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Abstract

Nicotinic acetylcholine receptors (nAChR) are pentameric ligand gated ion channels, critical to signaling across synapses and the neuromuscular junction. While sensitive to boundary lipids, nAChR have been shown to be functionally dependent on cholesterol. This dependence on cholesterol has led to the hypothesis that nAChR resides within the cholesterol rich liquid ordered domains. Using the MARTINI force field, coarse-grained molecular dynamic simulations were performed, with nAChRs in quasi-native ternary membranes. Native nAChR membrane composition has an abundance of polyunsaturated fatty acids (PUFAs), saturated fatty acids, and cholesterol. The two PUFAs chosen for these simulations were Docosahexaenoic acid and Linoleic acid.

These simulations display nAChR consistently residing in the PUFA enriched disordered domain, remaining nearby the liquid ordered domain. Analysis of boundary lipid composition confirms nAChR boundary lipids are enriched in PUFAs. Further analysis of nAChR subunit-domain interaction show alpha subunits preference for cholesterol rich domains, while beta subunits show preference for PUFAs. Lastly, analysis shows PUFAs and cholesterol binding non-annularly nAChR.

This study is being expanded to compare complex quasi-native synaptic and oocyte membranes. The oocyte membrane, in particular, is an optimal model for studying lipid-protein interactions, because it has a lower abundance of n-3 PUFAs compared to the neuron. From our simulations, we find that differences in membrane composition are especially noticeable around nAChRs. Given that nAChRs no longer exhibit partitioning preferences in oocyte membranes, our initial simulations suggest that oocytes do not provide a sufficiently native-like environment for nAChR.

Results

Equations

- ρ_a is defined as the density of lipid species a within a given bin of area (A^2). n_a number of lipid a in a bin
- $M_{a,b}$ compares measured and expected mixing values; a : reference lipid, b : local lipid; η : percent of a near b
- Q : boundary DPPC around protein, B : boundary DPPC. N_B : total boundary lipids. x_{DPPC} : concentration DPPC

$$\rho_a = \langle \frac{n_a}{A} \rangle$$

$$M_{a,b} = \frac{\langle \eta_{a,b} \rangle}{\langle \eta_{a,b} \rangle_{rand}} - 1$$

$$Q = \langle \frac{B_{DPPC}}{N_B \cdot x_{DPPC}} \rangle - 1$$

PUFA Demixing and Boundary Lipids

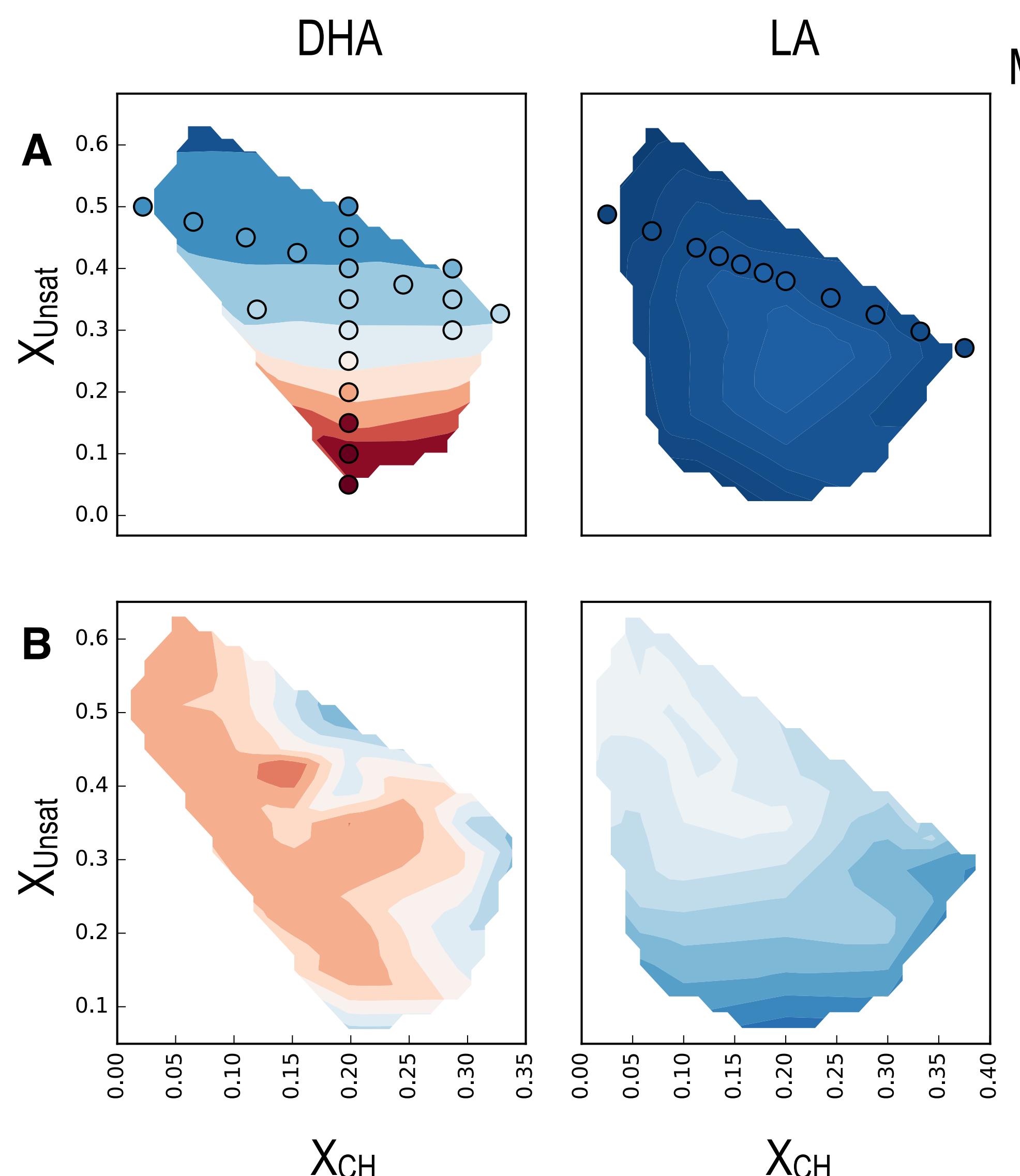


Figure 2:

A. Circles represent membrane only systems. Left shows the PUFA $M_{DHA,DHA}$. Right shows $M_{LA,LA}$. DHA helps promote domains formation, LA tends to remain mixed with the bulk membrane.

B. Plots show Q_{DPPC} . $Q>0$ DPPC enriched, $Q=0$ randomly mixed, $Q<0$ DPPC depleted. All interactions between the protein and lipid DPPC are below 1. Left: Systems with DHA-PE; Low Q_{DPPC} for DPPC at 40-50%. Right: Systems with DLiPC; shows fairly consistent DPPC protein interaction.

Introduction

- Nicotinic Acetylcholine Receptors (nAChR) are essential pentameric ligand gated ion channels (pLGICs) and is highly lipid sensitive
- nAChR is well studied but nAChR-neuronal like membrane interactions are not
 - Neuronal membranes and Torpedo have similar lipid compositions, both rich in n-3 polyunsaturated fatty acids (PUFAs)
 - Initial model membranes (based on Barrantes[3]) are formed of DPPC:PUFA:CHOL
- PUFAs used: n-6 Linoleic acid (LA), n-3 Docosahexaenoic acid (DHA) (prominent neuronal PUFAs)
- PUFAs form liquid disordered phases; saturated fatty acids and cholesterol form liquid ordered phases

Methods

- cryo-EM structure (PDB:2BG9) [5] used in these experiments (derived from Torpedo)
- Coarse-grained Molecular Dynamics simulation preformed with MARTINI force field 2.2 [2] and GROMACS[1] 5.0.6
- Run under NPT
- Ran for ~2 μ s

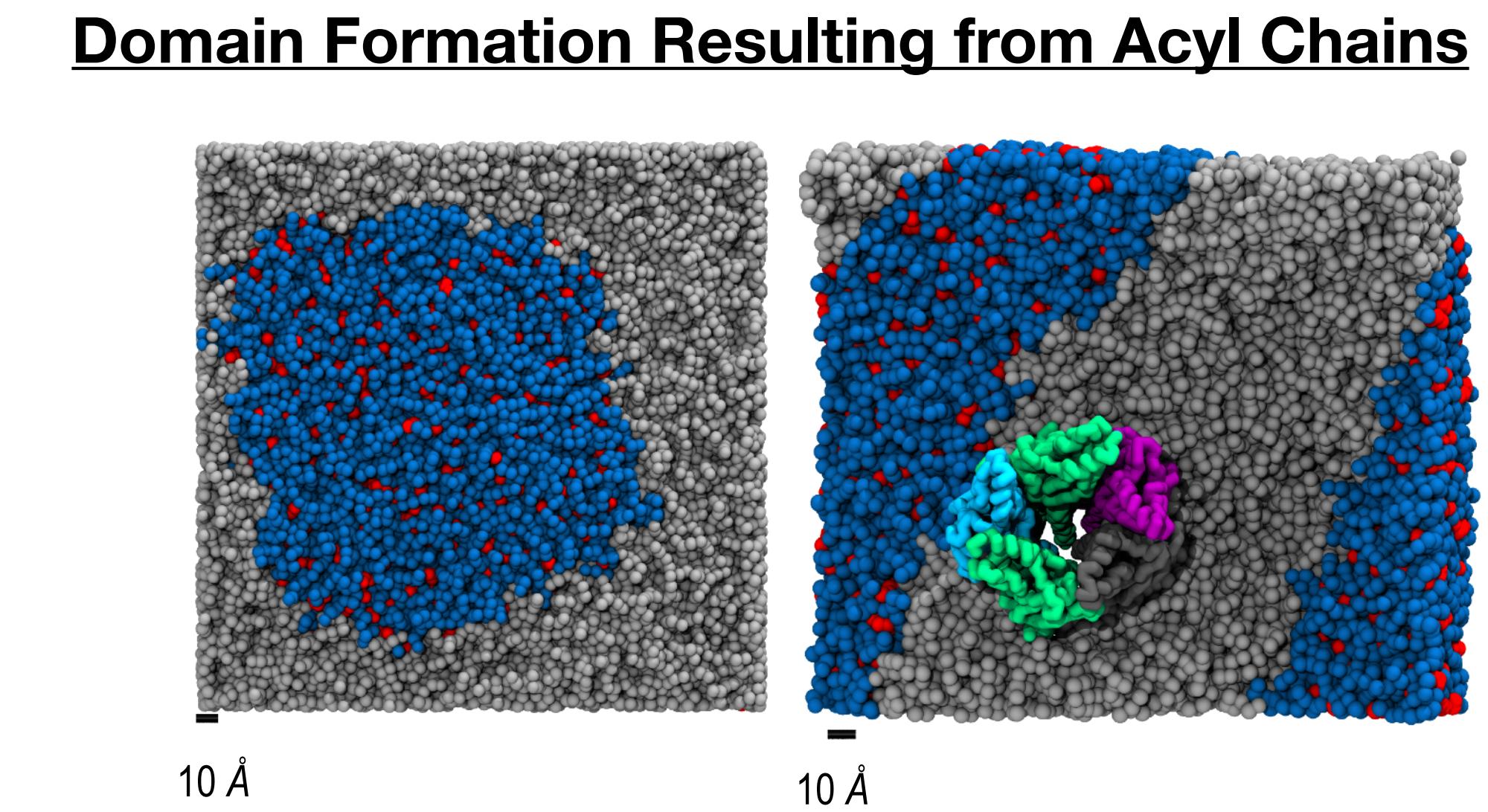
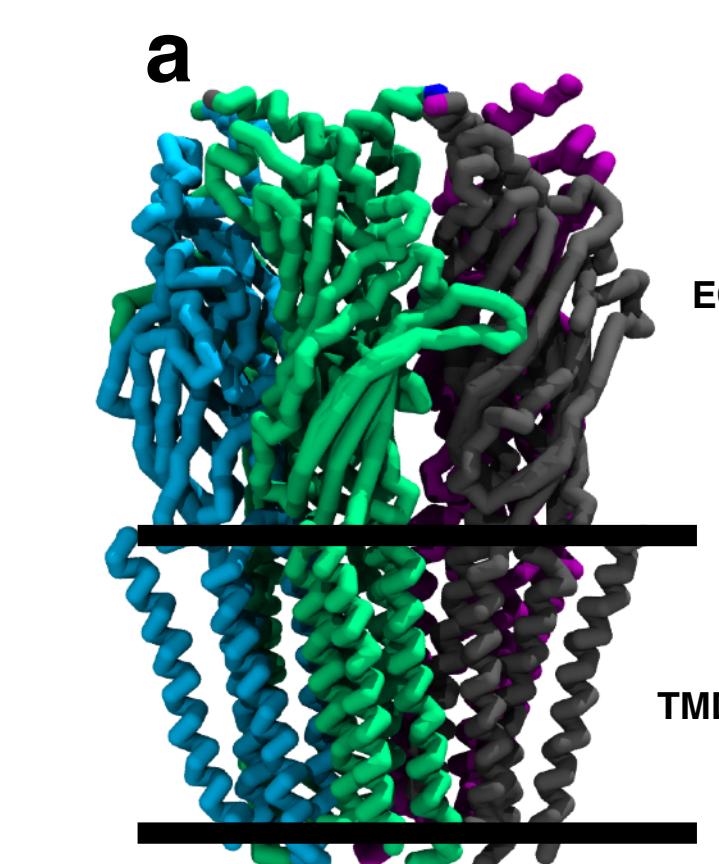


Figure 1:

nAChR is a pentameric ligand gated ion channel. The neuromuscular species are composed of α : seafoam, γ : cyan, δ : grey, β : purple. Protein colors are used throughout. Further broken down into an extra cellular domain (ECD), transmembrane domain (TMD)(area of interest), and intercellular domain (ICD) (not shown). Domain form based on acyl chains rather than proteins (DPPC:blue, Cholesterol:red, DHA-PE:silver); however, proteins may effect domain mixing.

Introductory Oocyte Simulations

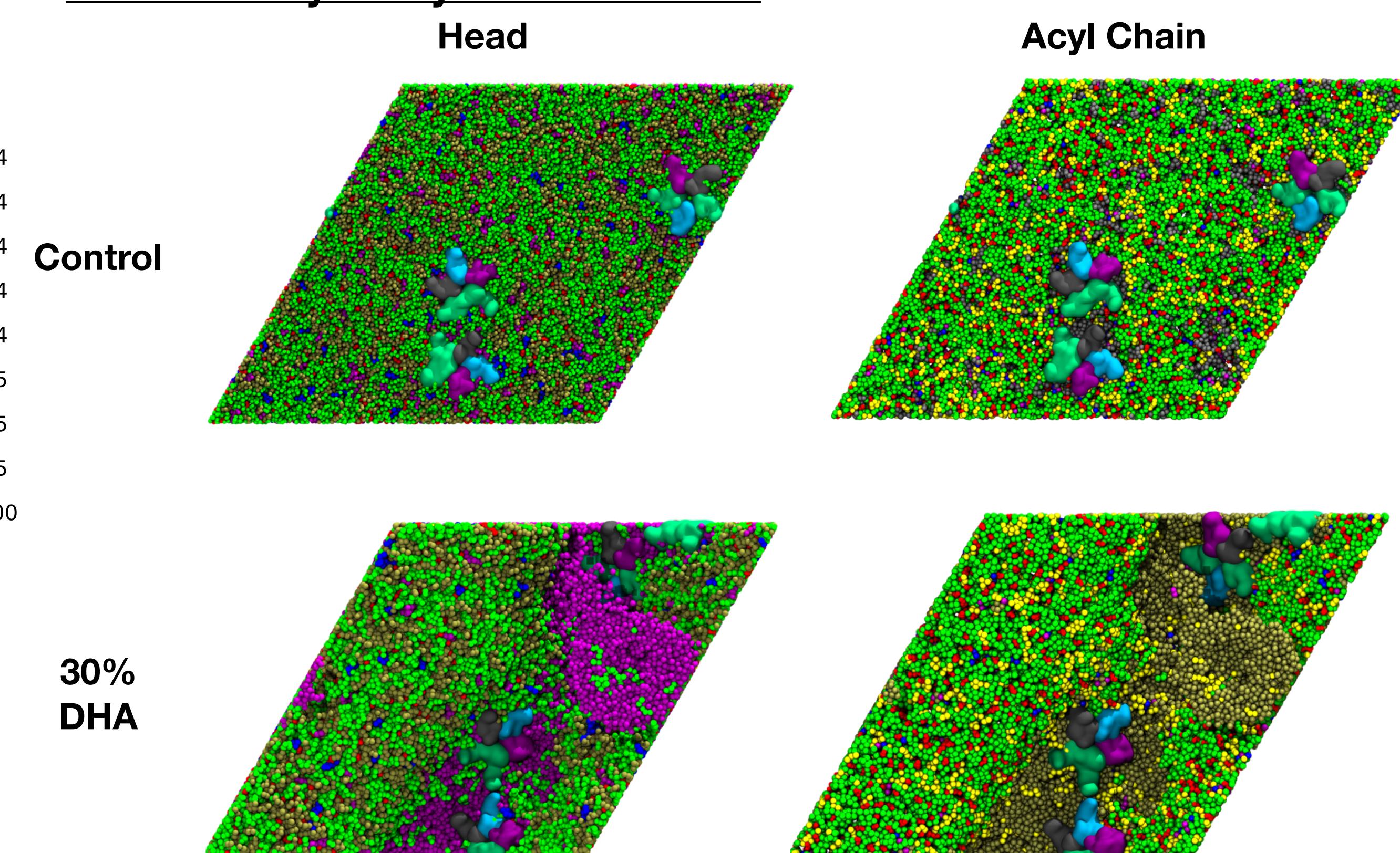


Figure 4:

A) Comparison averaged over three replicas DPPC:PUFA:CHOL 40:40:20. Row 1 to 3 show lipid density of lipid species. Left: System with DHA, Cholesterol densities are found predominantly in the Lo phase, with increased potentials at alpha and beta subunits. Right, LA system, cholesterol is found throughout most subunits and the bulk membrane.

B) Close up of row 1 and 3 of replicas containing DHA. Density scale is altered to better show cholesterol interaction. DHA encompasses bulk of protein with cholesterol being localized near the beta subunit and between alpha-delta, and gamma.

Oocyte modeled membranes are rich in saturated fatty acids and sphingomyelin. Left images show coloring by lipid head groups and are fairly randomized domain (PC:green, PE:purple, PS:blue, PI:yellow, PA:silver, SM:tan). Systems with 30% di-DHA-PE do show some scattered domain formation around nAChR. Right Coloring by acyl chain reveals domain formation (Sat:green, n9:purple, n7:blue, n6:yellow, n3:tan). There are areas around nAChR in controlled systems rich with PUFAs at ~30% DHA, forming a disordered phase containing all three proteins.

Conclusion

- Model-native Torpedo membranes de-mix into liquid order and liquid disorder phases
- nAChR consistently partitions into cholesterol poor domains; which are abundant in long chained PUFAs suggesting an annular dependency for PUFAs
 - This is interesting as nAChR is functionally dependent on cholesterol
 - nAChR's orientation is similar when position restraints are removed and membrane size is increased
- In the cholesterol poor domain, nAChR tends to position itself near the phase interfaces
- In cholesterol poor domains, DHA-PE occupies embeds throughout nAChR but prefers the β and γ subunits
- Cholesterol dependence may come from non-annular binding
- Small domains form around nAChR in oocyte membranes
 - Domains not rich in n-3 PUFAs and may not be optimal membranes

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