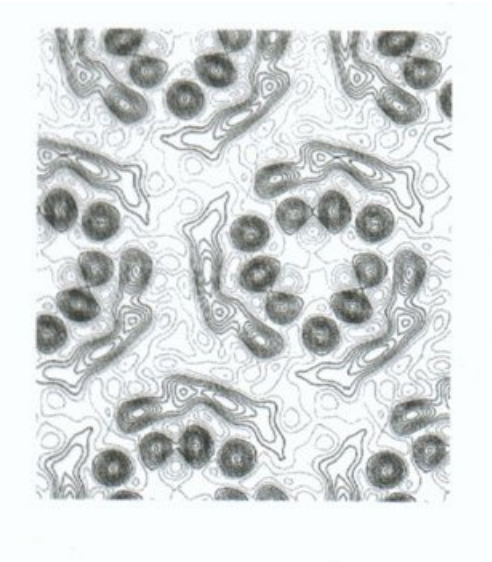
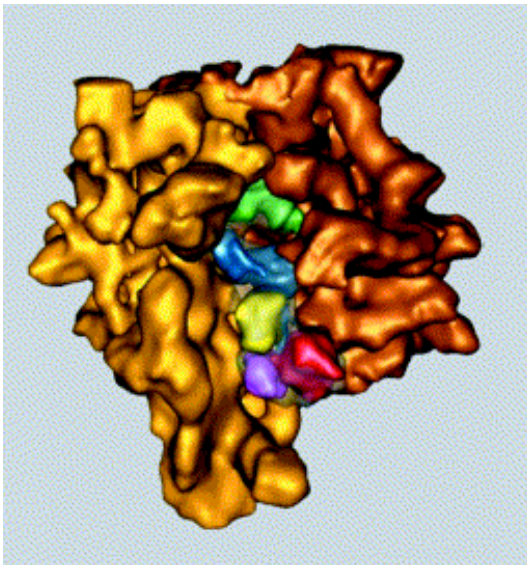
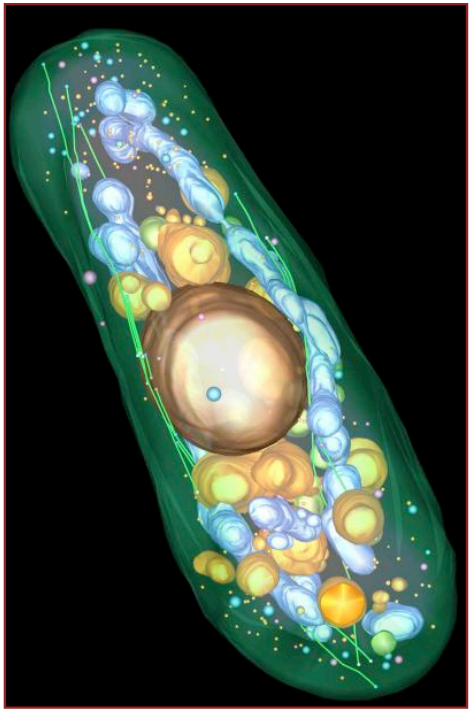
Cryo-electron microscopy
Cryo-EM







Garry Taylor

www.st-andrews.ac.uk/~glt2/BL3301

Electron has a wavelength

- de Broglie relationship:

$$m v = h / \lambda \qquad \text{or} \qquad \lambda = h / mv$$

- Accelerate e^- in a field of potential V, it gains energy eV which is converted to kinetic energy:

$$1/2\ mv^2 = eV \qquad \text{or} \qquad v = (2eV/m)^{1/2}$$

$$\therefore \quad \lambda = h / (2emV)^{1/2}$$

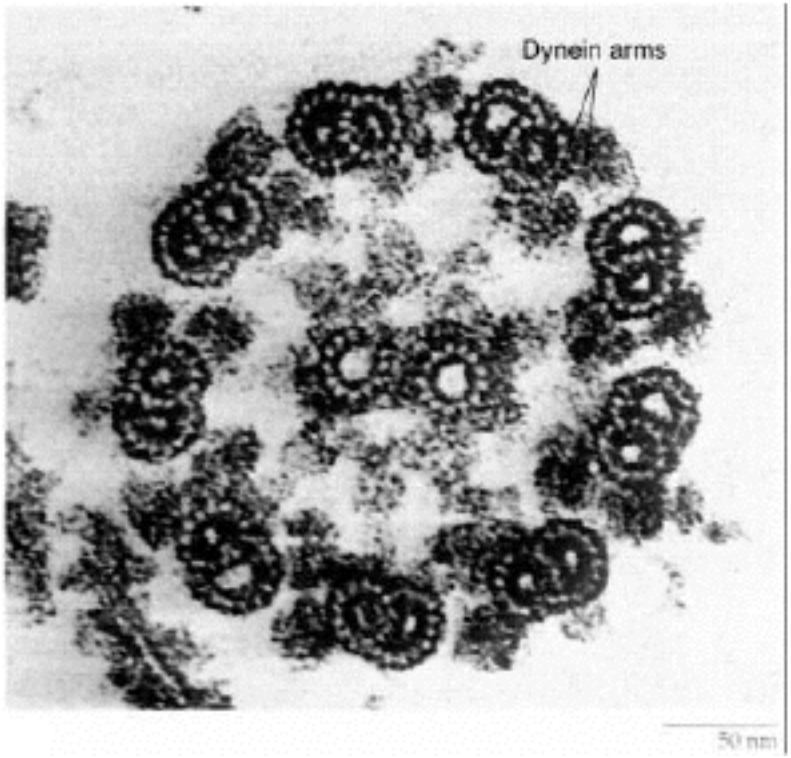
V (kV)	λ(Å)
50	0.054
100	0.037
1000	0.0087

Scanning electron microscopy
SEM

- ~100Å beam swept across the surface
- ~2000Å resolution

Transmission Electron Microscopy (TEM)

100Kev to 1 Mev electrons illuminate whole field of view. Image is a projection of the thin specimen.



TEM

electrons generated & focused

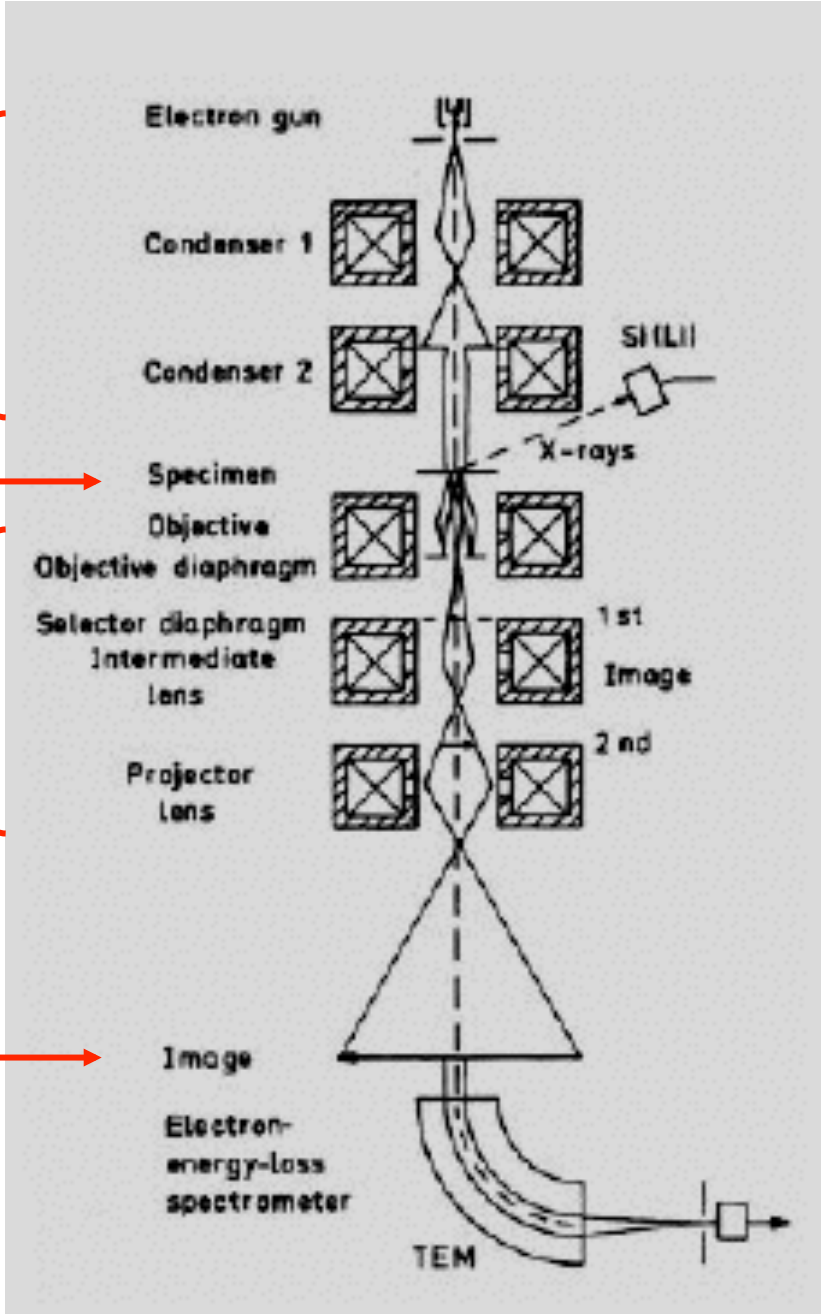
specimen

objective lens (forms image)

intermediate lens (switch between imaging and diffraction modes)

projector lens (magnifies image or diffraction pattern)

image

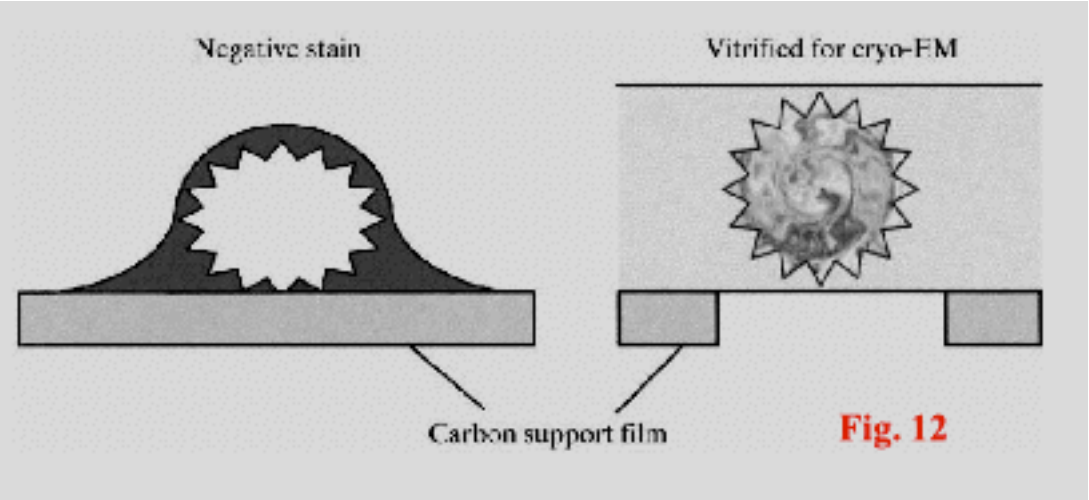


TEM

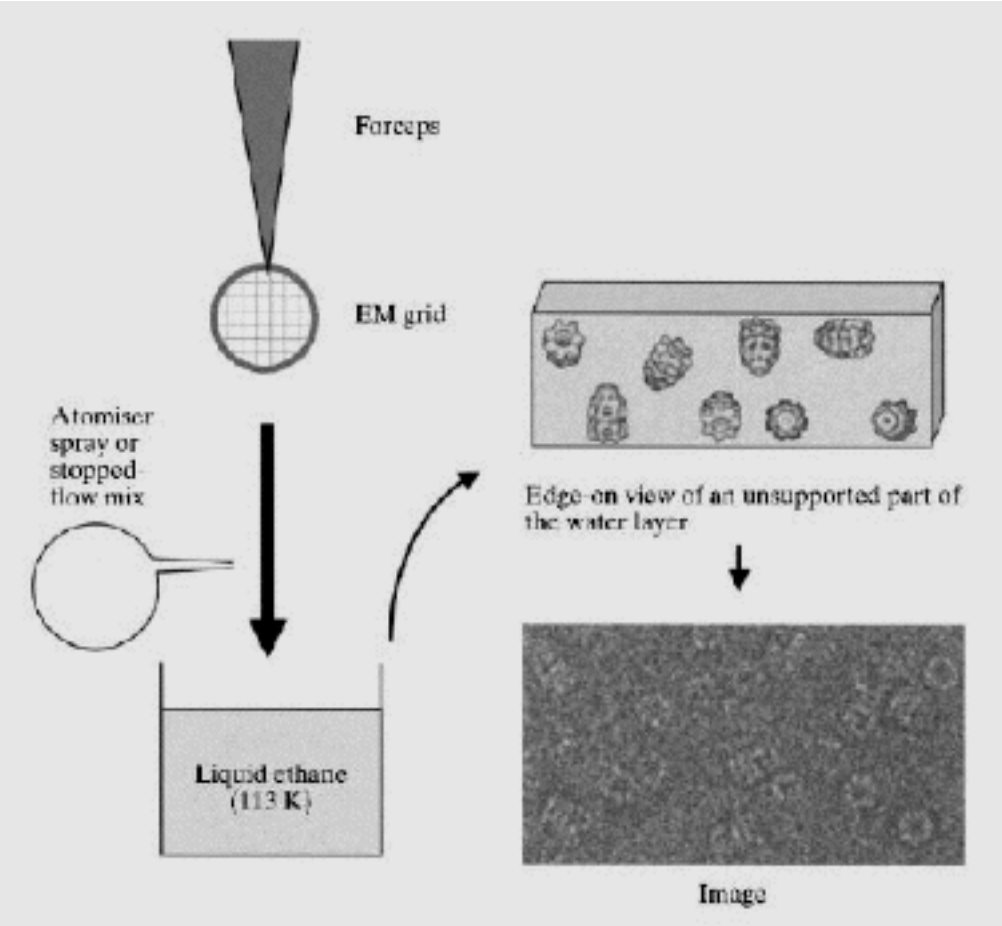
Interactions of electrons with matter

- 90% pass through
- Elastic scattering
  - Electron interacts with Coulomb potential of nucleus (2000 x heavier)
  - Bounces off, no energy loss, same λ
- Inelastic scattering
  - Electrons interact with electrons
  - Energy loss, different λ, focused at different place
  - Chromatic aberration
  - Radiation damage - ions & reactive species
- So, use very thin specimens

Negative stain v cryo-EM



Vitrification - rapid freezing



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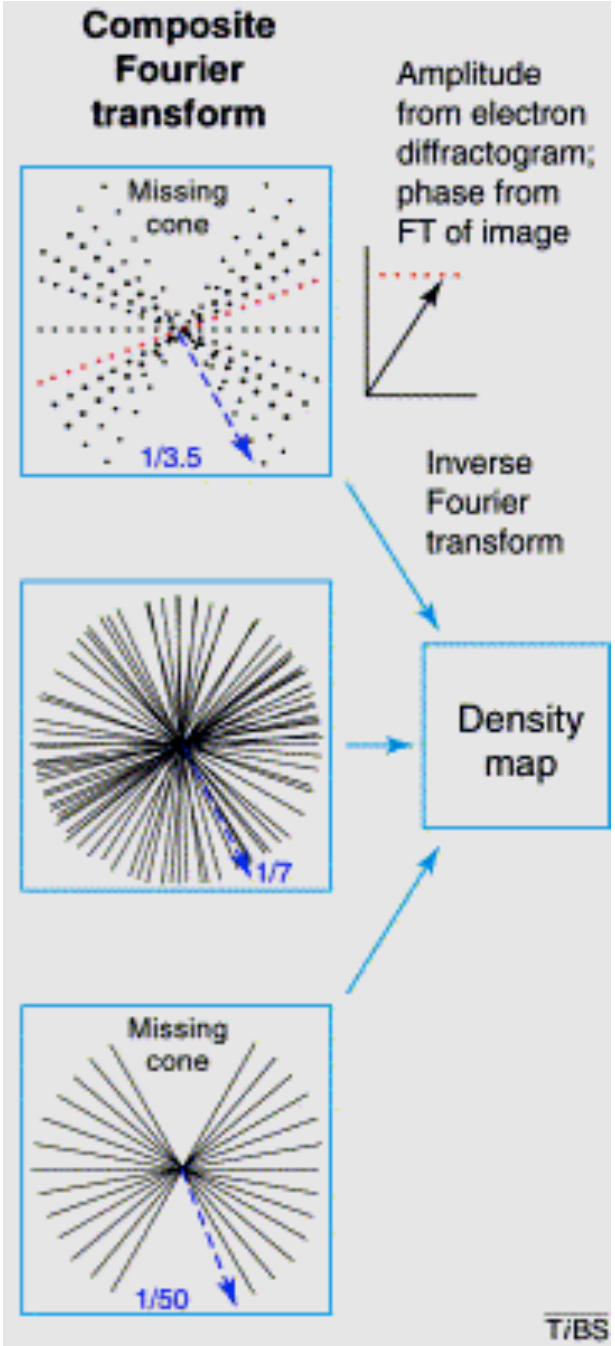
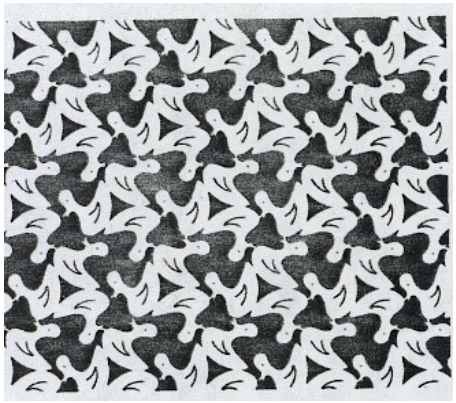
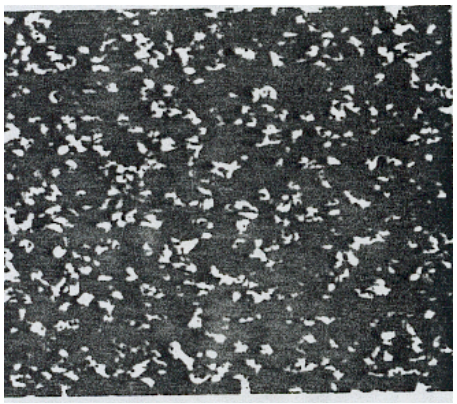


1. Electron crystallography – need a regular 2D array of molecules

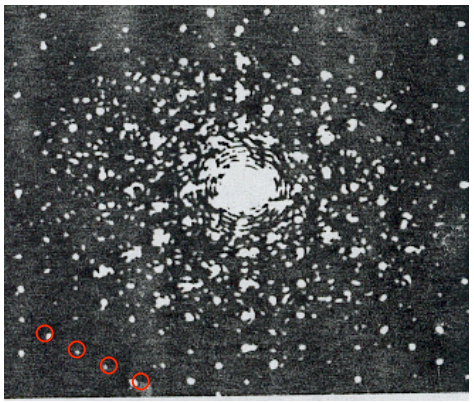
image enhancement by Fourier averaging



2D object with local 3-fold symmetry



Typical noisy image Fourier transform to get phases



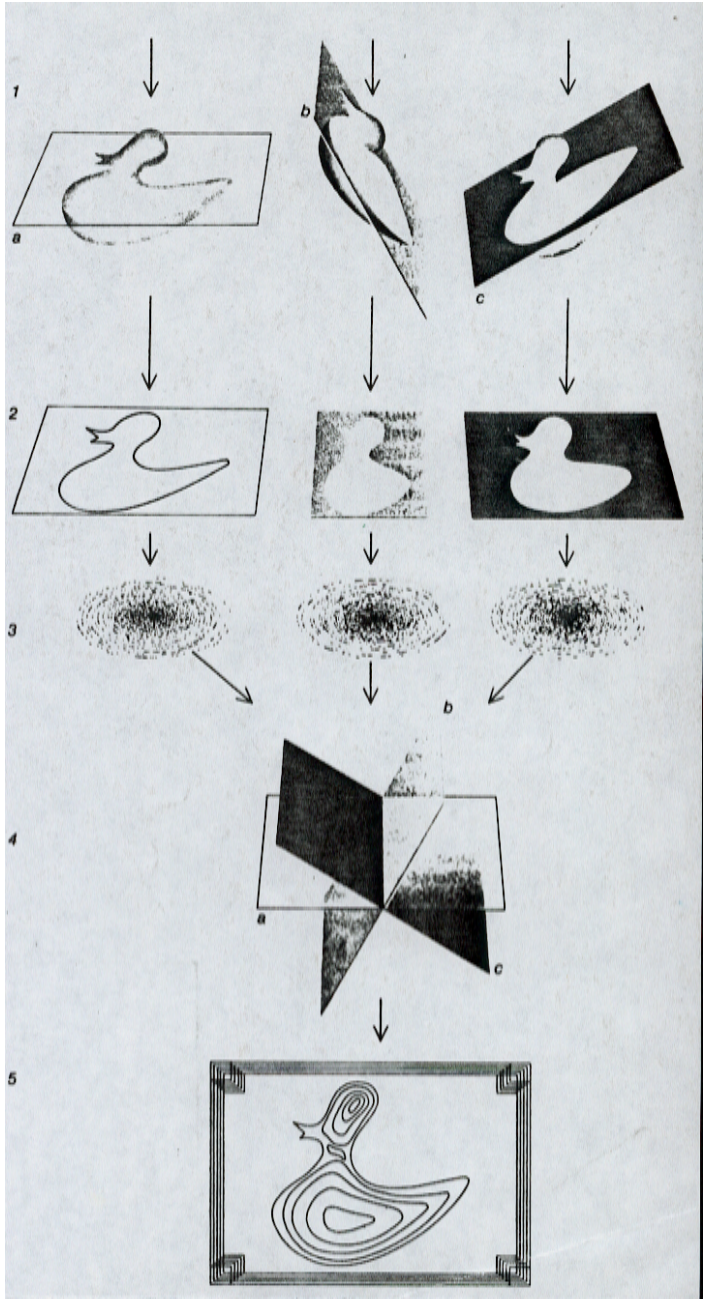
Electron diffraction of noisy image - measure intensities of peaks

Combine diffraction amplitudes with image phases

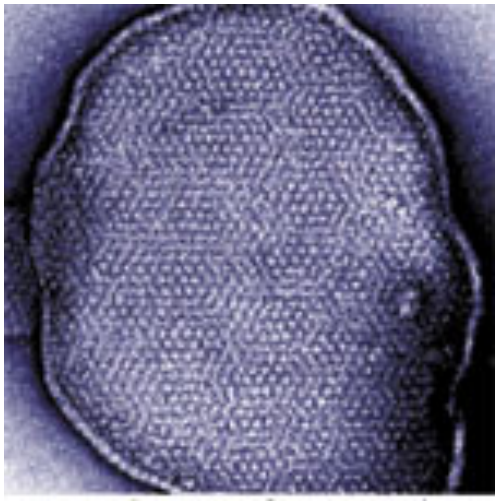
3-fold symmetry average image

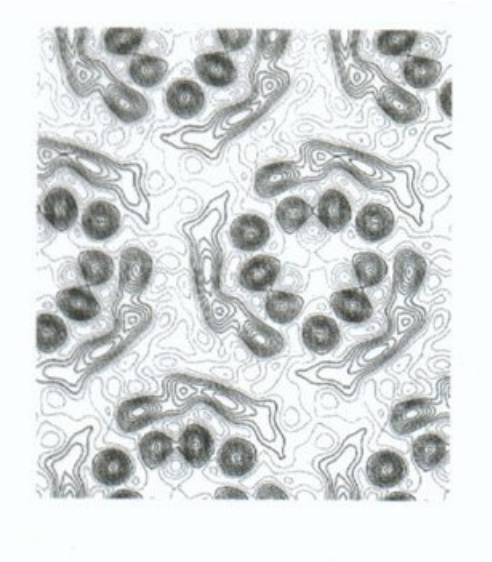
Electron crystallography from 2D to 3D

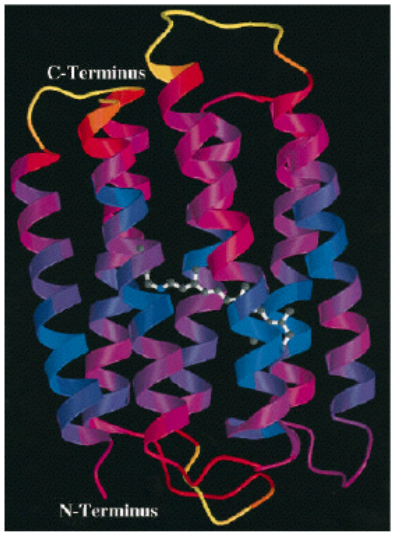
1. Tilt sample
2. Capture image
3. Collect electron diffraction
4. Build up 3D Fourier space (amplitudes from electron diffraction, phases from Fourier inversion of image)
5. Calculate 3D electron density map

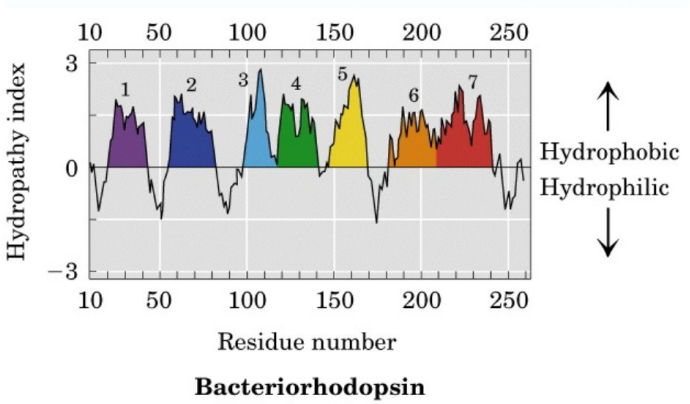


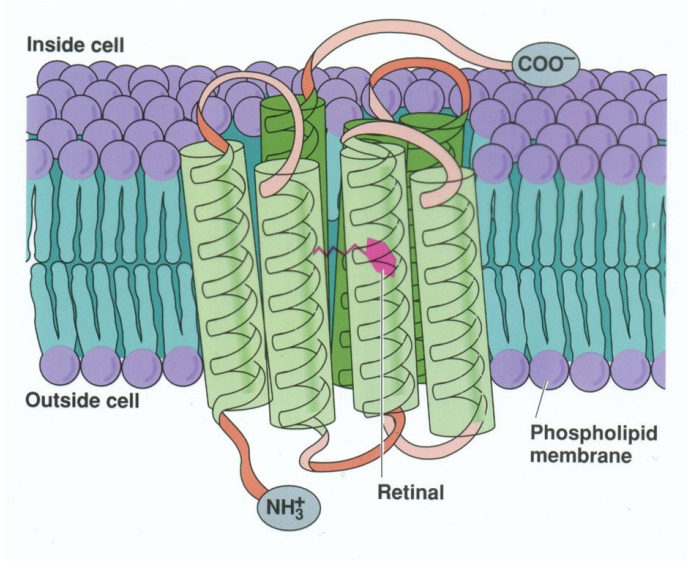
First success..bacteriorhodopsin



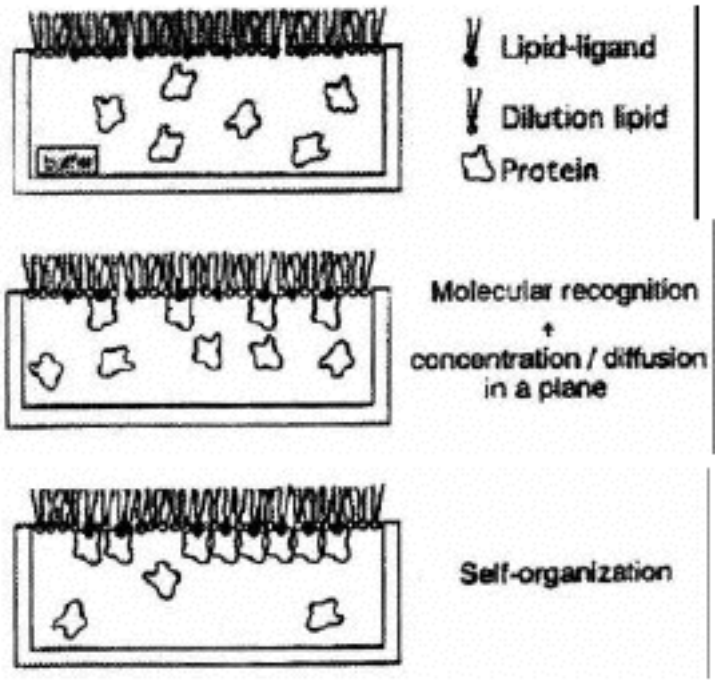






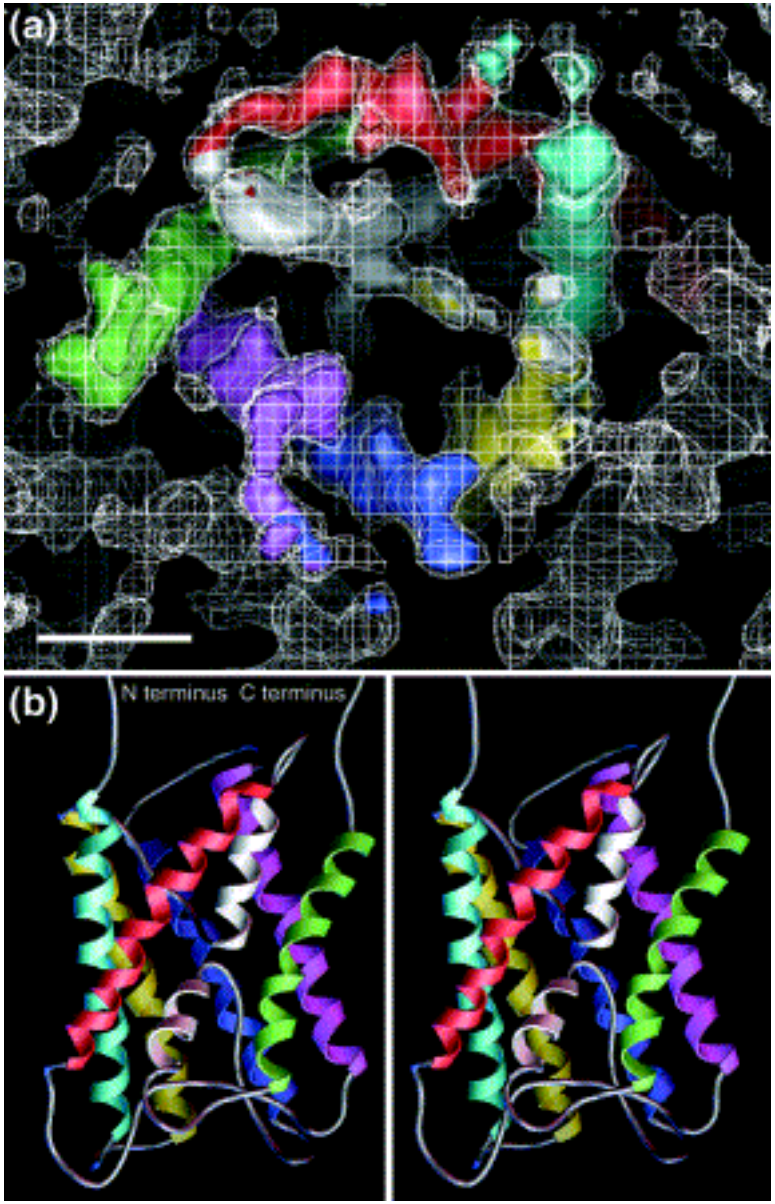


Extending the method..
inducing 2D arrays

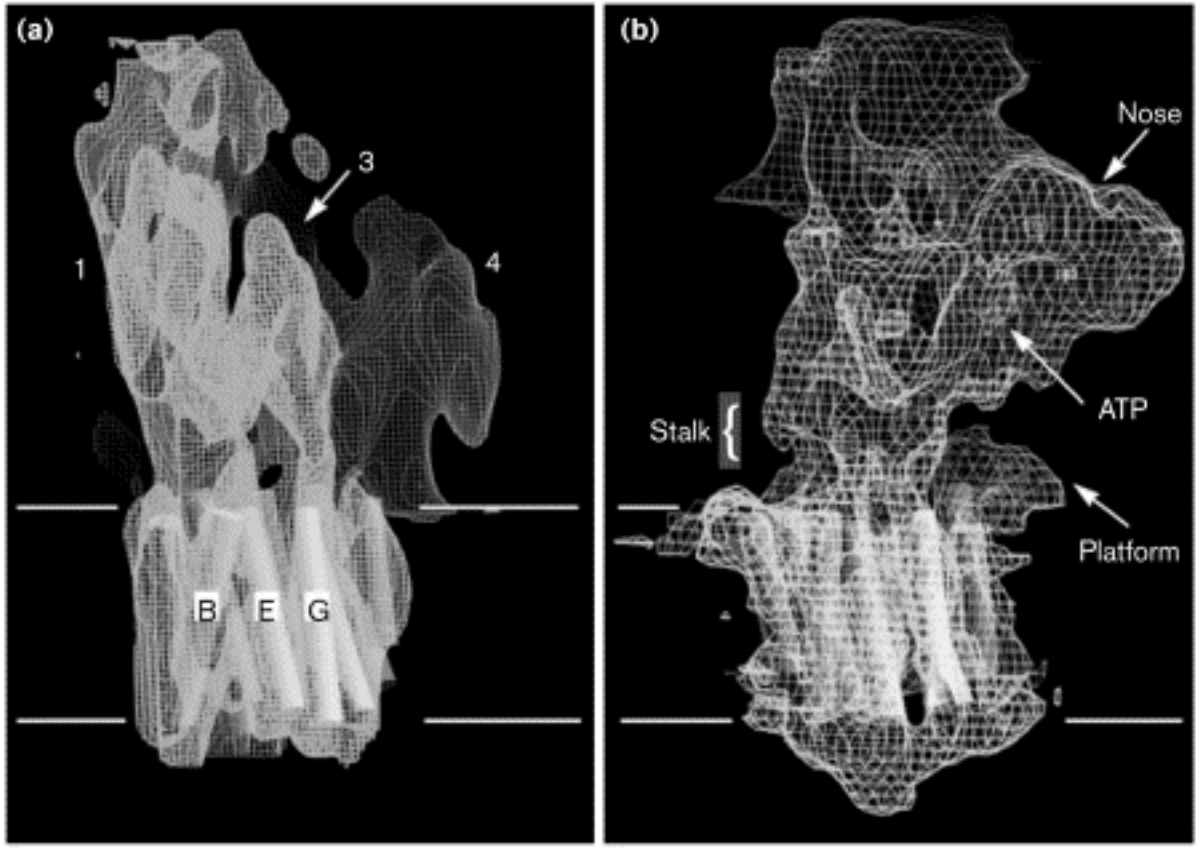


Human aquaporin

- 4Å electron diffraction of ice-embedded 2D crystals
- Stereo view of six tilted transmembrane α-helices

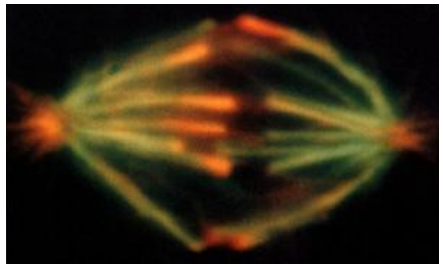


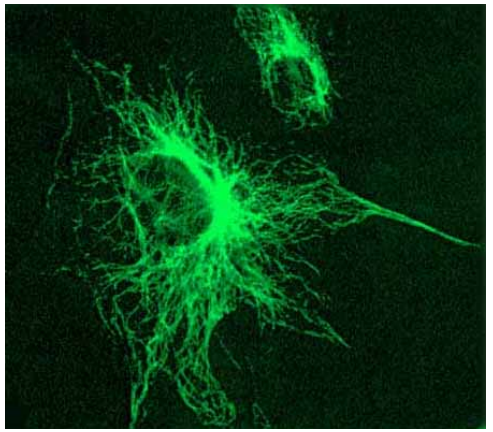
ATPase at 8Å resolution



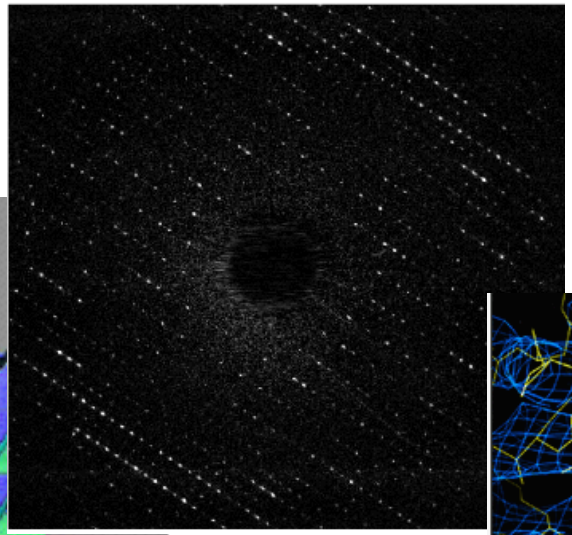
Current Opinion in Chemical Biology

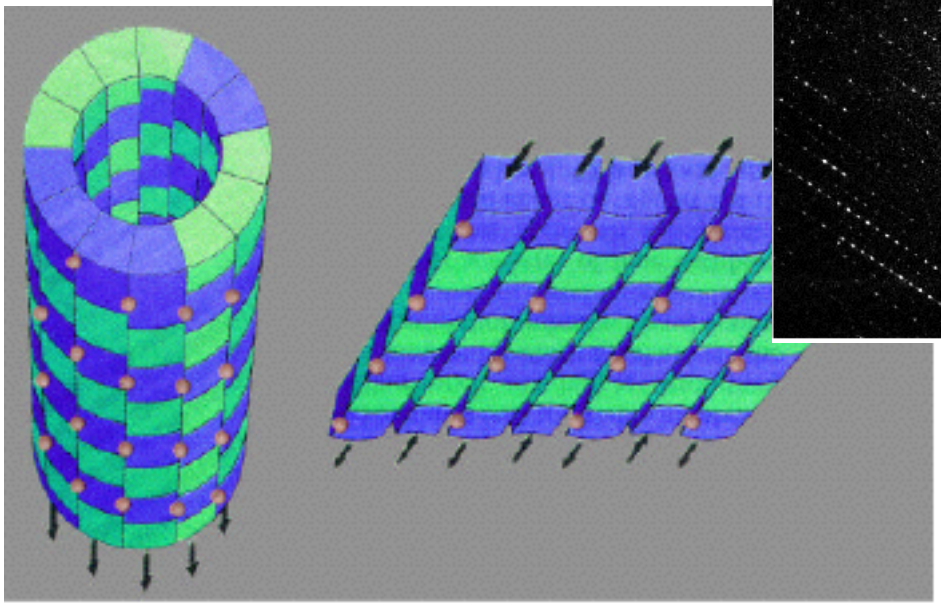
Tubulin
at 3.7Å resolution



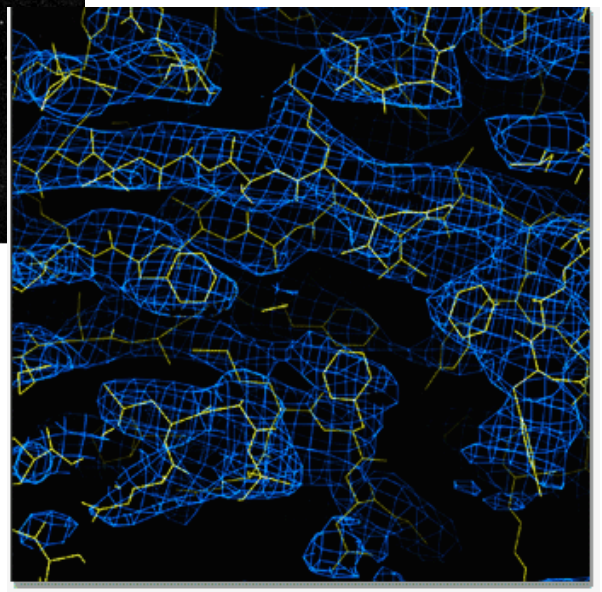


Electron Diffraction Pattern

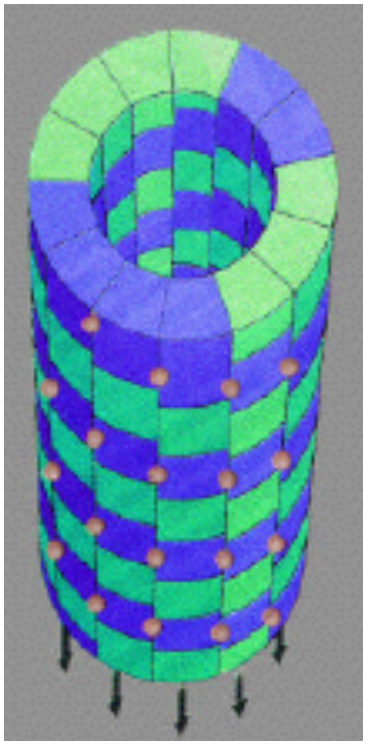


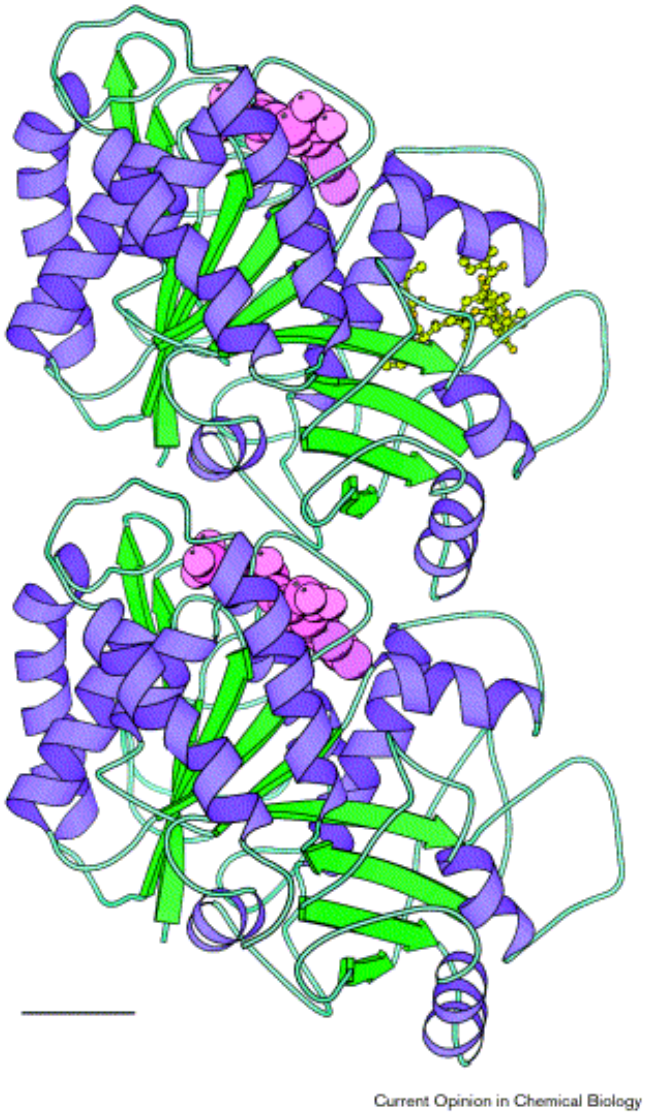


Zinc-Induced Tubulin Sheets



Tubulin





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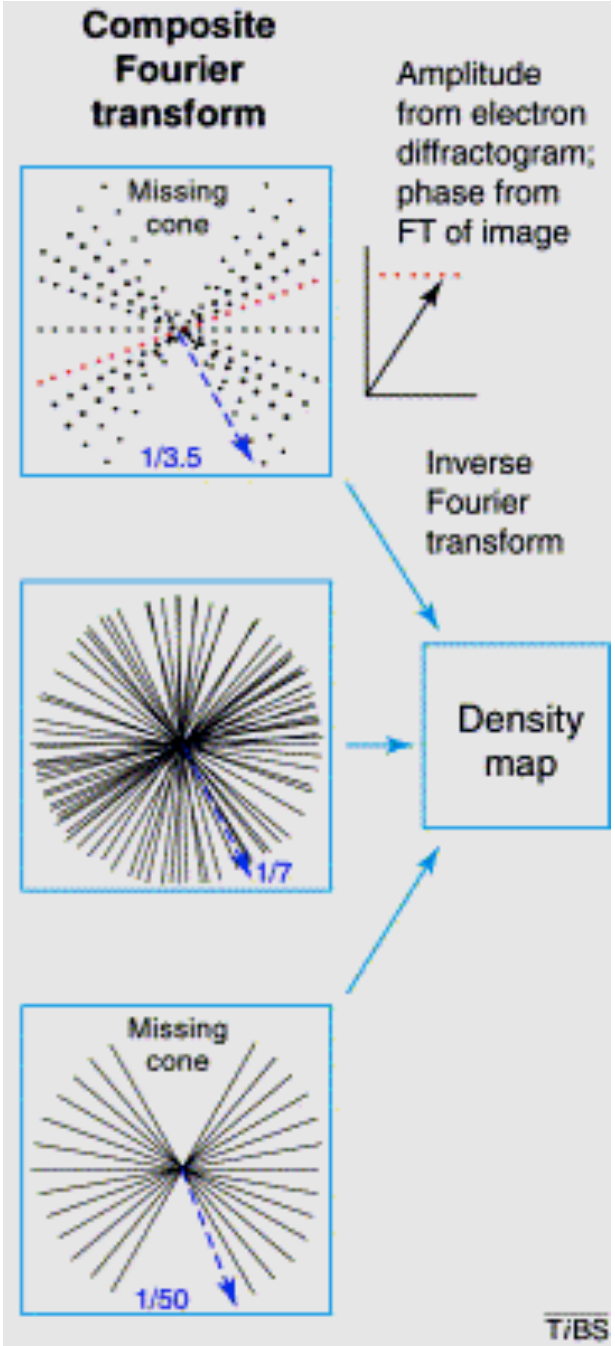
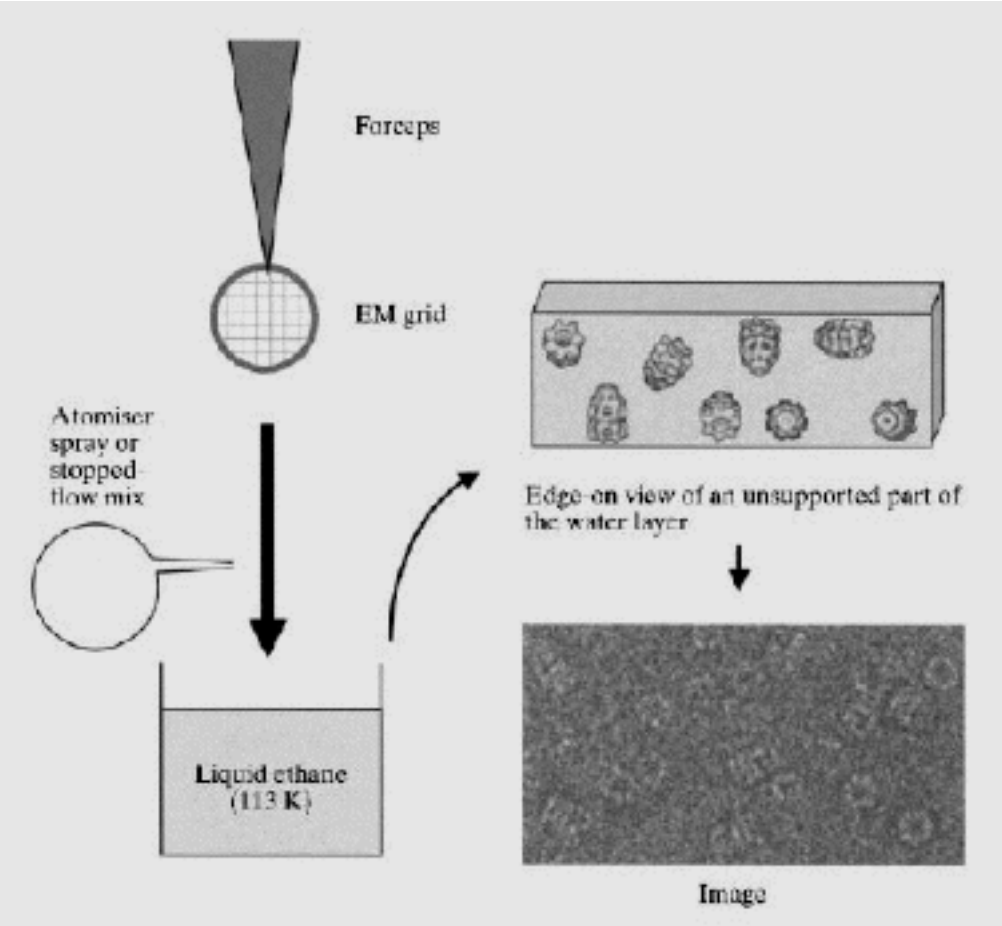


2. Single particle analysis



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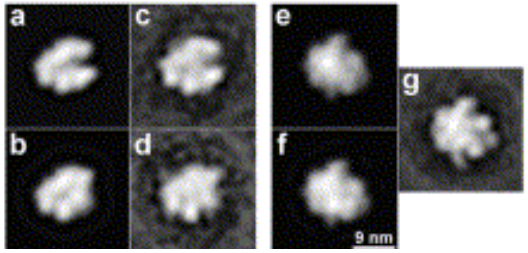
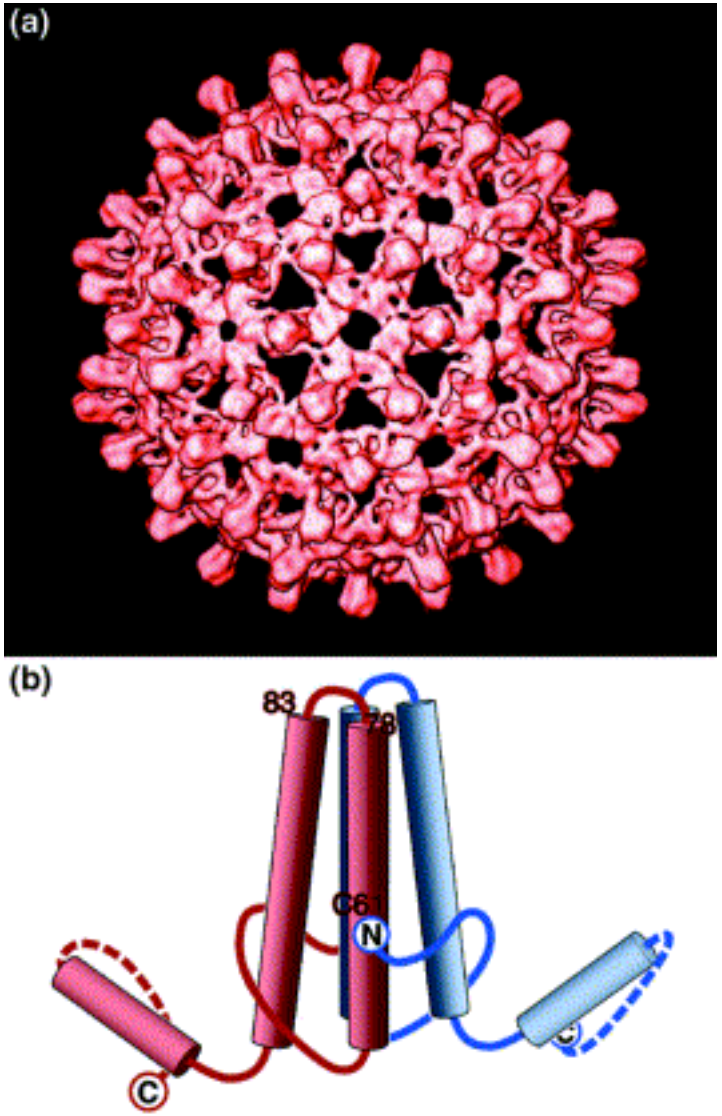


- Lower limit is 250 - 500 kDa.
- Missing cone not a problem as long as multiple views are present
- Actually 1000s of particles
- Problem: finding relative orientation of each particle
- First achieved with icosahedral viruses - 60 - fold symmetry reduces number of particles needed. 9Å resolution at best.

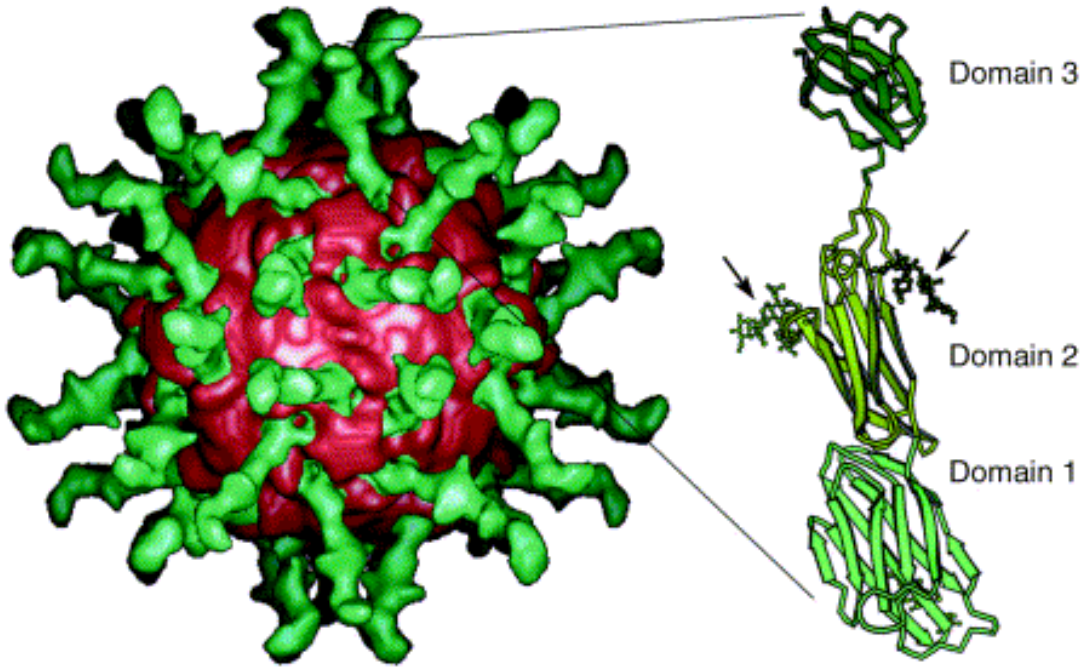
Hepatitis B virus capsid

- 9Å icosahedral 3D reconstruction from single particles
- Model of the 149-residue dimer that serves as its building block



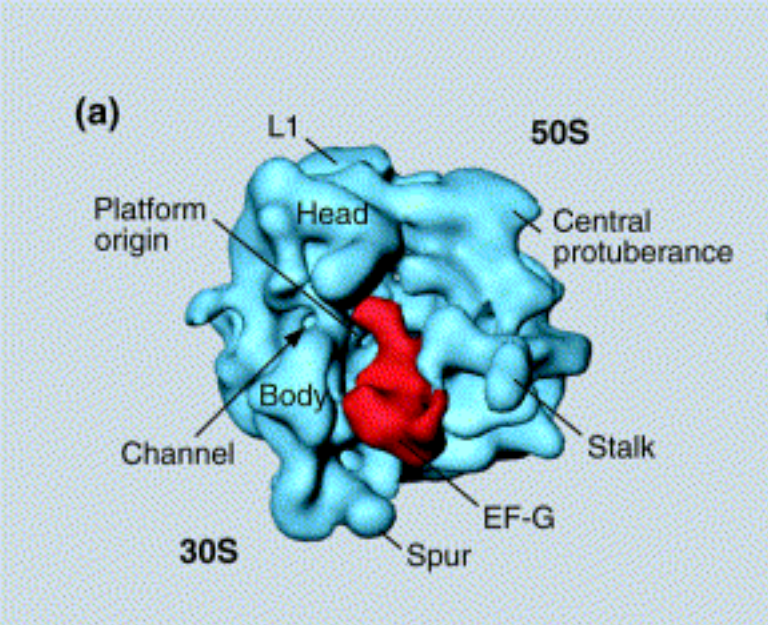
Poliovirus and its receptor

- 21Å resolution
- virus with water soluble ectodomain of its receptor
- revealed binding interactions
- allowed construction of quasi-atomic model of receptor, known to consist of 3 immunoglobulin domains

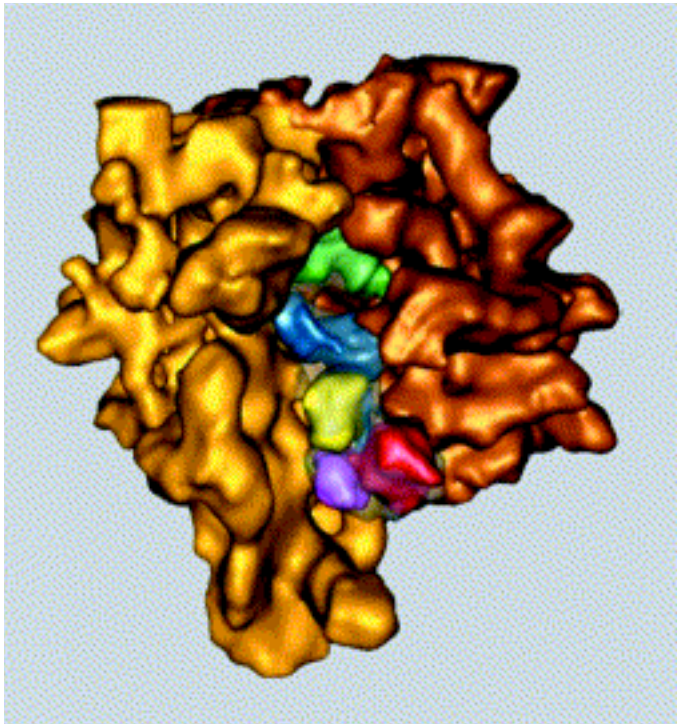


Interactions of the elongation factor with the 70S *E. coli* ribosome

20Å map of ribosome (blue) and EF-G in red, from difference map

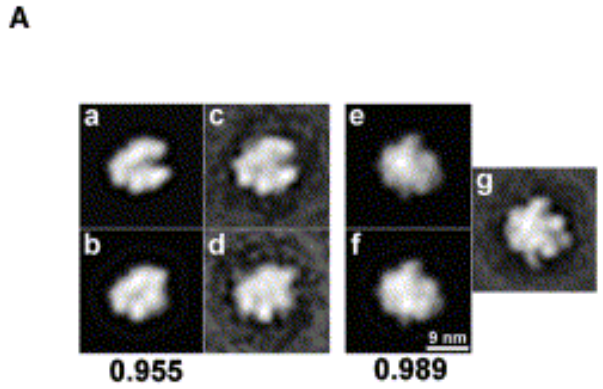


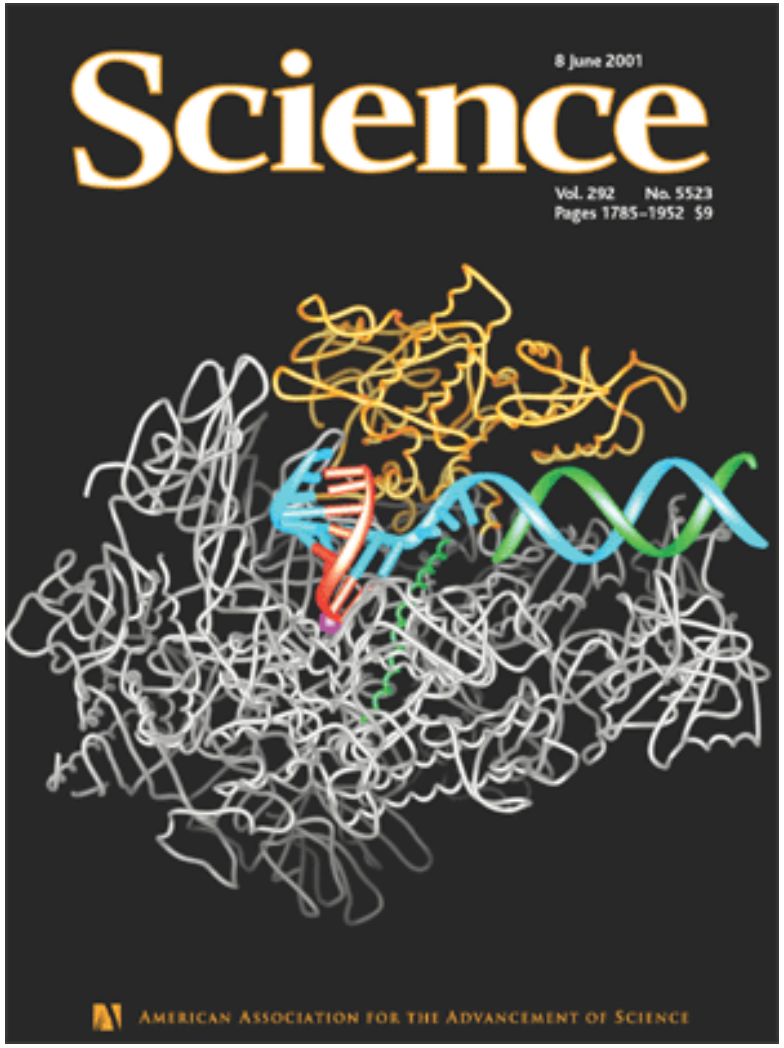
13Å map of ribosome with EF-G and tRNAs

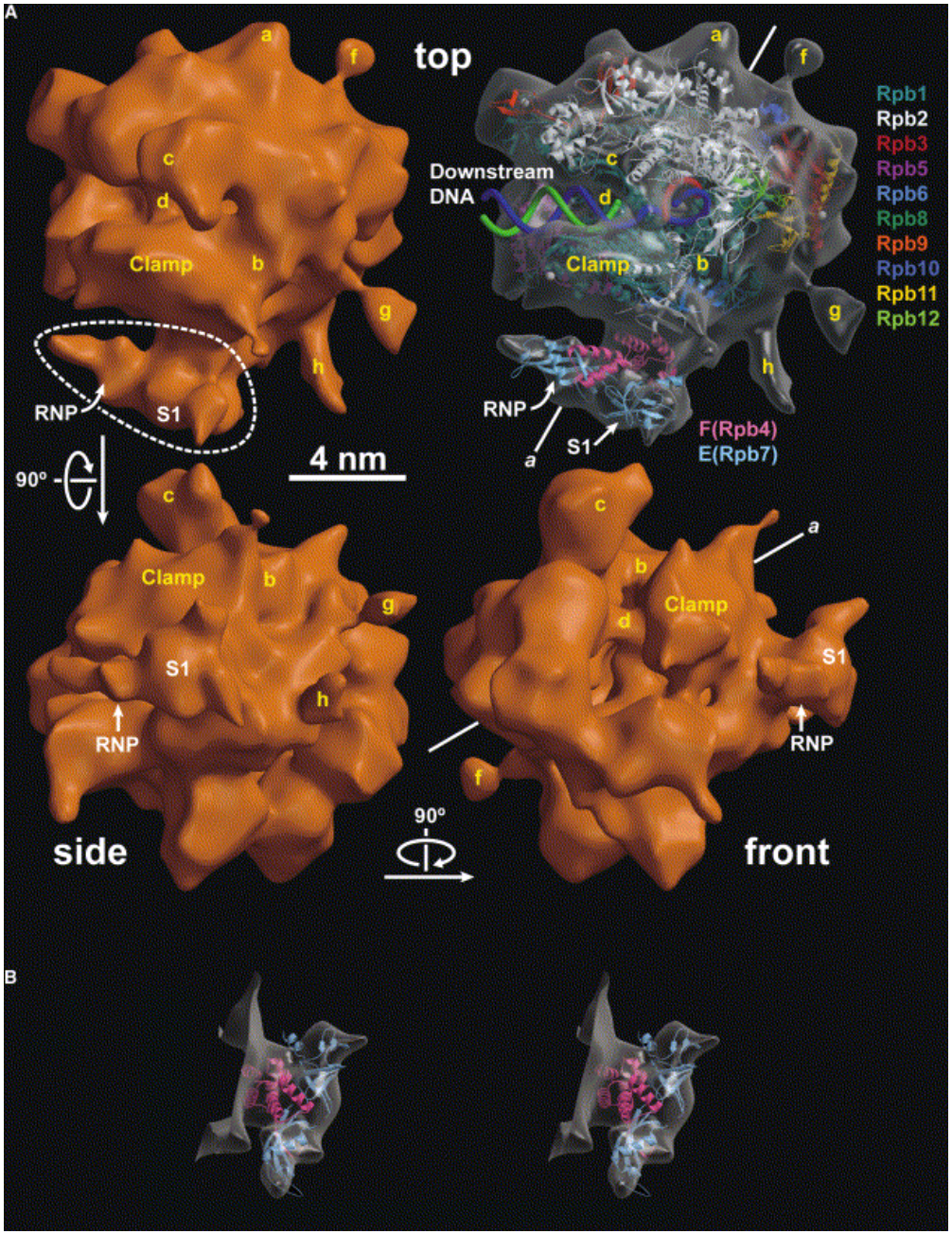


RNA polymerase

- 18Å resolution structure of the 12-subunit complex

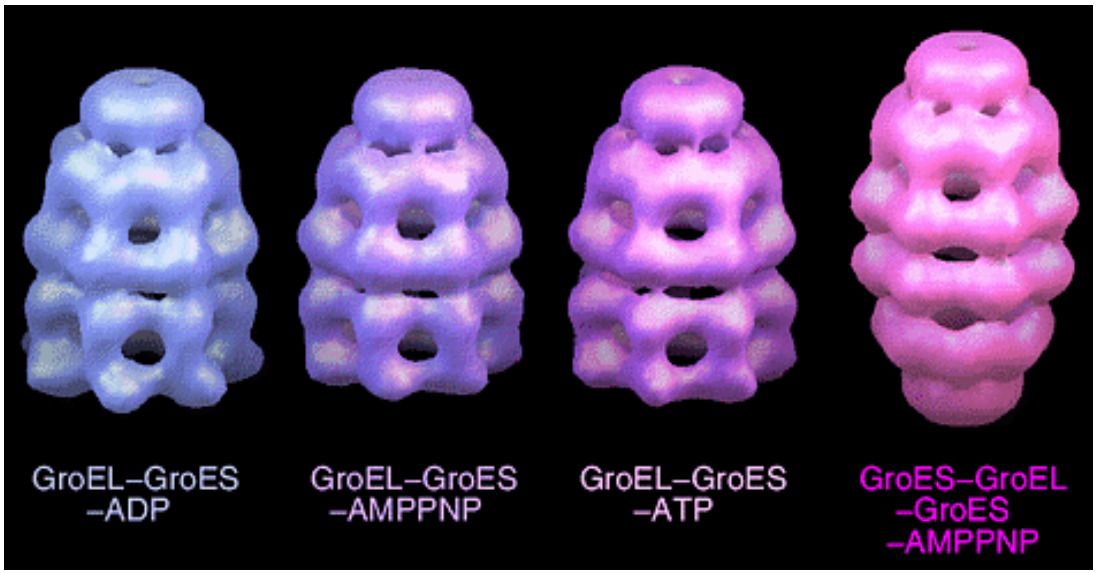


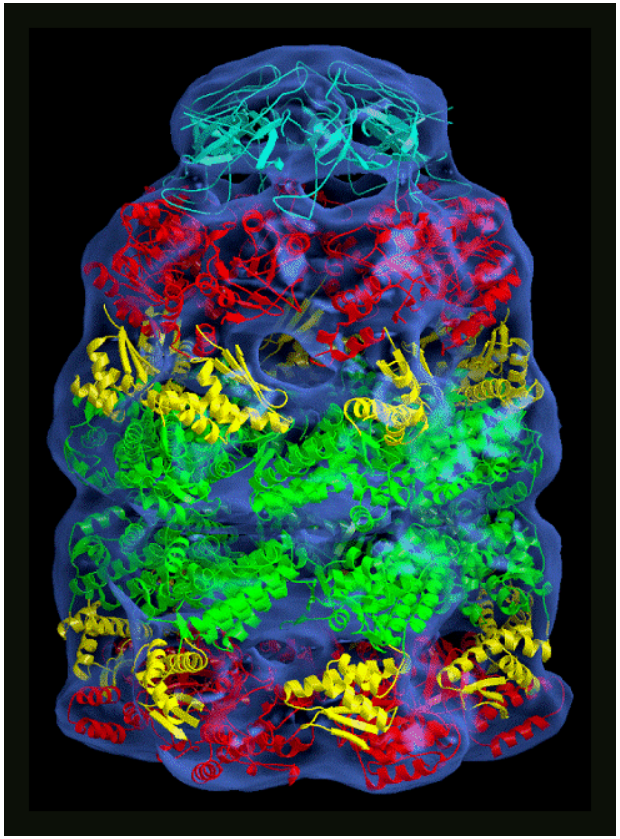



A
top
Rpb1
Rpb2
Rpb3
Rpb5
Rpb6
Rpb8
Rpb9
Rpb10
Rpb11
Rpb12
Downstream
DNA
Clamp
RNP
S1
F(Rpb4)
E(Rpb7)
4 nm
90°
side
front
B

Conformational changes studied by Cryo-EM

- http://people.cryst.bbk.ac.uk/~ubcg16z/chaperone.html





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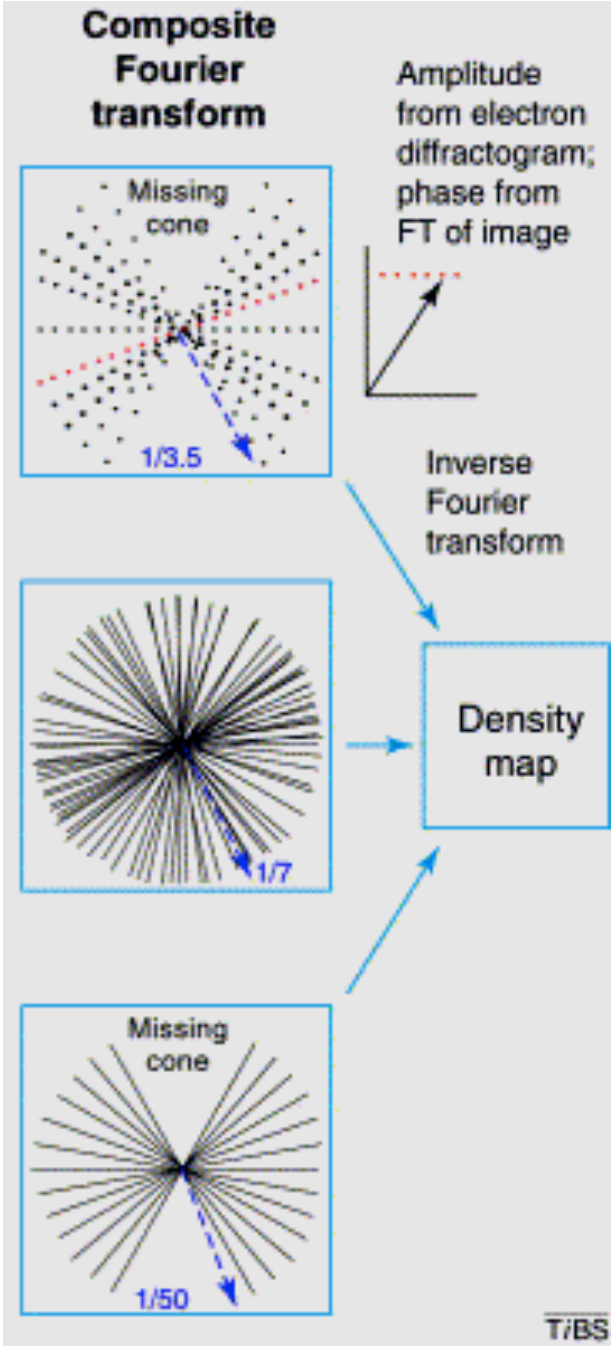
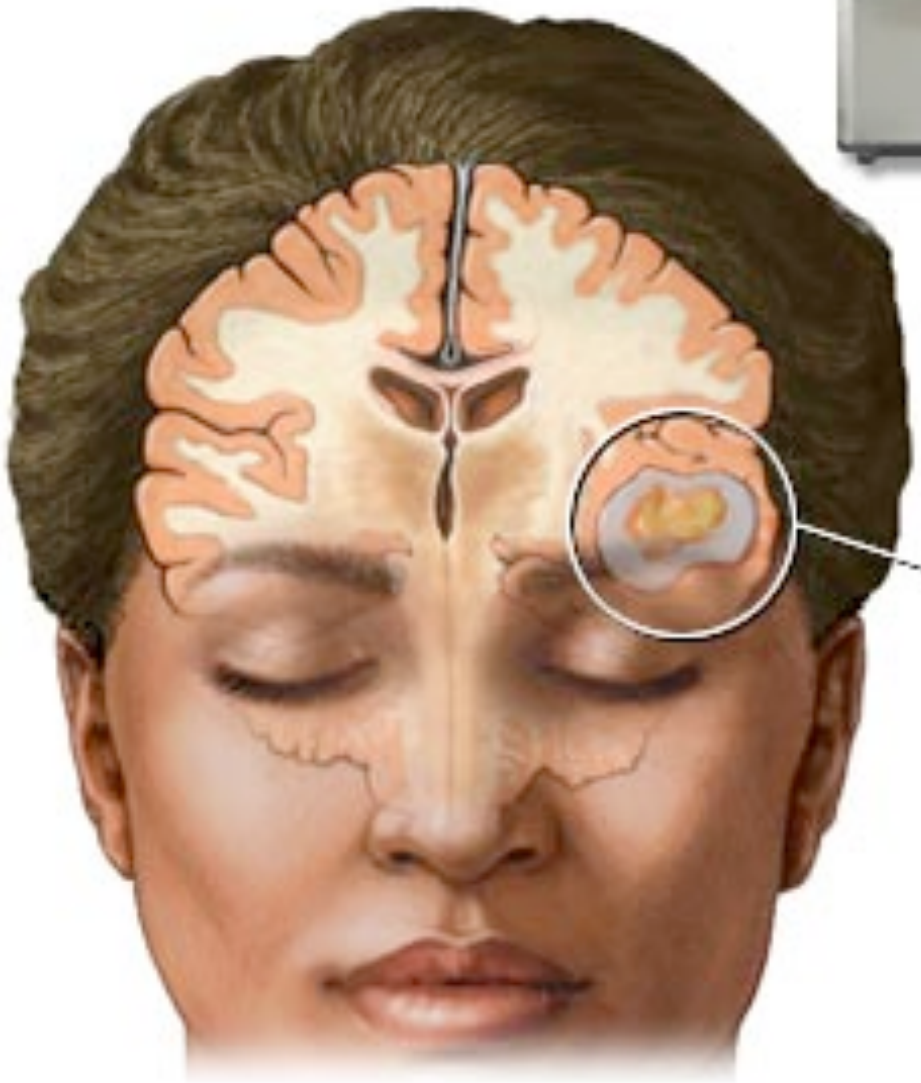
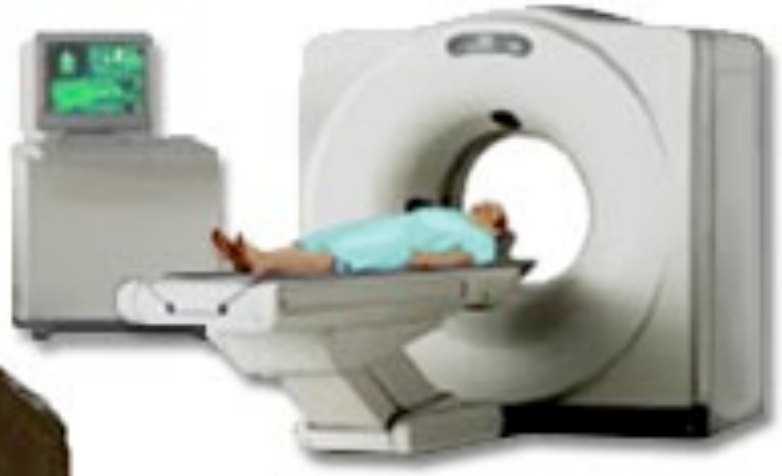
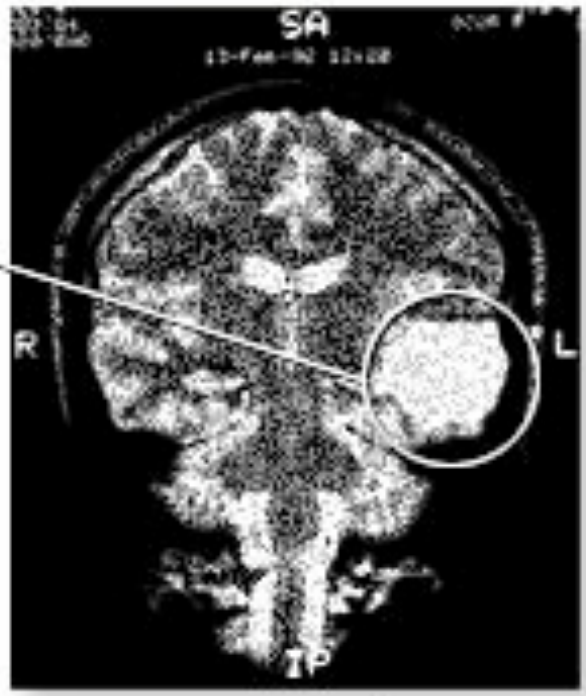


Computed tomography (CT or CAT scan) of the brain







A.D.A.M.

3. Electron tomography

- Multiple projections of a particle at different angles
- Problem: electron dose
- ~1000 e nm^{-2} for high resolution
- ~10,000 e nm^{-2} for medium resolution

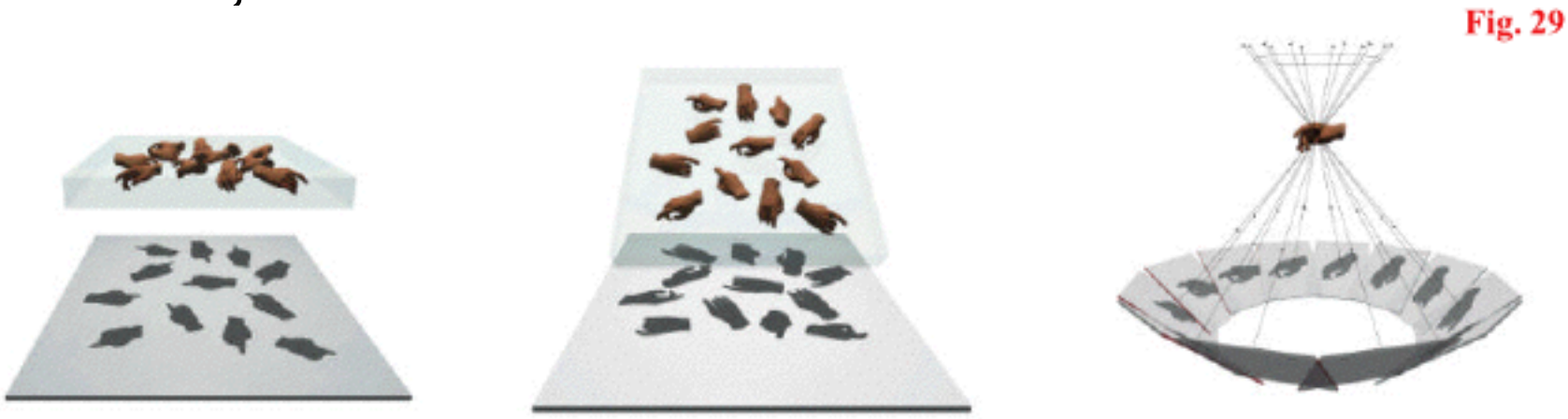
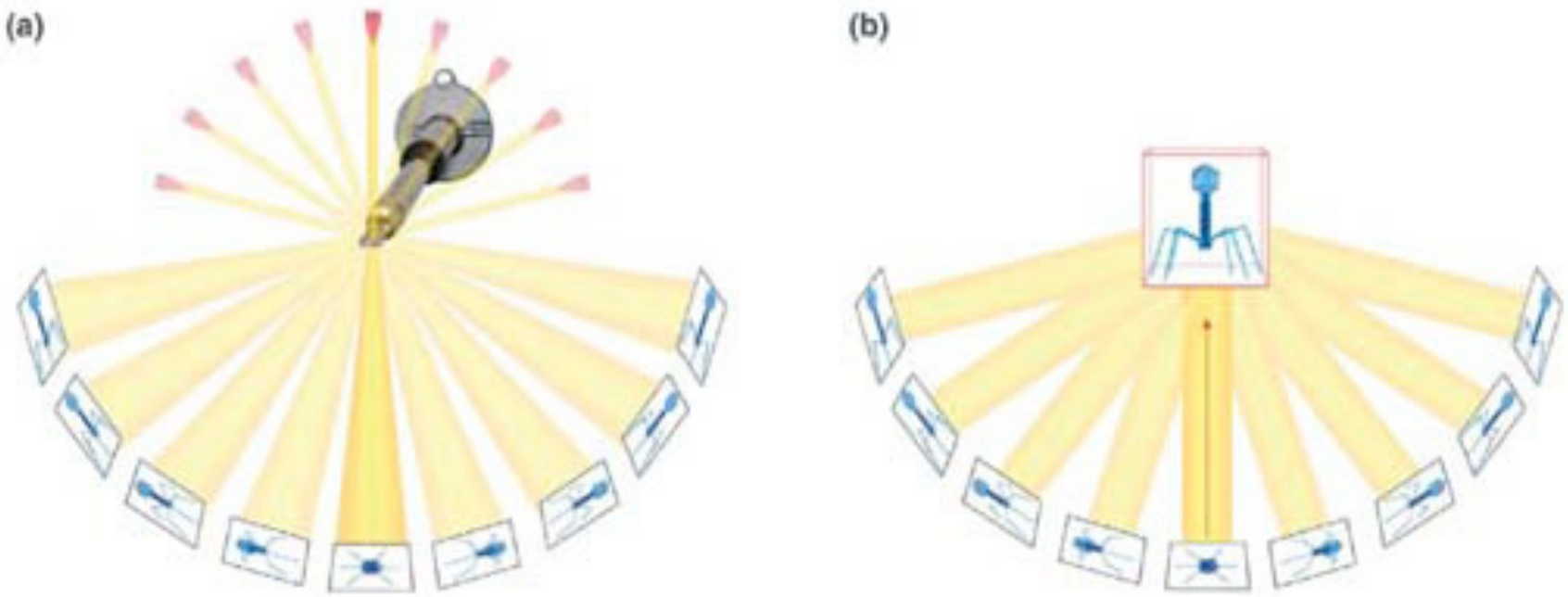
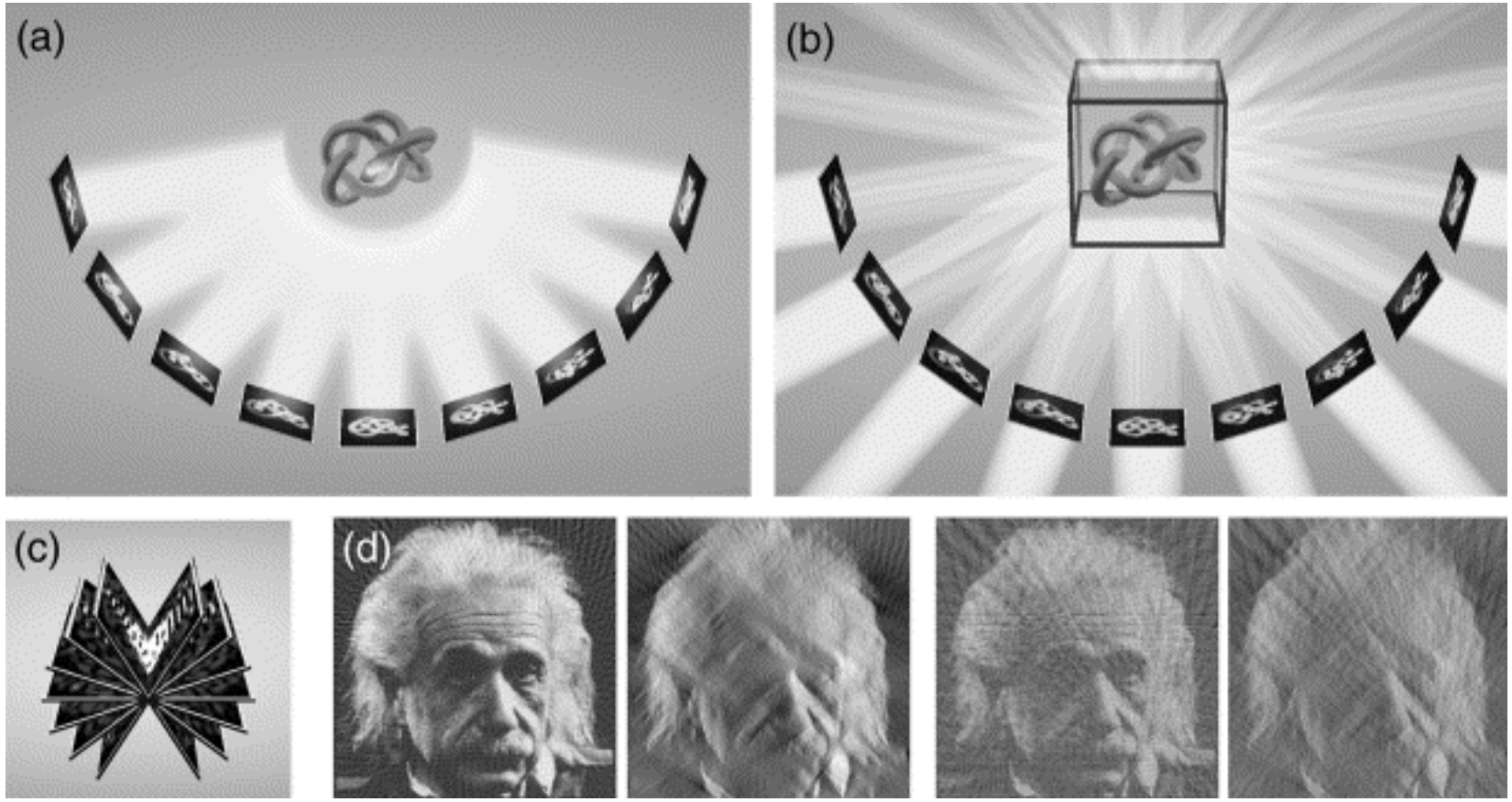


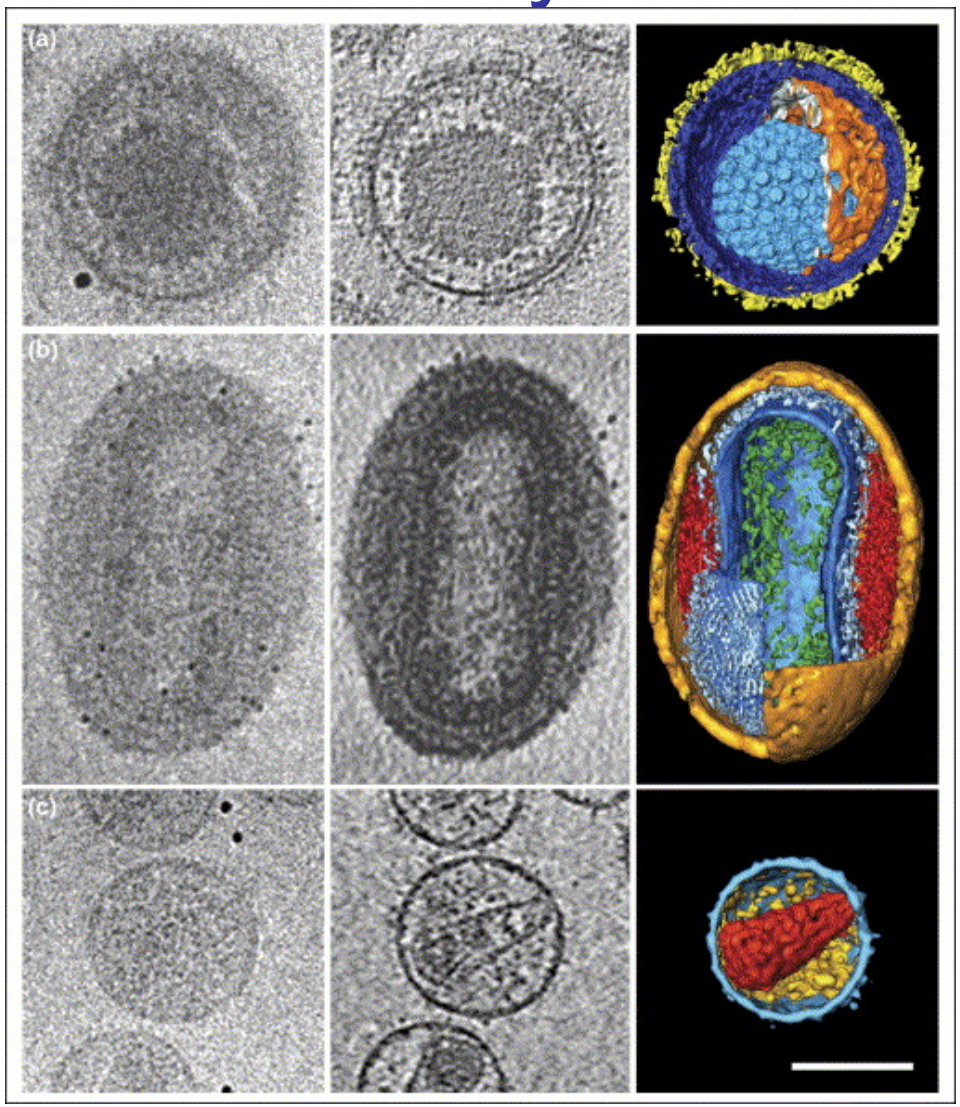
Fig. 29



Principles of Electron Tomography. (a) A biological specimen, in this case a bacteriophage contained in an EM sample holder, can be imaged from several orientations by tilting the holder in the electron microscope. (b) Process of computed backprojection, in which each tilted view is used to reconstruct to three-dimensional information of the original structure. [McIntosh, et al. (2005) Trends Cell Biol. 15:43-51].


(a)
(b)
(c)
(d)

Cryo-ET of viruses



Herpes simplex virus

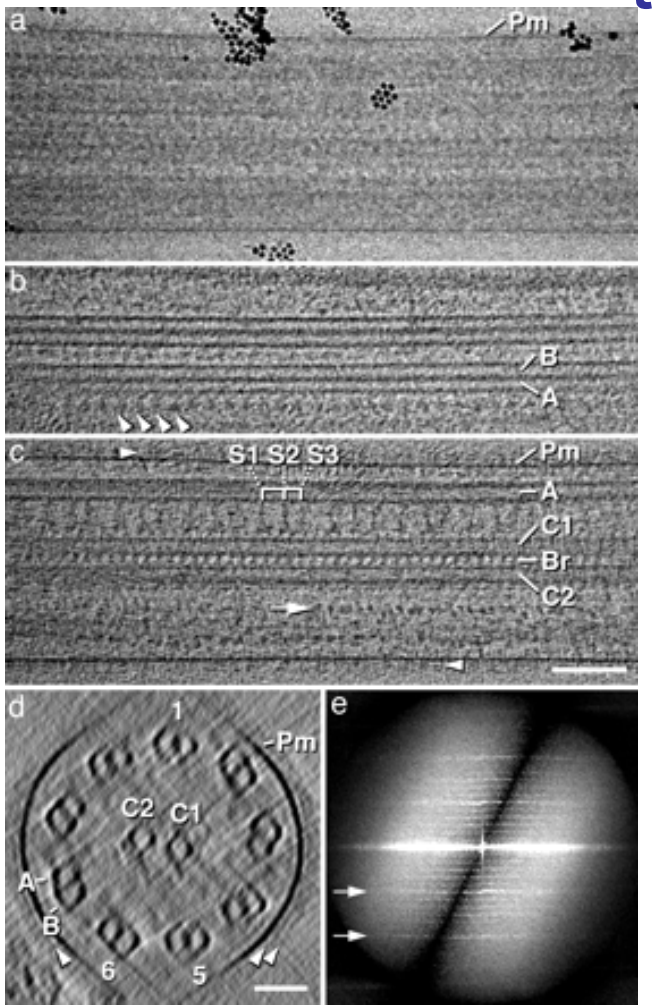
Vaccinia virus

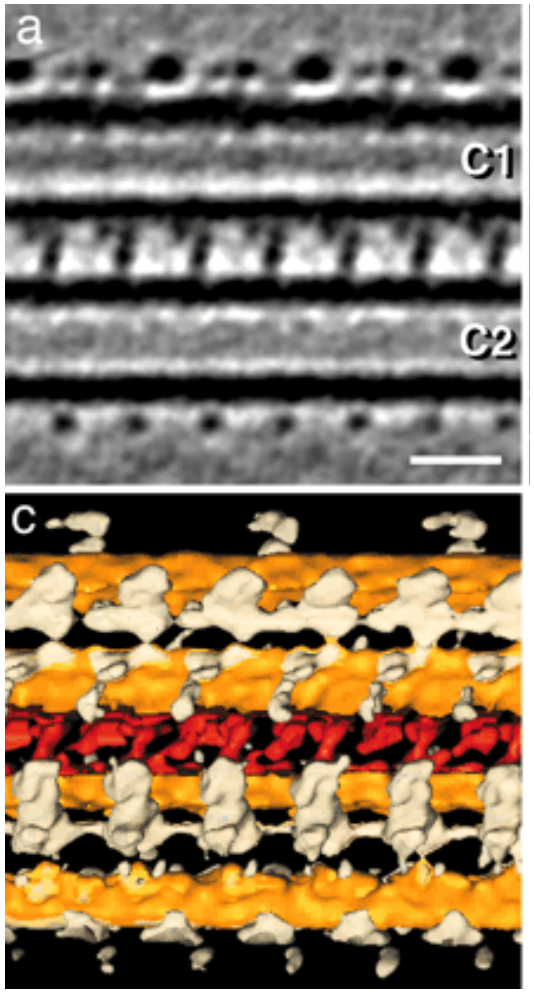
HIV virus

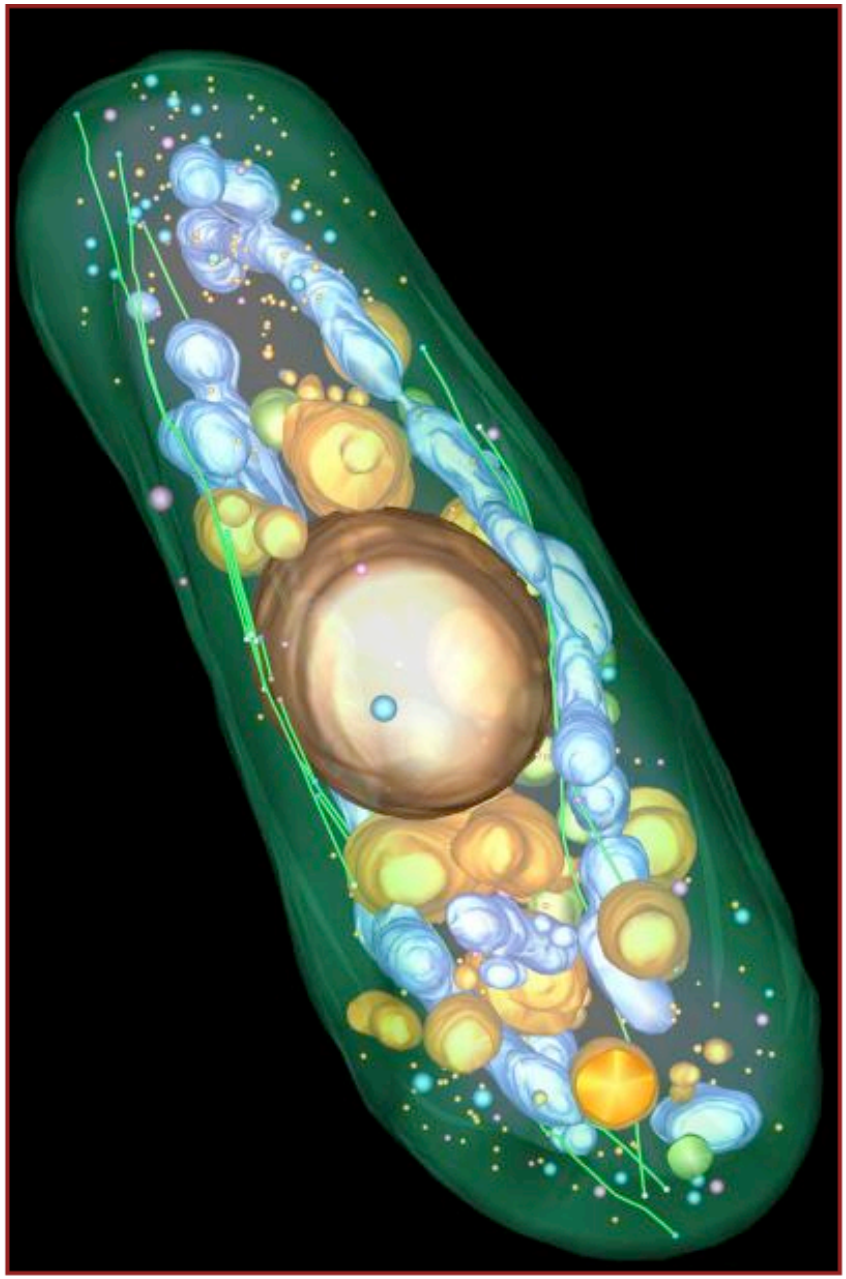
100nm

2-D image reconstructed tomogram slice colored cutaway 3-D image

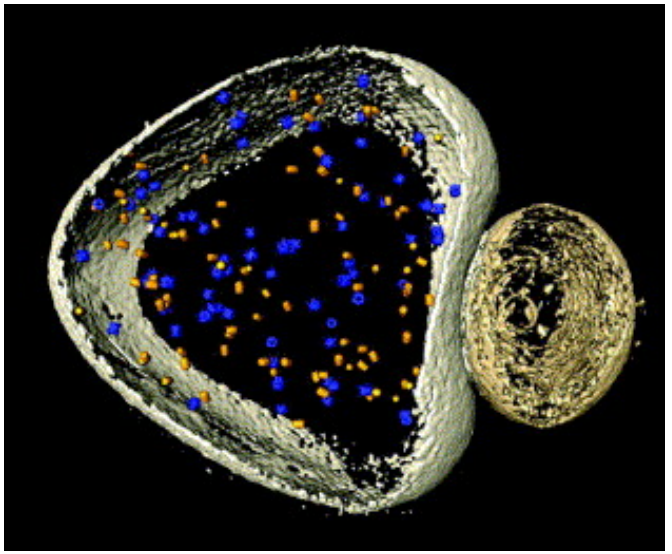
3D structure of eukaryotic flagella in a quiescent state revealed by cryo-electron tomography







The electron tomogram of a complete yeast cell reveals the cellular architecture. It shows plasma membrane, microtubules and light vacuoles (green), nucleus, dark vacuoles and dark vesicles (gold), mitochondria and large dark vesicles (blue) and light vesicles (pink).



Cell Lysate
Isolation of Complexes
FFE, nPAGE
Identification (MS)
Structure Determination
(X-ray Crystallography,
Single-Particle EM)
Cellular Tomogram
Library of Templates
Cellular Atlas
ψ
φ
θ
ψ
φ
θ
CCF(φ,θ,ψ,r)