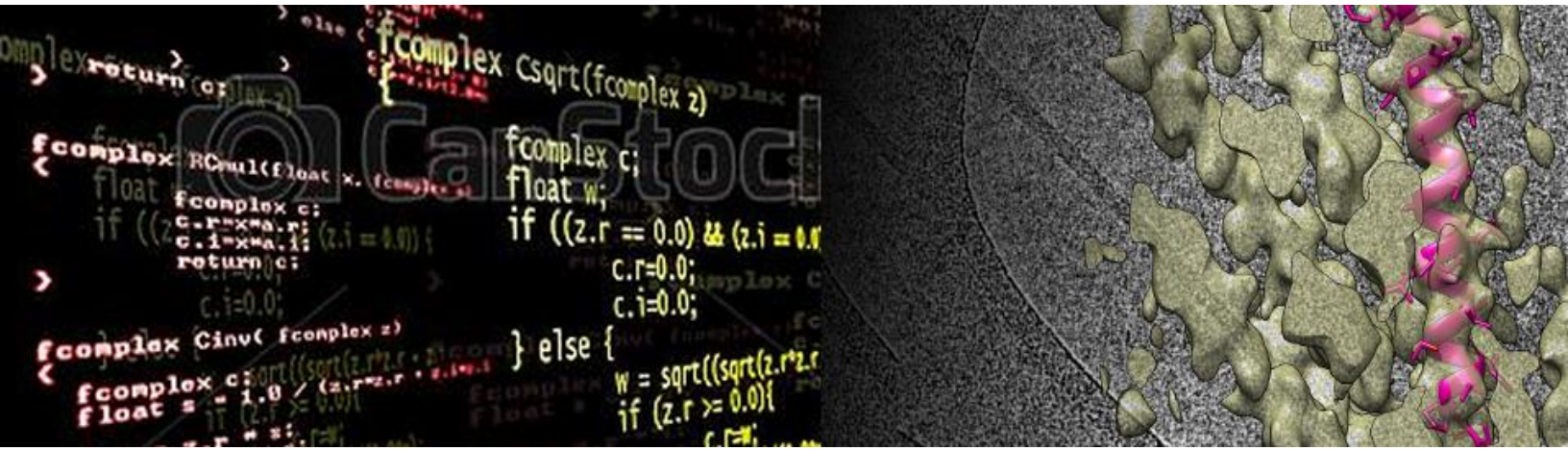
6TH INTERNATIONAL SYMPOSIUM ON CRYO-EM 3D IMAGE ANALYSIS 2026



March 18–21 2026
Granlibakken Conference Center & Lodge
Lake Tahoe, CA USA

Program Schedule
Abstracts

The Organizing Committee gratefully acknowledges financial support from the following sponsors:

SPONSORS

AGOURON INSTITUTE

MiTeGen
www.mitegen.com

SCINTILLON INSTITUTE
FOR BIOMEDICAL AND BIOENERGY RESEARCH

ThermoFisher SCIENTIFIC

JEOL

6th International Symposium on Cryo-3D Image Analysis 2026
March 18–21, 2026

Welcome to the 6th biennial CryoEM 3D Image Analysis Symposium to be held at Granlibakken in March 2026. As in the past, the symposium provides a GRC-like atmosphere with a strong focus on technical aspects of algorithms for image processing in CryoEM/CryoET. A significant amount of time in the schedule has been reserved for discussions, and there are several social events we encourage people to use for deeper discussions.

To help encourage open discussion and to avoid distractions during talks, please note that this meeting follows **a strict no photography and no audio/video recording policy.** The organizers will take a few non-science photos to document the event, and these will be shared on social media. If your vision prevents you from easily reading the screen, we encourage you to sit closer rather than pulling out your camera. The poster session, however, will permit opt-in photography. Each poster presenter will have the option of putting a tag on their poster indicating that photography of that specific poster is permitted. This should eliminate the need for participants to bring printed copies of posters they wish to disseminate more widely. If you see this tag (will include picture in schedule) on a poster, you may take pictures of it. Please respect the request of authors who request no photography. Non-photographic social media posts are also permitted. Please use the hashtag **#3DEMImgAn**.

ORGANIZERS

Dorit Hanein University of California Santa Barbara, CA USA

Steve Ludtke
Baylor College of Medicine
Houston, TX USA

ORGANIZING COMMITTEE

José-María Carazo Garcia
Centro Nacional de Biotecnología, Madrid, SPAIN

Edward Egelman
UVA Medical School
Charlottesville, VA, USA

Slavica Jonic
CNRS, Sorbonne Université, Paris, France

Masahide Kikkawa
University of Tokyo
Tokyo, JAPAN

Niels Volkmann
University of California Santa Barbara, CA USA

Table of Contents

Day 1 - Wednesday

March 18, 2026

Check-in (3:00 - 6:00 PM)

You will get your badge on check-in, there is no other on-site registration

Please put up your posters in the Bay Room before the evening session

No photography of scientific content is permitted during the sessions. The organizers will take photos which don't compromise this intent and share them.

5:00 - 6:00 PM Wine and Cheese Reception

Dinner (Granhall) 6:00 - 7:30

7:30 - 7:45 PM Welcome, Dr. Dorit Hanein (Meeting Chair)

7:45 - 8:45 PM Introduction, Dr. Ed Egelman (Session Chair)

Keynote: Dr. Bridget Carragher

Title: CryoEM and CryoET: Tools and Technologies from Origins to Outlook

Founding Technical Director

Chan Zuckerberg Imaging Institute

Social/Cash Bar (Cedar House) 8:45 - 11:00 PM

Day 2 - Thursday

March 19, 2026

Breakfast (Granhall) 7:00 - 8:00

Session 1 (Mountain Lake)

In vitro Compositional and Conformational Variability

8:00 - 8:15 AM **Introduction, Dr. Steve Ludtke (Session Chair)**

8:15 - 8:35 AM **Dr. Sonya Hanson**

The Inaugural Flatiron Institute Cryo-EM Conformational Heterogeneity Challenge

Flatiron Institute

8:40 - 9:00 AM **Dr. Roy Lederman**

On a Flaw in EM

Yale University

9:05 - 9:25 AM **Dr. Shigeyuki Matsumoto**

Identifying thermodynamically distinct structural states and their transition pathways from cryo-EM data using a deep isometric autoencoder

Graduate School of Medicine, Kyoto University

9:30 - 9:50 AM Dr. Joey Davis

Resolving structural heterogeneity in complex mixtures MIT

Coffee Break 10:00 - 10:30 AM

Session 2 (Mountain Lake)

In situ Compositional and Conformational Variability

10:30 - 10:40 AM Introduction, Dr. Slavica Jonic (Session Chair)

10:45 - 11:05 AM Dr. Alberto Bartesaghi

Studying structure heterogeneity in situ using nextPYP

Duke University, Durham, NC, USA

11:10 - 11:30 AM Dr. Friedrich Forster

PyTom-match-pick integrated into pipeline for structure determination in situ

Utrecht University, Utrecht, Netherlands

11:35 - 11:45 AM Dr. Jessica Heebner

Data-Driven Deep Learning for Robust Membrane Segmentation in Cryo-Electron Tomography

TFS (Vendor short talk)

11:50 - 12:10 AM Dr. Carsten Sachse

Quantification of membrane structures from cryo-EM micrographs and tomograms

Forschungszentrum Jülich, Jülich, Germany

Lunch (Granhall) 12:15 - 1:15

Afternoon Free Until Posters

4:00 - 6:00 PM Poster Session (posters should be up Wed - Fri)

Wine and Cheese Reception (Bay Room)

4:00 - 5:00 PM Round Table - Deep learning impact on community innovation
Masahide Kikkawa (Chair)
Panelists:
Jianlin Cheng, University of Missouri
Reinhard Heckel, Tecnical Univesity of Munich
Alberto Bartesaghi, Duke University

Dinner (Granhall) 6:00 - 7:30

Session 3 (Mountain Lake)

Automation

7:30 - 7:45 PM Introduction, Dr. Dorit Hanein (Session Chair)

7:45 - 8:05 PM Dr. Sebastian Tacke

Need for speed: cryo-plasma focused ion beam milling on the road to a high-throughput cryo-electron tomography pipeline

Max Planck Institute of Molecular Physiology, Dortmund Germany

8:10 - 8:30 PM Dr. Rado Danev

Advancing cryo-EM throughput and performance

The University of Tokyo, Tokyo Japan

8:35 - 8:55PM

Dr. Toshio Moriya

GoToCloud: optimization of cloud computing environments for accelerating cryo-EM structure-based drug design

High Energy Accelerator Research Organization (KEK), University of Tsukuba, Ibaraki, Japan

Social Hour/Cash Bar (Cedar House) 9:30 - 11:00 PM

Day 3 - Friday

March 20, 2026

Breakfast (Granhall) 7:00 - 8:00

Session 4 (Mountain Lake)

Modeling & Validation in CryoEM/ ET

8:00 - 8:15 AM — Introduction, Dr. José María Carazo (Session Chair)

8:15 - 8:35 AM — Dr. Pintilie, Greg

Q score as a *validation metric for 3DEM* maps *and models*

Department of Bioengineering, Stanford University

8:40 - 9:00 AM — Dr. Hemant Tagare

New Methods for Evaluating Resolution Anisotropy

Dept. of Radiology and Biomedical Imaging, Yale University.

9:05 - 9:25 AM — Dr. Jianlin Cheng

Deep Learning Approaches to Building Protein Structures from Cryo-EM Maps *and AlphaFold Predictions*

Department of Electrical Engineering and Computer Science, NextGen Precision Health, University of Missouri, Columbia, MO, USA

9:30 - 9:50 AM Dr. Arjen Jakobi

Confidence-weighted and context-aware cryoEM map optimisation

Kavli Institute of Nanoscience Delft & Delft University of Technology (The Netherlands)

Group Photo 10:00 - 10:10

Coffee Break 10:10 - 10:20 AM

Selected Posters Posted

If you have a poster, CHECK THE SCREEN

Session 5 (Mountain Lake)

Tomogram Segmentation and Interpretation

10:30 - 10:45 AM Introduction, Dr. Niels Volkmann (Session Chair)

10:45 - 11:05 AM Dr. Khanh Huy Bui

Uncovering Protein Domains at Intermediate Resolution McGill University (Canada)

11:10 - 11:30 AM Dr. William Wan

Geometric Approaches for Tilt-Series Refinement in STOPGAP

Vanderbilt University

11:35-11:55 AM

Dr. Reinhard Heckel

Towards Automated cryo-ET: Self-supervised Tomogram Reconstruction and Promptable Particle Picking

Technical University Munich (Germany)

12:00-12:10 PM

Dr. Jan Kosinski

Modeling of macromolecular-membrane systems from cryo-ET data

European Molecular Biology Laboratory (EMBL), Hamburg (Germany)

Lunch (Granhall) 12:20-1:20

Afternoon Free Until Posters

Wine and Cheese Reception (Bay Room) 4:00 - 6:00

4:00-5:15 PM

Poster Session (posters should be up Wed - Fri)

5:15-6:00 PM

Vendors' Session (Mountain Lake)

Advances in cryo-ET and data visualization (Dr. Eliza Nieweglowska and Dr. Mark McClendon, TFS)

The JEOL cryoARM for High-Throughput Cryo-EM (Dr. Emmanuel W. Smith, JEOL).

Dinner (Granhall) 6:00 - 7:30

Session 6 (Mountain Lake)

Selected Poster talks

7:30 - 9:30 Masahide Kikkawa (Chair)

Selected poster talks, each 10 min + 5 for questions

Social Hour/Cash Bar (Cedar House) 9:30 - 11:00 PM

Saturday- Departures

March 21, 2026

Breakfast (Granhall) 7:00 - 8:00

Check Out - Departures

Increasing signal in Biological Cryo-Lamella

Laina N Hall, Joshua Paul, Bronwyn Lucas

The application of focused ion beam (FIB) milling to frozen hydrated cells has made it possible to visualize thick cells and tissues with cryogenic electron microscopy (cryo-EM)1,2. However, standard protocols for Gallium liquid metal ion source (LMIS) and plasma FIB-milling cause damage extending ~30-60 nm from each lamella surface3–6, limiting the usable lamella volume. Limiting FIB damage is essential to realize the goals of in situ structural biology including unambiguously identifying single molecules in cells and determining the 3D structure of proteins to near-atomic resolutions. We sought to identify conditions that minimize FIB-damage in biological lamellae by using two-dimensional template matching (2DTM)7,8 to measure single molecule damage profiles and directly compare different ions and accelerating voltages. In contrast to prior reports we demonstrate that Xenon plasma FIB-milled lamellae show significantly reduced lamella damage relative to lamellae prepared with other ions. We further identify conditions to improve particle detection and increase signal in resulting lamellae by polishing with lower acceleration voltages. We show that the reduction in damage unlocks the benefit of thinner lamella.

Automated cryo-EM data processing of GPCRs with CryoSPARC

CryoSPARC Team - Structura Biotechnology Inc.

Single particle cryo-electron microscopy (cryo-EM) is increasingly used in structure-based drug design settings to determine the structure of a particular target molecule bound to various ligands. A key challenge in this context is automating structure determination in a manner that minimizes user intervention and the time spent on data processing steps that are common across multiple datasets, while making use of existing information about the target in a reliable and unbiased manner. We describe the algorithmic, software and workflow developments that were required to construct a processing system that is able to process nearly two dozen publicly deposited G protein-coupled (GPCR) datasets in a completely automated manner within CryoSPARC, with the goal of obtaining equal or better resolution and map quality as manually processed, deposited results. We present challenges and lessons learned, with the aim of enabling practitioners to construct their own automated workflows for repeat structure determination.

The graphene-based affinity cryo-EM grid for the endogenous protein structure determination

Sojin An, Eungjin Ahn, Tyler Koo, Soyoung Park, Boeon Suh, Krishna P. Rengasamy, Gaocong Lyu, Cheal Kim, Byungchul Kim, Hanseong Kim, Sangho Park, Dongyan Tan, and Uhn-Soo Cho*

Following recent advancements in cryo-electron microscopy (cryo-EM) instrumentation and software algorithms, the next bottleneck in achieving high-resolution cryo-EM structures arises from sample preparation. To overcome this, we developed a graphene-based affinity cryo-EM grid, the Graffendor (GFD) grid, to target low-abundance endogenous protein complexes. To maintain grid quality and consistency within a single batch of 36 grids, we established a one-step crosslinking batch-production method using genetically modified ALFA nanobody as affinity probe (GFD-A grid). Using low concentrations of β-galactosidase-2xALFA, we demonstrated the GFD-A grid's efficiency in capturing tagged proteins and resolving its cryo-EM structure at 2.71 Å. To test its application for endogenous proteins, we engineered yeast cells with a C-terminal tandem affinity tag (3xALFA-Tev-3xFlag: ATF) at Pop6, a shared component of RNase MRP and RNase P. Cryo-EM structures of RNase MRP and RNase P were resolved at 3.3 Å and 3.0 Å from cell lysates, and 3.6 Å and 3.9 Å from anti-flag elution, respectively. Notably, additional densities were observed in the structures obtained from cell lysates, which were absent in those from the anti-FLAG eluate. These findings establish the GFD-A grid as a robust platform for investigating endogenous proteins, capable of capturing transient interactions and enhancing the resolution of challenging cryo-EM structures with greater efficiency.

MissAlignment learns to straighten out tilt series

Marten Chaillet*, Joyce van Loenhout, Miguel R. Leung, Alister Burt, Dimitry Tegunov

Cryo-electron tomography (cryo-ET) and subtomogram averaging can reveal how proteins organize and interact in their native cellular environment at molecular resolution. Our ability to routinely extract structural insights from cryo-ET data is limited by the accuracy of initial tilt series alignment. Existing algorithms for reference-free alignment are prone to failure due to incorrect assumptions about the sample thickness and shapes of the features in a sample. Meanwhile, reference-based alignment algorithms require accurate initial particle pose estimates of evenly-distributed, bulky macromolecules. We introduce MissAlignment, a novel machine-learning approach for reference-free tilt-series alignment. Our method optimizes sample motion parameters by minimizing a misalignment score generated by a small 3D CNN that is trained contrastively to score alignment quality. Multiple rounds of inference followed by retraining the model, gradually improves the alignments of a dataset. We demonstrate using both simulated and experimental data that MissAlignment consistently produces near optimal rigid-body 3D reconstructions, outperforming standard methods in the field. Additionally, MissAlignment is capable of accurately modelling local sample deformations and propagation of this data to downstream subtomogram averaging improves the resolution of macromolecular structures reconstructed from the data. Our tool provides accurate automated tilt-series alignment for diverse cryo-ET samples.

Structural dissection of αβ- tubulin heterodimer assembly and disassembly by human tubulin–specific chaperones

Yeonjae Seong*, Hyunmin Kim, Kyumi Byun, Yeon-Woo Park, Soung-Hun Roh

Microtubule assembly requires a set of chaperones known as tubulin-binding cofactors (TBCs). We used cryo-electron microscopy to visualize how human TBCD, TBCE, TBCC, and GTPase Arl2 mediate αβ-tubulin assembly and disassembly. We captured multiple conformational states, revealing how TBCs orchestrate tubulin heterodimer biogenesis. TBCD stabilizes monomeric β-tubulin and scaffolds the other cofactors. GTP binding to Arl2 induces conformational changes that toggle the complex between assembly and disassembly. TBCD and TBCE guide α- and β-tubulin into a partially assembled interface, while TBCC, acting as a molecular clamp, completes the heterodimer. TBCD also functions as a GTPase-activating protein for β-tubulin. β-tubulin GTP hydrolysis is coupled to Arl2's GTPase activity, establishing a checkpoint that ensures only fully matured heterodimers proceed. These findings provide a structural framework for tubulin heterodimer biogenesis and recycling, supporting cytoskeletal proteostasis.

Mechanistic Study of Adenovirus Packaging via Cellular Cryo-Electron Microscopy and Mutant Virion Analysis

Jasmine Larrick*, Ruchao Peng, Matthew Charman, Tingting Chung, Yi-Wei Chang, Matthew Weitzman

Adenoviruses (AdV) rely on a coordinated process to package their double-stranded DNA (dsDNA) genome into infectious progeny for cell-to-cell transmission. Although the essential packaging proteins are known, the coordination between capsid formation and genome replication remains unclear. Two competing models have been proposed: the sequential and concurrent models. A growing body of evidence has favored the concurrent packaging model, where the capsid forms around the condensing dsDNA genome; however, the definitive mechanism of packaging remains a mystery. During infection, AdV dramatically remodels the host-cell nucleus to form viral replication compartments (VRCs) where dsDNA genomes are replicated. Our lab recently discovered another virus-mediated nuclear compartment, termed nuclear bodies (NBs), that border VRCs and recruit viral capsid proteins. Disruption of NBs leads to defective packaging, suggesting that NBs may serve as organizational hubs that bring capsid proteins into proximity with newly replicated viral genomes for packaging. Some evidence suggests that dense DNA bundles form in the periphery of VRCs near capsids, but these data are limited in their resolution and lack spatial context in relation to NBs. Thus, based on these data, I hypothesized that nascent virions are produced at the interface of NBs and VRCs via a concurrent assembly mechanism. The study of AdV packaging is challenging because purification-based approaches lose transient assembly intermediates, and no cell-free system has been developed due to the challenge of recapitulating the complex nuclear environment. To address these limitations, I am using advanced cryo-electron microscopy and purification methods to dissect the mechanism of AdV packaging. In Aim 1 of my project, I am using cryo-volume scanning electron microscopy and cryo-electron tomography (cryo-ET) to determine the location and detailed structure of nascent virions within infected nuclei. My preliminary findings have found heterogenous collections of virions beginning near NBs which likely represent viral packaging factories, a promising target for downstream high-resolution investigation. In Aim 2 of my project, I am addressing the challenge of losing transient intermediate virions by testing GraFIX, a unique purification approach which has not been applied to AdV, to isolate WT and mutant virions. Thus far, I have validated components of this alternative purification protocol in WT AdV, showing that I can isolate a spectrum of intermediate and mature virions from infected cells. I have also created, purified, and analyzed mutant viruses which are arrested during distinct steps in the packaging process. My initial findings suggest that capsid protein proteolysis is spatially or temporally partitioned, with further biochemical and structural characterization of intermediate virions underway. Through the completion of my project, I will utilize fluorescently labeled cells and viruses to perform in situ cryo-ET to directly visualize virions inside infected host cells. I will also explore the biochemical determinants of stalled mutant viral packaging. This integrated approach will help elucidate the mechanism of AdV packaging, laying the groundwork for future structure-guided therapeutic development.

GoToCloud Project: Development and Future of the Cloud-Based Platform for Cryo-EM Facilities in Japan

Toshio Moriya, Yusuke Yamada, Tsukasa Nakamura, Yuki Fujii, Satomi Inaba, Yasuomi Miyashita, Masato Kawasaki, Mikio Tanabe, Toshiya Senda

Cryo-EM Single-Particle Analysis (SPA) has revolutionized Structure-Based Drug Design (SBDD). This technique enables the visualization of proteins with atomic-level detail, including membrane proteins and supramolecular complexes that are often difficult to crystallize. While Cryo-EM facilitates the rational design of therapeutics through the detailed mapping of ligand-binding pockets and conformational changes, the field faces a significant bottleneck in computational throughput due to the massive, terabyte-scale data volumes involved. Conventional on-premise computing and manual workflows are increasingly unable to satisfy the speed and precision required for modern pharmaceutical research. To address these challenges, the Structural Biology Research Center at the High Energy Accelerator Research Organization (KEK-SBRC) has launched the "GoToCloud" project. This initiative establishes an Internet of Things (IoT) network connecting Cryo-EM facilities in Japan using the PREWS Cluster at The University of Osaka and a cloud-computing as a hub to achieve high-throughput, fully automated data analysis. By enabling the rapid construction of large-scale protein-compound databases and big data analysis, the GoToCloud platform is expected to serve as a common technical foundation to support all Cryo-EM facilities and accelerate the discovery of novel drugs. This presentation provides an overview of the GoToCloud project and its achieved milestones.

Unbend: Correction of local beam-induced sample motion in cryo-EM images using a 3D spline model

Lingli Kong, Ximena Zottig, Johannes Elferich, Nikolaus Grigorieff

The exposure of frozen biological samples to the high-energy electron beam in a cryo-electron microscope commonly leads to beam-induced sample motion and distortions. Previously, we described Unblur, which is part of our cisTEM software to correct for beam-induced motion based on the alignment of full frames in a movie collected during the beam exposure. However, Unblur cannot accommodate motion due to more localized sample bending and distortions. Here, we present Unbend, extending Unblur by incorporating local motion correction using a three-dimensional cubic spline model. The 3D spline model is constructed using cubic B-splines along the exposure time axis, and bicubic B-splines within movie frames. Unbend is integrated into our cisTEM software with a new local motion visualization panel within the cisTEM graphical user interface. We processed movie frames from various in-situ sample types, including whole cells, lamellae, and cell lysates, to analyze motion behavior across different specimen types. To quantify the improvement in high-resolution signal, we utilized the 2D template matching method, which operates independently of the motion correction process, to search large ribosomal subunits from the motion-corrected micrographs. Overall, the signal-to-noise ratio of detected particles improved by 3 ~ 8% across different samples compared with full-frame aligned micrographs, while the number of detected target particles increased by up to ~ 300%. The total and Von Mises equivalent strain shows a deformation scale of less than 1% in most of the samples, confirming that our model induces minimal additional distortion. Furthermore, we processed micrograph montages to study motion patterns across an entire sample, revealing considerable variance in distortion scale within the same sample, suggesting a complex underlying mechanism.

Improving Ligand Elucidation in Cryo-EM reconstructions

Federico P. de Isidro-Gomez*, Michael Saur, Carlos Oscar Sorzano, Jose María Carazo.

Structure and fragment-based drug discovery (SBDD/FBDD) are widely used techniques for the pharmaceutical industry, enabling the development of drugs to treat a wide range of diseases. Recent advancements in cryo-EM have enabled the high-resolution structure determination of many specimens that remained challenging for alternative techniques. This progress positions cryo-EM as a reliable structural method for supporting SBDD and FBDD projects, capable to both identify initial hits and guide structure-based design [1]. However, a major challenge for cryo-EM remains the accurate definition of ligands in reconstructions. Compromised elucidation restricts detailed protein–ligand interaction analysis and may hamper structure-guided design. Our research focuses on improving ligand detection and characterization to provide critical insights into ligand location, occupancy, and pose.

SNARE disassembly requires Sec18/NSF side loading

Yousuf A. Khan, K. Ian White, Richard A. Pfuetzner, Bharti Singal, Luis Esquivies, Garvey Mckenzie, Fang Liu, Katherine DeLong, Ucheor B. Choi, Elizabeth Montabana, Theresa Mclaughlin, William T. Wickner & Axel T. Brunger

SNARE (soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor) proteins drive membrane fusion at different cell compartments as their core domains zipper into a parallel four-helix bundle. After fusion, these bundles are disassembled by the AAA+ (ATPase associated with diverse cellular activities) protein Sec18/NSF and its adaptor Sec17/α-SNAP to make them available for subsequent rounds of membrane fusion. SNARE domains are often flanked by C-terminal transmembrane or N-terminal domains. Previous structures of the NSF–α-SNAP–SNARE complex revealed binding to the D1 ATPase pore, posing a topological constraint as SNARE transmembrane domains would prevent complete substrate threading as suggested for other AAA+ systems. Using mass spectrometry in yeast cells, we show N-terminal SNARE domain interactions with Sec18, exacerbating this topological issue. We present cryo-electron microscopy (cryo-EM) structures of a yeast SNARE complex, Sec18 and Sec17 in a nonhydrolyzing condition, which show SNARE Sso1 threaded through the D1 and D2 ATPase rings of Sec18, with its folded, N-terminal Habc domain interacting with the D2 ring. This domain does not unfold during Sec18/NSF activity. Cryo-EM structures under hydrolyzing conditions revealed substrate-released and substrate-free states of Sec18 with a coordinated opening in the side of the ATPase rings. Thus, Sec18/NSF operates by substrate side loading and unloading topologically constrained SNARE substrates.

Structural Characterization of Native RNA Polymerase II Transcription Complexes and Nucleosomes in Drosophila melanogaster

Structural studies of eukaryotic RNA polymerase II (Pol II) transcription often rely on in vitro assembly, which may not fully represent native conditions. To investigate Pol II transcription in metazoan cells, we developed a method to isolate native transcription complexes from Drosophila melanogaster embryos using FLAG-tag affinity purification and Micrococcal Nuclease treatment. Cryo-EM and proteomics studies revealed diverse transcription complexes and nucleosomes, including a metazoan Rpb4/Rpb7 stalk-less elongation complex and a hexameric nucleosome lacking an H2A/H2B dimer. Notably, nucleosome is found only downstream of the nucleosome elongation complex, underscoring it as a major energy barrier and a time-consuming step during Pol II progression through chromatin. Proteomics identified co-purified factors involved in transcription initiation, elongation, and RNA modification. This study provides a framework for investigations of transcription in cells, paving the way for future studies of transient and minor complexes.

Automated Detection of Bacterial Flagellar Motors

Eben Lonsdale*, Braxton Owens, Gus Hart

With advances in cryogenic electron tomography, the ability to study bacterial structures in their cellular context has improved. However, 3D images of bacteria, called tomograms, have a low signal-to-noise ratio. This makes annotating structures of interest difficult, as traditional computer vision models struggle with tomograms and manual annotation is time consuming. We build on the results of the BYU Kaggle competition to create an ensemble model capable of automatically annotating flagellar models with nearly 85% accuracy, showing that machine learning is a viable way to automatically annotate bacterial structures.

Twist and Scout: Analysis and Curation of Particles in Cryo-ET Using TANGO

Markus Schreiber*, Beata Turonova

Cryo-electron tomography (cryo-ET) enables the visualization of cellular structures in near-native environments, but its potential for spatial analysis has been underutilized due to a lack of versatile tools accommodating biological sample diversity. Available solutions often rely on case-specific or hypothesis-driven approaches, while holistic analyses remain challenging. In this work, we introduce TANGO (Twist-Aware Neighborhoods for Geometric Organization), a framework leveraging point cloud descriptors to analyze spatial arrangements of particles, such as macromolecular complexes, in cryo-ET. By encoding relative positions and orientations of particles as twist vectors, TANGO enables rotationally invariant feature extraction, including structured neighborhood occupancy, lattice topology, or angular deviations. Its modular design and user-friendly interface allow for customization of features, facilitating exploratory analyses of spatial patterns in diverse experimental datasets. With its open-source Python implementation, TANGO advances the ability to decode complex cellular architectures and their functional relationships, offering a particle data analysis tool for the cryo-ET community.

Embrella: Integrated Data Tracking and Workflow Orchestration for Cryo-Electron Tomography

Yue Yu*, David Dong, Michael Souza, Pallavi Khedle, Utz Ermel, Yongbaek Cho, Shawn Zheng, Ariana Peck, Jonathan Schwartz, Anchi Cheng†

Processing cryo-electron tomography (cryo-ET) data relies on multi-stage workflows. Ensuring metadata consistency and analysis reproducibility is crucial for scalable cryo-ET. Here, we present Embrella, an integrated data tracking and workflow orchestration platform for managing cryo-ET data from sample preparation through data processing and curation. In Embrella, biological samples are registered with ontology-aware metadata and linked to data collections with experimental parameters systematically tracked. Preprocessing parameters and results are logged throughout the pipeline, enabling data provenance and analysis reproducibility. Embrella supports both automated and human-in-the-loop curation by combining preprocessing-derived metrics with visual inspection of tomograms. Annotations are tracked using Copick as a backend, providing a cross-platform, storage-agnostic dataset API. The Embrella web interface unifies workflow execution by supporting job submission for preprocessing using aretomo3 and denoisET, as well as annotation through deep learning–based tools Octopi and MemBrain. By streamlining these steps within a single system, Embrella reduces operational overhead and enables consistent, traceable analysis workflows. We demonstrate its utility through a benchmark study evaluating the effect of the Volta Phase Plate (VPP) in cryo-ET, with all data processing and curation managed in Embrella. Ongoing development includes support for preparing deposition to public data repositories, as well as resource packaging and distribution of Embrella to the cryo-ET community.

Fine-tuning DINOv3 for a Foundational Model in CryoET

Doing traditional supervised learning in order to find parts of CryoET tomograms is a technically complex and time consuming task. Meta AI's DINOv3 is a cutting-edge computer vision model trained to notice naturally occurring patterns on a massive dataset of real-world images. Since no tomograms or similar images were included in its corpus, it fails to recognize the minute low-resolution differences that distinguish structures of interest. Fine-tuning DINOv3 on a dataset of tomograms from the CZII CryoET portal results in a model adept at extracting the signal from tomograms outside of its training set. This fine-tuned model can then be used on a new dataset to extract a small feature vector for each tomogram that can be processed and analyzed via simpler machine learning methods, drastically reducing training time and simplifying the technical process.

Leveraging particle denoising and high-order implicit neural representations for cryo-EM heterogeneous reconstruction

Jiahua He*, Yifan Cheng

Resolving conformational dynamics and compositional variability is central to understanding biomolecular mechanisms. Cryo-electron microscopy (cryo-EM) enables visualization of biomolecular ensembles, but recovering continuous structural variability and mixed compositions from noisy two-dimensional (2D) particle images remains challenging. In this study, we introduce DINHR (Denoising Implicit Neural High-order Representation), a deep learning framework that couples self-supervised particle denoising with a high-order implicit neural representation for heterogeneous reconstruction. DINHR jointly trains a spatial-spectral dual-domain U-Net denoiser and a high-order INR hypervolume to learn a noise-robust mapping from a low-dimensional latent space to particle-specific three-dimensional (3D) density volumes. On simulated CryoBench datasets with known ground truth, DINHR achieves accurate recovery of both latent geometry and 3D volumes and compares favorably with existing methods. We further apply DINHR to experimental datasets including the U4/U6.U5 tri-snRNP, the 50S ribosomal assembly intermediates, and the INO80-hexasome complex. In each case, the inferred ensembles reproduce dominant modes of variation or structural states consistent with prior analyses, remain stable in the presence of impurities, and reveal additional fine-scale variability.

SegSort: Interactive Object Discovery in Volumetric Imaging

Braxton Owens*, Jackson Pond, Gus Hart, Bryan Morse

We introduce SegSort, a human-in-the-loop framework for scalable segmentation and object discovery in volumetric imaging data. The approach combines automatic slice-wise segmentation with embedding-based representations to organize candidate objects across large datasets. By clustering these representations, the method enables users to efficiently explore and identify structures of interest through low-effort interaction, rather than exhaustive manual annotation. User selections are incorporated in real time to iteratively refine the system's priors, progressively focusing the search on relevant objects throughout the volume. This interactive loop allows rapid adaptation to new targets without task-specific retraining, supporting exploratory analysis in heterogeneous and noisy data. While motivated by challenges in cryo-electron tomography, SegSort is designed as a general-purpose, open-source tool applicable to a wide range of volumetric imaging modalities.

FactorEM: Fast and robust heterogeneity analysis in CryoEM though Orthogonal Group Synchronization

O.L. Zarrabeitia*, J.L. Vilas, M. Iceta, J.M. Carazo, C.O.S. Sorzano

Understanding structural heterogeneity is a key challenge in Cryo-Electron Microscopy (Cryo-EM). We propose a novel method that leverages the natural similarity between neighboring projection directions to perform a localized Principal Component Analysis (PCA) in directional subsets of the data. These local analyses are then globally synchronized using Orthogonal Group Synchronization, a novel field in applied mathematics. Our approach is designed to pose very few execution parametes, reducing the need for extensive user tuning. The parameters involved have clear physical and statistical interpretations, enhancing their usability and transparency. In addition, it is also computationally efficient, achieving significantly faster execution times compared to conventional heterogeneity analysis methods. We validate our approach on both simulated and experimental datasets exhibiting compositional and conformational heterogeneity, demonstrating its effectiveness in capturing structural variability.

Evaluation and operation of an automatic SPA data collection workflow with SmartScope

Hiroyuki Harigaya*, Fumiaki Makino, Naoki Hosogi

In this study, we performed data acquisition using SmartScope and compared it with SerialEM, which is widely used as image acquisition software. We report the results of this comparison and also introduce the strategies we implemented to fully leverage the capabilities of our newly developed cryo-electron microscope by taking advantage of SmartScope’s extensibility.

Locating Macromolecules within Crowded Cell Environments with Deep Representation Learning

Kithmini Herath*, Bronwyn Lucas

Studying macromolecules in their native cellular environment is crucial for understanding molecular function. Cryogenic electron microscopy (cryo-EM) images capture complexes directly inside frozen-hydrated cells and tissues and retain high-resolution features that are absent from tomograms with the benefit of faster data collection and processing. However, the crowded cellular environment and low signal-to-noise ratio in cryo-EM images make identifying macromolecules challenging. Supervised machine learning methods for object detection have been effectively applied to the problem of particle picking in single particle cryo-EM. However, we observed that when trained on previously identified ribosomes in images of untilted cellular lamellae, these methods fail to detect particles of interest, likely due to the effective loss of low-resolution contrast in crowded cell environments. Scaling supervised learning methods to new data faces the additional bottleneck of the high cost of annotation. To address these limitations, we developed a self-supervised, contrastive learning method for particle localization in situ. Our approach first pre-trains a deep neural encoder on large, unannotated datasets of patches of cellular cryo-EM images to learn relevant structural features. This pre-trained encoder is then finetuned with a minimal number of labels to classify image patches and identify particles of interest. We demonstrate that our self-supervised model performs on-par with, and in some cases outperforms, supervised methods in distinguishing ribosomes from background in in situ data. Crucially, our model generalizes better to unseen datasets and offers significantly higher throughput relative to 2D template matching (2DTM). By enabling fast, template-free particle localization directly within cellular images, this approach advances our ability to explore complex biological systems.

Cryo-electron tomography of Nipah virus structural protein complexes in virus-like particles

Viraj V. Upadhye*, Jean F. Lee, Nihan Ercanli, Clifton Ricana, Amy C. Hinsley, Martin Obr, Ludovic Autin, Florian K. M. Schur, Hector C. Aguilar, Robert A. Dick

Paramyxoviruses present major public health threats, yet the structures of their full-length viral proteins in virions remain obscure. The matrix protein M orchestrates virion assembly, hypothetically via lattice formation, bringing together transmembrane glycoproteins F and G/H/HN and cytoplasmic nucleocapsid protein N. Using cryo-electron tomography and systematic assembly of virus-like-particles, we visualized protein:protein and protein:membrane complexes of Nipah virus, a BSL-4 zoonotic paramyxovirus with ~75% human mortality. This analysis generated the first paramyxoviral M lattice structure, at 7Å resolution. Remarkably, the M lattice contains two alternating distinct tetramers of dimers, with F trimers occupying only one tetramer type. G and N promote M lattice curvature and particle pleomorphism. This first comprehensive visualization of full-length paramyxoviral structural proteins in virions also illuminates the origins of pleomorphism.

Synthetic data-driven deep learning for robust membrane segmentation in cryo-electron tomograpy

Rachida Seghiri, Juan Diego Gallego Nicolás, Robert Brandt, Miguel A. Meroño, Pierre Lefevre, Noushin Hajarolasvadi, Daniel Baum, Jessica Heebner*, Harold Phelippeau, Pascal Doux, Antonio Martinez-Sanchez

The quantitative analysis of biological membranes from cryo-electron tomography (cryo-ET) data is hindered by imaging artifacts, noise, and the complexity of membrane morphologies. The missing wedge remains a critical barrier because it erases regions of membranes oriented outside of the tilt range which prevents complete annotation. Current deep learning models can achieve strong results but rely on incomplete and error-prone manual annotations, leading to variable generalizability across biological systems and little recovery of the data lost from the missing wedge. Here, we introduce a nnUNet model trained exclusively on a novel synthetic dataset generated by an extended version of the PolNet simulator that can generate considerably more realistic membranes. Importantly, this nnUNet trained only on synthetic data achieves competitive or superior accuracy compared to models trained on manually annotated real data. Moreover, our model demonstrates enhanced robustness across diverse biological contexts and successfully recovers membranes degraded by the missing wedge artifact. This work establishes a framework for the systematic design of synthetic training data using PolNet, enabling more accurate, generalizable, and artifact-aware deep learning models for segmentation of cryo-ET data.

Pre-fusion AAA+ remodeling of target-SNARE protein complexes enables synaptic transmission

K. Ian White*, Yousuf A. Khan, Axel T. Brunger

Synaptic vesicles containing neurotransmitters fuse with the plasma membrane upon the arrival of an action potential at the active zone. Multiple proteins organize trans-SNARE complex assembly and priming, leading to fusion. One target membrane SNARE, syntaxin, forms nanodomains at the active zone, and another, SNAP-25, enters non-fusogenic complexes with it. Here, we reveal mechanistic details of AAA+ protein NSF (N-ethylmaleimide sensitive factor) and SNAP (soluble NSF attachment protein) action before fusion. We show that syntaxin clusters are conserved, that NSF colocalizes with them, and characterize SNARE populations that may exist within or near them using cryo-EM. Supercomplexes of NSF, α-SNAP, and either a syntaxin tetramer or one of two binary complexes of syntaxin—SNAP-25 reveal atomic details of SNARE processing and show how sequential ATP hydrolysis drives disassembly. These results suggest a functional role for syntaxin clusters as reservoirs and a corresponding role for NSF in syntaxin liberation and SNARE protein quality control preceding fusion.

The Low Voltage Method

Laina N. Hall, Bronwyn Lucas

The application of focused ion beam (FIB) milling to frozen hydrated cells has made it possible to visualize thick cells and tissues with cryogenic electron microscopy (cryo-EM). However, standard protocols for Gallium and plasma FIB-milling cause damage extending 30–60 nm from each lamella surface, limiting usable lamella volume and negating the benefit of thin samples. Limiting FIB damage is essential to realize the goals of in situ structural biology including unambiguously identifying single molecules in cells and determining the 3D structure of proteins to near-atomic resolution. To identify optimal conditions for generating thin lamellae with minimal damage, we use two-dimensional template matching (2DTM) to directly compare damage resulting from milling with different ions and accelerating voltages at single molecule resolution.. In contrast to prior reports, we demonstrate that ion choice significantly affects FIB damage. We detail a method to reproducibly generate thin (50-100nm) lamella while limiting FIB damage to <10 nm using readily available commercial hardware. By making it possible to generate thin lamellae with minimal damage we extend the sensitivity of 2DTM to reliably detect macromolecules of <300 kDa, expanding the visual proteome.

MOSAICS enables quantitative macromolecular structure characterization of rare complexes in situ

Matthew Giammar* and Bronwyn Lucas

Cryogenic electron microscopy (cryo-EM) and tomography (cryo-ET) are powerful tools for visualizing the structure of biological macromolecules in situ. However, current computational methods require tens- to hundreds-of-thousands of particles for reliable identification of small molecular features, thus limiting their application to highly abundant cellular complexes or requiring large datasets, precluding characterization of rare complexes. We present MOSAICS (molecular in situ atomic coordinate scanning) as a quantitative strategy that leverages 2D template matching (2DTM) to directly compare molecular models against cryo-EM images. MOSAICS can identify structural differences between molecular populations in situ using far fewer particles than required for classical 3D reconstruction techniques – extending in situ structural analysis beyond only highly abundant cellular complexes. Applying MOSAICS to a dataset of <300 nuclear 60S ribosome maturation intermediates in yeast, we recapitulate rRNA conformational changes and find that the order of ribosome biogenesis factor reorganization differs from in vitro models. MOSAICS is available as an open-source, easily installable Python package alongside Leopard-EM, an extensible Python package for running GPU-accelerated 2DTM and is compatible with other cryo-EM workflows. MOSAICS presents a new paradigm for structural cell biology by enabling spatially resolved, high-resolution structure probing of RNA and protein structures.

Learning to See Pilus Motors: Automated Detection in Cryo-Electron Tomograms

Jackson Pond*, Braxton Owens, Gus Hart

Determining the atomic structure of protein machines inside bacterial cells relies on accurately localizing these complexes within three-dimensional cryo-electron tomograms. This localization step has traditionally required manual annotation, creating a major bottleneck in the discovery pipeline. I developed a machine-learning–based pipeline for detecting Type IV pilus motors—protein complexes that play a central role in bacterial motility and adhesion—directly from low–signal-to-noise tomographic volumes.

Building on recent advances in 3D biomedical segmentation, I adapted and trained deep convolutional models to reliably identify pilus motors despite their small size and visual ambiguity. To support both model development and downstream biological analysis, I additionally designed a custom Napari plugin that integrates visualization, annotation, and prediction review into a unified workflow. Together, these tools reduce the need for manual annotation and help accelerate the process of mapping protein machinery toward high-resolution structural determination.

CryoEM Reconstruction and Refinement in a Gaussian Basis Improves Features and Reduces Artifacts

Anya Porter*, Phil Baldwin, Erik Anderson, Steven J. Ludtke

We have developed a reconstruction and refinement strategy for use with CryoEM and potentially CryoET that uses a Gaussian (Dirac delta) basis and leverages energy minimization strategies developed for use with Deep Learning. This method dramatically reduces artifacts caused by anisotropic orientation distributions. In several tests, we've demonstrated improved detail in 3-D reconstructions, resolving features which were unclear in traditional Fourier reconstructions. Due to the more efficient structure representation, the method also intrinsically performs denoising, somewhat analogous to PCA methods. The reconstruction method is easily extended to perform orientation refinement, and can readily incorporate difficult to correct microscopic artifacts such as Ewald sphere curvature and defocus through thick specimens.

FI-ManifoldEM: a modern Python implementation of ManifoldEM hosted at the Flatiron Institute

A. A. Ojha, R. Blackwell, E. R. Cruz-Chú, R. Dsouza, M. A. Astore, P. Schwander and S. M. Hanson*

Resolving continuous conformational heterogeneity in single-particle cryo-electron microscopy (cryo-EM) is a field in which new methods are now emerging regularly. Methods range from traditional statistical techniques to state-of-the-art neural network approaches. Such ongoing efforts continue to enhance the ability to explore and understand the continuous conformational variations in cryo-EM data. One of the first methods was the manifold embedding approach or ManifoldEM. However, comparing it with more recent methods has been challenging due to software availability and usability issues. Here we highlight a modern Python implementation that is user-friendly, orders of magnitude faster than its previous versions and designed with a developer-ready environment. This implementation allows a more thorough evaluation of the strengths and limitations of methods addressing continuous conformational heterogeneity in cryo-EM, paving the way for further community-driven improvements.

Quantifying Inter-Domain Motion in Cryo-EM via Relative Orientation Landscape

Chengmin Li*, Wooyoug Choi, Yifan Cheng

Inter-domain motions are central to the function of many macromolecular machines, yet they are difficult to quantify and visualize directly from cryo-EM data. Standard workflows use focused procedure to obtain high-resolution maps of individual domains that are combined into a composite map. However, information about relative motion between domains is lost in such composite maps. Here, we introduce cryoROLE (cryo-Relative Orientation LandscapE) to fill this gap. For each particle, one domain is treated as a reference and we compute the relative orientation (RO) of the other(s). RO is a single rigid-body rotation in SO(3) that captures only the true inter-domain rotation, along with any domain-specific uncertainties(e.g. one domain is less well resolved than the other) and provides a consistent, per-particle descriptor when domains behave approximately as “rigid bodies”. By mapping all ROS into a landscape, cryoROLE enables uncovering protein dynamics that are functionally important but are often missed or blurred by conventional classification or multibody refinement.

Building a Vaulted Bazaar with EMhub-Tomo: Standardized yet Flexible Environment for Collaborative Tomography Data Processing.

José Miguel De la Rosa Trevín*, Yunior Fonseca Reyna, Robert Nwokonko, Mohammad Zuhaib Qayyum, Tanvir Shaikh, Asfarul Haque, and Abhay Kotecha

Electron cryo-tomography (cryo-ET) is rapidly expanding in scale and complexity, yet its data-processing landscape remains fragmented, with heterogeneous tools, formats, and computational environments limiting reproducibility and collaboration. Building upon the EMhub framework for integrated, on-the-fly cryo-EM data handling, we introduce EMhub-Tomo, a “vaulted bazaar” environment that combines standardized workflow orchestration with the flexibility required for diverse tomography pipelines. EMhub-Tomo integrates state-of-the-art software for motion correction, tilt-series alignment, and tomogram reconstruction—primarily leveraging Warp for denoising and alignment and RELION for subtomogram averaging—while exposing these capabilities through a unified set of web visualization tools. The system provides a reproducible, modular, and shareable processing space where users can iteratively refine workflows, exchange metadata-rich processing histories, and collaboratively evaluate algorithmic choices. Together, these features position EMhub-Tomo as a scalable solution for cryo-ET facilities seeking to harmonize their data-processing infrastructure while maintaining the transparency and adaptability needed for methodological innovation. Some of the tools developed for EMhub-Tomo has already been used for the determination of the in-situ structure of apoferritin in E. coli at 2.3 Å resolution, establishing a robust, reproducible benchmark system for validating cryo-ET/STA workflows and advancing high-resolution in-cell structural biology.

Deconvolution Improves Single Particle Cryo-EM

Junrui Li, Wooyoung Choi, Yifei Chen, Shawn Zheng, Angus McDonald, John W. Sedat, David A. Agard and Yifan Cheng*

We present a procedure, AR-Decon, which stands for Anisotropic Resolution correction by Deconvolution, to improve maps with anisotropic resolution due to preferred particle orientation in vitreous ice. This procedure was developed based on ER-Decon, a deconvolution program developed to deconvolute light microscopy image in the presence of large amount of noise. Here, we demonstrate that, not only does AR-Decon mitigate the map's resolution anisotropy, but it also improves the high-frequency SNRs, leading to overall resolution improvement for the map.

High-Sensitivity 2D Template Matching via Differentiable Movie Realignment and CTF Optimization

Joshua L. Dickerson*, Matthew D. Giammar, Bronwyn A. Lucas

Two-dimensional template matching (2DTM) is a powerful method for identifying and localizing macromolecules within untilted cryo-EM micrographs of the crowded cellular environment. By leveraging high-resolution structural information, 2DTM has successfully been applied to characterize large in situ complexes, such as ribosomes and photosynthetic supercomplexes. However, extending this technique to smaller macromolecules requires significant improvements in detection sensitivity.

To meet this challenge, we developed Leopard-EM, an extensible Python-based framework for 2DTM. In this work, we have implemented a fully differentiable backend for Leopard-EM, enabling the application of gradient descent optimization to 2DTM workflows. We leveraged this differentiable architecture to develop two new algorithms that utilize abundant, large macromolecules to refine both micrograph quality and template projections. 2DTM provides a single molecule assessment of the agreement between a model template and the image, making it extremely sensitive to small errors in frame alignment and CTF parameter estimation.

To improve movie frame alignment, we model specimen motion using a cubic spline grid deformation field. Following patch-based alignment, the deformation field is further refined through Bayesian polishing, optimizing the 2DTM cross-correlation of the 60S ribosome. For Contrast Transfer Function (CTF) refinement, we optimize anisotropic magnification, beam tilt, per-micrograph astigmatism, and per-particle defocus. Together, these advancements improve the identification of macromolecules with a molecular mass < 1 MDa, as demonstrated by the improved detection of the 40S ribosomal subunit.

A continuous-rotation tomography pipeline of cellular specimens

Philip R Baldwin,* Erik D Anderson, Anya Porter, Benjamin Bammes, and Steven J Ludtke

Continuous-tilt cryo-electron tomography (cryo-ET) was originally proposed by Jensen et al. as a means to increase acquisition efficiency. However, early implementations required stopping at discrete angles during exposure due to stage stability limitations, preventing true continuous acquisition and limiting reconstruction quality. Here we demonstrate true continuous-tilt cellular cryo-ET enabled by an ultra-fast electron counting camera. By recording low-dose frames during uninterrupted stage rotation, we can improve data collection speed while extending resolution to at least 20 Å, with potential for further improvement. Continuous acquisition produces extremely low-dose individual frames, making alignment and reconstruction challenging. We have developed a functional processing strategy for motion correction, alignment, and tomographic reconstruction of these data. Although reconstruction remains computationally intensive on a single workstation, parallel processing across multiple machines makes real-time reconstruction at acquisition speed feasible. Notably, continuous-tilt reconstructions exhibit substantially reduced artifacts compared to conventional stop-and-tilt tomography, even without explicit missing-wedge compensation. These results demonstrate that true continuous-tilt cryo-ET is a practical high-throughput alternative to conventional tilt-series.

Circular Polysomes Revealed by Cryo-Electron Tomography

Jason J Hu*§, Jane KJ Lee§, Charlotte Meredith, Jennifer A Doudna#, Wah Chiu#, Jamie HD Cate#

Translational control is fundamental to the regulation of gene expression. In eukaryotes, communication between the 5′ and 3′ ends of mRNA has long been proposed to promote protein synthesis. According to the canonical “closed loop” model, interactions between cap- and poly(A)-binding factors bring the mRNA ends into proximity to functionally circularize mRNA. Although decades of biochemical and evolutionary studies have led to widespread acceptance of this model, direct structural evidence for stable closed-loop mRNAs and circular polysomes in cells remains remarkably scarce. Recent studies further challenge whether the closed-loop model uniquely accounts for 5′–3′ interactions, raising the possibility of alternative mechanisms. To address this knowledge gap, we leverage cryo-FIB/ET to visualize polysomes in situ, with a focus on ER-associated ribosomes in a model pituitary cell type. By using ribosomes as fiducials for actively translated mRNAs, we seek to map the polysome landscape on the ER within cells. Our preliminary data reveal ER-associated circular polysomes, whose abundance is modulated by stimulation with specific hormone factors. Ongoing biochemical analyses will identify the mRNA transcripts translated by circular polysomes and define the biological consequences of polysome circularization in these specialized cells.

HILL: a Web app for Fourier-Bessel layer line-based indexing of helical symmetry parameters

Xiaoqi Zhang, Wen Jiang*

Biological assemblies with helical symmetry are widespread across organisms and often play essential functional roles. Although recent advances in cryo-electron microscopy (Cryo-EM) have enabled near-atomic reconstruction of many helical structures, accurately determining the helical parameters, twist and rise, remains challenging, especially for previously uncharacterized structures. To address this need, we developed HILL, a web-based, user-friendly tool for Fourier–Bessel layer-line–based indexing of helical parameters from 2D projection images. HILL requires no software installation and provides an integrated interface featuring side-by-side visualization of power spectra (PS) and meridional phase differences (PD), with synchronized mouse-hovering to facilitate intuitive estimation of Bessel order ranges and discrimination between even and odd layer lines. Because PD patterns are sensitive to out-of-plane tilts, HILL also assists users in identifying tilted images that may lead to ambiguity or mis-indexing. Additionally, HILL includes automated 2D image-processing options, such as rotation, translation, masking, and computational straightening of curved filaments, enabling robust helical indexing for long, curved filament images.

Challenges in identifying proteins using 2D Template matching in crowded cellular environments

Raison Dsouza, Shashwat Shastri, Timothy Grant

Identifying the precise location and orientation of proteins within crowded cellular environments remains a major challenge in cell biology. While single-particle analysis in cryo-electron microscopy provides high-resolution structures, it offers a reductionist view that lacks native cellular context [1]. Two-dimensional template matching implemented in cisTEM (2DTM) is a widely used strategy for in situ localization, yet its performance is limited with complex protein geometries [2].

Membrane proteins are particularly difficult to detect because the strong and extended density of the lipid bilayer can dominate the local signal, biasing correlation scores and obscuring weaker protein features. In addition, anisotropic (non-spherical) templates interact differently with the surrounding cellular density depending on their orientation, leading to orientation-dependent score variations and reduced detection reliability. Small template mis-centering further alters the overlap between template and image, distorting correlation values and increasing the risk of false detections or missed true positives, especially when scores are aggregated across orientations.

To address these challenges, we propose treating distinct projection views independently using orientational aware strategies [3] to sample SO(3) space uniformly. By analyzing orientations separately rather than pooling all scores together, orientation-specific background effects can be better controlled, improving robustness for anisotropic templates and membrane-associated proteins.

These considerations highlight key limitations of conventional aggregated NCC approaches and support the development of orientation-aware strategies for reliable protein localization in crowded cellular environments [4].

[1].Bäuerlein, Felix JB, and Wolfgang Baumeister. "Towards visual proteomics at high resolution." Journal of Molecular Biology 433.20 (2021): 167187.

[2]. Lucas, Bronwyn A., et al. "Locating macromolecular assemblies in cells by 2D template matching with cisTEM." Elife 10 (2021): e68946.

[3].Gorski, Krzysztof M., et al. "HEALPix: A framework for high-resolution discretization and fast analysis of data distributed on the sphere." The Astrophysical Journal 622.2 (2005): 759.

[4]. Dsouza, Raison, and Timothy Grant. "Rotational Quantization: A Robust, Improved Analysis for Particle Identification in Single Molecule Cryo-EM Images." Microscopy and Microanalysis 31.Supplement_1 (2025): ozaf048-492.

BOE-ViT: Boosting Orientation Estimation with Equivariance in Self-Supervised 3D Subtomogram Alignment

Subtomogram alignment is a critical task in cryo-electron tomography (cryo-ET) analysis, essential for achieving high-resolution reconstructions of macromolecular complexes. However, learning effective positional representations remains challenging due to limited labels and high noise levels inherent in cryo-ET data. In this work, we address this challenge by proposing a self-supervised learning approach that leverages intrinsic geometric transformations as implicit supervisory signals, enabling robust representation learning despite data scarcity. We introduce BOE-ViT, the first Vision Transformer (ViT) framework for 3D subtomogram alignment. Recognizing that traditional ViTs lack equivariance and are therefore suboptimal for orientation estimation, we enhance the model with two innovative modules that introduce equivariance include 1) the Polyshift module for improved shift estimation and 2) MultiAxis Rotation Encoding (MARE) for enhanced rotation estimation. Experimental results demonstrate that BOE-ViT significantly outperforms state-of-the-art methods. Notably, at SNR 0.01 dataset, our approach achieves a 77.3% reduction in rotation estimation error and a 62.5% reduction in translation estimation error, effectively overcoming the challenges in cryo-ET subtomogram alignment.

Structural characterization of the cooperative binding mechanism of BI-TFR1 capsids to hTfR1

Tyler Brittain*, Camille PMA Chossis, Izabela Giriat, Timothy F. Shay, Pamela J. Bjorkman, Viviana Gradinaru

There has been great progress in the engineering adeno-associated virus (AAV) vectors for gene therapy applications targeting the brain. Recently, the capsid AAV BI-TFR1 was engineered specifically to target the human receptor TfR1 which is heavily localized on the blood brain barrier(BBB). The mechanism by which BI-TFR1 recognizes hTfR1 and its binding geometry remains unelucidated. To amend this, we solved the cryo-EM structures of BI-TFR1 and BI-TFR1_2 alone and complexed to hTfR1. We then employed eVLPs expressing hTfR1 to study the interaction between BI-TFR1 with hTfR1 in a natural membrane environment. Using subtomogram averaging we found a distribution of hTfR1 bound states on the AAV 3-fold face indicative of a cooperative binding mechanism. Furthermore, based on structural models we designed hTfR1 mutants that increased or decreased its cooperative binding to BI-TFR1. Overall, we characterized a new avenue for AAV rational engineering to inform the design of safer and more efficient capsids.

Laser phase plate for cryo-EM

Jessie T. Zhang*, Petar N. Petrov, Jonathan P. Remis, Jeremy J. Axelrod, Hang Cheng, Eric S. Cooper, Ian K. Hicklin, Shahar Sandhaus, Cooper Schnurr, Robert M. Glaeser, Holger Mueller

Cryo-EM imaging of vitrified biological specimens is fundamentally limited by weak phase contrast and electron radiation damage. In conventional defocus-based cryo-EM, the contrast transfer function has a weak signal at low spatial frequencies, which can limit particle detection, alignment, and interpretability under low-dose conditions. A laser phase plate (LPP) can increase the contrast in the low frequency components of the image by imparting a stable phase shift to the unscattered electron beam after the specimen. We have developed and integrated a laser phase plate into a state-of-the-art Krios equipped with a custom transfer-optics module and spherical aberration-corrector. In this poster, I will present recent results on single particle analysis (SPA) of small proteins using the LPP. By comparing SPA experiments with and without the LPP, we find a significant improvement in final resolution achieved with the LPP under otherwise similar experimental and processing conditions. We investigate in detail the mechanisms by which the LPP enables this improvement. In addition, I will provide an outlook on our current progress in spherical aberration-corrected in-focus imaging and the application of LPP in cryo-ET. These results pave the way towards applications of the laser phase plate in more complex and biologically relevant samples.

MiLoPYP2: iterative self-supervised membrane-aware localization of proteins in situ

Alperen Tupurtu*, Alberto Bartesaghi

Cryo-electron tomography (cryo-ET) enables in situ structure determination of proteins that play essential roles in cellular processes, but accurate particle picking remains a major bottleneck. Crowded cellular environments, missing-wedge distortions, and low signal-to-noise ratio limit the accuracy and practicality of existing particle picking methods. Membrane proteins are particularly challenging because their colocalization with strong membrane signals can hinder localization and alignment. Recent self-supervised machine learning approaches, including MiLoPYP, have improved throughput but often remain sensitive to highly prevalent background signals, still require substantial user effort, and are slow to train. Here, we introduce MiLoPYP2, a self-supervised, membrane-aware iterative framework for particle mining and localization that generalizes across diverse targets while substantially reducing manual effort and training time. Key advances include a surface-awareness component that leverages the local membrane geometry and an iterative mining scheme that progressively filters out unwanted features to focus learning toward the particles of interest. Across multiple tomographic datasets, MiLoPYP2 consistently detects diverse particle types and produces high-quality particle positions suitable for downstream high-resolution refinement, enabling scalable cryo-ET workflows and facilitating the structural analysis of membrane proteins along with other challenging targets in situ.

Comparing Synthetic and Authentic Tomograms with TomoTwin Embeddings

Joshua Blaser*, Gus Hart

Cryogenic electron tomography (Cryo-ET) relies on deep learning for macromolecular identification, but these methods require reliable ground-truth data, motivating the development of synthetic tomograms. However, quantitative evaluation of synthetic realism remains limited. Here, we use TomoTwin, a deep metric learning framework that embeds subvolumes into a 32-dimensional latent space, representing each tomogram as a point cloud in the embedding space. By comparing synthetic and authentic tomograms using Maximum Mean Discrepancy (MMD) and embedding variance, we observe a domain shift, with synthetic data exhibiting higher MMD and greater dispersion. These findings demonstrate that TomoTwin embeddings provide a quantitative measure of synthetic–authentic discrepancies and offer a practical framework for improving tomogram simulation fidelity.

New methods for the Initial model problem in Subomogram averaing

Jose L. Vilas, O. Lauzirika, C.O.S. Sorzano, J.M. Carazo

Obtaining a reliable initial reference remains a critical bottleneck in subtomogram averaging (STA). The iterative refinement process is prone to model bias and often trapped in local minima when starting from a poor initial volume. To address this, we present two novel, computationally efficient algorithms designed to generate robust initial volumes for STA without prior structural knowledge.

The first method revitalizes the Random Conical Tilt (RCT) strategy, adapting it to the specific constraints of cryo-ET. This algorithm maximizes efficiency by exploiting the fixed geometric constraints of the experimental tilt series. We explore two input strategies: utilizing reconstructed subtomograms and utilizing cropped particle stacks from the raw tilt series. The workflow performs 2D classification on subtomogram projections or the 0º tilt images from the cropped stacks. Once consistent 2D classes are identified and relative in-plane rotations are determined, we employ the known tilt geometry to perform Direct Fourier Reconstruction for each class. This approach is computationally inexpensive and rapidly generates multiple candidate volumes (one per 2D class). These candidates can subsequently be aligned to form a higher-quality consensus volume, followed by a final local refinement step using 2D projections.

The second method employs a reference-free approach based on Orthogonal Group Synchronization. Instead of aligning particles to a reference, subtomograms are aligned pairwise. While individual pairwise alignments are error-prone due to the low signal-to-noise ratio (SNR) inherent in cryo-ET, the method exploits redundancy within the dataset to estimate globally consistent orientations. To mitigate the high computational cost of all-against-all alignment, we introduce two optimizations: 1) accelerating the alignment process using Spherical Harmonics decomposition, and 2) defining a sparse, closed graph topology, which eliminates the need to align every possible pair while maintaining global connectivity. In fact, this second method can serve as alignment algorithm for the reconstructed volumes obtained in the previous algorithm based on RCT.

Both algorithms have been theoretically defined and experimentally validated using the 70S Ribosome dataset (EMPIAR-10304). Results demonstrate that both methods successfully yield initial volumes of sufficient quality to seed downstream refinement, offering a robust solution to the "missing reference" problem in cryo-ET.

Preserving Information and Integrity in Cryo-EM: A Fourier-Space Deposition Approach

Zbyszek Otwinowski, Yirui Guo, Raquel Bromberg, Dominika Borek

Current cryogenic electron microscopy single-particle reconstruction (cryo-EM SPR) deposition guidelines require submission of unfiltered, unmasked, unsharpened raw half-maps. Resolution can then be estimated from the Fourier shell correlation (FSC) between half-maps as a proxy for signal-to-noise ratio (SNR). However, reconstruction weights and related metadata are not retained in deposited half-maps, yet are required to reproduce reported resolution and validation statistics. As a result, FSC-based metrics derived from deposited half-maps are systematically biased.

We describe these limitations and propose a complementary deposition standard: deposit reconstruction results in reciprocal (Fourier) space together with mandatory molecular masks (or mask descriptors). Reciprocal-space deposition preserves direction-dependent signal and associated uncertainties for each Fourier position, improving interpretability of anisotropy and enabling more faithful downstream analyses, including atomic model refinement. FSC curves remain computable from the deposited information, and inclusion of masks improves FSC interpretation and resolution reporting while enhancing reproducibility.

Uncertainty normalization in cryo-EM remains incompletely solved because lens and detector behavior, resolution-dependent processing, and motion introduce complex, direction- and resolution-dependent effects. Uncertainty (sigma) calculated per point in reciprocal space preserves information already represented in current software and provides a practical foundation for future reanalysis and improved uncertainty modeling.

Validating conformational and compositional heterogeneity: a case study of the SARS-CoV-2 spike protein trimer receptor binding domains

Qinwei Wang, Spencer Chmielak, Geoffrey Woollard*, Sriram Subramaniam, Khanh Dao Duc

The entry of SARS-CoV-2 into cells is mediated by a trimeric spike protein that utilizes its receptor binding domain (RBD) to engage with the cell surface receptor ACE2, with each protomer capable of shuttling between an “up” conformation that can bind ACE2 and a “down” conformation that prevents ACE2 binding. To discover intermediate conformational states during this transition, we applied the recently reported linear subspace method RECOVAR to embed 2D projection cryo-EM images of ACE2-bound spike proteins into a low-dimensional latent space. From this embedding, multiple conformational states were reconstructed followed by an automated masking protocol, leveraging a topology representing network and optimal transport. A surprising result from our analysis is the finding that the dominant trajectory for the transition between conformations that have one-up and two-up RBD states involves an all-down RBD conformational state. The methods we present here could be generally applicable to other dynamic protein assemblies to uncover novel intermediate conformational states.

copick: An open API and toolkit for collaborative cryoET annotation and analysis

Utz Heinrich Ermel*, Jonathan Schwartz, Zhuowen Zhao, Daniel Ji, Ariana Peck, Yue Yu, Mohammadreza Paraan, Bridget Carragher, Kyle I. S. Harrington

Cryo-electron tomography (cryoET) enables visualization of macromolecular complexes within intact cellular environments. Continued improvements in instrumentation, sample preparation, and data-processing pipelines have increased both the scale and complexity of cryoET datasets, making manual analysis increasingly impractical. To support scalable, collaborative annotation, we developed copick, an open-source dataset API and accompanying tool suite for cryoET analysis. Copick provides standardized access to tomograms, segmentations, point annotations, meshes, and feature maps across local storage, HPC systems, cloud platforms, and public repositories. Multi-resolution image storage in OME-Zarr format supports responsive visualization and cross-platform interoperability. Plugins for napari and ChimeraX enable human-in-the-loop workflows for particle picking, segmentation, inspection of machine-learning outputs, and project-level collaboration. Additionally, copick exposes a Model Context Protocol (MCP) interface that enables automated generation of annotation-curation pipelines based on natural-language instructions in AI assistants. Together, these capabilities facilitate reproducible, scalable, and collaborative cryoET analysis.

Bayesian Perspective for Orientation Determination in Cryo-EM with Application to Structural Heterogeneity Analysis

Sheng Xu*, Amnon Balanov, Amit Singer, Tamir Bendory

Accurate orientation estimation is a crucial component of 3D molecular structure reconstruction, both in single-particle cryo-electron microscopy (cryo-EM) and in the increasingly popular field of cryo-electron tomography (cryo-ET). The dominant approach, which involves searching for the orientation that maximizes cross-correlation relative to given templates, is sub-optimal, particularly under low signal-to-noise conditions. In this work, we propose a Bayesian framework for more accurate and flexible orientation estimation, with the minimum mean square error (MMSE) estimator serving as a key example. Through simulations, we demonstrate that the MMSE estimator consistently outperforms the cross-correlation-based method, especially in challenging low signal-to-noise scenarios, and we provide a theoretical framework that supports these improvements.

When incorporated into iterative refinement algorithms in the 3D reconstruction pipeline, the MMSE estimator markedly improves reconstruction accuracy, reduces model bias, and enhances robustness to the Einstein from Noise artifact. Crucially, we demonstrate that orientation estimation accuracy has a decisive effect on downstream structural heterogeneity analysis. In particular, integrating the MMSE-based pose estimator into frameworks for continuous heterogeneity recovery yields accuracy improvements approaching those obtained with ground-truth poses, establishing MMSE-based pose estimation as a key enabler of high-fidelity conformational landscape reconstruction. These findings indicate that the proposed Bayesian framework could substantially advance cryo-EM and cryo-ET by enhancing the accuracy, robustness, and reliability of 3D molecular structure reconstruction, thereby facilitating deeper insights into complex biological systems.

Toward Physically Grounded Noise Models in Cryo-EM and Cryo-ET

Trent Llewellyn, Peter Van Blerkom, Ari Jacobs, Dorit Hanein, and Niels Volkmann

Signal-to-noise ratios in cryo-electron microscopy (cryo-EM) data are extremely low, yet most simulation approaches prioritize modeling signal while relying on simplified noise assumptions. Here, we present a framework for automated selection of signal-free patches as a first step toward deriving a realistic, data-driven noise model. The method uses a sliding-window root-mean-square deviation metric applied after denoising to identify regions containing structured image content, including biological signal, contaminants, and other non-noise features, and to exclude these regions from noise analysis. We evaluate limitations for low-contrast specimens and outline strategies to extend applicability. Using the resulting signal-free patches, we show that cryo-EM noise is well described by a composite distribution combining Poisson, Gaussian, and asymmetric Laplacian components, with parameters estimated from empirical noise statistics. Preliminary results demonstrate near-perfect agreement between modeled and empirical noise distributions, providing a robust foundation for noise-aware downstream cryo-EM processing pipelines.

Automation and benchmarking of subtomogram analysis pipelines

Joshua Hutchings*, Jonathan Schwartz, Mallak Ali, Ariana Peck, Bridget Carragher, Dari Kimanius, Yue Yu, Mohammadreza Paraan

Cryo-electron tomography (cryo-ET) combined with subtomogram averaging (STA) is a powerful approach for determining macromolecular structures in their native cellular context, but the complexity of data processing pipelines remains a significant barrier to routine adoption. Here we demonstrate an automated STA pipeline on benchmarking datasets, including a phantom dataset comprising mixtures of purified proteins and cell lysates, and an in situ dataset of E. coli over-expressing virus-like particles. We assess the impact of the Volta phase plate (VPP) on downstream subtomogram analysis by comparing equivalent datasets collected with and without VPP, highlighting future considerations for optimal processing. Additionally, we use a smaller benchmarking dataset to compare STA resolutions from RELION-5 and Warp/M. These comparisons aim to stimulate efforts to establish best practices in cryo-ET data processing.

Unsupervised Deep Learning Frameworks for Automated Cellular Cryo-Electron Tomography Analysis

Min Xu

This research introduces a suite of unsupervised and self-supervised deep learning frameworks designed to overcome fundamental challenges in cellular cryo-electron tomography (cryo-ET), specifically addressing extremely low signal-to-noise ratios, high data volumes, and the prohibitive cost of manual annotation. The studies propose novel methodologies for various stages of the cryo-ET workflow, including a J-invariant blind-spot network for denoising single noisy volumes , an unsupervised multi-scale segmentation pipeline that leverages features from the Stable Diffusion foundation model to identify subcellular objects , and disentangled representation learning architectures—such as "DualContrast" and SE(3) DRL—for identifying protein compositions and 3D macromolecular morphologies. By automating complex data processing tasks and improving structural preservation without requiring paired datasets or expert labels, these frameworks may potentially enable efficient large-scale mining of heterogeneous biological patterns.

Referecnces:

Liu X, Kassab M, Xu M, Ho Q. J-Invariant Volume Shuffle for Self-Supervised Cryo-Electron Tomogram Denoising on Single Noisy Volume. arXiv preprint arXiv:2411.15248. 2024 Nov 22.

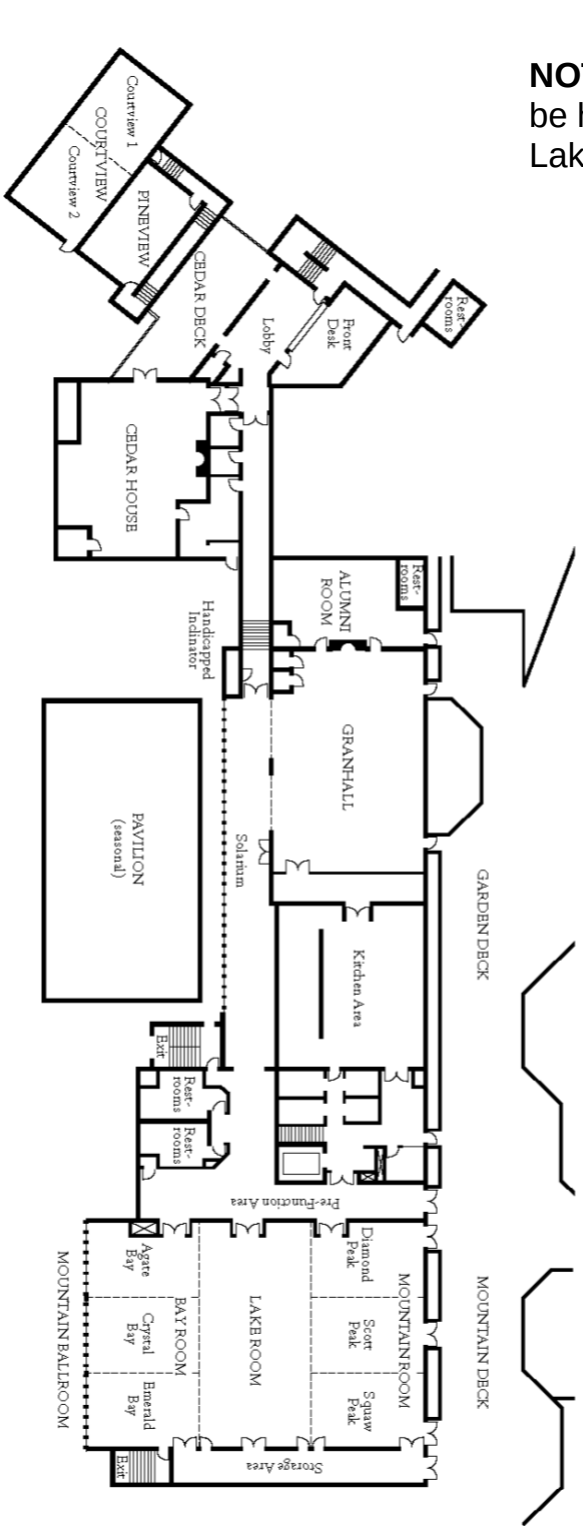
Uddin MR, Nguyen TH, Tabib HS, Gandhi K, Xu M. Unsupervised Multi-scale Segmentation of Cellular cryo-electron Tomograms with Stable Diffusion Foundation Model. CVPR 2026.

Uddin MR, Armouti J, Xu M. Unsupervised Identification of Protein Compositions and Conformations via Implicit Content-Transformation Disentanglement. ICCV 2025.

Uddin MR, Vora M, Wu Q, Chen M, Xu M. Unsupervised SE (3) Disentanglement for in situ Macromolecular Morphology Identification from Cryo-Electron Tomography. arXiv preprint arXiv:2601.01364.

Meeting Room Diagram

NOTE: All sessions will be held in the Mountain-Lake Room



MEETING ROOM DIAGRAM