Advances and developments in cryo-electron tomography for in situ structural biology

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The ultimate goal of cellular tomography is to map the landscape of cellular territories on a molecular level and to directly observe the interplay of molecules and macromolecular machines in their native environment, thus to study the molecular sociology *in situ* [1]. The main obstacle in here is the sample itself; even small cells are, by and large too 'thick' to be directly accessible for transmitting electrons and too thick to visualise them at the required resolution. To make a variety of differently sized cells or even whole organisms accessible, suitable sample preparation techniques are required to make them transparent enough for cryo-electron tomography (cryo-ET).

Fabrication of electron transparent lamellas, can be done gently and in a targeted fashion with the focused-ion-beam instrument. Originally designed for the quality control of silicon wafers, it was first explored for frozen-hydrated samples 10 years ago: A fine focused beam of ions ablating material of a cryo-specimen to make it transparent enough for cryo-ET, while at the same time keeping it frozen hydrated [2]. That pilot study was the start of further exploration of this methodology for *in situ* structural biology [3].

Perhaps it is too early to say that cryo-focused ion beam milling (cryo-FIB) is now the method of choice for obtaining 150-300 nm thin slices from a wide variety of cell types ranging from larger bacteria to neuronal primary cultures. However, the method is certainly gaining momentum and in combination with direct detection and phase-plate assisted tomography, we are now able to explore the 'wonders' of the inner space of cells at molecular detail. This lecture will present recent work in the field of cryo-ET and *in situ* structural biology and highlights technological developments, limitations and their opportunities. Furthermore, it will give a prospective towards obtaining structural insights from an *in situ* context, possibly at atomic resolution.

References

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