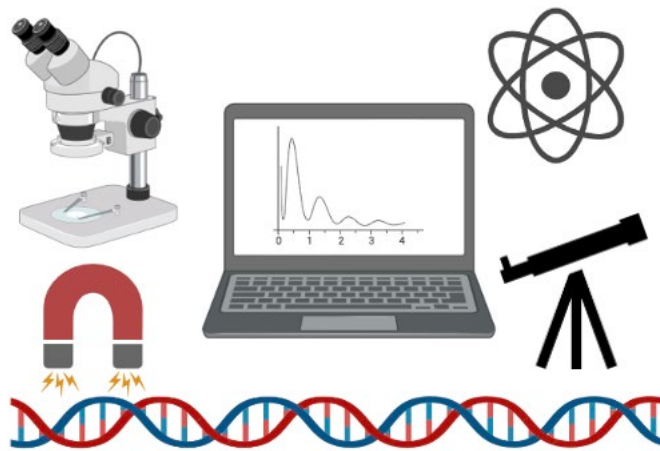


Yale

Science, Technology, and Research Scholars



STARSII Annual Symposium

April 24, 2023

5:30 PM - 8:00 PM

Marsh Lecture Hall
at the Yale Science Building
260 Whitney Avenue

Schedule of Presentations

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| 5:30-6:30 | STARSII Students | Poster Presentations |
| 6:30-6:45 | Dr. Sandy Chang <i>Associate Dean of Science and QR Education</i> | Welcoming Remarks |
| 6:45-6:55 | Laiba Akhtar <i>Dept. of Chemical and Environmental Engineering</i> | PACE polyplexes for encapsulation and delivery of gene editing materials |
| 6:55-7:05 | Julia Balch <i>Dept. of Molecular, Cellular, and Developmental Biology</i> | Indole glucosides act as a gut-to-brain signal to drive microbially-influenced behaviors |
| 7:05-7:15 | Jay Baptista <i>Dept. of Astronomy and Astrophysics</i> | Modeling cosmic feedback using Fast Radio Bursts |
| 7:15-7:25 | Peter Choi <i>Dept. of Molecular, Cellular, and Developmental Biology</i> | Determining how the autophagosome maintains organization for growth |
| 7:25-7:35 | Miriam Kopyto <i>Dept. of Molecular Biophysics and Biochemistry</i> | How your antibodies fight infection: what we know and what we don't |
| 7:35-7:45 | Jaida Morgan <i>Dept. of Molecular, Cellular, and Developmental Biology</i> | CAD as a potential therapeutic target for ARID1A-deficient gynecologic cancers |

Poster Presentations

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| Awa Cisse <i>Dept. of Molecular, Cellular, and Developmental Biology</i> | Evaluating the impact of genetic diversity on Pfs230: a transmission-blocking malaria vaccine candidate |
| Cecilia Chak <i>Dept. of Molecular, Cellular, and Developmental Biology</i> | Characterizing interspecies recognition between <i>Vibrio cholerae</i> and <i>Vibrio coralliilyticus</i> biofilms through protein-polysaccharide matrix interaction |
| Danielle Castro <i>Dept. of Molecular Biophysics and Biochemistry</i> | Investigating the activity of the drug Afatinib through analog synthesis |
| Eugene Shen <i>Dept. of Electrical Engineering</i> | Quantum implementation of a quantum-cognitive model of decision-making |
| Faiad Alam <i>Dept. of Molecular, Cellular, and Developmental Biology</i> | The role of ELF4 in CD4 T-cell immunity and inflammation |
| Jaweria Bakar <i>Dept. of Molecular, Cellular, and Developmental Biology</i> | Investigating the off-target toxicity of the cancer drug Ralimetinib and its role as an EGFR inhibitor |

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| Josh Nguyen <i>Dept. of Molecular, Cellular, and Developmental Biology</i> | Investigating the role of GJC2 in lymphedema |
| Aikaterini Kargioti <i>Dept. of Applied Physics</i> | Design and characterization of radiation shielding package for quantum superconducting circuits |
| Lynne Kim <i>Dept. of Biomedical Engineering</i> | Assessing hypertrophic cardiomyopathy risk using engineered heart tissue |
| Maxine Mackie <i>Dept. of Ecology and Evolutionary</i> | Enhancing immune responses in the tumor microenvironment |
| Sally Jiang <i>Dept. of Astronomy</i> | Influence of magnetic fields on formation of gas structures in the Orion Molecular Cloud |
| Tenzin Kunsel <i>Dept. of Biomedical Engineering</i> | 3D printing method for the articular cartilage of the patellofemoral joint |
| Angelica Lorenzo <i>Dept. of Biomedical Engineering</i> | Creation of a biomimetic microvascular device to model fibrosis |
| Daena S. Rodriguez Rueda <i>Dept. of Molecular, Cellular, and Developmental Biology</i> | Investigating the role of histone deacetylation in early critical periods of vertebrate neurodevelopment |
| Jackson Roberts <i>Dept. of Ecology and Evolutionary Biology</i> | Deciphering the variable relationship between overlapping and distinct foci of TBP-1 and PRDE-1 in piRNA biogenesis |
| Matthew Midy <i>Dept. of Molecular Biophysics and Biochemistry</i> | Engineering RNA aptamers by directed evolution from a natural TPP riboswitch scaffold |
| Noemi Guerra <i>Dept. of Molecular, Cellular, and Developmental Biology</i> | Exploring the role of Cysteine-Rich Protective Antigen (CyRPA) across Plasmodium species |
| Annalyse Olcott <i>Dept. of Earth and Planetary Sciences</i> | Investigating the relationship between Titan's methane lakes and its surface temperature to understand their role on variations in Titan's climate system |
| Sorah Park <i>Dept. of Economics</i> | Understanding Cilia Dysfunction Using SARS-CoV-2 ORF10 |
| Dina Garmroudi <i>Dept. of Molecular, Cellular, and Developmental Biology</i> | A serotonergic pathway mediating the effects of gut microbes on host feeding behavior |
| Andy Tang <i>Dept. of Molecular, Cellular, and Developmental Biology</i> | Comparative analysis of TP53 alleles in pancreatic ductal adenocarcinoma |
| Harper Lowrey <i>Dept. of Molecular, Cellular, and Developmental Biology</i> | The plant circadian clock imposes a morning state through CFH1-mediated degradation of PIF3 |
| Mikayla Labissiere <i>Dept. of Biomedical Engineering</i> | Endothelial targeting with improved nanoparticles for specific and localized use |
| Eric Stevens <i>Dept. of Molecular, Cellular, and Developmental Biology</i> | Identifying the driving mechanisms for the recurrent 1q aneuploidy in human cancers |

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| Lydia Tarekegn <i>Dept. of Molecular Biophysics and Biochemistry</i> | Detection of fold-switching members of the NusG superfamily |
| Austen Theroux <i>Dept. of Applied Mathematics</i> | Host spatial structure and viral cheating |
| Barkotel Zemenu <i>Dept. of Physics</i> | Characterizing the outgassing of electronegative impurities in nEXO |
| William An <i>Dept. of Molecular, Cellular, and Developmental Biology</i> | Investigating the effect of bacterial diversity on the evolution of <i>Pseudomonas aeruginosa</i> virulence in response to phage H6 |
| Akua Agyemang <i>Dept. of Molecular Biophysics and Biochemistry</i> | Persistence length of chromatin in the nuclear context |

PACE Polyplexes for Encapsulation and Delivery of Gene Editing Materials

Laiba Akhtar^{1,2}, Alexandra Suberi^{2,3}, Mark Saltzman^{2,3}

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Chemical & Environmental Engineering, Yale University, New Haven, CT

³Department of Biomedical Engineering, Yale University, New Haven, CT

Gene therapy shows great potential for the future of personalized medicine. RNA based therapies can alter protein expression or edit the genome; specifically, Cas9 mRNA and specific guide RNAs (sgRNA) can be co-delivered for gene editing. Cas9 mRNA is translated into the Cas9 nuclease that cleaves DNA while sgRNAs guide the nuclease to the DNA sequence of interest. Despite advanced gene editing systems, targeted delivery and cellular uptake of gene editing materials remains an obstacle. Polymer-based vehicles can encapsulate and protect nucleic acid cargos for *in vitro* and *in vivo* delivery and are highly customizable. We use poly(amine-co-ester) (PACE) polyplexes to encapsulate varying ratios of Cas9 mRNA and sgRNA and deliver them *in vitro* to Ai9 cells, which contain a premature stop codon upstream the gene coding for the fluorescent tdTomato protein. Using a plate reader to measure fluorescence and flow cytometry, we analyzed the levels of gene editing through editing kinetics and tdTomato expression. This research validates PACE polyplexes as viable delivery vehicles for gene editing as well as two *in vitro* models for assessing gene editing efficiency. Using PACE polyplexes to encapsulate Cas9 mRNA and sgRNAs creates a basis for future *in vivo* applications of gene editing.

Indole glucosides act as a gut-to-brain signal to drive microbially-influenced locomotory behavior

Julia Balch,^{1,2} Chester Wrobel,³ Madhumanti Dasgupta,² Jingfang Yu,³ Frank Schroeder,³ Michael O'Donnell²

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT

³Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY

In the study of animal-microbiome interactions, the common bacterial tryptophan metabolite, indole has been identified as a neuroactive compound involved in microbe-nervous system signaling in diverse animal systems. However, it is unclear how gut microbial indole metabolites are trafficked to and sensed by the nervous system. In the bacterivorous nematode *C. elegans*, bacterially produced indole is incorporated into carboxylesterase-diversified modular glucosides (MOGLs) produced in the lysosome-related organelles (LRO) of the intestine. We find that disruption of indole MOGL (*iglu*) biosynthesis via elimination of bacterial indole production or prevention of LRO formation results in behavioral defects in the locomotory escape response. Additionally, expression of the carboxylesterase CEST-1.2, which is responsible for the 2' acylation of *iglus*, in both the gut and a single olfactory neuron is necessary for producing sustained escape reversals. We propose a model by which *iglus* are assembled in the intestine and trafficked to the nervous system where they are hydrolyzed to release free indole. The neuronally expressed transient receptor potential ankyrin 1 (TRPA-1) channel is a putative target for neuronally released indole to act on locomotory behavior circuits. This system suggests that glycosylation of bacterial metabolites may represent a general mechanism by which microbiota regulate the nervous systems of their animal hosts.

Modeling Cosmic Feedback using Fast Radio Bursts

¹Jay Baptista, ²J. Xavier Prochaska, ²Alexandra Mannings, ³Marla Geha

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Astronomy and Astrophysics, University of California, Santa Cruz, CA

³Department of Astronomy, Yale University, New Haven, CT

The width of the Macquart relation (z-DM distribution) is sensitive to the distribution of baryons in the intergalactic medium (IGM) including those ejected from galactic halos through feedback processes. The width of this distribution has been parameterized by a feedback parameter F , where F is related to the cosmic DM variance by $\sigma_{DM} = \frac{F}{\sqrt{z}}$. In this work, we present a new measurement ($\log_{10} F = -0.48^{+0.48}_{-0.18}$) using 76 FRBs of which 16 have localizations. Our analysis simultaneously fits for the Hubble constant H_0 and the DM excess distribution due to the FRB host galaxy ($\sigma_{\text{host}}, \mu_{\text{host}}$). We find that the feedback parameter is degenerate with these parameters, most notably H_0 . Using a synthetic sample of 100 localized FRBs, the constraint on the feedback parameter is enhanced by a factor of ~ 2 . Comparing our F measurement to simulated predictions from cosmological simulations, we find agreement between $0.4 < z < 2$. However, the simulated measurement on F when $z < 0.4$ lies outside of the 68% confidence interval of our measurement and is likely attributed to the rapidly changing extragalactic DM excess distribution at low redshift. Measuring the feedback parameter using z-DM modeling represents a novel method for measuring the strength of galactic feedback in the IGM.

Determining How the Autophagosome Maintains Organization for Growth

Peter Choi^{1,2,3}, Taryn Olivas³, Thomas Melia³

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT

³Department of Cell Biology, Yale University School of Medicine, New Haven, CT

Autophagy, the cellular degradation of damaged cytoplasmic material and protein aggregates, is an essential process in maintaining cellular homeostasis; its dysfunction has been linked to neurological disease, cardiovascular disease, and cancer. Autophagy requires the formation of an autophagosome which traps cytoplasmic cargo material and fuses with the lysosome for subsequent degradation. Although the function of the autophagosome is known, the mechanism of its biogenesis has remained enigmatic for decades. Recently, we have shown that in mammalian cells lacking autophagy protein ATG2, there are clusters of vesicles positive for early autophagic markers such as p62, ATG9A and LC3B. Upon cleavage of LC3B-II from the ATG2 DKO compartment, we discovered smaller structures of p62 and ATG9A which mark the ATG2 DKO compartment. Hence, we hypothesize that liquid-liquid phase separation, a phenomenon where vesicles can condense into membraneless compartments, holds the ATG2 DKO compartment together and show that LC3B is partially responsible for organization of the compartment.

Identifying Novel Factors in Somatic Hypermutation

Miriam Kopyto^{1,2}, Lizhen Wu², Anurupa Yadavalli², David Schatz²

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Immunobiology, Yale University, New Haven, CT

Somatic Hypermutation (SHM) introduces mutations in immunoglobulin variable (IgV) regions in early B-cells. SHM requires activation induced deaminase (AID), yet the mechanisms underlying its targeting and regulation are poorly understood. Discovery and characterization of potential SHM factors can lead to greater understanding of basic cellular repair processes and related clinical fields. Identification of novel SHM regulatory factors was achieved through CRISPR-Cas9 knockout in Rapid Assay for Somatic Hypermutation (RASH) Ramos B-cells containing a GFP assay for SHM activity. Results demonstrated that retinol dehydrogenase 13 (RDH13) and leukocyte cluster member 1 (LENG1) knockouts correlated with a decrease in SHM. Serine and arginine rich splicing factor 10 (SRSF10) knockout correlated with an increase in SHM. This data indicates a possibly significant role for these proteins in SHM regulatory mechanisms. Whether these effects are due to global transcriptional effects or due to a specific role in SHM is still unknown and future directions aim to address their mechanistic impacts on SHM.

CAD as a Potential Therapeutic Target for ARID1A-Deficient Gynecologic Cancers

¹Jaida Morgan, ²Zainab Shonibare, ²Gloria Huang

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Obstetrics, Gynecology, & Reproductive Sciences, Yale School of Medicine, New Haven, CT

Gynecologic cancers are cancers of the female reproductive system. Ovarian cancer is the most fatal gynecological cancer affecting women in the United States and worldwide. Among the histological subtypes of ovarian cancer, there is an unmet need for targeted therapy for clear cell ovarian cancer. This cancer has the highest rates of *ARID1A* loss-of-function (LOF) mutations. *ARID1A* is a tumor-suppressor gene, encoding a subunit of the SWI/SNF chromatin remodeling complex. LOF of this complex has been linked to cancer initiation and progression. Our lab recently found that ARID1A-deficiency in cancer cells provokes metabolic reprogramming and greater reliance on the *de novo* pyrimidine DNA synthesis pathway, resulting in increased resistance to chemotherapy. To develop effective anti-cancer drugs targeting ARID1A deficiency, we will investigate the impact of the *de novo* pyrimidine biosynthesis pathway on cancer cell proliferation and viability. CAD is an enzyme that controls the first three steps of the *de novo* pyrimidine biosynthetic pathway. We hypothesize that CAD inhibition is an effective targeted approach to ARID1A-deficient cancers. Our preliminary data suggests that CAD inhibition preferentially reduces cell viability in ARID1A-deficient cancers. We have evidence that the novel inhibitor YD21 is a potent inhibitor of CAD, irrespective of ARID1A status.

Evaluating the Impact of genetic diversity on Pfs230: a transmission-blocking malaria vaccine candidate

¹Awa Cisse, ²Alessandra Orfano, ²Nicole Feriancek, ²Ife Desamours, ²Amy K. Bei

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Epidemiology of Microbial Diseases, Yale School of Public Health, Yale University, New Haven, CT

Despite having multiple tools to combat malaria, progress in malaria control has stagnated. To eradicate the disease, the WHO has set a goal of licensing a vaccine with 75% efficacy. Pfs230 is a protein expressed during the sexual development of *Plasmodium falciparum*, one of the five species of *Plasmodium* that causes malaria in humans. Studies have shown the promise of Pfs230 for inclusion in a malaria transmission-blocking vaccine, which would prevent transmission to the *Anopheles* mosquito vector, decreasing downstream transmission. Previous findings uncovered that monoclonal antibodies against Pfs230 have strong transmission-blocking activity. However, genetic variation in this locus may affect the transmission-blocking efficacy of Pfs230 antibodies. To determine the impact of genetic diversity on function, we combine genomic and experimental genetic approaches. Here, we employ targeted amplicon sequencing of Pfs230 from samples obtained in a high-transmission zone: Kédougou, Senegal. Using Illumina Next-Generation sequencing on a NovaSeq6000, we are sequencing *Pfs230* to identify non-synonymous mutations. By introducing prioritized mutations in domain I into an isogenic parasite background, a domain previously shown to contain the most potent inhibitory activity, we will analyze the impact of specific different SNPs on function combinations. Functional evaluation of transmission inhibition will be performed using monoclonal antibodies and the standard membrane-feeding assay. This approach will help identify specific polymorphisms in Pfs230 that can decrease potential efficacy. The findings from this study will permit the rational prioritization of new alleles and combinations of alleles in future iterations of transmission-blocking vaccines for malaria.

Characterizing interspecies recognition between *Vibrio cholerae* and *Vibrio coralliilyticus* biofilms through protein-polysaccharide matrix interaction

¹Cecilia Chak, ²Jing Yan

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT

Biofilms are surface-attached communities of bacterial cells embedded in an extracellular matrix of polysaccharides and proteins. Biofilm formation enhances bacteria's ability to survive in dynamic environments by conferring properties including stronger surface adhesion and resistance against stressors such as antibiotics. Thus, research on biofilm have has huge potential health and environmental implications. In *Vibrio cholerae* biofilms, matrix protein RbmC associates with the vibrio polysaccharide (VPS) and is responsible for cell-to-surface adhesion and contribution to the overall integrity of the biofilm structure. Highly similar homologs of RbmC have been found in several other *Vibrio* species, but it remains unknown whether these proteins are functional homologs. One such species is *V. coralliilyticus*, a coral and shellfish pathogen. In this study, we examine cross-species VPS-RbmC interactions – between *V. cholerae* and *V. coralliilyticus* – in order to characterize the specificity of the biofilm formation and recognition mechanisms across species. We approach this problem with the use of techniques such as bacterial transformation, fluorescent confocal microscopy, and microfluidics. We show preliminary evidence that despite the two species forming phenotypically distinct biofilms, *V. coralliilyticus* may be able to incorporate RbmC from *V. cholerae* into its own biofilm structure.

Investigating the Activity of the Drug Afatinib Through Analog Synthesis

Danielle Castro¹; Saul Jaime-Figueroa, PhD²; Craig Crews, PhD^{2,3,4}

¹Science, Technology, and Research Scholars, Yale College, New Haven, CT

²Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven CT

³Department of Chemistry, Yale University, New Haven CT

⁴Department of Pharmacology, Yale University, New Haven CT

Chordomas are a type of aggressive bone cancer typically found on the spine and skull, which are very difficult to treat. One area of interest is to target the transcription factor brachyury, which is overexpressed in all chordomas. However, since brachyury has multiple functions, a traditional small molecule inhibitor would probably not abrogate all of brachyury's functions. PROTeolysis TArgeting Chimeras (PROTAC)-mediated degradation of brachyury is a fitting alternative since PROTACs require only transient interactions with target proteins to cause degradation. PROTAC synthesis requires a ligand for target protein binding, and the small-molecule drug afatinib was discovered to bind brachyury as well as epidermal growth factors (EGFR), which would be undesirable for use in chordoma treatment. The Crews lab developed compound SJF-HI4601 which does not have EGFR activity but still binds brachyury covalently by mass spectrometry (MS), and preliminary data shows that SJF-HI4601 PROTACs have promising brachyury degradation activity. The aim of this study was to fully validate the affinity of SJF-HI4601 by synthesizing "bait proteins" of SJF-HI4601 conjugated to biotin which will be used in pull-down assays; secondly, to further investigate afatinib activity by synthesizing a very similar analog and evaluating whether the novel compound can still bind brachyury but not EGFR.

Quantum Implementation of a Quantum-Cognitive Model of Decision-Making

¹Yu Jun Shen, ²Raghavendra Pothukuchi, ²Bhattacharjee Abhishek

¹Science Research and Technology Scholar, Yale College, New Haven, CT

²Department of Computer Science, Yale University, New Haven, CT

Models of human decision-making have conventionally drawn inspiration from classical physics, such as the Drift Diffusion Model (DDM) based on Brownian motion. DDM has been used to model decision making in tasks like the Two Alternative Forced Choice (TAFC). However, models based on classical physics cannot account for some advanced aspects of observed behaviours. Our goal in this work is to develop the frameworks to deploy decision-making models based on quantum computing, focusing on the quantum potential wells model. In this approach, choices are modelled as potential wells and evidence favoring a choice is accumulated from quantum particles. One bottleneck in the calculation is finding the eigenstates and energies in order to estimate where the quantum particle lands. In our work, we deployed a Variational Quantum Eigensolver (VQE) algorithm in Qiskit which minimizes the cost of a parameterized quantum “ansatz” circuit within a classical optimizer feedback loop. The circuit, once reconfigured with the optimized parameters, outputs the desired eigenstates. VQE is a hybrid classical-quantum algorithm suitable for use on current quantum hardware. We investigated the impacts of various ansatz styles, the classical optimizer choice and measurement mappings between qubits and the potential well. Convergence is observed for multiple lowest energy states in the potential well Hamiltonian using the Qiskit model.

The Role of ELF4 in CD4 T-cell Immunity and Inflammation

¹Faiad Alam, ²Molly Bucklin, ²Dinesh Babu Uthaya Kumar, ²Sam Olyha, ²Carrie Lucas

¹Science, Technology, and Research Scholar, Yale College, New Haven, CT

²Department of Immunology, Yale School of Medicine, New Haven, CT

Autoinflammation occurs due to a dysregulated inflammatory response that can result in diseases such as Inflammatory Bowel Disease (IBD). The ELF4 gene encodes an ETS transcription factor that we have previously demonstrated to be a key regulator in the inflammatory capacity of several T-cell lineages; however, mechanisms are still unknown. Our lab strives to find novel ELF4 variants in IBD patients and explore how ELF4 impacts inflammatory regulation in T helper type 1 (Th1) cells. Our prior discovery of a novel autoinflammatory disorder, Deficiency in ELF4, X-linked (DEX) revealed that ELF4 is required in humans to protect from mucosal inflammation. To validate loss-of-function mediated by genetic variants in ELF4, we used a luciferase reporter assay and Western blotting. Despite robust protein expression detected, we found variants in the ETS-DNA binding domain or those that cause frameshift ablate transcription factor activity of ELF4. In addition, we used mice genetically engineered to delete ELF4 from T cells (Elf4-floxed; CD4-cre mice) and found that T cells produce elevated interferon gamma (IFN γ) in supernatants after in vitro Th1 differentiation. Mechanisms for upregulated cytokine expression in Th1 T-cells are currently being defined. Ultimately, our investigation of the ELF4 gene provides insight into unknown inflammatory processes.

Investigating the Off Target Toxicity of the Cancer Drug Ralimetinib and its Role as an EGFR Inhibitor

Jaweria Bakar^{1,2}, Debanjan Bhattacharjee³, Erin Sausville³, Brianna Mendelson³, Kaitlin Long³, Joan C. Smith³, Jason Sheltzer³

¹Science, Research and Technology Scholar, Yale College, New Haven, CT

²Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT

³Department of Surgery, Yale School of Medicine, New Haven, CT

Ralimetinib is a small-molecule oncology drug that was developed as a p38 α kinase inhibitor. Recent research from the Sheltzer Lab indicates that ralimetinib is effective at killing cancer cells even when its putative target, p38 α , was knocked out. Preliminary data on ralimetinib suggests it may act as an Epidermal Growth Factor Receptor (EGFR) inhibitor rather than a p38 α inhibitor. Gain of function mutations in the EGFR gene cause hyperactivation of EGFR signaling and can drive the development of multiple cancer types. *In vitro* kinase assays showed that ralimetinib was potent against wild-type EGFR (IC₅₀: 180 nM) and a common cancer-causing mutation EGFR-L858R (IC₅₀: 179 nM). This research aims to study the effects of ralimetinib as a potential EGFR inhibitor. Ralimetinib's IC₅₀ was determined in various cancer cell lines with or without EGFR mutations. Ralimetinib's inhibitory effect on the phosphorylation of EGFR at Tyr1068 and downstream EGFR target ERK Thr202 was determined by western blotting. Results indicate cancers driven by EGFR mutations are more sensitive to ralimetinib than cancers driven by other mutations, similar to what was observed with other known EGFR inhibitors. Identification of ralimetinib's target is crucial to ensure that the patients who will exhibit the most robust response are being treated with ralimetinib.

Investigating the Role of GJC2 in Lymphedema

¹Joshua Nguyen, ²Katherine Ellis, ²Keith Choate

¹Science Research and Technology Scholar, Yale College, New Haven, CT

²School of Medicine, Yale University, New Haven, CT

Lymphedema is a long-term condition presenting as serious swelling due to the accumulation of lymphatic fluid. Previous studies have identified mutations in *GJC2* as a cause of lymphedema; however, the role of the gene in lymphatic functionality remains unknown. *GJC2* encodes production of connexin-47, a gap junction connexin family protein. While previous studies indicate the expression of connexin in lymphatic endothelial cells, as well as a correspondence between gap junction functionality and lymphatic flow, there is limited characterization of gap junctions in lymphatic vessels. Recent genetic studies have linked monogenic lymphatic disorders to the RAS/MAPK and VEGFR3 pathways, which include *GJC2* as a pathway gene. Paired whole-exome sequencing has revealed two specific *GJC2* missense mutations within two unrelated subjects to the Choate lab. To investigate the pathogenesis of these mutations, their effects will be analyzed via in-vitro studies such as western blotting, immunofluorescence and functional assays. Specifically, the expression levels of common players within the MAPK and VEGFR3 signaling pathways will be examined. Through the evaluation of these studies, I aim to provide greater insight into how *GJC2* mutations result in lymphedema, potentially revealing important genetic drivers in gap junction functionality and lymphatic flow, thus opening avenues to targeted therapeutics.

Design and characterization of radiation shielding package for quantum superconducting circuits

Aikaterini Kargioti¹, Akshay Koottandavida¹, Cassady Smith¹, Ioannis Tsioutsios^{1,2}, Luigi Frunzio², and Michel H. Devoret²

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Applied Physics, Yale University, New Haven CT

Superconducting circuits allow the experimental study of fundamental aspects of quantum mechanics phenomena and constitute a promising method of enabling quantum computation and quantum information processing (Devoret & Schoelkopf, 2013). Susceptibility of the superconducting circuits to noise induced by thermal fluctuations and electromagnetic radiation may lead to irreversible information loss (Krinner et al., 2019). Therefore, minimization of undesired couplings to the environment is required to fully take advantage of the superconducting circuit properties. The main factor that improves the performance of quantum circuits is the measurement space, located in the dilution refrigerator at temperatures ranging between 10 and 20mK (NanoScience Oxford Instruments - Nanoscience, n.d.). However, non-ideal thermal resistance pathways and added heat and radiation loads from elements of higher-temperature stages and the environment increase the effective temperature of the superconducting circuits. In this project, we designed a new package for superconducting devices that aims to provide improved thermal anchoring of the devices to the dilution unit and shielding from electromagnetic noise. We showed that the new radiation shielding package improved the excited state population and the characteristic relaxation time of the qubit.

Assessing Hypertrophic Cardiomyopathy Risk Using Engineered Heart Tissue

¹Lynne Kim, ²Xia Li, ²Stuart G. Campbell

¹Science Research and Technology Scholar, Yale College, New Haven, CT

²Department of Biomedical Engineering, Yale University, New Haven, CT

Familial Hypertrophic Cardiomyopathy (HCM) is the most common inherited heart disease, affecting 1 in 500 Americans. It is characterized by abnormal thickening of the heart wall, affecting diastolic function and contractile mechanisms. Cardiomyocytes derived from human induced pluripotent stem cells (iPSCs) provide valuable insight into the pathophysiology of cardiac diseases. Specifically, human iPSCs are genetically matched to the patient, thus the derived cardiomyocytes exhibit analogous physiology at the cellular level. When cultured on an engineered heart tissue (EHT), they simulate contractile behavior *in vitro*, shedding light on the identification and characterization of heart disease phenotypes. Here, we use patient-derived EHTs in a blinded study consisting of 3 control groups and 3 patient groups with unknown status of HCM. A novel biomechanical instrument was used to measure the forces generated by the EHTs as a function of tissue length and stretch to plot the length-dependent activation (LDA). Cardiomyocytes typically produce higher contractile force in response to a higher preload, a phenomenon known as the Frank Starling mechanism. Consistent with previous literature, we hypothesize that HCM impacts the Frank Starling mechanism and will result in impaired LDA. Our investigation aims to compare the functional differences between patient groups and assess the reliability of discerning patient phenotypes using mechanical characterizations such as peak force and LDA.

Enhancing Immune Responses in the Tumor Microenvironment

¹Maxine Mackie, ²Curtis Perry, ²Jeffrey Ishizuka, ²Mackenzie Bender

¹Science Research and Technology Scholar, Yale College, New Haven, CT

²Yale Cancer Center and Yale School of Medicine, New Haven, CT

Within the immune system, sensing of double-stranded RNA (dsRNA) induces a type-I interferon (IFN) response. These pathways drive anti-tumor immunity within the tumor microenvironment (TME). Our project leverages this knowledge to drive our central questions: what happens to T-cell phenotypes after dsRNA sensor stimulation, and is there any synergy between dsRNA sensor stimulation and *Adar1* knockdown? *Adar1* encodes an adenosine deaminase that limits dsRNA sensing. When it is knocked down, IFN signaling is restored, thereby enhancing immune infiltration within the TME and suppressing tumor growth. After treatment with dsRNA sensor agonists, such as RIG-I agonist SLR14, human T-cells were analyzed via flow cytometry and single-cell RNA sequencing. The lymphocyte-activating gene 3 (LAG-3) was upregulated at both the protein and gene level after SLR14 stimulation. To address our second question, we continued to stimulate with SLR14 along with transfecting mouse melanoma cells both *in vitro* and *in vivo* using siRNA targeted against *Adar1* (siADAR). CellTiter-Glo® luminescent cell viability assays were used to gauge inhibition of tumor cell growth *in vitro*, while enzyme-linked immunosorbent assays (ELISAs) were used to quantify immune enhancement through IFN- β production. In both mouse melanoma systems, synergistic tumor suppression and immune enhancement was observed with combinatorial siADAR/SLR14 treatment.

Influence of Magnetic Fields on Formation of Gas Structures in the Orion Molecular Cloud

Sally Jiang^{1,2}, Hector Arce²

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Astronomy, Yale University, New Haven, CT

Star formation is an integral part of the life cycle of stars in the universe and thus it is important to understand what conditions and factors affect the formation of stars. Magnetic fields are believed to inhibit star formation by countering the collapse of gas or help produce star-forming structures like filaments by funnel material into clouds. The goal of this work is to study the role played by magnetic fields in star-forming regions in the formation of gas structures. We focus on the Orion Molecular Cloud (OMC), one of the closest active star-forming regions, at about 400 parsecs, and with extensively mapped gas and magnetic field structures. We have gas maps in OMC-1 of ^{12}CO , which encompass gas encompassing the outer shell of the cloud, ^{13}CO and C^{18}O , which detail denser regions within the clouds. We will study filamentary gas structures with the magnetic field orientation using thermal dust emission maps in the far-infrared and sub-millimeter. We find that certain structures found in all three maps related to the outflow of the cloud are often aligned with the magnetic field. In addition, from combining gas and magnetic field orientation maps, we find that structures in the ^{12}CO gas are, on average, more aligned with the magnetic field compared to ^{13}CO and C^{18}O gas, hinting at decreased magnetic field influence deeper within the cloud. Through closer examination, we analyze the structures of at all velocities of the gas cloud that is aligned with the magnetic field. We find that for ^{12}CO , ^{13}CO , and C^{18}O gas at all velocity ranges are aligned and misaligned, showing no preferentially aligned by velocity in the wider region.

3D Printing Method For The Articular Cartilage of the Patellofemoral Joint

Tenzin Kunsel^{1,2,4}; Brian Beitler⁴; Annie Wang, MD⁵; Steven Tommasini, PhD^{2,4}; John Fulkerson, MD⁴, Daniel Wiznia, MD^{3,4}

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Biomedical Engineering, Yale College, New Haven, CT

³Department of Mechanical Engineering, Yale University, New Haven, CT

⁴Orthopaedics and Rehabilitation, Yale School of Medicine, New Haven, CT

⁵Radiology and Biomedical Imaging, Yale School of Medicine, New Haven, CT

Dysfunction at the patellofemoral (PF) joint is a leading cause of knee pain, with up to 45% of cases attributed to altered mechanics between the patellar and trochlear articular surfaces. The resulting high focal contact stress can lead to developmental PF dysplasia, a structural malformation in the joint. While significant efforts have been made to understand the PF joint through 3D models of bone morphology from CT scans, a comprehensive understanding requires accurate models of the articular cartilage. In this study, 3D models of the articular cartilage of the PF joint were created to better understand the relationship between patella and trochlear morphology. Scans of the PF joint were obtained using a custom thin-section sagittal gradient echo MRI sequence, and areas of cartilage were marked with white contrast and split into three regions to create models of the femur, patella, and tibia cartilage. These models were manually edited and 3D printed using Formlabs printers. Congruence was found between the articular cartilages of the patella and femur in patients with PF dysplasia who had not undergone trochleoplasty. Trochleoplasty is a surgical procedure that deepens the groove of the trochlea in patients with PF dysplasia. Further research can provide evidence that trochleoplasty disrupts congruence, leading to discomfort in the joint. This novel method can help orthopedic research scientists study joint biomechanics and understand trochlear dysplasia and related pathologies, providing a more accurate model of the PF joint surface.

Creation of a Biomimetic Microvascular Device to Model Fibrosis

Angelica Lorenzo^{1,2}, Michelle Wu², Anjelica Gonzalez²

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Biomedical Engineering, Yale University, New Haven, CT

Fibrosis is the pathological scarring and thickening of tissue caused by the excessive deposition of extracellular matrix (ECM) components in response to injury. Fibrotic diseases can unfold in multiple organs and can lead to organ dysfunction and eventually total organ failure. Engineering an *in vitro* biomimetic microvascular model to simulate vascular dysfunction during fibrosis will allow us to study the mechanisms and propose treatments for the disease. To do so, we successfully created a polydimethylsiloxane (PDMS) device featuring three channels. Our next steps include adding the vascular cells and extracellular matrix to the channels of the device to finalize our *in vitro* model of human fibrosis.

Investigating the role of histone deacetylation in early critical periods of vertebrate neurodevelopment

Daena S. Rodriguez Rueda¹, Valerie A. Tornini², Antonio J. Giraldez²

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Genetics, Yale University School of Medicine, New Haven, CT

Histone acetylation is a critical epigenetic modification that works through the balanced addition of acetyl groups by histone acetyltransferases (HATs), and removal by histone deacetylases (HDACs). This balance regulates chromatin accessibility during several physiological and cellular processes in early development. Our lab screened zebrafish larvae for behavioral effects after treatment with HDAC inhibitors, as a readout for affected neurodevelopmental processes. We identified hyperactivity in zebrafish larvae after treating them with trichostatin A (TSA), a pan-histone deacetylase inhibitor. We hypothesized that the altered organismal behavior was due to the disruption to histone acetylation by TSA, which dysregulated normal gene expression and neural cell specification. To test this hypothesis, we are investigating the behavioral, cellular, and molecular phenotypes following TSA treatment. First, we characterized the behavioral parameters affected in the drug-treated zebrafish larvae in a quantitative manner using locomotor activity tracking. To understand the cell types that are most affected by TSA treatment, we are imaging different neural cell types, including glutamatergic and GABAergic cells, oligodendrocyte precursor cells, microglia, and astrocytes. Our ongoing work will provide insight into the effects of TSA on chromatin structure and will also elucidate the conserved roles of histone acetylation in normal vertebrate neurodevelopment and behavior.

Deciphering the variable relationship between overlapping and distinct foci of TBP-1 and PRDE-1 in piRNA biogenesis

^{1,2}C. Jackson Roberts, ³Nancy Sanchez, ³Valerie Reinke

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Ecology & Evolutionary Biology, Yale University, New Haven, CT

³Department of Genetics, Yale School of Medicine, New Haven, CT

The Piwi-interacting RNA (piRNA) pathway maintains germline integrity and fertility through the suppression of transposons and nonself nucleic acids. In *C. elegans*, the factors PRDE-1, SNPC-4, TOFU-5, and TOFU-4 are components of the upstream sequence transcription complex (USTC), which is found to promote piRNA expression. Recent literature demonstrated that TATA-Box-Binding Protein 1 (TBP-1) physically interacts with PRDE-1, however, the mechanism by which TBP-1 coordinates piRNA expression is unknown. To determine the spatial relationship between TBP-1 and PRDE-1, we performed super-resolution confocal imaging in living worms. We observed that TBP-1 forms multiple distinct foci in *C. elegans* germline nuclei. Surprisingly, we observe temporal variation in the level of colocalization of TBP-1 and PRDE-1 foci across germline development. We used Z-stack images to generate 3-D models to observe the extent of colocalization in multiple planes. By performing colocalization analysis, we find that TBP-1 and PRDE-1 foci colocalize in the distal tip and early pachytene stages of germline development, however in mid-pachytene we observe no colocalization. We will continue to investigate the observed temporal variation and its significance in piRNA biogenesis. This project will provide insight into piRNA biogenesis, germline-specific genomic regulation, and transcriptional mechanisms that have adapted to maintain germline integrity.

Engineering RNA Aptamers by Directed Evolution from a Natural TPP Riboswitch Scaffold

Matthew K. Midy^{1,2}, Michael G. Mohsen^{3,4}, Ronald R. Breaker^{2,3,4}

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT

³Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT

⁴Howard Hughes Medical Institute, Yale University, New Haven, CT

Current human gene therapy approaches pose safety issues due to a lack of precise, external control of gene expression levels. One solution is the use of riboswitches in gene therapy. Riboswitches are gene regulatory elements typically found in the 5' untranslated regions of messenger RNAs. With an aptamer domain that selectively binds a ligand and an adjacent expression platform that modulates gene expression, riboswitches provide a mechanism for ligand-induced gene control. Using Systematic Evolution of Ligands by Exponential Enrichment (SELEX), synthetic aptamers that bind different compounds can be discovered. Here, a thiamin pyrophosphate (TPP) riboswitch aptamer is used as a scaffold for SELEX. After exposing the RNA pool to a four-compound collection (compounds A, B, P, S) for fourteen generations, the pool displays affinity for Compounds P and A by elution profile. The fourth most abundant sequence in the pool (G14.4) has an affinity for Compound B as determined by in-line probing, with a dissociation constant (K_D) of $2.25 \pm 0.21 \mu\text{M}$. Optimizing aptamer affinity and grafting onto a viable expression platform could produce a device that could eventually prove useful as gene regulatory devices for therapeutic applications.

Exploring the Role of Cysteine-Rich Protective Antigen (CyRPA) across *Plasmodium* Species

¹Noemi Guerra, ²Amy K. Bei

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, CT

With an estimated 247 million cases and 619,000 deaths estimated worldwide in 2021 alone, malaria remains a major global health concern. To lessen malaria's burden, the WHO has recently begun recommending the pre-erythrocytic vaccine RTS,S which targets CSP on the sporozoite surface of *P. falciparum* to certain demographics. Unfortunately, this vaccine confers only partial protection, creating an ongoing search for a more potent vaccine candidate. CyRPA (Cysteine-Rich Protective Antigen) is a protein involved in the erythrocytic, schizont stage of *Plasmodium* infection. Within *P. falciparum*, CyRPA has been shown to be essential, highly conserved, and susceptible to cross-strain neutralizing antibodies. These characteristics have made PfCyRPA an attractive next-generation blood-stage malaria vaccine candidate that has progressed to phase I clinical trials. PfCyRPA is part of a multiprotein complex and forms a bridge between other essential erythrocyte invasion proteins PfRh5 (a vaccine candidate currently in Phase 1b/2a trials) and PfRipr (a vaccine candidate just entering Phase 1 trials). Interestingly, CyRPA exists in other *Plasmodium* species albeit in a different, unknown, yet essential complex. To better understand the still largely unknown role of CyRPA orthologues, we will determine whether CyRPA orthologues can complement PfCyRPA. We hypothesize that PfCyRPA, PkCyRPA, and PvCyRPA will be able to functionally complement each other. Completion of this research proposal will aid in identifying the role of CyRPA across plasmodium species and will offer insight into the potential of CyRPA as a species-transcendent malaria vaccine candidate.

Investigating the Relationship Between Titan's Methane Lakes and its Surface Temperature to Understand their Role on Variations in Titan's Climate System

Annalyse M. Olcott^{1,2}, Juan M. Lora^{1,2}

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Earth and Planetary Sciences, Yale University, New Haven, CT

In gaining a perspective on the global climate of Titan, particular focus must be paid to the polar regions where the moon's lakes, Kraken and Ligeia in the north and Ontario in the south, reside. These lakes are composed of methane and are part of a larger hydrological cycle on Titan. Mapping the seasonality in surface temperature at Titan's lakes over 10 Titan years simulated through the Titan Atmospheric Model reveals anomalous surface temperatures around each location. At Kraken there is a significant yet unprecedented drop in temperature to 91.7 K in the spring of the ninth mapped year. Ligeia shows highly variable surface temperatures throughout its spring and summer, occasionally dropping to temperatures as low as 91.2 K, which is also the coldest it gets during the northern winter. Ontario's spring and summer have the most variability, showing dramatic drops in temperature in the late summer of the first and eighth mapped year. Investigating these uncertainties will reveal important information about Titan's overall climate system and will be crucial to further understanding how hydrological cycles influence a planet or moon's climate and will help to explain the implications of these behaviors.

Understanding Cilia Dysfunction Using SARS-CoV-2 ORF10

¹Sorah Park, ²Svetlana Makova, ²Martina Brueckner

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Pediatrics, Yale School of Medicine, Yale University, New Haven, CT

To better understand diseases such as congenital heart disease, it is critical to understand heart development through structures such as cilia. While current research understands the significance of cilia in heart development, the pathological mechanisms of cilia dysfunction remain unknown. In a prior 2022 study on SARS-CoV-2, research found cilia dysfunction in response to SARS-CoV-2 ORF10 exposure. This research project expands on the research on SARS-Cov-2 ORF10 in impairment of cilia biogenesis and maintenance and will assess the ability of the SARS-CoV-2 gene ORF10 to be a genetic approach to removing cilia. ORF10 will be cloned into a mammalian expression vector, pCDNA. A V5 tag has been attached to the 3'end of ORF10 to detect ORF10 expression. C3 cells will be transfected with ORF10 and cilia growth will be studied through immunostaining and fluorescence microscopy. Conclusions from this research study are applicable to understanding cilia dysfunction on the genome level and paving the way towards understanding the root cause(s) of dysfunctional cilia expression and cilia dysfunction-related symptoms and diseases.

A serotonergic pathway mediating the effects of gut microbes on host feeding behavior

¹Dina Garmroudi, ²Madhumanti Dasgupta, ²Michael O'Donnell

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT

The gut-brain-axis can provide key insights into processes such brain development and neurogenesis, but also behavioral disorders and neurodegenerative disease. The nervous system is not an isolated system—microbes colonizing the gut may regulate neurons via the production of neuroactive compounds. For example, gut microbiota can produce neurotransmitters, some of which may alter animal behavior. There is evidence in animals that gut microbiota can impact host feeding decisions. However, the molecular mechanisms by which gut microbiota impact host feeding behavior are not well understood. We are interested in determining if and how naturally occurring gut commensals impact the rate of feeding in *C. elegans*. Pharyngeal pumping is a simple measure of feeding behavior in *C. elegans*. Serotonin regulates pharyngeal pumping via multiple mechanisms. When wild-type worms are colonized by *Providencia*, a naturally occurring gut commensal for *C. elegans*, feeding behavior is largely unchanged compared to worms fed laboratory *E. coli* strains. However, mutations in *ser-7*, which encodes a conserved serotonin receptor, result in reduced feeding rates specifically when colonized by *Providencia*. We hypothesize that *Providencia* either causes the worm to produce more serotonin or produces a serotonin receptor agonist. This increased serotonin signaling in the absence of SER-7 function then results in reduced feeding rates. We hypothesize that gut microbial influences on serotonin signaling may potentiate specific neuronal pathways to influence feeding.

Comparative Analysis of TP53 Alleles in Pancreatic Ductal Adenocarcinoma

^{1,2}Andy Tang, ³Sherry Agabiti, ^{3,4}Mandar Muzumdar

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT

³Yale Cancer Biology Institute, Yale University, West Haven, CT

⁴Department of Genetics, Yale University School of Medicine, New Haven, CT,

Pancreatic ductal adenocarcinoma (PDAC) is the most prevalent form of pancreatic cancer, a lethal disease with a 5-year survival rate of 12%. The gene TP53 that codes for the tumor suppressor p53 is mutated in 75% of pancreatic cancers, making it a potential target for developing novel therapies. The majority of TP53 mutations are point mutations, and in PDAC, point mutations allow for enhanced metastatic spread when compared to deletion mutations. However, functions of p53 point mutations are still unknown. Here, we will perform a rigorous comparison of in vivo functions of point (R172H) and deletion mutations of TP53 in PDAC in comparison to wild type TP53. We performed this comparison using the Mosaic Analysis with Double Markers (MADM) system in mouse models, which faithfully recapitulates human cancers while also fluorescently labeling mutant, wild-type, and knockout cells within the same mouse. Immunofluorescence was used to validate the presence of stabilized mutant p53. We show that early p53 point mutants act similarly to wild type p53 in initiating and expanding pancreatic cancer, however, the p53 point mutant resembles the p53 deletion mutation in observed survival studies.

The Plant Circadian Clock Imposes a Morning State Through CFH1 Mediated Degradation of PIF3

Harper Lowrey^{1,2}, Dr. Wei Liu², Christopher Adamcheck², Dr. Joshua Gendron²

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT

The circadian clock controls rhythmic gene expression and downstream processes that give plants essential adaptations to fluctuating environments. However, many mechanisms of circadian regulation remain unclear. Here, we identified a pure clock-regulated F-box protein, CLOCK F-BOX CONTROL of HYPOCOTYL 1 (*CFH1*), which operates within an E3 ubiquitin ligase complex to guarantee a morning for plants essential for consistent regulation independent of light conditions. *CFH1* expression is restricted to the early day, and both knockout and decoy exhibit long hypocotyl phenotypes, suggesting it's a morning-phased growth restrictor. The most dramatic hypocotyl defects are observed under red light, and *CFH1* interacts with both Cullin-1, an essential E3 ubiquitin ligase complex component, and PIF3, a transcription factor involved in light-dependent growth and red light signaling. These data suggest that *CFH1*'s hypocotyl regulation results from targeting PIF3, representing novel circadian regulation of the red light signaling pathway. Double mutant screens further support that *PIF3* is downstream of *CFH1*, and tobacco assays demonstrate that *CFH1* and PIF3 can interact in vivo. In summary, *CFH1* represents a pure clock output that imposes a morning state on plants by targeting PIF3 for degradation, illustrating a novel, post-translational integration of the circadian clock with downstream growth and light-sensing.

Endothelial Targeting with Improved Nanoparticles for Specific and Localized Use

¹Mikayla Labissiere, ²Dana Akiki, ²W. Mark Saltzman

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Biomedical Engineering, Yale University, New Haven, CT

In recent years, there has been a push for more innovative approaches to drug therapy. While there have been significant gains, major obstacles for drug delivery remain, including low circulation time, diminished uptake by target cell types, and drug degradation; these challenges are particularly important for nucleic acid drugs. Drug encapsulation in nanoparticles, which have a higher payload than the lone drug, better protection of cargo from physiologic environment, and surface tunability for different purposes, combats these issues. Our project focuses on a nanoparticle system to target endothelial cells while circulating in the bloodstream. We use two polymer types, poly(lactic acid)-block-poly(ethylene glycol) (PLA-PEG) and poly(amine-co-ester)-block-poly(ethylene glycol) (PACE-PEG), and fragmented antibodies on nanoparticle surfaces to create a targeted and tunable system for encapsulating multiple drugs and therapeutics. This approach has the potential for broad impact as it is tunable, precise in delivery, versatile, and can promote an increased quality of life for patients by decreasing off-target effects and complications. Here, we show that our antibody cleavage methodology and nanoparticle formulations are viable through a variety of characterization assays.

Identifying the Driving Mechanisms for the Recurrent 1q Aneuploidy in Human Cancers

^{1,2}Eric Stevens, ²Sarah Thompson, ^{2,3}Vishruth Girish, ²Asad Lakhani, ^{2,3}Erin L. Sausville, ²Brianna E. Mendelson, ³Monet L. Yuan, ^{2,3}Jason Sheltzer

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Surgery, Yale School of Medicine, New Haven, CT

³Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Current targeted therapies for human cancers present higher efficacy and lower toxicity than chemotherapies and radiation, but lack broad treatment application and long-term efficacy due to the heterogeneous nature of cancer. Aneuploidy, a state in which cells exhibit altered chromosome copy numbers, is observed in 90% of tumors and is not observed in somatic cells. Therefore, aneuploidy could provide novel cancer-specific targets for new treatments that could benefit a broad patient population, combining the efficacy of targeted therapies and the broad application of chemotherapies and radiation. Patient data shows that an extra copy of chromosome 1q is gained in nearly 30% of all solid tumors. Previous experiments demonstrate that 1q disomy clones generated from wild type 1q trisomy cancer cell lines display a fitness defect. Other experiments demonstrate increased activation of p53, indicating that the mechanisms in which p53 is involved in, such as apoptosis, senescence, and cell cycle delay may contribute to this recurrent aneuploidy. Here, we analyze the activation of these mechanisms through Western blotting, beta-Galactosidase staining, and cell cycle analysis. We show no activation of apoptosis, while senescence and cell cycle delays occur at higher frequencies in 1q disomy clones compared to their respective wild type lines.

Detection of Fold-Switching Members of the NusG Superfamily

¹Lydia Tarekegn, ²Ume Tahir, ²Nara Chung, ^{3,4}Lauren Porter, ²Caitlin Davis

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Chemistry, Yale University, New Haven, CT

³National Library of Medicine, National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD

⁴National Heart, Lung, and Blood Institute, Biochemistry and Biophysics Center, National Institutes of Health, Bethesda, MD

Though most proteins maintain only a single structure, proteins known as fold-switchers can adopt two different folds that perform distinct functions. However, fold-switchers are very difficult to identify. One such protein is the transcription regulator RfaH, which has a functional C-terminal domain that reversibly switches between an α -helical hairpin and a β -roll fold. NusG is a paralog of RfaH that cannot switch folds; it only adopts the β -roll. Using free RfaH and NusG, we developed a FRET assay that can distinguish between the two folds. Because the α -helical fold is more compact than the β -roll, the end-to-end distance measured by FRET was used to differentiate between the folds. We confirmed that labeling does not perturb the stability of the free proteins. Then, in vitro donor-acceptor ratios (D/A) were determined by FRET melts. Finally, mammalian cells were transfected with either FRET labeled NusG or RfaH, and the D/A was calculated from red and green fluorescence. RfaH had a lower D/A than NusG, validating that our FRET sensor can distinguish between the two folds. Preliminary data has been collected on 15 other variants of the NusG superfamily, 12 of which successfully had their fold-switching abilities predicted by D/A.

Host Spatial Structure and Viral Cheating

¹Austen Theroux, ²Asher Leeks, ²Paul Turner

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT

Viral replication often produces defective genomes that can reduce virulence, promote persistent infection, and trigger potent immune responses. Defective viral genomes (DVGs) harbor deletions in essential gene(s). With shortened genomes, ‘cheat’ DVGs replicate faster and can ‘steal’ public goods that fully intact, ‘cooperator’ viruses produce. Viruses navigate different spatial arrangements of host cells during infection, and understanding how spatial geometry impacts within-host virus evolution is crucial to improving clinical outcomes. Here, we model cheat-cooperator population dynamics on evolutionary graphs to simulate how spatial structure influences the evolution of viral cheating. We develop this using a game-theoretic model of social interactions generalized to study the behavior of populations on a network. We then intend to supplement model predictions with *in vitro* experimental evolution of cheating in phage M13 and tissue-specific genomics analysis of SARS-CoV-2 and influenza A clinical samples.

Characterizing the Outgassing of Electronegative Impurities in nEXO

^{1,2}Barkotel Zemenu, ²Sierra Wilde, ²Glenn Richardson, ^{2,3}Ako Jamil, ²Prof. David C. Moore

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Physics, Yale University, New Haven, CT

³Now at Department of Physics, Princeton University, Princeton, NJ

Neutrinoless double beta decay (0νββ) is a hypothetical rare nuclear process that can occur if neutrinos are their own antiparticles, a property that might explain why matter dominates antimatter in the universe. nEXO is an upcoming project searching for this decay in Xenon-136 using a detector filled with 5 tonnes of liquid xenon. When 0νββ occurs, two neutrons decay into two protons, emitting a pair of electrons that carry the entire decay energy. The more efficiently nEXO reconstructs the electron pair's energy, the more accurately the decay energy is known and 0νββ can be separated from backgrounds. However, the outgassing of electronegative impurities via diffusion from detector materials compromises this reconstruction. Our study aims to quantify this diffusion of impurities, compare it across candidate nEXO materials, and develop a model to ensure that nEXO meets its design goals for electronegative impurities.

Investigating the effect of bacterial diversity on the evolution of *Pseudomonas aeruginosa* virulence in response to phage H6

William An^{1,2}, Michael Blazanin¹, and Paul Turner¹

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT

Antibiotic-resistant bacterial pathogens are a growing threat to human health. In 2019, the US CDC identified *Pseudomonas aeruginosa* as one of the most serious antibiotic-resistant threats. *P. aeruginosa* is implicated in a variety of human infections, including frequently infecting the lungs of individuals with cystic fibrosis (CF). Phages (viruses that infect bacteria) are an emerging therapy for these antibiotic-resistant infections. However, phage treatment can have effects beyond simply reducing bacterial populations. For instance, it can drive bacterial evolution, including of virulence traits. Moreover, this evolution can be altered by numerous factors, including the presence of other bacterial species. Despite this, how bacterial diversity alters phage-driven evolution of a target pathogen remains unclear. Here, we study the effect of bacterial diversity on the bacterium *P. aeruginosa* in response to phage H6, using *Staphylococcus aureus*, *Enterococcus faecalis*, and *Achromobacter xylosoxidans*. These species are known to co-infect CF patients with *P. aeruginosa* but are not susceptible to H6. We experimentally evolved *P. aeruginosa* in the presence or absence of H6 and the three other bacterial species. We observed evolution in bacterial virulence by measuring biofilm formation, pyocyanin production, growth rates, and motility. Ultimately, this work helps reveal how bacterial diversity can affect pathogen evolutionary responses to phage therapy.

Persistence Length of Chromatin in the Nuclear Context

Akua A. Agyemang^{1,2,3}, Misheal Saah³, Emily Clarke⁴, Ryan Ogasawara³, Thomas Moss⁴, Ivan Surovtsev³, Megan C. King³

¹Science Technology and Research Scholar, Yale College, New Haven, CT

²Department of Molecular Biophysics and Biochemistry, Yale School of Medicine, New Haven, CT

³Department of Cell Biology, Yale School of Medicine, New Haven, CT

⁴NSF REU Summer Program, Yale University, New Haven, CT

Chromatin dynamics and organization has confounded researchers for decades. The entirety of the human genome—6 gigabases, over 2 meters of DNA in length resides in the nucleus with a diameter of only 10 micrometers. Within the nucleus, DNA is wrapped around histones, and takes on higher orders of organization to form chromosomes. Currently, the movement of chromatinized DNA is not fully understood, especially within the context of live cells. The stiffness of chromatinized DNA is of particular interest for analyzing the formation of loops and other structures within chromatin to allow for inter-loci interactions. The fission yeast *Schizosaccharomyces pombe* is a prime model organism for studying eukaryotic cell processes. With a 13.8 megabase-genome stored in a nucleus 2-3 μm in diameter, similar questions of genetic compaction and dynamic ability within the nucleus arise. Additionally, *S. pombe* allows for easy genetic manipulation and displays much of the chromosomal organization seen in eukaryotes. This project aims to determine the persistence length, or rigidity, of chromosomal DNA in live cells, which will give greater understanding of how chromatin structure relates to loci interactions.

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