KEYNOTE

9:30a (Frick Auditorium)

Justin W. Taraska Ph.D., Senior Investigator, NHLBI, NIH, Bethesda, MD

Imaging the structure of the plasma membrane with light and electron microscopy

The plasma membrane separates the cell's interior from the outside world. The exchange of signals and materials across this barrier is regulated by a multitude of channels, transporters, receptors, and trafficking organelles. Mapping the molecular structure and dynamics of the plasma membrane and these organelles is key to understanding how human cells function in health and malfunction in disease. We develop and use super-resolution light, EM, correlative light and EM (CLEM), and cryo-electron tomography to image protein complexes within the dense native environment of the cell. These methods are uniquely suited to determine the nanometer-scale structure of the plasma membrane in human cells. With these new tools we have been investigating the nanoscale structure, mechanics, and signaling properties of clathrin-mediated endocytosis—the central mechanism human cells use to internalize receptors, nutrients, membrane, hormones, and other cargo.

LIGHT TRACK TALKS

10:45a-11:30a (Frick Auditorium)

Danelle Devenport Ph.D., Associate Professor, Department of Molecular Biology, Princeton University, Princeton, NJ

Multiscale visualization of epidermal planar cell polarity

A striking feature of the skin is the uniform alignment of polarized structures (like hairs, feathers or scales) across the skin surface, an ancient and deeply conserved feature of epithelial tissues known as planar cell polarity (PCP). PCP is, by definition, a multiscale phenomenon where polarity organizes over several different length-scales, from the molecular, to multicellular, to tissue-scale. Here I will discuss how super-resolution and live imaging of the PCP dynamics has yielded fundamental insights into PCP establishment and its maintenance during tissue growth and turnover.

11:30a-12:15p (Frick Auditorium)

Stephanie Rudolph Ph.D., Assistant Professor, Dominick P. Purpura Department of Neuroscience and Department of Psychiatry and Behavioral Sciences, Albert Einstein College of Medicine, NY, NY

Pathways for non-canonical neuromodulation by cerebrospinal fluid

Neurotransmitters enable communication between neurons and can act at precise point-to-point contacts called synapses, or more diffusely via volume transmission. Synaptic and volume transmission serve distinct physiological roles: the former mediates local precisely timed communication, and the latter achieves more sustained global modulation via distribution in fluid-filled spaces of the brain. Although volume transmission is well-characterized in invertebrate nervous systems, its functions are poorly understood in mammals. However, evidence of axons in the ventricle lining, and neurotransmitters in cerebrospinal fluid (CSF) suggest that neurotransmitter is released into CSF and that CSF itself could also provide neuromodulation in mammalian systems. CSF is continuously produced in the choroid plexus and interacts with brain regions adjacent to the brain's ventricles and meningeal spaces near the brain surface. Here, we provide evidence that molecularly defined subpopulations of serotonergic neurons located in the caudal dorsal and medial raphe nuclei (cDR and MR) of the brainstem project axons to the meninges. We thus hypothesize that CSF constitutes a source of serotonergic neuromodulation that acts in a coordinated and physiologically purposeful manner to regulate brain activity. We employ a combination of anatomical tracing, single-cell RNA sequencing (scRNAseq) data analysis, *in situ* hybridization, whole-brain tissue clearing, and light sheet imaging to characterize meninges and ventricle-projecting serotonergic neurons. Our goal is to anatomically map these neurons and their projections within the meninges and ventricles to infer putative target regions in the brain that are modulated by CSF-mediated volume transmission.

EM TRACK TALKS

10:45a-11:30a (Icahn 101)

Yi-Wei Chang Ph.D., Assistant Professor of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA

In situ structural parasitology by cryo-electron tomography – using apicomplexan rhoptry secretion system as an example.

Parasites of the phylum Apicomplexa cause important diseases including malaria, toxoplasmosis and cryptosporidiosis. These intracellular pathogens inject the contents of an essential organelle, the rhoptry, into host cells to enable invasion and infection. However, the structure and mechanism of this eukaryotic secretion system remain elusive. In this presentation, I will show how we have used cryo-electron tomography to reveal the supramolecular architecture of this secretion system directly inside intact, frozen-hydrated *Cryptosporidium parvum* sporozoites, *Toxoplasma gondii* tachyzoites, and *Plasmodium falciparum* merozoites at a resolution of few nanometers in three dimensions. We identified membrane-bound helical filaments which appear to shape and compartmentalize the rhoptries. We uncovered a remarkable multi-component molecular assembly, which we named the rhoptry secretory apparatus (RSA), at the apical membrane of these parasites. The RSA anchors an enigmatic apical vesicle (AV) in the cytoplasm, and the AV docks the tip of rhoptries. We further resolved different

conformations of the RSA in *P. falciparum* corresponding to different rhoptry morphological states, supporting RSA and AV's roles in mediating rhoptry secretion. Moreover, we showed that *T. gondii* contains a line of AV-like vesicles which interact with a pair of microtubules and accumulate towards the AV, leading to a working model for AV-reloading and discharging of multiple rhoptries in some apicomplexan parasites. Together, our analyses provide a structural framework to elucidate how these important parasites regulate and conduct rhoptry discharge for their infection.

11:30a-12:15p (Icahn 101)

Ellen Zhong Ph.D., Assistant Professor, Department of Computer Science, Princeton University, Princeton, NJ

Machine learning for determining protein structure and dynamics from cryo-EM images

Major technological advances in cryo-electron microscopy (cryo-EM) have produced new opportunities to study the structure and dynamics of proteins and other biomolecular complexes. However, this structural heterogeneity complicates the algorithmic task of 3D reconstruction from the collected dataset of 2D cryo-EM images. In this seminar, I will overview cryoDRGN, an algorithm that leverages the representation power of deep neural networks to reconstruct continuous distributions of 3D density maps. Underpinning the cryoDRGN method is a deep generative model parameterized by a new neural representation of 3D volumes and a learning algorithm to optimize this representation from unlabeled 2D cryo-EM images. Extended to real datasets and released as an open-source tool, cryoDRGN has been used to discover new protein structures and visualize continuous trajectories of their motion. I will discuss various extensions of the method for broadening the scope of cryo-EM to new classes of dynamic protein complexes and analyzing the learned generative model. CryoDRGN is open-source software freely available at http://cryodrgn.cs.princeton.edu.

POSTERS

Anurag Sharma & Hilda Amalia Pasolli
The Rockefeller University Electron Microscopy Resource Center: An Overview

At The Rockefeller University Electron Microscopy Resource Center, we offer state-of-the-art instruments and competent expertise to support scientists with a broad range of electron microscopy (EM) studies, including sample preparation, transmission and scanning electron microscopy image acquisition and results interpretation for a variety of experimental models.We will show some examples of the results of some of our most challenging projects. Immunogold labeling of proteins in Drosophila brain: There are very few immunogold protocols that have been applied to Drosophila brain. They are long, complicated, and only work for a few antigens. We have developed a protocol with the Maimon Lab that allows to successfully label proteins in the brain while achieving good ultrastructure.

Scanning electron microscopy of different developmental stages of clonal raider ant (Ooceraea biroi) With the Kronauer Lab, we are studying the internal and external structure of clonal raider ants (Ooceraea biroi), a species never examined by EM before. Recently, the Kronauer lab has discovered the secretion of moulting fluid ("pupal milk"), which is essential to the ant colony's survival (Orli et al., 2022). Our EM studies contributed to elucidating the structures responsible for secreting the fluid. We are also working on their projects to understand how ants perceive and process external signals, by revealing the ultrastructure of sensory organs and pheromone-producing glands

Ultrastructural analysis confirmed the intimate proximity between lymphatics and crypt-base intestinal cells. Spatial transcriptomics, 3D imaging, and our TEM analysis revealed lymphatics as key crypt niche residents. With the Fuchs Lab (Niec et al, Cell Stem Cell, 2022).

Describing a new type of immune cell with the help of TEM. The Brown-Rudensky Labs (MSKCC), have recently identified a new class of RORγt+ antigen-presenting cells called Thetis cells, with transcriptional features of both medullary thymic epithelial cells and dendritic cells. Despite their scarce number, we succeeded in characterizing this novel cell type by TEM. (Nature, 2022).

https://www.nature.com/articles/s41586-022-05480-9#Sec6
https://www.nature.com/articles/s41586-022-05309-5
https://www.sciencedirect.com/science/article/pii/S1934590922002077?via%3Dihub

Eric Franklin, Princeton University

Global population growth is projected to outpace crop yields by 2050. This problem cannot be fully addressed by existing agricultural methods, as many crops are growth-limited not by farmer-provided resources (e.g. water, fertilizer), but by the amount of CO2 they can absorb from the atmosphere. Our lab studies a unique organelle — the pyrenoid — which many photosynthetic algae use to address this problem. Many single-celled algae use a pyrenoid to locally concentrate CO2 around Rubisco, the main enzyme responsible for algal and plant assimilation of CO2. Here, I aim to discover genes involved in the biogenesis of the membrane

tubules that run through the pyrenoid and deliver the CO2 to Rubisco. Using a modified co-immunoprecipitation assay, we pulled down intact tubule membranes and identified several novel tubule-localized proteins. Preliminary electron microscopy data support the hypothesis that these proteins are involved in tubule biogenesis. We plan to further characterize the function of these novel tubule proteins using cryo-electron tomography, ultimately aiming to understand how pyrenoid tubules are formed and engineer them — and eventually a fully-functional pyrenoid — into crop species.

Will Hofstadter, Princeton University

Organelles cooperate through inter and intra-organelle membrane contact sites to carry out nearly all cellular processes. Even though organelle contacts are pervasive and fundamental to basic cellular biology, they remain largely uncharacterized in the subcellular pathologies of disease progression. Our lab recently showed that diverse viruses modulate membrane contact sites to rewire cellular processes for infection progression. Excitingly, we found that Human cytomegalovirus (HCMV), a b-herpesvirus that infects ~80% of the world's population, globally changes organelle contact protein dynamics concomitant with extensive remodeling of the organelle landscape. However, the functional outputs of virus-driven changes to organelle contacts remain unclear. Here we aim to elucidate how HCMV infection remodels membrane contact sites to promote mitochondria fragmentation concurrent with increased respiration. This question is particularly intriguing given that mitochondria fragmentation is associated with decreased bioenergetics in many other disease states, such as Alzheimer's. We integrated super-resolution and cryo-electron imaging with proteomic and molecular virology assays to show how HCMV infection selectively fragments mitochondria and circumvents host autophagy to promote increased mitochondrial bioenergetics. First, we find that HCMV specifically promotes fission at the mitochondrial periphery. While peripheral fission was previously shown to decrease membrane potential and promote mitophagy in the smaller progeny, we show that during HCMV infection peripheral progeny maintain membrane potential and evade degradation. We further find that peripheral progeny form mitochondria-ER encapsulations (MENCs). MENCs are stable ER-mitochondria associations enriched with the membrane contact site protein PTPIP51 that we recently characterized and found to form during HCMV infection. Using IP-MS and live-cell metabolic sensors we show that PTPIP51 suppresses mitophagy while elevating mitochondrial respiration and calcium intake. We further show that MENCs facilitate stabilization of pro-viral mitochondria-mitochondria contact sites (MiMiCS), which accumulate as infection progresses and allow the visible exchange of bioenergetic potential. Cryo-electron tomography further reveals high-energy cristae alignments at MENC-MiMiCS. Moreover, the ability of MENCs to protect the bioenergetic capacity of aberrant mitochondria is not limited to virus infection, as we also observe MENC formation and stabilized metabolic potential at fragmented mitochondria in metastatic melanoma cells. Together, our findings highlight a mechanism whereby inter and intra-organelle contacts promote bioenergetic function in fragmented mitochondria, which we show is a relevant strategy in multiple disease states.

Rebecca Jones Ph.D., Princeton University

Metastatic melanoma is an invasive skin cancer that accounts for ~1% of all skin cancer cases but the vast majority of deaths: in 2022 it is estimated to cause over 7500 deaths in the USA alone. Despite this, the actual process by which metastasis occurs is poorly understood. To escape the epithelium into the underlying dermis and migrate through the body to form metastases, transformed melanocytes must first migrate across the epidermal basement membrane (BM). There are numerous hallmarks of cancer, yet only one factor clearly defines a metastatic cancer from a benign tumor, and that is invasion through a BM. Critically, it is difficult to predict which melanomas will breach the BM, as diagnostic biomarkers are lacking, because the process by which melanoma cells breach this otherwise impermeable barrier remains poorly understood. The key bottleneck in our understanding has been a lack of live imaging approaches in relevant model systems, with most studies being undertaken in cell culture or invertebrates. We have developed methods to perform long-term live imaging and automated cell tracking in embryonic mouse skin, and we propose to visualize mammalian BM breach, in vivo, live, for the first time. Using advanced CRISPR/Cas9 genome editing, we have developed a new mouse model expressing fluorescently tagged collagen IV, a key component of the BM from the endogenous locus. We will use live imaging techniques developed in our laboratory to directly visualize BM assembly and breach in the context of melanocyte infiltration during development and after BRafV600E-induced oncogenic transformation. In combination with scRNA-seq, we will identify BM breach associated gene signatures common to both WT and BRafV600E melanocytes, and finally, we will validate candidate invasion genes using in vivo lentiviral CRISPRi approaches. This work will not only allow us to visualize the dynamics of BM breach during a clinically important disease state, crucially, it will identify novel biomarkers, and potential therapeutic targets, for high risk/metastatic disease.

Angelo Kayser-Browne, Princeton University
Revealing the Nuanced Localization of Algal Pyrenoid Proteins via Super-Resolution Microscopy

The enzyme Rubisco drives the fixation of atmospheric CO2 into organic compounds and therefore serves as the origin of all photoautotrophically-derived biomass on Earth. Approximately ⅓ of global carbon assimilation occurs in the pyrenoid, a liquid-like algal organelle located within the chloroplast. In the green alga Chlamydomonas reinhardtii, proper function of the pyrenoid is dependent on a membranous, branching tubule network which traverses the organelle and is thought to deliver concentrated CO2 directly to Rubisco. The complex nanoscale architecture of this network has previously been elucidated via electron microscopy and electron cryotomography. However, its biogenesis and precise functional roles remain largely unexplored, in part because the diffraction limit of conventional confocal microscopy precludes the localization of tubule proteins from being determined with high fidelity. Using 2D STED and SoRa super-resolution imaging in conjunction with immunofluorescence and fluorescent tagging, respectively, we have determined the localization of numerous proteins thought to be associated with the tubule network to a previously unachieved degree of precision. Moreover, we show that many of these proteins have unique patterns of localization, suggesting that the tubule network has a high degree of heterogeneity that allows for location-specific functionality. An improved understanding of the tubule network and its protein components represents a crucial step toward engineering the

pyrenoid into higher plants to expedite crop growth. More broadly, our work demonstrates the viability of emerging super-resolution modalities as a method of probing the nuanced features of small organelles and their even-smaller subcompartments.

Arek Kulczyk Ph.D., Assistant Professor, Rutgers University

Laminin polymerization is the major step in basement membranes assembly. Its failures cause laminin N-terminal domain lamininopathies including Pierson syndrome. We have employed cryo-electron microscopy to determine a 3.7 Å structure of the trimeric laminin polymer node containing α1, β1 and γ1 subunits. The structure reveals the molecular basis of calcium-dependent formation of laminin lattice and provides insights into polymerization defects manifesting in human disease.

Megan Dilorio, Rutgers University

Recent advances in both instrumentation and image processing software have made single-particle cryo-electron microscopy (cryo-EM) the preferred method for structural biologists to determine high-resolution structures of a wide variety of macromolecules. Multiple software suites are available to new and expert users for image processing and structure calculation, which streamline the same basic workflow: movies acquired by the microscope detectors undergo correction for beam-induced motion and contrast transfer function (CTF) estimation. Next, particle images are selected and extracted from averaged movie frames for iterative 2D and 3D classification, followed by 3D reconstruction, refinement, and validation. Because various software packages employ different algorithms and require varying levels of expertise to operate, the 3D maps they generate often differ in quality and resolution. Thus, users regularly transfer data between a variety of programs for optimal results. This paper provides a guide for users to navigate a workflow across the popular software packages: cryoSPARC v3, RELION-3, and Scipion 3 to obtain a near-atomic resolution structure of the adeno-associated virus (AAV). We first detail an image processing pipeline with cryoSPARC v3, as its efficient algorithms and easy-to-use GUI allow users to quickly arrive at a 3D map. In the next step, we use PyEM and in-house scripts to convert and transfer particle coordinates from the best quality 3D reconstruction obtained in cryoSPARC v3 to RELION-3 and Scipion 3 and recalculate 3D maps. Finally, we outline steps for further refinement and validation of the resultant structures by integrating algorithms from RELION-3 and Scipion 3. In this article, we describe how to effectively utilize three processing platforms to create a single and robust workflow applicable to a variety of data sets for high-resolution structure determination.