Interactions Of Nicotinic Acetylcholine Receptors With Liquid-Disordered Domains Rich In Polyunsaturated Fatty Acids

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Abstract

Nicotinic acetylcholine receptors (nAChRs) are pentameric Ligand Gated Ion Channels critical to signaling across synapses and the neuromuscular junction. nAChR function is particularly sensitive to the surrounding lipids, with numerous experimental studies showing native function of reconstituted nAChRs only in membranes with cholesterol. It has been expected that cholesterol serves as a boundary lipid by binding to the nAChR in annular and possibly non-annular (embedded) sites; given the expectation of annular cholesterol it was further hypothesized that nAChR likely partitions into liquid-ordered (raft) phases of domain-separated membranes, but this has not been observed experimentally in simple domain-forming mixtures containing cholesterol. Furthermore, although n-3 polyunsaturated fatty acids (PUFAs) are abundant in both the native nAChR torpedo membrane and the neuron, the role of these acyl chains in nAChR has not been studied experimentally.

In the present research, we use Coarse-grained Molecular Dynamics Simulations via MARTINI to investigate spontaneous partitioning of nAChRs in domain-forming lipid mixtures based on the native torpedo lipid environment. We observe that, contrary to expectations, nAChR partitions into the liquid-disordered phase rich in n-3 PUFAs and low in cholesterol. When nAChR is partitioned into a cholesterol-poor liquid-disordered phase, binding of annular cholesterol is not observed, but cholesterol is stable in some non-annular embedded sites at some cholesterol concentrations. One origin of nAChR's preference for the liquid-disordered phase becomes clear upon examining the equilibrated systems: the more flexible liquid-disordered phase can accommodate the deformation induced by the cone-shaped nAChR.

Introduction

- Nicotinic Acetylcholine Receptors (nAChR) are essential pentameric ligand gated ion channels (pLGICs) Figure 1
- Functionality is dependent on cholesterol
- nAChR is well studied but nAChR-neuronal like membrane interactions are not
  - Neuronal membranes and *Torpedo* have similar lipid compositions, both rich in polyunsaturated fatty acids (PUFA's)
  - Membranes modeled from *Torpedo* membrane characterized by Barrantes[3] Table 1

Methods

- cryo-EM structure (PDB:2BG9) used in these experiments (derived from *Torpedo*)
- nAChR (2BG9) coarse grained and embedded in membrane with position restraints
- Solvated and 0.15 M of NaCl added
- Coarse-grained Molecular Dynamics simulation preformed with MARTINI force field 2.2 [2] and GROMACS[1] 5.0.6
- Ran for 2 μs

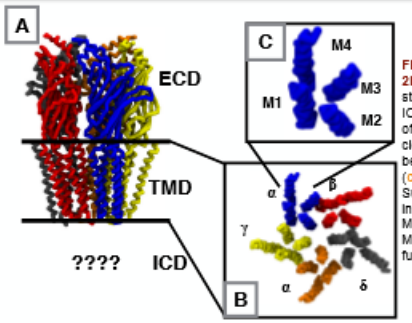

Figure 1. Breakdown of nAChR 2BG9. A) Side view of 2BG9 structure showing: ECD, TMD, and ICD. B) Topdown view of the TMD of 2BG9. Subunits are colored clockwise as follows: alpha (blue), beta (red), delta (gray), alpha (orange), gamma (yellow). C) Subunits are further broken down into helices M1-M4. M1,M2 and M2,M3 face neighboring subunits. M4 sits in the annular domain, further M2 makes up the pore.

	Torpedo[a]	Synapse[b]	Xenopus[c]	Mammalian[d]
PC	37	40	28	38
PE	44	36	17	22
PS+PI+PA	13	19	10	12
SM	3	5	20	20
Other PL	3	1	5	8
Saturated	42	49	44	53
Monounsaturated	26	15	39	20
Polyunsaturated	32	36	17	27
Cholesterol Mol Fraction	35	30	21	29

Table 1 Membrane composition over cell lines. a) Torpedo californica electric organ b) Rat brain Synaptosomes c) Xenopus oocytes d) "Idealized" (average) Mammalian Plasma Membrane[5]

Results

Qualitative effects of changing phospholipid species:

nAChR in membranes with composition:

15% CHOL + 42.5% DPPC + 42.5% _____

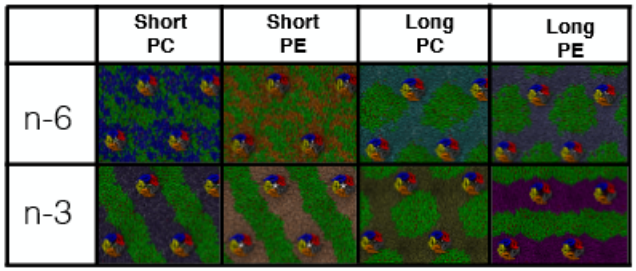

Figure 2. Mixed membranes containing phospholipids, cholesterol, and an nAChR, after 2 μs of MD simulation, viewed from the ECD. Multiple periodic images are shown. nAChR tends to partition into long chained PUFAs (n-3 and n-6) when present.

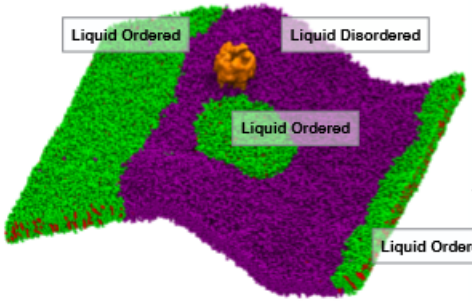

Figure 3. Initial Results Using Harmonic Restraints. 75nm x 75nm membrane. Protein position restraints have been removed and can move freely. Image taken at end of 2.18 μs.

Lipid Domain and Protein Interaction:

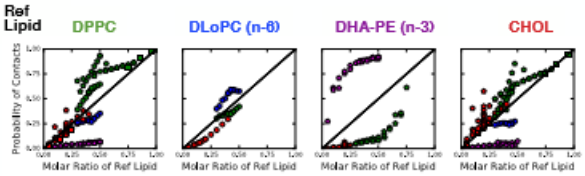

Figure 4. Probability of lipid species contact. Nearest neighbors are defined as the six closest lipids to a reference lipid (DPPC, DLoPC, DHA-PE, CHOL). Diagonal lines indicate expected values for a random mixture.

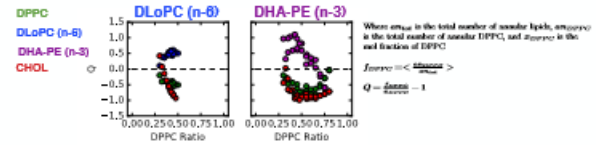

Figure 5. Likelihood of nAChR partitioning in the disordered domain We defined annular lipids as lipids within 35 Å but further than 10 Å away from the M2 helices. nAChR is more likely to partition into the disordered domain.

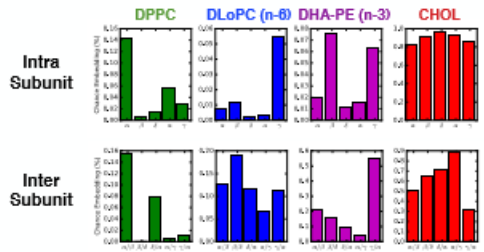

Figure 6. Distribution of Lipids in the Inter and Intra Subunits of nAChR. We define intra subunits as space between a single subunit's M1-M4 helices. Inter subunits are defined as space between two subunits.

Summary

- 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 in 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 dependance may come from non-annular binding

References

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Acknowledgments

Funding: Research Corporation, NIH P01GM55876-14A1
Computational resources: NSF XSEDE Allocation NSF-MCB110149 and local cluster funded by NSF-DBI1126052.