RUTGERS

The Winter-Spring 2022 EM Course

Introduction to Cryo-electron Tomography

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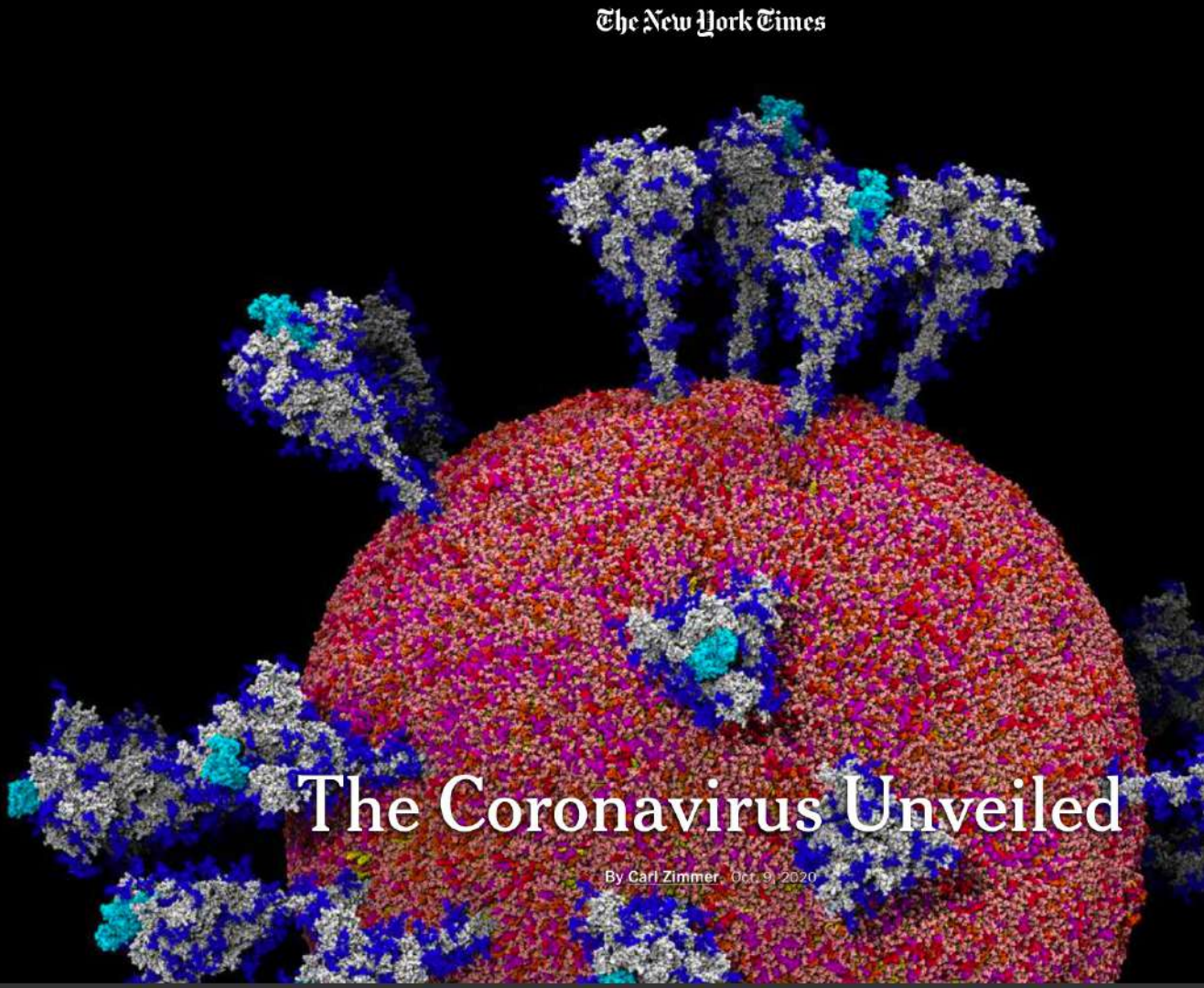
Institute for Quantitative Biomedicine

Rutgers University

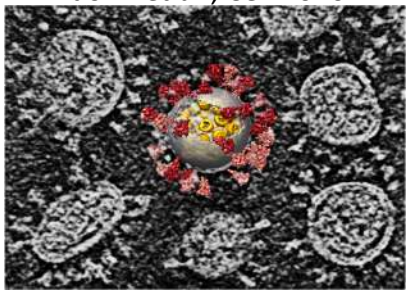
February 14, 2022

Resolving Structures to Drive Scientific Discoveries during the Pandemic



How does the virus look like?

Yao H. *et al.*, Cell 2020



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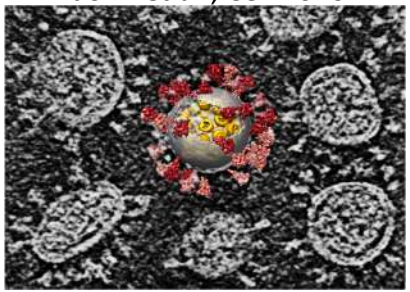

How does Remdesivir interact with SARS-CoV-2?

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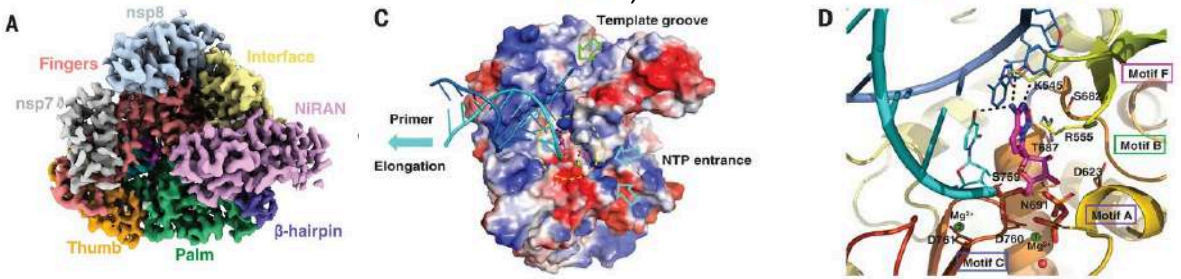

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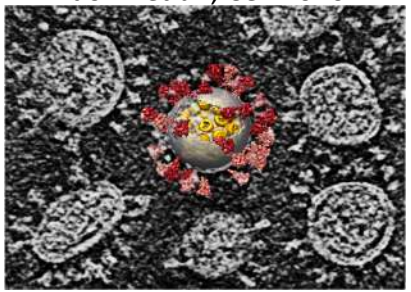

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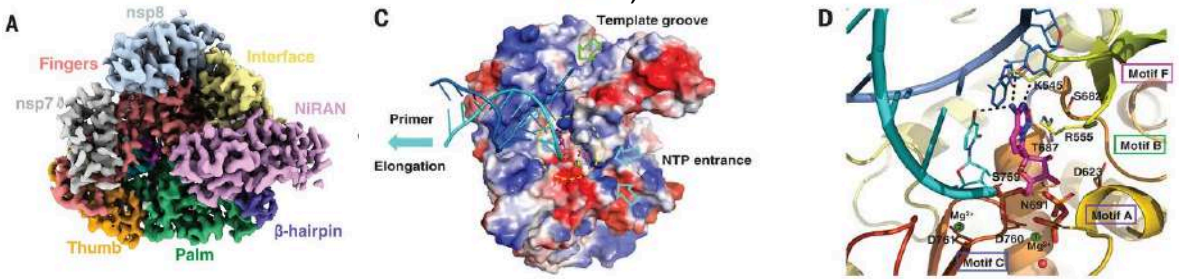
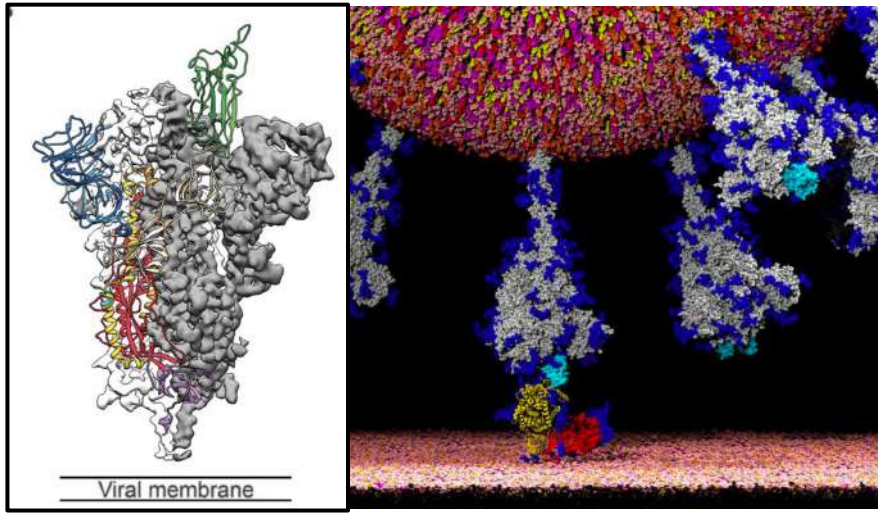


How does SARS-CoV-2 enter human cells?

Wrapp D. *et al*., Science 2020; Simulation by Amaro lab, UCSD



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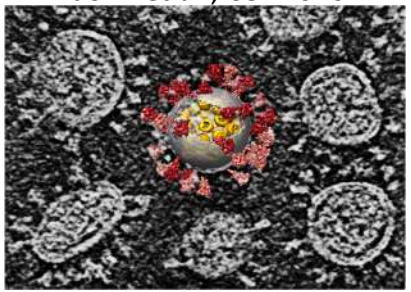

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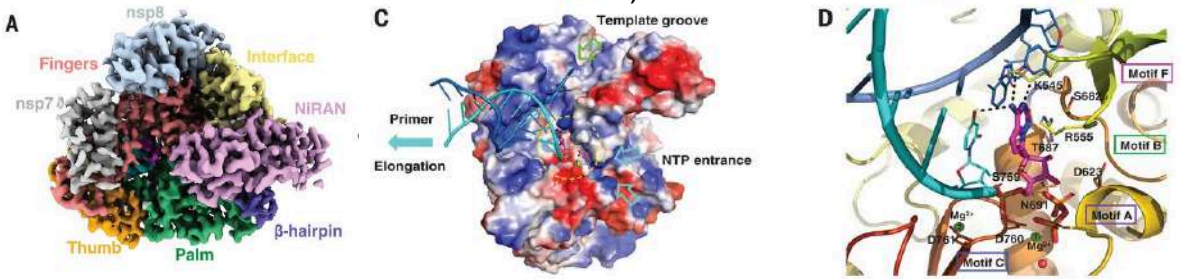

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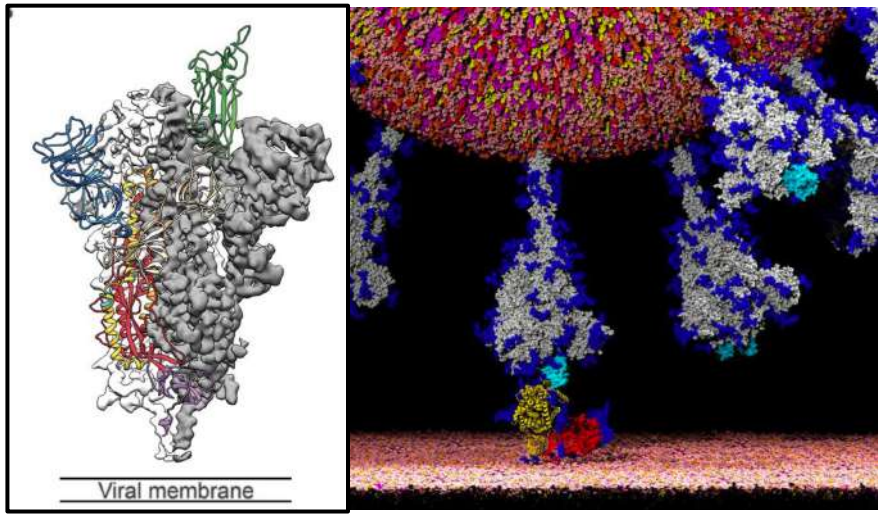
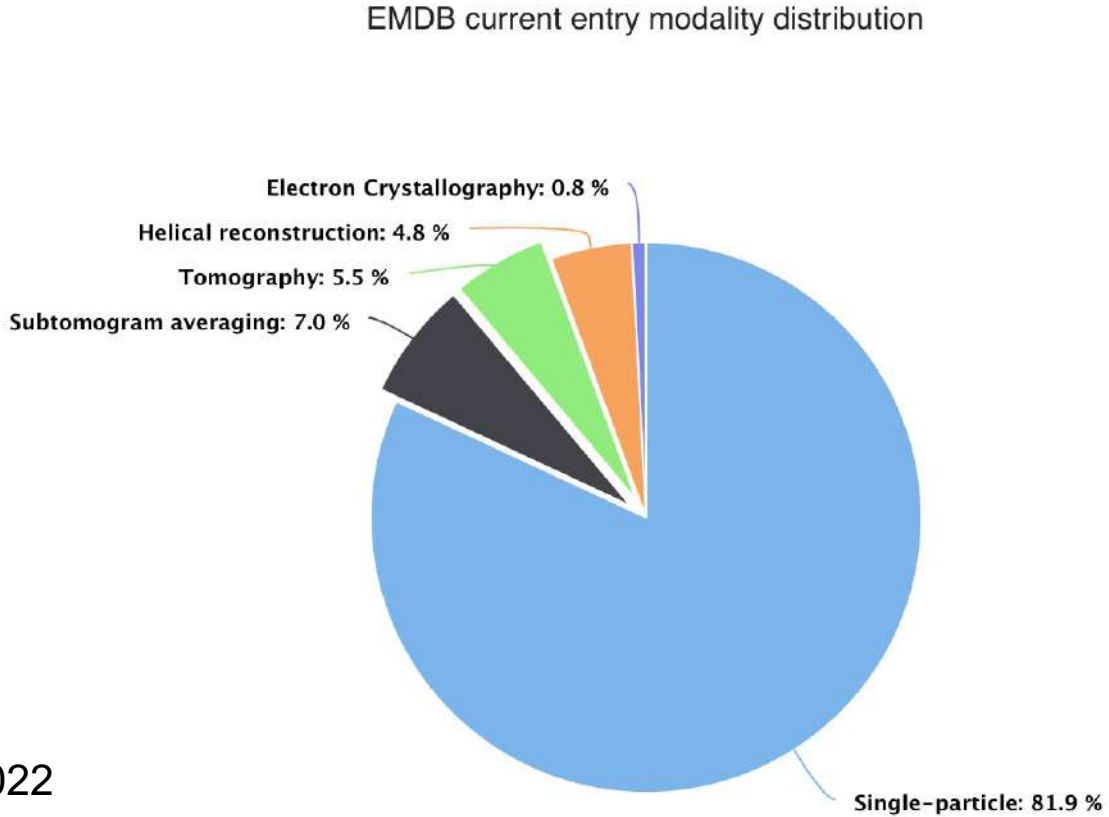


How does SARS-CoV-2 assemble inside cells?

Klein S. *et al*., Nature Communications 2020

Structures Resolved by Tomography in EMDB

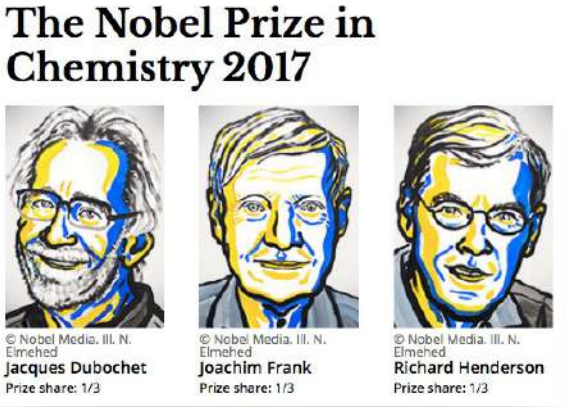


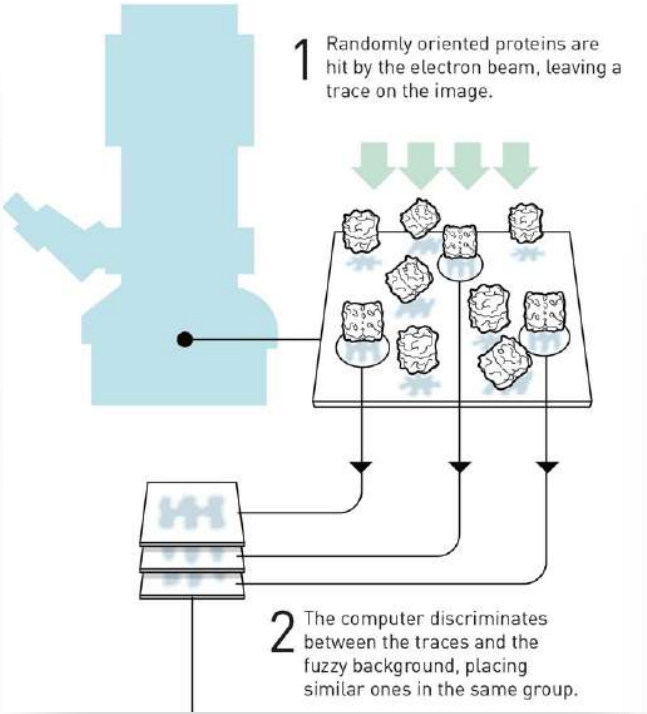
As of Feb. 4, 2022

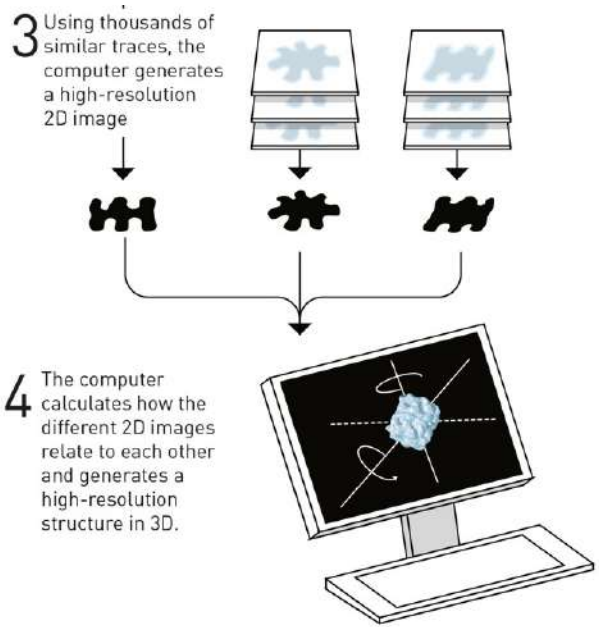
Outline

- What is cryo-electron tomography?
- Sample preparation: special considerations
- Data collection, alignment, and reconstruction
- Application of cryo-electron tomography in cell biology (structural cell biology)

Cryo-EM Single Particle Analysis

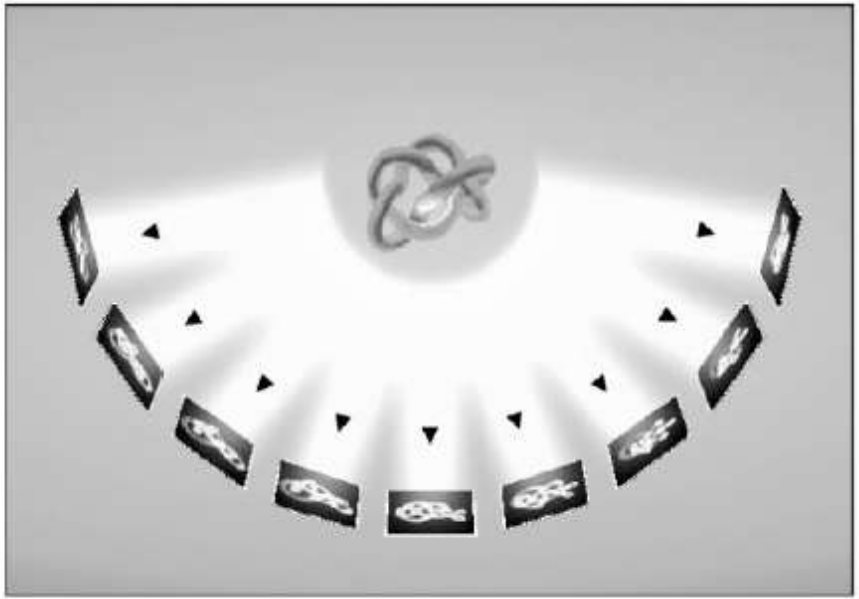






Cryo-electron tomography (Cryo-ET)

On a TEM: 3D structures → 2D images



On a computer: 2D images → 3D structures

Baumeister W. 1999 Trends in Cell Bio.

Why Cryo-electron Tomography (Cryo-ET)?

- Sample has a unique structure or heterogenous

- Sample in a complex environment

Concept Check Questions

Sample Preparation

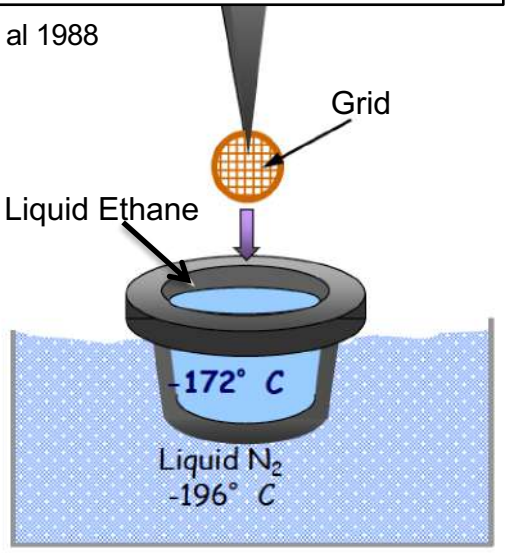
- Preserving various structural elements of the specimen in native structure in aqueous solution
- Good concentration
- Good thickness & good contrast
- Target tracking in their native environment

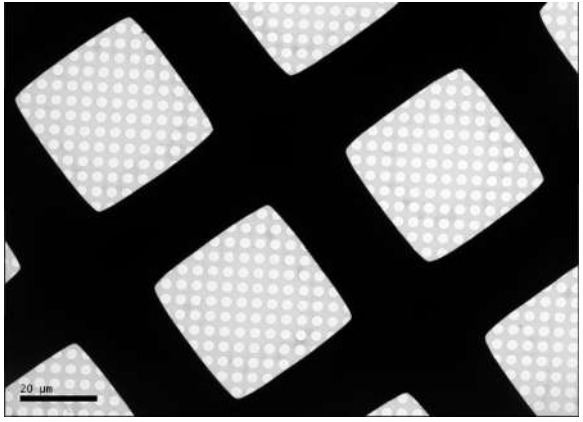
Plunge Freezing

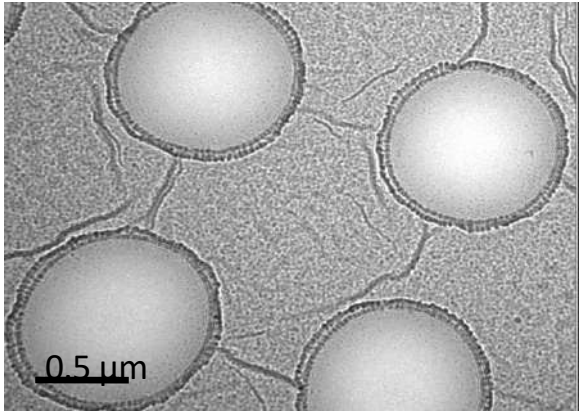
To vitrify water:

- Temperature drop > 10^5-10^6 K/s → liquid ethane
  - Liquid N_2 has poor cooling capacity
  - Water is a poor thermal conductor so sample thickness < 5 μm
- Gravity plunge at > 1 m/s

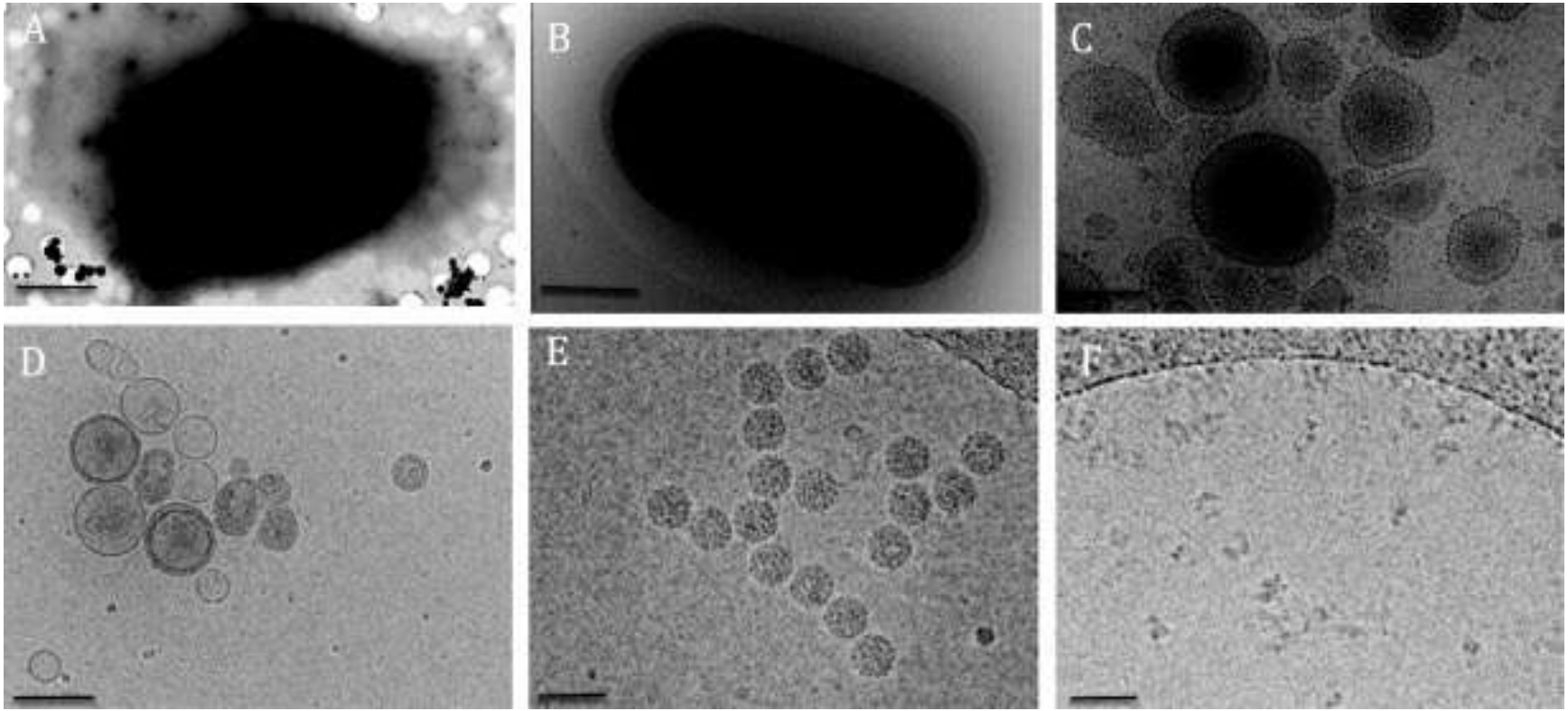
Dubochet et al 1988







Specimen Preserved by Plunge Freezing



R. F. Thompson, *et al.*, 2016 Methods, Vol. 100 , 3-15

Preparing Intact Mammalian Cells For Cellular Tomography

Prepare EM Grids
- Material: gold
- Mesh: 200 mesh
- Supporting film: carbon or SIO_2
- Hole size: support *vs* contrast

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Plate Cells on EM Grids
- Coating protein
- Plating density

↓

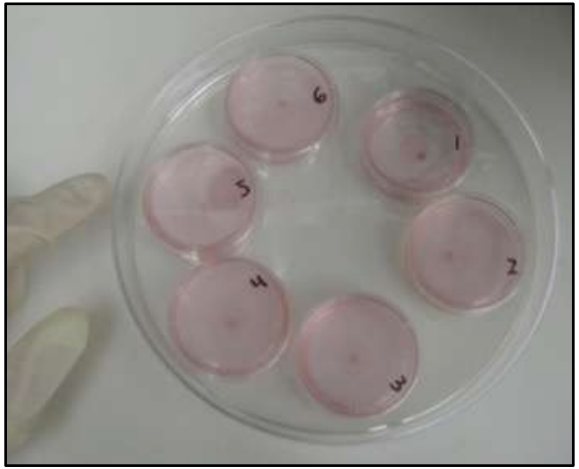
Allow Cells to Adhere/Grow

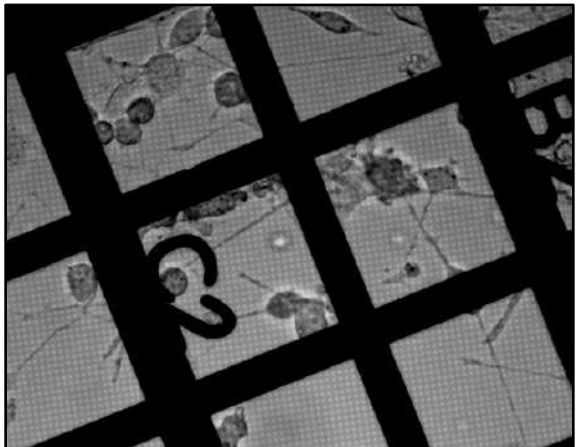
↓

Plunge Freeze Grids

↓

Imaging

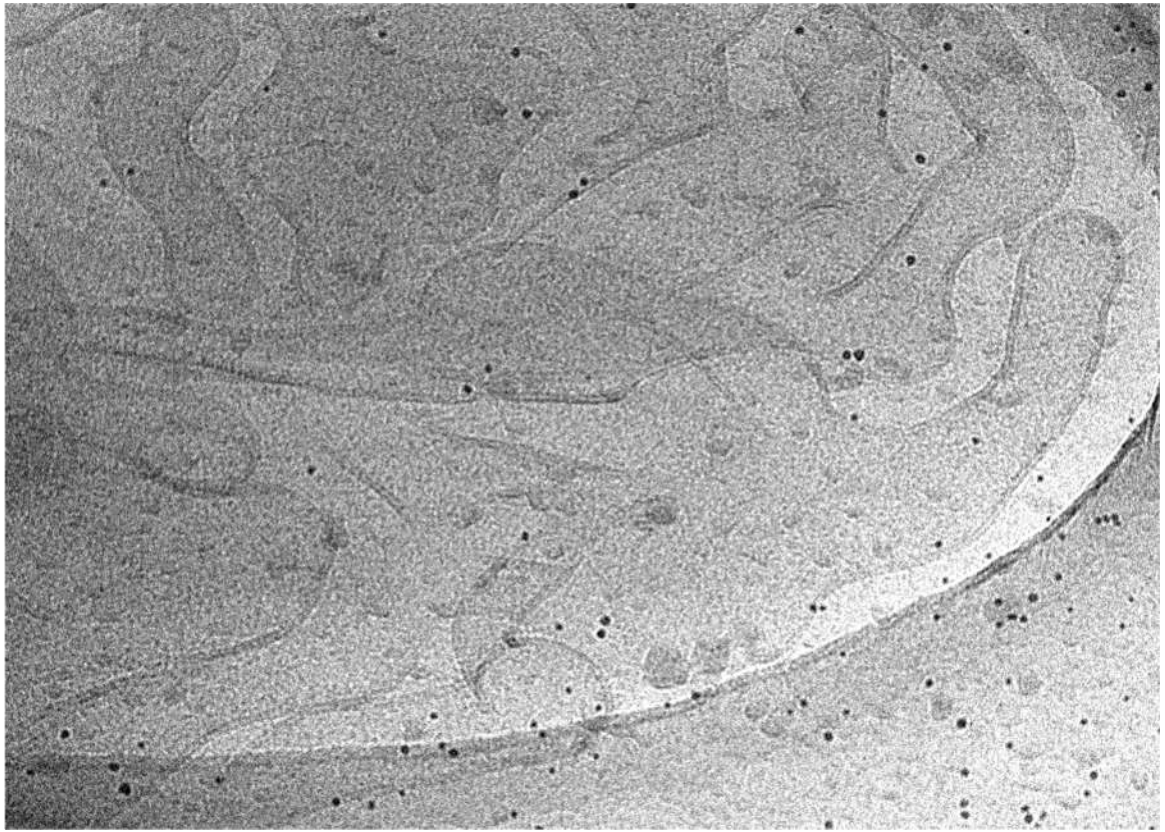




CryoET Data Collection

What is a “tilt-series”?

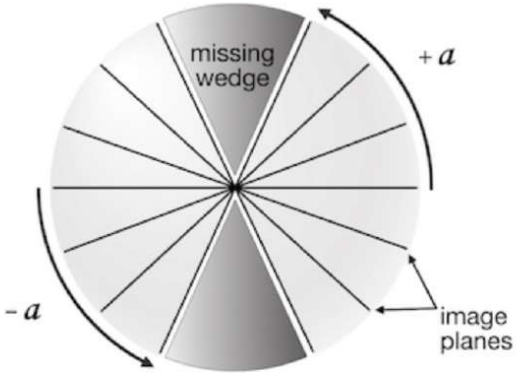
- Images taken when the sample is tilting about the tilt axis.



Tilt series of *C. glabrata* plasma membranes

CryoET Data Collection

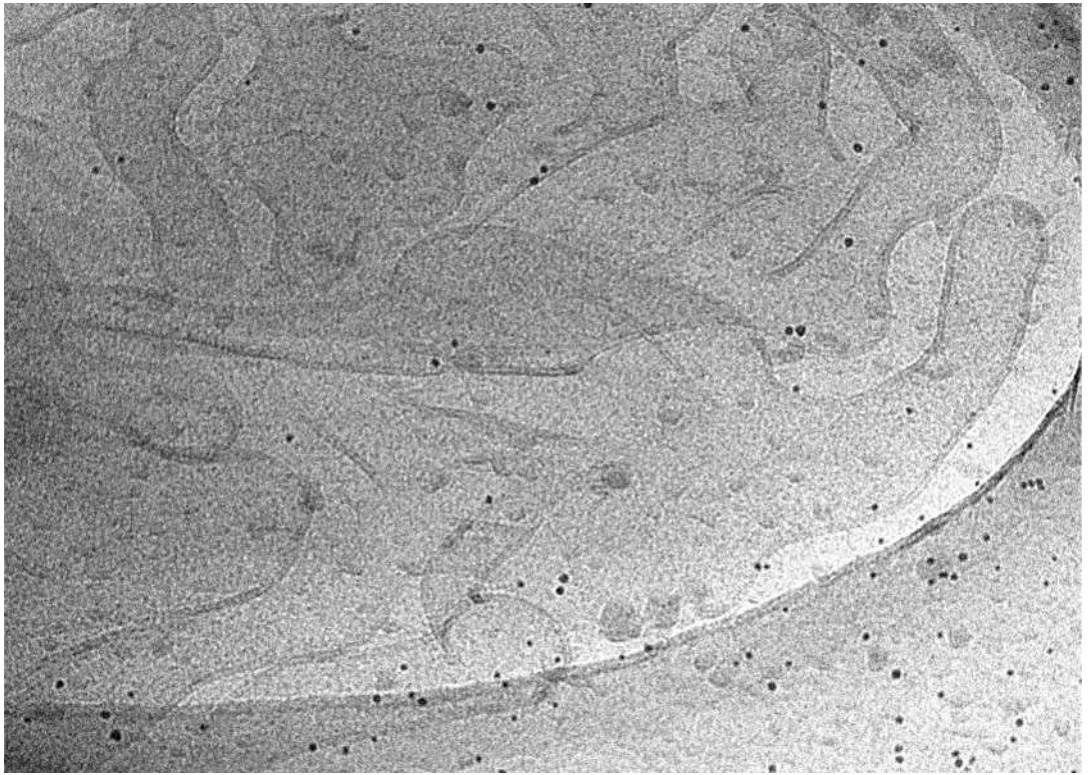
- There are many configuration parameters involved in data collection. Each is a balance between opposing considerations.
  - Defocus: contrast vs resolution
  - Total dose: signal vs radiation damage
  - Tilt range and increment:
    - goniometer mechanical limit (-70 <->+70)
    - goal of the project
    - sample geometry



Concept Check Questions

Data alignment and reconstruction

- Each image in a tilt series has to be “aligned”
  - x, y shift
  - Rotation (position of tilt axis)
  - Tilt angle
  - defocus



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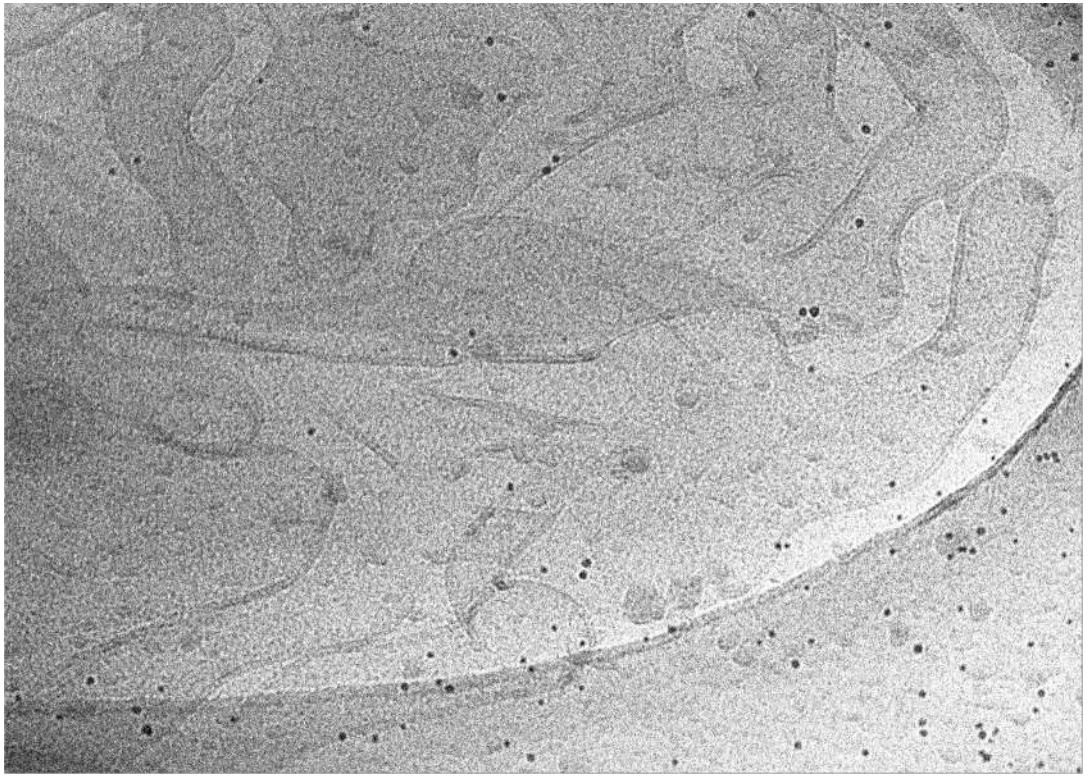

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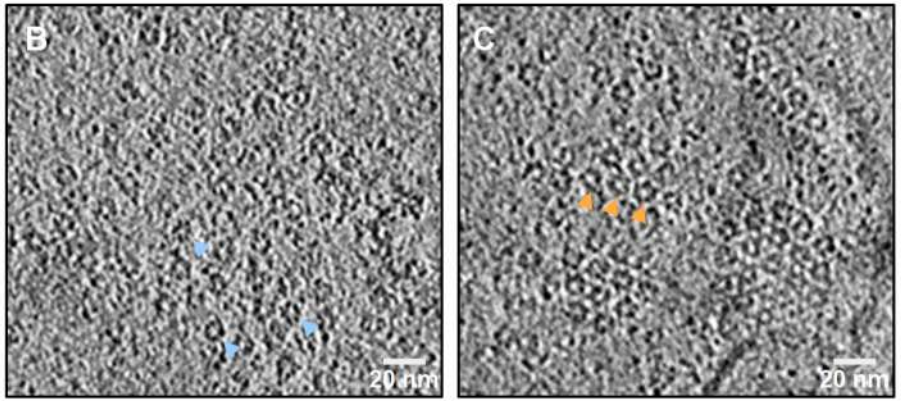
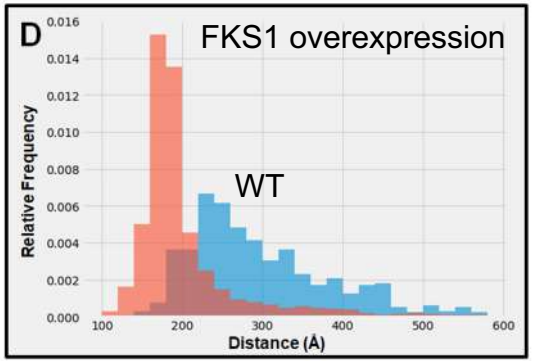
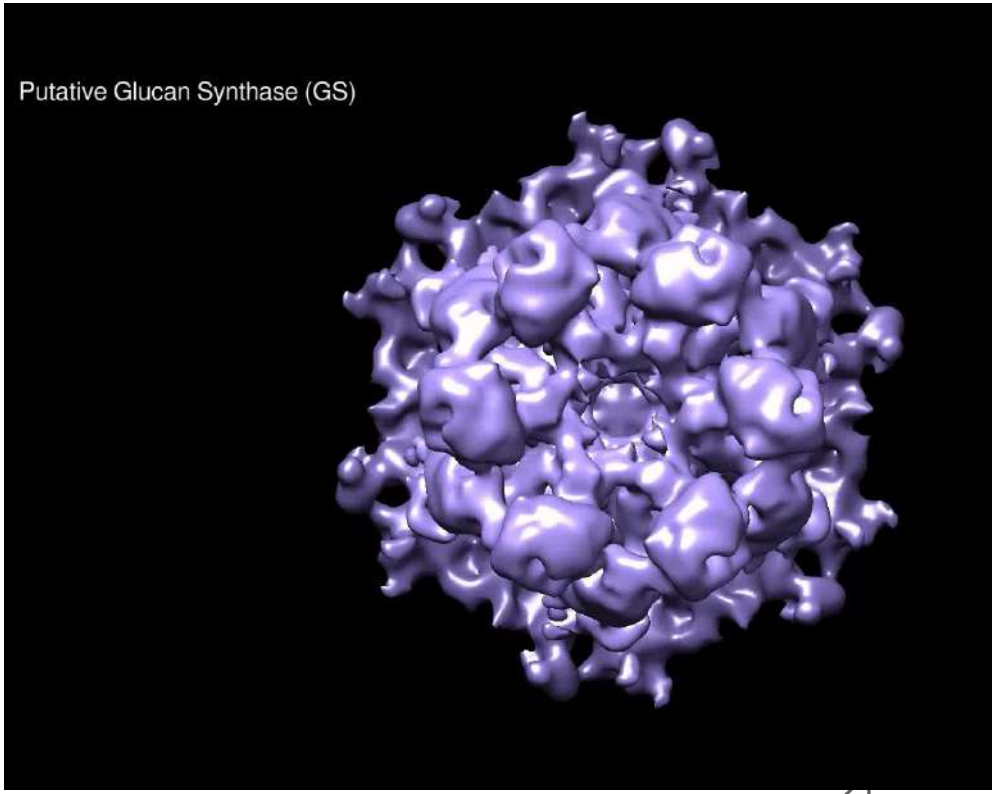
Data alignment and reconstruction

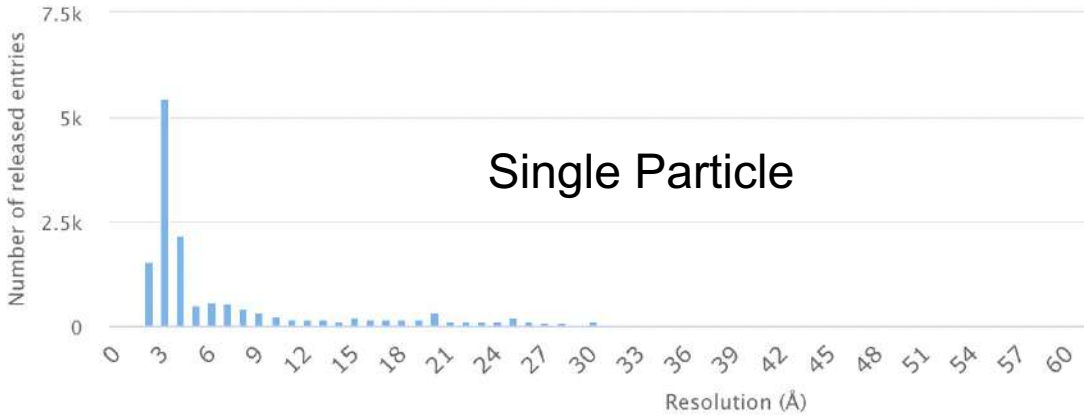
Data visualization, analysis and subtomogram averaging

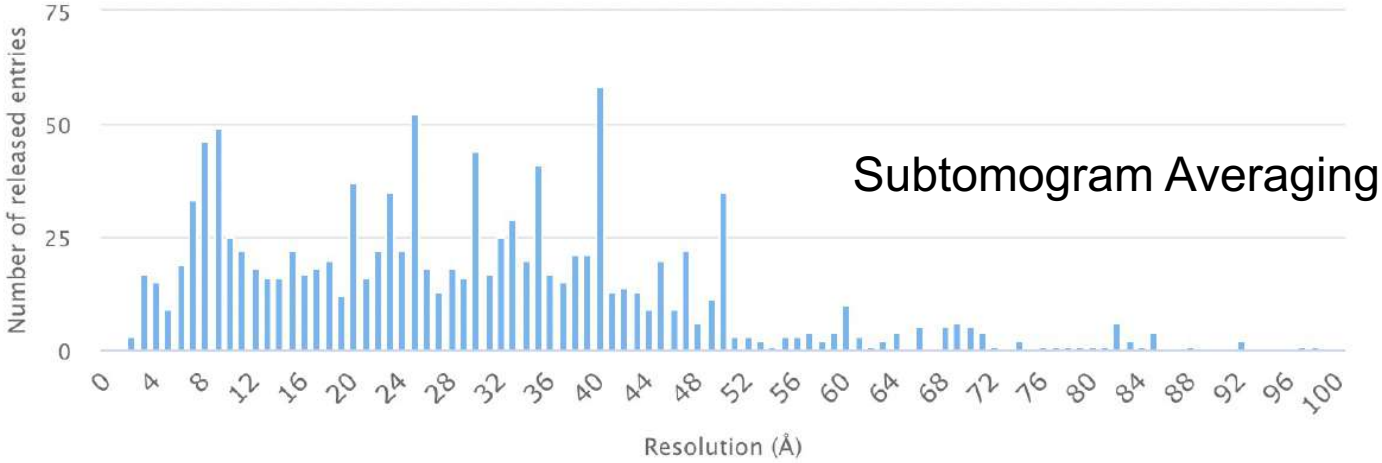


WT *FKS1* overexpression









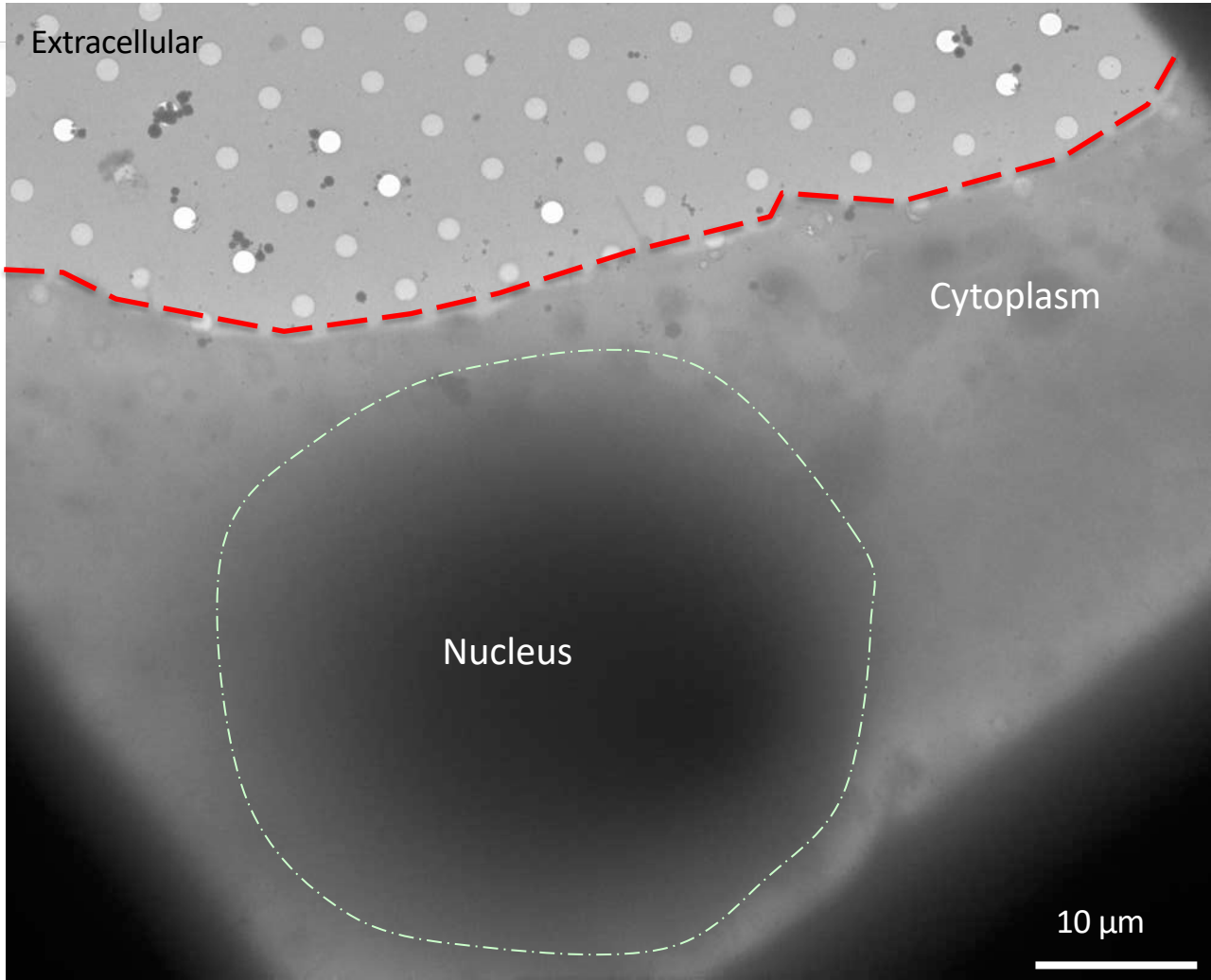
Concept Check Questions

Outline

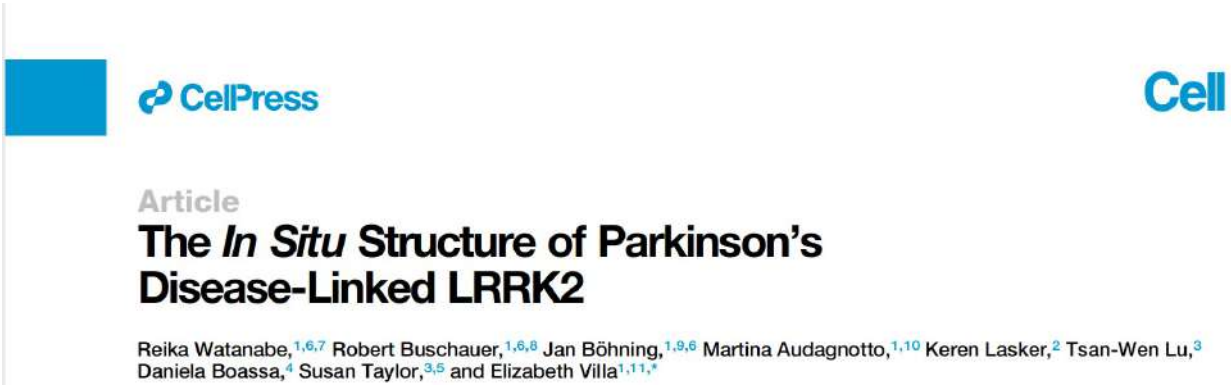
- What is cryo-electron tomography?
- Sample preparation: special considerations
- Data collection, alignment, and reconstruction
- Application of cryo-electron tomography in cell biology (structural cell biology)

Cellular Tomography:

- Cytoplasm: too thick for electrons to penetrate;
- How to find targets within a crowded cell?



Applying Cryo-ET to Reveal Protein Structure *in situ* – The Workflow



CellPress

Cell

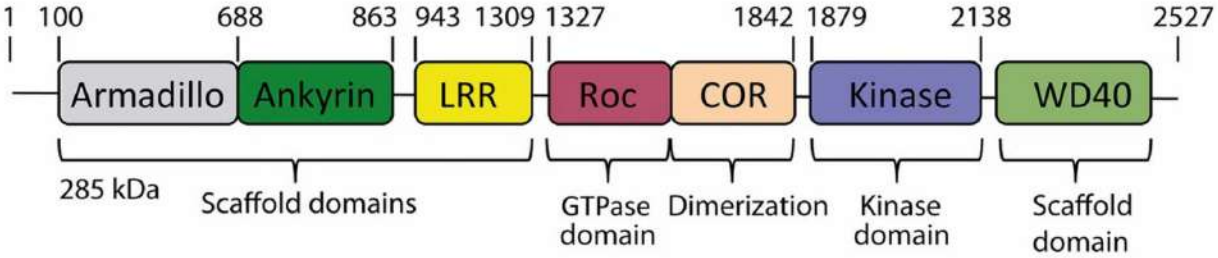
Article

The *In Situ* Structure of Parkinson's Disease-Linked LRRK2

Reika Watanabe,[1,6,7] Robert Buschauer,[1,6,8] Jan Böhning,[1,9,6] Martina Audagnotto,[1,10] Keren Lasker,[2] Tsan-Wen Lu,[3] Daniela Boassa,[4] Susan Taylor,[3,5] and Elizabeth Villa[1,11,*]

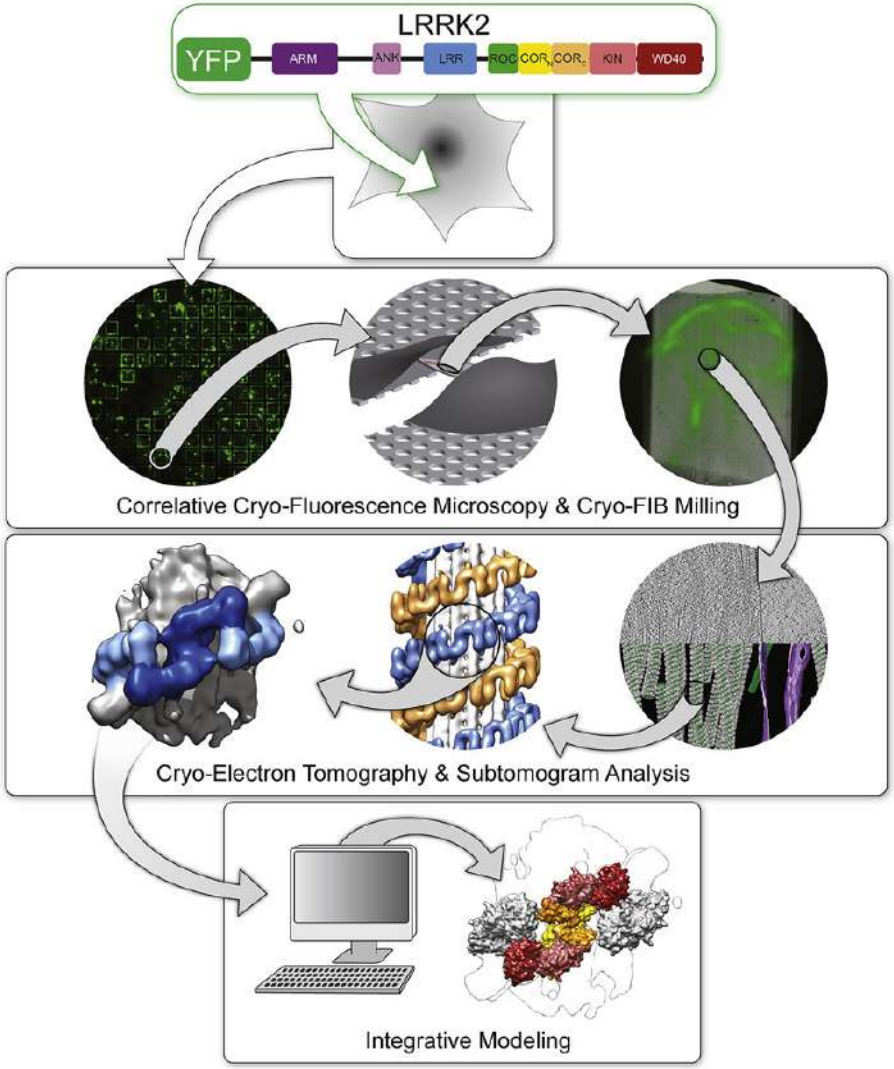
Structure of LRRK2

- LRRK2: (Leucine-rich repeat kinase 2) the most mutated gene in familial Parkinson's disease
- Functions in neurite outgrowth, membrane trafficking, autophagy
- Mutations or pharmacological inhibition of kinase activity recruit LRRK2 to microtubules
- Multi-domain protein; structure of the full-length protein is not available.



Guaitoli, G. *et al.*, PNAS 2016

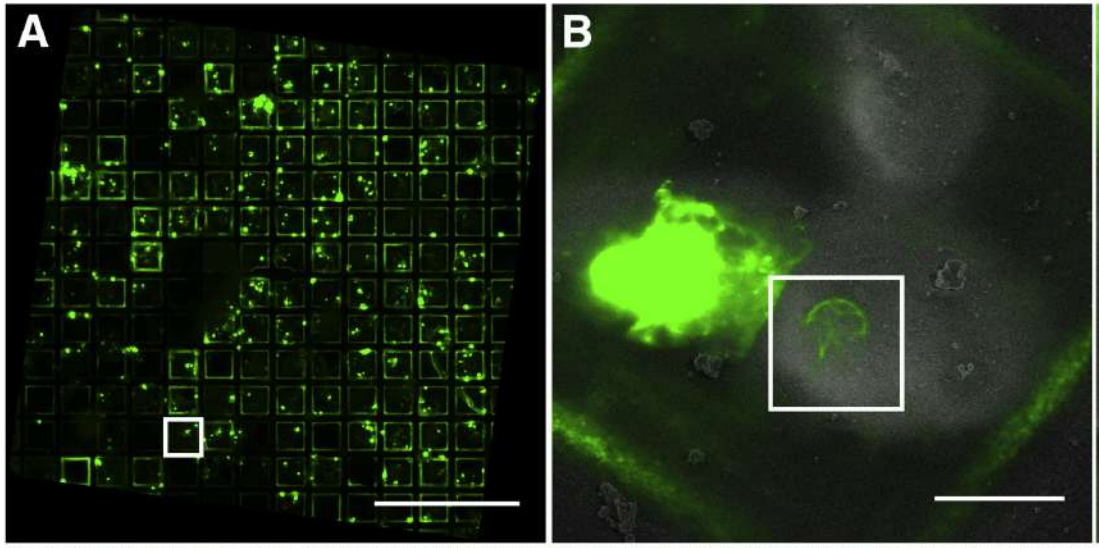
Workflow



Watanabe, R. *et al*., Cell 2020

Step 1: Design and Prepare Cells to Allow Detection of Targets in the Crowded Environment

- Add fluorescence tag; Increasing abundance for easy detection
- Correlative Light and Electron Microscopy (CLEM)



Watanabe, R. *et al*., Cell 2020

Concept Check Questions

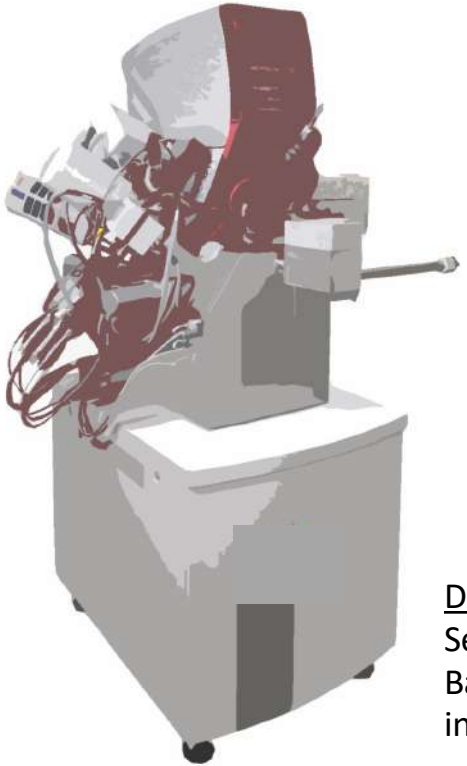
Step 2: Focused Ion Beam Milling to Generate Thin Cell Lamella for Cryo-ET

- Cells on grids: 1 - 5 µm
- Electron penetration power: 100– 300 nm

Watanabe, R. *et al*., Cell 2020

Focused Ion Beam

SEM column
~1nm resolution
Beam deceleration

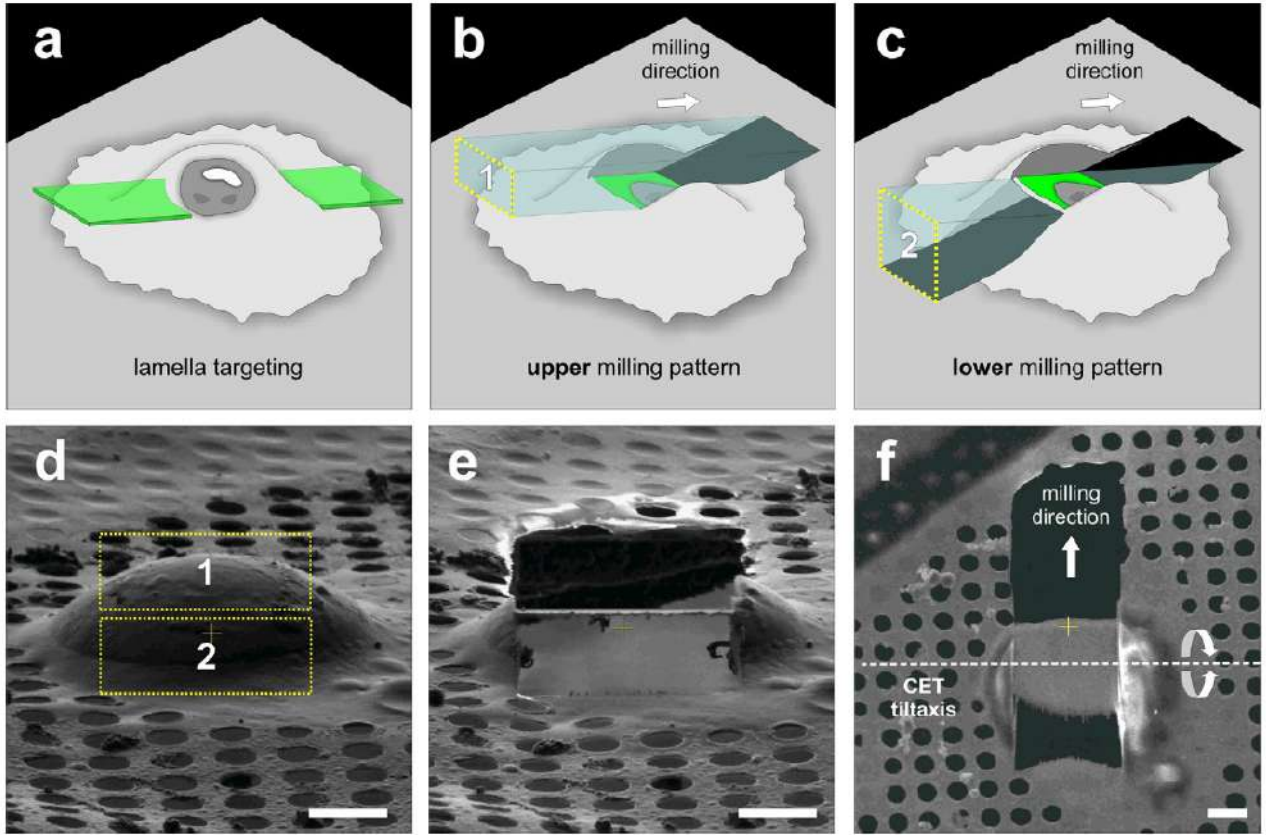


Gallium ion milling capability
20 nm milling precision
Fine milling (<1pA) to preserve specimen and high-current (>100nA) for large areas

Cryostage/cryotransfer
Accommodates autogrid cartridges for integration with cryoCLEM & cryoTEM
Stable operation below the devitrification point of water
Approaches liquid nitrogen temperature
Airlock for loading/unloading under cryo-conditions

Detectors
Secondary electron
Back-scattered electron
incl. in-lens detectors

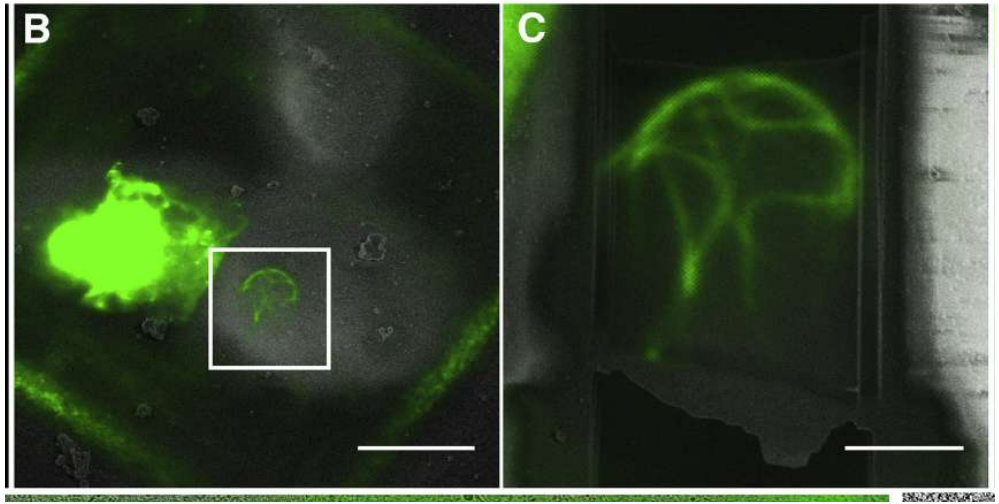
Focused Ion Beam



Rigort and Plitzko, 2015 *Arch Biochem Biophys.* 581: 122-130

Step 2: Focused Ion Beam Milling to Generate Thin Cell Lamella for Cryo-ET

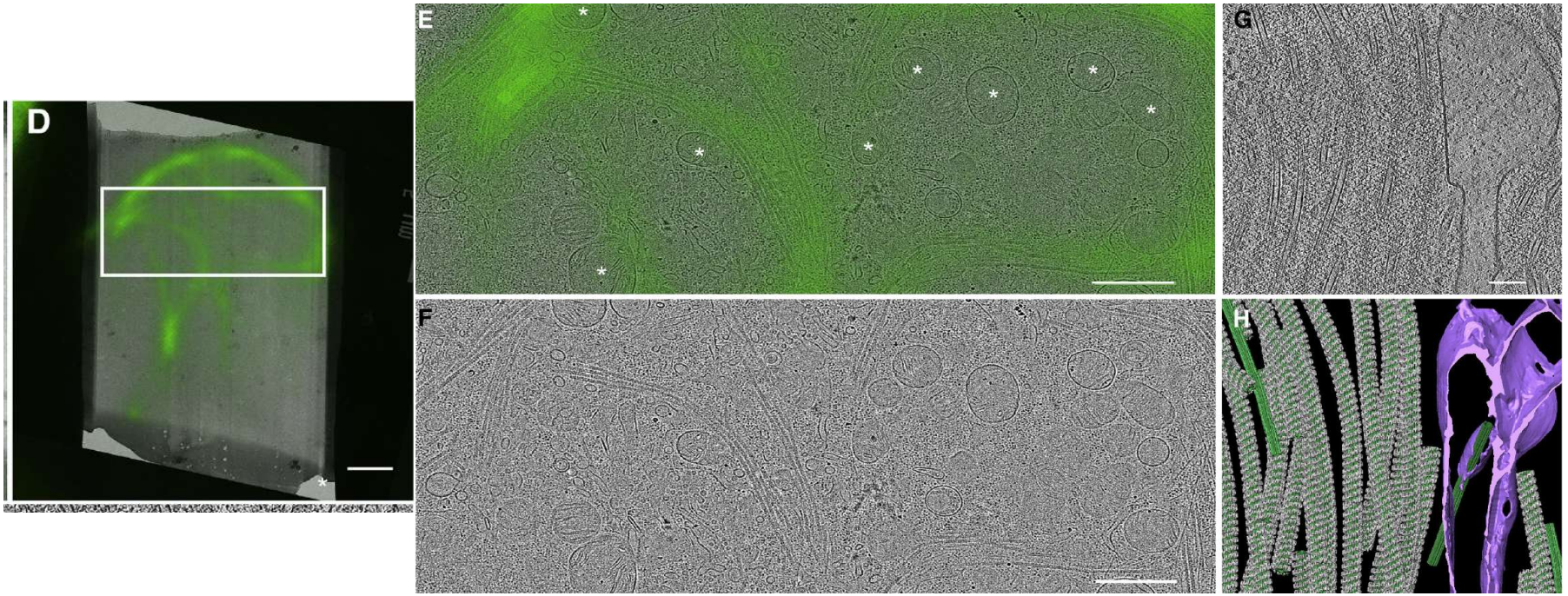
- Cells on grids: 1 - 5 µm
- Lamella: 100– 150 nm



Watanabe, R. *et al*., Cell 2020

Step 3: Cryo-ET Imaging and Tomogram Reconstruction

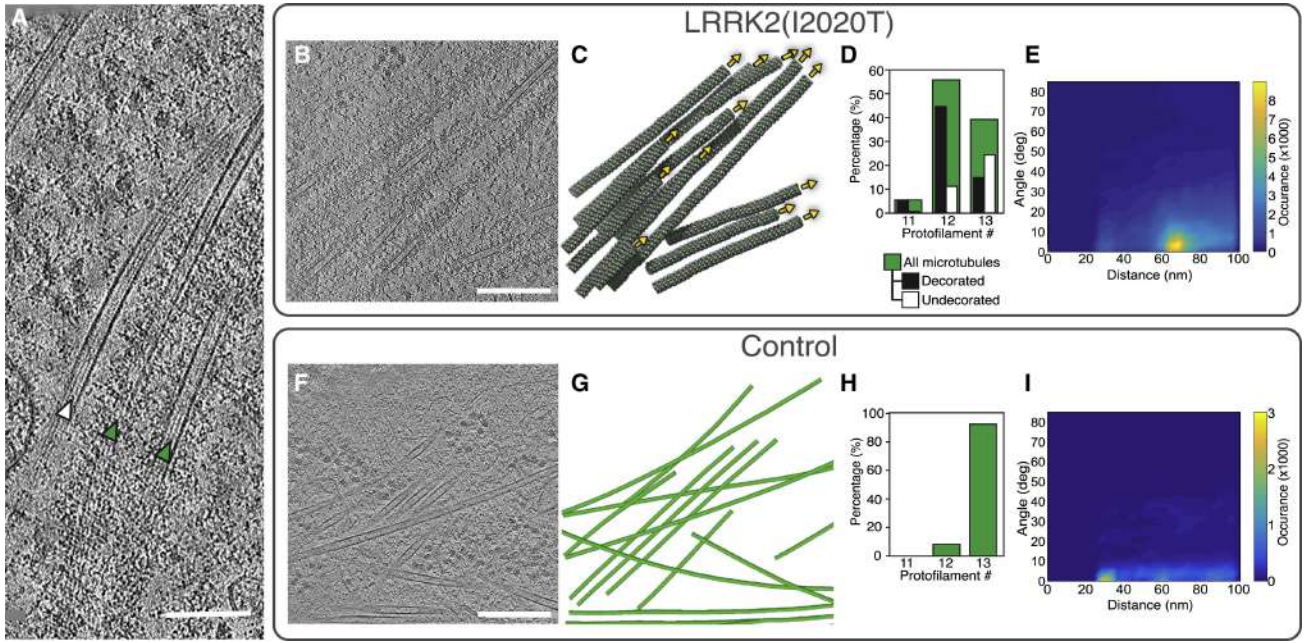
- Use CLEM to guide tilt series data collection



Watanabe, R. *et al*., Cell 2020

Step 4: *In situ* Structure Analysis

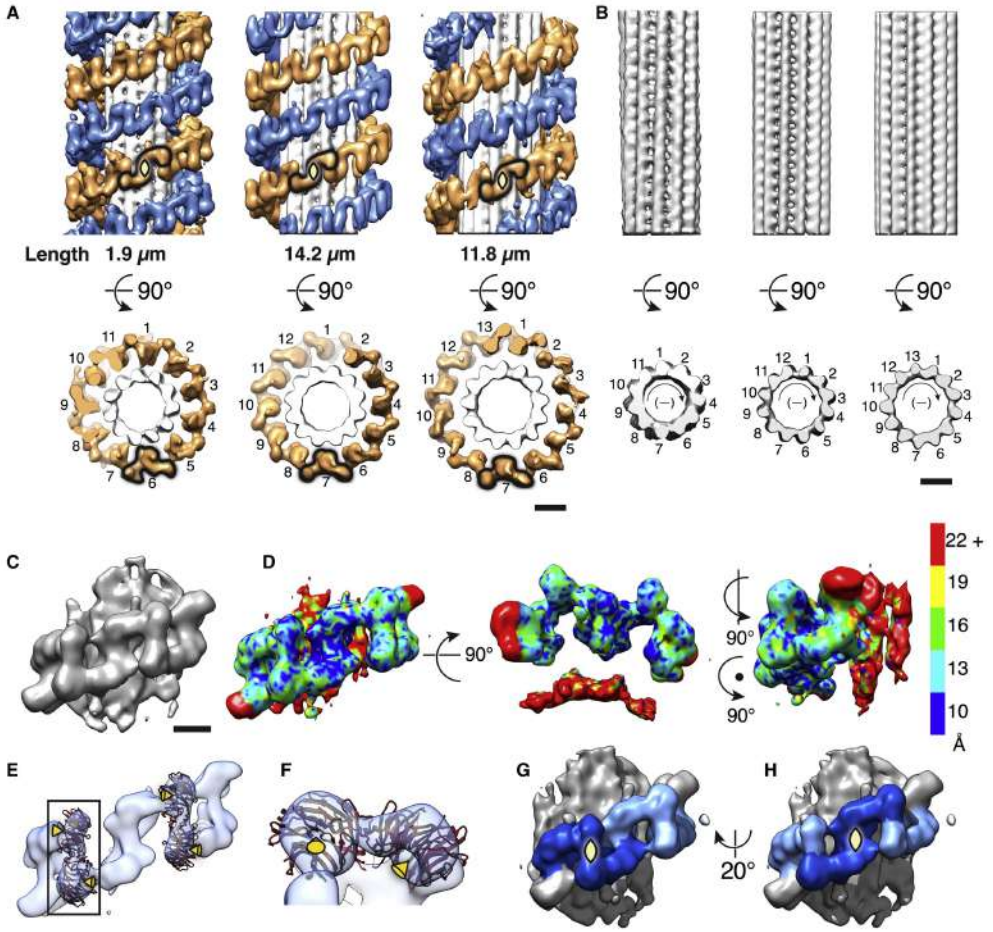
- Distribution and dynamics in cells



Watanabe, R. *et al*., Cell 2020

Step 5: Subtomogram Analysis

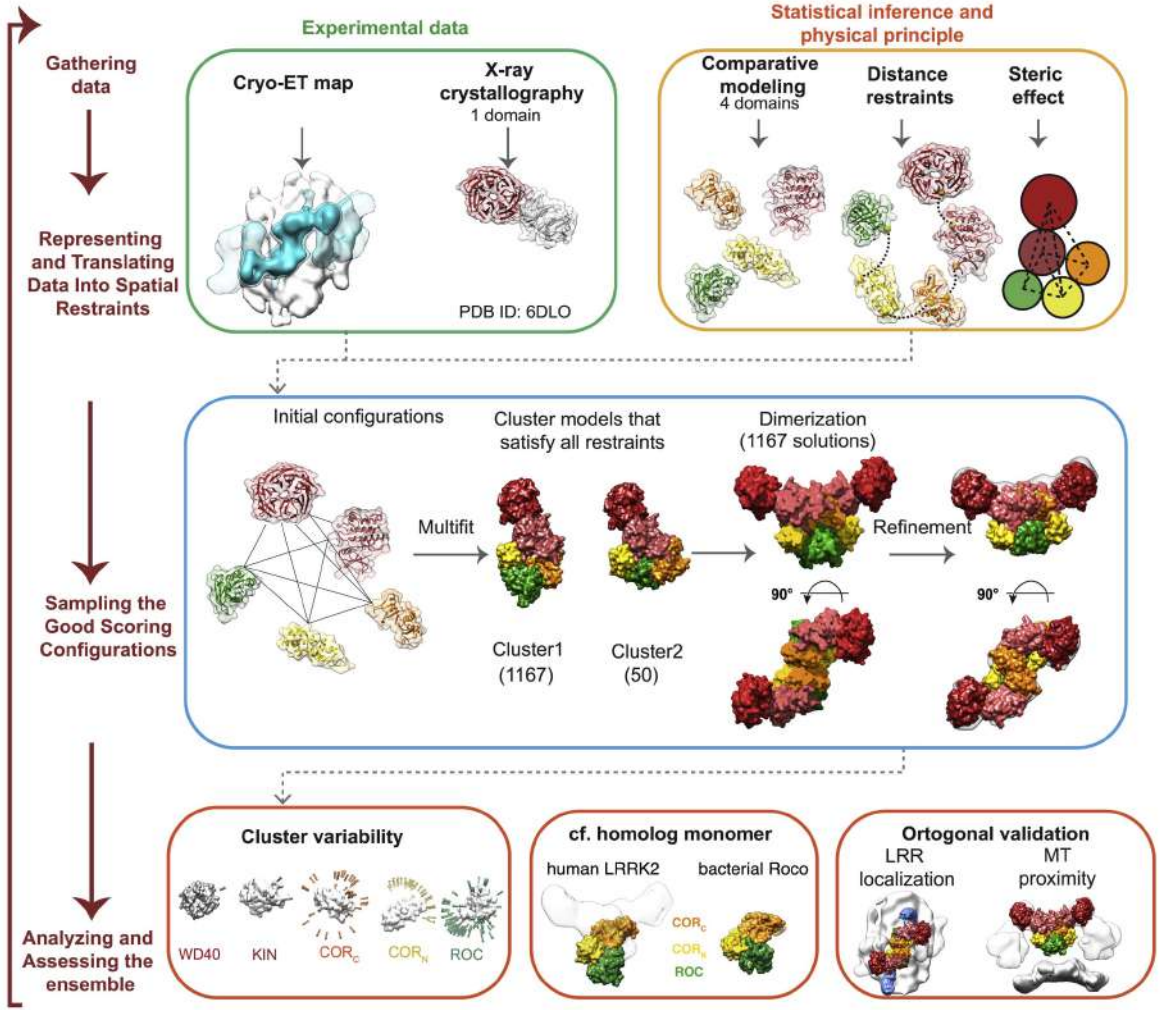
- Extraction
- Classification
- Averaging
- Model fitting



Watanabe, R. *et al*., Cell 2020

Step 6: Integrative Modeling

- Details in domain organization can be deduced from nanometer resolution maps



Watanabe, R. *et al*., Cell 2020

Step 7: Functional Analysis

Disturbing structure

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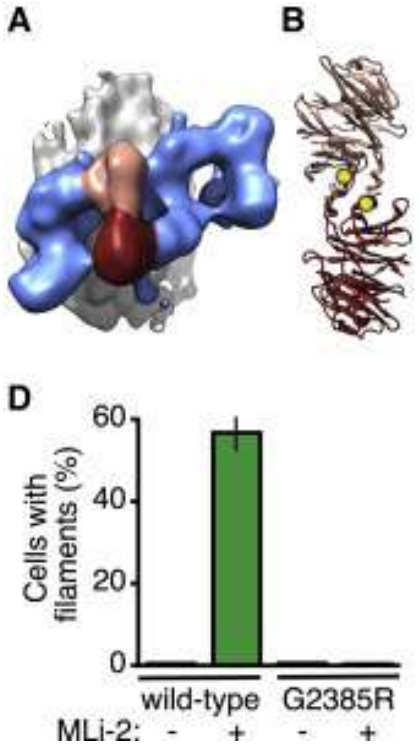
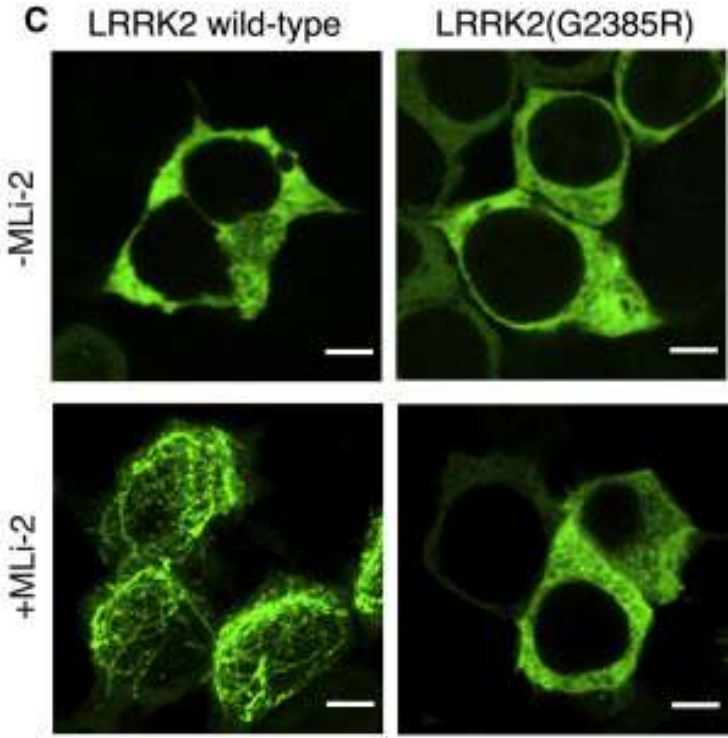
Variations of functions





Watanabe, R. *et al*., Cell 2020

Summary

- Introduction to cryo-electron tomography, correlative light and electron microscopy and focused ion beam milling
- Sample preparation for cryo-electron tomography
- Structures and distribution of protein complexes or aggregates in neurodegenerative diseases by multimodal bioimaging combining CLEM, cryoFIB and cryoET.

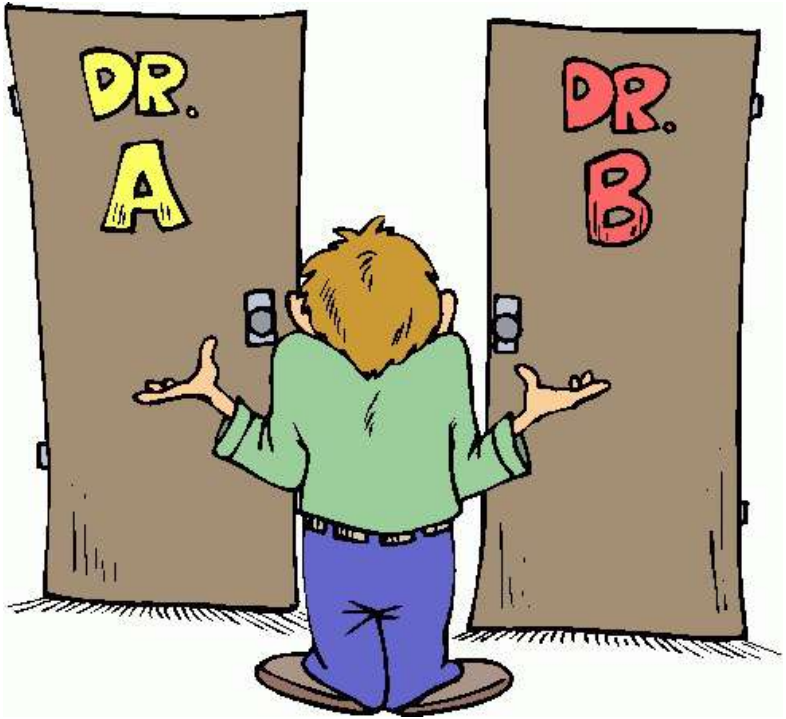
References

1. Wrapp, D. *et al.* Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* **367**, 1260-1263, doi:10.1126/science.abb2507 (2020).
2. Yao, H. *et al.* Molecular Architecture of the SARS-CoV-2 Virus. *Cell* **183**, 730-738 e713, doi:10.1016/j.cell.2020.09.018 (2020).
3. Klein, S. *et al.* SARS-CoV-2 structure and replication characterized by in situ cryo-electron tomography. *Nat Commun* **11**, 5885, doi:10.1038/s41467-020-19619-7 (2020).
4. Deniston, C. K. *et al.* Structure of LRRK2 in Parkinson's disease and model for microtubule interaction. *Nature* **588**, 344-349, doi:10.1038/s41586-020-2673-2 (2020).
5. Watanabe, R. *et al.* The In Situ Structure of Parkinson's Disease-Linked LRRK2. *Cell* **182**, 1508-1518 e1516, doi:10.1016/j.cell.2020.08.004 (2020).
6. Jiménez-Ortigosa C., Jiang J., Chen M., Kuang, X., Healey K. R., Castellano P., Boparai N., Ludtke S. J., Perlin D. S., and Dai W. (2021), Preliminary structural elucidation of β-(1,3)-glucan synthase from candida glabrata using cryo-electron tomography. *JOF* 7 (2), 120.

Concept Checking Questions

- Single particle vs Tomography
- Data collection configurations
- The missing wedge artifact
- Factors limiting the resolution of subtomogram averages
- Identifying objects in information-rich tomograms
- Factors affecting correlation in CLEM

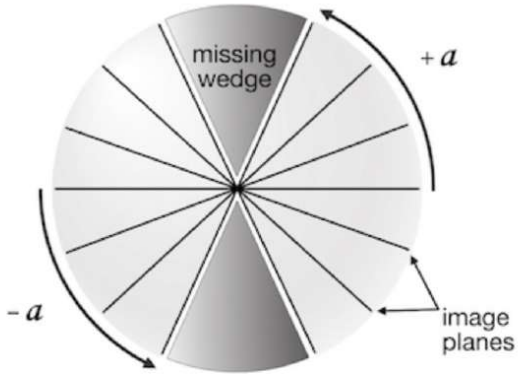
Single Particle *or* Tomography



https://clipart-library.com

CryoET Data Collection

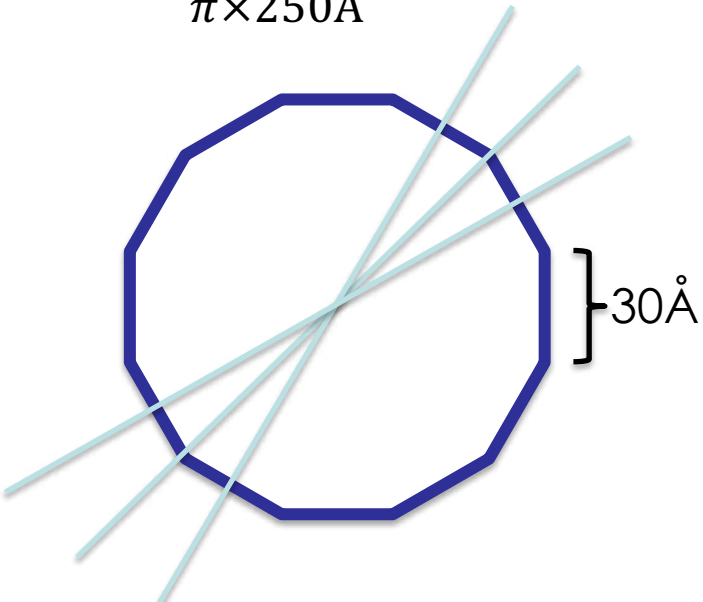
- There are many configuration parameters involved in data collection. Each is a balance between opposing considerations.
  - Defocus: contrast vs resolution
  - Total dose: signal vs radiation damage
  - Tilt range and increment:
    - goniometer mechanical limit (-70 <->+70)
    - goal of the project
    - sample geometry



- "Crowther criterion":

$$\frac{180° \times resolution}{\pi \times diameter} = angular\ step$$

$$\frac{180° \times 3Å}{\pi \times 250Å} = 0.69°$$

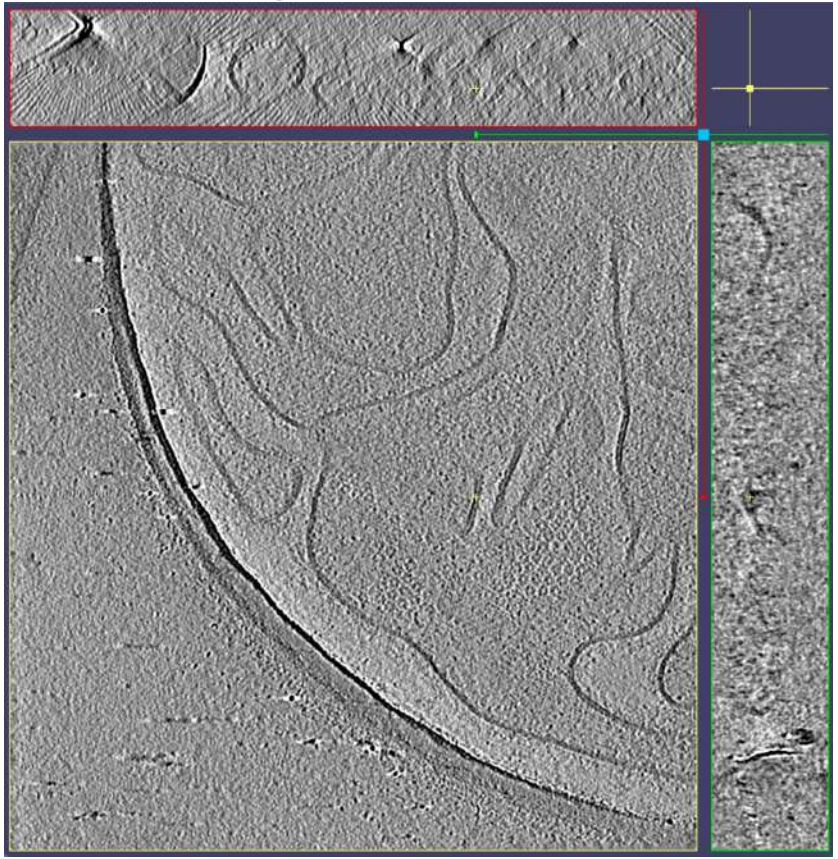


radius (Å)	res (Å)	# views	angular step (°)
125	3	259	0.69
125	10	79	2.292
125	40	20	9.167
10000	3	20944	0.009
10000	10	6283	0.029
10000	40	1571	0.115

Courtesy of Jason Kaelber

- The Missing Wedge Artifact

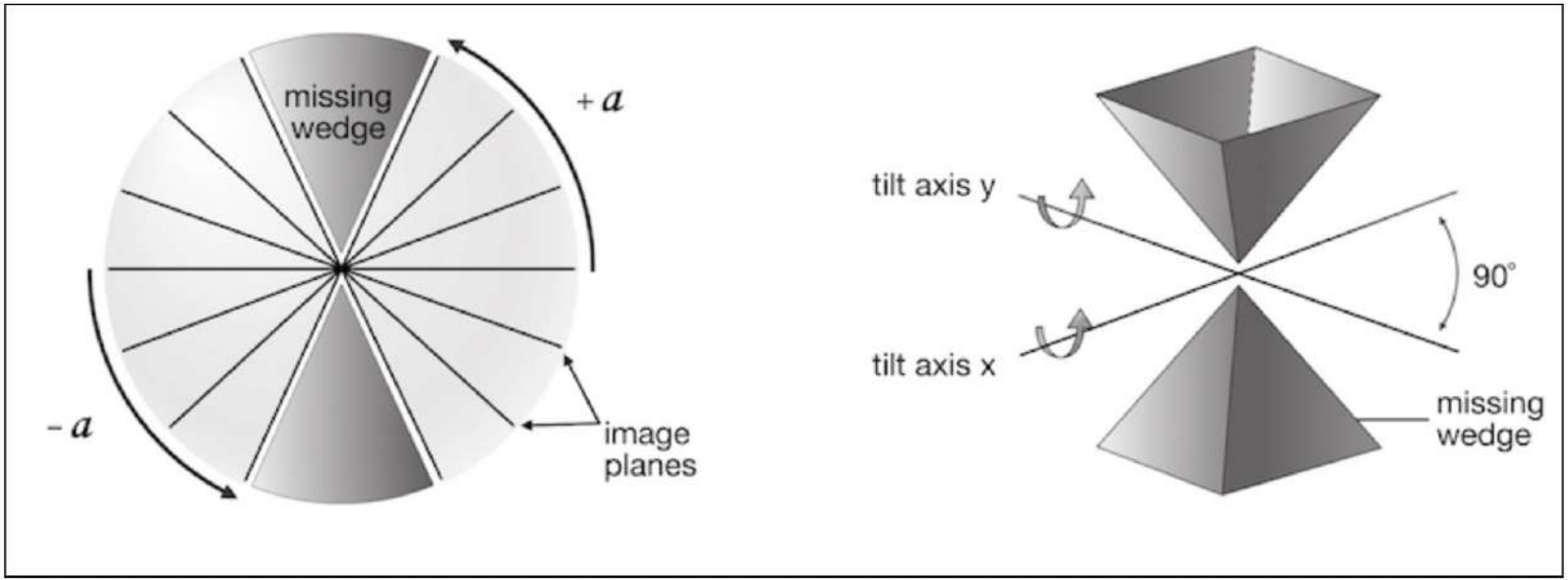
Data alignment and reconstruction



- What is the “missing wedge”?
  - Missing data that are not recorded because of limited tilt range

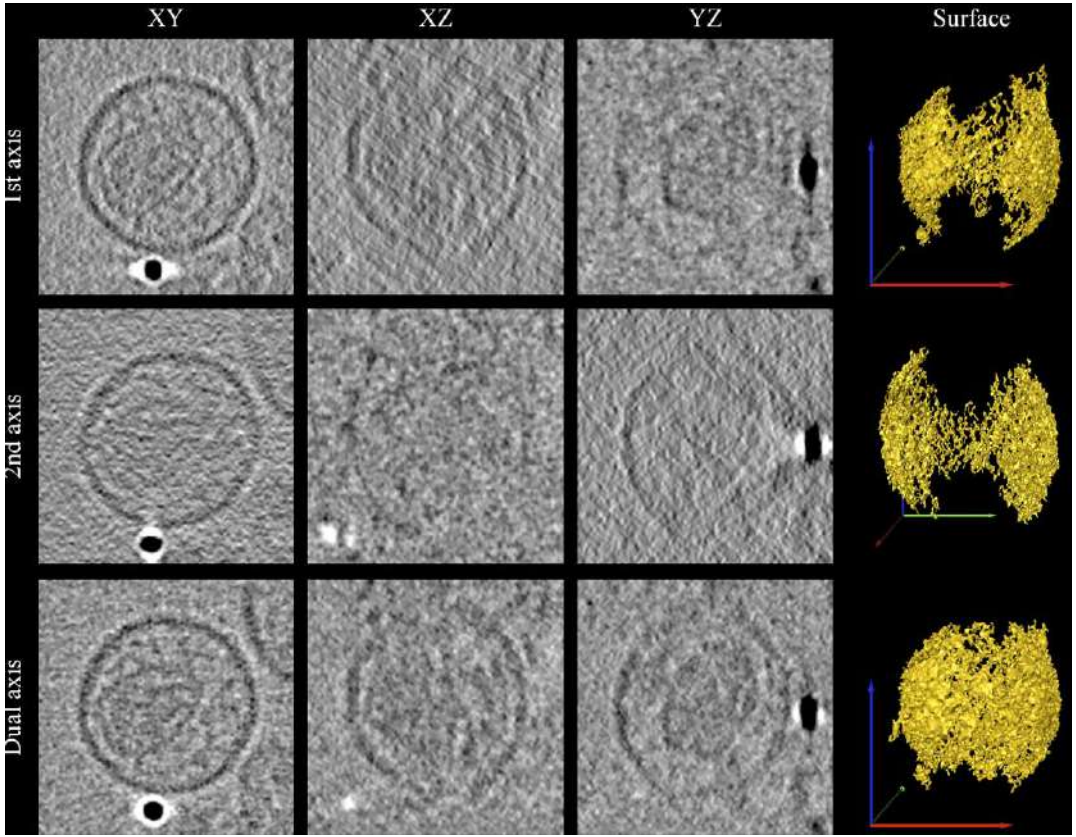
Dual tilt reduces missing wedge

A holder that “flips” in the microscope so x-tilt will image a second axis



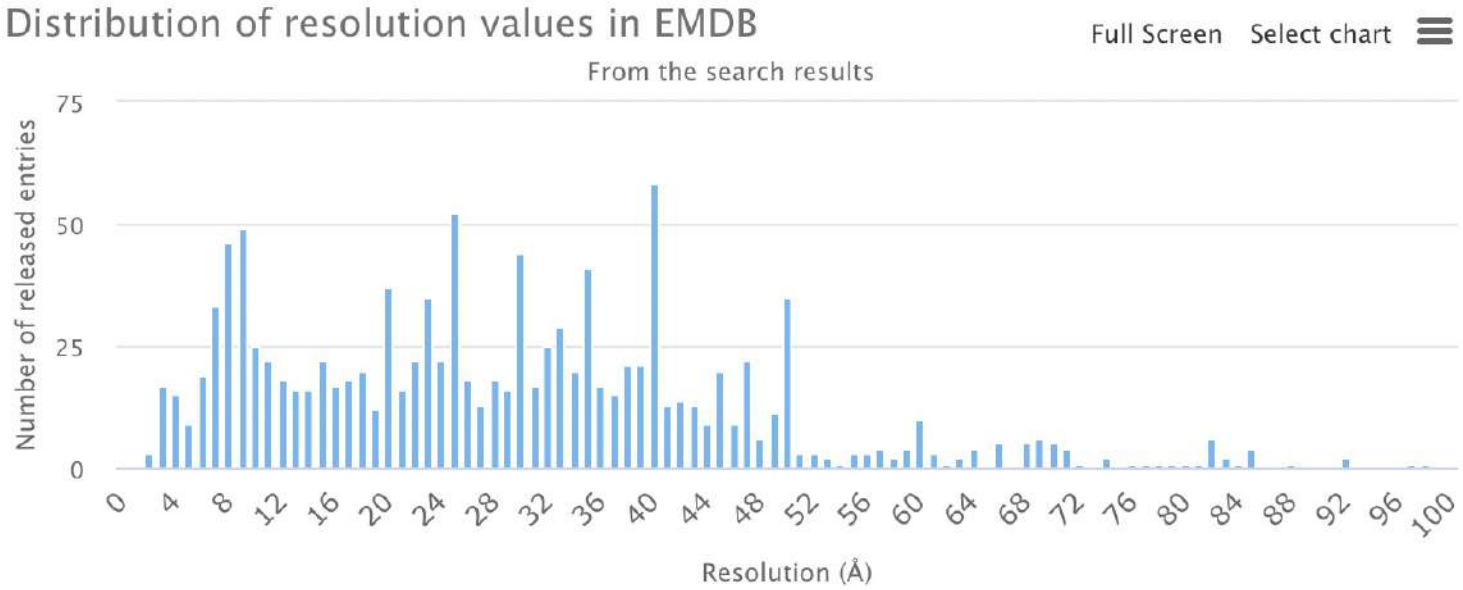
https://www.wormatlas.org/EMmethods/ETmethods.htm

Dual tilt reduces missing wedge



Iancu, Wright &al. *JSB* 2005

What are the factors limiting the resolution of subtomogram averages?



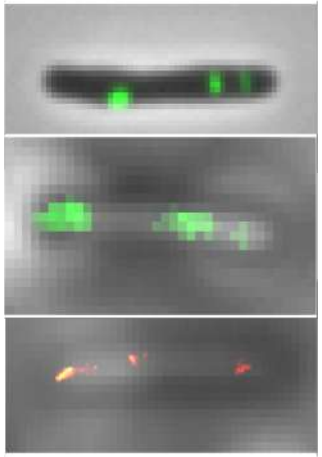
Identifying objects in tomograms

- Structure signature
- CLEM
  - Perturbing abundance or structure
  - Template matching
  - Heavy metal tag

CLEM:

- What are the advantages and disadvantages of doing light microscopy at room temperature and cryo-temperature?
  - Room temperature: oil immersion objective lens, large NA
  - Cryo temperature: samples stop moving

CLEM: many factors affect correlation

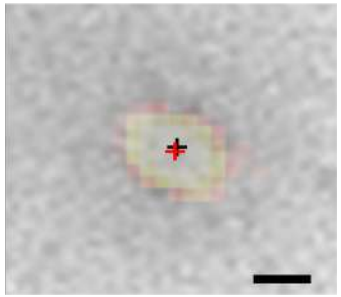


GFP RT
100x NA1.4
oil immersion objective lens

GFP 80K
60x NA 0.7
air objective lens

PA-GFP 80K
cryo-PALM

Fluorophore & objective lens



Distance between EM/cryo-PALM
bead centers around 9±2 nm

Correlation of images

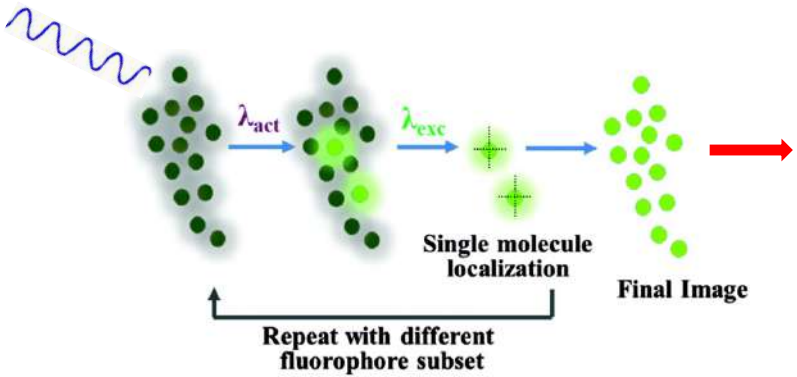
Chang, Yi-Wei, et al., Correlated cryogenic photoactivated localization microscopy and cryo-electron tomography. Nature methods. 2014

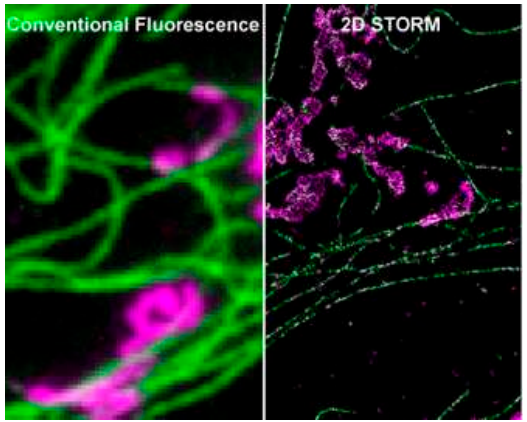
Tomography - Identifying objects in tomograms concept check questions:

- What is “cryo-PALM”?

- PALM: photoactivated localization microscopy

PALM (Photo Activated Light Microscopy)





In PALM, small bursts of UV light are used to excite subsets of fluorophores. Since these fluorophores are excited in small sets, their diffraction patterns are less likely to overlap, allowing the location of the actual molecules to be located through back calculation

http://huanglab.ucsf.edu/STORM.html