Towards optimized sample preparation for cryo-electron tomography of cellular lamellas

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Cryo-electron microscopy is a dynamically developing method with increasing utilization in structural biology research. Recent advances in the detector technology enabled near-atomic structural studies of protein complexes including the molecular machines such as RNA polymerases or ribosomes. One advantage of the cryo-electron microscopy with respect to the other high-resolution structural biology methods lies in its capability to structurally characterize pleiomorphic objects and molecular assemblies which are difficult to characterize under in vitro conditions by cryo-electron tomography (cryo-ET). Therefore, cryo-ET can provide detailed structural information about macromolecular complexes in their native cellular environment. Since most of the eukaryotic cells are not transparent for transmission electron microscopy and thus not directly accessible for cryo-ET data collection, additional pre-processing of the cellular samples is necessary prior. Focused ion beam micromachining (FIBM) under cryo-conditions has been developed to generate 100-300 nm thick cellular cross-sections suitable for cryo-ET. The complete sample preparation protocol comprises adhesion of the single layer of the cells to the transmission electron microscopy (TEM) grids, fixation of the cells in frozen hydrated state by vitrification, and FIBM of cellular lamella. Since the sample undergoes multitude transfers and processing in several instruments, the throughput of the whole process in not in general very high and rarely reaches 50%. We systematically study individual steps of the process primarily focussing on the cell adhesion, sample vitrification, and FIBM in order to determine the crucial aspects and parameters which are seminal for increasing the throughput of the lamella preparation workflow.