Research presentation abstract listed

Session 1:

Name: Hongjia Li
Title: Systematic Characterization of Wavefront Distortion Improves Cryo-FLM Localization Fidelity
Abstract: Correlative light and electron microscopy (CLEM) has become a transformative approach in structural biology by combining the molecular specificity of fluorescence microscopy with the high-resolution context of electron microscopy. However, high-precision localization of fluorescent molecules in cryogenic fluorescence light microscopy (cryo-FLM) remains challenging due to complex and unpredictable optical aberrations that distort the point spread function (PSF), potentially introducing localization errors exceeding 100~nm, and compromising downstream cryo-electron tomography (cryo-ET) analysis. Here, we present a robust strategy and an open-source MATLAB toolbox for quantitative aberration characterization and PSF modeling in cryo-FLM systems. Using maximum likelihood-based phase retrieval on experimental bead image stacks, we resolve both system- and sample-induced aberrations across the field of view (FOV). Our analysis reveals that mismatched PSF models are a major source of localization bias, and that using spatially matched or adaptive PSF models significantly improves localization accuracy. This work establishes a generalizable framework for aberration analysis and aberration-aware molecular localization in cryo-FLM. It provides critical insight into the impact of aberrations on localization precision and lays the foundation for more accurate molecular interpretation within complex cellular environments in cryo-CLEM workflows.

Name: Genki Terashi
Title: Global and Local Model-Map Fitting of Large Macromolecular Structures in Cryo-EM Maps Using Diffusion Models and Point Clouds
Abstract: Cryo-EM has become a fundamental technique for determining large macromolecules. With the growing popularity of modeling tools like AlphaFold, more accurate atomic models have become available. As a result, model fitting into cryo-EM maps has become a common strategy for interpreting structures. However, accurately fitting models remains challenging, particularly in intermediate or low-resolution maps. Here, we present our two advanced methods, DiffModeler and DMcloud, that address these challenges by integrating diffusion models to enhance the backbone position and local point cloud-based matching.
DiffModeler employs a diffusion model to trace protein backbones in cryo-EM maps at resolutions of 5-10 Å. DiffModeler also integrates AlphaFold for individual protein chains and the map-model alignment method (VESPER). DiffModeler demonstrates exceptional accuracy and outperforms other state-of-the-art fitting methods.
DMcloud introduces a novel point cloud matching-based fitting method for protein and DNA/RNA structures. DMcloud converts both molecular models and cryo-EM maps into point clouds for precise alignments. Our benchmarking shows that DMcloud outperforms traditional methods without providing explicit chain information.
DiffModeler and DMcloud provide significant advancements in structure modeling, especially for large macromolecules. These tools achieve automated, accurate solutions for fitting

protein and DNA/RNA structures into cryo-EM maps. Our programs are available on https://em.kiharalab.org.

Session 2:

Name: Siddhi Shetty
Title: Functional coupling between TPC2 and RyR1 augments intracellular Ca2+ signals
Abstract: The endoplasmic reticulum (ER) is the largest intracellular Ca2+ store. Ryanodine receptors (RyR) and inositol-1,4,5-trisphosphate receptors (IP3R) are the major Ca2+ release channels located in the ER membrane. In addition to the ER, acidic organelles such as lysosomes and endosomes also regulate intracellular Ca2+ signaling. Two pore channels (TPCs) are a class of ubiquitously expressed ion channels that reside in the endo-lysosomal membrane. These channels switch their ion permeability in a ligand-dependent manner. They toggle between a Ca2+ permeable state when stimulated with nicotinic acid adenine dinucleotide phosphate (NAADP)and a highly Na+ -selective state upon direct activation with phosphatidylinositol-3,5-bisphopshate (PI(3,5)P2). The discovery of cell-permeable, specific, TPC agonists that mimic NAADP and PI(3,5,)P2 has made it possible to study signaling through TPCs in an endogenous setting. TPC2-A1-N mimics NAADP promoting selective Ca2+ currents while TPC2-A1-P mimics activation by PI(3,5,)P2 leading to robust Na+ currents. We hypothesize that NAADP-evoked Ca2+ signals from TPCs sensitize ER Ca2+ release channels resulting in global Ca2+ signals within the cell. Recent work from our lab indicates that TPC2 on the lysosomes couple to IP3R on the ER, but it is unknown if such crosstalk exists between TPC2 and RyR. To test this interplay between TPC2 and RyR1, we utilize the lipophilic, synthetic, TPC2 agonists and investigate whether local Ca2+ signals evoked from TPC2 are amplified by RyR1 on the ER, leading to global Ca2+ signals.

Name: Leon Laskowski
Title: Determine the mechanism by which the D630Y mutation constitutively activates PLCb4
Abstract: Uveal Melanoma (UM) is the most prevalent malignant intraocular tumor and is an extremely deadly disease. 50% of patients develop liver metastases with fewer than 15% surviving past 12 months. 90% of all UM cases are driven by a constitutively activating mutation (Q209X) in the heterotrimeric G protein subunit Gaq/11. Gaq/11 activates two signaling pathways: PLCb4-PKC-ERK and Trio- RhoA-YAP. The latter was identified as a driver of tumorigenesis and becoming the focus of drug discovery efforts. However, these efforts fail to benefit the cohort of UM patients wherein the cancer is caused by a constitutively activating mutation in PLCb4 (D630Y). PLCb4D630Y was shown to be maximally active without being further activated by Gaq/11. This mutation is located on a solvent- exposed surface of the catalytic domain but does not interact with the active site or with known autoregulatory elements. Introduction of the D-to-Y at the equivalent position in other PLC enzymes also results in constitutive activity, suggesting a conserved function of this position. I determined high-resolution structures of PLCb4 and PLCb4D630Y using cryo-EM SPA, and structural-based hypotheses will be tested with In cellulo and in vitro functional assays to identify how D630Y maximally activates PLCb4.

Name: Yasmin Aydin

Title: Unlike Twins: Arrestin2 and Arrestin3 Engage the Membrane Differently
Abstract: Arrestin2 and Arrestin3 are versatile adaptor proteins that regulate over 800 G-protein coupled receptors (GPCRs), the largest family of membrane receptors in the human body. By recognizing cues such as receptor phosphorylation patterns, conformational changes, and membrane properties, arrestins selectively bind to activated and phosphorylated GPCRs. Recent studies have highlighted the membrane itself as a key player in shaping arrestin function. Here, we use biophysical and live-cell assays to investigate how different membrane environments influence Arrestin2 and Arrestin3. We find that these highly similar proteins engage with membranes in distinct ways, driven by subtle differences in their membrane-binding regions. Our findings underscore the membrane as a critical modulator of arrestin function and reveal a previously underappreciated layer of regulation in GPCR signaling.

Name: Johanna Bovill
Title: Using Cryo-EM to Understand Gaq-Dependent Activation of PLCb
Abstract: Phospholipase C (PLC) enzymes regulate numerous physiological processes. The PLCb subfamily is critical for normal cardiovascular function, and when dysregulated, can cause cardiovascular disease. The best characterized activator of PLCb is the heterotrimeric G protein subunit Gaq, which binds directly to C-terminal regulatory domains in the lipase. High-resolution structures of PLCb, alone and in complex with Gaq, have been determined, but in all cases, known autoinhibitory interactions within PLCb persist. Thus, the structure of an active PLCb or an active Gaq–PLCb complex remain to be determined. To address this gap, I am using cryo-electron microscopy single particle analysis (cryo-EM SPA) to determine structures of PLCb in complex with Gaq in its fully activated, GTP-bound state. To investigate this state, I am using site-directed mutagenesis and cell-based activity assays. This work will provide crucial new insights into how the Gaq-PLCb complex is activated and deactivated, which can be used to provide further understanding and develop new therapeutic strategies for treating cardiovascular disease.

Session 3:

Name: Uttara Jayashankar
Title: SARS-CoV-2 inhibitors found to activate main protease from human-infecting canine coronavirus through ligand-induced dimerization.
Abstract: Coronaviruses are divided into 4 genera- alpha, beta, gamma and delta. The last decade has witnessed an increase in the zoonotic transmission of these coronaviruses. The 3C-like protease (3CLpro), is essential for coronaviral replication and is highly conserved across multiple coronaviral genera making it an attractive drug target for the development of broad-spectrum, anti-coronaviral drugs. In this study, we focus on investigating the role of dimerization in a recently discovered human-infecting canine alpha-coronavirus, CCoV-HuPn-2018 (CCoV), identified in patients in Malaysia and Haiti. Using a combination of steady-state kinetic approaches along with native nanoelectrospray mass spectrometry (nESI-MS), we show that the 3CLpro from CCoV is a weakly associated dimer. In the absence of substrates or inhibitors, the Kd value for the dimer is ~ 14 μM, over 100-fold greater than that of SARS-CoV-2. However, in the presence of inhibitors such as nirmatrelvir (active component of Paxlovid) and its analogs, we observe a shift in the equilibrium towards the formation of the active dimer, demonstrating ligand-induced dimerization. Moreover, we

found that at low inhibitor concentrations, activation of CCoV-HuPn-2018 3CLpro occurs suggesting that the use of such inhibitors at low concentrations could promote viral replication. We determined high-resolution X-ray structures of CCoV 3CL in complex with different inhibitors to reveal structural elements driving dimerization. We found that unlike SARS-CoV-2, CCoV 3CLpro has a Glycine residue at position 122 along the dimer interface and upon mutation to a Serine, as found in SARS-CoV-2, the dimer affinity increased by approximately 50-fold (Kd ~ 0.3). These results improve our understanding of inhibitor (drug) induced dimerization of 3CLpro across different coronaviral genera, helping us design better broad-spectrum anti-coronaviral drugs.

Name: Barsha Bhowal
Title: A mutational analysis of the Ebola virus matrix protein VP40 to assess effects on assembly and budding of Ebola virus
Abstract: Ebola virus (EBOV) is a filamentous lipid-enveloped virus that causes a highly fatal hemorrhagic fever. EBOV harbors a negative sense single stranded RNA and codes for seven different proteins. The matrix protein VP40 is responsible for assembly and budding of the virus from the host cell plasma membrane, from where it acquires its lipid envelope. VP40 forms a high affinity dimer that oligomerizes upon binding lipids at the plasma membrane inner leaflet. The VP40 dimer structure is necessary for proper lipid binding and viral budding. VP40 also can form an RNA-binding ring octamer. The octameric form is thought to be involved in the RNA transcription process and has no role in assembly. VP40 consists of a N-terminal domain with a dimerization interface and a C-terminal domain that has a basic patch that interacts with the anionic membrane lipids. Multiple residues in the dimer interface as well as in the membrane binding patch mediate the dimerization process. Per-residue energy decomposition and site-saturation mutagenesis have identified multiple keystone residues important for this dimerization and membrane binding process. Some mutations have also been identified from patient samples during previous EBOV outbreaks. In this project, we are analyzing a few such mutations with major focus on Arg204 and His269. Integration of computational, in vitro and in cellulo assays have identified changes in membrane binding, plasma membrane interactions, VP40 oligomerization and egress from the host cell.

Name: Swati Mundre
Title: Elucidating the structure and function of the zinc uptake regulator (Zur) protein of Neisseria gonorrhea
Abstract: Transition metals, such as zinc, are essential for the survival of all life forms. Humans have evolved to have nutritional immunity whereby metal ions like zinc are sequestered by proteins to limit their access by opportunistic bacteria. *Neisseria gonorrhea*, a gram-negative bacterium, overcomes this nutritional immunity by expressing metal acquisition systems on its surface to mediate piracy of zinc from the host metal sequestering proteins. Zinc is essential for the survival of bacteria as it serves numerous functions for the cell including gene regulation and enzyme activation. Nevertheless, excess zinc is toxic to the cells and, therefore, their levels need to be maintained. Zinc uptake regulator (Zur) is a cytosolic protein belonging to the Fur (ferric) family of proteins that senses metals and regulates the expression of genes involved in metal homeostasis. Zur has an N-terminal DNA-binding domain and a C-terminal dimerization domain with two zinc binding sites. Binding of zinc to Zur leads to its association to its cognate DNA binding sequence called the Zurbox, which leads to the repression of genes involved in zinc homeostasis, zinc uptake,

and zinc transport. The only known structure of Zur bound to its cognate DNA sequence is from *E. coli*. Despite 31% sequence identity to *Ec*Zur, there are distinct features in *Ng*Zur that suggest additional regulatory roles. We aim to characterize the structure of *Ng*Zur in complex with its Zurbox, which would bring us closer to developing therapeutics against this obstinate pathogen.

Name: Jacqueline S. Anderson
Title: Importance of Capsid Stability in Enterovirus Capsid Assembly and Maturation
Abstract: Enterovirus D68 (EV-D68) is a respiratory illness implicated in recent outbreaks of acute flaccid myelitis, a neurologic flaccid paralysis like poliomyelitis, yet no therapeutic or vaccine is currently available. During EV-D68 infection, individual structural proteins VP1, VP0, and VP3 are translated and self-assemble into protomers for use in capsid assembly. As capsid assembly progresses, protomers form an unstable immature particle. Immature particles subsequently undergo maturation, a conformational shift initiated by cleavage of precursor protein VP0 in to separate structural proteins VP4 and VP2 producing a stabilized native virion. Based on high VP4 conservation among enteroviruses, we hypothesize that VP4 has critical stabilizing interprotomer interactions, and that alteration of these interactions impair capsid assembly and maturation. Structural analysis identified an interprotomer interaction between VP4 Lysine 33 (K33) and VP3 aspartic acid (D18). VP3 D18 amino acid substitution to glutamic acid (D18E) produces non-infectious virus following RNA transfection. Preliminary cryo-EM imaging showed VP3 D18E can form particles yet VP2 was absent in western blots, suggesting a capsid maturation defect. To delineate the type of particles being assembled, virus purification and discontinuous 10-50% sucrose gradient fractionation was performed. Each fraction was collected and checked for the presence of VP0 and VP2 by western blot and qRT-PCR. In wild type EV-D68, mature particles were present in the 40-45% fractions confirmed by the presence of VP2 and viral genome. In contrast, VP3 D18E VP2 was present in lower density 20-30% fractions, yet RNA was absent suggesting the presence of mature empty capsids. Cryo-EM imaging confirmed the presence of particles in the VP3 D18E 30% sucrose fraction, suggesting that capsid destabilization causes VP3 D18E genome loss following maturation. Further analyses are ongoing, but this work highlights the importance of interprotomer interactions in capsid assembly and maturation mechanisms and reveals important insights into icosahedral particle architecture.

Session 4:

Name: Kratika Singhal
Title: Mechanistic insight into SHIP1's interaction with immune receptors
Abstract: Late – onset Alzheimer's disease (LOAD) is a complex neurodegenerative disorder with limited treatment options due to an incomplete understanding of its molecular mechanisms. Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) has emerged as a potential therapeutic target for LOAD due to its high expression on microglia, the brain's immune cells, as it helps in clearing out amyloid plaques via the PI3K pathway. TREM2 signaling is negatively regulated by Src homology 2-containing-inositol-phosphatase 1 (SHIP1) phosphatase, which dephosphorylates Phosphatidyl Inositol (3,4,5) triphosphate [PIP3] to Phosphatidyl Inositol (3,4) diphosphate [PIP2] thereby preventing downstream PI3K activation. SHIP1 is a large multidomain protein with poorly understood characteristics,

which is overexpressed in LOAD, and this makes it a crucial target for further analysis. In this work, we have cloned, expressed and purified various truncated forms of SHIP1 phosphatase. Since previous lab efforts identified C-terminal truncated protein to be dimer, we are also investigating the biological significance of SHIP1 oligomerization. Our current working hypothesis is that SHIP1's SH2 domain binds phosphorylated ITIM while facilitating the second subunit of SHIP1 to interact with ITAM motif on DAP12 transmembrane receptor, stabilizing SHIP1 at the membrane and enhancing its ability to dephosphorylate PIP3, thus regulating TREM2 signaling. Additionally, we are screening small molecule inhibitors to understand their interactions with SHIP1 and to explore whether ITIM and ITAM peptides modulate SHIP1's accessibility to these inhibitors. These findings may contribute to the design of SHIP1-targeting inhibitors with therapeutic potential for AD.

Name: Sarah Stanhope
Title: One-carbon metabolism enzyme Ahcy is a redox sensor that modulates gene expression to protect against light-stress induced retinal degeneration.
Abstract: One-carbon metabolism influences epigenetics by providing the methyl donor S-adenosylmethionine (SAM). The methylation byproduct S-adenosylhomocysteine (SAH) is a methyltransferase inhibitor that is hydrolyzed by S-adenosylhomocysteinase (Ahcy). Here, we show Ahcy is a redox-sensitive enzyme that is inhibited by oxidation of a conserved cysteine. Ahcy oxidation correlates with increased SAH in a Drosophila light stress model, and Ahcy knockdown protects against light stress-induced retinal degeneration. Ahcy knockdown also suppresses light stress-induced changes in gene expression, suggesting that redox sensing by Ahcy enables rapid transcriptional responses to altered redox homeostasis to regulate neuronal adaptation. Thus, Ahcy connects metabolic redox sensing to transcription. These findings advance our understanding of how metabolic pathways integrate with redox homeostasis to regulate neuronal adaptation, with broad implications in neurobiology and disease.

Name: Trung Thach
Title: Mechanistic Insights into Selective P2X3 Inhibition: A Structural and Functional Study
Abstract: P2X3 receptors are ATP-gated ion channels essential for sensory neurotransmission and are implicated in pathological conditions such as chronic cough. Despite their therapeutic relevance, the molecular mechanisms underlying selective P2X3 inhibition remain incompletely understood. Here, we present high-resolution cryo-electron microscopy (cryo-EM) structures of the receptor in both agonist- and antagonist-bound states, revealing a previously uncharacterized allosteric binding pocket within the extracellular domain. Antagonist binding induces conformational changes in the upper body region, including the turret, which propagate to the transmembrane helices and stabilize a closed, non-conducting channel conformation. This state is characterized by a narrowed ion conduction pathway (~0.7 Å), effectively preventing cation permeation. Molecular dynamics simulations and electrophysiological assays support a model in which turret dynamics play a critical role in channel gating and desensitization. These findings offer detailed structural insight into the allosteric modulation of P2X3 and provide a molecular framework for the rational design of selective P2X3 inhibitors.

Name: Luying Chen
Title: Structural basis for nucleolin recognition of MYC promoter G-quadruplex

Abstract: The G-quadruplex structure found in the promoter region of the MYC oncogene (referred to as MycG4) plays an important role in regulating gene transcription and is commonly present in immortalized cells. Nucleolin, a key protein that binds to MycG4, shows a much stronger preference for MycG4 than for its usual RNA target, the nucleolin recognition element (NRE). However, although nucleolin was discovered to be the major MycG4 binding protein, it's molecular recognition is unknown.
In our study, we determined the crystal structure of the nucleolin–MycG4 complex at 2.6-angstrom resolution. This strong interaction depends on all four of nucleolin's RNA-binding domains (RBDs).The structure reveals a well-folded, parallel G-quadruplex stabilized by two potassium ions. This G4 structure interacts specifically and extensively with nucleolin's RBD domains. Our structure demonstrate that nucleolin recognizes the G-quadruplex shape through multiple contact points, highlighting a specific structural basis for this interaction. Our findings suggest that G4s are nucleolin's primary cellular substrates and indicate a G4-based epigenetic transcriptional regulation. These insights also provide a valuable foundation for developing drugs that target G4 structures.