

## Abstract

The nicotinic acetylcholine receptor (nAChR) is a neurotransmitter receptor and pentameric ligand gated ion channel (pLGIC) critical for signaling across synapses, including the neuromuscular junction. In reconstituted membranes, the nAChR function depends heavily on cholesterol, leading to the hypothesis that nAChR will partition into cholesterol-enriched liquid-ordered domains ("rafts"). Native nAChR membranes are rich in lipids with saturated fatty acid (like palmitic acid) or polyunsaturated fatty acid (PUFA) acyl chains (like docosahexaenoic acid (DHA)). Using coarse-grained molecular dynamics simulations (CG-MD) we characterized preferential lipid interactions and partitioning behavior of nAChR in binary membranes (cholesterol and lipids with two palmitic acid acyl chains) ternary membranes (cholesterol, a lipid with two palmitic acid acyl chains, and lipids with either two chains composed of the long-chain omega-3 PUFA docosahexaenoic acid (DHA) or of the omega-6 PUFA linoleic acid).

We quantify occupation of non-annular and annular regions by cholesterol and saturated and polyunsaturated lipids. In the absence of PUFAs, cholesterol is enriched in the nAChR annulus in a concentration-dependent manner. Cholesterol is also distributed throughout the non-annular (embedded) sites, while palmitic acyl chains persistently occupy only one interface between the beta and alpha subunits. When lipids containing long-chain PUFA acyl chains are introduced, they displace cholesterol from two additional interfaces. Contrary to expectations, in domain-forming membranes containing PUFAs, the nAChR is observed to consistently partition into PUFA-rich, cholesterol-poor domains. Saturated lipids with either phosphatidylcholine (PC) and phosphatidylethanolamine (PE) head groups are significantly depleted in such systems, although the extent of depletion is reduced for PE. While nAChR consistently partitions into the cholesterol poor domain, the alpha-gamma and delta-beta faces interact more than other faces with the cholesterol-rich domains. We extend this approach to more complex membranes of interest, including more realistic synaptic membranes and the Xenopus oocyte membranes used for electrophysiology.

## Introduction

- Nicotinic Acetylcholine Receptors (nAChR) are essential pentameric ligand gated ion channels (pLGICs) and highly lipid sensitive
- While nAChRs are well studied they have not been well studied in native or quasi-native membranes
  - nAChR native membranes, such as neuronal membranes and *Torpedo* electric organs, have similar lipid compositions, both rich in n-3 polyunsaturated fatty acids (PUFAs) and cholesterol (~20% of membrane DHA and upwards of 35% of cholesterol per lipids)[3]
  - nAChR functional studies are performed in *Xenopus* Oocyte which is poor in n-3 PUFAs and cholesterol compared to native membranes (~5% of membrane DHA and ~15% Cholesterol per lipid)[6]
    - Xenopus Oocytes require external lipid "doping" for nAChR to achieve native function
  - PUFAs form liquid disordered phases; saturated fatty acids and cholesterol form liquid ordered phases

## Methods

- cryo-EM structure (PDB:2BG9) [5] used in these simulations (derived from *Torpedo*)
- Systems for quasi-*Torpedo* are composed of saturated:cholesterol:PUFA
  - PUFAs used are Docosahexaenoic acid (DHA) and linoleic acid (LA) as both lipids are common n-3 and n-6 in *Torpedo* electric organ
- Quasi-*Xenopus* oocyte membranes[6] used to determine nAChR partitioning in potential experimental membranes (~15 lipid species used)
- Coarse-grained Molecular Dynamics simulation preformed with MARTINI force field 2.2 [2] and GROMACS[1] 5.0.6 and 5.1.2
- Run under constant pressure and temperature (1 atm and 323 K)
- Ran for 2 ns to 10 μs
- Small boxes had ~1400 lipids (~25x25 nm<sup>2</sup>). Large boxes had ~8300 lipids (40x40 nm<sup>2</sup>)

## Equations

$$M_{A,B} = \frac{\langle n_{A,B} \rangle}{6x_B} - 1$$

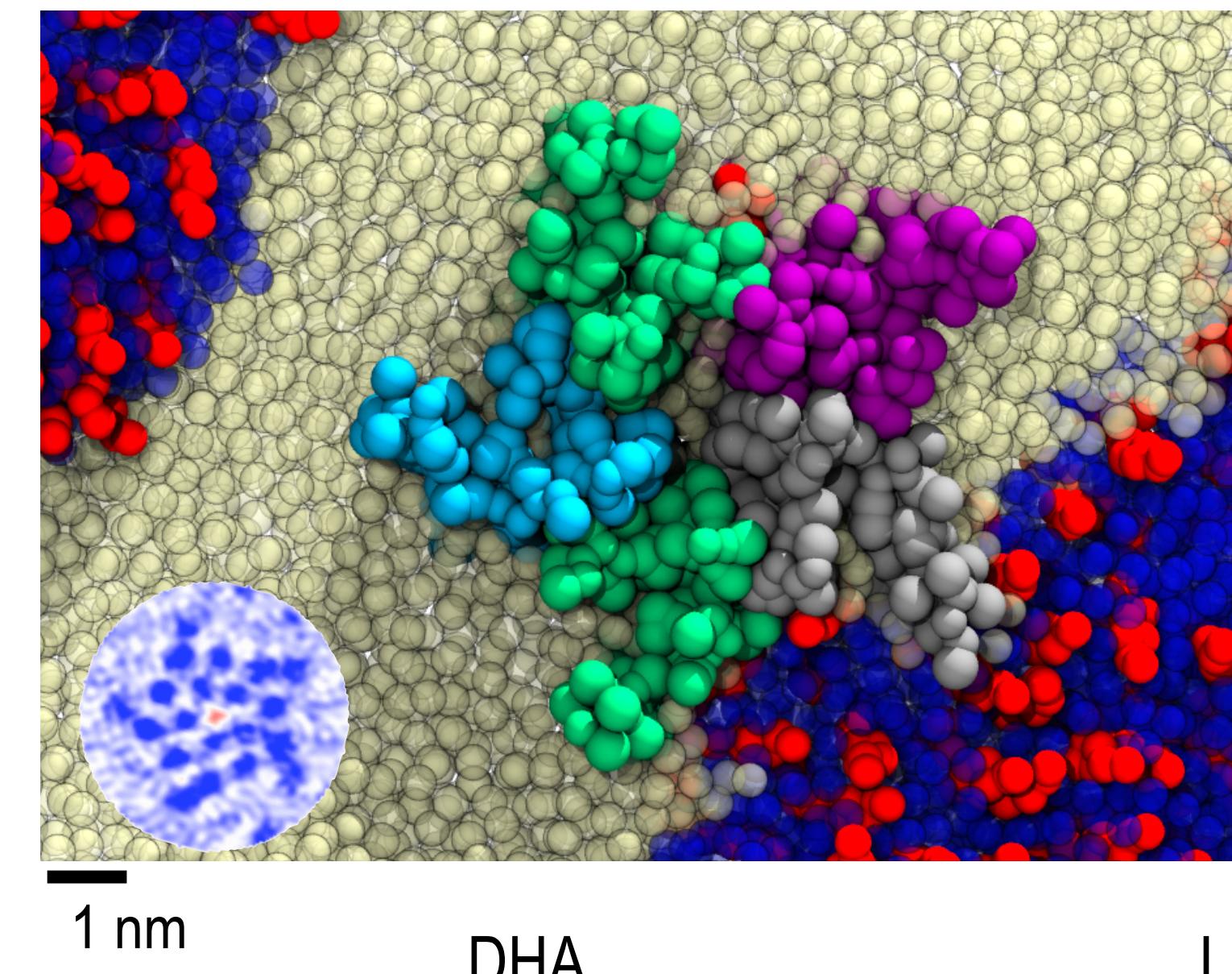
$$Q_{sat} = \frac{1}{x_{sat}} \langle \frac{b_{sat}}{b_{tot}} \rangle - 1$$

$$\rho_B(r_i, \theta_j) = \frac{\langle n_B(r_i, \theta_j) \rangle}{r_i \Delta r \Delta \theta}$$

$$\bar{\rho}(r_i, \theta_j) = \frac{\rho_B(r_i, \theta_j)}{x_B s_B N_L / (L^2)}$$

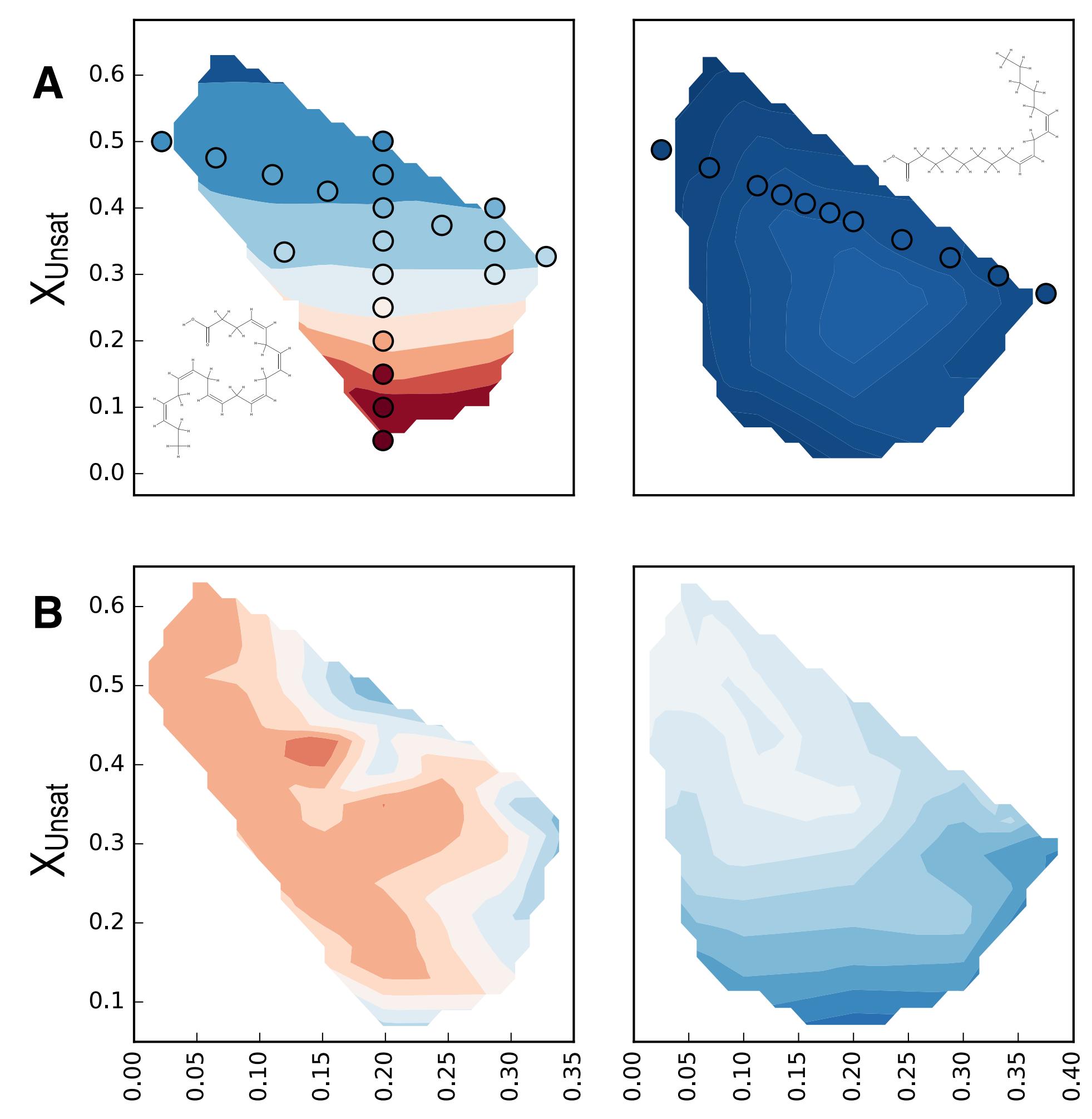
## Results

### Boundary Lipids in quasi-Native Membranes



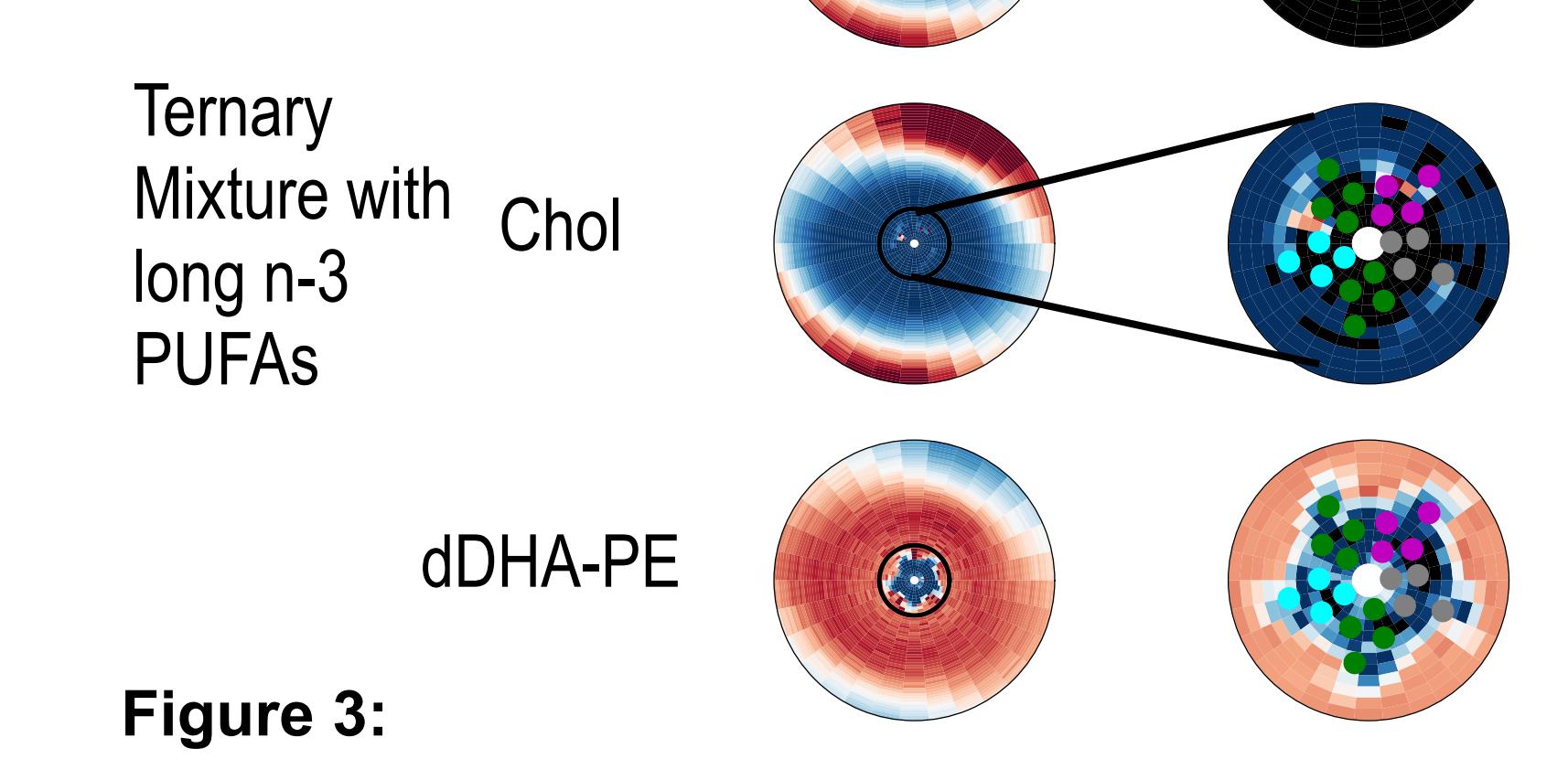
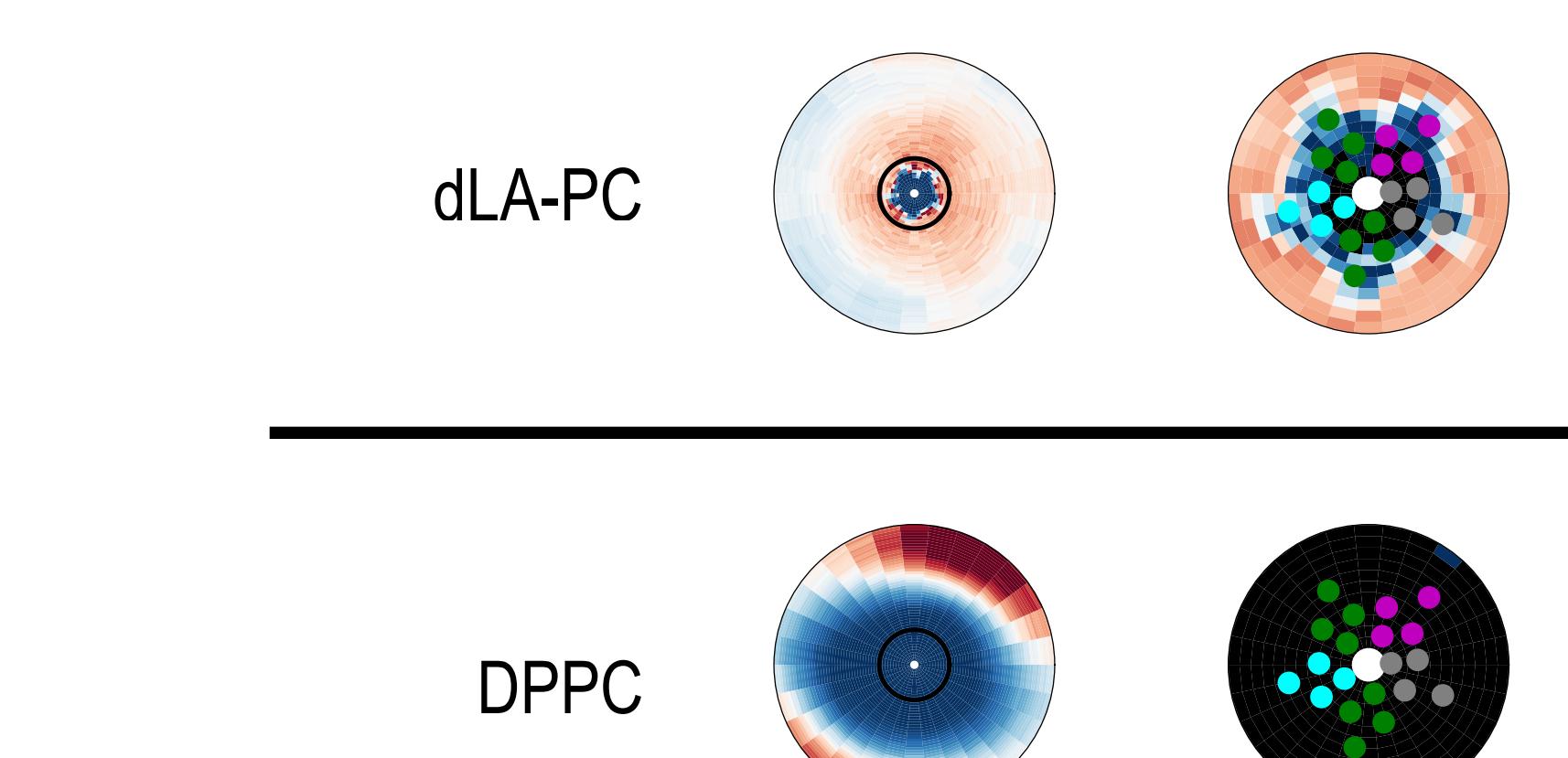
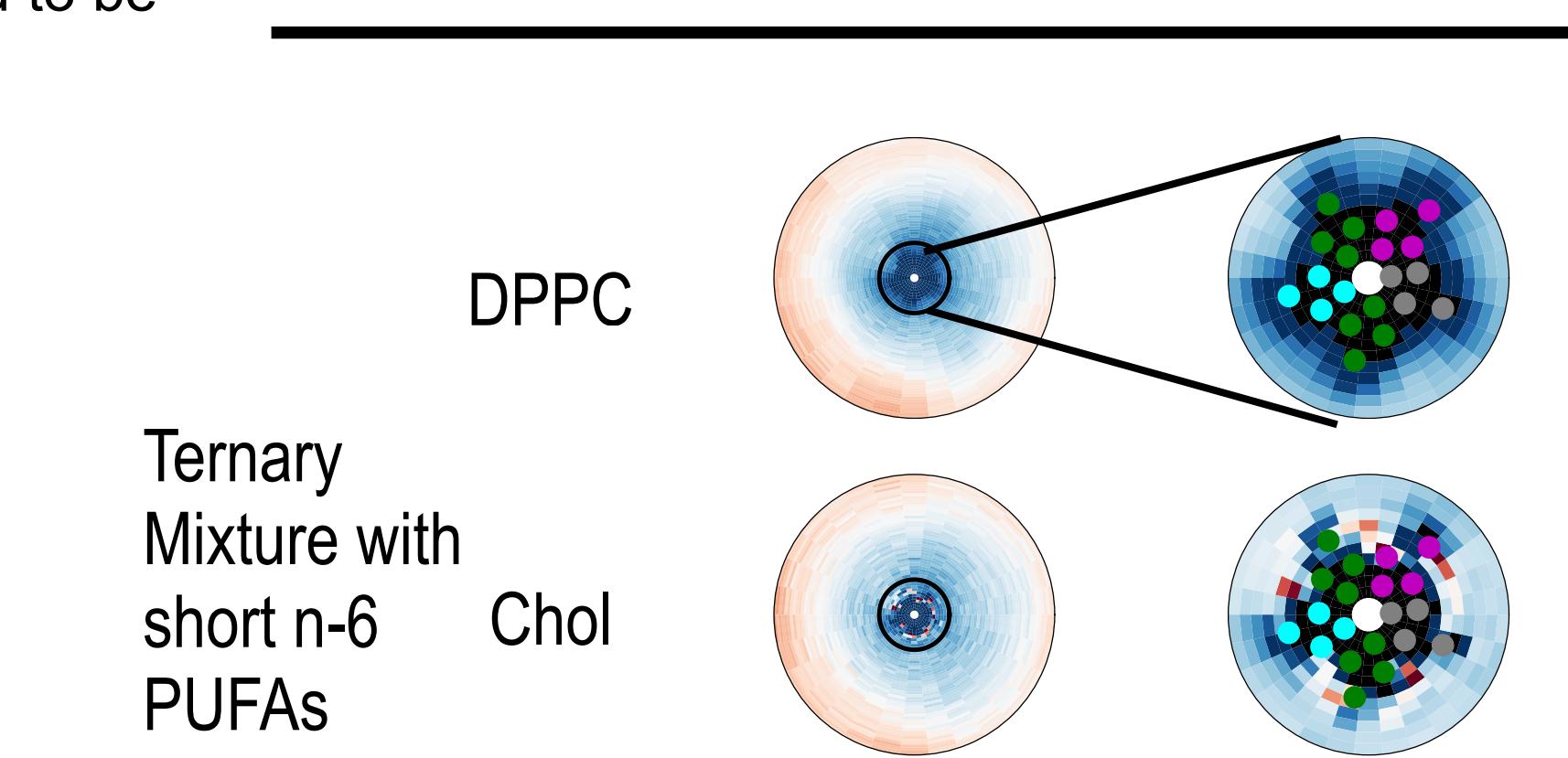
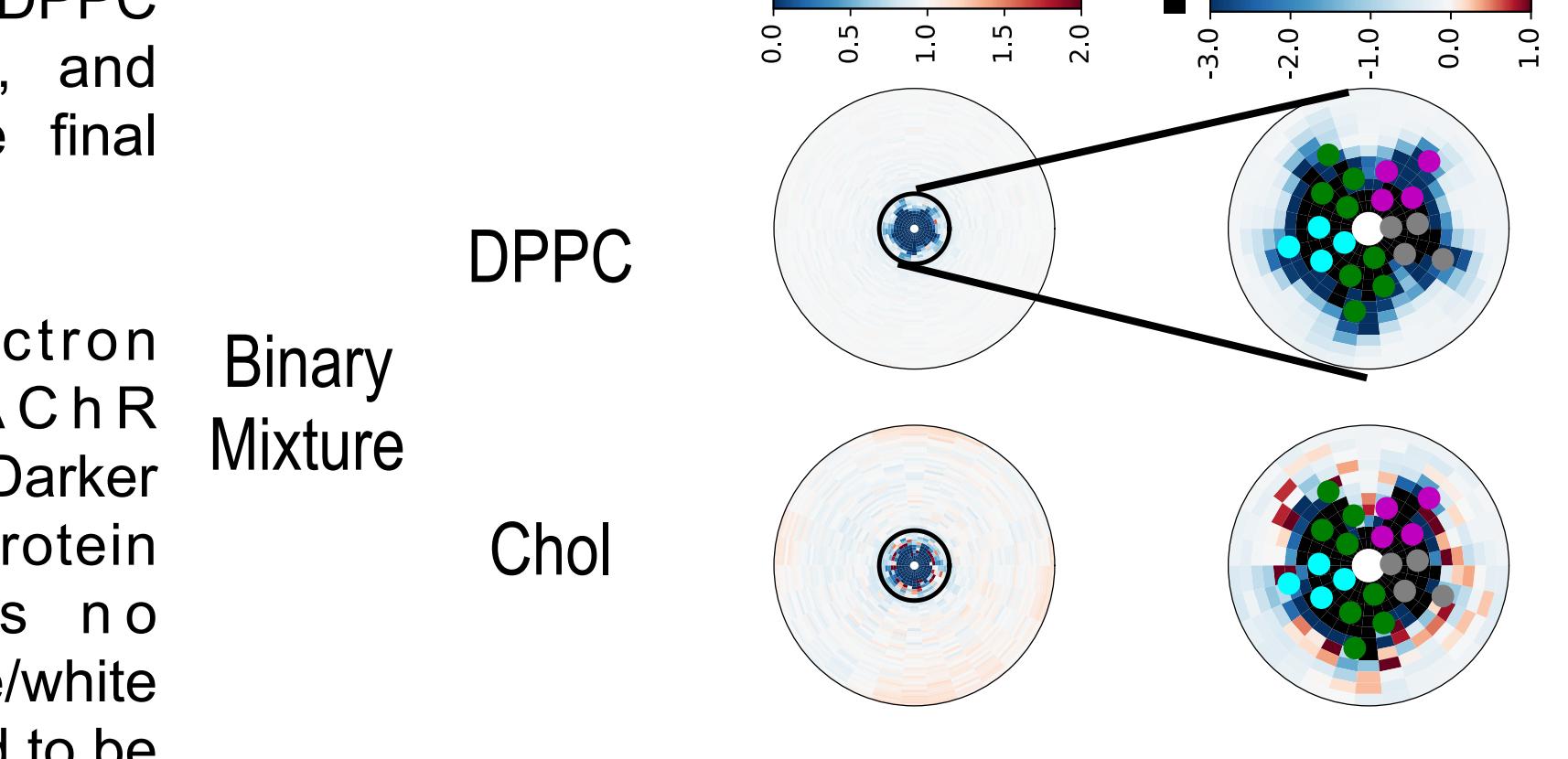
**Figure 1:**  
Transmembrane domain of nAChR in a membrane of DPPC (blue), cholesterol (red), and DHA-PE (cream). The final frame of a 2 μs simulation.

Subfigure shows electron density map of nAChR transmembrane region. Darker blue the higher the protein density, red shows no density[3,5]. The light blue/white regions were hypothesized to be filled with cholesterol [3].



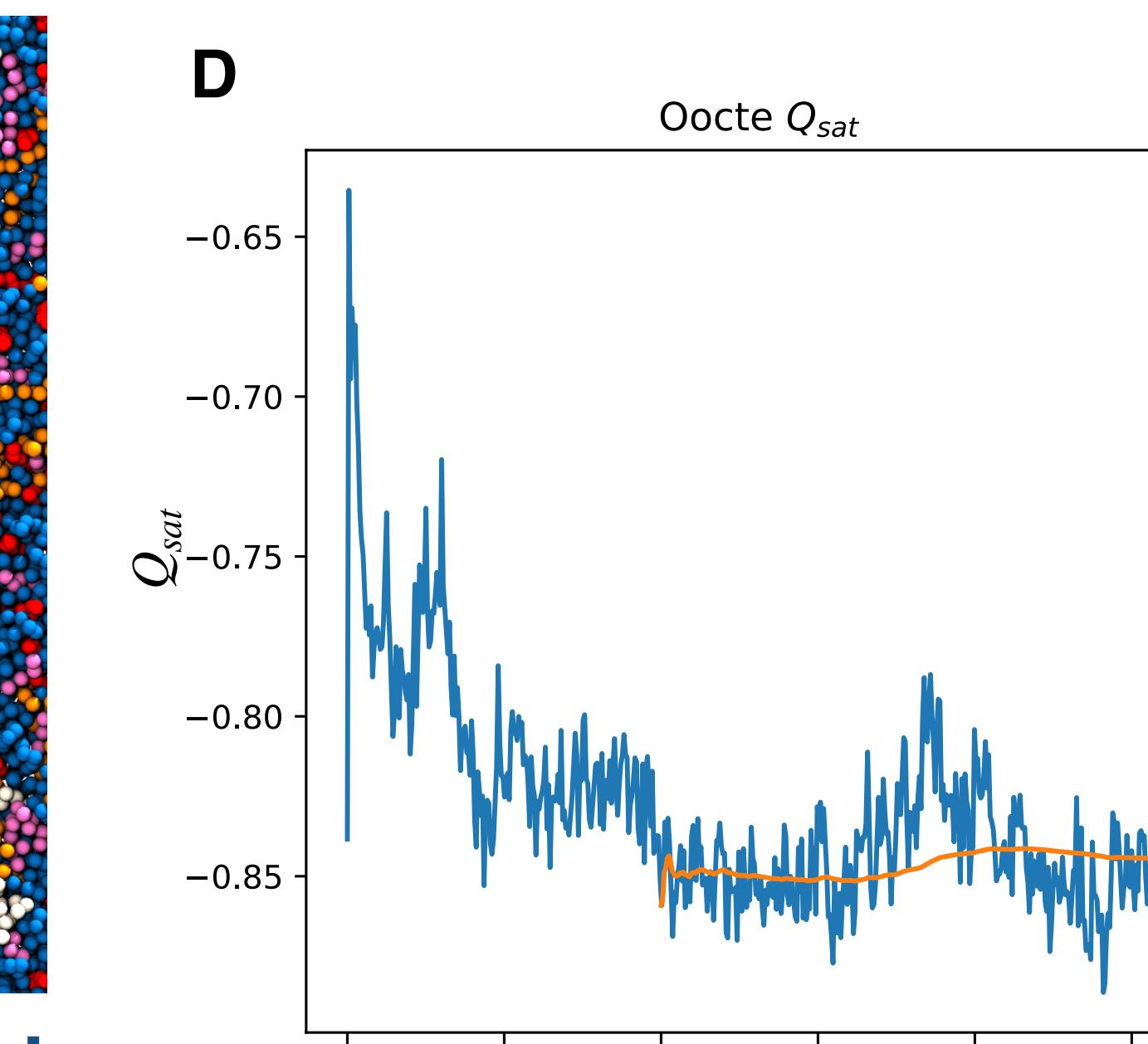
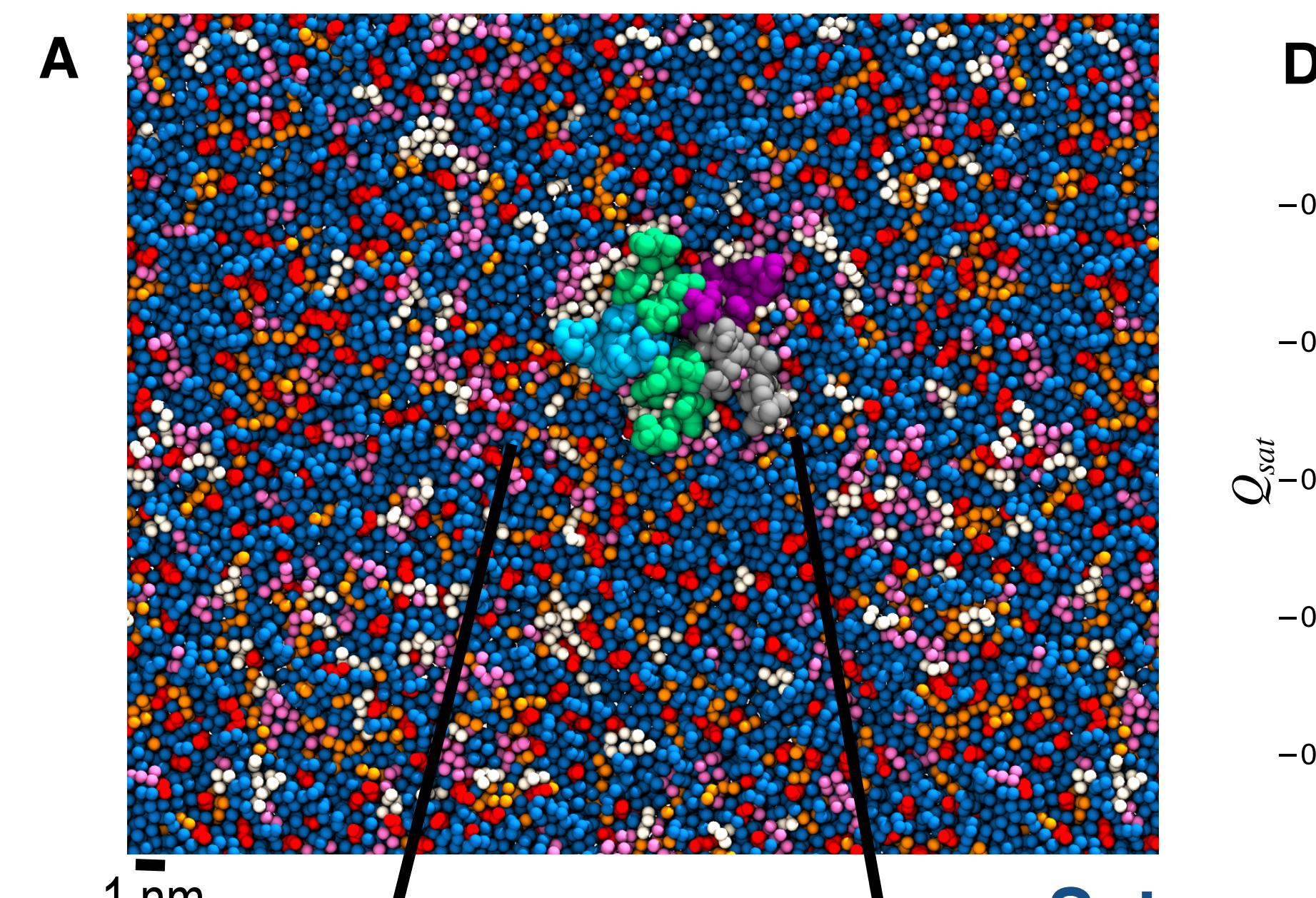
**Figure 2:**  
Subplots A and B use the same ternary systems of DPPC:Cholesterol:PUFA. All simulations run for 2 μs in small boxes.

- Self association of n-3 and n-6 PUFAs across a variety of ternary membranes. The mixing metric M is used to show self mixing with itself. More red, the greater PUFA-PUFA interaction. More blue, the less PUFA-PUFA interaction.
- Quantification of saturated lipids within the proteins boundary region. Using the metric Q, we determine how strong a presence of saturated lipids. Q>0 saturated enrichment, Q=0 random mixing, Q<0 depletion. Red shows strong sat expected, and blue shows weak sat depletion

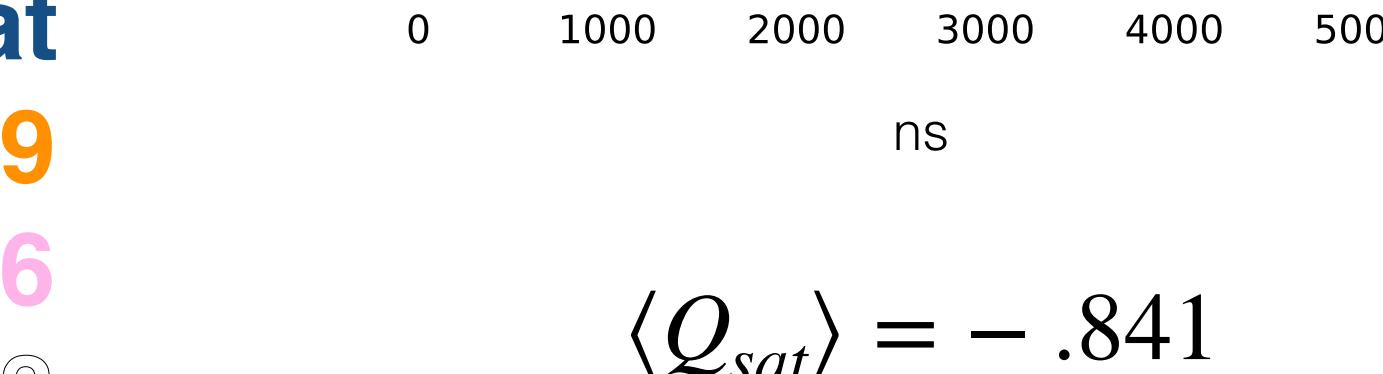


**Figure 3:**  
Normalized probability of lipid organization in large boxes, averaged over 5 μs. Membranes are comprised of DPPC:PUFA:CHOL at 2:2:1 ratio. Left column shows full membrane, right column is a zoomed in (5 nm from center of protein). Top row shows binary system of DPPC:cholesterol; the middle and bottom rows show ternary systems of DPPC:cholesterol:DLaPC and DPPC:cholesterol:Di-DHA-PE respectively. White represents expected, red shows the below expected, and blue above the expected probabilities of a lipid species.

### Boundary Lipids in Xenopus Oocyte Membranes



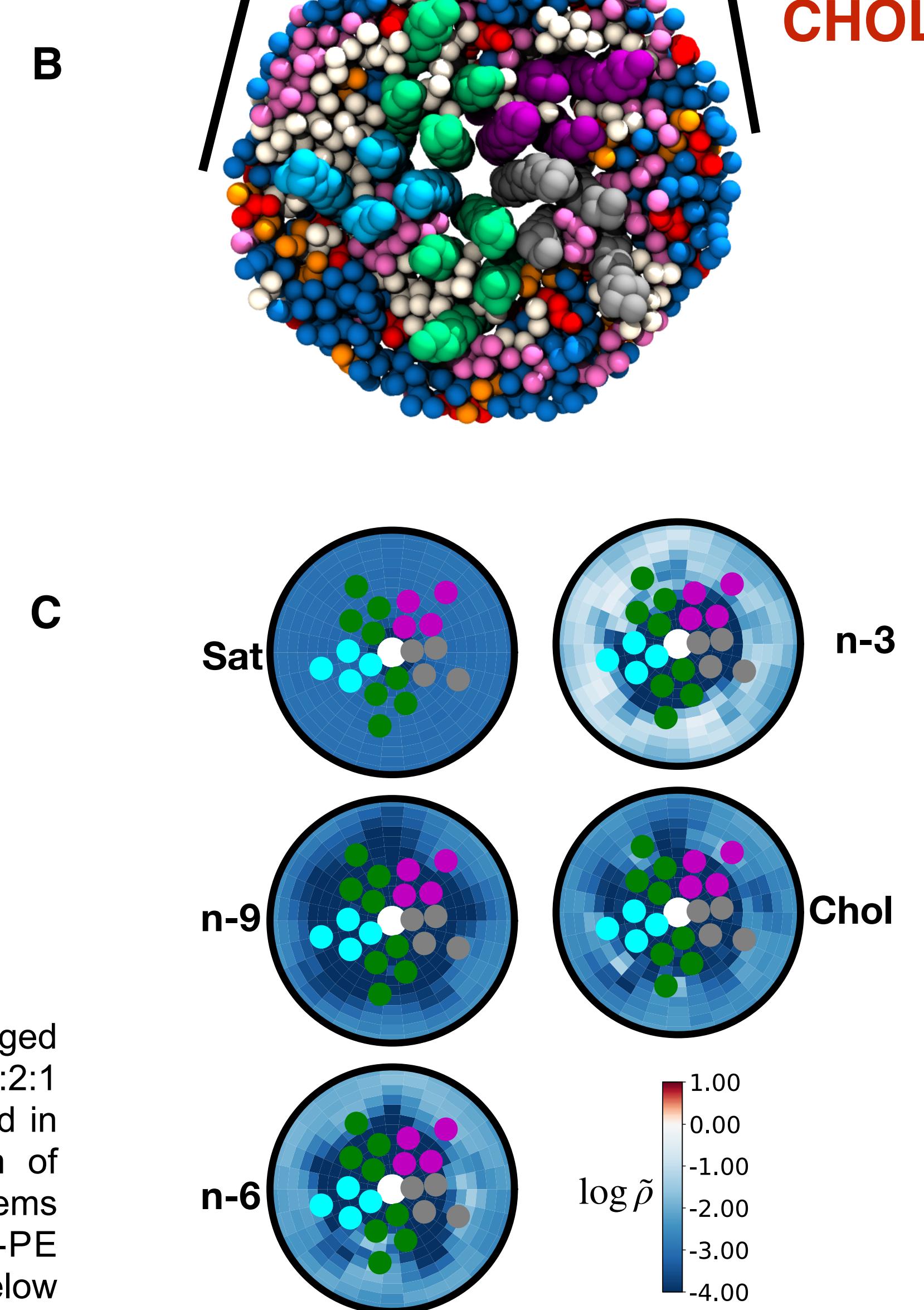
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$$\langle Q_{sat} \rangle = -.841$$

**Figure 4:**  
nAChR within quasi-Xenopus oocyte membranes. **A** and **B**: Saturated lipids:blue, PUFAs: n-9:white, n-6:pink, n-3:orange, cholesterol:red in a small box.

- Final frame of a 5 μs simulation using quasi-oocyte membrane (~15 lipids species) at 15% cholesterol.
- Close up at ~3.5 nm from the pore forming helices. PUFAs form most of the annulus with some saturated lipid interaction.
- Lipid densities acyl chain saturation within 5 nm of protein. White represents expected, red more than expected, and blue less than expected probabilities
- Quantification of boundary saturated lipid near protein using the Metric  $Q_{sat}$ .  $Q>0$  enrichment,  $Q=0$  expected,  $Q<0$  depletion. Average value taken over the last ~2.5 μs.



## Conclusion

- Model-native *Torpedo* membranes de-mix into liquid order and liquid disorder phases
  - Domain formation is a result of acyl chain saturation, dependent of nAChR
  - nAChR consistently partitions into cholesterol poor domains; which are abundant in long chained PUFAs
    - If Fig 2, showed similar trends between metric M and Q, it would suggest lipid driven organization
    - This is interesting as nAChR is functionally dependent on cholesterol
    - Fig 3, shows despite nAChR's preferred domain, cholesterol still interacts with nAChR annularly and non-annularly
  - Small boundary domains form around nAChR in oocyte membranes
    - Fig 4, despite a lack of n-3 PUFAs, n-3 PUFAs had the greatest chance of protein interaction
    - Membranes lacking n-3 PUFAs and may not be optimal membranes

## Reference

- [1]Berendsen, et al. (1995) Comp. Phys. Comm. 91: 43-56. [2]Brannigan GB et al. Embedded cholesterol in the nicotinic acetylcholine receptor PNAS 2008;105:14418-14423. [3]Barrantes, JB. The Lipid Environment of Nicotinic Acetylcholine Receptor in Native and Reconstituted Membrane. Critical Reviews in Biochemistry and Molecular Biology 1989;24:5:437-478. [4]Daily MD. Improved Coarse-Grained Modeling of Cholesterol-Containing Lipid Bilayers. Journal of Chemical Theory and Computation 2014; 10 (5), 2137-2150 [5]Unwin N. Refined structure of the nicotinic acetylcholine receptor at 4 Å resolution. Journal of Molecular Biology 2005; 397:989. [6]Warren G, Hill et al. (2005) Am J Physiol Renal Physiol.

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