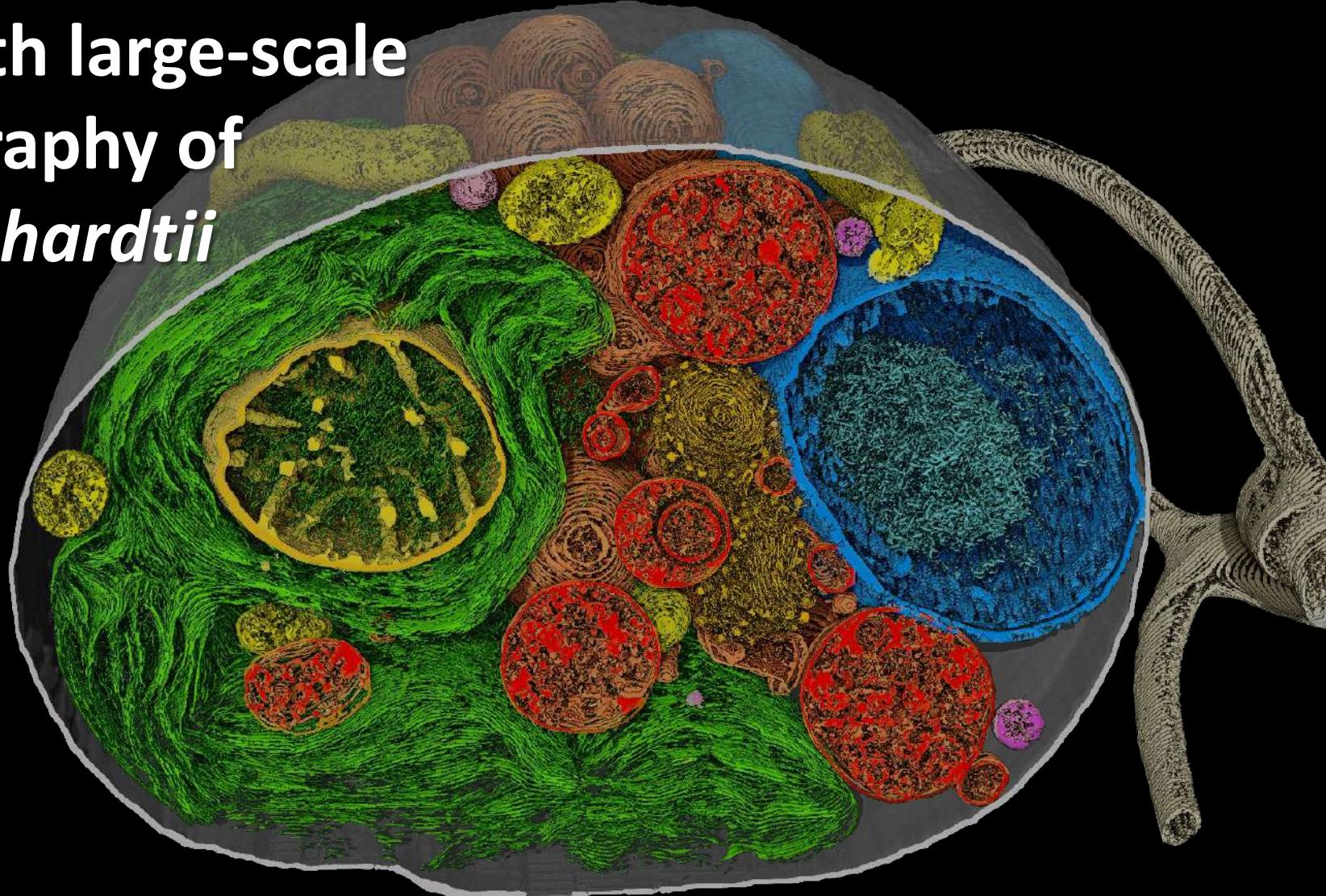


# Towards community-driven visual proteomics with large-scale cryo-electron tomography of *Chlamydomonas reinhardtii*

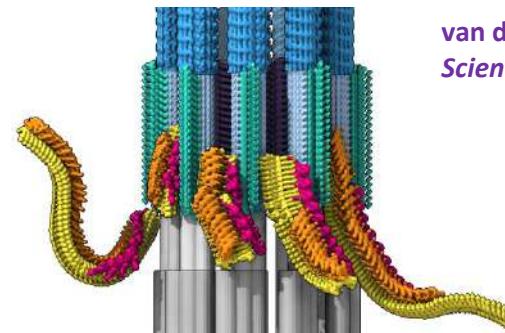
Ricardo D. Righetto

CellArchLab (Engel group)



# Chlamydomonas: meet the PLANIMAL

**ANIMAL:** Centrioles and Cilia

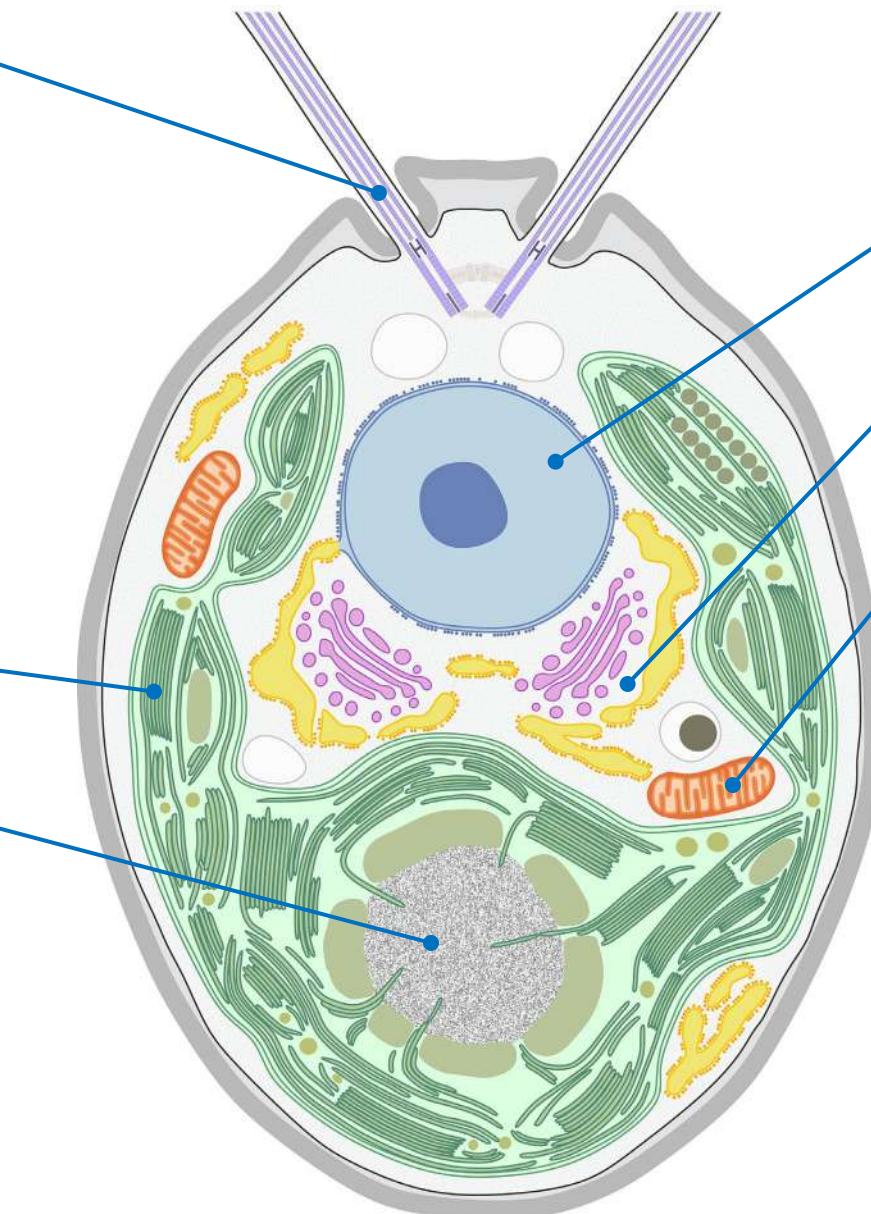
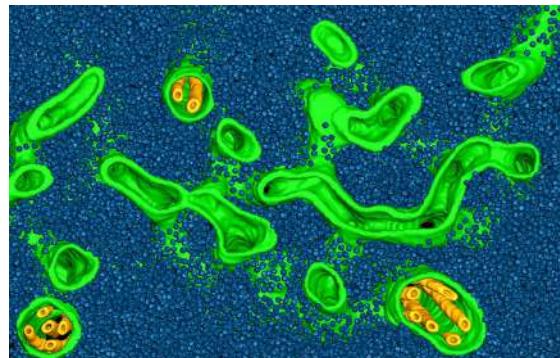


van den Hoek et al.  
*Science* (2022)

**PLANT:** Chloroplast

Thylakoids: Light Harvesting

Pyrenoid: Carbon Fixation

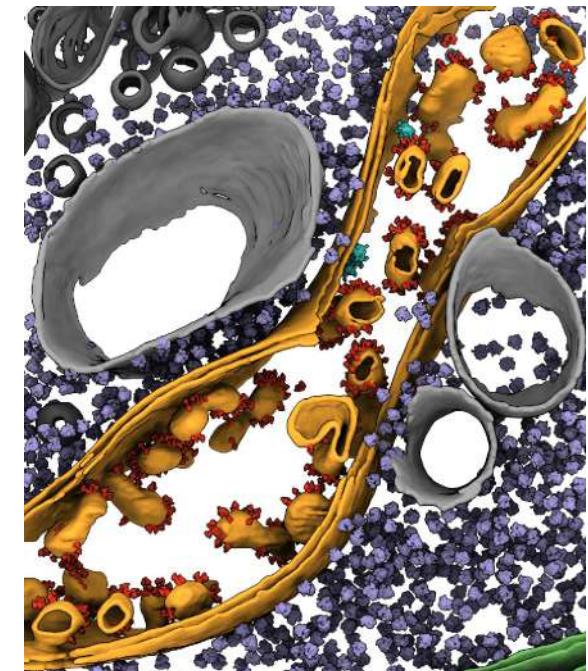


**“Core” Eukaryotic Organelles**

Nucleus

Endoplasmic Reticulum & Golgi Apparatus

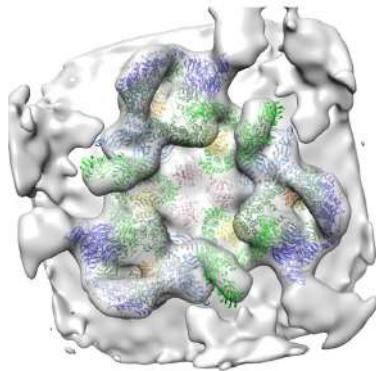
Mitochondria



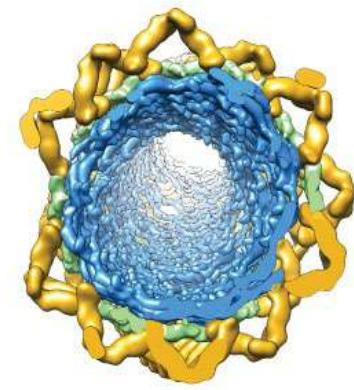
# Chlamy is perfect for cryo-ET



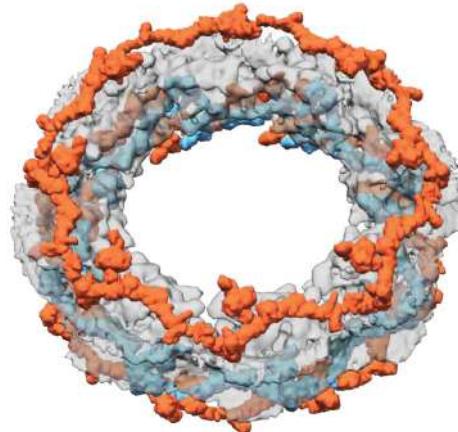
COPI Coat



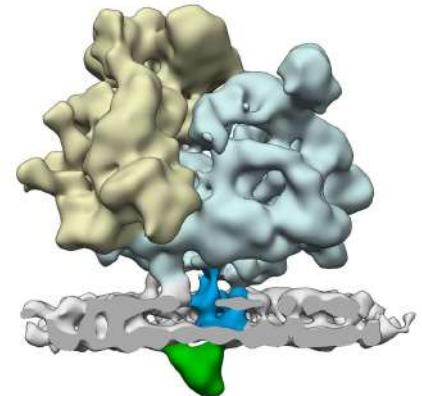
Retromer Coat



Nuclear Pore Complex



ER-bound Ribosome



Bykov *et al.*, eLife (2017)

Mosalaganti *et al.*, Nat Comm (2018)

Kovtun *et al.*, Nature (2018)

Pfeffer *et al.*, Nat Comm (2017)

# Cryo-ET of FIB-milled cells

Lamella preparation



FIB-SEM

Data acquisition

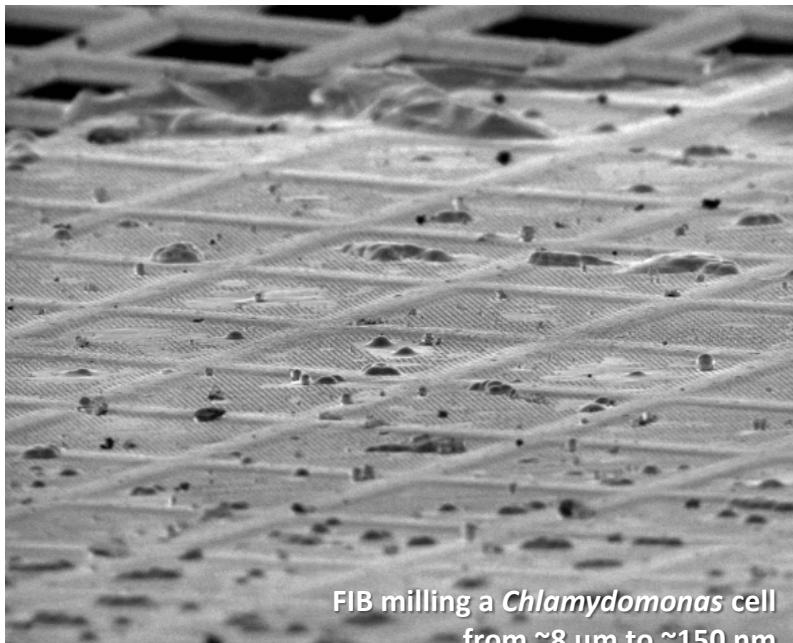


Cryo-TEM

Tomogram reconstruction and  
subtomogram averaging



Computer

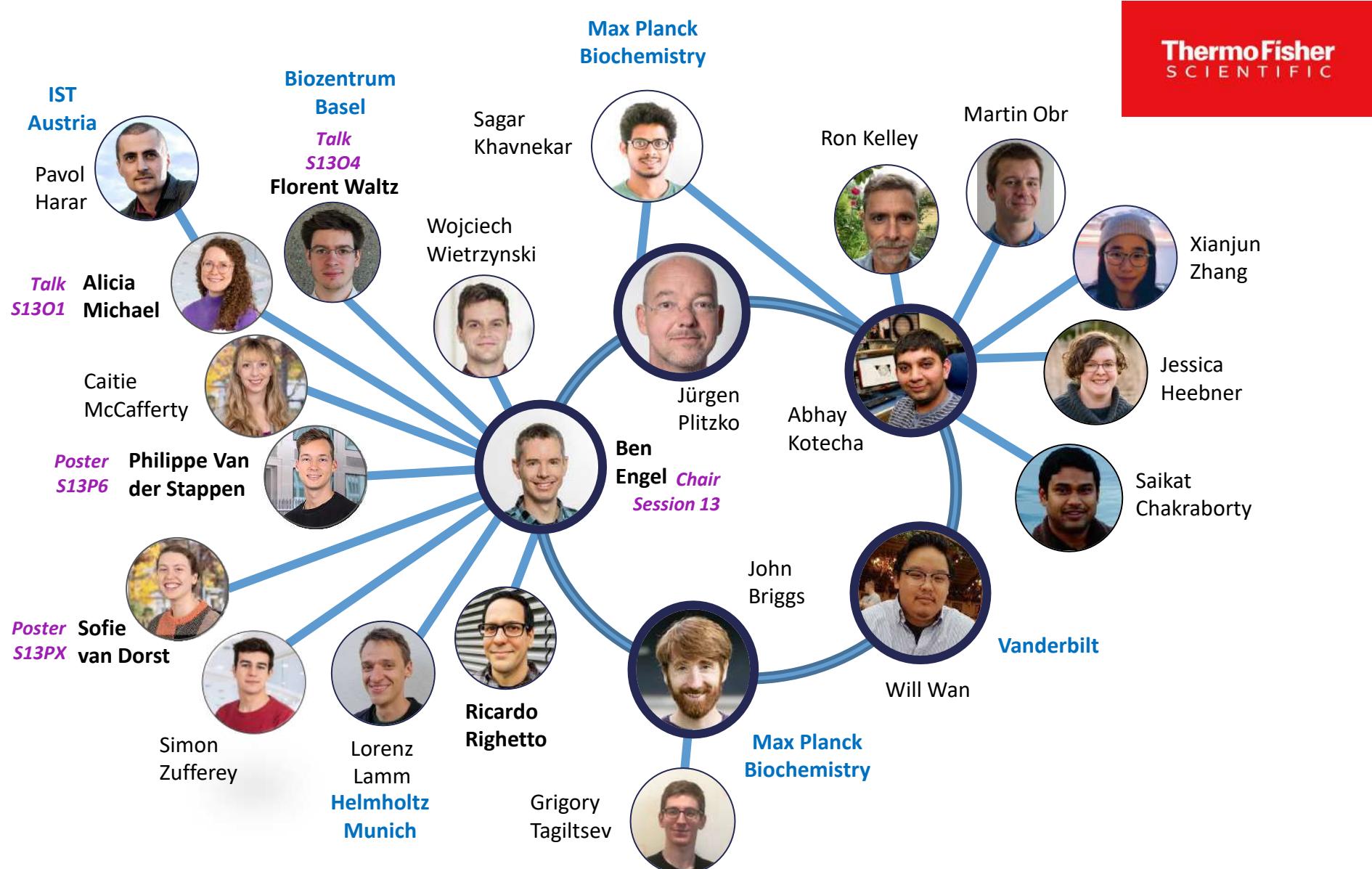


HV | curr | dwell | HFW | WD | tilt | 100 µm  
30.0 kV | 10.3 pA | 1 µs | 379 µm | 19.1 mm | 17° | MPI für Biochemie



High-resolution cryo-electron tomography

# The Chlamy Project consortium



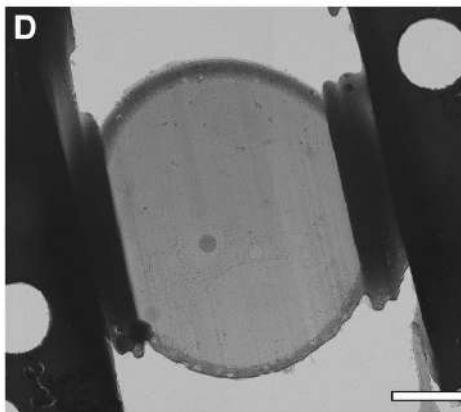
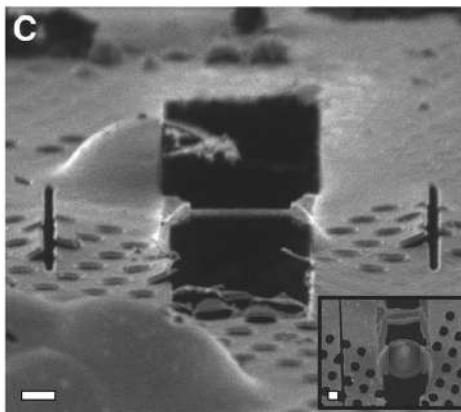
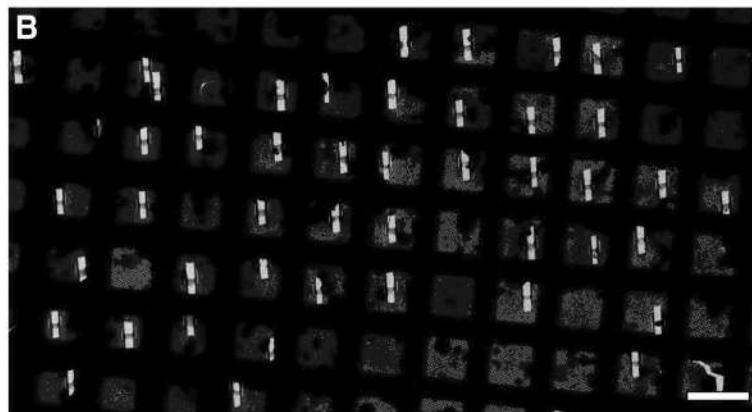
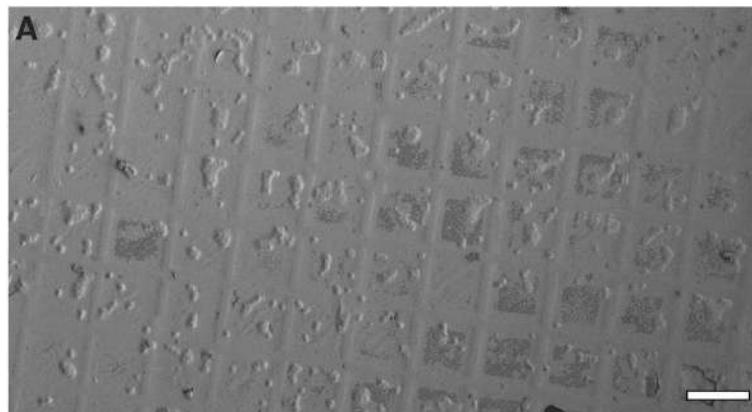
# Generating a large-scale cryo-ET dataset of Chlamy

*C. reinhardtii* strain mat3-4 (CC3994)

*Chlamydomonas Resource Center, University of Minnesota*

Smaller phenotype: ~6 µm cell size

TAP media, constant shaking and white light



Arctis cryo-plasma-FIB:  
high throughput milling

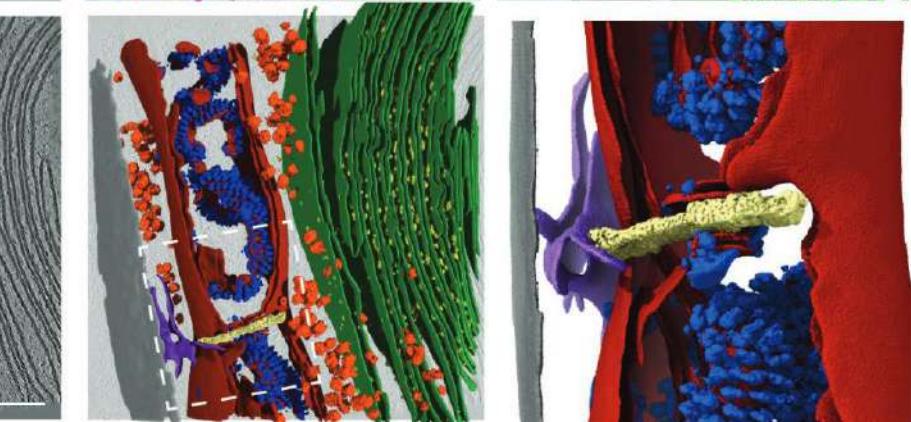
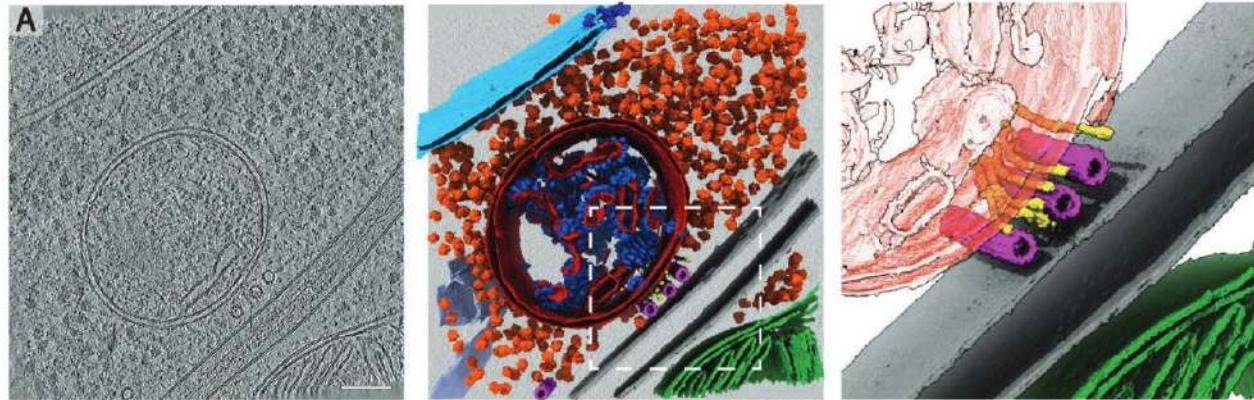
- >300 lamellae
- 55 grids
- 42 overnight sessions
- Titan G4, Falcon 4i, Selectris X
  - 1.96 Å/px
- 2,991 tilt series acquired
  - Manually curated
- **1,829 tilt series released**

# Catching “rare” cellular events



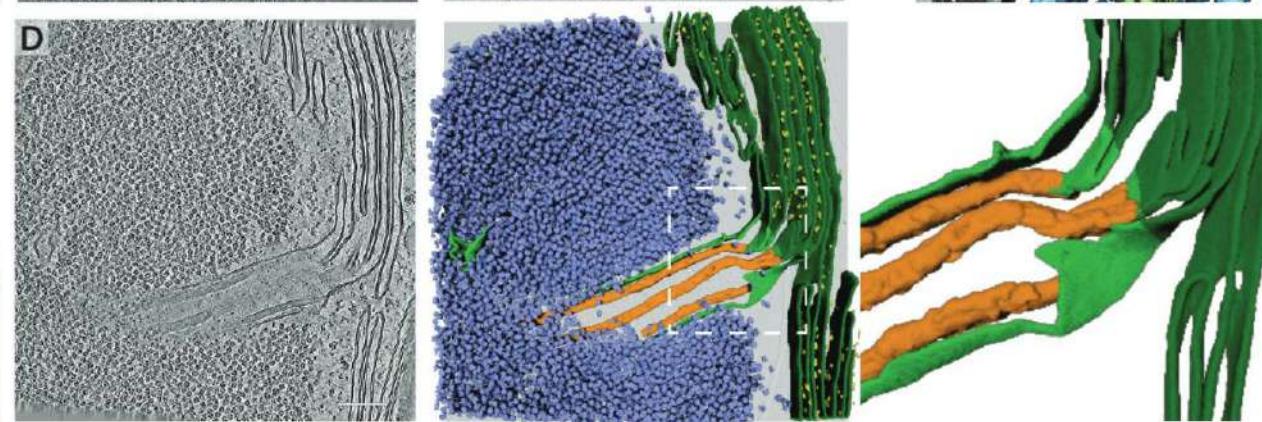
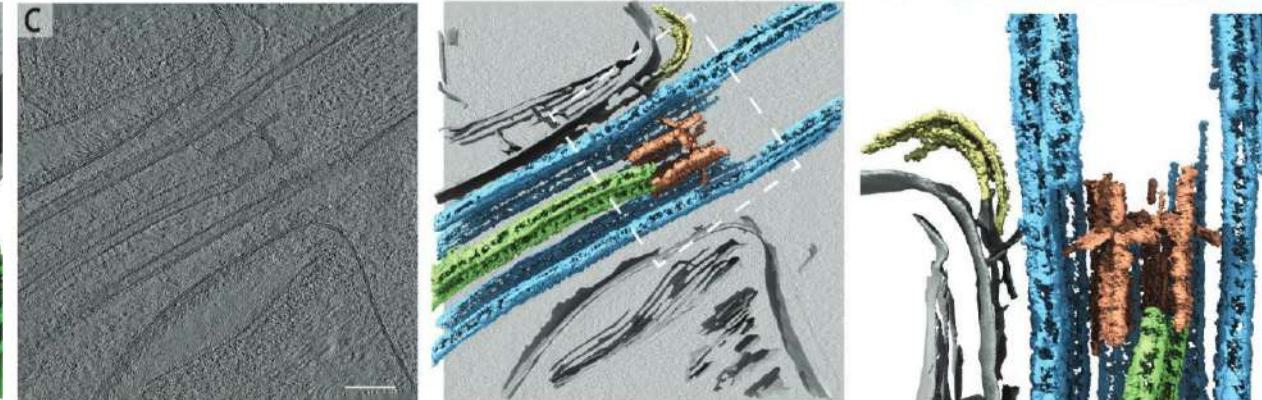
Jessica  
Heebner

Mitos on a roll



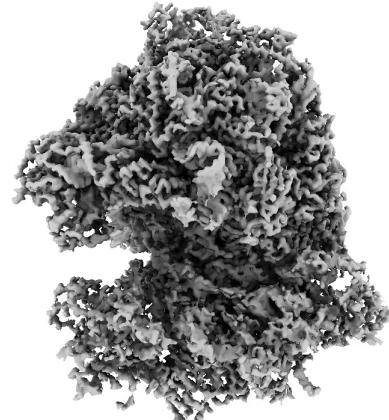
Mitochondrial fission

IFT trains assembling at the ciliary TZ

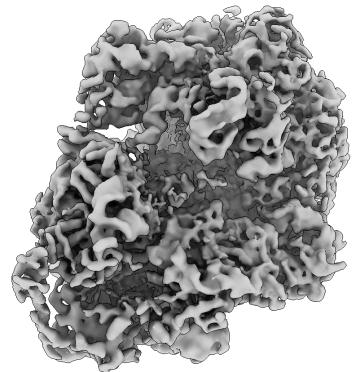


Pyrenoid tubules extending from thylakoids

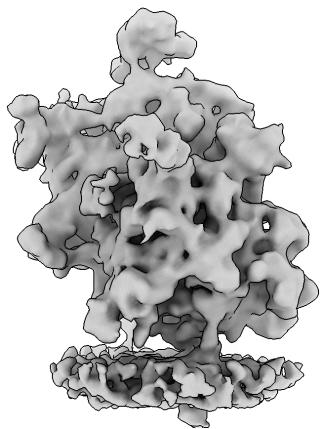
# Building an atlas of Chlamy structures



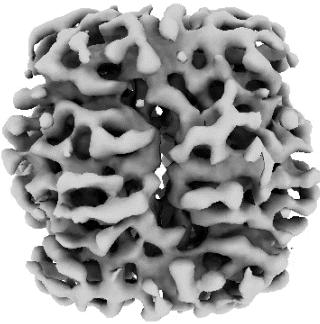
Cyto-Ribosome, 4 Å



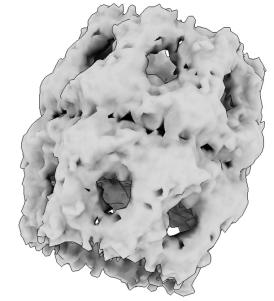
Chloro-ribosome, 6 Å



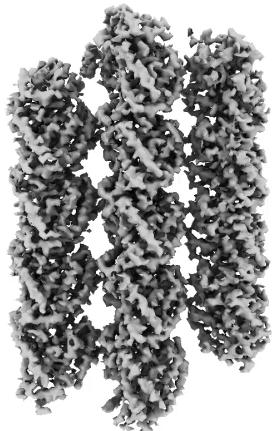
Mito-ribosome, 22 Å



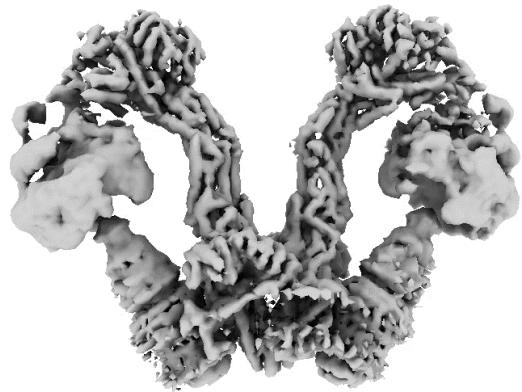
RuBisCO, 7.5 Å



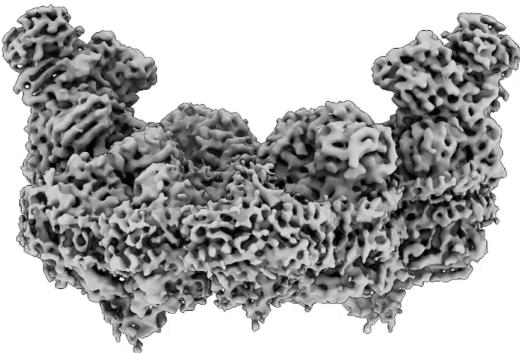
HSP60, 20 Å



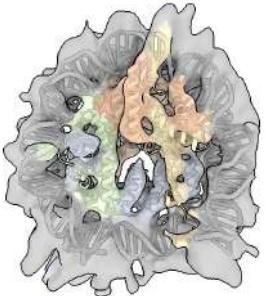
Microtubules, 4.7 Å



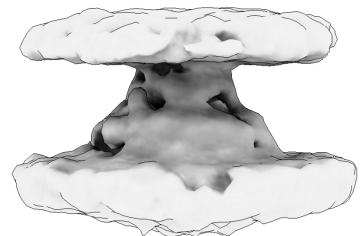
ATP synthase, 5.7 Å



Respirasome, 5.6 Å

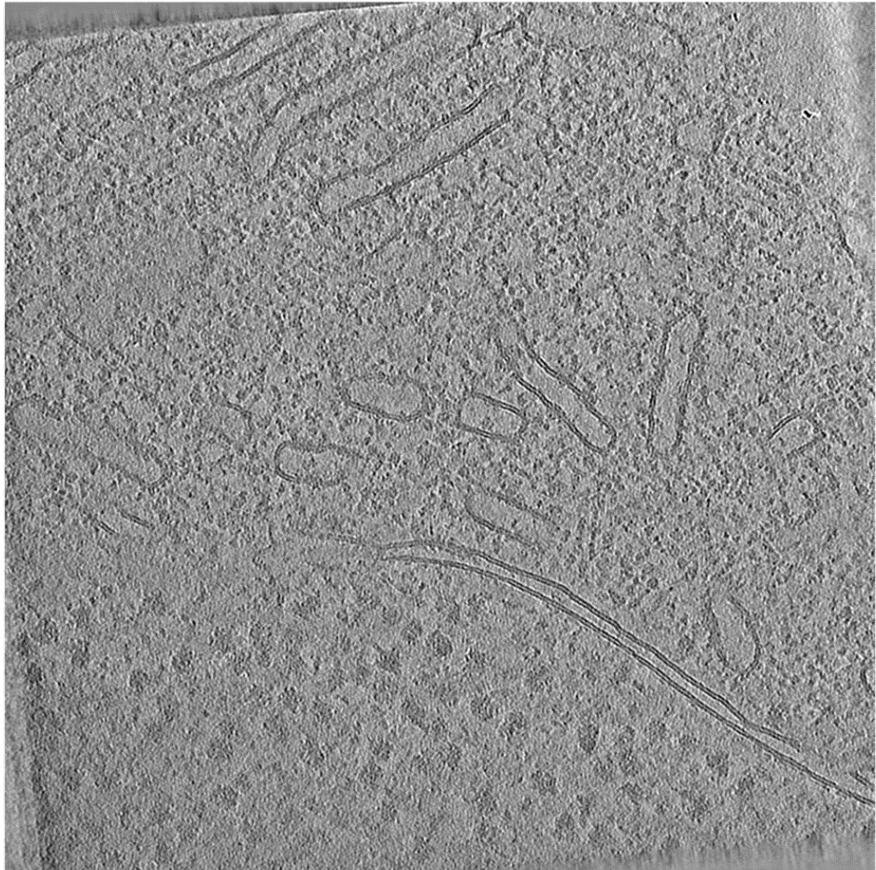


Nucleosome, 9.6 Å

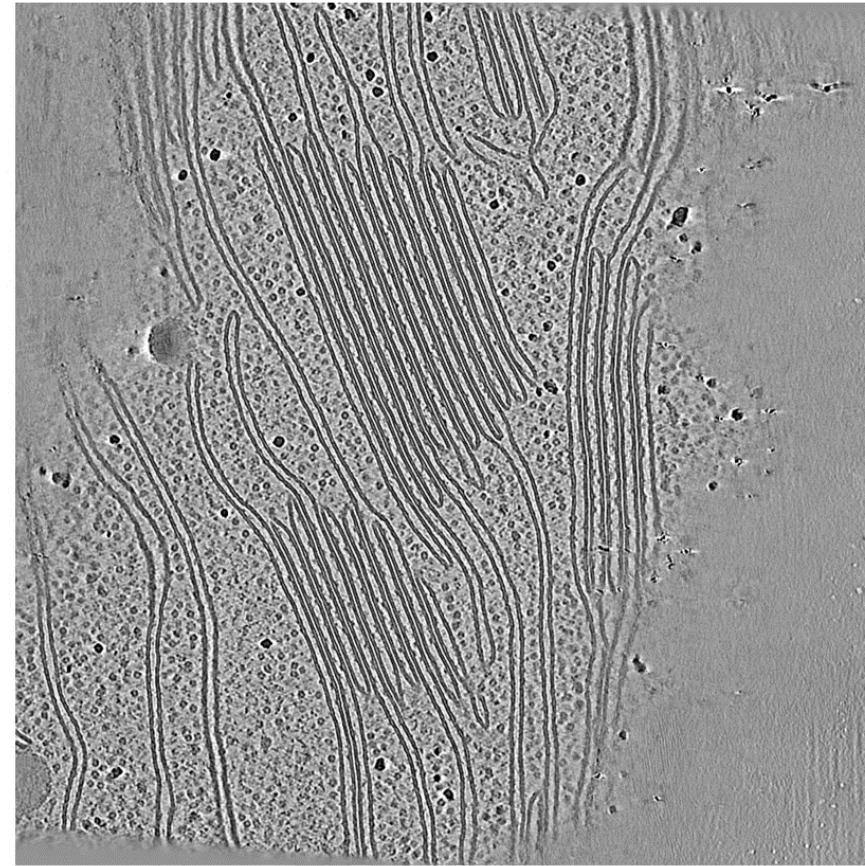


Unknown protein, 25 Å

# Developing methods: MemBrain-seg



*Chlamy mito*



*Spinach chloroplast*



Lorenz  
Lamm



Simon  
Zufferey

<https://github.com/teamtomo/membrain-seg>

See also

**MemBrain-pick**

**MemBrain-stats**

Lamm et al., bioRxiv (2024)

# For beginners: learn cryo-ET with the Chlamy dataset

<https://tomoguide.github.io/>

The screenshot shows the homepage of the TomoGuide website. At the top left is a logo of a green electron microscope icon. Next to it is a search bar with the placeholder "Search TomoGuide". On the far left is a sidebar with a light gray background and a dark gray header. The sidebar contains several sections with expandable arrows:

- Welcome to TomoGuide
- Some Words about Cryo-Electron Tomography
- Datasets, Software, and Resources
- Tutorial
  - Preprocessing in AreTomo3
  - Preprocessing in Scipion
  - Preprocessing in RELION5
  - Particle Picking
  - STA in RELION5
  - Check Handedness
- Acknowledgements

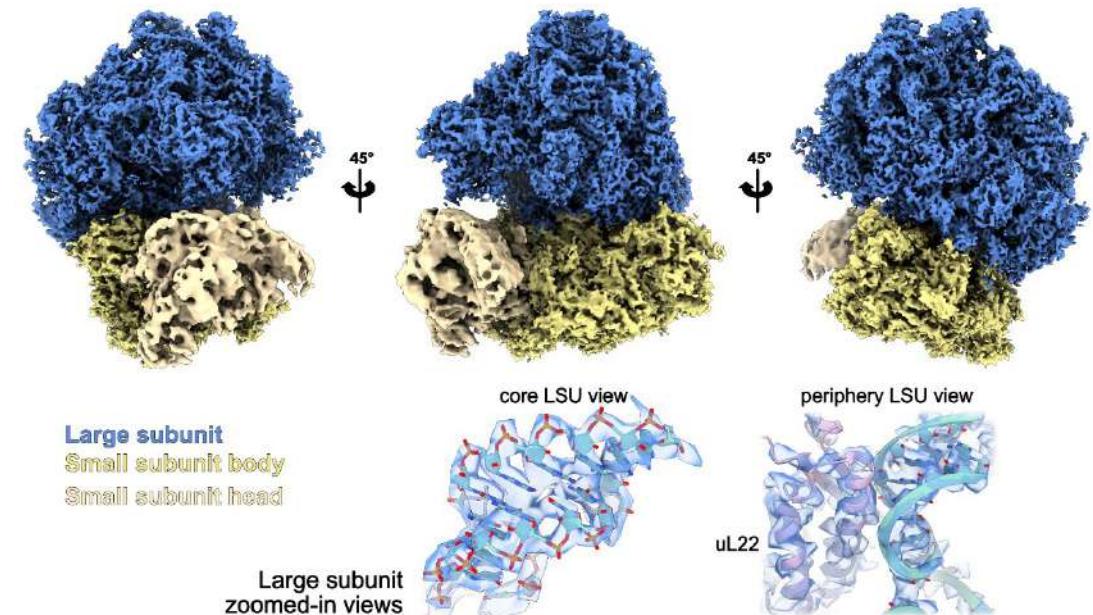
The main content area features a large image of numerous small, colored particles (green and purple) arranged in a grid-like pattern. Below this image is a heading "Welcome to TomoGuide" with a 3D cube icon. A descriptive paragraph follows, explaining the website's purpose: "This website serves as a resource for both beginners and experienced researchers in cryo-ET, offering an overview of the technique and data processing workflows. You'll find practical guidance, software recommendations, and a step-by-step tutorial for preprocessing and sub-tomogram averaging (STA), along with a test dataset to practice and explore the workflow." At the bottom of the main content area is a call-to-action: "Start by selecting a topic from the sidebar! You have multiple options to choose from!" with a list of preprocessing steps.



Florent  
Waltz



Philippe  
Van der Stappen



# For experts: raw data, metadata, annotations, scripts



## Data resources

- Raw files
- Reconstruction files
- Denoised tomos
- CTF-corrected tomos



**EMPIAR  
11830**



CZII CryoET Data Portal  
DS-10302

Contributors 5

Pavol Harar



<https://github.com/Chromatin-Structure-Rhythms-Lab/ChlamyAnnotations>

particle	coordinates	density	density_EMDB	resolution	representative_tomogram
ATPase	<a href="#">atp.star</a>	<a href="#">atp.mrc</a>	<a href="#">EMD-51802</a>	5.2 Å	tomo_1963
Clathrin	<a href="#">clathrin.star</a>	<a href="#">clathrin.mrc</a>	<a href="#">EMD-51789</a>	8.7 Å	tomo_2276
Microtubule	<a href="#">microtubule.star</a>	<a href="#">microtubule.mrc</a>	<a href="#">EMD-51804</a>	4.7 Å	tomo_2050
Nucleosome	<a href="#">nucleosome.star</a>	<a href="#">nucleosome.mrc</a>	<a href="#">EMD-19906</a>	9.6 Å	tomo_2173
Photosystem II	<a href="#">ps2.star</a>	<a href="#">ps2.mrc</a>	<a href="#">EMD-51731</a>	19 Å	tomo_0573
Rubisco	<a href="#">rubisco.star</a>	<a href="#">rubisco.mrc</a>	<a href="#">EMD-51848</a>	7.5 Å	tomo_0349
Ribosome 80S	<a href="#">ribosome80S.star</a>	<a href="#">ribosome80S.mrc</a>	<a href="#">EMD-51847</a>	4.0 Å	tomo_0017

- Particle coordinates & angles
- How to visualize in ArtiaX
- Script to import into RELION-5
- Manual tomo annotation info
- Community contributions & derived work

**Presentation will be available here – link in preprint!**

# For everyone: searching the dataset with cryoPom



Mart Last

Using automated segmentations to measure the **composition** of tomograms

## Dataset summary

The table below lists measurements of the fraction of a tomogram's volume occupied by each of 15 ontology segmentations and 4 macromolecule segmentations.

Click a header element to sort by that feature, or a **tomogram name** to inspect that volume.

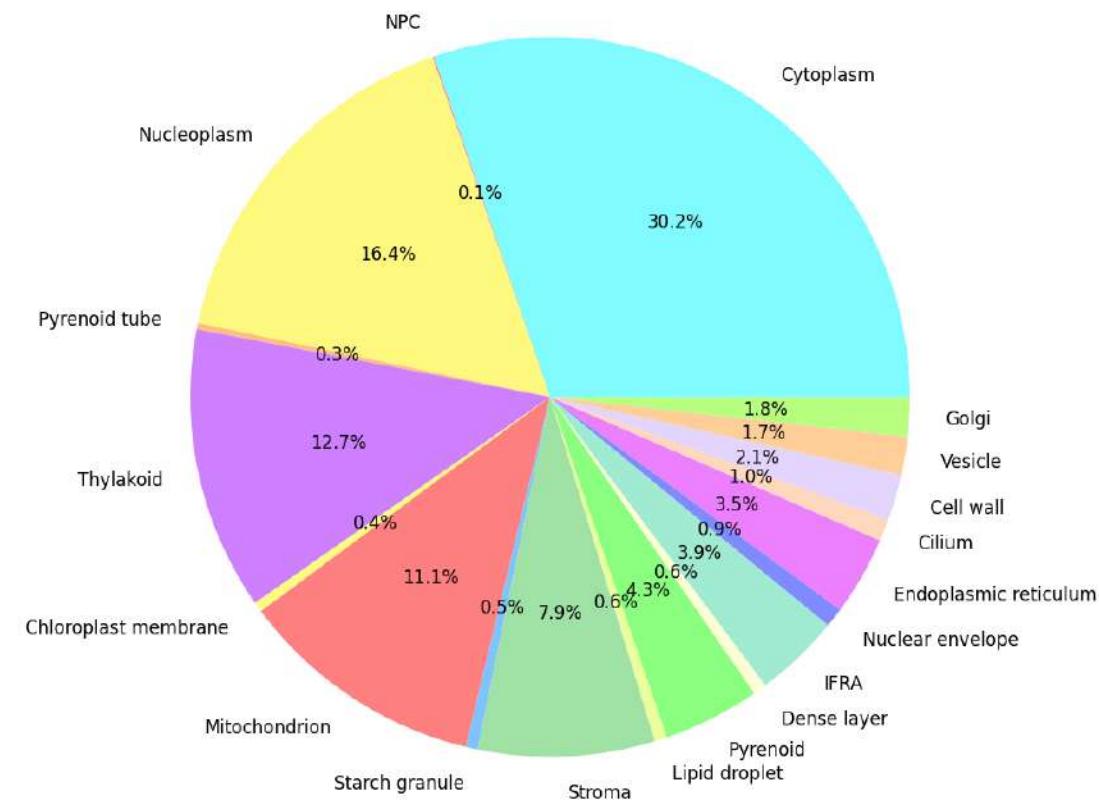
Search Tomogram by name

Filters

Tomogram	Thylakoid	Lipid ...	Vesicle	Golgi	Stroma	Pyren...	Endoplasm...	Pyrenoid	Cytoplasm	Mitoch...
01082023_BrnoKrios_Arcitis_WebUI_Position_11_bin2	0	0.1	1.6	0	0.1	0	9.1	0	2.5	
01082023_BrnoKrios_Arcitis_WebUI_Position_12_bin2	0	0	9.9	0.1	0	0	1.8	0	54	
01082023_BrnoKrios_Arcitis_WebUI_Position_13_bin2	0	0	1.6	0.2	0	0	1.7	0	10.7	
01082023_BrnoKrios_Arcitis_WebUI_Position_14_bin2	0	20.4	0.3	0	0	0	0.6	0	0.1	
01082023_BrnoKrios_Arcitis_WebUI_Position_15_bin2	21.2	11.7	0.3	0	7.3	0.1	0	0.1	5.2	
01082023_BrnoKrios_Arcitis_WebUI_Position_17_bin2	16.5	4.2	0	0.1	5	0	0.1	0.2	11	
01082023_BrnoKrios_Arcitis_WebUI_Position_19_bin2	0.1	0	0.1	0	0.1	0	1.9	0	11.1	
01082023_BrnoKrios_Arcitis_WebUI_Position_1_bin2	12.9	0.2	3	1.5	4.2	0	4.3	0	21.5	
01082023_BrnoKrios_Arcitis_WebUI_Position_20_bin2	42.7	0.1	0	0	14.7	0	0	0	14.3	
01082023_BrnoKrios_Arcitis_WebUI_Position_21_bin2	0	7.4	1.9	0.3	0.1	0	7	0	45.8	
01082023_BrnoKrios_Arcitis_WebUI_Position_22_bin2	27	0.9	0	0.1	12.8	0	0.3	0	9	
01082023_BrnoKrios_Arcitis_WebUI_Position_23_bin2	0	4.6	0.3	0	0.7	10	0.1	43.5	0.4	
01082023_BrnoKrios_Arcitis_WebUI_Position_24_bin2	26.5	2.6	0	0	19.4	0	0.1	0	11.6	
01082023_BrnoKrios_Arcitis_WebUI_Position_25_bin2	4.2	4.5	0.1	0.6	1	0	0.6	0	22.4	
01082023_BrnoKrios_Arcitis_WebUI_Position_26_bin2	0	3.6	0.6	0.7	0.1	0	1.7	0	16.1	
01082023_BrnoKrios_Arcitis_WebUI_Position_27_bin2	0	2.1	4.8	0	0	0	3.2	0	10.5	
01082023_BrnoKrios_Arcitis_WebUI_Position_28_bin2	0	3.1	0.1	0	0	0	0.9	0	0.3	
01082023_BrnoKrios_Arcitis_WebUI_Position_29_bin2	39.7	5.4	0.1	0	12.8	0	0.1	0	16.8	
01082023_BrnoKrios_Arcitis_WebUI_Position_2(bin2)	0.3	0.6	2.3	0	0.5	10.6	0.5	26.4	2.4	
01082023_BrnoKrios_Arcitis_WebUI_Position_3(bin2)	41.1	3.1	0.1	0.3	23.1	0	0.1	0	10.6	
01082023_BrnoKrios_Arcitis_WebUI_Position_35_bin2	0.4	9.2	0.2	0	0	0	4.4	0	30	
BrnoKrios_Arcitis_WebUI_Position_36_bin2	48.1	0.2	0	0.1	20.3	0	0	0	6.5	
Krios_Arcitis_WebUI_Position_39_bin2	0.1	0	0.1	0	0.1	0	5.4	0	9.3	
Krios_Arcitis_WebUI_Position_3(bin2)	19.6	0.2	0.1	0.6	7.4	0	0.1	0	16.6	
Krios_Arcitis_WebUI_Position_40_bin2	4	2.6	0.6	0	0.3	0	0.6	0	12.2	
Krios_Arcitis_WebUI_Position_42_bin2	23.7	0.3	0	0	12.2	0	0.4	0	26.7	
Total Volume (nm³)	77.4	1.6	0.1	0.7	16.7	0	0.3	0	19.7	



<https://cryopom.streamlit.app/>

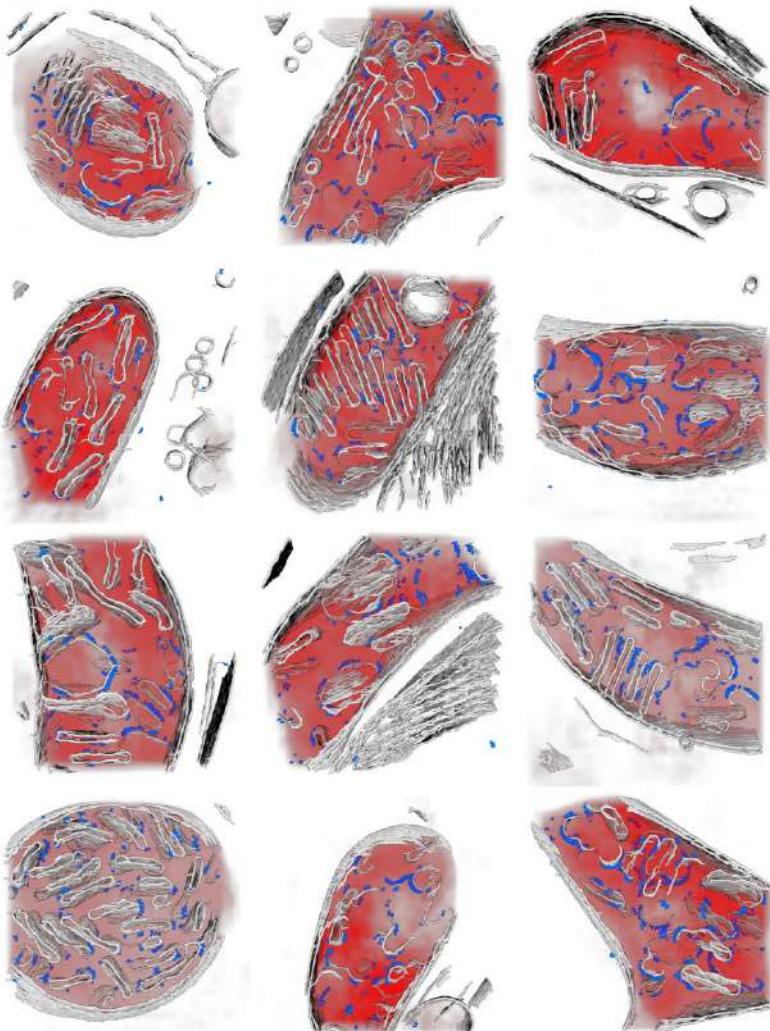


Last et al., bioRxiv (2025)

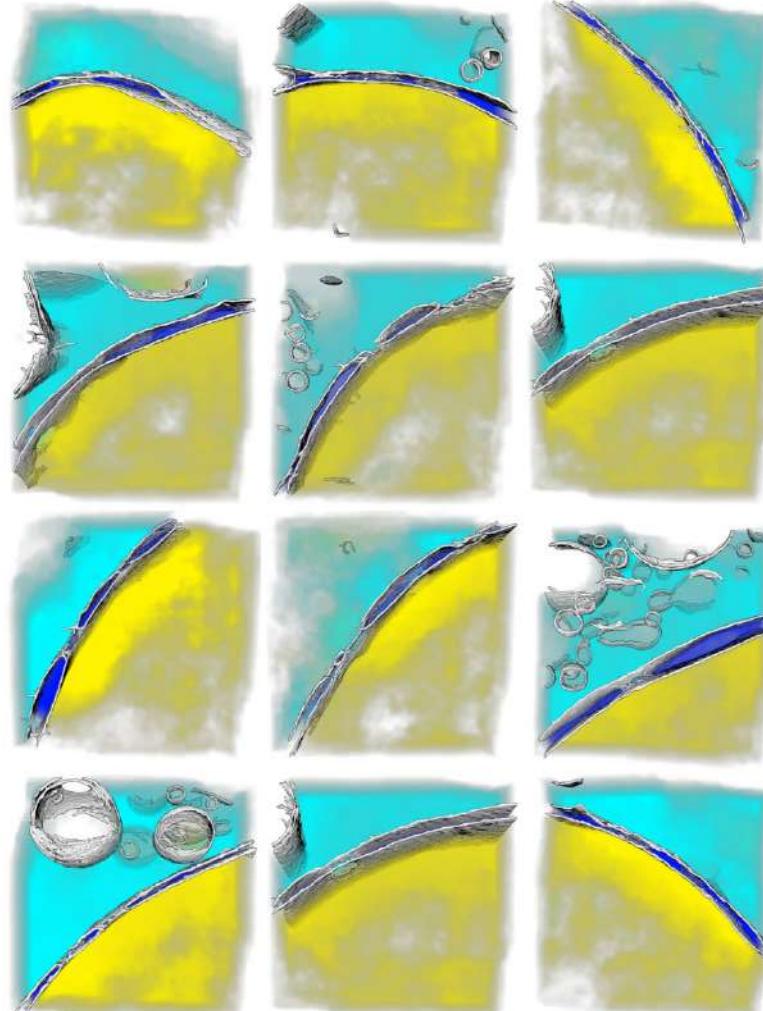
# cryoPom: automated annotation



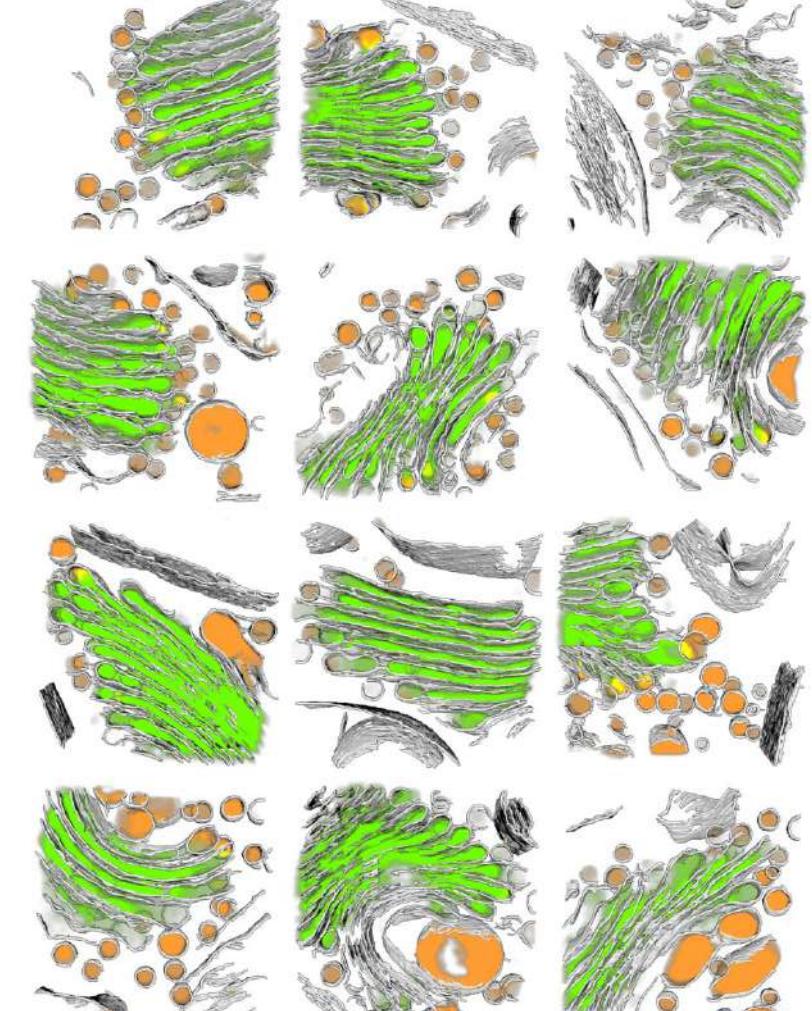
Example subset 1:  
>50 ATP synthase particles



Example subset 3:  
>10% cytoplasm and >10% nucleoplasm



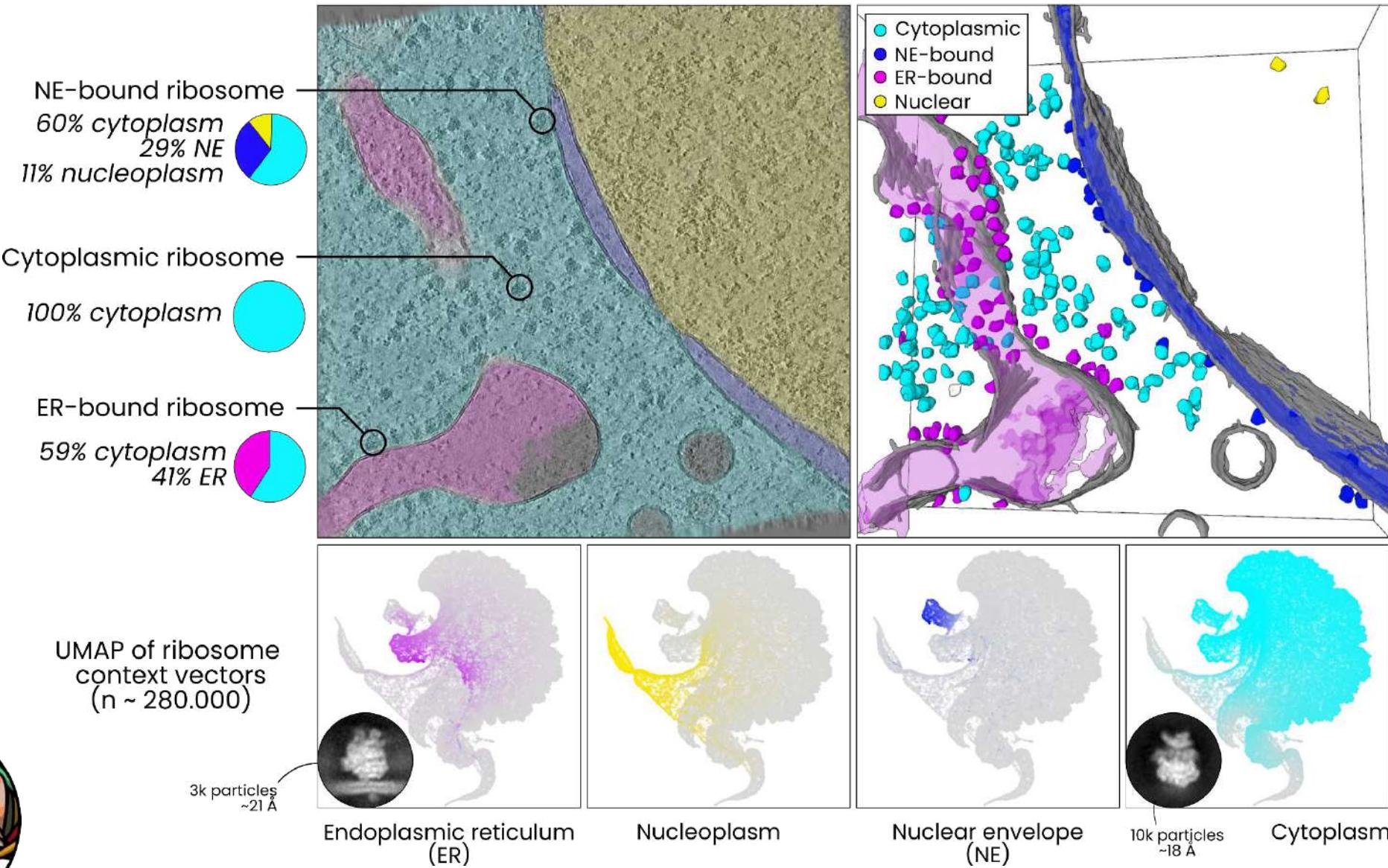
Example subset 2:  
>10% Golgi



# cryoPom: contextual search



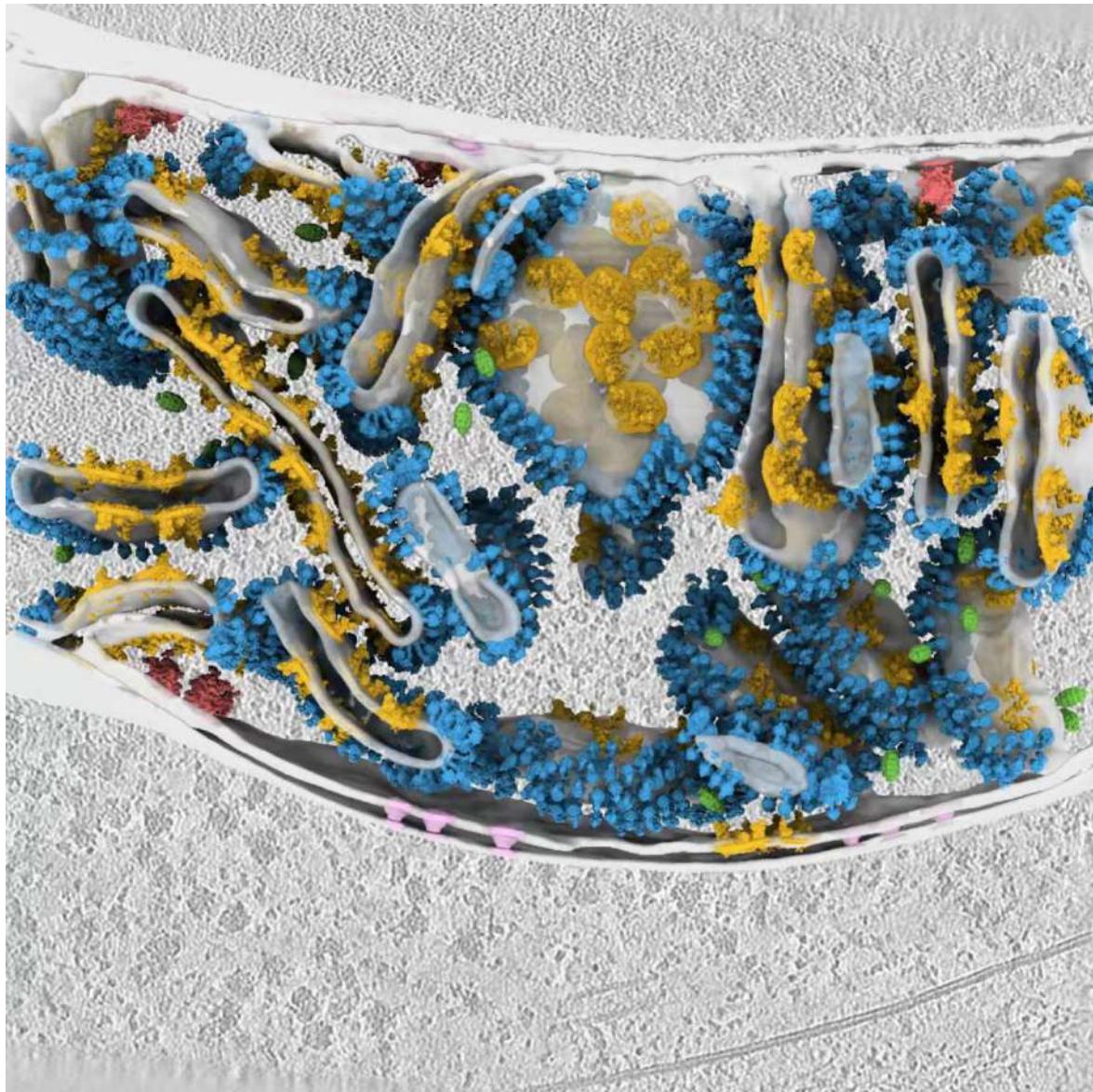
Mart Last



<https://cryopom.streamlit.app/>

Last et al., bioRxiv (2025)

# Building a molecular atlas of mitochondria



**Tomorrow!**

**S13O4**



*Poster  
S13P5*

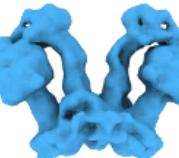
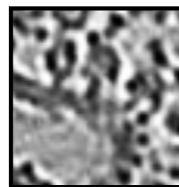
Maria  
Melnik



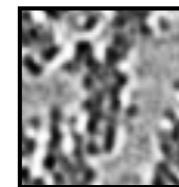
Florent  
Waltz

**Native Complexes**

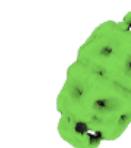
ATP  
Synthase



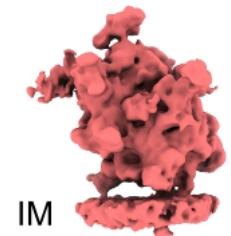
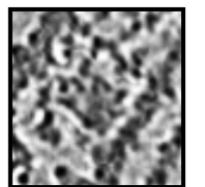
Respiratory  
Complexes



Chaperone  
HSP60



Mitochondrial  
Ribosome



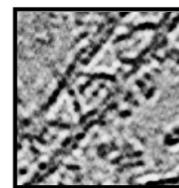
IM

OM  
IM

*Unknown complexes*



'Lightbulb'



'Volcano'



(probably Prohibitin)

# Summary

- The Chlamy dataset is the result of a large team effort towards *visual proteomics* by cryo-ET
- 1,829 tomos are available to the community, *from raw data to annotations*
- Resources for learning & working with the data are available
- High-resolution “known” structures were solved: Ribosomes, Rubisco, Nucleosome, Microtubules, PSII, Clathrin, ATP synthase...

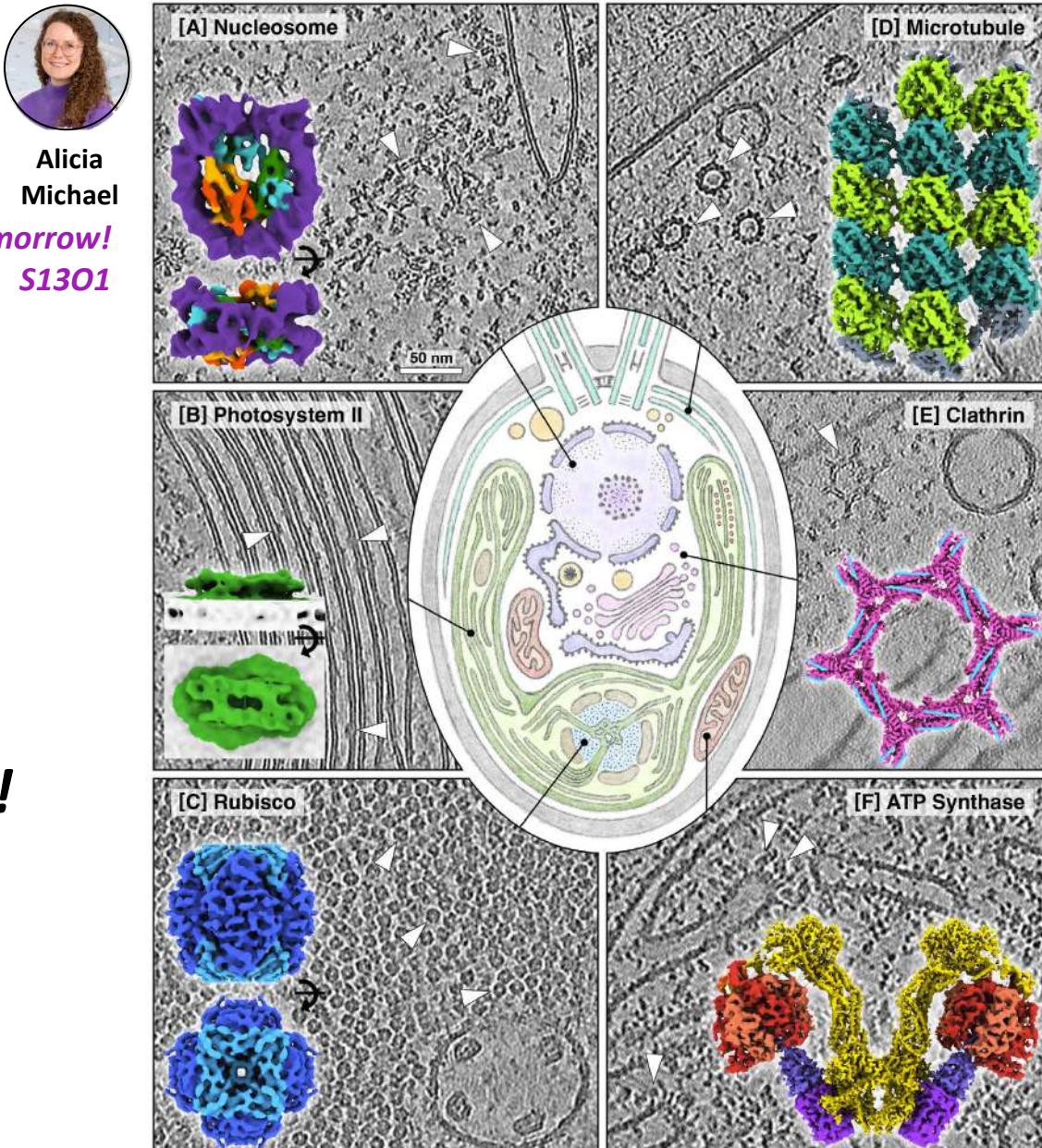
*...but many more are to be discovered!*

**Structural biology must be done inside the cell!**



Kelley, Khavnekar, Righetto *et al.*, bioRxiv (2024)

<https://github.com/Chromatin-Structure-Rhythms-Lab/ChlamyAnnotations>



# Thank you!

**Ben Engel**  
Florent Waltz  
Wojciech Wietrzynski  
Sofie van Dorst  
Philippe Van der Stappen  
Caitie McCafferty  
Lorenz Lamm  
Simon Zufferey  
Hugo van den Hoek  
Manon Demulder  
Annemarie Perez  
Fanny LeBlanc  
Davide Tamborrini  
Adrià Nogales  
Daniil Litvinov  
Maria Melnik  
Jacquot Schiltz

**Abhay Kotecha**  
Ron Kelley  
Martin Obr  
Xianjun Zheng  
Jess Heebner  
Saikat Chakraborty  
**Jürgen Plitzko**  
Sagar Khavnekar  
**John Briggs**  
Grigory Tagiltsev  
**Alicia Michael**  
Pavol Harar  
**Will Wan**

*Slides*

Ben Engel  
Florent Waltz  
Mart Last



@lifeonthewedge.bsky.social

**BIOZENTRUM**

The Center for  
Molecular Life Sciences



ricardo.righetto@unibas.ch



**max planck institute  
of biochemistry**