32nd Keck Annual Research Conference: Structural Biology: Past, Present and Future

October 21, 2022

Conference Chair: Irina Serysheva, UT Health Science Center
Xiaodong Cheng, MD Anderson Cancer Center
Co-chair: Mariah Baker, UT Health Science Center
Gundeep Kaur, MD Anderson Cancer Center

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Structural Biology: Past, Present and Future

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Irina Serysheva, PhD
Structural Biology Imaging Center, UT Health Science Center at Houston

Xiaodong Cheng, PhD
Molecular and Cellular Oncology, MD Anderson Cancer Center

Conference Co-Chairs

Mariah Baker, PhD
Biochemistry and Molecular Biology, UT Health Science Center at Houston

Gundeep Kaur, PhD
Epigenetics and Melecular Carniogenesis, MD Anderson Cancer Center

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The Keck Center

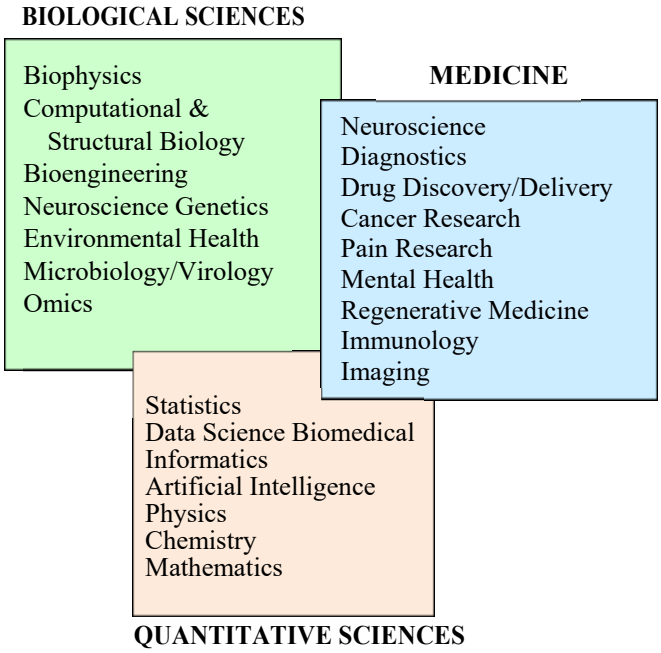
The Keck Center, established in 1990 with support from the W. M. Keck Foundation, celebrates its 32nd year of supporting predoctoral and postdoctoral trainees and their mentors. From the founding institutions, Baylor College of Medicine and Rice University, the Keck Center grew in its first 10 years to six major public and private institutions in the Houston/Galveston area, including University of Houston, The University of Texas Health Science Center at Houston, The University of Texas Medical Branch at Galveston, and The University of Texas MD Anderson Cancer Center. The Institute of Biosciences and Technology of Texas A&M Health Science Center joined in 2015. The Houston Methodist Research Institute joined in 2022. Guiding the formation of this collaboration was the realization that significant advances in the biological sciences, such as the DNA sequencing of the human genome, would be driven by the integration of biology and computer science. The partners realized, however, that most biological scientists were not prepared to capitalize on novel approaches to visualization, analysis and interpretation of experimental data made possible by rapid advances in computing technology. Moreover, most researchers in computer programming and analysis systems did not have adequate knowledge about biology and biological systems. The Keck Center was explicitly designed to bridge this gap between biological and computational sciences by fostering collaborations among scientists through specially designed research and training programs.

Building on its expertise in interdisciplinary, inter-institutional programs, the Keck Center's focus has evolved to the quantitative biomedical sciences. Participants are drawn from various disciplines such as biophysics, chemistry, bioengineering, neuroscience, computer science, biochemistry, genetics, physics, mathematics, data science, biomedical informatics, environmental health, biology and statistics. Currently, the Keck Center administers training programs in biomedical informatics and data science, molecular biophysics, pharmacological sciences, computational cancer biology, precision environmental health, antimicrobial resistance, cancer therapeutics, and infectious diseases.

Gulf Coast Consortia

In March 2001, the presidents of each of the six-member institutions of the Keck Center signed an unprecedented agreement establishing the Gulf Coast Consortia (GCC), explicitly designed to coalesce institutional strengths in order to:

1. train new scientists at the intersection of biological sciences with quantitative and physical sciences
2. build cutting-edge research infrastructure and facilities
3. cultivate a supportive atmosphere for the collaboration of basic and translational scientists, researchers, clinicians and students in both biological and non-biological fields
4. apply the resulting knowledge to prevent and treat diseases.



While the Keck Center serves as the training arm of the GCC, the research arm consists of individual, topic-focused research, including translational pain research, antimicrobial resistance, cellular and molecular biophysics, regenerative medicine, drug discovery and development, mental health research, single cell omics, immunology, translational imaging, artificial intelligence in health care, and theoretical and computational neuroscience. These consortia and newly forming clusters provide a supportive environment for the encouragement and development of research that might otherwise be beyond the reach of any one institution. New consortia form when faculty come together around a common interest, establishing a working vision and engaging a broad faculty community to pursue interinstitutional research, present conferences, acquire shared equipment and research cores and/or develop training, research or curriculum grants.
https://www.gulfcoastconsortia.org/

2022 Logo Design Information

Logo Design by:

Gabriel Tukeman

Predoctoral Student,
Molecular and Cellular Biology
Training in Precision Environmental Health Sciences
Baylor College of Medicine

Acknowledgements

The Keck Center thanks the following for their generous support:

National Library of Medicine (NLM)
NLM Training Program in Biomedical Informatics and Data Science T15 LM007093
Program Director: Lydia E. Kavraki, PhD, Rice University

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Training Interdisciplinary Pharmacology Scientists T32 GM139801
Program Director: Carmen W. Dessauer, PhD, UT Health Science Center at Houston

Houston Area Molecular Biophysics Program T32 GM008280 Program Director: Theodore G Wensel, PhD, Baylor College of Medicine

National Institute of Environmental Health Sciences (NIEHS)
Training in Precision Environmental Health Sciences T32 ES027801
Program Director: Cheryl L. Walker, PhD, Baylor College of Medicine

National Institute of Allergy and Infectious Diseases (NIAID)
Molecular Basis of Infectious Diseases T32 AI055449
Program Director: Theresa M. Koehler, PhD, UT Health Science Center at Houston

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Program Director: Anthony R. Flores, MD, PhD, UT Health Science Center at Houston

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32nd Keck Annual Research Conference

Structural Biology: Past, Present, and Future

Chairs:
Irina Serysheva, PhD, Professor and Director, Structural Biology Imaging Center, UTHealth
and
Xiaodong Cheng, PhD, Professor, Epigenetics and Molecular Carcinogenesis, MD Anderson Cancer Center

Co-Chairs:
Mariah Baker, PhD, Assistant Professor, Biochemistry and Molecular Biology, UTHealth
and
Gundeep Kaur, PhD, Postdoctoral Fellow, Epigenetics and Molecular Carcinogenesis, MD Anderson Cancer Center

Friday, October 21, 2022

8:15 Welcome: Keck Center Overview and Conference Day Overview
Irina Serysheva and Xiaodong Cheng

SESSION 1 Chairs: Irina Serysheva, PhD & Mariah Baker, PhD

8:25-9:25 **Keynote Speaker**: (50 Min + 10 Min Q&A)
Edward Egelman, PhD, Professor, Biochemistry and Molecular Genetics, University of Virginia
CryoEM of Polymers: From Proteins to Peptides

9:25-10:05 **Guest Speaker:** (30 min + 10 Min Q&A)
Youxing Jiang, PhD, Professor, Physiology, UT-Southwestern Medical Center
Structural Insights into the Assembly, Gating, and Selectivity of Mitochondrial Calcium Uniporter

10:05-10:15 Keck Center Trainee Speaker 1 (10 min)
Mauricio Menegatti Rigo, PhD, Computer Science, Rice University; Computational Cancer Biology Training Program
Developing Structural Tools for Immunotherapy Improvement

10:15-10:25 Keck Center Trainee Speaker 2 (10 min)
Jordan Johnson, Biology and Biochemistry, University of Houston; Houston-Area Molecular Biophysics Program
Single-Molecule Studies of EF-Tu Accommodation and Proofreading Mechanism

10:25-11:20 Poster Session and Coffee Break

11:20-11:30 Keck Center Trainee Speaker 3 (10 min)
Astrid Manuel, School of Biomedical Informatics, The University of Texas Health Science Center at Houston; NLM Training Program in Biomedical Informatics and Data Science
Innovating Drug Repositioning Strategies for Multiple Sclerosis via Translational Bioinformatics Approaches

11:30-11:40 Keck Center Trainee Speaker 4 (10 min)
William Shropshire, PhD, Infectious Diseases and Infection Control, The University of Texas MD Anderson Cancer Center; Training Program in Antimicrobial Resistance
Elucidation of Molecular Mechanisms Underlying Successful Adaptation to Carbapenem Antimicrobials in High Risk Carbapenem Resistant Escherichia coli Lineages

11:40-12:20 **Guest Speaker:** (30 min + 10 min. Q&A)
Naoko Mizuno, PhD, Structural Cell Biology Lab, National Heart, Lung, Blood Institute,
Molecular Neurobiology by Cryo-ET

12:20-12:50 Lunch Break

SESSION 2 Chairs: Xiaodong Cheng, PhD and Gundeep Kaur, PhD

12:50-1:50 **Keynote Speaker:** (50 min + 10 Min Q&A)
Scott Stagg, PhD, Professor, Chemistry and Biochemistry, Institute of Molecular Biophysics, Florida State University
Determining the Structures of Challenging Membrane Remodeling Complexes

1:50-2:30 **Guest Speaker** (30 min + 10 min. Q&A);
Gabrielle Rudenko, PhD, Professor, Toxicology and Pharmacology, Sealy Center for Structural Biology and Molecular Biophysics, UT Medical Branch at Galveston
Drugging the Redox Switch in the Transcription Factor ΔFosB - a Potential Therapeutic Target for Neuropsychiatric and Neurological Disorders

2:30-2:40 Keck Center Trainee Speaker 5 (10 min)
Miranda Lewis, Molecular Virology and Microbiology, Baylor College of Medicine; Training Interdisciplinary Pharmacology Scientists
Evaluation of Nitazoxanide as an Antiviral for Human Norovirus Using Human Intestinal Organoids

2:40-2:50 Keck Center Trainee Speaker 6 (10 min)
Hannah Wilson, Microbiology and Infectious Disease, The University of Texas Health Science Center at Houston; Molecular Basis of Infectious Diseases
Characterizing C. albicans Morphogenesis Regulation in the Context of Host Macrophages

2:50-3:45 Poster Session and Coffee Break

3:45-3:55 Keck Center Trainee Speaker 7 (10 min)
Rachel Keuls, Development, Disease Models and Therapeutics Graduate Program, Baylor College of Medicine; Training in Precision Environmental Health Sciences
Yolk Sac-Derived Cells Are Required for Brain Development

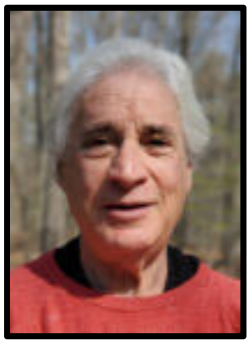
3:55-4:05 Keck Center Trainee Speaker 8 (10 min)
Kehinde Idowu, PhD, Center for Biomedical and Minority Health Research, Texas Southern University; Cancer Therapeutics Training Program
The Design and Development of GMC1 Analogues: Targeting the Regulation of FKBP52 and Hormonal Receptors in Prostate Cancer Cells

4:05-4:45 **Guest Speaker:** (30 min + 10 min. Q&A):
Tom Terwilliger, PhD, Laboratory Fellow, Los Alamos National Lab
AlphaFold Changes Everything

4:45-5:00 Awards and Closing / Reception Following

Speaker Abstracts
(in order of appearance)

CryoEM of Polymers: From Proteins to Peptides

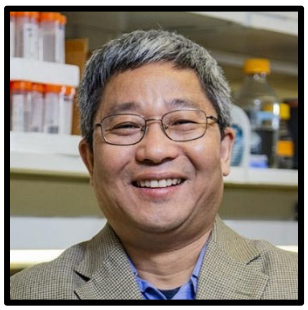


Edward Egelman, PhD
Professor
Biochemistry and Molecular Genetics
School of Medicine
University of Virginia
https://med.virginia.edu/faculty/faculty-listing/ehe2n/

Dr. Egelman is a biophysicist known for his work on the structure and function of protein and nucleoprotein polymers. He developed the algorithm that is now widely used in cryo-electron microscopy for the three-dimensional reconstruction of helical filaments and tubes. His research has ranged from studies of actin to bacterial pili to viruses that infect hosts living in nearly boiling acid. He was born in New York and graduated from Brandeis University in 1976 with a B.A. in physics. He started as a Ph.D. student in experimental high energy physics at Harvard, but changed fields and received his Ph.D. from Brandeis University in 1982 in biophysics. He was a Jane Coffin Childs postdoctoral fellow at the Medical Research Council Laboratory of Molecular Biology in Cambridge, UK, and became an Assistant Professor at Yale University in 1984. In 1989, he moved to the University of Minnesota where he was an Associate and Full Professor, and in 1999, moved to the University of Virginia where he is now a Harrison Distinguished Professor. He has been president of the Biophysical Society and Editor-in-Chief of Biophysical Journal, and is a Fellow of both the Biophysical Society and the American Academy of Microbiology. In 2019, he was elected to the National Academy of Sciences.

Abstract: Cryo-EM has emerged as the main technique for determining the atomic structure of macromolecular assemblies. I will discuss our applications of cryo-EM to a range of polymers, including bacterial and archaeal pili, bacterial and archaeal flagella, extracellular cytochrome filaments that conduct electrons over long distances (“microbial nanowires”) and filamentous viruses that infect hosts living in nearly boiling acid. Watson and Crick observed in 1956 that almost all viruses were spherical (icosahedral) or rod-like (helical) and explained this in terms of symmetry: both allowed many copies of a single protein to encapsulate a genome. The basis for spindle-shaped viruses that infect archaea has therefore been a puzzle, as they cannot be explained by either icosahedral or helical symmetry. We show how they actually evolved from helical archaeal viruses, driven by the need to package a larger genome, and that these capsids can be explained by the idea of quasi-equivalence, first postulated by Caspar and Klug in 1962 with regard to icosahedral viruses. The powerful methods that have been developed in cryo-EM of biological complexes can now be readily applied to assemblies of peptides and small molecules. Cryo-EM is thus beginning to make a real impact in areas such as materials science, soft matter and chemistry.

Structural Insights into the Assembly, Gating, and Selectivity of Mitochondrial Calcium Uniporter



Youxing Jiang, PhD
W.W. Caruth, Jr. Scholar in Biomedical Research
Rosewood Corporation Chair in Biomedical Science
Physiology
University of Texas-Southwestern Medical Center
https://profiles.utsouthwestern.edu/profile/58081/youxing-jiang.html

Dr. Youxing Jiang is a chaired professor of physiology (Rosewood Corporation Chair in Biomedical Science) and a W.W. Caruth, Jr. Scholar in Biomedical Research at The University of Texas Southwestern Medical Center (UT Southwestern) in Dallas. In 2008, Dr. Jiang became an investigator of the Howard Hughes Medical Institute. He received his Bachelor of Science degree in 1992 from Peking University and his Ph.D. in chemistry from Yale University in 1997. Dr. Jiang completed his postdoctoral training at Rockefeller University with Nobel Laureate Dr. Roderick Mackinnon, during which time he was at the forefront of groundbreaking research in the ion channel field. In 2003, Dr. Jiang was recruited to the Department of Physiology at UT Southwestern to launch his independent research program. Dr. Jiang was promoted to associate professor with tenure in 2008 and full professor in 2012. Dr. Jiang is a recipient of the Searle Scholar Award, the David and Lucile Packard Fellowship, and the Edith and Peter O'Donnell Award.

Abstract: Mitochondria can take up large amounts of Ca^{2+} from their environment, a process that can modulate ATP production, alter cytoplasmic Ca^{2+} dynamics, and trigger cell death. Ca^{2+} enters the mitochondrial matrix through the mitochondrial calcium uniporter, a highly selective Ca^{2+} channel that is localized to the inner mitochondrial membrane. In humans, the uniporter is a protein complex or uniplex consisting of at least four components: the ion conducting pore MCU, the essential membrane spanning subunit EMRE, and the Ca^{2+}-sensing gate-keeping proteins MICU1 and MICU2. While MCU is found in all major eukaryotic taxa, EMRE is metazoan-specific and is required for the conductivity of MCU in these organisms. On the contrary, the pore-forming MCU is the only component of the uniporter in most fungi based on genome sequence analysis and likely represents the minimal channel component of the uniporter for Ca^{2+} uptake. My lab aims to address the fundamental questions about the uniporter's assembly, gating, and ion permeation properties by determining the structure of the uniporter, focusing first on the MCU component and then, ultimately, the uniplex.

Molecular Neurobiology by cryo-ET

Naoko Mizuno, PhD
Senior Investigator
Laboratory of Structural Cell Biology
National Heart, Lung, Blood Institute
https://irp.nih.gov/pi/naoko-mizuno

Naoko Mizuno graduated from the University of Tokyo in 1999, and received her Ph.D. in biophysics from the University of Tokyo/University of Texas Southwestern Medical Center in 2005. Prior to joining the NHLBI, she spent 8 years as an independent group leader at the Max Planck Institute of Biochemistry, Martinsried in Germany. Dr. Mizuno received several European awards and honors, including EMBO Young Investigators award, Boheringer Ingelheim Plus3 programme, and ERC consolidator grant. Dr. Mizuno serves as an editor of FEBS letter, evaluator for H2020 program of European Union.

Abstract: Neurons are highly polarized cells forming an intricate network of dendrites and axons. They are shaped by the dynamic reorganization of cytoskeleton components and cellular organelles. Axon growth is controlled by the external stimuli. Axon branching allows the formation of new paths and increases circuit complexity. Finally, neuronal maintenance, the process of regeneration and degeneration are critical points for the remodeling of the circuit. However, our understanding of the neuronal morphogenesis is sparse at a molecular level due to the lack of direct in-depth observations. Using in situ cellular cryo-electron tomography on primary neurons, we aim to understand the molecular actions of the remodeling of organelles and cytoskeleton structures during the critical events of neuronal morphogenesis. In this talk, I will discuss about our recent molecular findings of neuronal regeneration.

Determining the Structures of Challenging Membrane Remodeling Complexes

Scott Stagg, PhD
Professor
Chemistry and Biochemistry
Institute of Molecular Biophysics
Florida State University
https://www.bio.fsu.edu/faculty.php?faculty-id=sstagg

Scott Stagg received his PhD in biochemistry from the University of Alabama at Birmingham. He has over a decade of experience in 3DEM. He is a developer of the Leginon and Appion software packages for automated cryo- EM, and his lab has used that software to determine the structures of COPII complexes and to systematize the data acquisition and processing to drive the EM reconstruction process to higher resolution. His lab had one of the first sub 3Å 3D reconstructions at the advent of the cryo-EM in 2015. On the biological side, his lab is interested in determining the structures and mechanisms of complexes in the secretory pathway. They have been studying the structures of COPII complexes assembled in vitro, the structures of tubular complexes that are involved in membrane remodeling, and the structures of natively assembled clathrin coated vesicles.

Abstract: Membrane remodeling complexes, by their nature, are pleiomorphic, forming coats of different shapes and sizes in order to capture deformations in membranes or bend membranes into specific shapes. These features can make membrane remodeling complexes challenging targets for structure determination by cryogenic electron microscopy (cryo-EM). Structure determination by single particle cryo-EM depends on imaging identical copies of the specimens of interest in different orientations. Coat proteins usually form repeating units on membrane often with only relatively small differences from subunit to subunit. Therefore, while the overall complexes are different from one to the next, the individual subunits that make them up are quite similar. We will discuss the strategies we have used to identify the alignable units on membrane remodeling complexes and will discuss the biological insights we have discovered by using these techniques on COPII cages, clathrin coats, and Drp1.

Drugging the Redox Switch in the Transcription Factor ΔFosB - a Potential Therapeutic Target for Neuropsychiatric and Neurological Disorders

Gabrielle Rudenko, PhD
Professor
Toxicology and Pharmacology
Sealy Center for Structural Biology and Molecular Biophysics
University of Texas Medical Branch at Galveston
https://www.utmb.edu/phtox/faculty-staff-students/faculty-and-staff/gabrielle-rudenko

Dr. Rudenko received her Ph.D. training at the Rijksuniversiteit Groningen (Netherlands) and University of Washington (USA). Subsequently, Dr. Rudenko carried out postdoctoral training at UT Southwestern Medical Center (USA) mentored by Professor Johann Deisenhofer collaborating with Professors Thomas Südhof, Michael Brown and Joseph Goldstein on the three-dimensional structure of cell surface receptors and synaptic adhesion molecules.

Dr. Rudenko's laboratory focusses on proteins implicated in various neuropsychiatric disorders. The long-term goal is to leverage their 3D structural information to develop novel therapeutic strategies. Targets under study include proteins implicated in autism spectrum disorder, schizophrenia, drug addiction, cognitive decline, as well as other neuropsychiatric disorders. They use a combination of structural biology, biochemical and biophysical methods, proteomics, and chemical genomics.

Abstract: ΔFosB is an unusually stable transcription factor that accumulates uniquely to very high levels in specific regions of the brain following insults such as chronic exposure to drugs of abuse. ΔFosB accumulation drives locomotor responses to drugs of abuse, drug reward, drug self-administration and relapse. ΔFosB also accumulates in brain in response to Alzheimer's disease where it contributes to cognitive decline, and Parkinson's disease where it is involved in mediating dyskinesia. ΔFosB is an attractive drug target. However, it is also a highly challenging one because it lacks typical 'druggable' molecular features like a deep active site found in enzymes, and because it is generally difficult to identify compounds that can interfere with DNA binding. In addition, the molecular basis for ΔFosB action is not understood well yet.

We are delineating the molecular mechanism of ΔFosB and de-risking ΔFosB as a highly novel drug target. We have characterized molecular and structural features of ΔFosB, in particular its DNA binding site, and discovered a redox switch that controls DNA binding. The redox switch renders ΔFosB susceptible to small molecule binding, and we have leveraged it to identify compounds that disrupt ΔFosB's ability to bind to DNA, demonstrating that ΔFosB is indeed 'druggable'. By manipulating ΔFosB with small molecules, it may be possible to exert selective effects on gene transcription, and guide neural and behavioral outcomes in a therapeutically beneficial manner, for example, following exposure to drugs of abuse. We will report on our recent progress.

Alphafold Changes Everything (And Nothing)

Tom Terwilliger, PhD
Laboratory Fellow
Los Alamos National Lab
https://solve.lanl.gov/terwilliger/

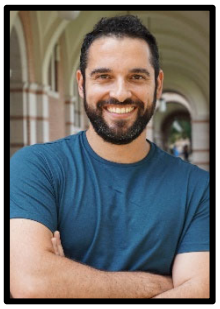
Tom Terwilliger received his PhD in Molecular Biology from the University of California at Los Angeles. He is a member of the Phenix team and has helped structural biologists carry out macromolecular structure determination with X-ray crystallography and cryo-EM by creating easy-to-use automated tools. His SOLVE software incorporated decision-making algorithms to solve structures by X-ray crystallography with experimental phasing, and his RESOLVE software optimized density maps with likelihood-based density modification and automatically interpreted the maps to yield atomic models. Recently he has created structure determination tools for fully automatic analysis of cryo-EM maps and for using AlphaFold predictions in structure determination. In addition to his work developing methods, he helped found the field of Structural Genomics, led the International Structural Genomics Organization from its inception, and was the leader of two large-scale structural genomics projects.

Abstract: The ability to create an AlphaFold model for any sequence in a few minutes changes every protein crystal structure determination into a molecular replacement problem and every protein cryo-EM structure determination into a docking problem. Making this even more transformative is the ability to iteratively improve AlphaFold modeling by docking an AlphaFold model into density, rebuilding it, and using the rebuilt model as a template for further AlphaFold model generation (Terwilliger et al., 2022). These features of AlphaFold will make structure determination by crystallography and cryo-EM easier and more powerful than ever before, but do not fundamentally change the importance of the experiment. Anyone can carry out these steps easily using Phenix and free cloud-based Google Colab notebooks.

Trainee Speaker Abstracts

(In order of appearance)

Developing Structural Tools for Immunotherapy Improvement



Mauricio Menegatti Rigo, PhD
Postdoctoral Research Associate
Computer Science
Rice University
Computational Cancer Biology Training Program
Developing Structural Tools for Immunotherapy Improvement

Mauricio M. Rigo graduated with a major in Biomedical Sciences. He completed his PhD in Genetics and Molecular Biology at Federal University of Rio Grande do Sul (Brazil). His research interests focus on developing in silico tools for the study of protein structures that can ultimately guide the success of new immunotherapies. His goal is to better understand the recognition patterns involving Human Leukocyte Antigens and T Cell Receptors interaction in cellular immunity. Mauricio conducts his research co-advised by mentors Dr. Lydia E. Kavraki and Dr. Gregory Lizée.

Abstract: Immunotherapy treatments have revolutionized the clinical approach for patients with cancer. Contrary to conventional approaches such as chemotherapy, immunotherapy is designed to improve the competence of immune cells against cancer cells. In this scenario, T-cell lymphocytes are able to recognize tumor-derived peptides displayed at the surface of cancer cells by Human Leukocyte Antigens (HLAs), promoting the tumor elimination. Although there exist computational pipelines designed to identify and suggest new tumor-specific peptide-targets that activate specific T-cell lymphocytes receptors (TCRs), undesired outcomes such as low affinity T-cells and immune evasion hamper progress in this field. We hypothesize this happens mainly because computational methods do not take into account the protein flexibility between the peptide-HLA complex (pHLA) and the TCR. Our goal is to create a platform that allows practitioners with different expertise to perform protein-protein molecular docking using ensembles of pre-computed conformations from pHLAs and TCRs.

Single-Molecule Studies of EF-Tu Accommodation and Proofreading Mechanism

Jordan Johnson
PhD Student
Biology and Biochemistry
University of Houston
Houston Area Molecular Biophysics Program

Jordan Johnson is a third-year biochemistry graduate student at University of Houston and a fellow under the Molecular Biophysics Training Program (HAMBP). She works under Dr. Yuhong Wang in her lab focusing on the ribosome and translation. Her current work is assessing the effects of mutated elongation factors on ribosomal translation and how that can contribute to the development of neurological disorders.

Abstract: Delivery of aminoacyl-tRNA to the ribosome during translation is assisted by elongation factor thermal unstable (EF-Tu). During this process the EF-Tu ternary complex detects the correct codon and initiates delivery at the cost of GTP. Although the translation process is meticulously monitored, errors can occur such as when assisting factors have mutations. Localized mutations EF-Tu, more specifically eukaryotic homolog eEF1A, have been linked to various health issues such as Huntington's disease, ADHD, epilepsy, intellectual disability, and depression; however, as factors of EF-Tu's mechanism is highly debated, it is not well understood how these mutations contribute to these diseases. To observe and understand EF-Tu's mechanism and conformational changes, mutations at D81 were introduced into the GTP binding pocket and will be observed using single-molecule studies, such as FRET and FIRMS, to determine the conformational changes and interactions with the ribosome. Ternary complex formation and GTPase activity will also be assessed to determine the effect of the mutations on enzymatic activity. Once the single molecule studies have been analyzed, we can survey antibiotics to test their effects on mutant EF-Tu function and conformation to help gather useful information for the further development of these antibiotics.

Innovating Drug Repositioning Strategies for Multiple Sclerosis via Translational Bioinformatics Approaches

Astrid M. Manuel
PhD Student
School Informatics and Data Science
The University of Texas Health Science Center at Houston
NLM Training Program in Biomedical Informatics and Data Science

Astrid M Manuel earned her Bachelor of Science (B.Sc.) degree in Biological Sciences from Florida International University in Miami, Florida. She is now a 5th year PhD student at the School of Biomedical Informatics, University of Texas Health Science Center (UTHealth) in Houston, Texas. She is also a Predoctoral Fellow of the National Library of Medicine (NLM) Training Program in Biomedical Informatics and Data Science, under the supervision of primary mentor Dr. Zhongming Zhao and secondary mentor Dr. Assaf Gottlieb. Her fellowship project aims to pinpoint drug repositioning strategies for multiple sclerosis by linking genetic risk factors to drug target genes via network-based bioinformatics approaches.

Abstract: Multiple sclerosis (MS) is an autoimmune disease in which immune-mediated demyelination of the central nervous system (CNS) leads to serious neurological deficits. Investigators postulate that MS is a genetically predisposed disease, onset after a suspected environmental trigger. Despite recent advancements in MS treatment, it remains that several patients still experience treatment-resistant MS. However, drug development is a time-consuming and risky process; on the other hand, drug repositioning strategies, which find new indications for existing drugs, have had higher rates of success and lower investment costs. We hypothesize that better understanding of the genetic risk factors of MS may aid to discover underlying biological pathways of MS pathology and to pinpoint potential repurposable drug candidates. The most recent and largest genome-wide association study (GWAS) of MS, which quantitatively measured the genotypes of MS cases and healthy controls, identified 233 genetic variants associated with MS with genome-wide significance. We use network-assisted methods to integrate this GWAS data with other disease-specific molecular (proteomic, transcriptomic and epigenomic) datasets to

investigate the complex genetic architecture of MS. We pinpoint potential gene-environment interactions in MS and link genetic risk factors of MS to existing drug target genes. Based on the enrichment of drug target genes in MS-associated gene networks, we propose potential repurposable drug candidates for MS, including the leukotriene receptor antagonist montelukast, *HDAC1* inhibitor vorinostat, and *ELANE* inhibitor sivelestat. Currently, we are using administrative health claims data of the Optum UTHealth database to evaluate the potential clinical outcomes of these drug repositioning strategies.

Elucidation of Molecular Mechanisms Underlying Successful Adaptation to Carbapenem Antimicrobials in High Risk Carbapenem Resistant Escherichia coli Lineages

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William Shropshire earned his B.A. in Biochemistry from the University of Texas at Austin in 2010, completed a postbaccalaureate fellowship at the NIH in 2013, and obtained his PhD in Epidemiology from the UTHealth School of Public Health in 2020. His PhD training focused on investigating the molecular epidemiology of carbapenem resistant Enterobacterales and their antimicrobial resistance mechanisms within the Houston, TX region. Currently, Dr. Shropshire is a second-year postdoctoral research fellow at the University of Texas MD Anderson Cancer in the Department of Infectious Diseases and Infection Control under the guidance of Dr. Samuel Shelburne. As part of his T32 training program, his work has shifted towards experimental investigation of clinical Enterobacterales isolates to understand the evolutionary pathways that underlie the successive adaptation to carbapenem selective pressures.

Abstract: Escherichia coli is a leading cause of human infection and a major contributor to the epidemic of antimicrobial resistant (AMR) bacteria. Thus, there is an urgent need to understand how certain E. coli populations successfully adapt to antibiotic treatments in clinical settings. One of the most challenging AMR E. coli infections are those that are resistant to carbapenems, which are considered as last-resort antibiotic treatments. While there has been extensive research on carbapenem resistant E. coli, there remains a knowledge gap of how particular high-risk E. coli lineages are able to adapt to initial antibiotic exposure, which can be conceptualized as a ‘pre-resistant’ phase. One of the most prolific high-risk E. coli lineages is sequence type 131 (ST131), which includes certain sub-populations that readily develop carbapenem resistance. We used a combination of multiple experimental evolution platforms and computational biology techniques to analyze the early adaptive response of ST131 E. coli to carbapenems. We identified evidence of early phenotypic changes predicted to reduce carbapenem entrance into the E. coli cell prior to a fully carbapenem resistant phenotype. Moreover, we found that at the same time ST131 E. coli also rapidly responds to carbapenem exposure by increasing the copy number of antimicrobial resistant genes, which in combination with reduced carbapenem entrance could allow for cell survival in the presence of a carbapenem. Importantly, these changes occur prior to fixed genetic mutations that are ultimately found in fully carbapenem resistant strains. The long-term goals of our holistic investigation of the central tenets of these pre-resistant isolates are to identify which ST131 E. coli strains have the capacity to develop carbapenem resistance following carbapenem exposure and to

fully understand the adaptive strategies of these high-risk bacteria to assist with the development of novel preventative approaches.

Evaluation of Nitazoxanide as an Antiviral for Human Norovirus Using Human Intestinal Organoids

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Miranda Lewis received her B.S. in Microbiology and Infectious Diseases at the University of Texas at Austin and is currently a 5th-year Molecular Virology and Microbiology graduate student. Her research aims to evaluate antiviral drug toxicity in human intestinal organoids and characterize antiviral traits of the compound nitazoxanide. Using the pathogen human norovirus as her model, she aims to determine activity, mutagenesis capability, and possible mechanisms of action of nitazoxanide. Miranda is training in the laboratory of Dr. Mary Estes and has completed 2 years of her TIPS fellowship training program with Dr. Timothy Palzkill as her co-mentor.

Abstract: Human noroviruses (HuNoVs) are the leading cause of acute vomiting and diarrhea. In healthy people, symptoms usually resolve within three days; however, in immunocompromised persons, HuNoV infection can become persistent, debilitating, and life-threatening. There are currently no licensed therapeutics for HuNoV due to a near half-century delay in its cultivation. Treatment for chronic HuNoV infection in immunosuppressed patients includes off-label nitazoxanide (NTZ), a broad-spectrum antimicrobial. Nitazoxanide shows antiviral activity in an in vitro RNA replicon model of genotype GI.1 HuNoV RNA expression. However, this drug has not been evaluated in infections with commonly circulating HuNoVs. Nontransformed, multicellular human intestinal organoids (HIOs) are a physiologically relevant cell culture system derived from intestinal stem cells that support replication of HuNoV. HIOs have great potential for antiviral studies, as they are permissive for several HuNoV strains and can be generated from different donors, allowing evaluation of the diversity of human responses. Despite these advantages, few studies have used HIOs for antiviral research. A pipeline for NTZ testing was established to inoculate a standard viral dose using 100 half maximal tissue culture infectious doses (TCID0s) and treat cells with 5 ascending drug doses in tandem with cytotoxicity testing. Antiviral activity of NTZ was measured based on viral RNA replication 24-48 hours after infection of HIOs with or without drug treatment. Cell viability was measured to demonstrate that replication inhibition was not due to cytotoxicity across the therapeutic range of the compound. NTZ showed antiviral activity in HIOs; strain-specific responses were observed and mechanisms for these differences are under investigation. HIOs provide a pre-clinical platform to test antivirals against HuNoVs and develop therapeutics to treat norovirus disease.

Characterizing C. albicans Morphogenesis Regulation in the Context of Host Macrophages

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Hannah completed her B.S. in Microbiology at Texas A&M University and is now a fifth year Ph.D student in the Microbiology and Infectious Diseases program at UTHealth Houston. Her research interests involve uncovering the molecular mechanisms that underly deadly fungal infections caused by the opportunistic yeast Candida albicans in immunocompromised hosts. Specifically, she is seeking to understand the signals and mechanisms by which C. albicans escapes from macrophages (innate immune cells) after engulfment. Hannah is advised by Dr. Michael Lorenz.

Abstract: Candida albicans is the most clinically relevant fungal pathogen. Because existing treatment options are limited and exhibit poor efficacy, rigorous mechanistic work characterizing the virulence of this microorganism is crucial in order to develop new treatments that improve clinical outcomes. Innate immune cells such as macrophages are the primary defense against invading fungal cells and serve as a robust model ex vivo for understanding virulence mechanisms. C. albicans undergoes a morphological transition after macrophage engulfment, which allows the fungus to rupture the immune cell and escape the compartment designated for microbial killing. The molecular signals that trigger the morphological change of C. albicans in this context are unknown, although several have been implicated. In this study, we have tested the hypothesis that either CO_2 or alkaline transformation of the phagolysosome/fungal cytosol serve as the primary hyphal inducing signal within the macrophage. C. albicans mutants deficient in components of the CO_2-sensing pathway (a null mutant of the Nce103 carbonic anhydrase and a bicarbonate-insensitive point mutant of the Cyr1 adenylyl cyclase) were evaluated in their ability to change morphology once engulfed by host macrophages. These mutants do not exhibit significant morphological defects inside of the macrophage compared to the wild-type strain. Further, using the pH sensor pHluorin, we have demonstrated that the cytosolic pH of C. albicans is unchanged during morphogenesis, and others have recently shown that phagolysosomal pH does not contribute to hyphal formation. Thus, neither CO_2 sensing nor pH changes are required for the induction of morphogenesis in this context. Work is ongoing to reveal the signal(s) driving this process.

Yolk sac-derived cells are required for brain development

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Rachel is a 4th year graduate student in the laboratory of Dr. Ronald Parchem and is in her second year of the TPEHS training program with Dr. Richard Finnell as a co-mentor. Rachel studies how information from the maternal environment is communicated to the early embryo and how metabolic misregulation results in severe birth defects.

Abstract: The maternal environment experienced by the embryo in utero has long-lasting impacts on brain development. Understanding how the embryonic brain is influenced by environmental insult is important to prevent birth defects and neurodevelopmental disorders. Neural tube closure, the first step of brain development in which the bilateral halves of the neuroepithelium converge and form the closed neural tube, occurs early in embryonic life, before the placenta is established for maternal-fetal nutrient exchange. Instead, the yolk sac supports this period of rapid morphogenesis by transporting nutrients from the maternal to the embryonic environment and generating cells that migrate into the early brain to promote neuronal differentiation during neural tube closure. How yolk sac-derived cells regulate neuroepithelial development is poorly understood. Further it is unknown whether defects in yolk sac-derived cells contribute to neurodevelopmental disorders. Here we demonstrate that miR-290 is expressed in the yolk sac where it is required for proper processing of metabolites from the maternal environment. Additionally, we find cells expressing miR-290 present within the embryo adjacent to the cranial neuroepithelium. RNA sequencing of miR-290+ cells within the embryonic cranial region revealed expression of genes involved in cell migration and neurogenesis and identified Cd200 as a unique cell surface marker. Upon miR-290 deletion we find a significant reduction in the number of CD200+ cells in the cranial region of mir-290-/- embryos and a significant reduction in the ability of mir-290-/- cranial CD200+ cells to induce neurogenesis. Similarly, mir-290-/- embryos have a significant reduction in neurogenesis and expansion of neuroepithelial progenitors that persists until late gestation. This reduction of mir-290-/- neurogenesis results in a reduction of embryonic-born dopaminergic interneurons and is accompanied by an increase in activated microglia that is reminiscent of neurodegeneration. These findings suggest that defects in yolk sac-derived cells can have long-lasting impacts on brain development and may serve to prevent both neurodevelopmental disorders and neurodegeneration. Understanding how yolk sac-derived cells contribute to early brain development will present a new therapeutic approach to prevent neurodevelopmental disorders.

The Design and Development of GMC1 Analogues: Targeting the Regulation of FKBP52 and Hormonal Receptors in Prostate Cancer Cells

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Dr. Kehinde Idowu is a virologist and biochemist by orientation, with his research centered on viral (such as HIV, SARS-CoV-2) and cancer diseases. He is also passionate about the applications of computational methods and artificial intelligence (AI) algorithms to the field of molecular biology and drug discovery.

Abstract: Prostate cancer (PC) is a proliferative disorder characterized by abnormal cell growth that originates in the prostate gland. An effective way of treating castration-resistant PC (CRPC) is androgen deprivation therapy (ADT). Earlier research reported GMC1 effectively inhibits androgen receptor (AR) and glucocorticoid receptor (GR) activities in a variety of PC lines. However, the poor solubility of GMC1 in water and lipid has made it desirable and necessary to design and develop new pharmacophores/analogues with suitable water solubility, liquid stability, and therapeutically potent against CRPC. This study is aimed at designing and developing new analogues of GMC1, and we employed both computational and in vitro methods to identify compounds with inhibitory potentials against CRPC related proteins and PC cells. A search of the databases identified over 7000 analogues of GMC1, out of which, 231 were predicted to show better solubility in lipid and water than GMC1. And the results of the molecular docking analysis revealed 27 compounds exhibited higher docking scores toward the FK1 domain of FKBP52 protein compared to the reference drug (FK506) and GMC1. For the AR and GR, 35 and 40 analogues respectively exhibited higher docking scores towards their ligand binding domain (LBD) than the reference drugs (AV6 and RU486, respectively) and GMC1. A further molecular dynamic simulations study of the best docked compounds showed 8, 4 and 7 compounds showed better binding affinities and stable conformation at the binding sites of GR, FKBP52 and AR, respectively. In vitro evaluation of the EC50 of the identified compounds (25 μM) using luciferase induction assay against AR and GR in MDA cells revealed two compounds, RJ3 and RJ11, showed 45 and 90 % inhibition, respectively. However, the toxicity assay showed the two compounds lowered the reporter's expression. Further identification of lead compounds is under investigation by using in vitro inhibitory activity against MDA cells.

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Abouelniaj, Sara **Poster #1**

Simpler Approach to Make Fresh Graphene Grids for Cryo-EM

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Cryo-EM has become one of the most powerful and sought techniques for revealing the high-resolution structures of biological macromolecules. Graphene grids, aiming at preserving a thin and uniform ice while also improving protein absorption and sample preparation as well as data acquisition in cryo-EM. However, making graphene grids remains challenging for structural biology labs. In addition, we noticed that the graphene grids in the market usually lose their integrity very soon after minimal exposure to air. Instead, we propose that PMMA-covered graphene grids would maintain their cleanliness and integrity for a very long time. Our work provides proof to the manufacturers that the PMMA covered graphene grids are the better option for the biological community so they can buy it and save it until needed. This approach provides the biological community the simplest way to attain fresh graphene grids whenever needed, by simply following a twostep cleaning process to remove the PMMA using solutions found in every lab.

Supported by a training fellowship from the Gulf Coast Consortia, on the Houston Area Molecular Biophysics Program (HAMBP), (Grant No. T32 GM008280).

Aloi, Macarena **Poster #2**

Identifying Brain Regions Relevant in Sleep Dysregulation in OUD Patients Using Resting State Functional Connectivity and Machine Learning

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Sleep affects mechanisms that are involved in opioid abuse, including mood regulation, stress, risk-taking behavior, and pain perception. Over 80% of people with opioid use disorders (OUD) report having disrupted sleep. In turn, disrupted sleep is linked to depression, obsessive compulsive disorder, panic disorder, post-traumatic stress disorder, and relapse. This link suggests a strong association between circadian rhythm disfunction, OUD treatment outcomes, and psychiatric disorders. The overall goal of our work is to shed light on the relationship between sleep and circadian rhythm dysregulation and psychiatric comorbidities in OUD patients undergoing Opioid Maintenance Treatment (OMT). We are employing a two-pronged approach to address this gap in knowledge. First, are using wearable devices and brain imaging to understand impairments in sleep quality and altered brain connectivity in OUD patients undergoing treatment. Second, we propose to develop an AI model capable of providing personalized sleep outcomes for patients undergoing OUD treatment. Ultimately, we will combine wearable device data - like circadian rhythms and sleep (duration, regularity, and stage) - with brain imaging and clinical outcomes using advanced machine learning techniques. Our model will perform unsupervised clustering, time series forecasting, and classification tasks to take advantage of the multiple scenarios offered by the collected data. We will focus on developing an explainable model aiming to provide evidence of the relationship between sleep patterns and brain connectivity in OUD patients.

This work is supported by the Veteran Health Administration (VHA I01CX000994, VHA I01CX001937). Dr. Aloi was funded by the Gulf Coast Consortia and National Library of Medicine NLM Training Program in Biomedical Informatics and Data Science (5T15LM007093-30).

Aramburu, Sofia **Poster #3**

The Hunt for Molecular-Shield Proteins That Prevent DNA Damage

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First world countries have seen an increase in the incidence of age-related diseases such as cancers and neurodegenerative disorders including Alzheimer's Disease. Despite the multiplicity of causes underlying these diseases, we can compare them to a system that has accumulated too much disorder and is not functioning properly. As cells age, they accumulate damage and the resulting impaired cell functions lead to an environment prone to disease development.

DNA damage causes mutations. Endogenous cellular processes are the main culprit behind mutations, protein aggregation and other molecular entropy. Cells are exposed to over 70,000 DNA-damaging events that require repair every day. Although the DNA repair machinery is usually able to cope with this damage, if repair activity is overwhelmed or error-prone, higher mutation rates result. Preventing DNA damage in the first place would then enable the cell to reduce this rate of mutation accumulation, thus reducing the risk of cancer or age-related diseases.

We aim to identify proteins that can protect DNA from damage caused by both endogenous and exogenous sources. These proposed 'molecular-shield' proteins could act by directly shielding DNA from damaging agents, or indirectly by maintaining cellular homeostasis and preventing proteins or other molecules from being damaged.

We have identified human molecular-shield candidate proteins by a bioinformatic screen for analogs of stress proteins of other organisms and have expanded our pool of candidates by targeting proteins previously shown in the lab to be poor DNA damage inducers. Using flow cytometry, we screened for proteins that significantly reduced γH2AX (double-strand break, DNA damage response marker) levels in HEK-293T and MRC5-SV40 cells. We also assayed phosphorylated p53 (Ser15) as a second independent marker of activation of the DNA damage response in HEK-293T cells. Reduction in both γH2AX and p-p53 levels indicate a highly promising candidate. To identify which of these proteins protect against exogenous DNA damage, cells were exposed to ionizing radiation prior to analysis of DNA damage markers by flow cytometry.

Through this rigorous screening, *we have identified two highly promising molecular-shield candidate proteins, several more γH2AX reducers and five promising exogenous DNA damage reducing candidates.*

This work was supported by a National Institutes of Health Director's Pioneer Award (DP1 AG072751-01) and a Fellowship in Precision Environmental Health funded by the NIEHS (T32 ES 027801-05) through the Gulf Coast Consortia.

Armendariz, Lois **Poster #4**

Elucidating the Role of Host Lipid Metabolism in Pathogenic Infection of *Caenorhabditis elegans*

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The development and spread of antimicrobial resistance have dramatically increased in recent decades, with over 2 million antimicrobial-resistant infections occurring each year. Simply relying on existing (or even new) antimicrobials will not solve this crisis. One innovative solution that has been proposed is to modulate host and bacterial pathways to push the host-pathogen interaction into the host's favor. This would require extensive understanding of the specific pathways involved in host defense. One popular model used to study host-pathogen interactions is the small bacterivorous nematode, *Caenorhabditis elegans.* Its small size and short generation time facilitate high-throughput experiments and substantial automation, providing robust data. Additionally, the virulence mechanisms employed by many human pathogens are also used to infect and kill *C. elegans*. Finally, innate immune features show significant conservation between *C. elegans* and mammals.

Previous research has suggested that lipid metabolism could be involved in host response to pathogenic infection. It was shown that the monounsaturated fatty acid, oleate, is needed to activate innate immune effectors. Additionally, it was demonstrated that the evolutionarily conserved mediator subunit MDT-15/MED15, is involved in inducing an immune response against Gram-negative pathogens by regulating the activation of xenobiotic detoxification genes. Interestingly, MDT-15/MED15 is also an important regulator for lipid metabolism.

We compared transcriptional profile of *C. elegans* during infection with *P. aeruginosa* in liquid and on agar. Using Gene Ontology analysis, we identified host lipid metabolism as the most enriched category during infection with *P. aeruginosa* in liquid, indicating this process might play a role in host defense. Through our well-established Liquid killing assay with *P. aeruginosa* we were able to demonstrate that the lipid-metabolism genes *mdt-15*/*MED15*, *lpin-1*/*LPIN1-3*, *nhr-49*/*HNF4*, *pde-2*/*PDE2A*, and *smg-1*/*SMG1* are required for host defense against *P. aeruginosa*. This work establishes the involvement of lipid metabolism in defense response against Gram-negative bacterial pathogens and it sets the groundwork for the discovery of candidate genes that can be enhanced for the host's benefit in host-pathogen interactions.

Supported by a training fellowship from the Gulf Coast Consortia, on the Molecular Basis of Infectious Diseases Training Program (MBID), (NIH Grant No. T32AI055449).

Balsells, Alex "Cito" **Poster #5**

Click Based Semi-Automatic Image Segmentation

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Image segmentation is the task of taking an image and producing a mask, or segmentation, of some region of interest. Many machine learning models rely upon large amounts of pre-labeled data for training. Such models are fully-automatic and solely use the training data.

However, this does not take into account the user's expert knowledge. To do this, we turn to interactive image segmentation. The key assumption being that a human user can provide unique insights to improve model performance. Here, the user provides additional input to the convolutional neural network in the form of mouse clicks on the image. The clicks indicate regions which either belong to the region of interest, a positive click, or the background, a negative click. Models vary in their degree of interaction and the number of interactions recorded.

This research takes a simple scenario: the task of segmenting tumors from 3D MRI brain scans, from the BraTS 2020 dataset, with a single positive and a single negative click. This corresponds to the case with minimal user input. We investigate the importance of click placement on model performance. To address this, we ran two experiments. In the first, we simulate a user clicking randomly through the 3D scans. In the second, the user is more judicious and places clicks in the center, with high probability, of the corresponding regions. The *center* of a region is found by assigning each voxel a probability of being selected that is proportional to the exponential of the Euclidean distance from it to the boundary.

We ran fivefold cross validation over 295 multimodal 3D MRI scans with testing done on a separate group of 59 scans. We used a four-layer pocket U-Net with batch size two over 200 epochs.

Findings suggest models trained with centered clicks perform no better or worse than models trained with randomly placed clicks. This means the user can spare the effort of identifying the center and instead provide clicks at random without losing any predictive power.

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Beruldsen, Finn **Poster #6**

Enabling Large-Scale Modeling of Immune Complexes with Webserver Automation Using Selenium

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Efficient computational of protein structures and macromolecular complexes will continue to be progressively more and more integrated within research pipelines as the technology and speed of such modeling methods increases. Such ongoing proliferation of modeling methods addresses the depth and breadth of modeling needs respectively. While increasingly powerful AI-based methods such as AlphaFold2 increase our ability to predict the 3-dimensional structures of proteins and protein complexes, there are still important limitations. For instance, AlphaFold2 leverages data from multiple sequence alignment (MSA) of related proteins to make inferences about amino acid distances and protein folding. While very informative for the modeling of structurally conserved protein domains, such MSA-derived data cannot inform the modeling of hypervariable protein regions (e.g., variable domains of antibodies and T Cell Receptors), or the modeling of short peptide-ligands displayed by Major Histocompatibility Complexes (MHCs). Given the importance of these molecules for immunological responses, customized methods have been proposed to model specific components of this system. However, the efficient connection between these tools becomes yet another limitation for modeling at scale, as needed for personalized immunotherapy applications. For example, the use of a specialized software might be restricted to the manual input of a text file through the authors' website. Without access to an API nor local download, scientists interested in such a tool might be forced to manually input dozens or even hundreds of files one at a time and model them sequentially. This dramatically increases both the time spent on modeling and the chance for human error. Here, we discuss the use of Selenium — a tool designed to aid programmers in unit-testing but which can also be used to automate a web browser as part of a larger workflow. We show this tool is able to turn hours of focused work into an automated process which does not depend upon direct supervision once it is initiated. Further, taking advantage of the overlapping but distinct capabilities of multiple web servers allows for data processing pipelines including analyses not available through any single tool alone. Specifically, we implemented a pipeline making use of the web servers TCRmodel and TCRpMHCModels for building up complete 3D models of TCR-peptide-MHC complexes using only the gene name and the sequences of the peptide-target and the CDR3 loops. Such scripts can work together with local software for model preparation, refinement, or post-processing (e.g., molecular dynamics simulation) and could be customized for different workflows to string together and synergize powerful scientific software, increasing efficiency while decreasing error.

Bishop, Anthony **Poster #7**

AKR1C3 Inhibitors Induce Allosteric Changes in Ligand Binding Interactions - Implications for Androgen Receptor Coactivation

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Prostate cancer (PC) is among the most common cancers affecting men in the United States with over 200,000 new cases annually. Despite the general efficacy of androgen deprivation in PC treatment, PC tumors often develop resistance to androgen deprivation therapies. This disease subtype, termed castration-resistant prostate cancer (CRPC) has a poor prognosis with few available treatment options. Thus, novel targets for inhibiting the androgen signaling axis are of significant interest. One such target Aldoketoreductase 1C3 (AKR1C3) is a promising candidate. In addition to being overexpressed in CRPC tumors, AKR1C3 is known to activate the androgen receptor through both the enzymatic production of potent androgens and via direct coactivation of the receptor itself. A novel compound (GTx-560) has been the first identified that is capable of inhibiting both activities, but the precise mechanism by which the molecule inhibits coactivation remains poorly understood. We hypothesize that GTx-560 induces allosteric changes in the structure of AKR1C3 in a manner that is distinct from other enzymatic inhibitors (i.e. indomethacin) and that these structural changes render the protein coactivation-incompetent. To test this hypothesis, solution NMR was employed to observe potential allosteric effects of ligand binding. AKR1C3 backbone assignments were achieved via the acquisition of 15N HSQC, HNCA, HN(CO)CA, HNCB, HN(CO)CB and HNCO triple resonance experiments. HNCO spectra reveal a multitude of cross peaks in slow exchange, indicating the presence of multiple conformations in solution. The addition of GTx-560 or indomethacin eliminates the presence of slow exchange in HNCO spectra, suggesting conformational selection upon ligand binding. Furthermore, analysis of chemical shift perturbations (CSPs) in HNCO spectra due to Gtx-560/Indomethacin binding, reveal many large CSPs distant from the binding site. In summary, these data provide strong evidence for the ability of these ligands to act as allosteric modulators of AKR1C3 structure. Current work underway includes the collection of residual dipolar coupling (RDCs) experiments to characterize ligand-induced changes in the structure of AKR1C3 as well as solving the AKR1C3-GTx-560 complex via NMR.

Supported by a Cancer Therapeutics Training Program fellowship (CTTP), (CPRIT Grant No. RP210043).

Bobkov, Georg **Poster #8**

The Role of Arsenic Exposure in Tumor Cell Clustering of Triple-Negative Breast Cancer

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Inorganic arsenic is the most significant chemical contaminant in drinking-water globally and a confirmed carcinogen. However, as its impact on cells can be multifactorial and depend on exposure strength, concentration and affected cell type, its impact on specific aspects of cancer such as cancer metastasis remains largely understudied. In this study, I will investigate whether arsenic exposure enhances the ability of circulating tumor cells (CTCs) to cluster together which has been shown to drastically increase their metastatic potential.

Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer (BC). TNBC has the highest rate of metastasis, which is the major cause of mortality in BC patients. Metastatic lesions originate from CTCs, which detach from the primary tumor and use the bloodstream to migrate to distant organs. As most CTCs become inert or die during this process, cancer metastasis is generally incredibly inefficient. It is therefore imperative to understand the cellular and environmental cues that enable a small subset of CTCs to overcome the barriers that naturally prevent metastasis.

CTCs can exist in the bloodstream as single cells or as CTC clusters and both preclinical and clinical data demonstrated an increased metastatic potential for clustered CTCs. Utilizing a newly developed, shear force mimicking, tumor cell clustering assay, I show that TNBC cell clustering is initiated through the interaction of cellular protrusions. Upon first contact, these protrusions bind to and slide along each other to pull neighboring cells closer. The resulting mature cell-cell interaction site stably connects TNBC cells across a large membrane surface area.

Using various microscopy techniques, I visualized the extracellular matrix component hyaluronic acid (HA) at the top of these protrusions. Its peculiar localization and the fact that experimental depletion of HA completely abolishes CTC clustering in TNBC indicates that HA acts as a molecular glue that enables cells to stably bind to each other. In agreement with these results, both HA and its major producer HA synthase 2 (HAS2) are upregulated in BC and correlate with poor survival and metastasis status of BC patients.

Previously, we showed that PI3K/Akt signaling results in increased HAS2 expression in BC cells. Intriguingly, arsenic-induced alterations of the PI3K/Akt signaling pathway are also well documented and my preliminary data show that the PI3K/Akt inhibitor LY-294002 perturbs tumor cell cluster formation. Building upon these results, I am currently testing whether arsenic exposure can induce HA-mediated CTC clustering through altered PI3K/Akt-signaling.

This work is funded by the Training In Precision Environmental Health Sciences (TPEHS) Training Grant (Grant No. T32ES027801-05).

Bonomo, Melia **Poster #9**

Using Informatics and Deep Learning to Optimize Cochlear Implant Signal Processing of Music

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In the U.S., 38 million adults and 5 in every 1000 children experience hearing loss. Pediatric patients especially are at risk of developing a communication disorder, and hearing impairment is a risk factor for Alzheimer's and other neurological diseases later in life. Over 96,000 of children and adults who are deaf or severely hard-of-hearing use cochlear implants (CI), which are devices surgically inserted to directly stimulate the auditory nerve and convey acoustic information as a neural signal to the brain. While CI signal processing is optimized for speech, it is limited in processing complex auditory signals, such as music, and individual differences in hearing performance are not well understood but are thought to be related to neural health. One parameter that could be reengineered as a tunable setting during music listening is the range of frequencies that are filtered into each CI channel. Here, we are using graph signal processing and deep learning methods in conjunction with a large medical audiology database to predict personalized frequency allocations for improved music perception.

For the first part of this project, we used graph signal processing to quantify differences in music processing for patients with normal hearing and hearing impairment. Sound encoding in the cochlea has traditionally been understood to be the product of individual hair cells being excited at characteristic frequencies, which has been the inspiration for CI design: the hardware reduces the excitation of about 4,000 tonotopic cells to 12-22 frequency channels that stimulate tonotopic electrodes. A graph model of the cochlea is hypothesized to help to recover melody and harmony information that would otherwise be lost between channels. To develop this graph model, patient audiograms from the AudGenDB dataset were input into a MATLAB toolbox (UR EAR 2020b) to compute auditory hair cell response profiles for various chords and music excerpts. Graph signal processing is then used to learn how the hair cells are functionally connected. By calculating various graph-theoretic properties, we show that these cochlea graphs carry distinguishing information based on a patient's hearing loss diagnosis.

For the second part of this project, we will develop a graph deep learning model that uses the AudGenDB dataset to predict the optimal allocation of frequencies to CI channels for music processing. A graph neural network will be designed to cluster the 4,000 hair cells that act as auditory filters into the replacement 12-22 CI channels, based on a musical input and a user's personal audiology data after implant. The graph edges for CI users will be those determined using the individualized method developed in the first aim. This model will be used to predict, based on a user's neural health and the type of musical input, a personalized CI programming setting such that melody information is not lost between channels.

This clinical research informatics project will lead to the creation of a music signal processing algorithm that predicts the optimal allocation of frequencies to each CI channel based on a user's audiology data. CI device companies would be able to implement this as a setting that users could turn on when listening to music.

This work is supported by a training fellowship from the Gulf Coast Consortia, on the NIH National Library of Medicine Training Program in Biomedical Informatics and Data Science (T15LM007093).

Burns Casamayor, Violeta **Poster #10**

Interaction Between NTE and Pops Headgroups Are Key for Lipid Anchor Capabilities

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Cancer is the second leading cause of death in the US[1], and while there has been some great strides in recent years to combat certain types of cancers, others remain elusive. Three of these evasive types of cancers are pancreatic, lung and ovarian cancers, and are predominantly driven by localized mutations of the oncogenic RAS protein. RAS functions as a signaling protein that regulates cell growth through an “on” and “off” switch. When mutations of the amino acid sequence occur, RAS is unable to deactivate in an effective manner and therefore, it produces uncontrollable tumor growth. Previous studies have shown that binding of oncogenic RAS to DIRAS3 prevents clustering and disrupts cell growth signaling[2]. DIRAS3 protein is a member of the RAS superfamily, and possesses 60% amino acid homology to the oncogenic RAS variants (H-, K-, N-RAS), but with the distinction of a characteristic N-Terminus Extension (NTE). We hypothesize that the NTE acts as a lipid anchor by embedding itself into the plasma membrane. To examine this hypothesis, we performed equilibrium molecular dynamics (MD) simulations of the NTE with a myristoyl tail bound on Gly2 and slightly inserted into an asymmetrical POPC/POPS membrane. Our results show that NTE requires the presence of POPS lipids in the bound leaflet in order to retain secondary structure, and that interactions between POPS and NTE basic amino acids are crucial to allow for continuous proximity of NTE to the membrane surface. These results suggest that NTE does act as a lipid anchor for DIRAS3 through interactions with the plasma membrane.

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1. 2022 Cancer Facts & Figures Cancer: Cancer Death Rate Drops, ACS Medical Content and News Staff, https://www.cancer.org/latest-news/facts-and-figures-2022.html.
2. Sutton, M. N., Lu, Z., Li, Y.-C., Zhou, Y., Huang, T., Reger, A. S., Hurwitz, A. M., Palzkill, T., Logsdon, C., Liang, X., Gray, J. W., Nan, X., Hancock, J., Wahl, G. M., & Bast, R. C. (2019). Diras3 (ARHI) blocks Ras/MAPK signaling by binding directly to Ras and disrupting Ras Clusters. Cell Reports, 29(11). https://doi.org/10.1016/j.celrep.2019.11.045.

Chang, Caleb **Poster #11**

Structural Basis of Cytarabine-Mediated Inhibition on DNA Synthesis

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Nucleoside analogues are DNA building block mimics commonly used as drugs against fast-growing cancers. Although many are effective at treating tumors, they cause lethal side effects like hair loss and digestive problems. After DNA polymerases incorporate and move these drug compounds to the primer terminus region during DNA replication, because many lack a functional 3'-OH group to facilitate nucleophilic attack, they act as chain terminators for the next round of incorporation. Cytarabine is a nucleoside-analogue drug for leukemia with a 2'-OH chemical group pointing in the beta direction of its 3'-OH. Although it contains a functional 3'-OH, it effectively inhibits the extension reaction of DNA synthesis. Time-resolved crystallography studies with polymerase η have revealed important transient events that occur during DNA synthesis such as the separated roles of the three metal ions, C2'-endo to C3'-endo sugar pucker change, and primer alignment. It was recently revealed that primer alignment plays a major role in polymerase fidelity and substrate discrimination. When the incorrect nucleotide binds within the polymerase active site, the DNA primer terminus misaligns and exists too far to induce a nucleophilic attack. Further studies that changed the DNA primer terminus to RNA found increased misincorporation efficiency and correlated such fidelity changes to lower barriers in sugar pucker change and enhancement of primer alignment. It remains unknown how the 2'-OH chemical group pointing in the beta direction on cytarabine affects the active site and whether these factors are the basis for cytarabine's inhibitory mechanism. Here, the steady-state kinetics data show that polymerase η is able to efficiently incorporate cytarabine but inefficiently extends from cytarabine. The time-resolved X-ray crystal structures show that during correct nucleotide incorporation, cytarabine at the primer terminus remains in a C2'-endo sugar pucker and exists at an equilibrium between an aligned and misaligned conformation, explaining the decrease in extension activity.

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Chen, Hu **Poster #12**

An Upgrade on DrBioRight, a Next-Generation Analytics Platform for Analyzing Omics Data

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An increasing amount of omics data has expedited and revolutionized biomedical research. Numerous packages, tools, and platforms have been developed to make use of the data. However, using these tools still requires additional knowledge and expertise in bioinformatics and statistics which presents a major challenge. Furthermore, the bioinformatics workflows often have hundreds of hidden parameters, thus making the results difficult to reproduce.

To solve this problem, we have developed DrBioRight, an analytics platform that is empowered by natural language processing and artificial intelligence. DrBioRight allows users to explore, analyze and visualize publicly available omics data directly through human languages. Specifically, users first ask a biological question through the online chat interface. DrBioRight will translate the question into bioinformatics tasks and return the results to the user in interactive tables and figures. Finally, users can submit feedback and rate each analysis to help retrain and improve the backend machine learning models.

To maximize the utility of DrBioRight, we have recently developed a few new modules, including (a) an NVIDIA NeMo NLP module that can identify intents and slots for better understanding questions; (b) A workflow-language-based pipeline processing module that reassembles commonly used tools to analyze omics data; (c) An analytic report module uses markdown language to provide every detail from data preprocessing, normalization, and quality control to downstream analysis and data interpretation, which can be used to reproduce the analysis and generate publication-quality figures; and (d) a Shiny app-based analytic module integrates multiple tools for users to perform their customized analyses.

With this major update, we expect that the improved capability, transparency, and user-friendliness of DrBioRight will further enhance our user experience and maximize its utility in facilitating biomedical cancer research.

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Choi, Sae Hee **Poster #13**

Molecular Modeling Reveals Alternative Dimer Possibilities for HLA-G7 Molecules

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The non-classical human leukocyte antigen G (HLA-G) is involved in several immunoregulatory roles, including the mediation of immune evasion and the suppression of adaptive and innate immune responses. Different from classical HLAs, HLA-G can be expressed as four alternatively spliced membrane-bound isoforms (e.g., HLA-G1 to HLA-G4) and three soluble HLA-G isoforms (e.g., HLA-G5 to HLA-G7). These different isoforms could have distinct immunosuppressive potentials. Interestingly, some of these isoforms are known to form dimers through a disulfide bridge involving the residue Cys-42, which is conserved across all HLA-G isoforms. Dimer formation provides additional opportunities for the regulation of protein stability and binding specificity. Unfortunately, very little is known about the dynamics and function of dimeric forms for most HLA-G isoforms. For instance, the HLA-G7 isoform contains only the α1 domain of the HLA structure, and it is not clear if it is secreted as a monomer or a dimer. In addition, it is not clear if it can bind a peptide ligand (as observed for HLA-G1), or if such complex would be unstable. In order to assess the stability of alternative structural arrangements for G7, we modeled different possible constructs and simulated their dynamics in solution. The studied constructs included the G7 monomer (containing a peptide-ligand or not), and 2 alternative dimeric forms (containing a peptide or not). One of these forms had the disulfide bridge in position 42 (G7-Cys42), as previously described for the HLA-G1 dimer. Interestingly, both ClusPro and AlphaFold2 predicted an alternative dimer structure which does not require a disulfide bridge and resembles the HLA cleft fold (a.k.a., HLA-like dimer). All alternative constructs were subjected to Molecular Dynamics (MD) simulations with Gromacs 2020 package, using a previously described protocol with Charmm36 force field and explicit solvent (TIP3 water). Simulations were performed at the UH high performance computing clusters. After MD, structure integrity and stability of constructs was accessed by a combined analysis, including Root Mean Square Deviation (RMSD of all heavy-atoms), Root Mean Square Fluctuation (RMSF per residue) and the Dictionary of Protein Secondary Structure (DSSP). Both constructs for the HLA-G7 monomer were stable in solution, with higher stability observed for the peptide-loaded structure. Among the dimer constructs, the G7-Cys42 dimer presented higher flexibility when compared to the HLA-like dimer, mostly due to the rotational freedom of individual protomers linked by the disulfide bond. The peptide loaded G7-Cys42 dimer was shown to be slightly more unstable. Interestingly, the opposite was observed for the HLA-like dimer, with the peptide-loaded construct being the most stable dimer across all constructs included in this study. This work contains the first MD evaluation of the isoform HLA-G7 and its possible dimers, and fosters new testable hypothesis into the function and regulation of HLA-G7 dimers.

Conev, Anja **Poster #14**

3pHLA-Score: Improved Structure-Based Peptide-HLA Binding Affinity Prediction

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Improved understanding of the molecular mechanisms that drive human immune response has enabled development of state-of-the-art cancer immunotherapy. While FDA-approved immune checkpoint blockade inhibitors are considered its most notable success, many other treatments such as therapeutic cancer vaccines have undergone a resurgence in the past decade. Binding of peptides to Human Leukocyte Antigen (HLA) receptors is a key mechanism and a prerequisite for triggering immune response. Understanding structural aspects of this binding is of high importance when designing cancer vaccines. Most existing computational tools for peptide-HLA binding affinity (BA) prediction use sequence-based data and do not give structural insights. Large-scale structural analysis of proteins relevant to cancer therapeutics (such as HLA receptors) will be enabled by the Proteomics Toolkit for Ensemble Analysis in Cancer Research (PROTEAN-CR). In this work, we developed a novel machine learning (ML) structure-based protocol to predict BA of peptides to HLA receptors: 3pHLA-score. Using Rosetta's ref2015 BA predictor as a baseline, we engineered novel input features for the ML models that are specific to the peptide-HLA target system. The use of the proposed features led to an increase from 0.82 to 0.99 of the area under the precision-recall curve on the test set. The 3pHLA-score outperformed widely used scoring functions (AutoDock4, Vina, Dope, Vinardo, FoldX, GradDock) in a structural virtual screening task. The 3pHLA-score is now a part of the customizable target-specific BA predictors which will represent a key component of the PROTEAN-CR toolkit. This work showcases the power of target-specific BA predictors and brings structure-based methods one step closer to the pipelines for cancer vaccine development.

Conrad, James **Poster #15**

Cytosine Deamination in I-Motif DNA and its Potential for Hot Spot Mutations in the Human Telomerase Reverse Transcriptase Promoter

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Human telomerase reverse transcriptase (hTERT) maintains telomer length in healthy cells and elevated expression is associated with numerous cancers. Two hot spot mutations in the hTERT core promoter are C>T mutations at -124 and -146 are associated with gliomas and increased *TERT* expression. Deamination of cytosine to uracil can result in C>T mutations if unrepaired. The core promoter consists of a guanine (G) rich DNA strand which can fold into the non-canonical quadruplex leaving the cytosine (C) rich strand exposed. This single stranded DNA is more susceptible to deamination or may fold into an i-motif forming C:C+ bonds. We hypothesized that the formation of an i-motif increases the susceptibility to deamination by creating a positively charged cytosine or by selectively increasing the C4 surface area of some cytosines to water attack and subsequent deamination. The objectives of this research were to: determine if and where i-motifs in the C-rich strand form, determine the susceptibility of cytosines to water attack and deamination by calculating the solvent accessible surface area (SASA), compare SASA predicted sites of potential water attack to next generation sequencing (NGS) data of hTERT DNA exposed to conditions favoring i-motif formation and deamination.

Methods. The hTERT core promoter was divided into nine potential i-motif forming sequences and tested by measuring the circular dichroism (CD) under a range of pH (5-8). The potential for water attack was determined using the solvent accessible surface area (SASA) of an i-motif crystal structure (PDB: 1ELN) modified for the stable hTERT i-motif sequences. A single stranded oligo consisting of the full-length C-rich sequence was constructed and exposed to a range of temperature (37-95 °C) at pH 5 or 7, for varying lengths of time (24-168 h) to induce i-motif formation and deamination. NGS was then used to determine if random or sequence specific deaminations occurred in the full-length hTERT sequence.

Results. Most of the nine hTERT partial sequences formed i-motifs but three formed strong i-motifs with a pKa >6. SASA analysis of static i-motifs indicate that the cytosine C4 is shielded from water by C5 and C6. However, loop cytosines may be vulnerable to attack.

Conclusions. It was unknown if the hTERT C-rich strand can form i-motifs. We have demonstrated that multiple partial sequences are capable of folding into stable i-motifs with pKa close to physiological pH. Potentially, multiple i-motifs are present simultaneously in the full-length sequence which complicates CD interpretation. We had hypothesized that an i-motif would increase the susceptibility of deamination by water attack. However, in a static model the C4 position within core cytosines is shielded from water by C5 and C6. Additional simulations allowing for DNA flexibility will elucidate when the core becomes accessible to water. Loop cytosines may present more vulnerable targets due to a lack of C:C pairing which contributes to C4 shielding.

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Coronado, Ivan **Poster #16**

Deep Learning-Based Segmentation of the Foveal Avascular Zone Using Fundus Photographs

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Optical coherence tomography angiography (OCT-A) is a relatively novel modality allowing visualization of retinal characteristics using near-infrared light. Compared to other retinal modalities, OCT-A enables detailed visualization of the foveal avascular zone (FAZ), which has been associated with various retinal disorders. Unfortunately, OCT-A is not widely available, and OCT-A cameras have an elevated cost. Contrary to OCT-A, fundus photography is a widely available modality, frequently used in population studies, that could potentially be used to capture FAZ information as allowed by OCT-A. In this work, we have investigated a deep learning-based approach using a fully convolutional neural network following the U-Net architecture to segment the FAZ from fundus photos. We call this method FUN-FAZ-SEG. We assessed the effectiveness of this approach by using OCT-A images as a baseline to produce ground truth FAZ segmentations. These segmentations were manually made by two raters (rater MB & rater HD) who looked at the OCT-A images and produced an estimation of the FAZ by delineating a mask over the FAZ area. High inter-rater agreement was demonstrated, given a DICE index of ~0.77 between raters' segmentations. Our dataset included 151 fundus photos and a corresponding OCT-A macula image. We trained FUN-FAZ-SEG using a fraction (60% for training, 15% validation) of the images and masks made by rater MB; then, we compared the model's output segmentations against two test sets. The first test set used the remaining 25% of data from rater MB not used for training the model, and the second set used all of the masks made by rater HD. We observed high agreement between network and raters, given a DICE index of 0.66 for rater MB masks and 0.70 for rater HD masks. High agreement between network and raters suggests that fundus photographs could potentially be used to estimate the FAZ area bypassing OCT-A acquisition.

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Dvorak, Nolan **Poster #17**

FGF14 Is a Downstream Effector of TNFR1 That Regulates Hippocampal Excitability and Sickness Behavior in Experimental Cerebral Malaria

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In the brain, the pro-inflammatory cytokine tumor necrosis factor (TNF) can exert opposing functions, with basal or elevated levels contributing to neurogenesis or neurodegeneration, respectively. TNF exerts these effects by regulating activity of ligand- and voltage-gated ion channels that control neuronal excitability. Pertinent to the latter, TNF has been shown to regulate transient sodium currents (I_{Na}) mediated by voltage-gated Na^+ (Na_v) channels. Here, we investigate signaling molecules upstream of the $Na_v1.6$ channel and downstream of tumor necrosis factor receptor 1 (TNFR1) that might converge to produce these regulatory effects of TNF on I_{Na}. To that end, we performed a high-throughput screening of kinase inhibitors against the $Na_v1.6$ and fibroblast growth factor 14 (FGF14) complex, the latter of which is a Na_v channel auxiliary protein. We found that TNF treatment increases FGF14:$Na_v1.6$ complex assembly through a Janus Kinase 2 (JAK2)-dependent mechanism. We next characterized the functional implications of our proposed TNFR1-JAK2-FGF14-$Na_v1.6$ signaling by performing patch-clamp recordings in slices from experimental cerebral malaria (eCM) mice, an animal model chosen on account of eCM causing increased levels of TNF in the hippocampus. We found that infection augmented the amplitude of the $Na_v1.6$-mediated I_{Na} of CA1 pyramidal cells, an effect blocked by inhibiting TNFR1, JAK2, and FGF14. Correspondingly, infection induced a hyperexcitability phenotype in CA1 pyramidal cells that was mitigated via inhibition of TNFR1, JAK2, and FGF14. Having provided evidence of a TNFR1-JAK2-FGF14-$Na_v1.6$ signaling network *ex vivo*, we next sought to investigate if *in vivo* manipulation of the signaling cascade could block the hyperexcitability phenotype induced by infection. To that end, we first showed that *in vivo* modulation of TNF was necessary and sufficient to block the hyperexcitability phenotype induced by infection, as *in vivo* administration of an anti-TNF antibody restored CA1 neuron firing to a level comparable to the uninfected condition. Secondly, we showed that *in vivo* genetic silencing of FGF14 in the CA1 region was necessary and sufficient to mitigate the hyperexcitability phenotype induced by eCM, as CA1 neurons in slices from infected mice stereotaxically injected with AAV-shFGF14 prior to infection displayed firing comparable to CA1 neurons in slices from uninfected mice. To investigate behavioral correlates of these electrophysiological changes, infected mice injected with a control vector (AAV-shCTRL) or AAV-shFGF14 in the CA1 region of the hippocampus were assessed using a SHIRPA protocol previously shown to capture behavioral symptoms of cerebral malaria. We found that infection caused a significant change in body temperature, as evidenced by infected mice treated with AAV-shCTRL displaying hypothermia compared to uninfected mice. This consequence of infection was blocked by AAV-shFGF14 treatment, as infected mice treated with AAV-shFGF14 displayed no change in body temperature compared to control mice. At the circuital level, these effects of AAV-shFGF14 treatment in the eCM model were shown to be accompanied by infected mice treated with AAV-shFGF14 displaying reduced release of glutamate onto the hypothalamus, a key brain region controlling thermoregulation, compared to infected mice treated with AAV-shFGF14. Collectively, these studies provide evidence for a TNFR1-JAK2F-FGF14-$Na_v1.6$ signaling network that controls hippocampal excitability and confers thermoregulatory effects in eCM by modulating hippocampal-hypothalamic circuity.

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Fang, Shaohai **Poster #18**

Remodeling DNA Methylation Landscapes to Prolong CAR T-cell Activity to Overcome Cancer Relapse

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Objectives: Although chimeric antigen receptor (CAR) T-cell based immunotherapy has achieved promising outcomes in patients with lymphoma and leukemia over the past five years, antigen escape and gradual reduction of T cell activity led to cancer relapse in nearly 50% of patients after initial treatment. Recent studies uncovered the critical role of DNA methylation in boosting CAR T activity, hence pointing to the possibility of overcoming cancer relapse through precise control over the DNA methylation regulatory pathways in therapeutic T cells.

Methods: Novel classes of DNMT enzyme inhibitors and TET protein engineering technologies are used to remodel the DNA methylation landscapes in CAR T-cells. In vitro co-culture of CD19 expressed Raji lymphoma cells and CAR T-cells and in vivo injection of CAR T-cells into tumor bearing mice systems are applied to test the CAR T-cell activities after remodeling their DNA methylation landscapes.

Results: We identified two classes of small molecules which might have inhibitory effects on DNMT1 enzyme. In vitro enzymatic activity assay indicated that several candidate compounds had strong DNMT1 inhibition. The inhibitory effect of these candidates was further tested in a co-culture system composed of CD19-positive Raji lymphoma cells and engineered CAR T-cells. Luciferase assay showed that some candidates could significantly increase luciferase level and enhance CAR T-cell activity. To modulate TET activity, we developed more than 15 nanobodies against TET2. Immunofluorescence images showed that two nanobodies showed strong colocalization with TET2, suggesting that they can recognize TET2 efficiently and can be used in the following experiments to modulate TET2 activity in CAR T-cells.

Conclusions: We identified candidates that can specifically inhibit DNMT1 activity and boost CAR T-cell activity in vitro and produced TET2-specific nanobodies, the latter of which can be used to modulate TET2 activity. The CAR T-cell modulating activities of both DNMT1 inhibitors and TET2 nanobodies will be tested in mouse models of CD19-positive tumor, thereby establishing the preclinical rationale of manipulating the DNA methylation landscapes to benefit CAR T-cell therapy.

Shaohai Fang is the Cancer Therapeutics Training Program fellow (supported by CPRIT Grant No: RP210043).

Fasoulis, Romanos **Poster #19**

APE-Gen2.0: Conformational Ensemble Generation of Peptides Bound to MHC Receptors

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The recognition of peptides bound to Major Histocompability Complexes (MHC) receptors by T-cell Receptors (TCRs) is a determinant to trigger the adaptive immune response. The exact interaction features that drive the immune response when TCRs bind are still unknown. However, studies have suggested that the geometry of the joint peptide-MHC (pMHC) structure plays an important role in T-cell recognition. In an effort to contribute to this field we previously developed APE-Gen, a tool for generating conformational ensembles of pMHC complexes. However, predicting the fine-grained structural effects that certain peptide modifications (such as single-point mutations or post-translational modifications) will have on the pMHC complex is still a very difficult task. Additionally, there is a need for accurate modeling of pMHC complexes that exhibit these modifications, as there is evidence that they are excellent targets for cancer therapy. To this end, we have developed APE-Gen2.0, a tool that improves upon its predecessor. Some of the improvements include: (i) the ability to model peptides that have different types of post-translational modifications such as phosphorylation, nitration and citrullination; (ii) a new and improved anchor identification routine in order to identify and model peptides that exhibit a non-canonical anchor conformation; (iii) a web server that provides a platform for easy and accessible pMHC modeling, which will be freely available at https://apegen.kavrakilab.org. The core of APE-Gen2.0 will be part of PROTEAN-CR, a proteomics toolkit for ensemble analysis in cancer research.

Feinberg, Mandi **Poster #20**

Interaction of West Nile Virus Stem-loop A (SLA) with Flaviviral Polymerases NS5

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West Nile virus (WNV) is a single stranded, positive-sense RNA virus in *Flaviviridae*. Flavivirus also includes dengue (DENV), Zika (ZIKV), and Japanese encephalitis virus (JEV), all of which have been implicated in human disease and are considered etiologies of emerging infectious diseases. WNV causes West Nile fever in humans, the most common mosquito-borne disease in the United States. Flaviviral replication depends upon the presence of the stem-loop A (SLA) structure in the 5' untranslated region of the genome. The viral polymerase NS5 interacts with the 5'SLA and initiates synthesis of the negative strand. The SLA secondary structure and NS5 protein are both highly conserved across flavivirus, and the DENV NS5 is shown to recognize the WNV SLA and replicate the WNV genome and vice versa. To test if DENV and WNV NS5 recognize virus-specific 5'SLA, we determined interaction between various flavivirus 5'SLA and NS5 using an electrophoretic mobility shift assay. We show that WNV NS5 binds SLA of WNV, DENV, and JEV, and not with ZIKV, and that DENV NS5 binds SLA of WNV, DENV, ZIKV, and JEV, consistent with the interchangeability of SLA between DENV and WNV. Next, we determined the NS5-binding site in SLA using WNV SLA mutants. Interestingly, WNV and DENV NS5 do not bind WNV SLA on the same sites. Finally, we determined that the presence of excess WNV SLA during an *in vitro* infection reduces viral replication, indicating that WNV SLA can act as an inhibitor of WNV infection.

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Ferdous, Salma **Poster #21**

Spatial Organization of the Mouse Retina at Single Cell Resolution

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Purpose: Single-cell omics studies have identified more than 120 different neuronal subtypes in the mouse retina, each with a unique functional role within the circuitry. One of the major drawbacks in the current technologies is that they require the dissociation of the tissue, resulting in the loss of spatial information. While the neuronal cell body position of major cell types is well established and critical for proper retinal circuitry, little is known about the spatial organization of retinal cells on the subtype level. The purpose of this study is to establish the first single cell spatial atlas of the mouse retina using spatial transcriptome technology.

Methods: To generate the spatial atlas of the mouse retina, we performed single cell spatial transcriptomics analysis on the wild type C57Bl/6J mouse retina using multiplexed error-robust fluorescence in situ hybridization (MERFISH). Based on single cell RNA-seq (scRNA-seq) data from the mouse retina, probes against a panel of 368 cell subtype marker genes were designed and synthesized. To achieve accurate cell segmentation in the highly packed retina, a set of oligo-conjugated antibodies specific to a cell membrane protein was co-stained with MEFISH probes. Deep-learning segmentation algorithms were then used to identify cell boundaries and assign transcripts to single cells. Using single-cell analysis tools such as scVI, tangram, and Giotto, cell type annotation and further downstream spatial analysis were performed.

Results: Six MERFISH experiments each containing 4-9 tissue sections were performed to generate spatial transcriptomic profiles of over 100,000 cells in total. We identified all major retinal cell types based on UMAP clustering and these cells are present in the expected proportions and locations based on previous literature. Looking specifically at amacrine cells, we found 12 amacrine cell subtypes that have >25% of their cells in the ganglion cell layer (GCL) compared to the canonical location in the inner nuclear layer (INL). A subset of these preferentially displaced subtypes (AC17, AC21, AC37, AC44, AC46, and AC48) were validated via RNAscope.

Conclusion: In short, we are working to generate the first comprehensive spatial single cell reference map of the mouse retina. We hope that this map will be a valuable resource for the entire vision community and serve as an essential step towards gaining a comprehensive understanding of the mechanism of retinal function.

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Ferreira de Mesquita, Ana **Poster #22**

Transcriptional Patterns of Allorecognition Mediated by RasD

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Cells and complex organisms depend on the ability to recognize each other to interact and develop together. This phenomenon is called allorecognition. Understanding allorecognition among cell types is crucial in areas like cancer biology, tumor microenvironment, and transplantation where dynamic interaction between cells can affect cell growth, proliferation and cooperation. Our lab discovered that in *Dictyostelium discoideum* allorecognition is mediated by the genes *tgrB1* and *tgrC1*. *Dictyostelium* is a simple model organism with characteristics that make it ideal to study cell dynamics. *Dictyostelium* typically lives as a single cell but upon starvation it is capable of recognizing others of the same species and interact with them to form a multicellular organism. This process takes about 24 hours and culminates with the formation of a fruiting body composed of a spheric top full of spores (80% of the cells) on top of a stalk (20%) composed by dead cells. As in any other social interaction there is the possibility that one of the interacting partners benefits disproportionally from the interaction. In *Dictyostelium*, these cells, which are called cheaters, make more spores in detriment of their victims. We have recently discovered that *rasD*, a member of the Ras oncogene family, is part of the *tgrB1-tgrC1* signaling pathway, implying that it plays a role in allorecognition. Others have shown that *rasD* activation promotes strain segregation from wild type and affects cell fate. To test this hypothesis, we knocked out *rasD* and analyzed its effects on development, cell type proportioning and cooperation with other strains. Mixing the $rasD^{-}$ cells in equal proportions with a wild-type partner shows that $rasD^{-}$ generates significantly more than its fair share of the spores. By contrast, when we generated an activated *rasD* strain, $rasD^{G12T}$, we observed an increase in the production of prestalk cells. These results suggest that *rasD* is a key element in the developmental process as well as in social behavior in *Dictyostelium* and refute the previous observation that activated *rasD* causes segregation. We are currently studying the direct effects of *rasD* on cell-type proportioning and cheating. Following these analyses, we will use standard RNA-seq and single cell RNA-seq (scRNA-seq) to study the differences in global gene expression in the *rasD* strains both in pure and mixed populations. We will also use scRNA-seq to analyze differential gene expression in mixes of tagged cells, such as $rasD^{-}$ cells tagged with GFP mixed with wild-type cells tagged with RFP. These analyses will reveal how the transcriptomes of the cheater and the victim differ over time during the cheating process. These studies will provide a broader understanding of the role of *rasD* in development, sociality, and allorecognition, and a new outlook on the role of ras signaling in eukaryotes.

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Fonseca, Andre **Poster #23**

Leveraging Structural Data on Contact Maps to Improve T-cell Cross-Reactivity Predictions

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The interaction between the peptide-loaded major histocompatibility complex (pMHC) and the T-cell receptor (TCR) is a key determinant for the cellular immune response. This interaction reveals a contact map associated with biophysical bonds that ultimately reflects binding affinity, stability, and antigen-specificity. Additionally, TCRpMHC contact maps can uncover position-specific hotspots related to T-cell specificity and cross-reactivity potential, a major safety concern for immunotherapy applications. Therefore, molecular analysis of such interactions can hold the key to the development of better and safer T-cell-based immunotherapies. In this context, we hypothesize that contact maps can dramatically improve peptide-based T-cell cross-reactivity (CR) predictions. To evaluate the contact map impact on CR predictions, we capitalized on crystallography data to highlight peptide-centered hotspots, i.e., preferential residues recognized by TCR. The well-known MAGE A3A TCR crystal (PDB: 5BRZ) was selected due to biomedical interest. A3A TCR has enhanced affinity to the MAGE-A3 antigen (EVDPIGHLY) but was also found to be cross-reactive with the Titin antigen (ESDPIVAQY), resulting in cardiovascular damage. Next, we characterize the molecular interactions between A3A TCR and MAGE-A3 antigen. This process provides a contact map derived from a static structure, where positions 1, 4, 5, 7, and 8 are preferential residues recognized by A3A TCR. In particular, position 4 (Proline) holds the highest amount of interactions, including backbone-mediated hydrogen bonds, hydrophobic, and Van der Waals. The MAGE-A3 antigen contact map was validated using molecular dynamics, considering incremental simulation times, e.g., 10, 20, 50, 80, and 100 ns. The overall profile of interactions is conserved across all time points. In addition, our contact map is also supported by curated interactions from IEDB Portal. Finally, the contacts were normalized and integrated into our software for cross-reactivity prediction, Crossdome. We run MAGE-A3 antigen predictions against a database, including Titin antigen, 60 synthetic peptides recognized by A3A TCR, and 23,000 HLA-A*01:01-eluted peptides. In short, our experiment covers two distinct scenario predictions, i) with and ii) without contact map weights. The contact maps improved the Crossdome predictions, with 60% to 82% true-positive cases related to high-similarity peptides, i.e., potentially cross-reactive peptides. Thus, leveraging structural data on contact maps represents a promising approach to improve T-cell cross-reactivity predictions. Further, we demonstrated that static crystal structures could be a feasible source for producing such contact maps. Finally, we believe that data from the TCR repertoires are a valuable resource for building and integrating contact maps on Crossdome.

Gonzalez, Cuauhtemoc Ulises **Poster #25**

Building a Model System for the Delta Receptor Activation

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The delta receptor is a member of the ligand-gated ionotropic glutamate receptor (iGluR) family due to its sequence homology and structure similarity. It has been implicated in neurological disorders like autism and schizophrenia as well as motor deficits like ataxia, making it noteworthy as a pharmacological target. Interestingly, the delta receptor does not demonstrate isolated ligand-gated currents as the other iGluRs do, making it challenging to target. Our lab discovered that the delta receptor is capable of having glycine-gated activity when in complex with its trans-synaptic partners, presynaptic adhesion protein Neurexin1β (NRXN1) and soluble scaffolding protein cerebellin (Cbln). Furthermore, using FRET/FLIM and chemical linkers our findings suggest that this glycine-induced activity is triggered by coupling of the extracellular domains of the delta receptor. Although there is no full structure of this trans-synaptic complex, structures depicting the interaction between the delta receptor and Cbln along with the interaction between Cbln and NRXN1 suggest that the stoichiometry of the trans-synaptic complex between NRXN1, Cbln, and the delta receptor is 2 NRXN1: 2 Cbln hexamers: 1 delta receptor. Therefore, I hypothesize this minimal synaptic complex is required for glycine -induced activity of the delta receptor. To test this, I have created a dimeric NRXN1 by using coiled-coil interactions and investigated the structure-function of the delta receptor using single-channel patch clamp and single molecule FRET to measure the conformational changes of the extracellular domains. The findings obtained from these experiments will 1) elucidate the conformational changes required for delta receptor ionotropic activity and 2) describe a novel methodology which facilitates studies of the activity of delta receptor in vitro. These findings will ultimately reduce the challenges of targeting the delta receptor.

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Greenway, Harmon **Poster #26**

Anti-Tumor Efficacy of mRNA Splicing Modulators as Antibody-Drug Conjugates

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Although tumors demonstrate diverse mechanisms of immune evasion, the resistance of breast cancers to immunotherapy has been attributed to low neoantigen burden. The presentation of neoantigens is an important driver of antitumor immunity and a significant correlate of the clinical outcome for checkpoint blockade and adoptive T-cell therapies. Dysregulation of pre-mRNA splicing is implicated in many cancers and induces a class of neoantigen that is recognized by immune cells. Small-molecule modulators of alternative splicing have been shown to enhance T-cell mediated antitumor immunity by increasing neoantigen burden. These treatments inhibit tumor growth and may significantly improve patient response to immunotherapy. However, splicing modulators have demonstrated adverse off-target tissue effects in patients and are also known to inhibit proliferation of T-cells in vitro. To improve the safety and efficacy of these treatments, we have developed the spliceosome modulator pladienolide B as an antibody-drug conjugate targeted to HER2+ and triple-negative breast cancer. Antibody-drug conjugates are a class of cancer therapeutics designed to enable the targeted delivery of cytotoxic agents via covalent attachment to a monoclonal antibody. The anti-tumor efficacy of pladienolide B conjugates will be evaluated in breast cancer mouse models as a monotherapy and in combination with anti-PD1. Development of pladienolide B as an antibody-drug conjugate will enhance the safety of an antitumor drug with proven clinical efficacy and yield a novel therapeutic for further pre-clinical research.

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Guo, Shirley **Poster #27**

Development of Selective ENL Inhibitors and PROTACS for Acute Myeloid Leukemia Therapy

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Acute myeloid leukemia (AML) is the most diagnosed and the deadliest subtype of leukemia. It is characterized by uncontrolled proliferation of abnormal myeloblasts that lacking the ability to further differentiate into normal blood cells and usually linked to gene mutations or chromosomal rearrangements in myeloblasts. Recently genetic loss-of-function studies have demonstrated that a human YEATS domain-containing protein named eleven-nineteen-leukaemia (ENL) functions as a transcriptional coactivator and is essential for the proliferation of AML and acute lymphoblastic leukemia that harbour oncogenic multiple lineage leukemia (MLL) rearrangements. Our previous studies have demonstrated a series of ENL inhibitors (**1**, **7**-**15** and **24**) displaying significant and specific inhibitory effects targeting ENL YEAST domain. In this study, we developed a NanoBRET system which allows the analysis of cellular permeability, potency, selectivity, and stability of synthesized ENL inhibitors. Followed by *in vitro* metabolic stability and cell growth inhibition studies, we identified a potent and specific ENL YEATS domain inhibitor **13** with both high *in vitro* metabolic stability and strong anti-proliferation ability on MLL-fusion leukemia cell lines. Cumulatively, this study established **13** as a promising inhibitor to disrupt the pathogenic functions of ENL for acute myeloid leukemia treatment.

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References

1. Wan L, Wen H, Li Y, *et al*. ENL links histone acetylation to oncogenic gene expression in acute myeloid leukaemia. *Nature*, 2017, 543:265-9.
2. Xinyu Ma, Longxia Xu, *et al*. Discovery of Selective Small-Molecule Inhibitors for the ENL YEATS Domain. *Journal of Medicinal Chemistry*, 2021, 64.15: 10997-11013.

Ha, Yin Yuen **Poster #28**

A Glycine-based Tripeptide Linker for Maximizing the Therapeutic Index of Antibody–Drug Conjugates

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Valine-citrulline (VCit) is an industrial-standard protease-cleavable linker commonly used in antibody-drug conjugates (ADCs) for cancer therapy. However, its in vivo linker instability can cause various clinical adverse effects including neutropenia and hepatotoxicity. In phase II and III studies of Adcetris (VCit-based ADC), neutropenia and hepatotoxicity were observed in 16%–22% and 7% of patients respectively, leading to dose delay or treatment discontinuation. Here, we report that a glycine-based tripeptide linker sequence, glutamic acid-glycine-citrulline (EGCit), have the potential to solve these clinical issues without compromising the ability of traceless drug release and ADC therapeutic efficacy.[1] We demonstrate that our EGCit ADC resists human neutrophil protease-mediated degradation and spares differentiating human neutrophils. Notably, our anti-HER2 ADC shows almost no sign of blood and liver toxicity in healthy mice at 80 mg kg^{-1}. Our EGCit ADCs also exert greater antitumor efficacy in multiple xenograft tumor models compared to the FDA-approved ADCs including Kadcyla® and Enhertu®. Because of the linker simplicity, desirable physicochemical properties and independence from conjugation modality and payload type, EGCit linker is transferable to a wide range of ADC design and other drug delivery agents. We believe that the EGCit linker technology will help expand the repertoire of effective, safe targeted drug delivery systems. This may provide clinicians and patients with cancer with access to otherwise unrealistic treatment options such as high-dose ADC therapy.

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References

1. S. Y. Y. Ha, Y. Anami, C. M. Yamazaki, W. Xiong, C. M. Haase, S. D. Olson, J. Lee, N. T. Ueno, N. Zhang, Z. An, K. Tsuchikama, An enzymatically cleavable tripeptide linker for maximizing the therapeutic index of antibody-drug conjugates. *Mol. Cancer Ther.* **21**, 1449–1461 (2022).

Hall-Swan, Sarah **Poster #29**

A Peptide-HLA Similarity Score for Prediction of T-cell Cross-reactivity

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T-cell-based cancer immunotherapy harnesses our bodies' immune systems to pinpoint and eliminate cancer cells. The basis of this therapy is that peptide-HLA (pHLA) complexes on the surface of tumor cells can be targeted by cytotoxic T-cells to eliminate the tumor. However, there exist cases where therapeutic T-cells directed towards tumor pHLA complexes may also recognize pHLAs from healthy normal cells, causing autoimmune side effects. The process where the same T-cell clone recognizes more than one pHLA is referred to as T-cell cross-reactivity and it is driven mainly by the similarity between pHLAs.

Our objective is to develop a computational method for predicting T-cell cross-reactivity. We developed a novel similarity score that uses structural and biochemical features of the pHLAs as well as the sequence of the peptide to determine the similarity between pHLAs. We first use APE-Gen, a recent docking tool, to model the three-dimensional structure of each pHLA, and then represent the solvent-accessible surface of the T-cell interacting region using a point cloud. Each vertex of the point cloud is annotated with biochemical features (i.e., electrostatic potential, hydrophobicity, and hydrogen bond potential). We perform a pairwise alignment of the point clouds using Iterative Closest Point and score the resulting alignments based on their structural and biochemical similarity. We also calculate the pairwise similarity between the peptide sequences using HLAthena and the BLOSUM62 matrix, and we combine the scores of these three methods to get our final similarity scores. These scores can be used to cluster the peptides based on similarity using data science clustering techniques. We tested this method on a dataset of 180 peptides, 60 of which are recognized by the same T-cell. The final clustering result has 93.22% sensitivity and 99.16% specificity.

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Hamor, Clark **Poster #30**

Solving the Capsid Structure of an RNA Virus That Infects Key Coral Symbionts

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Symbiodiniaceae, a family of photosynthetic microbes, establish symbioses with stony corals that are essential for coral health and survival. In stressful environmental conditions, Symbiodiniaceae are lost from coral tissues, and corals experience a diseased state known as “bleaching”. Climate change and warming sea temperatures are driving recurrent, large-scale coral bleaching and mortality events. Such events are contributing to the decline of coral reef ecosystems worldwide, which poses major ecological and economic risks. Mitigating these risks through reef conservation and restoration efforts will require a fuller understanding of the mechanisms that cause coral bleaching.

Viral lysis of Symbiodiniaceae has been hypothesized to contribute to bleaching, but this hypothesis has not been thoroughly tested. Evidence suggests that dinoRNAVs, a group of Symbiodiniaceae-infecting +ssRNA viruses, could contribute to bleaching by reducing Symbiodiniaceae thermal tolerance. However, little is known about dinoRNAVs outside of transcriptomic data, making it difficult to assess the impacts of dinoRNAVs on Symbiodiniaceae. The goal of our work is to characterize dinoRNAVs by solving the dinoRNAV capsid structure, characterizing the dinoRNAV non-structural genes and by culturing dinoRNAVs in Symbiodiniaceae.

Thus far, we have obtained transmission electron micrographs of recombinantly produced dinoRNAV capsids. These micrographs show dinoRNAVs to have icosahedral capsid that is ≈ 30 nm in diameter. We now plan to analyze dinoRNAV-infected Symbiodiniaceae through thin-sectioning and electron microscopy, as well as obtain a high-resolution structure of the dinoRNAV capsid through cryo-EM.

Through our work, we hope to inform reef conservation efforts by providing a foundational step in understanding the role viruses play in coral health and bleaching events.

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Hoang, Anthony **Poster #31**

Mechanism of Anion Transport and Inhibition in Pendrin

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Pendrin is an anion transporter expressed in the inner ear, thyroid gland, kidneys, and inflamed lungs. Mutations in pendrin cause Pendred syndrome and a closely related enlarged vestibular aqueduct syndrome (EVAS), both of which are genetic disorders characterized by early hearing loss in children and account for 5-10% of hereditary hearing loss. Pendrin is also a validated drug target for attenuating airway hyperresponsiveness in asthma and for treating hypertension, and several clinically prescribed drugs such as niflumic acid are found to inhibit pendrin. It is known that pendrin transports iodide ions (I^-) or bicarbonate ions (HCO_3^-) in exchange of chloride ions (Cl^-), but our understanding of anion transport and inhibition in pendrin remains rudimentary due to a lack of precise functional studies and an absence of pendrin structures. Here we report structures of pendrin from *Sus scrofa* in the presence of I^-, Cl^- or HCO_3^-. The structures show two anion binding sites that are common to all three substrates. We measured HCO_3^- and I^- transport in proteoliposomes reconstituted with pendrin and validated the anion binding site residues by mutagenesis. The structures also reveal additional ion and lipid binding sites, which may have important functional impacts. Further, we have determined the structure of pendrin in complex with niflumic acid and identified two binding sites and the mechanism for inhibition. These results provide a foundation to address questions regarding anion substrate recognition and inhibition by small molecule compounds, which will expand understanding of pendrin as an anion transporter and facilitate development of novel therapeutics.

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Hood, Kara **Poster #32**

Topology of Enterococcus faecalis LiaF Suggests Complex Interaction with Enterococcal-specific Regulator LiaX

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Background and Objective: VRE (Vancomycin Resistant Enterococci)-associated infections are prevalent in healthcare settings and pose a significant therapeutic challenge. The lipopeptide antimicrobial daptomycin (DAP) is one of the last resort options to treat these organisms. Among VRE, mutations that confer DAP resistance (DAP-R) arise in proteins in the three-component signaling system LiaFSR, which is highly conserved in other Gram-positive pathogens. Similar to Enterococcus faecalis (Efs), LiaFSR in Bacillus subtilis (Bsu) is specifically activated by DAP and, thus, serves as a proxy for studying mechanisms of DAP-R. In Bsu, LiaF inhibits LiaR phosphorylation; however, the current model for DAP-R in Efs positions LiaF as an activator of LiaFSR directly regulated by the enterococcal-specific protein LiaX. The mechanism of interaction between LiaX and LiaF is still unknown, thus we mapped Efs LiaF topology in vivo to identify potential interaction sites with LiaX. In addition, we compared structure predictions of Efs and Bsu LiaF to determine if conformational differences between them could account for its opposing roles in regulation.

Methods: LiaF protein predictions were determined through the RoseTTAFold algorithm (RoBetta online web server, David Baker, University of Washington). Structural comparisons were conducted using the MatchMaker function in UCSF Chimera. Sequence alignments were performed using EMBOSS Needle Pairwise Alignment (EMBL-EBI). Efs LiaF topology was mapped experimentally in E. coli DH5a with 2 assays: first, an in vivo LacZ alpha-complementation assay wherein the LacZ-alpha fragment was fused to truncations of LiaF in between its predicted transmembrane domains and analyzed through a blue/white colony screen. Second, a similar blue/white colony screen assay was performed with the same LiaF truncations fused to E. coli PhoA.

Results and Conclusions: Predicted structures of LiaF from Efs and Bsu exhibited similar domain organization, with 4 N-terminal transmembrane (TM) helices connected to a C-terminal ß-sheet domain by a flexible linker of variable length. When analyzed for structural similarity, the C-terminal domains were the most like one another. Our analysis of the N-terminal domains revealed distinct differences in the orientation of TM helices 2 and 3 in LiaF from Efs compared to Bsu. In vivo topological mapping of Efs LiaF showed that the N- and C-termini are intracellular, and the 4 predicted transmembrane domains likely thread through the membrane as expected. The absence of an obvious binding region for LiaX suggests that the communication between Efs LiaF and LiaX is much more complex than direct physical interaction. Moreover, the similarity between LiaF from Bsu and Efs suggests that other enterococcal-specific proteins like LiaX may account for the contrasting functions LiaF serves in LiaFSR regulation.

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Idowu, Kehinde **Poster #33**

The Design and Development of GMC1 Analogues: Targeting the Regulation of FKBP52 and Hormonal Receptors in Prostate Cancer Cells

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Prostate cancer (PC) is a proliferative disorder characterized by abnormal cell growth that originates in the prostate gland. An effective way of treating castration-resistant PC (CRPC) is androgen deprivation therapy (ADT). Earlier research reported GMC1 effectively inhibits androgen receptor (AR) and glucocorticoid receptor (GR) activities in a variety of PC lines. However, the poor solubility of GMC1 in water and lipid has made it desirable and necessary to design and develop new pharmacophores/analogues with suitable water solubility, liquid stability, and therapeutically potent against CRPC. This study is aimed at designing and developing new analogues of GMC1, and we employed both computational and *in vitro* methods to identify compounds with inhibitory potentials against CRPC related proteins and PC cells. A search of the databases identified over 7000 analogues of GMC1, out of which, 231 were predicted to show better solubility in lipid and water than GMC1. And the results of the molecular docking analysis revealed 27 compounds exhibited higher docking scores toward the FK1 domain of FKBP52 protein compared to the reference drug (FK506) and GMC1. For the AR and GR, 35 and 40 analogues respectively exhibited higher docking scores towards their ligand binding domain (LBD) than the reference drugs (AV6 and RU486, respectively) and GMC1. A further molecular dynamic simulations study of the best docked compounds showed 8, 4 and 7 compounds showed better binding affinities and stable conformation at the binding sites of GR, FKBP52 and AR, respectively. *In vitro* evaluation of the EC_{50} of the identified compounds (25 μM) using luciferase induction assay against AR and GR in MDA cells revealed two compounds, RJ3 and RJ11, showed 45 and 90 % inhibition, respectively. However, the toxicity assay showed the two compounds lowered the reporter's expression. Further identification of lead compounds is under investigation by using in vitro inhibitory activity against MDA cells.

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Jacob, Joan **Poster #34**

Targeting Colorectal Cancer Cell Plasticity Using Novel Epiregulin Antibody-Drug Conjugates

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Colorectal cancer (CRC) is the third leading cause of cancer related deaths worldwide. This is largely due to metastasis, tumor relapse, and the vast differences in CRC tumors leading to treatment failure. Cancer stem cells (CSCs) are a small population of tumor-initiating cells thought to act as immortal seeds that form metastases and contribute to tumor relapse by transitioning between a differentiated (metastatic) and undifferentiated (tumor-initiating, immortal) state. Epiregulin (EREG) is a protein highly expressed in both states, as well as in treatment resistant CRCs of various mutation statuses, providing a target for combating cellular plasticity in CRC. Therefore, I hypothesize that EREG can mediate CRC tumor growth and metastasis and an EREG-targeted antibody-drug conjugate (ADC) can act as a guided missile to deliver cytotoxic drugs to EREG-expressing tumors for targeting treatment resistant CRC. To develop a novel EREG ADC, I cloned an EREG monoclonal antibody (mAb) construct based on patented variable regions of a humanized EREG mAb and tested for binding affinity, the ability to internalize, and to block exogenous and endogenous EREG activity. Using the EREG mAb as well as a non-targeting IgG1 control antibody (cAb) we performed radiolabeling with Zr89 to trace antibody tumor uptake in vivo using a CRC cell line xenograft model. Our EREG antibody showed high binding affinity, internalization and lysosomal colocalization in vitro and tumor specificity in vivo. We then conjugated the EREG mAb and cAb to potent cytotoxic agents using either a cleavable, or non-cleavable peptide linker. Our EREG ADCs were evaluated in vitro against a wide panel of CRC cell lines of various *KRAS*, *BRAF*, and *PI3CKA* statuses and various EREG expression. Drug efficacy was determined using cytotoxicity assays to measure percent cell survival given varying doses of the cAb and EREG mAb as well as control ADC (cADC) or EREG ADC. While the EREG mAb alone does not produce significant cytotoxicity, conjugated to a chemical payload, the EREG ADC has a much higher efficacy at lower concentrations in causing cytotoxicity compared to cADC. Ongoing studies include large-scale ADC production with the lead anti-EREG ADC and cADC for cytotoxicity profiling in patient derived organoid culture and safety and therapeutic efficacy studies in vivo. By targeting EREG, we can develop an ADC capable of acting as a potent clinical drug for targeting cellular plasticity and eliminate colorectal cancer resistance and recurrence.

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Jiang, Xianli **Poster #35**

Structural Method-Based Prediction of the T Cell Receptor (TCR)-Antigen Interaction and Specificity

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The antitumor function of T cells is activated upon the TCR recognition of cancer related antigens presented with major histocompatibility complex (MHC) on cancer cells. Accurate prediction of the interaction greatly aids in the development of cancer therapy. However, the high diversity of TCR clonotypes, peptides, and MHC molecules and the high specificity of interaction between TCR and its recognizing peptide, together with limited data sources, lead to the great challenges in predicting the binding specificity for novel TCRs or peptides, especially the cancer-related antigens. Current computational approaches are limited in relying on sequence data and using only complementarity-determining region 3 information of TCR beta chain. Here, we leveraged computational structural modeling tools to predict full TCR-pMHC complex structures in a high throughput manner and then build a deep learning model using structural features that could represent comprehensive interactions within the complex to predict functional TCR-pMHC.

Our dataset contained TCR-pMHC complexes obtained from public datasets, including VDJdb, PDB and highly multiplexed antigen-TCR profiling data from 10x genomics. A structural modeling pipeline was built by integrating multiple computational tools to predict the structures of these complexes from sequences of TCR alpha and beta chains, peptides, MHCs. In addition to the distance matrix for TCR-peptide, MHC-peptide extracted from the predicted structures, other structural and binding surface features, such as atomic interactions, binding energies, were estimated by computational scoring tools. All features were used to train a deep learning model to classify TCR-pMHC interactions.

The complex modeling pipeline predicted structural conformations comparable to native structures for 126 PDB complexes. Around 40,000 TCR-pMHC complexes with peptide length at 9 were predicted, with 31% true interaction complexes. The cross-validation accuracy on the testing dataset of the deep learning model outperformed current sequence-based computational approaches. The model was further tested for the ability of predicting oncovirus protein-originated peptides interacting with TCRs from T cells that could kill oncovirus containing tumor cells.

Our study built a fast and accurate structural modeling pipeline that could deal with growing data from public TCR-pMHC interactome databases and high throughput data generated by multiplexed pMHC tetramer sequencing techniques. It enables the using of structural features to build deep learning models for the prediction of specific TCR-pMHC interaction from a TCR repertoire or peptide pools, highlighting its potential in recognizing immunogenic neoantigens, tumor reactive TCRs, and evaluating TCR cross-reactivity during real cancer therapy development.

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Johnson, Jordan **Poster #36**

Single Molecule Studies of EF-Tu Accommodation and Proofreading Mechanism

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Delivery of aminoacyl-tRNA to the ribosome during translation is assisted by elongation factor thermal unstable (EF-Tu). During this process the EF-Tu ternary complex detects the correct codon and initiates delivery at the cost of GTP. Although the translation process is meticulously monitored, errors can occur such as when assisting factors have mutations. Localized mutations EF-Tu, more specifically eukaryotic homolog eEF1A, have been linked to various health issues such as Huntington's disease, ADHD, epilepsy, intellectual disability, and depression; however, as factors of EF-Tu's mechanism is highly debated, it is not well understood how these mutations contribute to these diseases. To observe and understand EF-Tu's mechanism and conformational changes, mutations at D81 were introduced into the GTP binding pocket and will be observed using single-molecule studies, such as FRET and FIRMS, to determine the conformational changes and interactions with the ribosome. Ternary complex formation and GTPase activity will also be assessed to determine the effect of the mutations on enzymatic activity. Once the single molecule studies have been analyzed, we can survey antibiotics to test their effects on mutant EF-Tu function and conformation to help gather useful information for the further development of these antibiotics.

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Jonnakuti, Venkata **Poster #37**

PolyAMiner-Bulk: A Machine Learning Based Bioinformatics Method to Infer and Decode Alternative Polyadenylation Dynamics from Bulk RNA-seq Data

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Alternative polyadenylation (APA) is a post-transcriptional regulatory mechanism that appends adenosine residues at one of the potentially several cleavage and polyadenylation sites (C/PASs) of a pre-mRNA, resulting in multiple mRNA isoforms with varying 3'UTR lengths. By controlling the length of the 3'UTR region, APA allows for differential inclusion of binding sites specific for miRNAs and RNA-binding proteins. However, the current generation of bioinformatics tools for identifying C/PASs and quantifying 3'UTR length changes from bulk RNA-seq data are hampered by poor C/PAS annotations that do not converge with other C/PAS databases, intrinsic limitations of de novo C/PAS detection, and inability to detect intra-distal or intra-proximal APA changes. Here, we develop a bioinformatics method, PolyAMiner-Bulk, that addresses these concerns. In brief, PolyAMiner-Bulk detects de novo C/PASs, merges them with a priori C/PAS databases like PolyA_DB and PolyASite, and filters these candidate C/PASs using a C/PAS-BERT machine learning model to create an accurate and comprehensive C/PAS collection to examine APA dynamics in all genic regions. Using PolyAMiner-Bulk and previously published RBM17 knockdown data, we unravel APA changes previously underappreciated by existing APA methods.

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Kapoor, Kshipra **Poster #38**

Morphological Diversity of Extracellular Vesicles Revealed by Cryo-Electron Microscopy

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Introduction: Exosomes are extracellular vesicles 80-150 nm in diameter, containing proteins, mRNAs, microRNAs, and lipids reflecting the parent cell. While there has been an extensive characterization of the cargo incorporated in exosomes, a detailed morphological analysis of exosomes purified by various isolation techniques has not been performed.

Objective: We aimed to determine the heterogeneity of exosomes morphology and if such morphological features are conserved across sample types.

Methods: Our study used Cryogenic Electron Microscopy (Cryo-EM) to examine exosome size and morphology.

Results: Our results revealed significant diversity in extracellular vesicle morphology independent of the isolation method, suggesting that morphological subpopulations of these vesicles exist. Based on their shape, our analysis classified exosomes into seven categories. In addition, we developed a semi-automatic image analysis framework to accurately characterize exosome attributes and distribution to facilitate reliable quantification of specific bio-nanoparticle features in Cryo-EM micrographs.

Conclusions: Morphological features of exosomes inform their biophysical properties, which influence both biodistribution and biological activity in vivo. Our data demonstrating the innate morphological diversity of exosomes may have implications for improving the specificity and precision of exosome-delivered therapeutics.

Conflict of interest: R.K. and MD Anderson Cancer Center hold patents in exosome biology and are stock equity holders in Codiak Biosciences Inc. R.K. is a consultant and a scientific advisor of Codiak Biosciences Inc.

Keuls, Rachel **Poster #39**

Yolk Sac-Derived Cells Are Required for Brain Development

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The maternal environment experienced by the embryo in utero has long-lasting impacts on brain development. Understanding how the embryonic brain is influenced by environmental insult is important to prevent birth defects and neurodevelopmental disorders. Neural tube closure, the first step of brain development in which the bilateral halves of the neuroepithelium converge and form the closed neural tube, occurs early in embryonic life, before the placenta is established for maternal-fetal nutrient exchange. Instead, the yolk sac supports this period of rapid morphogenesis by transporting nutrients from the maternal to the embryonic environment and generating cells that migrate into the early brain to promote neuronal differentiation during neural tube closure. How yolk sac-derived cells regulate neuroepithelial development is poorly understood. Further it is unknown whether defects in yolk sac-derived cells contribute to neurodevelopmental disorders. Here we demonstrate that miR-290 is expressed in the yolk sac where it is required for proper processing of metabolites from the maternal environment. Additionally, we find cells expressing miR-290 present within the embryo adjacent to the cranial neuroepithelium. RNA sequencing of miR-290+ cells within the embryonic cranial region revealed expression of genes involved in cell migration and neurogenesis and identified *Cd200* as a unique cell surface marker. Upon miR-290 deletion we find a significant reduction in the number of CD200+ cells in the cranial region of *mir-290*$^{-/-}$ embryos and a significant reduction in the ability of *mir-290*$^{-/-}$ cranial CD200+ cells to induce neurogenesis. Similarly, *mir-290*$^{-/-}$ embryos have a significant reduction in neurogenesis and expansion of neuroepithelial progenitors that persists until late gestation. This reduction of *mir-290*$^{-/-}$ neurogenesis results in a reduction of embryonic-born dopaminergic interneurons and is accompanied by an increase in activated microglia that is reminiscent of neurodegeneration. These findings suggest that defects in yolk sac-derived cells can have long-lasting impacts on brain development and may serve to prevent both neurodevelopmental disorders and neurodegeneration. Understanding how yolk sac-derived cells contribute to early brain development will present a new therapeutic approach to prevent neurodevelopmental disorders.

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Kille, Bryce **Poster #40**

Algorithmic Improvements to Long Read Mapping and Their Effect on the Human Reference Pangenome Analysis

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The completion of the human reference genome has paved the way for the Human Pangenome Reference Consortium (HPRC) to construct a human reference pangenome, a high-quality reference genome that includes a representation of global genomic variation in addition to just the reference sequence. Concomitant with the population-scale long-read sequencing projects aimed at yielding such pangenomes is the need for scalable tools which are able to align massive numbers of long reads back to reference genomes. MashMap is a tool capable of such tasks and achieves substantial scalability by only identifying where in the reference a read should be mapped to without providing a base pair alignment. MashMap is able to efficiently identify segmental duplication in the human genome and is currently used in the HPRC pipeline for computing genome alignments. However, MashMap has been proven to have statistical bias and also struggles to accurately align repetitive regions. In our work, we remove the bias from MashMap by replacing the core alignment kernel and in the process improve the accuracy, speed, and memory of MashMap. With our algorithmic improvements to MashMap, we will pursue identifying previously undiscovered structural variants in the human genome as well as measure the improvement of alignment accuracy in the HPRC pipeline with our updated alignment kernel.

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Kunin, Alexander **Poster #41**

Hierarchical Modularity in Large Scale Brain Circuits

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Brain function relies on the organization of neural circuitry in the brain. Historically, investigating neural connectivity involved a trade off in scale and resolution -- connectivity in the whole brain could only be described at the level of brain regions or large neural populations, while studying specific synaptic connections could only be carried out at the scale of tens or hundreds of neurons. Recently, reconstructions of tens to hundreds of thousands of neurons at synaptic resolution have been published, enabling investigations into the interplay between global, modular organization, and cell type-specific wiring. These data sets are large and complex; realizing their promise of multi-scale investigations into brain circuitry requires new methods and tools for the analysis of large networks. We present a study using such tools to study large-scale brain circuits.

We applied novel community detection methods to the Hemibrain data set, a synapse-level reconstruction of 21 thousand neurons and over 10 million synaptic connections in the brain of an adult female fruit fly (*Drosophila melanogaster*). By optimizing a modularity score at multiple scales of resolution, we revealed structure from the level of brain regions, comprising thousands of neurons, down to wiring patterns involving as few as three neurons. We validated our results by comparing to known anatomical features and functional networks of the fly brain. For example, our method automatically identifies the layered structure of the fan-shaped body, a structure in the central complex of the fly brain that plays a role in spatial navigation and the modulation of internal state. The communities we found also revealed cell type wiring patterns, both large and small scale. We found that the most common cell types in the Hemibrain tended to cluster together in large, densely-connected networks. Moreover, the smallest, most densely-connected networks revealed cell-type-specific wiring patterns, such as in the pooling of visual inputs to the central brain.

These methods show that the fine-scale network reconstruction made possible by modern experimental methods are sufficiently detailed to identify both large-scale, global organization of the brain and small-scale, local circuit structure. They moreover enable automated, large-scale generation of novel predictions of brain organization.

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Le, Nhu **Poster #42**

Alternative Recognition Patterns Driving Cross-genotype Response of Hepatitis E Virus-specific T Cells

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Cytotoxic T-cells' specificity and efficacy in clearing diseased cells make them a potent option for immunotherapies. Chronic hepatitis E virus (HEV) infections are in principle treatable with ribavirin (off-label), but side effects limit its use. In addition, there are different genotypes of HEV with different patterns of distribution across human populations (genotypes 1 and 3 - GT1 and GT3 - are the most prevalent), and the genetic variability between these genotypes represents an additional challenge for the development of effective immune treatments. Our group has previously proposed the use of a $CD8^+$ T cell-based immunotherapy as a novel treatment option for chronic GT3 HEV infection in patients who have exhausted current treatment options or are unsuitable for them. Usually, T cell receptors (TCR) recognize only peptides displayed by Major Histocompatibility Complex (MHC) molecules. Each TCR is specific to one peptide-MHC (pMHC) complex, and this specificity is important to prevent off-target toxicity in cellular responses. When such off-target toxicity is observed, it is usually associated with molecular mimicry between the recognized peptide-targets. Here, our preliminary data has demonstrated cross-reactivity between peptides derived from GT1 and GT3. To further understand this cross-genotype response, we used the sequences of the most prevalent T cell clonotypes recognizing GT1 and GT3 in a previous experiment, alongside the sequences of the peptide and the MHC, in order to model the 3D structure of the corresponding TCR-peptide-MHC complex. In turn, this structure was used to identify shared molecular interaction patterns in molecular dynamics (MD) simulations. To our surprise, we observed alternative recognition patterns for the same TCR, when interacting with the GT1- or GT3-derived peptide. This result highlight how subtle sequence variations among peptide-targets can affect their recognition by the same TCR, and how T-cell cross-reactivity can be driven by alternative recognition, rather than molecular mimicry of peptide-targets.

Lee, Kibaek **Poster #43**

Development of Substrate-specific mAbs inhibiting MMP-14

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Matrix metalloproteinases (MMPs) play pivotal roles in controlling a wide variety of physiological and pathological procedures, and thus abnormal MMPs expression or substrate proteolysis leads to many disorders. Among these MMPs, membrane bound MMP-14 has been regarded as the most significant regulator in cancer progression and metastasis. MMP-14 overexpression is associated with breast cancer, therefore inhibiting MMP-14 proteolytic function is an attractive approach for cancer treatment. As regulators of pathological and physiological processes, MMPs have both detrimental and beneficial effects. Intriguingly, these paradoxical roles have been observed on single MMP through the cleavage of different substrates. Especially, cleavage of syndecan-1 by MMP-14 stimulates tumor cell migration, invasion, and metastasis, therefore its inhibition provides a promising strategy for cancer treatment. In contrast, MMP-14 also cleavages monocyte chemotactic protein 3 (MCP3), which results in dampening inflammatory responses, and thus degradation of MCP3 by MMP-14 is beneficial and should not be inhibited. Consequently, there is a requirement to develop substrate-specific inhibitors blocking the cleavage of syndecan-1 by MMP-14 not MCP3. However, there are technical challenges associated with functional selection of substrate-specific mAbs. In this study, we aim to address these issues and develops mAbs with high potency, target/substrate-specific, and stability.

To facilitate proteases inhibitory mAb discovery, our previous works developed convex paratope antibody libraries and functional genetic selection (positive selection) method. This combination is an effect method for the development of mAbs blocking syndencan-1 degradation from MMP-14. However, for isolating mAbs that do not impede MMP-14-catalyzed degradation of MCP3, a counterselection (negative selection) method is highly required. As a GH68 fructosyltransferase, levansucrase hydrolyzes sucrose and synthesizes levan, leading to accumulation of levan in periplasm of gram-negative bacteria and cell death. Based on our previous experiences for periplasmic expression of target protein, we confirmed that *Escherichia coli* cells carrying levansucrase were not grown in liquid media supplemented with 1 mM sucrose or more. In addition, we inserted a cleavable peptide sequence (QPVGINTSTT of MCP3) between N405 and D406, resulting in minimal disruption to catalytic function of levansucrase. By co-expression with or without MMP-14, cells only survived in media supplemented with 290 mM sucrose when MMP-14 was periplasmically co-expressed with modified levansucrase carrying a cleavable peptide insert, and thus rounds of such positive and negative selections led to the generation of mAbs exhibiting substrate-specific inhibition. We expect that technological developments within this study allow us to systematically discover substrate-specific mAbs targeting protease, which otherwise is not able to be achieved by current antibodies selection/screening technologies.

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Lewis, Jaila **Poster #44**

Evaluation of the Ranking Power of Peptide-HLA Scoring Functions

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Cellular immunity is a complex process that requires the participation of diverse proteins, cells, and mechanisms to combat infections and cellular diseases. Human-Leukocyte Antigen (HLA) receptors play a vital role in cellular immunity due to their ability to bind to intracellular peptides and present them to T-cell lymphocytes. The recognition of peptide-HLA (pHLA) complexes by T-cells gives rise to T-cell activation, elimination of the diseased cell (e.g., cell presenting *non-self* peptides, derived from tumoral or viral proteins), and the generation of immunological memory against the recognized peptide-target. This mechanism can be used to boost the cellular immunity against a specific peptide-target, through innovative immunotherapy methods like cancer vaccines and T-cell-based immunotherapy. In this context, several computational methods have been used for the identification of the peptides that bind to HLAs, which, in turn, can be used in treatments for cancer and viral infection. Sequence-based approaches, trained on available datasets of known HLA-binders, are the gold-standard in the field. However, these methods have limitations due to the lack of data for less prevalent HLA alleles. On the other hand, structure-based methods like molecular docking have been proposed as a more general approach for studying pHLA interactions, including the uncommon HLA alleles. Structure-based methods are still limited by the efficiencies of their sampling algorithms, and the accuracy of the scoring functions used to rank predicted conformations. To address the sampling challenge, our group developed the meta-docking approach DINC (Docking INCrementally). DINC's sampling method outperformed regular docking tools, but DINC's predictions still depend on popular scoring functions that were designed to rank small, drug-like ligands. Here, we tested several existing scoring functions to identify the best option for ranking peptide conformations produced by DINC. In order to benchmark the scoring functions, we selected a dataset of 50 pHLA complexes for which we have (i) an experimentally-determined crystal structure and (ii) an ensemble of 100 alternative conformations that were sampled by DINC in a self-docking experiment. The experimentally-determined crystal structures provide us with a reference to rank all predicted conformations in terms of their distance to the *correct answer* (e.g., compute the Root Mean Square Deviation, RMSD, for all heavy atoms of the peptide). This RMSD-based ranking was used to determine the accuracy of the ranking power for each scoring function. Our preliminary benchmark included 7 alternative scoring functions: Vina, Vinardo, AutoDock4, 3pHLA, DOPE, GradDOCK, and FoldX. Among those, 3pHLA and AutoDock4 stood out as the best options, but there is clear room for improvement. In the future, we will expand our dataset to a total of 836 pHLAs (e.g., all available pHLA crystal structures). We will also include more scoring functions and evaluate the use of consensus scoring methods. If successful, this project will have direct applications to antiviral vaccine development and cancer immunotherapy, by providing the means for accurate structure-based screening of HLA binders.

Lewis, Miranda **Poster #45**

Evaluation of Nitazoxanide as an Antiviral for Human Norovirus Using Human Intestinal Organoids

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Human noroviruses (HuNoVs) are the leading cause of acute vomiting and diarrhea. In healthy people, symptoms usually resolve within three days; however, in immunocompromised persons, HuNoV infection can become persistent, debilitating, and life-threatening. There are currently no licensed therapeutics for HuNoV due to a near half-century delay in its cultivation. Treatment for chronic HuNoV infection in immunosuppressed patients includes off-label nitazoxanide (NTZ), a broad-spectrum antimicrobial. Nitazoxanide shows antiviral activity in an *in vitro* replicon model of genotype GI.1 HuNoV RNA expression. However, this drug has not been evaluated in infections with commonly circulating HuNoVs. Nontransformed, multicellular human intestinal organoids (HIOs) are a physiologically relevant cell culture system derived from intestinal stem cells that support replication of HuNoV. HIOs have great potential for antiviral studies, as they are permissive for several HuNoV strains and can be generated from different donors, allowing evaluation of the diversity of human responses. Despite these advantages, few studies have used HIOs for antiviral research. A pipeline for NTZ testing was established to inoculate a standard viral dose using 100 half maximal tissue culture infectious doses ($TCID_{50}$s) and treat cells with 5 ascending drug doses in tandem with cytotoxicity testing. Antiviral activity of NTZ was measured based on viral RNA replication 24-48 hours after infection of HIOs with or without drug treatment. Cell viability was measured to demonstrate that replication inhibition was not due to cytotoxicity across the therapeutic range of the compound. NTZ showed antiviral activity in HIOs; strain-specific responses were observed and mechanisms for these differences are under investigation. HIOs provide a pre-clinical platform to test antivirals against HuNoVs and develop therapeutics to treat norovirus disease.

This work was supported by the GCC Training Interdisciplinary Pharmacology Scientists T32 (T32-GM139801).

Liu, Andi **Poster #46**

Identifying Candidate Genes and Drug Targets for Alzheimer's Disease by an Integrative Network Approach Using Genetic and Brain Region-Specific Proteomic Data

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Genome-wide association studies (GWAS) have identified more than 75 genetic variants associated with Alzheimer's disease (AD). However, how these variants function and impact protein expression in brain regions remain elusive. Large-scale proteomic datasets of AD postmortem brain tissues have become available recently. In this study, we used these datasets to investigate brain region-specific molecular pathways underlying AD pathogenesis and explore their potential drug targets. We applied our new network-based tool, Edge-Weighted Dense Module Search of GWAS (EW_dmGWAS), to integrate AD GWAS statistics of 472 868 individuals with proteomic profiles from two brain regions from two large-scale AD cohorts [parahippocampal gyrus (PHG), sample size n = 190; dorsolateral prefrontal cortex (DLPFC), n = 192]. The resulting network modules were evaluated using a scale-free network index, followed by a cross-region consistency evaluation. Our EW_dmGWAS analyses prioritized 52 top module genes (TMGs) specific in PHG and 58 TMGs in DLPFC, of which four genes (*CLU*, *PICALM*, *PRRC2A* and *NDUFS3*) overlapped. Those four genes were significantly associated with AD (GWAS gene-level false discovery rate < 0.05). To explore the impact of these genetic components on TMGs, we further examined their differentially co-expressed genes at the proteomic level and compared them with investigational drug targets. We pinpointed three potential drug target genes, *APP*, *SNCA* and *VCAM1*, specifically in PHG. Gene set enrichment analyses of TMGs in PHG and DLPFC revealed region-specific biological processes, tissue-cell type signatures and enriched drug signatures, suggesting potential region-specific drug repurposing targets for AD.

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Luu, Trang **Poster #47**

Identifying Proteomic Responses to Acute Sphingolipid Synthesis Inhibition

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Sphingolipids and their metabolites modulate numerous cellular processes, such as cell proliferation, death and differentiation. Recent findings have implicated alterations in sphingolipid levels are associated with various pathologies, such as Alzheimer's disease, asthma, autoimmune diseases, Gaucher disease, diabetes, chronic heart failure, and cancer. Targeting sphingolipid metabolism pathways have becoming promising strategies for the development of therapeutics in the treatment of these diseases. However, most of the current drugs show either low efficacy or resistance after initial responses. To improve current therapies, it is therefore crucial to understand the early cellular responses to the inhibition of sphingolipid metabolism, which is more likely to reveal direct and efficient targets for combinational therapy. We hypothesize that one type of early cellular responses to sphingolipid synthesis inhibition are alterations in the abundance or subcellular localization of some proteins. Here, we performed mass spectrometry analysis to compare cytosolic, membrane and nuclear proteome in control and myriocin-treated HeLa cells. We found that levels of proteins in several pathways are changed in response to acute sphingolipid synthesis inhibition by myriocin treatment, such as cholesterol synthesis, and mitochondrial protein import. We plan to confirm the changes in some candidate proteins by Western blot in these two pathways, such as DNAJC19, PMVK, and MSMO1. We will also evaluate whether inhibition of one of these candidates alters the sensitivity to myriocin. Our finding will provide a better understanding of the mechanism of *de novo* sphingolipid synthesis, and may help to develop a new treatment strategy for sphingolipid-imbalanced diseases.

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Ma, Rulong **Poster #48**

The Regulation Mechanism of the C-terminal Extension on SERCA2b Activity

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The sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) maintains the cellular calcium homeostasis by transporting Ca^{2+} from the cytosol to the ER. SERCA2b is a housekeeping protein in human cells, and its loss or mutations lead to Darier's disease. Comparing to other members in SERCA family, SERCA2b possesses a 49-residue C-terminal extension (CTE) with inhibitory effect. The CTE consists of a loop (L10/11), a transmembrane helix (TM11) and luminal extension (LE). Both the mutations (F1018G/V1029G) on TM11 and LE deletion (T1032stop) can weaken the inhibitory effect of the CTE. Further, some point mutants (e.g., E917K and S920Y) out of CTE can significantly weak or remove the inhibitory effect of the CTE. However, the inhibitory regulation mechanism of the CTE is not clear.

Since the cryo-EM structure of SERCA2b does not have the L10/11 structure, the L10/11 was added via homology modeling. We used molecular dynamics simulations to study CTE's behavior in the WT and mutants. In the WT simulation, we found that Helix-1002 formed in the L10/11 binds L8/9 and that Helix-1002 is in the way of the tilting motion of the cytosolic extension of TM5 (ceTM5). When the tilting angle of ceTM5 becomes larger, the binding between Helix-1002 and L8/9 was significantly degraded. When the tilting angle of ceTM5 becomes smaller, the binding between Helix-1002 and L8/9 was recovered. There is no helix formed in L10/11 that binds L8/9 in the mutants, because these mutants can degrade (F1018G/V1029G or T1032stop) or block (E917K or S920Y) their binding.

Herein, we propose a novel regulation mechanism of the CTE on SERCA2b activity. L10/11 plays a critical role in the inhibitory effect of the CTE. Helix-1002 formed in the L10/11 binds L8/9. This binding impedes the tilting motion of ceTM5 through L6/7. This can retard the intermediate transition of SERCA2b in the catalytic cycle and decrease its activity. This regulation mechanism can explain the variants that can weaken (or remove) the inhibitory effect on of the CTE on SERCA2b activity, such as variants E917K, S920Y, F1018G/V1029G, and T1032stop.

Manuel, Astrid **Poster #49**

Gene Regulatory Network Synchronizes Genetic and Epigenetic Signals, Prioritizes GWAS SNPs, and Identifies Repurposable Drug Candidates for Multiple Sclerosis

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In multiple sclerosis (MS), immune-mediated demyelination of the central nervous system leads to chronic neurological disability. The etiology of MS is poorly understood; nevertheless, both genetic and environmental risk factors have been determined. More than 200 single nucleotide polymorphisms (SNPs) have been identified with genome-wide significant associations in MS by genome-wide association studies (GWAS). Differentially methylated CpG sites in the genomes of MS patients have also been pinpointed by epigenomic studies. Here, we investigated the interplay between genetic factors and epigenetic regulation in MS. We employed a network model to integrate GWAS summary statistics of 14,802 MS cases and 26,703 controls with DNA methylation profiles from 140 MS cases and 139 controls and the human interactome. We acquired differentially methylated genes by aggregating the effects of differentially methylated CpG sites within respective promoter regions. Literature-curated transcription factor (TF) interactions were used to reconstruct a gene regulatory network (GRN). Colocalization of the MS GWAS and methylation quantitative trait loci (mQTL) was performed to assess the genes of GRN. Cell-type specificity of the GRN was also investigated by the Web-based Cell-type Specific Enrichment Analysis of Genes (WebCSEA). Lastly, we performed drug target enrichment analysis using the Therapeutic Target Database (TTD). The prioritized MS-associated GRN was comprised of 25 genes and several TF interactions. We highlight genome-wide significant SNPs with GWAS-mQTL colocalization pairs: rs6032663, rs6065926, and rs2024568 of *CD40* locus, rs9913597 of *STAT3* locus, and rs887864 and rs741175 of *CIITA* locus. Interestingly, aligned mQTL and eQTL signals were identified at the rs6065926 variant of the *CD40* promoter, suggesting epigenetic changes at this locus may also lead to gene expression alteration. WebCSEA analysis showed that the GRN was enriched (p-value = 0.0016) in T follicular helper cells (p-value = 1.11×10^{-8}) and memory B cells (p-value = 1.57×10^{-7}). Conjectural cell-type-specific gene-environment mechanisms of MS etiology were introduced based on these results. Moreover, the GRN was enriched with drug target genes (p-value = 3.89×10^{-4}), which revealed repurposable candidates for MS treatment: vorinostat (*HDAC1* inhibitor), napabucasin (*STAT3* inhibitor), and others. The repurposable drug candidates proposed warrant further investigation.

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Marcus, Joshua **Poster #50**

Quality Control of Arsenic Induced Stress Granules by the Endoplasmic Reticulum

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Millions of people around the world are exposed to arsenic-contaminated drinking water. Arsenic exposure has negative effects on human health, including an increased risk of developing cancer, diabetes, and neurodegenerative disease. Cells respond to arsenic exposure through a program called the integrated stress response (ISR). The ISR halts normal cellular functions to conserve energy and resources until the stress is mitigated. One mechanism to conserve energy is to inhibit bulk protein synthesis by sequestering mRNAs into an inducible membrane-less organelle called the stress granule (SG). SGs form shortly after exposure to arsenic and disassemble after arsenic is washed away, but the quality control mechanisms that dictate the timing of these events remain unknown. SGs interact with the endoplasmic reticulum (ER) and marks sites of SG fission, but it is not yet known whether the ER are important for SG quality control. By genetically and pharmacologically altering ER morphology and function, we can test the dependence of the ER on the SG disassembly process. We demonstrate that increasing the abundance of ER sheets and decreasing ER tubules in cells leads to a decrease in SG disassembly rate. Expansion of ER sheets blocks SG fission, leading to larger SG with decreased surface area per SG volume. Proteomic analysis of SG interacting proteins at the time of disassembly identifies ER-associated degradation (ERAD) machinery as potential SG disassembly factors. Our data suggest that ER-directed fission of SGs is a novel component of the SG disassembly mechanism. We propose a model for SG disassembly where fission by the ER both increases the surface area of SGs and couples them to the ER-localized disassembly machinery. Investigating the stress granule quality control mechanism will identify new potential therapeutic targets for diseases that arise from arsenic exposure and may identify risk factors for these diseases that are linked to defects in SG quality control.

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Menegatti Rigo, Mauricio **Poster #51**

TCRdock: A Protein-Protein Ensemble Docking Tool for Immunotherapy Applications

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Cancer has a major impact on society and represents one of the biggest challenges for healthcare. Despite projections pointing to an expected annual increase in new cancer cases worldwide, the overall death rate and incidence decreased in the last years. This decrease was driven mainly due to advances in cancer treatments, especially with the arising of cancer immunotherapy. Cancer immunotherapy involves the "training" of the patient's own immune system to fight cancer cells, and one way to achieve better results is through the recognition of tumor-derived peptides and neoantigens by the T cell lymphocytes. These peptides will be displayed by Human Leukocyte Antigen class I (HLA-I) molecules at the surface of cancer cells and recognized by T cell receptors (TCRs). There are pivotal features that govern the immune response against cancer cells coded into the peptide:HLA-I (pHLA-I) binding and in the pHLA-I:TCR interaction. These features can be extracted from structural attributes (e.g., topography, electrostatic potential) and amino acids sequence composition (e.g., hydrophobicity, pKa, size). Structural homology modeling and sequence-based computational algorithms are currently employed to deal with the myriad of information obtained from these interactions to predict pHLA-I binding modes and affinities. However, most of these methods do not consider the flexible nature of the residues involved in both pHLA-I and pHLA-I:TCR interaction. To address this, we focus our work in three aims: *(i)* to develop a method (TCRDock) for ensemble generation of pHLAs, *(ii)* and ensemble generation of TCRs, *(iii)* to provide a tool for pHLA-TCR modeling using the aforementioned ensembles to account for protein flexibility. For the first aim, we improved APE-Gen, a tool for generating conformational ensembles of pHLA complexes. The second version of APE-Gen allows for modeling peptides with different post translational modifications and provides a better way to model peptides that present non-canonical anchor conformations. For the second aim, we retrieved TCR sequence information from single cell experiments contained in different databases (e.g., VDJdb). We then created pipelines to model the TCRs, renumber them according to IMGT nomenclature, and use the Randomized Coordinate Descent (RCD) algorithm to model various CDR loop conformations. For the third aim, combining the different conformations to create ensembles of TCR-pMHC complexes, we are testing protocols with homology modeling using modeler, and docking via a modified version of PIPER, the core algorithm behind the ClusPro server. Validation of the structures will be done by comparing the Root Mean Square Deviation (RMSD) and the difference in energy between generated conformations and known pHLA-TCR structures from Protein Data Bank. Finally, data science techniques, such as clustering and feature extraction, will be employed to facilitate the gleaning of information from the large ensembles of generated conformations. By doing this, we plan to leverage the knowledge on the pHLA-I:TCR interaction by using different conformations to unveil molecular aspects that would not be possible to observe by analyzing only static crystal structures. We expect our method can be used to further guide the selection of higher affinity T-cells for cancer immunotherapy and to better understand the molecular features underlying the TCR recognition.

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Nair, Achuth **Poster #52**

Patient Specific Corneal Biomechanics Guided by Machine Learning

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The mechanical properties of the cornea may provide an important biomarker for evaluating ocular health. Alterations in the stiffness of the cornea have been implicated in the development of several diseases including keratoconus and post-LASIK ectasia. Optical coherence elastography (OCE), a functional extension of optical coherence tomography (OCT), presents one method for measuring the mechanical properties of the cornea and may be a useful tool for disease diagnosis and treatment planning. Briefly, the technique involves using OCT to detect a sample's response to an induced force, which relates to its material properties. Despite the effectiveness of OCE, the technology is currently limited by slow processing times that restrict rapid interpretation of results. In this work, a deep-learning approach is used to improve mechanical property estimation from raw OCE data.

Initial development and testing of the deep learning approach is performed using synthetic representation of raw quasistatic OCE data. OCE data is typically represented in complex form with both an amplitude and phase component. 50,000 images of random Gaussian surfaces of size 128 x 128 pixels are formed and wrapped to pi. Gaussian noise is added to each image to simulate the OCE speckle noise. Each wrapped phase map represents the motion between successive images that is captured in the OCE phase data. In the typical quasistatic OCE workflow, motion detected using OCE is translated to displacement and used to calculate strain using an axial spatial derivative. The same procedure was implemented here to obtain a strain map for each synthetic phase map, prior to the addition of gaussian noise. The phase map and strain map were input into a four-layer U-net convolutional neural network as input and labelled data respectively. The trained network was then tested on new synthetic wrapped phase maps for validation. Sample input and labelled data, as well as network results, are shown in Figure 1.

The results suggest that the CNN network can effectively estimate the strain with comparable results to more established strain estimation algorithms. Furthermore, the network shows a 30x increase in in computation speed compared to more established strain estimation algorithms. Future work will focus on application of this technique to real data obtained on patient corneas for rapid biomechanical property estimation.

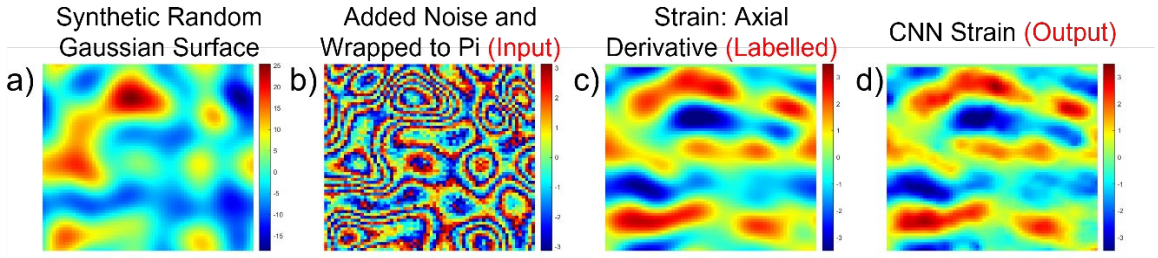

Figure 1: a) Generated random gaussian surface, representing the displacement detected between OCE frames. b) After wrapping and adding noise, input image into CNN c) Strain calculated from a) using axial derivative, used as the labelled data for the CNN. d) Strain calculated from the CNN.

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Neja, Sultan **Poster #53**

Metabolomics of Acute vs. Chronic Spinach Intake in an Apc-Mutant Genetic Background: Linoleate and Butanoate Metabolites Targeting HDAC Activity and IFN-g Signaling

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There is growing interest in the crosstalk between the gut microbiome, metabolomic features, and disease pathogenesis. Colorectal cancer is a major health burden worldwide, linked in part to modifiable risk factors associated with diet and lifestyle (1). The differential roles of the metabolites in targeting Wnt/b-catenin signaling were recently reported (2). The current investigation compared long-term (26 week) and acute (3 day) dietary spinach intake in a genetic model of colorectal cancer. Metabolomic analyses in the polyposis in rat colon (Pirc) model and in wildtype animals corroborated key contributions to anticancer outcomes by spinach-derived linoleate bioactives and a butanoate metabolite linked to increased a-diversity of the gut microbiome (3). Combining linoleate and butanoate metabolites in human colon cancer cells revealed enhanced apoptosis and reduced cell viability, paralleling the apoptosis induction observed in colon tumors from rats given long-term spinach treatment. Mechanistic studies in cell-based assays and *in vivo* implicated the linoleate and butanoate metabolites in targeting histone deacetylase (HDAC) activity and the interferon-g (IFN-g) signaling axis. Clinical translation of the findings from this investigation to at-risk patients might provide valuable quality-of-life benefits by delaying surgical interventions and drug therapies with adverse side effects (4,5).

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References

1. Xi, Y., Xu, P. Global colorectal cancer burden in 2020 and projections to 2040. *Transl Oncol* 2021;14: 101174
2. Liu, F. *et al*. Suppression of membranous LPR5 recycling, Wnt/b-catenin signaling, and colon carcinogenesis by 15-LOX-1 peroxidation of linoleic acid in PI3P. *Cell Rep* 2020;32:108049.
3. Chen, Y.-S. *et al*. Dietary spinach reshapes the gut microbiome in an Apc-mutant genetic background: mechanistic insights from integrated multi-omics. *Gut Microbes* 2021;13:1972756.
4. Ulusan, A.M. *et al.* Optimization of erlotinib plus sulindac dosing regimens for intestinal cancer prevention in an Apc-mutant model of Familial Adenomatous Polyposis (FAP). *Cancer Prev Res (Phila)* 2021;14:325-336.
5. Samadder, N.J. *et al.* Association of sulindac and erlotinib vs placebo with colorectal neoplasia in Familial Adenomatous Polyposis: secondary analysis of a randomized clinical trial. *JAMA Oncol* 2018:4:671-677.

Pizzini, Jason **Poster #54**

Microbial Therapeutics to Prevent ExPEC Colonization and Disease

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Extraintestinal pathogenic *Escherichia coli* **(ExPEC)** are the most common cause of extraintestinal infections worldwide. ExPEC are versatile bacteria, able to infect nearly all sites of the body, including: blood, urinary tract, and the brain. Global rates of ExPEC morbidity and mortality are steadily increasing, predominantly driven by the emergence of multidrug resistant strains. To date, there are no vaccines or non-antibiotic treatments available and novel therapeutic options are gravely needed to combat this emerging threat. ExPEC behave as commensals while in the gastrointestinal tract **(GIT)** and only cause disease upon dissemination. To disseminate to non-intestinal sites and cause infection, ExPEC must first outcompete and coexist with the native microbial community of the GIT. The necessity of GIT establishment as a precursor to extraintestinal infections provides an opportunity to target ExPEC colonization as a preventative measure to disease.

We predict that administration of microbial communities that enhance colonization resistance against ExPEC establishment in the GIT will dramatically reduce ExPEC infections. To achieve these goals, we will use simplified human microbial communities previously generated through dilution of fecal samples. Using a method developed in our laboratory, *in vitro* minibioreactor array **(MBRA)** platforms, we can rapidly screen these simplified microbial communities for inhibition of ExPEC colonization.

To date, we have identified two simplified microbial communities which successful inhibit ExPEC colonization in our MBRAs. These communities will be further tested in a humanized microbiota mouse model of ExPEC colonization. Following identification of simplified communities which successfully and unsuccessfully provide colonization resistance in vivo we will use a comparative genomic sequencing approach to identify the major organisms common among resistant communities. **We hypothesize that select simplified microbial communities will prevent *in vitro* and *in vivo* ExPEC colonization.**

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Rutherford, Jacob **Poster #55**

Characterization of *Fusobacterium nucleatum* Fatty Acid Synthesis Using Chemical and Molecular Genetics

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Fusobacterium nucleatum is the most commonly isolated microorganism from colorectal cancer (CRC) tissue and mediates CRC progression. Specifically, *F. nucleatum* has been shown to contribute to tumor cell proliferation, immune suppression, and chemoresistance. While broad spectrum antibiotics can target *F. nucleatum*, they also contribute to gut dysbiosis which can have severe adverse health effects. Thus, there is a clinical need for *Fusobacteria* specific antimicrobials. Using chemical inhibitors to demonstrate druggability, and molecular genetics to confirm essentiality, we aim to validate the enoyl-ACP reductase (ENR) FabK as a narrow-spectrum drug target against *F. nucleatum*. ENR is an essential component of the bacterial fatty acid synthesis pathway; analysis of the *F. nucleatum* genome showed that it possesses FabK and none of the other ENR isoenzymes, which are typically present in commensal microbiota. We hypothesize that inhibition of FabK will inhibit the growth of *F. nucleatum,* while having minimal effect on commensal bacteria. We evaluated the druggability of FabK through three separate approaches: 1) we tested a panel of analogs of a known FabK inhibitor against *F. nucleatum* and identified a potent inhibitor of the enzyme, called 681 (MIC 0.4µg/ml); 2) we tested the analogs against isogenic strains of *Enterococcus faecalis* independently possessing FabK or its isoenzyme, FabI; 3) we generated and sequenced *F. nucleatum* mutants that are resistant to 681. Growth of *E. faecalis* was inhibited by 681 only when FabK was the sole ENR, and not when FabI was present, indicating 681 acts specifically on FabK. Ongoing characterization of the 681-resistant mutants revealed multiple mutations that map to *fabK*. We verified FabK essentiality in *F. nucleatum* with two different methods: 1) we attempted to delete *fabK* from the genome; 2) we developed an RNAi based knockdown system to deplete *fabK*. Multiple attempts to delete *fabK* have been unsuccessful, suggesting that the enzyme is essential for growth. In support of that, the RNAi knockdown system shows a loss of growth when *fabK* is depleted. Additionally, supplementing the media with fatty acids is insufficient to protect against *fabK* knockdown. Currently, we are working to create a strain of *F. nucleatum* that has controllable expression of *fabK* to evaluate how increased *fabK* expression changes the MIC of 681. Taken together, our chemical and molecular genetics demonstrate that FabK is a promising drug target for the development of narrow-spectrum antimicrobials.

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Seely, Savannah **Poster #56**

Structural Basis for HflXr-Mediated Ribosome Recycling by Time-Resolved Cryo-EM

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The ribosome, which translates mRNA and synthesizes proteins in all organisms, is the target of most antibiotics currently used to treat bacterial infections. However, pathogenic bacteria have evolved strategies to evade the effects of antibiotics. One such strategy of through rescue of antibiotic-stalled ribosomes, in which ribosome-binding factors restore the ribosome to its functional state. The universally conserved protein HflX rescues stalled ribosomes caused by heat shock and manganese stress, and its mechanism of action involves splitting of the 70S ribosome into its individual small and large subunits. The expression of one variant in *Listeria monocytogenes*, HflXr, is upregulated by sub-lethal concentrations of ribosome-targeting antibiotics, leading to resistance. The molecular bases by which HflXr recycles the ribosome and confers antibiotic resistance are unknown. Here, we use time-resolved cryo-electron microscopy (cryo-EM) to reveal the molecular mechanism of HflXr-mediated ribosome splitting. The structures show that the N-domain of HflXr locates proximal to helix H69 in the large subunit within the 70S ribosome and alters the conformation of the essential inter-subunit bridge B2a, promoting dissociation of ribosomal subunits. The helix-loop-helix domain of HflXr overlaps with the binding site of multiple antibiotics that target the peptidyl transferase center (PTC) in the large subunit, elucidating the molecular basis by which HflXr confers resistance to ribosome-targeting antibiotics.

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Sharkey, Rory **Poster #57**

Mitochondrial DNA Damage Response: TFAM Binding Affinity Is Increased by the Presence of DNA Mismatches or Lesions

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Improper maintenance of the mitochondrial genome (mtDNA) has been linked to many diseases such as neurodegenerative diseases (e.g., Alzheimer's), cancer, and premature aging. While several pathways of DNA damage response are known in the nucleus, the mechanisms of mtDNA damage sensing, response, and repair remain poorly understood. A potential mtDNA damage sensor is transcription factor A (TFAM) due to its abilities to bind and slide along DNA and induce DNA bending upon binding. TFAM consists of two high-mobility group (HMG) box domains, which kink DNA, connected by a flexible linker. Additionally, TFAM is the most abundant dsDNA binding protein in the mitochondria and has roles in transcription through promoter specific binding and compaction of mtDNA through non-specific binding. We have combined biochemistry and structural biology approaches to explore the interaction of TFAM with damaged or mismatched DNA. We found that the binding affinity of TFAM to non-specific DNA is increased due to the presence of a G:T mismatch. The location of the mismatch was also significant as a greater increase in affinity was observed when the mismatch was placed at a kink position rather than in the middle of the two HMG boxes. A still larger increase in affinity was observed when lesions such as 8-oxoGuanine or abasic site were present at the kink position. A similar effect was observed for TFAM specific binding to the heavy strand promoter sequence. We have determined the structure of TFAM bound to non-specific DNA with a G:T mismatch at the kink position. Comparison of our structure to previously solved structures of TFAM bound to non-specific DNA yielded the structural basis for the increase in binding affinity and highlighted key residues interacting with the DNA lesion. This work shows that TFAM binds preferentially to DNA lesions and supports that it may act as a sensor of mtDNA damage. Our work also implies that DNA damage may have a profound impact on mtDNA transcription and compaction.

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Sheffield, Logan **Poster #58**

Molecular Dynamics Simulations Provide Insight into Stability of Hyperthermophilic Endoglucanases

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Endoglucanases play a key role in the industrial production of bioethanol, but the most efficient method requires the utilization of high temperatures and is currently limited by the thermostability of endoglucanases. For this reason, it would be beneficial to discover more high-efficiency, thermostable enzymes to utilize in the hydrolytic process. In this study molecular dynamics simulations were performed on structurally similar endoglucanases with varying levels of thermostability to gain insight on what factors contribute to thermostability in endoglucanases. RMSD, RMSF, PCA, hydrogen bonding and salt bridges were analyzed. Finally, protein energy networks were constructed from nonbonded interaction potentials and analysis was performed using hub population, cluster population, largest community transition profiles and LCC profiles. It was found that the more thermostable endoglucanases exhibited a greater number of hydrogen bonds along with fewer, more segregated electrostatic interactions and a larger network of low-energy van der Waals interactions – likely responsible for providing adequate rigidity to withstand high-temperature conditions while still allowing the flexibility needed for proper catalytic function.

Shropshire, William **Poster #59**

Elucidation of Molecular Mechanisms Underlying Successful Adaptation to Carbapenem Antimicrobials in High Risk Carbapenem Resistant Escherichia coli Lineages

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Escherichia coli is a leading cause of human infection and a major contributor to the epidemic of antimicrobial resistant (AMR) bacteria. Thus, there is an urgent need to understand how certain *E. coli* populations successfully adapt to antibiotic treatments in clinical settings. One of the most challenging AMR *E. coli* infections are those that are resistant to carbapenems, which are considered as last-resort antibiotic treatments. While there has been extensive research on carbapenem resistant *E. coli*, there remains a knowledge gap of how particular high-risk *E. coli* lineages are able to adapt to initial antibiotic exposure, which can be conceptualized as a 'pre-resistant' phase. One of the most prolific high-risk *E. coli* lineages is sequence type 131 (ST131), which include certain sub-populations that readily develop carbapenem resistance. We used a combination of multiple experimental evolution platforms and computational biology techniques to analyze the early adaptive response of ST131 *E. coli* to carbapenems. We identified evidence of early phenotypic changes predicted to reduce carbapenem entrance into the *E. coli* cell prior to a fully carbapenem resistant phenotype. Moreover, we found that at the same time ST131 *E. coli* also rapidly responds to carbapenem exposure by increasing the copy number of antimicrobial resistant genes, which in combination with reduced carbapenem entrance could allow for cell survival in the presence of a carbapenem. Importantly, these changes occur prior to fixed genetic mutations that are ultimately found in fully carbapenem resistant strains. The long-term goals of our holistic investigation of the central tenets of these pre-resistant isolates are to identify which ST131 *E. coli* strains have the capacity to develop carbapenem resistance following carbapenem exposure and to fully understand the adaptive strategies of these high-risk bacteria to assist with the development of novel preventative approaches.

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Trimmer, Kenneth **Poster #60**

TOR and Insulin Signaling Regulate a Nutritional G2/M Checkpoint in the *C. elegans* Adult Hermaphroditic Germline Progenitor Zone Cells

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To produce fully viable gametes, germ cells must go through the energy and nutrient intensive processes of proliferation, differentiation, and meiosis. Therefore, it is vital that germ cells sense and respond to external nutritional cues to prevent any of these processes from proceeding under conditions of insufficient energy or nutrients. This is exemplified by the *C. elegans* adult germline progenitor zone cells which enter a cell cycle arrest at G2 during acute starvation (Seidel et al. 2015), though the major regulators of this starvation-induced G2 arrest remain unknown. We investigated the regulation of this arrest and found a dependence on *wee-1.3* and *cdk-1*, effectors of the G2 DNA damage checkpoint, while retaining independence from the core DNA damage kinases *atm-1* and *atl-1* (ATM and ATR respectively). Instead, we find that a *let-363* (mTOR) weak loss-of-function mutant displays decreased cell division, though not as much as acute starvation. While decreased *daf-2* (IGF1R) function alone produced a decrease in cell division as well, the *let-363;daf-2* double mutant was further reduced in cell divisions, suggesting that a combination of TOR and Insulin signaling may help impose the starvation-induced G2 arrest. To investigate this model, we attempted to create conditions with increased activity for each pathway. For Insulin signaling, we used a loss-of-function of the pathway inhibitor *daf-18* (PTEN). TOR signaling, however, is split into two different complexes (mTORC1, mTORC2), each of which responds to different stimuli. As mTORC2 mostly responds to signaling, including Insulin signaling, we chose to generate mTORC1 mutations which had been shown in vertebrates to increase mTOR activity: *raga-1* Q63L (RagA Q66L) for amino acid signaling, and *daf-15* S907A, S948A (RAPTOR S722A, S792A) for cellular energy signaling. These mutations were generated through CRISPR-editing and combined with *daf-18(ok480)* to generate a triple mutant with putatively higher TOR and Insulin signaling. Functionally, we find that the starvation-induced G2 arrest is partially rescued in the triple mutant, and the germ cells no longer impose the block at G2. Together, we propose that this starvation-induced cell cycle arrest is indicative of a TOR and Insulin signaling regulated G2/M checkpoint, independent of the DNA damage checkpoint, which protects the adult germline progenitor zone cells while energy or nutrients are limiting.

References

SEIDEL, H. S. & KIMBLE, J. 2015. Cell-cycle quiescence maintains *Caenorhabditis elegans* germline stem cells independent of GLP-1/Notch. Elife, 4.

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Tukeman, Gabriel **Poster #61**

Mouse Embryonic Fibroblast Cellular Response to HIV Integrase Inhibitor Dolutegravir

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In 2018, the Tsepamo Study, a surveillance of human immunodeficiency virus (HIV) antiretroviral therapy birth outcomes in Botswana, reported an increased risk of neural tube defects (NTDs) in infants whose mothers were treated with the integrase inhibitor dolutegravir (DTG) from the time of conception. Botswana is a country that does not mandate food fortification with folic acid, which is used widely used to reduce folate deficiency and the risk of birth defects. Understanding the teratogenic mechanism of action for dolutegravir is expected to identify potential rescue strategies that may then be used to mitigate risk for women of child-bearing age taking the medication. As maternal folate supplementation is an established modifier of NTD risk and DTG is a reported partial antagonist of folate receptor 1 (FOLR1), we evaluated the cellular response of Folr1 wildtype, heterozygous, and knockout mouse embryonic fibroblasts with DTG and differing levels of folic acid and 5-methyltetrahydrofolate (5-MTHF), a naturally derived reduced folate. Mouse embryonic fibroblasts were cultured in media supplemented with 100nM or 1μM of either folic acid or 5-MTHF. Following the addition of 0, 5μM, 10μM, or 20μM DTG, cells were allowed to incubate for 24 hours before being fixed and stained with DAPI (DNA) and phalloidin (actin). These experiments show notable cellular differences between supplementation with folic acid and 5-MTHF. Folic acid supplementation increased proliferation of cells and also impacted the cytoskeleton, as actin staining revealed increased intensity and networks. In contrast, 5-MTHF supplementation produced similar proliferation rates, but lacked the remodeled cytoskeletal network. A ten-fold increase in the concentration of 5-MTHF yielded a cytoskeleton network visually similar to the baseline folic acid concentration, which may be due to the ten-fold decrease in affinity between FOLR1 and 5-MTHF compared to FOLR1 and folic acid. As DTG is a FOLR1 antagonist, a further reduction of cytoskeletal activity from 5-MTHF may negatively impact the cell biomechanics needed for neural tube closure, offering a potential mechanism for DTG-related failure.

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Van Riper, Justin **Poster #62**

Characterization of the Human FASTKD4 Post-transcriptional Regulator

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Mitochondria are multifunctional organelles responsible for vital biological processes such as energy production and programmed cell death. Mitochondrial plasticity and homeostasis are largely orchestrated by nucleic acid binding proteins tasked with regulating the mitochondrial genome, transcriptome, and proteome. Tight control of mitochondrial RNA processing events is especially important since aberrant mitochondrial RNA causes a variety of pathologies including cancer. The FASTK protein family are an emerging class of mitochondrial post-transcriptional regulators linked to diverse mitochondrial RNA processing events including RNA maturation, modification, and turnover. The FASTKD4 member is functionally linked to RNA maturation at non-canonical junctions, an important RNA processing event that liberates select RNA transcripts from its polycistronic RNA precursor. Despite its central role in RNA maturation and mitochondrial activity, FASTKD4 function and regulation remains unclear. This knowledge gap is largely due to the complete absence of biophysical and biochemical studies describing the molecular properties of FASTKD4. To address this hurdle, we reconstituted human FASTKD4 and launched a comprehensive structure-function program to identify its intrinsic molecular properties. We performed size exclusion chromatography to determine FASTKD4 is monomeric in solution. Using a quantitative fluorescence-based assay, we establish that FASTKD4 has a strong preference for single stranded RNA and correlated binding affinity with substrate length. Together, these data begin to elucidate the molecular principles driving FASTKD4's function in mitochondrial RNA maturation.

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Wilson, Hannah **Poster #63**

Characterizing *C. albicans* Morphogenesis Regulation in the Context of Host Macrophages

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The opportunistic pathogen *Candida albicans* is the leading cause of systemic fungal infections globally. Disseminated disease carries a fatality rate approaching 40-50%, despite treatment with antifungal drugs. The main predisposing factor for these infections is an impaired innate immune response. Thus, characterizing the interaction between *C. albicans* and innate immune cells is critical in order to understand the mechanisms by which *C. albicans* is able to disseminate in compromised hosts. In particular, the interaction between *C. albicans* and host macrophages is dynamic and complex. Once phagocytosed, *C. albicans* engages alternative metabolic pathways and rapidly adapts to the phagosomal environment. The fungus neutralizes the acidic phagosome and induces morphogenesis, generating hyphae that physically rupture and kill the macrophage. The molecular signals responsible for hyphal growth in this context have not yet been identified, although it is thought that the phagosome is neutralized prior to hyphal growth via fungal metabolic byproducts, and the neutral pH then serves as the inducing signal for morphogenesis. Recent work has suggested that the neutralization is instead the result of hyphal damage to the phagosomal membrane, which occurs after morphogenesis is triggered by an alternative signal (CO_2 has been proposed). These conflicting ideas warrant more rigorous investigation.

OBJECTIVES: The objective of this study is to identify the molecular signals that regulate the hyphal switch inside of the macrophage. Combining the strongest elements of published models, we hypothesize that alkaline metabolic byproducts arising from alternative metabolism cause a transient alkalinization of the *C. albicans* cytosol, which serves as a local signal for the induction of hyphal growth inside of the macrophage phagosome. Here we also test the alternative hypothesis that CO_2 is the hyphal-inducing signal.

METHODS: The interaction between *C. albicans* and macrophages was monitored using live-imaging microscopy. Two mutants defective in components of the CO_2-sensing pathway were generated: a carbonic anhydrase mutant (unable to enzymatically convert CO_2 to bicarbonate), and an adenylyl cyclase point mutant (unable to generate cAMP in response to bicarbonate). These strains were assessed in their ability to form hyphae inside of macrophages. *C. albicans* strains expressing the ratiometric protein pHluorin2 cytosolically were used to track the intracellular pH of wild-type *C. albicans* during hyphal morphogenesis in macrophage co-culture as well as in a variety of conditions *in vitro.*

RESULTS AND CONCLUSIONS: The carbonic anhydrase mutant and the bicarbonate-insensitive adenylyl cyclase mutant were both able to form hyphae inside of macrophages, indicating that CO_2 sensing is not required for this process. Further, using the pH sensor pHluorin, we observed that the intracellular pH of wild-type *C. albicans* is tightly regulated within a physiological range during the process of hyphal morphogenesis, which contradicts previously published work. Concurrently, others have shown that phagolysosomal pH does not contribute to hyphal formation. Thus, neither CO_2 sensing nor pH fluctuations are required for the induction of morphogenesis in this context. Work is ongoing to reveal the signal(s) driving this process.

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Yu, Evan **Poster #64**

Combining Transfer Learning with Graph Attention Models for HIV Risk Prediction

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Objective
In the United States (U.S.), approximately 1.2 million people are living with human immunodeficiency virus (HIV), with up to 13% unaware of being infected. While graph neural networks have been used to predict an individual's HIV status, no studies examine the generalizability of these models when combining HIV datasets or populations of similar settings. We aim to implement a transfer learning strategy of combining two HIV datasets and evaluating the predictive power of these graph neural networks.

Methods
For two study cohorts from 2 U.S. cities (Houston and Chicago), we train logistic regression (LR) and random forest (RF) models to serve as baseline models. We compare them to graph attention models, specifically GAT (Graph Attention Networks) and RGAT (Relational Graph Attention Networks). We implement a transfer learning strategy in which we combine node samples from Houston and Chicago. We use a train-test split of 75/25 from both cities and performed ten-fold cross validation while using AUC (area under the receiver operating curve) as our metric.

Results
The transfer learning strategy of combining the Houston and Chicago datasets for HIV study populations from two cities leads to better performance in graph neural network models when compared to training on individual datasets. The transfer learning strategy does improve the generalizability of models in predicting HIV status.

Conclusion
Combining datasets for the study populations from two cities can lead to better performance in graph neural network models than training on individual datasets. Such a framework is especially valuable in HIV studies where data is limited and sparse. Future research can incorporate other transfer learning techniques like domain adaptation and training models on one dataset and using that model (with optimally tuned hyperparameters) on the other dataset.

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Zhang, Min **Poster #65**

Development of ADAR1 Inhibitors to Improve Cancer Immunotherapy

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Cancer immunotherapies have achieved unprecedented clinical responses and are revolutionizing cancer treatments. Recent genetic screens identified ADAR1 as a promising target for improving immunotherapies. ADAR1 catalyzes the posttranscriptional conversion of adenosine to inosine in double-stranded RNA. Editing levels of dsRNA are elevated in most tumor types relative to normal tissue. So far, no inhibitors that specifically target ADAR1 have been developed due to two major challenges. First, the currently available assays for ADAR1 activities are low throughput, rendering high throughput drug screening impossible and hindering medicinal chemistry optimization. Second, there is no structural information for either the deaminase or the RNA binding domain of ADAR1, hampering structure-based drug design. We are the in the process of developing a high throughput ADAR1 activity assay based on fluorescence quenching and will leverage the High Throughput Research and Screening Center at TAMU-IBT to perform drug screening to identify hits. At the same times, we are also performing high throughput docking study using the homology modeling and AlphaFold2 predicted ADAR1 structure. However, applying AlphaFold2 to structure-based virtual screening is challenging. We will combine molecular dynamics simulation with induced fitting docking to improve the performance of virtual screening. Overall, effective synergy of wet and dry laboratory work will help us quickly discover effective hits and perform medicinal chemistry studies.

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Zhao, Yongyi **Poster #66**

Real-time Algorithms for Non-Invasive Imaging through the Skull

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Epilepsy affects over 2.5 million people in the US and leads to $15.5 million in annual medical costs [1]. Detection of early seizure activity or even "pre-seizure" signals could lead to earlier interventions, thereby reducing the likelihood of injuries or death. Many technologies exist for detecting seizures, including electrocardiogram and motion sensors; however neuroimaging possesses the highest sensitivity and is often considered the "gold standard" [2]. The ideal neuroimaging device for seizure detection would achieve millimeter-scale resolution and sub-second temporal resolution [3]. In addition, it should be comfortable and portable for patients. Thus, time-of-flight diffuse optical tomography ToF-DOT, is one of the most promising candidates. ToF-DOT uses an array of light sources and time-domain sensors in the visible/NIR spectrum and an associated reconstruction algorithm to image deep into biological tissue. It can be built into a compact form factor and is safe for patients since it does not require a surgically implanted device nor harmful ionizing radiation. Unfortunately, one limitation of ToF-DOT is that it exhibits a tradeoff in the spatial and temporal resolution. This is because achieving the desired millimeter-scale resolution requires a long measurement acquisition time and a long algorithm runtime to process large volumes of captured data. We propose an unrolled algorithm to increase the algorithm and measurement acquisition speed without compromising high spatial resolution. We tested our learning-based algorithm on real-world data from our experimental prototype and demonstrated an order of magnitude improvement in both the algorithm runtime and image reconstruction quality compared to traditional algorithms. This technology could lead to wearable, fast, high-resolution functional neuroimaging for applications in not only seizure detection but also brain-computer interfaces and detection of other neurological disorders.

[1] M. M. Goldenberg, "Overview of drugs used for epilepsy and seizures: etiology, diagnosis, and treatment," *P T*, vol. 35, no. 7, pp. 392–415, Jul. 2010, [Online]. Available: https://pubmed.ncbi.nlm.nih.gov/20689626.
[2] A. Ulate-Campos, F. Coughlin, M. Gaínza-Lein, I. S. Fernández, P. L. Pearl, and T. Loddenkemper, "Automated seizure detection systems and their effectiveness for each type of seizure," *Seizure*, vol. 40, pp. 88–101, 2016, doi: https://doi.org/10.1016/j.seizure.2016.06.008.
[3] Zhang T, Zhou J, Carney PR, Jiang H. Towards real-time detection of seizures in awake rats with GPU-accelerated diffuse optical tomography. J Neurosci Methods. 2015 Jan 30;240:28-36. doi: 10.1016/j.jneumeth.2014.10.018. Epub 2014 Nov 1. PMID: 25445250; PMCID: PMC4284948.

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Zhu, Catherine **Poster #67**

GWAS Meta-Analysis Identified Novel Genetic Risk Variants Associated with Myositis

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Background

Idiopathic inflammatory myopathies (IIMs), also known as myositis, are heterogeneous, systemic autoimmune diseases influenced by genetic factors. Previous genome-wide association studies (GWAS) have identified a few susceptibility loci at the human leukocyte antigen (HLA) region. Non-HLA loci are also associated with IIMs, but investigations on these chromosomal regions are still limited. Here, we performed multiple GWAS meta-analysis in 15,350 Caucasian *individuals* (3,528 cases and 11,822 controls) and identified novel variants associated with myositis.

Methods

Genotypes from the Immunochip cohort and the GWAS cohort were provided by the Myositis Genetics Consortium (MYOGEN). We imputed genotypes against the Trans-Omics for Precision Medicine reference panel (TOPMed) and HLA region against the Multi-ethnic HLA v2 reference panel. Both the genotyped and imputed data were included for association analysis. Meta-analysis was conducted on the total IIM and subgroups of myositis. Conditional and joint analyses were performed on the meta-analysis result to identify independent signals.

Results

Regions within the HLA genes had the strongest associations with IIM (Figure 1A). Independent variants in FCRLA, STAT4, IRF4, TLR6, SH2B3, and DCAKD reached genome-wide significance ($P < 5.0 \times 10^{-8}$). Novel INDEL variants, C4B and PDE4D genes were significantly associated with myositis. C4B and rs1265764, and rs9269032 (Figure 1B) also jointly contribute to the risk of myositis.

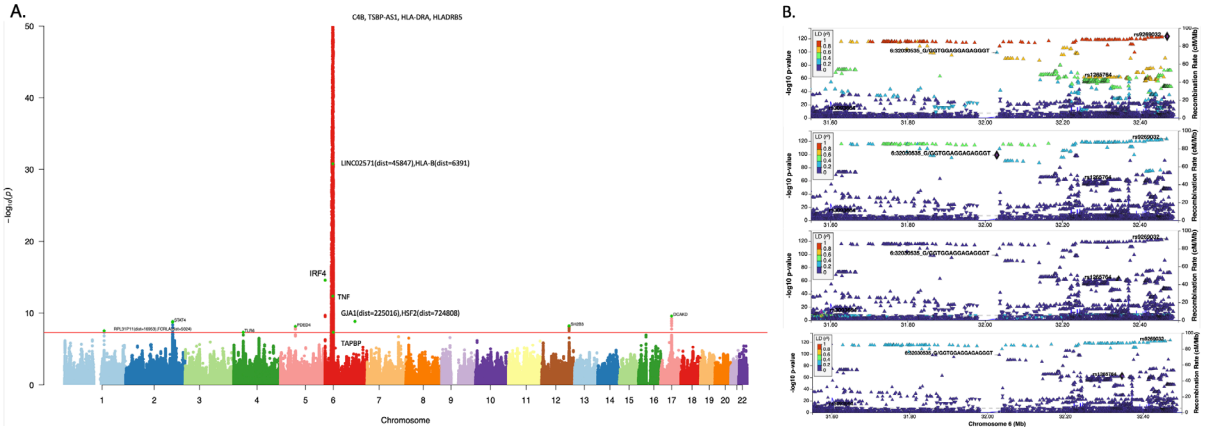

Figure 1 A. Manhattan Plots of the total IIMs. Signals reaching GWAS significance level ($P < 5 \times 10^{-8}$, red line) were highlighted in green. B. Regional plots of rs3093664 (TNF), rs1775857346 (C4B), rs1265764 (TSBP1-AS1), and rs9269032 (HLA-DRA (dist=24931), HLA-DRB5 (dist=47376)). Reference signals are highlighted in bold diamond.

Conclusion:

Our study identified novel signals that contribute to the genetic predisposition of myositis. These variants were highly expressed in lymphocytes, lung, and the whole blood cells, and were enriched in immune function related biological processes, such as antigen processing and presentation and immune response-regulating signaling pathway.

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