Cryo-Electron Microscopy and Tomography: The past, the present and the future

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The structural elucidation of isolated macromolecules with cryo-EM (i. e. *ex situ*) has been awarded with the nobel prize in chemistry in 2017 and can be seen nowadays as an established method. Although cryo-EM is not yet one of the high-throughput methods, the requirements in terms of user experience and measurement time are becoming ever less demanding. Equivalent to the beamlines in protein crystallography the first cryo-EM facilities are being build up and put into operation. Of course, there is still room for further improvements in cryo-EM. The latest development of the Volta phase plate is a good example of this. The clear phase contrast improves the selection and classification of the individual particles and thus also enables the structural determination of very small proteins that were previously inaccessible.

However, proteins have their biological function in the complex environment of the cell and interact with other macromolecules. The exciting potential of cryo-EM therefore lies in cryo-electron tomography, the three-dimensional analysis of biological structures in the undisturbed cellular context (i. e. *in situ*). This method closes the gap between molecular and cytological structural research.
Thin samples are nevertheless mandatory for any high-resolution tomographic study. 'Slicing' in the 21st century, to obtain electron transparent lamellas, can be done gently and in a targeted fashion with the focused-ion-beam instrument. 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.

After a brief look back this lecture will present our recent work in the field of cryo-electron tomography 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.