ScopeM

Cryo Electron Microscopy (CryoEM)

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CCMX 2022
5th November 2024

ScopeM

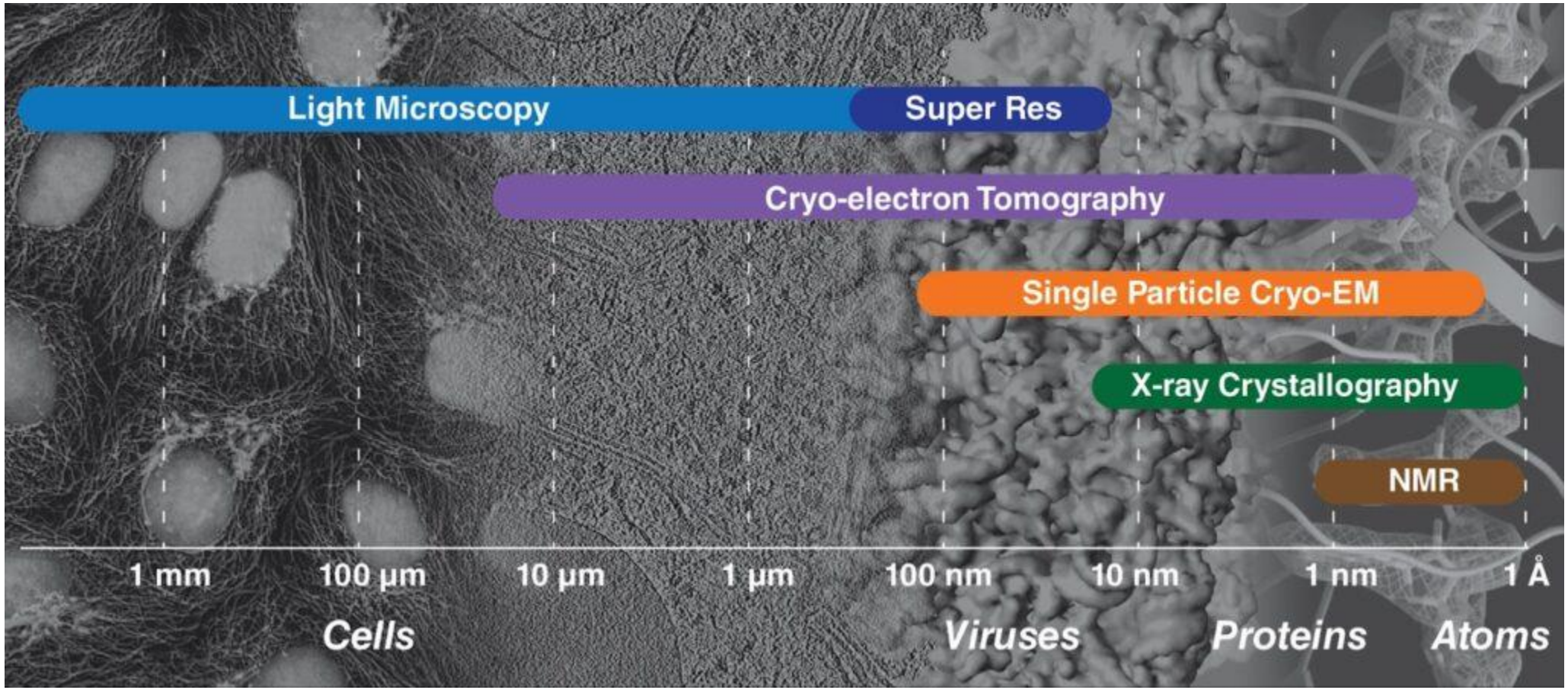
Index

- Why cryoEM?
- Radiation damage and low dose EM
- Representative projects:
  - Soft matter
  - Protein complexes
  - hard matter/nanoparticles

- See Demo on Instruments for details about (with Stephan Handschin, Miroslav Peterek):
  - Vitrification
  - Microscopy at cryogenic conditions
  - High throughput and automation

ScopeM

Visualization of biological or soft materials



https://cryoem101.org/chapter-1-et/

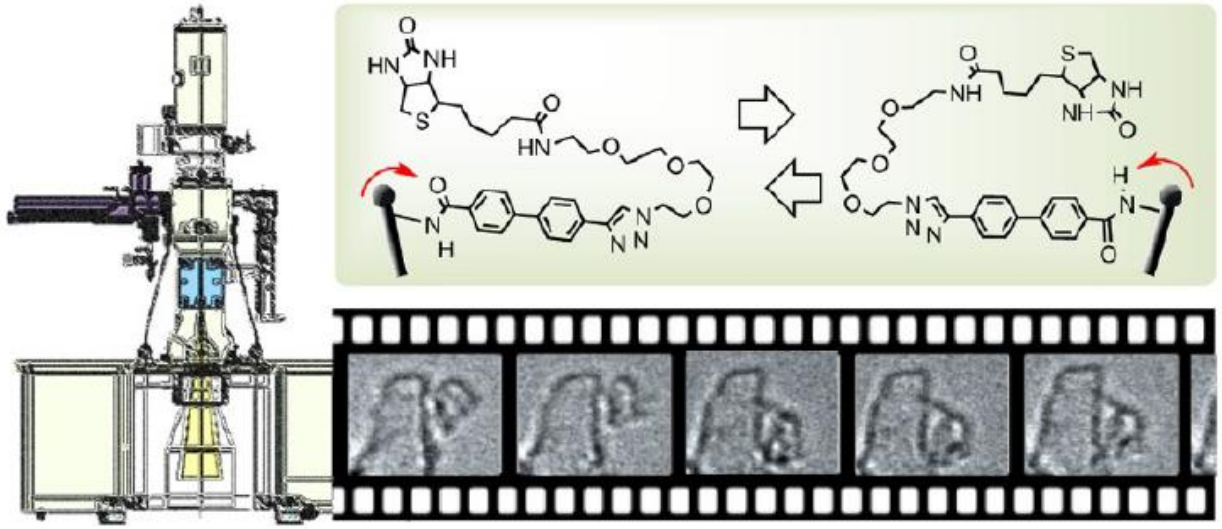
Why CryoEM

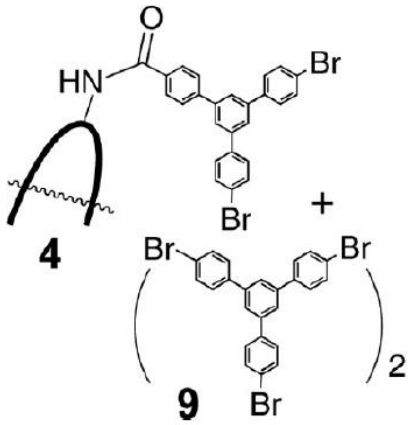
Cryogenic (transmission) Electron Microscopy

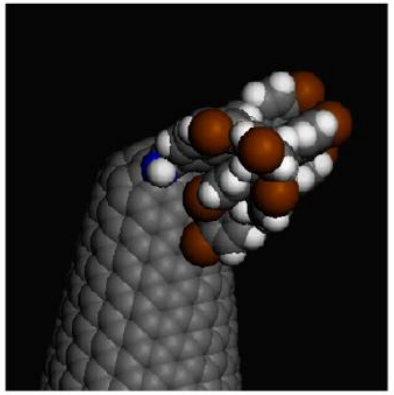
Why EM: The wavelength of electron is very short und thus EM has high resolving power

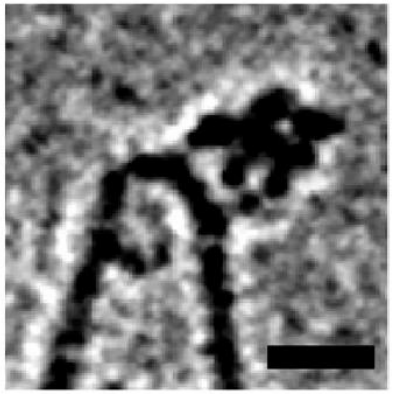
Why Cryo: Water in biological/softmaterial samples is retained by rapid freezing (vitrification) making the samples compatible with high vacuum of an electron microscope. Moreover, vitrification preserves near native conformation (structure) of such samples

EMs have high resolving power

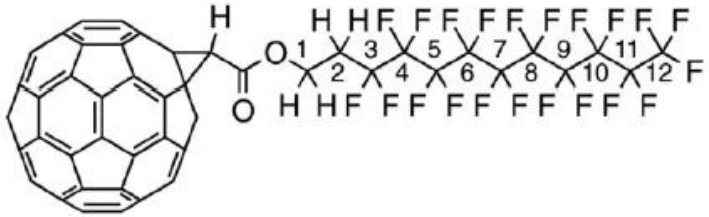








(a)



(b)

Nakamura (2017) *Acc. Chem. Res.* 50, 1281

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Are biological/soft matter samples suitable for electron microscopy?

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Liquids will evaporate in vacuum of TEM
→

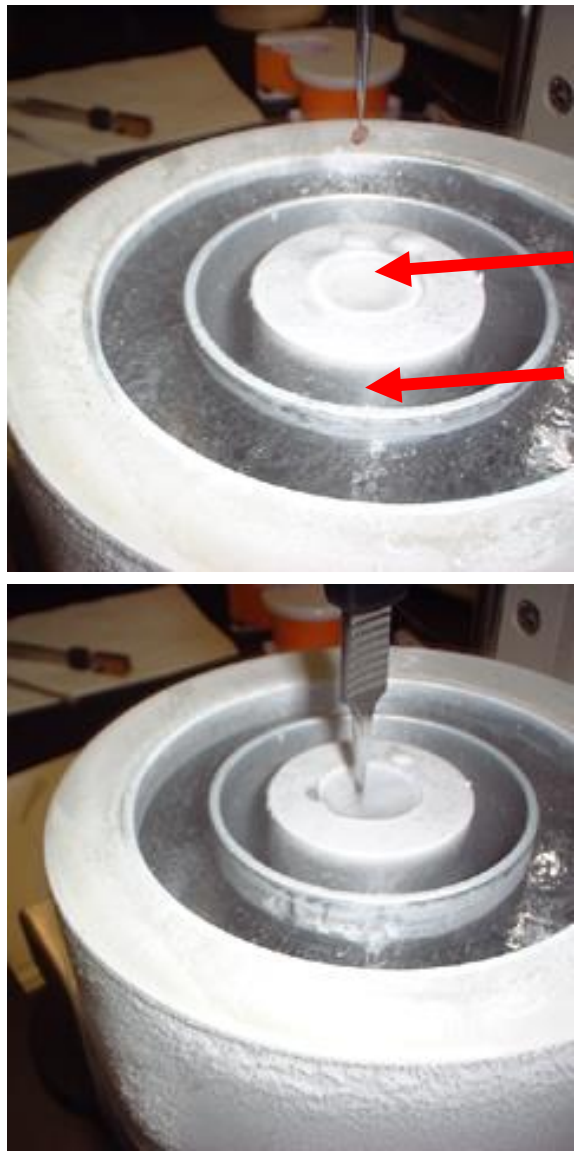
Beam will destroy sample
(temperatures as on the sun)
→

Are biological/soft matter samples suitable for electron microscopy?

Liquids will evaporate in vacuum of TEM
→ Requires fixing of samples

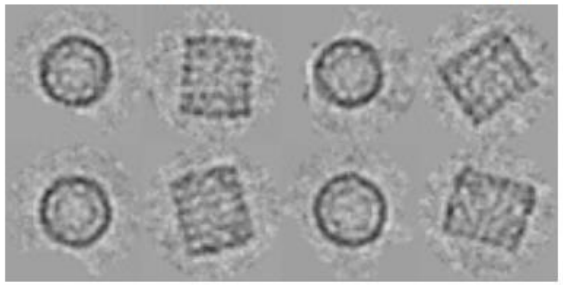
Beam will destroy sample
(temperatures as on the sun)
→ Requires (i) vitrification, (ii) low-dose and (iii) rapid data collection (movie mode) before beam damage

Vitrification and beam damage

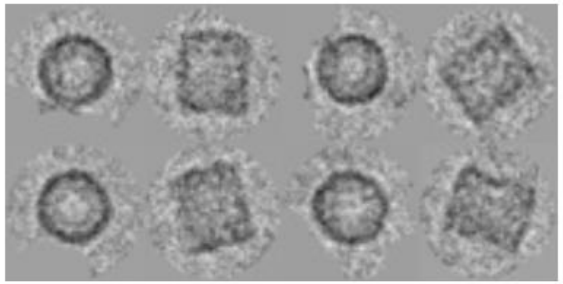


82 K (liquid N2 cooling)

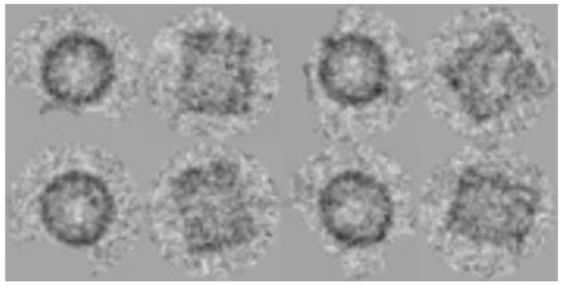
10 or 20 e^-/A^2



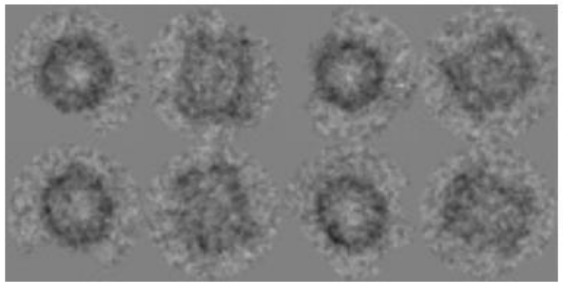
120 e^-/A^2



200 e^-/A^2



350 e^-/A^2

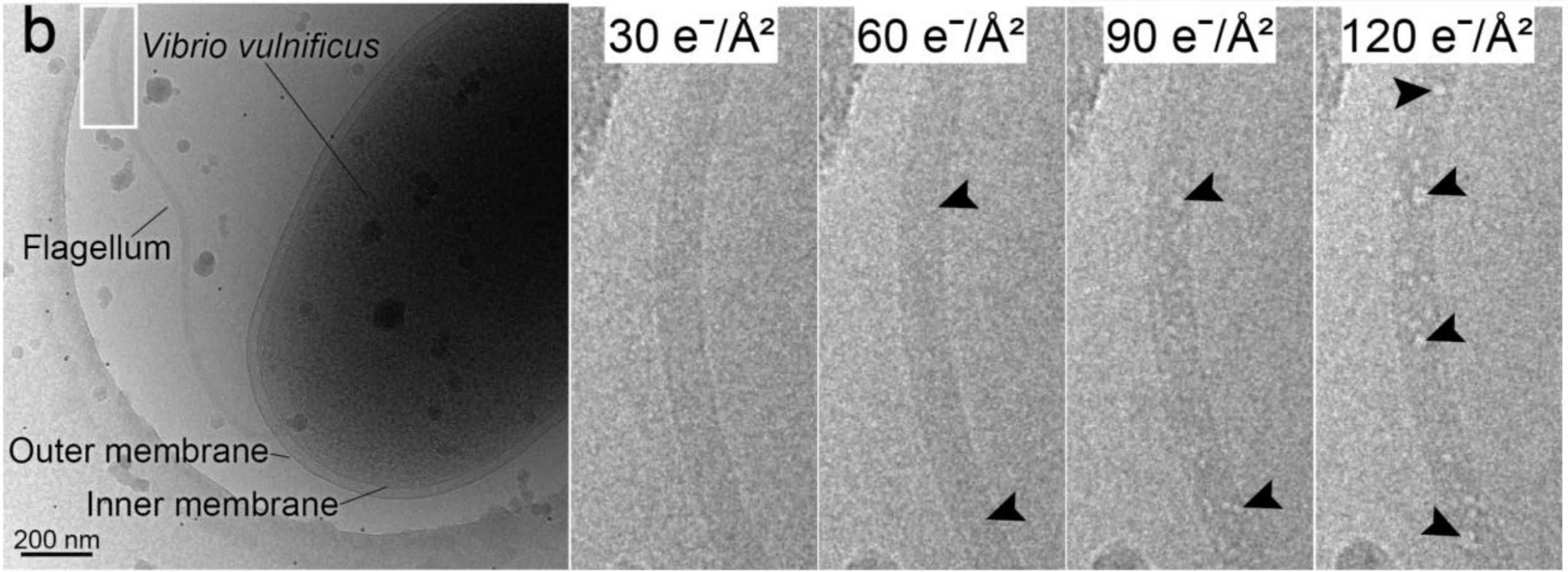


EM for dummies by H. Niederstrasser

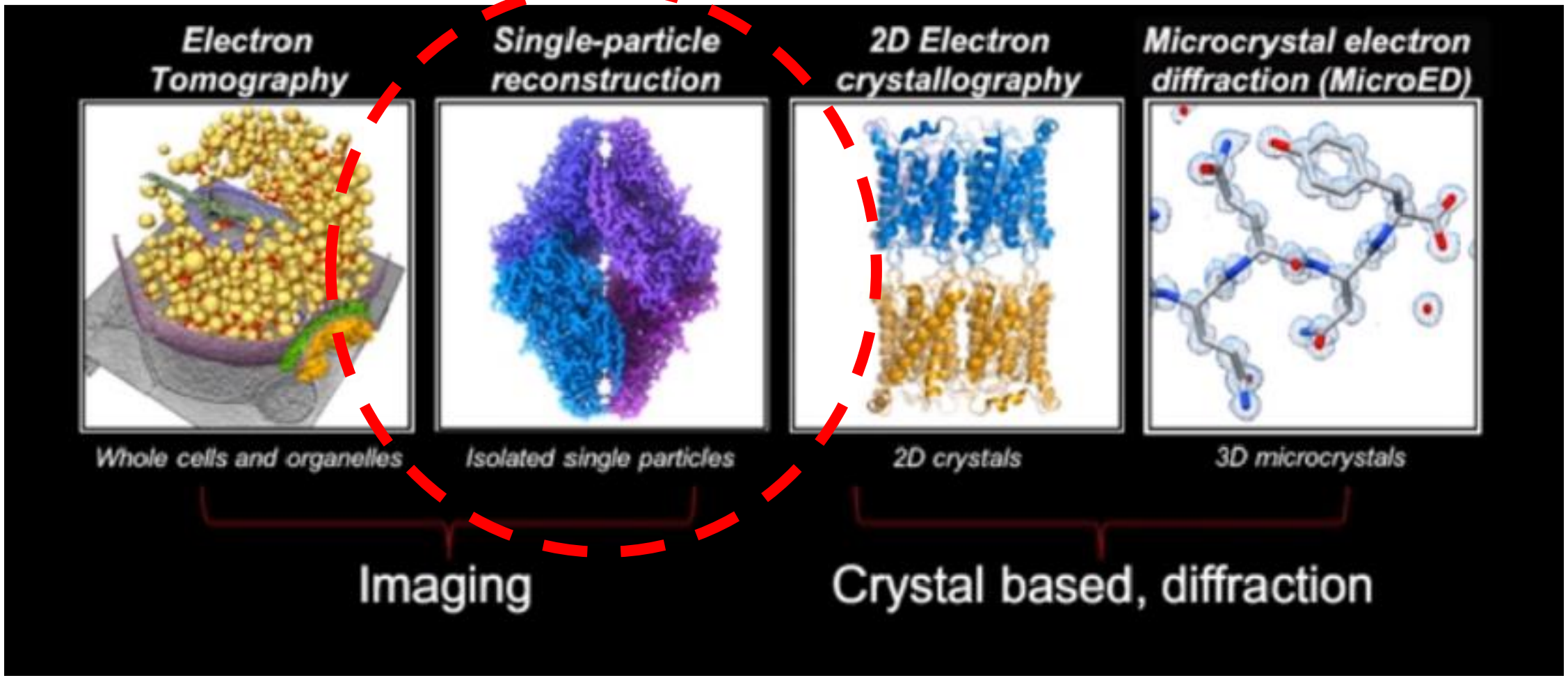
Courtesy: Grant Jensen: http://cryo-em-course.caltech.edu/outline

ScopeM

Vitrification and beam damage



CryoEM: four basic “3D techniques” plus some variations!



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ScopeM

Representative projects…

Project 1: Vesicles

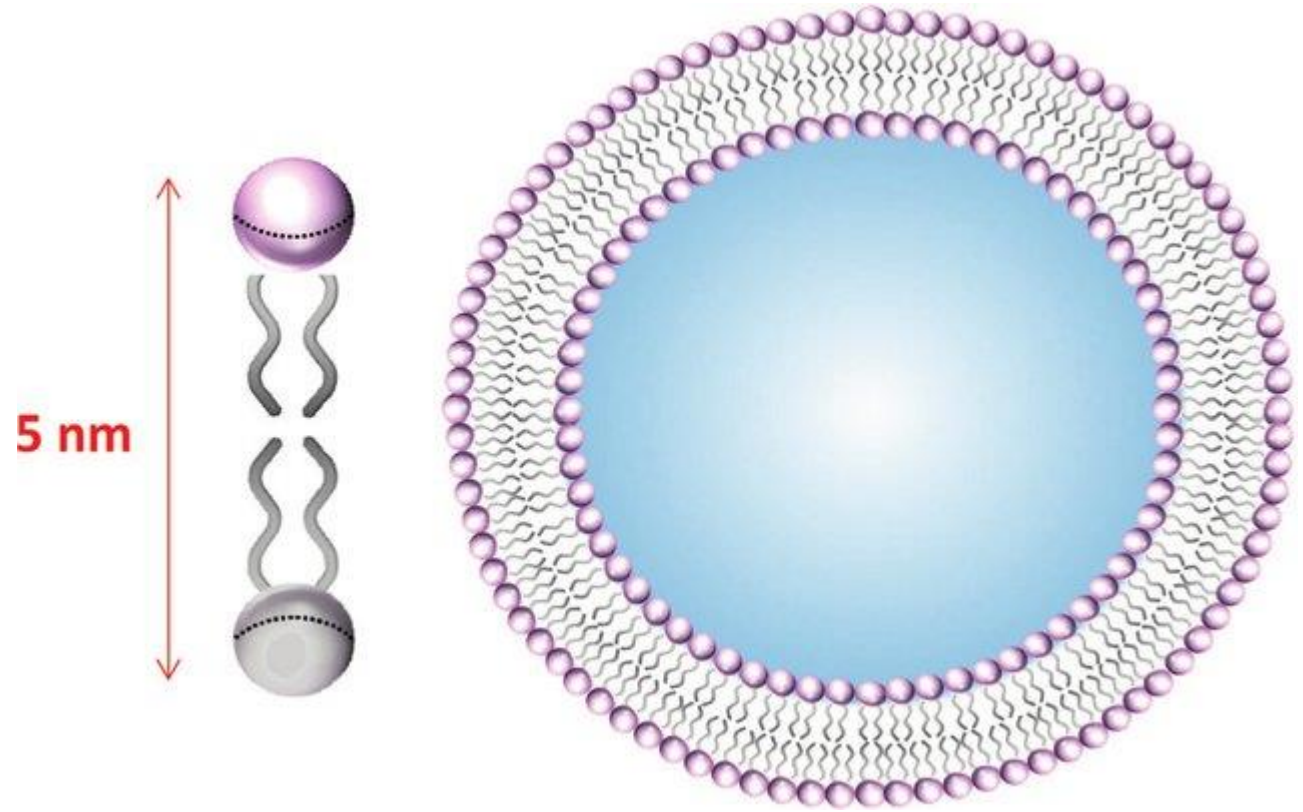
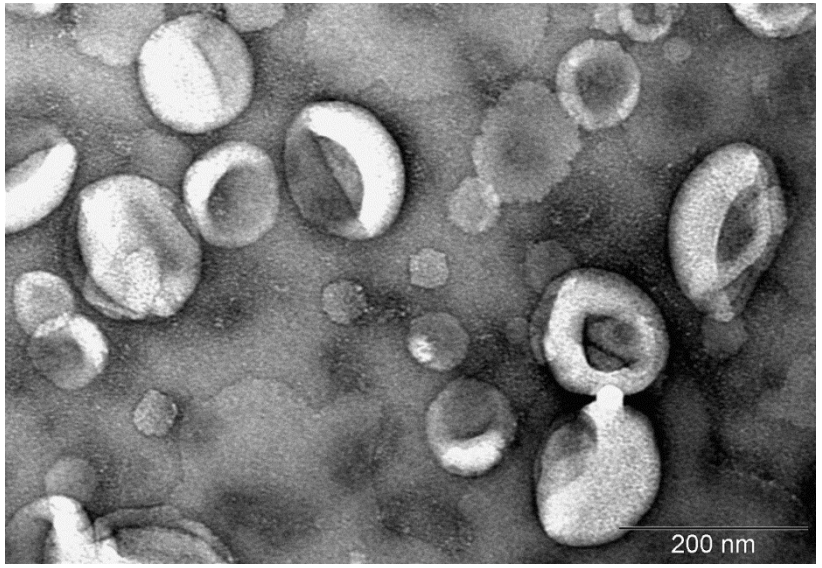
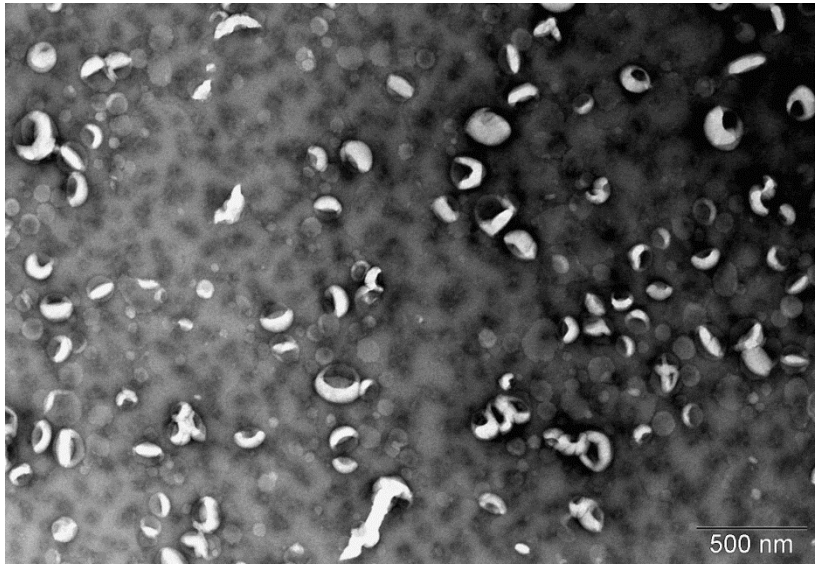


Image from Yamashita (2018) Recent Dispersion Technology Using Liquid Crystal (book chapter)

Project 1: Vesicle structural analysis

Room temperature/stained





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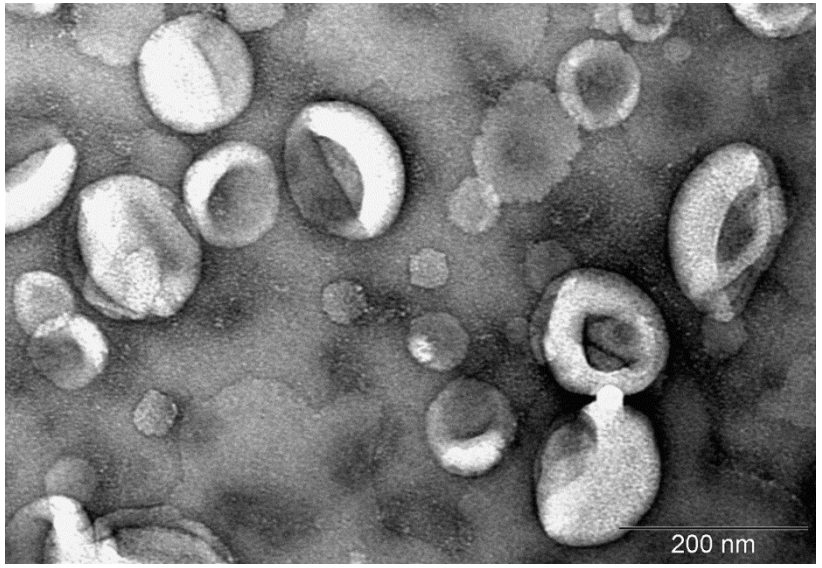
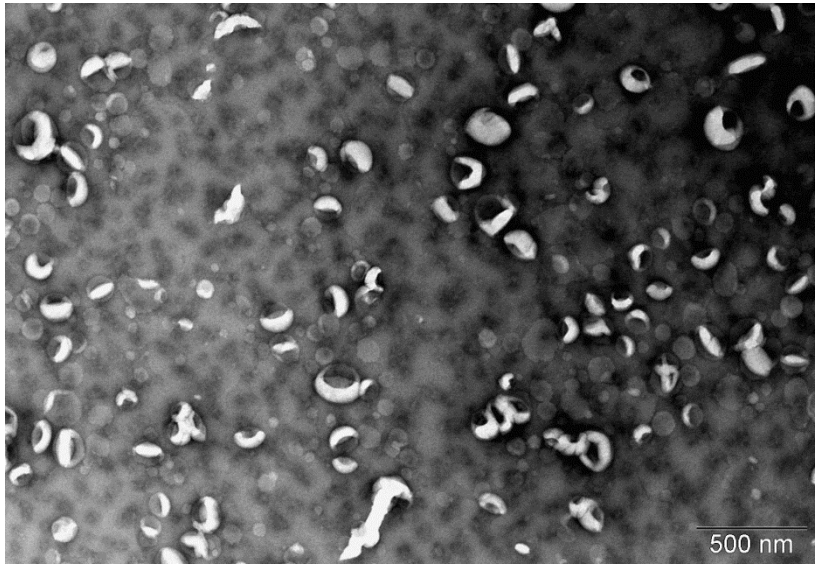
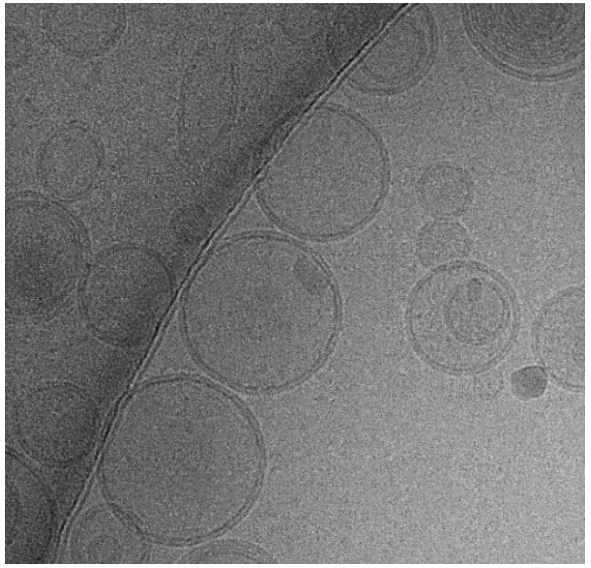
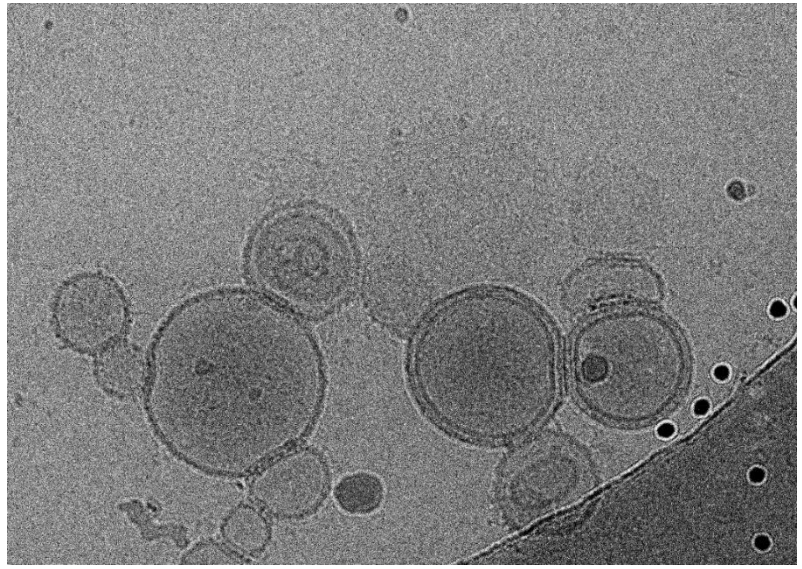


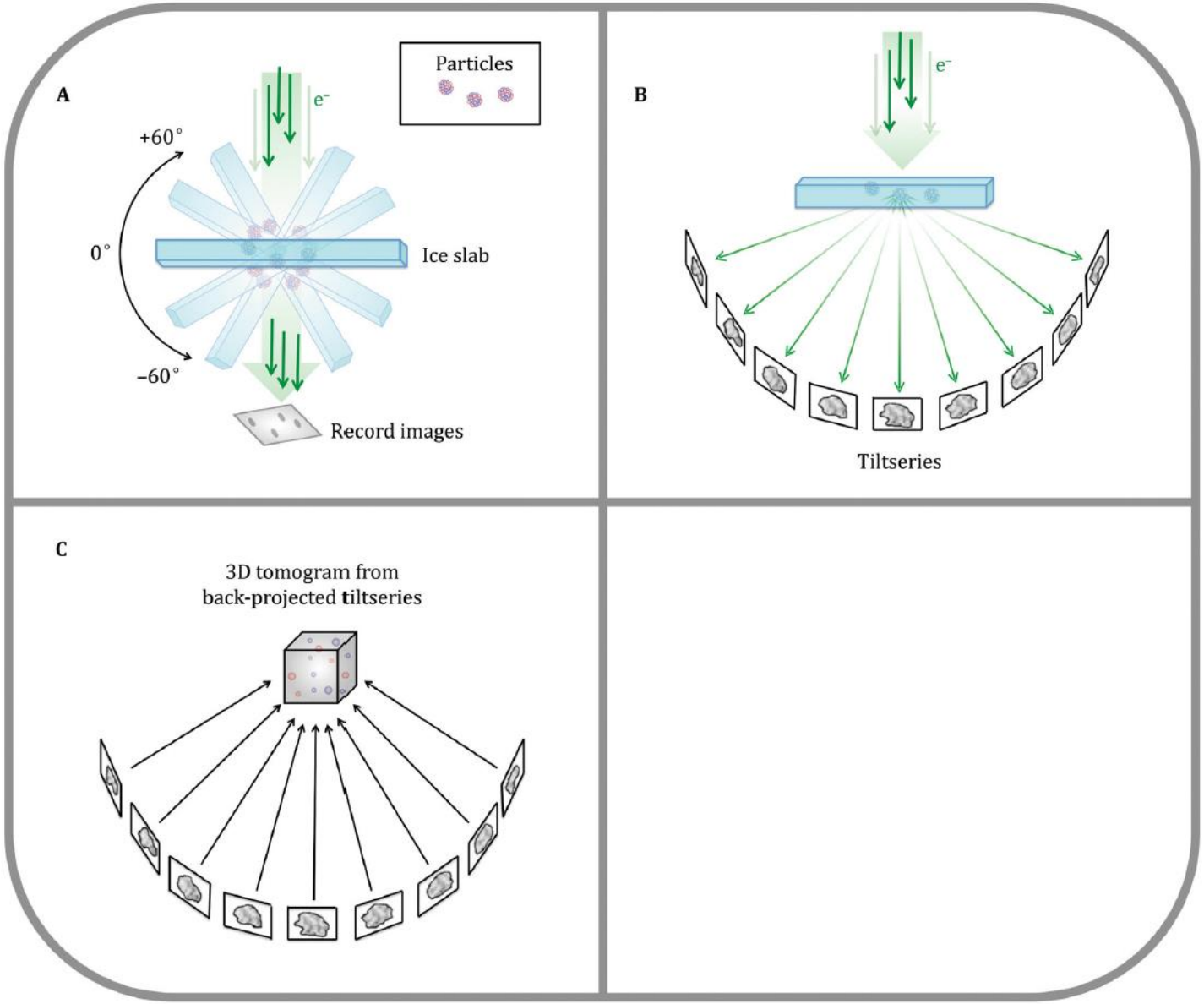


CryoEM





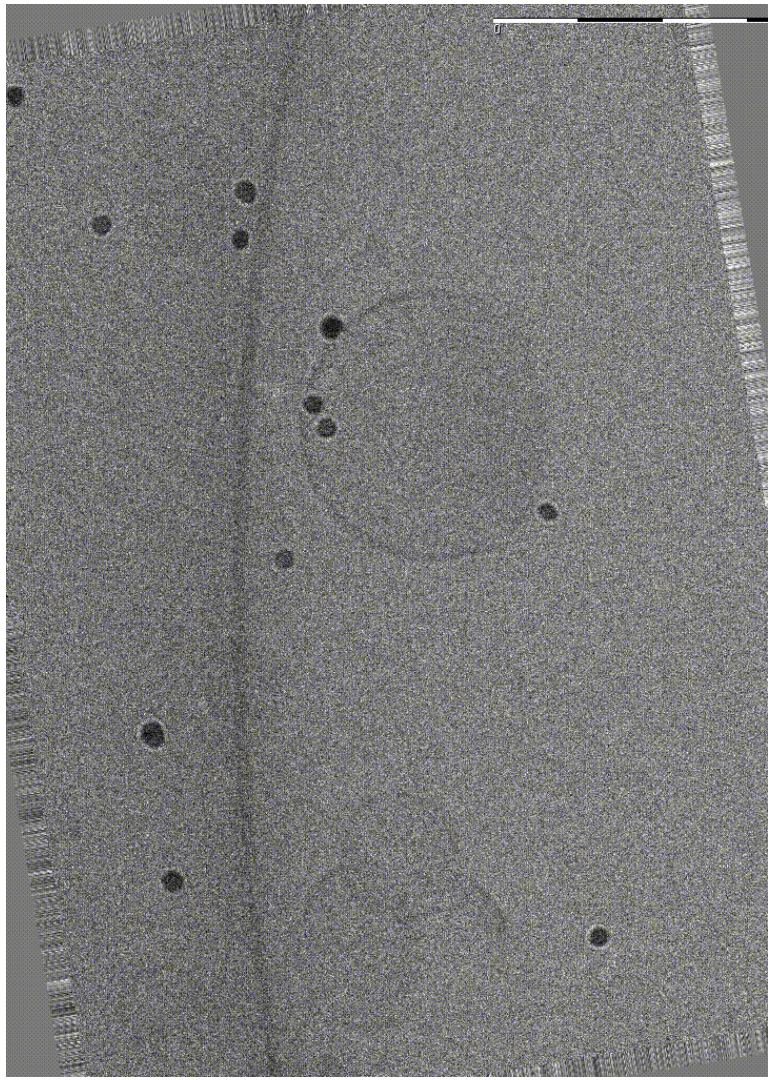
Cryo tilt series & electron tomography



Galaz-Montoya and Ludtke (2017) *Biophys. Rep.* 3, 17

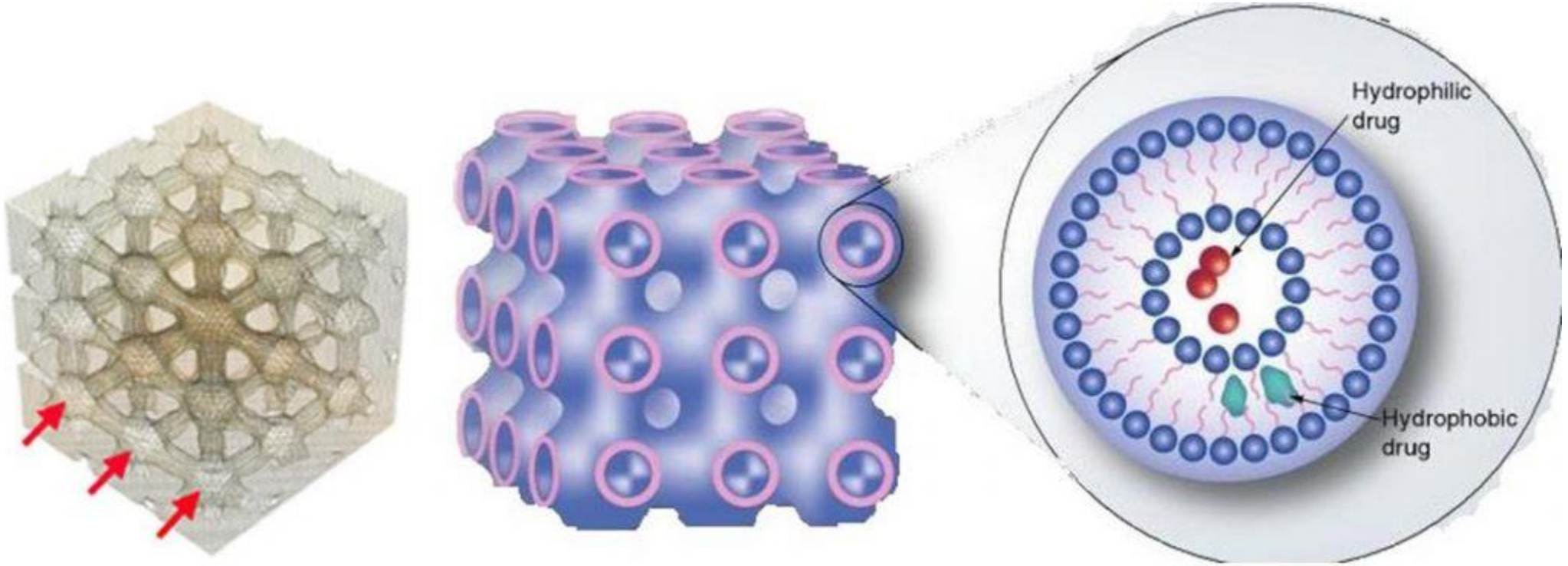
Project 1: Vesicle structural analysis

Movie1



Movie2

Project 2: Cubosomes

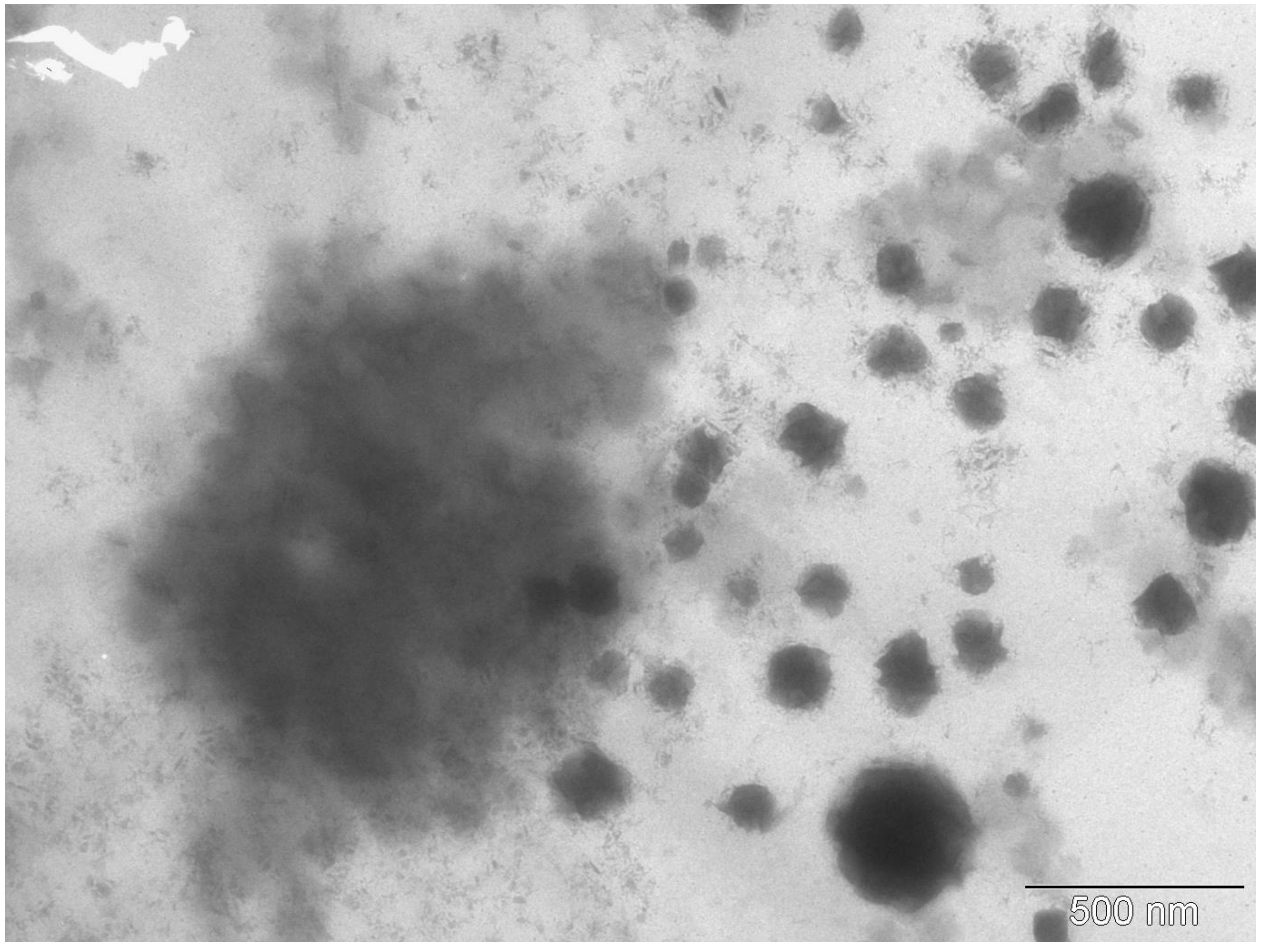


Cubosomes are discrete, sub-micron, nanostructured particles of the bicontinuous cubic liquid crystalline phase. The term "bicontinuous" refers to two distinct hydrophilic regions separated by the bilayer (Wikipedia)

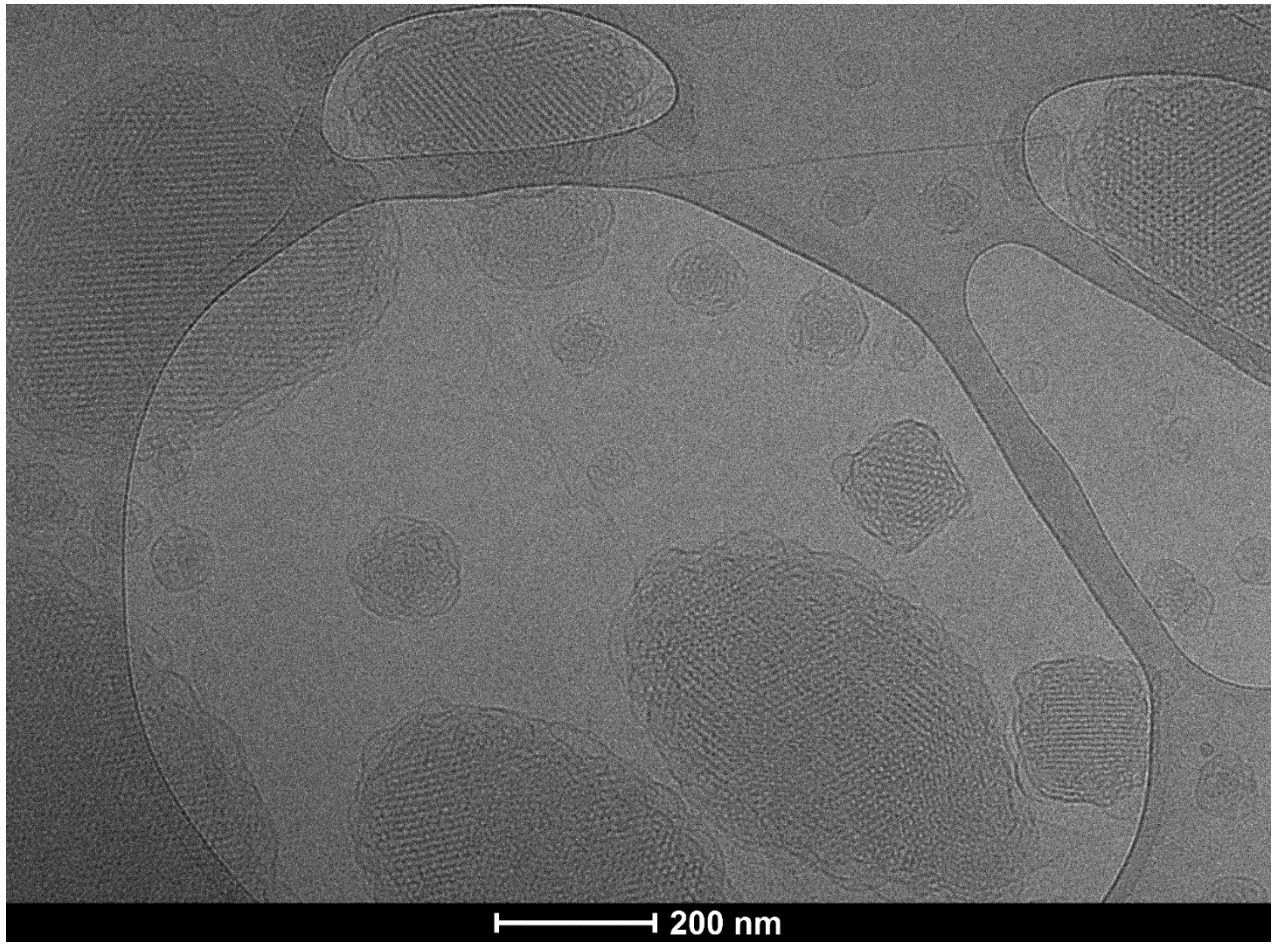
https://www.labiotech.eu/trends-news/cubosomes-nanomedicine-drug-delivery-epfl-coimbra-nestle-cime/

Project 2: Cubosome structural analysis

Room temperature/stained



CryoEM



Project 3: Proteins

Protein shake (source: Wikipedia)

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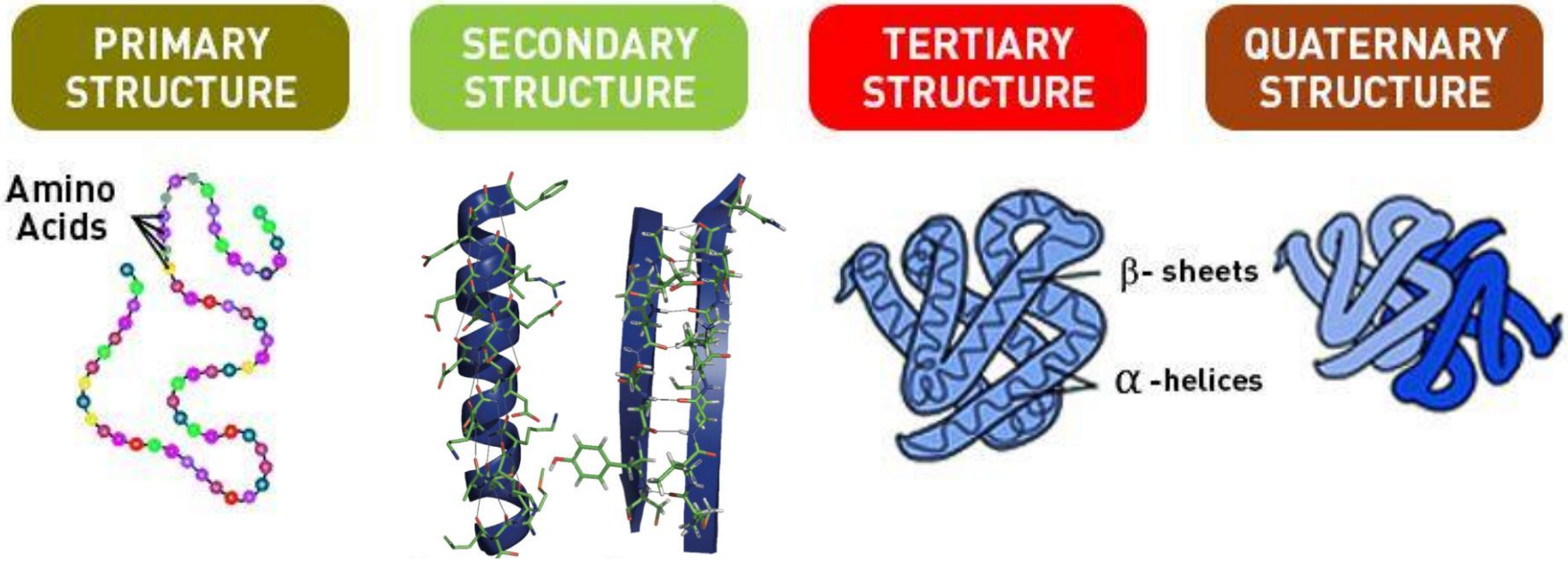
Protein shake (source: Wikipedia)

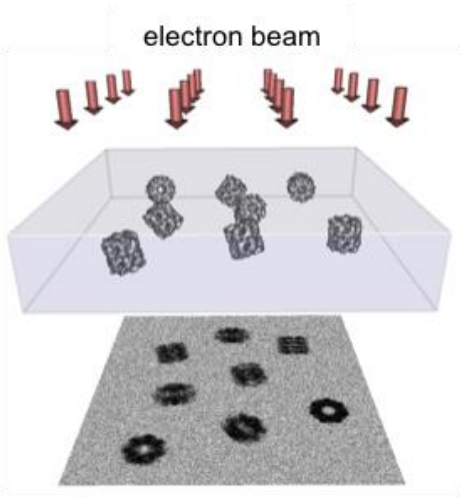
https://byjus.com/biology/proteins-structure-and-functions/

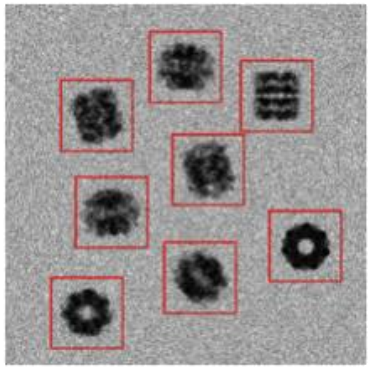


α-helix β-sheet

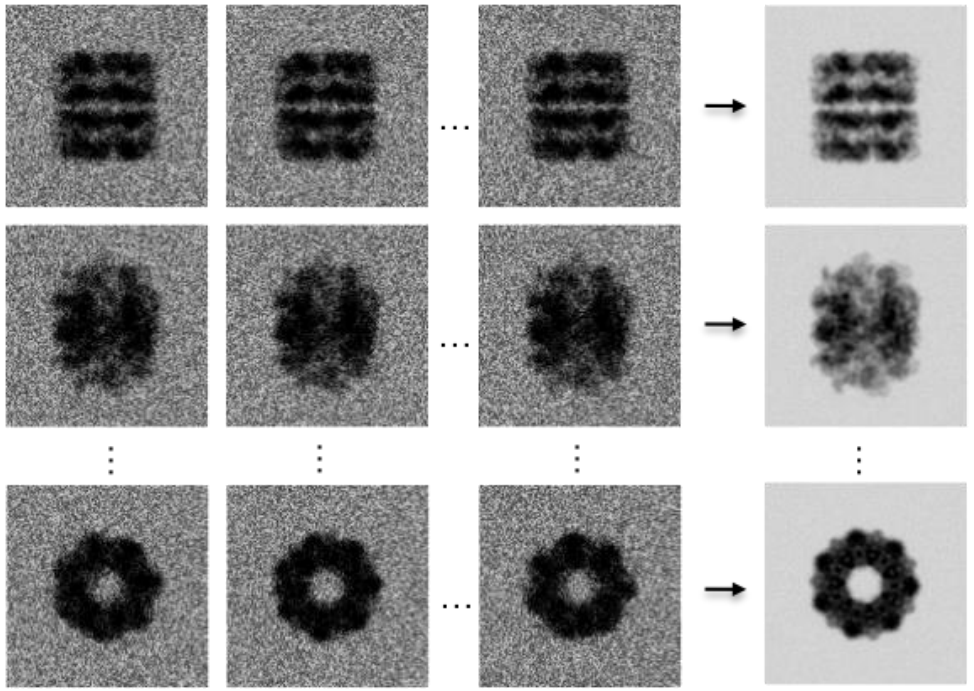
https://www.chemtube3d.com/bc-26-3-2/

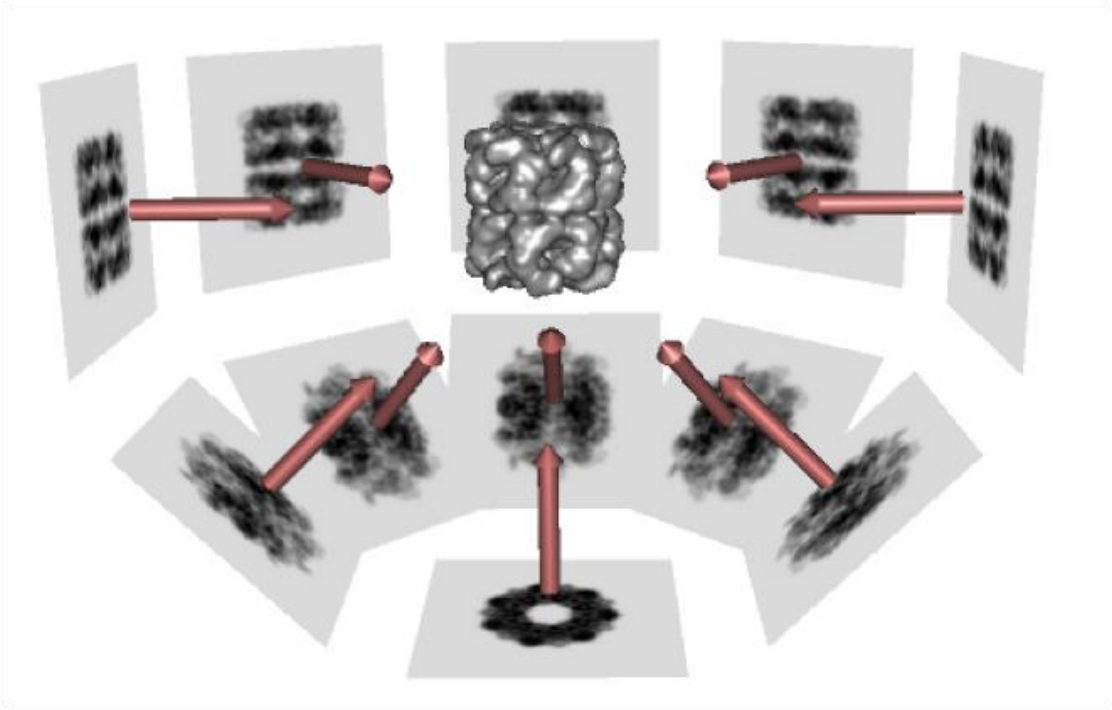
Project 3: Single Particle Analysis (SPA)





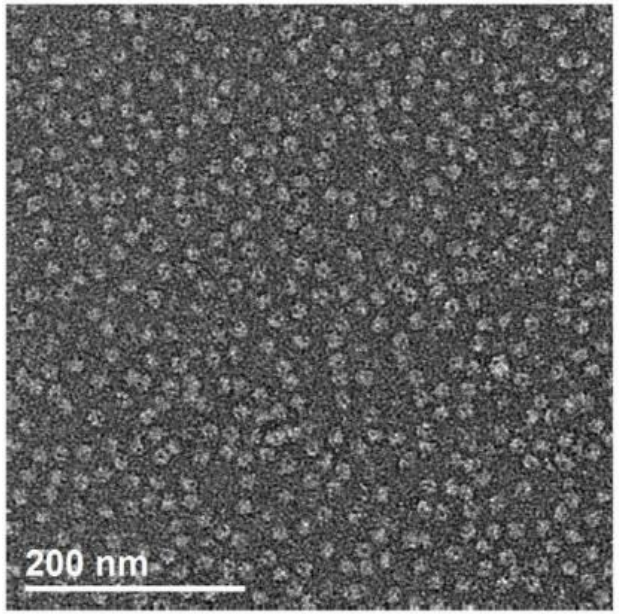
→ **Reconstruct 3D map from "back projected" 2D projections**

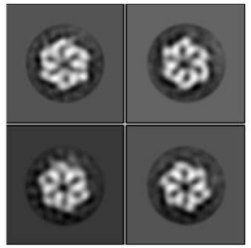




https://people.csail.mit.edu/gdp/cryoem.html

Project 3: Single Particle Analysis (SPA)

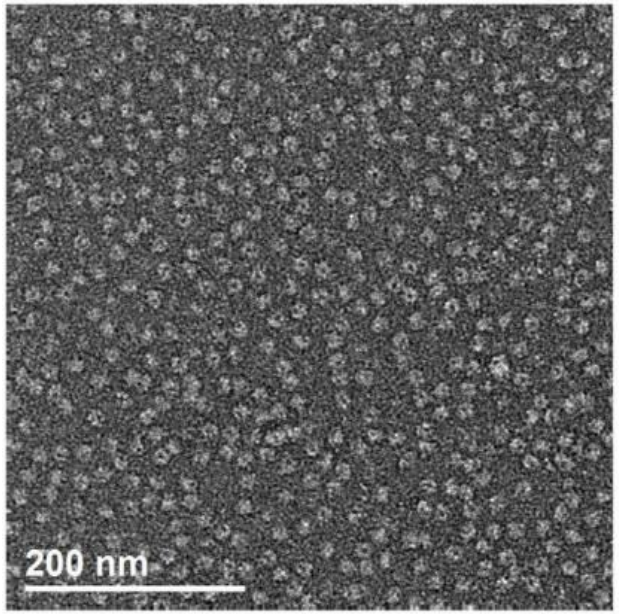


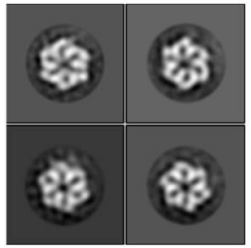


B. Huntington et al.,… & **B. M. Qureshi** (2022) *Frontiers in Molecular Biology* 9, 890390

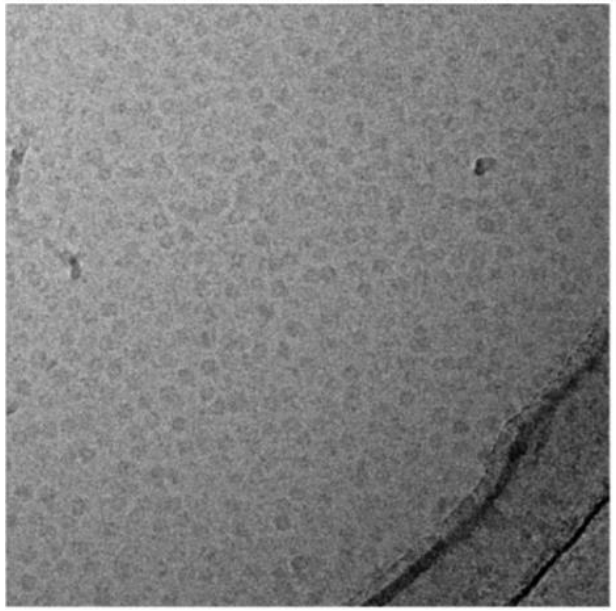
Project 3: Single Particle Analysis (SPA)

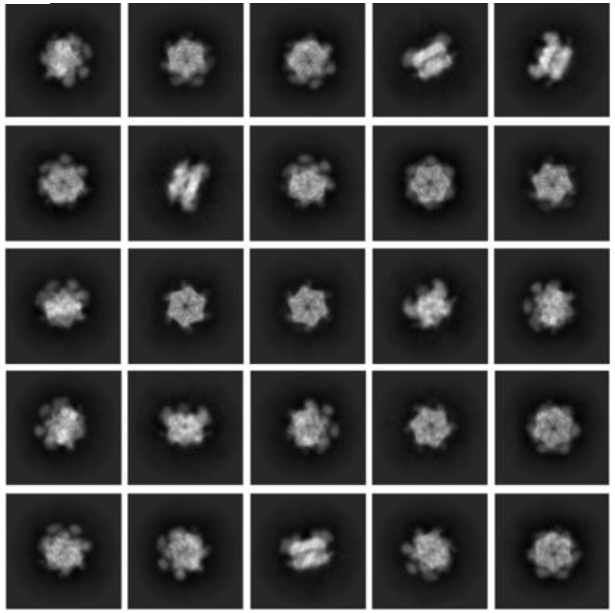
Room temperature/stained

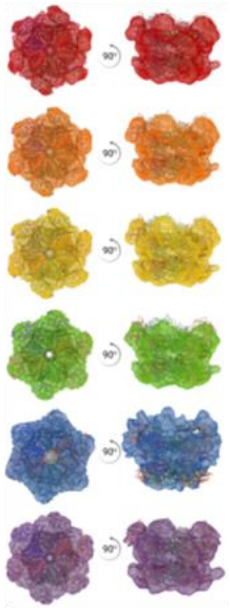




Cryo



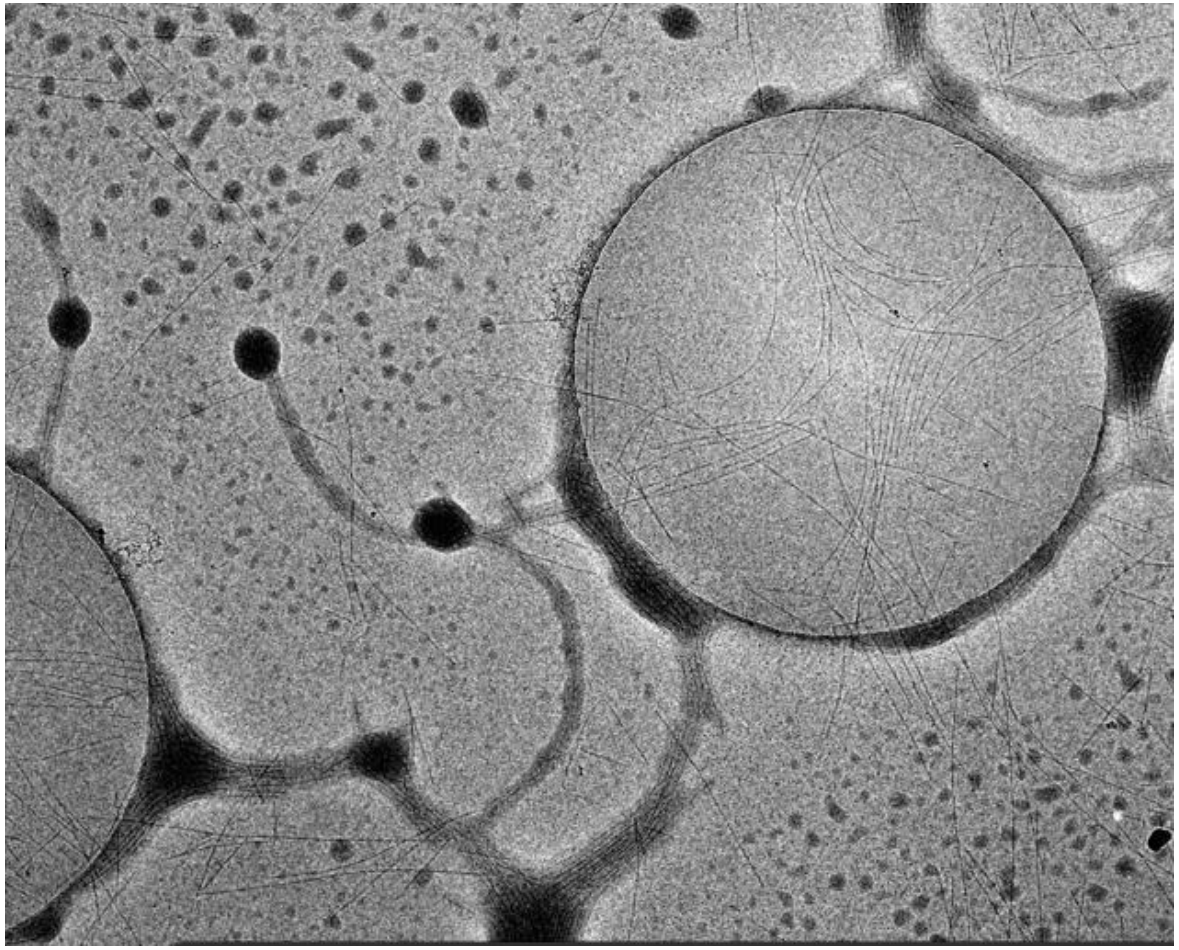


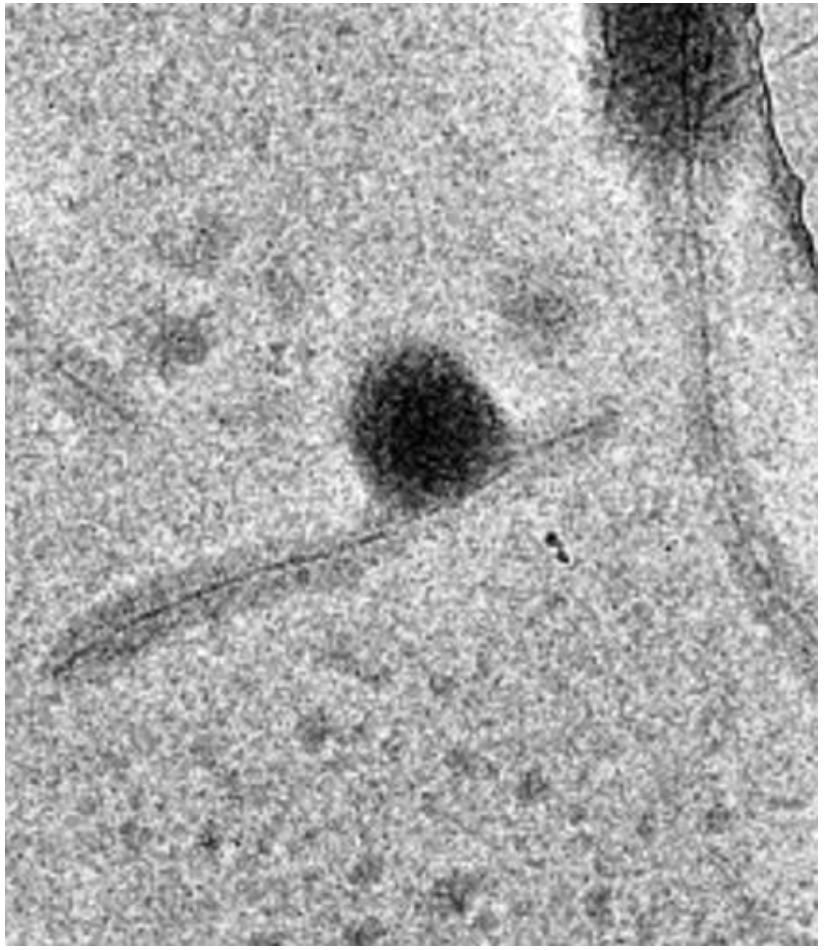


Huntington et al.,… & **Qureshi** (2022) *Frontiers in Molecular Biology* 9, 890390

ScopeM

Project 4: Helical Reconstruction





See: Frey, Gosh, **Qureshi** et al. eLife 2023;12:RP93562

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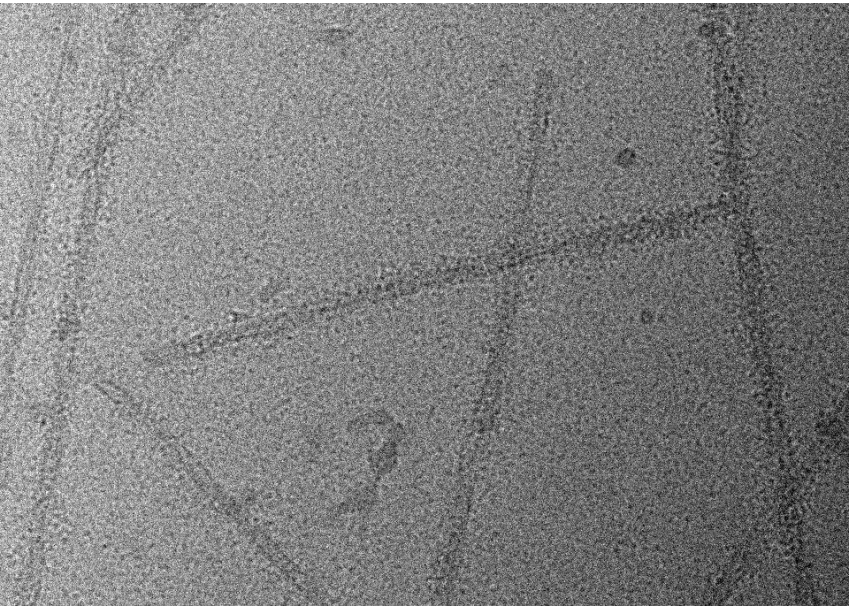
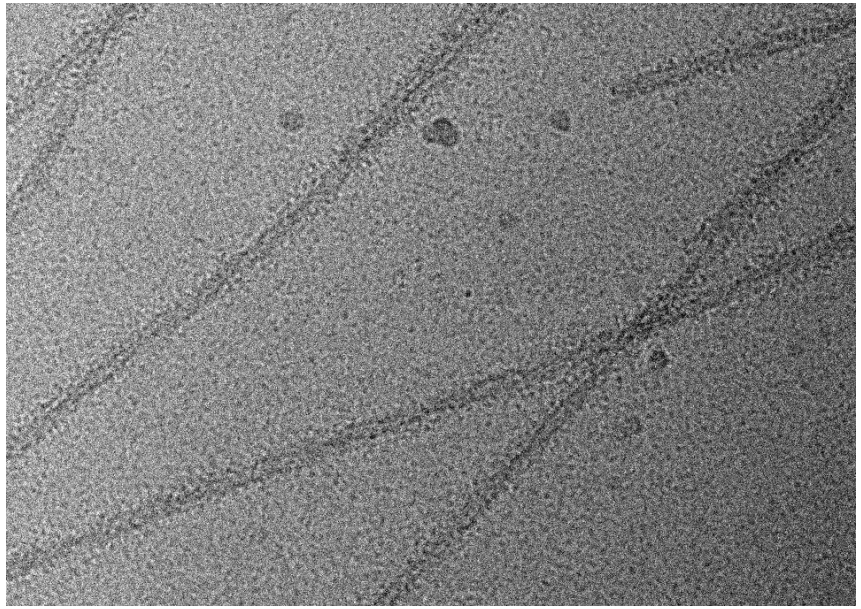
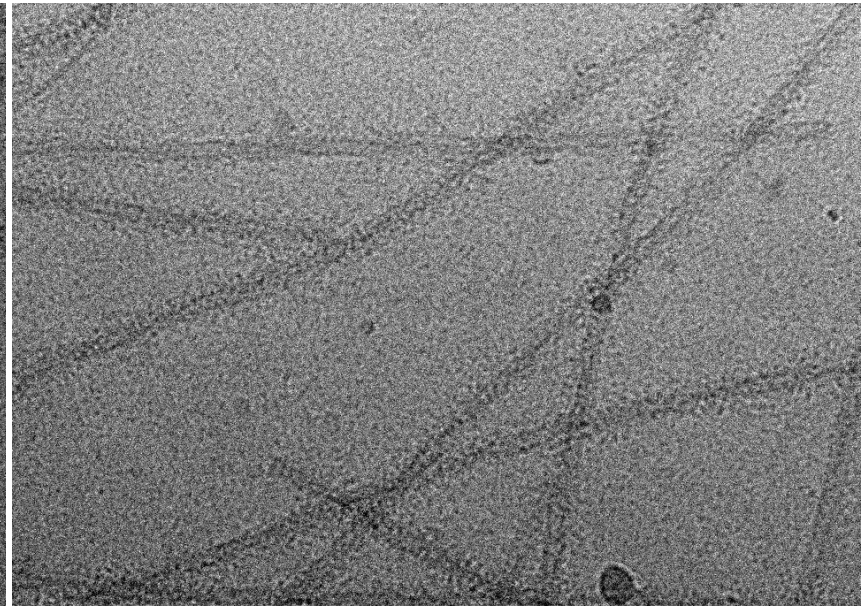
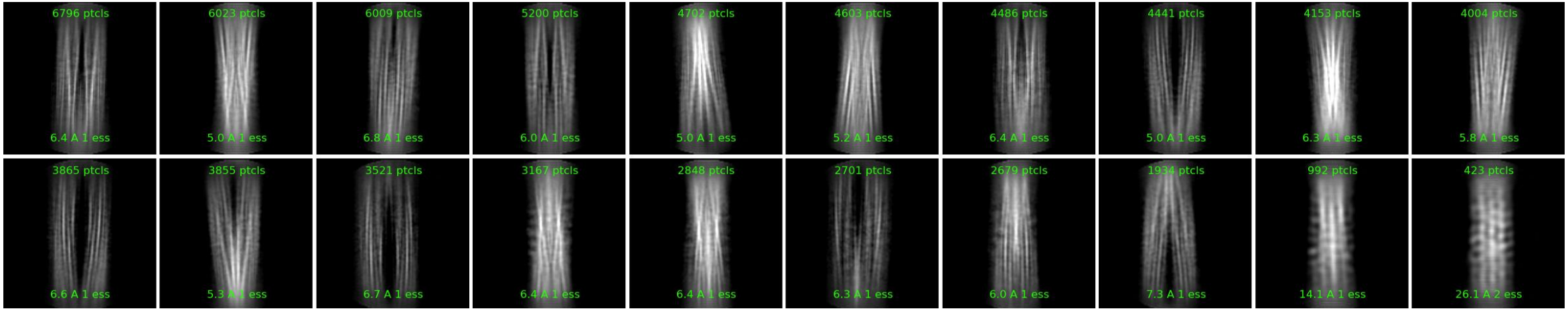
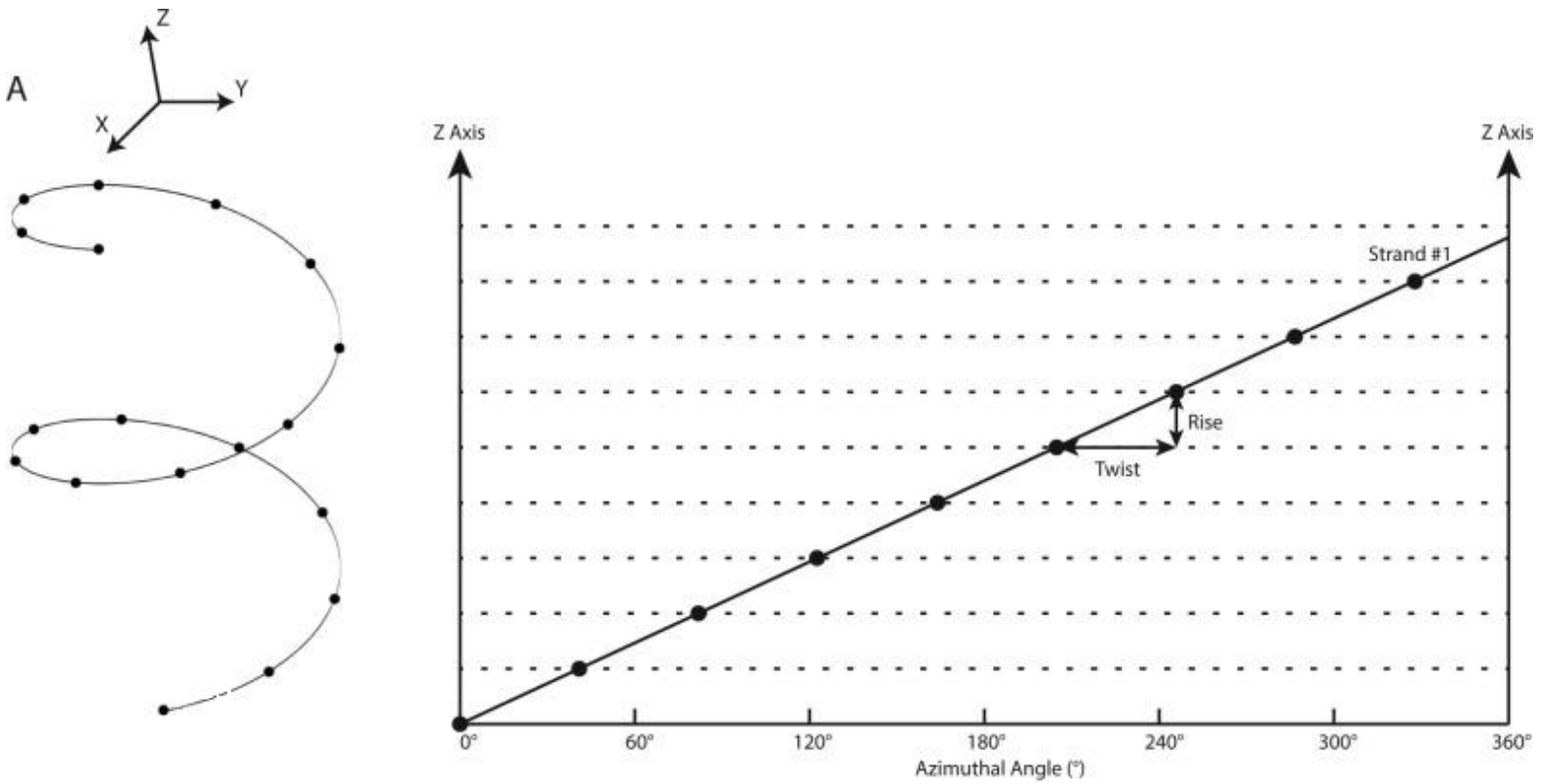






See: Frey, Gosh, **Qureshi** et al. eLife 2023;12:RP93562

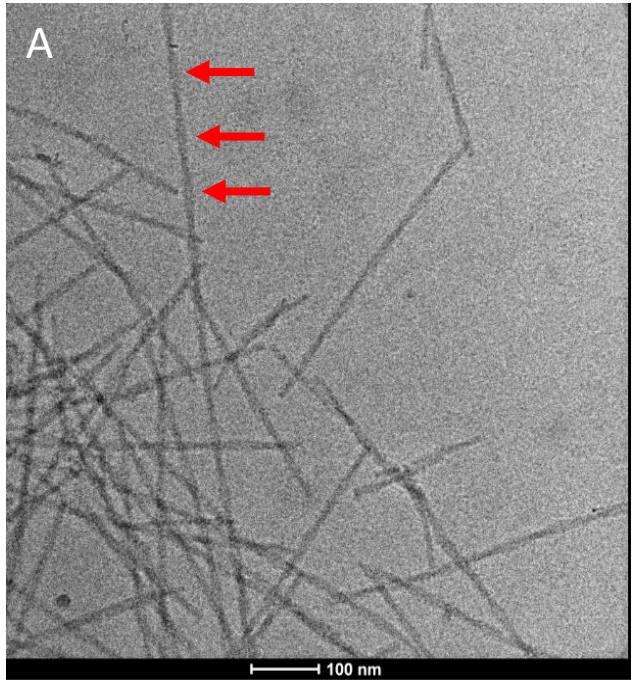
Twist and Rise parameters



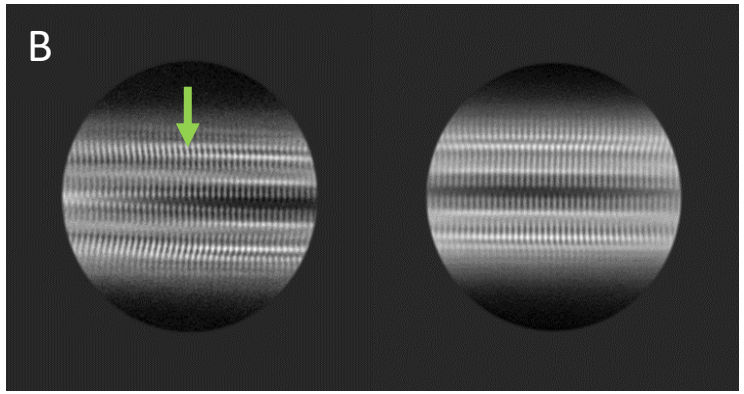
→ **Symmetry operations of translation and rotation in a screw like fashion reconstruct the helix**

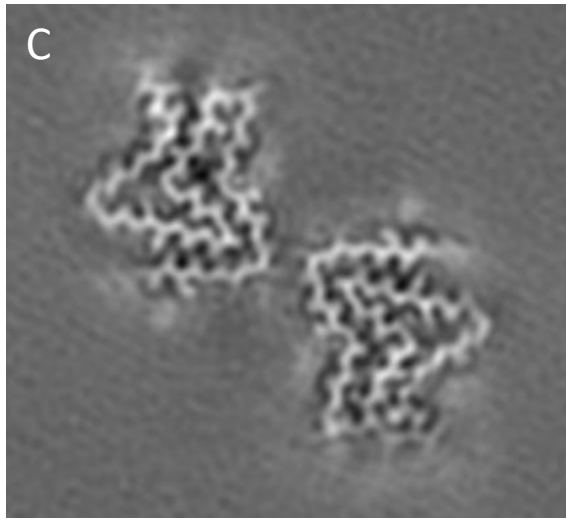
He & Scheres (2017) *J Stru Biol* 198, 163-76

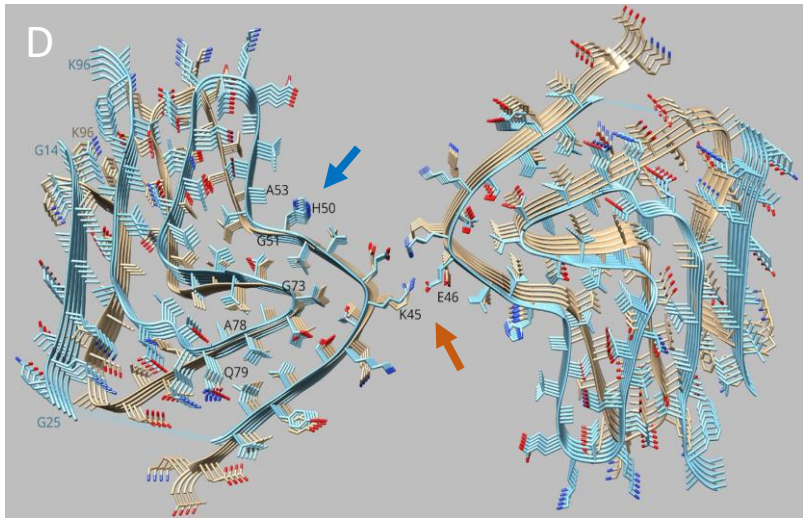
Collaboration with Riek Group, ETH Zurich



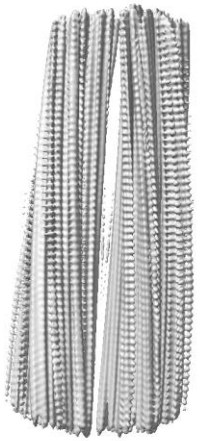
A Representative cryo-EM image of the α-synuclein fibrils showing twist of the two strands (red arrows), B Representative 2D class-average images showing striations visible due to helical arrangement of the monomers (green arrow pointing to a monomer) in the two protofibrils, C Cross-section of cryo-EM map showing the relative orientation of the monomers D Model structures (gold) compared with published structure (aquamarine)





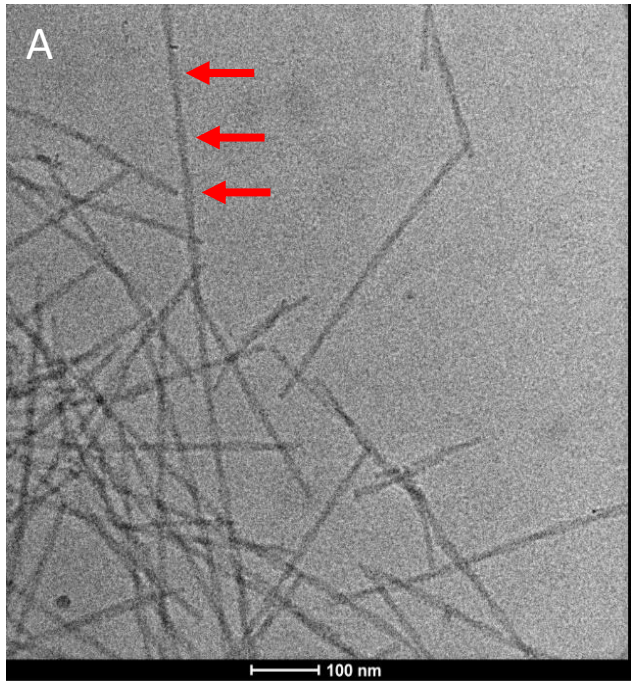


Movies

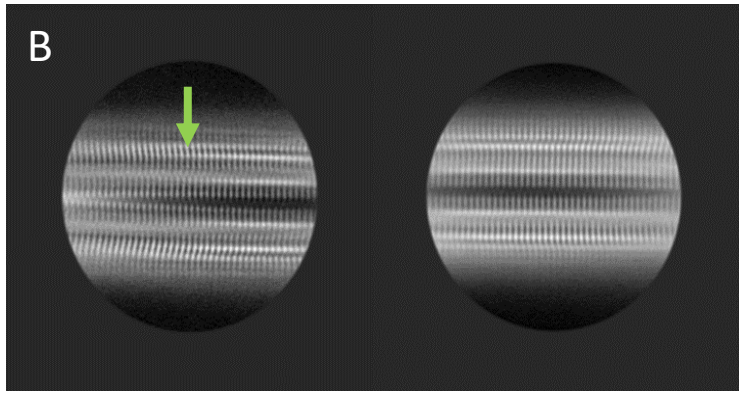


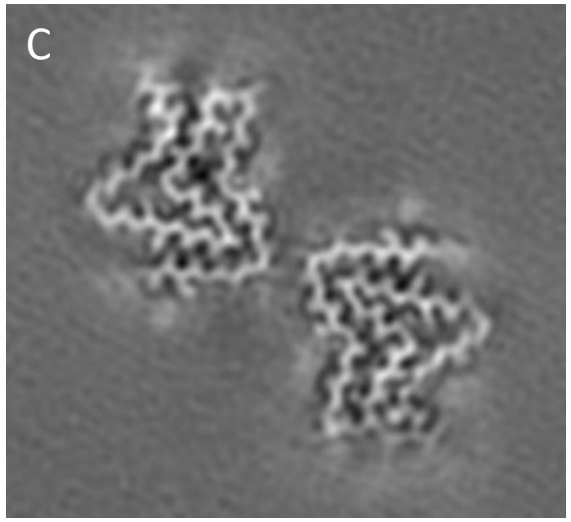
See: Frey, Gosh, **Qureshi** et al. eLife 2023;12:RP93562

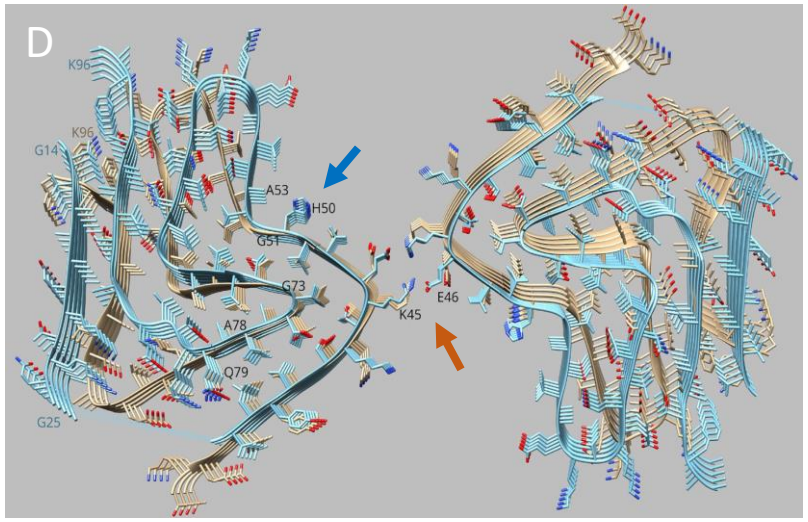
Collaboration with Rolad Riek Group, ETH Zurich



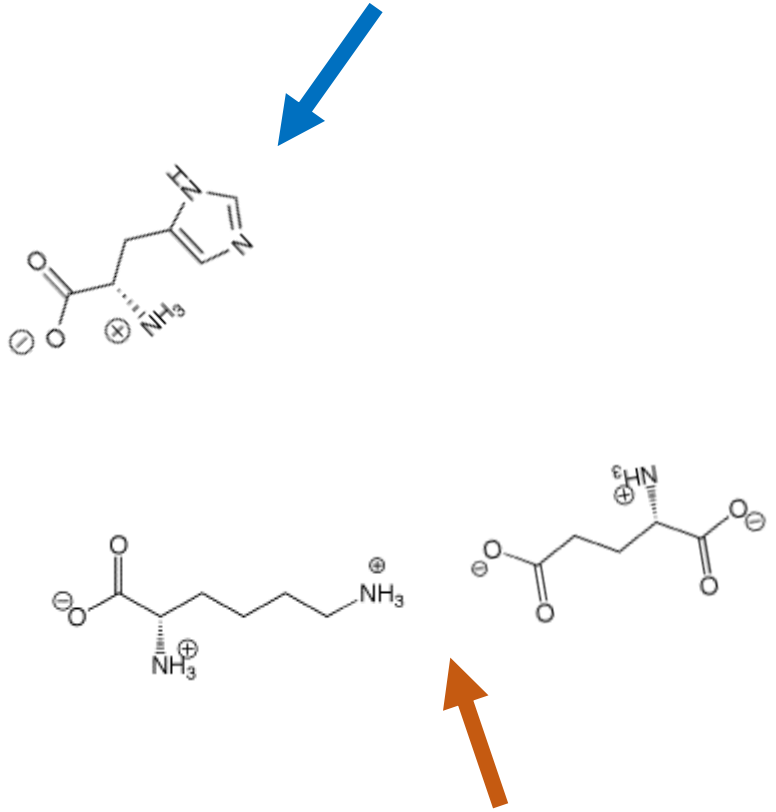
A Representative cryo-EM image of the α-synuclein fibrils showing twist of the two strands (red arrows), B Representative 2D class-average images showing striations visible due to helical arrangement of the monomers (green arrow pointing to a monomer) in the two protofibrils, C Cross-section of cryo-EM map showing the relative orientation of the monomers D Model structures (gold) compared with published structure (aquamarine)





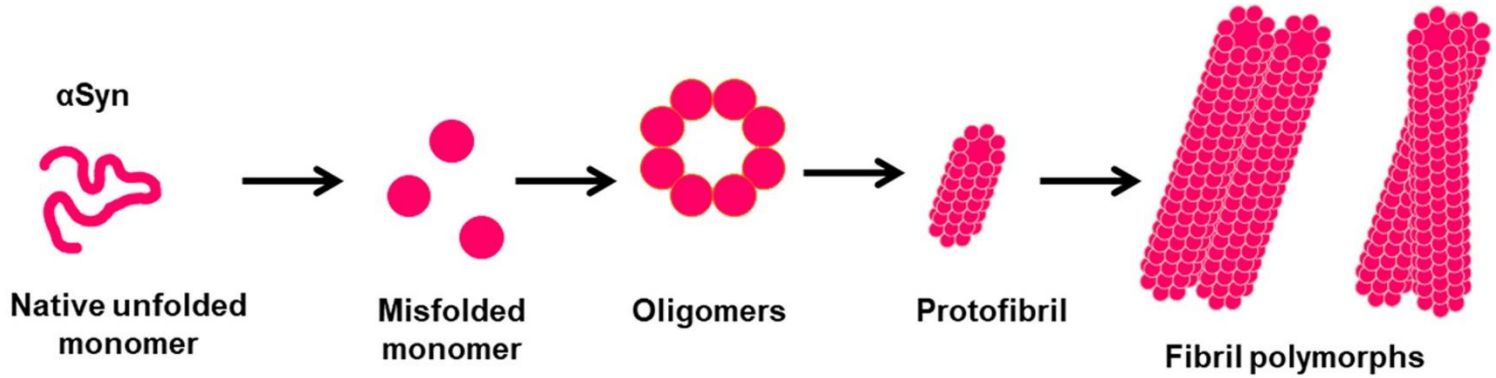


Resolving power at atomic level:
histidine, lysine, glutamate residues depicted



See: Frey, Gosh, **Qureshi** et al. eLife 2023;12:RP93562

α–synuclein has a role health and disease (synucleinopathies)



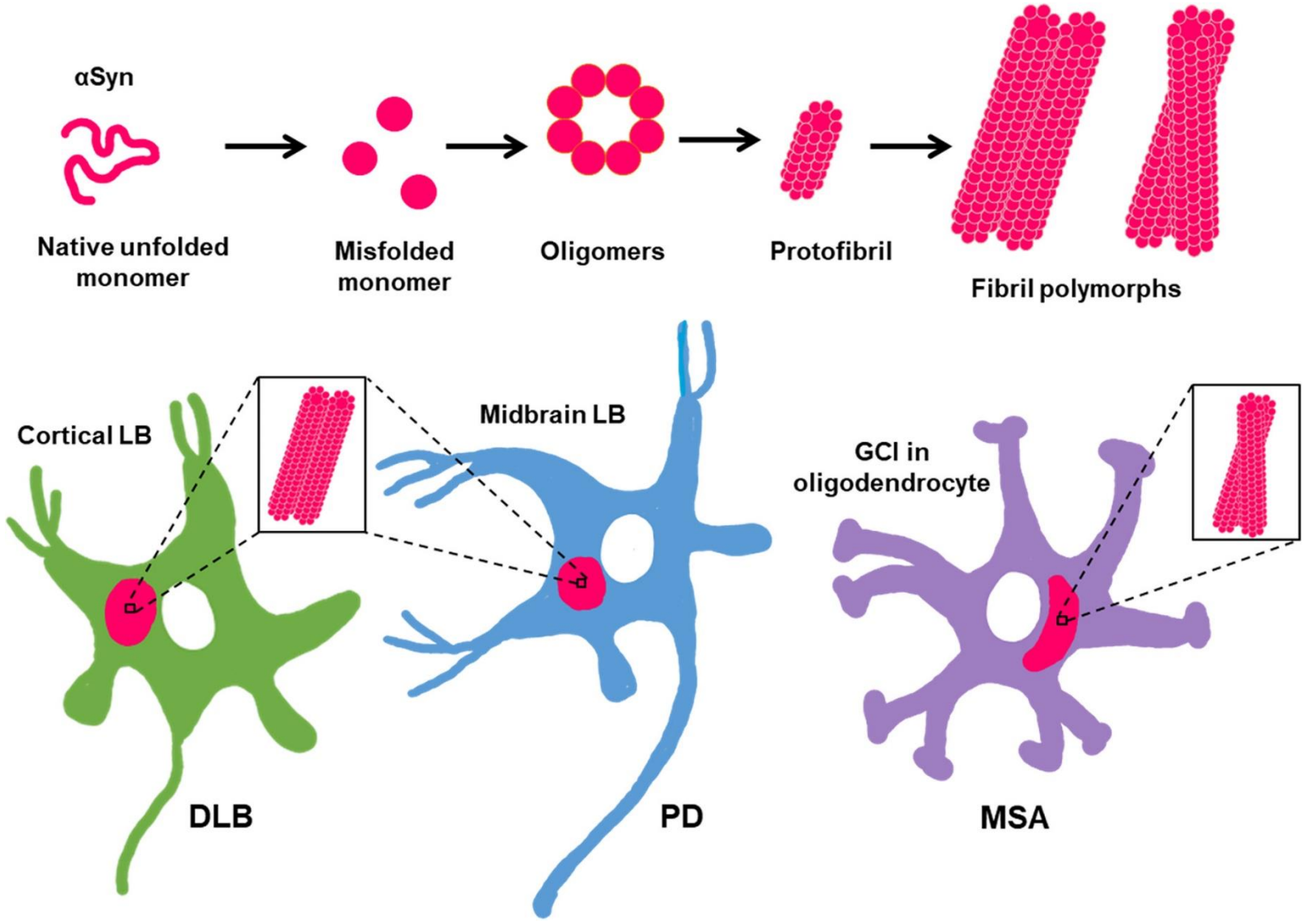
Choong & Mochizuki (2022) *Neuropathalogy* 42, 93-103

α–synuclein has a role health and disease (synucleinopathies)

Health:

Vesicle trafficking

…



Disease:

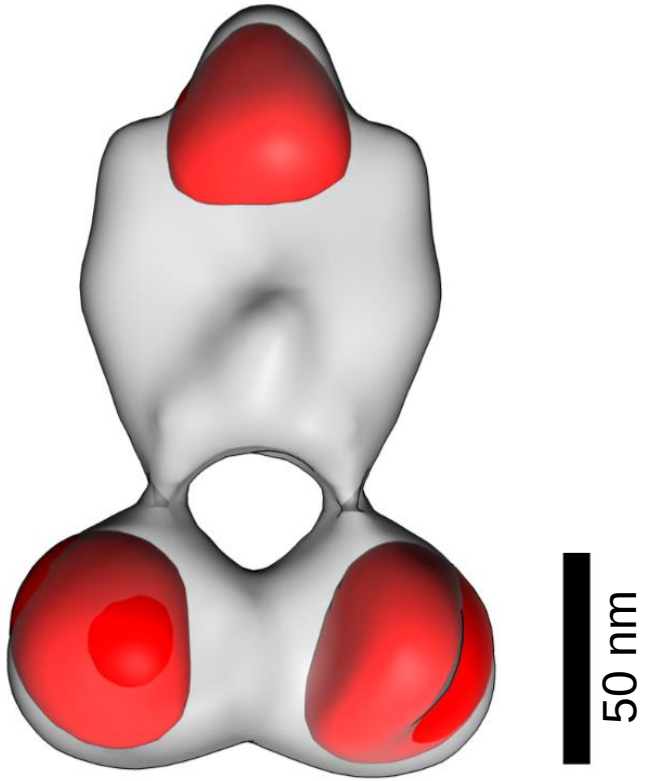
Parkinson`s disease

…

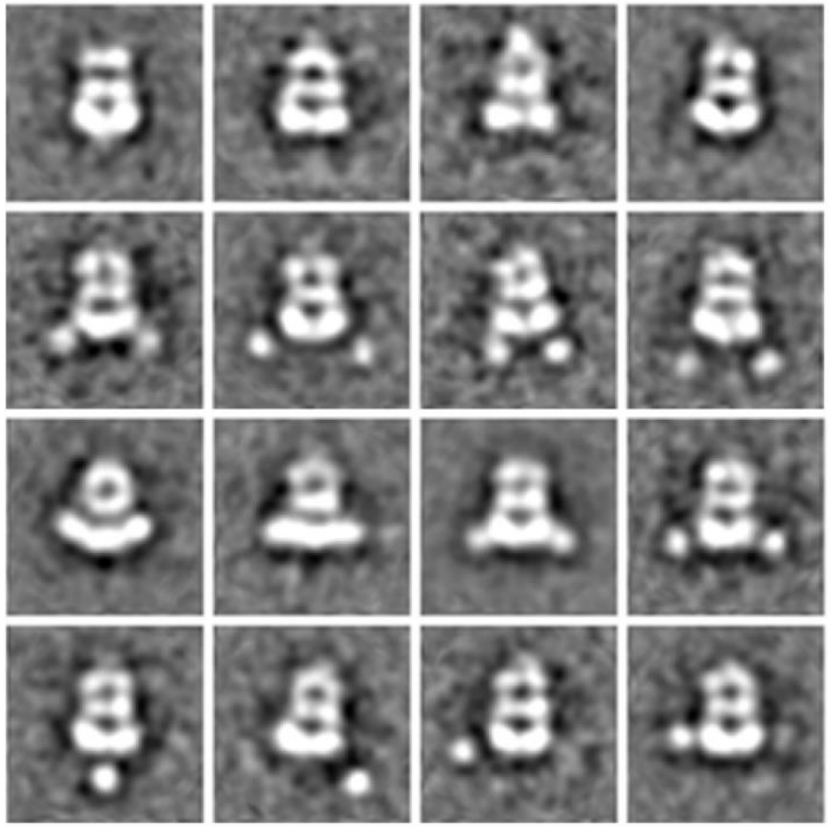
Choong & Mochizuki (2022) *Neuropathalogy* 42, 93-103

Project 6: Domain mobility or co-factor occupation

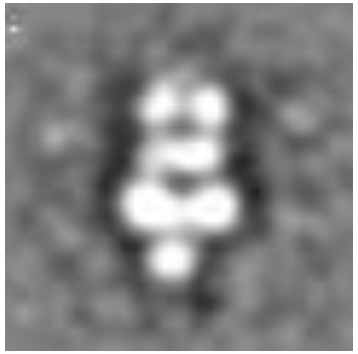
cryo-EM structure (tPDE):
3D variability



negative stain:
holo-PDE6 in complex with PrBP/δ



Movie



Qureshi, Schmidt et al (2018) *Nat Comms* 9 (1), 90

Qureshi, Behrmann et al (2018) *Open Biology* 8, 18075

ScopeM

Thank you for you attention!

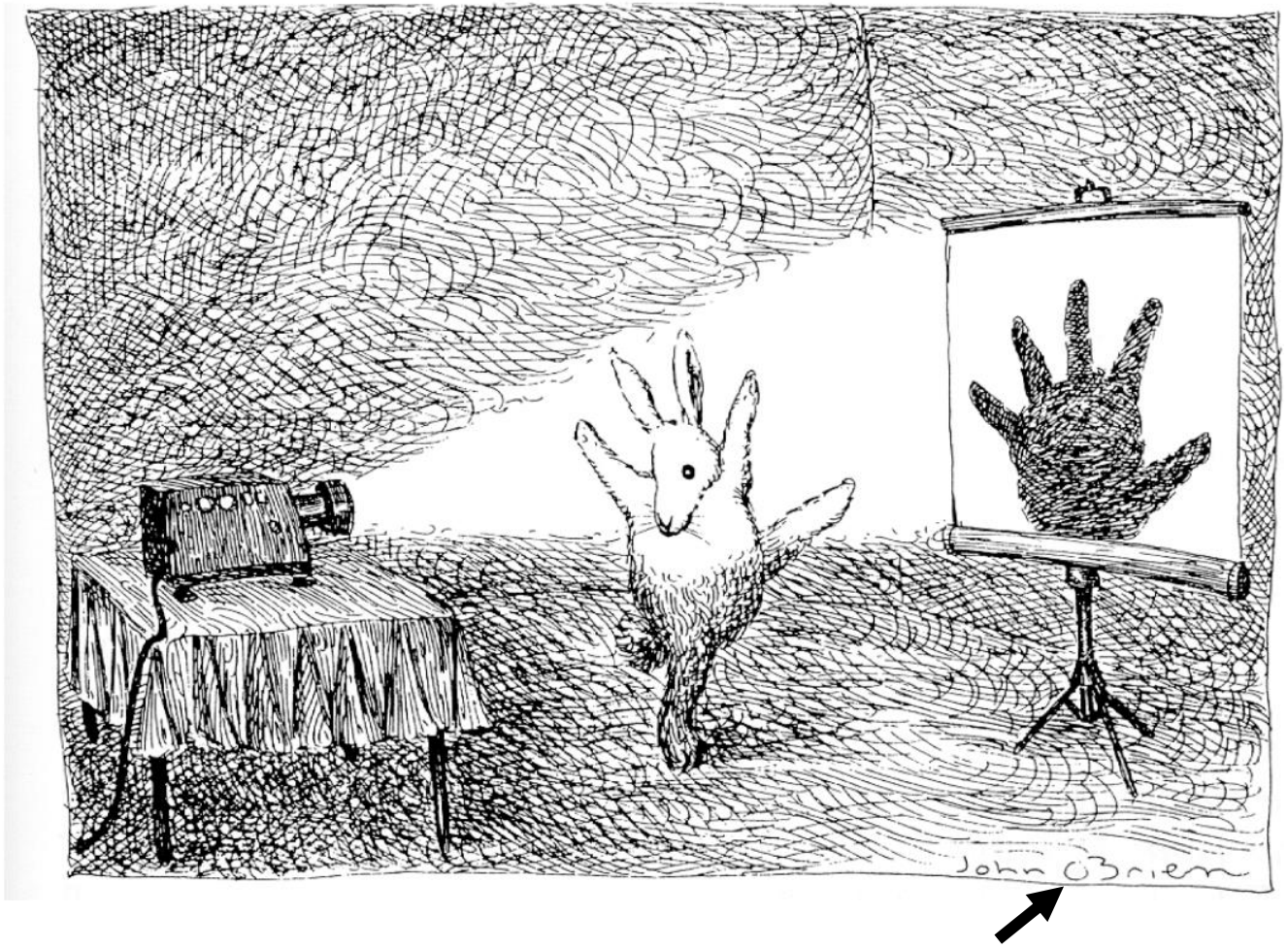
Questions?

ScopeM

Additional slides for potential questions?

ScopeM

2D images (shadows/photos vs. projects) of 3D objects



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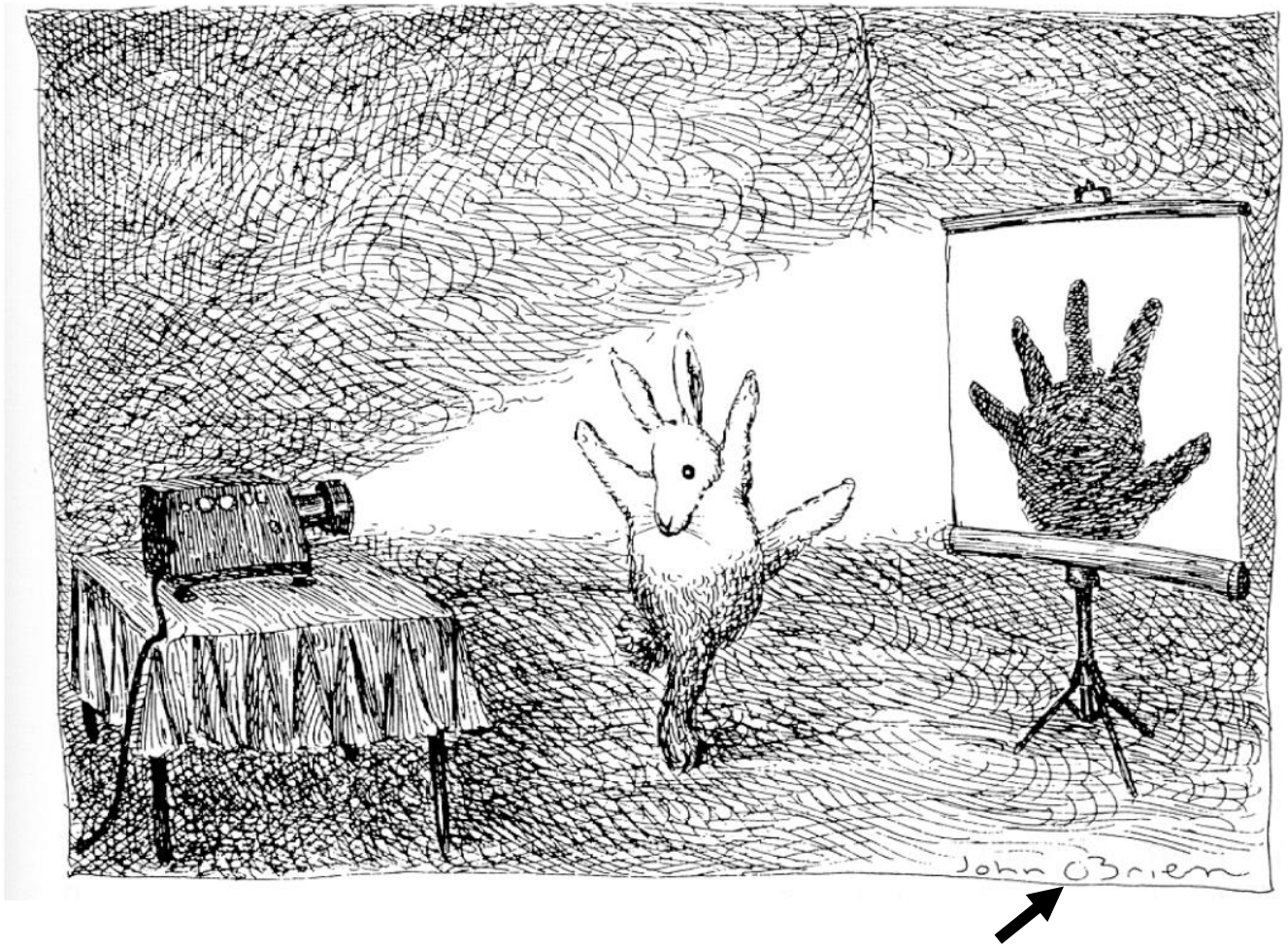
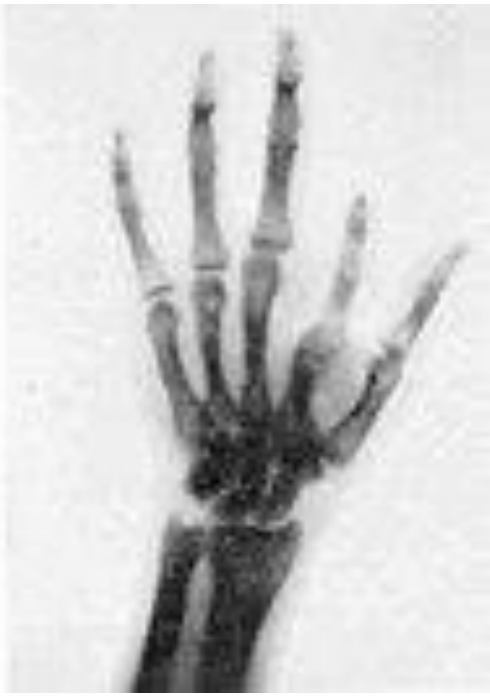
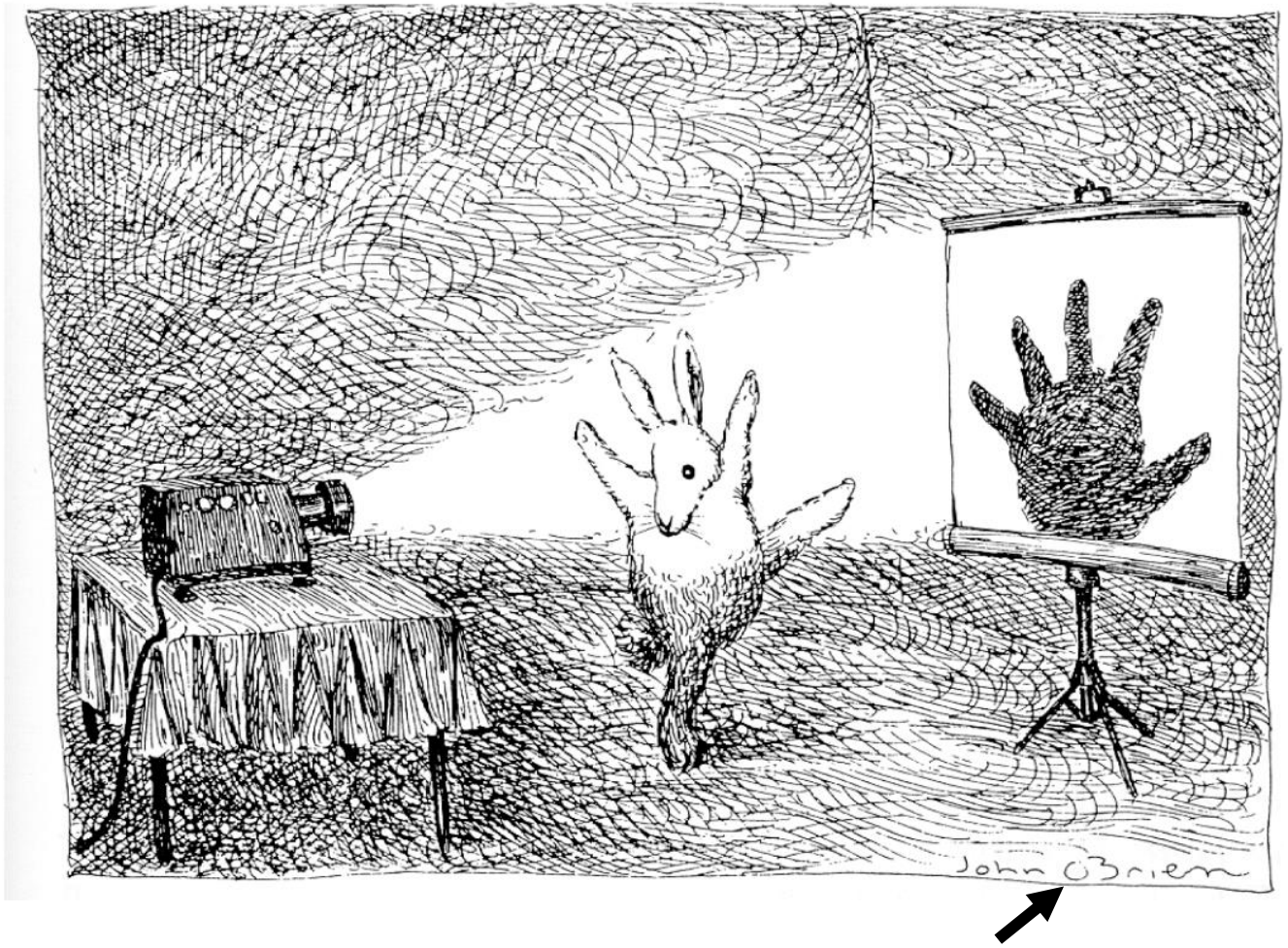
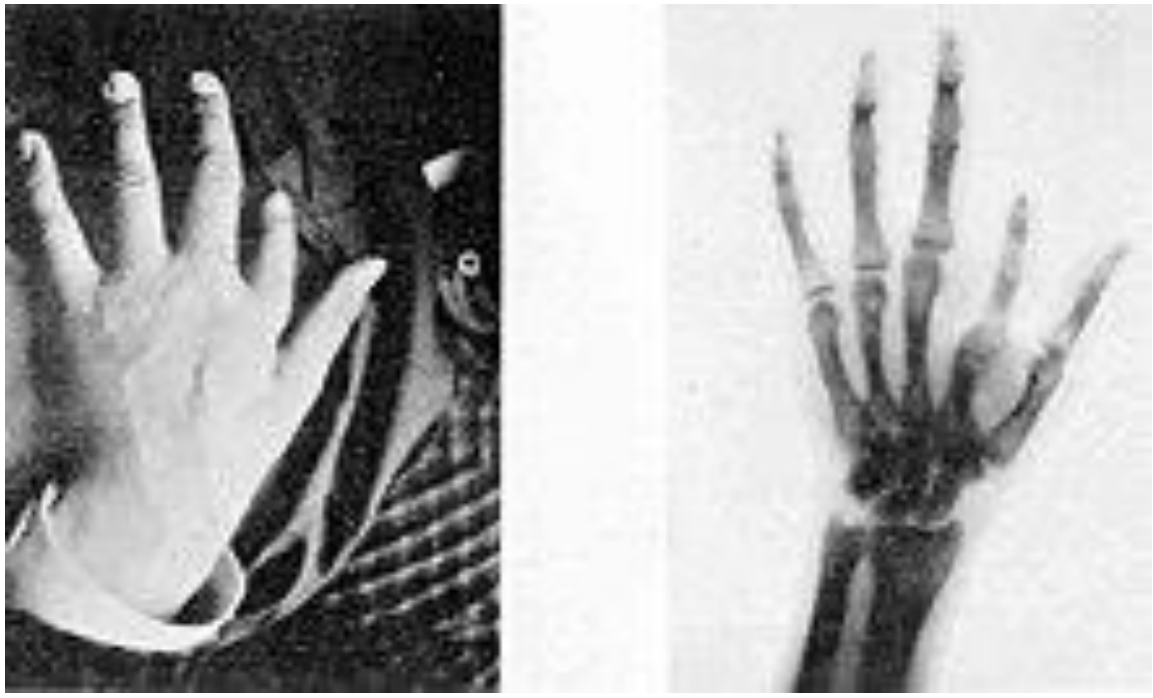




Courtesy: Wikipedia

2D images (shadows/photos vs. projects) of 3D objects





Courtesy: Wikipedia

→ No transmission (shadow, photo) vs. transmission in projection image i.e., internal structure

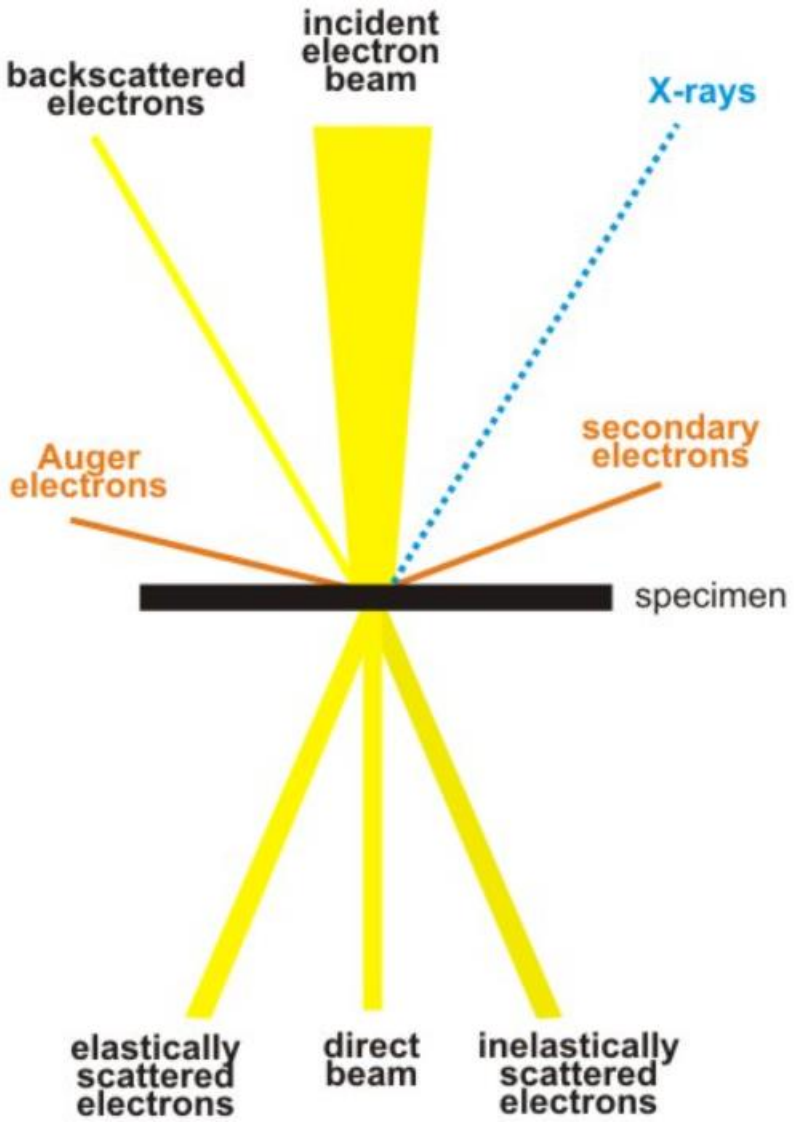
Electron interactions with matter in TEM

Two different types of interactions appear:

Elastic Interactions
No energy is transferred from the electron to the sample. The electron either passes without any interaction (direct beam) or is scattered by the electrostatic attraction of the positive potential inside the electron cloud. The arising signals are mainly exploited in TEM and electron diffraction.

Inelastic Interactions
Energy is transferred from the incident electrons to the sample producing secondary electrons, phonons, UV quanta or cathodo-luminescence. Ionization of atoms by removing inner shell electrons leads to the emission of X-rays or Auger electrons. These signals are used in analytical electron microscopy.

A script giving an introduction can be downloaded here: interactions.



https://www.microscopy.ethz.ch/interactions.htm

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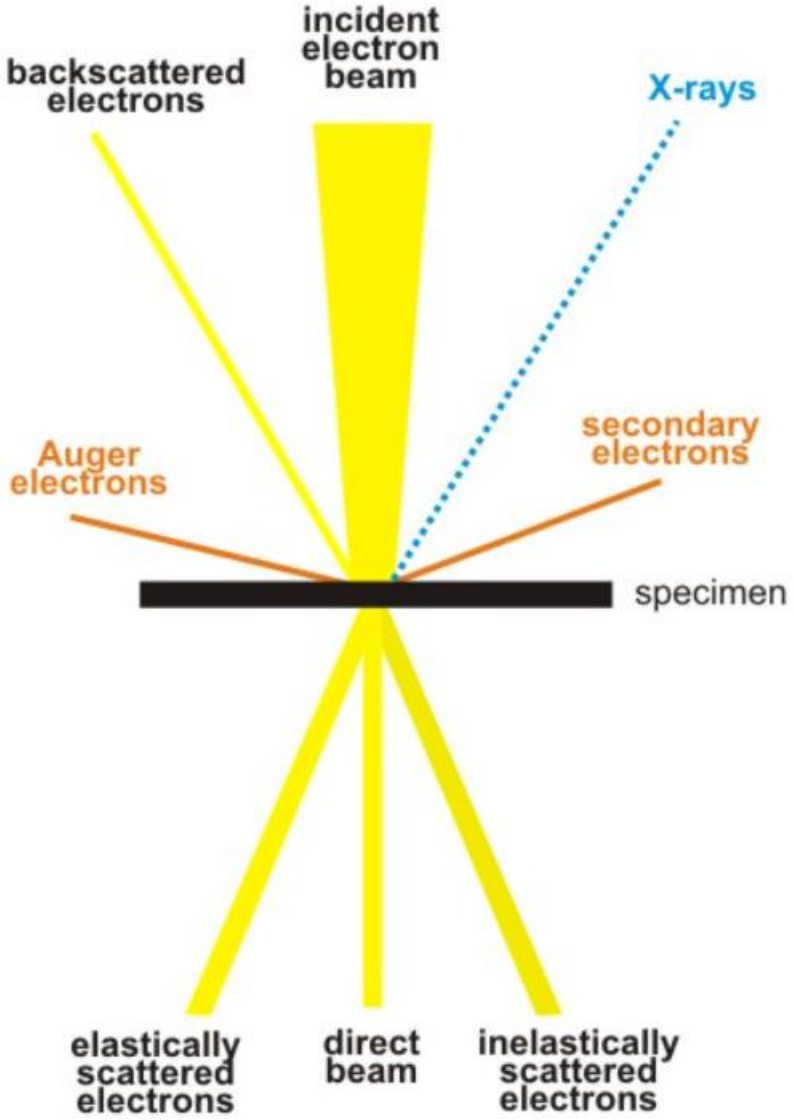

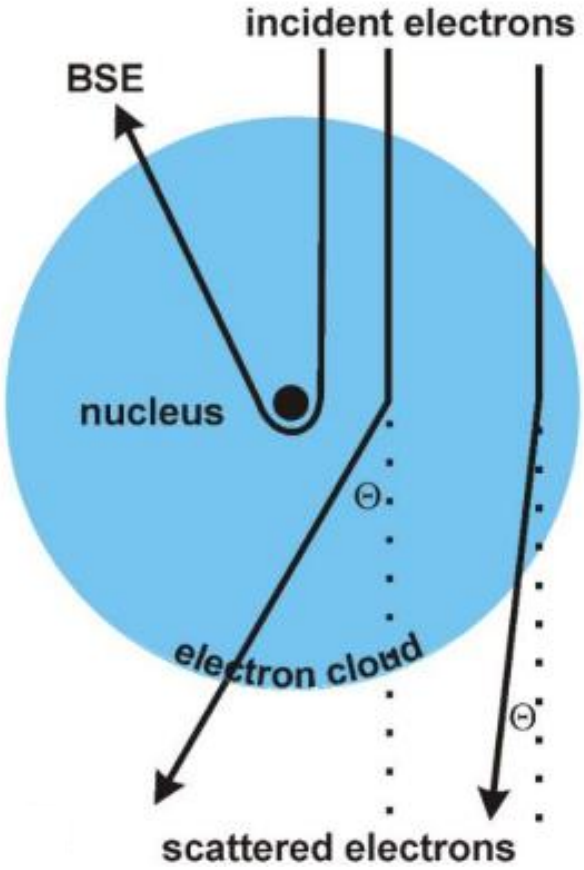

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