SIMONS ELECTRON MICROSCOPY CENTER

NEW YORK STRUCTURAL BIOLOGY CENTER

Simons Electron Microscopy Center EM course

Challenges in Biological EM & Sample Prep

2016/02/08

STRUCTURAL BIOLOGY INITIATIVE

CUNY ADVANCED SCIENCE RESEARCH CENTER

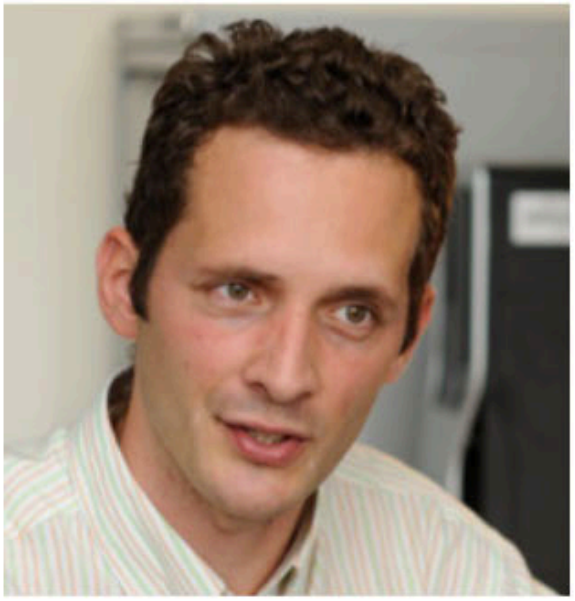
hosted in conjunction with: SIMONS ELECTRON MICROSCOPY CENTER
NEW YORK STRUCTURAL BIOLOGY CENTER

SBI seminar on:

How cryo-EM is revolutionizing structural biology

Sjors Scheres, PhD

MRC-Laboratory of Molecular Biology, Cambridge, UK



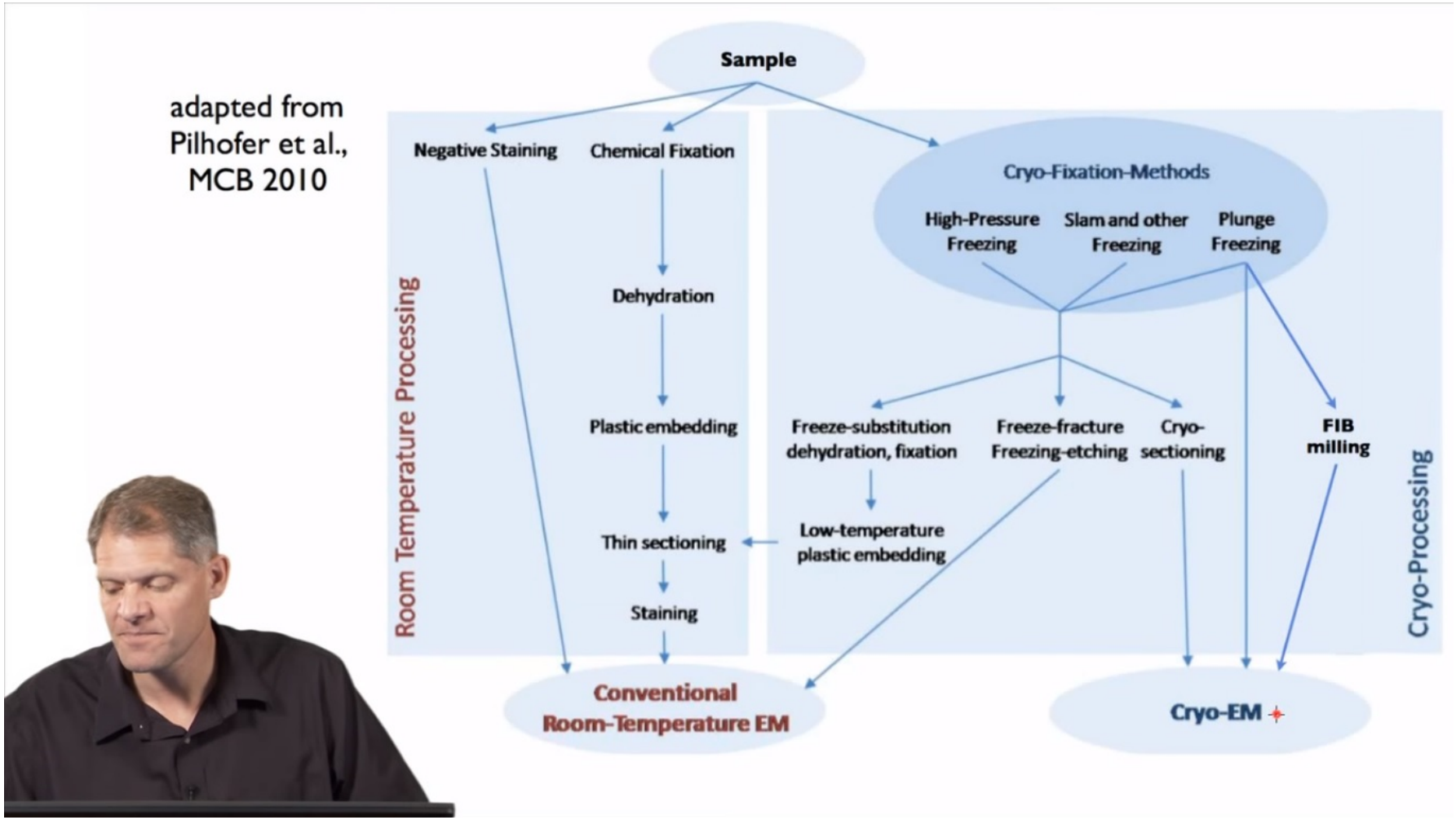
Recent advances in direct-electron detectors and advanced image processing algorithms have resulted in cryo-EM structure determination to near-atomic resolution for a wide range of macromolecular complexes. I will discuss these advances and illustrate their potential by presenting our results on gamma-secretase. This intra-membrane protease cleaves many substrates, and is perhaps best known for producing amyloid-beta peptides, abundant deposits of which in the brain are a defining characteristic of Alzheimer's disease.

Date:
Wednesday, February 24, 2016

Time:
3:00pm, followed by informal coffee hour until 5:00pm

Location:
CUNY ASRC Auditorium
85 Saint Nicholas Terrace
New York, NY 10031

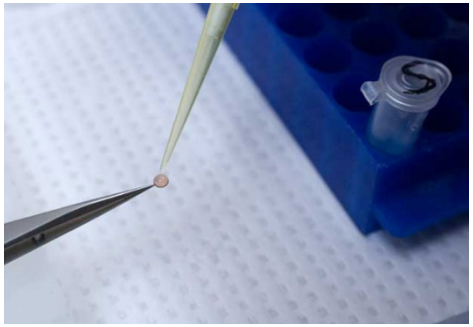
RT & Cryo Sample Prep Methods

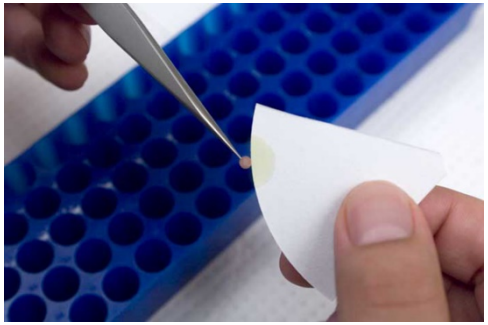


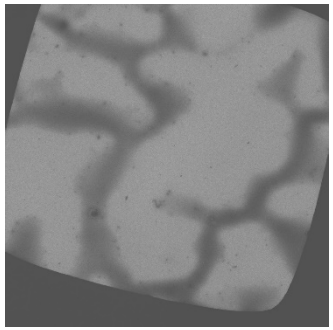
Negative Staining

Heavy metal salt solution surrounds sample
Continuous carbon support film
Protocol: glow discharge, sample, wash, stain
SEMC: UA/UF, PTA, ammonium molybdate

Advantages: high contrast, easy to learn, high SNR, radiation resistant, 3D reconstruction possible
Disadvantages: structural collapse & flattening artifacts, non-native environment, ~20 Å max resolution





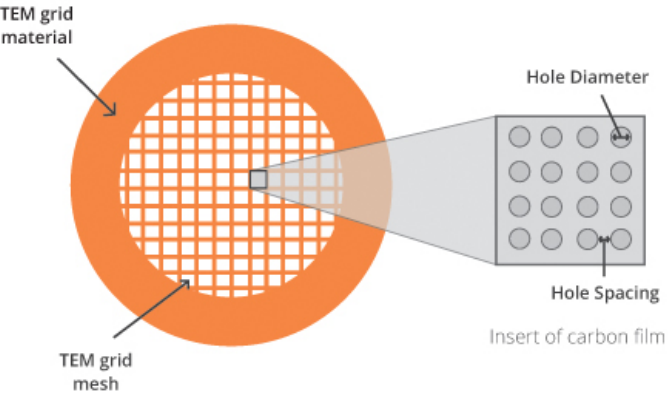


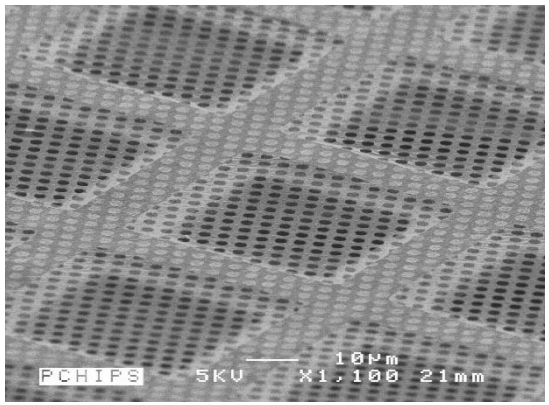
Baker, 2007

Plunge Freezing

Sample suspended in physiological buffer
Holey carbon support film: C-flats, Quantifoil
Protocol: glow discharge, sample, blot, plunge freeze
SEMC: Gatan CryoPlunge Freezer 3, FEI Vitrobot, manual plunge freezer

Advantages: no fixation/dehydration/staining artifacts, learning curve, random orientation, higher resolution than stain
Disadvantages: low contrast, low SNR, radiation sensitive, difficult to visualize <100 kD, freezing artifacts





Protochips.com

www.mcb.ucdavis.edu/cryoem/microscop

Gold Grids

Holey gold foil on gold mesh grid

dvantages:

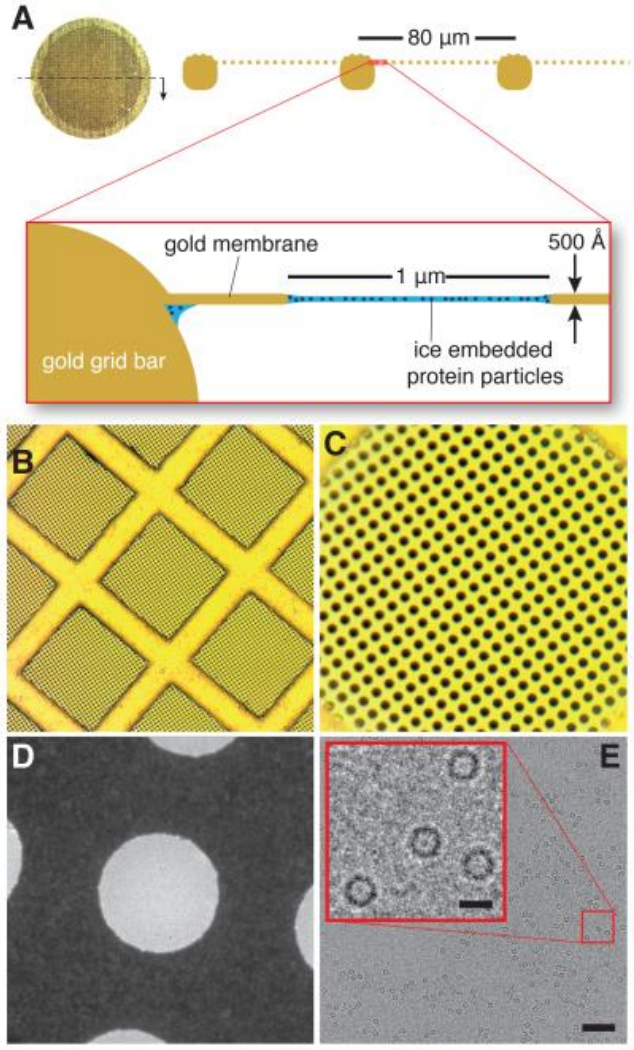
Prevents differential thermal contraction when freezing

Reduces beam-induced specimen movement

Combined with direct detector technology allows for near atomic resolution

isadvantages:

Difficult to find focus due to lack of amorphous substrate

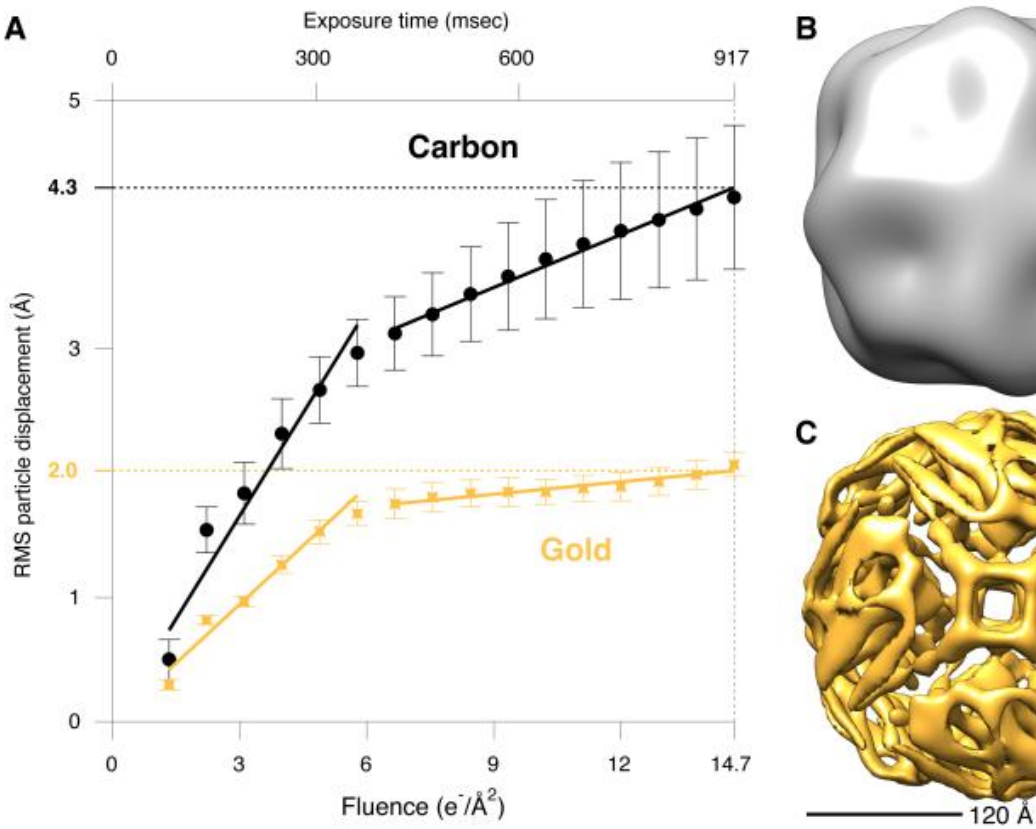


Russo & Passmore, 2015

Gold Grids

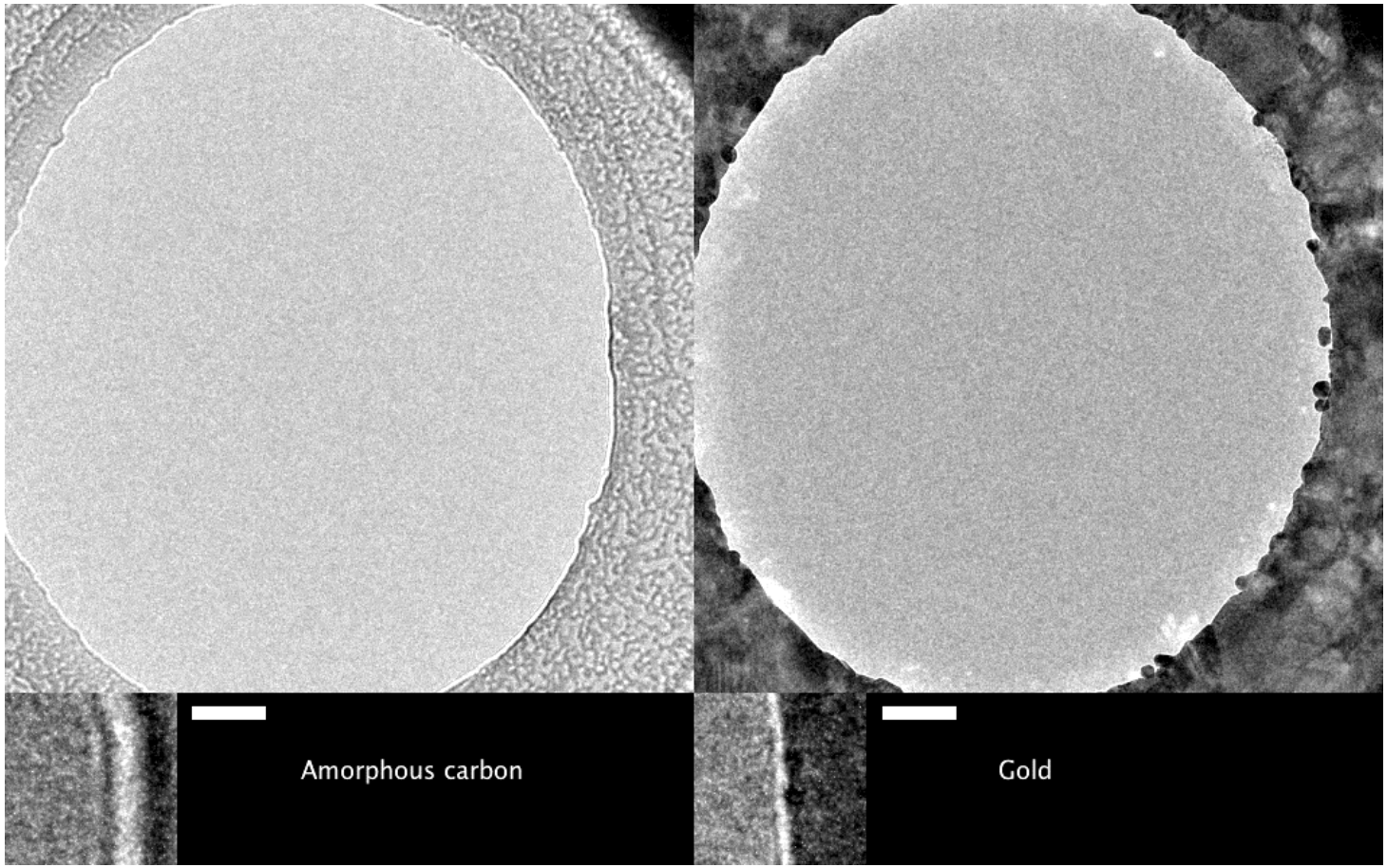
. 80S ribosome movement during irradiation
upported by amorphous carbon and gold using same
maging conditions.

poferritin density maps using same imaging conditions
nd identical processing for **B.** carbon and **C.** gold
ubstrates. **B.** is at 25 Å and **C.** 8 Å resolution.



Russo & Passmore, 2015

Gold Grids



Russo & Passmore, 2015

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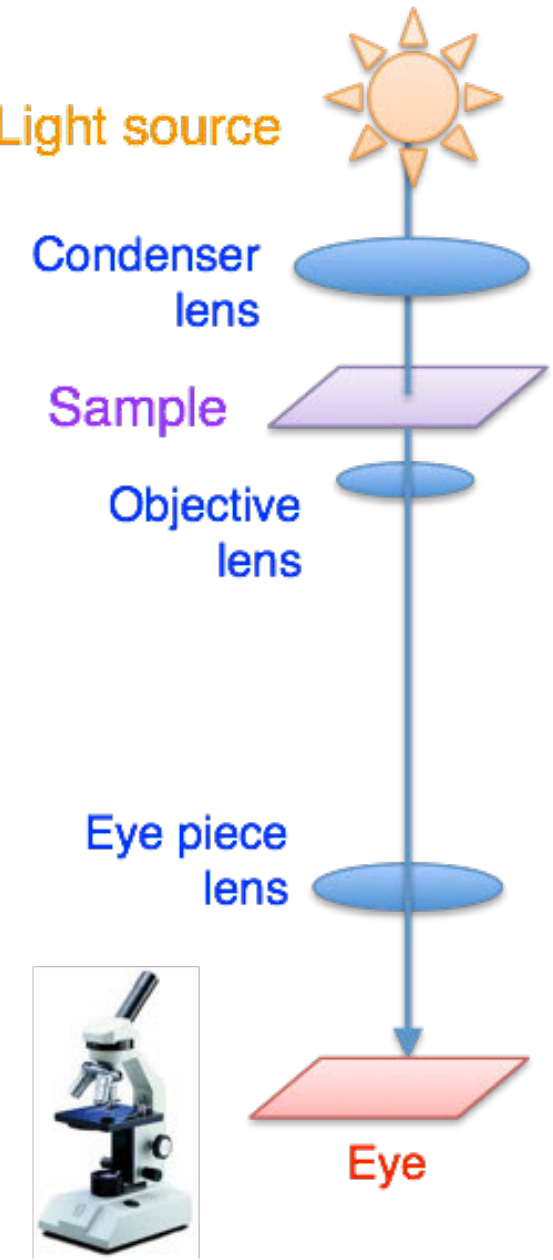
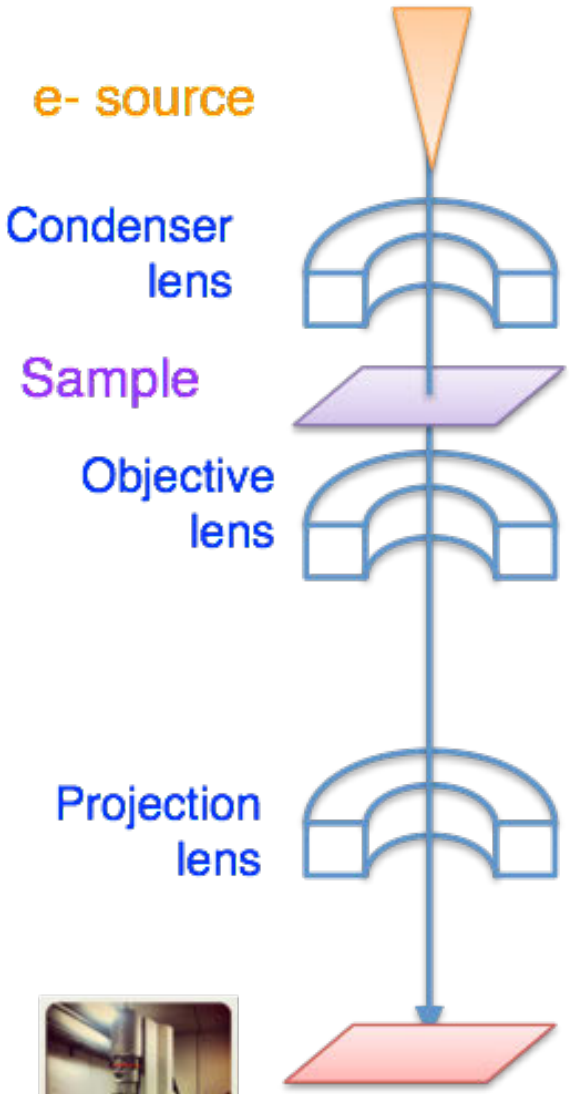
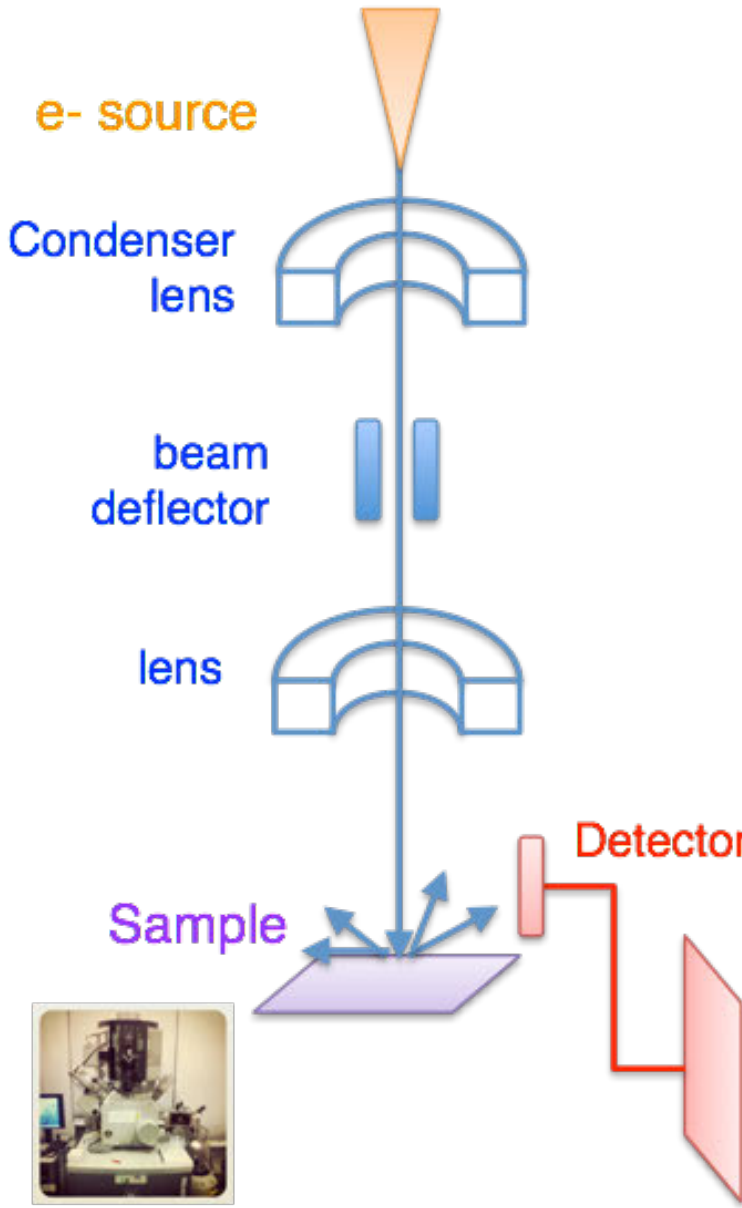
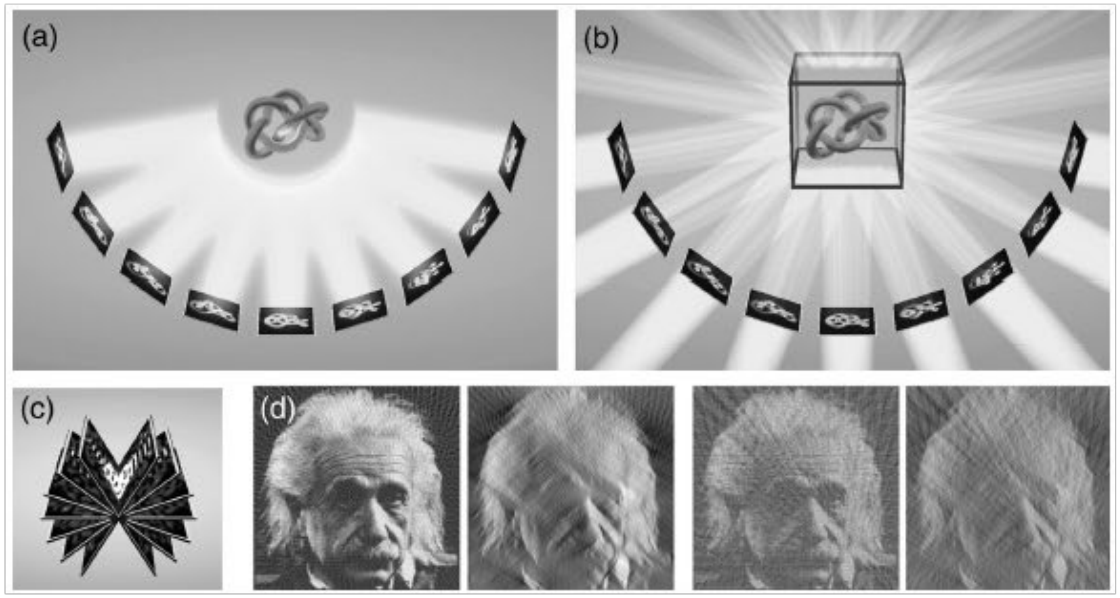
Comparison of a light microscope, TEM & SEM

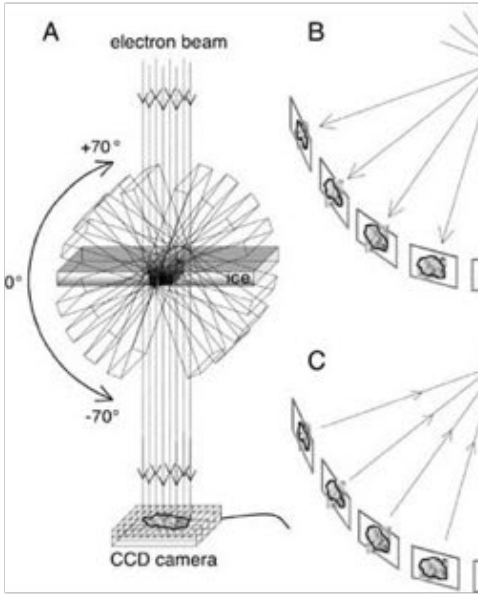






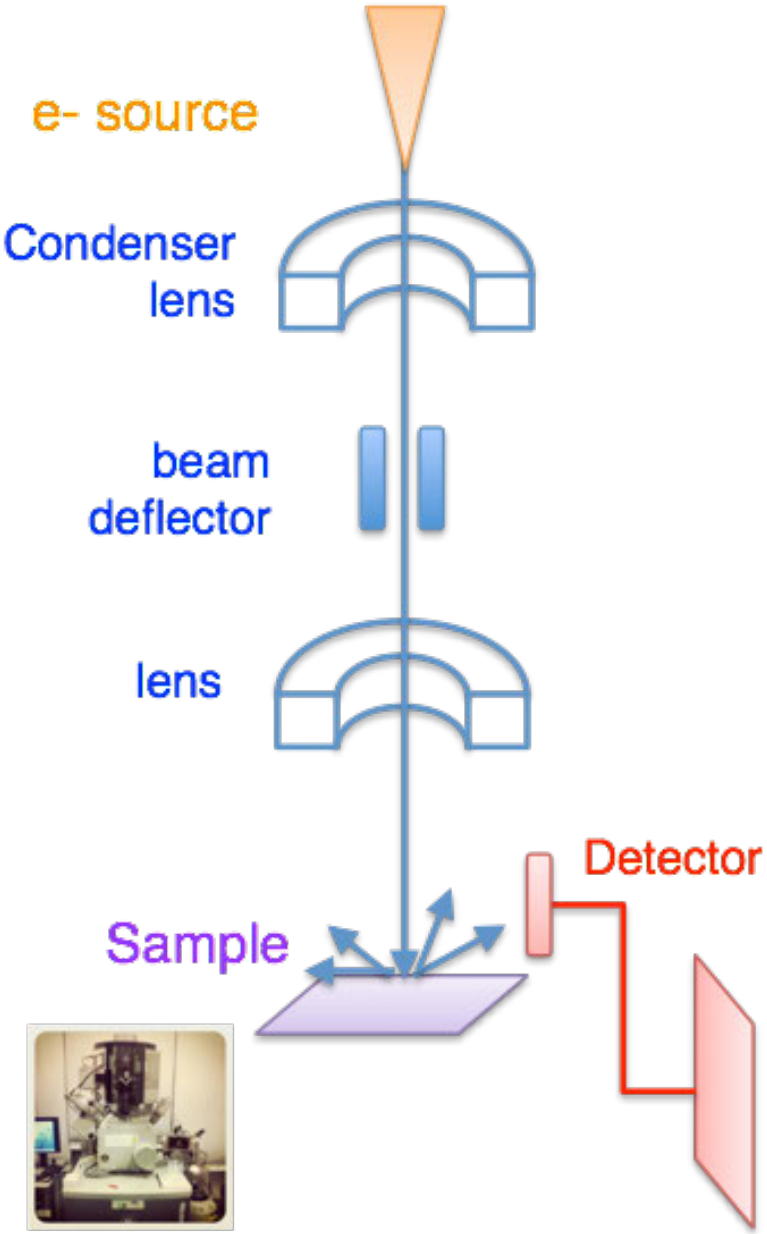
Obtaining a 3D structure from a 2D image

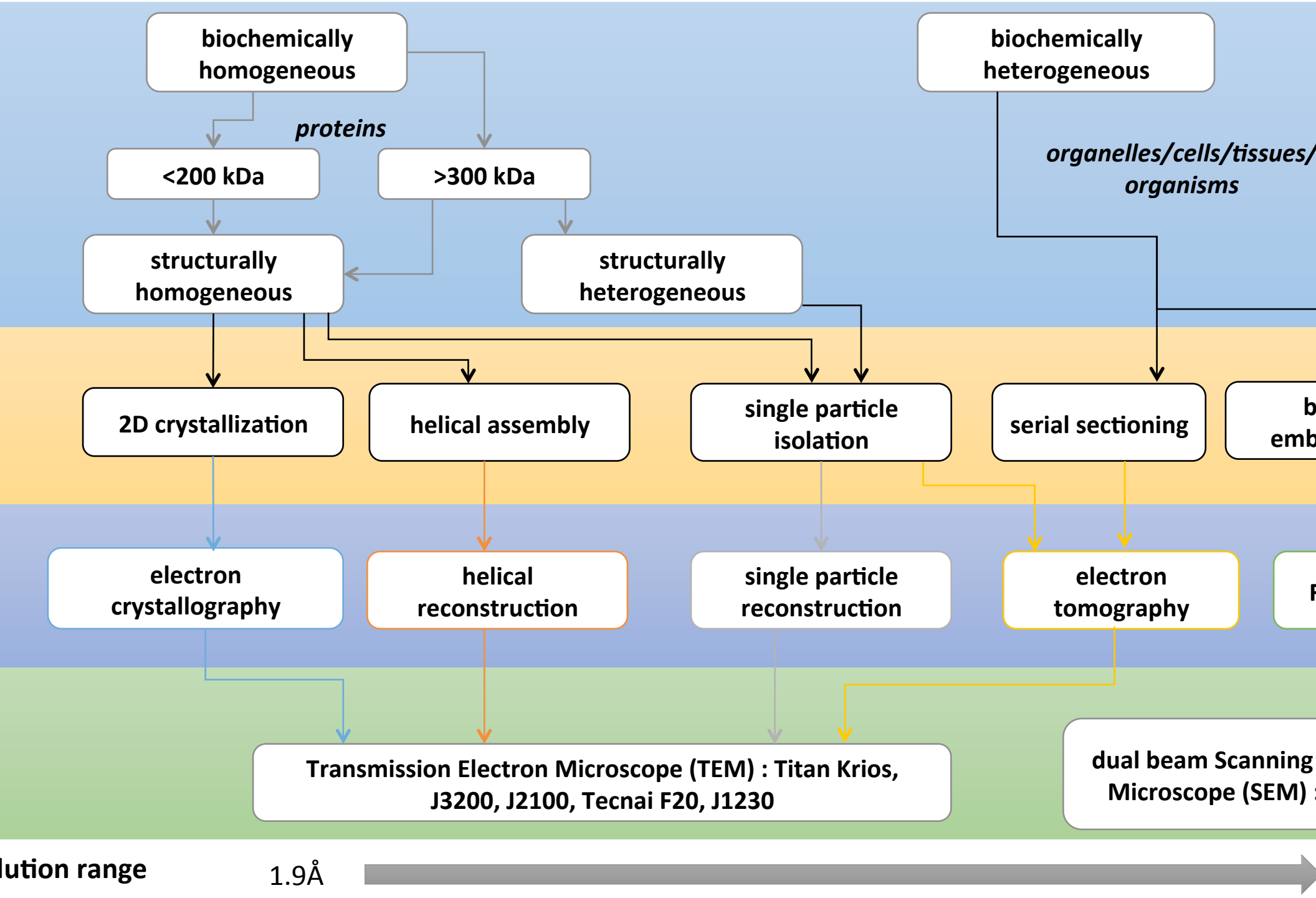




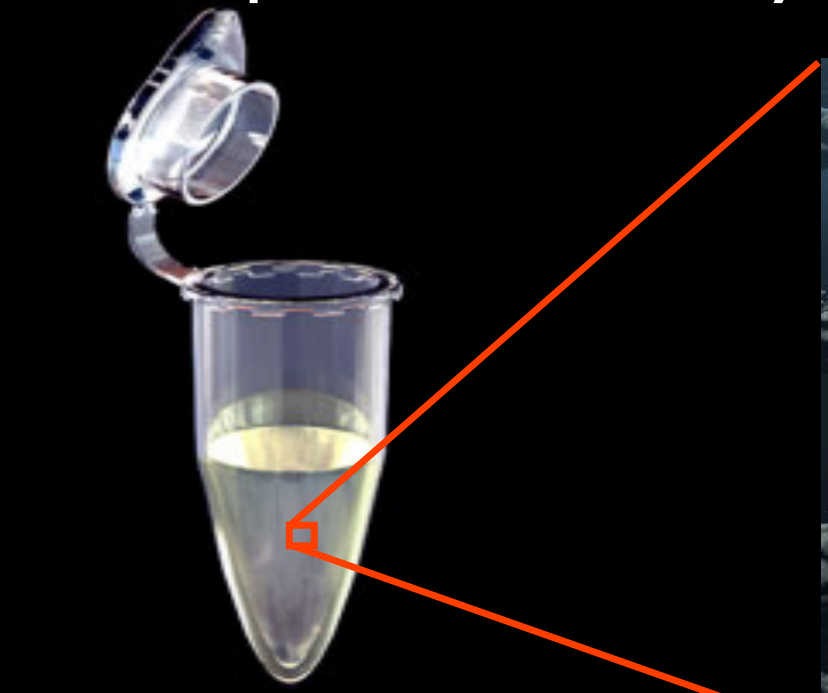
From W. Baumeister et al. Trend in Cell Biology 9(1999)81

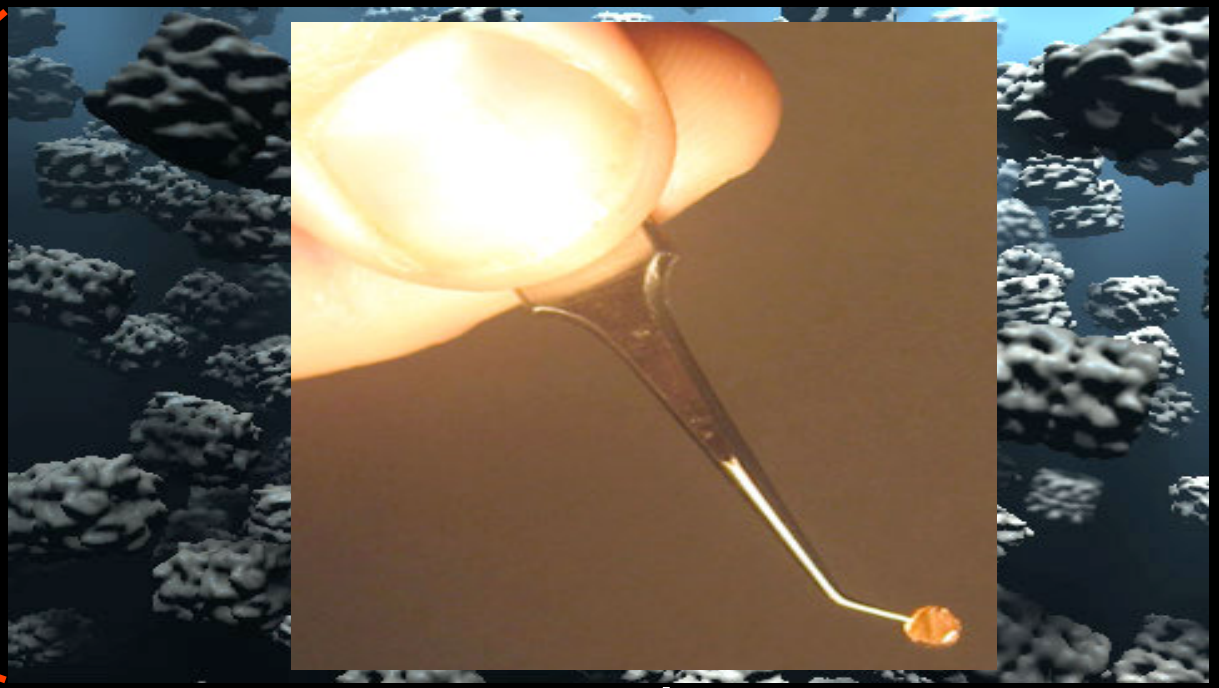
Obtaining a 3D structure from a 2D image

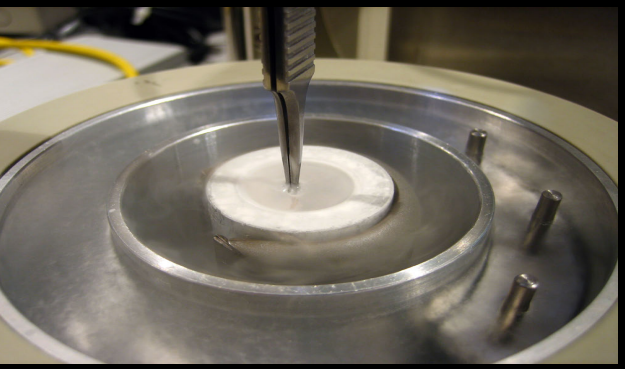


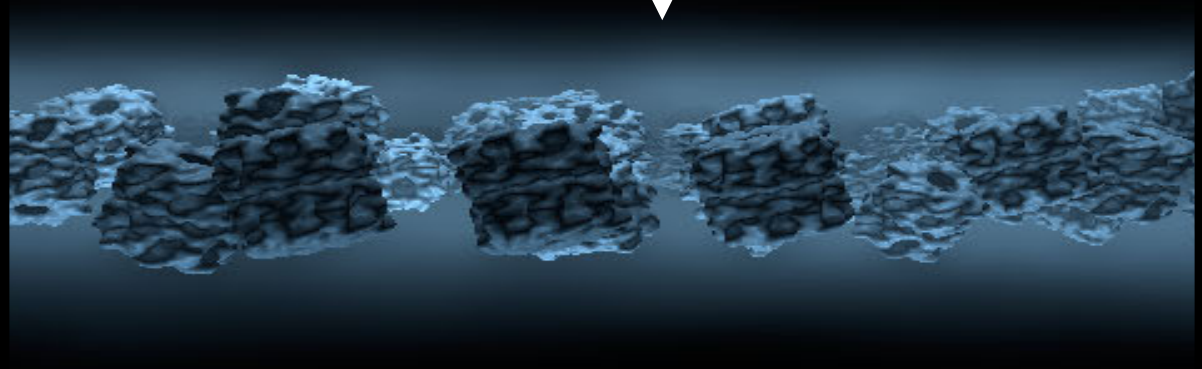

biochemically homogeneous
biochemically heterogeneous
proteins
organelles/cells/tissues/ organisms
<200 kDa
>300 kDa
structurally homogeneous
structurally heterogeneous
2D crystallization
helical assembly
single particle isolation
serial sectioning
electron crystallography
helical reconstruction
single particle reconstruction
electron tomography
Transmission Electron Microscope (TEM) : Titan Krios, J3200, J2100, Tecnai F20, J1230
dual beam Scanning Microscope (SEM)
lution range
1.9Å

cation process for CryoTEM

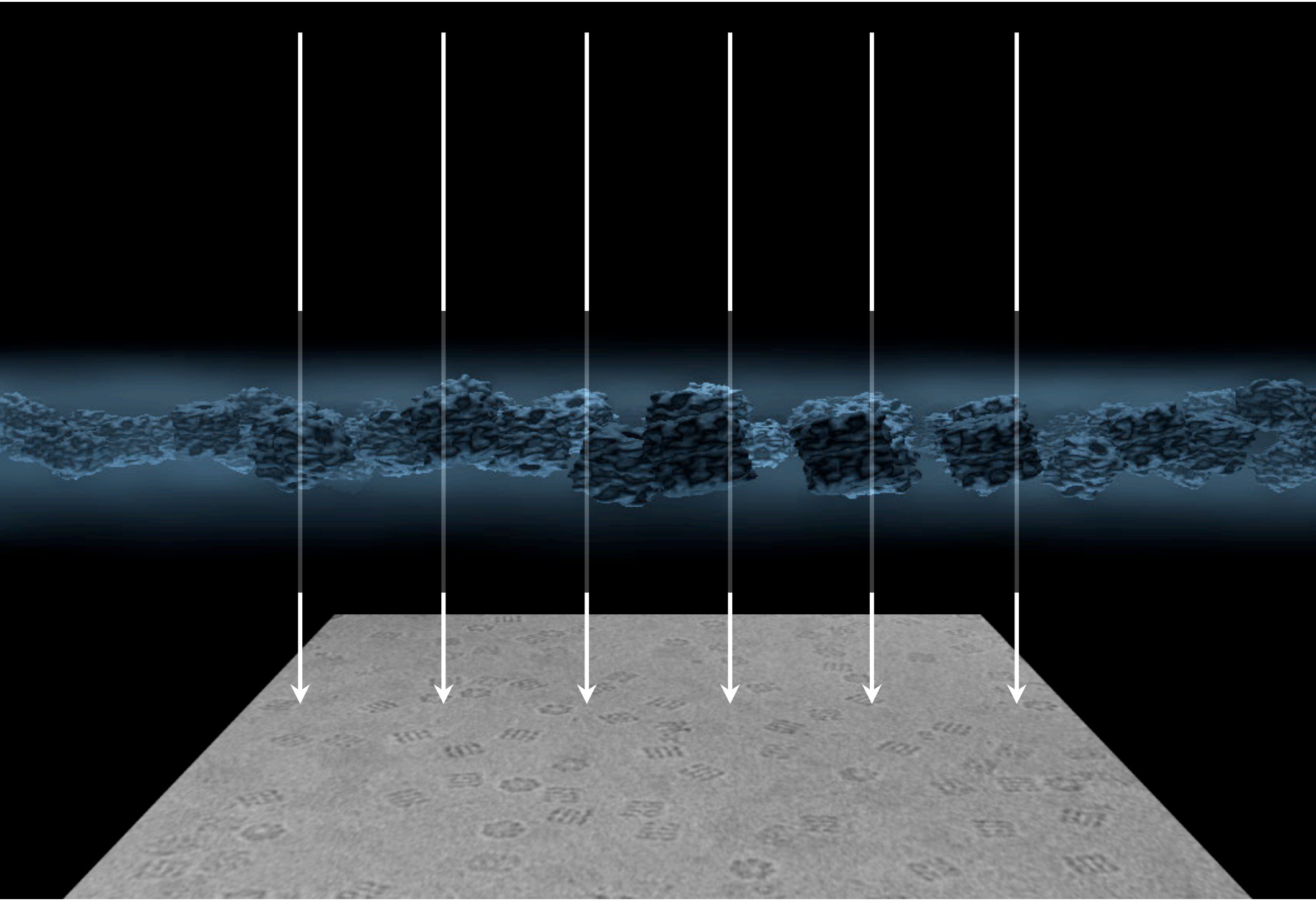


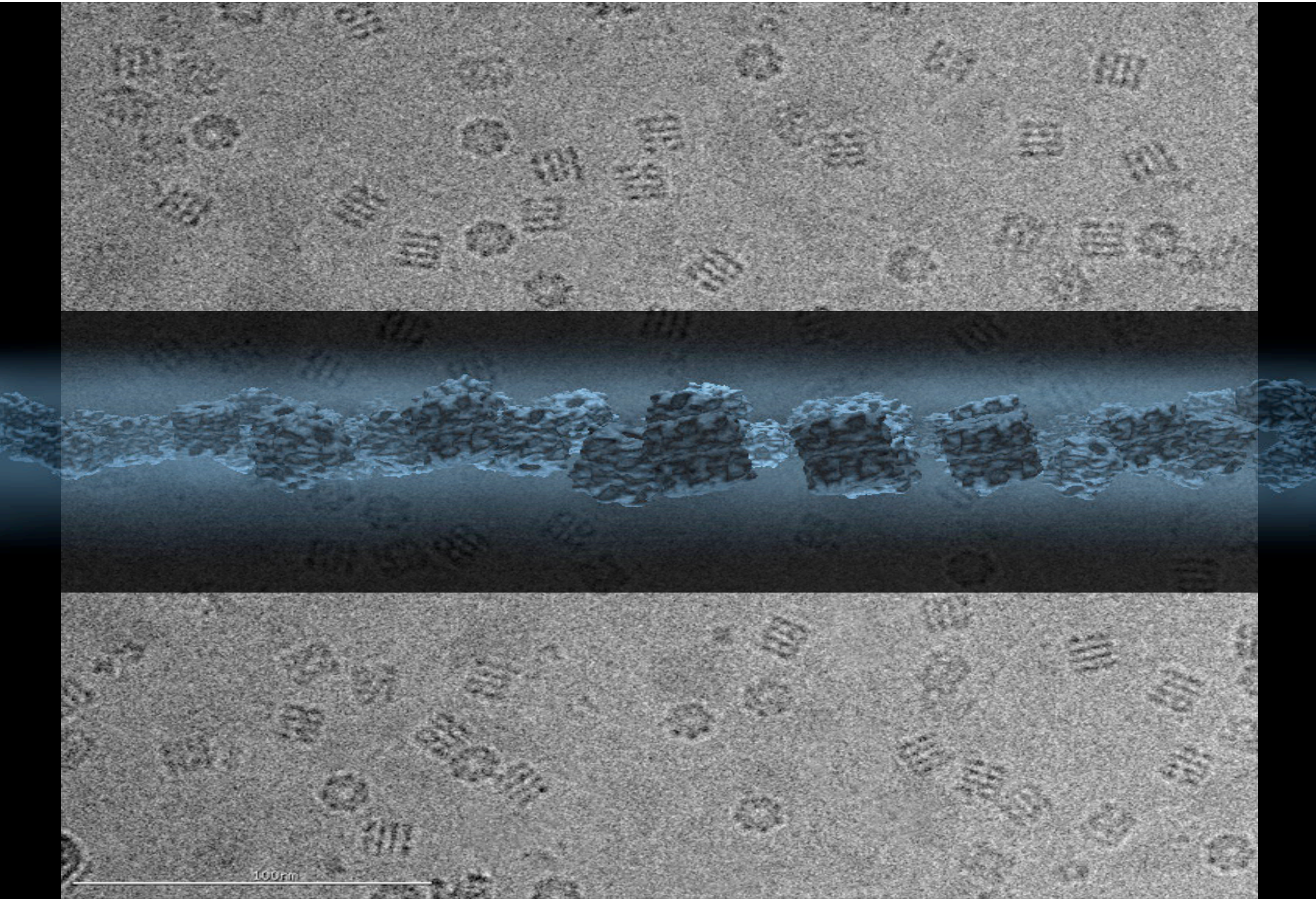


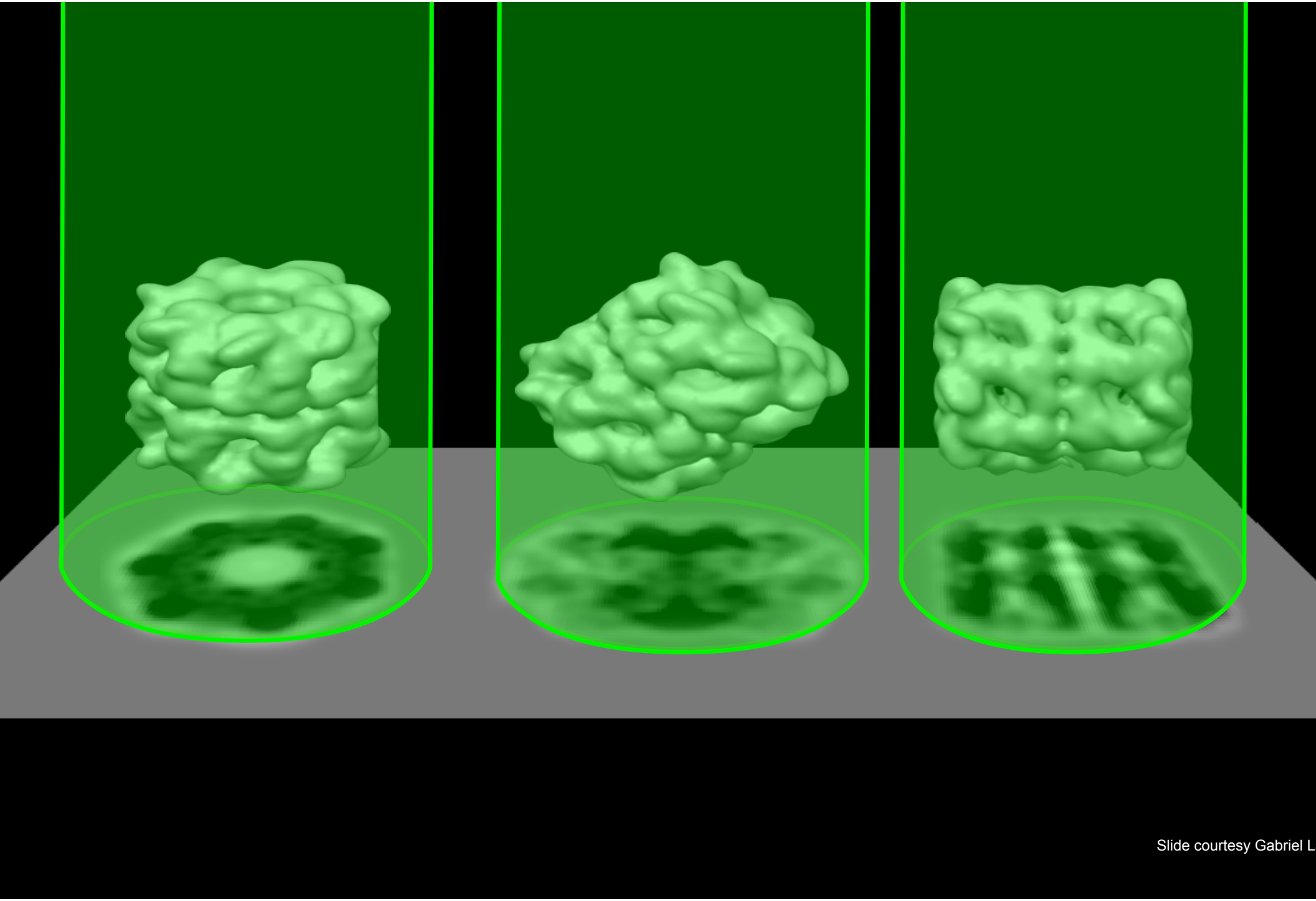


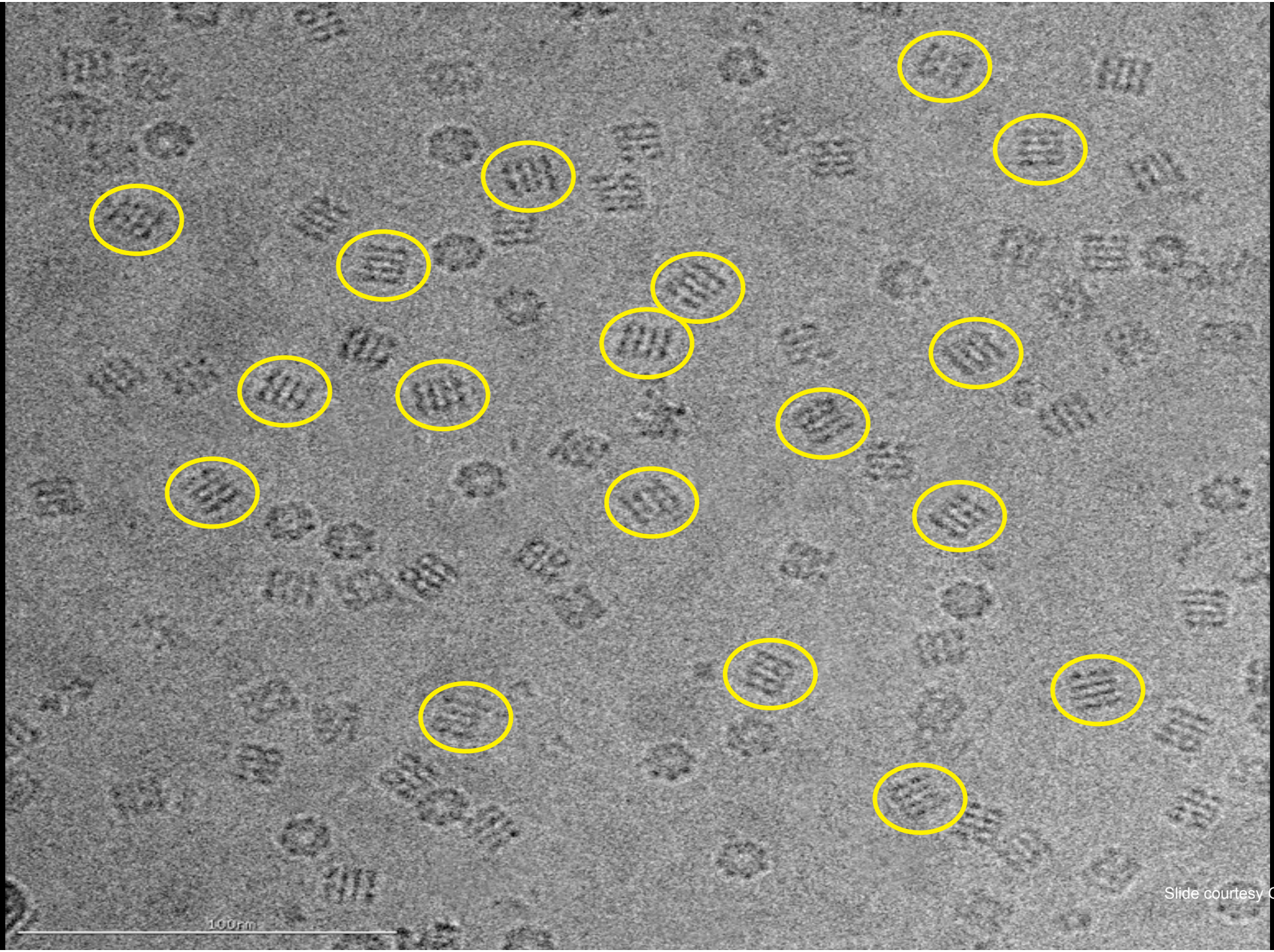


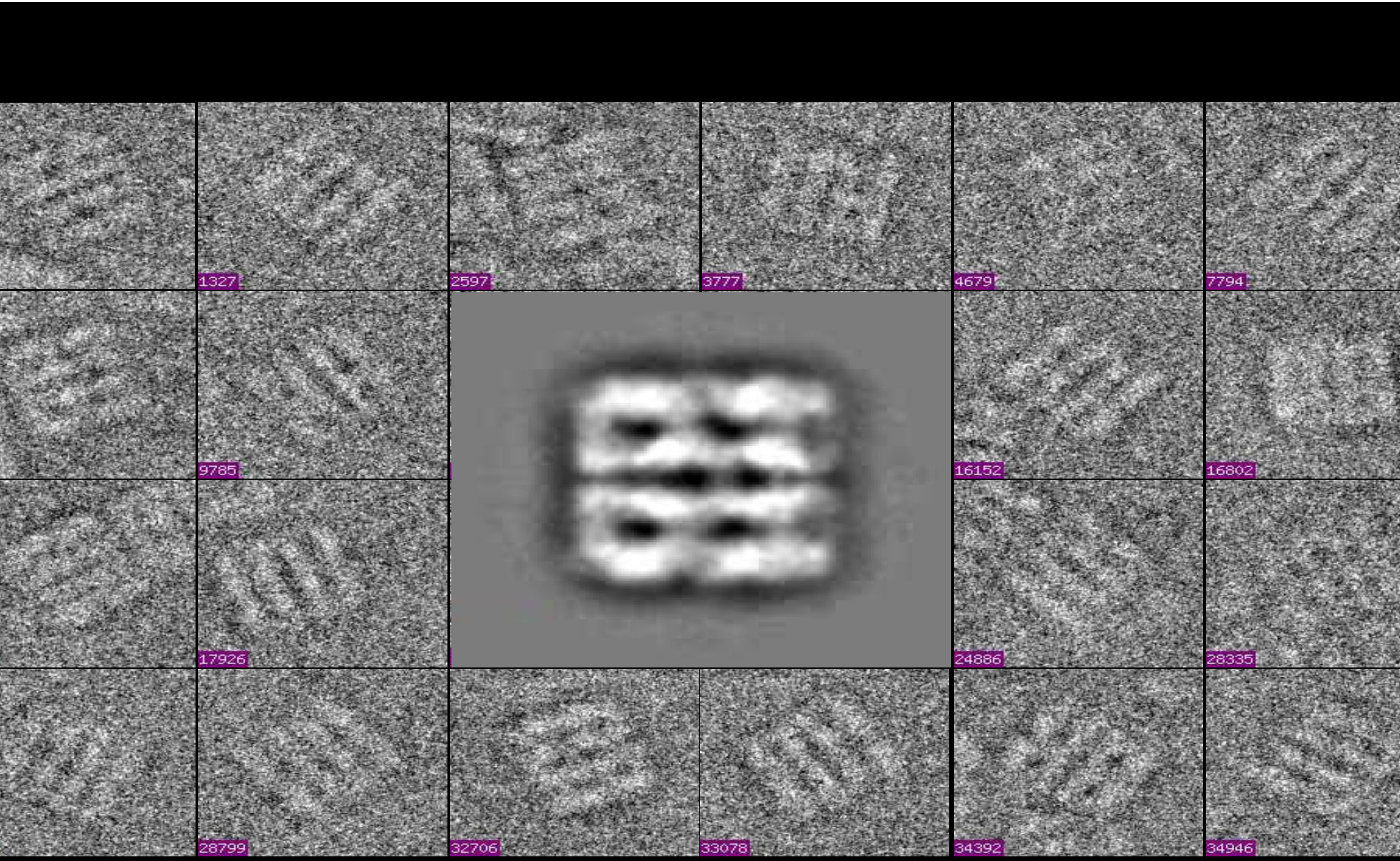
Slide courtesy Gabrie



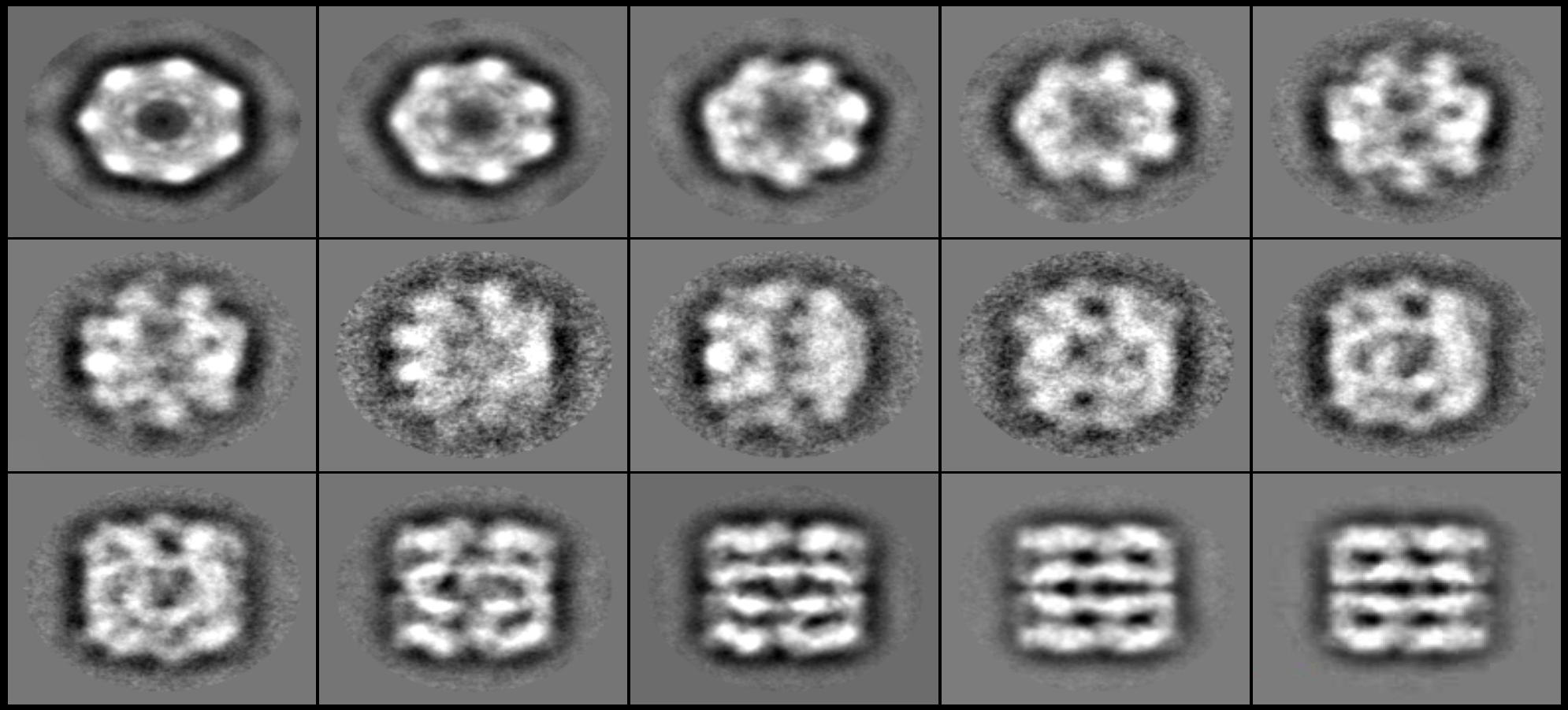

100nm


Slide courtesy Gabriel L


100nm
Slide courtesy Gabriel L



Slide courtesy Gabriel L



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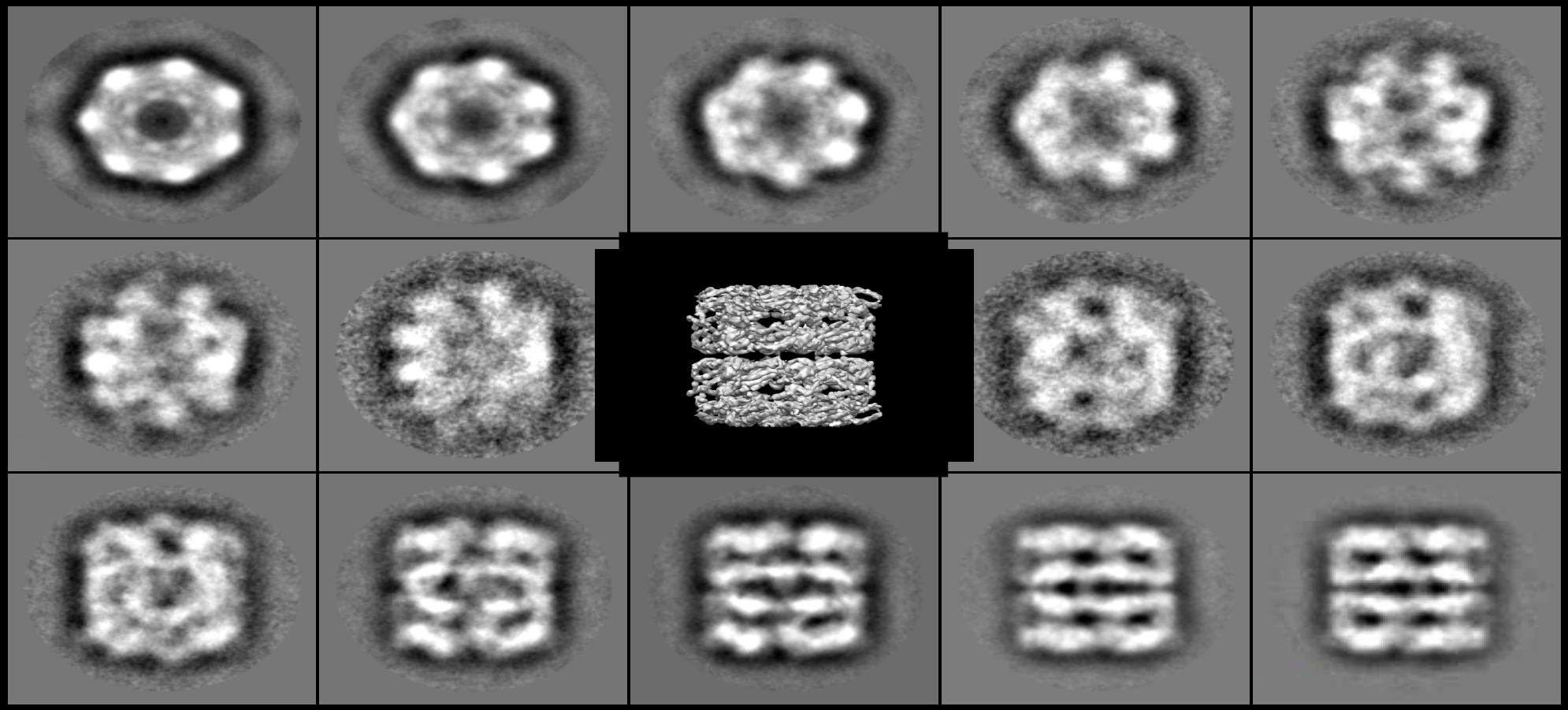
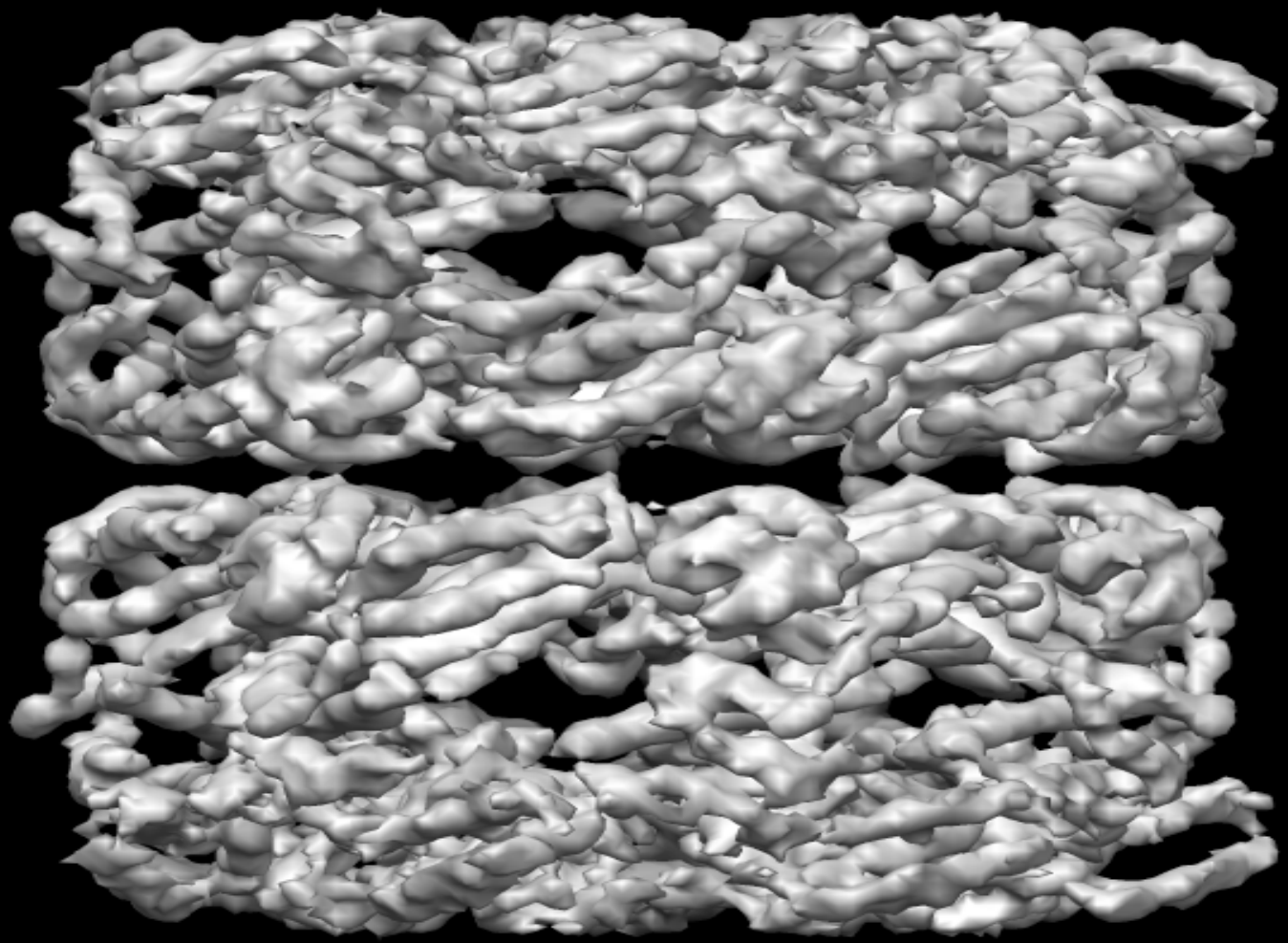
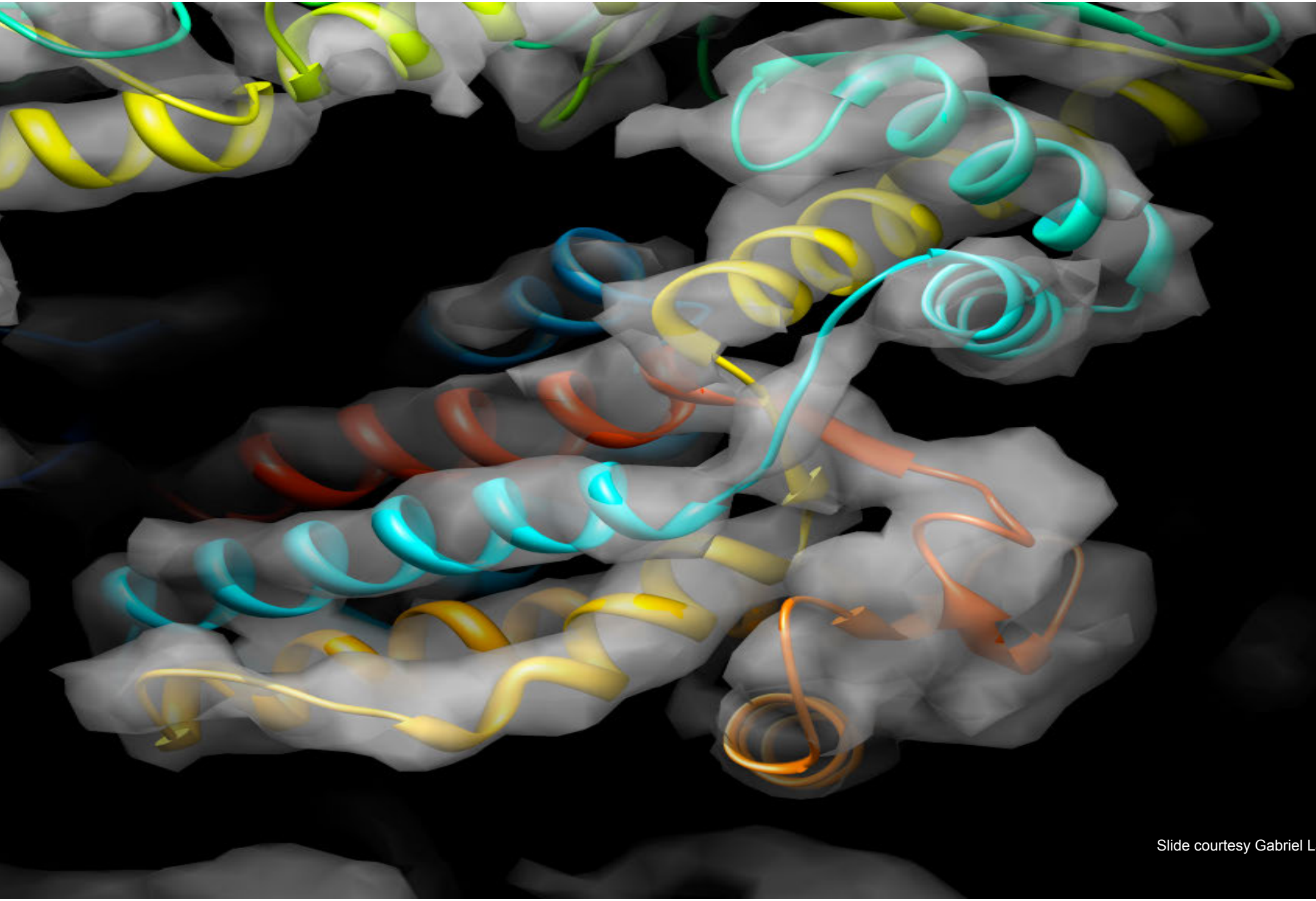


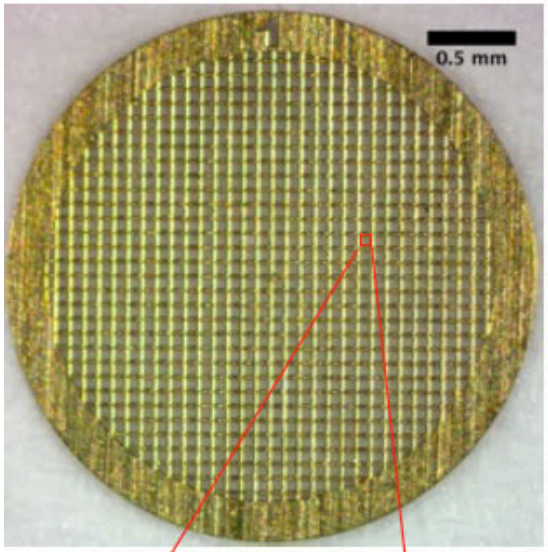
Slide courtesy Gabriel L

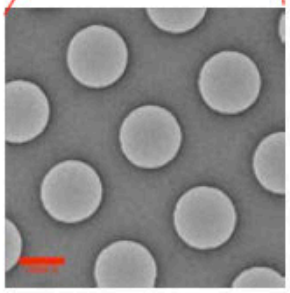


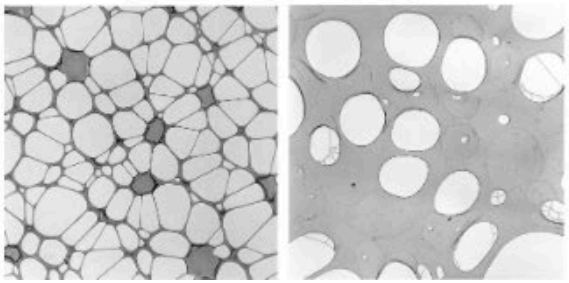
Slide courtesy Gabriel L

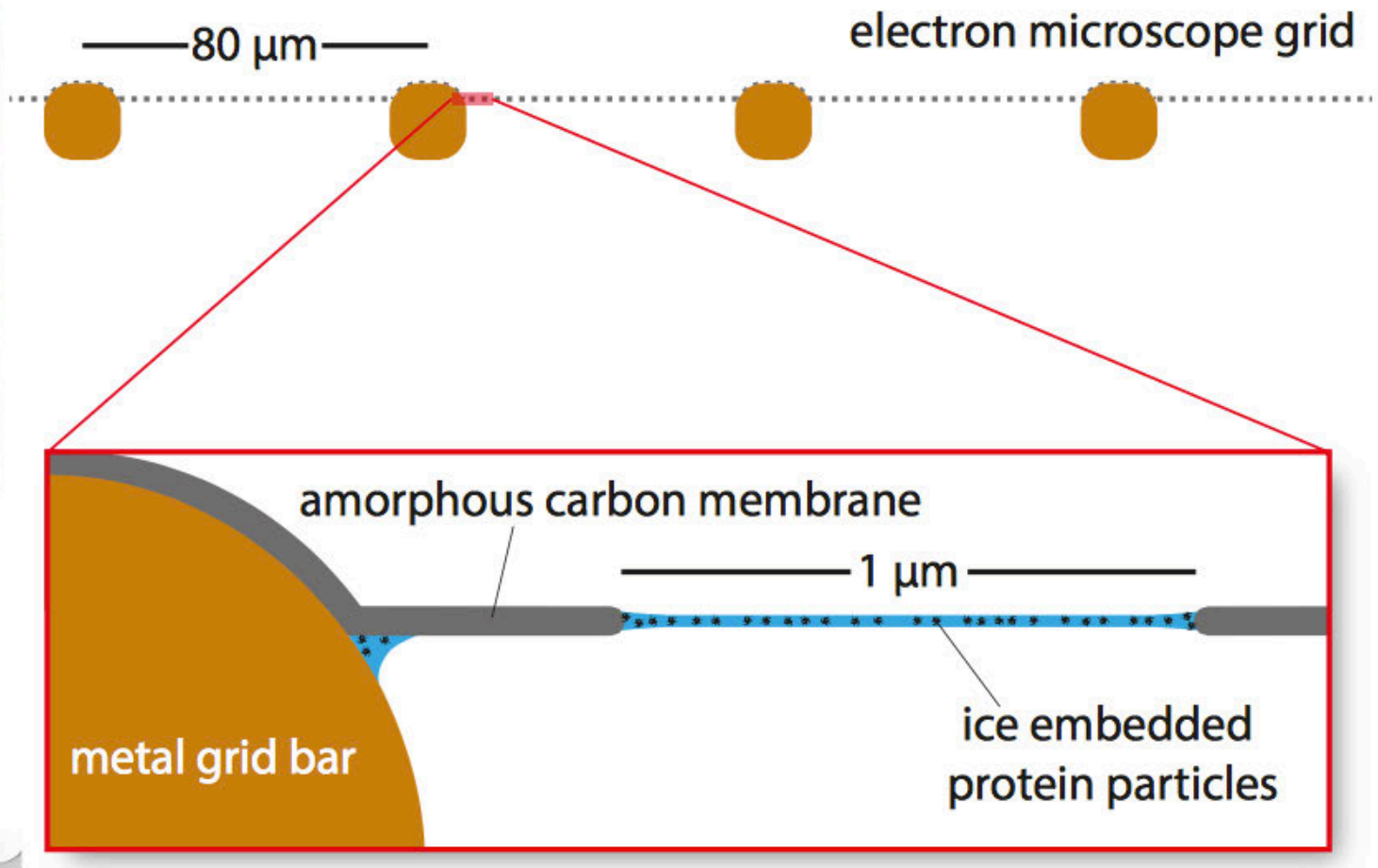

Slide courtesy Gabriel L

Traditional substrates for cryo-EM









Quantifoil, C-flat
Cryomesh

Passmore, 2

Traditional substrates for cryo-EM

- Proteins interact with surfaces present during the blotting process

  ➡ Denaturation of proteins, preferential orientations

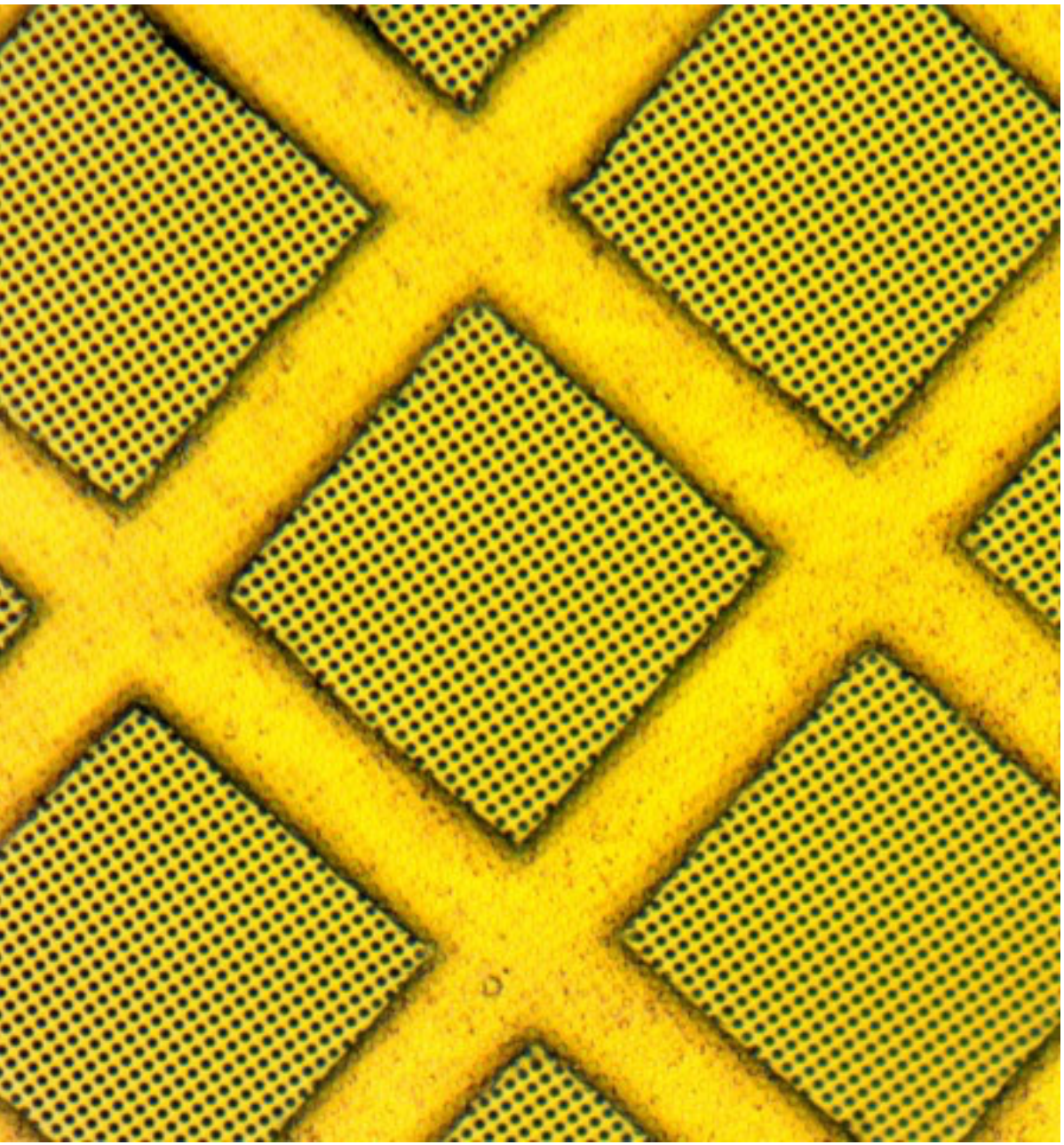
- Electron radiation induces motion of the particles and substrates

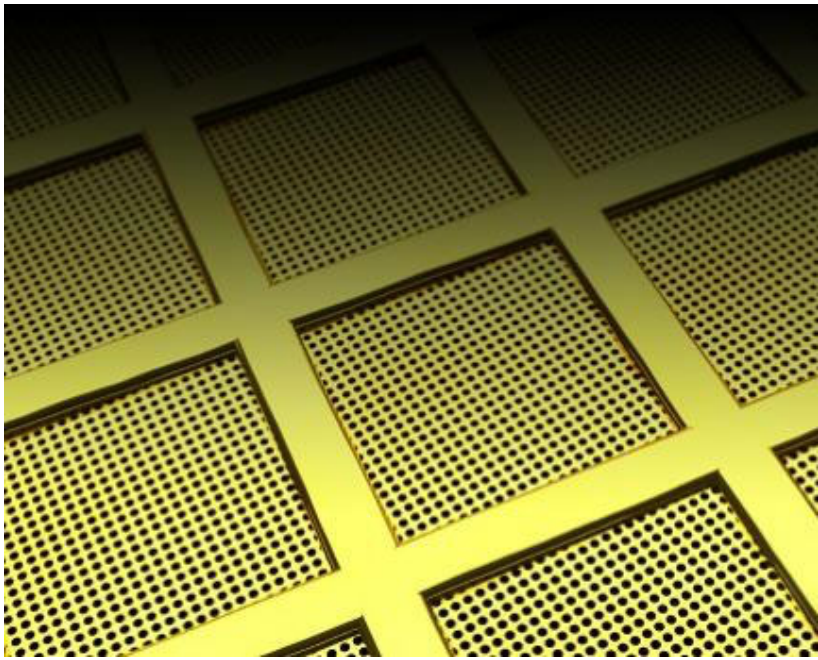
  ➡ Image blurring

- Additional layer of carbon reduces signal to noise per particle

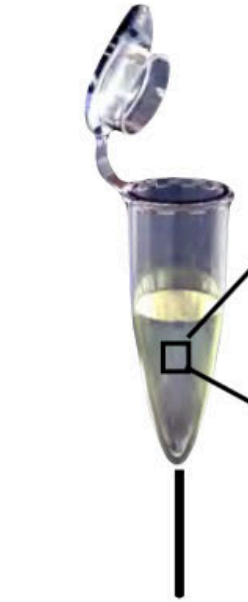
  ➡ alignment more difficult

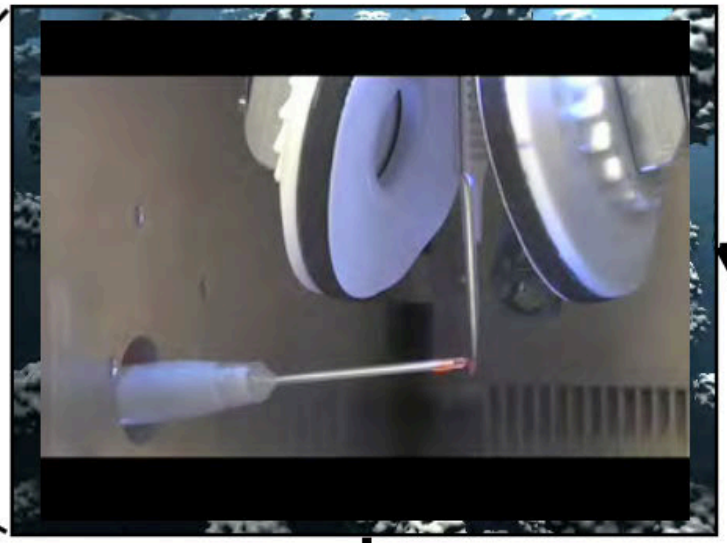
- Overall lack of reproducibility from grid to grid





Current CryoTEM Specimen Preparation

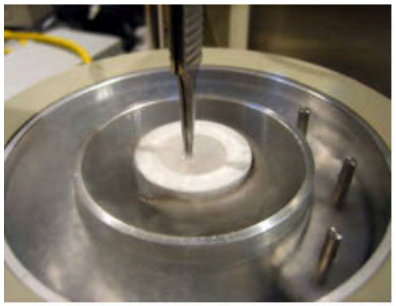


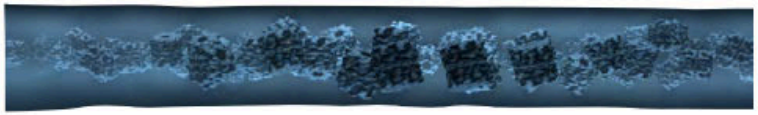


3µl

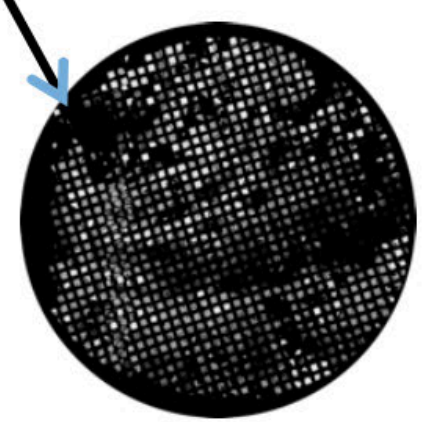
99.9%

0.1%



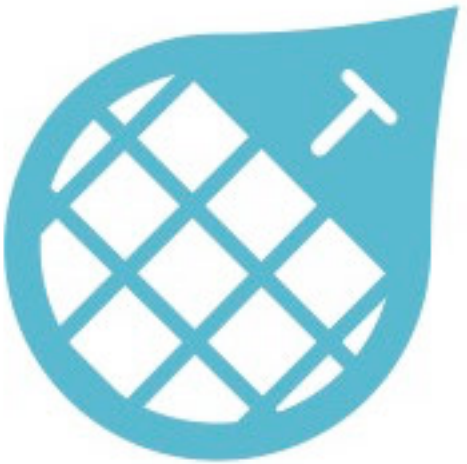


3nl

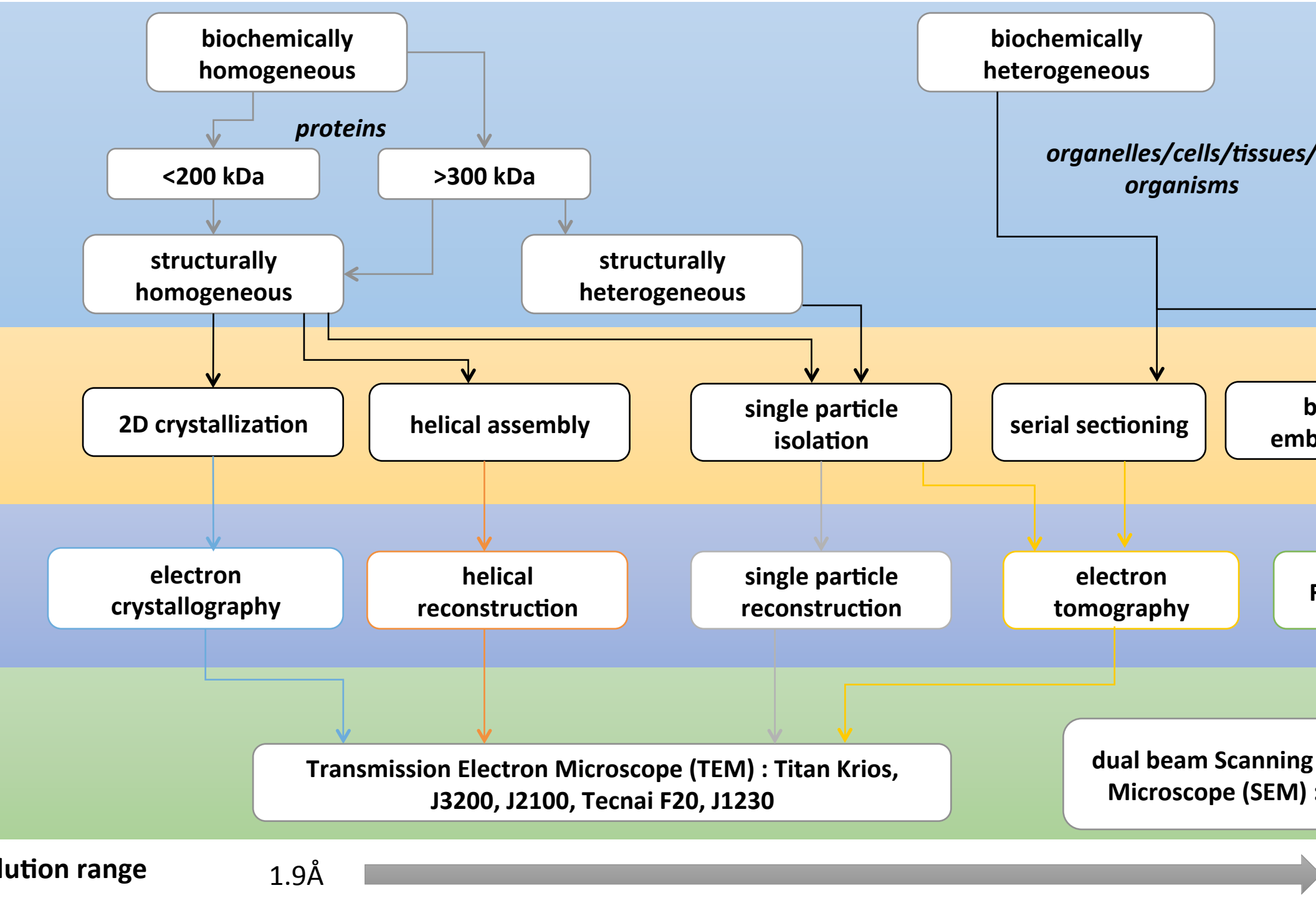


>100,000 potential targets for imaging; most of them are not usable.

Spotiton



Typhon


biochemically homogeneous
biochemically heterogeneous
proteins
organelles/cells/tissues/ organisms
<200 kDa
>300 kDa
structurally homogeneous
structurally heterogeneous
2D crystallization
helical assembly
single particle isolation
serial sectioning
electron crystallography
helical reconstruction
single particle reconstruction
electron tomography
Transmission Electron Microscope (TEM) : Titan Krios, J3200, J2100, Tecnai F20, J1230
dual beam Scanning Microscope (SEM)
lution range
1.9Å