



Tri-Institutional PhD Program
Chemical Biology

**17TH ANNUAL TRI-INSTITUTIONAL
CHEMICAL BIOLOGY SYMPOSIUM**

POSTER SESSION

Tuesday, August 31st, 2021

1:15 – 3:15 pm

Virtual Event

Broadcast by MSK via Zoom

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Memorial Sloan Kettering
Cancer Center



SCIENCE FOR THE BENEFIT OF HUMANITY



**Weill Cornell
Medicine**

Abstract Booklet

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SESSION 1, 1:15 – 1:45 pm

1. Chen, Chen

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

Using Nanoparticulated PLX8394 to Overcome the Delivery Challenge in Fast-metabolism

ERK signaling pathway remains one of the most important therapeutic targets in cancer. Small molecule RAF inhibitors often selectively binds to monomeric RAF (like B-RAFV600E), but constitutively active RAF dimers became a major mechanism for acquired drug resistance. Previous work on PLX8394, a potent inhibitor for both monomeric and dimeric BRAF tumor models has shed a light on the resistance dilemma. However, in vivo results and recent clinical trials showed that PLX8394, while showing efficacy, required intense dose regime, or co-administration of cytochrome P450 inhibitors to combat the fast-metabolism nature of the molecule. We are interested in encapsulating PLX8394 into nanoparticles to engage targeted delivery to solid tumors. Expanding the chemical space of potential therapeutics can significantly increase our options in drug discovery, and overcome the barriers of potent compounds with insufficient pharmacokinetics (PK) properties from being efficacious in biological systems. We seek to utilize nanoparticles to address absorption, distribution, metabolism, and excretion (ADME) properties separately from the functionality of a molecule, and thus permit structural diversity and competent PK in vivo.

2. Huang, Hsin-Che

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

Characterizing the Role of the INO80 Complex in DPP8/9 Inhibition-Induced Pyroptosis

Inflammasomes are multiprotein complexes that detect pathogen or danger associated signals and trigger inflammatory osmolysis defined as pyroptosis. Inhibition of dipeptidyl peptidases (DPP) 8 and 9 activates NLRP1 and CARD8 inflammasomes via a proteasome-mediated degradation pathway; however, the exact danger signals and regulations remain unknown. Proteasome plays important roles in amino acid recycling by degrading proteins into short peptide fragments. The DPP8/9 and their downstream aminopeptidases process post-proteasome peptides containing a N-terminal penultimate proline residue and liberate the amino acids. Therefore, we hypothesize that the danger signal is in fact a perturbation in amino acid recycling or related metabolites that communicates a precarious cellular energy state. In line with this, using genome-wide CRISPR-Cas9 screens, we identified several proteins of the INO80 chromatin remodeling complex to be involved in VbP-induced pyroptosis. Since the INO80 remodeler regulates metabolic cycles in yeast, we hypothesize the disruption of INO80 chromatin remodeler confer resistance to VbP by altering the metabolic state of the cells. To that end, we will generate knockout cell lines and access the changes in metabolism, gene expression, and cell cycle progression. In addition, we will also determine the role of INO80 subunits on proteasome regulation.

3. Impastato, Anna

Graduate Student, New York University

Lab: Dirk Trauner, PhD

Optical Control of Mitosis with a Photoswitchable Eg5 Inhibitor

Eg5 is a kinesin motor protein that is responsible for bipolar spindle formation and plays a crucial role during mitosis. Loss of Eg5 function leads to the formation of monopolar spindles, followed by mitotic arrest, and subsequent cell death. Several cell-permeable small molecules have been reported to inhibit Eg5 and some have been evaluated as anticancer agents. We now describe the design, synthesis, and biological evaluation of photoswitchable variants with five different pharmacophores. Our lead compound AzoEMD is a cell permeable azobenzene that inhibits Eg5 more potently in its light-induced form. This activity decreased movement in microtubule gliding assays, promoted formation of monopolar spindles, and led to mitotic arrest in a light dependent way.

4. Khanye, Setshaba, PhD

Academic Staff Scientist, Rhodes University

Serendipitous identification of Novel Ferrocenyl Aminocresols Targeting Hemozoin Inhibition and DNA Interaction

The private public partnerships exemplified by GlaxoSmithKline laboratories (Tres Cantos) and Medicine for Malaria Venture (MMV) pioneered the creation of open access malaria box in the quest of innovative antimalarial drug scaffolds [1]. Through this partnership, a plethora of small molecules were released to public to fast track the rapid identification of lead compounds, which can be translated into clinical drug candidate, and ultimately, new tools for treatment of malaria, a protozoan parasitic disease that has claimed millions of lives, especially in the continent of Africa. Among the scaffolds identified from the malaria box is α -amino cresol. Compounds containing α -amino cresol motif has been shown to be promising as potent antimalarial agents [2]. However, the activity of their organometallic derivatives remains scarce in literature. Investigation of ferrocenyl 1,3-benzoxazines [3] as anti-infective agents by our group led to serendipitous identification of ferrocenyl α -aminocresol analogues [4] as side products. We pursued the synthesis of newly discovered ferrocenyl α -aminocresol derivatives and demonstrated their potential biological effects against two strains of the Plasmodium falciparum parasite, a causative agent for malaria. The resultant compounds were also assessed for hemozoin inhibition and DNA binding studies.

5. Lapinsky, David, PhD

Professor, Duquesne University School of Pharmacy

Appendage- and Scaffold-Diverse Electrophilic and Photoreactive Fully Functionalized Small-Molecule Probes for Integrated Phenotypic Screening-Target Identification Campaigns Via Minimalist Trifunctional Building Blocks

One of the grand challenges for chemical biology is identifying a small-molecule modulator for the individual functions of all proteins within a proteome. To expand the variety and number of ligandable proteins that could impact future drug target selection, the objective of our work is to synthesize and evaluate the protein target profiles of electrophilic and photoreactive fully functionalized small-molecule probes (FFSMPs) featuring increased scaffold- and appendage-diversity versus previously reported FFSMP libraries. FFSMPs contain: 1.) a protein-binding motif, 2.) an electrophile or photoreactive functional group for target protein capture, and 3.) a terminal alkyne for click chemistry-based proteomic applications. These compounds can be directly applied in phenotypic screening programs to identify ligand-protein pairs in cells in an unbiased manner. Essential to the synthesis of our FFSMPs are new minimalist trifunctional building blocks in an isocyanide-based multicomponent reaction-amine unmasking-arming synthetic sequence. Evaluation of protein labeling profiles of our FFSMPs demonstrates engagement of diverse subsets of protein targets by individual library members. Phenotypic screening is used to identify FFSMPs with potent activity in cells, followed by chemoproteomic target identification methods. In conclusion, our work supports the continued use of minimalist trifunctional building blocks for preparing structurally diverse FFSMPs for integrated phenotypic screening-target identification campaigns.

6. Neugroschl, Atara

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

A Ubiquitin-Independent Proteasome Pathway Activates the CARD8 Inflammasome

In response to danger-associated or pathogen-associated signals, intracellular pattern recognition receptors (PRR) assemble into macromolecular protein complexes called inflammasomes. When activated, these inflammasomes cause a lytic form of programmed cell death termed pyroptosis. CARD8 is a PRR known for undergoing auto-proteolysis, an event that breaks the polypeptide chain into C- and N-terminal (CT and NT) fragments that remain non-covalently associated. Upon activation of CARD8 by a danger signal, a proteasome degrades the NT. The freed CT then oligomerizes, assembles into an inflammasome, and executes pyroptosis. Currently, the identity of the specific proteasome and the mechanism of how it degrades the NT is unknown. Here, we demonstrate that the 20S proteasome degrades CARD8's N-terminal intrinsically disordered region (IDR). While this degradative process does not depend on the sequence identity, it does require a structurally disordered region. It can also degrade CARD8's IDR internally when the protein lacks an accessible terminus, demonstrating the machinery's endoproteolytic capability. Lastly, this process is independent of the ubiquitin-proteasome system (UPS). Overall, these data suggest that the 20S proteasome is sufficient to degrade CARD8's IDR. Further investigation is needed to discover whether the 20S proteasome, when activated, is necessary or sufficient to generate the CARD8 inflammasome complex.

7. Orth-He, Elizabeth

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

M24B aminopeptidase inhibitors selectively activate the CARD8 inflammasome

CARD8 and NLRP1 are homologous innate immune sensors that detect pathogen associated molecular patterns. When activated, CARD8 and NLRP1 form an inflammasome, a multiprotein complex that triggers an immune response. Both NLRP1 and CARD8 are restrained from activation by their binding to the dipeptidyl peptidase DPP9, and both become activated in response to DPP9 inhibition by a potent inhibitor, Val-boroPro. Here, we report that an inhibitor of the M24B family of aminopeptidases, CQ31, selectively activates the CARD8 but not the NLRP1 inflammasome. CQ31 specifically inhibits the peptidases Prolidase and XPNPEP1, resulting in accumulation of proline-containing dipeptides in cells, which in turn weakly inhibit DPP9 activity. This inhibition of DPP9 by dipeptide buildup activates the CARD8 inflammasome.

8. Ralls, Jack

Graduate Student, Weill Cornell Pharmacology
Lab: Alex Kentsis, MD, PhD

Designing Novel Antibody Conjugates for Therapeutic Genome Editing

Antibody-drug conjugates represent a relatively new, but highly effective, field of therapeutics. Antibody-drug conjugates have a number of advantages over traditional small molecule drugs; one of the most significant advantages is target specificity, lowering off-target effects. Since the first antibody-drug conjugate, Mylotarg™, was approved by the FDA, a total of 9 ADCs have been approved, all for cancer treatment. Strategies for macromolecular delivery and treatment of non-oncological ailments, however, has lagged. Recent interest in Cas9 delivery for therapeutic gene editing has been hindered by issues with viral transfection, including random genomic integration and constitutive expression of Cas9, increasing the chance of off-target editing. We propose to develop a novel Cas9-delivery method through antibody conjugation, and we hypothesize that the endonuclease Cas9 can be delivered via antibody conjugation to cells for therapeutic genome editing. We aim to design a tripartite construct, composed of an antibody and recombinant Cas9 cargo conjugated to one another by a cell penetration peptide. The antibody and recombinant Cas9 will each be conjugated to one terminus of the CPP, with the peptide sequence between them. The antibody will be directed against human CD117, a transmembrane receptor heavily expressed on hematopoietic stem and progenitor cells. The Cas9 will be preloaded with sgRNA targeting an erythroid-specific enhancer of BCL11A; disruption of this gene by Cas9 has been shown to be a potent treatment for β —hemoglobinopathies, and we hope this ADC will advance the treatment of such diseases.

9. Repeta, Lucas

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

MTREC: Exploring a Unique RNA Exosome Cofactor That Determines RNA Fates

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

The ubiquitous Mtr4 helicase is conserved throughout eukaryotes as an essential component of RNA exosome cofactor complexes. The recent discovery of a novel Mtr4-like protein (Mtl1) containing complex, MTREC, in *Schizosaccharomyces pombe* has expanded the catalogue of known exosome functions. Degradation of mRNAs and cryptic transcripts through MTREC-mediated pathways is reported to assist in the formation and maintenance of facultative heterochromatin at their originating genomic loci, a system not yet observed in other organisms.

This study proposes to reconstitute MTREC in vitro. Helicase activity assays will characterize the kinetic and thermodynamic properties of MTREC and elucidate how the complex selects and processes substrates. To date, limited biochemical or structural information on MTREC has been published, complicating the interpretation of in vivo observations. These experiments, combined with data from cryo-electron microscopy and X-ray crystallography will establish a foundation to understand how MTREC and the exosome cooperate to regulate target genes, as well as the factors that distinguish MTREC from canonical Mtr4-containing exosome cofactors.

10. Vandana, Jeya

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

The development of smart functional beta cells with enhanced insulin secretion via PC1/3 overexpression in human embryonic stem cells (hESCs)

Proglucagon, produced from the Gcg gene in pancreatic α -cells and enteroendocrine cells, is differentially processed by a class of enzymes, proprotein convertases (PCs). PC1/3 modulates the conversion of proglucagon into GLP-1, GLP-2 and its oxyntomodulin derivatives whereas PC2 regulates its conversion to glucagon. Maturation of hESCs into β -cells is broadly encompassed into the four key stages of endoderm formation, pancreas specification, endocrine specification, and β -cell maturation. To date, several protocols utilizing a variety of appropriate proteins, growth factors and small molecules to direct the differentiation of hESCs into β -cells have been adopted. However, the protocols that have been developed thus far are lacking, usually producing immature β -cells which are not able to exhibit adequate glucose stimulated insulin secretion (GSIS). We hypothesize that overexpressing PC1/3 in hESC-derived β -like cells would effectively direct their differentiation into monohormonal mature β -cells with an enhanced insulin producing potential due to increased GLP-1 levels.

11. Vogt, Kristen

TPCB Graduate Student, Memorial Sloan Kettering
Labs: Daniel Heller, PhD & David Scheinberg, MD, PhD

A Delivery Strategy for Targeted Protein Degraders

Proteolysis-targeting chimeras (PROTACs) are next-generation small molecule therapeutics that exploit the ubiquitin-proteasome system to selectively degrade target proteins. These degraders have shown promise in pre-clinical models but their translation to the clinic may be hindered by their un-druglike characteristics (> 500 Da molecular weight) and on-target off-tumor toxicities. Recent work in the Heller lab has enabled the design of nanoparticles that target solid tumors by binding to P-selectin, a cell adhesion molecule upregulated in the tumor vasculature. These self-assembling nanoparticles encapsulated drugs at high loadings of up to 90% by mass and showed improved tumor regression, fewer side effects and longer survival in vivo compared to free drug.

In these studies, we aim to expand the utility of PROTACs in the clinic by developing a predictive and broadly useful nanoformulation for targeted protein degraders. We engineered self-assembling PROTAC nanoparticles (nanoPROTACs) to target various solid tumors by incorporating polysaccharides with high affinity to P-selectin. We demonstrated that a variety of PROTACs can be predictably encapsulated with high drug loadings and therapeutically relevant sizes (~100-200 nm). Initial tumor regression studies showed therapeutic benefit of nanoformulated dBET6, a degrader of the oncogenic regulator BRD4, in nut midline carcinoma xenografts. We next aim to validate other nanoPROTACs in vivo by investigating biodistribution, PK/PD, tumor regression and overall survival. The successful development of a nanoformulation for targeted protein degraders will expand the utility of this exciting next-generation therapeutic for the treatment of solid tumors in the clinic.

SESSION 2, 1:45 – 2:15 pm

12. Baca, Christian

TPCB Graduate Student, The Rockefeller University

Lab: Luciano Marraffini, PhD

Surveying Soil Metagenomic Libraries for Novel Phage Defense Systems

Bacteria and other microorganisms face constant threat by parasitic your phages that outnumber them. Bacteria have evolved numerous defense systems to resist these invaders, CRISPR being among them. Our current knowledge on phage defense systems has so far been limited to those present in publicly available genomic databases. We aim to identify and characterize novel phage defense systems using in-house metagenomic libraries derived from soil microorganisms, which carry vast genomic information that are yet to be surveyed for defense systems. To identify phage defense systems, we will employ a functional screening approach: we will take cosmid libraries containing millions of DNA inserts from soil or gut microorganisms, transform them into *Escherichia coli*, and infect these with different phages to screen for cosmids containing inserts that confer phage resistance. Currently, we have been able to enrich for and narrow down multiple genes that provide resistance to a variety of *E. coli* phages. Expanding our knowledge on phage defense systems will provide further insight into the basic biology of bacterial immunity against parasitic elements. Furthermore, novel defense systems open up possibilities for new biotechnological tools in medicine and science.

13. Boyer, Jake

Graduate Student, Memorial Sloan Kettering
Lab: Neal Rosen, MD, PhD

EIF4A Promotes Estrogen Receptor Alpha Translation and Represents a Novel target in Breast Cancer

The majority of human breast cancers are dependent on Estrogen Receptor Alpha (ER) and sensitive to its inhibition. In advanced, ER+-dependent breast cancers, resistance usually develops and is associated with insensitivity of estrogen receptor to inhibition. Mutations that activate PI3K signaling occur in 40% of ER-driven breast cancers. The PI3K pathway regulates cap-dependent protein translation by controlling mTOR complex I (mTORC1). Inhibitors of PI3K/mTOR are effective in this setting when given with anti-estrogens, but induce ER activity. We now show that despite reducing global cap-dependent translation, PI3K/mTOR inhibition does not reduce ER translation or expression. Translation of ER instead depends on EIF4A helicase, a translation initiation factor. Inhibitors of EIF4A significantly reduce the expression of WT and mutant ER and block the growth of breast cancer models in vivo, including models driven by estrogen-independent ER fusions that are unaffected by estrogen receptor antagonists. The utility of EIF4A inhibition can be enhanced when combined with Fulvestrant, a degrader of ER. Combining inhibition of ER translation and induction of ER degradation causes synergistic deep and durable inhibition of ER expression and tumor growth. Inhibition of ER translation represents a new potent strategy for treating ER-dependent breast cancers with acquired resistance to current therapies.

14. Digwal, Chander, PhD

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

Epichaperomics: a platform to decipher stressor-to-phenotype interactome alterations in Alzheimer's disease

The recognition of the importance of complicated stressor-to-phenotype associations in human disease demands a greater understanding of how specific stressors affect interactomes, especially in the context of late onset neurodegenerative disorders such as Alzheimer's disease (AD). Currently untreatable diseases arise due to variations in, and through a combination of, multiple stressors of genetic, epigenetic, and environmental nature. Unfortunately, how these stressors lead to a specific disease phenotype or inflict a vulnerability to some cells and tissues but not others, remains largely unknown and unsatisfactorily addressed. This concept 'selective vulnerability' is especially relevant in AD and related neuropathological conditions, where specific neuronal populations and circuits degenerate while others do not, making treatment a vexing problem. Accordingly, analysis of cell- and tissue-specific interactome networks is posited to shed light on organization of biological systems and subsequently to disease vulnerabilities. To date, deriving human interactomes across different cell- and disease-contexts remains a challenge. We provide evidence linking stressor-induced protein interactome network perturbations to the formation of pathologic scaffolds termed epichaperomes. A specialized 'omics platform, epichaperomics is a viable and reproducible experimental solution to obtaining rigorous context-dependent interactomes. Epichaperomics can be performed as a standalone approach as well as complement and enhance currently available conventional approaches to aid the scientific community in defining, understanding, and ultimately controlling interactome networks of complex diseases such as AD. Ultimately, this approach may aid the transition from a limited single-alteration perspective in disease to a comprehensive network-based mindset, which we posit will provide an evidence based view how these maladaptive changes from stressor to phenotype provide unique precision medicine opportunities for diagnostic and therapeutic development, especially in the context of neurodegenerative disorders such as AD where treatment options are currently limited.

15. Gao, Yijun, PhD

Postdoctoral Fellow, Memorial Sloan Kettering
Lab: Neal Rosen, MD, PhD

A novel RAF inhibitor BGB-3245 durably inhibits BRAF mutant-driven tumors despite feedback reactivation of RAS

Current RAF inhibitors potently inhibit BRAF V600E monomers, but not RAF dimers. They inhibit RAF activation of ERK signaling transiently and then inhibition is attenuated by relief of ERK dependent feedback, reactivation of RAS and induction of RAS-dependent RAF dimers that are insensitive to current inhibitors of RAF monomers. The rebound in ERK signaling that ensues is sensitive to blockade of reactivation of RAF dimers by either inhibition of upstream RTK signaling or knocking out CRAF expression. Rebound is modest in BRAF V600E melanomas, which are sensitive to inhibitors of RAF monomers and much greater in BRAF driven colorectal and thyroid carcinomas, which are usually not.

Here we show that BGB-3245, a drug that inhibits both RAF monomers and dimers can durably inhibit ERK signaling in BRAF V600 melanomas, colorectal and thyroid carcinomas. Inhibition of both BRAF V600E monomers and induced RAF homo- and hetero dimers results in reduced pathway rebound, greater inhibition of pathway output and effective treatment of BRAF mutant-driven colorectal and thyroid cancer models. However, modest rebound of ERK but not MEK phosphorylation still occurs. This rebound is due to inhibition of the ERK dependent expression of the ERK phosphatase DUSP6. Any residual MEK activation of the pathway is amplified by the decrease in DUSP6. This rebound may be reduced by combining RAF inhibition with MEK or ERK inhibitors which results in even greater antitumor activity.

16. Luwang, Johnson, PhD

PhD Graduate, Indian Institute of Science Education and Research, Thiruvananthapuram
Natesh Structural and Molecular Biology Lab

Impingement of the p53 stability by virtue of tetramerization

The p53 protein has been known to exist structurally in three different forms inside the cells. Earlier studies have reported the predominance of the lower oligomeric forms of p53 over its tetrameric form inside the cells, although only the tetrameric p53 contributes to its transcriptional activity. However, it remains unclear the functional relevance of the existence of other p53 oligomers inside the cells. In our studies, we characterize the stability and conformational state of tetrameric, dimeric and monomeric p53 that spans both DNA Binding Domain (DBD) and Tetramerization Domain (TD) of human p53 (94-360 amino acid residues). Intriguingly, our studies reveal an unexpected drastic reduction in tetrameric p53 thermal stability in comparison to its dimeric and monomeric form with a higher propensity to aggregate at physiological temperature. Our EMSA study suggests that tetrameric p53, not their lower oligomeric counterpart, exhibit rapid loss of binding to their consensus DNA elements at the physiological temperature. This detrimental effect of destabilization is imparted due to the tetramerization of p53 that drives the DBDs to misfold at a faster pace when compared to its lower oligomeric form. This crosstalk between DBDs is achieved when it exists as a tetramer but not as dimer or monomer. Our findings throw light on the plausible reason for the predominant existence of p53 in dimer and monomer forms inside the cells with a lesser population of tetramer form. Therefore, the transient disruption of tetramerization between TDs could be a potential cue for the stabilization of p53 inside the cells.

17. Payne, Alexander

TPCB Graduate Student, Memorial Sloan Kettering
Labs: John Chodera, PhD & Richard Hite, PhD

Pursuing Allosteric Activators of TMEM175 on the Road to a Parkinson's Disease Therapeutic

TMEM175 is a lysosomal potassium channel whose mutation is associated with early onset and increased rate of cognitive decline in Parkinson's Disease (PD) patients through an unknown mechanism. Recent work has demonstrated the potential allosteric activation of TMEM175 by the kinase Akt and indicated that the wild-type activity of TMEM175 is not optimized for preventing PD-associated pathologies. As such, a small molecule activator which increases the open probability of TMEM175 could be viable as a PD therapy. The Hite lab has previously solved structures of human TMEM175 in putative conducting ("open") and non-conducting ("closed") states. Molecular dynamics ensembles of these two states will be analyzed to propose sites which are predicted to allosterically regulate channel opening. In silico docking of commercially synthesizable compounds against these sites will be performed and potential hits will be tested by a liposome flux assay. Here, we present flux assay results for novel inhibitors of TMEM175 as well as the initial simulation setup for TMEM175 in a lipid membrane.

18. Peyear, Thasin

Graduate Student, Weill Cornell Medicine

Lab: Olaf Anderson, MD

Screening for Lipid Bilayer-Modifying Compounds using Gramicidin Based Simulation Assays, A Scaffold Based Approach

The lipid bilayer is a regulator of membrane protein function and therefore of many physiological processes. This is important because small molecules that target membrane and intracellular proteins interact with the bilayer, which may produce changes in bilayer properties (thickness, curvature, and elasticity), that in turn may cause indiscriminate changes in membrane protein function—and thereby disrupt cell function and, eventually, become cytotoxic. (We know that the likelihood of cytotoxicity increases with bilayer—modifying potency.) To assess drug effects on bilayer properties, we used gramicidin channels, which has been widely used to detect changes in bilayer properties, in an *in silico* approach to examine the drugs' bilayer-modifying potencies, quantified as changes in the gramicidin monomer↔dimer equilibrium as estimated using coarse-grained molecular dynamics simulations. We developed a scaffold-based drug library that includes quinoline (S1), isoflavone (S2), benzodiazepine (S3), sterol (S4) and clozapine-like antipsychotics (S5). Each base scaffold has three R-group attachment sites, where we placed ten distinct R-group substitutions (0 - 9). Systematic insertion of each R-group at each attachment site gave rise to 1000 unique molecules per base-scaffold and 4996 compounds in total. Across the scaffolds, we found that certain functional groups increased the likelihood a molecule is bilayer perturbing. We are currently using cheminformatics to identify key physicochemical properties that allow us to predict a molecule's bilayer-modifying potency, with the goal of using machine learning techniques to better understand and quantify drug-induced bilayer perturbations, which we hope will help guide the development of drugs with fewer bilayer-mediated off-target effects.

19. Rufa, Dominic

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

Improving protein:ligand binding affinity predictions with machine-learned quantum chemistry

Relative binding free energy calculations (RBFEs) are routinely employed in campaigns to design and optimize high-affinity, specific ligands to interrogate—and often inhibit—the biochemical functionality of protein targets. RBFEs often deliver protein:ligand binding affinity predictions within ~1-2 kcal/mol RMSE to experiment [1] at a fraction of the time and cost of compound synthesis/in vitro assays on a per-compound basis. This makes them an integral part of a medicinal chemist's toolkit for structure-enabled programs [2]. The limited accuracy afforded to free energy calculations by molecular mechanics (MM) force fields is a major limitation to their predictive capacity [3], however. Recent work in machine-learned (ML) potentials has been able to recover near-quantum mechanical (QM) energetic accuracy at a sufficiently low computational cost to employ them in RBFEs [4]. Our recent work [5] demonstrates that accuracy of RBFEs can be significantly improved using hybrid ML/MM potentials in a computationally cheap post-processing procedure.

20. Shea, Christie

TPCB Graduate Student, Weill Cornell Medicine
Lab: John Blenis, PhD

Establishing Histone H3 variant H3.3 as a Therapeutic Target for Metastatic Cancer

Metastasis is responsible for 70-90% of cancer-related deaths, therefore, it is important to understand the mechanisms controlling this process so that more therapeutic agents can be developed. While cancer is usually thought to be a disease of the genome, epigenetic changes, such as chromatin remodeling, are required for almost every step of tumor progression. Our lab has demonstrated that incorporation of H3.3, a noncanonical H3 variant, onto chromatin at specific genic regions by the HIRA chaperone complex, is critical for tumor progression and the ability of breast cancer cells to effectively form metastasis. However, it is still largely unclear how H3.3 promotes metastasis whereas canonical H3 does not. Therefore, my goal is to elucidate how the HIRA complex and wildtype H3.3 plays a role in tumorigenic process. While it is known that the protein level of HIRA increases during EMT, there was no change in its mRNA level; therefore, it is still unclear how it is regulated during the process. Using qPCR, I found that while HIRA mRNA level remained similar, there was an increase in UBN1 mRNA level when the cells were treated with TGF β . My preliminary result suggests that the increase of HIRA protein level during EMT may be a consequence of the increase of UBN1 expression level. My data also shows that there is an overall decrease in the acetylation of lysine by the same metastatic signals that drive the incorporation of H3.3 onto pro-aggressive genes, suggesting that histone deacetylase may play a role during metastasis.

21. Stella, Gianna

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

Targeting flexibility in Type III CRISPR-Cas Immunity

Prokaryotes have evolved multiple defense strategies to fight invading genetic elements such as plasmids and bacteriophages. CRISPR, which stands for Clustered Regularly Interspaced Short Palindromic Repeats, and CRISPR-associated (cas) genes act as an adaptive immune system in 40% of bacteria and 80% of archaea. During the Type III CRISPR-Cas bacterial immune response, recognition of an RNA molecule complementary to the CRISPR RNA (crRNA) within the Cas10-Csm complex triggers three distinct activities: (i) non-specific ssDNA degradation by the Cas10 HD nuclease domain, (ii) conversion of ATP into cyclic-oligoadenylate (cOA) second messengers by the Cas10 Palm cyclase domain, which activates CARF-containing nucleases that in turn degrade RNA in a nonspecific manner, and (iii) cleavage of the complementary target RNA, which deactivates both domains of Cas10. Previous work has shown that Type III immunity tolerates mismatches between the target transcript and crRNA *in vivo*, but it is not understood if and how mismatches disrupt the three biochemical events linked to immunity. Studying the flexibility of the Type III system *in vitro* will reveal how the system overcomes constantly evolving bacterial invaders.

22. Strauss, Alexa

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

Domain Coupling in Metabotropic Glutamate Receptors

Metabotropic glutamate receptors (mGluRs) are synaptic G protein coupled receptors (GPCRs), which respond to the excitatory neurotransmitter glutamate. mGluRs are characterized by constitutive dimerization and the presence of a large extracellular ligand binding domain (LBD). In mGluRs, the LBD is connected via a cysteine-rich domain (CRD) to a transmembrane domain (TMD). The TMD couples directly to intracellular G proteins. Recent structural characterization has provided a breakthrough in defining the overall architecture of this multi-domain receptor and suggesting a mechanism by which agonist binding in the LBD is coupled to activation of the TMD. For this mechanism, residues in the extracellular loop two of the TMD interact with residues on the CRD and a short CRD-TMD linker. However, the precise role of each of these regions in the activation mechanism and the extent each component plays in coupling remains an open question. To probe the role of these domains, we design constructs with targeted insertions and deletions to assess the effect of perturbing this loop-linker interaction. The effects of these constructs are assessed through functional assays and measures of conformational dynamics. Taken together, this data supports the proposed coupling mechanism as one component of the allosteric domain coupling and suggests an additional inhibitory role for the CRD.

SESSION 3, 2:15 – 2:45 pm

23. Chua, Gabriella

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

Single-stranded nucleic acid sensing and enhanced phase separation by linker histone H1

The linker histone H1 is well known for its role as a nucleosome compactor and essential component of higher order chromatin structure in eukaryotes. However, the biophysical nature of the H1-DNA interaction and whether H1 is able to distinguish between different DNA substrates present in cells remain unclear. Here we used single-molecule correlative force and fluorescence microscopy to visualize the behavior of H1 on single-stranded and double-stranded DNA in real time. Unexpectedly, we observed that H1 preferentially coalesces around nascent single-stranded DNA, forming condensate-like complexes. Further characterization showed that longer single-stranded DNA increased the propensity of H1 to phase separate into condensates. Satisfyingly, molecular dynamics simulations revealed that multivalent and transient engagement of H1 with unpaired DNA strands drives their enhanced phase separation. Controlled condensate fusion using optical tweezers showed that single-stranded nucleic acids mediate the formation of more viscous, gel-like H1 droplets. Finally, we found the number of H1 puncta in cells increased under stressed conditions, suggesting H1 may function to aid in DNA repair. Overall, our results provide evidence for a new role of H1 to sense different forms of nucleic acids and may orient the field towards viewing H1 as a more diverse player in genome organization in maintenance.

24. Corless, Broderick

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

EpoxySEAKER and onSEAKER: CAR T-Cell Models for Targeted Local Biosynthesis of Therapeutic Warheads

The development of CAR (Chimeric Antigen Receptor) T-cell therapies for B-cell cancers has emerged as paradigm-shifting option for patients. Our labs have developed proof-of-concept SEAKER (Synthetic Enzyme-Armed KILLER) CAR T-cells that recognize tumor cells and express enzymes that cleave masking groups from prodrugs. Enzymes EpnF and TsnB9 have been characterized to catalyze the final step in biological synthesis of the natural products Eponemycin (proteasome inhibitor) and Trichostatin A (HDAC inhibitor), respectively. Several proteasome and HDAC inhibitors have been approved by the FDA to treat multiple myeloma. These enzymes will be harnessed to convert analogs of the above-mentioned natural products into protease and HDAC inhibitors in situ. In vitro experiments carried out previously have demonstrated the enzymes' ability to install warheads on precursor compounds. Prodrugs for Epoxomicin and Trichostatin A have been designed and synthesized. Preliminary in situ enzyme assays with EpnF and TsnB9 show generation of desired products via UPLC monitoring. FDA-approved proteasome and HDAC inhibitors such as Carfilzomib or Panobinostat harness the same warheads to elicit the mechanistic inhibition of their targets. We are generating prodrugs that mimic the biological precursors that may be acted on by the aforementioned enzymes to yield cytotoxic compounds. Further progression of these technologies will yield potent cell mediated prodrug therapies for multiple myeloma.

25. Fu, Ziao, PhD

Postdoctoral Fellow, The Rockefeller University
Lab: Roderick MacKinnon, MD

Structure of human GABA B receptor in an inactive state

The human GABAB receptor—a member of the class C family of G-protein-coupled receptors (GPCRs)—mediates inhibitory neurotransmission and has been implicated in epilepsy, pain and addiction. A unique GPCR that is known to require heterodimerization for function, the GABAB receptor has two subunits, GABAB1 and GABAB2, that are structurally homologous but perform distinct and complementary functions. GABAB1 recognizes orthosteric ligands, while GABAB2 couples with G proteins. Each subunit is characterized by an extracellular Venus flytrap (VFT) module, a descending peptide linker, a seven-helix transmembrane domain and a cytoplasmic tail. Although the VFT heterodimer structure has been resolved, the structure of the full-length receptor and its transmembrane signalling mechanism remain unknown. Here we present a near full-length structure of the GABAB receptor, captured in an inactive state by cryo-electron microscopy. Our structure reveals several ligands that preassociate with the receptor, including two large endogenous phospholipids that are embedded within the transmembrane domains to maintain receptor integrity and modulate receptor function. We also identify a previously unknown heterodimer interface between transmembrane helices 3 and 5 of both subunits, which serves as a signature of the inactive conformation. A unique 'intersubunit latch' within this transmembrane interface maintains the inactive state, and its disruption leads to constitutive receptor activity.

26. Harper, Nathan

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

Late maturation events of the human mitochondrial small subunit captured 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 rRNA folding, modification, and scaffolding, to generate translationally-competent subunits with high fidelity. While a number of assembly factors for mitoribosomes have been identified, the molecular details of how they function within the context of assembly intermediates, and the chronology of association events, is unclear. To address this, we aimed to purify native assembly intermediates from human cells and structurally analyze them by cryo-EM. Using an endogenous tagging system developed in our lab to tag a putative methyltransferase involved in mitochondrial small subunit assembly, we purified native assembly intermediates and obtained reconstructions of multiple states along the native assembly pathway by cryo-EM, identifying five bound assembly factors and elucidating their interactions with mitochondrial small subunit intermediates. 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. This work highlights conserved mechanisms for the late stages of small subunit maturation across ribosome assembly systems, and provides an initial framework for targeted biochemical experiments to mechanistically interrogate the function of identified assembly factors.

27. Hou, Qian

TPCB Graduate Student, Weill Cornell Medicine
Lab: Samie Jaffrey, MD, PhD

Turn on eukaryotic mRNA translation with drug-regulated RNA switches

Being able to regulate protein expression swiftly and reversibly is a necessary tool in biological research. It also has great potential in the rapidly evolving gene therapy world. Conventional gene regulatory tools such as the endogenous steroid receptor-derived systems and the tetracycline repressor protein-derived systems operate on transcriptional level. Transcriptional regulators give big change in protein expression due to accumulation of large quantity of mRNA transcripts. However, these systems are difficult to engineer, have prolonged lag time after induction, and are prone to triggering immune response. I am interested in designing a translational RNA switch embedded in the 5'-untranslated region (UTR) of mRNA. This RNA switch is evolved from encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES). The inactive form of IRES turns off translation of the target gene. When small-molecule drug acyclovir is introduced, IRES goes through conformational re-arrangement – translation is enabled. The acyclovir-regulated IRES is selected through an in vitro selection technique called mRNA display which combines the advantages of in vitro selection with the power of cell-free lysate environment. This IRES-based translational switch is likely to have better temporal control as it does not require over-accumulation of mRNA transcript. Further, this system does not require expression of exogenous trans-acting proteins which are usually immunogenic. This technology sets itself apart from existing tools and can potentially transcend gene therapy in the translation regulation field.

28. Hsieh, David

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

Discovery of novel bioactive aromatic amino acid biotransformation in the gut microbiota
by mass spectrometry

The human microbiota is a collection of over one thousand species of bacteria. These bacteria outnumber human cells by a factor of ten and are implicated in diverse aspects of host physiology and diseases. Recent studies about human microbiota have focused on characterizing species composition and achieved some success in treating inflammatory bowel disease by fecal microbiota transplantation. 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 has the potential to improve the development of probiotic therapies.

The human gut microbiota actively metabolizes aromatic amino acids (Phe, Tyr, and Trp, abbreviated here as AAA) but these metabolized products have not yet been systematically explored across different microbiota species. Many important human signaling molecules such as serotonin and dopamine are derived from AAA. Analogs of these signaling molecules tend to be active against their respective receptors. Given the genetic capacity of the human microbiota, I hypothesize that the human microbiota can modulate the host physiology by metabolizing AAA into human ligand analogs.

29. Jain, Richa

Assistant Professor, Motilal Nehru College
Department of Physics

Tailoring of structural, magnetic, electrical, and optical properties of magnetite nanopowders by gadolinium doping for biomedical applications

Effect of gadolinium doping on the structural, magnetic, and electrical properties of magnetite nanoparticles has been studied. $\text{Fe}_{3-x}\text{Gd}_x\text{O}_4$ ($x=0$ to 0.10) nanoparticles have been synthesized using facile co-precipitation method. With doping rod like structure appears along with round shape particles. Magnetization has increased with doping till $x=0.04$. Resistivity and band gaps have decreased with doping. These particles could have potential applications in biomedical science.

30. Rosenzweig, Adam

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

Total Synthesis of a Novel Bioinformatically Predicted HPG Rich Lipodepsipeptide

Secondary metabolites from bacterial and fungi have long been a source of bioactive molecules for therapeutic application as well as structural inspiration for medicinal chemistry. Traditional culture-based methods of natural product discovery from microbes has dwindled in its ability to produce novel natural products over the last several decades. Bioinformatic methods have emerged as a reliable method to investigate a bacteria's secondary metabolome and predict the molecular structures of products that are coded within an organism's genome. Molecules that can be predicted from biosynthetic gene clusters (BGCs) and then built via total synthesis constitute a class of molecules called synthetic bioinformatic natural products (syn-BNP). Non-ribosomal peptide synthases (NRPSs) are particularly good candidates for this approach as the order and composition of the short polypeptides are easily predictable and can be synthesized using peptide synthesis. Assembling a phylogenetic tree of the condensation start domain gene sequences of many bacterial species, the Brady lab has identified two novel hydroxyphenylglycine (HPG) rich, lipodepsipeptide syn-BNPs, from gene clusters related to Ramoplanin and Enduracidin. These syn-BNPs are predicted to inhibit Lipid 1 synthesis based on their phylogenetic and structural similarities to Ramoplanin and Enduracidin. Using bioinformatic analysis we can predict the proposed gene product of these BGCs and we have designed a total synthesis route to build one of the predicated products, HPG_01. Once built, the syn-BNP can be evaluated for its bioactivity and inhibitory potential.

31. Škuta, Ctibor, PhD

Postdoctoral Fellow, Institute of Molecular Genetics of the Czech Academy of Sciences
Lab: Petr Bartůněk, PhD

State of chemical probes in 2021: sources, structures, scoring and targets

In 2005, the NIH Molecular Libraries Program (MLP) undertook the identification of tool compounds to expand biological insights, now termed small-molecule chemical probes. This inspired other organisations to initiate similar efforts from 2010 onwards. However, a decade later it is very difficult to get an overview of exactly what chemical probes are available and what proportion of the human druggable proteome they cover. As a central focus of the Probes & Drugs portal (P&D), we have standardised, integrated and compared sets of declared probe compounds harvested from 12 different sources. Our results address key questions including; a) individual and total standardised structure counts, b) overlaps between sources, c) comparisons with selected PubChem sources and d) the probe proteome coverage. In addition, we developed new high-level scoring schemes to filter collections down to probes of higher quality. This generated 548 high-quality chemical probes (HQCP) covering 447 distinct protein targets. This collection has been added to the P&D portal and is regularly updated as established sources expand and new ones release data.

32. Ye, Linzhi

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

Novel Antimicrobial Compounds from Human Microbiome

The human microbiome is a collection of diverse microorganisms that produce a variety of bioactive small molecules, including antimicrobials. Microbes that produce antimicrobial compounds benefit by outcompeting surrounding microbes, and in some cases defend the host against infection by pathogenic microbes. Indeed, people colonized by antimicrobial-producing microbes are protected from certain multidrug-resistant pathogens. However, our knowledge of antimicrobial compounds produced by human-associated commensal bacteria remains limited. The goal of my project is to discover novel antimicrobials from human microbiome by thin-layer chromatography screening of a library of extracted bacterial metabolites. Subsequently, specific antimicrobial compounds will be isolated using bioactivity-guided fractionation, and their structure will be elucidated using analytical chemistry. Following isolation of these molecules, I plan to study their distribution in human population and their ecological roles in human microbiome evolution.

33. Zhang, Yuxi

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

Correlation between structure and function in phosphatidylinositol lipid-dependent Kir2.2 gating

Inward rectifier K⁺(Kir) channels regulate cell membrane potential. Different Kir channels respond to unique ligands, but all are regulated by phosphatidylinositol 4,5-bisphosphate (PI(4,5)P₂). Using planar lipid bilayers we show that Kir2.2 exhibits bursts of openings separated by long quiescent inter-burst periods. Increasing PI(4,5)P₂ concentration shortens the Kir2.2 inter-burst duration and lengthens the burst duration without affecting dwell times within a burst. From this, we propose that burst and inter-burst durations correspond to the CTD-docked and CTD-undocked conformations observed in the presence and absence of PI(4,5)P₂ in atomic structures. We also studied the effect of different phosphatidylinositol lipids on Kir2.2 activation and conclude that the 5' phosphate is essential to Kir2.2 pore opening. Other phosphatidylinositol lipids can compete with PI(4,5)P₂ but cannot activate Kir2.2 without the 5' phosphate. PI(4)P, which is directly interconvertible to and from PI(4,5)P₂, might thus be a regulator of Kir channels in the plasma membrane.

SESSION 4, 2:45 – 3:15 pm

34. Berman, Adi

TPCB Graduate Student, The Rockefeller University

Lab: Tarun Kapoor, PhD

Using Chemical and Cell Biology Tools to Investigate the Role of VCP in Mitosis

The AAA-ATPase, Valosin-Containing-Protein (VCP/p97) is a key regulator of ubiquitin-dependent protein quality control in the cell. VCP utilizes ATP hydrolysis to process protein for recycling or proteasomal degradation. Several cellular contexts, such as ER-associated protein degradation, autophagy, and endosomal trafficking, are dependent on proper VCP function. RNAi-based depletion of VCP has also been shown to result in mitotic abnormalities, but given the short time course of mitosis (~2 hours) and the long incubation time needed to achieve RNAi knockdown (~72 hours), it is unclear if VCP is directly involved in mitotic progression, or if mitotic errors are a downstream result of the loss of VCP during other contexts in the cell cycle. To address this question, we used acute chemical inhibition of VCP and examined any changes in mitotic cellular phenotype. After a 2-hour inhibition of VCP with either an ATP-competitive inhibitor or an allosteric inhibitor, mitotic cells displayed disrupted mitotic spindles, with disorganized microtubules and misaligned chromosomes. Furthermore, nocodazole washout experiments revealed that VCP inhibition decreased non-centrosomal microtubule formation in mitotic cells. Thus, the use of chemical inhibitors in combination with cell biological methods to examine individual sites of microtubule growth show that VCP plays a direct role in cell division.

35. Carl, Ayala

TPCB Graduate Student, The Rockefeller University
Lab: Gregory Alushin, PhD

Structural Characterization Of F-actin under Force

The ability of cells to sense mechanical forces in their microenvironments influences cellular functions including migration, proliferation, and apoptosis. The actin cytoskeleton, a network consisting of actin filaments (F-actin), myosin motor proteins, and over 150 Actin Binding Proteins (ABPs), plays a central role in cellular mechanics. F-actin has been reported to transduce mechanical stimuli into downstream biochemical pathways (“mechanotransduction”) through force-regulated interactions with mechanosensitive ABPs. As multiple actin subunit conformations have been reported to co-exist in filament populations in the absence of force, we hypothesized that actin’s inherent structural plasticity facilitates the formation of force-regulated structural states which can be recognized by mechanosensitive ABPs. To test this hypothesis, we developed a novel reconstitution system utilizing surface-immobilized myosin motor proteins to study the conformational response of F-actin to compressive and tensile forces with cryo-electron microscopy (cryo-EM). Utilizing this system for cryo-electron tomography (cryo-ET) studies, we observe that F-actin adopts a super helical spiral conformation in local regions along the filament (which we term “squiggles”) in the presence of both tension and compression. Analysis of the distribution of subunit positions in tomograms suggest that squiggles form due to asymmetric longitudinal promotor spacing between the two strands that compose the filament. We furthermore observe the frequent presence of dislocated subunits in squiggles, which we posit are the initiating sites of lattice defects that propagate along a strand. In addition to providing the first direct visualization of a force-induced rearrangement of F-actin, our studies identify a potential binding platform for mechanosensitive ABPs.

36. Hebert, Jakob

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

The SWI/SNF related protein SMARCA3 is a histone H3K23 ubiquitin ligase that regulates H3K9me3 in cancer

Histone ubiquitination is a key post-translational modification (PTM) that mediates diverse chromatin functions. However, our understanding of the enzymes that regulate ubiquitination on histones, along with their relationships to human disease, remains limited. Here, we identify the SWI/SNF-related protein SMARCA3 as a new histone H3K23 E3 ubiquitin ligase that functions through a distinct mechanism from its characterized role in post-replication repair. We show that cellular depletion of SMARCA3 results in reduced H3K9me3 levels and genomic redistribution of this mark, as well as of DNA methylation. These correlate with an increase in chromatin accessibility of cancer associated genes and enhancers in cells depleted with SMARCA3, leading to a 'rewiring' of the cellular transcriptional program. Using both cell lines and patient-derived organoids, we demonstrate that colorectal cancer (CRC) tumors, where SMARCA3 is substantially downregulated, also display a decrease in H3K9me3 levels, as well as alterations in the expression of similar cancer-promoting genes. Taken together, our data indicate critical roles for SMARCA3 E3 ligase activity in maintaining chromatin integrity and provide a new mechanism linking DNA-methylation, chromatin structure and cancer.

37. Horioka, Mizuho

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

Cellular Phenotypes of All Human Missense Variants and SNPs of the GPCR Oncogene CYSLTR2

A mutation in CYSLTR2, the gene for the G protein-coupled receptor (GPCR) cysteinyl-leukotriene receptor 2 (CysLTR2), causes uveal melanoma and is a significantly mutated GPCR in several other cancers as well. We identified >100 CYSLTR2 missense variants of unknown significance (VUS) in human cancer genomes from available cancer databases, as well as another >100 CYSLTR2 single-nucleotide polymorphisms (SNPs) from exome sequence data. We characterized expression level, basal and agonist-stimulated G protein signaling, and basal and agonist-stimulated β -arrestin recruitment using cells from a single transfection for each of the VUS and SNPs. The CysLTR2-L129Q mutant that causes uveal melanoma has a unique phenotype among all cancer-associated CYSLTR2 variants. It is highly constitutively active with gain-of-function in basal Gq/11-PLC β signaling, while only poorly recruiting β -arrestin. Approximately 70% of the >200 CYSLTR2 variants show a loss-of-function phenotype in at least one of assays employed. While many loss-of-function mutations were located at canonical functional sites (G protein or β -arrestin binding interfaces, ligand binding sites, and microswitch regions), others were at sites not known to be necessary for CysLTR2 function, highlighting the need for phenotypic evaluation of GPCR VUS and SNPs in human cancers. Our strategy introduces a scalable pipeline for a rapid, high throughput characterization of the phenotypic landscape of cancer-associated mutations in GPCRs.

38. Kotliar, Ilana

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

Multiplexed analysis of the secretin-like GPCR-RAMP interactome by suspension bead array

Receptor activity-modifying proteins (RAMPs) have been shown to modulate the functions of several different G protein-coupled receptors (GPCRs), but the potential for direct interactions among the three known RAMPs and hundreds of GPCRs has not been measured. Focusing on the secretin-like family of GPCRs, we engineered 23 epitope-tagged GPCRs and three epitope-tagged RAMPs. We used these constructs in a multiplexed suspension bead array (SBA) immunoassay that we developed for detection of GPCR-RAMP complexes. The SBA assay results elucidate the complete interactome for secretin-like GPCRs with RAMPs and validate polyclonal Human Protein Atlas antibodies against 19 of the receptors tested. A subset of GPCR-RAMP interactions that were detected using the SBA assay were verified in cell membranes using the semi-quantitative in situ proximity ligation assay (PLA). The PLA indicates a range of interactions that is consistent with the results of the SBA assay. In total, we found GPCR-RAMP interactions for the majority of the 23 GPCRs tested, suggesting that GPCR-RAMP interactions are more common than previously appreciated. Our SBA approach to proteomics analysis of membrane protein interactions can be scaled up to elucidate the entire GPCR-RAMP interactome. Since RAMPs may regulate GPCR pharmacology and biology, characterization of GPCR-RAMP interactions will provide essential information to advance GPCR-targeted drug development.

39. Mattheisen, Jordan

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

Targeting cryptic drug-binding pockets in GPCRs using genetic code expansion technology

G protein-coupled receptors (GPCRs) are heptahelical transmembrane receptors that bind to ligands and activate cellular signaling pathways. Endogenous ligands bind to orthosteric sites, but drugs and other ligands can bind to allosteric sites as well. Some allosteric-binding sites are “cryptic” which are often conformation-dependent and difficult to identify. Targeting cryptic binding sites in drug discovery using traditional high-throughput screening and GPCR cell-based signaling assays is challenging. We sought to develop a robust approach to facilitate the discovery of GPCR allosteric ligands in live cells. We hypothesized that covalent tethering of low-affinity drug fragments adjacent to allosteric sites in GPCRs would enable fragment-based drug screening. Using CCR5 (human chemokine receptor 5) and its allosteric modulator maraviroc as a model system, we employed genetic code expansion technology to site-specifically introduce unnatural amino acids as functional handles in the receptor at sites of interest based on crystal structures and molecular dynamics (MD) simulations. In parallel, we designed and synthesized a library of bivalent maraviroc analogues containing a reactive moiety capable of undergoing bioorthogonal reactions, and subsequently tested two different bioorthogonal chemistries and several different reactive substrates. Functional assays in live cells, including calcium flux monitoring, were used to screen for affinity enhancement of the analogues due to covalent tethering at engineered unnatural amino acids. Several analogues displayed an increase in potency for engineered CCR5 constructs. Modulation of the affinity of the drug-receptor interaction demonstrates the ability of tethering to enhance the pharmacology of low affinity drug fragments. We plan to expand the approach to other GPCRs with the aim of identifying site-specific covalent modulators to enable high-throughput drug screens of novel ligands that target cryptic binding pockets.

40. Ou, Arnold, PhD

Postdoctoral Fellow, The Rockefeller University
Lab: Viviana Risca, PhD

The development of protein-targetable DNA cleavage probes to investigate chromatin structure and compaction

The nucleosome-nucleosome interactions which govern the accessibility of chromatin to transcriptional machinery are of importance to study, as they play a large role in gene regulation in healthy and diseased cells. Although accessibility can be measured directly by ATAC-seq, MNase-seq and new methyltransferase-based methods, structural insight into the mechanisms by which nucleosomes regulate accessibility requires probing DNA folding directly. Radiation-induced correlated cleavage with sequencing (RICC-seq) and micro-C (a modified chromosome conformation capture using micrococcal nuclease) generate DNA fragments that are then sequenced to infer 3D chromatin structure with the aid of modelling. Both methods require large cell numbers and billions of sequencing reads to obtain enough coverage and depth across the genome.

We envisioned that using protein targetable probes with iron/copper complexes, we can produce hydroxyl radicals via a Fenton reaction in proximity to nucleosomes which could then produce spatially correlated cleavage that reports on how DNA is locally folded in chromatin by variable nucleosome-nucleosome interactions. Localizing the probe to a histone or another chromatin binding protein will not only increase cleavage events but also allow us to probe specific protein interactions or influences on chromatin structure or compaction. To achieve this, we developed cell lines with H2B-HaloTag and macroH2A-HaloTag fusion protein knock-in, and modified HaloTag ligands bearing iron/copper complexes. Preliminary results shows that the probes can cleave DNA to give nucleosome sized fragments, however further work is required.

41. Palate, Kleo

Graduate Student, University of York
Lab: Will Unsworth, PhD

Expanding the Scope of Successive Ring Expansion

Most cyclic compounds are synthesized via the cyclization of a linear precursor, typically carried out towards or at the end of a synthesis. However, this approach often requires high dilution conditions to avoid undesired intermolecular reactions leading to dimer or oligomer formation. Successive Ring Expansion (SuRE) chemistry developed in the Unsworth group can be utilized to 'grow' macrocyclic lactams or β -keto esters via sequential ring enlargement reactions.

Ring expansion reactions occur over two sequential steps. A cyclic molecule is first acylated with an amino, hydroxy, or thio acid derivative. Following this, a spontaneous ring expansion reaction proceeds via a transient bicyclic intermediate. Crucially, this process yields a product with the same functionality as the starting material, allowing the product of the reaction to undergo iterative ring expansions.

My works expands the scope of successive ring expansion chemistry through three different approaches with a common aim to introduce new functionality into medium sized rings and macrocycles in order to be explored as novel scaffolds in medicinal chemistry. A variety of N-Fmoc N-alkylated amino acids were synthesized and implemented in ring expansions to access functionalized medium sized rings and macrocycles with a peptoid like structure.

Chem. Eur. J. 2020, 26, 12674–12683

SuRE has recently been demonstrated to be compatible with thiol-tethered carboxylic acids, with novel thioester containing macrocycles accessible for the first time to synthesize a variety of thiolactones.

Org. Biomol. Chem. 2021, 19, 1404–1411

Finally, an exciting new cascade reaction approach to SuRE has allowed the synthesis of functionalized medium sized rings directly from primary amines in just two steps.

42. Prescott, Nicholas

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

Reconstitution of the Hepatitis B Virus minichromosome reveals a novel mechanism for viral transcription

Chronic Hepatitis B Virus (HBV) infection is a global public health threat responsible for almost half of all cases of hepatocellular carcinoma. The key replicative intermediate and transcriptional template of HBV is a viral minichromosome named after the covalently closed circular (ccc) DNA structure of the genome. While existing antiviral treatments prevent viral proliferation, an inability to target cccDNA allows the minichromosome to persist and support chronic infection. Host histones populate cccDNA, and studies have shown they are heavily enriched with activating post-translational modifications (PTMs) compared to bulk host chromatin. Moreover, the primary host effector protein encoded by HBV, Hepatitis B protein X (HBx), has been shown to degrade the Smc5/6 complex, which otherwise silences cccDNA transcription. These data lead to a somewhat paradoxical relationship in which HBx is required for viral transcription, but cccDNA must be transcribed in order to generate HBx in newly-infected cells. In order to dissect this paradoxical relationship, I have developed a platform to reconstitute the minichromosome *in vitro*. This has opened the door for biophysical and biochemical interrogations of the system, which I have used to characterize the compaction state, nucleosome phasing, and transcriptional output of cccDNA in the presence and absence of histones and host and viral effectors. Unexpectedly, *in vitro* transcription of naked or chromatinized cccDNA revealed a dependence on chromatinization for the generation of viral RNA encoding HBx, but not other viral transcripts. This data, together with recently reported insights into the establishment of cccDNA, suggest a model whereby concurrent histone incorporation and transcription enable HBx expression prior to Smc5/6-mediated viral silencing.

43. Ray, Devin

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

Harnessing Split-Inteins as a Tool for the Selective Modification of Membrane Proteins in Cells

Membrane proteins are often not suitable for most in vitro assays due to their structure and function being strongly connected to the fold, integration, and location in the plasma bilayer. Intein technology represents an innovative way to study membrane proteins in their native environment. Inteins are a family of proteins that catalyze their own excision from a flanking peptide sequence. A subset of these inteins are expressed as a split pair, which can be manipulated to site-specifically modify proteins in a traceless manner. I have adapted these split-inteins towards modifying the extracellular terminals of membrane proteins with small chemical probes to allow for study of these proteins in live cells. Taking advantage of the site specificity this methodology allows, I will also use split-inteins to introduce post-translational modifications. Finally, instead of small molecule probes, I will utilize this method to incorporate whole protein domains by splicing on an scFv domain to a CAR protein, reconstituting the entire receptor to restore functionality that will ultimately allow temporal control of CAR-T cell activation.

44. Thakur, Kalyani

Graduate Student, Indian Institute of Science Education and Research, Bhopal
Lab: Vishal Rai, PhD

LDM® platform: precise and modular engineering of lysine in native proteins

The precise modification of native proteins plays a crucial role in regulating their function.¹ Such a toolbox becomes essential for meeting diverse technological demands at the interface of chemistry, biology, and medicine. In this perspective, we have developed a linchpin-directed modification or LDM® platform for chemoselective, site-selective, and modular engineering of native proteins, enzymes, and antibodies.^{2,3} The synchronized display of attributes between three key components enables the LDM reagent to achieve precision. In one such example, the *o*-hydroxybenzaldehyde reacts with surface accessible lysine and primary amine to form a linchpin capable of on-demand reversibility. The rapid and intermolecular linchpin formation is chemoselective. Subsequently, the LDM reagent offers an activated ester to react with a proximal lysine to form a stable amide bond. This intramolecular second step is also chemoselective and offers the site-selectivity and thus single-site labeling of a protein. Besides, this functional group is tuned to ensure no intermolecular non-selective background reaction. Further, the spacer design connecting the two functional groups also contributes to the regulation of site-selectivity while offering modularity to switch the bioconjugation site. In other words, the spacer design allows the modification of different sites within the same protein. After demonstrating precise labeling of a structurally diverse set of native proteins, we used the *o*-hydroxybenzaldehyde handle for late-stage installation of probes (NMR, affinity, fluorophore). Moreover, the method renders single-site tagged analytically pure protein. The method provides an excellent platform for synthesizing homogeneous antibody-fluorophore or antibody-drug conjugates (AFCs and ADCs). For example, LDMK-K ADC outperforms Kadcyra and exhibits highly selective anti-proliferative activity towards HER-2 expressing SKBR-3 breast cancer cells.

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[3] LDM for Lys modification: Adusumalli, S. R.; Rawale, D. G.; Thakur, K.; Purushottam, L.; Reddy, N. C.; Kalra, N.; Shukla, S.; Rai, V. *Angew. Chem. Int. Ed.* 2020, 59, 10332. (Video abstract)

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A Chemical Proteomics Approach to Reveal Direct and Context-Dependent VCP Substrates in Living Cells

Valosin-containing protein (VCP/p97) belongs to the AAA (ATPases associated with diverse cellular activities) family of enzymes and plays an essential role in proteostasis pathways by extracting ubiquitinated proteins from macromolecular complexes. Through interaction with a series of cofactors, ubiquitinated substrates are recruited to the VCP homoheptamer where they are unfolded and threaded through the central pore. Previous studies have used biochemical and proteomic methods to identify novel VCP cofactors and substrates, however, direct VCP protein-protein interactions have not been characterized in a cellular context due to their dynamic and transient nature. Here, we plan to employ an approach relying on photo-crosslinking, amber suppression, and quantitative proteomics to capture and identify VCP substrates in living cells. This work will likely reveal a collection of direct VCP-substrate interactions, a subset of which will be validated through functional studies. Furthermore, this approach will be used to characterize context-dependent VCP substrates (i) during interphase and mitosis and (ii) upon incorporation of a disease-associated VCP mutation. Evaluation of VCP substrates in these contexts is likely to reveal interactions important for VCP function in cell division and proteostasis.