in the field. Furthermore in addition to other involvements and honors Dr. Segal is also currently part of the inaugural UC Davis Faculty Academy of Graduate Student Well-Being.

# 2022 CHEMICAL BIOLOGY PROGRAM RETREAT ABSTRACT COLLECTION



# **KEYNOTE ABSTRACT**

#### TRANSLATIONAL SCIENCE Paul A. Wender Department of Chemistry Department of Chemical and Systems Biology Stanford University <u>https://web.stanford.edu/group/pawender/</u>

Our research integrates chemistry, computer-based design, synthesis, biology, medicinal chemistry and material science into studies directed at unsolved clinical problems including HIV/AIDS eradication (Nature Commun. 2022, 13:121; Cell Reports Medicine 2020, 100162; Blood Advances 2020, 4244; Virology 2018, 83), Alzheimer's disease (ACS Chemical *Neuroscience* **2020**, 1545), antigen-enhanced CAR T and NK cell therapies (*Nature* Commun 2020, 1879), resistant and metastatic cancer (Cancer Research 2019, 1624; *Gynecologic Oncology* **2012**, 118), therapeutic and prophylactic vaccination and immunooncology (Proc. Natl. Acad. Sci. 2018, E9153) including a Covid vaccine (ACS Central Science 2021, 1191), resistant infectious diseases (J. Am. Chem. Soc. 2018, 16140; ACS Chemical Biology 2019, 2065; Antimicrob Agents Chemother 2021, 65:e02416), cell/organ rejuvenation, multiple sclerosis (Cell Chemical Biology 2021, 537) and other neurological disorders. This research is driven by over 30 collaborations and has figured in the creation of several new companies. This lecture will focus on an overview of cell modulators (Science 2017, 218; Proc. Natl. Acad. Sci. 2020, 10688; J. Org. Chem. 2020, 15116) and new drug delivery systems for small molecules, siRNA, mRNA, DNA and other polyanions (J. Am. Chem. Soc. 2019, 8416; Proc. Natl. Acad. Sci. 2017, E448; PNAS 2018, E5859).

# CBP TRAINER PRESENTATION ABSTRACT

Structures of Tetrahymena's respiratory chain reveal the diversity of eukaryotic core metabolism James Letts Assistant Professor Department of Molecular and Cellular Biology College of Biological Sciences University of California, Davis

Respiration is a core biological energy-converting process whose last steps are carried out by a chain of multi-subunit complexes in the inner mitochondrial membrane. To probe the functional and structural diversity of eukaryotic respiration, we examined the respiratory chain of the ciliate Tetrahymena thermophila (Tt). Using cryo-electron microscopy on a mixed sample, we solved structures of a supercomplex between Tt-complex I (CI) and Tt-CIII2 (Tt-SC I+III2) and a structure of Tt-CIV2. Tt-SC I+III2 (~2.3 MDa) is a curved assembly with structural and functional symmetry breaking. Tt-CIV2 is a ~2.7 MDa dimer with over 52 subunits per protomer, including mitochondrial carriers and a TIM83-TIM133-like domain. Our structural and functional study of the T. thermophila respiratory chain reveals divergence in key components of eukaryotic respiration, expanding our understanding of core metabolism.

# **STUDENT SPEAKERS**

#### Characterization of the Hydrogenase Maturase Enzyme HydG by EPR and Mössbauer Spectroscopy David Villarreal / Britt lab / CBP Trainee

Owing to their unique organometallic cofactors, hydrogenase enzymes are efficient bidirectional catalysts for the consumption and production of molecular hydrogen, H2. However, a complete understanding of how nature can construct such remarkable cofactors is lacking. In the biological maturation of the active site H-cluster in [FeFe] hydrogenases, the radical S-adenosylmethionine (SAM) enzyme HydG has been shown to produce an [Fe(Cys)(CO)2(CN)] organometallic intermediate (Complex B) that is diamagnetic (i.e., EPR silent). We observed the reaction through a combination of EPR and Mössbauer spectroscopy. We report new spectroscopic signals associated with Complex B as well as its precursor, Complex A. Spectroscopically derived structural features of the intermediates will be discussed.

#### IDNA glycosylase NEIL1 demonstrates lesion specificity from RNA editing Elizabeth Lotsof / David lab

Conditions of oxidative stress produced from exogenous toxins and toxicants and endogenous cellular processes can produce modifications to all four nucleobases. Such oxidative DNA damage can result in mutations or disrupt normal cellular processes including replication and transcription. The DNA glycosylase, NEIL1, is able to excise lesions arising from all four nucleobases from a variety of DNA contexts. Additionally, the pre-mRNA of NEIL1 is subject to modification by the Adenosine Deaminase Acting on RNA (ADAR1) that leads to a recoding event that converts a lysine to arginine in the lesion recognition loop of NEIL1. This leads to the presence of two isoforms of NEIL1 under different cellular conditions. Notably, the two isoforms display different enzymatic properties on Thymine Glycol (Tg), where the unedited (K242) isoform showed a significantly faster rate of excision compared to edited NEIL1 (R242). We have performed detailed examinations of lesion processing by the two NEIL1 isoforms on a large number of substrates, and many interesting trends have emerged. Notably, unedited NEIL1 demonstrates better excision of oxidized pyrimidines than the edited isoform, but the differences in excision are not as striking as those observed previously with Tg. Calculations were performed in the gas phase to examine lesion tautomer stability and proton affinity, and there is a correlation between the isoform specific excision to the N3 proton affinity of the most stable tautomer. These results suggest that enzyme promoted tautomerization affects the cleavage of the glycosidic bond and the enhanced excision observed with the unedited enzyme. Thus, the differences in activity between the two isoforms of NEIL1 imply a unique regulatory mechanism for DNA repair.

#### *Effects of a high fat diet on hepatic copper metabolism* Nate Harder / Heffern lab

Non-alcoholic fatty liver disease (NAFLD) is an increasingly prevalent disease impacting around 30% of the US population. Characterized by an increase in hepatic fat, the disease is predicted to become the most prevalent liver disease within a decade. Recent literature has observed changes in copper metabolism with the onset of NAFLD in rodent models. My research seeks to define the connections between copper regulation and NAFLD. This work will facilitate the discovery of biomarkers, diagnostic strategies, and therapeutic interventions for NAFLD and related disorders. A variety of molecular biology techniques have been employed to investigate changes in protein levels, gene expression, and subcellular protein localization. Through protein and gene expression analysis, we have noted an initial state of intracellular copper overload, followed by a decline towards a copper-deficient status over a 24-hour stimulation. Immunofluorescence data, however, shows that despite protein and expression levels reflecting an overall status of copper deficiency, localization of the copper transporter ATP7B is akin to copper overload states. In addition, we have observed nonparadigmatic extracellular copper metabolism. In normal copper export, Cp is secreted in the extracellular media loaded with copper and functions as a copper-dependent enzyme. However, while intracellular data points to increased copper export we found that palmitate stimulation decreased Cp enzyme activity while eliciting no change in Cp concentration. Taken together, our data points to a mechanism wherein palmitate induces a copper imbalance that appears as functional overload, resulting in the cell exporting copper by non-traditional mechanisms towards a copper-deficient state.

# POSTER PRESENTERS

# Substrate and process engineering for biocatalytic synthesis and facile purification of human milk oligosaccharides (HMOs) Yuanyuan Bai / Chen lab

Oligosaccharides are important ingredients in human milk that support the healthy development of breast-fed infants. Many beneficial roles of human milk oligosaccharides (HMOs) have been discovered but the detailed information of specific structure-function relationship is lacking. Accessing structurally defined individual HMOSs in sufficient amounts for laboratory research and clinical studies is an actively pursued objective by us and others. We have developed a highly efficient chemoenzymatic strategy for synthesizing structurally complex HMOs. Starting with a lactoside readily obtained by chemical derivatization, HMOs with up to nonasaccharides have been successfully synthesized using sequential one-pot multienzyme (OPME) synthesis with C18column purification. The efficiency of the strategy has been demonstrated for gram-scale synthesize of a pentasaccharide and preparative-scale synthesis of twenty HMOs.

#### Insights into the Elusive Base Excision Repair Glycosylase NEIL2's Substrate Scope and Specificity Joshua Bumgarner / David lab

DNA damage can be induced by oxidative stress and is associated with cancer, aging, neurodegenerative diseases, and metabolic disorders. Reactive oxygen and nitrogen species produced by exogenous and endogenous sources of oxidative stress can modify DNA nucleobases, compromising the integrity of the genome. DNA glycosylases initiate the base excision repair pathway by locating and cleaving modified nucleobases from DNA. While most glycosylases work on a single specific substrate, the NEIL family of DNA glycosylases (NEIL1, NEIL2, NEIL3) work on a wide range of substrates and can remove several lesions from alterative DNA contexts, including single-stranded DNA and R-loops. NEIL2 is implicated in transcription-coupled repair and is associated with a wide range of disease phenotypes. However, the molecular basis of how NEIL2 dysfunction impacts these diseases is unclear, and there are substantial gaps in our understanding of the basic biochemical properties of NEIL2. We evaluated the ability of human and opossum NEIL2 to remove substrates from a panel of 12 lesions in single-stranded and double-stranded DNA. We identified four key lesions where NEIL2 is active: guanidinohydantoin, 5-hydroxyuracil, uracil glycol and abasic sites, with a demonstrated preference for the abasic site. Detailed analysis reveals unique structural features of the enzyme and lesions which may play critical roles in NEIL2 recognition and removal. An uracil-like face is required for base excision but can be influenced by additional substituents around the heterocycle. Most of the lesions tested have never been previously studied and are not commercially available providing valuable insight into NEIL2's biochemistry.

#### The Impact of Metals on the Structure and Dynamics of the Intermediate Filament Protein Vimentin Estely Carranza / Heffern/Murray labs / CBP Trainee

Vimentin intermediate filaments (IFs) are an integral component of the cell cytoskeleton. Disruptions in the organization and assembly of vimentin filaments have been implicated in numerous human intermediate filament diseases including cataracts, neuromuscular disorders, and metastatic cancer. The binding of metal ions to the tail domain of vimentin has shown to modulate filament assembly, organization, and interactions. Despite the known metal ion interactions of vimentin IFs, the structure and protein-metal interactions of tail domain in the full-length filaments of vimentin have not been thoroughly characterized at the atomic-level. This project aims to provide structural insight of the full-length vimentin IFs by investigating how the tail domain interacts with metals and how these interactions affect the functional higher-order organization of full-length vimentin filaments. This work employs a 'divide and conquer' approach to investigate the impact of metals on the structure and dynamics of peptides and protein fragments to understand these interactions in the full-length vimentin protein. To determine the amino acid-specific interactions of metals with peptide and protein fragments of the tail domain, various spectroscopic methods are being pursued including circular dichroism and UV-visible absorption spectroscopy. The outcomes of this work will provide an experimental framework for characterizing the structural properties and metal-protein interaction of other IFs, to help elucidate how these interactions may play a role in the various human IF diseases.

#### Identification and removal of oxidatively modified bases in G-quadruplexes by DNA glycosylase enzymes NEIL1 and NEIL3 Natasha Deo (undergraduate); Savannah Conlon / David lab

DNA base modifications resulting from reactions with exogenously and endogenously produced reactive oxygen and nitrogen species can contribute to genomic instability. In order to protect genomic integrity, cells employ the base excision repair (BER) pathway to repair DNA damage. The endonuclease VIII-like (NEIL) family of repair enzymes are DNA glycosylases that initiate the BER pathway by cleaving the N-glycosidic bond between the base and the sugar when abnormal bases are detected. NEIL1 and NEIL3 preferentially excise modified bases from double-stranded and singlestranded DNA respectively, and exhibit high removal activity toward the hydantoin lesions, guanidinoand spiriminodi- hydantoin (Gh and Sp), which block DNA polymerases and lead to mutagenesis. More recently, we have demonstrated the removal of the Gh base modification from G-quadruplex (G4) structures by NEIL1 and NEIL3. Notably, because G4 structures are found in oncogene and repair enzyme promoter regions, the unique ability of the NEIL enzymes to remove DNA damage from G4s could reveal a role beyond genome maintenance. To further investigate the ability of the NEIL enzymes to remove DNA damage from G4 structures, we performed kinetic assays under singleturnover (STO) conditions with the G4 DNA sequence from the KRAS promoter region. We evaluated the ability of NEIL1 and NEIL3 to remove various base modifications from the G4, including 2,6diamino-4-hydroxy-5-formamidopyrimidine (FapyG), 5-hydroxyuracil (5-OHU), and an abasic site (AP). Our results indicate that both NEIL enzymes are capable of excising FapyG, 5-OHU, and AP from the KRAS promoter region G4. This showcases that the NEIL family of enzymes are involved in the recognition and excision of multiple modified bases from G4s and may play an important role in protection of genomic integrity as well as gene regulation.

#### Rational Design of Oligonucleotide Guide Strands for Site-Directed RNA Editing Erin Doherty / Beal lab / CBP Trainee

Adenosine Deaminases Acting on RNA (ADARs) convert adenosine to inosine in double stranded RNA. Inosine is recognized by cellular machinery as guanosine, affording an A-to-G transition. Therefore, endogenous human ADARs can be used to correct disease-causing point mutations at the RNA level by directing them to target sites in the transcriptome via complementary guide strands. The use of endogenous editing enzymes can minimize barriers associated with off-target editing and enzyme delivery, and presents a potentially safer alternative to genome editing for therapeutic applications. This strategy requires the use of guide oligonucleotides that can stimulate efficient directed editing, leading to efforts aimed at optimizing guide RNAs. Here we use structural information for ADAR2-RNA complexes to guide the design of nucleoside analogs for the position in the guide strand that contacts a conserved glutamic acid residue in ADARs (E488 in ADAR2), where mutation of this residue to glutamate (E488Q) results in enzyme hyperactivity. We designed and evaluated chemical modifications that mimic this activating mutation, and found that Benner's Z nucleotide (a cytidine analog) increased the observed rate when compared to the preferred canonical base. We obtained a high-resolution X-ray crystal structure of ADAR2 complexed with a duplex containing the Z nucleotide, which revealed its effect on the contact with E488. Finally, we report that this single nucleotide modification increased directed editing yields in human cells and mouse primary liver fibroblasts. Our results show that modification of the guide RNA can mimic the effect of hyperactive mutants of ADARs, and lays the groundwork for additional rational design of ADAR guide strands.

#### Protein Fibril Structure Determination: Solid-State Nuclear Magnetic Resonance and Cryo-Electron Microscopy Blake Fonda / Murray lab

TAR-DNA Binding Protein-43 (TDP43) is an essential human transcription factor protein involved in many different biological processes related to transcriptional modulation. In some neurodegenerative disease, including amyotrophic lateral sclerosis and frontal-temporal dementia, TDP43 forms aggregates within neuronal tissue. It was previously observed that the C-terminal region of TDP43 is particularly important in driving this aggregation behavior. The discovery of TDP43 structure in disease-like aggregated states has the potential to provide a better understanding for disease mechanism and classification. In this work, TDP43 C-terminal aggregates are formed from recombinantly expressed protein, and the structure of these aggregates is investigated with solid-state NMR. These results demonstrate a specific sub-region of the C-terminal domain adopts β-strand structure in aggregates.

In a separate work, the structure of a Tropomyosin alternative isoform (Tm1 I/C) is investigated. This isoform, essential to Drosophila cellular organization, contains a C-terminal region without well-defined secondary structure. In both Tm1 I/C filaments and with fibrils formed by the Tm1 I/C C-terminal region in isolation, this C-terminal "tail-domain" adopts a  $\beta$ -strand conformation. Tm1 I/C tail-domain fibrils are studied via cryo-electron microscopy to attempt a high-resolution reconstruction. Preliminary work from 2D Classification demonstrates amyloid spacing of 4.8 A, and suggests near C2 symmetry.

#### Inhibition of ADAR1 editing by an RNA duplex bearing 8-azanebularene Herra Grajo / Beal lab

Though essential for proper cellular function, A-to-I editing by ADARs has also been implicated in various cancers and other metabolic disorders. Studies have shown that ADAR1 deletion results in increased tumor sensitivity to immunotherapy and in cell death for some cancers characterized by elevated levels of interferon-stimulated genes. However, there are no potent, targeted inhibitors of ADARs to date. Here, we designed an ADAR1 inhibitor from a short RNA duplex derived from HER1, a yeast RNA which we previously found to be preferentially edited by human ADAR1. The duplex inhibitor also bears the adenosine analog, 8-azanebularene (8-azaN), at the edited A position; a feature that we've previously shown to help in trapping the base-flipped conformation of ADAR2-dsRNA structures. In vitro deamination experiments in the presence of the duplex inhibitor showed inhibition of 5-HT2C and NEIL1 editing by ADAR1, with an IC50 of  $13.2 \pm 2.1$  nM and  $8.9 \pm 0.9$  nM, respectively. We found that this inhibition is 8-azaN-dependent and that the potency is affected by the length of the oligonucleotide relative to 8-azaN. Additionally, we have shown that this duplex does not inhibit 5-HT2C or NEIL1 editing by ADAR2, and hence appears to be ADAR1-selective.





N = 8-azanebularene

#### *Identifying and Characterizing Effectors of Low Complexity Protein Domain Assembly* Khaled Jami / Murray lab

TDP-43 is an RNA and DNA processing protein with a highly conserved, C-terminal low-complexity domain. In vitro, the TDP-43 low complexity domain can form liquid-liquid phase separations which readily mature into amyloid fibrils. Fibrils containing C-terminal fragments of TDP-43 have been identified within cellular inclusions of post-mortem neuronal tissue in a considerable range of neurodegenerative diseases, including ALS, FTD, and Alzheimer's. Nearly all ALS-associated mutations are found in the low complexity domain, further highlighting its significance to disease. Accumulating research indicates that aberrant phase transition and fibril propagation play an important role in the progression and severity of these diseases. However, the molecular mechanisms of TDP-43 phase separation, fibril formation, and the reversibility of these processes are not known. Furthermore, there are no existing tools to quantify TDP-43 aggregation in disease. This work aims to bridge this gap by identifying molecular effectors of functional and pathological TDP-43 assembly. Currently, we are investigating a library of nitroxide-functionalized amyloid-binders, metal cations, and protein chaperones. These potential effectors are screened by mass spectrometry, fluorescence, and turbidity assays for their ability to bind and perturb assembly. Complementary solution and solidstate NMR techniques are then used to extract binding sites, residue contacts and structural changes in both soluble monomeric and fibrillar states of TDP-43. These experiments will also lay the foundation for future work in understanding and regulating assembly behavior in other lowcomplexity domains.

#### Noncovalent Interactions Involving Sulfur Atoms in Thiopeptide Antibiotics Glycothiohexide α and Nocathiacin I Volga Kojasoy / Tantillo lab

Noncovalent interactions involving sulfur atoms play essential roles in protein structure and function by significantly contributing to the protein and peptide stability, the overall folding and biological activity. Sulfur being a highly polarizable atom with filled 3p and empty 3d orbitals participates in strong van der Waals interactions as well as hydrogen bonding, electrostatic interactions and hydrophobic effect and the impact of these interactions on molecular recognition and drug design is profound. For instance, hydrogen bonding has a fundamental role on secondary structure motifs and sulfur atoms have been known to accept and donate hydrogen bonds. Moreover, S····π, S····X and S•••C=O interactions are important contributors controlling the overall protein framework. Despite, the unique attributes of noncovalent interactions, they are being unappreciated. Although, only one of these interactions contributes only a few kcals energetically, as the system size grows this contribution increases dramatically. Understanding the nature of these noncovalent interactions involving sulfur atoms could pave the way for the drug design and development. Therefore, we chose naturally occurring complex molecules Glycothiohexide  $\alpha$  and its close structural analog Nocathiacin I as our model systems and deeply investigate the intramolecular sulfur contacts that are important for the structures by quantum chemical calculations. Glycothiohexide α and Nocathiacin I are sulfurcontaining polycyclic thiopeptide antibiotics with fascinating bioactivities. They are highly potent against gram-positive bacteria and have features of both small drugs and proteins. We validate our structures by computational NMR calculations. We identify the strength of the intramolecular noncovalent interactions involving sulfur atoms both quantitatively and qualitatively by natural bond orbital (NBO) analysis, non-covalent interaction (NCI) analysis and energy decomposition (EDA) analysis as measures to understand which type of interaction contributes more to the overall stability.

# *Targeted perfluorocarbon nanoprobes for 19F MRI macrophage phenotype identification* Lauren E. Ohman / Louie lab / CBP Trainee

Macrophages play an integral role in the body's defense system and have recently been appreciated for their roles in disease progression. In tumor environments specifically, macrophages can undergo differentiation of phenotypes leading to either tumor promotion, M2-type, or active immune response to fight growth, M1-type. These phenotypes are correlated to surface biomarkers on the macrophages that emerge upon cytokine expression. Given the large impact macrophage phenotype can have on disease progression, characterization of these distinct biomarkers in vivo can greatly increase patient outcome. Current clinical practice involves characterization of biomarkers from tissue biopsy, which is invasive and can miss tumor heterogeneity. Though the targeting of biomarkers in in vivo imaging has been explored as a non-invasive option, we propose the use of hotspot 19F MRI (magnetic resonance imaging) for its high signal-to-noise, high resolution capabilities, and ability for contrast and anatomical view with paired 19F/1H imaging. In this work, we describe a 19F MR probe design for in vivo imaging that can be readily modified with differing targeting ligands through copper-free click chemistry. We propose the targeting of four different biomarkers, two for M1-type and two for M2-type macrophages, through different perfluorocarbon cores and targeting ligands, for future multiplexed imaging.

We demonstrate the ability to synthesize perfluorocarbon nanoemulsions (PFC-NE) with the incorporated dibenzocyclooctyne (DBCO) click moiety for future functionalization of azide-containing ligands. The PFC-NE design was also further modified for in vitro work to include a stealth molecule, polyethylene glycol (PEG), for less non-specific uptake in the macrophages, and future longer circulation times when used in in vivo models. We also showed preliminary cellular uptake studies for the two tumor promoting biomarkers, CD204 and CD206. Dextran sulfate (SD) azide was incorporated in order to target the CD204 marker and showed preferential uptake of the SD-NE over a control dextran clicked NE. For the CD206 biomarker, a preliminary targeting study employing exclusively ligand was completed to demonstrate the ability of an α-D-mannopyranosyl azide to be taken up by macrophages. An overlap of fluorescent signal was shown of the receptor and ligand when immunostained, but further characterization of competitive binding and uptake is needed to confirm specific targeting and uptake of the ligand through receptor binding. Future work will continue on the characterization of the CD206 targeting, as well as characterizing all of the four proposed biomarkers for eventual multiplexed imaging in vivo.

#### A nanomaterials sensing platform for chemical fingerprinting of cancer odor Hannah O'Toole / Carney lab / CBP Trainee

My research aims to address the need for a non-invasive, sensitive, specific, and rapid tool for the detection and monitoring of cancer via readout of tumor-associated volatile organic compounds (VOCs) generated from the underlying disease metabolic processes. The gold standard for VOCs analysis is mass-spectrometry (MS), but there are practical limitations for its use in a clinical setting. In MS, the sample must be pre-concentrated to detect the needed parts-per-million to parts-perbillion concentration range of tumor VOCs. MS also requires a trained user, requires offline data processing, is only semi-quantitative, and is expensive. An attractive alternative for VOC discovery and testing is via Raman spectroscopy, a non-destructive, label-free chemical spectroscopic technique that fingerprints complex mixtures of analytes. Amplification of the typically weak Raman scattering signal can be achieved via surface-enhanced Raman scattering (SERS), a highly sensitive extension of Raman which utilizes localized surface plasmon resonance effects of metallic nano-features to enhance Raman signal by factors >106 times. Thus, VOCs detection via SERS addresses the need for sensitive detection. However, there are downsides to SERS, as enhancement is orientation and affinity dependent between the metal and analyte of interest, making bulk analysis difficult. To improve VOC localization to the metal surface, I have devised several coatings of metal-organic frameworks (MOFs) at the nanoparticle surface, tailored to selectively adsorb tumor-associated VOCs. MOFs are crystalline porous materials with ultrahigh surface area, composed of metal-oxide units joined by organic linkers that have selective affinity to different functional groups. By utilizing the ultra-sensitive readout of SERS in combination with the selectivity of tuning the organic linkers of MOFs, tumor-associated VOCs can be detected and quantified via this chemical sensing spectral readout platform called a SERS-MOF array. The project overview can be visualized in the figure below. Thus far, I have begun to form a spectral library of known tumor-associated VOCs, as well as explore multiple synthetic approaches to incorporate plasmonic gold nanoparticles into ZIF-8, a common MOF for use in bio-applications. I am exploring different nanoparticle morphologies and their effect on the limit of detection of the tumor-associated VOCs, as well as investigating preliminary binding affinity models for the selective adsorption of these VOCs to the organic linkers of the MOF structure. My poster will present this preliminary data, as well as my projections for the future of this project.



SERS-MOF Array Overview. (Top) Metal NPs of various sizes and shapes can be incorporated into (right) MOF architectures that can be (bottom) tuned via organic ligand incorporation for (left) selective capture and readout of targeted analytes, with focus on tumor-associated VOCs. (Center) Taken together, these pieces enable realization of a new multiplexed VOC sensing platform for cancer detection and diagnosis, where arrays of SERS-MOF materials can be optically readout using Raman spectroscopy to build advanced models using patient samples.

#### Exploring Drug Arrythmogenicity Via Atomistic Simulations Of State-Specific hERG-Channel Block Kyle Rouen / Vorobyov lab / CBP Trainee

Expressed primarily in cardiac myocytes, the hERG channel (Kv11.1) is a voltage-gated potassium channel that facilitates a repolarizing K+ current during the cardiac action potential (CAP). In response to changes in membrane potential, the hERG channel undergoes transitions from closed to open and inactivated states. Utilizing atomistic molecular modeling and docking simulations, we aim to assess the state-specific binding of drugs to the hERG channel utilizing structural models of open and inactivated hERG channels. To overcome the prohibitively long run times of all-atom molecular dynamics (MD) simulations, we utilize Site Identification by Ligand Competitive Saturation (SILCS), a pre-compute ensemble molecular docking technique, to predict hERG-channel binding poses and free energies of binding of relevant drug molecules. Furthermore, we present modifications to the SILCS functional group affinity map (FragMap) generation protocol that improve its applicability to ion channels like hERG. To validate and improve the SILCS results, we perform enhanced-sampling MD simulations to obtain reference values for SILCS docking predictions and use Bayesian machine learning to improve the accuracy of the SILCS in-silico methodology. The docking poses generated by SILCS agree with these MD simulations for the drugs dofetilide and moxifloxacin as well as with astemizole as observed in a recent cryo-EM structure. Data from these simulations provide parameters for multi-scale functional models of cardiac cells and tissue that predict the drugs' effects on the CAP and heart rhythm. This methodology allows us to computationally screen and differentiate open vs. inactivated-state hERG-drug binding for potentially safe and efficacious drugs that would otherwise be rejected by regulators using only hERG block and QT prolongation as markers of pro-arrhythmia risks. Finally, electrophysiology recordings of cultured cells expressing hERG channels will provide experimental validation for the state-dependent binding of selected hERG blockers.

#### Structural and Phenotypic Characterization of a Mycobacterial Sulfate Activating Complex Sam Hartanto / Fisher lab / CBP Trainee

The respiratory disease tuberculosis (TB), caused by the pathogen Mycobacterium tuberculosis (Mtb), is an ongoing worldwide epidemic which necessitates the identification of novel drug targets. Sulfur is an essential element for the growth, virulence, and survival of Mtb, and thus disruption of sulfur metabolism may be a potential means of combating TB. To address this, we are studying CysDNC, a key trifunctional sulfate activating complex which lies at the beginning of mycobacterial sulfur metabolism. CysDNC is a heterodimer formed by the subunits CysD and CysNC. The subunit CysD is an ATP sulfurylase (ATPS) which catalyzes the reaction between imported sulfate and ATP, forming adenosine-5'-phosphosulfate (APS). This reaction is energetically unfavorable and only proceeds when coupled to GTP hydrolysis by the CysN subunit. CysN is fused to CysC, a kinase which phosphorylates APS, forming 3'-phosphoadenosine-5'-phosphosulfate (PAPS). Both APS and PAPS serve as precursors for downstream sulfur-containing biomolecules. Despite its requisite role in sulfur metabolism, little structural information exists about this complex. To probe the physiological relevance of the complex, we used the closely related model organism M. smegmatis to create a knockout mutant deficient in the gene encoding CysD. In vitro growth curve experiments show that the  $\Delta$ cysD mutant cannot grow using sulfate as its sole sulfur source, and instead is auxotrophic for reduced sulfur compounds including sulfite, sulfide, cysteine, and methionine. Interestingly, this mutant also cannot grow with APS as the sole sulfur source, despite the CysNC gene left intact. We have also studied the in vitro biochemistry of CysDNC. While the forward ATPS reaction is energetically unfavorable, the reverse reaction is spontaneous and easily measured. Using a spectrophotometric reverse ATPS assay, we have seen that CysDNC exhibits reverse ATPS activity, but the CysD subunit on its own does not. These data suggests that the interaction between CysD and CysNC subunits is critical in the overall function of the complex, extending beyond just an energetic requirement. To further investigate molecular basis of this interaction, we aim to obtain highresolution 3D structures of CysDNC using cryogenic electron microscopy (cryo-EM). Current efforts are focused on optimizing plunge-freezing conditions to obtain grids with homogeneous, welldispersed protein molecules. Such grids will allow for large scale data collection and, ultimately, single particle reconstruction.

#### Calculated oxidation potentials predict reactivity in Baeyer–Mills reactions Robert J. Tombari / Olson lab / CBP Trainee

Azobenzenes are widely used as dyes and photochromic compounds, with the Baeyer–Mills reaction serving as the most common method for their preparation. This transformation is often plagued by low yields due to the formation of undesired azoxybenzene. Here, we explore electronic effects dictating the formation of the azoxybenzene side-product. Using calculated oxidation potentials, we were able to predict reaction outcomes and improve reaction efficiency simply by modulating the oxidation potential of the arylamine component.





# *Quinone binding in respiratory complex I: the squeeze-in mechanism of passing the narrow entrance of the quinone site* Panyue Wang / Stuchebrukhov lab

We are trying to investigate the binding mechanism of quinone in the complex I quinone binding cavity. We used molecular dynamics to simulate protein dynamics and principal component analysis to find protein conformation changes. We found that the crystal structure is too narrow for quinone to pass the entrance, and thermal fluctuations can open up the entrance. Therefore, it's not possible for quinone to pass the quinone cavity entrance of the crystal structure unless some thermal fluctuations happen.

#### Structural Characterization of Fibrils Formed by the Low Complexity Domain of the RNA-binding Protein TIA-1 Yuuki Wittmer / Murray lab

Proteins containing low complexity sequence domains harbor mutations genetically linked to neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) and frontal temporal dementia (FTD). Not much is known about the structures these protein domains adopt functionally and what role the mutations play in causing disease. One of the key proteins associated with both ALS and FTD is T-cell restricted intracellular antigen-1 (TIA1). TIA1 contains a low complexity domain (LCD) which is known to be an important factor in the formation of fibril-like structures, often with mutations, seen in disease. The two main focuses of this work are to determine an atomic resolution structure for fibrils formed by the wild-type LCD of TIA1 using solid state nuclear magnetic resonance, as well as determining the kinetic stability and liquid-liquid phase separation propensity of the TIA1 LCD fibrils with the mutations found in disease. Quiescently seeded sample preparation methods of wild-type TIA1 yield assemblies similar to amyloid fibrils based on transmission electron microscopy images and increased thioflavin T fluorescence. Initial solid state nuclear magnetic resonance spectra reveal which amino acid types compose the rigid core of the protein fibrils and suggest a structure determination is highly promising. The first major hurdle for the structure determination is to make sequence specific assignments of the observed signals. We present the results of our computational efforts to make these assignments. Although a high resolution structure is still forthcoming, our data provide a low-resolution characterization of the TIA1 low complexity domain conformation in fibril form. Once complete, the lens of a high-resolution structure for TIA1 fibrils will allow for a better understanding of low complexity sequence domain self-assembly and its role in disease. In addition, work with the TIA1 LCD with mutations will provide a better understanding of how the mutations factor into the stability and structure of the fibrils and ultimately aid in understanding these neurodegenerative diseases.

# One bacterial glycosyltransferase that is highly efficient in catalyzing the synthesis of legionaminic acid-terminated glycosides Xiaohong Yang / Chen lab

Legionaminic acid (Leg5,7diNAc) is a bacterial monosaccharide belonging to the nine-carbon alphaketo acid family called nonulosonic acid. It has been found in bacterial capsular polysaccharide and lipopolysaccharides that can be potential bacterial vaccine candidates. Limited information is available about glycosyltransferases that are responsible for catalyzing the transfer of Leg5,7diNAc. We identified and characterized a bacterial glycosyltransferase that is highly efficient in catalyzing the synthesis of Leg5,7diNAc-glycosides. Its properties and applications in glycan synthesis will be presented and discussed.

#### Novel Perfluorocarbon-loaded nanolipoprotein particles for 19F MRI imaging Daniel Zoltek / Louie lab

19F has drawn renewed attention recently, for its potential in cell tracking applications to monitor cell-based therapies. 19F nuclei are suitable for MRI imaging, while also having a lack of natural fluorine background in the body, which allows for very high contrast-to-noise compared to 1H MRI. The most common contrast agents are perfluorocarbon (PFC) nanoemulsions, containing a PFC core surrounded by a surfactant/lipid layer, however, synthetic techniques often yield large particles (≥160 nm) which are rapidly cleared from the body. Our objective is to develop a new series of perfluorocarbon-containing nanolipoprotein particles (PFC-NLPs) which are composed of a scaffold encompassing a lipid bilayer. Native NLPs contains a hydrophobic core suitable for the incorporation of PFCs and assemble in the range of 6-25 nm, ideal for longer circulation times and escape from nonspecific phagocytosis. We propose novel methods for the synthesis of PFC-NLPs including self-assembly and ultrasonic assembly with both protein and polymeric scaffolds. While lipid choice will affect membrane mobility and PFC incorporation, the scaffold mediates the assembly process. Results suggest that PFC choice has the largest impact on 19F MRI signal intensity, while lipid mobility impacts loading capabilities during self-assembly. Select PFC-NLPs showing optimal structural characteristics will be used for future imaging in mouse models.