

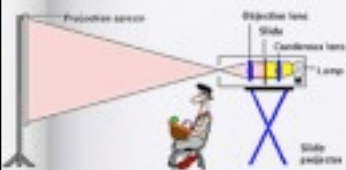
# Introduction of Molecular Electron Microscopy

Yifan Cheng

May 5, 2010



## Tecnai F30 Polara from FEI company



## Outlines

- Basics of molecular electron microscopy
  - electron microscope
  - electron cryo-microscopy
  - 3D reconstruction
- Three different techniques of molecular EM
  - electron crystallography of membrane proteins
  - single particle analysis
  - electron tomography
- Applications of molecular EM
- Current status of molecular EM
- What can you get out of molecular EM?

## Wave-particle duality of electron

De Broglie's hypothesis:  $\lambda = \frac{h}{p}$

$\lambda$  is wavelength,  $h$  is Planck's constant, and  $p$  is momentum.

$$eE_0 = \frac{h^2}{2m\lambda^2} \quad \lambda = \frac{h}{\sqrt{2meE_0}}$$

$E_0$  = acceleration voltage

$\lambda$  = wavelength

$m$  = electron mass

$e$  = electron charge

$h$  = Planck's constant

- Electron scattering - particle character of electron
- Image formation - wave character of electron

## Electron v.s. X-ray

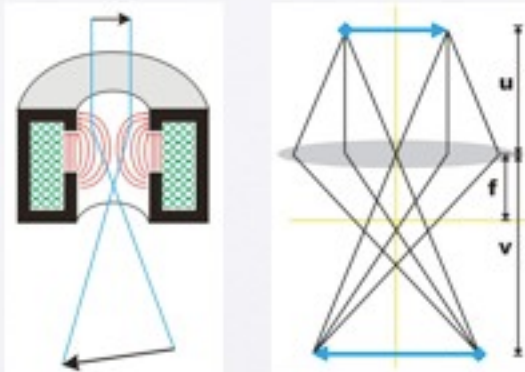
As particles:

- Electrons interact with an atom's shell electrons and nucleus, X-rays interact with only shell electrons.
- Electrons have much larger scattering cross-sections than X-rays. Thus, electron scattered much stronger than x-ray.

As wave:

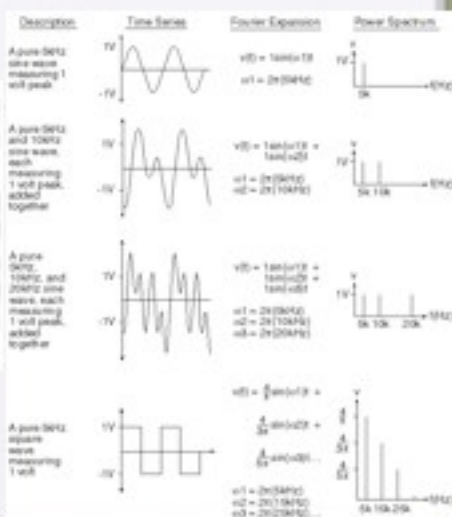
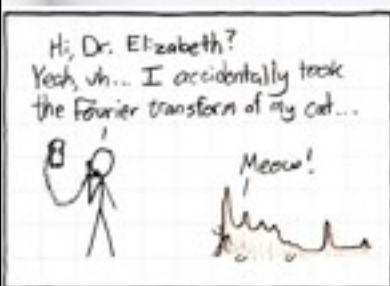
- Electrons can be focused by electromagnetic lens, X-ray can not be focused by lens;

## Electromagnetic lens



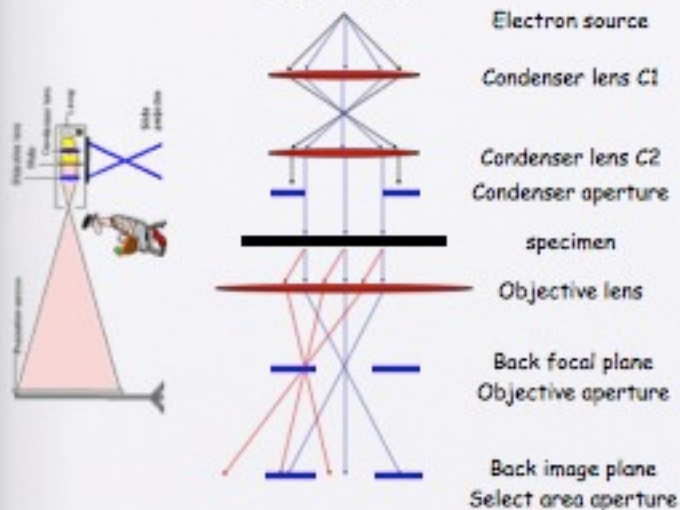
- The focal length of a electromagnetic lens can be easily adjusted by changing the lens current.

## Fourier Transform



From web: <http://mathworld.wolfram.com/FourierTransform.html>

## Optic system in an electron microscope



## It is a microscope!

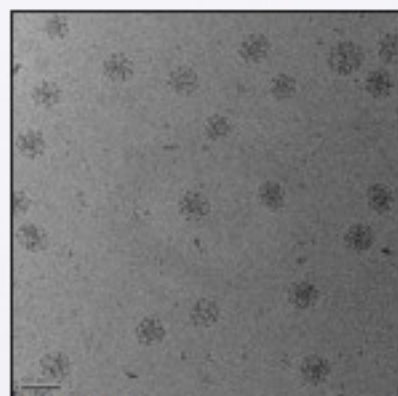
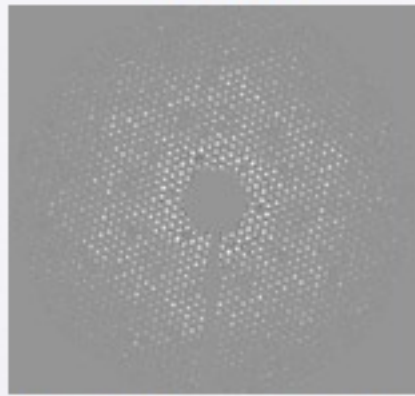
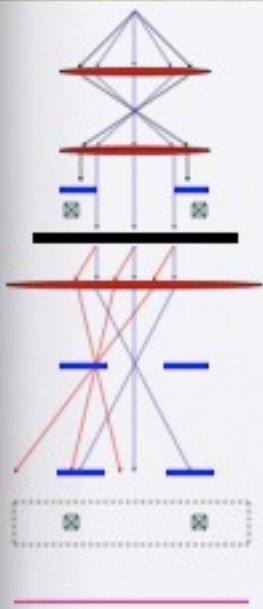


Image mode

Clathrin coat

It is also a  
diffractometer!



Diffraction mode

bacteriorhodopsin

### Observing biological sample by electron microscopy

Strong scattering power means two things:

- 1 high vacuum of microscope column;
- 2 strong scattering with protein sample;

Problems:

- 1 dehydration of biological sample;
- 2 radiation damage by high energy beam;

### Cellular Electron Microscopy

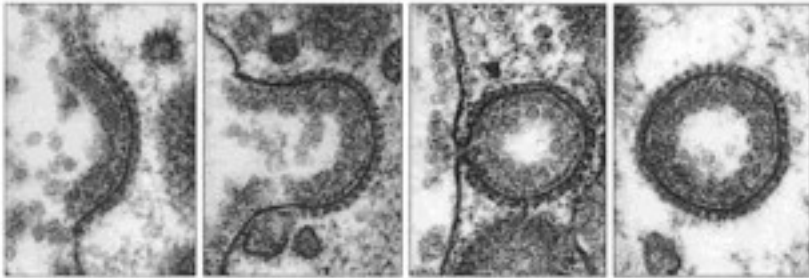
To look at cellular organization by using electron microscope:

Sample are prepared by fixation, dehydration, plastic embedding, thin section and staining;



A typical electron micrograph of a biological sample  
(plastic embedding/thin section/negative staining)

## Formation of Clathrin-Coated Vesicles



- 2500 every minute
- CCV uncoat within seconds

## Cellular Electron Microscopy

To look at cellular organization by using electron microscope:  
Sample are prepared by fixation, dehydration, plastic embedding, thin section and staining;

## Molecular Electron Microscopy

To look at structures of individual molecules:  
look at the purified sample directly by electron microscope  
\* negative staining;  
\* embedding sample in vitreous ice (frozen hydrated sample);

## Electron cryomicroscopy - cryoEM

Taylor K and Glaeser RM  
(1974) "Electron diffraction  
of frozen, hydrated protein  
crystals" *Science* **186**,  
1036-1037



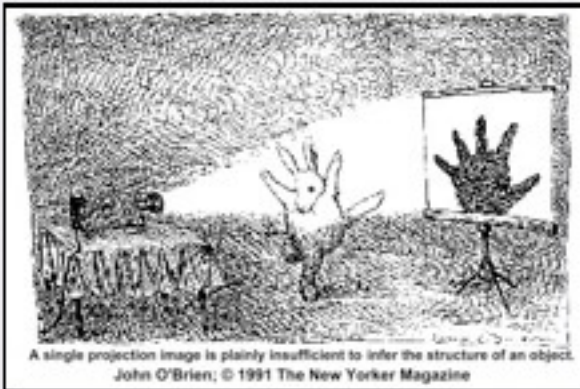
Fig. 1. Electron diffraction pattern of a catalase crystal which was frozen in liquid nitrogen and observed on a specimen stage cooled with liquid nitrogen. The resolution of the photographic reproduction is 4.5 Å, although that of the diffraction pattern on the original plate was 3.4 Å.

Taylor and Glaeser (1974)

## Cryo-electron microscopy

- Against dehydration:
  - glucose/trehalose embedding: using glucose to substitute water, thus maintain hydration in the high vacuum.
  - frozen hydration: using plunge freezing to avoid crystal ice. Mostly for single particle;
- Against radiation damage:
  - Low-temperature: LN2 (~80K) or LHe (~10K); Challenges to the instrumentation;
  - Low-electron dose: Low-dose imaging; Results in extremely noisy images, challenges for the data processing;

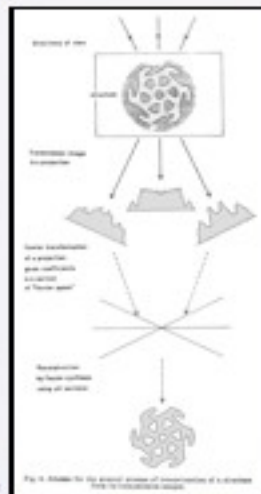
Images obtained from electron microscope are projections of three dimensional object



## Principle of 3D reconstruction of biological object from projection images

**Central Section Theorem :**  
Fourier transform of a 2D projection equals the central section through its 3D Fourier transform perpendicular to the direction of projection.

DeRosier, D. and Klug, A. (1968)  
"Reconstruction of three dimensional structures from electron micrographs" *Nature* 217 130-134



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  - 3D reconstruction
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## 3D electron microscopy

There are three distinct 3DEM techniques:

- \* Electron crystallography: to study membrane proteins that forms 2D crystal;
- \* Single particle averaging: to study protein that have defined (homogeneous) shapes;
- \* Electron tomography: to study large assemblies or organelles with unique shapes that can not be averaged;

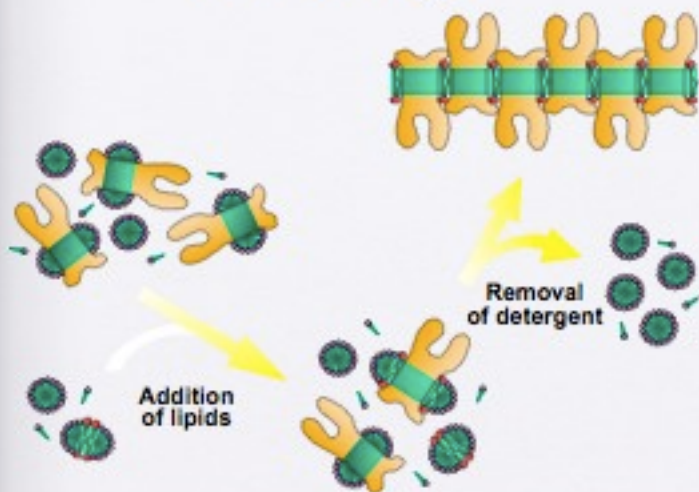
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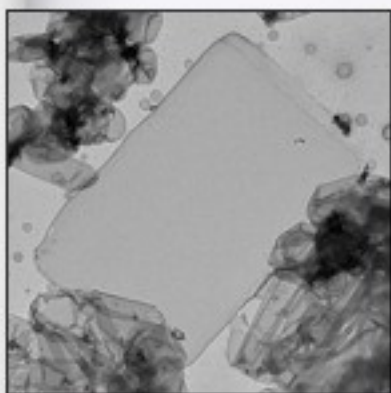
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## Crystallization of a membrane protein into 2-dimensional crystal



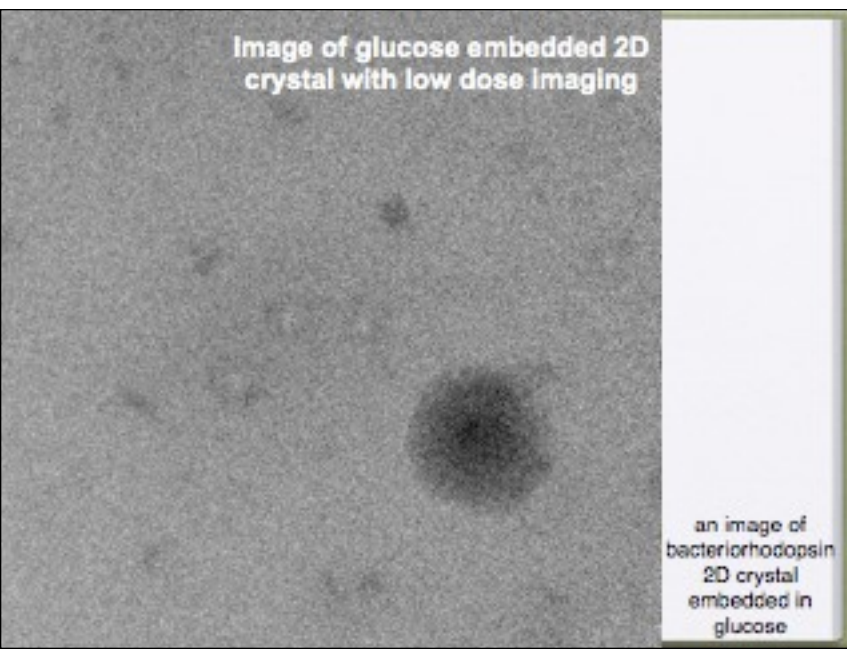
## 2D crystal



A good 2D crystal!

Typically, only one image or one diffraction pattern can be taken from one single 2D crystal.

## Image of glucose embedded 2D crystal with low dose imaging

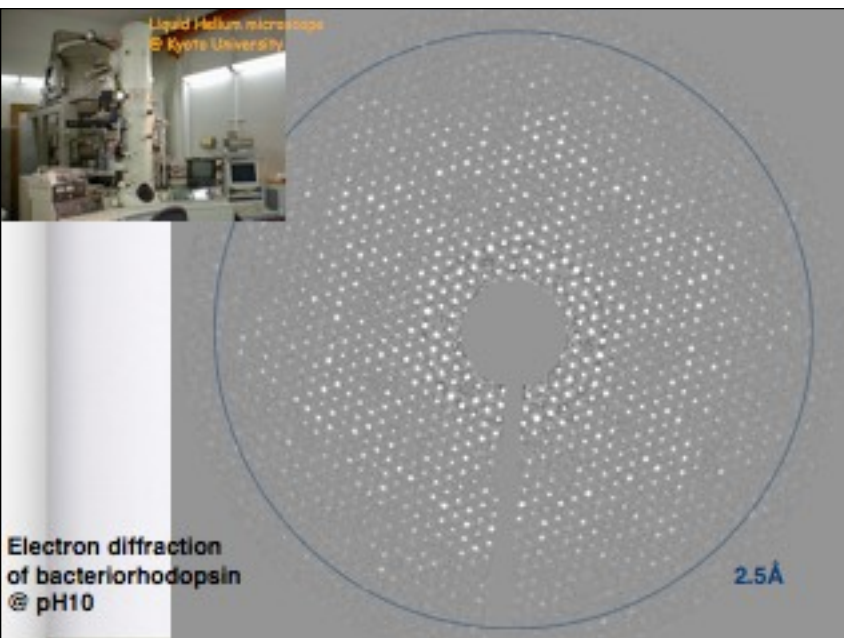


an image of bacteriorhodopsin 2D crystal embedded in glucose

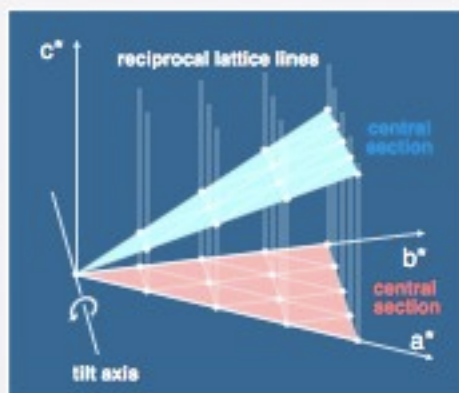


## Fourier transform gives both phase and amplitude

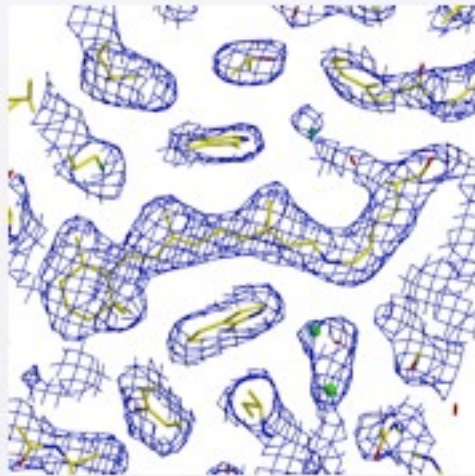
Fourier transform  
from image in  
previous slide.



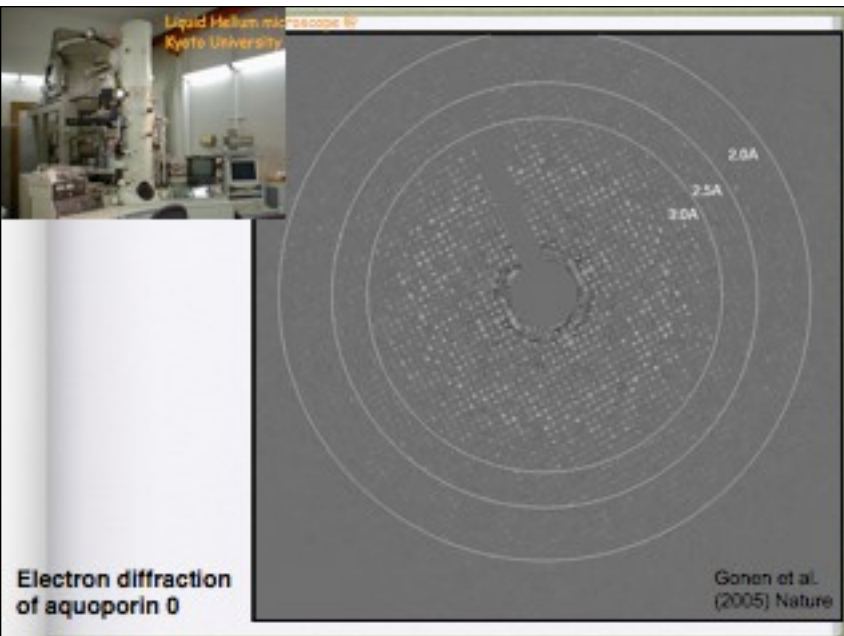
## 3D reconstruction of 2D crystals



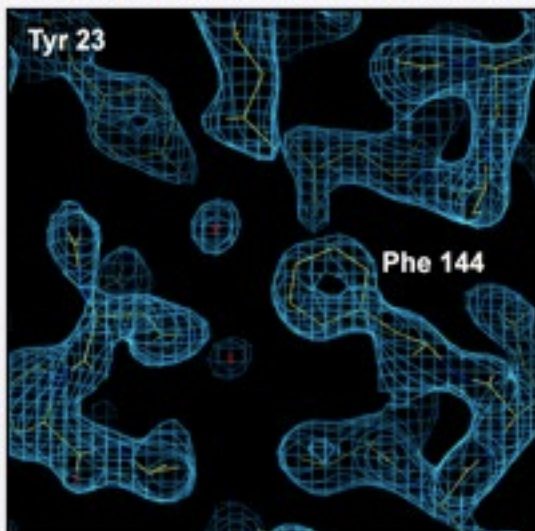
**A part of the density map of bacteriorhodopsin  
at a resolution of 2.5Å**



$(2F_o - F_c)$  map



**Density map of AQP0 at a resolution of 1.9Å**



Gonen et al.  
(2005) Nature

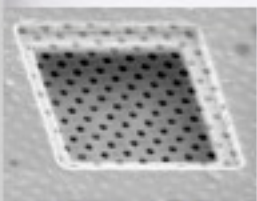
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There are three distinct 3DEM techniques:

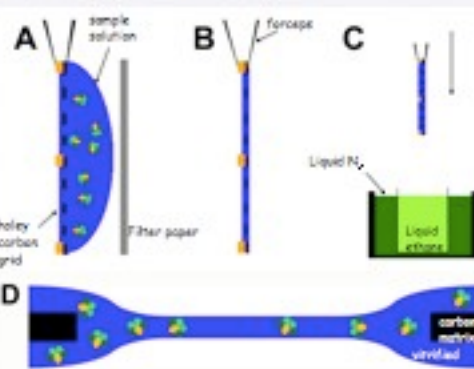
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### Frozen hydrated sample preparation for single particle cryoEM

Adrian M, Dubochet J, Lepault J & McDowell AW (1984) Cryo-electron microscopy of viruses. *Nature* 308, 32-36.

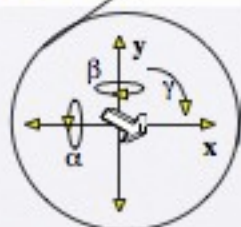


Quantifoil grid



Plunge freezing

### Single particle cryoEM of macromolecular assemblies

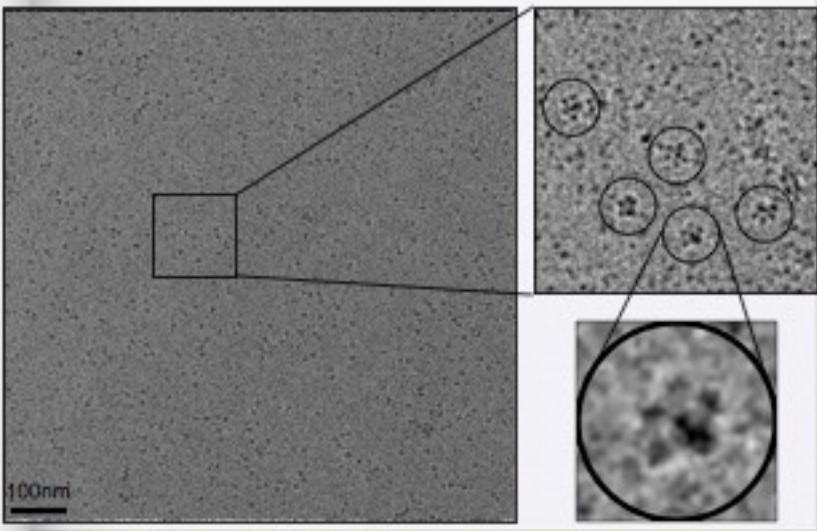


The geometry of each particles are determined by 5 parameters: three Euler angles and two in plane shifts.

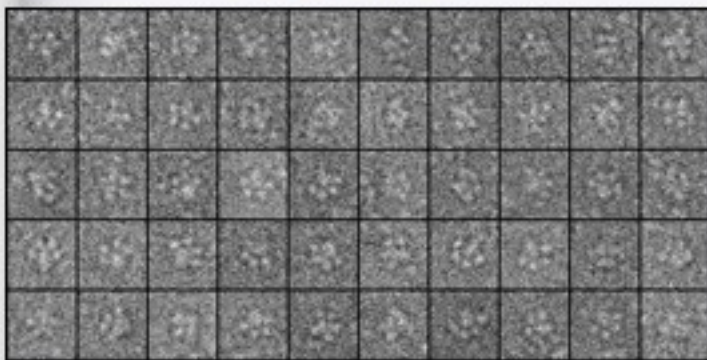
Single particles are randomly oriented in vitreous ice



### Human TfR-Tf complex in vitrified ice



### Individual TfR-Tf Complexes in Vitrified Ice



50 out of 36,266 particles

### Single particle EM

- \* Classifications and multi-reference alignment to produce class averages that have higher signal-to-noise ratio;

- \* 3D reconstruction using 2D class averages;



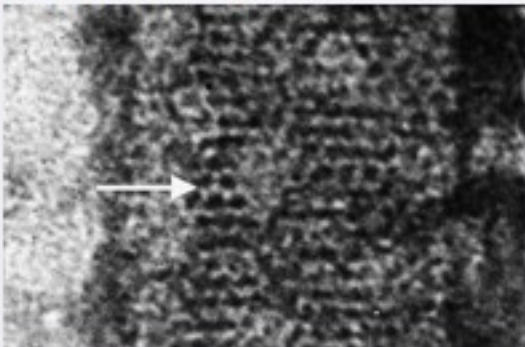


Image averaging  
is powerful and  
interesting!

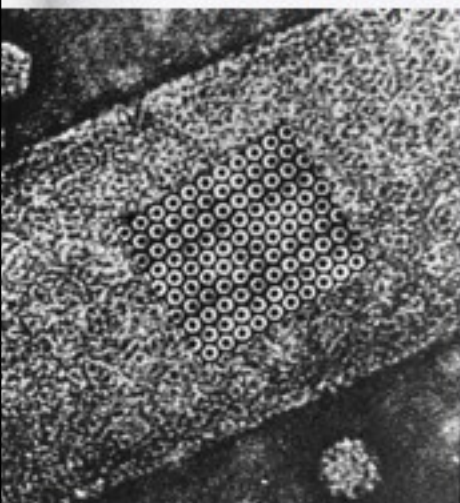
From Joachim Frank

### Image averaging

Cryo-EM images are very noisy; have extremely low signal-to-noise ratio. Averaging of a large number of images are necessary to improve the SNR.



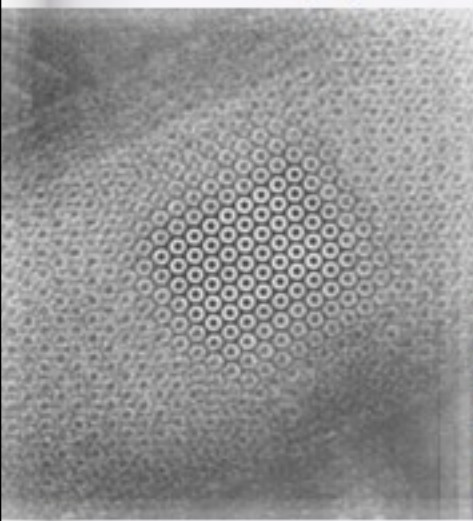
### Averaging in a darkroom



Photographic image  
superposition  
(averaging) by Roy  
Markham, who shifted  
image and added to the  
original in darkroom.

The trick is to know how  
much and along which  
direction to shift the  
image for superposition.

## Averaging in a computer!



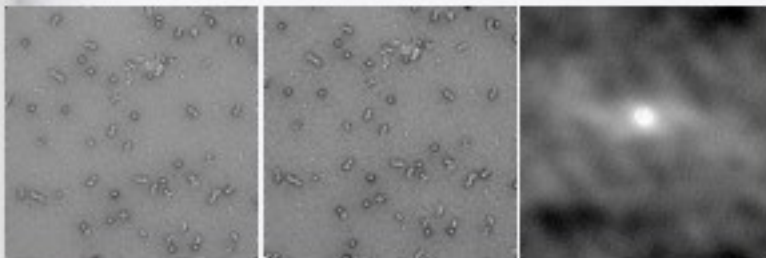
David DeRosier used Markham's lattice to determine how much to shift by, and performed averaging by using Adobe Photoshop.

## What about single molecules

A single particle image data set is a collection of images, each contains projection images of one molecules. The orientations and position of particles in all images are different. Before averaging, one needs to:

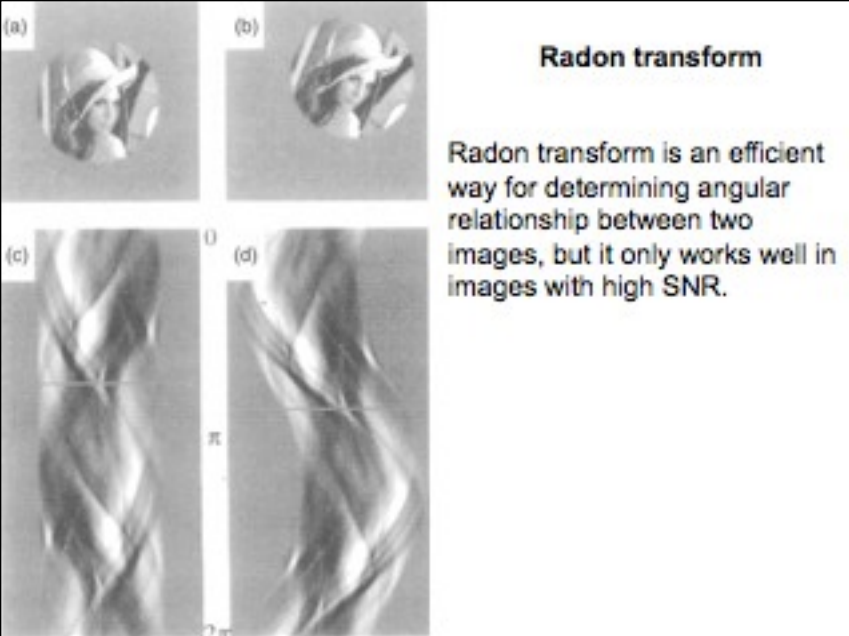
- judge how similar is the two particles: *cross-correlation coefficient*;
- shifts/rotates one particle to match another by maximizing ccc: *alignment*;
- separate different particles: *classification*;

## How cross-correlation looks like

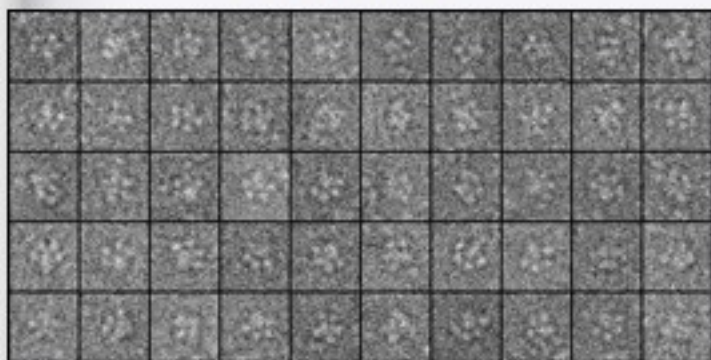


CCF

The image size is 1024X1024. The peak in the CCF is at (445,500). How much is the shift?

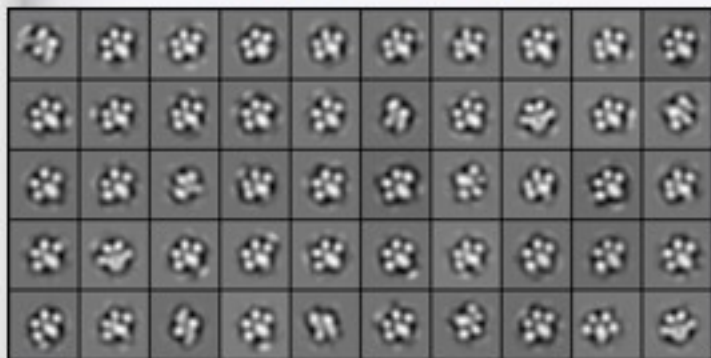


**Individual TfR-Tf Complexes  
in Vitrified Ice**



50 out of 36,266 particles

**Class Averages of  
Vitrified TfR-Tf Complexes**



50 out of 200 classes



### Demonstration of reference induced bias

Note: The averaged image after reference based alignment is strongly biased towards the reference.



100 images

1000 images

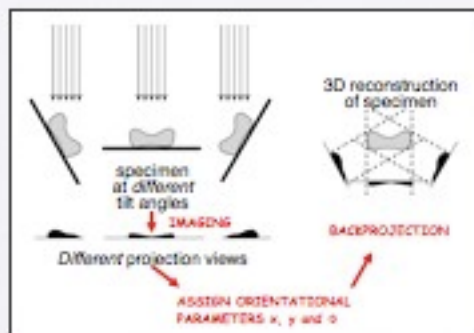
reference

From Niko Gorioeff

### 3D reconstruction

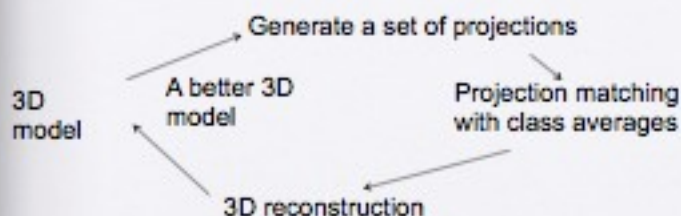
Assume we have already a number of class averages, they represent the projections of a 3D object in different orientations. And we know (can determine) these relative orientations of each class averages. We can reconstruct the 3D object - 3D reconstruction.

Back projection:



### How to determine the relative orientations?

Model based projection matching: by matching of class averages with the calculated projections of the 3D object in known directions - a refinement procedure.

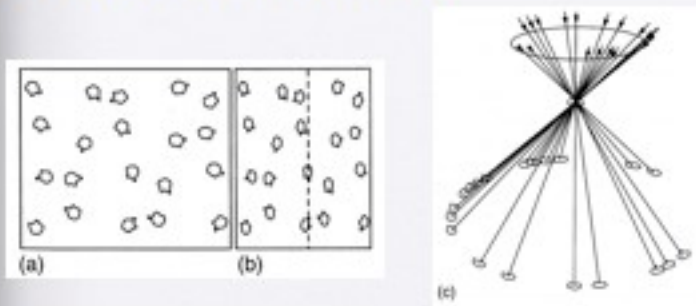


Where do you get the first model?



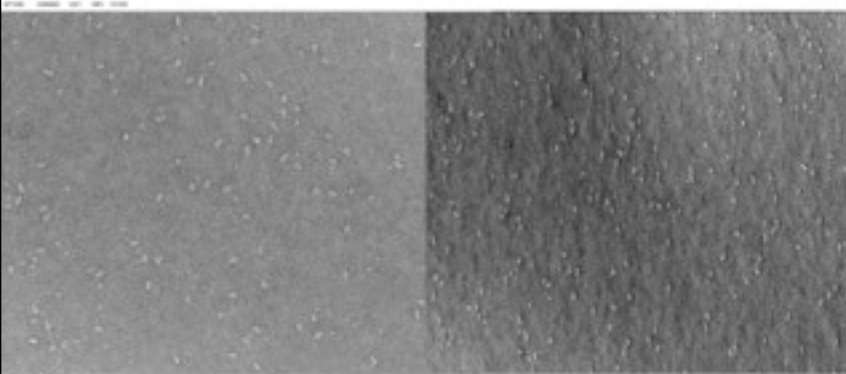
### Random conical tilt

A pair of images are taken from the same specimen area for the random conical tilt 3D reconstruction.



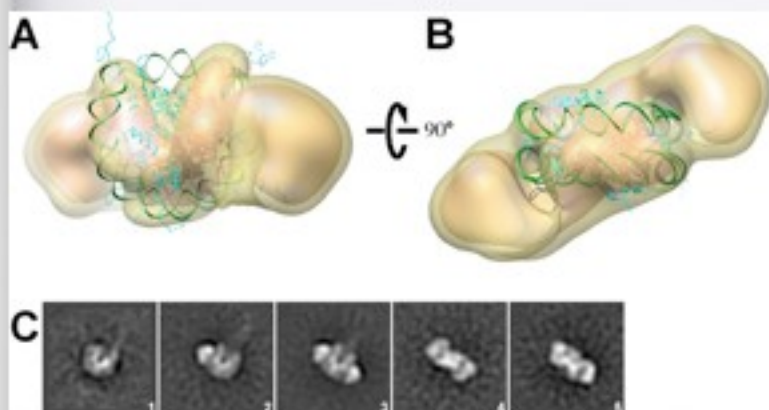
From Joachim Frank

### Random conical tilt



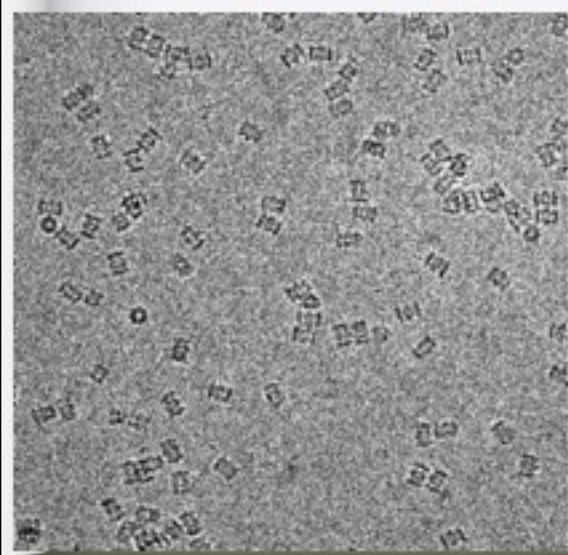
A tilt-pair image of SNF2h-nucleosome complex

### Random conical tilt



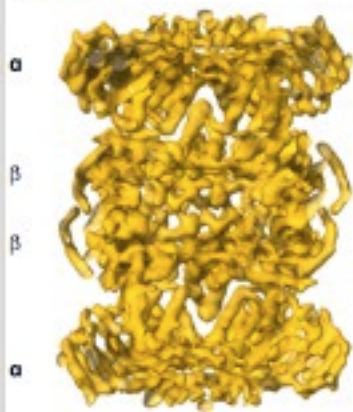
A tilt-pair image of SNF2h-nucleosome complex

*T. acidophilum* 20S proteasome

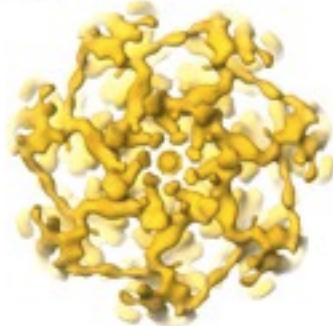


Structure of archaeal 20S CP

Side view



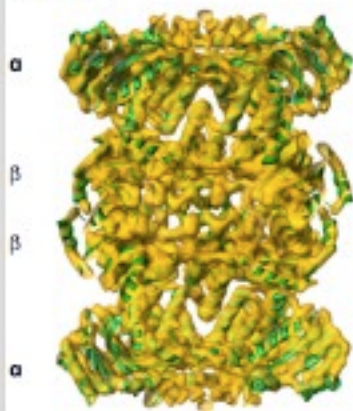
Top view



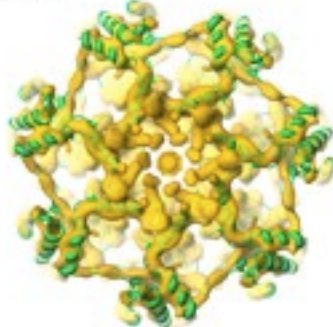
\* Density map filtered to 6.8 Å;

Structure of archaeal 20S CP

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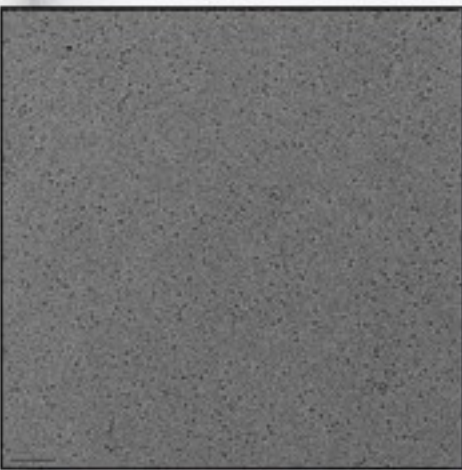


Top view

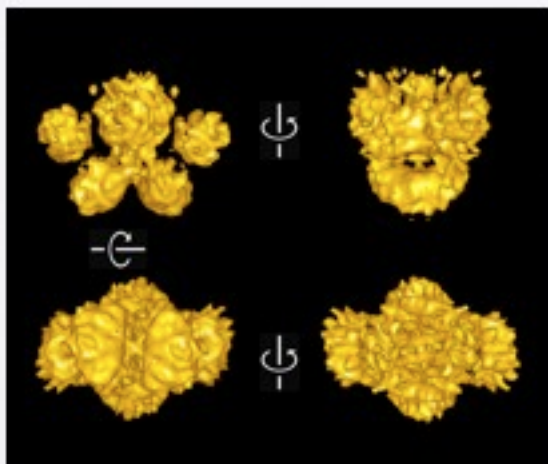


\* Density map filtered to 6.8 Å;

## Human Transferrin receptor - transferrin complex

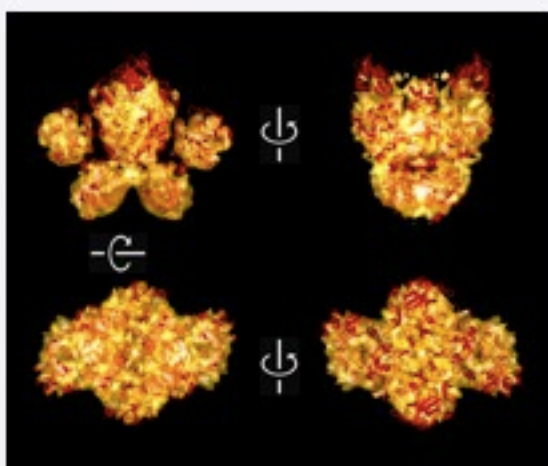


## Structure of TfR-Tf complex at a 7.5Å resolution



Cheng et al.  
(2004) Cell

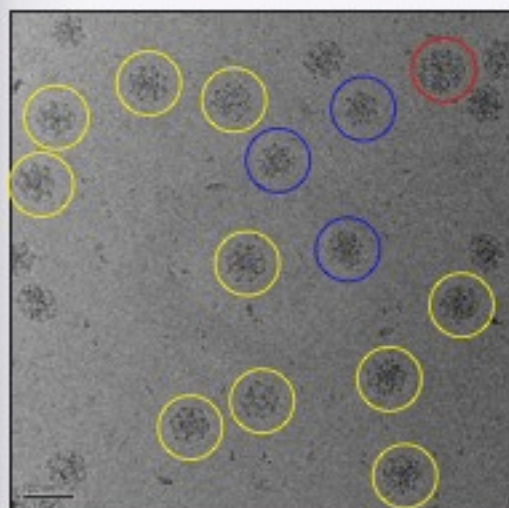
## Docking the atomic structures



Cheng et al.  
(2004) Cell



# In vitro assembled clathrin coats in vitrified ice



Tetrahedral coats

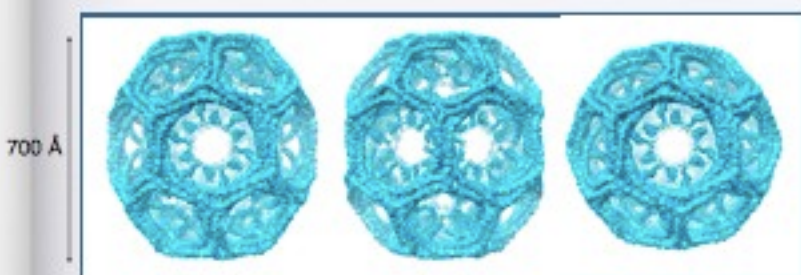


D6 barrel coats



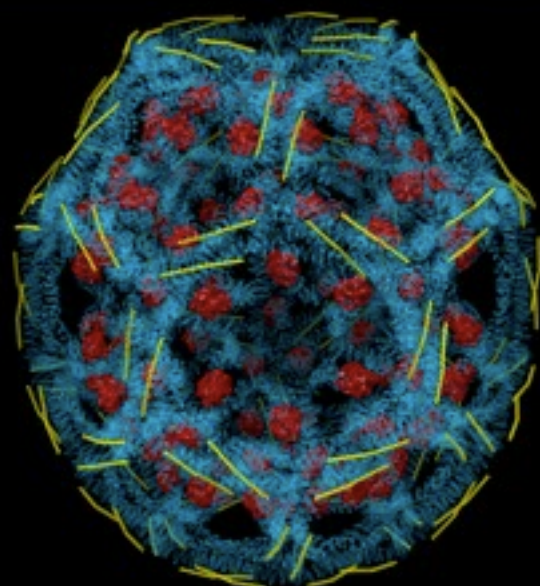
Icosahedral coats

## Structure of a clathrin coat at 7.9 Å resolution



Fotin et al.  
(2004) Nature

## The complete clathrin coat with light chain and auxilin



Fotin et al.  
(2004) Nature

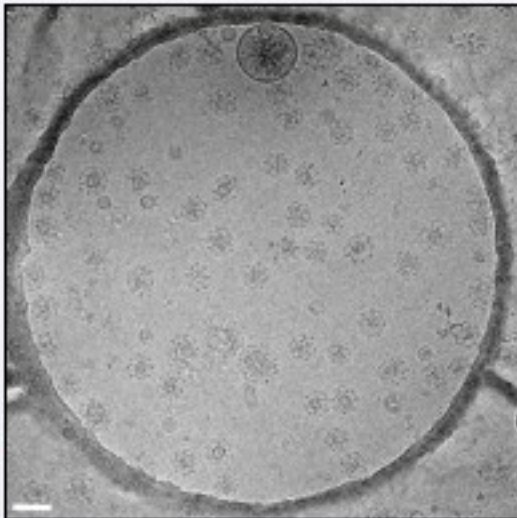


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#### Clathrin coated vesicles in vitrified ice



Clathrin coated vesicle purified from calf brain

100nm

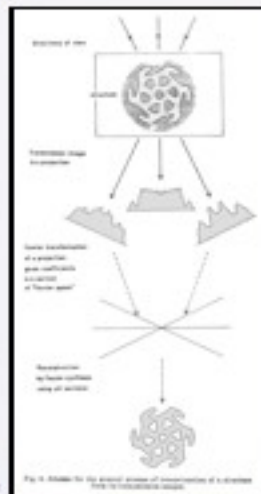
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Fourier transform of a 2D projection equals the central section through its 3D Fourier transform perpendicular to the direction of projection.

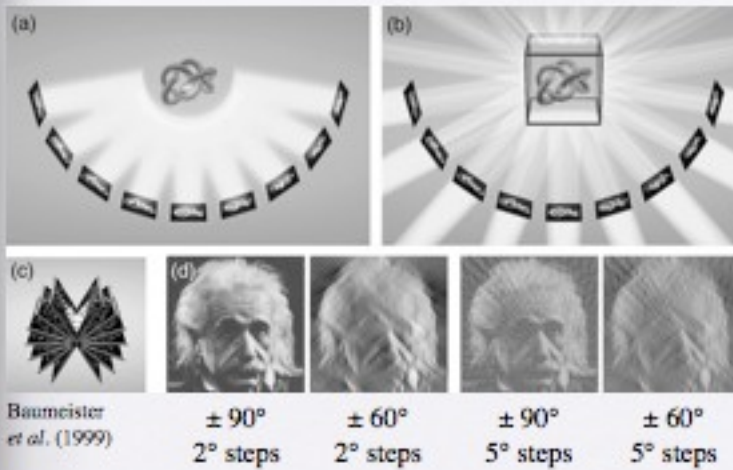
DeRosier, D. and Klug, A. (1968)  
"Reconstruction of three dimensional structures from electron micrographs" *Nature* **217** 130-134

Hart, R.G. (1968) "Electron microscopy of unstained biological material: the polytopic montage" *Science* **159** 1464-1467

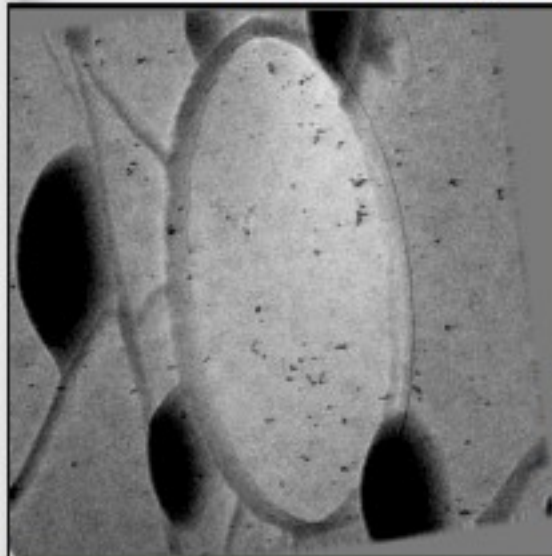


DeRosier and Klug (1968)

## Electron Tomography



### A tilt series after alignment

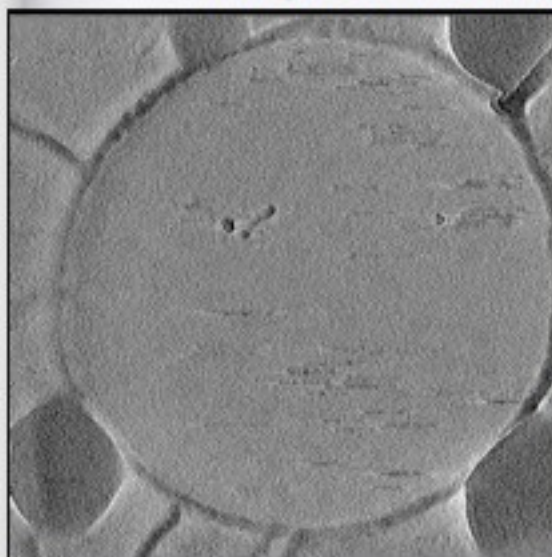


#### Aligned tilt series of clathrin-coated vesicles

- \* 10nm colloid gold;
- \* tilt range:  $-55^\circ$  --  $+67^\circ$
- \* defocus:  $-7\mu\text{m}$ ;
- \* magnification: 29kX;
- \* 7.75Å/pixel in CCD;
- \* binning 2x2;

Cheng *et al.*  
(2007) JMB

### Tomogram of coated vesicles

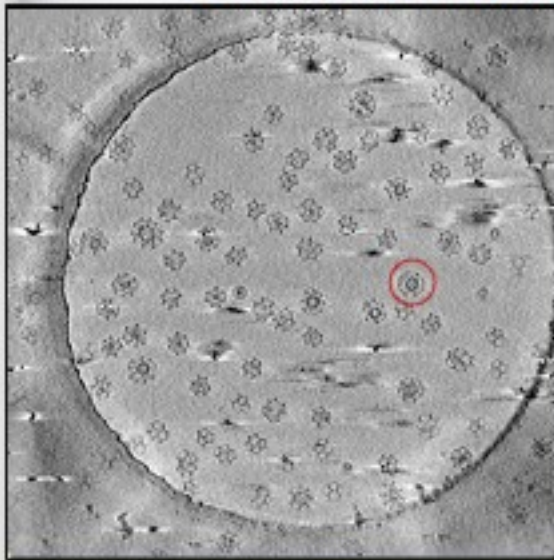


#### Slice view of tomogram

- \* IMOD;
- \* pixel size: 15.5Å;
- \* thickness: 1,550Å;

Cheng *et al.*  
(2007) JMB

### Central section of tomogram



Central slice of  
15.5 nm thick  
from tomogram

Cheng et al.  
(2007) JMB

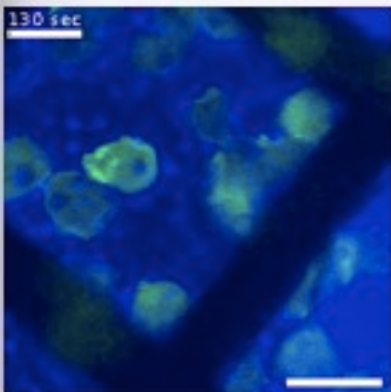
### Reconstruction of coated vesicles



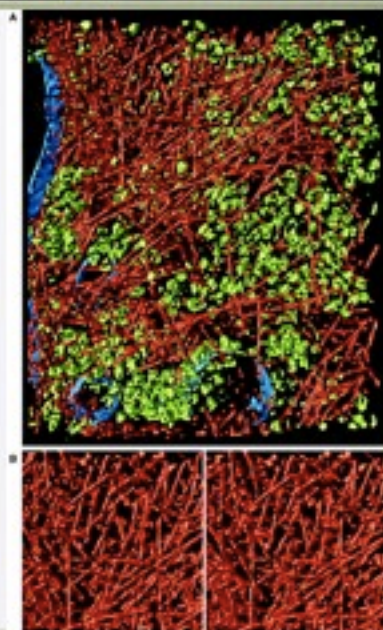
3D density map of  
clathrin-coated  
vesicle filter by  
3x3x3 median filter

Cheng et al.  
(2007) JMB

### Electron cryo-Tomography of whole cell



Medalia et al. (2002) Science





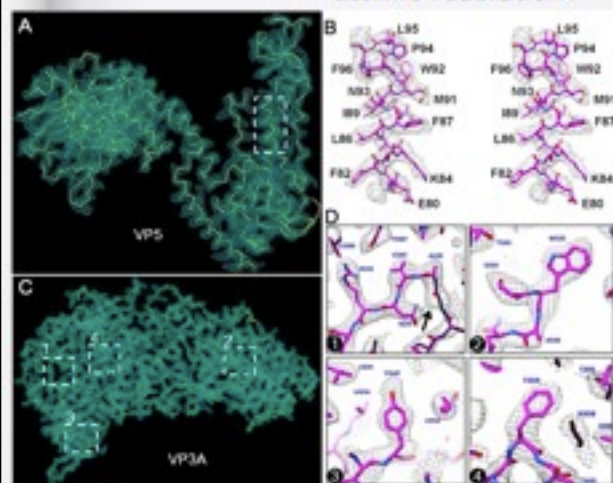
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## Molecular electron microscopy

- \* Electron crystallography: atomic resolution;
- \* Single particle EM: sub-nanometer level for most large and stable complex, reveal secondary structural features; For icosahedral virus, ATOMIC resolution, ~3.3Å;
- \* Electron tomography: can look at individual assembly or cell, but at lower resolution (~ 2nm);

## Single particle cryoEM reached near atomic resolution





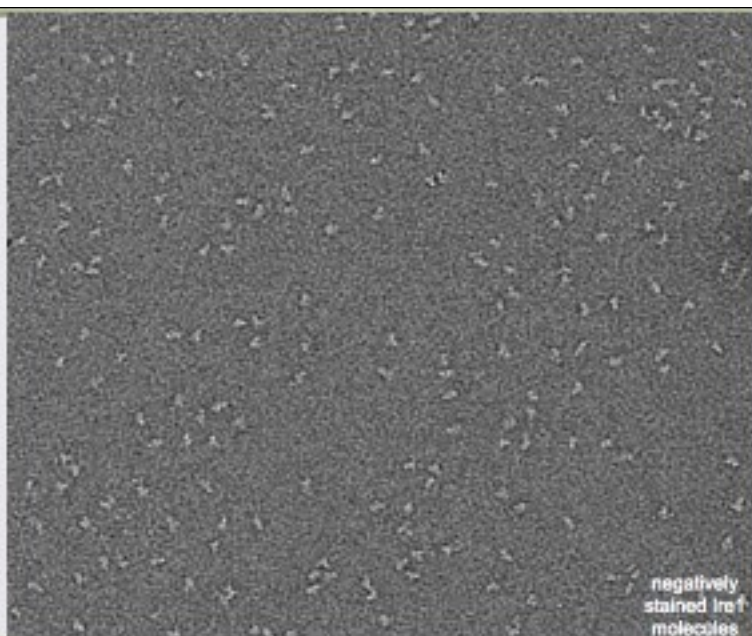
## What do we want to achieve by molecular electron microscopy?

To achieve near atomic resolution of macromolecular complexes/machineries by the molecular single particle cryoEM and routinely;

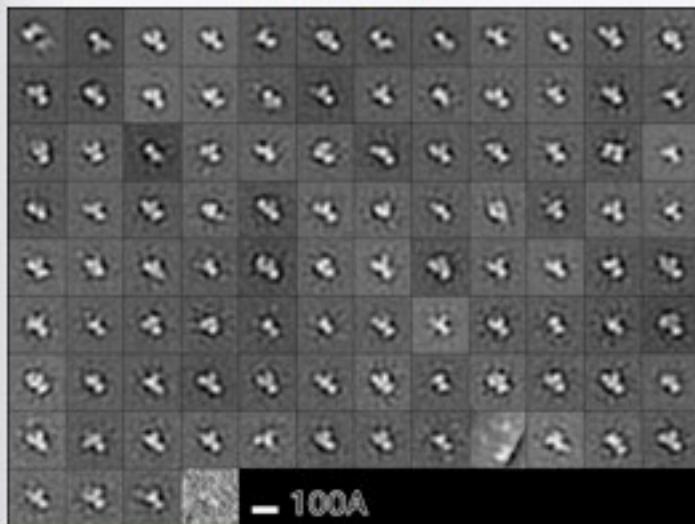
- \* To determine structures of macromolecules that are too large to be crystallized for x-ray crystallography and NMR spectroscopy.
- \* To understand complex biological processes in molecular/atomic details.

## Outlines

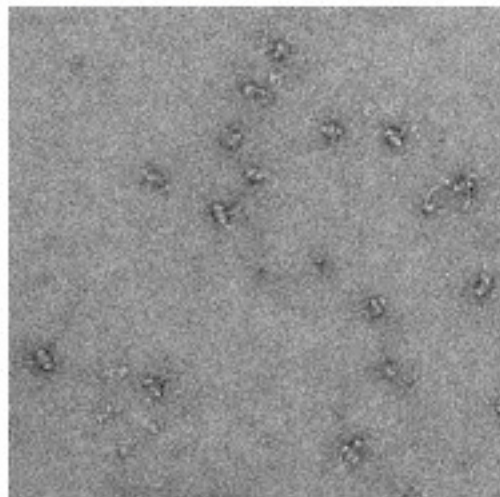
- Basics of molecular electron microscopy
  - electron microscope
  - electron cryo-microscopy
  - 3D reconstruction
- Three different techniques of molecular EM
  - electron crystallography of membrane proteins
  - single particle analysis
  - electron tomography
- Applications of molecular EM
- Current status of molecular EM
- **What can YOU get out of molecular EM?**



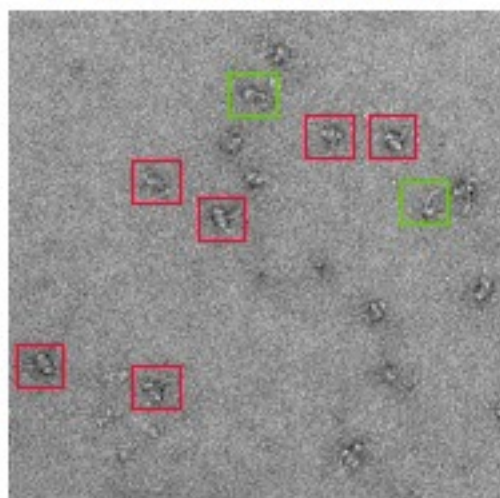
class averages after multi-reference alignment and classification



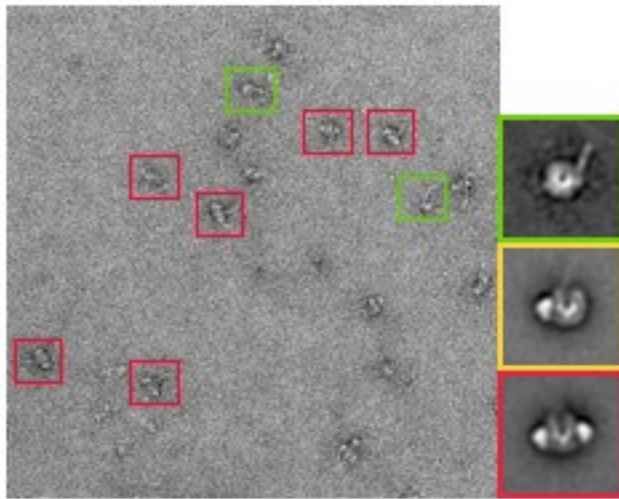
SNF2h-nucleosome complex



SNF2h-nucleosome complex

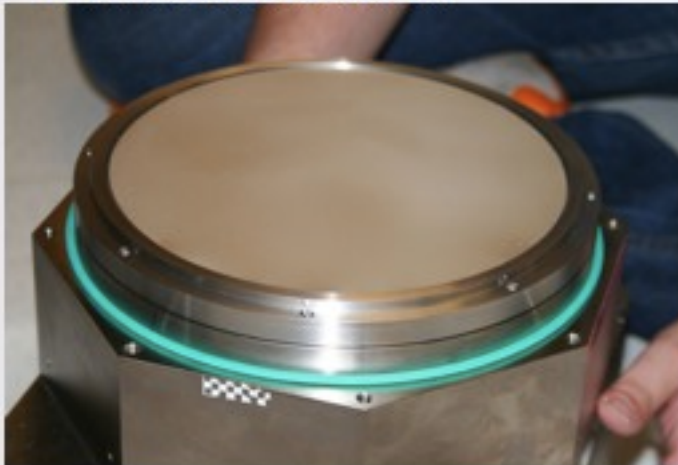


### SNF2h-nucleosome complex



### Recent cryoEM technology development at UCSF

- Instrumentation: 8K x 8K camera



### Recent cryoEM technology development at UCSF

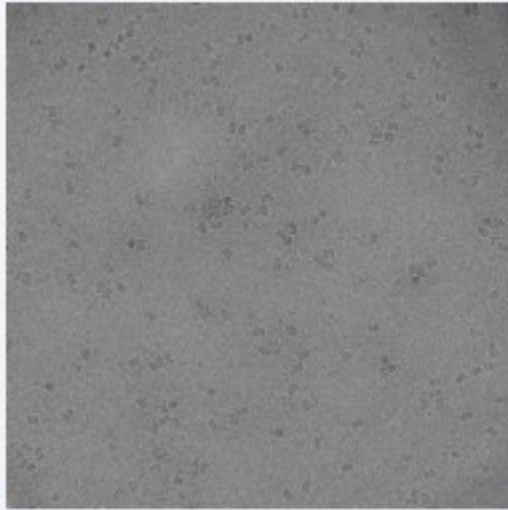
- Instrumentation: 8K x 8K camera on a TF20





## Recent cryoEM technology development at UCSF

- Instrumentation:  
A first image from  
the 8K x 8K camera



yeast 20S proteasome

## Use molecular EM in your research

- exam the quality of your purification
- verify your hypothesis
- obtain addition information about your proteins: such as oligomeric status of your protein, formation of complex, etc
- Or if you are really really serious, get high resolution structure by cryoEM!

### Facility at UCSF:

Keck Advanced Microscopy Laboratory

Yifan Cheng - S312B ([yfcheng@ucsf.edu](mailto:yfcheng@ucsf.edu))

David Agard - S412D ([agard@msg.ucsf.edu](mailto:agard@msg.ucsf.edu))