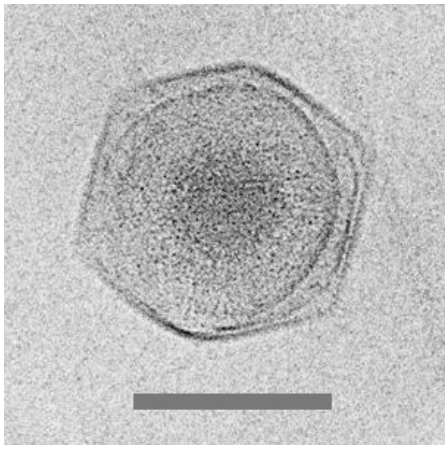
Cryogenic electron microscopy

Cryogenic electron microscopy (**cryo-EM**) is an electron microscopy (EM) technique applied on samples cooled to cryogenic temperatures and embedded in an environment of vitreous water. An aqueous sample solution is applied to a grid-mesh and plunge-frozen in liquid ethane or a mixture of liquid ethane and propane.[2] While development of the technique began in the 1970s, recent advances in detector technology and software algorithms have allowed for the determination of biomolecular structures at near-atomic resolution.[3] This has attracted wide attention to the approach as an alternative to X-ray crystallography or NMR spectroscopy for macromolecular structure determination without the need for crystallization.

In 2017, the Nobel Prize in Chemistry was awarded to Jacques Dubochet, Joachim Frank, and Richard Henderson "for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution."[4] *Nature Methods* also named cryo-EM as the "Method of the Year" in 2016.[5]

A transmission electron microscope (2015).

Contents



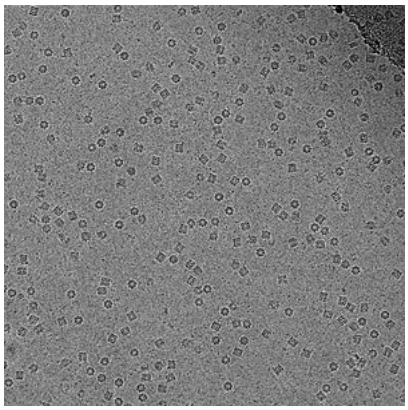
Cryo-electron micrograph of the CroV giant marine virus (scale bar represents 200 nm)[1]

Transmission electron cryomicroscopy

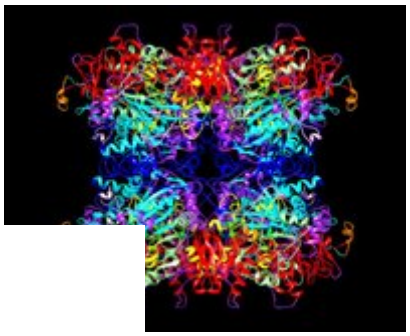
Cryogenic transmission electron microscopy (cryo-TEM) is a transmission electron microscopy technique that is used in structural biology and materials science.

- Cryogenic electron tomography (Cryo-ET), a specialized application of where samples are imaged as they are tilted
- Electron crystallography, method to determine the arrangement of atoms in solids using a TEM
- MicroED,[6] method to determine the structure of proteins, peptides, organic molecules, and inorganic compounds using electron diffraction from 3D crystals[7][8][9]

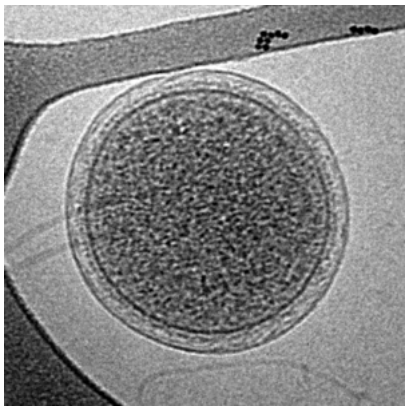
- Single particle analysis cryo-EM, an averaging method to determine protein structure from monodisperse samples[10]



Cryo-TEM image of GroEL suspended in amorphous ice at 50 000× magnification



Structure of alcohol oxidase from *Pichia pastoris* by Cryo-TEM



Cryogenic transmission electron microscopy (cryoTEM) image of an intact ARMAN cell from an Iron Mountain biofilm. Image width is 576 nm.

History

Early development

In the 1960s, the use of transmission electron microscopy for structure determination methods was limited because of the radiation damage due to high energy electron beams. Scientists hypothesized that examining specimens at low temperatures would reduce beam-induced radiation damage.[11] Both liquid helium (−269 °C or 4 K or −452.2 °F) and liquid nitrogen (−195.79 °C or 77 K or −320 °F) were considered as cryogens. In 1980, Erwin Knapek and Jacques Dubochet published commenting on beam damage at cryogenic temperatures sharing observations that:

> Thin crystals mounted on carbon film were found to be from 30 to 300 times more beam-resistant at 4 K than at room temperature... Most of our results can be explained by assuming that cryoprotection in the region of 4 K is strongly dependent on the temperature.[12]

However, these results were not reproducible and amendments were published in *Nature* just two years later informing that the beam resistance was less significant than initially anticipated. The protection gained at 4 K was closer to "tenfold for standard samples of L-valine",[13] than what was previously stated.

In 1981, Alasdair McDowall and Jacques Dubochet, scientists at the European Molecular Biology Laboratory, reported the first successful implementation of cryo-EM.[14] McDowall and Dubochet vitrified pure water in a thin film by spraying it onto a hydrophilic carbon film that was rapidly plunged into cryogen (liquid propane or liquid ethane cooled to 77 K). The thin layer of amorphous ice was less than 1 μm thick and an electron diffraction pattern confirmed the presence of amorphous/vitreous ice. In 1984, Dubochet's group demonstrated the power of cryo-EM in structural biology with analysis of vitrified adenovirus type 2, T4 bacteriophage, Semliki Forest virus, Bacteriophage CbK, and Vesicular-Stomatitis-Virus.[15]

2017 Nobel Prize in Chemistry

In 2017, three scientists, Jacques Dubochet, Joachim Frank and Richard Henderson, were awarded the Nobel Prize in Chemistry for developing a technique that would image biomolecules.[4]

Potential rival to X-ray crystallography

As of October 27, 2020 X-ray crystallography has been used to image 150,494 biological samples and is the dominant technique in biological microscopy, with Cryo-EM far behind at just 6016.[16]

However, according to *Nature*, advancements in direct electron detectors (often referred to as a direct detection devices or DDDs) at the University of Cambridge[17] and automation of sample production by SPT labtech[18] has led to an increase in use in biological fields,[19] making Cryo-EM a potential rival.

The resolution of X-ray crystallography is limited by crystal purity,[20] and creating these samples is very time consuming, taking up to months or even years.[19] Also, some proteins are hard to crystalize.[19][21] Although sample preparation for Cryo-EM is still laborious,[22] it does not have these issues as it observes the sample in its “native state”.[21]

According to Proteopedia, the median resolution achieved by X-ray crystallography (as of May 19, 2019) on the Protein Data Bank is 2.05 Å,[20] and the highest resolution achieved on record (as of October 27, 2020) is 0.48 Å.[23] As of 2020, the majority of the protein structures determined by Cryo-EM are at a lower resolution of 3–4 Å.[24] However, the best Cryo-EM resolutions are approaching 1.5 Å,[25] making it a fair competitor in resolution in some cases.

Correlative light Cryo-TEM and Cryo-ET

In 2019, correlative light Cryo-TEM and Cryo-ET were used to observed tunnelling nanotubes (TNTs) in neuronal cells.[26]

Scanning electron cryomicroscopy

Scanning electron cryomicroscopy (cryoSEM) is a scanning electron microscopy technique with a scanning electron microscope's cold stage in a cryogenic chamber.

See also

- Cryofixation
- Electron tomography (ET)

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