Electronic Supplementary Material (ESI) for Soft Matter.


**Supporting Information For:**

# Investigating the cut-off effect of *n*-alcohols on lipid movement: a biophysical study

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**Table S1:** Liposomal system conditions, hydrodynamic particle radius, and polydispersity. All measurements were measured at 30 °C using dynamic light scattering.

| System | [a]C# | [b]$X_{alc}$ | [Alc] (mmol/L) | Radius (nm) | Polydispersity % | [c]$t_{mixing}$ |
|---|---|---|---|---|---|---|
| h-DMPC | 0 | 0 | 0 | 70.1 ± 5.9 | 8.4 | 0 |
| d-DMPC | | 0 | 0 | 68.1 ± 4.4 | 6.4 | |
| h-DMPC | 10 | 0.10 | 2 | 61.8 ± 4.7 | 7.6 | |
| d-DMPC | | 0.10 | 2 | 68.0 ± 7.0 | 10.2 | |
| h-DMPC | | 0.30 | 9 | 62.5 ± 4.1 | 6.6 | |
| d-DMPC | | 0.30 | 9 | 63.4 ± 4.0 | 6.4 | |
| h-DMPC | 14 | 0.10 | 2 | 68.5 ± 5.2 | 7.5 | |
| d-DMPC | | 0.10 | 2 | 59.4 ± 4.9 | 8.2 | |
| h-DMPC | | 0.30 | 9 | 72.4 ± 7.5 | 10.4 | |
| d-DMPC | | 0.30 | 9 | 73.1 ± 3.3 | 4.5 | |
| h-DMPC | 18 | 0.10 | 2 | 52.9 ± 7.2 | 13.7 | |
| d-DMPC | | 0.10 | 2 | 57.0 ± 5.3 | 9.3 | |
| h-DMPC | | 0.30 | 9 | 74.1 ± 5.2 | 7.0 | |
| d-DMPC | | 0.30 | 9 | - | - | |
| DMPC | 0 | 0 | 0 | 67.2 ± 9.3 | 13.8 | >3 days |
| | 2 | 0.10 | 2 | 71.2 ± 9.4 | 13.2 | >2 days |
| | | 0.30 | 9 | 71.2 ± 3.9 | 5.5 | |
| | | 0.54 | 25 | 71.4 ± 6.8 | 9.5 | |
| | | 0.83 | 100 | 70.6 ± 6.9 | 9.8 | |
| | | 0.90 | 200 | 70.7 ± 5.6 | 8.0 | |
| | | 0.98 | 855 (5 % v/v) | 71.0 ± 2.3 | 3.2 | |
| | 6 | 0.1 | 2 | 72.7 ± 7.1 | 6.3 | |
| | | 0.3 | 9 | 71.9 ± 5.8 | 8.0 | |
| | 10 | 0.1 | 2 | 50.1 ± 6.0 | 12.0 | |
| | | 0.3 | 9 | 57.6 ± 5.6 | 9.7 | |
| | 14 | 0.1 | 2 | 67.6 ± 5.8 | 8.6 | |
| | | 0.3 | 9 | 70.2 ± 7.8 | 11.1 | |
| | 18 | 0.1 | 2 | 45.8 ± 5.6 | 12.2 | |
| | | 0.3 | 9 | 70.2 ± 8.4 | 12.0 | |

[a]alcohol carbon chain length

[b]alcohol mole fraction to the lipid bilayer total

[c]time after mixing of the two isotopic DMPC populations: h-DMPC and d-DMPC

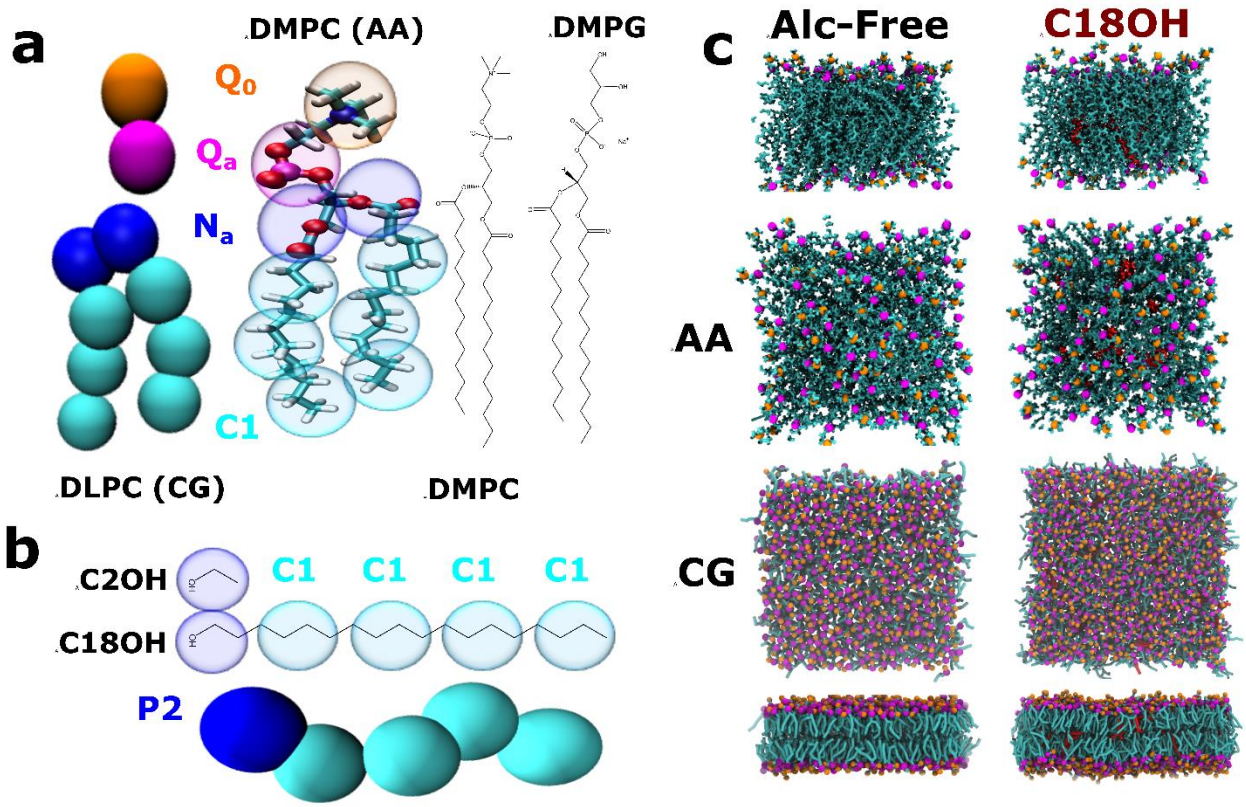
**Table S2:** Molecular make-up and simulation details of the alcohol-free and alcohol-doped systems.

| **All-Atomistic (AA)** | [a]**C#** | [b]$X_{alc}$ | **# of Alcohols** | **# of DMPC** | **# of DMPG** | [c]**# of TIP3** | [e]**t (ns)** |
|---|---|---|---|---|---|---|---|
| Alcohol-Free DMPC | 0 | 0 | 0 | 114 | 6 | 7800 | 500 |
| + C2OH | 2 | 0.05 | 6 | | | | |
| | | 0.1 | 14 | | | | |
| + C6OH | 6 | 0.05 | 6 | | | | |
| | | 0.1 | 14 | | | | |
| + C10OH | 10 | 0.05 | 6 | | | | |
| | | 0.1 | 14 | | | | |
| + C14OH | 14 | 0.05 | 6 | | | | |
| | | 0.1 | 14 | | | | |
| + C18OH | 18 | 0.05 | 6 | | | | |
| | | 0.1 | 14 | | | | |
| **Coarse-Grained (CG)** | | | | | | [d]**# of W** | **t (μs)** |
| Alcohol-Free DMPC | 0 | 0 | 0 | 486 | 26 | 16110 | 2.0 |
| + C2OH | 2 | 0.1 | 57 | | | 16099 | 2.0 |
| + C6OH | 6 | | | | | 16079 | 2.0 |
| + C10OH | 10 | | | | | 16066 | 7.0 |
| + C14OH | 14 | | | | | 16039 | 2.3 |
| + C18OH | 18 | | | | | 16031 | 3.3 |

[a]alcohol carbon chain length
[b]alcohol mole fraction to the lipid bilayer total
[c]AA bulk-phase was ionized with 25 sodium and 19 chlorine ions - extra 6 sodiums to neutralize the negative DMPG headgroup charge
[d]CG bulk-phase was ionized with 209 sodium and 183 chlorine ions - extra 26 sodiums to neutralize the negative DLPG (DMPG) headgroup charge
[e]total time for the simulation production run

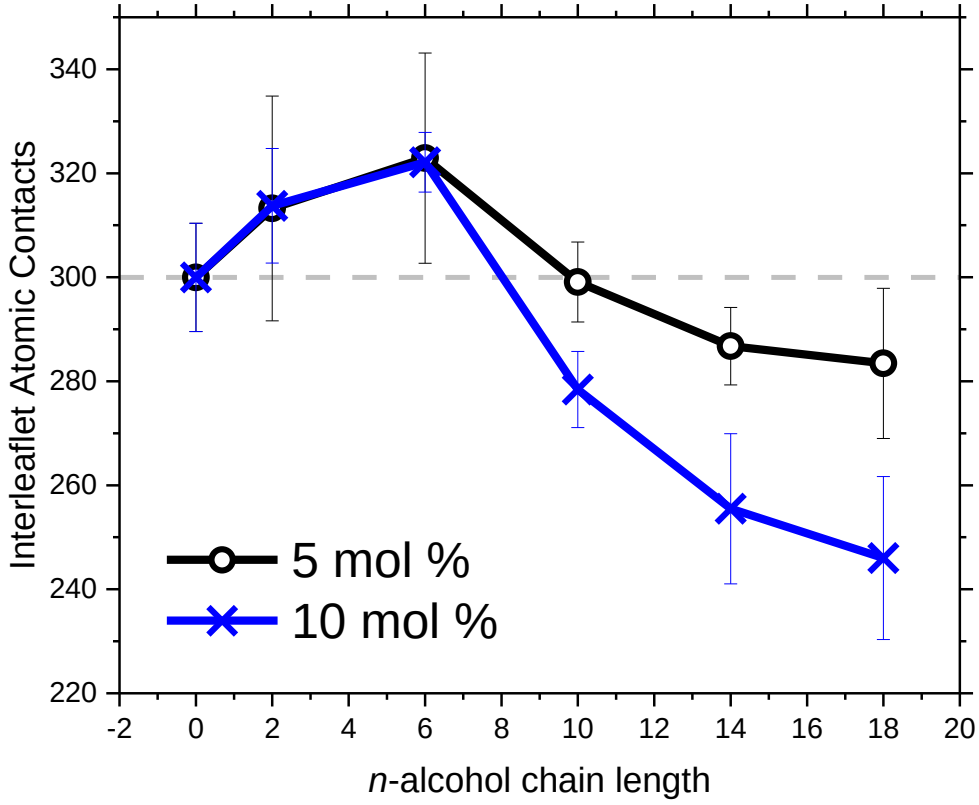
All simulations were conducted at 310 K to mirror the experimental data and simulate physiological temperature. CG simulations were conducted until all alcohol molecules were embedded within the bilayer for at least 1 μs, save for ethanol, C2OH, as it favors the bulk-phase.

**Figure S1:** Chemical structures and illustrations of the DMPC (or DLPC, same as DMPC, in CG) simulation systems.



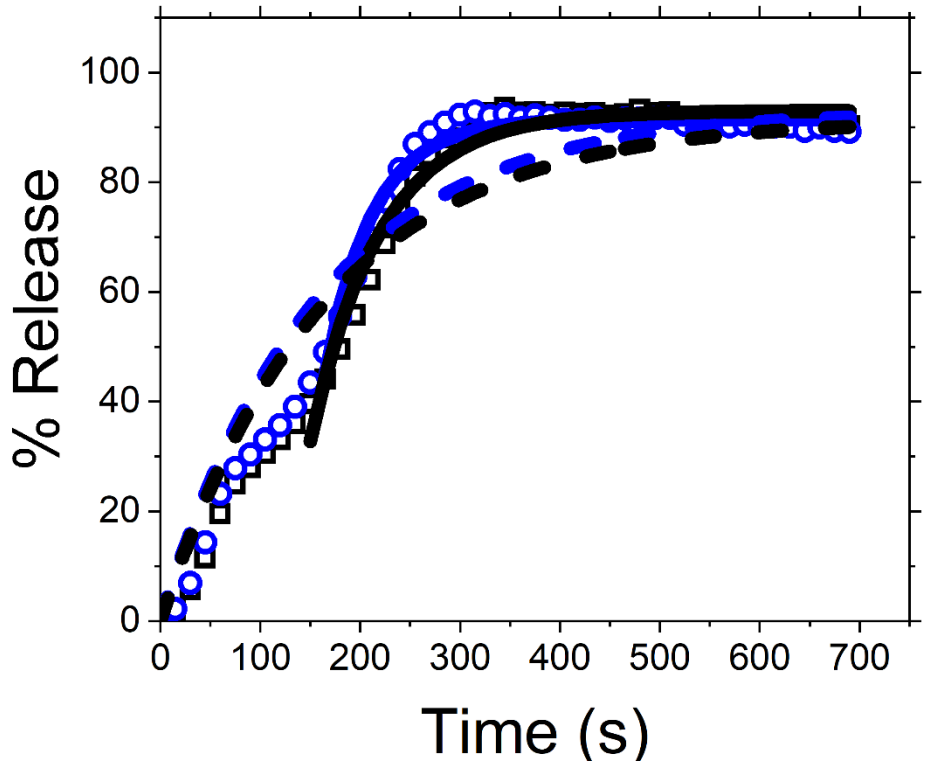

a) The bead mapping that is required for CG simulations is shown as transparent beads transposed onto the AA DMPC structure. Roughly 3-4 large atoms are replaced by these single beads to yield the structure seen on the left of (a). Alcohol chemical structures are shown in (b) and the corresponding coarse grained C18OH molecule below. P2 and C1 were chosen to represent these atomic groupings. Final frame snapshots of the side and top view of alcohol-free and C18OH-doped systems (AA, top; CG, bottom) are shown in (c). Phosphate/phosphorus atoms (magenta), choline/nitrogen atoms (orange), C18OH molecules (red) and the remaining lipid components (teal) are visually represented for clarity.

**Figure S2:** The number of interleaflet atomic contacts as a function of *n*-alcohol carbon chain length to measure the degree of leaflet interdigitation.




*n*-Alcohols at 5 mol % (black open circles) and 10 mol % (blue crosses) were added to neat molecular systems composed of DMPC and 5 mol % DMPG. The number of interleaflet atomic contacts was calculated using MEMBPLUGIN[1]. The cut-off distance required for a contact was set to 4 Å. Grey dashed line represents the alcohol-free control: above the dashed line signifies increased interdigitation, while below the dashed line represents fewer interdigitation events.

**Figure S3:** Percent calcein leakage from DMPC vesicles under ethanolic conditions.




Percent calcein release from DMPC vesicles in the presence of 9 mmol/L (black) and 200 mmol/L (blue) C2OH measured at 37˚C. The calculated % $RF^T$ are shown as open symbols. Fitting the whole calculated % $RF^T$ curve with a single exponential (Equation 7 in the main text) yields the ill-fitted dashed lines. Omitting the initial process, i.e. the first humped centred at 100 s, yields a decent fit but potentially fails to capture the true leakage rate.

**References**:

[1] Guixà-González, R., Rodriguez-Espigares, I., Ramírez-Anguita, J. M., Carrió-Gaspar, P., Martinez-Seara, H., Giorgino, T., & Selent, J. (2014). MEMBPLUGIN: studying membrane complexity in VMD. *Bioinformatics*, *30*(10), 1478–1480.