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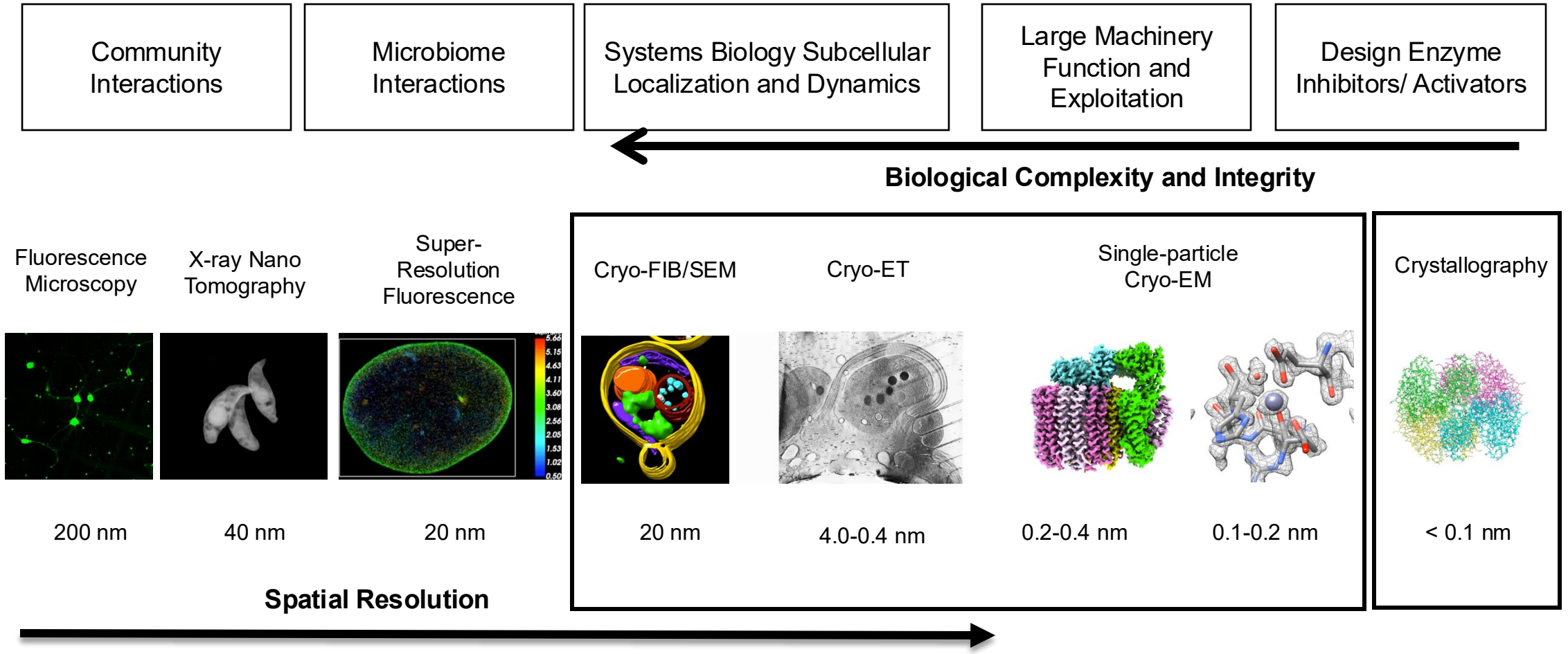
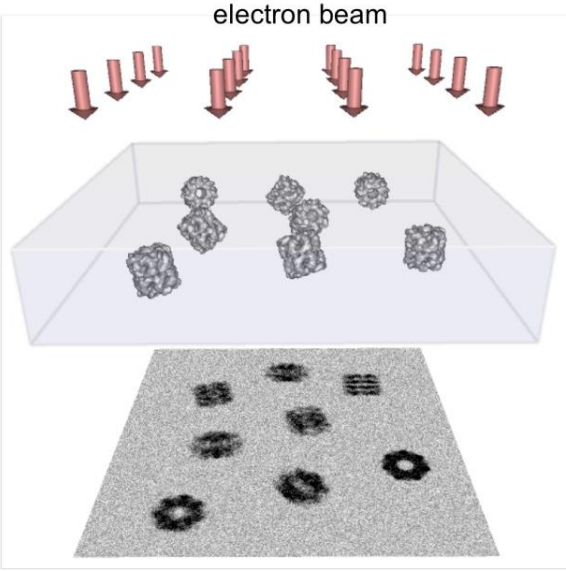
# Multi-Scale Imaging: A Key to Understanding and Regulating Biological Processes for Human and Planetary Health



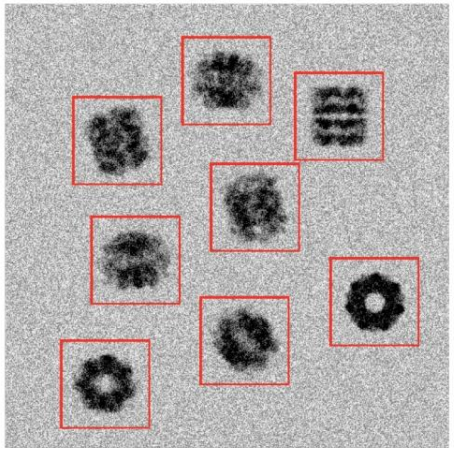
# Atomic Resolution CryoEM for Purified Macromolecules

Imaging

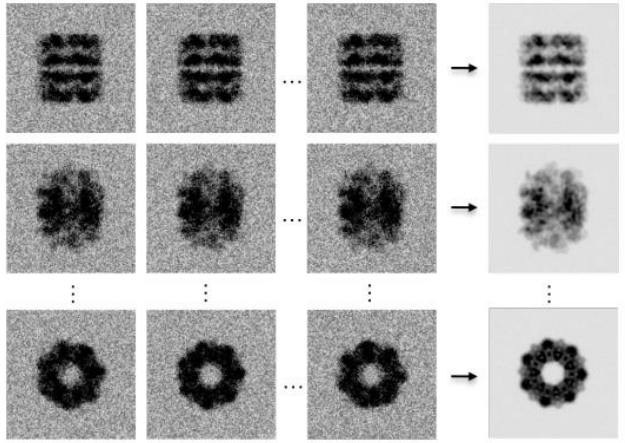



*(Motion Correction)*

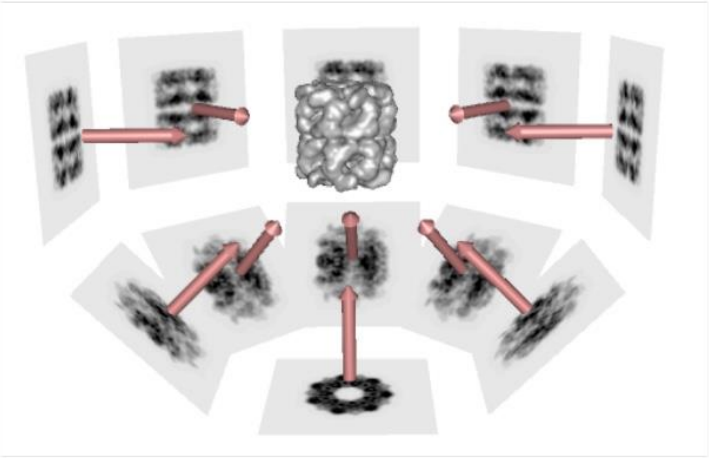
Particle Picking



2D Classification



3D Pose Estimation and Back-projection



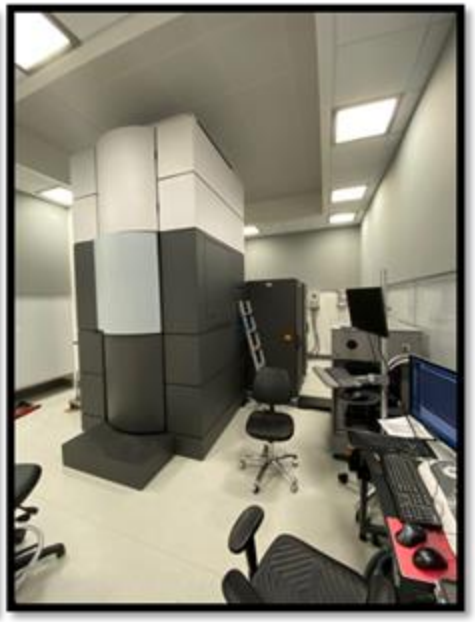
*(3D Classification)*

# Stanford-SLAC Cryo-EM Center ($S^2C^2$)

https://s2c2.slac.stanford.edu/

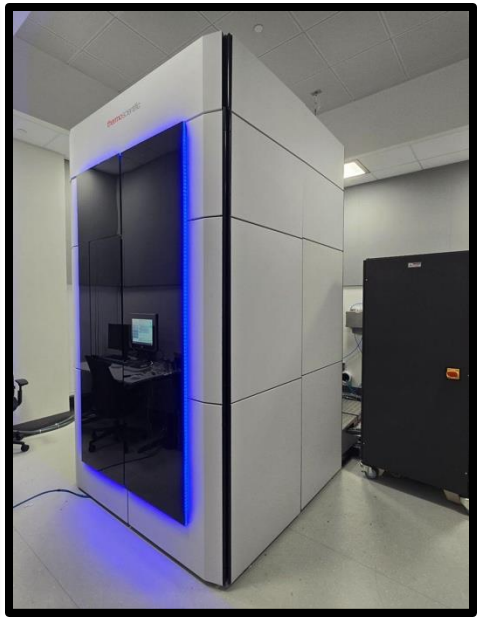
NIH R24GM154186

G3i, CFE, Selectris X, Falcon4i



G3i, BioQuantum, K3

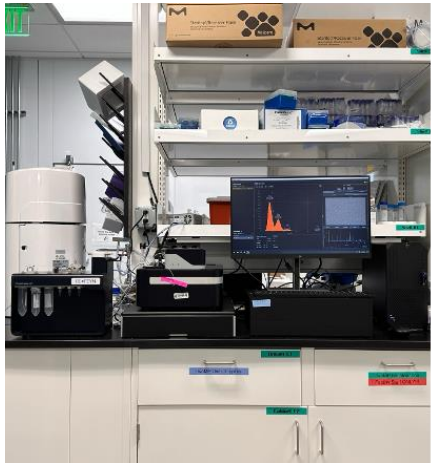
G3i, CFE, Selectris X, Falcon4i



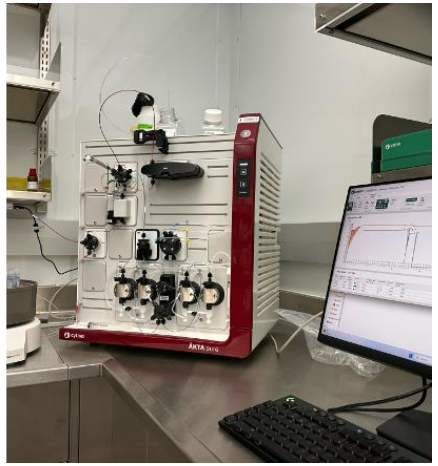
G4 CFE, Selectris X, Falcon4i

G2, Selectris, Falcon4i

Cryo-specimen preparation
Low Humidity Lab



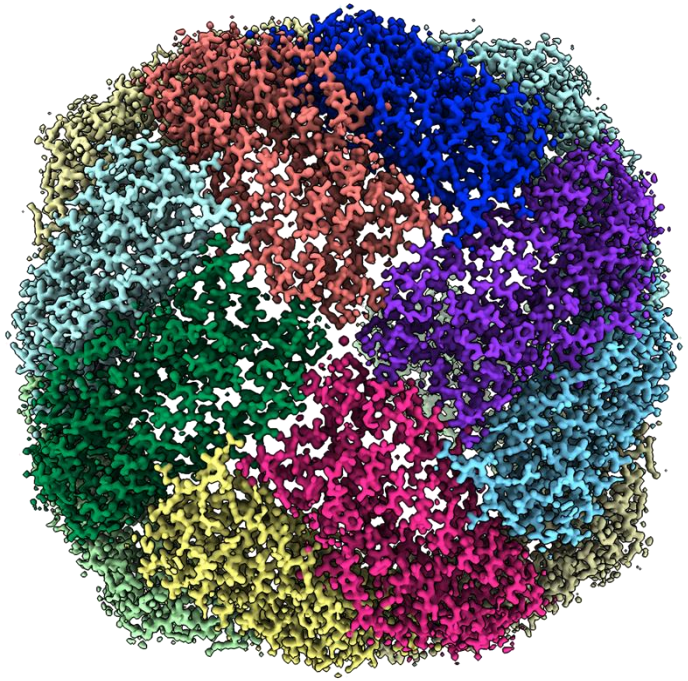
Refyne TwoMP mass photometer



GE AKTA Pure 25 chromatography system

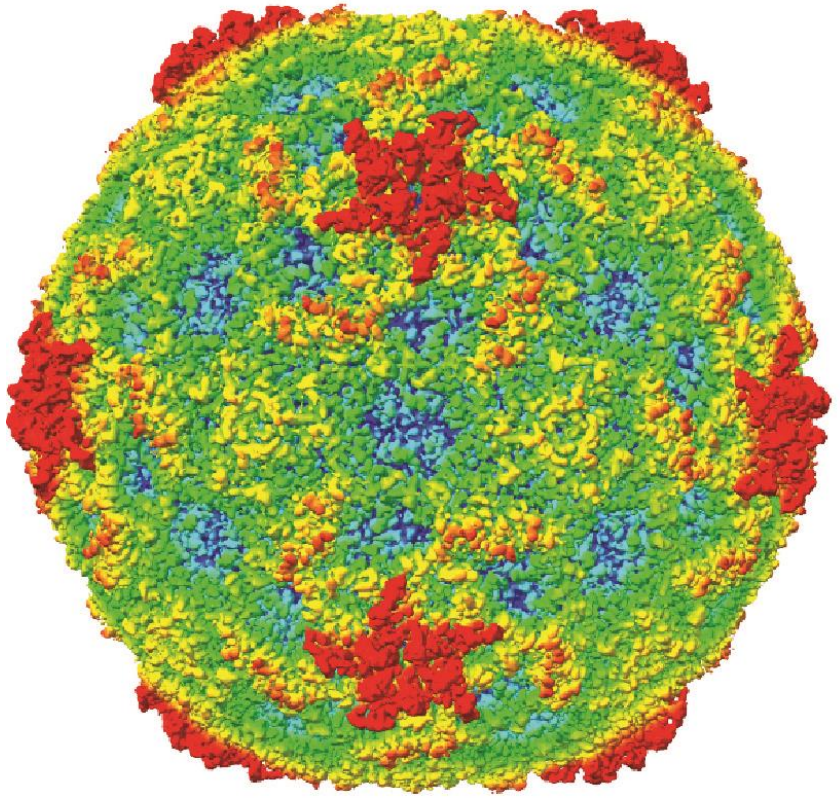
Tundra, Falcon C, Ceta F

# Atomic Resolution CryoEM Single Particle Structures



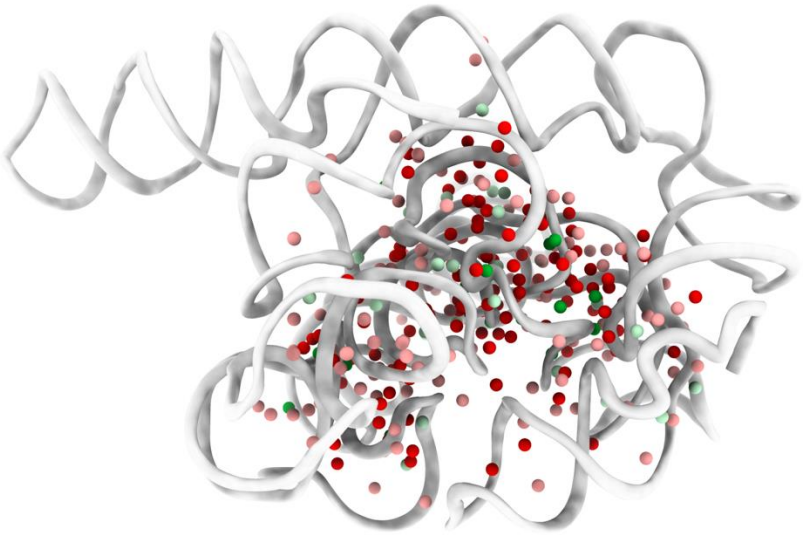
1.27 Å ApoFerritin
PDB: 7rrp

Zhang, Pintilie



2.0 Å Enterovirus EV-68
PDB: 9mwz

Varanese, Xu, Pintilie et al, *Nature* (2025)

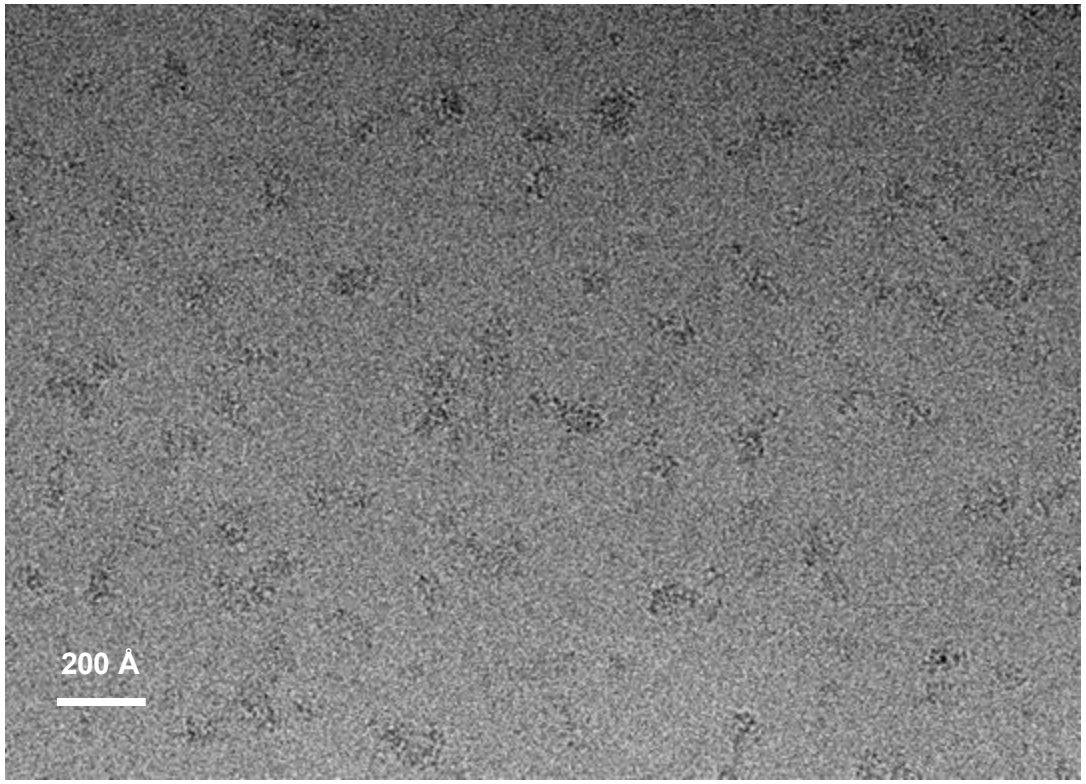


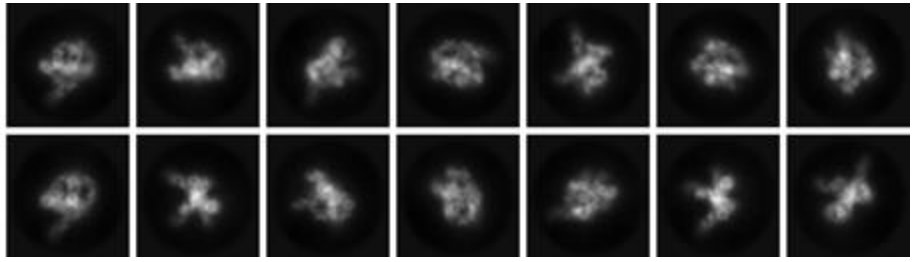
2.2 Å Ribozyme
PDB: 9cbw

Kretsch et al, *Nature* (2025)

# CryoEM Can Resolve Water in Ribozyme Structure @2.2 Å

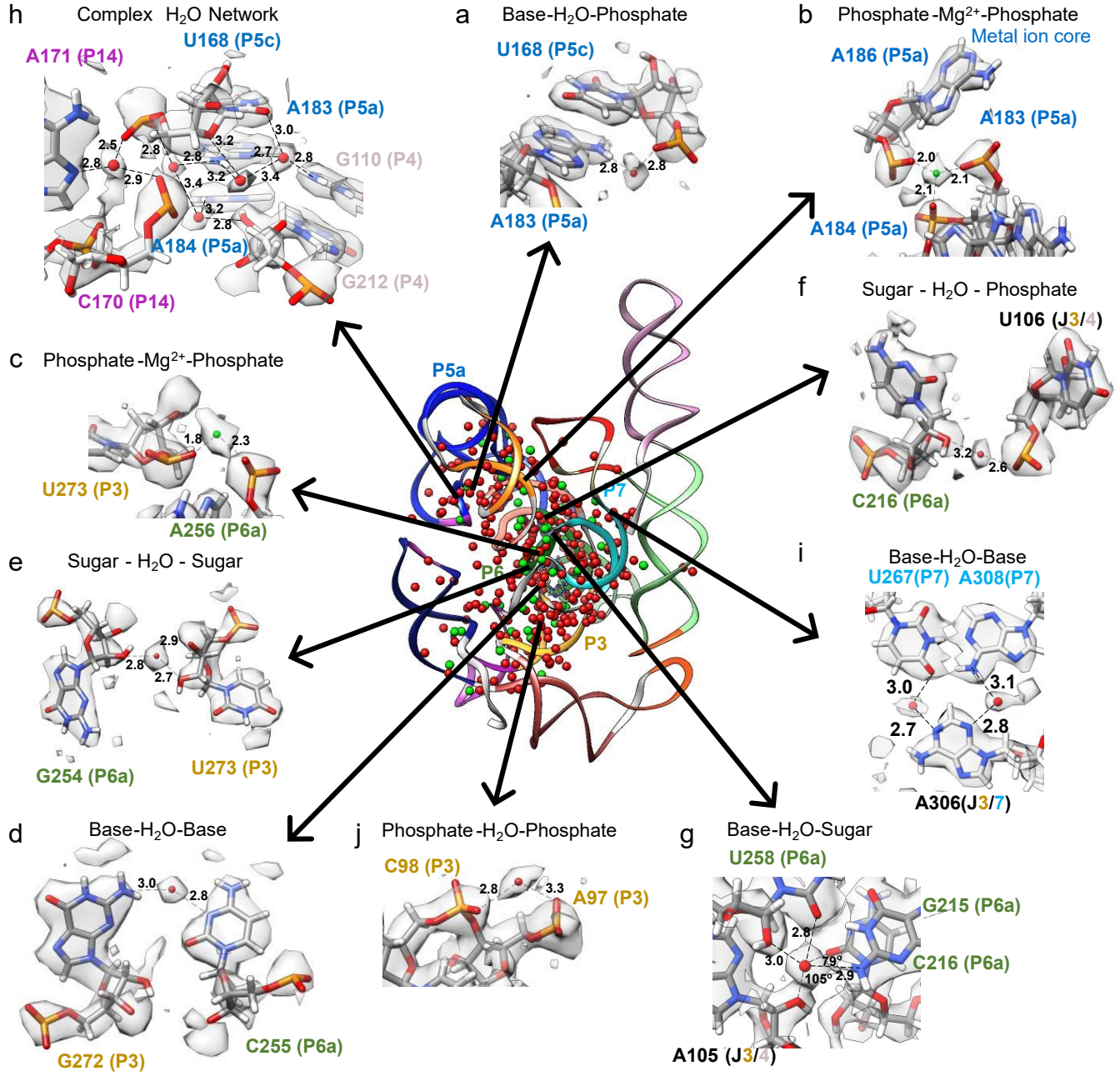
*Tetrahymena* Ribozyme (128 kDa, 387 nt)



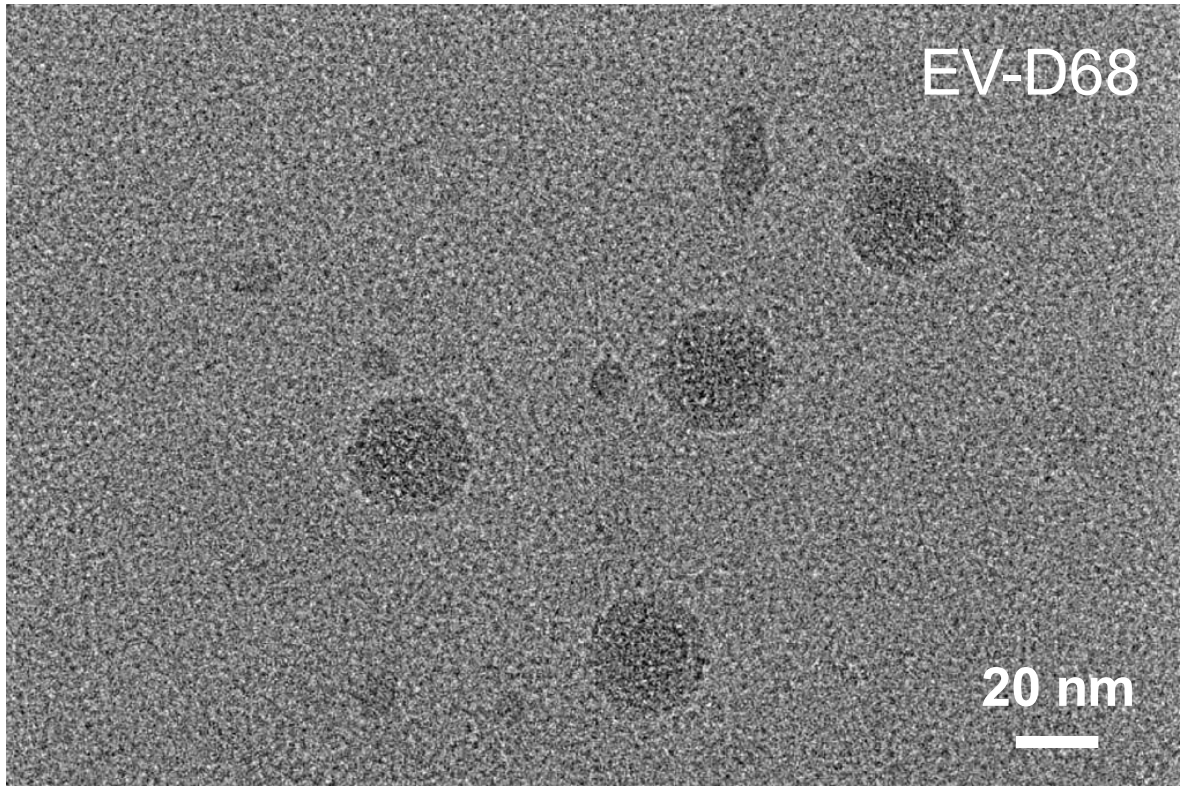
1.2M/3.8M particles
18,365 movie stacks

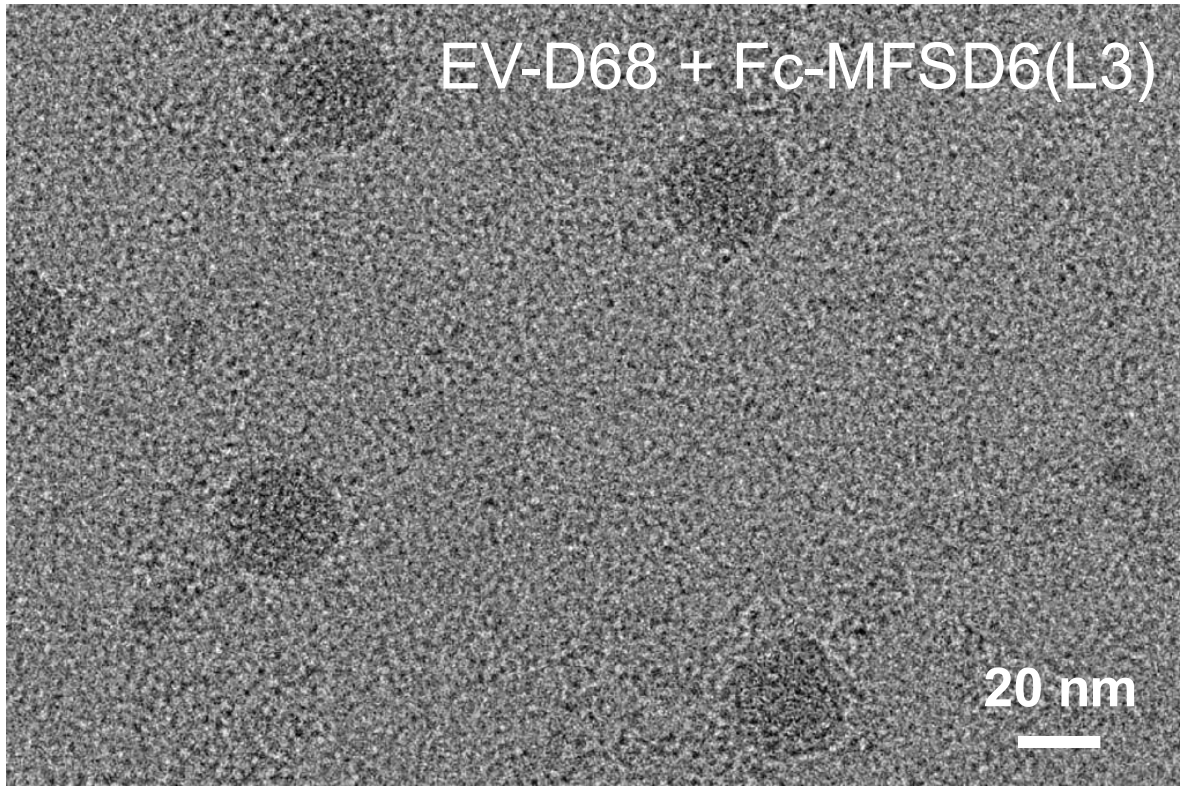
Kretsch et al, ***Nature*** (2025)



# CryoEM of Enterovirus D68 and Entry Receptor MFSD6(L3)

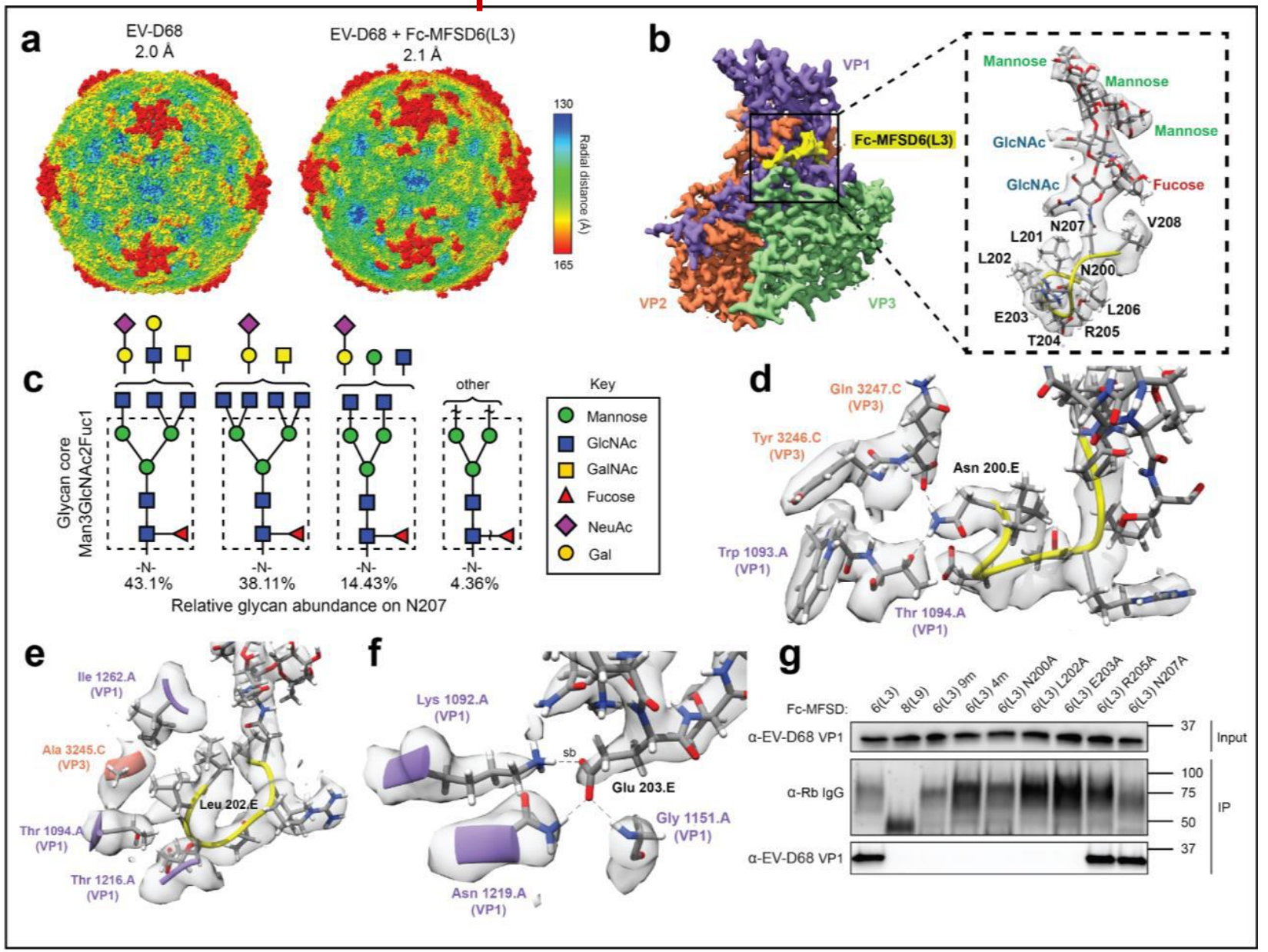
- Enteroviruses (EVs) are a large genus of single-stranded, positive-sense RNA viruses related to the poliovirus
- Recent global outbreaks of EV-D68 were associated with respiratory disease and polio-like paralysis in kids
- A decoy receptor, engineered by fusing MFSD6(L3) to Fc, blocked EV-D68 infection of human primary lung epithelial cells, and provided near complete protection in a lethal mouse model of EV-D68 infection




L Varanese, L Xu, C Peters, G Pintilie, C Bertozzi, J Carette *et al*, *Nature* (2025)

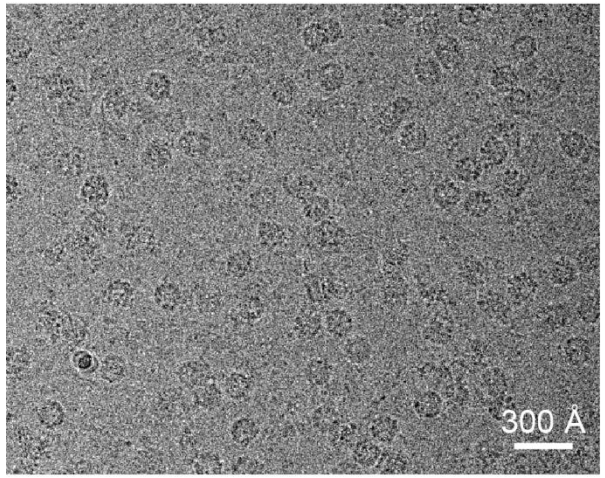
# Structure of EV-D68 in Complex with Fc-MFSD6(L3) Reveals Virus-Receptor Interaction Interface

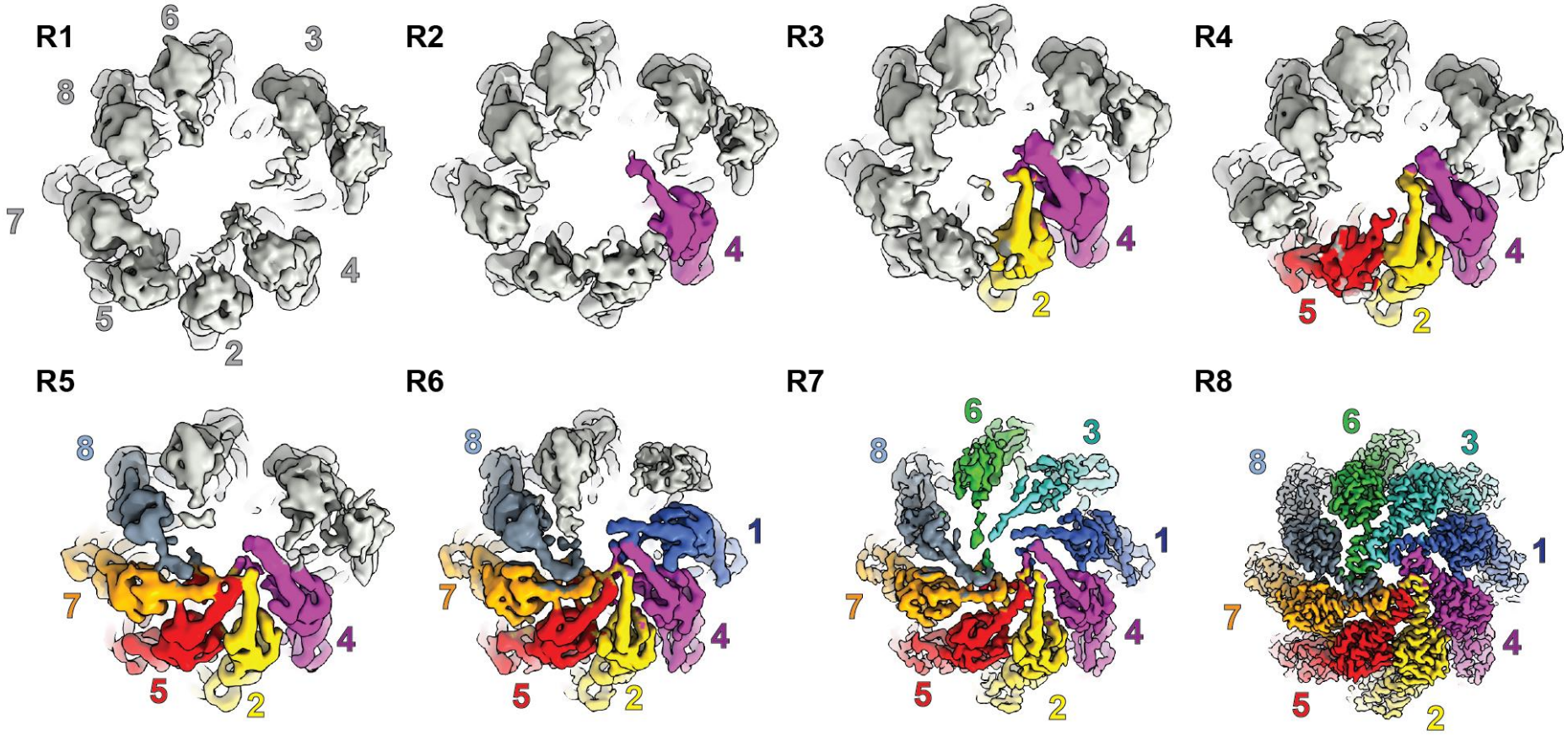



matching MFSD6 residues 200-208 (chain E), along with a complex type N-glycan located at N207 (chain F)

L Varanese, L Xu, G Pintilie, D Roberts, C Bertozzi, J Carette, *Nature* (2025)

# Structural Heterogeneity of Human Chaperonin TRiC +ATP

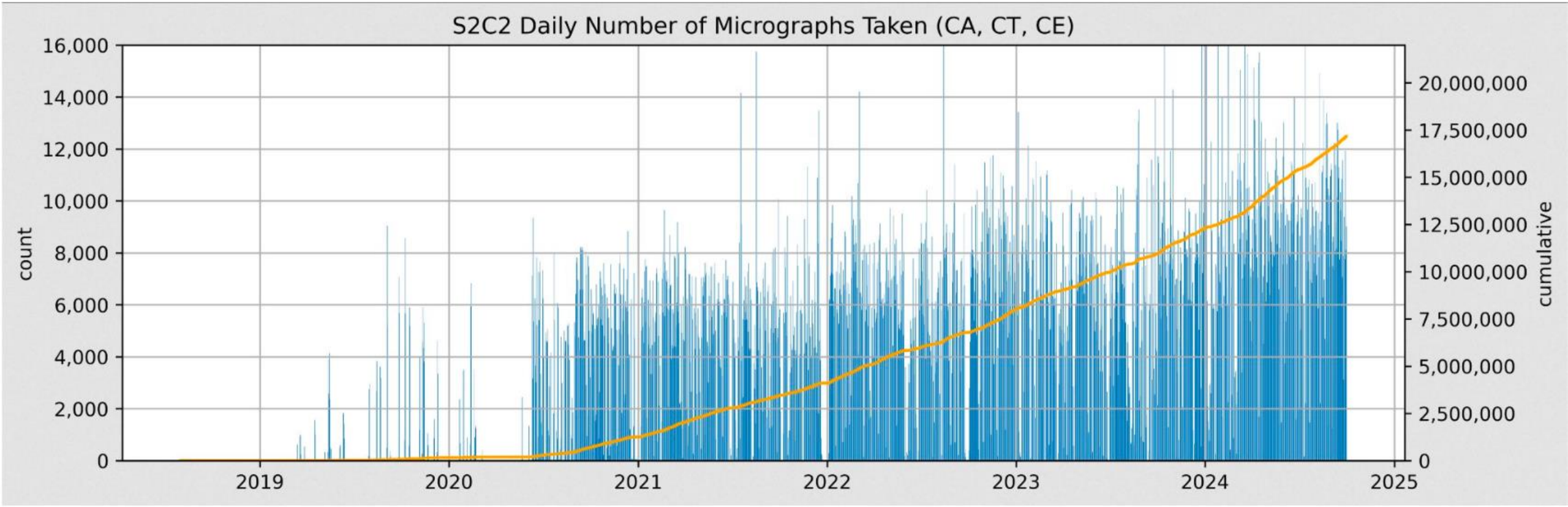


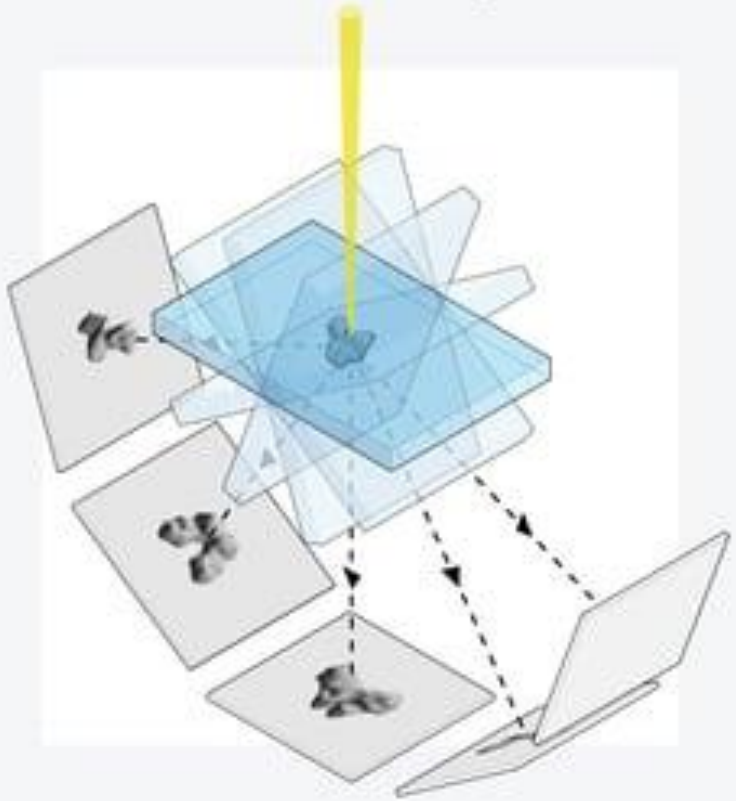

Yanyan Zhao, unpublished

# $S^2C^2$ Activity Thus Far… (since 2018)

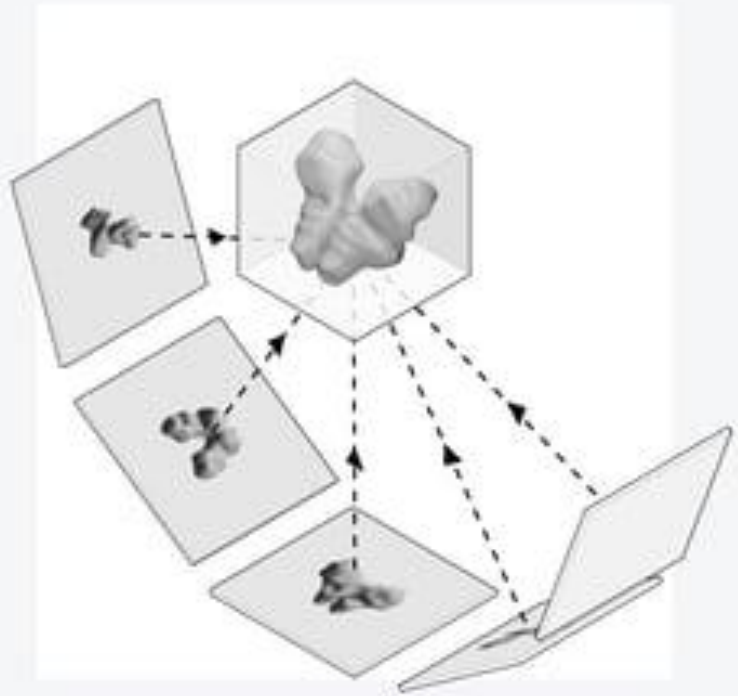
- 1200+ experimental sessions
- ~11PB+ total data volume
- 17M+ micrographs
- Typical SPI experimental session >12,000 movies (~8TB)



## Cryo-electron tomography (Cryo-ET)



Tilt series of 2D projections

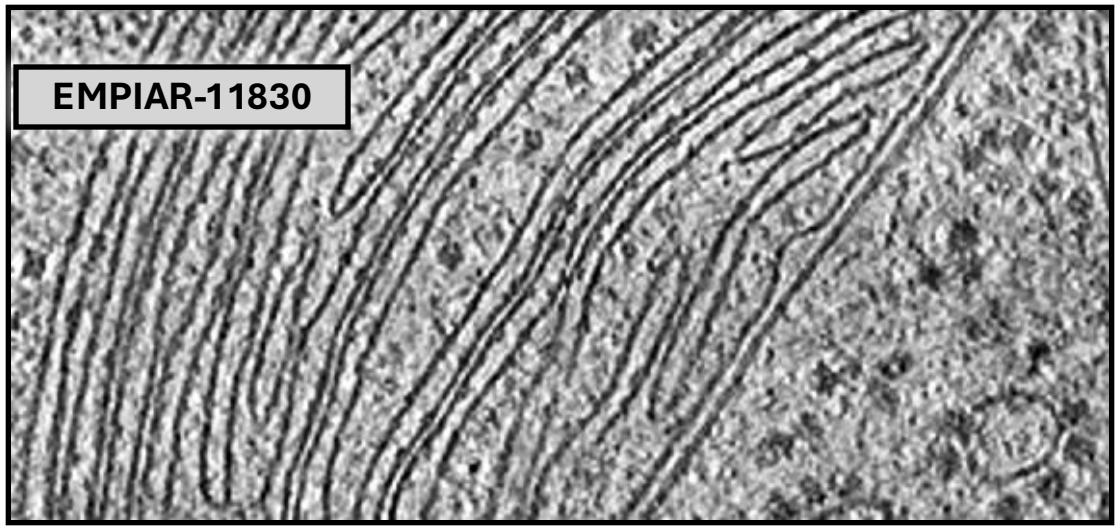


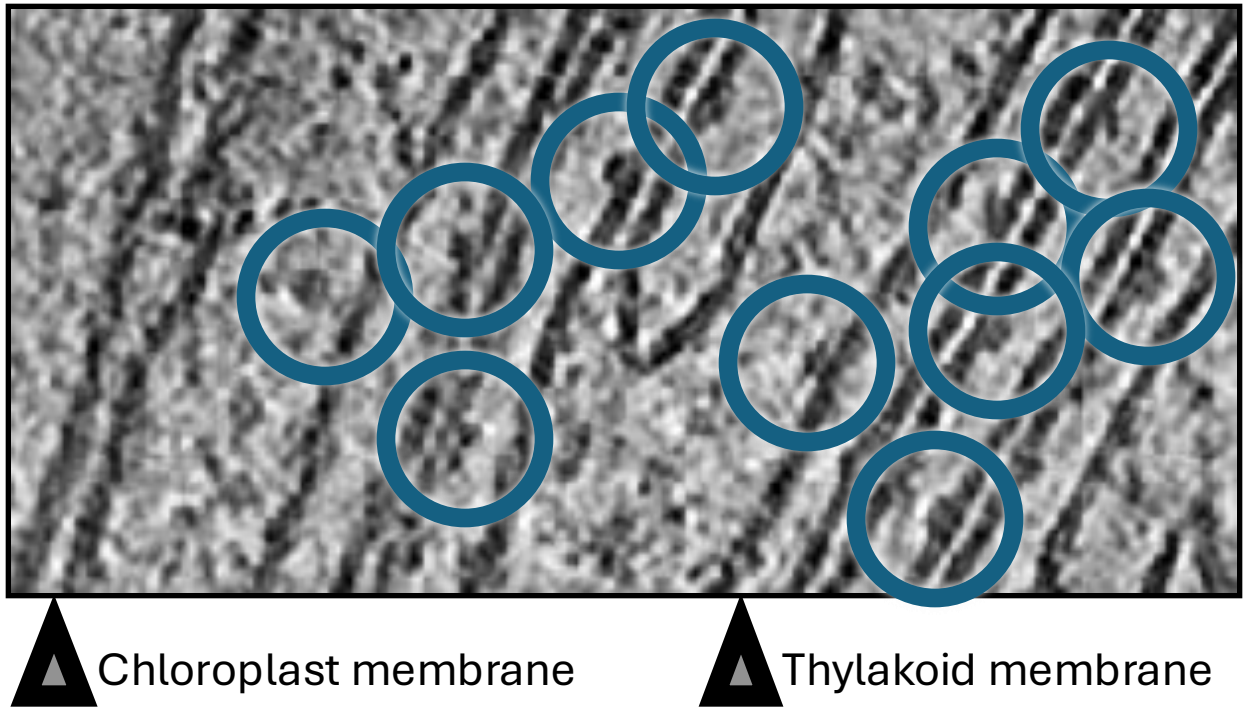
Tomogram 3D reconstruction

# Visual Proteomics Inside Cells with Cryo-PFIB and Cryo-ET

**Sorting out photosynthesis-related proteins on the thylakoid membranes of Chlamydomonas**

- A limited set of target proteins with known structures
- Membranes serving as anchor for alignment, making classification (slightly) easier



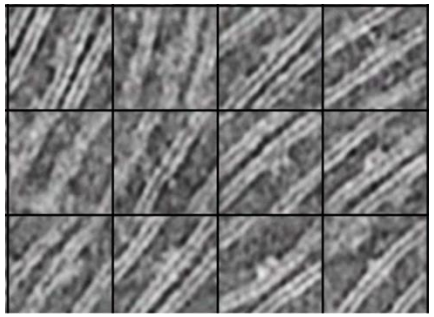



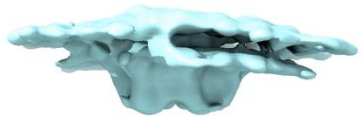

**Particle selection:**

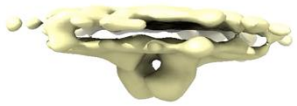
- Convolutional neural networks with iteratively updated training set
- Targeting any density protruding from membrane inside chloroplast

# Visual Proteomics Inside Cells with Cryo-PFIB and Cryo-ET

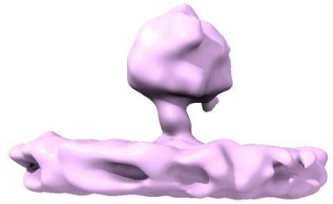
**55k particles from 68 tomograms**

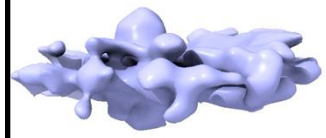








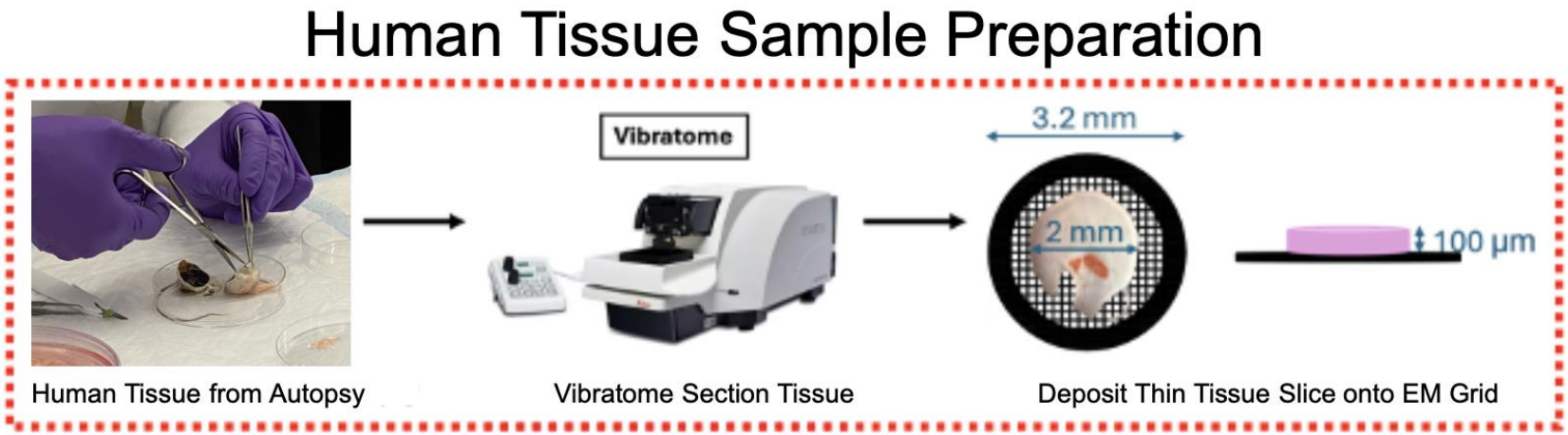


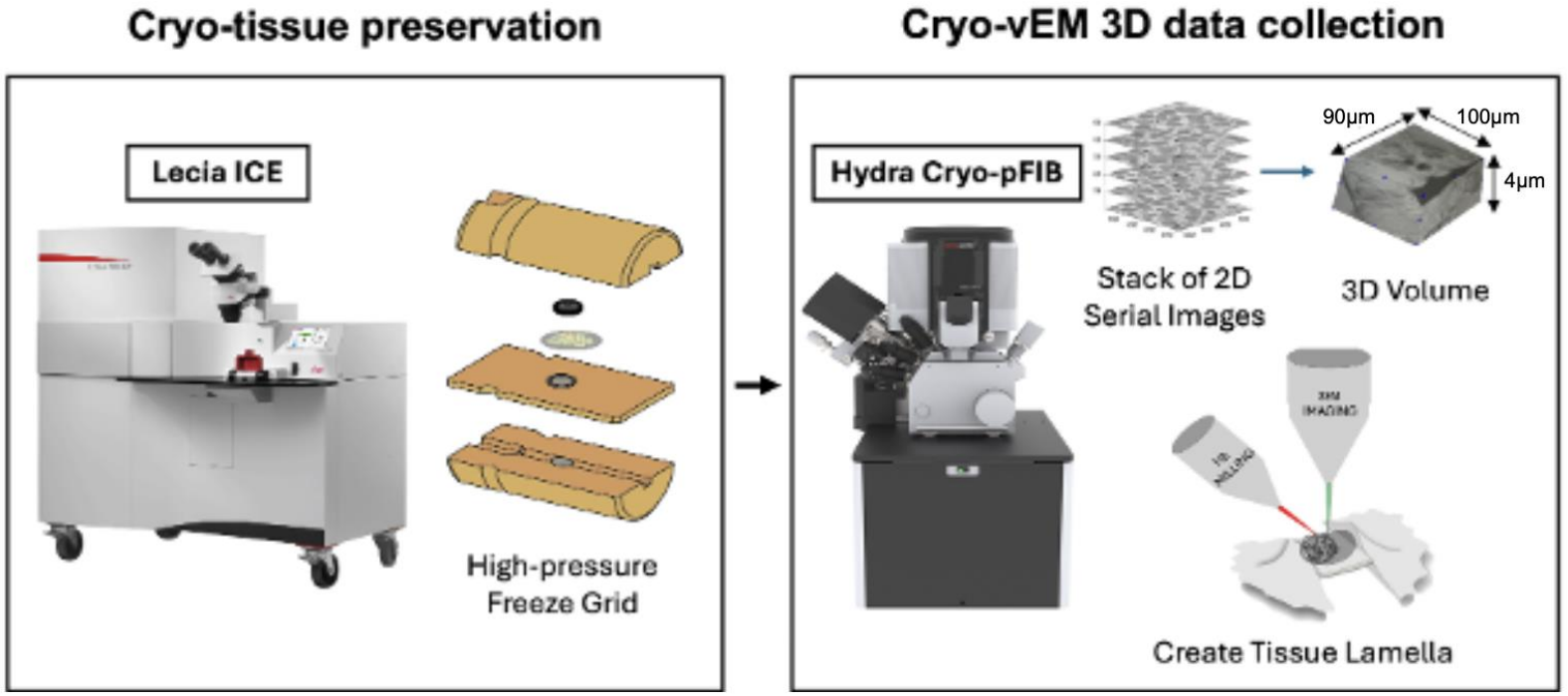
**Manifold embedding:**

- Unsupervised variational auto-encoder (VAE) with maxout to encode structure features and ignore orientation differences
- Particles mapped to a low dimensional latent space corresponding to their structures
- Subtomogram averaging to refine 3D structure

Muyuan Chen, unpublished

# Cryo-vEM of Organoids and Tissues



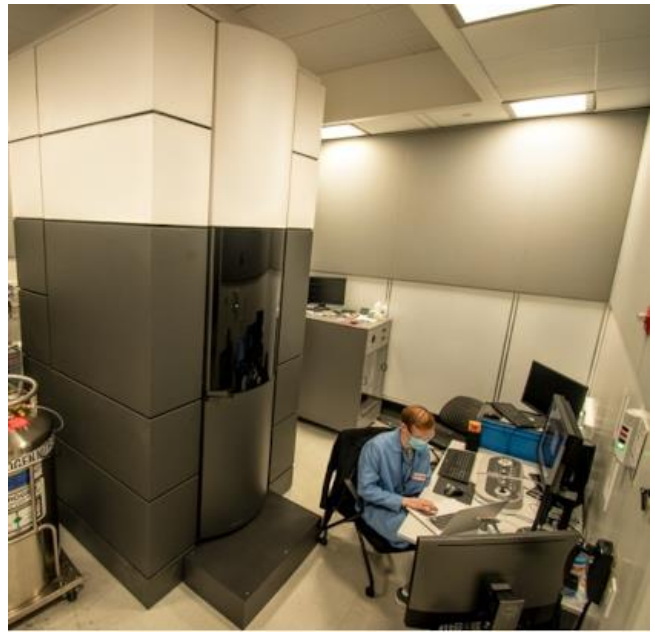
# SCSC | Stanford-SLAC CryoET Specimen Preparation Center

https://scsc.slac.stanford.edu/

U24GM139166

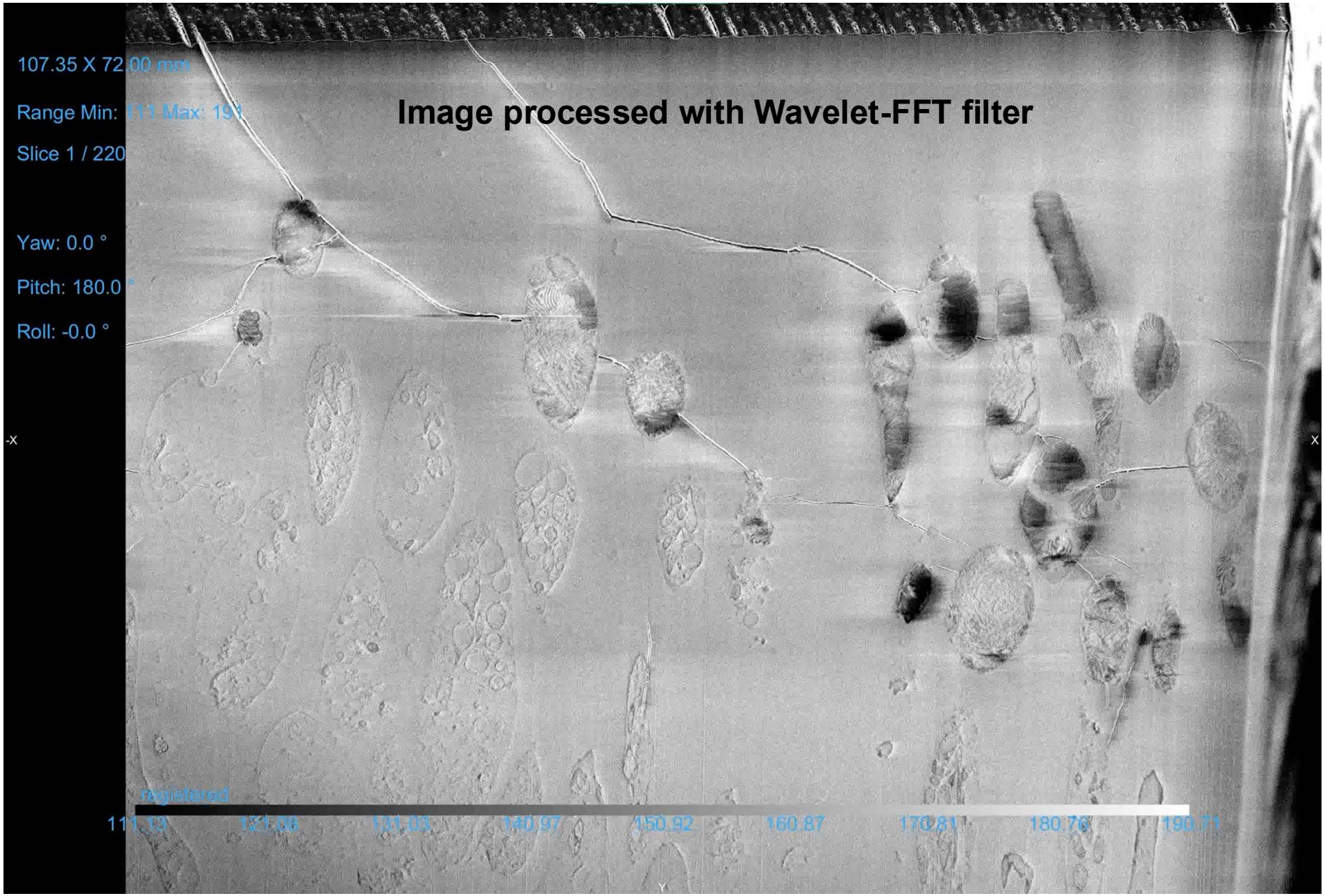

High Pressure Freezing Device

Hydra-Bio and Aquillos 2 Cryogenic Focused Ion Beam - Scanning Electron microscope



Titan Krios TEM

# Cryo-vEM of Postmortem Human Retina



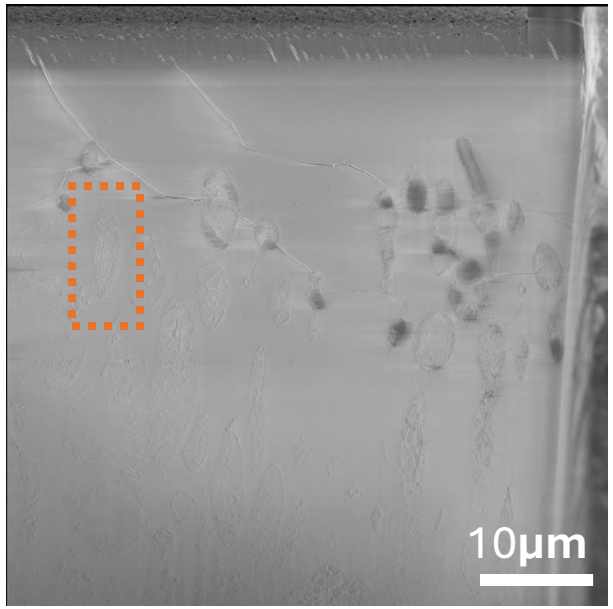

SCSC Hydra-Bio pFIB-SEM

- 255 slices (5.1 μm)
- pixel size
  5 nm X 5 nm X 20 nm
- Entire width 50 μm

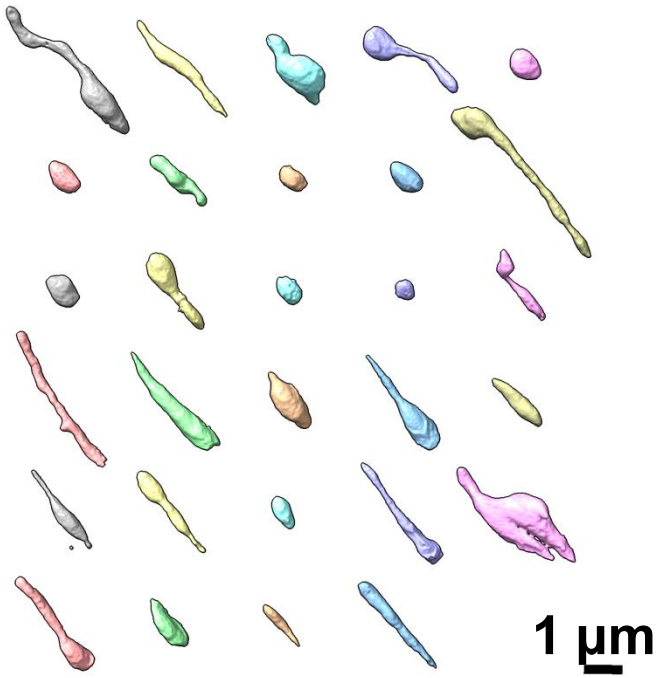
1. 58 years old
2. Woman
3. cancer

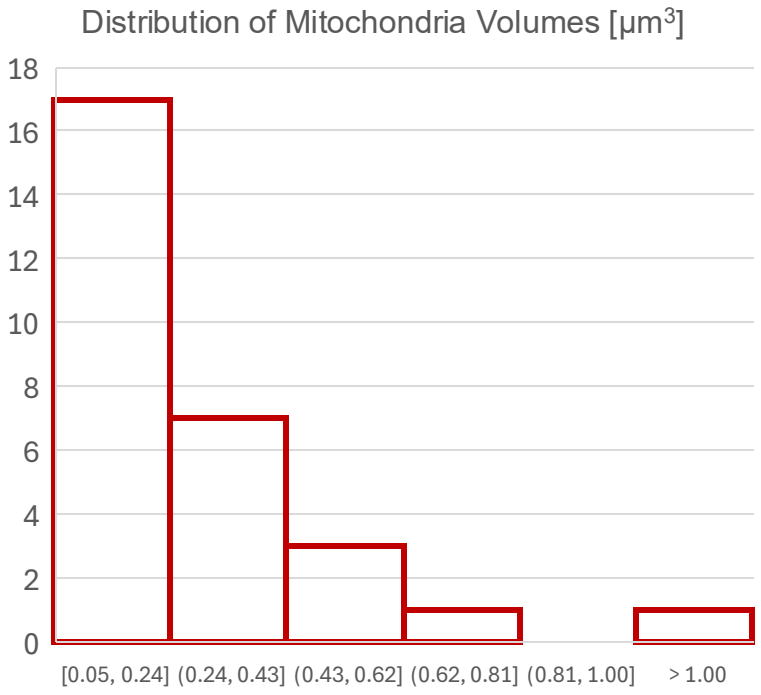
**Stanford University/SCSC**
Gong Her Wu, Pingting Liu, Lydia Joubert, Risa Kawasaki, Greg Pintilie, Hiren Patel, **Jody Hooper**, **Joyce Liao**

# Annotation of Mitochondria in a Single Rod Cell from Postmortem Retina








Risa Kawasaki,
Greg Pintilie
Unpublished

# Future Developments in Quantitative CryoEM/ET/vEM

- Advanced and robust image processing tools: Machine-Learning based algorithms for structure determinations of isolated and in situ macromolecule with structural and/or compositional heterogeneity
- Establish rigorous structure validation criteria to provide quantitative assessment of cryoEM structures across broad range of resolution
- CryovEM and cryoET data will be integrated with various –omic and clinical data to understand basic biological process, drug and guide vaccine design, diagnostics and therapeutics administration and planetary health
- Provide computational infrastructure and data management to serve the community

# Acknowledgements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**Risa Kawasaki**
Pingting Liu
Lydia Maria Joubert
Muyuan Chen (SLAC)

Stanford University

Mike Palo, **Rhiju Das**
Lauren Varanese, **Jan Carrett**
C.E. Peters, **Carolyn Bertozzi**
Hirenkumar Patel, **Joyce Liao**
**Jody Hopper**

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Stanford University and SLAC

Rachael Kretsch
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Hirenkumar Patel, Joyce Liao
Jody Hopper

Rutgers University

David Case

NIH, NSF