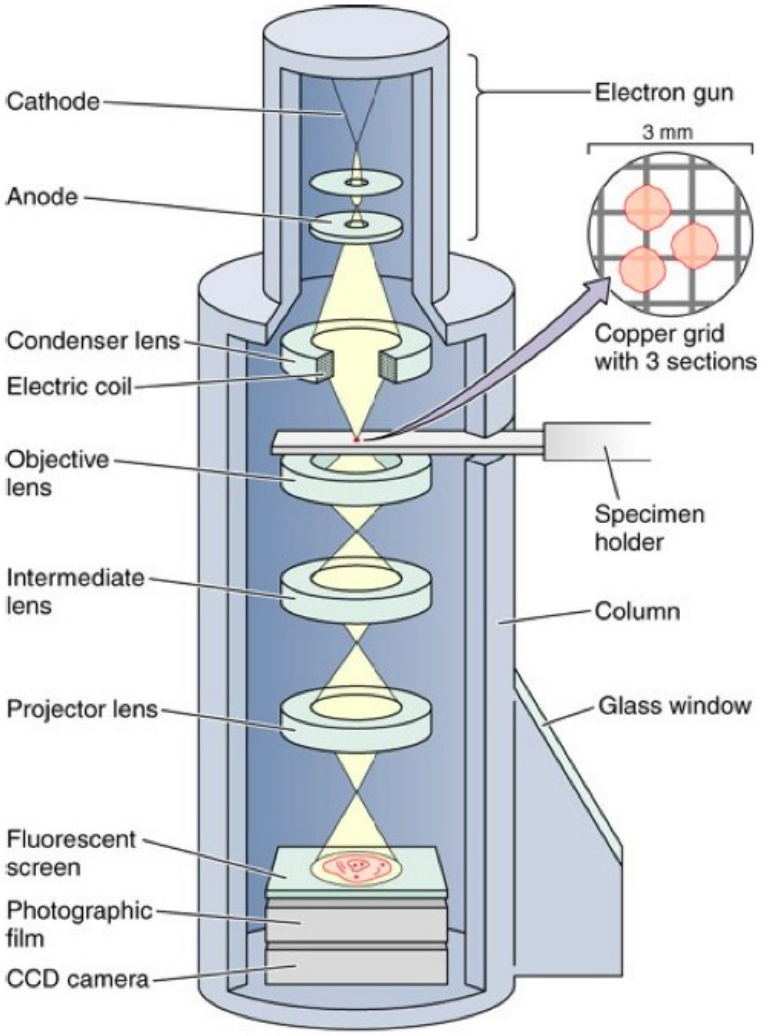
Cryo EM: From organelles to atomic-resolution structures of molecules inside the cell

Credit to Jiří Nováček

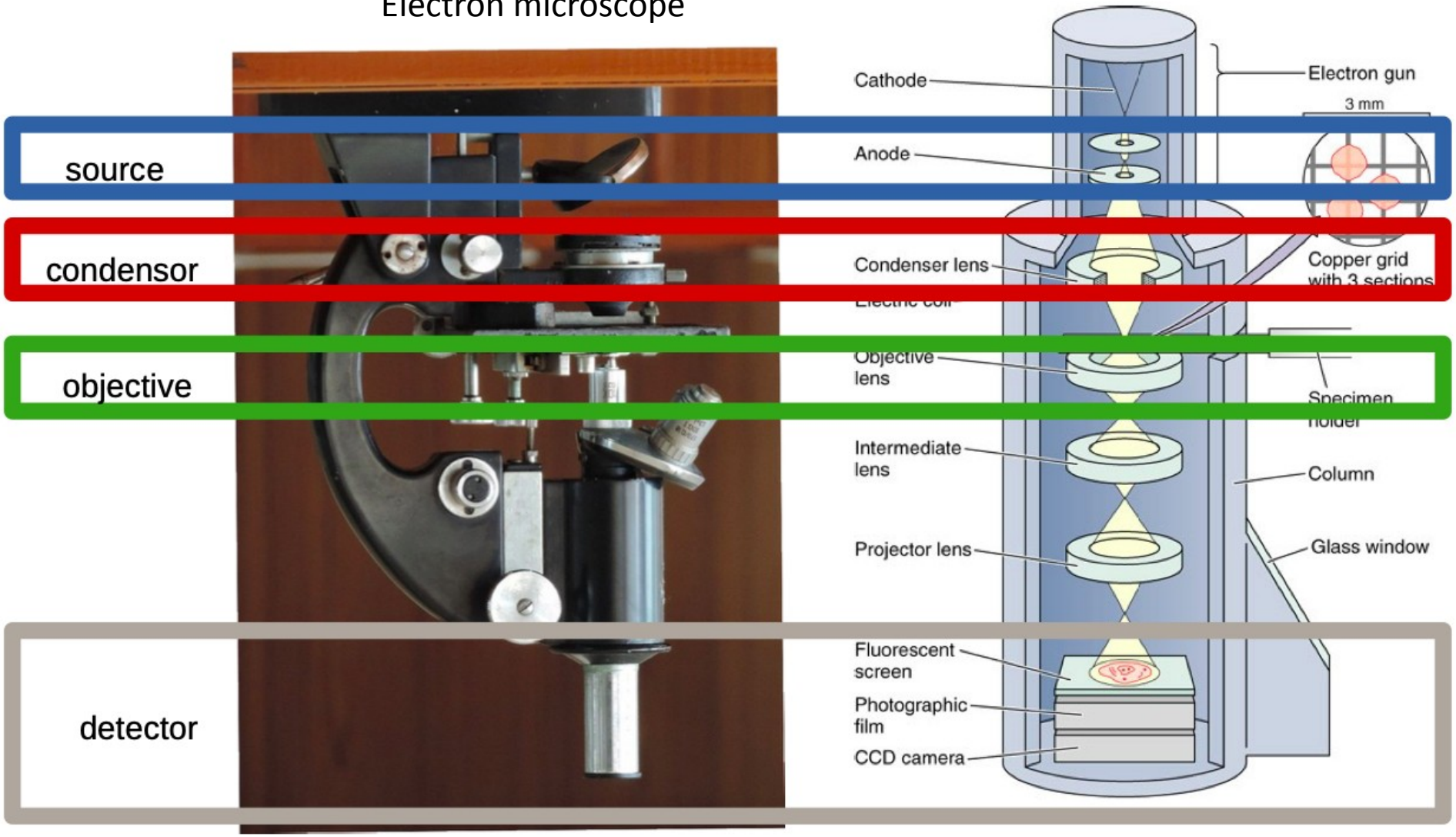
Optical microscope

Electron microscope

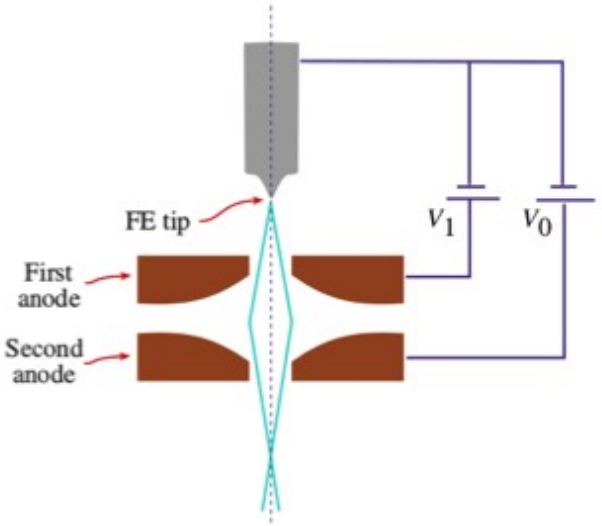


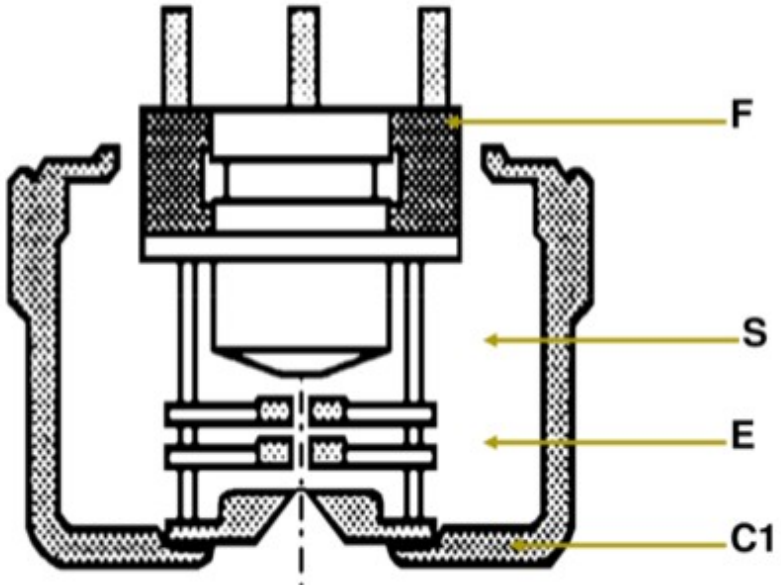
Optical microscope

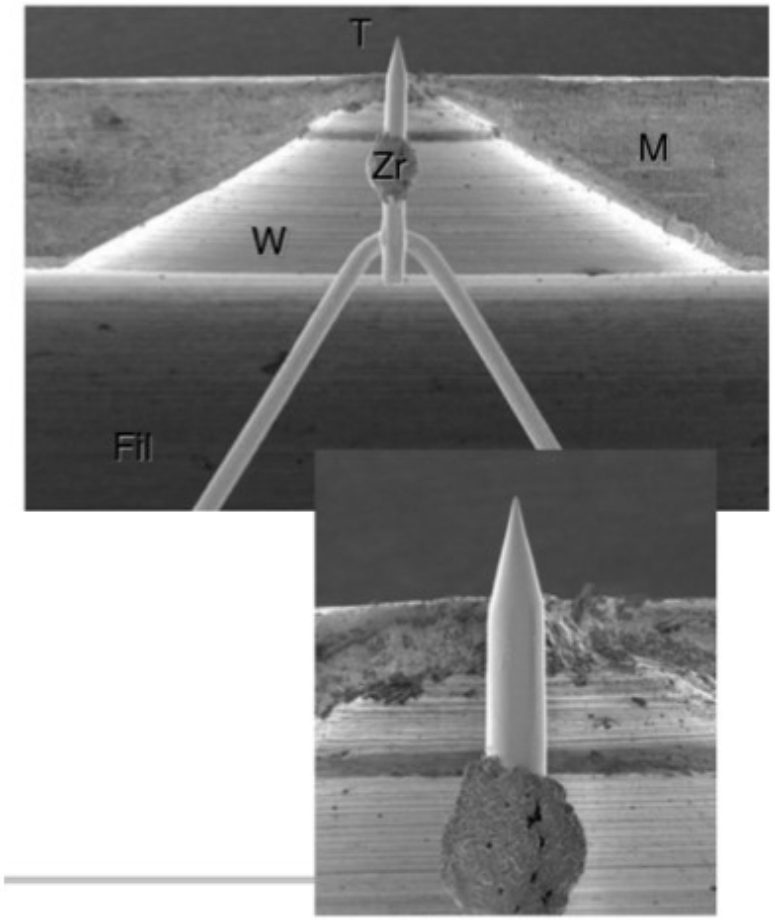
Electron microscope



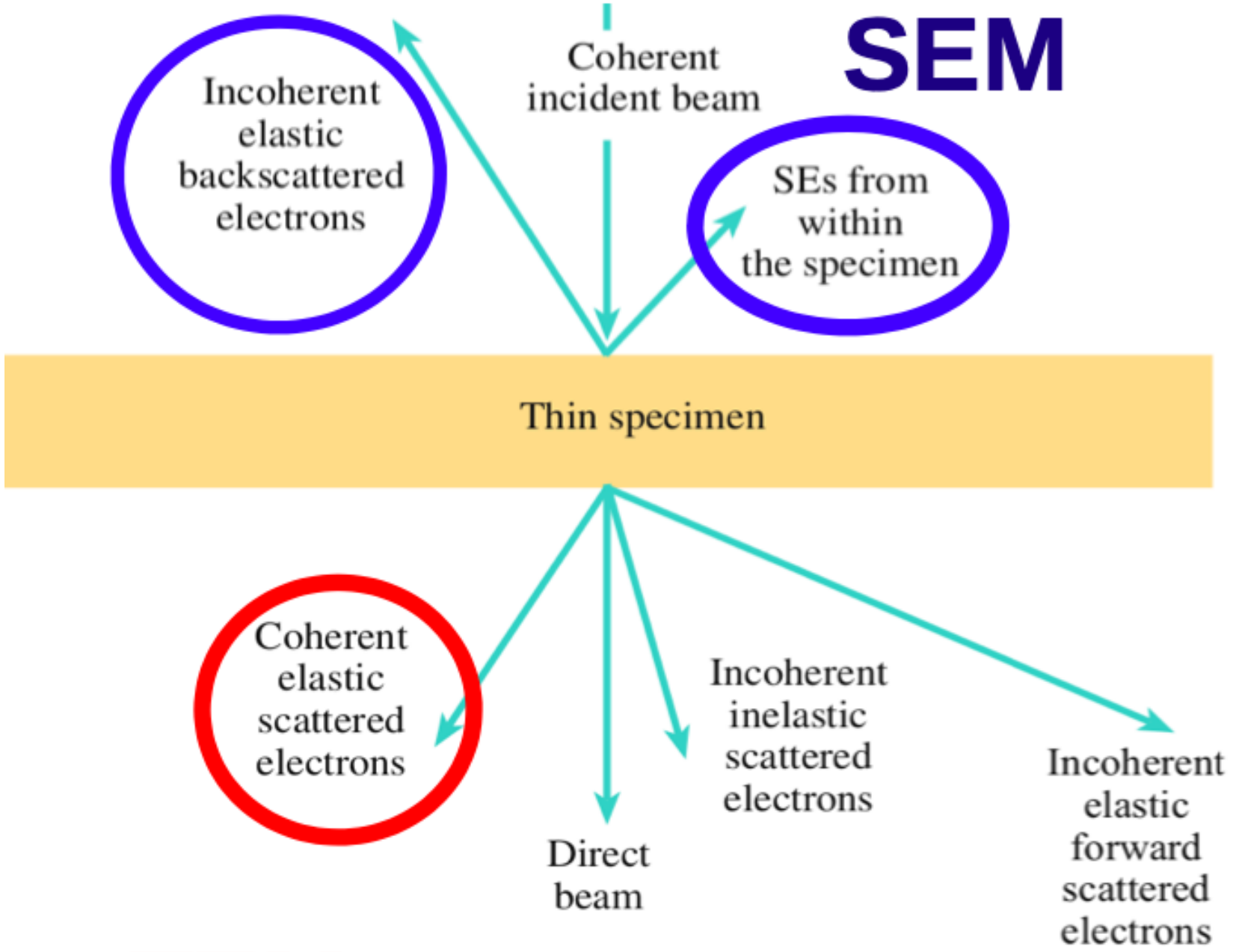
Electron source - FEG





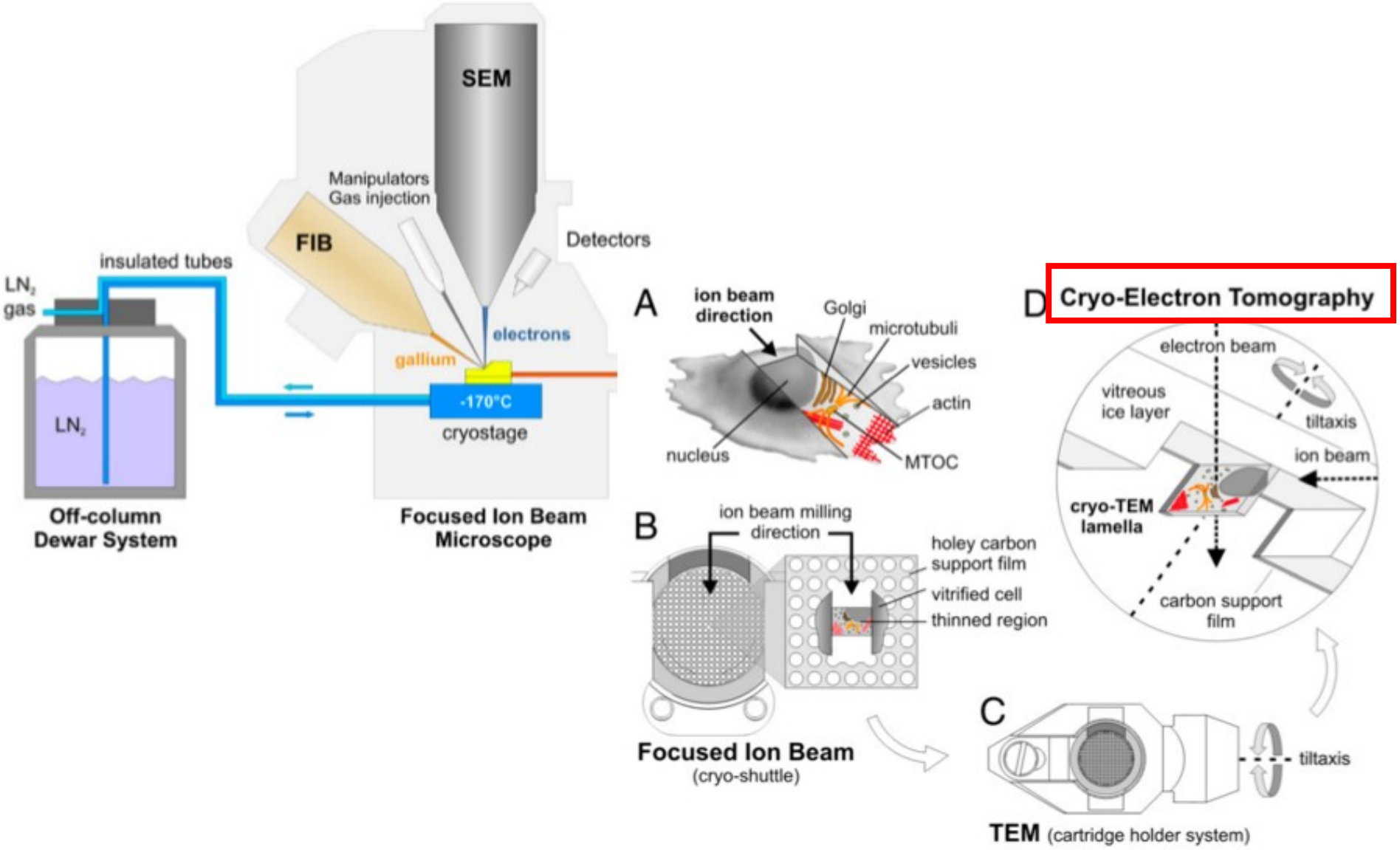


FEG - Field Emission Gun



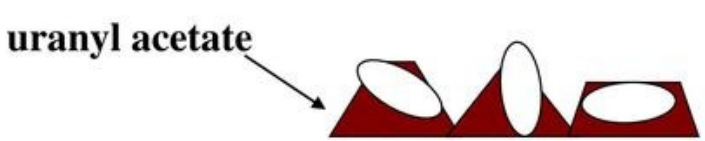
SEM – skenovací EM, TEM – transmisní/prozařovací EM

Focused Ion Beam (FIB) + SEM



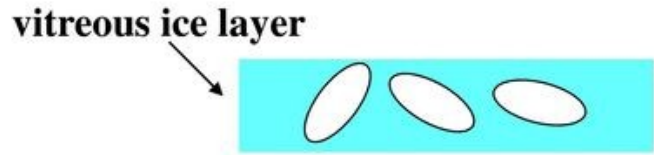
Negative Stain vs. Vitreous Ice

Specimen in Stain



- High contrast image
- No special temperature control
- Essentially no radiation damage
- Particle distorted
- Image = stain “shell” around the particle
- Low resolution method: 20-15 Å
- Great choice for initial sample screening

Cryogenic Specimen

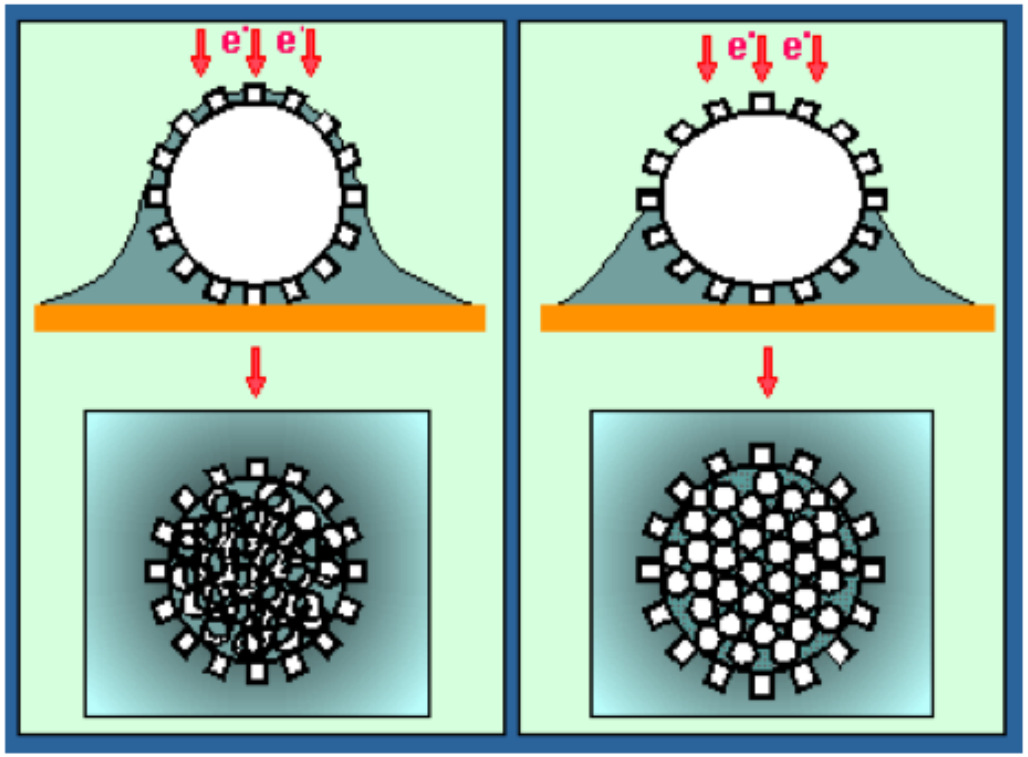


- Low contrast image
- Sample maintained at cryogenic temperature (85 K)
- High radiation damage
- Particle undistorted
- Image is of the actual particle
- Higher resolution obtained: 15-4 Å
- Best choice for reconstruction

test

TEM – Sample preparation

Negative staining

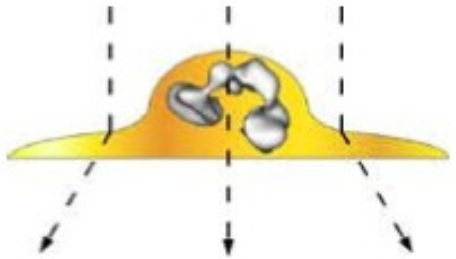


Pros: quick sample screening
high amplitude contrast
less prone to beam damage

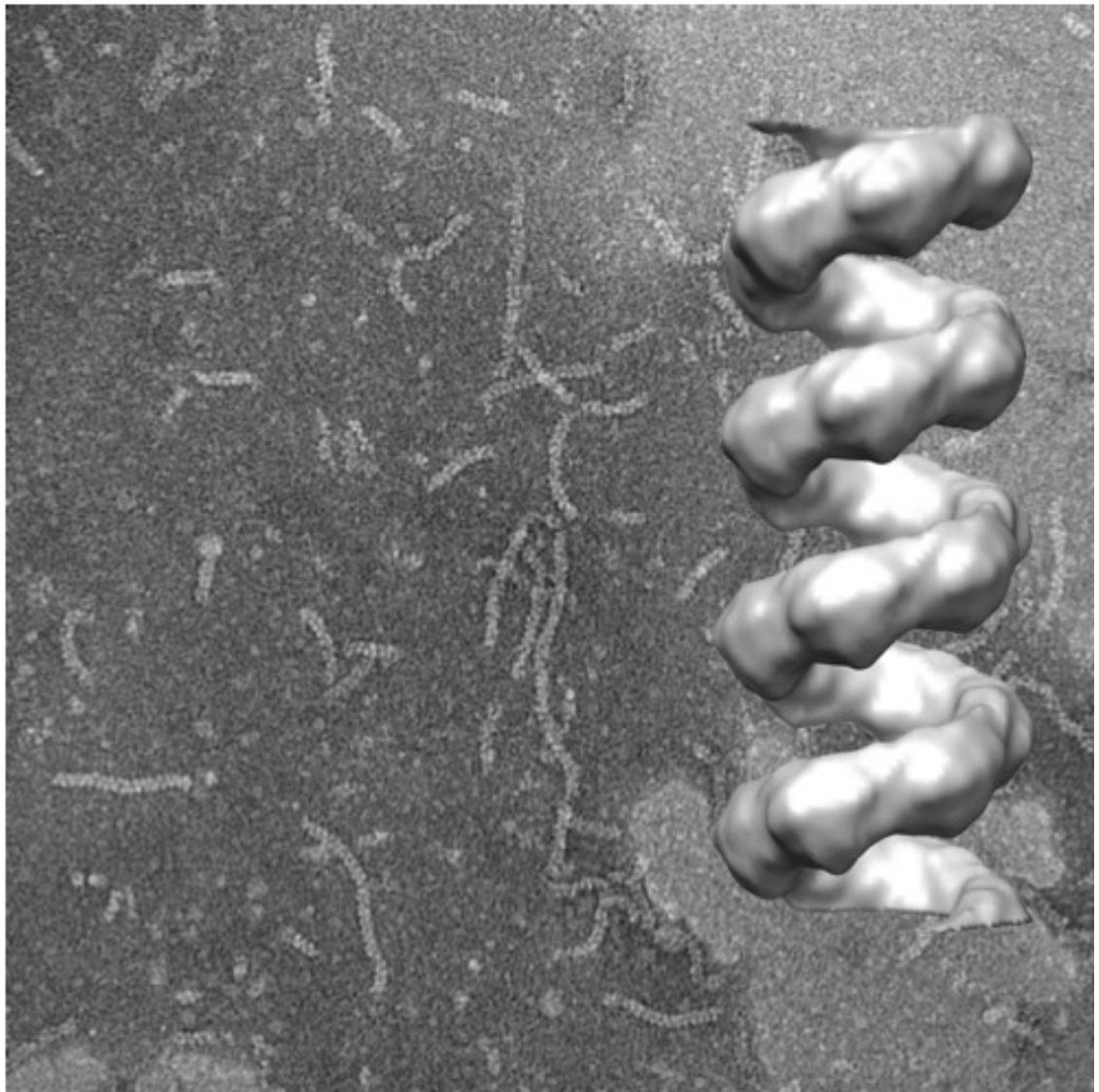
Cons: limited resolution (20A)
flattening artefacts
denaturation of proteins

Heavy metal staining

e.g. uranyl acetate

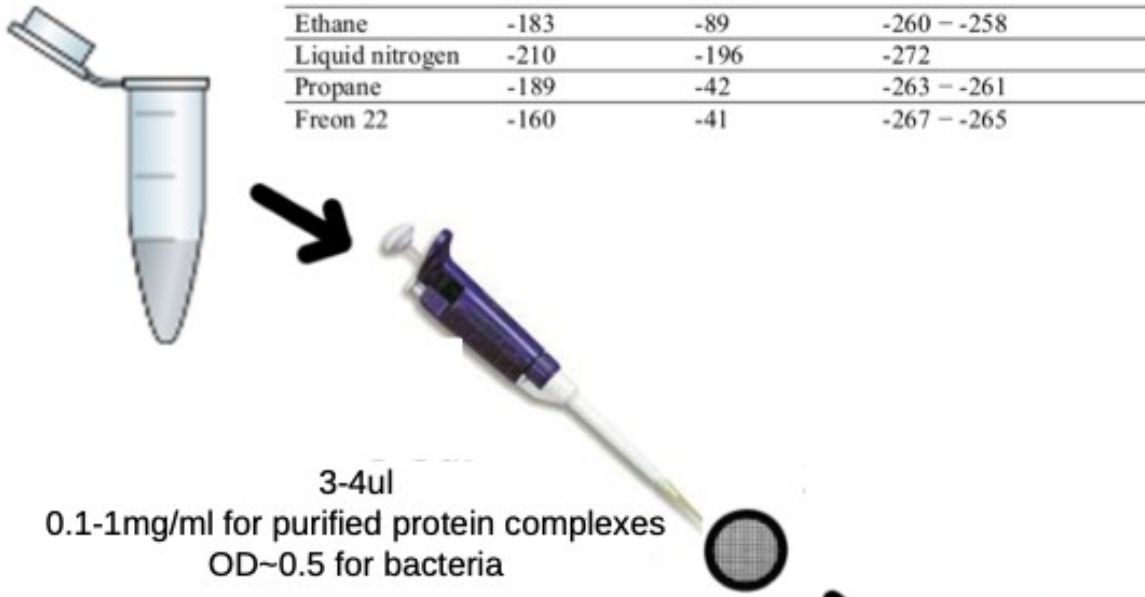


Negative staining

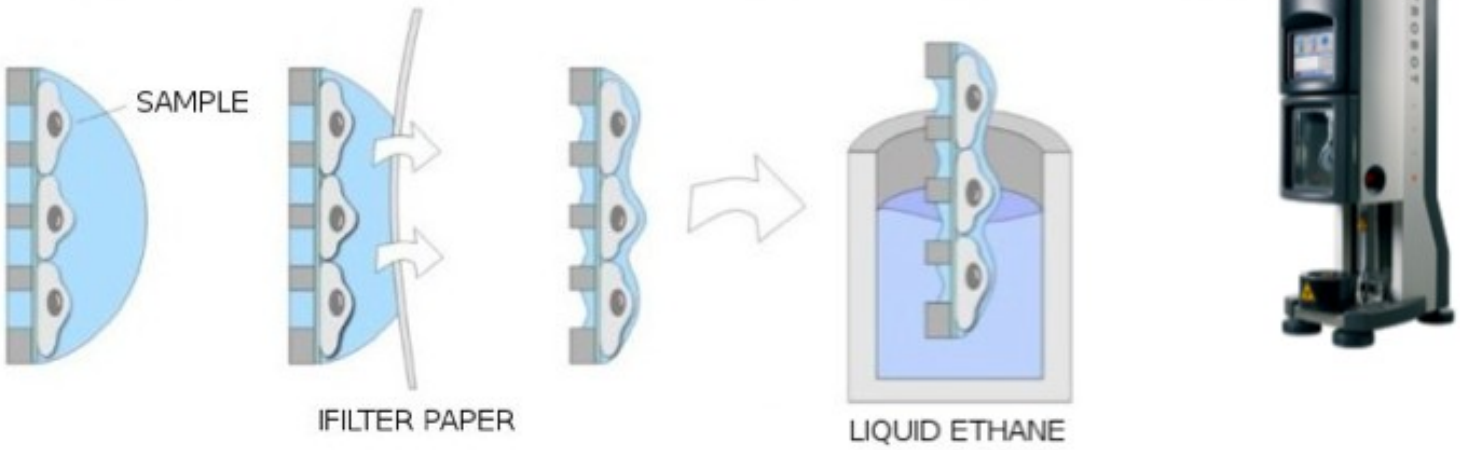


Sample vitrification

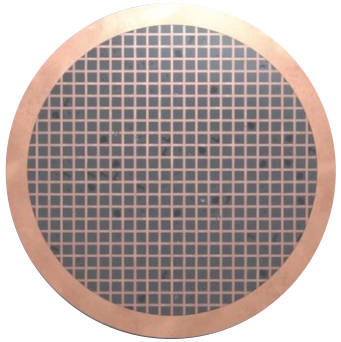
Cryogens	Melting point (°C)	Boiling point (°C)	Cooling rate (10^3 °C/s)	Relative cooling efficiency*
Ethane	-183	-89	-260 – -258	1.3
Liquid nitrogen	-210	-196	-272	0.1
Propane	-189	-42	-263 – -261	1.0
Freon 22	-160	-41	-267 – -265	0.7



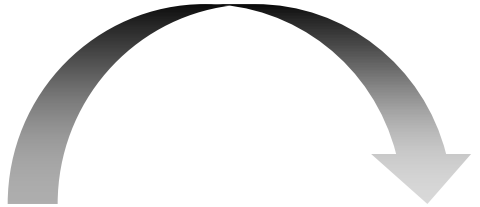
3-4ul
0.1-1mg/ml for purified protein complexes
OD~0.5 for bacteria



Grid

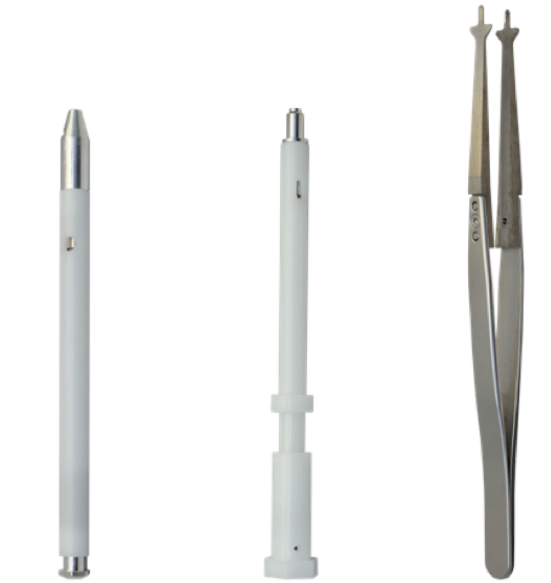


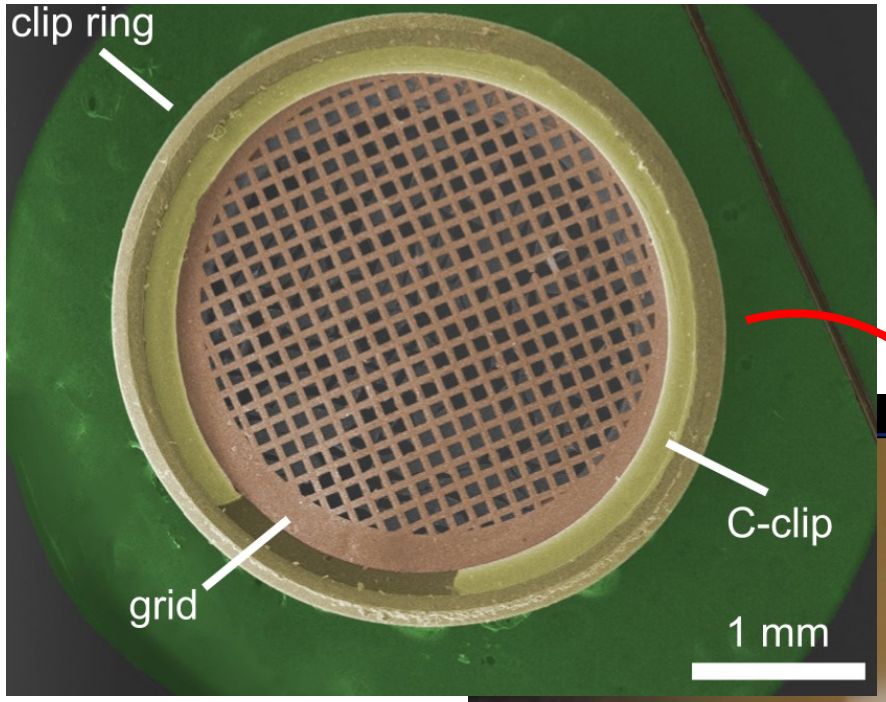
C-clip



AutoGrid

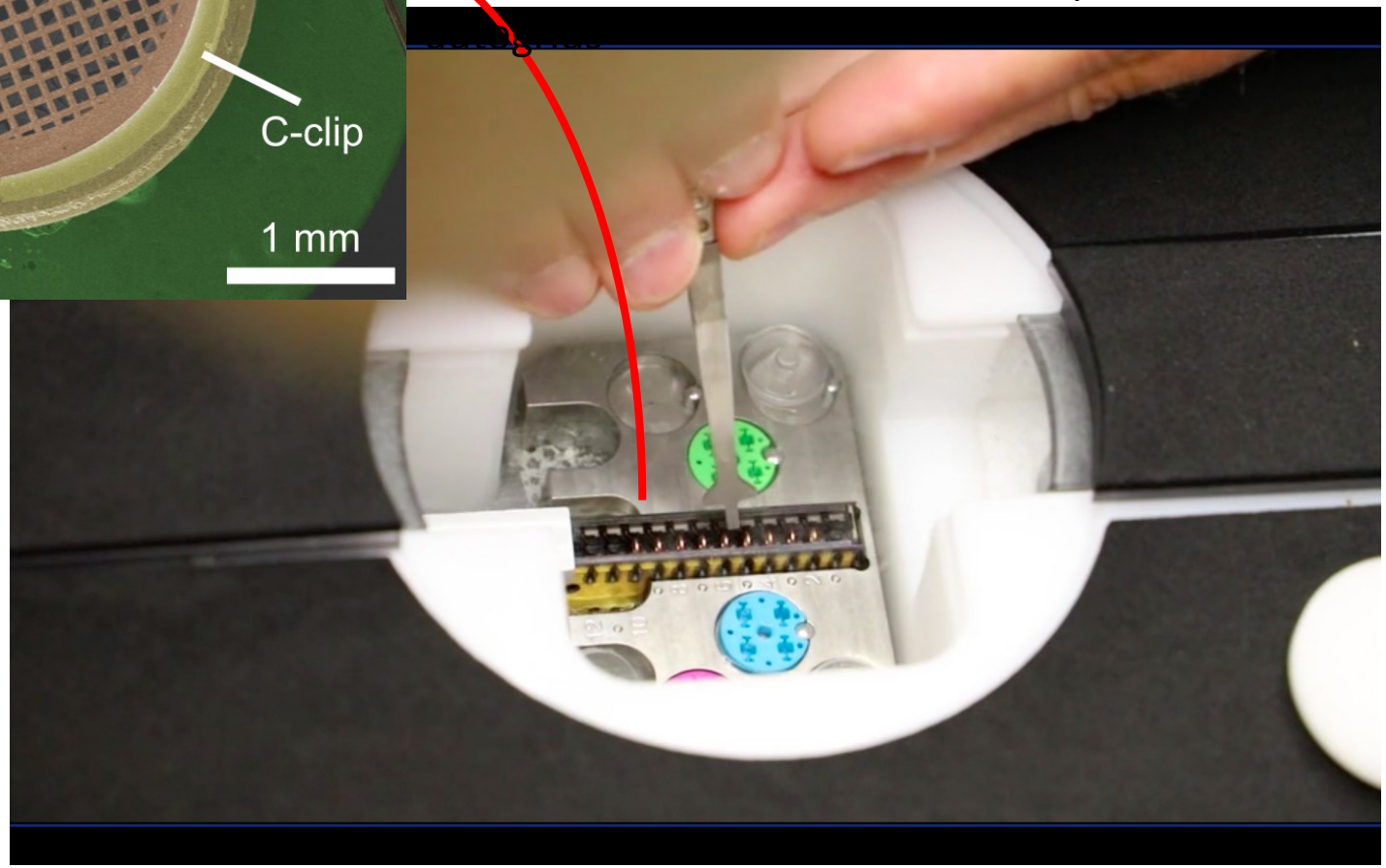
ThermoFisherScientific


Grid Box Tool
Clipping Tool
Autogrid Tweezer



Autogrid:
grid + C-shape spring (C-clip) + clip ring

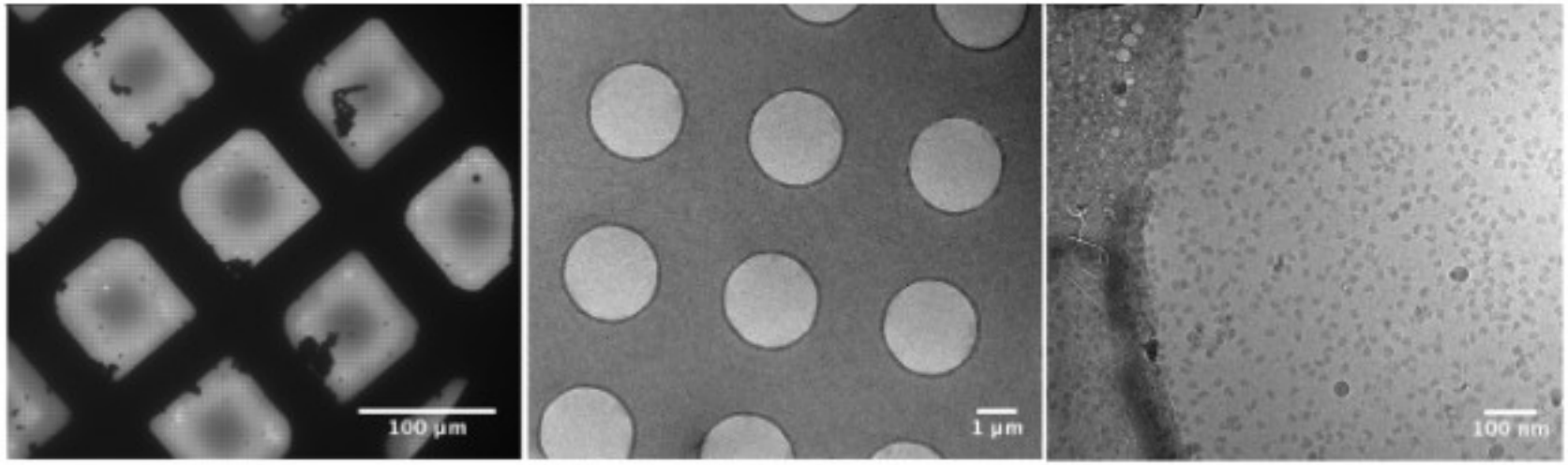
Auto-loader: up to 12

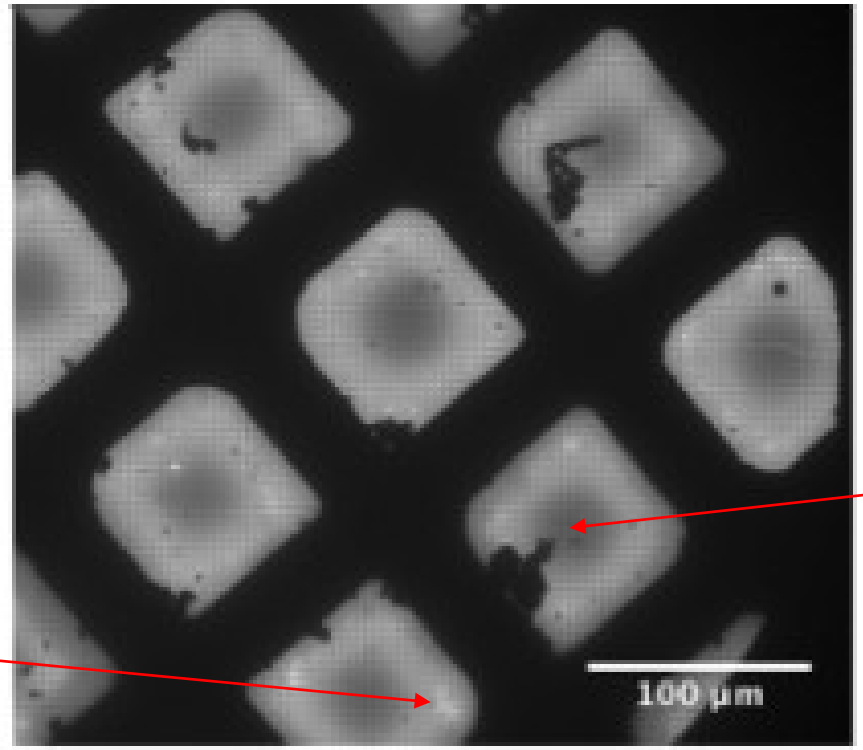


cryoem101.org

AutoGrid Container

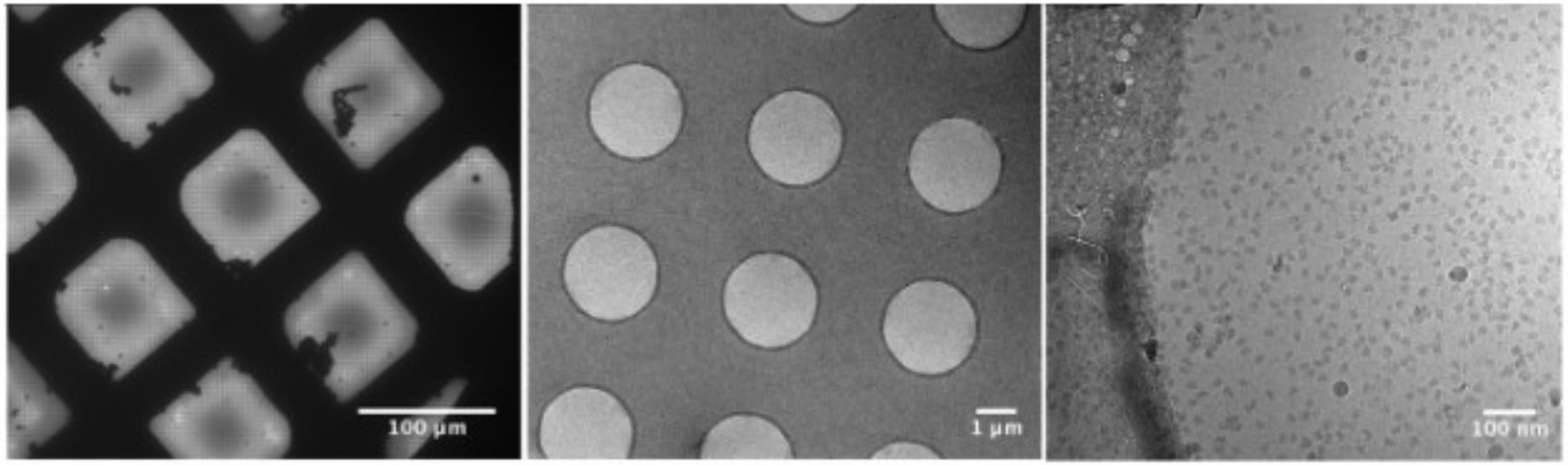
ThermoFisherScientific; MiTeGen

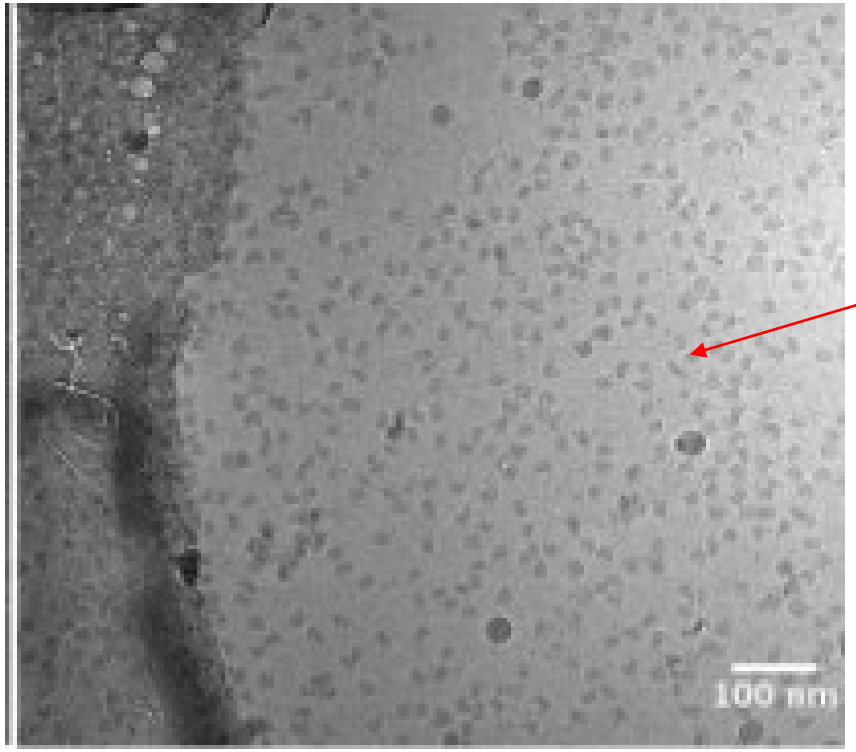




Note the ice thickness

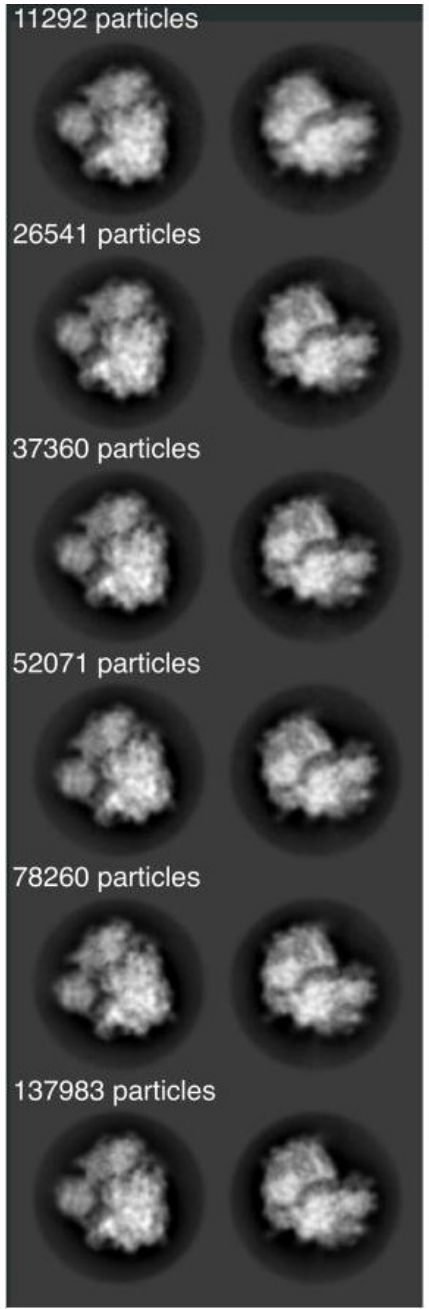
Grids get damaged

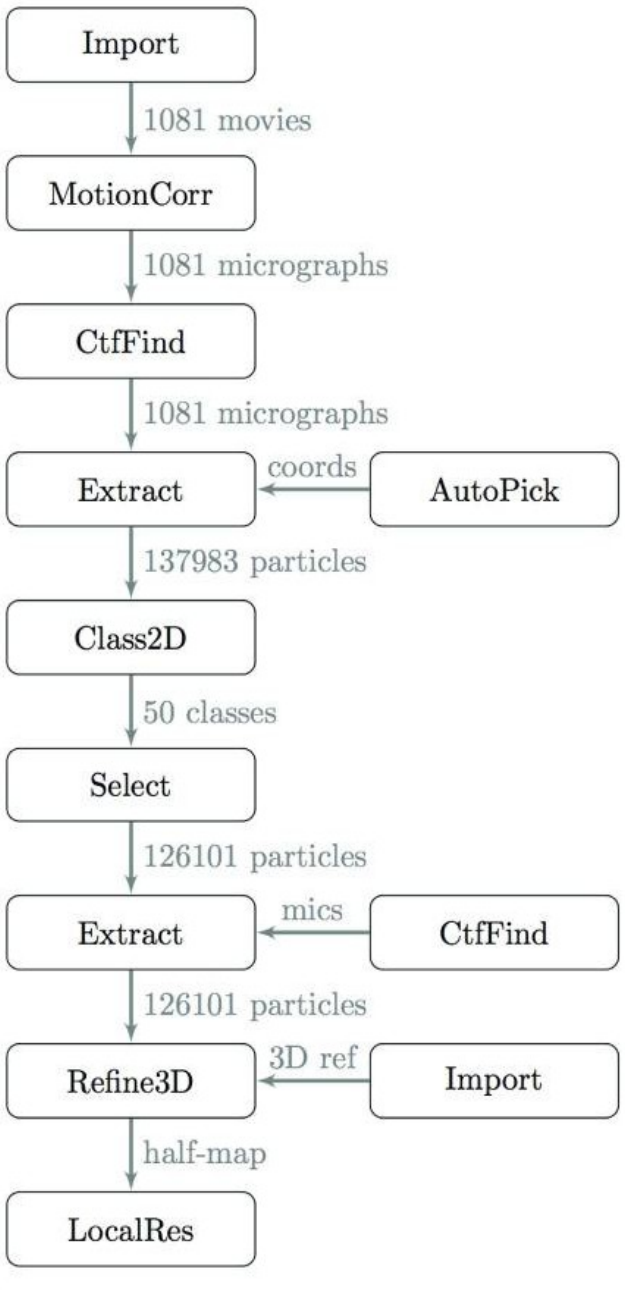


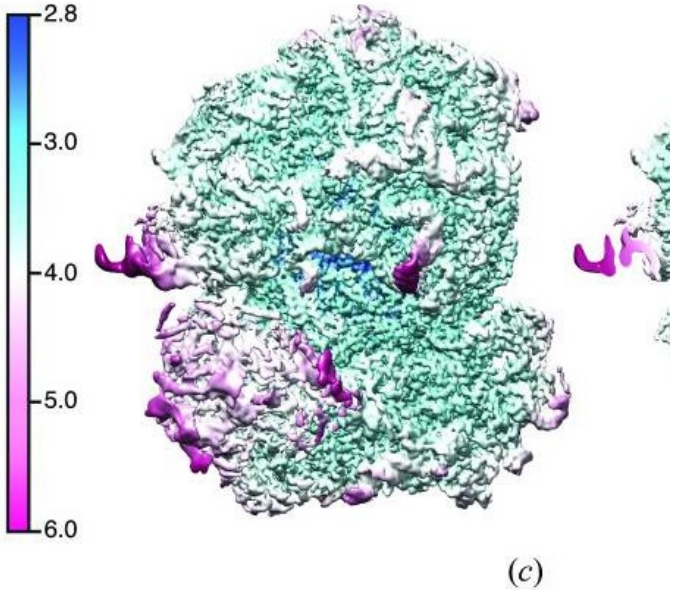


single particles

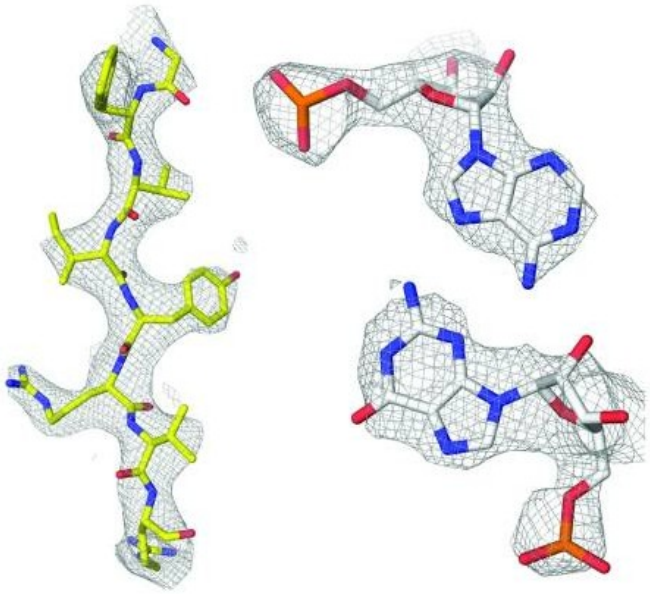
Structure determination of biomolecules using TEM







(c)



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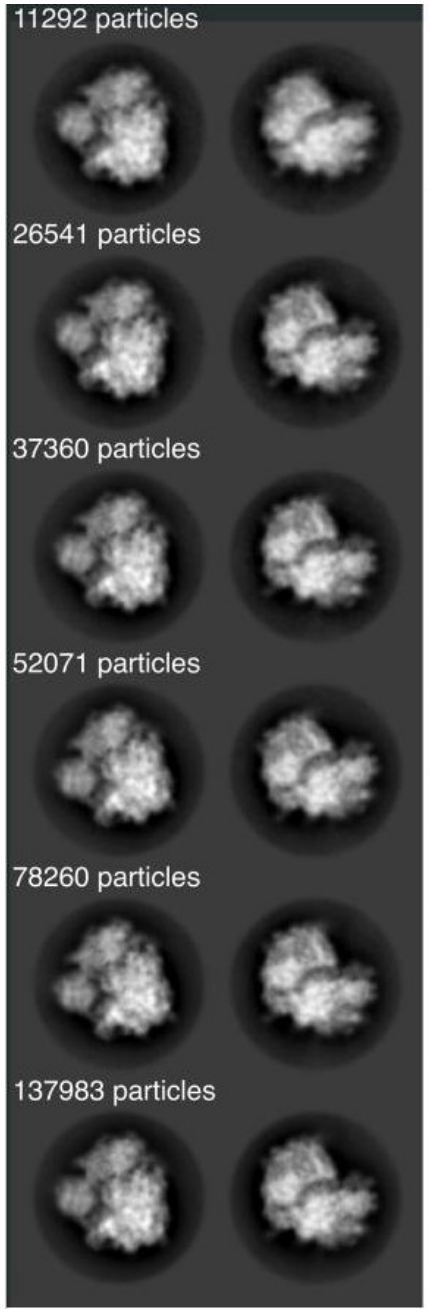

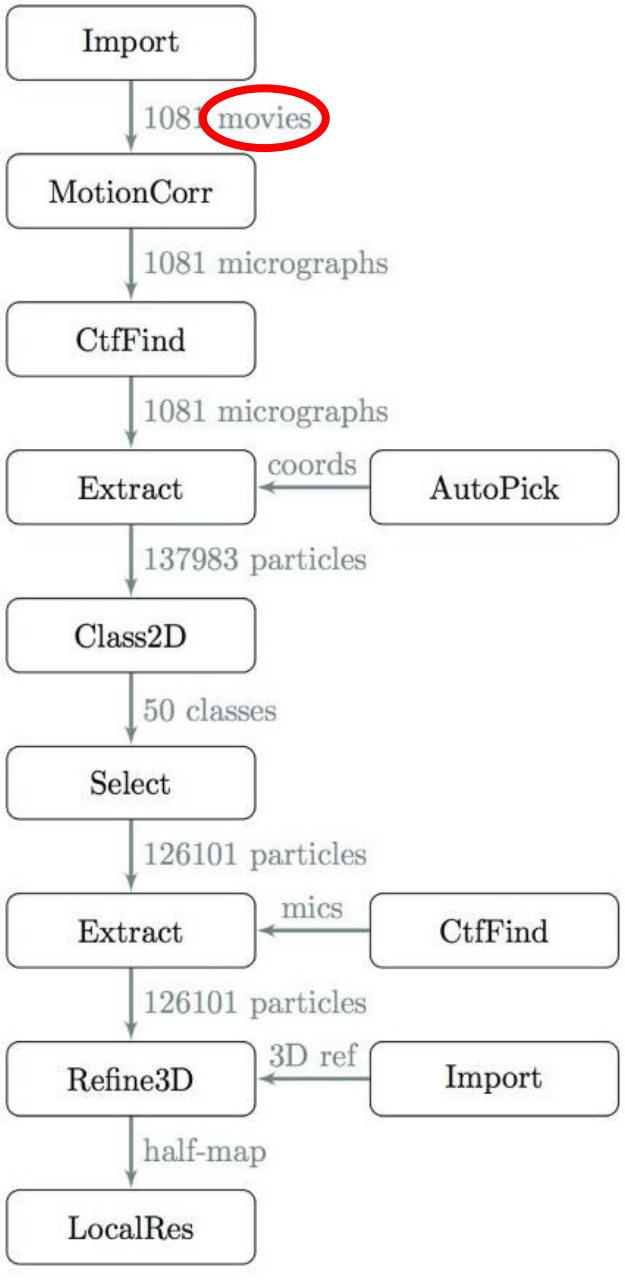

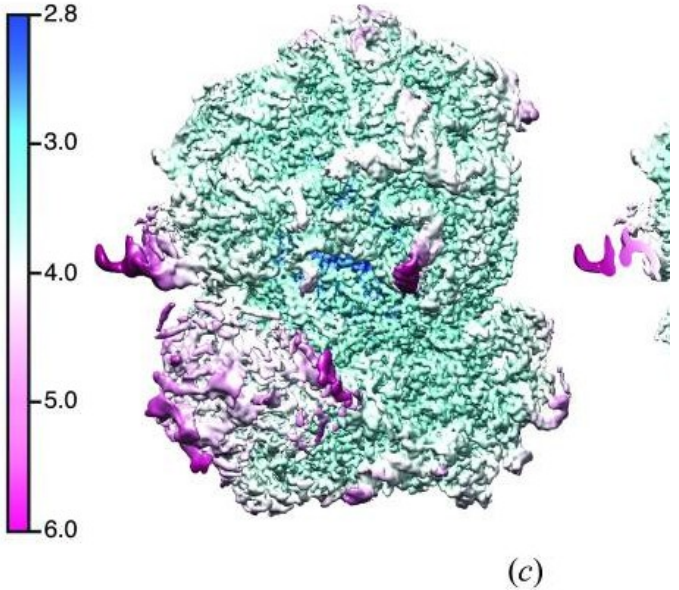

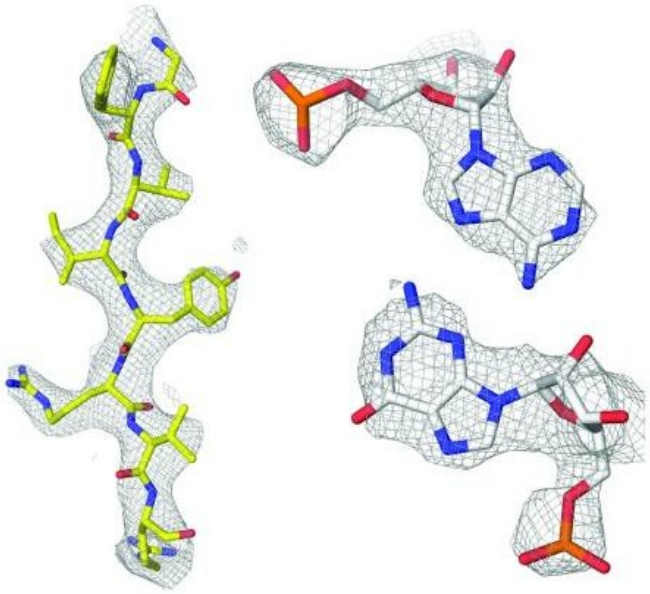

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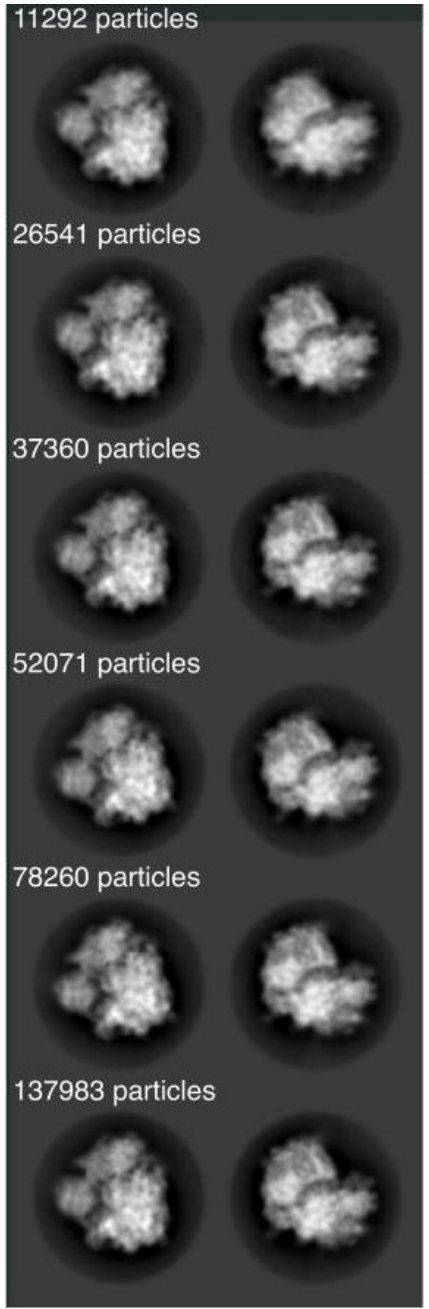
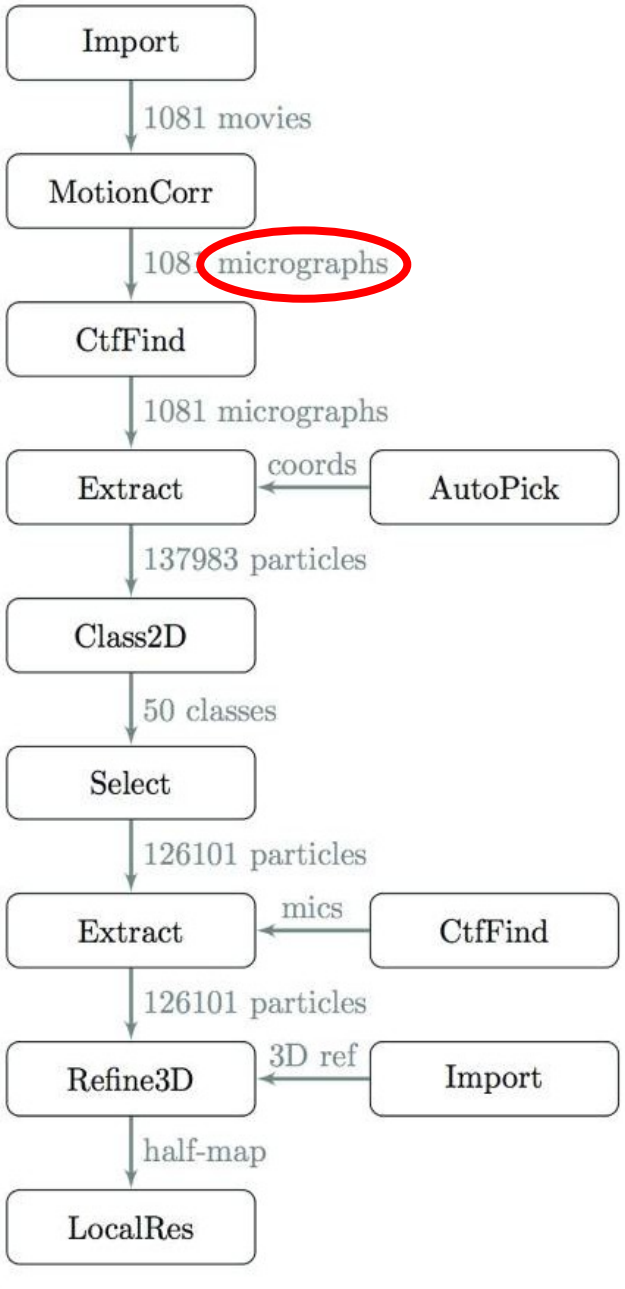
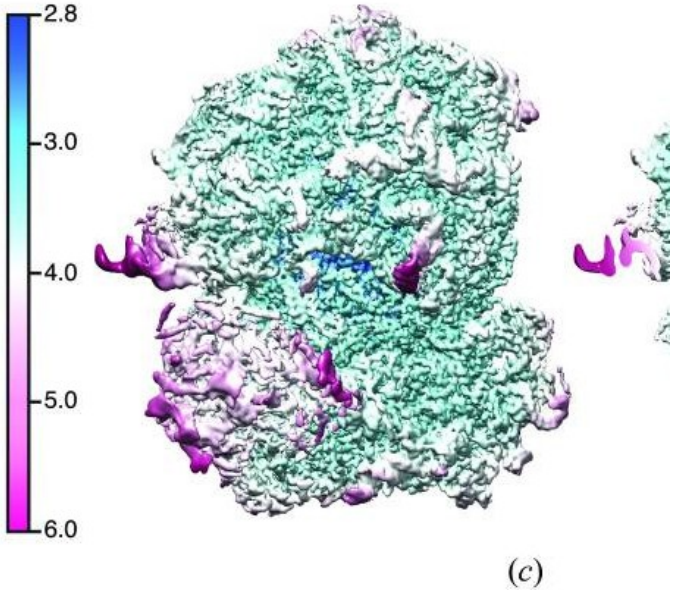
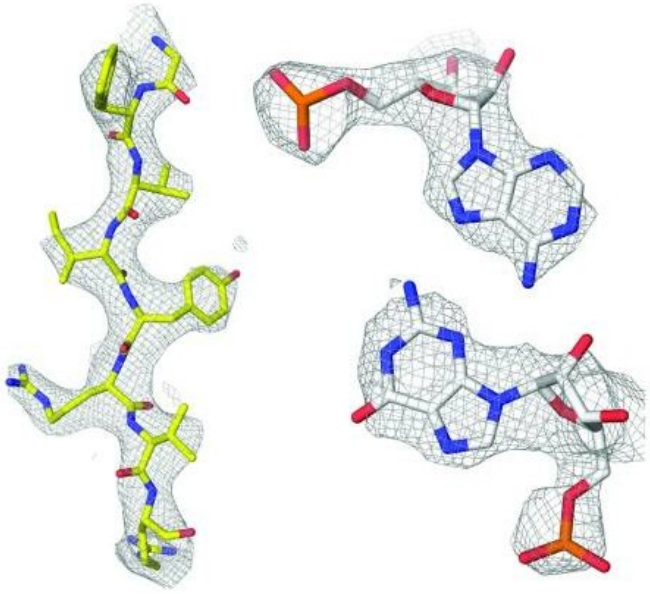
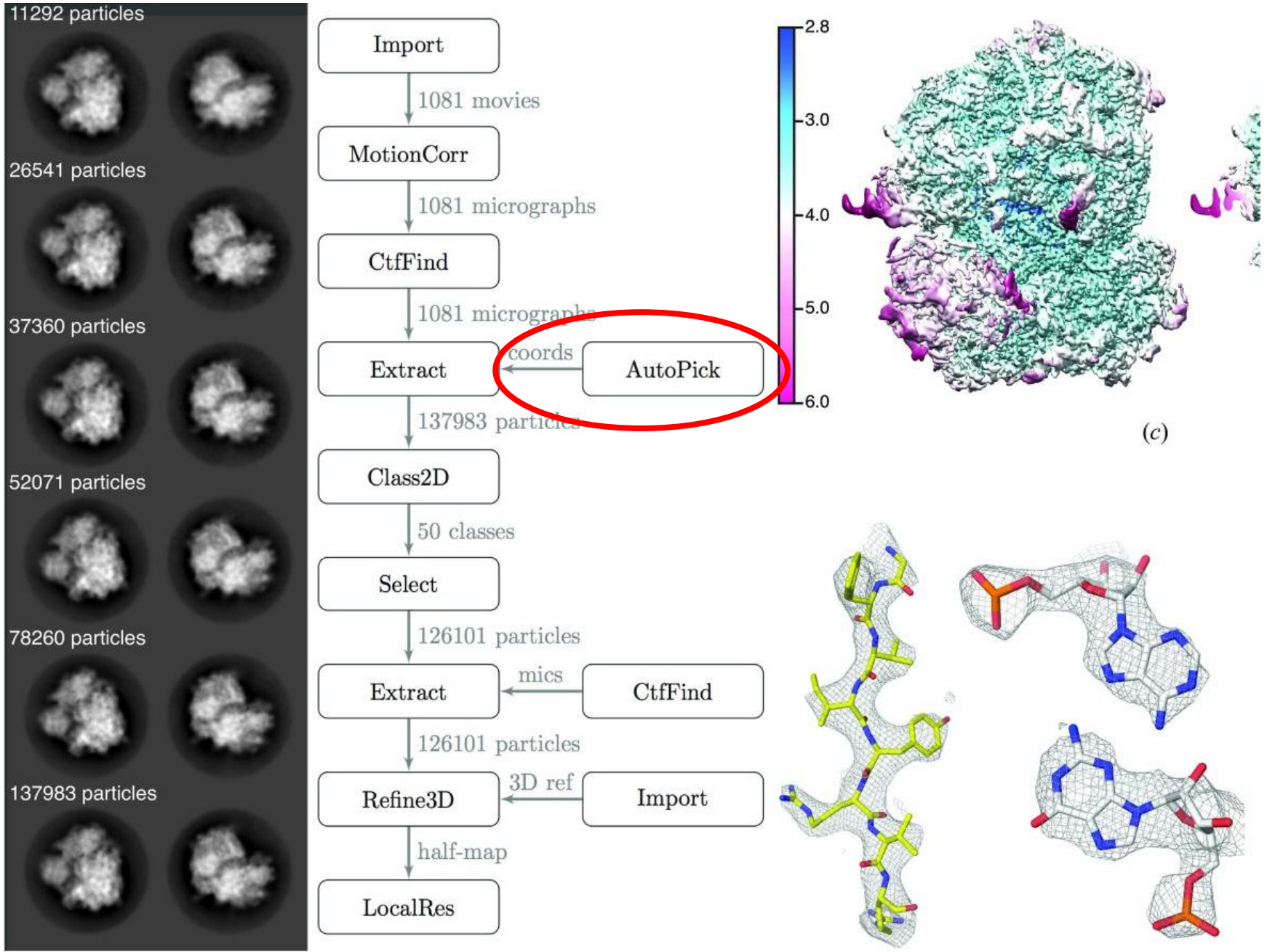






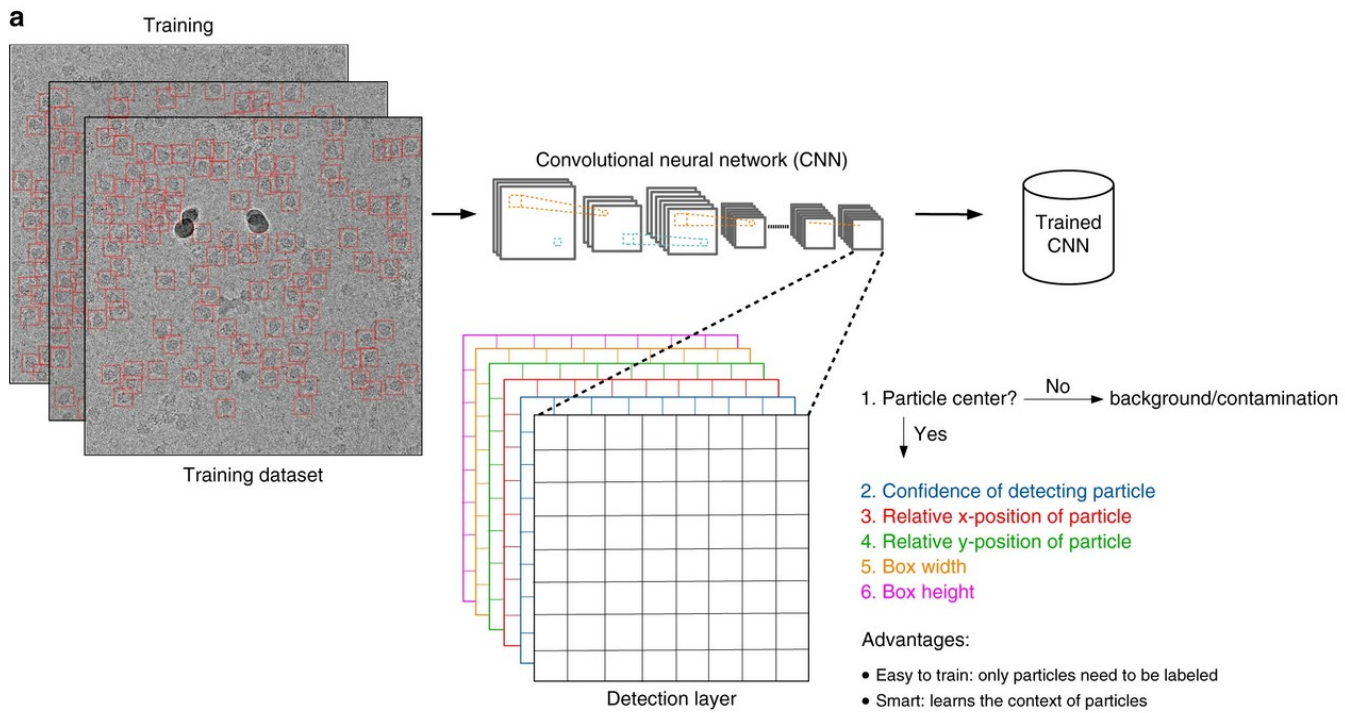


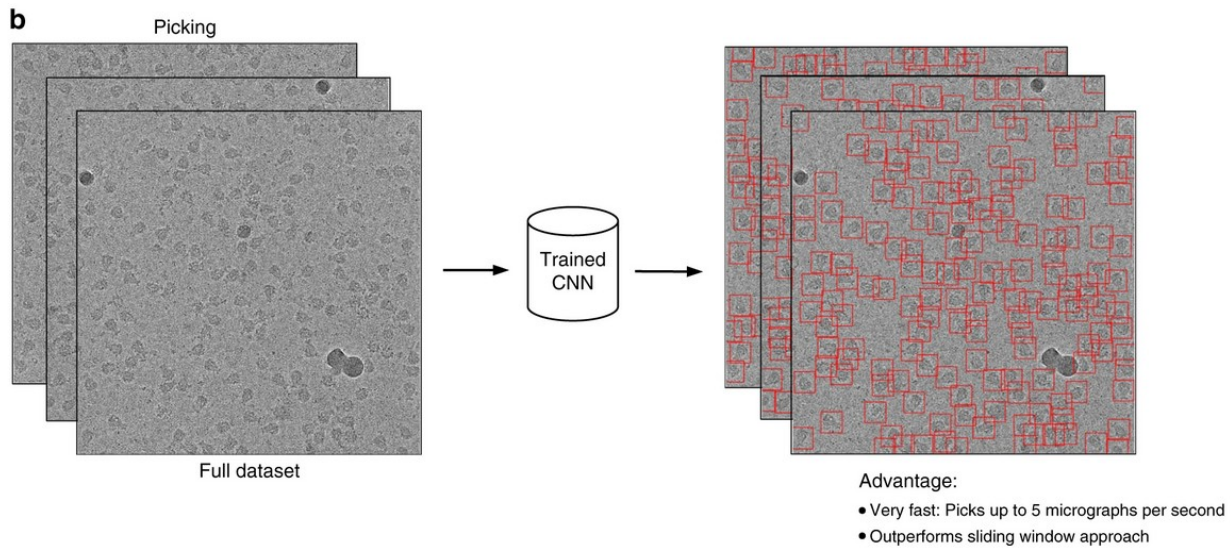
Structure determination of biomolecules using TEM



crYOLO - an application for fast and accurate cryo-EM particle picking

You
Only
Look
Once





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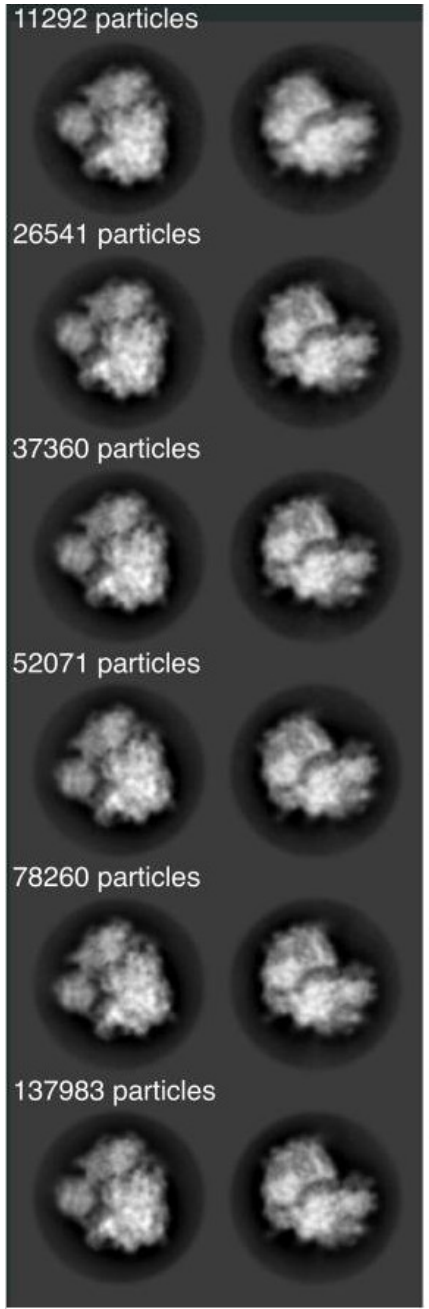
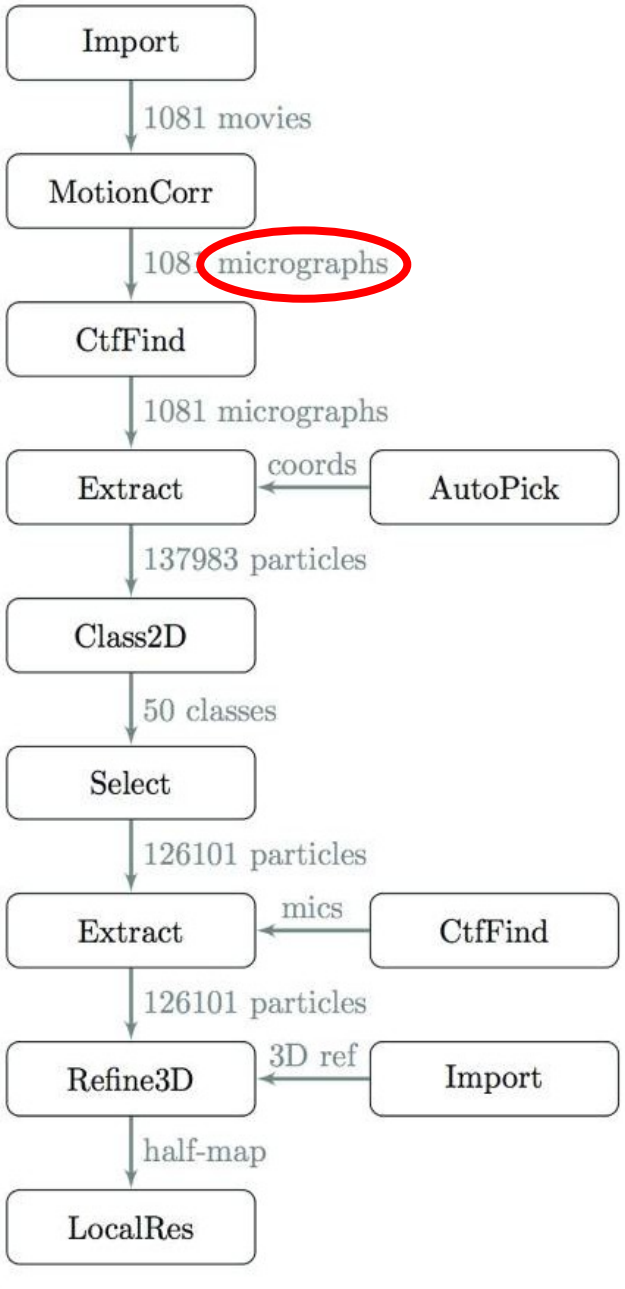
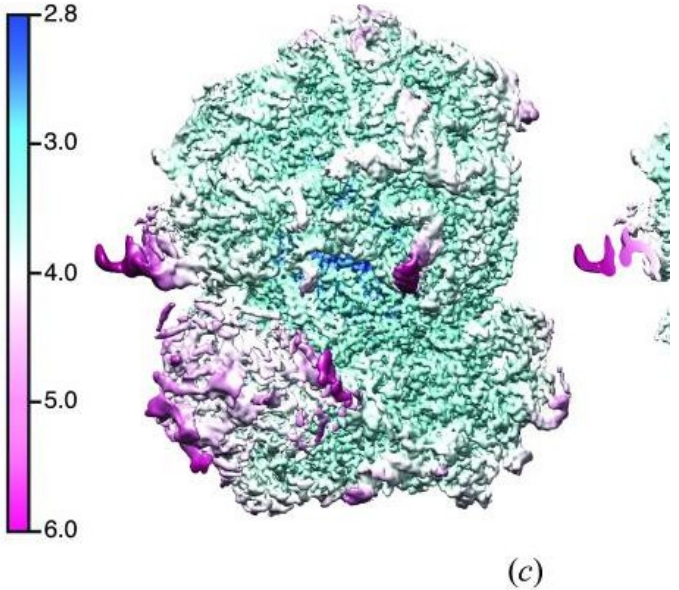
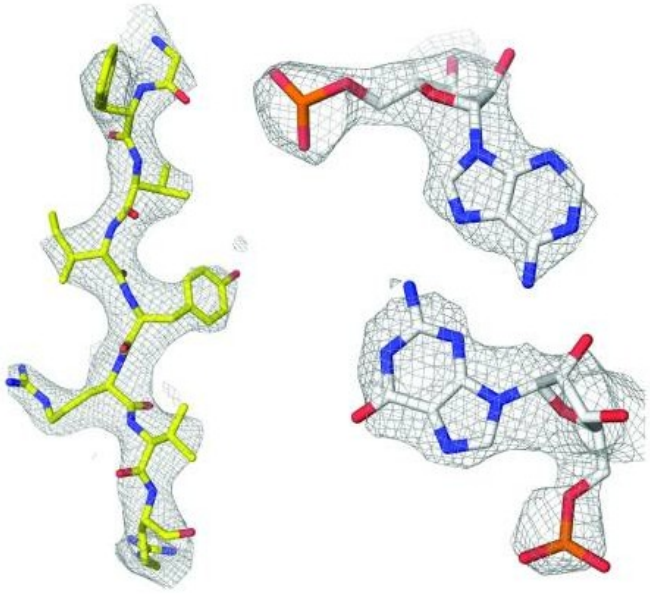


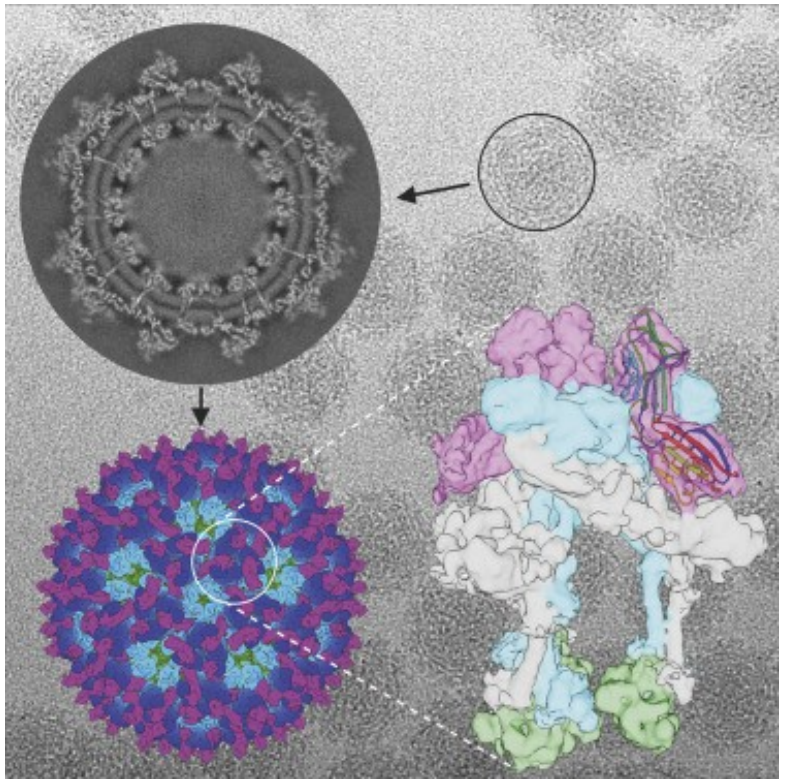


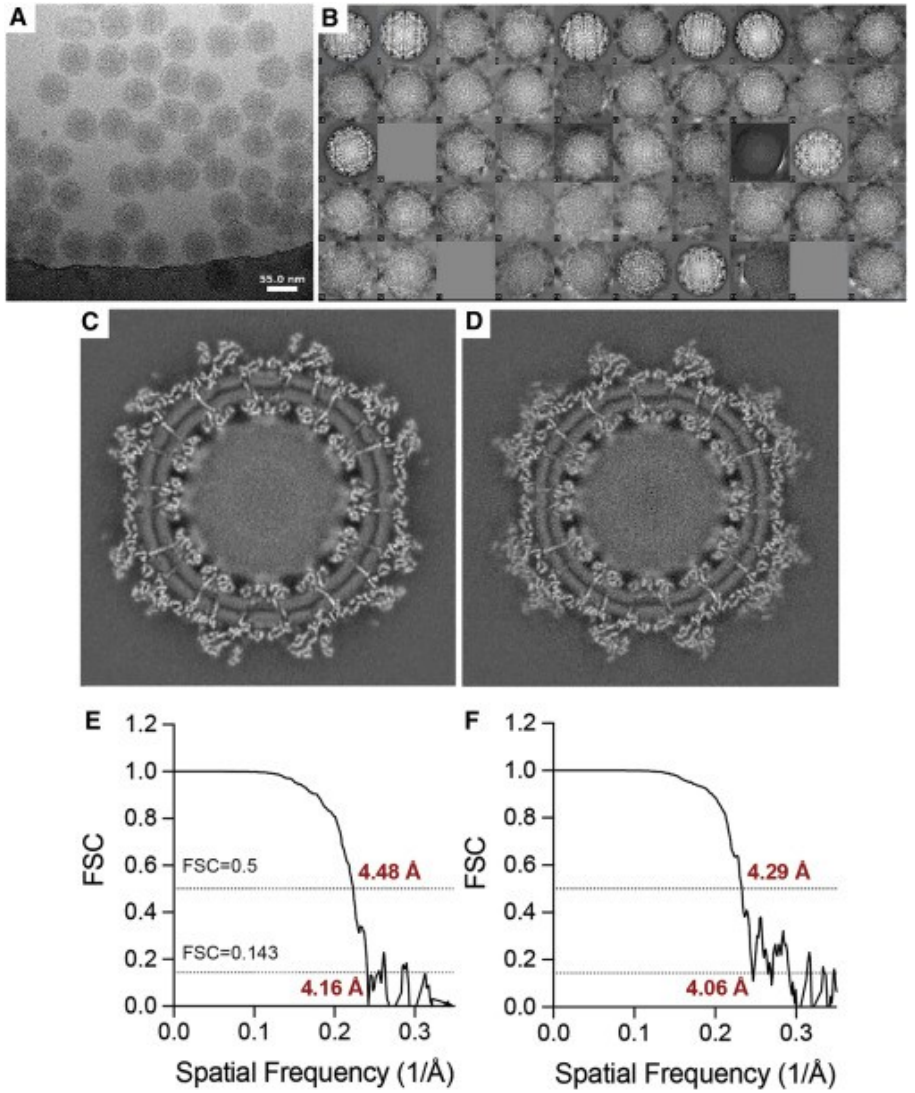




Structure determination using single particle cryoEM

1) Sample preparation – 2) vitrification – 3) measurement – 4) particle picking – 5) 2D classification – 6) 3D model reconstruction





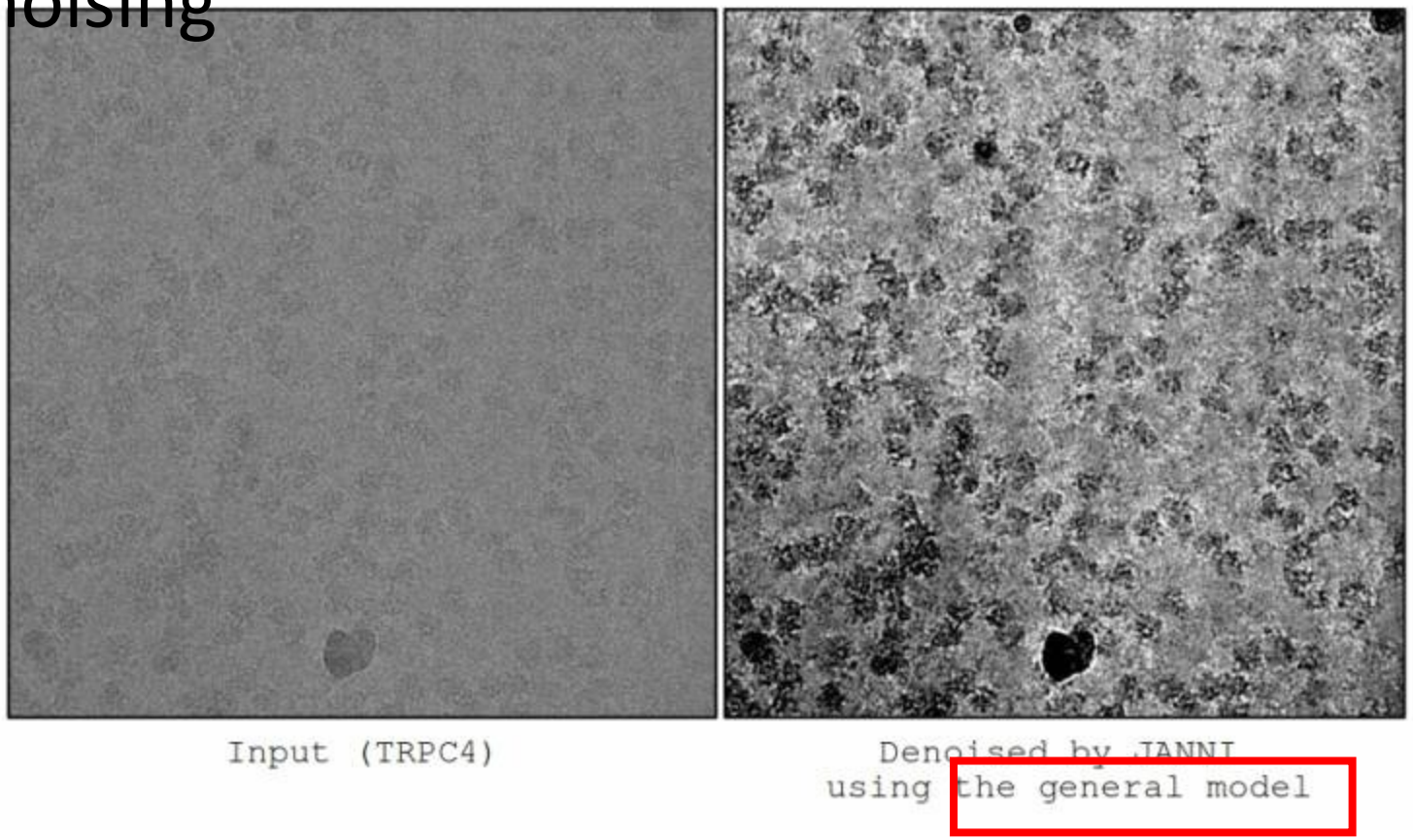
https://doi.org/10.1016/j.cell.2019.04.006

Micrographs processing

1) Denoising
    i. Just Another Noise 2 Noise Implementation (JANNI)
    ii. TOPAZ

2) Lowpass filtering

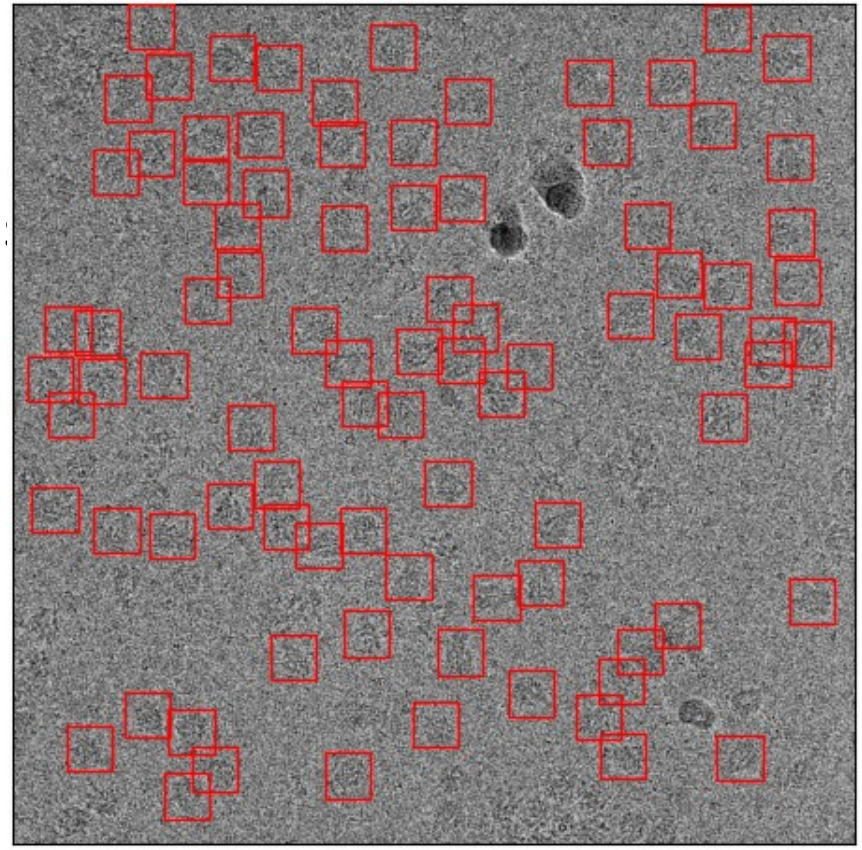
Original data* After denoising



*each micrograph is average of a movie of ~40..60 frames, motion corrected and dose-weighted

Particle picking

1. Initial Manual Picking (cyYOLO, Relion, Topaz, cryoSPARC …)
    i. Selected micrographs (10-100)
    ii. Fully picked micrographs (80-100 % of particles present)
2. Model Training
3. Full Dataset Picking
    (X00.000 – X.000.000 particle



Particle Extraction

i) Creates separate image for each individual picked particles

ii) Large particles can be reduced by so called binning
=> speeds up initial calculations

iii) Once dataset is “cleaned” and only ”good” particles selected, calculations are repeated using full-scale particles

2D Classification

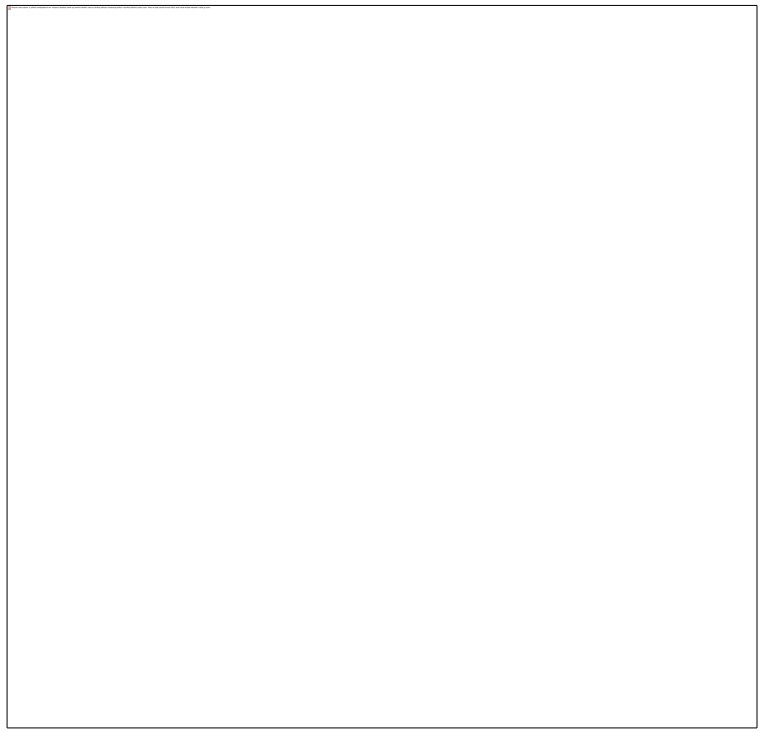
i) .**alignment** (a translation and an in-plane rotation) to map one image onto another

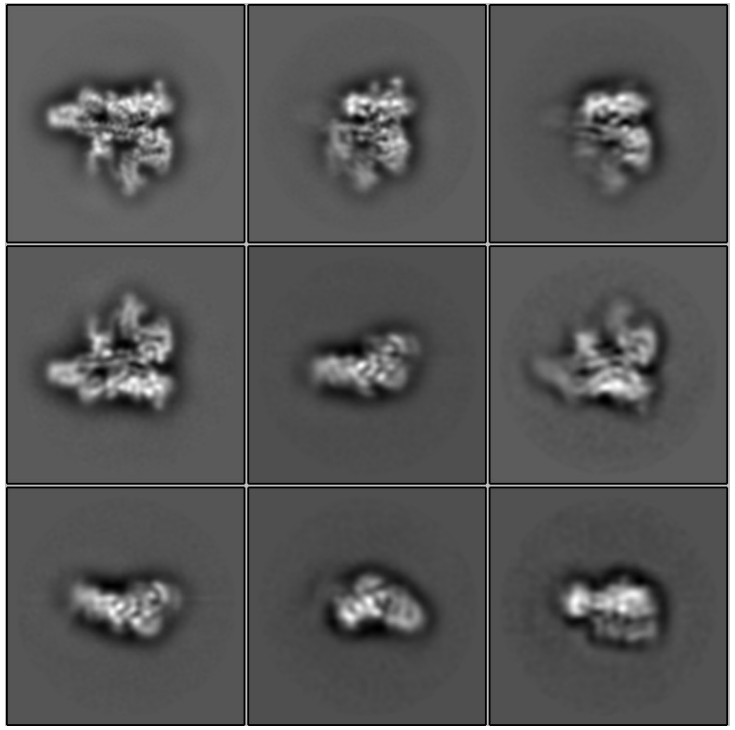
ii) images have high noise relative to the signal of particles

iii) aligning several similar images to each other then averaging them => image with higher signal to noise ratio

iv) the **noise is mostly randomly distributed** and the underlying **image features constant**

v) averaging the intensity of each pixel over several images only the constant features are reinforced.





2.995.892 particles picked from **13.807** micrographs using **cryoSPARC template picker**

718.639 particles selected after **2D classification**

3D Reconstruction

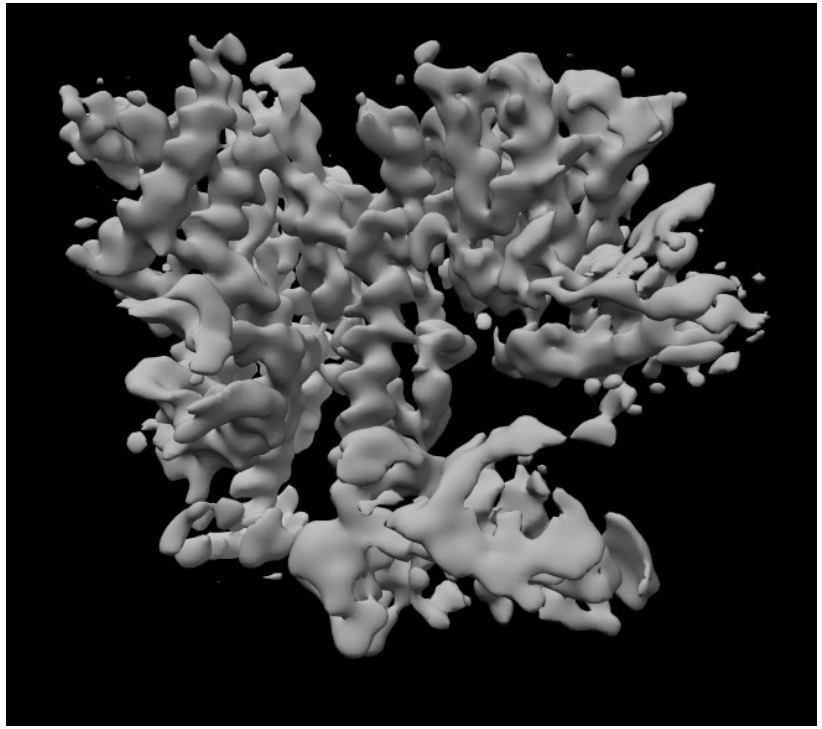
i) Initial model

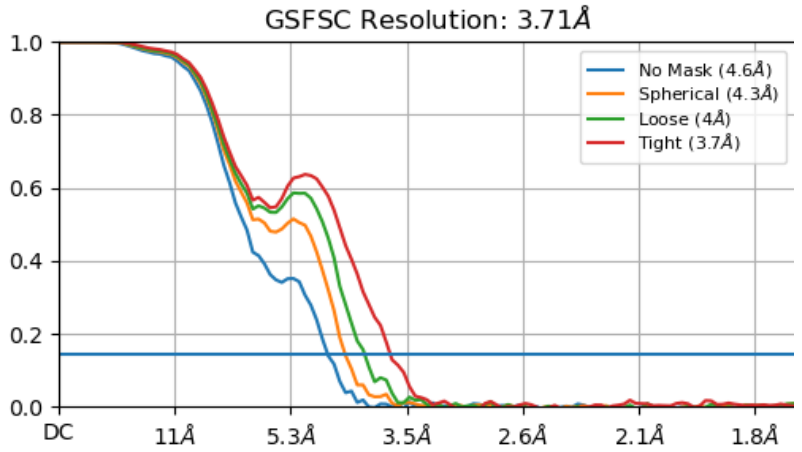
ii) 3D model reconstruction

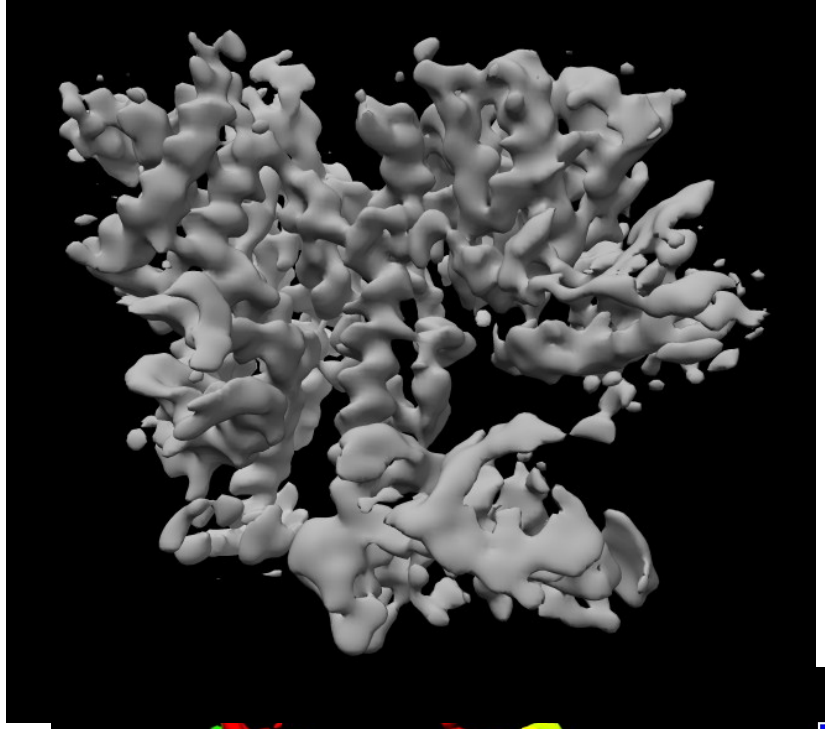
=> only a 3D electron density map will be provided

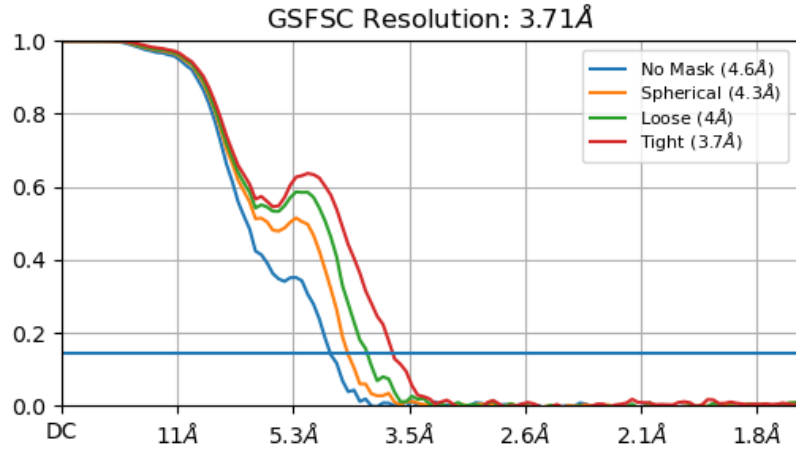
3D structure reconstruction is another (tedious) work:

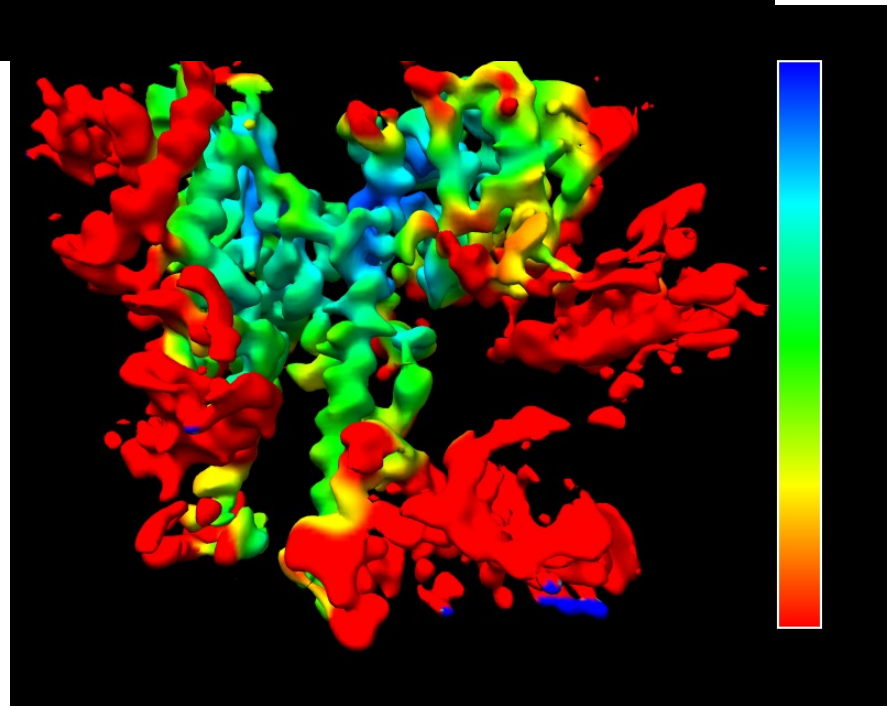
1. rigid body docking
2. manual model building (semi-automatic) – Coot, Isolde …

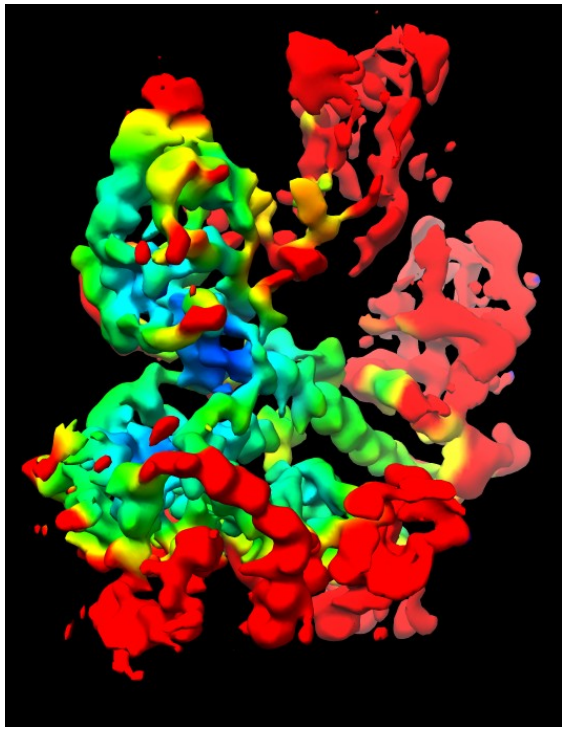



GSFSC Resolution: 3.71Å
1.0
0.8
0.6
0.4
0.2
0.0
DC
11Å
5.3Å
3.5Å
2.6Å
2.1Å
1.8Å
No Mask (4.6Å)
Spherical (4.3Å)
Loose (4Å)
Tight (3.7Å)




GSFSC Resolution: 3.71Å
No Mask (4.6Å)
Spherical (4.3Å)
Loose (4Å)
Tight (3.7Å)
1.0
0.8
0.6
0.4
0.2
0.0
DC
11Å
5.3Å
3.5Å
2.6Å
2.1Å
1.8Å

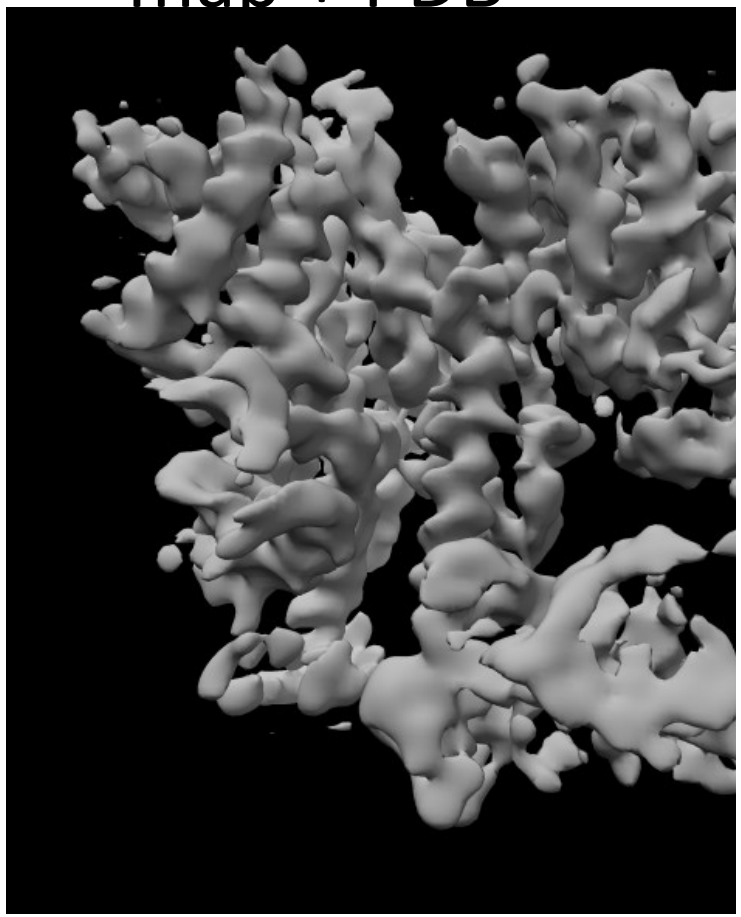


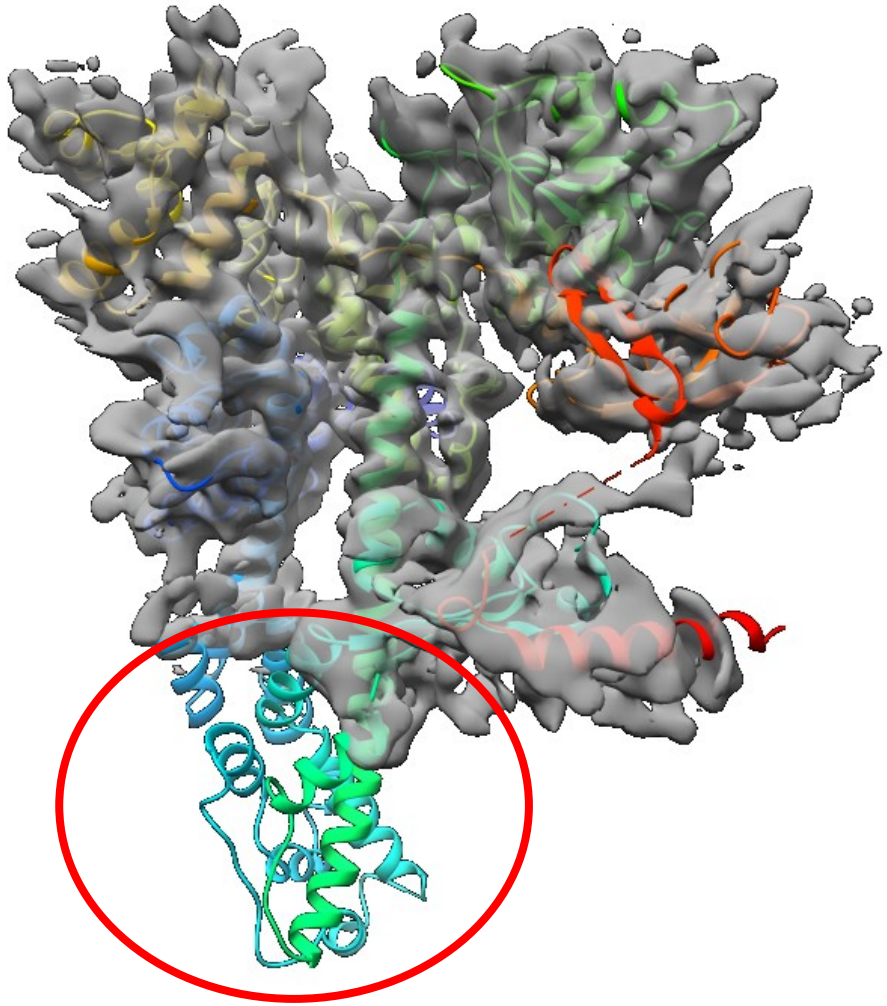


EM map

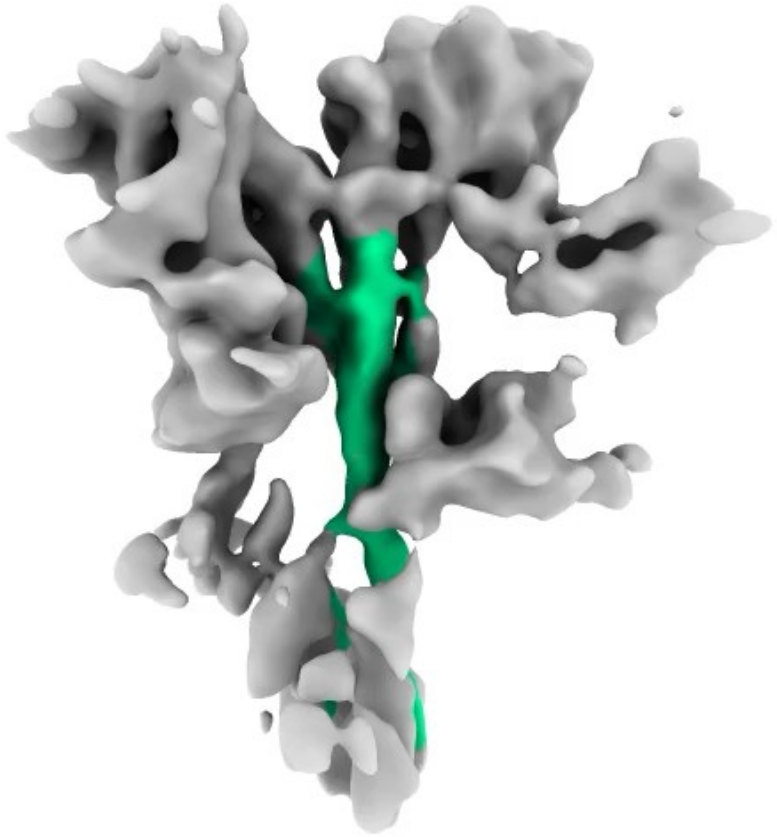
EM

map + PDB

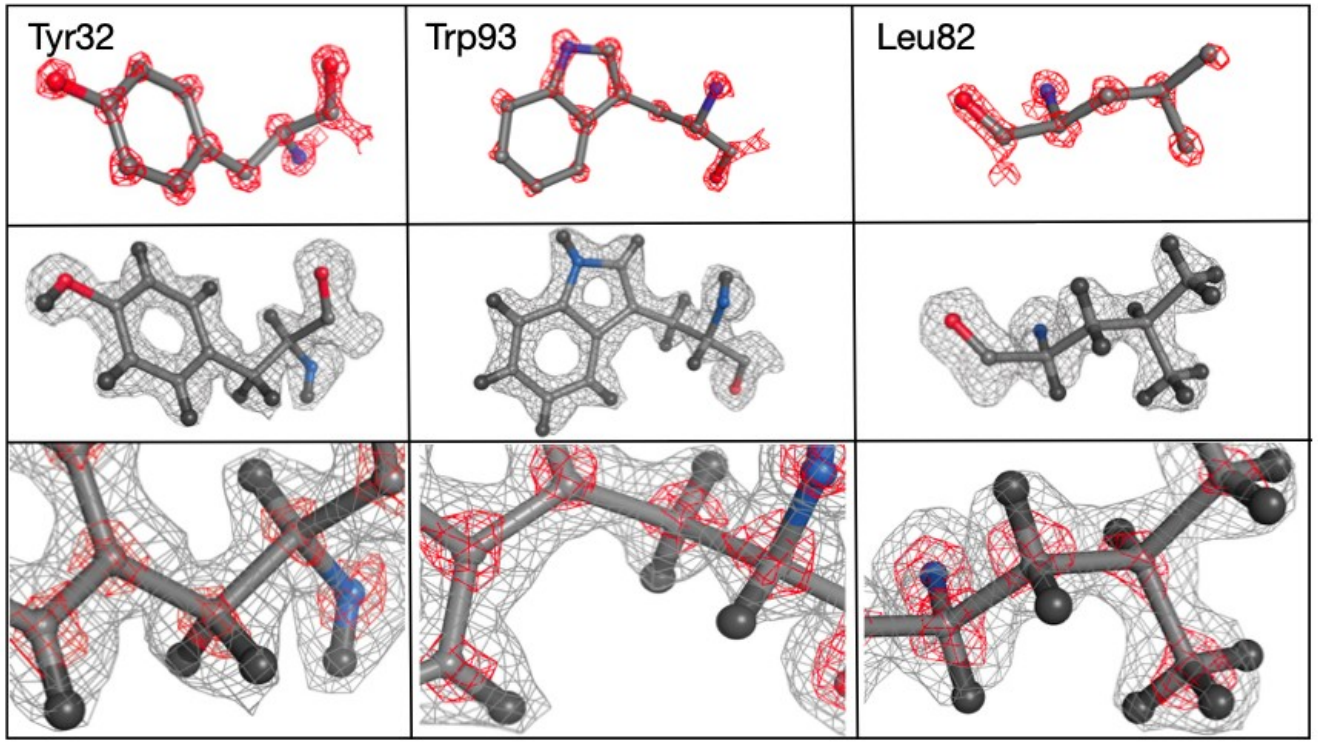




Dynamics in EM



State of the art in single particle EM – 1.25 Angstr.



Article

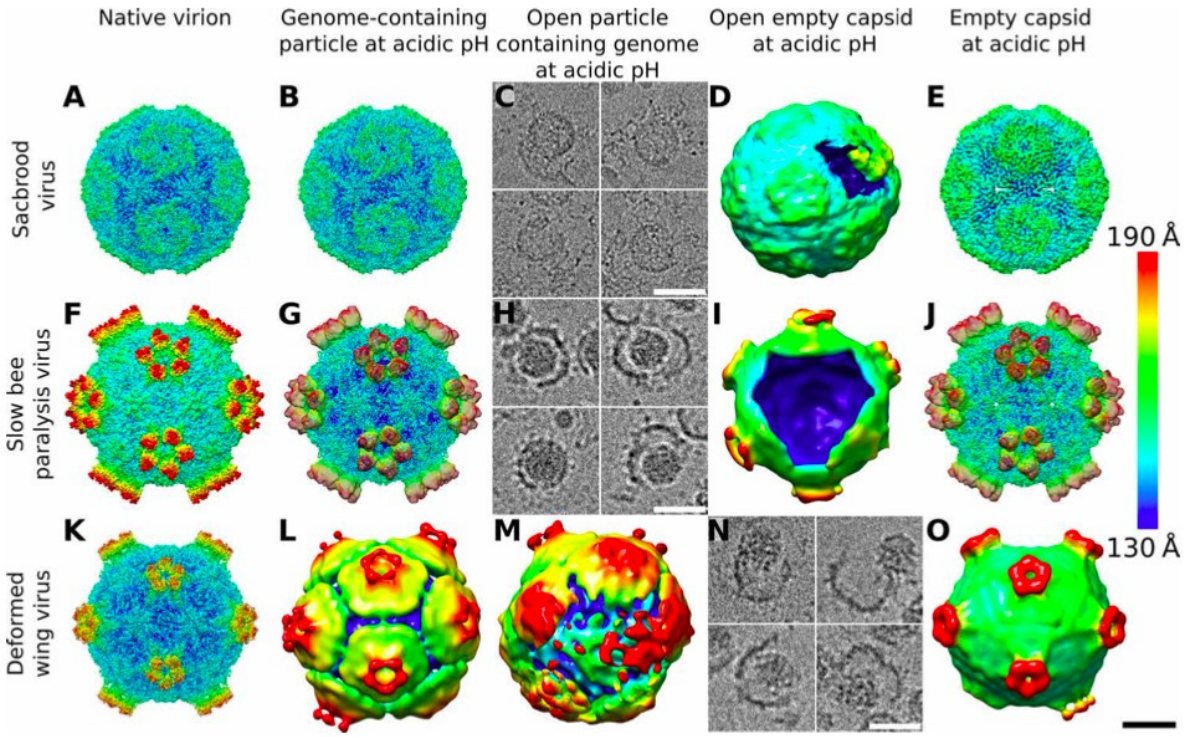
Atomic-resolution protein structure determination by cryo-EM

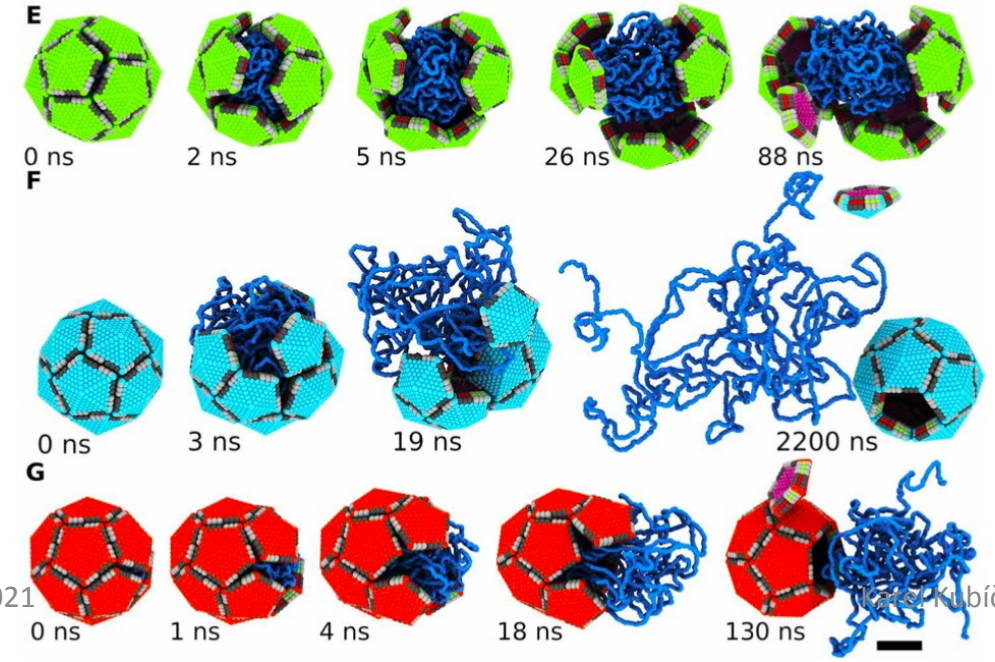
https://doi.org/10.1038/s41586-020-2833-4

Ka Man Yip[1], Niels Fischer[1], Elham Paknia[1], Ashwin Chari[1] & Holger Stark[1]✉

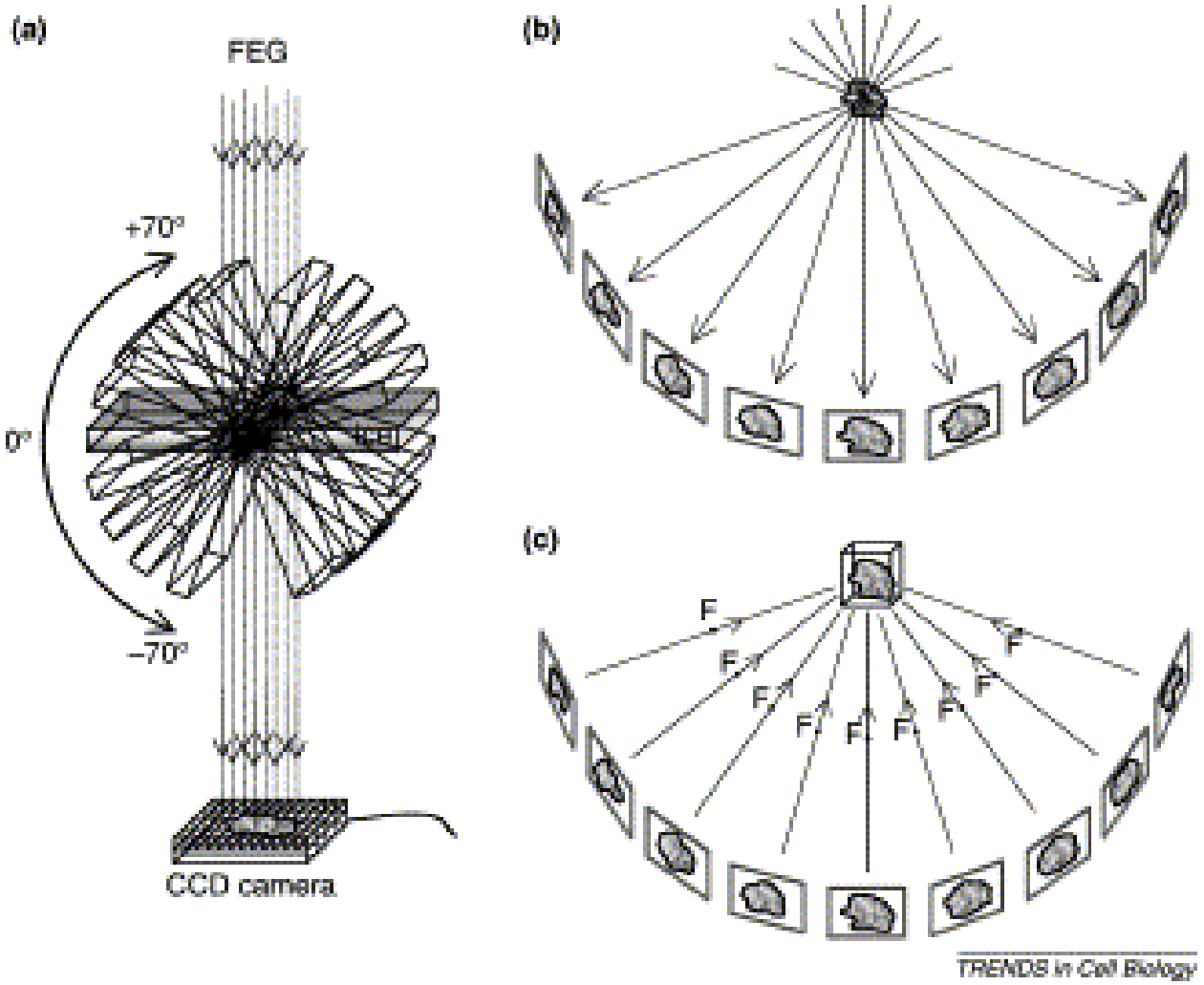
Nature, 2020

Computational + experimental methods



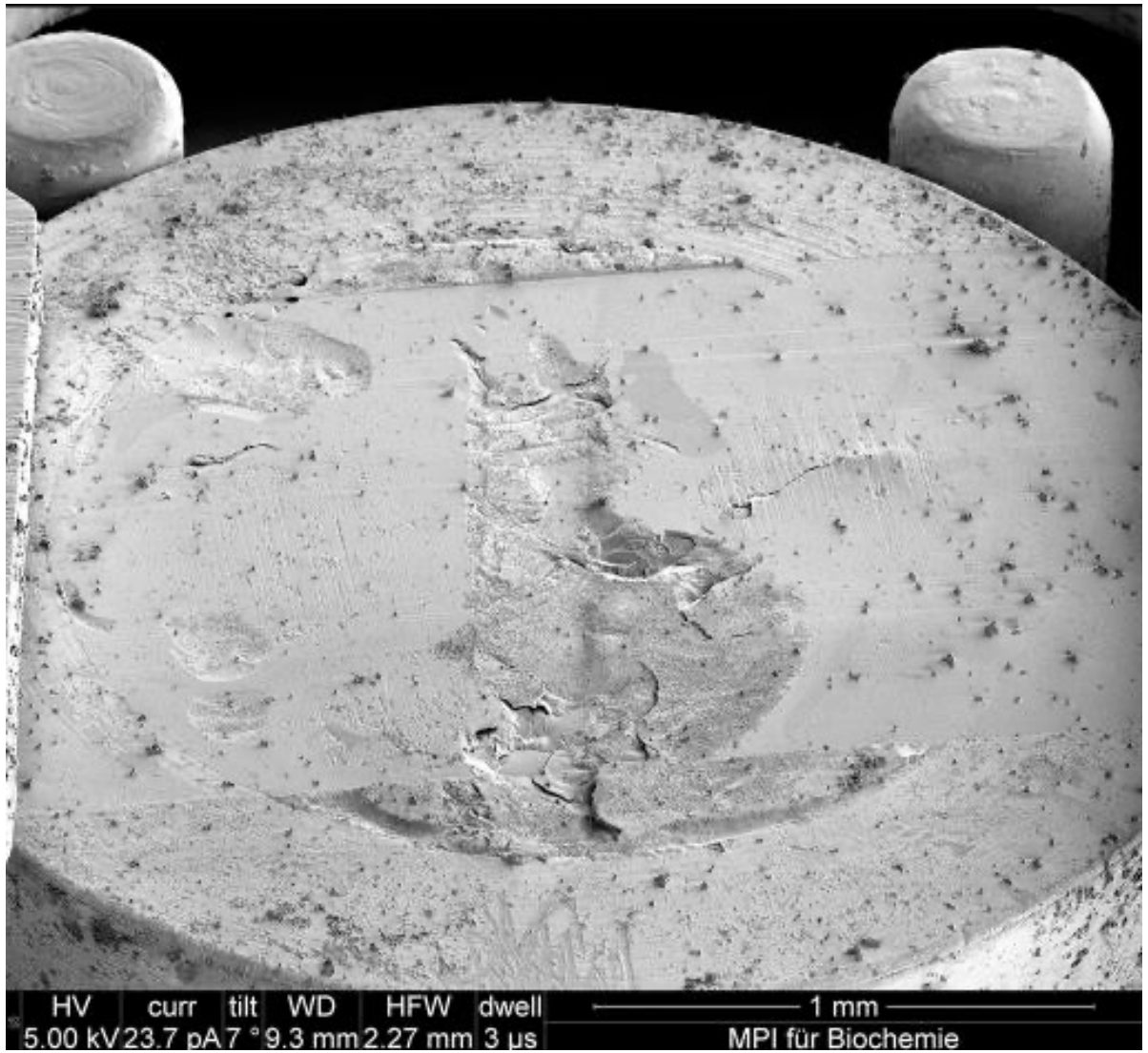


Skubnik, K; Sukenik, L; Buchta, D; Fuzik, T; Prochazkova, M; Moravcova, J; Smerdova, L; Pridal, A; Vacha, R; Plevka, P, 2021: Capsid opening enables genome release of iflaviruses. *SCIENCE ADVANCES*

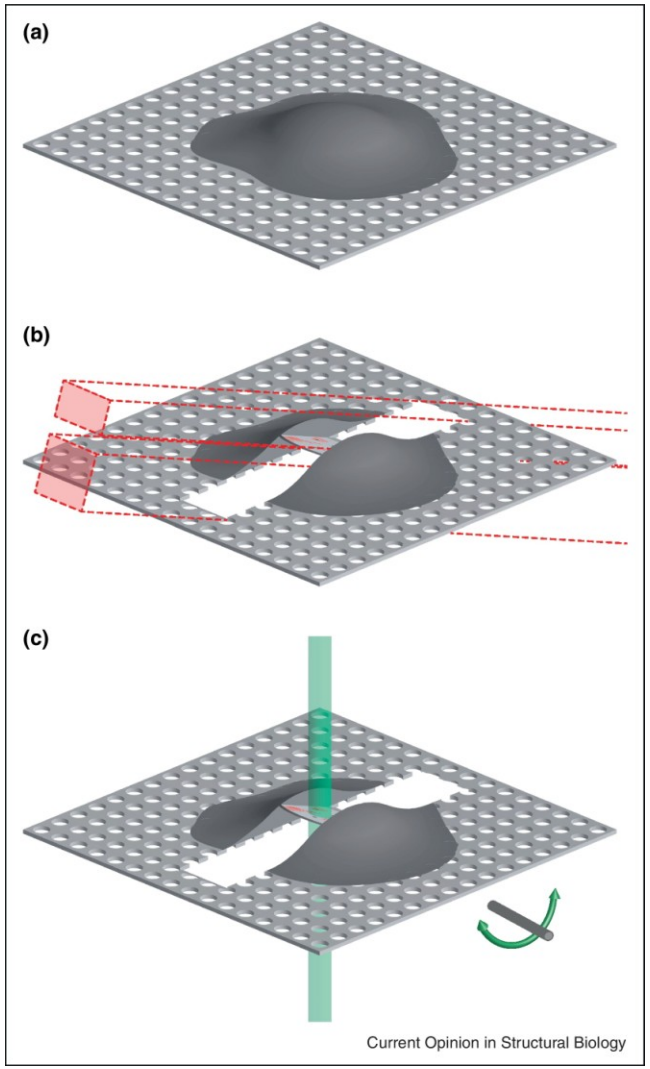


Trends in Cell Biology, 13(3), 2003, 107-110

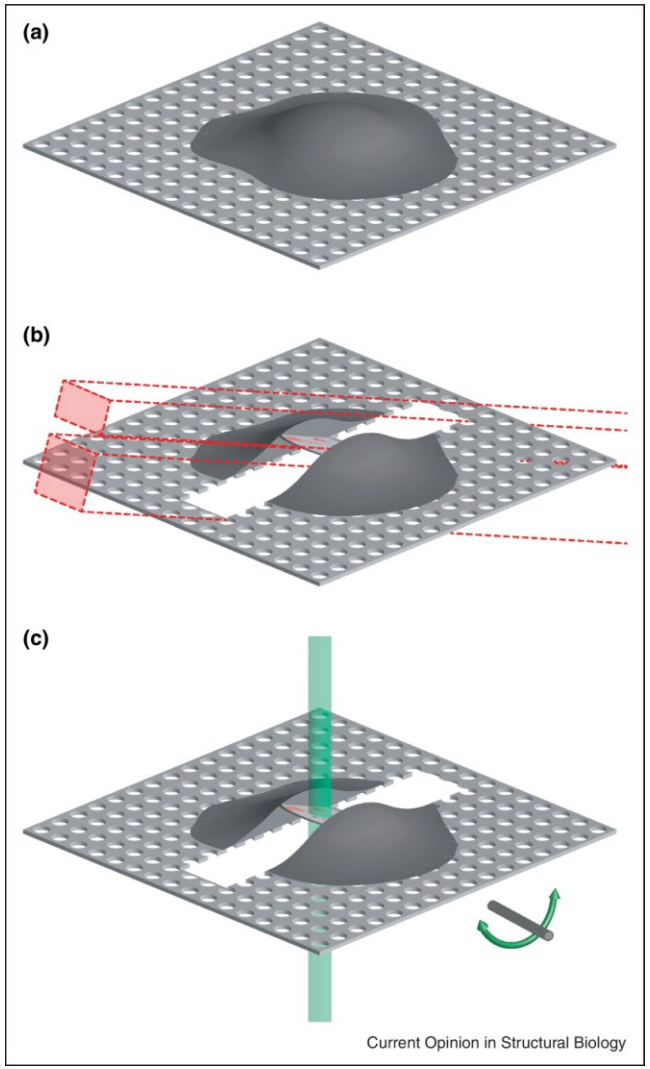
CLEM - Correlative light and electron microscopy

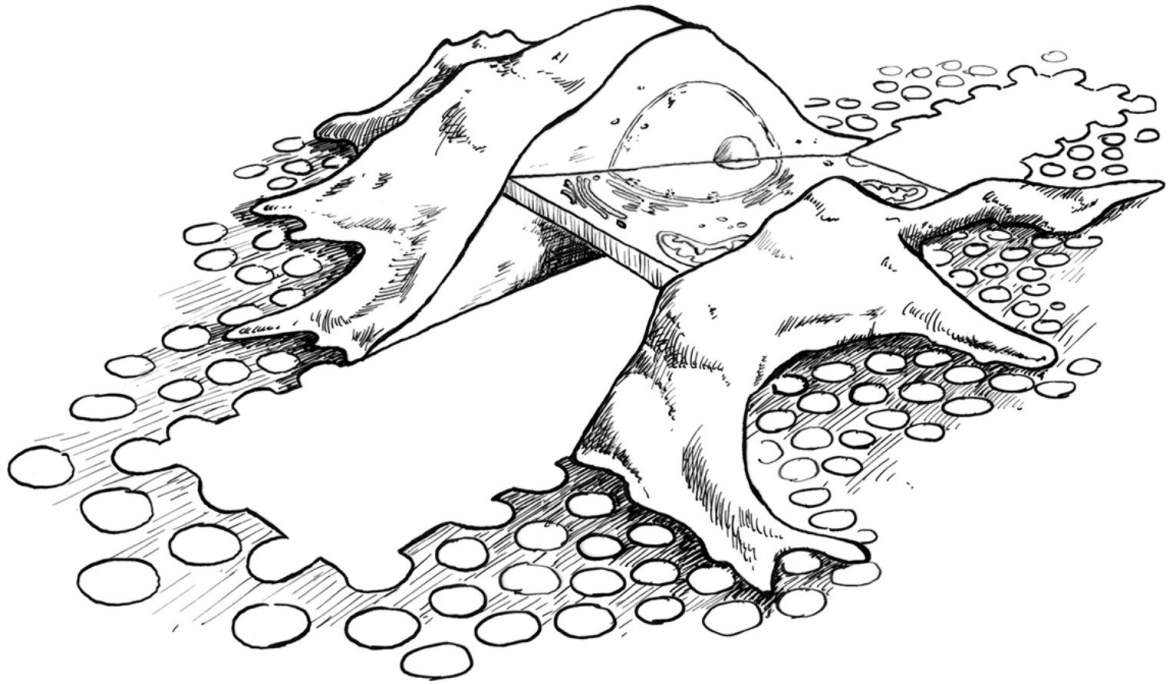


A cryo-FIB lift-out technique enables molecular-resolution cryo-ET within native *Caenorhabditis elegans* tissue, Nature methods, 2019

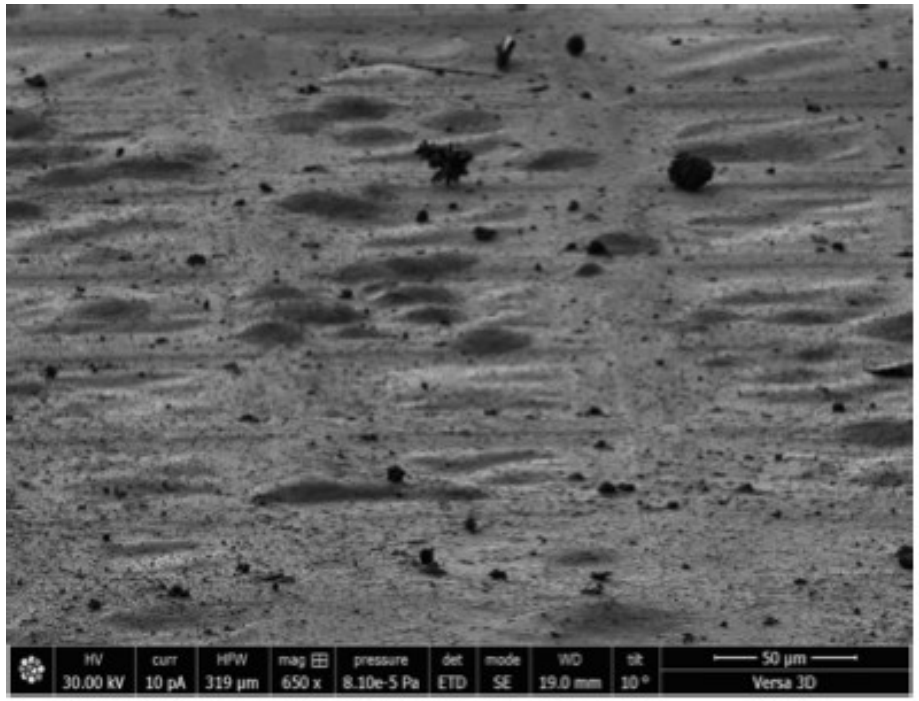


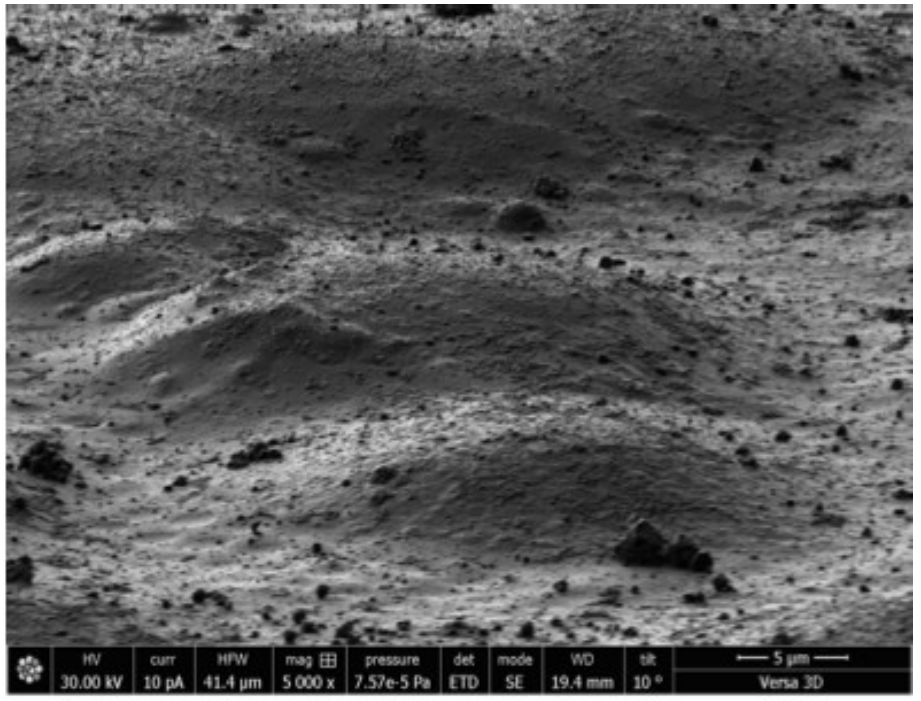
Villa et al. Current Opinion in Structural Biology, 23(5), 2013, 771-7





Villa et al. Current Opinion in Structural Biology, 23(5), 2013, 771-7


HV
30.00 kV
curr
10 pA
HFW
319 µm
mag
650 x
pressure
8.10e-5 Pa
det
ETD
mode
SE
WD
19.0 mm
tilt
10 °
50 µm
Versa 3D


HV
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curr
10 pA
HFW
41.4 µm
mag
5 000 x
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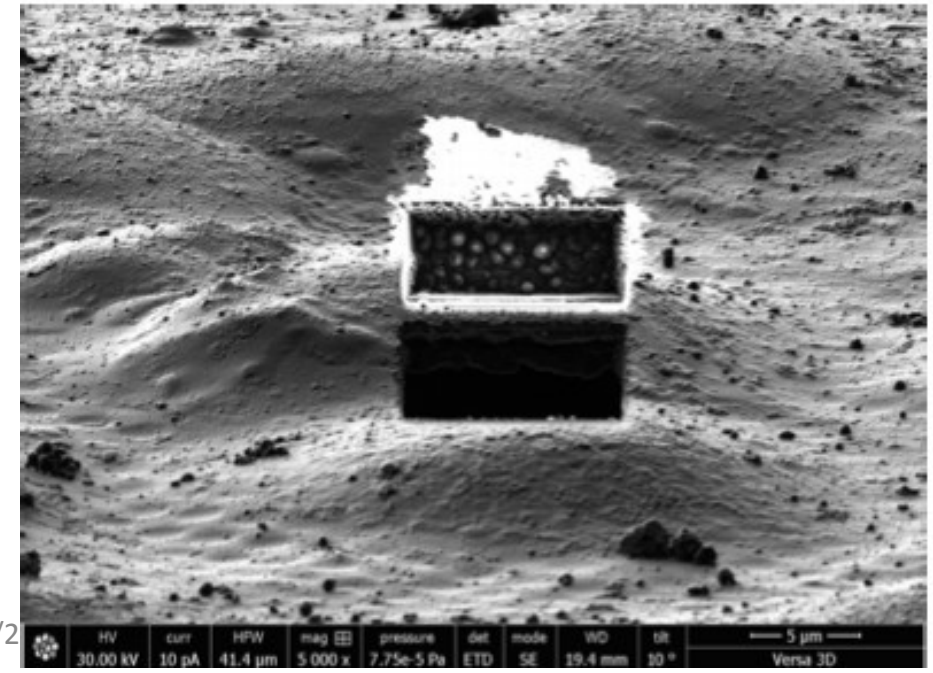
WD
19.4 mm
tilt
10 °
5 µm
Versa 3D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curr
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HFW
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mode
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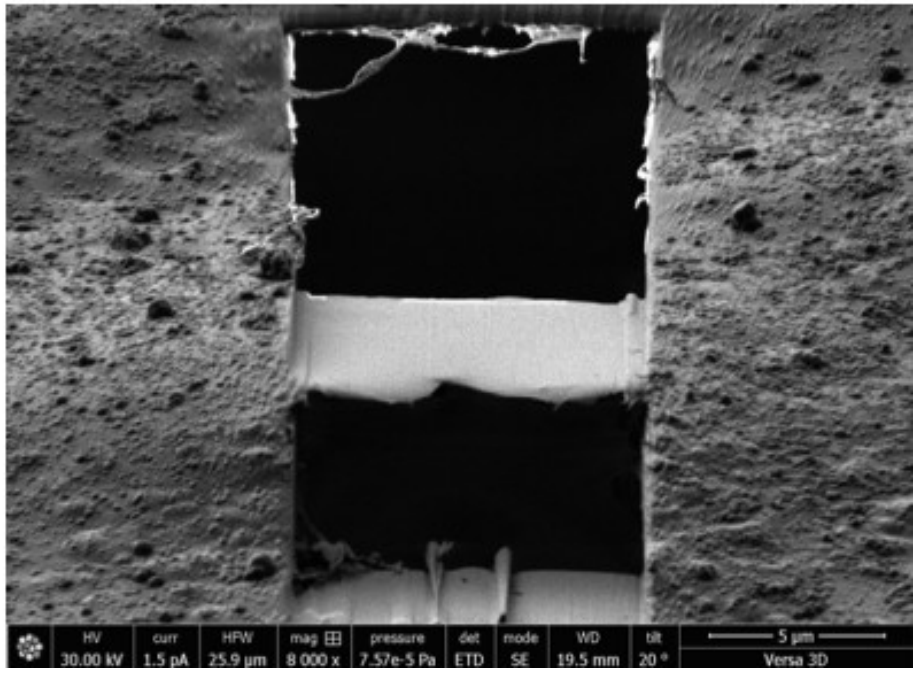
WD
19.4 mm
tilt
10 °
5 µm
Versa 3D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19.5 mm
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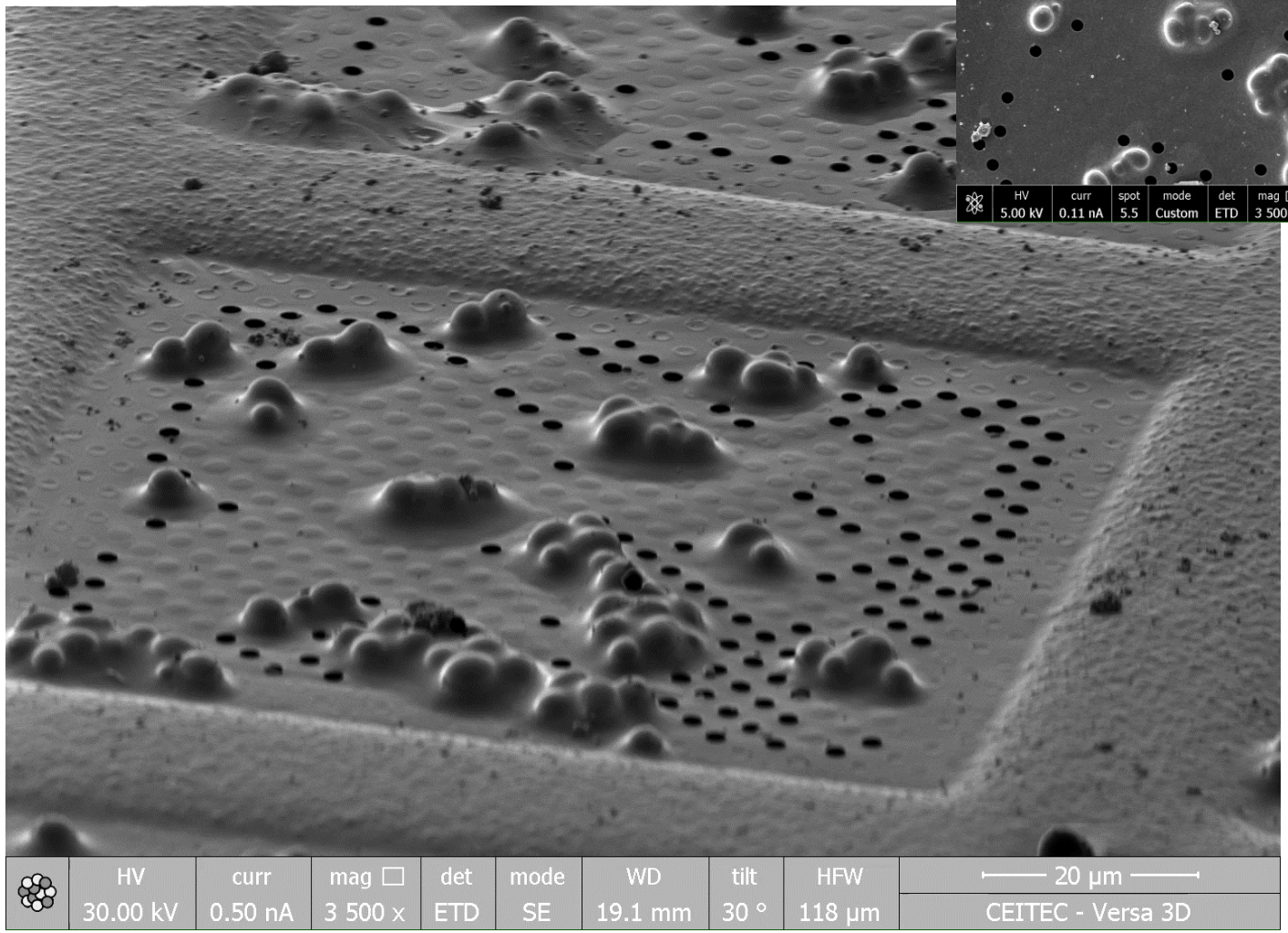
20 °
5 µm
Versa 3D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curr
0.50 nA
mag
3 500 x
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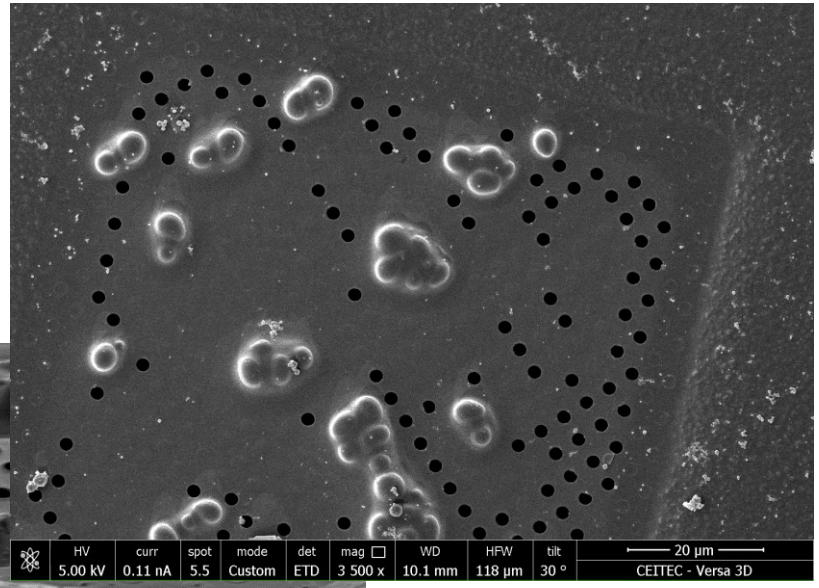
ETD
mode
SE
WD
19.1 mm
tilt
30 °
HFW
118 µm
20 µm
CEITEC - Versa 3D


HV
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spot
5.5
mode
Custom
det
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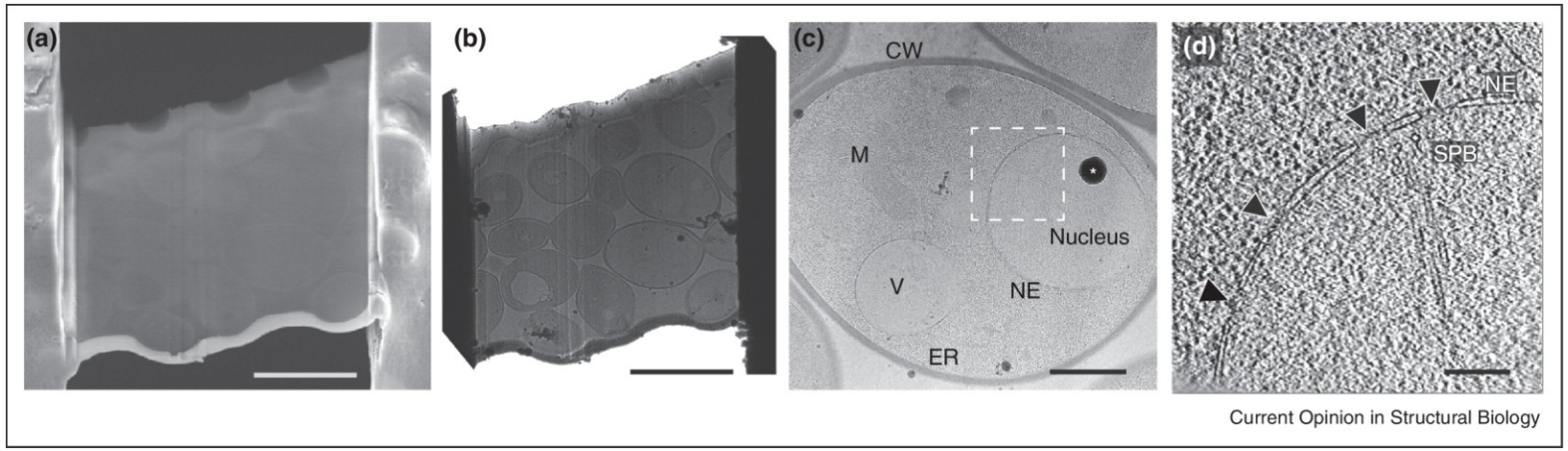
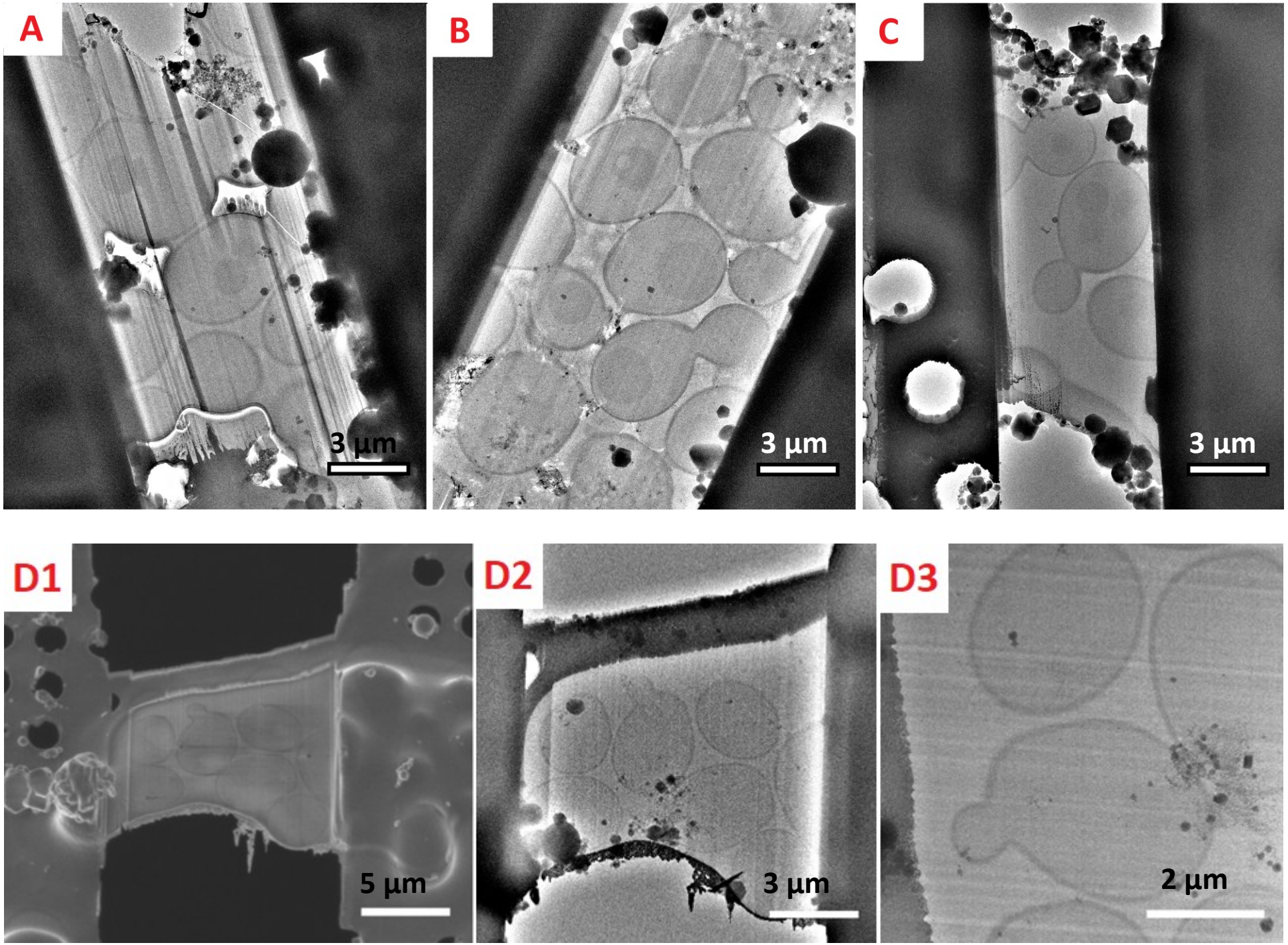
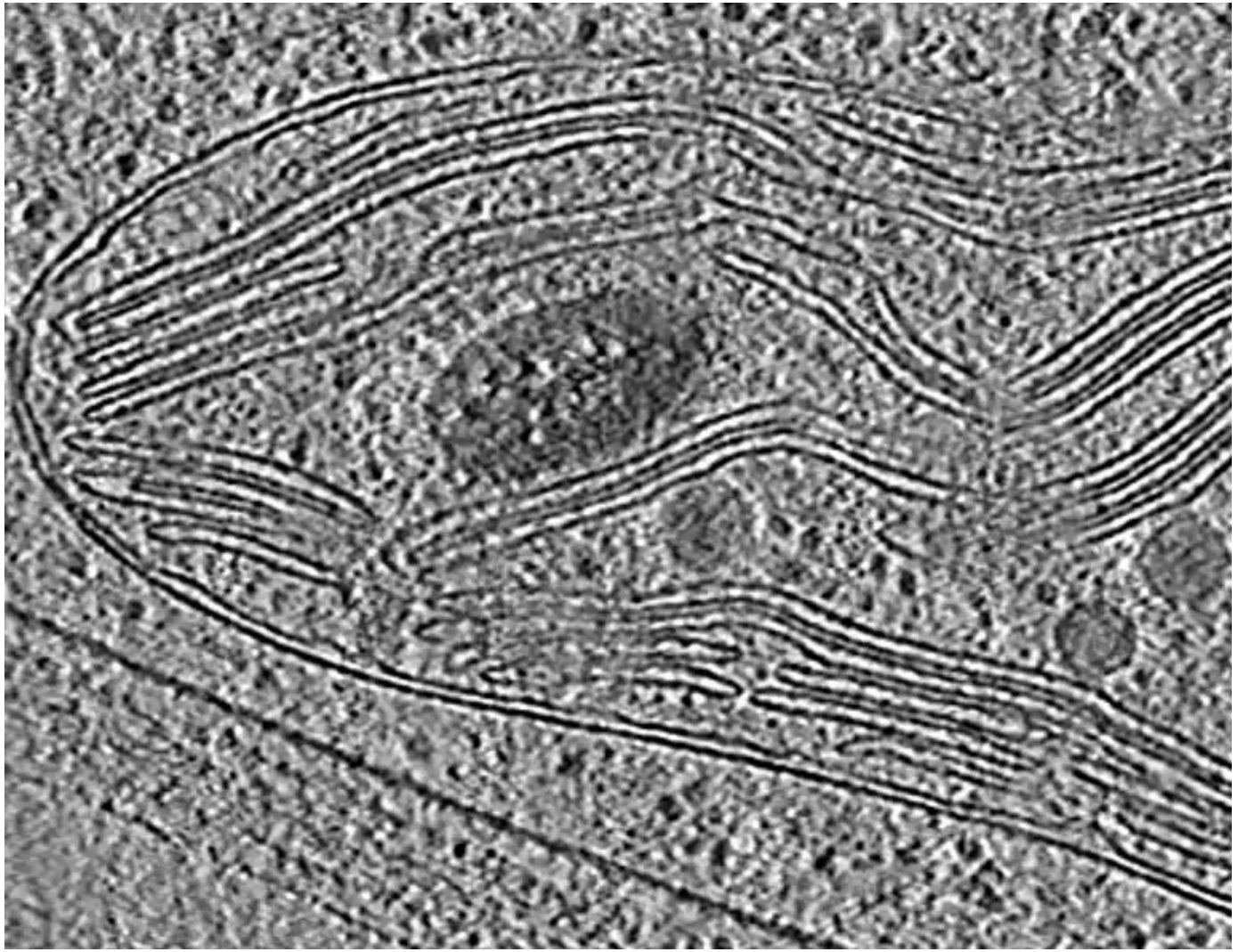
mag
3 500 x
WD
10.1 mm
HFW
118 µm
tilt
30 °
20 µm
CEITEC - Versa 3D



Villa et al. Current Opinion in Structural Biology, 23(5), 2013, 771-7

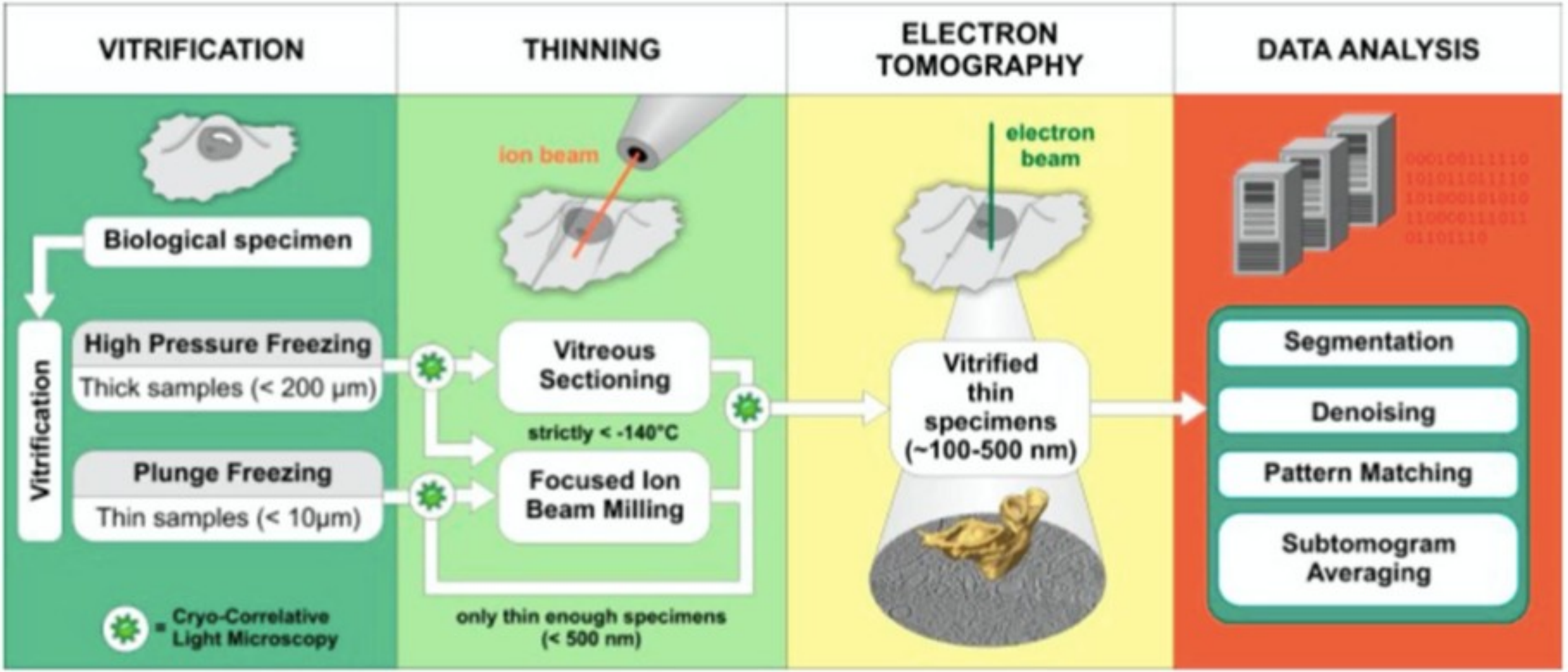

A
3 µm
B
3 µm
C
3 µm
D1
5 µm
D2
3 µm
D3
2 µm

thylakoid tip convergence zone at the rim of the chloroplast cup



Native architecture of the Chlamydomonas chloroplast revealed by in situ cryo-electron tomography, *Elife* 2015

EM Tomografie

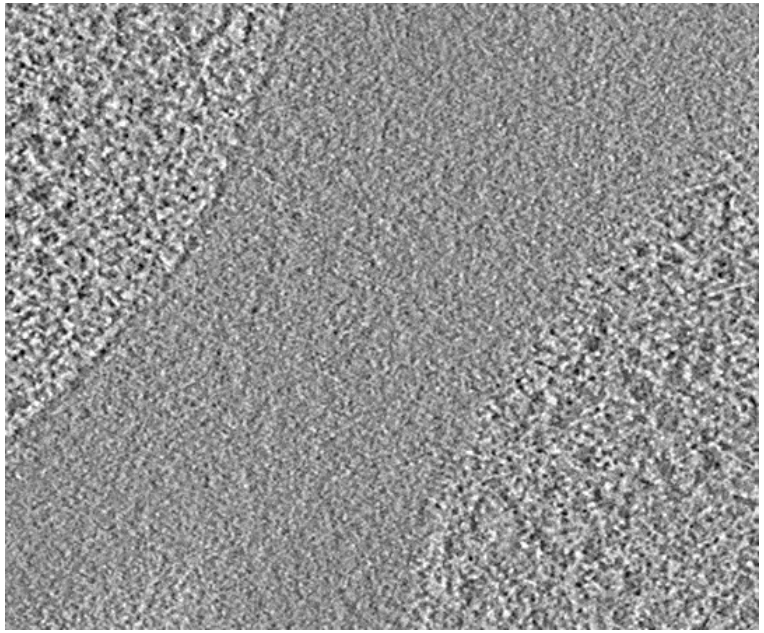


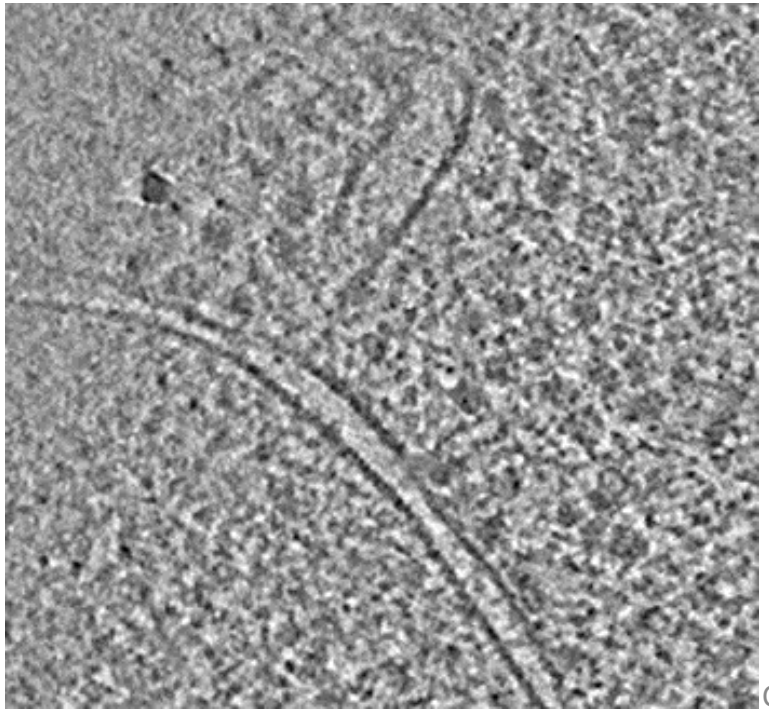
MUNI SCI

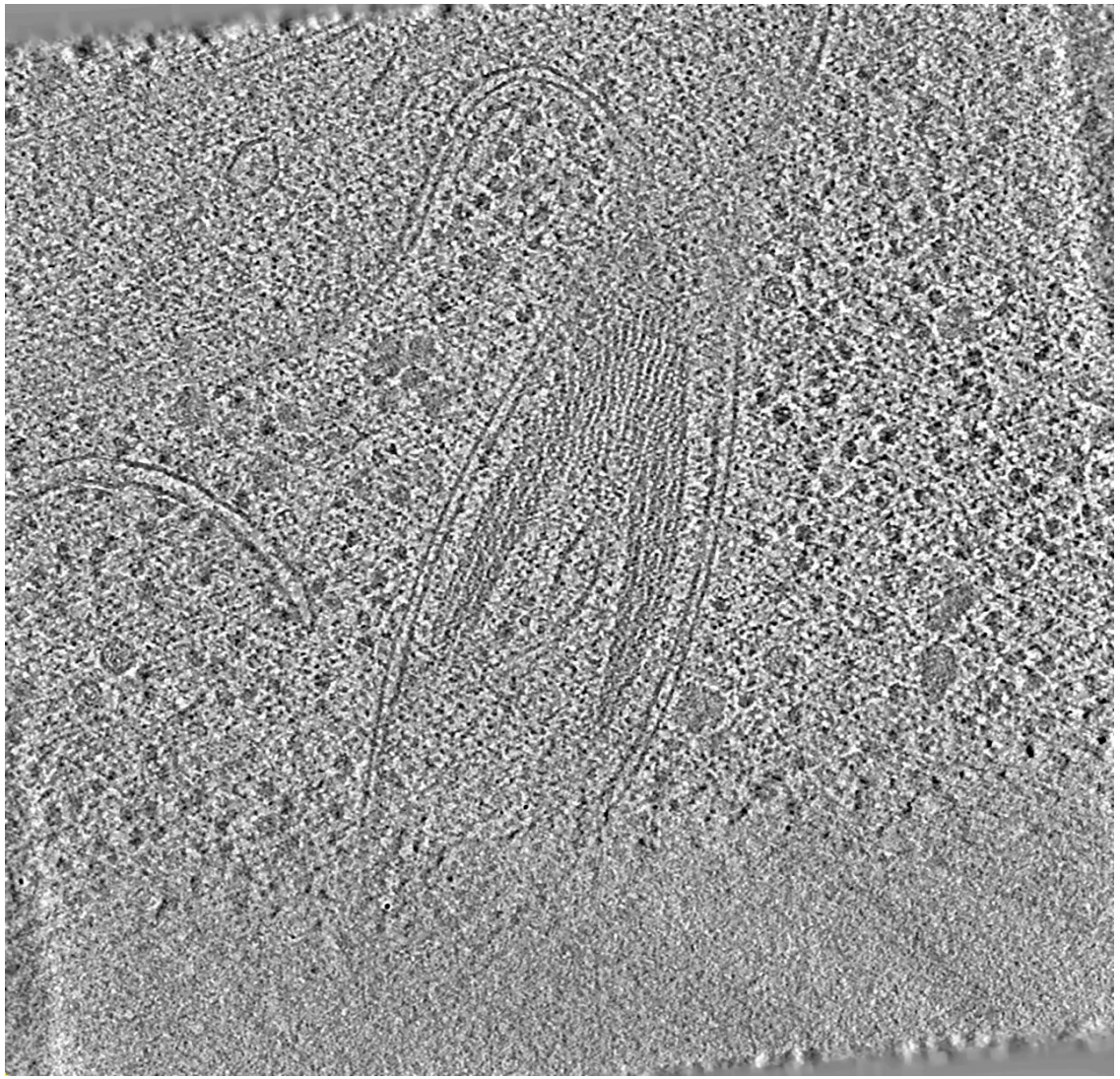
Take home message:

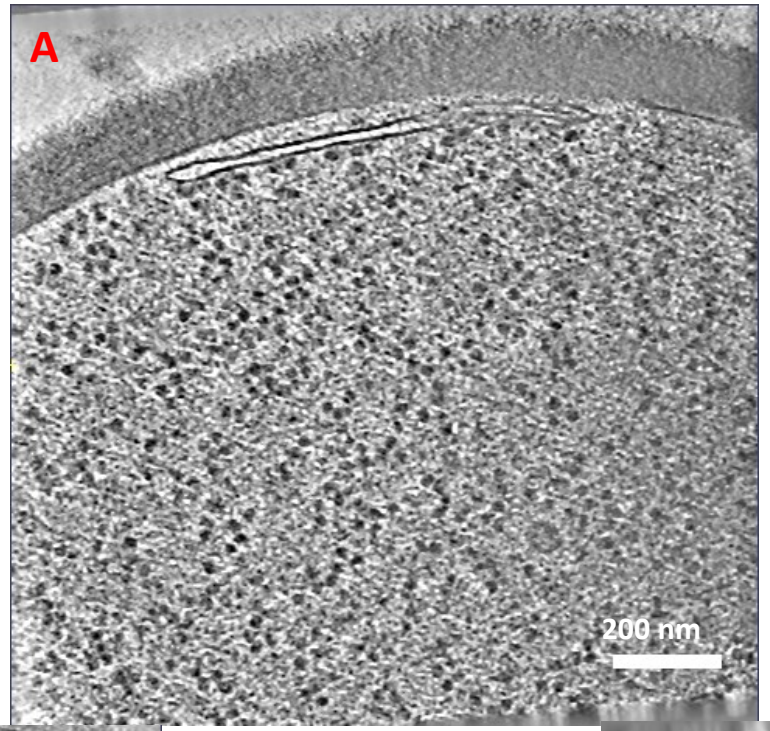
1. Negative stain
2. Single particle reconstruction
3. Dynamics
4. Tomography
5. CLEM

MUNI
SCI



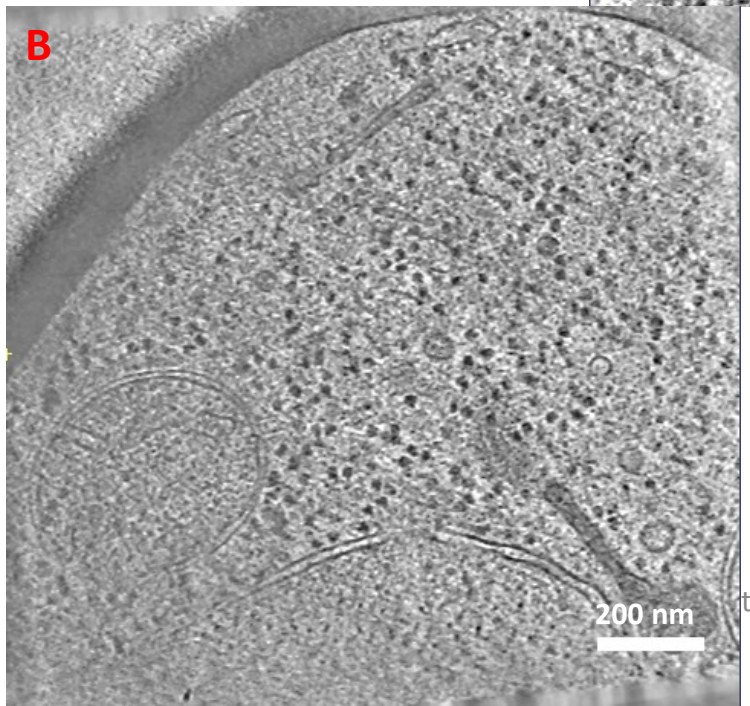




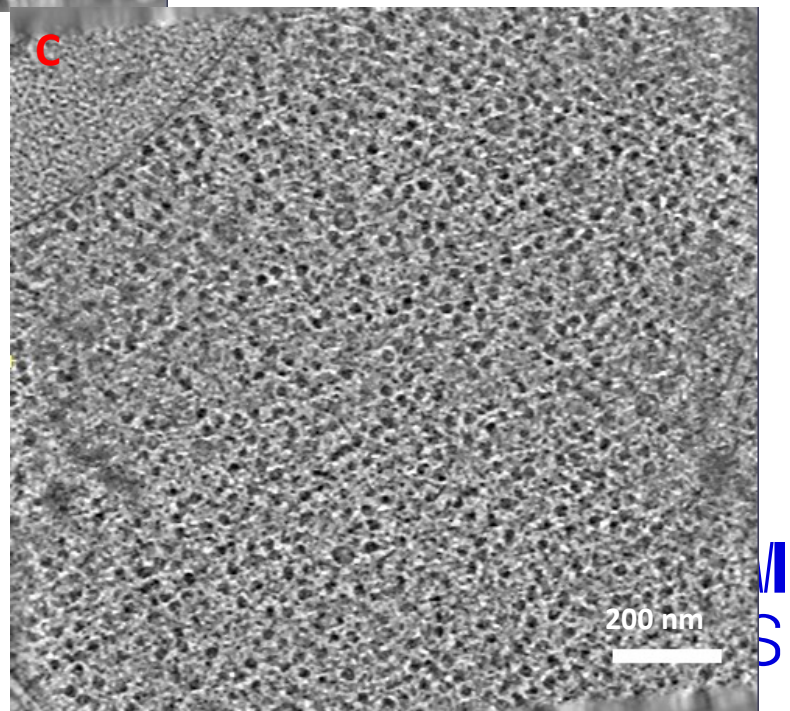


Glucose-free
(low polysome content)

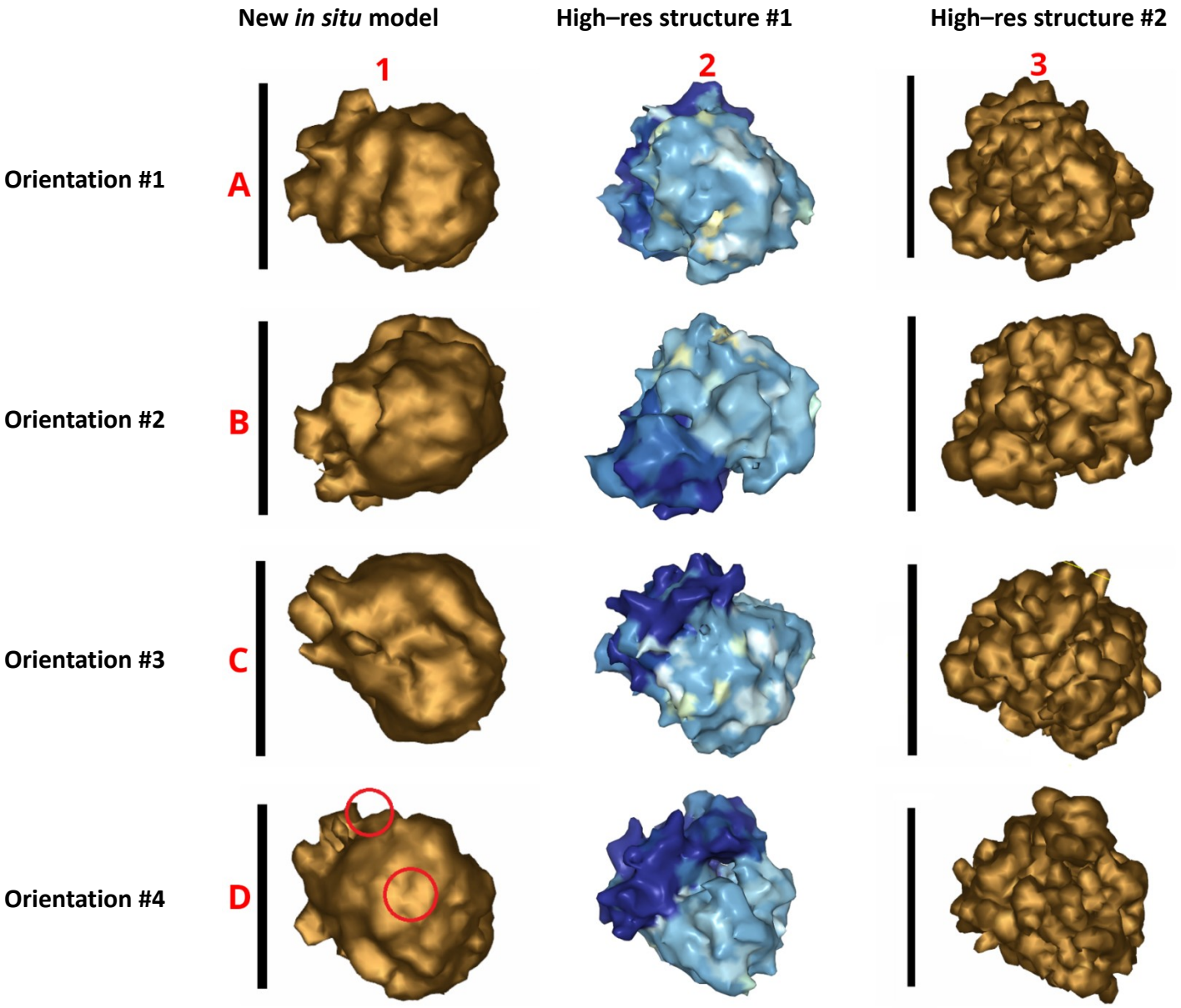
Heat shock 47° C
(low polysome content)



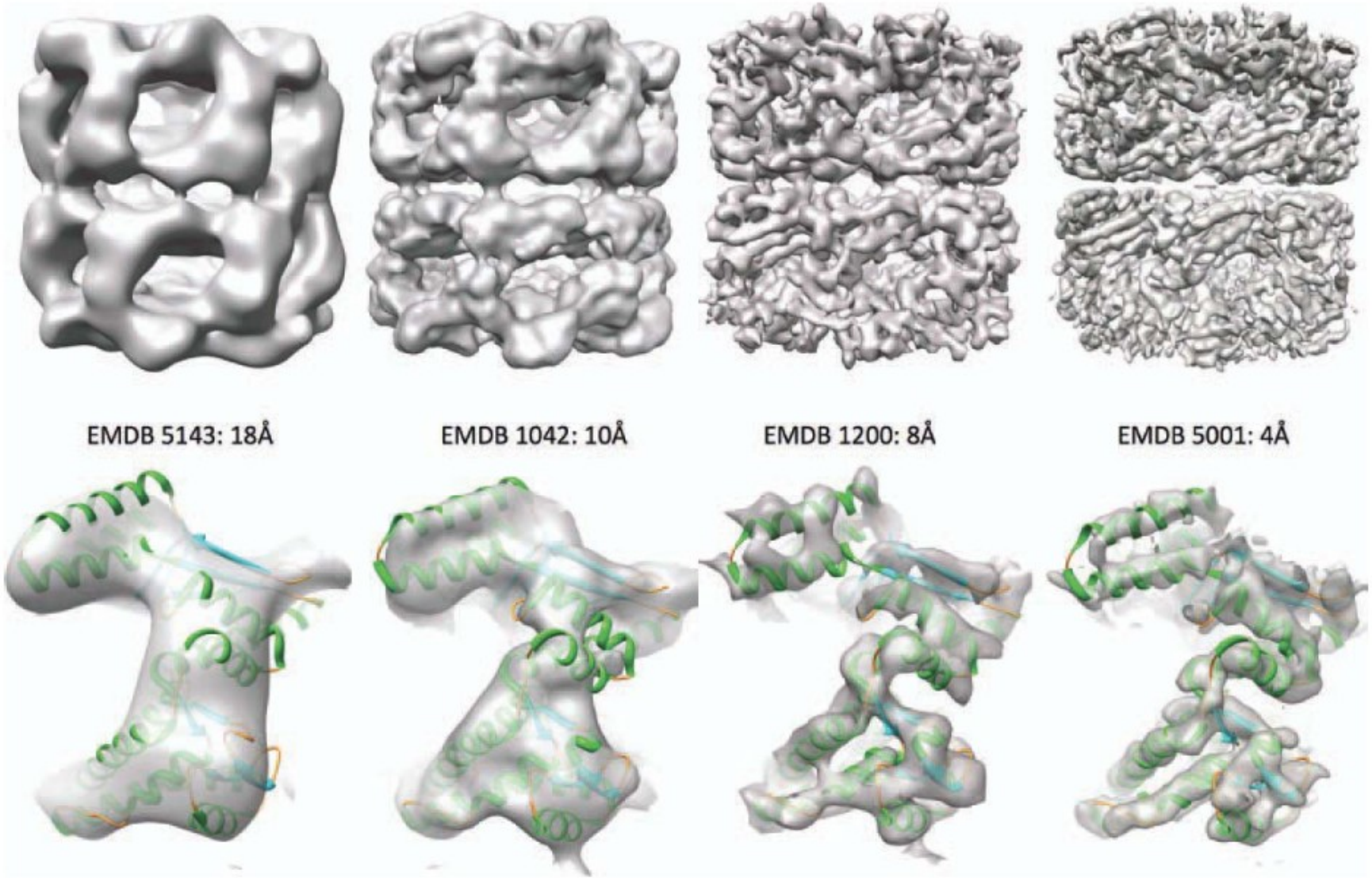
Standard
(high polysome content)



A structure of the 80S ribosomes in situ



GroEL at different resolutions (levels of detail)

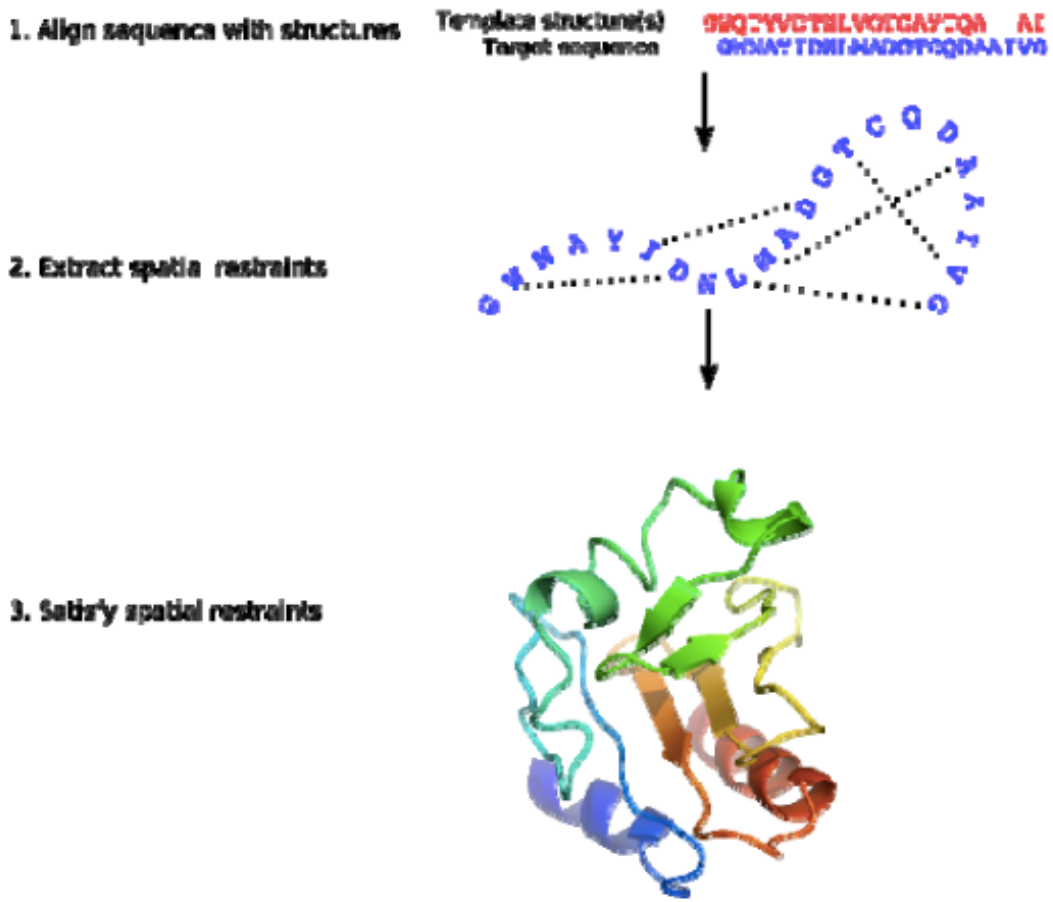


Fitting of known structures (rigid body fitting)

Flexible fitting of known structures

Building of de novo models

- Many more sequences available than structures
- Many applications rely on structural information
- Structure is often more conserved than sequence
  (evolution preserves function)

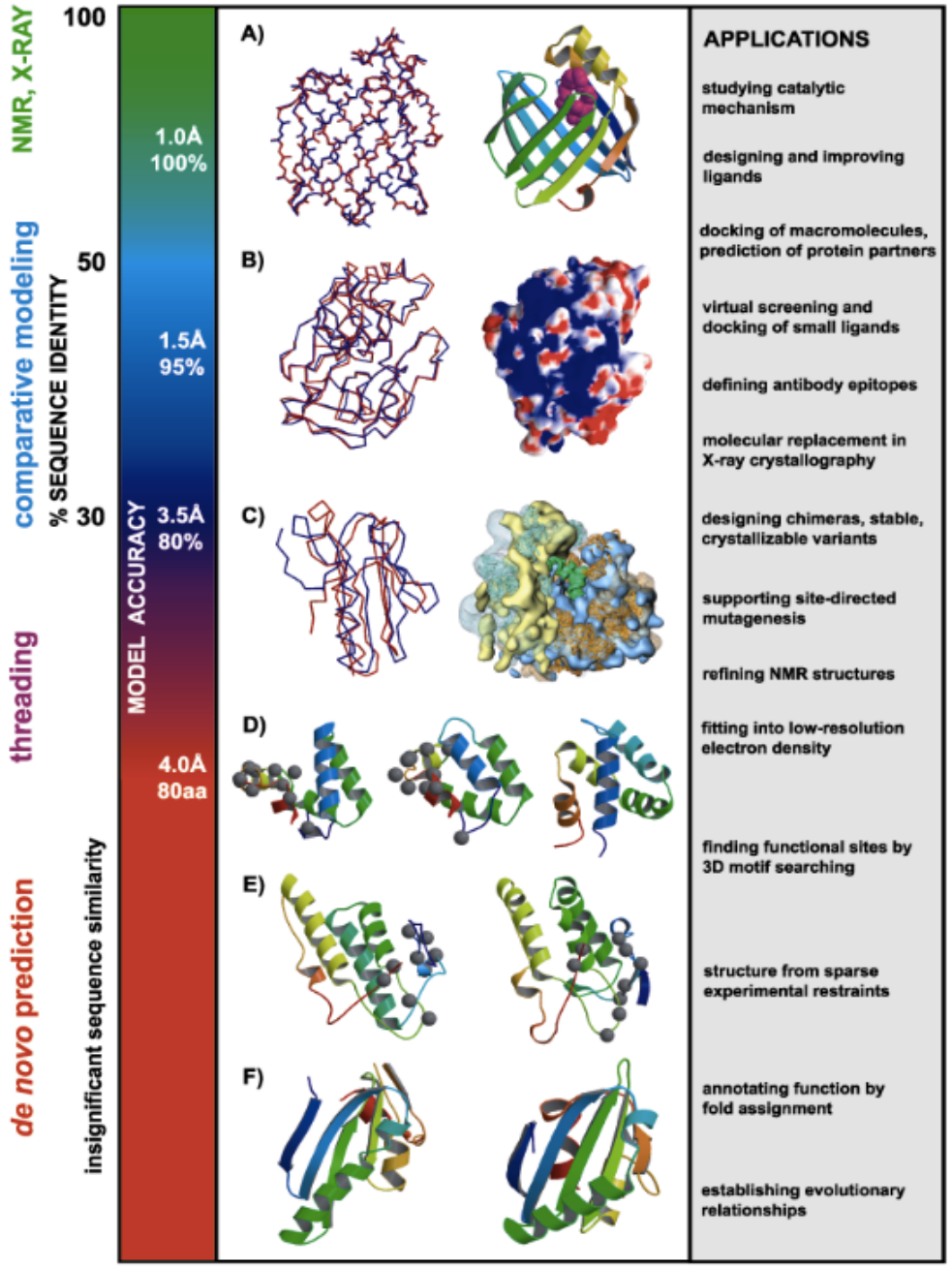


1) Assembly of rigid bodies
   (core, loops, sidechains)

2) Segment matching

3) Satisfaction of spatial restraints

A. Šali & T. Blundell. J. Mol. Biol. 234, 779, 1993.
J.P. Overington & A. Šali. Prot. Sci. 3, 1582, 1994.
A. Fiser, R. Do & A. Šali, Prot. Sci., 9, 1753, 2000.



D. Baker & A. Sali. Science 294, 93, 2001.

1. Model building and fitting into EM maps
2. Comparative and homology modeling
3. Rigid body fitting of atomic models
4. Flexible fitting of atomic models
5. Building models, hybrid methods
6. De novo model building