Tri-Institutional PhD Program
Chemical Biology

16TH ANNUAL TRI-INSTITUTIONAL CHEMICAL BIOLOGY SYMPOSIUM

Tuesday, September 1, 2020
9:00 am – 6:30 pm

POSTER ABSTRACTS

Virtual Event
Broadcast by MSK via Zoom

Memorial Sloan Kettering Cancer Center

The Rockefeller University — Science for the Benefit of Humanity

Weill Cornell Medicine

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SESSION 1

Cheekatla, Subb Rao, PhD

Research Associate at the Indian Institute of Technology Bombay
Lab: Sambasivarao Kotha, PhD

Molecular Acrobatics in Polycyclic Frames: A New Entry to Decorated D3-Trishomocubanes via Rearrangement Approach

D3-Trishomocubane (C11H14) is known to be a stable hydrocarbon with planar chiral cage structure with high symmetry. Its enhanced lipophilicity, propeller chirality combined with enormous cage size (5.5 Å) and rigid architecture makes it a worthwhile scaffold for further investigation. Several trishomocubane derivatives are found to be exhibit improved pharmacokinetic properties as drugs and they are also useful substrates for organocatalysis. Here in, an efficient and new synthetic route to generate D3-trishomocubanones as well as oxa- D3-trishomocubane framework has been established by acid-promoted rearrangement in the presence of BF3.OEt2, Zn/AcOH, and fuming HNO3 using cage [4.3.2] and [4.4.2]propellane systems.

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Chowdhury, Farhan

Research Associate at Memorial Sloan Kettering
Lab: Derek Tan, PhD

Total synthesis of a diisonitrile metallophore natural product from Mycobacterium tuberculosis

Chalkophores are bacterial natural products that bind and transport copper from extracellular space into the bacteria. Recently, diisonitrile natural products have been identified as a new class of putative chalkophores. Herein, we report the modular total synthesis of a recently discovered diisonitrile metallophore from Mycobacterium tuberculosis.

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Ciftci, Didar

TPCB Graduate Student at Weill Cornell Medicine
Lab: Olga Boudker, PhD

Activation pathways for faster aspartate/glutamate uptake: Lessons learned from an aspartate transporter

Glutamate transporters, also known as excitatory amino acid transporters (EAATs), transport the most abundant neurotransmitter 'glutamate' across cells using electrochemical gradients of Na and K ions. Much of our knowledge on the structure and molecular mechanism of glutamate transporters come from structural and functional studies of an archaeal homolog GltPh. Here, we use single-molecule Fluorescence Resonance Energy Transfer (FRET) based TIRF microscopy imaging to follow conformational dynamic and aspartate transport in GltPh. Our results show that GltPh proteins function at rates that are orders of magnitude different from each other. Transport cycle has two rate-limiting steps; large conformational changes from outward to inward facing conformation and substrate release. Both of these steps can be independently enhanced by mutations at critical sites to have up to 30 fold higher transport rates.

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Hebert, Jake

TPCB Graduate Student at 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 histone ubiquitination, along with their relationships to human disease, remains limited. Here, we identify the SWI/SNF related tumor suppressor SMARCA3 as a 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 levels of H3K9me3 and an altered genomic distribution of this mark, leading to a cancer-associated 'rewiring' of the transcriptional program. Using both cell lines and patient-derived organoids, we demonstrate that colorectal cancer tumors driven by downregulation of SMARCA3 display similar decreases in H3K9me3, as well as alterations in the expression of cancer-promoting genes. Taken together, our data indicate a critical role for SMARCA3 in maintaining chromatin integrity and provide a mechanism through which loss of this protein in its normal tumor-suppressor function may drive colorectal cancer.

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Hou, Qian

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

Control eukaryotic mRNA translation with small-molecule acyclovir

Regulating protein expression swiftly and reversibly is not only invaluable for biomedical research but also for therapeutic purposes. Regulatory systems such as the endogenous steroid receptor derived systems and the tetracycline repressor protein derived systems operate on transcriptional level. These systems are difficult to engineer, have prolonged lag time after induction, and are prone to triggering immune response. To overcome the limitations, we aim to develop a translational regulator based on encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES). The inactive form of IRES turns off translation of the target gene completely. When small molecule drug acyclovir is introduced, IRES-dependent translation turns back on. 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. Using the tool we are developing, translational regulation is likely to have better temporal control comparing to induced response through mRNA transcription. 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 translation regulation field.

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Jones, Natalie

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

Chemical probe development for katanin, a microtubule remodeling AAA protein

AAA (ATPases associated with diverse cellular activities) proteins have been generally well studied biochemically and structurally, but many of their cellular functions remain difficult to dissect. Chemical probes are useful tools for studying these proteins in their native environments, but selective inhibitors for only a few AAA proteins have been reported. The high degree of structural and sequence conservation within the AAA ATP-binding site has made the design of selective inhibitors particularly challenging. Here, we employ RADD (resistance analysis during design), an approach that combines computational modeling with analyses of inhibitor activity against alleles with biochemically silent mutations, to develop a model for how a heterocycle-based small molecule binds katanin, a AAA protein that regulates microtubule organization. This model guided the design of a potent and selective inhibitor for a sensitized katanin mutant allele. We demonstrate the cellular activity of this probe by recapitulating a previously observed katanin knockdown phenotype with an engineered cell line. Our results suggest the broader utility of RADD paired with chemical genetics as an inhibitor design approach that may be generalizable to other AAA proteins.

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Lee, Jonghan

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

Targetable micropharmacies: cells that produce cytotoxic small molecule drugs in situ

Current chimeric antigen receptor-T cell (CAR-T) therapy has been limited by short durations of response, frequent relapse from cancer antigen loss, and the general resistance of solid tumors. These limitations have led to efforts to enhance CAR T cell activity, by equipping them with potentiating biologic agents. In distinction, we now describe an alternative CAR-T cell technology that creates for the first time, small molecule drugs in situ at the tumor site. Such drugs are not affected by the immunosuppressive tumor microenvironment nor antigen loss, and do not require direct engagement of the T cell with the cancer cell. These Synthetic Enzyme-Armed Killer (SEAKER) cells are genetically encoded with prodrug-unmasking enzyme. SEAKER cell expansion at the tumor results in high local concentration of enzyme. After systemic administration of the inert prodrug, SEAKER cells unmask high concentrations of chemotherapeutic drug, which can diffuse into tumors and killing cells, regardless of antigen expression. We provide two SEAKER enzyme systems and several prodrug classes, effectively creating a modular therapeutic platform for a variety of cancers or other diseases.

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Luo, Joanna

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

Mechanism of gamma-secretase modulators

Amyloid-beta (Aβ) plaques are believed to be integral to Alzheimer's disease (AD) pathogenesis through their role in the "amyloid cascade hypothesis," in which the accumulation of Aβ peptides initiates a cascade of pathological events leading to neurodegeneration and AD. γ-Secretase is a transmembrane aspartyl protease which cleaves APP to generate Aβ peptides, making γ-secretase an attractive drug target. However, inhibitors failed in clinical trials due to their unwanted side effects on other γ-secretase substrates such as Notch. ɣ-Secretase modulators (GSMs) selectively reduce levels of the pathogenic Aβ species without affecting Notch and overall APP processing. However, the precise mechanism of action of GSMs is still unclear. From cross-competition studies using photoaffinity labeling, we have reported that active site-directed inhibitor L-686, 458 enhances the labeling of GSM photoprobe E2012-BPyne to presenilin-1, the catalytic subunit of γ-secretase. Our goal is to determine their exact peptide interactions and elucidate the mechanism of action for GSMs. We strategized several photoafffinity labeling approaches to achieve the most robust labeling of the target protein. We demonstrated that E2012-BPyne can be conjugated to the hydrazine-labile Dde linker, which can be cleaved to efficiently release the photolabeled and affinity-captured presenilin-1. Currently we are progressing towards a large-scale photolabeling experiment to pull down samples for LC-MS/MS. From there we aim identify the insertion site of the photoprobe and model the probe binding site. Precise mapping of the precise binding site will clarify the molecular mechanism of GSMs, leading to a better understanding of their modulation for AD drug development.

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Mei, Lin

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

Molecular Mechanism of direct actin force sensing by α-catenin

Cells in the body must perceive and appropriately respond to mechanical stimuli in their local microenvironments during development and to maintain homeostasis. Dysfunction of mechanical signal transduction (”mechanotransduction”) pathways is correspondingly implicated in a wide variety of human diseases, including metastatic cancer, muscular dystrophy and cardiomyopathy, yet therapeutics targeting these pathways are largely absent due to the lack of knowledge of the underlying molecular mechanisms. The actin cytoskeleton plays a central role in mechanotransduction through the regulated interactions between actin filaments (F-actin) and more than 150 actin-binding proteins (ABPs). Research at the cellular and tissue levels have implicated mechanical regulation of actin-ABP interactions in mechanotransduction, but how force regulates critical actin-ABP interactions at the molecular level is largely unknown. Here, using a novel myosin-motor based TIRF reconstitution assay, we show that a pair of homologous ABPs which are essential for cell-cell and cell-matrix adhesion, α-catenin and vinculin, have distinct mechanosensitive F-actin binding. The actin-binding domain (ABD) of α-catenin preferentially engages F-actin in the presence of mechanical load across actin filaments (“mechanoaccumulation”), while vinculin’s ABD does not. Simultaneous optical trapping and confocal microscopy experiments demonstrate that a load of ~1 pN across single filaments activates α-catenin ABD binding. Atomic resolution cryo-EM structures of the metavinculin ABD-F-actin (2.9 Å) and α-catenin ABD-F-actin (3.2 Å) complexes demonstrate both ABDs undergo major conformational changes upon actin engagement, prominently at their N- and C-termini, and their C-terminal regions differentially refold to bind distinct sites on the filament surface. A C-terminal truncation of α-catenin’s ABD constitutively binds F-actin regardless of force, and a chimeric protein of vinculin’s ABD featuring α-catenin’s flexible termini gains mechanoaccumulation activity, suggesting the α-catenin C-terminus-F-actin interaction is necessary and sufficient for mechanically regulated binding. This work, for the first time, establishes a force-regulated actin-binding mechanism in structural detail, and lays the groundwork for the rational design of therapeutics targeting cytoskeletal mechanotransduction pathways.

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Panagoda, Navanjalee

Graduate Student at Stony Brook University
Lab: Nicole Sampson, PhD

Elucidating the Role of the Transcriptional Regulator Mce3R and mel2 Encoded Genes in Mycobacterium tuberculosis Drug Resistance.

Mycobacterium tuberculosis (Mtb) which causes Tuberculosis (TB) is a major worldwide threat due to the high prevalence of infection and the emerging burden of multi-drug resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB). Developing new drugs and discovering new drug targets will play a key role in controlling TB infection. We are investigating a TetR like repressor Mce3R (Rv1963c). The Mce3R regulon comprises three operons: EchA13/FadE17/FadE18 (Rv1933c-Rv1935c), Mel2 (Rv1936-Rv1941), and Mce3 (Rv1964-Rv1971). The encoded genes are indirectly regulated by cholesterol which is the main carbon and energy source of Mtb. The focus of this study is the elucidation of the structure and function of the Mce3R repressor and Mel2-encoded genes. Previous studies have shown that Mce3R is self-regulated and not required for Mtb replication in macrophages, whereas the mel2 genes are important for the survival of Mtb in the presence of Reactive Oxygen Species (ROS). We have demonstrated using an electrophoretic mobility gel shift assay (EMSA) that Mce3R binding motifs are present in both intergenic regions of mce3R-yrbE3A and echA13(Rv1935c)-Rv1936. MelK (Rv1941), which is one of the six gene products encoded in the Mel2 operon belongs to the short-chain type dehydrogenase/reductase family. Sequence alignments and thermal shift binding assays (TSA) demonstrated that MelK preferentially binds NADH compared to NADPH. Experiments to determine the structure of Mce3R and the identity of the MelK substrate function are in progress.

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Payne, Alexander

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

Beyond High Resolution: Mining Cryo-EM Datasets for Functional Conformational Heterogeneity

Advances in cryo-EM single particle analysis have enabled the visualization of protein structures in physiological conditions at atomic resolution. Most pipelines for doing this involve performing 2D and 3D classifications to select a stack of homogenous particles which can be aligned to high resolution. However, in contrast to X-ray crystallography, cryo-EM has the potential to reveal a continuity of structures whose relative probabilities follow a Boltzmann distribution. Several existing methods approach this goal, namely manifold embedding, cryoSPARC's 3D Variability Analysis, and cryoDRGN. The application of such algorithms to dynamic proteins hints at a future of high-resolution dynamic structural biology, in which the precise details of structure, dynamics, and function are integrated.

A framework for integrating these kinds of dynamic structural data is naturally found in the field of molecular dynamics, which must compress the complexity of the dynamic motions of thousands to millions of particles into interpretable reaction coordinates. Several attempts have already been made to incorporate cryo-EM density maps into molecular dynamics simulations, generally by using them to generate a guiding force field. Here, I will outline a method in which a set of discrete conformational states from a cryo-EM dataset are used to seed massively parallel molecular dynamics simulations on Folding@Home. These simulations are analyzed via Markov State Modeling, in which the time-evolution of a series of discrete structural states is determined. This framework is amenable to integration with alternative experimental approaches, making it a powerful way to connect structure, dynamics, and function.

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Repeta, Lucas

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

MTREC: Exploring a Unique Exosome Cofactor That Determines RNA Fates

Cells have evolved sophisticated systems for gene regulation that exert dynamic control over RNA expression and stability. Advances in transcriptomics have revealed an enigmatic class of eukaryotic RNAs arising from inter- and intragenic regions. These 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.

Mtr4 helicase containing complexes are required for targeting and processing of RNA substrates for degradation by the exosome. The discovery of an Mt4-like protein (Mtl1) containing complex, MTREC, in Schizosaccharomyces pombe has the potential to redefine the exosome's role in the cell. MTREC is reported to regulate cryptic transcripts and its interaction with the exosome appears to be essential for the controlling cell cycle progression and heterochromatin formation. How MTREC's targeting of cryptic transcripts relates to cell cycle control and chromatin remodeling, and whether similar pathways exist in other organisms remains unknown.

This study proposes to reconstitute the MTREC complex in vitro. Helicase activity assays will establish kinetic and thermodynamic parameters for describing the activities of Mtl1 and MTREC. These data, combined with structural characterization by cryo-EM and X-ray crystallography can be used to interrogate specific functions of MTREC subunits. Together, the proposed experiments will establish a foundation to better understand functions for MTREC and the exosome in gene regulation.

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Strauss, Alexa

TPCB Graduate Student at Weill Cornell Medicine
Lab: Joshua Levitz

Domain Coupling in Metabotropic Glutamate Receptors

Metabotropic glutamate receptors (mGluRs) are synaptic G-protein coupled receptors (GPCRs), which respond to the excitatory neurotransmitter glutamate. They belong to the class C family of GPCRs, which is 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. While recent structural characterization has helped to define the overall architecture of this multi-domain receptor, the mechanism by which agonist binding in the LBD is coupled to activation of the TMD is unknown. Two possible mechanisms have been proposed. In the “rigid pulling” mechanism, the LBD exerts a mechanical force on the TMD to cause its rearrangement and subsequent activation. In the “interdomain interaction” mechanism, intramolecular interactions between residues in the CRD and in the extracellular loop 2 of the TMD are required for activation. To probe the contribution of these two mechanisms, we have developed a series of mGluR constructs with targeted deletions or mutations to perturb the proposed coupling interactions. The effects of these constructs are assessed through functional assays, including electrophysiology, and measures of conformational dynamics, such as intersubunit FRET. Taken together, these data support a mechanism in which the LBD exerts mechanical force on the TMD to activate it, with specific interdomain interactions serving as a secondary, modulatory form of coupling.

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SESSION 2

Barman, Dipti

Research Associate at Memorial Sloan Kettering
Lab: Derek Tan, PhD

AMSN library development for compound accumulation in gram-negative bacteria

The antibiotic resistance is a growing public health crisis. The CDC predicts every year 2 million Americans contract drug-resistant bacterial infections which causes $10-20 billion cost to US healthcare industry. Gram(-) bacteria causes a wide spectrum of disease that includes antibiotic resistance. Gram(-) bacteria has an inner membrane of phospholipid, a thin peptidoglycan layer, plus an outer membrane. The outer membrane is an asymmetric bilayer of glycerol phospholipids and lipopolysaccharide (LPS) LPS and phospholipids and that makes the wall rigid hence slow the passive diffusion of hydrophobic compounds and narrow pores limit by size the penetration of drugs. In our SPEAR-GN project, (Small-Molecule Penetration & Efflux in Antibiotic-Resistant Gram-Negatives bacteria) we are looking for a correlation between chemical structure and bacterial permeability. Our lab has an interest on sulfonyladenosine (AMS) compounds, we are looking for a synthesis of unique AMS derivative to do accumulation study. The chemo informatics analysis of these type of the compounds derivatized from structure activity relationship (SAR) data will predict the permeability and efflux sensitivity of small molecules in pathogenic bacteria. That will give us an idea of developing predictive tools to accelerate antibiotic drug discovery to tackle the growing threat.

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Carl, Ayala

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

Mechanically Regulated Structural States of F-Actin

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.

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Chen, Chen

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

Real-time, In Vivo Monitoring of Pharmacodynamics of Autophagy Activators Using Organic Color Centers

Regulations of endolysosomal pH play an important role in protein degradation pathways and cellular homeostasis. The ability to continuously monitor lysosomal pH is beneficial to understand many disease progressions, respond to current treatment, and optimize the experimental therapeutics. However, it is challenging to quantify lysosomal pH in biological systems where high sensitivity, selectivity, and spatial resolution (sub-micrometers) are simultaneously required for in vivo measurements. Covalent attachment of an aminoaryl functional group to a single-walled carbon nanotube produces an sp3 defect, or "organic color center" (OCC) that fluoresces brightly in the near-infrared. Benefit from the unique electronic and optical properties of OCC, we develop an optical reporter of endolysosomal pH whose fluorescence wavelength sensitively responds to local pH. We utilize the optical reporter to real-time monitor the endolysosomal pH upon the treatment of the small molecules that disrupt autophagic flux and lysosomal physiology. We aim to apply our nanosensors to investigate the pharmacodynamics and pharmacokinetics of the small molecule drugs in vivo.

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Corless, Broderick

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

epoxySEAKER and onSEAKER: CAR T-CELL SYSTEMS FOR TARGETED LOCAL BIOSYNTHESIS OF THERAPEUTIC WARHEADS

Our labs have developed novel SEAKER (Synthetic Enzyme-Armed KillER) CAR T-cells that express enzymes that cleave masking groups from systemically administered non-toxic prodrugs. The localization of SEAKER cells to tumors allows for specific conversion of the prodrug to anticancer agent at the site of interest. These SEAKER cells overcome limitations of CAR T-cell therapy such as T-cell exhaustion, immunosuppression and antigen variance by creating a cascade effect through proliferation of T-cells, constitutive secretion of enzymes and catalytic generation of the active drugs. The enzymes continue to produce cytotoxic compounds at the tumor site even after the T-cells functionally exhaust. We will engineer this system to produce novel “epoxySEAKER” and “onSEAKER” cells that express bacterial biosynthetic enzymes and convert non-toxic prodrug substrates into cytotoxic compounds through an enzyme-mediated therapeutic warhead installation. Limiting the bulkiness of the prodrug by eliminating the need for masking moieties we expect greater pharmacological properties of the prodrugs. Installing the warhead moiety onto the prodrug rather than cleaving a masking group will eliminate the non-specific activation of cytotoxic agents and reduce the on-target, off-site toxicity associated with the target compounds.

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Freage, Lina

Postdoctoral Fellow at The City University of New York
Lab: Prabodhika Mallikaratchy, PhD

Homodimeric variant of an aptamer generated from LIGS activates TCR-CD3ε complex

Nucleic acid aptamers represent a broad class of nucleic acids that reveal specific binding properties towards various ligands, including low molecular-weight substrates as well as macromolecules. Through binding specific classes of molecules, aptamers can be targeted towards cells displaying such substrates of interest on their surface. We recently introduced an aptamer named ZUCH-1 against the target Tcell receptor-cluster of differentiation three epsilon (TCR-CD3ε), expressed on human T cells utilizing the method called Ligand Guided Selection (LIGS). The aptamer ZUCH-1 showed high affinity and specificity towards the desired target, T-cell receptor Complex-CD3ε. In our current work, herein we report systematic truncation followed by modification utilizing synthetic nucleic acids, notably locked nucleic acid (LNA) and a 2'OMe RNA base in anti-TCR-CD3ε to improve the aptamer's affinity without compromising specificity.

Furthermore, dimerization of the modified aptamer showed higher avidity, and the observed avidity is comparable to corresponding monoclonal antibody. Functional studies using dimeric anti-TCR-CD3ε aptamers against TCR-CD3 expressed in cultured cells demonstrated that dimeric variants can activate TCR-CD3 and this activation is comparable to its corresponding monoclonal antibody. Thus, we will introduce a first-of-a-kind aptamer-based TCR-CD3ε activator that provides a synthetic tool to investigate TCR-CD3 mediated immunological mechanisms and synthetic immunotherapeutic development.

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Garst, Emma

TPCB Graduate Student at The Rockefeller University
Lab: Howard Hang, PhD

Site-specific lipidation enhances IFITM3 membrane interactions and antiviral activity

Interferon-induced membrane proteins (IFITMs) are S-palmitoylated proteins in vertebrates that restrict a diverse range of viruses including influenza, Dengue, Ebola, Zika and coronaviruses. IFITM3 in particular directly engages incoming virus particles, prevents virus entry and accelerates pathogen clearance in host cells. However, the precise mechanisms of action for IFITM-mediated restriction are still unclear. To further investigate how site-specific S-palmitoylation controls IFITM3 antiviral activity, here we employed chemical strategies to install stable S-palmitoylation analogs in vitro and in mammalian cells to investigate the impact of site-specific lipidation on IFITM3 structure and activity. Using chemical and computational approaches, we show that site-specific lipidation of IFITM3 at highly conserved Cys72 modulates its conformation and interaction with lipid membranes as well as enhances the antiviral activity of IFITM3 in mammalian cells. Together, these data suggest that site-specific S-palmitoylation of IFITM3 can directly alter its biophysical properties to prevent virus infection.

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Gunawardhana, Nipuni

Graduate Student at Stony Brook University
Lab: Scott Laughlin, PhD

Astrocyte specific delivery of methyl-pyridinium tagged small-molecule cargo

Neurons were the big players in neuroscience research until recent efforts identified that astrocytes too are active players in brain function. Astrocytes are the most abundant type of non-neuronal cells in the brain. In addition to maintaining blood-brain barrier integrity and providing nutrients to the surrounding cells, astrocytes form a specialized structure with pre- and post-synaptic neurons known as the tripartite synapse where they take part in the uptake and release of neurotransmitters. Astrocytes have been implicated in many neurological diseases. Accordingly, to understand how the brain circuitry functions it is essential to study astrocytes. However, only a limited number of strategies are available for astrocyte visualization and manipulation. These existing methods lack specificity and versatility. A new class of methyl-pyridinium tagged fluorescent markers has been developed in our lab that shows specific targeting and visualization of astrocytes. The positive charge of the methyl-pyridinium targeting moiety of this probe allows active transport of diverse cargo such as Ca2+ sensors, small molecule drugs and transcriptional activators to astrocytes through the astrocyte resident Organic Cation Transporter (OCT). I am currently synthesizing a methyl-pyridinium tagged cyanodoxycycline to activate transcription of specific genes in astrocytes using the tetracycline-inducible gene expression (Tet-On) system. My work is also focused on the drug tamoxifen to create a traceless astrocyte delivery strategy. These molecules will help us to evaluate the ability of the targeting moiety to deliver drugs specifically to astrocytes. This strategy will be beneficial for exploring the therapeutic aspects of astrocytes in various neurological diseases and will improve our understanding of the functions of astrocytes in the brain.

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Jiang, Ting

Graduate Student at Stony Brook University
Lab: Scott Laughlin, PhD

Modular Enzyme- and Light- Activatable Cyclopropene-Tetrazine Ligation for Spatiotemporal Imaging of Biological Systems

Bioorthogonal chemistry has been optimized to provide a new scenario for imaging and perturbing the function of biomolecules like lipids, proteins and nucleic acids in cells and organisms. Generally, these optimizations have focused on developing new bioorthogonal pairs and improving reaction rates. Less well explored are reactions that permit control when and where the reactions occur. Here we report our newly developed 3-N spirocyclopene, which can be modularly caged via diverse enzyme-labile or photolabile protecting groups. These caged cyclopropenes are activated to react with tetrazine upon the removal of the bulky groups by uncaging enzymes or UV/visible illumination. Here we are glad to present synthesis of the novel cyclopropene scaffold, a panel of diverse photo-caged and enzyme-caged cyclopropenes, efficiency of uncaging, and analysis of reactivity between the caged and liberated cyclopropenes, and further the applications in diverse biological imaging. We expect this to be a powerful tool to control cyclopropene-tetrazine biorthogonal ligation in living mammalian cells and organisms.

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Liu, Jiacheng

Graduate Student at Weill Cornell Medicine
Lab: Bjorn Kafsack, PhD

Identifying targets of histone reader domains in malaria parasites

Histone post-translational modifications (PTMs) play a central role in controlling chromatin accessibility, DNA-protein interaction and gene expression. Histone modification writers and erasers add or remove PTMs, in order to change or maintain chromatin states. However, the recognition of these marks by proteins with histone reader domains is what gives these marks biological meaning by recruiting other modifiers, remodelers, or transcription complexes. Most of our existing understanding of histone reader specificity is derived from higher eukaryotes that diverged about 1 billion years ago from malaria parasites. Understanding the epigenetics regulation of Plasmodium cycles will greatly advance the prevention of disease transmission. As a proof-of-principle experiment, I have selected a subset of potential histone lysine methylation reader domains for analysis, including PHD, Chromo, and Zn-CW domains, with human and malaria parasite heterochromatin protein 1 (HP1) serving as positive controls. Candidate reader domains were expressed in E. coli and well-folded reader domains were then assayed for binding against a panel of single modified nucleosome covering the possible methylation states of lysine residues on histones H3 & H4 using the dCypher assay based on AlphaScreen technology (Epicypher / Perkin-Elmer). We hope to identify the binding between histone modifications and reader domains, and proceed to further characterize histone modification functions in a biological context.

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Rufa, Dominic

TPCB Graduate Student at Memorial Sloan Kettering
Lab: John Chodera

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—especially torsions—can be modeled, as poor modeling of torsional profiles and coupling with other valence degrees of freedom 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).

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Tsukidate, Taku

TPCB Graduate Student at The Rockefeller University
Lab: Howard Hang, PhD

Nuclear receptor chemical reporter enables domain-specific analysis of ligands in mammalian cells

The characterization of specific metabolite–protein interactions is important in chemical biology and drug discovery. For example, nuclear receptors (NRs) are a family of ligand-activated transcription factors that regulate diverse physiological processes in animals and are key targets for therapeutic development. However, the identification and characterization of physiological ligands for many NRs remains challenging due to limitations in domain-specific analysis of ligand binding in cells. To address these limitations, we developed a domain-specific covalent chemical reporter for peroxisome proliferator–activated receptors (PPARs) and demonstrated its utility to screen and characterize the potency of candidate NR ligands in live cells. These studies demonstrate that targeted and domain-specific chemical reporters provide excellent tools to evaluate endogenous and exogenous (diet, microbiota, therapeutics) ligands of PPARs in mammalian cells as well as additional protein targets for further investigation.

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Williams, Nicole

Graduate Student at the City University of New York
Lab: Prabodhika Mallikaratchy, PhD

Multi target Ligand-Guided Selection (LIGS) to generate aptamers against B-cell biomarkers

Nucleic Acid Aptamers (NAAs), are single-stranded ribo- and deoxyribo- oligonucleotide molecules that fold into complex functional three-dimensional structures. These three-dimensional structures can bind to target molecules with high affinity and specificity. Nucleic Acid Aptamers are selected through an in vitro iterative process called Systematic Evolution of Ligands by Exponential enrichment (SELEX). Here, we implemented Ligand-Guided Selection (LIGS), a variant of SELEX, to elute aptamers against multiple biomarkers expressed in B-cells, i.e., CD19 and CD20. During the LIGS step, specific aptamers against CD19 and CD20 were competitively eluted using two specific monoclonal antibodies against the same target at 25°C. The eluted LIGS libraries were then sequenced using the Illumina high-throughput (HT) DNA sequencing platform. The resulting sequences were analyzed using a novel bioinformatics workflow designed using GALAXY, an online bioinformatics server. A total of 18 candidate sequences against CD19 and 34 candidate sequences against CD20 were identified based on defined enrichment values. The potential hit sequences were subsequently synthesized and screened against positive and negative cell lines to identify aptamers specific for their respective biomarkers. This study collectively establishes LIGS as a state-of-the-art screening technology that can be used to generate highly specific aptamers against multiple receptor-proteins in their native state expressed on one cell population.

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Zinder, John

Postdoctoral Fellow at The Rockefeller University
Lab: Titia de Lange, PhD & Thomas Walz, PhD

Structure and Biochemistry of Shelterin and Associated Factors

The human shelterin complex binds telomeres, the ends of chromosomes, and prevents them from being recognized as DNA damage. Additionally, it recruits factors that aid in telomere replication, maturation, and elongation. Despite its central role in telomere biology and genome stability, little is known regarding the structure and DNA binding activities of the intact complex. To address this, we have recombinantly expressed and purified human shelterin and and subjected it to structural analysis using negative staining and cryo-electron microscopy. Extensive conformational heterogeneity within the complex has limited 3D reconstructions to low-resolution, however chemical and biochemical strategies are being employed to mitigate these issues. These studies will illuminate molecular aspects of shelterin's diverse functions and further our understanding of its dysfunction in human disease.

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SESSION 3

Bhattacharya, Appy

Graduate Student at The New York University
Lab: Jin Montclare, PhD

The engineering and characterization of engineered PET-traceable multifunctional protein drug delivery agent in cancer

Theranostic agents are being developed for their ability to diagnose various diseases and improve therapeutic delivery. Traditional drug delivery has been inefficient due to factors such as drug insolubility, tissue indiscriminate cytotoxicity, their inability to stimulate release and their lack of direct monitoring. Recently, we have engineered a protein-based theranostic agent fluorinated thermo-responsive assembled protein (F-TRAP) bearing fluorinated amino acids. F-TRAP self-assembles into micellar structures and consist of hydrophobic pores capable of encapsulating the chemotherapeutic drug molecule, doxorubicin. Moreover, F-TRAP is traceable via 19F magnetic resonance imaging (MRI). To increase the sensitivity for imaging, we are developing a positron emission tomography (PET) visible agent, 18F-TRAP. We employ residue-specific incorporation of an azide-bearing methionine analog, azidohomoalanine to give rise to clickable azide-functionalized TRAP that can be subsequently conjugated to a 18F bearing alkyne analog of boron-dipyrromethene (BODIPY) dye, imparting it an ability to be used as a dual modality PET probe.

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Burnside, Chloe

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

Structural Characterisation of Assembly Intermediates of the Yeast Mitochondrial Ribosome

Ribosomes are large macromolecular assemblies that exist within all cells and are responsible for the translation of messenger RNA into proteins. Within the mitochondria of eukaryotic cells, dedicated ribosomes (mitoribosomes) are responsible for translating a handful of proteins involved the oxidative phosphorylation pathway. The correct assembly and function of mitoribosomes within cells is crucial for mitochondrial respiration, cell viability, growth and differentiation in all eukaryotic organisms. Putative and known factors involved in the assembly and function of mitoribosomes are implicated in human disease. Although the structure and function of the mitoribosome has been well documented across species, knowledge surrounding its assembly and the factors that regulate this process is severely lacking. Here we present preliminary data showing the isolation of assembly intermediate(s) of the small subunit of the mitoribosome in Saccharomyces cerevisiae. Isolated intermediates implicate a number of novel proteins in mitochondrial ribosome assembly and provide promise for future structural studies that may elucidate the stepwise mechanisms of mitochondrial ribosome assembly.

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Cervasio, Danielle

Graduate Student at Stony Brook University
Lab: Scott Laughlin, PhD

Targeting the Brain's Astrocytes using Diverse Small Molecules

Astrocytes are glial cells that tile the entire central nervous system. Within the brain, they control metabolism, blood flow, and water flux, as well as dynamic processes like synaptic formation, maintenance, and pruning. Targeting astrocytes using gene therapies could be an attractive therapeutic option for seizure, epilepsy, Parkinson's or Alzheimer's disease, where there exists an imbalance in ion, neurotransmitter, or protein clearance—processes all modulated by astrocytes. We have recently developed chemical tags that ferry small molecule cargo into astrocytes. Here, we adapt these astrocyte targeting tags to control transcription, with the ultimate goal of using them for regulated gene therapy. Essentially, we utilize a modified transcription activator, doxycycline, to turn on the tetracycline-inducible gene expression (Tet-On) system—obtaining temporal control over transcription in astrocytes. Astrocyte specificity is attained by attaching a permanently positive astrocyte targeting moiety via organic chemistry to the small molecule drug doxycycline, which retains its Tet-On activating capabilities. Our first probe, doxycycline methyl pyridinium (doxyMP) can successfully activate transcription in primary astrocytes under the strong, mammalian cytomegalovirus (CMV) promoter, eliminating the need for a cell-specific promoter. In classical gene therapies, constitutive systems suffer from off-target effects or variable gene expression of cell-specific promoters. Using an astrocyte-targeted transcription activator, we eliminate the need for these variably expressed, cell-specific promoters, while obtaining specific and robust gene expression in astrocytes. Despite the continued development of gene therapies, there are few that utilize regulated gene expression systems. We hope to employ these molecules therapeutically where current constitutive gene therapies for Parkinson's or glioblastoma, for example, could benefit from regulated gene expression.

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Chen, Yu-Ching

Graduate Student at Stony Brook University
Lab: Nicole Sampson, PhD

The influence of melH epoxide hydrolase b in Mycobacterium tuberculosis response to oxidative stress and drug resistance

Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), is the leading bacterial cause of death in humans worldwide. TB treatment is long-term and complicated. TB control is threatened by the continued spread of drug resistance, which is often associated with failure to complete the lengthy regimen of TB chemotherapy. Therefore, drugs that kill Mtb more quickly while also preventing development of drug resistance will significantly improve TB chemotherapy. The reactive oxygen species (ROS) generated by host macrophages and antioxidant defense mechanisms-mediated by Mtb play significant roles during Mtb infection and treatment. In silico analysis identified mel genes as close homologs of lux genes involved in bioluminescence production. These genes are believed to have evolved to facilitate detoxification of oxidizing species. MelH is an enzyme in the a/b hydrolase family and catalyzes hydrolysis of epoxides. Interestingly, we found that the Mtb melH mutant is more susceptible to oxidants such as CHP and H2O2 than wild type, suggesting that Mtb melH plays a role in Mtb's defense against ROS, perhaps by detoxifying epoxide intermediates that result from ROS reaction with alkenes. Mtb can utilize different carbon sources for its survival. Interestingly, the melH mutant growth rate was impaired in minimal media containing glycerol compared to other carbon sources, indicating a potential link of melH to carbon metabolism during Mtb growth. Mtb has multiple mechanisms to modulate its lipid biosynthetic pathways, and to ensure survival within the host. Here, we demonstrate that Mtb melH contributes to withstanding ROS stress, which may be generated by the host, and is linked to Mtb primary metabolism.

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Das, Tandrila

TPCB Graduate Student at The Rockefeller University
Lab: Howard Hang, PhD

Functional analysis of S-palmitoylated IFITM3 antiviral activity and regulation

To defend against infectious agents, the human immune system expresses several host restriction factors. The interferon-induced transmembrane proteins (IFITM1, 2 and 3) inhibit infection by diverse enveloped viruses. Our lab discovered S-palmitoylation of IFITMs and demonstrated its importance for IFITM3 antiviral activity. Recently, through site-specific fluorophore tagging, live cell imaging and virus trafficking studies, we show that IFITM3 localized on endocytic vesicles fuse with incoming virus particles and accelerates shuttling of these pathogenic cargos to lysosomes. We also found trafficking to incoming virus particles and antiviral activity requires S-palmitoylation at highly conserved Cys72. However, the cellular and molecular determinants of site-specific S-palmitoylated IFITM3 trafficking to incoming viruses are unknown. Previously, it has been suggested that perturbation of cellular cholesterol homeostasis is associated with IFITM3 antiviral activity, but this model is highly contentious. However, IFITM3 cholesterol physical interaction has not been explored before. This is interesting since palmitoylation of integral membrane proteins has been shown to regulate cholesterol rich raft association. Moreover, presence of putative cholesterol binding domain in IFITM3 suggests potential cholesterol interaction. Therefore, to probe S-palmitoylated IFITM3 cholesterol interaction, we developed a bifunctional cholesterol reporter (x-alk-chol). Crosslinking in interferon stimulated HeLa cells and label-free quantitative proteomics identified IFITM3 as a cholesterol interacting protein. We further demonstrate that IFITM3 cholesterol interaction is S-palmitoylation dependent and decrease in this interaction reduces IFITM3 antiviral activity.

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Delos Reyes, Avelyn Mae

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

Use of bis-electrophilic probe to obtain snapshots of the ubiquitin cascade

Ubiquitin is a small regulatory protein comprised of 76 amino acids. Ubiquitination is a type of post-translational modification that can signal for various biological functions. Although ubiquitin is best known for its role in proteasomal degradation, ubiquitin also plays a role in DNA repair, endocytosis, gene expression, and kinase activation. Protein ubiquitination involves 3 enzymes, E1, E2, and E3. The ubiquitin cascade involves several transthioesterification steps that result in the covalent addition of ubiquitin to the protein substrate. In order to better understand the ubiquitin cascade, we have developed a bis-electrophilic probe, Gly-PDAE, to form non-hydrolyzable mimics of the E1-E2 and E2-E3 transthioesterification reactions that can be used for structural studies (Fig. 1). The large scale synthesis of Gly-PDAE became challenging at the penultimate step, the DCC coupling. Purification of the coupling reaction was problematic because the product was unstable on silica gel. Alternative purification methods and coupling reactions were screened, ultimately an optimized purification protocol for the DCC reaction was established to isolate the product in appreciable yield.

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Horioka, Mizuho

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

Biased constitutive signaling of CysLTR2-L129Q

A mutation in CYSLTR2, the gene for the G protein-coupled receptor (GPCR) cysteinyl-leukotriene receptor 2 (CysLTR2), causes uveal melanoma. We identified >100 additional CYSLTR2 missense variants of unknown significance in human cancer genomes. We characterized all variants for expression level, basal and agonist-stimulated G protein signaling, and β-arrestin recruitment. The CysLTR2-L129Q mutation causing uveal melanoma has a unique phenotype among all cancer-associated variants. It is highly constitutively active with gain-of-function in basal Gq/11-PLCβ signaling and loss-of-function in agonist-dependent signaling. Although highly constitutively active in Gq/11-PLCβ signaling, CysLTR2-L129Q only poorly recruits β-arrestin and escapes β-arrestin-mediated downregulation. We show using long-time-scale molecular dynamics simulations that CysLTR2-L129Q forms a novel hydrogen-bond network that stabilizes an active state through interactions of the mutant glutamine with a highly conserved tyrosine in helix 5. CYSLTR2 is the first known example of a GPCR oncogene with highly biased constitutive signaling and the ability to escape cellular downregulation.

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Kotliar, Ilana

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

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 RAMP-GPCR 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 semi-quantitative in situ proximity ligation assays (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. In addition, the SBA methodology can be applied to measure GPCR-GPCR heterodimer interactions in cell membranes.

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Malone, Brandon

Graduate Student at The Rockefeller University
Lab: Seth Darst, PhD

Structural Basis of Helicase-Polymerase coupling in the SARS-CoV-2 Replication-Transcription Complex

SARS-CoV-2, the causative agent of the ongoing COVID-19 pandemic, has led to a tremendous loss of life worldwide since arising in late December 2019. Ongoing and new drug discovery programs are much needed to help identify compounds that target key aspects of SARS-CoV-2 pathogenesis. SARS-CoV-2, like other Coronaviridae (CoV) family members, possesses one of the largest viral RNA genomes which is close to 30 kb in length. To successfully replicate this genome, the virus requires several replicative proteins in addition to the holoenzyme (holo) RNA dependent RNA polymerase (RdRp nsp12 with co-factors nsp7 and nsp8) which form the Replication-Transcription Complex (RTC). Structural characterization of the RTC can identify druggable protein-protein interaction interfaces. Herein, we use Cryo-EM to characterize the interaction between the nsp13 RNA helicase and the holo RdRp on an RNA substrate. The structure illustrates the basis of interactions between the holo-RdRp and the helicase. A Nidovirus-order-specific N-terminal domain of nsp13 interacts with the N-terminal 'sliding pole' of nsp8 as well as the tip of the nsp12 thumb domain. A second interaction occurs between one of the canonical RecA ATPase domains of the helicase and nsp7/nsp8. The structure places the nucleic acid-binding ATPase domains of the helicase directly in front of the replicating-transcribing holo-RdRP. These constraints allow one to propose models for how the helicase unwinding is coupled to the replication-transcription activity of the holo-RdRp that are testable by more incisive experiments going forward. The structure also identifies a new nsp12 binding pocket for anti-viral therapeutic development.

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Miller, Linamarie

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

Towards a mechanistic understanding of Dhr1 helicase activation by Utp14

Assembly of the small ribosomal subunit in eukaryotes requires the specific activity of the DEAH-helicase Dhr1. Dhr1 acts upon the small subunit (SSU) processome, an early precursor of the small subunit. Another component of the small subunit processome, Utp14, is responsible for recruiting Dhr1 to the SSU processome and for stimulating Dhr1 activity. Previously, we structurally characterized the Dhr1 core and observed the Dhr1-specific auto-inhibitory loop, which keeps the enzyme in an open conformation and prevents enzymatic catalysis by occupying the same path as substrate RNA. More recently, we have begun characterizing the interaction between Dhr1 and its co-activator Utp14 in the yeast S. cerevisiae. We have identified the minimal region of Utp14 necessary for stimulation of Dhr1 RNA unwinding. Our current efforts focus on using this minimal region to understand the mechanism of Dhr1 activation by Utp14.

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Orth, Elizabeth

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

The fate of proline in peptide catabolism.

The dipeptidases DPP8 and DPP9 (DPP8/9) and their immediate downstream peptidase prolidase (PEPD) process peptides containing a second-position proline residue from post-proteasomal peptides. Together, these enzymes liberate proline from peptides. Since the proteasome and most aminopeptidases cannot cleave Pro-Xaa bonds, we hypothesize that the DPP8/9-PEPD axis is rate-limiting for the recycling of proline from degraded proteins into the cell's cytosolic amino acid pools. To that end, we performed a variety of metabolomics and genetics experiments to determine the fate of proline in peptide catabolism.

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Prescott, Nick

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

Reconstitution and biophysical characterization of the Hepatitis B Virus minichromosome

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 composed of covalently closed circular (ccc)DNA, which supports chronic infection despite existing antiviral treatments. Host histones populate cccDNA, and studies have shown they are heavily enriched with activating post-translational modifications (PTMs) compared to bulk host chromatin. However, the ensemble of PTMs composing the chromatin landscape of cccDNA remains unclear. Moreover, the sole effector protein encoded by HBV, Hepatitis B protein X (HBx), has been shown discriminate cccDNA from host chromatin and differentially modulate their histone PTM landscapes. In order to dissect the mechanism by which HBx recognizes cccDNA, I have developed a method to reconstitute the minichromosome in vitro. This has opened the door for biophysical and biochemical studies of cccDNA, which I have used to characterize both its compaction state and nucleosome phasing in the presence and absence of HBx. Though ongoing, this work has already yielded the tools necessary for the in-depth study of this crucial intermediate in the viral life cycle, and revealed the HBx-nucleosome interaction as a potential drug target to treat chronic infections.

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Rao, Sahana

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

N-terminal degradation activates the NLRP1 inflammasome

Intracellular pathogens and danger signals trigger the formation of inflammasomes, which activate inflammatory caspases and induce pyroptotic cell death. The anthrax lethal factor metalloprotease, the apicomplexan parasite Toxoplasma gondii and small molecule DPP8/9 inhibitors activate the Nlrp1b inflammasome, but the molecular mechanism of Nlrp1b activation is not known. Here, we used genome-wide CRISPR/Cas9 knockout screens to identify genes involved for NLRP1B-mediated pyroptosis by Anthrax Lethal Toxin and DPP8/9 inhibitors. Anthrax Lethal factor is a metalloprotease that directly cleaves NLRP1B. This induces the N-end rule-mediated degradation of the NLRP1B N-terminus and frees up NLRP1B C-terminus to activate caspase-1 and cause pyroptosis. Val-boroPro also activates NLRP1B inflammasome and degrades the N-terminus via an unknown mechanism and causes pyroptosis. We discovered that N-terminal degradation is the universal mechanism for NLRP1 activation.

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Stone, Samantha

Research Associate at Memorial Sloan Kettering
Lab: Derek Tan, PhD

Cheminformatic Comparison of Synthetic and Natural Product-based Drugs and Novel Chemical Probes

Despite a declining emphasis on natural product discovery in the pharmaceutical industry, approximately 50% of all novel drug approvals are still structurally linked to natural product scaffolds. Herein, we perform a principal component analysis (PCA) of the structural and physicochemical properties of synthetic and natural product-based drugs, as well as a selection of recently discovered chemical probes. Our results illustrate that the chemical diversity of small-molecule drug discovery may be increased through the incorporation of the structural features of natural products.

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SESSION 4

Ahn, Yong-Mo, PhD

Staff Scientist at Rutgers University
Lab: Joel Freundlich, PhD

A Small Molecule Inhibitor, JSF-3285, as a Preclinical Candidate Targeting Mycobacterium tuberculosis KasA

Cell wall biosynthesis inhibitors have proven highly effective for treating tuberculosis (TB). We have previously discovered and validated members of the indazole sulfonamide class of small molecules as inhibitors of Mycobacterium tuberculosis KasA—a key component in the biosynthesis of the mycolic acid layer of the bacterium's cell wall. To deliver on the promise of KasA as a TB drug target, we utilized a structure-based approach to optimize the existing KasA inhibitor DG167 to identify compounds with cidal efficacy in mouse models of acute and chronic M. tuberculosis infection. Our efforts ultimately afforded a promising preclinical candidate for tuberculosis, indazole JSF-3285, shown through multiple approaches to potently target KasA. Impressively, JSF-3285 offers a 30-fold increase in mouse plasma exposure over DG167, and once-daily oral activity in acute and chronic models of infection worthy of further translational study.

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Berman, Adi

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

Characterizing cellular functions of the γ-TuRC

The antibiotic resistance is a growing public health crisis. The CDC predicts every year 2 million Americans contract drug-resistant bacterial infections which causes $10-20 billion cost to US healthcare industry. Gram(-) bacteria causes a wide spectrum of disease that includes antibiotic resistance. Gram(-) bacteria has an inner membrane of phospholipid, a thin peptidoglycan layer, plus an outer membrane. The outer membrane is an asymmetric bilayer of glycerol phospholipids and lipopolysaccharide (LPS) LPS and phospholipids and that makes the wall rigid hence slow the passive diffusion of hydrophobic compounds and narrow pores limit by size the penetration of drugs. In our SPEAR-GN project, (Small-Molecule Penetration & Efflux in Antibiotic-Resistant Gram-Negatives bacteria) we are looking for a correlation between chemical structure and bacterial permeability. Our lab has an interest on sulfonyladenosine (AMS) compounds, we are looking for a synthesis of unique AMS derivative to do accumulation study. The chemo informatics analysis of these type of the compounds derivatized from structure activity relationship (SAR) data will predict the permeability and efflux sensitivity of small molecules in pathogenic bacteria. That will give us an idea of developing predictive tools to accelerate antibiotic drug discovery to tackle the growing threat.

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Boby, Melissa

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

The Development of Novel Antibiotics: Rational Design of MenE Inhibitors

As the prevalence of antibiotic resistance continues to rise, we are presented with an urgent need to develop antibiotics that function through novel pathways and mechanisms. We seek to address this problem by developing a new class of antibiotics targeting the enzyme MenE, a key acyl-CoA synthetase in the menaquinone biosynthetic pathway. Menaquinone is the sole electron carrier in the electron-transport chain of many clinically relevant bacteria, such as M. tuberculosis and S. aureus, and is required for cellular function. MenE converts o-succinyl benzoate (OSB) to OSB-CoA via an OSB-AMP intermediate. We have previously reported the first mechanism-based inhibitors of MenE, which employ an acyl-sulfamate motif as an acyl-phosphate bioisostere. While the inhibitors are potent in biochemical assays, they show relatively low antimicrobial activity due to poor bacterial compound accumulation. Herein we describe the use of crystallographic data, computational docking studies, and bacterial compound accumulation assays to design a series of analogues that use m-phenyl ethers as novel, non-traditional acyl-phosphate bioisosteres. This combination of techniques allows us to guide further analogue development towards structures that are not only better inhibitors, but also show improved compound accumulation.

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Chongsaritsinsuk, Joann

Undergraduate Student at The University of Richmond
Lab: Tarun Kapoor, PhD

Structural parallels between the ribosome biogenesis factor Mdn1 and integrin

Mdn1 is an essential ATPase composed of a hexameric AAA ATPase ring, an alpha helical linker region and a C-terminal metal ion-dependent adhesion site (MIDAS). Mdn1 converts chemical energy from ATP hydrolysis into force to dislodge the ribosomal assembly protein 4 (Rsa4) from the pre-60S ribosome. Integrins are transmembrane receptors which contain an inserted domain in their alpha subunits homologous to the MIDAS in Mdn1 and exhibit catch bonding. Catch bonds are a biological interaction enhanced by a force pulling a ligand-receptor complex apart. Mdn1 may similarly exhibit catch bonding with Rsa4. Here, we identify one amino acid interaction (Gln-Ser) which may play a role in catch bonding between Mdn1 and Rsa4. Catch bonding may allow Mdn1 to regulate the dynamic attachment and detachment of Rsa4 during ribosome biogenesis.

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Leicher, Rachel

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

SINGLE-MOLECULE INVESTIGATION OF PRC2 NON-ADJACENT NUCLEOSOME BRIDGING

Epigenetic marks play a key role in the regulation of gene expression. These post-translational modifications contribute to heterochromatin and euchromatin states, which control gene expression profiles that can determine cellular differentiation and development. Failure to maintain the proper epigenetic landscape during cell differentiation and division can result in disease states such as cancer. The players that mediate the maintenance of these reversible chromatin marks are often essential proteins in the cell. Uncovering the mechanisms of these molecular complexes and their dynamic interplay with chromatin is imperative for understanding how epigenetics contribute to cellular homeostasis. Polycomb repressive complex 2 (PRC2) maintains transcriptionally silent heterochromatic states in vivo by spreading the repressive hallmark H3K27me3 mark along chromatin. Despite extensive research into the catalysis, the way PRC2 binds and engages chromatin substrates remains unclear. To uncover this, we utilized single-molecule force spectroscopy, computer simulations, and electron microscopy to dissect the wide array of PRC2 binding configurations. Specifically, we found that PRC2 was able to bridge non-adjacent nucleosomes, a new phenomenon, that can lead to chromatin compaction. We also uncovered how PRC2 binding states are regulated by accessory subunits, oncogenic histone mutations, and chromatin methylation states. Overall, this work provides a new paradigm for understanding the physical basis of heterochromatic chromatin maintenance by PRC2.

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Maksimovic, Igor

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

Developing a chemical toolbox to investigate histone glycation

Reactive cellular metabolites can modify macromolecules and form adducts known as nonenzymatic covalent modifications (NECMs). The dissection of the mechanisms, regulation, and consequences of NECMs, such as glycation, has been challenging due to the complex and often ambiguous nature of the adducts formed. Specific chemical tools are required to directly track the formation of these modifications on key targets in order to uncover their underlying physiological importance. Here, we present the novel chemoenzymatic synthesis of an active azido-modified ribose analog, 5-azidoribose (5-AR), as well as the synthesis of an inactive control derivative, 1-azidoribose (1-AR), and their application toward understanding protein ribose-glycation in vitro and in cellulo. With these new probes we found that, similar to methylglyoxal (MGO) glycation, ribose glycation specifically accumulates on histones. In addition to fluorescent labeling, we demonstrate the utility of the probe in enriching modified targets, which were identified by label-free quantitative proteomics and high-resolution MS/MS workflows. Finally, we establish that the known oncoprotein and hexose deglycase, fructosamine 3-kinase (FN3K), recognizes and facilitates the removal of 5-AR glycation adducts in live cells, supporting the dynamic regulation of ribose glycation as well as validating the probe as a new platform to monitor FN3K activity. Altogether, we demonstrate this probe's utilities to uncover ribose-glycation and deglycation events as well as track FN3K activity toward establishing its potential as a new cancer vulnerability.

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Martin, Kirsten

Graduate Student at Stony Brook University
Lab: Eszter Boros, PhD

In situ excitation of luminescent lanthanide complexes: Towards enabling in vivo bioimaging

Optical imaging probes are indispensable tools for visualizing biomarkers of disease without perturbing the biological system. Ideal optical imaging probes provide low limits of detection, minimal photobleaching and emit in the first or second biological window (550-800 nm or 1000-1350 nm). Luminescent lanthanides exhibit many of these aforementioned properties; however, as f-f transitions are Laporte-forbidden, organic chromophores absorbing in the 200-300 nm range, known as antennae, are necessary for efficient excitation of the metal ion. These excitation wavelengths are not compatible with biological applications due to the strong absorbance of blood and tissues at wavelengths below 550 nm. To circumvent this, we propose that lanthanide-based complexes can be excited in situ using Cherenkov radiation. In situ excitation of lanthanide complexes with Cherenkov radiation energy transfer (CRET) eliminates the need for external high energy and short wavelength excitation, making them amenable for in vivo imaging applications.

Following initial proof of concept studies, it has been demonstrated that CRET is suitable to excite discrete Tb and Eu complexes with a detection limit of 5 nmol and 10 nmol, respectively. Here, we demonstrate our efforts towards the application of these luminescent lanthanide complexes for multiplexed imaging and targeted in vitro and in vivo imaging applications. The targeted and nontargeted europium and terbium-based probes produce moderate quantum yields (10-47%) and are efficiently excited with Cherenkov radiation with radioisotopes F-18 and Zr-89. This system represents the first application of discrete lanthanide complexes for optical imaging without an external excitation source.

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Mattheisen, Jordan

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

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. These so-called “cryptic binding sites,” which are often conformation-dependent, are difficult to identify. Targeting cryptic binding sites in drug discovery using traditional high-throughput screening and GPCR cell-based signaling assays is also challenging. We aim to develop a robust methodology to identify and study cryptic allosteric sites in GPCRs. We hypothesize that tethering of low-affinity fragments into allosteric sites in GPCRs can 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 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. We 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. Several analogues displayed an increase in potency for CCR5 engineered to contain unnatural amino acids. In future experiments, 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.

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Ray, Devin

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

Developing a Robust and Flexible Method for the Selective Modification of Membrane Proteins In Vivo

Membrane proteins make up over 30% of the genome, and represent key regulators of information and communication for cells. The ability to tracelessly modify integral membrane proteins in their cellular environment has the potential to both significantly expand the avenues for investigation and increase their potential manipulation for therapeutic value. Many effective protein engineering strategies have been less successful when applied to membrane proteins due to the added complexity of a membrane protein's interaction to the plasma membrane. Protein trans splicing (PTS) systems have previously been used for the manipulation of cytosolic and nuclear proteins, introducing multiple modifications. Utilizing a split intein system, we have been able to modify the Epidermal Growth Factor on live cells. This methodology will allow for the traceless introduction of a library of physiologically-relevant as well as synthetic modifications to membrane proteins in live cells, thus permitting more extensive study in biologically relevant contexts.

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Rundlet, Emily

TPCB Graduate Student at Weill Cornell Medicine/St. Jude Children's Research Hospital
Lab: Scott Blanchard, PhD

Pre-hydrolysis state of EF-G on the ribosome during tRNA translocation.

The ribosome, along with amino acyl tRNA and the translational GTPases EF-Tu and EF-G, is responsible for rapid and accurate mRNA decoding and maintenance of the codon reading frame during the peptide chain elongation cycle. Despite over 60 years of mechanistic analysis, our understanding of the basic mechanism of tRNA translocation through the inter-subunit space remains to be fully elucidated. We have employed in vitro-reconstituted platforms to probe the reaction coordinate of tRNA translocation using multi-perspective single molecule fluorescence resonance energy transfer (smFRET) to identify three kinetic intermediates of translocation. Guided by smFRET, we have sought to rectify open questions regarding the translocation mechanism by visualizing EF-G on the ribosome by cryo-electron microscopy (cryo-EM) during different intermediate states of translocation. In strong agreement with the predictions of our multi-perspective smFRET studies, we identified eight distinct ribosome conformations ranging in resolution from 2.2-2.8 Å, including classical and hybrid Pre-translocation (PRE), Post-translocation (POST), and translocation intermediate states. This work suggests the need for specific revisions to canonical models of translocation, particularly as it pertains to the role and timing of GTP hydrolysis and inorganic phosphate (Pi) release.

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Ser, Zheng

TPCB Graduate Student at Memorial Sloan Kettering
Lab: Alex Kentsis, PhD

Elucidation of structure and interactions of DNA binding protein complexes through mass spectrometry proteomics

Interactions between DNA and proteins at the replication fork are essential processes of the cell. Using two different proteomics approaches, we report investigations of two different protein complexes involved at the replication fork. First, the DNA transposase piggy-bac transposable element derived 5 (PGBD5), which induces genomic rearrangements, has recently been reported to be an oncogenic mutator. Inhibition of Ataxia telangiectasia mutated and RAD3-related (ATR) was reported as a potential therapeutic strategy for cancers expressing high levels of PGBD5. To investigate the potential mechanism and interaction between PGBD5 and ATR function at the replication fork, isolation of proteins at nascent DNA (iPOND) with isotope labels was performed on PGBD5 knock-in and knock-out cells. The quantitative proteomic results reveal a set of PGBD5 associated proteins at the replication fork with possible interactions with ATR related proteins. Second, among the structural maintenance of chromosome (Smc) family of proteins, Smc5 and Smc6 have reported functions in maintaining replication fork stability, involvement in DNA repair and homologous recombination. Smc5 and Smc6 function together with 6 non-Smc elements (Nse), Nse1-6, and certain structural aspects of the Smc5-Smc6 protein complex, such as the placement of Nse5-Nse6 in relation to the other components of the complex, remain poorly understood. Using cross-linking mass spectrometry, we were able to assign distance constraints between cross-links and determine the interaction site between Nse6 and the coiled-coil regions of Smc5-Smc6 and between Nse2 and the coiled-coil region of Smc5. These structural insights will help us better understand the function of the Nse proteins in relation to the Smc5-Smc6 protein complex. Overall, the different proteomic approaches allow us to better understand the protein complexes involved with different functions at the replication fork.

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Shea, Christie

TPCB Graduate Student at 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 development and progression. Our lab has demonstrated that incorporation of H3.3, an 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. Our findings suggest that targeting the HIRA complex can be a viable therapeutic target for advanced carcinomas. However, it is still largely unclear how H3.3 promotes metastasis whereas canonical H3 does not. Therefore, my goal is to investigate how H3.3 specifically promote metastasis. My preliminary data 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 an active role during metastasis.

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Warren, Charles

Undergraduate Student at University of North Carolina at Chapel Hill
Lab: Daniel Bachovchin, PhD

Using Homology Modeling to Understand an Inflammasome Licensing Interaction

Inflammasomes are multiprotein complexes that detect intracellular pathogens or molecular danger signals, recruit and activate pro-caspase-1, and trigger a lytic, pro-inflammatory form of programmed cell death called pyroptosis. NLRP3 is the most extensively studied inflammasome-forming sensor protein. Several recent studies have demonstrated that the NAHCT-LRR domains of NLRP3 bind NEK7, a kinase involved in cell cycle regulation, and this binding is required to license inflammasome formation and pyroptosis induction. NACHT-LRR domains are conserved in many other inflammasome proteins, but it is unknown if these motifs also have regulatory binding partners. Intriguingly, we have identified a binding partner for NLRP1's NACHT-LRR (NLRP1BP) that, like the NLRP3-NEK7 system, may similarly regulate its activation state. To better understand the NLRP1-NLRP1BP interaction, we constructed a homology model (HM) of NLRP1's NACHT-LRR based on the NLRP3-NEK7 cryo-EM structure, which indicated that known-disease-associated mutations in NLRP1 are regions structurally analogous to those critical for the NLRP3-NEK7 interaction. Fascinatingly, these mutations result in weaker affinity between NRLP1 and NLRP1BP. We will next probe the nature of the NLRP1-NLRP1BP interaction surface through mutagenesis of HM-predicted residues and assess the downstream consequences for NLRP1 inflammasome formation and activation. Overall, this work aims to understand how NACHT-LRR interaction(s) regulate the NLRP1 inflammasome and establish whether NACHT-LRR binding proteins are a conserved feature used to license pyroptotic behavior under certain cellular contexts.

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