SIMONS ELECTRON MICROSCOPY CENTER

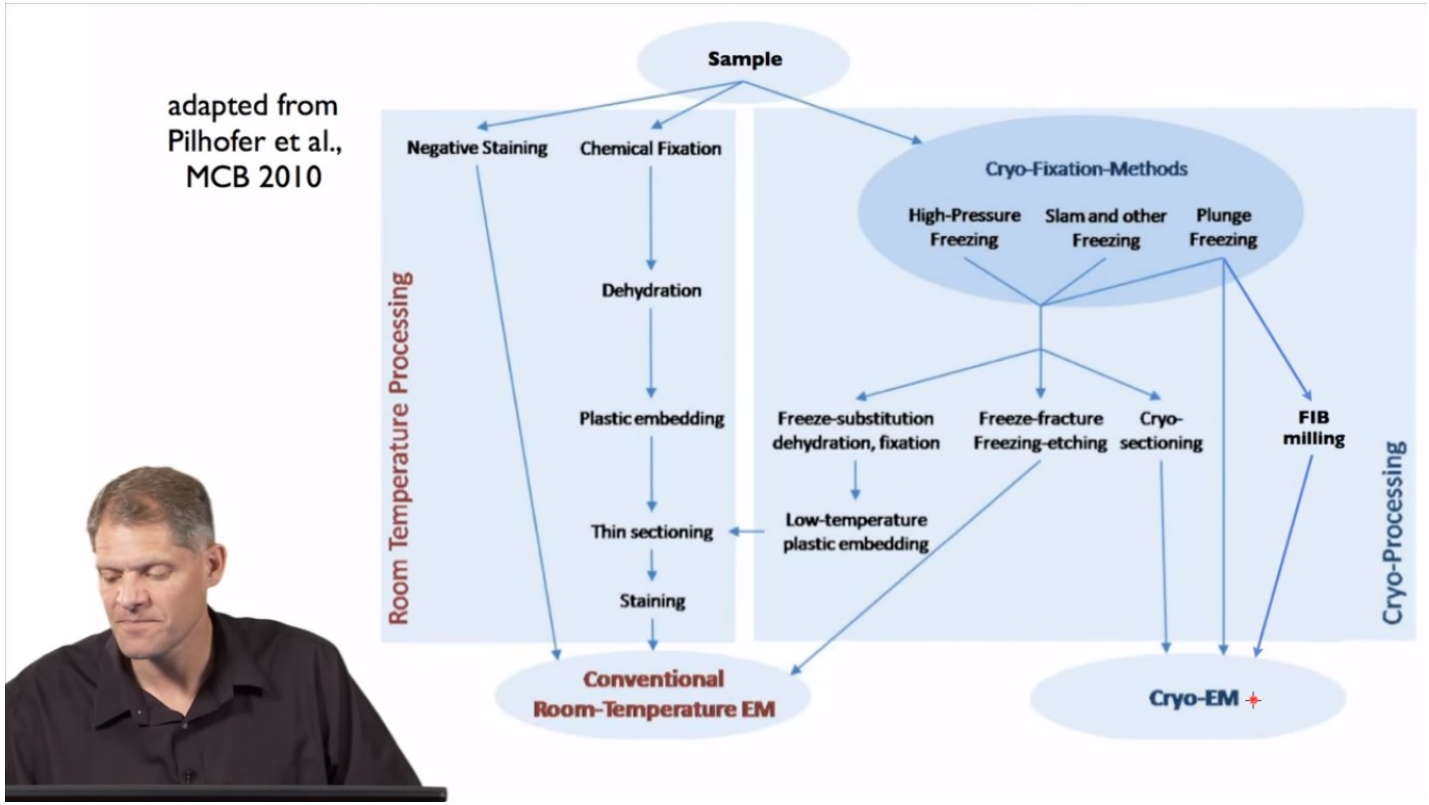
NEW YORK STRUCTURAL BIOLOGY CENTER

Simons Electron Microscopy Center EM course

Challenges in Biological EM & Sample Prep

2017/01/18

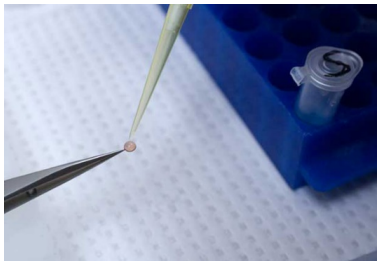
RT & Cryo Sample Prep Methods

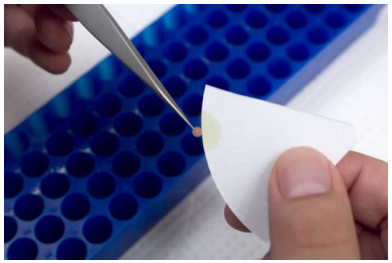


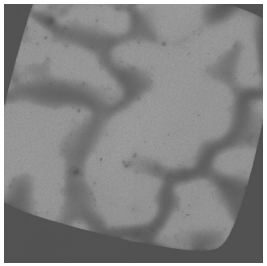
Negative Staining

- 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







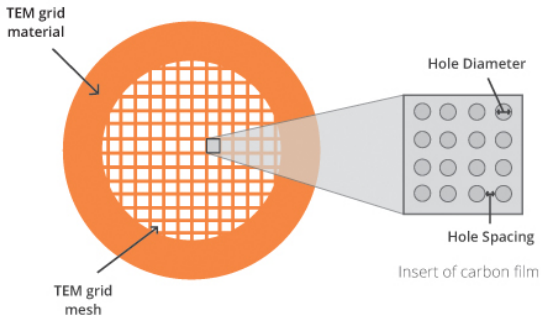
Baker, 2007

www.mcb.ucdavis.edu/cryoem/microscopy101.html

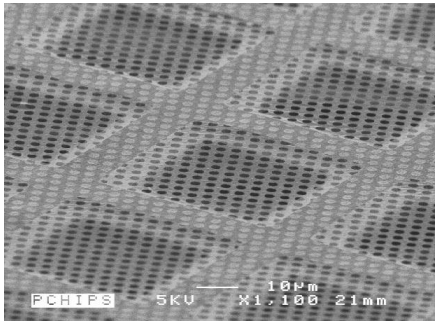
Plunge Freezing

- 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



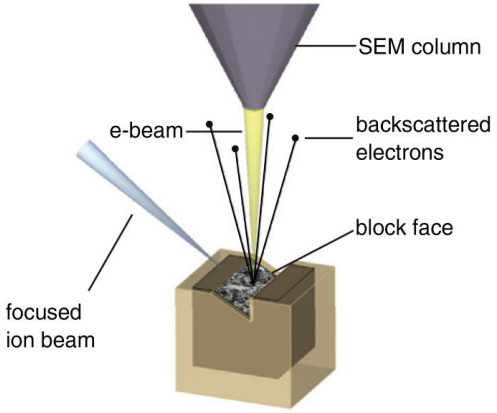
www.mcb.ucdavis.edu/cryoem/microscopy101.html

FIB/SEM vs Thin Section Sample Prep

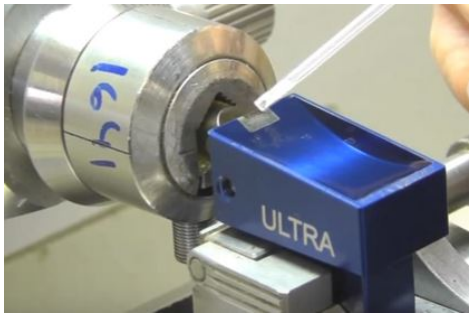
- Chemical fixation
- Staining
  - En bloc, enhanced contrast and electrical conductivity
- Dehydration
- Embedding

- Au/Pd coat
  - Conductivity

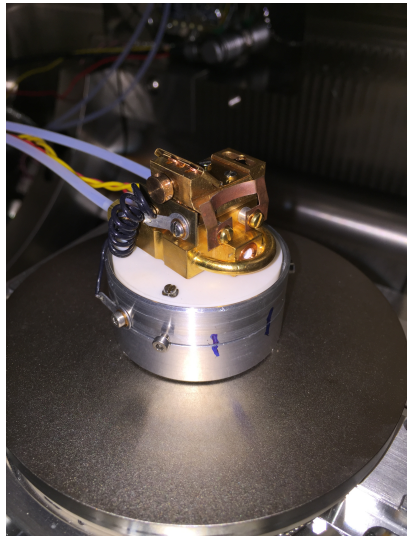
Cryofixation: High pressure freezing
Dehydration: Freeze substitution

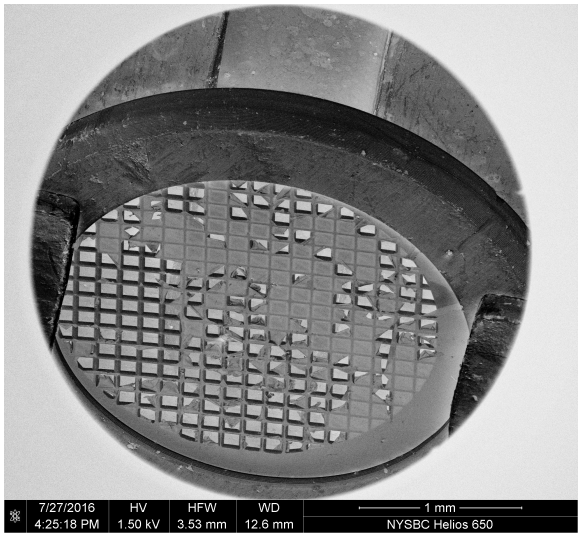


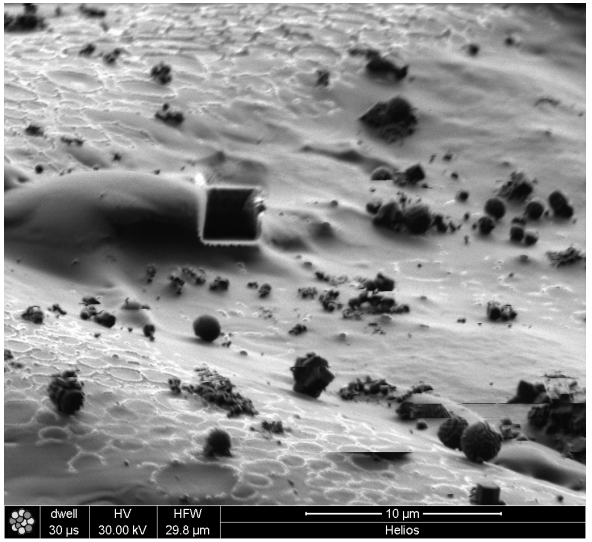
- Chemical fixation
- Dehydration
- Embedding
- Sectioning
- Staining



Cryo FIB Milling







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

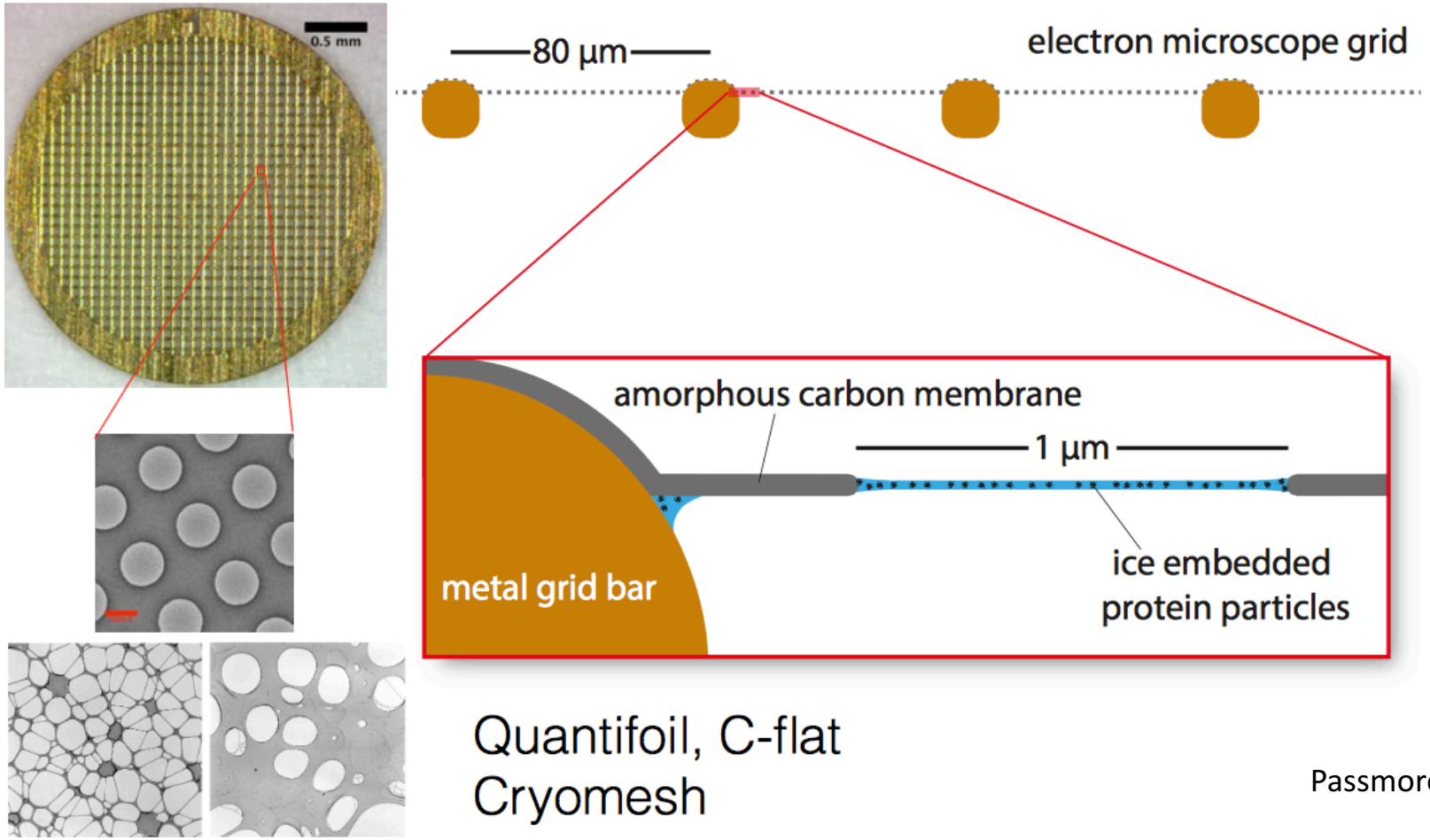
  ➡ Image blurring

- Additional layer of carbon reduces signal to noise per particle

  ➡ alignment more difficult

- Overall lack of reproducibility from grid to grid

Traditional substrates for cryo-EM



Quantifoil, C-flat
Cryomesh

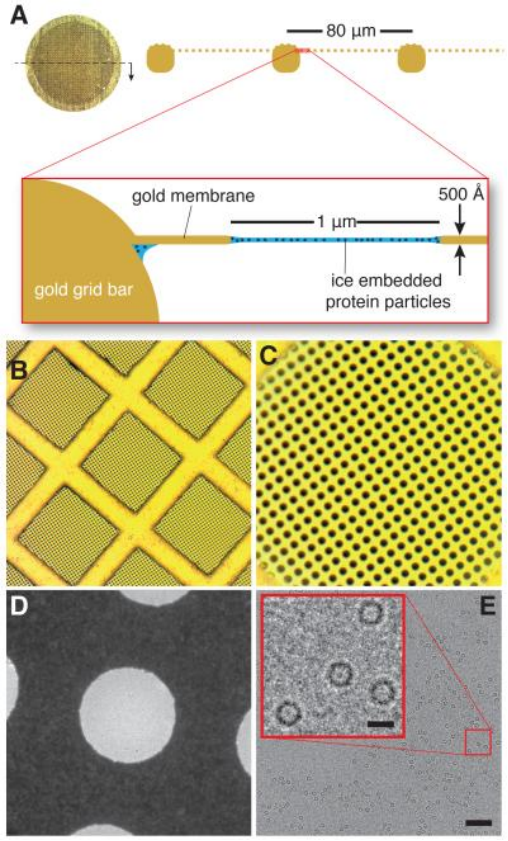
Passmore, 2014

Gold Grids

- Holey gold foil on gold mesh grid

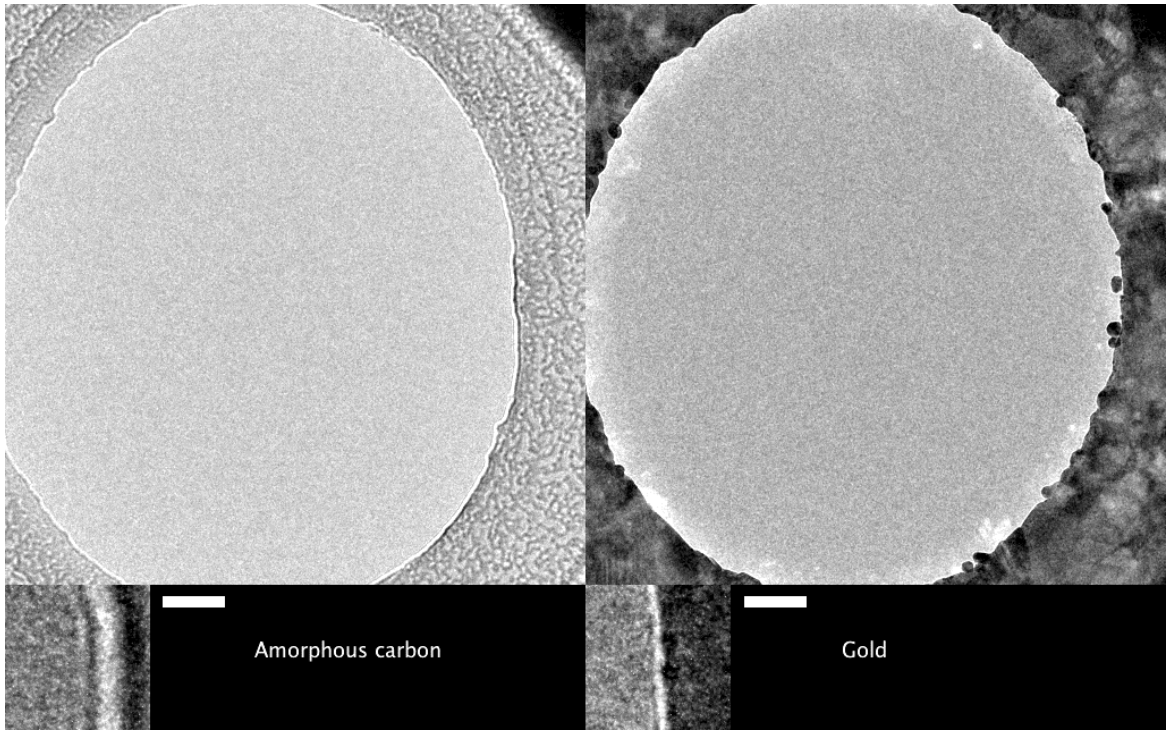
Advantages:
- Prevents differential thermal contraction when freezing
- Reduces beam-induced specimen movement
- Combined with direct detector technology allows for near atomic resolution

Disadvantages:
- Difficult to find focus due to lack of amorphous substrate



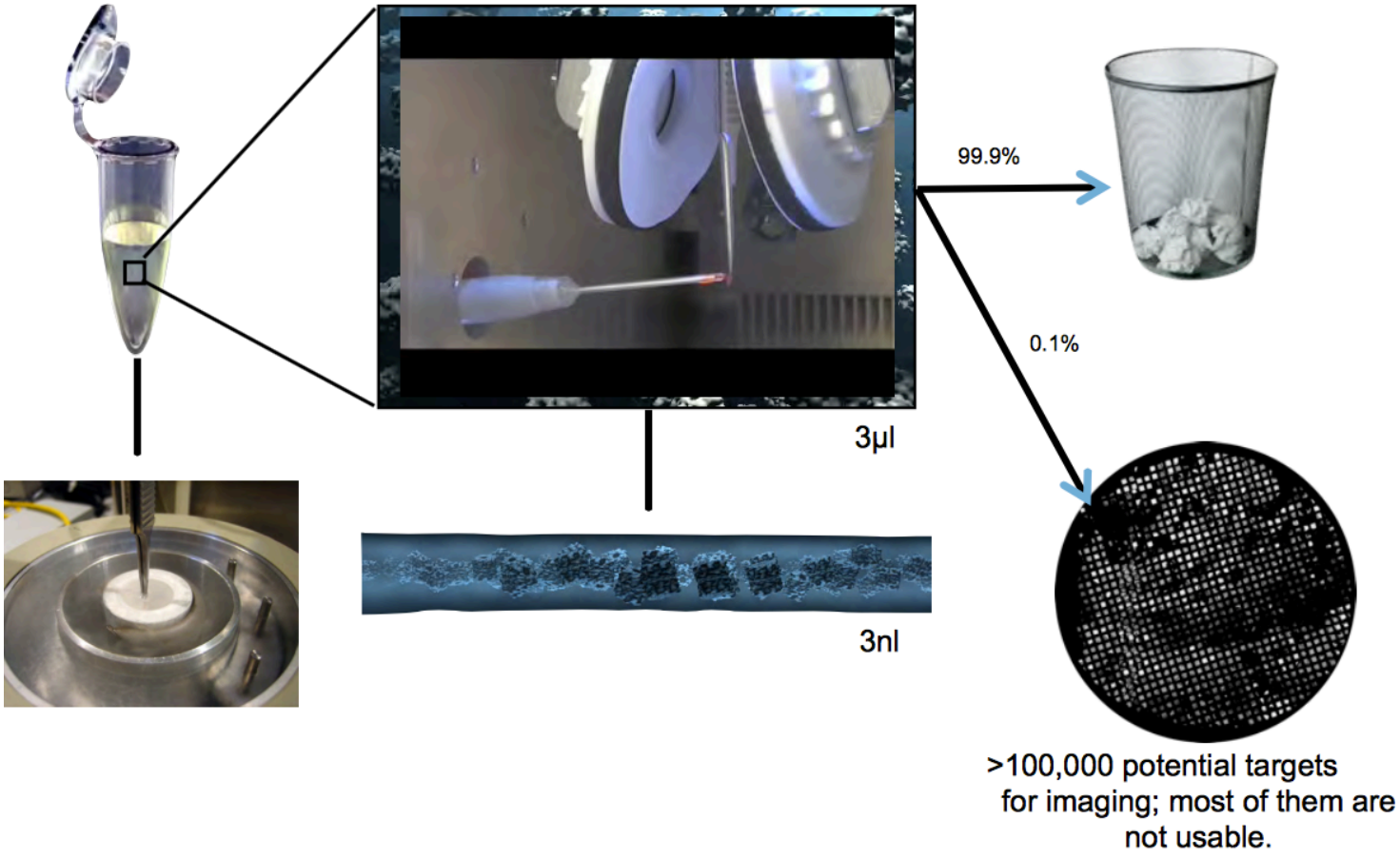
Russo & Passmore, 2015

Gold Grids



Russo & Passmore, 2015

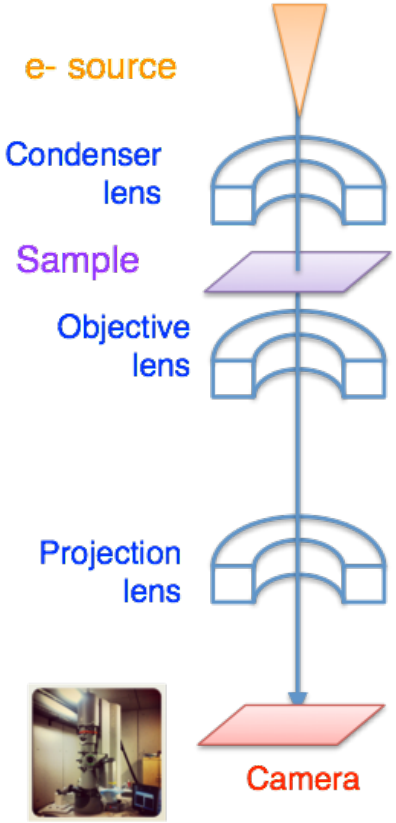
Current CryoTEM Specimen Preparation

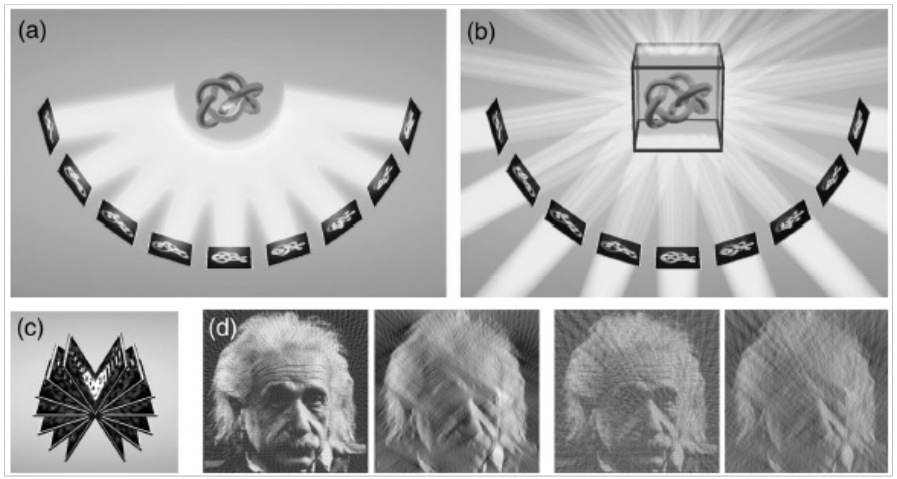


Spotiton

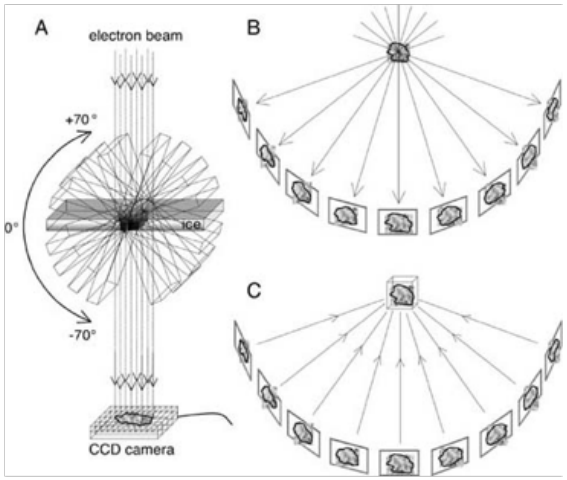
Typhon

Obtaining a 3D structure from a 2D image

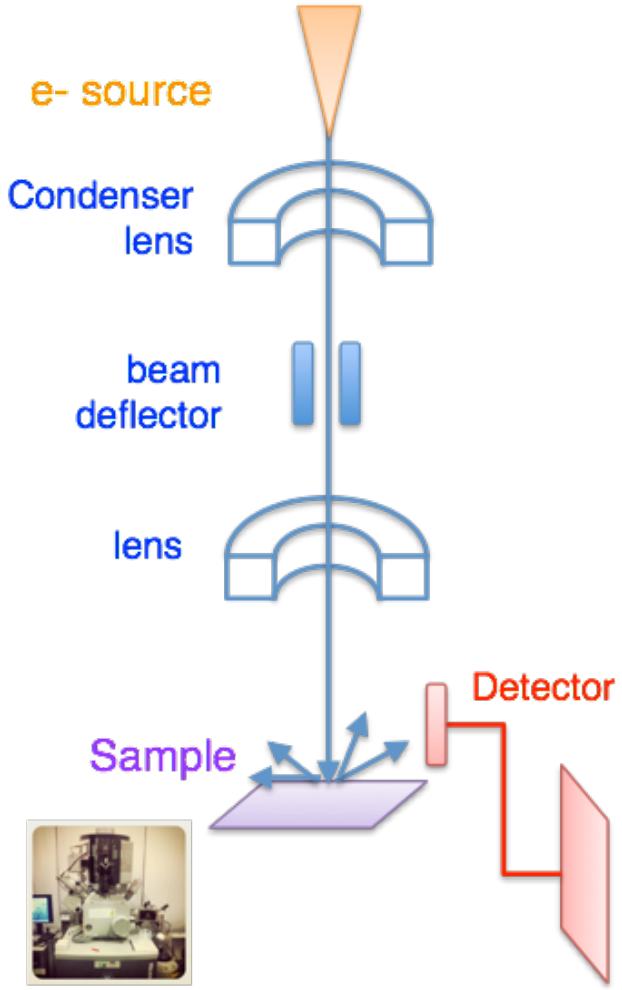


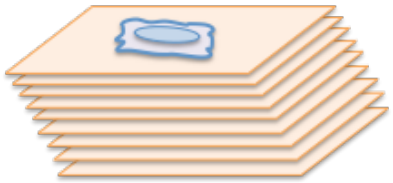


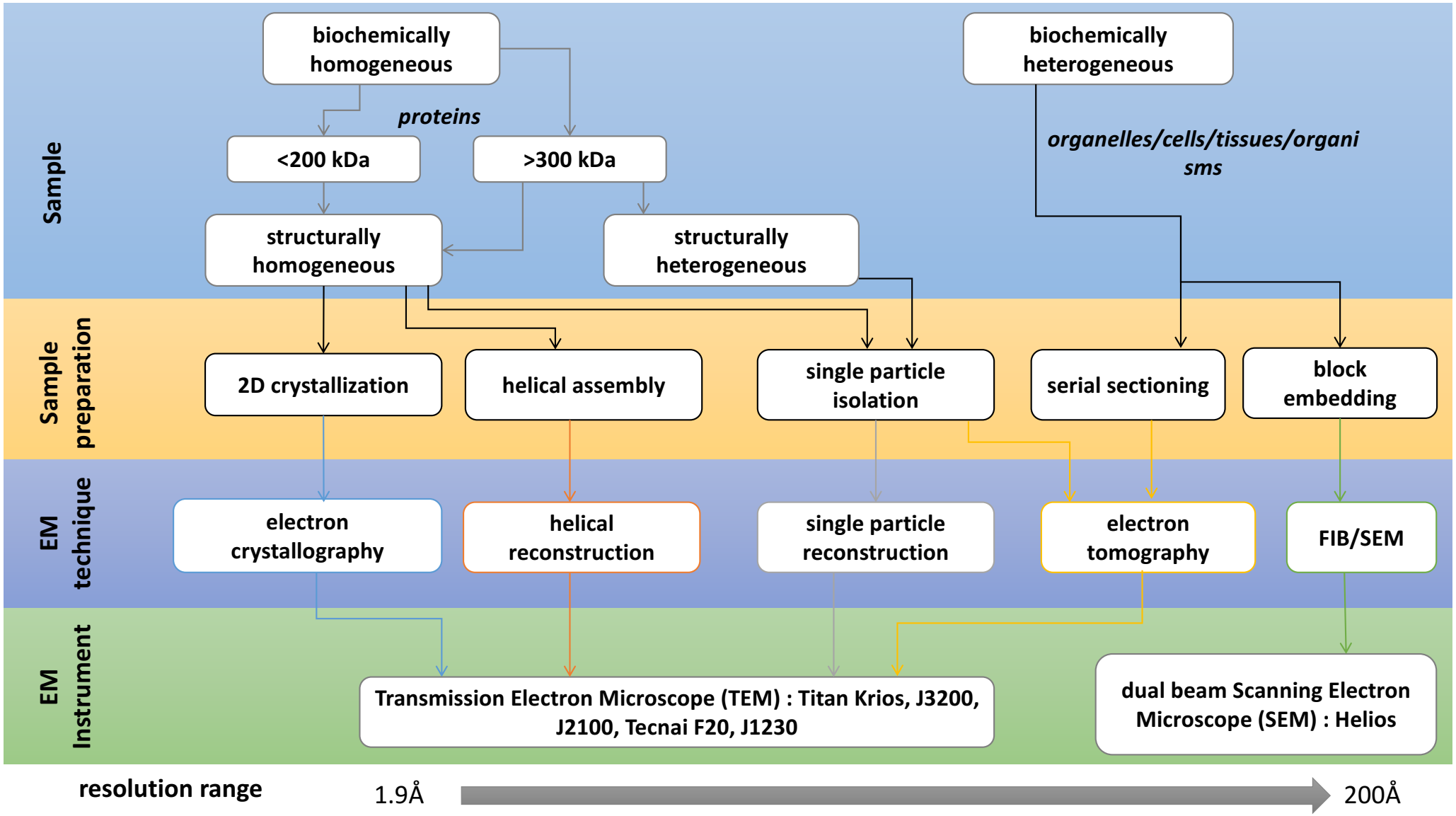
From W. Baumeister et al. Trend in Cell Biology 9(1999)81



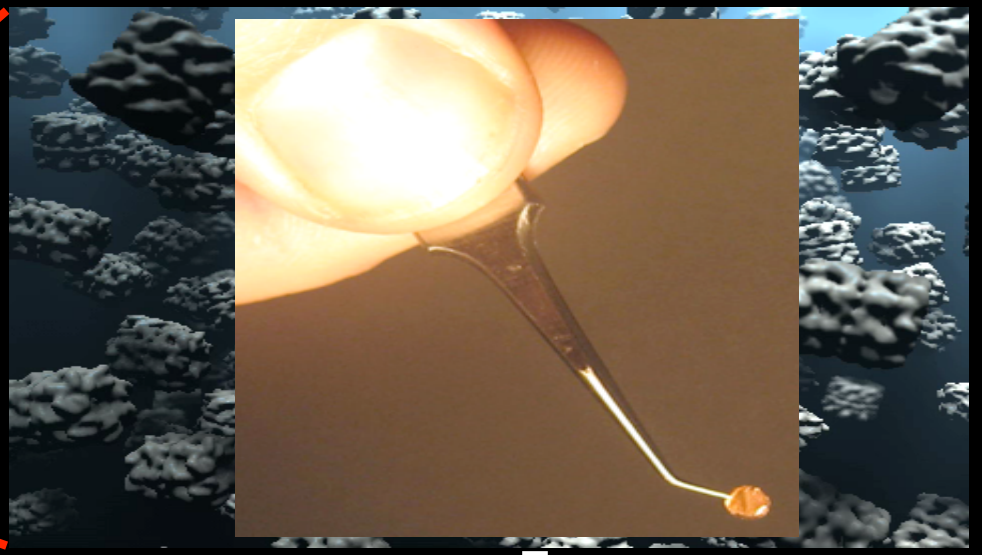
Obtaining a 3D structure from a 2D image

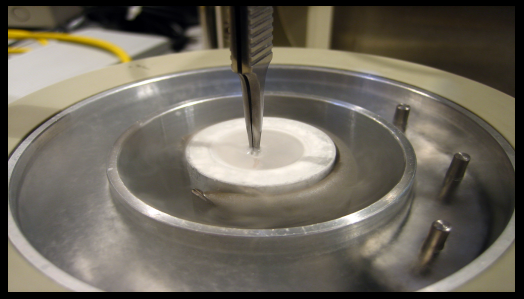


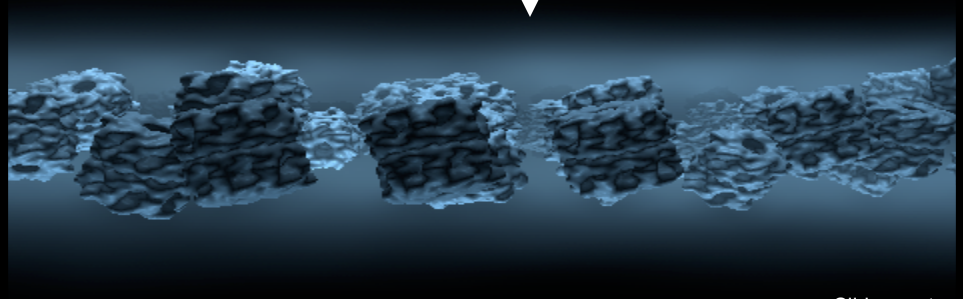



Sample
Sample preparation
EM technique
EM Instrument
biochemically homogeneous
biochemically heterogeneous
proteins
<200 kDa
>300 kDa
organelles/cells/tissues/organisms
structurally homogeneous
structurally heterogeneous
2D crystallization
helical assembly
single particle isolation
serial sectioning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

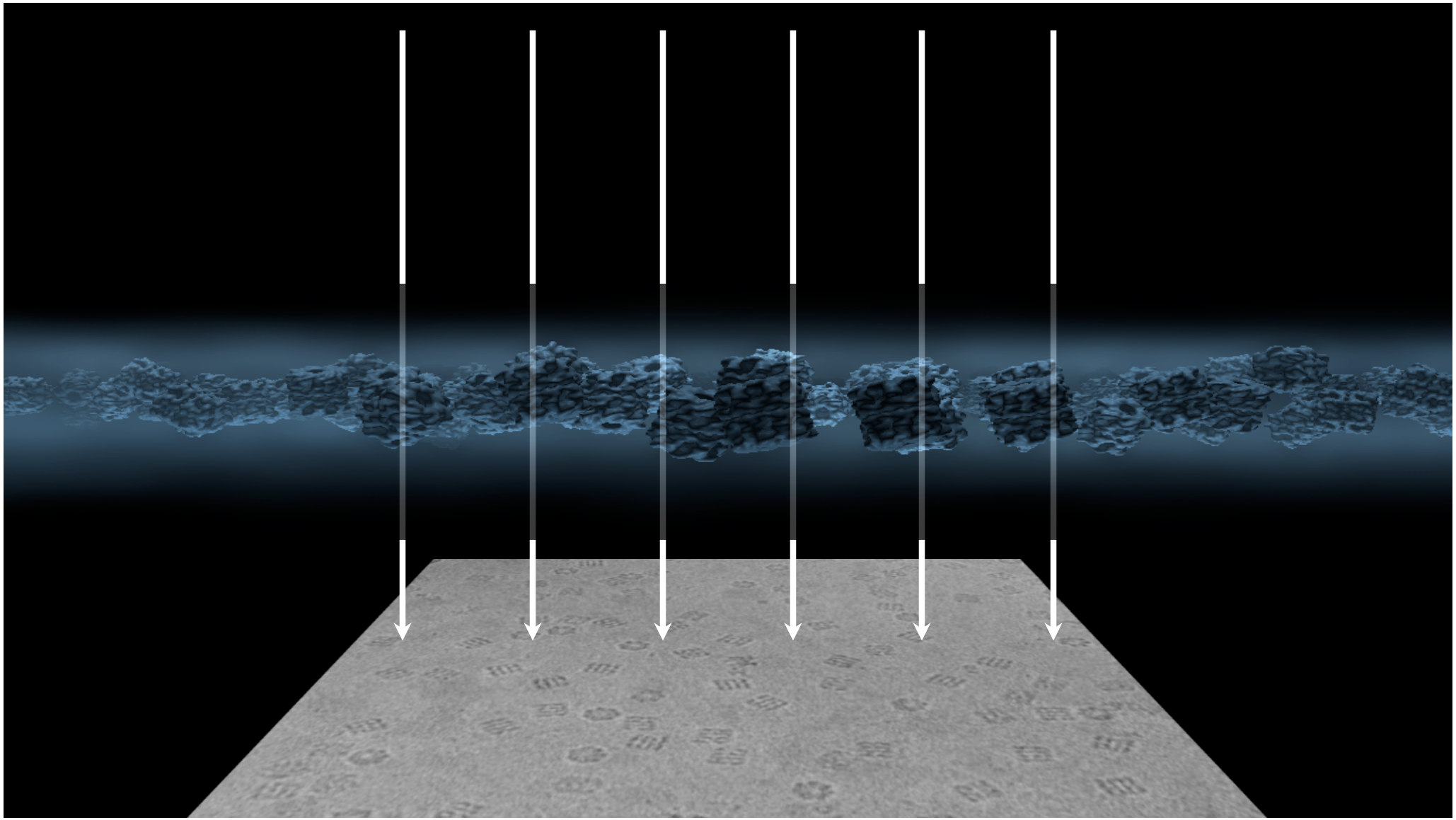
Vitrification process for CryoTEM

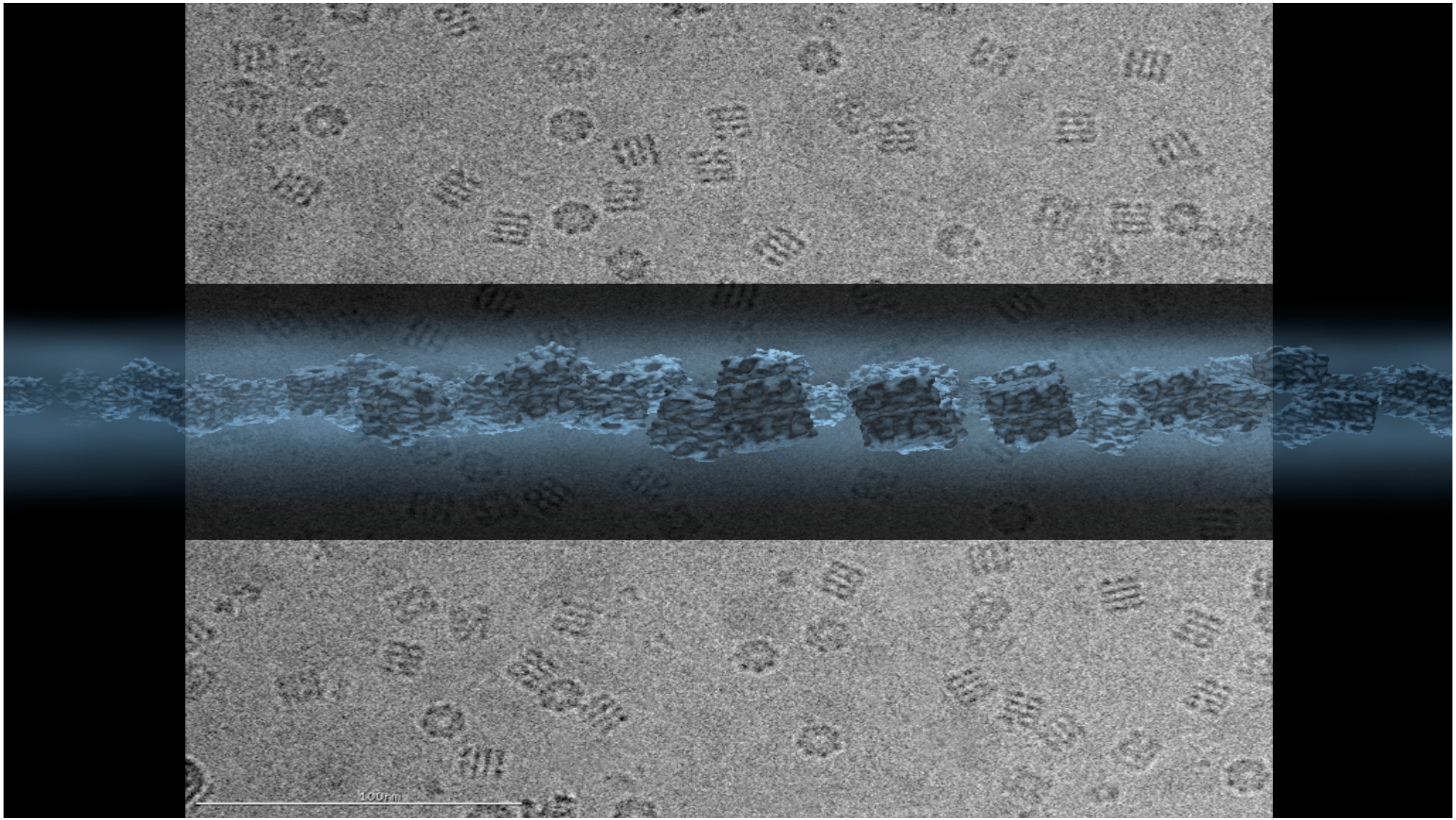


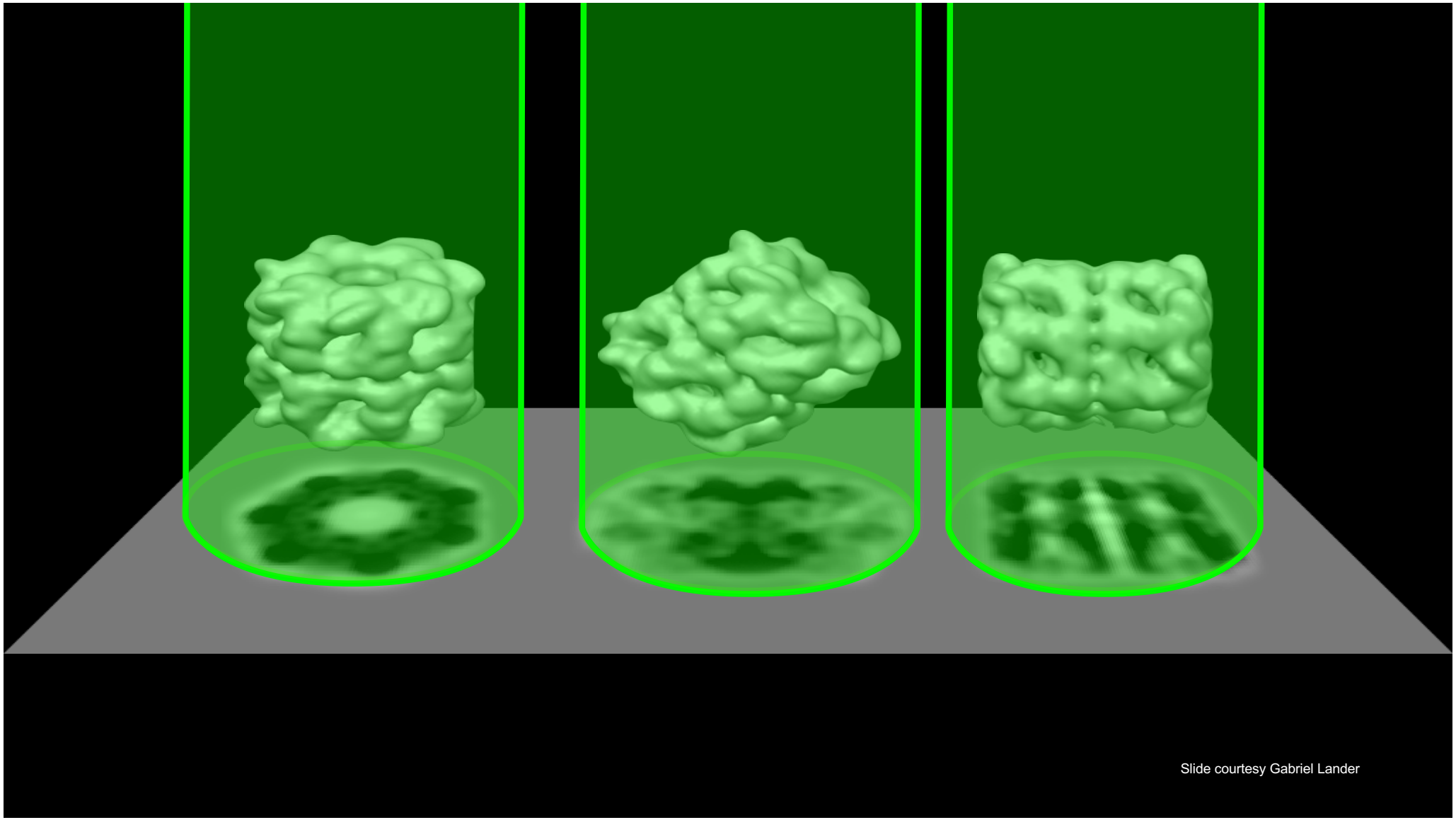


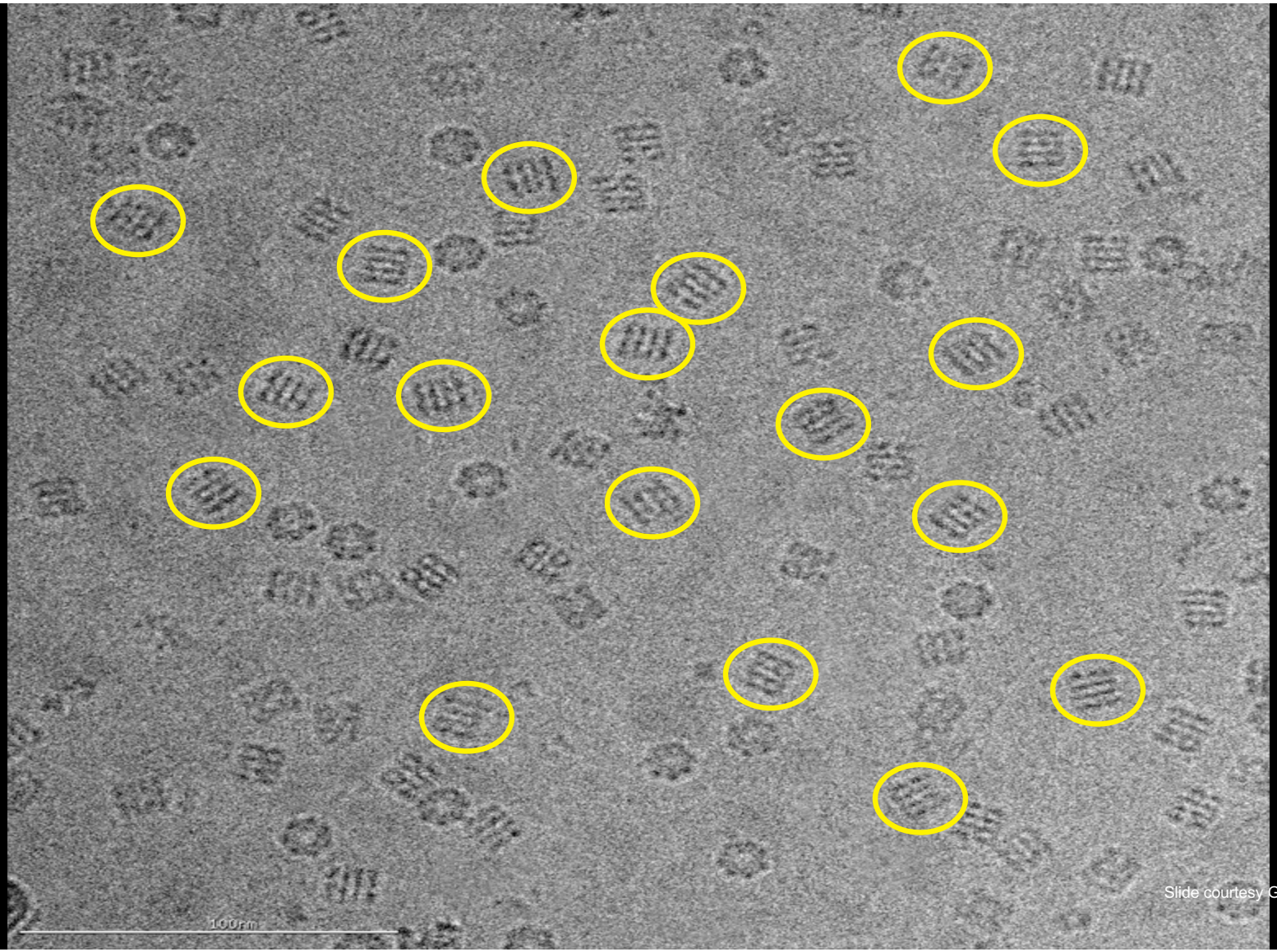


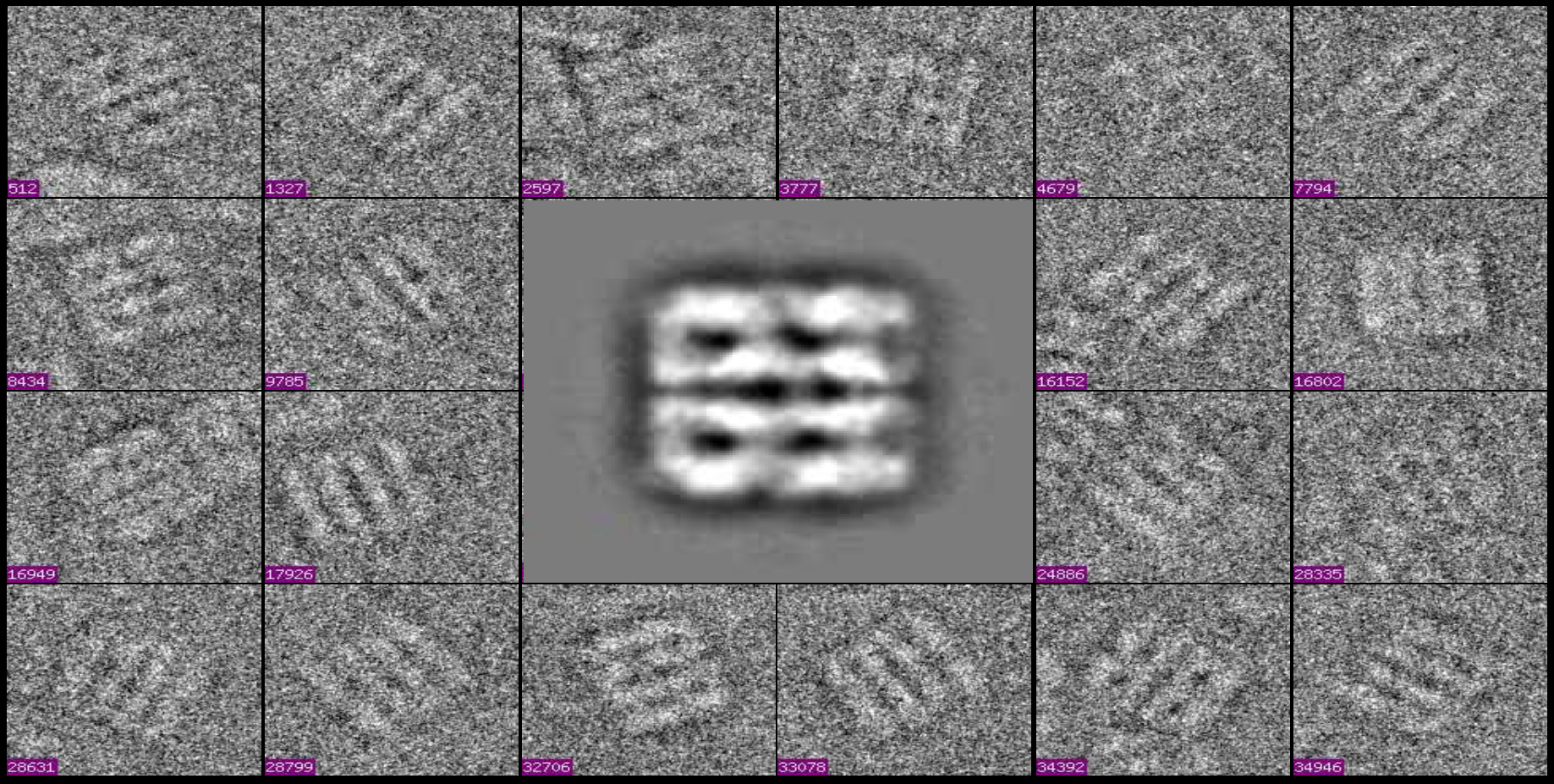
Slide courtesy Gabriel Lander



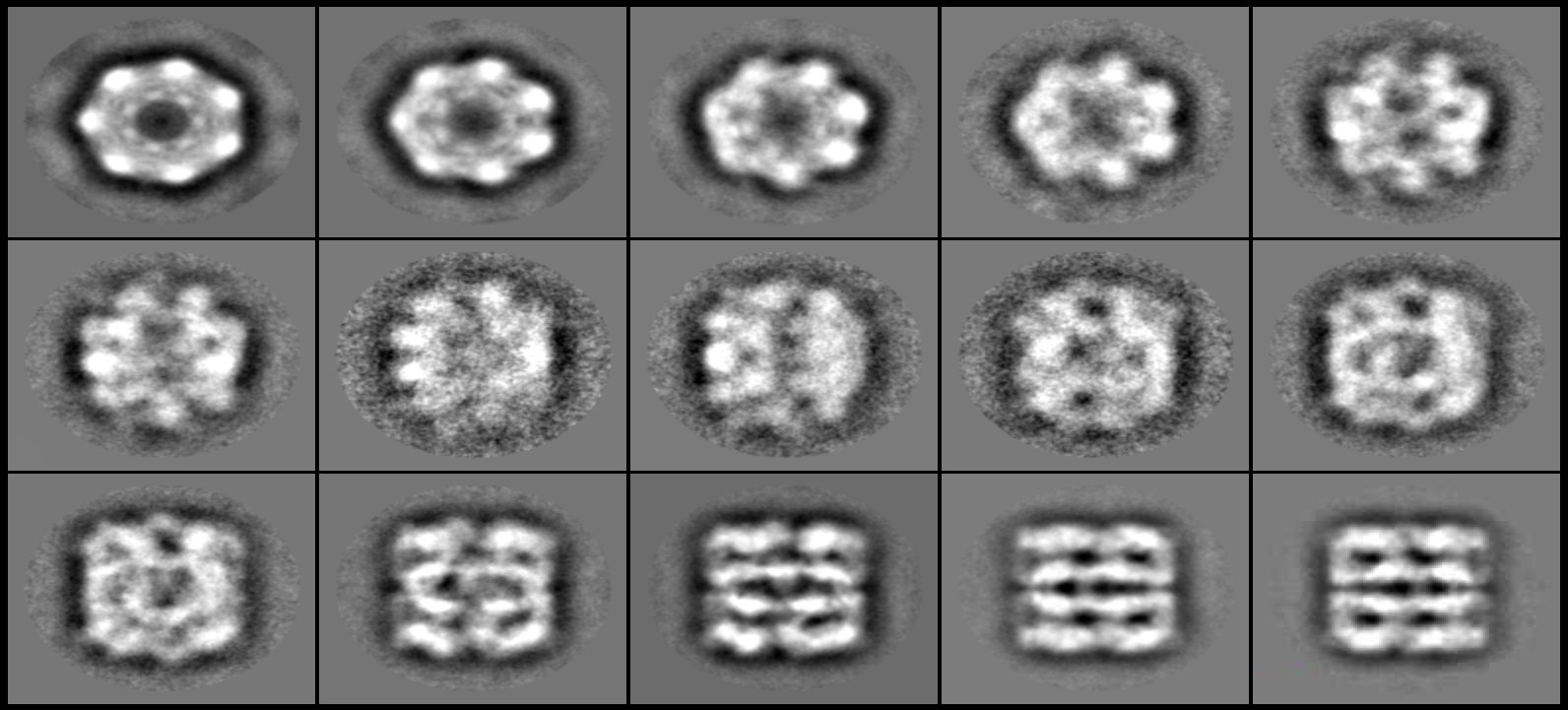

100nm


Slide courtesy Gabriel Lander

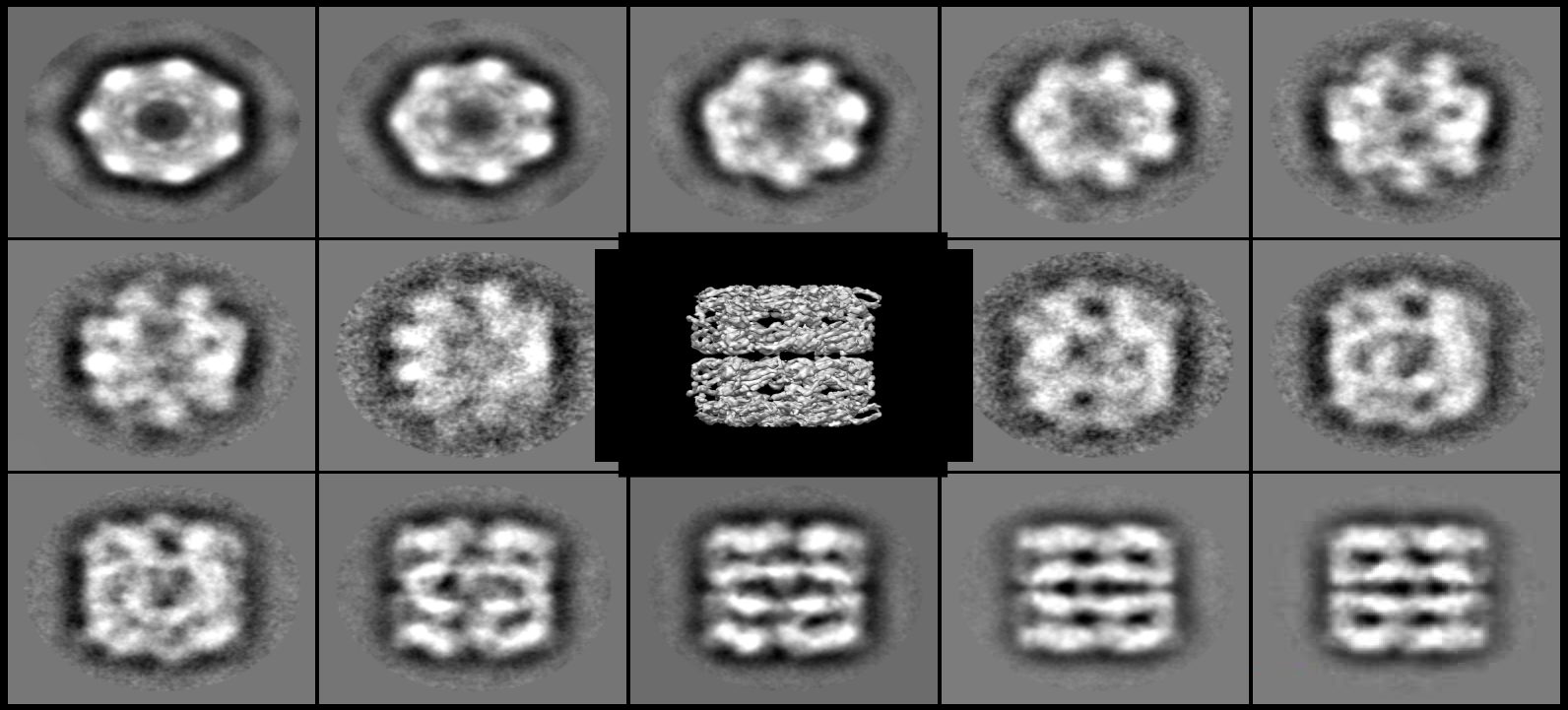

100nm
Slide courtesy Gabriel Lander



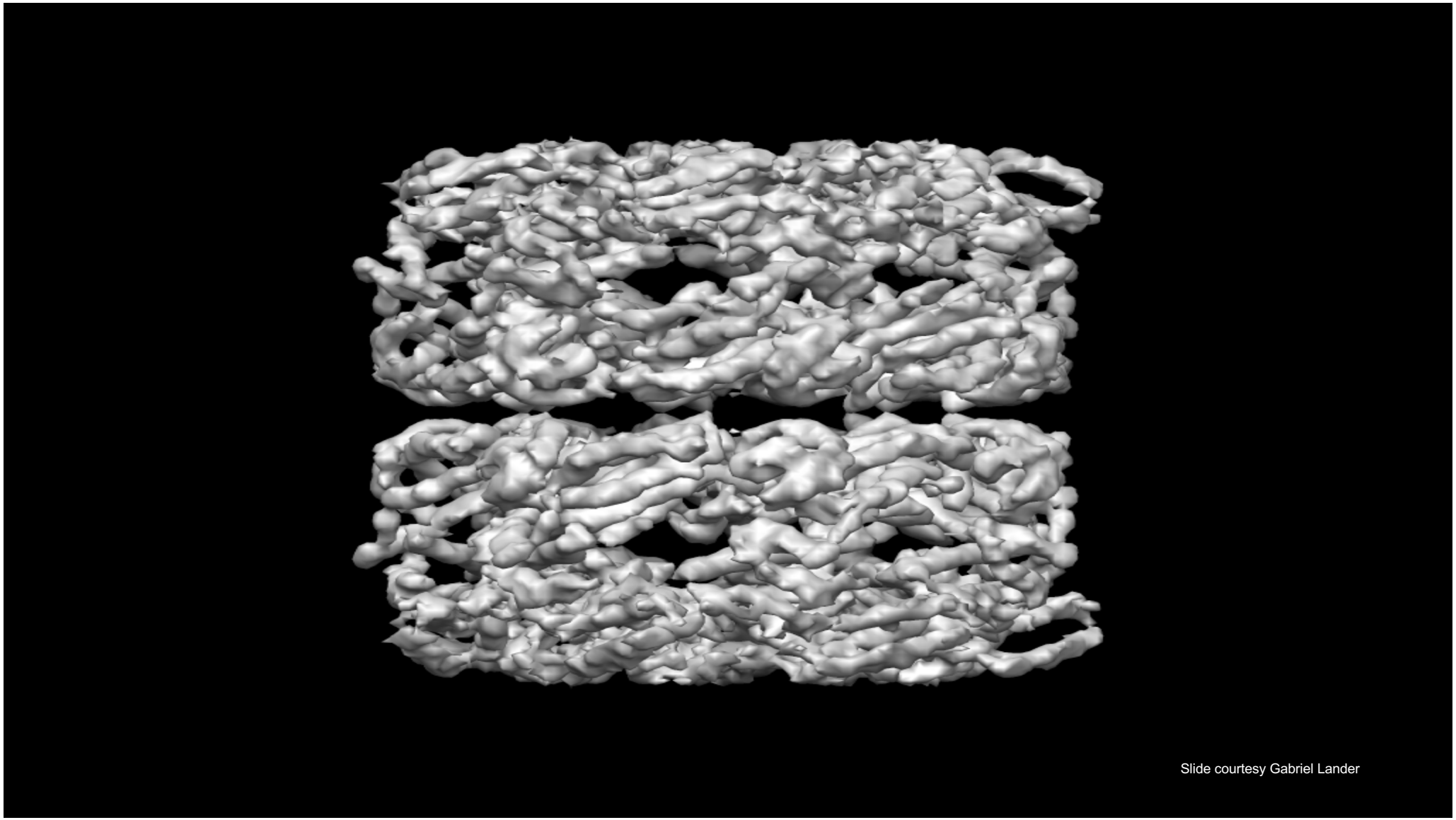
Slide courtesy Gabriel Lander



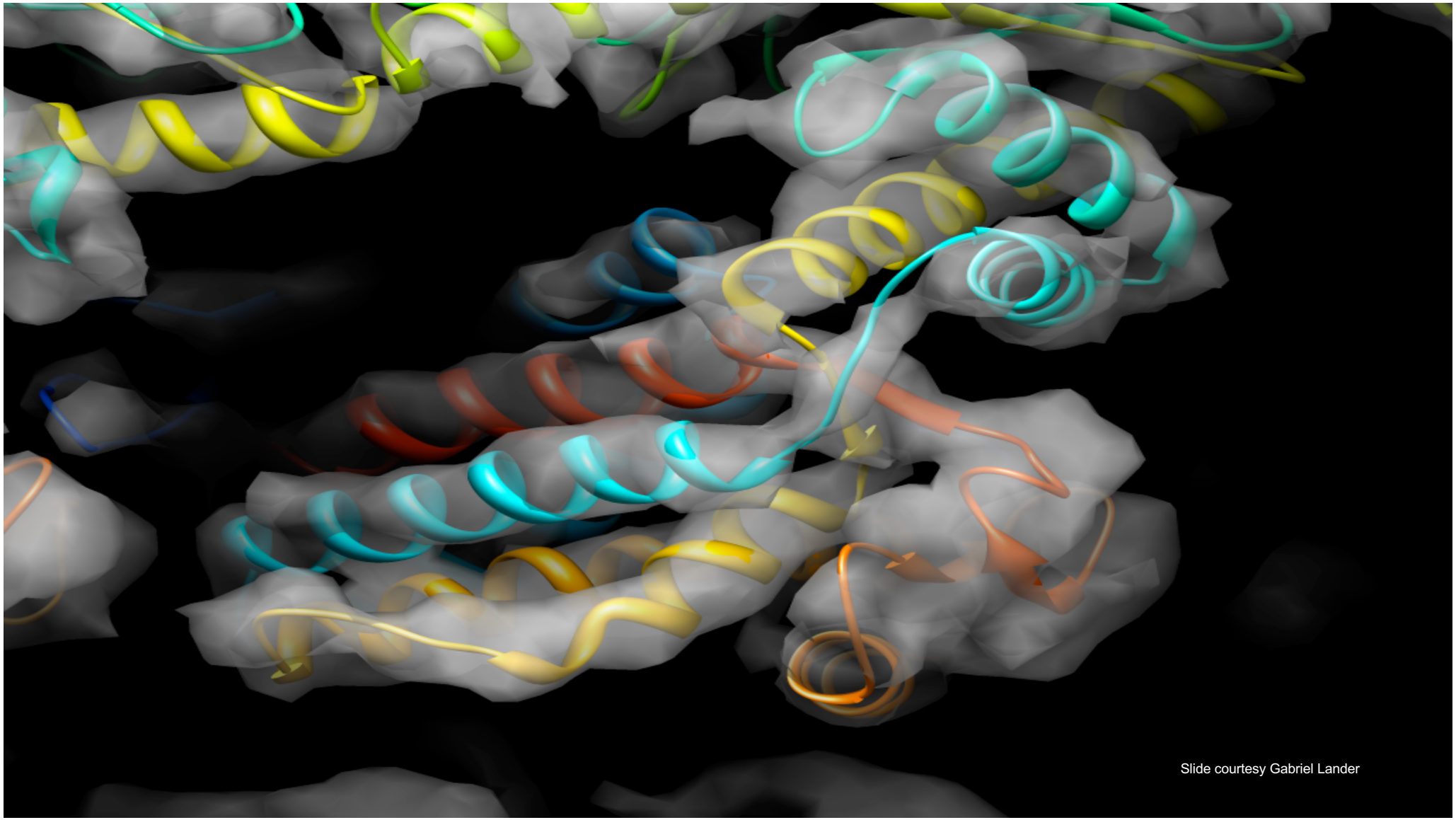
Slide courtesy Gabriel Lander



Slide courtesy Gabriel Lander



Slide courtesy Gabriel Lander


Slide courtesy Gabriel Lander