RUTGERS

2025 Tomo Short Course

Introduction to Cryo-electron Tomography

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Rutgers University

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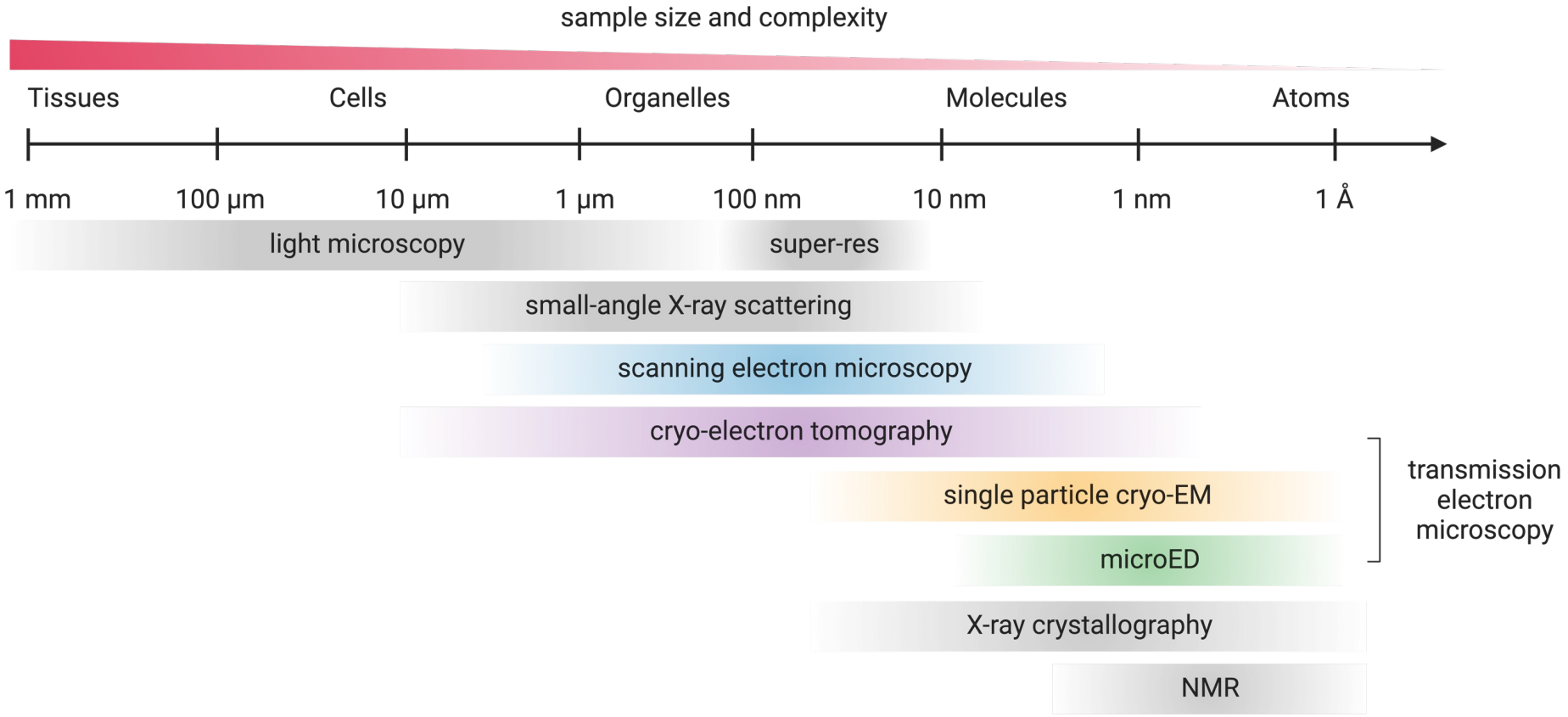
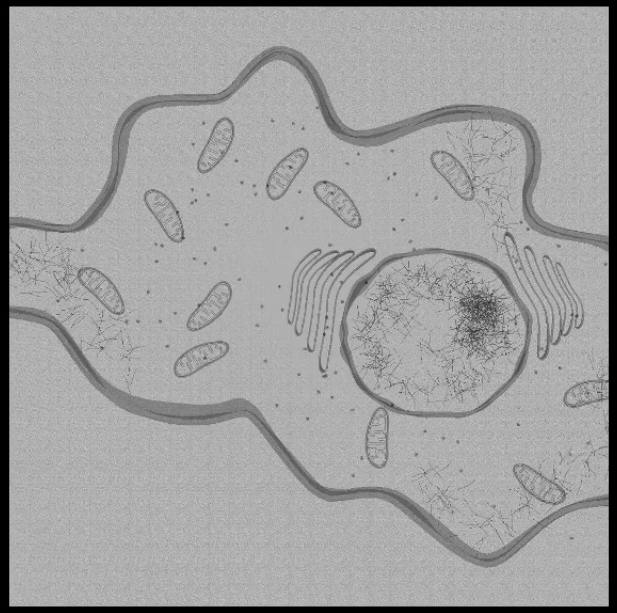
Learning Goals

- Understand the history and applications of cryoET in structural biology.
- Understand how technological and computational advances expand cryoET applications in Cell Biology.
- Examine how cryoET reveals the molecular landscape of fungal plasma membrane proteins.

Multi-scale Bioimaging to Address Dynamics in Cell Biology

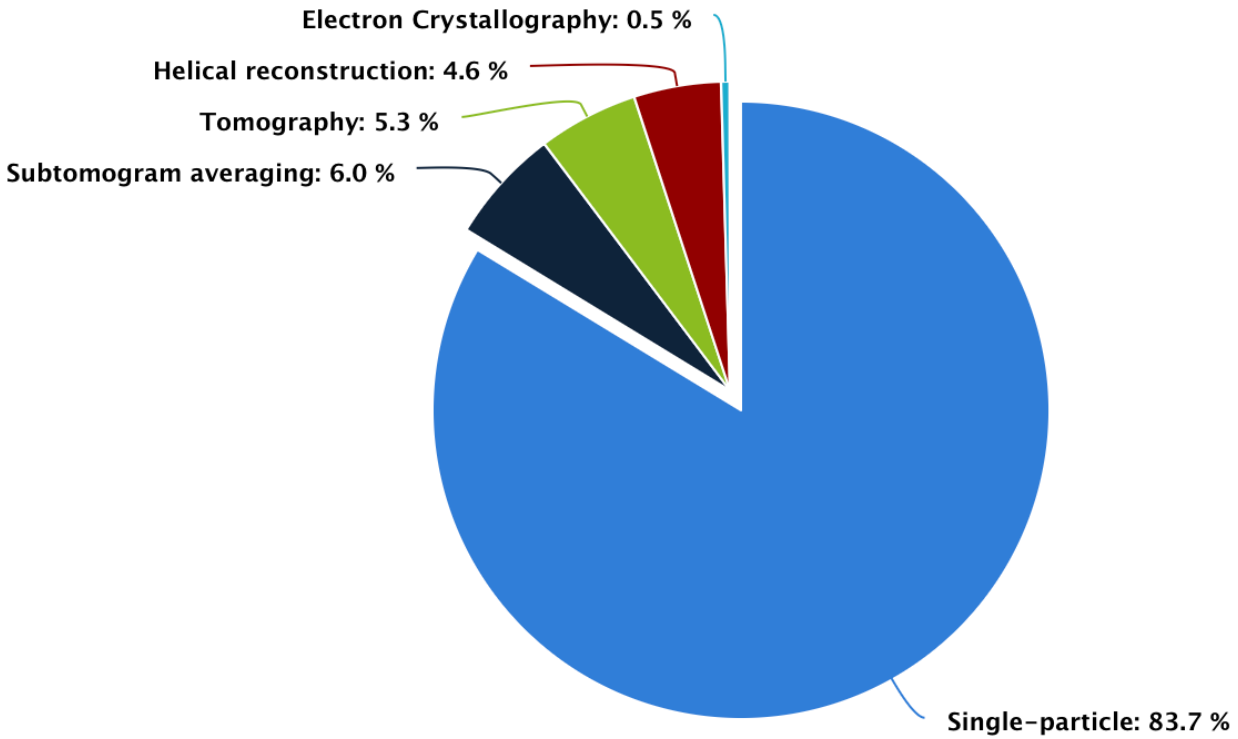


Tilt series acquisition and tomogram reconstruction



Diamond Light Source

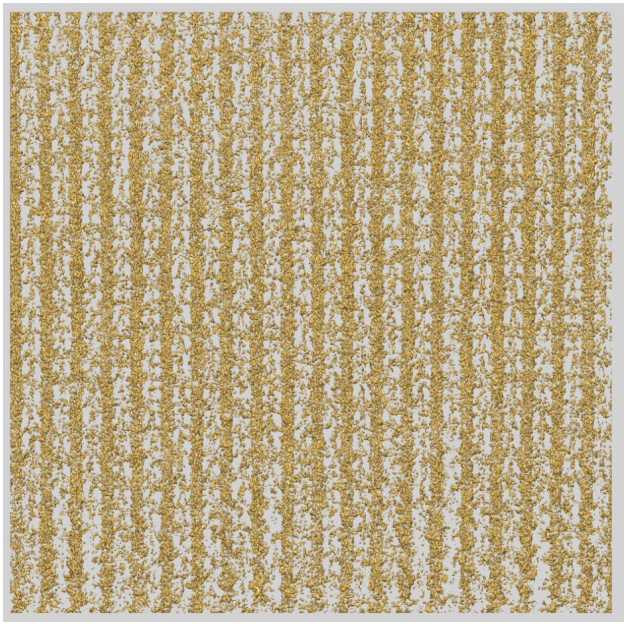
EMDB: Entry Modality Distribution



https://www.ebi.ac.uk/emdb/statistics/

Electron Tomography: Early Days

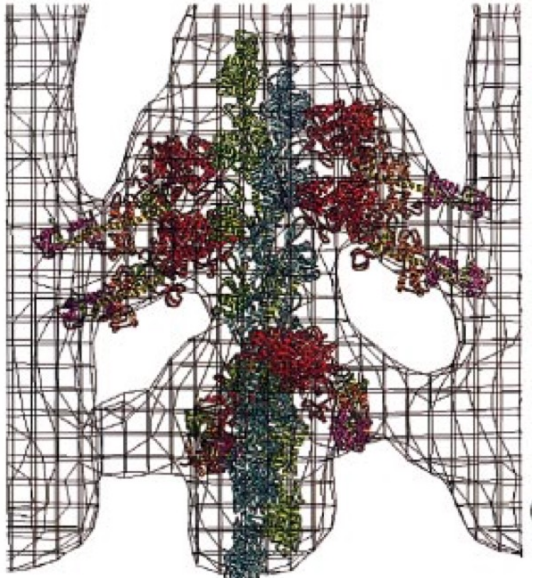
Tomogram of thin-sectioned, plastic-embedded insect flight muscle



EMD-1001

Chen *et al.* JSB 2001 https://doi.org/10.1006/jsbi.2000.4321

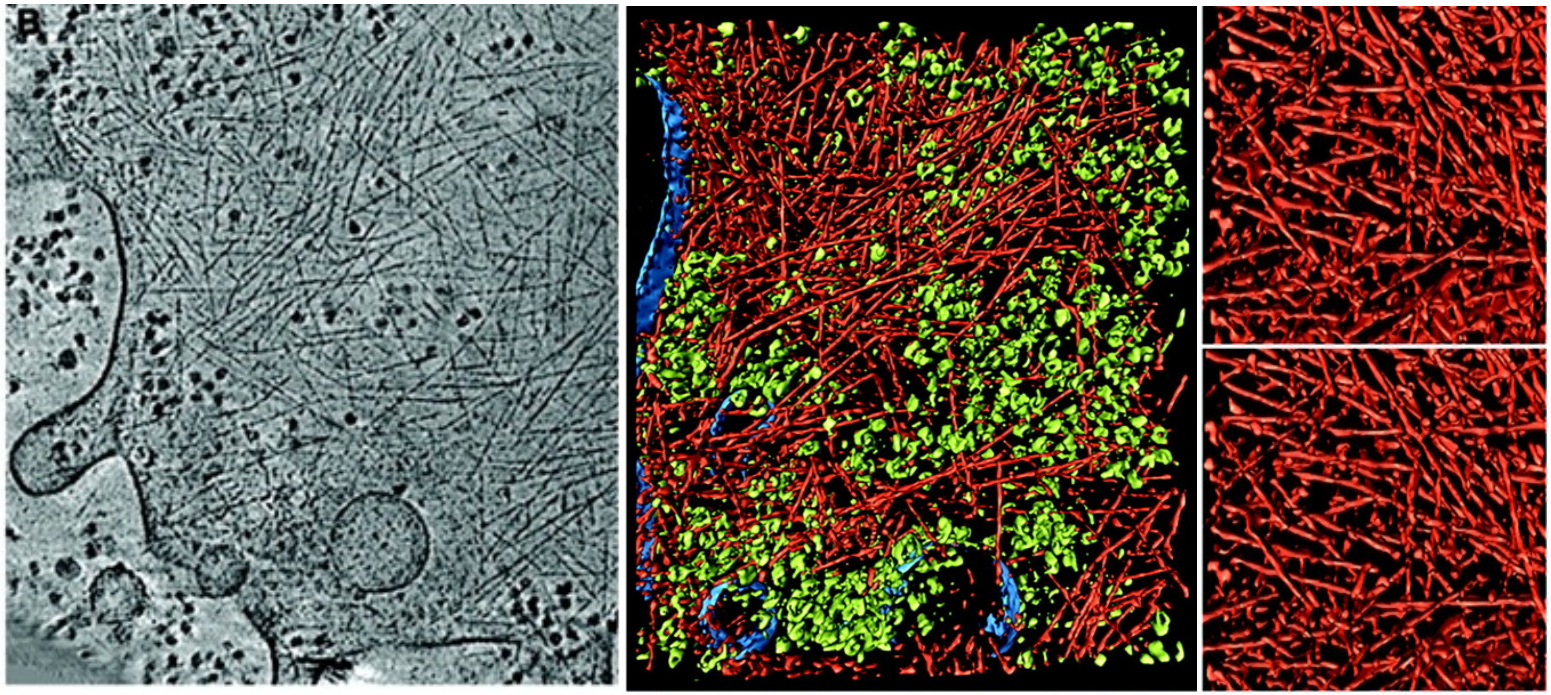
Fitting of atomic models to the 3D average of rigor insect muscle cross-bridges



PDB 1M8Q: atomic model of rigor crossbridges

Electron Tomography: The Ice-Age

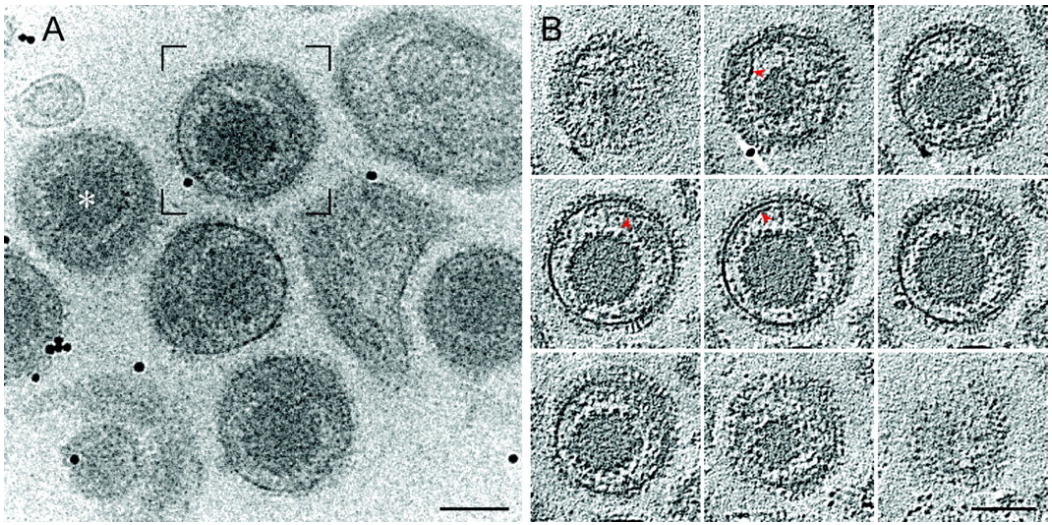
The periphery region of *Dictyostelium discoideum* cells

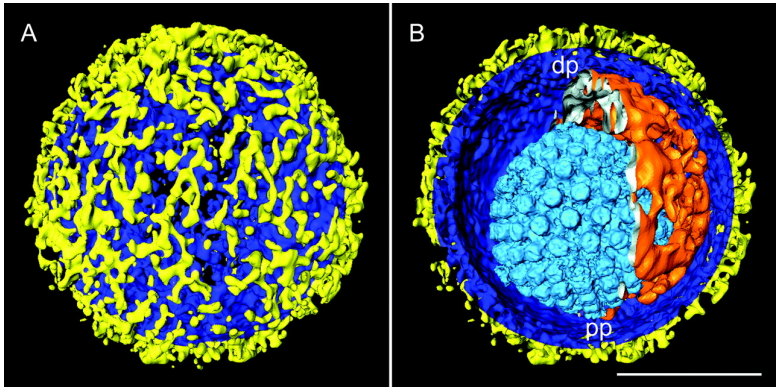


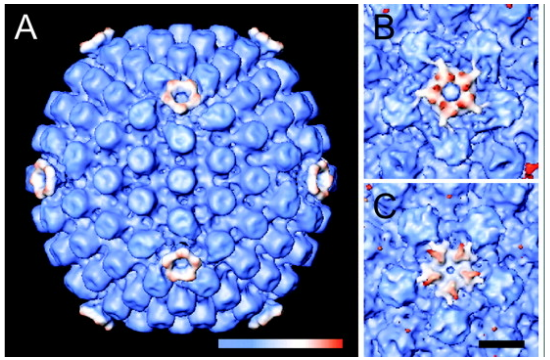
Medalia et al. Science 298, 2002

Electron Tomography: To Study the Polymorphic Elements

Tegument layers and asymmetric elements in HSV virions





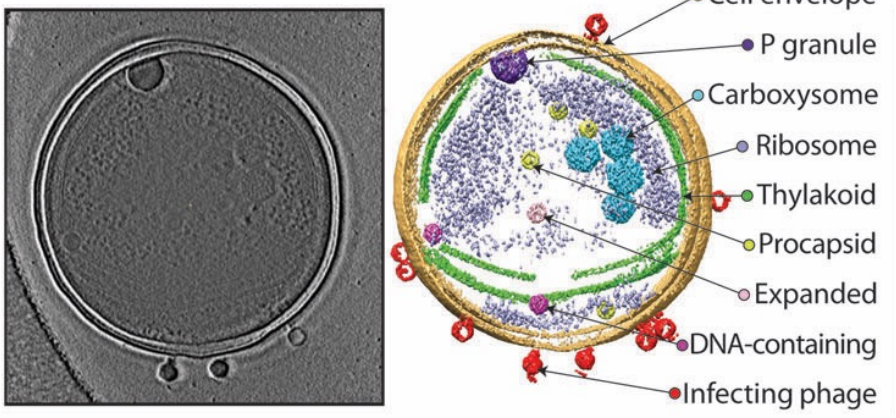


K. Grünewald *et al.* Science 2003 https://www.science.org/doi/10.1126/science.1090284

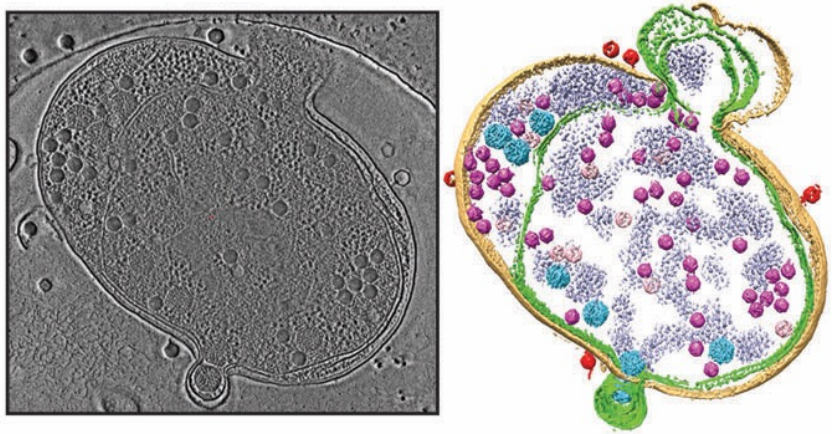
Cellular CryoET to Directly Visualize Biological Processes

Visualizing the phage maturation process by cellular phase contrast cryoET

Early stage



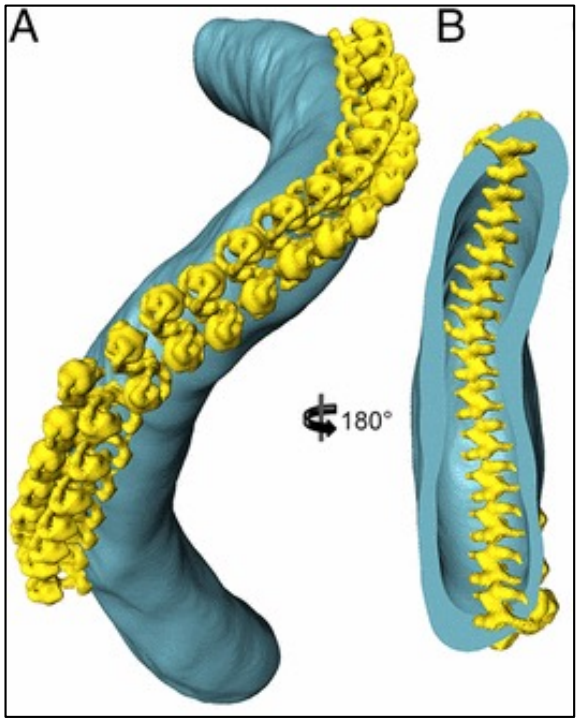
Late stage

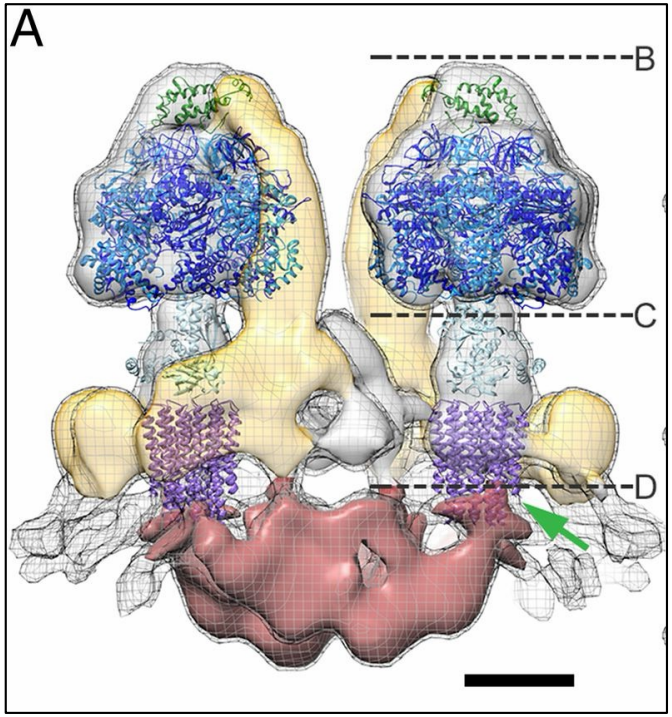


Dai *et al.* Nature 2013

CryoET to Visualize Subcellular Structures and Organelles

ATP synthase dimer on mitochondria cristae

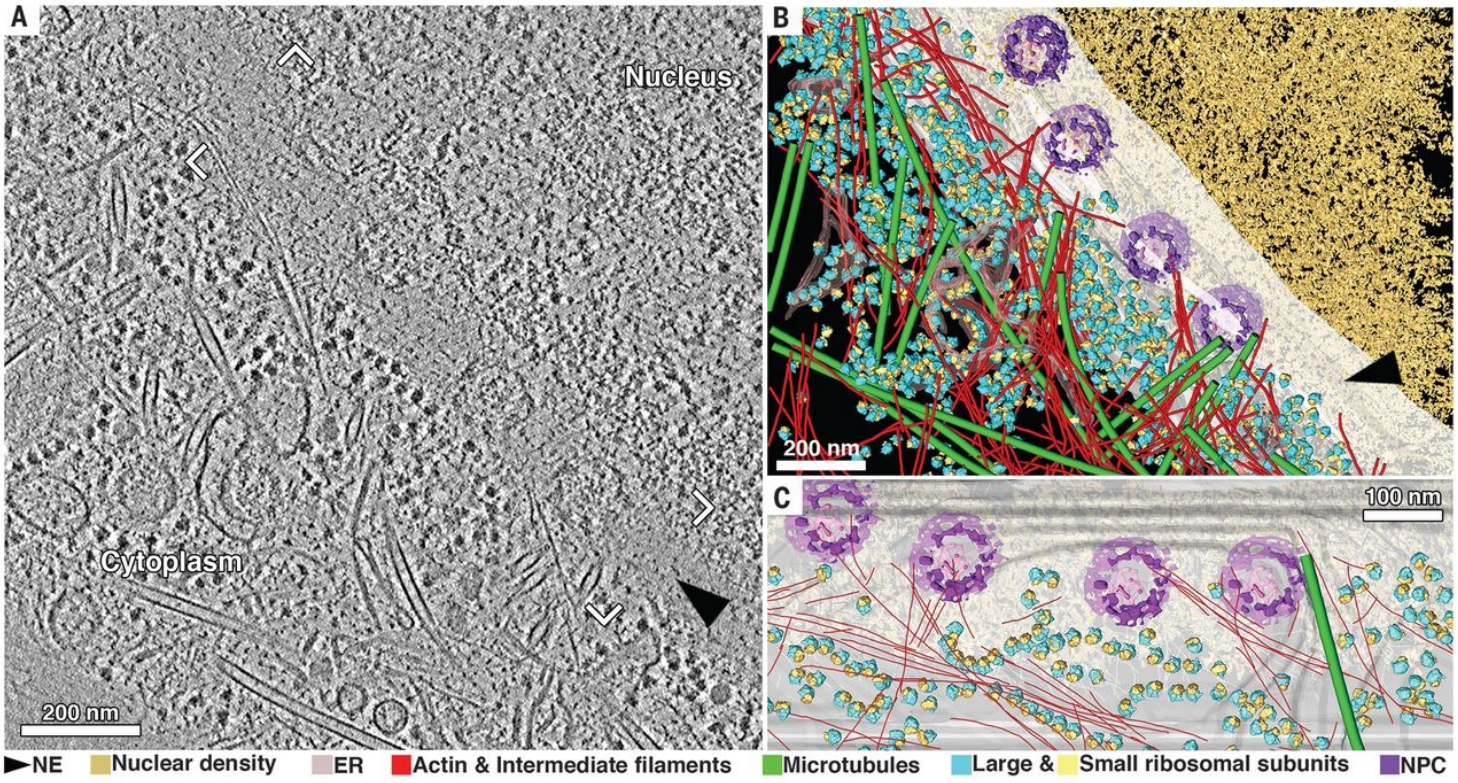




Alexander W. Mühleip et al. PNAS 2016;113:8442-8447

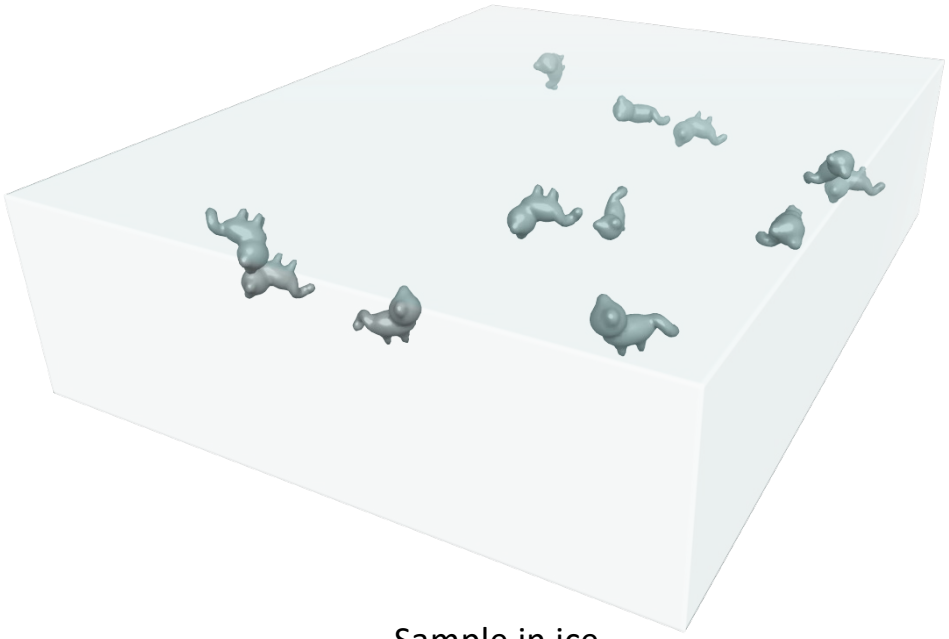
Cryo-focused Ion Beam: The Beginning of *In Situ* Structural Biology

The Nuclear periphery by cryoET of FIB-milled of a Hela cell

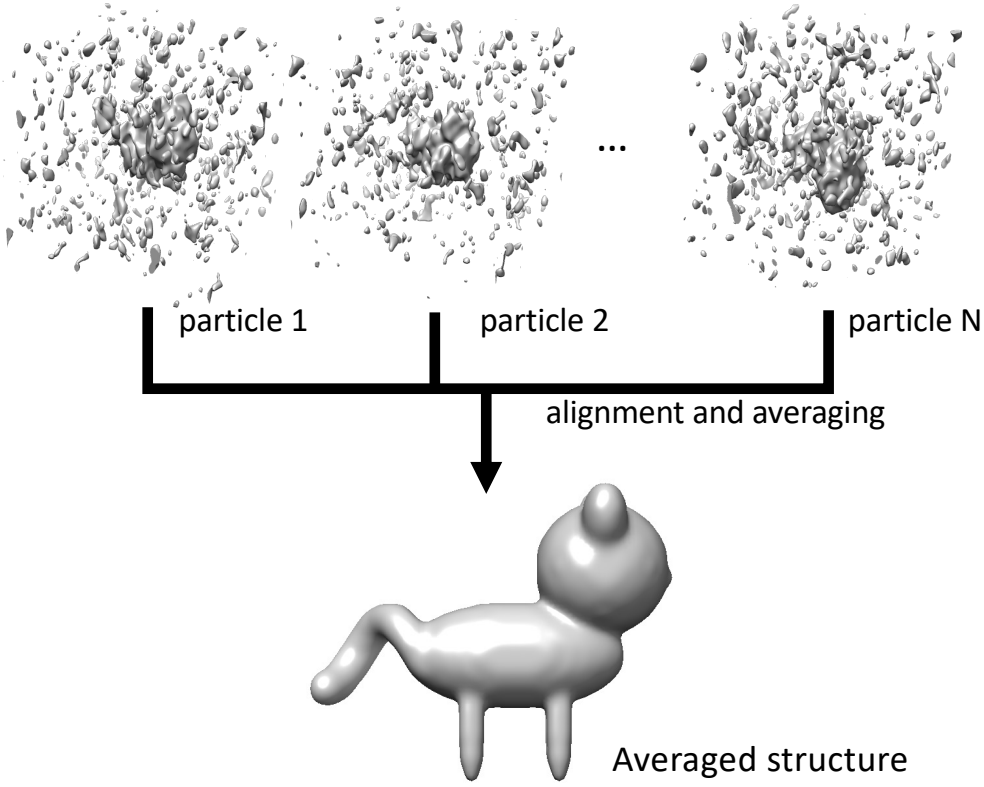


J. Mahamid, et al 2016, Science, 351 (6276) 969-972

Subtomogram Averaging – Computationally Isolate Particles for High(er) Resolution Structure Determination



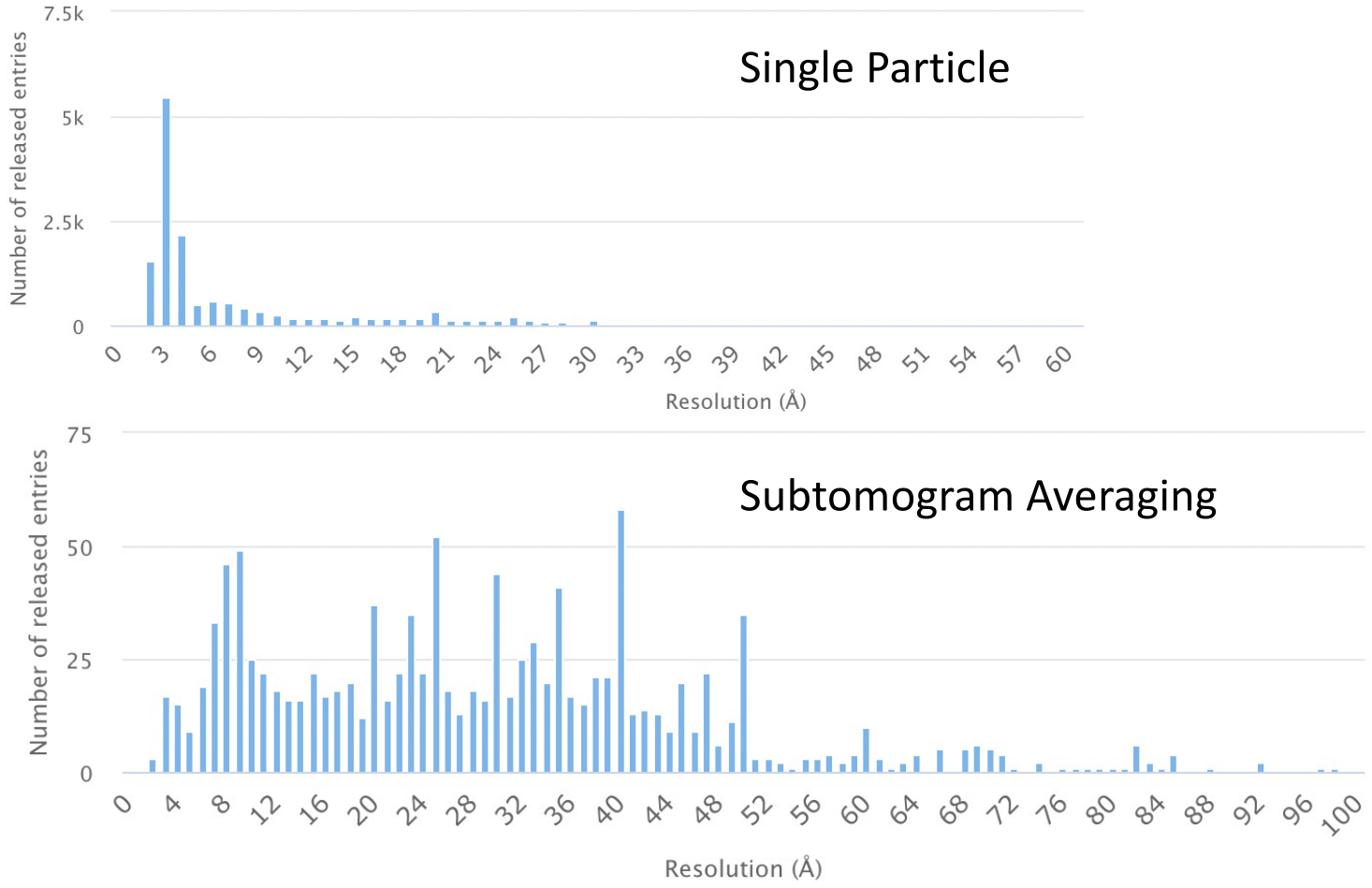
Sample in ice



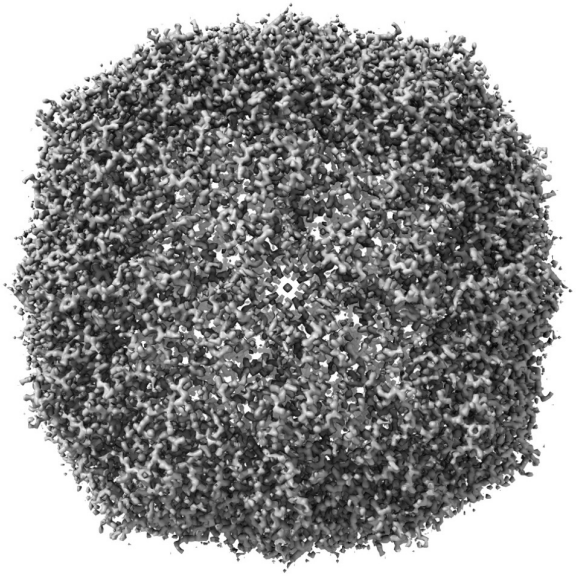
Averaged structure

Courtesy of Muyuan Chen

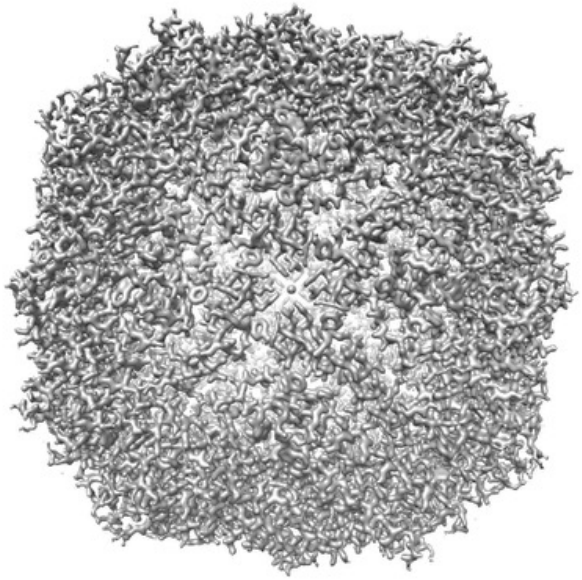
Subtomogram Averaging vs CryoEM Single Particle Analysis

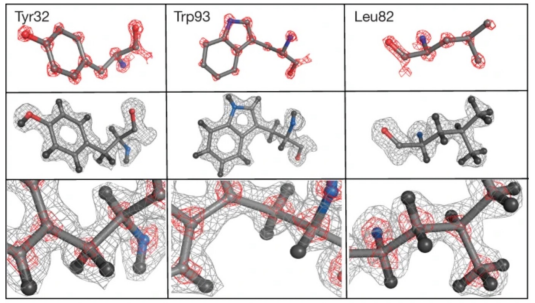


Subtomogram Averaging vs CryoEM Single Particle Analysis



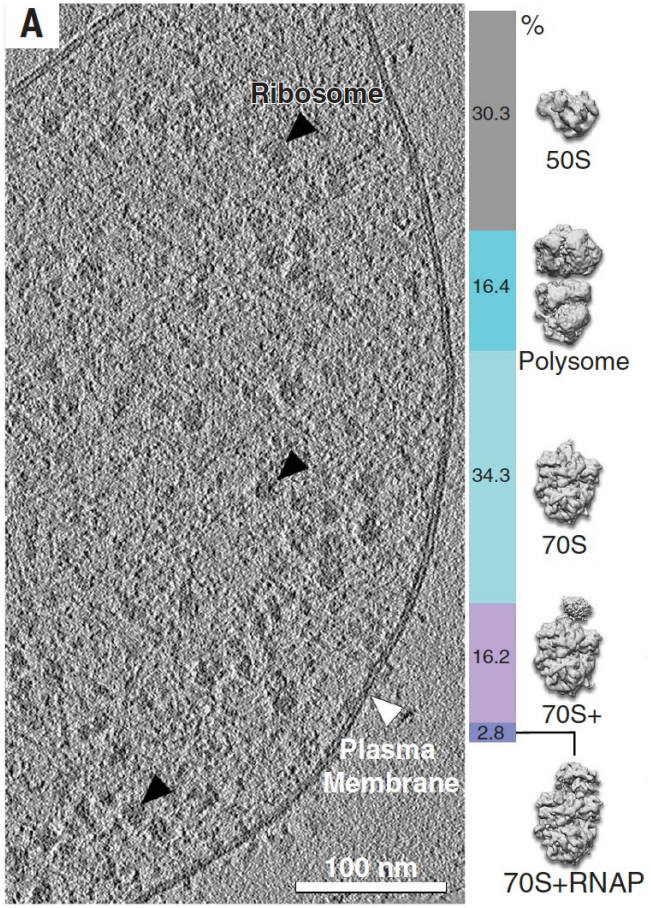
Apoferritin at 1.6Å by subtomogram averaging
EMD-16032 Obr M. *et al*., (2023)





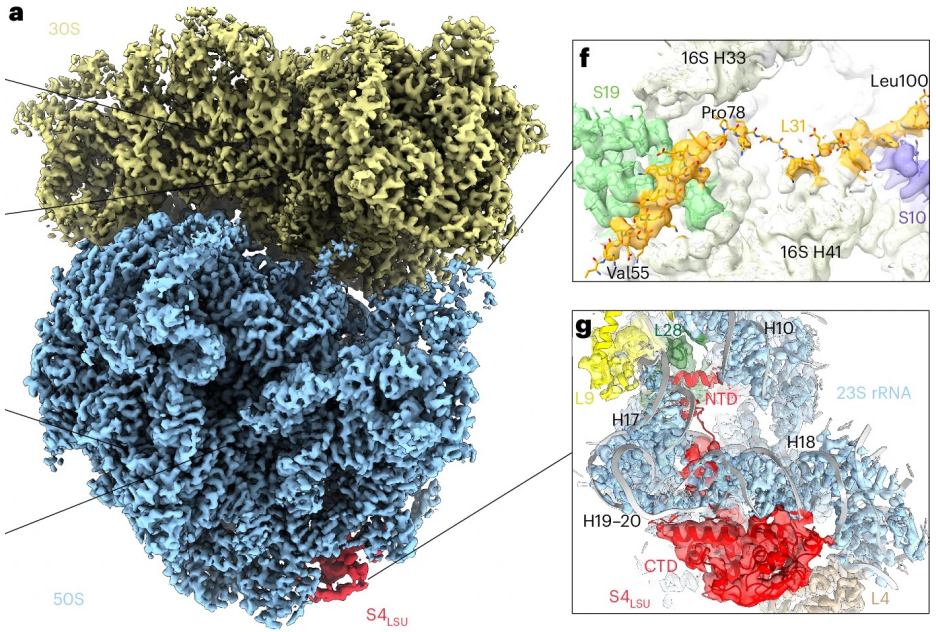
Human apoferritin at 1.15Å by cryoEM single particle analysis
Yip K. *et al*., Nature (2020)

In situ Structural Biology at High Resolution

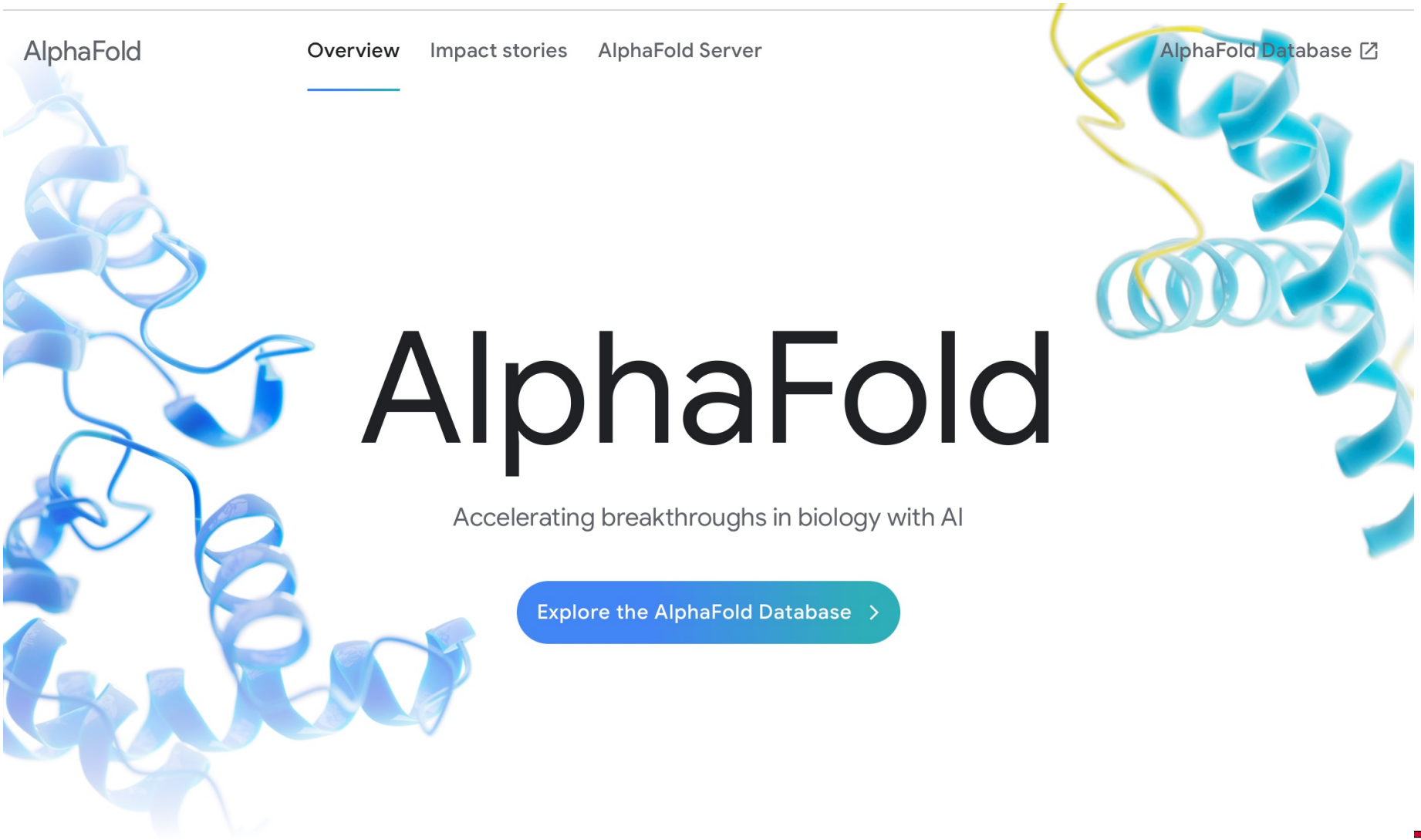


O'Reilly, F. J. *et al.*, Science 2020

2.9Å resolution structure of ribosomes in chloramphenicol-treated *Mycoplasma pneumoniae* cells



L. Xue *et al.* Nat Struct Mol Biol (2024)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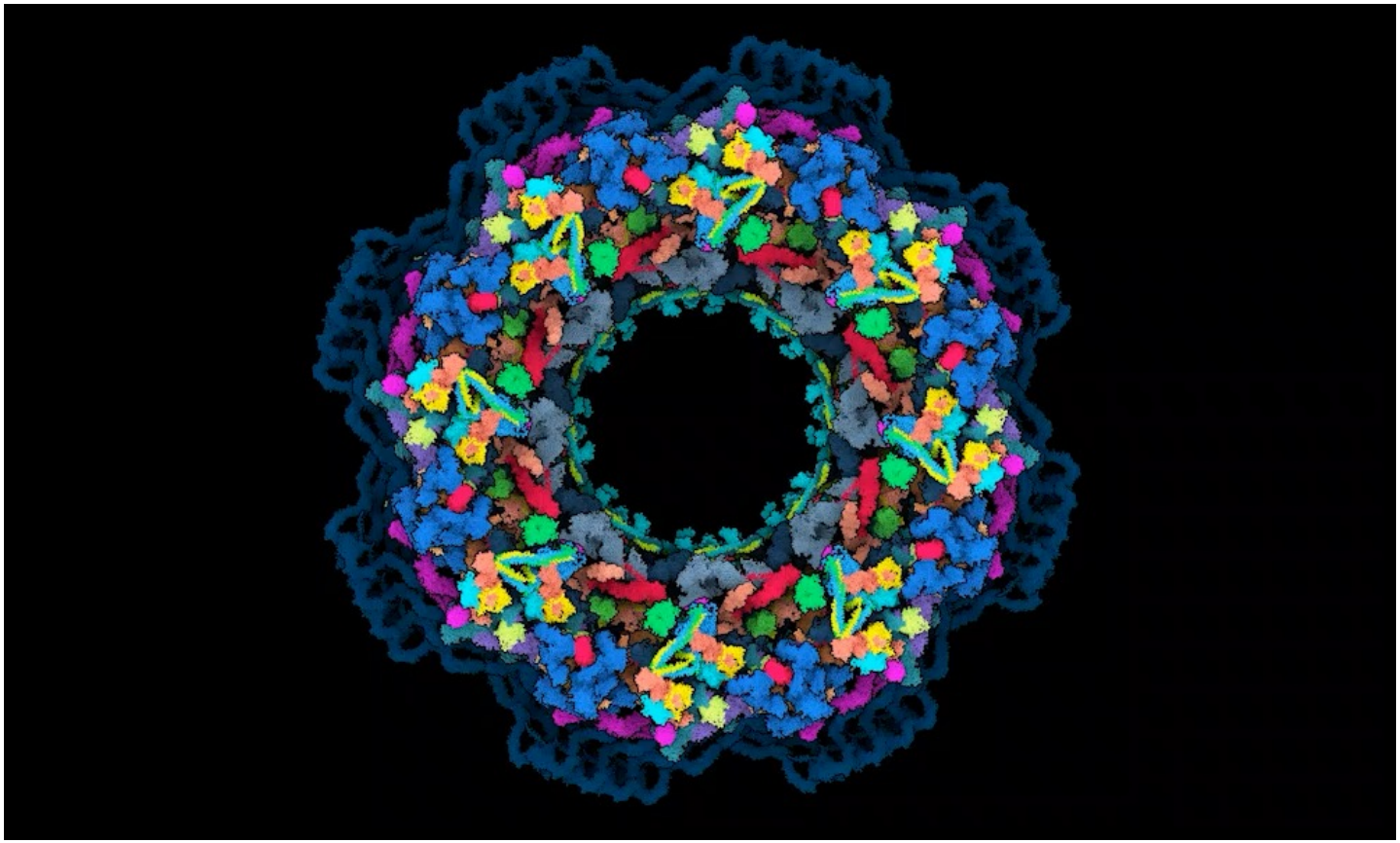
Accelerating breakthroughs in biology with AI
Explore the AlphaFold Database

AI-based Structure Prediction Empowers Integrative Structural Analysis of Human Nuclear Pores



Mosalaganti *et al*. (2022) *Science 37*6 6598

Summary - Why CryoET?

- Visualize the unique features of biological samples
- Understand the dynamics and organizations of protein complexes and organelles involved in fundamental biological processes
- Resolve *in situ* structures under the cellular context

Mapping the Molecular Landscape of the Fungal Plasma Membrane by Integrative Structural Biology

A Silent but Deadly Crisis

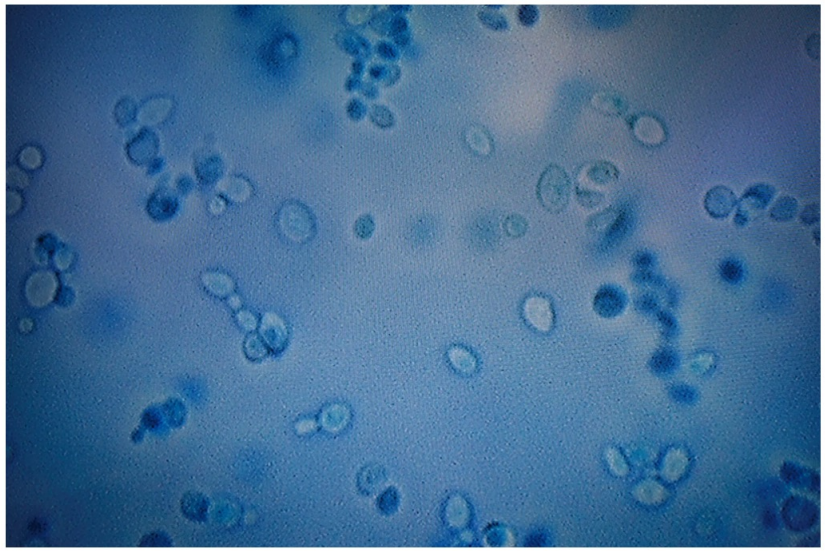
- Fungal infections affect more than 1 billion people each year and kill >1.5 million globally.
- In the US, direct medical costs are estimated at $6.7 - $7.5 billion annually.
- The number of deaths from fungal infections has increased during the COVID-19 pandemic.

https://www.nytimes.com/2023/03/20/health/candida-auris-us-fungus.html

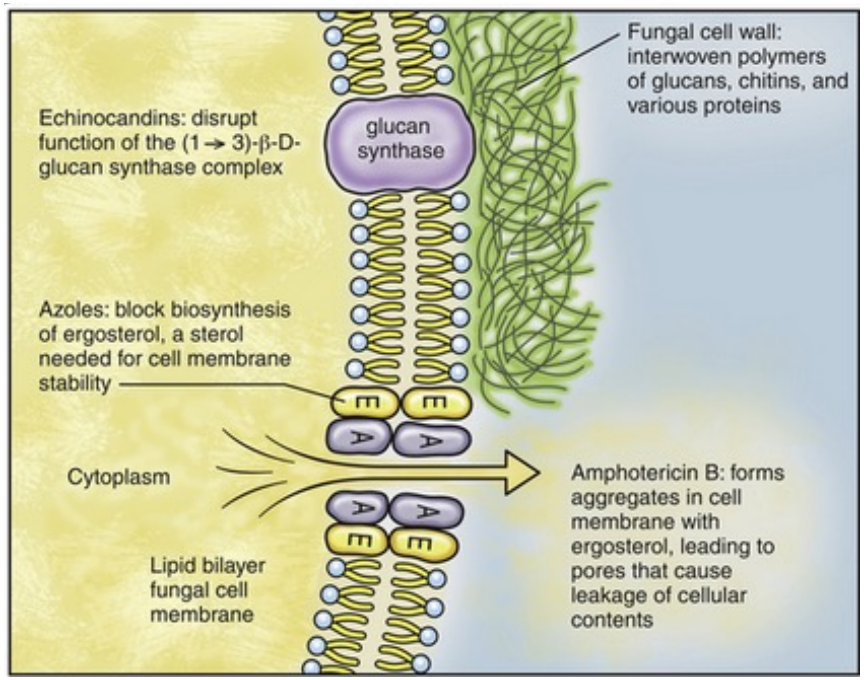
Deadly Fungus Spread Rapidly During the Pandemic, C.D.C. Says

Candida auris, a drug-resistant fungus that health officials hoped to contain is now in more than half the 50 states, according to a new research paper.

Share full article



The Fight Against Fungi



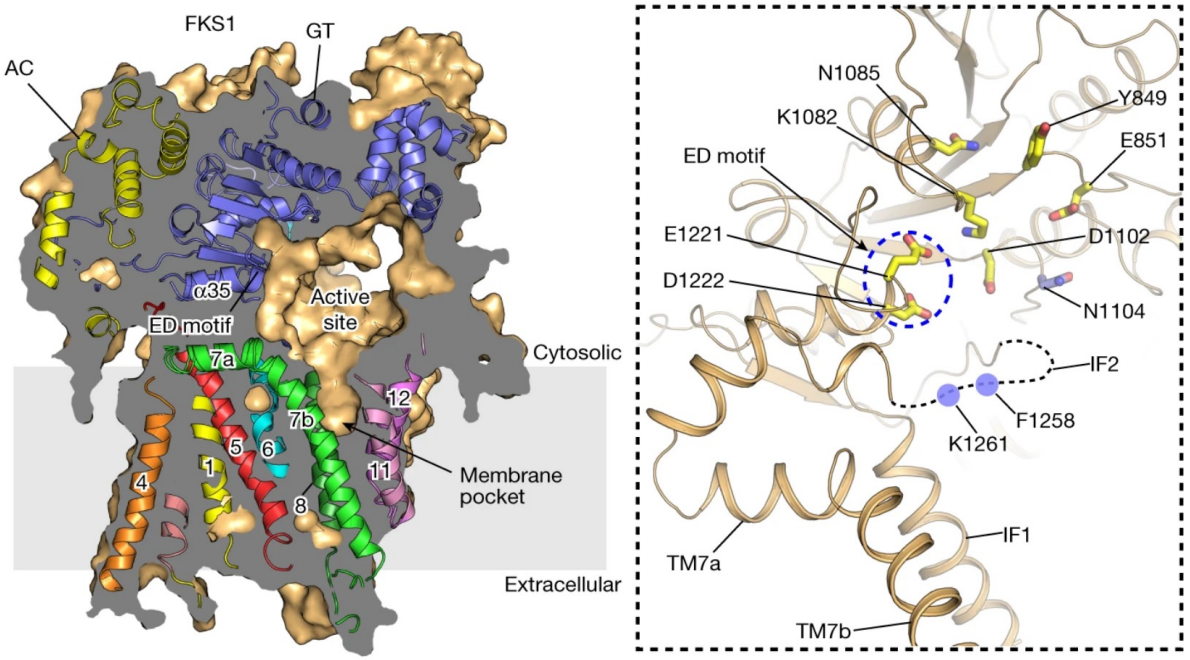
- Fungal cells:
  - Eukaryotic
  - Unique membrane lipid compositions
  - Have a cell wall that is critical for fungal growth, survival, and pathogenesis
- Antifungal drugs
  - **Polyenes (Amphotericin B):** bind to ergosterol in the plasma membrane
  - **Azoles**: inhibit ergosterol biosynthesis
  - **Echinocandins:** inhibit beta glucan synthase activities

https://veteriankey.com/antifungal-drugs-2/

Fungal Glucan Synthase

- Multi-subunit complex with two main subunits: a large, membrane embedded catalytic subunit encoded by *FKS* genes, and a regulatory subunit Rho1
- The membrane embedded catalytic subunit is the target for the echinocandin drugs
- First 3D structure resolved by cryoEM in 2023

Fungal Glucan Synthase – CryoEM Structure

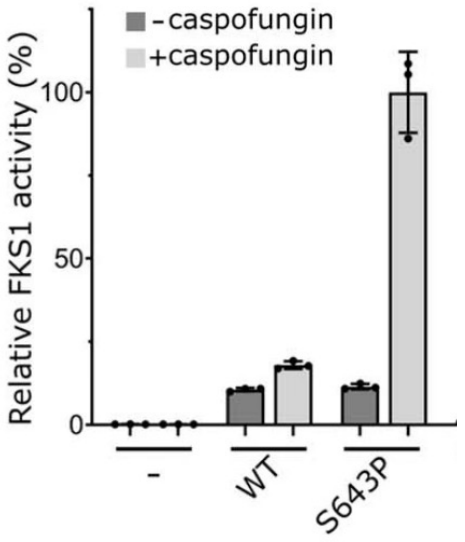


- Has a conserved cellulose-synthase-like fold
- Has FKS1-specific features, notably at the membrane-cytosolic interface
- A solvent-exposed chamber as the active site, and conserved residues for substrate binding and catalysis

Hu *et al*. (2023) *Nature* 616 190-198

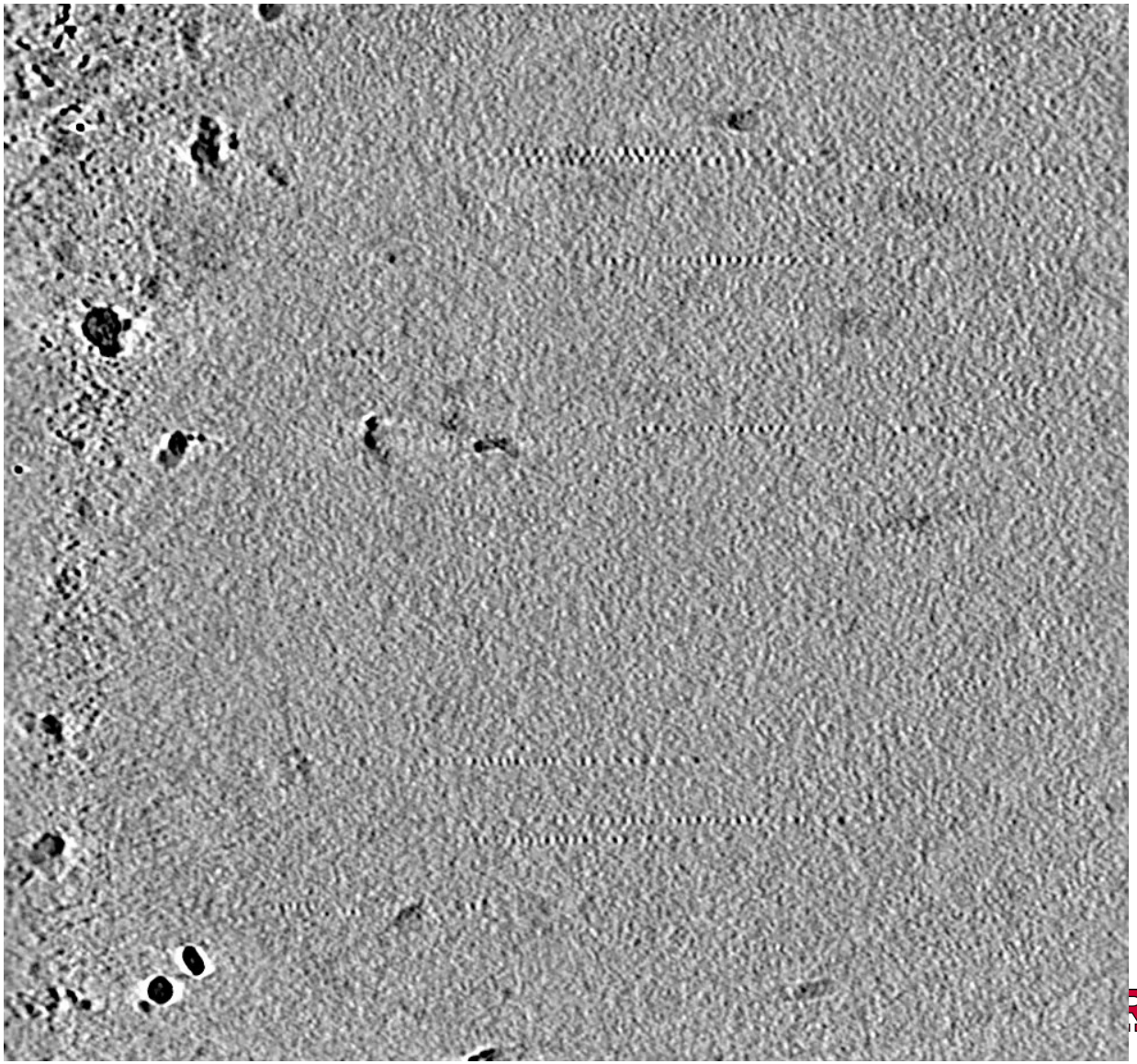
Lipids May be Involved in GS Catalytic Activities & Drug Interactions

- GS purified by different detergents
  - Displayed conformational differences at the active site
  - Showed varied susceptibilities to CSF exposure.
    - CSF treatment increases the activities of GDN-purified GS *in vitro*

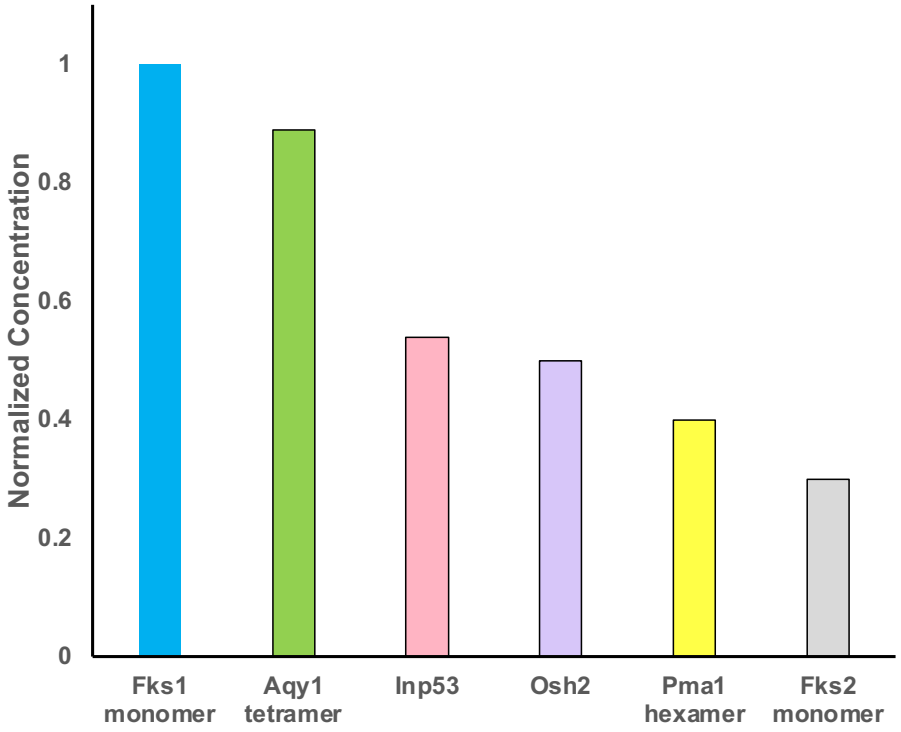


Hu *et al*. (2023) *Nature*, Zhao et al. (2023) *Science Advances*

Tomograms of *C. glabrate* Plasma Membranes



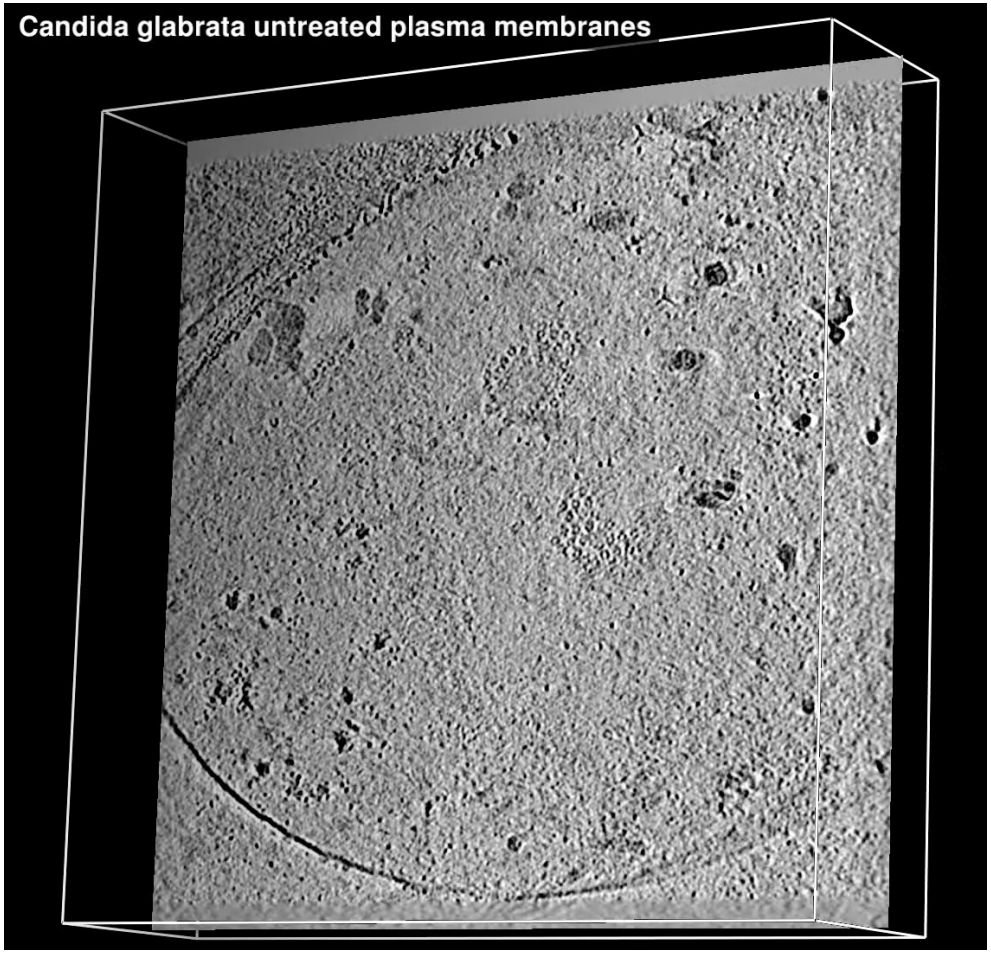
Proteomics Profiled the Abundance of Fungal Membrane Proteins



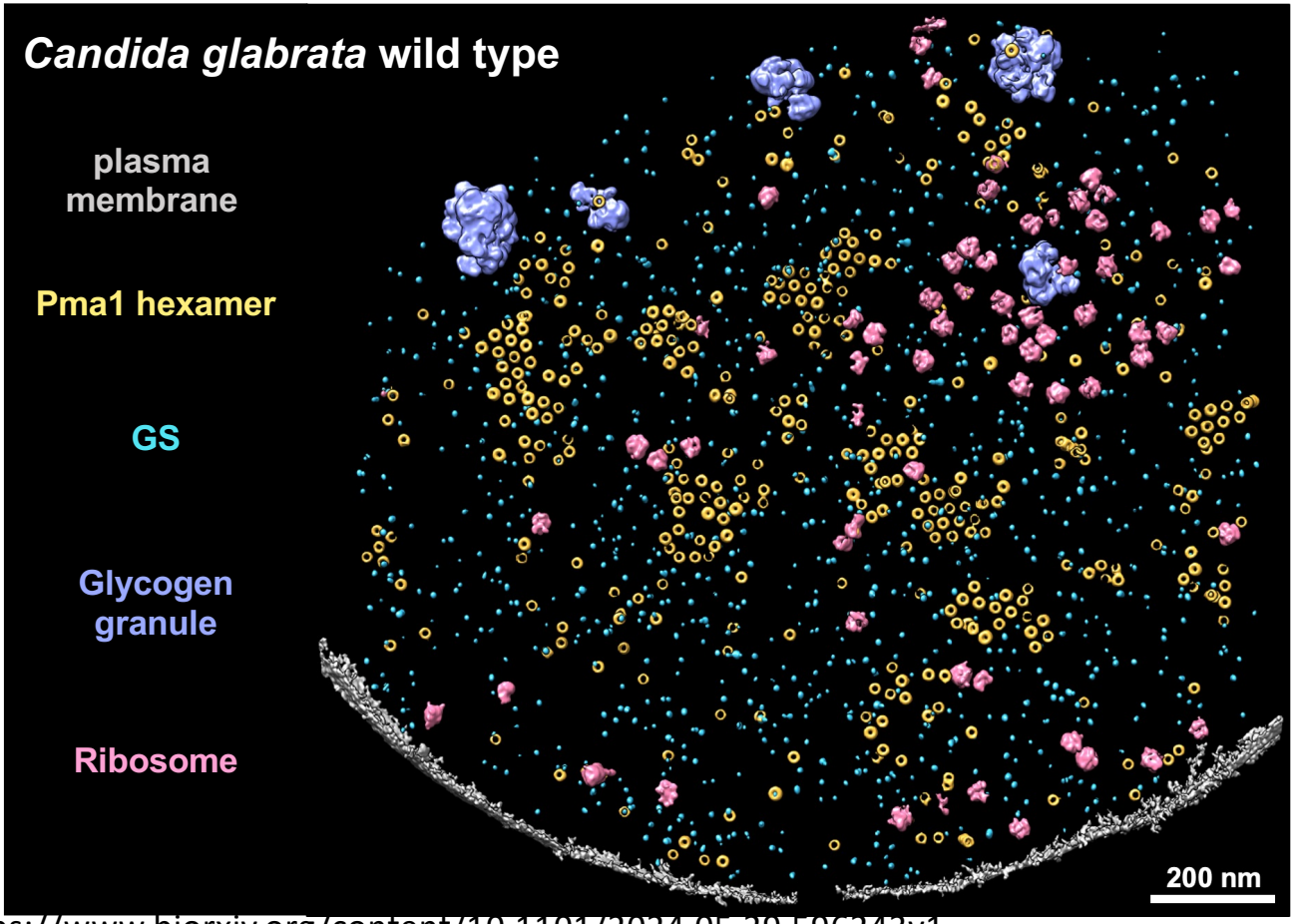
- Mass spectrometry identified 3,905 proteins.
- Fks1 (GS) and Pma1 (proton pump) are among the most abundant and detectable membrane proteins.

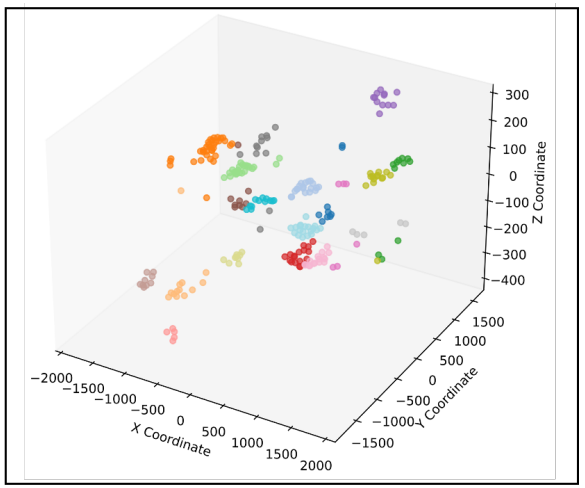
https://www.biorxiv.org/content/10.1101/2024.05.29.596243v1

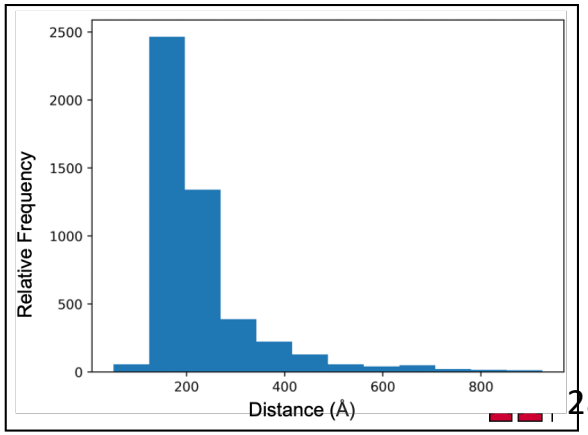
Annotation of the Fungal Plasma Membrane



Fungal Plasma Membrane Proteins Segregate into Microdomains

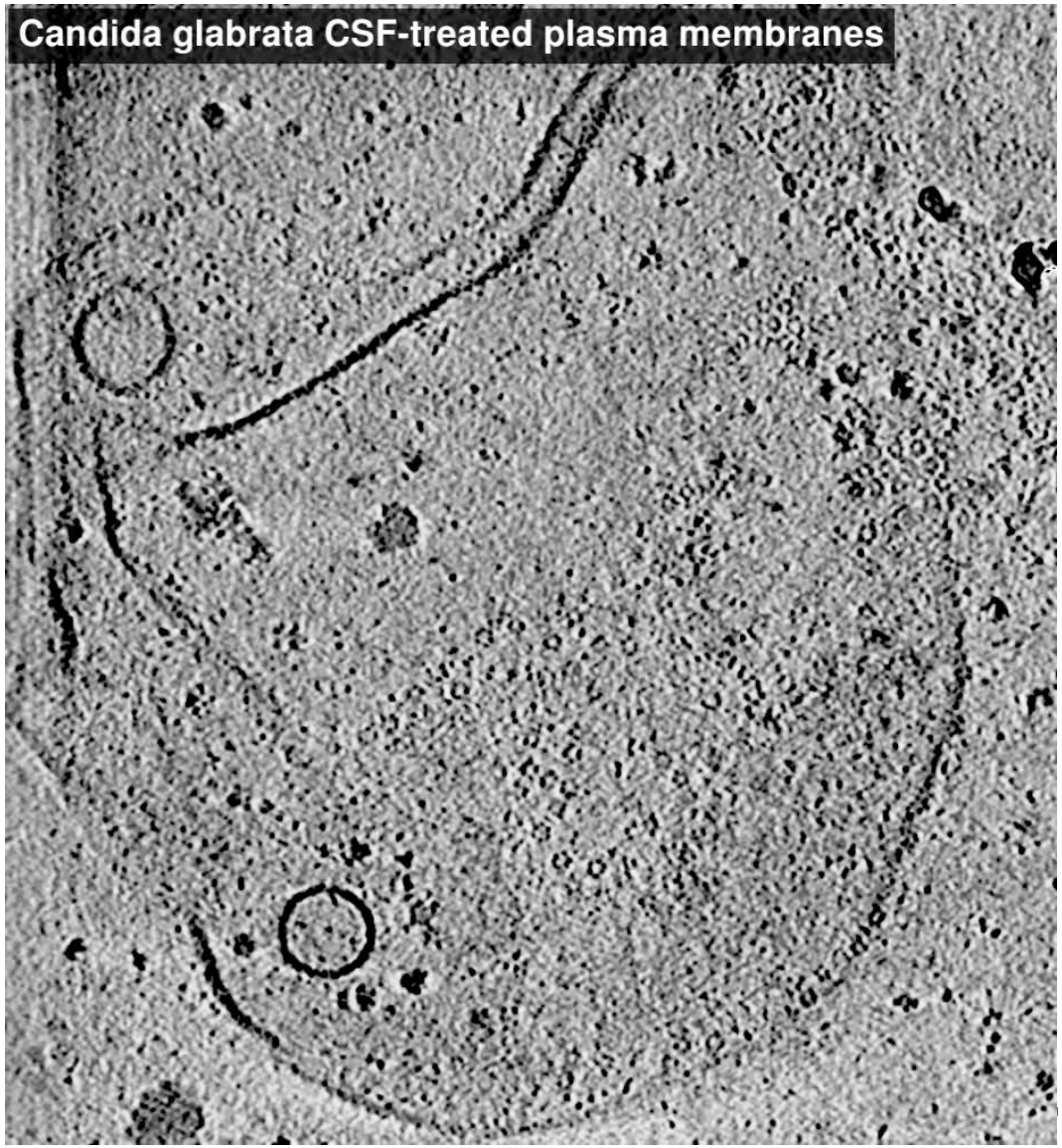






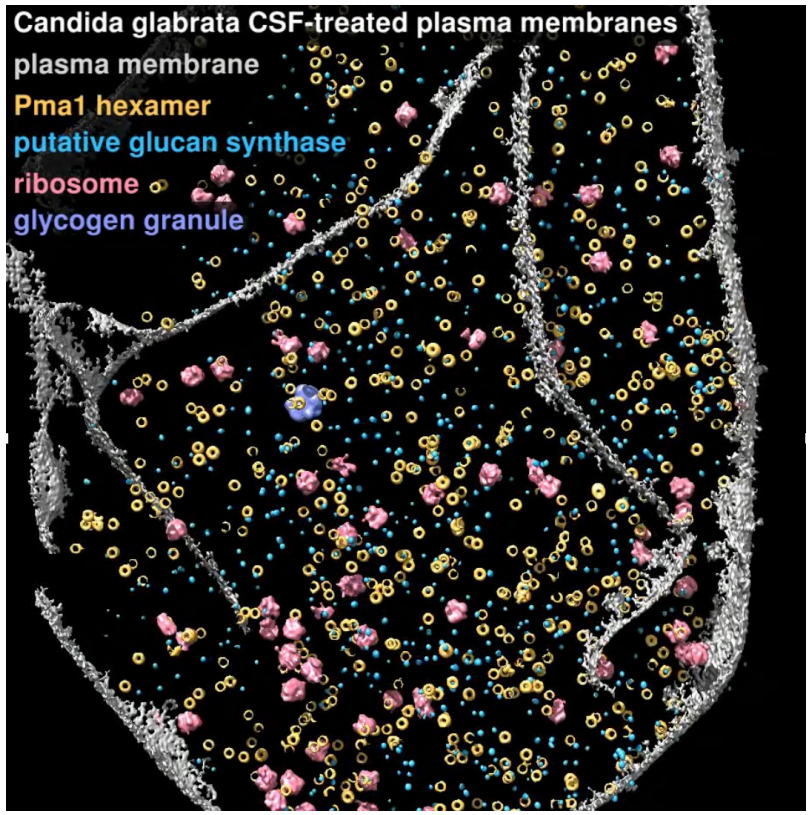
https://www.biorxiv.org/content/10.1101/2024.05.29.596243v1

Annotation of an Echinocandin-treated Plasma Membrane

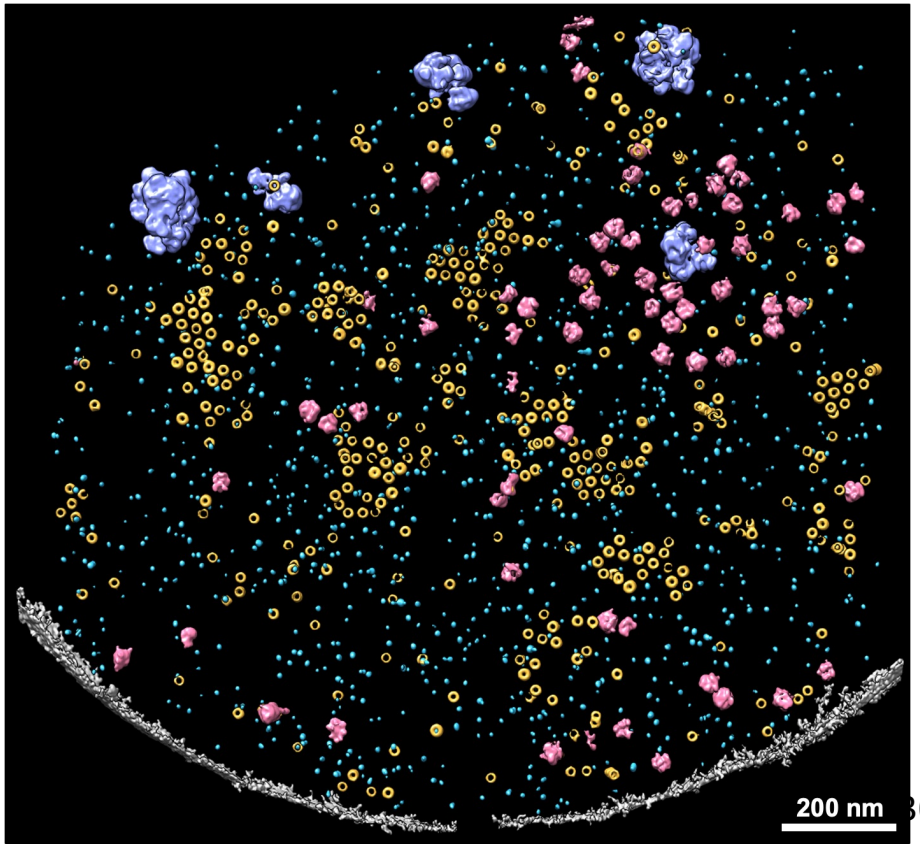


Echinocandin Treatment Disrupts Membrane Protein Microdomain Organization

Treated

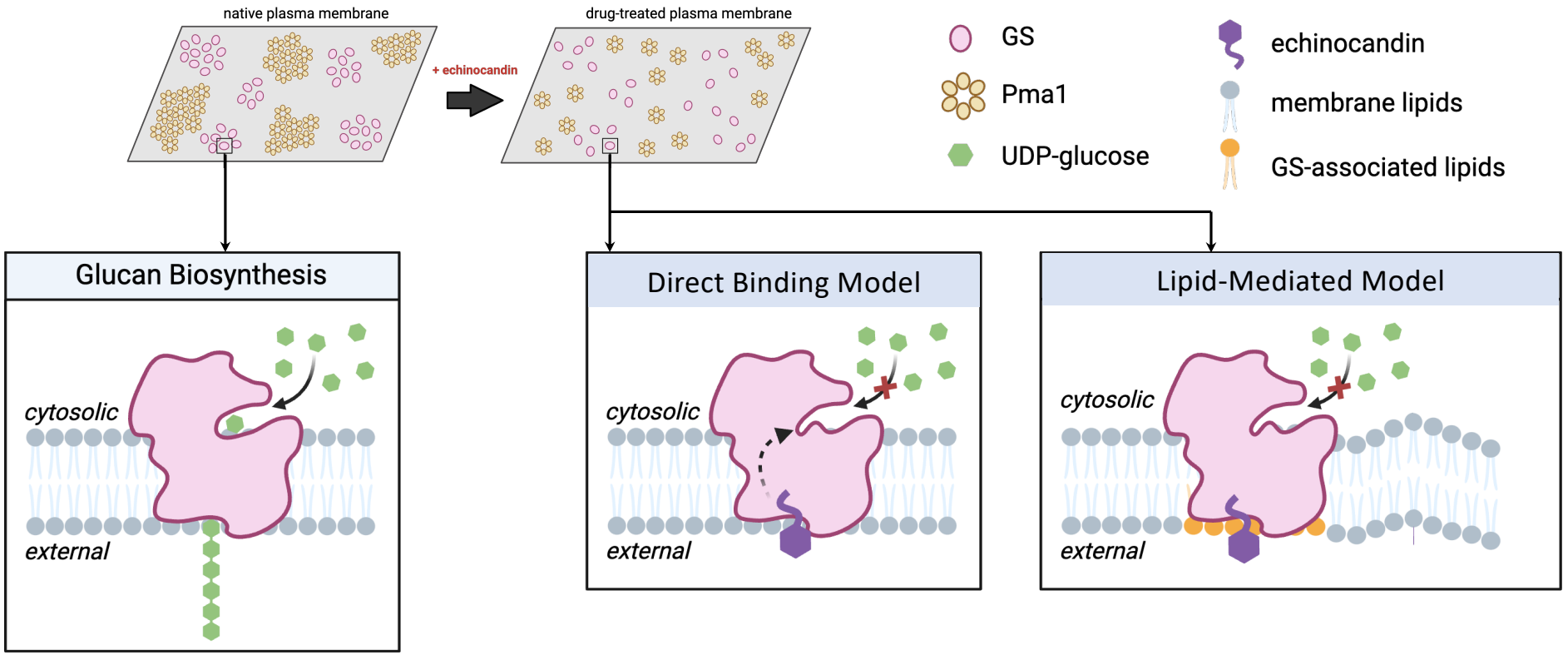


Untreated



https://www.biorxiv.org/content/10.1101/2024.05.29.596243v1

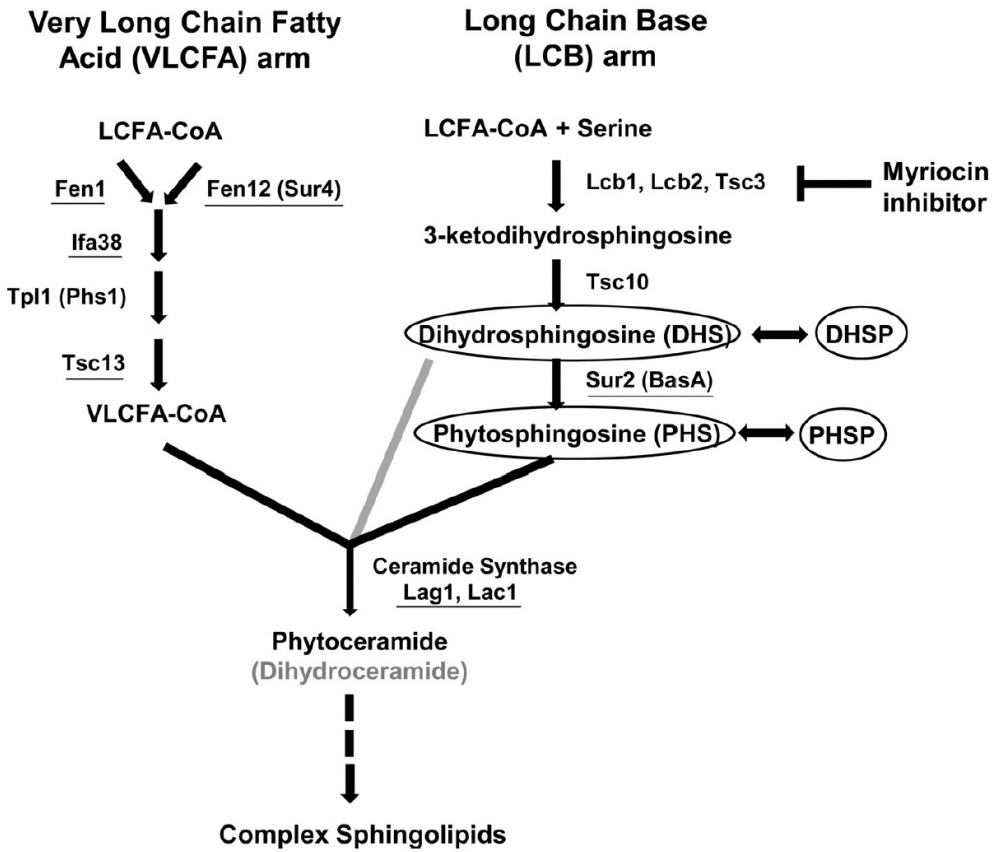
Model: Echinocandin Inhibition Mediated by the Integral Lipids



https://www.biorxiv.org/content/10.1101/2024.05.29.596243v1

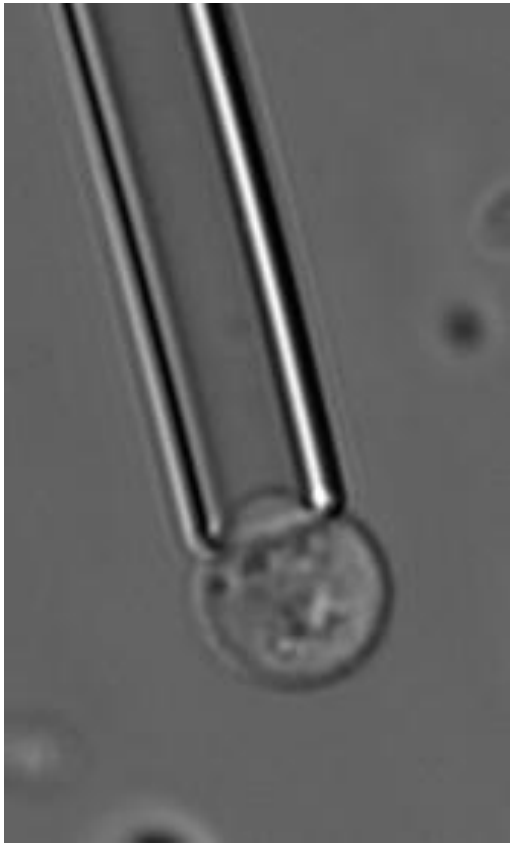
Will Changes to Lipid Compositions Affect Echinocandin Inhibition?

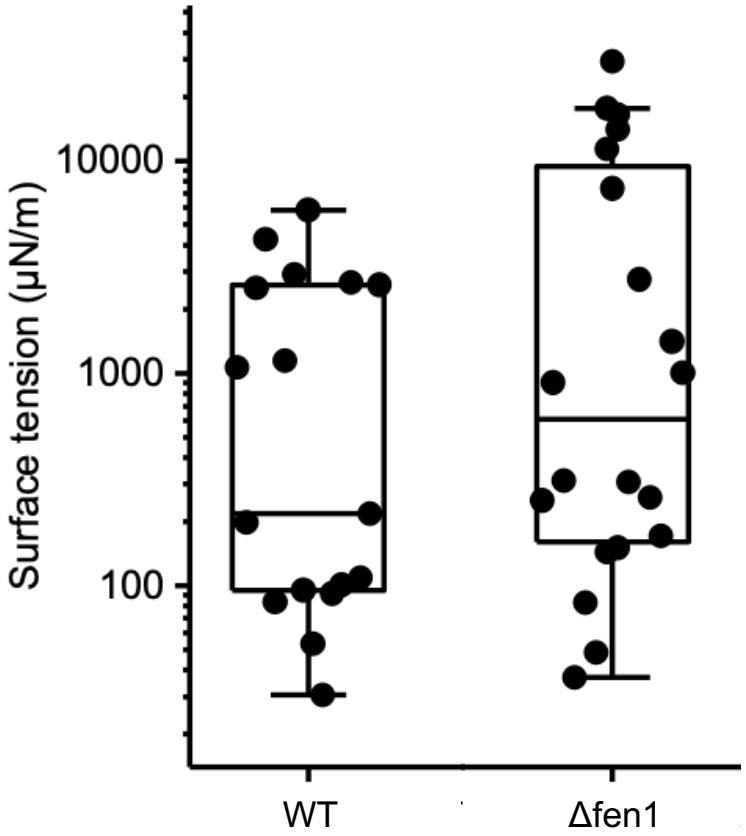
FEN1 **encodes a fatty acid elongase involved in early steps in sphingolipid biosynthesis**



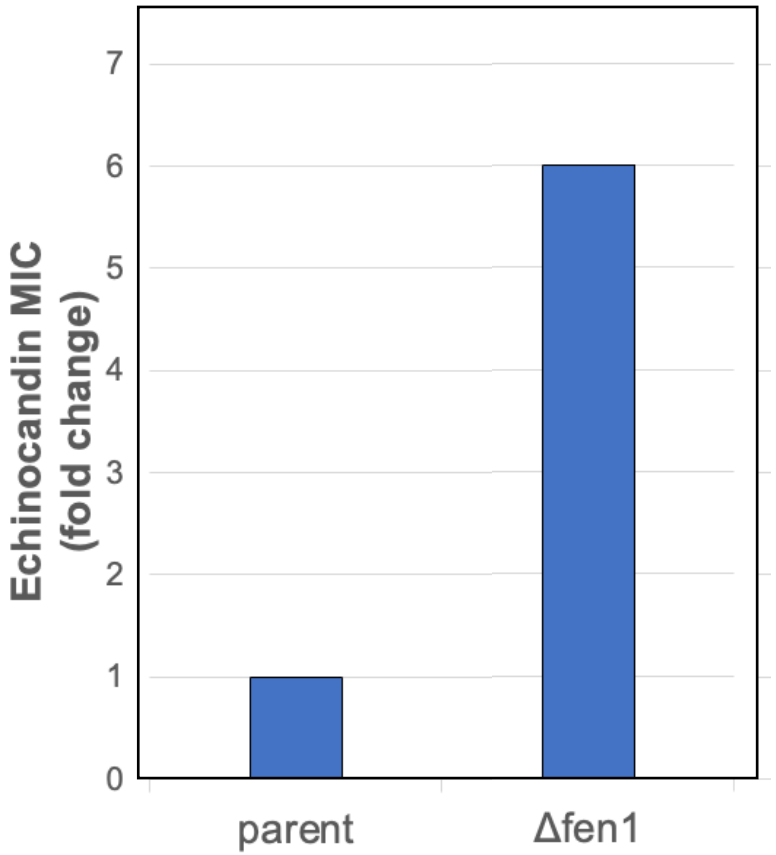
K. Healey *et al*. *AAC*. (2015)

Δfen1 Exhibit Altered Biophysical Properties of the Membrane





Δfen1 Exhibit Altered Echinocandin Susceptibility



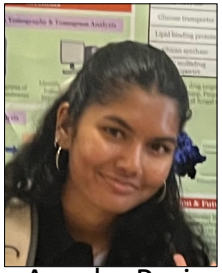
Summary

- Integrative cell biology, combining mass spectrometry, biophysical analyses, and structural studies supported by cryo-ET, provides a comprehensive understanding of membrane protein structure and dynamics within the cellular context.

- The membrane environment plays a pivotal role in GS function and drug responses and presents a promising avenue for drug development.

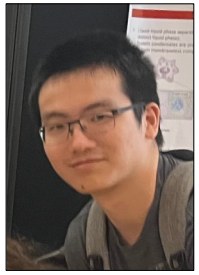
Acknowledgments

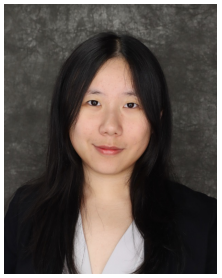

Jennifer Jiang


Anusha Puri


Cris Jimenez-Ortigosa


Mikhail Keniya


Jeff Cheng


Cynthia Pang


Yunkyung Lee

RUTGERS

CDI Center for Discovery & Innovation
Member of Hackensack Meridian *Health*

Sanghyuk Lee Group

Zhen Shi Group

Huan Wang

Rutgers CryoEM & Nanoimaging Facility

Jason Kaelber, Ph.D.

Rutgers Mass Spectrometry Core Facility

Haiyan Zheng, Ph.D.

Rutgers Busch Biomedical Research Grant

Perlin Group

Cristina Jimenez-Ortigosa Ph.D.

Mikhail Keniya

Stanford University

Muyuan Chen, Ph.D,

Carnegie Mellon University

Min Xu Group

Xueying Zhan, Ph.D.

The National Network for CryoET

NSF