Session 3: Talk 1

Anika Jain
Purdue University
Biology, Graduate Student (5th Year)
PI: Daisuke Kihara

Emap2Lig: Predicting and Modeling Ligands in Cryo-EM Maps Using Deep Learning

Cryogenic electron microscopy (cryo-EM) has become increasingly valuable for structure-based drug discovery by enabling the characterization of interactions between macromolecules and small-molecule ligands. However, computational modeling of ligands into cryo-EM densities remains challenging, particularly when ligand locations are unknown or when local map resolution is weak. Existing methods typically require a well-resolved macromolecular structure and predefined binding sites, limiting their applicability in early-stage structure determination where ligands must first be identified directly from the map.

Here, we present Emap2Lig, a two-stage deep-learning framework for automated ligand detection and atomic modeling directly from cryo-EM maps. Emap2Lig-Find employs a U-Net-based network to detect ligand densities, remaining effective for maps with resolutions as low as 6 Å. Emap2Lig-Build then isolates the detected densities and uses a diffusion-based structure model to generate three-dimensional atomic models of small-molecule ligands. Together, Emap2Lig provides a unified solution for ligand discovery and modeling across a broad range of resolutions, facilitating early-stage structure-based drug discovery from cryo-EM data alone.

Session 3: Talk 2

Laura Lukov
Purdue University
Chemistry, Graduate Student (3rd Year)
PI: Chi (Jesse) Zhang

Direct Quantification of Crystalline Insecticide Penetrative Ability in Soybean Foliage

Understanding the foliar uptake and distribution of insecticides is essential for improving agrochemical formulations and managing insects in crop systems. Conventionally, chemical uptake has been measured using mass spectrometry or radiotracer studies combined with autoradiography. However, these approaches require sample sectioning, which can damage tissue structure and result in carry-over of surface chemicals.

Here, we use multimodal Stimulated Raman Scattering (SRS) microscopy to directly visualize the penetrative behavior of two chemically distinct insecticides as well as Pluronic-type surfactants in soybean foliage. Label-free hyperspectral SRS enables quantitative distribution mapping through the cuticle, epidermis, and mesophyll, while SHG identifies crystalline deposits. We demonstrate that one insecticide remains largely confined above the cuticle, whereas the second penetrates deep into the mesophyll when formulated with Pluronic surfactants. Together, these findings provide the first in situ, chemically specific visualization of surfactant and crystalline insecticide penetration pathways in intact plant tissue.

Session 3: Talk 3

Samuel Hartzler

Purdue University
Biology, Graduate Student (3rd Year)
PI: Lauren Ann Metskas

Cellular Tomography of In Situ Ultrastructural Dynamics of a Carbon-Fixing Bacterial Microcompartment

Intracellular compartmentalization of metabolic pathways is essential for cellular homeostasis. Unlike eukaryotic membrane-bound organelles, prokaryotes sequester specific metabolic pathways within protein-based structures known as bacterial microcompartments (BMCs). One of the best-characterized BMCs is the Rubisco-containing α-carboxysome (α-CB), a protein shell that encapsulates carbon-fixation machinery.

To date, most studies have focused on mature, purified α-carboxysomes rather than transient intracellular assembly intermediates. Using cryo-electron tomography (cryo-ET) and subtomogram averaging (STA), we investigate the molecular interactions governing α-carboxysome assembly and maturation in vivo. Our work reveals extensive ultrastructural remodeling of the α-carboxysome cellular locus during these processes. Ongoing analyses aim to define the molecular mechanisms of cargo condensation and shell assembly. These findings will provide insights into bacterial organelle biogenesis and support future efforts to engineer microcompartments for biomedical and bioengineering applications.

Session 3: Talk 4

Alex Xiao

Purdue University
Biology, Graduate Student (4th Year)
PI: John Tesmer

Structural Insights into G Protein-Coupled Receptor Kinase 5 Phosphorylation on NTSR1

G protein-coupled receptor kinase 5 (GRK5) plays important roles in both cancer progression and cardiovascular disease. GRKs regulate GPCR signaling by selectively phosphorylating activated receptors, leading to arrestin recruitment and receptor downregulation. Despite decades of study, the mechanisms by which a limited number of GRKs recognize hundreds of activated GPCRs remain incompletely understood.

My research aims to elucidate the structural and dynamic aspects of the GRK5–NTSR1 complex using cryo-electron microscopy (cryo-EM) and hydrogen-deuterium exchange mass spectrometry (HDX-MS). To promote complex formation, we incorporated NanoBiT tags onto GRK5 and NTSR1 and utilized Fab13 to stabilize the complex. Initial cryo-EM analyses reveal recognizable GPCR–kinase architecture and support low-resolution three-dimensional reconstruction. Complementary HDX-MS studies indicate that phosphatidylinositol 4,5-bisphosphate (PIP2) enhances GRK5–NTSR1 interactions and stabilizes a more active kinase conformation. These studies provide critical insights into GRK5 regulation and may inform future therapeutic strategies targeting cancer and cardiovascular disease.

Session 3: Talk 5

Genki Terashi

Purdue University
Biology, Postdoctoral Researcher
PI: Daisuke Kihara

DAQplugin: Deep Learning-Based Real-Time Model Evaluation Plugin for ChimeraX

Accurate model building in cryo-EM maps remains challenging, particularly in regions with ambiguous or low-resolution density. As a result, structural models may contain residue misassignments or sequence-register shifts that are difficult to identify during model building and refinement.

Here, we present DAQplugin, a UCSF ChimeraX plugin for real-time deep-learning-based validation of protein models against cryo-EM maps. DAQplugin computes residue-wise DAQ scores interactively during model building, enabling immediate identification of potential modeling errors without requiring post-processing analysis. In addition to visualizing residue-level model-map agreement, the software provides sequence register-shift suggestions and generates positional restraints compatible with ISOLDE-guided refinement. We demonstrate that DAQplugin can identify local positional and sequence-register errors that are difficult to recognize using conventional validation metrics alone, providing a powerful new tool for interactive model refinement.

Session 3: Talk 6

Ketaki Mahurkar

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PULSe/Chemistry, Graduate Student (5th Year)
PI: Angeline Lyon

Regulation of Phospholipase Cε by Ras GTPases

Phospholipase C epsilon (PLCε) is a critical signaling enzyme involved in cellular signal transduction and is required for normal cellular function. Dysregulation of PLCε has been implicated in the development of cardiovascular disease and cancer. PLCε hydrolyzes PIP2 to generate second messengers that elevate intracellular calcium levels and activate protein kinase C, thereby regulating numerous downstream signaling pathways.

PLCε is activated by Ras GTPases, which are themselves linked to cardiac disease and cancer. Although previous studies have demonstrated that activated Ras promotes PLCε translocation to the plasma membrane, membrane localization alone is insufficient for maximal activation, suggesting an important role for allosteric regulation. Cell-based and biochemical assays are being used to investigate isoform-specific activation of PLCε by Ras proteins, while structural studies aim to define the molecular interactions underlying this process. This work will provide fundamental insights into the regulation of two critical signaling proteins and may facilitate the development of small-molecule modulators targeting their interaction.