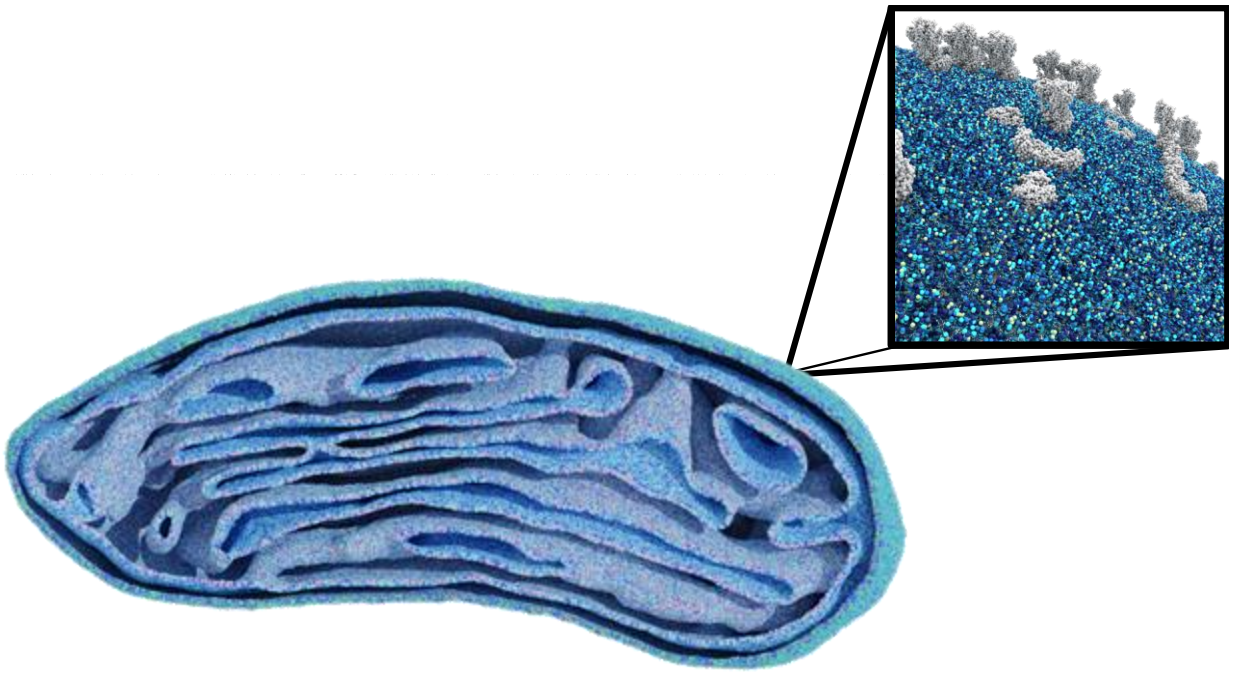
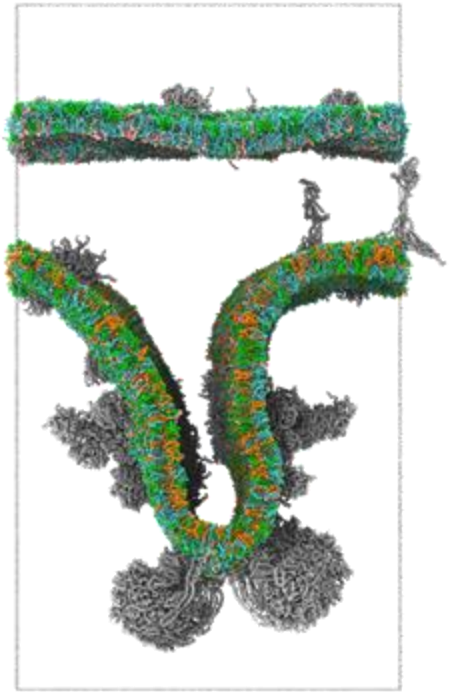
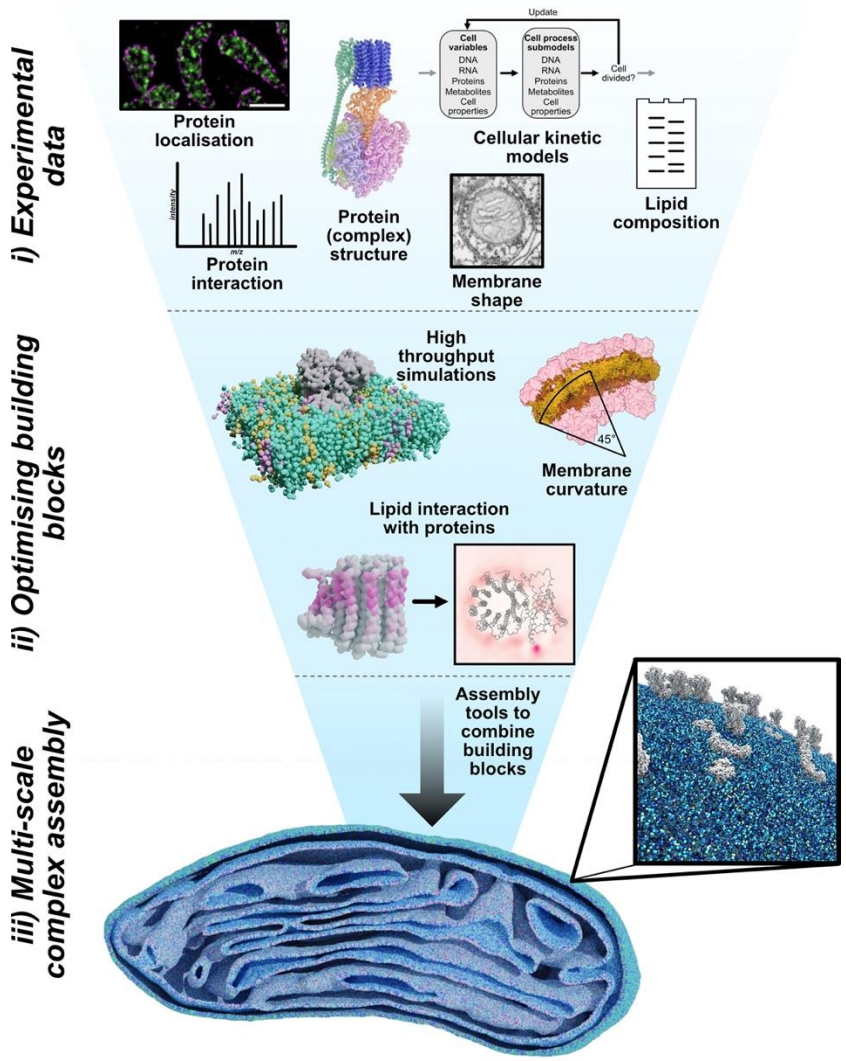
university of groningen





Using integrative modelling to build a representative membrane

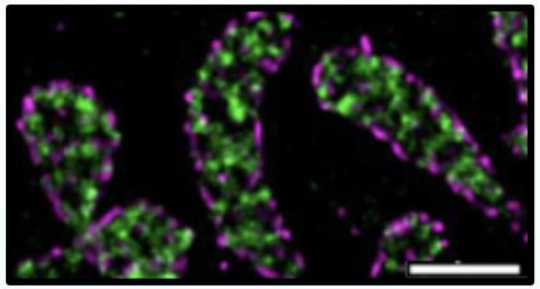
Dr. Chelsea M. Brown Marrink research group Martini Workshop



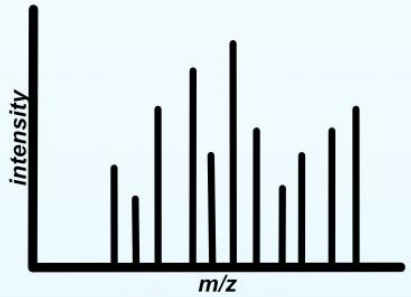
What is integrative modelling?

Brown, Chelsea M., and Siewert J. Marrink. "Modeling membranes in situ." *Current Opinion in Structural Biology* 87 (2024): 102837.

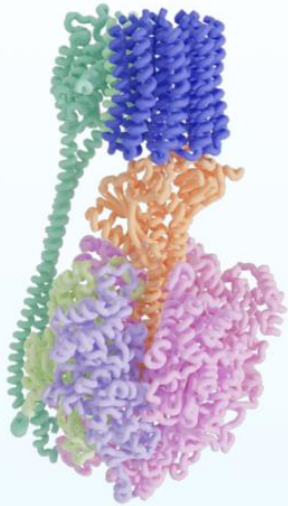
i) Experimental data



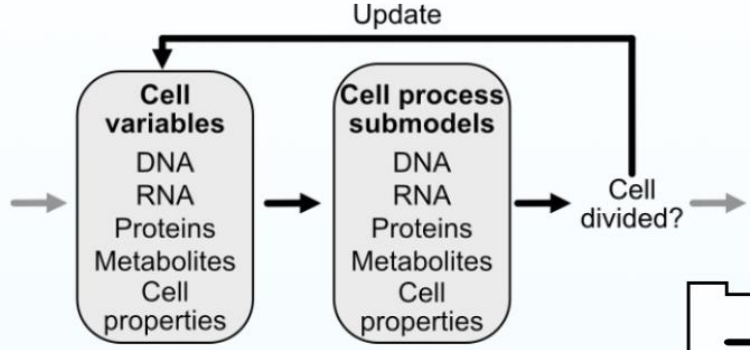
Protein localisation



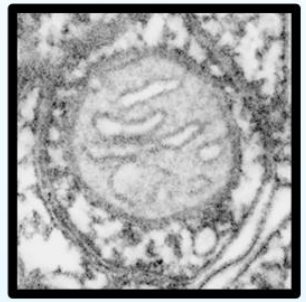
Protein interaction



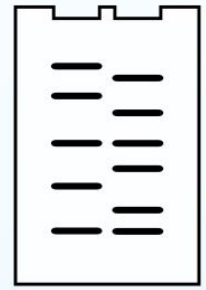
Protein (complex) structure



Cellular kinetic models

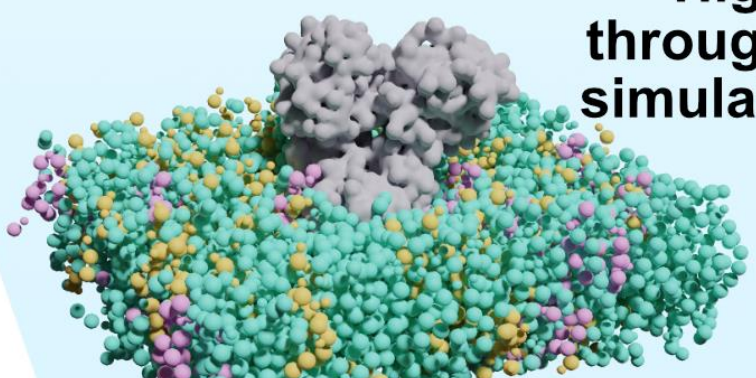


Membrane shape

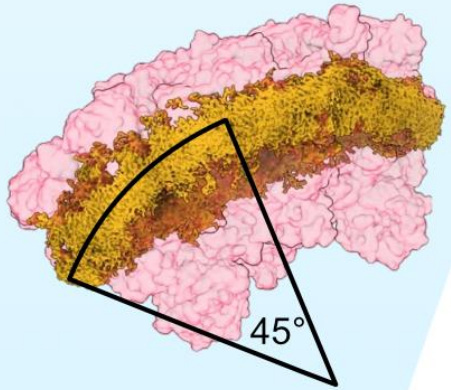


Lipid composition

ii) Model building



High throughput simulations

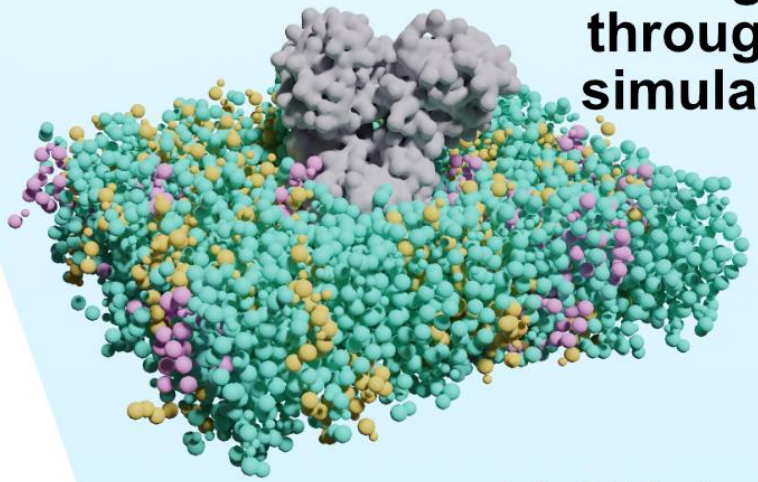


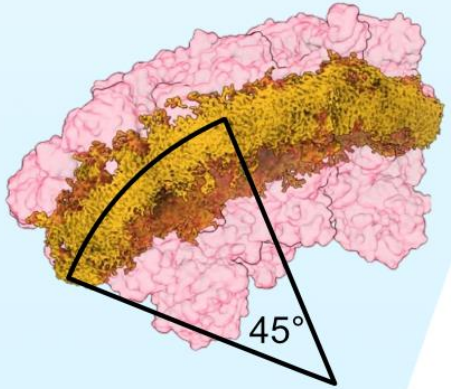
Protein interaction

Membrane shape

ii) Optimising building blocks

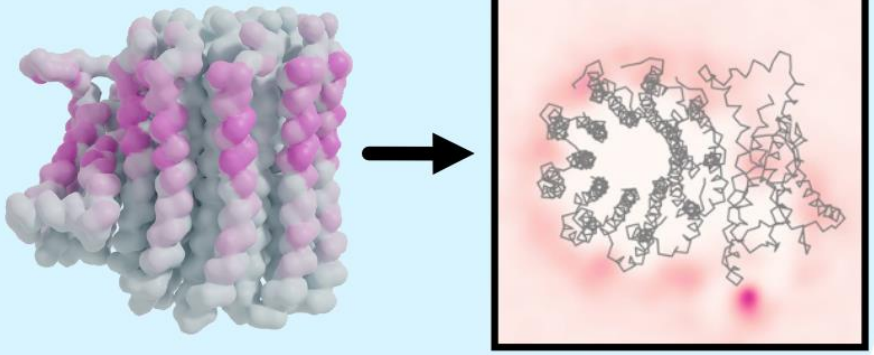
High throughput simulations





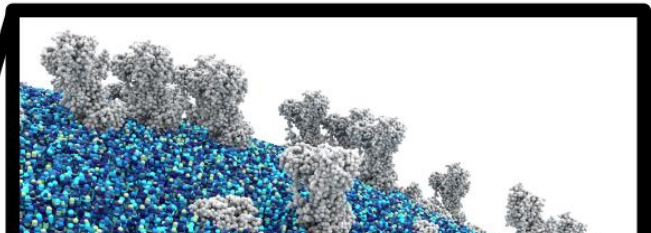
Membrane curvature

Lipid interaction with proteins



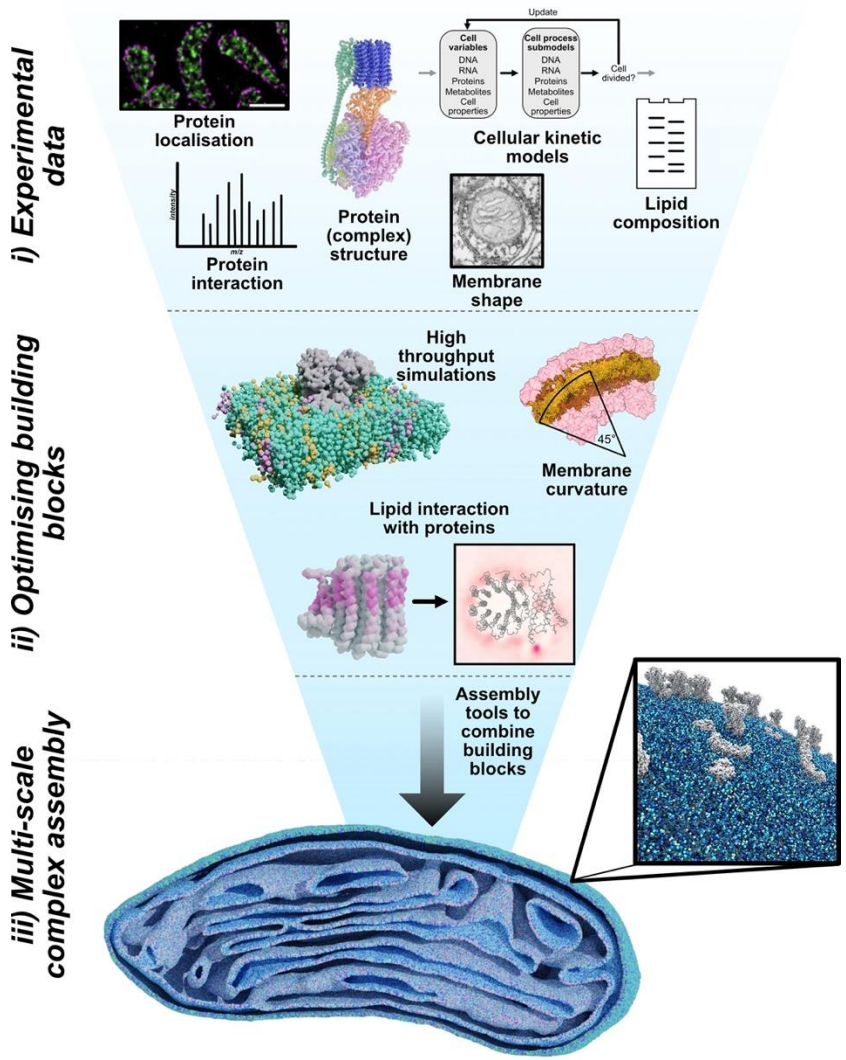
iii) Assembly

Assembly tools to combine



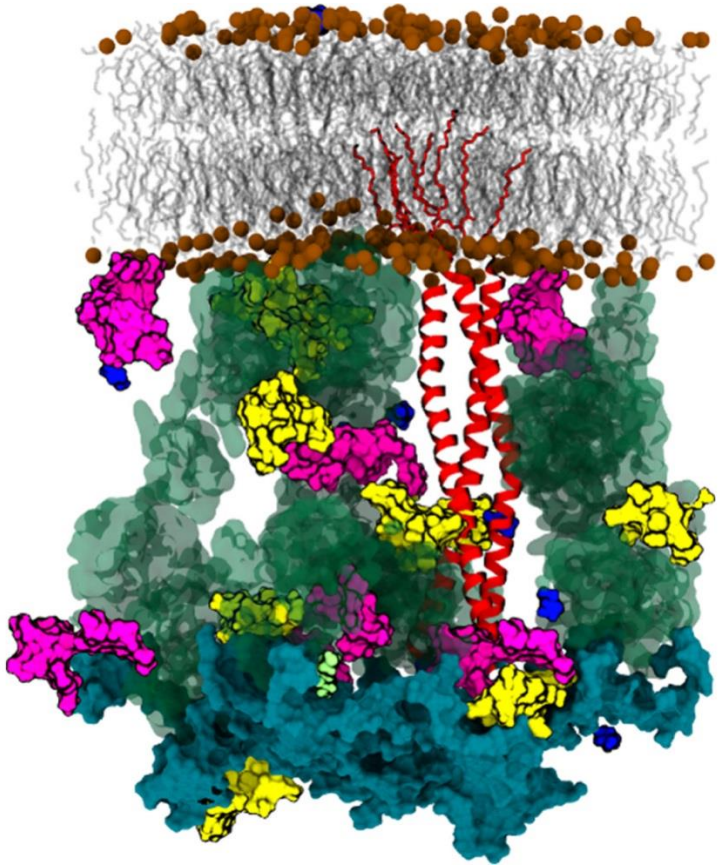
iii) Multi-scale complex assembly

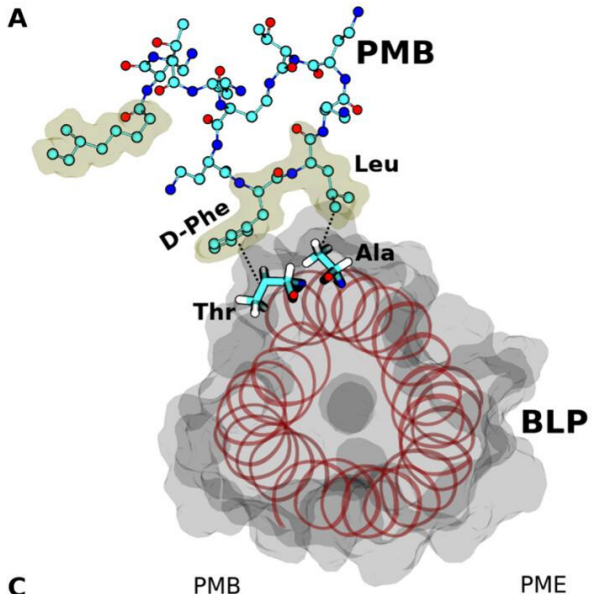
Assembly tools to combine building blocks

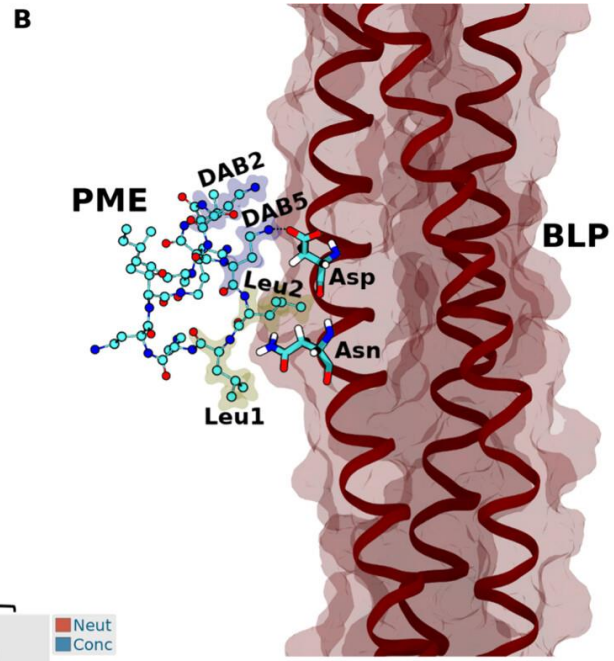


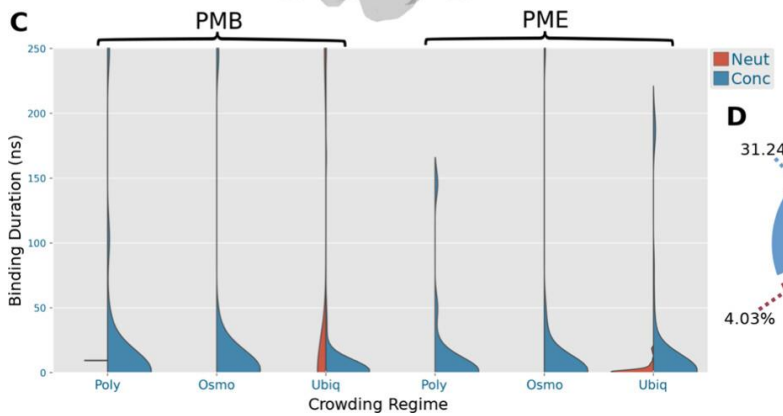
Why is it important to get these things right?

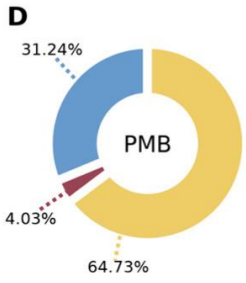
Behaviour changes with different system components/set-ups

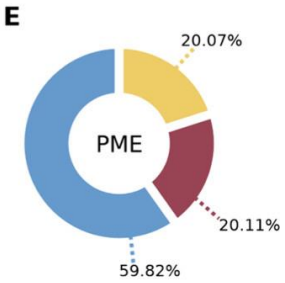






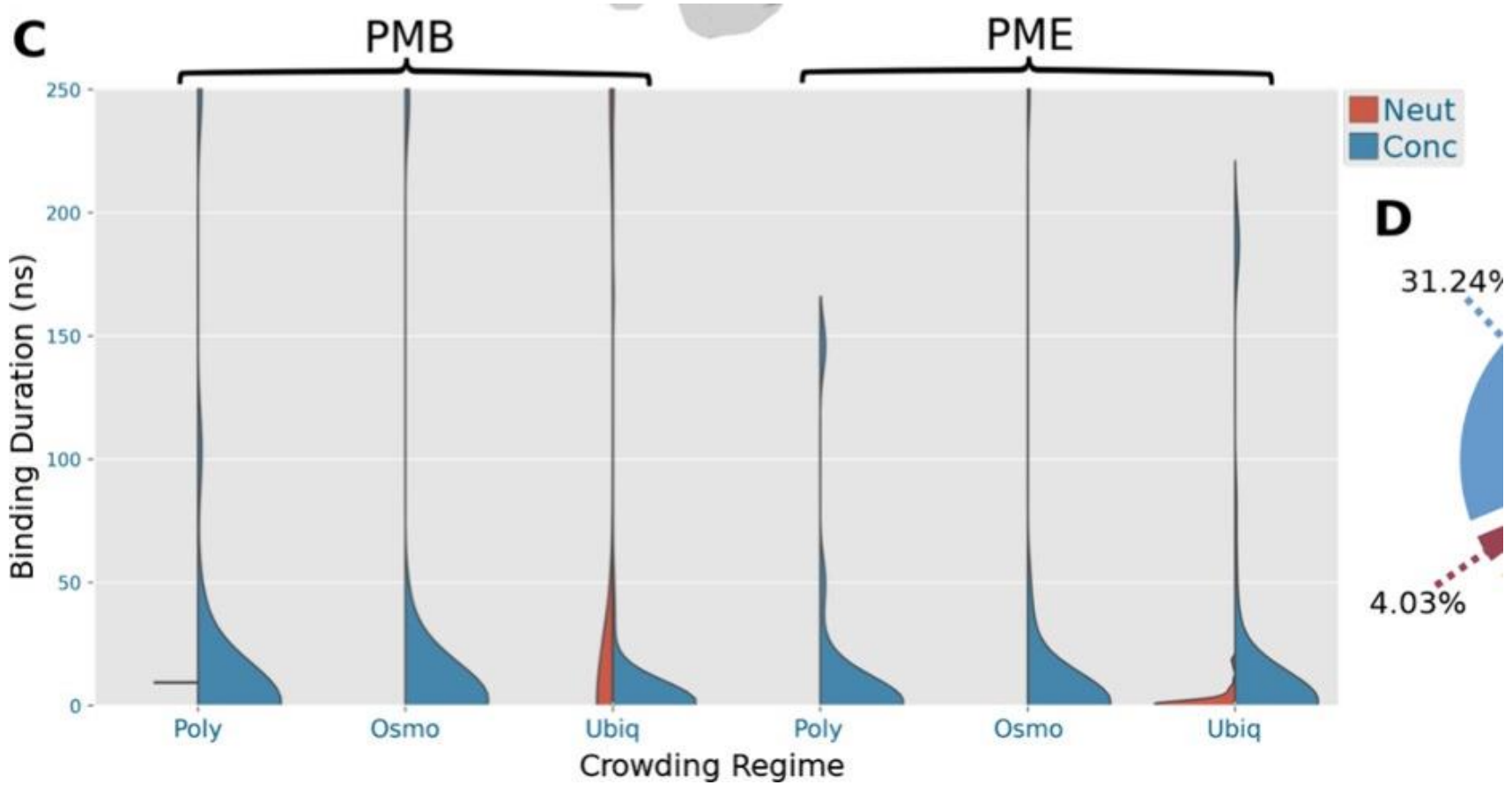






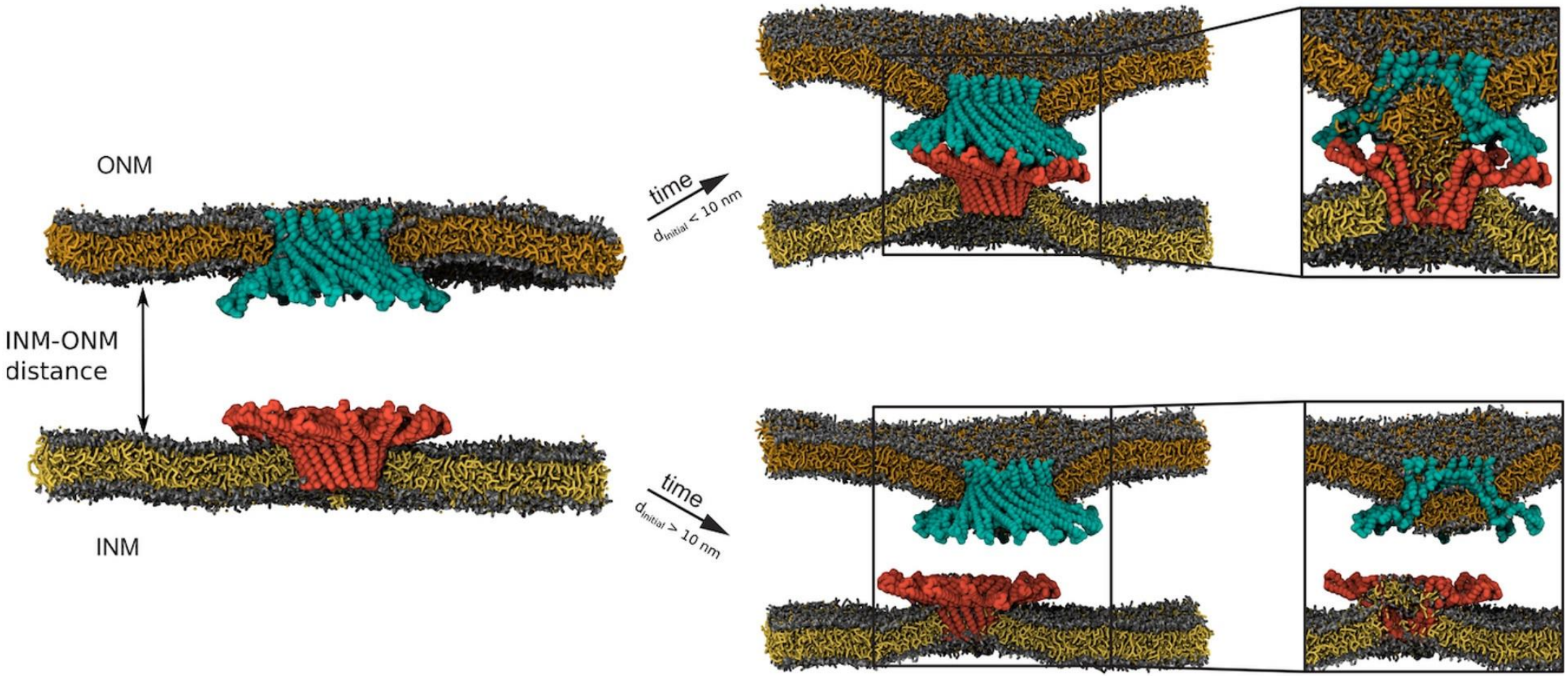
Smith, I. P., Pedebos, C., & Khalid, S. (2024). Molecular crowding alters the interactions of polymyxin lipopeptides within the periplasm of E. coli: insights from molecular dynamics. *The Journal of Physical Chemistry B*, *128*(11), 2717-2733.

Behaviour changes with different system components/set-ups

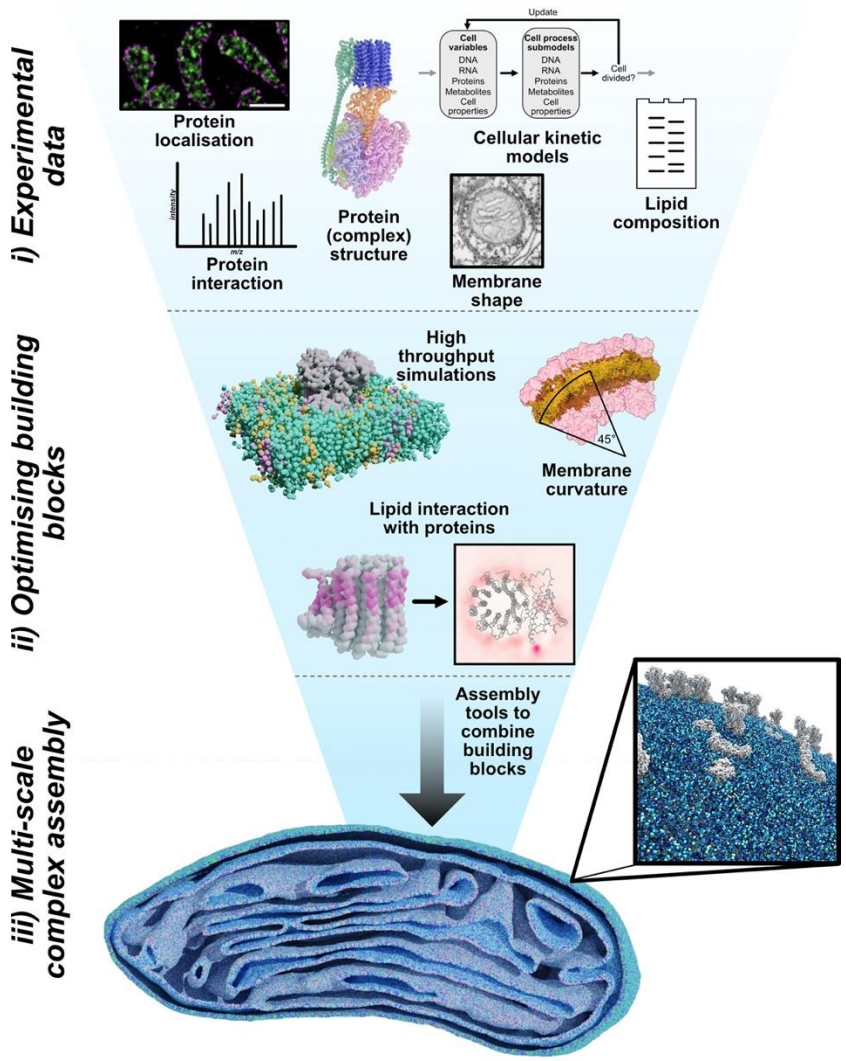


Smith, I. P., Pedebos, C., & Khalid, S. (2024). Molecular crowding alters the interactions of polymyxin lipopeptides within the periplasm of E. coli: insights from molecular dynamics. *The Journal of Physical Chemistry B*, *128*(11), 2717-2733.

Behaviour changes with different system components/set-ups



Fischer, J. S., Wojtynek, M., Kumar, A., Baird, H., Radilova, K., Maslennikova, D., ... & Weis, K. (2025). A conserved mechanism of membrane fusion in nuclear pore complex assembly. *bioRxiv*, 2025-07.



How do you know what is in your system?

Brown, Chelsea M., and Siewert J. Marrink. "Modeling membranes in situ." *Current Opinion in Structural Biology* 87 (2024): 102837.

Lipids

Contents lists available at ScienceDirect

Progress in Lipid Research

journal homepage: www.elsevier.com/locate/plipres

Review

Lipids of mitochondria

Susanne E. Horvath [1], Günther Daum *

Institute of Biochemistry, Graz University of Technology, Petersgasse 12/2, A-8010 Graz, Austria

nature communications

Article

https://doi.org/10.1038/s41467-024-53975-y

A tuneable minimal cell membrane reveals that two lipid species suffice for life

Received: 17 December 2023

Accepted: 25 October 2024

Published online: 08 November 2024

Check for updates

Isaac Justice [1], Petra Kiesel [2], Nataliya Safronova [1], Alexander von Appen [2] & James P. Saenz [1,3]

All cells are encapsulated by a lipid membrane that facilitates their interactions with the environment. How cells manage diverse mixtures of lipids, which dictate membrane property and function, is experimentally challenging to address. Here, we present an approach to tune and minimize membrane lipid composition in the bacterium *Mycoplasma mycoides* and its derived 'minimal cell' (JCVI-Syn3A), revealing that a two-component lipidome can support life. Systematic reintroduction of phospholipids with different features demonstrates that acyl chain diversity is more important for growth than head group diversity. By tuning lipid chirality, we explore the lipid divide between Archaea and the rest of life, showing that ancestral lipidomes could have been heterochiral. However, in these simple organisms, heterochirality leads to impaired cellular fitness. Thus, our approach offers a tunable minimal membrane system to explore the fundamental lipidomic requirements for life, thereby extending the concept of minimal life from the genome to the lipidome.

PNAS

Mycobacterial outer membrane is a lipid bilayer and the inner membrane is unusually rich in diacyl phosphatidylinositol dimannosides

Ritu Bansal-Mutalik and Hiroshi Nikaido[1]

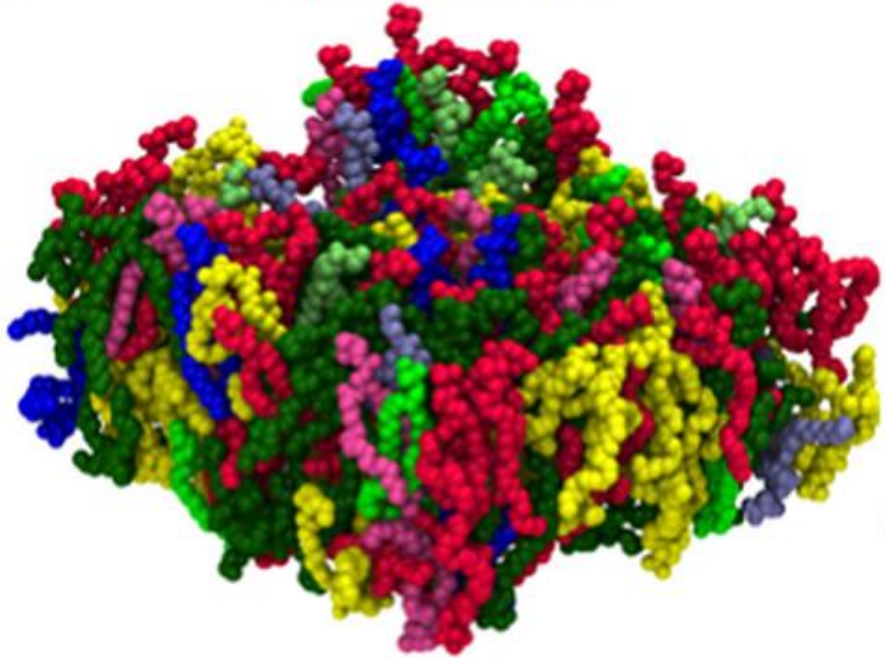
Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720

Contributed by Hiroshi Nikaido, February 18, 2014 (sent for review January 13, 2014)

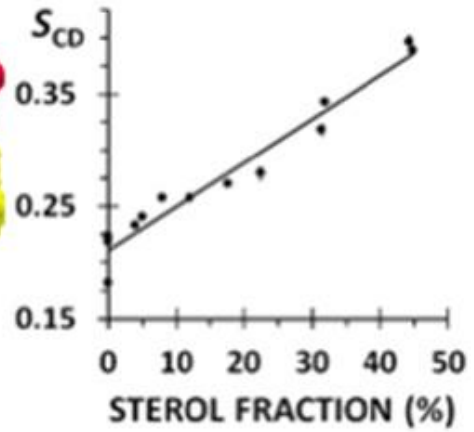
Lipids

CHARMM-GUI *Membrane Builder* → MD simulations → Comparative analysis

Native-like
18 Biomembranes:
14 eukaryotic
1 archaeal
3 bacterial



D_{P-P}, APL, Θ, S_{CD}, K_A
Correlations:



Pogozheva, I. D., Armstrong, G. A., Kong, L., Hartnagel, T. J., Carpino, C. A., Gee, S. E., ... & Im, W. (2022). Comparative molecular dynamics simulation studies of realistic eukaryotic, prokaryotic, and archaeal membranes. *Journal of chemical information and modeling*, *62*(4), 1036-1051.

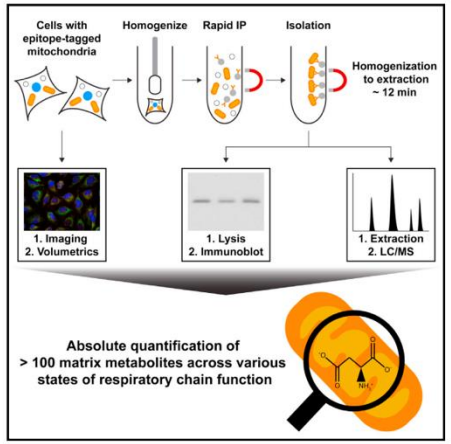
Metabolites

Resource

Cell

Absolute Quantification of Matrix Metabolites Reveals the Dynamics of Mitochondrial Metabolism

Graphical Abstract



Authors

Walter W. Chen, Elizaveta Freinkman, Tim Wang, Kıvanç Birsoy, David M. Sabatini

Correspondence

sabatini@wi.mit.edu

In Brief

Metabolite profiling of intact mammalian mitochondria captures dynamics of mitochondrial metabolism not revealed by whole-cell analysis.

Highlights

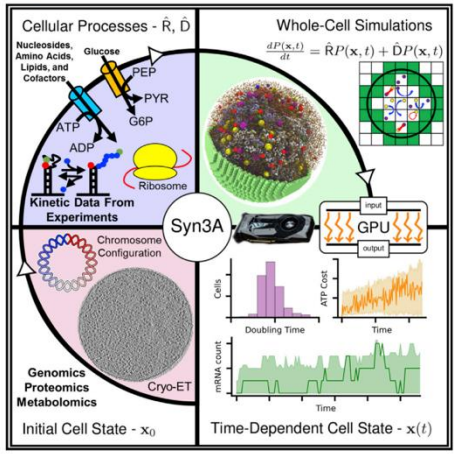
- A workflow for absolute quantification of mitochondrial matrix metabolites
- Rapid and specific isolation of mitochondria from cells for metabolite profiling
- Profiling guided by MITObolome, a set of all predicted mitochondrial metabolites
- Dynamics of mitochondrial metabolism revealed by quantification of >100 metabolites

Article

Cell

Fundamental behaviors emerge from simulations of a living minimal cell

Graphical abstract



Authors

Zane R. Thornburg, David M. Bianchi, Troy A. Brier, ..., Hamilton O. Smith, John I. Glass, Zaida Luthey-Schulten

Correspondence

zan@illinois.edu

In brief

A kinetic model for a minimal bacterial cell offers quantitative insight into how the cell balances processes from metabolism to gene expression to growth.

Highlights

- 3D spatial resolution of a fully dynamical whole-cell kinetic model
- Detailed single-reaction, single-cell accounting of time-dependent ATP costs
- Genome-wide mRNA half-lives emerge from length-dependent kinetics and diffusion
- Connections among metabolism, genetic information, and cell growth are revealed

ARTICLES

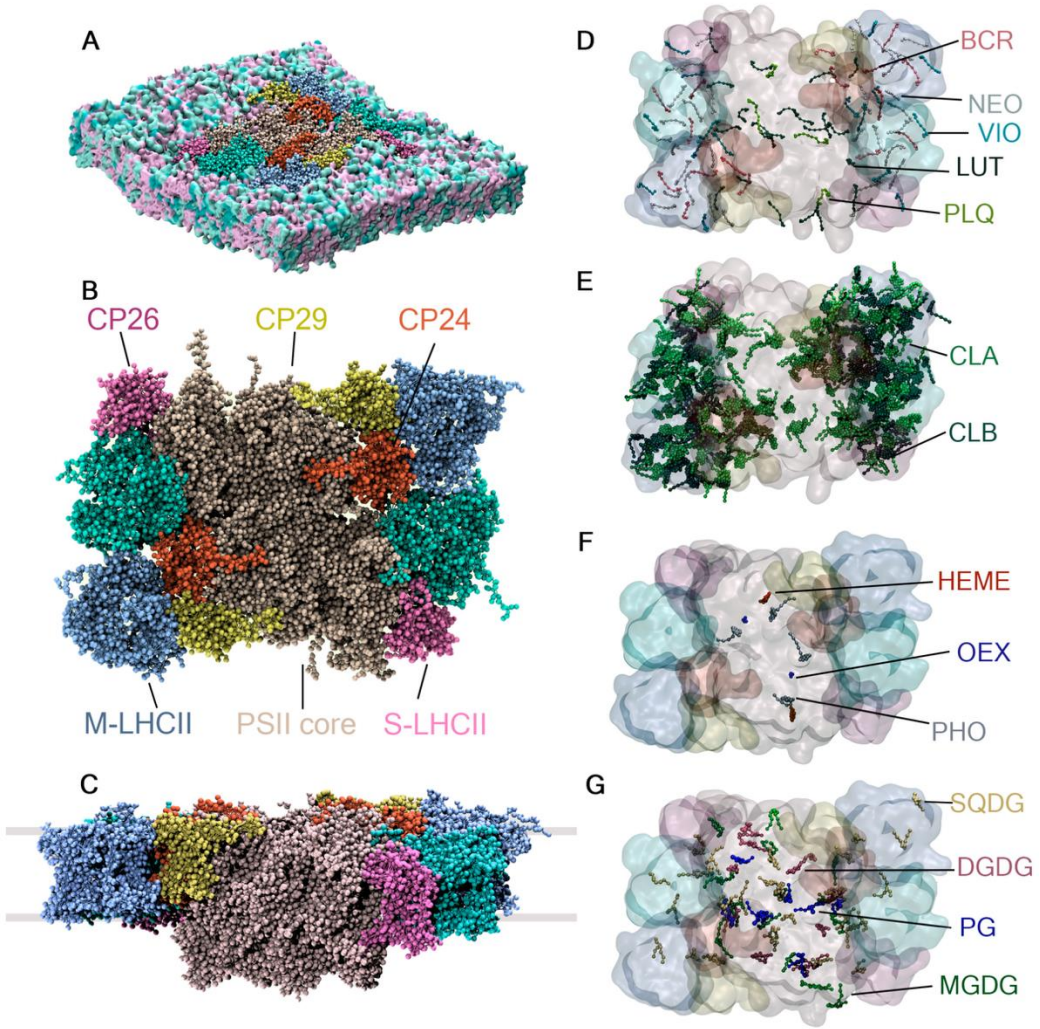
nature chemical biology

Absolute metabolite concentrations and implied enzyme active site occupancy in *Escherichia coli*

Bryson D Bennett[1], Elizabeth H Kimball[1], Melissa Gao[1], Robin Osterhout[2], Stephen J Van Dien[2] & Joshua D Rabinowitz[1]

Absolute metabolite concentrations are critical to a quantitative understanding of cellular metabolism, as concentrations impact both the free energies and rates of metabolic reactions. Here we use LC-MS/MS to quantify more than 100 metabolite concentrations in aerobic, exponentially growing *Escherichia coli* with glucose, glycerol or acetate as the carbon source. The total observed intracellular metabolite pool was approximately 300 mM. A small number of metabolites dominate the metabolome on a molar basis, with glutamate being the most abundant. Metabolite concentration exceeds K_m for most substrate-enzyme pairs. An exception is lower glycolysis, where concentrations of intermediates are near the K_m of their consuming enzymes and all reactions are near equilibrium. This may facilitate efficient flux reversibility given thermodynamic and osmotic constraints. The data and analyses presented here highlight the ability to identify organizing metabolic principles from systems-level absolute metabolite concentration data.

Metabolites



Chiariello, M. G., Zarmiento-Garcia, R., & Marrink, S. J. (2024). Martini 3 coarse-grained model for the cofactors involved in photosynthesis. *International Journal of Molecular Sciences*, *25*(14), 7947.

Proteins

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MitoCarta3.0: An Inventory of Mammalian Mitochondrial Proteins and Pathways

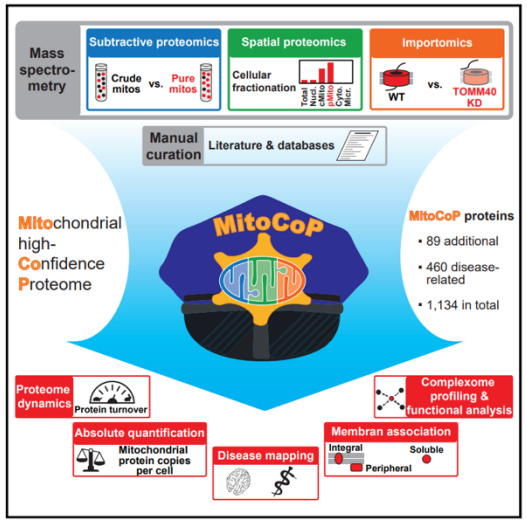
MitoCarta3.0 is an inventory of 1136 human and 1140 mouse genes encoding proteins with strong support of mitochondrial localization, now with sub-mitochondrial compartment and pathway annotations. To generate this inventory, we performed mass spectrometry of mitochondria isolated from fourteen tissues, assessed protein localization through large-scale GFP tagging/microscopy, and integrated these results with six other genome-scale datasets of mitochondrial localization, using a Bayesian approach. MitoCarta3.0, released 2020, uses manual literature curation to revise the previous MitoCarta2.0 inventory (78 added and 100 removed genes), provide annotation of sub-mitochondrial localization, and assign genes to a custom ontology of 149 mitochondrial pathways.

Cell Metabolism

Resource

Quantitative high-confidence human mitochondrial proteome and its dynamics in cellular context

Graphical abstract



Authors

Marcel Morgenstern, Christian D. Peikert, Philipp Lübbert, ..., Nikolaus Pfanner, Nils Wiedemann, Bettina Warscheid

Correspondence

nils.wiedemann@biochemie.uni-freiburg.de (N.W.), bettina.warscheid@biologie.uni-freiburg.de (B.W.)

In brief

Mitochondria are crucial for cellular energy metabolism and human health. Morgenstern et al. present a high-confidence protein compendium of human mitochondria including mitochondria-specific protein copy numbers and half-lives. They identify interactors of key mitochondrial protein machineries and link >40% of the mitochondrial proteome to human diseases.

Highlights

- Human mitochondrial high-confidence proteome with >1,100 proteins (MitoCoP)
- Mitochondria-specific protein copy numbers and half-lives

Proteins

RESOURCE

nature biotechnology

The quantitative and condition-dependent *Escherichia coli* proteome

Alexander Schmidt[1], Karl Kochanowski[2], Silke Vedelaar[3], Erik Ahrné[1], Benjamin Volkmer[2], Luciano Callipo[2], Kèvin Knoops[4], Manuel Bauer[1], Ruedi Aebersold[2,5] & Matthias Heinemann[2,3]

Measuring precise concentrations of proteins can provide insights into biological processes. Here we use efficient protein extraction and sample fractionation, as well as state-of-the-art quantitative mass spectrometry techniques to generate a comprehensive, condition-dependent protein-abundance map for *Escherichia coli*. We measure cellular protein concentrations for 55% of predicted *E. coli* genes (>2,300 proteins) under 22 different experimental conditions and identify methylation and N-terminal protein acetylations previously not known to be prevalent in bacteria. We uncover system-wide proteome allocation, expression regulation and post-translational adaptations. These data provide a valuable resource for the systems biology and broader *E. coli* research communities.

Proteins

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Status

Reviewed (Swiss-Prot) (4,191)

Unreviewed (TrEMBL) (4,368)

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B. subtilis (8,559)

Taxonomy

224308 ✖

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Gene Ontology

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Proteins with

3D structure (754)

Active site (1,029)

UniProtKB 8,559 results or restrict to reference proteome UP000001570

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Entry	Entry Name	Protein Names	Gene Names	Organism	Length
C0SPC1	CCRZ_BACSU	**Cell cycle regulator CcrZ**[...]	**ccrZ**, ytmP, BSU29920	Bacillus subtilis (strain 168)	269 AA
O05512	MANB_BACSU	**Mannan endo-1,4-beta-mannosidase**[...]	**gmuG**, ydhT, BSU05880	Bacillus subtilis (strain 168)	362 AA
O06724	YISK_BACSU	**Oxaloacetate tautomerase YisK**[...]	**yisK**, BSU10750	Bacillus subtilis (strain 168)	301 AA
O08394	CYPD_BACSU	**Bifunctional cytochrome P450/NADPH--P450 reductase 1**[...]	**cypD**, cyp102A2, yetO, yfnJ, BSU07250	Bacillus subtilis (strain 168)	1,061 AA
O31616	GLYOX_BACSU	**Glycine oxidase**[...]	**thiO**, goxB, yjbR, BSU11670	Bacillus subtilis (strain 168)	369 AA
O31644	MANR_BACSU	**Transcriptional regulator ManR**[...]	**manR**, BSU12000	Bacillus subtilis (strain 168)	648 AA
O31645	PTN3B_BACSU	**PTS system mannose-specific EIIBCA component**[...]	**manP**, yjdD, BSU12010	Bacillus subtilis (strain 168)	650 AA
O31691	GLCT_BACSU	**PtsGHI operon antiterminator**[...]	**glcT**, ykwA, BSU13880	Bacillus subtilis (strain 168)	281 AA

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Help

Proteins

UniProt BLAST Align Peptide search ID mapping SPARQL UniProtKB organism_id:224308 Advanced | List Search Help

Motif (5)
Mutagenesis (49)
Natural variant (3)
Pathway (70)
Propeptide (4)
PTM comments (24)
Region (151)
Repeat (2)
Signal peptide (44)
Subcellular location (1,599)
Subunit structure (198)
Topological domain (125)
Transmembrane (1,992) ✖
Turn (41)
Fewer items

Protein existence
Predicted (829)
Homology (783)
Protein level (260)

UniProtKB 1,992 results

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Entry	Entry Name	Protein Names	Gene Names	Organism	Length
O31645	PTN3B_BACSU	**PTS system mannose-specific EIIBCA component** [...]	**manP**, yjdD, BSU12010	Bacillus subtilis (strain 168)	650 AA
O34525	SPPA_BACSU	**Putative signal peptide peptidase SppA**[...]	**sppA**, yteI, BSU29530	Bacillus subtilis (strain 168)	335 AA
O34952	LTAS2_BACSU	**Lipoteichoic acid synthase 2**[...]	**ltaS2**, yflE, BSU07710	Bacillus subtilis (strain 168)	649 AA
P13801	SP2G_BACSU	**Sporulation sigma-E factor-processing peptidase** [...]	**spoIIGA**, BSU15310	Bacillus subtilis (strain 168)	309 AA
P20166	PTG3C_BACSU	**PTS system glucose-specific EIICBA component** [...]	**ptsG**, crr, ptsX, BSU13890	Bacillus subtilis (strain 168)	699 AA
P26937	SP4FB_BACSU	**Stage IV sporulation protein FB**[...]	**spoIVFB**, bofB, BSU27970	Bacillus subtilis (strain 168)	288 AA
P28628	LEPS_BACSU	**Signal peptidase I S**[...]	**sipS**, BSU23310	Bacillus subtilis (strain 168)	184 AA
P39215	MCPB_BACSU	**Methyl-accepting chemotaxis protein McpB**[...]	**mcpB**, BSU31260	Bacillus subtilis (strain 168)	662 AA

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Proteins

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Toward the Complete Functional Characterization of a Minimal Bacterial Proteome

Published as part of The Journal of Physical Chemistry virtual special issue "Jose Onuchic Festschrift".

David M. Bianchi, James F. Pelletier, Clyde A. Hutchison, III, John I. Glass, and Zaida Luthey-Schulten*

Cite This: *J. Phys. Chem. B* 2022, 126, 6820–6834 Read Online

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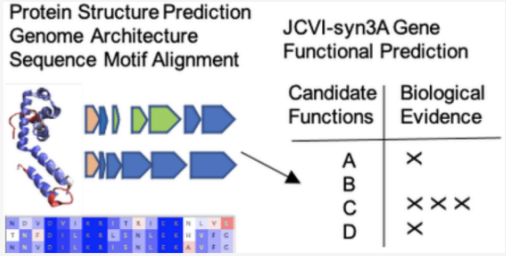
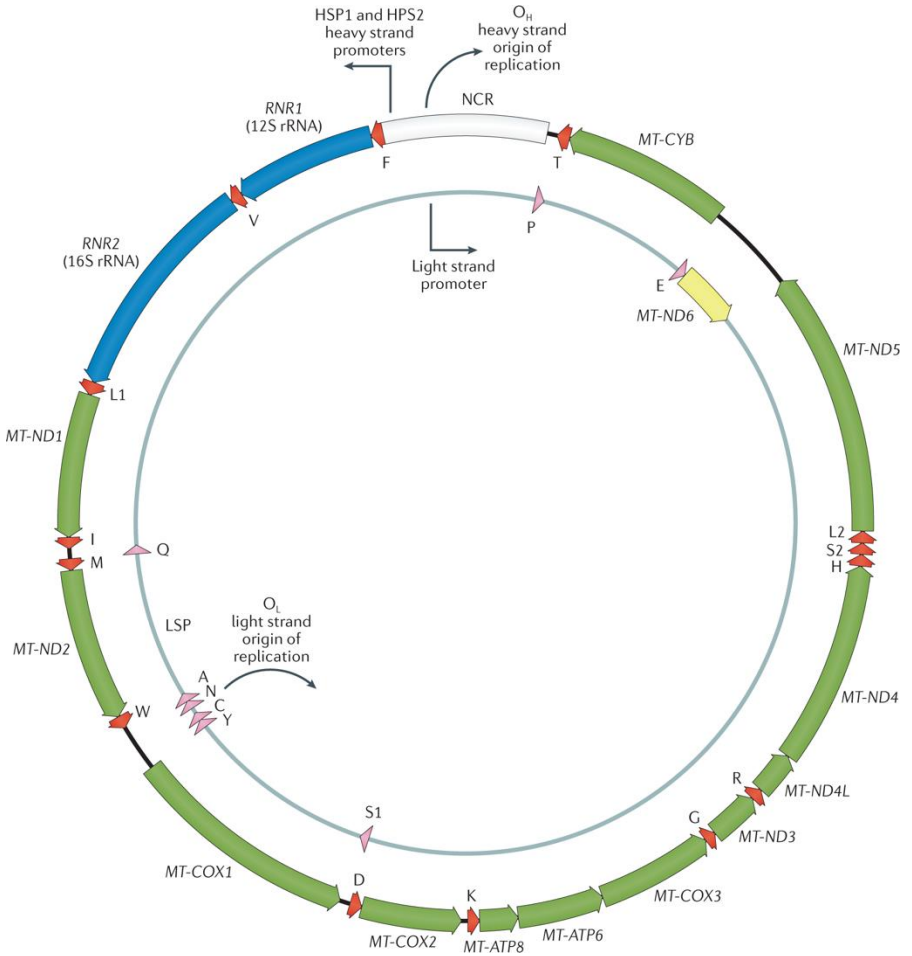
ABSTRACT: Recently, we presented a whole-cell kinetic model of the genetically minimal bacterium JCVI-syn3A that described the coupled metabolic and genetic information processes and predicted behaviors emerging from the interactions among these networks. JCVI-syn3A is a genetically reduced bacterial cell that has the fewest number and smallest fraction of genes of unclear function, with approximately 90 of its 452 protein-coding genes (that is less than 20%) unannotated. Further characterization of unclear JCVI-syn3A genes strengthens the robustness and predictive power of cell modeling efforts and can lead to a deeper understanding of biophysical processes and pathways at the cell scale. Here, we apply computational analyses to elucidate the functions of the products of several essential but previously uncharacterized genes involved in integral cellular processes, particularly those directly affecting cell growth, division, and morphology. We also suggest directed wet-lab experiments informed by our analyses to further understand these "missing puzzle pieces" that are an essential part of the mosaic of biological interactions present in JCVI-syn3A. Our workflow leverages evolutionary sequence analysis, protein structure prediction, interactomics, and genome architecture to determine upgraded annotations. Additionally, we apply the structure prediction analysis component of our work to all 452 protein coding genes in JCVI-syn3A to expedite future functional annotation studies as well as the inverse mapping of the cell state to more physical models requiring all-atom or coarse-grained representations for all JCVI-syn3A proteins.



Anything else?

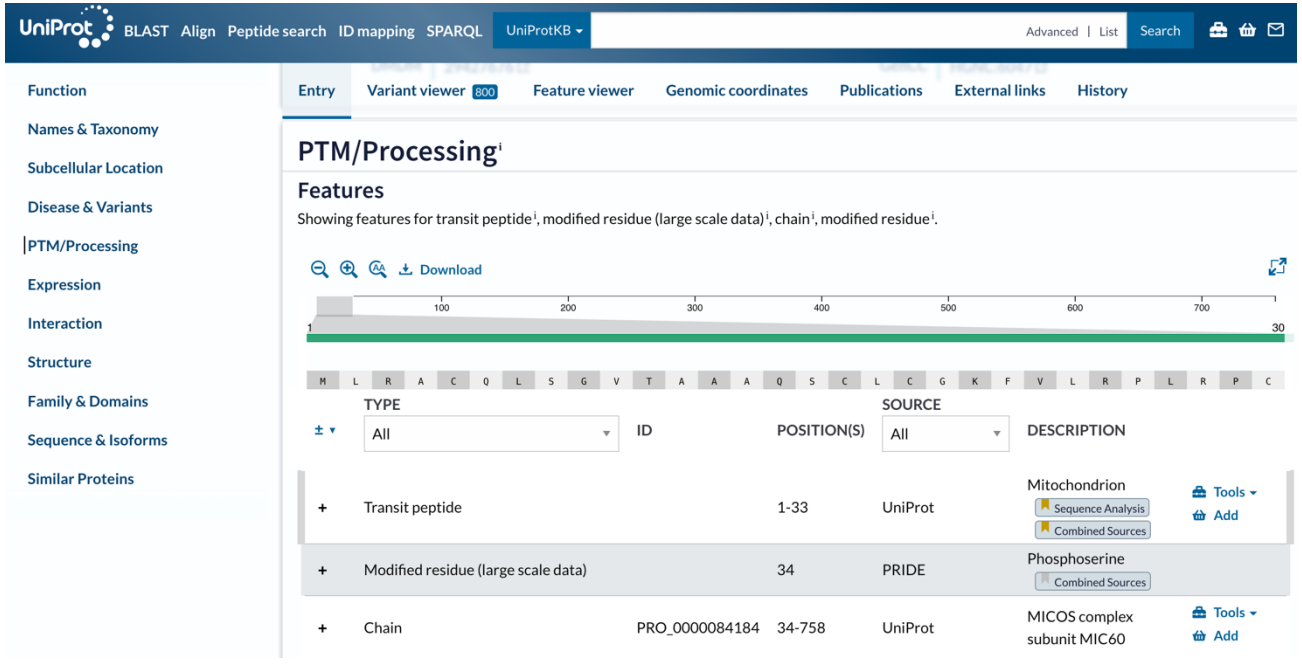
- DNA



Stewart, J. B., & Chinnery, P. F. (2021). Extreme heterogeneity of human mitochondrial DNA from organelles to populations. *Nature Reviews Genetics*, *22*(2), 106-118.

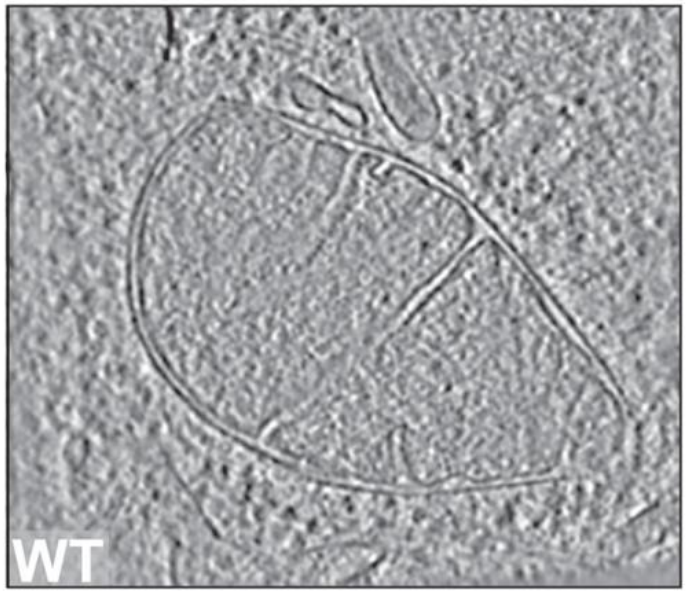
Anything else?

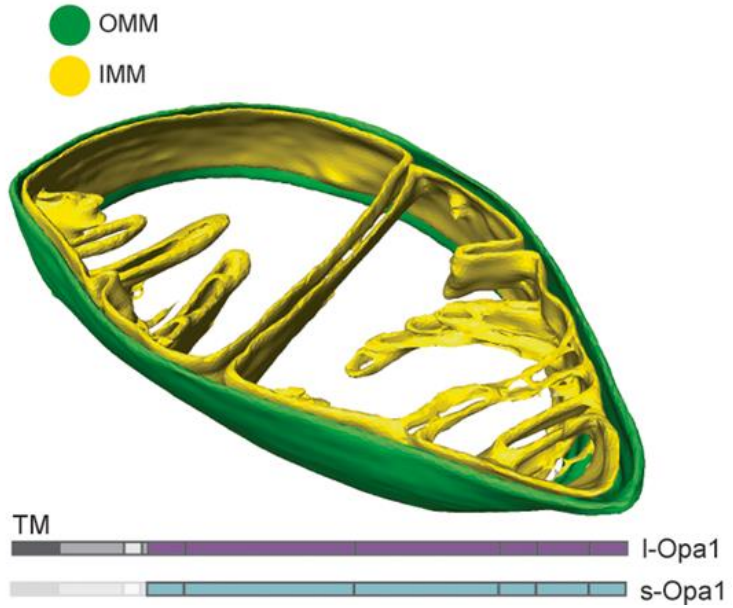
- DNA
- Post-translational modifications
  - Lipidation, phosphorylation, cleavage of signal peptides…
- Membrane shape



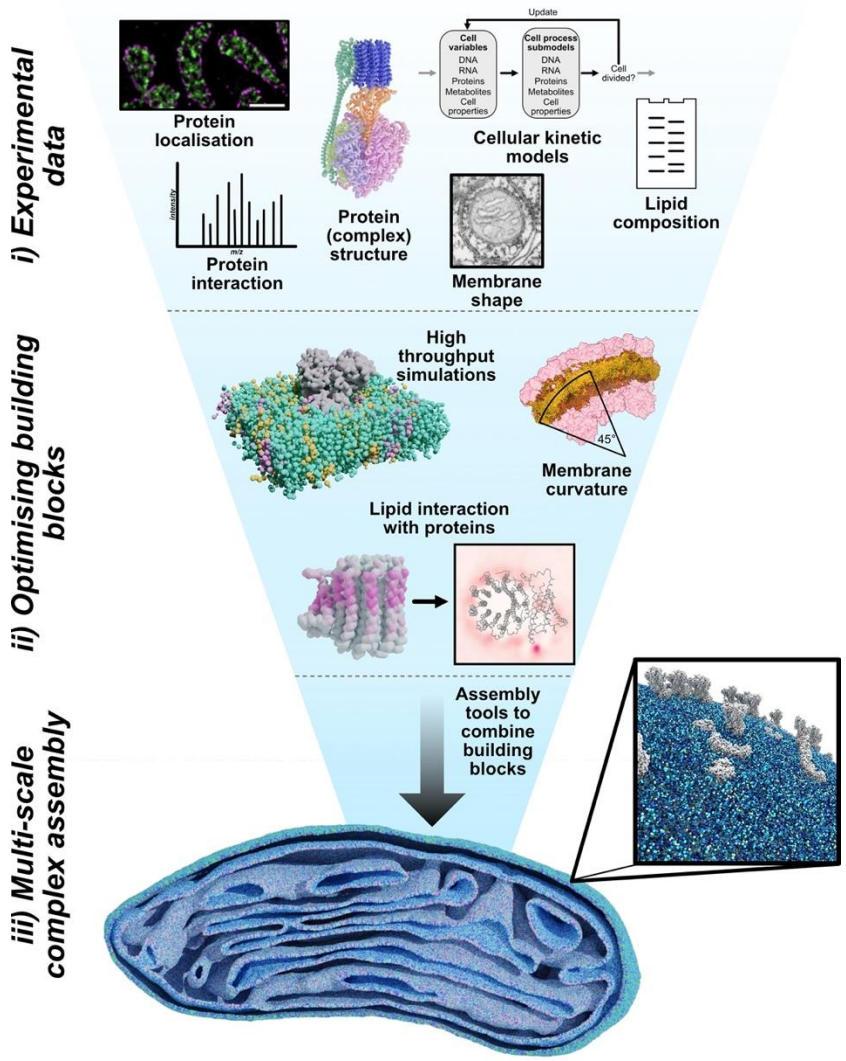
Anything else?

- DNA
- Post-translational modifications
  - Lipidation, phosphorylation, cleavage of signal peptides…
- Membrane shape





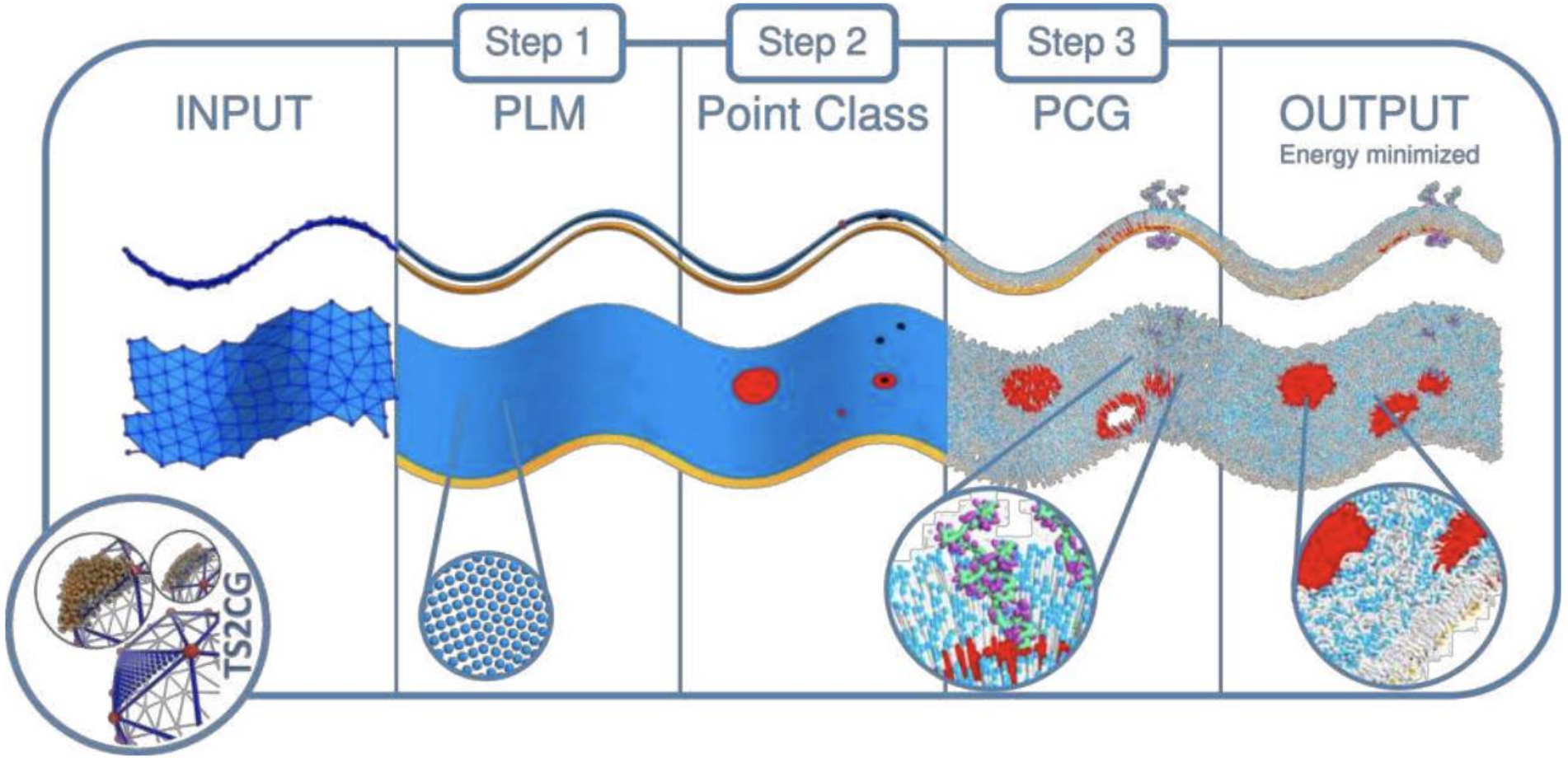
Fry, M. Y., Navarro, P. P., Hakim, P., Ananda, V. Y., Qin, X., Landoni, J. C., ... & Chao, L. H. (2024). In situ architecture of Opa1-dependent mitochondrial cristae remodeling. *The EMBO journal*, *43*(3), 391-413.



How do you put all of this together?

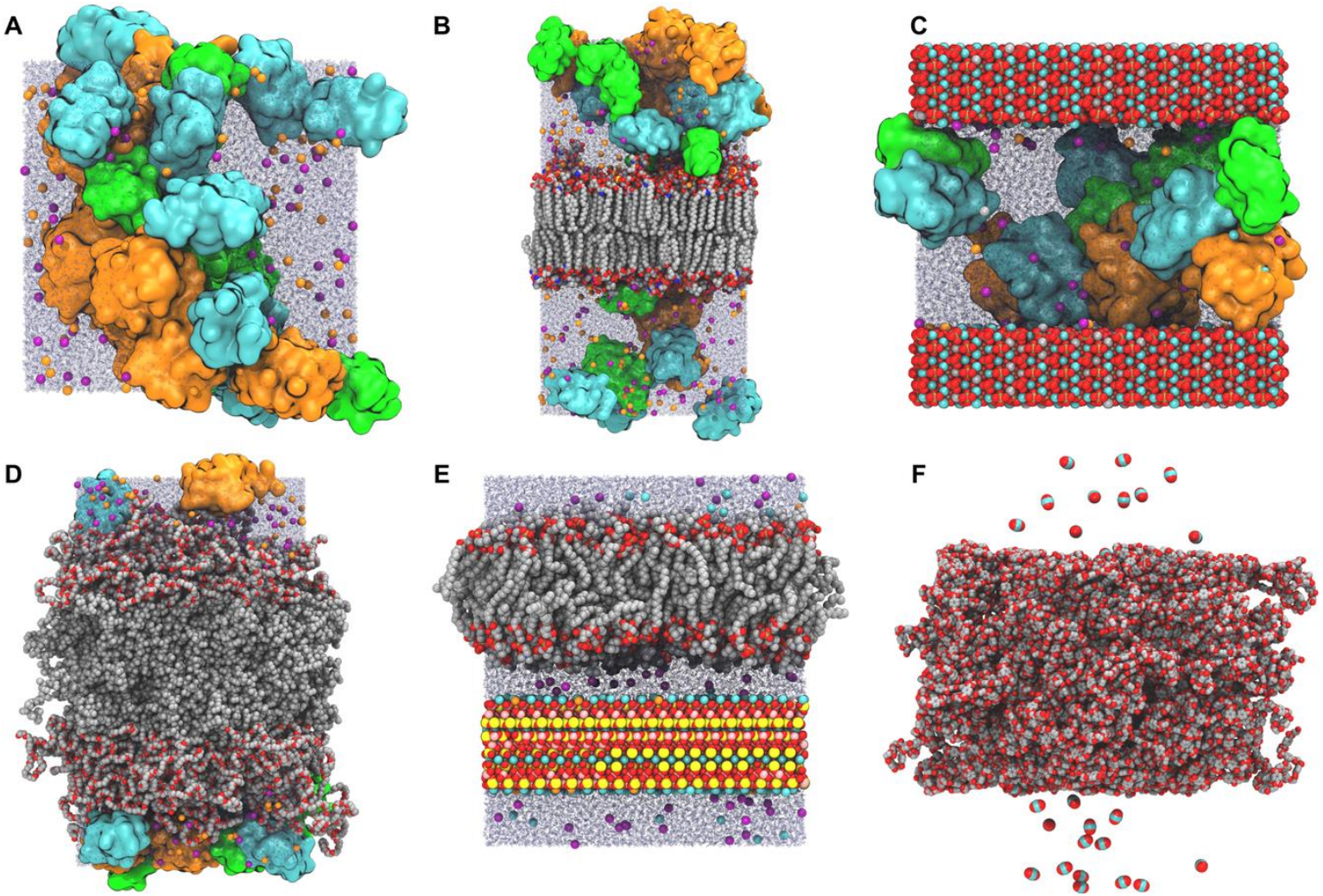
Brown, Chelsea M., and Siewert J. Marrink. "Modeling membranes in situ." *Current Opinion in Structural Biology* 87 (2024): 102837.

System building



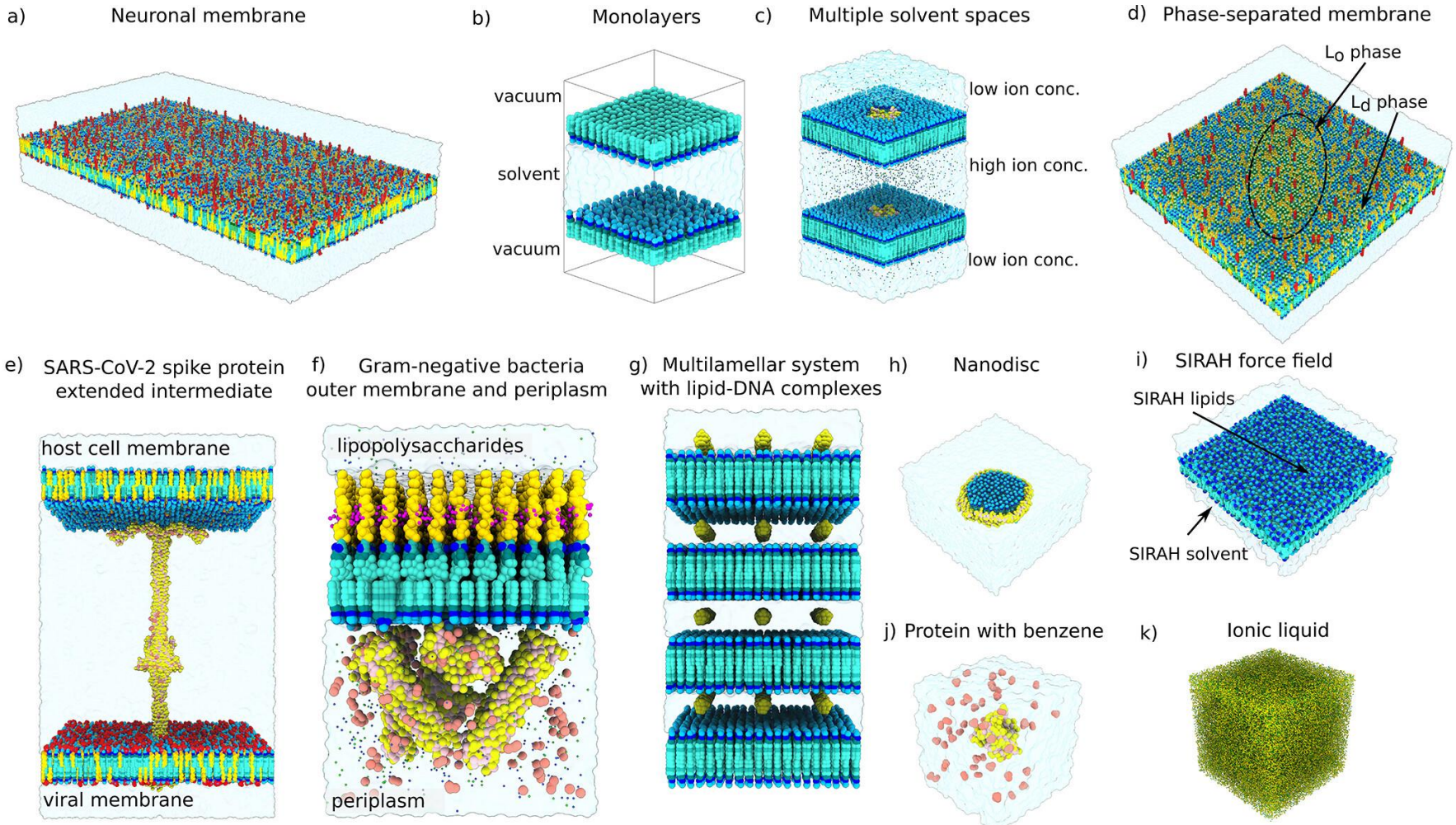
Schuhmann, F., Stevens, J. A., Rahmani, N., Lindahl, I., Brown, C. M., Brasnett, C., ... & Pezeshkian, W. (2025). TS2CG as a membrane builder. *bioRxiv*, 2025-04.

System building

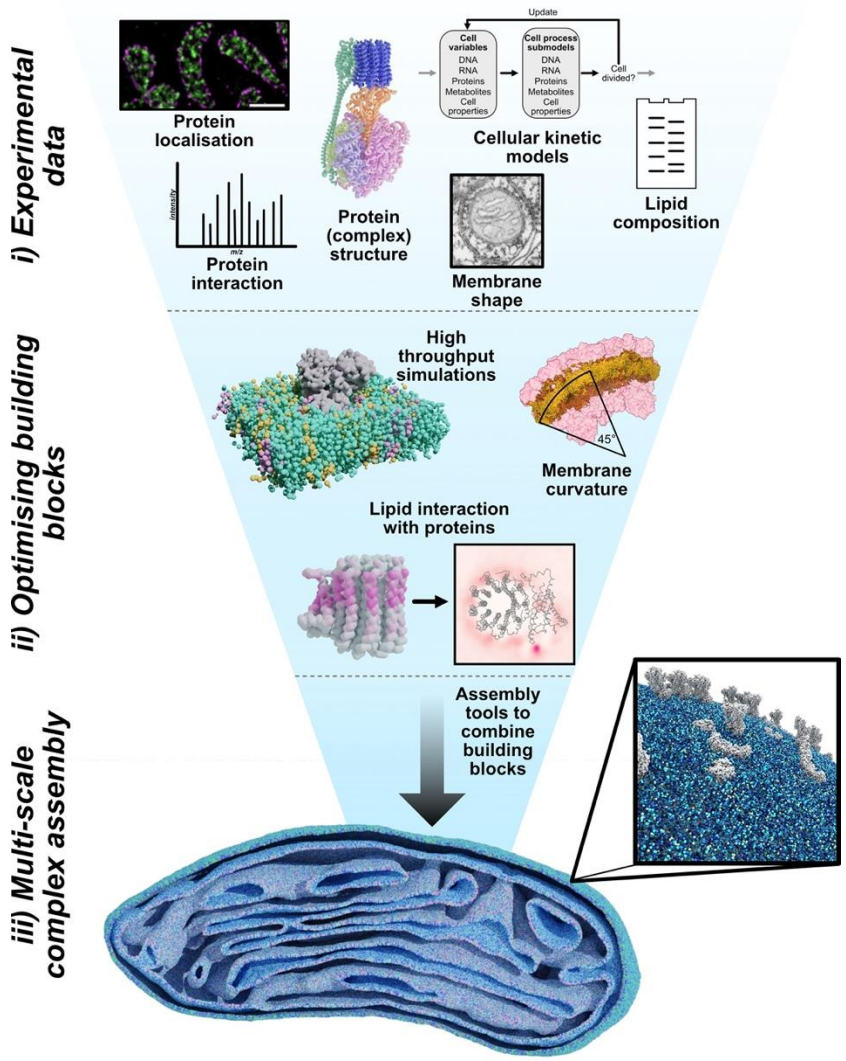


Kern, N. R., Lee, J., Choi, Y. K., & Im, W. (2024). CHARMM-GUI Multicomponent Assembler for modeling and simulation of complex multicomponent systems. *Nature Communications*, *15*(1), 5459.

System building

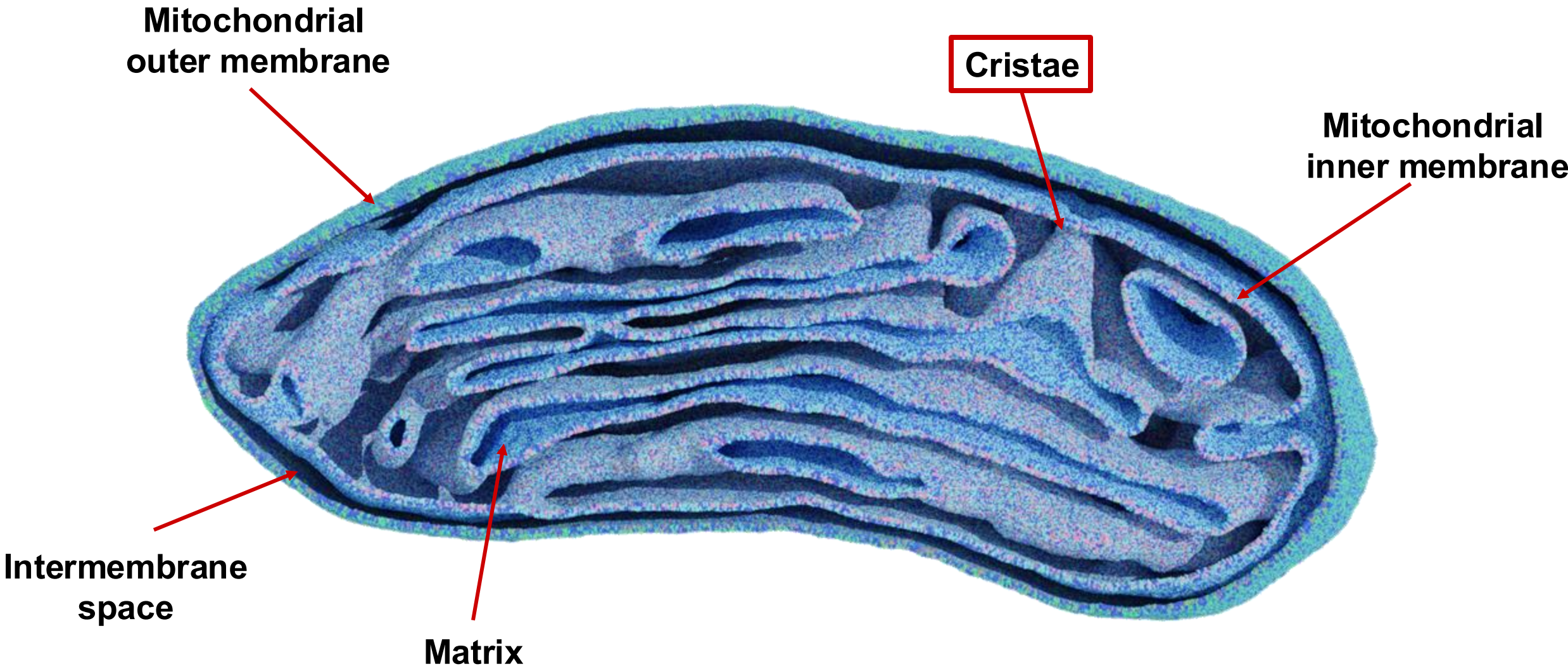


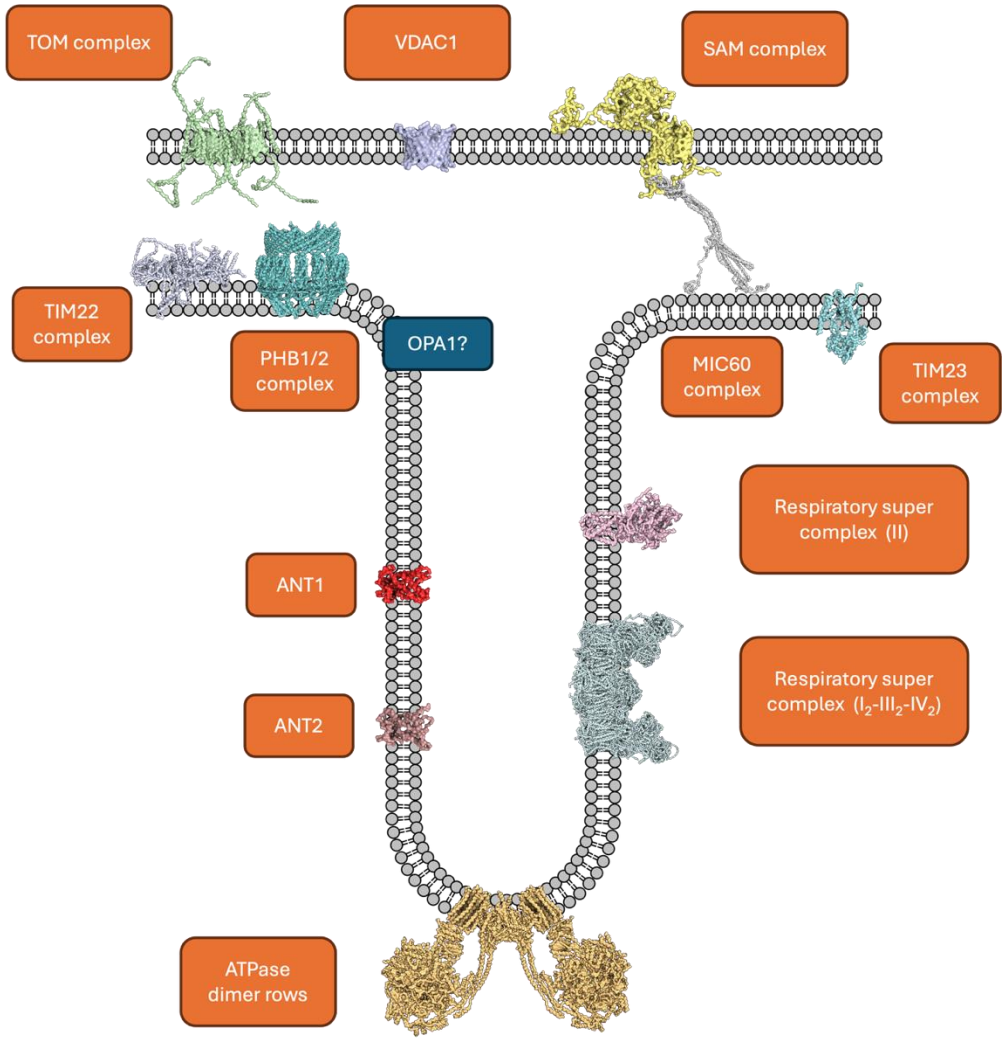
Andreasen, M. D., Souza, P. C., Schiøtt, B., & Zuzic, L. (2025). Creating Coarse-Grained Systems with COBY: Toward Higher Accuracy of Complex Biological Systems. *Journal of Chemical Information and Modeling*, *65*(10), 4760-4766.



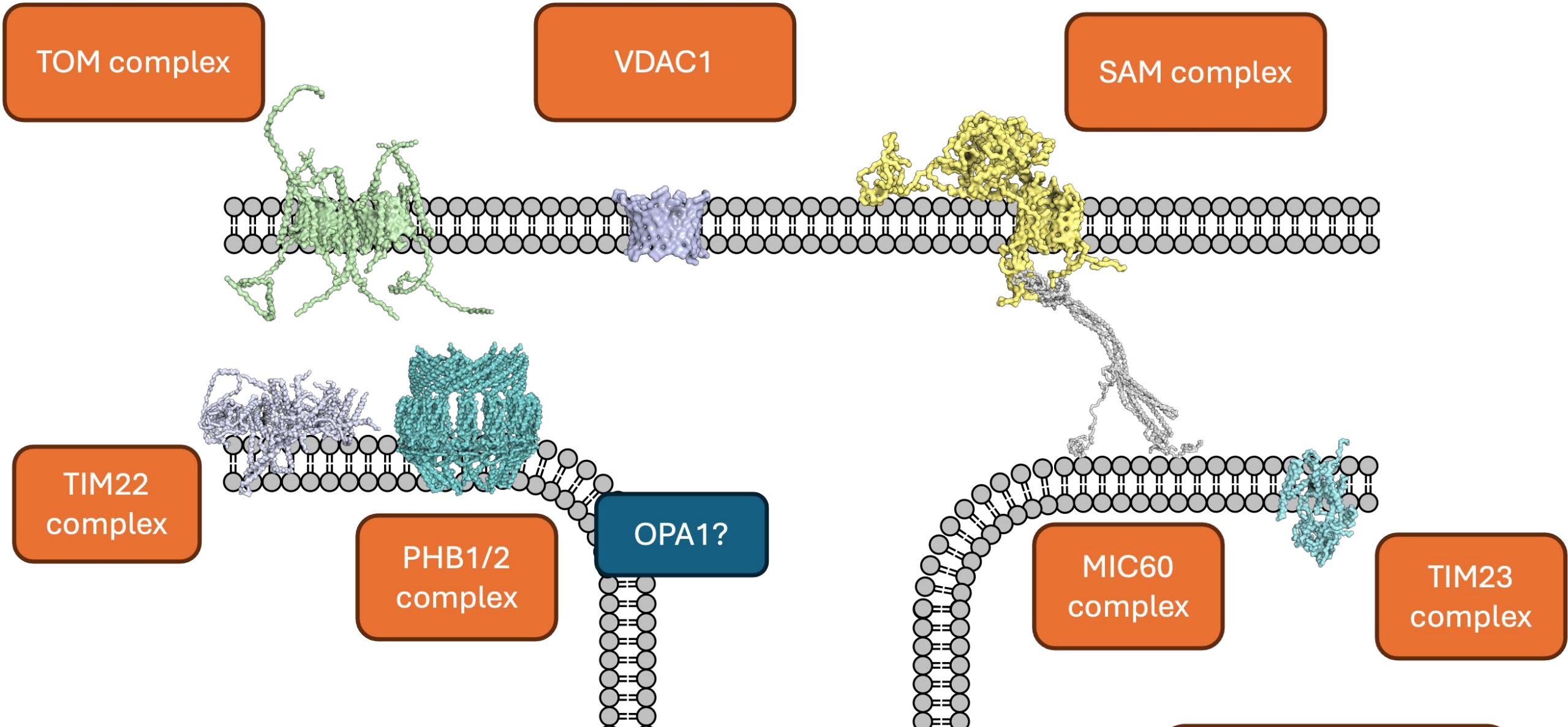
But how would we build a model of the mitochondria?

Brown, Chelsea M., and Siewert J. Marrink. "Modeling membranes in situ." *Current Opinion in Structural Biology* 87 (2024): 102837.


Mitochondrial outer membrane
Cristae
Mitochondrial inner membrane
Intermembrane space
Matrix



Model of a cristae junction


TOM complex
VDAC1
SAM complex
TIM22 complex
PHB1/2 complex
OPA1?
MIC60 complex
TIM23 complex

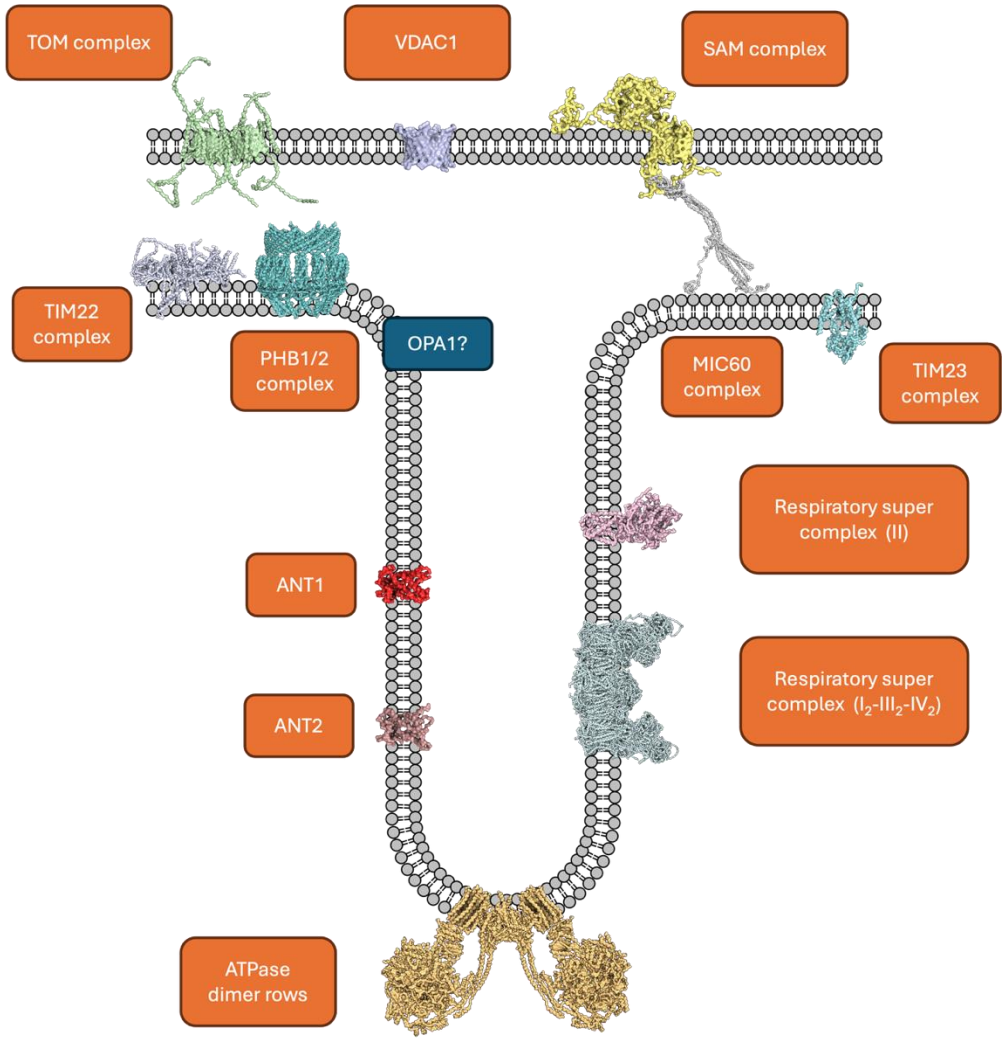
ANT1

ANT2

ATPase
dimer rows

Respiratory super
complex (II)

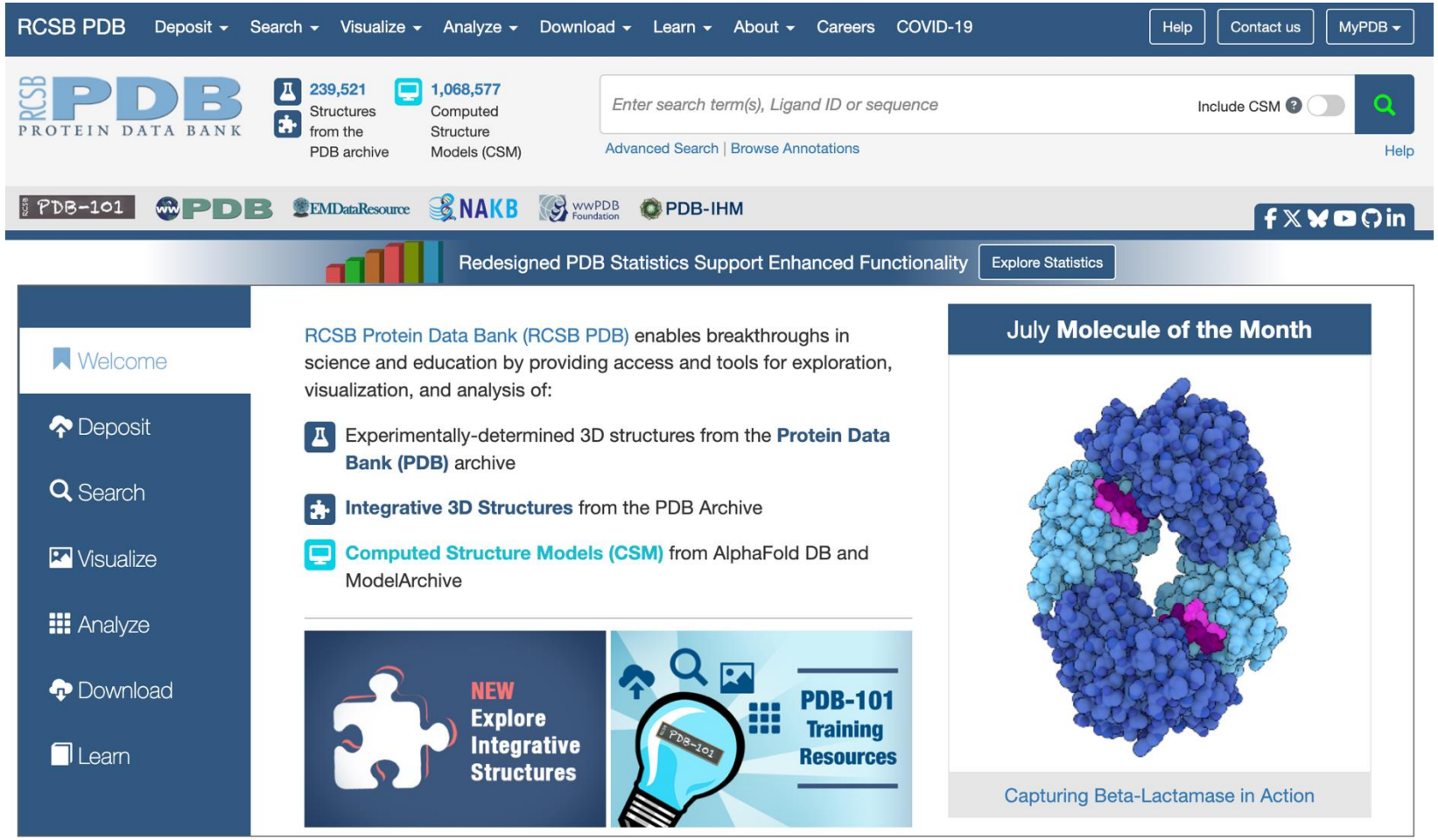
Respiratory super
complex (I_2-III_2-IV_2)



Need *structures*!

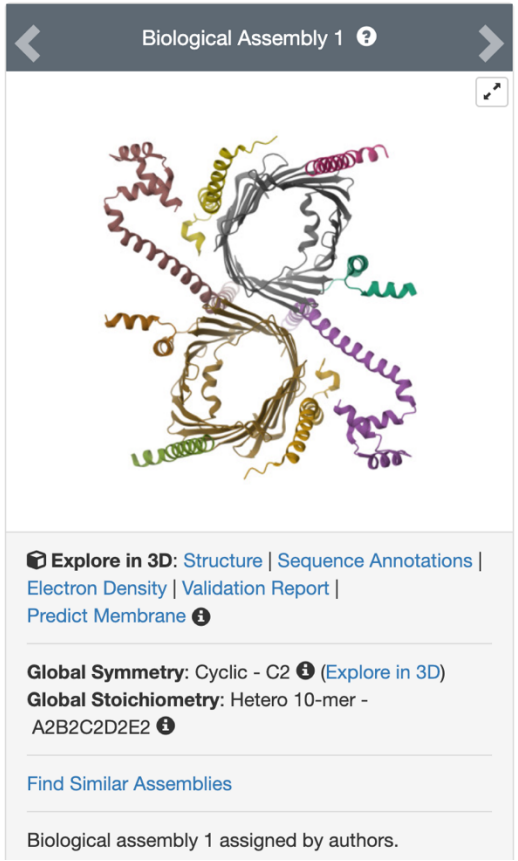
include all native residues from human proteins

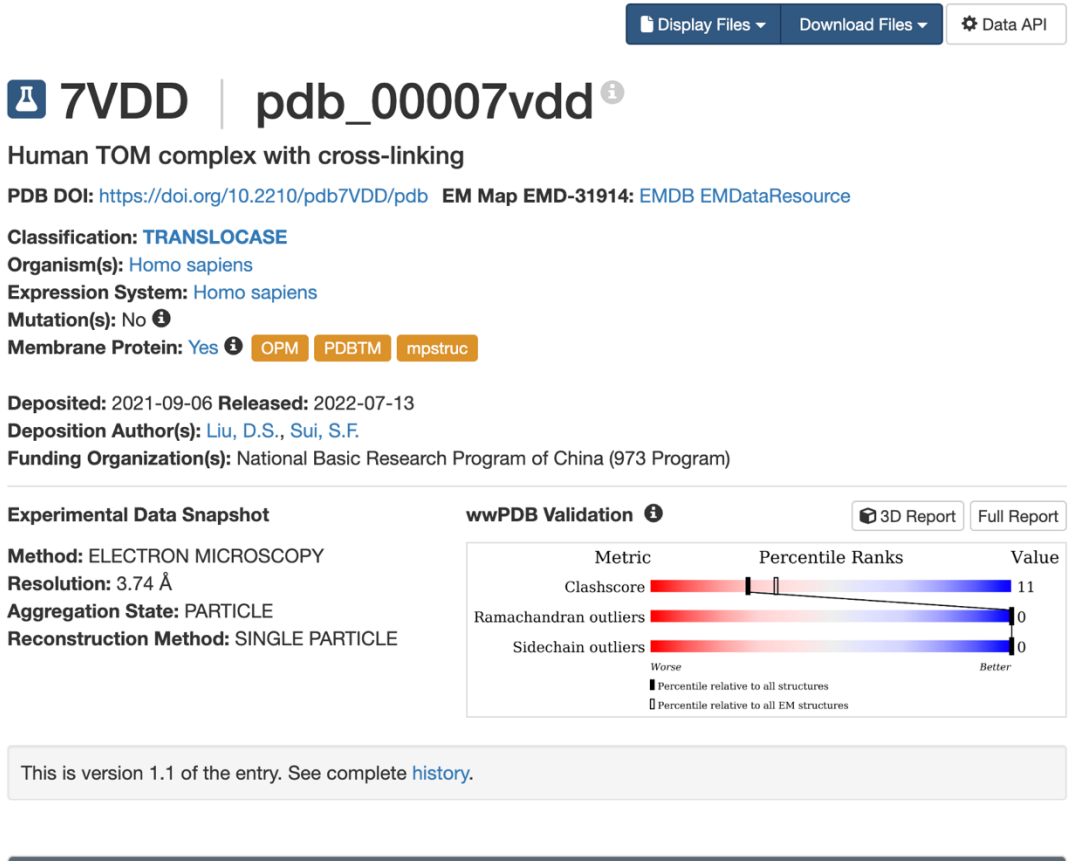
How to get ‘complete’ protein structures



How to get ‘complete’ protein structures

- Missing termini
- Missing chains
- Missing loops

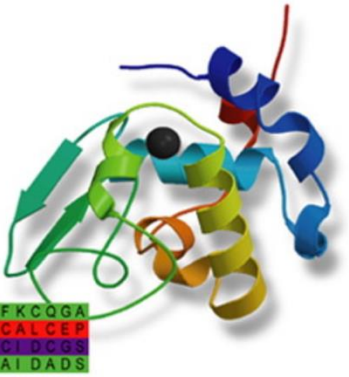




Missing loops:

Modeller

Program for Comparative Protein Structure Modelling by Satisfaction of Spatial Restraints

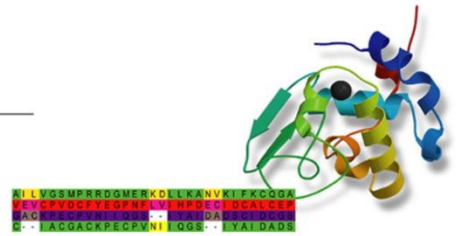


About MODELLER

MODELLER is used for homology or comparative modeling of protein three-dimensional structures (1,2). The user provides an alignment of a sequence to be modeled with known related structures and MODELLER automatically calculates a model containing all non-hydrogen atoms. MODELLER implements comparative protein structure modeling by satisfaction of spatial restraints (3,4), and can perform many additional tasks, including de novo modeling of loops in protein structures, optimization of various models of protein structure with respect to a flexibly defined objective function, multiple alignment of protein sequences and/or structures, clustering, searching of sequence databases, comparison of protein structures, etc. MODELLER is available for download for most Unix/Linux systems, Windows, and Mac.

Several graphical interfaces to MODELLER are commercially available. There are also many other resources and people using Modeller in graphical or web interfaces or other frameworks.

Missing loops:



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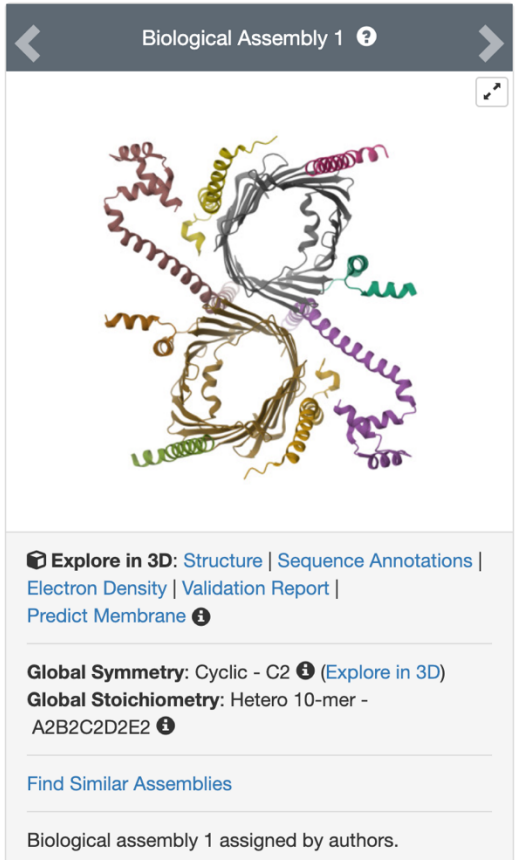
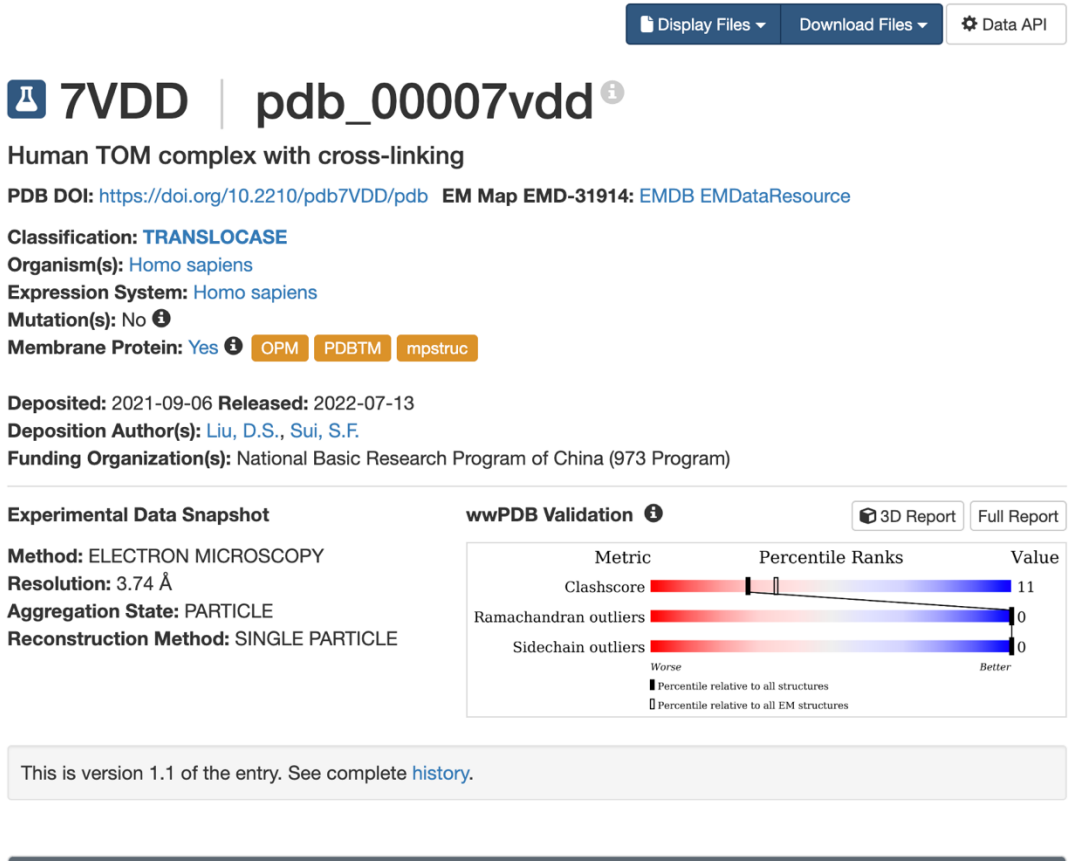
```
from modeller import *
import sys
from modeller.automodel import *    # Load the AutoModel class

log.verbose()
env = Environ()

class MyModel(AutoModel):
    def select_atoms(self):
        return Selection(self.residue_range(sys.argv[2], sys.argv[3]),
                         self.residue_range(sys.argv[4], sys.argv[5]))

a = MyModel(env, alnfile = sys.argv[1]+'_alignment.ali',
                 knowns = sys.argv[1], sequence = sys.argv[1]+'_fill')
a.starting_model= 1
a.ending_model  = 1
a.md_level      = refine.fast

a.make()
```

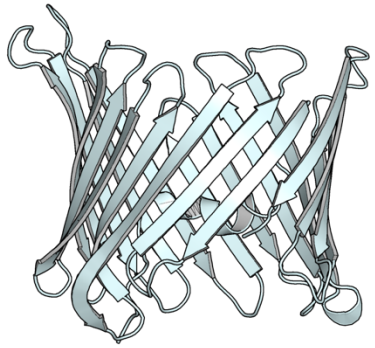
Missing termini:



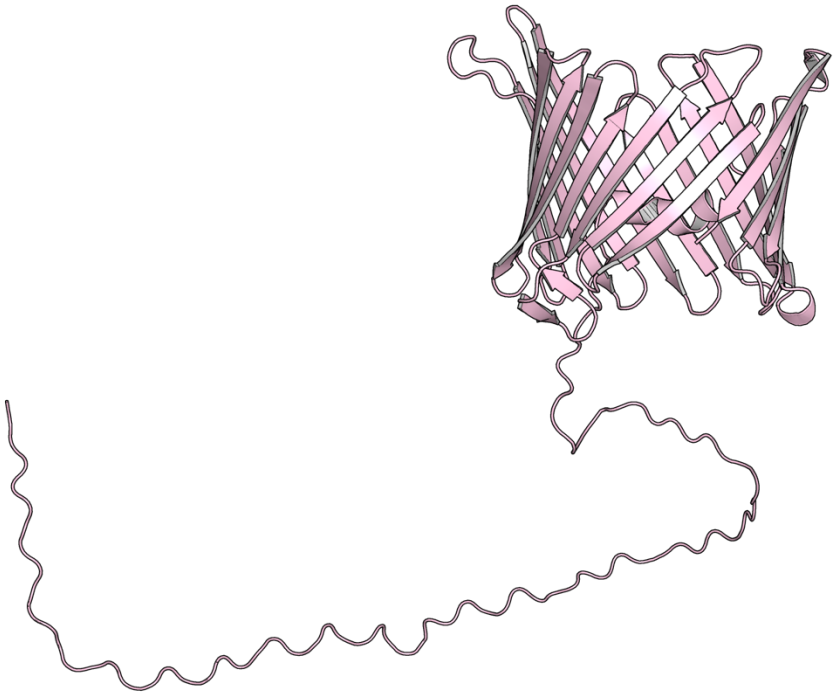


Missing termini:

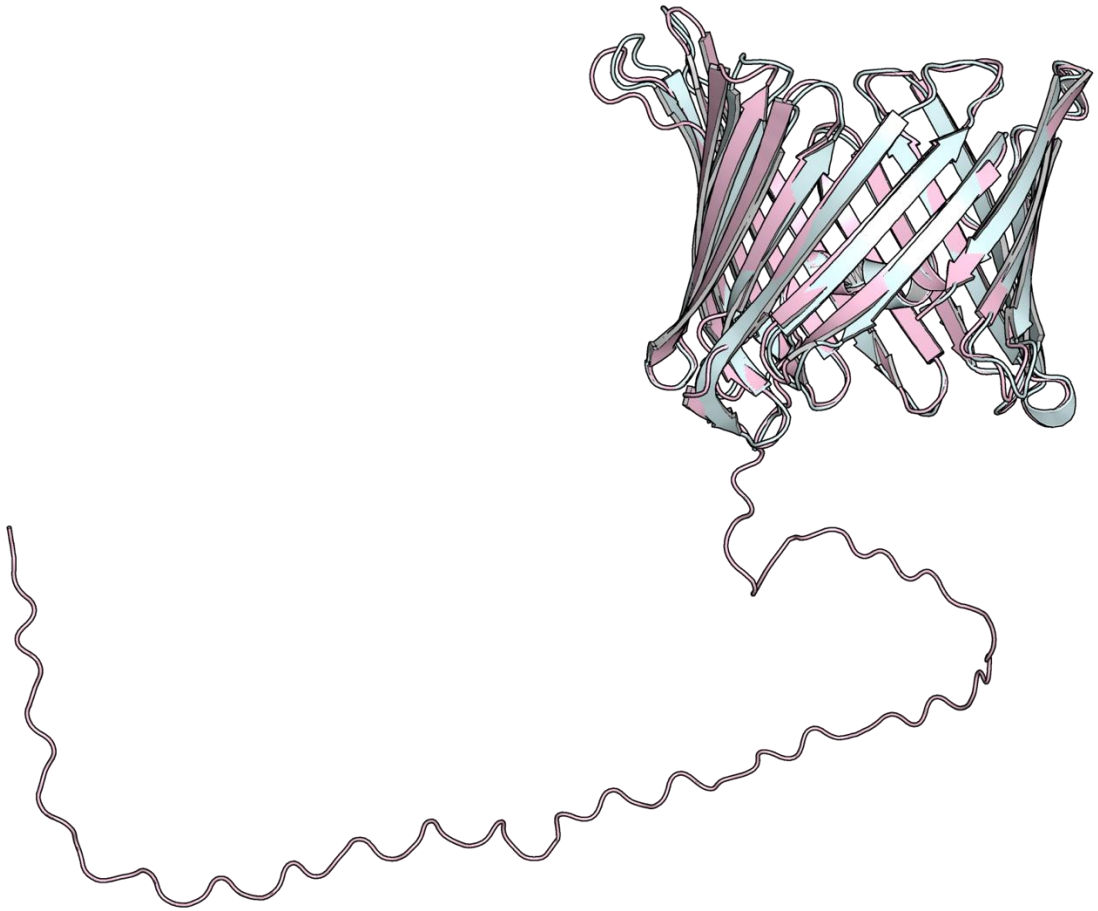
TOM20 – 7VDD



TOM20 – AlphaFold

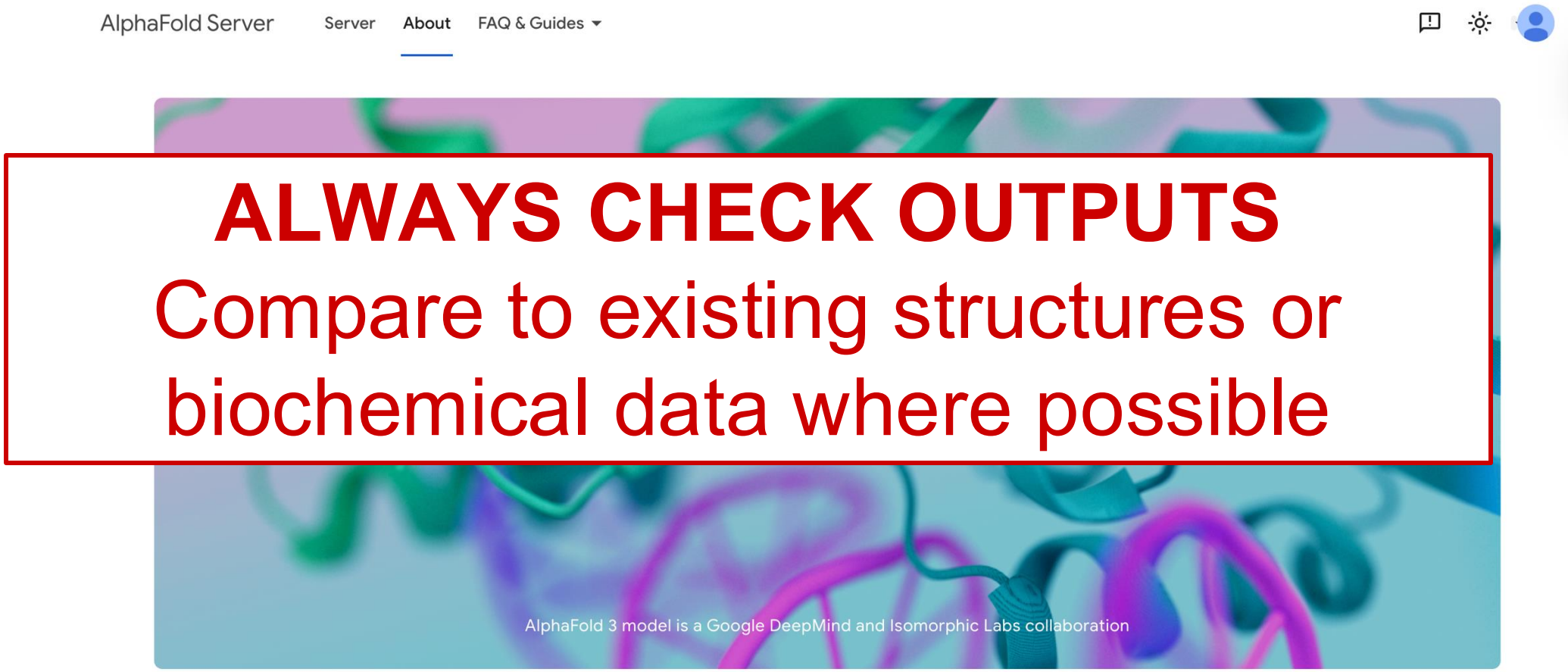


Missing termini:



RMSD – 0.85 Å (5 cycles of refinement, 1525/1844 atoms)

Missing chains:



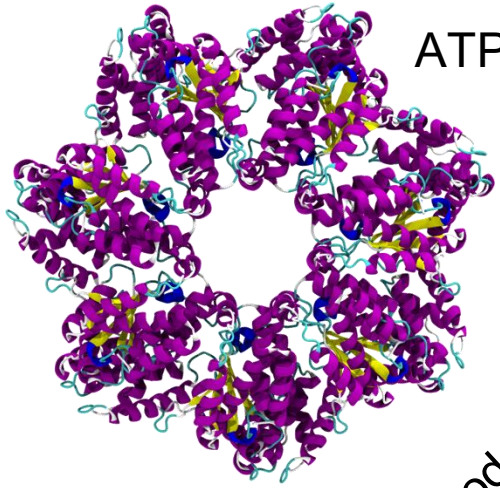
ALWAYS CHECK OUTPUTS

Compare to existing structures or biochemical data where possible

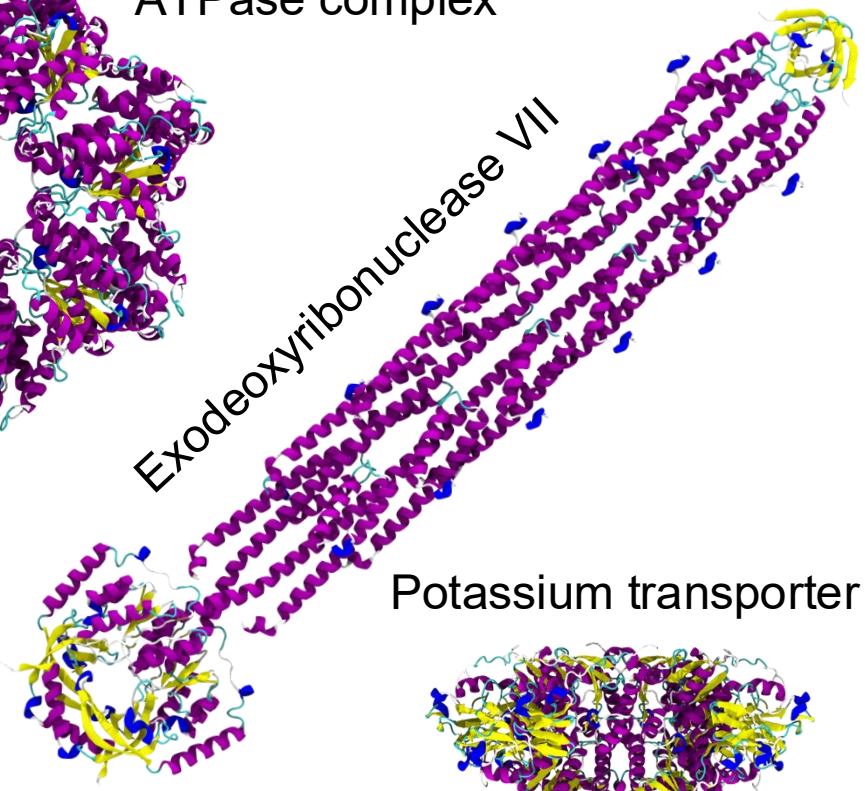
Proteins – Minimal cell!!

Syn3A genome consists of **452 protein-coding genes** where 20% remains uncharacterised.

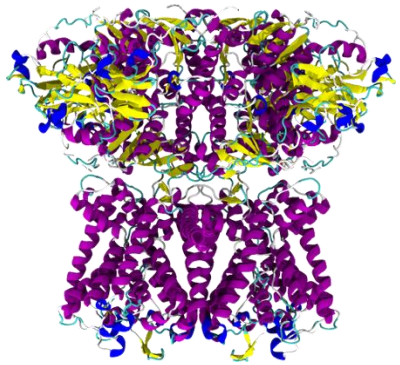
Can we predict the protein complexes?



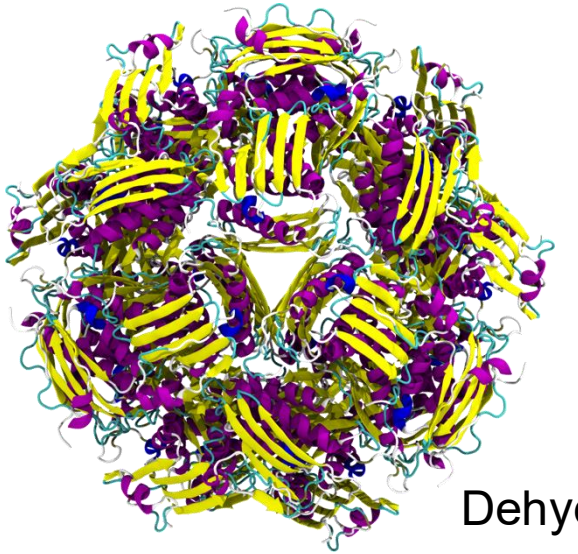
ATPase complex



Exodeoxyribonuclease VII



Potassium transporter

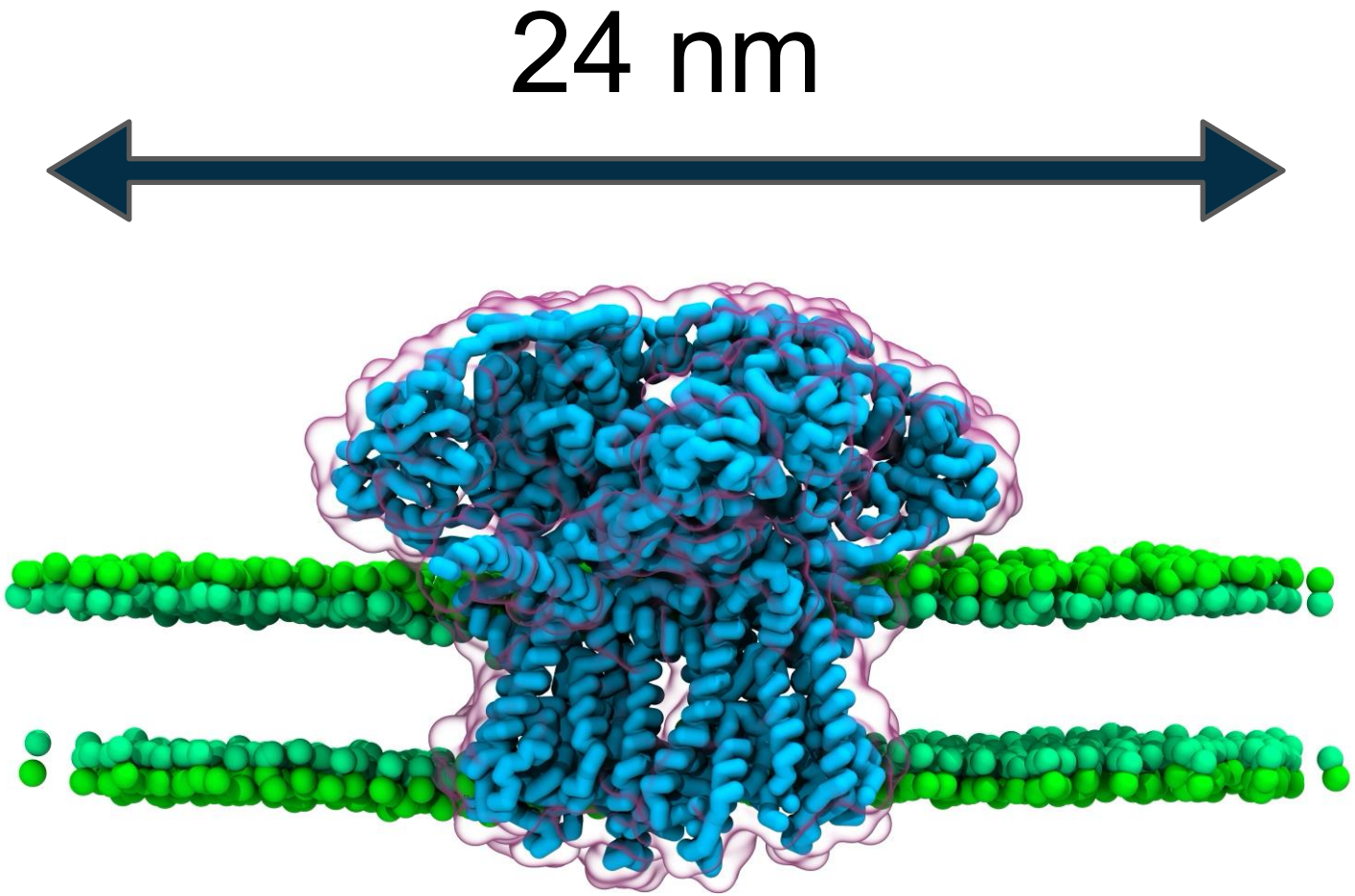


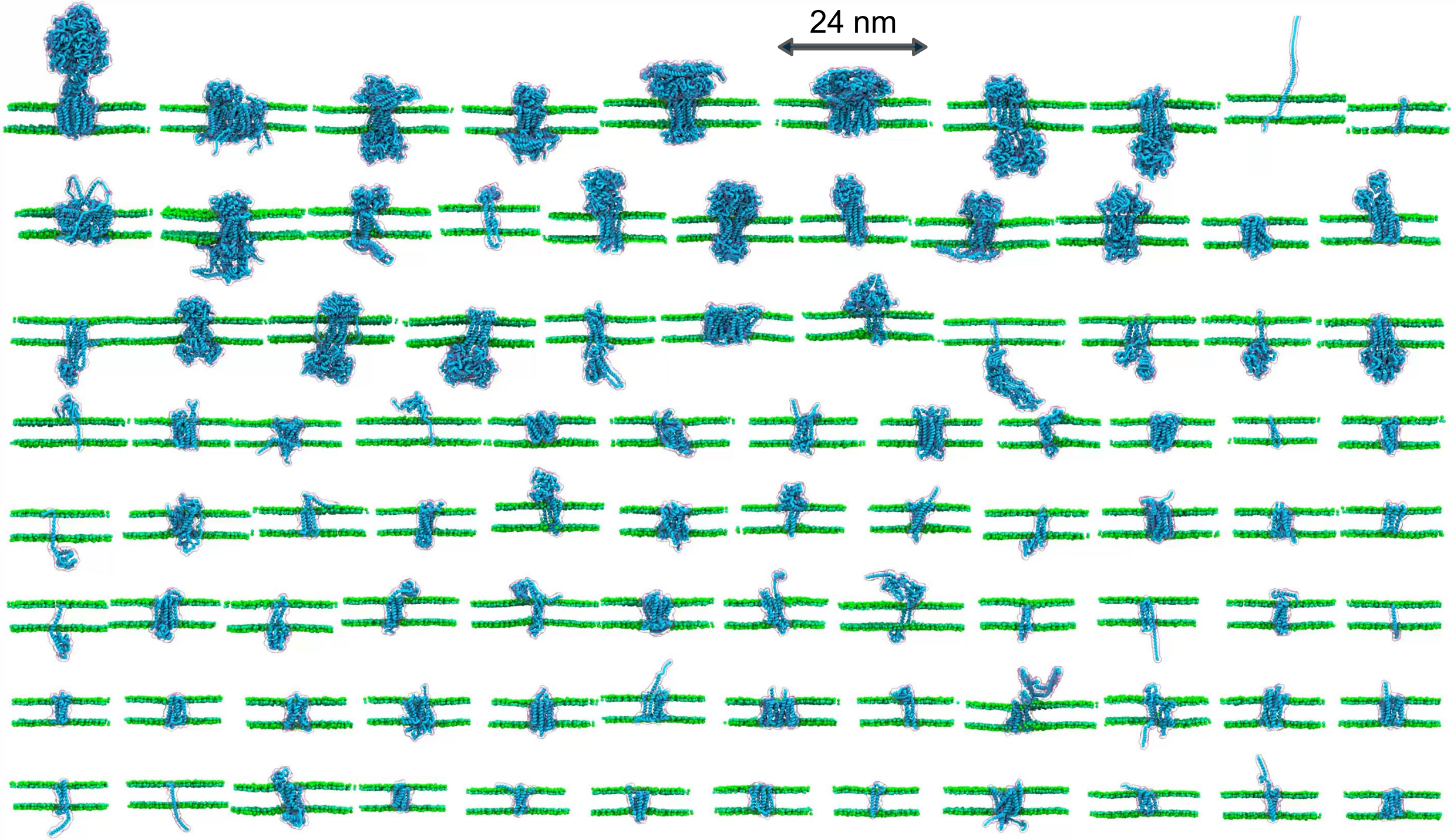
Dehydratase complex

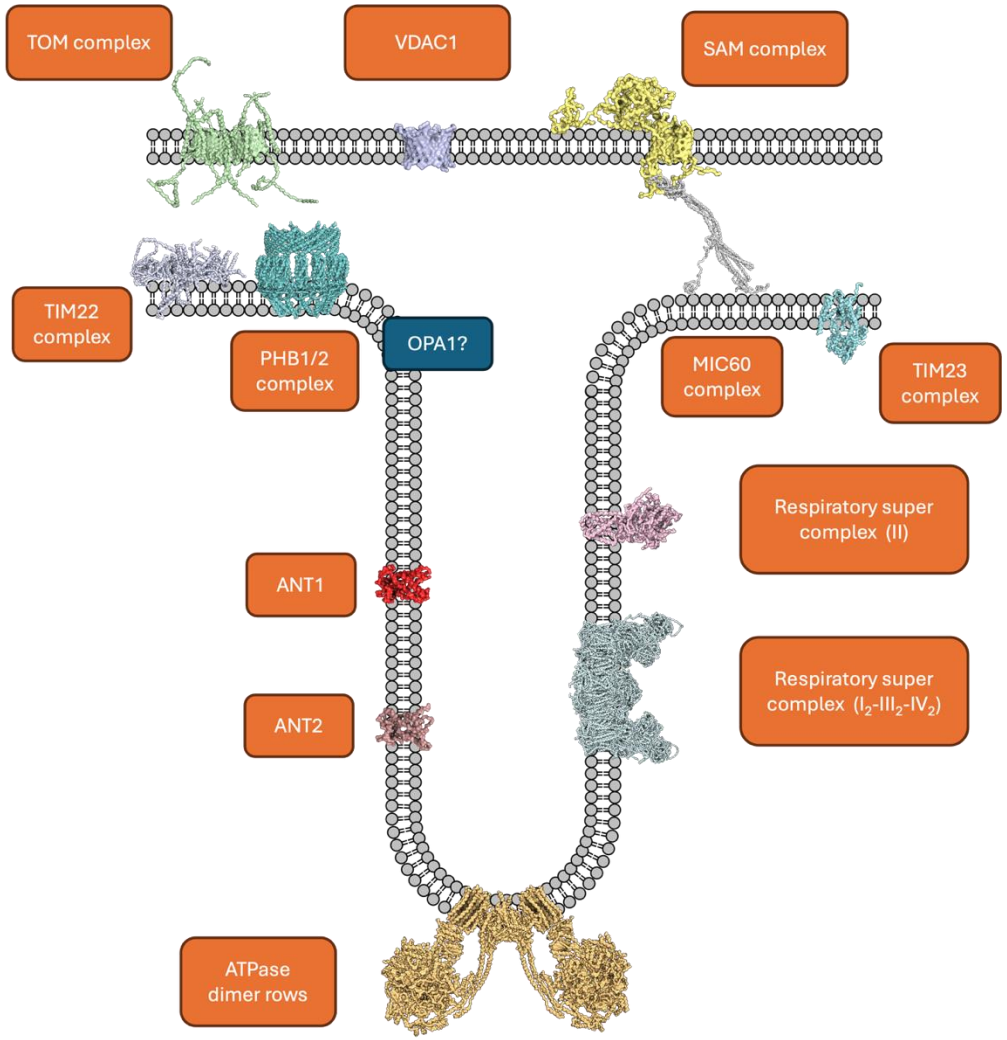
- Found a diverse set of complexes

  452 monomers, 81 dimers, 9 trimers, 20 tetramers, 39 ‘larger’ complex

Proteins – Minimal cell!!



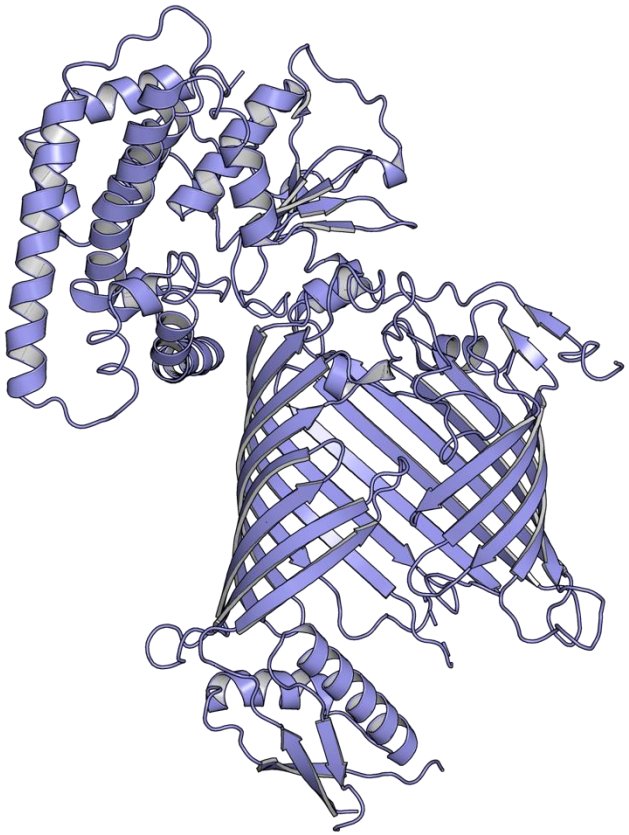

24 nm

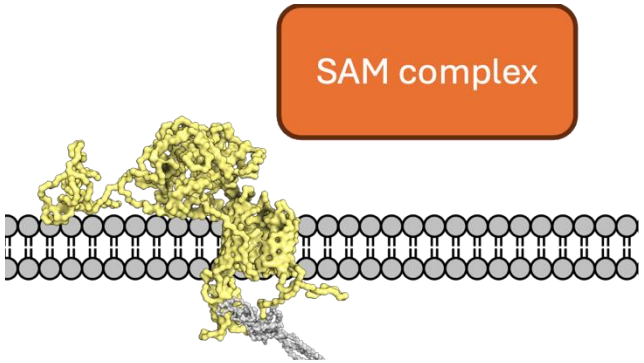


Need *structures*!

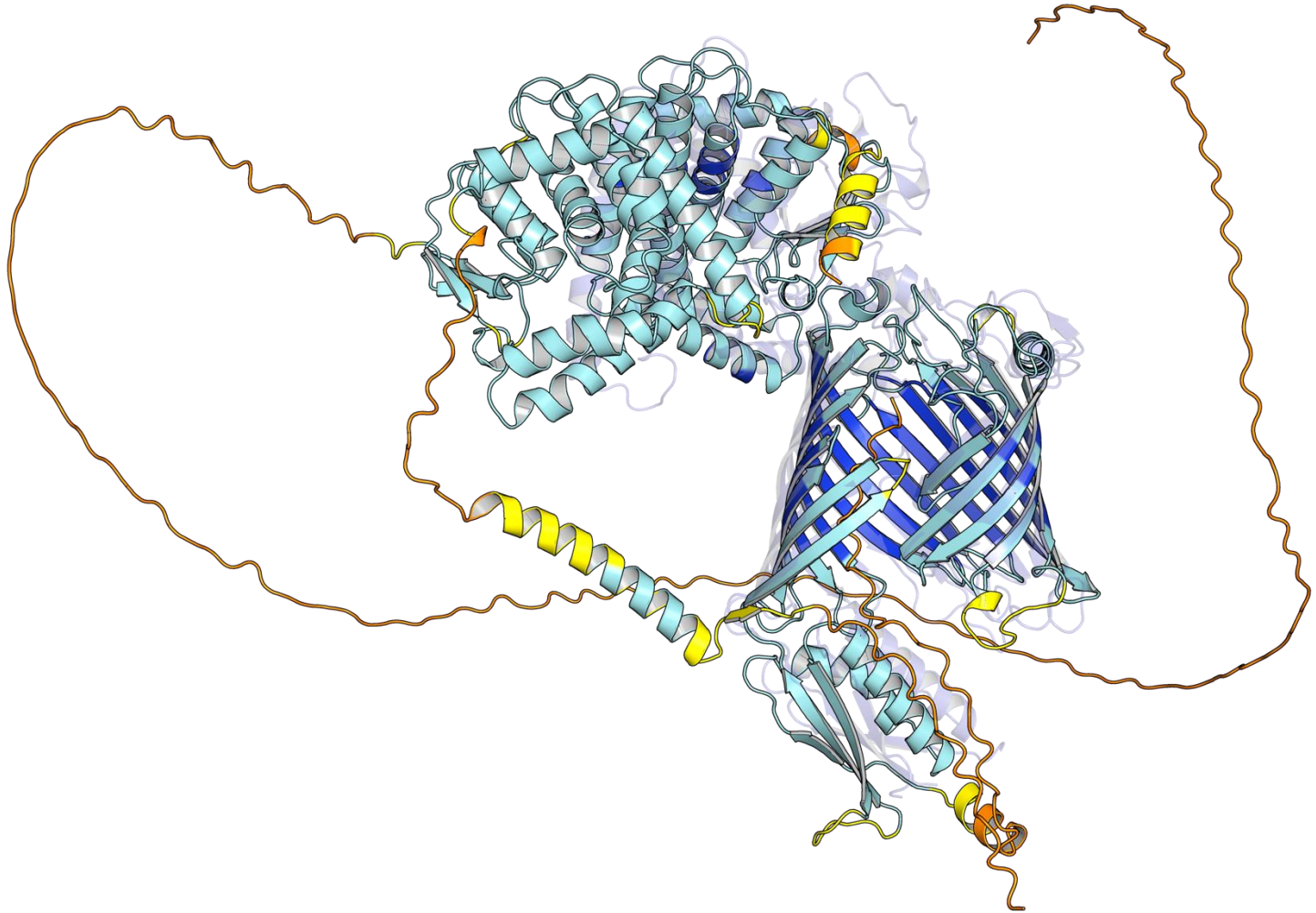
include all native residues from human proteins

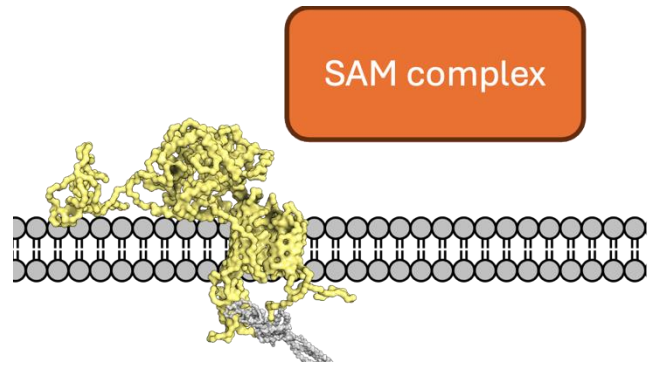
SAM complex – 7E4H from Yeast

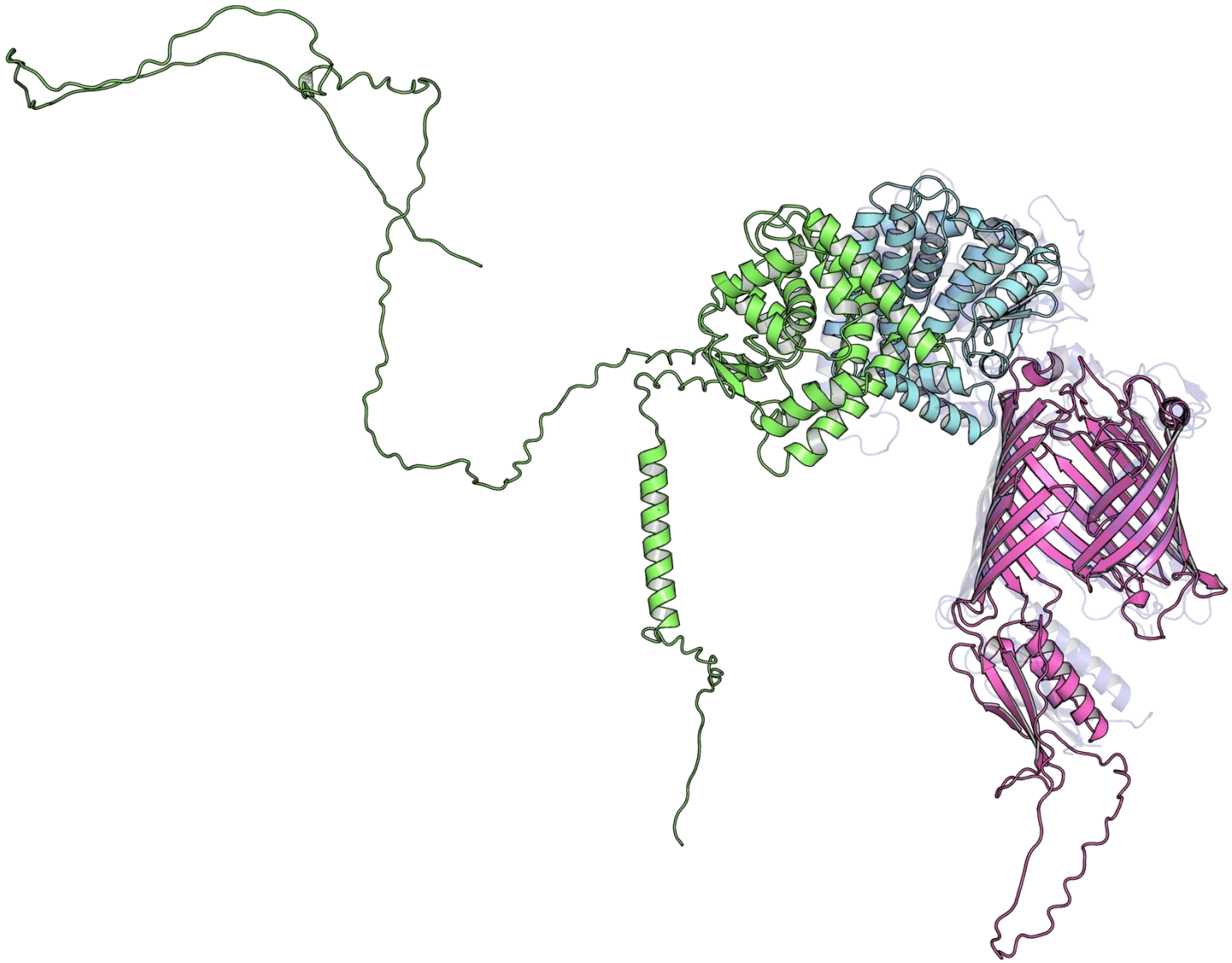




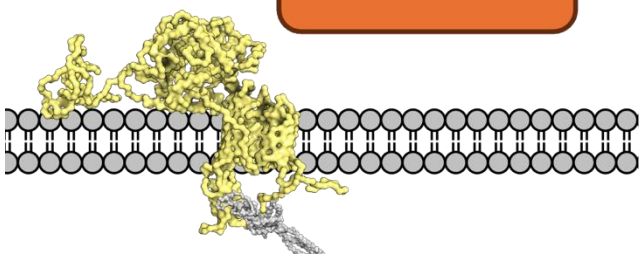
AlphaFold 3 complex prediction

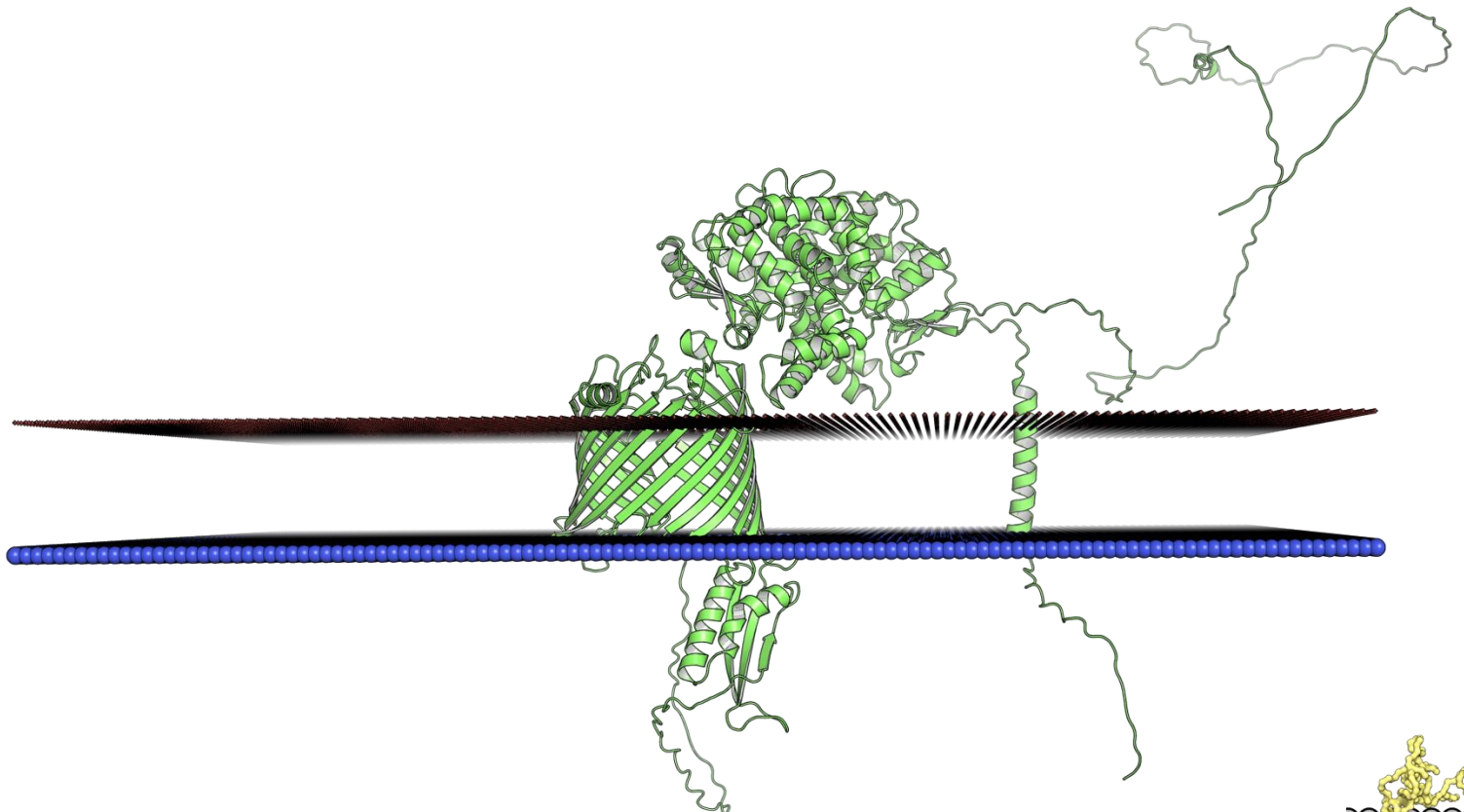




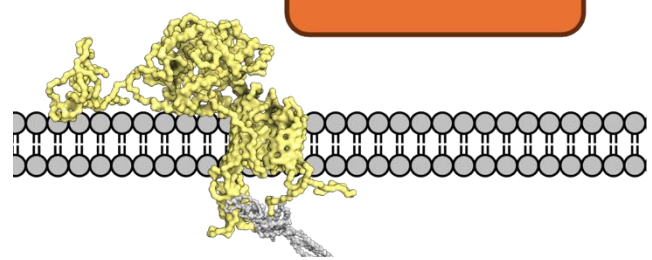


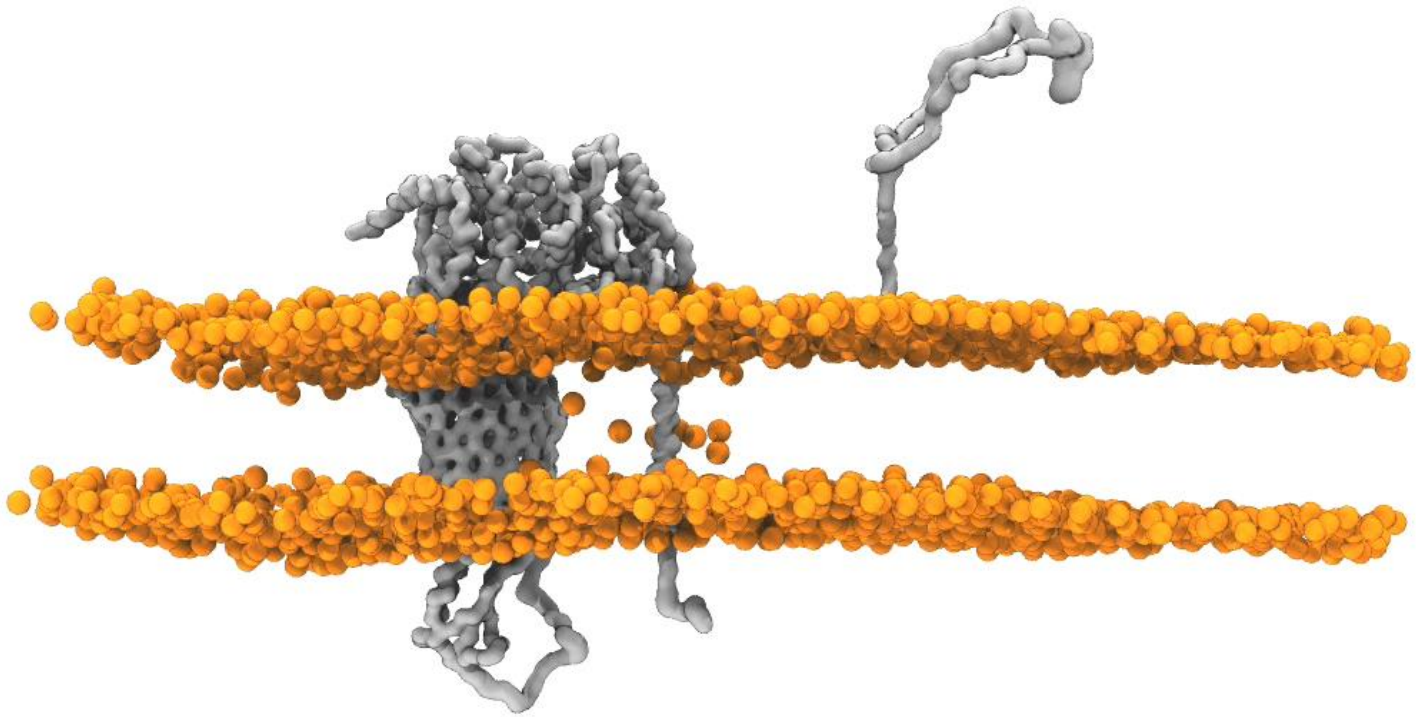
SAM complex



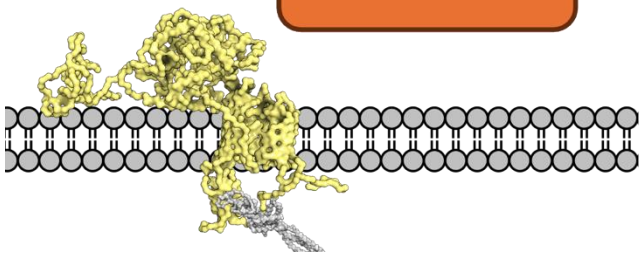


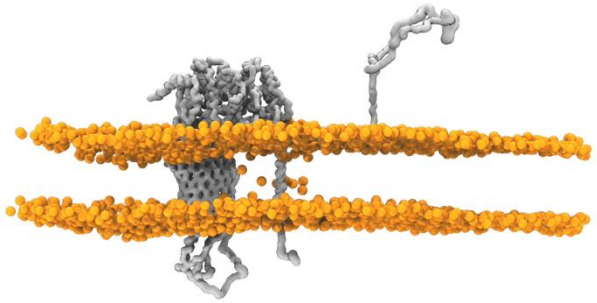
SAM complex



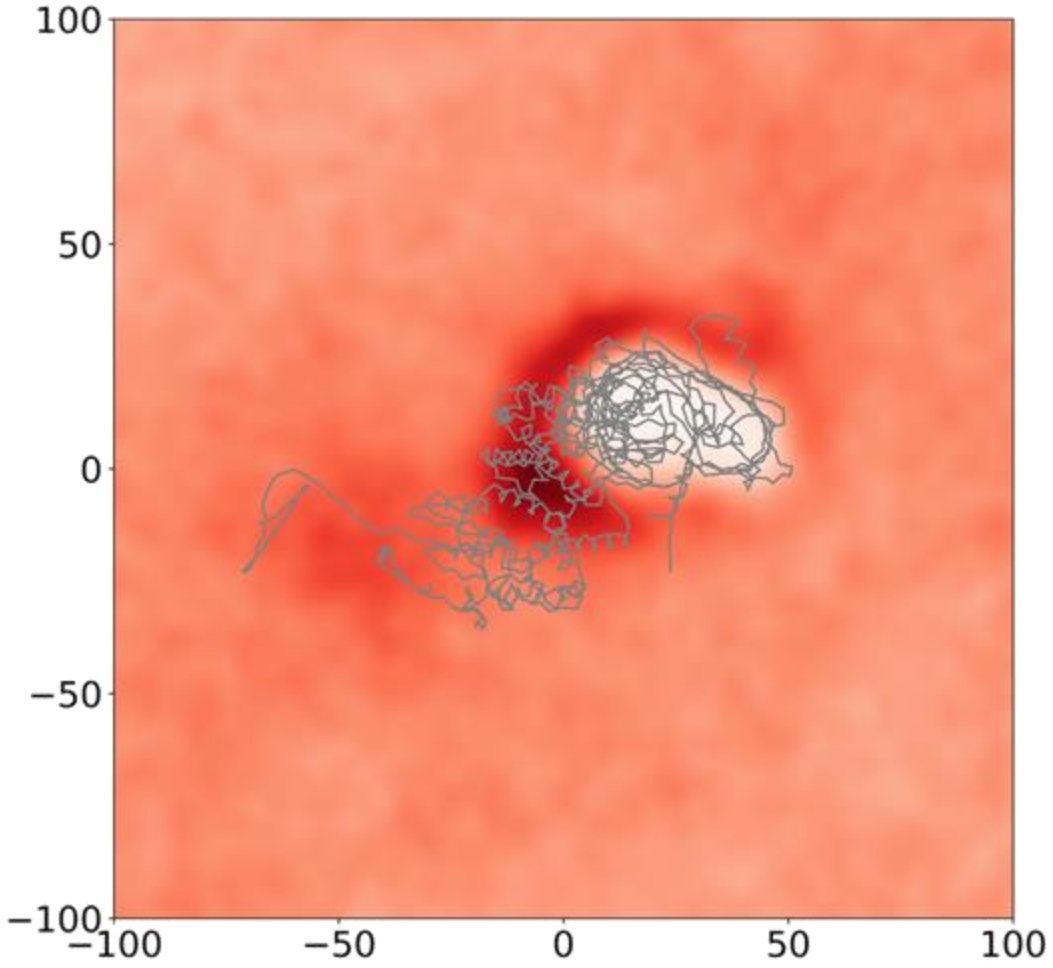


SAM complex

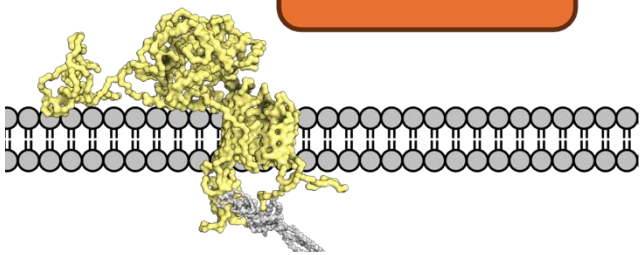


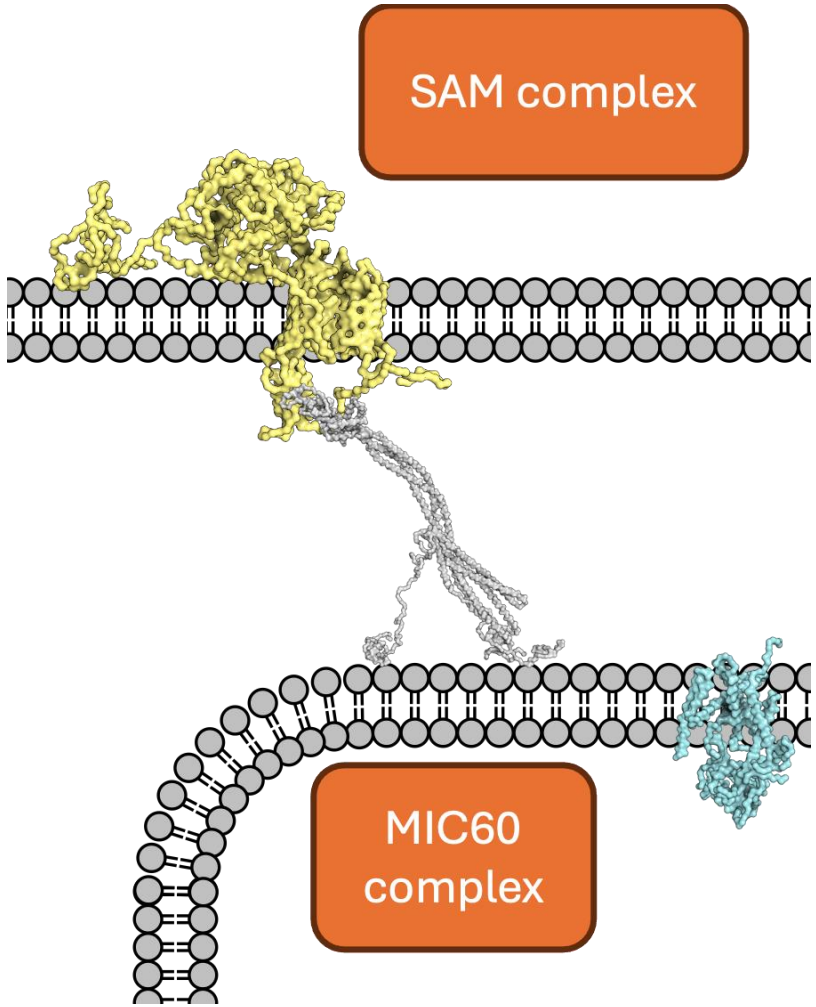


PAPI density around SAM complex



SAM complex





Cell Death & Differentiation (2020) 27:146–160
https://doi.org/10.1038/s41418-019-0345-2

Cell Death & Differentiation

ARTICLE

Sam50–Mic19–Mic60 axis determines mitochondrial cristae architecture by mediating mitochondrial outer and inner membrane contact

Junhui Tang[1] · Kuan Zhang[1,4] · Jun Dong[1] · Chaojun Yan[1] · Chao Hu[1] · Hongchao Ji[1] · Liangyi Chen[2] · Shi Chen[3] · Huabin Zhao[1] · Zhiyin Song[1]

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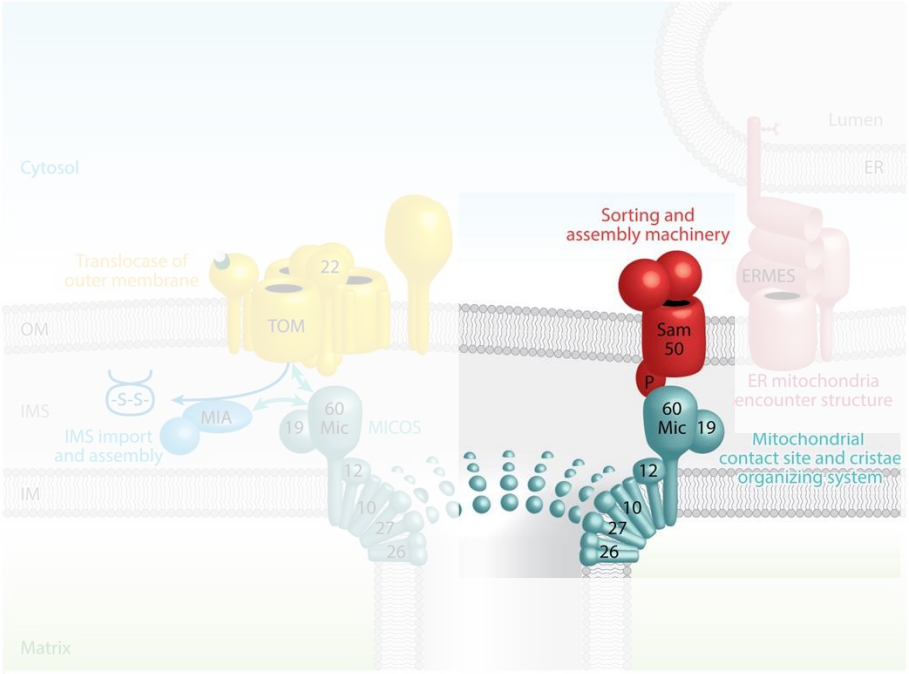
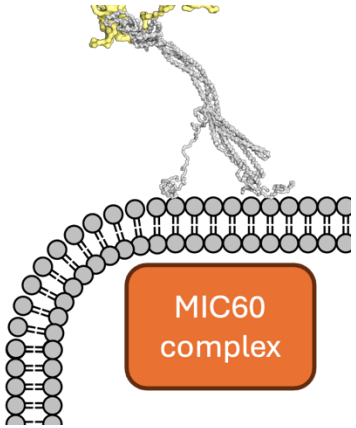
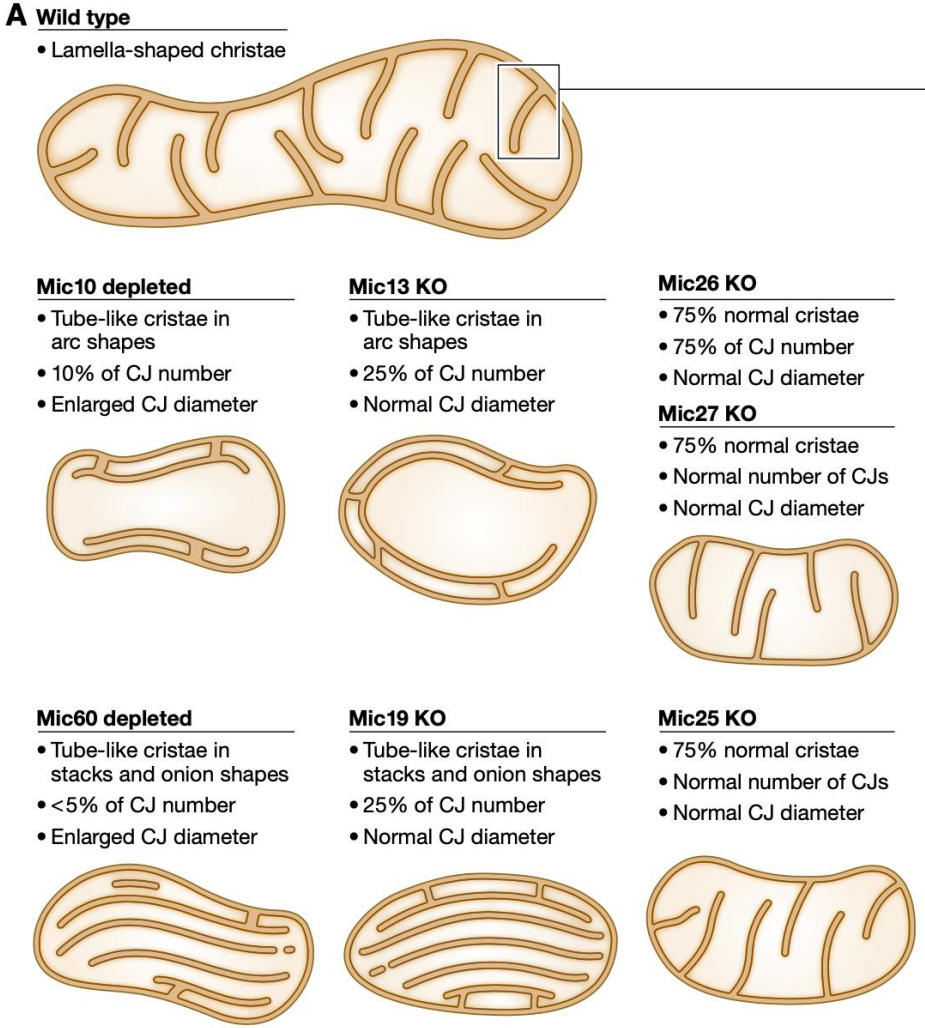
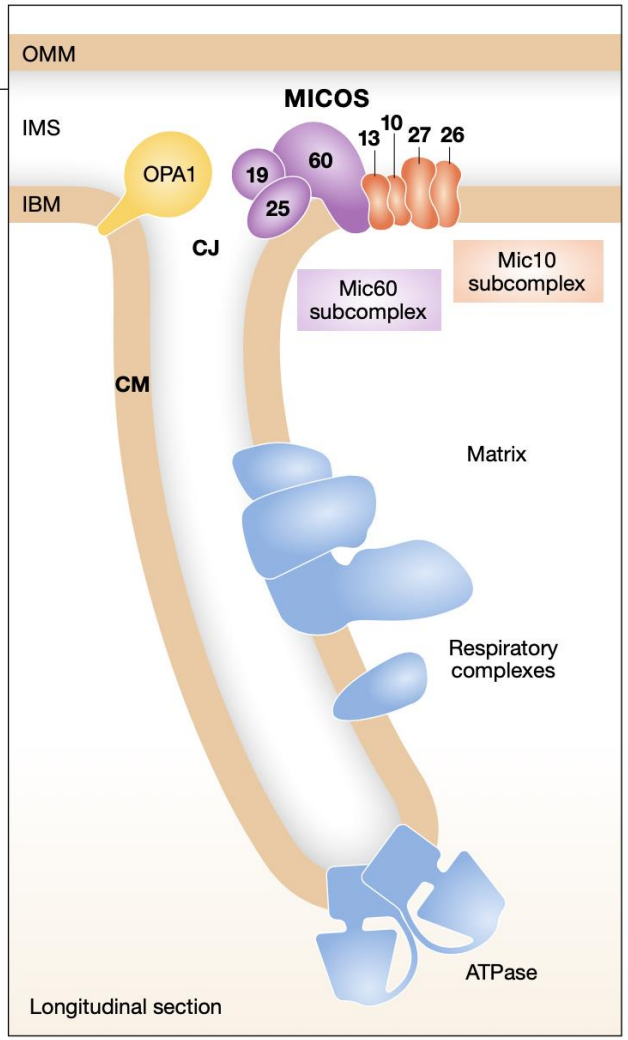


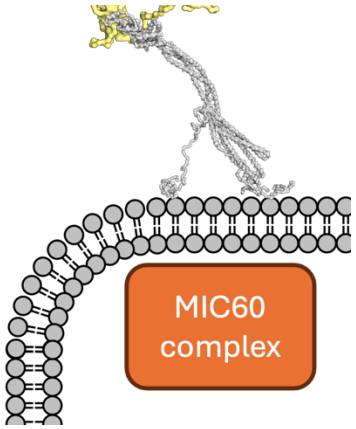
Benaroya, Haym. "Mitochondria and MICOS–function and modeling." *Reviews in the Neurosciences 0* (2024).



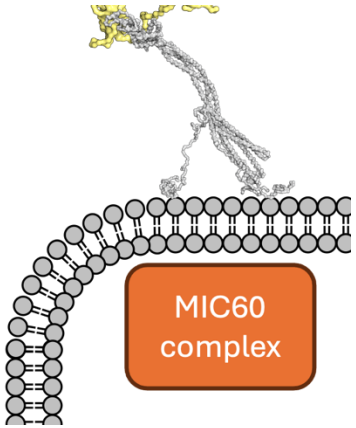


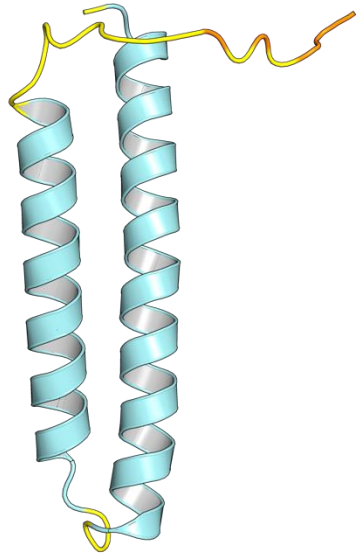


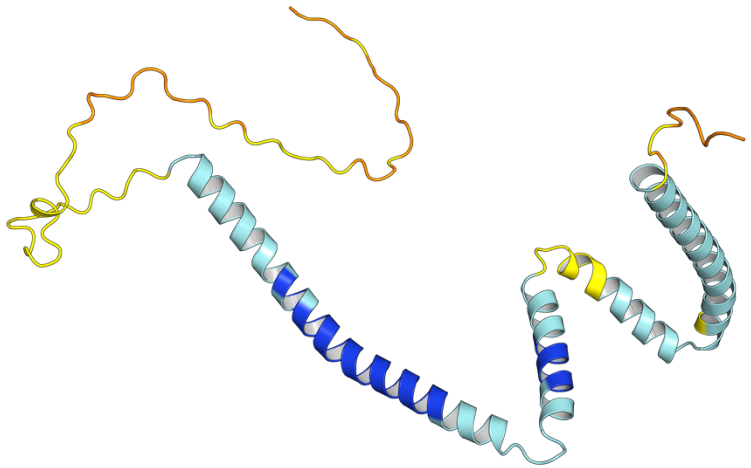
McArthur, Kate, and Michael T. Ryan. "Resolving mitochondrial cristae: introducing a new model into the fold." *The EMBO Journal* 39.14 (2020): e105714.



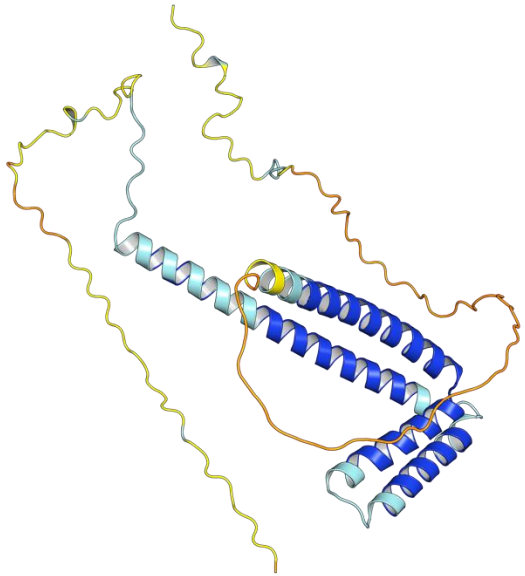
But no complete structures even of homologues







colored by chain

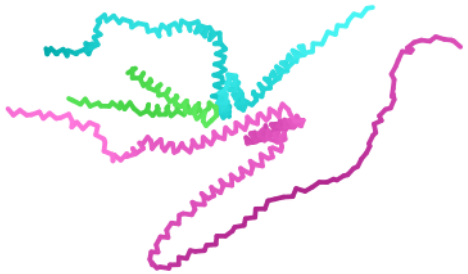


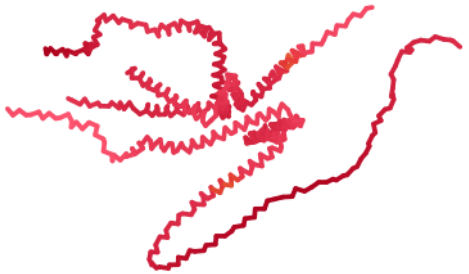
colored by pLDDT

Model Confidence

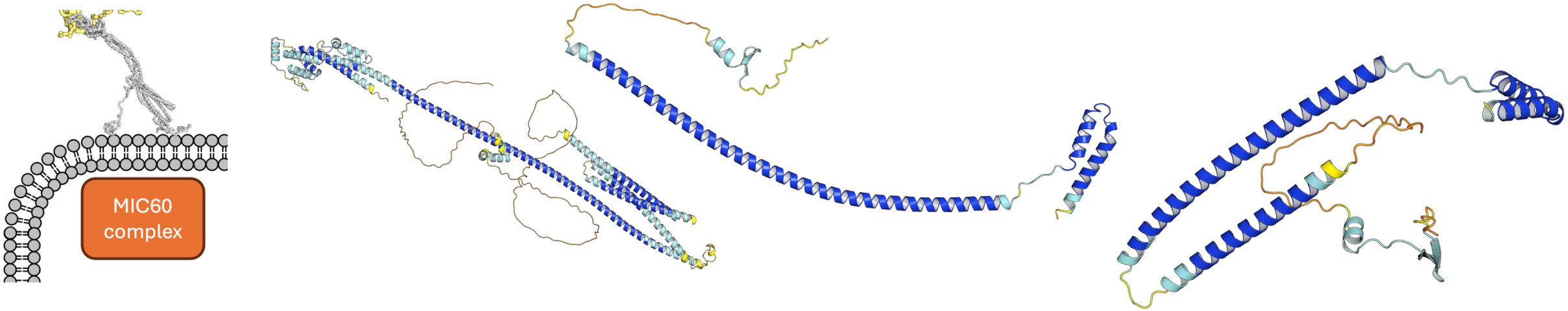
- Very high (pLDDT > 90)
- High (90 > pLDDT > 70)
- Low (70 > pLDDT > 50)
- Very low (pLDDT < 50)

AlphaFold produces a per-residue model confidence score (pLDDT) between 0 and 100. Some regions below 50 pLDDT may be unstructured in isolation.





Bryant, Patrick, Gabriele Pozzati, and Arne Elofsson. "Improved prediction of protein-protein interactions using AlphaFold2." *Nature communications* 13.1 (2022): 1265.



Too big to fold together with AF2

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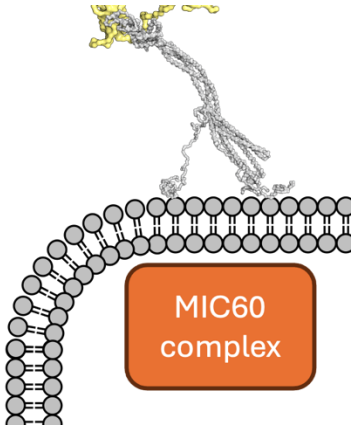

Article

Accurate structure prediction of biomolecular interactions with AlphaFold 3

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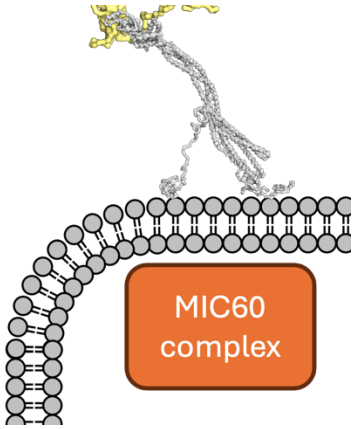

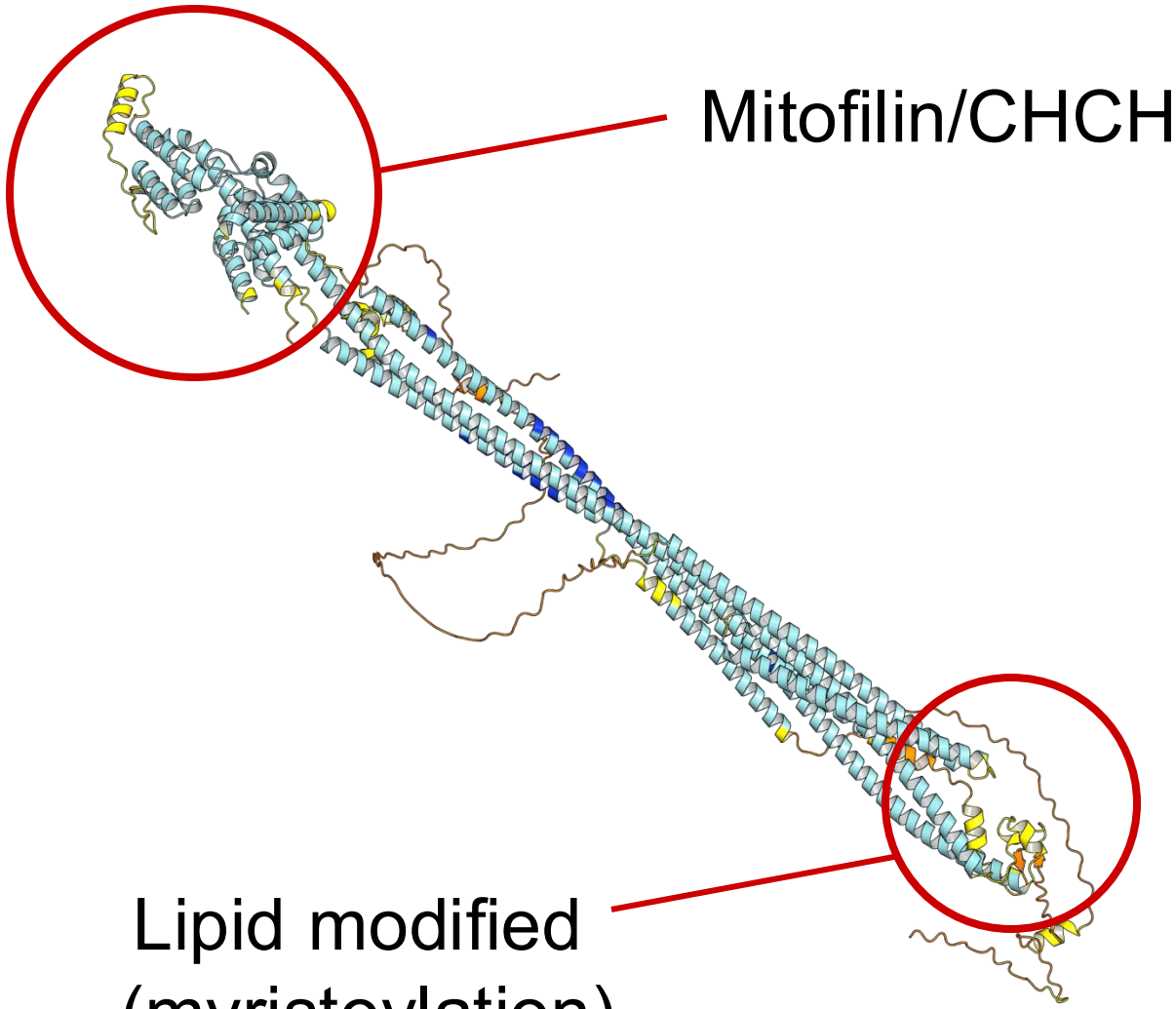

Mitofilin/CHCH domains

Lipid modified
(myristoylation)

Model Confidence

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- High (90 > pLDDT > 70)
- Low (70 > pLDDT > 50)
- Very low (pLDDT < 50)

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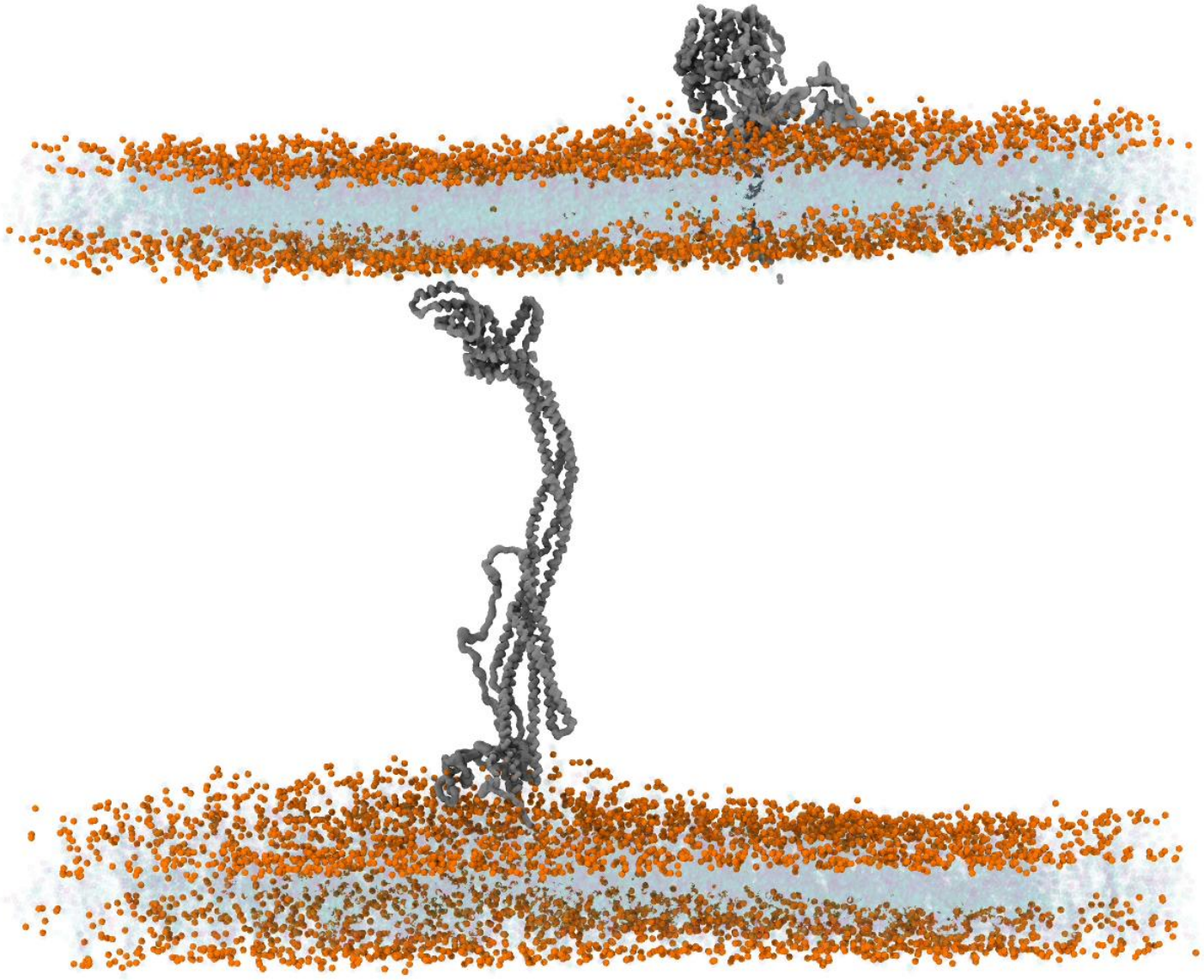

nature COMMUNICATIONS

ARTICLE

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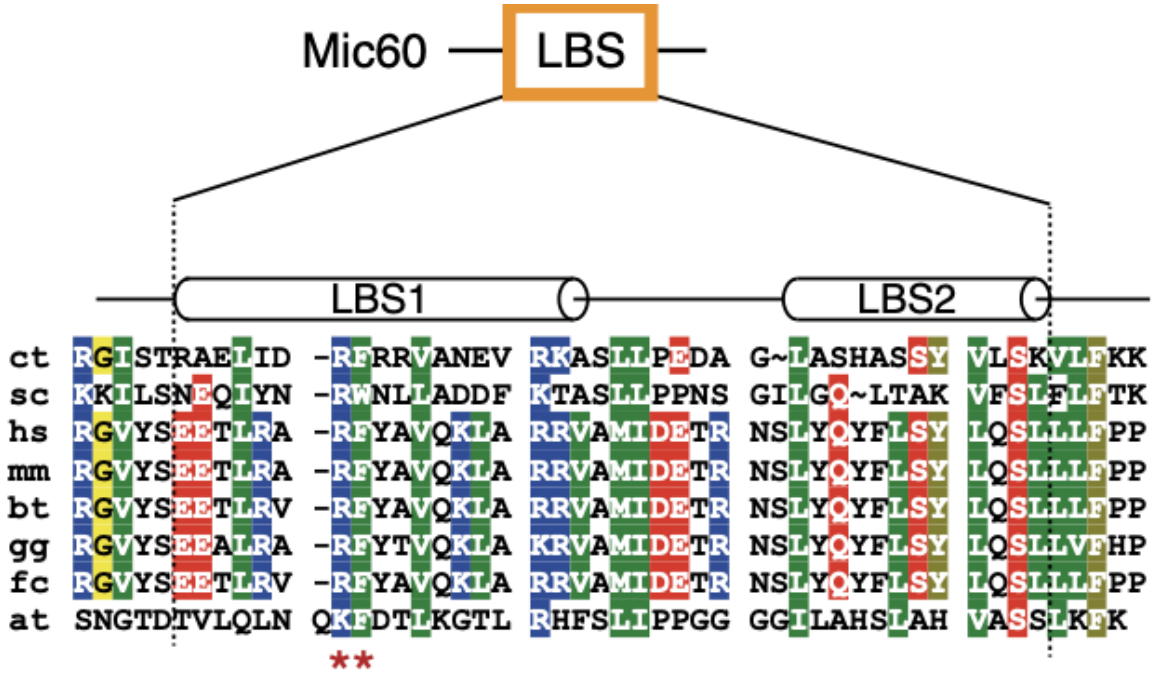
DOI: 10.1038/ncomms15258 OPEN

Regulated membrane remodeling by Mic60 controls formation of mitochondrial crista junctions

Manuel Hessenberger[1,2], Ralf M. Zerbes[3,4], Heike Rampelt[3], Séverine Kunz[1,5], Audrey H. Xavier[1,2], Bettina Purfürst[5], Hauke Lilie[6], Nikolaus Pfanner[3,7], Martin van der Laan[8] & Oliver Daumke[1,2]



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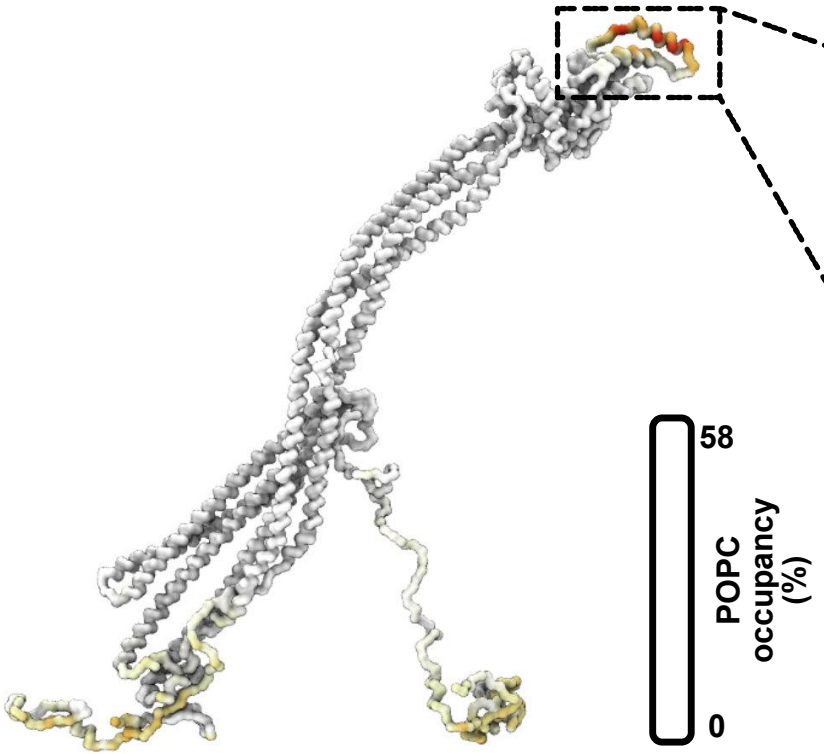
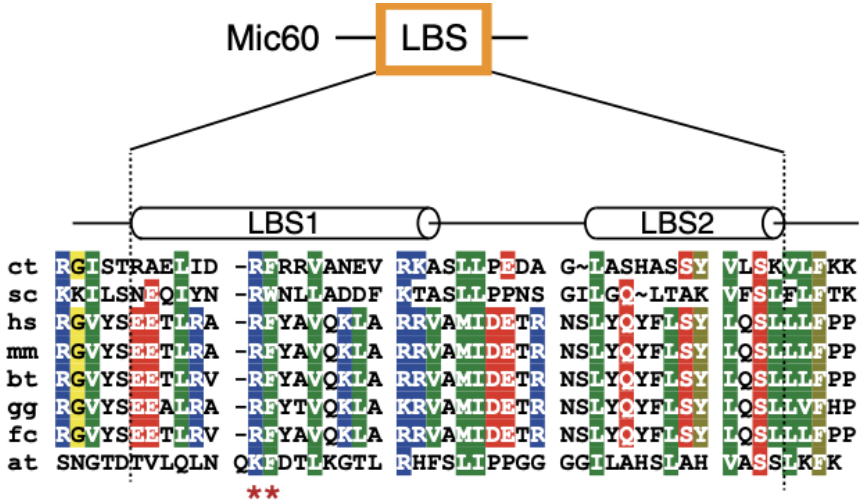
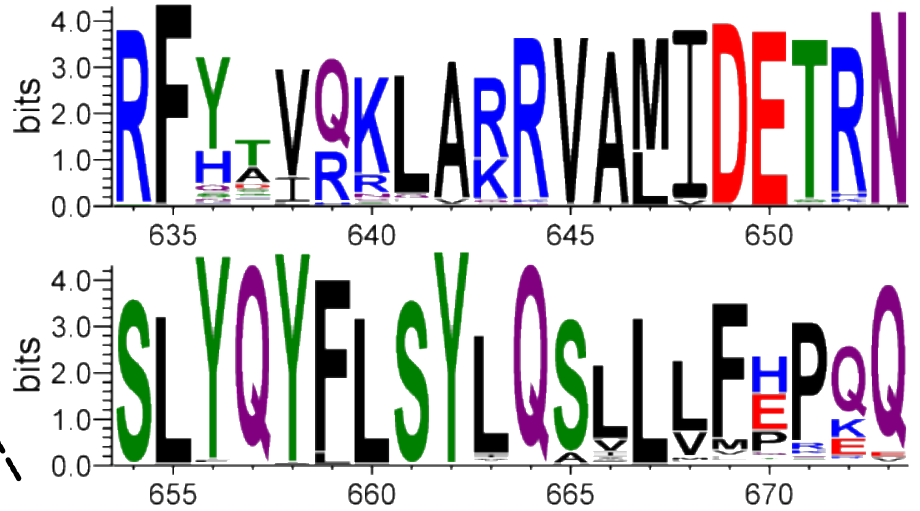
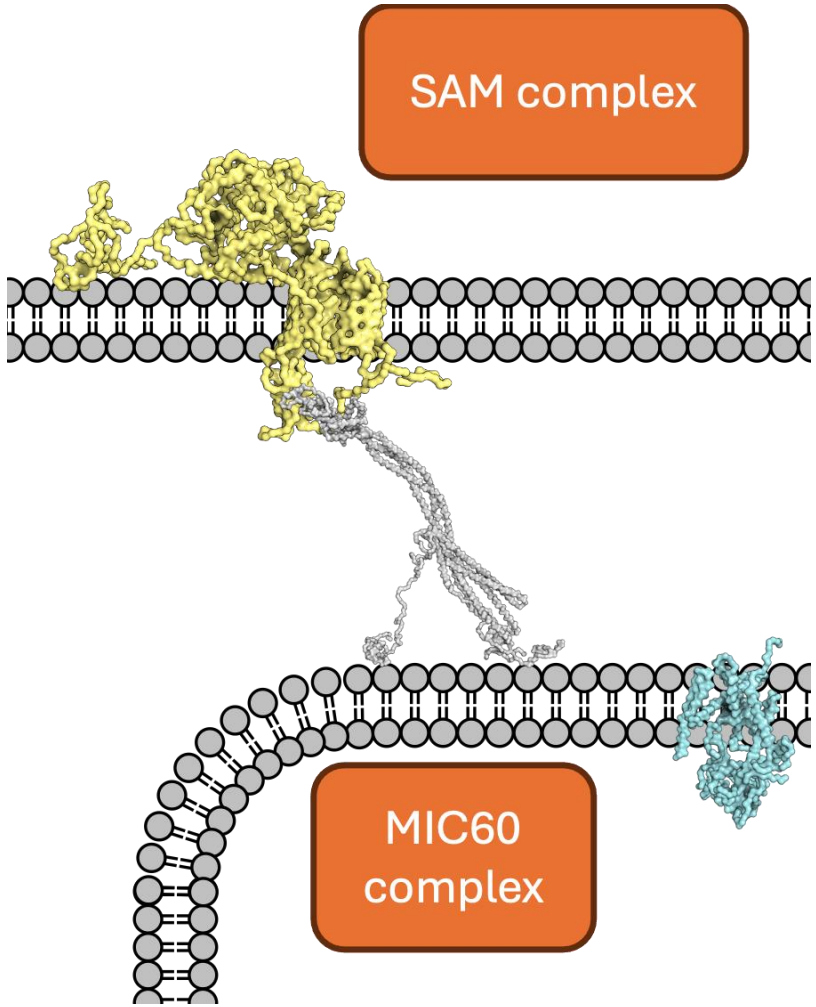




MIC60 conservation

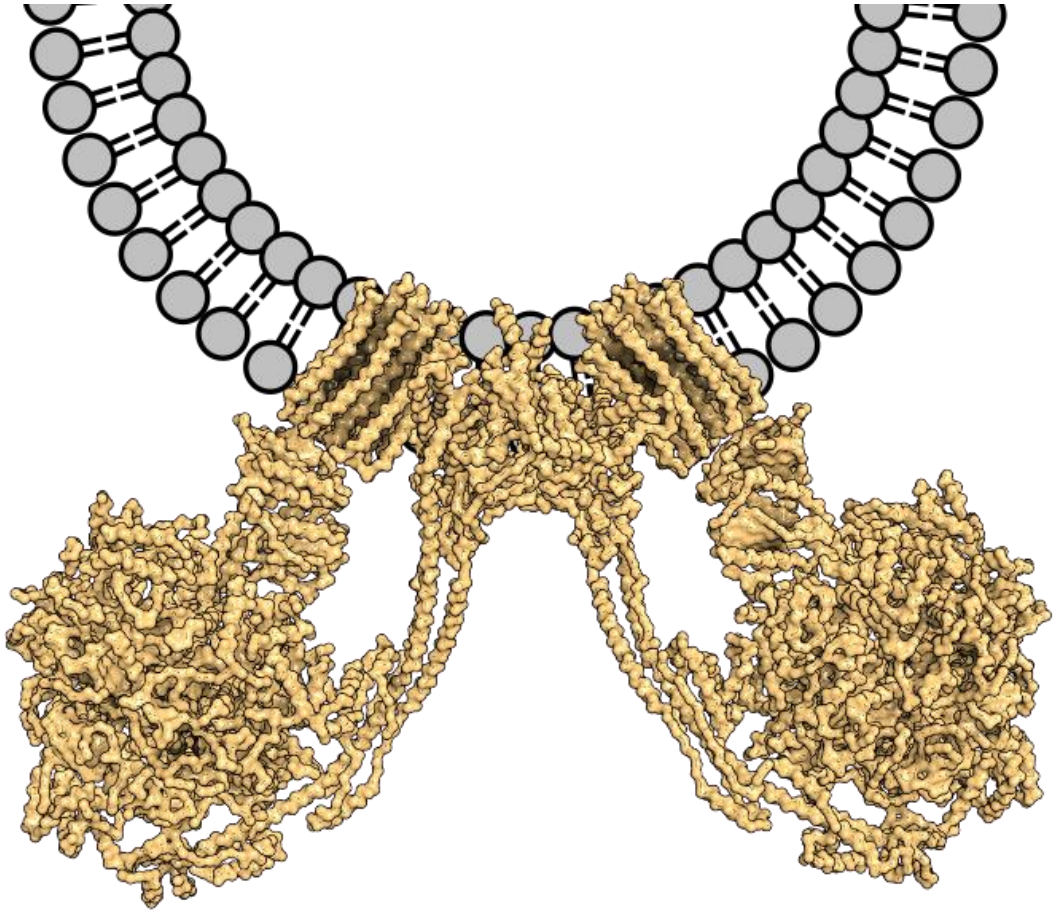


Song, Wanling, et al. "PyLipID: a python package for analysis of protein–lipid interactions from molecular dynamics simulations." *Journal of Chemical Theory and Computation* 18.2 (2022): 1188-1201.

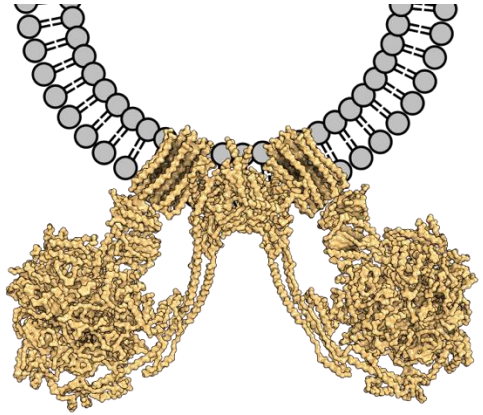


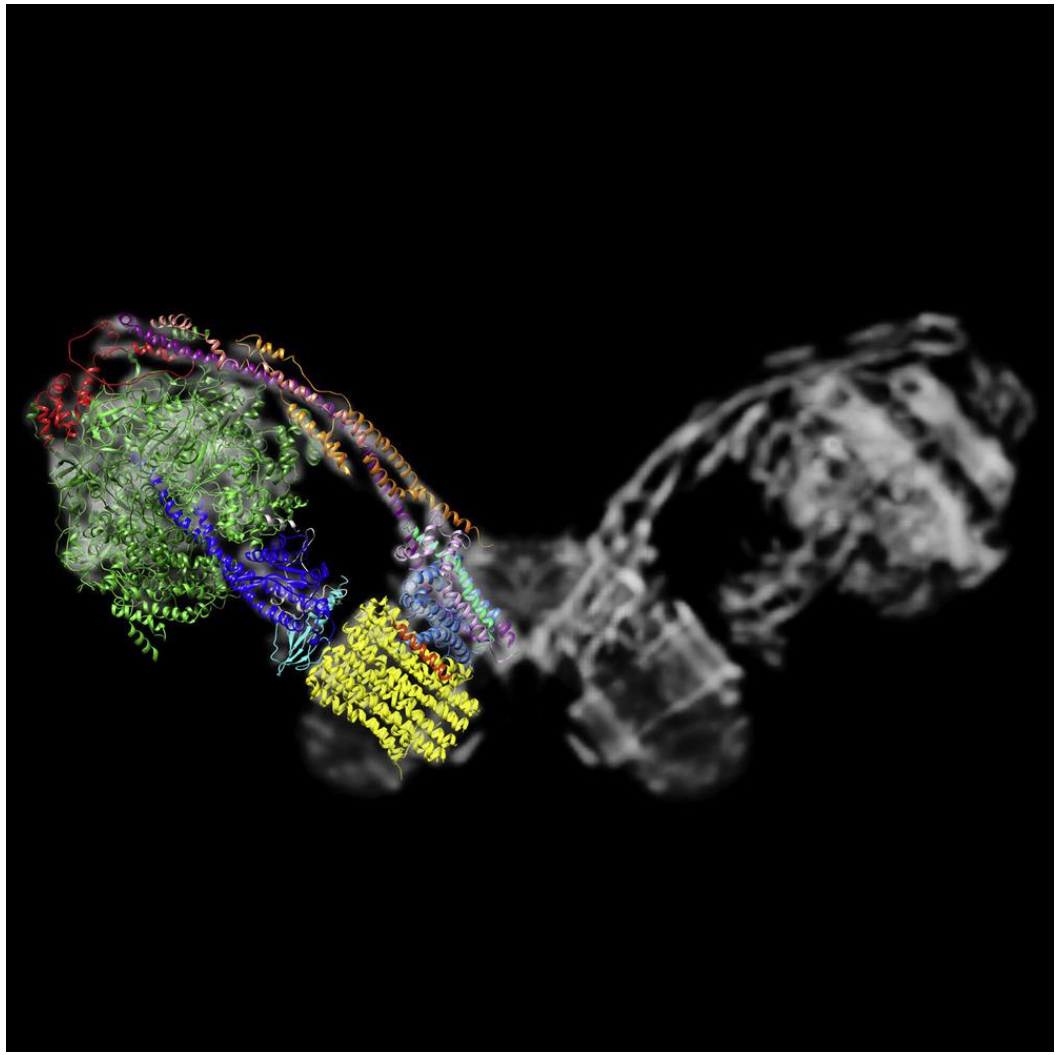
✅ Mitochondrial bridging site

ATPase dimer rows



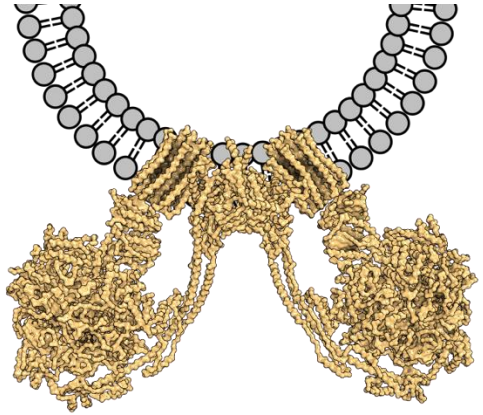
ATPase dimer rows

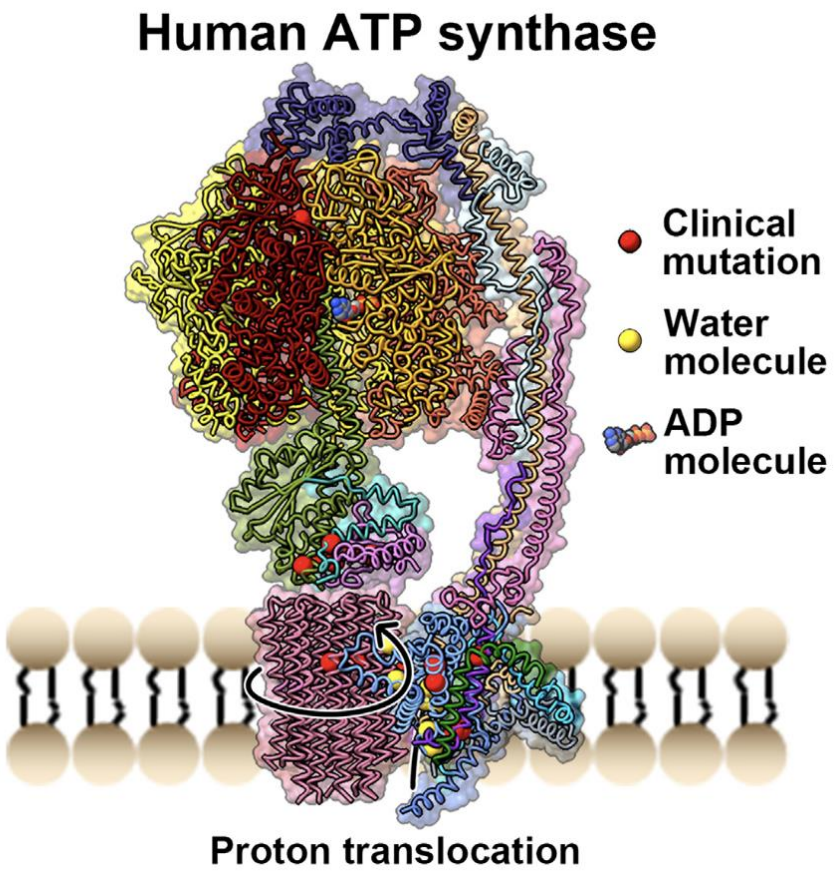




Hahn, Alexander, et al. "Structure of a complete ATP synthase dimer reveals the molecular basis of inner mitochondrial membrane morphology." *Molecular cell* 63.3 (2016): 445-456.

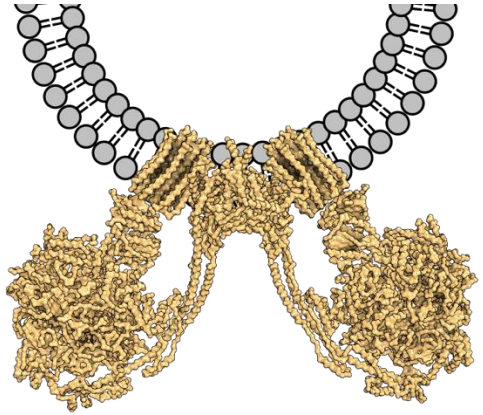
ATPase dimer rows

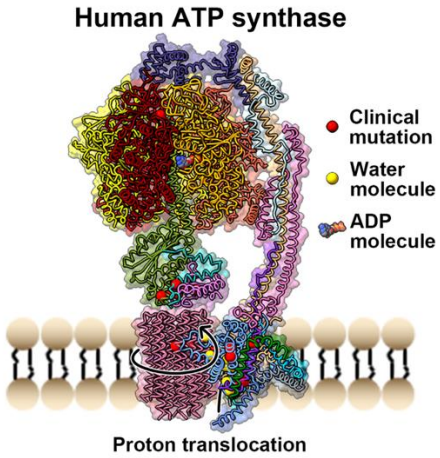




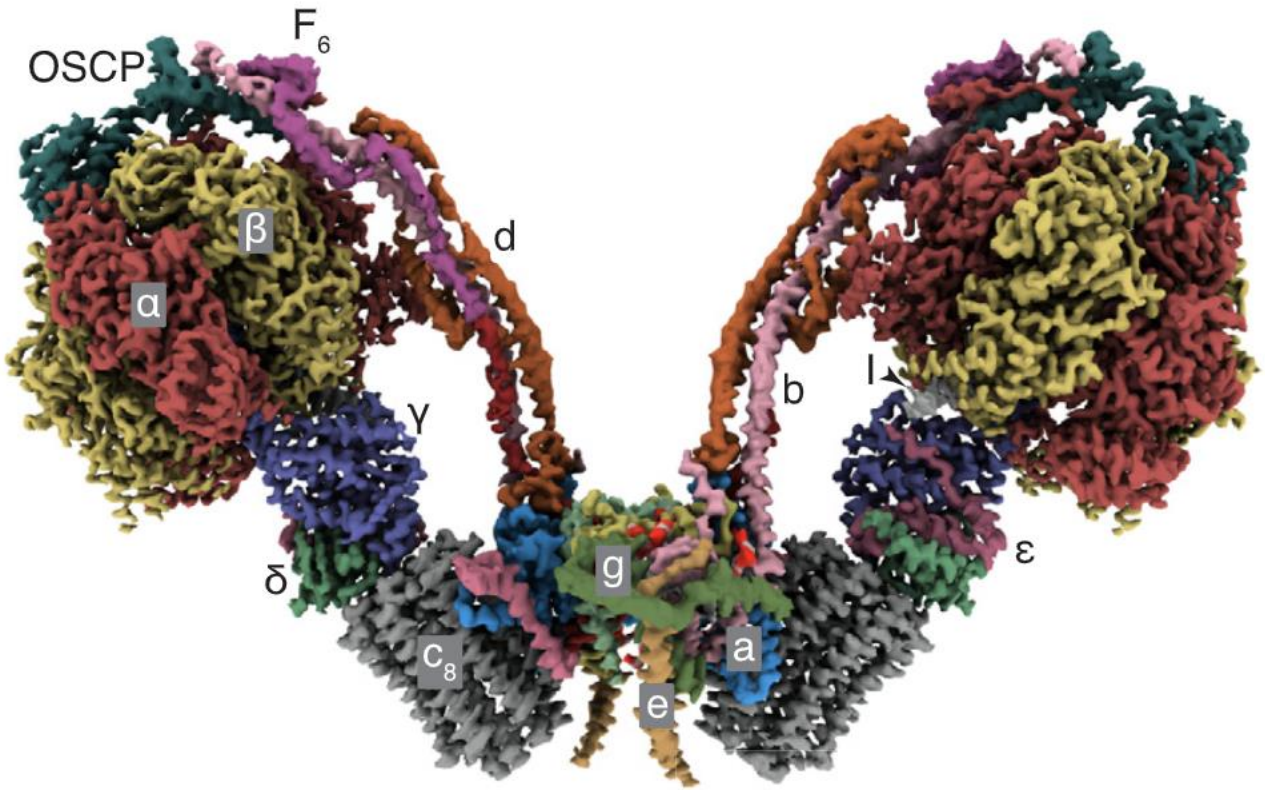
Lai, Yuezheng, et al. "Structure of the human ATP synthase." *Molecular Cell* 83.12 (2023): 2137-2147.

ATPase dimer rows





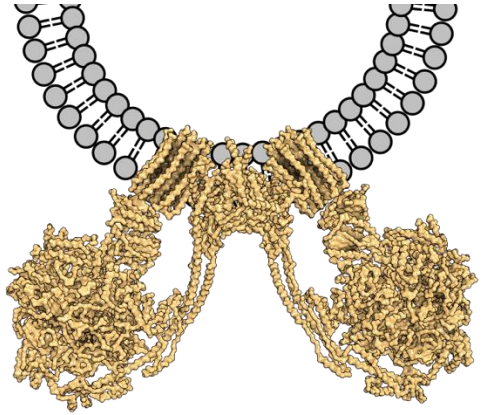
Bovine ATP synthase

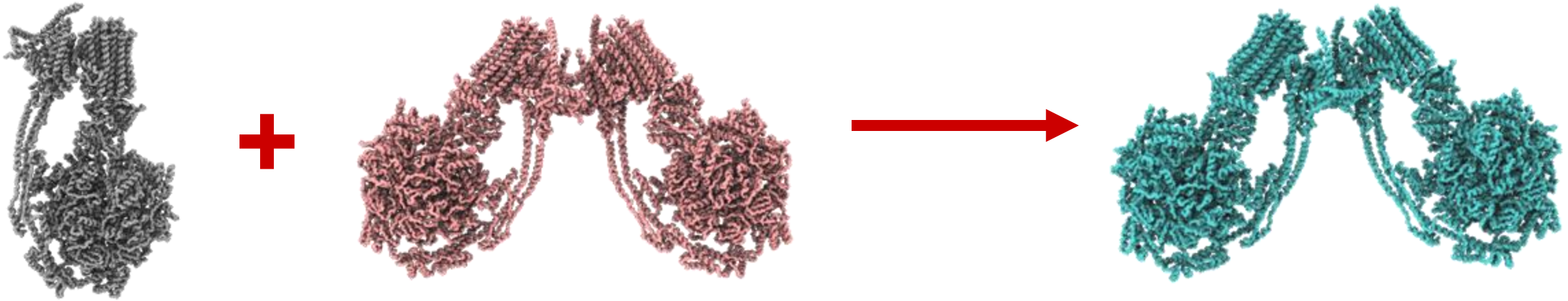


Lai, Yuezheng, et al. "Structure of the human ATP synthase." *Molecular Cell* 83.12 (2023): 2137-2147.

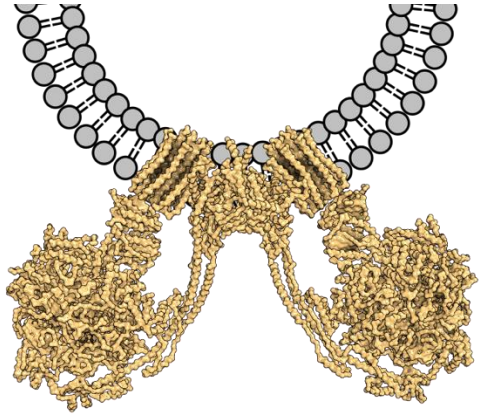
Spikes, Tobias E., Martin G. Montgomery, and John E. Walker. "Structure of the dimeric ATP synthase from bovine mitochondria." *Proceedings of the National Academy of Sciences* 117.38 (2020): 23519-23526.

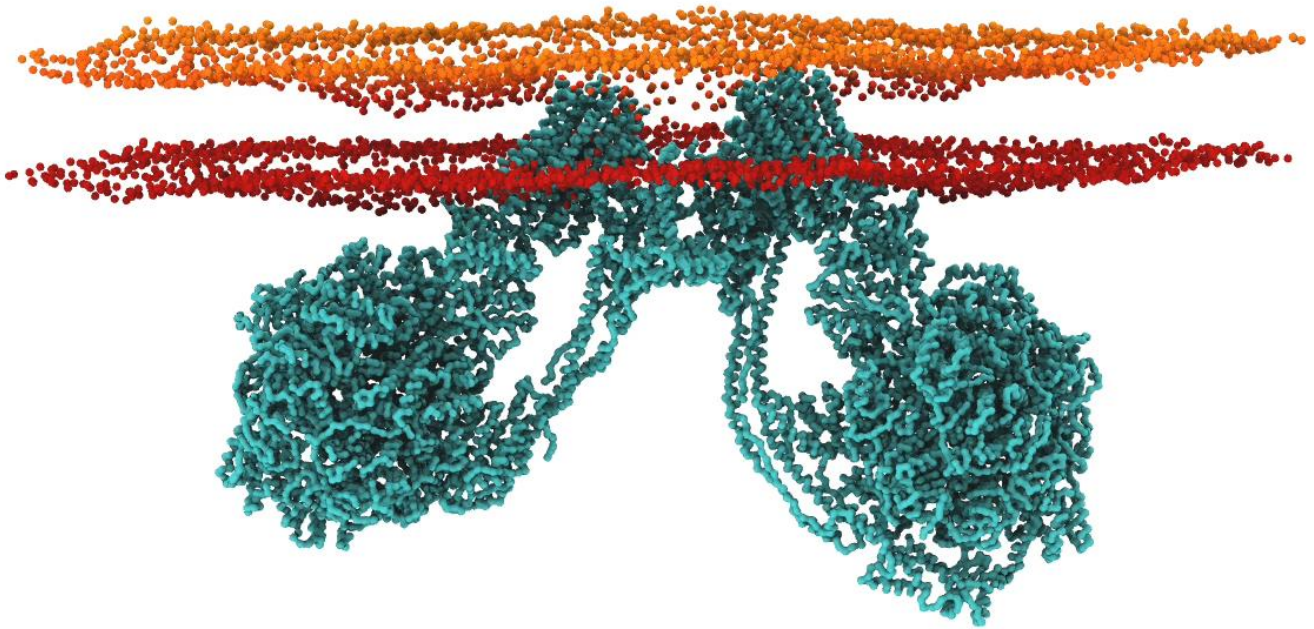
ATPase dimer rows



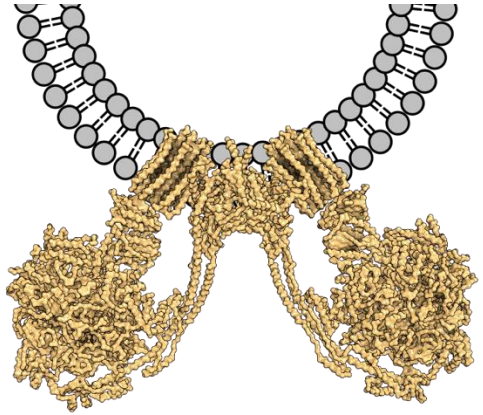


ATPase dimer rows





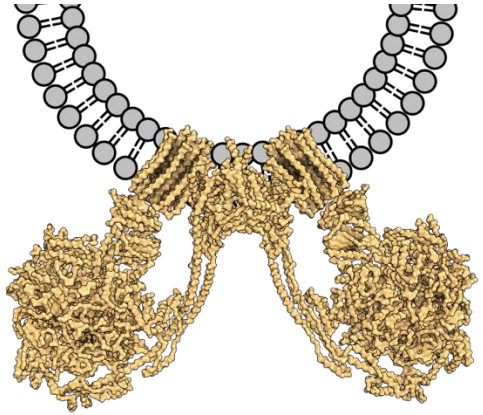
ATPase dimer rows



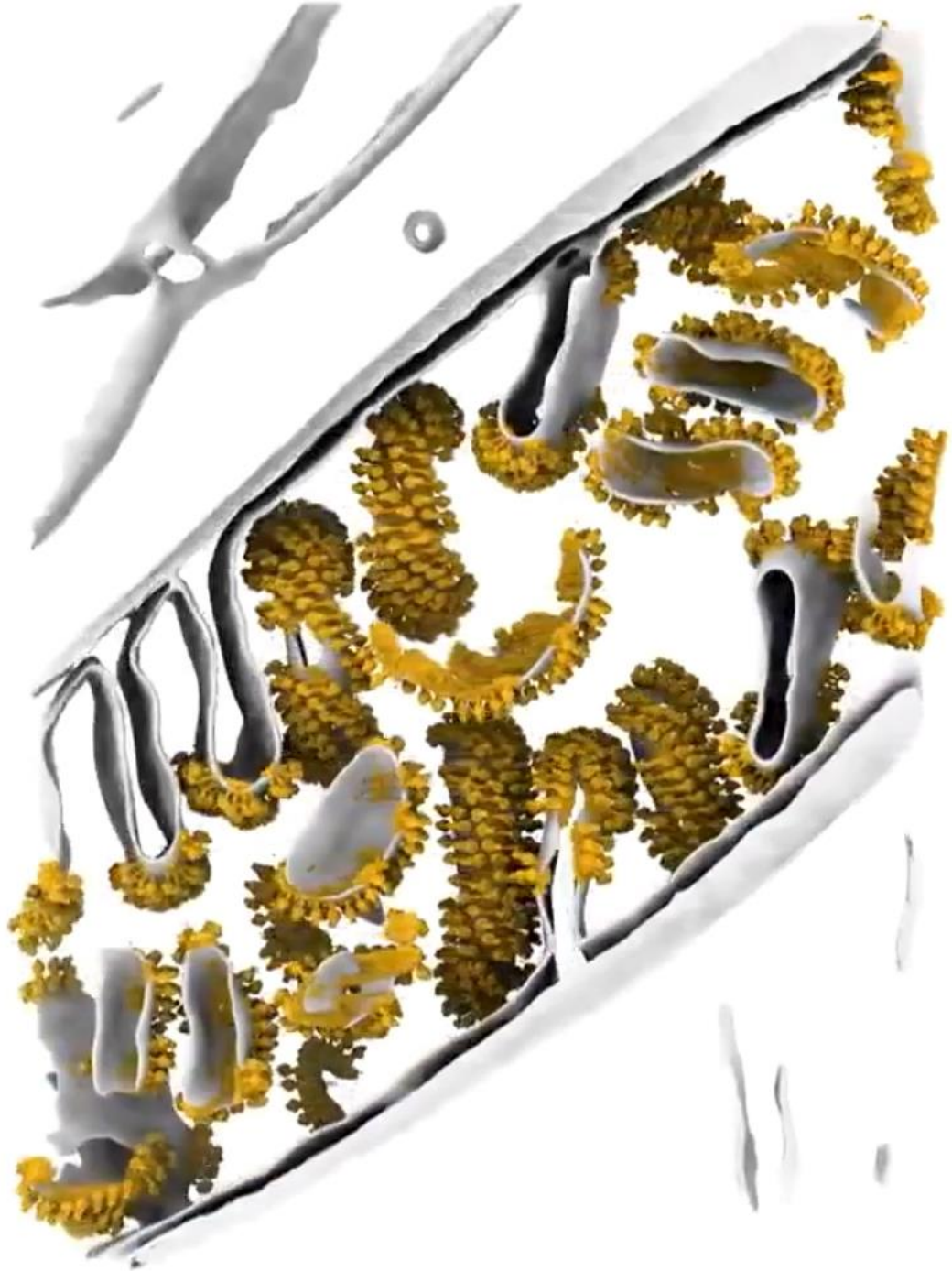
But dimers do not only exist by themselves

university of groningen

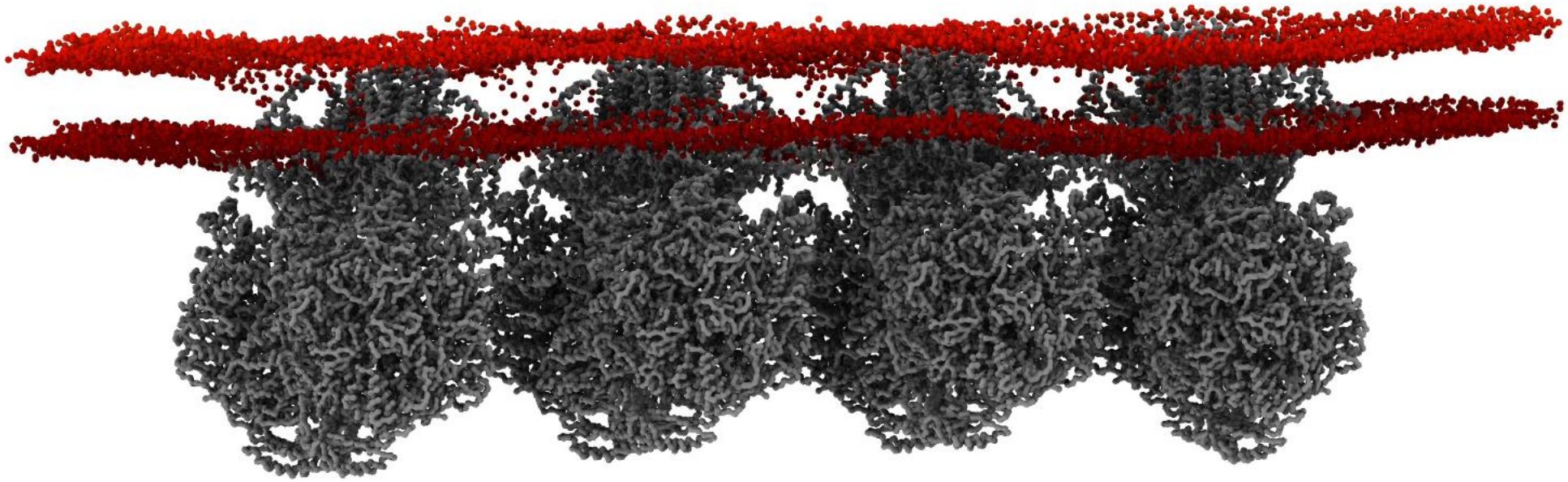
ATPase dimer rows



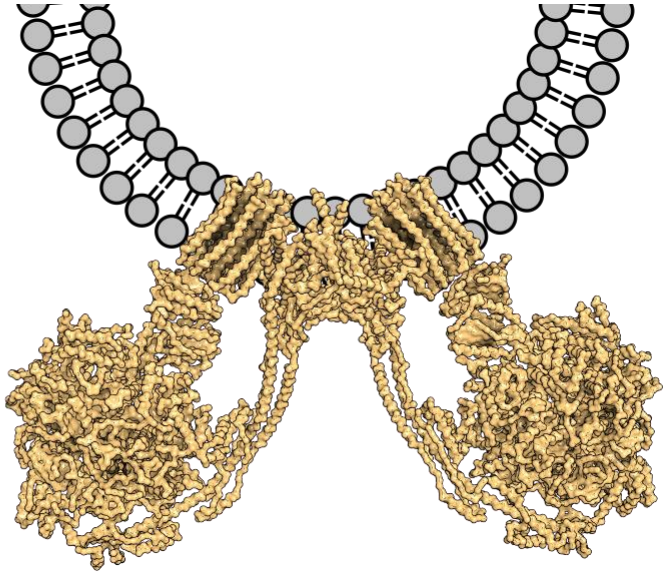
Dimers form rows in mammals



Dietrich, L., Agip, A. N. A., Kunz, C., Schwarz, A., & Kühlbrandt, W. (2024). In situ structure and rotary states of mitochondrial ATP synthase in whole Polytomella cells. *Science*, 385(6713), 1086-1090.

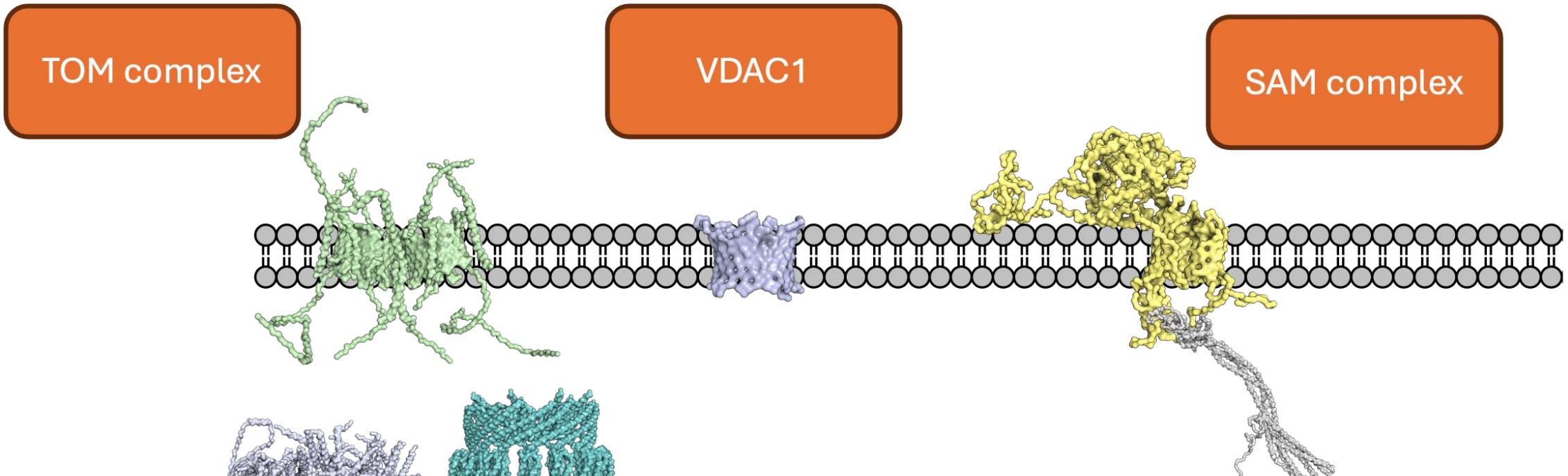


ATPase dimer rows

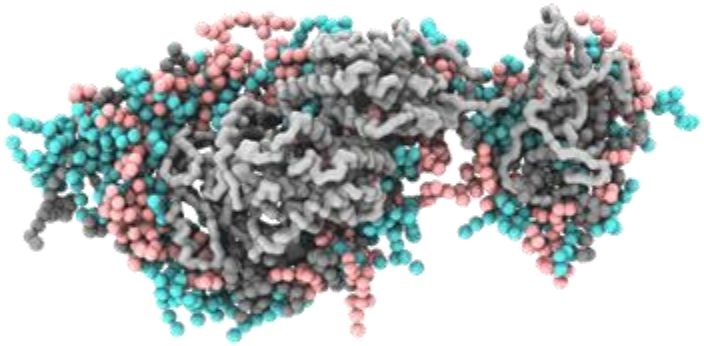


✅ Curvature inducing proteins

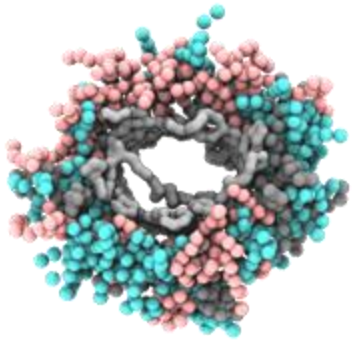
Building the outer membrane of a cristae junction



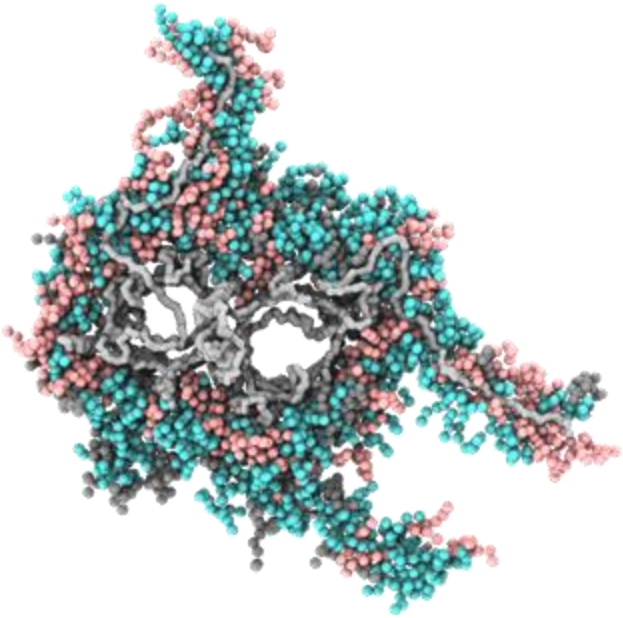
SAM complex



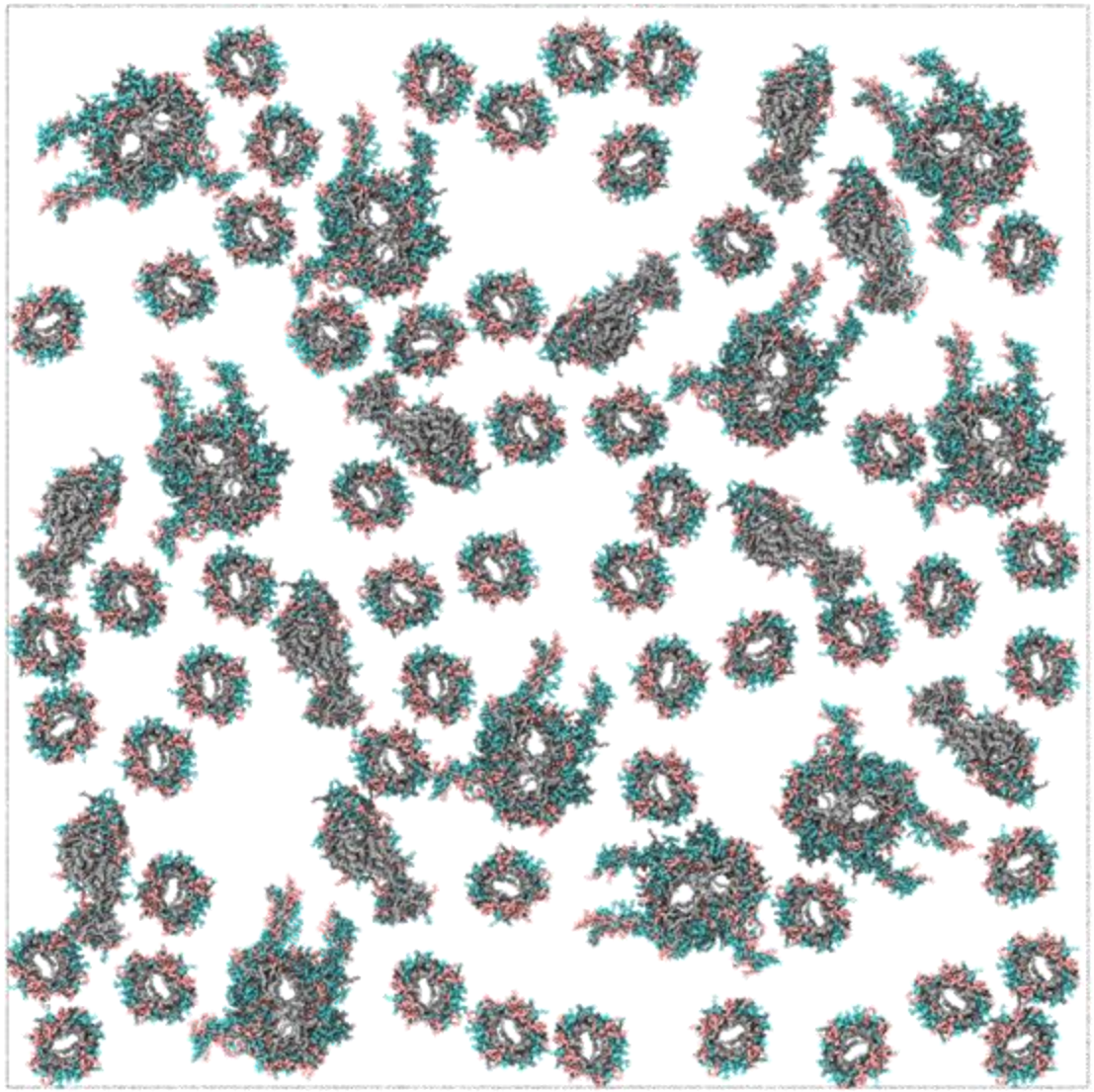
VDAC1



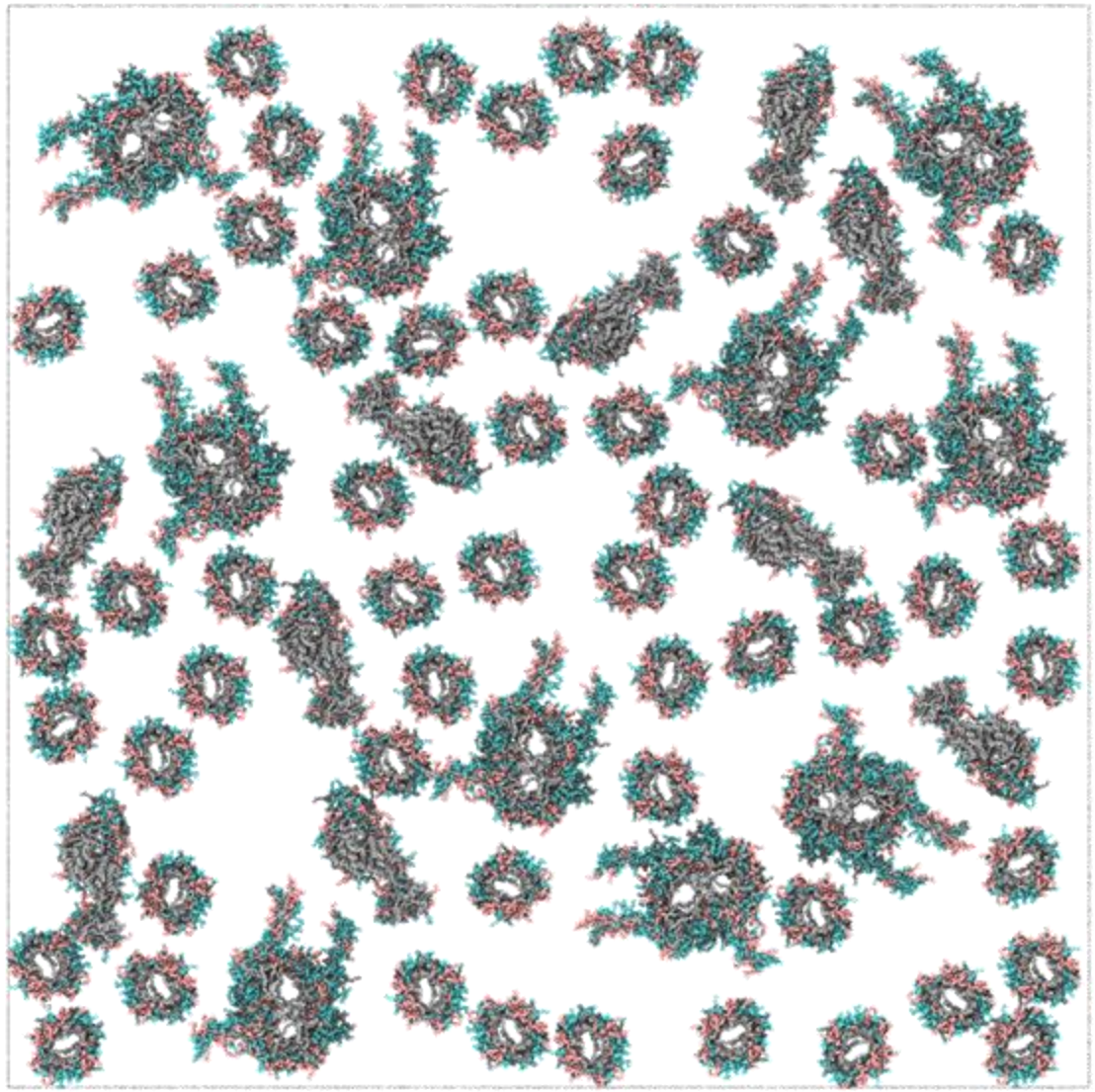
TOM complex



VDAC1: 53
SAM complex: 10
TOM complex: 10



Built with *bentopy* (Westendorp, M. S. S., Stevens, J. A., Bruininks, B. M. H., & Marrink, S.-J. (2024). Bentopy: building molecular dynamics simulations with cellular complexity and scale. Zenodo. https://doi.org/10.5281/zenodo.13818758)



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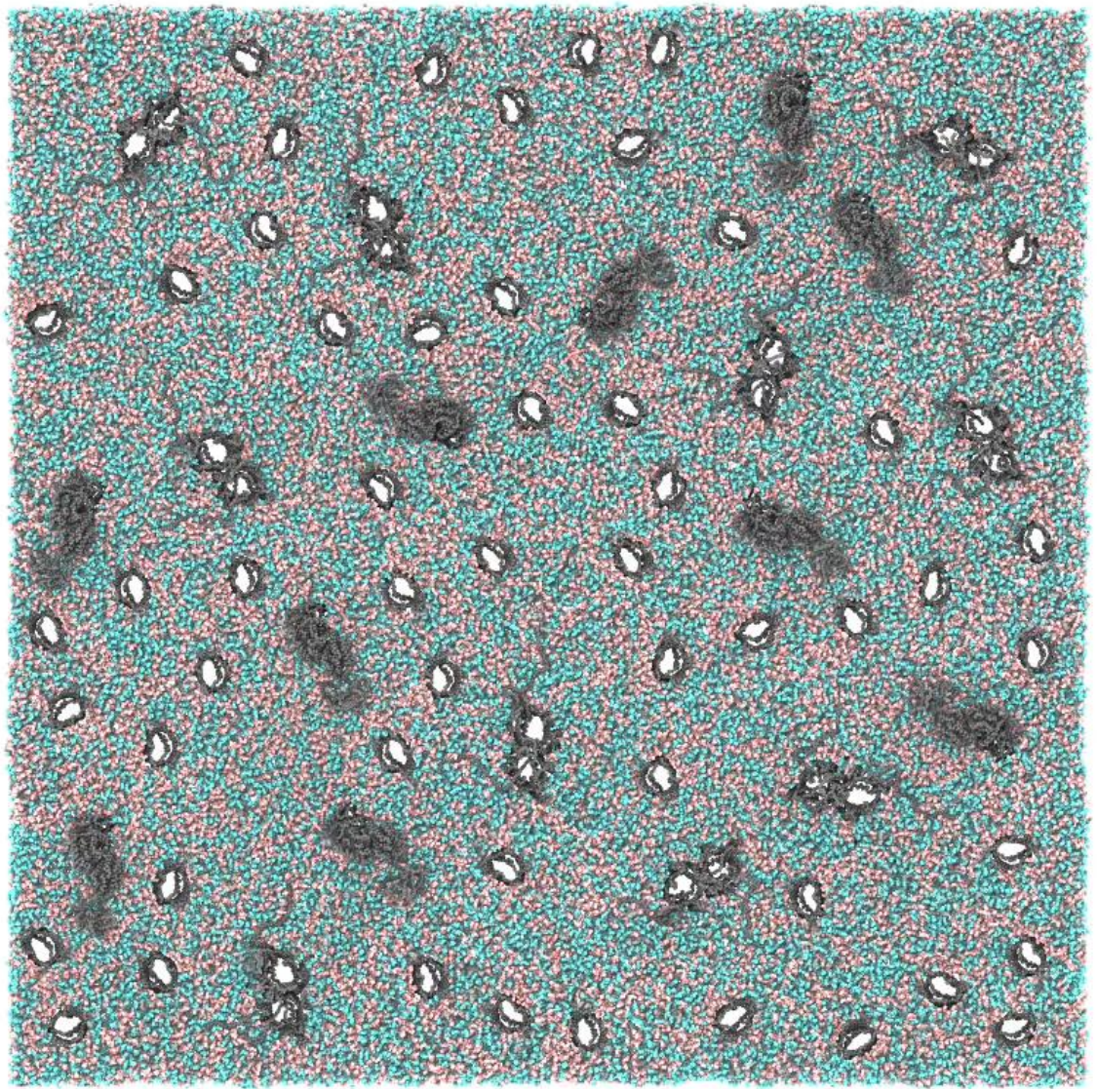
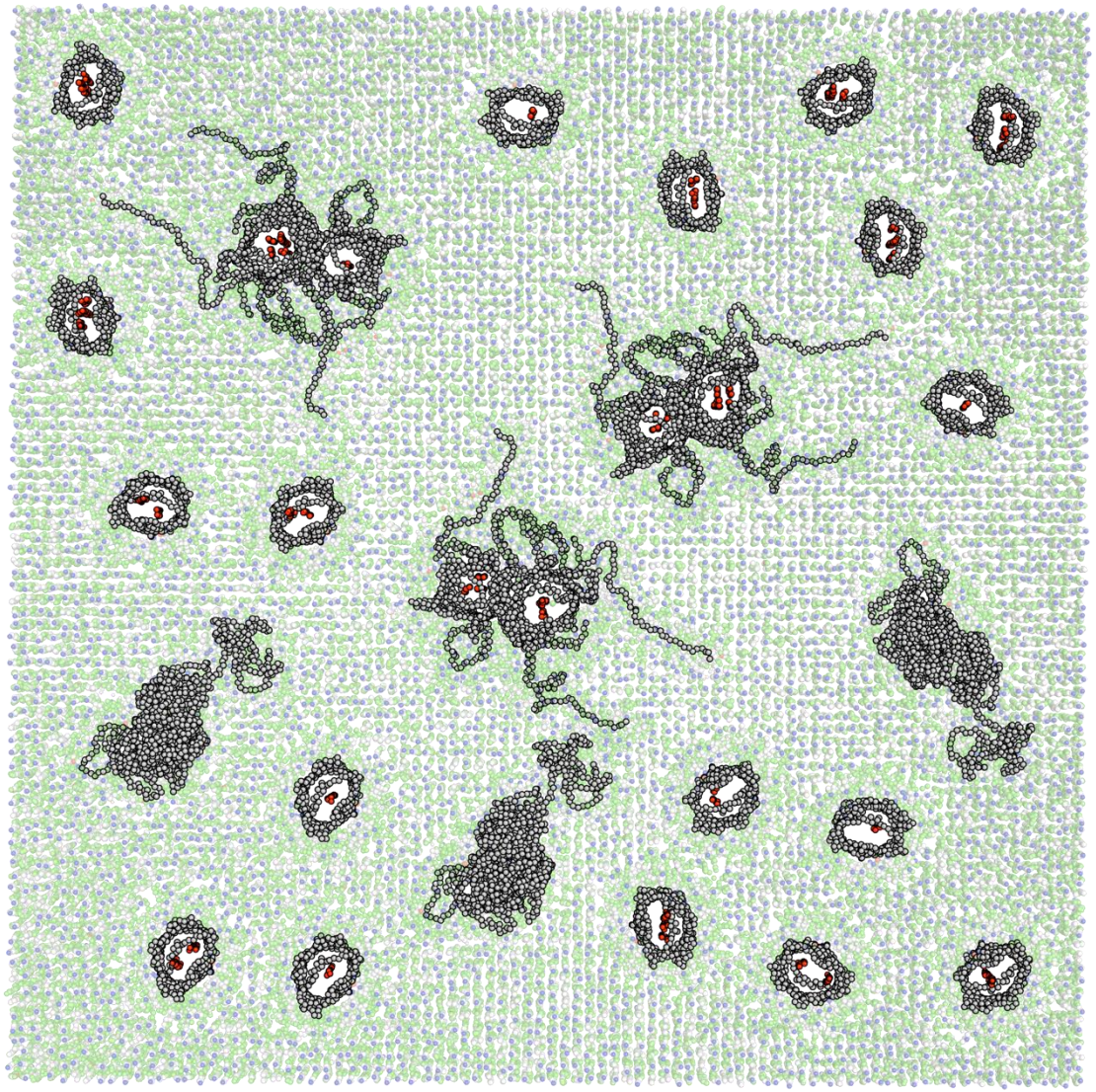
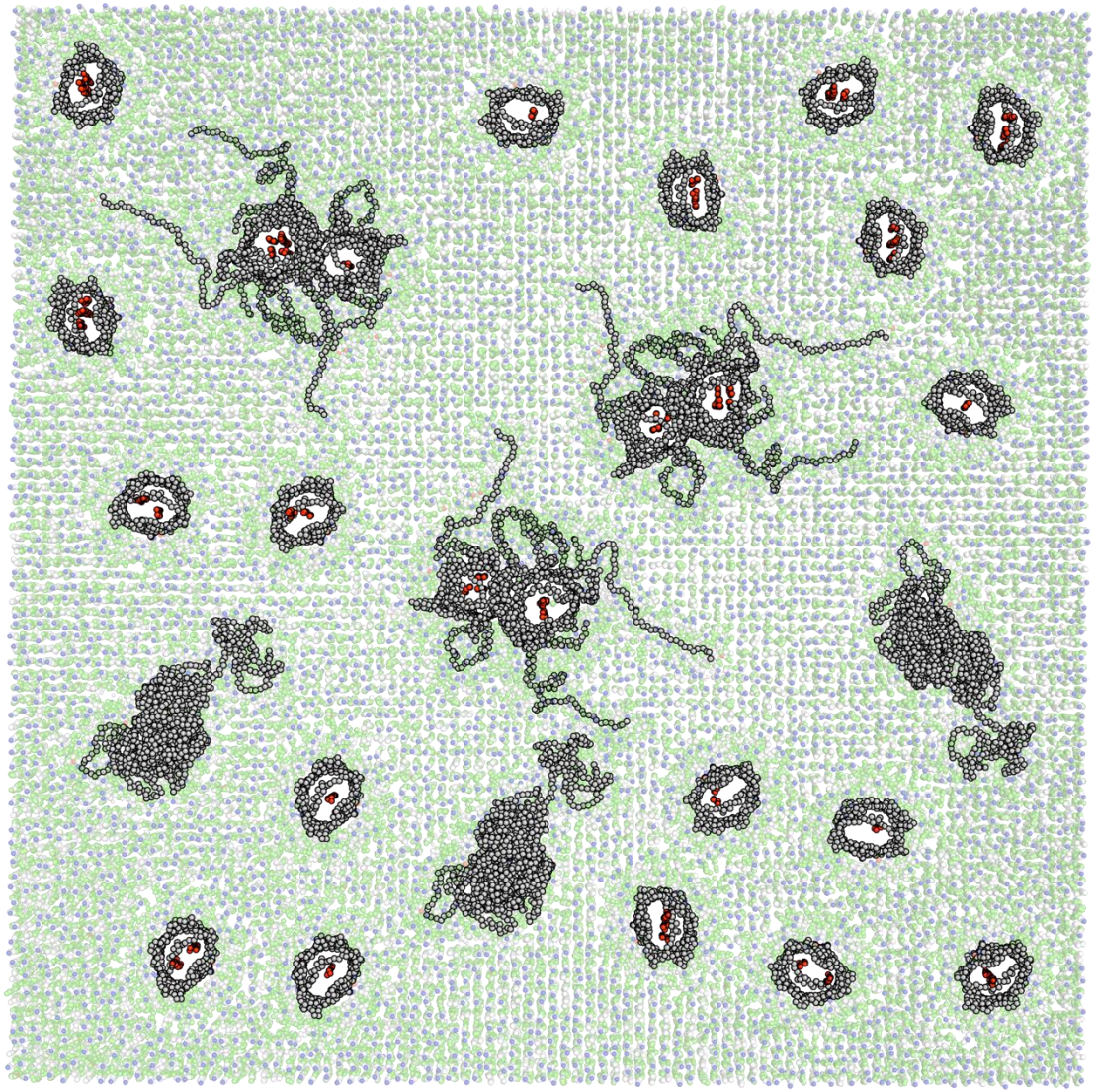
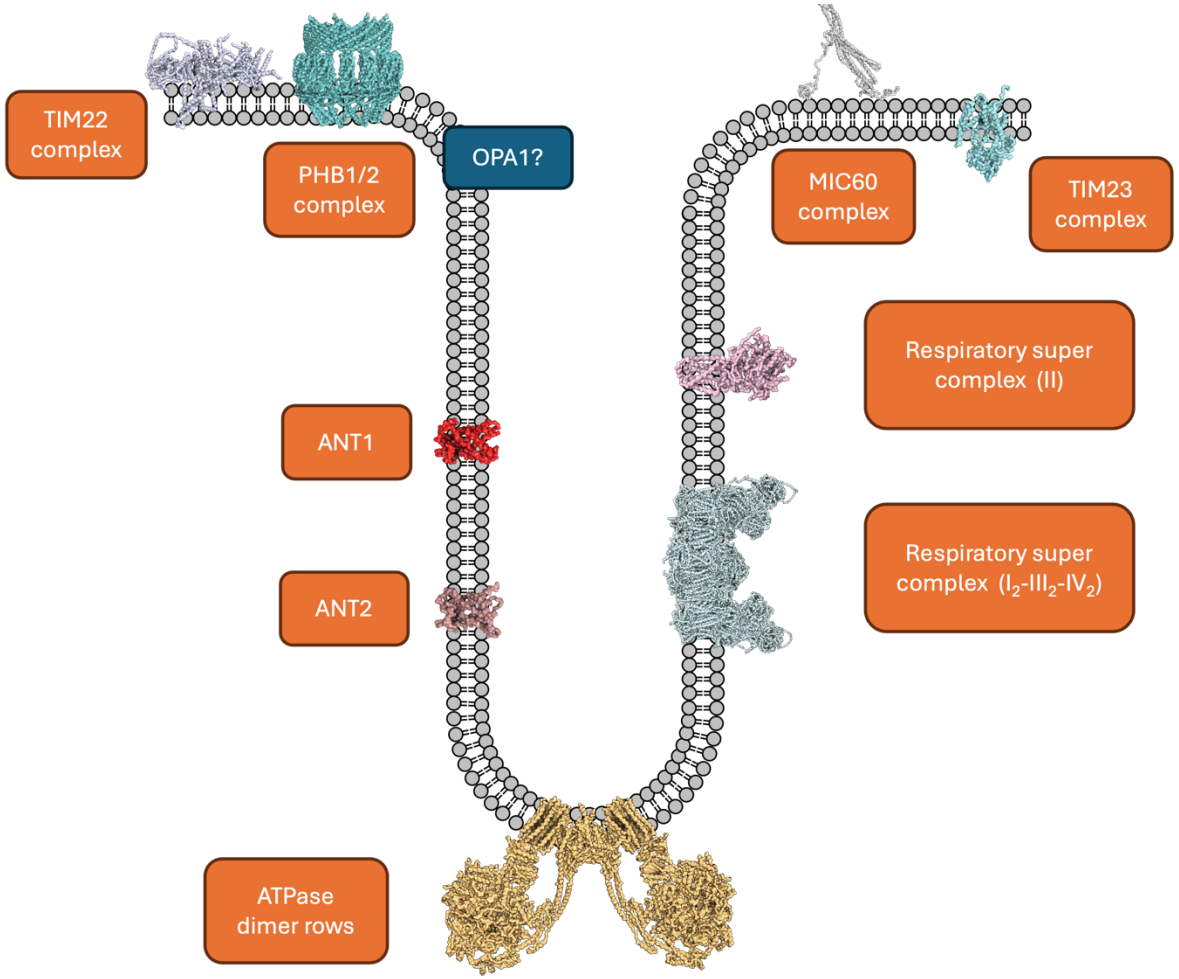


Wassenaar, Tsjerk A., et al. "Computational lipidomics with insane: a versatile tool for generating custom membranes for molecular simulations." *Journal of chemical theory and computation* 11.5 (2015): 2144-2155.

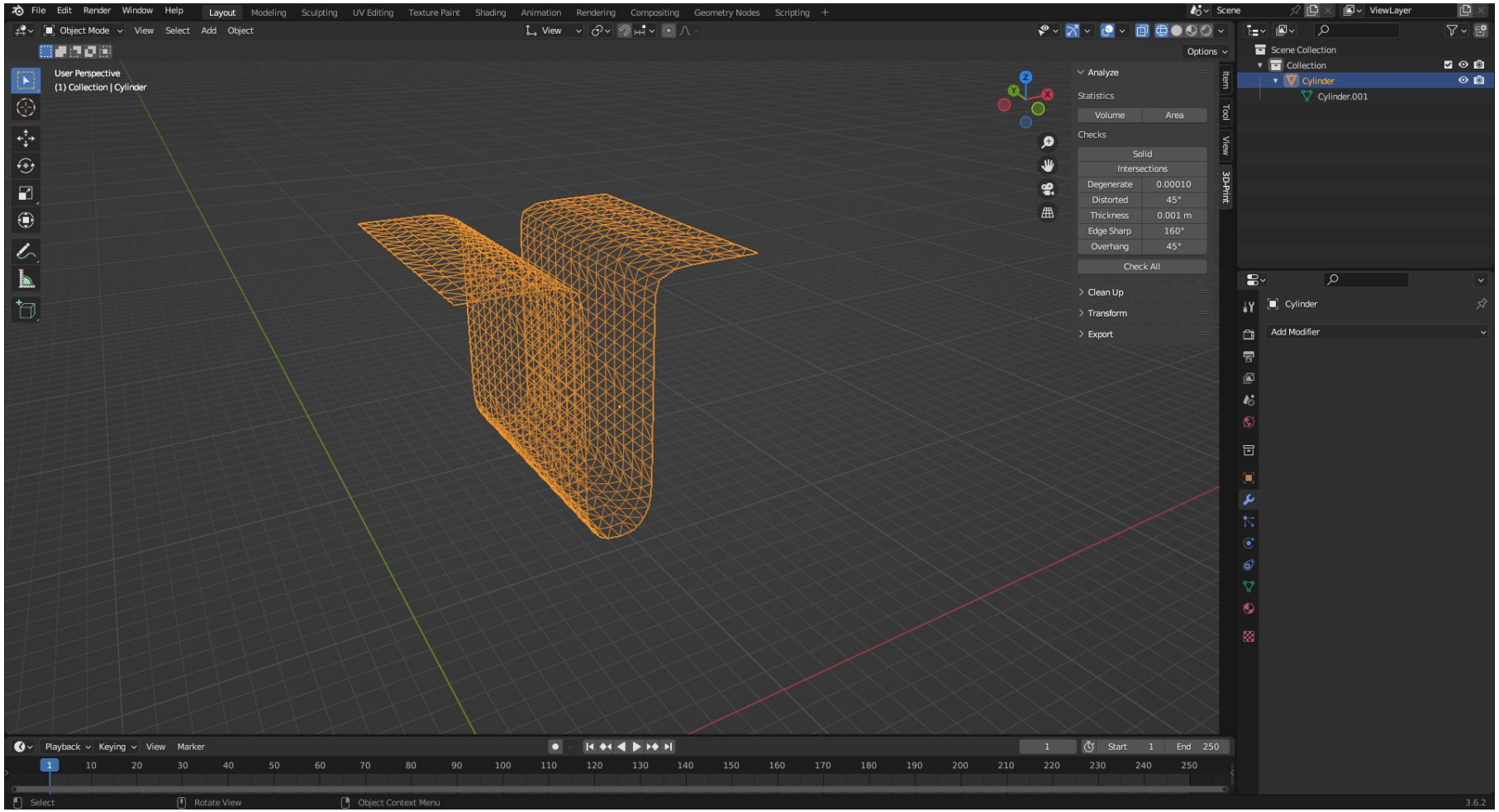


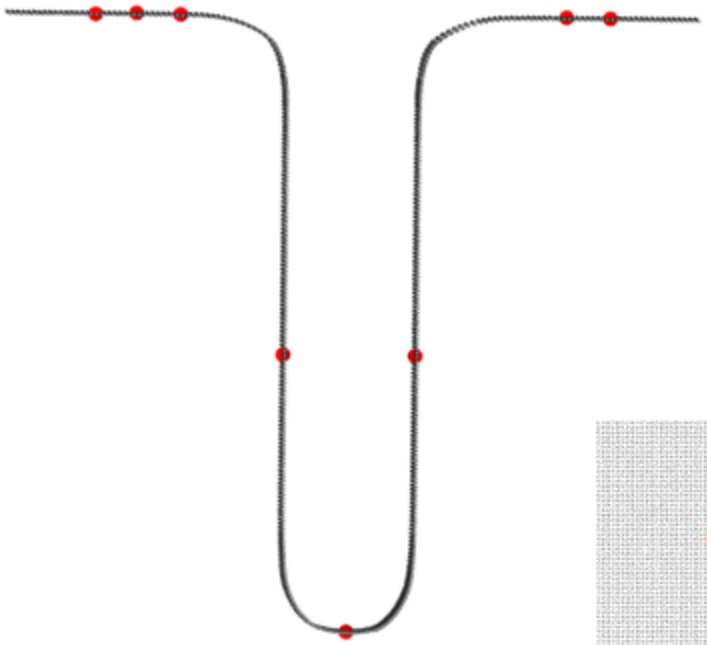


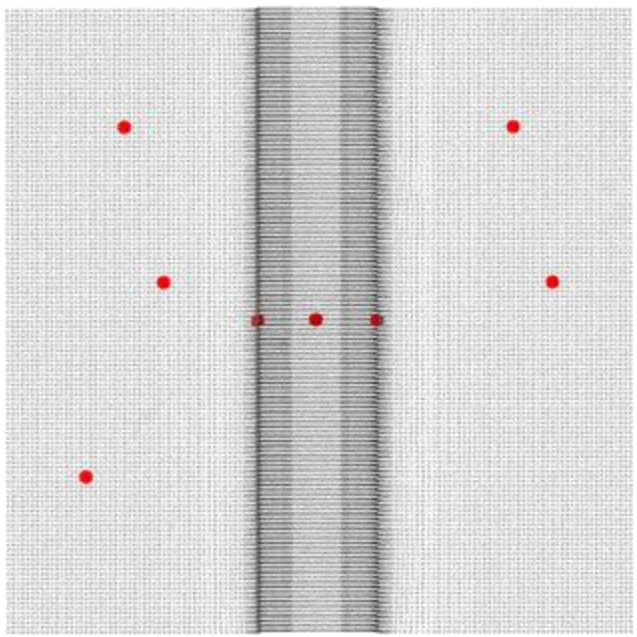
Use TS2CG or another method?

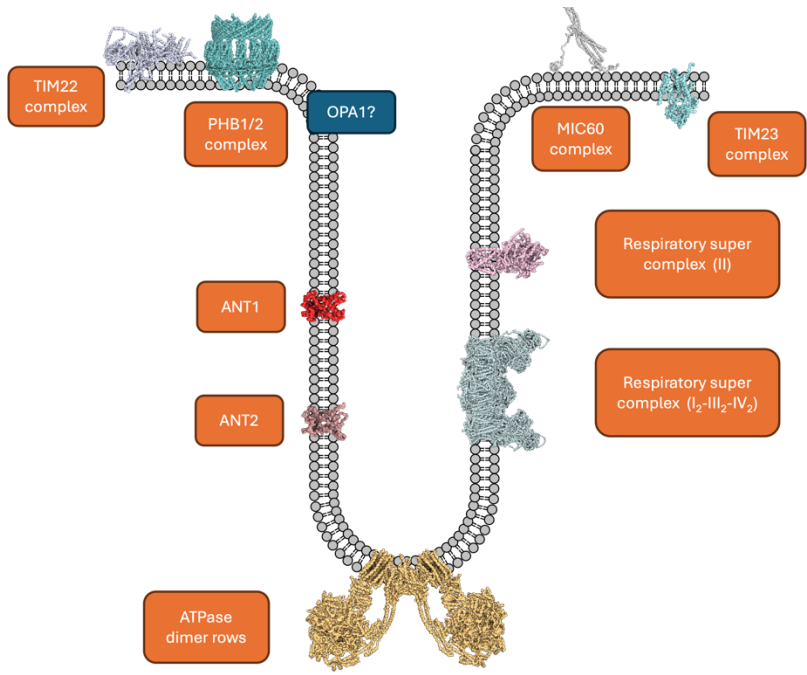


Building the inner membrane of a cristae junction

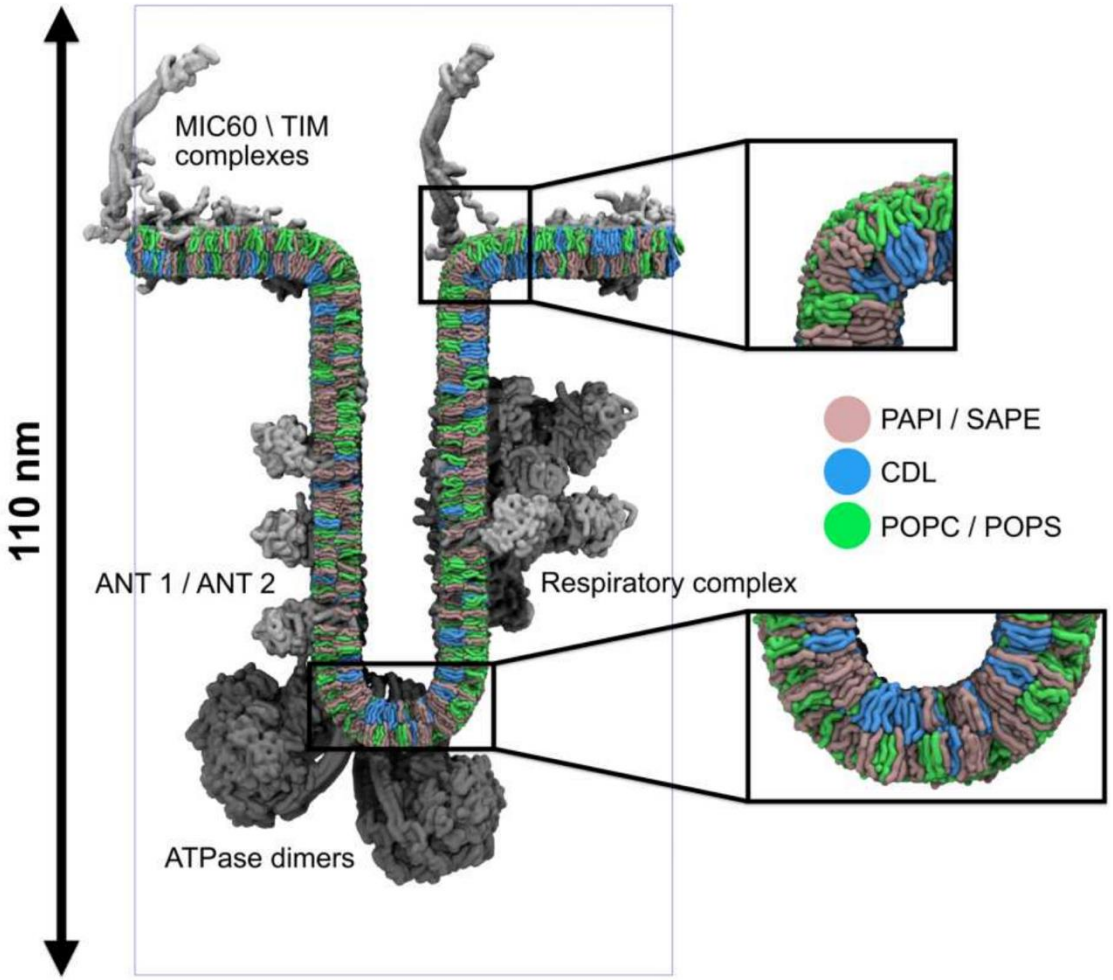

File Edit Render Window Help
Layout Modeling Sculpting UV Editing Texture Paint Shading Animation Rendering Compositing Geometry Nodes Scripting
Object Mode View Select Add Object
User Perspective
(1) Collection | Cylinder
Options
Analyze
Statistics
Volume Area
Checks
Solid
Intersections
Degenerate 0.00010
Distorted 45°
Thickness 0.001 m
Edge Sharp 160°
Overhang 45°
Check All
Clean Up
Transform
Export
Item Tool View 3D-Print
Scene
ViewLayer
Scene Collection
Collection
Cylinder
Cylinder.001
Add Modifier
Playback Keying View Marker
Start 1 End 250
Select
Rotate View
Object Context Menu
3.6.2

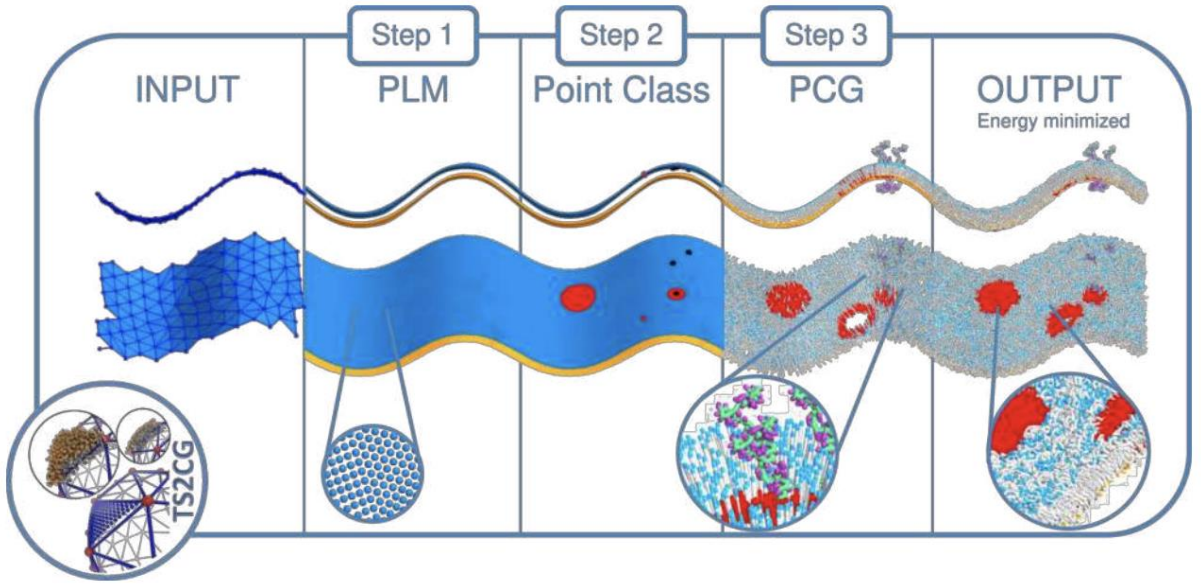




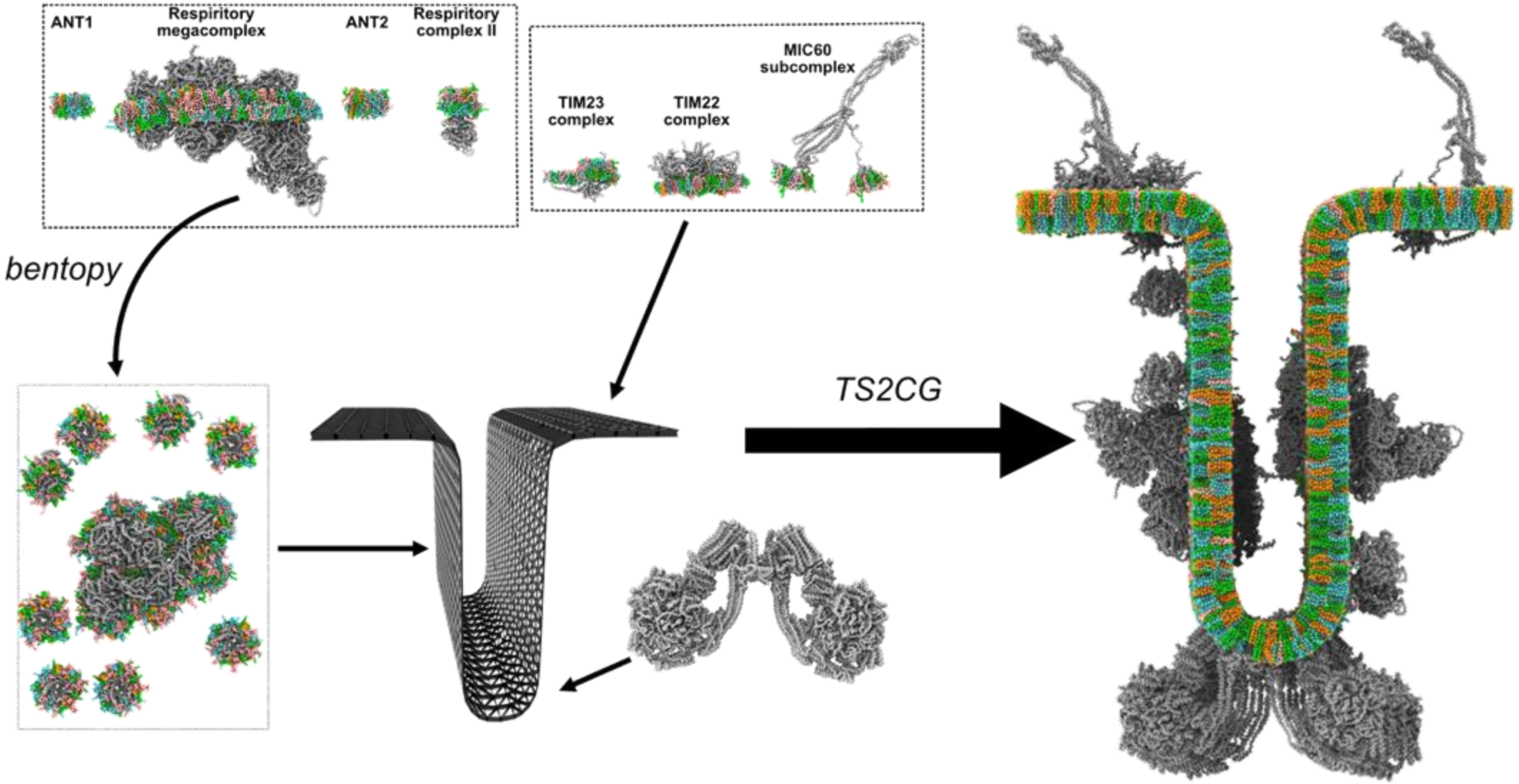

TIM22 complex
PHB1/2 complex
OPA1?
MIC60 complex
TIM23 complex
Respiratory super complex (II)
ANT1
Respiratory super complex (I_2-III_2-IV_2)
ANT2
ATPase dimer rows

INU: (‘**IN**clusion **U**pdater’) Facilitates easy collision-free protein placement based on local membrane curvature, using the same probabilistic assignment as DOP.

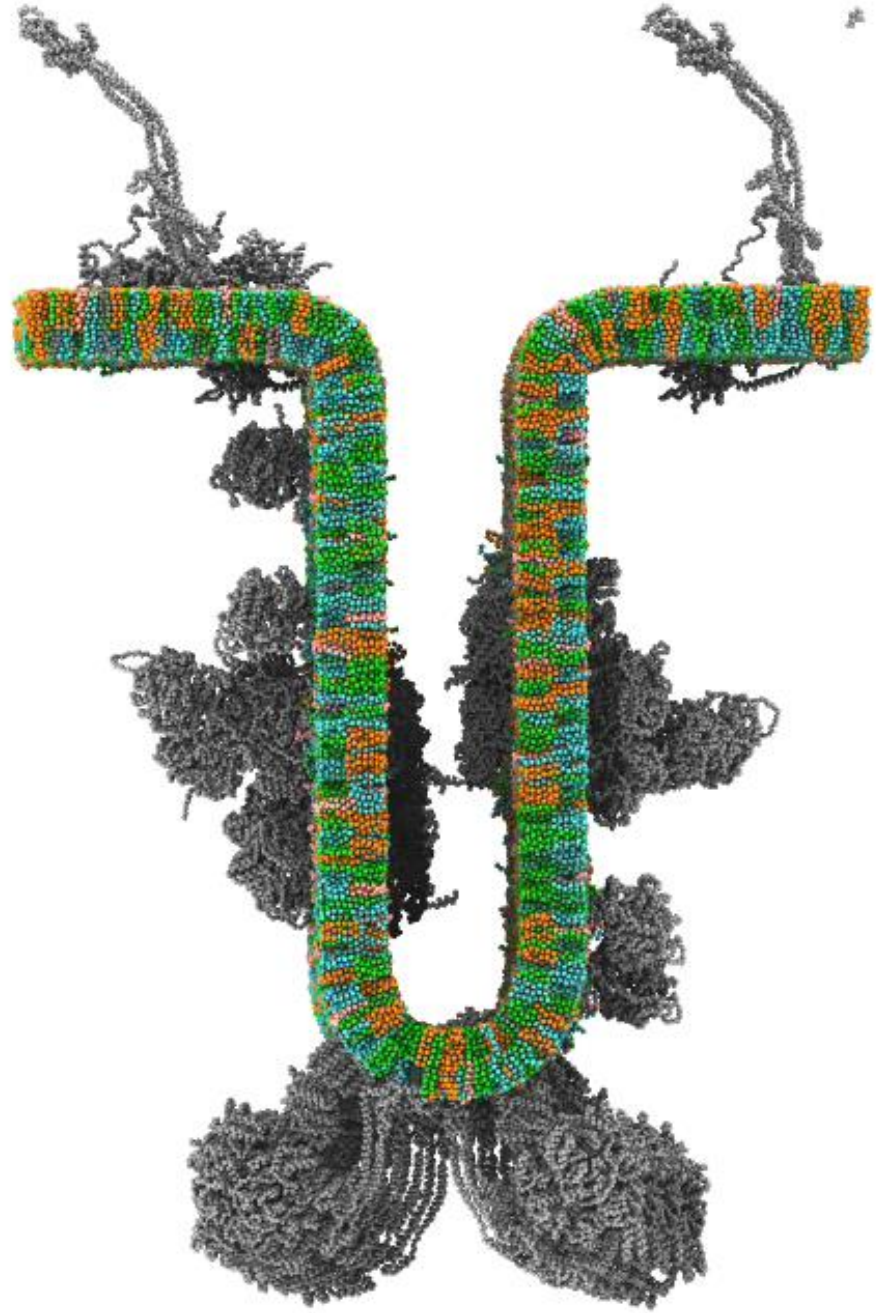


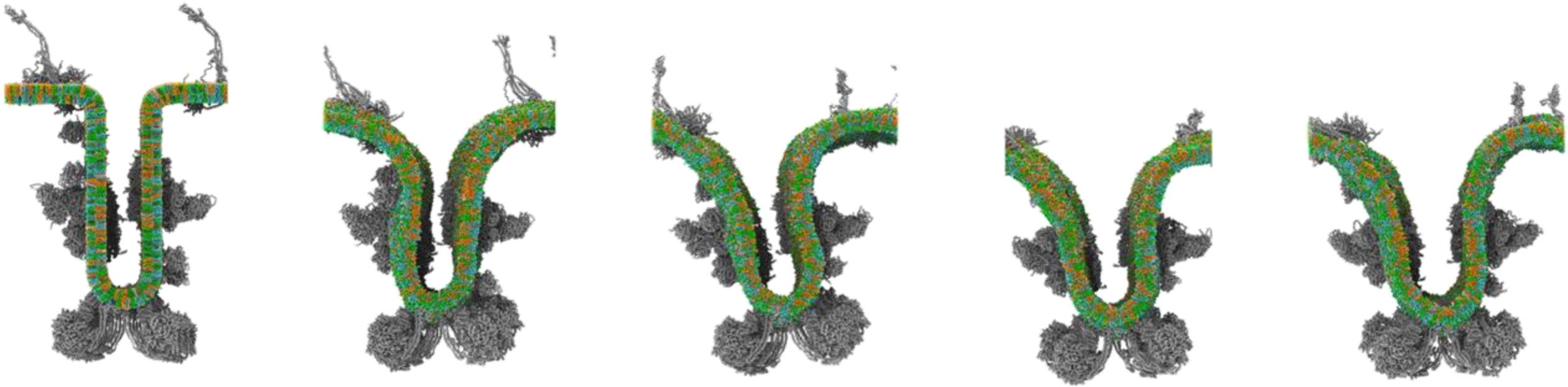


Schuhmann, F., Stevens, J. A., Rahmani, N., Lindahl, I., Brown, C. M., Brasnett, C., ... & Pezeshkian, W. (2025). TS2CG as a membrane builder. *bioRxiv*, 2025-04.

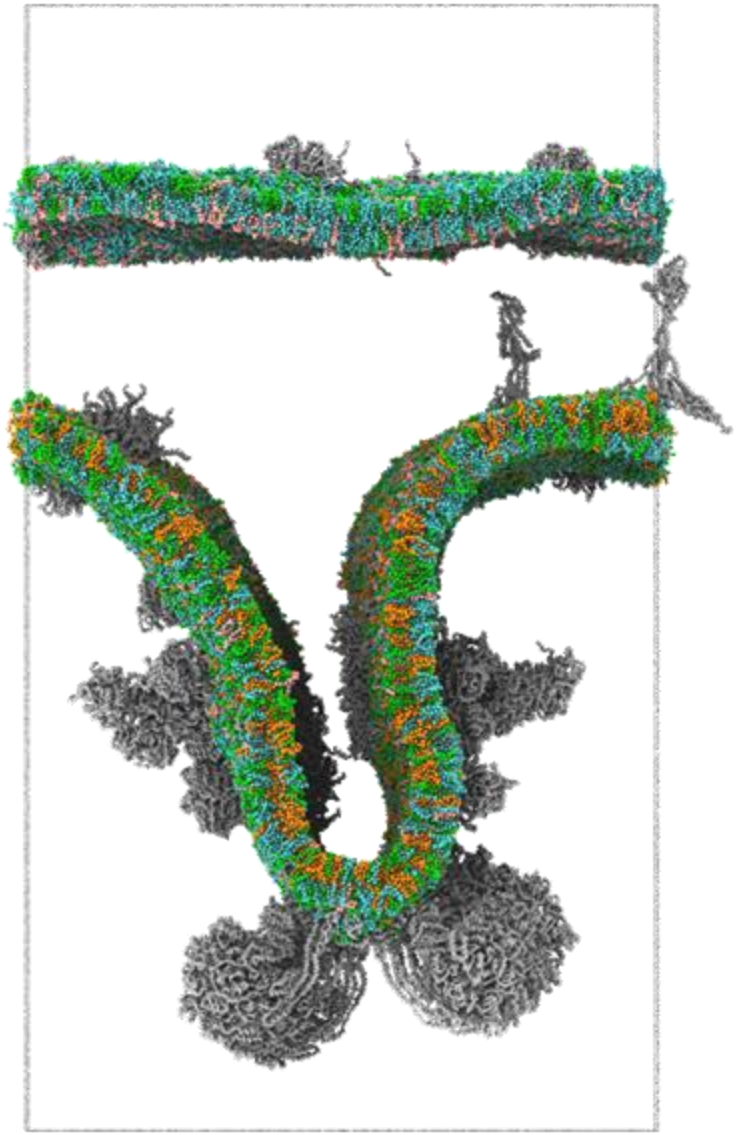


Pezeshkian, W., König, M., Wassenaar, T. A., & Marrink, S. J. (2020). Backmapping triangulated surfaces to coarse-grained membrane models. *Nature communications*, *11*(1), 2296.

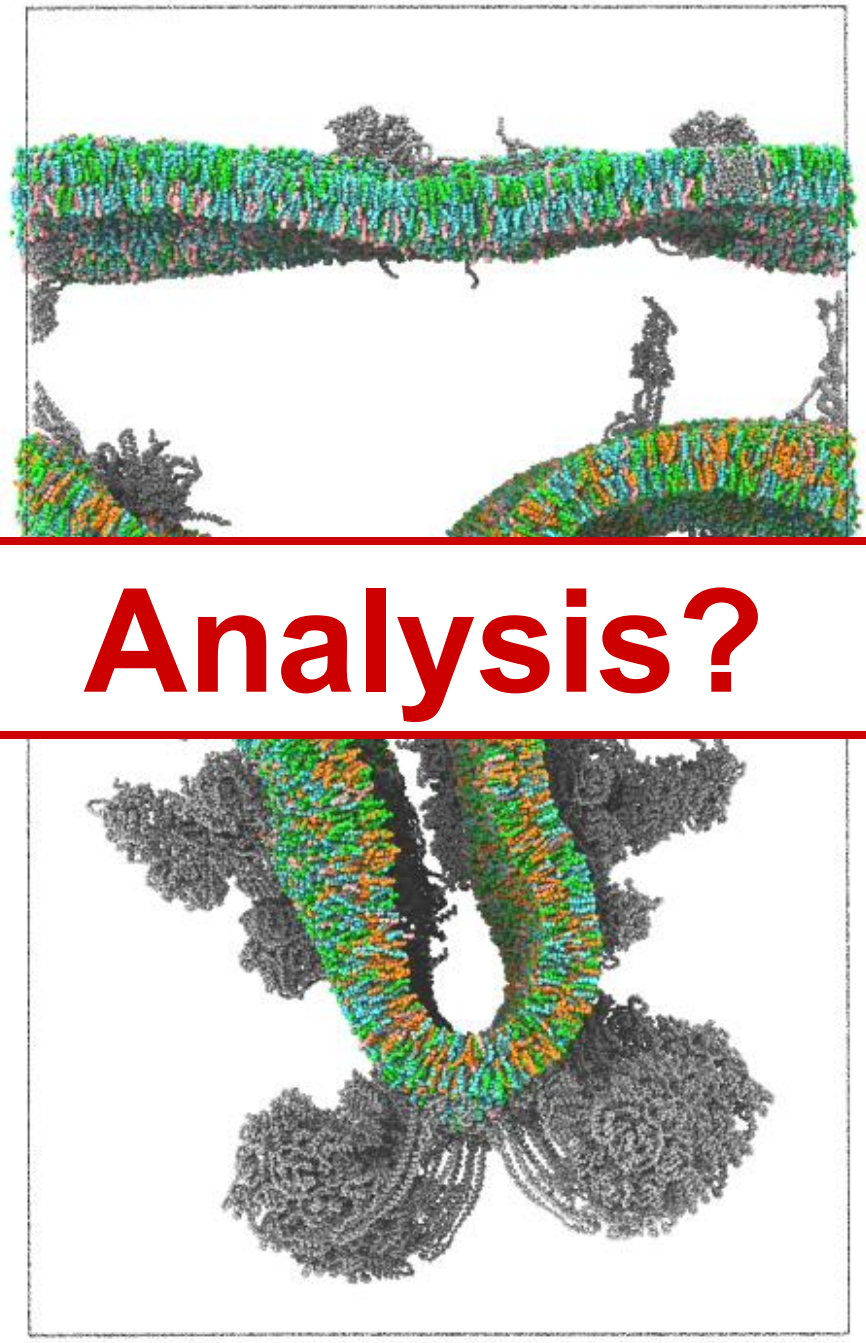




0 ns 250 ns 500 ns 750 ns 900 ns

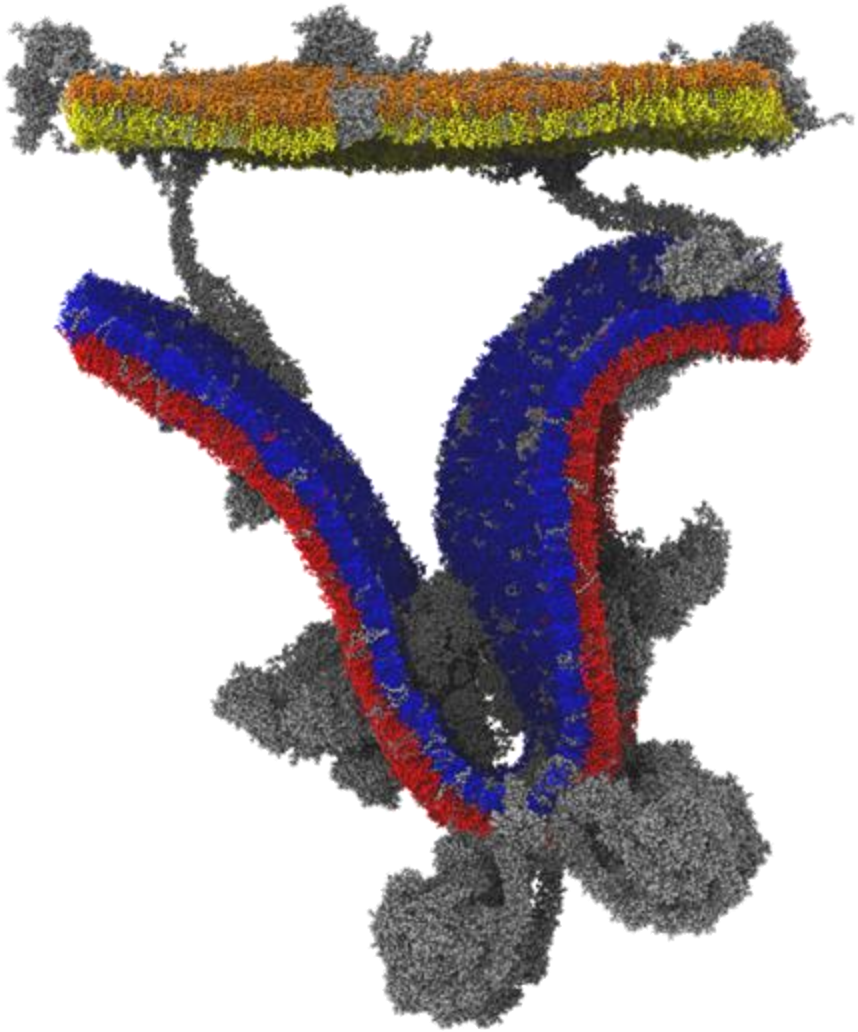


- 651 protein chains
  - 13 unique protein complexes
- 31,094 lipids
- 3,092,859 water and ion particles
- 3,769,862 total particles



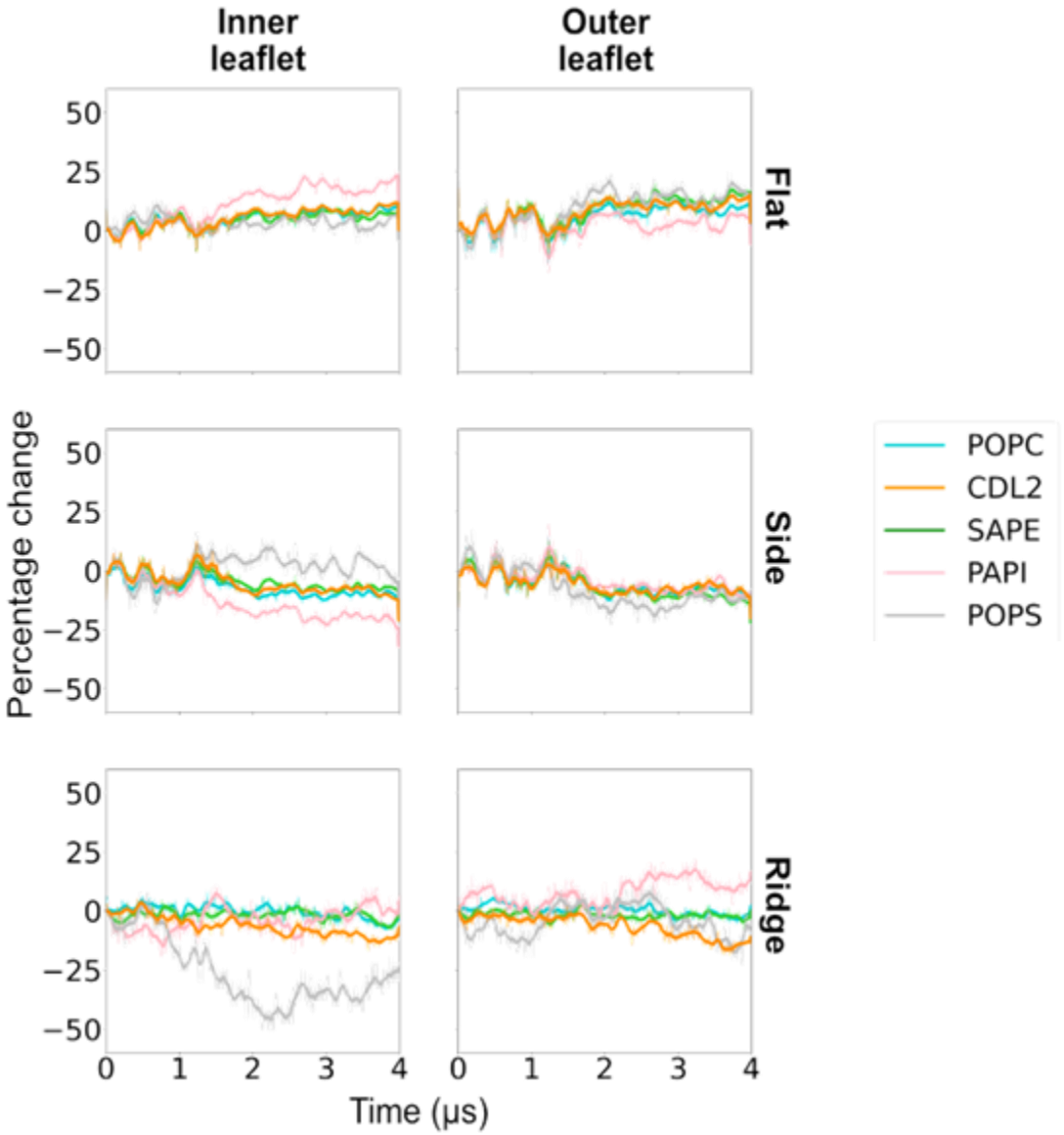
Analysis?

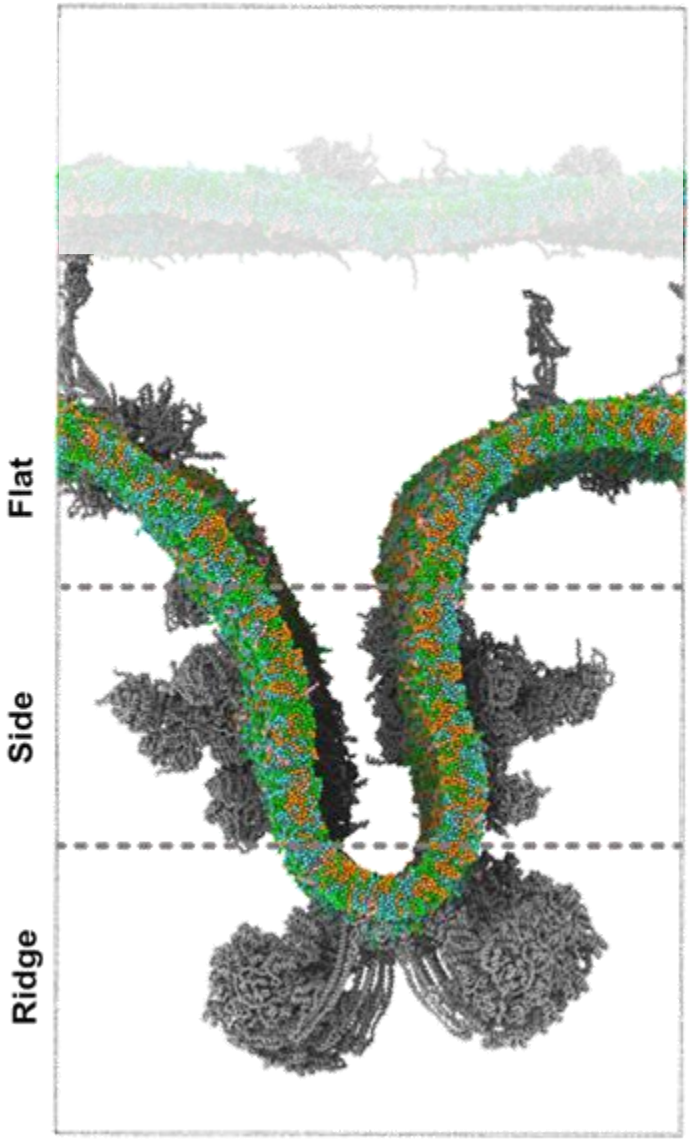
```
mdvseg -f md.gro -x md.gro -res 1 -hg water -tg none
       -eg nwater -fs 0 -hres 0 -nt 1
```

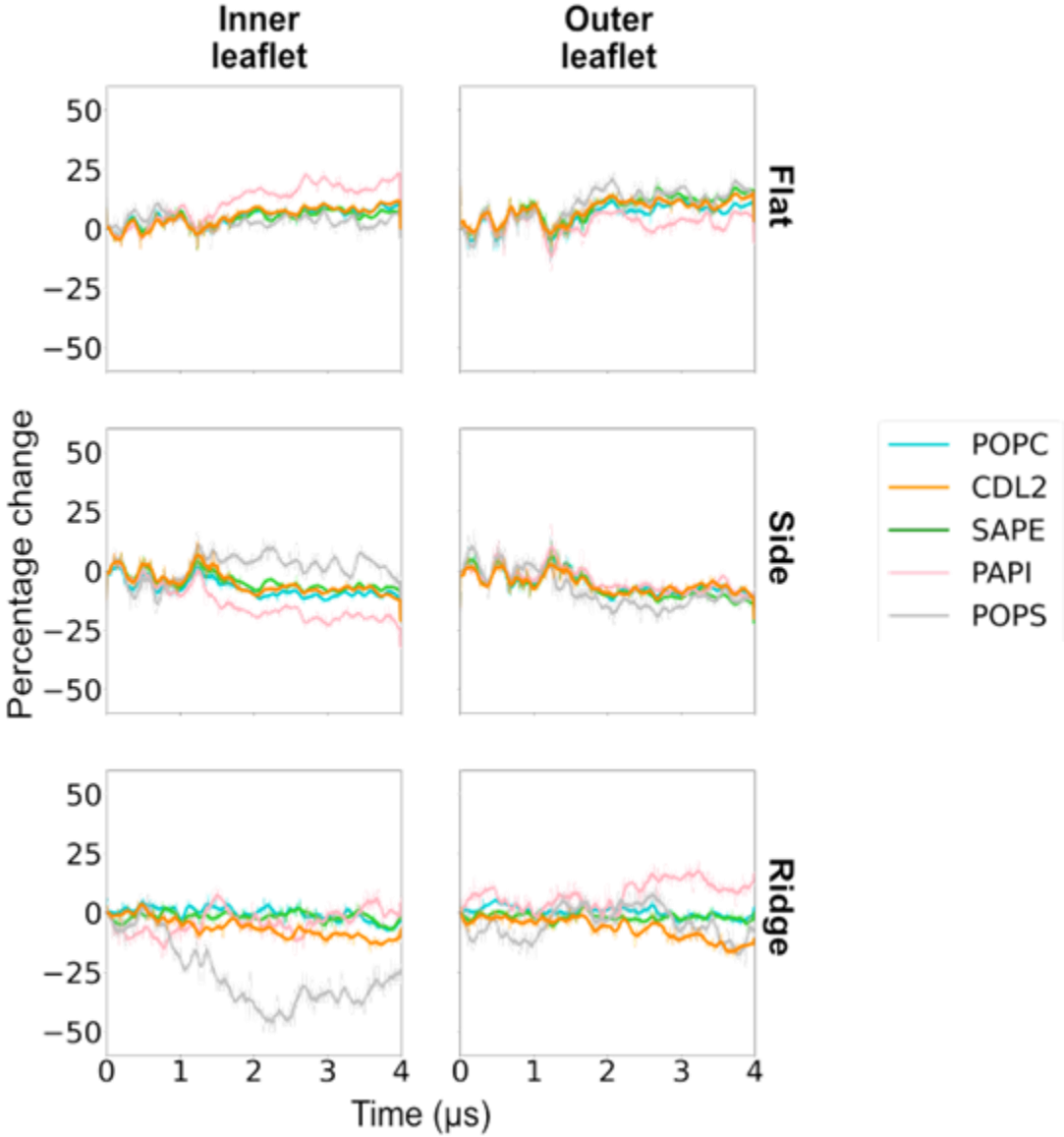


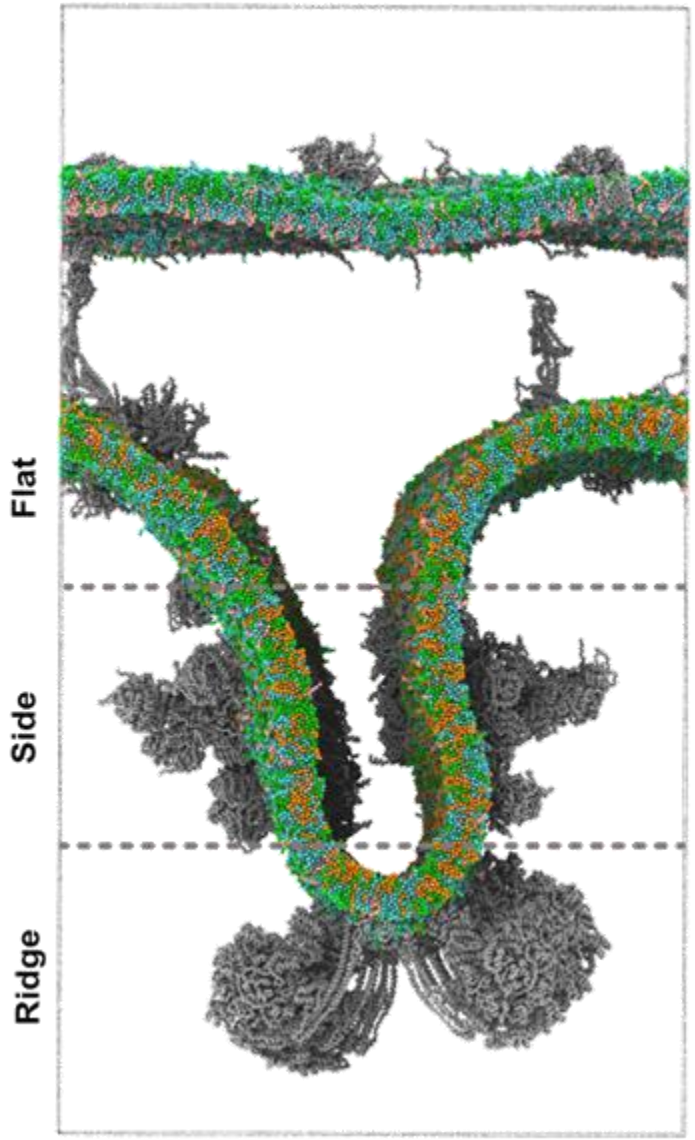
Bruininks, Bart MH, et al. "Sequential voxel-based leaflet segmentation of complex lipid morphologies." *Journal of Chemical Theory and Computation* 17.12 (2021): 7873-7885.

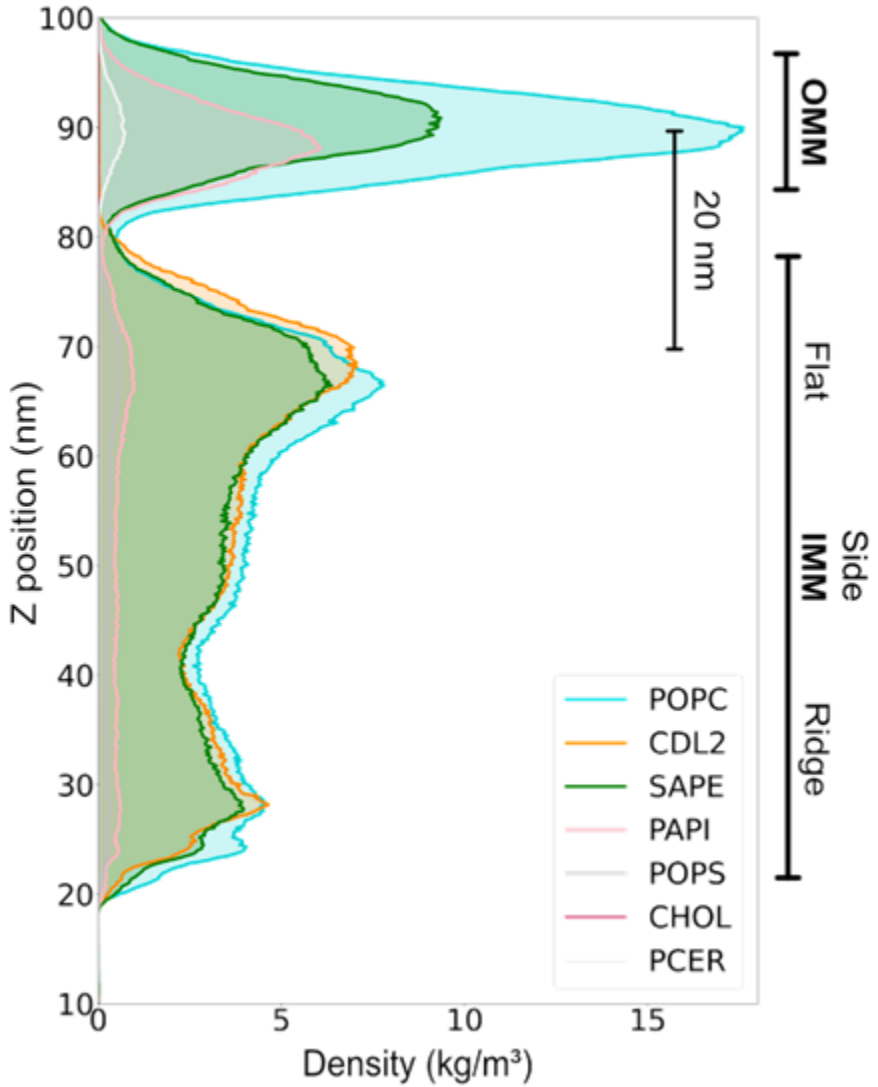
```
universe = mda.Universe('md.gro', 'md.xtc')

clusters = 'clusters.npy'
betafactors = np.load(clusters)[0]
universe.add_TopologyAttr(mda.core.topologyattrs.Tempfactors(betafactors))
```

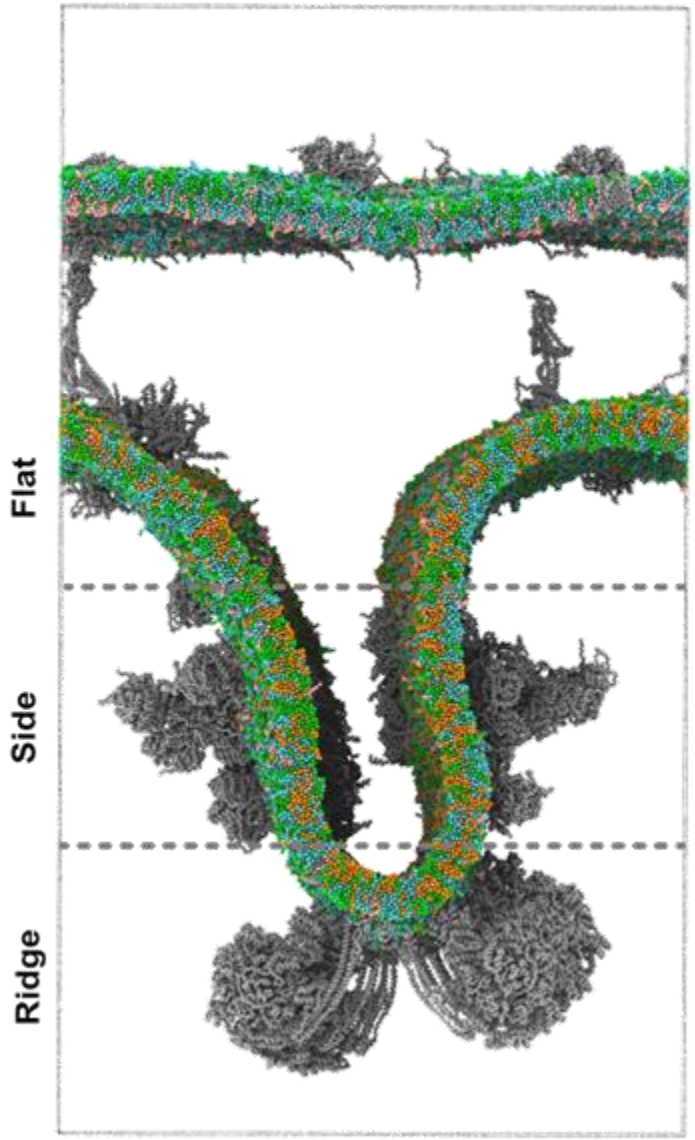


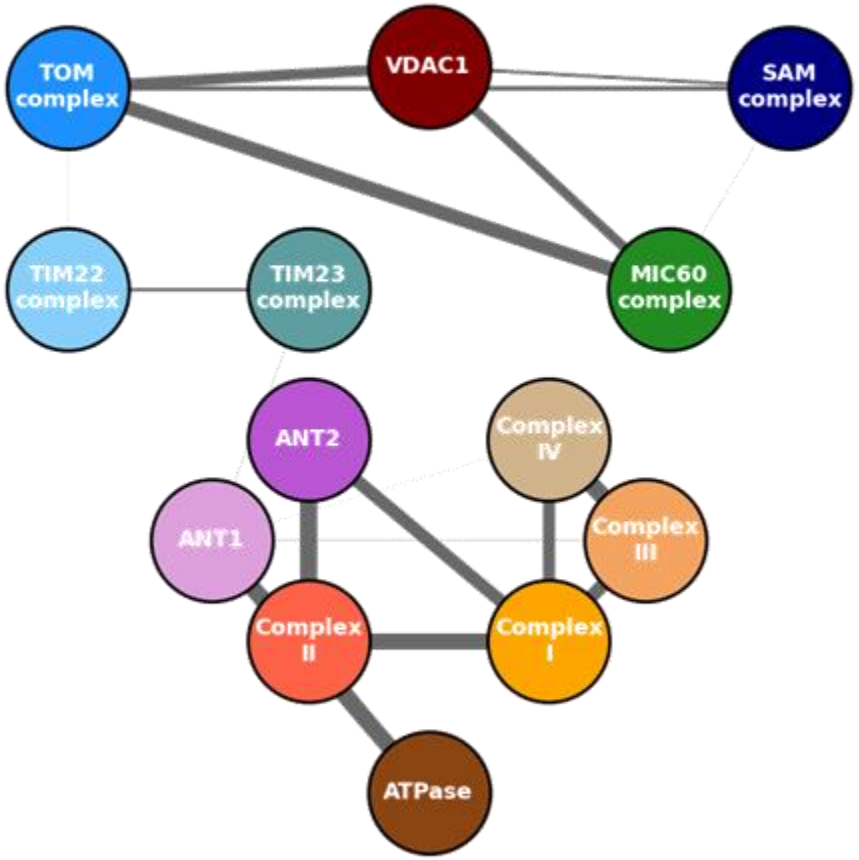

Flat
Side
Ridge

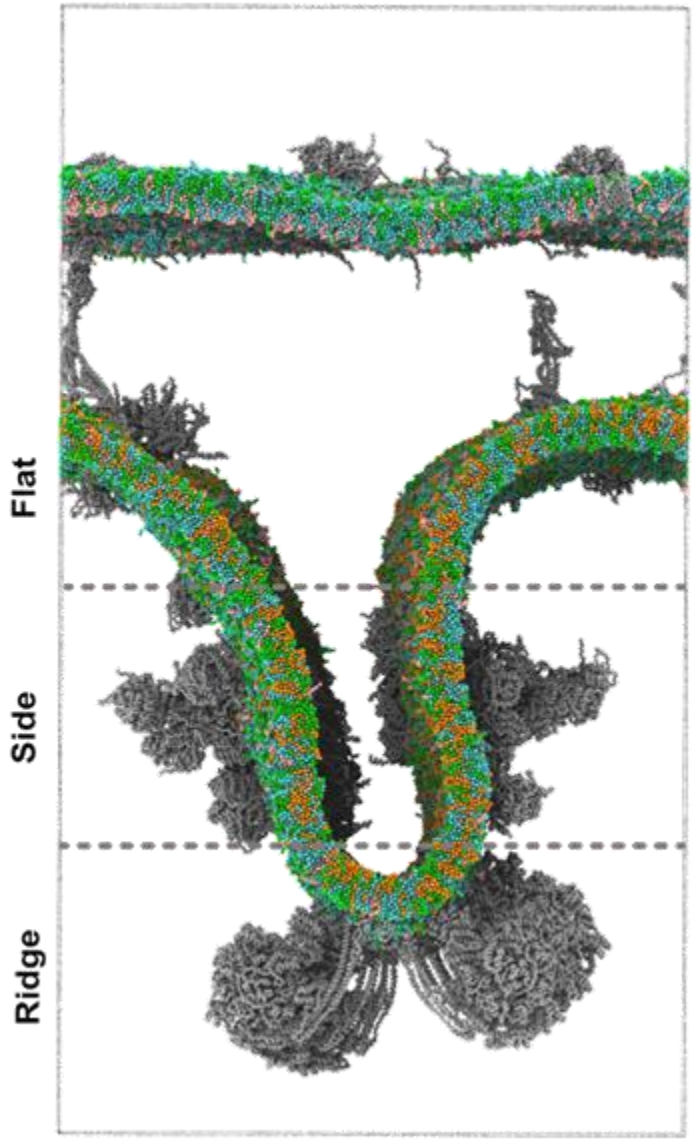

Inner leaflet
Outer leaflet
Flat
Side
Ridge
Percentage change
50
25
0
−25
−50
0
1
2
3
4
Time (μs)
POPC
CDL2
SAPE
PAPI
POPS

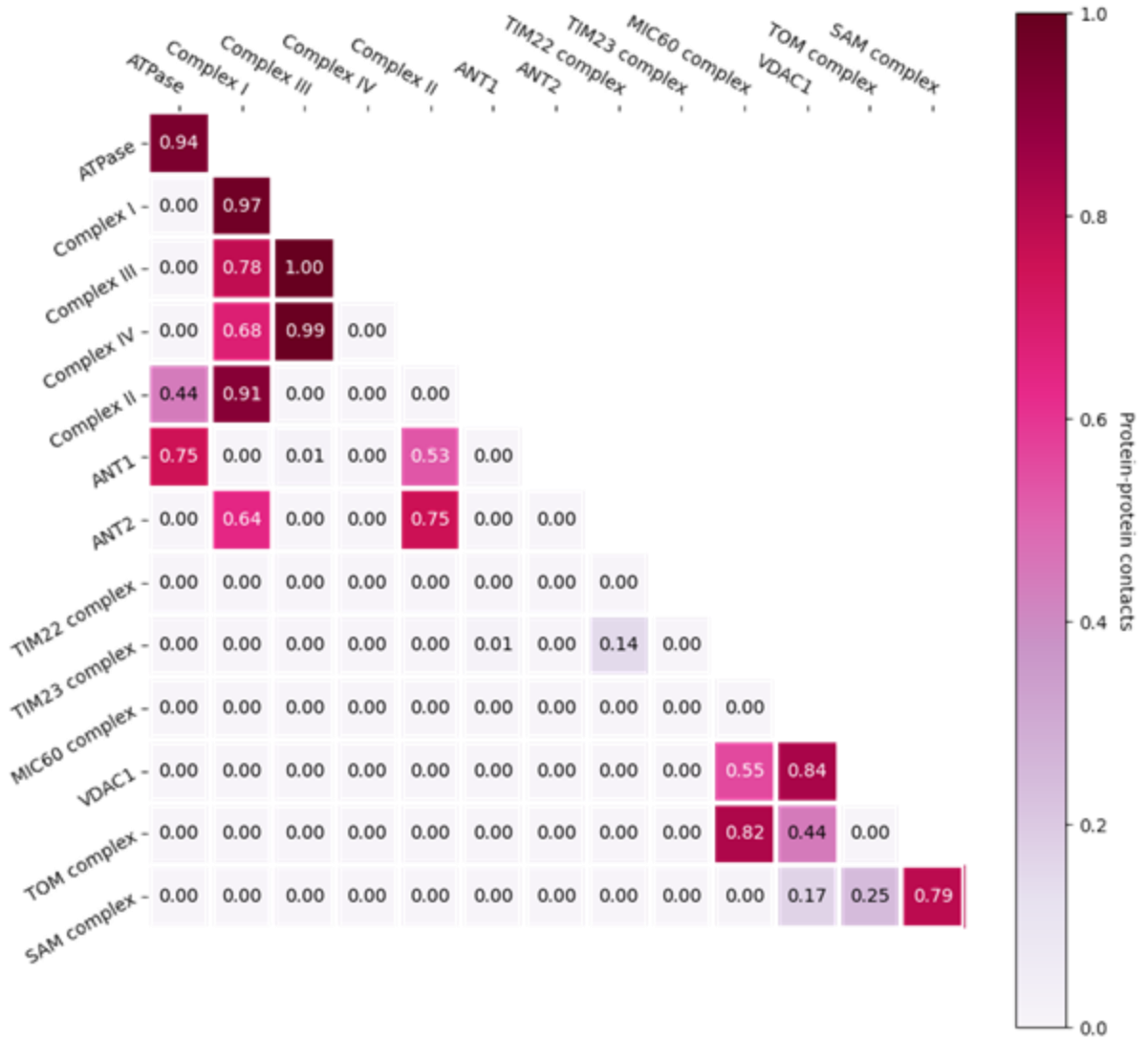

Flat
Side
Ridge

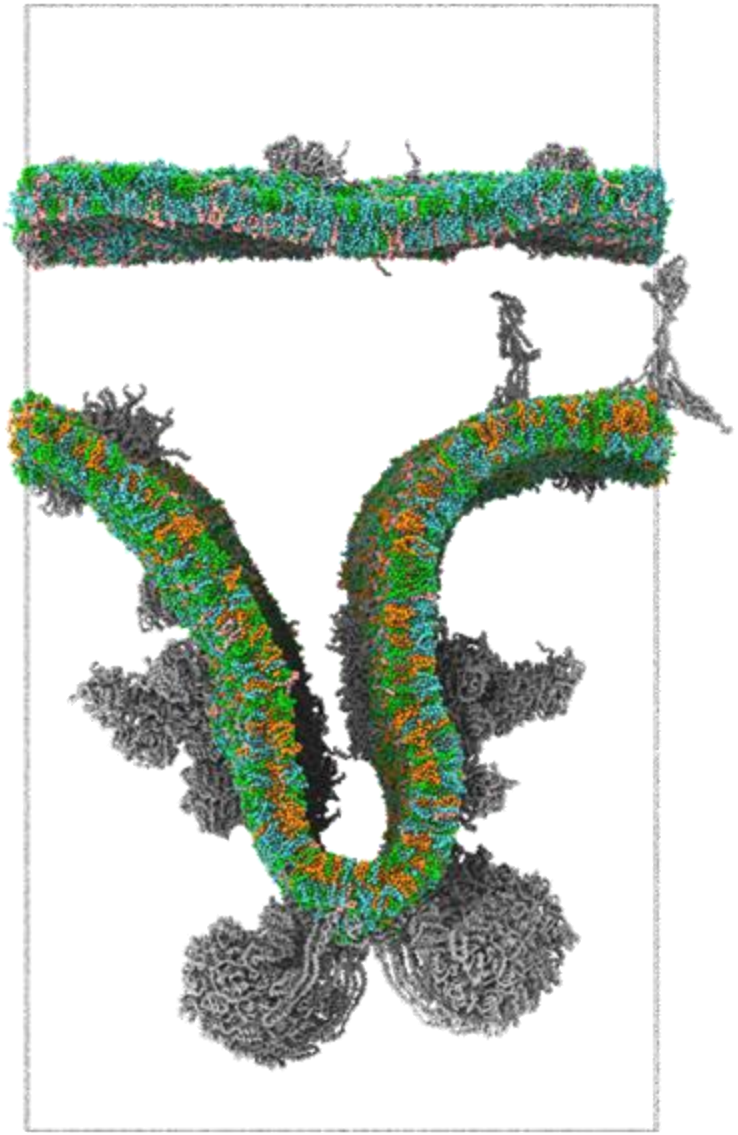

100
90
80
70
60
50
40
30
20
10
Z position (nm)
0
5
10
15
Density (kg/m³)
POPC
CDL2
SAPE
PAPI
POPS
CHOL
PCER
20 nm
OMM
Flat
Side
IMM
Ridge


Flat
Side
Ridge

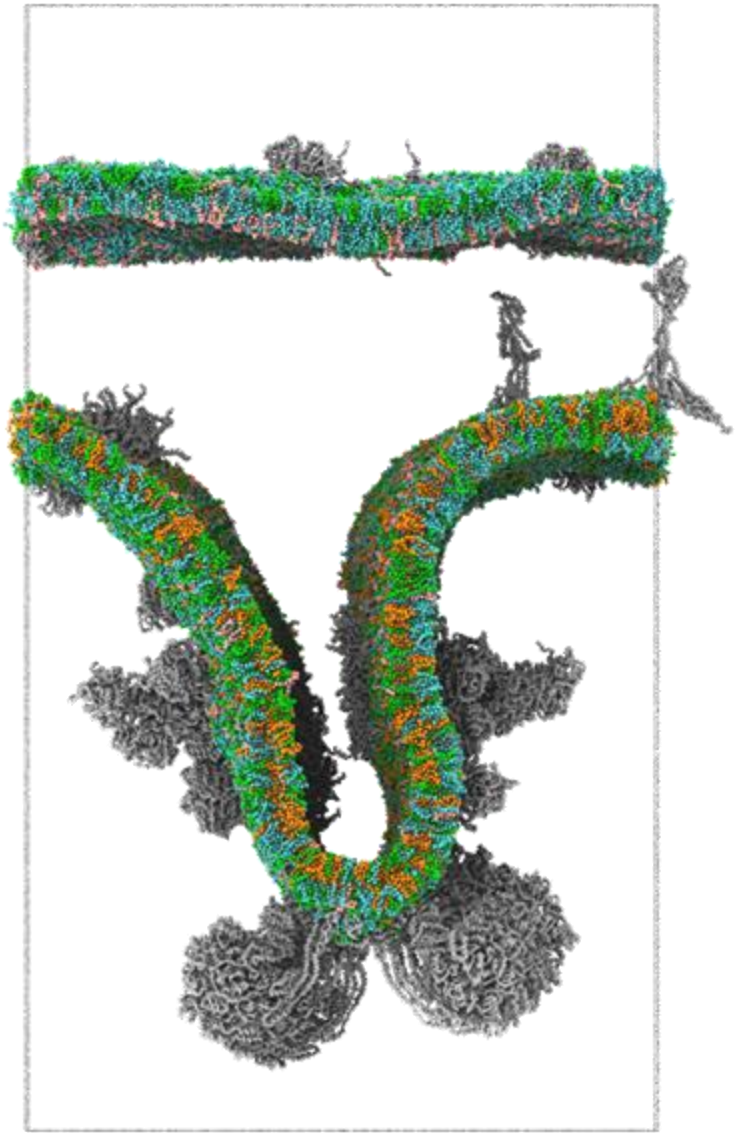

TOM complex
VDAC1
SAM complex
TIM22 complex
TIM23 complex
MIC60 complex
ANT2
Complex IV
ANT1
Complex III
Complex II
Complex I
ATPase

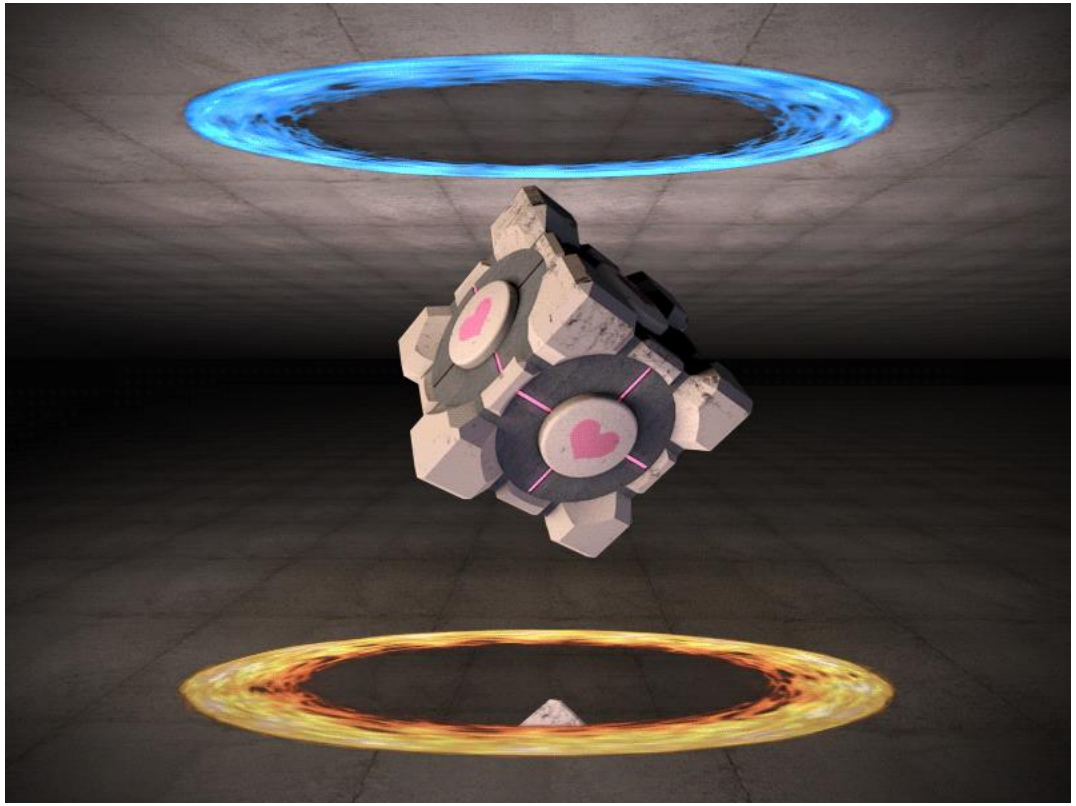

Flat
Side
Ridge


ATPase
Complex I
Complex III
Complex IV
Complex II
ANT1
ANT2
TIM22 complex
TIM23 complex
MIC60 complex
VDAC1
TOM complex
SAM complex
0.94
0.00 0.97
0.00 0.78 1.00
0.00 0.68 0.99 0.00
0.44 0.91 0.00 0.00 0.00
0.75 0.00 0.01 0.00 0.53 0.00
0.00 0.64 0.00 0.00 0.75 0.00 0.00
0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00
0.00 0.00 0.00 0.00 0.00 0.01 0.00 0.14 0.00
0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00
0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.55 0.84
0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.82 0.44 0.00
0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.17 0.25 0.79
Protein-protein contacts
1.0
0.8
0.6
0.4
0.2
0.0

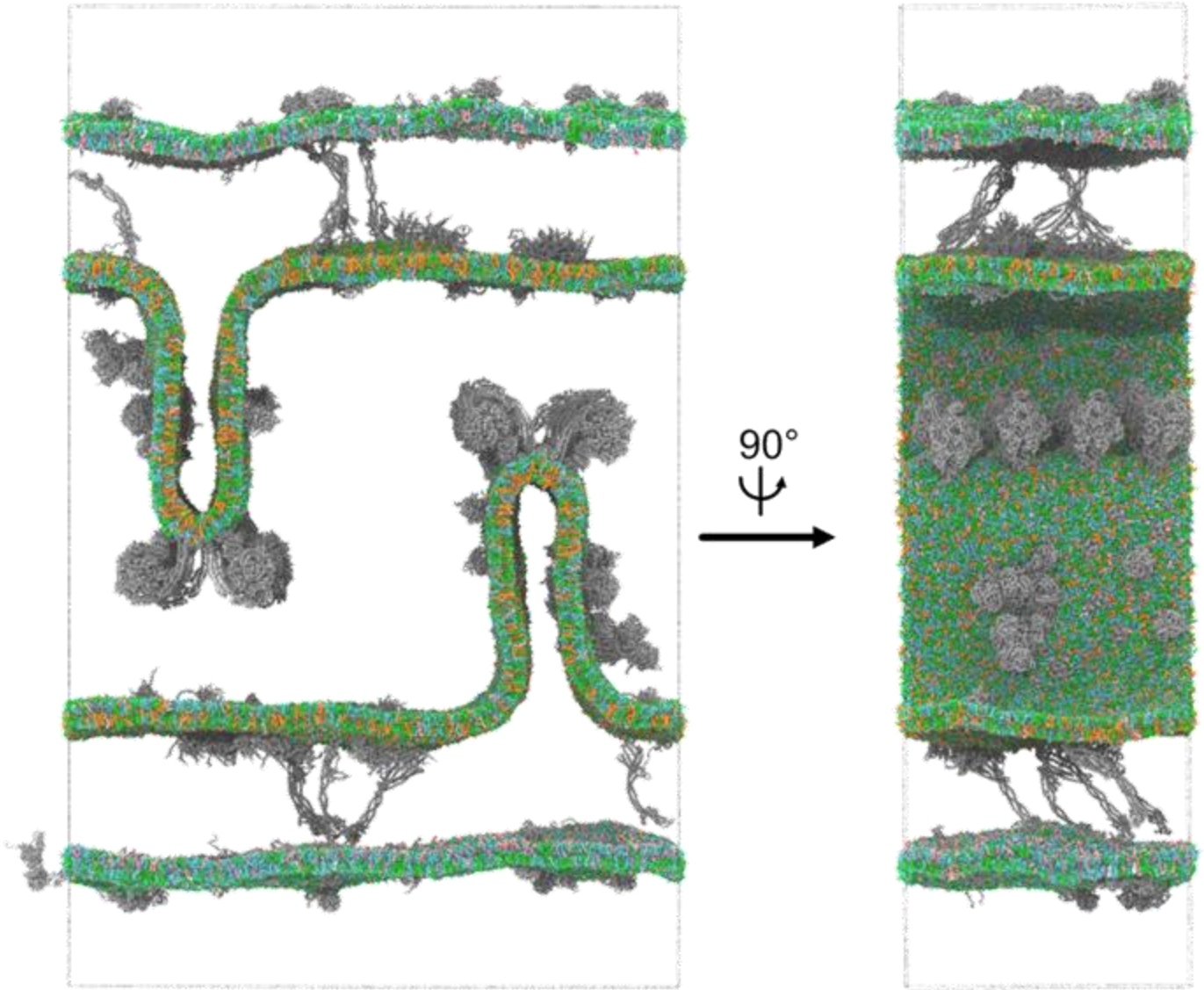


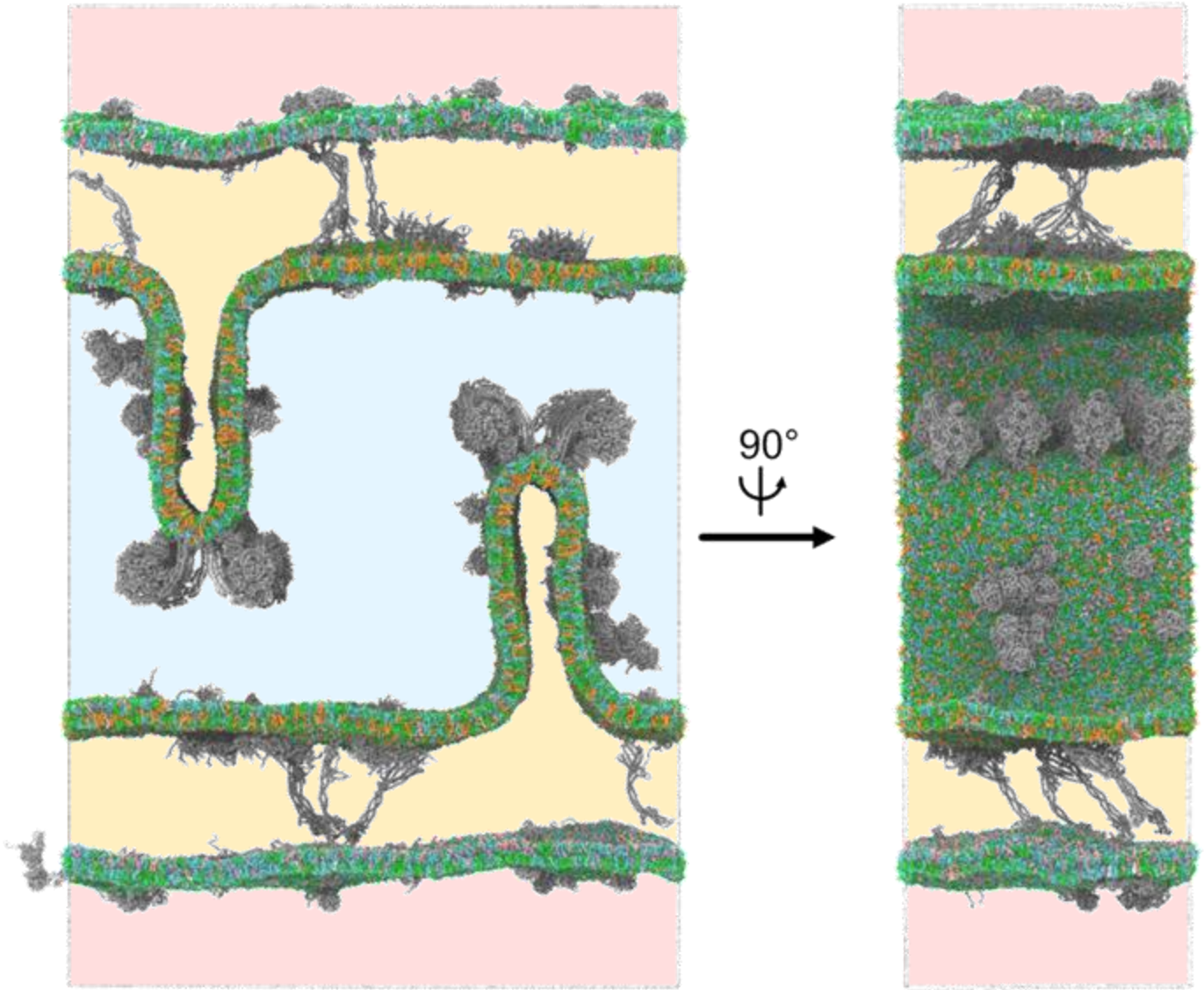
But still flaws in the system set up





Portal 2 Wheatley Gif – Chris Baker, https://ru.pinterest.com/pin/740983563615182 49/

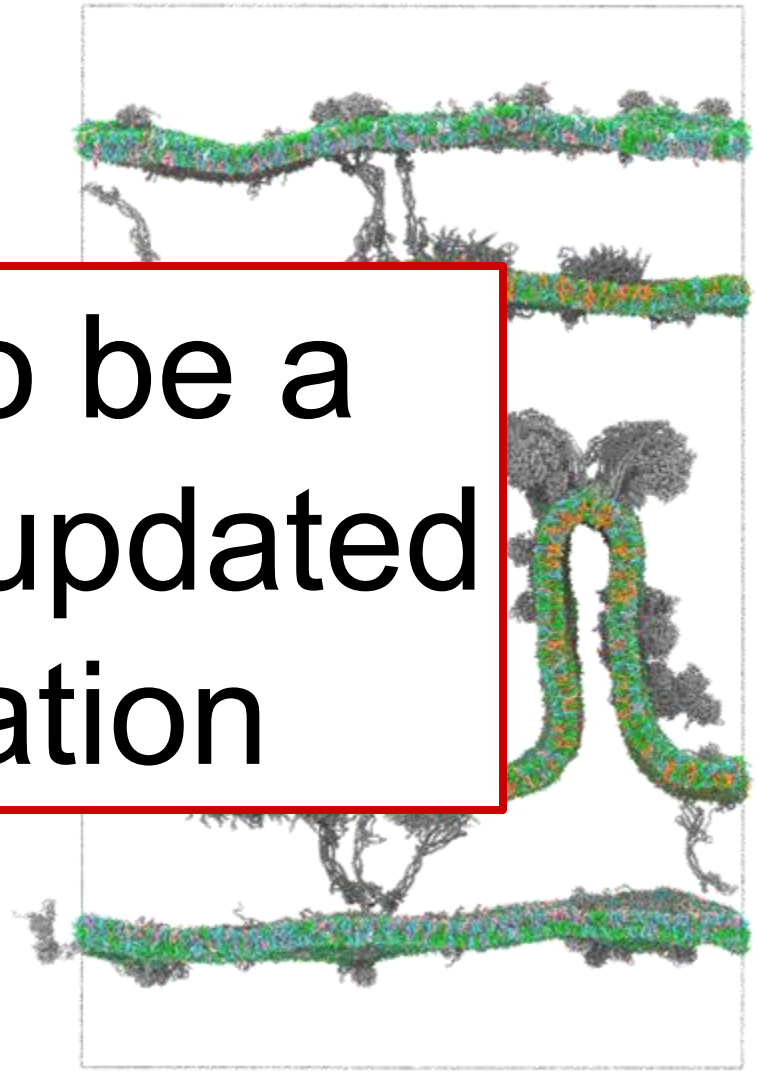

90°


90°

Future goals:

- Build a
  - Mem
  - Solu
  - Meta
  - mtDI

This is intended to be a ‘**living model**’ and updated with new information



Rath, Sneha, et al. "MitoCarta3.0: an updated mitochondrial proteome now with sub-organelle localization and pathway annotations." *Nucleic acids research* 49.D1 (2021): D1541-D1547.

Acknowledgements

MD group

Siewert-Jan Marrink

Tsjerk Wassenaar

Jan Stevens

Rubi Zarmiento Garcia

Marieke Westendorp

Bart Bruininks

and everyone else!

erc

SURF

Thank you for your attention!
Any questions?

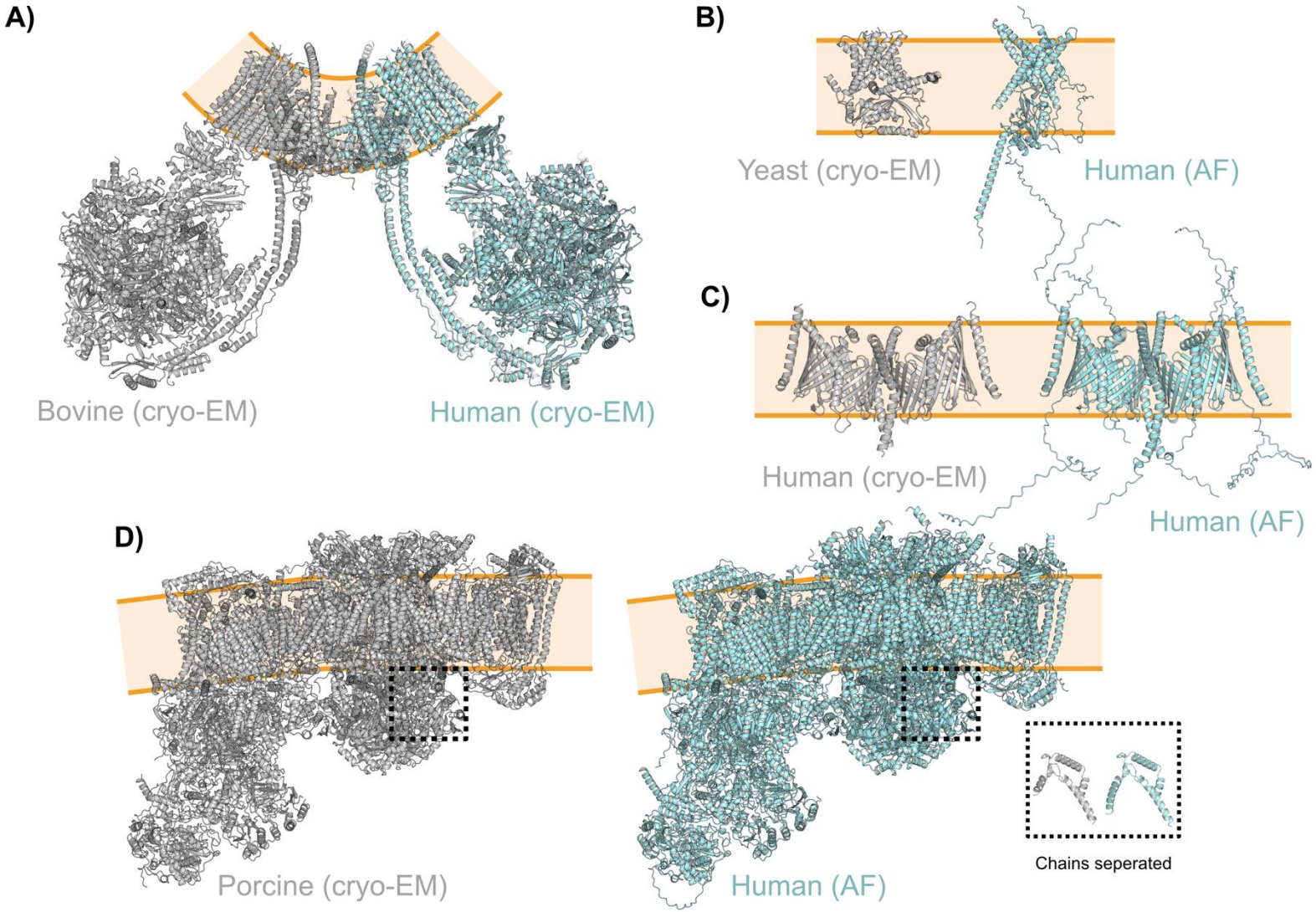
GitHub repo here!

Check out the paper here!

Brown, C.M., Westendorp, M.S.S., Zarmiento-Garcia, R. *et al.* An integrative modelling approach to the mitochondrial cristae. *Commun Biol* **8**, 972 (2025). https://doi.org/10.1038/s42003-025-08381-5

Supplementary slides

Protein (complex)	Original structure (PDB ID): organism	Method used in this study
ATPase	8H9S: Human (overlayed with 7AJB: Bovine)	Overlaid human monomer on bovine dimer
Respiratory supercomplex	8UGH: Porcine	AlphaFold2 replacement
Respiratory complex II	8GS8: Human	AlphaFold2 replacement
ANT1	2C3E: Bovine	AlphaFold2 Database
ANT2	N/A	AlphaFold2 Database
MIC60 subcomplex	N/A	AlphaFold 3 online server
TIM22 complex	7CGP: Human	AlphaFold2 replacement
TIM23 complex	8SCX: Yeast	AlphaFold2 online server
TOM complex	7VD2: Human	AlphaFold2 replacement
SAM complex	7E4H: Yeast	AlphaFold2 replacement
VDAC1	6TIQ: Human	Use original structure


A)
Bovine (cryo-EM)
Human (cryo-EM)
B)
Yeast (cryo-EM)
Human (AF)
C)
Human (cryo-EM)
Human (AF)
D)
Porcine (cryo-EM)
Human (AF)
Chains seperated