Bio 5357 Lecture
(2) Electron Microscopy & Biomolecular Assemblies

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Assistant Professor
Dept. of Biochemistry and Molecular Biophysics

Washington University in St. Louis
School of Medicine
Outlines

- Transmission Electron Microscope (TEM)
- Fourier Transform
- Contrast Transfer Function (CTF)
- 3D Reconstruction
- **Cryo-EM Sample Preparation**
- Cryo-EM Techniques
- The Future of Single Particle Cryo-EM
Cryo-EM Sample Preparation

Cryo-electron microscopy of viruses

Marc Adrian, Jacques Dubochet, Jean Lepault & Alasdair W. McDowall
European Molecular Biology Laboratory, Postfach 10.2209, D-6900 Heidelberg, FRG

Thin vitrified layers of unfixed, unstained and unsupported virus suspensions can be prepared for observation by cryo-electron microscopy in easily controlled conditions. The viral particles appear free from the kind of damage caused by dehydration, freezing or adsorption to a support that is encountered in preparing biological samples for conventional electron microscopy. Cryo-electron microscopy of vitrified specimens offers possibilities for high resolution observations that compare favourably with any other electron microscopical method.

Cryo-electron microscopy of vitrified specimens


Vitreous = glass, amorphous
NOT crystalline

Jacques Dubochet

JACQUES DUBOCHEL, MARC ADRIAN, JIIN-JU CHANG, JEAN-CLAUDE HOMO, JEAN LEPault, ALASDAIR W. MCDOwALL AND PATRICK SCHULTZ
European Molecular Biology Laboratory (EMBL), Postfach 10. 2209, D-6900 Heidelberg, FRG
Cryo-EM Sample Freezing by Vitrobot
Outlines

- Transmission Electron Microscope (TEM)
- Fourier Transform and convolution
- Contrast Transfer Function (CTF)
- 3D Reconstruction
- Cryo-EM Sample Preparation
- **Cryo-EM Techniques**
- The Future of Single Particle Cryo-EM
Cryo-EM Techniques

- Electron crystallography (2D crystal)
- Single particle cryo-EM
  - Single particle helical reconstruction
- Cryo-electron tomography (Cryo-ET)
2D crystallization of membrane proteins

Two-dimensional Crystallization of a Membrane Protein

Addition of lipids

Removal of detergent
Lipid–protein interactions in double-layered two-dimensional AQPO crystals

Tamir Gonen¹, Yifan Cheng¹, Piotr Sliz²,³, Yoko Hiroaki⁴, Yoshinori Fujiyoshi⁴, Stephen C. Harrison²,³ & Thomas Walz¹

Lens-specific aquaporin-0 (AQPO) functions as a specific water pore and forms the thin junctions between fibre cells. Here we describe a 1.9 Å resolution structure of junctional AQPO, determined by electron crystallography of double-layered two-dimensional crystals. Comparison of junctional and non-junctional AQPO structures shows that junction formation depends on a conformational switch in an extracellular loop, which may result from cleavage of the cytoplasmic amino and carboxy termini. In the centre of the water pathway, the closed pore in junctional AQPO retains only three water molecules, which are too widely spaced to form hydrogen bonds with each other. Packing interactions between AQPO tetramers in the crystalline array are mediated by lipid molecules, which assume preferred conformations. We were therefore able to build an atomic model for the lipid bilayer surrounding the AQPO tetramers, and we describe lipid–protein interactions.
Cryo-EM Techniques

- Electron crystallography (2D crystal)
- **Single particle cryo-EM**
  - Single particle helical reconstruction
- Cryo-electron tomography (Cryo-ET)
Geometry of Cryo-EM Samples

Repeating assemblies

2D crystals
(high tilts needed for 3D)

Helical filaments or tubes
(no tilts needed)

Single particles
(no tilting needed, if randomly oriented)

Icosahedra (or other symmetrical particles)

Asymmetric
(e.g. ribosomes)
Medium-sized membrane protein structures

(a) V-type ATPase
(b) Complex I
(c) Anthrax pore

Large virus structures

(a) Rotavirus
(b) BMV
(c) TMV

Subramaniam, S., Kuhlbrandt, W. & Henderson, R. (2016) IUCrJ
# OF CRYO-EM STRUCTURES OF SMALL COMPLEXES IS INCREASING

EMDB entries: Size vs. Resolution

- SAM-IV riboswitch RNA:
  - 39 kDa
  - 3.7 Å

- Streptavidin:
  - 52 kDa
  - 2.6 Å

- PfCRT+Fab:
  - 103 kDa
  - 3.3 Å

- NavPaS channel:
  - 191 kDa
  - 2.6 Å

- OSCA1.2 channel:
  - 178 kDa
  - 3.1 Å

- Neurotensin receptor-
  - G_{i1} complex:
  - 135 kDa
  - 3 Å

© Mengyu Wu & Gabe Lander

Cryo-EM has achieved true atomic resolution!

apoferitin (pictured on the cover) at ~1.22 Å resolution

© Sjors Scheres
Image classification enables the study of macromolecular dynamics.

Cryo-EM Techniques

- Electron crystallography (2D crystal)
- Single particle cryo-EM
  - Single particle helical reconstruction
- Cryo-electron tomography (Cryo-ET)
An extensively glycosylated archaeal pilus survives extreme conditions

Fengbin Wang, Virginija Cvirkaite-Krupovic, Mark A. B. Kreutzberger, Zhangli Su, Guilherme A. P. de Oliveira, Tomasz Osinski, Nicholas Sherman, Frank DiMaio, Joseph S. Wall, David Prangishvili, Mart Krupovic and Edward H. Egelman

(a) Image of a pilus structure. (b) Electron microscopy image with diffraction pattern. (c) Graph showing rise vs. rotation. (d) 3D reconstruction of the pilus structure. (e) Cross-sectional view of the pilus.
EB3-decorated GTPγS-MTs

- FEI Titan microscope
- K2 direct detector

β-tubulin
α-tubulin
EB3

Single Particle Approach
Cryo-EM Techniques

- Electron crystallography
- Single particle cryo-EM
  - Single particle helical reconstruction
- Cryo-electron tomography (Cryo-ET)
Cryo-electron tomography methodology: Tilt-series

Cryo-electron tomography (cryo-ET) of an intact *Bdellovibrio bacteriovorus* cell

Cryo-ET structure of the human nuclear pore complex

Cryo-focused ion beam (cryo-FIB) milling to analyze structures in situ

Outlines

- Transmission Electron Microscope (TEM)
- Fourier Transform and convolution
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- 3D Reconstruction
- Cryo-EM Sample Preparation
- Cryo-EM Techniques
- Cryo-EM and alphafold2
Cryo-EM Won the Nobel Prize in Chemistry 2017

Joachim Frank, New York, U.S.
Richard Henderson, Cambridge, Britain
Jacques Dubochet, Lausanne, Switzerland
PROTEIN POWER
AI network predicts highly accurate 3D structures for the human proteome
AlphaFold DB provides open access to protein structure predictions for the human proteome and 20 other key organisms to accelerate scientific research.
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<th>Species</th>
<th>Common Name</th>
<th>Reference Proteome</th>
<th>Predicted Structures</th>
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<td>Arabidopsis thaliana</td>
<td>Arabidopsis</td>
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<td>Glycine max</td>
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- *Glutamate dehydrogenase* (GDH) 334 kDa, 1.8 Å
- *Isocitrate dehydrogenase* (IDH) 93 kDa, 3.8 Å
- *LDH* 145 kDa, 2.8 Å
- *β-galactosidase* 465 kDa, 2.2 Å
- *Ribosome* 2,300 kDa, 2.9 Å
- *Dengue Virus* 11,200 kDa, 3.6 Å

Difficulty of cryo-EM analysis
Structural Study of Microtubule Doublet by Single Particle Method
(Our protocol for *Chlamydomonas*)

1. ATP
2. subtilisin
3. protease inhibitor

Axoneme Purification

180 nm

negative stain EM
Atomic Structure of Microtubule Doublet

Identified 38 associated proteins including 33 MIPs!

**Molecular Cell Biology**

Ninth Edition  ©2021

Harvey Lodish; Arnold Berk; Chris A. Kaiser; Monty Krieger; Anthony Bretscher; Hidde Ploegh; Kelsey C. Martin; Michael Yaffe; Angelika Amon

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