

Preparation of biological samples for cryo-electron microscopy using the HPF "Waffle" method

Mrs. Jana Moravcová¹, Petra Reznickova², Martin Polak¹, Jiri Novacek¹

¹Masaryk University, Central European Institute of Technology, Brno, Czech Republic, ²Thermo Fisher Scientific, Brno, Czech Republic

Background incl. aims

Cryo-electron microscopy (cryo-EM) has emerged as a pivotal technique in structural biology, offering unparalleled insights into the architecture of macromolecules at near-atomic resolution.

A crucial requirement for acquisition and collection of high quality data is properly vitrified and highly concentrated specimen. However, sample preparation still presents challenges in thicker specimens as bigger cells or cellular clusters, those are frozen by conventional plunge freezing method and may suffer with improper vitrification. Another problems could be low concentration or inadequate distribution of sample on electron microscopy grid or preferred orientation of the specific sample [1]. Here, we focus on the recently introduced "Waffle" method [1] and show it potential for preparation of various types of sample used in cryo-EM.

Methods

The waffle method is based on sample vitrification within the thickness of the TEM grid bars and it combines plunge freezing on the electron microscopy grid with a technique of high pressure freezing, that provides an advantage of proper vitrification of specimens thicker than 15µm.

Thus, a 20-30µm thick layer is prepared which needs to be further processed by cryo-focused ion beam micromaching (cryo-FIBM) to final thickness ~200nm before cryo-EM imaging.

Results and conclusions

We show benefits and limitations of the waffle method for vitrification of purified proteins, protein crystals, bacterial cell suspensions and eukaryotic cells.

Keywords:

waffle, vitrification, electron microscopy

Reference:

[1] Kelley K, Raczkowski AM, Klykov O, Jaroenlak P, Bobe D, Kopylov M, Eng ET, Bhabha G, Potter CS, Carragher B, Noble AJ. Nat Commun. 2022 Apr 6;13(1):1857. doi: 10.1038/s41467-022-29501-3.