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Hosted by Associate Professor Sun Ji

In-situ Visual Proteomics and Molecular Tissue Imaging with Cryo-ET

By Abhay Kotecha

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In situ cryo-electron tomography (cryo-ET) has become the method of choice to investigate structures of biomolecules in their native cellular environments. Despite its transformative potential, key challenges remain in achieving high-throughput data collection, high-resolution data processing and in enabling broad community access to these rich datasets.

Here, we demonstrate the use of a cryogenic plasma-focused ion beam (cryo-PFIB) for high-throughput lamella preparation from *Chlamydomonas reinhardtii, E. coli*, and high-pressure–frozen (HPF) brain tissue samples. Using recombinantly expressed apoferritin (apoF) in *E. coli* and native ribosomes from *C. reinhardtii*, we show that radiation damage from PFIB milling is minimal. *In situ* subtomogram averaging (STA) of apoF yielded a 2.2 Å reconstruction, representing one of the highest-resolution cryo-ET structures obtained directly within cells.

For *C. reinhardtii*, we combined cryo-PFIB milling with recent advances in automated cryo-ET data acquisition and image processing, producing 1,829 reconstructed and annotated tomograms. This dataset, which we released as a community resource, supports both method development and biological discovery. Subtomogram averaging of native complexes, including ribosomes, ATP synthase, Respirasome, Rubisco, photosystem II, microtubules, and nucleosomes, achieved sub-nanometer resolution for most of these protein molecules directly inside the cells, highlighting the quality and utility of this dataset for visual proteomics.

Finally, we extend this workflow to HPF brain tissue, showing lamella preparation and high-resolution cryo-ET of molecular assemblies in their native tissue context. Together, these results illustrate how emerging cryo-ET workflows are transforming *in situ* structural biology across scales.

About the Speaker

Abhay Kotecha is a structural biologist specializing in cryo-electron microscopy (cryo-EM) and cryo-electron tomography (cryo-ET). He currently serves as Senior Director of Technologies for Cryo-EM and Cryo-ET at St. Jude Children's Research Hospital, where he leads the design and implementation of the newly formed Center of Excellence for Structural Cell Biology (CoE-SCB), with a mission to bridge multiscale imaging from tissues to atoms using cryo-ET, volume-EM, and automation technologies. Dr. Kotecha earned his DPhil in Structural Biology from the University of Oxford and holds a BSc in Cell and Molecular Biology from Oxford Brookes University. Prior to joining St. Jude, he was a group leader at Thermo Fisher Scientific, where he contributed to advancing high-throughput and high-resolution cryo-EM imaging technologies. His expertise includes structure-based vaccine design, epitope mapping, antiviral development, drug design and high-resolution imaging using cryo-EM and focused ion beam (FIB) milling.