

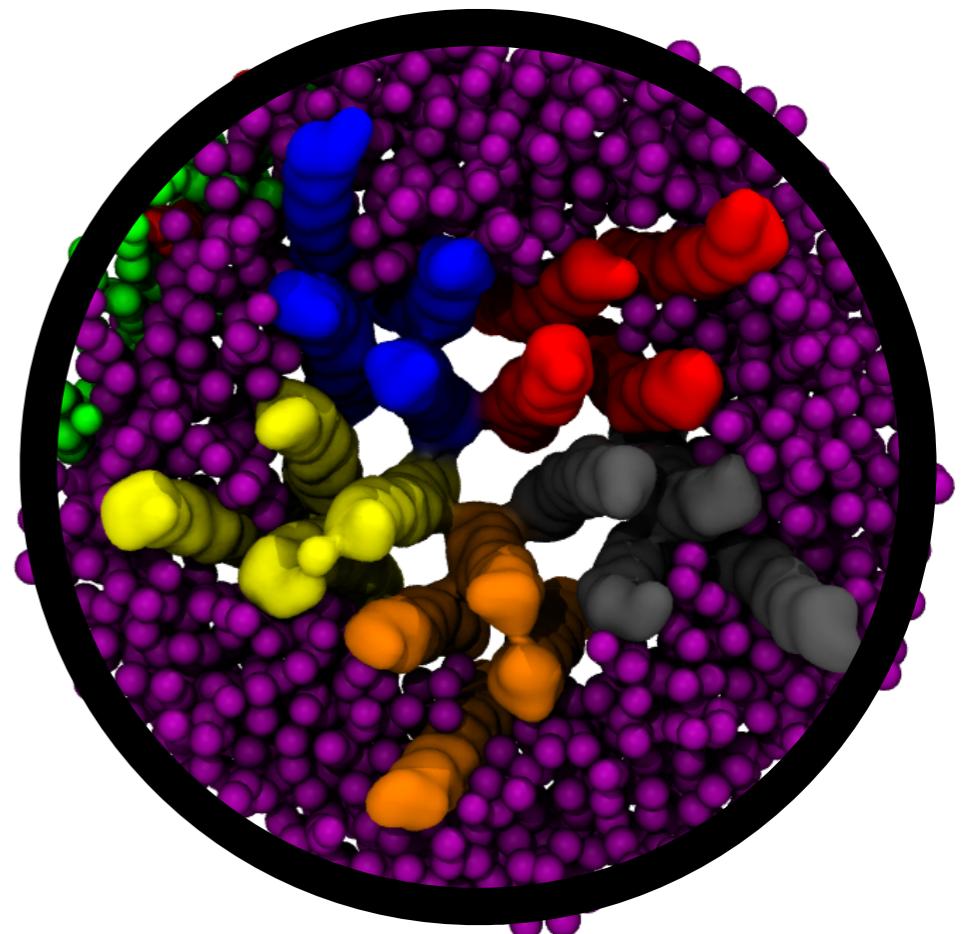
A Coarse Grained Study of Nicotinic Acetylcholine Receptor-Lipid Interactions

Liam Sharp of Brannigan Lab

Overview

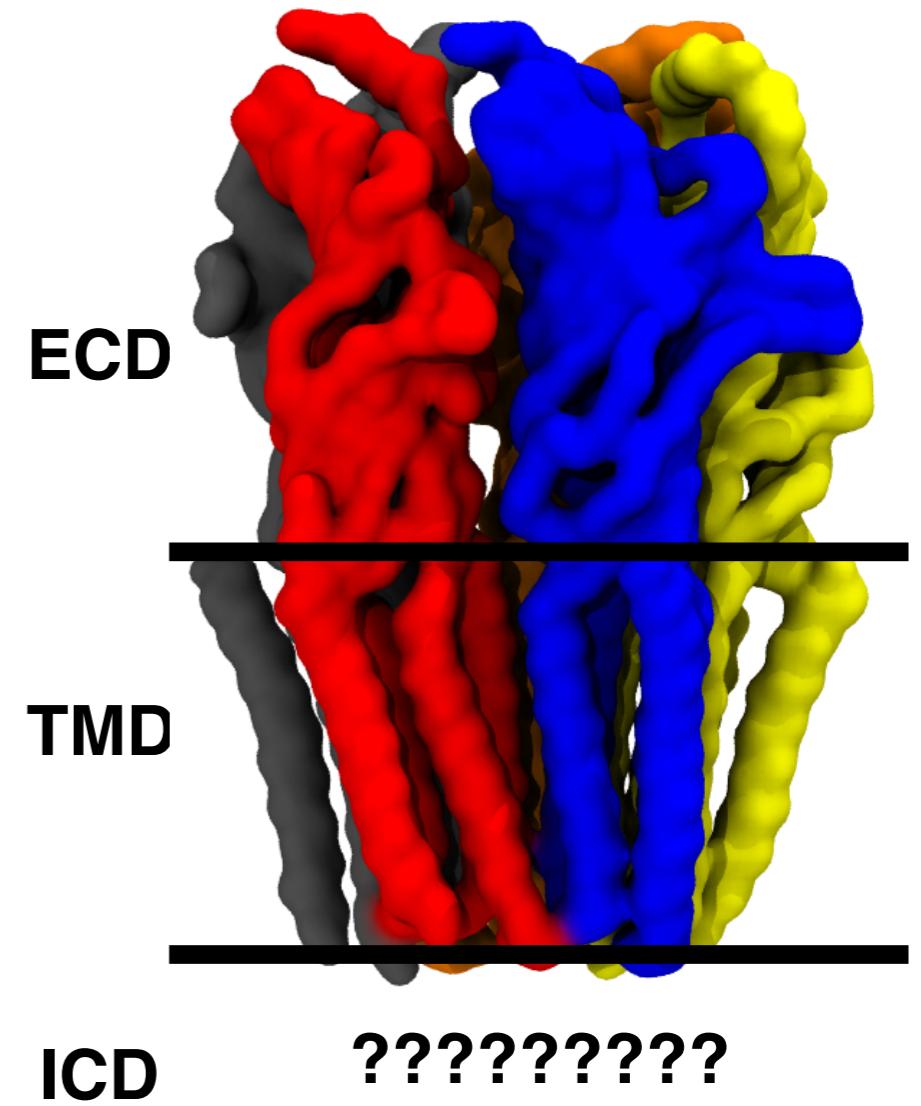
- Little to no information on nAChR-lipid interactions in **neuronal membrane**
 - What lipids are in the annular domain?
 - Where do lipids bind if they bind?
 - Is nAChR lipid specific?
- **Initial hypothesis:** nAChR functional dependency on cholesterol suggesting cholesterol and saturated lipids form the annular domain
 - Marchand (2002), Zhu (2006), Campagna (2006) show nAChR clustering is dependent on lipid rafts

Annular Domain

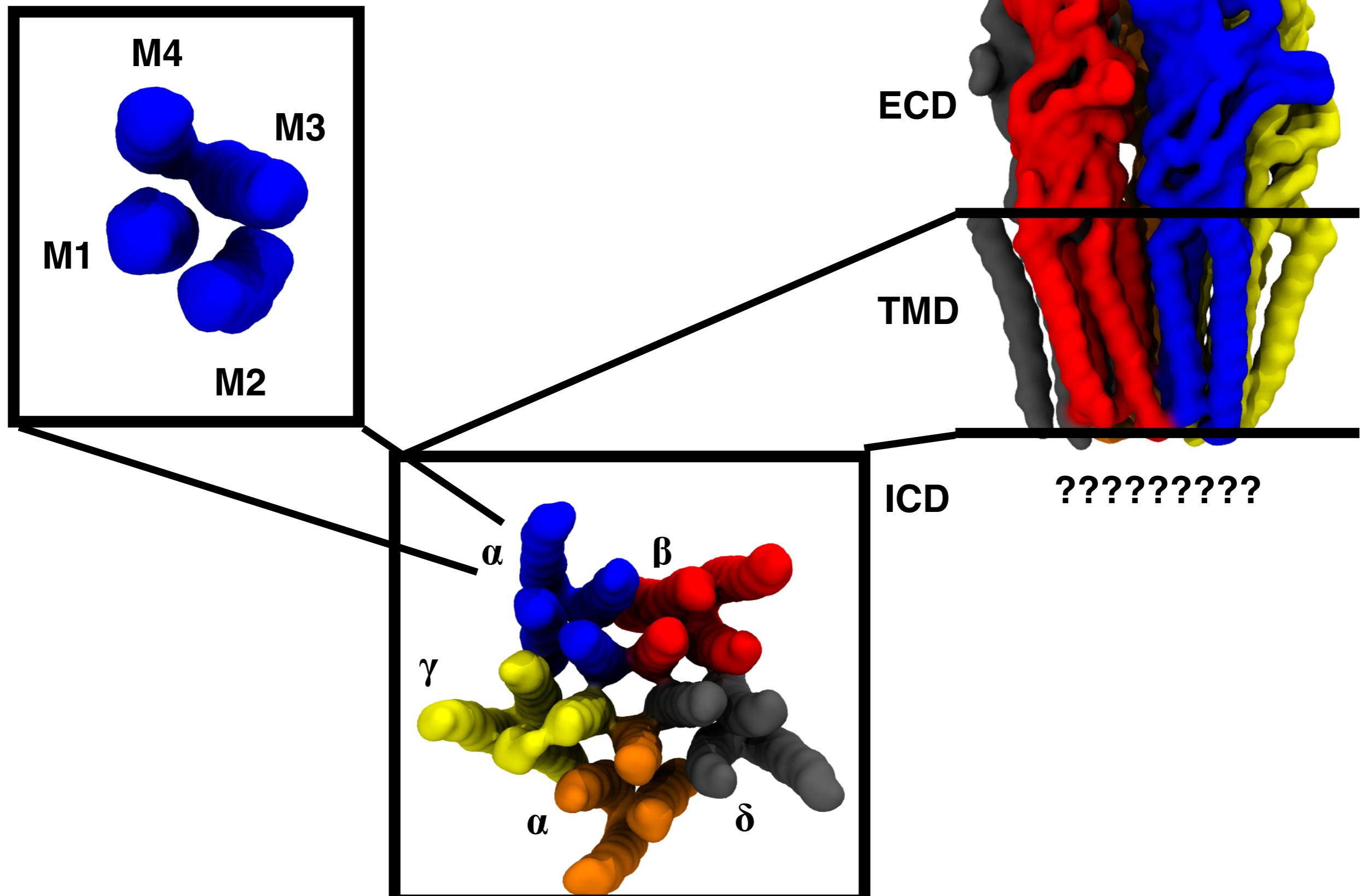


Nicotinic Acetylcholine Receptor (nAChR)

- Pentameric ligand gated ion channel gated by binding of acetylcholine
- Found through out the central and peripheral nervous system
- Roll in neurological disorders, addiction, anesthetic binding
- Contributes to neuronal and muscular function by stimulating an action potential across post-synaptic membrane
- Has various subunit types and hetero-pentamer configuration.

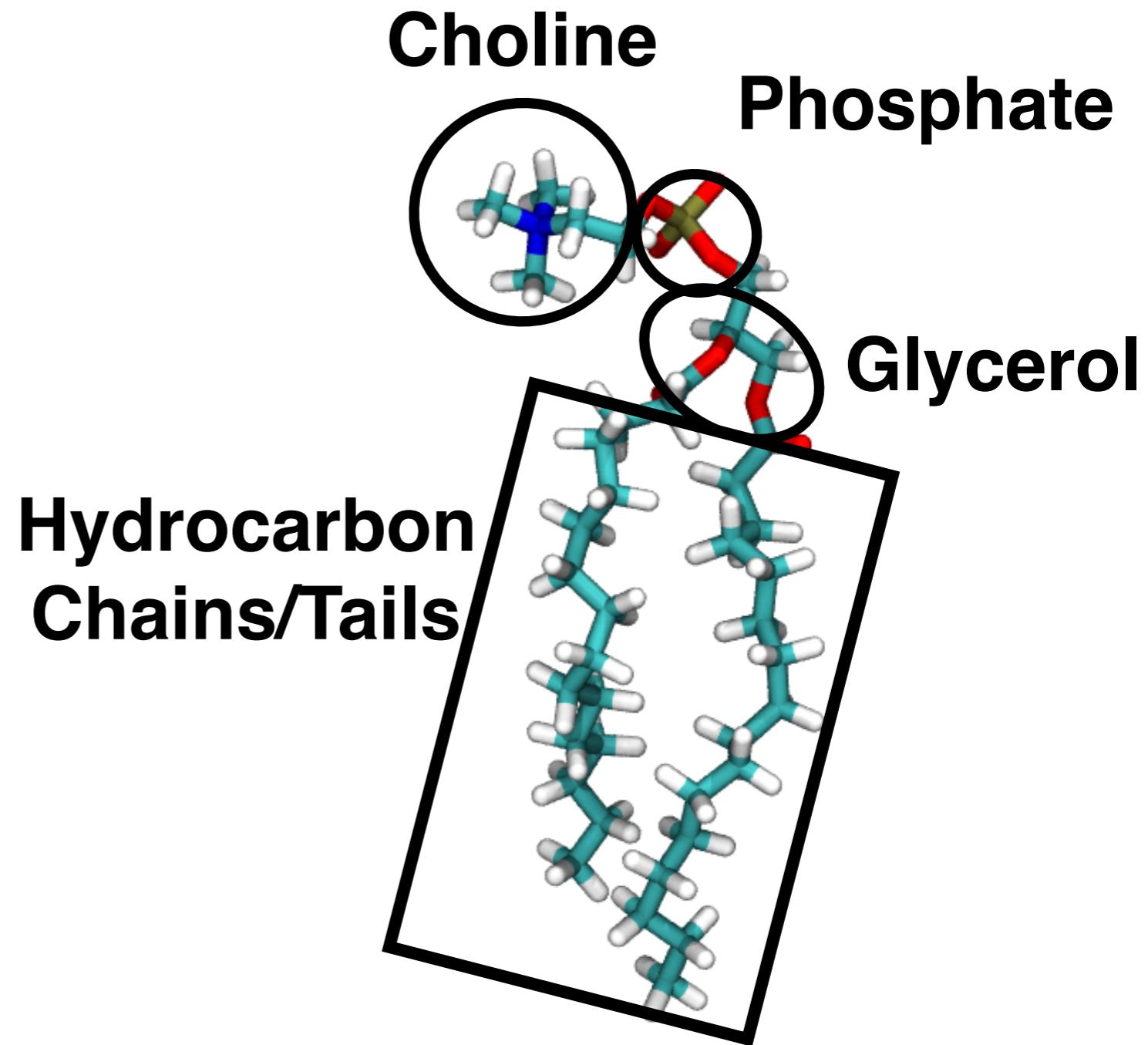


Nicotinic Acetylcholine Receptor (nAChR)



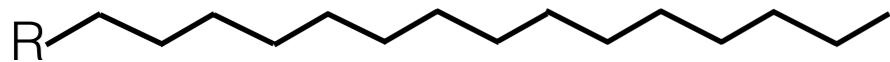
Phospholipids

- Hydrophilic Head
 - Choline, ethanolamine, serine, glycerol, myo-inositol, phosphate, and glycerol backbone
- Hydrophobic Chains
 - Hydrocarbon chains of various lengths and hydrogen saturation
- Amphiphilic nature promotes spontaneous membrane formation



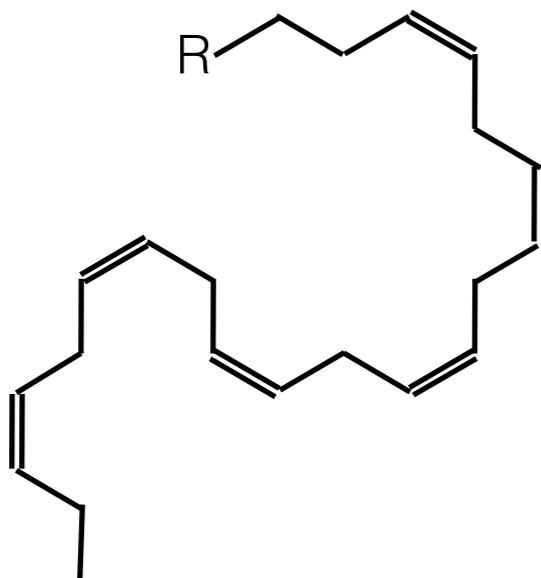
Phospholipids

Sat



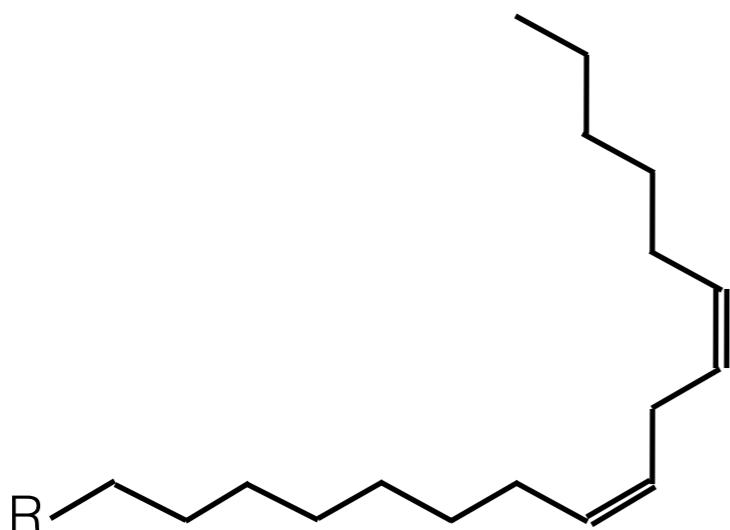
<https://pubchem.ncbi.nlm.nih.gov/compound/985#section=Top>

n-3



<https://pubchem.ncbi.nlm.nih.gov/compound/445580#section=Top>

n-6



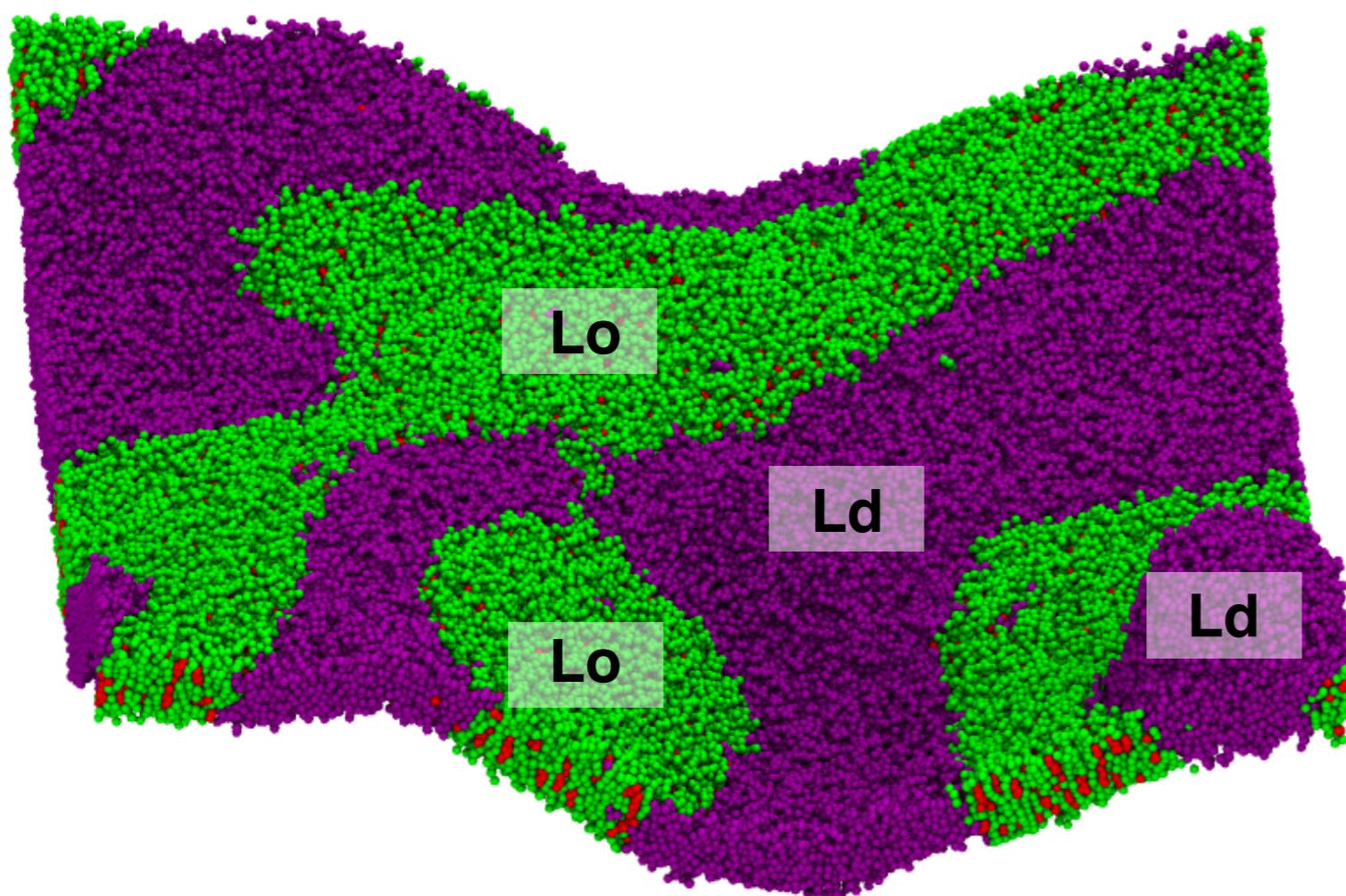
<https://pubchem.ncbi.nlm.nih.gov/compound/5283446#section=Top>

- Focus on three types of Lipids:

- Lipids with saturated fatty acid chains
- Lipids with poly-unsaturated fatty acid (PUFAs) chains

- We look at n-3 and n-6
- Cholesterol

Domain Formations

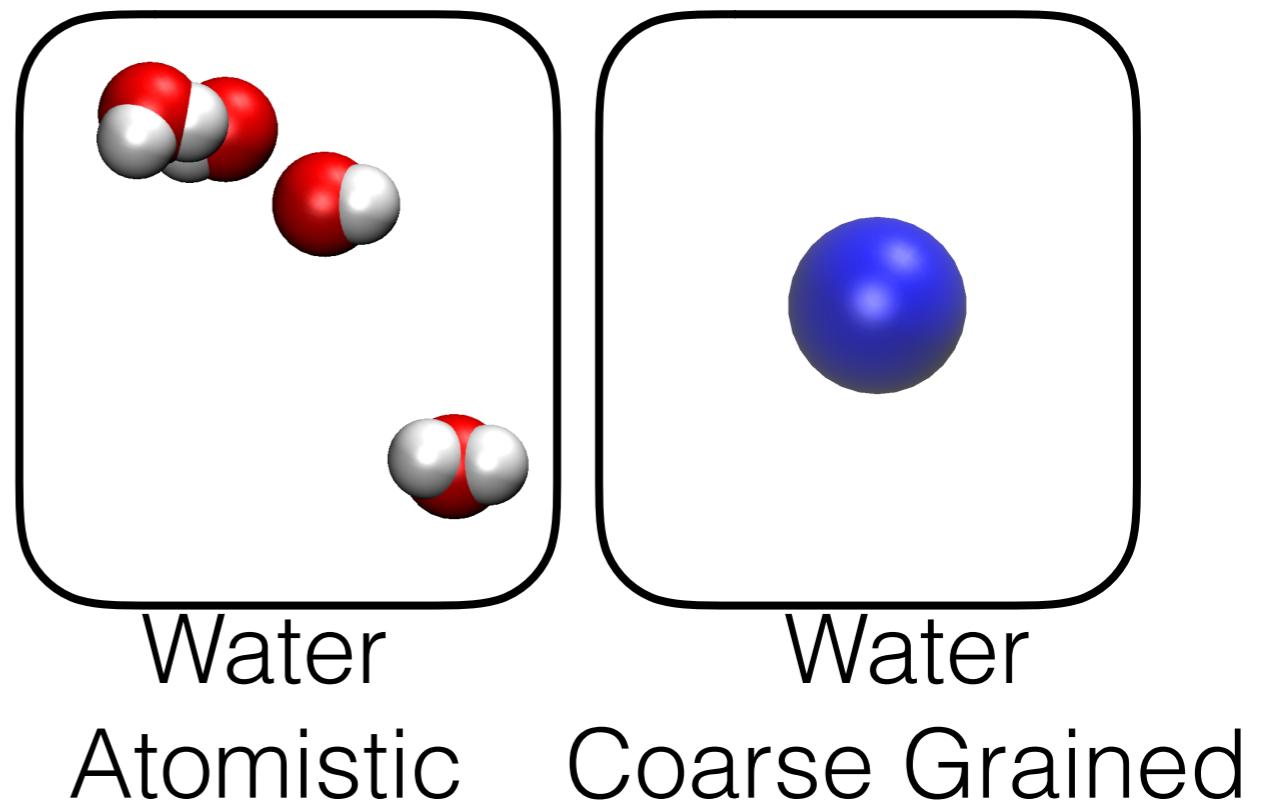


- Membranes with at least 3 lipids can form two distinct fluid phases
 - Liquid ordered (Lo or lipid raft):
 - Saturated Lipids and cholesterol
 - Stable, rigid, flat, compressed, low melting point
 - Liquid disordered (Ld):
 - Unsaturated Lipids
 - Flexible, fluid, high melting point

Saturated **Cholesterol** Unsaturated

Methods

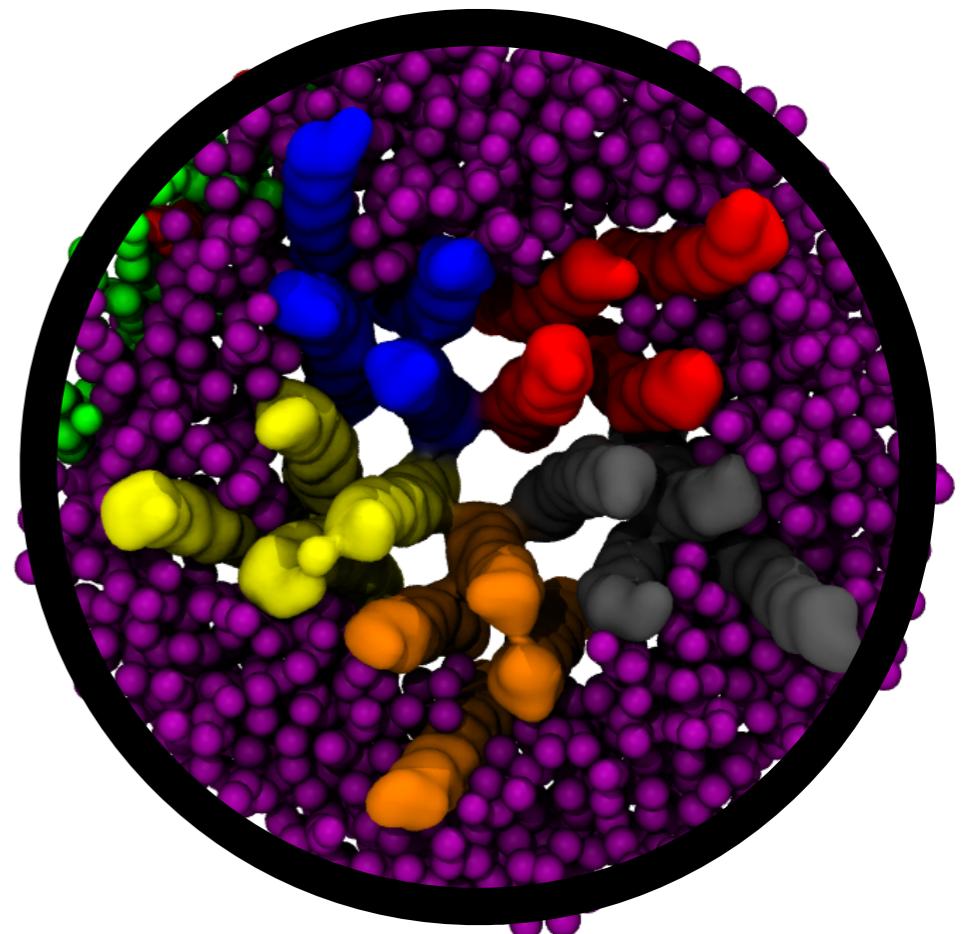
- Molecular Dynamics (Gromacs 5.0.6)
- Martini (ver 2.2) Coarse Grained Force Field
 - Longer time scales
 - Larger system
 - Domain Formation



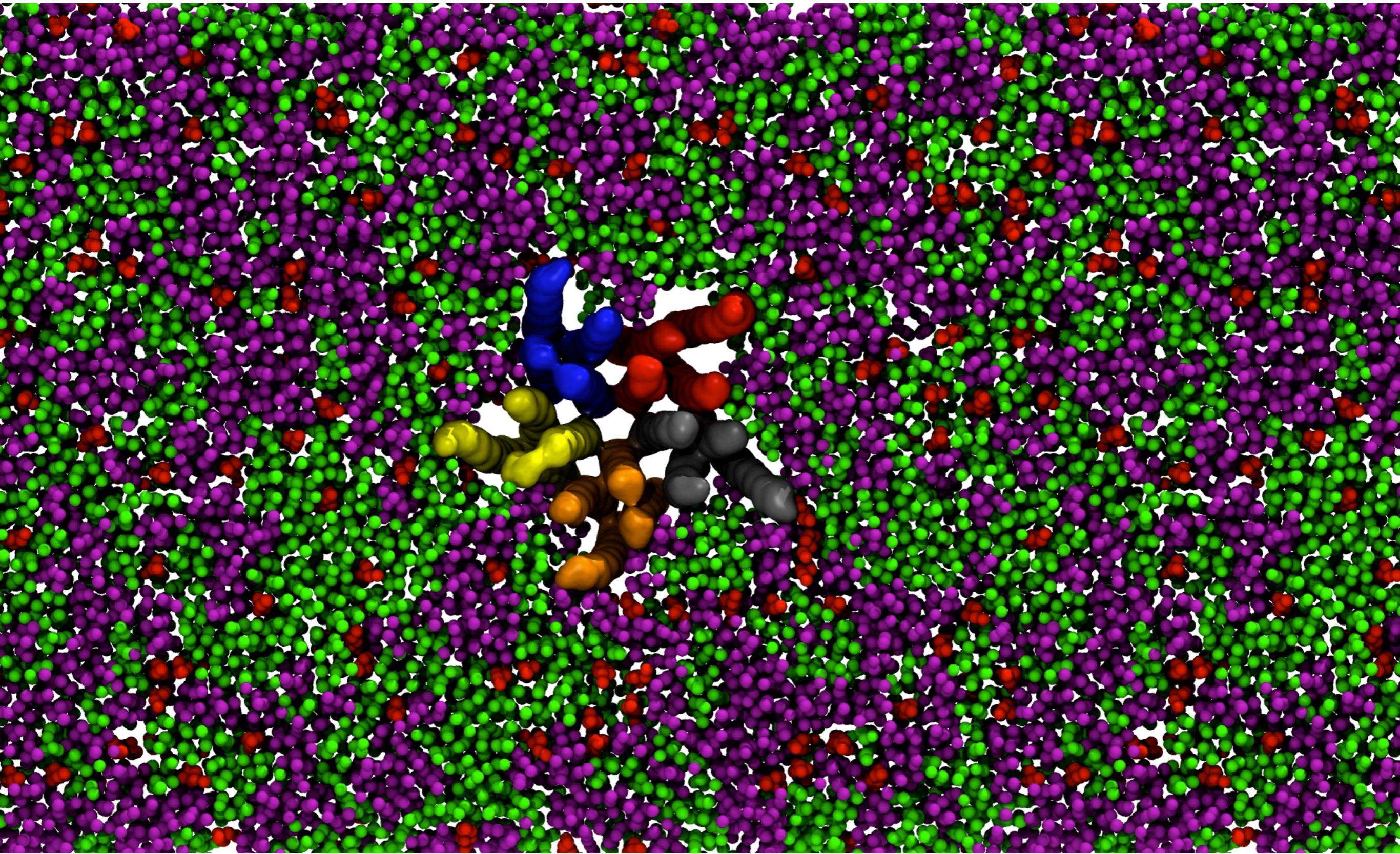
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Annular Domain



nAChR Torpedo Model Membrane



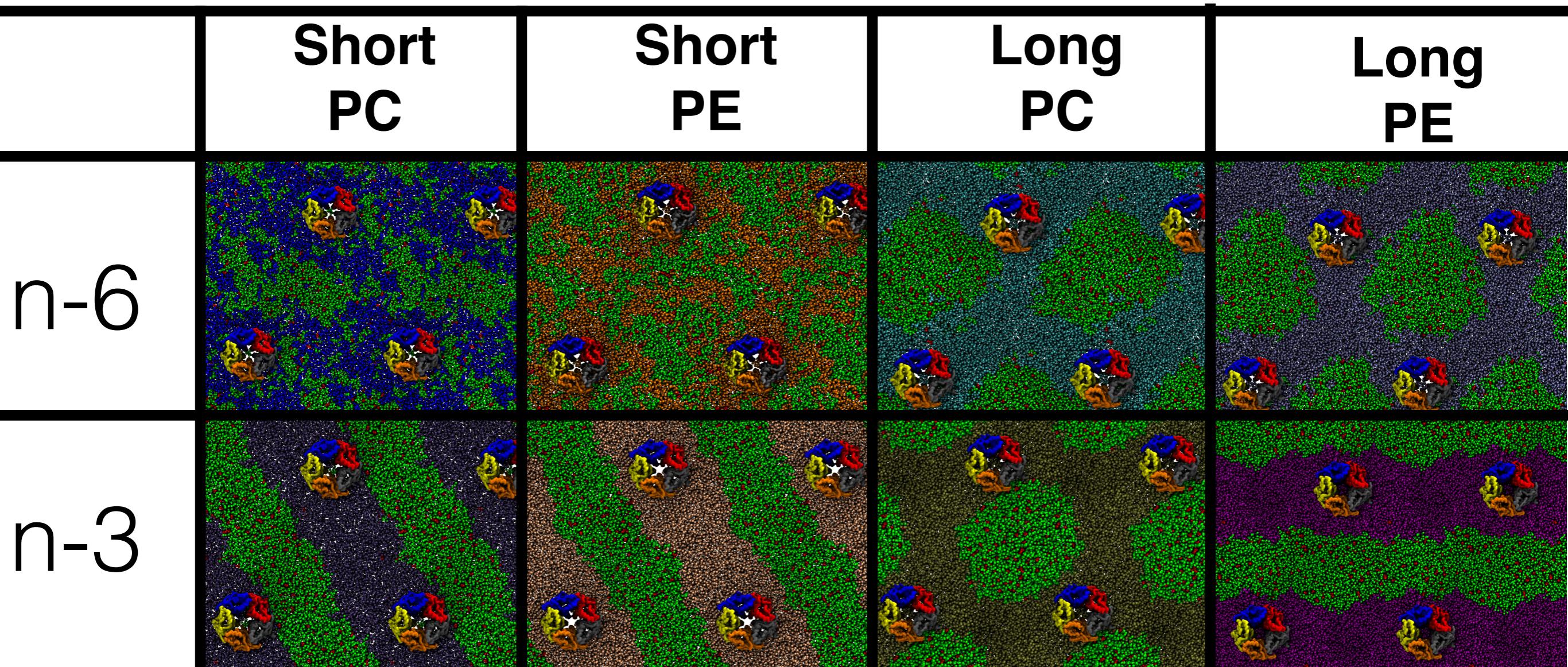
Saturated Cholesterol Unsaturated

~ Initial 50 ns

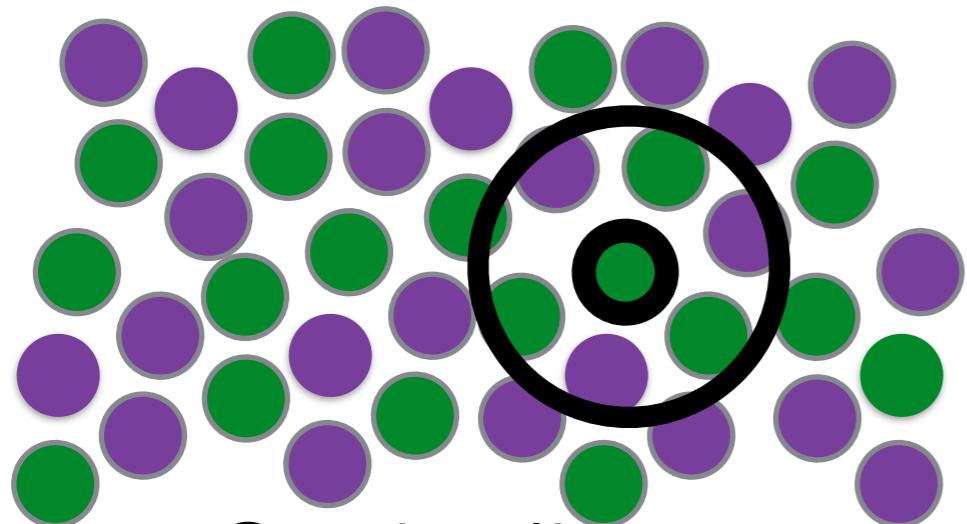
nAChR Torpedo Model Membrane

Final snapshot of simulations lasting 2 us

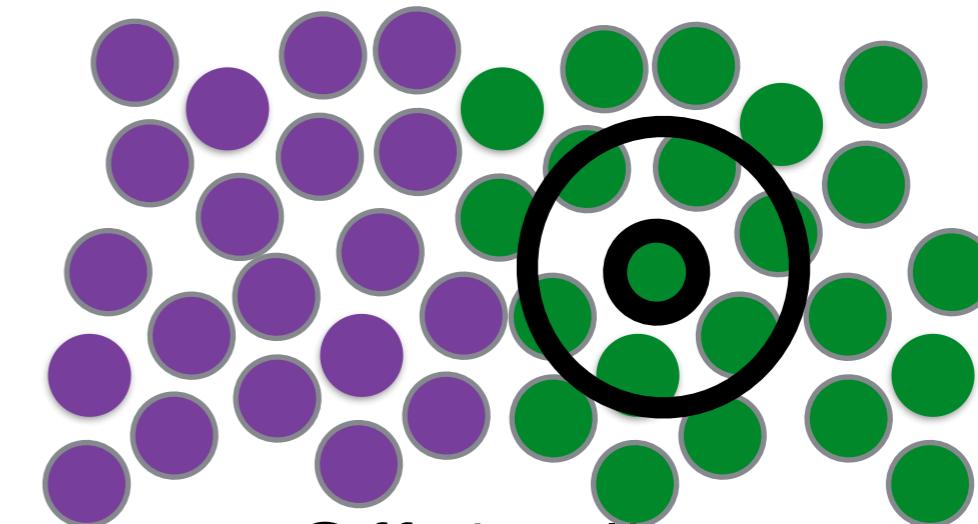
42.5% Saturated 15% Cholesterol 42.5%



nAChR *Torpedo* Model Membrane Lipid-Lipid Contact



On the line



Off the line

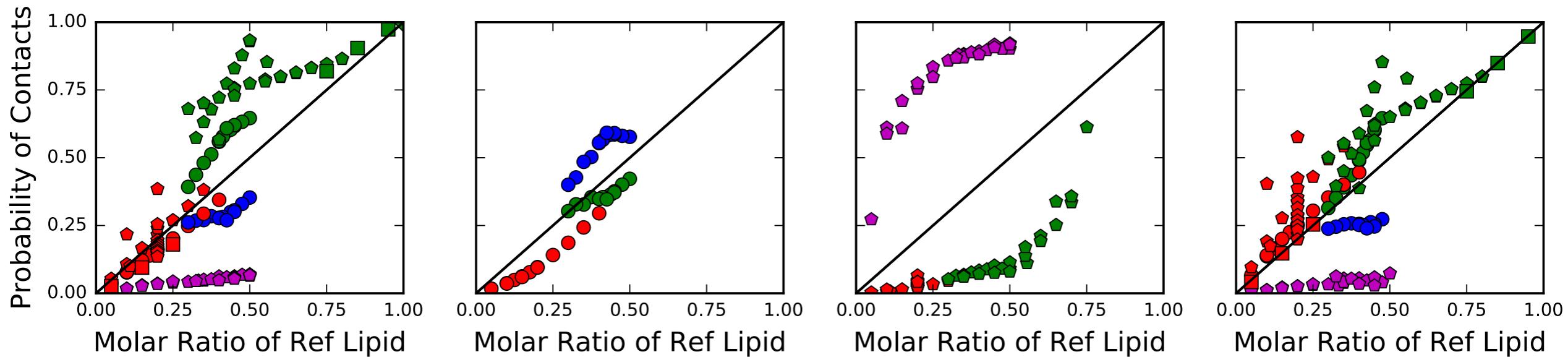
**Ref
Lipid**

DPPC

DLoPC (ω -6)

DHA-PE (ω -3)

CHOL

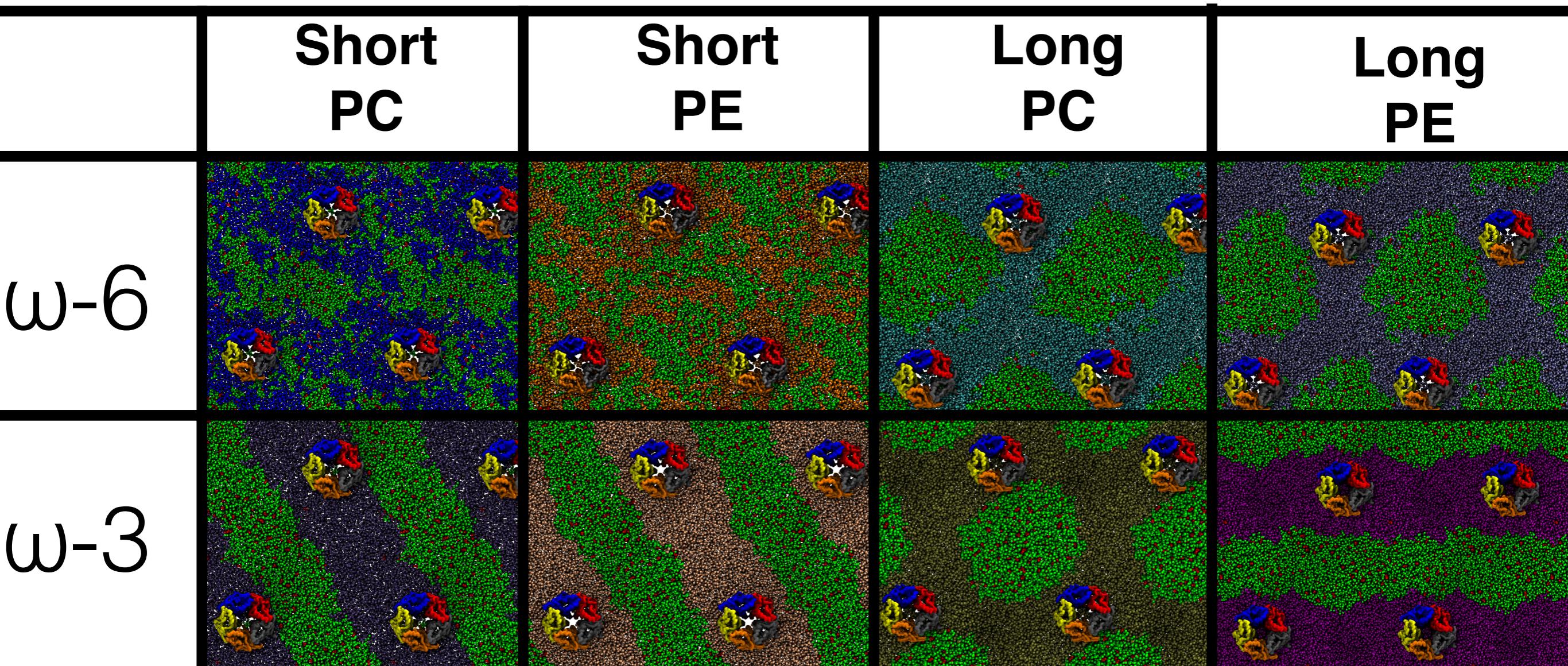


nAChR *Torpedo* Model Membrane

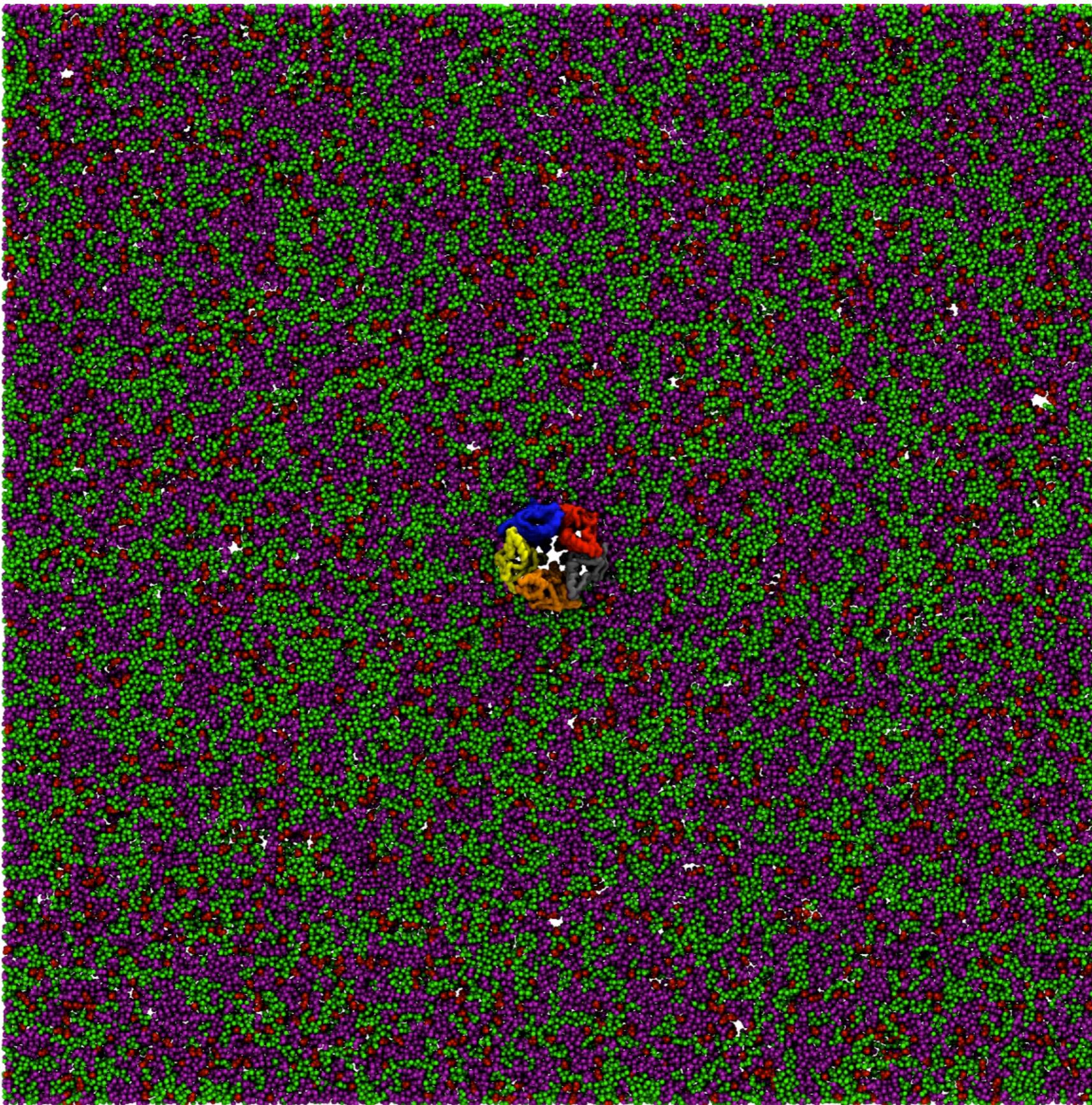
Final snapshot of simulations lasting 2 us

nAChR partitions into liquid-disordered domains

42.5% Saturated 15% Cholesterol 42.5%



nAChR Torpedo Model Membrane



Saturated Cholesterol Unsaturated

~ 2.3 us

nAChR *Torpedo* Model Membrane Experimental Comparison

- Multiple experimental studies show lipid raft's importance to clustered nAChR organization

Partition profile of the nicotinic acetylcholine receptor in lipid domains upon reconstitution^S

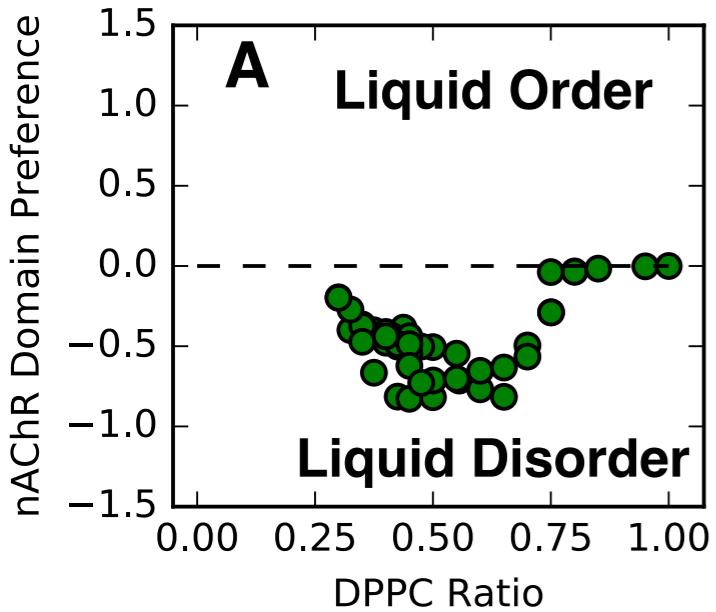
Vicente Bermúdez, Silvia S. Antolini,¹ Gaspar A. Fernández Nievas, Marta I. Aveldaño, and Francisco J. Barrantes

Instituto de Investigaciones Bioquímicas de Bahía Blanca, Consejo Nacional de Investigaciones Científicas y Técnicas, and UNESCO Chair of Biophysics and Molecular Neurobiology, Universidad Nacional del Sur, Buenos Aires, Argentina

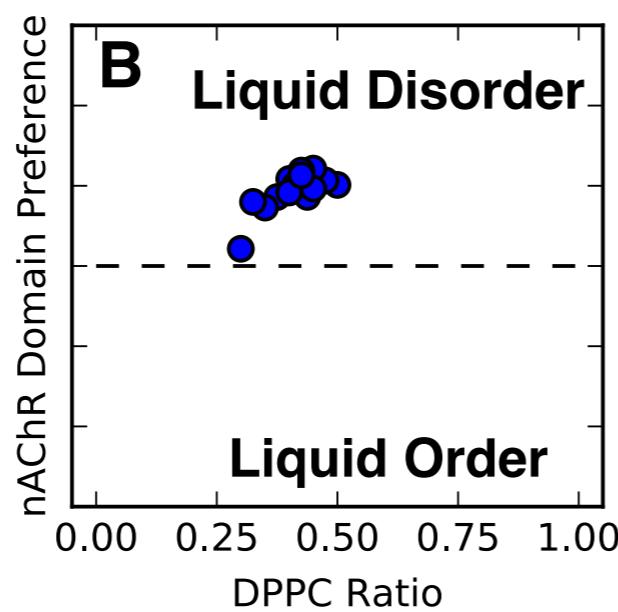
- Clustered nAChR do not have domain preference (based on model membrane) which is surprising due to cholesterol dependance

nAChR *Torpedo* Domain Preference

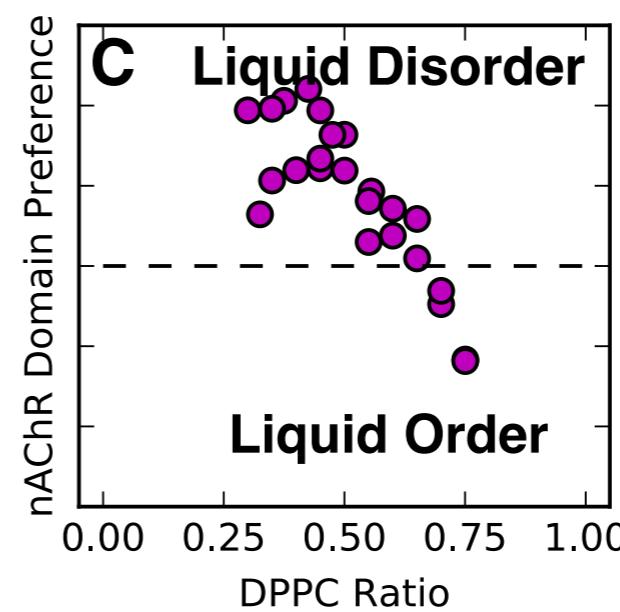
DPPC



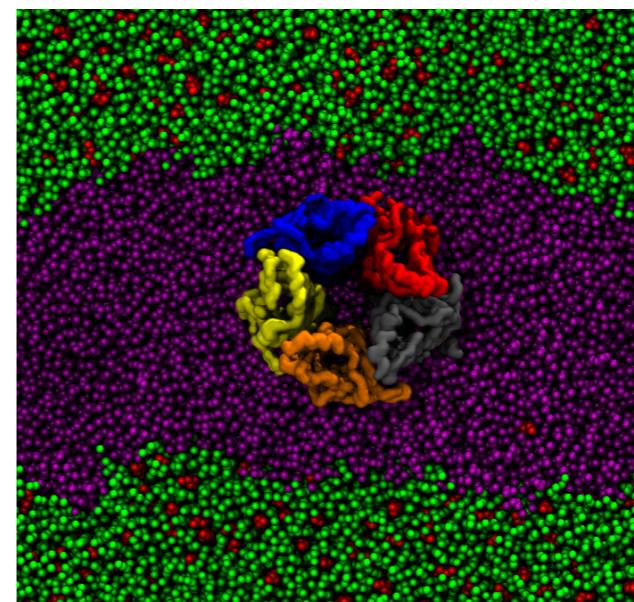
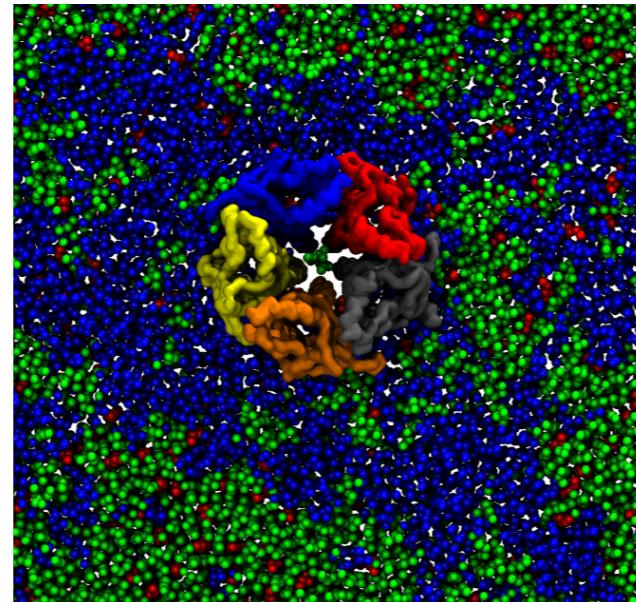
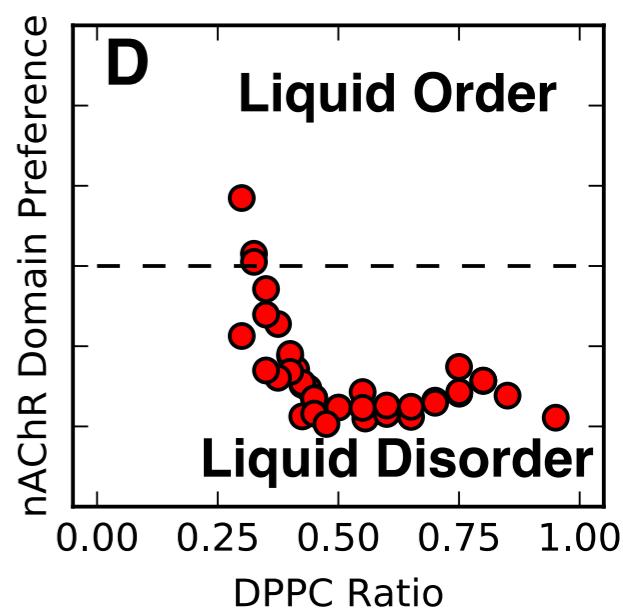
DLoPC (ω -6)



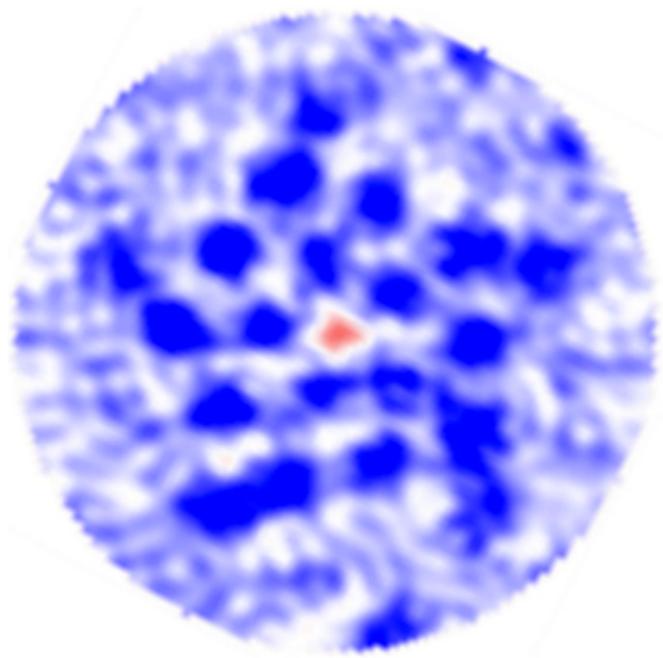
DHA-PE (ω -3)



CHOL

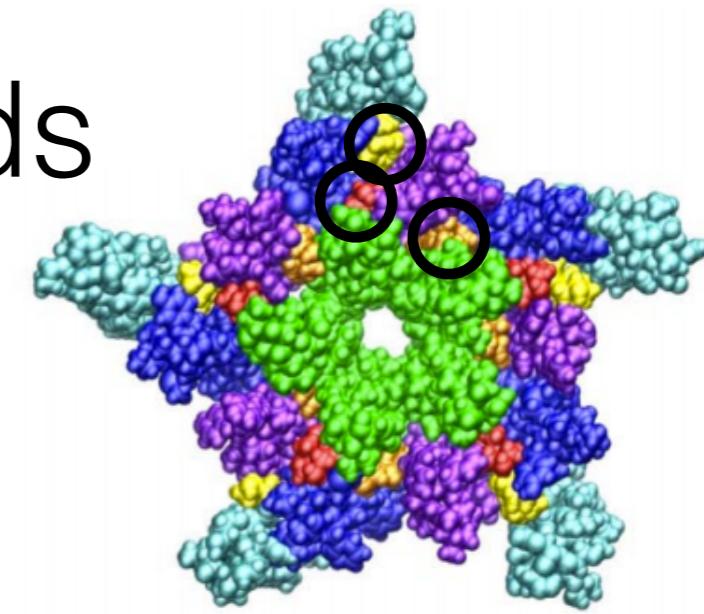


nAChR *Torpedo* Model Membrane

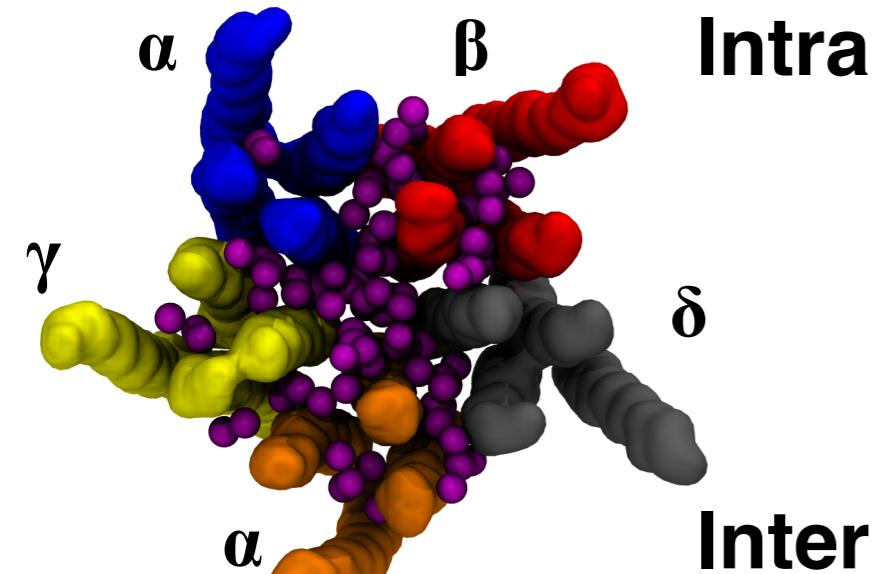


Embedded cholesterol in the nicotinic acetylcholine receptor-Grace Brannigan

Embedded Lipids



Embedded cholesterol in the nicotinic acetylcholine receptor-Grace Brannigan



Intra

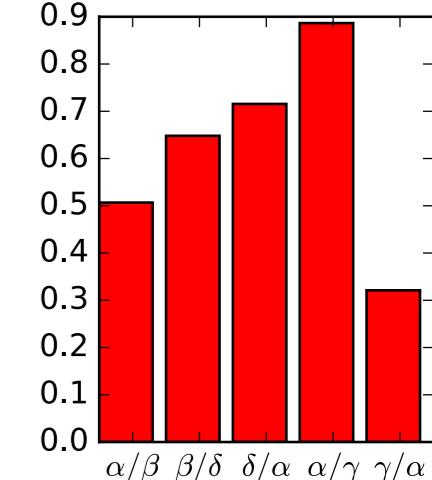
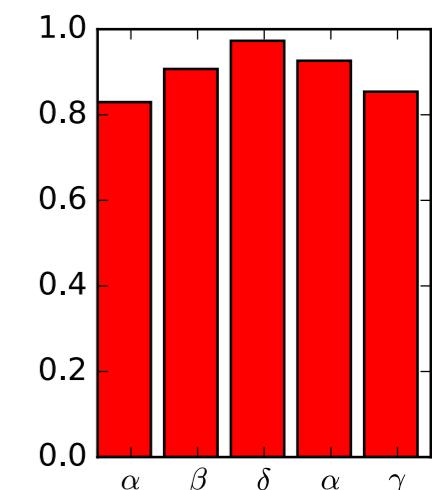
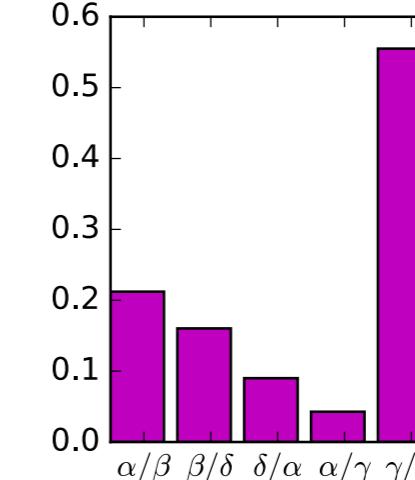
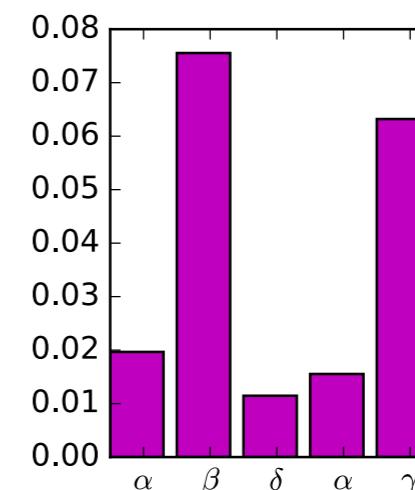
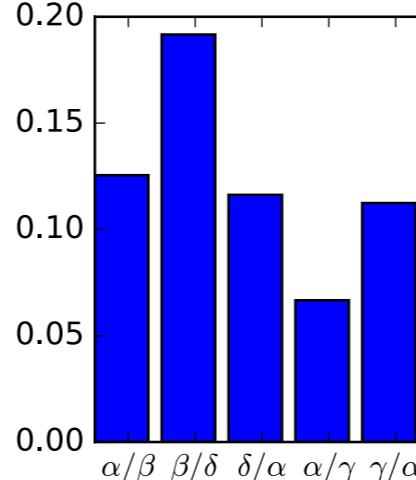
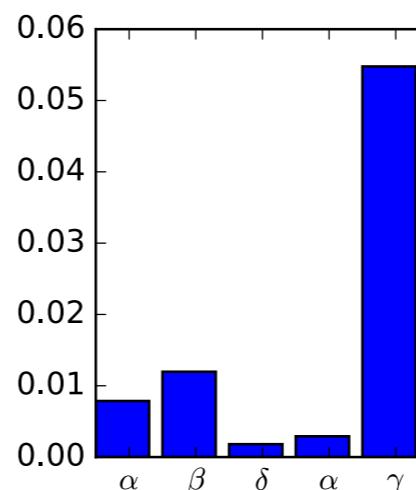
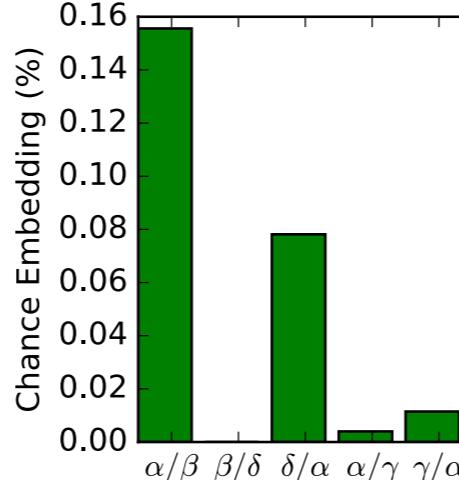
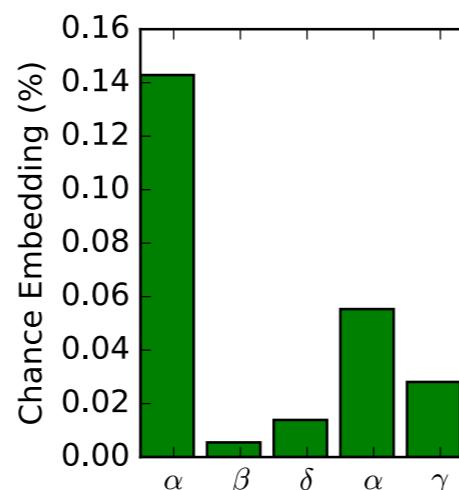
Inter

DPPC

DLoPC (ω -6)

DHA-PE (ω -3)

CHOL



Summary

- nAChR consistently partitions into disordered domains regardless of lipid head group
 - We were surprised by the direct interactions with PUFAs
- In the disordered domain, nAChR tends to position itself near the phase interfaces
- Cholesterol effects may come from non-annular binding

Acknowledgment

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 - Dr. Martin
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 - Sruthi
 - Ruchi
 - Kristen
 - Shashank
 - Dr. Salari



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