Tri-Institutional PhD Program
Chemical Biology

20TH ANNUAL TRI-INSTITUTIONAL CHEMICAL BIOLOGY SYMPOSIUM

POSTER SESSION

Wednesday, August 14th, 2024
1:15 pm – 3:15 pm

Abby Aldrich Dining Hall
Rockefeller University
1230 York Ave, New York, NY 10065

Memorial Sloan Kettering Cancer Center

SCIENCE FOR THE BENEFIT OF HUMANITY

Weill Cornell Medicine

Schedule

12:00 – 1:00 pm Lunch

Session 1 presenters hang posters according to assigned poster board number.

1:15 – 2:05 pm Session 1

2:05 – 2:25 pm Coffee Break

Session 1 presenters remove their posters.
Session 2 presenters hang their posters.

2:25 – 3:15 pm Session 2

Session 2 presenters remove their posters.
Poster boards will be taken away at 3:30 pm.

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SESSION 1 (1:15 - 2:05 pm)

1. Andrews, Olivia
Undergraduate Student, Memorial Sloan Kettering
Lab: Daniel A. Heller, PhD

Design and Characterization of Nanocarrier-Based Estrogen Receptor Degrading Synergistic Drug Pairs (NERDS)

Estrogen receptor positive (ER+) metastatic breast cancer (MBC) is an incurable condition affecting approximately 168,000 women annually in the US. Fulvestrant, an Estrogen Receptor Degrader, is a standard treatment for ER+ MBC but is limited by dose-related toxicity and the development of drug resistance. To address these issues, we aim to develop a tumor-targeted nanoparticle formulation that enhances the efficacy of fulvestrant while reducing side effects.

Fulvestrant has shown to work well as a stand alone nanoparticle and is predicted to be an ideal candidate as a combinatorial nanocarrier. Combination indocyanine-based nanoparticles (INPs) are a feasible approach to deliver drugs with normally poor pharmacokinetics or poor PD (toxic). INPs encapsulating fulvestrant were created via nanoprecipitation in an aqueous solution and characterized using dynamic light scattering (DLS), transmission electron microscopy (TEM), and high-performance liquid chromatography (HPLC). Drug encapsulation efficiency and nanoparticle stability was monitored over time at various temperatures (4°C, RT, 37°C). Additionally, seventeen rational drug combinations were evaluated for nanometasynergy in co-encapsulated nanoparticle systems, with five combinations demonstrating successful co-assembly as confirmed by HPLC. This approach aims to leverage the benefits of drug synergy and reduce the likelihood of acquired resistance.

Among these, we characterized a combinatorial nanoparticle, Fulvestrant-Olaparib (Nano-Fulv-Ola). We further demonstrated the efficacy of Nano-Fulv and Nano-Fulv-Ola *in vitro* using MCF7 cells. By combining fulvestrant with rational drug pairs in a targeted nanoparticle system, we report the development of a novel class of targeted nanoparticle estrogen receptor degraders (NERDs).

SESSION 1 (1:15 - 2:05 pm)

2. Banerjee, Anoosha
TPCB Graduate Student, Rockefeller University
Lab: Seth Darst, PhD and Elizabeth Campbell, PhD

Elucidating the Mechanism of Ribosome Stalling by Nascent HBV 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.

SESSION 1 (1:15 - 2:05 pm)

3. Barin, Ersilia
Graduate Student, Memorial Sloan Kettering
Lab: Heeseon An, PhD

Rapid degradation of ribosomal proteins, Ribo-DART, determines the role of r-proteins after ribosome biogenesis

Human ribosomes are homogenous organelles comprised of 4 ribosomal RNAs (rRNAs) and 80 ribosomal proteins (r-proteins). While the rRNAs catalyze peptide bond formation, the specific contributions of individual r-proteins to ribosome structure and function within mature ribosomes remain poorly understood. To define the role of r-proteins in human cells, we have developed a Ribo-DART system that combines CRISPR-gene editing and targeted proteolysis. Using a combination of Ribo-DART system, multiplexing chemical proteomic approaches, and cryo-EM analysis, we show that rapid depletion of RPL29/eL29 selectively increases synthesis of YBX1 and 3 proteins without altering ribosome structure or global translation efficiency. In contrast, removing RPL24/eL24 reduces global translation, activates mTORC1 signaling, and accelerates ribosome turnover by inducing ribosome degradation and biogenesis. The Ribo-DART system thus provides a framework to determine the biochemical characteristics of Δr-protein ribosomes in cells and to establish the functional role of r-proteins.

SESSION 1 (1:15 - 2:05 pm)

4. Cabo, Maurelio Jr.
Graduate Student, The University of North Carolina at Greensboro
Lab: Dennis Lajeunesse, PhD

Influence of Ultraviolet Radiation on the Growth of Bacterial Nanocellulose Hydrogel from *Gluconacetobacter hansenii*

This study examines the impact of ultraviolet (UV) radiation on bacterial nanocellulose (BNC) production by *Gluconacetobacter hansenii* using three media: Hestrin and Schramm (HS), Yeast/Peptone/Mannitol (YPM), and Yeast/Peptone/Dextrose (YPD). After a 10-minute UV exposure and 28 days of growth, the YPM medium yielded the highest BNC production yield, reaching 296 g/L , which was significantly higher than the yields from HS and YPD media. This suggests that YPM-grown cells may exhibit greater UV resistance, possibly due to genetic adaptation or mutations. The pH and optical density (OD) measurements supported these findings, with YPM cultures maintaining stable pH levels and OD values indicative of high BNC production. The enhanced BNC production in YPM medium under UV stress has potential implications for biomedical applications such as tissue engineering and wound healing. The UV-resistant strain could be valuable for developing durable biomaterials for use in UV-exposed environments. Further research into the underlying mechanisms of this UV resistance could improve BNC production processes and expand its applications in advanced biomedical fields.

SESSION 1 (1:15 - 2:05 pm)

5. 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.

SESSION 1 (1:15 - 2:05 pm)

6. Chen, Yuanhuang

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

Multiplexed Mapping of the GPCR-RAMP Interactome Using Suspension Bead Array

Receptor activity-modifying proteins (RAMPs) form complexes with certain G protein-coupled receptors (GPCRs) to regulate their cellular trafficking and pharmacology. Human tissues ubiquitously express at least one of three RAMPs, which are single-span transmembrane proteins. Although around 50 GPCRs have been identified to interact with at least one RAMP, detailed studies on specific GPCR-RAMP complexes are limited. To elucidate a comprehensive GPCR-RAMP interactome, we developed a library of 215 Dual Epitope-Tagged (DuET) GPCRs representing all GPCR subfamilies and co-expressed each GPCR with each of the three known human RAMPs. Using the customized multiplexed suspension bead array (SBA) immunoassays, we identified 122 GPCRs that showed strong evidence for interaction with at least one of the RAMPs. We also screened for native GPCR-RAMP interactions in three cell lines and employed a proximity-based assay to validate endogenous GPCR-RAMP2 interactions in SK-N-MC cells for eight GPCRs identified from the SBA assay screen. Mapping of the GPCR-RAMP interactome and validating GPCR-RAMP complexes in cell lines enhances the current system-wide understanding of RAMP-interacting GPCRs and facilitates the design of selective therapeutics targeting GPCR-RAMP complexes.

SESSION 1 (1:15 - 2:05 pm)

7. Chua, Gabriella

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

Eukaryotic clamp loading visualized at the single-molecule level

Sliding clamps are ring-shaped protein complexes that are essential for processive DNA synthesis across all organisms. In eukaryotes, the proliferating cell nuclear antigen (PCNA) clamp is loaded onto DNA by its loader, replication factor C (RFC), enabling it to scaffold other proteins for DNA replication and genomic maintenance. The textbook model of loading, suggested mainly by bulk biochemical studies, depicts a rigid sequence of events ordered as RFC engaging and opening PCNA, finding a DNA junction, and disengaging, leaving PCNA encircled around the DNA in an ATP-dependent manner. However, recent structural data indicate that the PCNA/RFC interaction with DNA is more flexible and dynamic than what had been previously predicted. To directly test this, we used a single-molecule platform combining fluorescence microscopy with optical trapping to directly visualize the clamp loading process on an Okazaki fragment DNA mimic using purified PCNA and RFC proteins. Contrary to the textbook model, we observed that loader-clamp complexes (LCCs) rarely decouple and frequently co-diffuse on duplex DNA. We also observed that such co-diffusion is dependent on the BRCT domain of the RFC1 subunit. The flap endonuclease FEN1, mainly known to function during Okazaki fragment maturation, competes for PCNA binding against RFC, which uncouples the LCC. Finally, we found that diffusing LCCs are active for initiating fill-in synthesis by DNA polymerase ⍰, often proceeding together as a ternary complex. Together, our findings reveal an adaptable, fail-safe mechanism for factor coordination and recycling during clamp loading.

SESSION 1 (1:15 - 2:05 pm)

8. Do, Stephanie

Post-Baccalaureate Research Assistant, Memorial Sloan Kettering
Lab: Thomas Vierbuchen, PhD

A Pluripotent Stem Cell Platform for *in Vitro* Systems Genetics Studies of Mouse Development

To understand the effect of natural genetic variation on cells and tissues in development, genetically diverse models of pluripotent stem cells (PSCs) are differentiated to mimic the different stages of embryonic development. By using the Diversity Outbred mouse stock, we establish new PSC lines and develop experimental approaches to model development *in vitro*. Here we show that epiblast stem cells (EpiSCs) can be maintained in the primed pluripotent state and contribute to genetic variation to differences in phenotype in gene regulation and directed differentiation. We produce and characterize a panel of 230 Diversity Outbred PSC lines through *in vitro* fertilization and form “cell villages” for genetic screens of large-scale differentiations. We corroborate the pluripotency of the Diversity Outbred stock by successfully differentiating the three germ layers (n=8), microglia (n=2), and cortical organoids (n=8). These findings contribute to a larger body of research within the stem cell biology and human genetics community, demonstrating the effect of natural genetic variation on phenotypic variation and disease-risk.

SESSION 1 (1:15 - 2:05 pm)

9. Fox, Nina

Post-baccalaureate Research Assistant, Memorial Sloan Kettering
Lab: Omar Abdel-Wahab, PhD

Therapeutic modulation of surface CD47 abundance via targeting the dual-specificity kinase DYRK1A

CD47 is a transmembrane protein that allows cells to evade macrophage-mediated phagocytosis. Targeting of CD47 via blocking antibodies has been heavily pursued for cancer therapy but no such therapies have been clinically successful to date.

In hopes of identifying positive and negative regulators of surface CD47 expression in acute myeloid leukemia (AML), we performed CD47 surface antigen-guided genome-wide CRISPR knockout (KO) screens. These screens were performed in three human AML cell lines *in vitro* as well as in the setting of macrophage pressure *in vivo*.

One of the top hits downregulating CD47 across all three cell lines was CD47 itself, confirming robustness of the screens. Interestingly, another recurrent hit was the dual-specificity serine/threonine and tyrosine kinase DYRK1A. We validated that deletion of DYRK1A led to CD47 downregulation. Moreover, DYRK1A KO induced phagocytosis of AML cells by mouse macrophages *in vitro. In vivo*, deletion of either CD47 or DYRK1A promoted survival of mice engrafted with AML.

Overexpression of DYRK1A upregulated surface CD47 expression in AML, an effect not seen with overexpression of kinase-dead mutants of DYRK1A. *In silico* predictions ranked the E3 ubiquitin ligase CBL as a top DYRK1a substrate. Consistent with this, DYRK1A inhibition-induced downregulation of CD47 required CBL.

Given the functional role of DYRK1A in modulating surface CD47 abundance, we tested the first examples of small molecules that are highly selective for DYRK1A over other DYRK or CLK family kinases. These compounds downregulated surface CD47 expression and potently inhibited the growth of leukemia cells *in vivo*.

SESSION 1 (1:15 - 2:05 pm)

10. Guo, Ruiyang (Michelle)

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

In Vitro Profiling of CD8+ T cell Proteome Changes During Target Killing

As the cytotoxic effector cells of the adaptive immune system, cytotoxic T lymphocytes (CTLs) recognize pathogen-infected and cancerous cells and induce their apoptosis. The mechanism by which CTLs exert their effector function upon a target cell is a complex, multi-step process involving multiple signaling pathways and physical reorientation of multiple components of the T cell. To gain insight into the proteome-level regulation of this process, previous studies have profiled the total proteome of CTLs during activation. However, CTL activation is only one step of the process by which a CTL kills a target cell. Here, we use mass spectrometry-based proteomics in the more biologically relevant context of in vitro differentiated CTLs isolated from OT-1 transgenic TCR mice cultured with antigen-presenting target cells to profile the proteomic landscape of CTLs during the killing process. To enable isolation of the CTL proteome from that of the target cells' and simultaneously decouple protein synthesis and degradation, we combine incorporation of L-homopropargylglycine, an unnatural amino acid probe, with proteomics, allowing us to study the contribution that each process makes to overall protein homeostasis and regulation. This method provides new insights into both degradational and translational paradigms of regulating T cell cytotoxicity.

SESSION 1 (1:15 - 2:05 pm)

11. 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.

SESSION 1 (1:15 - 2:05 pm)

12. Hiotis, Giorgos

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

Different interactions with the pocket lipids explain the poor mechanosensitivity of YnaI compared to MscS

YnaI is a member of the family of bacterial MscS (mechanosensitive channel of small conductance)-like channels. MscS protects cells from hypoosmotic stress by opening a transmembrane ion-conducting pathway in response to membrane tension. While MscS has been extensively characterized, YnaI is much less well understood. It has the archetypal MscS-like core structure but features two additional transmembrane helices. It requires near-lytic membrane tension to open but then creates only a small current. Here, we present a cryo-EM structure of nanodisc-embedded YnaI in the closed conformation that reveals well-defined pocket lipids. Use of β-cyclodextrin (to mimic membrane tension), a thin lipid bilayer (to simulate hydrophobic mismatch) and lyso-phosphatidylcholine (previously used to open YnaI) all failed to yield the structure of nanodisc-embedded YnaI in an open conformation. All maps revealed essentially the same density representing the pocket lipids. Molecular dynamics simulations of YnaI and MscS showed that the two additional helices of YnaI result in several more interactions with the pocket lipids compared to MscS. Single-point mutations aimed at weaking these interactions did not substantially increase the mechanosensitivity of YnaI. However, deletion of the two additional helices resulted in increased conformational heterogeneity, except for the pore-forming helices that remained in the closed conformation, and rendered the channel unresponsive to membrane tension. Our results suggest that the two additional transmembrane helices of YnaI make it more difficult for its pocket lipids to dissociate, thus stabilizing the closed conformation of the channel and explaining its weaker mechanosensitivity compared to MscS.

SESSION 1 (1:15 - 2:05 pm)

13. Huang, Hsin-Che

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

Hypoxia potentiates NLRP1 inflammasome activation

NLRP1 is a pattern-recognition receptors (PRRs) that detect pathogen-associated danger signals and form inflammasome that trigger immune responses. Thioredoxin (TRX1) in its oxidized form was found to bind to NLRP1 and restrain its inflammasome activation, demonstrating the potential role of NLRP1 in monitoring the cellular redox state. However, the exact danger signal that activates NLRP1 remains unknown. Here, we discovered that hypoxia, a condition known to alter cellular redox, potentiates NLRP1 activation in mouse macrophages. We further showed that HIF1A, the master regulator of cellular adaptation to hypoxia, is required for hypoxia-potentiated inflammasome activation. The mechanism of hypoxia-potentiated NLRP1 activation remains unclear, but evidence from RNA-seq and immunoblotting showed a mild upregulation of NLRP1B protein and an increased level of NLRP1 C-terminal fragment which forms the inflammasome. Additional investigations are ongoing to characterize NLRP1 binding proteins that regulate such phenotypes, potentially through the disruption of TRX1 binding and subsequently licensing NLRP1 for activation.

SESSION 1 (1:15 - 2:05 pm)

14. Lemmon, Abigail

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

Recognition of SUMO and polyubiquitin by p97 cofactors

The ubiquitin and ubiquitin-like protein SUMO are conjugated to protein targets in response to signals and stress. In addition to proteins that are individually conjugated to SUMO or ubiquitin, SUMO-targeted ubiquitin ligases (STUbLs) add a further layer of recognition and regulation by conjugating ubiquitin to SUMOylated proteins. In yeast, the Ufd1/Npl4(UN)-Cdc48 complex (UNC) recognizes SUMO and ubiquitin via ubiquitin binding sites in Npl4 and Ufd1 in conjunction with a C-terminal SUMO interaction motif (SIM) in the final seven residues of Ufd1. UNC is proposed to target dually modified proteins for recycling or subsequent proteasomal degradation. We biochemically reconstituted this process in the yeast system by generating a substrate modified with SUMO-polyubiquitin that is unfolded in a manner that is partly dependent on SUMO and the Ufd1 SIM. While dually modified proteins and UNC are conserved in humans, human Ufd1 does not contain a C-terminal SIM. The human complex has been shown to unfold ubiquitinated substrates, but it remains unclear if it can unfold substrates modified with both SUMO and ubiquitin. Further, it is unclear if human UNC interacts with SUMO through undetermined SIMs or if another p97 (Cdc48 human homolog) cofactor is involved in SUMO recognition. In support of this latter possibility, ubiquitin-interacting p97 cofactor Fas-associated factor 1 (FAF1) was recently shown to interact with SUMO. However, the mechanism of ubiquitin recognition and subsequent unfolding by FAF1 containing complexes remains unclear. Here we reconstitute FAF1-Ufd1/Npl4-p97 (FUN-p97) and FAF1-p97 complexes and test their relative ATP hydrolysis activity in the presence of ubiquitin-containing substrates. Using a fluorescence-based unfolding assay we also assess the ubiquitin-directed unfolding activity of FAF1-containing complexes.

SESSION 1 (1:15 - 2:05 pm)

15. 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"). An additional level of Ub code regulation is based on poorly understood PTMs of Ub itself including 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 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 readers of nine 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. pUb^{Ser65} 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. Initially, Ser65 was tested along with two other phospho-serines and later our method was expanded to six phospho-threonine residues. Globally, this initial dataset and method provide a first step for investigating the role of phosphorylation within ubiquitin biology.

SESSION 1 (1:15 - 2:05 pm)

16. Nambiar, Deepika

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

Investigating ribosome composition during neurogenesis

Neurons are highly polarized cells with unique biological and morphological features that enable them to issue chemical and electrical signals to regulate the body. Once terminally differentiated, neurons can function without undergoing cell division, a key process in mitotic cells that newly produces 50% of the proteome per division. As such, post-mitotic neurons can only replenish their initial set of proteins through protein turnover, a notably lengthy process to produce ribosomal proteins. While mitotic cells produce 50% of their ribosomes every 16 hours, it takes neurons 8-10 days. During this long lifespan, neuronal ribosomes can be altered or damaged, potentially leading to variations in r-protein stoichiometry. Interestingly, my preliminary data suggests that some ribosomal proteins differ in their stoichiometric compositions as neurons mature. Specifically, RPS29, RPL22, and RPL22L1 appear to be expressed at significantly lower levels compared to most ribosomal proteins from a total proteomics study comparing d12 and d24-induced neurons (iNeurons). This novel observation raises several questions: What is the biological mechanism responsible for this observation? Are these ribosomes still functional? If so, do they have specialized functions in iNeurons? I aim to address these questions using cell biological methods in an iNeuron model. As subtle alterations in translation efficiency have been linked with aging and neurodegenerative diseases, our findings will highlight the significance of ribosome composition.

SESSION 1 (1:15 - 2:05 pm)

17. Oh, SeCheol, PhD

Postdoctoral Fellow, Memorial Sloan Kettering
Lab: Richard Hite, PhD

Discovery of selective inhibitors for the lysosomal Parkinson's disease channel TMEM175

TMEM175 is a lysosomal potassium and proton channel that is associated with the development of Parkinson's disease. Advances in understanding the physiological roles of TMEM175 have been hampered by the absence of selective inhibitors, and studies involving genetic perturbations have yielded conflicting results. Here, we report the discovery and characterization of the first reported TMEM175-selective inhibitors, 2-phenylpyridin-4-ylamine and AP-6. Cryo-EM structures of human TMEM175 bound by 2-PPA and AP-6 reveal that they act as pore blockers, binding at distinct sites in the pore and occluding the ion permeation pathway. Acute inhibition of TMEM175 by 2-PPA or AP-6 increases lysosomal macromolecule catabolism, thereby accelerating macropinocytosis and other digestive processes. These inhibitors may serve as valuable tools to study the roles of TMEM175 in regulating lysosomal function and provide useful templates for future therapeutic development in Parkinson's disease.

SESSION 1 (1:15 - 2:05 pm)

18. Pandya, Pooja

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

Synthesis of Copper-Chelating Diisonitrile Natural Products from *M. tuberculosis*

Tuberculosis (TB), the disease caused by *Mycobacterium tuberculosis*, is a leading cause of death worldwide. While first-line treatments such as isoniazid and rifampin are effective for many patients, multidrug-resistant tuberculosis poses a continued threat to global public health. New approaches are needed to provide effective chemotherapy against these resistant pathogens. Chalkophores are diisonitrile natural products that regulate copper homeostasis and virulence in *M. tuberculosis*. Our aim is to understand the fundamental metal-binding properties of these molecules and to determine their functions in bacterial physiology and virulence. Toward this end, we have developed a synthetic platform that allows rapid and diverse construction of chalkophore natural products and analogues. We are now using these synthetic chalkophore analogues to characterize the metal-binding properties of these molecules, to identify the bacterial proteins involved in chalkophore-mediated Cu transport, and to elucidate their roles in virulence.

SESSION 1 (1:15 - 2:05 pm)

19. Payne, Alex

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

What is the minimum amount of experimental data needed to accurately triage molecules for the development of a SARS-CoV-2 main protease inhibitor?

In the pursuit of novel small molecule drug candidates targeting coronaviruses such as SARS-CoV-2 and MERS, the ASAP Antiviral Drug Discovery Center has produced an unprecedented number of protein : ligand crystal structures, providing a unique opportunity to assess several strategies for generating docked poses for related molecular designs in both retrospective and prospective analyses. In this poster, we explore the tradeoff between the cost of obtaining crystal structures and their utility for accurately predicting poses of newly designed molecules. We find that a simple strategy using molecular similarity to identify relevant template structures and a docking protocol which leverages the template ligand structure to pose and score provides a method with useful accuracy in predicting poses for the SARS-CoV-2 main viral protease. We show that the pose prediction accuracy is highly dependent on the similarity of the query molecule to the reference molecule; when docking each molecule to ten reference structures, using the most similar reference ligands vastly outperforms using the least similar reference ligands. We show that for ~10% of the ligands, the failure is caused not by failing to generate the best pose, but rather to score it favorably, suggesting that an accurate machine-learning model trained to identify the best pose from a set of posed molecules could make a significant impact on pose prediction accuracy. Future work will analyze the consequences of the pose-prediction choices on the accuracy of free energy calculations.

SESSION 1 (1:15 - 2:05 pm)

20. Peng, Zicong

Undergraduate Student, Rockefeller University
Lab: Viviana Risca, PhD

Development of CBRN-PROTACs for Targeted Degradation of TEAD Transcription Factors

Transcription factors (TFs) play a fundamental role in regulating gene expression; they can control the regulation of entire gene networks, including those involved in essential processes such as cell proliferation and survival that are frequently dysregulated in tumorigenesis. However, TFs have historically been difficult to drug with conventional methods due to the lack of small molecule ligand binding sites. Proteolysis targeting chimeras (PROTACs) have emerged as a powerful tool to degrade a protein interest selectively. Such tools can be leveraged to selectively target TFs by coupling them to DNA oligonucleotides containing the corresponding consensus sequence motif for the target TF (oligo-PROTACs). We aim to synthesize a library of Cereblon attached to an azide moiety using various PEG linker lengths and conjugate them with alkyne-functionalized decoy oligonucleotides containing consensus motifs for the TFs TEAD, CTCF, and NF-kB using click-chemistry. These oligo-PROTACs will then be delivered via liposome-based transfection to breast cancer cells. TF degradation will be assessed using Western blots, mass spectrometry, and RNA-seq or qPCR against TF target genes. Successful development of these oligo-PROTACs will provide a valuable tool to dissect gene regulatory networks involved in breast cancer and its response to treatments.

SESSION 1 (1:15 - 2:05 pm)

21. Perea, Ana Marie

Post-Baccalaureate Research Assistant, Memorial Sloan Kettering
Lab: Daniel Heller, PhD

Endolysosomal Sequestration Effects Controlled Release of BRAF Paradox Breaker Nanoparticles

BRAF remains one of the most important therapeutic targets in cancer, but BRAF inhibitors can cause “paradoxical” pathway activation and drug resistance through RAF dimerization. A clinical “paradox breaker” inhibitor of BRAF monomers and dimers can potentially evade drug resistance. However, patients are required to receive a high oral daily drug dose to achieve the target therapeutic window. Co-administration of a cytochrome P450 blocker can improve drug exposure, but the combination can lead to drug-drug interactions. We investigated delivery via fucoidan-based nanocarriers to improve pharmacologic properties. We found that the nanoparticles extended BRAF inhibition in cancer cells due to sequestration into endolysosomes, followed by controlled release from a lysosomal depot. Following intraperitoneal administration, nanoparticles improved drug pharmacokinetics *in vivo* without inhibiting cytochrome P450 and also resulted in substantial improvements in anti-tumor efficacy. This work describes a general nanotherapeutic strategy to improve the pharmacologic properties of drugs via intracellular depot formation.

SESSION 1 (1:15 - 2:05 pm)

22. Rabadi, Dina

Post-Baccalaureate Research Assistant, Rockefeller University
Lab: Sanford Simon, PhD

HLA Class I Immunopeptidomics in Fibrolamellar Hepatocellular Carcinoma

Fibrolamellar hepatocellular carcinoma (FLC) is a rare, usually fatal pediatric liver cancer. There are no FDA-approved therapies. If it has not metastasized, surgery is the only option in the clinic. Alas, FLC is often diagnosed only after metastasis. This cancer is driven by a fusion of exon 1 of heat shock protein DNAJB1 to exons 2-10 of PRKACA, the catalytic subunit of PKA. This fusion, DNAJB1::PRKACA (chimera), has been identified in nearly all FLC patients (Honeyman 2014) and is the oncogenic driver of FLC (Kastenhuber, 2017; Neumayer 2023; Neumayer 2024).

Despite efforts, FLC shows limited response to immune checkpoint inhibitors (ICIs), often attributed to factors such as low tumor mutational burden (1.85 mut/MB, Chen 2022), minimal PD-L1 expression by IHC (Chen 2022), and an immunosuppressive tumor microenvironment (Daniel 2024).

Recent studies, however, propose a potential immunotherapeutic avenue through a peptide vaccine strategy targeting the chimera (Bauer 2022; Kirk 2024). Therefore, there is a critical need to determine if the chimera is presented on HLA and to identify additional tumor-specific targetable neoantigens. This study aims to characterize HLA Class I and II expression in FLC using immunofluorescence (IF) and to optimize an immunopeptidomics workflow for analyzing HLA-presented peptides in FLC. This approach may unveil novel neoantigens for potential immunotherapeutic targeting.

SESSION 1 (1:15 - 2:05 pm)

23. Roychowdhury, Tanaya, PhD

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

From Chaperone to Epichaperome: A Phosphorylation triggered functional shift of HSP90B

The sophisticated network of client-chaperone interaction is crucial for normal cellular function. However, under stressful conditions the existence of specialized long-lived multimeric higher molecular weight chaperone complexes referred to as “epichaperomes”, have been discovered. These protein assemblies orchestrate the rewiring of protein-protein interaction networks, thereby enhancing cellular adaptability and proliferation, promoting diseases. The present work delves deeper into the structural and regulatory aspects of epichaperomes, with special emphasis on the significance of post-translational modifications in shaping their structure and function. A key finding of this study is the identification of specific PTMs on HSP90B, at residues Ser226 and Ser255 situated within an intrinsically disordered region, the charged linker, as critical determinants in epichaperome assembly. Our data demonstrate that the phosphorylation of these serine residues promotes HSP90's interaction with other chaperones and co-chaperones, leading to the formation of these long-lived multimeric epichaperome complexes. Furthermore, this study establishes a direct link between epichaperome function and cellular physiology, especially in contexts where robust proliferation and adaptive behavior are essential, such as stem cell maintenance and cancer. These findings not only provide mechanistic insights but also hold promise for the development of novel therapeutic strategies targeting the chaperone complexes in diseases characterized by epichaperome formation, thus aiding the transition from bench to bedside.

SESSION 1 (1:15 - 2:05 pm)

24. Rufa, Dominic

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

Toward chemical accuracy for protein:ligand binding free energy with hybrid machine-learned/molecular mechanics potentials

Alchemical free energy methods with molecular mechanics (MM) force fields are now 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. A major limitation is the accuracy with which ligand intramolecular energetics can be modeled, as poor modelling can have a significant impact on binding free energies. Here, we demonstrate how a new generation of hybrid machine learning / molecular mechanics (ML/MM) potentials can deliver significant accuracy improvements in modeling protein-ligand binding affinities. Using a nonequilibrium perturbation approach, we can correct a standard, GPU-accelerated MM alchemical free energy calculation in a simple post-processing step to efficiently recover ML/MM free energies and deliver a significant accuracy improvement with small additional computational effort. To demonstrate the utility of ML/MM free energy calculations, we apply this approach to a benchmark system for predicting kinase:inhibitor binding affinities—a congeneric ligand series for non-receptor tyrosine kinase TYK2 (Tyk2)—wherein state-of-the-art MM free energy calculations (with OPLS2.1) achieve inaccuracies of 0.93±0.12 kcal mol^{-1} in predicting absolute binding free energies. Applying an ML/MM hybrid potential based on the ANI2x ML model and AMBER14SB/TIP3P with the OpenFF 1.0.0 (“Parsley”) small molecule force field as an MM model, we show that it is possible to significantly reduce the error in absolute binding free energies from 0.97 [95% CI: 0.68, 1.21] kcal mol^{-1} (MM) to 0.47 [95% CI: 0.31, 0.63] kcal mol^{-1} (ML/MM).

SESSION 1 (1:15 - 2:05 pm)

25. Sharer, David

Undergraduate Student, Hunter College
Lab: Mande Holford, PhD

Characterization of NK-92 Activation by Interleukin-12

Abstract: The activation of the NK-92 cell line by interleukin-12 (IL-12) has been under-examined in immunology. Interleukins are glycoprotein cytokines released by leukocytes such as CD4 helper T-cells, macrophages, and monocytes in response to pathogenic infections. They bind to interleukin receptors on natural killer cells to stimulate the release of cytotoxic perforins and granzymes (cytotoxicity upregulation) and/or cytokines such as interferon-γ (cytokine signaling upregulation). Assessing the activation of NK-92 cells by IL-12 would provide a model for studying the immune activation of natural killer cells *in vitro*. This model can then be exploited to further understand the transcriptional landscape that contributes to natural killer cell activation.

Methods: RT-qPCR was used to measure the expression of the serine protease granzyme b and cytokine interferon-γ. An ELISA was also performed to measure interferon-γ expression in NK-92 cells. Furthermore, flow cytometry-based killing assays will also be performed to assess the cytotoxicity of NK-92 cells in the presence and absence of IL-12.

Results: RT-qPCR experiments revealed that 24-hour incubation of NK-92 cells with IL-12 resulted in an approximately 10-fold increase in interferon-γ expression compared to untreated cells. Therefore, NK-92 cells exposed to IL-12 likely demonstrate an activated profile via upregulation of cytokine signaling. Furthermore, an ELISA experiment demonstrated a significant increase in IFN-γ release in IL-12 treated NK-92 cells relative to untreated NK-92 cells, thus further supporting IL-12 inducing NK-92 cell activation through the upregulation of cytokine signaling.

Conclusion: Although NK-92 activation by IL-12 via upregulated cytokine signaling was determined, NK-92 activation by IL-12 via increased cytotoxicity is inconclusive. The cytotoxicity of IL-12-activated NK-92 cells will be further assessed through performing a flow-cytometry-based cytotoxicity assay. Consequently, NK-92 activation by IL-12 is an informative model for studying the activation of natural killer cells *in vitro*. Using this model, putative transcripts of novel innate immune-related micropeptides (<100 amino acids long) can be identified to better elucidate the natural killer cell immune response.

SESSION 1 (1:15 - 2:05 pm)

26. Spotton, Kaylyn

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

Dodecacilagicin, an Optimized Antibiotic Development Candidate that Evades Resistance by Dual Polyprenyl Phosphate Binding

Cilagicin is a dual polyprenyl phosphate binding lipodepsipeptide antibiotic with strong activity against clinically relevant Gram-positive pathogens while evading antibiotic resistance. Cilagicin showed high serum binding that reduced its *in vivo* efficacy. Cilagicin-BP, which contains a biphenyl moiety in place of the N-terminal myristic acid found on cilagicin, showed reduced serum binding and increased *in vivo* efficacy, but decreased potency against some pathogens. Here, we manipulated the acyl tail and the peptide core of cilagicin to identify an optimized collection of structural features that maintain potent antibiotic activity against a wide range of pathogens in the presence of serum. This led to the identification of the optimized antibiotic dodecacilagicin, which contains an N-terminal dodecanoic acid. Dodecacilagicin exhibits low MICs against clinically relevant pathogens in the presence of serum, retains polyprenyl phosphate binding and evades resistance development even after long-term antibiotic exposure, making dodecacilagicin an appealing candidate for further therapeutic development.

SESSION 1 (1:15 - 2:05 pm)

27. Wu, You (Emily)

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

Tau is a microtubule-associated protein that is believed to play a critical role in stabilizing microtubules in neuronal cells. In Alzheimer's disease (AD), tau undergoes pathological changes that lead to its abnormal aggregation into insoluble filaments and neurofibrillary tangles (NFTs). The aggregation of tau is a complex, multi-step process involving its transition from soluble monomers to oligomers, then to filaments and eventually to tangles. This aggregation process is influenced by various isoforms of tau and post-translational modifications (PTMs), such as phosphorylation, truncation, and ubiquitination, which contribute to the heterogeneity of tau pathology in AD. Techniques that selective isolation of pathogenic tau from complex brain tissue would greatly facilitate our understanding in complexity and heterogeneity of the tauopathies and developing targeted therapies.

Over the last decade, advancements in positron emission tomography (PET) imaging techniques enables non-invasive visualization and quantification of NFTs in living patients using tau specific radiotracers. ^{18}F-THK523 was one of the first generation of tau PET tracers. [5-8] Subsequently, in 2020, ^{18}F-flortaucipir (AV-1451) became the first FDA-approved tau targeting PET tracer.[9] Those probes have been previously demonstrated high specificity and affinity for NFTs in both biochemical and clinical settings. Photoaffinity labeling allows for the specific tagging and isolation of target protein complexes in their native state, preserving their biological integrity and activity. In this study, we aim to develop a novel tau labeling and pulldown approach using photoaffinity labeling probes. Based on the structures of reported tau PET tracers, we have designed and synthesized two tau photolabeling chemical probes, Tau-2 and Tau-4. Next, we evaluated and confirmed the probe engagement with tau aggregates in AD brain tissues through an autoradiography competition binding study. By utilizing two different tau cell model, we successfully label and pull-down cellular tau protein using the tau-2 probe. Furthermore, the fluotaucipir based probe, Tau-4, demonstrated the capability to label the pathogenic tau in AD brain tissues, allowing subsequent click conjugation with biotin for affinity pulldown. Lastly, the proteomics analysis of pulldown product demonstrated the specific labeling and isolation of tau from AD samples. Collectively, these results suggest such probes are promising tools for studying the tau and NFTs in Alzheimer's Disease.

SESSION 1 (1:15 - 2:05 pm)

28. Xiang, Ruijie

Post-Baccalaureate Research Assistant, Rockefeller University
Lab: Paul Cohen, PhD

Cargo identification and regulatory mechanisms of endolysosome exocytosis in adipocytes

Adipose tissue is known as a secretory organ that regulates whole body homeostasis. It is estimated that ten percent of the adipocyte secretome under basal state is comprised of unconventional protein secretion (UcPS) cargos that lack signal peptides. Numerous UcPS pathways were associated with pathophysiological settings like inflammation, diabetes, and cancer. However, the exact UPS pathways these cargos exit, their identities and how they get sorted into specific organelles are largely unknown. One of the first well characterized UPS in adipocytes is Fatty acid binding protein 4 (FABP4), which leads to insulin resistance. FABP4 is secreted via endolysosomal pathway, and it can be induced by forskolin and inhibited by chloroquine (CQ). We hypothesized that there are many more cargos using this route to get secreted in adipocytes. To identify these cargos, we utilize multiple methods like silver stain-Mass spectrometry and Bio-orthogonal noncanonical amino acid tagging (BONCAT). To date, we have determined the optimal condition for pharmacological manipulation. However, standard BONCAT setting masked the inhibitory effect of CQ, revealing needs for further optimization. Alternatively, using silver stain-MS, we identified at least five bands that show similar patterns as FABP4 in response to FSK and CQ, and these bands were excised for MS analysis. Several candidates were identified and to be validated. Mechanistically, UPS cargos get sorted into organelles either through membrane remodeling or a translocator. By FABP4-DHFR fusion, I found that FABP4's entry into endolysosomal pathway does not require complete linearization, suggesting that FABP4 may get sorted via membrane remodeling mechanisms.

SESSION 1 (1:15 - 2:05 pm)

29. Xiao, Yang

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

Deciphering Histone Glycation in Cancer Using Novel Site-Specific Antibodies

Cancer cells undergo metabolic reprogramming to rely mostly on aerobic glycolysis, which raises the intracellular levels of reactive sugar metabolites that can covalently modify macromolecules in a process termed glycation. Our lab showed that methylglyoxal, a reactive by-product of glycolysis, modifies histones and induces changes in chromatin structure and function. However, due to a combination of rapid rearrangements, multiple sites, and a lack of analytical tools, it remains a challenge to study the physiological effects of histone glycation. To investigate histone glycation in various *in vivo* settings, we developed first-in-class target- and site-specific monoclonal antibodies against H3R17MG-H1, one of the most abundant histone glycation sites carrying the most prevalent adduct. These antibodies were validated in various applications and used to detect histone glycation in cellular systems. With these antibodies, we observed that the amount of H3 glycation in cells depends on glucose availability, methylglyoxal content in the growth medium, and the presence of glyoxalase enzymes in the cells. Excitingly, we observed higher basal H3 glycation levels in certain breast cancer cell lines, suggesting the potential of using histone glycation as a biomarker in cancer diagnostics. Moreover, these antibodies enable the first comprehensive genome-wide mapping of H3R17MG-H1 that provides crucial insights into the genomic localization of glycation and its interplay with other epigenetic systems. Taken together, these novel site-specific antibodies against new and understudied H3 modification open opportunities for investigating the molecular ramifications of histone glycation and establishing further regulatory systems and mechanisms that link histone glycation to cancer progression.

SESSION 1 (1:15 - 2:05 pm)

30. Yardeny, Noah

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

Development of a Novel, Bioorthogonal Photocatalyst for Proximity Labeling

μMap is a proximity labeling strategy that uses an iridium photocatalyst and visible light to sensitize diazirines into reactive singlet carbenes. The carbene generated labels biomolecules in a diffusion-limited manner within a fixed radius of a protein of interest (POI) enabling enrichment of the POI and its interactors under variable conditions. Photocatalyst conjugation is amenable to proteins, antibodies, and small molecules allowing both interaction discovery and drug target identification. Though μMap is an attractive technology its widespread implementation has been impeded by the photocatalyst's physical properties. Hydrophobic catalysts cause high background signal from nonspecific interactions and more hydrophilic complexes are unstable and irreversibly decompose upon exposure to light. To address these issues, I synthesized and characterized a library of heteroleptic $[Ir(C^\wedge N)_2(N^\wedge N)]^+$ complexes and assessed their catalytic ability to activate diazirine warheads. These efforts yielded a novel photocatalyst that is neutral, water-soluble, and stable. Comparing this bioorthognal catalyst to previous generations proved its superior ability to identify the binding targets of various small molecules, including weak interactions missed by other methods. Recently, we leveraged this improved technology to move beyond qualitative target ID data and instead quantitate binding affinities of molecules for the entire proteome in a single experiment. We are developing this into a platform that can be used to rapidly probe the affinity of small molecules and peptides across the proteome which will be of great utility to the drug discovery field.

SESSION 1 (1:15 - 2:05 pm)

31. 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.

SESSION 2 (2:25 - 3:15 pm)

1. Baca, Christian

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

The bacterial effector Cad1 provides antiviral immunity

Type III CRISPR-Cas systems provide immunity against genetic invaders through production of cyclic oligo-adenylate (cAn) molecules that bind to and activate effector proteins that contain CARF domains. Previously, CARF effectors harboring adenosine deaminase (ADA) domains have been reported to be genetically linked to type III CRISPR-*cas* loci; however, whether and how they provide immunity has not been explored. Here we characterized the function and structure of CRISPR-associated adenosine deaminase 1 (Cad1), A CARF-ADA effector. We show that upon binding of cA4 or cA6 to its CARF domain, Cad1 converts ATP to ITP, both *in vivo* and *in vitro*. Cryo-EM structural studies on full-length Cad1 reveal an hexameric assembly composed by a trimer of dimers, with bound ATP at inter-domain sites required for activity, and ATP/ITP within deaminase active sites. Upon synthesis of cAn during phage infection, Cad1 activation leads to a growth arrest of the host that prevents viral propagation. Our findings reveal that CRISPR-Cas systems employ a wide range of molecular mechanisms beyond nucleic acid degradation to provide adaptive immunity in prokaryotes.

SESSION 2 (2:25 - 3:15 pm)

2. Bay, Sadik, PhD

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

Illuminating Epichaperomes at the Single-Cell Level: Tools and Methods for Studying Neurodegenerative Disorders

Stressors associated with disease trigger the remodeling of protein-protein interaction networks, converting chaperones into epichaperomes. These structures are long-lived assemblies and disease-associated pathologic scaffolds comprising tightly bound chaperones, co-chaperones, and other factors. Unlike chaperones, which are ubiquitous proteins functioning through dynamic one-on-one complexes, epichaperomes act as pathologic scaffolds, leading to improper interactions and organization of thousands of proteins inside cells. This negatively impacts neuronal function. Notably, the ability of epichaperome disruptors to reverse disease-related phenotypes highlights their critical role in regulating functions underlying disease pathology, suggesting a novel therapeutic approach. To deepen our understanding of epichaperomes in neurodegenerative disorders and gain important mechanistic insights into their context-dependent composition, structure, and function, we developed chemical probes and methods suitable for use in confocal and single-molecule super-resolution imaging approaches. As a demonstration of the utility of these probes in investigating biology, we characterized an epichaperome click probe along with a relevant negative control. Our study includes high-resolution imaging of epichaperomes at the single-cell level in a transgenic mouse model. We demonstrate the successful detection of cell-specific vulnerability to epichaperome formation using the click probe, highlighting its potential as valuable tool for dissecting the intricate cellular responses underlying neurodegenerative diseases. The probe shows promise for understanding epichaperome formation, composition, and localization in different biological contexts, representing a significant step towards realizing its potential for diagnosing and treating neurodegenerative disorders. Importantly, the versatility of this click probe allows for multiplexing with antibodies against specific disease markers, offering the opportunity to investigate intricate mechanistic details.

SESSION 2 (2:25 - 3:15 pm)

3. Eum, Lucy

TPCB Graduate Student, Memorial Sloan Kettering
Lab: Arvin Dar, PhD

Small molecule modulation of inactive-to-active state transitions of RAF-family complexes

Ras proteins, including H-RAS, N-RAS, K-RAS4A, and K-RAS4B, are small GTPases that play critical roles in cell signaling pathways such as RAF-MEK-ERK/MAPK (RAS/MAPK). Mutations in this pathway are prevalent in various human cancers, making each component a significant drug target. RAF kinases, including B-RAF, C-RAF, and A-RAF, interact with activated RAS via a conserved RAS-binding domain (RBD), transitioning from inactive to active states through complex assemblies with proteins like KSR, MEK, and 14-3-3. This complexity generates numerous biochemical entities driving RAS-RAF signaling, complicating the understanding of drug-target interactions. I hypothesize that drug target engagement levels on specific RAF kinases and complexes correlate with drug sensitivity in these cancer models. To test this, I am optimizing a live-cell target engagement platform to quantify the transition of RAF kinases from monomer (inactive) to dimer (active) states. Additionally, I am evaluating current RAS-pathway inhibitors and novel small molecule inhibitors in K-RAS and B-RAF mutant cancer models, focusing on specific RAF paralogs linked to drug resistance.

SESSION 2 (2:25 - 3:15 pm)

4. Hsieh, David

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

Aromatic amino acids metabolism in the gut microbiota

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. Using a combination of isotope tracing and untargeted metabolomics, we identified a variety of N-acyl amino acids from *C. difficile*.

SESSION 2 (2:25 - 3:15 pm)

5. Hu, Yingying

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

Regulation of Ferroptosis by PPARG Through PLA2G2F

Ferroptosis is a regulated cell death resulting from the imbalance of iron, lipid metabolism, and redox homeostasis, leading to oxidative cell death. It has been shown to play a vital role in impacting tumor progression. In a recent CRISPR screen performed by the Jiang lab, PLA2G2F was identified as a potential inhibitor of ferroptosis. PLA2G2F belongs to the phospholipase A2 family and is involved in lipid metabolisms, which is known to be an important factor in ferroptosis susceptibility. Here, we confirmed the role of PLA2G2F in ferroptosis inhibition and identified its regulation by PPARG, a transcription factor that regulates lipid metabolism. We aim to investigate the mechanisms of PLA2G2F and PPARG regulation in ferroptosis inhibition. These findings could offer insights for novel therapeutic strategies targeting ferroptosis in cancer treatment.

SESSION 2 (2:25 - 3:15 pm)

6. Kan, Lijuan, PhD

Academic Staff Scientist, Memorial Sloan Kettering
Lab: Eric Lai, PhD

The m6A pathway regulates neuronal function in Drosophila

N6-methyladenosine (m6A) is a widespread and conserved mRNA modification with diverse regulatory effects. Most studies of m6A have been done in mammalian cell culture, which has limited capacity to inform *in vivo* biology. Accordingly, we developed the Drosophila model to study Mettl3-dependent m6A, including via nuclear (YTHDC1) and cytoplasmic (YTHDF) readers.

We identified a new role for nuclear m6A in reproductive biology. In particular, *mettl3* and *ythdc1* mutants are defective in choosing optimal substrates for egg-laying. This is a specific defect, since *ythdf* mutants were normal, and all of these mutants are largely normal with respect to other behavioral parameters relevant to egg-laying. Spatially-controlled manipulations show that these factors are needed in neurons for this behavior. We conclude that gene regulation via neural, nuclear m6A is important for a higher-order cognitive decision process.

To understand its regulatory basis, we mapped the m6A landscape in fly heads using miCLIP, and annotated high-confidence, Mettl3-dependent sites. We also generated RNA-seq data from mutant heads and S2 cells and found that both datasets harbor a substantial set of altered splice isoforms. Moreover, YTHDC1 iCLIP data in S2 cells shows its occupancy at a strong proportion of m6A targets with altered splicing in mettl3/ythdc1 mutants.

We are currently using S2 cell reporter assays to determine if YTHDC1 directly mediates alternative splicing choices, and how it might affect spliceosome activity. Ultimately, we hope to integrate these mechanistic data on how m6A/YTHDC1 regulate splicing with knowledge of discrete neural circuits that regulate egg-laying site choice.

SESSION 2 (2:25 - 3:15 pm)

7. Lengyel, Miklos, PhD

Postdoctoral Fellow, Memorial Sloan Kettering
Lab: Philipp Niethammer, PhD

The G-protein coupled receptor OXER1 is a tissue redox sensor essential for intestinal epithelial barrier integrity.

Barrier tissues are continuously exposed to various levels of oxidative stress. Increased oxidative stress can have physiological benefits but with the tradeoff of damaging healthy tissue. Inadequate adaptation to oxidative stress has been implicated in the pathogenesis of various inflammatory diseases such as asthma and ulcerative colitis. Oxidative stress increases levels of the arachidonic acid metabolite 5-oxo-ETE in mucosal tissues. However, despite its potential pathophysiological relevance, the biology of 5-oxo-ETE is poorly understood, because in contrast with humans (and zebrafish), rodents do not express the receptor of 5-KETE, OXER1. Here we show that OXER1 is required for maintaining the integrity of the intestinal epithelial barrier. Loss of OXER1 leads to increased intestinal epithelial apoptosis, barrier defects and increased inflammatory leukocyte recruitment. Mechanistically, OXER1 protects the gut epithelium from oxidative stress by regulating the expression of Nudix hydrolase enzymes involved in the sanitization of the cellular nucleotide pool. Understanding the biology of this new redox sensing pathway in mucosal inflammation is the first step required for targeting it for clinical benefit.

SESSION 2 (2:25 - 3:15 pm)

8. Lin, Karl

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

Engineering the NanoTag – a nanobody-based, multifunctional genetically encoded protein tag

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.

SESSION 2 (2:25 - 3:15 pm)

9. Neugroschl, Atara

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

The Effects of MBOAT2 Regulated Ferroptosis on Immune Response in Pancreatic Ductal Adenocarcinoma

Pancreatic ductal adenocarcinoma (PDAC) is the most common form of pancreatic cancer and is projected to become the second-leading cause of cancer-related death by 2030. Currently, researchers are interested in the prospect of inducing ferroptosis as a cancer treatment. There is also debate as to whether inducing ferroptosis can lead to an immune response. This is particularly of interest in PDAC because the levels tumor infiltrating lymphocytes are low, and the cancer does not respond to immunotherapy. Additionally, the Jiang lab recently characterized membrane-bound o-acyltransferase domain containing 2 (MBOAT2) as a negative ferroptosis regulator. This protein has been found to be upregulated in PDAC compared to normal pancreatic cells, which is also correlated to lower survival rates and a poorer overall prognosis. Here, we explore how modulation of MBOAT2 activity influences ferroptosis in PDAC and whether this can induce an immune response against the tumor. Specifically, we aim to provide insights into the potential of targeting MBOAT2 and inducing ferroptosis to enhance the efficacy of immunotherapies in pancreatic cancer treatment.

SESSION 2 (2:25 - 3:15 pm)

10. Pham, Brittany

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

mTORC1 regulates the pyrimidine salvage pathway by controlling UCK2 turnover via the CTLH-WDR26 E3 ligase

One critical aspect of cell proliferation is increased nucleotide synthesis, including pyrimidines. Pyrimidines are synthesized through both *de novo* and salvage pathways. Prior studies established that the mammalian target of rapamycin (mTOR) promotes pyrimidine synthesis by activating the *de novo* pathway for cell proliferation. However, the involvement of mTOR in controlling the salvage pathway remains unclear. Here, we report that mTORC1 controls the half-life of Uridine cytidine kinase 2 (UCK2), the rate-limiting enzyme in the salvage pathway. Specifically, UCK2 is degraded in the cytoplasm via the CTLH-WDR26 E3 complex during mTORC1 inhibition. However, this degradation is prevented when mTORC1 signaling is active, during which cells require nucleotides to proliferate. We also show that maintaining sufficient levels of UCK2 is crucial for the efficacy of pyrimidine analog prodrugs, as UCK2 converts them into active drugs. Therefore, our discovery of mTORC1-CTLH E3-mediated degradation of UCK2 reveals a new role of mTORC1 in the pyrimidine pathway and has implications for pyrimidine analog chemotherapeutics.

SESSION 2 (2:25 - 3:15 pm)

11. Philip, Kara

Undergraduate Student, Memorial Sloan Kettering
Lab: Daniel Heller, PhD

Design and Characterization of Nanoparticle Estrogen Receptor Degraders (NERDs)

Estrogen receptor positive (ER+) metastatic breast cancer (MBC) is an incurable condition affecting approximately 168,000 women annually in the US. Fulvestrant, an Estrogen Receptor Degrader, is a standard treatment for ER+ MBC but is limited by dose-related toxicity and the development of drug resistance. To address these issues, we aim to develop a tumor-targeted nanoparticle formulation that enhances the efficacy of fulvestrant while reducing side effects. Fulvestrant has been shown to work well as a stand-alone nanoparticle and is predicted to be an ideal candidate as a combinatorial nanocarrier. Combination indocyanine-based nanoparticles (INPs) are a feasible approach to deliver drugs with normally poor pharmacokinetics or poor PD (toxic). INPs encapsulating fulvestrant were created via nanoprecipitation in an aqueous solution and characterized using dynamic light scattering (DLS), transmission electron microscopy (TEM), and high-performance liquid chromatography (HPLC). Drug encapsulation efficiency and nanoparticle stability was monitored over time at various temperatures (4°C, RT, 37°C). Additionally, seventeen rational drug combinations were evaluated for nanometasynergy in co-encapsulated nanoparticle systems, with five combinations demonstrating successful co-assembly, as confirmed by HPLC. This approach aims to leverage the benefits of drug synergy and reduce the likelihood of acquired resistance, as seen in prior studies of meta-synergistic nanomedicines. Among these, we characterized a combinatorial nanoparticle, Fulvestrant-BSJ-03-096 (Nano-Fulv-Pro. We further demonstrated the efficacy of Nano-Fulv and Nano-Fulv-Pro *in vitro* using MCF7 cells. By combining fulvestrant with rational drug pairs in a targeted nanoparticle system, we report the development of a novel class of targeted nanoparticle estrogen receptor degraders (NERDs).

SESSION 2 (2:25 - 3:15 pm)

12. Rajkumar, Sandy

Graduate Student, Weill Cornell Medicine
Lab: Yicheng Long, PhD

The role of accessory proteins in PRC2-mediated epigenetic repression

The Polycomb Repressive Complex 2 (PRC2) catalyzes H3K27me3 and is essential for normal embryonic cell fate specification and the regulation of key developmental genes. How mammalian PRC2 is spatiotemporally regulated and recruited to target genes remains an open question. Recent studies have demonstrated that certain sub-stochiometric proteins bind PRC2, creating distinct PRC2 subcomplexes. The PRC2.1 subcomplex is composed of one of three Polycomb-like proteins (PCLs): PHF1, MTF2, or PHF19. Knockout of PCLs in mESCs causes a substantial decrease in PRC2 targeting on chromatin indicating that the PCLs enhance PRC2 occupancy at target genes. However, the mechanism and functional relevance of how the PCLs regulate PRC2 specificity to target genes remains poorly understood.
In hiPSCs, MTF2 is the most abundant PCL protein. Key residues for the interactions between the MTF2-PRC2 core, MTF2-DNA and MTF2-H3K36me3 were disrupted in hiPSCs using the CRISPR/Cas9 method. This separation-of-function approach is an elegant system to study functional relevance of these interactions in the epigenetic regulation of PRC2 target genes without disrupting the entire protein. Disrupting the PRC2 core and DNA-binding interface of MTF2 decreases PRC2 binding and H3K27me3 at the cardiac TF NKX2-5. Additionally, perturbing the MTF2-H3K36me3 interaction results in the gain of H3K36me3 at another cardiac TF TBX5. These results indicate that the different binding interfaces of MTF2 affect distinct cardiac TF genes. In conclusion, MTF2 acts as a hub for sensing chromatin elements including DNA sequences and histone modifications to regulate cardiac TFs in stem cells.

SESSION 2 (2:25 - 3:15 pm)

13. 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 substrates. These enzymes have key roles in essential cellular processes, such as genome replication and maintenance. Helicases with essential functions in certain cancer cells have been identified, and helicases expressed by many viruses are required for their pathogenicity. Therefore, helicases are important targets for chemical probes and therapeutics. However, only a limited number of selective helicase inhibitors have been reported. Here, we develop a function-first approach, combining enzymatic assays, enantiomeric probe pairs and mass spectrometry to develop a covalent inhibitor that selectively targets an allosteric site in SARS-CoV-2 nsp13. We assayed electrophilic probes known as “scout fragments” and identified an inhibitor starting point, compound 1, that covalently binds and inhibits nsp13. Systematic mass spectrometry-based analysis of cysteine mutants indicated that the C556 residue of nsp13 can be liganded to inhibit helicase activity. We generated analogs of compound 1 and found that compound 4b is a site-selective and more potent inhibitor of nsp13 (Ramsey *et. al*., *JACS*, 2024). In ongoing work, we have identified a chemical scaffold with attenuated ‘warhead’ reactivity that ligands C556 and inhibits nsp13 activity. Our results demonstrate how enantiomeric and diastereomeric covalent probes can be leveraged to develop site-selective inhibitors. Together, these findings provide a starting point for nsp13-targeting therapeutics, and also suggest a platform for the development of small molecule inhibitors of helicase mechanoenzymes.

SESSION 2 (2:25 - 3:15 pm)

14. Repeta, Lucas

TPCB Graduate Student, Memorial Sloan Kettering
Lab: Christopher D. 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 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 a role 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 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 vitroreconstituted 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 exosome cofactors, as well as notable differences. Helicase activity assays and equilibrium binding experiments have demonstrated that Mtl1 exhibits properties that distinguish it from Mtr4, suggesting that Red1 alters how Mtl1 engages its substrates. These experiments establish a foundation to understand how MTREC and the exosome cooperate to regulate target genes.

Supported in part by HHMI and the National Institutes of Health (GM118080)

SESSION 2 (2:25 - 3:15 pm)

15. Rout, Michael, PhD

TPCB Faculty, Rockefeller University

Structural analysis and inhibition of human LINE-1 ORF2 protein reveals novel adaptations and functions

The LINE-1 (L1) retrotransposon is an ancient genetic parasite that has written around one third of the human genome through a “copy-and-paste” mechanism catalyzed by its multifunctional enzyme ORF2p. ORF2p reverse transcriptase (RT) and endonuclease activities have been implicated in the pathophysiology of cancer, autoimmunity, and aging, making ORF2p a potential therapeutic target; however, a lack of structural and mechanistic knowledge hampers efforts to rationally exploit it. We have determined structures of the ORF2p by cryo-EM and X-ray crystallography in multiple conformational states that reveal two novel folded domains and a dynamic closed ring conformation with extensive contacts to RNA template. We characterized ORF2p biochemical activities *in vitro* and explore inhibition by retroviral RT inhibitors. We demonstrate via imaging and biochemical experiments that non-canonical cytosolic ORF2p RT activity can produce inflammatory RNA:DNA hybrids which activate innate immune signaling via cGAS/STING. Finally, we present an evolutionary analysis that both reveals ORF2p structural conservation and uncovers relationships of specific domains to other RNA- and DNA-dependent polymerases. These data provide key mechanistic insights into L1 polymerization and insertion, shed light on the evolutionary history of L1 elements, and enable rational L1 RT inhibitor development.

SESSION 2 (2:25 - 3:15 pm)

16. Saca, Victoria

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

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 fusion receptor expression constructs in which HiBiT and HaloTags are added to the cytoplasmic tail of Gq-coupled GPCRs. 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 used the fusion constructs for a proof-of-concept study to evaluate the potential feasibility of using PROTACs to target GPCRs, such as CysLTR2 – a GPCR implicated as a driver oncoprotein in uveal melanoma.

SESSION 2 (2:25 - 3:15 pm)

17. Sharma, Sahil, PhD

Senior Research Scientist, Memorial Sloan Kettering
Lab: Gabriela Chiosis, PhD

Development of epichaperome imaging probes for precision medicine in Alzheimer's disease

Alzheimer's disease (AD) is influenced by genetic, epigenetic, and environmental factors, resulting in individualized brain circuitry changes and cognitive decline. Our team found that AD-related stressors affect protein-protein interaction (PPI) networks through epichaperomes, pathologic scaffolds composed of chaperones and other factors, providing a unifying AD mechanism. Epichaperomes thus represent a promising target for detection and reversal of functional imbalances associated with AD. Developing effective small molecule probes for epichaperomes presents a challenge. These probes should selectively target epichaperomes over physiologic chaperones. In this study, we present our work on the design, synthesis, and characterization of small molecule epichaperome probes. Through various biochemical and functional assays, both *in vitro* and *in vivo*, including studies on mice and humans, we demonstrate how small molecule HSP90 binders can kinetically select and distinguish the small fraction of HSP90 in epichaperomes from the abundant HSP90 pools found in the same cell and throughout the body. We provide proof-of-principle evidence from mouse models, showing how an epichaperome imaging probe can reveal the region- and age-dependent formation of epichaperomes in disease-relevant areas. Additionally, we present the results of a pilot feasibility clinical study demonstrating that epichaperomes can be imaged and quantified in human patients using PET scans. In conclusion, epichaperome imaging probes have significant diagnostic applications in AD. When combined with other neuroimaging techniques and plasma biomarkers, they can be used to diagnose and quantify molecular changes underlying functional decline in the AD brain before the onset of tau and amyloid pathology.

SESSION 2 (2:25 - 3:15 pm)

18. Simon, Marcell

TPCB Graduate Student, Weill Cornell Medicine
Lab: Aaron Leconte, PhD (Claremont McKenna College)

Discovery of Red-Shifting Mutations in Firefly Luciferase Using High-Throughput Biochemistry

Photinus pyralis luciferase (FLuc) has proven a valuable tool for bioluminescence imaging, but much of the light emitted from the native enzyme is absorbed by endogenous biomolecules. Thus, luciferases displaying red-shifted emission enable higher resolution during deep-tissue imaging. A robust model of how protein structure determines emission color would greatly aid the engineering of red-shifted mutants, but no consensus has been reached to date. In this work, we applied deep mutational scanning to systematically assess 20 functionally important amino acid positions on FLuc for red-shifting mutations, predicting that an unbiased approach would enable novel contributions to this debate. We report dozens of red-shifting mutations as a result, a large majority of which have not been previously identified. Further characterization revealed that mutations N229T and T352M, in particular, bring about unimodal emission with the majority of photons being >600 nm. The red-shifting mutations identified by this high-throughput approach provide strong biochemical evidence for the multiple-emitter mechanism of color determination and point to the importance of a water network in the enzyme binding pocket for altering the emitter ratio. This work provides a broadly applicable mutational data set tying FLuc structure to emission color that contributes to our mechanistic understanding of emission color determination and should facilitate further engineering of improved probes for deep-tissue imaging.

SESSION 2 (2:25 - 3:15 pm)

19. Turque, Oliver, PhD

Academic Staff Scientist, CUNY Baruch
Lab: Edyta Greer, PhD

Singlet Oxygen-Mediated Conformational Flexibility and Antioxidant Studies

Simple organic disulfide molecules can serve as substrates to quantify peroxy intermediates with subsequent departure of oxygen in its singlet or triplet form. This kind of departure of oxygen is non-oxidative, and non-oxidative processes have been sparsely addressed. *We are hypothesizing that singlet oxygen adds in this non-oxidative fashion, and lowers the barrier of rotation around the disulfide bond.* In our second study, we explore a possible mechanism for the pro- versus antioxidant behavior of disulfides. Single sulfur persulfoxide intermediates have been previously studied, but we are interested in exploring the possibility that the sulfur atom as a carrier could enhance antioxidant action. α-Lipoic acid is a prevalent antioxidant found in all of the kingdoms of life. Lipoic acid's reactivity with $^1O_2$ has biochemical importance,[2] but the mechanism has remained unknown. *We hypothesize that in peroxy disulfide intermediates, α-CH availability, governed by dihedral CS–SC, determines anti- versus prooxidant behavior.* We used 1,2-dithiolane as a biomimetic entity to study lipoic acid's mechanism.

SESSION 2 (2:25 - 3:15 pm)

20. Vandana, J Jeya

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

Dissecting key signaling pathways of gut development for the derivation of mature intestinal organoids from human embryonic stem cells

Human embryonic stem cells (hESCs) can be differentiated into a variety of cell types and organoids including intestinal organoids. Several protocols have been developed for the derivation of intestinal organoids including foregut and colonic organoids in recent times. However, these protocols are limited in that they produce organoids that are immature or exhibit “fetal-like” behavior. Hence, we study the trajectory of gut development, analyzing fetal and adult gut tissues derived from patients of different ages in order to dissect important signaling pathways and transcriptional networks that may play a key role in contributing to gut development. In this manner, we discover that cAMP signaling is a key regulator of gut development, biasing differentiation towards either the foregut or colonic lineage. Moreover, we also focus on key signaling pathways that are critical for the generation of a rare population of enteroendocrine cell types such as L cells and K cells, responsible for the release of incretins such as GLP-1 and GIP, which have been implicated in weight loss in recent studies. We discover that RhoA signaling as well as cAMP signaling may be integral to the derivation of L cells and K cells respectively. Lastly, we also find that STAT3 signaling may be an integral pathway to the generation of goblet cells, the main secretory cell type in the gut. The dissection of these key signaling pathways allowed us to select chemical modulators of these key pathways to derive more mature intestinal organoids from hESCs *in vitro*.

SESSION 2 (2:25 - 3:15 pm)

21. Vogt, Kristen

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

Microenvironment actuated CAR T cells improve solid tumor efficacy without toxicity

A major limiting factor in the success of CAR T cell therapy for the treatment of solid tumors is targeting tumor antigens also found on normal tissues, which poses significant risks to patients. We found that CAR T cells against GD2, a tumor-associated antigen highly expressed in neuroblastoma, induced rapid, fatal neurotoxicity due to CAR recognition of GD2 positive normal mouse brain tissue. To improve selectivity of the CAR T cell for the cancer only, we engineered a synthetic Notch receptor that selectively expresses the CAR upon binding to a cell adhesion target upregulated in solid tumor neovasculature with minimal expression in normal brain and peripheral nerve tissue. These tumor microenvironment actuated T cells restricted expression of the GD2 CAR to tumor tissue, preventing fatal neurotoxicity while maintaining anti-tumor efficacy. We also found that conditional CAR expression improved CAR T cell anti-tumor efficacy by improving metabolic fitness and maintaining a less differentiated memory phenotype. This approach increases the repertoire of targetable solid tumor antigens by restricting CAR expression and killing to malignant tissue only and provides a proof-of-concept model for other difficult solid tumor targets.

SESSION 2 (2:25 - 3:15 pm)

22. Walker, Nicole

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

The methylglyoxal detoxifier DJ-1 enhances the anti-tumor function of BCMA-targeted CAR T cells

Methylglyoxal (MGO), a reactive dicarbonyl produced as a byproduct of glycolysis, non-enzymatically modifies proteins in a process known as glycation. Cancer cells, which exhibit increased reliance on glycolytic or Warburg metabolism, have significantly higher intracellular concentrations of MGO and levels of protein glycation. Specifically, our lab has shown that histones within cancer cells are glycated, leading to epigenetic changes. Importantly, cancer cells overexpress MGO detoxifying proteins such as DJ-1, staving off the worst of the cellular damage by converting MGO to lactate. Here, we show that activated T cells, which rely on Warburg-like metabolism, accumulate glycation adducts while failing to upregulate expression of MGO detoxifiers. Furthermore, we show that increased concentrations of MGO inhibit T cell activation and function while accelerating the life cycle of T cells. Continuous activation of T cells drives differentiation into a unique phenotype known as exhausted T cells (T_{ex}), which are characterized by a higher glycolytic flux than effector T cells, decreased cytokine production, decreased proliferation, and increased inhibitory receptor expression. Thus, we overexpressed the MGO detoxifier DJ-1 within a chimeric antigen receptor T model system which we used to show that detoxification staves off the aforementioned T_{ex} characteristics as well as making T cells more resistant to MGO and within the tumor microenvironment.

SESSION 2 (2:25 - 3:15 pm)

23. Warren, Charlie

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

Global Protein-Ligand Affinity Mapping Using Proximity Labeling

Interactions between biomolecules give rise to regulatory networks that govern complex biological processes. Disruption of these finely tuned interactions result in imbalanced cellular states that drive disease. Therefore, studying these interactions in detail will both uncover mechanisms of disease progression and enable effective therapeutic intervention. The development of proximity labeling technologies has been invaluable for identifying novel biological interactions. However, existing techniques often provide little to no quantitative biophysical readout with discovery being dictated by arbitrary false discovery cutoffs. Here, we describe AffinityMap, a high throughput proteomics platform that makes use of the proximity labeling technology μMap to profile small molecule, protein, and peptide binding affinities across the proteome. By measuring protein-ligand K_d values *in situ*, we unambiguously discover known and novel biomolecule interactions.

SESSION 2 (2:25 - 3:15 pm)

24. Ye, Linzhi

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

Fusobacteria induce inflammatory responses via simultaneous release of ADP-heptose and ribonucleotides

Fusobacteria are common bacterial species that colonize oral, gastrointestinal, and genital tracts. Their prevalence is correlated with diverse diseases including cancers, adverse pregnancy outcomes, and a number of inflammatory diseases. *Fusobacterium nucleatum*'s strong association with colorectal cancer (CRC) is particularly troubling as CRC is the second cause of cancer deaths in the United States. The mechanisms underlying the correlation between Fusobacteria and disease remain poorly understood; however, several studies have implicated nuclear factor kappa-B (NF-κB) transcription factor in their pathogenesis. Here we discovered that Fusobacteria uniquely release a mixture of ADP-heptose (ADPH) and ribonucleotides that together activate NF-κB and induce cancerous and inflammatory pathways in human cells.

SESSION 2 (2:25 - 3:15 pm)

25. Young, Paul

Graduate Student, Rockefeller University
Lab: Jue Chen, PhD

Structural basis of CFTR inhibition by CFTRinh-172

The cystic fibrosis transmembrane conductance regulator (CFTR) is an essential regulator of fluid and electrolyte homeostasis throughout the body. Although loss of function leads to cystic fibrosis, hyperactivation of CFTR results in secretory diarrhea and ADPKD. Thus, selective inhibition of CFTR has been posited as a potential treatment for these diseases. Several CFTR inhibitors have been developed, including the thiazolidinone $CFTR_{inh}$-172. Here, using a combination of structural, electrophysiological, and biophysical methods, we show how this molecule inhibits CFTR by binding within the pore. We demonstrate that $CFTR_{inh}$-172 inhibits anion flux by stabilization of a unique conformation in which the nucleotide binding domains (NBD) are dimerized, but the channel is closed. This conformation entails both constriction of the pore and collapse of the anion selectivity filter. We also show that binding of $CFTR_{inh}$-172 inhibits ATP turnover through interaction with transmembrane helix 8 (TM 8) without affecting NBD dimerization. These findings reveal the mechanism of inhibition by $CFTR_{inh}$-172 while also highlighting the pharmacological importance of TM 8. Taken together, the observations discussed herein expand our understanding of CFTR pharmacology and lay the groundwork for development of novel CFTR inhibitors.

SESSION 2 (2:25 - 3:15 pm)

26. Zenge, Colin

Graduate Student, Memorial Sloan Kettering
Lab: Alban Ordureau, PhD

Induced protein degradation to identify substrates of deubiquitylating enzyme USP8

Endosomes are critical organelles in eukaryotic cells that facilitate the sorting of endocytosed molecules and membrane proteins. These junction points rely on post-translational modification by ubiquitin to decide between recycling the uptaken proteins or degrading them. USP8, a deubiquitylating enzyme (DUB) localized to endosomes, is proposed to regulate the stability of endocytosed membrane proteins. However, the scope of USP8 activity within the cell has not yet been fully characterized. USP8 is one of the few essential DUBs and mutations in USP8 are associated with cancer and hypercortisolism due to aberrant membrane protein signaling. Since USP8 is an essential gene, I developed an $FKBP12^{F36V}$-USP8 fusion cell line where the addition of the heterobifunctional degrader dTAG induces rapid USP8 depletion within 2 hours. Using global proteomic analysis following a USP8 degradation time course, I identified which proteins are destabilized by the loss of the deubiquitinating enzyme. Components of the endosomal sorting complex ESCRT-0 are among the first proteins to decrease following USP8 degradation, followed by selective autophagy receptors. Consistently, phosphoproteomic analysis following USP8 degradation identified many key events involved in selective organellar turnover. This work provides an initial characterization of the consequences of USP8 loss and identifies a link to USP8 function and endosomal complex stability. Future work to identify changes in protein ubiquitylation will narrow the list of potential USP8 substrates and aid in understanding the role of endosomal ubiquitin balance in membrane protein homeostasis.

SESSION 2 (2:25 - 3:15 pm)

27. Zhang, Yuxi

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

M2R and GIRK channel distribution and communication

The M2R GIRK channel signaling pathway is important in regulating heart rate and many features of it have been characterized, such as the major components in the pathway. To learn more about this signaling pathway, here we used EM to look at the distribution of M2R and GIRK in a cardiac cell line. We found both proteins form their own clusters. M2R clustering is important to build up local Gβγ concentration and activate GIRK channels close to M2R clusters. We also found a bias between GIRK and M2R clusters. Both the bias and the protein clustering are important to the M2R GIRK channel signaling.

SESSION 2 (2:25 - 3:15 pm)

28. Zhang, Tiffany

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

Proteomic Characterization of Macrophage Polarization State

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.

SESSION 2 (2:25 - 3:15 pm)

29. Zhao, Nan, PhD

Academic Staff Scientist, Memorial Sloan Kettering
Lab: Arvin Dar, PhD

Targeted Degradation of ERK2/MAPK1

The mitogen-activated protein kinase (MAPK) pathway, also known as the RAS-RAF-MEK-ERK pathway, regulates a variety of physiologic cell functions, including cell proliferation and survival. This physiologic pathway is frequently hijacked by cancer cells through activating mutations in RAS, RAF, and MEK genes. Targeting ERK provides potential therapeutic opportunities for a broad spectrum of cancers bearing RAS, RAF and MEK mutations as well as those with acquired resistance to RAF and MEK inhibitors (RAFi and MEKi) [1]. Compared to the traditional 'occupancy-driven pharmacology' of inhibitors, 'event-driven pharmacology' of degraders, targeted protein degradation, offers distinct benefits, mainly including high potency at low doses, particular sensitivity to drug-resistant targets, and targeting at "untargetable" proteins [2]. In this study, we discovered that ERK1/2 inhibitors (ERKi) can specifically trigger the ubiquitination and proteasome-degradation of ERK2/MAPK1, a more abundant isoform of ERK, in the cancer cells line with RAS mutations. This ERKi-induced specific ERK2 depletion may require the engagement of E3 SUMO ligase, RanBP2:RanGAP1-SUMO1:UBE2I complex. This discovery provides a novel therapeutical insight into developing selective ERK2 degraders for the purpose of treating cancers with RAS, RAF and MEK mutations.

SESSION 2 (2:25 - 3:15 pm)

30. Zhou, Anqi (Nora)

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

Deciphering the Role of NEK7 in NLRP3 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. NLRP3 remains caged before sensing an activation signal, and it is tightly regulated by NIMA related kinase 7(NEK7), a protein which is important in spindle assembly during early mitotic state. However, NEK7's exact role in activating NLRP3 inflammation is disputed. Previous studies have used toxins or synthetic compounds to trigger rapid NLRP3 inflammation, which may overlook natural regulators because of the resulting drastic cell death. Here we use genetic methods to create NLRP3 mutations, in order to specifically investigate the role of NIMA-related kinase 7 (NEK7) in NLRP3 activation. I wish to answer if NEK7 is solely responsible for breaking up the cage, and if there is a secondary universal signal on top of NEK7 that controls NLRP3 activation.

SESSION 2 (2:25 - 3:15 pm)

31. Pimentel Marcelino, Leandro

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

Strategies to Stabilize Human VPS4 Hexamer for Structural-Based Drug Design

Vascular Protein Sorting 4 (VPS4) is a crucial ATPase involved in regulating the Endosomal Sorting Complexes Required for Transport (ESCRT) machinery in cells. Inhibiting VPS4 has therapeutic potential, as it could target approximately one-third of all cancers. Despite its importance, there is a lack of structural information on VPS4, particularly in its active hexameric form, and no structures of the human VPS4 hexamer have been characterized. Current structural data are limited to yeast, and none of these structures are bound to known inhibitors. Here, we used transmission electron (EM) negative stain (NS) and Mass Photometry (MP) to determine conditions where VPS4 could form stable hexamers, with the long-term goal of elucidating the structure of the hexameric complex with known and in-house inhibitors. Understanding the binding mechanisms of these inhibitors may facilitate the design of improved VPS4 inhibitors.