# SARS-CoV-2 E Protein Induces Asymmetric Hydrophobic Mismatch in Saturated Membranes

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## Background & Approach

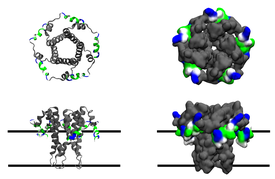


**Fig 1:** The E protein of SARS-CoV (pdb id 5x29) shown in extracellular (top) and membrane/lateral (bottom) view. Black bars indicate approx. membrane position.

- 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

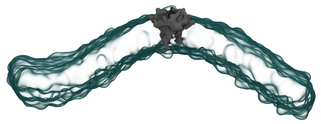
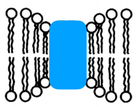


**Fig 2:** Still from MD trajectory of E protein (grey, center) embedded in 100% POPC membrane (cyan, transparent).

**Ultimate Aim: How does the E protein bend membranes?**

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

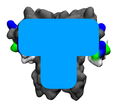
## Membrane Bending 101



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:*

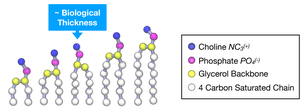
Varying thickness should vary the level of mismatch; we would expect membrane properties to vary as a function of thickness

*If contact angle:*

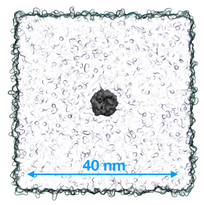
Varying thickness should have no effect on the contact angle exerted on the membrane; we would expect membrane properties to be invariant

## Methods & Analysis

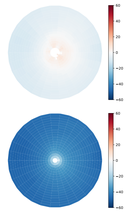
- Five coarse-grain molecular dynamics (CG-MD) simulations
- Each system has 100% composition of PC lipid with different equilibrium thickness
- E protein is embedded in large (40 nm x 40 nm) membrane to control for finite size effects
- Lipid number asymmetry is present, but kept constant across simulations



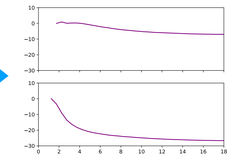

Saturated lipids with PC head groups in Martini 2 representation




The E protein is embedded in a membrane and simulated for 2 $\mu s$ 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 $\theta$ dimension to produce 1d plots of the quantity of interest - in this case membrane height - as a function of distance from the protein

## Asymmetric Thickness Deformations

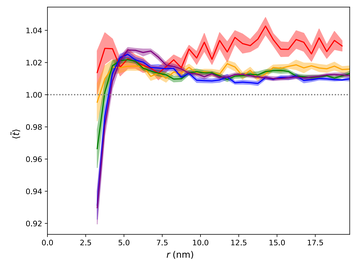



**Fig 3a:** Average normalized thickness $\tilde{t}$ shows lipids are compressed close to the protein.

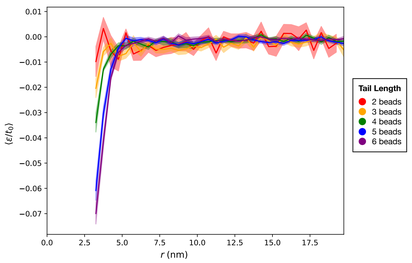



**Fig 3b:** Average normalized leaflet asymmetry $\varepsilon/t_0$ shows that leaflets are differentially compressed close to the protein.

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

This new expression adds two terms that contain an explicit measure of local leaflet asymmetry: $\epsilon$.

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

## Large Deviations in Elastic Terms Local to Protein

**Fig 4:** Average normalized mean curvature $(\tilde{H}^+)^2$ shows larger curvature values close the interface between protein and membrane. It also shows that as membranes grow thinner they experience higher curvature in the bulk.

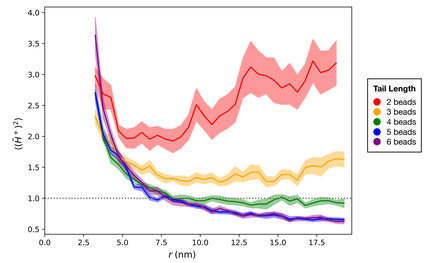
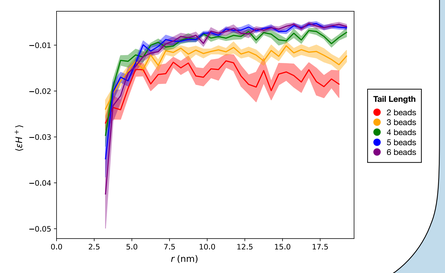



**Fig 5:** Average leaflet asymmetry times mean curvature ($\epsilon H^+$) is negative everywhere, especially where there is high curvature (see Fig. 4). This shows that areas with high curvature are energetically compensated by the combination of curvature and leaflet asymmetry.



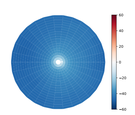

## Conclusions

- 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

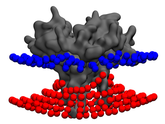
## Next Steps

- 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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CAREERS Cyberteam

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