Investigation of the relative importance of pLGIC sequence versus shape in determining preferred lipid domain

Neuronal signaling relies heavily on transmembrane proteins such as ion channels and receptors, which are embedded in membranes with distinctive lipid compositions. The multitude of polyunsaturated fatty acids (PUFAs) within nAChR native membranes does not necessarily discount them; in fact they may be critical to functionality. Such lipid dependence offers the organism numerous possibilities for lipid based regulation [5].

Separation of cholesterol and saturated phospholipids from unsaturated phospholipids, into liquid-ordered l_o ("raft") and liquid-disordered l_{do} domains respectively, is detected even in simple ternary lipid mixtures. Increasing both cholesterol concentration and acyl chain unsaturation, as in neuronal membranes, increases the propensity of the membrane to form sharply-defined domains relative to other mammalian membranes.

As one example, neurotransmitter receptors must cluster at high density for efficient neurotransmission, and effects of lipid composition on membrane organization may serve an important modulatory role for an organism's ion channel functionality. The high density of nAChR clusters found in the postsynaptic membrane of the mature neuromuscular junction ($10^4 \mu m^{-2}$) is well-established to be stabilized by dimerization of nAChRs via binding of the cytoplasmic peripheral membrane protein rapsyn [9]. This process is also sensitive to membrane composition, particularly cholesterol. It has been frequently hypothesized [8, 2] that initial stages of clustering may require clustering via lipid domains, but experiments investigating whether nAChRs partition into lipid domains have been inconclusive [1, 6].

Such experiments have focused primarily on detecting partitioning into liquid-ordered (l_o) domains, which is often detected differently than partitioning into l_{do} domains; in my preliminary simulations we observe partitioning of nAChRs into the l_{do} domain. Partitioning into l_{do} domains would also cluster receptors and be cholesterol dependent, but would be a less effective mechanism in low cholesterol membranes, such as oocytes.

If partitioning of pLGICs within domain-forming membranes is driven by membrane elasticity and the requirement for a flexible membrane around the cone-shaped protein, partitioning will be strongly sensitive to changes in lipid composition that affect flexibility or spontaneous curvature of l_o and/or l_{do} phases, and only weakly sensitive to pLGIC sequence, since pLGICs are structurally conserved. If partitioning is instead driven by specific interactions with lipids, it will be far more sensitive to pLGIC sequence, particularly the presence of bulky versus small residues in the TMD.

My approach will involve determining the relative partitioning effects of (1) multiple mutations to nAChR M1, M3, and M4 helices, to sequences of other pLGICs , including the GABA(A) receptor, glycine receptor, 5HT-3 receptor, and prokaryotic pLGICs such as GLIC or ELIC. (2) Adjusting relative membrane elasticity parameters by e.g. increasing or decreasing membrane asymmetry or increasing or decreasing chain length.

As an example, comparing the sequence of TMDs for α subunits of neuromuscular nAChR and GABA(A) receptor $\alpha1\beta3\gamma2$ shows $\sim$ 20% identity [3]. Potentially suggesting that GABA(A) may not partition into the l_{do}. Interestingly the initial ternary DPPC:PUFA:CHOL membranes simulations show GABAAR with similar partitioning as nAChR. GABA(A) is currently running in an oocyte composition as a comparison.

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