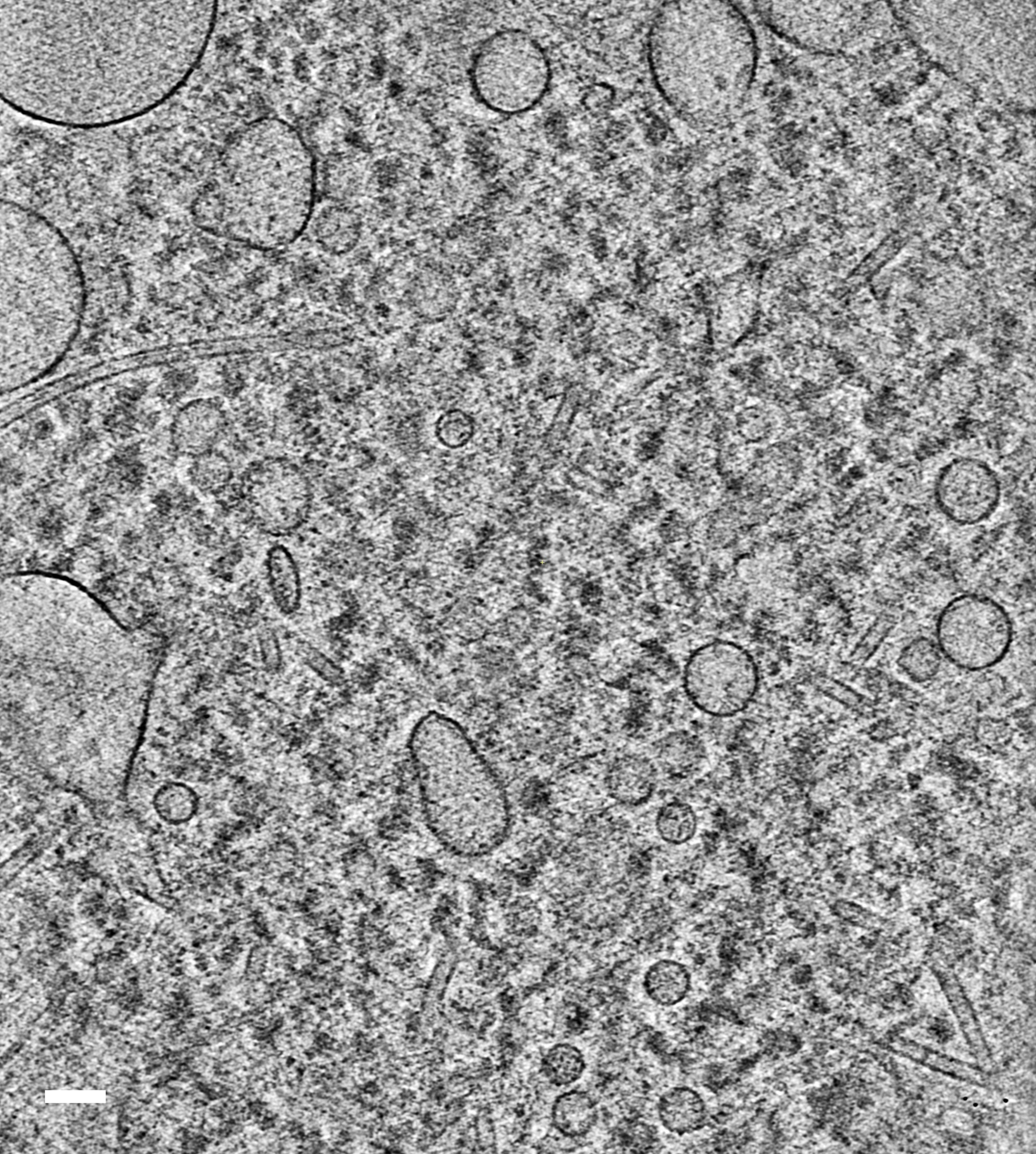
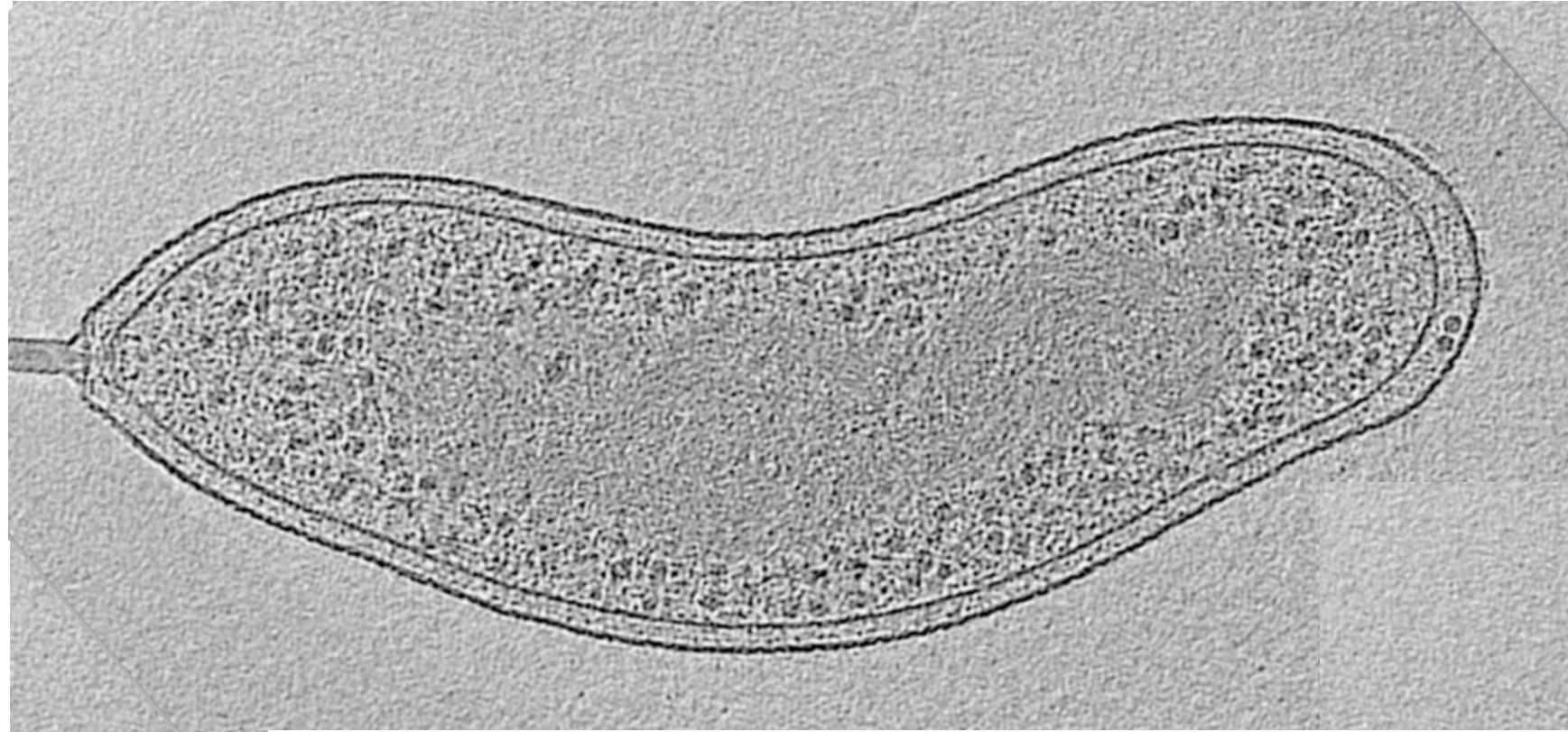




Electron cryotomography

Grant Jensen
BYU

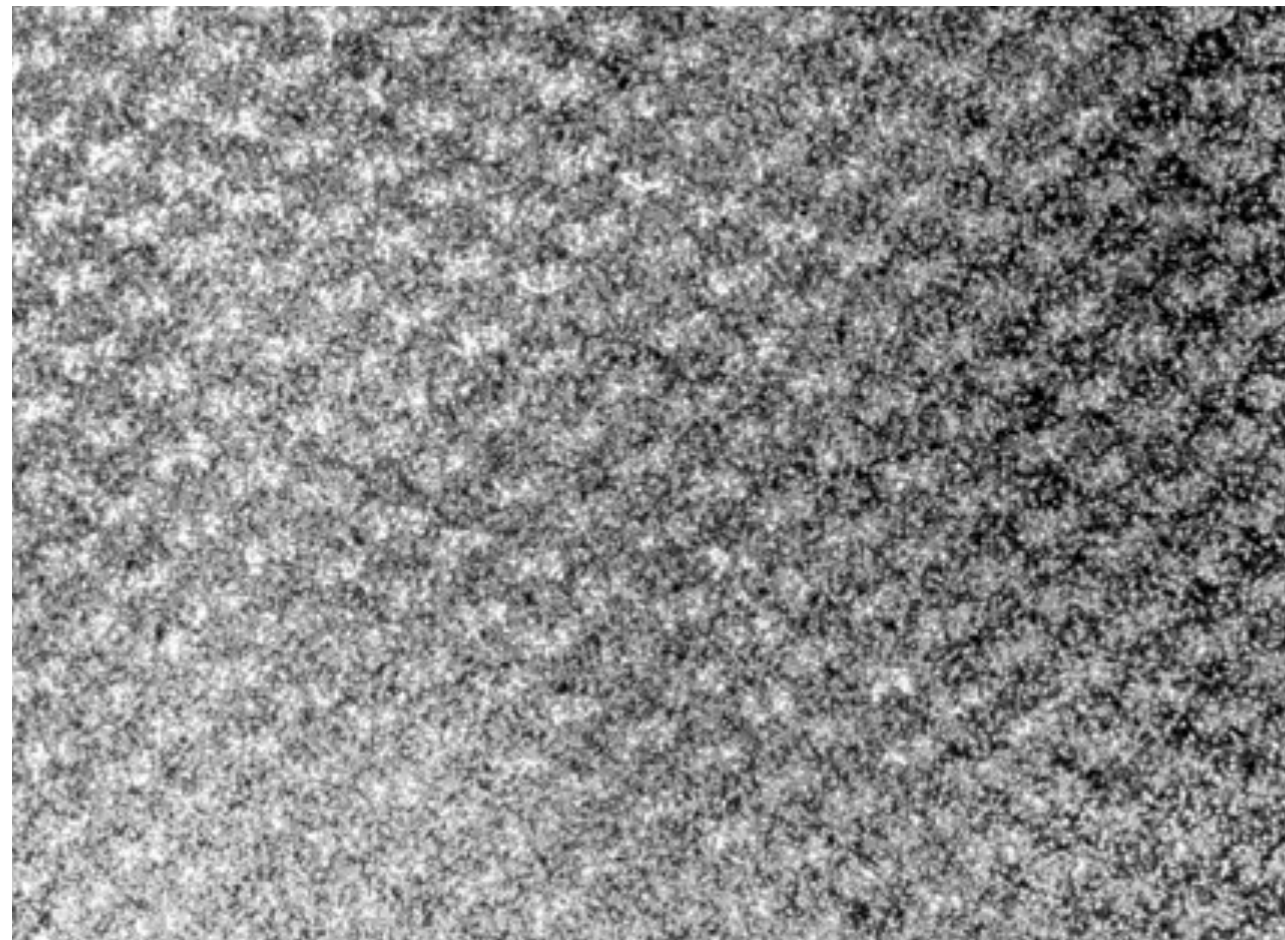
Basic approaches in cryo-EM



Tomography

1 object

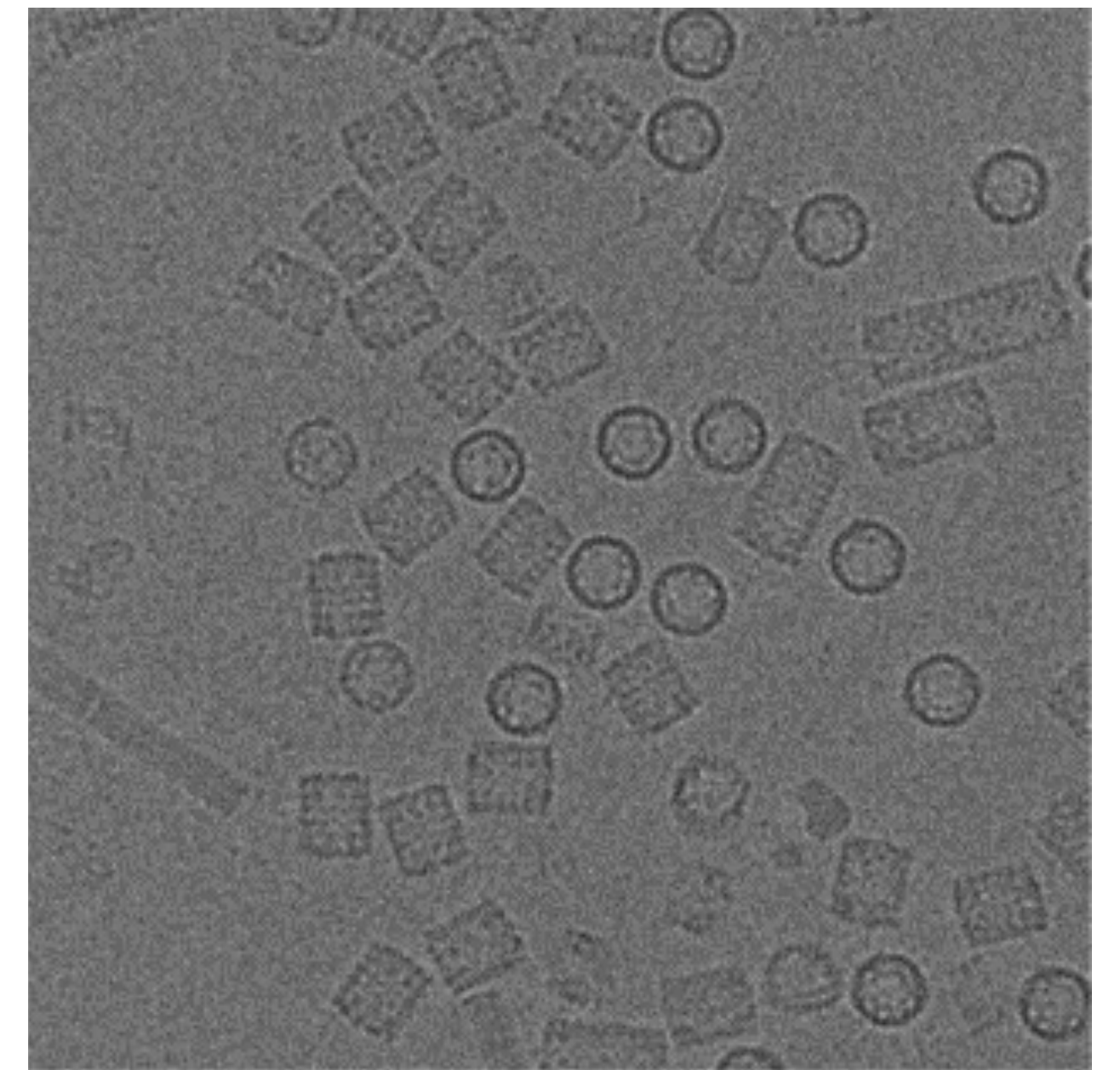
~4 nm resolution



Single particle analysis

Average 10^4 objects

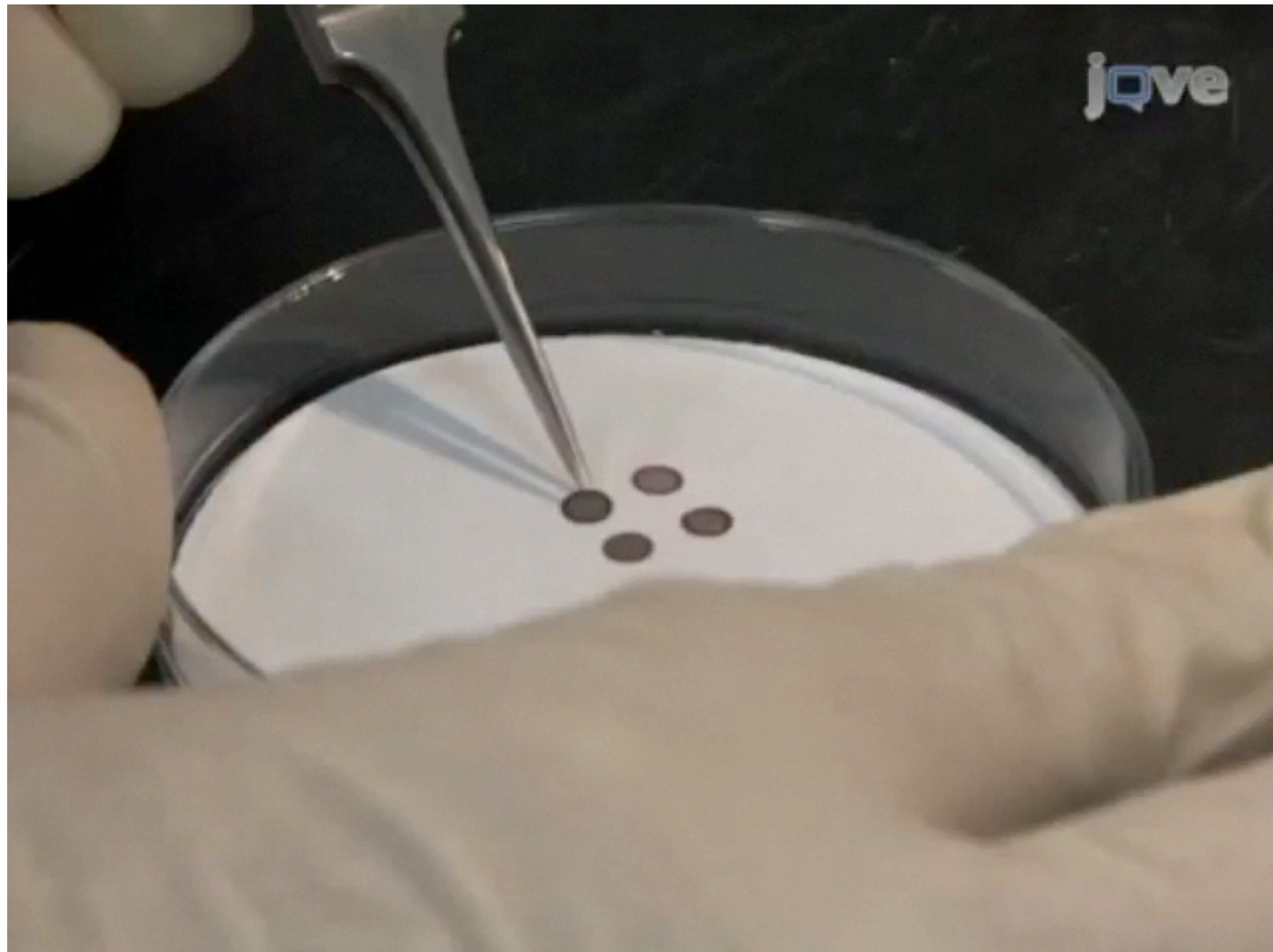
1-4 Å resolution

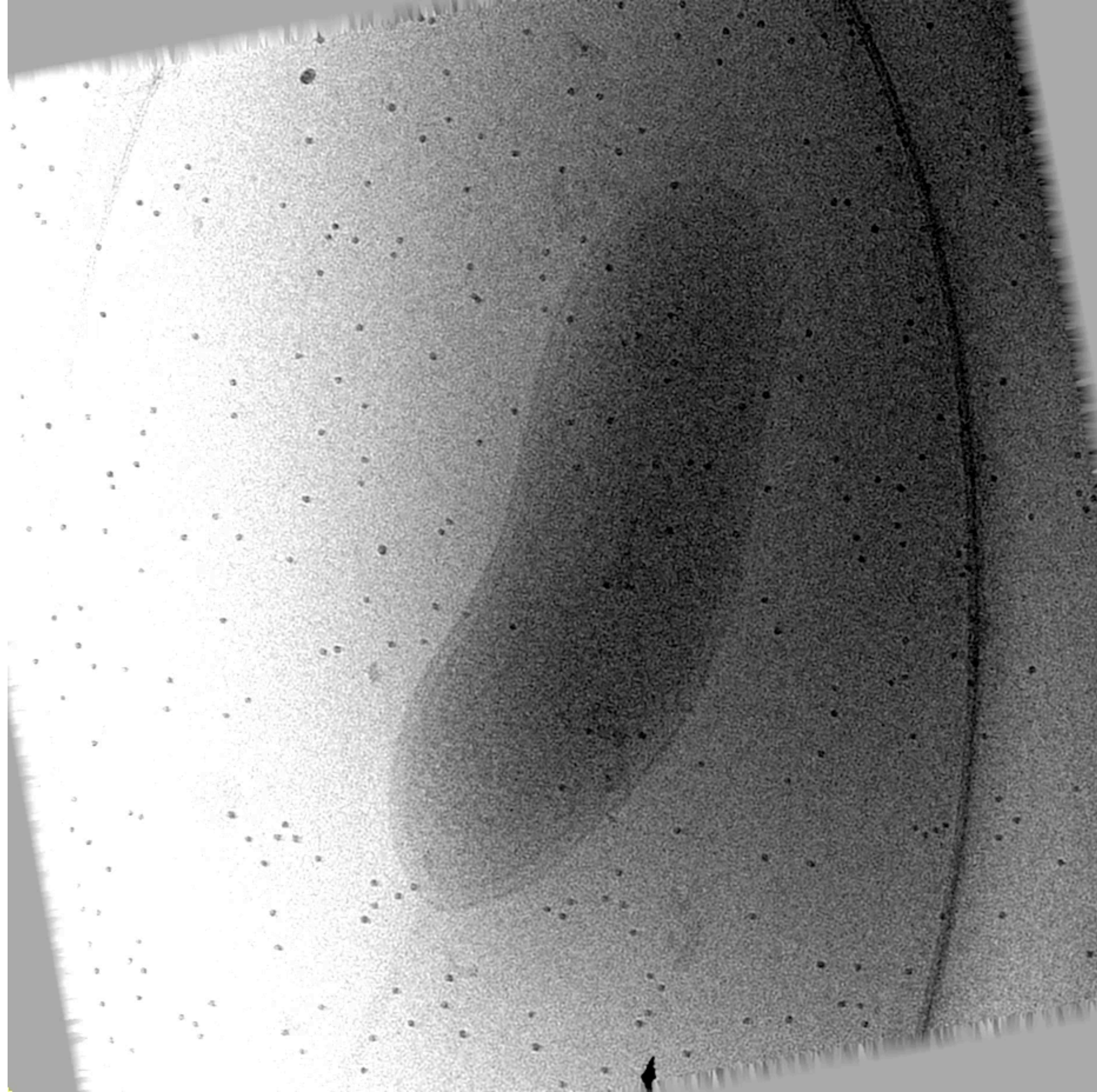


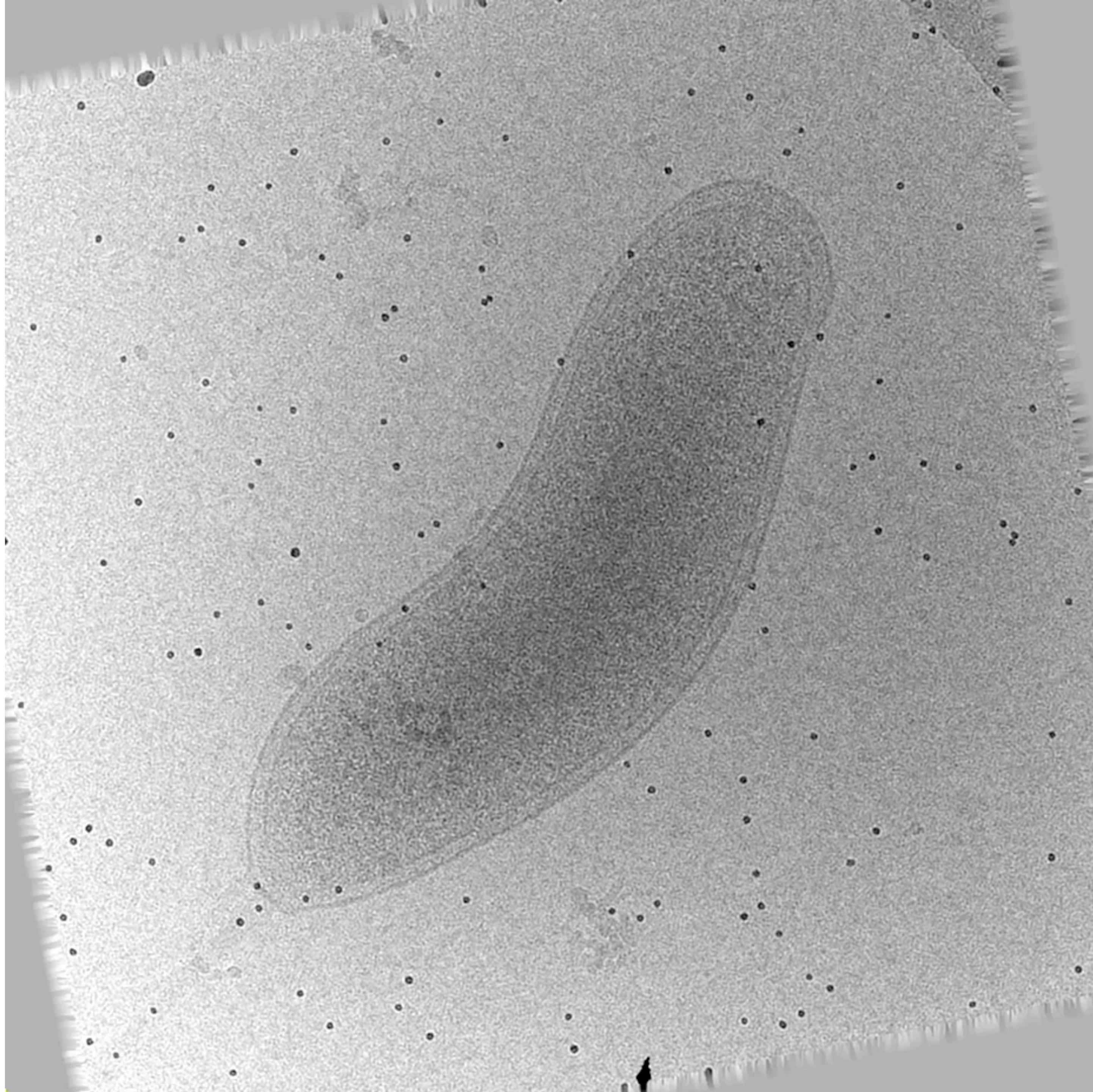
Electron crystallography

Average 10^7 objects

0.5-3 Å resolution







Aligning tilt series

For each image, determine

x,y shifts

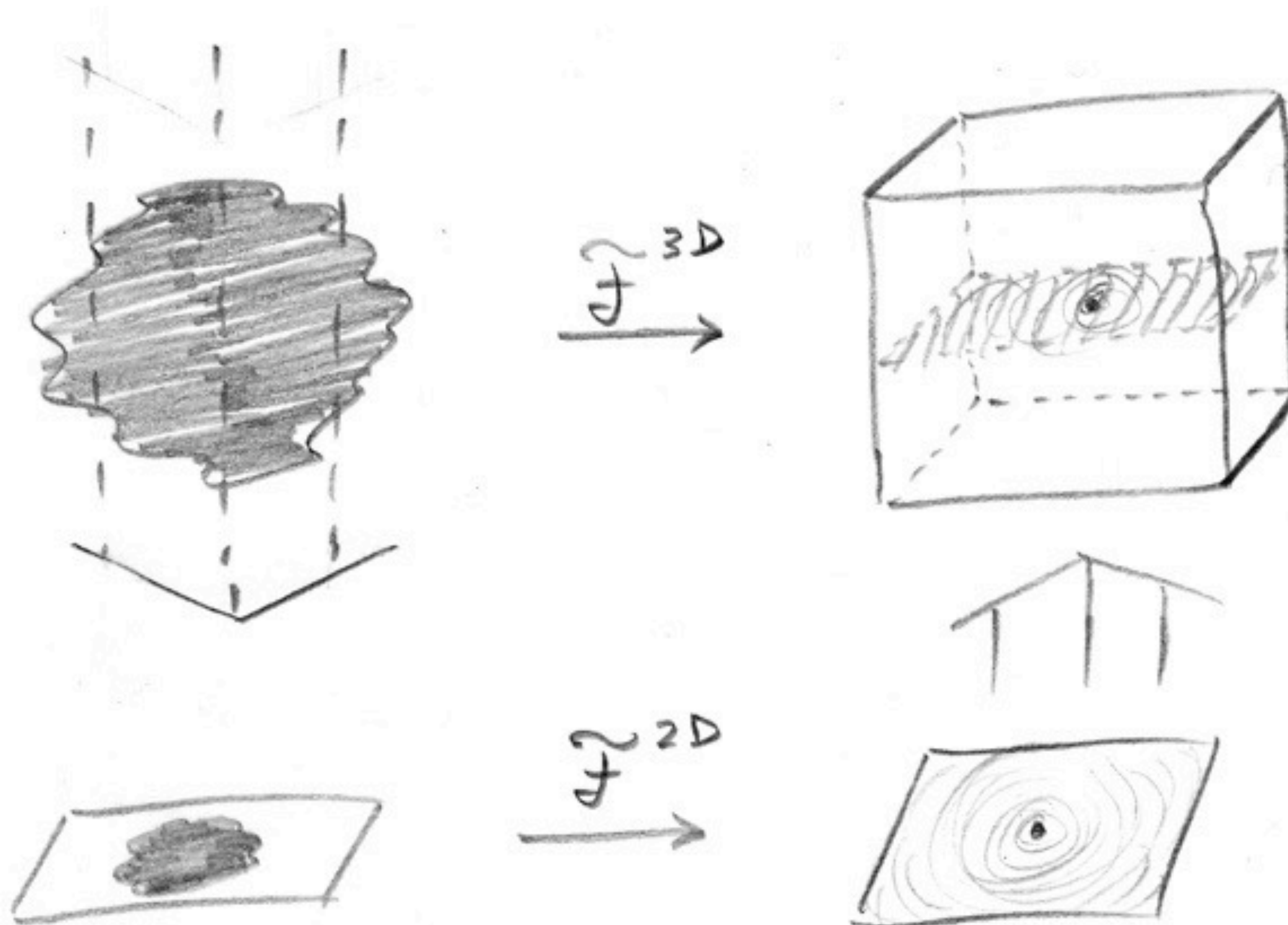
rotation (position of tilt axis)

tilt angle

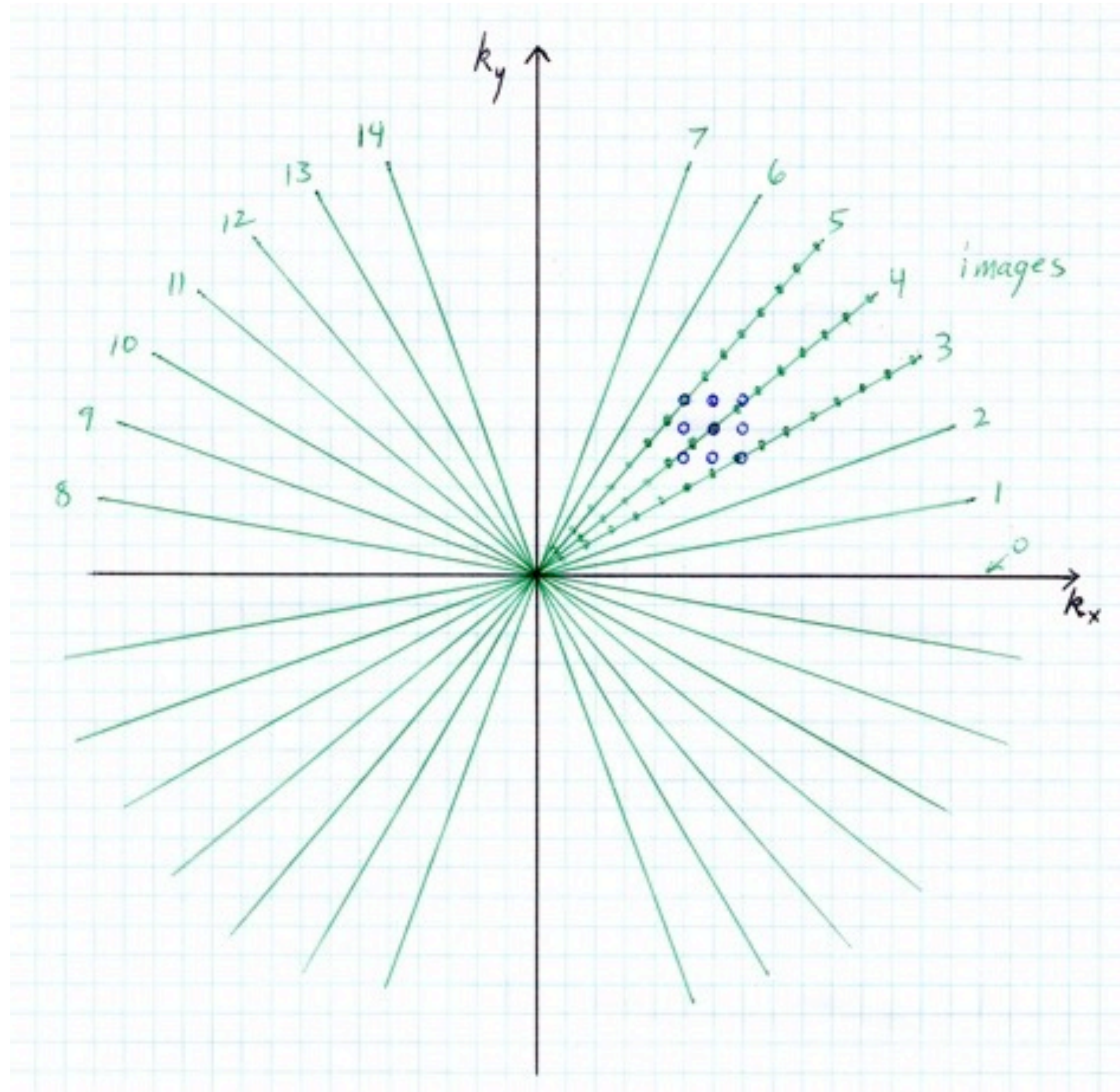
magnification

defocus

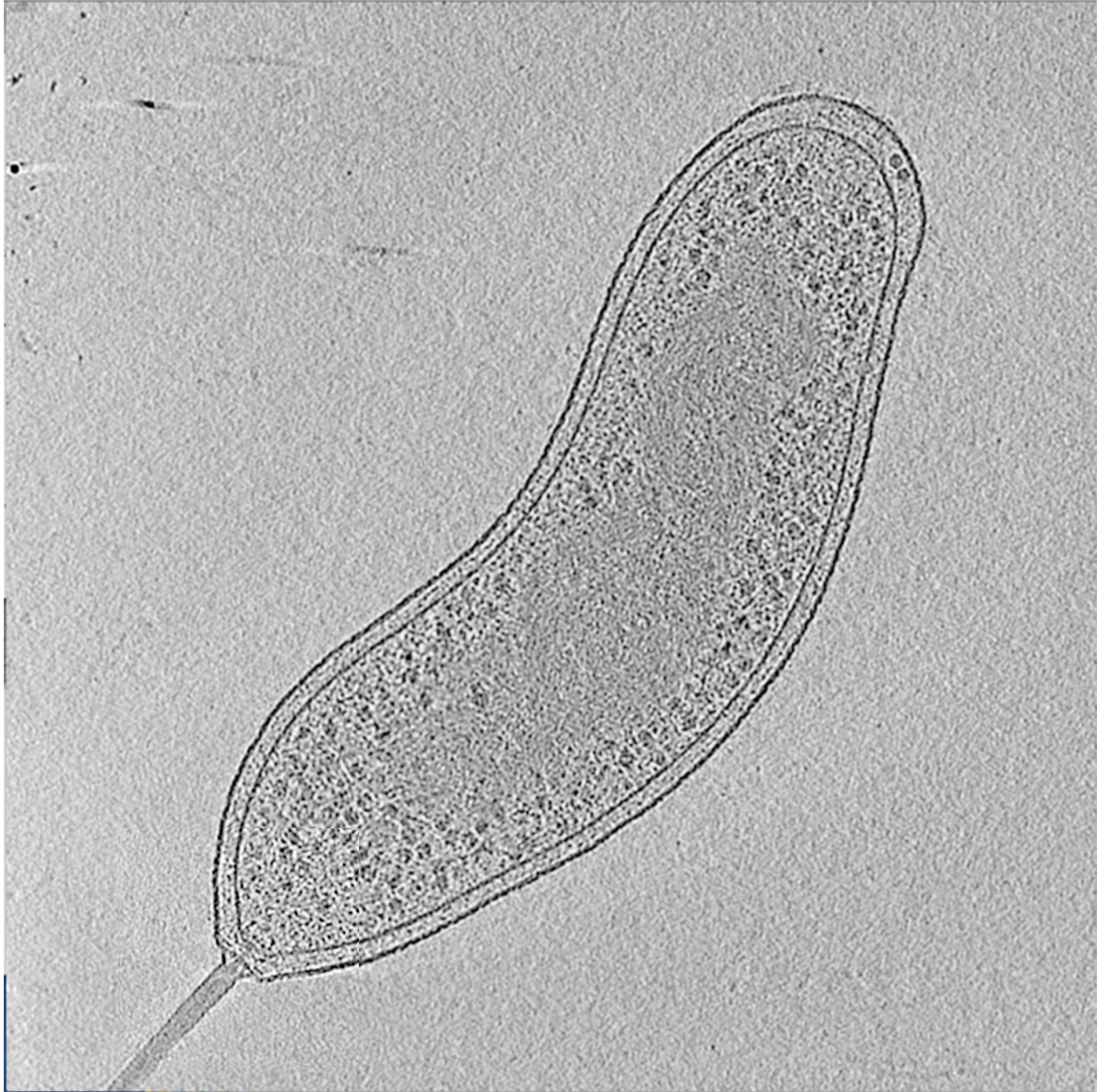
The projection theorem



Tilt series are typically merged in reciprocal space

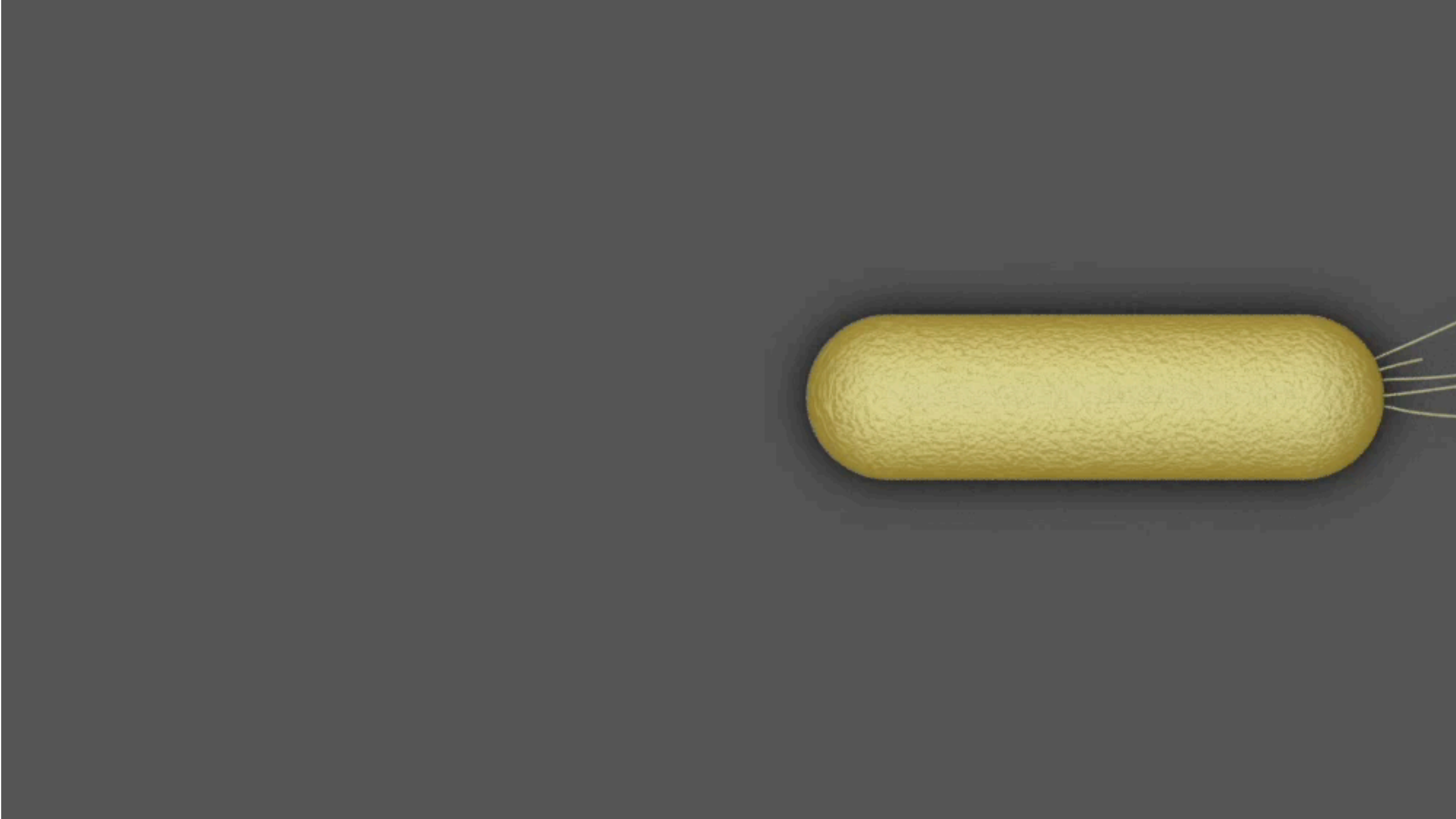


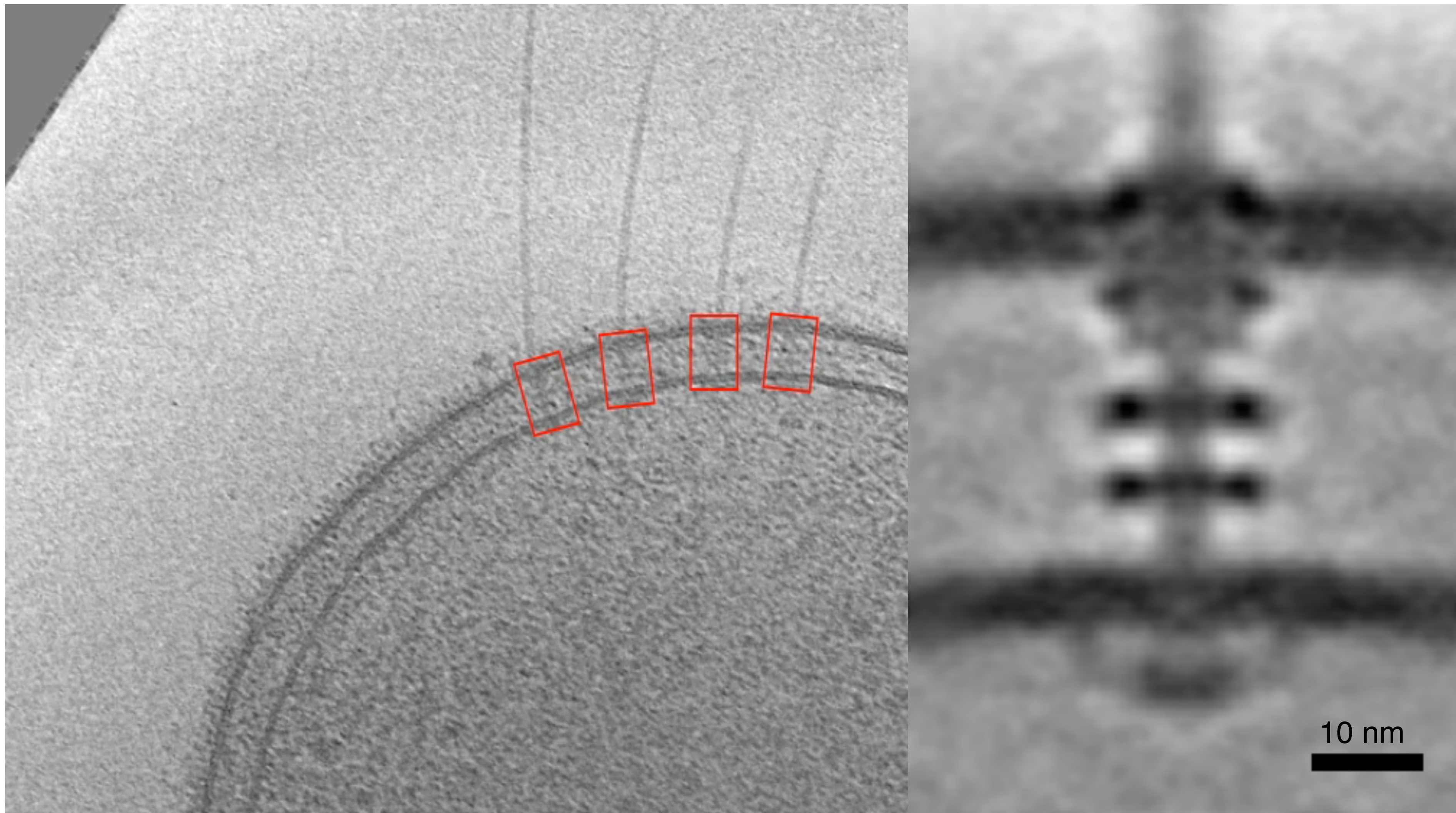


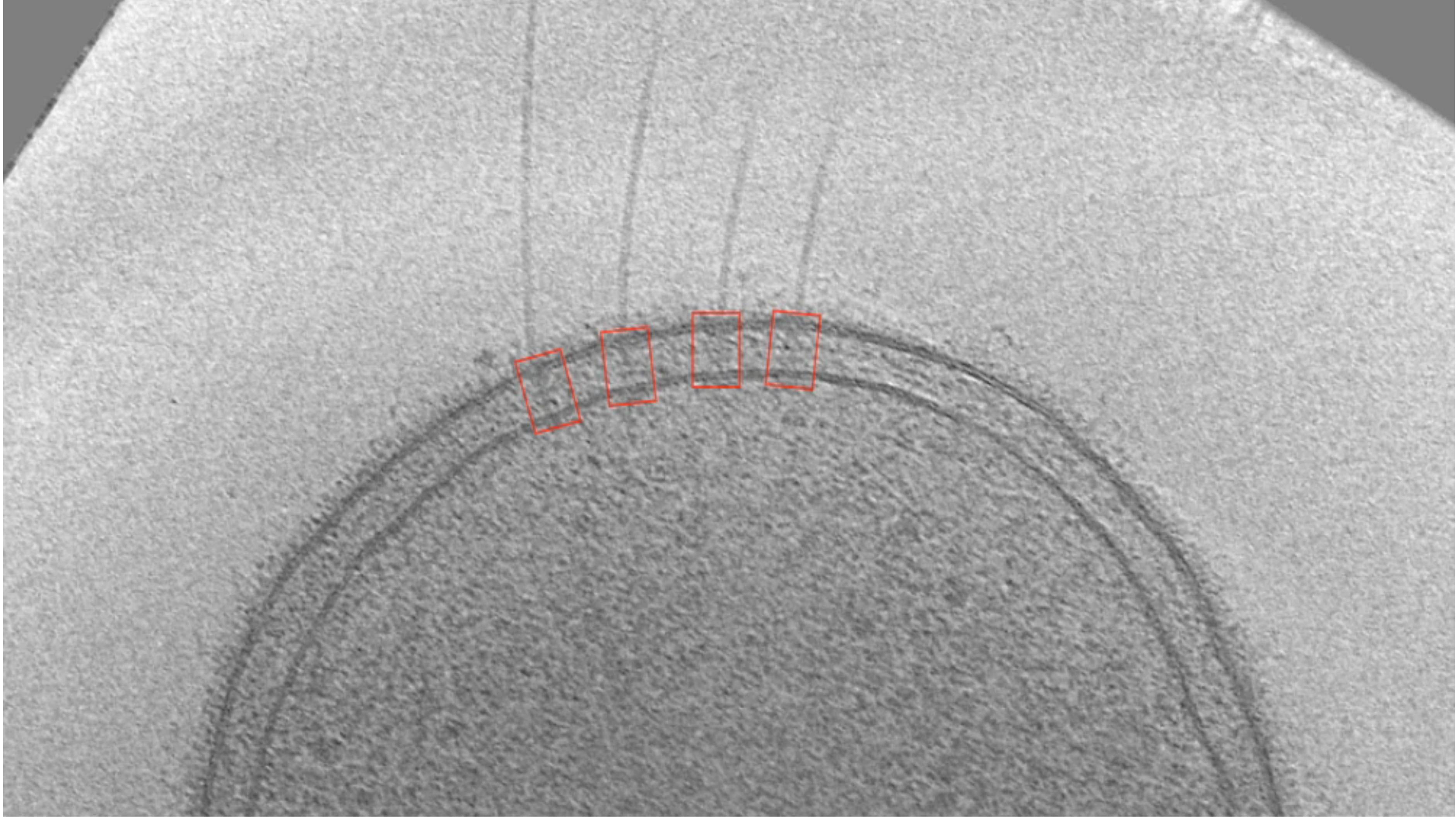


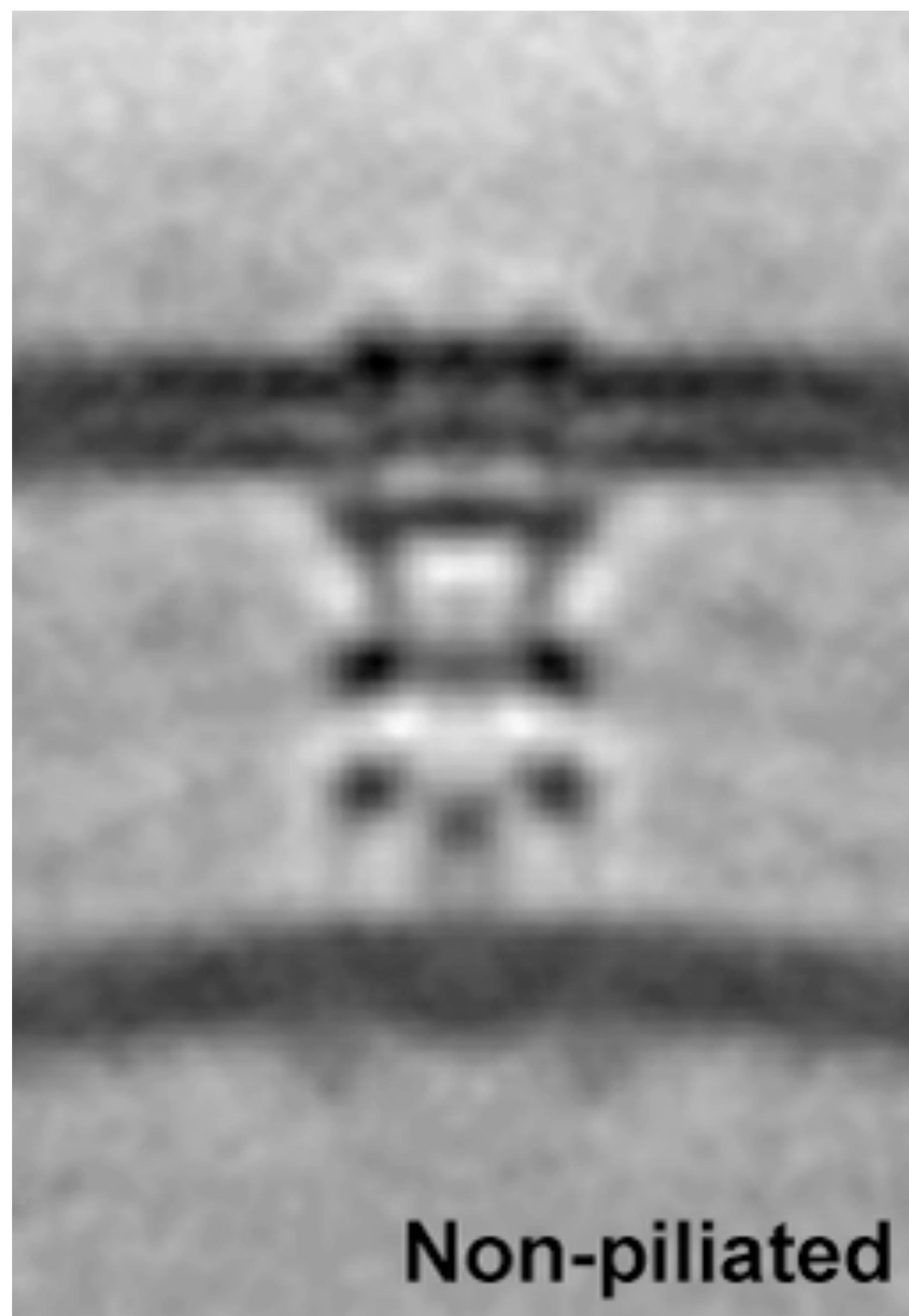
Myxococcus xanthus

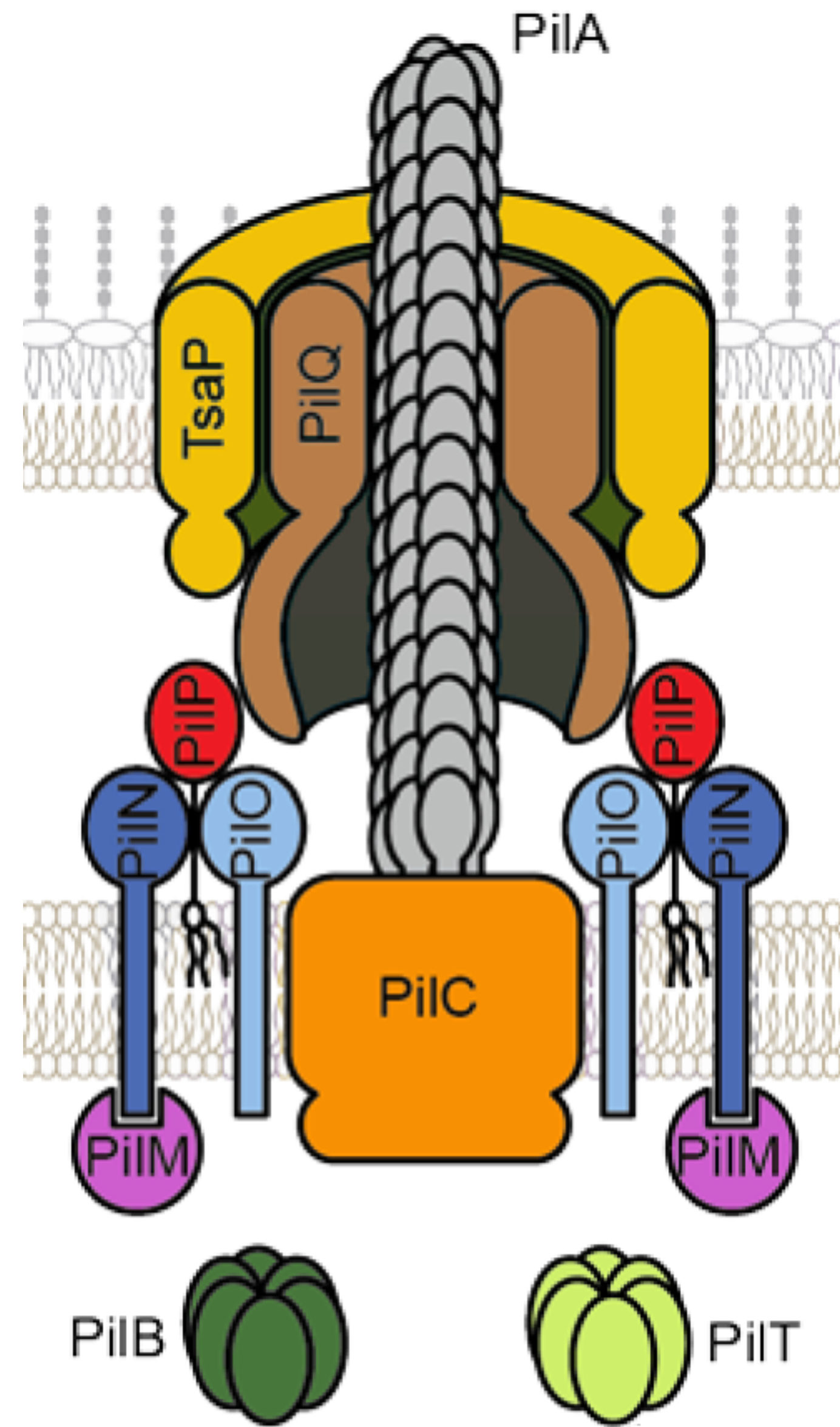






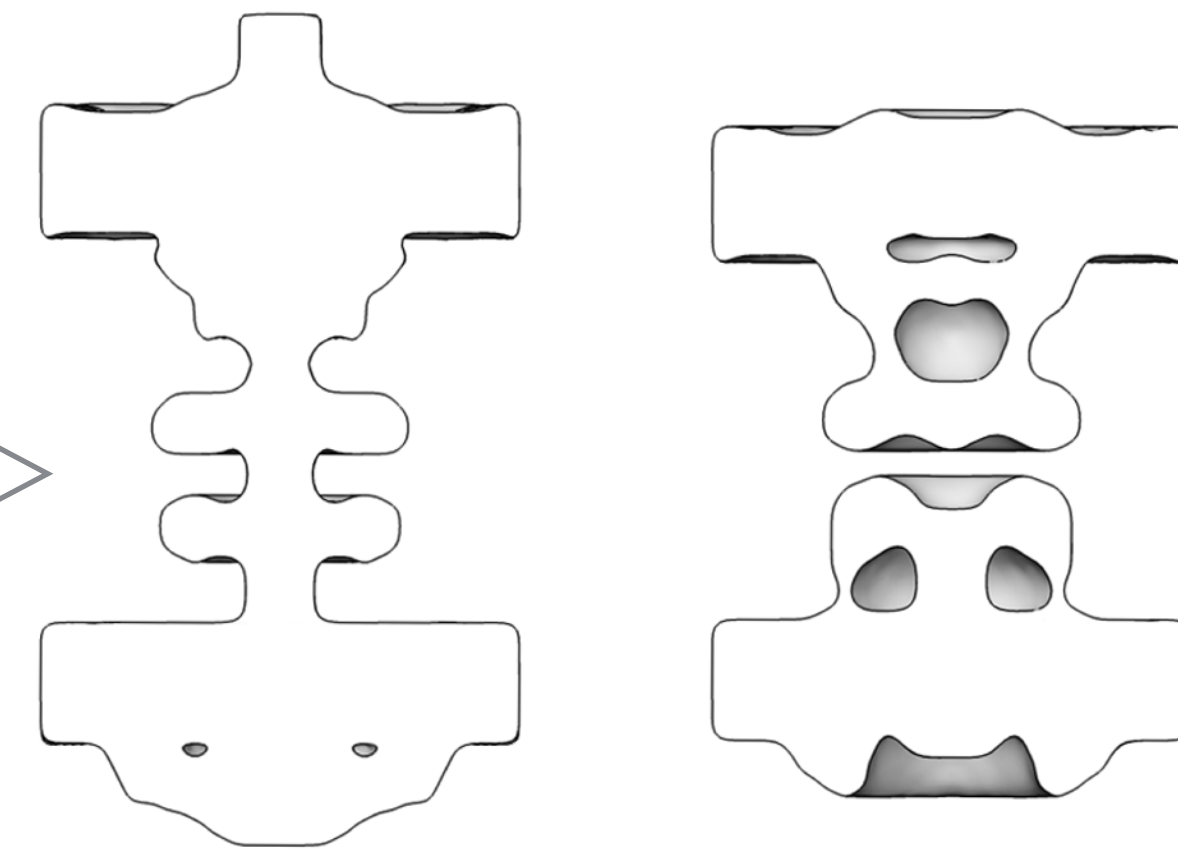
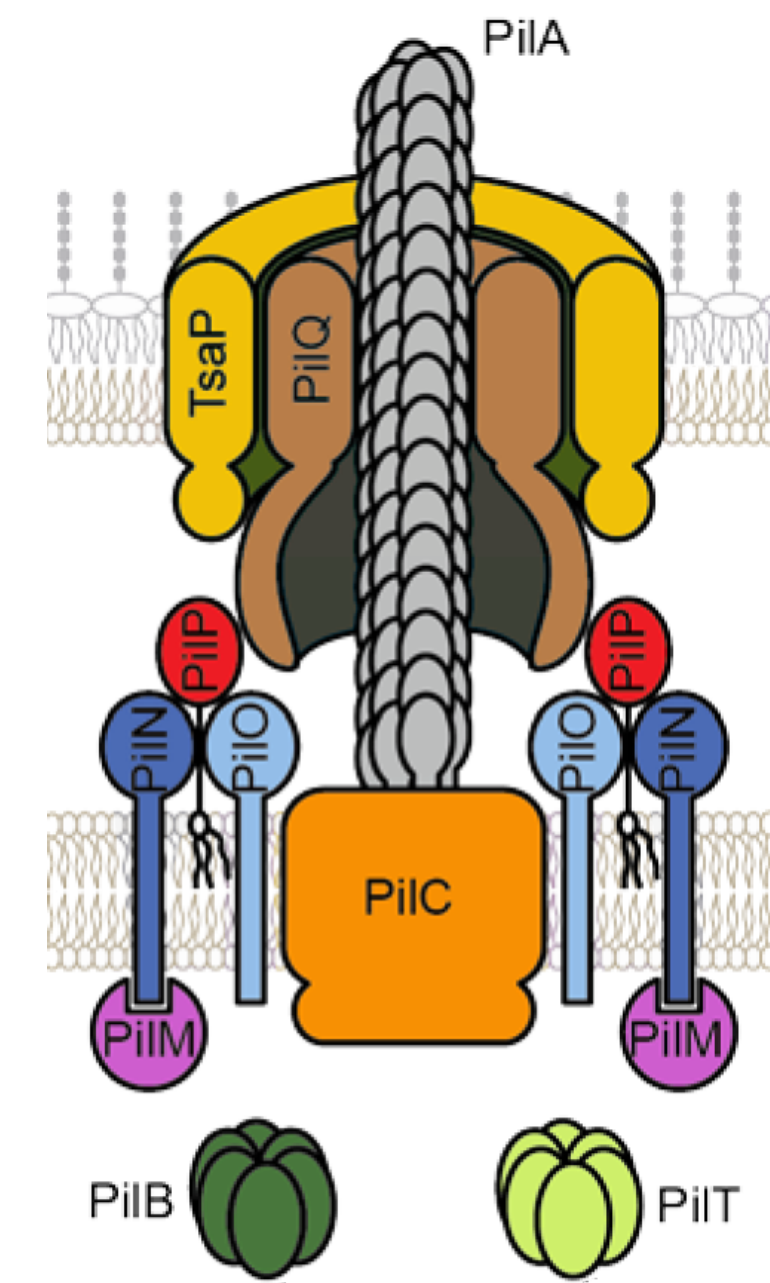








strategy: image knockouts and tagged strains

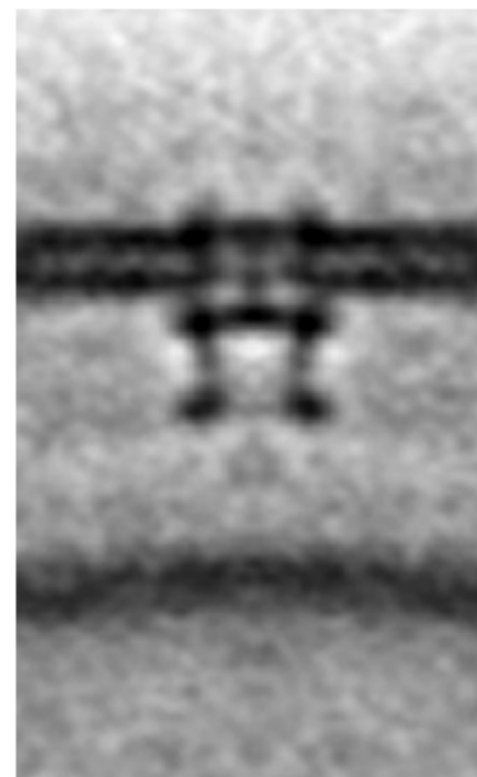




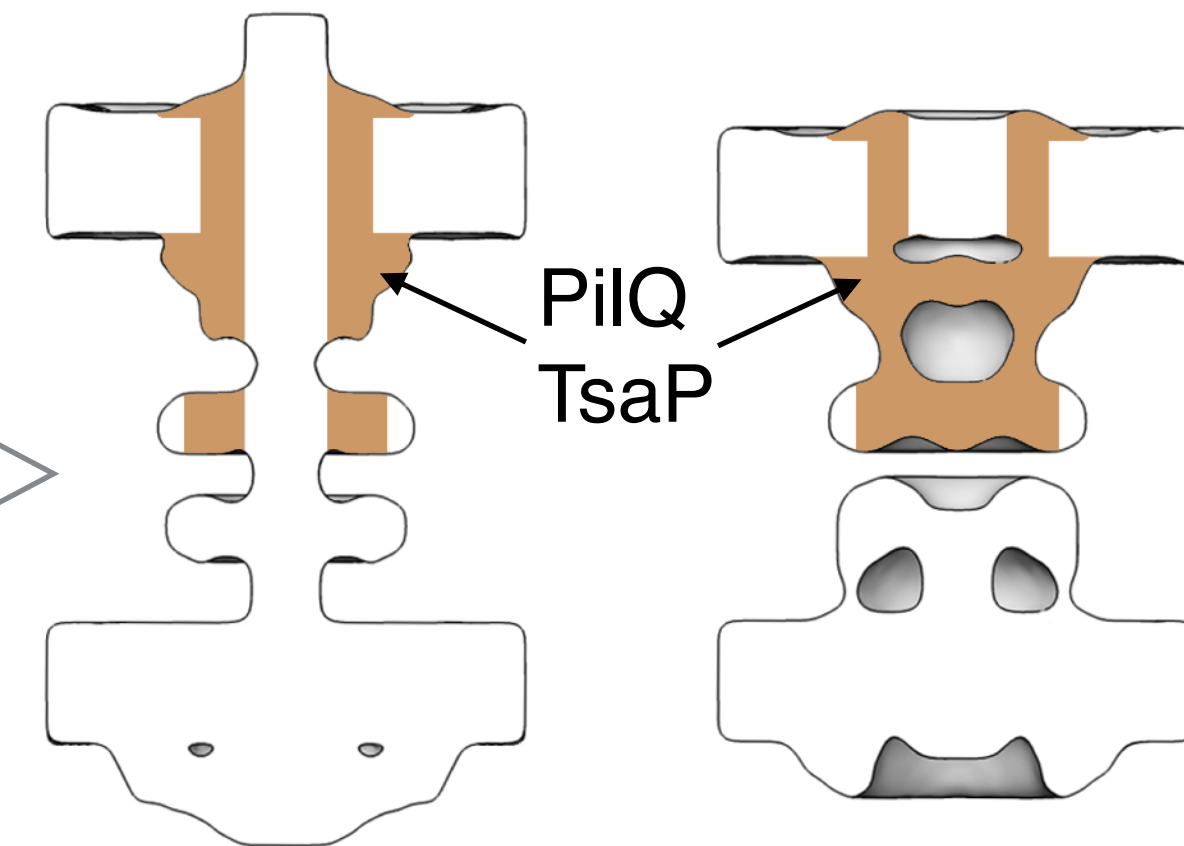
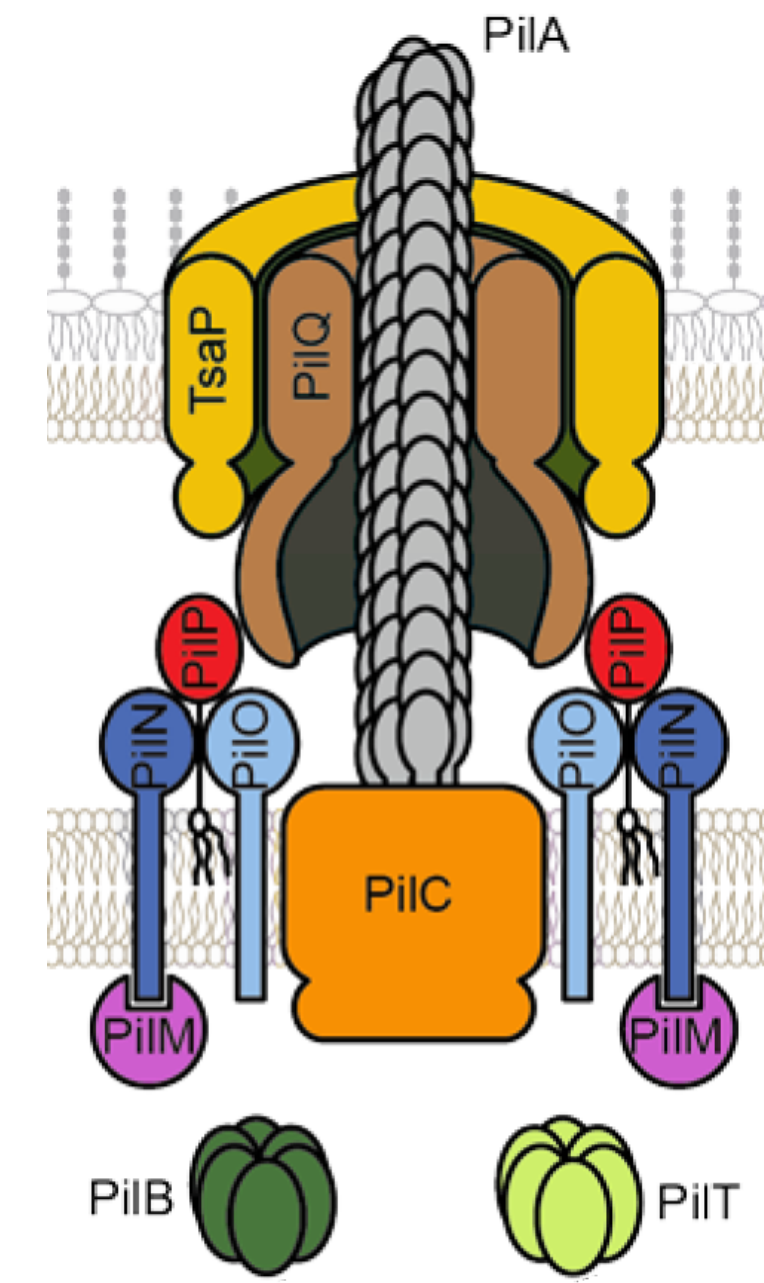
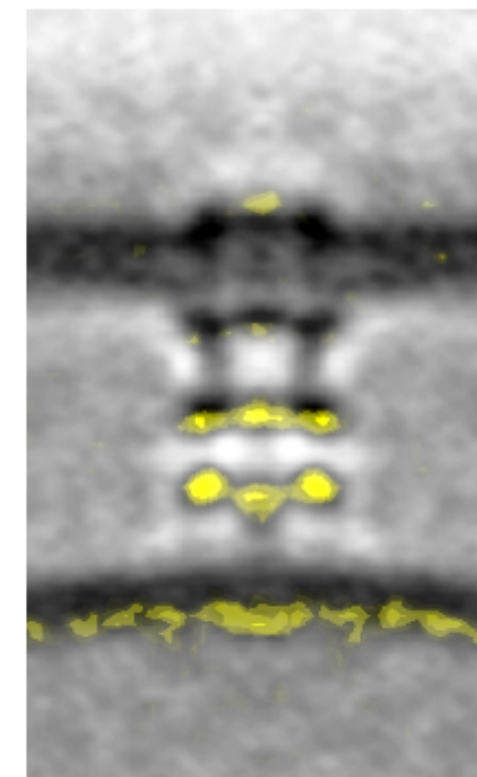
WT



WT



$\Delta pilP$





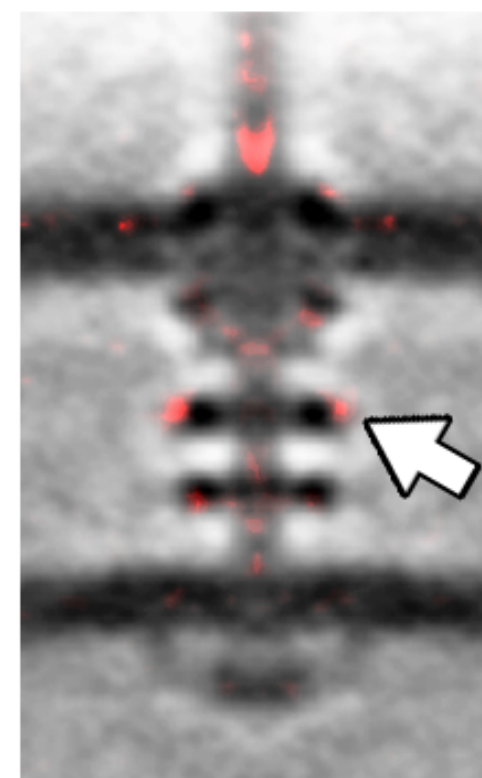
WT



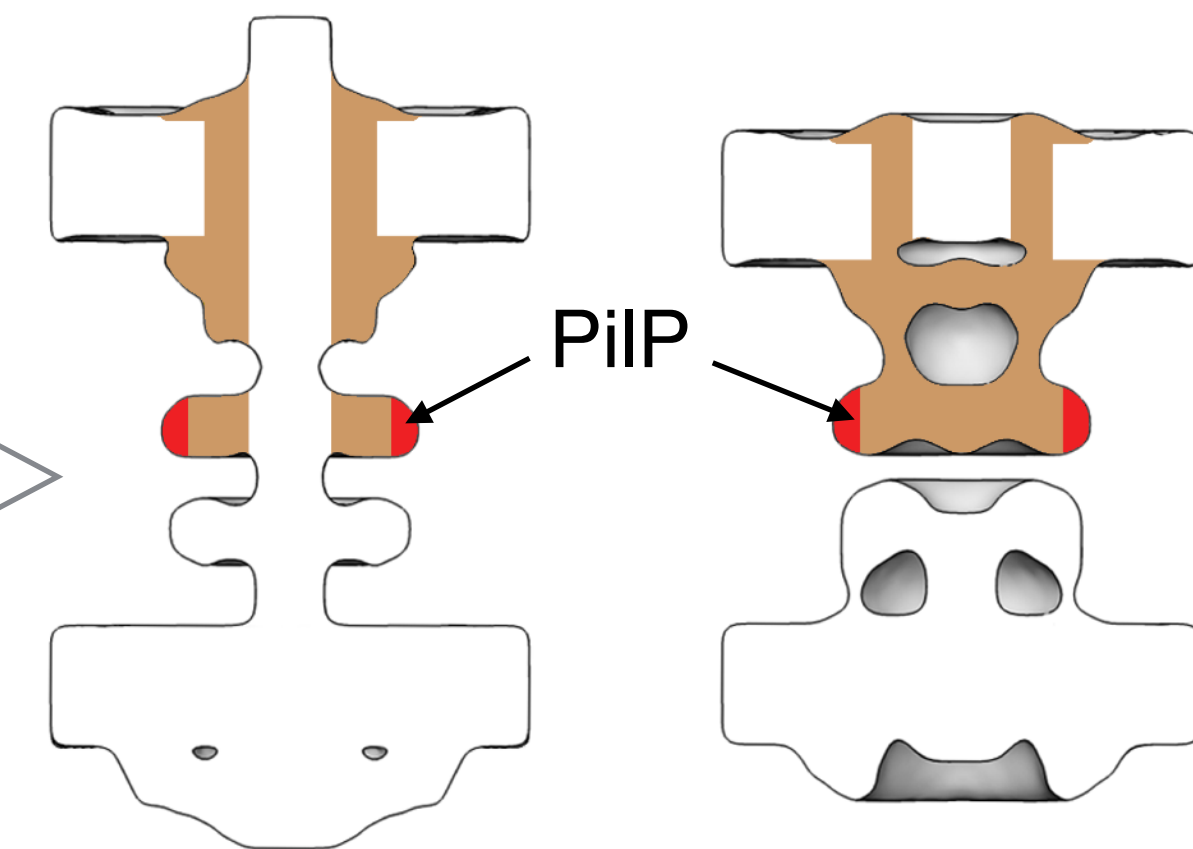
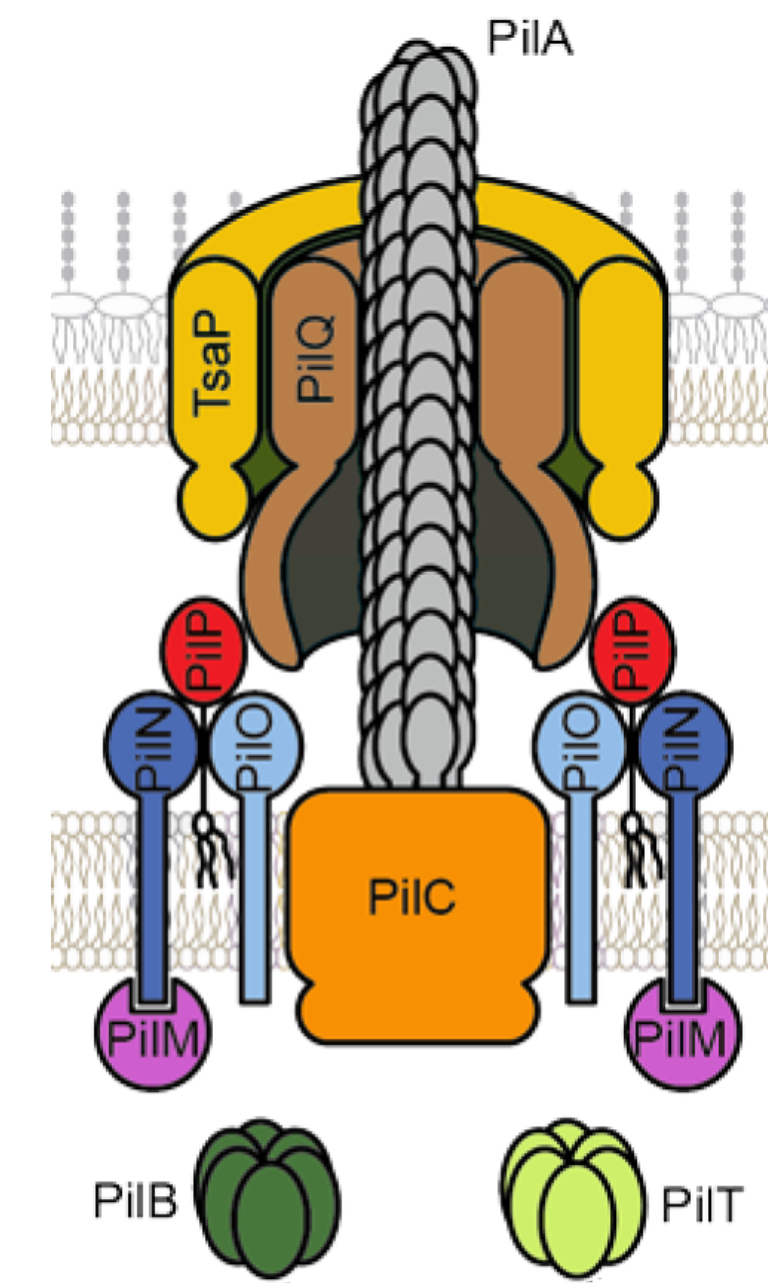
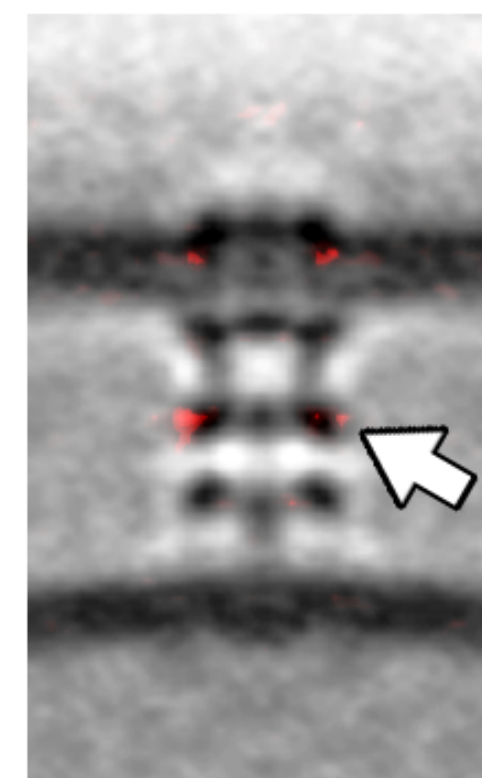
WT

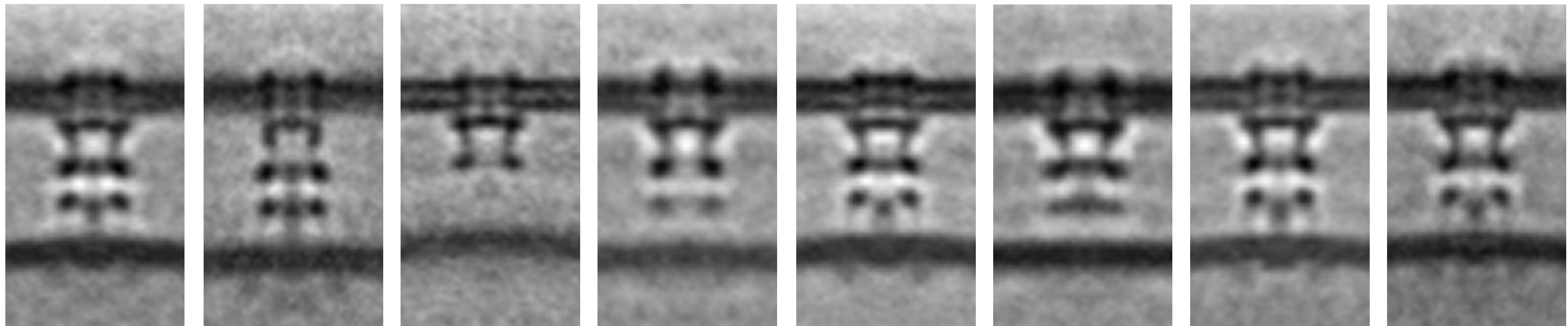


PilP-sfGFP



PilP-sfGFP





WT

$\Delta tsaP$

$\Delta pilP$

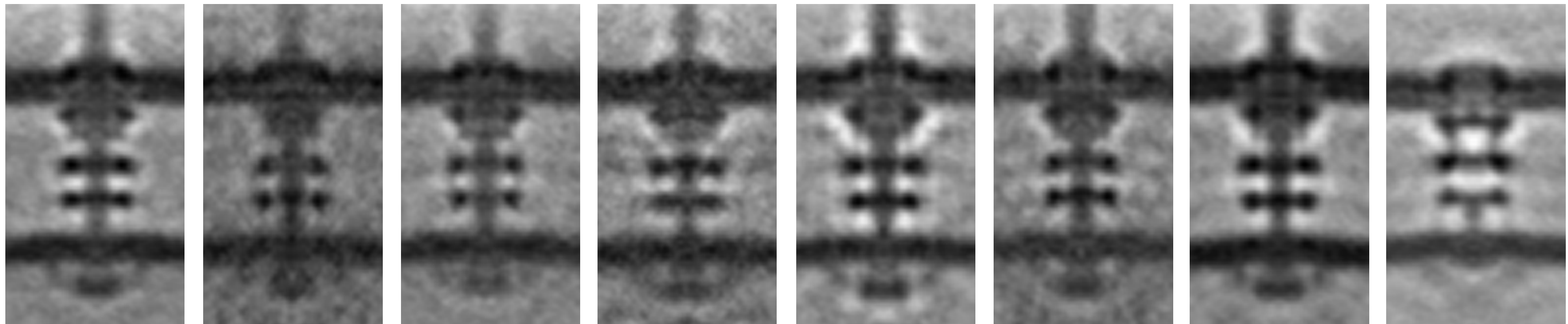
$\Delta pilC$

$\Delta pilB$

$\Delta pilA$

$\Delta pilB/T$

$\Delta pilQ_{\beta 1-\beta 2}$



WT

$\Delta tsaP$

$\Delta pilT$

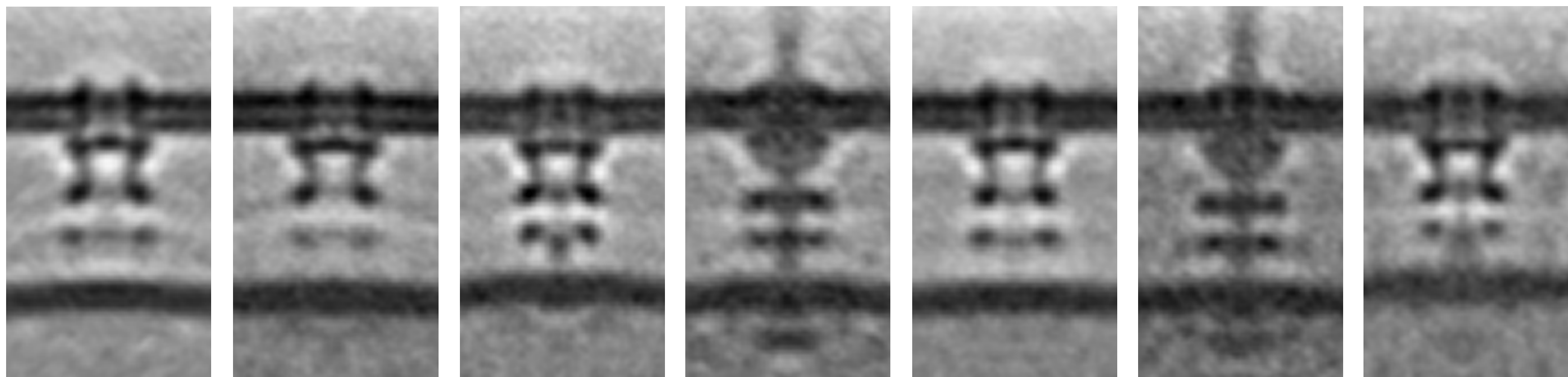
$\Delta pilQ_{\beta 1-\beta 2}$

PilP-sfGFP

PilO-sfGFP

PilM-YFP

PilP-sfGFP



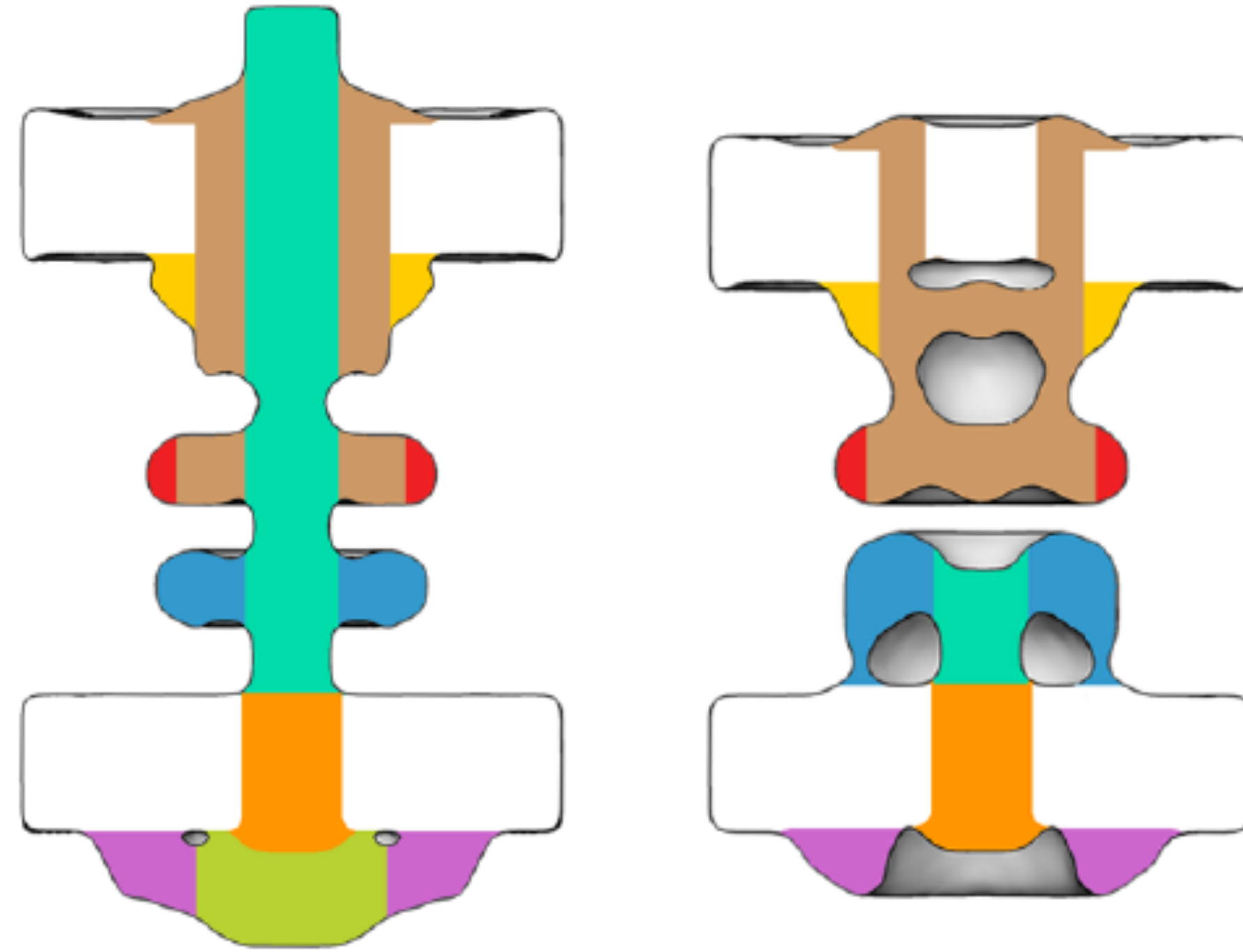
$\Delta pilV,pilW,fimU_{1-3}$

$\Delta pilY_{1-3}$

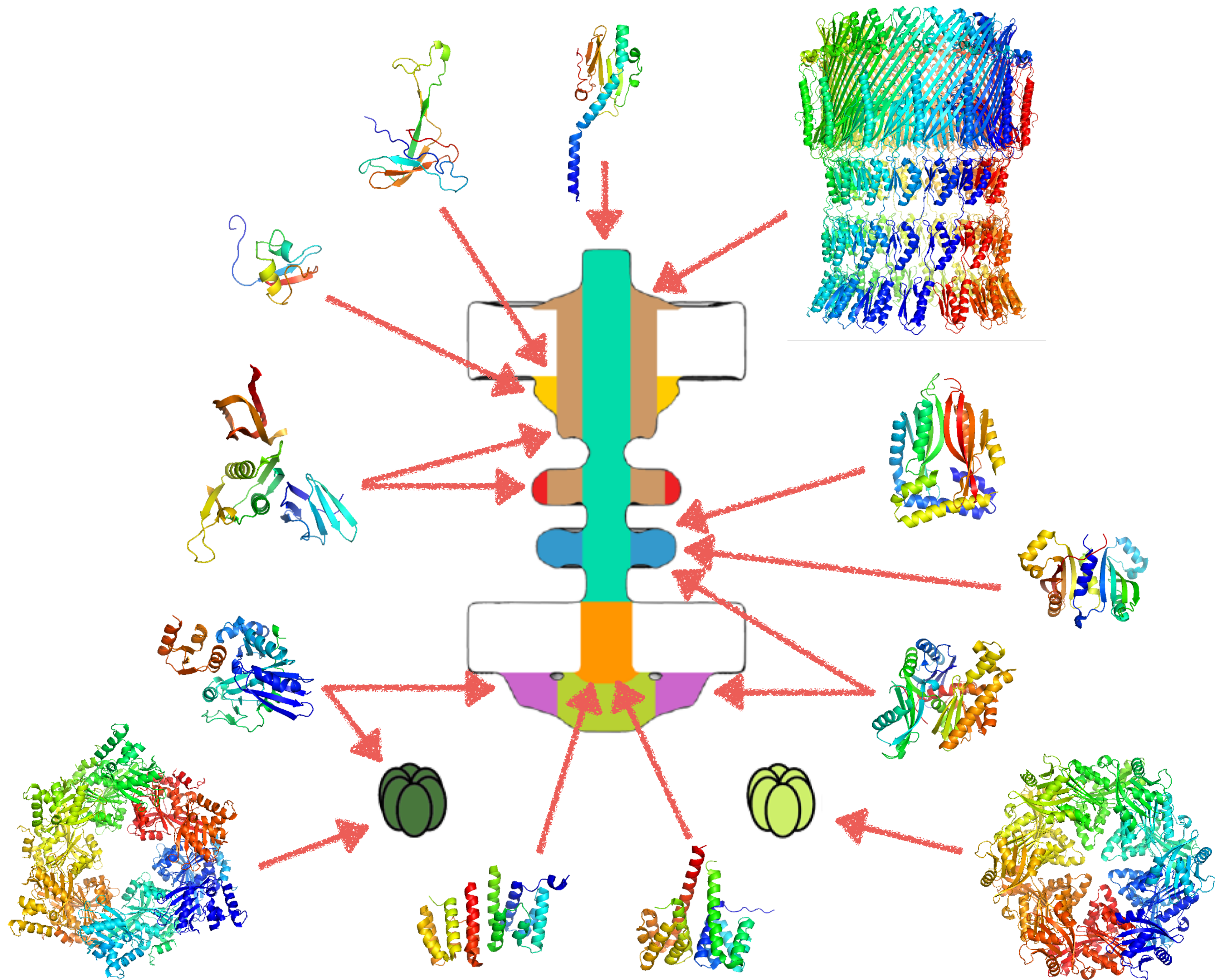
$\Delta \text{minor pilins clusters}_{1-2}$

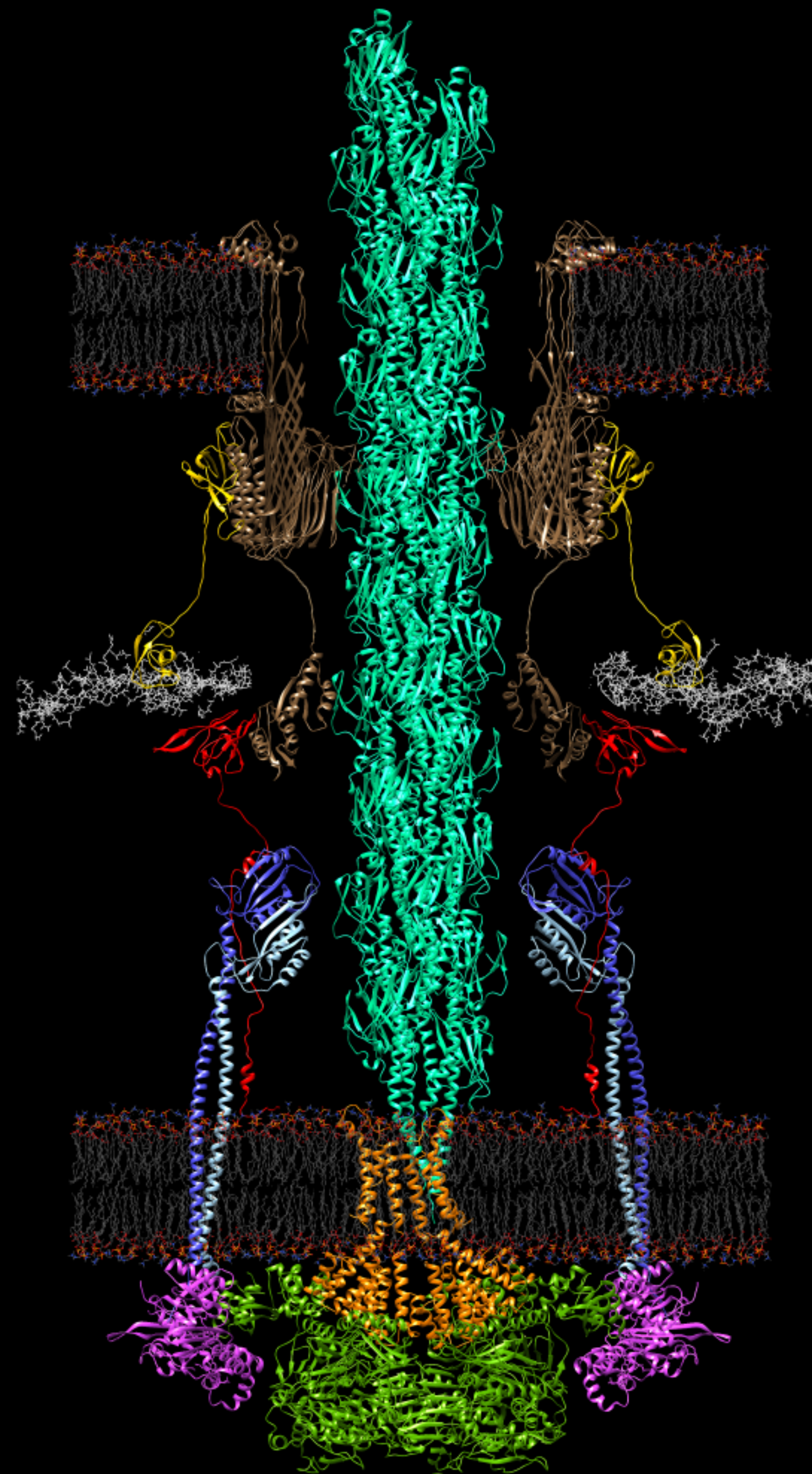
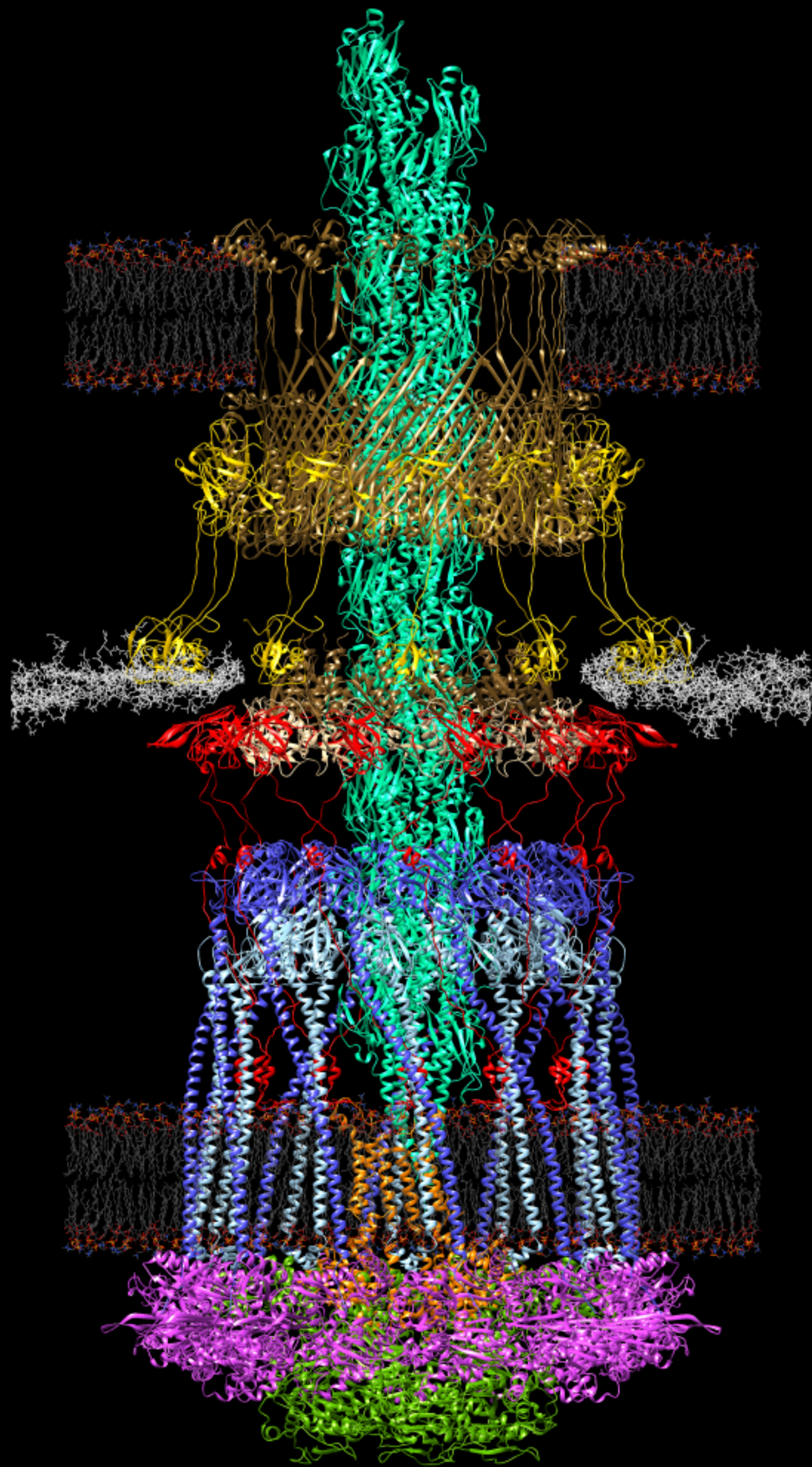
$\Delta \text{minor pilins clusters}_{2-3}$

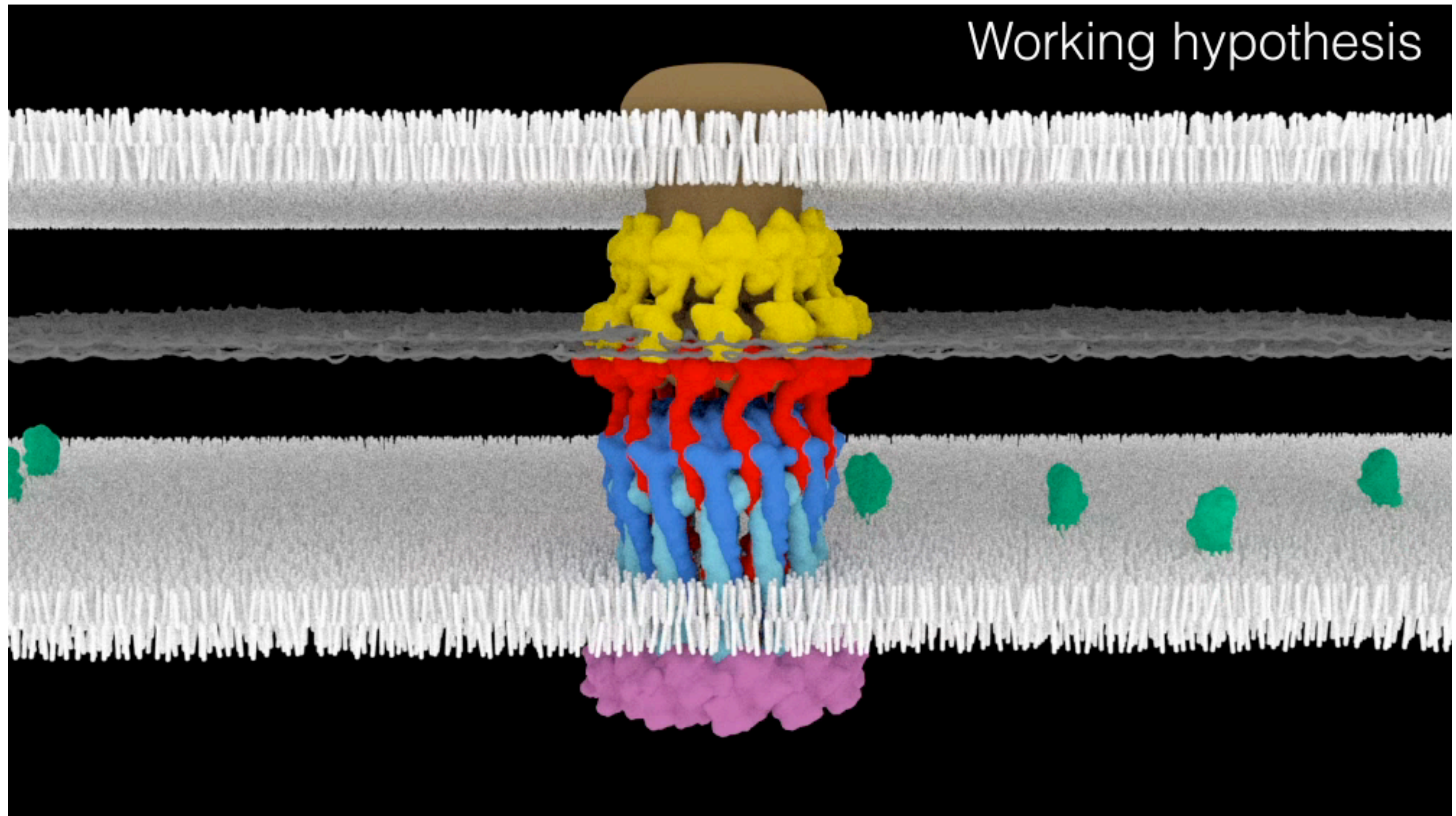
PilO-sfGFP



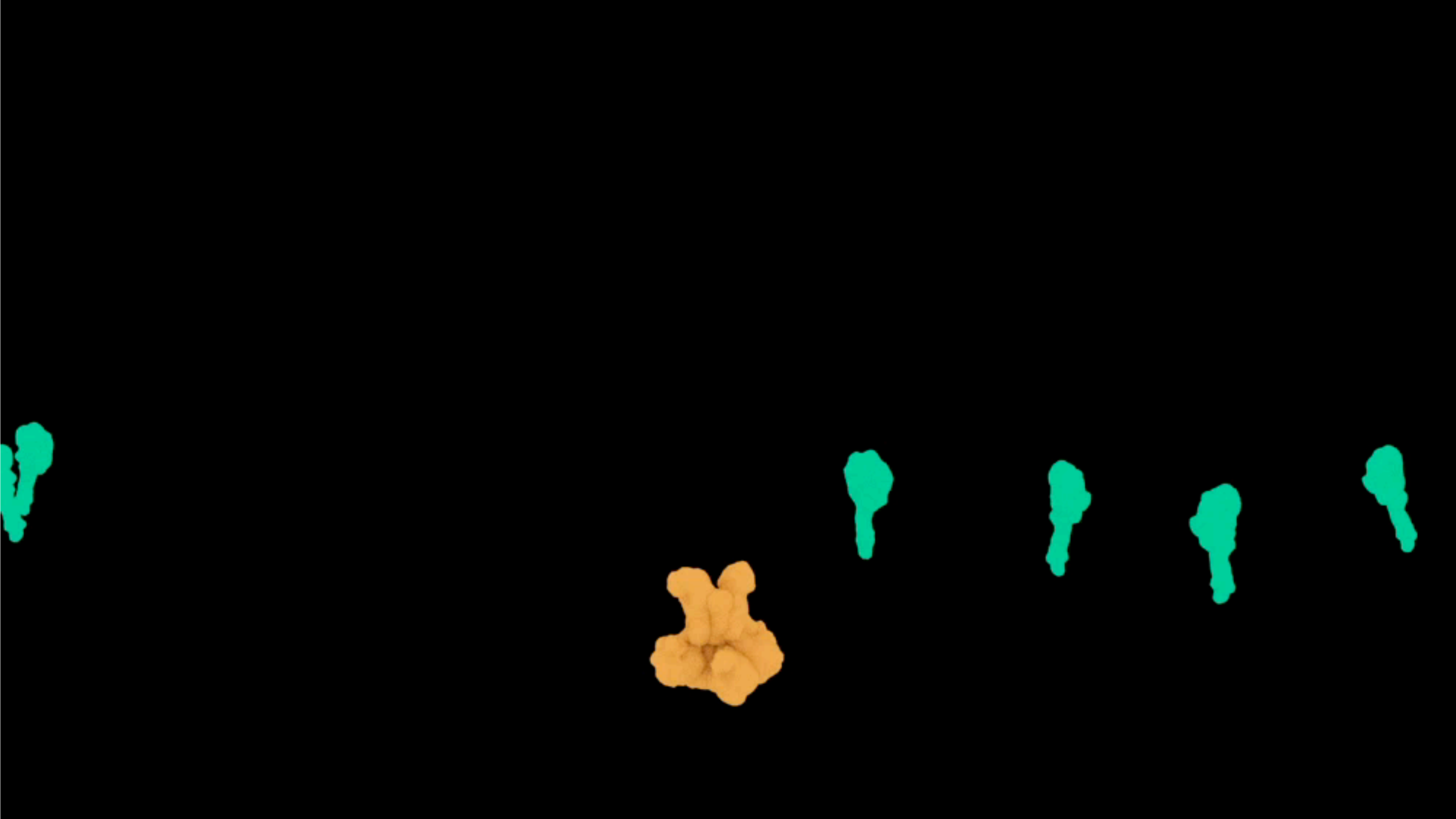
- | | | |
|--|---|--|
|  PiQ |  PiA |  PiN&PiO |
|  TsaP |  PiC |  PiB/PiI |
|  PiP |  PiM |  Membrane |



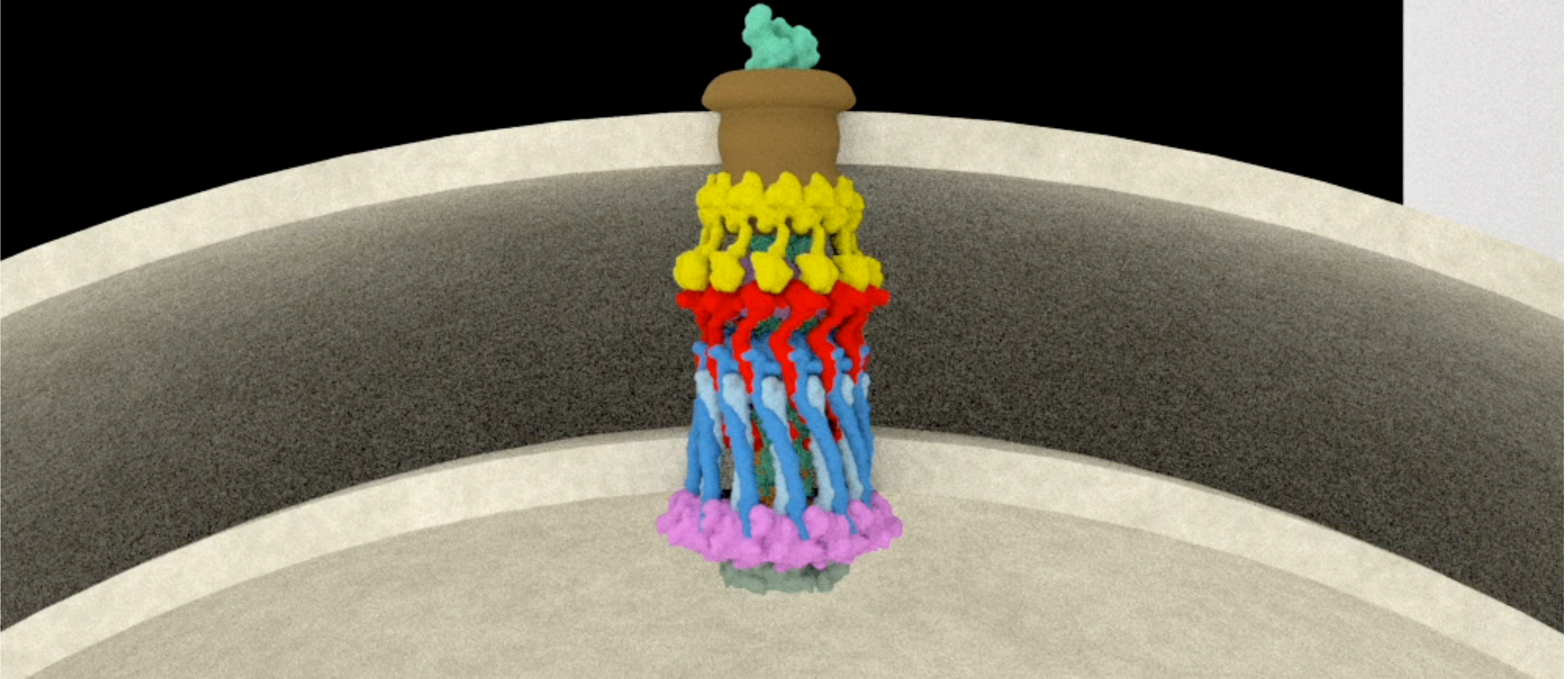




Animation by Janet Iwasa



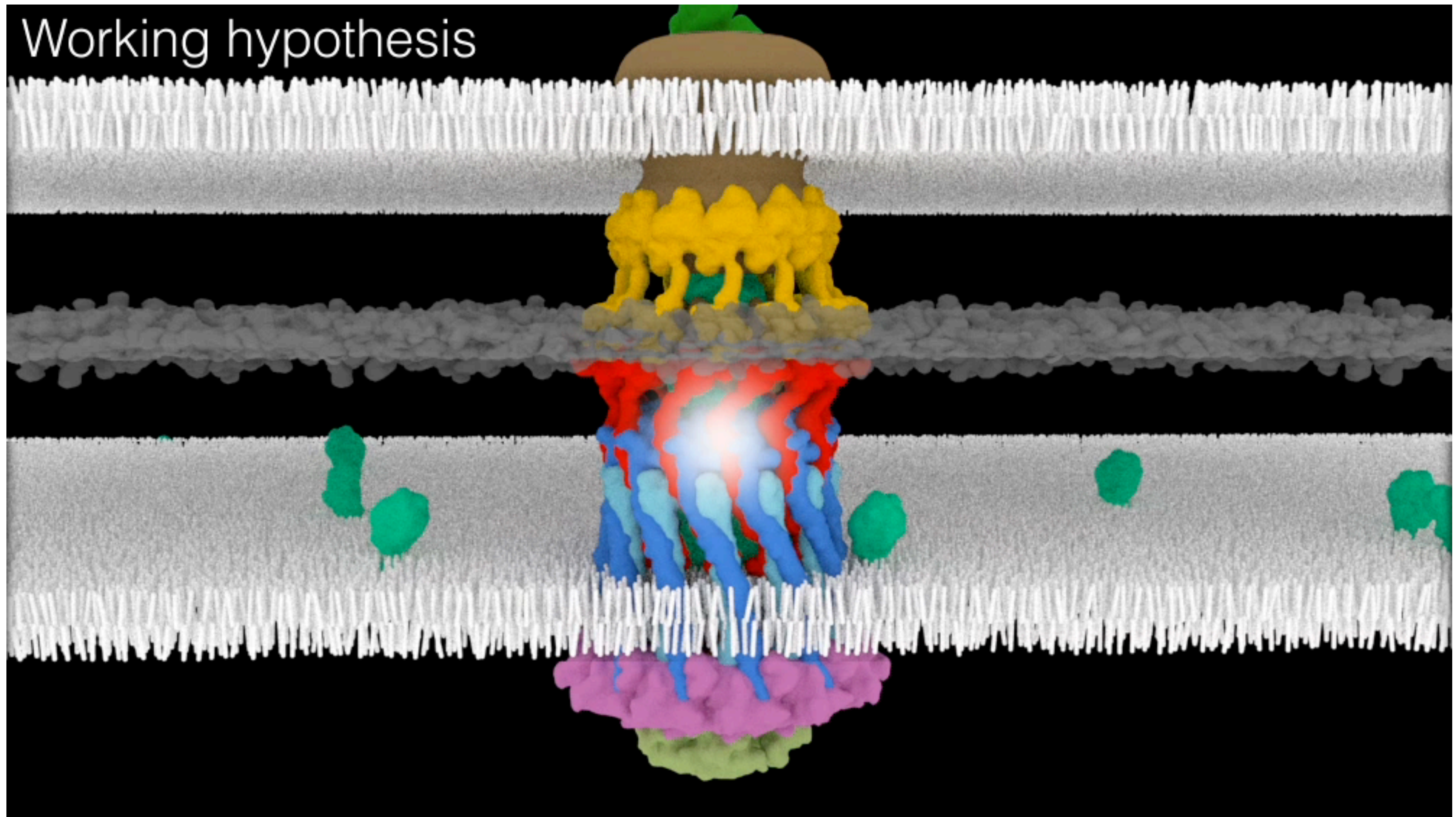
Working hypothesis

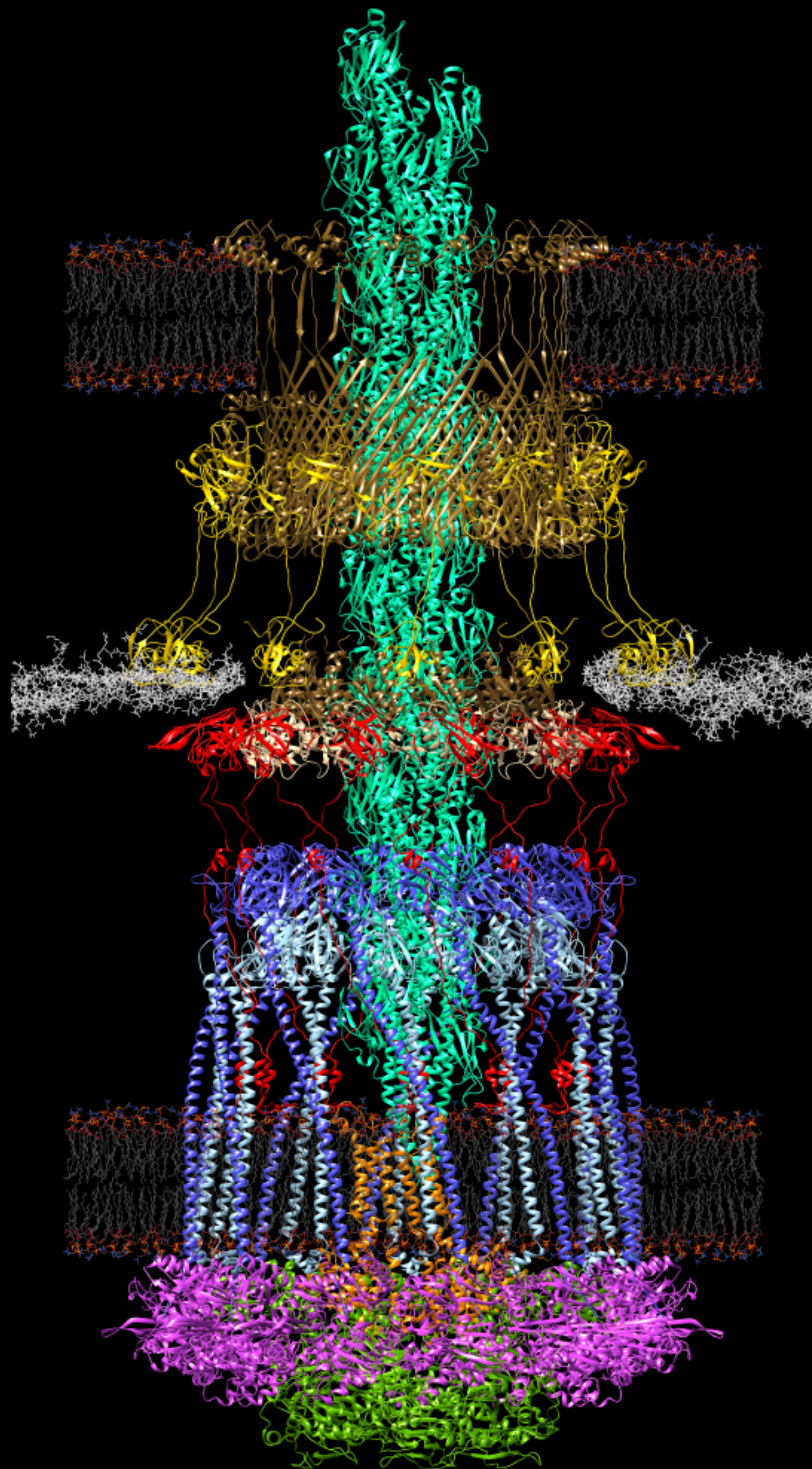


Working hypothesis



Working hypothesis





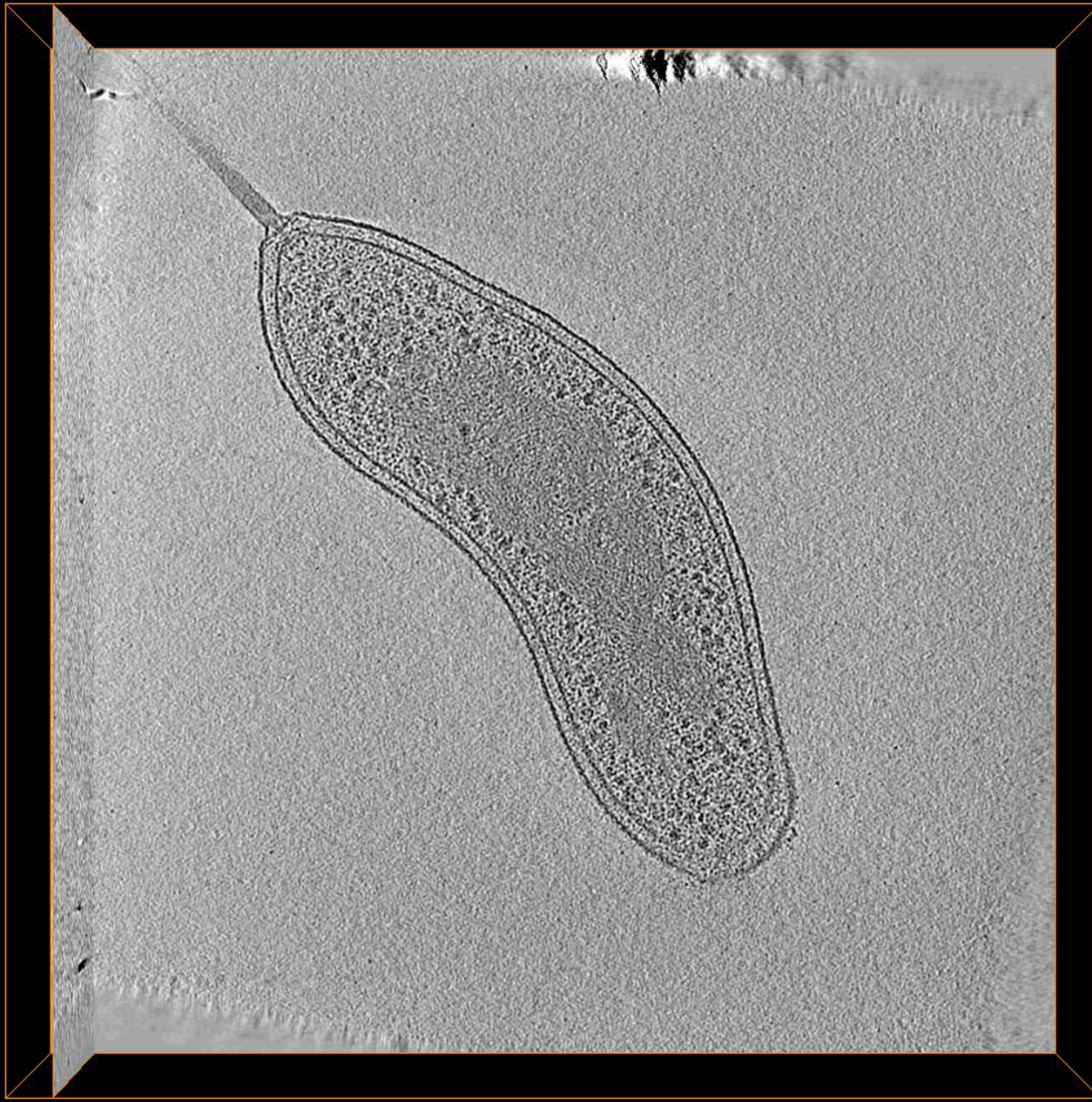
Yi-Wei
Chang

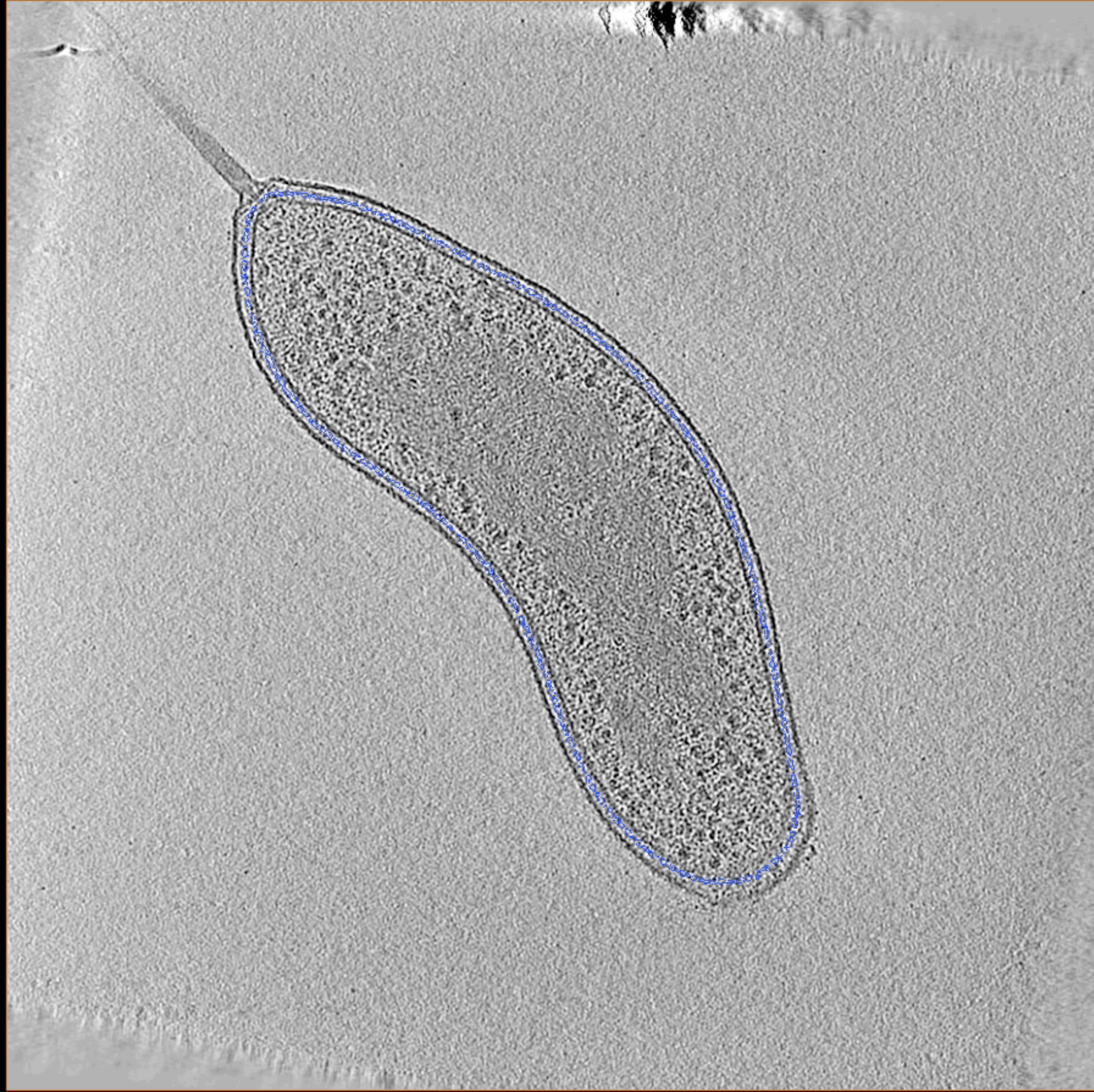


Sara
Weaver

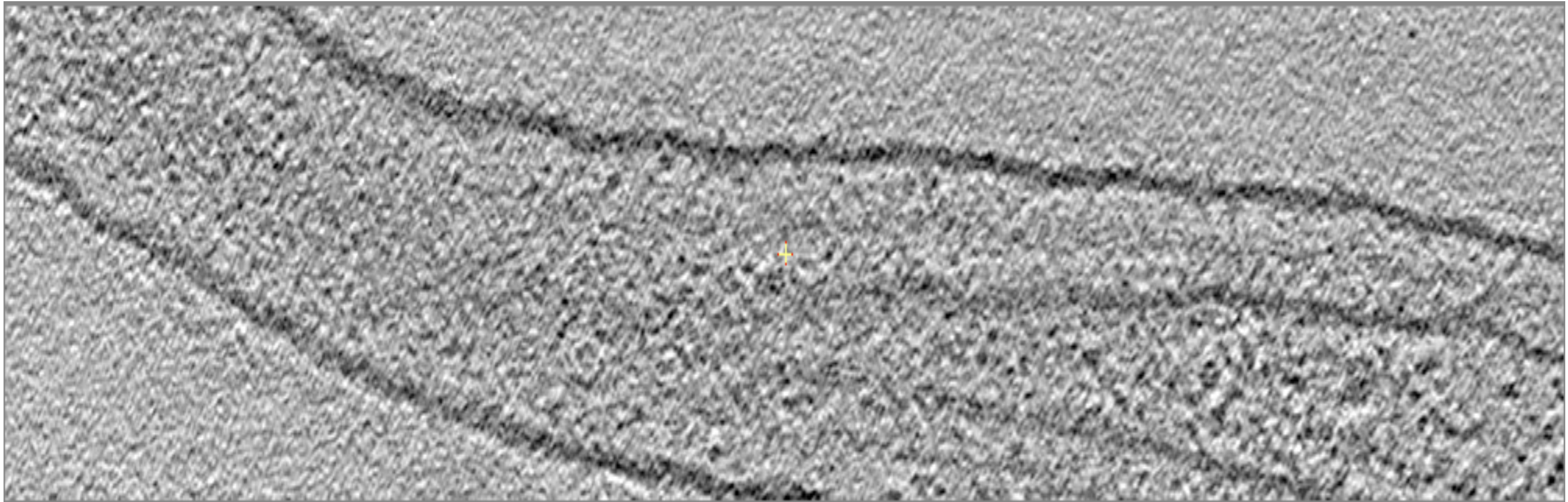


Lotte
Søgaard-Andersen







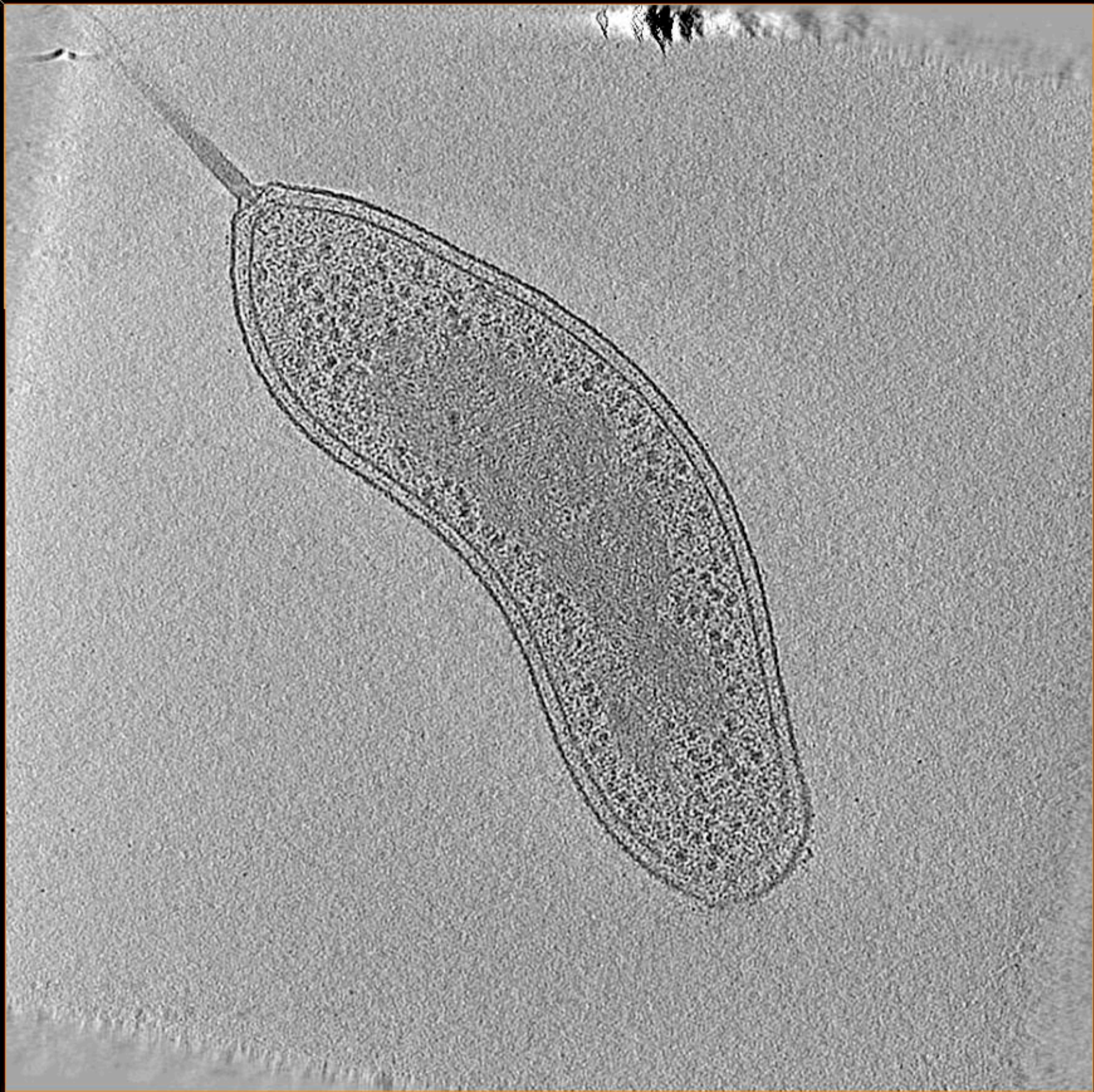












The Atlas of Bacterial & Archaeal Cell Structure

by Catherine M. Oikonomou & Grant J. Jensen

This open access digital textbook offers a tour of microbial cells guided by cutting-edge 3D electron microscopy.

 **READ ONLINE NOW**

<https://www.cellstructureatlas.org/>

The Atlas of Bacterial & Archaeal Cell Structure

by Catherine M. Oikonomou & Grant J. Jensen

This open access digital textbook offers a tour of microbial cells guided by cutting-edge 3D electron microscopy.

▶ [READ ONLINE NOW](#)

The Atlas of Bacterial & Archaeal Cell Structure

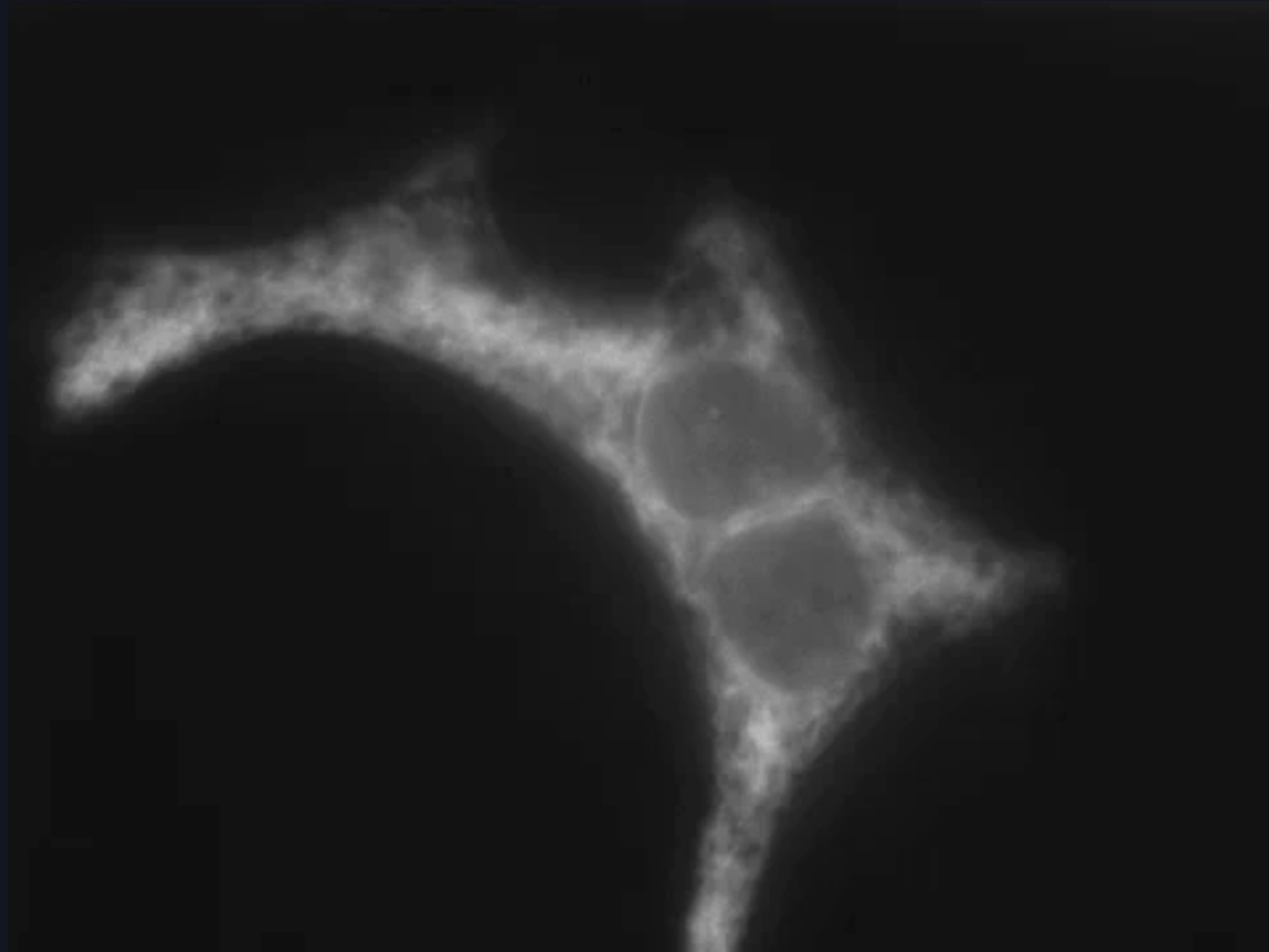
by Catherine M. Oikonomou & Grant J. Jensen

This open access digital textbook offers a tour of microbial cells guided by cutting-edge 3D electron microscopy.

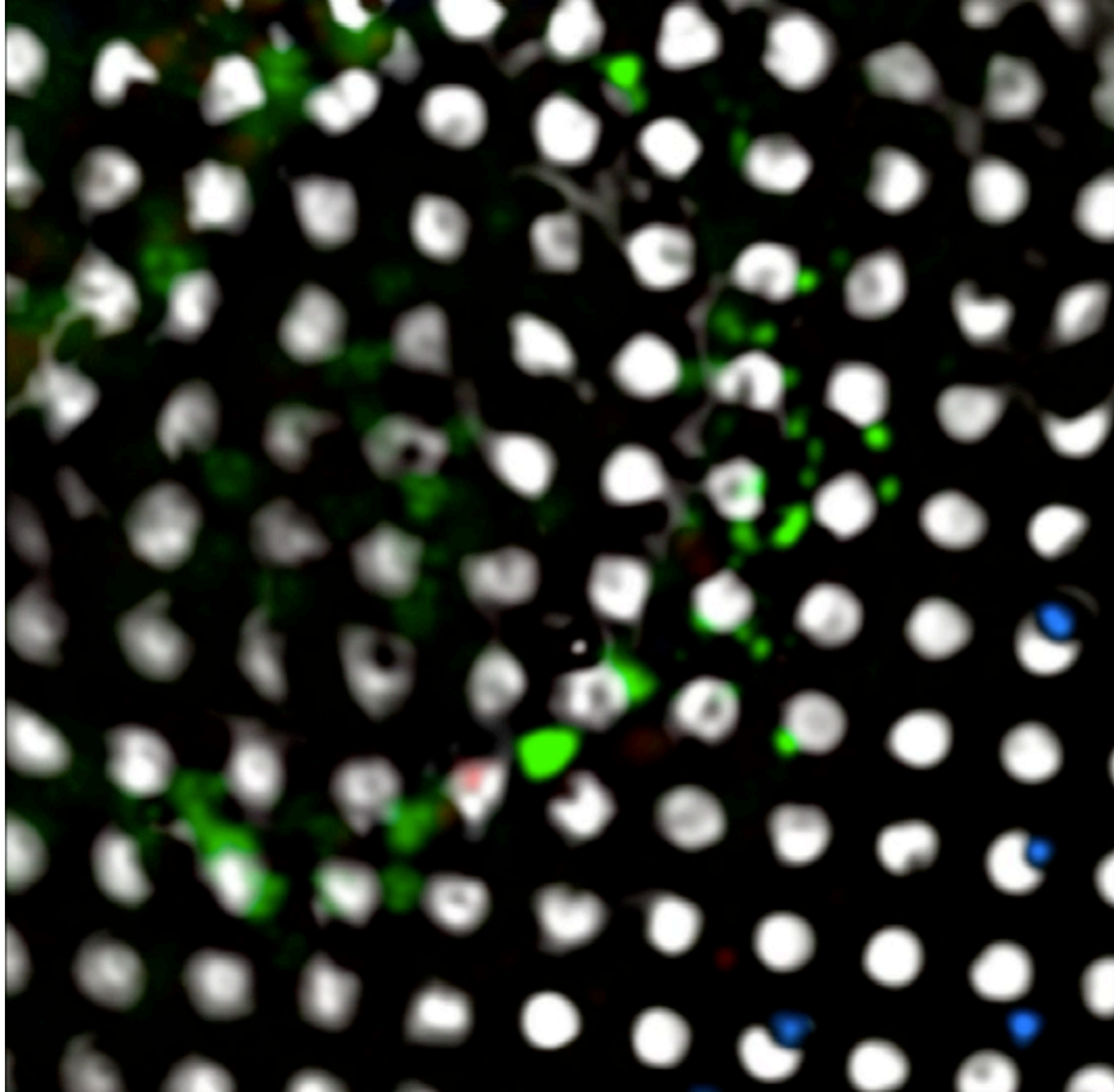
 READ ONLINE NOW

 <https://www.cellstructureatlas.org/> 

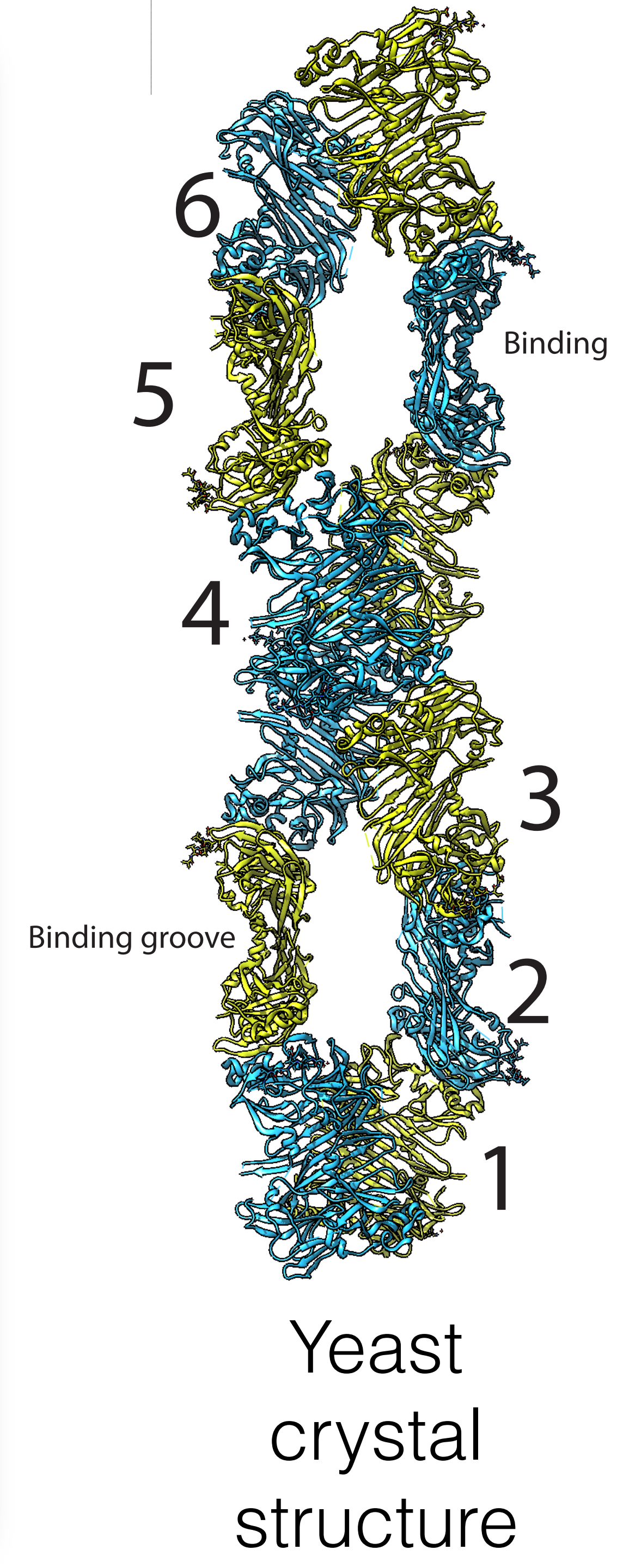
Evidence of IRE1 α oligomerization *in vivo*

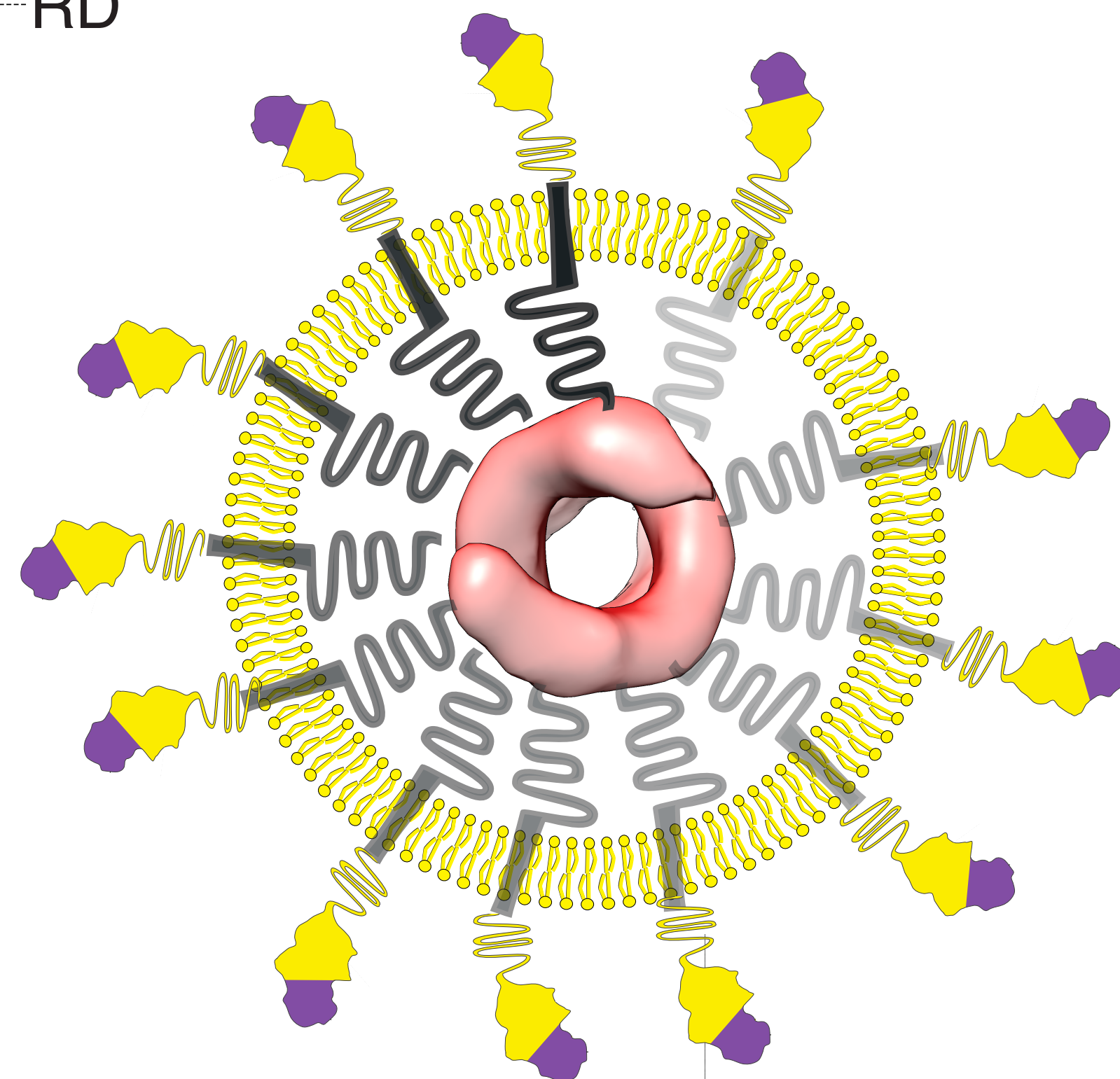
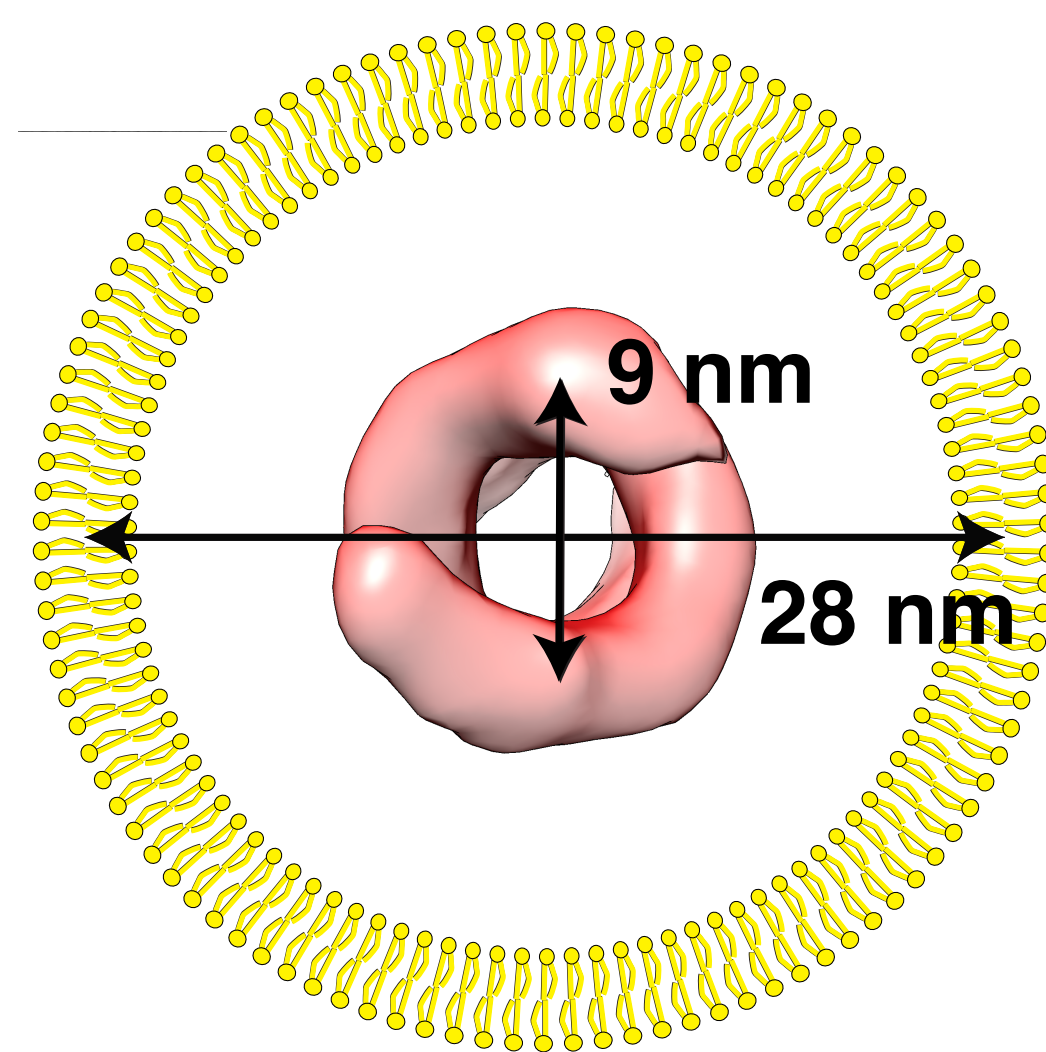
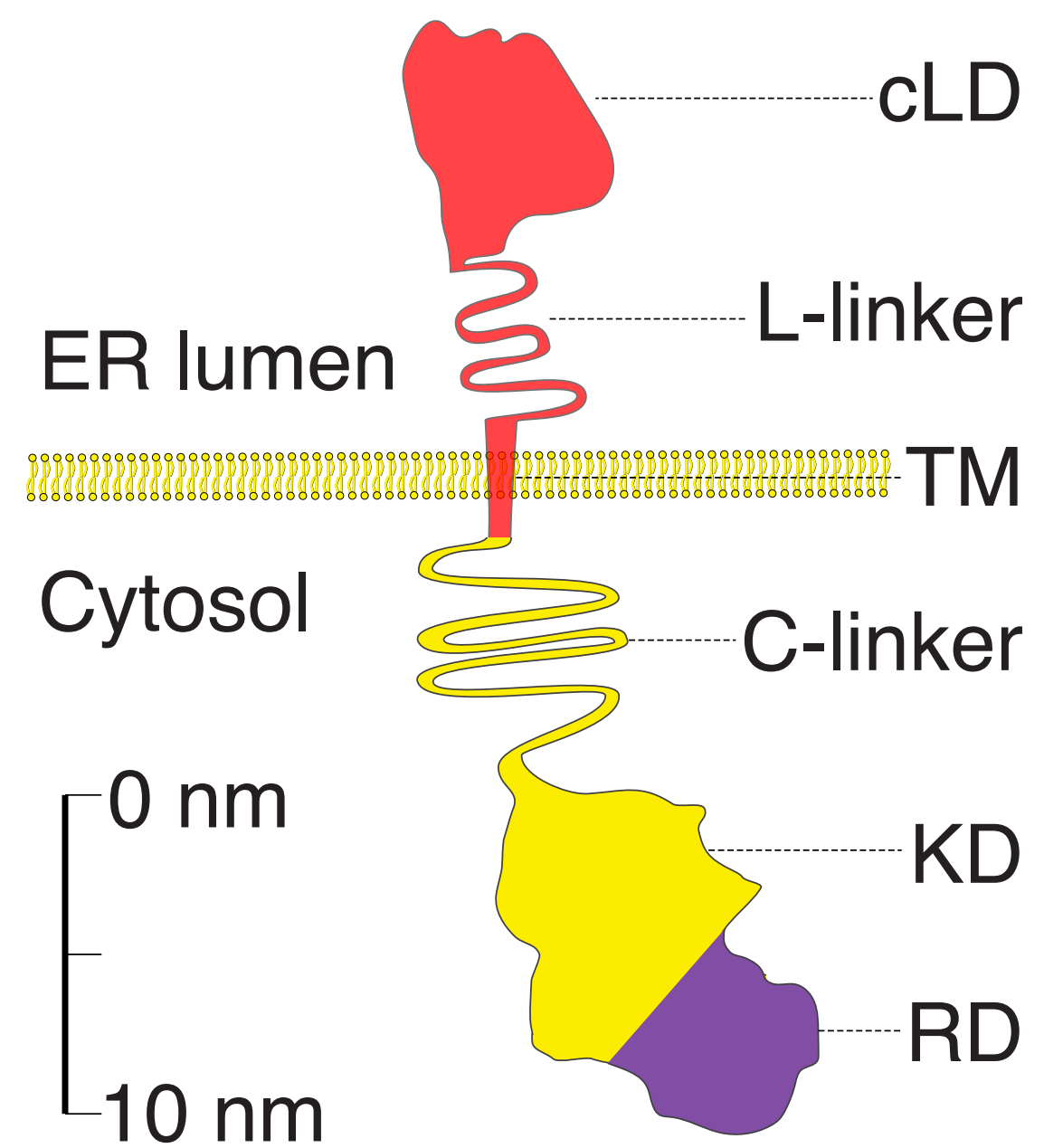


Walter and Ron, **2011**; Li *et al*, **2010**; Aragón *et al*, **2009**











Stephen
Carter
Caltech

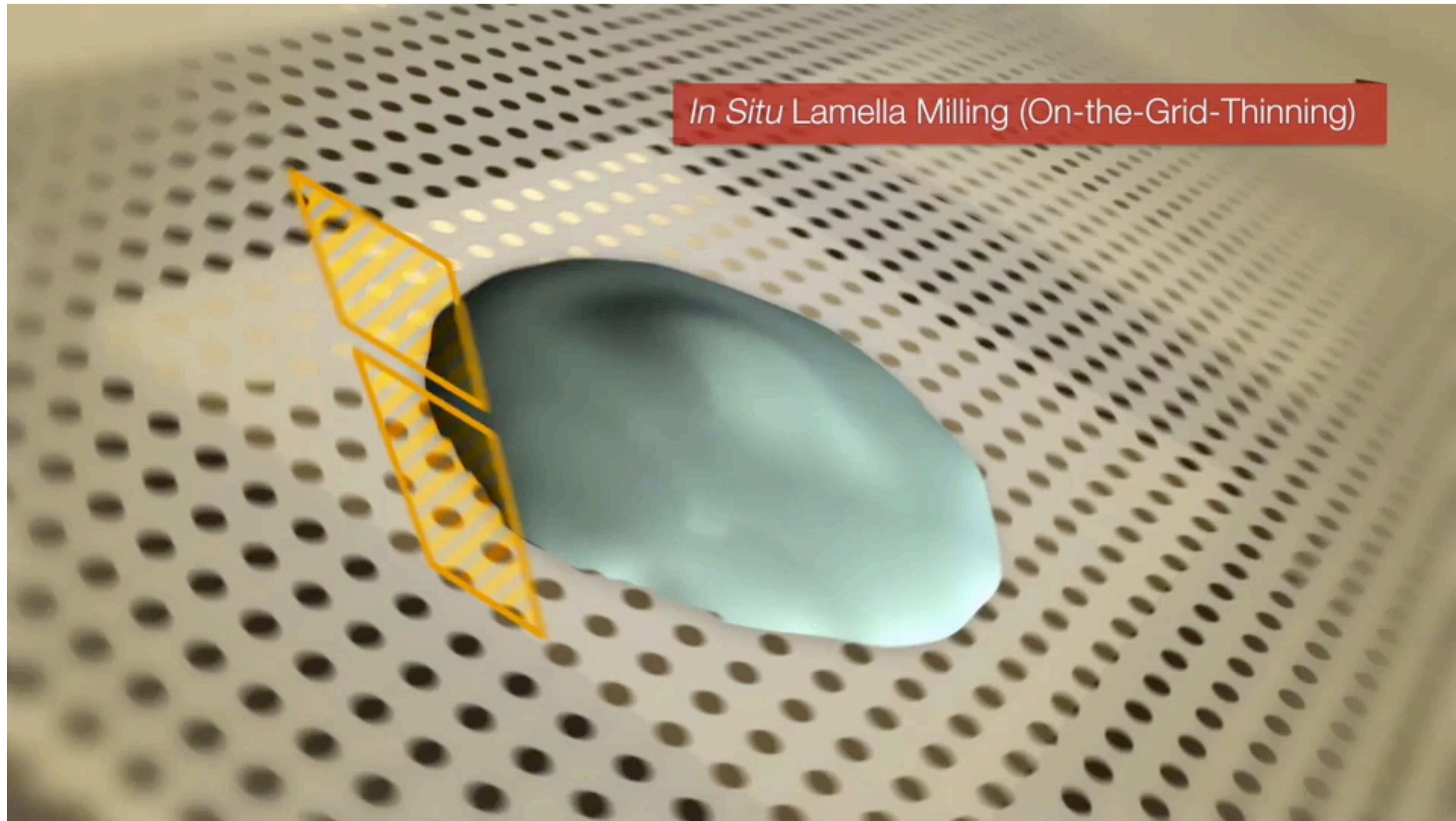


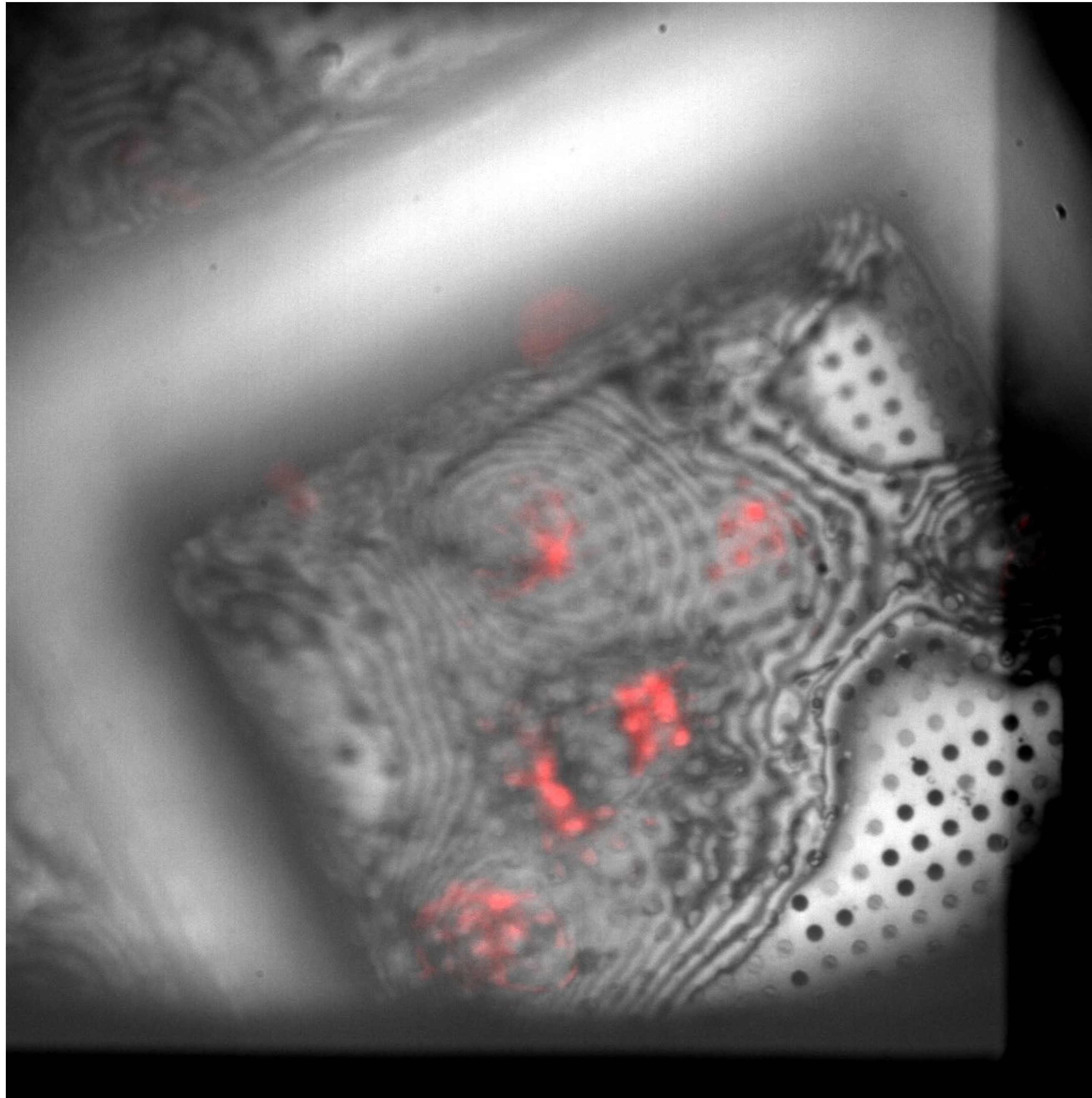
Han
Tran
UCSF



Peter
Walter
UCSF

Focused ion beam (FIB) milling

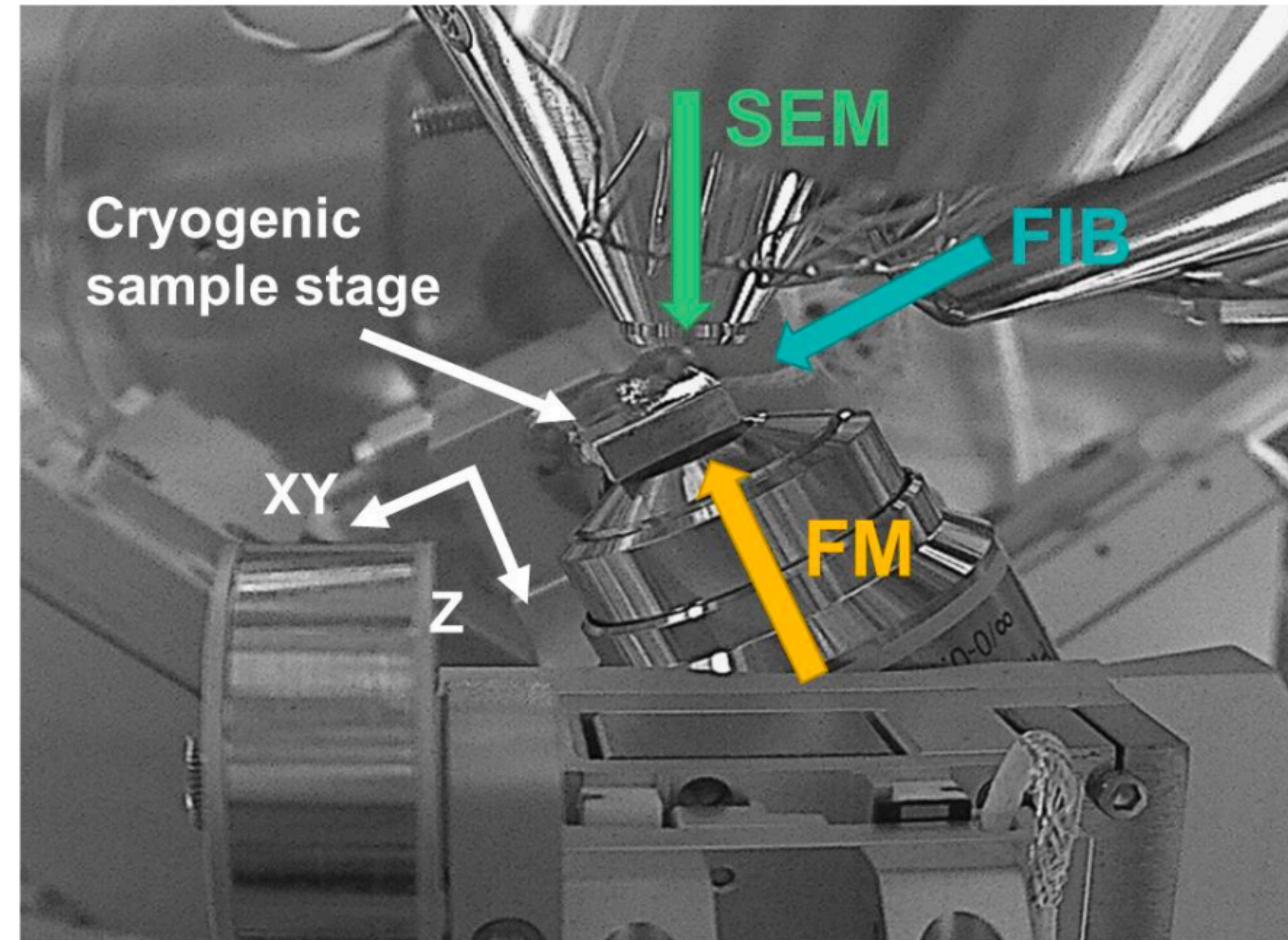
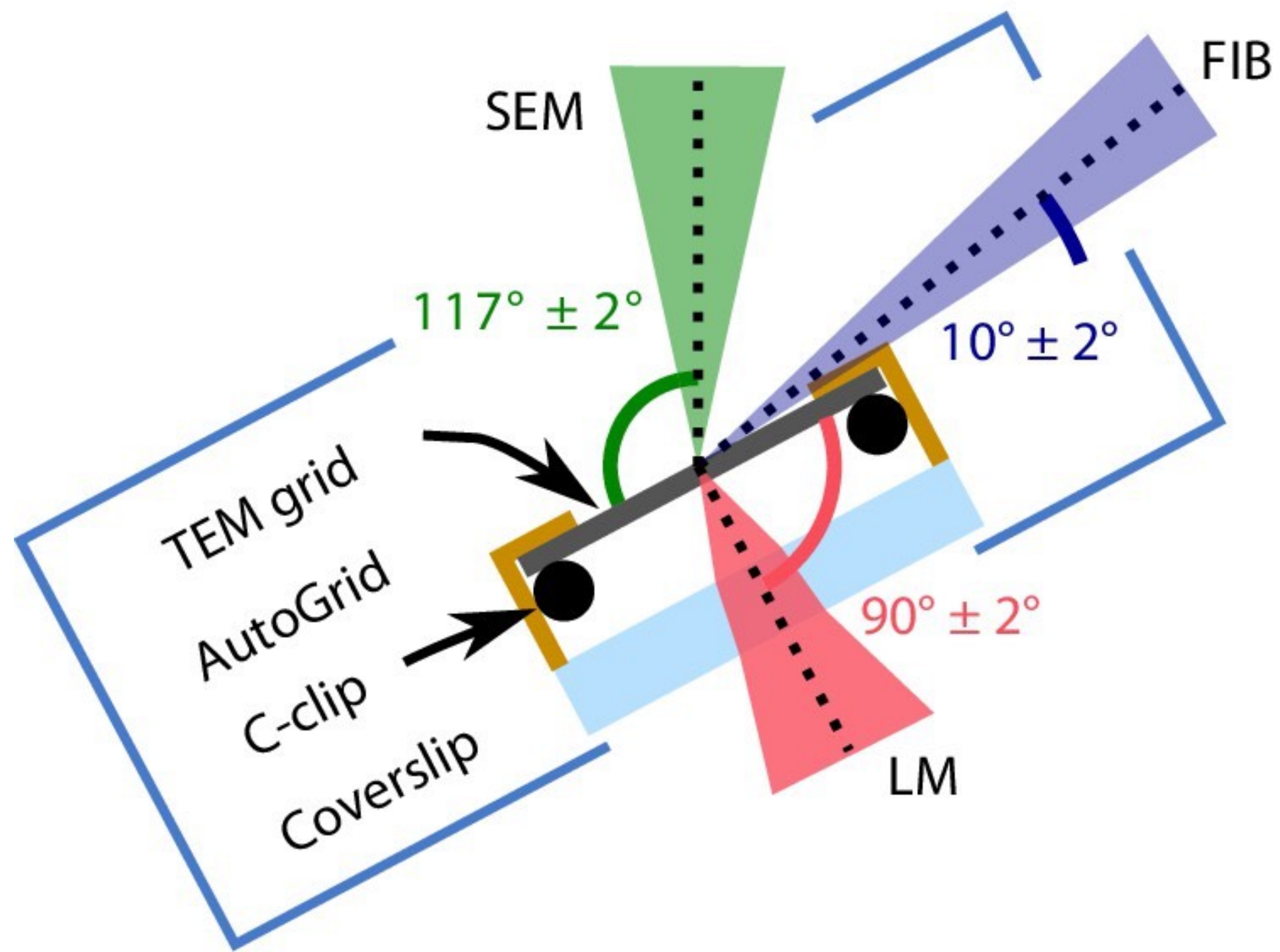


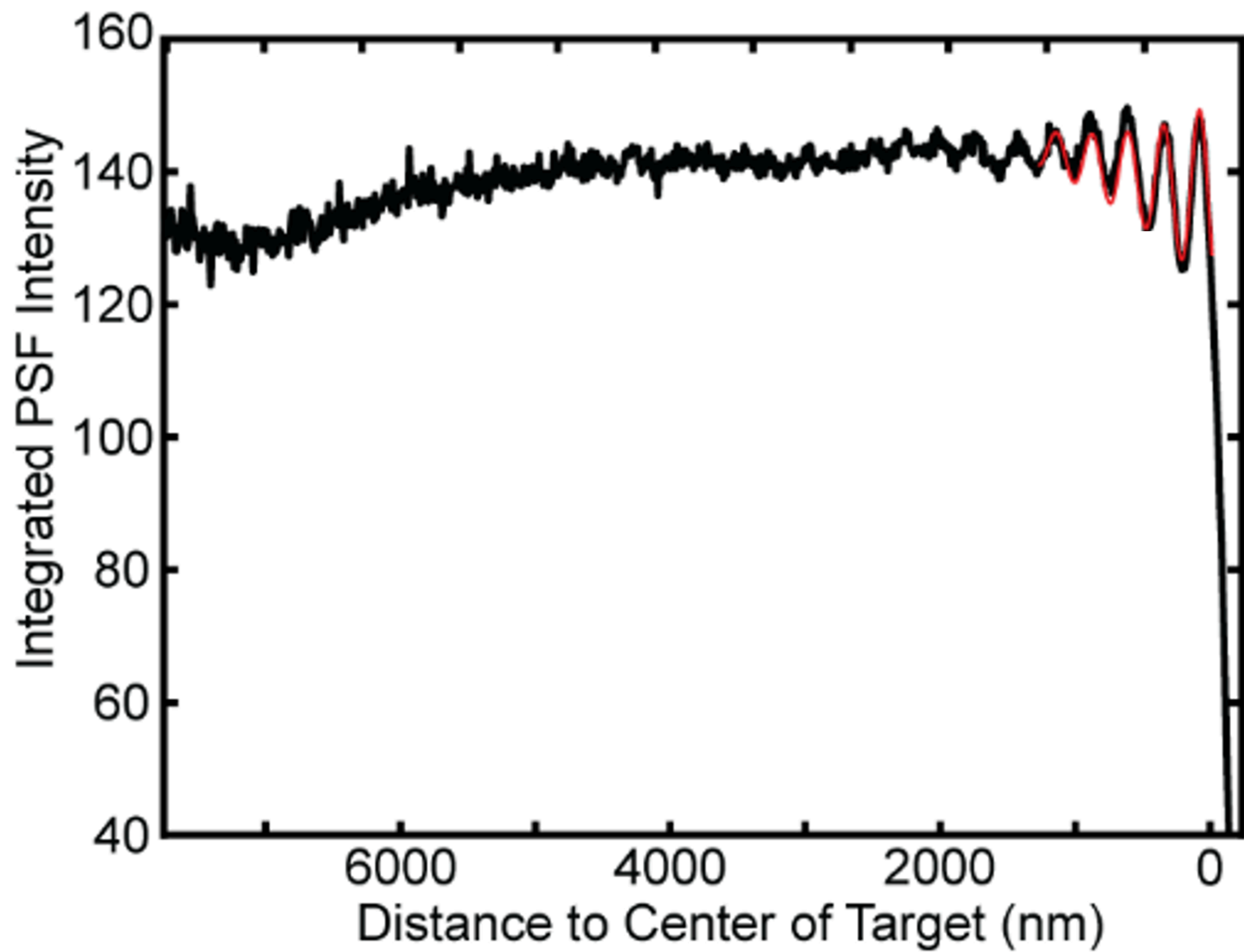
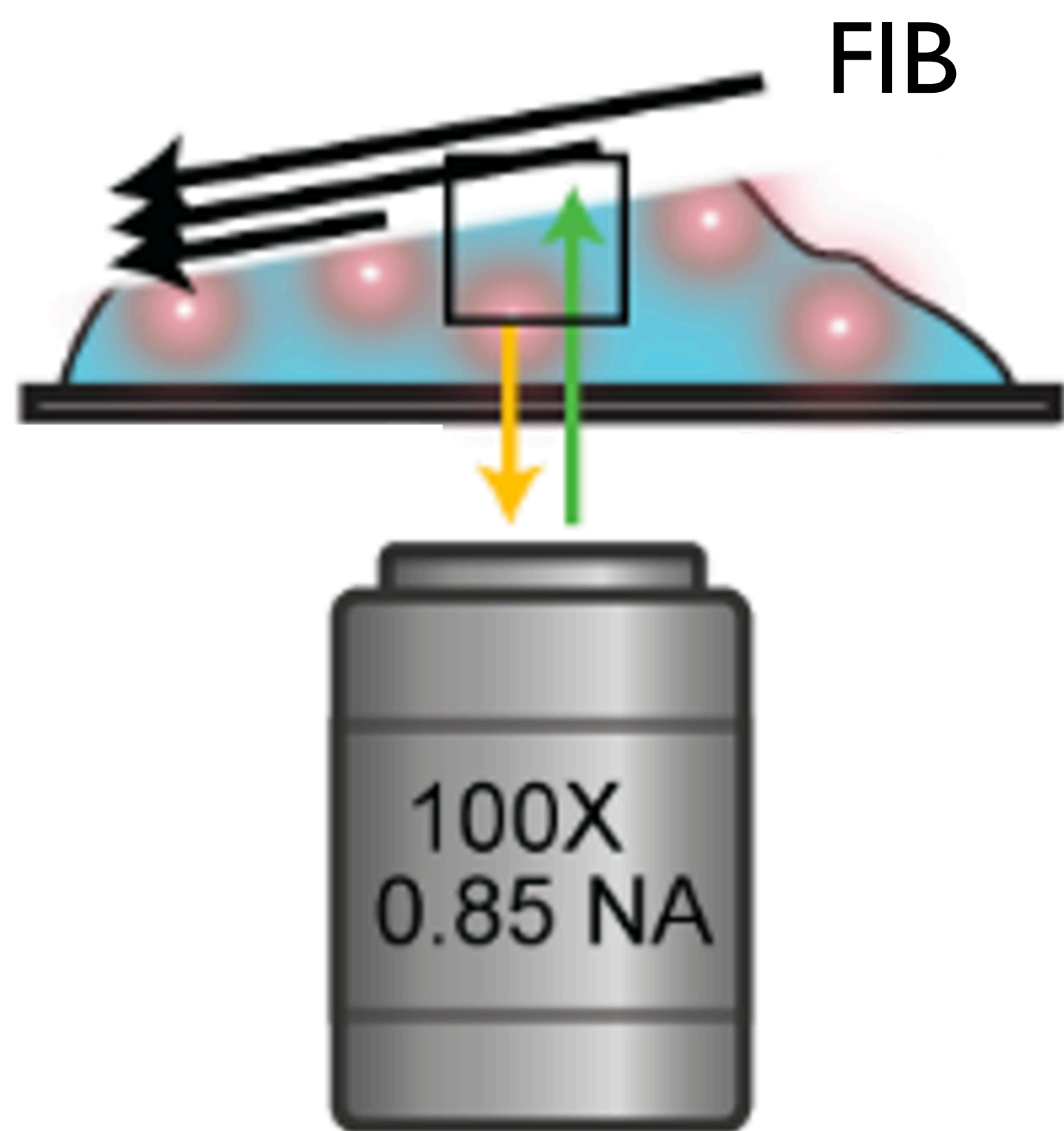


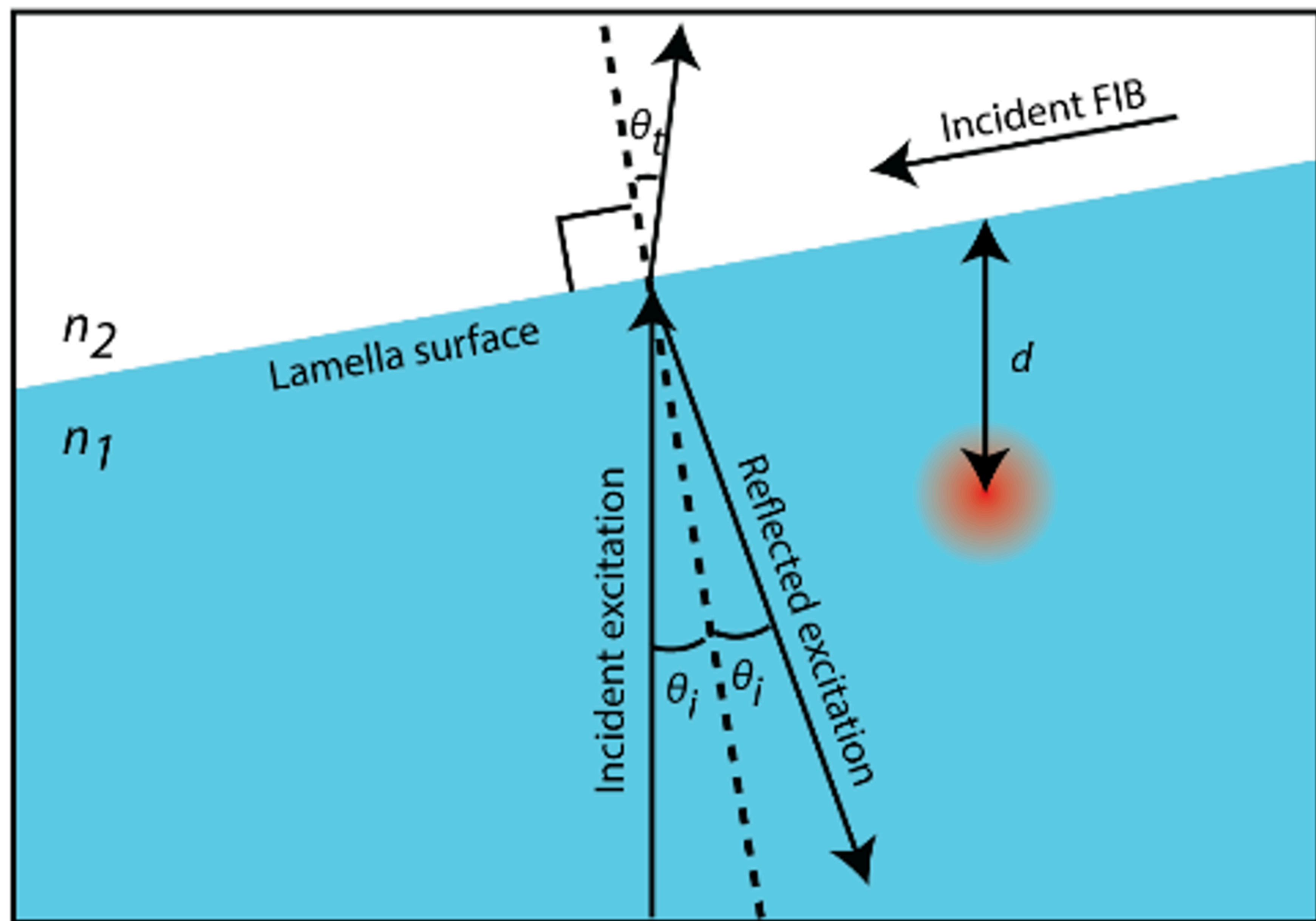
It's easy enough to target
milling in x and y,
but z is hard

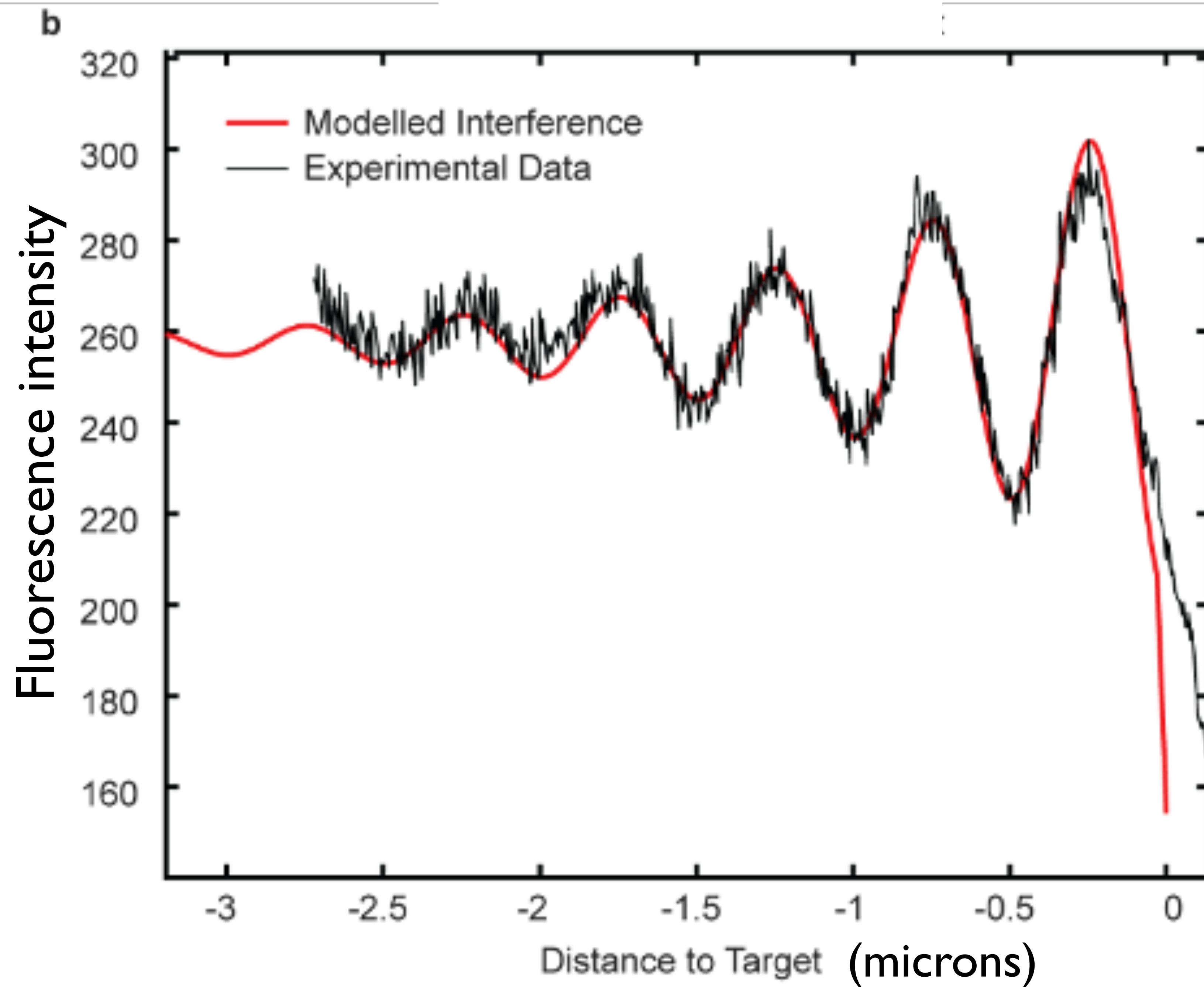
ENZEL

Cryo-FIB stage









Strategy:
Mill from top until close
Finish milling from below



Peter Dahlberg
Stanford

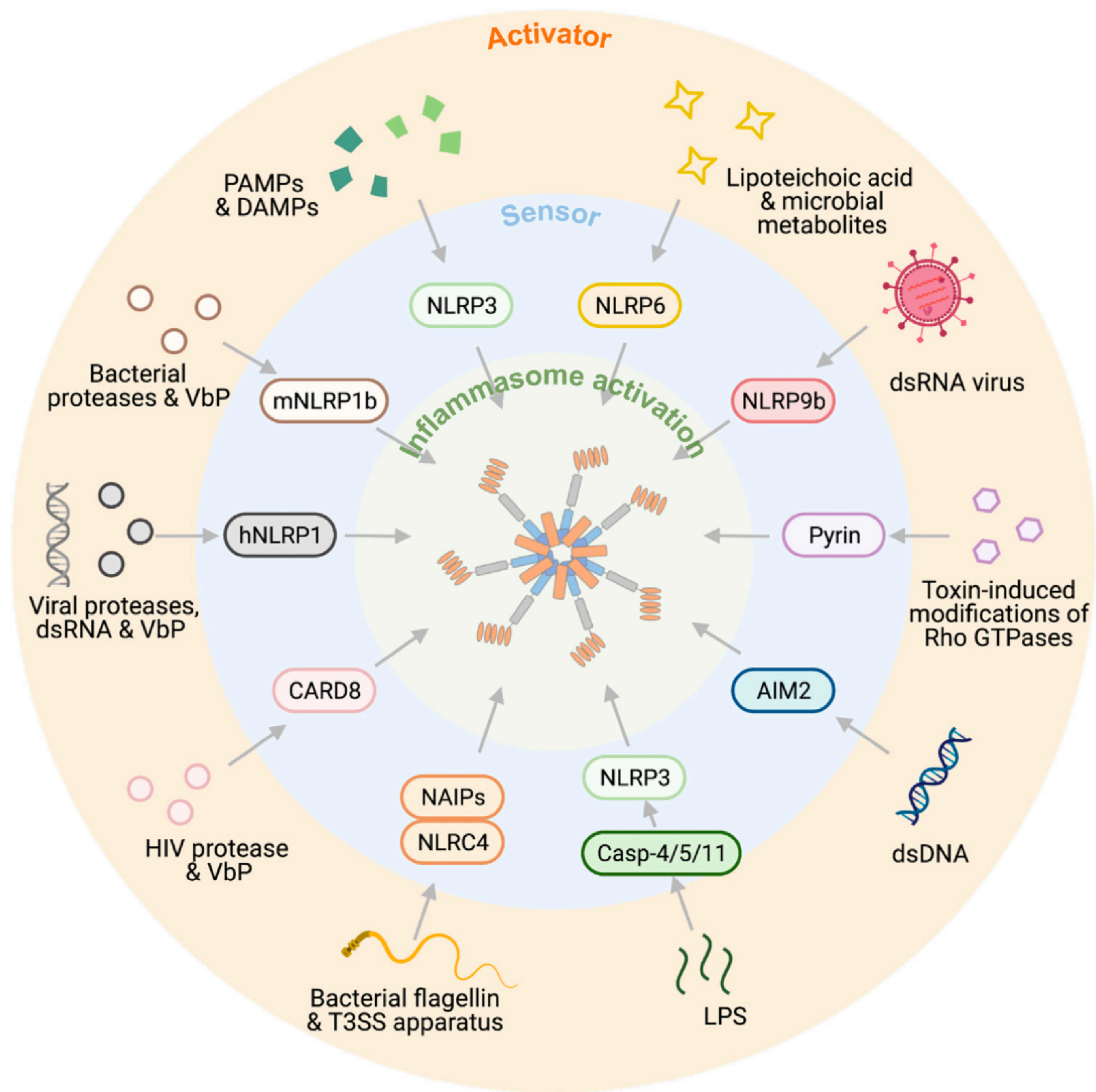


Phyllis Wang
Caltech/BYU/Stanford



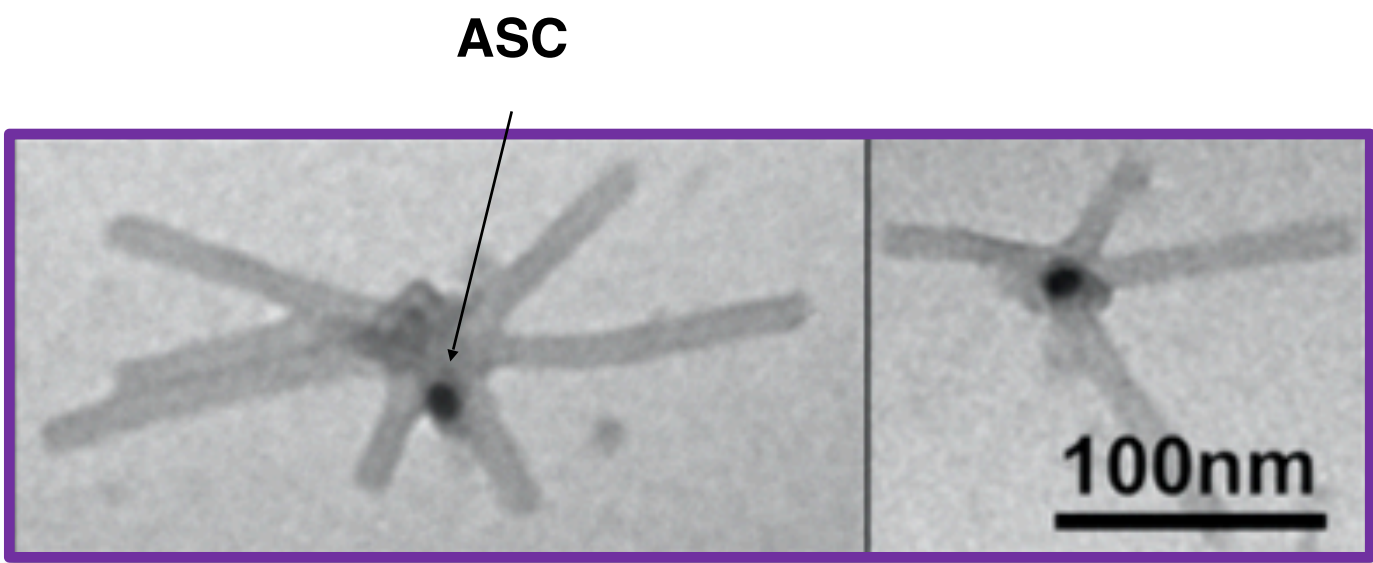
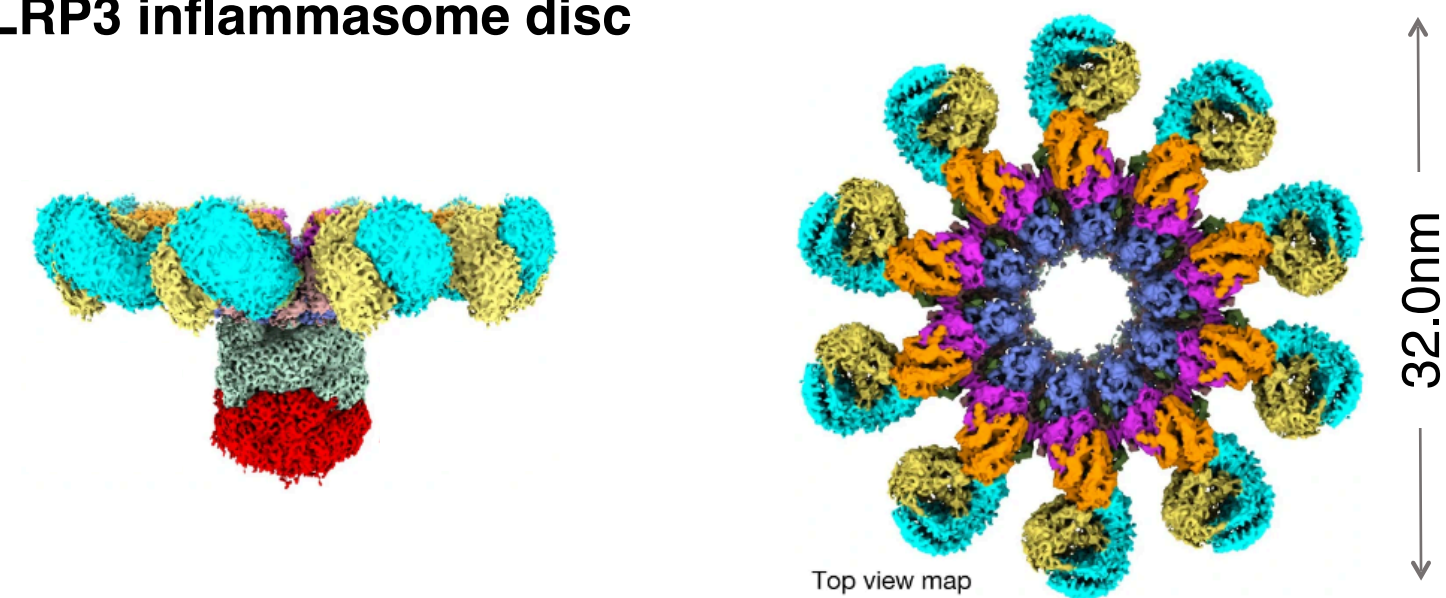
Anthony Sica
Stanford

NLRP3 inflammasome

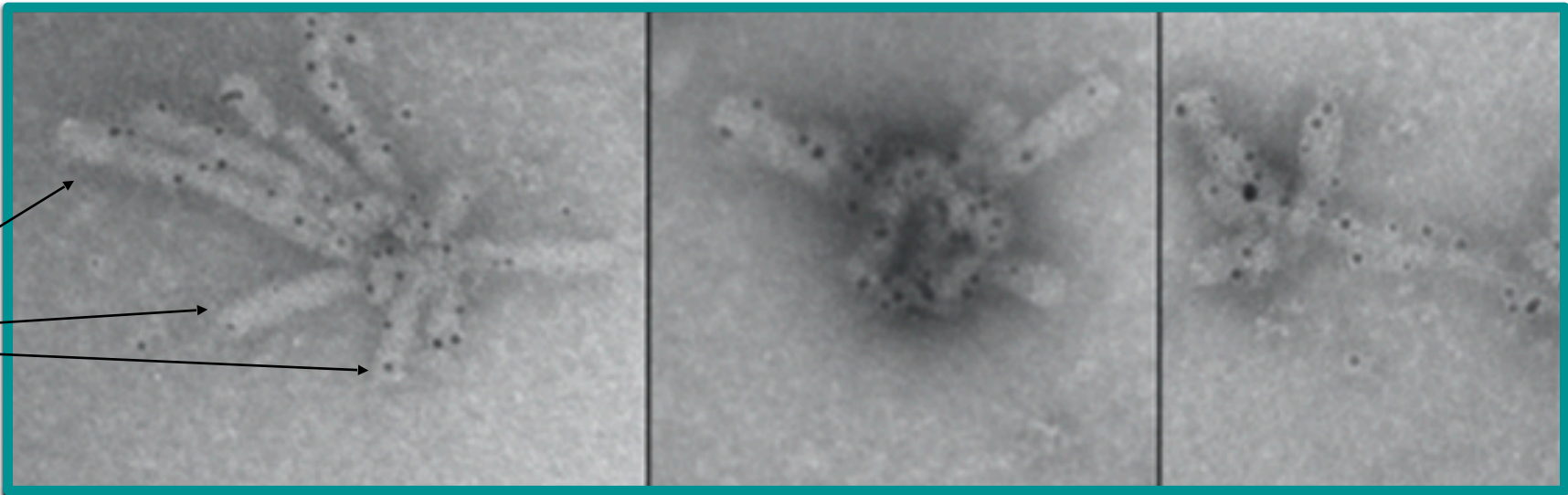


Structures of the NLRP3 inflammasome

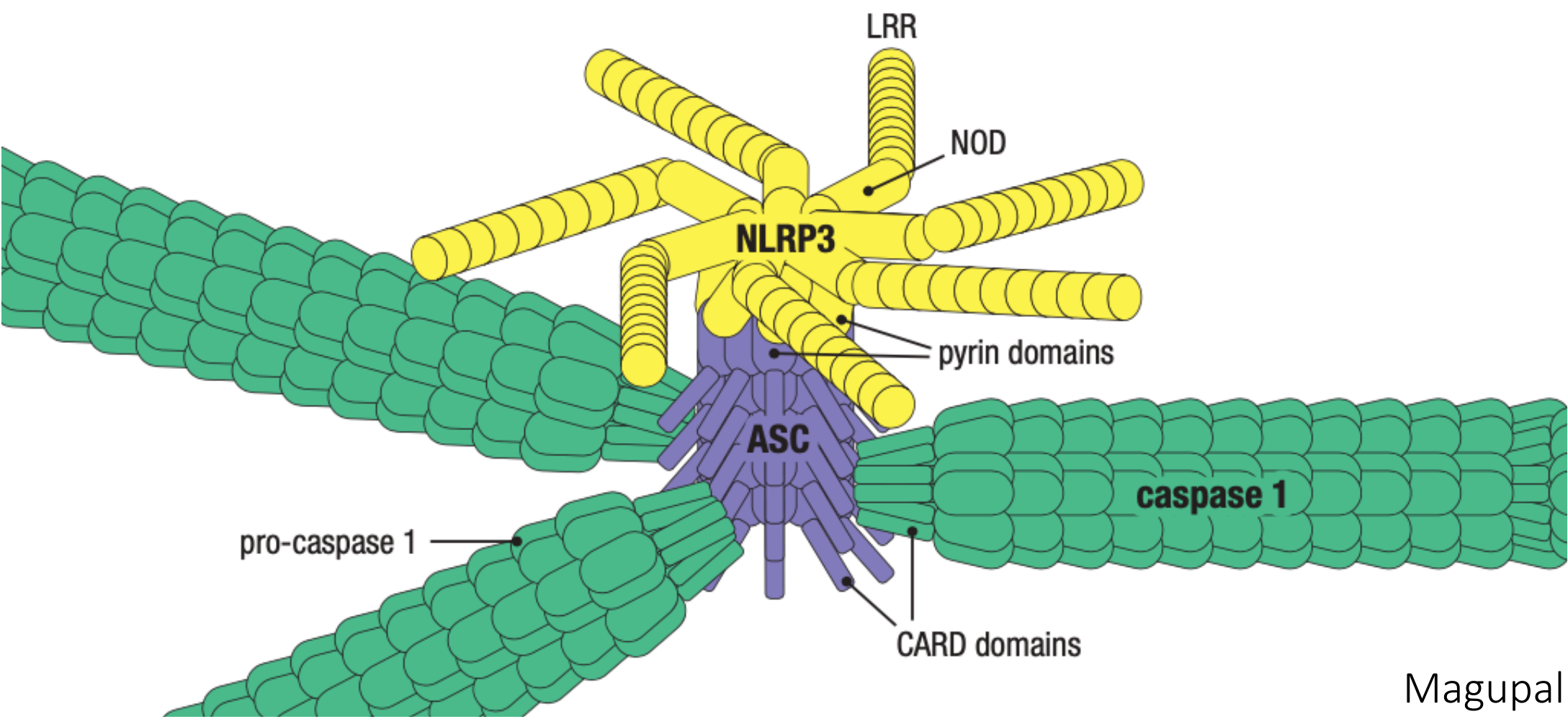
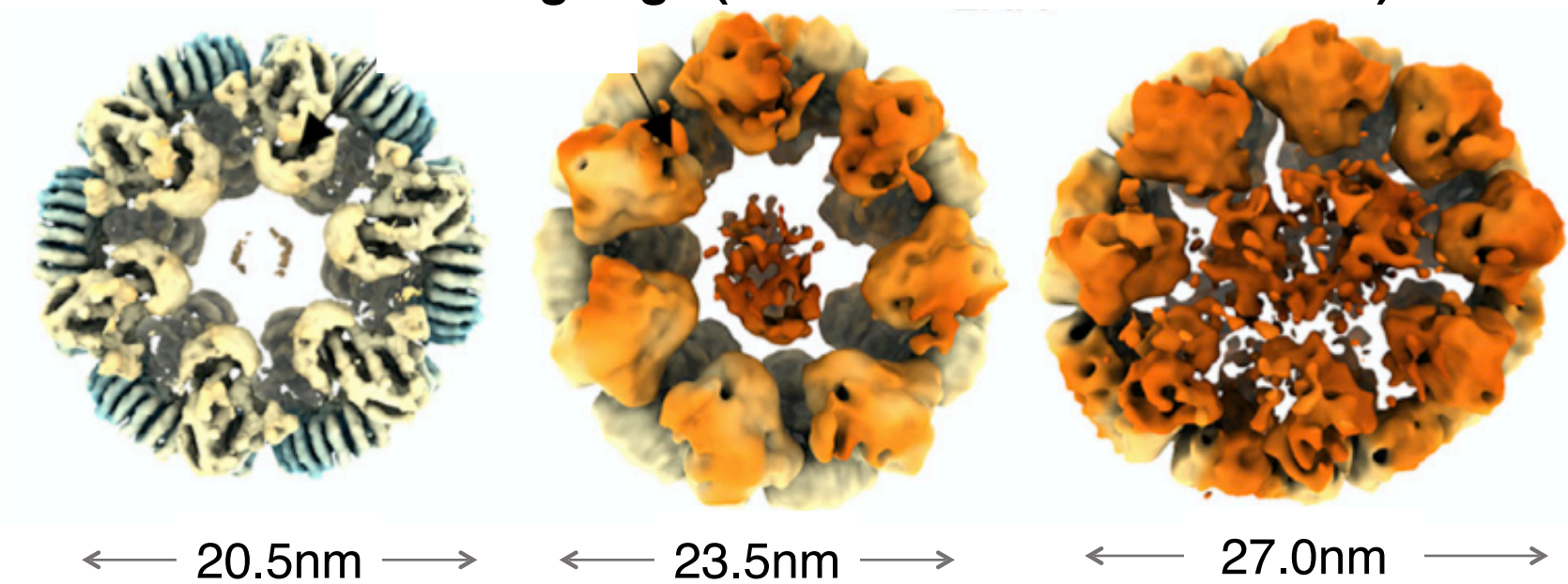
NLRP3 inflammasome disc



Pro-Caspase-1



NLRP3 double-ring cage (autoinhibited conformation)



Magupalli, V.G. et al., *Science*. 2020
Lu, A. et al. *Cell*. 2014
Zhang, L. et al. *Science*. 2015
Andreeva, L. et al. *Cell*. 2021
Xiao, L. et al. *Nature*. 2022

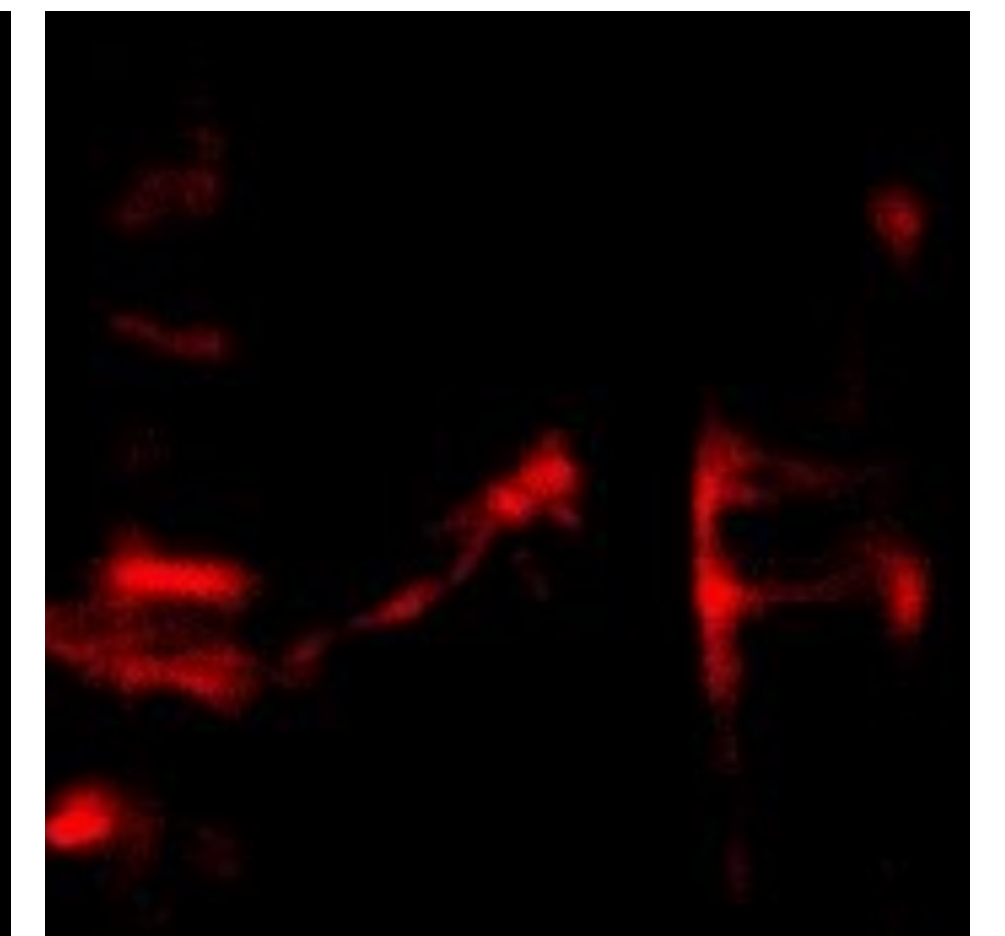
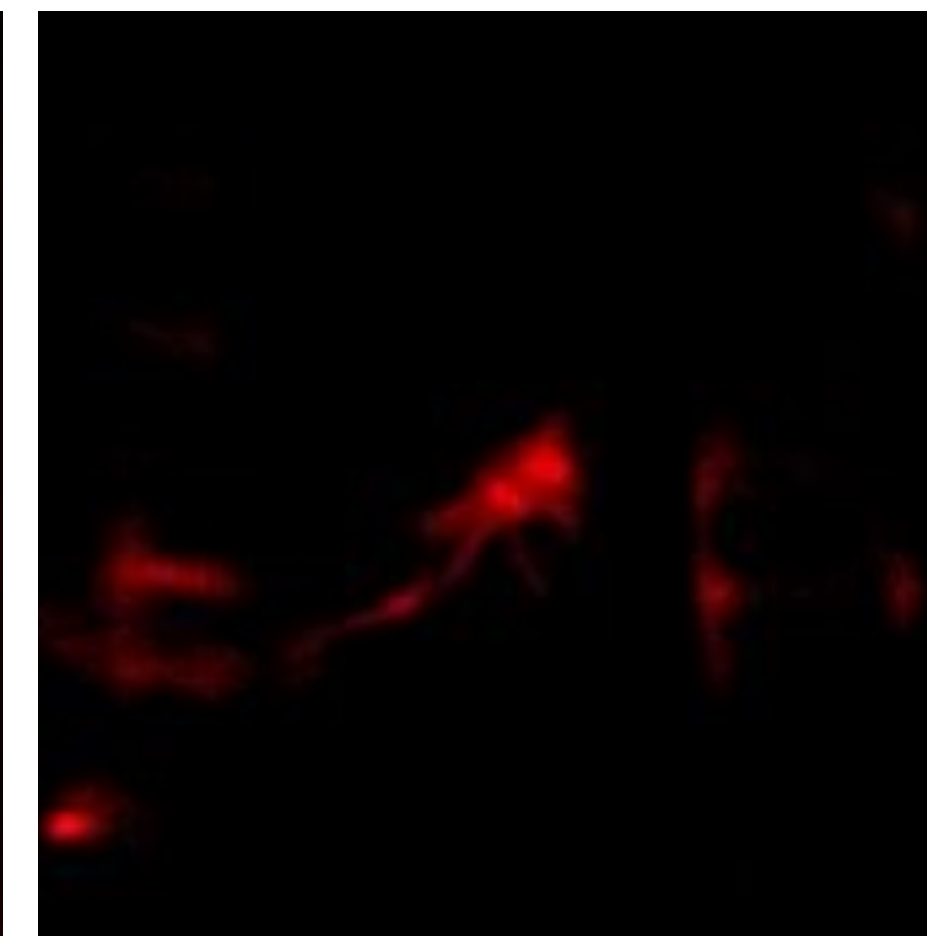
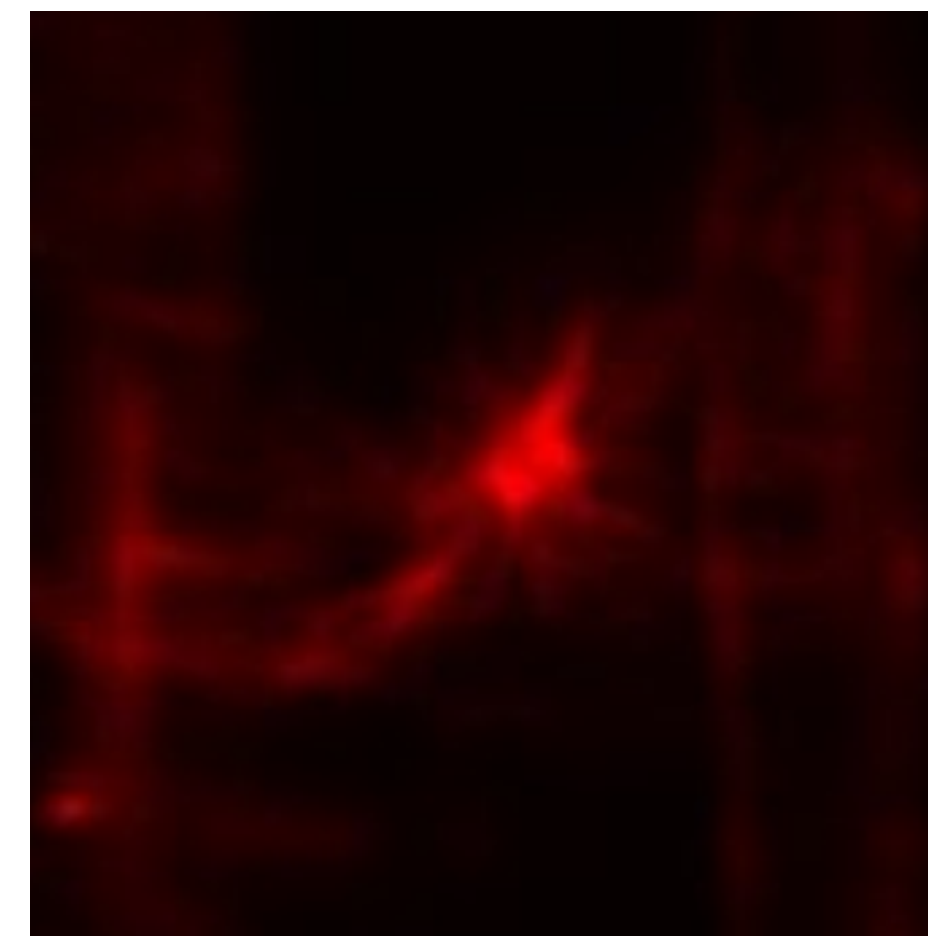
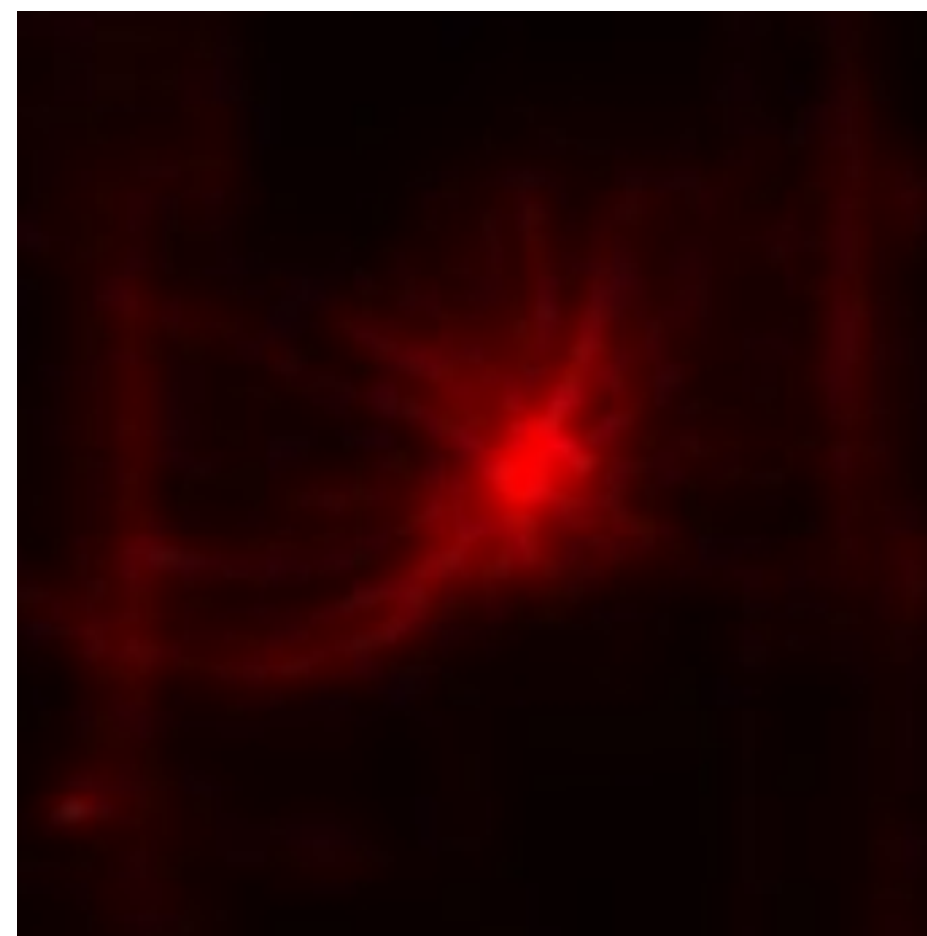
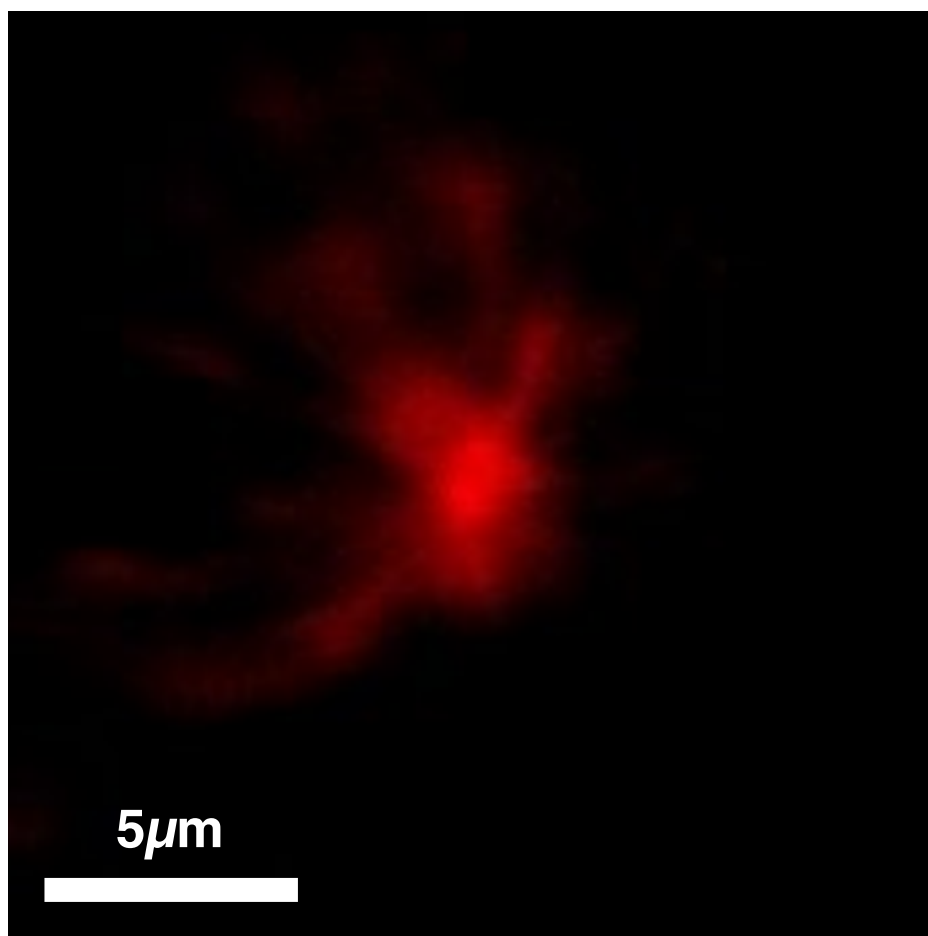
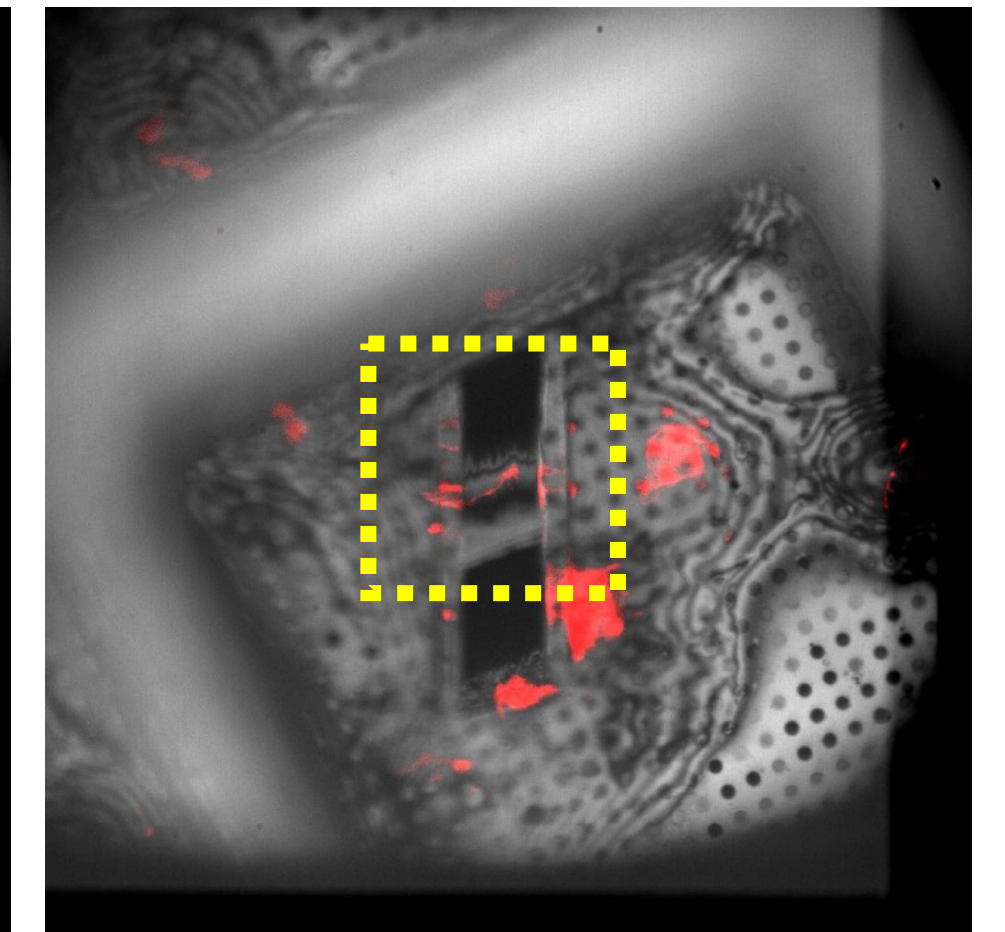
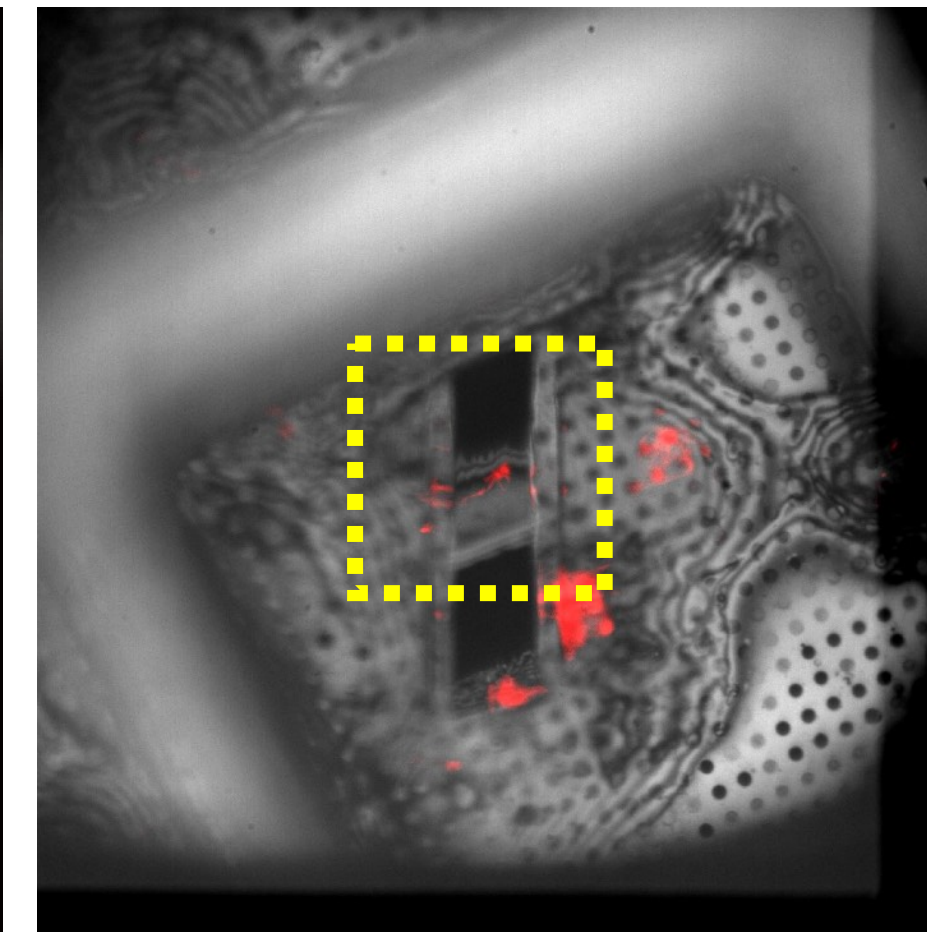
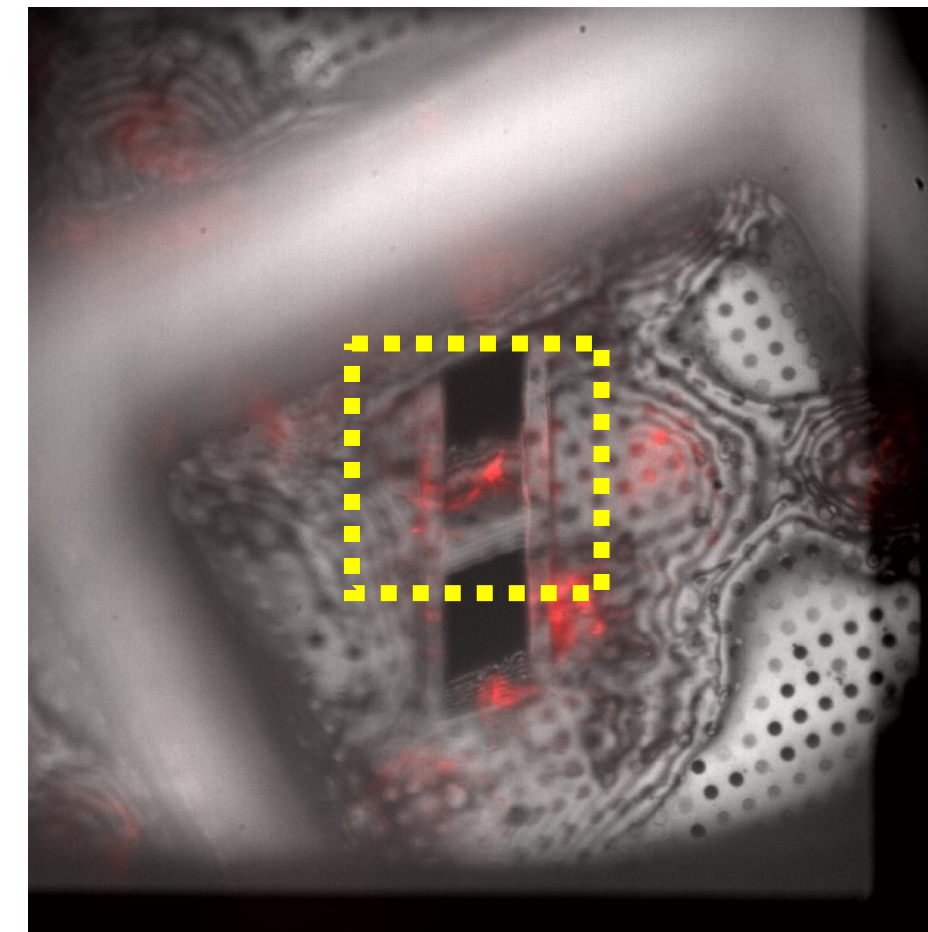
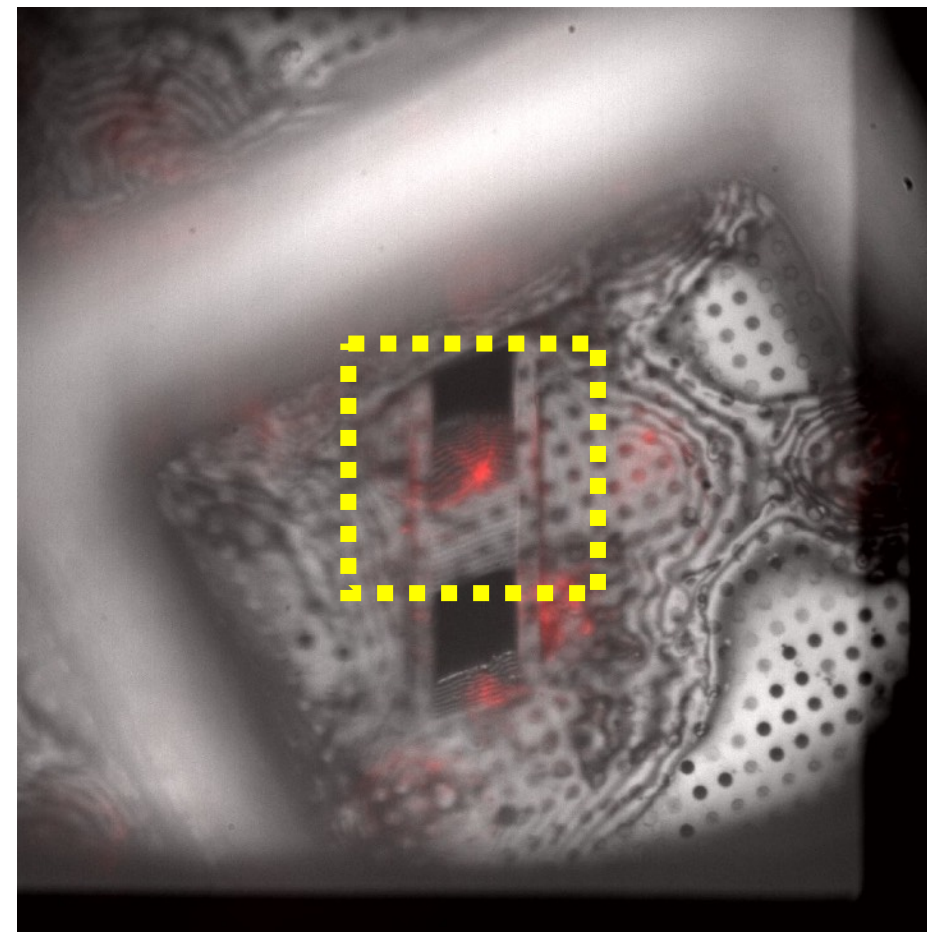
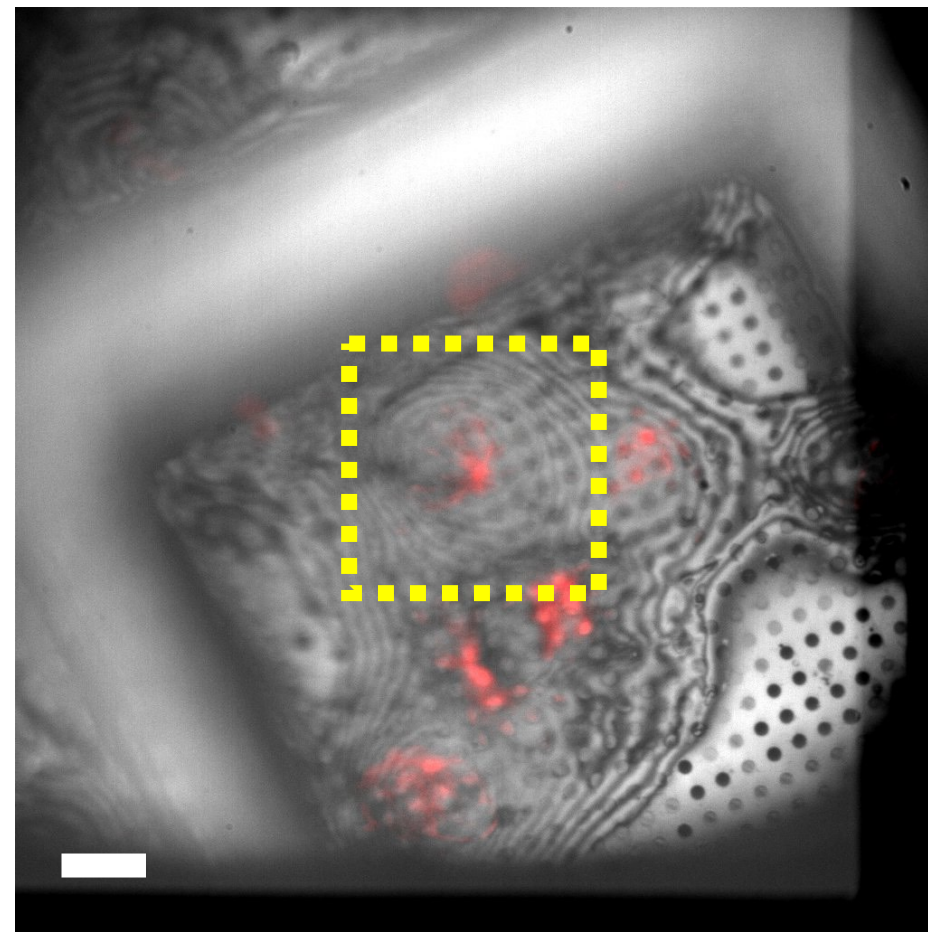
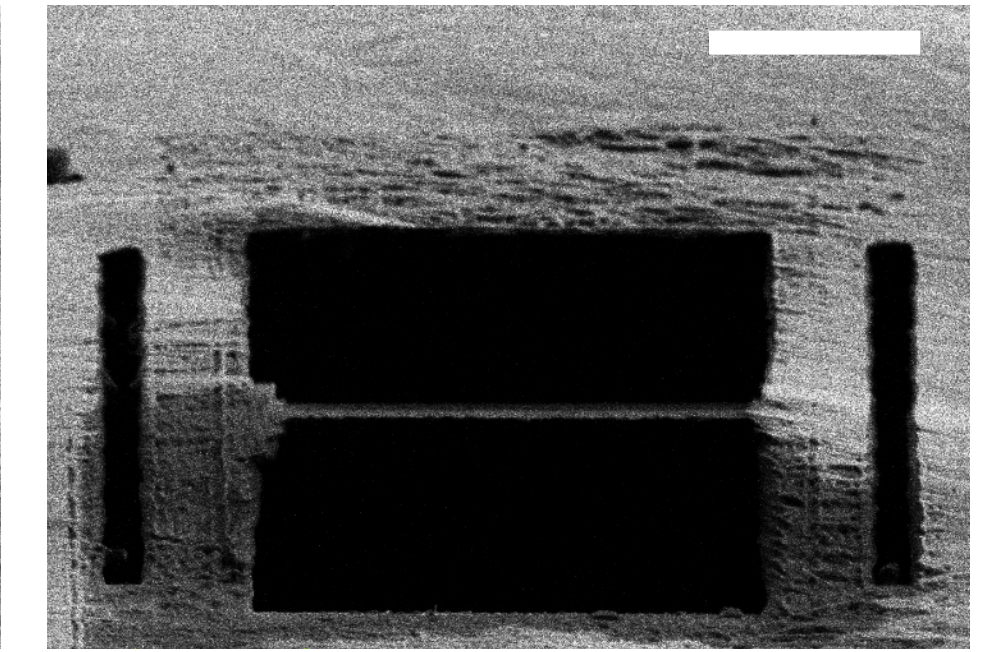
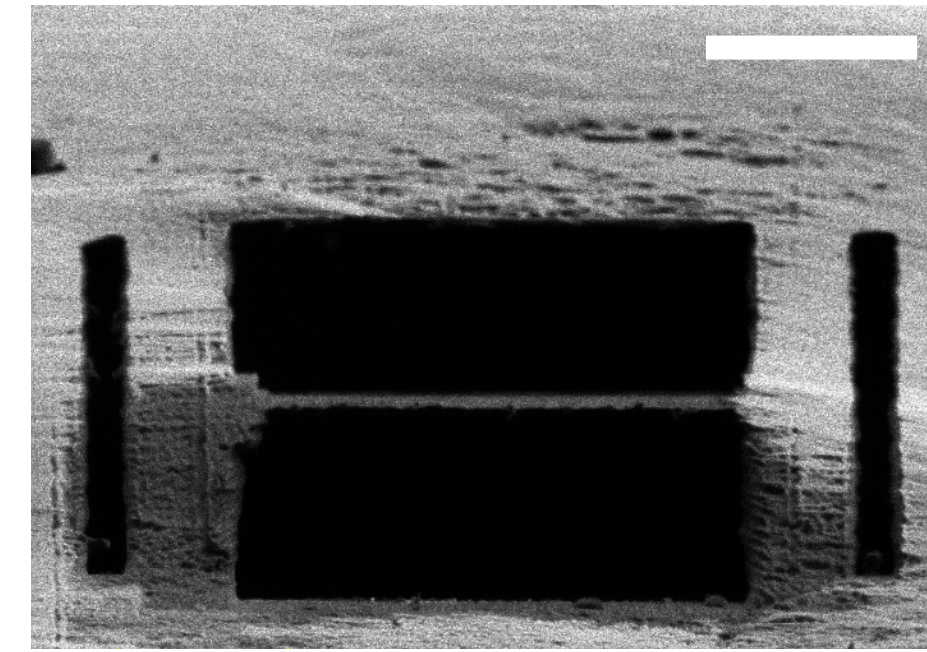
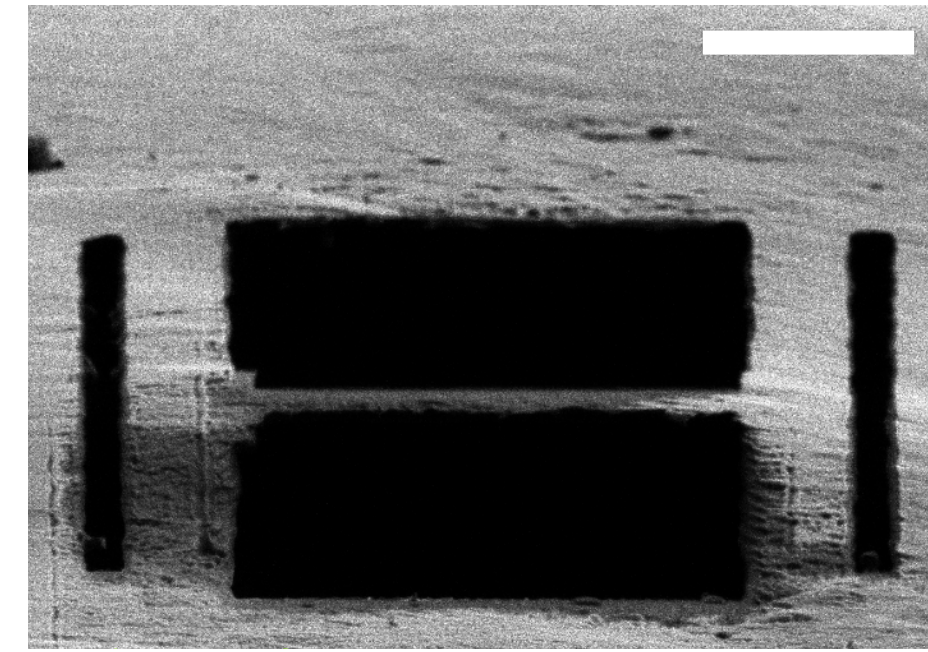
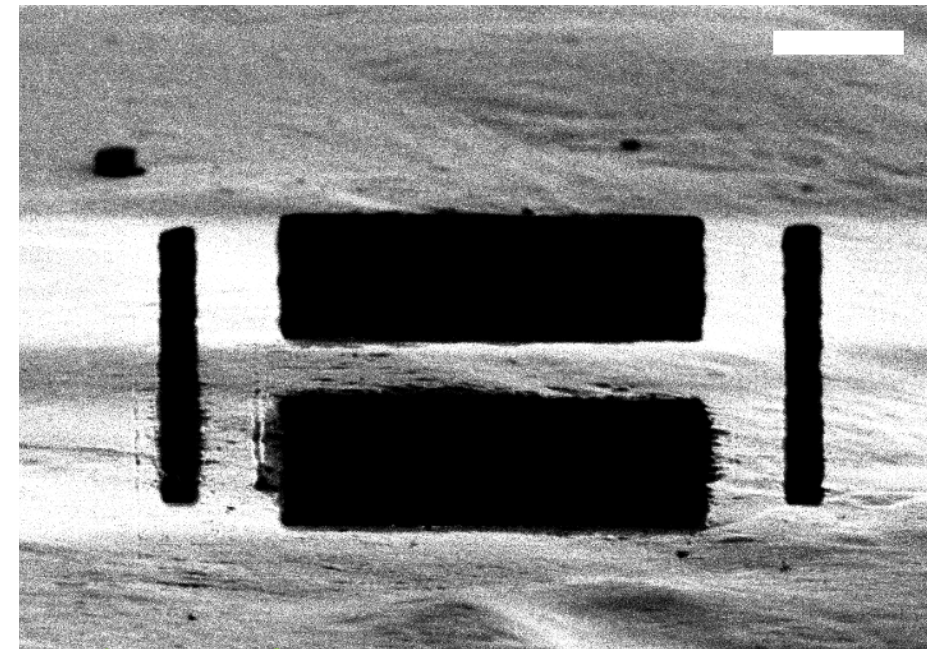
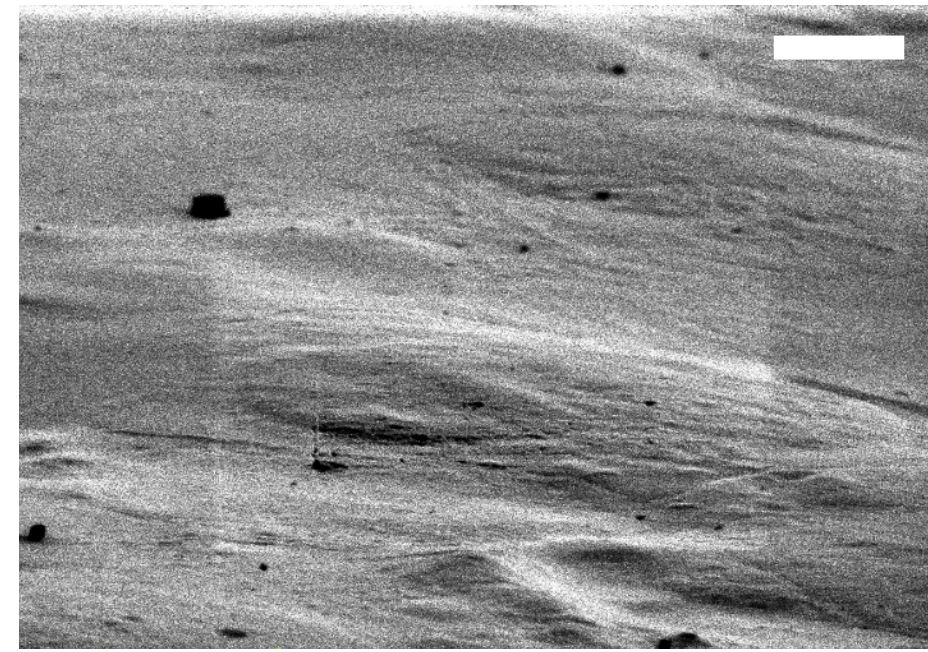
Pre-milling

2 μ m

800nm

500nm

180nm

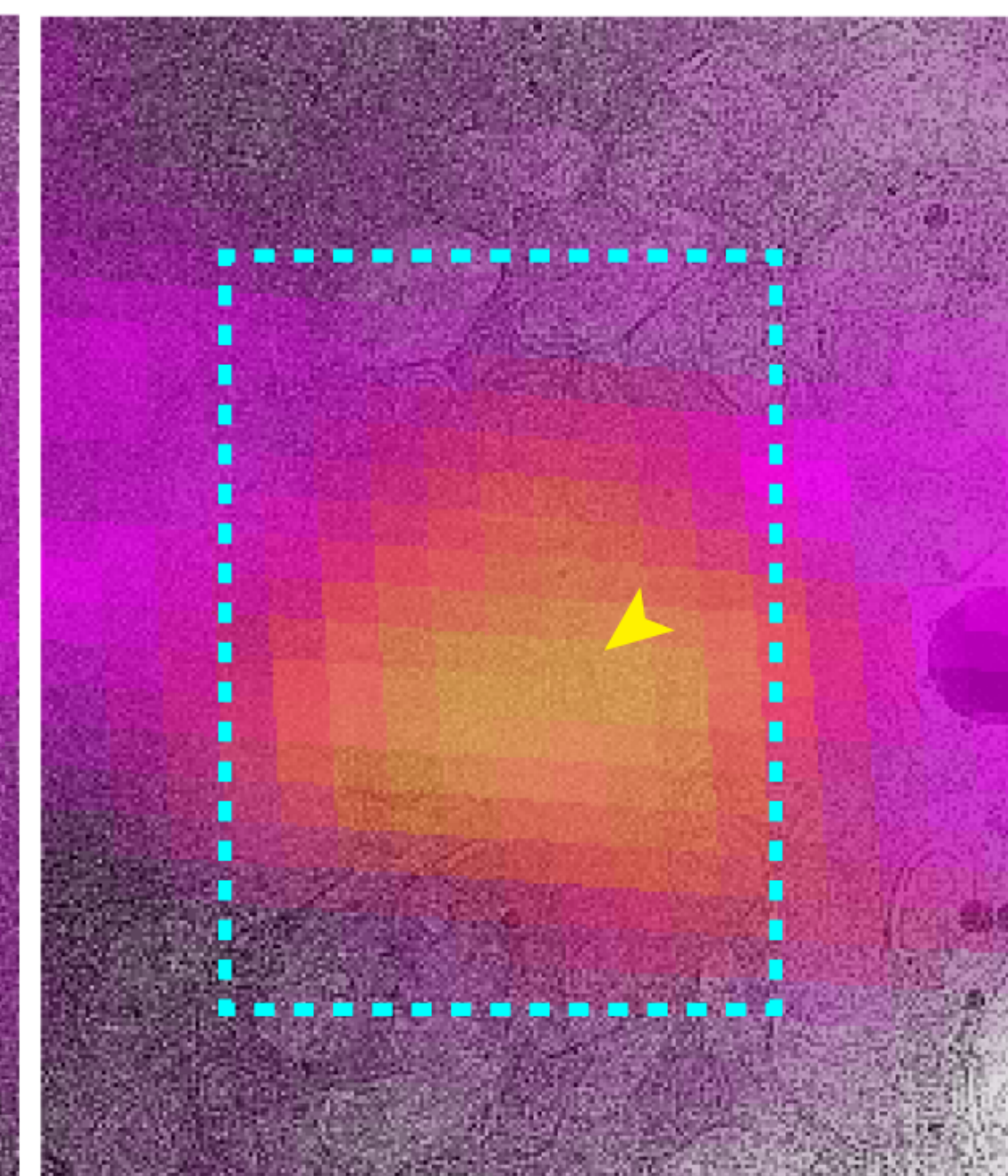
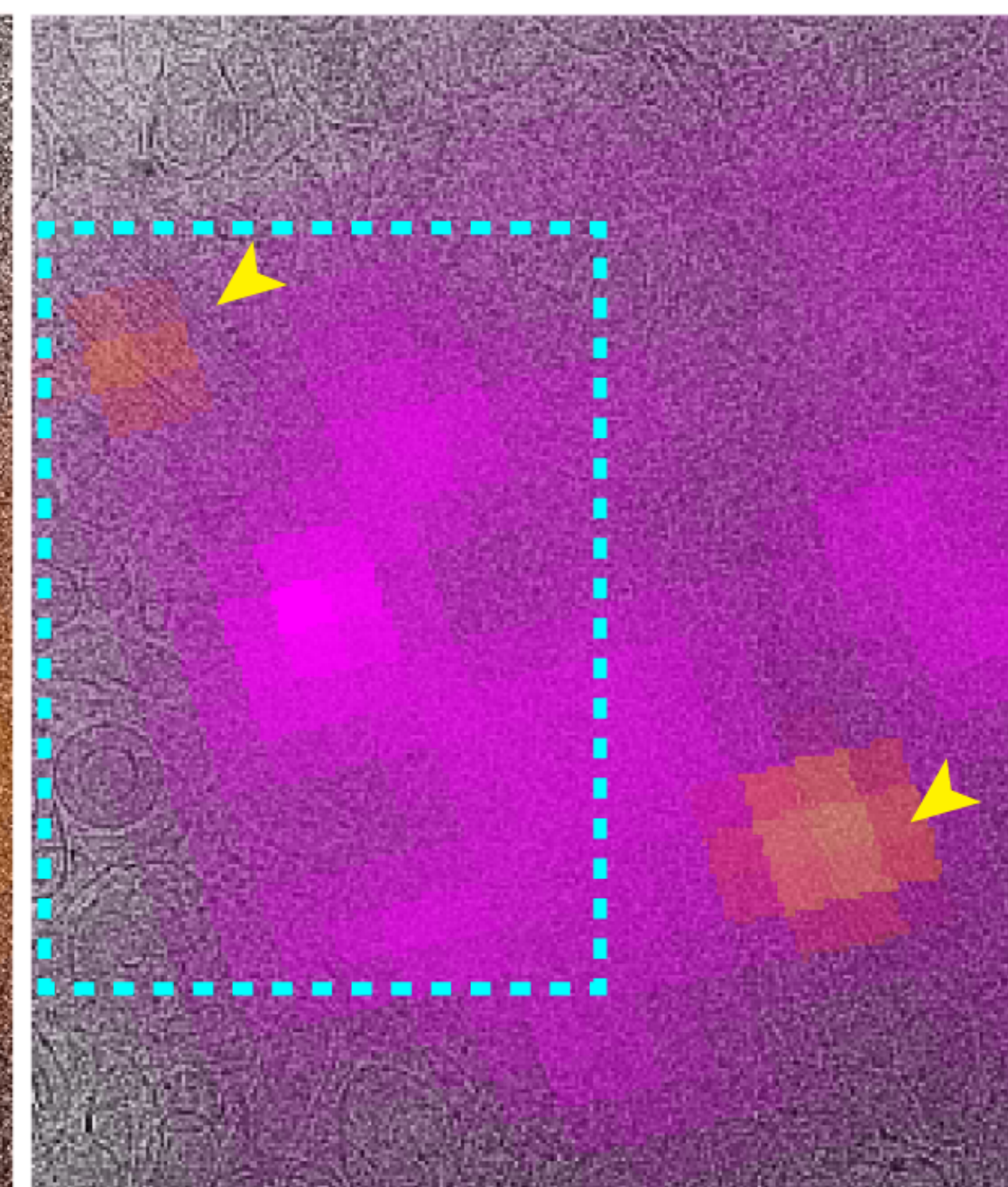
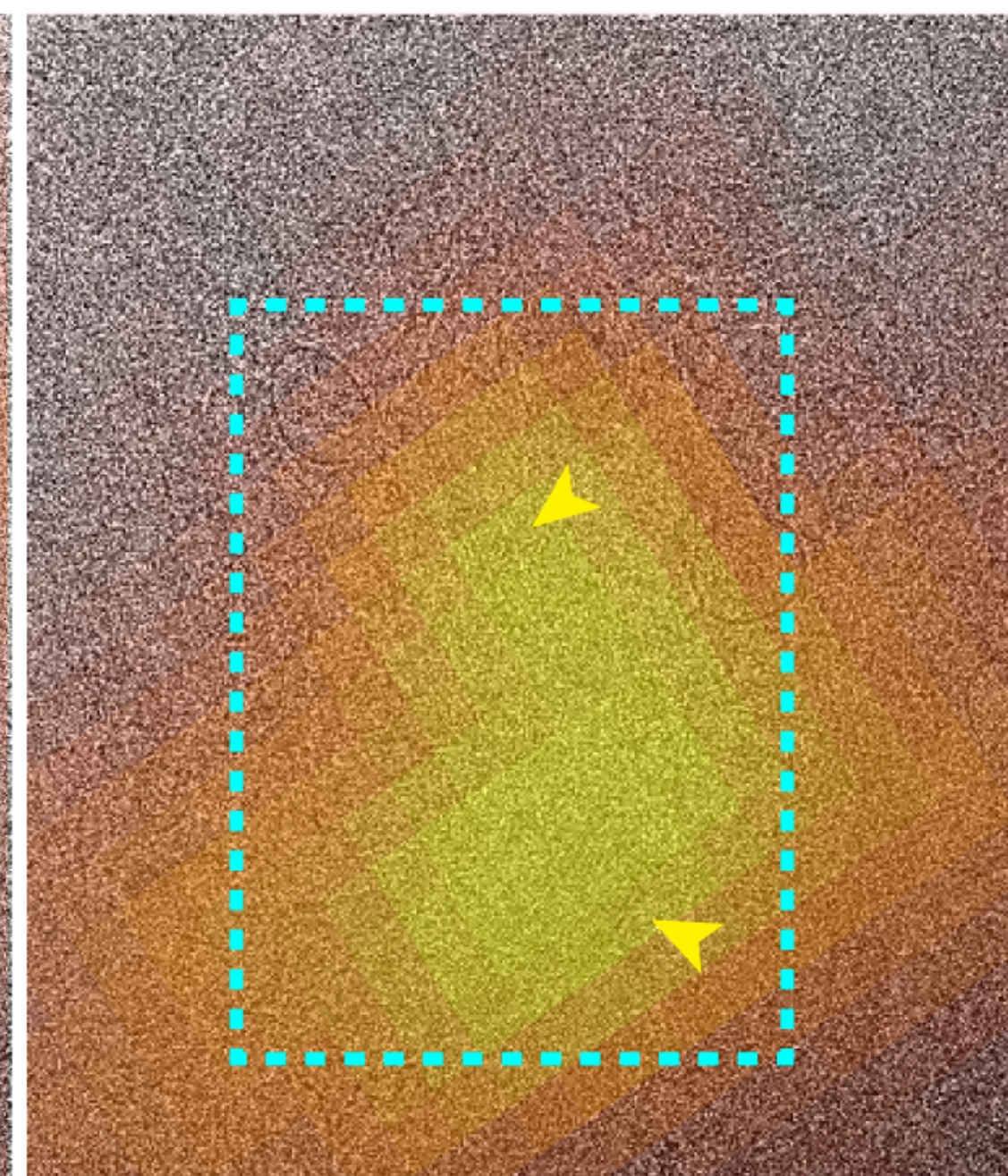
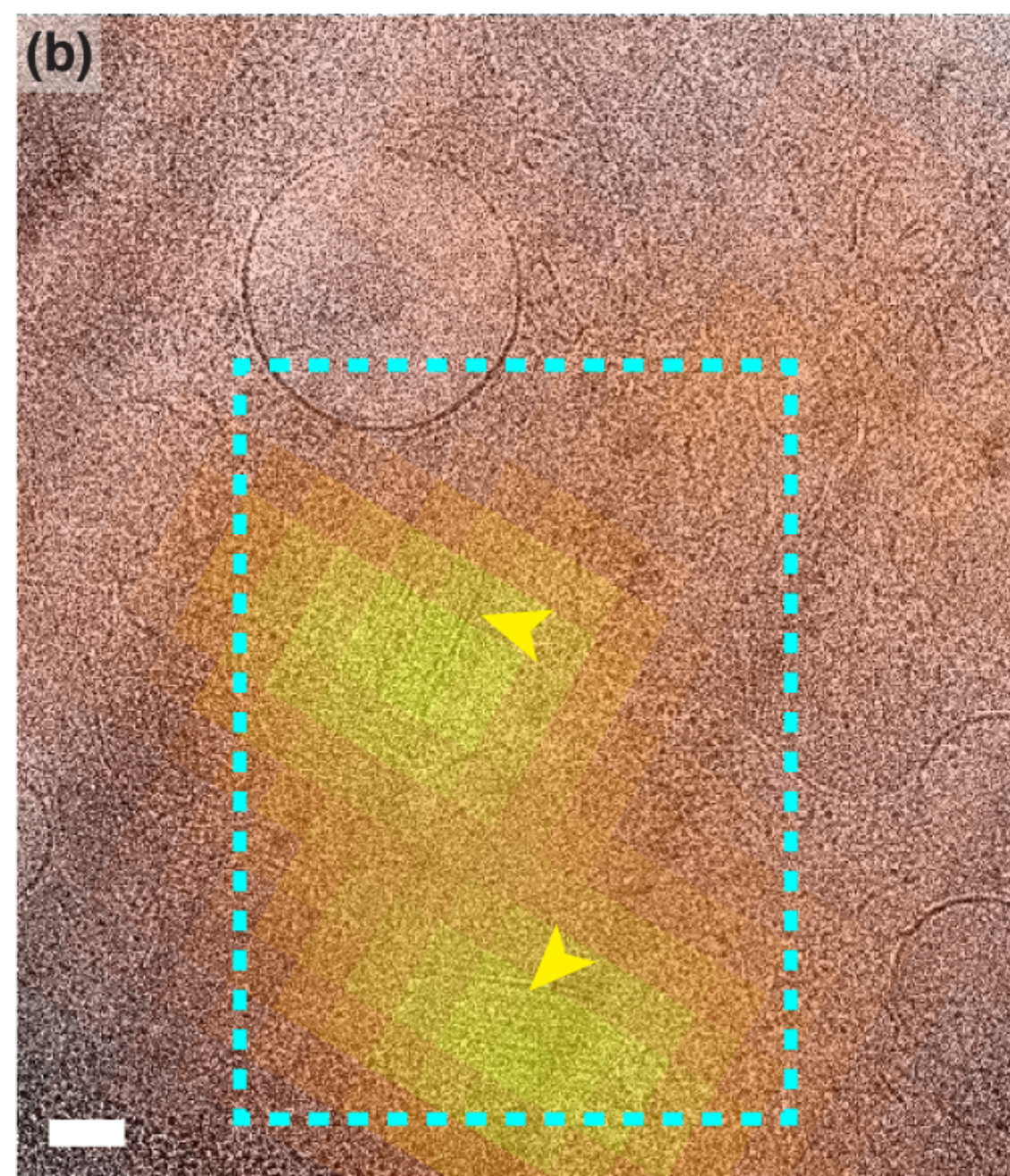
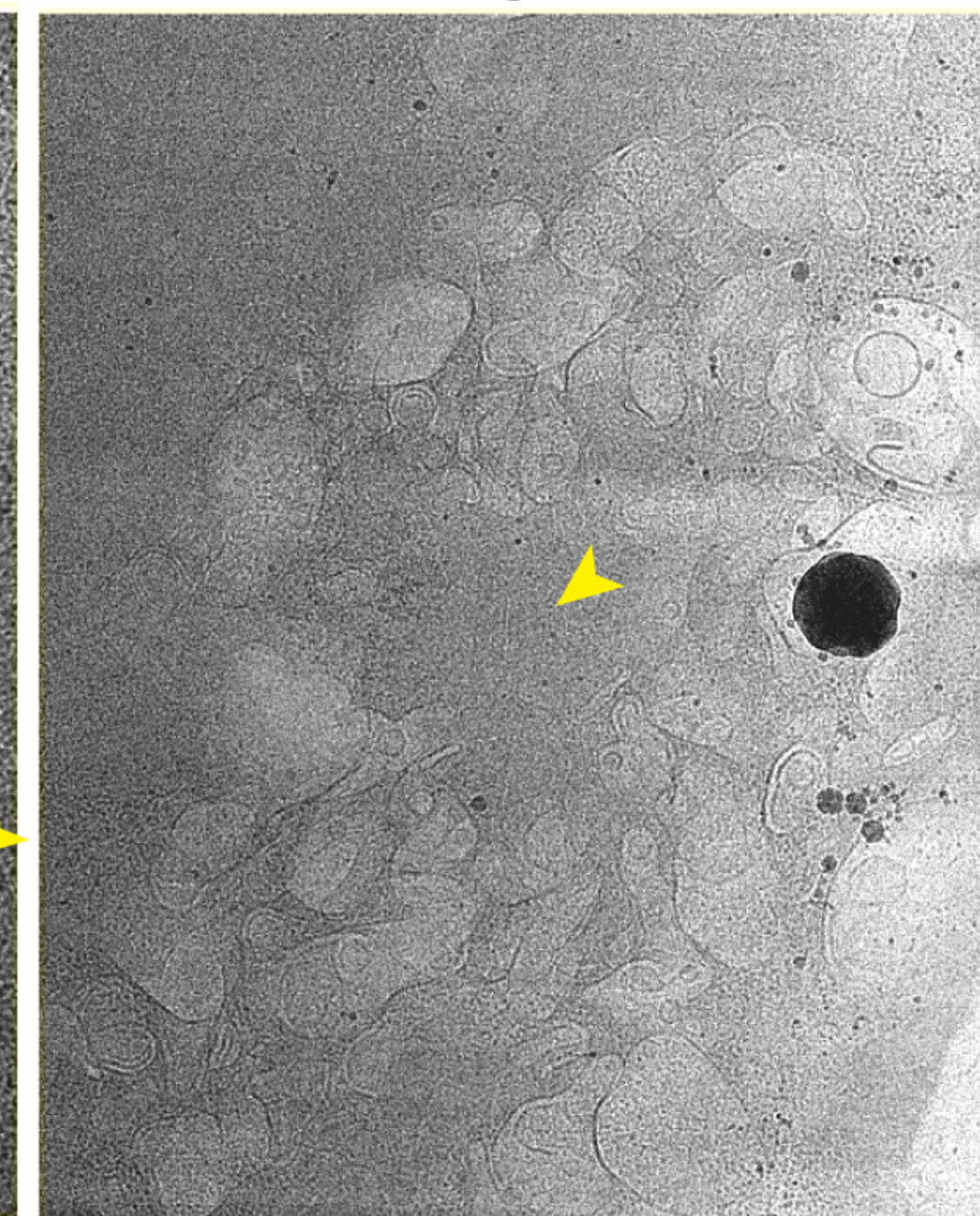
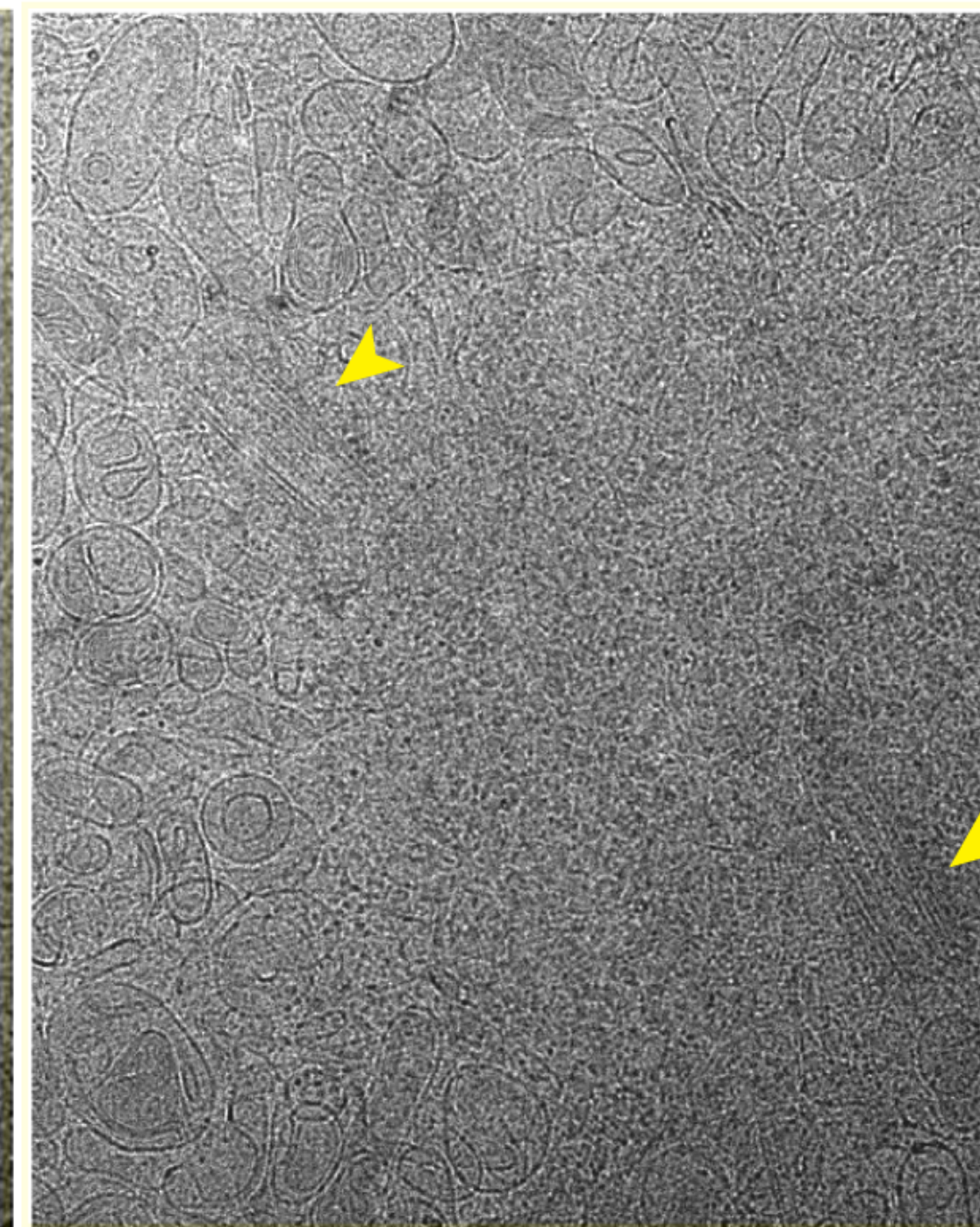
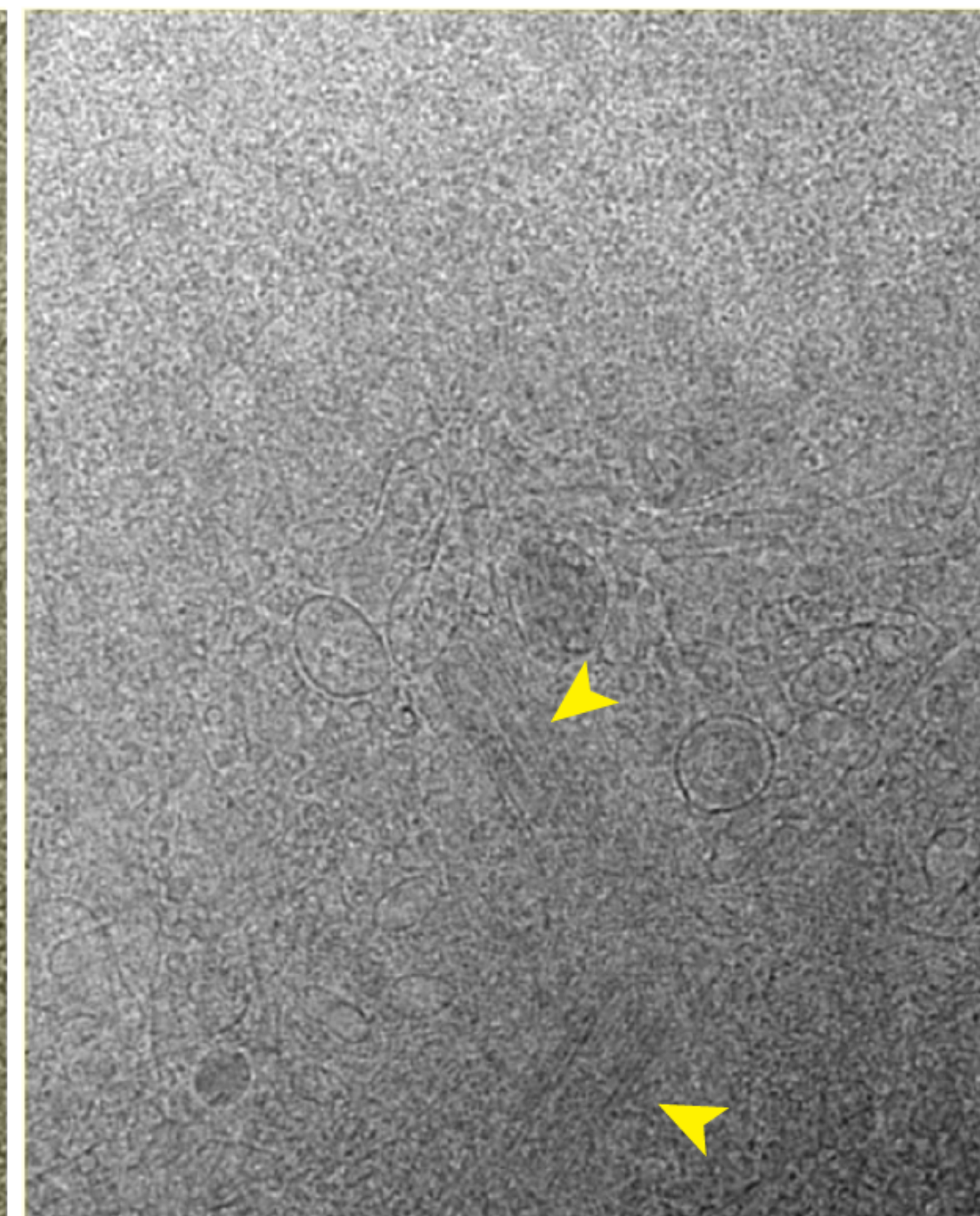
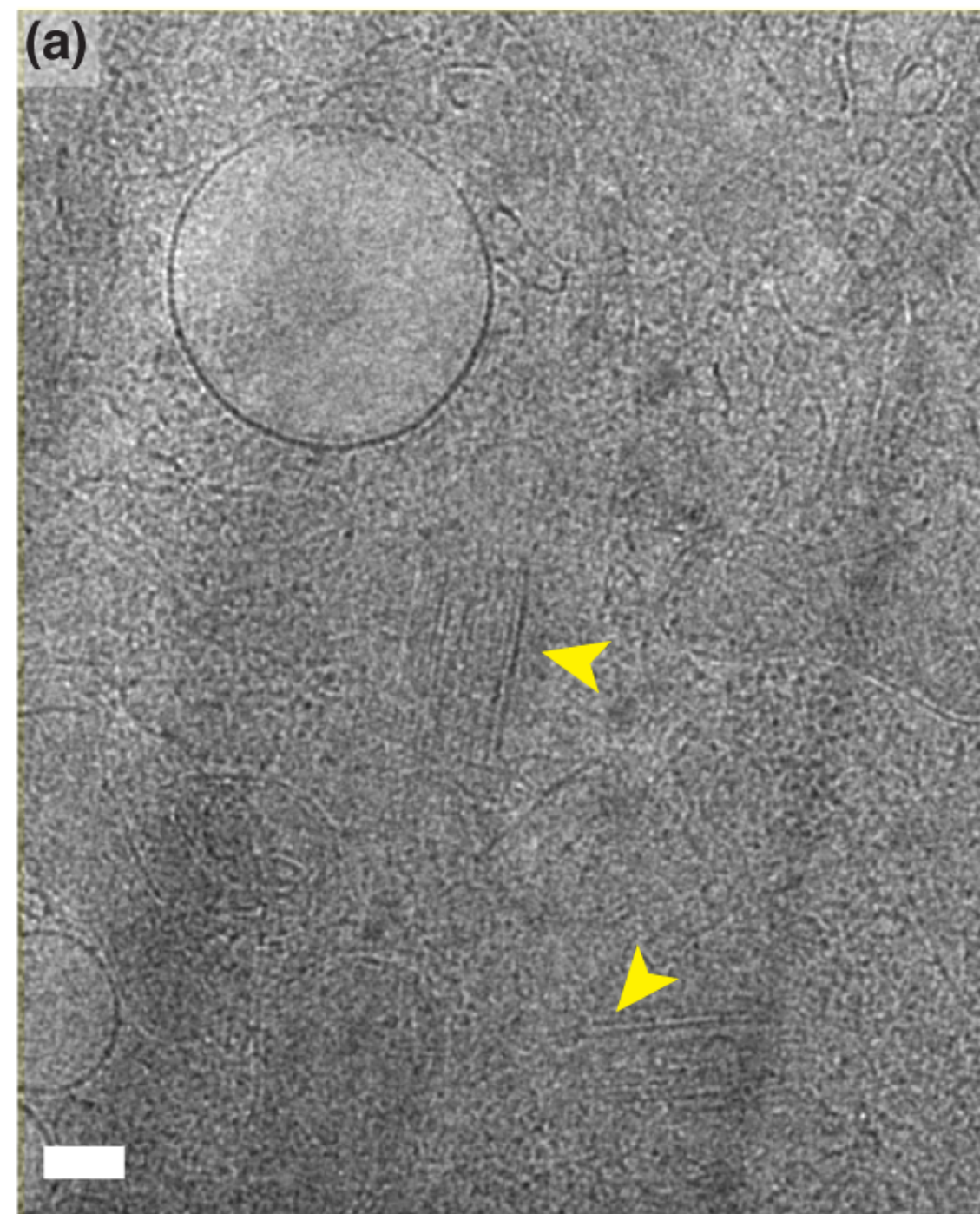
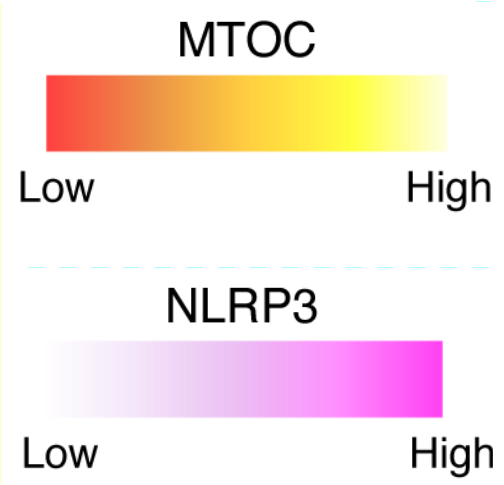


Untreated

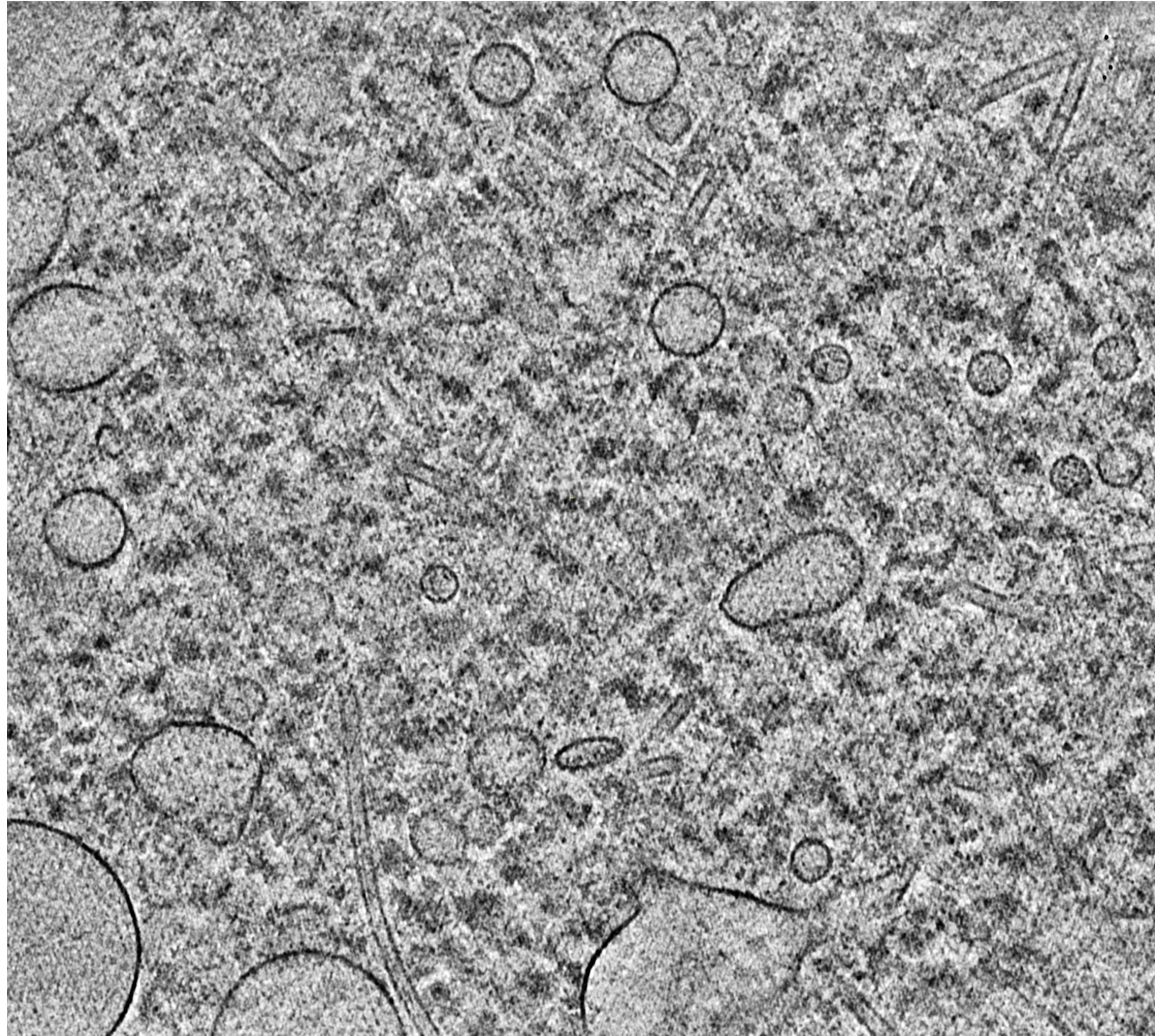
LPS

LPS+Nig 10 min

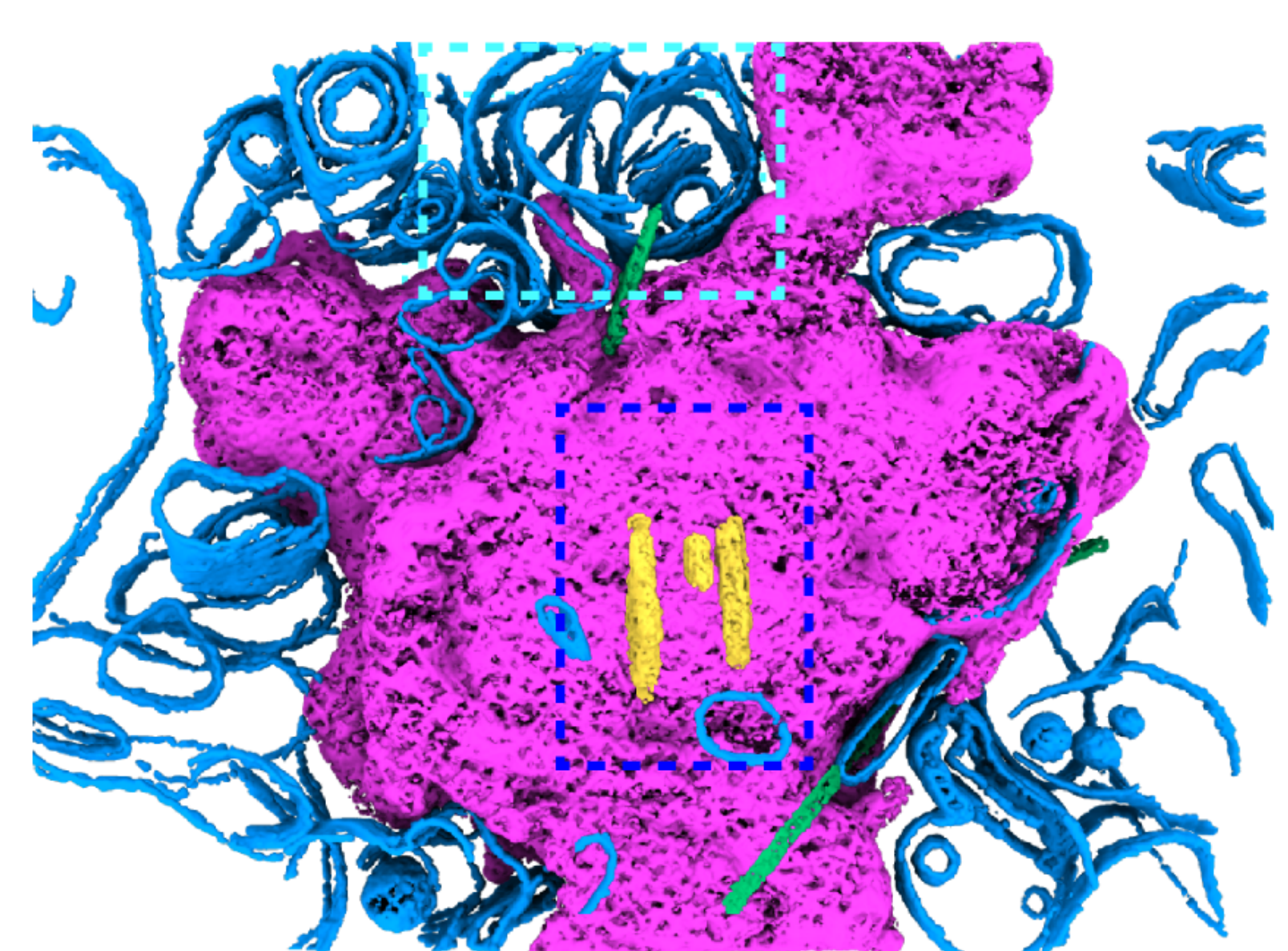
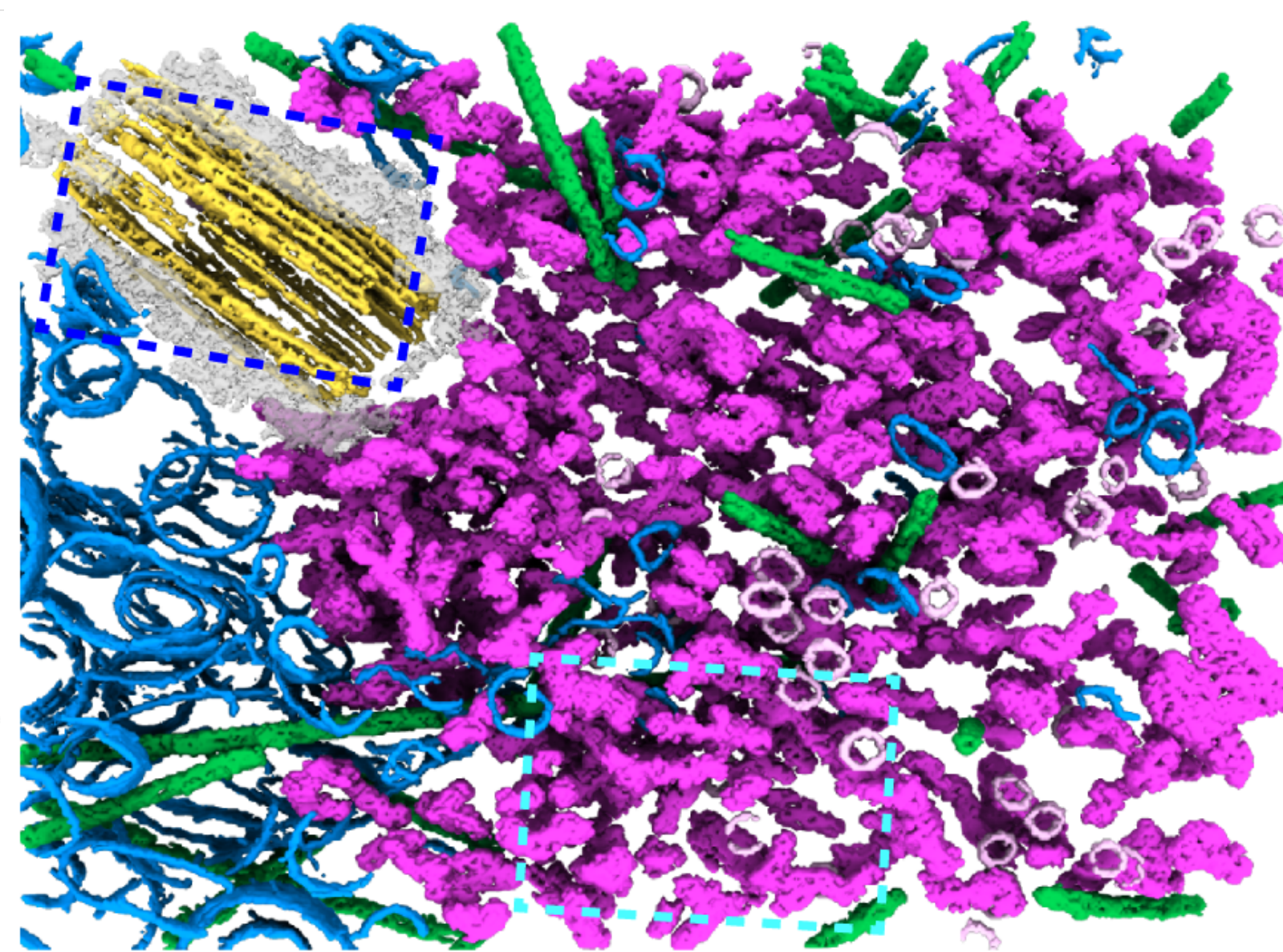
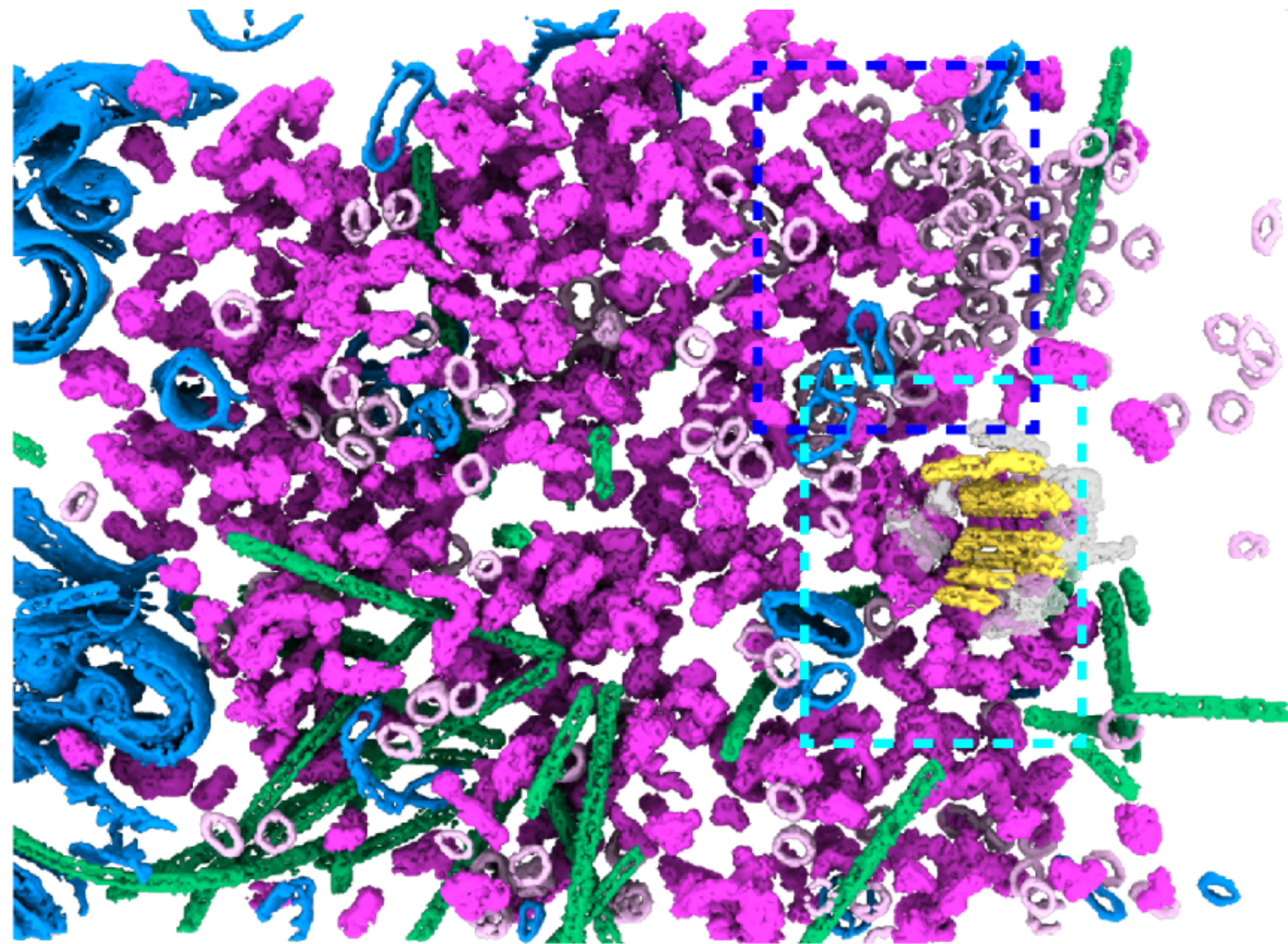
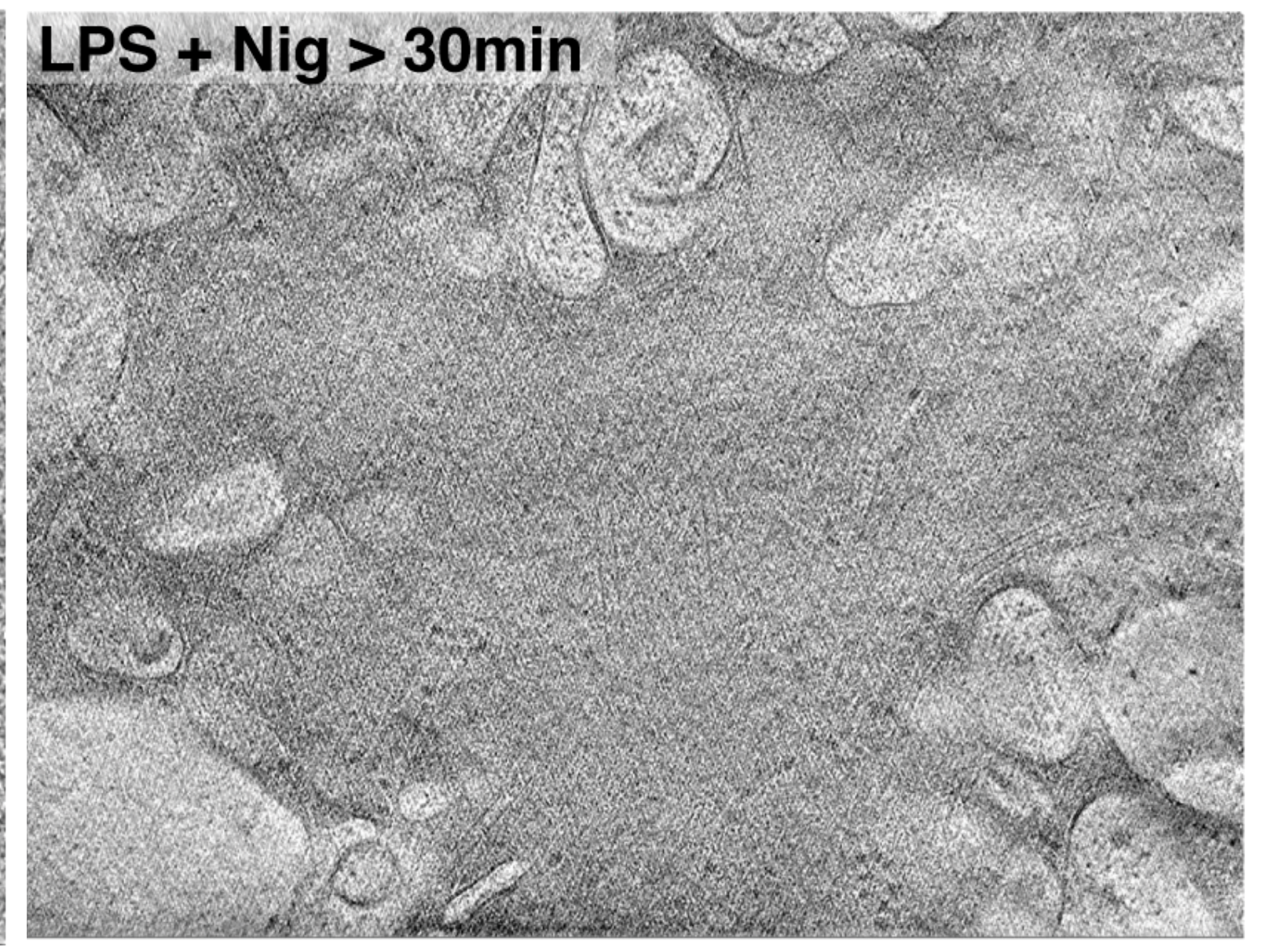
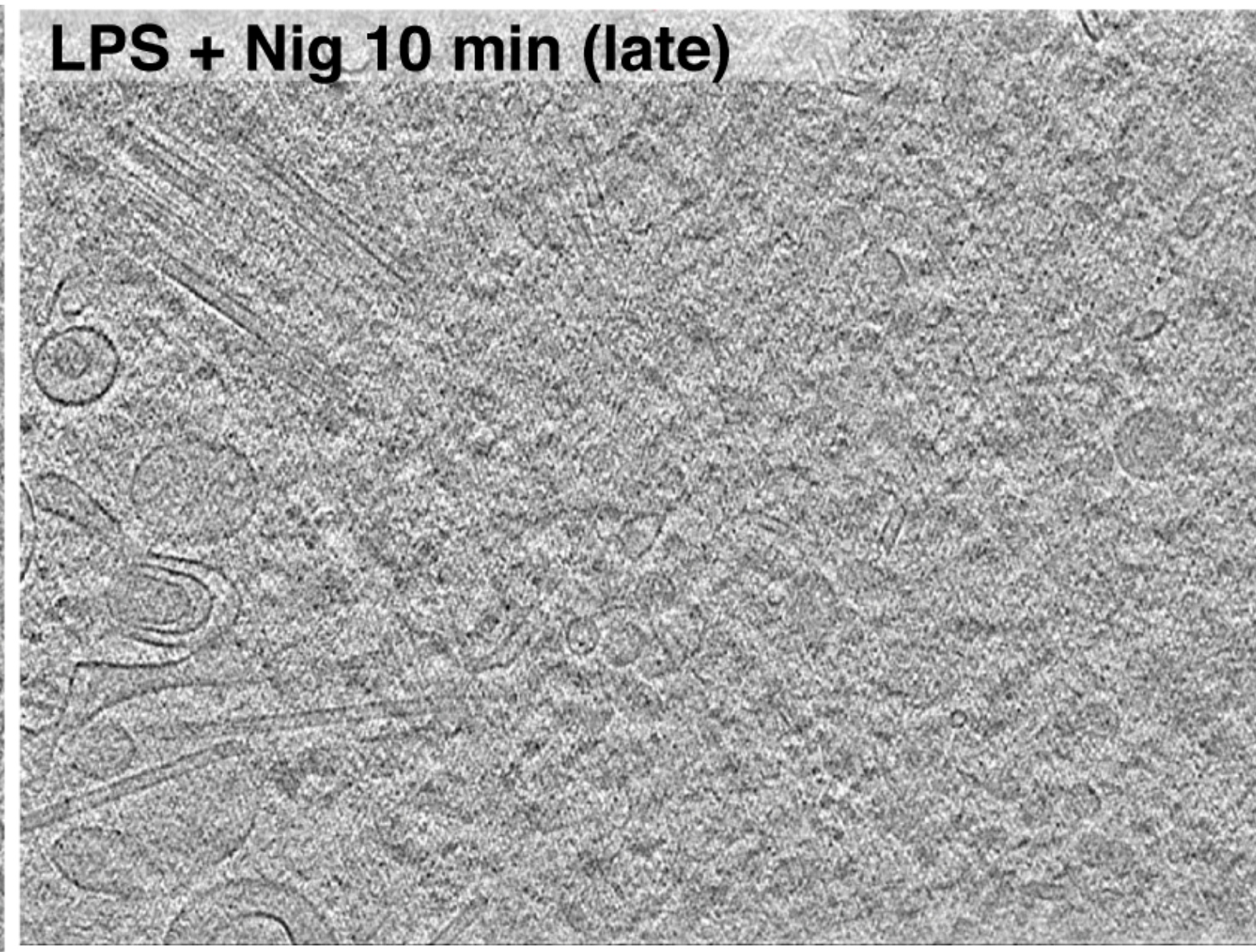
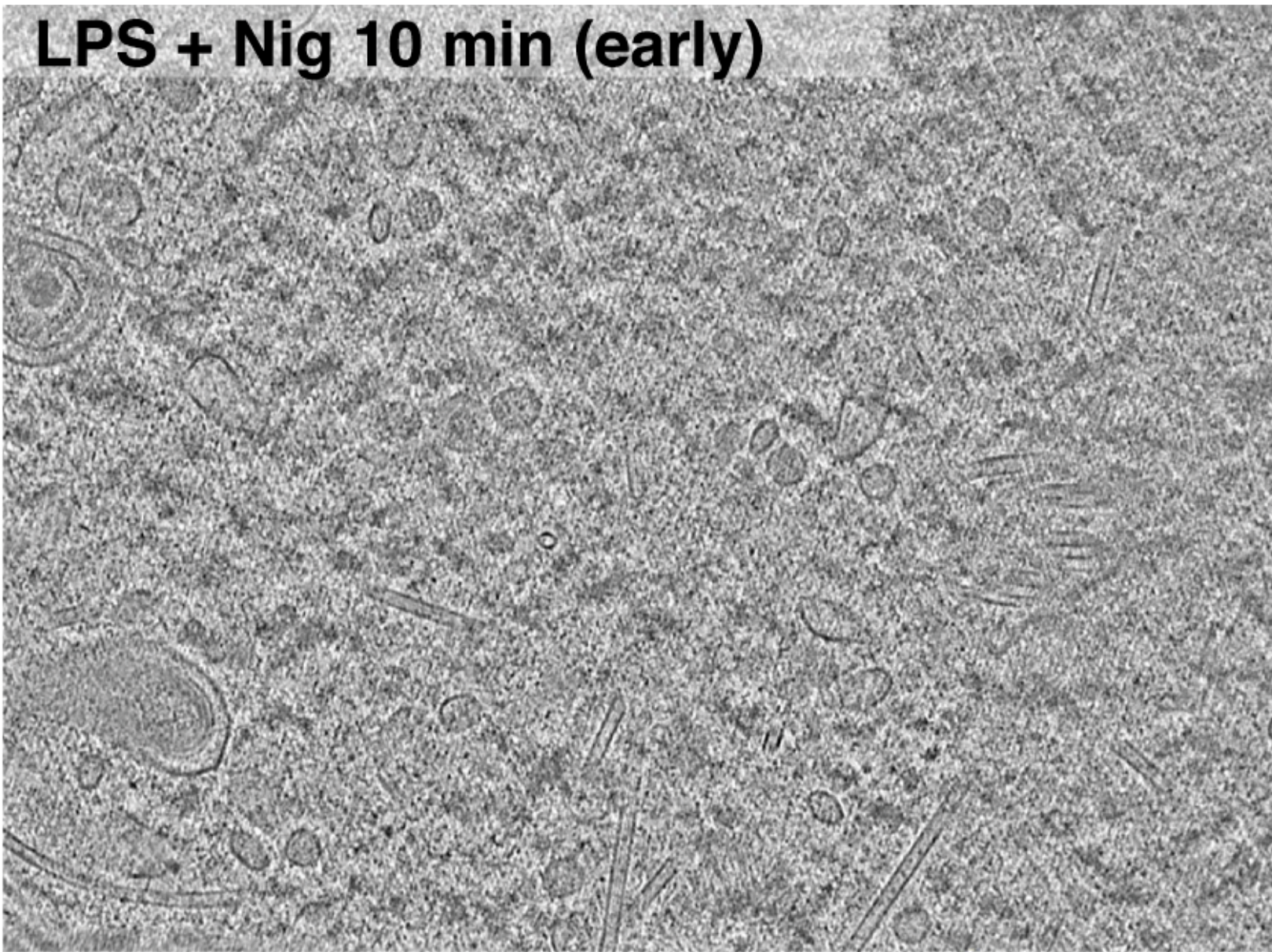
LPS+Nig > 30min



Activated human NLRP3 inflammasomes are unstructured condensates



The NLRP3 condensate grows denser





Phyllis Wang
Caltech/Stanford



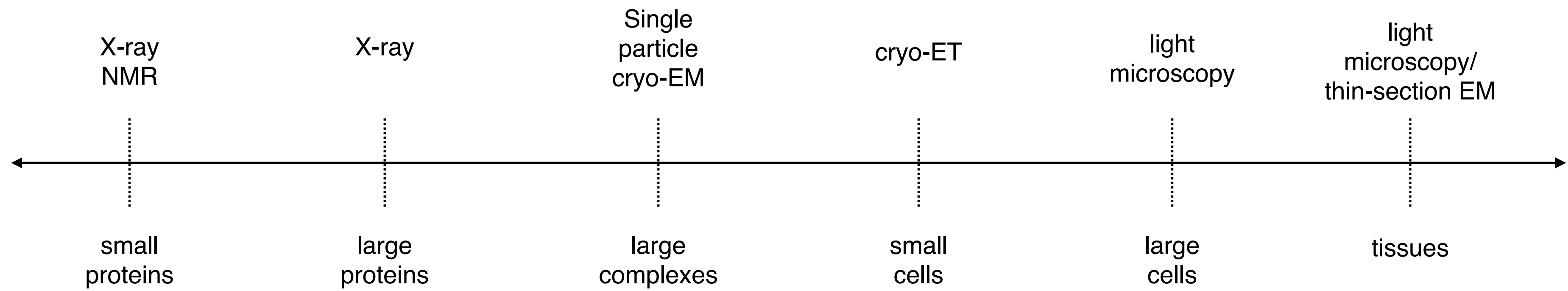
Hao Wu
Harvard

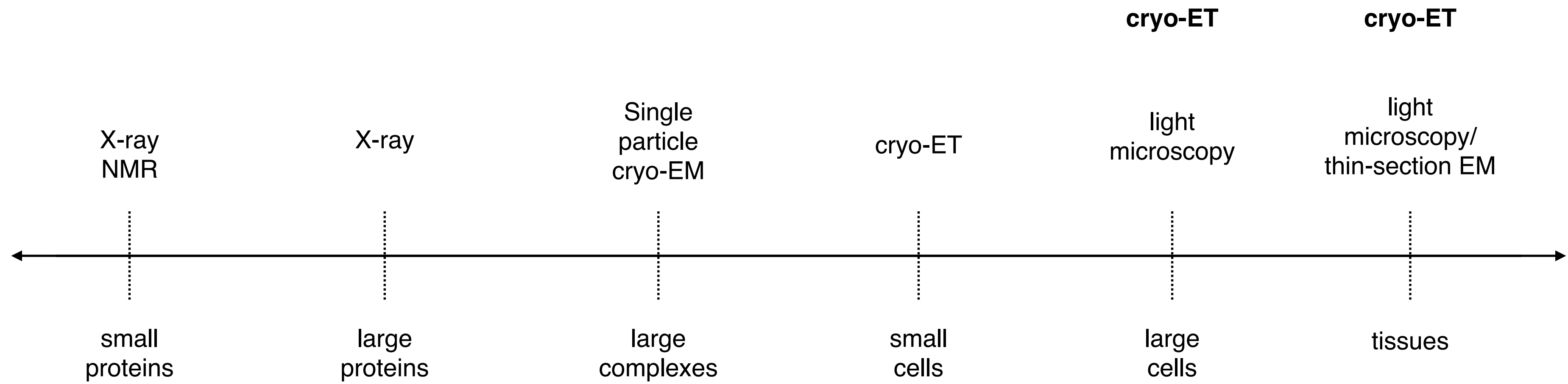


Peter Dahlberg
Stanford

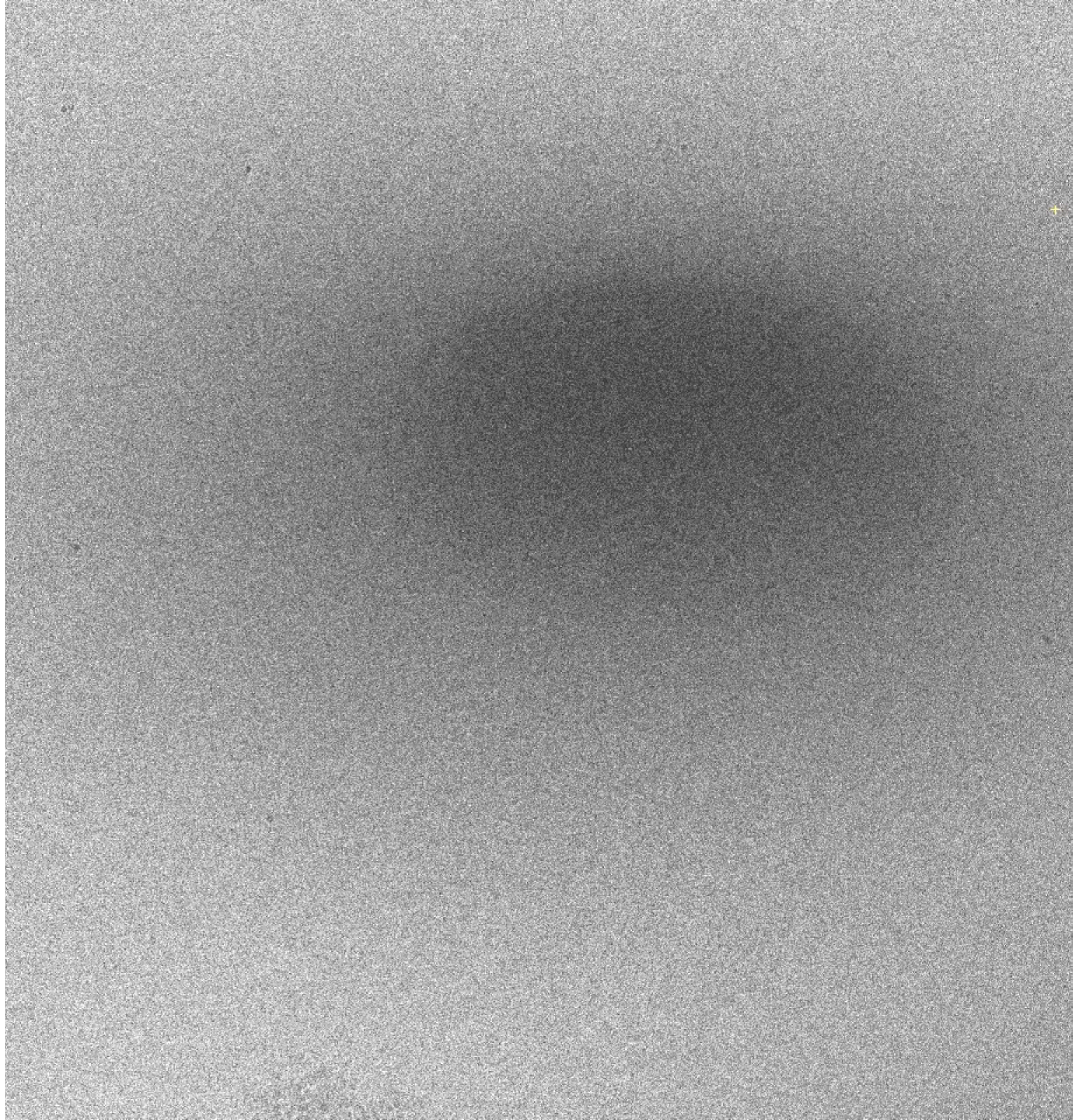
Future technical advances

- Direct detectors: quantum efficiency/MTF, size, speed
- Phase plates
- Lower temperatures with liquid helium-cooling
- Better images at high tilt - what is the problem?
- Larger areas: montage imaging
- Faster - continuous tilting?
- Automation (cryo-FIB milling, data collection, image processing)
- Super-resolution CLEM
- Routine serial-section lift-out

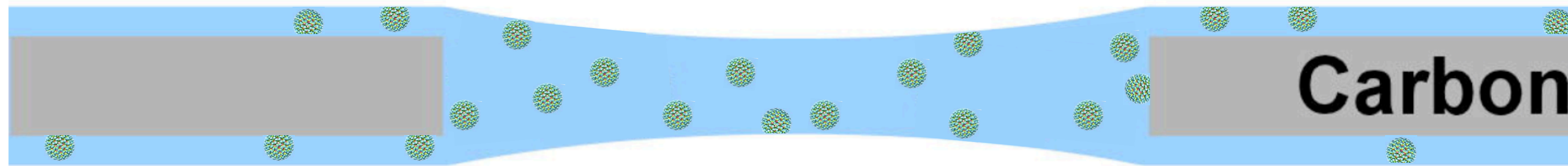




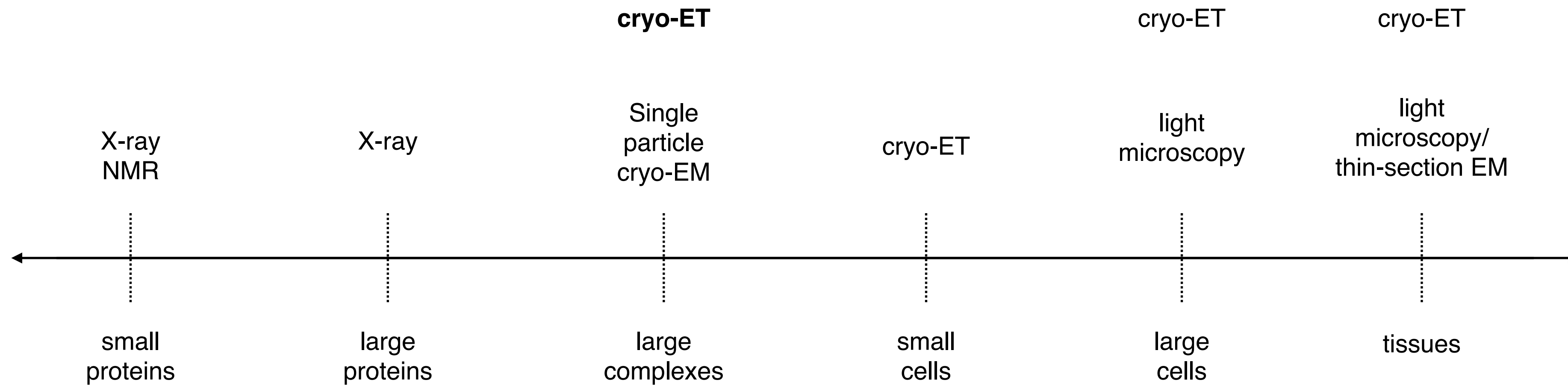
1. Fluorescence-guided FIB-milling



How fast tilt-series could supplement single particle projections

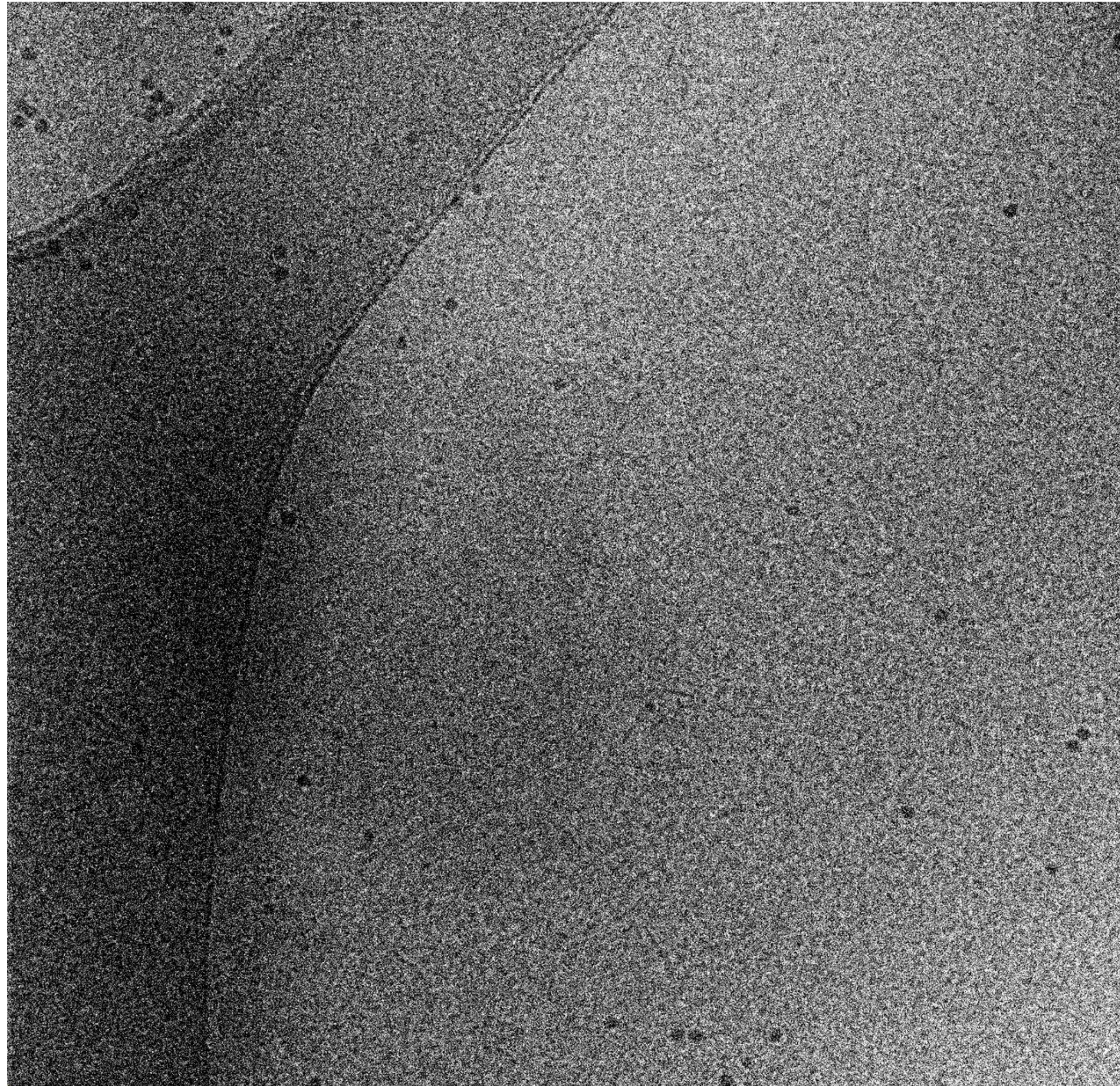


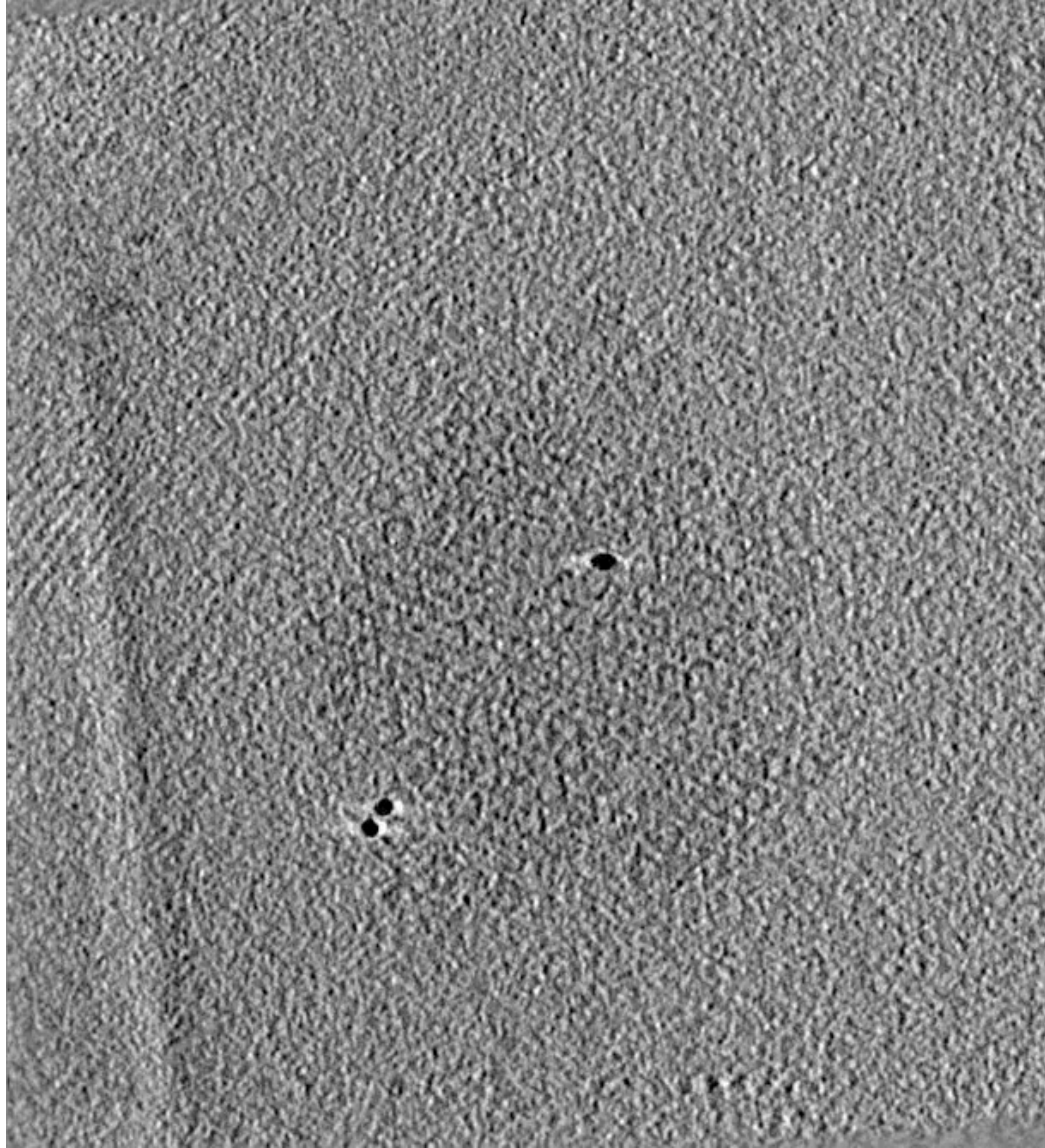
- detect and exclude particles on the air/water interface (which are likely damaged)
- improve per-particle CTF-refinement by determining each particle's z-coordinate within the ice
- disambiguate conformational changes from differences in orientation
- start alignment searches very near the true 3-D orientation



1. Fluorescence-guided FIB-milling
2. Rapid tilt-series

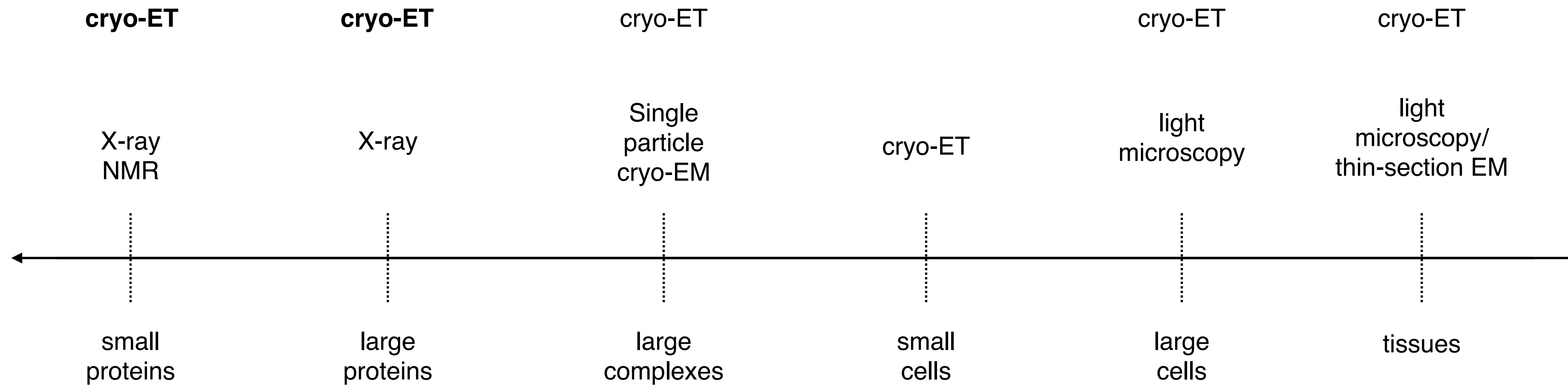
Tomography of nanocrystals





Electron tomography of nanocrystals

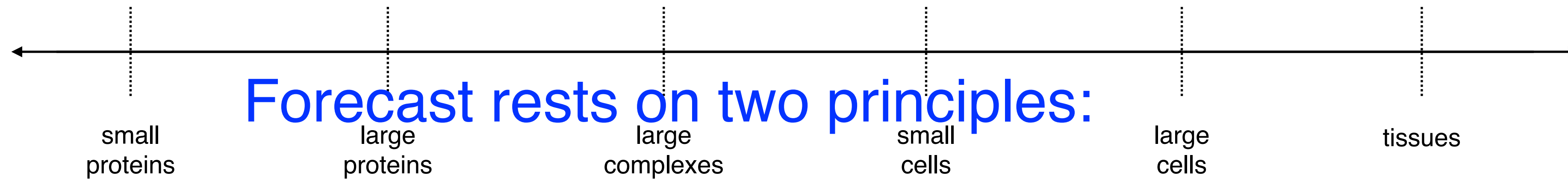
- Uses crystals only a few hundred nanometers thick
- Could use poorly-ordered crystals
- Could resolve twinning and joints
- Fast, accurate phases with no need for heavy atom derivatives or a molecular replacement model
- Cheaper (no synchrotron needed)



1. Fluorescence-guided FIB-milling
2. Rapid tilt series
- 3. Tomography of nanocrystals**



cryo-ET



1. Electrons are better than X-rays

- they can be focused
- give more info for the same damage

2. 3D is better than 2D

The physics is clear, if the technical challenges can be solved!