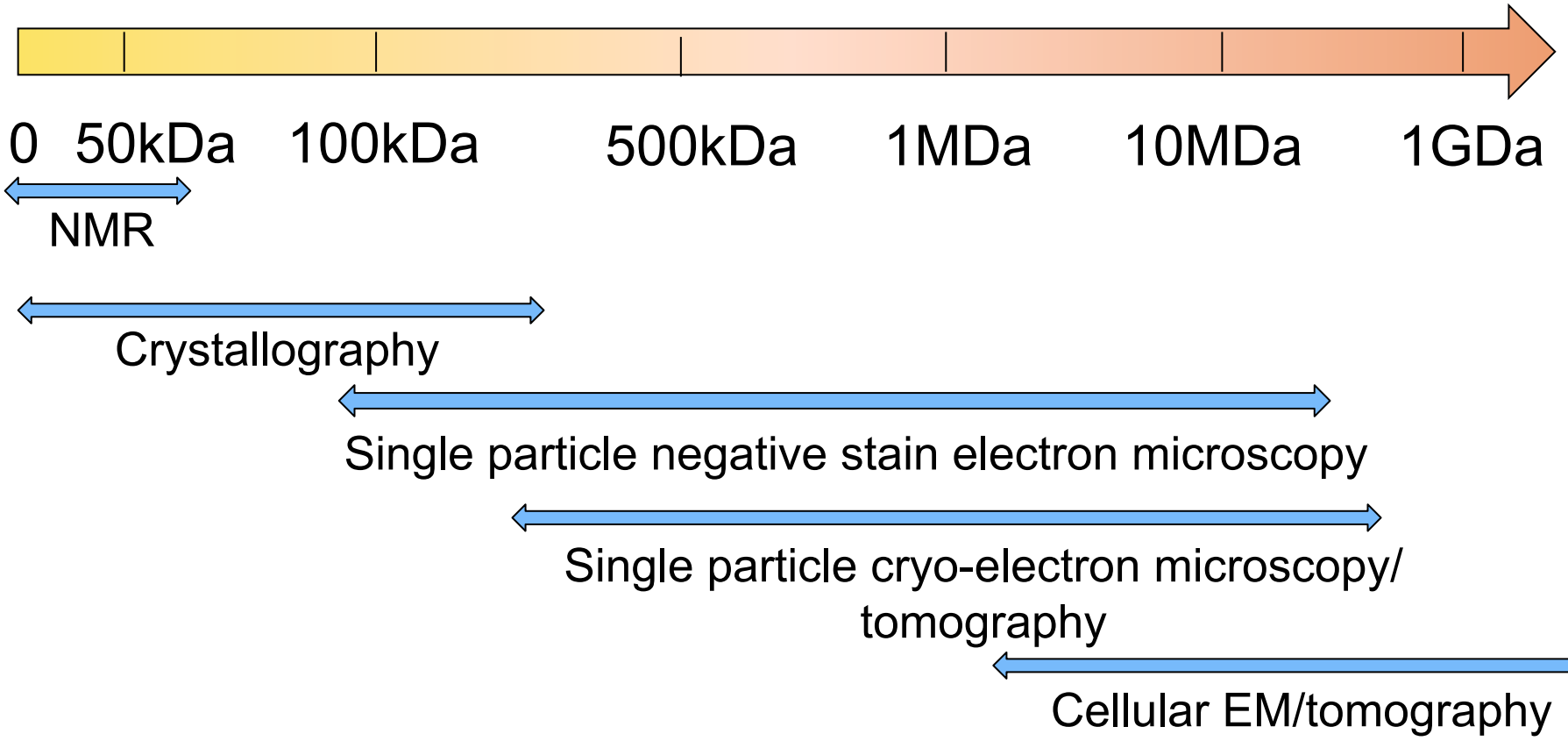
Electron microscopy: new opportunities

Helene Malet

Electron microscopy group, IBS

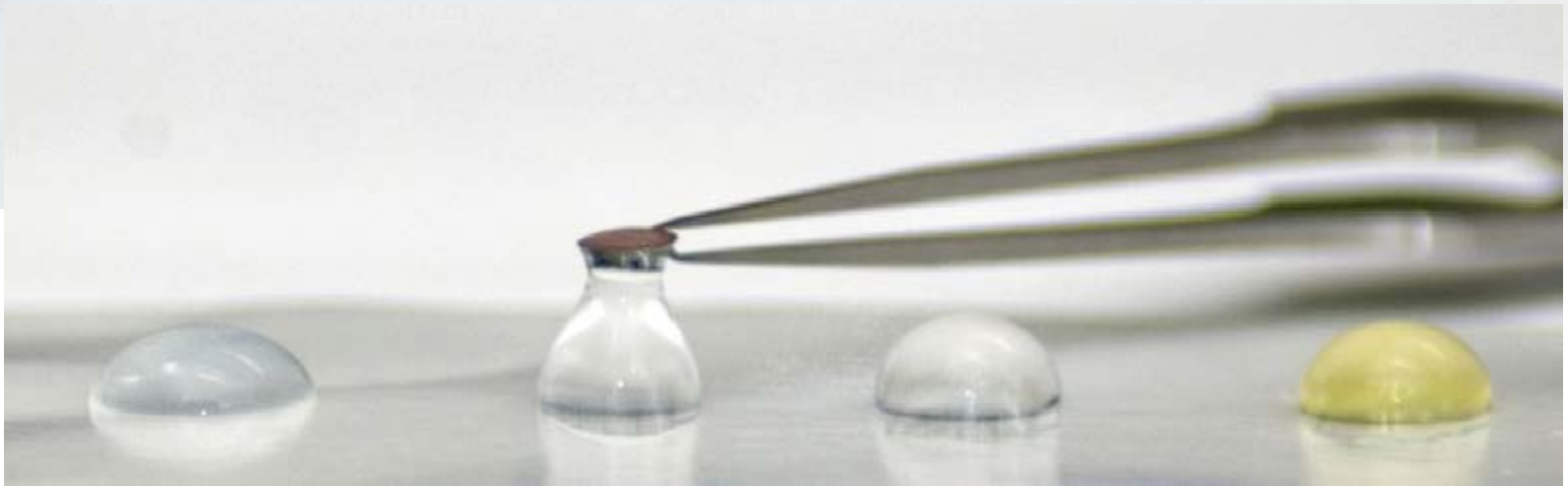
Electron microscopy in structural biology: Which objects can be analysed

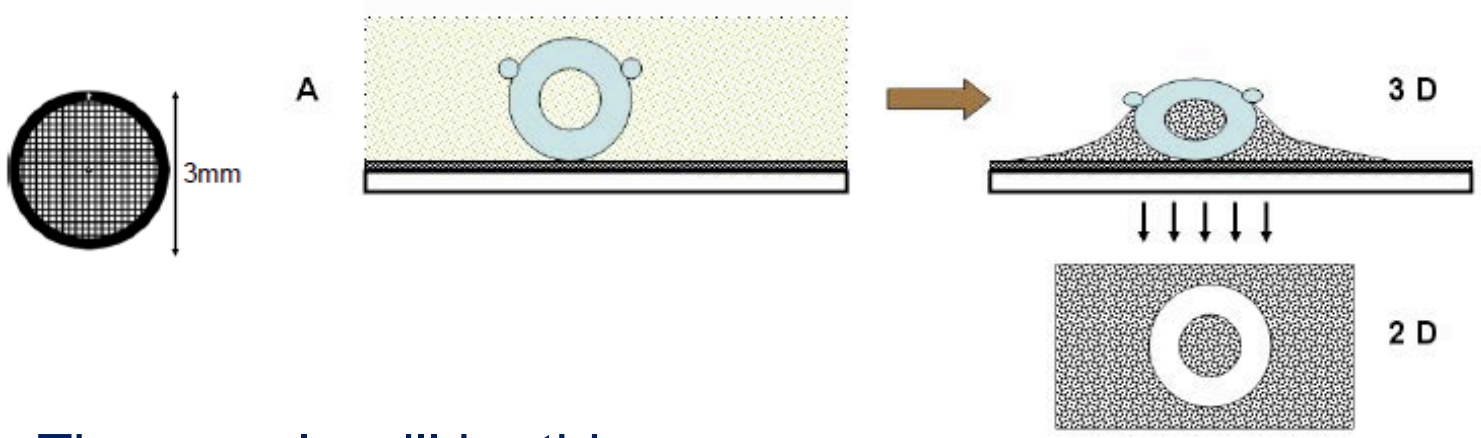


Method combinaison to study a biological process

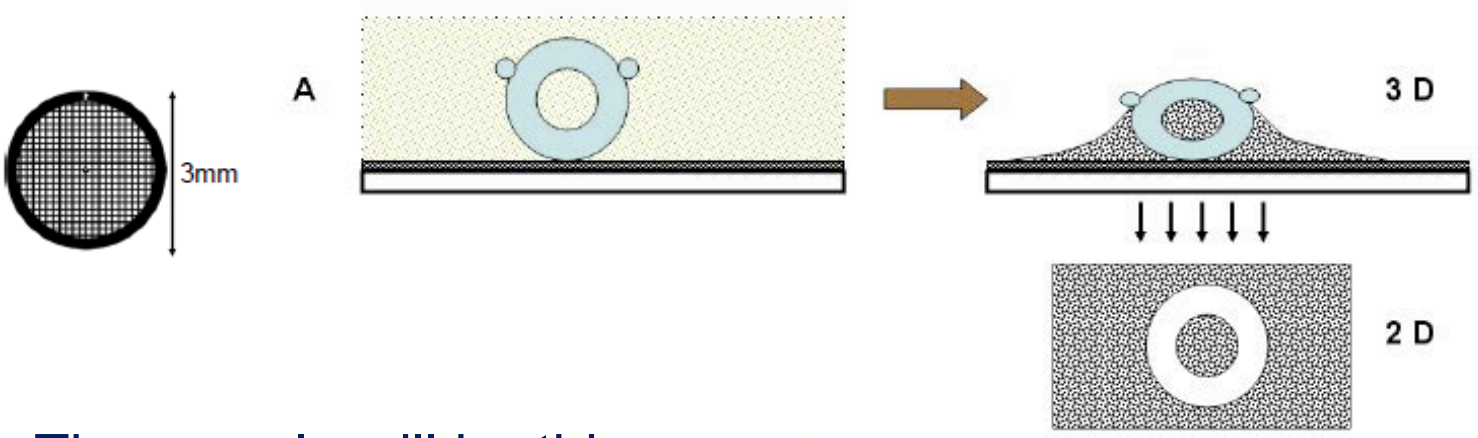
What is negative stain electron microscopy ?

Sample preparation





- The sample will be thin so electrons will be able to go through.
- We will see the stain as it is made of heavy atom which will interact with electrons.
- The stain will be more present where the protein is not: “negative stain”



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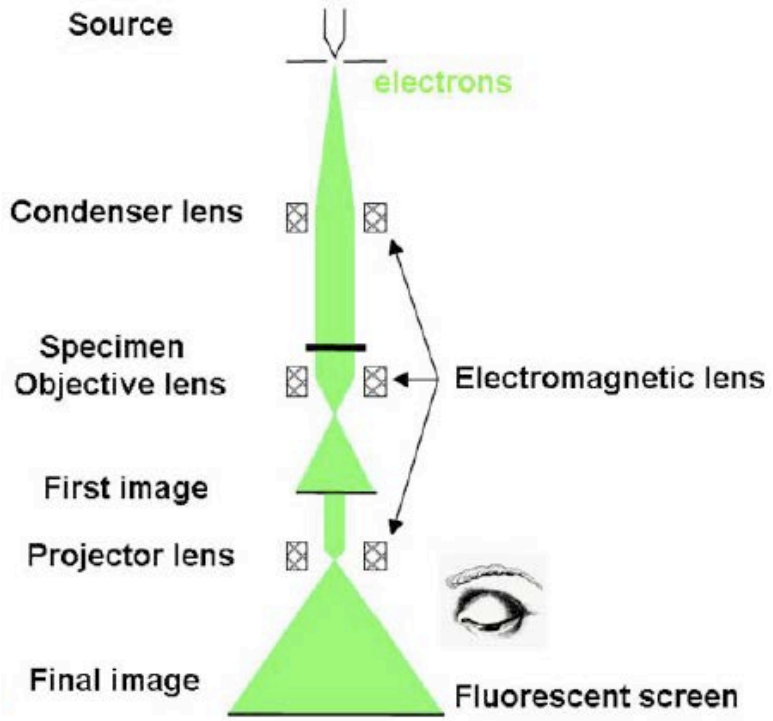
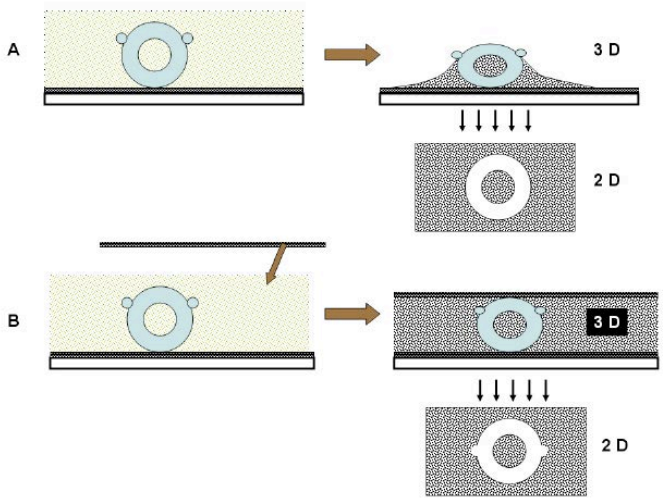


Advantages/drawbacks

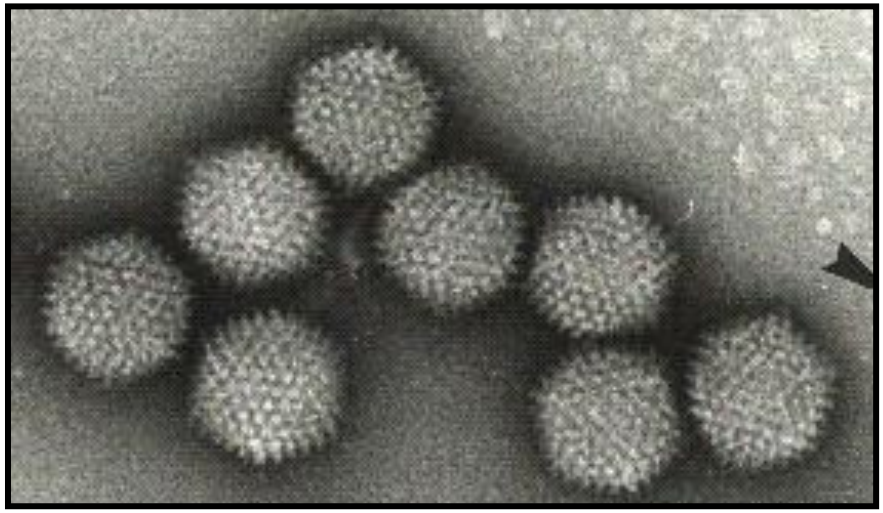
- Advantages:
  - Fast
  - Small amount of protein
    (concentration around 0.01-0.1mg/ml, few ul)
  - Small proteins visible (>50-100kDa)
  - High contrast

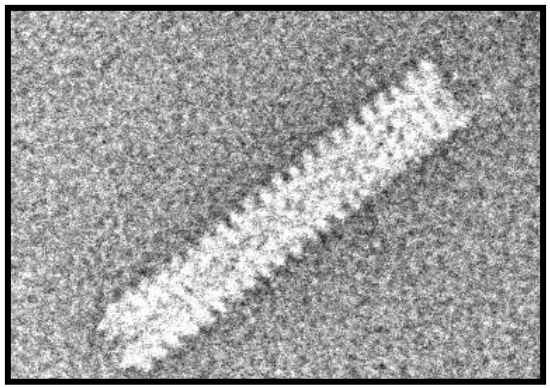
- Drawbacks:
  - Flattening and drying of the sample.
  - Artifacts due to the stain.

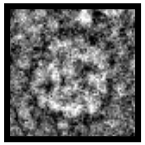


What we see in negative stain

Single particles visualized by negative stain EM: examples

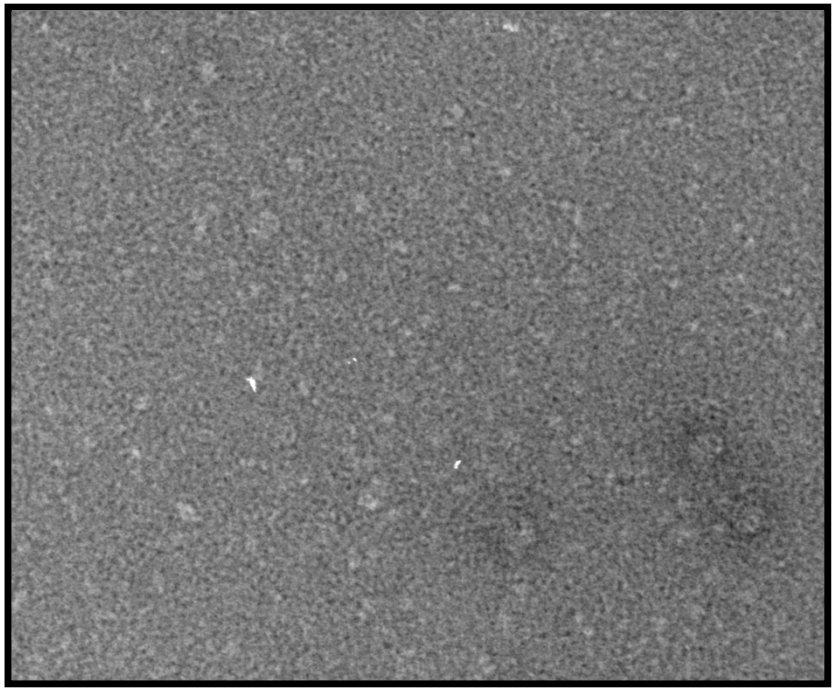


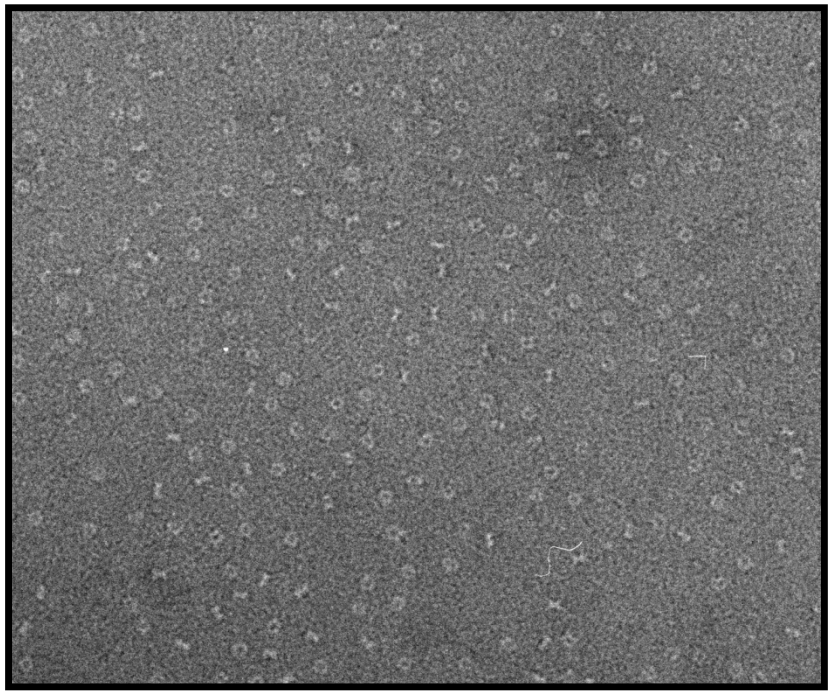




What is negative stain EM used for ?

- Quality control



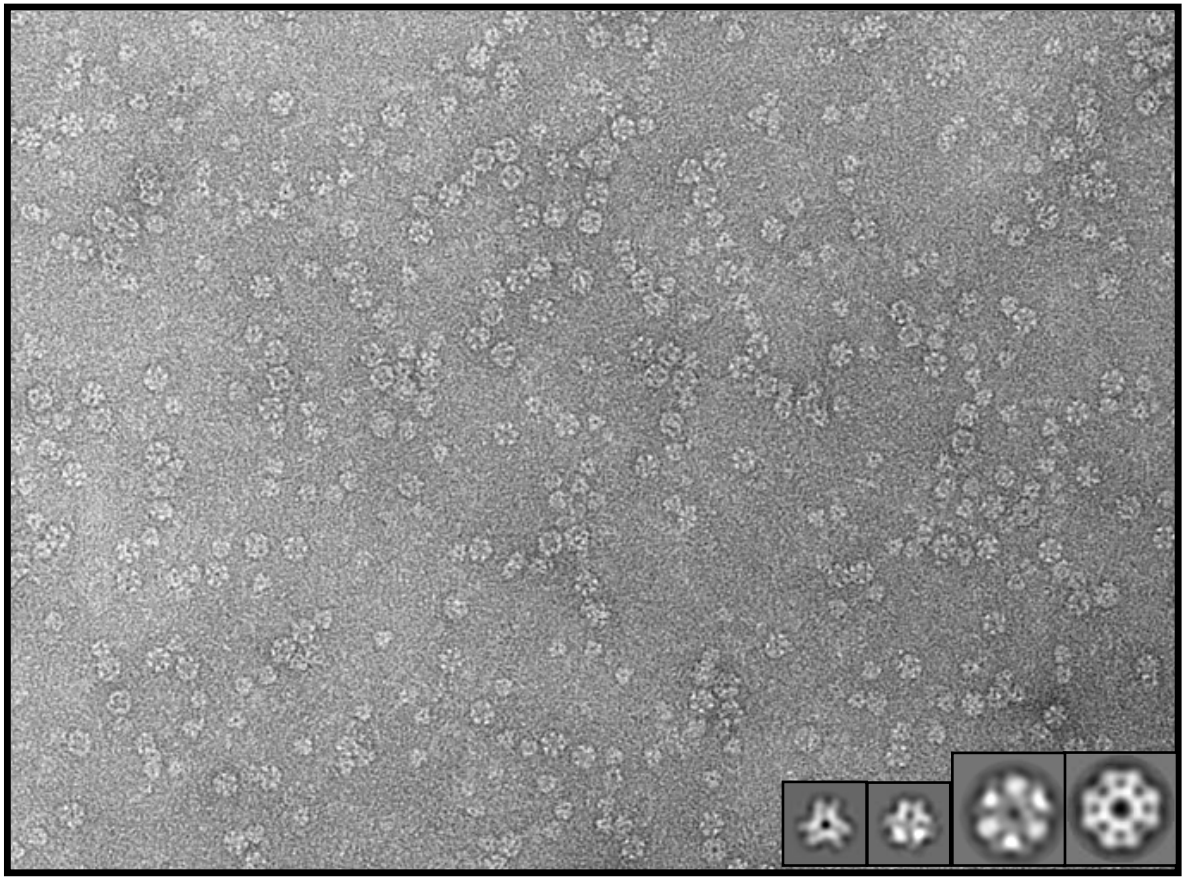


Heterogeneous

150 kDa

Homogeneous
(quite)

- Define oligomerization states

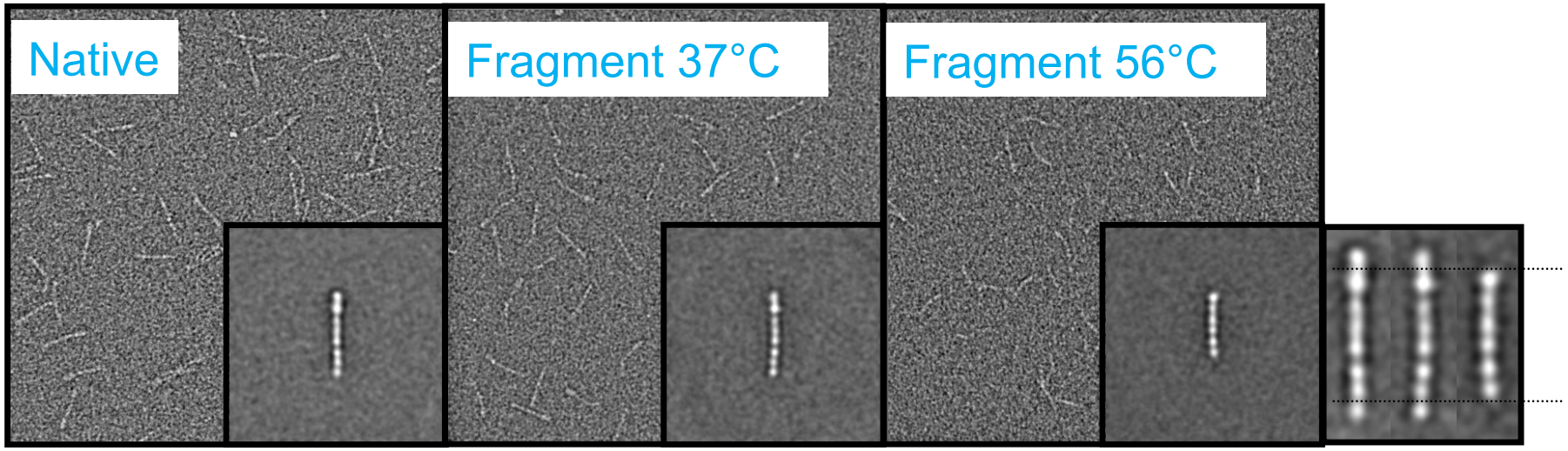


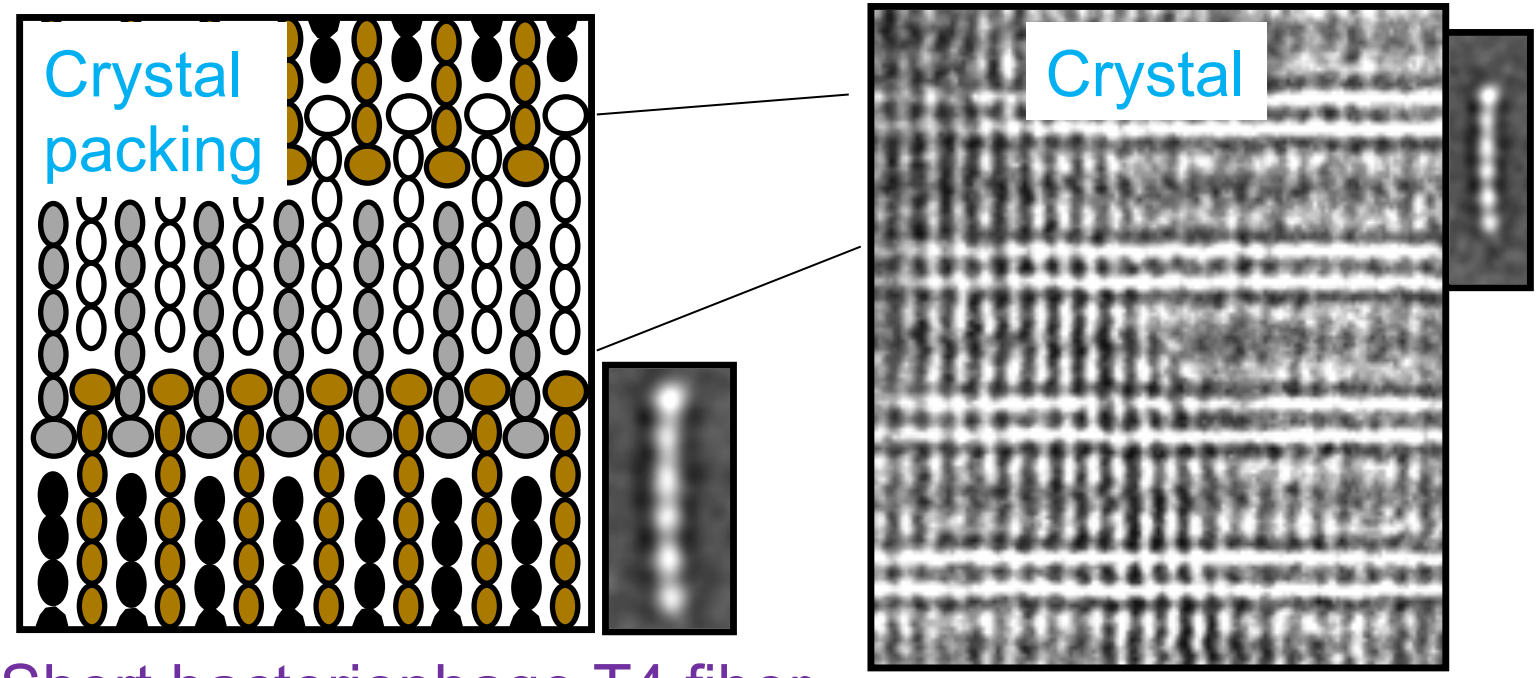
1 band on gel, 2 size
→ 2 oligomerisation state

After fine gel filtration
→ Crystal of the 2 types

➢ Organization of the protein in the crystal

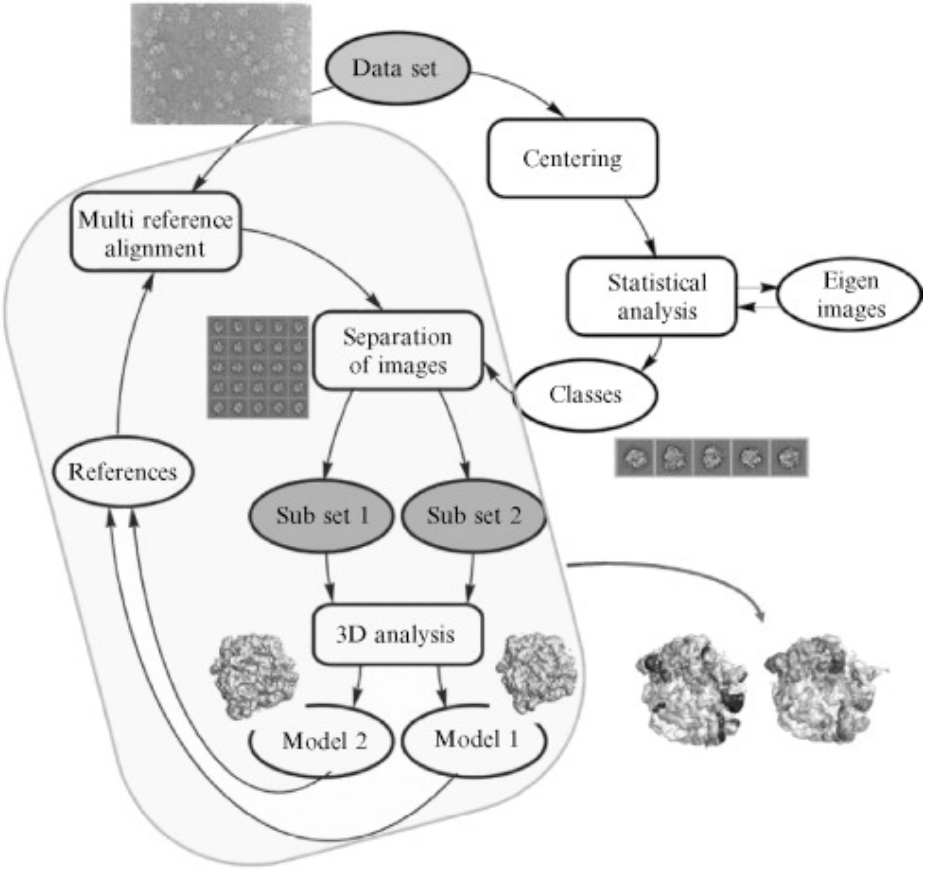
- Scratch the crystal with a potter so that it becomes thin enough for the beam to go through.
- Image the small crystals obtained in the microscope in order to see the crystal lattice

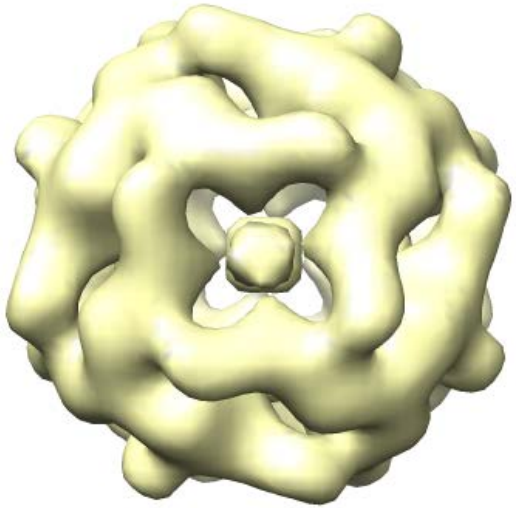




Short bacteriophage T4 fiber

➢ 3D reconstruction

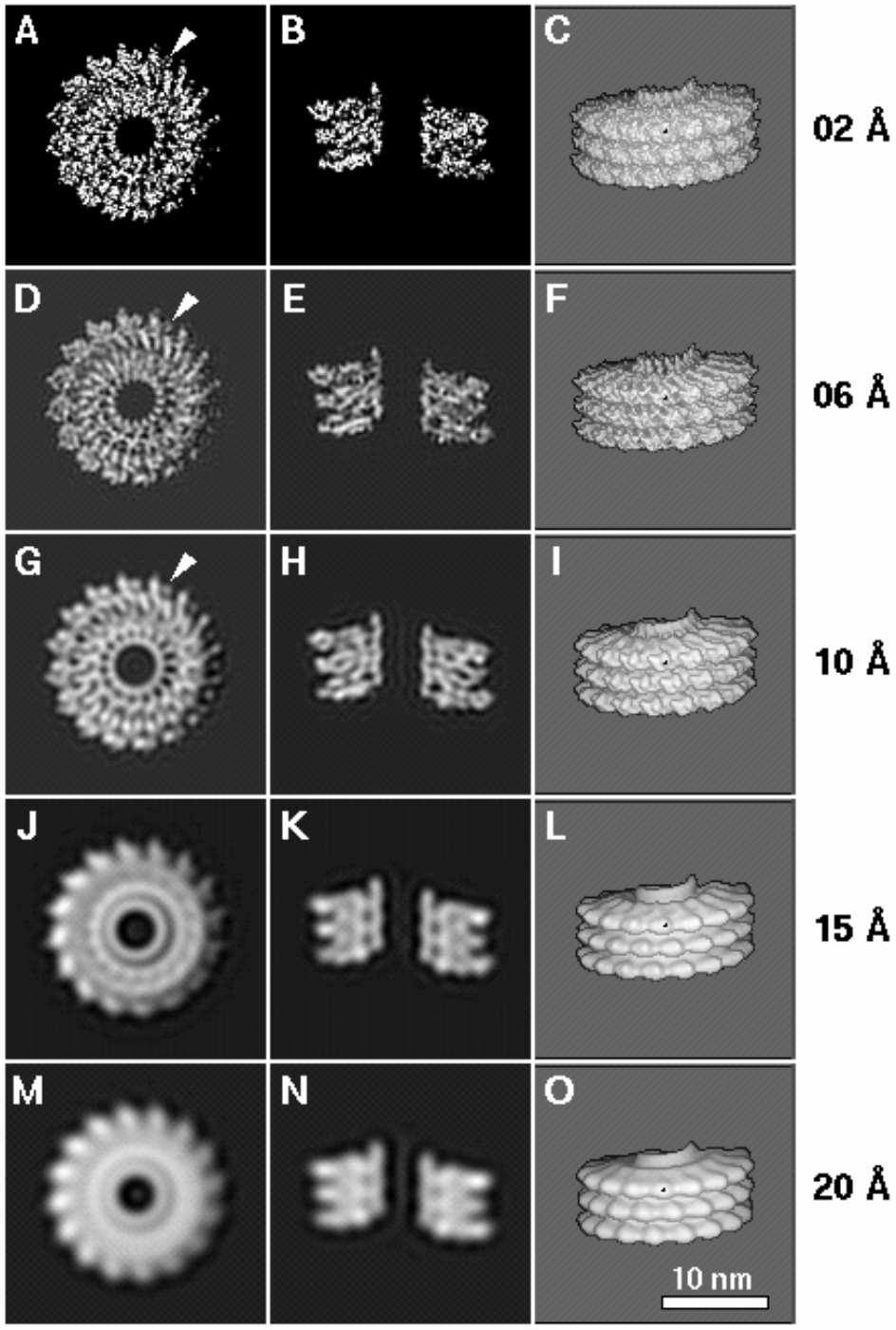




Negative stain-EM

Envelope of the molecule, resolution limited to 15-20 A

Resolution: what do we see ?



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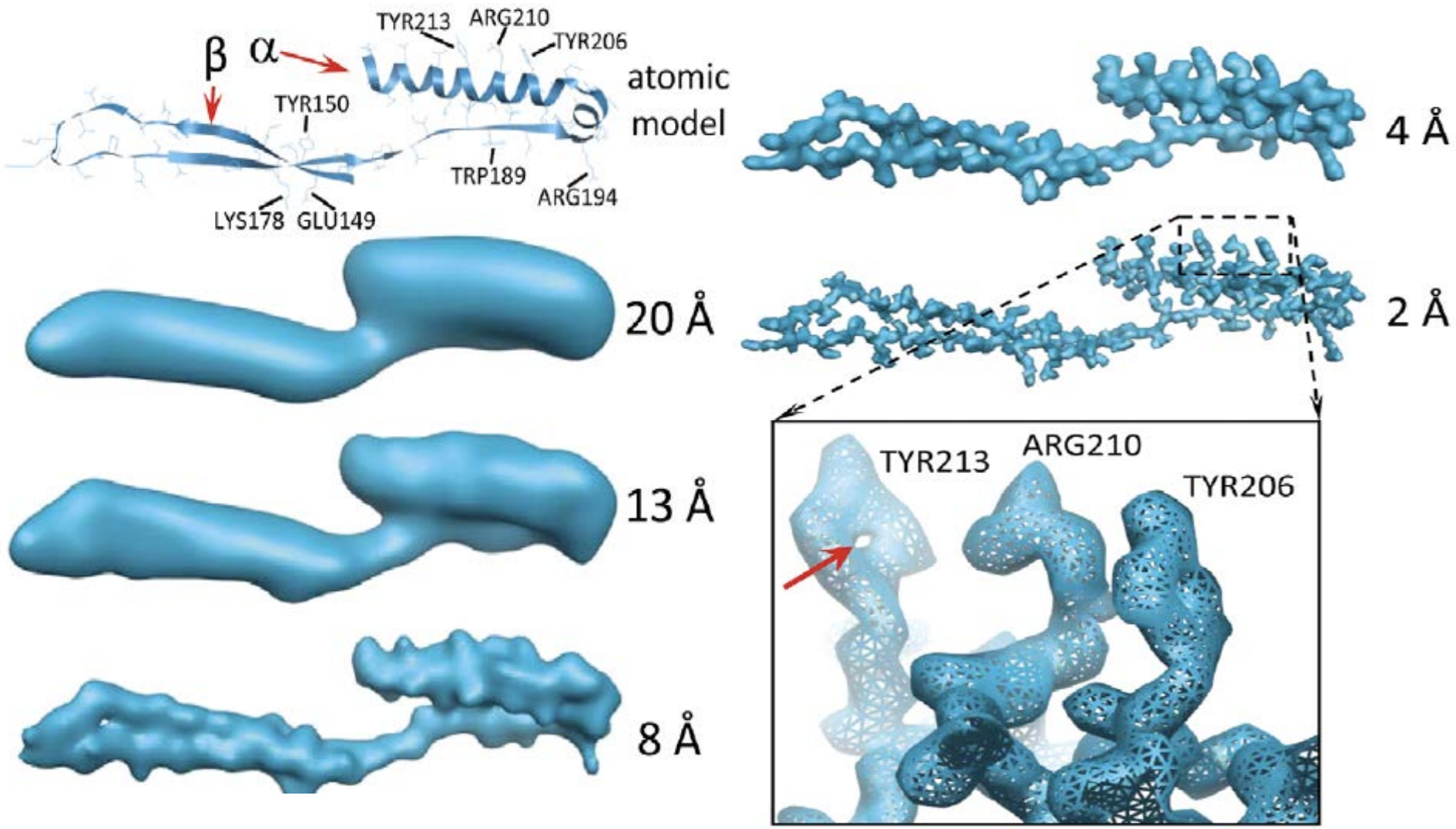


So, negative stain EM can give important information quickly, but:

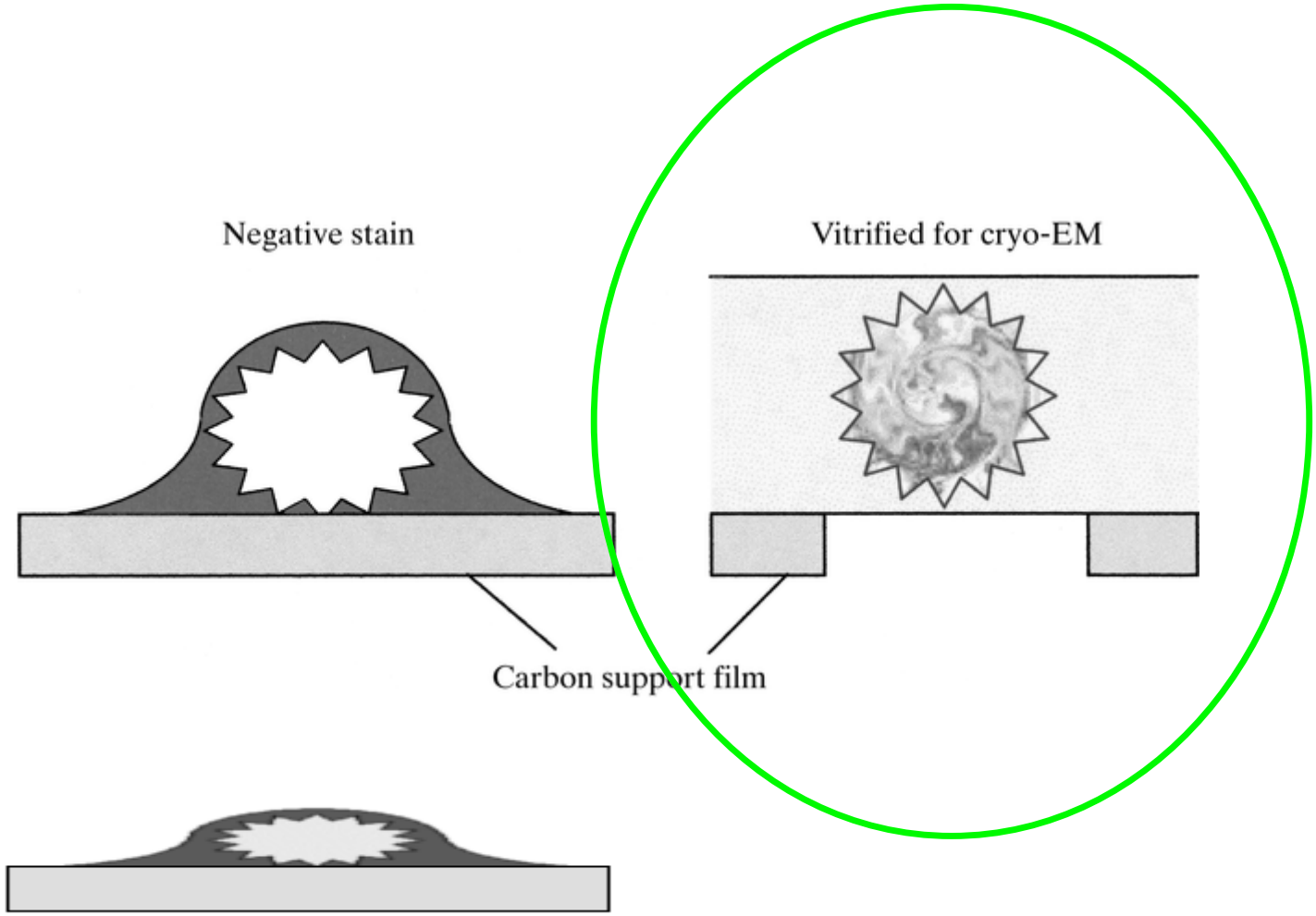
- the object is not in its native environment
- Can be flattened
- resolution is limited to 15 A.

Can we do better ??

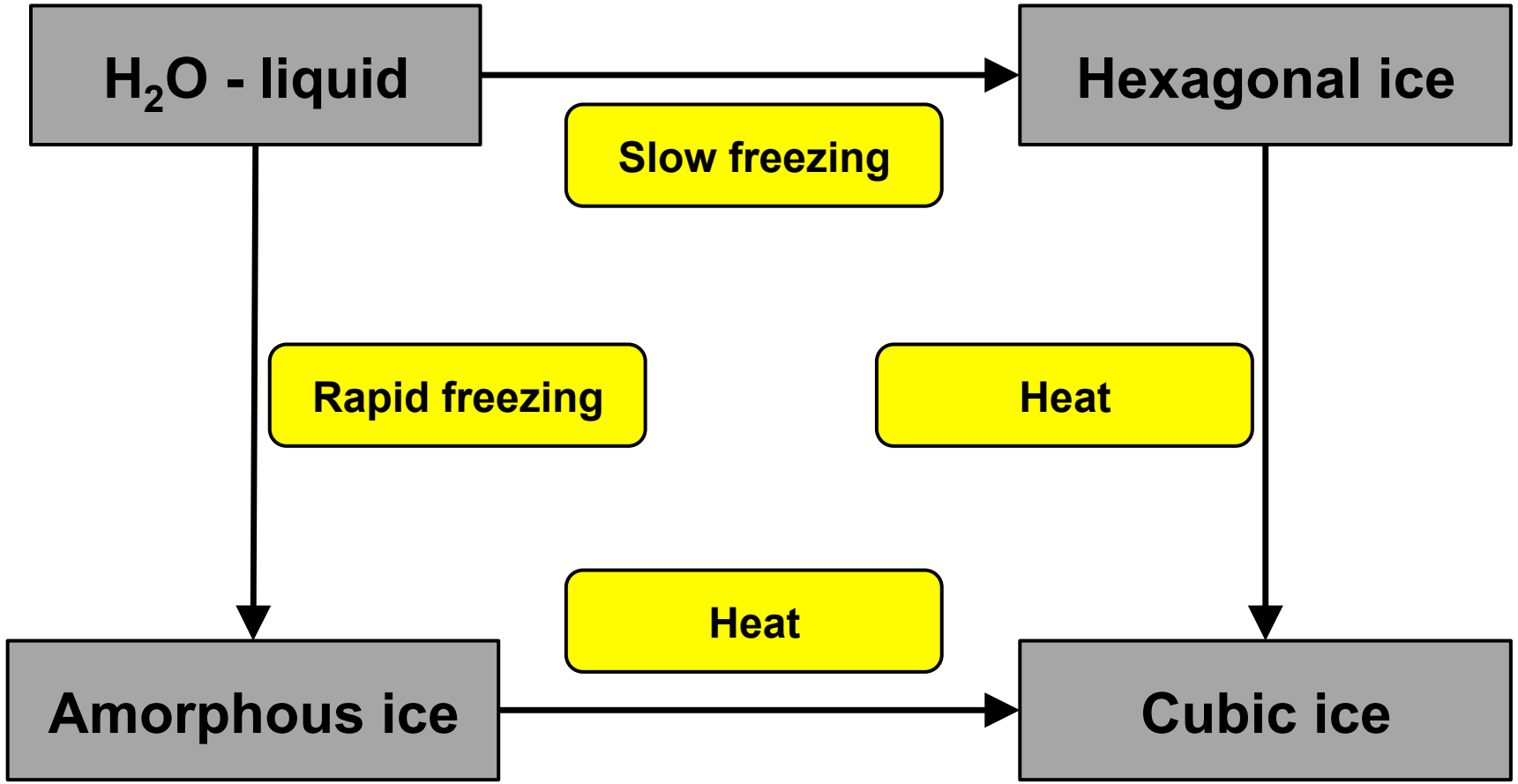
Object seen in negative stain

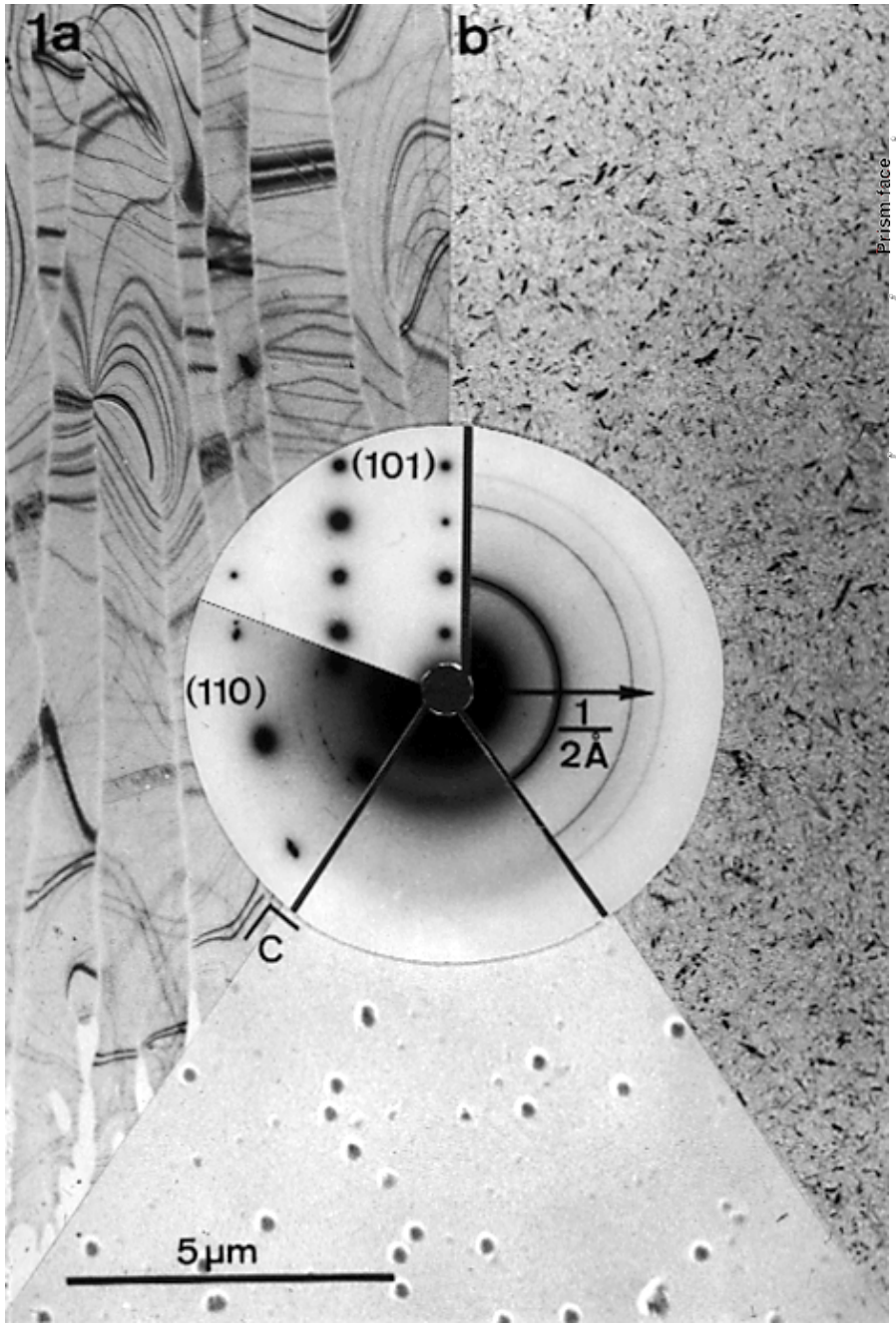
Real object to be visualized

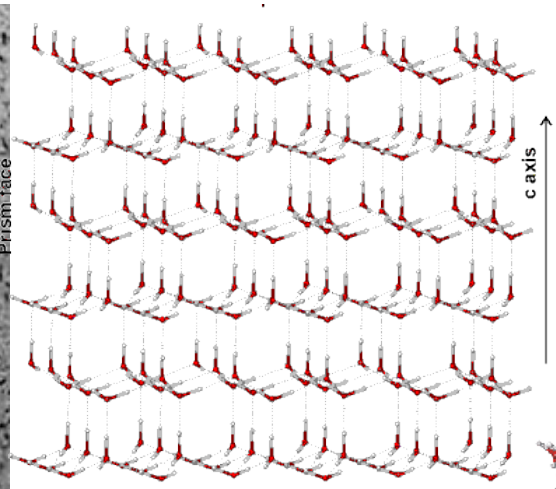
Cryo-electron microscopy !



- We need to freeze our sample as a thin layer:
  - ✓ to resist to the vacuum
  - ✓ to allow electrons to be transmitted



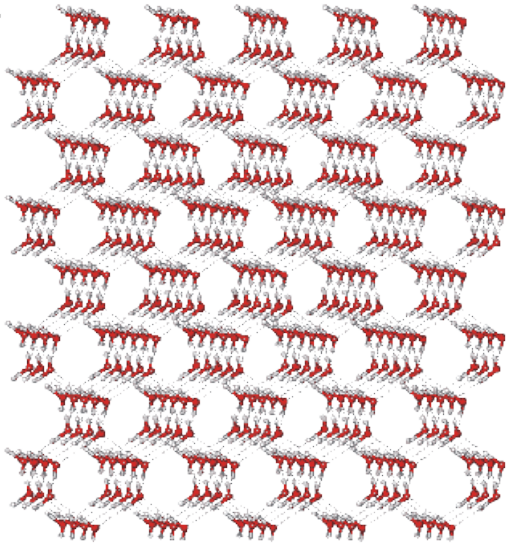




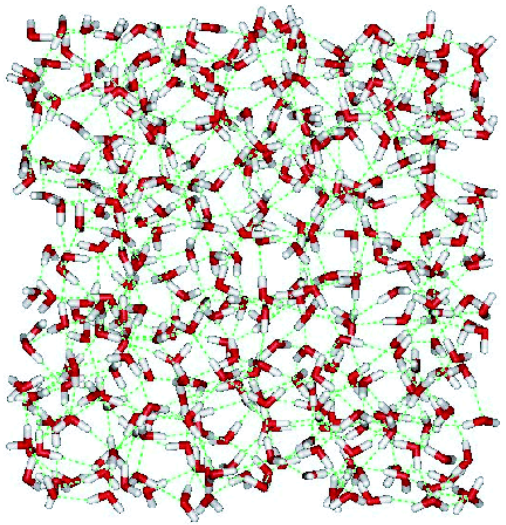
Dubochet et al., (1982)

a)

b)

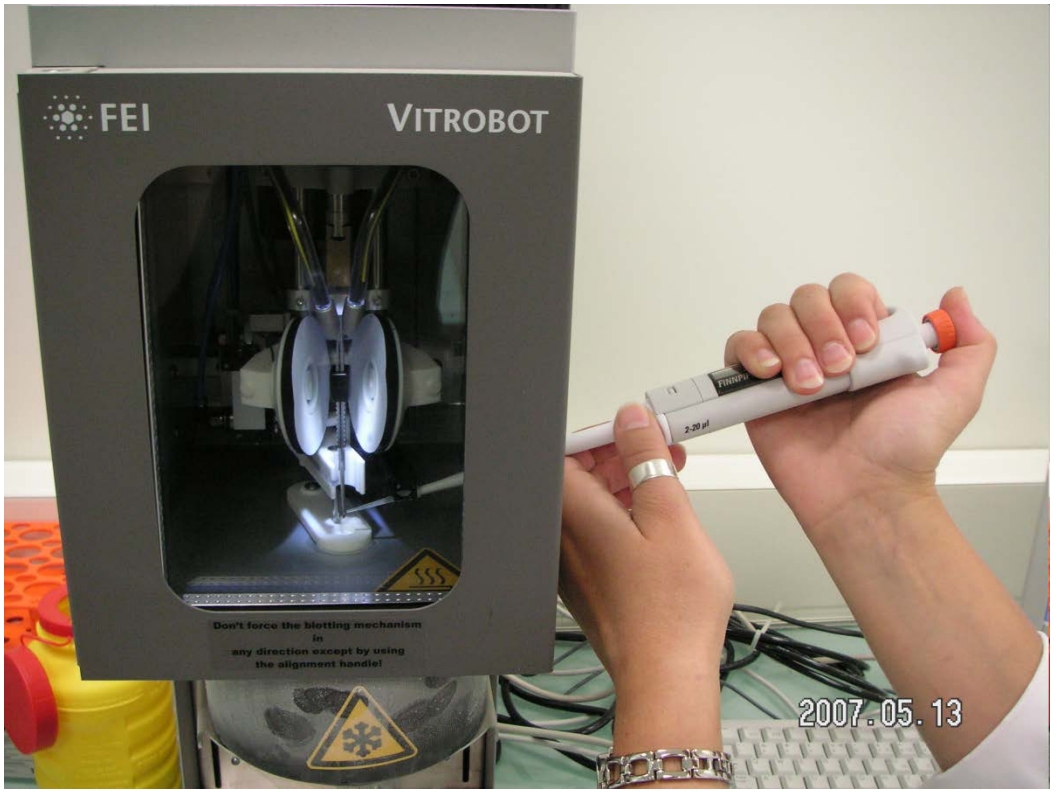


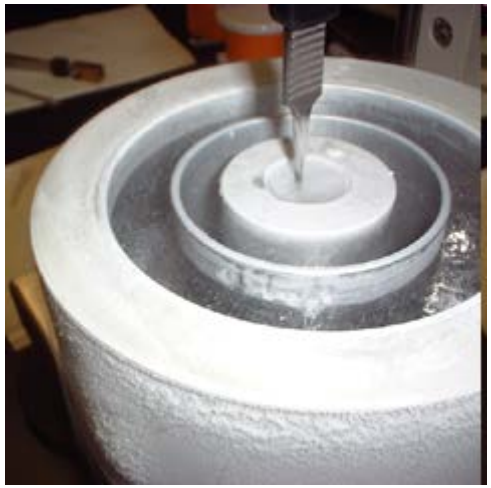
c)



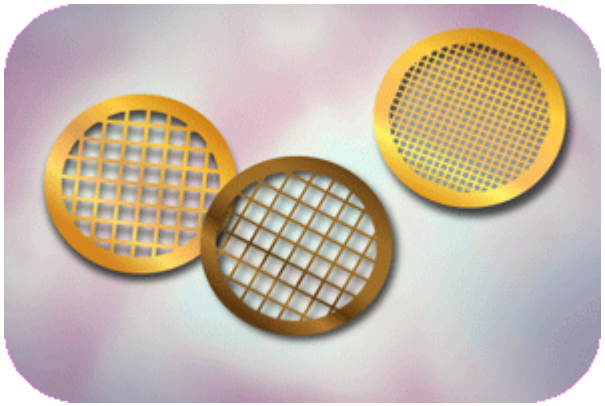
a) hexagonal b) cubic c) vitrified

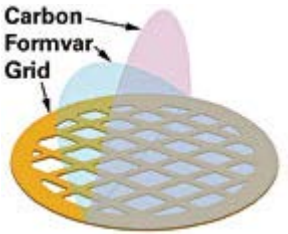

VITROBOT

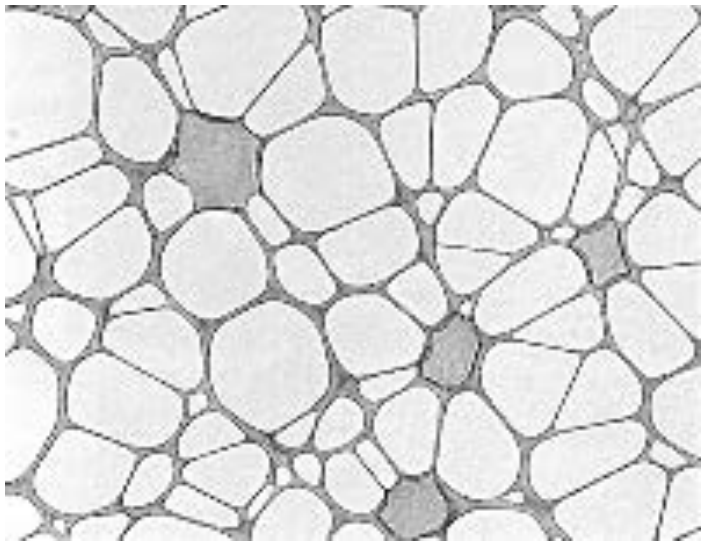

FEI
VITROBOT
Don't force the blotting mechanism
in
any direction except by using
the alignment handle!
2007.05.13

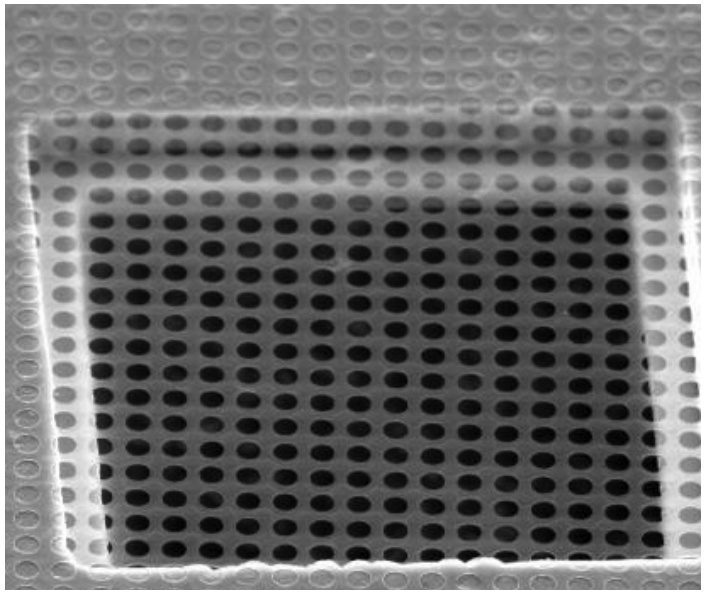


Grids used for cryo-EM

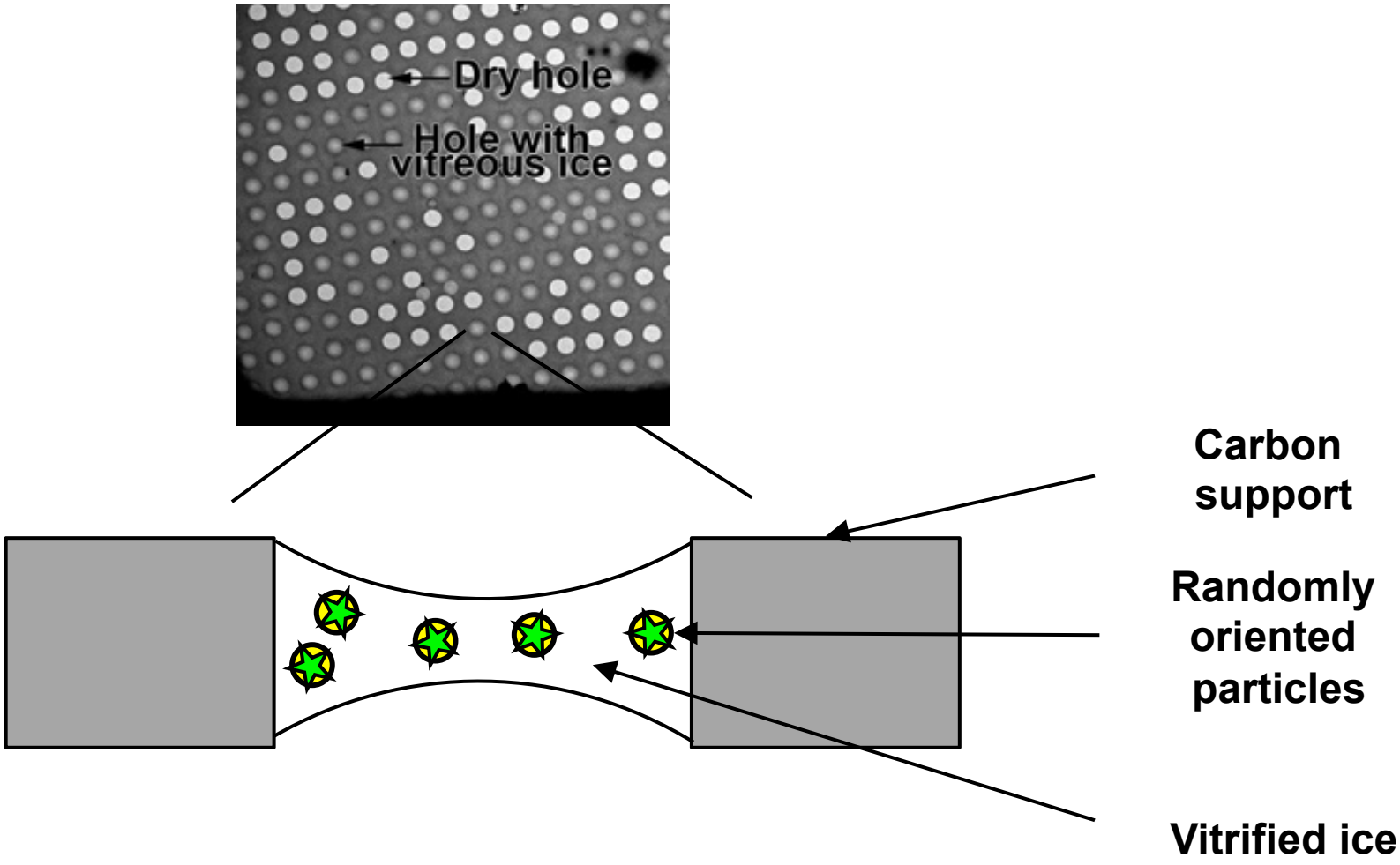




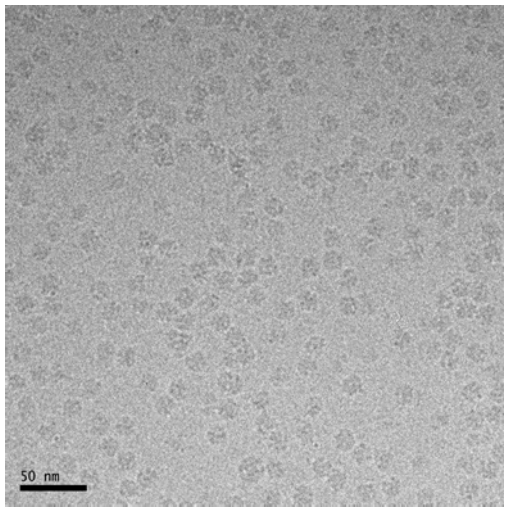


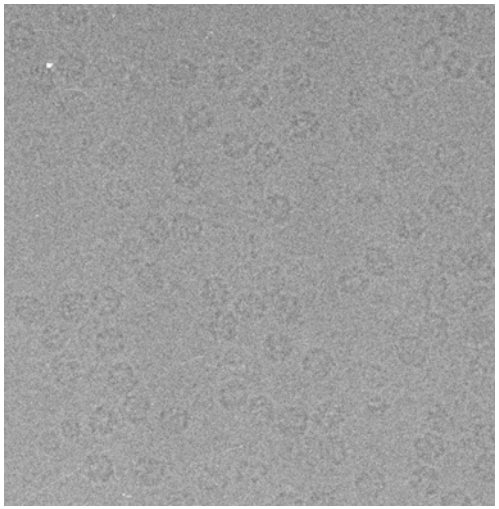


A frozen grid



Cryo-EM: advantages/drawbacks

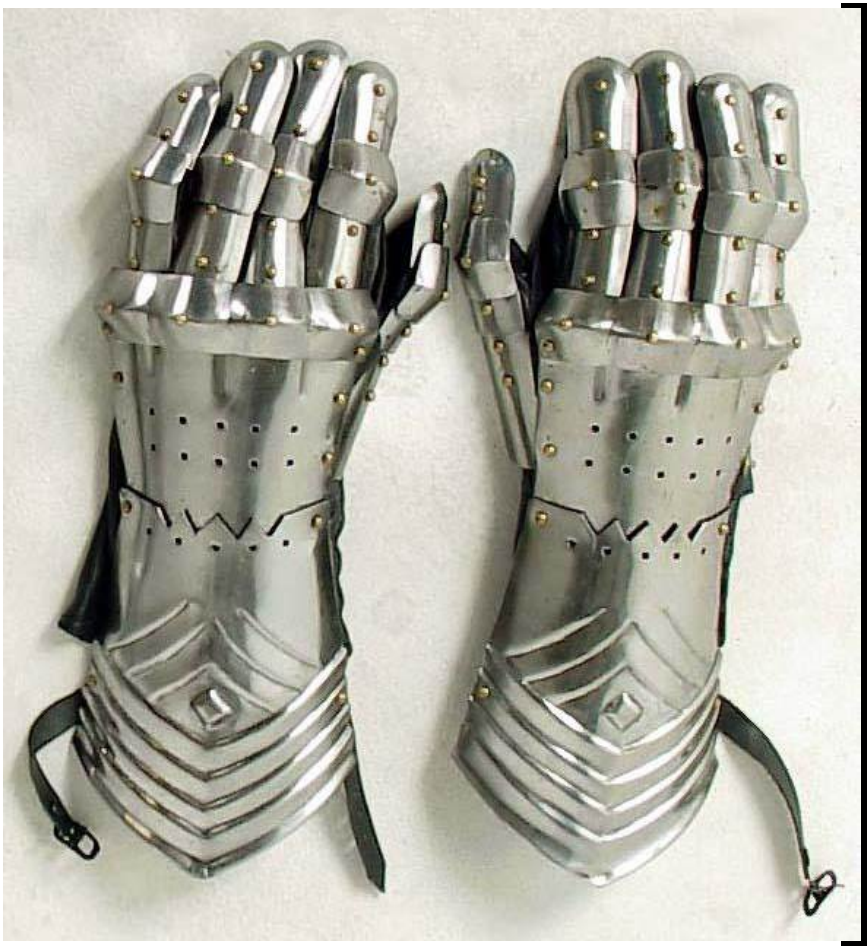


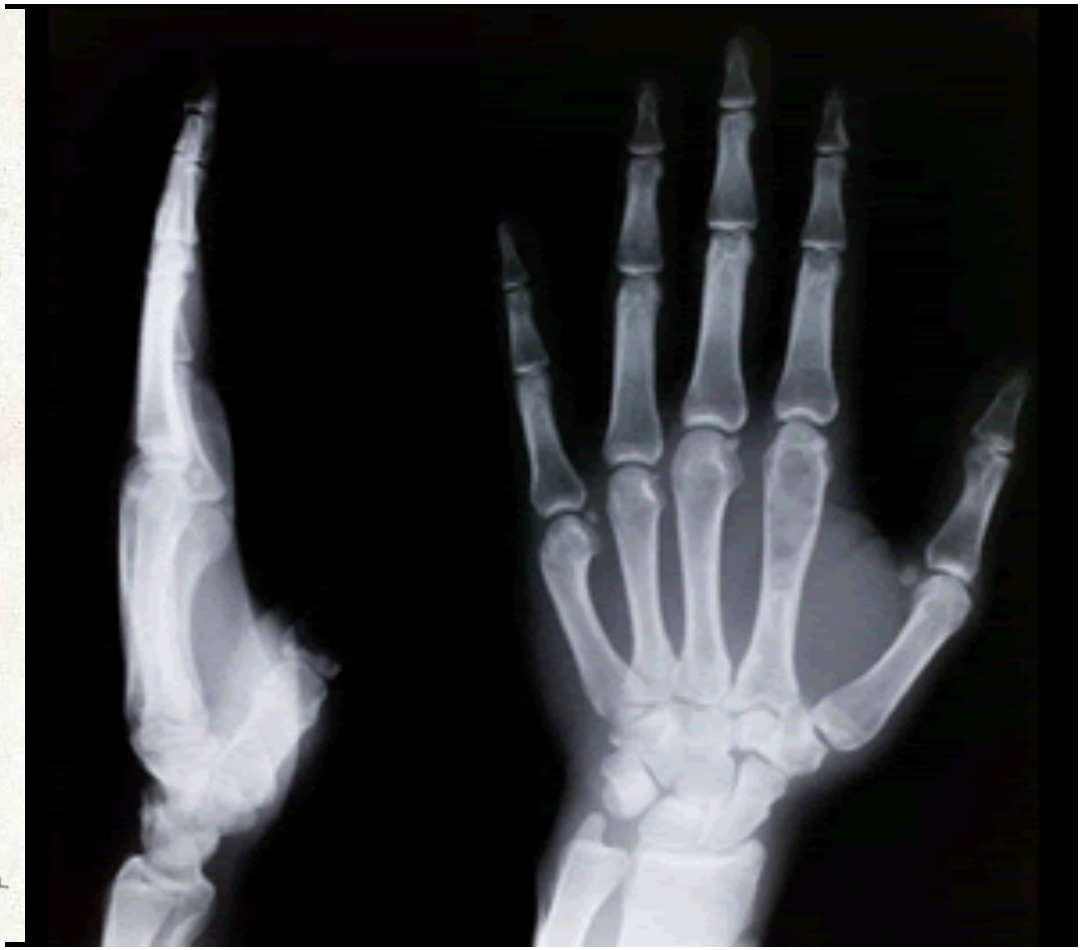


- Advantages:
  - Native state
  - Higher resolution
  - Small amount of protein (1 grid = 4 ul at 0.1-1mg/ml)

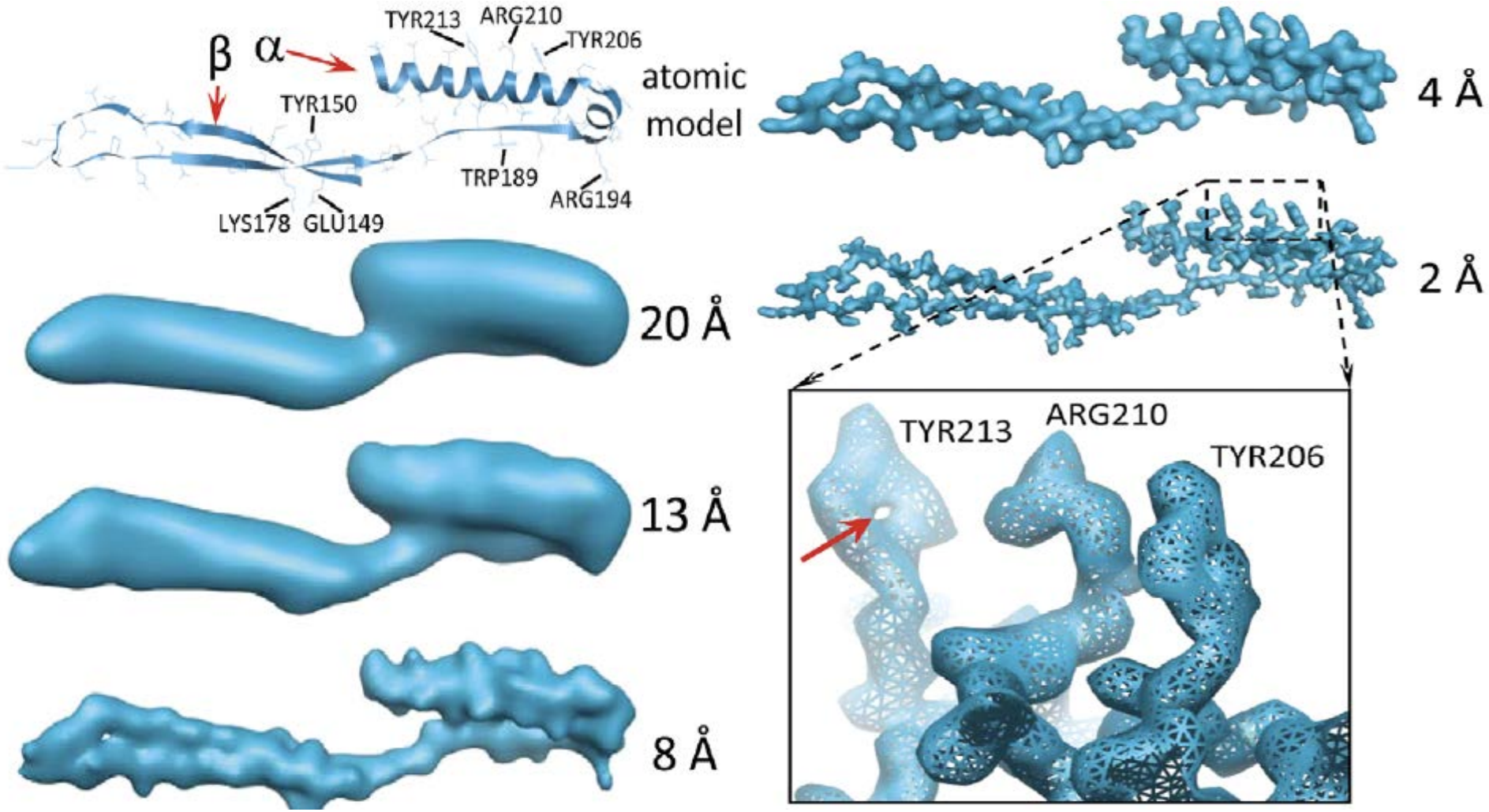
- Disadvantages:
  - Low contrast
  - Highly sensitive to radiations
  - Minimal size limit: 250 kDa
  - Difficult

Negative stain vs. Cryo-EM





Cryo-EM: native state, so atomic resolution can (in theory) be reached!

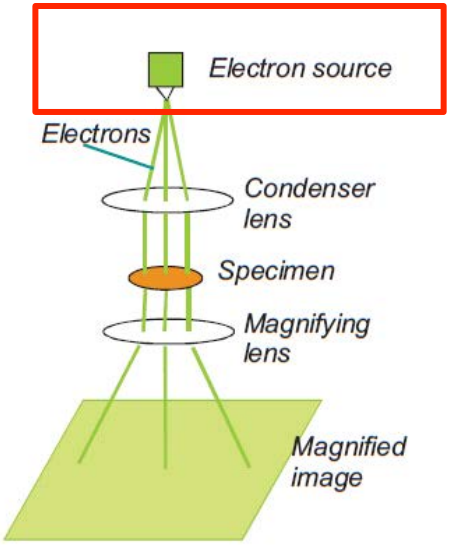


For high-resolution, you will need to collect on a state-of-the art electron microscope

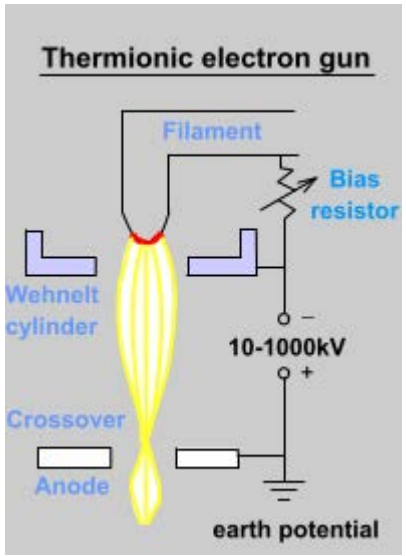
FEI Polara

FEI Titan Krios

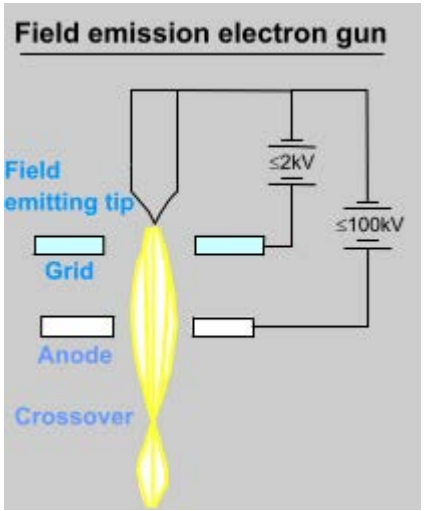
Electron source



- Purpose: generation of electrons that can be accelerated by high tension to obtain the illuminating electron beam



Thermionic gun: W or LaB6
Electrons come out when the emitter is heated

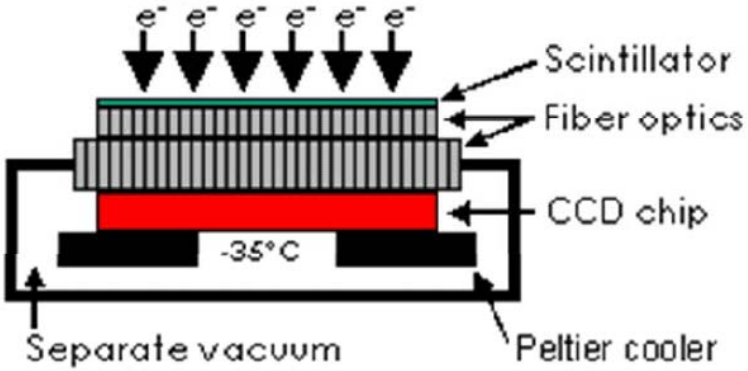


Field emission gun

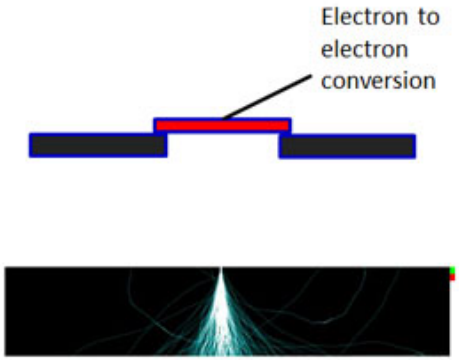
Detection

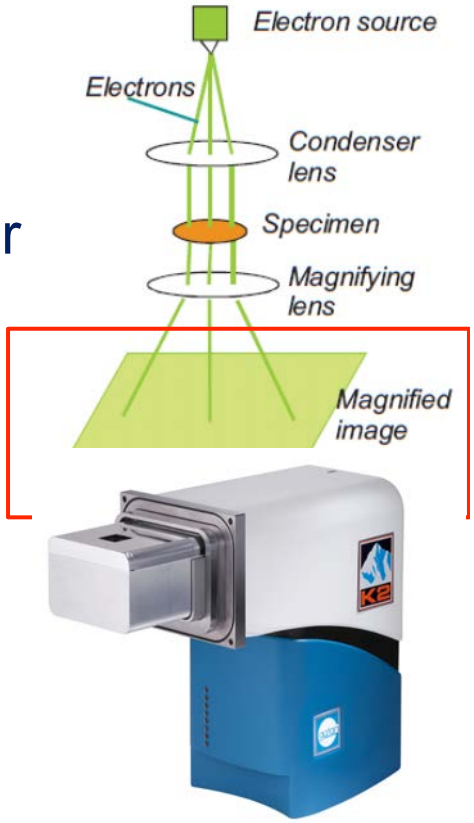
- On films that need to be developed and digitized

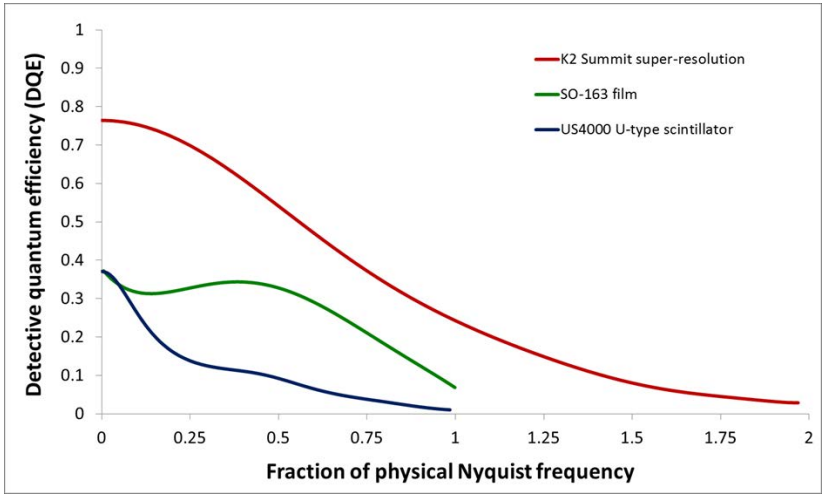
- On CCD camera



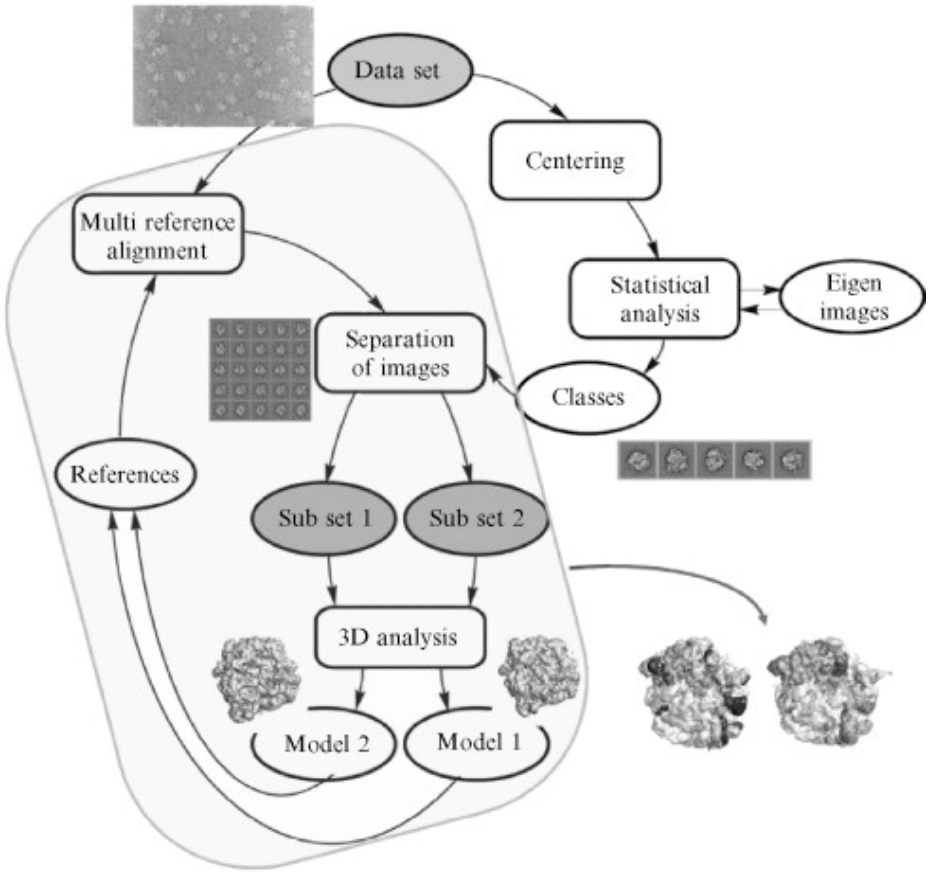
- On direct detector

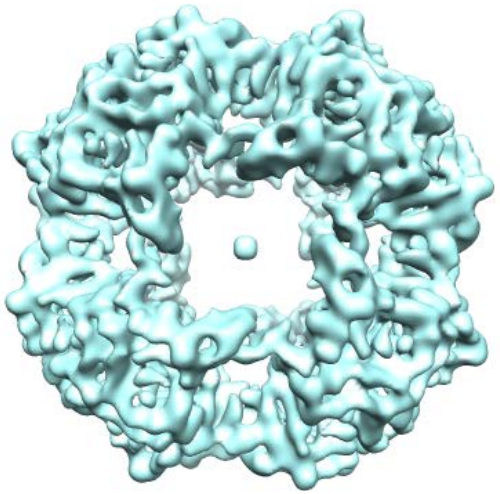




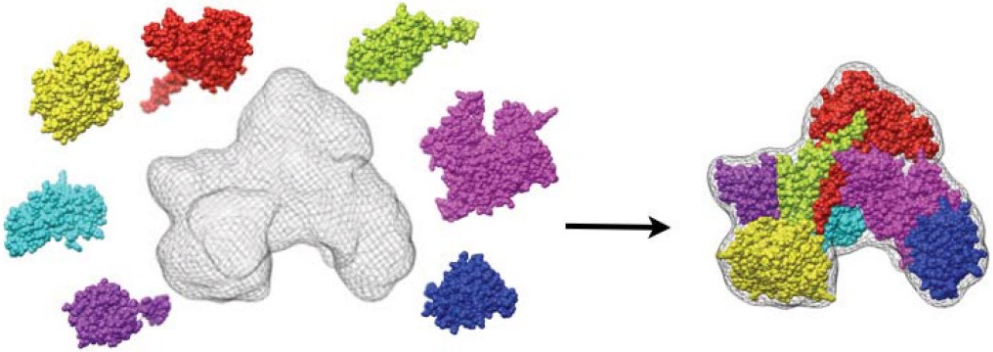


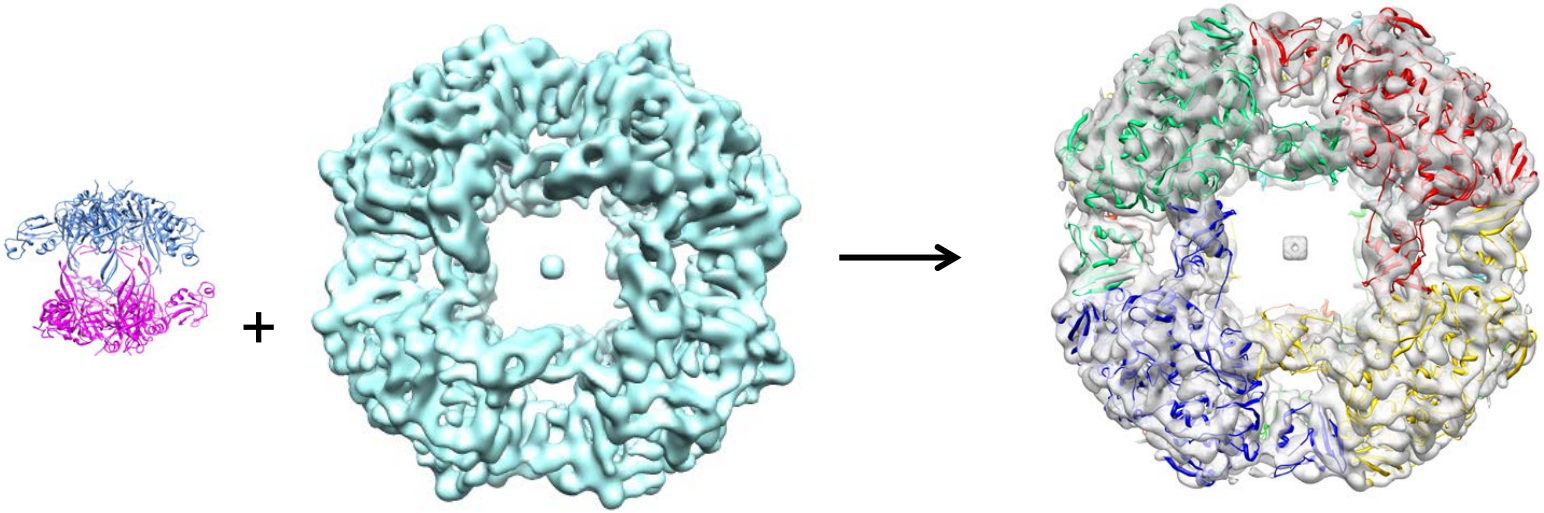
For high-resolution, you will need to do a lot of image processing



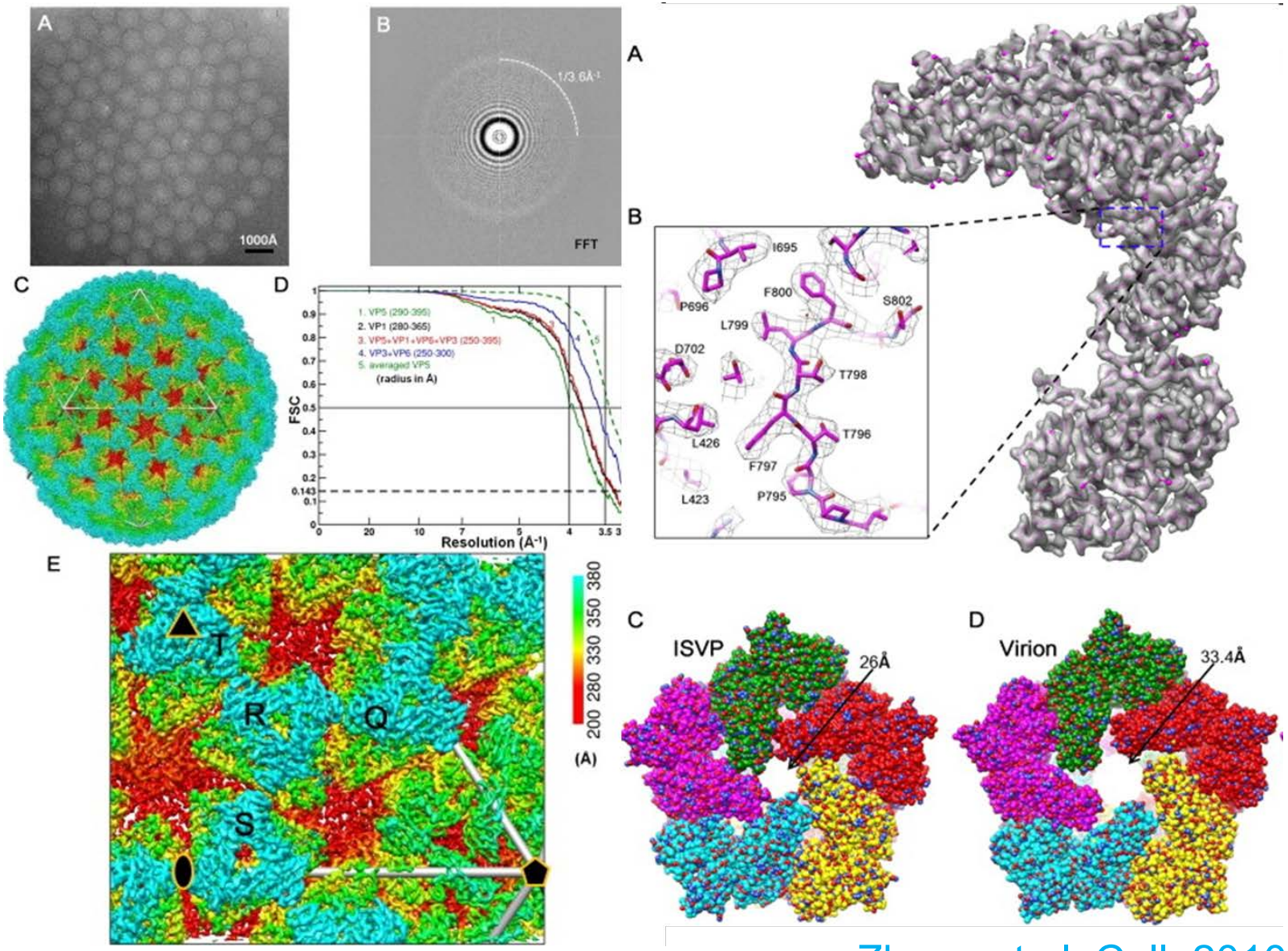


Combination EM-crystallography





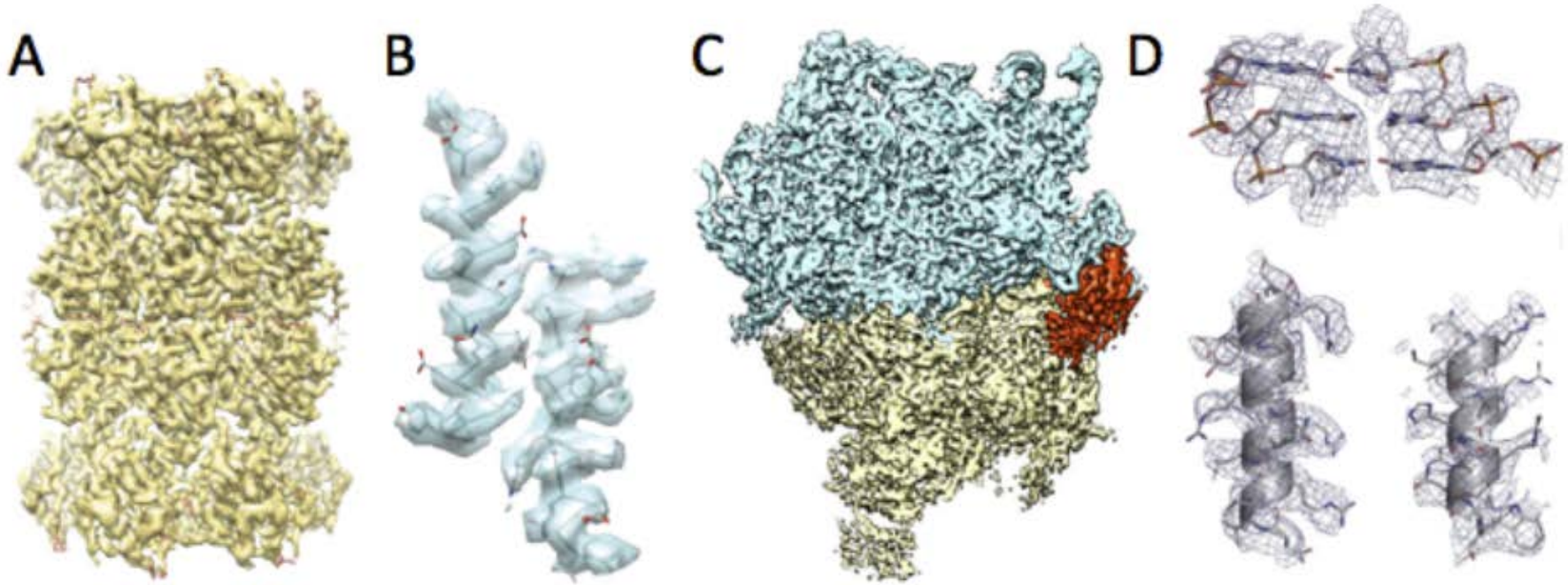
Or… high resolution EM



Zhang et al, Cell, 2010

Or… high resolution EM

- Smaller complexes less and less symetric



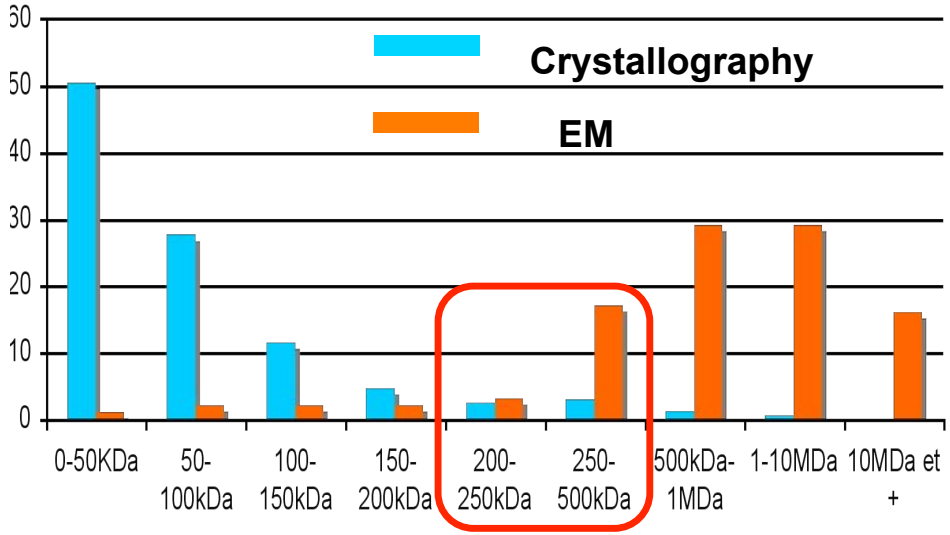
Proteasome (D7 symmetry, 700kDa)

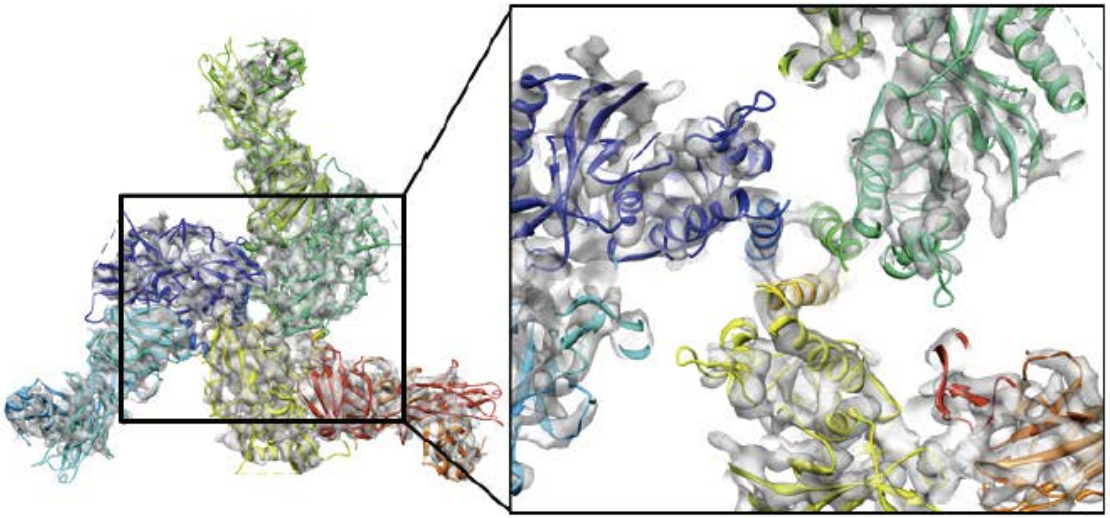
Li et al, Nature methods, 2013

Ribosome (no symmetry, 4MDa, 4 Å resolution)

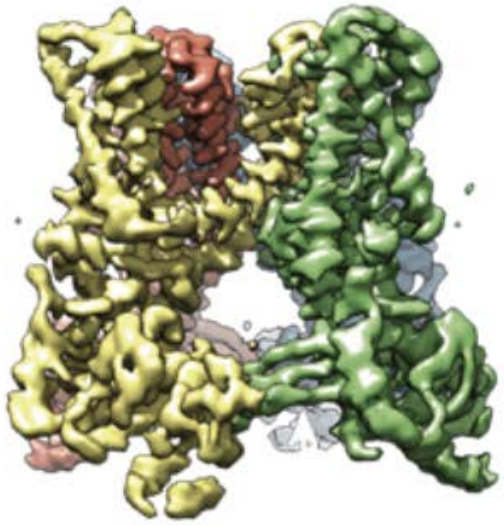
Bai et al, eLife, 2013

Towards high resolution of smaller complexes

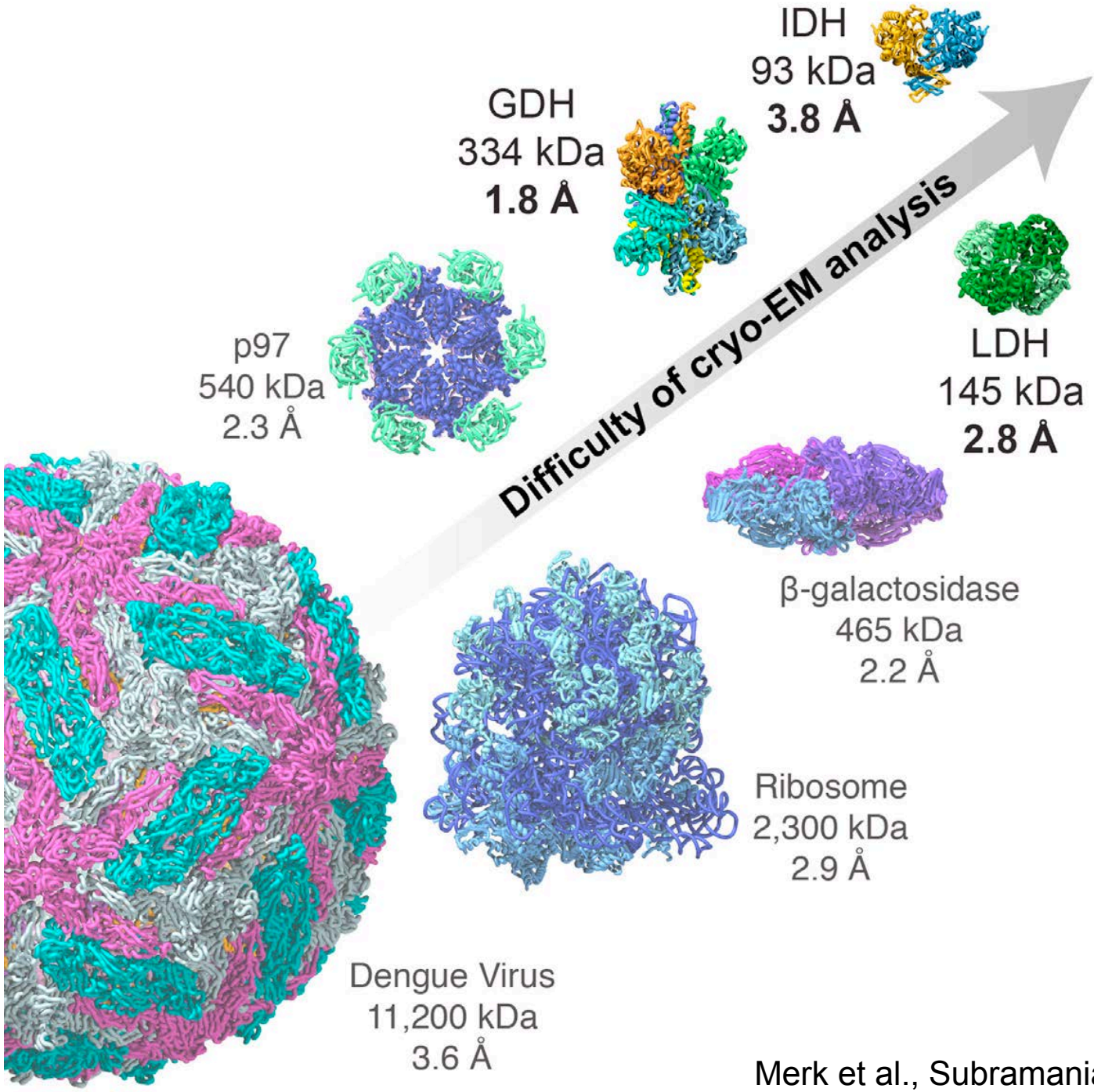




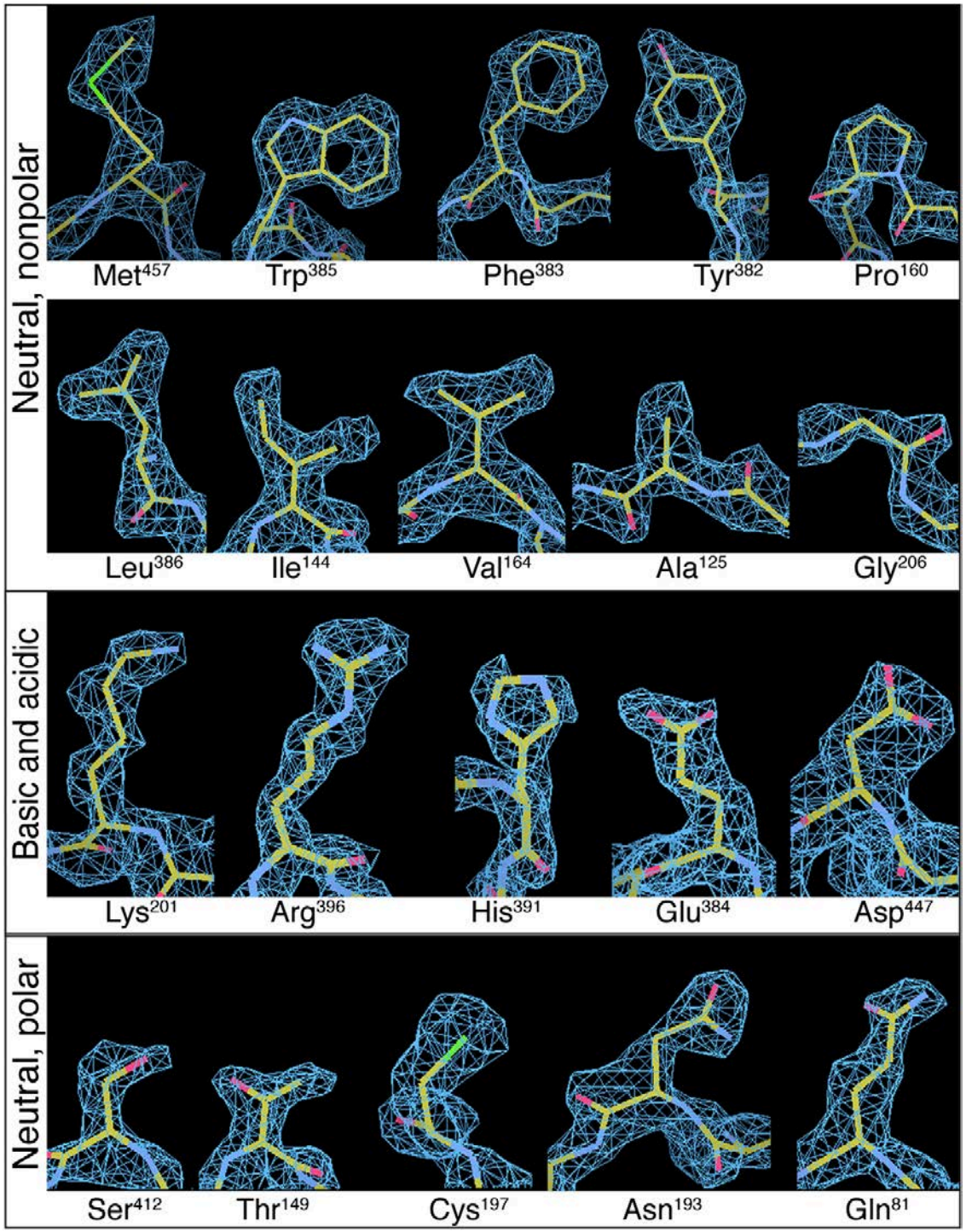
Lyumkis et al, Science, 2013



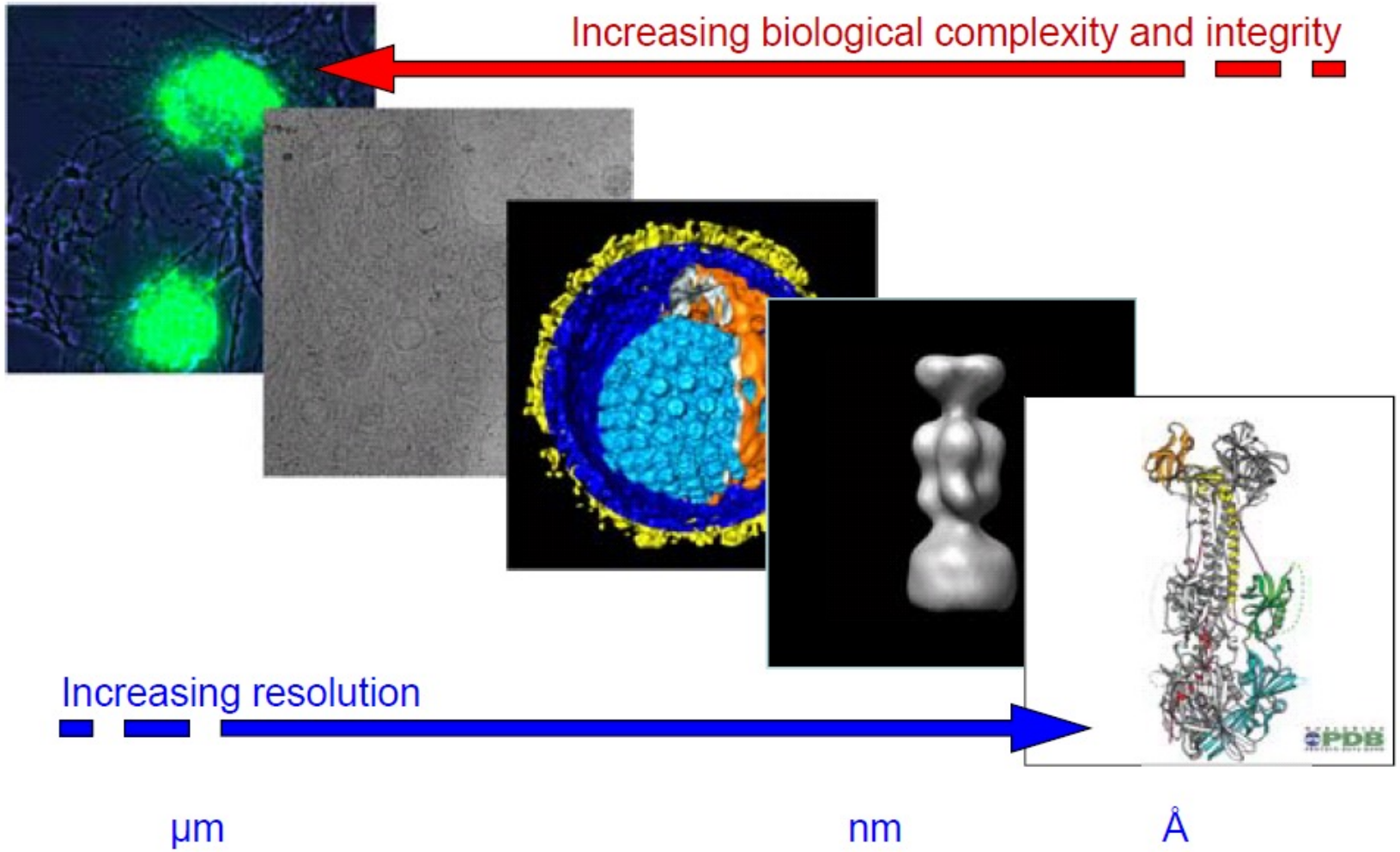
Liao et al, Nature, 2013



Merk et al., Subramaniam, Cell 2016

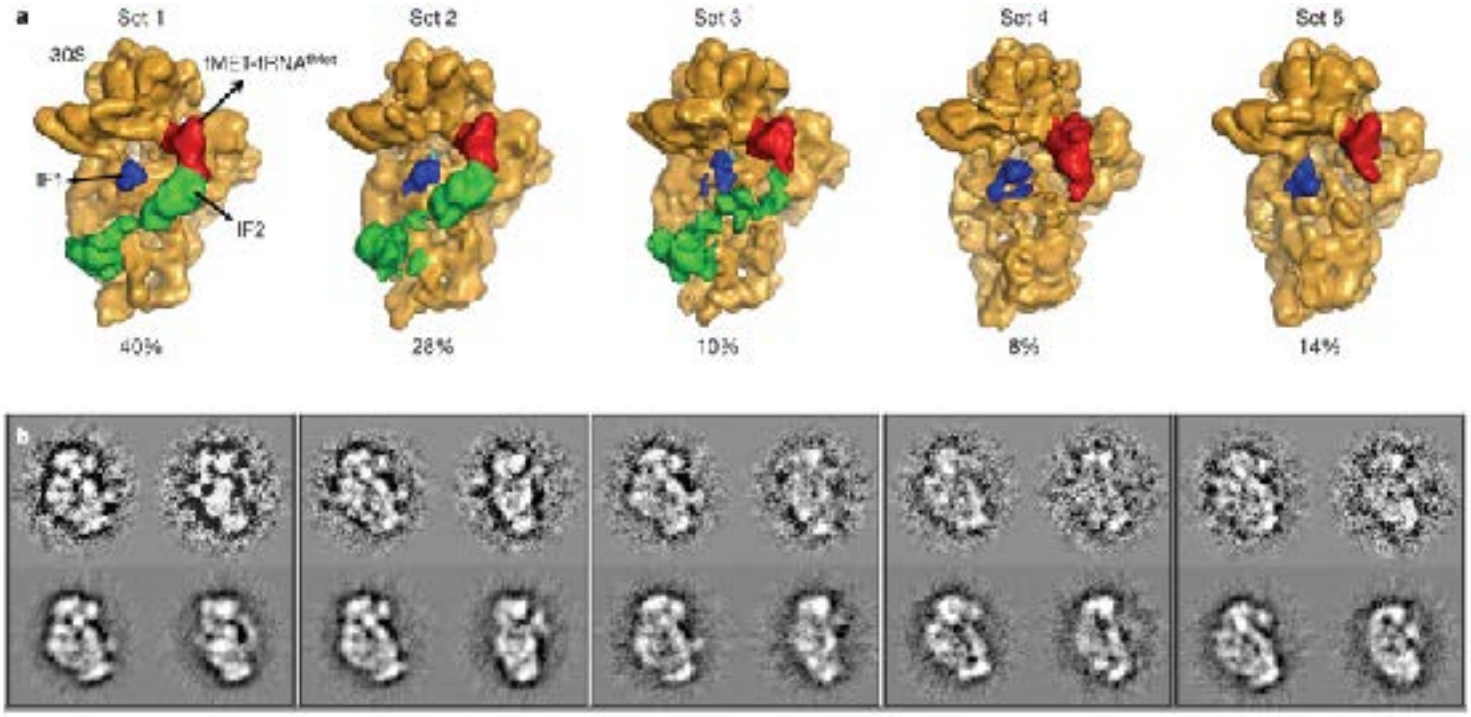

Neutral, nonpolar
Met457
Trp385
Phe383
Tyr382
Pro160
Leu386
Ile144
Val164
Ala125
Gly206
Basic and acidic
Lys201
Arg396
His391
Glu384
Asp447
Neutral, polar
Ser412
Thr149
Cys197
Asn193
Gln81

The dream: understanding a system from the cell context to the atomic level



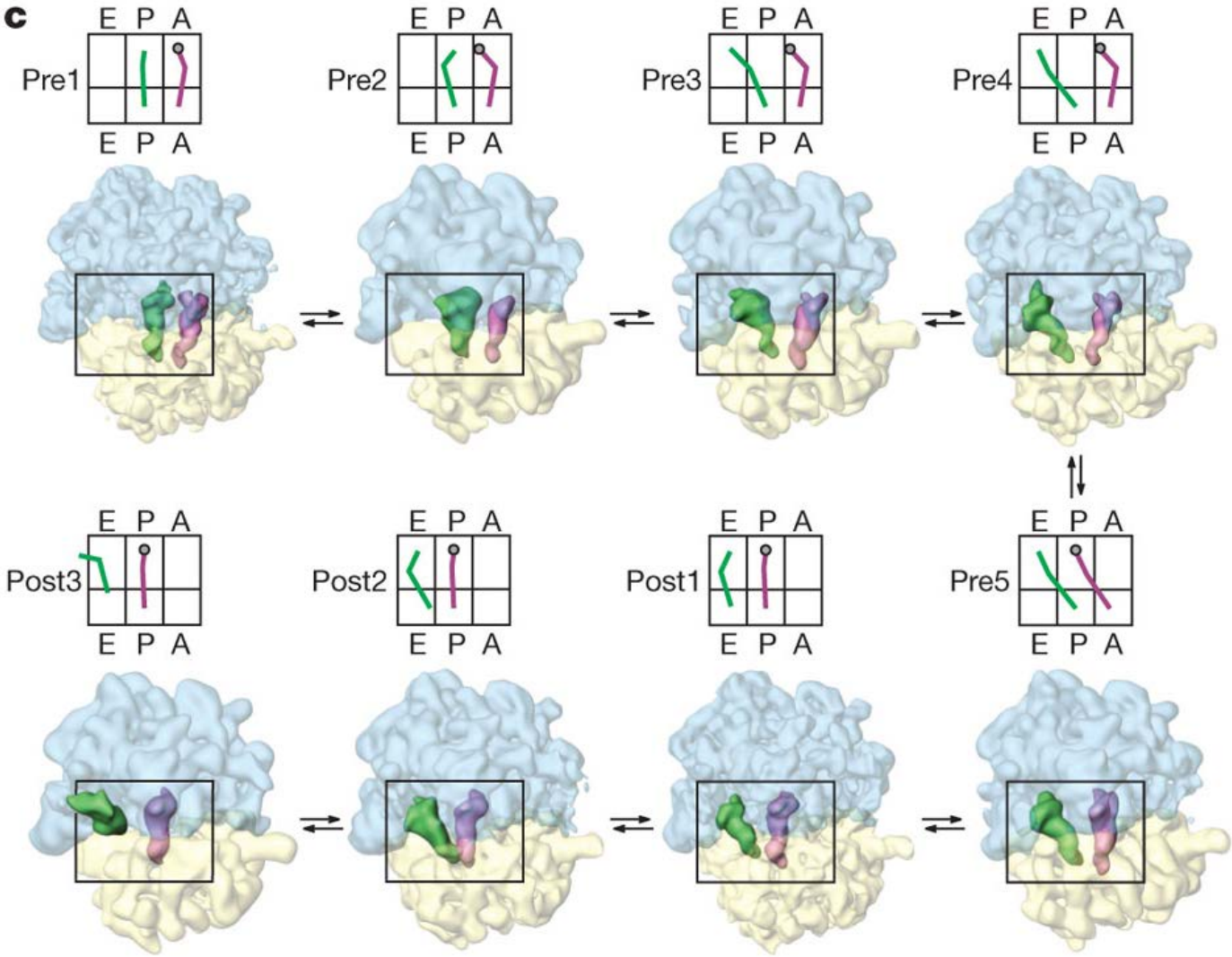
If complexes heterogeneous: separation of different populations within the same sample

- Different 3D volumes with particles chosen randomly.
- Competitive alignment to determine to which structure each particle belongs



Simonetti et al, Nature 2008

If complexes heterogenous: separation of time-resolved states



Fischer et al, Nature 2010