# Biophysical Study of the Dependence of Fusion of Dengue Virus with Host Membranes on Lipid Composition

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## Background

This work focuses on the dependence of fusion on membrane composition for Dengue (DEN) virus. Recently it has been reported that full fusion only occurs with membranes containing anionic lipids, i.e. late endosomes.[1] The reason for this strong dependence on annionic lipids is currently unknown. Regarding cholesterol, different conclusions have been reported.[2-4] We used neutron reflectivity and a quartz crystal microbalance (QCM) to study the depth of insertion of E into lipid membranes of different composition, the relative binding affinities, and the reversibility of the interaction.

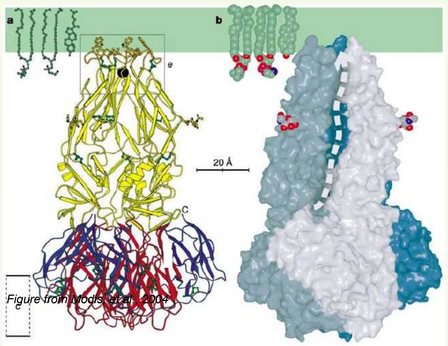



Figure from Modis, et al., 2004

*E Trimer Structure*

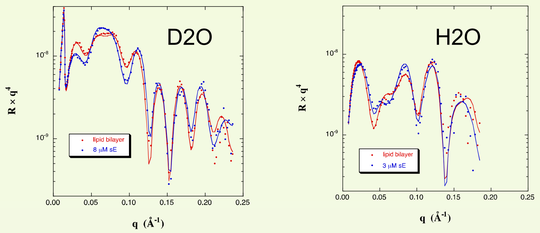
## Objectives

- *Determine if the depth of insertion varies with mambrane composition*
- *Determine the relative importance of negatively charged lipids and cholesterol on E protein-membrane binding*
- *Compare virus-like particle (VLP)-liposome fusion for liposomes containing negatively-charged lipids oversuss neutral lipids with choleestrol*

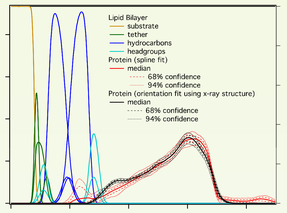
## Results and Discussion

### *Neutron Reflectometry*

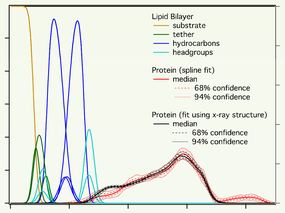
49:21:33 PC:PE:Chol (8 μM sE)




49:21:33 PC:PE:Chol (8 μM sE)




70:30 PC:PG (3 μM sE)

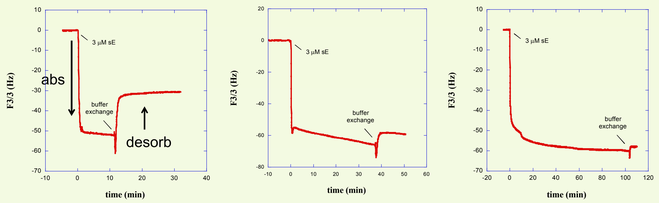



*These data show that the final state of the inserted protein is very similar for the two membrane compositions. E lis inserted to a depth such that the fusion loop coincides with the interface between the lipid headgroups and the hydrophobic tails. The tip-inserted protein is tilted with respect to the membrane on average by 30° in each case.*

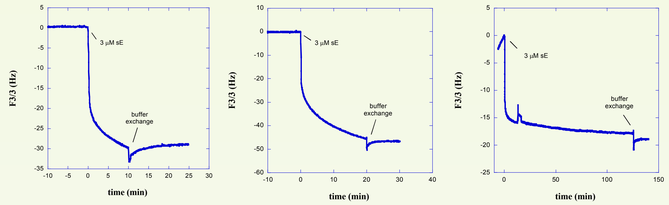
## Results and Discussion

### *QCM*

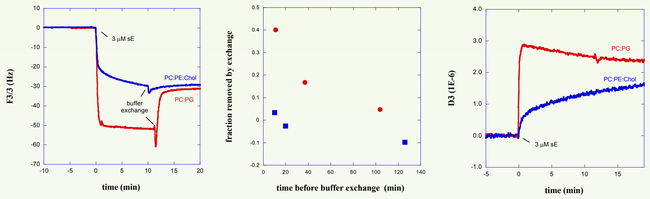
70:30 PC:PG



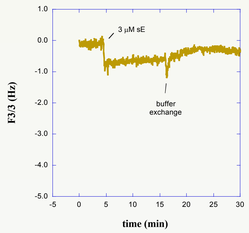

49:21:33 PC:PE:Chol




Comparing 70:30 PC:PG with 49:21:33 PC:PE:Chol

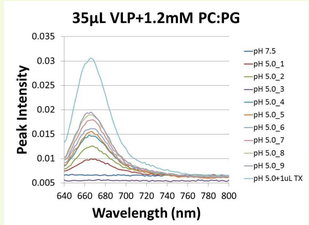


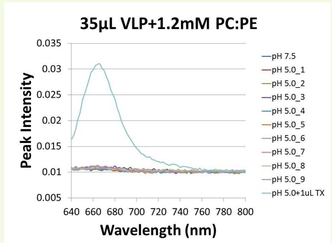

70:30 PC:PE




*The initial affinity of E for the membrane is stronger for PC:PG (due to electrostatic interactions) than for PC:PE:Chol. E has very little affinity for PC:PE. For PC:PG there is a slow process leading to strong anchoring into the membrane, whereas for PC:PE:Chol strong anchoring occurs much more rapidly. We propose that strong electrostatic interactions trap E in a metastable partially inserted adsorbed state such that the transition to the final state occurs more slowly than for*

## VLP-liposome fusion




*VLPs labeled with a self-quenching conc. of DiD emitted an increasing fluorescence signal over time when mixed with PC:PG liposomes, but not PC:PE liposomes. Fusion studies for PC:PE:Chol are in progress.*

## Conclusions

- *In the final state, E inserts to nearly the same position within the membrane for 70:30 PC:PG and 49:21:33 PC:PE:Chol.*
- *The absorption affinity of E with the membrane is in the following order: 70:30 PC:PG > 49:21:33 PC:PE:Chol >> 70:30 PC:PE*
- *E anchors irreversibly to both 70:30 PC:PG and 49:21:33 PC:PE:Chol*
- *For PC:PG, E binds rapidly but reversibly, and slowly undergoes a transition to a strongly anchored state.*

## References

(1) Zaitseva et al PLoS Pathog 2010, 6, e1001131
(2) Stiasny et al J. Virol. 2003, 77, 7856.
(3) Lee, et al J. of Virol. 2008, 82, 6470.
(4) Umashankar et al J. of Virol. 2008, 82, 9245

## Acknowledgement

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