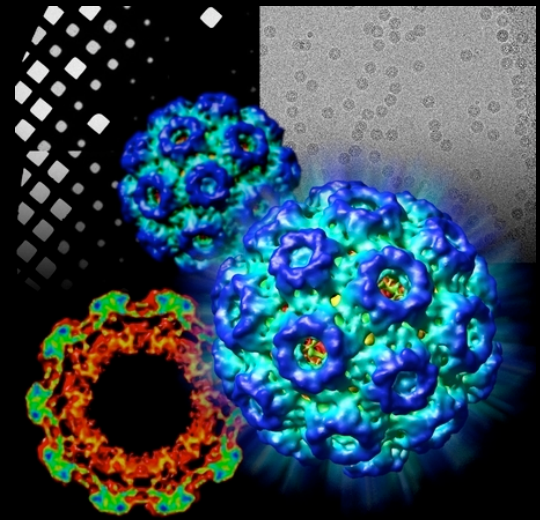
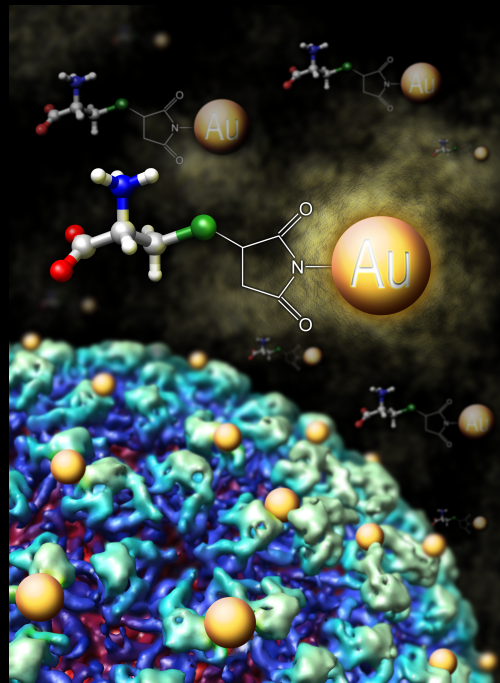
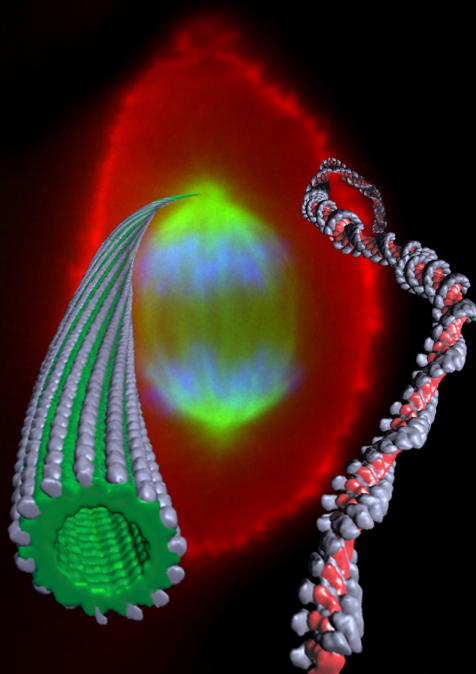
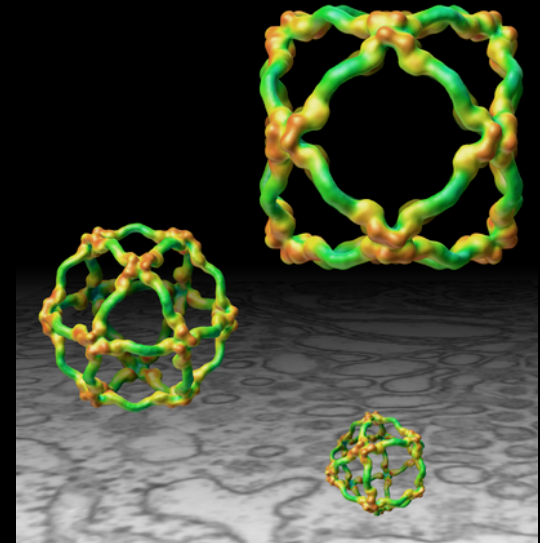
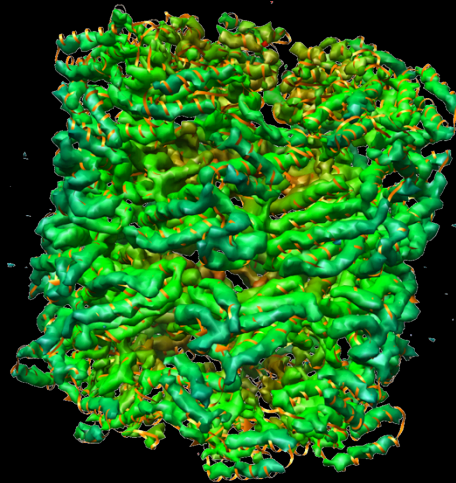
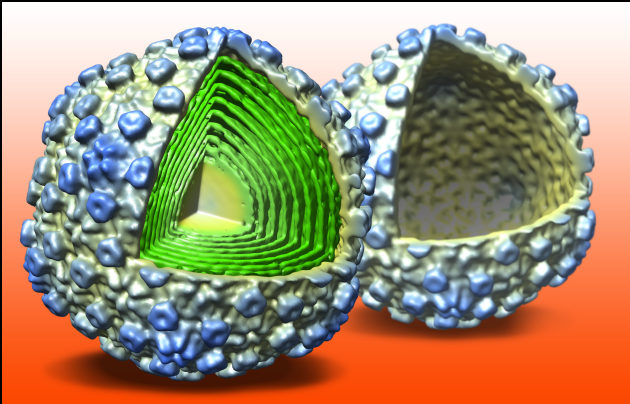


Visualizing Biological Processes with CryoEM



"It is very easy to answer many of these fundamental biological questions; you just look at the thing!... Make the microscope one hundred times more powerful, and many problems of biology would be made very much easier. I exaggerate, of course, but the biologists would surely be very thankful to you"

Richard P. Feynman.

**from: *There's Plenty of Room at the Bottom*,
a lecture given to the American Physical Society in 1959.**

The Scale of Biological Structures

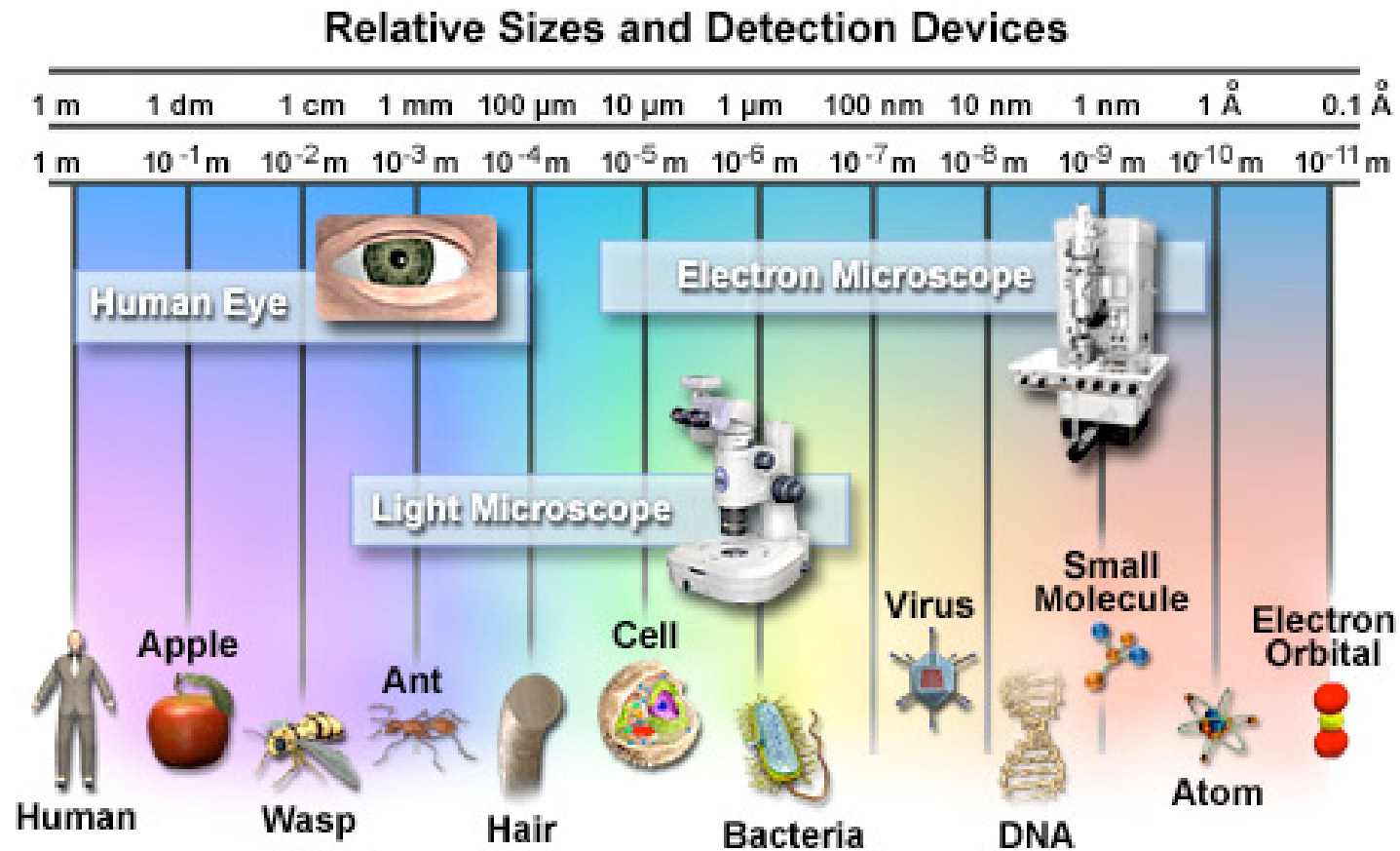
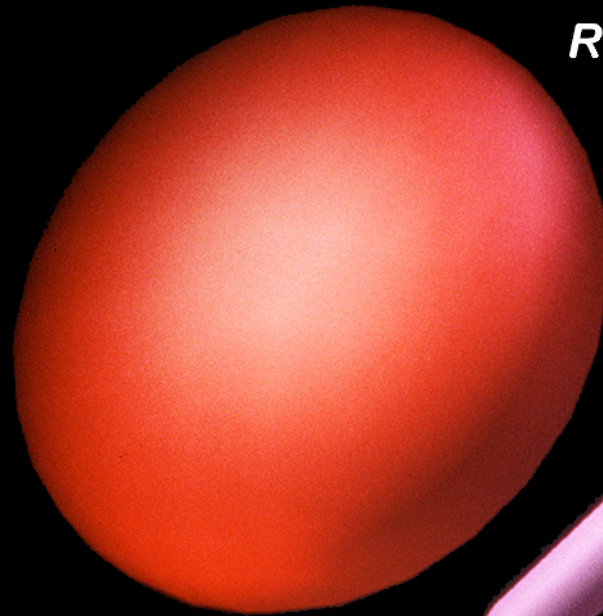


Figure 1

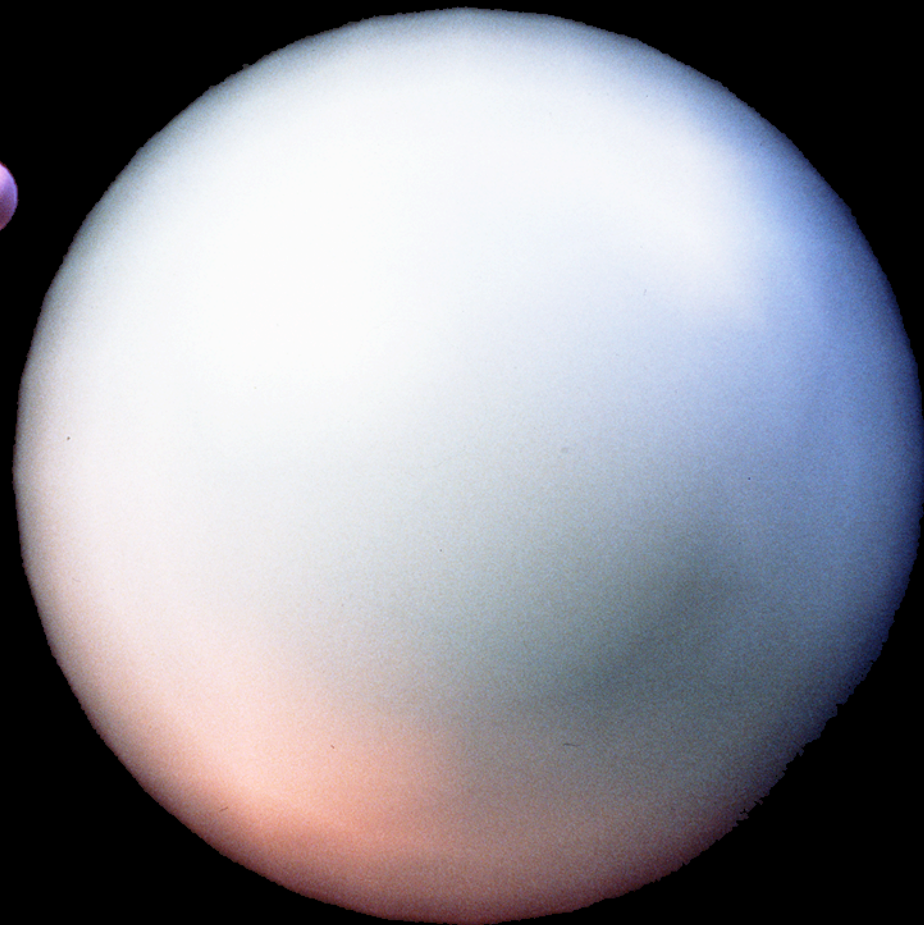


Red blood cell



E. coli

Bacteria



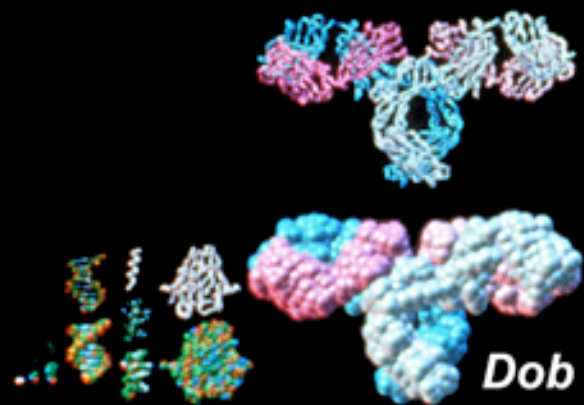
White blood cell

Human Cells

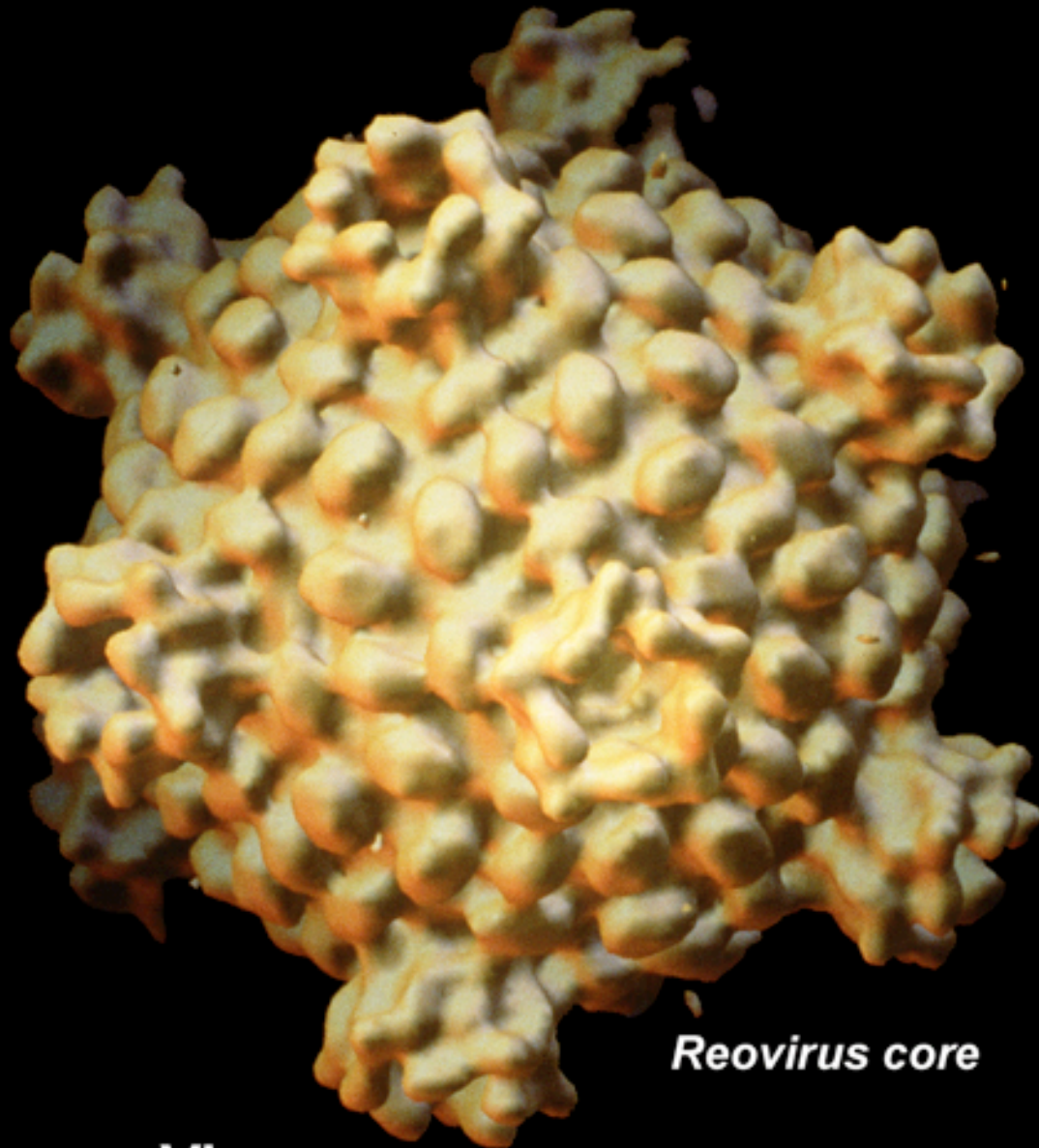
E. coli

Bacteria

:  Reovirus core
Virus

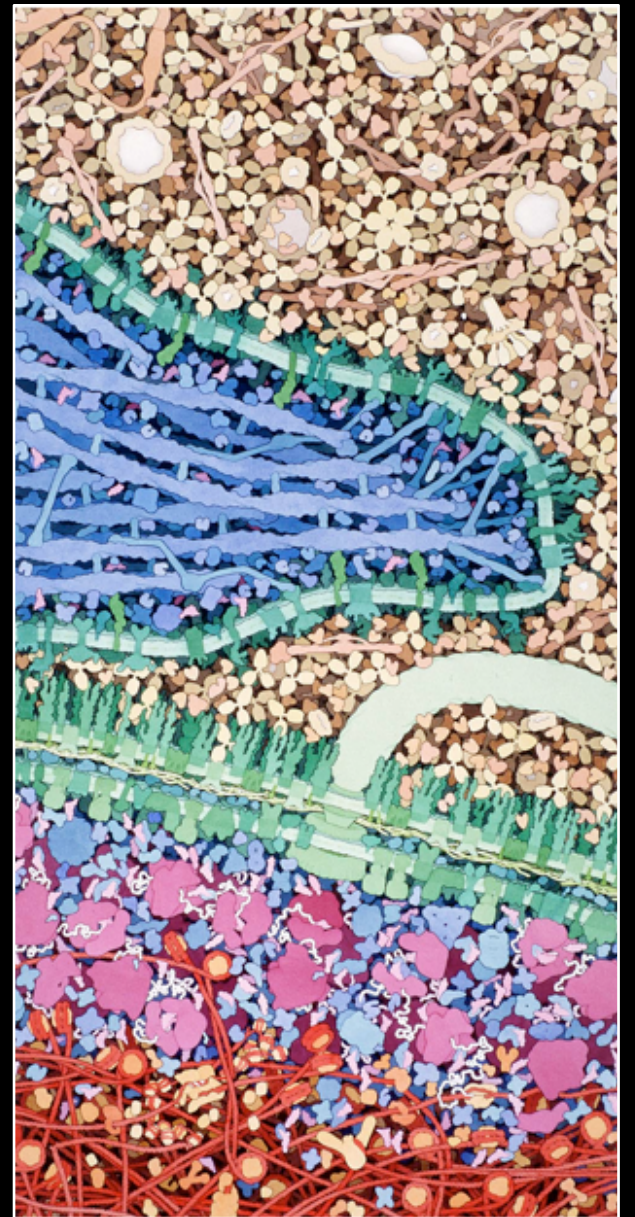
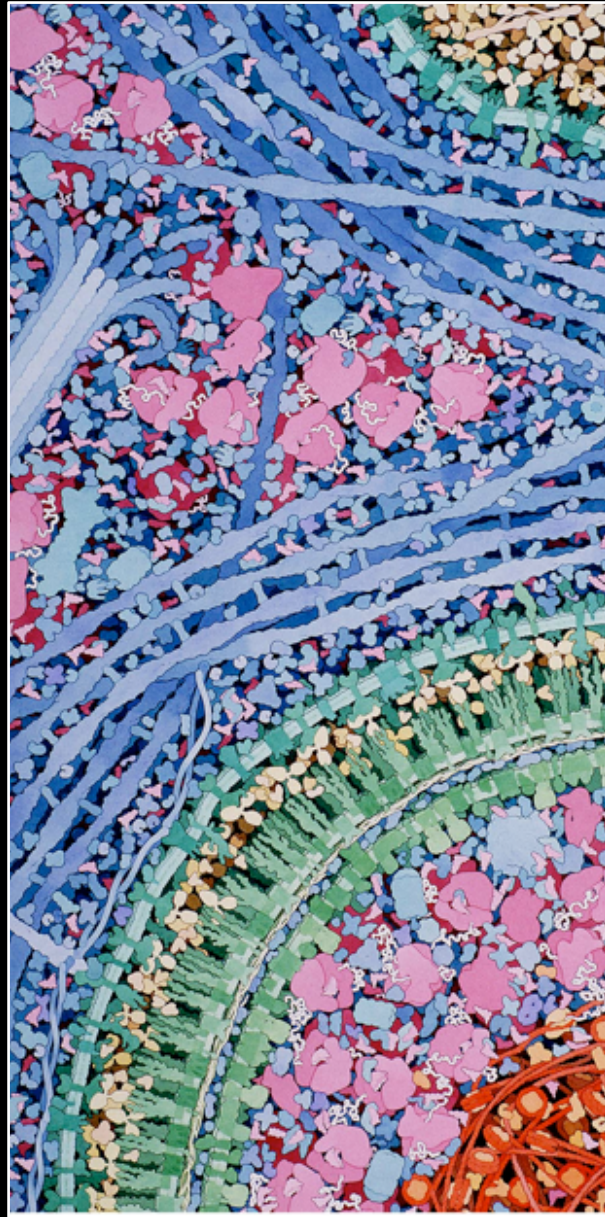
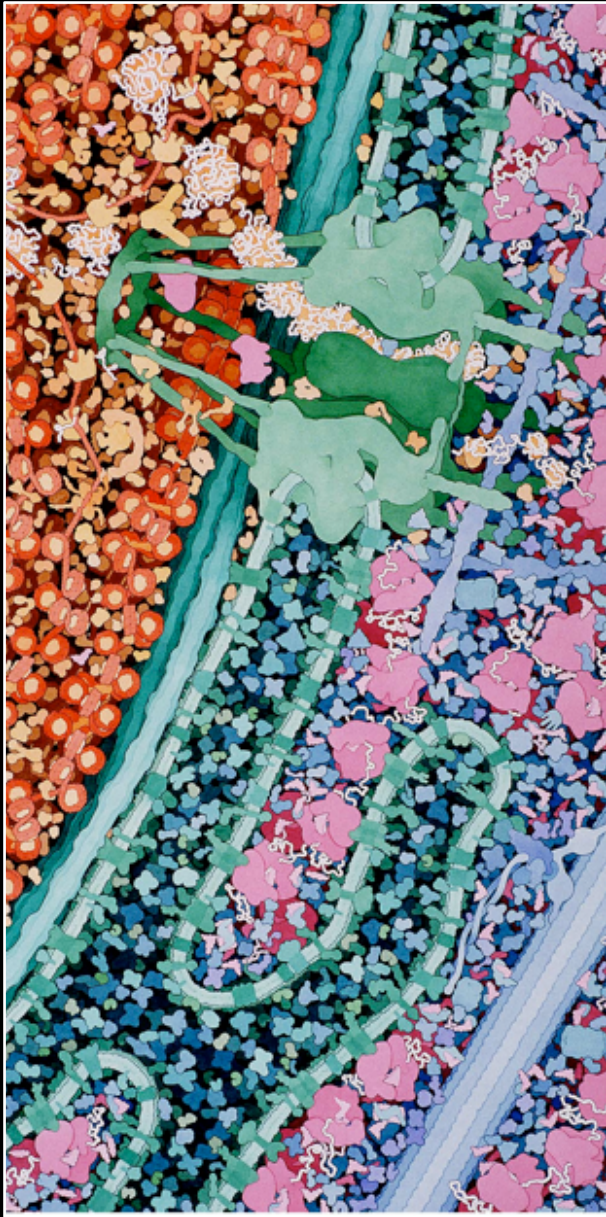


Antibody



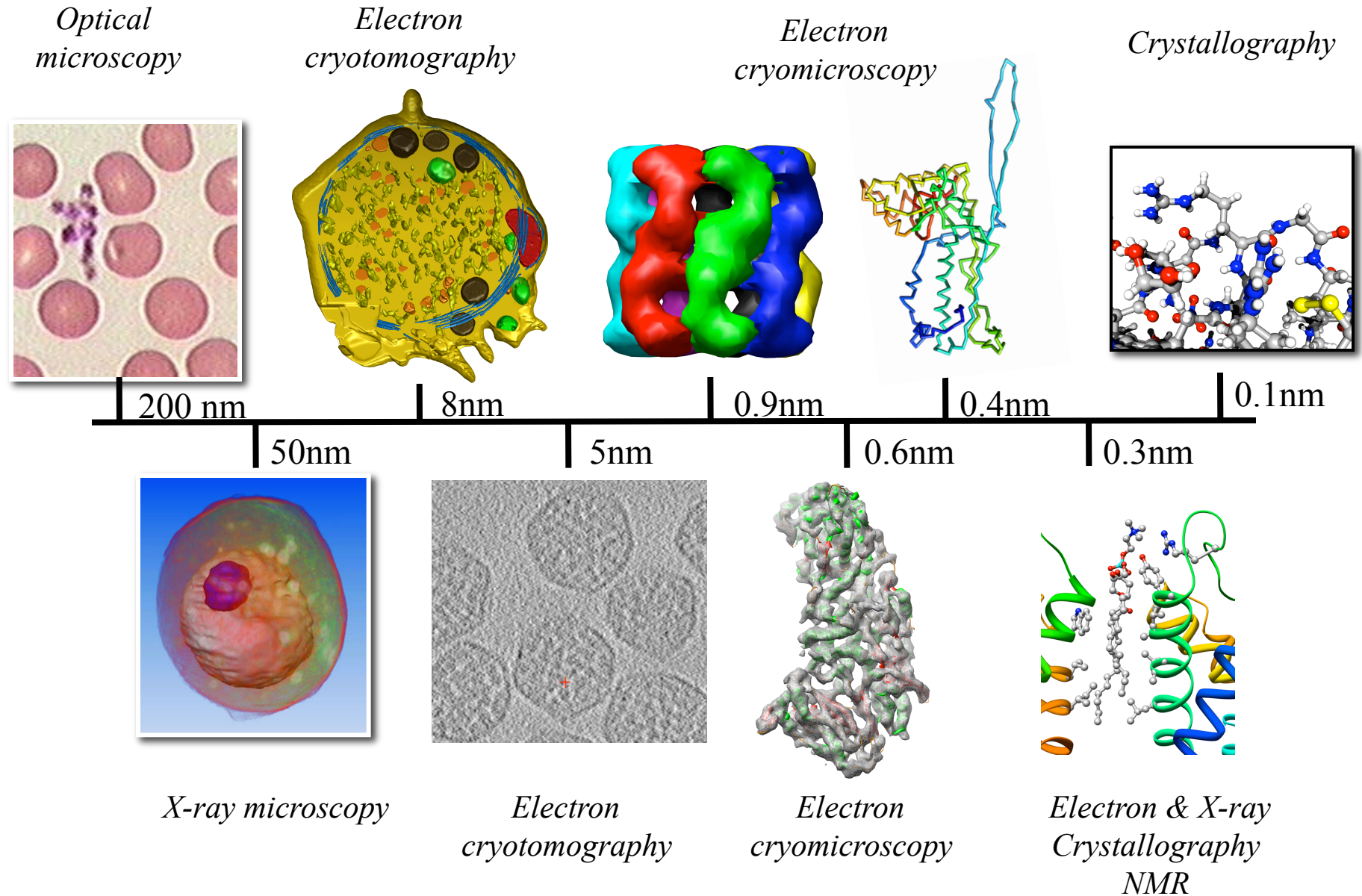
Reovirus core

Virus



Images by David Goodsell, Scripps Research Institute

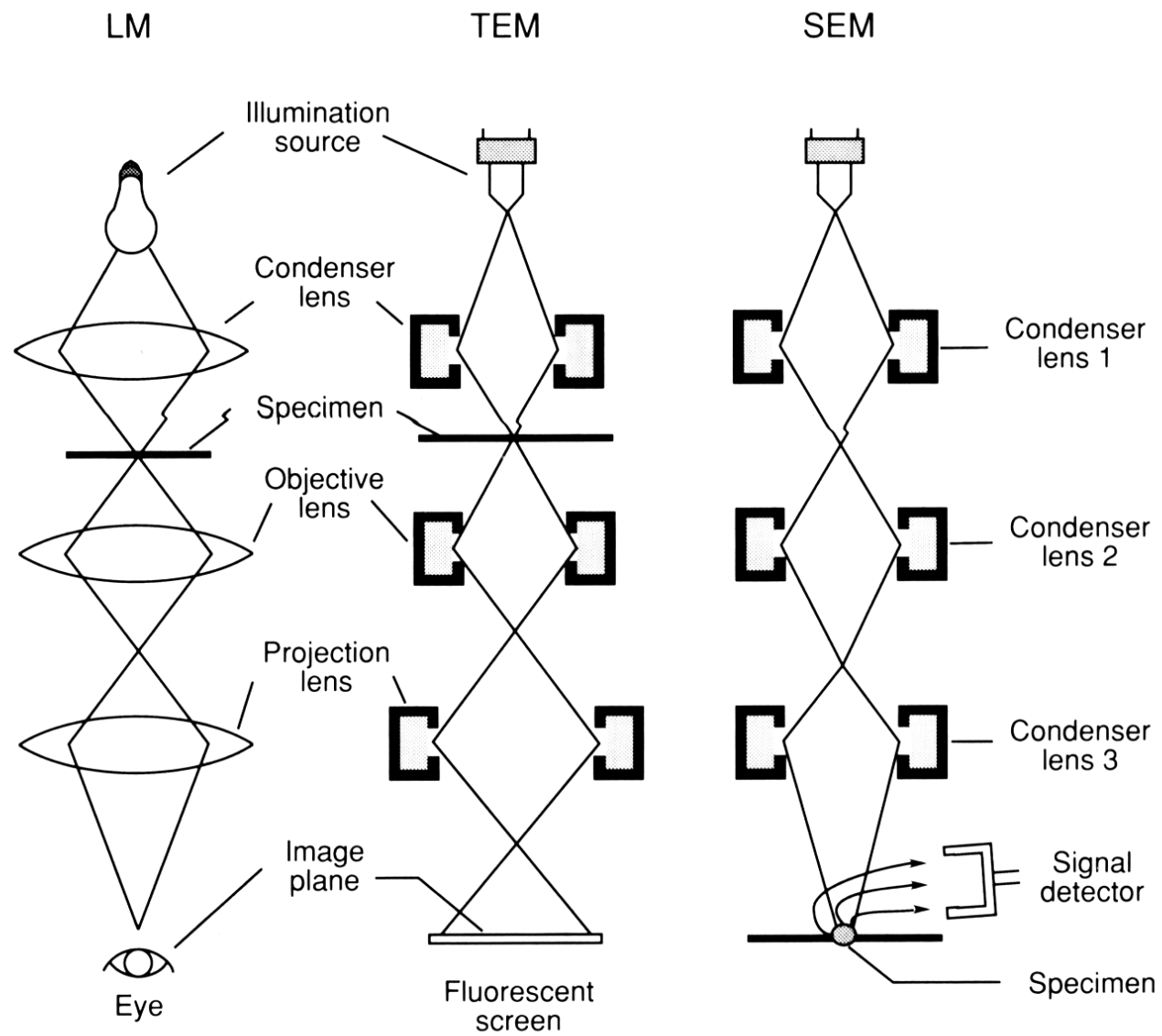
Structural Biology from Cells to Atoms



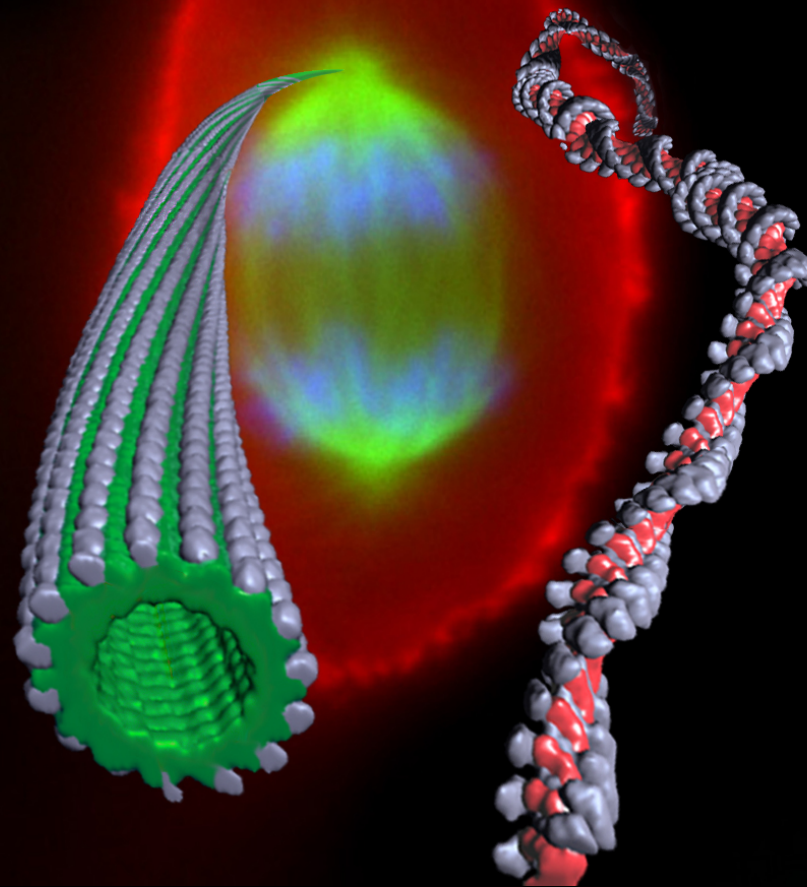
Slide courtesy Wah Chiu

The Transmission Electron Microscope



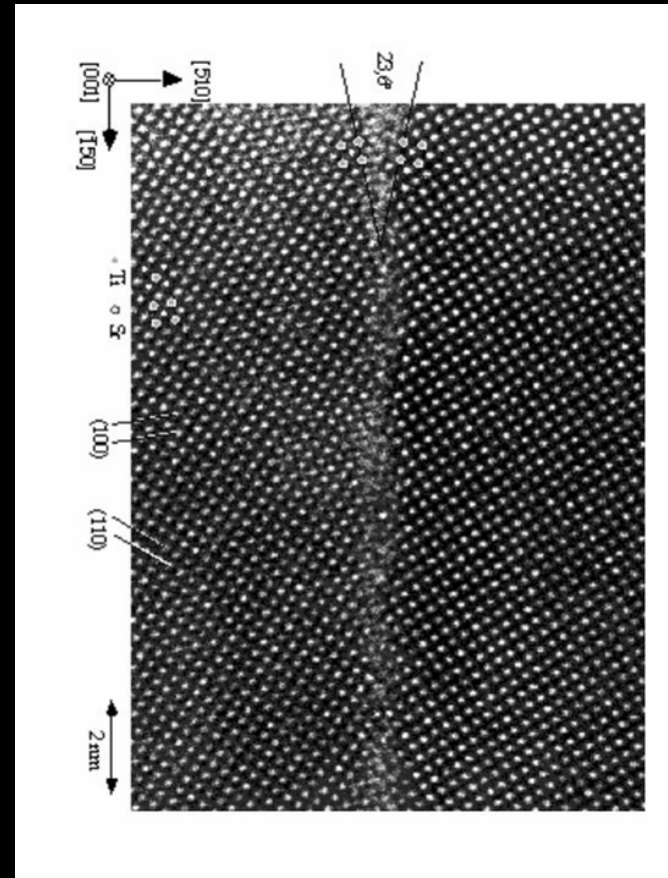


What distinguishes EM AND LM ?



Resolution!

The Transmission Electron Microscope

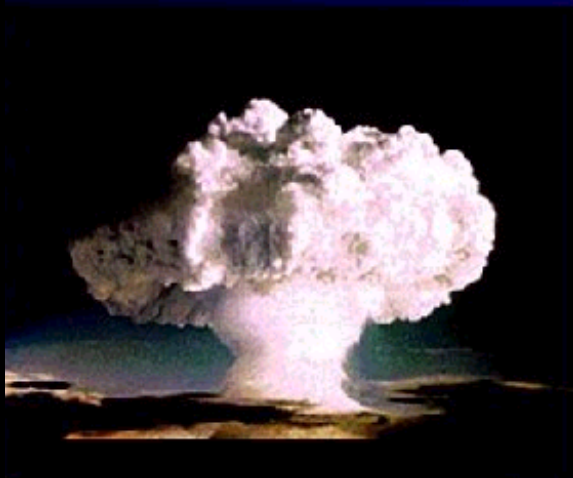


Resolution: ~ 0.2 nm

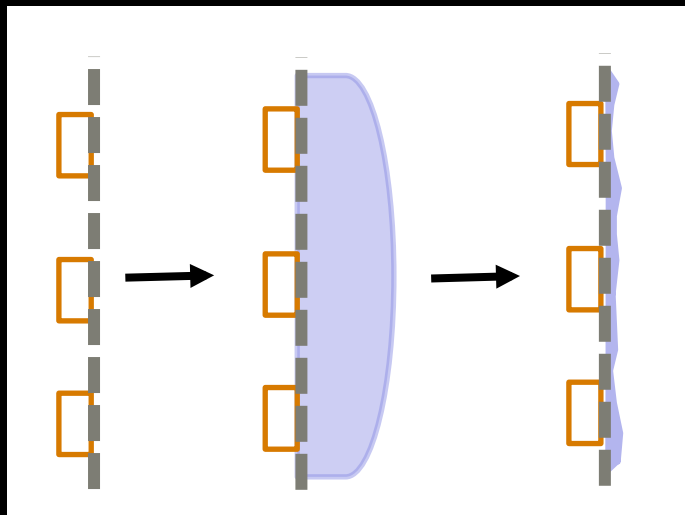
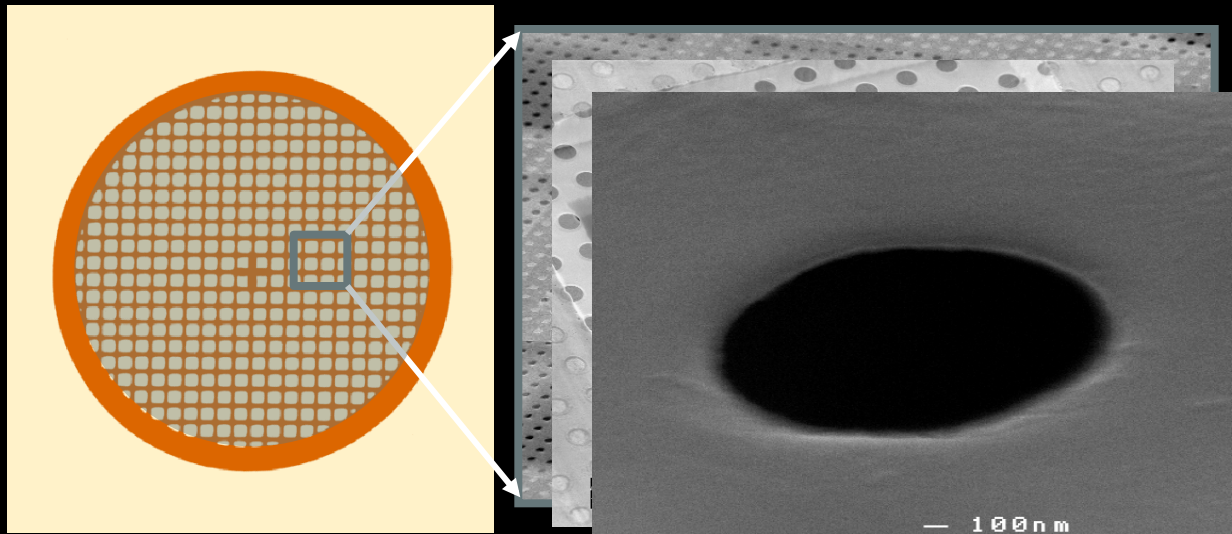
So why can't we just look at biological specimens and see details to 0.2 nm resolution?



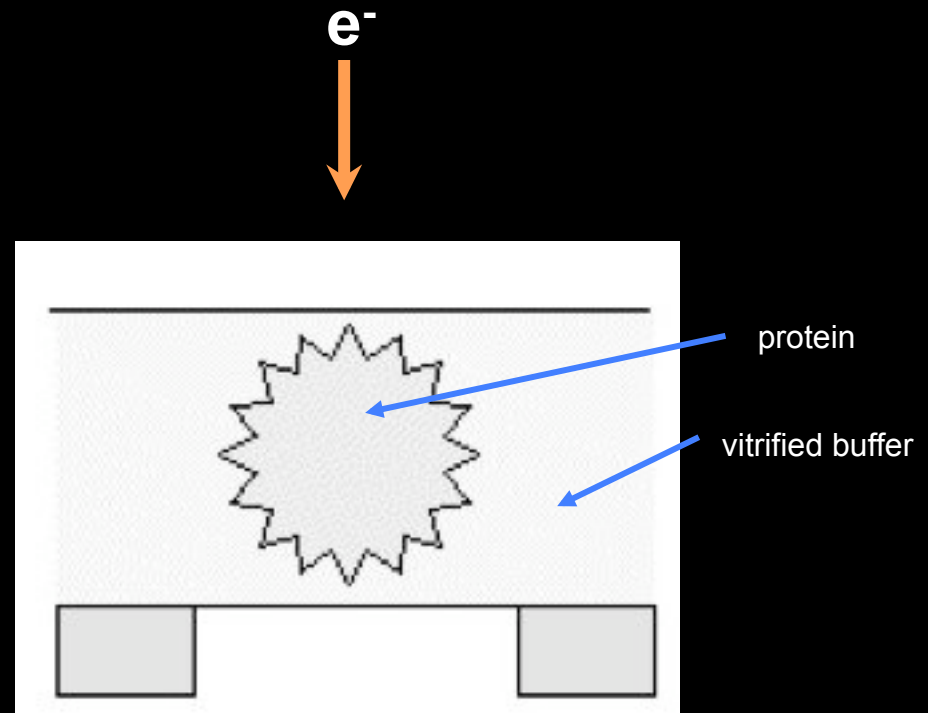
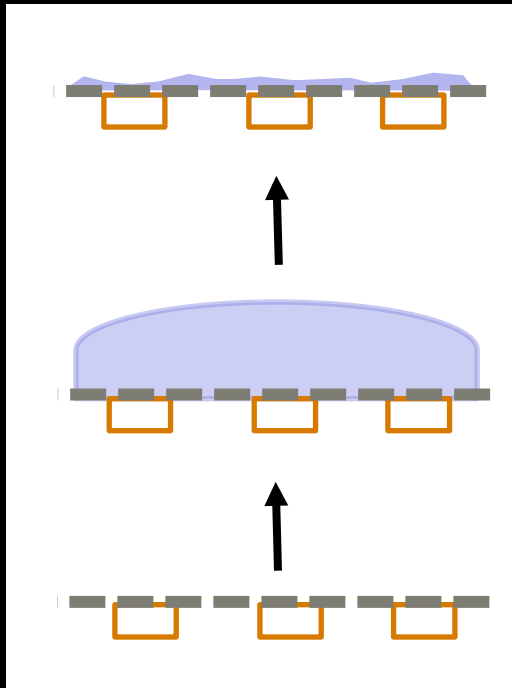
TEM requires a *thin* specimen that can withstand a *vacuum* and *high energy electrons*.



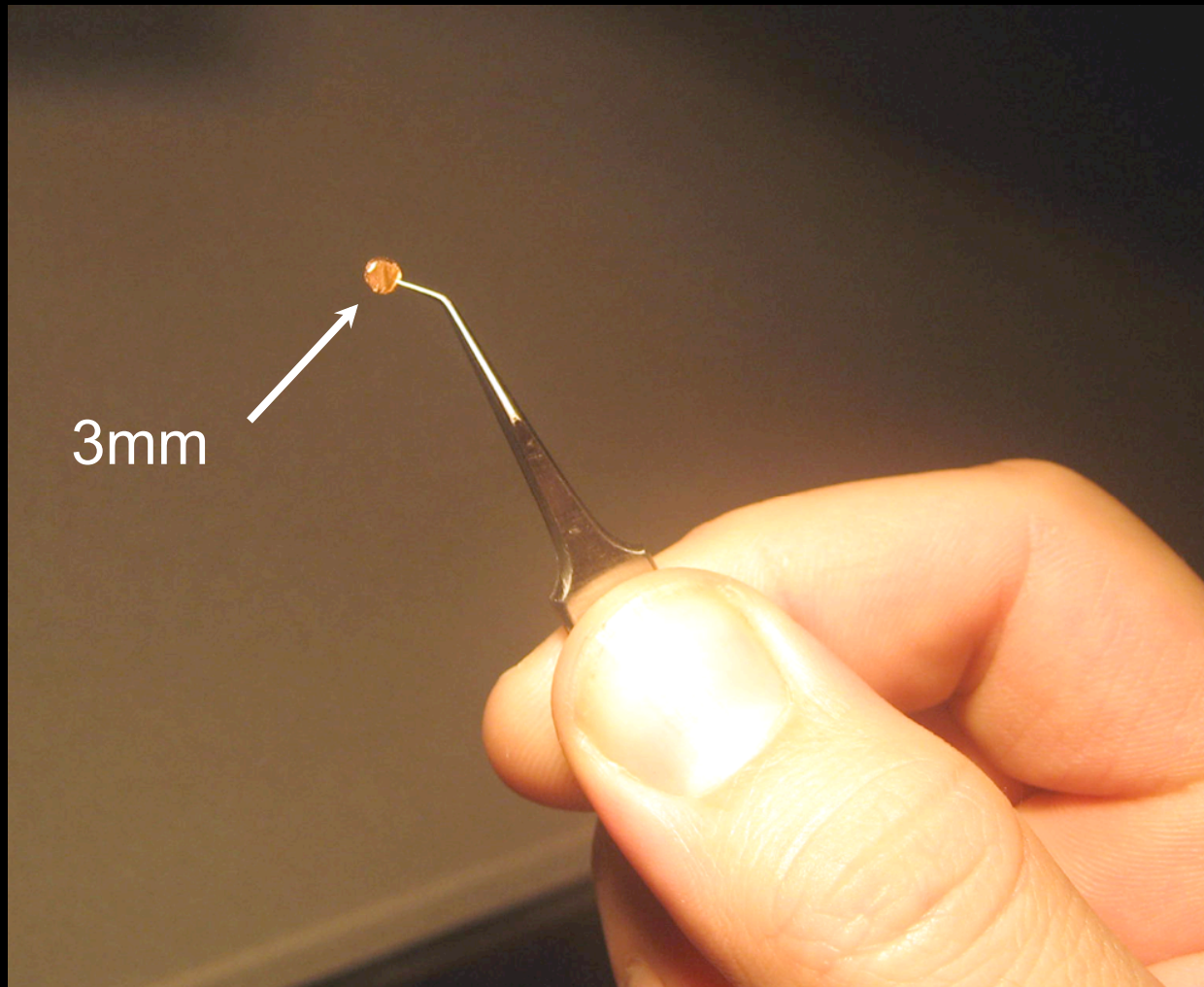
CryoEM – embedding specimens in vitreous ice

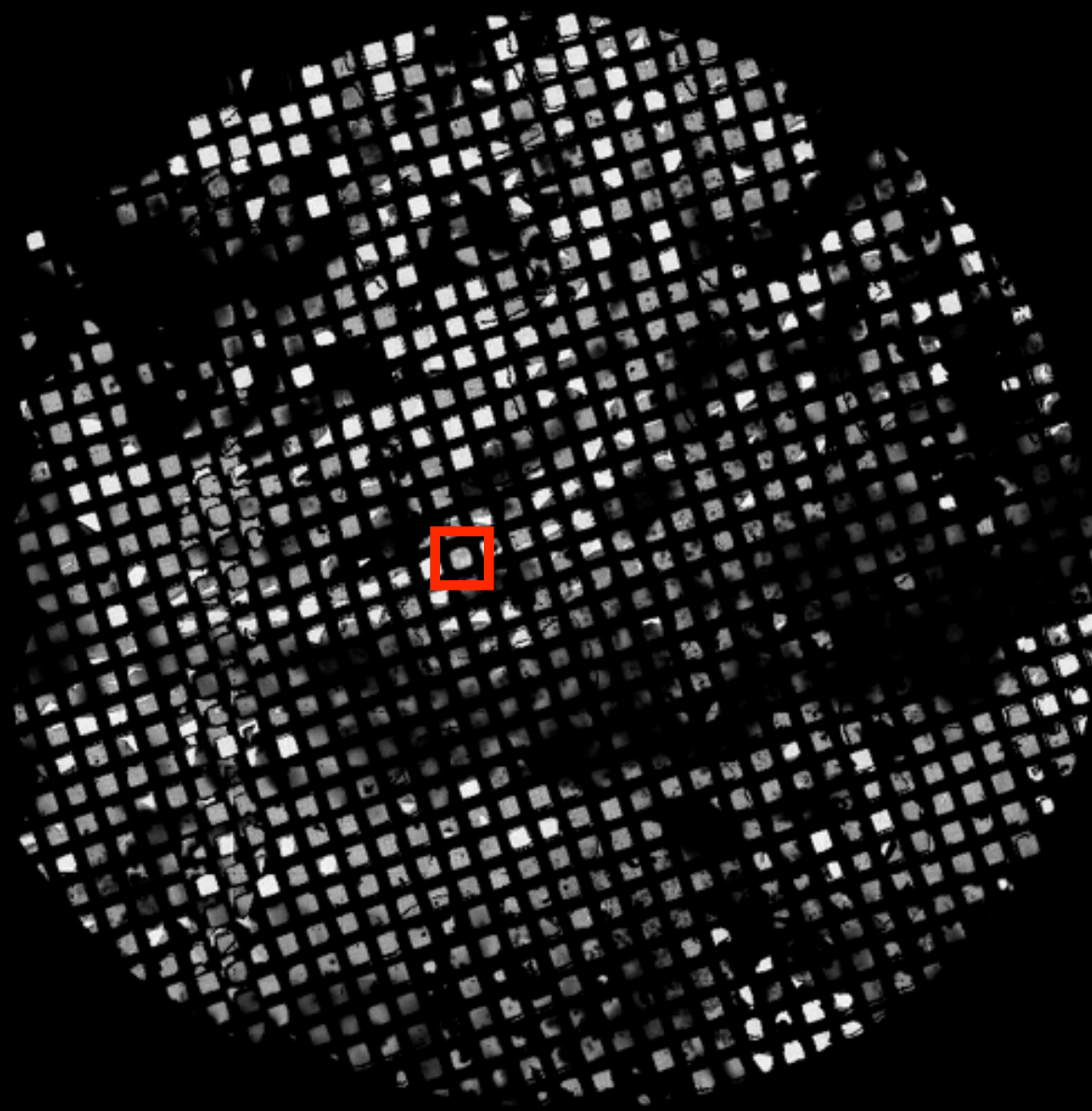


CryoEM – embedding specimens in vitreous ice

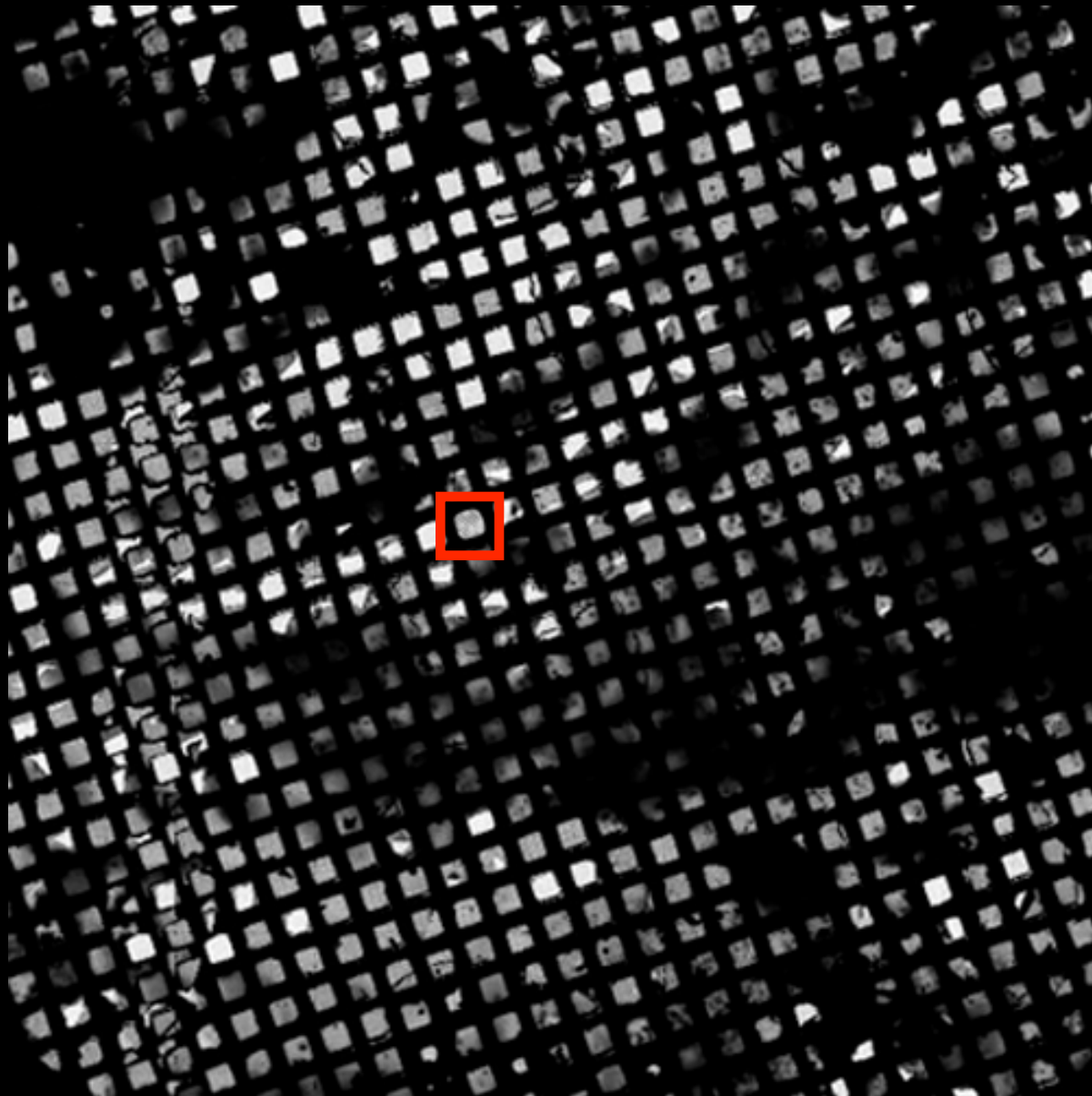


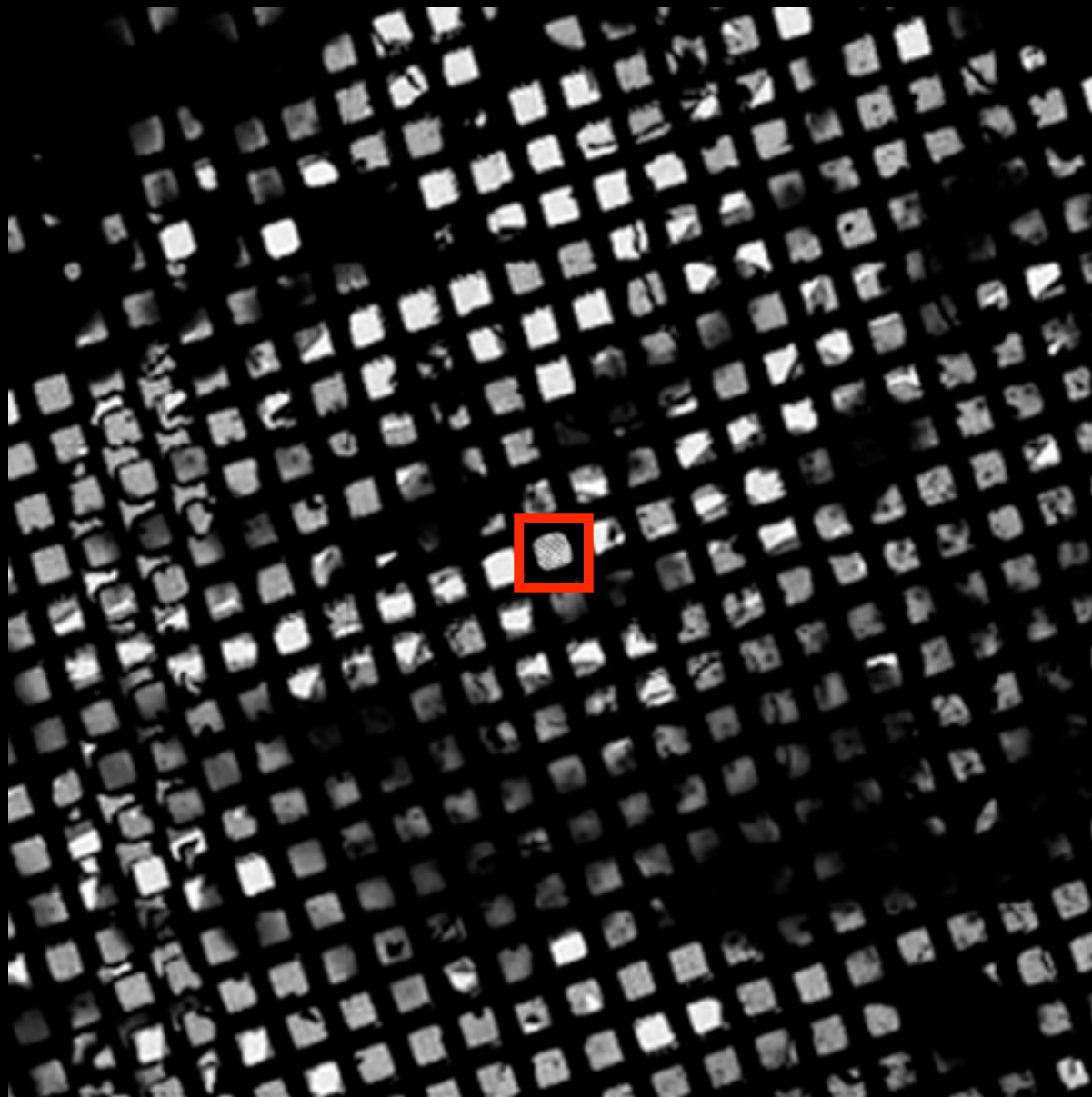
What does a vitrified specimen look like?

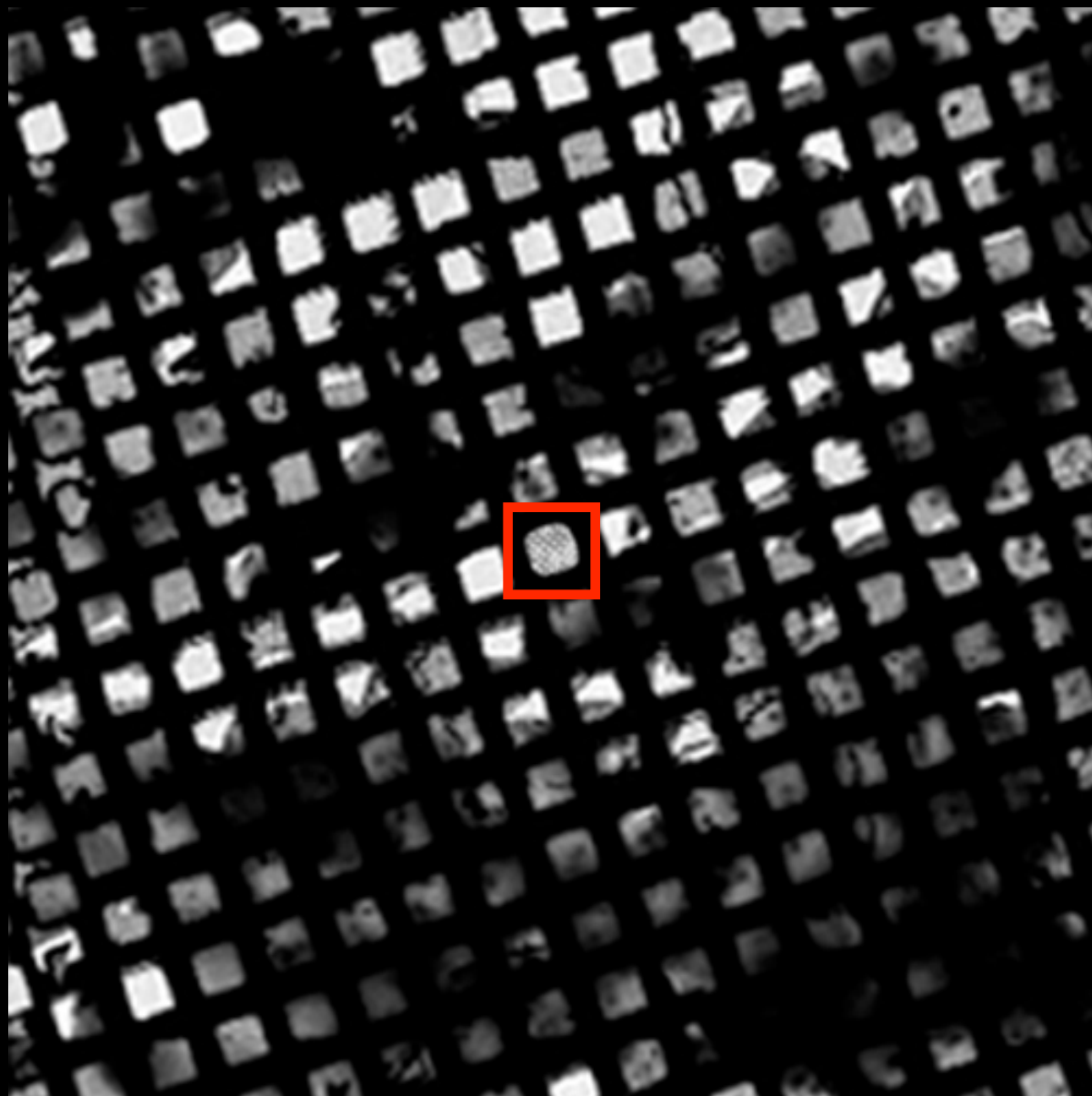


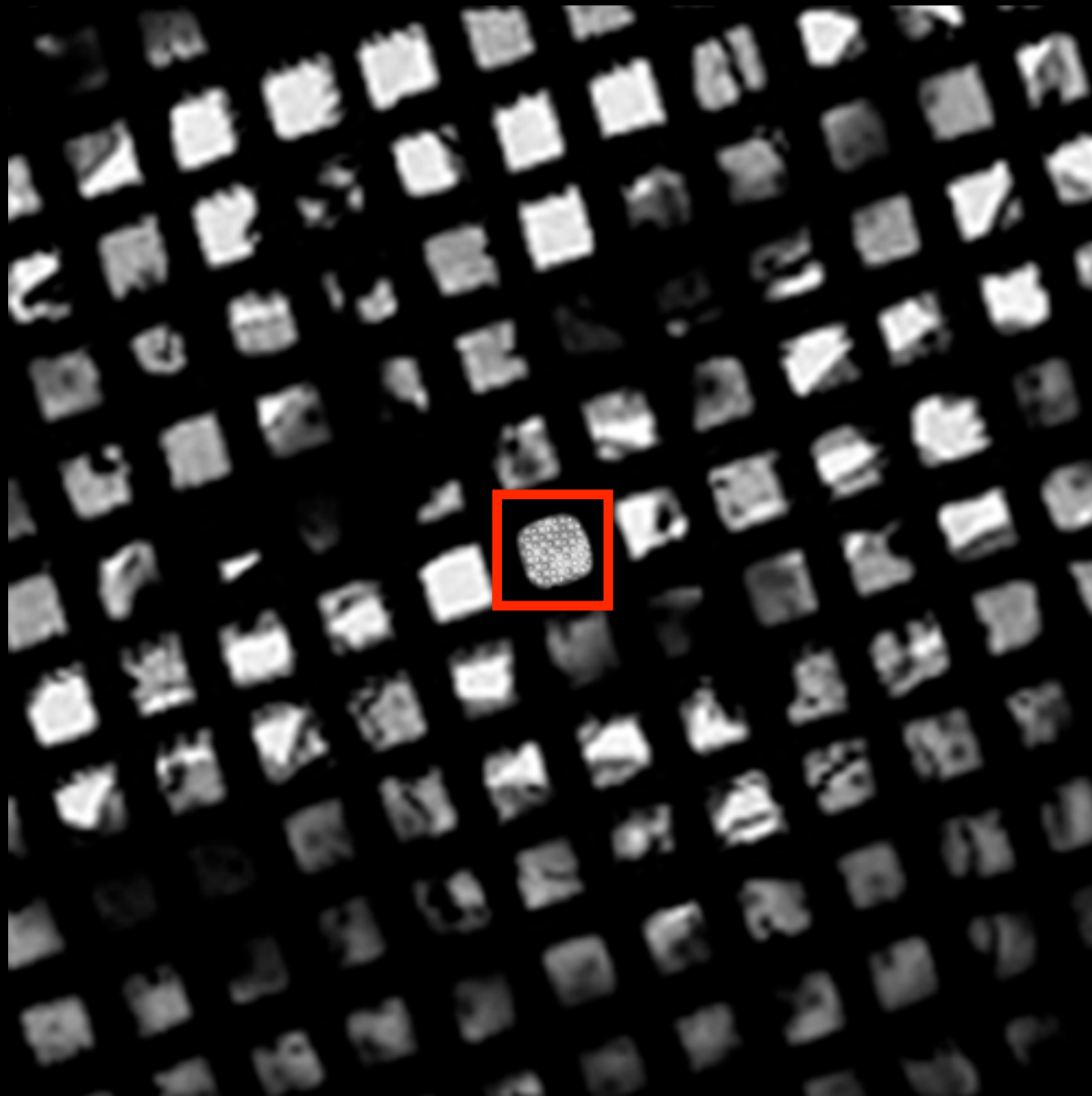


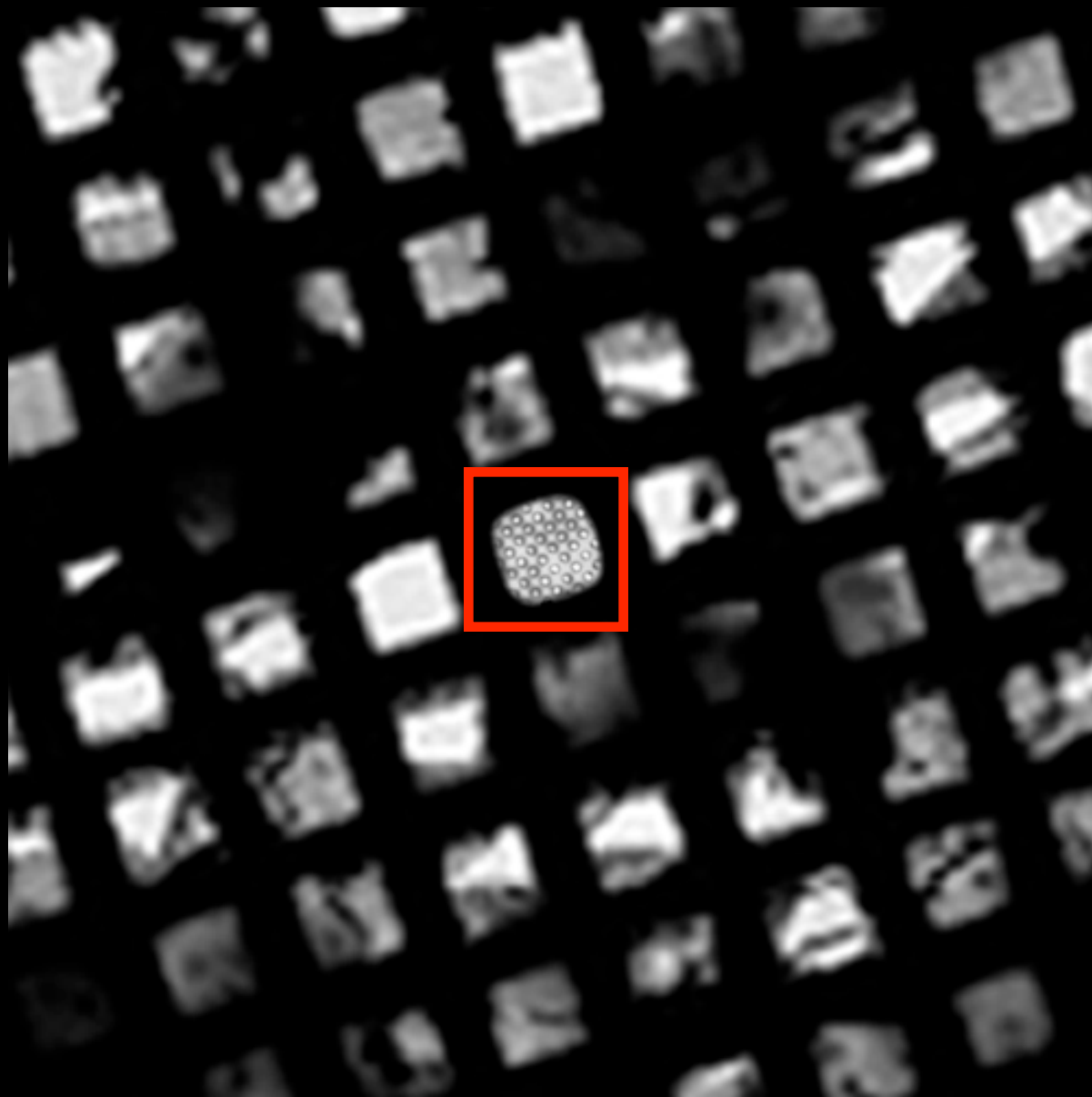
Slide courtesy Gabe Lander

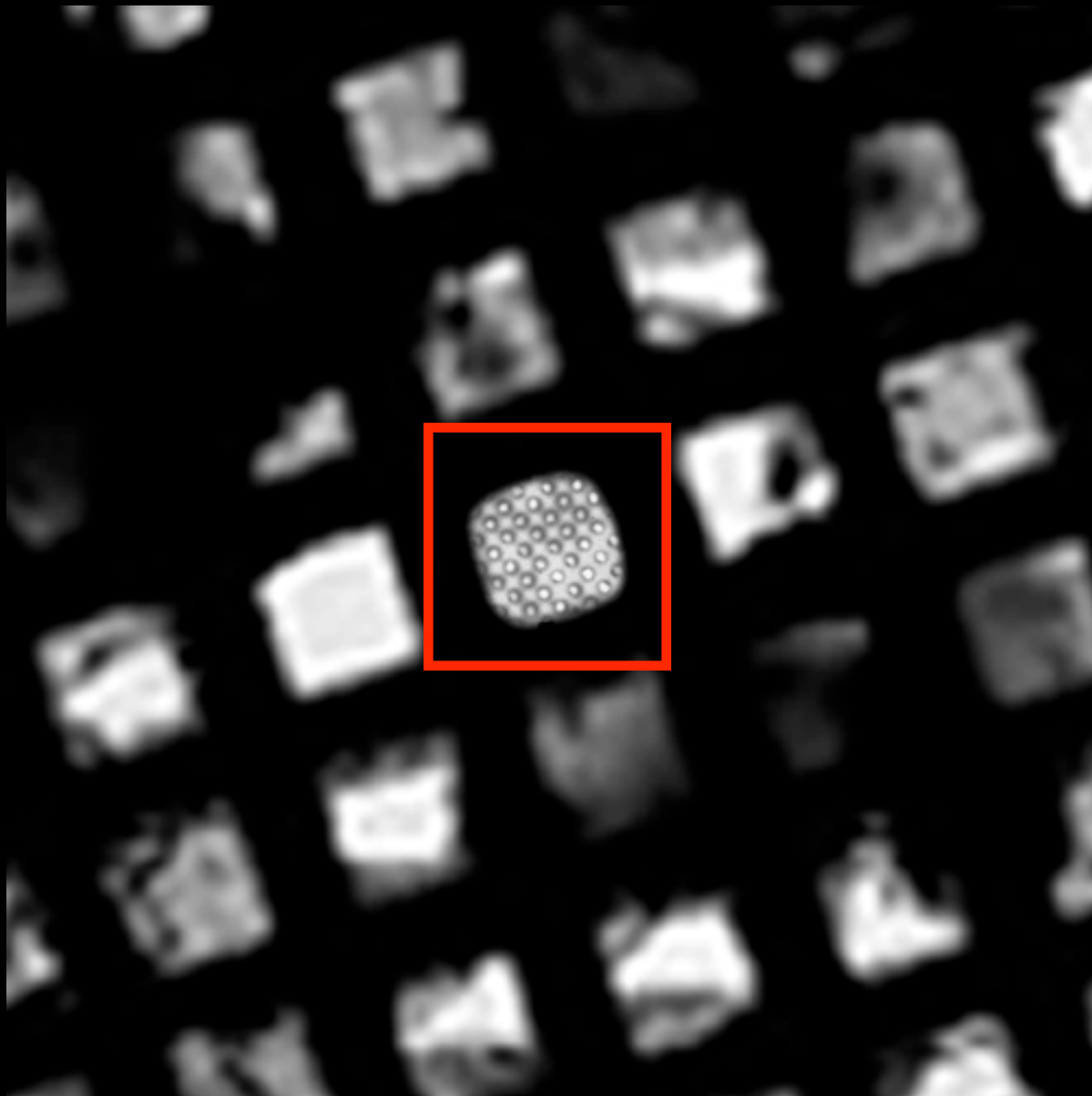


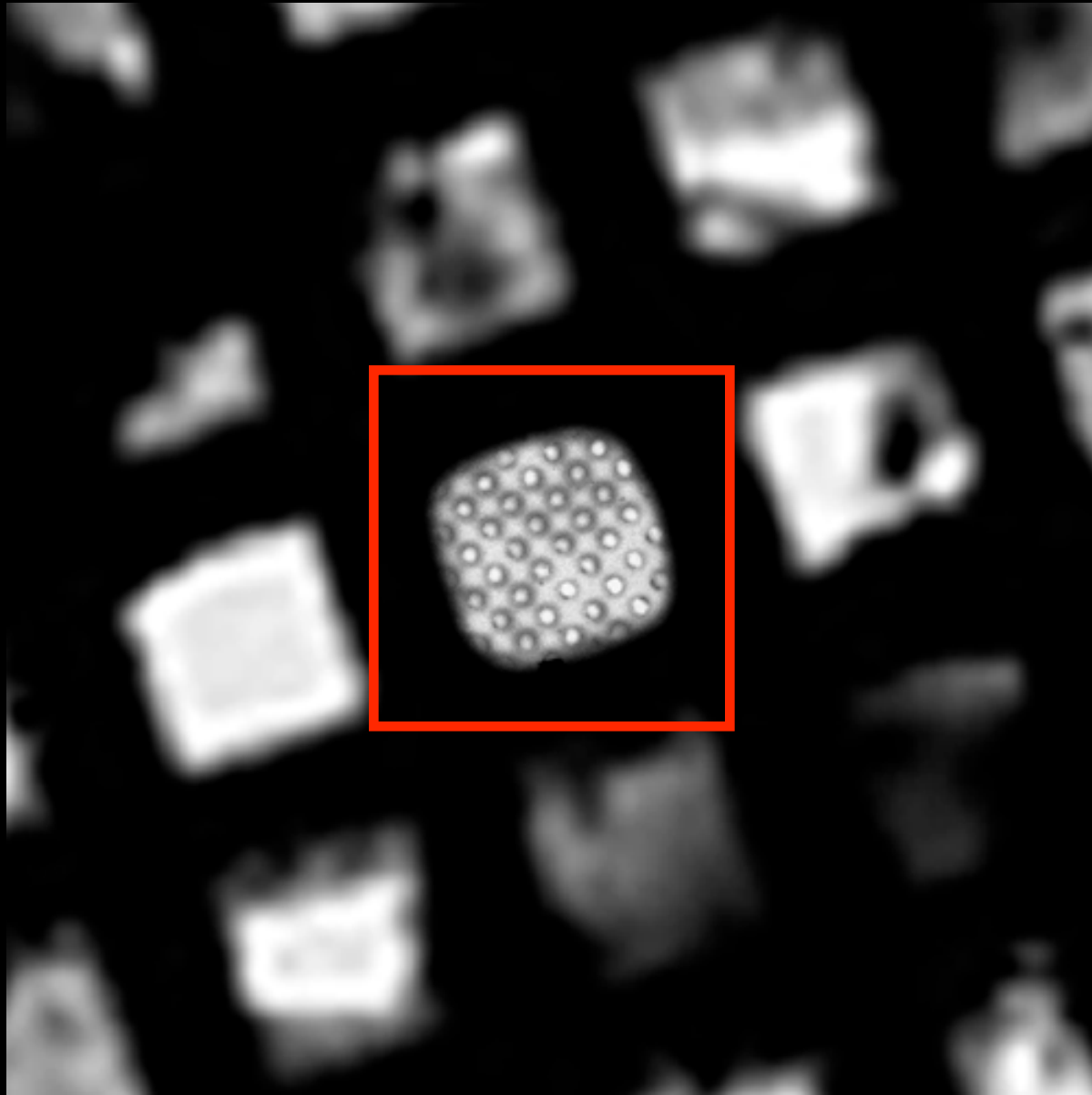


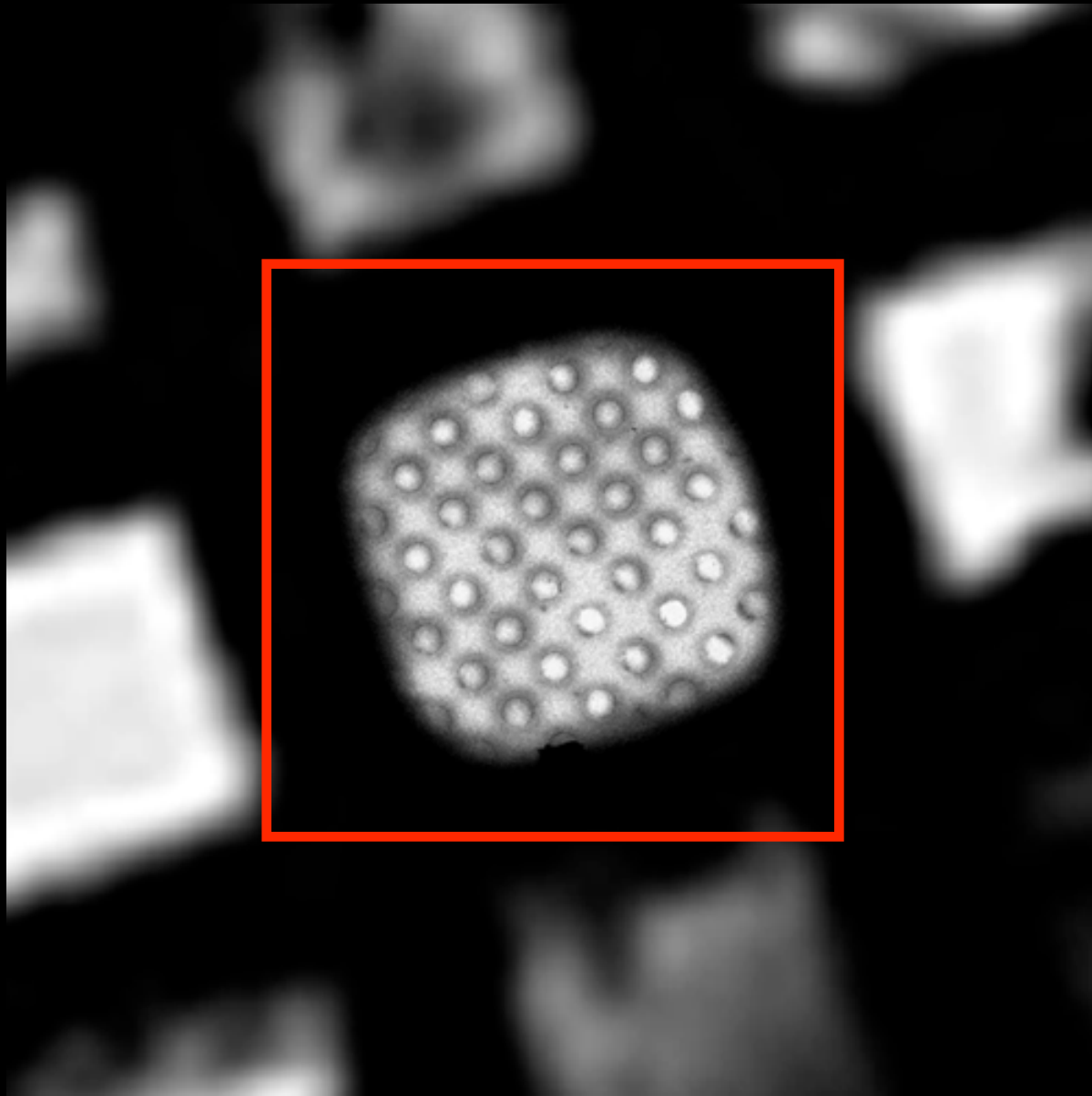


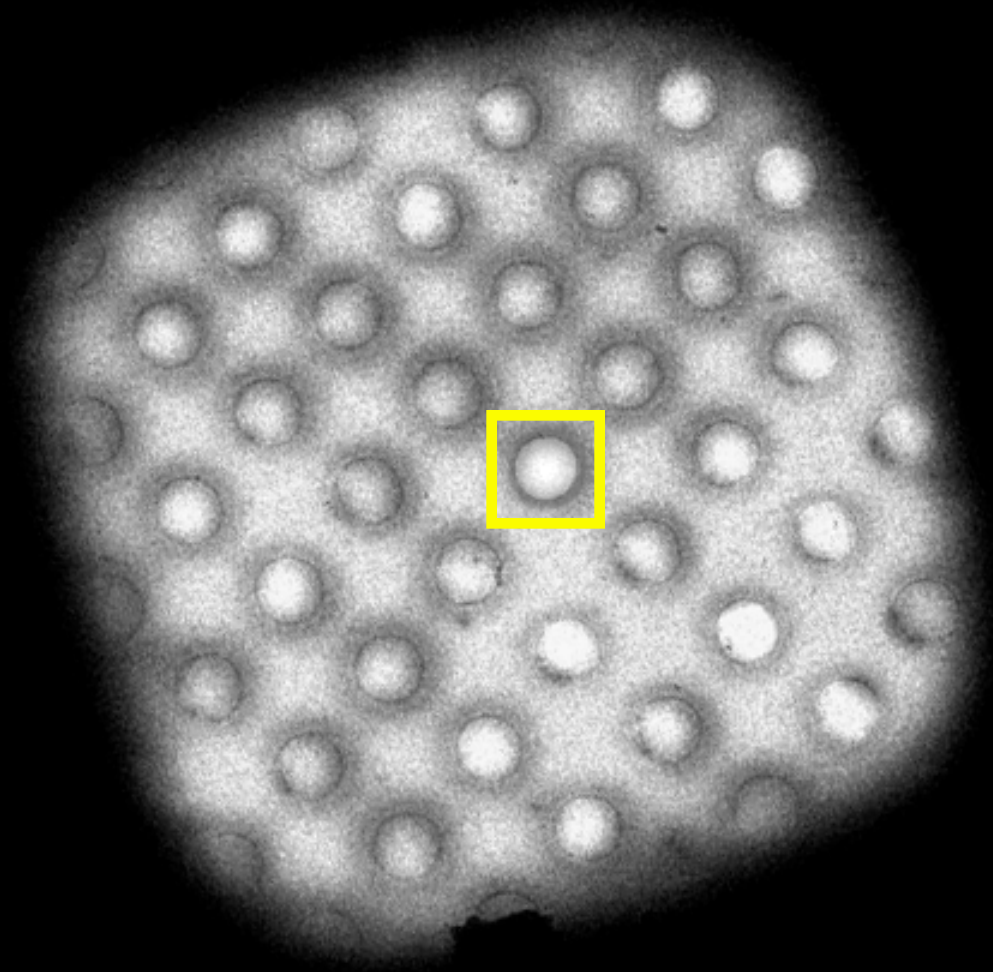


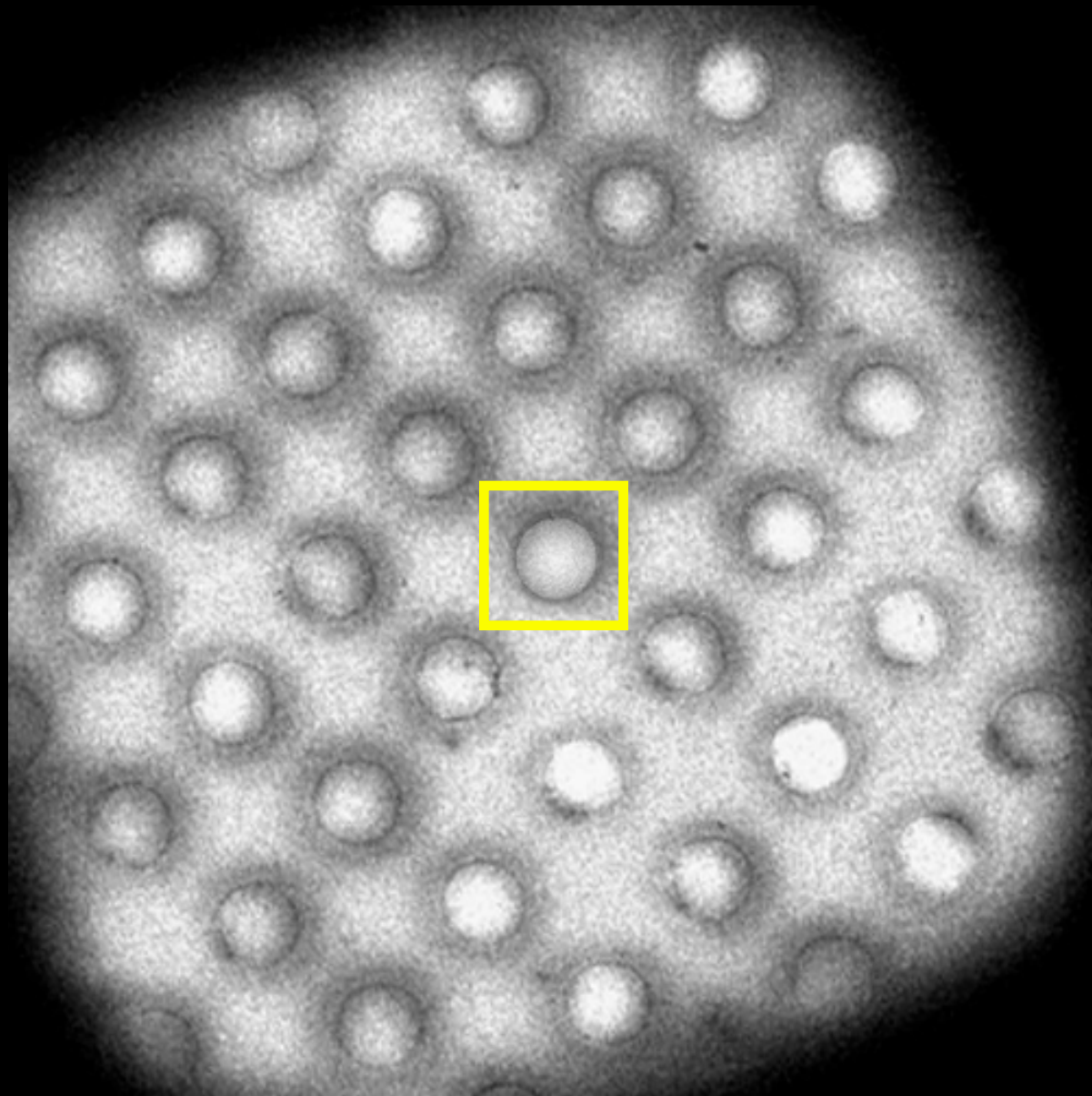


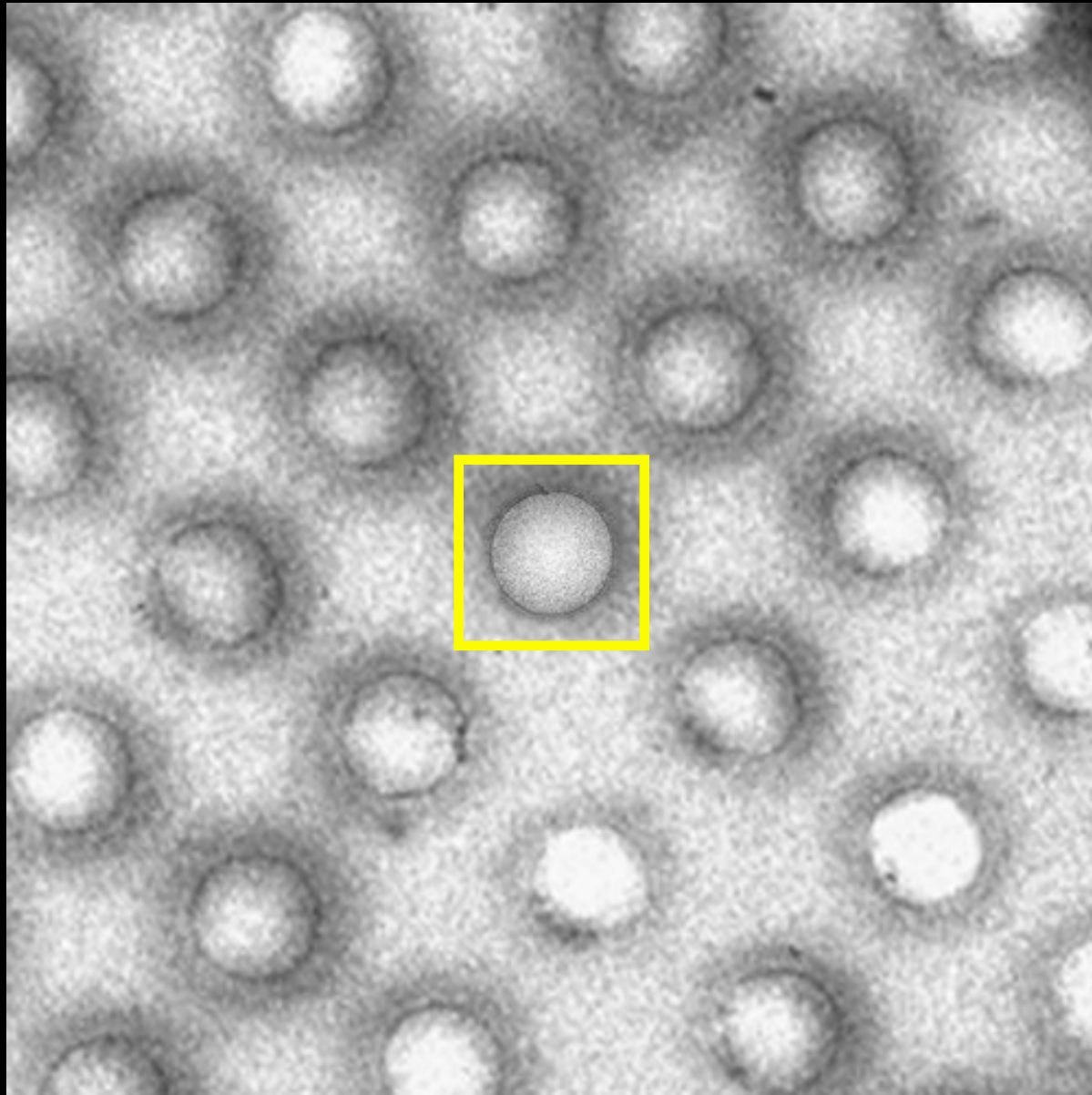


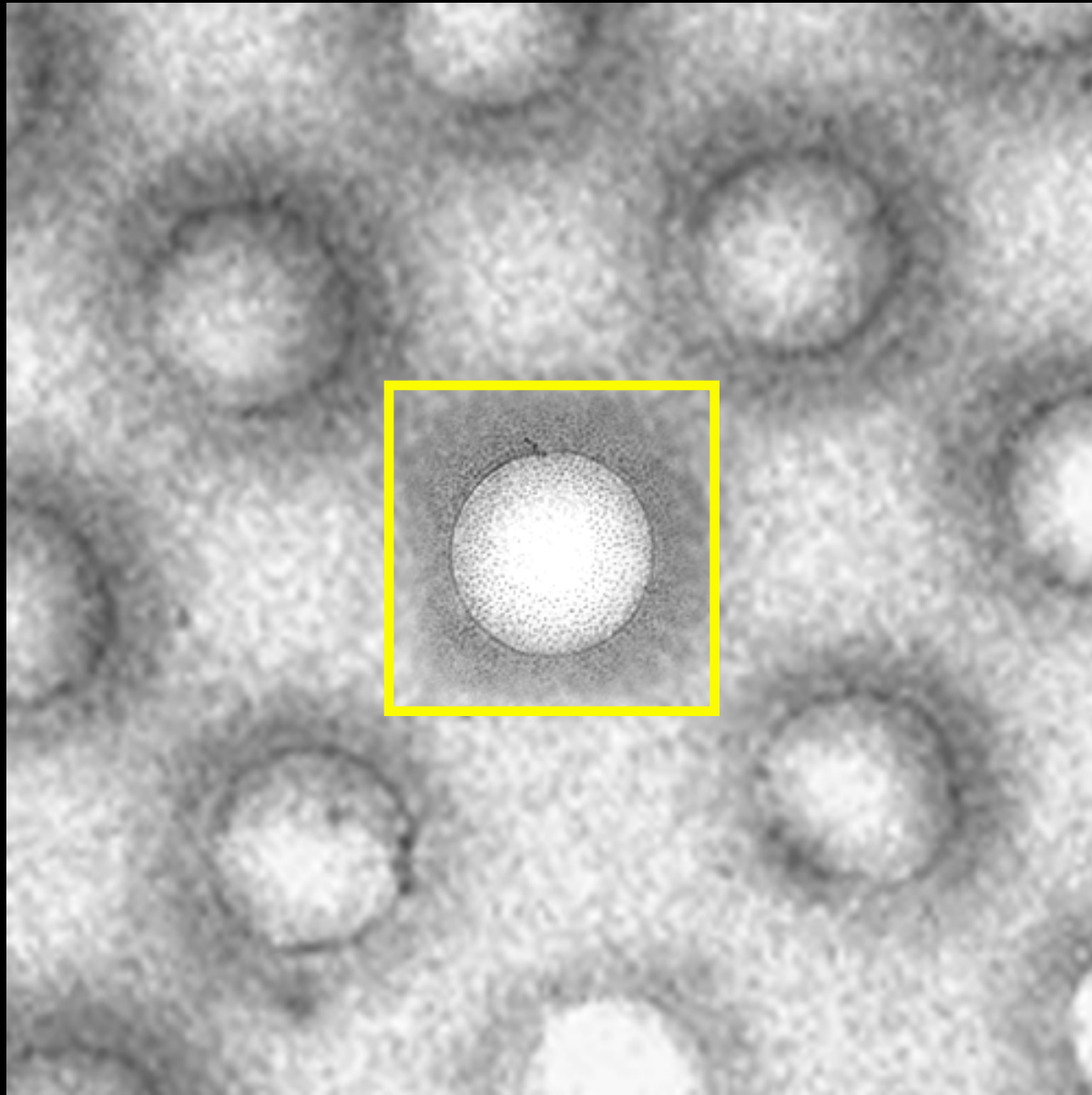


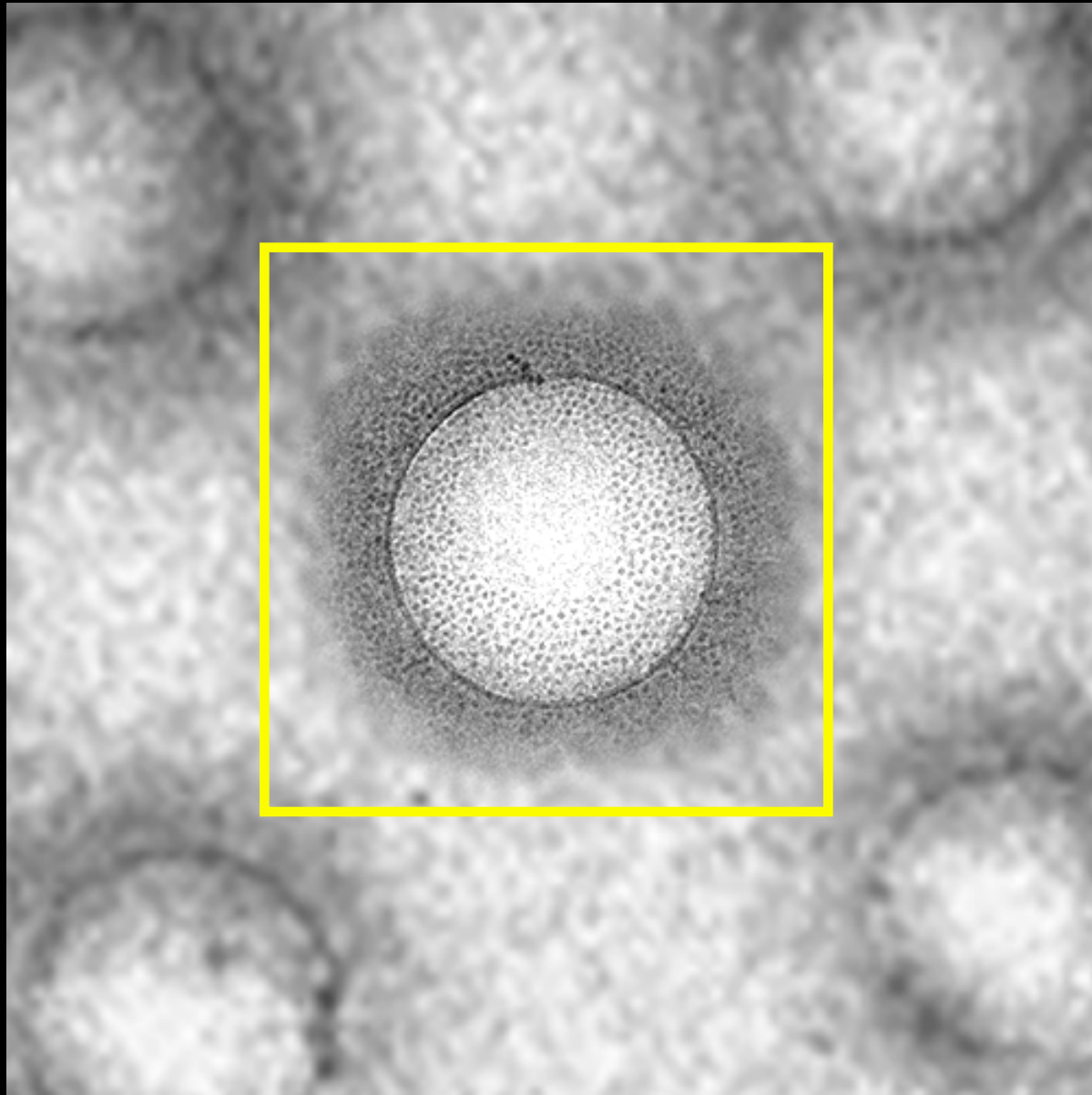


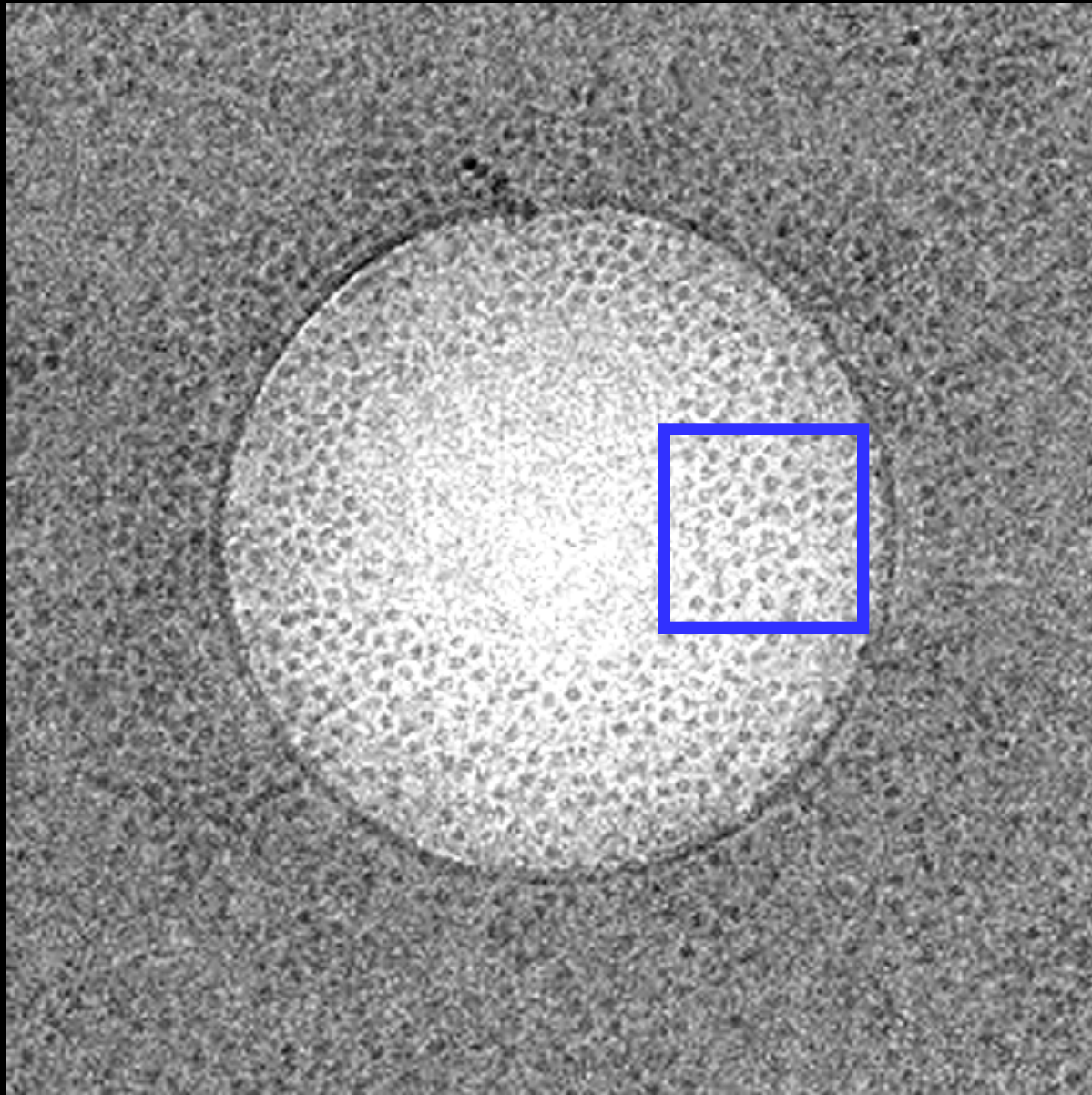


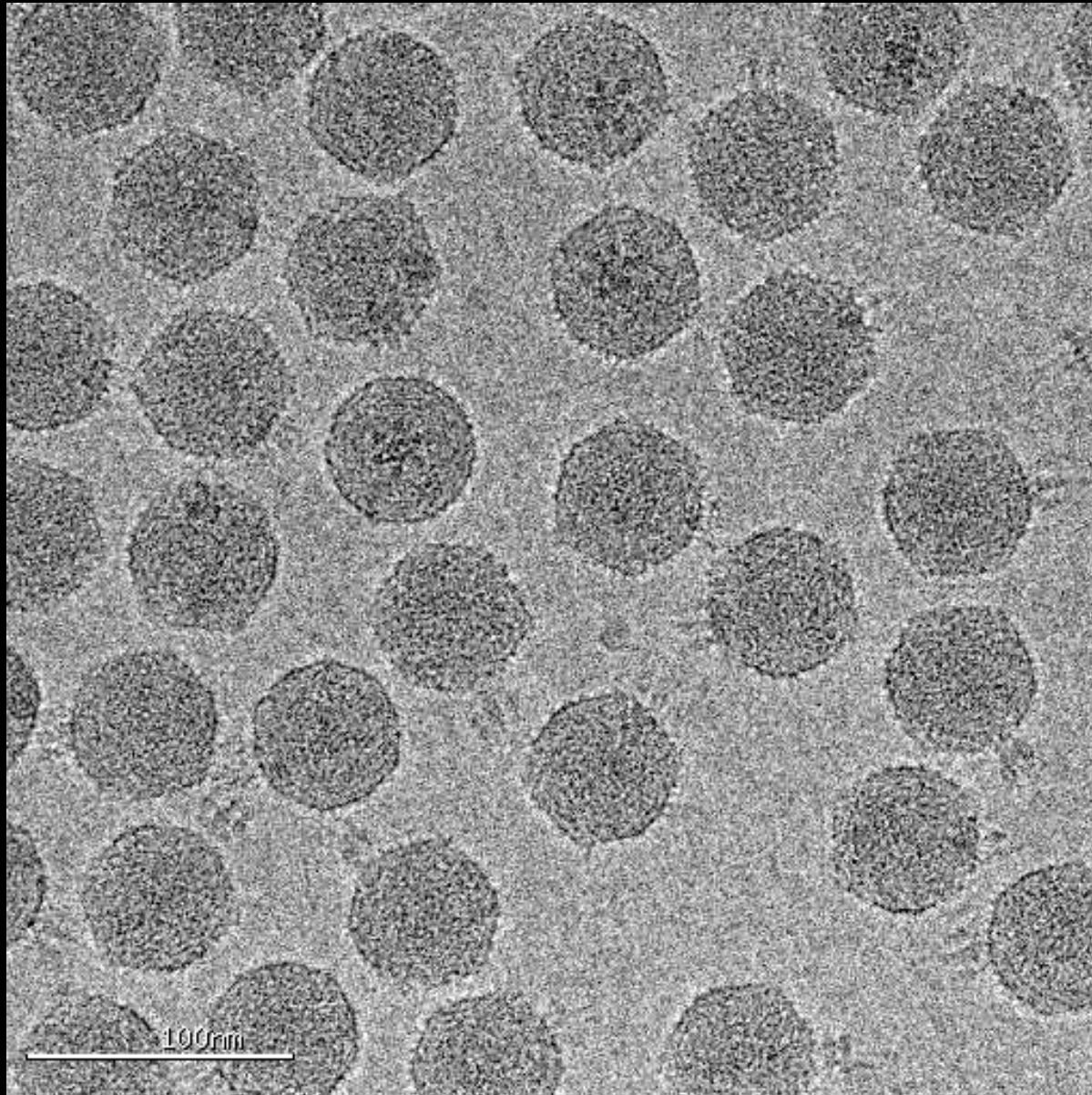




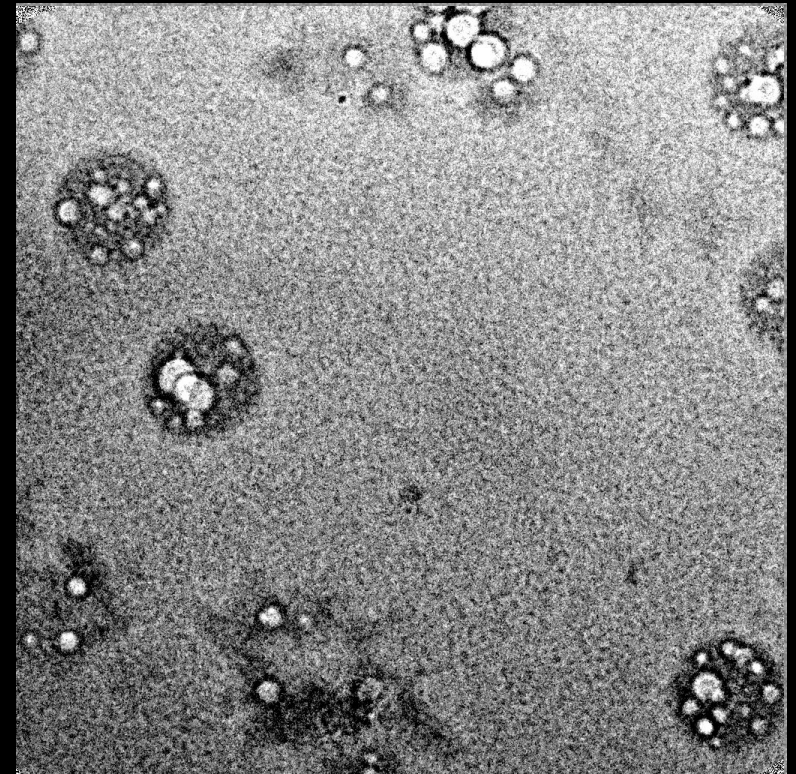
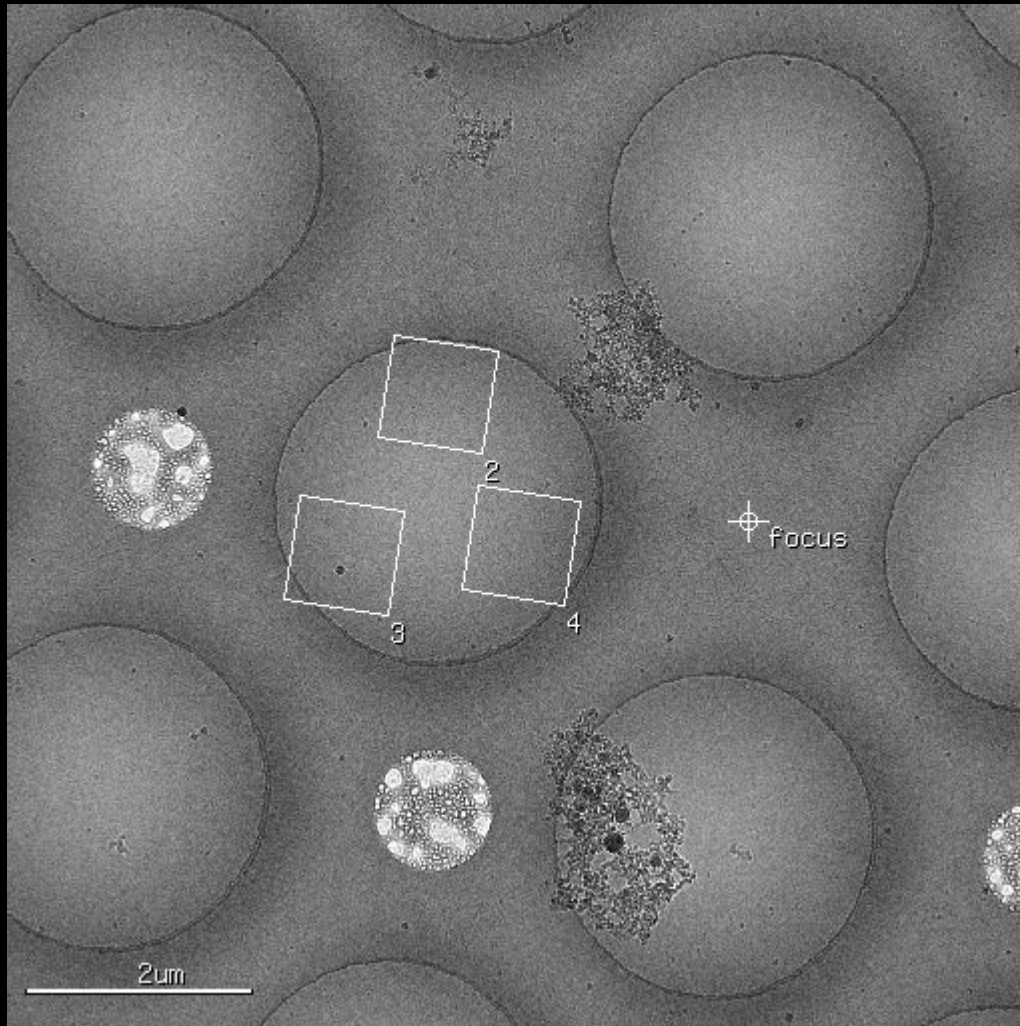






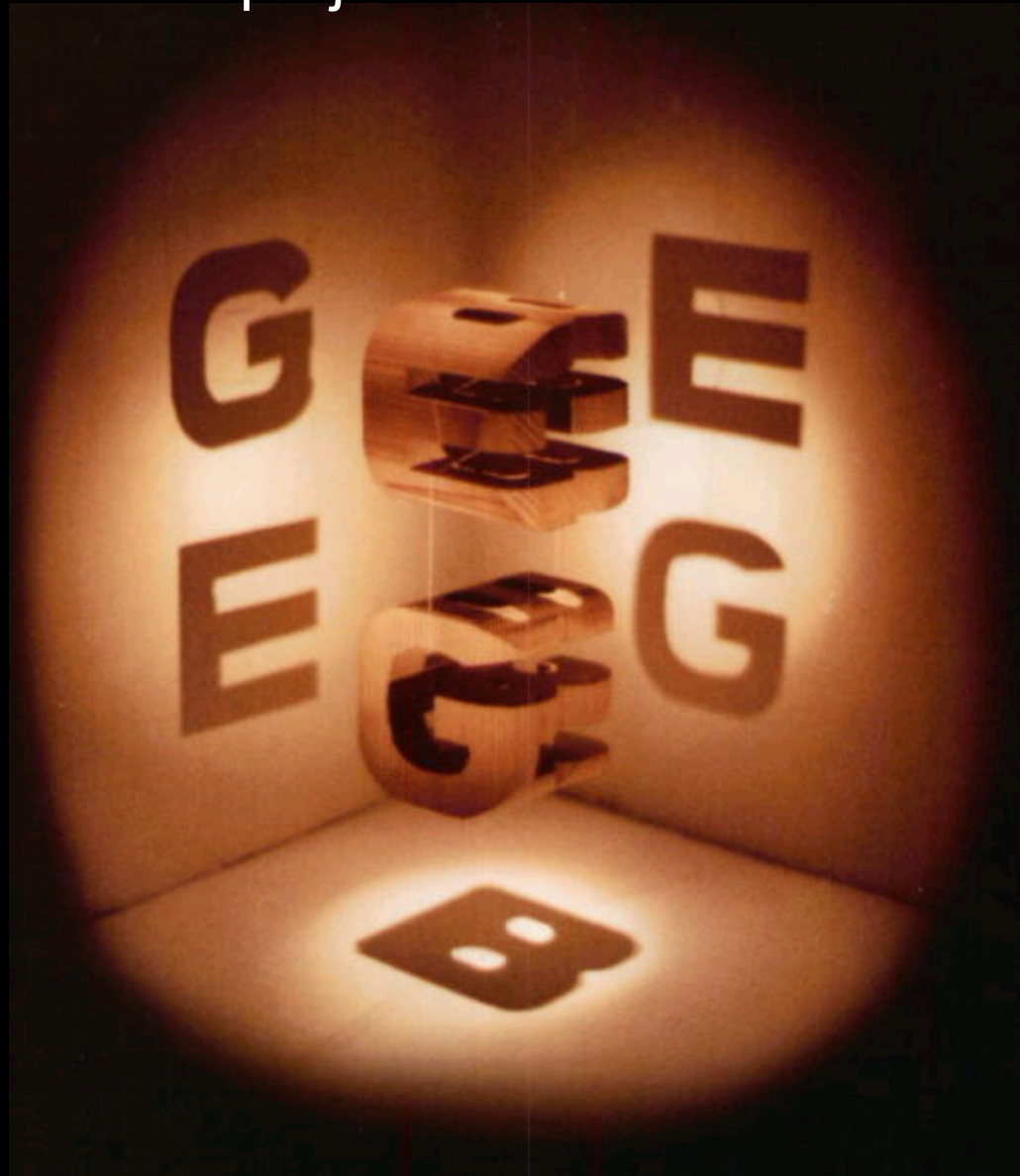


The physical challenge of CryoEM - Radiation damage



“Low-dose” imaging

Plus... The mathematical challenge of cryoEM:
2D projections to 3D volumes

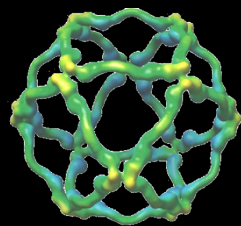


From: Gödel, Escher, Bach: An Eternal Golden Braid By Douglas R. Hofstadter

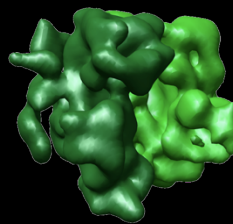
3D Structure of Macromolecular Machines: “electron crystallography” or “single particle” reconstruction methods



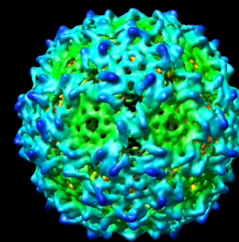
HBV



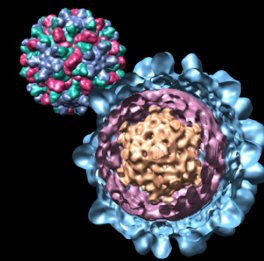
COPII



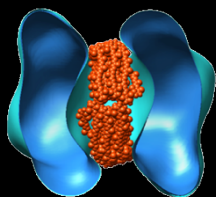
Ribosome



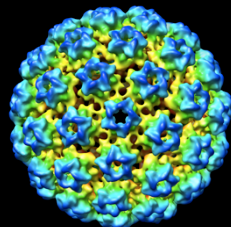
Qbeta



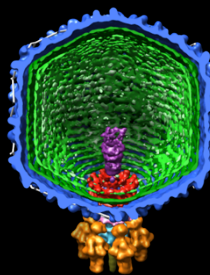
CNV



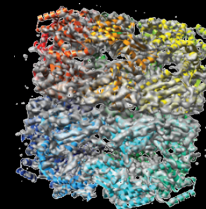
LCP



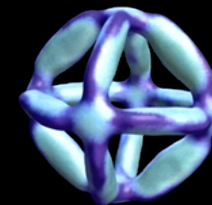
HPV



P22



GroEL



DNA

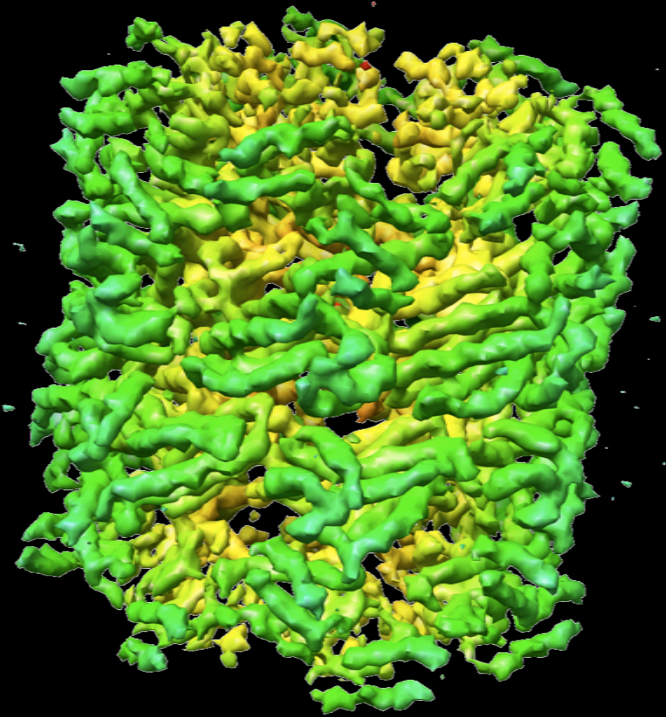
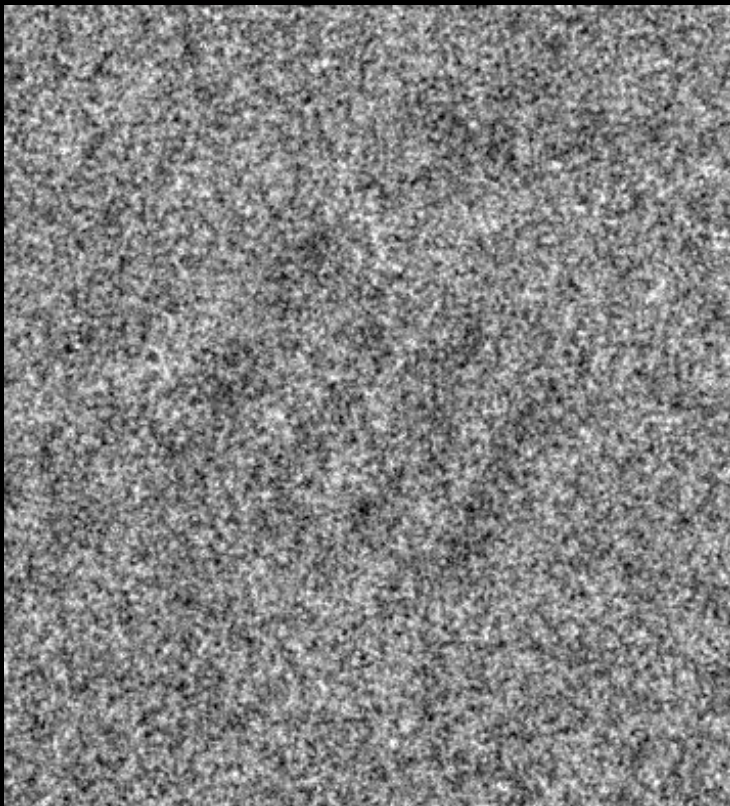
The challenge is:

How to we go from....

this

to...

that



The first step: Improving Signal to Noise using Averaging



1 copy



10 copies



50 copies



100 copies



200 copies



400 copies

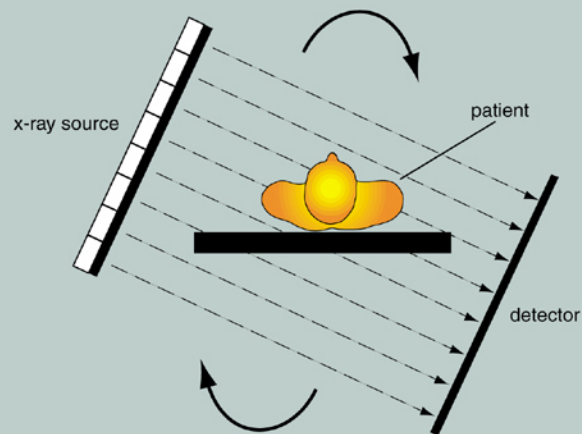


600 copies



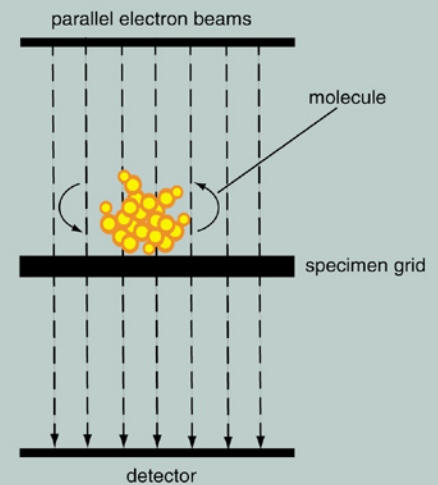
1000 copies

Plus the mathematics of tomography...



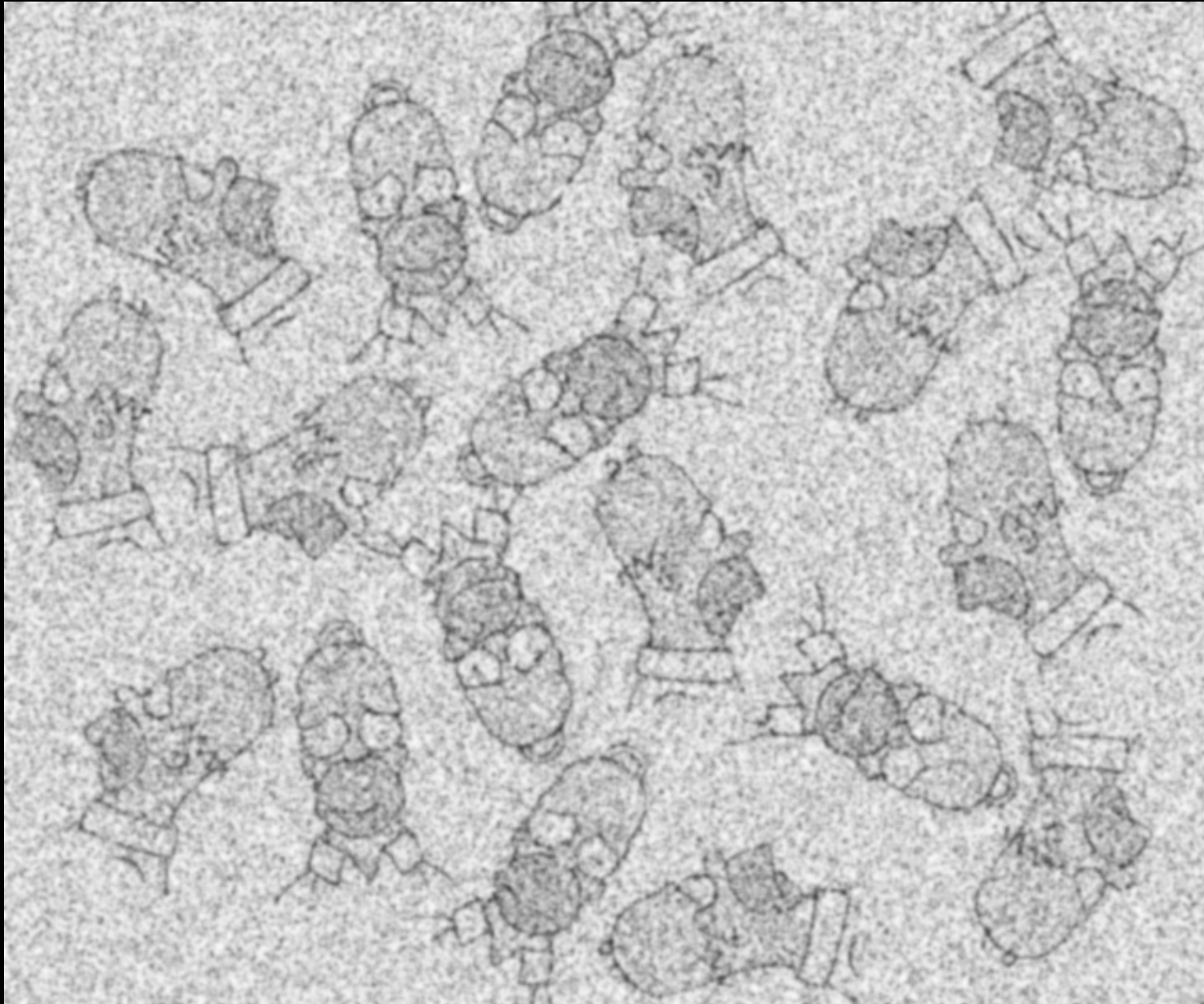
CAT - scan

- beam rotating
- patient stationary

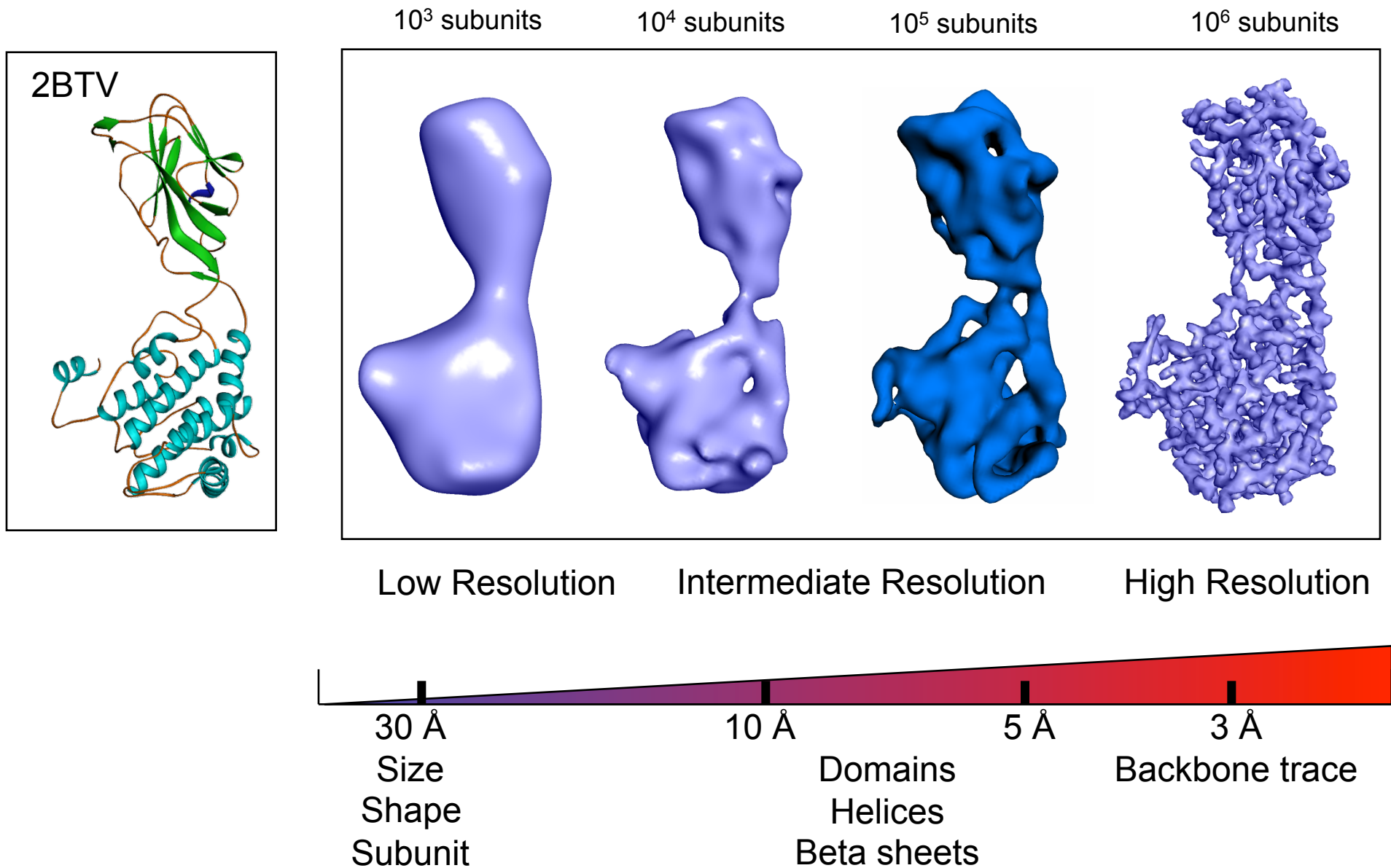


Single particle reconstruction

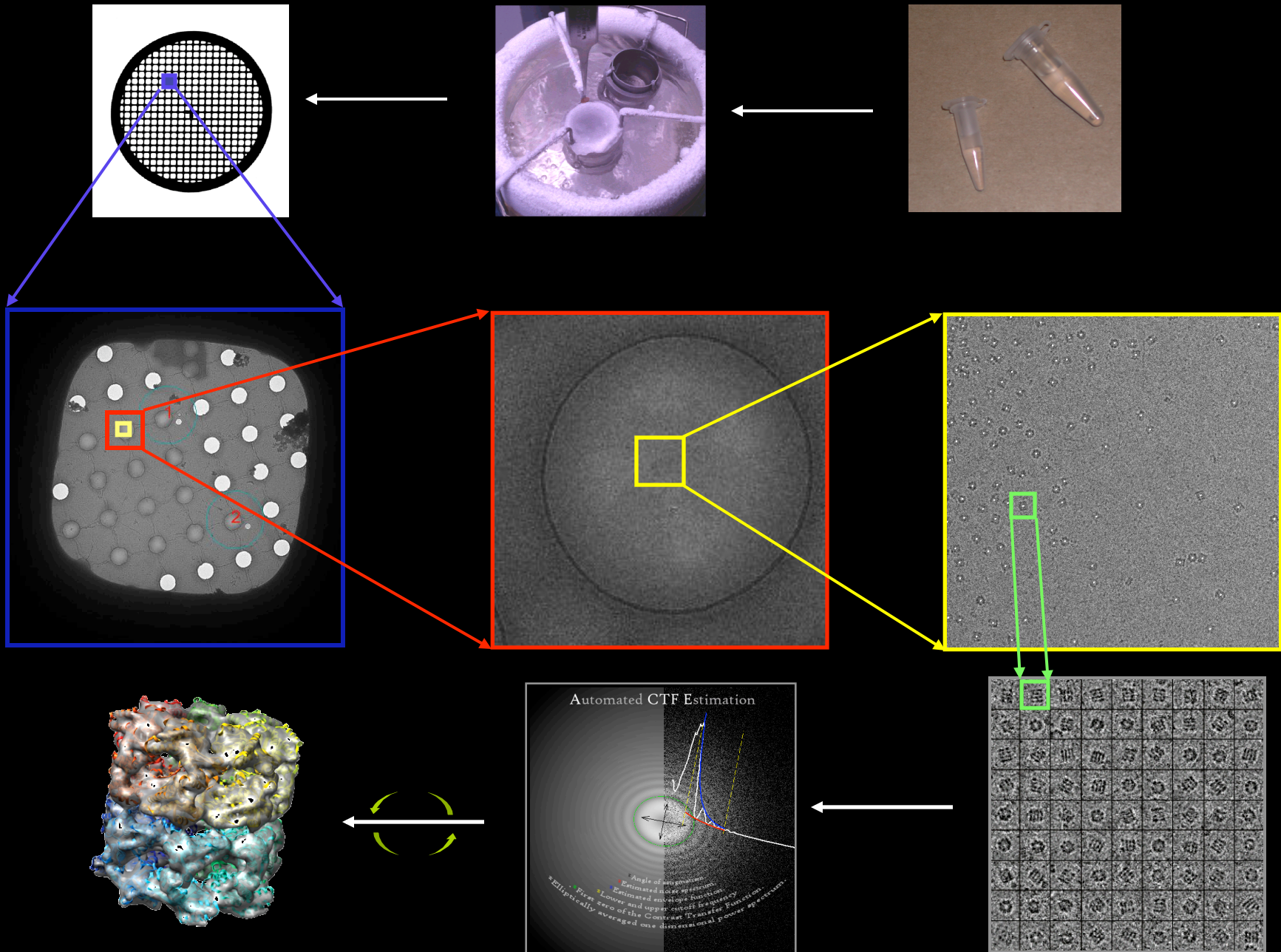
- molecule "rotating"
- beam stationary



The relation between averaging and resolution:



Single particle reconstruction: structure of macromolecular machines



NRAMM

National Resource for Automated Molecular Microscopy

The overall mission of NRAMM is to develop, test and apply technology aimed towards automating and streamlining cryo-electron microscopy (cryoEM) for structural biology.

Automation goals

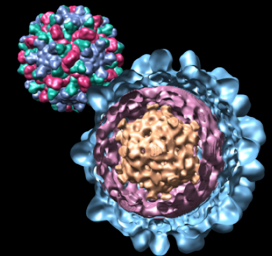
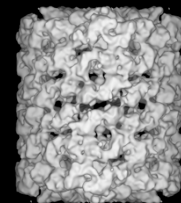
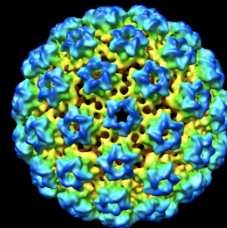
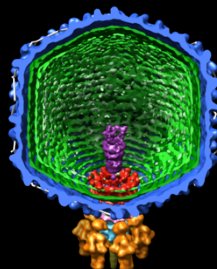
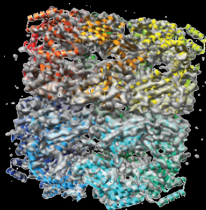
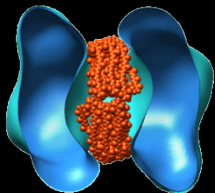
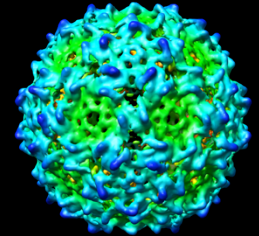
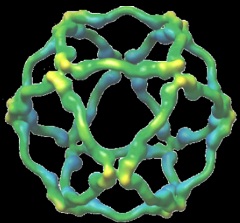
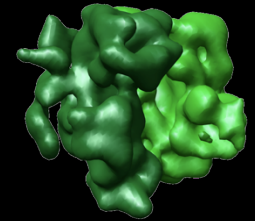
Facilitate the process

Increase throughput

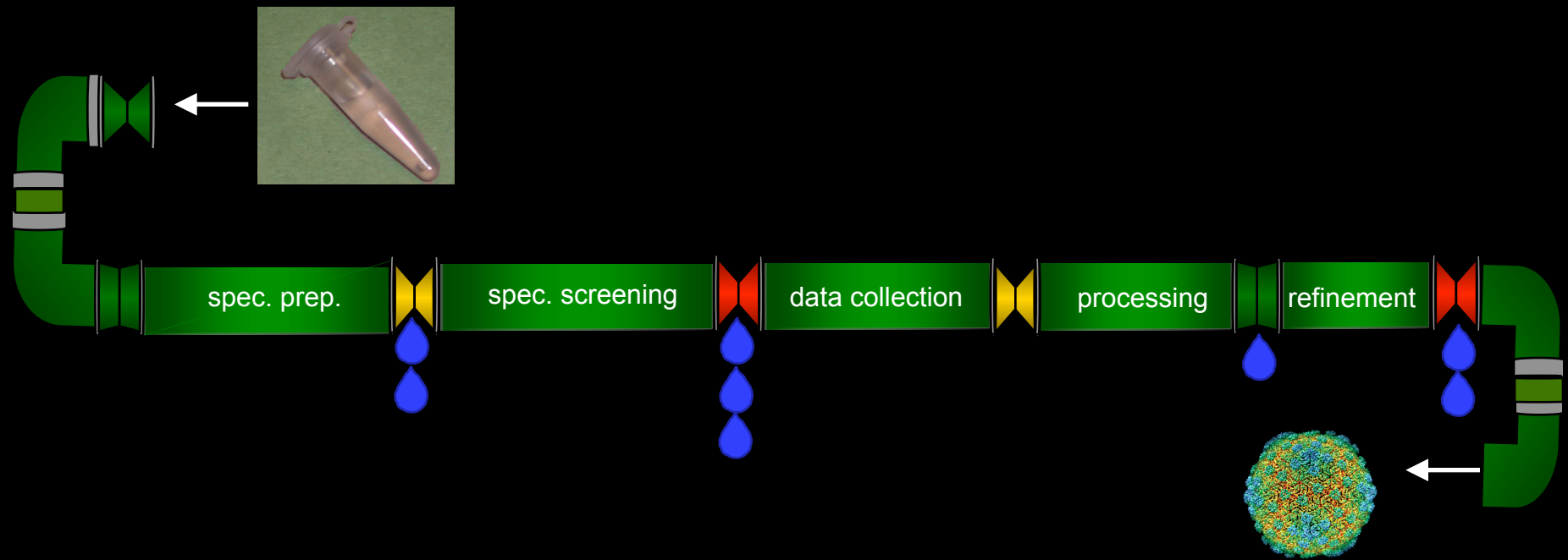
Optimize resolution

Expand the possibilities

Open the technology to wider audience



Automated Pipeline for Molecular Microscopy

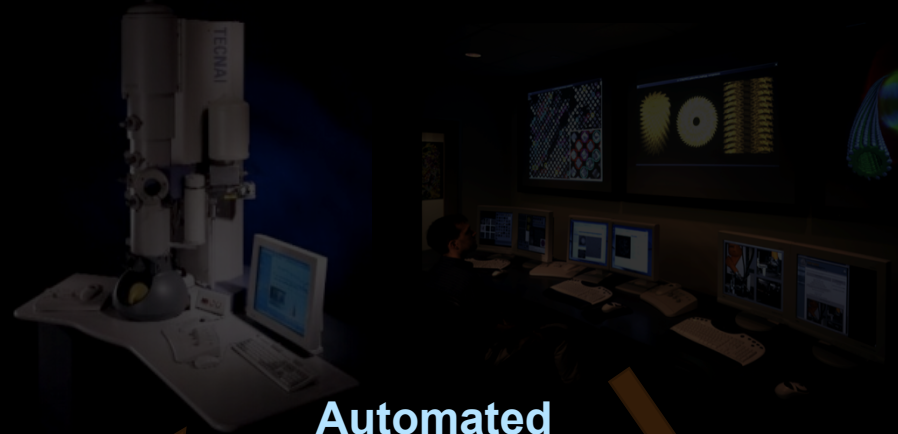


Adapted from a slide courtesy of: Peter Kuhn, Scripps-PARC Institute for Advanced Biomedical Sciences, TSRI

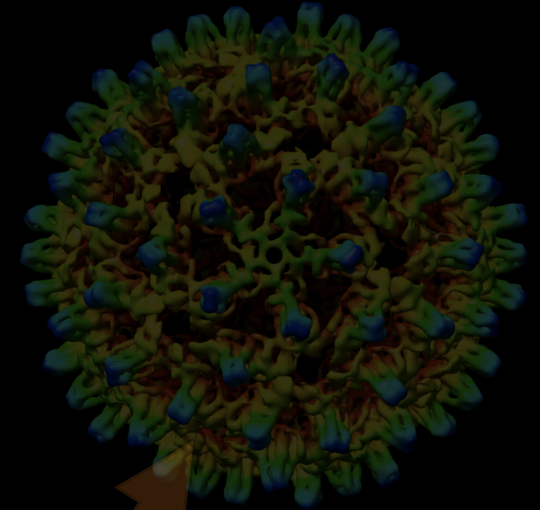
CryoEM Pipeline at NRAMM



Sample



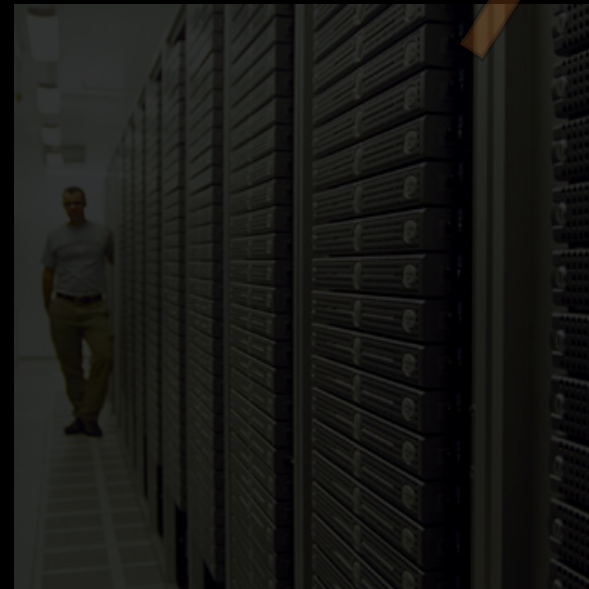
**Automated
Data Collection
(LEGION)**



3D EM Density



Specimen Vitrification

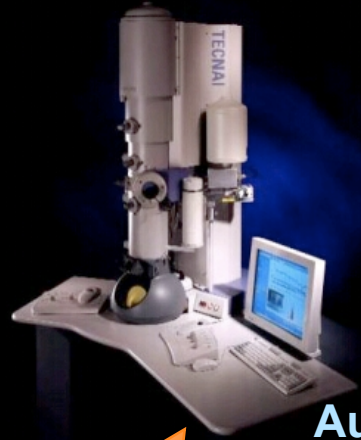


**Streamlined
Processing
(Appion)**

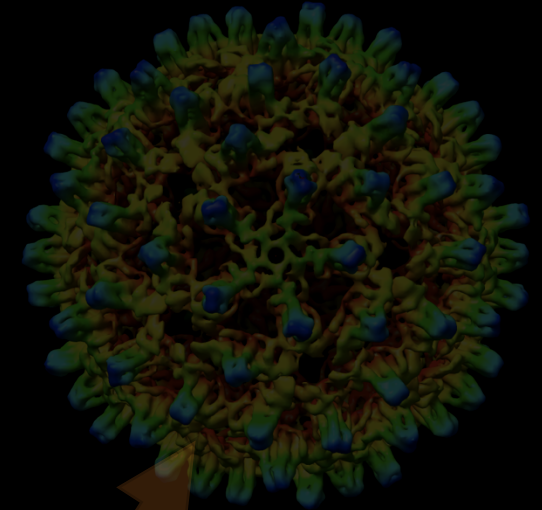
CryoEM Pipeline at NRAMM



Sample



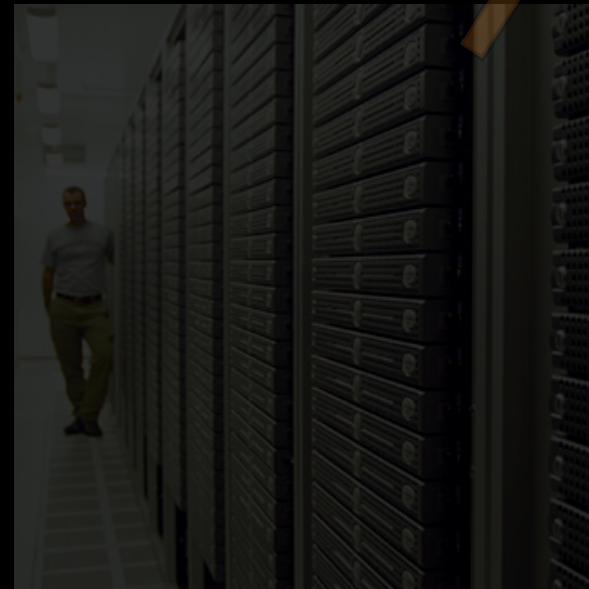
**Automated
Data Collection
(LEGION)**



3D EM Density



Specimen Vitrification

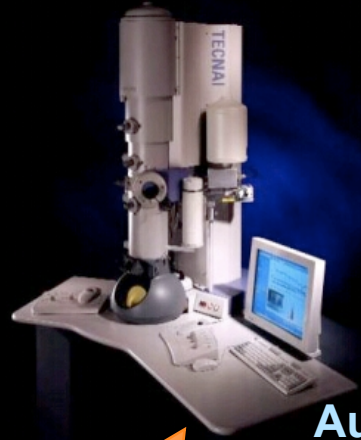


**Streamlined
Processing
(Appion)**

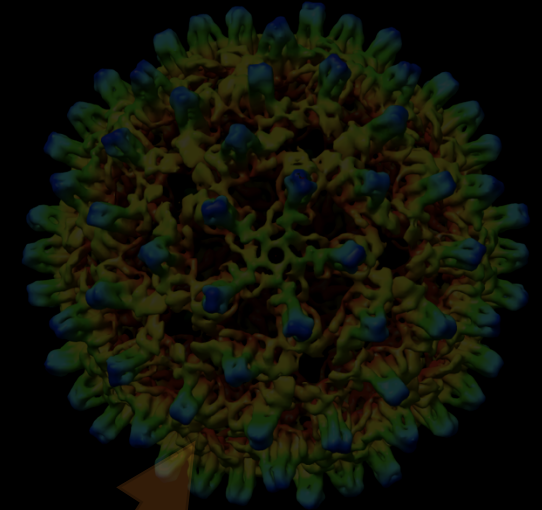
CryoEM Pipeline at NRAMM



Sample



**Automated
Data Collection
(LEGION)**



3D EM Density



Specimen Vitrification

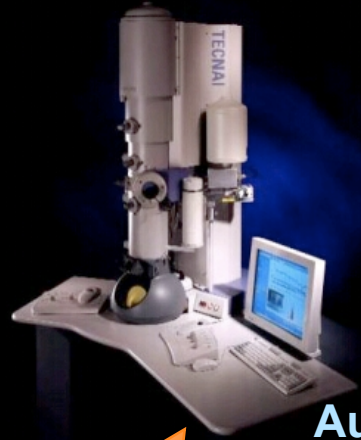


**Streamlined
Processing
(Appion)**

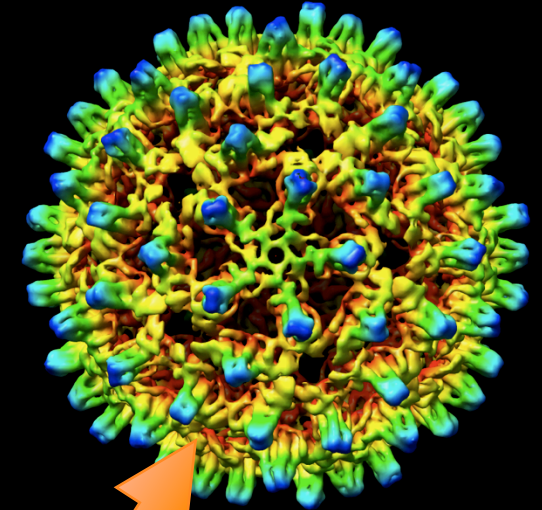
CryoEM Pipeline at NRAMM



Sample



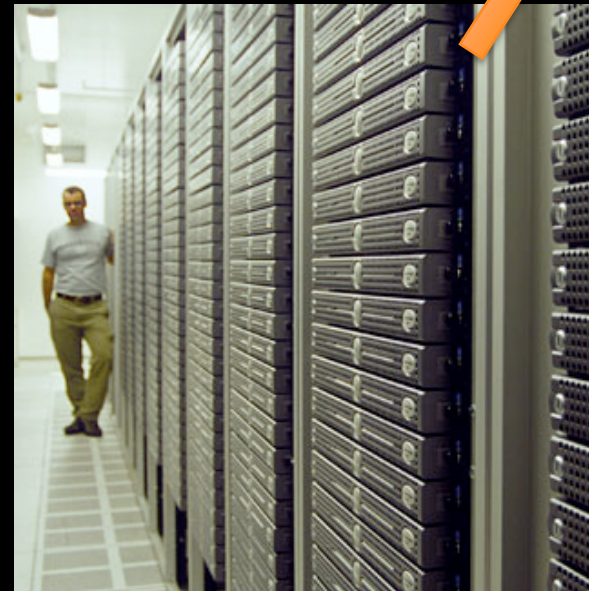
**Automated
Data Collection
(LEGION)**



3D EM Density



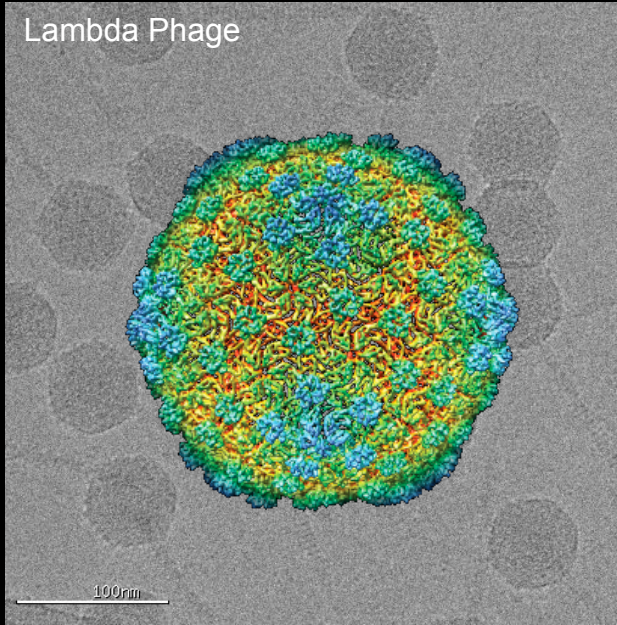
Specimen Vitrification



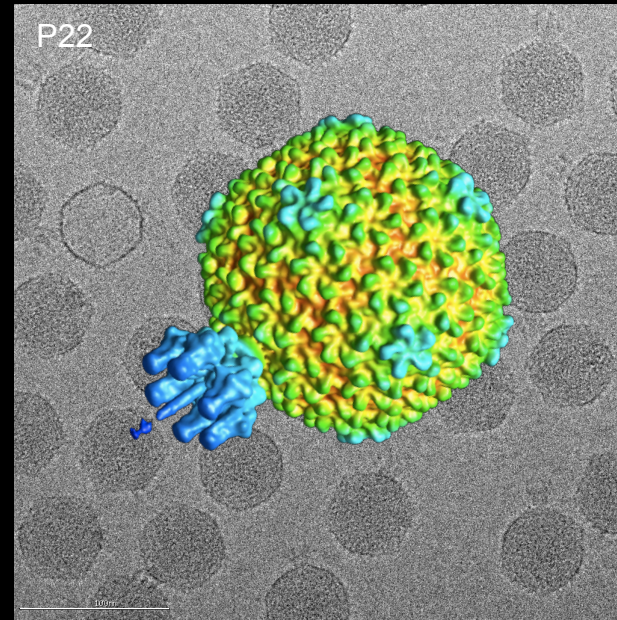
**Streamlined
Processing
(Appion)**

Four case studies to illustrate the pipeline....

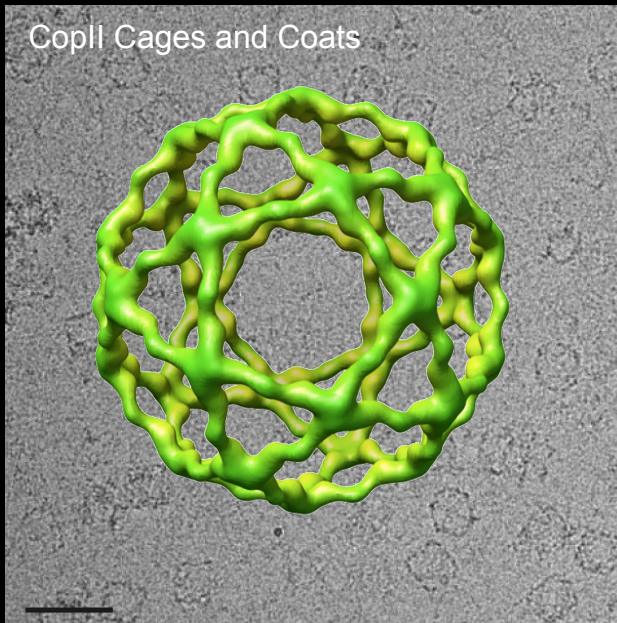
Lambda Phage



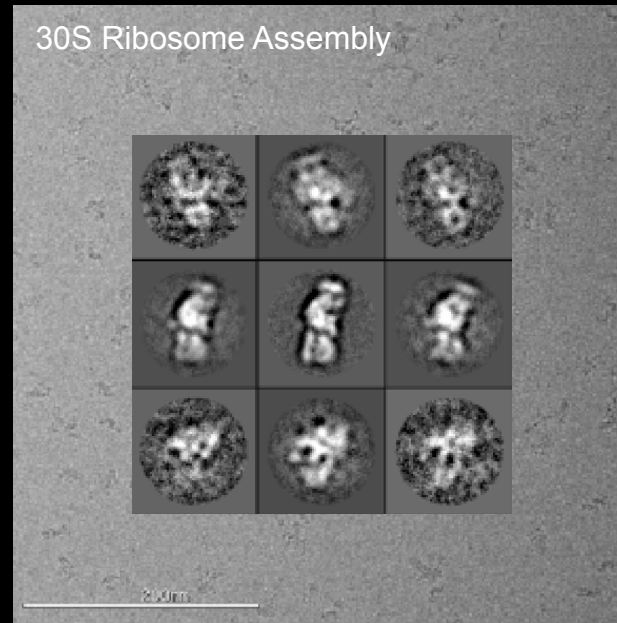
P22



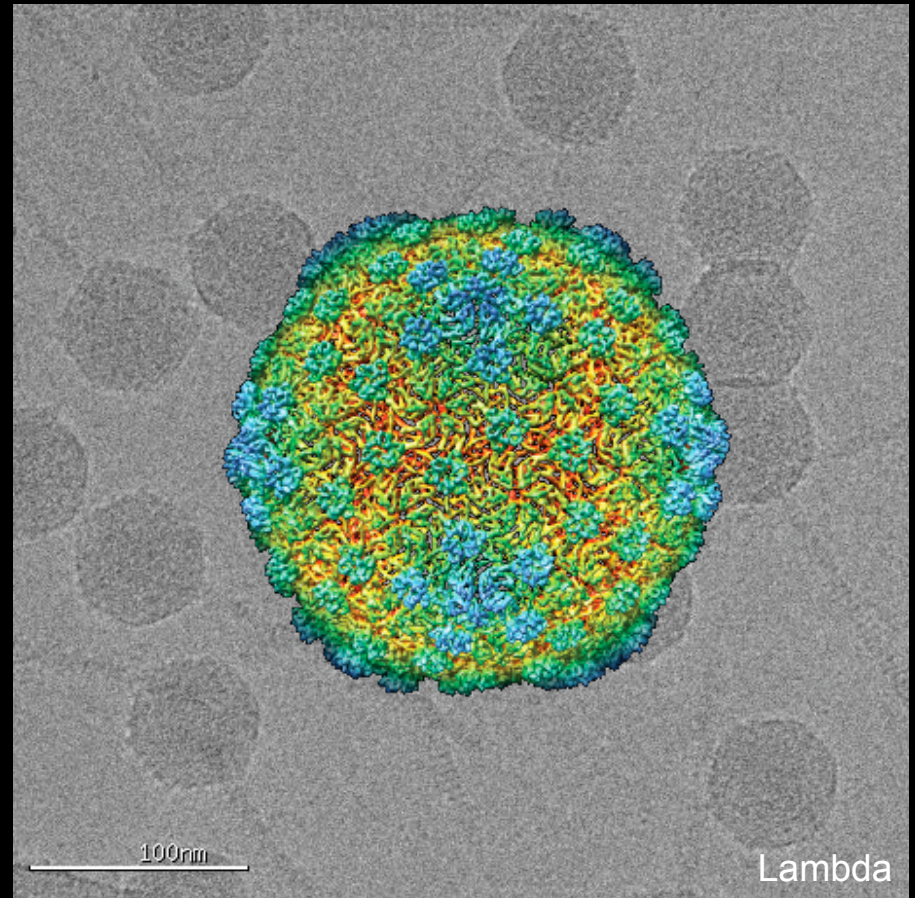
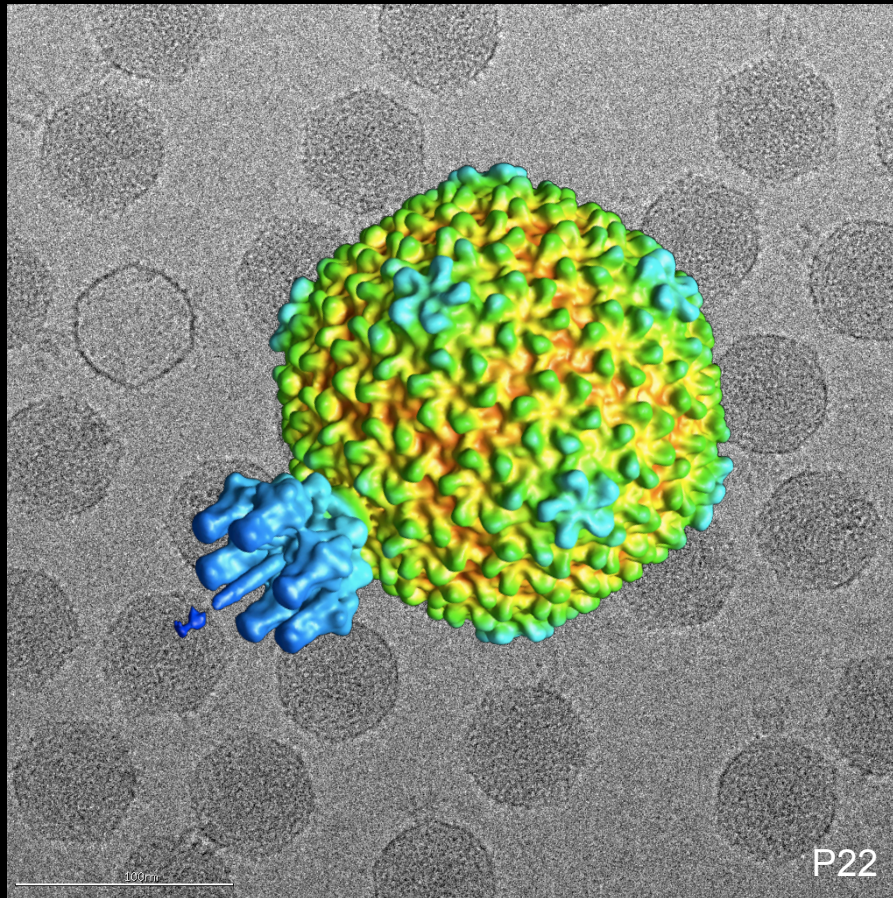
CopII Cages and Coats



30S Ribosome Assembly



DNA packaging and delivery machines

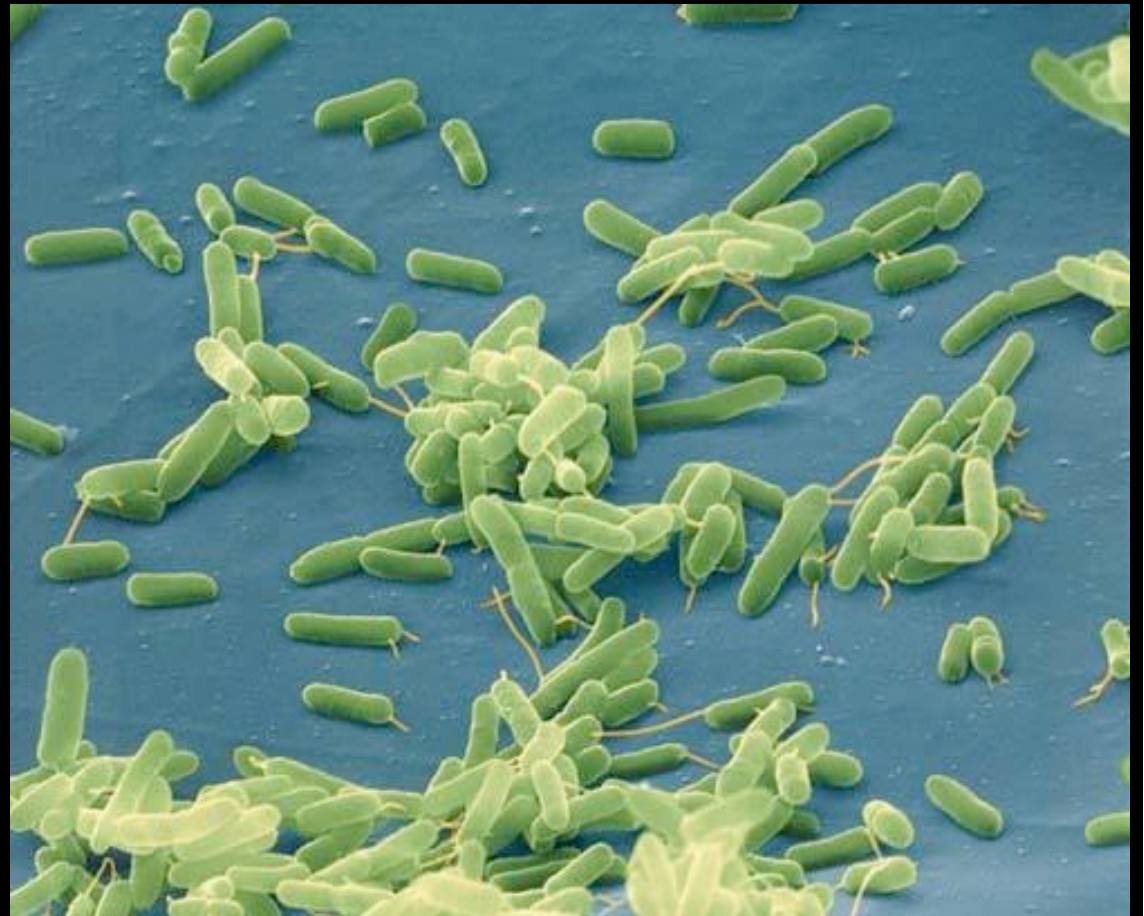
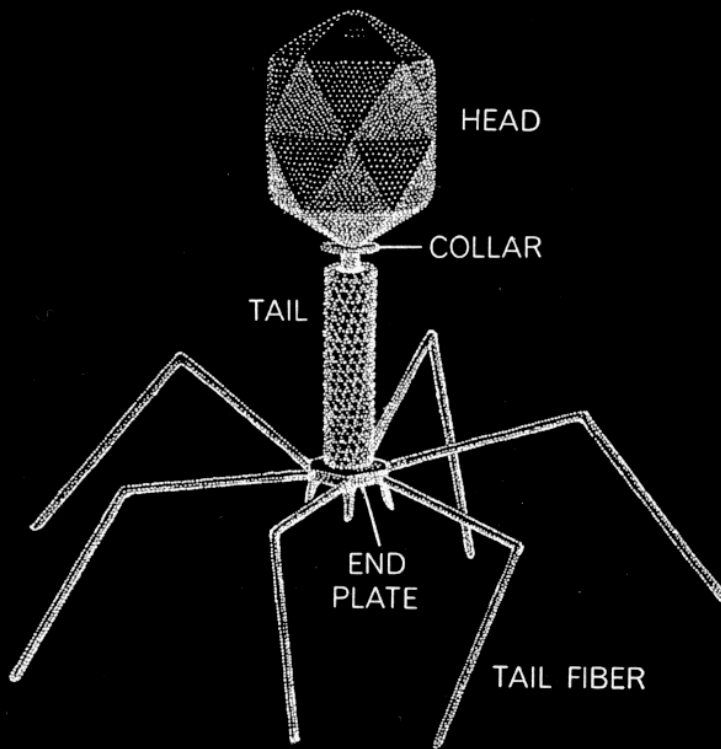


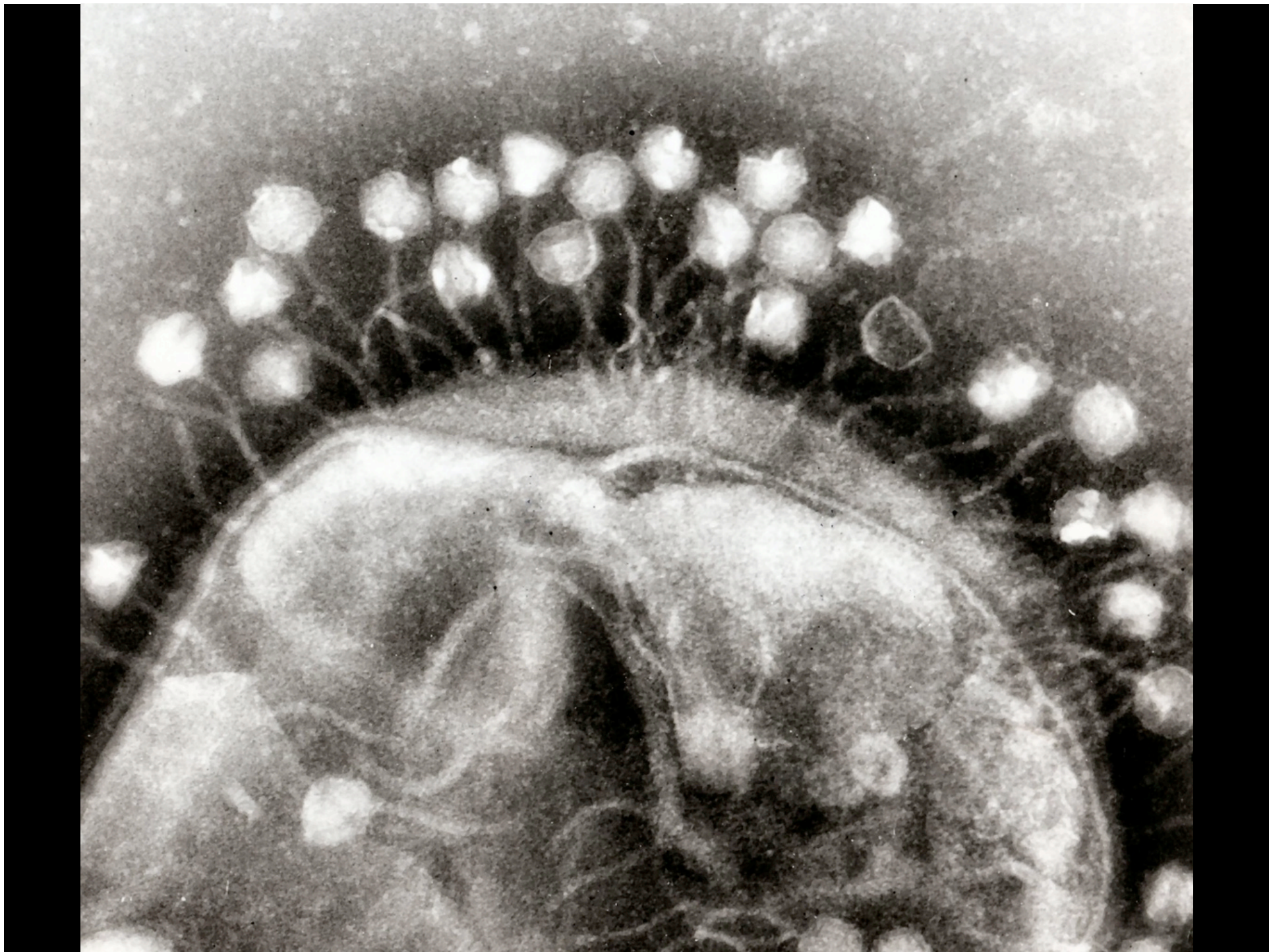
Gabe Lander



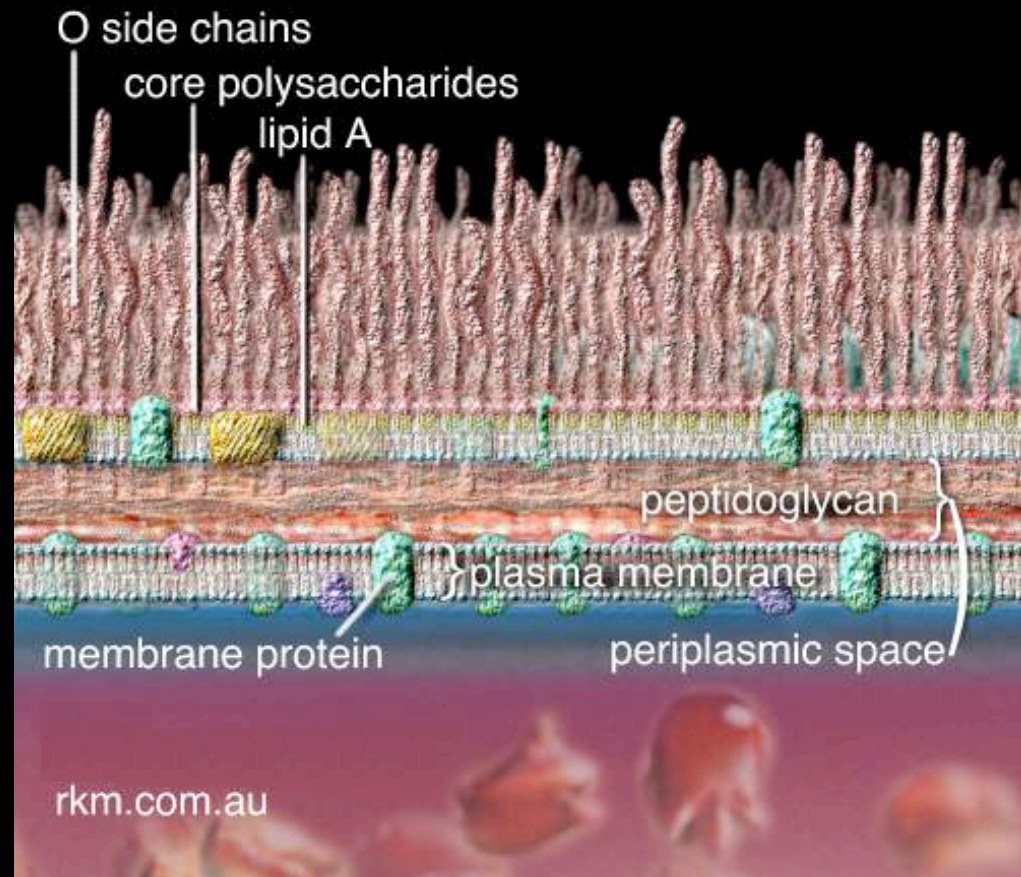
In Collaboration with Alex Evilevitch, Sherwood Casjens, Peter Prevelige, and Jack Johnson.

Viruses vs. Bacteria: An Arms Race

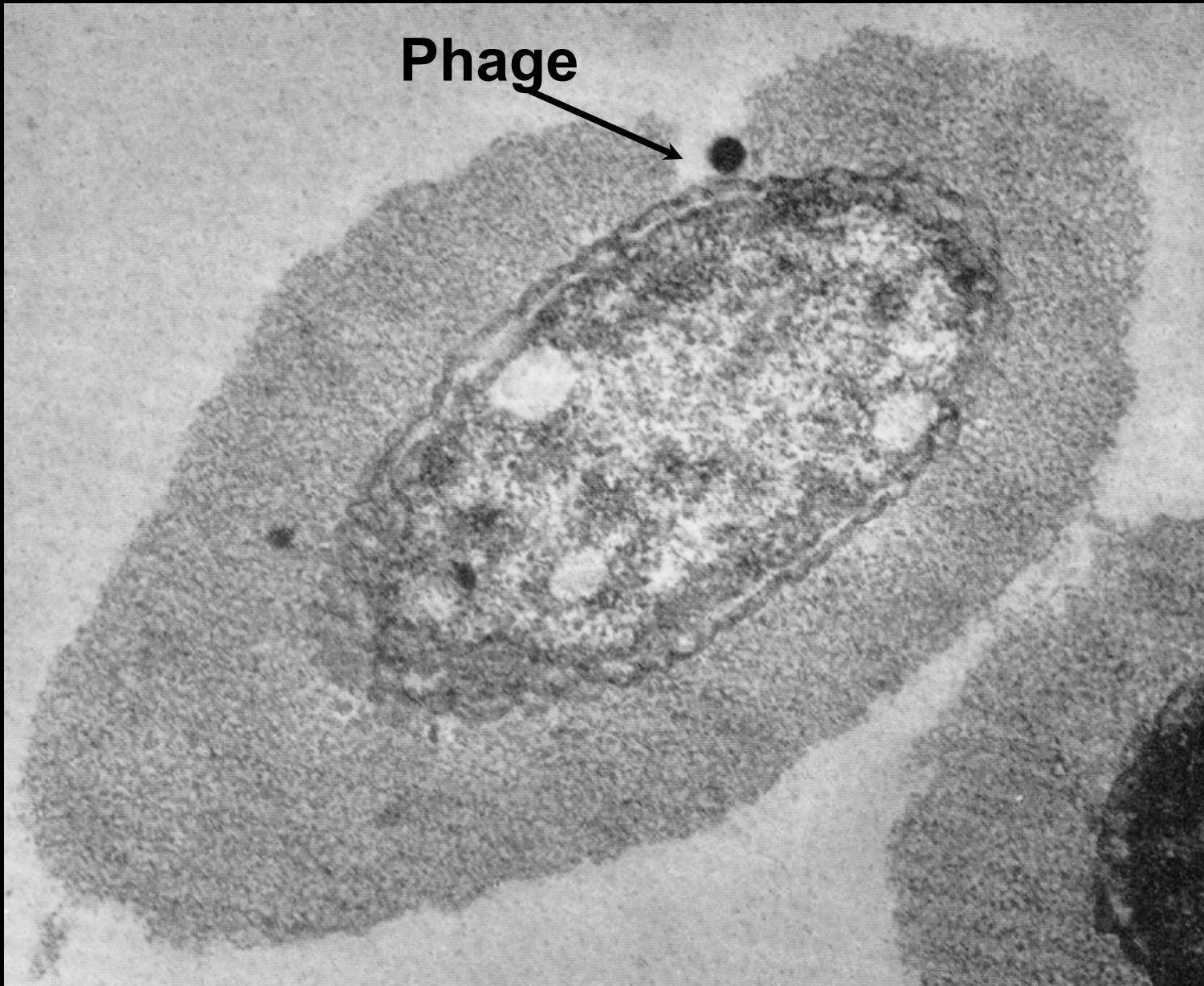




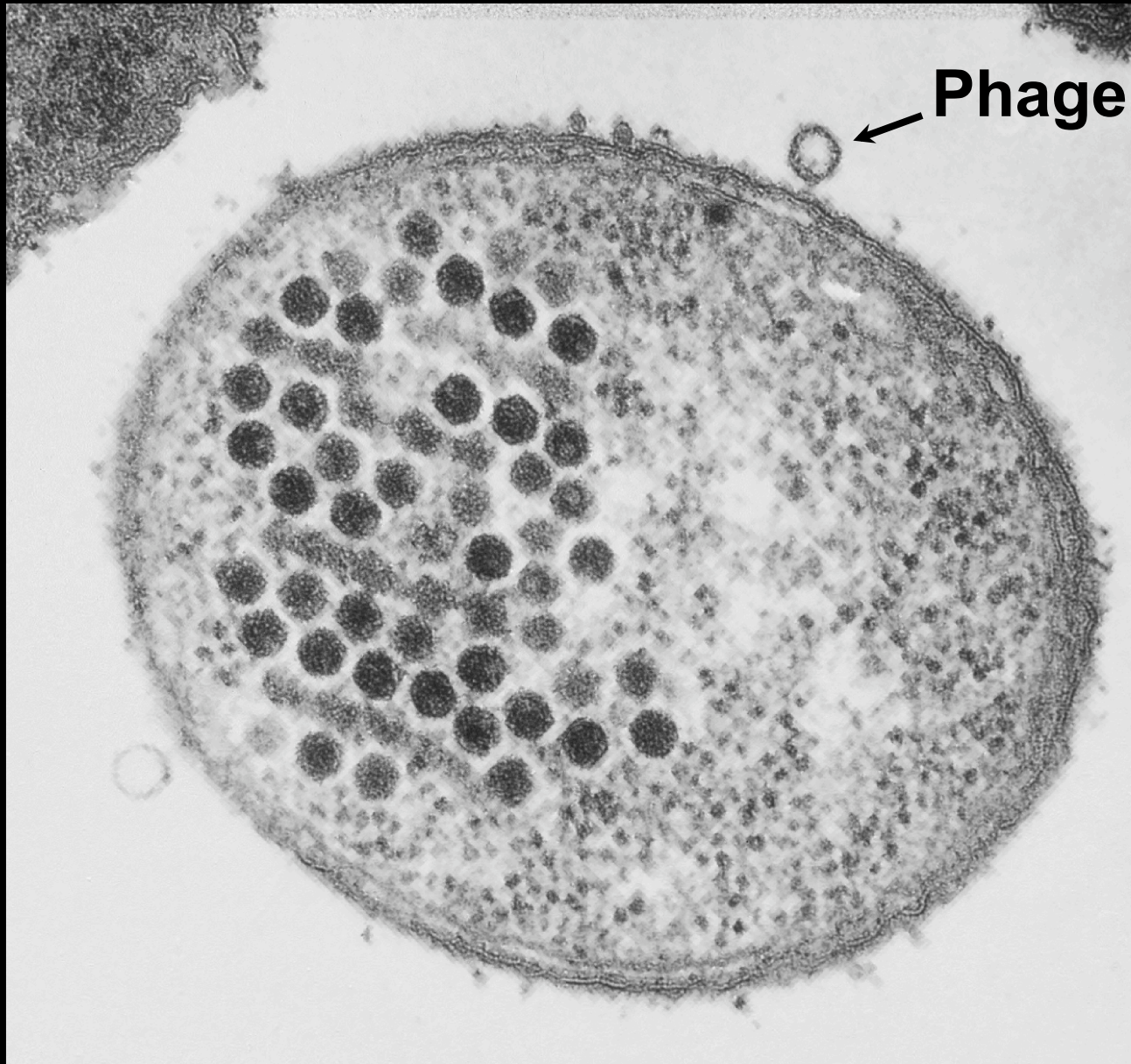
Podoviridae Infection of Gram-Negative Cells



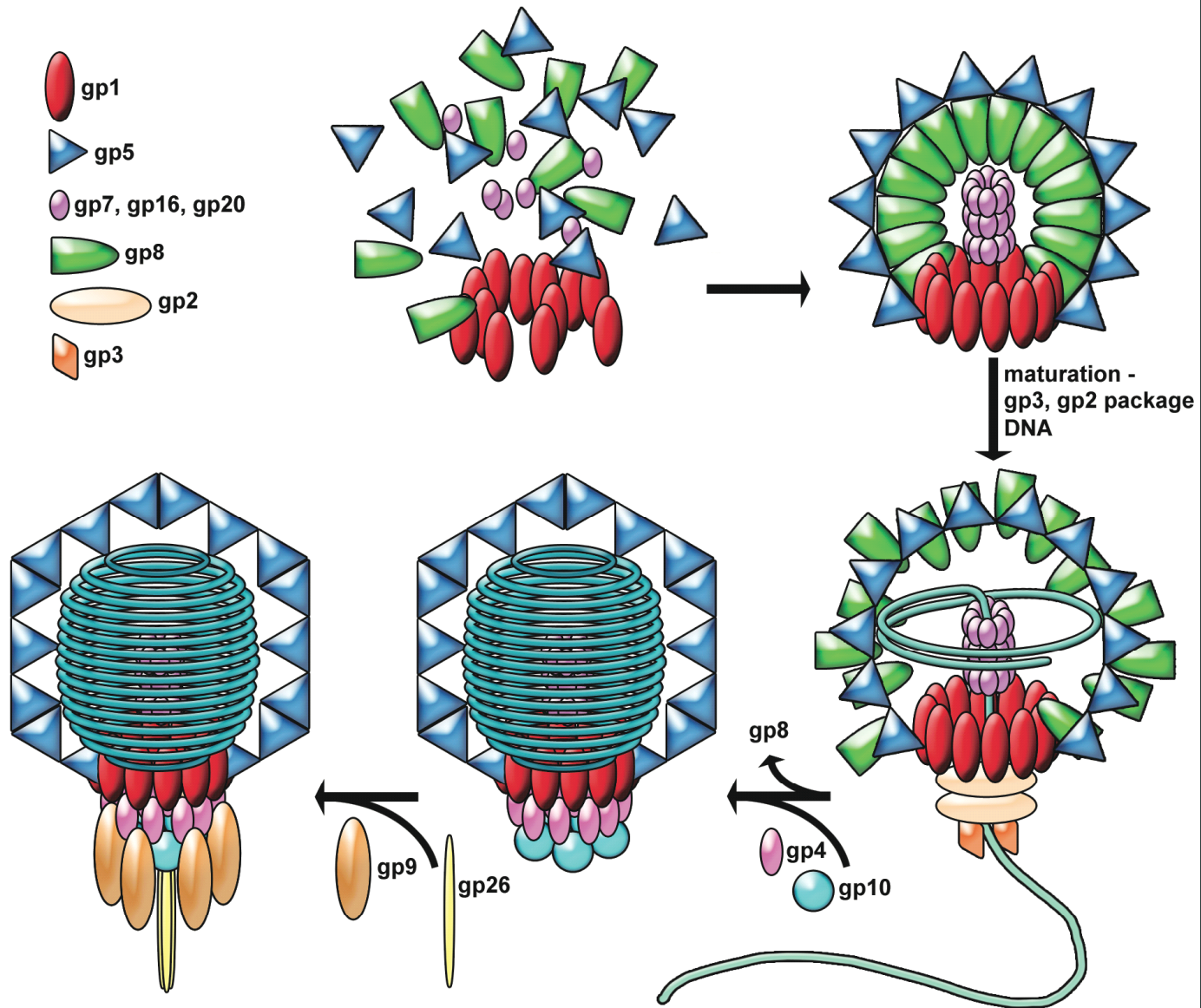
Podoviridae Infection of Cells



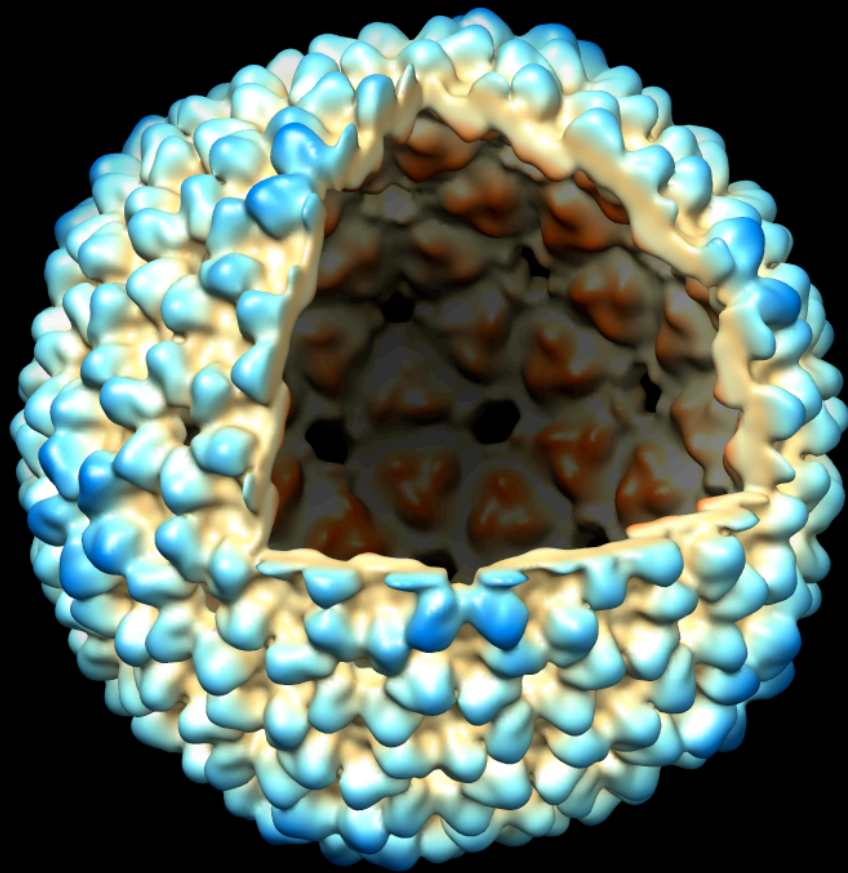
Podoviridae Infection of Cells



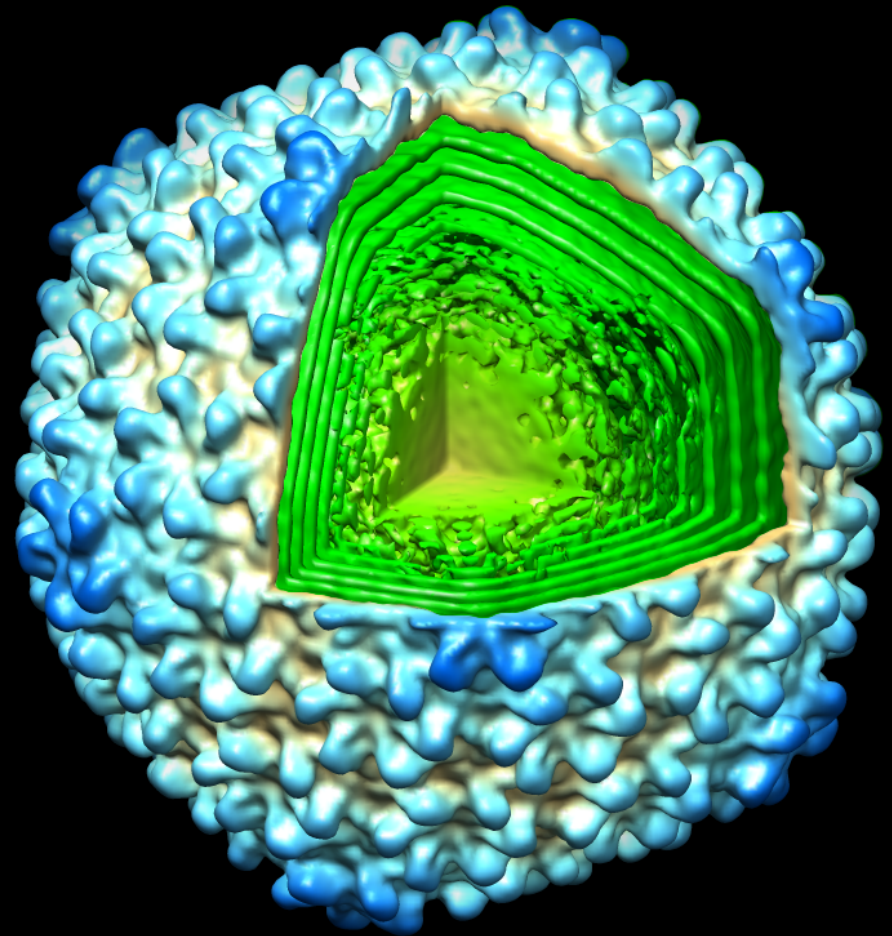
Virus Assembly and Maturation



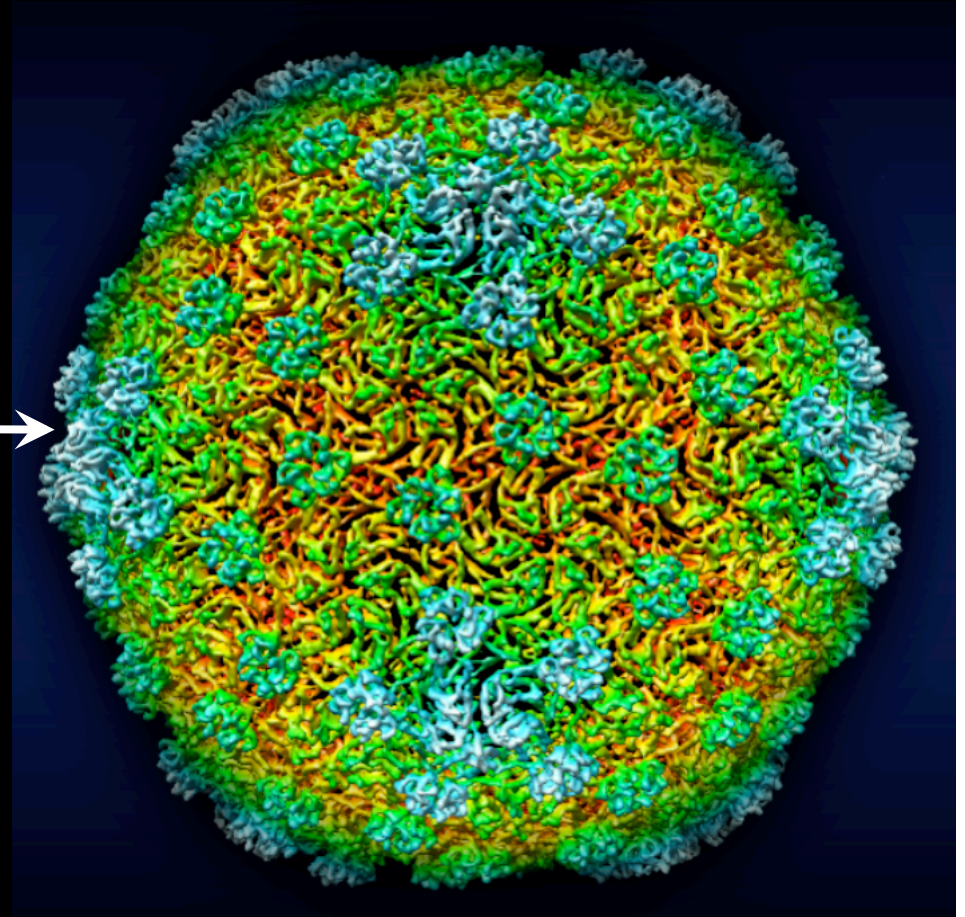
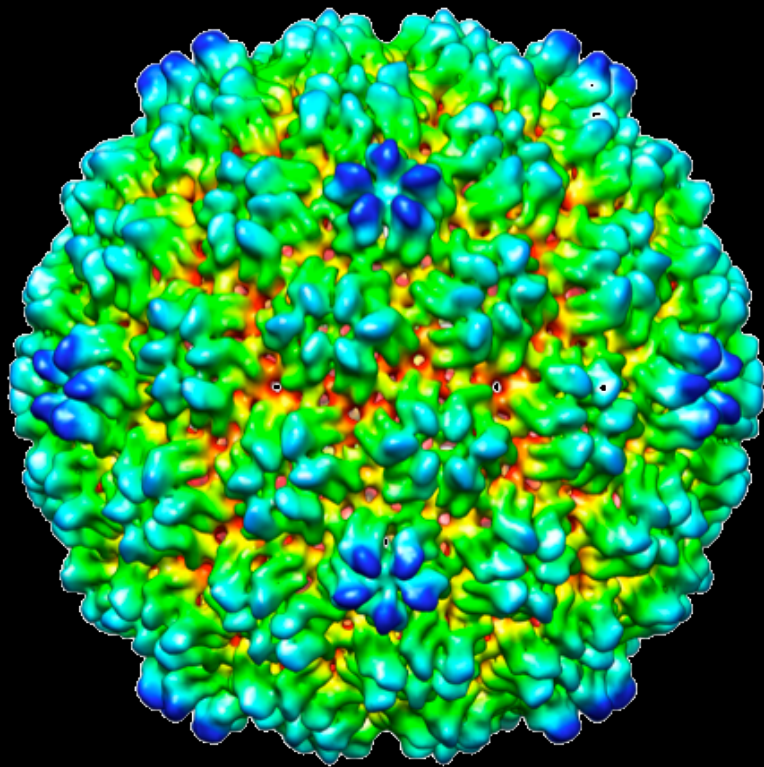
procapsid



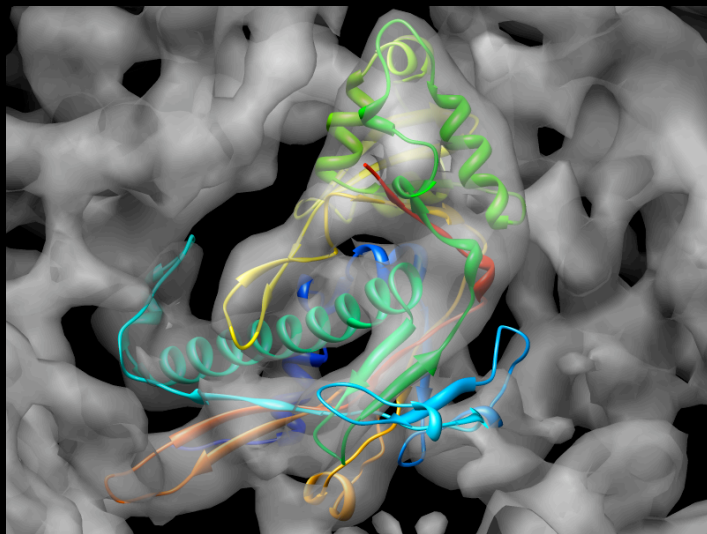
mature capsid



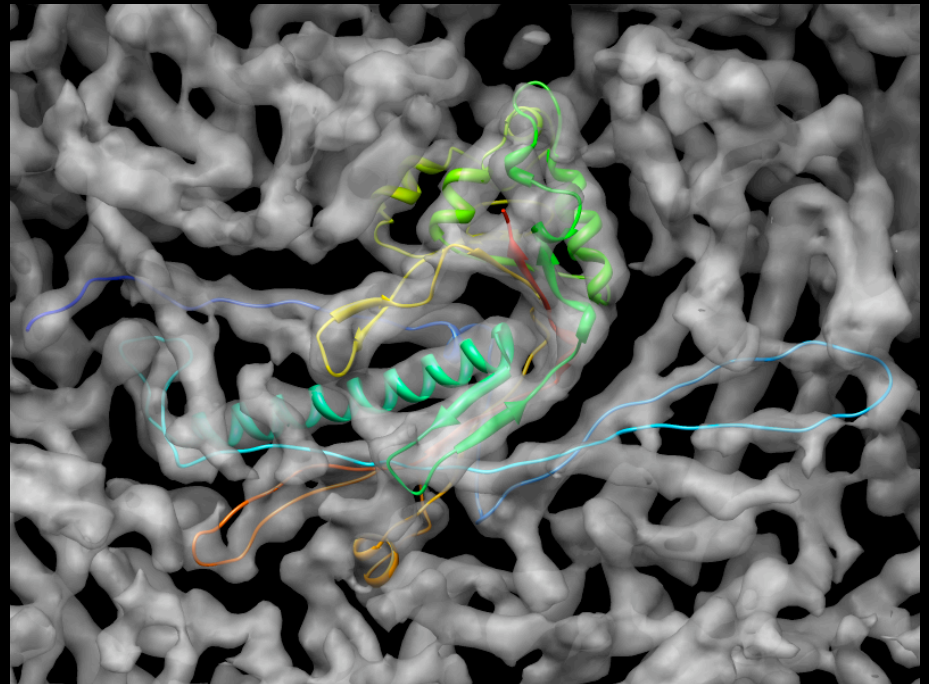
Lambda virus maturation

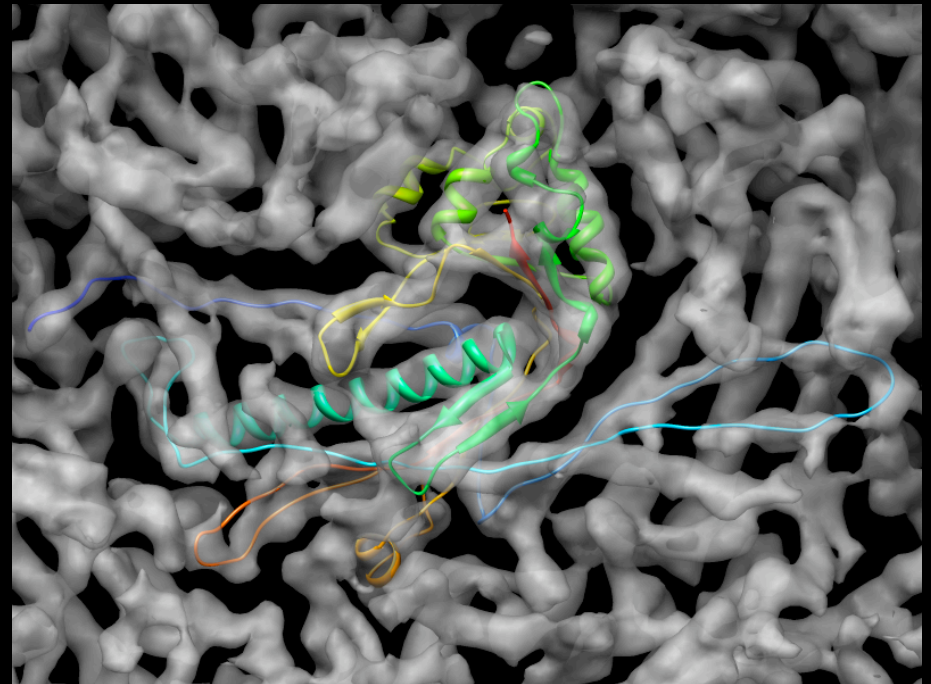


procapsid



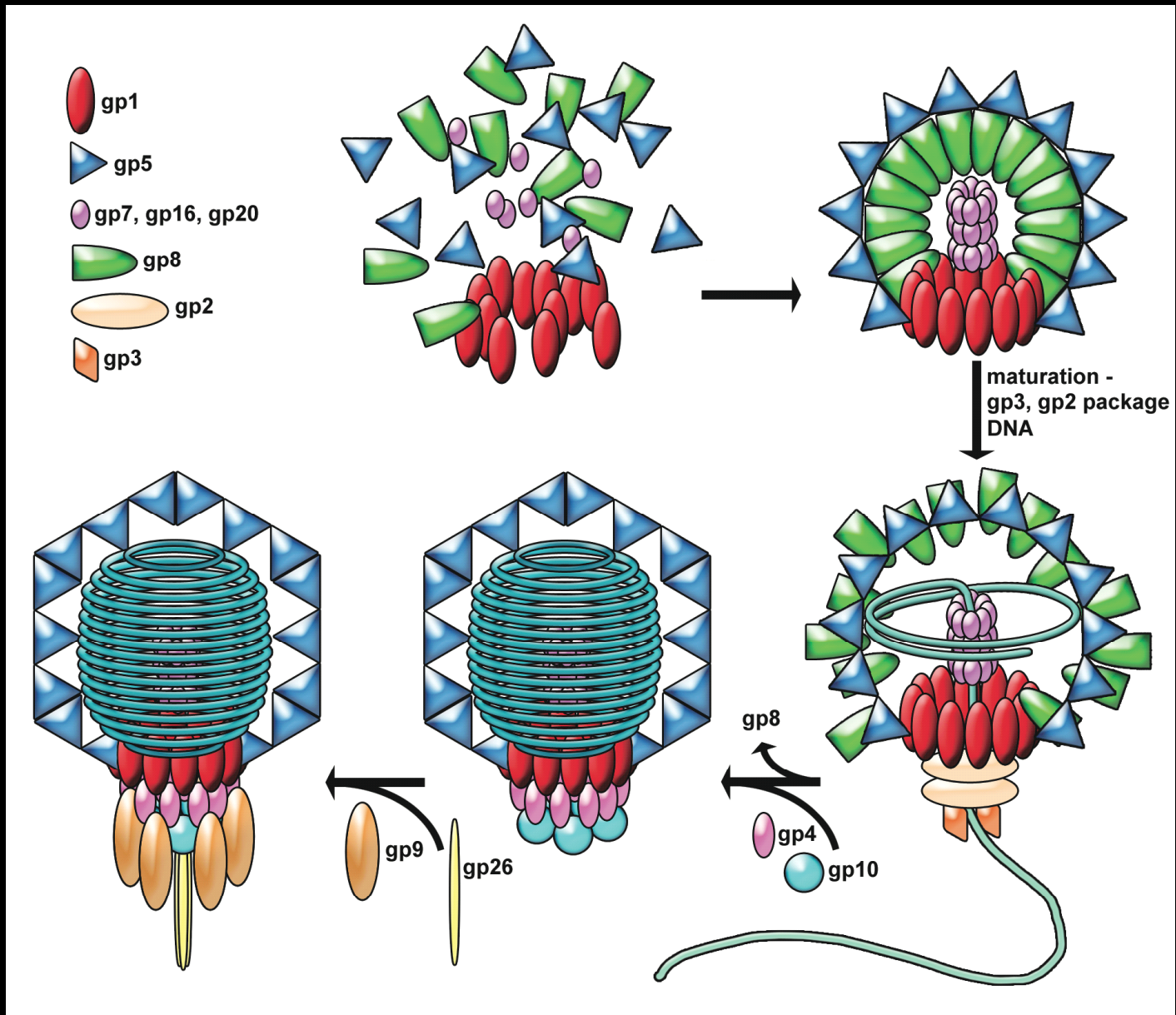
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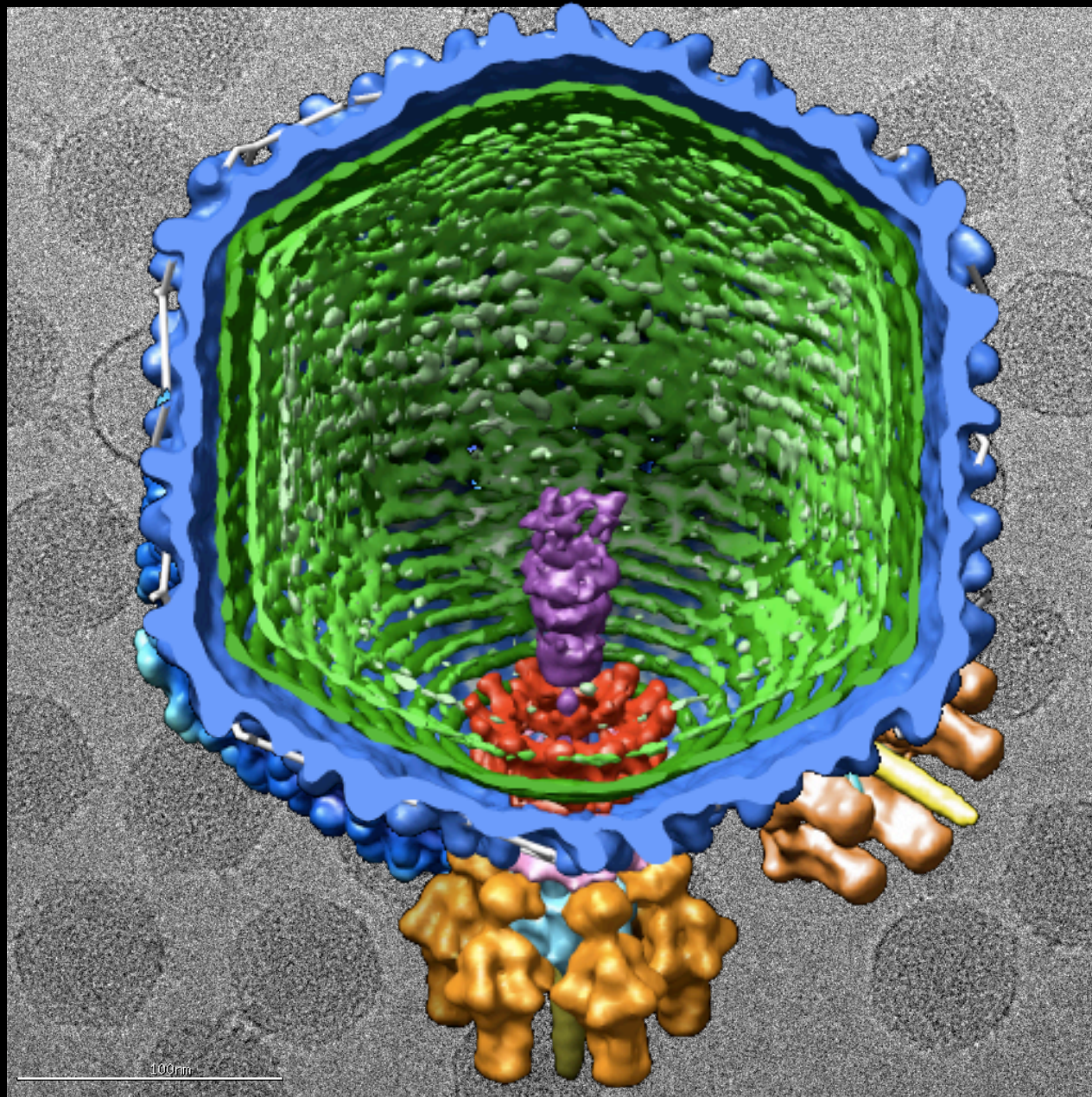




Lander, Gabriel C., et al. "Bacteriophage Lambda Stabilization by Auxiliary Protein Gpd: Timing, Location, and Mechanism of Attachment Determined by Cryo-Em." *Structure* 16.9 (2008): 1399-406.

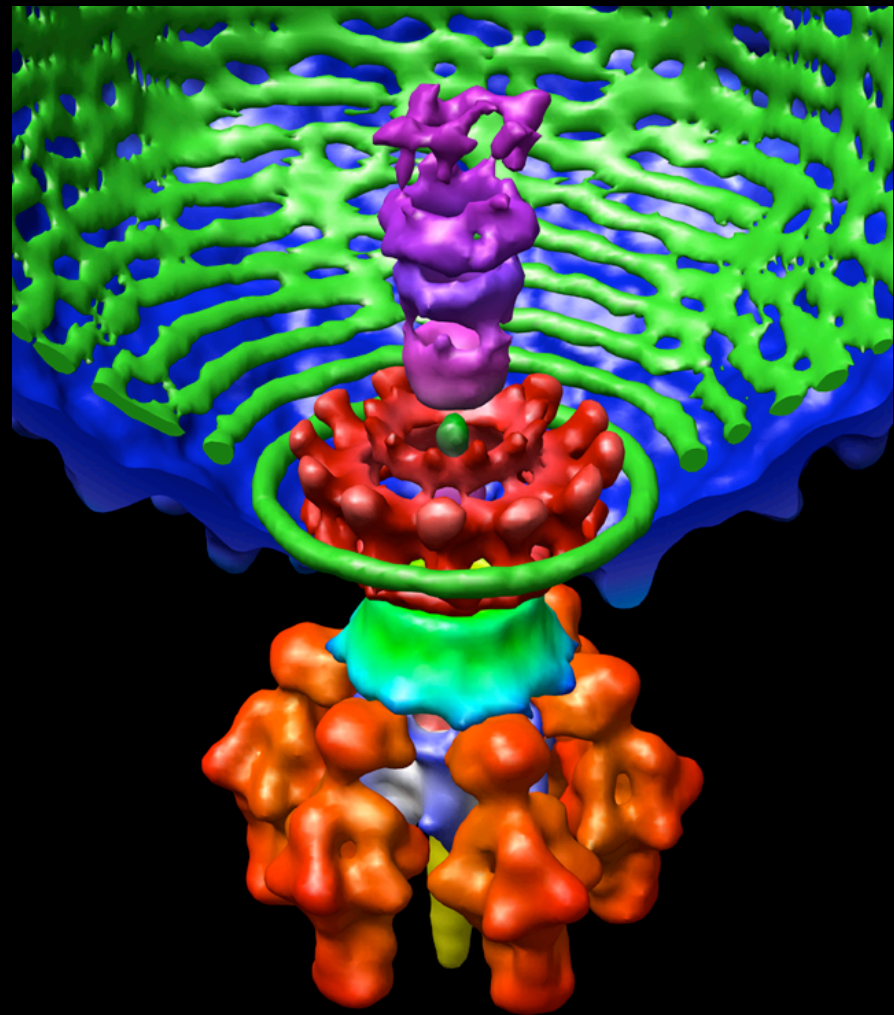
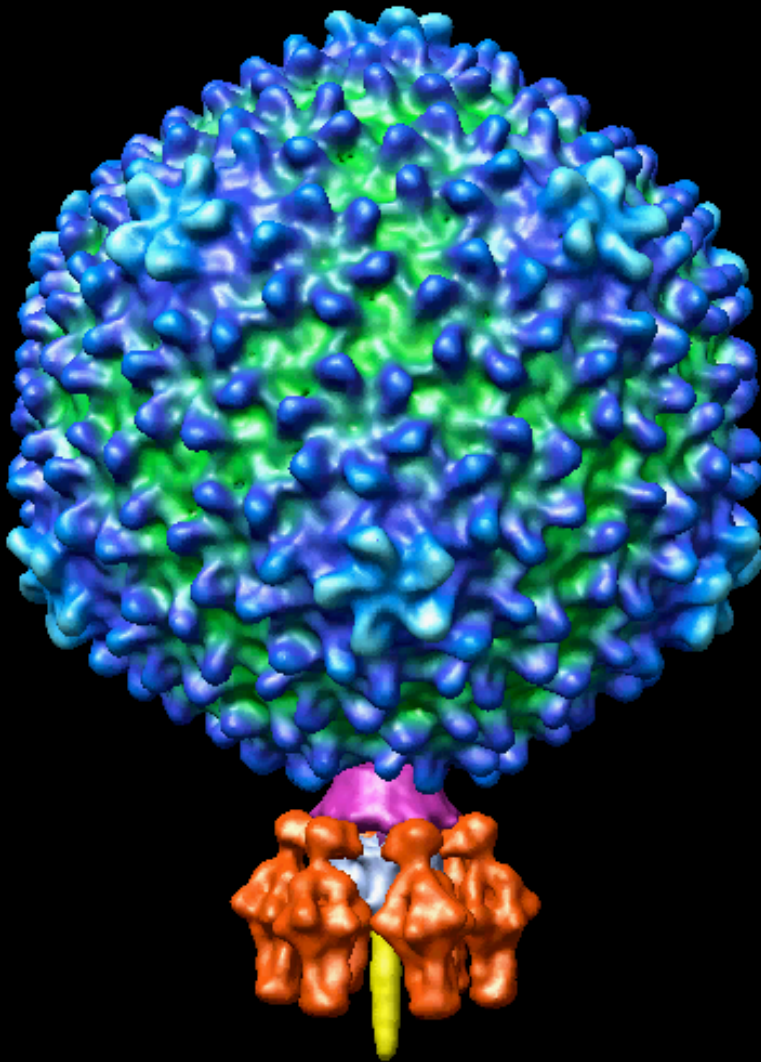
Virus Assembly and Maturation





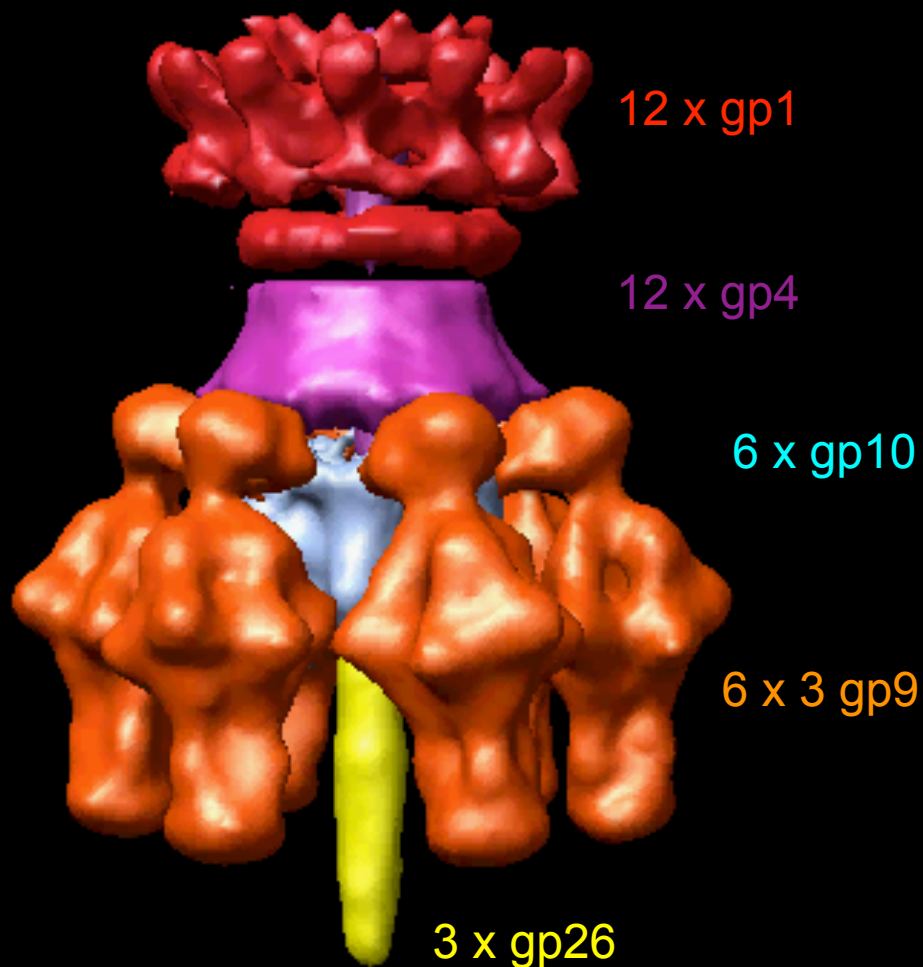
4,899 images; 25,793 particles

Phage P22 Infection Machinery

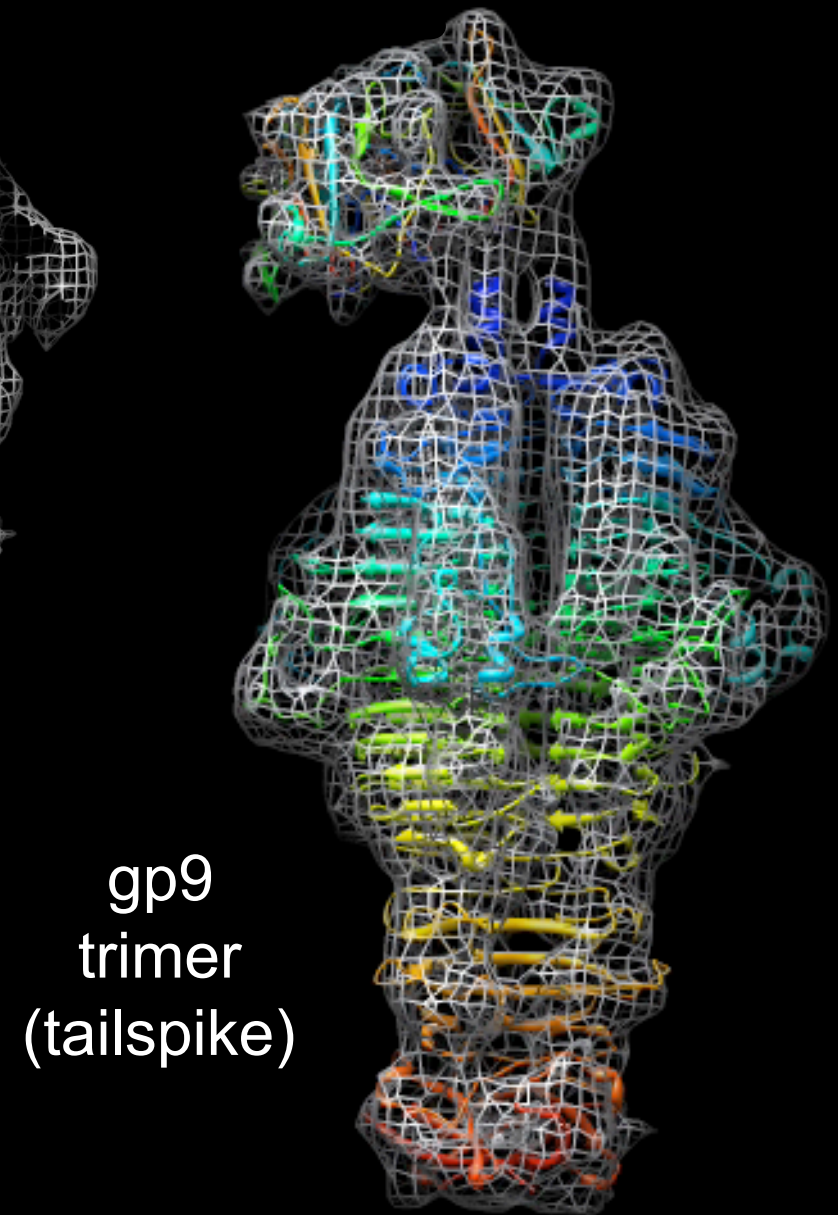
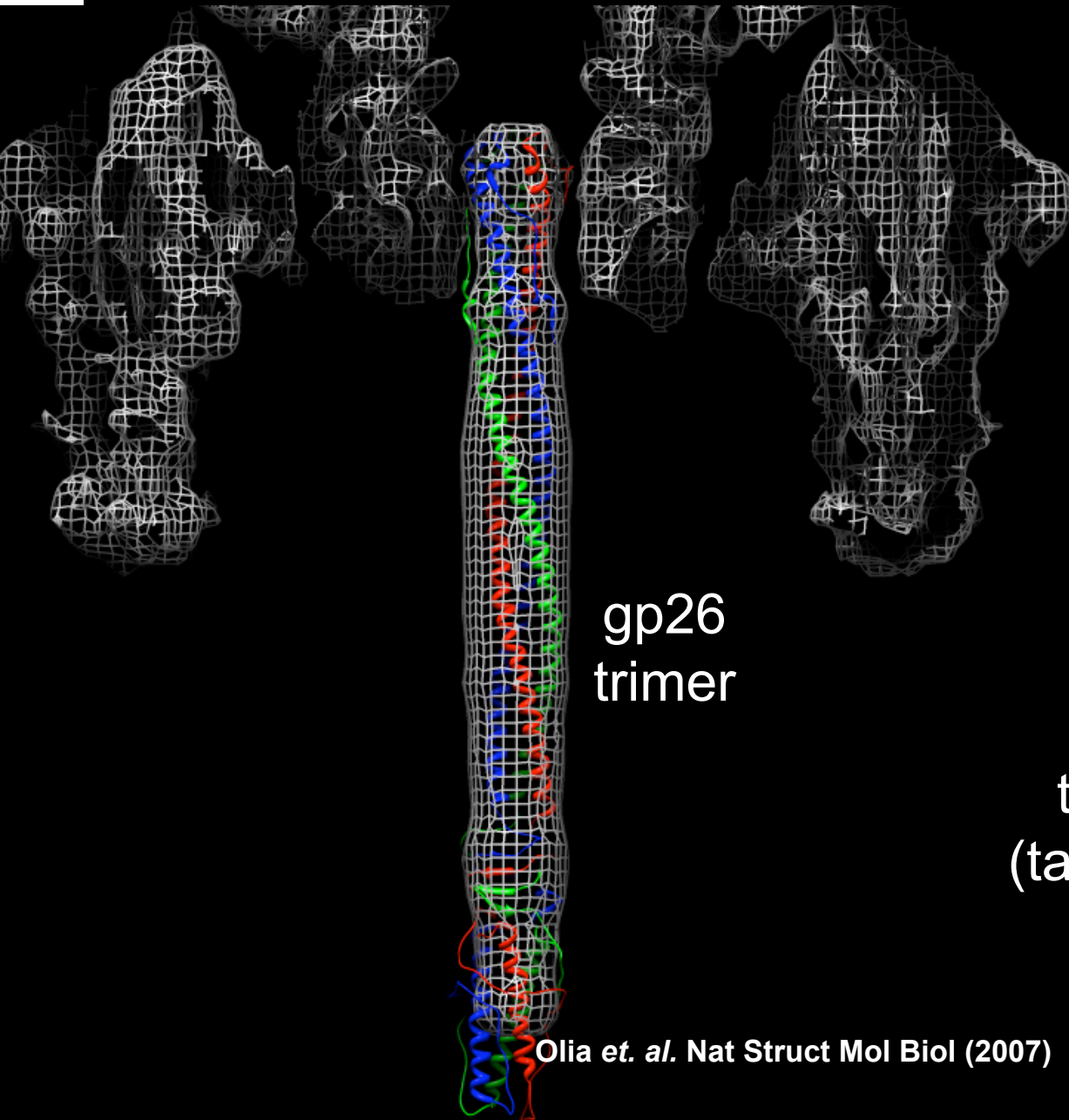


Lander, G. C., et al. "The Structure of an Infectious P22 Virion Shows the Signal for Headful DNA Packaging." *Science* 312.5781 (2006): 1791-5.

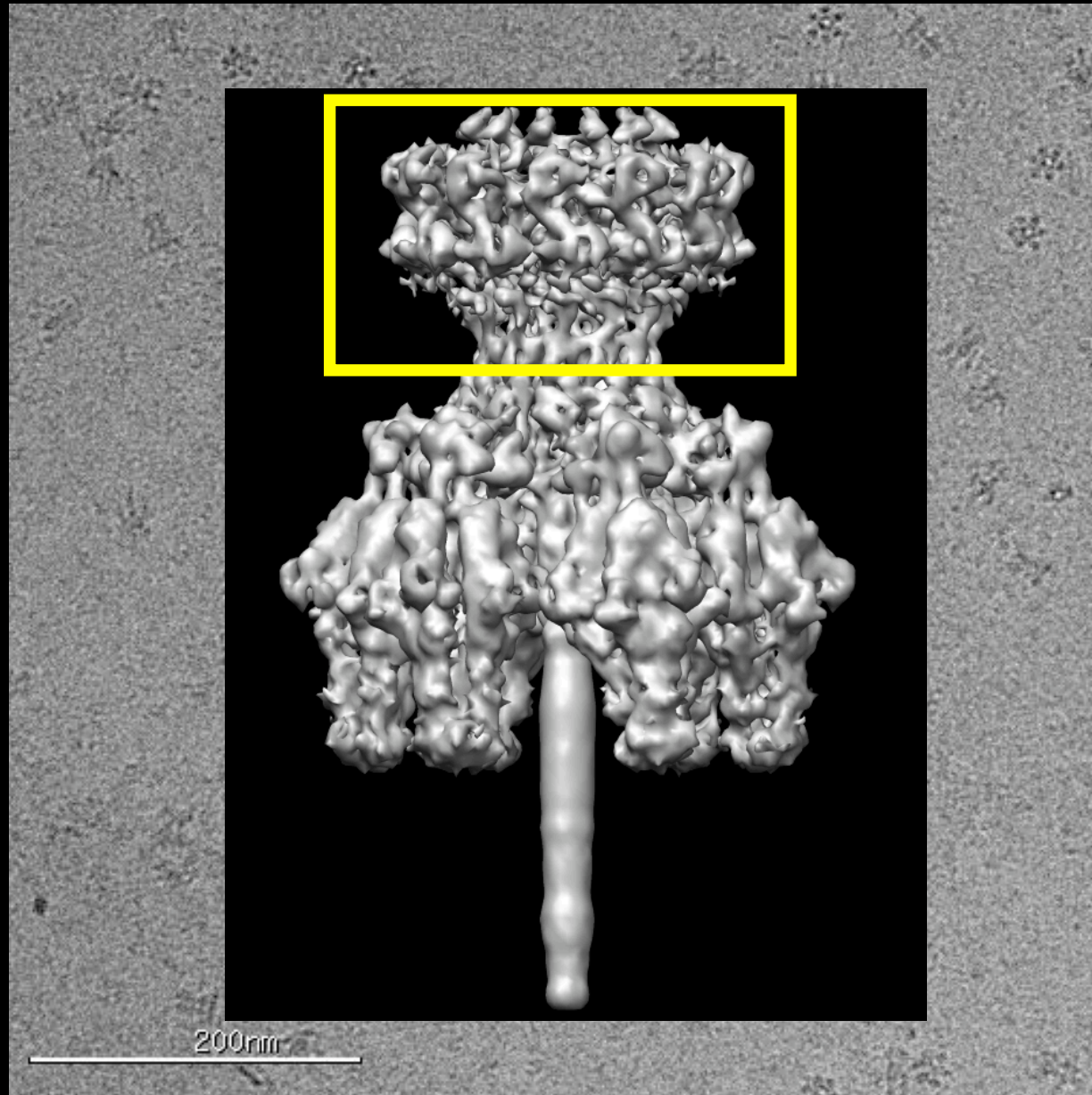
P22 Tail Machine



- 2 megadaltons
- 5 types of proteins
- 51 subunits

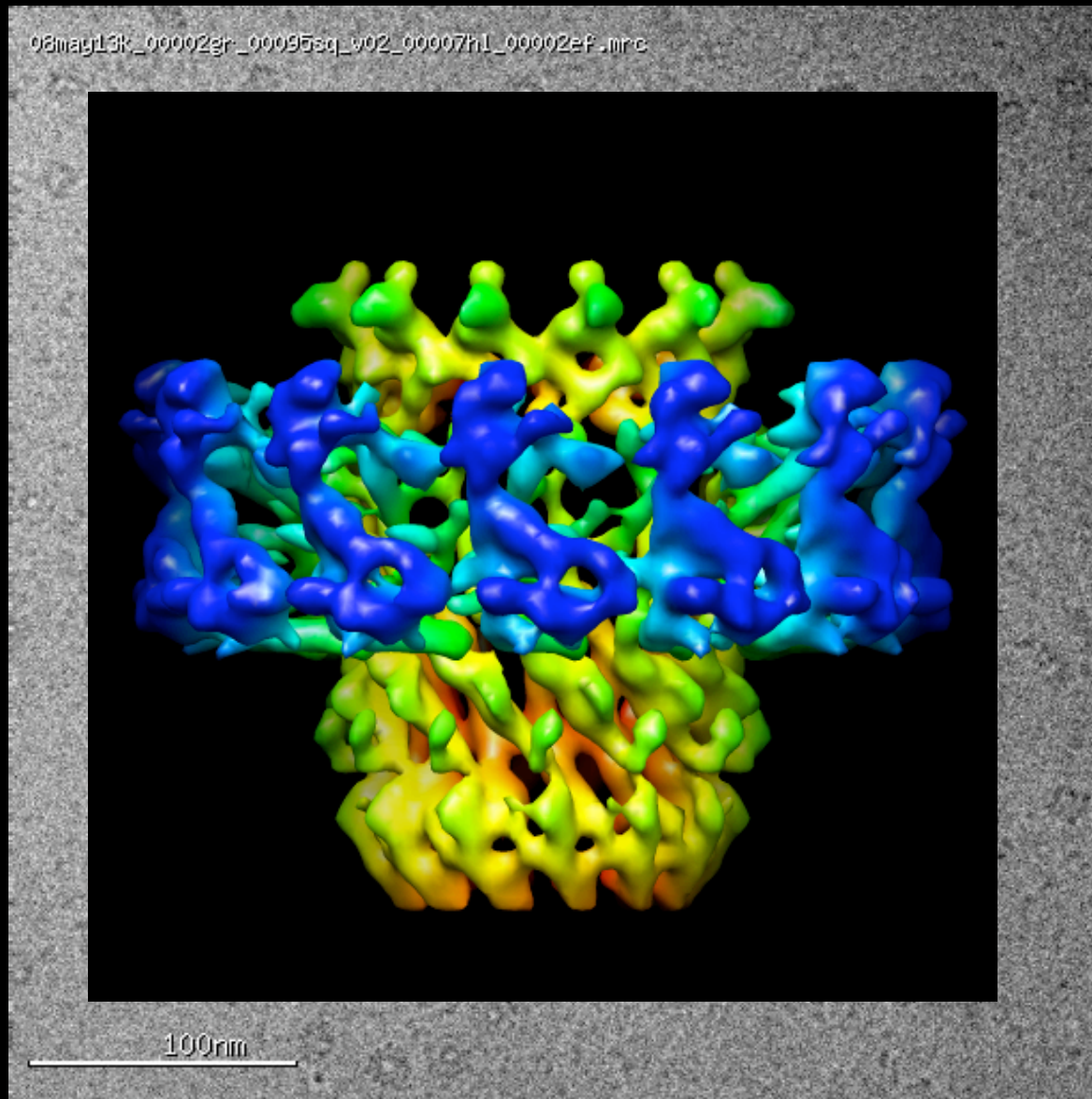


Isolated P22 Tail Machines

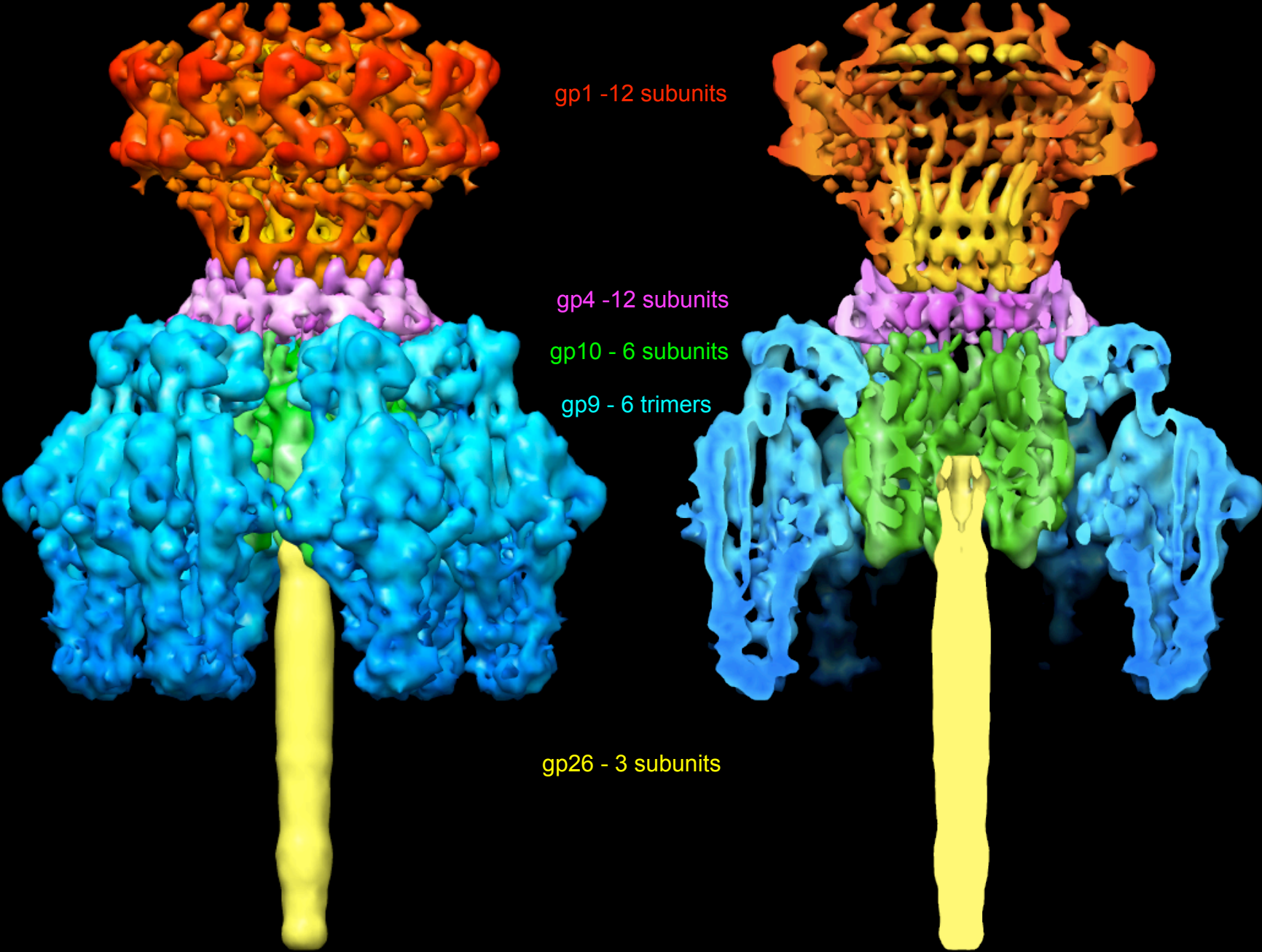


~350,000 particles
resolution ~ 9 Å

Isolated P22 Portals



~50,000 particles
resolution ~ 10 Å



gp1 -12 subunits

gp4 -12 subunits

gp10 - 6 subunits

gp9 - 6 trimers

gp26 - 3 subunits

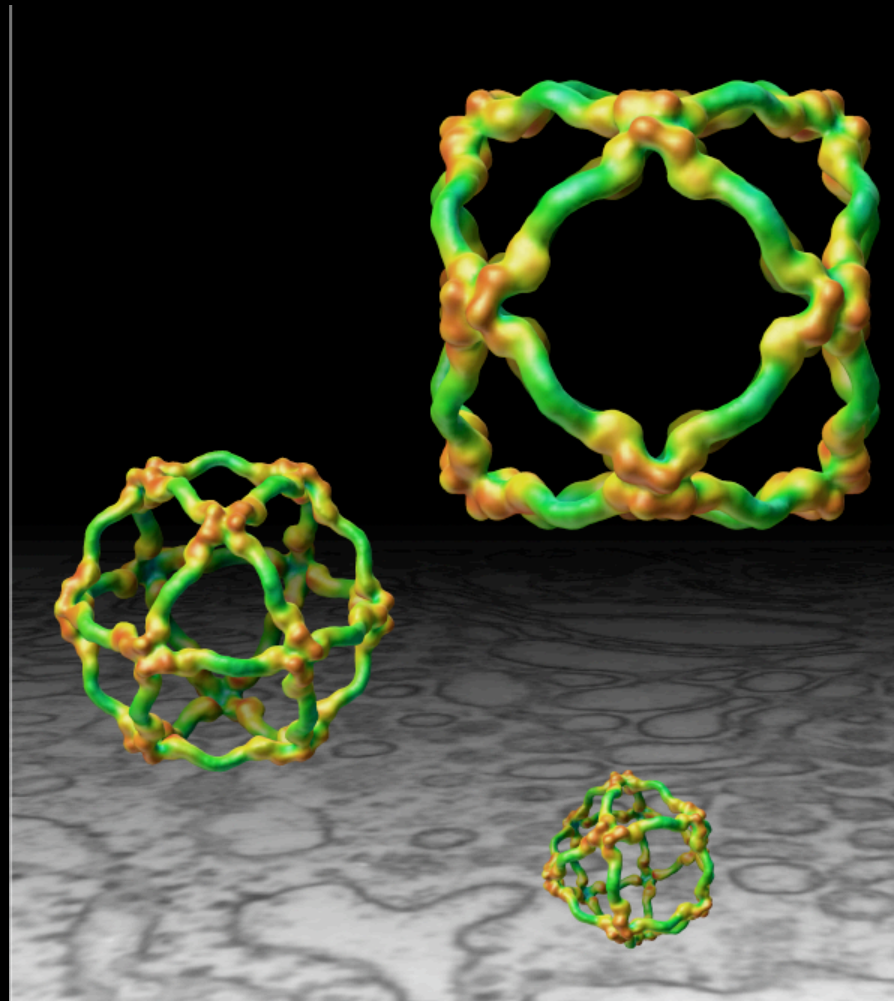
Case Study

Structure of COPII coats and cages

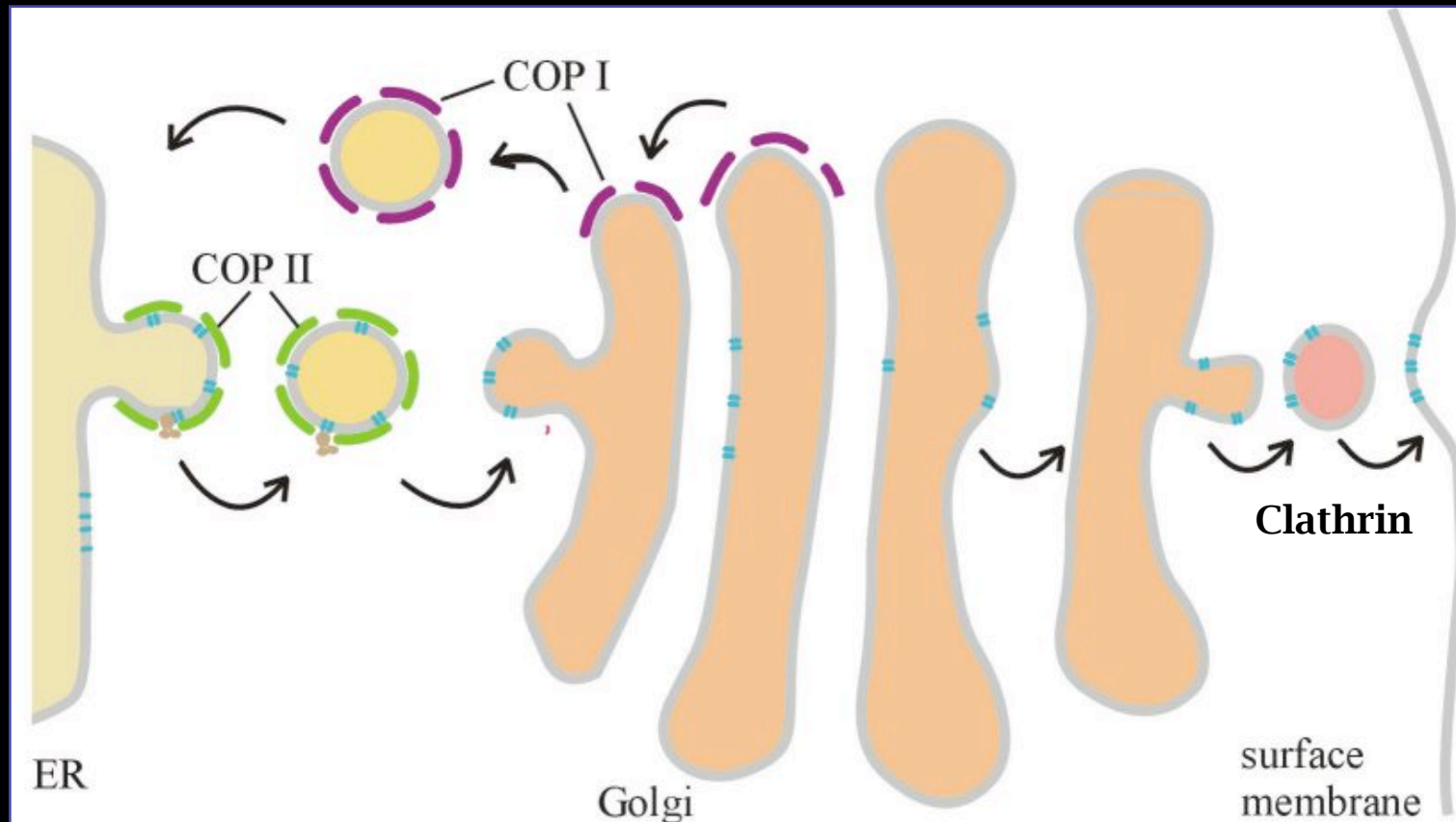
Scott Stagg



In Collaboration with
Paul LaPoint, Cemal
Gurkan, Douglas Fowler,
Ted Foss, Bill Balch.



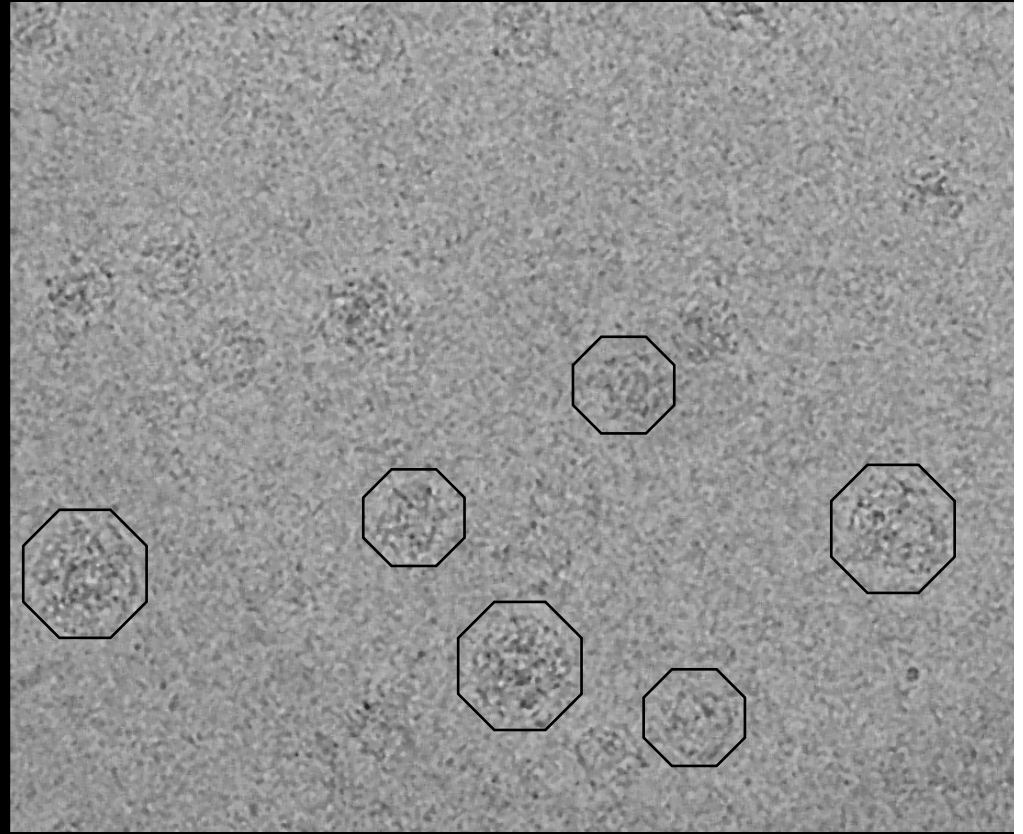
Vesicular trafficking





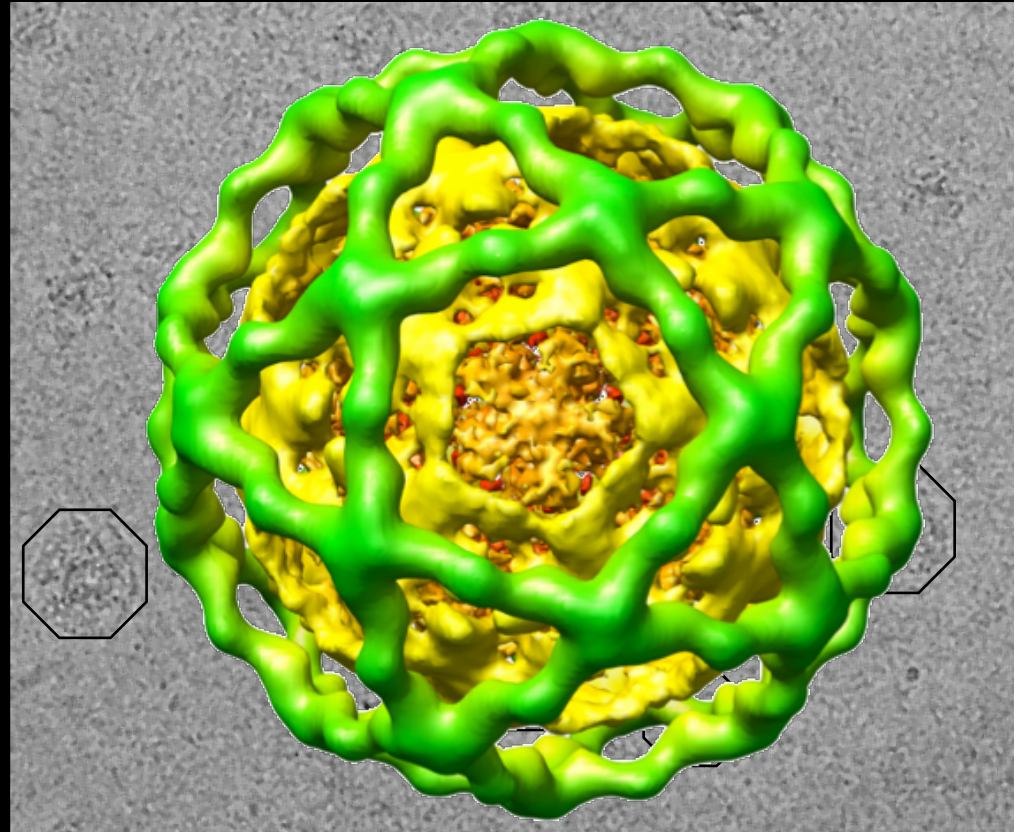
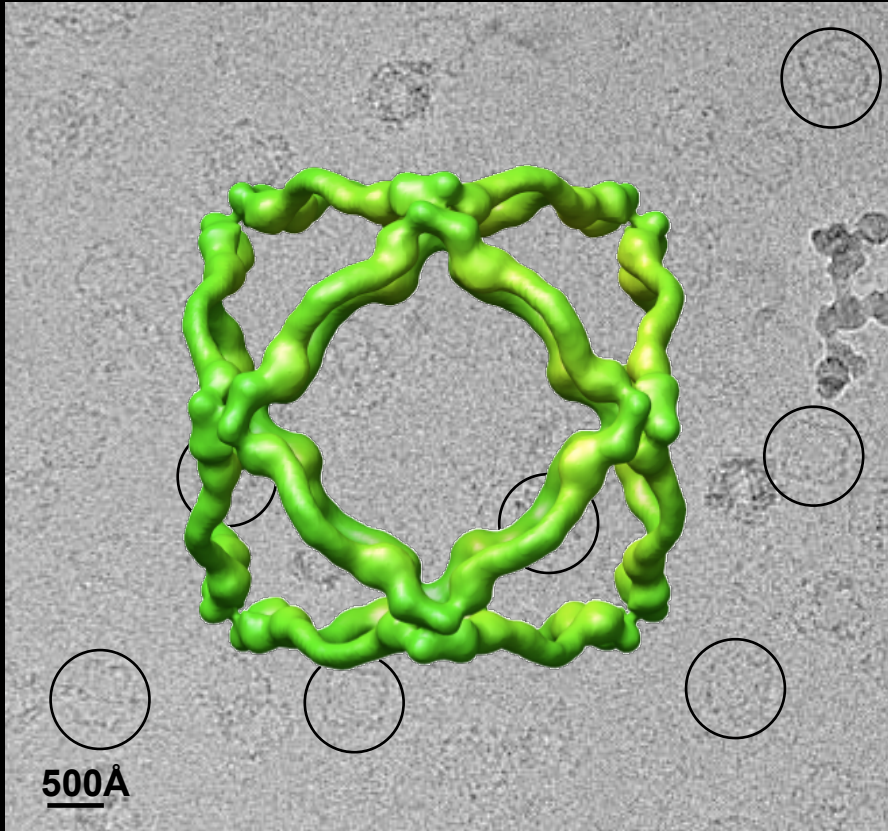
Sec13/31 self-assembles into cages

Sec13/31 and Sec23/24 together
assemble into a variety of structures of
varying sizes and shapes



Sec13/31 self-assembles into cages

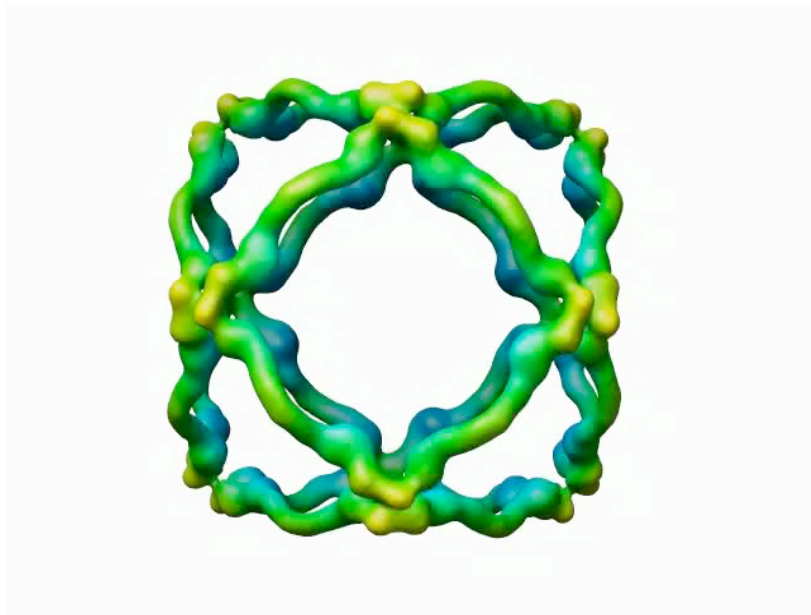
Sec13/31 and Sec23/24 together
assemble into a variety of structures of
varying sizes and shapes



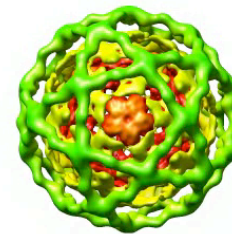
Stagg, S. M., et al. "Structure of the Sec13/31 Copii Coat Cage." *Nature* 439.7073 (2006): 234-8.

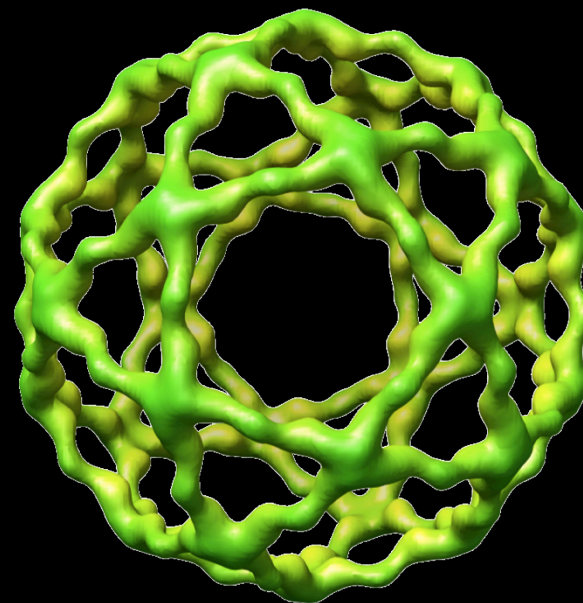
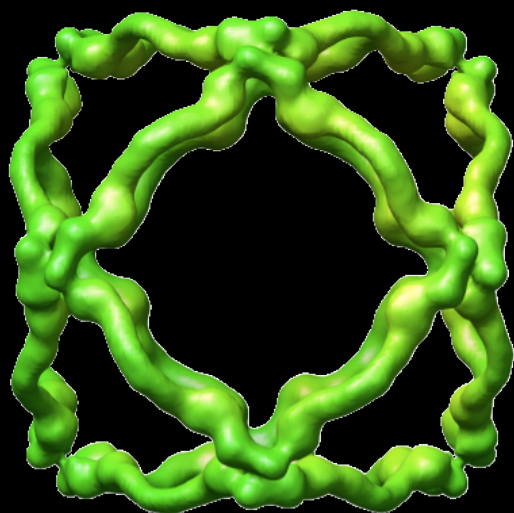
Stagg, S. M., et al. "Structural Basis for Cargo Regulation of Copii Coat Assembly." *Cell* 134.3 (2008): 474-84.

Sec13/31 cage

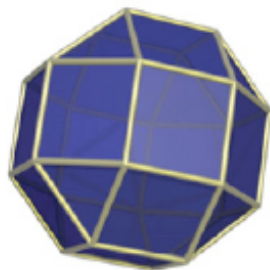


Sec13/31 and Sec23/24 coat

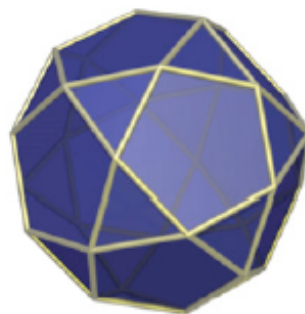




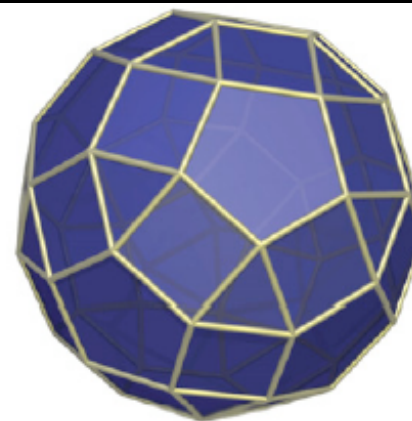
cuboctahedron



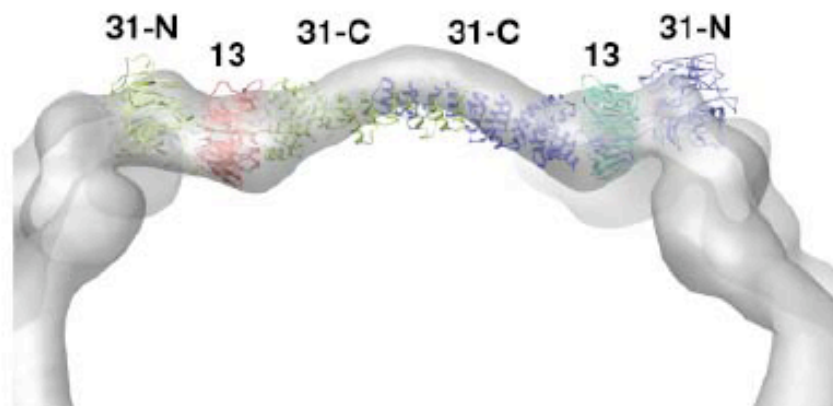
small
rhombicuboctahedron



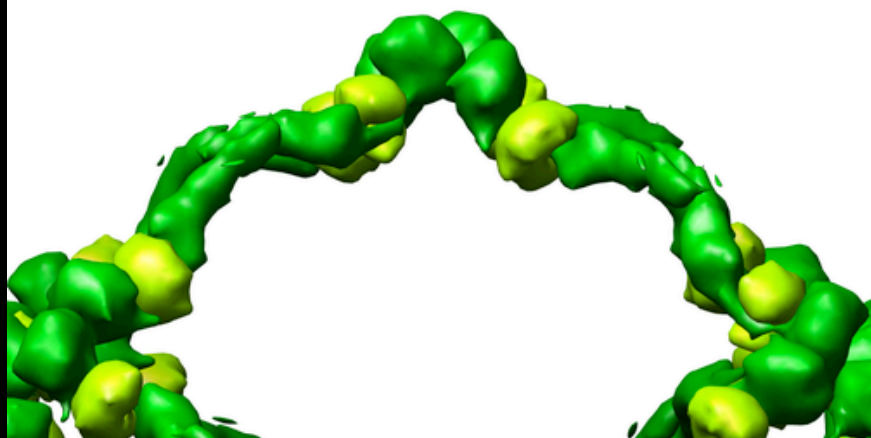
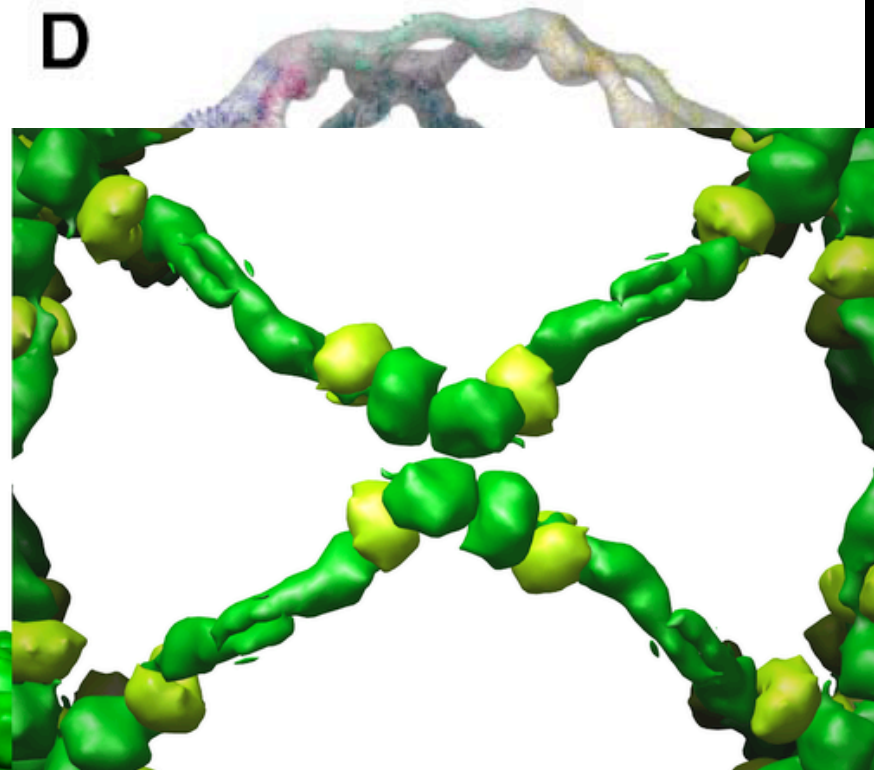
icosidodecahedron

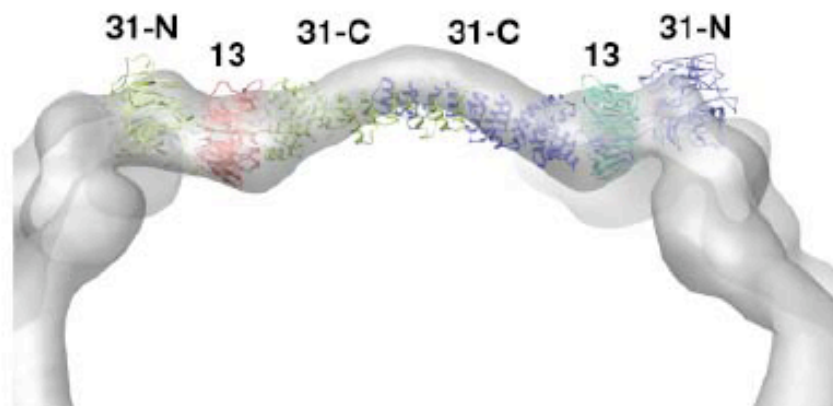


small
rhombicosidodecahedron

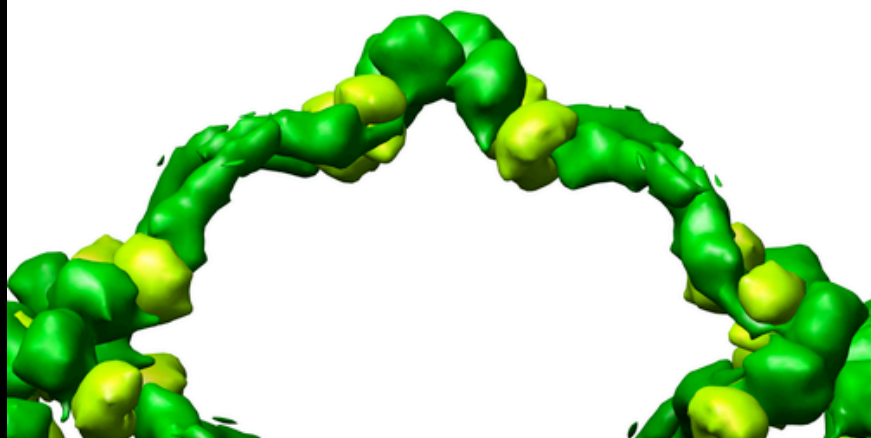
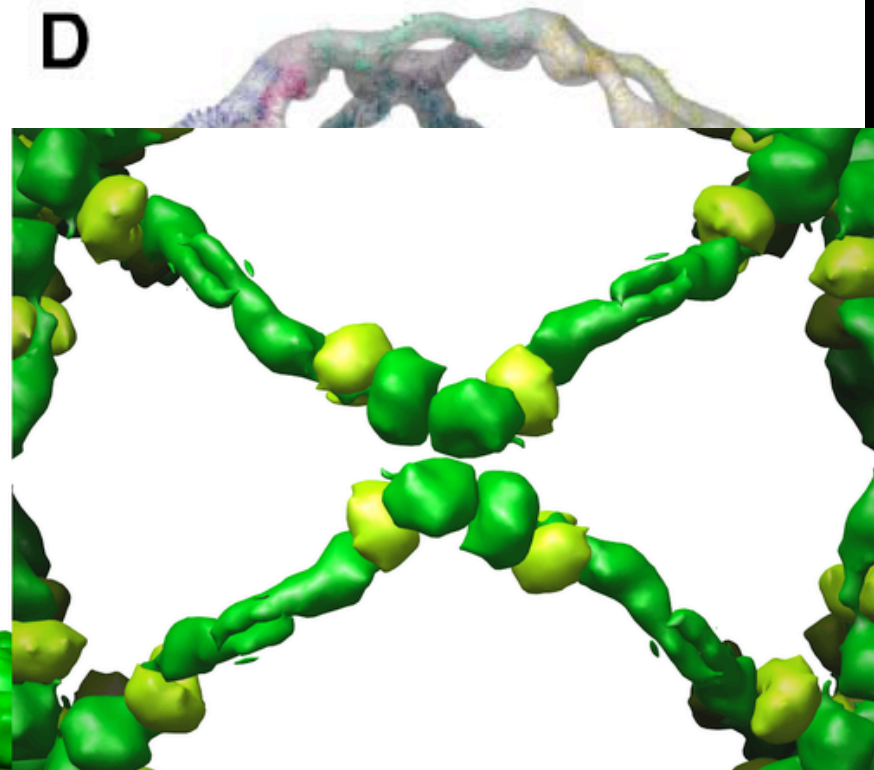
A**B****C**

cub

**D**

A**B****C**

cub

**D**

Case Study

30S ribosome assembly



Craig Yoshioka

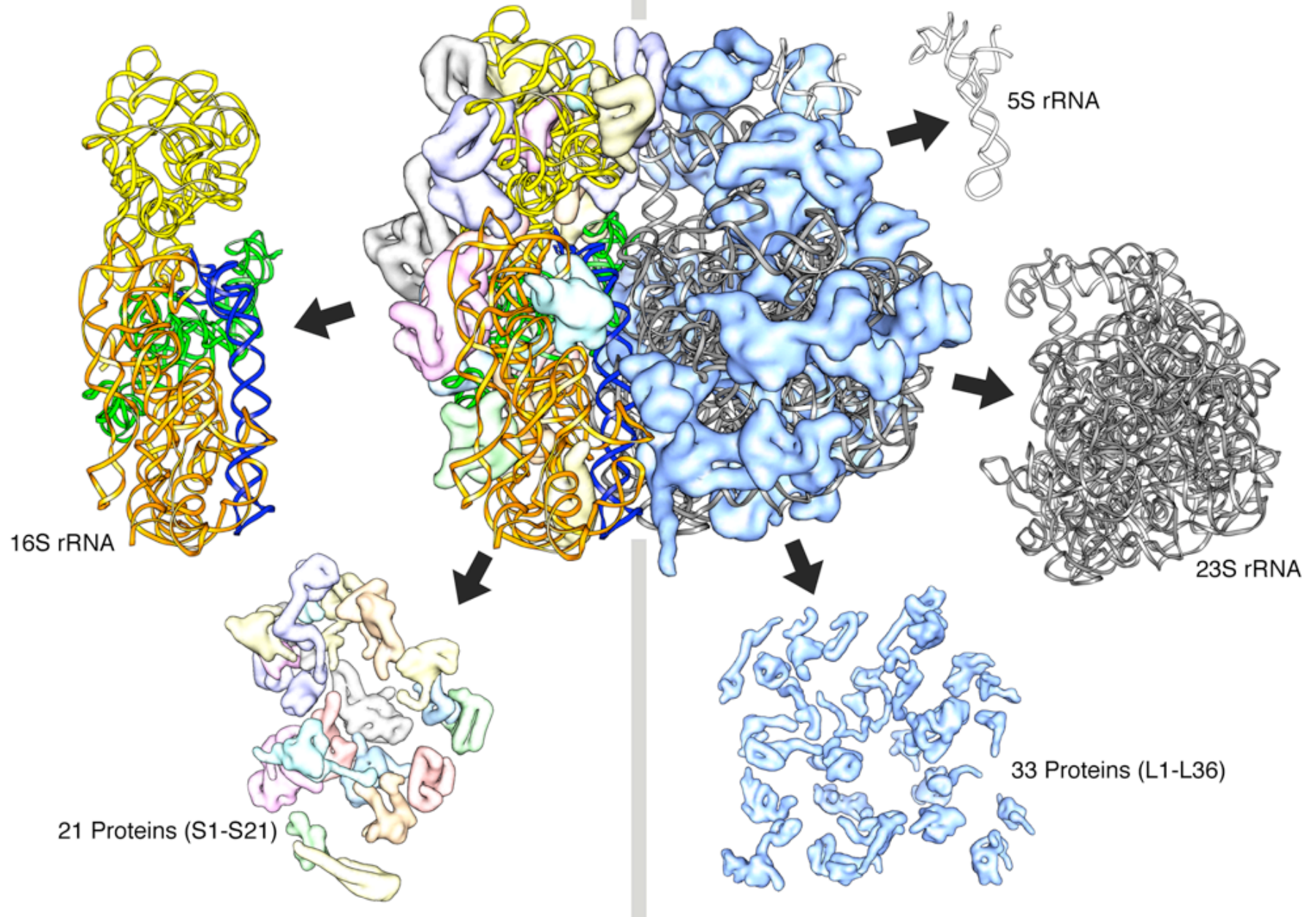


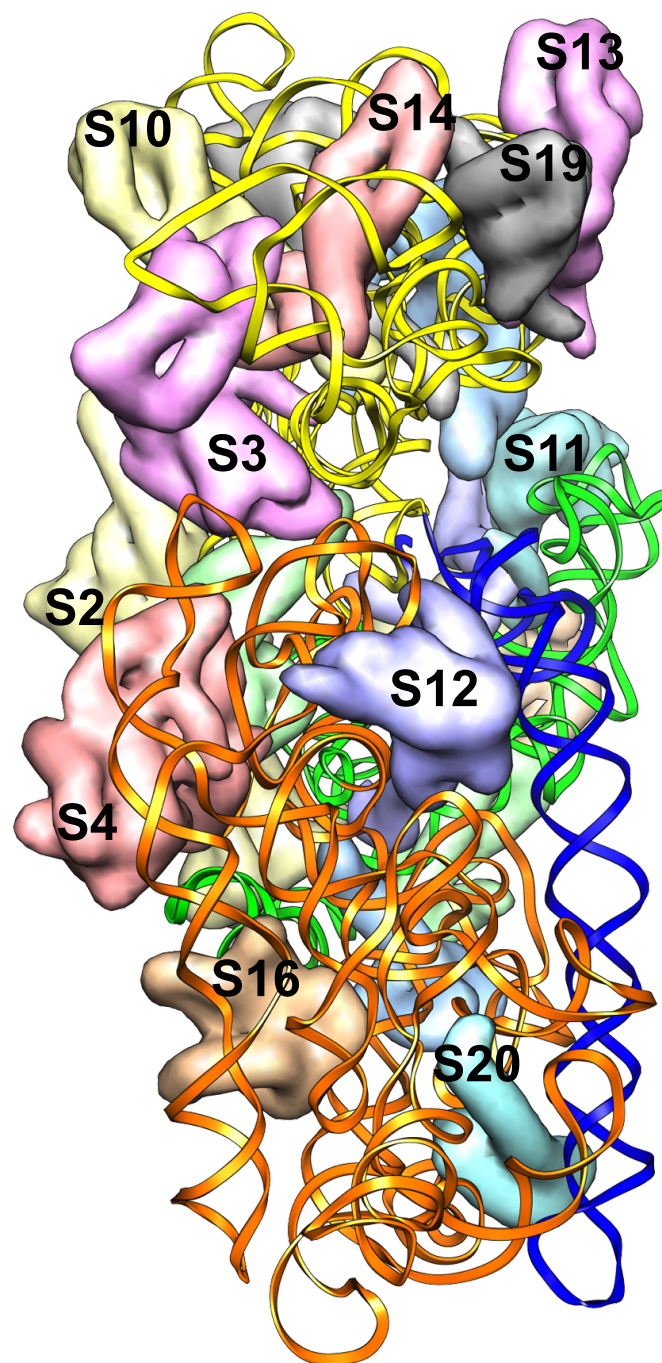
In Collaboration with
Megan Talkington and
James Williamson

Ribosome Assembly

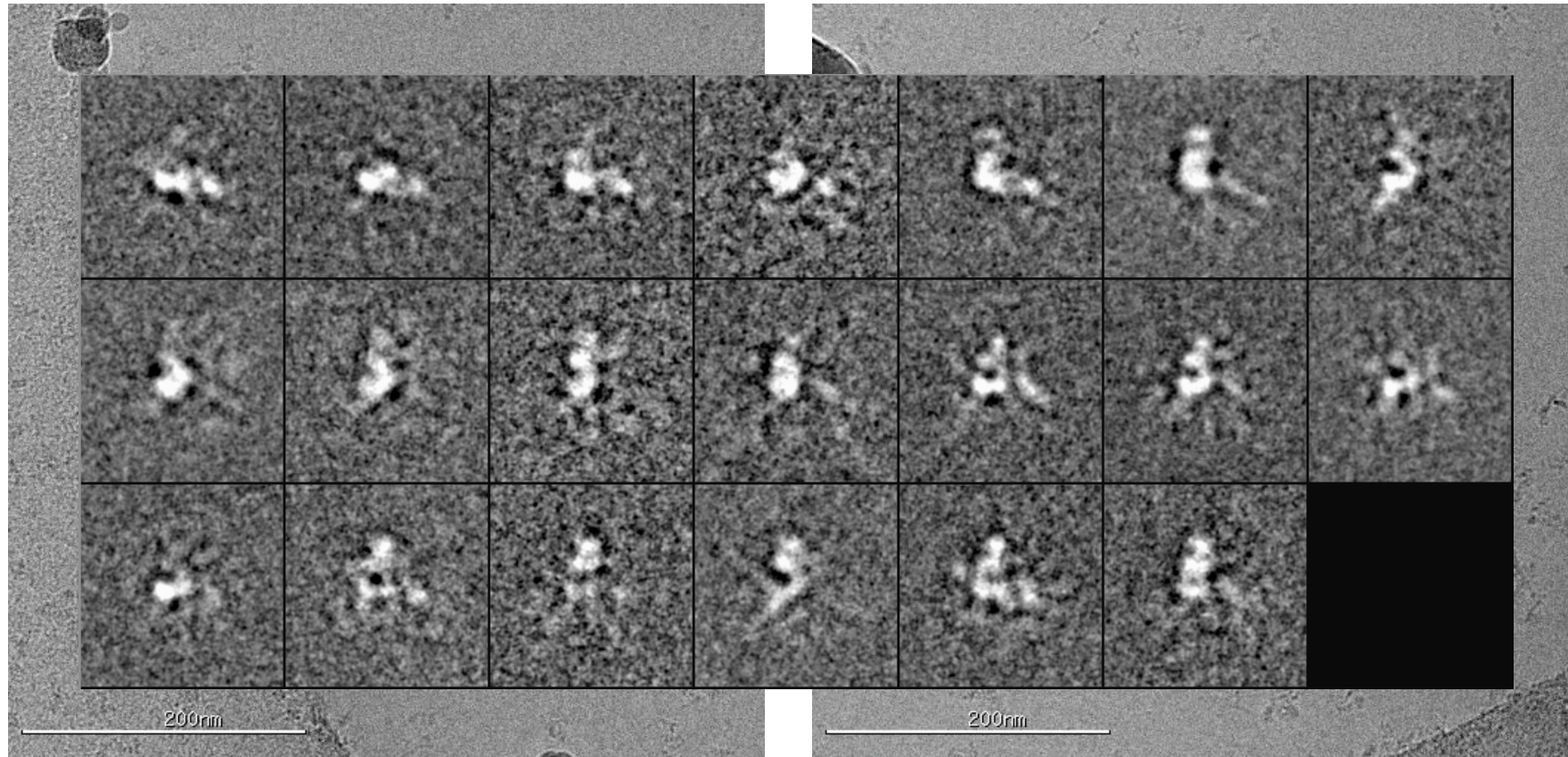
Small Subunit (30S)

Large Subunit (50S)

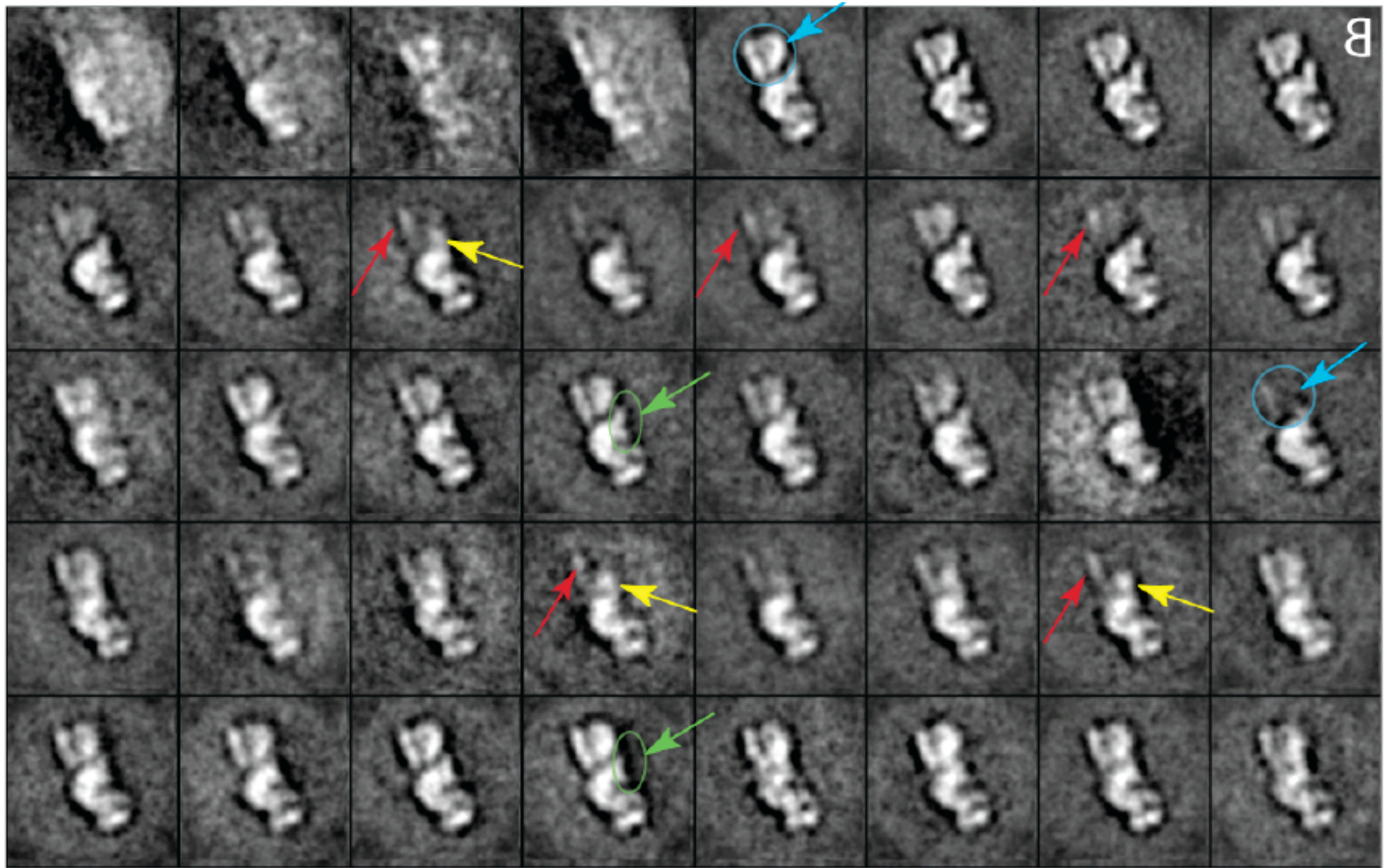




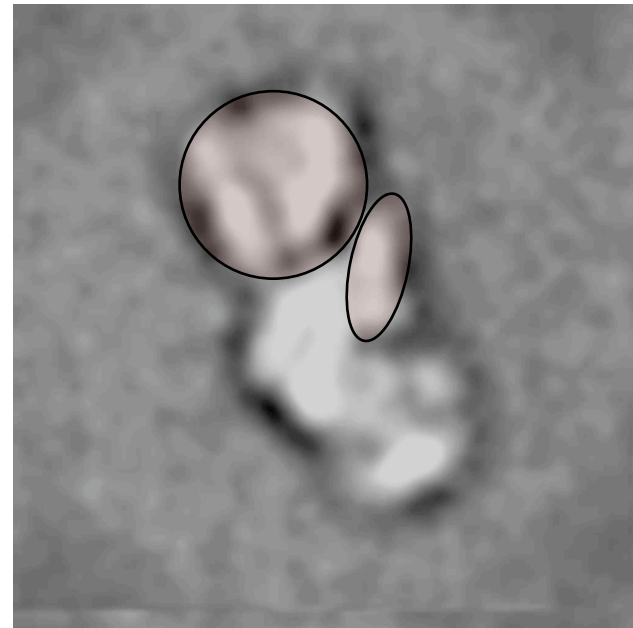
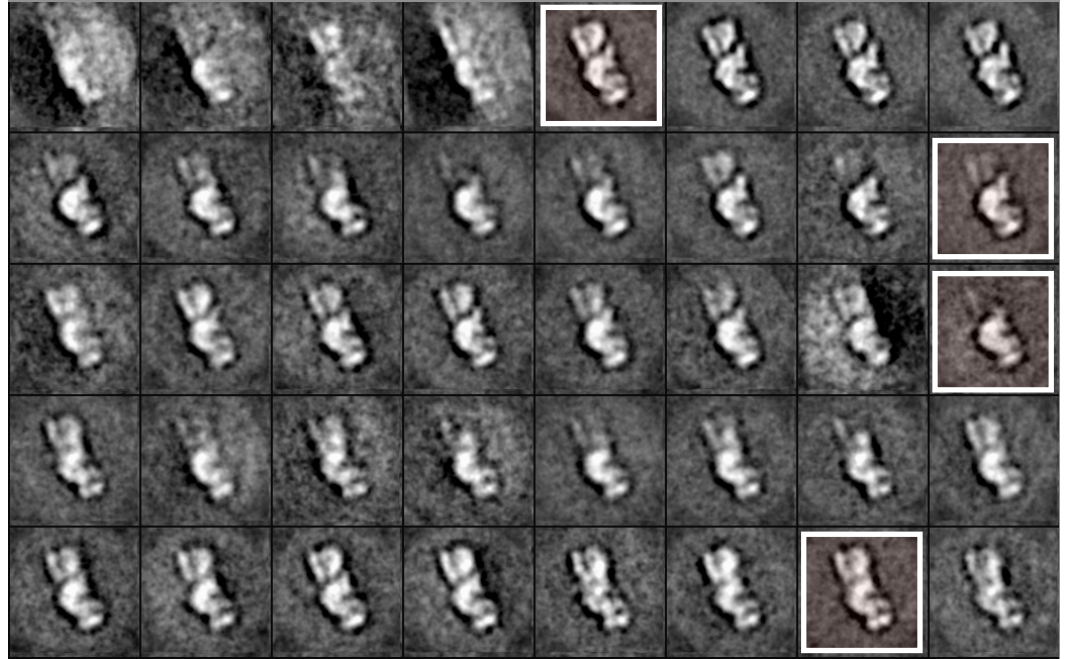
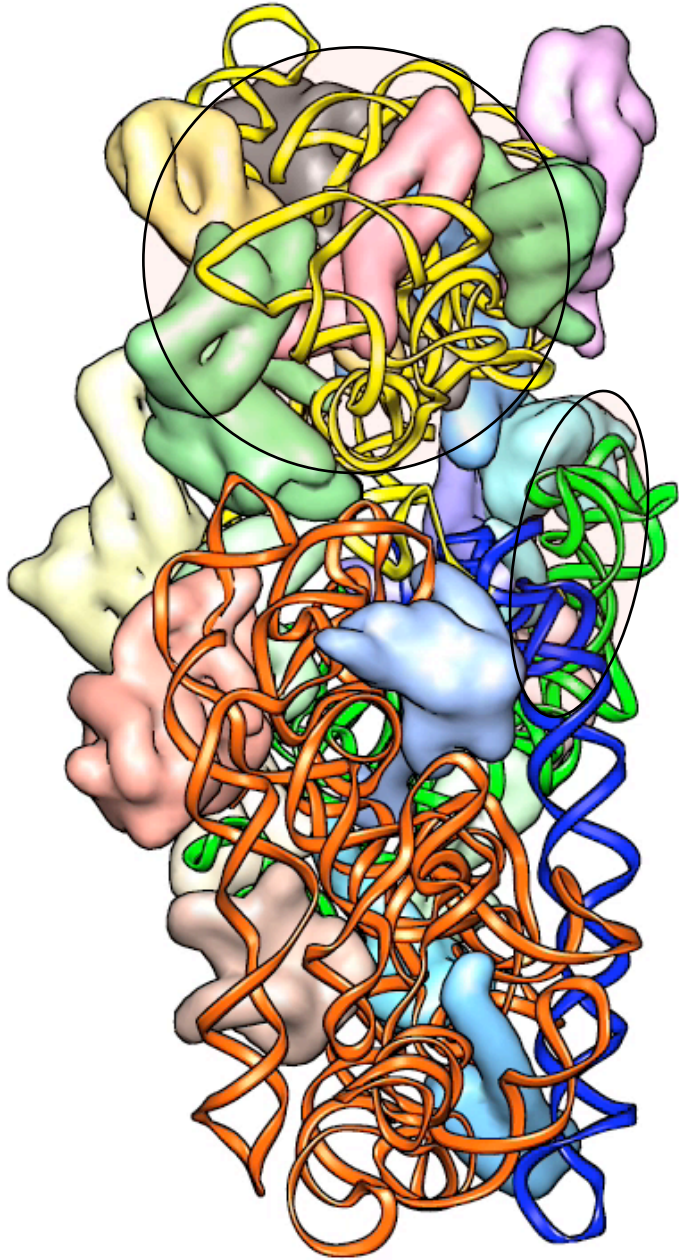
Ribosome Assembly, Time: ~42 sec

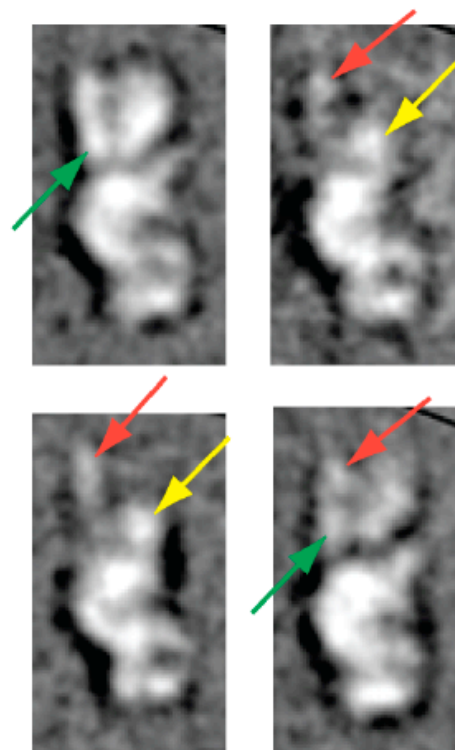
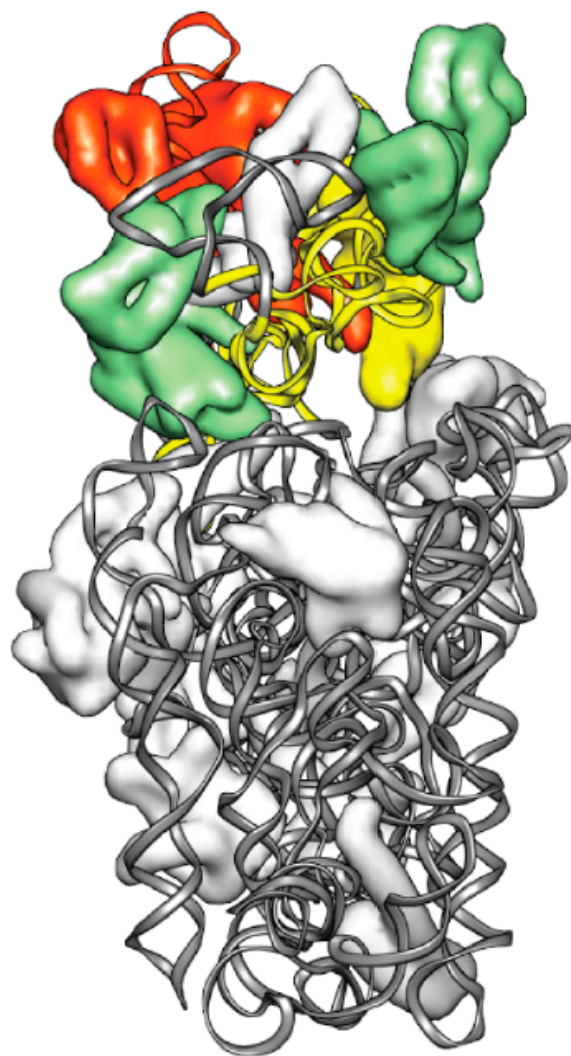


Ribosome Assembly, Time: 3 min

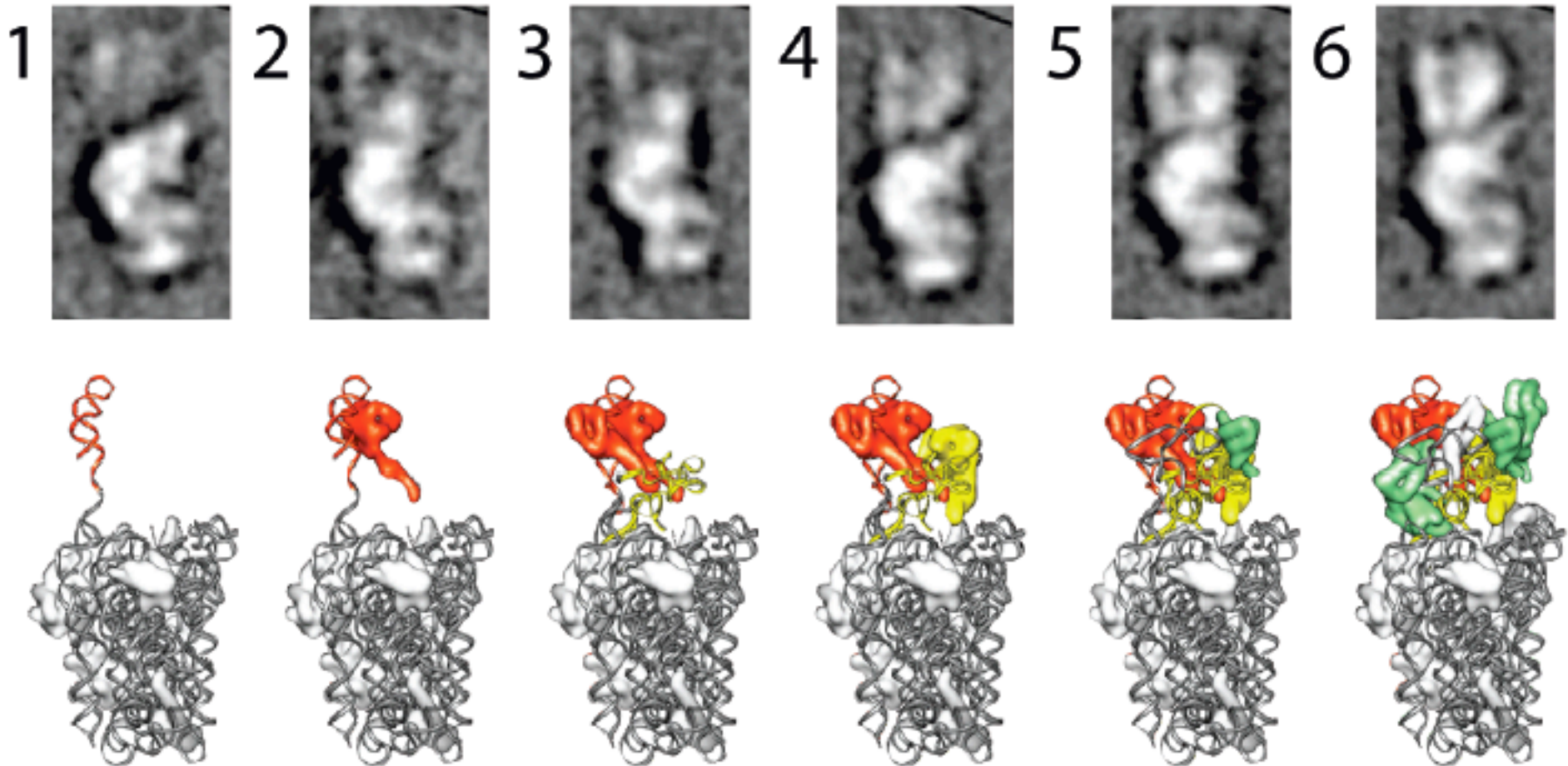


Ribosome Assembly, Time: 3 min





A potential folding pathway?



Automated Molecular Imaging Group at TSRI:



Jim Pulokas



Denis Fellmann



Joel Quispe



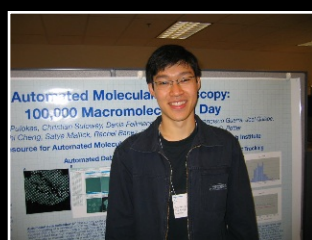
Anchi Cheng



Mark Palmer



Gabriel Lander



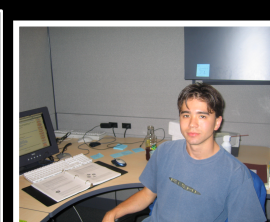
Pick-Wei Lau



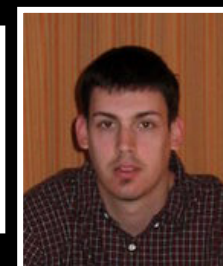
Anke Mulder



Dmitry Lyumkis



Craig Yoshioka



Neil Voss



Christopher Irving



Lorraine Lathrop

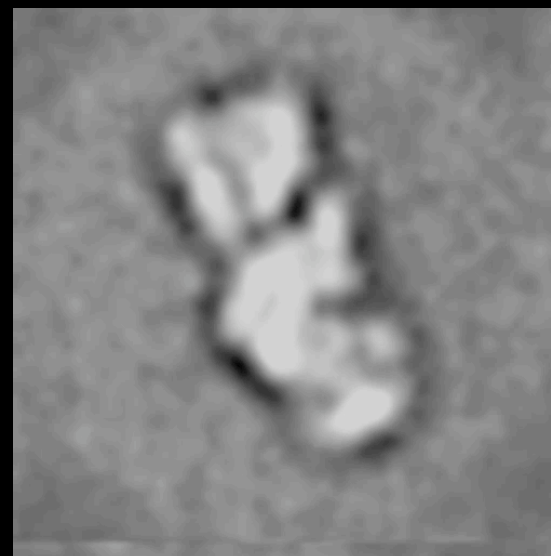
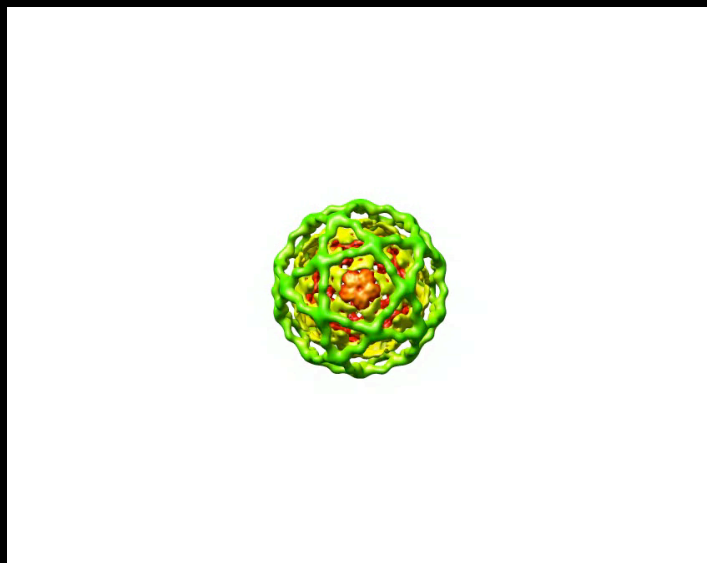
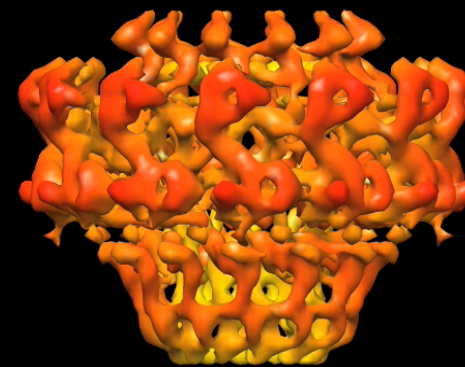
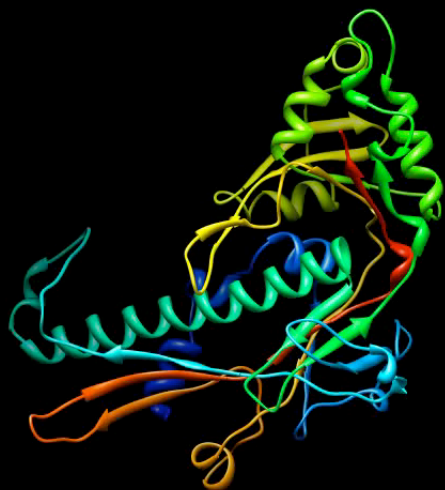


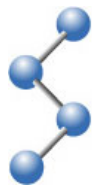
Clint Potter



Bridget Carragher







Workshop on Advanced Topics in EM Structure Determination
November 10 -16, 2007
National Resource for Automated Molecular Microscopy
The Scripps Research Institute, La Jolla, CA



Participants

Xabier Agirrezabala; Jussi Aittoniemi; Teddy Ajero; Cinzia Ambrosi; Chris Arthur; Francisco Asturias; Tim Baker; Albion Baucom; David Belpap; Stefan Bohn; Chris Booth; Edward Brignole; Bridget Carragher; Joshua Chappie; James Chen; Shaoxia Chen; Songye Chen; Xiaocheng Chen; Anchi Cheng; Wah Chiu; So-Hye Cho; Jack Coats; Julia Cope; Pierre-Damien Coureux; Alexandra Deaconescu; Erik Debler; Frank Depoix; David DeRosier; Amedee des Georges; Kenneth Downing; Edward Egelman; Mary Evans; Denis Fellmann; Joachim Frank; Yoshinori Fujiyoshi; Carrie Gabaldon; Lu Gan; Christoph Gerle; Robert Glaeser; Thomas Goddard; Niko Grigorieff; Irina Gutsche; Stephen Harrison; Richard Henderson; Christopher Irving; Grant Jensen; Qiu-xing Jiang; Jack Johnson; Debbie Kelly; Christopher Kennaway; Reza Khayat; Kevin Koehntop; Justin Kollman; Anselm Kusser; Meindert Lamers; Gabriel Lander; Lorraine Lathrop; Pick-Wei Lau; Catherine Lawson; Jyh-Yeuan Lee; Kelly Lee; Kyung Eun Lee; Sukyeong Lee; Andres Leschziner; Hazel Levy; Yen-Chywan Liaw; Steven Ludtke; Jason Mears; Ulrich Meissner; Linda Melanson; Stephen Mick; Anna-Clare Milazzo; Ron Milligan; Crystal Moran; Anke Mulder; David Nackashi; Sunita Nayak; Eva Nogales; Pawel Penczek; Steven Pfeiffer; Clint Potter; Jim Pulokas; Joel Quispe; Ludovic Renault; Klaus Schulten; Paul Shao; Fred Sigworth; Thomas Smith; Daniel Southworth; Scott Stagg; Raymond Stevens; Min Su; Haixin Sui; Dongyan Tan; Kenneth Taylor; Dennis Thomas; Vincenzo Unger; Nigel Unwin; Auke van Balen; Sangita Venkataraman; Neil Voss; Thomas Walz; Hongwei Wang; Andrew Ward; Liz Wilson-Kubalek; Chuan Xiao; Yi Xing; Fei Xu; Alevtyna Yakushevskaya; Mark Yeager; Craig Yoshioka;