Institut Européen de Chimie et Biologie

I E C B

Opening Windows into the Cell: The Molecular Architecture of Nuclear Transport.

Elizabeth VILLA
Max Planck Institute for Biochemistry, Martinsried, GERMANY

Cryo-electron tomography (cryo-ET) is a biophysical tool that provides unprecedented insights into the 3-D organization of cells in their native state at molecular resolution, free of artefacts such as fixation, staining, or labelling. To date, the main limitation of cryo-ET is the thickness of most cells, rendering them inaccessible to intermediate-voltage TEM. I will describe how we have adapted focused ion beam (FIB) milling to prepare 200-500 nm lamellae from intact cells, effectively opening large windows into the cell's interior at molecular resolution. Through image processing, macromolecular complexes can be localized and identified, enabling quantitative analysis of the organization and structure of molecular complexes in situ. Cryo-ET and FIB milling were used to study the nuclear pore complex, one of the largest macromolecular machines in the cell that selectively controls all traffic between the nucleus and the cytoplasm. Due to its sheer size, its local environment and its dynamic nature, determining its structure at molecular resolution remains a challenge for conventional techniques, and studies of nuclear transport lack a molecular scaffold onto which models can be interpreted. Combining FIB milling, cryo-ET, and image processing enable the study of the NPC in its native environment, performing its function, and free of the distortions caused by purification. This approach has produced the yeast NPC architecture at unparalleled resolution and revealed the structural dynamics of the NPC in action. Other uses of cryo-FIB/ET to study diverse cellular environments at molecular detail will be presented, including actin networks and the distribution of macromolecular complexes within organelles. Finally, I will discuss new emerging technologies in EM, including direct detector devices and phase plates, and their impact on cryo-ET.