Contribution ID: **24** Type: **Invited**

Perspectives and limitations in high-resolution cryo-EM

Wednesday 4 June 2025 11:30 (30 minutes)

Single particle cryo-EM has become a powerful technology in structure determination of macromolecular complexes. The technique benefits from the decades of investment in the development of high-end electron microscopy hardware, powerful computational hard- and software and the fact that samples do not need to be crystalized. Over the past decade, the number of structures solved by cryo-EM has consequently increased and soon a point will be reached when more structures will be solved in academia by cryo-EM than by X-ray crystallography. Simultaneously, the maximum resolution limit of the technology has been improved and at least for the model protein apoferritin even atomic resolution has been achieved [1].
In spite of this success story, the mean resolution of single particle cryo-EM structures deposited in the database (EMDB), is still >4 Angstrom even until today. The important question is therefore, why can this technology potentially obtain even atomic resolution structures and what are the limiting factors that prevent the user from obtaining this level of resolution on a regular basis?
A major limiting factor in cryo-EM is the biochemical quality of a purified macromolecular complex. Macromolecules are known to be prone to suffer in quality during biochemical purification but also during electron microscopical grid preparation and often it isn't even an easy task to distinguish between the two effects. Other limitations can be attributed to electron optical limitations but also limitations in computational image processing.
Achieving routine atomic resolution depends on parallel improvements in all three pillars of the technology. For example, even a small amount of sample degradation during biochemical purification or grid preparation can negate the gains made by state-of-the-art electron microscopes. Similarly, poor electron optics can limit the usefulness of pristine samples. Overcoming these challenges requires a holistic approach: refining biochemical workflows to preserve macromolecular integrity, optimising electron optics to minimise imaging artefacts, and developing adaptive software pipelines to extract maximum information from complex datasets.

References

[1] Yip K.M., Fischer N., Paknia E., Chari A., Stark H. (2020) Nature, Atomic-resolution protein structure determination by cryo-EM, 157-161.
[2] Nakane T, et al. (2020) Nature, Single-particle cryo-EM at atomic resolution. 152-156.

Presenter: STARK, Holger (MPI Göttingen)

Session Classification: Radiation Damage in Electron Crystallography and Microscopy