1 THESIS ABSTRACT: DISSERTATION AWARD

Pentameric ligand-gated ion channels (pLGIC) are a family of channels essential in synaptic function. pLGICs are shown to be functionally dependent on their environment's lipid composition. The local interactions and direct lipids-proteins contacts are poorly understood.

While the role of lipids is essential to pLGIC, the accessible boundary lipids have not been identified. Experimental studies have provided mixed results determining boundary lipids in model membranes, and have largely ignored native membranes. We simulate the pLGIC the nicotinic acetylcholine receptor (nAChR) in neuronal membranes (membranes nAChR resides in), and *Xenopus* oocyte membranes (a membrane used in experimental studies). nAChR native membranes have approximately 3 times the ω-3's polyunsaturated fatty acids (PUFAs) and 1.5 times the cholesterol compared to *Xenopus* oocytes; yet, ω-3 PUFAs are infrequently used in pLGIC experimental studies.

pLGIC are conserved across structures and have been observed to function in similar lipid compositions. To better understand the interplay between pLGICs and ω-3 PUFAs we simulate a series of ternary membranes using saturated fatty acids, ω-6 PUFAs, and ω-3 PUFAs using various pLGICs with existing crystal or cryo-EM structures. We hypothesize one of two outcomes: 1) all pLGICs form a similar boundary distribution of PUFAs, suggesting lipid organization is a result of a shared structure. 2) Lipid distributions are dissimilar, and lipid organization is driven by protein sequence over the structure.

CG-MD simulations play the role of a "computational microscope" to visualize lipid diffusion and various feasible lipid-protein arrangements in thermodynamically equilibrated systems over microseconds, allowing observations not readily seen experimentally.

2 RESEARCH NARITIVE

2.1 *Nicotinic Acetylcholine Receptors Lipid Preferences Within Simulated Coarse-Graned Native Membranes and Experimental Membranes*

Simulations are running.

Significance of the work: *Xenopus* oocytes are used in pentameric ligand-gated ion channel (pLGIC), nAChR in particular, functional studies [papers to show this]; they are large single cells and easily manipulated. However, reconstituted pLGICs do not reach native function without the inclusion of cholesterol [cite], anionic lipids [cite], asolectin [cite], or pLGIC native membrane [steve's paper]. Of interest, native anionic lipids, asolectin and many of these native membranes are rich in polyunsaturated fatty acids. Combined with our previous prediction nAChR has a high affinity for ω-3 PUFAs, we hypothesize nAChR requires not simply annular PUFAs and cholesterol, but a sizable required area of PUFAs and cholesterol to pro-

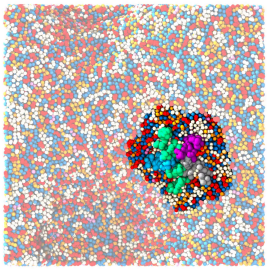

Figure 1: Trajectories of ternary mixtures at ratios of 2:2:1 DPPC:PUFA:Chol. A and B: Trajectories of simulation systems with a single nAChR embedded within small membranes, using lipids containing DHA acyl chains or LA acyl chains. Both simulations were run for 2 μs. C: Final snapshot of 4 μs trajectory of a system within a large $\sim$ 75x75 nm^2 membrane with the same composition as in A. Subunits are colored: α: green, β: purple, δ: gray, γ: cyan. Lipids are colored: Chol: red, DPPC: blue, dDHA-PE: white, dLA-PC: tan.

mote function. Comparing neuronal membranes and *Xenopus* oocytes show, distinct differences in lipid compositions that have not been evaluated.

Goal: Quantify the overall differences of nAChR boundary lipids to make suggestions for lipid additives for experimental research.

2.2 *Lipid Variations Between Pentameric Ligand Gated Ion Channels: Is Boundary Lipid Organization Driven by Structure or Sequence*

Simulations Running.

*Significance of the work:*Pentameric ligand-gated ion channels (pLGICs) sequence vary across species and even within individual species, despite all pLGICs sharing a common structure. It is unclear whether boundary lipids organization is driven by a protein sequence or its structure. We hypothesize the shared structure promotes an affinity for long-chained PUFAs.

Goal: Predict the role of protein sequence and structure play on membrane organization.

3 EFFECTIVENESS OF THE AWARD

Being awarded this fellowship would alleviate financial stressors for the summer. This summer will be challenging to be paid through the Center for Computational Biology. The award would allow me to continue working on my finishing my projects and beginning my dissertation. Without this honor, it would require me to find work elsewhere, pushing off my defense. I have school year funding until the end of December 2020.

4 TIMELINE TO COMPLETION

- April-May 2020:
  - *Nicotinic Acetylcholine Receptors Lipid Preferences Within Simulated Coarse-Graned Native Membranes and Experimental Membranes* Analysis finished
  - Start Manuscript
  - Simulations for *Lipid Variations Between Pentameric Ligand Gated Ion Channels: Is Boundary Lipid Organization Driven by Structure or Sequence* finish
- June-July 2020
  - Committee meeting
  - *Analyze Lipid Variations Between Pentameric Ligand Gated Ion Channels: Is Boundary Lipid Organization Driven by Structure or Sequence*
  - Submit *Nicotinic Acetylcholine Receptors Lipid Preferences Within Simulated Coarse-Graned Native Membranes and Experimental Membranes*
- August-September
  - Begin Disertation
  - Defend