

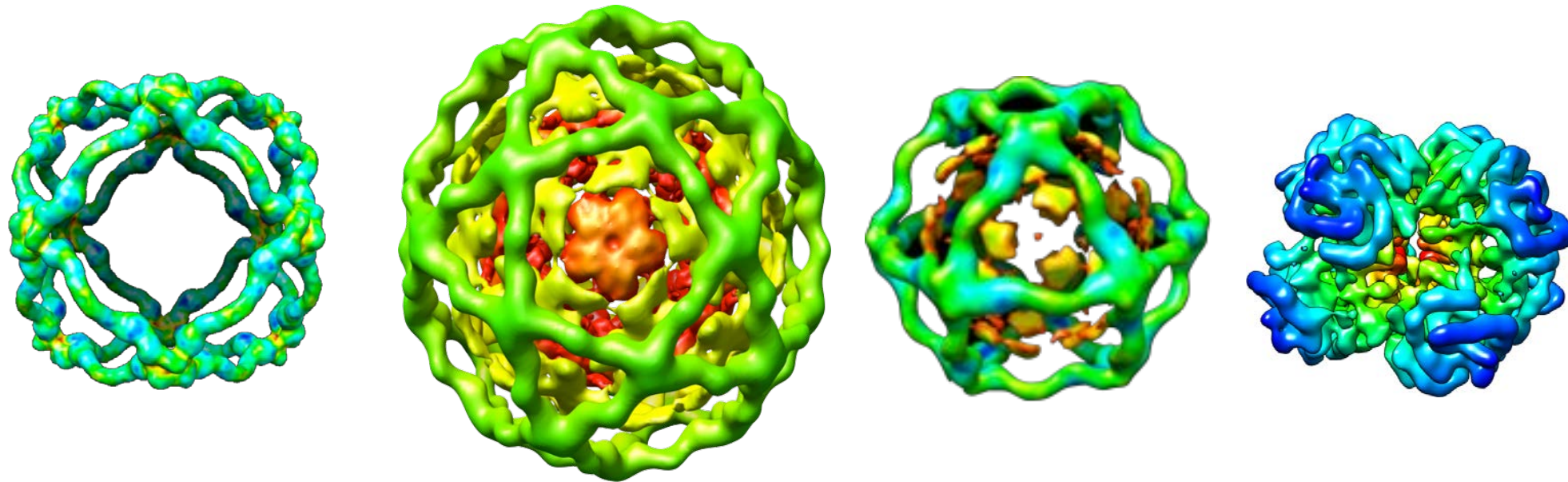
Using High-Throughput High-Resolution Cryo-EM to Address Challenging Questions in Structural Biology

Scott M. Stagg

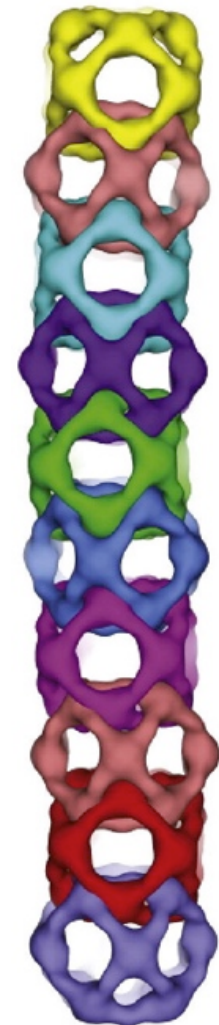
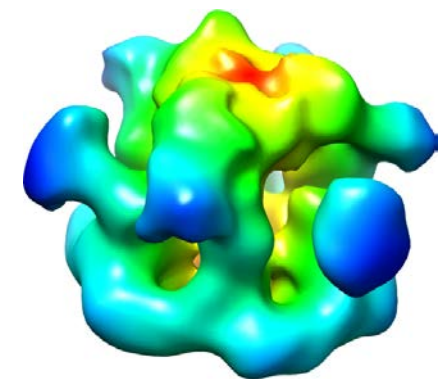
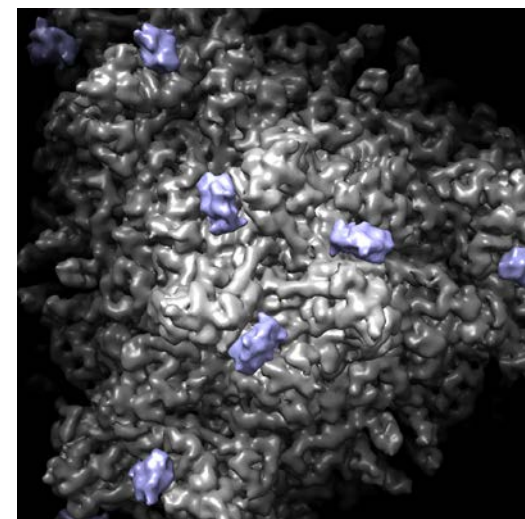
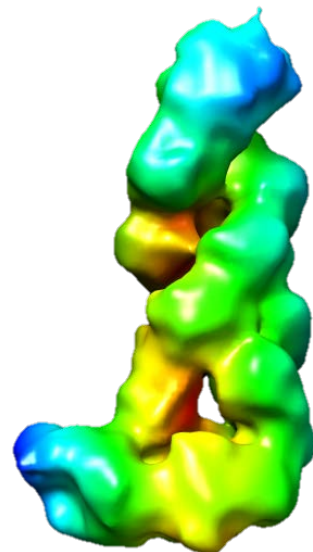
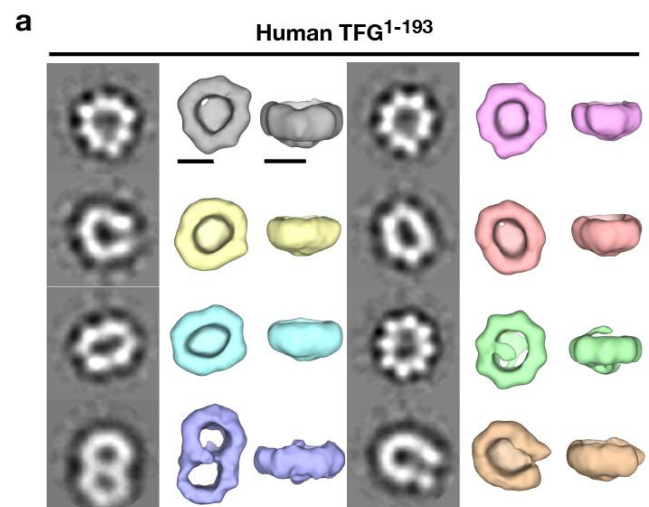
**Institute of Molecular Biophysics
Department of Chemistry and Biochemistry
Florida State University**

Research in the Stagg lab

Structural biology of vesicle trafficking



Tools for high-throughput high-resolution cryo-EM





The revolution will not be crystallized: a new method sweeps through structural biology

Move over X-ray crystallography. Cryo-electron microscopy is kicking up a storm by revealing the hidden machinery of the cell.

[Ewen Callaway](#)

09 September 2015



PDF



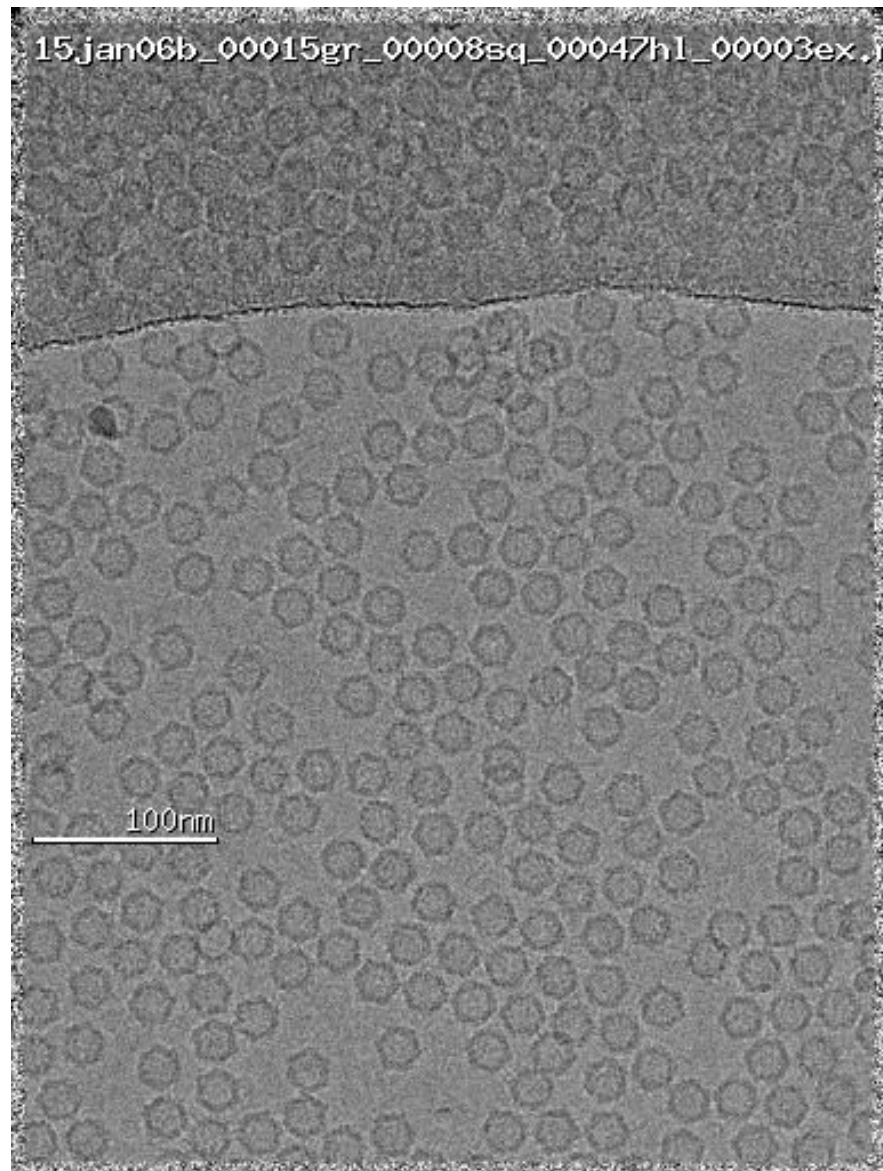
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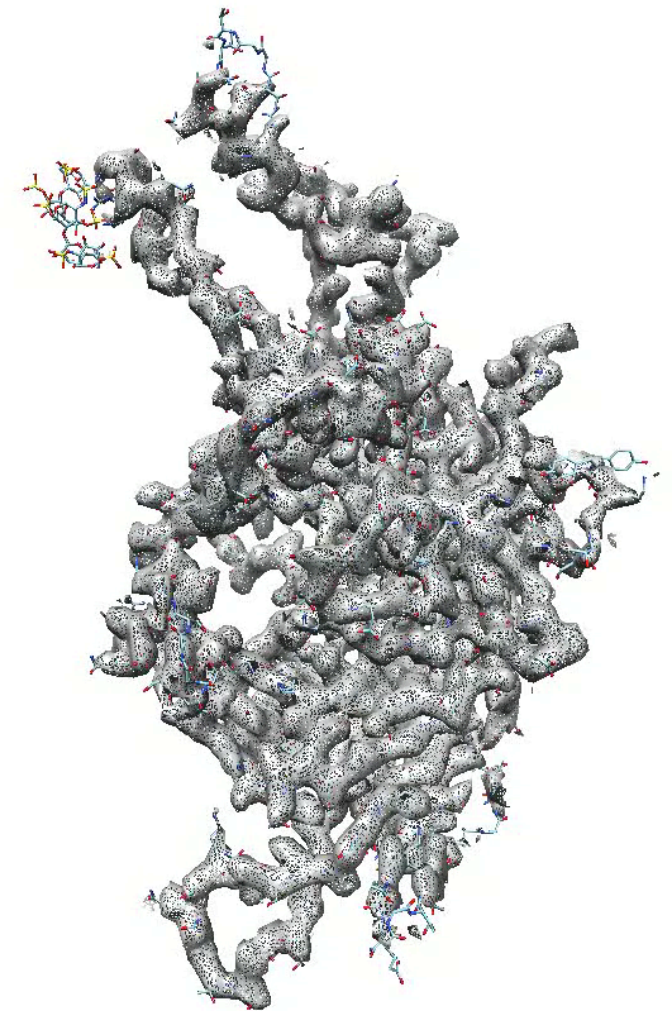
Illustration by Viktor Koen

Tools for high-throughput high-resolution cryo-EM

Single particle reconstruction



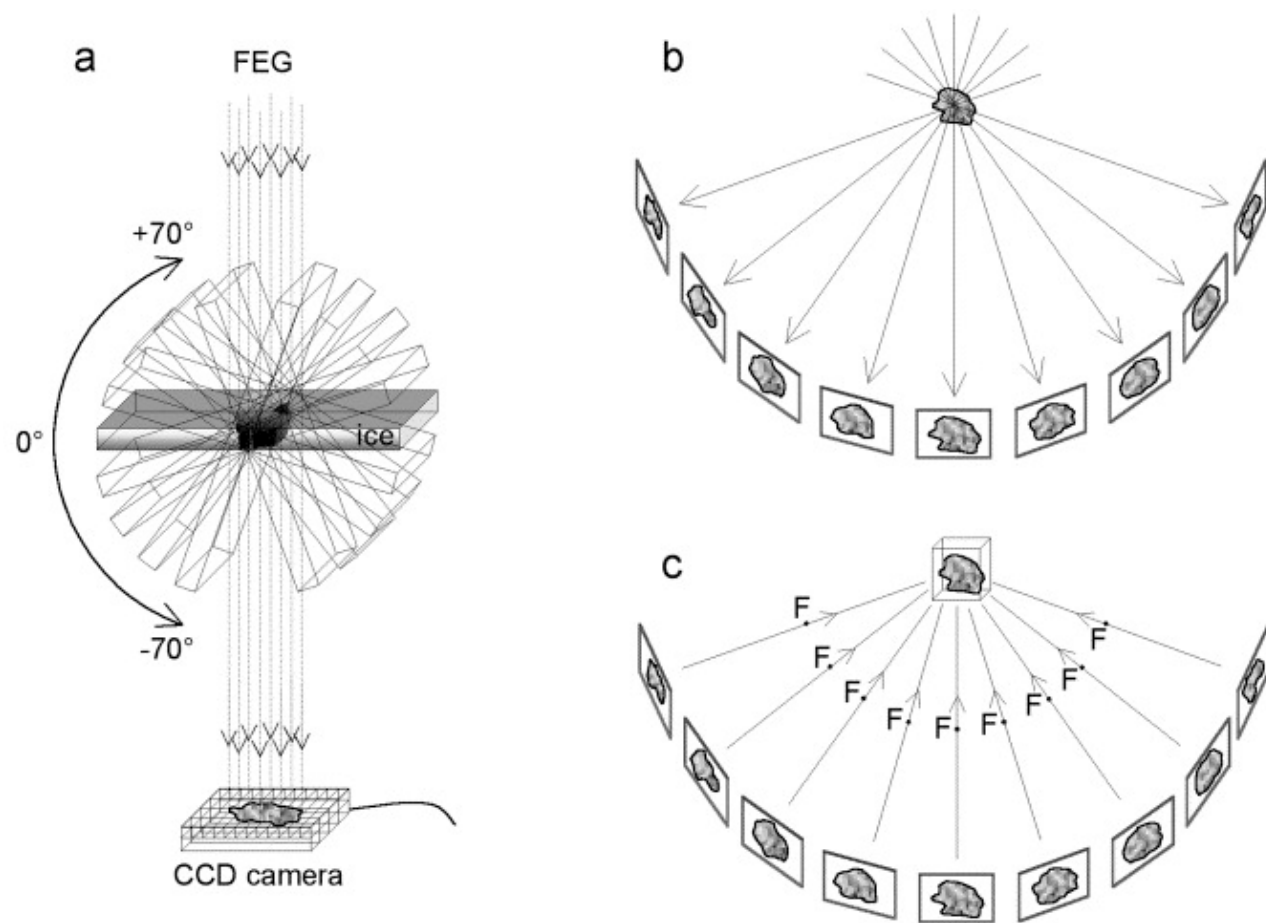
Alignment,
Classification,
Angular assignment



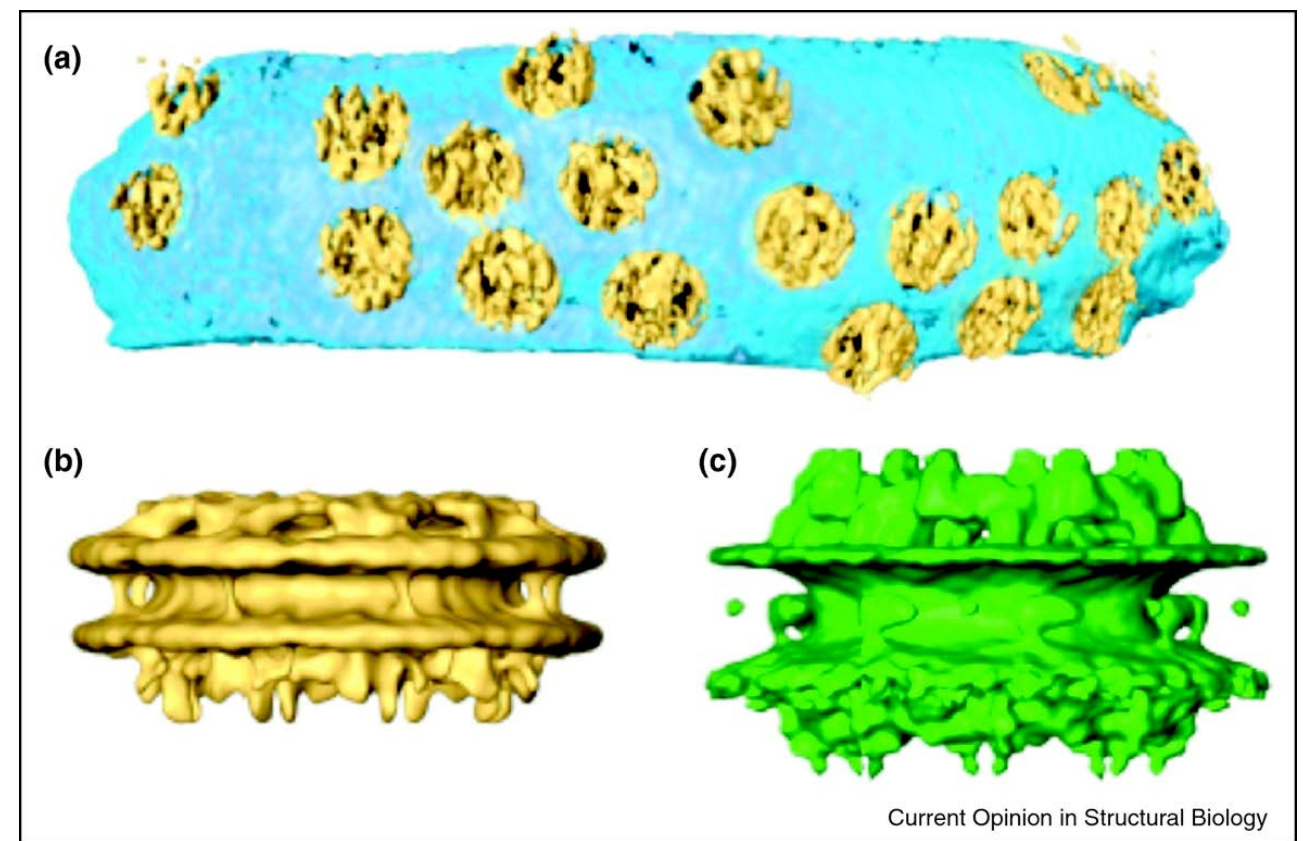
3D reconstruction

- ~1000 images
(inherently atomic resolution)
- 10^4 - 10^6 particles

Electron Tomography

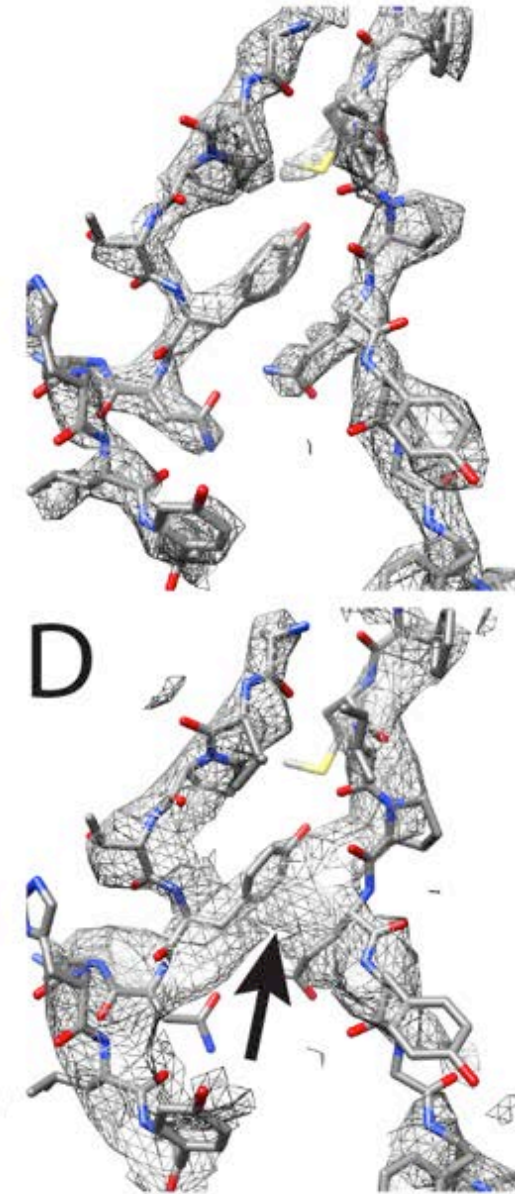
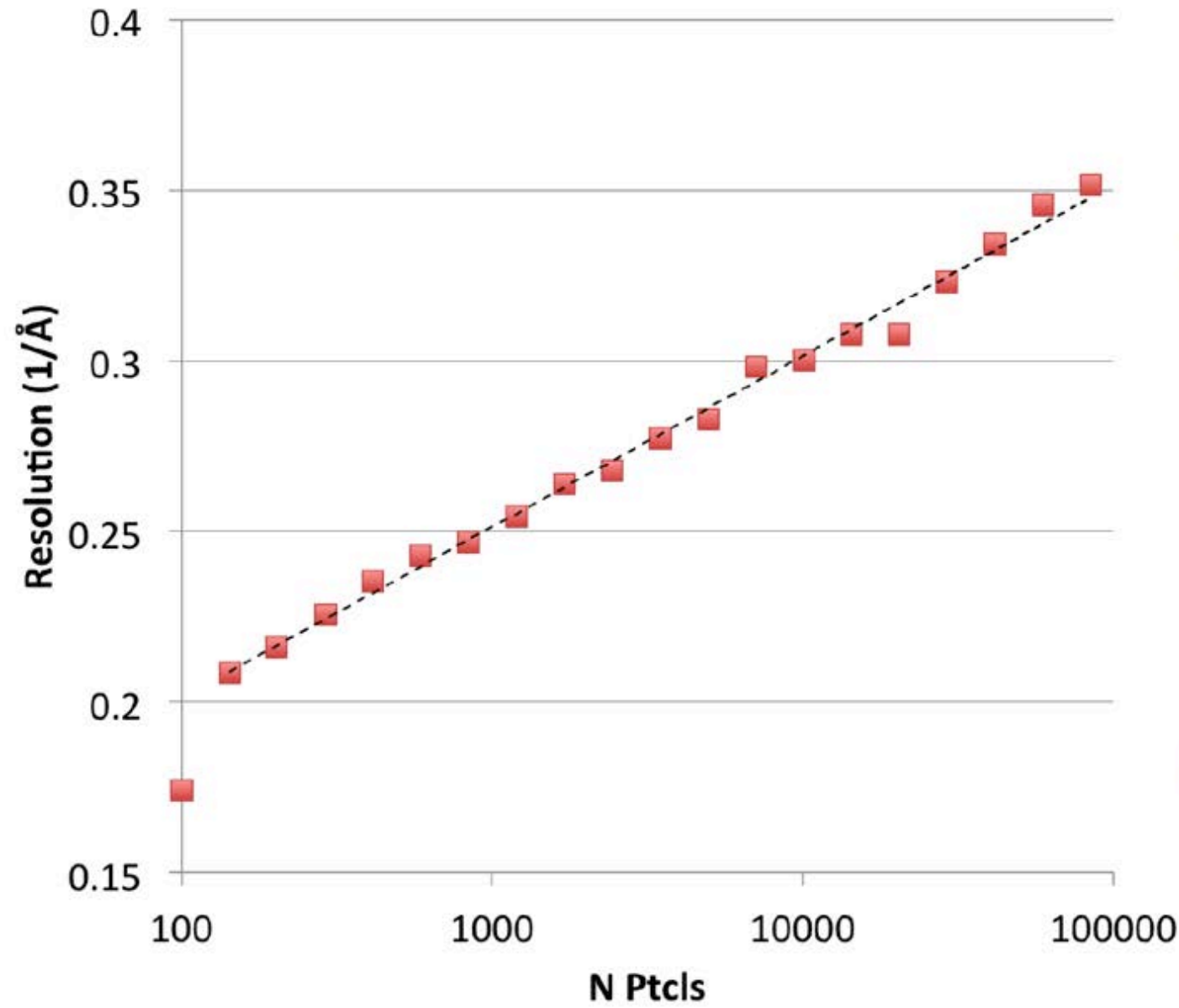


[Grünwald](#) *et. al.*, Biophys Chem. 2003

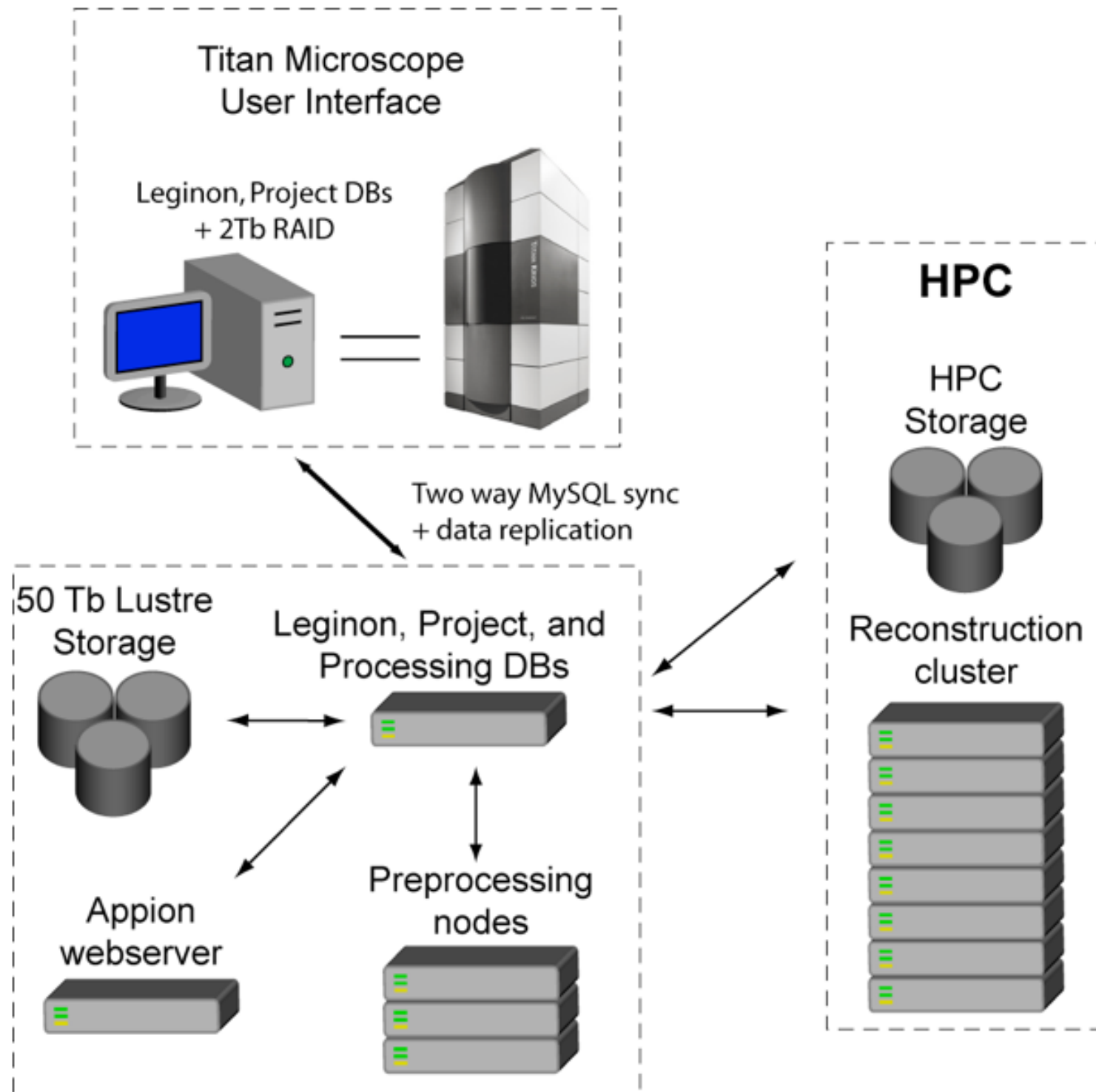


[Yahav](#) *et. al.*, Curr Op Struct Biol. 2011

Resolution depends on # of particles



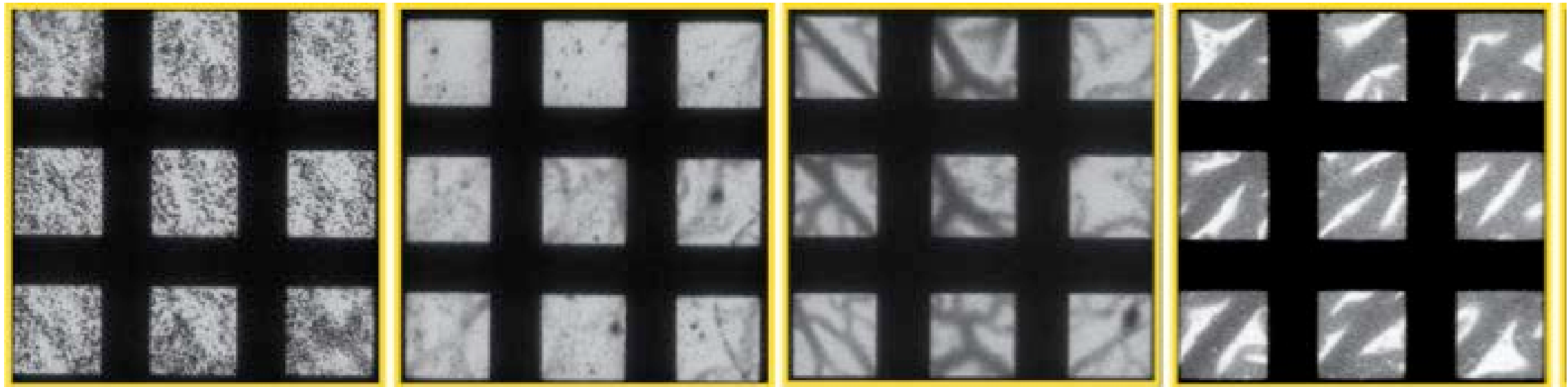
The FSU EM pipeline



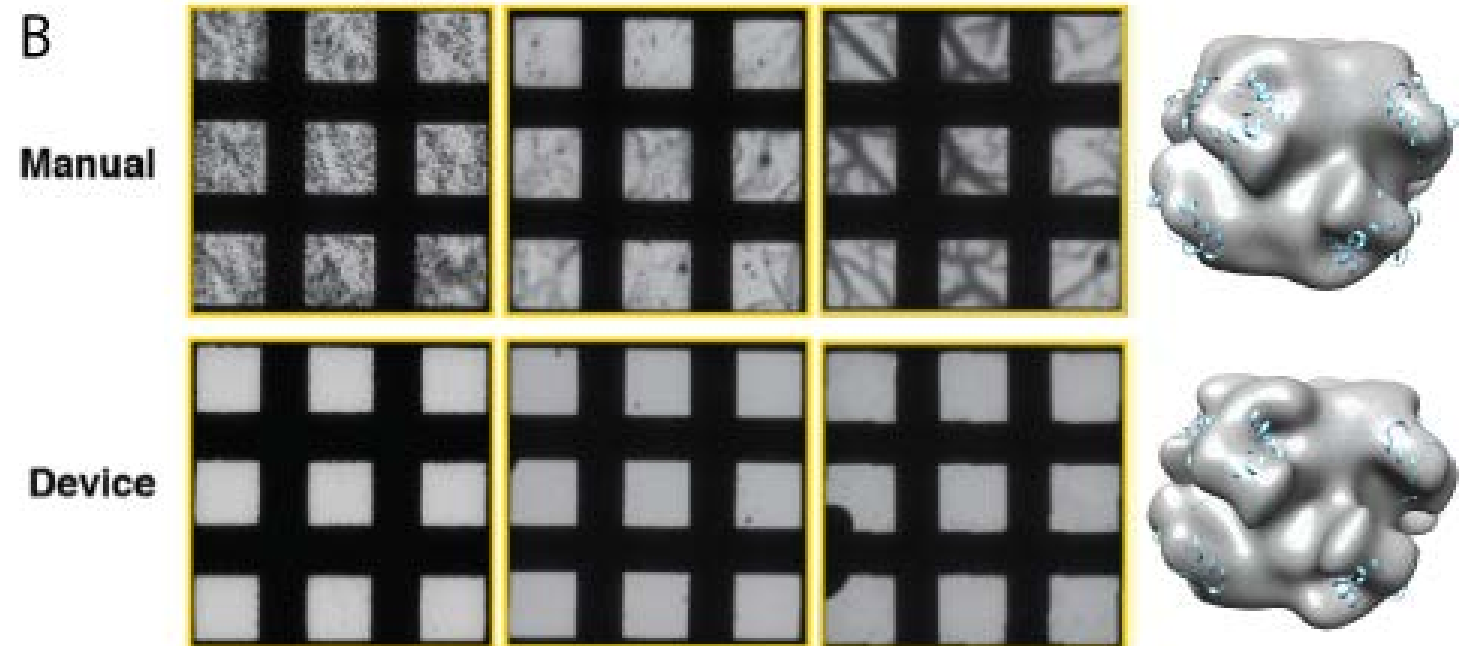
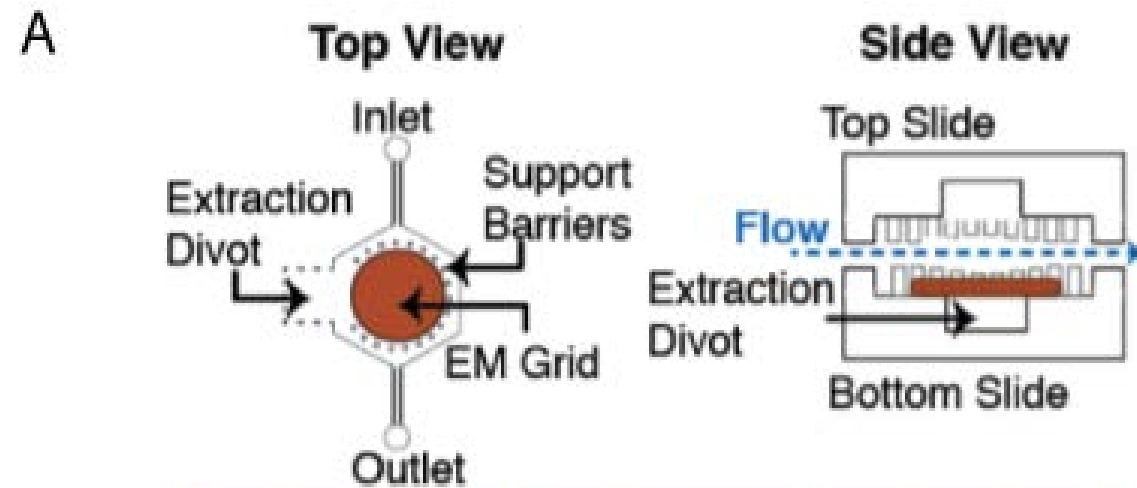
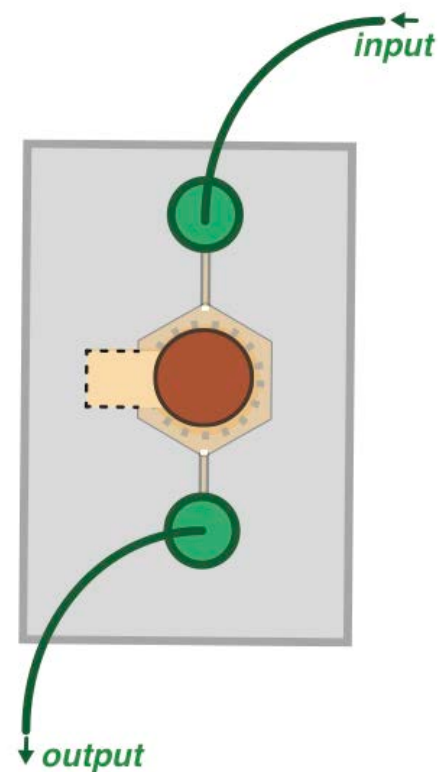
- Leginon/Appion pipeline facilitates high-throughput data collection
 - 85 projects
 - Dozens of users
 - ~2,000,000 images
 - ~50,000,000 particles
 - Backbone for the NIH U24 SECM4 consortium

Sample preparation is the next frontier in 3DEM

- The quality of sample preparation for TEM varies widely from researcher to researcher and sample to sample



Microfluidics for reproducible specimen preparation

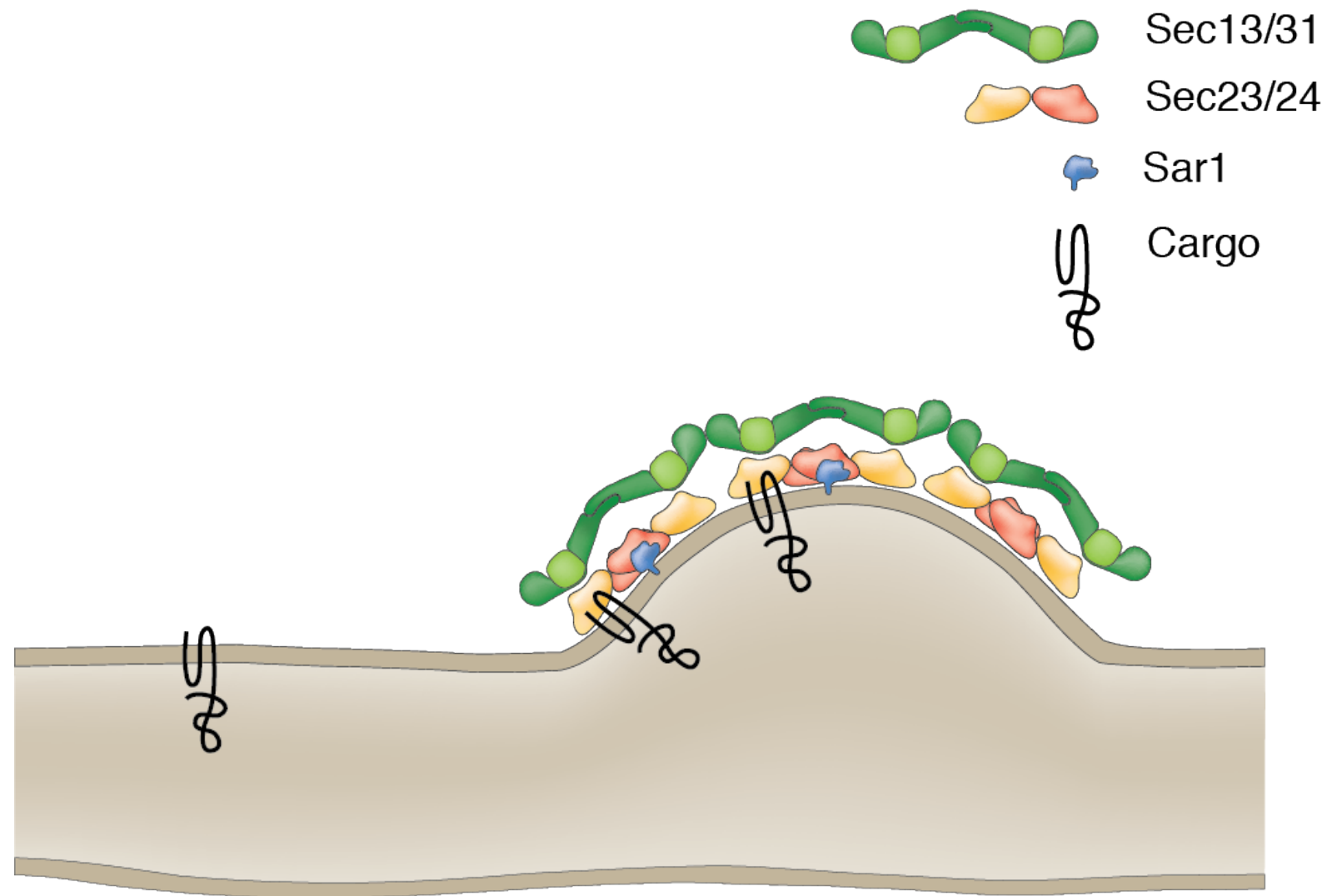


Structural biology of vesicle trafficking

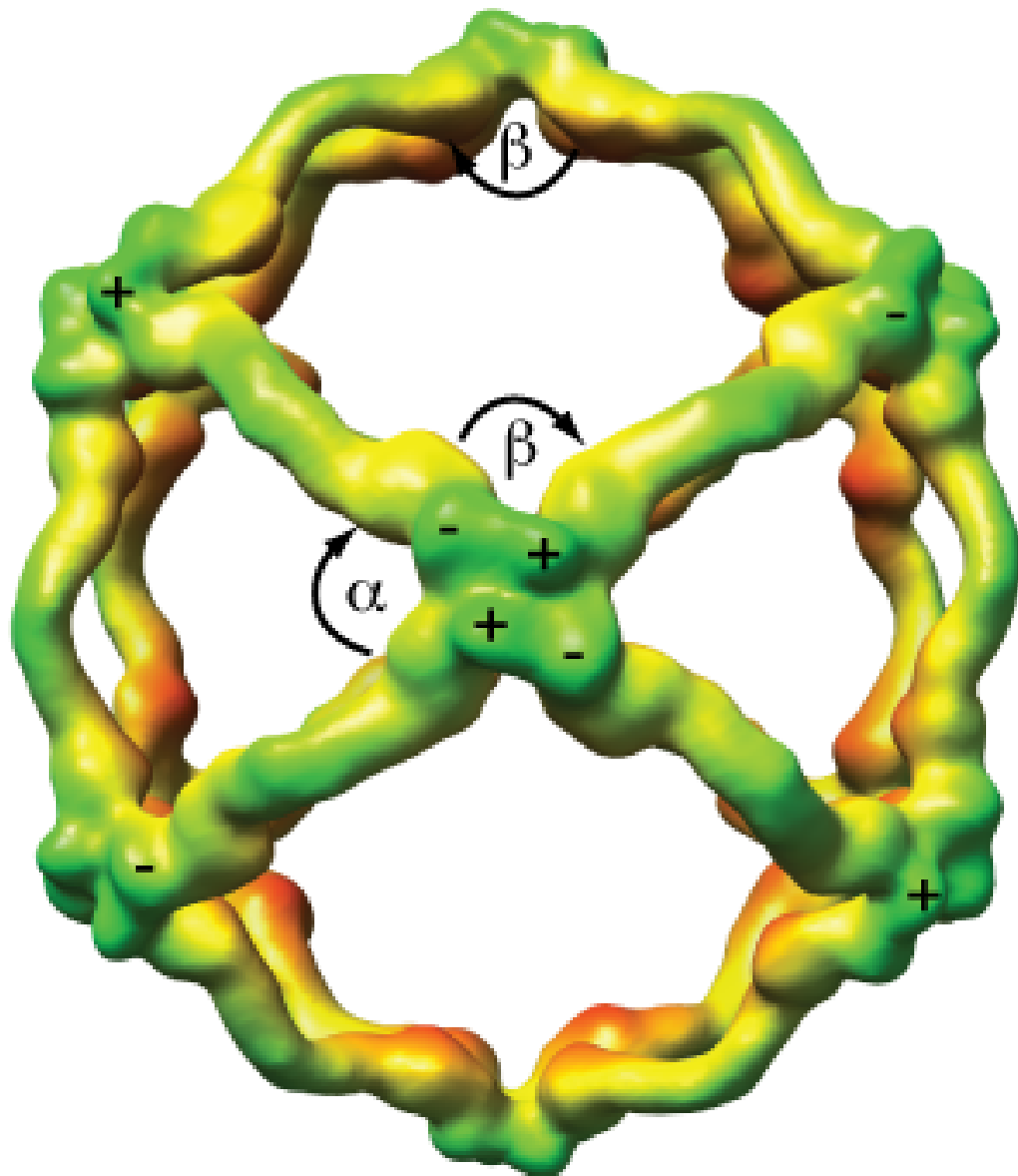
Do all the things

COPII proteins form vesicles from the ER

- Sar1 - regulatory GTPase
 - 22 kDa
- Sec23/24
 - Sec23 - Sar1 GTPase activating protein (GAP)
 - 80 kDa
 - Sec24 - cargo recognition
 - 120 kDa
- Sec13/31 - promotes coat assembly
 - Sec13 - 35kDa
 - Sec31 - 135 kDa



Sec13/31 assembles into a cage

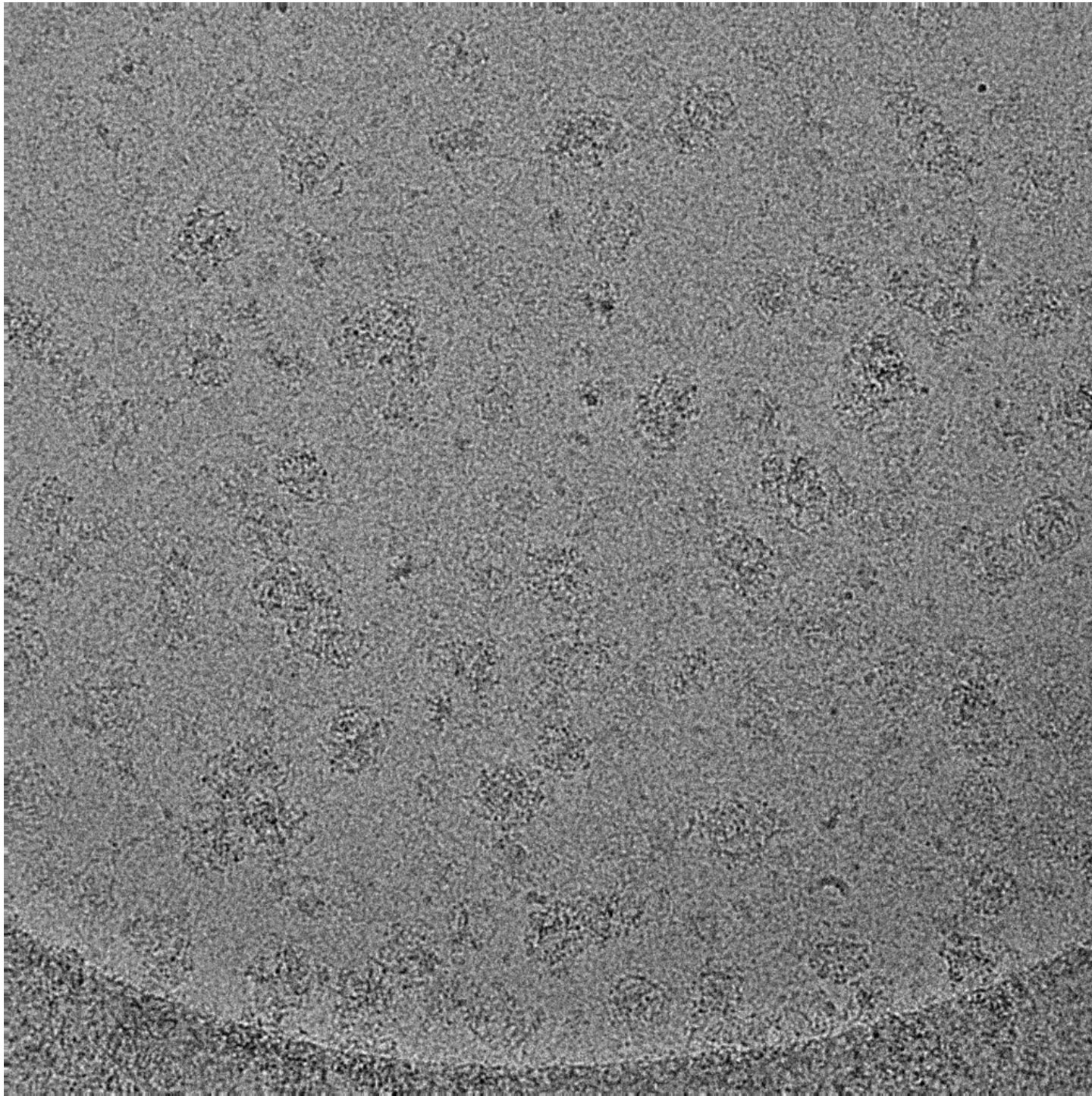


Cage diameter
determined by β angle

$$\alpha = 60$$

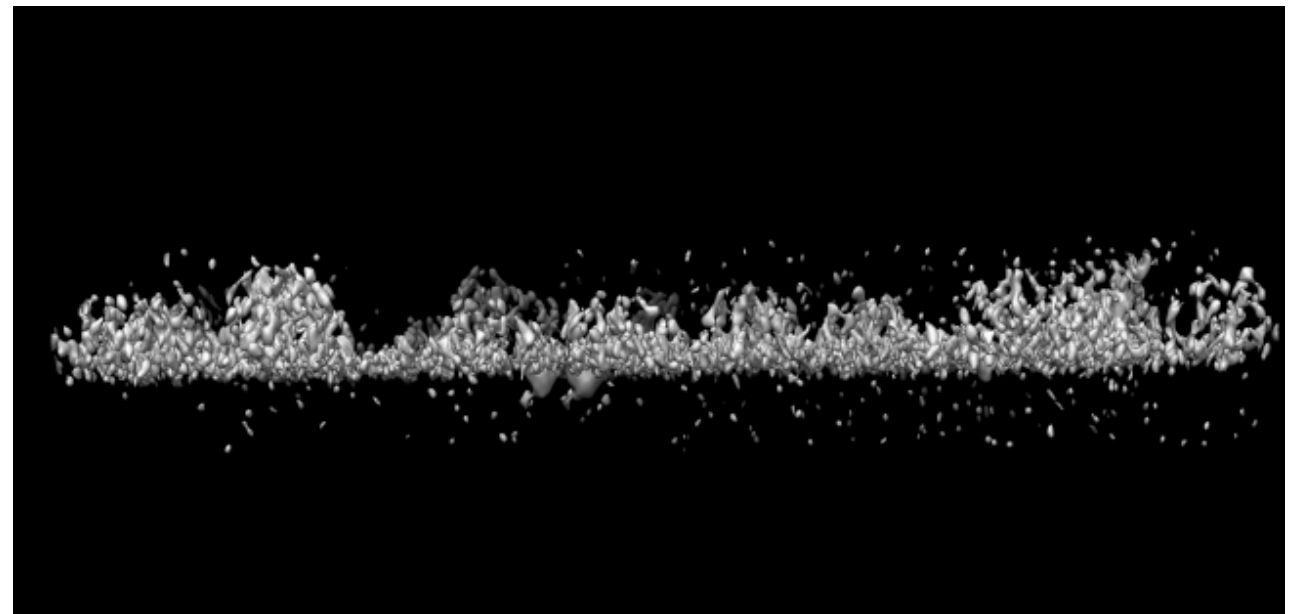
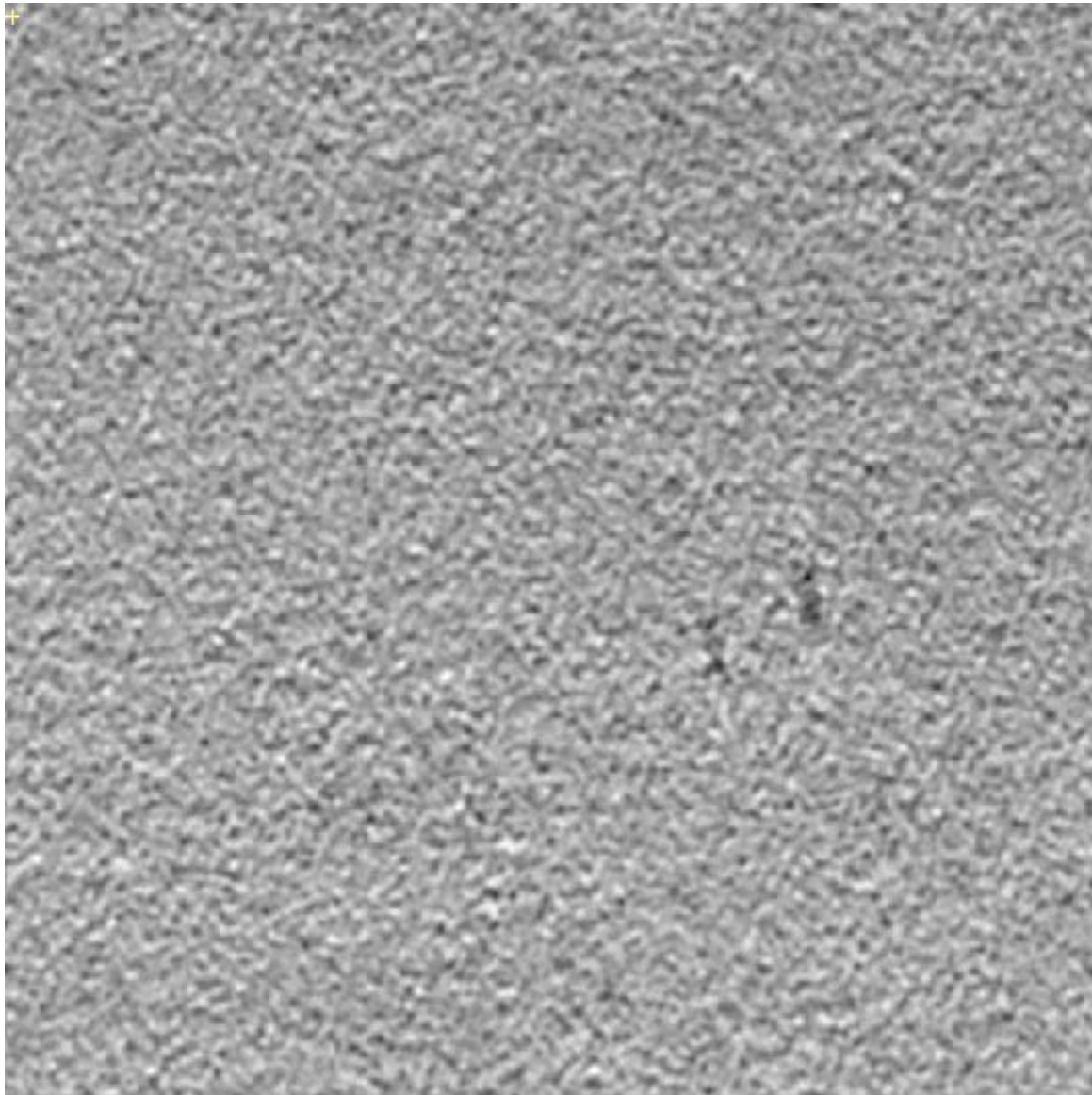
$$\beta = 90, 108$$

Attempt at high resolution COPII cages

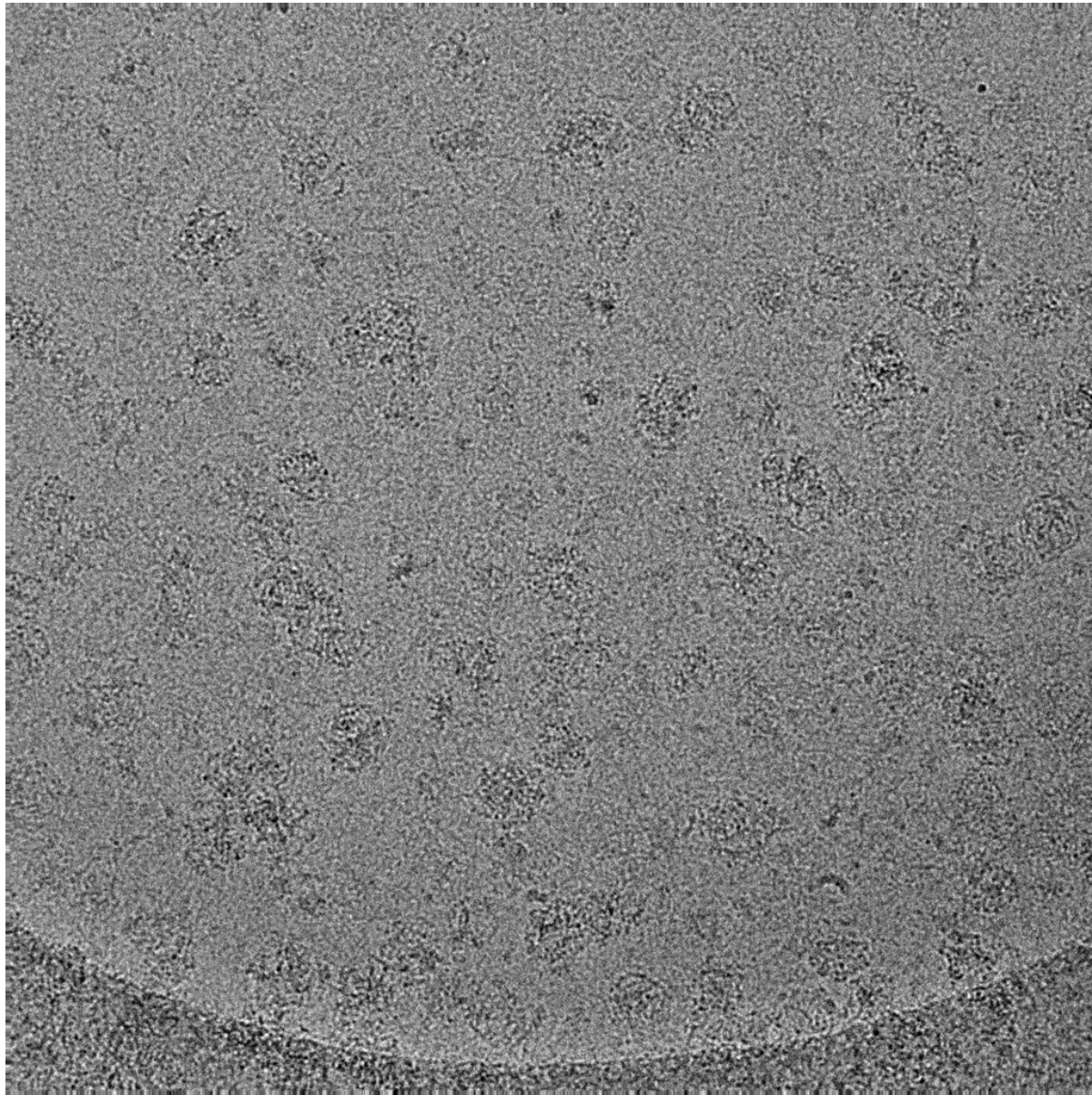


Data went nowhere!

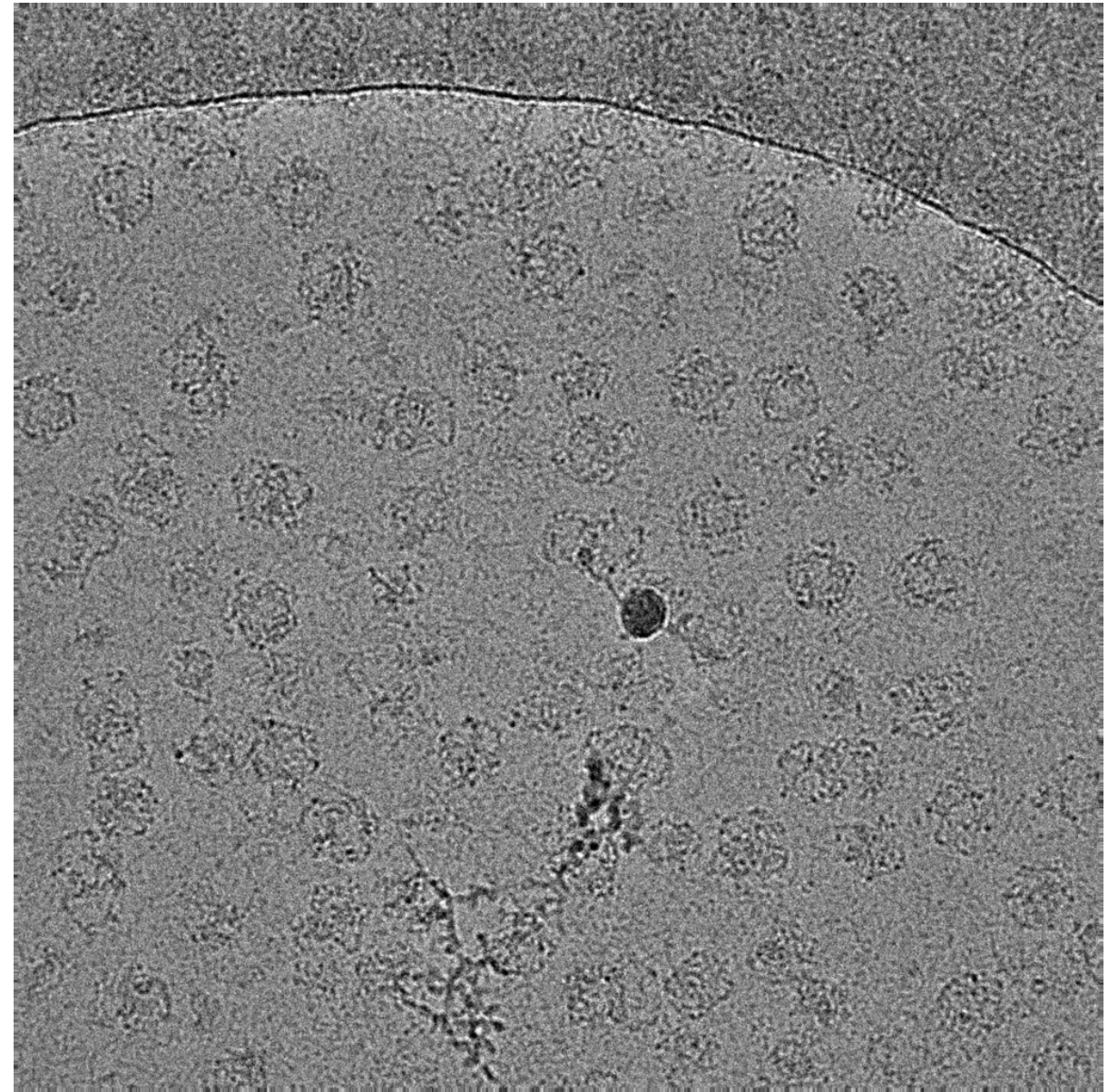
Tomography of COPII cages



After mild fixation, cages are much better preserved

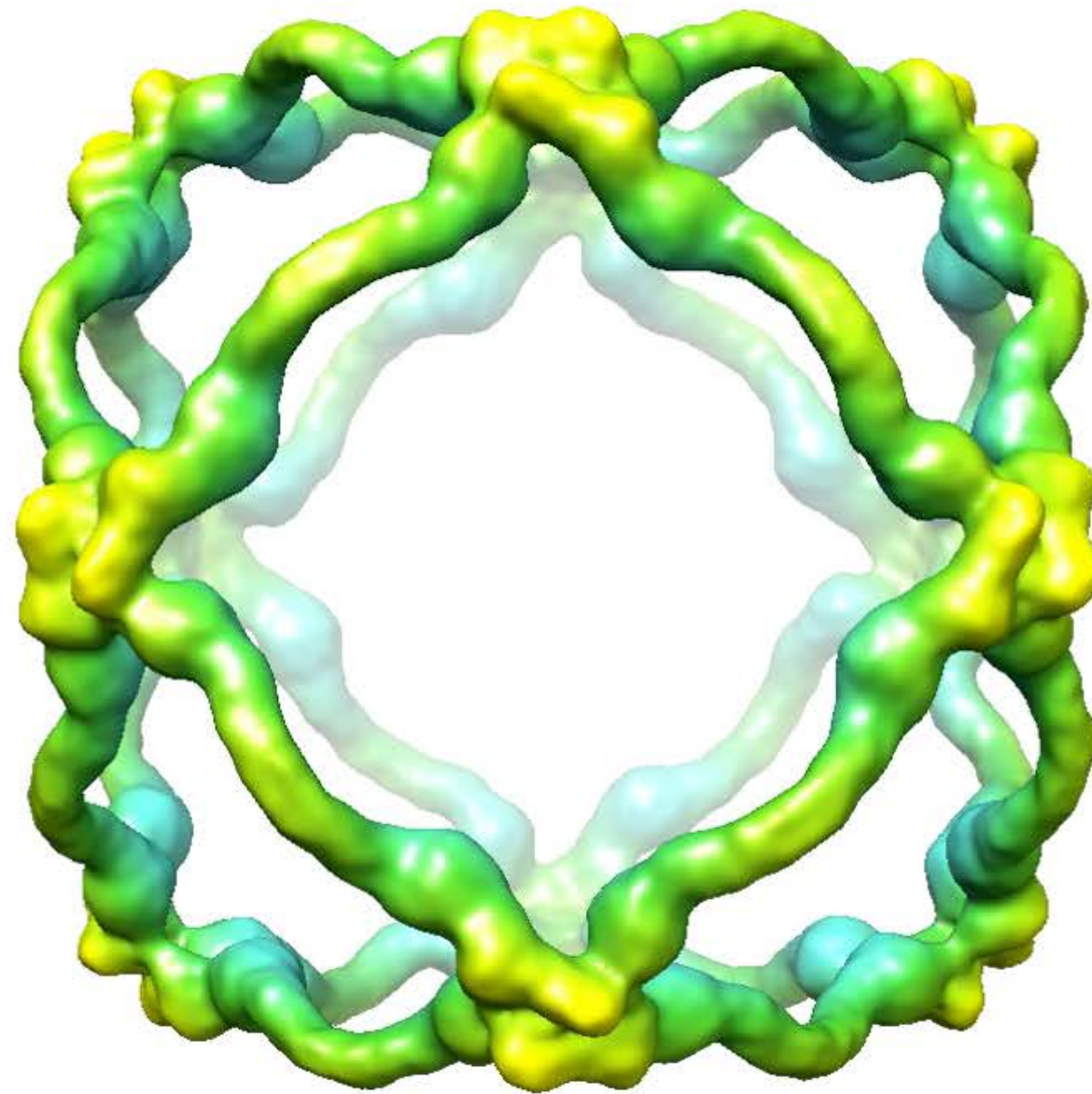


Before



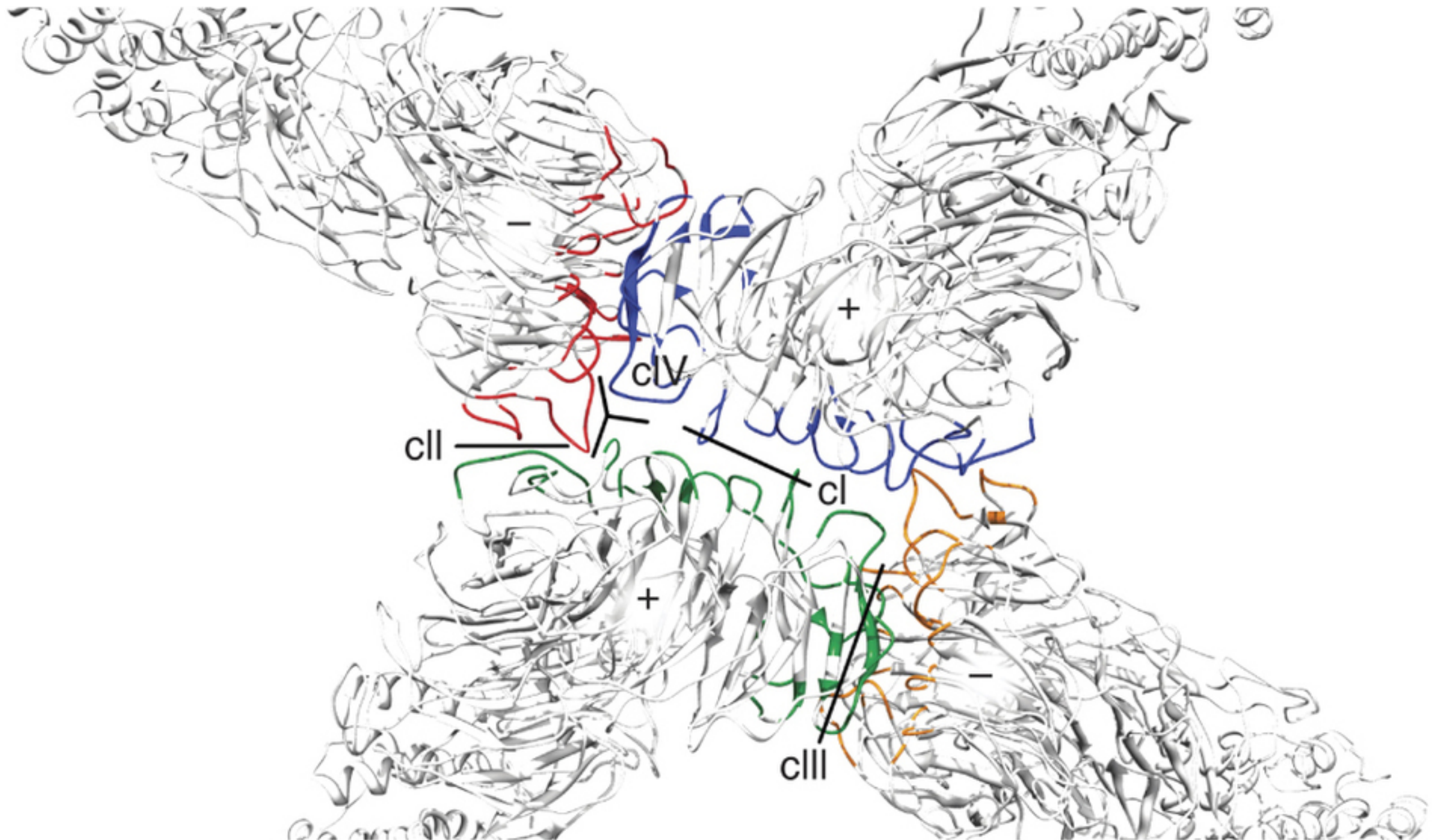
After GRAFIX

Fitting Homology Model to EM density

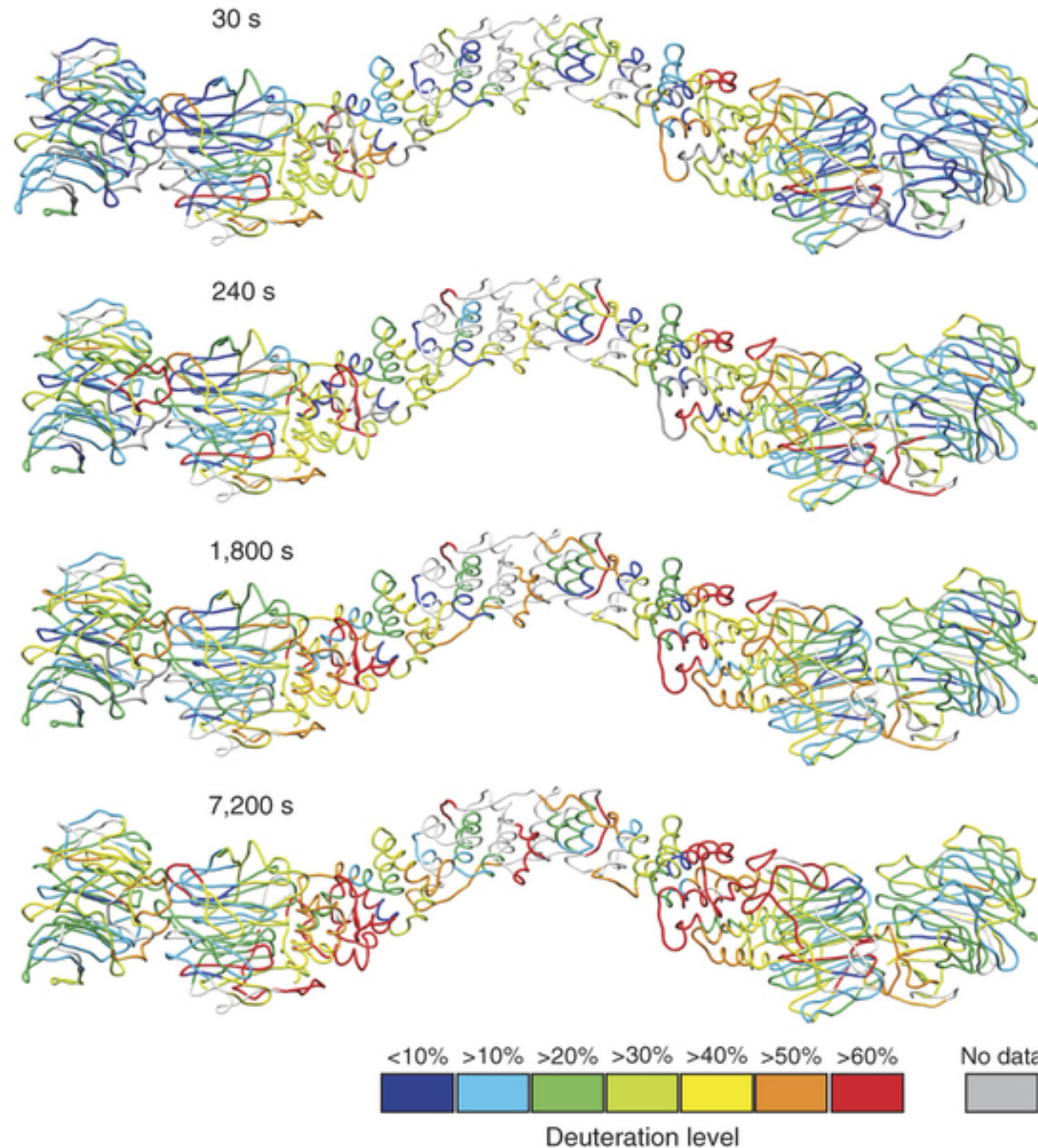


Resolution: 12 Å
(FSC 0.143)

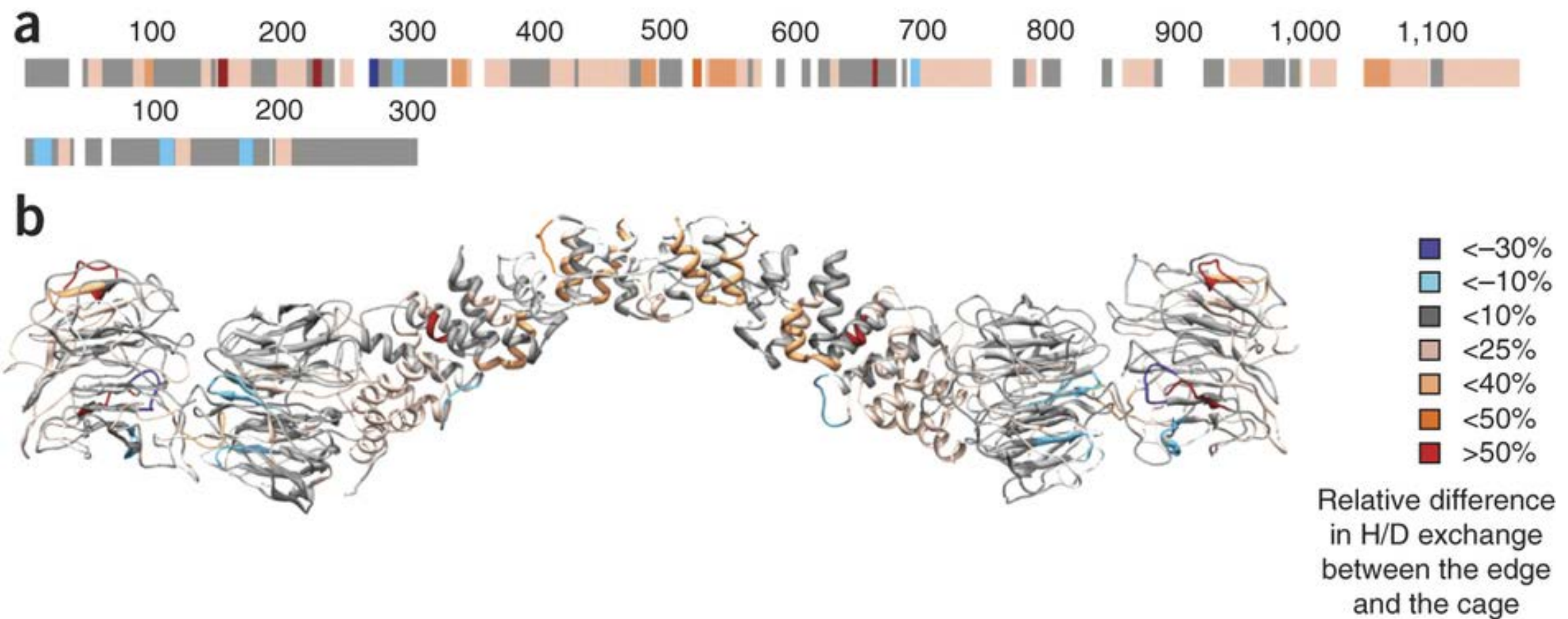
Predicted interactions



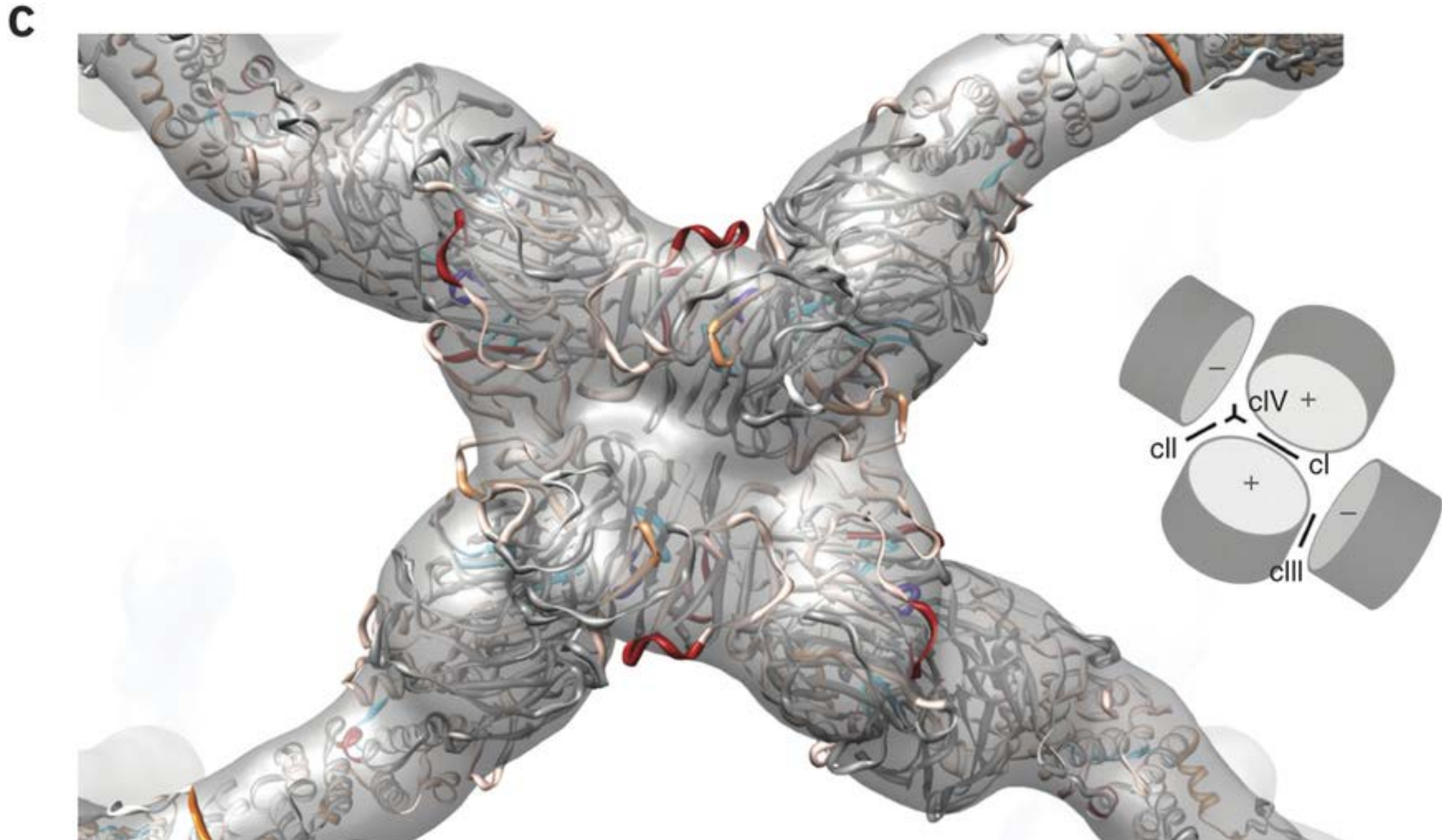
HDX-MS mapped on Sec13/31



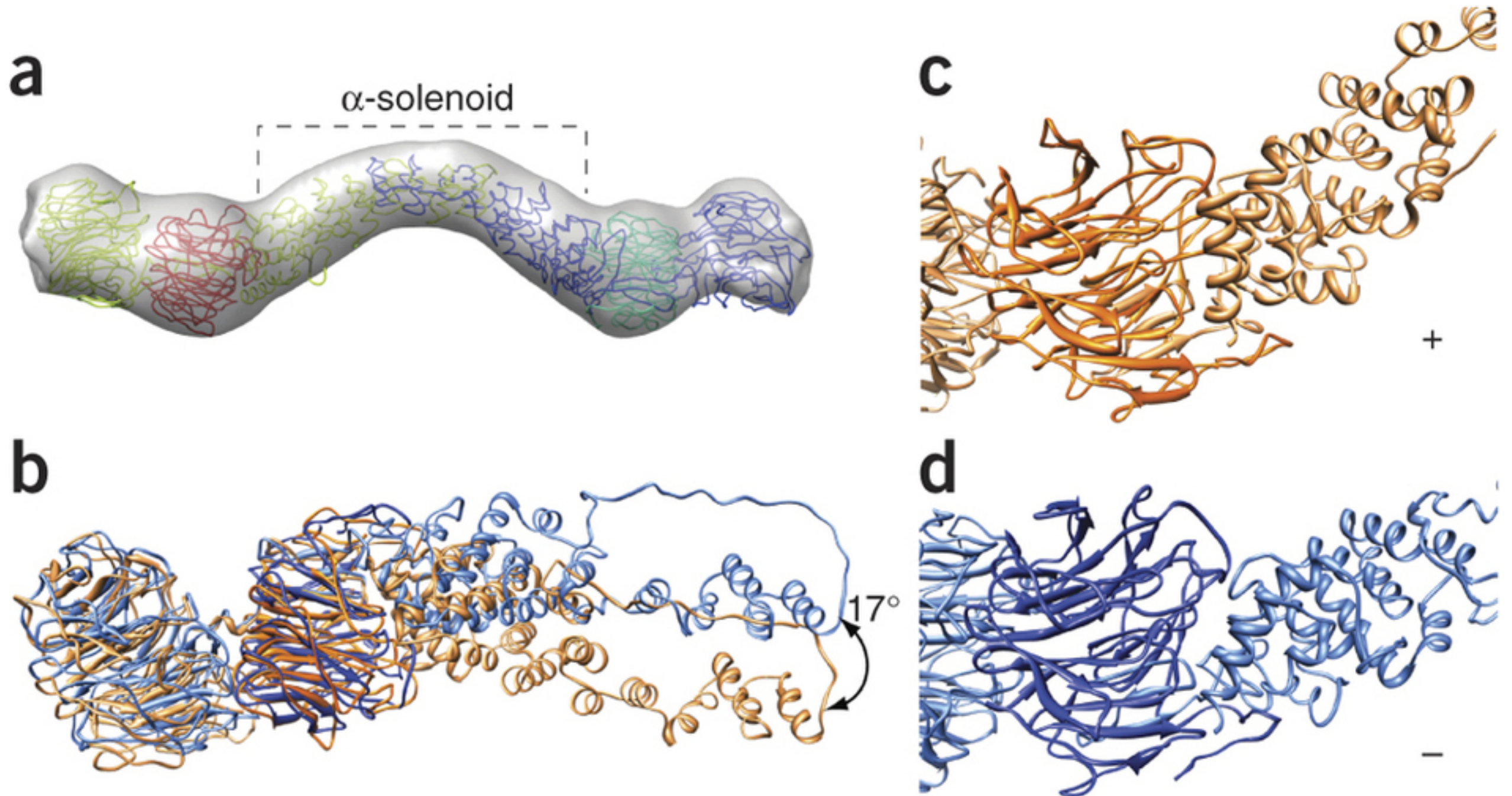
HDX-MS identifies conformational changes and interacting loops



HDX-MS identifies interacting loops



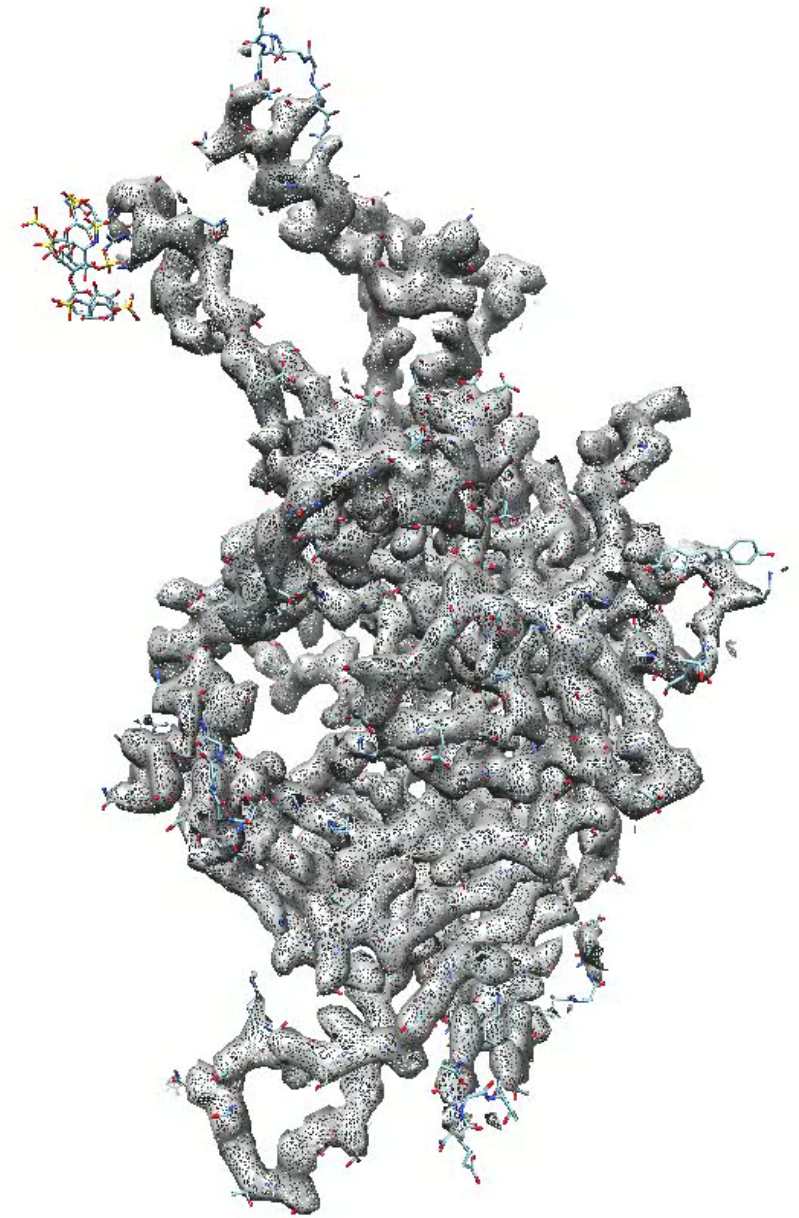
Modeling unveils a hinge between Sec13 and Sec31



Modeling is validated by HDX-MS



Structural biology of the gene therapy vector adeno-associated virus

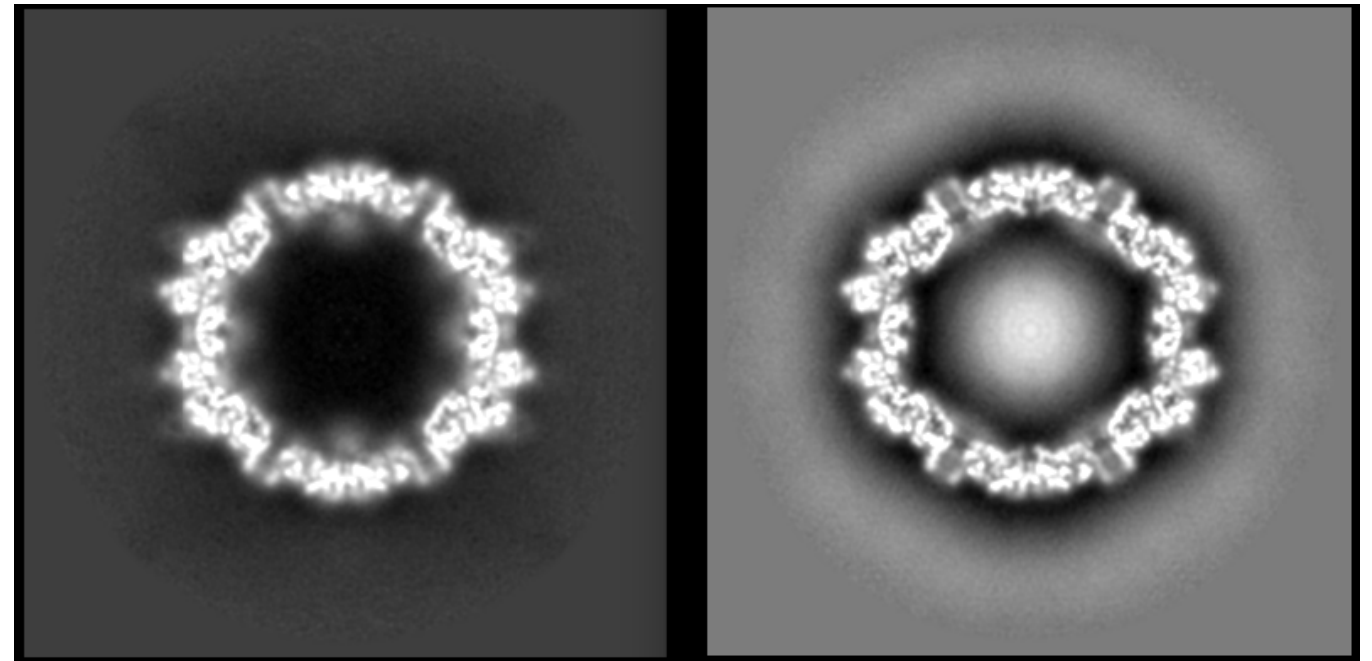
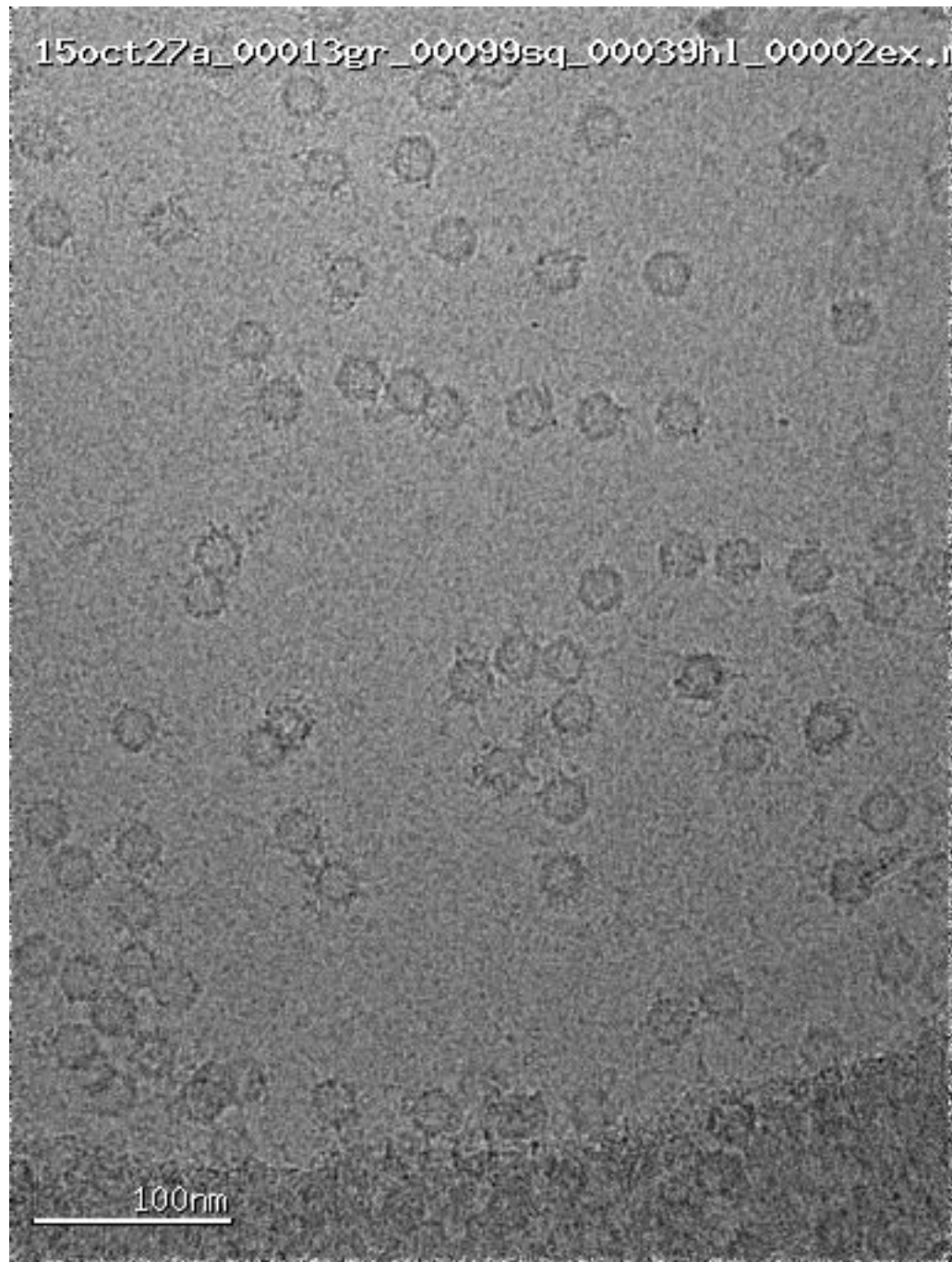


AAV-DJ/Arixtra at 2.8 Å
resolution

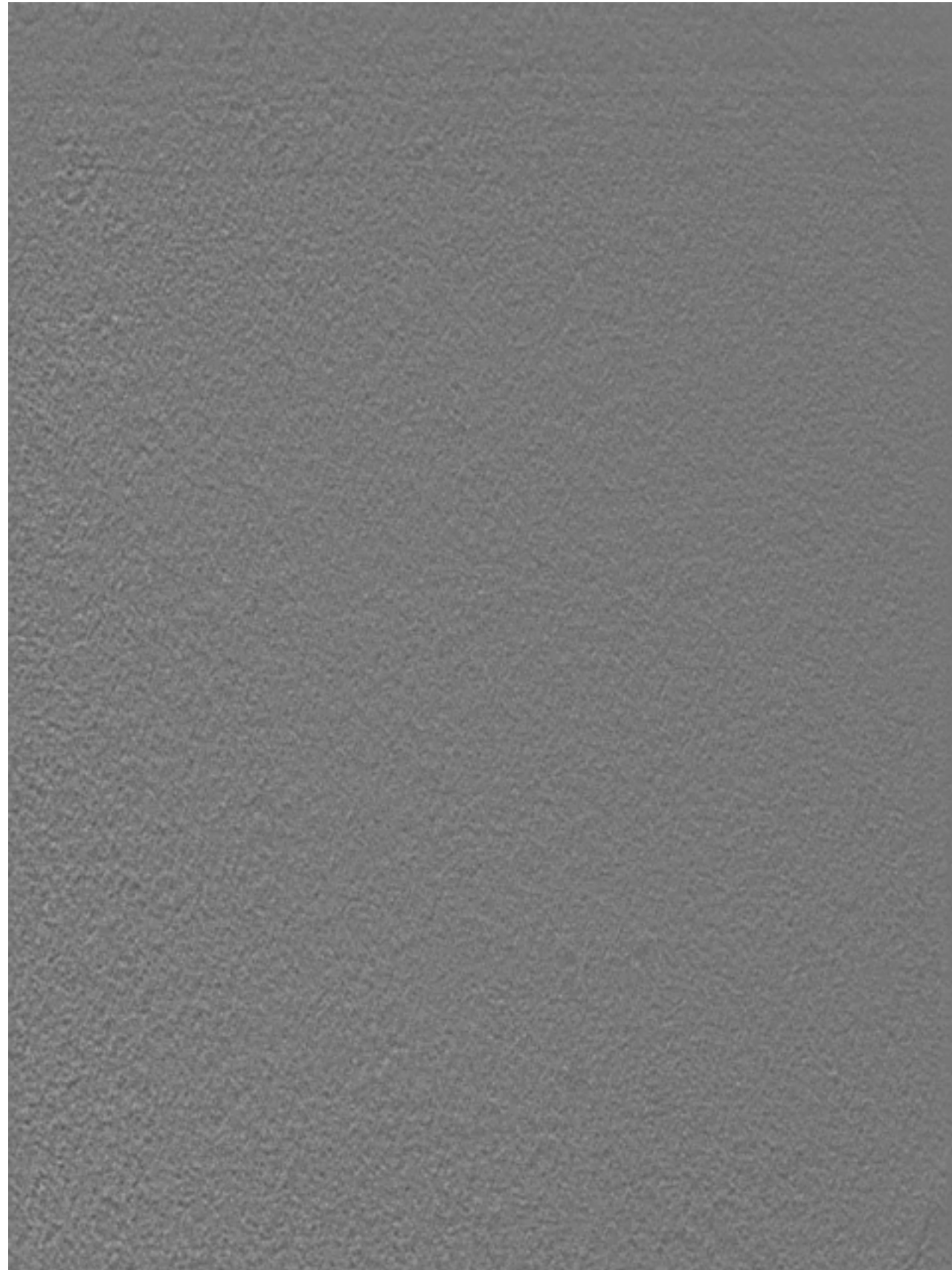
AAV with newly discovered protein receptor AAVR

- In 2016, Chapman and Carette labs determined a novel protein receptor that was critical for AAV uptake
 - Named AAVR

The structure of AAV/AAVR



AAVR tomography

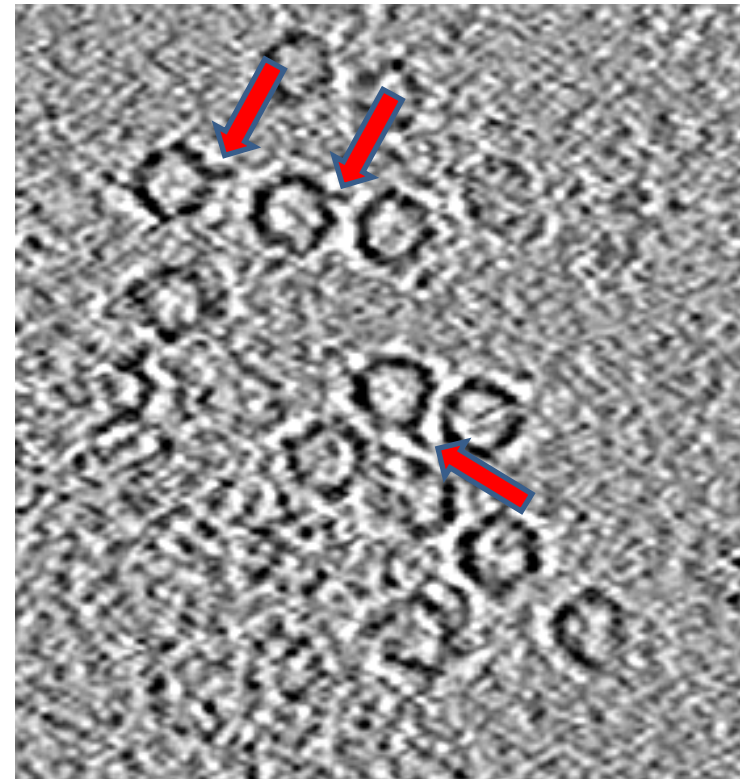
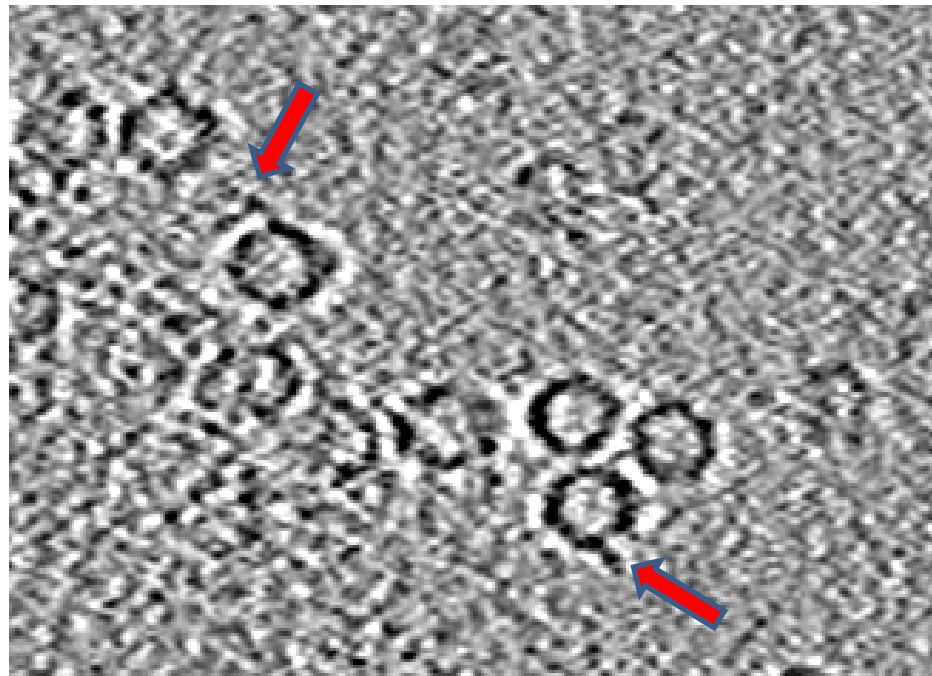


250 nm

Z-Slice: 25/154

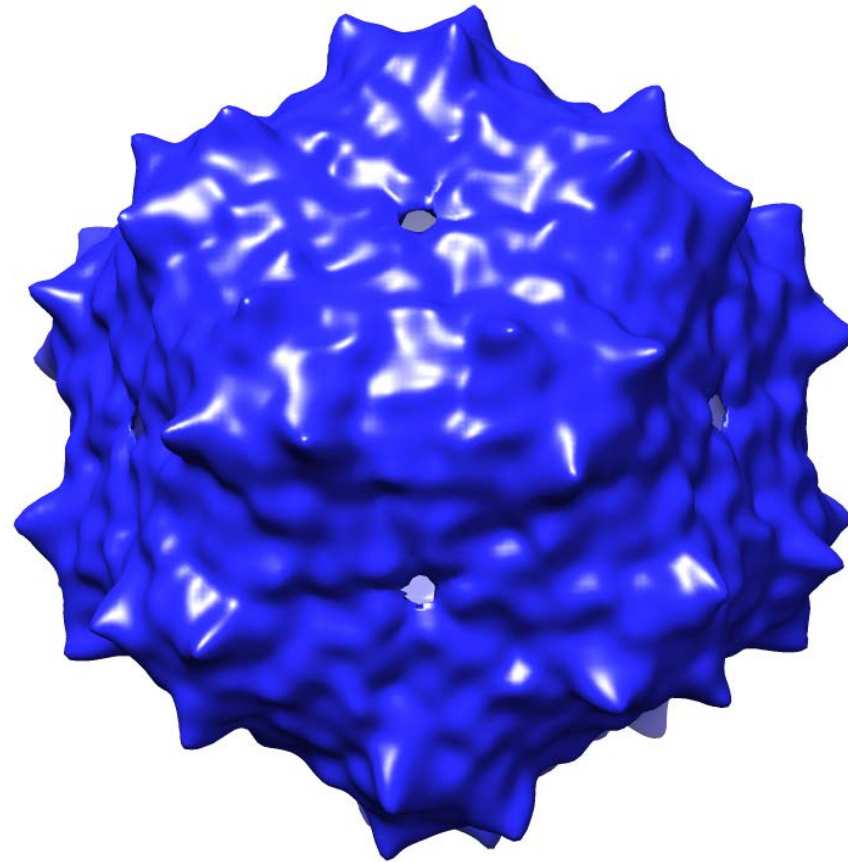
bin=8, lp=31.3, thick=1250

AAVR tomograms



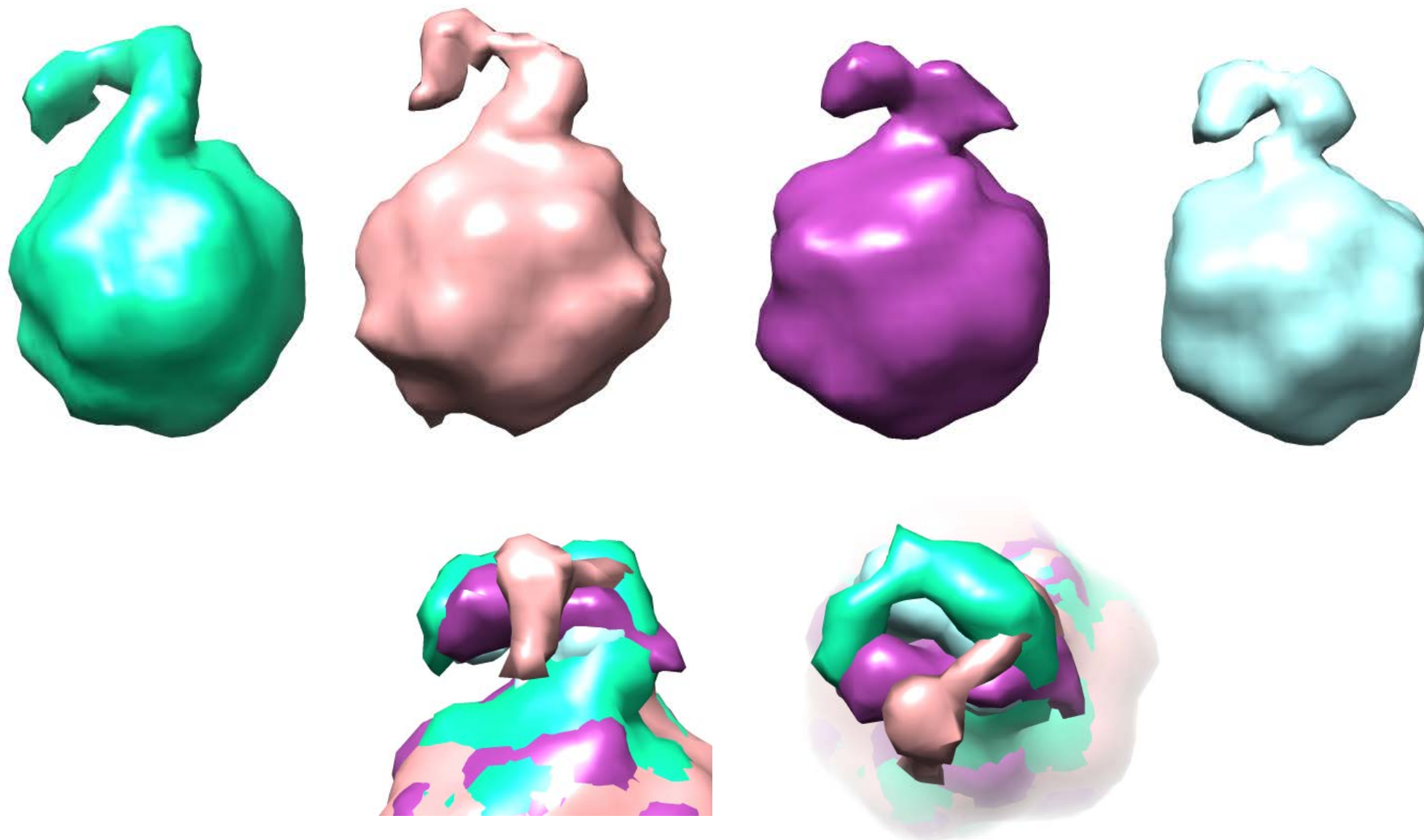
AAVR is clearly visible from the raw tomogram
(red arrow)

Subvolume averaging of AAVR



~1600 individual AAVR particles aligned and averaged – no receptor density visible due to low occupancy

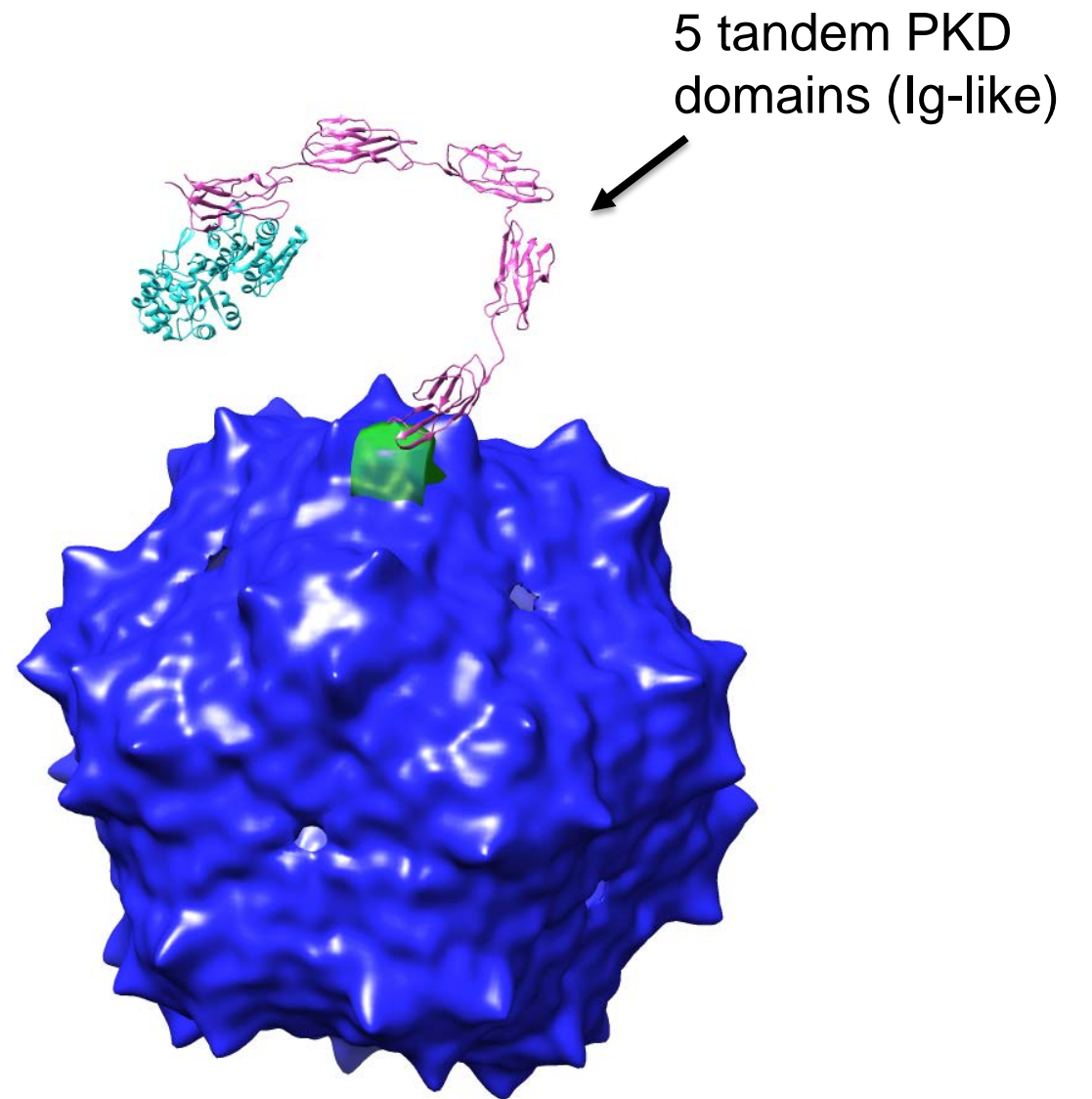
AAVR heterogeneity



Modeling AAVR

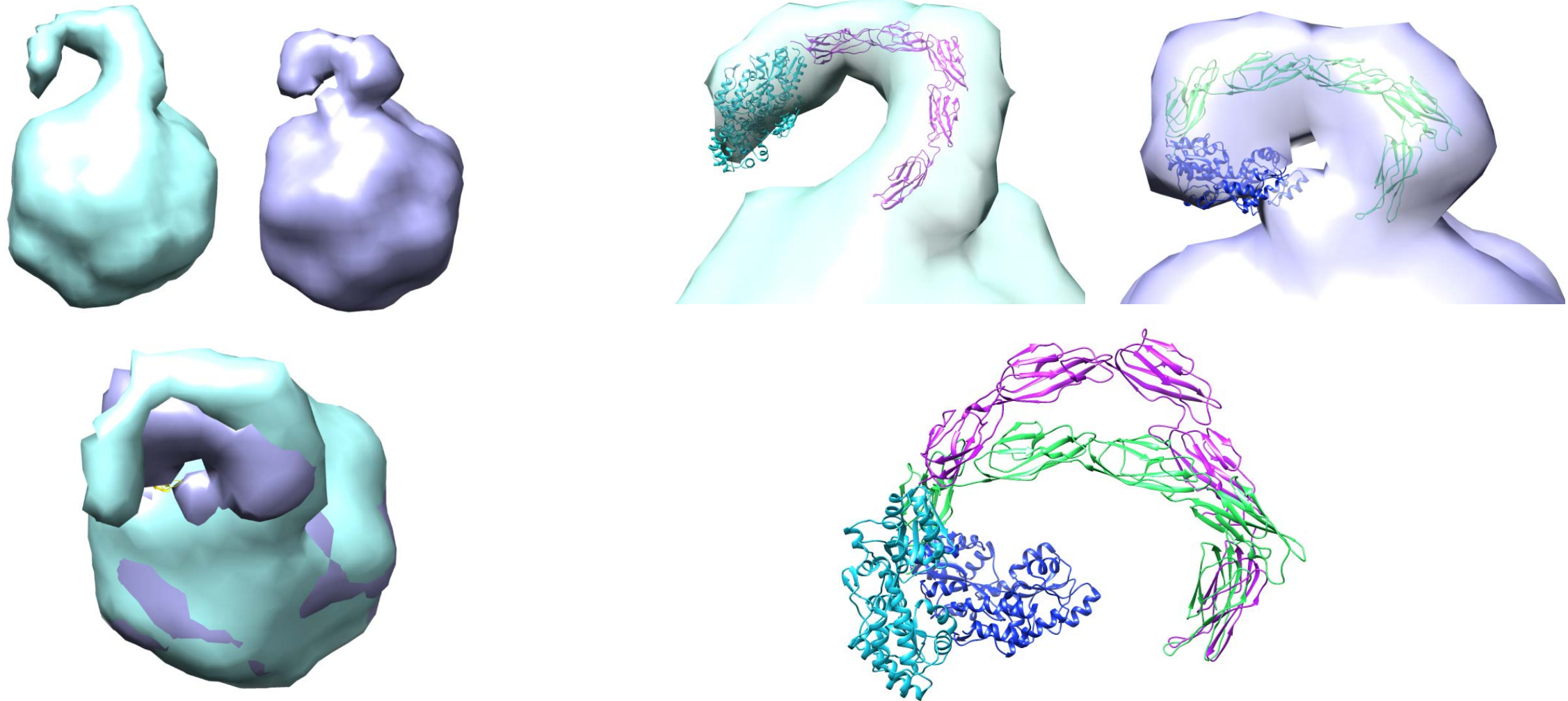


~200 subvolumes averaged



AAVR binds outside of threefold spike

AAVR flexibility



Different subvolume reconstructions demonstrate the range of AAVR flexibility.

AAVR conclusions

- AAVR saturates AAV with ~ 3 molecules per virion
- Flexibility and low occupancy too extreme for single particle reconstruction
- Tomography reveals binding site for AAVR
- AAVR is highly flexible
 - Flexibility is likely related to its function

Acknowledgements

- Stagg Lab
 - John Spear
 - Alex Noble
 - Jason O’Donnell
 - Hanaa Hariri
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- COPII
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 - Qian Zhang
- AAV
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