Tri-Institutional PhD Program
Chemical Biology

19TH ANNUAL TRI-INSTITUTIONAL CHEMICAL BIOLOGY SYMPOSIUM

POSTER SESSION

Wednesday, September 6th, 2023
1:15 pm – 3:15 pm

Zuckerman Research Center Lobby
Memorial Sloan Kettering Cancer Center
417 E 68th St, New York, NY 10065

Memorial Sloan Kettering Cancer Center
The Rockefeller University — Science for the Benefit of Humanity
Weill Cornell Medicine

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SESSION 1 – 1:15pm-2:15pm

1. Banerjee, Anoosha

TPCB Graduate Student, Rockefeller University
Lab: Seth Darst, PhD

Elucidating the Mechanism of Ribosome Stalling by Nascent Hepatitis B Virus Polymerase

Hepatitis B Virus (HBV) is a hepatotropic DNA virus that chronically infects 296 million people, causing almost one million deaths per year due to increased risk of liver cirrhosis and hepatocellular carcinoma. HBV and other Hepadnaviridaes replicate by reverse transcription, whereby the virus' pregenomic RNA (pgRNA), transcribed by host RNA polymerase II, is converted back to its partially double-stranded DNA genome by HBV polymerase's (HBV Pol) reverse transcriptase domain. HBV Pol also has a terminal protein (TP) domain responsible for binding of the polymerase to the pgRNA, priming reverse transcriptase activity and triggering re-encapsidation of the viral genome. Deep mutational scanning of HBV Pol for viral fitness shows conservation of two prolines adjacent to the polymerase's termination codon. These conserved prolines have been implicated in ribosome stalling, a key feature of how HBV pol achieves cis preference for pgRNA encapsidation and an important detail in understanding how a rapidly mutating virus is able to positively select for functional polymerases. While the importance of the two C-terminal prolines in ribosome stalling is established, the potential presence of other stalling motifs within the nascent protein sequence and the exact mechanism of stalling is still unknown. In order to gain structural insight into the mechanism of stalling and how that relates to conserved elements within HBV Pol, I am working towards purifying and obtaining cryo-electron microscopy (cryo-EM) structures of the HBV Pol-stalled ribosome nascent chain (RNC) complexes. Using an in vitro translation system, I introduce capped mRNA that encodes for N-terminally tagged HBV Pol that remains stuck to the translating ribosome, resulting in accumulation of HBV Pol-stalled RNCs. Currently, I am optimizing the biochemical purification of these complexes via affinity purification for downstream structural analysis using cryo-EM.

3. Bay, Sadik, PhD

Postdoctoral Fellow, Memorial Sloan Kettering
Lab: Gabriela Chiosis, PhD

Visualization of Epichaperomes: Composition, Structure and Function Through Advanced Imaging Techniques

Stressors associated with disease states remodel protein-protein interaction networks (PPIs) through the switch of chaperones into epichaperomes, long-lived assemblies and disease-associated pathologic scaffolds composed of tightly bound chaperones, co-chaperones, and other factors. Not to be confused with chaperones, ubiquitous proteins which fold and act through one-on-one dynamic complexes, epichaperomes act as pathologic scaffolds that form under disease conditions, notably cancer and neurodegenerative disorders including Alzheimer's disease (AD). Epichaperomes have thus emerged as a conceptually innovative and translationally significant gateway to target, detect and control dysfunctional interactomes, now translated to clinic in cancer and Alzheimer's disease, for both treatment and diagnosis (eg. Zelavespib, Icapamespib, PU-PET, PU-FITC). To advance our understanding of epichaperomes in cancer as well as neurodegenerative diseases and learn important mechanistic insights we developed chemical probes and methods for use in confocal and single molecule super-resolution imaging approaches. Here we present probe synthesis and specificity in cultured cancer cells, glutamatergic neurons and human tissues and provide proof-of-principle in the use of these probes to address key questions related to the context-dependent composition of the epichaperomes, their cellular localization and change in response to cellular events and/or stressors. In sum, these preliminary studies propose our imaging probes as unique and important reagents to study cancer and AD biology.

5. Burnside, Chloe

TPCB Graduate Student, Rockefeller University
Lab: Sebastian Klinge, PhD

Structural Characterisation of Assembly Intermediates of the Yeast Mitochondrial Ribosome

Ribosomes are large macromolecular assemblies that exist within all cells and are responsible for the translation of messenger RNA into proteins. Within the mitochondria of eukaryotic cells, dedicated ribosomes (mitoribosomes) are responsible for translating a handful of proteins involved the oxidative phosphorylation pathway. The correct assembly and function of mitoribosomes within cells is crucial for mitochondrial respiration, cell viability, growth and differentiation in all eukaryotic organisms. Although the structure and function of the mitoribosome has been well documented across species, detailed knowledge surrounding its assembly and the factors that regulate this process is lacking. Here we present 3 high resolution cryo-EM structures of assembly intermediates of the small subunit of the mitoribosome in Saccharomyces cerevisiae. These structures, together with biochemical studies of yeast specific assembly factors, illustrate the mechanisms of the late stages of small subunit assembly in the mitochondria of yeast.

7. Cahir, Clare

TPCB Graduate Student, Rockefeller University
Lab: Luciano Marraffini, PhD

Screening Environmental DNA Libraries for Inhibitors of the Type III-A CRISPR-Cas System

Bacteria and their viruses, called bacteriophages (phages), are in a constant arms race for survival. To defend against phages, bacteria have evolved numerous defense mechanisms. One such defense system is the bacterial adaptive immune system called CRISPR-Cas, which consists of clustered, regularly interspaced short palindromic repeats (CRISPRs) and a set of genes that encode CRISPR-associated (Cas) proteins. CRISPR-Cas is a robust immune system present in over 40% of bacteria and 80% of archaea. Nonetheless, phages have evolved mechanisms to counteract CRISPR-Cas; many viruses express anti-CRISPR (Acr) proteins that interact directly with Cas proteins and inactivate them. While over 40 Acrs have been discovered for type II CRISPR systems, very few have been found for the type III CRISPR systems. Therefore, to identify novel Acrs of the type III-A CRISPR system, I will screen environmental DNA (eDNA) libraries from Dr. Sean Brady's lab at The Rockefeller University. These libraries consist of environmental DNA extracted from soil that are introduced into E. coli, and they make genetic material from unculturable bacteria accessible for screening. By identifying novel Acrs, this study will contribute to understanding the mechanisms by which phage evade the highly complex type III CRISPR immunity.

9. Chen, Chen

TPCB Graduate Student, Memorial Sloan Kettering
Lab: Daniel Heller, PhD

Fragment-Based Drug Nanoaggregation Reveals Drivers of Self-Assembly

Drug nanoaggregates can substantially modulate drug action, due to spontaneous formation during screening and in cells, and as a delivery strategy to improve pharmacokinetics. However, the structural features of molecules that drive nanoaggregate formation remain elusive. Therefore, prediction of intracellular aggregation and rational design of nanoaggregate-based carriers are still challenging. We investigated the self-assembly mechanism using small molecule fragments to identify the critical molecular forces that contribute to self-assembly. We found that aromatic groups and hydrogen bond acceptors/donors are essential for nanoaggregate formation, suggesting that both π-π stacking and hydrogen bonding are drivers. We applied structure-assembly-relationship analysis to the drug sorafenib and discovered that nanoaggregate formation can be predicted entirely using drug fragment substructures. We also found that drug nanoaggregates are stabilized in an amorphous core-shell structure. These findings demonstrate that rational design can address intracellular aggregation and pharmacologic/delivery challenges in conventional and fragment-based drug development processes.

11. Chua, Gabriella

TPCB Graduate Student, Rockefeller University
Lab: Shixin Liu, PhD

Differential Dynamics Specify MeCP2 Function at Nucleosomes and Methylated DNA

Methyl-CpG-binding protein 2 (MeCP2) is an essential chromatin-binding protein whose mutations cause Rett syndrome (RTT), a leading cause of monogenic intellectual disabilities in females. Targeted therapeutic intervention against RTT is still lacking, in large part due to insufficient understanding of the heterogenous molecular behavior of MeCP2. In this work, we used correlative single-molecule fluorescence and force microscopy to directly visualize the dynamics and distribution of MeCP2 on DNA and chromatin. We discovered that MeCP2 exhibits distinct diffusion kinetics when bound to unmethylated and CpG methylated bare DNA and exploits these differences to fulfill methylation-specific activities such as the recruitment of co-repressors. We showed that on chromatinized DNA, nucleosomes titrate MeCP2 away from bare DNA, modulating its genomic distribution. MeCP2 was also found to stabilize nucleosomes from mechanical perturbation. Moreover, we showed that RTT mutations differentially alter the biophysical properties of MeCP2-chromatin interaction, explaining their heterogeneous clinical phenotypes in disease. Our work reveals the molecular mechanisms underlying MeCP2's DNA methylation- and nucleosome-dependent functions as well as dosage sensitivity, demonstrating its multifaceted regulation of chromatin structure and gene expression.

13. Gao, Qing

Post-Baccalaureate Research Assistant, Memorial Sloan Kettering
Lab: Yael David, PhD

Dissecting the Link Between the SWI/SNF-Related Protein SMARCA3 and H3K9me3 Maintenance in Cancer

Histone ubiquitination is a key post-translational modification that mediates diverse chromatin functions. However, our understanding of the enzymes that regulate ubiquitination on histones, along with their relationships to human disease, remains limited. Here, we identify SMARCA3 as a novel histone H3K23 E3 ubiquitin ligase that functions through a distinct mechanism from its previously characterized role in post-replication repair. We find that SMARCA3 recruits histone methyltransferase enzymes such as SUV(39)H1, and that depletion of SMARCA3 in HCT116 colorectal cancer (CRC) cells results in reduced levels of heterochromatic H3K9me3. Using both CRC cell lines and patient derived organoids, we demonstrate a correlation between SMARCA3 expression and relative levels of H3K9me3. Complementary immunofluorescence data depicts co-localization between SMARCA3 and H3Kme3, suggesting that SMARCA3 may play a role in the stabilization or deposition of H3K9me3 in HEK 293T cells. Taken together, our data offer a novel epigenetic function for SMARCA3 involved in the maintenance of chromatin integrity.

15. Goldenberg, Isaac

Undergraduate Student, CUNY Queens College
Lab: Sanjai Kumar Pathak, PhD

Development of Dual-Action Inhibitory Agents of Nek2 and EGFR Kinases for Highly Metastatic Cancers

The overexpression and dysregulation of Nek2, a Ser/Thr kinase, is implicated in a wide variety of aggressive cancers, such as triple-negative breast, cervical, and ovarian cancers. Therefore, it is considered a valuable target for the development of anti-cancer therapeutics. Currently, there are no targeted inhibitory agents of Nek2 that have advanced to clinical trials. Using a Nek2 overexpression model in Drosophila melanogaster, our laboratory has identified a novel, non-toxic, quinoline-based pharmacophore from a library of EGFR inhibitor candidates that inhibits Nek2 activity in a competitive manner. EGFR is another important receptor tyrosine kinase with overexpression in several metastatic cancers, overexpressing Nek2 kinase. We hypothesize that a small molecule dual-action inhibitor of both Nek2 and EGFR will yield potent anti-cancer activities. In this work, we test this hypothesis and develop a synthetic strategy for generation of a library of quinoline-based inhibitors of both Nek2 and EGFR kinases. We predict that our library of dual-action inhibitors will yield a greater therapeutic index in cancers with both Nek2 and EGFR overexpression.

17. Harper, Nathan

TPCB Graduate Student, Rockefeller University
Lab: Sebastian Klinge, PhD

Early Stages of Human Mitochondrial Small Subunit Assembly Revealed by Cryo-EM

The mitochondrial ribosome (mitoribosome) is a specialized molecular machine responsible for the translation of OXPHOS subunits encoded in the mitochondrial genome, and thus is a cornerstone of eukaryotic cellular energy production. Assembly of ribosomes involves the coordination of many trans-acting assembly factors which aid in ribosomal RNA folding, modification, and scaffolding, to generate translationally-competent subunits with high fidelity. To visualize early stages of mitochondrial small subunit (mtSSU) assembly in human cells, we used an endogenous tagging system and affinity purification to isolate native assembly intermediates and obtain cryo-EM reconstructions of 6 intermediates at high resolution. These structures elucidate how a network of assembly factors, including GTPases, methyltransferases, and rRNA chaperones, promote the initial stages of the formation of the decoding center, the region of rRNA responsible for translational fidelity. In addition to controlling decoding center formation, these assembly factors also couple these steps to nearby rRNA compaction events, controlling integration of the free 3' rRNA end, ligand-based stabilization of rRNA structure, and methyltransferase activation. Together, these structures illustrate how assembly factors control stepwise ribosomal RNA folding events to control maturation of the central functional site of the small subunit, the decoding center, to ensure proper assembly and prevent premature translation initiation.

19. Hsieh, David

TPCB Graduate Student, Rockefeller University
Lab: Sean Brady, PhD

Discovery of Novel Signaling Molecules in the Gut Microbiome by Mass Spectrometry

The human microbiota is a collection of over one thousand species of bacteria. These bacteria outnumber human cells by a factor of ten, with the majority of them residing in the gastrointestinal tract (GI tract). Because the bacteria in the GI tract (termed the gut microbiota) are close to epithelial, neuronal, and immune cells, they are implicated in diverse aspects of host physiology and diseases. Recent studies about human microbiota have focused on characterizing the species composition and host responses. Despite the importance of human microbiota, very little is known about the mechanisms by which they impact host physiology. A large part of their impact on the host is likely mediated by the molecules they produce. Characterizing these molecules, their biosynthetic pathways and their targets have the potential to improve the development of probiotic therapies.

21. Hu, Yingying

TPCB Graduate Student, Memorial Sloan Kettering
Lab: Xuejun Jiang, PhD

The Effect of mTORC1 Inhibition on Ferroptosis in PI3K Pathway-Activated Endometrial Cancer

Endometrial cancer frequently activates the PI3K-AKT-mTOR pathway via oncogenic co-mutations of PI3K or PTEN. This pathway regulates a wide range of cellular activities including cell proliferation, cell growth, and metabolism, and is often dysregulated in most cancers, making it a prime target for cancer therapies. However, the co-mutation in this pathway makes endometrial cancer insensitive to these therapies. Recent research by the Jiang lab discovered that cells with PI3K pathway-activating mutants are resistant to ferroptosis, and the inhibition of mTORC1 sensitizes them to ferroptosis through the transcriptional factor SREBP1 regulating SCD1, an enzyme involved in lipogenesis. We aim to investigate whether this approach of sensitizing cells to ferroptosis is viable in endometrial cancer, especially those with PI3K pathway hyperactivation. We showed that the combination of RSL3 (an inhibitor of anti-ferroptotic enzyme GPX4) and mTORC1 inhibitors sensitizes endometrial cancer cells with PI3K pathway mutations to ferroptosis. These results will offer a potential direction of clinical treatment of endometrial cancers and cancers with PI3K-activating mutations.

23. Huang, Hsin-Che

TPCB Graduate Student, Memorial Sloan Kettering
Lab: Daniel Bachovchin, PhD

Protein Folding Stress Potentiates NLRP1 and CARD8 Inflammasome Activation

NLRP1 and CARD8 are related pattern-recognition receptors (PRRs) that detect intracellular danger signals and form inflammasomes. Both undergo autoproteolysis, generating N-terminal (NT) and C-terminal (CT) fragments. The proteasome-mediated degradation of the NT releases the CT from autoinhibition, but the stimuli that trigger NT degradation have not been fully elucidated. Here, we show that several distinct agents that interfere with protein folding, including aminopeptidase inhibitors, chaperone inhibitors, and inducers of the unfolded protein response, accelerate NT degradation. However, these agents alone do not trigger inflammasome formation because the released CT fragments are physically sequestered by the serine dipeptidase DPP9. We show that DPP9-binding ligands must also be present to disrupt these complexes and allow the CT fragments to oligomerize into inflammasomes. Overall, these results indicate that NLRP1 and CARD8 detect a specific perturbation that induces both protein folding stress and DPP9 ligand accumulation.

25. Jordan, Victoria

TPCB Graduate Student, Memorial Sloan Kettering
Lab: Heeseon An, PhD

Characterizing the Cellular Role and Regulation of the Novel MINDY1 Deubiquitinase

The small regulatory protein Ubiquitin (Ub) plays an important role in almost all cellular processes. To achieve a diverse set of functions, ubiquitin signaling is regulated by a series of spatiotemporally regulated enzymes: two Ub activating enzymes (E1), ~40 conjugating enzymes (E2), and ~600 ligation enzymes (E3), which facilitate the covalent addition of Ub. To reverse signaling, humans have ~100 deubiquitinating (DUBs) enzymes responsible for removing Ub residues by proteolysis. Recently, a new subfamily of DUBs called the motif interacting with Ub (MIU)-containing novel DUB family (MINDY1-4), has been classified. MINDY1 and 2 proteins share 52% sequence homology and are evolutionarily conserved. Importantly, MINDY1 and 2 proteins have been shown to play important roles in organismal and cellular functions. Knock-out (KO) of the MINDY1 gene in mice resulted in skeletal and neurological abnormalities, and KO mice of MINDY2 showed cardiovascular system abnormalities. Despite the significance of MINDY1-2, there are limited reports of MINDY to understand the mechanism of these KO phenotypes and their direct substrates in cells. In vitro, the deubiquitinating activity of MINDY enzymes has been characterized to target long K48 chains, suggesting a role in regulating substrates for degradation. In addition, MINDY1 and 2 both contain a C-term CaaL prenylation motif, which suggests their functions involve membrane-bound protein regulation. Here, we present preliminary data toward identifying the role of prenylation and to identify the deubiquitinating substrates of MINDY1. This work will elucidate cellular function and regulation of MINDY enzymes and their role in disease pathways.

27. Lemmon, Abigail

TPCB Graduate Student, Memorial Sloan Kettering
Lab: Christopher Lima, PhD

Unfolding SUMO-Polyubiquitin Substrates by Ufd1/Npl4/Cdc48

The Ufd1/Npl4/Cdc48 complex is a universal segregase that targets polyubiquitylated proteins for clearance or removal. Prior studies suggest that the complex also recognizes substrates modified with SUMO. Here, we show that interactions between the C-terminal SUMO interaction motif (SIM) of Ufd1 and SUMO enhance the unfolding of SUMO-polyubiquitin-modified substrates by the budding yeast Ufd1/Npl4/Cdc48 complex compared to substrates modified by polyubiquitin-only chains. Incubating Ufd1/Npl4/Cdc48 with a substrate modified by a SUMO-polyubiquitin hybrid chain produced a series of single particle cryo-EM structures that reveal new features of interactions between Ufd1/Npl4/Cdc48 and ubiquitin prior to and during unfolding of ubiquitin. These results are consistent with cellular roles for and interplay between SUMO and ubiquitin modifications and support a physical model wherein Ufd1/Npl4/Cdc48, SUMO and ubiquitin conjugation pathways converge to promote clearance of proteins modified with SUMO and polyubiquitin. While a C-terminal SIM is not apparent in human Ufd1, the p97 (Cdc48 human homolog) co-factor FAF1 has been implicated in SUMO recognition. Ongoing work is focused on recognition of SUMO-polyubiquitin hybrid chains as a conserved function for human p97 complexes through cofactors that collaborate with p97 to direct metabolism of modified substrates.

29. Meyer, Alex

Undergraduate Student, Lehigh University
Lab: Damien Thévenin, PhD

Development of a pH-Dependent Epitope to Stimulate Immune-Cell Mediated Cytolysis

One of the fastest-growing fields of cancer therapeutics involves directing components of the immune system to recognize cancer cells. However, current methods rely on targeting self-antigens (biomarkers present on healthy cells) that are only found on a small number of cells across all cancer types. This problem is only compounded by the very nature of cancer itself, with its high level of mutations and heterogeneity within tumors. These qualities greatly reduce the already small number of targetable cancers. However, there is an often overlooked yet relevant biomarker that holds for a majority of solid tumors: their acidified extracellular microenvironment. Previous work has shown that the pH (low) insertion peptide (pHLIP), is capable of selectively targeting this acidified micro-environment for a wide range of cancers in vivo. In this project, we aim to exploit this unique targeting ability of pHLIP to display on the surface of tumor cells the SunTag peptide epitope. We hypothesized that cancerous cells decorated with SunTag should be recognized by engineered chimeric antigen receptor natural killer cells (CAR-NKs). This recognition by CAR-NK should promote NK cell-mediated cytolysis of the decorated cancerous cell, resulting in cell death. Here, we describe techniques for synthesizing this SunTag-pHLIP conjugate through bacterial expressions as a fusion protein, and through peptide synthesis with subsequent conjugation reactions. Recently, we demonstrated the ability of this conjugate to selectively insert in cancer cells via flow cytometry experiments.

31. Peyear, Thasin

Graduate Student, Memorial Sloan Kettering
Lab: Kayvan Keshari, PhD

Small Volume Coils for Hyperpolarized Micromagnetic Resonance Spectrometry

Magnetic resonance (MR) spectroscopy is powerful tool that allow for characterizing metabolic profiles without affecting chemical or biological processes. Current methods (MRI/NMR) incorporate large coils and require large samples (large number of detectable nuclei) to produce a quantifiable signal. By decreasing the volume of the coil, we can increase MR sensitivity while lowering the number of cells needed for quantification. However, this comes at the cost of magnetic homogeneity which is crucial for MRS. When coupled with dynamic nuclear polarization (DNP), sensitivity of the system can be substantially improved by ~105. Using hyperpolarized micromagnetic resonance spectrometer (HMRS) with DNP allows for measuring the real-time metabolic fluxes in living cells. The success of these small coils is determined by how well they produce a homogenous magnetic field and if they can detect/characterize metabolites produced. Our current microcoil requires 2 μL of cells, we have developed a smaller microcoil which uses 0.2 μL, and Dresden University of Technology has also created a 1.5 μL nanocoil for high resolution NMR. By integrating these coils into our HMRS platform we can characterize the metabolic fluxes in a wide range of cells. To determine sensitivity of the coils, we used [1-13C]pyruvate as our metabolic probe, as pyruvate enters the cell through the monocarboxylate transporters (MCTs). Pyruvate mainly converts to lactate through lactate dehydrogenase (LDH); it can also convert to bicarbonate by pyruvate dehydrogenase (PDH). By quantifying the changes in the pyruvate to lactate in cells we have a much better understanding of cell function.

33. Ramsey, Jared

TPCB Graduate Student, Rockefeller University
Lab: Tarun Kapoor, PhD

Designing a Covalent Inhibitor of the SARS-CoV-2 Helicase, nsp13

Helicases are conformationally dynamic enzymes that utilize energy derived from ATP hydrolysis to remodel DNA and RNA structures. These enzymes have key roles in essential cellular processes, such as genome replication and maintenance. Additionally, helicases are required for the proliferation of pathogenic viruses and for tumor growth of certain cancers. As a result, helicases are considered to be important targets for chemical probes and therapeutics. However, only a limited number of selective chemical inhibitors for helicases have been reported. Here, we combine the use of biochemical assays and enantiomeric chemical probe pairs to develop a site selective covalent inhibitor of nsp13, the SARS-CoV-2 helicase. We assayed electrophilic probes known as 'scout fragments' and identified an inhibitor starting point, compound 1, that can covalently bind and inhibit nsp13. Systematic testing of cysteine mutants combined with mass spectrometry techniques indicated that the C556 residue of nsp13 can be covalently modified to induce functional inhibition. We generated analogs of compound 1 and found that compound 10 is a site-selective and more potent inhibitor of nsp13. Importantly, analysis of compound 10 and its enantiomer suggested that potency is derived not only from electrophilic reactivity but also from non-covalent binding contacts at the C556 site. Our results demonstrate how enantiomeric covalent probes can be leveraged to develop a site-selective helicase inhibitor. Together, these findings provide a starting point for nsp13-targeting therapeutics, and also suggest a platform for the development of small molecules targeting additional helicase mechanoenzymes.

35. Rasmussen, Victoria

TPCB Graduate Student, Rockefeller University
Lab: Thomas Sakmar, MD

Developing a PROTAC to Degrade the Constitutively Active Onco-GPCR in Uveal Melanoma

G protein-coupled receptors (GPCRs) comprise a super-family of transmembrane signal transducers. GPCRs are common drug targets, but only one-third of non-olfactory GPCRs have been “drugged” to date and many GPCRs are considered “undruggable”. Aberrant GPCR signaling is implicated in the pathophysiology of many cancers, but there are only a few anti-cancer drugs available that target GPCRs. New strategies are required to develop novel therapeutic agents for cancer-associated GPCRs. Proteolysis-targeting chimeras (PROTACs) exploit the ubiquitin-proteasome protein degradation system to target previously undruggable targets. PROTACs are bifunctional molecules with a ligand for the target protein linked to a warhead ligand that binds an E3 ubiquitin ligase. PROTACs bring the target protein into proximity of the E3 ligase, which enables the transfer of ubiquitin from an E3 ubiquitin-conjugating enzyme to the target protein, which can then be degraded at the proteasome. Our hypothesis is that PROTAC-dependent ubiquitination of GPCRs can trigger their proteasomal and lysosomal degradation. To test the hypothesis we designed a fusion receptor expression construct in which HiBiT and HaloTags are added to the cytoplasmic tail of CysLTR2 – a GPCR implicated as a driver oncoprotein in uveal melanoma. The HaloTag provides a binding site for the HaloPROTAC3 ligand, which recruits the von Hippel-Lindau (VHL) tumor suppressor – an E3 ligase often used for PROTACs. The HiBiT tag allows quantification of HaloPROTAC3-dependent degradation through a nanoluciferase protein complementation assay. We will use the CysLTR2 fusion constructs for a proof-of-concept study to evaluate the potential feasibility of using PROTACs to target GPCRs.

37. Repeta, Lucas

TPCB Graduate Student, Memorial Sloan Kettering
Lab: Christopher Lima, PhD

MTREC: Activities and Architectures of an RNA Exosome-Associated Helicase Complex

Cells have evolved sophisticated gene regulation systems that exert dynamic control over RNA transcription and stability. Advances in transcriptomics have revealed an enigmatic class of eukaryotic RNAs arising from inter- and intragenic regions. These cryptic RNAs are constitutively transcribed and targeted for rapid degradation by the RNA exosome, a 3'-5' exoribonuclease that serves as a focal point for RNA surveillance and decay across all domains of life. What function this degradation serves remains unclear, but evidence suggests that it participates in transcription regulation and post-transcriptional gene silencing pathways.

The ubiquitous Mtr4 helicase is conserved throughout eukaryotes as an essential component of RNA exosome cofactor complexes. The recent discovery of a novel Mtr4-like protein (Mtl1) containing complex, MTREC, in Schizosaccharomyces pombe has expanded the catalogue of known exosome functions. Degradation of mRNAs and cryptic transcripts through MTREC-mediated pathways is reported to assist in the formation and maintenance of facultative heterochromatin at their originating genomic loci, a strategy that may be conserved in higher organisms.

To date, limited biochemical or structural information on MTREC has been published. Using in vitro reconstituted MTREC, this study has revealed the architecture of the MTREC complex using cryo-electron microscopy. Structural data indicates several conserved features between MTREC and other known exosome cofactors, as well as notable differences. Helicase activity assays as well as equilibrium binding experiments have demonstrated that Mtl1 exhibits properties that distinguish it from Mtr4, and suggest that Red1 alters how Mtl1 engages its substrates. These experiments have established a foundation to understand how MTREC and the exosome cooperate to regulate target genes, as well as the factors that distinguish MTREC from canonical Mtr4-containing exosome cofactors.

39. Rosenzweig, Adam

TPCB Graduate Student, Rockefeller University
Lab: Sean Brady, PhD

Discovering a Family of Antibiotics That Evades Resistance by Binding Polyprenyl Phosphates

Identification of new antibiotics that can avoid resistance is critical to combating the growing antibiotic resistance crisis. Cilagicin is a recently discovered Gram-positive active antibiotic that has a dual polyprenyl phosphate (C55:P, C55:PP) binding mechanism that impedes resistance development. Here, we screened bioinformatically predicted non-ribosomal poly-peptide synthetase biosynthetic gene cluster (BGC) products in an effort to identify antibiotics that might similarly avoid resistance development. The total synthesis and bioactivity screening of natural products predicted to arise from the BGCs we identified led to the identification of three antibiotics that are active against multidrug-resistant Gram-positive pathogens, two of which (paenilagicin and virgilagicin) sequester both C55:P and C55:PP and, like cilagicin, did not develop resistance even after prolonged antibiotic exposure.

41. Sun, Zhen, PhD

Postdoctoral Fellow, Memorial Sloan Kettering
Lab: Charles Sawyers, MD

Chromatin Regulation of Transcriptional Enhancers and Cell Fate by the Sotos Syndrome Gene NSD1

Nuclear-receptor-binding SET-domain protein 1 (NSD1), a methyltransferase that catalyzes H3K36me2, is essential for mammalian development and frequently dysregulated in diseases including Sotos syndrome, a multisystemic developmental disorder. Despite impacts of H3K36me2 on H3K27me3 and DNA methylation, a direct role of NSD1 in transcriptional regulation remains largely unknown. Using mouse ESCs and directed differentiation, we show that NSD1 and H3K36me2 are enriched at cis-regulatory elements, particularly enhancers, in a cell type-specific manner. NSD1 enhancer association is conferred by a unique tandem quadruple PHD-PWWP module, which selectively recognizes p300-catalyzed enhancer mark H3K18ac and is a hotspot for Sotos syndrome missense mutations. By combining degron-mediated acute NSD1 depletion with time-resolved epigenomic and nascent transcriptomic analyses, we demonstrate that NSD1 promotes enhancer-dependent gene transcription by facilitating RNA polymerase II pause release. Specifically, NSD1 promotes the recruitment of SPT5 at enhancers and promoters. Notably, genetic rescue studies revealed that NSD1 can act as a transcriptional coactivator independent of its catalytic activity. Consistently, our system, which enables high temporal resolution, reveals a decoupling of the rapid reduction in transcription upon NSD1/H3K36me2 loss and slow changes in H3K27me3 and DNA methylation, demonstrating that the primary coactivator role of NSD1 is independent of its functional interplay with PRC2 and DNMT3A/B. Moreover, using embryoid body formation and forebrain organoid differentiation, we show that NSD1 enables activation of developmental transcriptional programs associated with Sotos syndrome pathophysiology and controls ESC multilineage differentiation. Collectively, we have identified NSD1 as an enhancer-acting transcriptional coactivator that contributes to cell fate transition and Sotos syndrome development.

43. Tornow, Nicolai

TPCB Graduate Student, Rockefeller University
Lab: Jeremy Rock, PhD

Identification of Differentially Vulnerable Genes in an In Vitro Cystic Fibrosis Infection Model of Mycobacterium abscessus

Lung infections by non-tuberculous mycobacteria (NTM) are increasing worldwide and present a considerable healthcare problem. Mycobacterium abscessus (Mab) is an opportunistic pathogen often referred to as one of the most antibiotic resistance mycobacteria, frequently manifesting as lung disease. Its abundance in the environment, such as soil and water sources, results in a high rate of human-pathogen contact. Infection is predominant but not exclusive in immunocompromised hosts. Cystic fibrosis patients present a large patient population with up to ~23% suffering from NTM lung disease. No common treatment exists and infection with Mab is often managed instead of cured.

Previous work from the Rock lab focused on Mycobacterium tuberculosis has shown that genome-wide expression tuning by CRISPR interference (CRISPRi) allows quantitative correlation of gene expression levels with resulting bacterial fitness, a relationship commonly referred to as vulnerability. Due to the nature of methods available, essentiality of genes has been viewed and studied as a binary variable – genes can either be essential or non-essential for fitness in a given growth condition. Hitherto restricted to the genome-wide study of non-essential genes or laborious pooling of hypomorphs, titratable gene expression by CRISPRi shifted the paradigm of drug discovery, genomics and target prioritization in M. tuberculosis.

Developing high throughput methodology to identify vulnerable genes in a cystic fibrosis model and resistance mechanisms to commonly used antibiotics is indispensable for the prioritization for drug targets among the enormous genetic variation within the Mab species and to enable discovery of desperately needed new chemical matter.

45. Yardeny, Noah

TPCB Graduate Student, Weill Cornell Medicine
Lab: Jacob Geri, PhD

High-Throughput Target ID of Endogenous Biomolecules

Proximity labeling uses reactive chemical species to label biomolecules within a fixed radius of a protein of interest (POI). These labels can be used to enrich proteins proximal to a POI to study its interactors or environment under variable conditions. Map is a proximity labeling strategy that uses an iridium photocatalyst and visible light to sensitize diazirines into reactive carbenes. The incredibly short half-life (< 1 ns) of the singlet carbene in aqueous environments limits diffusion and allows for labeling with unprecedented spatial resolution (< 20 nm). Photocatalyst conjugation is amenable to small molecules, proteins, and antibodies and can be used to label both intracellular and extracellular targets in live cells or cell lysate. I am using Map to study the binding targets of endogenous cryptic peptides in human cells. To do this, I am first developing new photocatalysts to improve the underlying chemistry of Map. I am then using high-throughput chemistry and purification to conjugate the photocatalyst to peptides of interest. In addition, our group has developed a rapid, efficient 96-well-plate based proteomic platform for large-scale target ID. I plan to perform this workflow on the library of peptide-catalyst conjugates to elucidate the binding targets of hundreds of understudied peptides. This knowledge will be leveraged to unlock novel natural products which will serve as leads for drug development.

SESSION 2 – 2:15pm-3:15pm

2. Cai, Sarah

Graduate Student, Rockefeller University
Lab: Titia de Lange, PhD and Thomas Walz, PhD

POT1 Recruits and Regulates CST–Polα/Primase at Human Telomeres

Telomere maintenance requires extension of the G-rich telomeric repeat strand by telomerase and fill-in synthesis of the C-rich strand by Polα/Primase. Telomeric Polα/Primase is bound to Ctc1-Stn1-Ten1 (CST), a single-stranded DNA-binding complex. Like mutations in telomerase, mutations affecting CST–Polα/Primase result in pathological telomere shortening and cause a telomere biology disorder, Coats plus (CP). We determined cryogenic electron microscopy structures of human CST bound to the shelterin heterodimer POT1/TPP1 that reveal how CST is recruited to telomeres by POT1. Phosphorylation of POT1 is required for CST recruitment, and the complex is formed through conserved interactions involving several residues mutated in CP. Our structural and biochemical data suggest that phosphorylated POT1 holds CST–Polα/Primase in an inactive auto-inhibited state until telomerase has extended the telomere ends. We propose that dephosphorylation of POT1 releases CST–Polα/Primase into an active state that completes telomere replication through fill-in synthesis.

4. Gonzalez-Hernandez, Alberto, PhD

Postdoctoral Fellow, Weill Cornell Medicine
Lab: Joshua Levitz, PhD

Differential Desensitization and Beta-Arrestin Coupling of Presynaptic Metabotropic Glutamate Receptor Homo- and Hetero-Dimers

Metabotropic glutamate receptors (mGluRs) comprise a diverse family of class C GPCRs that play fundamental neuromodulatory roles across a range of excitatory and inhibitory synapses. Within this family of receptors, the presynaptic group II (mGluR2, mGluR3) and group III (mGluR4, mGluR7, mGluR8) subtypes play key inhibitory roles by signaling via the Gi/o subtype of G-proteins. Despite their importance in mediating short and long-term forms of synaptic plasticity and as drug targets for a variety of neurological and psychiatric disorders, little is known about how these mGluR subtypes are regulated. Our group has recently shown that, after exposure to agonists, a subset of presynaptic mGluRs (mGluR3, 7, 8) undergo GRK and beta-arrestin dependent desensitization and internalization, while other subtypes (mGluR2, 5) are resistant to homologous desensitization. Here we extend this analysis to dissect the distinct arrestin-coupling properties, trafficking itineraries, and other regulatory partners of distinct mGluR subtypes using a combination of live cell imaging and single molecule fluorescence. We find that different mGluR subtypes either form transient beta-arrestin complexes which enable endosomal recycling or stable beta-arrestin complexes which undergo lysosomal degradation. We find that the strength of this interaction is controlled by determinants within the intracellular C-terminal domains and can be further tuned by different orthosteric or allosteric agonists. Importantly, the trafficking profiles observed in heterologous systems are conserved in presynaptic compartments where distinct recycling and lysosomal degradation behavior are observed. Moreover, we find that the formation of heterodimers further extends and tunes the desensitization, trafficking, and pharmacological properties of group II and III mGluRs. Together, this work gives new insights into the regulation of presynaptic mGluRs, a key step toward a deeper understand of their synaptic physiology.

6. Hiotis, Giorgos

TPCB Graduate Student, Rockefeller University
Lab: Thomas Walz, PhD

Dynamic HIV-1 Spike Motion Creates Vulnerability for its Membrane-Bound Tripod to Antibody Attack

Vaccines targeting HIV-1's gp160 envelope spike protein are stymied by high viral mutation rates and structural chicanery. gp160's membrane-proximal external region (MPER), which is highly conserved amongst clade strains, is the target of naturally arising broadly neutralizing antibodies (bnAbs), yet MPER-based vaccines fail to generate bnAbs1,2,3. Here, nanodisc-embedded spike protein was investigated by cryo-electron microscopy and molecular-dynamics simulations, revealing spontaneous ectodomain tilting that creates vulnerability for HIV-1. While each MPER protomer radiates centrally towards the three-fold axis contributing to an occluded membrane-associated tripod structure in the upright spike,
tilting provides access to the opposing MPER. Structures of spike proteins with bound 4E10 bnAb Fabs reveal that the antibody binds exposed MPER, altering ectodomain tilt, and imposes strain on the viral membrane and spike's transmembrane segments to disrupt fusion, thereby informing future vaccine development.

8. Juray, Alison

Undergraduate Student, Weill Cornell Medicine
Lab: Despina Siolas, MD, PhD

Site Specific p53 Mutagenesis Through Inducible CRISPR Base Editing in Pancreatic Organoids

Pancreatic cancer, the third-deadliest cancer in the United States, is associated with a poor prognosis. KRAS is the main oncogenic driver in pancreatic adenocarcinoma (PDA) followed by TP53, however, the cooperative phenotypes produced by distinct KRAS and TP53 mutations are not well characterized. TP53 is mutated in up to 70% of PDA, which may be gain-of-function mutations that bestow neomorphic capabilities or loss-of-function mutations silencing its tumor-suppressor activity. Various p53 mutations have been identified in PDA, although the specific effects they have on the tumor microenvironment, immune evasion, and resistance to cancer therapy remain poorly understood. Here, we use a novel inducible CRISPR/Cas 9 gene editing system to introduce specific Trp53 mutations into Kras mutated pancreatic organoids to study their impact on the tumor-immune microenvironment. Organoids mimic the function and behavior of 3D tissues in vitro, allowing us to determine the impact of the six most common p53 mutations in a physiological setting. KrasG12D mutated pancreatic organoids with a doxycycline inducible CRISPR/Cas9 editing enzyme were nucleofected with different Trp53 mutant oligonucleotides. To remove p53 wildtype cells, pancreatic organoids were selected with nutlin, causing apoptosis. DNA from nutlin surviving cells were extracted, followed by PCR sequencing of the p53 gene to confirm the mutational status. Ongoing experiments include orthotopic implantation of p53 mutated pancreas cell lines into the pancreata of wild type syngeneic mice for tumor formation and histological analysis. These experiments aim to understand the impact of p53 mutant alleles on pancreatic tumor development and the surrounding immune microenvironment.

10. Li, Jennie (Xiang)

Graduate Student, Weill Cornell Medicine
Lab: Hagen Tilgner, PhD

Real-Time Transcript Enrichment By Targeted Nanopore Sequencing

To facilitate the study of isoforms using single-nuclei RNA sequencing (snRNA-seq) data, we have previously developed a set of chemical procedures to remove intronic reads and enrich for exon-spanning reads. However, this three-day method is laborious and time consuming. Readfish is an open-source software that performs rapid identification of on-target reads and signals to the Nanopore sequencer to selectively reject unwanted reads in real-time. To achieve more cost-efficient exon enrichment, we used Readfish to enrich 30,673 transcripts from 3,630 human genes implicated in brain diseases in real-time on the latest MinION R10.4.1 flowcell. Optimization is performed by manipulating parameters and input format to maximize enrichment for our sgRNA samples.

Preliminary results have shown that reads sequenced with nanopore sequencing enrichment have 76% on-target rate, compared to 31% on-target rate of reads sequenced without enrichment. Using straightforward targeted Nanopore sequencing, we can effectively enrich for low abundance exonic reads collected from snRNA-seq, improving statistical power of downstream computational analysis beyond traditional gene level analysis. Elucidating cell type specific isoform signatures provides potentially clinically relevant discriminants, enabling targeted therapies at increased granularity. Real-time exon enrichment is an affordable and time-saving approach to harvest low abundance transcripts of interest and allow for impactful discoveries.

12. Lin, Karl

TPCB Graduate Student, Memorial Sloan Kettering
Lab: Heeseon An, PhD

Evolving the NanoTag: The All-in-One Tag System

Genetically encoded protein tags are widely used tools in numerous contexts, including protein purification (peptide tags) and cell imaging (fluorescent proteins). Conventionally, tagged proteins have been expressed transiently by plasmid DNA at artificial levels, which may interfere with biological processes wherein protein stoichiometry is critical, such as signaling pathways. Genomic engineering by the CRISPR approach can achieve expression at endogenous levels; however, generating cell lines for several tagged variants can become laborious and time-consuming. Here, we report our progress in developing a multifunctional protein tag system that enables immunoprecipitation, high-resolution cell imaging, and chemically induced degradation. Since fused tags may disrupt native protein function by steric hindrance, we use camelid single-domain antibody fragments, or “nanobodies,” as a potential small, yet versatile scaffold for our platform. Like traditional antibodies, nanobodies consist of a single 15-kDa immunoglobulin VHH domain that specifically recognizes and binds antigens with high affinity. We are engineering our tag to recognize a bioorthogonal small molecule antigen, to which a functional group (e.g., biotin, fluorophore, or E3 ligase binding moiety) is tethered. Using magnetic- and fluorescence-activated cell sorting on a yeast surface display synthetic nanobody library, we are isolating candidate constructs that bind to select antigens. Subsequently, we are applying random mutagenesis to enhance the binding affinity of our constructs and developing a mammalian expression system for proof-of-concept assays. Ultimately, we will apply our nanobody-antigen constructs to study protein homeostasis through a combination of live-cell imaging, co-immunoprecipitation, and degradation assays.

14. Murphy, Thomas

Post-Baccalaureate Research Assistant, Memorial Sloan Kettering
Lab: Alban Ordureau, PhD

Toolbox Development for Mapping the Phospho-Ubiquitin Interactome

The Ubiquitin (Ub) system controls almost all cellular processes using the 8.5 kDa protein Ub as a modifier. Research in the field has revealed that Ub ligases ("writers") use eight specific locations in the Ub molecule for chain extension, which can then be read by Ub-binding proteins ("readers") or removed by deubiquitylating enzymes ("erasers"). While the complexity of the current Ub code is not fully deciphered, a new Ub language is now emerging. This new code is based on poorly understood PTMs of Ub itself, including, for example, phosphorylation of several residues. Phosphorylation of Ub has been detected on multiple residues in phospho-proteomic databases, but only the modification of Ser65 in Ub by the PINK1 kinase is currently understood mechanistically. This code is read by the Parkin Ub ligase, thereby promoting its activation. Considering the involvement of protein ubiquitylation in a wide swath of cellular processes, Ub phosphorylation has great potential as a regulatory switch for specific signaling pathways. Here, we utilized proteomics to search for cellular receptors (‘readers’) of three phospho-ubiquitin (pUb) proteoforms. Recombinant pUb was first produced with unnatural amino acid incorporation and used as bait in pull-down assays against various cell lysates. pUbSer65 was used as a positive control and its strong interaction with Parkin was observed by MS and immunoblotting, validating our workflow strategy. Additional site-specific interactions were detected by MS and immunoblot. Globally, this initial dataset and method provide a first step for investigating the role of phosphorylation within ubiquitin biology.

16. Nambiar, Deepika

TPCB Graduate Student, Memorial Sloan Kettering
Lab: Heeseon An, PhD

Decoupling the Role of RPS19 During and After Biogenesis to Study Ribosomopathies

Ribosomopathies are genetic disorders caused by mutations in ribosomal proteins (r-proteins) or biogenesis factors that typically result in tissue-specific phenotypes such as craniofacial deformities, severe anemia, or growth failure. While a mechanism linking these mutations to the disorders has not been fully established, two theories have been proposed. One theory suggests that the heterozygous mutations in r-protein genes can inhibit ribosome biogenesis and, therefore, produce fewer ribosomes per cell, resulting in preferential translation of certain genes. Alternatively, these mutations can cause the formation of ribosomes lacking a r-protein (Δr-protein ribosomes), leading to variations in translation. To unbiasedly investigate the two hypotheses, it is critical to decouple the role of r-proteins 'during' and 'after' ribosome biogenesis. Recently, the An lab has established the 'Ribo-DART' system, which can selectively and acutely degrade a single r-protein from the mature ribosomes, forming Δr-protein ribosomes. Since RPS19 is mutated in 25% of Diamond Blackfan Anemia (DBA) cases, I have modified the Ribo-DART system to incorporate RPS19, allowing me to study the mechanism of this ribosomopathy. By using chemical proteomics, biochemical, and cell biological approaches, I aim to investigate whether RPS19 is considered "essential" or "non-essential" for protein translation, as well as the biological output of the ΔS19 ribosomes. Our study will define the role of RPS19 during and after the ribosome biogenesis and provide fundamental mechanistic insight on the relationship between RPS19 haploinsufficiency and DBA.

18. Nieves, Christopher

TPCB Graduate Student, Memorial Sloan Kettering
Lab: Derek Tan, PhD

Photoredox-Mediated Direct Conversion of Non-Steroidal Anti-Inflammatory Drugs to 3-Oxetanol Bioisosteres

Carboxylic acids are motifs commonly found in drugs. However, the presence of this functional group in drugs is often deemed as a liability that can lead to undesired effects in terms of toxicity and ADME properties. One strategy to overcome these pharmacological limitations is the use of bioisosteres, chemical group substitutes that mimic the physicochemical properties of the parent motif while avoiding undesired pharmacological properties. Recently, 3-oxetanols have been reported as potential bioisosteres of carboxylic acids. However, this concept has not been studied extensively due to the lack of efficient methods to synthesize this scaffold. Current methods to access these compounds employ harsh conditions that are incompatible with other moieties and/or require the early-stage introduction of the functionality by multistep de novo synthesis. Therefore, to facilitate the exploration and application of these promising bioisosteres, my goal is to develop synthetic methods to convert carboxylic acids directly to 3-oxetanol biososteres of carboxylic acids and evaluate their pharmacological properties. Herein, the visible-light mediated photocatalytic conversion of aryl acetic acid derivatives to their corresponding 3-oxetanol derivatives is reported. The optimized reaction conditions were then used to access the 3-oxetanol bioisosteres of a variety of non-steroidal anti-inflammatory drugs (NSAIDs), which were then analyzed for inhibition of cyclooygenase (COX) enzymes. Our work demonstrates the effectiveness and utility of this new synthetic transformation in medicinal chemistry.

20. Roychowdhury, Tanaya, PhD

Postdoctoral Fellow, Memorial Sloan Kettering
Lab: Gabriela Chiosis, PhD

Analyzing Systems-Level Protein-Protein Interaction Dysfunctions Through the Lens of Epichaperomics

Assessments of the systems-level protein-protein interaction (PPI) network dysfunctions are presently beyond reach because approaches that enable proteome-wide identification, analysis, and modulation of context-specific PPI changes in native (unmodified) cells and tissues are lacking.

To this end, a solution is provided by discoveries in disease biology that link stressor-induced interactome perturbations to the formation of epichaperomes, pathologic scaffolds composed of tightly bound chaperones, co-chaperones, and other factors. Epichaperomes mediate how thousands of proteins anomalously interact and organize inside cells, which aberrantly affects the function of protein networks, and in turn, cellular phenotypes. Therefore, capturing epichaperomes and the proteome at large negatively impacted by these critical scaffolds provides informative clues for direct access to interactome perturbations in diseases, and to the functional outcome of such changes in native biological systems, providing previously unattainable systems level insights into disease-specific stressor adaptation mechanisms. We introduced the term “epichaperomics” to describe the affinity-purification method that uses epichaperomes as baits to analyze context-specific alterations in protein connectivity and study disease specific interactomes. We here present an overview of the platform, from epichaperomics probes to bioinformatics pipelines, and provide proof-of principle applications of the method in investigating the biology of cancer and neurodegenerative diseases and in deriving new treatment paradigms for cancer.

22. Rufa, Dominic

TPCB Graduate Student, Memorial Sloan Kettering
Lab: John Chodera, PhD

Assessing Protein:ligand Binding Affinity Predictions With Machine-Learned Quantum Chemistry

Alchemical free energy calculations with molecular mechanics (MM) force fields are widely used in the prioritization of small molecules for synthesis in structure-enabled drug discovery projects because of their ability to deliver 1–2 kcal mol−1 accuracy in well-behaved protein-ligand systems. Surpassing this accuracy limit would significantly reduce the number of compounds that must be synthesized to achieve desired potencies and selectivities in drug design campaigns. However, MM force fields pose a challenge to achieving higher accuracy due to their inability to capture the intricate atomic interactions of the physical systems they model. Here, we assess the accuracy of and challenges posed by leveraging hybrid machine learning / molecular mechanics (ML/MM) to perform MM-to-ML corrections on a pharmaceutically-relevant protein:ligand benchmark congeneric series. In this pioneering study, we identify many key takeaways associated with performing molecular dynamics simulations and replica exchange with machine learning potential.
Among many insights, we discover that ML potentials (namely ANI2x) yield simulations characterized by spurious energetic minima and highly-correlated ligand motion which even aggressive enhanced sampling methods struggle to make converge. Furthermore, we find that these ML potentials cannot outcompete state-of-the-art publically available MM forcefields on our benchmarks.

24. Stella, Gianna

TPCB Graduate Student, Rockefeller University
Lab: Luciano Marraffini, PhD

Searching for Novel Anti-phage Defense Systems in Environmental DNA Libraries

Bacteria and the viruses which infect them called bacteriophage (phage) are the most abundant entities on the planet. Bacteria have evolved multiple defense systems to combat phage including CRISPR-Cas which disables phage through cleavage of DNA or RNA and restriction-modification (RM) systems which distinguish foe from self through modifications on DNA. Screens for novel anti-phage defense systems have revealed that there is great complexity and variety in these systems. Many screens rely on bioinformatic searches using publicly available databases which search for clusters of genes related to anti-phage defense known as “defense islands”. While these searches are adept at identifying new systems, they are limited by the diversity of available sequences and thus may only scratch the surface of anti-phage defense systems found in nature.

To address this issue, we utilized environmental DNA metagenomic libraries generated by the Brady Lab to access a plethora of genomic information. These libraries are derived from soil samples collected from across the United States and provide access to DNA of unculturable microorganisms. We can enrich these libraries for anti-phage defense systems using a functional screening pipeline and characterize hits to determine the molecular mechanism of defense.

26. Strauss, Alexa

TPCB Graduate Student, Weill Cornell Medicine
Lab: Joshua Levitz, PhD

Structural Basis of Allosteric Modulation of Metabotropic Glutamate Receptor Activation and Desensitization

The metabotropic glutamate receptors (mGluRs) are neuromodulatory family C G protein coupled receptors, which assemble as dimers and allosterically couple extracellular ligand binding domains (LBDs) to transmembrane domains (TMDs) to drive intracellular signaling. Pharmacologically, mGluRs can be targeted either at the LBDs by glutamate and synthetic “orthosteric” compounds or at the TMDs by allosteric modulators. Despite the potential of allosteric TMD-targeting compounds as therapeutics, an understanding of the functional and structural basis of their effects on mGluRs is limited. Here we use a battery of approaches to dissect the distinct functional and structural effects of orthosteric versus allosteric ligands. We find using electrophysiological and live cell imaging assays that both agonists and positive allosteric modulators (PAMs) can drive activation and desensitization of mGluRs. The effects of PAMs are pleiotropic, including both the ability to boost the maximal response to orthosteric agonists and to serve independently as desensitization-biased agonists across mGluR subtypes. Conformational sensors reveal PAM-driven inter-subunit re-arrangements at both the LBD and TMD. Motivated by this, we determine cryo-electron microscopy structures of mGluR3 in the presence of either an agonist or antagonist alone or in combination with a PAM. These structures reveal PAM-driven re-shaping of intra- and inter-subunit conformations and provide evidence for a rolling TMD dimer interface activation pathway that controls G protein and beta-arrestin coupling.

28. Van Dongen, David

Graduate Student, Memorial Sloan Kettering
Lab: Stephen Long, PhD

Purification and Biochemical Characterization of the CaaX Prenyl Protease RCE1

RCE1 is an integral membrane protein that catalyzes proteolytic removal of the C-terminal 'aaX' motif of RAS and other prenyl lipid modified proteins as part of the protein lipidation pathway known as "protein prenylation". Cleavage of the RAS C-terminus affects the subcellular localization of RAS, making RCE1 a potential target in cancers that harbor RAS mutations. The mechanisms of catalysis, substrate specificity, and the effect of the lipid membrane on these processes are unclear, in part, because of difficulties associated with purification of the enzyme and because it is distinct from other cellular proteases. We have purified RCE1 and characterized the catalytic properties of the purified protein. Using a fluorescence-based assay, we find that the purified enzyme is comparably active to the enzyme in purified and reconstituted membranes. The enzymatic activity of RCE1 is resistant to protease inhibitors of water-soluble aspartic, cysteine, and serine proteases which suggests that it has a distinct active site and mechanism from these enzymes. Finally, we find that RCE1 is reversibly inhibited by Zn^{2+}.

30. Vandana, Jeya

TPCB Graduate Student, Weill Cornell Medicine
Lab: Shuibing Chen, PhD

A Human Pluripotent Stem-Cell Based Platform to Investigate the Biological Role and Function of Chromogranin A in Human Cells and Organoids

Human neuroendocrine cells secrete hormones and peptides in response to neurological and chemical signals. NE cells have many functions, which include controlling the release of hormones to maintain blood glucose levels, air, and blood flow through the lungs, etc. In addition, neuroendocrine tumor, a rare tumor, is developed from NE cells. Human pluripotent stem cells provide unlimited resources to generate NE cells. Recently, we established efficient strategies to derive pancreatic endocrine organoids, intestinal organoids, and lung organoids. NE cells were identified in these organoids. In addition, we created a knock-in Chromogranin A (CHGA):mcherry human embryonic stem cell (CHGA):mcherry human embryonic stem cell (hESC) reporter line. CHGA, belongs to a family of acidic proteins and is co-stored and co-released with catecholamines and hormones from the secretory granules of NE cells. We aim to investigate the transcriptome and epigenome of CHGA:mcherry NE cells in hESC-derived organoids at the single cell level by performing snRNA/ATAC sequencing. Our snRNA/ATAC sequencing will ultimately enable a greater understanding of transcriptional and epigenetic mechanisms controlling NE cell generation in different tissues.

32. Vostal, Lauren

TPCB Graduate Student, Rockefeller University
Lab: Tarun Kapoor, PhD

Analyzing Site-specific and Direct Interactions of AAA Proteins with Cofactors and Substrates in Living Cells

Members of the ATPases associated with diverse cellular activities (AAA) protein family are macromolecular machines that require interaction with cofactors or adaptors to remodel target substrates across a variety of cellular processes, including cytoskeleton regulation, membrane biogenesis, and proteostasis. In particular, valosin-containing protein (VCP/p97) is a hexameric AAA protein that interacts with ~30 cofactors that recruit poly-ubiquitinated and non-ubiquitinated substrates. Previous studies have used proteomic and structural approaches to characterize VCP- cofactor and VCP-substrate interactions in vitro. However, the binding modes of VCP interactors to the AAA protein hexamer have not been analyzed in living cells. Here, we adapt iCLASPI, an approach relying on amber suppression, photo-crosslinking, and quantitative proteomics, to probe site-specific and direct VCP protein-protein interactions in living cells. We have focused on examining VCP-cofactor interactions, which structural studies map to a hydrophobic groove in the VCP N-terminal domain or above the entrance into the VCP central pore. We find that a known cofactor, Npl4, crosslinks directly to VCP L278 positioned at the entrance to the central pore, but not within the central pore near residues E314 and D592 in living cells. Additional cofactors, p47 and p37, crosslink to the same region of VCP as Npl4, consistent with a model in which mutually exclusive regulation of the AAA protein can be achieved in cells. Our modified iCLASPI approach will be used to map the native binding sites of VCP interactors and to examine the temporal dynamics of these interactions during cell division. In ongoing work we are applying our approach to VPS4, a AAA protein involved in membrane remodeling. Together, our findings introduce a robust method for probing site-specific and direct AAA protein-protein interactions in living cells, where these interactions are essential for several cellular processes.

34. Walker, Nicole

TPCB Graduate Student, Memorial Sloan Kettering
Lab: Yael David, PhD

The Effect of Methylglyoxal on Tumor-Associated Macrophage Function

Within the tumor microenvironment (TME), tumor-associated macrophages (TAMs) exist as a heterogenous population of cells, starting as anti-tumoral, pro-inflammatory TAMs and progressively polarizing to pro-tumoral, anti-inflammatory TAMs. This progression corresponds to changes in metabolic phenotype as well as TAM function within the TME. Increased levels of these terminally differentiated, purine metabolic TAMs have been associated with poor patient prognosis as they promote tumor angiogenesis and metastasis. However, what causes these progressive changes in TAM polarization remains unknown. Here, we look at the role that methylglyoxal (MGO), a glycolysis byproduct, whose concentration is increased within both cancer cells due to their reliance on Warburg metabolism and in early-stage glycolytic TAMs, plays on TAM function and polarization. Flow cytometry showed increases in TREM2 surface expression upon MGO treatment, suggesting that TAMs were acquiring a purine metabolic, pro-tumoral phenotype. Furthermore, ELISA data showed that the increased TREM2 expression was accompanied by a decreased secretion of pro-inflammatory cytokines and increased secretion of anti-inflammatory cytokines as well as increased secretion of growth factor VEGF. These data begin to establish a paradigm in which MGO within the TME modulates macrophage function to be more pro-tumoral.

36. Warren, Charles

TPCB Graduate Student, Weill Cornell Medicine
Lab: Jacob Geri, PhD

High Throughput Interactome Profiling of Small Bioactive Peptides

Interactions between individual biomolecules give rise to emergent signaling and regulatory networks that govern complex and varied biological processes. Disruption of these finely tuned interactions can result in imbalanced cellular states that may drive diseases including many cancers that become desensitized to checkpoints encoded by these interactions. Still, many interaction networks remain unmapped within the cell, namely those between cryptic endogenous peptides and their protein targets. This is primarily due to the lack of investigation around translation events that occur from “noncoding” genetic material and limited knowledge of how translated polypeptides can mature into functional proteolytic fragments. Moreover, technologies to map protein-peptide interactions in an efficient and reliable manner are limited, leaving a gap in knowledge of how many bioactive peptides regulate cellular homeostasis. We aim to map the interactions of short (30 ≤ amino acid), bioactive peptides that are (1) translated from noncanonical open reading frames or (2) generated through proteolytic cleavage of nascent proteins. To achieve these goals, we are leveraging the recently developed MicroMap technology and label-free mass spectrometry-based proteomics. A scalable and reliable platform to discover peptide binding targets will uncover the elusive functional roles of understudied endogenous peptides in cell signaling and regulatory networks.

38. Wu, You

TPCB Graduate Student, Memorial Sloan Kettering
Lab: Yueming Li, PhD

In Situ Labeling of Neurofibrillary Tangles in Alzheimer's Disease Using a Photo-Affinity Chemical Probe

Alzheimer's disease (AD) is a progressive neurodegenerative disorder mainly characterized by cognitive decline, memory impairment, and neuronal loss. One of the key pathological hallmarks of AD is the presence of neurofibrillary tangles (NFTs) within the patient's brain. Over the last decade, advancements in positron emission tomography (PET) imaging techniques enables non-invasive visualization and quantification of NFTs in living patients using specific radiotracers that target and bind to tau aggregates. Based on the structures of reported tau PET tracers, we have designed and synthesized a panel of tau photolabeling chemical probes. Next, we evaluated and confirmed the probe engagement with tau aggregates in AD brain tissues through an autoradiography competition binding study. Furthermore, the fluotaucipir based probe, Tau-4, demonstrated the capability to covalently label the NFTs in AD brain sections, allowing subsequent click conjugation with reporter tags – either biotin for affinity pulldown or fluorophore for microscopic imaging. Collectively, these results suggest such probes are promising tools for studying the tau and NFTs in neurodegenerative diseases.

40. Xiao, Yang

TPCB Graduate Student, Memorial Sloan Kettering
Lab: Yael David, PhD

Deciphering Biomolecule Glycation Using Chemical Biology Tools

Within the structural diversity of the human metabolome exist molecules capable of reacting and modifying large biological macromolecules such as proteins, lipids, or nucleic acids without enzymatic assistance forming non-enzymatic covalent modifications (NECMs). These NECMs have been shown to have detrimental structural effects and have been linked to aging as well as pathologies such as cancer, diabetes, and neurodegeneration. Methylglyoxal (MGO) is a highly reactive dicarbonyl molecule that is formed as a byproduct of the glycolysis pathway. Our lab showed that MGO modifies histone proteins in vivo and induces changes in chromatin structure and function. However, due to a combination of rapid rearrangements, multiple sites, and a lack of antibody tools available, it is difficult to track glycation in an in vivo setting. In order to study MGO glycation on histones as well as other proteins, DNA, and RNA, we generated an alkyne-tagged MGO proxy (AlkMG) as well as a site-specific glycation antibody (H3R17MG-H1). Preliminary results confirm that both chemical biology tools can be used to detect and enrich protein and nuclei acid glycation in cells. We plan to apply both tools in the profiling of glycation substrates in vivo as well as ChIP-seq and Click-seq to determine the specific genomic localization of glycation.

42. Ye, Linzhi

TPCB Graduate Student, Rockefeller University
Lab: Sean Brady, PhD

Discovery of Novel Bioactive Metabolites From Human Microbiome

Human-associated microbiome is an essential part in human health and disease. However, the underlying mechanisms remain largely unknown. Through in vitro functional screening of bacterial fermentation broth, less than 5 out of 85 commensal species were found to significantly activate NF-κB pathway, and active components in all positive hits were confirmed to be small molecules. To isolate and characterize the chemical structures of these bioactive metabolites, bioactivity-guided fractionation is in progress. On the other hand, knock-out of potential biosynthetic genes in the NF-κB active bacteria is also being carried out as a supplement to the direct bioactivity-guided fractionation.

44. Zhang, Yuxi

TPCB Graduate Student, Rockefeller University
Lab: Roderick MacKinnon, MD

Distribution of M2 Muscarinic Acetylcholine Receptors (M2Rs) on the Cell Membrane

M2 muscarinic acetylcholine receptors (M2Rs) belong to the G protein-coupled receptor (GPCR) superfamily, which regulates various physiological events. For example, activation of M2Rs in cardiac pacemaker cells opens GIRK channels, which hyperpolarizes the cell and slows the heart rate. In the M2R-GIRK channel signaling pathway, the neurotransmitter acetylcholine (Ach) binds and activates M2Rs. The activated receptors then generate free Gβγ subunits and free GTP-bound Gαi subunits. Free Gβγ subunits then diffuse on the membrane surface until they bind and open GIRK channels. Many features of this pathway have been characterized quantitatively, such as the M2R density in the cell membrane. Base on our calculation, at this reported receptor density, neither uniformly distributed nor randomly distributed M2Rs can generate a high enough Gβγ concentration to activate GIRK channels, as M2Rs are too sparse to build up a sufficient Gβγ concentration. To explain this inconsistency, we hypothesize that M2Rs form clusters with high receptor density on the cell membrane. Therefore, within these clusters, a sufficiently high Gβγ concentration could be achieved to activate GIRK channels. To test our hypothesis, I studied the distribution of M2Rs in a cardiomyocyte cell line which preserves the M2R-GIRK channel signaling.

46. Zhang, Tiffany

TPCB Graduate Student, Rockefeller University
Lab: Ekaterina Vinogradova, PhD

Proteomic Characterization of Macrophage Polarization States

Macrophages are professional antigen presenting cells of the innate immune system. These cells exhibit a high degree of plasticity that allows them to adapt to environmental cues and effectively perform functions such as antigen presentation via MHC molecules, secretion of pro- and anti-inflammatory chemokines, and phagocytosis of infected cells and pathogens. Toll-like receptors (TLRs) on cell surfaces and endosomes of macrophages allow the cells to recognize different components of invading pathogens and mediate inflammatory responses. We used a combination of advanced mass-spectrometry platforms, including unenriched proteomics and reactivity-based protein profiling, to characterize the proteomes of various TLR activation states in macrophages and identified several targets showing unexpected state-dependent reactivity changes with cysteine-reactive chemical probes. Ongoing studies are aimed at understanding the biochemical events preceding these reactivity changes, associated changes in protein structure and function, as well as signaling pathways regulated by these changes.

48. Zhou, Anqi (Nora)

TPCB Graduate Student, Memorial Sloan Kettering
Lab: Daniel Bachovchin, PhD

Exploring the Correlation Between pH and NLRP3 Inflammasome Activation

The innate immune system recognizes infection through germline-encoded receptors, some of which can oligomerize into inflammasomes and trigger a rapid form of cell death called pyroptosis. Nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) is one inflammasome-forming receptor, and it is associated with multiple autoimmune diseases. However, the danger signal that NLRP3 recognizes is unknown. Decades of research have revealed that NLRP3 responds to various compounds with different biological functions and chemical structures. We hypothesize that these compounds might all induce a unifying change in cell homeostasis, for example, lowering intracellular pH, which is sensed by NLRP3's polybasic linker region as an activation signal. To test our hypothesis, we will use small molecule probes as well as pH-sensitive proteins to measure the pH change upon treatment of different NLRP3 triggers. And we will attempt to trigger NLRP3 under low intracellular pH by using different proton pump inhibitors and through utilizing the CO2 buffering systems. In addition, we will also investigate the impact of pH on NLRP3's linker region, especially on its interaction with phospholipid, through both microscopy and lipid strips.