



Effects Of Quasi-Native Lipid Composition On Membrane Domain Formation Induced By Nicotinic Acetylcholine Receptors

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Master's Thesis Defense

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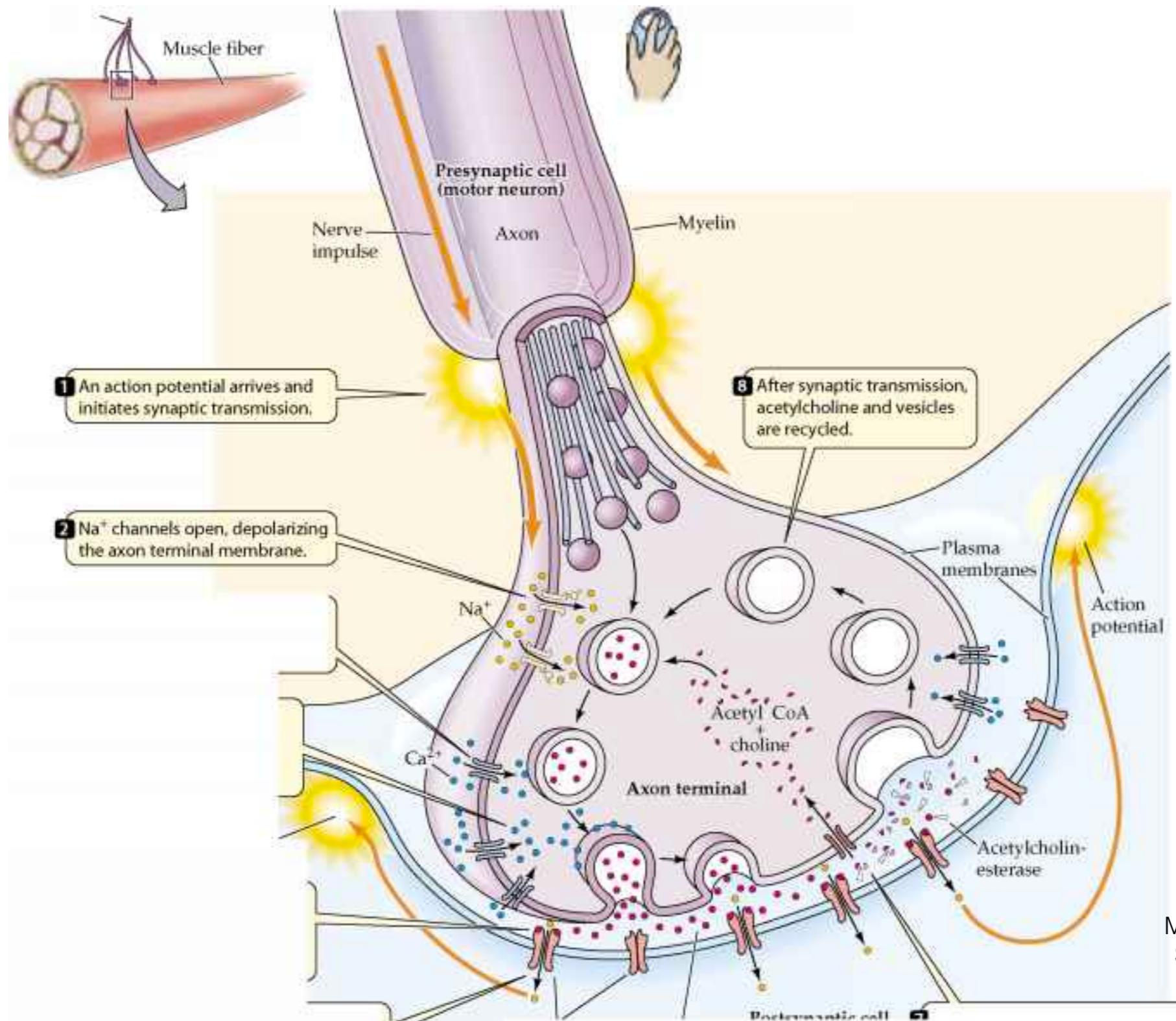
Outline

- **Introduction**
 - Nicotinic Acetylcholine
 - Lipids
- **Motivation**
- **Methods**
- **Results**
 - Embedded Lipids Analysis
 - Inter-Intra Subunit Analysis
 - Lipid Domain Formation
- **Conclusion**

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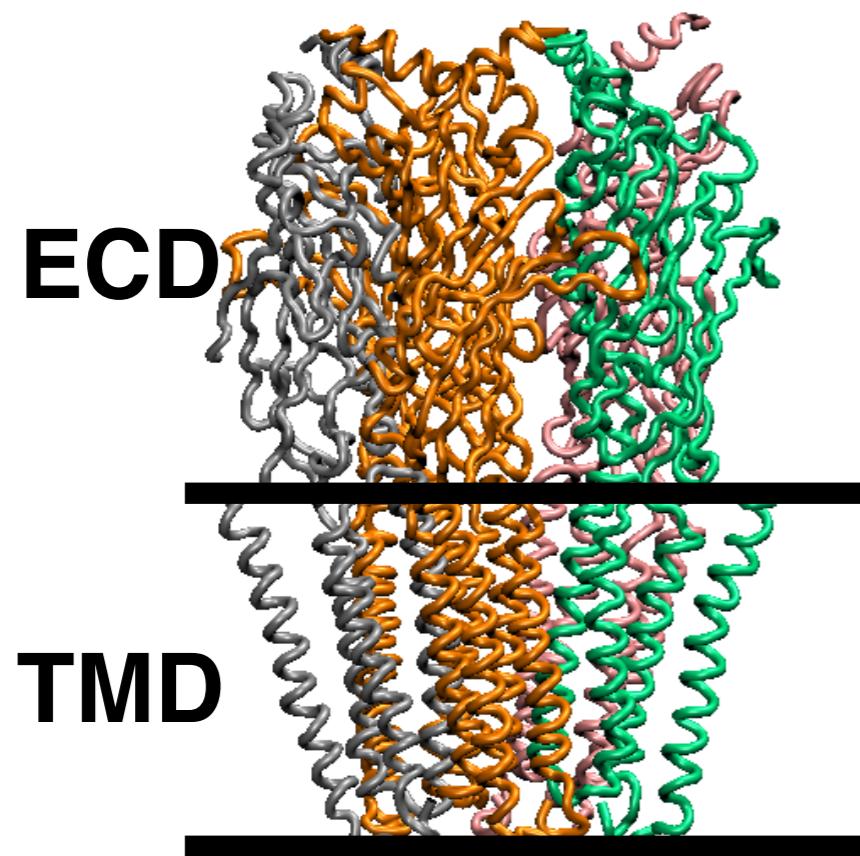
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Nicotinic AChR Is Important For Trans-synaptic signal transduction



Purves, William K., and Jürgen Markl. Biologie. München: Elsevier, Spektrum Akad. Verl, 2006. Print.

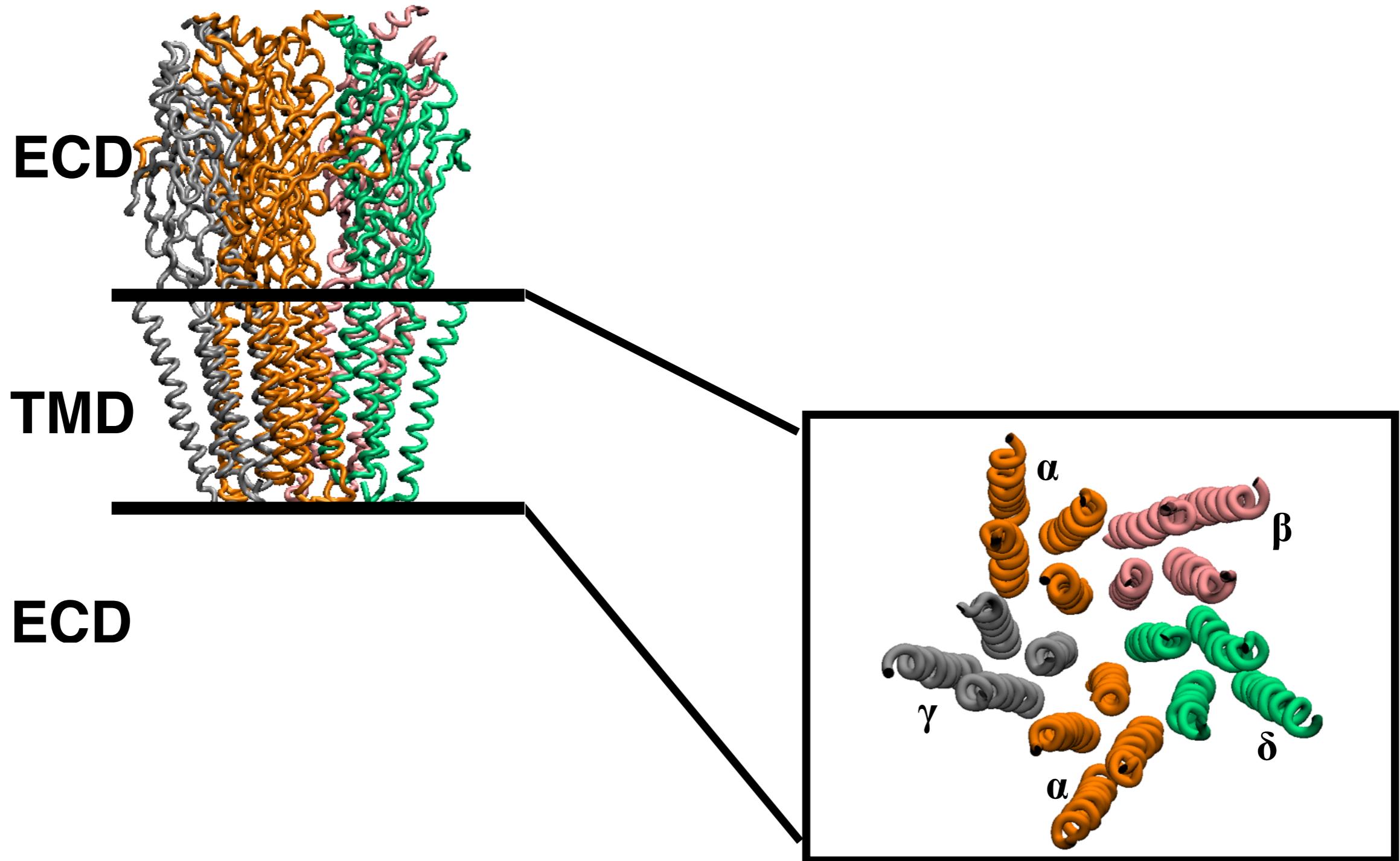
nAChR



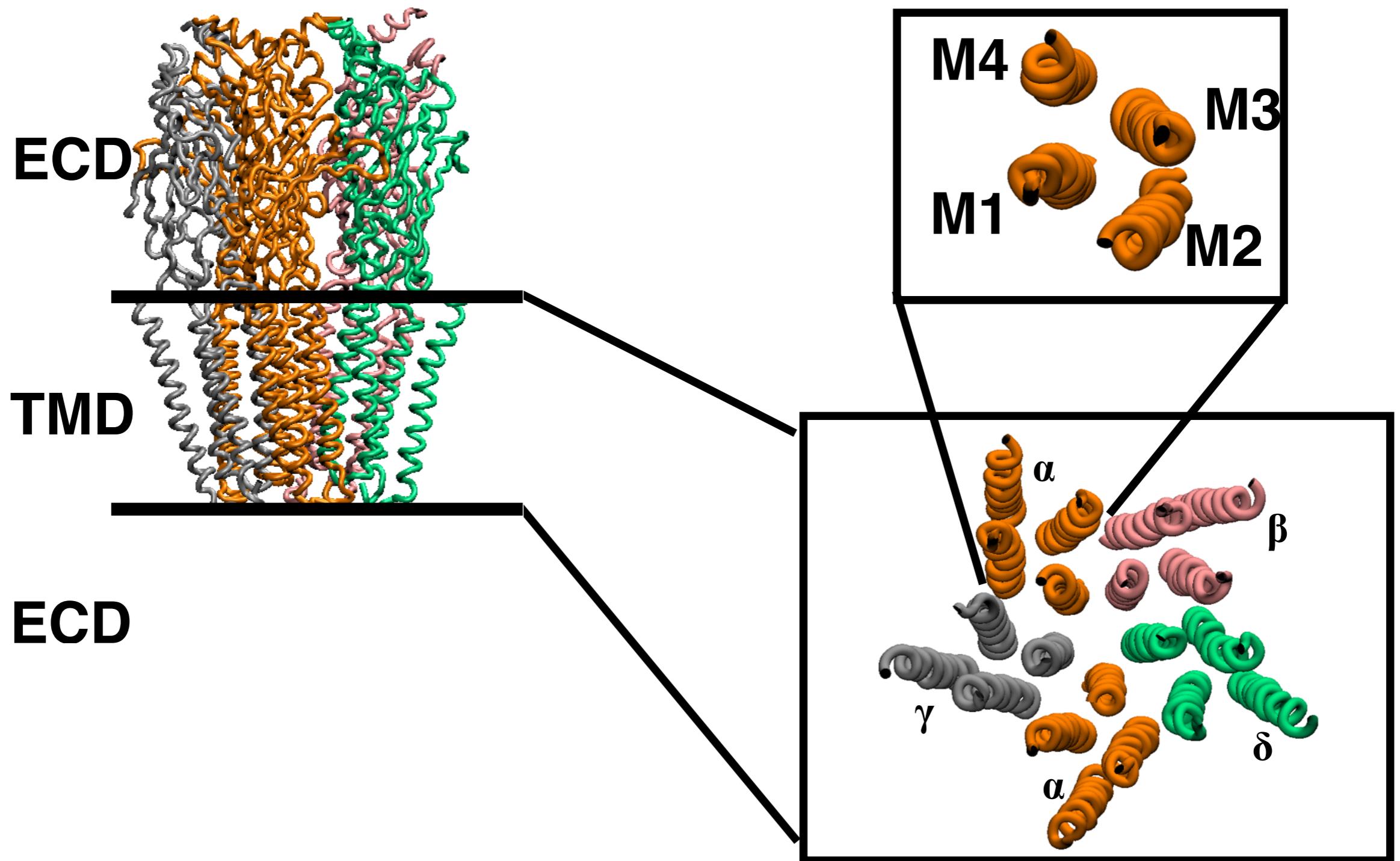
ECD

- A pentameric ligand gated ion channel found through out the central and peripheral nervous system
- Has various subunit types and general in hetero-pentamer configuration.
- Contributes to neuronal and muscular function by converting a chemical signal released by an excited pre-synaptic neuron into action potential in the post-synaptic neuron

nAChR



nAChR

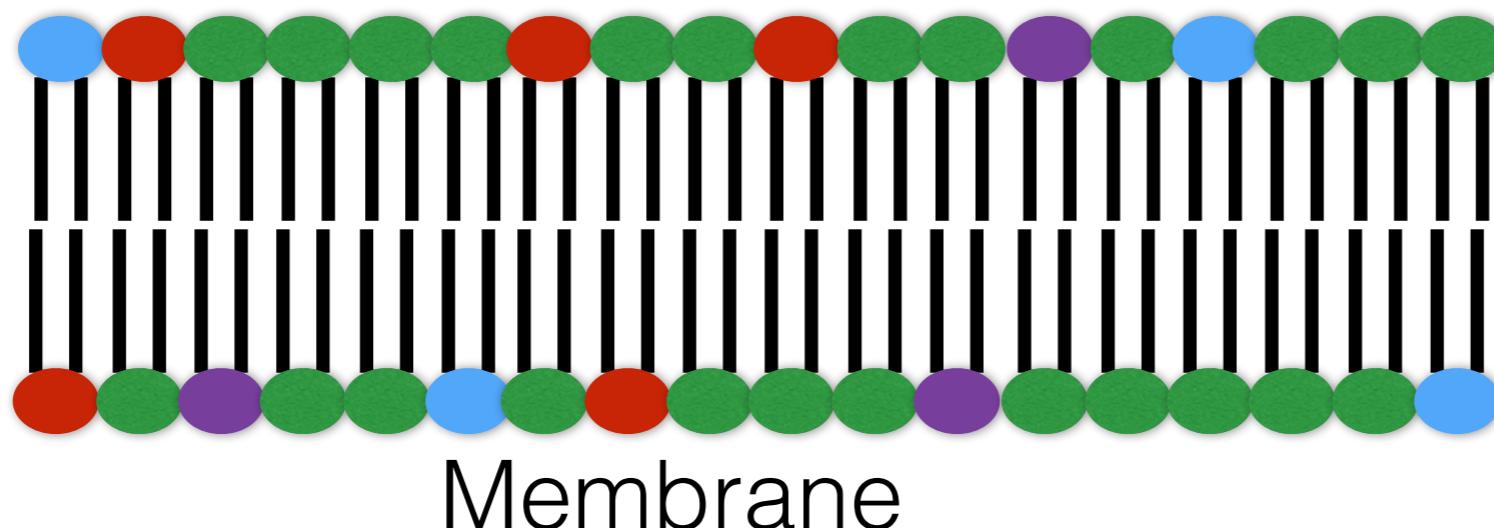


nAChR Requires Specific Membrane Composition To Function

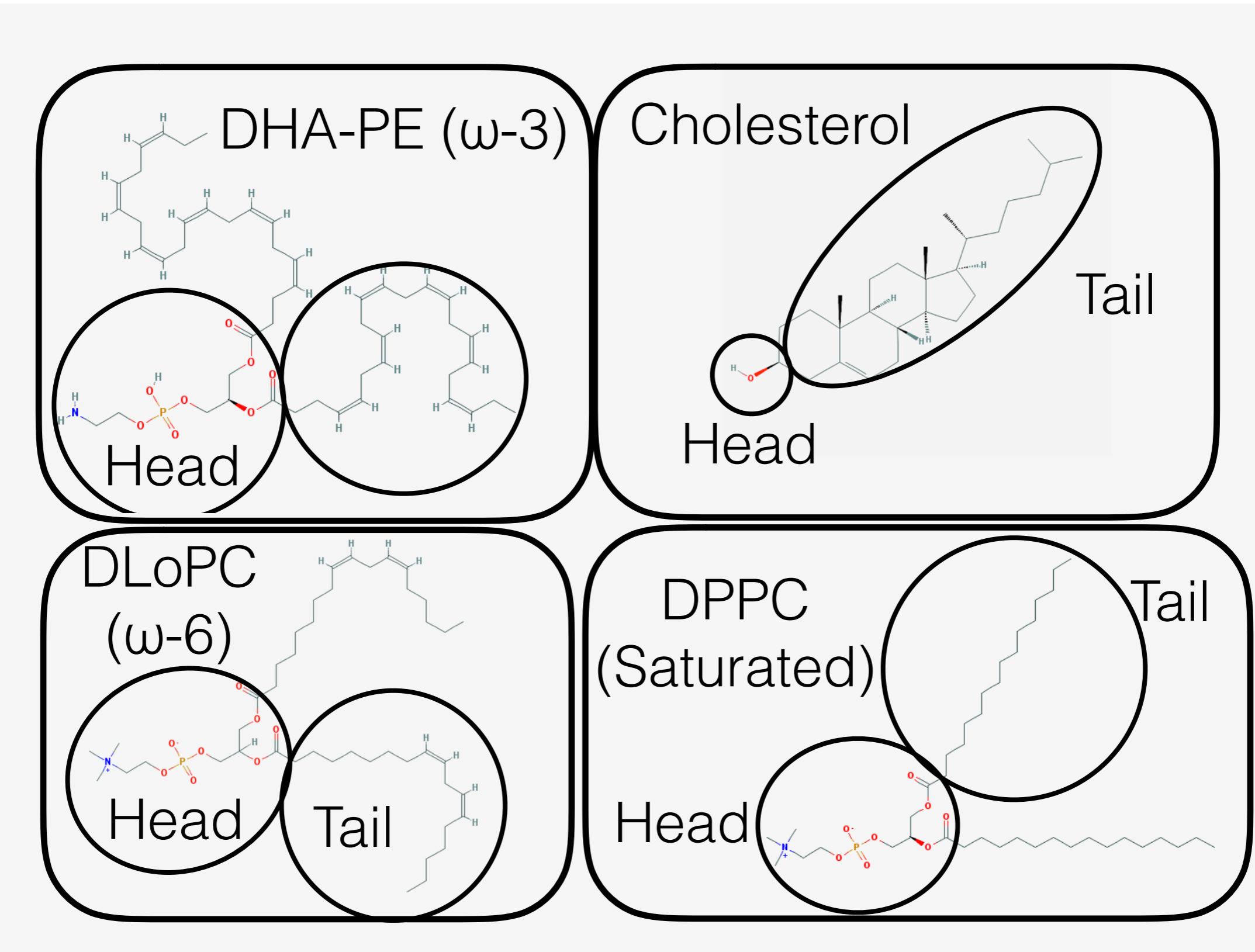
- Cholesterol is required for nAChR functionality
 - The mechanism for this requirement is still unknown.
 - One would expect nAChR to partition into cholesterol rich domains to function properly.
- Partitioning of nAChR in membranes is poorly studied.
- Several experimental studies suggest direct cholesterol interaction with nAChR as a potential mechanism of modulation, although the location of such interactions is largely uncharacterized.

Membrane Bilayer

- nAChR is located in membrane bilayer which is made of various lipids.
- Lipids are comprised of a head group and tail(s)
- The head is hydrophilic and the tails are hydrophobic

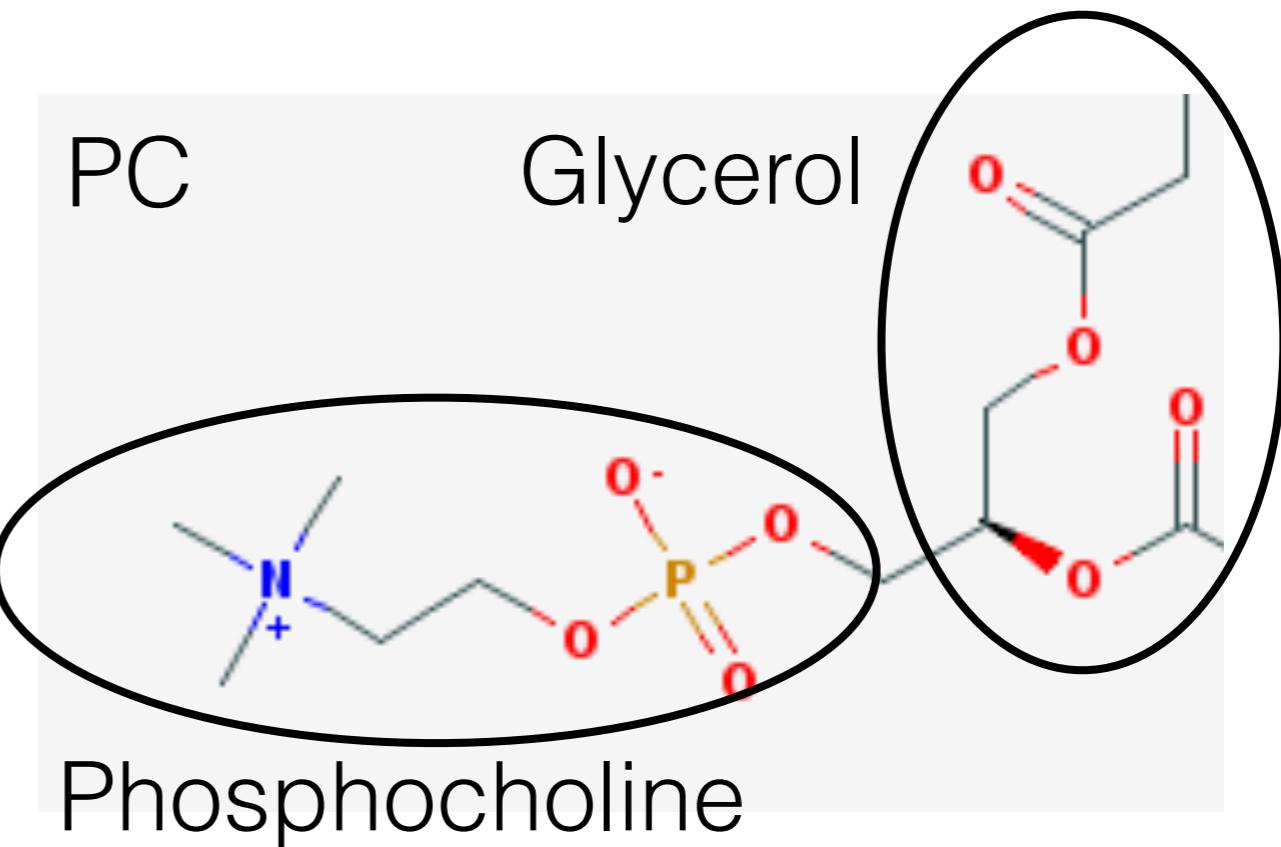


Anatomy of Membrane Lipids



Lipid Head Groups

- Head group:
 - Choline ($C_5H_{14}NO$) or Ethanolamine $C_{10}H_{20}NO_8P$
 - Phosphate (P_0_4)
 - Glycerol ($C_3H_8O_3$)



Lipid Tails

- Lipid tails are hydrocarbon chains of various lengths
- Can be divided to several groups based on saturation:
 - Saturated
 - Polyunsaturated (ω -3)
 - Polyunsaturated (ω -6)

Ordering of Lipids in Membrane

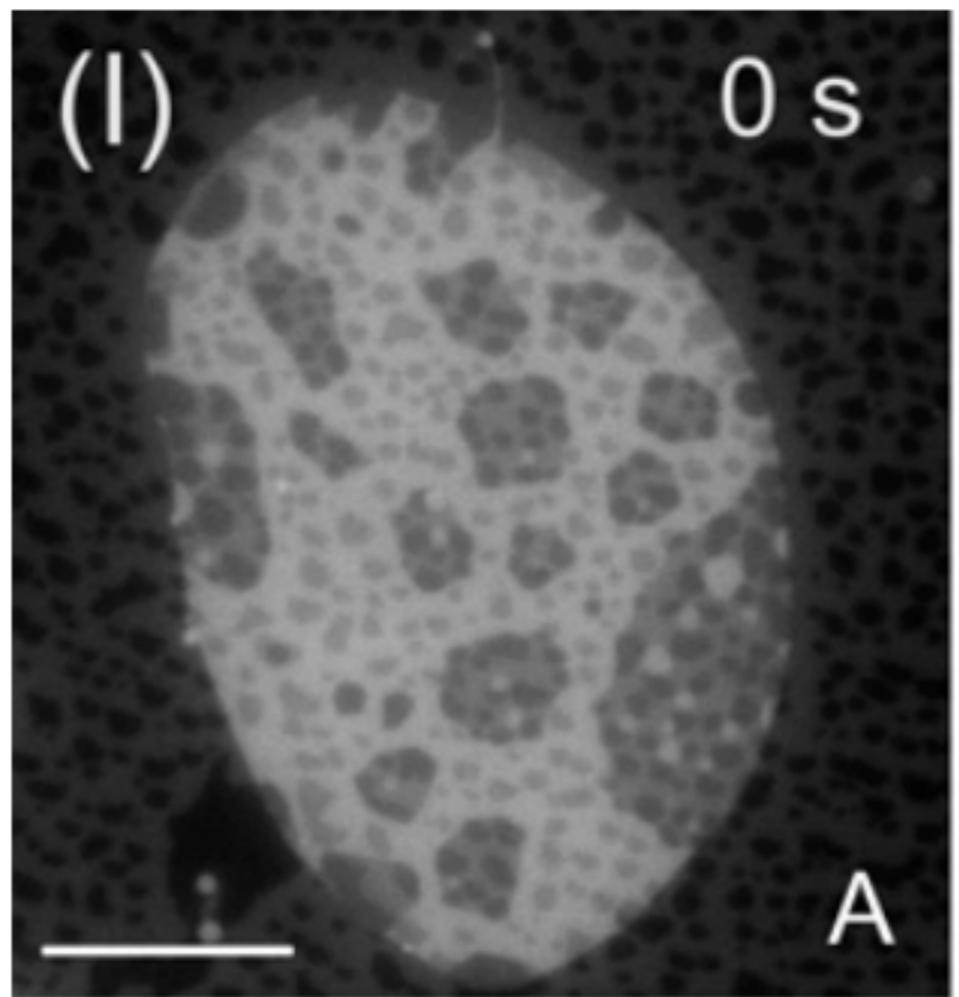
Lipids in biological membranes are not randomly distributed and partition into different phases:

- **Liquid ordered**: stable domain with higher melting temperature
 - Saturated lipids and cholesterol
- **Liquid disordered**: fluid domain with lower melting temperature
 - Unsaturated lipids

Ordering of Lipids in Membrane

Lipids in biological membranes are not randomly distributed and partition into different phases:

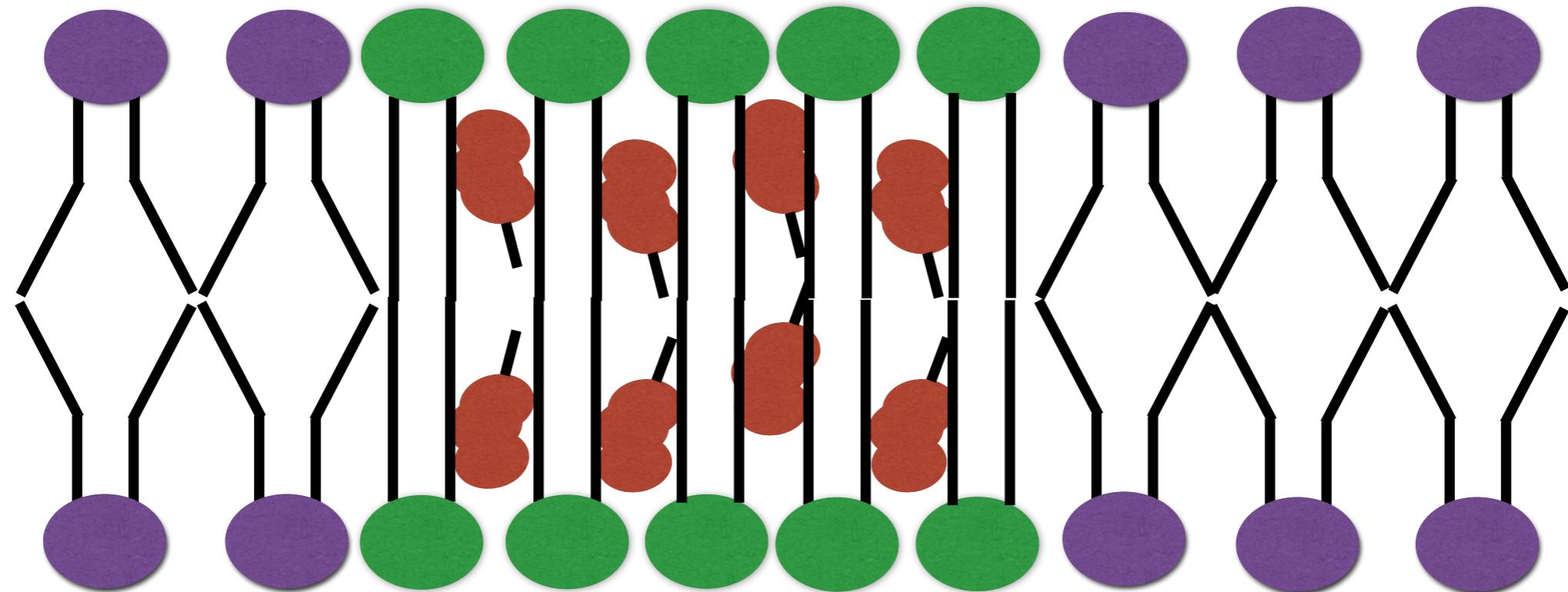
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Simonsen Biophys J. Vol 94 May 2008 3966–3975
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2367196/pdf/3966.pdf>

Ordered & Disordered Phases

Ordered



Disordered

Disordered

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Motivation

- Organization of nAChRs, including partitioning behavior in membranes containing distinct lipid domains, is poorly characterized.
- We investigate this problem by employing coarse Grained Force Fields and Molecular Dynamics Simulations of nAChRs embedded in complex model membranes.

Outline

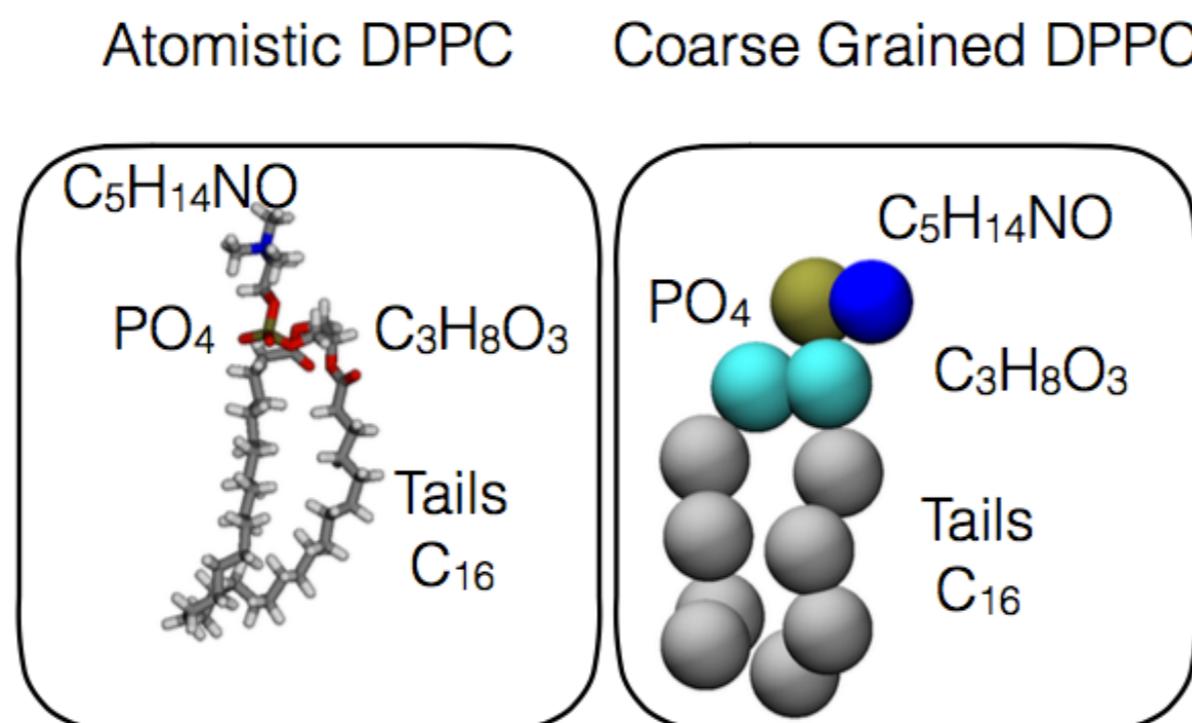
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Molecular Dynamics Simulations

- MD is a computer simulation technique that allows modeling the time-evolution of a system of interacting particle (e.g. atoms or coarse grain beads)
- The interaction potentials between particles (the ForceField) is used to derive forces
- The Newton's law of motion is then employed to move particles based on exerted forces

Coarse Grained Models

- Why do we care about CG models?
 - They reduce the number of simulated particles, so they become more efficient and allow reaching longer-timescales faster
 - Therefore we can study larger systems and slower processes over longer timescales
 - For example studying lipid diffusion and domain formation
- In MARTINI Coarse Grained model, each bead is equal to four atoms



Protein Structure

- CryoEM structure (PDB 2BG9) was determined by Unwin in 2005
 - Not a crystal structure
- nAChR was in native Torpedo membrane
- Protein has gaps in density in TM inter/intra subunits

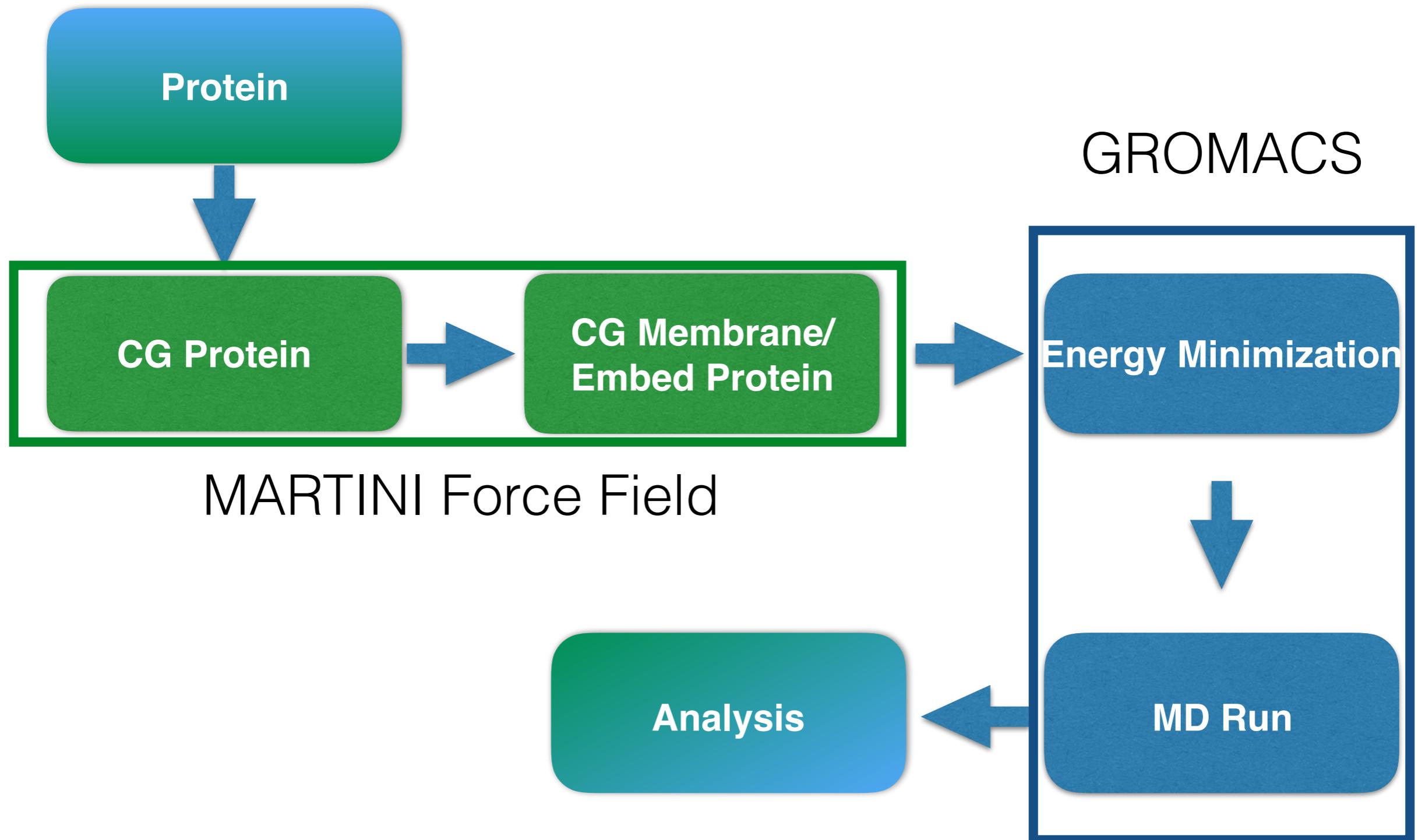
Model Membranes

Head	Tail	Percentage
PC	di-C14:0	~1.5
	di-C16:0	~60
	di-C18:0	~9
	di-C18:1	~15
	di-C20:4 n-6	~1.5
	di-C22:5-6 n-3	~10
PE	di-C16:0	~15
	di-C18:0-18:1	~40
	di-C20:4 n-6	~6.5
	di-C22:5-22:6 n-3	~35

	Short (16-18)		Long (20-22)	
	PC	PE	PC	PE
Saturated	DPPC	DPPE	DBPC	DBPE
ω-6	DLoPC	DLoPE	DA-PC	DA-PE
ω-3	ALA-PC	ALA-PE	DHA-PC	DHA-PE
Sterol	Cholesterol	---	---	---

- Model membrane compositions were based off of Barrantes proposed ratios of Torpedo membrane lipids
- We study membranes containing three lipids
 - DPPC, Cholesterol, and one other lipid

Approach



Outline

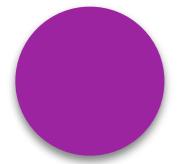
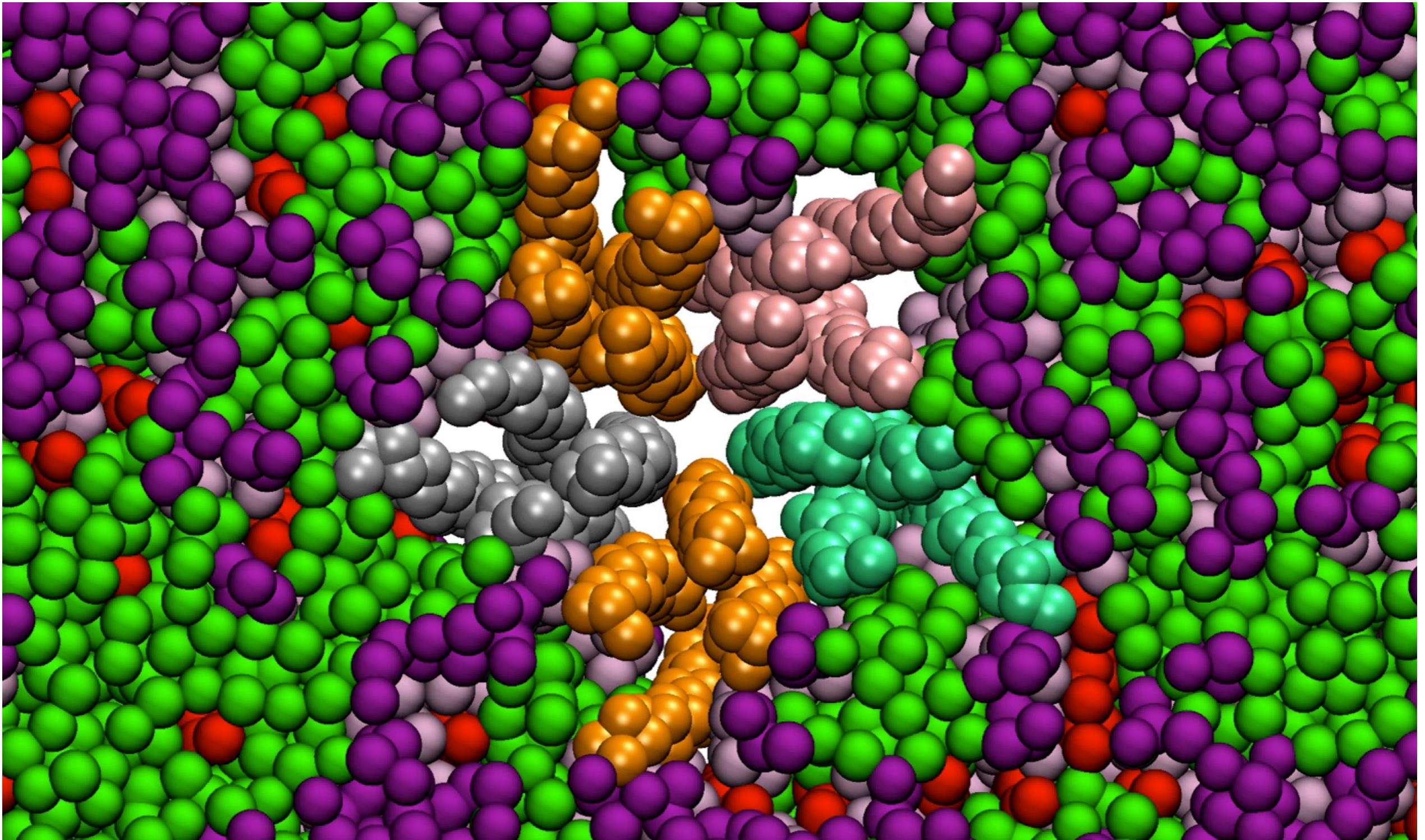
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Overview



DPPC DHA-PE Cholesterol

Embedding



Head

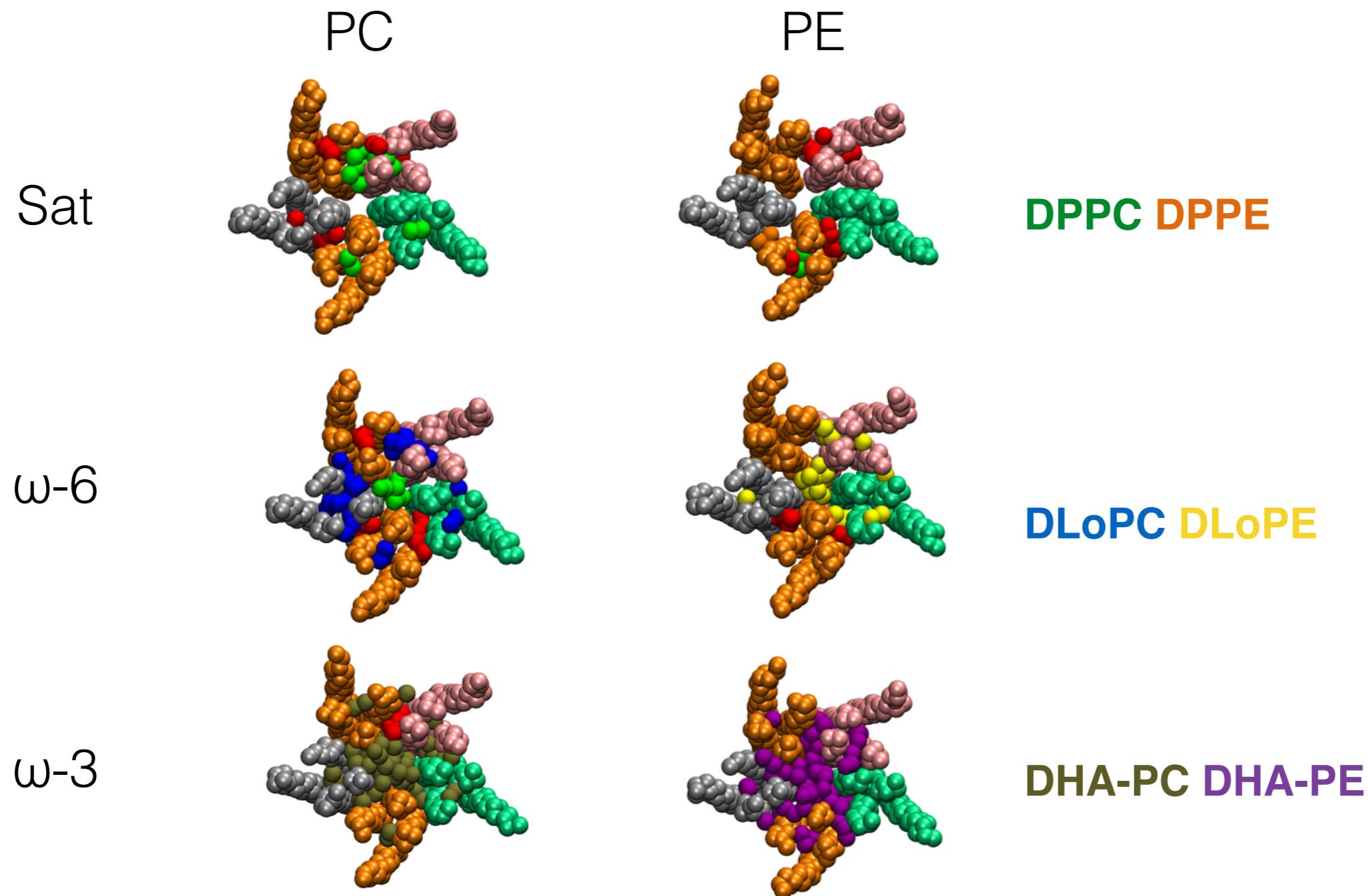


Tail

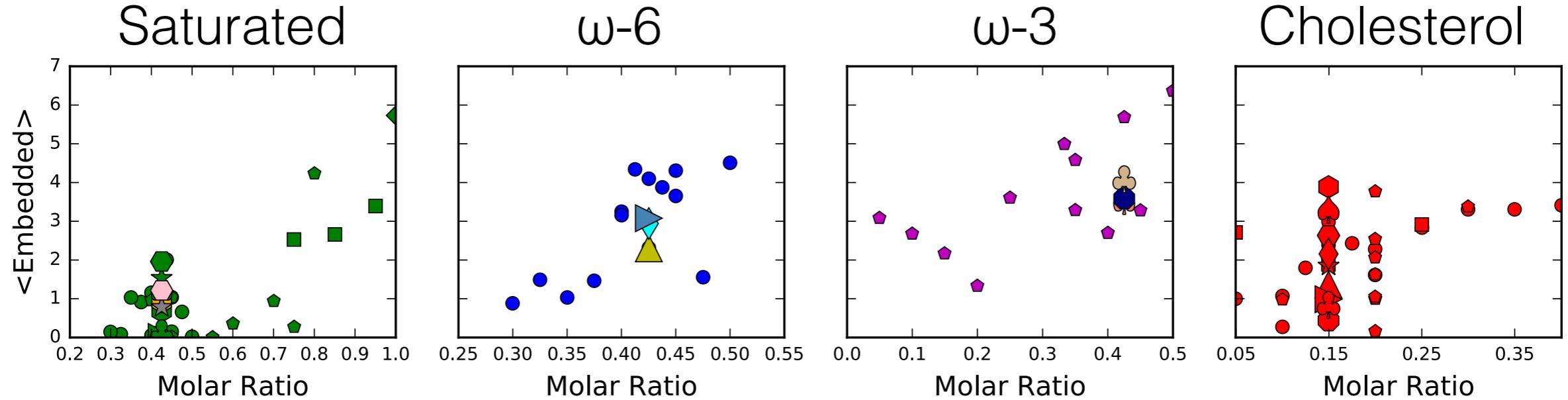
DPPC DHA-PE Cholesterol

Lipid Embedding

15% Cholesterol

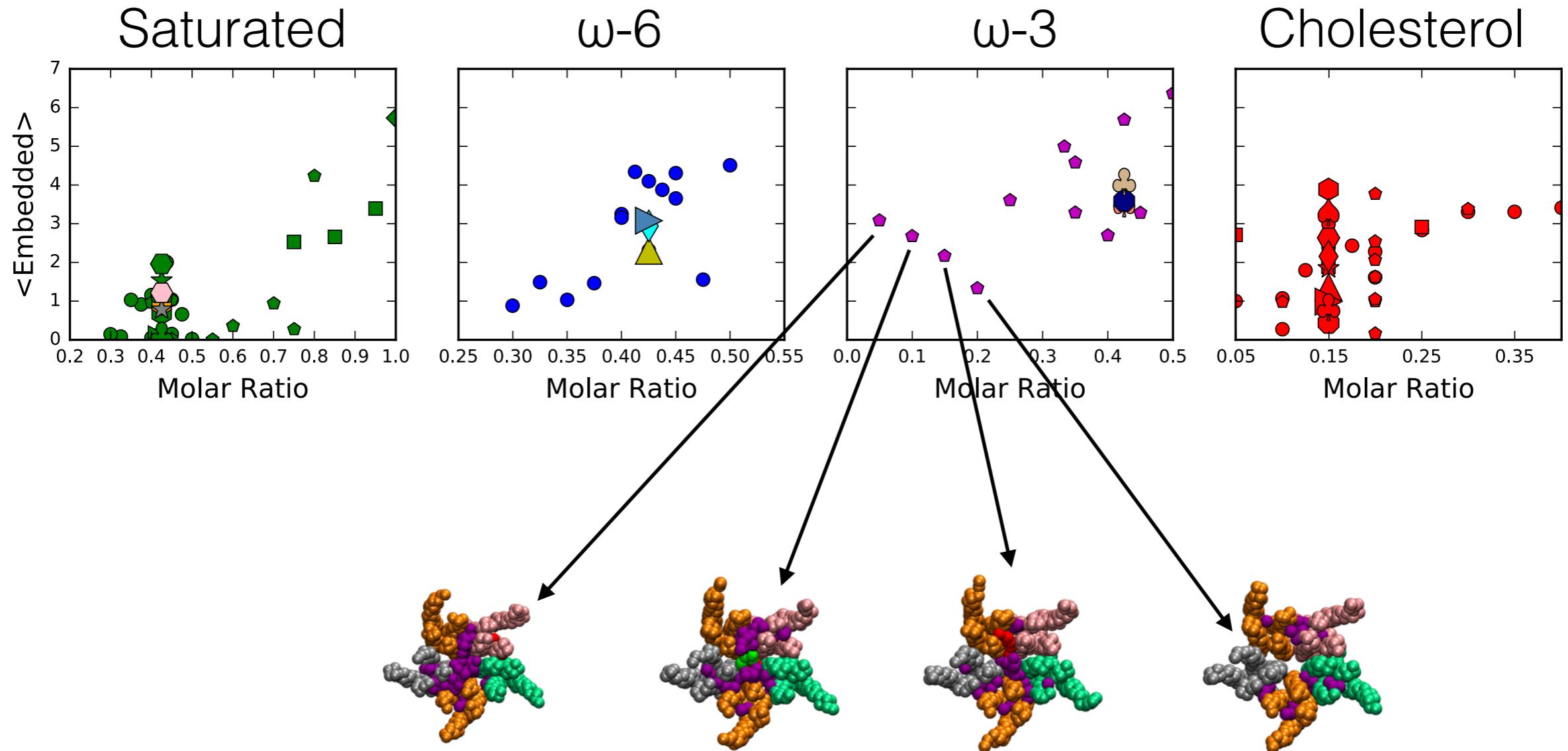


Lipid Embedding

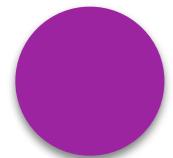
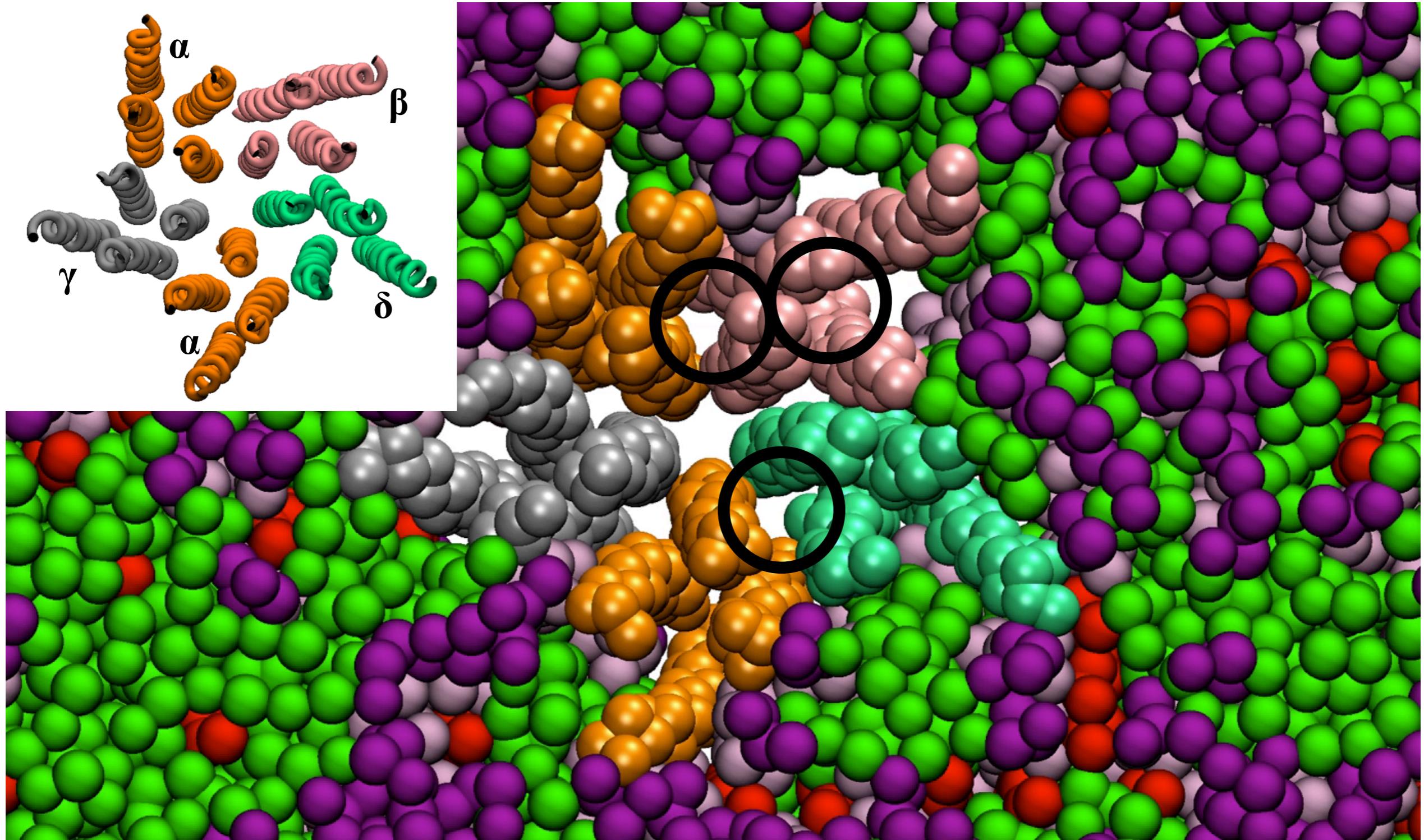


- **Expectation:** Higher concentration => more embedded lipids
- Saturated Lipids avoid embedding in nAChR
- Simulations with cholesterol at 15% and 20% do not follow what we anticipate
- DHA-PE (ω -3) below 20% does not follow what we anticipate

Lipid Embedding



Embedding



Head

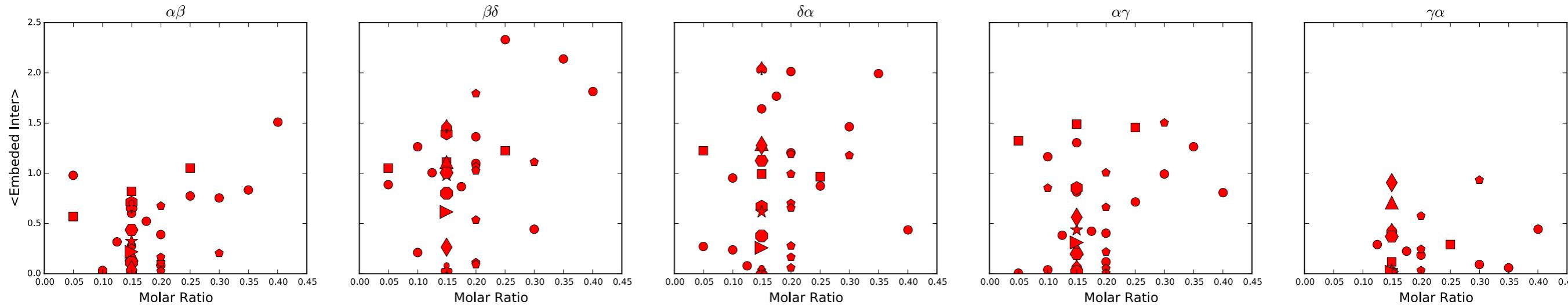


Tail

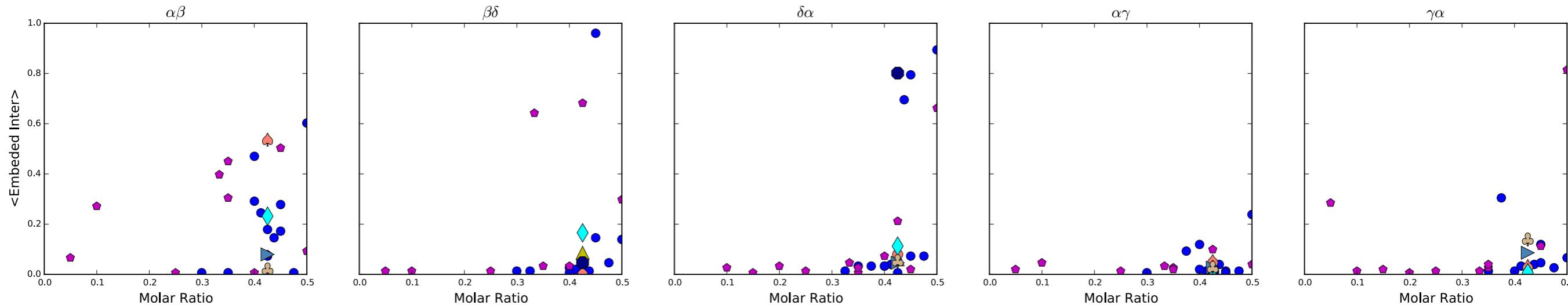
DPPC DHA-PE Cholesterol

Inter Subunit Embedding

Cholesterol



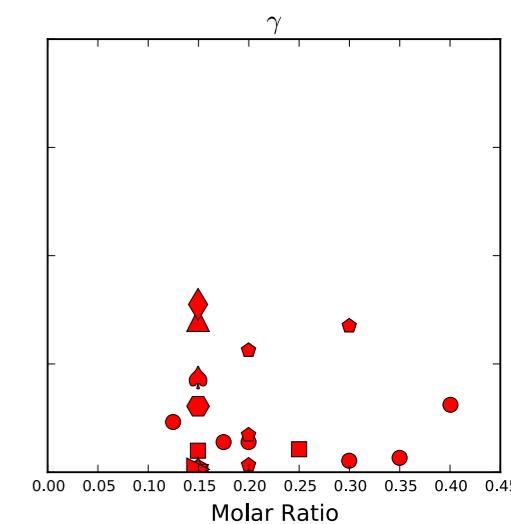
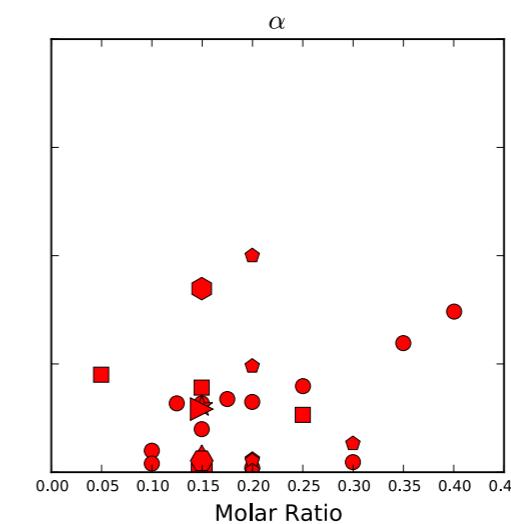
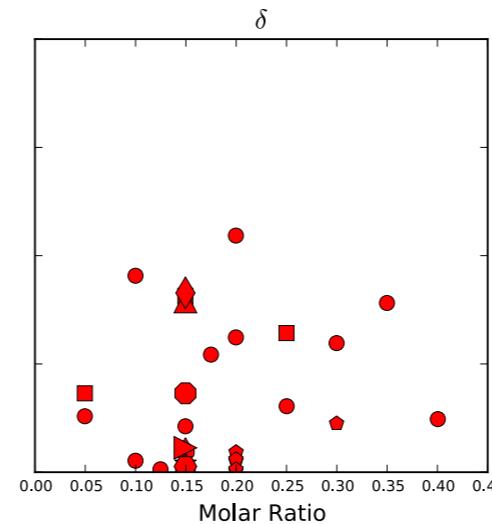
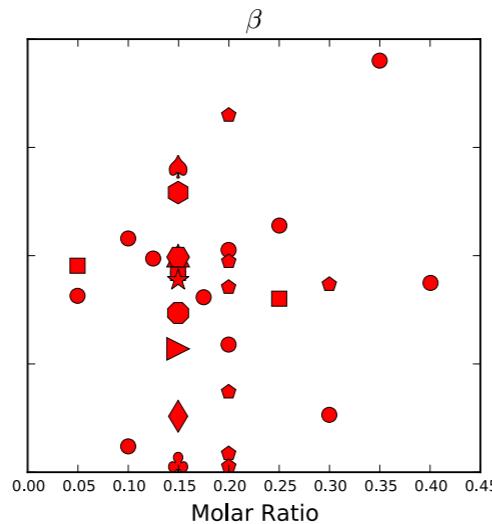
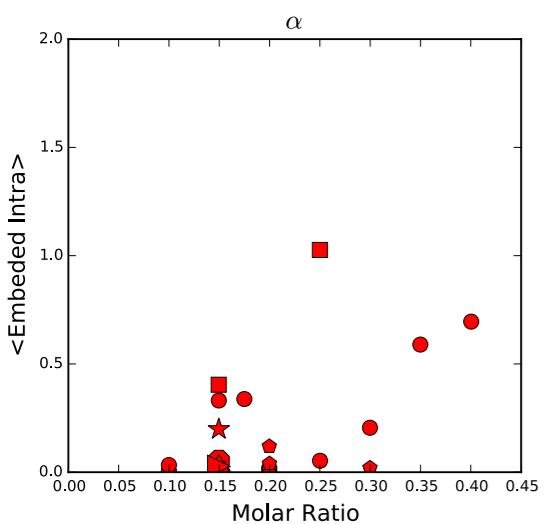
PUFAs



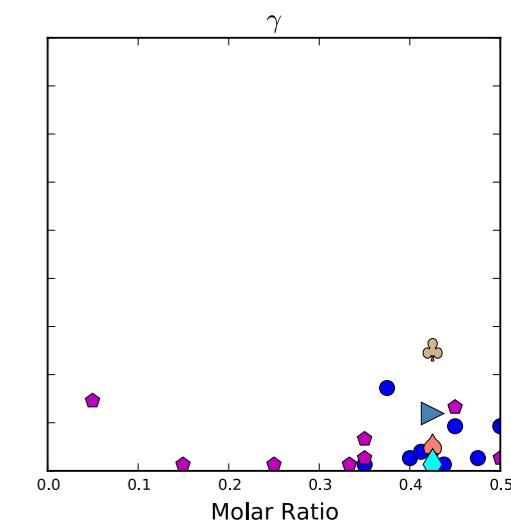
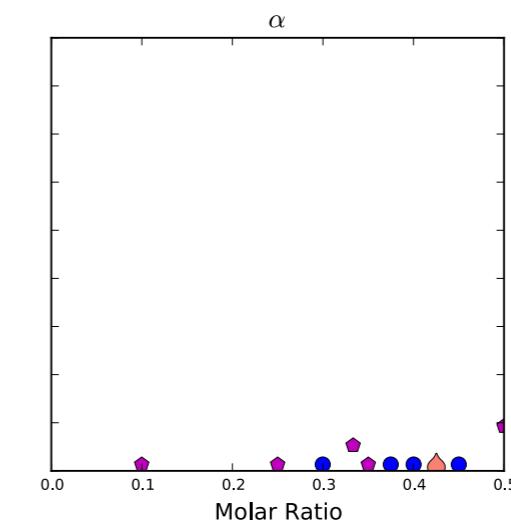
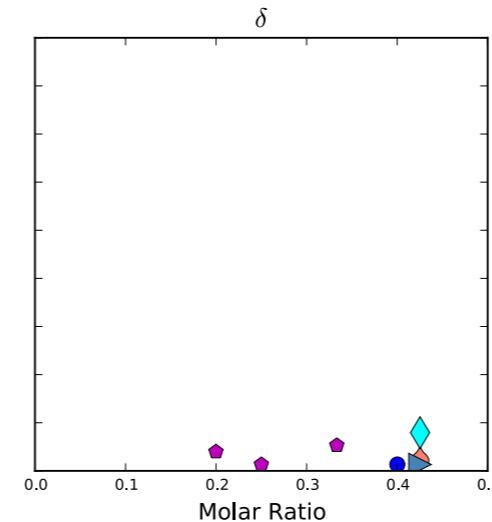
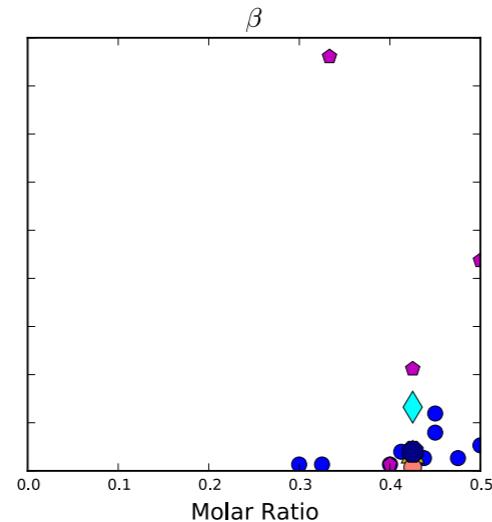
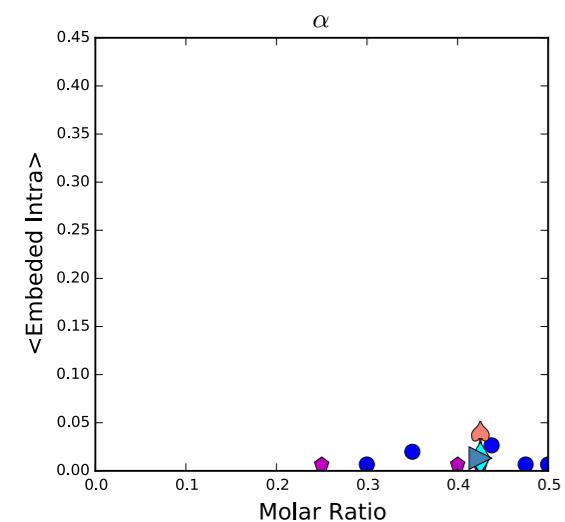
- If Lipids had no preference to subunits there would be even distribution
- Lipids embedded in the α/β , β/δ , and δ/α inter-subunits

Intra Subunit Embedding

Cholesterol



PUFAs



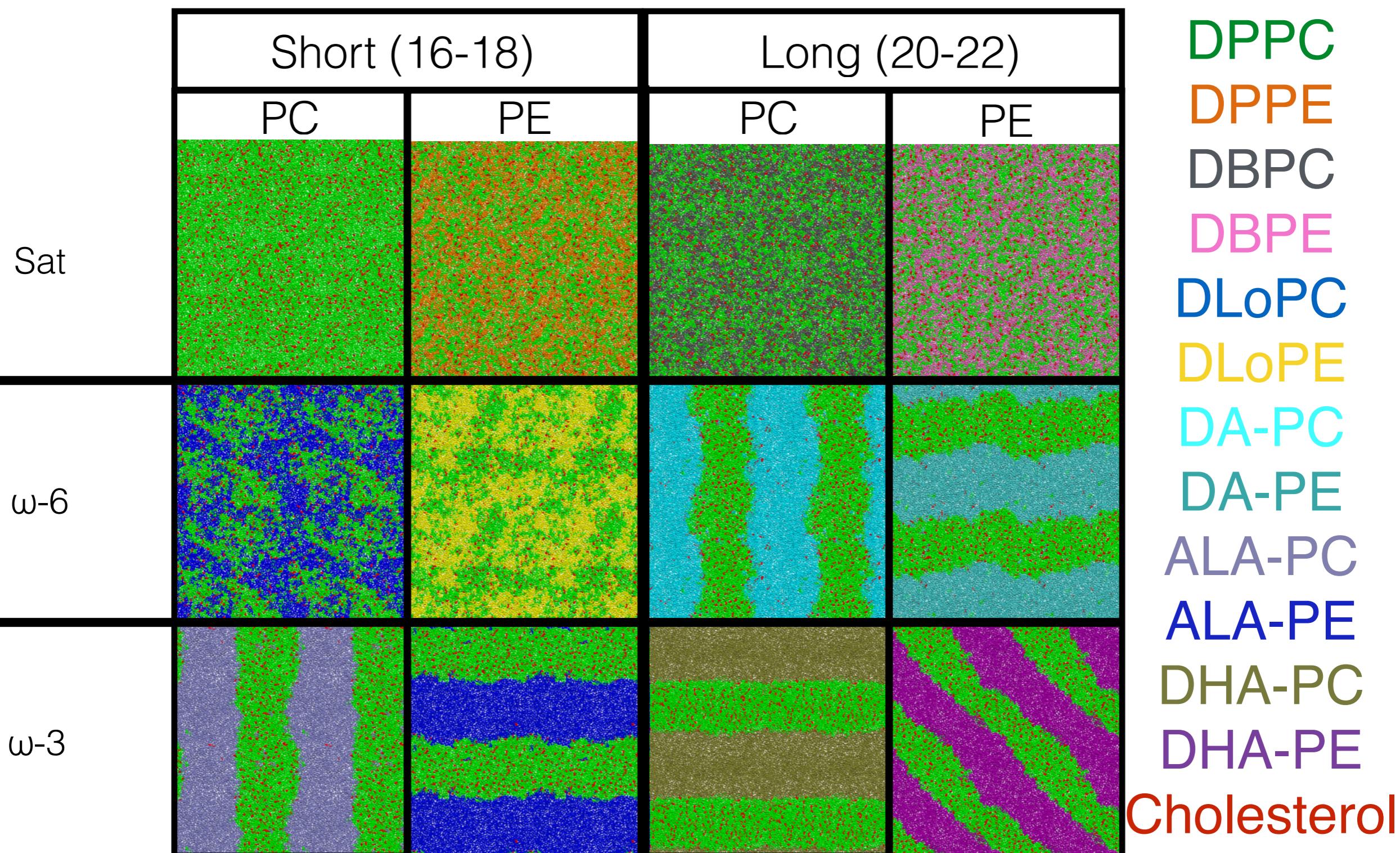
- Lipids embedded in the β subunit
- Lipids embed within subunits and show subunit-specificity

Domain Formation

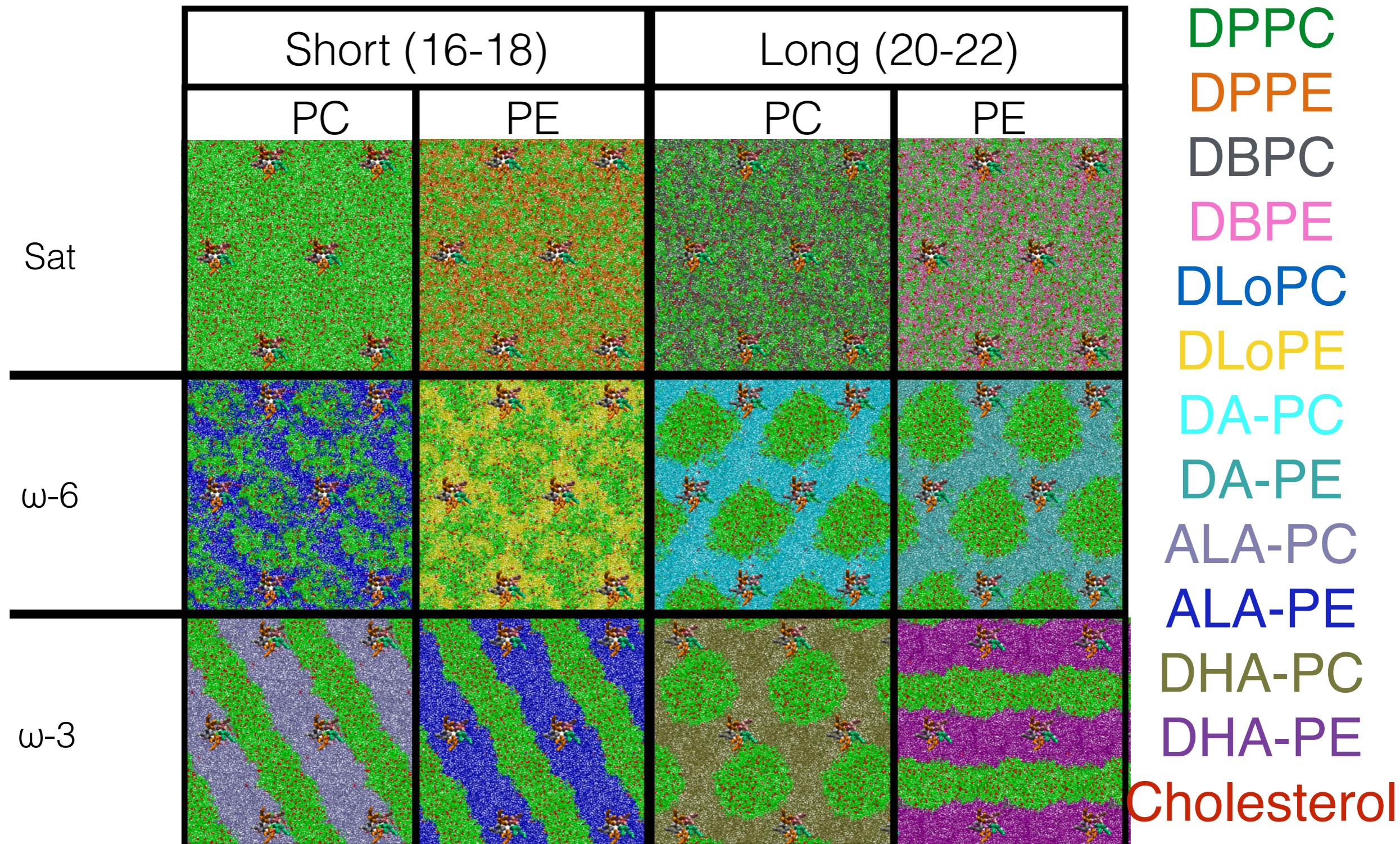


DPPC DHA-PE Cholesterol

Domain Formation - No nAChR

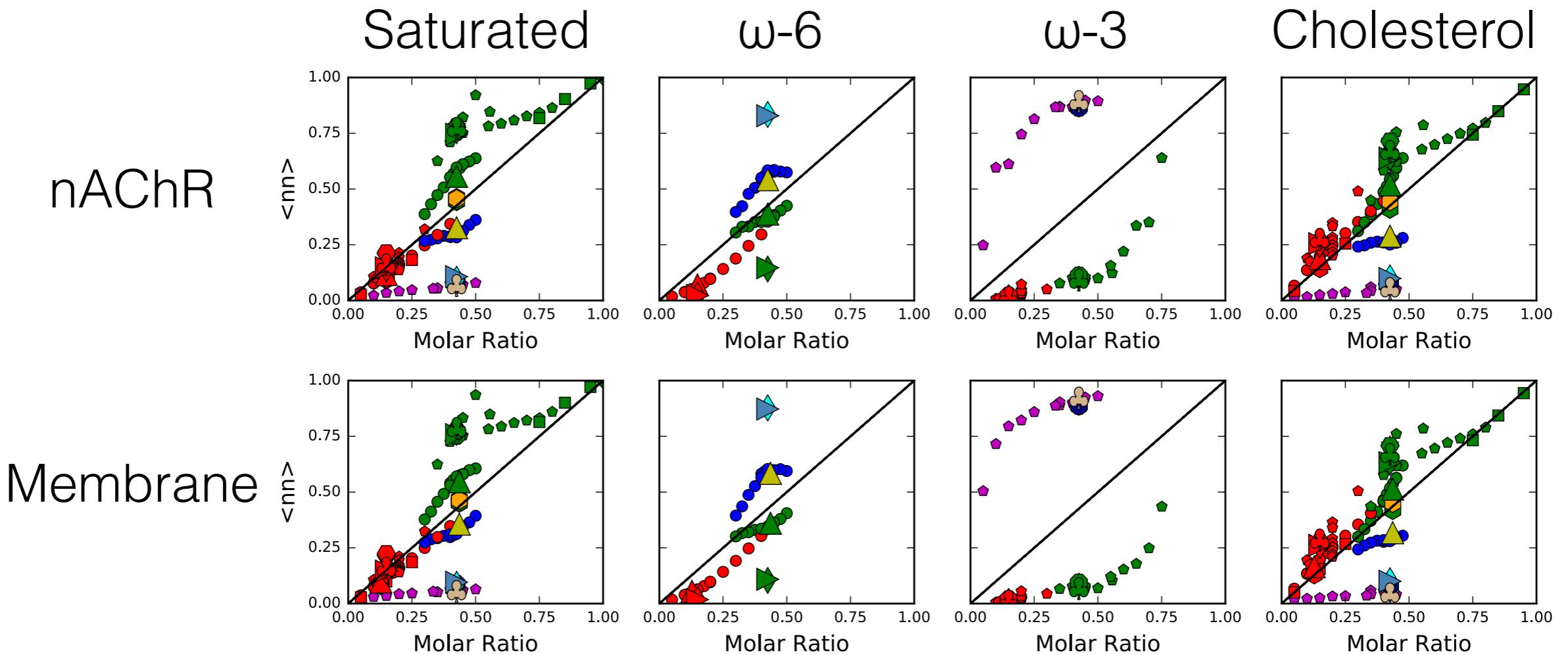


Domain Formation - with nAChR



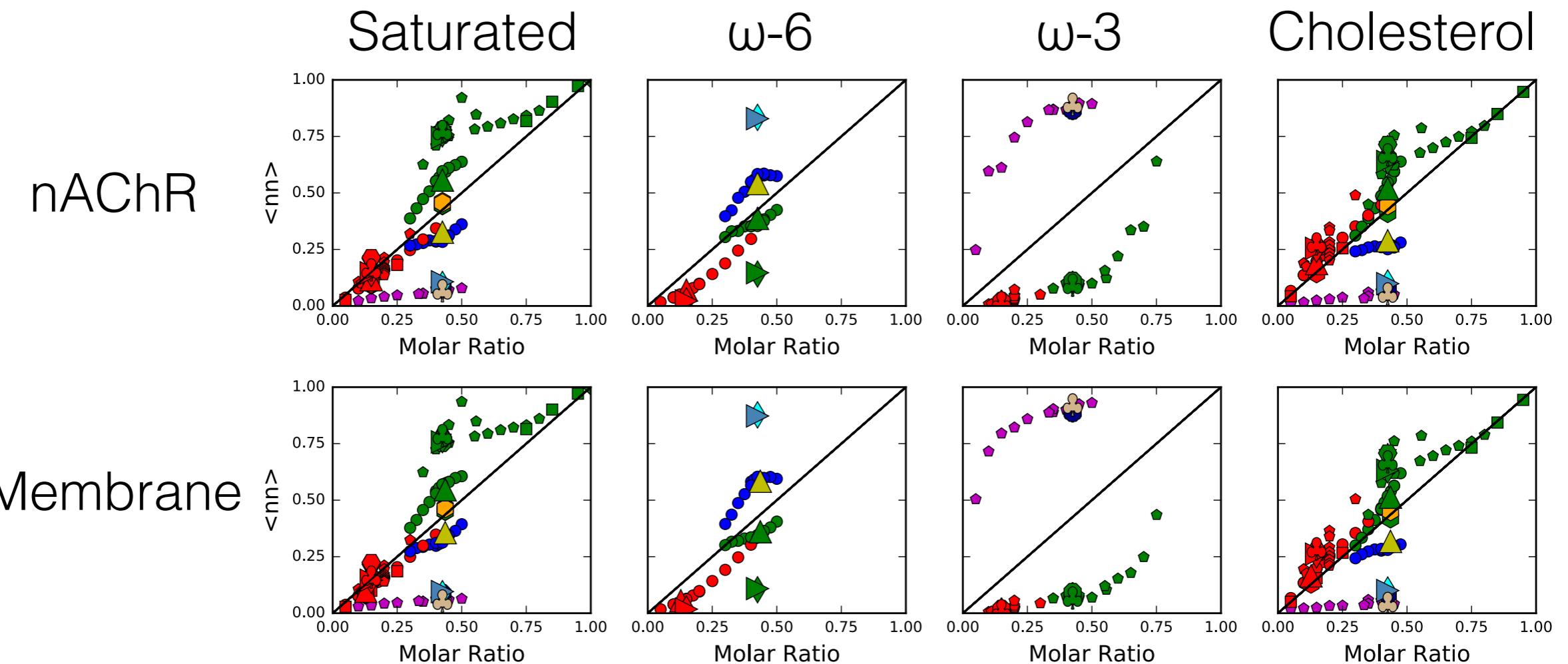
- **Expectation:** nAChR partitions into cholesterol rich domain
- We observe nAChR partitioning into cholesterol poor domains

Domain Formation

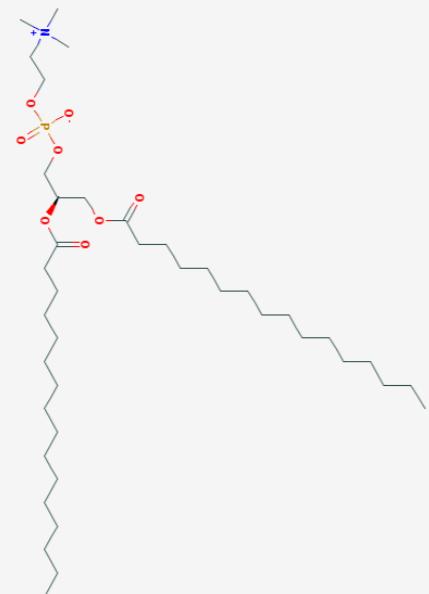


- Saturated lipids and cholesterol mix well
- Unsaturated lipids prefer to self mix

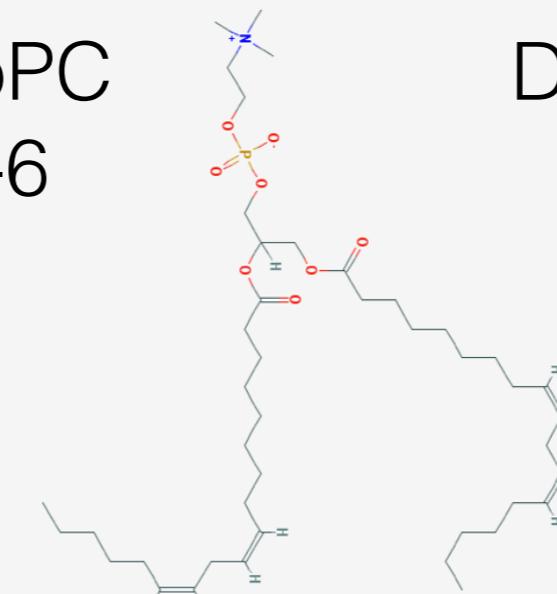
Domain Formation



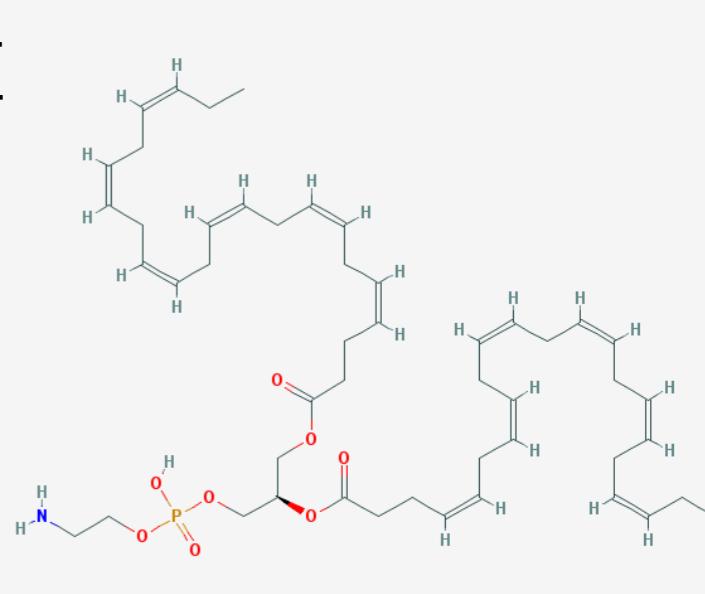
DPPC
Saturated



DLoPC
 ω -6



DHA-PE
 ω -3



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Conclusion

- nAChR partitions consistently into cholesterol poor phases
 - Due to interaction with cholesterol it was anticipated to partition into cholesterol rich domains
- nAChR has a clear affinity for polyunsaturated fatty acids
 - These lipids have been much less studied compared to cholesterol
- Lipids have a subunit specificity. Cholesterol and polyunsaturated fatty acids prefer the β subunit
- nAChR had a minimum effect on membrane domain formation

Acknowledgments

- **Dr. Grace Brannigan**
- **Committee Members**
- **Brannigan Lab Members**
 - Dr. Reza Salari
 - Sruthi Murlidaran
 - Ruchi Lohia
 - Shashank Chavali
- **Funding**
 - Research Corporation, NIH P01GM55876-14A1
- **Computation Resources**
 - NSF XSEDE Allocation NSF-MCB110149 and local cluster funded by NSF-DBI1126052