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

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Abstract

Nicotinic acetylcholine receptors (nAChRs) are pentameric Ligands-Gated Ion Channels critical to signaling across synapses and the muscarinic system. nAChR function is particularly sensitive to the surrounding lipids, with numerous experimental studies showing native function of nicotinic acetylcholine receptors (nAChRs) in membranes and cholesterol. It has been speculated that cholesterol serves as a boundary lipid to bind the nAChR in an annular and possibly non-annular conformation, given the exposition of annular cholesterol. We add further hypothesis that nAChR’s partition into liquid-ordered (Lo) domains of cholesterol-containing membranes, but this has not been observed experimentally in simple conformational studies containing cholesterol. Furthermore, although nAChRs have been observed to associate with lipids, the role of these associations 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 non-bilayer type membranes based on the native torus lipid environment. We observe that, contrary to expectations, nAChR partitions into the liquid-disordered phase rather than the liquid-ordered phase of lipid membranes. Binding of annular cholesterol is not observed, but cholesterol is stable in some non-bilayer embedded sites at reduced cholesterol concentrations. This result of the coarse-grained simulations for the liquid-disordered phase becomes clear upon examining the equilibrated systems. The hexagonal liquid-disordered phase was used to incorporate the information induced by the coarse-grained approach.

Methods

- Cryst-EM structure (PDB 2BCE) was used in all simulations (extracted from Tomoko).
- nAChR (2BCE) was inserted and embedded in membranes with position restraints.
- The system of 315 M of NaCl added.
- Coarse-grained molecular dynamics simulation performed with MARTINI force field 2.2 (G2 and G2A) and NAMD 2.8.3.
- PMF for 2 ps.

Results

Qualitative effects of changing phospholipid species: nAChR in membranes containing
- 15% CHOL + 42.5% DPPC + 42.5% lipids
- Short PC
- Short PE
- Long PC
- Long PE
- n-6
- n-3

Lipid Domain and Protein Interaction:

- Nearest neighbors are defined as six neighboring lipids in a reference lipid (DPPC, DPPC, DPPC, DPPC, DPPC).
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Figure 3. Initial results using harmonic restraints, T = 300 K and 500 ns; These results show that nAChR is highly ordered and mobile in these simulations.

Figure 4. 3D arrangement of lipids in the nAChR domain.

Figure 5. Lipid distribution in the nAChR domain.

Figure 6. Distribution of lipids in the nAChR domain.

Figure 7. Distribution of lipids in the nAChR domain.

Table 1. Membrane composition over cell lines. a) Torpedo californica simple organ b) Rat brain synaptosomes c) Xenopus colpus d) "idealized" average mammalian plasma membrane.

<table>
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<th>Lipids</th>
<th>Torpedo</th>
<th>Synapto</th>
<th>Xenopus</th>
<th>Mammalian</th>
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</table>

Summary

- nAChR mainly partitions into liquid disordered phase of lipid membranes.
- nAChR consistently partitions into cholesterol poor domains which are abundant in long chained FPPA suggesting an annular dependency on FPPAs.
- This is interesting as nAChRs are functionally dependent on cholesterol.
- nAChRs orientation is similar when position restraints are removed and membrane size is increased.
- In the cholesterol poor domain, nAChRs positions itself near the phase membrane.
- In cholesterol poor domain, DHA-PE occupies mediate across nAChR but prefers the β and γ subunits.
- Cholesterol dependency may come from non-annular binding.

References


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