UC San Diego

MBTG 2022 Annual Retreat

Friday, April 8th, 2022

Program Book

mbtg.ucsd.edu

UC San Diego

Molecular Biophysics Training Grant (MBTG)
2022 Annual Spring Retreat

Friday, April 8th, 2022, 1:00PM
Institute of the Americas

12:45PM — **Poster set-up**
Arango Foyer

1:00-1:05PM — **Welcome Remarks**: Betsy Komives
Deutz Room

1:05-1:35PM — **Keynote Address Introduced by Christine Stephen**
Deutz Room
Johannes Schoeneberg, Assistant Professor, Pharmacology
"4D Cell Biology: Adaptive optics lattice light-sheet imaging and AI powered big data processing of live stem cell-derived organoids."

1:35-2:35PM — **Poster Presentations: MBTG Trainees**
Arango Foyer

1. Lannah Abasi
2. Max Bachochin
3. Chelsea Blankenship
4. Gillian Gadbois
5. Simone Hall
6. An Hsieh
7. Israel Juarez Contreras
8. Marc Morizono
9. Xandra Nuqui
10. Emily Pool
11. Brandon Rawson
12. Jacob Vance
13. Kailash Venkatraman
14. Megan Young
15. Hoang Nguyen
16. Calvin Lin

2:35-3:30PM — **Career Panel Moderated by An Hsieh**
Deutz Room
Don Johnson, Principal Scientist at Bristol Myers Squibb
Jamie Schiffer, Senior Scientist at Takeda
Hannah Baughman, Postdoctoral Scholar at Komives Lab, UC San Diego
Stephanie Truhlar, Associate Vice President at Eli Lilly and Company

3:30PM — **Lightning Talks**
Deutz Room

1. Kira Podolsky
2. Bryce Ackermann
3. Joshua T. Arriola
4. Adarsh Balaji
5. Aileen Button
6. Quinn Cowan
7. Mounir Fizari
8. Ximena Garcia Arceo
9. Alexander Hoffnagle
10. Evan K. Kobori
11. Dominic McGrosso
12. Elizabeth Porto
13. Christine Stephen
14. Bryce M. Timm
15. Ryan Weeks
16. Douglas Zhang

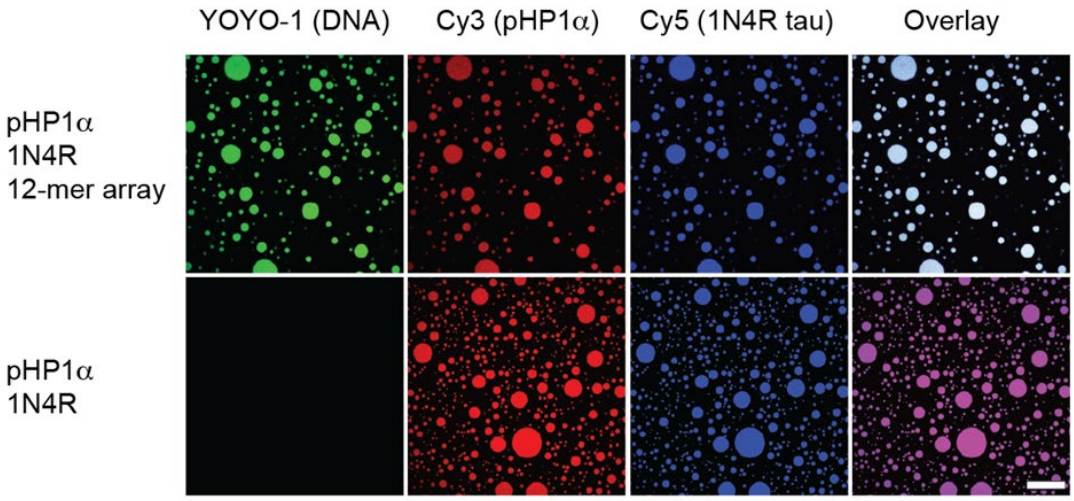
4:50-5:30PM — **Ethics Dilemmas Session moderated by Aileen Button**
Deutz Room
We will be breaking into small groups to discuss a case study dealing with pressures to complete work and publish in high impact journals (the case is located at the back of your Retreat Book)

5:30PM — **Barbeque Dinner**
Friend Plaza

5:45PM — **Poster Awards:** Betsy Komives
Friend Plaza

Poster #1
Investigations into the phase separation and nuclear role of tau
Lannah Abasi
Debelouchina Lab

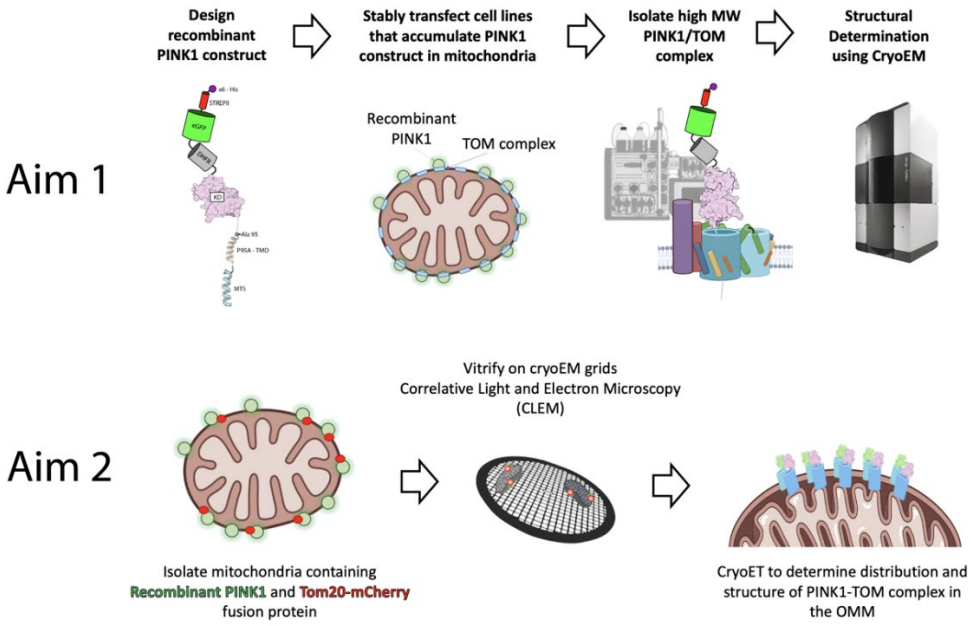
Tau is a microtubule-associated protein and pathological hallmark of Alzheimer's disease (AD), most infamous for becoming hyperphosphorylated and fibrilizing into neurofibrillary tangles (NFTs). Beyond this role, mounting evidence suggests that tau localizes into the nucleus and plays unknown roles in DNA protection and heterochromatin regulation. Intriguingly, frontotemporal dementia mutants (P301L) of tau show loss of genetically silent heterochromatin clusters. This has been associated with aberrant expression of heterochromatic genes and in other studies, of transposable element activation in AD patient brain tissue. Similar effects of heterochromatin relaxation and gene dysregulation have been observed in tau knockouts, suggesting that loss of tau is pathologically relevant. Recent literature showed that tau undergoes liquid-liquid phase separation (LLPS). Studies have not fully described the functional role of nuclear tau in gene regulation, and whether this involves LLPS. My work demonstrates that tau has an intrinsic ability to phase separate with, compact, and oligomerize chromatin, likely through its DNA-binding domain, and this is regulated by phosphorylation. In addition, it phase separates with heterochromatin protein 1α (HP1α), an essential heterochromatin constituent. We hypothesize that tau phase separates in heterochromatin domains in cells, and that dysfunction of this phase separation leads to heterochromatin relaxation and gene dysregulation.



LLPS studies of pHP1α, tau (1N4R) and chromatin (12-mer nucleosome arrays). The location of chromatin is visualized with the YOYO-1 dye, pHP1α is labeled with Cy3 and 1N4R tau is labeled with Cy5 (scale bar: 50 μm).

Poster #2
Structural insights into PINK1 activation in the TOM complex
Maxwell Bachochin
Herzik Lab

The mitochondria are responsible for the bulk production of cellular ATP and the biosynthesis of various macromolecular precursors and are thus central in multiple physiological roles. Predictably, mitochondrial health has been implicated in many disease pathologies, including chronic illnesses like diabetes, cancer, and neurodegenerative disease. In the disease state, mitochondria display an array of phenotypic abnormalities, one of which is a characteristic dysregulation of mitophagy. One mitophagic path is the PTEN-induced kinase 1 (PINK1)/Parkin relay system. In the outer mitochondrial membrane, PINK1 accumulates in the translocase of the outer membrane (TOM) complex and recruits and phosphorylates the E3 ubiquitin ligase Parkin, triggering a signal cascade to induce mitophagy. Though rigorous biochemical and structural study has been performed on both the TOM complex and PINK1 individually, details regarding the structure of this complex remain unknown. Using cryogenic electron microscopy (cryoEM) and cryogenic electron tomography (cryoET) I will identify the structural correlates of the PINK1-TOM complex in vitro and in situ. Both approaches utilize a recombinant PINK1 construct designed for ease of identification and purification, followed by specialized isolation procedures regarding each respective methodology. In Aim 1, I will use cryoEM to answer how PINK1 is activated in the TOM complex at an atomistic level of detail—an ambiguous process in the PINK1/Parkin mitophagy pathway. In Aim 2, cryoET methods will contextualize the atomistic information obtained from cryoEM experiments into a cellular environment, answering how PINK1-TOM complexes are organized across the outer mitochondrial membrane—biological details that are occluded using in vitro methods. Both aims will increase our collective biological understanding of a crucial step in PINK1/Parkin mitophagy.



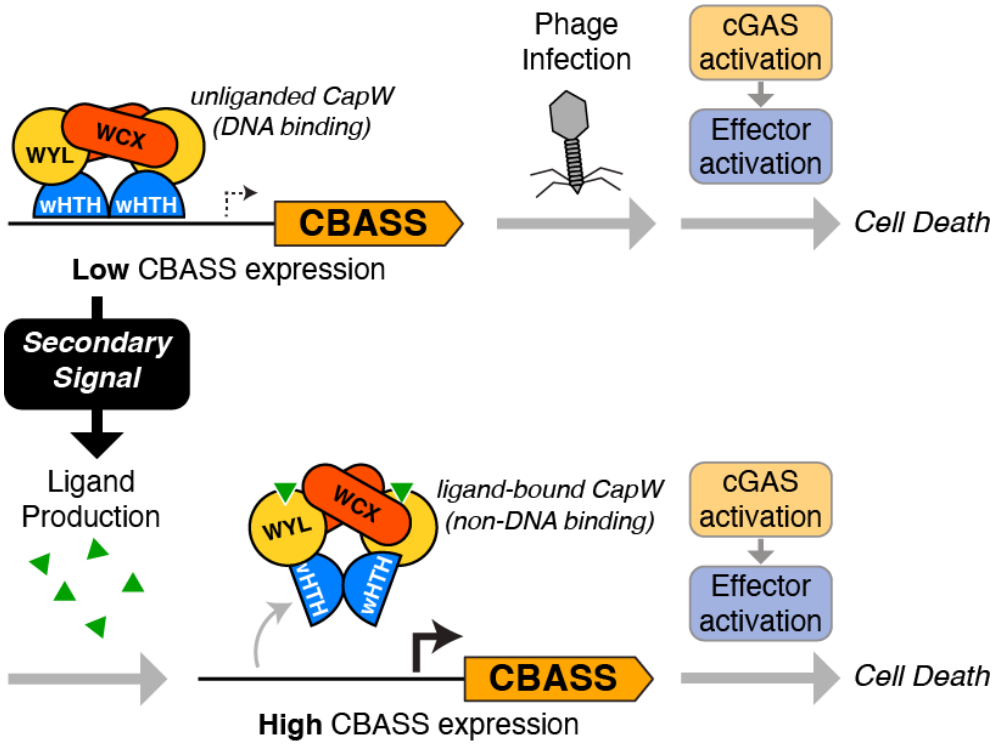
Poster #3

Control of bacterial immune signaling by a WYL domain transcription factor

Chelsea Blankenchip

Corbett Lab

Bacteria use diverse immune systems to defend themselves from ubiquitous viruses termed bacteriophages (phages). Many anti-phage systems function by abortive infection to kill a phage-infected cell, raising the question of how these systems are regulated to avoid cell killing outside the context of infection. One widespread abortive infection system is CBASS (Cyclic oligonucleotide-Based Anti-phage Signaling System), in which nucleotide-based second messenger molecules are produced in response to infection that trigger cell death by activating diverse effector proteins. Here, we identify a transcription factor, CapW, associated with hundreds of distinct CBASS systems in bacteria. Using three model systems from *Escherichia coli*, *Stenotrophomonas maltophilia*, and *Pseudomonas aeruginosa*, we find that CapW forms a homodimer and binds a palindromic DNA sequence in the CBASS operon's promoter region. Two crystal structures of CapW suggest that the protein switches from an unliganded, DNA binding-competent state to a ligand-bound state unable to bind DNA. Using a GFP reporter system, we show that CapW strongly represses CBASS gene expression in uninfected cells and drives increased CBASS expression upon phage infection. Unexpectedly, this CapW-dependent increase in CBASS expression is not required for robust anti-phage activity, suggesting that CapW may mediate CBASS activation and cell death in response to signals other than phage infection. Our results parallel concurrent reports on the structure and activity of BrxR, a transcription factor associated with the BREX anti-phage system, suggesting that CapW and BrxR are members of a broad family of defense signaling proteins.

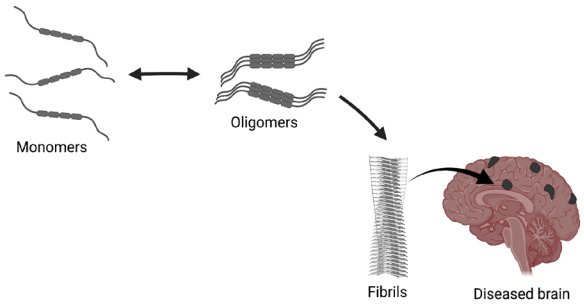


Poster #4
Expanding the druggable target space in Parkinson's Disease

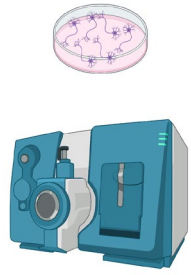
Gigi Gadbois and Fleur Ferguson

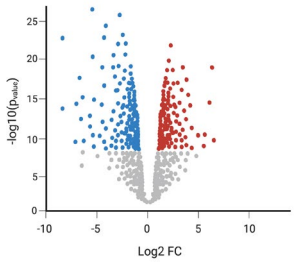
Abstract: Parkinson's disease (PD) is a neurodegenerative disease that is associated with the death of dopaminergic neurons. No current disease interventions currently exist for Parkinson's disease. A major barrier to treating Parkinson's disease is the lack of knowledge surrounding the cause and mechanism of the observed neurodegeneration. Aggregates of misfolded alpha-synuclein are a hallmark of the disease, but it is unclear whether the aggregates are a cause of the disease or a result of the disease. Currently, there is a lack of tools for scientists to specifically study the aggregated form of alpha-synuclein to explore this question. Another barrier to studying Parkinson's disease is the lack of known ligandable targets within the disease. Our project will address both issues by developing a strategy to degrade aggregated alpha-synuclein and by surveying the reactivity and ligandability of cysteines within neurons. A library of proteolysis targeting chimeras (PROTACs) designed to degrade aggregated alpha-synuclein has been synthesized by our lab. The small molecules within the library will soon be subjected to a series of assays to screen for cellular toxicity, cellular target engagement, dose-response activity, and target selectivity. From these series of screens, we will identify a small molecule that selectively degrades aggregated alpha-synuclein. In parallel, this project will investigate cysteine reactivity and ligandability within induced pluripotent stem cell (iPSC)-derived dopaminergic neurons. Another characteristic of Parkinson's disease is an altered reactive oxygen species environment and increased vulnerability to oxidative stress. We will explore whether the difference in the oxidative environment of iPSC-derived neurons from PD patients compared to healthy neurons translates to differences in reactivity and ligandability. Activity-based protein profiling experiments will allow us to investigate the cysteine profiles within the different neuron populations. These findings will allow for a greater understanding of the environment within PD neurons and potentially allow for new targets to be identified. Our work will generate new tools and data sets and will serve as a resource for other scientists to further study and develop therapeutics for Parkinson's disease.

Protein Dysfunction in Parkinson's Disease

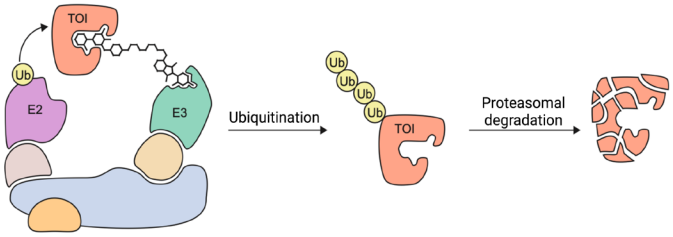


Activity-Based Protein Profiling





Targeted Protein Degradation



Outcomes

Identify an active alpha-synuclein degrader

Characterize the consequences of removing insoluble alpha-synuclein

Cysteine reactivity profiles of healthy and PD neurons

Cysteine ligandability profiles of healthy and PD neurons

SIMONE HALL, McHUGH LAB - RESEARCH ABSTRACT – MBTG SPRING RETREAT

Title: The long non-coding RNA linc00883 controls human colon cancer cell growth

Background: Long non-coding RNAs (lncRNA) are finely tuned regulators of gene expression that are important for human cell growth and cancer progression. This class of macromolecules interacts directly with proteins and chromatin to mediate their function. The long intergenic non-coding RNA 00883 (linc00883) is a novel lncRNA which is spliced, polyadenylated and abundant in human cells as evaluated by RNA sequencing data. Histone modifications often found near promoters and active regulatory elements suggest that linc00883 transcription occurs in many different cell types. High expression levels of this lncRNA are predictive of poor patient outcome in colon adenocarcinoma, suggesting that linc00883 may be involved in controlling cancer growth, yet there is no reported mechanism for the lncRNA in colon cancer.

Methods: Here we report that linc00883 is a regulator of human colon cancer cell survival. Knockdown of linc00883 transcript levels results in a dramatic loss in cell viability in the human adenocarcinoma cell line HCT116. We characterized the functional transcripts of linc00883 in colon adenocarcinoma cells and then performed RNA-sequencing and differential gene expression analysis to identify cellular pathways regulated by linc00883. Additionally, RNA antisense purification (RAP) techniques coupled with mass spectrometry and high-throughput DNA sequencing were used to identify the endogenous protein and chromatin regions interacting with linc00883. Mutational studies and rescue experiments will be utilized to identify the functional regions and protein/chromatin binding sites that allow this lncRNA to control human cell growth.

Significance: Our results indicate that linc00883 plays a vital role in colon cancer progression, and we are currently investigating the mechanism by which this RNA controls cancer cell growth. This research identifies a potential target for anti-colon cancer therapeutics and will also elucidate complex lncRNA/protein/chromatin interactions which allow for cell growth regulation.

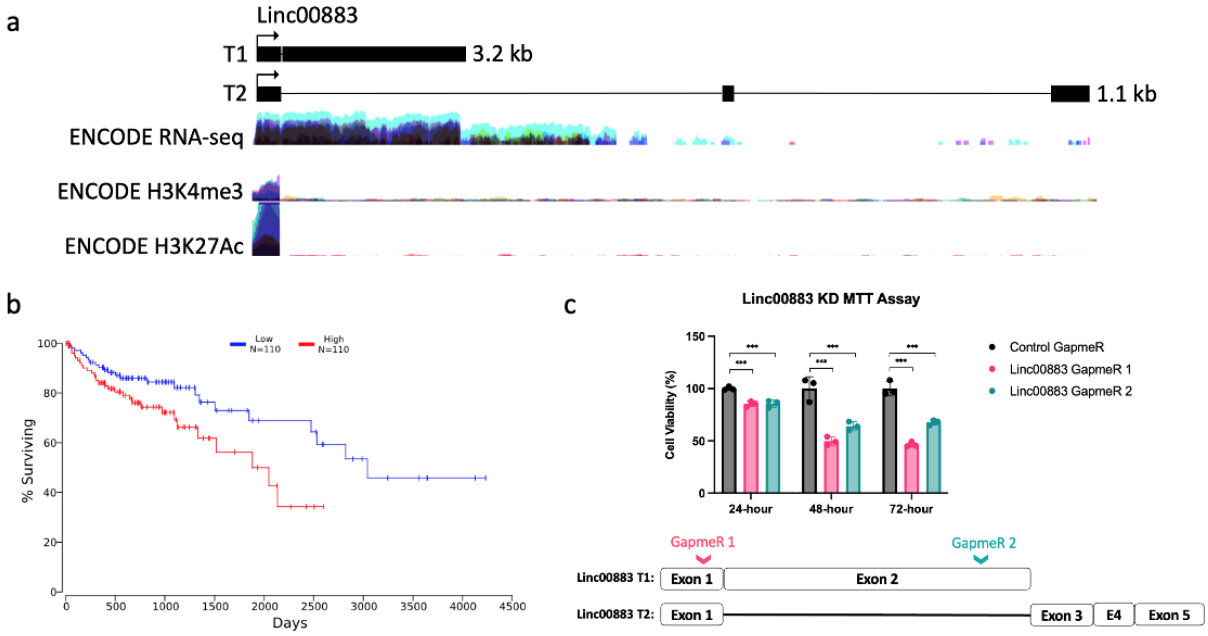


Figure 1. a) Linc00883 transcript 1 (T1) and transcript 2 (T2) relative abundance and histone modifications at promoter. b) High linc00883 T1 expression is predictive of poor patient outcome in colon adenocarcinoma TCGA data set (p = 0.0204). c) Knockdown of linc00883 T1 results in dramatic decrease in HCT116 cell viability (N = 3, * p<0.05, ** p<0.01, *** p<0.005).

Poster #6
Structure and Function of ABC Transporters in Oceanic Species
An Hsieh
Tatiana Lab

Abstract

The pollution of the world's oceans is a mounting problem that threatens the safety and well-being of our planet. Many marine organisms and ecosystems are exposed to pollutants with currently unknown toxicity and consequences. Membrane proteins that act as xenobiotic – foreign molecule – exporters are an important line of defense against pollutants in the organisms' environment. One of the most ubiquitous membrane proteins are the ATP binding cassette transporters (ABC). Found in organisms across all domains of life and in many tissue types, ABC transporters selectively export many different small molecules from within the cell, preventing the toxic accumulation of xenobiotics within the organism. Many of the small molecules exported by ABC transporters are pollutants. However, some small molecule pollutants may act as inhibitors of ABC transporters by interacting with their binding site and impeding transport. For example, a study conducted on one transporter – ABCB1 or P-glycoprotein – in mice found that transport activity was significantly reduced in the presence of DDD, DDE, and DDT (Nicklisch, 2016). In the case of ABC transporters in oceanic species, many reagents found polluting the ocean may inhibit ABC transporters' ability to export their wide range of substrates. This would lead to detrimental effects to the health of organisms in the oceanic food chain (e.g. yellowfin tuna) and to embryonic development (e.g. purple sea urchin). A thorough molecular and structural investigation of these transporters expressed in marine species is necessary to determine which chemicals inhibit transport. We propose to solve the structure(s) of ABC transporters to determine how they interact with substrates. We will also determine the binding constants of pollutants by calculating their IC_{50} values using established ATPase assays.

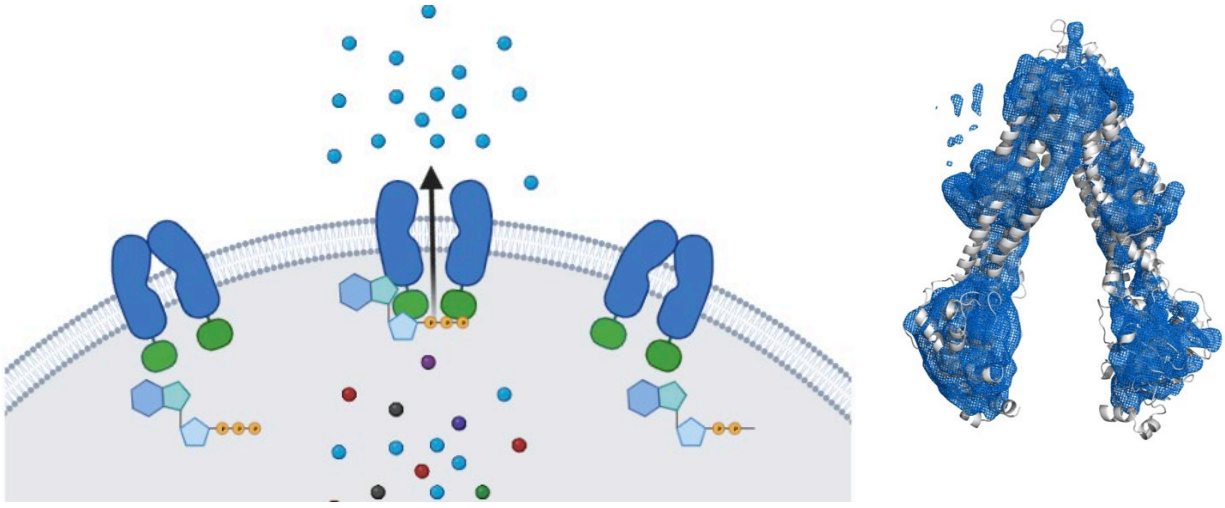


Figure: (Left) ABC transporters composed of a transmembrane domain and nucleotide binding domain hydrolyze ATP to catalyze transport of small molecules/xenobiotics out of the cell. (Right) Current cryo-EM data collected from sample of tuna ABCB1 (blue mesh) using model of mouse ABCB1 (white cartoon).

Poster #7

Dissecting the contributions of sterol structure to vacuole membrane phase separation

Israel Juarez Contreras, Budin Lab

The Bloch hypothesis states that sterol properties are gradually optimized for function along the biosynthetic pathway. Here, we compare the ability of sterol intermediates in the ergosterol biosynthesis pathway to support phase separation in the membranes of vacuoles, the lysosomal organelle in *Saccharomyces cerevisiae* (budding yeast). Manipulation of the ergosterol biosynthesis pathway in engineered yeast strains allowed for accumulation of sterol intermediates. To visualize the vacuole membrane, we used the well-established protein marker Pho8, an alkaline phosphatase, that localizes to the vacuole membrane. If the Bloch hypothesis holds true, then we expect to observe a trend, where intermediates found in the early stages in the ergosterol biosynthesis pathway exhibit little to no ability to promote phase separation whereas late-stage intermediates should exhibit a moderate ability to promote phase separation in comparison to ergosterol, the final product. Surprisingly, we observed that certain late-stage intermediates show little to no phase separation.

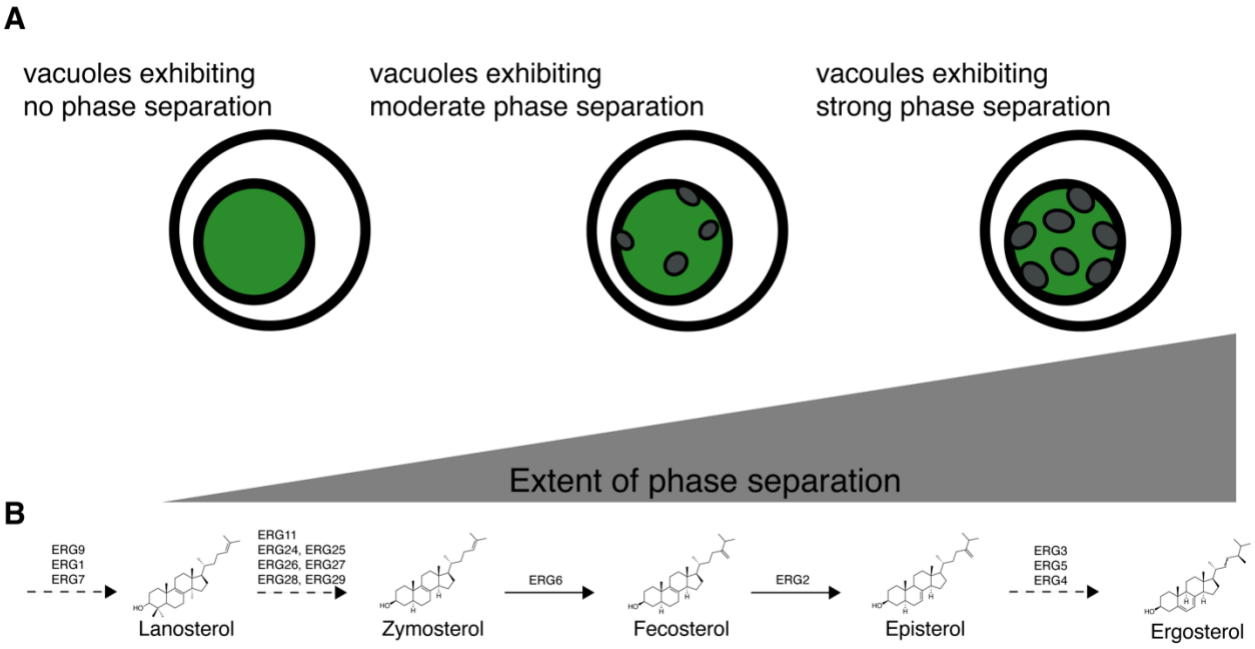
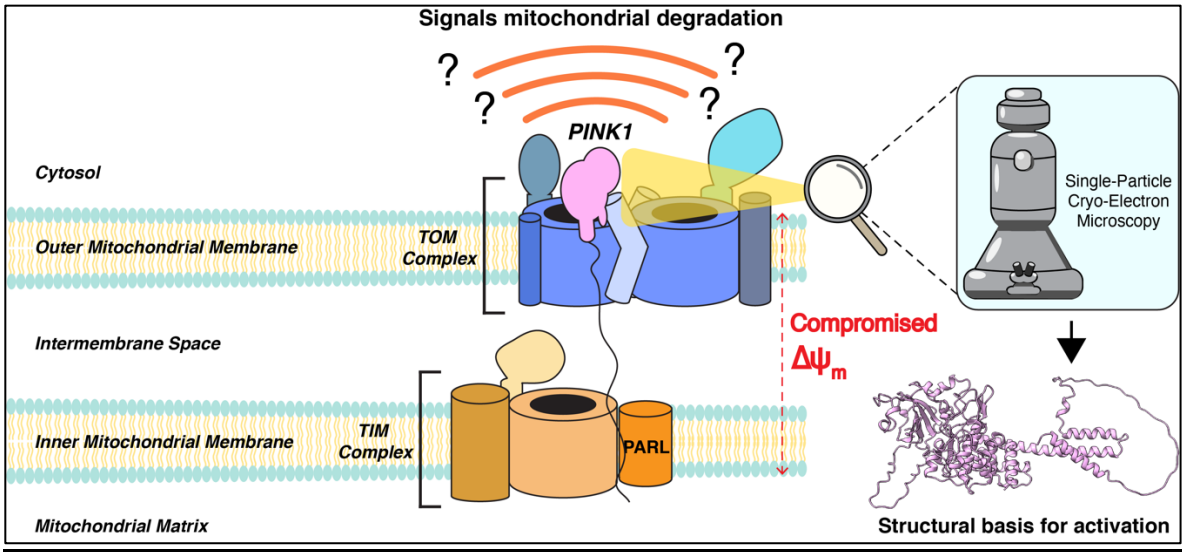


Figure 1. (A) The protein marker, Pho8-GFP, allows for visualization of phase separation of the vacuole membrane in live cells. In growing cells, Pho8-GFP is uniformly distributed on the vacuole membrane. However, when cells enter stationary phase, the vacuole membrane undergoes phase separation, resulting in Pho8-GFP localizing to the liquid disordered regions (L_d) of the membrane. Regions devoid of Pho8-GFP are the liquid ordered regions (L_o), enriched in sterols (ergosterol in wild-type cells) and sphingolipids. (B) Expected outcome of sterol intermediates by their ability to influence phase separation of the vacuole membrane according to the Bloch hypothesis. Early-stage intermediates such as lanosterol should exhibit little to no phase separation whereas late-stage intermediates such as fecosterol should exhibit moderate phase separation. Dashed arrows indicate multiple steps precede the intermediate shown. The names of the biosynthetic enzymes involved in producing the intermediates shown are listed.

Poster #8

Structural Insights into Mitochondrial Health Regulation using CryoEM

Marc Morizono
Herzik Lab



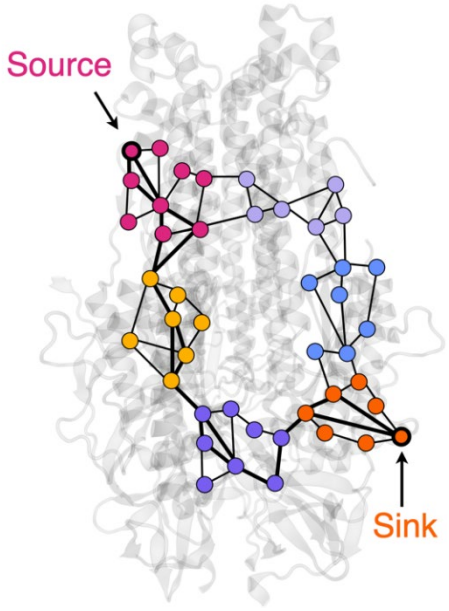
Mitochondria are essential eukaryotic organelles that generate cellular energy, regulate metabolism, and serve as master integrators of numerous cellular processes. For many of these processes to function properly, mitochondria must maintain an electrochemical potential gradient spanning both inner and outer mitochondrial membranes. Importantly, loss of this inner membrane potential causes these damaged mitochondria to be flagged for degradation in a process known as mitophagy. This programmed clearance of impaired mitochondria is vital to maintain cellular health as the accumulation of dysfunctional mitochondria is associated with various diseases including cancers, diabetes, and neurodegeneration.

In humans, the PTEN-induced kinase 1 (PINK1)-Parkin signaling relay serves as a mitochondrial health sensor and the principal regulator of mitophagy. In healthy mitochondria, PINK1 is partially translocated across the outer mitochondrial membrane (OMM) via the translocase of the outer membrane (TOM) complex before being proteolytically processed and released back to the cytoplasm for subsequent clearance. However, upon loss of the inner mitochondrial membrane (IMM) potential, PINK1 accumulates at the outer membrane, recruiting the E3 ubiquitin ligase Parkin for the covalent tagging of impaired mitochondria for clearance by mitophagy. Although PINK1 and Parkin have been identified as key players in the mitophagy pathway, a molecular understanding of PINK1 processing, activation, and subsequent Parkin recruitment remains elusive. Using single-particle cryo-electron microscopy (cryoEM), my project aims to elucidate the molecular underpinnings of PINK1-mediated mitophagy. By obtaining the first structure of *hs*PINK1, I will be able to interrogate the structural features necessary for PINK1 activation and regulation. My project will further explore how disease mutants structurally alter *hs*PINK1 and how these deficiencies coincide with deficiencies in the mitophagy pathway.

Poster #9

Abstract: Evaluation of stabilizing mutations in the SARS-CoV-2 spike (S) protein

Xandra Nuqui
Amaro Lab



The SARS-CoV-2 spike (S) protein is a key target for antibody-mediated SARS-CoV-2 neutralization and is the major antigenic component of vaccines. These vaccines produce a strong response against the S1 subunit. Unfortunately, S1 is under significant immune-selective pressure resulting in deletions and substitutions that impact neutralizing antibody binding. In contrast, the S2 subunit is the most conserved region of the spike and is capable of eliciting antibodies that bind to diverse β-coronavirus spikes. However, a major challenge to using S2 immunogens as a potential vaccine strategy is the general instability of S2 in the absence of S1. In this study, we evaluate the effects of distal mutations that stabilize antigenic conformations of S2. We use all-atom molecular dynamics (MD) simulations, a variety of computational tools, and dynamical network analysis to characterize protein dynamics and the correlated motions of residue networks. Examining the dynamics of stabilized conformations may provide valuable insight into the principles underlying site-specific allosteric stabilization, informing design strategies for future SARS-CoV-2 vaccines.

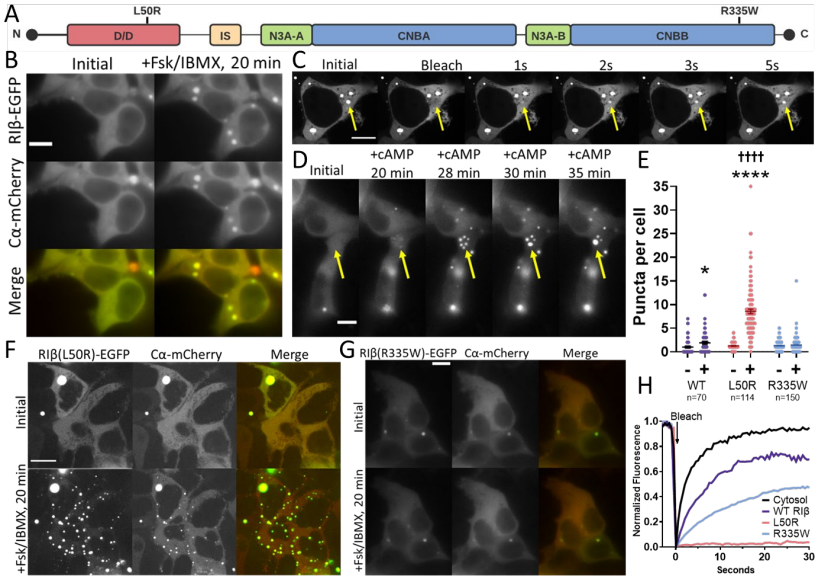
Poster #10

Characterization and molecular analysis of PKA RIβ liquid-liquid phase separation

Emily Pool

Zhang Lab and Taylor Lab

Cyclic AMP (cAMP) is a ubiquitous second messenger which regulates many cellular processes including cell growth, survival, metabolism, proliferation, and mobility. Given its critical role in so many processes, how does cAMP signaling achieve specificity? We previously showed that PKA regulatory (R) subunit isoform RIα undergoes liquid-liquid phase separation (LLPS), forming RIα bodies that dynamically buffer cAMP, enabling phosphodiesterases to maintain cAMP compartments that are essential for the signal specificity of the cAMP/PKA pathway. There are three other PKA R-subunit isoforms with distinct expression patterns and functions, and their contributions to cAMP compartmentation is not known. Here we use live-cell imaging approaches to show that RIβ, a neuronally expressed R-subunit, forms liquid-like phase separated bodies in cells. To evaluate the molecular basis of RIβ LLPS, we generated several RIβ mutants and tested their ability to form phase-separated puncta when expressed in cells under both basal and cAMP-elevating conditions. Our data indicates RIβ dimerization is required for LLPS and reveals the intrinsically disordered linker region plays a role in promoting RIβ solubility. Dementia-related disease mutations, L50R and R335W, alter the properties of RIβ LLPS. We show that upon cAMP stimulation, RIβ L50R forms more puncta which are less liquid-like than the wild-type, reminiscent of the assemblies observed in neurodegenerative disease. Our data indicate RIβ R335W retains its phase separation ability but lacks the cAMP-stimulated dynamics seen with wild-type RIβ. We propose that the R335W variant cannot bind cAMP, preventing dissociation of the catalytic subunit from the PKA holoenzyme. This work establishes that RIβ undergoes LLPS and describes the molecular basis for this property. The data suggest an unexplored mode of neuronal cAMP/PKA signaling regulation through RIβ LLPS. Future work will explore how dysregulated RIβ LLPS affects cAMP/PKA signaling in dementia, focusing on the L50R and R335W mutants.



RIβ-EGFP forms phase-separated condensates in HEK293 cells. (A) Domain architecture of RIβ. (B) RIβ-EGFP forms puncta following cAMP stimulation with Fsk/IBMX, and RIβ-EGFP and Cα-mCherry co-localize in puncta. RIβ-EGFP and Cα-mCherry were co-overexpressed in RIα KO HEK293 cells. (C) RIβ-EGFP puncta recover after photobleaching. (D) RIβ puncta fuse over time. (E) The number of RIβ-EGFP puncta per cell increases following cAMP stimulation (*), the number of RIβ(L50R) puncta increases after treatment (****), and the R335W variant does not respond to cAMP stimulation. The number of L50R puncta after cAMP stimulation was greater than the wild-type of the same condition (††††). Statistics calculated by unpaired Student's t-test with Welch's Correction. RIβ-EGFP and Cα-mCherry were co-overexpressed in HEK293 cells, stimulated with Fsk/IBMX for 20 minutes, and puncta counted per cell before (-) and after (+) Fsk/IBMX. (F) RIβ(L50R)-EGFP forms co-localized puncta after Fsk/IBMX. (G) RIβ(R335W)-EGFP does not form additional puncta following Fsk/IBMX stimulation. (H) Representative FRAP curves for wild-type, L50R, and R335W puncta compared to the bulk cytosol. For the L50R variant, RIβ(L50R)-EGFP was co-expressed with Cα-mCherry, puncta formation stimulated with Fsk/IBMX, puncta bleached, and recovery monitored. Scale bars = 10 μm.

Poster #11

Probing the phage λ packaging motor's grip on DNA with optical tweezers and rapid solution exchange

Brandon Rawson – Smith Lab, Department of Physics, University of California San Diego

Many dsDNA viruses, including numerous bacteriophages, utilize powerful ATP-fueled motors that repeatedly grip and translocate DNA to package viral genomes into pre-assembled capsids. Here we report preliminary results of single-molecule optical tweezers measurements of DNA gripping by the bacteriophage λ motor in different nucleotide conditions. Measurements were made by an improved rapid solution exchange technique using a laminar, dual-solution flow cell. Similar to the behavior observed previously for the phage T4 motor, binding of g-S-ATP causes near continuous DNA gripping and ADP causes highly intermittent gripping. Occasional slips occurring with g-S-ATP again notably have slow transient velocity, suggesting that multiple non-gripping subunits exert friction on the DNA to limit the slip rate. Unexpectedly, unlike the behavior observed with T4, there is significant pausing during DNA exit in the absence of nucleotides even at low capsid filling. Pauses having an average duration of 2 s occur before rapid slipping resumes. Possible explanations are transient gripping of DNA by the motor even in the absence of ATP or transient jamming/entanglement of the tightly confined DNA inside the capsid or portal-motor channel. Irrespective of nucleotide condition, λ, like other phage, ceases slipping when the end of the DNA is close to exiting the capsid. The presence of this end-clamp mechanism makes the acquisition of substrate essentially irreversible, improving the efficacy of viral packaging.

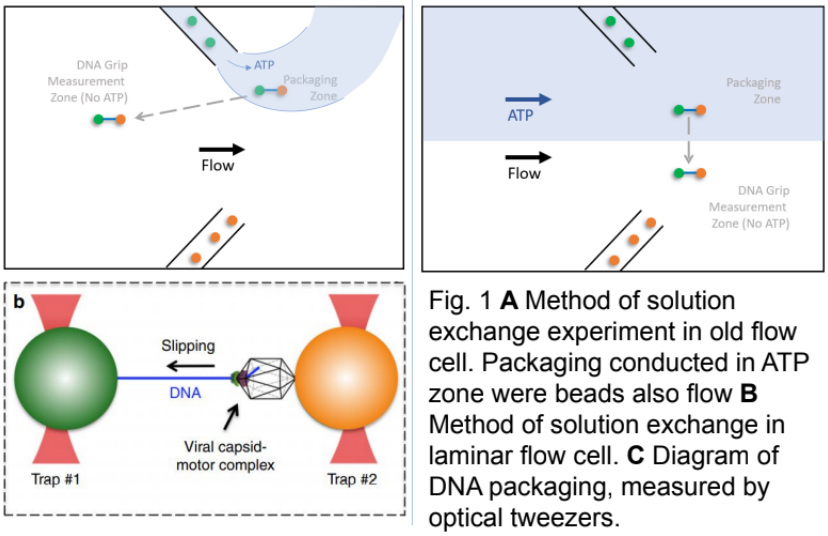
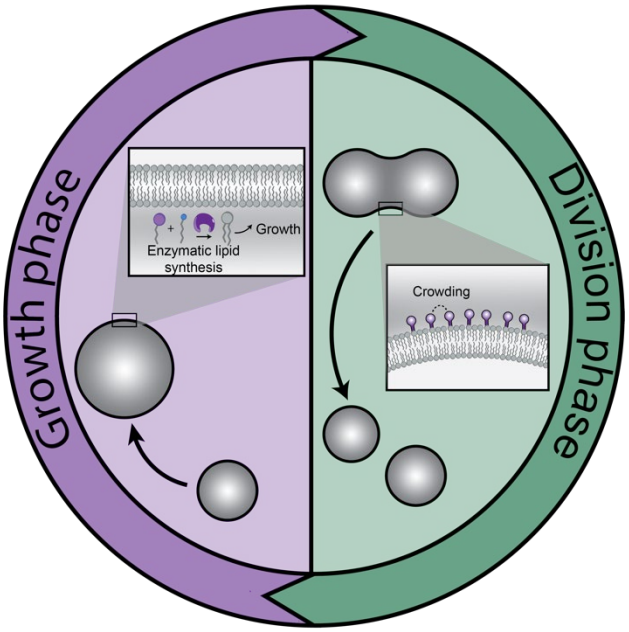


Fig. 1 **A** Method of solution exchange experiment in old flow cell. Packaging conducted in ATP zone were beads also flow **B** Method of solution exchange in laminar flow cell. **C** Diagram of DNA packaging, measured by optical tweezers.

Poster #12
Minimal biochemical methods for generating and propagating artificial cells
Jacob Vance
Devaraj Lab



Simple Artificial Cell Cycle

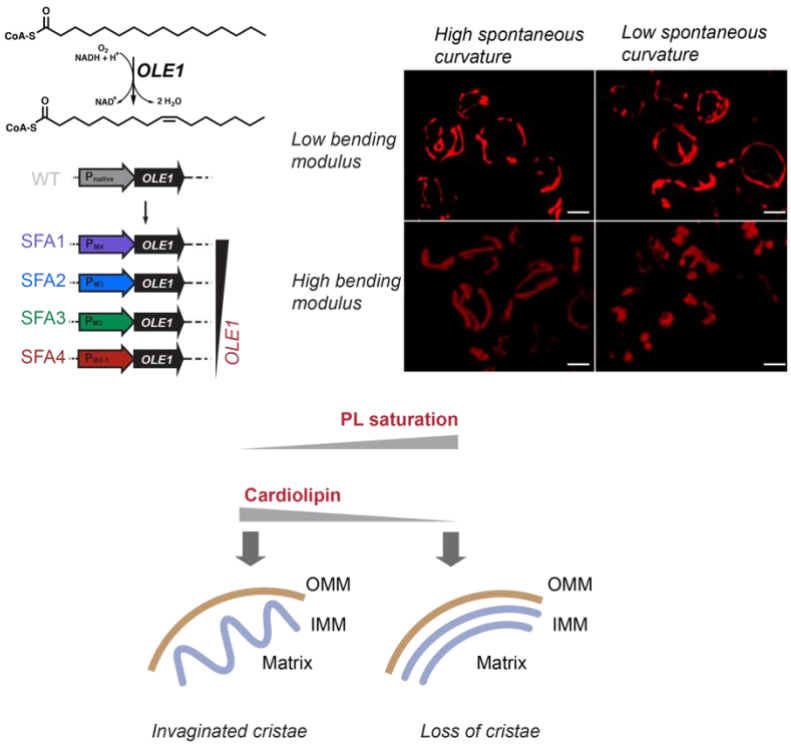
Abstract: The bottom-up construction of an artificial cell promises to revolutionize understanding of what life is and will lead to significant advances in multiple fields, from materials science to medicine. To realize the technical achievement of a dynamic, continuously propagating artificial cell, lipid bilayer-based compartmentalization is a likely requirement. Due to our inability to create dynamic and functional lipid-based compartments, the biochemical capabilities of current cell-mimics and cell-free reaction systems lag far behind those of living cells. State-of-the-art membrane reconstitution relies on rehydration of dried lipid films or microfluidic techniques to form lipid vesicles. Vesicles are then used in synthetic cell models merely as passive containers or scaffolds for reconstituted cellular machinery, making them poor approximations of cell membranes. In our lab, we have developed chemical methods to generate lipids *in situ*, leading to *de novo* formation of membranes that continuously grow and, at low efficiencies, undergo fission. My work will focus on harnessing the power of enzymes to generate membranes *in situ* at unprecedented efficiencies while modulating membrane lipid composition, leading to exquisite control of membrane curvature and shape. Specifically, I will use unconventional lipid-synthesis enzymes, such as the soluble lysophosphatidic acid acyltransferase Ict1 from *S. cerevisiae*, to produce membranes *in situ*. Preliminary data suggest that Ict1 can perform chemical transformations on lipid molecules *in vitro*. I will also utilize light-activated biochemical systems to control the shape of artificial cell membranes by taking advantage of known biophysical properties of membranes. Data from several groups, including our own, have suggested that protein crowding at the cell membrane can affect curvature and even lead to fission. I will therefore employ the engineered protein iLID (improved light-induced dimer), which binds the protein Nano (a variant of stringent starvation protein B) upon illumination with blue light and dissociates rapidly in the dark. We hypothesize that by immobilizing iLID on the surface of liposomes, we will be able to induce membrane crowding and subsequent deformation and promote fission of artificial cells by illumination with blue light in the presence of Nano-mCherry fusion proteins. Preliminary results have shown that liposomal membranes can be functionalized with iLID-GFP and used to recruit mCherry-Nano proteins with 420 nm light. We anticipate that these simple biochemical systems will be a significant advance over previous purely chemical methods used in our lab to generate dynamic lipid compartments for artificial cells. Ultimately, our aim is that these efforts will lead to a semi-autonomous artificial cell cycle, enabling the bottom-up design of self-replicating, self-contained reaction systems.

Poster #13

Dissecting the lipidic determinants of inner mitochondrial membrane mechanics

Kailash Venkatraman, Keunyoung Kim, Sebastien Phan, Guy Perkins, Guadalupe Garcia, Amalia Piscoli, Mark Ellisman, Padmini Rangamani, and Itay Budin*

Eukaryotic organelles are defined by membranes with unique and tightly regulated stoichiometries of different lipid building blocks. Lipids are thought to broadly control the morphology and function of membranes, but we lack specific physicochemical mechanisms by which they interact in the homeostasis of most organelles. Here we demonstrate how a data-derived continuum model can predict the compositional-dependence of membrane morphology in the mitochondria. The inner mitochondrial membrane (IMM) is folded into cristae, whose structure is central to respiratory function. Tuning phospholipid saturation in engineered budding yeast strains revealed a surprising breakpoint associated with a loss of cristae structure and a loss of mitochondrial respiration. To understand this phenomenon, we developed a mechanical model for cristae formation based on membrane bending energy. The model predicted epistasis between the membrane bending modulus, controlled by lipid saturation, and lipid spontaneous curvature, controlled by the synthesis of cardiolipin, a cone-shaped phospholipid enriched in the IMM. We tested this prediction through orthogonal manipulation of cardiolipin synthase (Crd1p) in yeast, whose deletion was previously described as having a minimal phenotype in optimal growth conditions. We found that Crd1p is essential for cristae assembly when membrane stiffness is increased, either genetically through *OLE1* modulation or by mild hypoxic conditions that are hallmarks of natural yeast environments. Taken together, these results show how IMM structure depends on multiple aspects of lipid chemistry, which then dictate mitochondrial morphology and function.



Poster #14
Structure and Function of ABC Transporters in Oceanic Species
Megan Young, Chang Lab

Abstract
The pollution of the world's oceans is a mounting problem that threatens the safety and well-being of our planet. Many marine organisms and ecosystems are exposed to pollutants with currently unknown toxicity and consequences. Membrane proteins that act as xenobiotic – foreign molecule – exporters are an important line of defense against pollutants in the organisms' environment. One of the most ubiquitous membrane proteins are the ATP binding cassette transporters (ABC). Found in organisms across all domains of life and in many tissue types, ABC transporters selectively export many different small molecules from within the cell, preventing the toxic accumulation of xenobiotics within the organism. Many of the small molecules exported by ABC transporters are pollutants. However, some small molecule pollutants may act as inhibitors of ABC transporters by interacting with their binding site and impeding transport. For example, a study conducted on one transporter – ABCB1 or P-glycoprotein – in mice found that transport activity was significantly reduced in the presence of DDD, DDE, and DDT (Nicklisch, 2016). In the case of ABC transporters in oceanic species, many reagents found polluting the ocean may inhibit ABC transporters' ability to export their wide range of substrates. This would lead to detrimental effects to the health of organisms in the oceanic food chain (e.g. yellowfin tuna) and to embryonic development (e.g. purple sea urchin). A thorough molecular and structural investigation of these transporters expressed in marine species is necessary to determine which chemicals inhibit transport. We propose to solve the structure(s) of ABC transporters to determine how they interact with substrates. We will also determine the binding constants of pollutants by calculating their IC_{50} values using established ATPase assays.

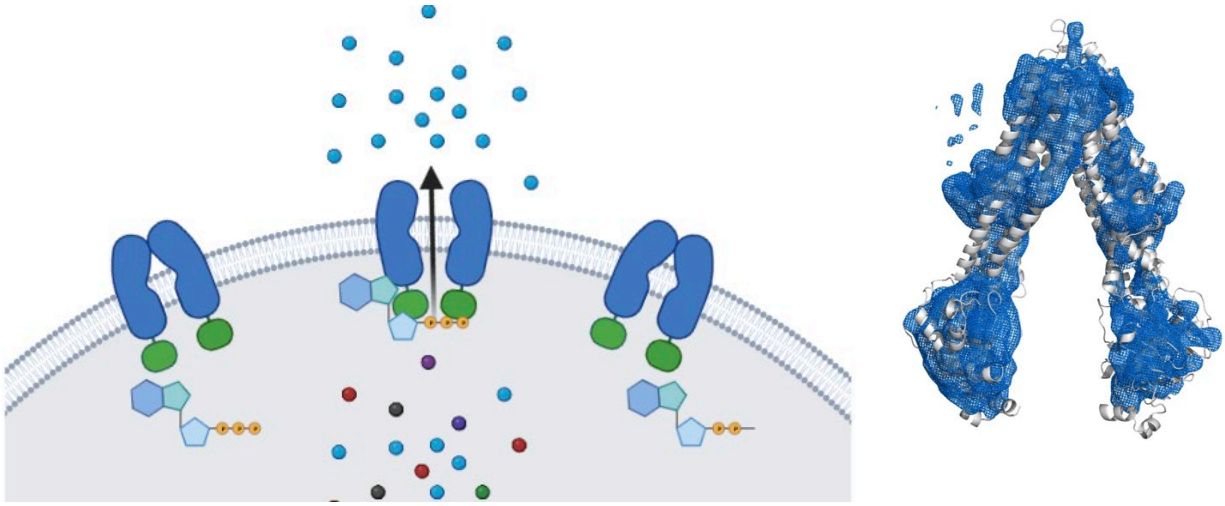


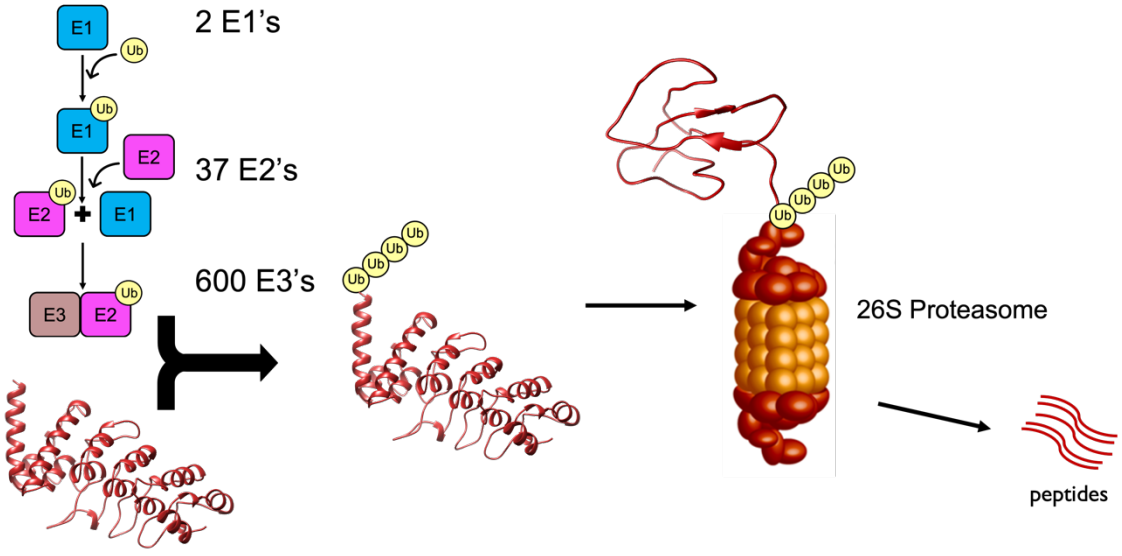
Figure: (Left) ABC transporters composed of a transmembrane domain and nucleotide binding domain hydrolyze ATP to catalyze transport of small molecules/xenobiotics out of the cell. (Right) Current cryo-EM data collected from sample of tuna ABCB1 (blue mesh) using model of mouse ABCB1 (white cartoon).

Poster #16

Identification and characterization of Cullin-Ring Ligase 5 as a novel histone E3 ligase

Calvin Lin, Komives Lab

The ubiquitin proteasome system serves as the primary means by which proteins are degraded within our cells. This process begins with the ubiquitylation cascade, involving a series of enzymatic reactions, that post-translationally modify proteins with ubiquitin. Ubiquitin can then form chains that are recognized by the proteasome causing substrate degradation. Ubiquitylation is carried out by 3 proteins; the activating enzyme (E1), conjugating enzyme (E2), and ubiquitin ligase (E3). A wide variety of E3s are present in cells, each recognizing different substrate proteins. The multi-subunit Cullin-RING ligase (CRLs) family is the largest class of E3 ligases and is comprised of 8 different Cullins that recruit an array of substrates through various substrate receptors. All CRLs share a similar architecture in which the Cullin scaffold and its bound ring box (Rbx) protein recruits the E2 and substrate. Substrates are recruited through Cullin-bound substrate receptor proteins via adaptor proteins. Our lab has shown that ubiquitylation of creatine kinase brain type (CKB) by CRL5 is a concerted effort by an 8-protein complex in which neddylation of Cul5 recruits ARIH2, an additional E3 ligase. I have now shown that CUL5 can ubiquitylate and interact with other putative substrates that were originally identified as substrate-receptor interacting proteins – namely monomeric histones. I have demonstrated ubiquitylation of histones *in vitro* and used mass spectrometry to show specifically which lysines are modified. I have also shown interaction between ligase and substrate through various binding assays. Together, these studies indicate that CRL5 interact with and ubiquitylate histones.



Lightning Talks

1) Kira Podolsky (Devaraj lab) “Assembling artificial cells and organelles”

2) Bryce Ackerman (Debelouchina lab) “An improved bio-resistant polarization agent for dynamic nuclear polarization”

3) Josh Arriola (Muller lab) “GTP synthesis by a catalytic RNA”

4) Adarsh Balaji (McHugh lab) “MALAT-1 lncRNA regulates TDP-43 splicing of SAT1 mRNA”

5) Aileen Button (McHugh lab) “Mechanistic investigation of RNA-protein complexes in human gene regulation”

6) Quinn Cowan (Komor lab) “Engineering a Multiplexed Orthogonal Base Editing System”

7) Mounir Fizari (Smith lab) "Intramolecular friction in viral DNA ejection".

8) Ximena Garcia-arceo (Zid lab) “Simulated Cerevisiae: Predictive modeling of mRNA localization in brewer's yeast.”

9) Alex Hoffnagle (Tezcan lab) “Metal-Directed Protein Docking for the Design of Metalloprotein Assemblies”

10) Evan Kobori (Taylor lab) "Network Analysis of the p90 RSK1:ERK2 complex"

11) Dominic McGrosso (Gonzalez lab) “Microproteins, Does Size REALLY matter?"

12) Elizabeth Porto (Komor lab) “Investigations of Non-Traditional Tools for Precise and Therapeutically Relevant Genome Engineering”

13) Christine Stephan (Mishanina lab) “Investigating the folding mechanism of a pH-responsive riboswitch in *E. coli”*

14) Bryce Timm (Godula lab) “An improved ELISA model for investigating endo-6-O-sulfatase activity and specificity”

15) Ryan Weeks (Zhang lab) “RasAR: A Novel Probe For Ras Activity Measurements”

16) Doug Zhang (Hermann Lab) “Metallation of nucleic acid nanostructures”
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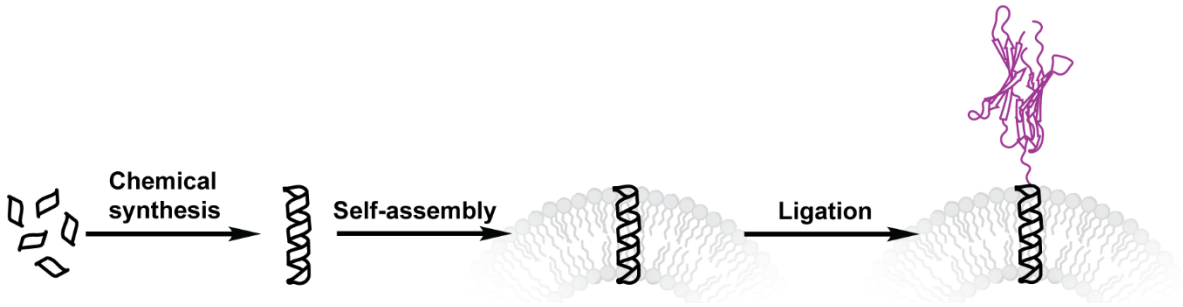
Lightning Talk #1
Assembling artificial cells and organelles

Kira Podolsky
Devaraj Lab

The bottom-up development of an artificial cell would provide a minimal system with which to understand the transition between non-living matter and life. As lipid membranes are necessary components to the emergence of life, I have explored different chemical approaches to making cell-mimetic compartments. Using synthetic strategies to drive membrane formation, including bioorthogonal ligations and dissipative self-assembly, I have recreated life-like cellular and organellar structures and functions. My current work has focused specifically on the development of a novel method for the construction of protein-incorporated lipid membranes. Cellular lipid membranes are embedded with transmembrane proteins crucial to cell function. Elucidating membrane proteins' diverse structures and biophysical mechanisms is increasingly necessary due to their growing prevalence as a therapeutic target and sheer ubiquity in cells. Most biophysical characterization strategies of transmembrane proteins rely on the tedious overexpression and isolation of recombinant proteins and their reconstitution in model phospholipid bilayers. Unfortunately, membrane protein reconstitution depends on the use of denaturing and unnatural detergents that can interfere with protein structure and function. I have developed a detergent-free method to reconstitute transmembrane proteins in model phospholipid vesicles. By utilizing split intein ligations, single-pass transmembrane proteins can be synthesized on liposomes. Split inteins are natural or engineered protein trans-splicing domains. By leveraging the biorthogonality and chemoselectivity of split inteins, overexpressed soluble domains are ligated to synthetic transmembrane peptides to build semisynthetic membrane proteins directly on phospholipid vesicles. This one-pot method bypasses the painstaking expression of recombinantly expressed integral membrane proteins and the multistep process of detergent-based protein reconstitution, making it easier to study these important biomolecules in an isolated system.



Cartoon schematic of transmembrane protein semisynthesis in phospholipid membranes from synthetic (black) and expressed (magenta) components.

Lightning Talk #2
An improved bio-resistant polarization agent for dynamic nuclear polarization
Bryce Ackermann, Debelouchina lab

The increasingly recognized influence of the cellular environment on structure, dynamics, and interactions of macromolecules has driven the field of NMR into direct observation of biological samples. NMR coupled with dynamic nuclear polarization (DNP) is a promising route to enhance the NMR signal of intracellular protein which function in the nano to micromolar range. Several successful in-cell DNP projects have showcased exceptional signal enhancement (Narasimhan et al.), multidimensional protein NMR(Narasimhan et al.), preservation of cell integrity (Overall and Barnes, 2021; Xiao et al., 2022), and are starting to tackle biological questions (Narasimhan et al.; Overall et al., 2020). One limitation to these endeavors is the DNP requirement for high concentrations of biradical molecules, or polarization agents. Polarization agents are heavily quenched by the reducing environment of the cellular milieu, greatly dulling the effect of DNP. Our lab has taken the approach of developing new polarization agents with greater resistance to the reducing agents of the cell.

Building from the prior literature of nitroxide stability, we have constructed a polarization agent that uses 5-member ring nitroxides. We have compared the *in vitro* and HEK cell lysate reduction rates between our new polarization agent, M5-TOTAPOL, and other commercial polarization agents, revealing M5-TOTAPOL to perform the best. We have also used HPLC to compare the cell entry rates of each polarization agent, as the ability to enter cells is required for in-cell DNP. Finally, we have compared the actual DNP performance of each polarization at different timepoints along HEK cell incubation, thus gauging the effect of bio-resistant on true signal enhancement. While M5-TOTAPOL is only marginally better than similar polarization agents, it serves as a signal towards a viable future design avenue.

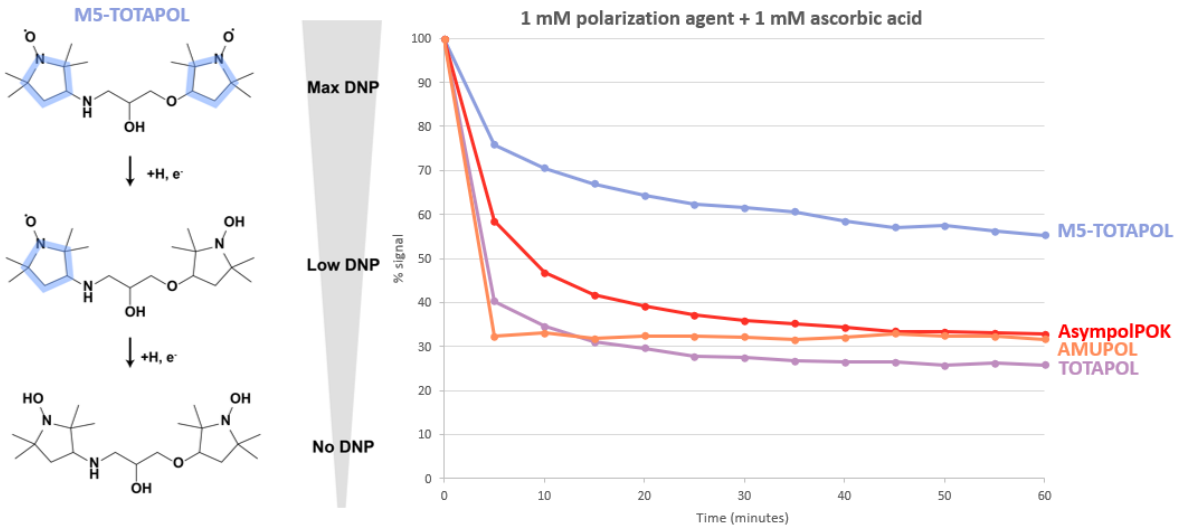


Figure 1: Reduction profile of M5-TOTAPOL compared to commercially available biradicals. Diagram (left) shows how biradical polarization agents become ineffective upon reduction. Graph (right) depicts the reduction rate by ascorbic acid of common polarization agents.

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Lightning Talk #3

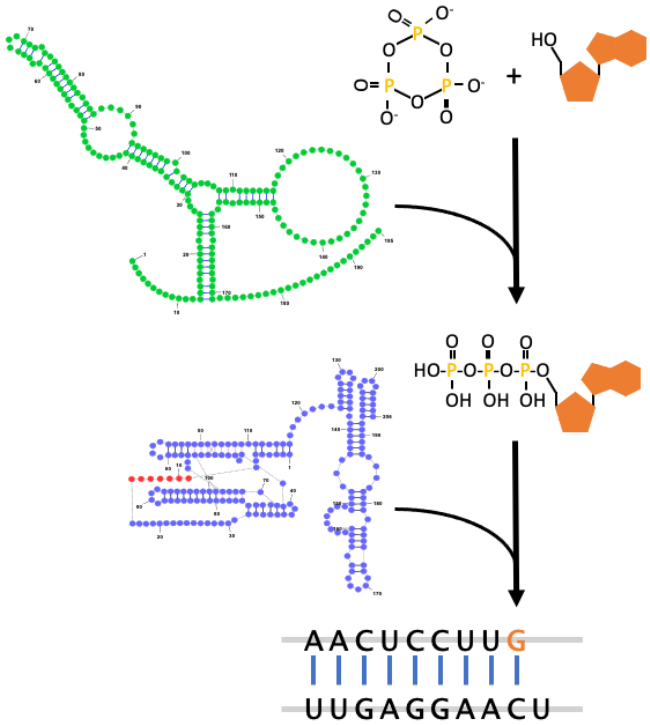
GTP synthesis by a catalytic RNA

Arvin Akoopie[1], Joshua T. Arriola[1], and Ulrich F. Müller[1],
[1]Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0340.

An early stage of life likely used catalytic RNAs (ribozymes) to support self-replication and a metabolism. Like in today's organisms, nucleoside 5'-triphosphates (NTPs) may have been used as central building blocks for self-replication and in metabolism. In early stages of life, NTPs could have been generated by reacting nucleoside 5'-hydroxyl groups with the prebiotically plausible molecule cyclic trimetaphosphate (cTmp) (Etaix & Orgel 1978; Pasek et al. 2013; Pasek et al. 2008). We previously showed that this chemistry can be catalyzed by ribozymes (Moretti & Muller 2014) but these ribozymes generated only RNA 5'-triphosphates and not free NTPs.

To identify ribozymes that generate free NTPs from free nucleosides and Tmp we set up an in vitro selection system in emulsion. Pool molecules with a randomized sequence of 150 nucleotides were incubated with cTmp and the thio-modified nucleotide 6-thio guanosine (6sGsn). Pool molecules catalyzing nucleoside triphosphorylation generated the corresponding 6-thio guanosine triphosphate (6sGTP). The 6sGTP in turn was ligated to the 3'-terminus of successful pool molecules by a polymerase ribozyme variant (Akoopie & Muller 2018). After 13 rounds of selection, pool activity increased 1,500-fold. The selected pools were analyzed by high throughput sequencing analysis.

Individual sequences from the most highly enriched sequence clusters were analyzed biochemically. These sequences were challenged to synthesize GTP from free guanosine and cTmp. The sequence with the strongest average signal was chosen for further analysis. Ribozyme mediated GTP formation was confirmed by LC-MS. Chemical probing was used to determine the secondary structure of the ribozyme. Under optimal reaction conditions, the catalytic rate enhancement of the ribozyme was 18,000-fold, with a turnover number of 1.7. We are currently working to increase the turnover number. GTP generated by the ribozyme could be incorporated into an RNA polymer using an RNA polymerase ribozyme, thereby coupling ribozyme-mediated synthesis of GTP to its use in ribozyme-mediated polymerization, and establishing a minimal metabolic system (Akoopie et al. 2021).



Metabolic coupling of a guanosine triphosphorylation ribozyme (GTR) to an RNA polymerase ribozyme. The GTR (green) identified in this study could generate GTP from cTmp (yellow) and guanosine (orange). GTP generated by the GTR could then be incorporated into an RNA polymer by an RNA polymerase ribozyme (blue, red).

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Lightning Talk #4

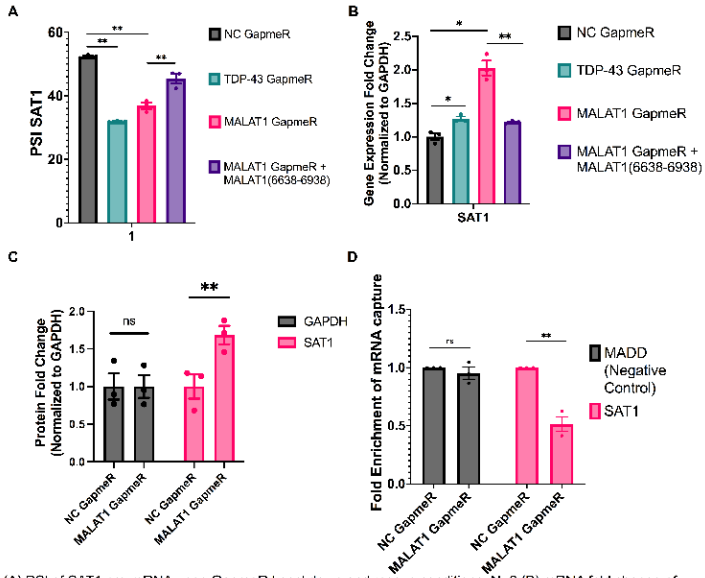
MALAT-1 lncRNA regulates TDP-43 splicing of SAT1 mRNA

Adarsh Balaji and Colleen A. McHugh, PhD.

The accumulation of aberrant protein splicing factors contributes to neurodegenerative diseases including frontotemporal dementia (FTD) in humans. The binding of splicing factor TDP-43 to UG-rich RNAs has been shown to mediate protein stability, preventing aggregation and disease phenotypes. The McHugh Lab has identified the long non-coding RNA MALAT-1 as a direct binder of TDP-43 in human cells using RNA antisense hybridization capture. One of the most significant increases in TDP-43 protein-RNA binding in FTD patient samples compared to control brain samples is to the long non-coding RNA MALAT-1. We analyzed crosslinking and immunoprecipitation (CLIP) data from the ENCODE project to identify a 300-nucleotide binding region of TDP-43 protein on the MALAT-1 lncRNA (MALAT-$1_{6638\text{-}6938}$). However, the details of these molecular interactions and the function of this complex have not yet been investigated. Understanding the contribution of MALAT-1 to TDP-43 splicing may lead to new treatments for neurodegenerative diseases.

We hypothesized that the MALAT-1/TDP-43 complex is involved in splicing regulation, and that the binding of MALAT-1 to TDP-43 may directly influence the ability of TDP-43 to splice target mRNAs. To identify candidate mRNA targets, we re-analyzed previous RNA-sequencing and microarray data to reveal six spliced genes regulated by both MALAT-1 and TDP-43. We focused our studies on the splicing of the Spermidine/Spermine N1-Acetyltransferase 1 (SAT1) gene, which encodes a rate-limiting enzyme involved in the acetylation of polyamines. Polyamines are a biomarker for neurodegenerative diseases, and their maintenance by SAT1 prevents the activation of autophagy pathways. Alternative splicing of SAT1 results in the inclusion of Exon X containing three premature stop codons, resulting in mRNA degradation through nonsense-mediated decay (NMD).

We performed splicing assays to determine the ratio of the inclusion product to both inclusion and exclusion products, expressed as percent of splice inclusion (PSI). A higher PSI indicates increased inclusion of the NMD-inducing Exon X. MALAT-1 lncRNA and TDP-43 mRNA transcripts were targeted with specific antisense LNA GapmeRs, and knockdown of both MALAT-1 and TDP-43 resulted in reduced PSI **(Fig 1A)**. MALAT-1_{KD} effects were rescued by ectopic expression of MALAT-$1_{6638\text{-}6938}$, highlighting the role of this RNA region in modulating TDP-43 splicing activity. These PSI changes were reflected in SAT1 mRNA expression levels, with increased mRNA levels in knockdown conditions and reduced mRNA levels in the rescue condition **(Fig. 1B)**. Increased mRNA expression in MALAT-1_{KD} resulted in increased SAT1 protein levels **(Fig. 1C)** which is of particular interest due to the role of SAT1 in maintaining steady-state levels of polyamines. As SAT1 is a known splicing target of TDP-43, and TDP-43 splicing interactions seem to be driven by physical interactions with target pre-mRNA, we believe that MALAT-1 may regulate TDP-43 binding to SAT1 by acting as a scaffold for mediating TDP-43/RNA interactions. We tested this hypothesis through IP pulldown of TDP-43 complexes and analyzed the fold enrichment of RNA capture of SAT1 compared to negative controls. MALAT-1_{KD} reduced TDP-43 binding to SAT1 **(Fig. 1D)**, which supported our hypothesis of MALAT-1 acting as a mediator for TDP-43 binding.



(A) PSI of SAT1 pre-mRNA upon GapmeR knockdown and rescue conditions. N=6 (B) mRNA fold change of SAT1 upon GapmeR knockdown and rescue conditions. N =3 (C) Protein fold change of SAT1 normalized to GAPDH in NC and MALAT-1 GapmeR conditions. N=3(D) Change in percentage of SAT1 mRNA capture by TDP-43 in IP with NC and MALAT-1 GapmeR conditions. N=3. All data are plotted with SEM error bars. * = $p<0.05$, ** = $p<0.01$.

We are currently investigating the biological relevance of the MALAT-1/TDP-43 complex in the neuronal cell line SH-SY5Y. We are also evaluating the changes in complex formation between TDP-43, MALAT-1, and target mRNAs using sucrose density ultracentrifugation. Determining the functional relevance of this complex is important, given the aberrant expression of MALAT-1 and TDP-43 in disease phenotypes. The information gained from these studies will provide a foundation for the design of antisense oligonucleotides or small molecules, which may have therapeutic effects in neurodegenerative diseases by regulating the interaction of MALAT-1 and TDP-43.

Lightning Talk #5

Mechanistic investigation of RNA-protein complexes in human gene regulation

Aileen C. Button and Colleen A. McHugh, Ph.D.

Long non-coding RNA (lncRNA) and protein interactions play important roles in cellular function, particularly in gene regulation and expression. LncRNA and their protein partners can change gene expression through a number of different pathways including altering histone modifications and recruiting transcription factors. However, more detailed characterization of these complexes is needed to determine the molecular basis of their interactions, and to understand how these molecules achieve specificity in the complex cellular environment. A major challenge in the field is that we still do not understand the rules governing lncRNA-protein interactions and how these lead to cellular phenotypes. To address this question, I am studying the interaction between the lncRNA XIST (X-inactive specific transcript) and the transcriptional repressor SHARP (SMART/HDAC1-Associated Repressor Protein), which is responsible for initiating X chromosome inactivation during early development.

The archetypical long non-coding RNA in humans is XIST that functions to silence one entire X chromosome in female development. Its interaction with SHARP was recently identified by RAP-MS (RNA affinity purification with mass spectrometry) and has been shown to be required for X chromosome inactivation in female mammals, including humans. Interestingly, SHARP also binds another lncRNA, the steroid receptor RNA activator (SRA). Furthermore, SHARP contains four RNA recognition motifs (RRM), which are well-established domains for RNA binding. For these reasons, the XIST-SHARP and SRA-SHARP interactions are an excellent gateway into understanding what makes a lncRNA-protein interaction specific.

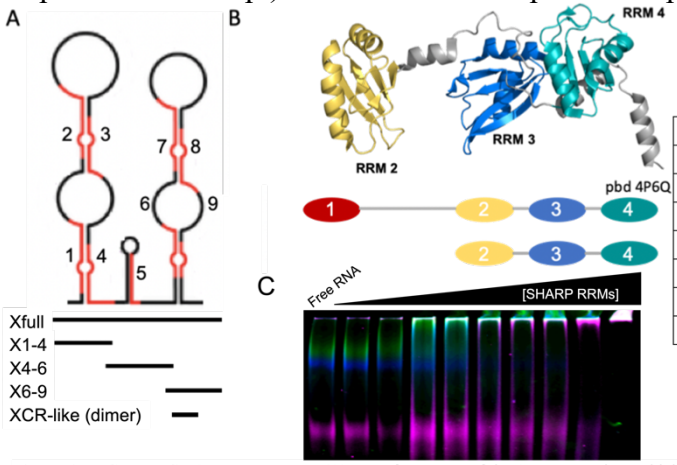


Table 2: Krel of XIST constructs with SHARP RRM 1-4

	K_{rel} (nM)
XCR-like	320
X1-4	360
X4-6	270
X6-9	270
X_full	250
SRA	270
tRNA	520

Figure 1: XIST and SHARP constructs. (A) Four fragments of the A repeat region, which contains 8.5 repeats in total, have been designed: full length (Xfull), repeats 1 to 4 (X1-4), repeats 4-6 (X4-6), and repeats 6-9 (X6-9). **(B)** I have made two constructs of SHARP: one containing all four RRMS and one with RRMs 2-4. **(C)** mEMSA of X full, SRA, XCR-like with SHARP RRM 1-4.

While it is known that SHARP binds in the A repeat region of XIST no precise binding site has been identified. Many potential structures of the region have been proposed by NMR or secondary structure probing, but no studies have examined protein-bound RNA. To narrow down specific binding sites, I have tested the binding of SHARP to several fragments of the A repeat region of XIST using the multiplex EMSA method I created with former lab technician Ellen Lavorando. These XIST fragments were designed from structural units predicted from secondary probing of the A repeat region as well as from a proposed minimal binding region consisting of a dimer of repeats (Fig. 1A). I have also produced two different SHARP constructs (Fig. 1B). The RRM2-4 construct has been used throughout the literature and I have now characterized the previously unstudied RRM1 of SHARP. The purified RRM1-4 protein appears to bind its target RNA more tightly than the previously studied proteins. This construct is likely more biologically relevant since it includes all known RRM domains of SHARP. For these studies, I used SRA, an established target of SHARP as a positive control and tRNA-Cys to examine the strength of nonspecific interactions (Fig. 1C and 1D).

For my next steps, I plan to examine both the bound and unbound RNA structures *in vitro*, using SHAPE-MaP and have begun working with the IGM core at UCSD on examining differences in the structure of the full A repeat region bound to each SHARP construct and without protein. I am also working with Ethan Ashley, an undergraduate student in our lab, on mutational studies wherein I perform alanine scanning on the putative binding residues of SHARP (i.e., the aromatic residues involved with base stacking).

As for the molecular details of the XIST-SHARP complex, I have used my findings from the multiplex EMSAs (table 2) to find a minimal RNA sequence (XCR-like) that can be used for cryo-electron microscopy. I have also tried the X6-9 construct which shows some of the tightest binding to SHARP (table 2). I am currently focusing on the SHARP RRM 1-4 construct as this shows the tightest binding *in vitro*. I have seen promising results in negative stain, and I am now optimizing conditions for cryo-EM. Carbon grids and crosslinking have been successful for other similar complexes and show promising initial results with low resolution (~10 Å) reconstruction.

Engineering a Multiplexed Orthogonal Base Editor System

Although advances in technology have made human genome sequencing increasingly accessible, our inability to interpret consequences of genetic variants remains a major obstacles to scientific progress.[1] As single nucleotide variants (SNVs) account for 55% of human pathogenic variation, the development of programmable tools to efficiently install multiple point mutations would be transformative for modelling and functionally characterizing genetic variation in human disease.[2,3] New precision genome editing tools have been engineered that avoid generating DSBs, including base editors (BEs). Two classes of base editors have been developed that use cytosine and adenine deamination chemistries to catalyze the conversion of C•G base pairs to T•A (CBEs), and A•T base pairs to G•C (ABEs), respectively.[4,5] Base editors are uniquely situated to enable multiplexed genome editing as they have high efficiencies and low byproduct formation. However, attempts to multiplex CBEs and ABEs via nucleic acid-mediated delivery (i.e. plasmid transfection or viral transduction) result in gRNA "crosstalk." The degree of separation between the nucleobase-specific modifier and the gRNA (encoding the genomic locus to be converted) is responsible for the lack of orthogonality in the current system.

Utilizing RNA aptamer technologies, we have recruited the DNA modifier directly to its gRNA via an aptamer-binding protein interaction, which provides a modular system to fix the crosstalk issue Structures of the Cas9:gRNA complex show that the tetraloop, stem-loop 2 (SL2), and 3'end of the gRNA physically protrude from the complex and tolerate additional sequences without affecting RNA stability or DNA binding.[6] We generated constructs where an aptamer is embedded within the gRNA at these locations, and the complimentary coat protein (CP) fused to either the CBE or ABE modifier (Fig. 1A). Initial experiments showed that aptamer-recruited base editors could install targeted point mutations, but with lower editing efficiencies than their covalently fused counterparts (established base editors). To increase editing, we chose to focus on poorly edited genomic sites and aptamer fusion locations closest in space to the target nucleobase, toward the 3'end of the gRNA. We employed recently described "next-generation" modifier enzymes with faster deamination kinetics: evo-APOBEC1 for CBE and TadA-8e/-8.20 for ABE.[7–9] Because the MS2 aptamer had precedence for cytosine deaminase recruitment,[10] we also modulated the aptamer-ABE identity between three orthogonal systems (PP7, boxB, and Com) while holding the corresponding CP-modifier architecture (N-terminal CP) and linker length (93aa) constant. Editing efficiencies dramatically improved with evolved TadA-8 deaminases and the Com aptamer fused to the 3'-end of the gRNA, and these constructs were selected for further optimization.

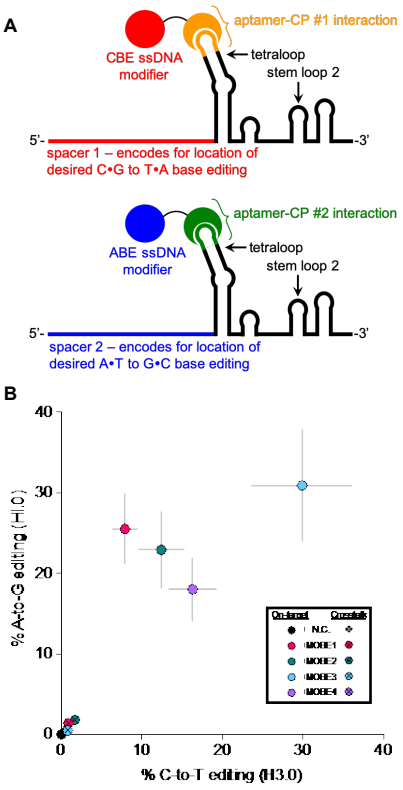


Figure 1. A) Overview of aptamer-based strategy for MOBE system. B) Mean editing efficiency of MOBEs at distinct genomic targets: ABE on the Y-axis and CBE on the X-axis (n=3).

To optimize aptamer-BEs architecture, we created constructs with the CP fused to either the N- or C-terminus of the deaminase with flexible linkers (93aa or 32aa) taken from established base editors.[11,12] Between all CP-modifiers tested for the ABE system (with 3'-Com aptamer gRNA), two repeatably showed higher A-to-G editing across genomic sites and were selected for characterization. For aptamer-CBE system, we tested similar variations of CP-modifiers and an additional MS2 aptamer-gRNA with stem-loop 3 (SL3) fusion from a recent study.[13] Unexpectedly, this new SL3 fusion yielded the highest C-to-T editing at certain sites, while the more established 3' fusion outperformed it at others. Each of the selected aptamer-BEs were tested at 16 genomic targets and analyzed for editing efficiency, editing window size, and sequence preference.

Finally, combining the each of the best aptamer-ABEs/-CBEs, we created four multiplexed orthogonal base editing (MOBE) systems and simultaneously targeted genomic sites. MOBEs demonstrate decent levels of on-target editing while avoiding almost all crosstalk. MOBE3 show the highest activity across multiple target site combinations (Fig. 1B). We are currently working to analyze these data for co-occurring edits (made in the same cell). We have also developed a reporter plasmid that expresses a 2x-dead GFP, harboring two point mutations, that requires correction by both aptamer-BEs to fluoresce and the gRNA-aptamer fusions required for GFP editing. This plasmid can be used to quantify simultaneous edits by the OBEs or to enrich for cells with high editing when co-transfected with genomic targeting gRNAs. Due to the relatively modest level of OBE activity, this enrichment strategy will be crucial at difficult to edit targets or cell types. In summary, by recruiting the nucleobase-specific modifier directly to each genomic site, multiplexed orthogonal base editing can enable the functional investigation of disease-relevant genetic variation.

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Lightning Talk #7
Intramolecular friction in viral DNA ejection
Mounir Fizari (Smith lab)

Many viruses use molecular motors to package DNA to extremely high density. Large forces resist packaging - arising from DNA electrostatic self-repulsion, bending rigidity, and entropy loss – but help drive DNA ejection into the host cell. Single molecule fluorescence measurements of DNA ejection in T5 and lambda found a non-monotonic filing dependence of ejection velocity, explained by competition between changing mobility and internal force. Gabashvili and Grosberg hypothesized that the reduced mobility is due to hydrodynamic friction forces that have a Stokes Law-type linear dependence on velocity, stemming from a reptation model. However, given the extremely tight DNA packing, it is unclear if bulk hydrodynamic theory is valid and if "solid-like" sliding friction between DNA segments could play a role.

To investigate the physical origin of the reduced mobility in packaged viral DNA, we conducted experiments to probe the mobility of tightly confined DNA in phi29 proheads by pulling out the DNA in using optical tweezers. In this experiment, DNA is pulled out of a fully packed prohead in the absence of ATP (Fig 1A).Although the pressure in the prohead is highest at high filling, the exit dynamics are the slowest, and the velocity increases with decreasing filling. This effect demonstrates that the mobility of the confined DNA is the lowest when it is tightly packed. Additionally, complexes exhibit stochastic pausing during DNA exit, reminiscent of jamming events predicted by MD simulations. Additionally, we conducted measurements with increased charge screening and found slowed ejection and increased pause occurrence, in agreement with the known effects of divalent cations on DNA arrays (Fig 1B, 1C).

To test the hypothesis that the source of friction is hydrodynamic in origin, we preformed measurements at a higher applied force. If friction were linear in velocity, the ratio of exit velocities with high and low applied force would be equal to the ratio of driving forces. Using the known internal force, I calculated these ratios and found that that the frictional force scales less than linearly with the velocity (Fig 1D). This result is explained by previous studies of friction at the atomic scale, where a 1D model that incorporates discrete thermally activated hopping of monomers between periodic potential wells predicts a logarithmic dependence of the frictional force on the velocity, which has been experimentally validated for sliding biomolecules (actin filaments) under osmotic compression.

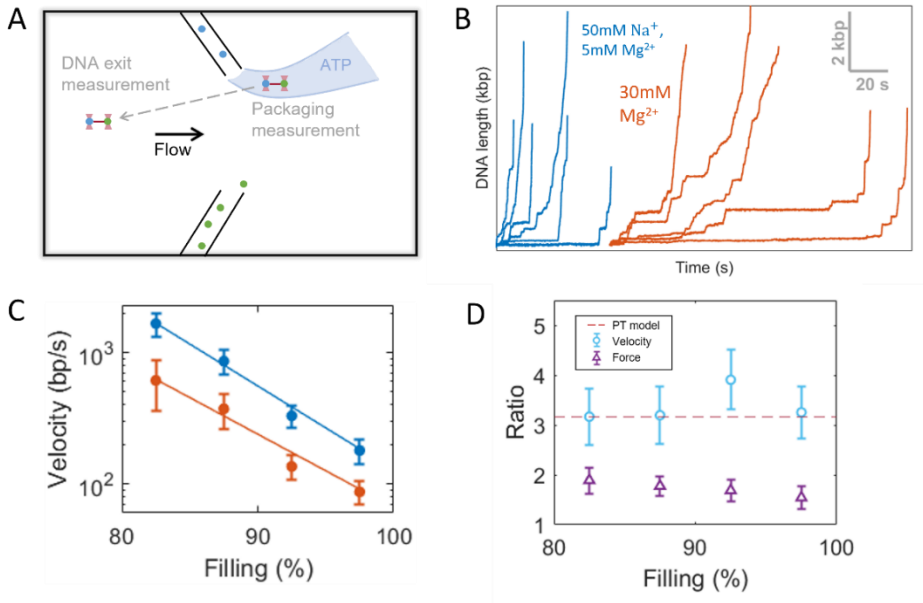


Figure 1: (A): Experimental schematic (B): Raw DNA exit trajectories in high and low screening conditions (C): Comparison of exit velocity in high and low screening (D): Comparison of ratio of exit velocities to ejection forces in high and low applied force, with the theoretical prediction marked (for monomer spacing of 0.34 nm).

Lightning Talk #8

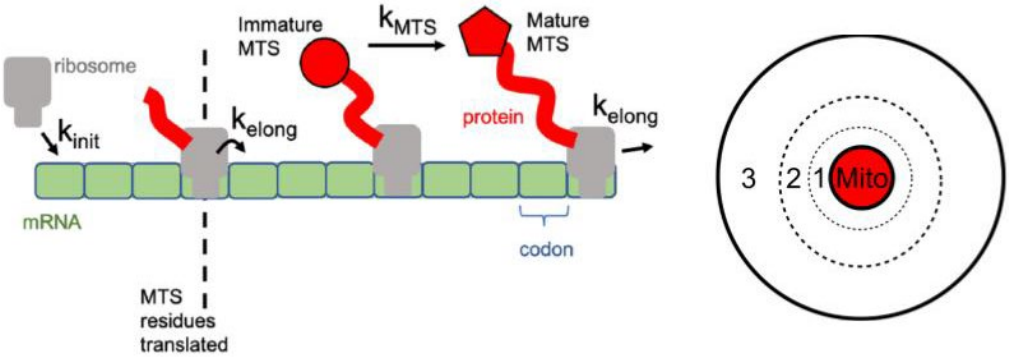
Title: Simulated Cerevisiae: Quantitative modeling of mRNA localization in brewer's yeast

Trainee: Ximena Garcia Arceo

P.I.: Brian Zid

Abstract:

Mitochondria are dynamic organelles that must precisely control their protein composition according to cellular energy demand. One way in which gene expression can be modulated post-transcriptionally is through localization of nuclear-encoded mRNAs to the mitochondrial surface. For many mRNAs, mitochondrial localization is facilitated by co-translational association of a mitochondrial targeting sequence (MTS) on a nascent polypeptide with the mitochondrial import complex. While mRNA localization to the mitochondria has been known for almost 50 years, there are many unanswered questions on the mechanisms of control and the functional impact of this localization. We find that the localization of a subset of mRNAs can be dynamically regulated across metabolic states in yeast. mRNAs that are conditionally localized during respiratory conditions are enriched for TCA cycle and aerobic respiration functionality. Through both experimental perturbations and computational modeling, we find mRNA localization to the mitochondria to be a nonequilibrium process, with translation kinetics combining with diffusive search times to control localization, which are simulated according to our figure below. As yeast switch to respiratory metabolism, there is an increase in the fraction of the cytoplasm that is mitochondrial (red circle in the figure), decreasing diffusive search time, and increasing the localization of conditionally localized mRNAs. Through artificial tethering of reporter mRNAs to the mitochondrial surface we find that localization is sufficient to increase protein expression. Overall, our work points to a mechanism by which cells are able to use translation elongation and the geometric constraints of the cell to fine-tune mitochondria-specific gene expression through mRNA localization.



Lightning Talk #9
Metal-Directed Protein Docking for the Design of Metalloprotein Assemblies
Alexander M Hoffnagle, Tezcan lab

Metals can play key roles in stabilizing protein structures, but ensuring their proper incorporation is a challenge when a metalloprotein is overexpressed or expressed in a non-native cellular environment. For proteins with structural metal cofactors, stabilizing the apo form of the protein can improve the ease of its expression and expand its potential applications. I have used computational protein design tools to redesign cytochrome b_{562} (cyt b_{562}), which relies on the binding of its heme cofactor to achieve its proper fold, into a stable, heme-free protein with high structural similarity (Fig. 1A). The resulting protein, ApoCyt, features only four mutations and no metal-ligand or covalent bonds, yet has improved stability over cyt b_{562} and is nearly as stable as cyt cb_{562}, a covalently stabilized cytochrome variant. Mutagenesis studies and x-ray crystal structures reveal that the increase in stability is due to the computationally prescribed mutations, which stabilize the protein fold through a combination of hydrophobic packing interactions, hydrogen bonds, and cation-π interactions. Upon installation of the relevant mutations, ApoCyt is capable of assembling into a previously reported, cytochrome-based trimeric assembly, demonstrating that ApoCyt retains the structure and assembly properties of cyt b_{562}. The successful design of ApoCyt therefore enables further functional diversification of cytochrome-based assemblies and demonstrates that structural metal cofactors can be replaced by a small number of well-designed, non-covalent interactions.

With the ApoCyt protein building block in hand, I have developed a computational approach to designing protein assemblies with predefined metal binding sites. Our lab has developed the use of metal coordination to drive protein assembly, but the challenge of predicting protein-metal interactions limits the extent to which this approach can be used to design metal coordination environments. To address this challenge, I have used geometric parameters of metal binding sites to guide the docking of individual ApoCyt chains into oligomeric protein structures which position metal binding residues to satisfy those geometric parameters (Fig. 1B). The resulting oligomers can then be stabilized by the design of favorable non-covalent interactions along the protein interfaces. While the characterization of initial designs with a Zn-His_3 metal binding site is ongoing, the early results are promising. Of seven tested variants, two form stable trimers at 50-75 μM concentration (the minimum concentration that can be tested by analytical ultracentrifugation). These two variants both have high thermal stability and are capable of binding Zn(II) with sub-micromolar affinity. Furthermore, a crystal structure of one of the designs without metal reveals good structural similarity to the design model (Fig. 1C). These designs may be promising starting points for exploring metal-based enzymatic activity in *de novo* designed protein scaffolds, while the computational approach can be expanded to enable the design of protein binding sites for more complex metal clusters.

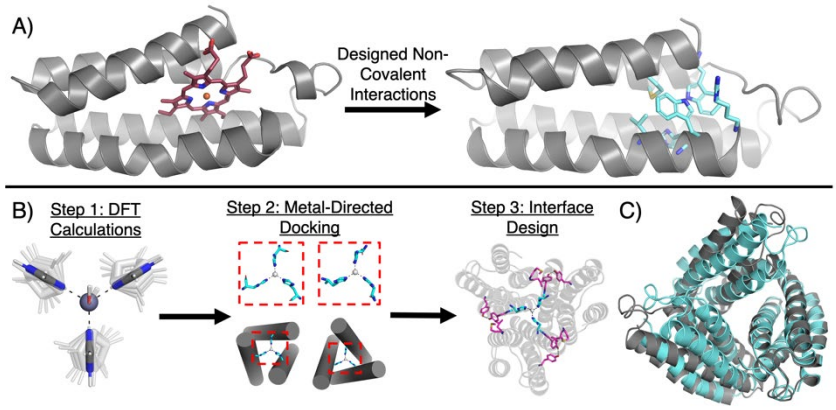


Fig. 1. A) Design and structures of ApoCyt (right) from cyt b_{562} (left). B) Computational metal-directed protein self-assembly. C) Crystal structure of a computationally designed assembly (gray) compared to the design model (cyan).

Lightning Talk #10
Network Analysis of the p90 RSK1:ERK2 complex
Evan Kobori, Taylor lab

Abstract

Protein protein interfaces play fundamental roles in a variety of biological processes. The study of these interfaces, and how can they be modulated either directly or allosterically remains of great interest. In this paper, we use molecular dynamics simulations, free energy perturbation, and a graph theory/network analysis approach to analyze the p90 RSK1 CTK-ERK2 heterodimeric kinase complex and better understand its interfaces and identify key residues that mediate communication between the two kinases. The CTK-ERK2 complex is primarily held together by two separate interfaces: between the C-terminal D-motif of the CTK and DRS binding site of ERK2, and the CTK APE/aF helix and the glycine rich loop of ERK2. Network analysis of the CTK-ERK2 complex enriched known, conserved residues necessary for catalysis and regulation, and identified known interface residues. This approach also predicts that the extended αF helix of the CTK is important, particularly R588 and E593. R588 is a cancer mutation site, while E593 is not. In silico mutation of R588 to cancer variants leucine caused the corresponding networks to have an increase in connections within the complex interfaces, suggesting that this mutation would enhance the stability of the complex. Whereas mutation of E593 to alanine disrupted the connections within the whole complex and the complex interfaces as well, indicating that this mutation would weaken the complex affinity. SPR experiments confirmed that E593A weakens the affinity of the complex, but R588L had no effect. We hypothesized that these mutations may impact the ability of the CTK to act as a substrate, therefore, we also performed mass spectrometry of the CTK following phosphorylation by ERK2. E593A resulted in a similarly efficient substrate, whereas R588L appears to be a more efficient substrate than WT. These results indicate that the extended aF helix of the CTK, in the case of E593, allosterically fine-tunes the affinity, or as for R588, the substrate efficiency of the complex. Furthermore, a combined approach of MD, network analysis, and FEP are capable of identifying critical residues, whose importance would be difficult to appreciate using other methods, and predict the functional consequences of these sites.

Lightning Talk #11

Microproteins, Does Size REALLY matter?

Dominic Mcgrosso

Gonzalez Lab

Microbial pathogenesis is a complex process involving many host and pathogen factors, often interacting directly with one another. We have much to learn in our understanding of how bacterial microproteins contribute to virulence. For example, the human pathogen *Staphylococcus aureus* is known to produce a diverse array of factors that contribute to its pathogenicity, yet many of these virulence factors, including microproteins, remain uncharacterized or unrecognized by classical alignment algorithms. To address this gap, we designed a peptido-genomic workflow to define the secreted microproteins of *S. aureus*. Overall, we identified 57 high-confidence, non-redundant microproteins detected as 120 microproteoforms. Within this group, we discovered two microproteins regulated by the *S. aureus* accessory gene regulator (*agr*) system.

The first microprotein discovered using our method, termed *S. aureus* microprotein 1 (SAM1) is a highly conserved, 2.6kDa amphipathic microprotein with a formylated initiator methionine. SAM1 does not show cytolytic activity towards keratinocytes nor does it interact with either formyl peptide receptor on neutrophils. Further investigation showed SAM1 disrupted keratin and desmosome networks which we found to be further supported by a greater degree of bacterial penetration deeper within the host. The mechanism of this novel interaction is still under investigation, though we believe SAM1 directly interacts with the keratin networks to enable deeper wound penetration.

The second microprotein discovered appears to act as a canonical cytolysin of the phenol soluble modulin (PSM) family. It is a highly conserved 2.5kDa amphipathic microprotein with a formylated initiator methionine which interacts with the formyl peptide receptor 2 of neutrophils. We have termed this microprotein PSM-ε, as we find diverging mechanisms of action between the canonical PSM-α3 and PSM-ε. Our investigation shows that PSM-ε is also a potent cytolysin for keratinocytes. Our in-vivo work shows that both PSM-α3 and PSM-ε result in large lesions, though analysis of the lesions indicate diverging pathways of pathogenesis; PSM-a3 causes an increase in proteasome and Rab-related proteins whereas PSM-ε perturbation results in increased prohibitins and karyopherins.

Our investigation has shown that pathogenesis of *S. aureus* is enhanced both in-vitro and in-vivo through distinct mechanisms of microprotein interaction and highlights the value of studying the microproteome, a largely unexplored space, and its contribution to bacterial virulence.

Lightning Talk #12

Investigations of Non-Traditional Tools for Precise and Therapeutically Relevant Genome Engineering

Elizabeth Porto

Komor Lab

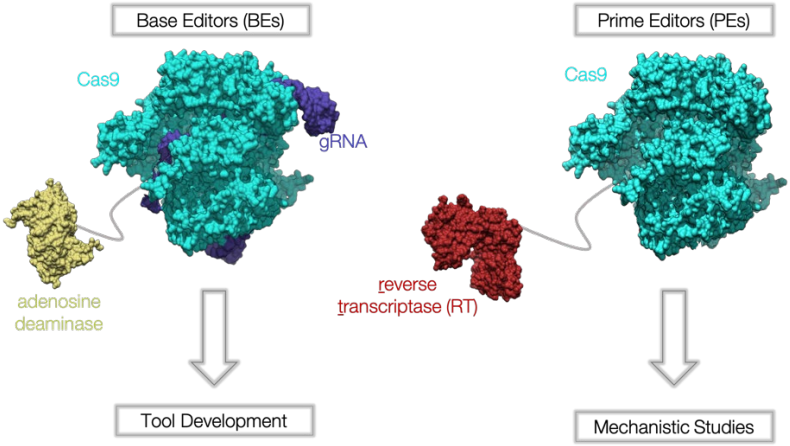
Genome editing, the introduction of a user-defined change to the sequence of chromosomal DNA, is quickly becoming an indispensable tool for scientific research. The optimal genome editing tool would irreversibly edit any chromosomal position with high specificity and efficiency, and with zero undesired edits. Use of the traditional clustered regularly interspaced short palindromic repeats (CRISPR)/ CRISPR-associated protein 9 (Cas9) system has become the standard method for specific genome editing. This system relies on the ability of the Cas9 endonuclease to introduce a double strand break (DSB) at a desired DNA sequence, which is then resolved through homology-directed repair (HDR) or non-homologous end joining (NHEJ). Under most conditions, these two repair processes are in direct competition, with NHEJ oft-winning, leading to high levels of undesired, random insertions and deletions at the site of the DSB. Therefore, DSB-reliant genome editing results in stochastic mixtures of unwanted genome modifications, making it less reliable for therapeutic and commercial use.

Base editing is one example of a non-traditional tool that enables the direct, irreversible conversion of a single target DNA base in a precise, programmable manner without introducing a toxic DSB or requiring an exogenous donor template. The current mechanistic model employs a catalytically dead Cas9 enzyme (dCas9), where only DNA binding capability is maintained, complexed with a short guide RNA (sgRNA) fused to a single stranded DNA (ssDNA) modifying enzyme. Similar to CRISPR methodology, the sgRNA is used for complementary base pairing to the protospacer, which can be located and bound to through recognition of the protospacer adjacent motif (PAM). After the base editor binds to its editing target, a small stretch of ssDNA is exposed, allowing for nucleotides within this editing window to be modified by the tethered enzyme. *See figure.*

As it stands, base editing technology is limited in its scope by only facilitating C•G to T•A or A•T to G•C base pair conversions through cytidine deaminase and adenine deaminase DNA modifying enzymes, as well as limited by its size compatibility with standard therapeutic delivery vehicles due to the presence of the large Cas9 protein. This project seeks to expand the base editor toolkit by addressing the second concern: formulating a base editor compatible with the size limitations of gold-standard AAV viral capsids, which will close the gap between this scientific tool at the bench and its potential as a viable therapeutic to treat genetic diseases.

Another non-traditional tool that circumvents DSBs are prime editors. Prime editors function similarly to base editors, albeit using a more complicated prime editor guide RNA (pegRNA) system. As a collective field, our understanding of this recently reported technology is limited, and the second component to my research project is to assess and elucidate the underlying mechanisms by which prime editors leverage canonical DNA damage and repair pathways to implement user-defined genetic changes.

This dual-component project will not only accelerate novel tool development efforts, but also have a profound impact on our understanding of non-traditional genome editing tools and their place in developing genetic disease therapies.



Lightning Talk #13

Title: Investigating the folding mechanism of a pH-responsive riboswitch in *E. coli*

Authors: Christine Stephen and Tatiana V. Mishanina

Riboswitches are 5'-untranslated regions of mRNA that change their conformation in response to a ligand-binding event, allowing post-transcriptional gene regulation. This ligand-based model of riboswitch function has been expanded since the discovery of a "pH-responsive element" (PRE) riboswitch at the *alx* gene locus in *E. coli*, which encodes a putative metal transporter. At neutral pH, the PRE folds into a translationally inactive structure with an occluded ribosome binding sequence, whereas at alkaline pH, the PRE adopts a translationally active structure. This unique riboswitch system does not rely on binding of a ligand in a traditional sense (typically a small-molecule metabolite) to modulate its alternative folding outcomes. Rather, pH controls riboswitch folding by either regulating the transcription rate of RNA polymerase (RNAP) or by acting on the RNA itself – the two possible modes of pH action that are yet to be distinguished. Previous work suggested that RNAP pausing is prolonged by alkaline pH at two strategic sites, stimulating folding of the PRE into the active structure. To distinguish between pH acting on RNAP versus RNA, I investigated RNAP pausing kinetics at key sites for PRE folding under different pH conditions. I find that alkaline pH does not slow RNAP pause escape, suggesting that the RNA itself has a role in detecting cellular pH.

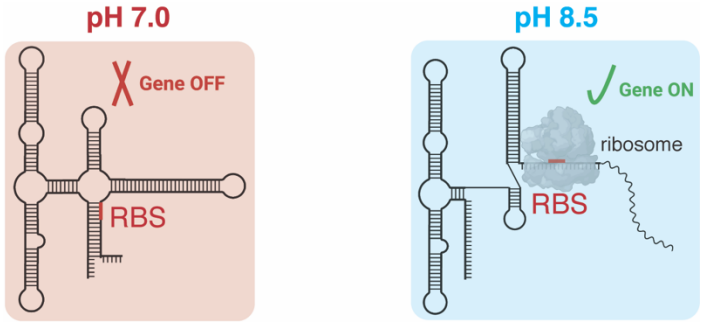
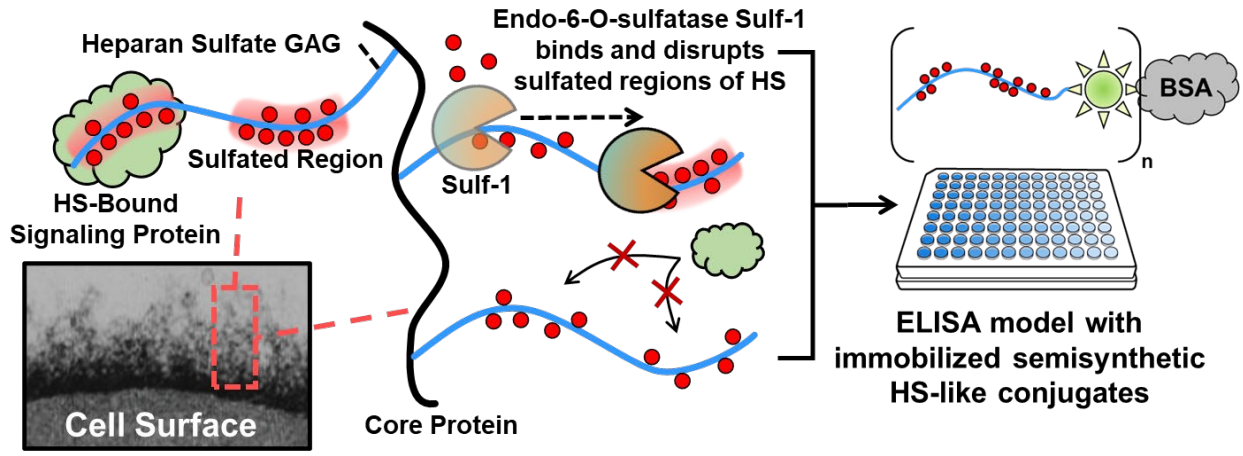


Figure 1. Overview of the pH-responsive riboswitch folding outcomes under different pH conditions. When the PRE is synthesized under conditions of alkaline pH, a translationally active structure is formed with the ribosome binding site (RBS) available for ribosome binding and subsequent translation, turning on the downstream gene (*alx*). When the PRE is synthesized under conditions of neutral pH, a translationally inactive structure is formed with the RBS occluded, turning off the *alx* gene.

Lightning Talk #14
Title: An improved ELISA model for investigating endo-6-O-sulfatase activity and specificity
Bryce Timm
PI: Kamil Godula



In the extracellular environment, a network of proteoglycans project from the cell surface and form components of the extracellular matrix (ECM). Some, like heparan sulfate proteoglycans (HSPGs), have unique sulfated glycan chains (glycosaminoglycans – GAG) that bind signaling proteins through interactions driven by the charge and structure of GAGs. Once secreted to the extracellular environment, HSPGs can be processed by unique extracellular endo-6-O-sulfatases, Sulf-1 and Sulf-2, which selectively hydrolyze 6-O-sulfates on glucosamine (GlcN) sugar residues of HSPG GAG chains. As the only known extracellular endosulfatases, Sulf-1 and Sulf-2 importantly influence HS-associated signaling pathways by neutralizing sulfated regions and disrupting binding interactions between HS and signaling proteins. The complex nature of both the Sulfs and their substrates has prevented a nuanced study of the relationship between HS, the Sulfs, and signaling proteins/pathways.

With difficulty in both the preparation and characterization of the Sulfs, we developed powerful chemical tools to improve on an ELISA platform previously used for Sulf analysis. Traditionally, this assay involves immobilizing heparin to a 96-well plate, treating with Sulf at 37 °C overnight to exhaustively desulfate, and evaluating the effect on signaling protein binding. While effective, prior studies using heparin do not represent the heterogeneity of HS structures found throughout the body, and therefore can't be used to interrogate subtle factors contributing to specificity.

To address the lack of specialized tools, we developed a synthetic method to conjugate bovine serum albumin (BSA) and recombinant HS of varying structure and sulfation. The resulting library was used to examine how structural features of HS influence the binding of signaling proteins and the activity of the Sulfs. Furthermore, we discovered that the Sulfs can exert a passive blocking effect that significantly impacts signal factor binding, beyond the canonical effect of 6-O-sulfate removal. These tools provide a foundation to interrogate Sulf-1/Sulf-2 specificity and how specific sulfation motifs or structural characteristics contribute to their mechanism of action.

Lightning Talk #15

RasAR: A Novel Probe For Ras Activity Measurements.

Ryan Weeks
Zhang Lab

The small GTPase Ras is a critical regulator of cell growth and proliferation. Its activity is frequently dysregulated in cancers, prompting decades of work to pharmacologically target Ras . The functional roles of different isoforms of Ras are tightly coupled with their subcellular distribution and regulation, yet the spatiotemporal regulation of Ras is not still well understood. We developed a ratiometric Ras activity reporter (RasAR) that reports cellular Ras activity with a change in Förster resonance energy transfer (FRET). We showed that this biosensor can provide quantitative measurement of live-cell activities of all the primary isoforms of Ras and capture the spatiotemporal dynamics of Ras activity. Utilizing subcellularly targeted RasAR, we investigated the spatiotemporal regulation of HRas and uncovered the mechanism underlying HRas inhibition by Src. We further demonstrated RasAR's ability to detect KRasG12C inhibition by several KRasG12C inhibitors in living cells and discovered a residual Ras activity that lingers for hours in the presence of these inhibitors. RasAR represents a powerful molecular tool to enable live-cell interrogation of Ras activity and facilitate the development of Ras inhibitors.

Lightning Talk #16
Metallation of nucleic acid nanostructures
Douglas Zhang
Hermann Lab

Owing to their ability to self-assemble according to well defined rules, nucleic acids have received considerable interest as a material for nanotechnology. We have previously designed nanostructures that consisted of both RNA and DNA. The RNA served as an architectural joint while the DNA served as a functional module. To functionalize these structures, we attempted to bind silver to the structures through mismatches in the nucleotide sequence that could be stabilized by silver ions. In doing so, we discovered an RNA motif with silver binding properties. Using this motif, we were able to design a mixture of polygonal nanostructures that form in the presence of silver ions.

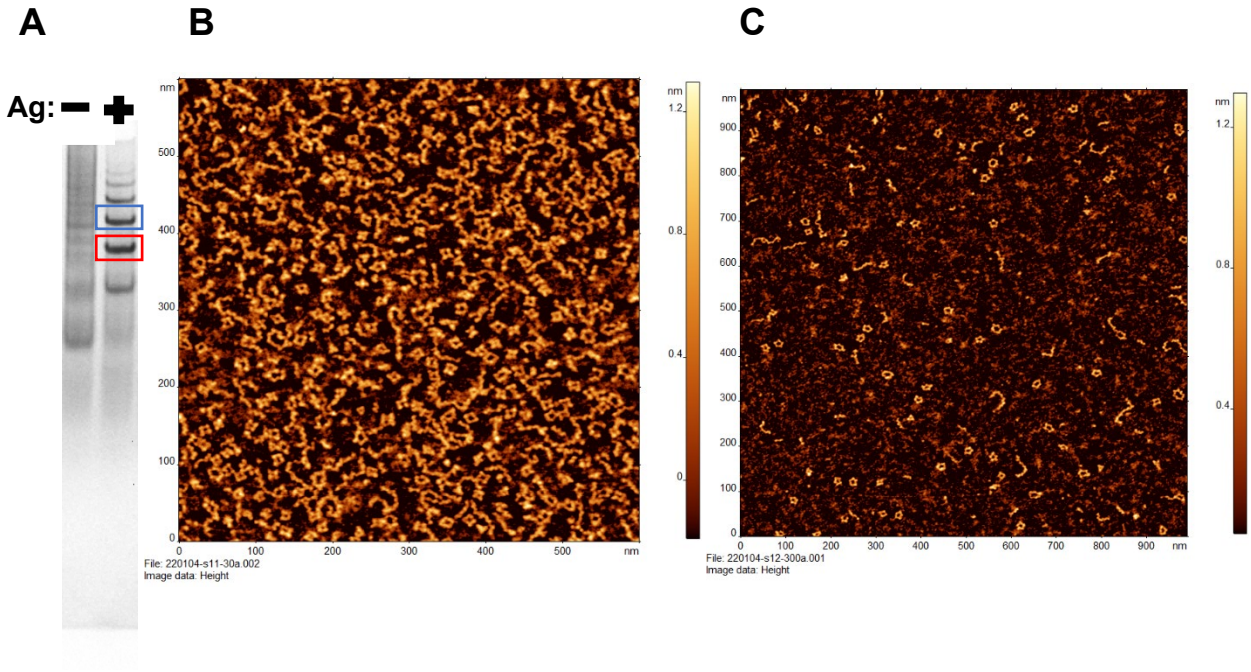


Fig 1. Silver responsive RNA-DNA hybrid nanoshapes. **A**) Native PAGE analysis of RNA/DNA oligonucleotides in the absence or presence of silver ions. Only in the presence of silver ions does the mixture of RNA and DNA form discrete bands. **B**) Atomic force microscopy of the red boxed band in **A**. The band shows that silver ions enable the oligonucleotides to self-assemble into square nanoshapes. **C**) Atomic force microscopy of the blue boxed band in **A**. The band represents pentagonal nanoshapes.

Introduction to the Ethics Case Study: Under Pressure

Pressure surrounds all of us in scientific settings. Depending on our respective roles within hierarchies in the laboratory, clinic, and other research groups, those pressures can come from different sources: Principal Investigators (PI) or group leaders; peers whom we compete with; journal editors and reviewers; our families and those who support us during what can be a long training/career trajectory; time itself in regards to the time limits of position appointments; and of course, ourselves. The nature of a career in research is that the output or "rewards" that we receive in the short term may not be proportional to the degree of effort we put in, which can be vexing for those whose career success is dependent on high-quality publications. Those in training positions may be particularly vulnerable to feeling pressure, even when those in supervisory roles do not consciously exert it. Trainees often feel that they must rely on positive opinions from supervisors to translate into glowing letters of recommendation that may be seen as required for successful career advancement. As a result, trainees may unduly focus on maintaining their supervisors' positive opinion of their performance, generating self-imposed pressure with potentially harmful outcomes.

The effects of being constantly surrounded by the many pressures to perform can manifest themselves in myriad ways. Comparison and competition with those in similar career stages lead to complex interpersonal dynamics in research groups. The research group leader must balance the needs and goals of all their group members along with their own position's requirements and pressures — achieving tenure, receiving continued positive scientific reviews, and maintaining one's own scientific reputation both within our institutes and in the broader scientific community. Yet, it is critical that the group leader not exert pressure on research group members resulting from unrealistic expectations inconsistent with the career goals of the research staff.

This year's case study explores the potential impact of several of these pressures in our research settings — its various sources and effects on group members, as well as the consequences when pressure is implied or direct, and when gaps in communication cause those in supervisory positions to send unclear messages about expectations. It is critical to consider this as we perform our research in a group, being aware of the competing needs and pressures of those around us as we work together on the common goal of pursuing scientific truth.

Under Pressure

As you go through the case, keep in mind that some key details are intentionally missing or left vague in order to encourage everyone to think through how the scenario might play out differently depending on some of the further case details you might want to consider.

Dr. Sam Best is a post-doctoral fellow who has worked in Dr. Taylor Jones's lab for almost 5 years. Best is now working on a project investigating how cells respond to a particular stimulant. Dr. Jones is a Tenure-Track Investigator coming up for tenure consideration within the year, who established the cell stimulation response system upon arrival at NIH, but Best later modified and perfected it. Best reported the development of the system and proof-of-principle data in two peer-reviewed publications, including one as first author. Their new research showed that the cellular response to the stimulant Invigorin was initially low but then steadily increased over time, accompanied by expression of a particular protein within a subset of the cells. Best found that adding specific chemicals inhibiting expression of that protein eliminated the cellular response. Best and Jones conclude that the protein mediates the effect and that they have uncovered a novel mechanism by which cells respond to this class of stimulants.

They draft the manuscript and send it to a high-impact journal. Dr. Jones believes their findings represent a major advance that could increase the likelihood of achieving tenure. The journal responds that while reviewers believed the work is exciting and potentially impactful, they want more direct evidence to prove the model through additional experiments, implying that the paper will be accepted if the new experimental data support the model.

1. Is publication in a 'high-impact' journal important for career success? Should it be?
2. What kind of message do reviewers send when they ask for evidence to 'prove' a model? What are the pitfalls of trying to 'prove' a hypothesis?

Meanwhile, Dr. Best is reaching the end of their NIH appointment and begins a geographically restricted job search in an effort to join their partner, who had moved for a job months earlier. Luckily, Best receives an interview invitation from Innovative Pharma, a prestigious company in the targeted area. Best also makes the short list of applicants for a position at World's Fabulous Research Institute, which provides opportunities for exciting scientific collaborations. The institute position is a dream job but requires preparing for a research proposal and an in-person interview within the next few weeks. Because the institute job is the first choice, Best delays the pharmaceutical company interview process until hearing from the research institute, even though the company position has a higher salary and is an excellent backup option.

Dr. Jones really wants to complete the reviewers' suggested experiments quickly and publish the study because it would increase the potential of achieving tenure, but Dr. Best is concerned about not being able to finish the work while applying for the institute position. Best relays these concerns to Jones and suggests that they ask Dr. Kai Ettero-Sanson, a new post-doctoral fellow that Best trained over the past year, to conduct the experiments, saying Ettero-Sanson would be eager to work on the project. However, Jones asserts that Ettero-Sanson needs more experience because the system is 'finicky' and implies that Ettero-

Sanson has lesser lab skills because of training outside the United States. Jones tells Best not to worry because even if neither position comes through, more offers will come, and compliments Best again for being "very gifted at the bench," a comment Jones has made many times. Jones adds that Best will be able to stay at NIH for an additional sixth year without a problem and that having a first-author paper in the *Journal of Fantastic Results* will greatly improve job prospects.

3. Is it fair to ask Dr. Ettero-Sanson to become involved with the project at this point? What are the advantages/disadvantages of having another researcher perform these experiments?
4. Is the advice from Dr. Jones about Dr. Best's job search reasonable? What would prompt Jones to offer this advice?
5. How should a lab handle systems that tend to be 'finicky'; i.e., a system that is reliable but requires extremely strict adherence to the protocol?
6. Do you think Dr. Jones has a bias against Dr. Ettero-Sanson? How could a bias (or the perception of one) affect lab relationships, pressure, and career development?

Dr. Best reluctantly agrees to ask the institute to postpone the in-person visit and convinces Dr. Jones to allow Dr. Ettero-Sanson to help with the experiments. It takes weeks for Best and Ettero-Sanson to finish their work, but the results are confusing and in one case, contradictory to what they predicted. Best shows the data to Jones, who concludes that the results must be incorrect and that perhaps Ettero-Sanson had misread reagent bottles or protocols. Jones suggests that Best repeat the experiments, but Best reminds Jones that the institute has been trying repeatedly to schedule the on-site interview ASAP. Jones then asks: "Do you think this institute position is a good fit for you? I say this because it is a very competitive environment, and I've found that success in places like that depend on one's ability to think broadly and develop novel and creative ideas." Dr. Best is troubled by these remarks because they imply that Best might not succeed as an independent scientist. It reminded Best of a previous comment by Dr. Jones that fellows who received PhDs from "certain types of universities" are typically better suited for non-academic positions. Best also realizes that aside from repeated compliments on technical skills, Jones has never commented on Best's potential to be a PI/group leader or suggested additional training or experience that would help with achieving a leadership position. Best is now worried about the recommendation letter that Jones had written, what had been communicated privately to professional colleagues, and whether successfully completing revisions of the paper would affect future letters.

7. Are Dr. Best's concerns legitimate? How could Dr. Best address them?
8. How might mentoring/communicating be improved in this interaction?
9. What do you think Dr. Jones meant when referring to 'certain types of places'? Do PIs/group leaders have preconceived ideas about particular schools and career paths? How do these ideas affect trainees?
10. What should take place during a conversation in which a trainee asks their PI/group leader for a letter of recommendation? What is the role of the PI/group leader in that conversation?

Dr. Best works day and night, mostly alone in the lab, repeating the experiments and finishes them faster than any of the previous experiments. This time, the data trended as expected. Dr. Jones is happy and immediately encourages Best to write up the results without Dr. Ettero-Sanson as a co-author and to resubmit the paper, commenting how this will help both of their careers. Best is relieved. While both potential job opportunities had granted interview delays, they were clear that no further delays would be acceptable.

11. Is it proper to remove Dr. Ettero-Sanson as an author? How and when should Dr. Jones have communicated how authorship on this paper would be decided?
12. Is running experiments 'day and night' appropriate in this case? What issues can arise from this behavior?

Dr. Ettero-Sanson learns of the new results and is skeptical. A meticulous experimentalist, Ettero-Sanson does not believe the new results could differ so substantially from the data obtained together with Best. After learning about the change in authorship, Ettero-Sanson tries to move on but cannot and decides to investigate further. One day, after everyone has left the lab, Ettero-Sanson looks through Dr. Best's lab notebooks and electronic files and uses the Excel data to try to replicate the results, without realizing that doing so would destroy the integrity of the spreadsheet. From the analysis, Ettero-Sanson concludes that Best ran the most recent experiment multiple times but presented only results from the three best experiments to Dr. Jones.

13. Is Dr. Ettero-Sanson justified to suspect Dr. Best's results? If so, what should Dr. Ettero-Sanson do?
14. Why is the integrity of primary data so important? How can the integrity of computer files be maintained?
15. Is it ever ok to look through a colleague's notebook and data files?
16. How should primary and analyzed data be stored?
17. Is it acceptable to present data selectively? Under what conditions, if ever, can specific data sets be removed from an analysis?

Dr. Ettero-Sanson is worried about the consequences of coming forward and questioning the experimental results, but out of great concern, speaks with Dr. Jones about the possible misconduct. Jones brushes off the concerns, saying that Ettero-Sanson must be mistaken and implies that Ettero-Sanson misunderstood Best's lab notebook and files, perhaps because of language issues. Jones begrudgingly agrees to a formal meeting to discuss the issue further but neglects to schedule one. Ignored and upset, Ettero-Sanson contacts the NIH Agency Intramural Research Integrity Officer (AIRIO). A preliminary assessment indicates that a misconduct inquiry is warranted.

18. How should Dr. Jones respond to Dr. Ettero-Sanson's concerns?
19. What type of signals is Dr. Jones sending to Dr. Ettero-Sanson by bringing up 'language issues' and by not scheduling the meeting?
20. What role does trust play in mentor-mentee relationships? How do you think the outcomes would differ if Dr. Jones trusted Dr. Ettero-Sanson more and Dr. Best less?

During the misconduct inquiry, Dr. Jones worries that rumors will spread, required external reference letters will be tainted, and the tenure committee will not recommend promotion. Jones blames Dr. Ettero-Sanson for the entire situation and begins to wonder if another lab would be a better fit. Dr. Ettero-Sanson worries that relationships within the lab are irreparably harmed. Dr. Best is extremely distressed and concerned about reputational damage. Unable to concentrate on the job proposal, Best withdraws from consideration for the institute position, but does interview with the pharmaceutical company as the inquiry progresses.

The inquiry ends and concludes that no further investigation is practical because Dr. Ettero-Sanson's handling of the original Excel file compromised its integrity. The pharmaceutical company selects a different candidate, and when Best asks for feedback, the recruiter responds that Best seemed distracted during the interview.

21. Do you see ineffective communication taking place in this case? If so, where and how might better communication from the PI/group leader to either trainee have changed the outcomes?
22. What choices could have been made differently that would have led to positive outcomes for everyone in this case?
23. Have you ever encountered or heard about any other situations related to the themes of this case study?
24. What types of services are available to the various parties involved here to get help dealing with high levels of stress?

Tell Us What You Think

The NIH Committee on Scientific Conduct and Ethics (CSCE) welcomes your voluntary, anonymous feedback on any aspect of the 2021 ethics case study. To provide feedback, please scan the QR code (a quick response code that can be read by cell phone cameras) or click the link below – each will take you to the same anonymous survey. Please provide feedback by December 31, 2021. All comments will be aggregated to generate a summary document for review. Any personal identifiers provided in the responses (e.g., names, position titles/types, etc.) will be removed prior to sharing the results outside the CSCE.

Open your cell phone camera application and focus on the QRC above, and you will be directed to Survey Monkey to leave anonymous feedback. You may choose to identify your IC and or Laboratory/Branch, if relevant to your feedback, but please do not identify any person by name or position (names will be redacted).

You may also access the survey by clicking on this URL:
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