SARS-CoV-2 E Protein Induces Asymmetric Hydrophobic Mismatch on Surrounding Membrane

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Background & Approach

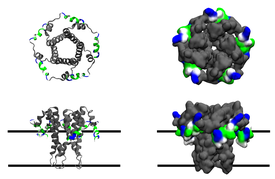


Fig 1: The E protein of SARS-CoV (pdb id 5x29) shown in extracellular (top) and membrane/lateral (bottom) view. Black bars indicate approx. membrane position.

- Pentameric, weakly-selective ion channel
- Highly conserved across many coronaviruses, including SARS-CoV and SARS-CoV-2[1]
- Primarily found in the ER-Golgi Intermediate Complex (ERGIC) of infected host cells
- Known to induce membrane curvature, allowing a new virion to bud out and escape the host cell[2]
- Knock-out or mutation produces weakened virions that are unable to infect new cells[3]
- Key role plus high conservation makes the E protein an important target for further study

- Coarse-grained Molecular Dynamics (CG-MD) simulations confirm that E protein bends membranes, but the precise mechanism is unknown
- In order to understand the global bending effect, we sought first to explore the *local* effect E protein has on membrane leaflets
- The following investigation focusses solely on saturated lipids, as they bend less than unsaturated lipids and therefore offer a more tractable first step in our study

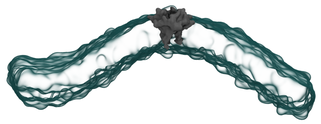


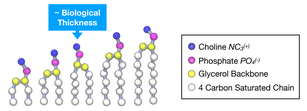
Fig 2: Still from MD trajectory of E protein (grey, center) embedded in 100% POPC membrane (cyan, transparent).

Ultimate Aim: How does the E protein bend membranes?

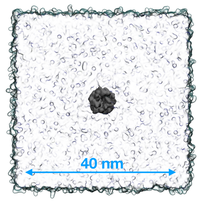
First Milestone: How do lipids behave near the E protein?

Methods & Analysis

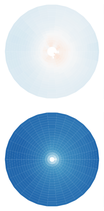
- Five coarse-grain molecular dynamics (CG-MD) simulations
- Each system has 100% composition of PC lipid with different equilibrium thickness
- E protein is embedded in large (40 nm x 40 nm) membrane to control for finite size effects
- Lipid number asymmetry is present, but kept constant across simulations



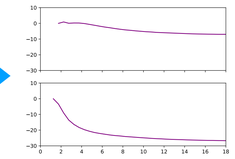
Saturated lipids with PC head groups in Martini 2 representation



The E protein is embedded in a membrane and simulated for 2 μs using the Martini 2 forcefield and Gromacs 2016



Analysis suite *nougat* bins and averages membrane quantities of interest over the course of the trajectory for both leaflets



Output from *nougat* is averaged across the θ dimension to produce 1d plots of the quantity of interest - in this case membrane height - as a function of distance from the protein

Large Deviations in Elastic Terms Local to Protein

Fig 4a: Average mean curvature (*H*) of outer leaflet shows non-zero values close the interface between protein and membrane. It also shows slightly positive curvature at points distant from the protein - indicating the membrane is bending downward slightly.

Fig 4b: Average mean curvature (*H*) of inner leaflet shows non-zero values close the interface between protein and membrane. It also shows correlation between lipid length and degree of curvature close to the interface.

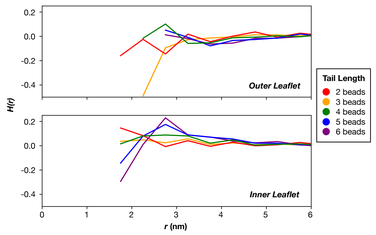
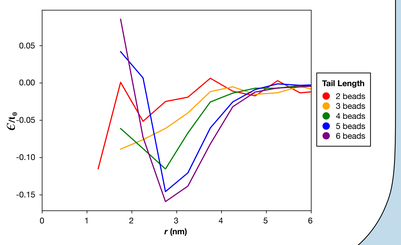
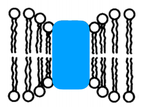


Fig 5: Average normalized leaflet asymmetry (ϵ/t_0) shifts drastically away from zero as the membrane approaches the protein. There is also a strong correlation between lipid length and degree of asymmetry.



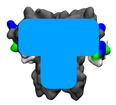
Membrane Bending 101



Classical hydrophobic mismatch occurs when the hydrophobic surface of the protein differs from the equilibrium thickness of the membrane, causing inward or outward symmetrical bending[4].

Figure adapted from: Mouritsen & Bloom, Biophysical Journal, 1984

Membrane bending also occurs in the presence of a wedge-shaped inclusion because the membrane will align to intersect the inclusion at a 90 degree angle. This is known as a contact angle mechanism.



"Which one am I?"

Potential Test: What if we altered membrane equilibrium thickness?

If hydrophobic mismatch:

Varying thickness should vary the level of mismatch; we would expect membrane properties to vary as a function of thickness

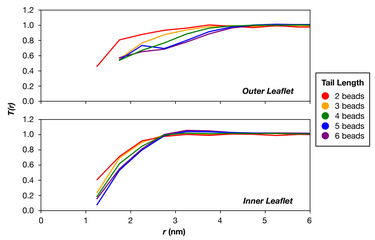
If contact angle:

Varying thickness should have no effect on the contact angle exerted on the membrane; we would expect membrane properties to be invariant

Asymmetric Thickness Deformations

Fig 3a: Average normalized thickness *T(r)* of outer leaflet shows lipids are compressed to approximately half their equilibrium thickness close to the protein. It also shows correlation between lipid length and ability to 'heal' back to equilibrium thickness.

Fig 3b: Average normalized thickness *T(r)* of inner leaflet shows severe leaflet compression close to the protein. Values show slight correlation with lipid length and are not identical to outer leaflet values - invalidating a common assumption in elastic theories of bending energy.



$$f_z = \frac{1}{2}K_C(H^-)^2 + 2K_C c_0 H^- + \frac{2K_C\zeta z^- H^-}{t_0} + \frac{K_A(z^-)^2}{2t_0^2} + \frac{1}{2}K_C(H^+)^2 + \boxed{\frac{2K_C\epsilon\zeta H^+}{t_0} + \frac{K_A\epsilon^2}{2t_0^2}}$$

To account for the asymmetric thickness deviations close to the protein, we revised an earlier expression [5] for the bending energy of the membrane to allow for local leaflet asymmetry.

This new Hamiltonian adds two terms that contain an explicit measure of local leaflet asymmetry: ϵ.

The penultimate term couples curvature (H+) and leaflet asymmetry together, theoretically allowing for the type of unidirectional membrane produced by the E protein.

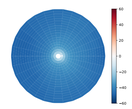
Conclusions

- Simulation series show clear variation of membrane properties as a function of equilibrium thickness
- This suggests that hydrophobic mismatch is present in the system
- Despite this we have not been able to eliminate bending altogether, suggesting other factors may be present that need to be elucidated
- Local leaflet thickness symmetry assumption not supported in systems with asymmetric protein inclusions
- Preliminary results from continuum simulations (not shown) using new Hamiltonian match bending profile from MD simulations, indicating local leaflet asymmetry may have outsize effect on membrane

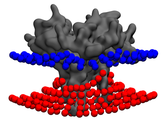
Next Steps

- Investigate effect of lipid asymmetry on bending
- Include degrees of unsaturation
- Explore lipid recruitment by E protein (tail length, head group)
- Run further continuum simulations with new Hamiltonian to compare agains MD results
- Further characterize lipid ordering at interface with protein
- Understand how local lipid effects create global bending pattern seen in simulations

Try *nougat*!



Heat maps in polar and cartesian coordinates



Average surface pdb file generated for direct visualization in VMD

github.com/BranniganLab/nougat

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