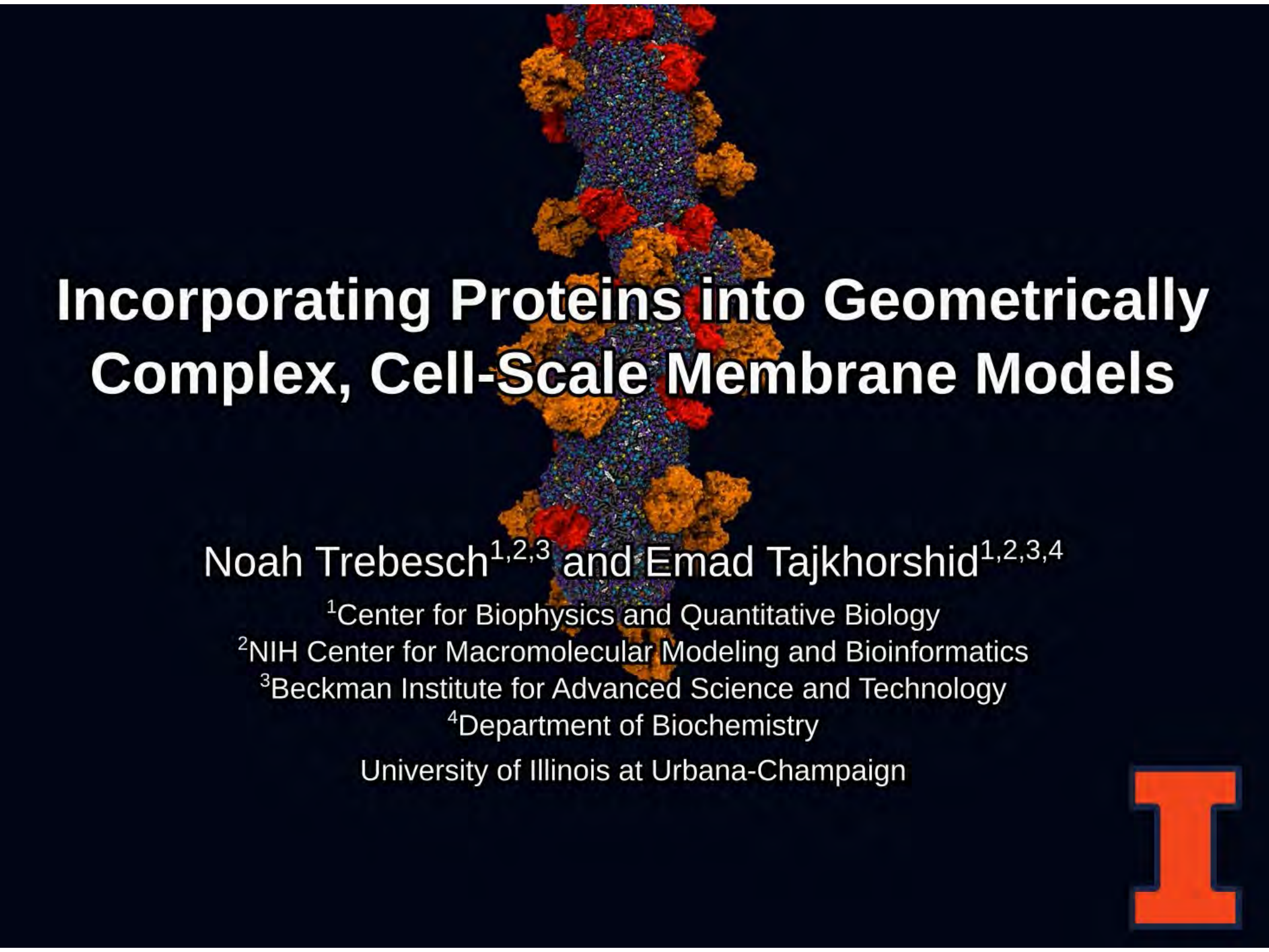

Incorporating Proteins into Geometrically Complex, Cell-Scale Membrane Models

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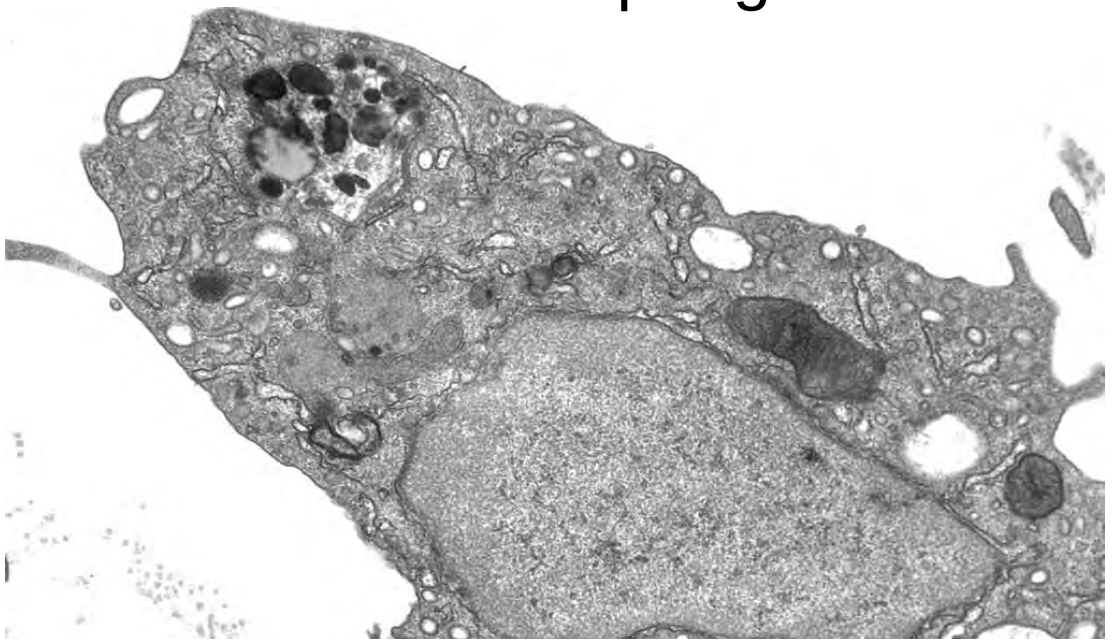
[3]Beckman Institute for Advanced Science and Technology
[4]Department of Biochemistry

University of Illinois at Urbana-Champaign

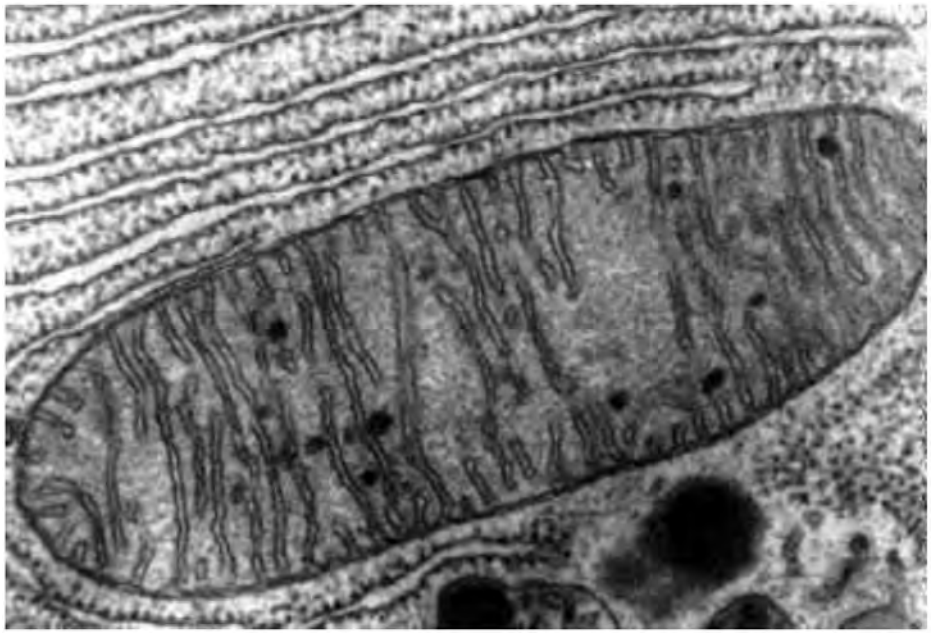
Cellular Membranes are Highly Complex

Macrophage



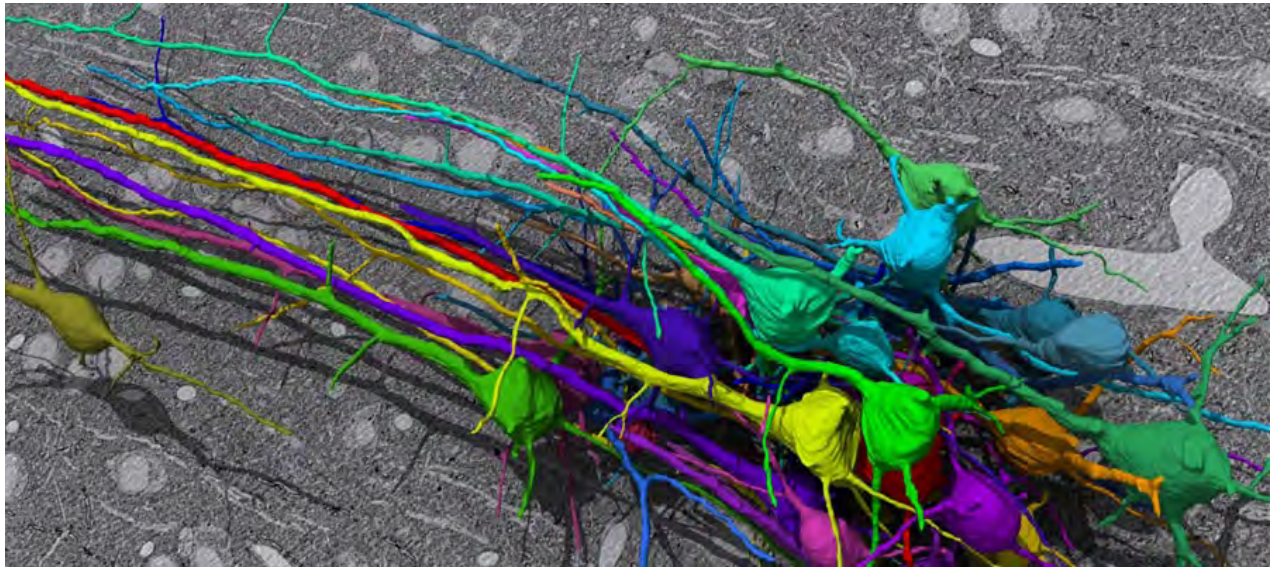
Yale University

Mitochondrion



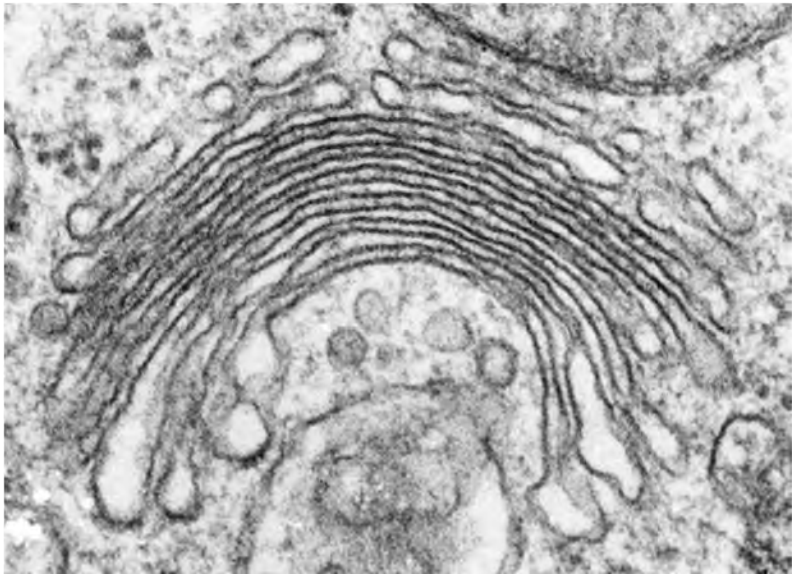
G. Angus McQuibban

Neurons



Daniel Berger

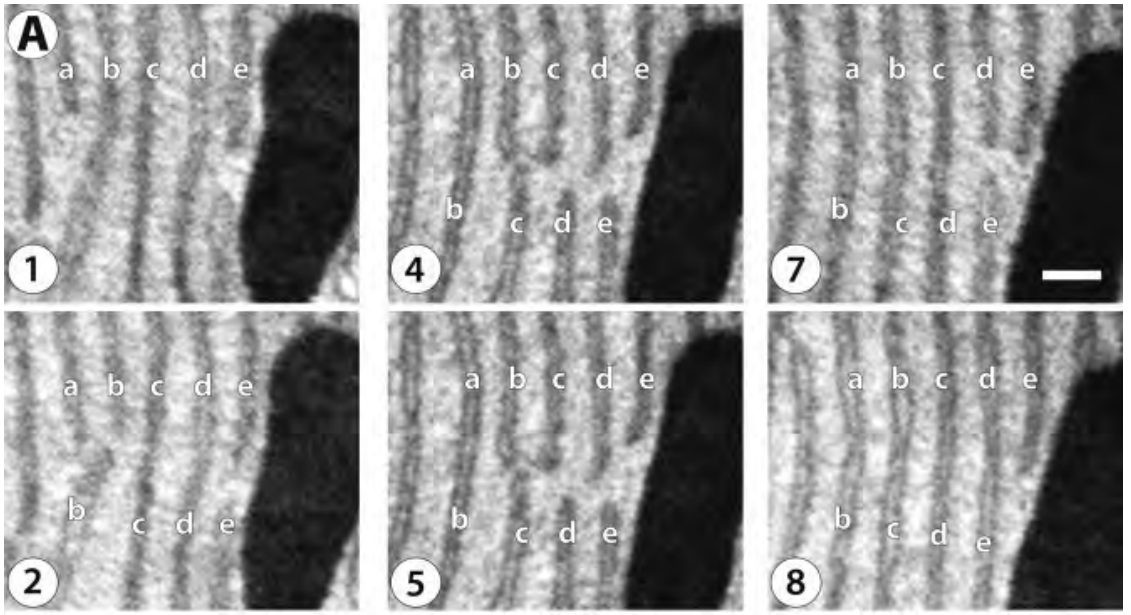
Golgi Apparatus

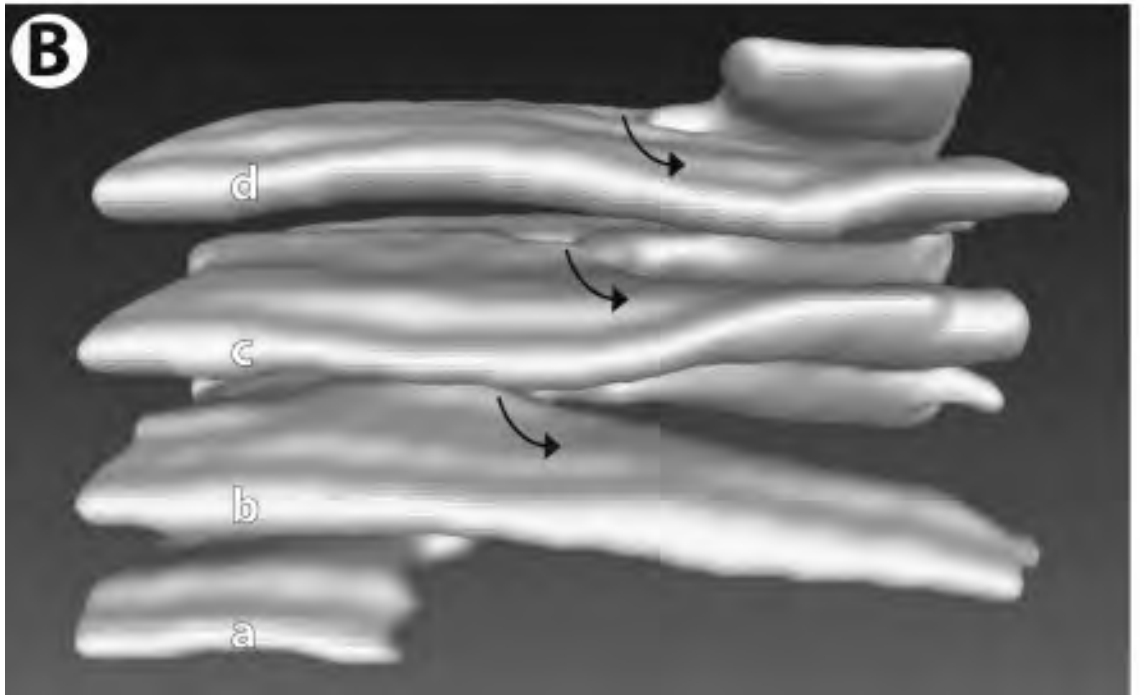


Yale University

ER Consists of Representative Cell Membranes

Structure: Electron Microscopy





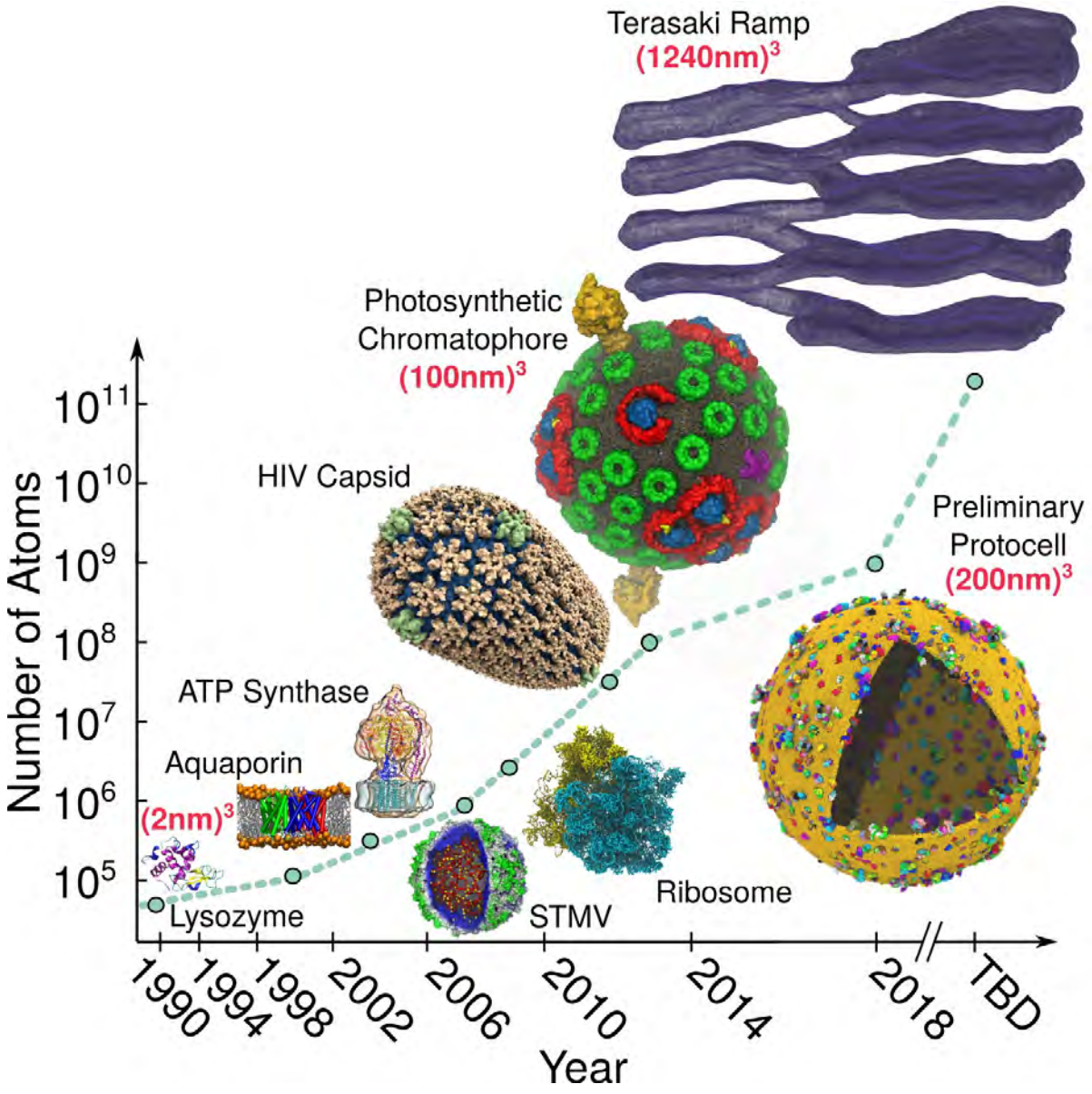
Terasaki *et al*. *Cell*. **154**:2, 285-296 (2013).

Lipid Composition: Chromatography

14.19%	Cholesterol
49.65%	POPC
23.01%	POPE
4.38%	POPI
3.46%	POPS
5.21%	Sphingomyelin
0.09%	Cardiolipin

Keenan and Huang. *J Dairy Sci*. **55**:11, 1586-1596 (1972).

Classical Molecular Dynamics (MD) Simulations



Classical MD Simulations Performed by the NIH Center for Macromolecular Modeling and Bioinformatics at the University of Illinois at Urbana-Champaign

Define Potential Energy Function (Force Field)

$$U_{CHARMM} = \begin{array}{c} U_{bond} + U_{angle} + U_{UB} + \\ U_{dihedral} + U_{improper} + U_{CMAP} + \\ U_{LJ} + U_{elec} \end{array}$$

$$U_{bond} = \sum_{bonds} K_b(b - b^0)^2$$

$$U_{angle} = \sum_{angles} K_\theta(\theta - \theta^0)^2$$

$$U_{UB} = \sum_{Urey-Bradley} K_{UB}(b^{1-3} - b^{1-3,0})^2$$

$$U_{dihedral} = \sum_{dihedrals} K_\varphi((1 + \cos(n\varphi - \delta))$$

$$U_{improper} = \sum_{impropers} K_\omega(\omega - \omega^0)^2$$

$$U_{CMAP} = \sum_{residues} u_{CMAP}(\Phi, \Psi)$$

$$U_{LJ} = \sum_{nonb.pairs} \varepsilon_{ij} \left[\left(\frac{r_{ij}^{min}}{r_{ij}} \right)^{12} - 2 \left(\frac{r_{ij}^{min}}{r_{ij}} \right)^{6} \right]$$

$$U_{elec} = \sum_{nonb.pairs} \frac{q_i q_j}{\epsilon r_{ij}}$$

Integrate Newton's Equations of Motion (Velocity Verlet)

$$\vec{r}_i(t + \delta t) = \vec{r}_i(t) + \vec{v}_i(t)\delta t + \tfrac{1}{2}\vec{a}_i(t)(\delta t)^2$$

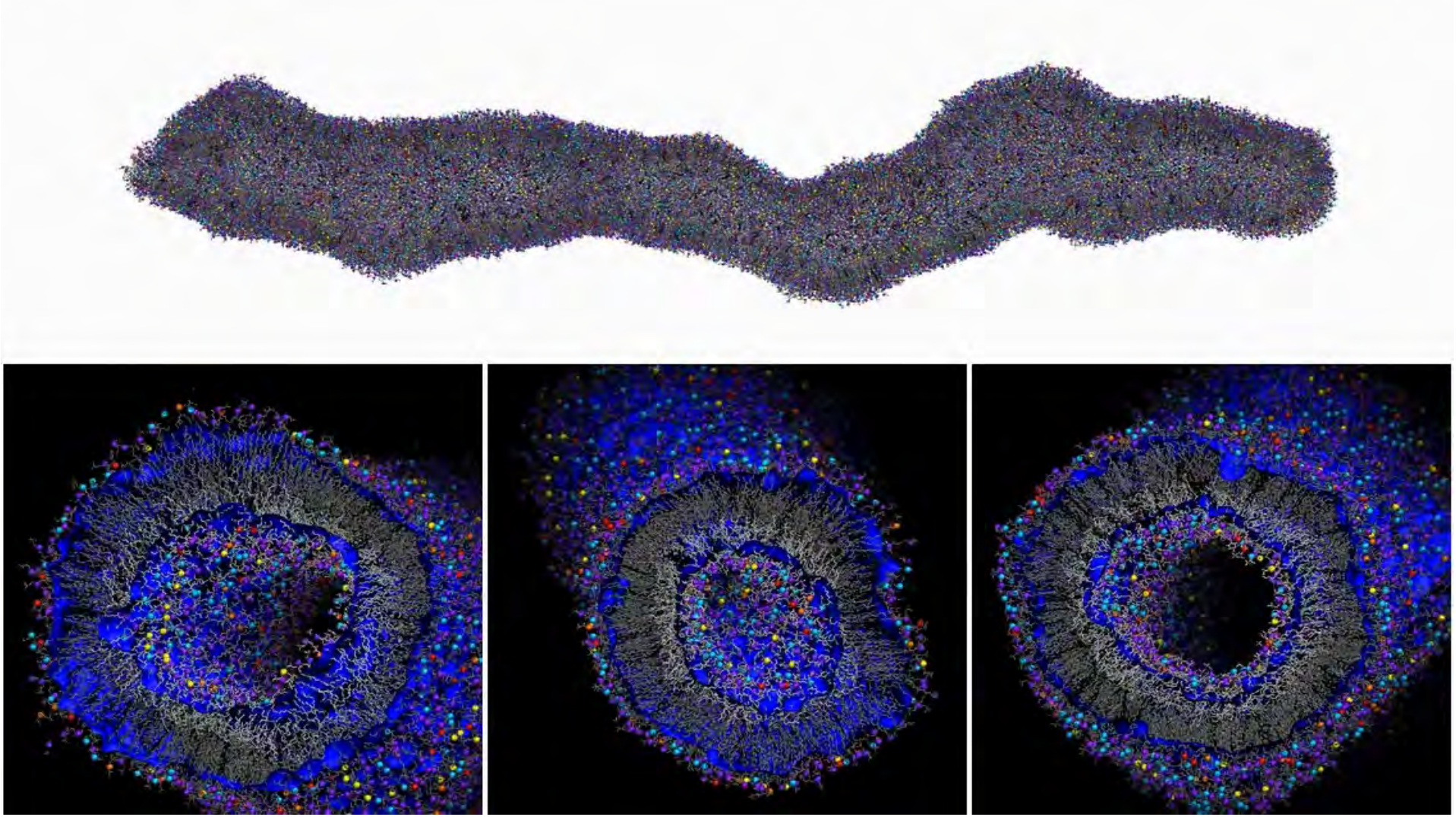
$$\vec{a}_i(t + \delta t) = \frac{\vec{F}_i(t+\delta t)}{m_i} = -\frac{1}{m_i}\vec{\nabla}_i U(\vec{r}_i(t + \delta t))$$

$$\vec{v}_i(t + \delta t) = \vec{v}_i(t) + \tfrac{1}{2}(\vec{a}_i(t) + \vec{a}_i(t + \delta t))\delta t$$

Methodological Overview of xMAS Builder

(E**x**perimentally-Derived **M**embranes of **A**rbitrary **S**hape)

Membrane Stable During Unbiased Simulation

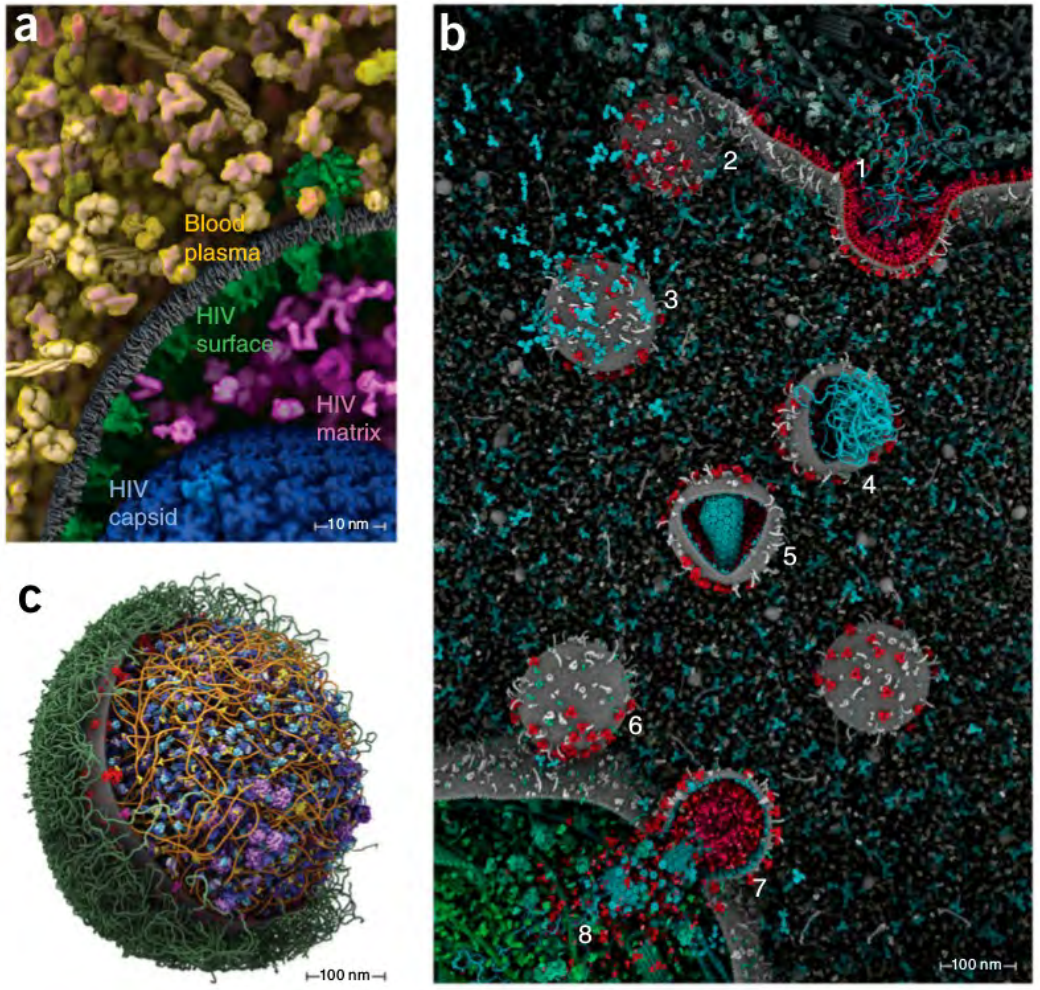


16,754 Lipids
~2.1 Million Lipid Atoms
~12.0 Million Atoms Total

~180ns Simulation

Generate Protein Packing

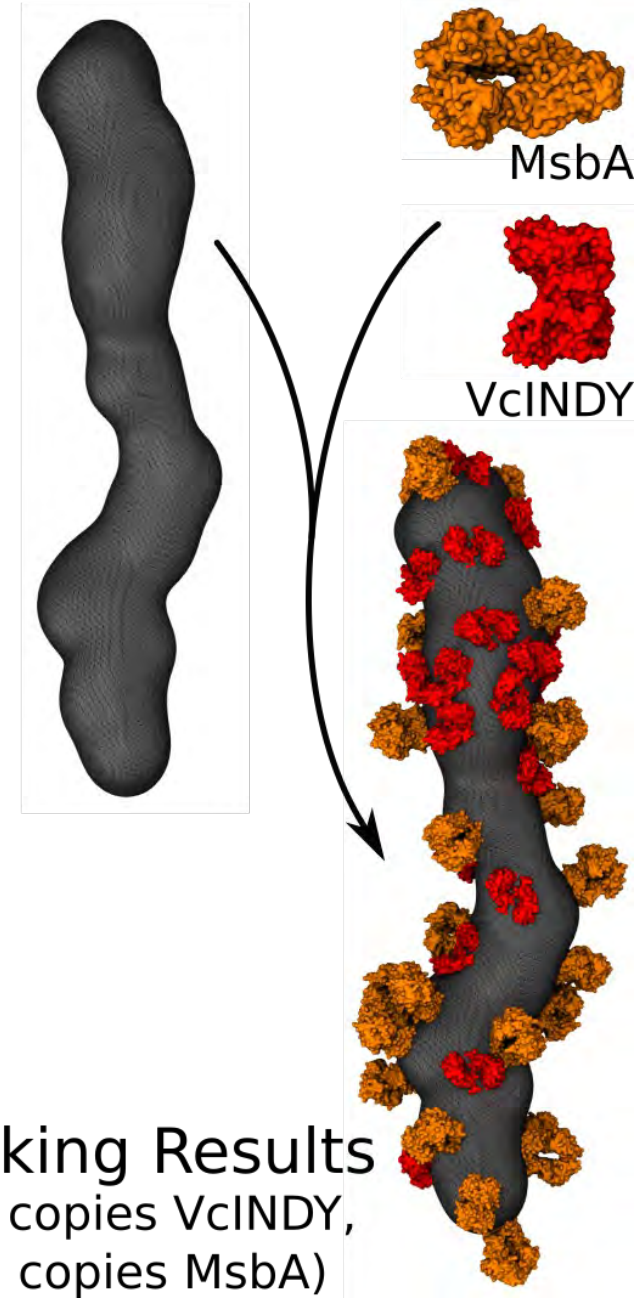
cellPACK



Johnson *et al*. *Nat Methods*. **12**:1, 85-91 (2015).

Membrane Mesh

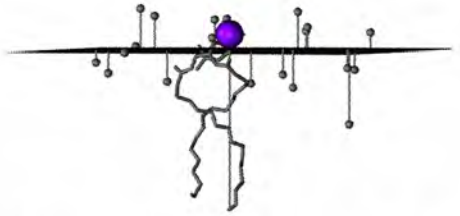
Protein Meshes



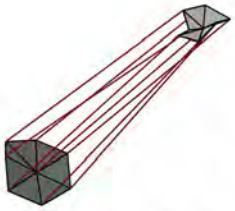
Packing Results
(25 copies VcINDY,
25 copies MsbA)

Methodological Overview of xMAS Builder

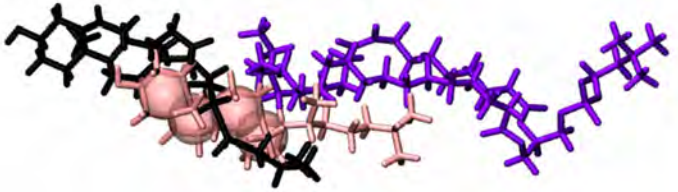
Obtain 3D mesh from an experimental technique



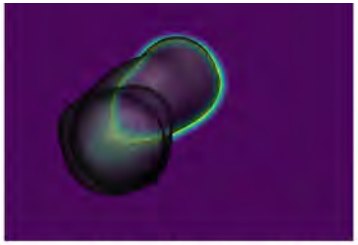
Replace particles with atomistic lipids



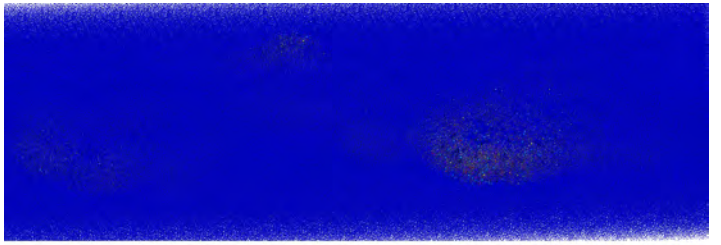
Construct inner leaflet mesh



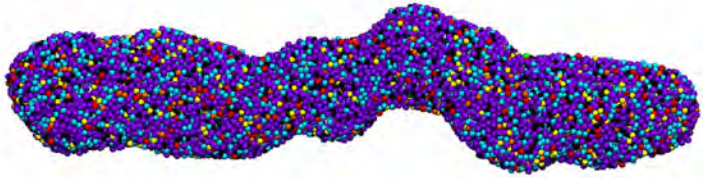
Fix ring piercings



Generate attractive grid densities from inner and outer leaflet meshes

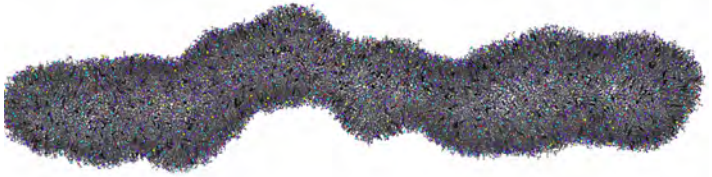


Solvate bilayer



Optimize lipid placement

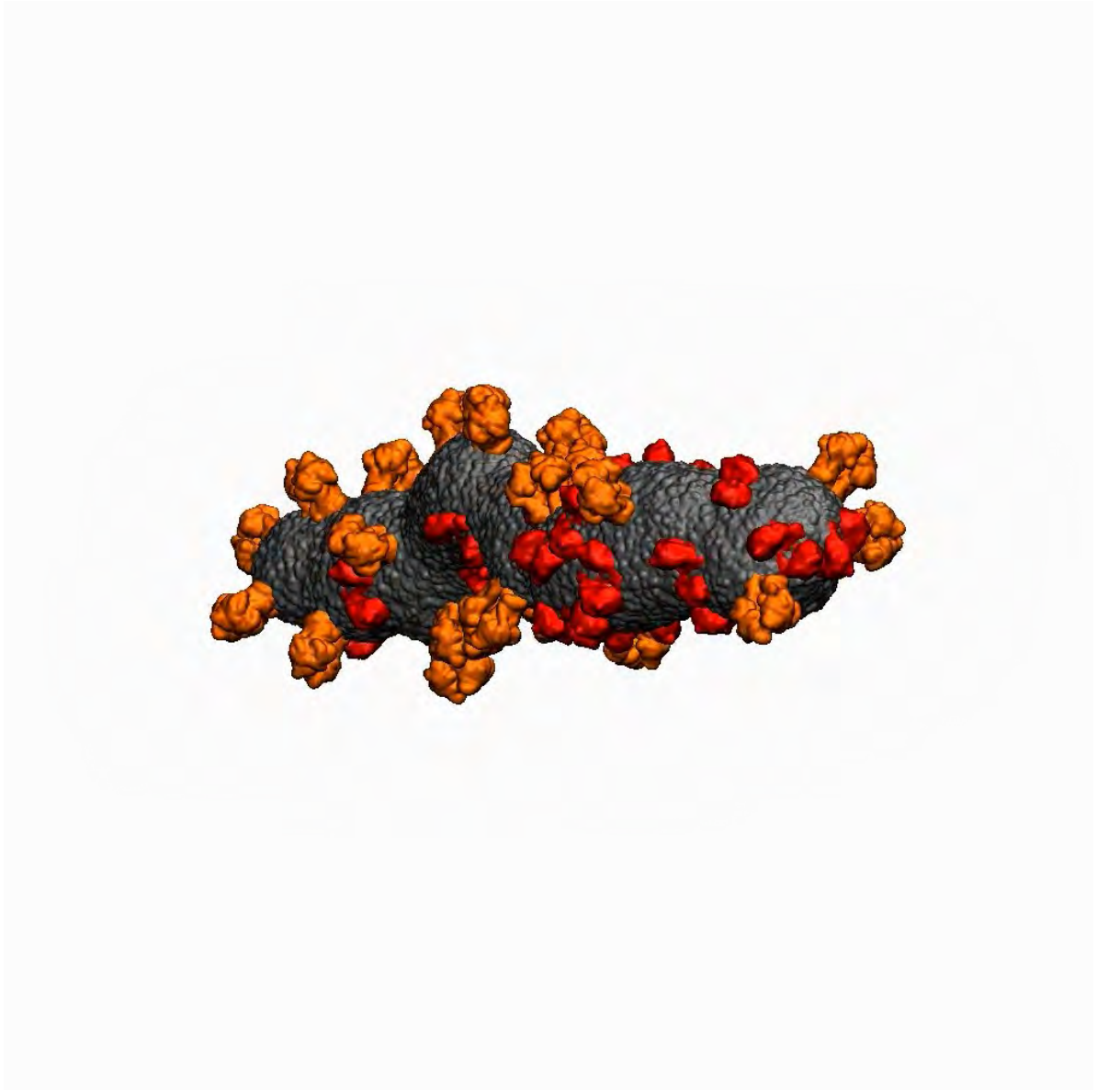
Simulate particles while restrained to attractive densities



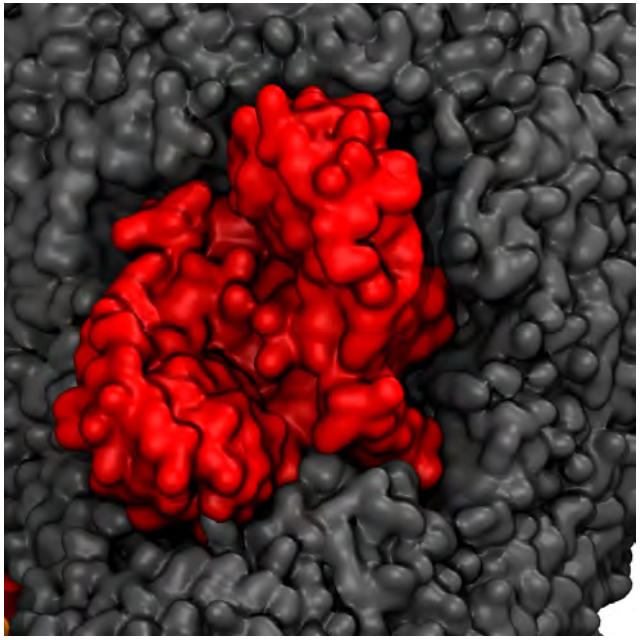
Run simulation

Generate a Membrane with Proteins

Overlay Packed Proteins with Lipids

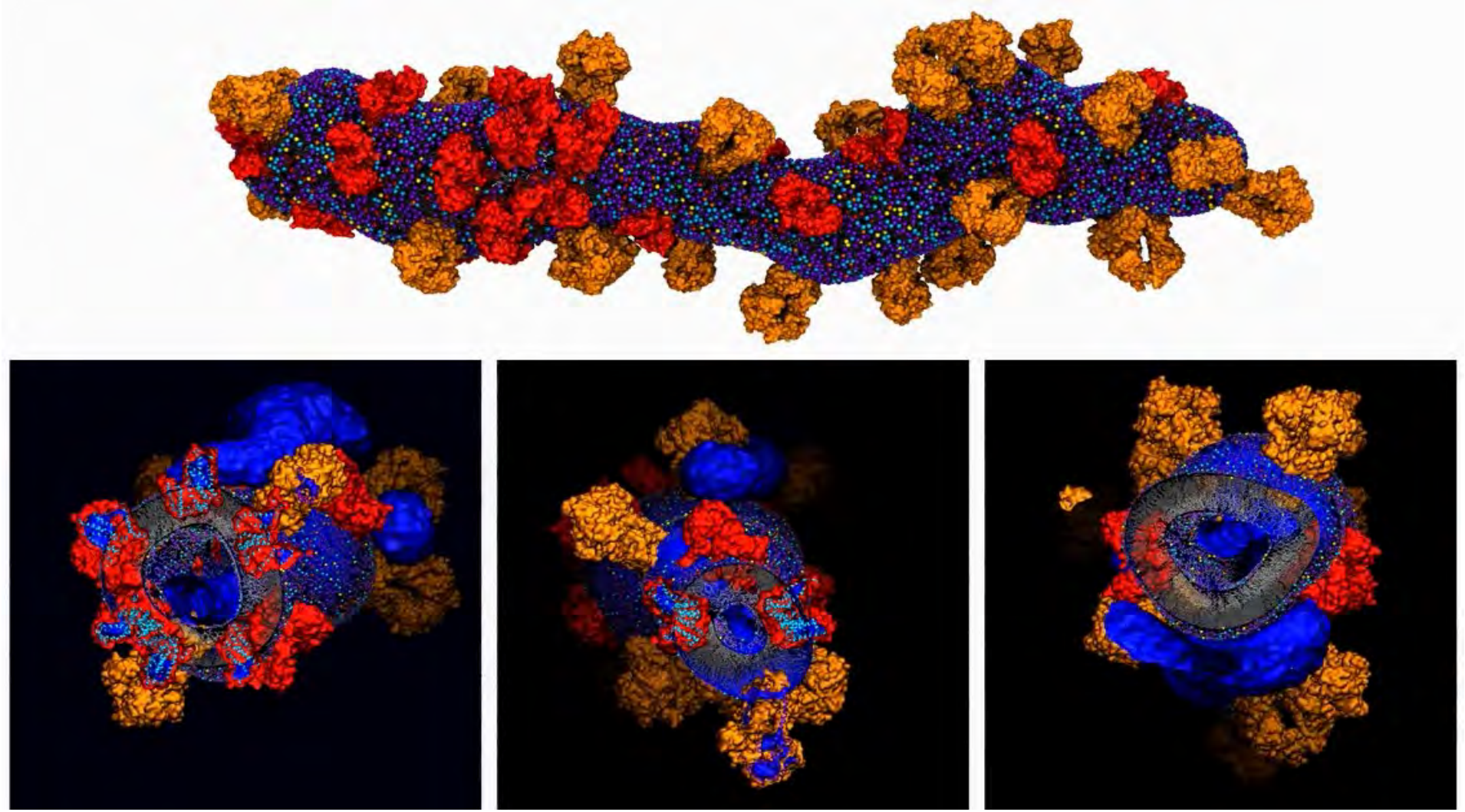


Delete Overlapping Lipids



- Removes too many lipids
- Does not maintain lipid composition

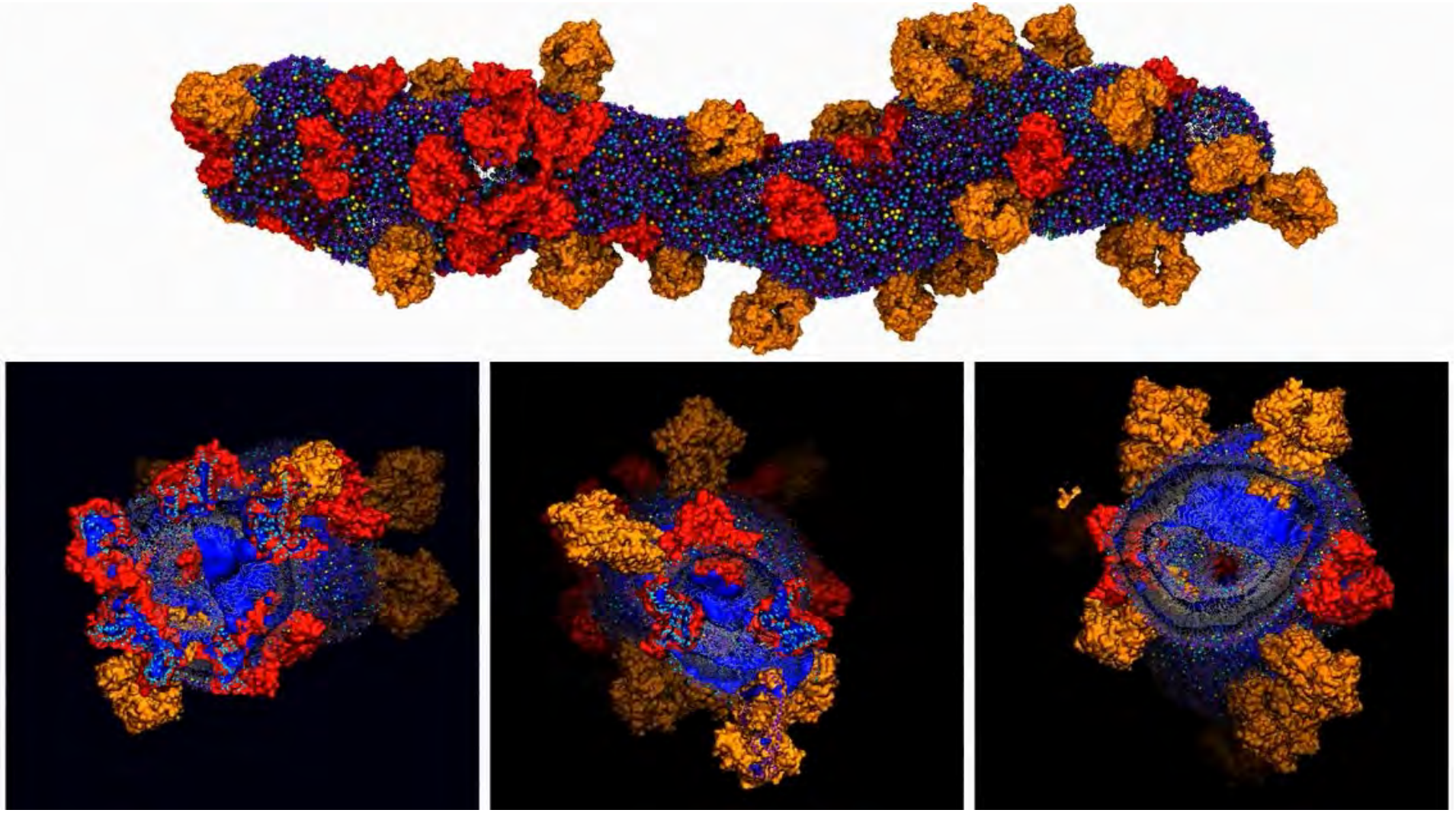
Apply Restrained Equilibration



~23.8 Million Atoms
5ns Simulation

Water Restrained
Membrane Restrained
Protein Cα Fixed

Apply Unrestrained Equilibration

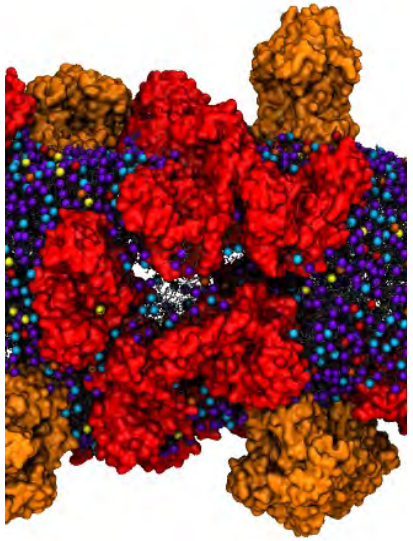


~24.5 Million Atoms
10ns Simulation

Water Unrestrained
Membrane Unrestrained
Protein Cα Free

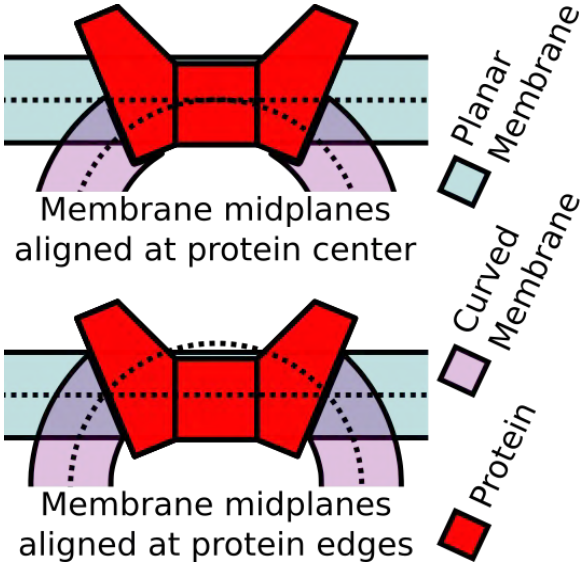
Modeling Problems Revealed by Simulation

Proteins Packed Too Close Together

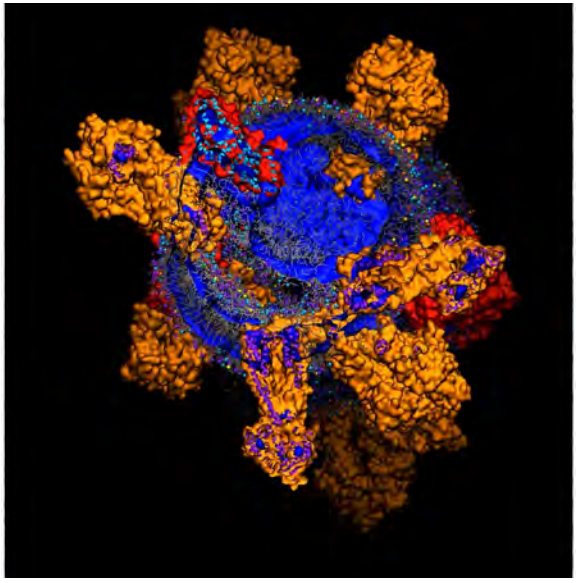


Few Lipids Between Proteins

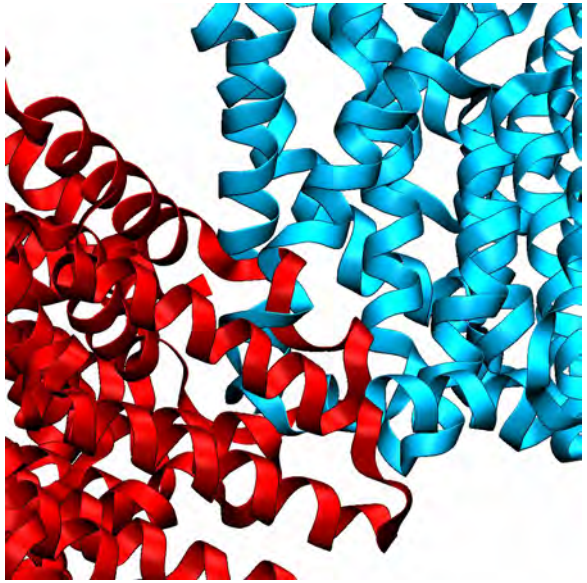
Inner and Outer Leaflets Unzip



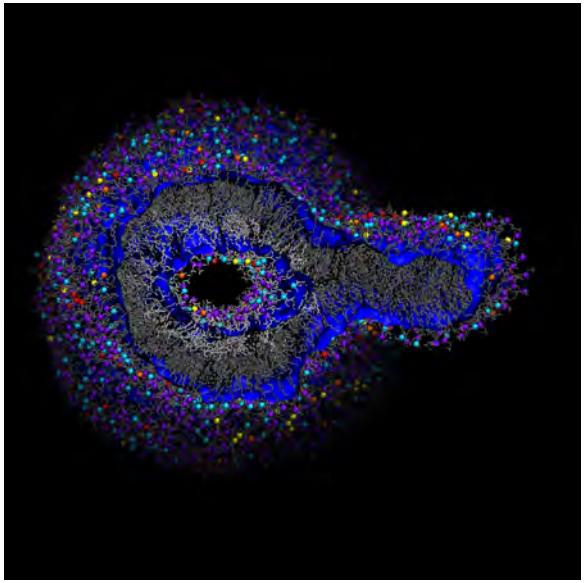
Proteins Inserted Too Shallowly



After 10ns Unrestrained Equilibration

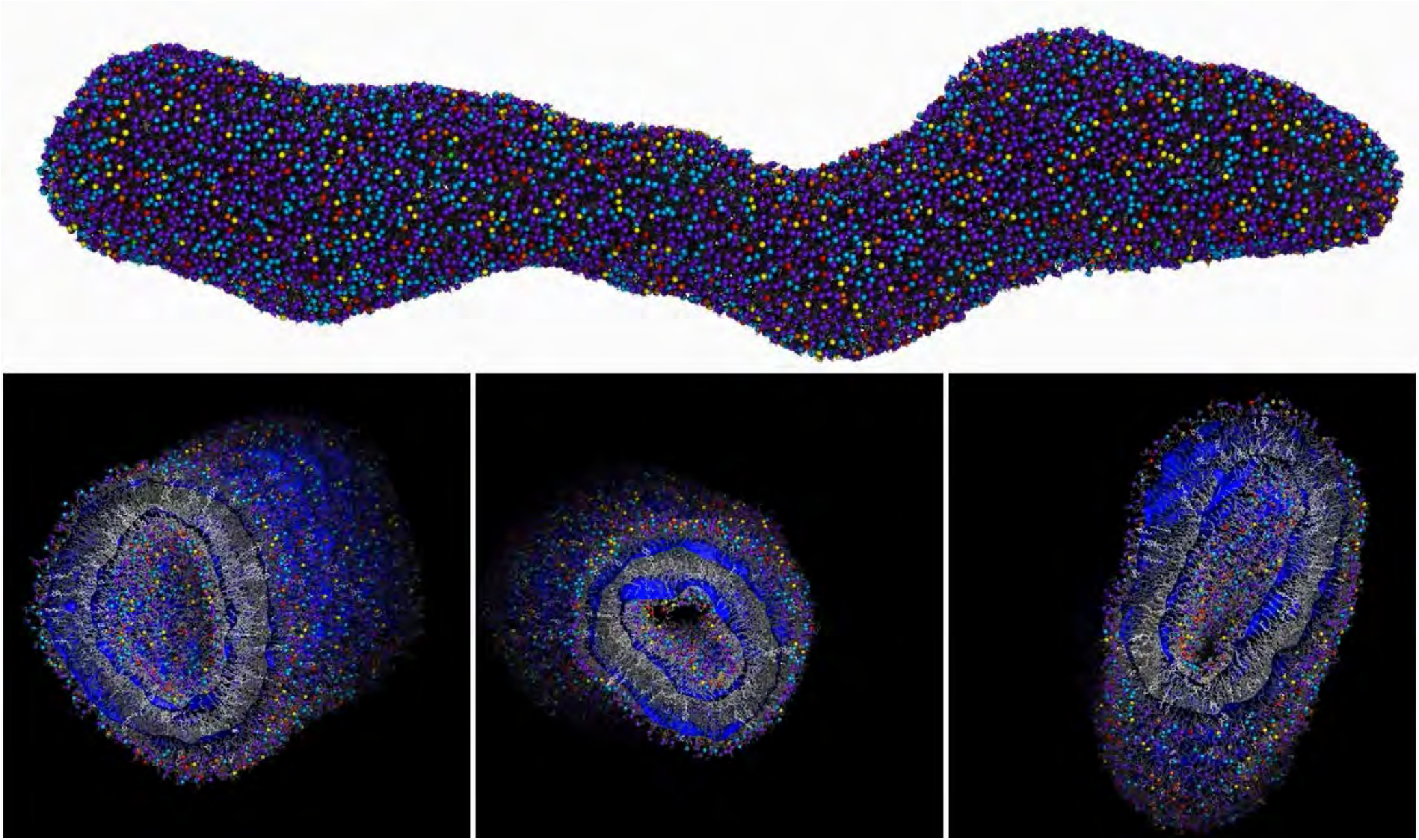


Polypeptide Chains Linked



Outer Leaflet Overfilled

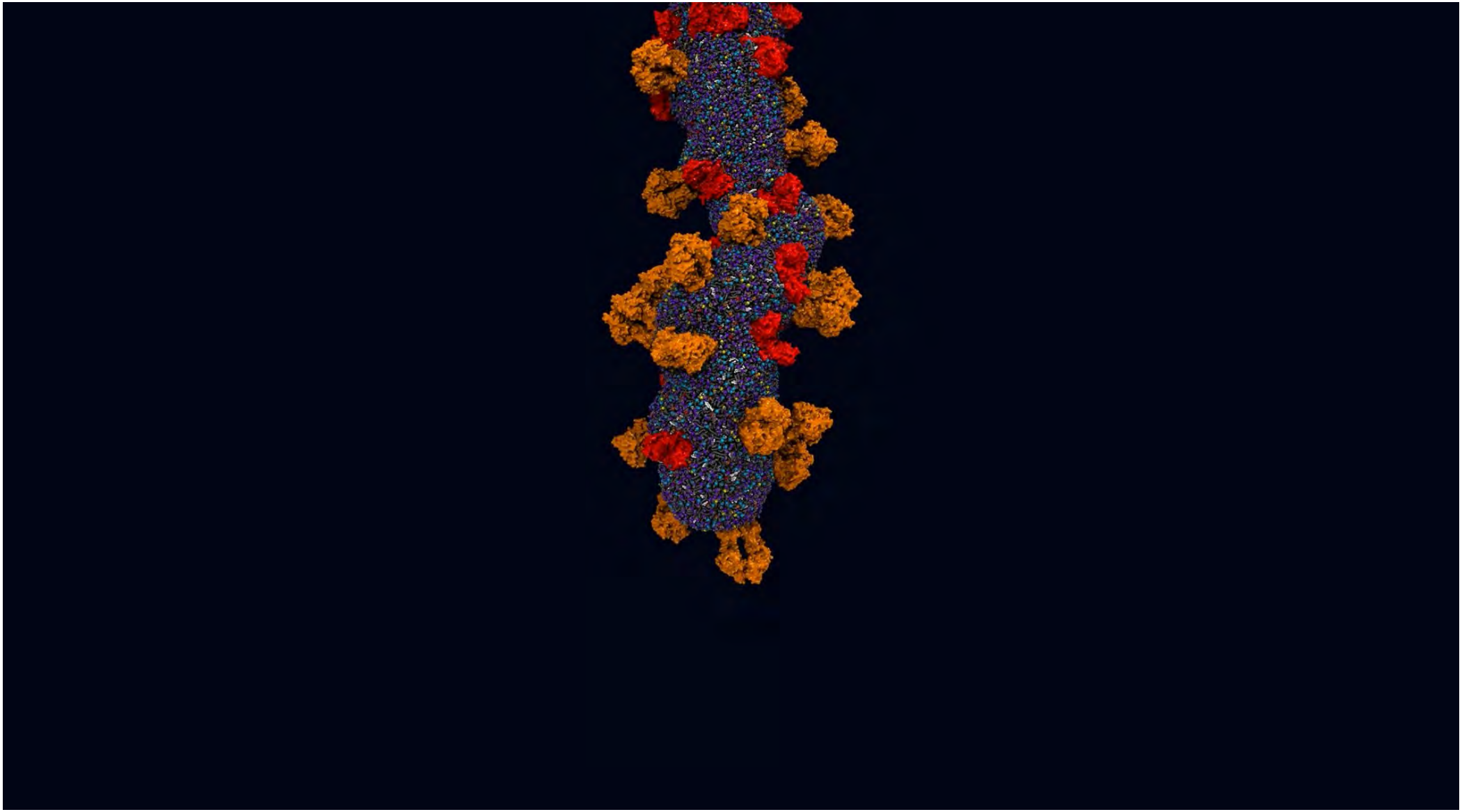
Revisiting Calculation of Lipids per Leaflet



16,100 Lipids
~2.0 Million Lipid Atoms
~11.5 Million Atoms Total

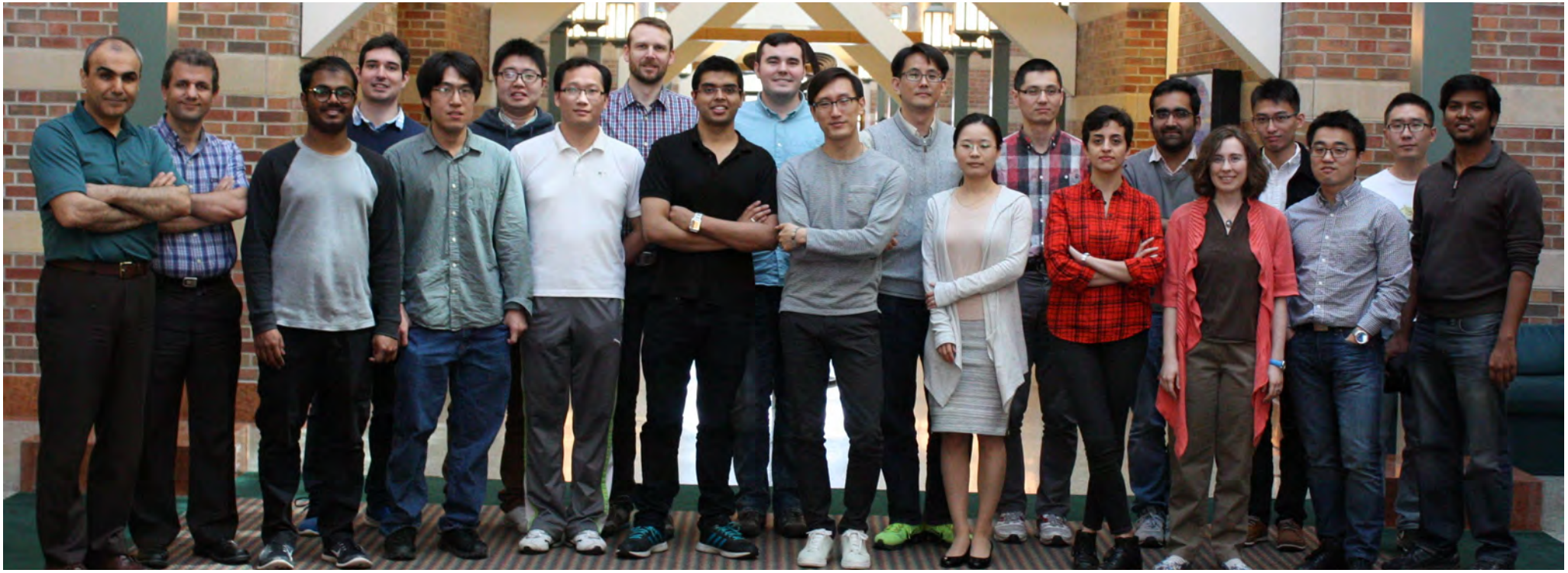
~225ns Simulation

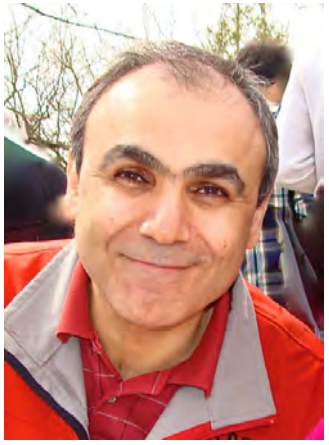
Future Work



- Implement full modeling protocol
- Fix existing modeling problems
- Choose different membrane proteins
- Run simulation again

Acknowledgements





Emad Tajkhorshid, Tajkhorshid Group
NIH Center for Macromolecular Modeling and Bioinformatics

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