Polyunsaturated fatty acids bind an intersubunit site on the nicotinic acetylcholine receptor

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Background & Motivation

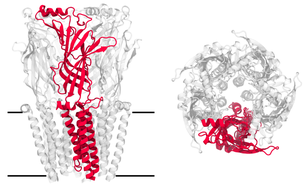


Fig 1: The α-7 nicotinic acetylcholine receptor (nAChR) (pdb id 8v89)[4] with intracellular domain removed, shown in membrane/lateral (left) and extracellular (top) view. Black bars indicate approx. membrane position.

- Pentameric Ligand-Gated Ion Channel (pLGIC)
- Primarily found in the post-synaptic junction and neuromuscular junction in the human central and peripheral nervous systems
- Implicated in many important neural processes and diseases[1]
- Acetylcholine or nicotine binding to extracellular domain serves as primary gating mechanism
- Highly responsive to lipid environment[2,3]
- Lipids may bind to the transmembrane domain (TMD) and modulate protein function allosterically

- Polyunsaturated fatty acids (PUFAs) are implicated in many neurological processes and diseases[5]
- Voltage clamp recordings indicate addition of PUFAs arachidonic acid (AA) and docosahexanoic acid (DHA) to oocyte membrane causes change in nAChR response (Fig 2, unpublished)
- Mutagenesis experiments (results not shown) suggest polar head group of neutral PUFAs interact with nAChR amino acid residues in outer leaflet

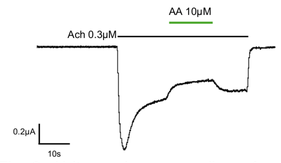
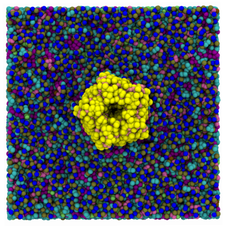


Fig 2: Voltage clamp recording of α-7 nAChR in *xenopus* oocyte after exposure to agonist acetylcholine (Ach, black bar) and candidate modulator arachidonic acid (AA, green bar).

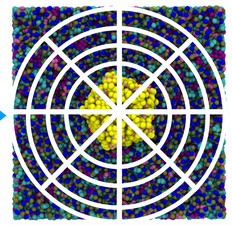
Do lipids 'bind' to the nAChR? If so, where and how strongly?

Do computational predictions correspond with experimental results?

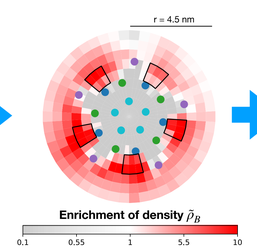
The Density-Threshold Affinity (DTA): Binding affinity from unbiased simulation[6,7]



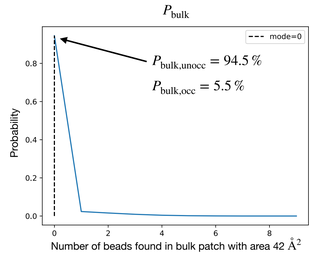
nAChR is embedded in oocyte-mimetic membrane and simulated for 10 μs. First half of trajectory is removed to ensure equilibration.



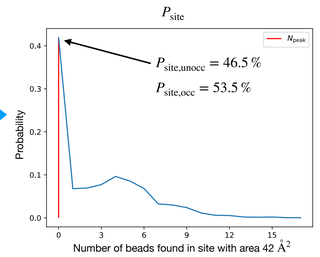
Polar lattice is constructed over membrane surface. Each "bin" will be used for subsequent analysis



Enrichment of lipid B density, $\tilde{\rho}_B$, is calculated for each bin. Lipid 'hotspots' may be observed and putative binding sites selected (black highlighted regions constitute one five-fold symmetric site).



Lipid bead counts from bulk site of equal area to binding site are histogrammed and normalized. Resulting probability distribution used in calculation of ΔG_{ref}. The mode of this distribution serves as the occupancy criterion for the site.



Lipid bead counts in binding site are histogrammed and normalized. Resulting probability distribution is used in calculation of ΔG_{calc}

Simulation Details

- Simulated nAChR in oocyte-mimetic membrane with 5% AA or 5% DHA present (2.5% anionic, 2.5% neutral)
- *Xenopus* oocyte-mimetic membrane composition adapted from Hill et al[6]
- Simulated using the MARTINI forcefield and Gromacs 2024 with GPU support for 10 μs each
- Additional protein-less simulations for bulk estimation used same settings

Bead Density in Bin i,j

$$\rho_B(r_i,\theta_j) = \frac{\langle n_B(r_i,\theta_j)\rangle}{A(r_i,\theta_j)}$$

Enrichment of Density in Bin i,j

$$\tilde{\rho}_B(r_i,\theta_j) = \frac{\rho_B(r_i,\theta_j)}{\langle \rho_B \rangle}$$

Binding Affinity

$$\Delta G_{\text{bind}} = \Delta G_{\text{calc}} - \Delta G_{\text{ref}}$$

Occupancy Criterion

$$N_{\text{peak}} \equiv \text{mode}(P_{\text{bulk}})$$

Uncorrected Site Affinity

$$\Delta G_{\text{calc}} = -RT \ln \frac{P_{\text{occ,site}}}{P_{\text{unocc,site}}}$$

Bulk correction factor

$$\Delta G_{\text{ref}} = -RT \ln \frac{P_{\text{occ,bulk}}}{P_{\text{unocc,bulk}}}$$

$$\Delta G_{\text{bind}} = -RT \left(\ln \frac{0.535}{0.465} - \ln \frac{0.055}{0.945} \right)$$

-1.9 kcal/mol

PUFAs bind M1/M3 outer leaflet site with 1-2 kcal/mol affinity

Enrichment of Density

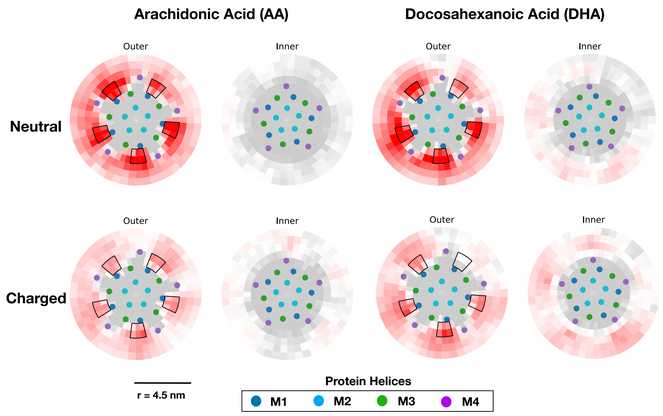


Fig 3: Enrichment of density plots for the outer and inner leaflets of neutral (top row) and charged (bottom row) moieties of AA (left two columns) and DHA (right two columns). Colored dots indicate approximate position of nAChR TMD helices. Black highlighted region indicates the binding site selected for DTA measurement.

Binding Affinities

Table 1: Binding affinities ΔG_{bind} of PUFAs for outer leaflet intersubunit site (black highlighted region, Fig 3). Arranged by PUFA species (columns) and charge state (rows).

	AA	DHA
Neutral	1.7 ± 0.3 kcal/mol	1.9 ± 0.3 kcal/mol
Charged	1.2 ± 0.3 kcal/mol	1.4 ± 0.3 kcal/mol

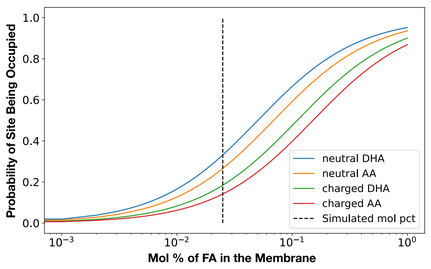


Fig 4: Titration curve of FA moieties as percentage of membrane composition.

Additional Correspondence with Mutagenesis Results

Mutagenesis studies show potential interaction between the polar head groups of neutral PUFAs and three specific amino acid residues: **N214, A275,** and **M253**

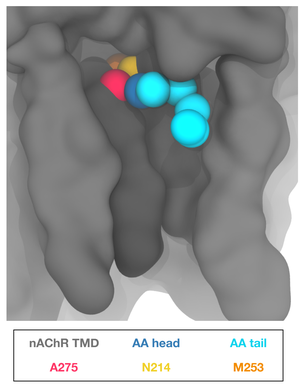


Fig 5: Representative image of the head group of neutral AA interacting with the specific amino acids identified in mutagenesis studies. M253 is not interacting directly with AA, but its side chain is flipped to face N214.

Conclusions

- DHA and AA both bind an intersubunit site of the nAChR
- In both species, neutral FAs have approximately 0.5 kcal/mol higher affinity compared to the charged moiety
- Specific interactions appear to occur between neutral FA head groups and N214, A275, and M253.
- Computational predictions from unbiased coarse-grained MD using the DTA match experimental results from electrophysiology and mutagenesis studies

Next steps

- Extend current simulations to ensure proper convergence
- Run replicas to provide better error estimate
- Compare results to atomistic ΔG_{bind} calculation
- Quantitatively characterize FA interactions with specific amino acids
- Investigate effect of competition (other fatty acids, cholesterol, polyunsaturated phospholipid tails may all wish to occupy the site)
- Investigate effect of cooperativity (not all sites occupied on average)

Measure your own DTA!

Read our protocol paper

Use our code for analysis

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