

Electron cryo-microscopy at 100 and 200 kV: from screening to structures

Dimple Karia¹, Adrian Koh²

¹*Materials and Structural Analysis Division Thermo Fisher Scientific Netherlands* ²*Materials and Structural Analysis Division, Thermo Fisher Scientific*

dimple.karia@thermofisher.com

Electron cryo-microscopy (cryo-EM) single particle analysis (SPA) method has become one of the dominating methods for high resolution structure determination of a wide variety of biological macromolecules. Such high resolution structures facilitate understanding of their functions, mechanism of action and protein ligand/drug interactions. With an increase in the popularity of cryoEM, the need for accessibility, ease of use and improved efficiency has also increased. Here we describe two cryo-transmission electron microscopes (cryo-TEMs), that are equivalent to home source X-ray diffractometers, but for cryo-EM.

The first is the Thermo Scientific Tundra cryo-TEM operating at 100kV with a semi-automated grid loading system and automated data collection for SPA. Tundra allows users to load the cryo grids in an effortless and robust way. Using this new microscope, we solved structures of several soluble and membrane proteins. For standard sample such as apoferritin (equivalent to lysozyme for X-ray crystallography) we solved the structure to 2.6 Å resolution. We then solved the structure of a challenging homo-pentameric human GABAA (gamma-aminobutyric acid type A) receptor to 3.4 Å resolution. The GABAA receptor is a small membrane protein and ligand-gated ion channel that mediates neurotransmission. These are important therapeutic drug targets and hence it is vital to understand the molecular mechanism by which these receptors mediate neurotransmission. After decades of efforts, in 2014, this same sample of GABAA receptor was crystallized and structure resolved to 3.0 Å. With cryo-EM on Tundra, we obtained similar resolution without the need of crystallization and in near native conditions.

To further push for resolution, automation and high-throughput, we used the Thermo Scientific Glacios™ cryo-TEM. Glacios has an Autoloader™, with a robotic arm which can load 12 grids simultaneously and switch the grids automatically. To push for higher resolution, Glacios is also equipped with direct electron detector, Falcon 4, and can be combined with Selectris energy filter. Using this system, we achieved a 2.4 Å resolution cryo-EM map for the same GABAA receptor. Both these microscopes are not only good for sample screening and optimization but are also capable of generating high resolution structures comparable to those obtained from X-ray crystallography experiments.