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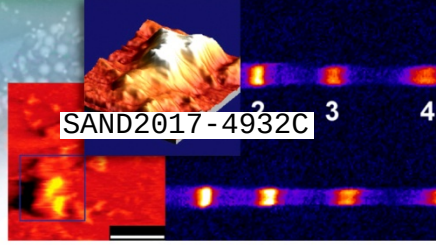
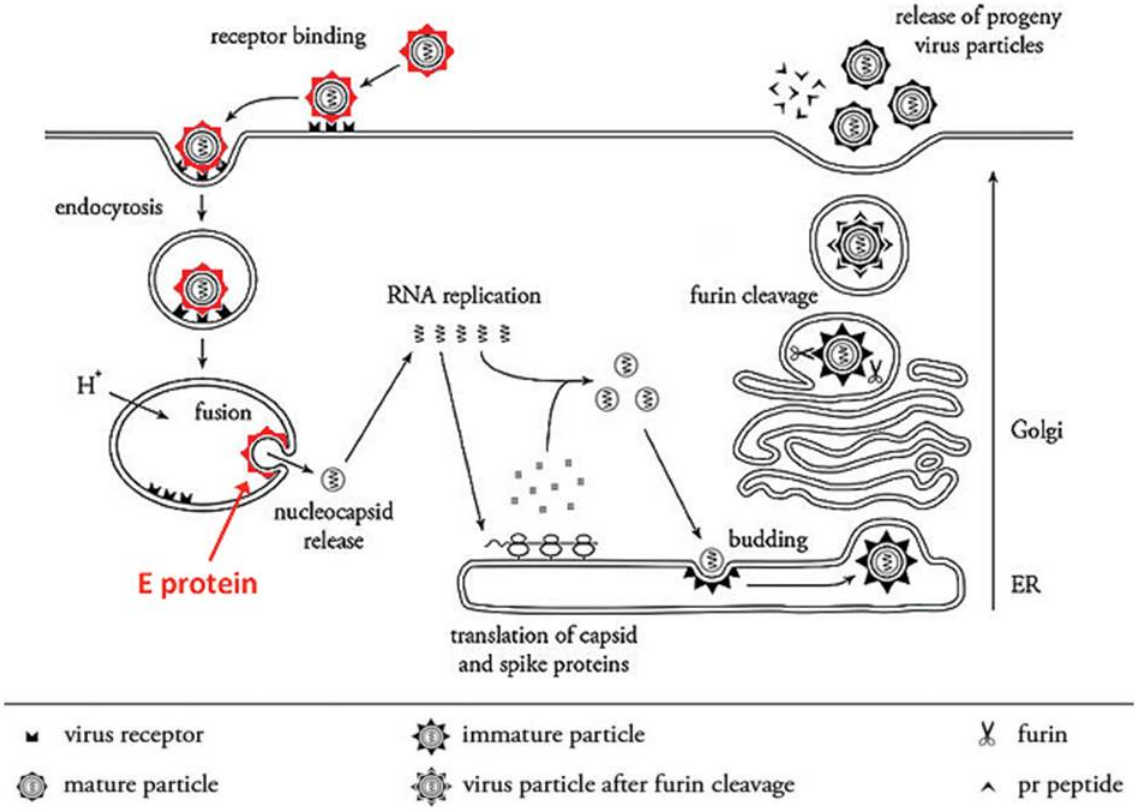


Study of Insertion of Dengue E into Lipid Bilayers by Neutron Reflectivity and Molecular Dynamics Simulations

Life cycle of Dengue Virus



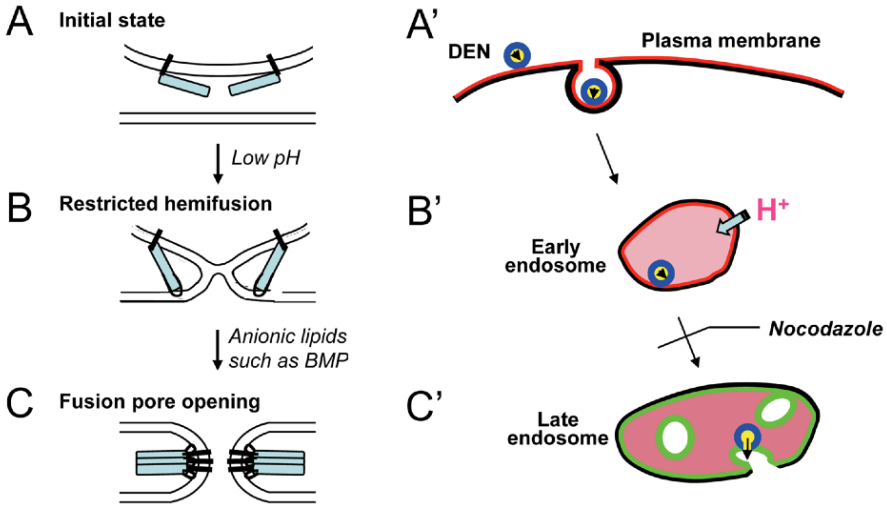
Fusion is critical to infectivity

Fusion highly dependent on lipid composition

Anionic lipids (AL) versus cholesterol:

Different concl. reported for chol.

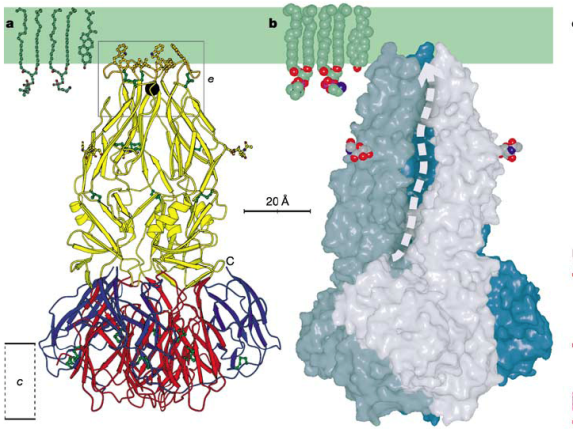
Chernomordik: Fusion only occurs with AL!



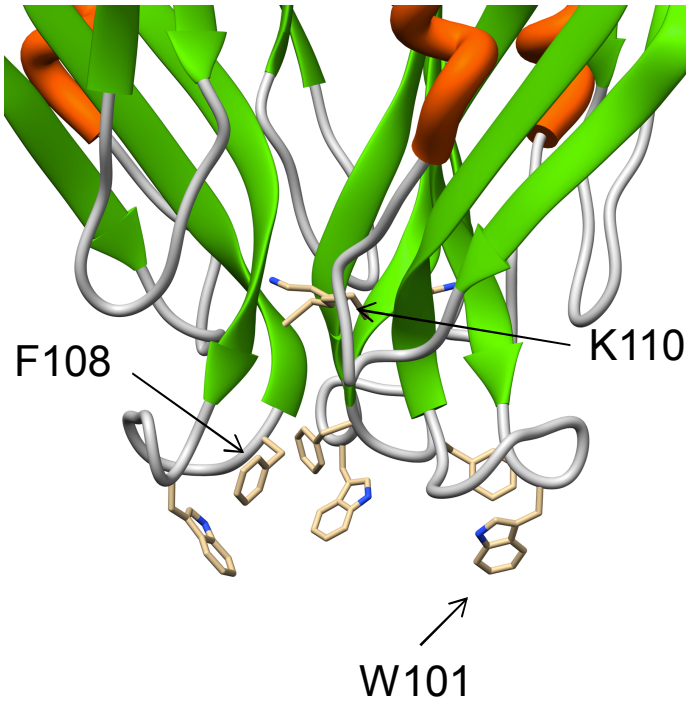
(Chernomordik et al PLoS PATHOGENS 2010)

Hypothesis: AL required for a) insertion of fusion loop
b) trimerization of E
c) alleviating restricted hemifusion

Fusion loop is critical



Fusion loop: residues 97-110



Residues W101, F108, K110 of fusion loop are conserved among 33 different flavivirsues

Mutational studies: severe restrictions for aa at 102, 104, 106, also W101F abolishes fusion

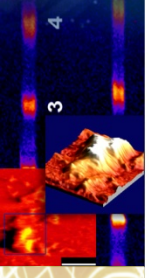
But no one knows why! anchoring?

modeling indicates no!

Outline

1. Insertion of E into membranes (anionic lipids, cholesterol) by neutron reflectivity

2. Insertion of E into membranes by molecular dynamics simulations



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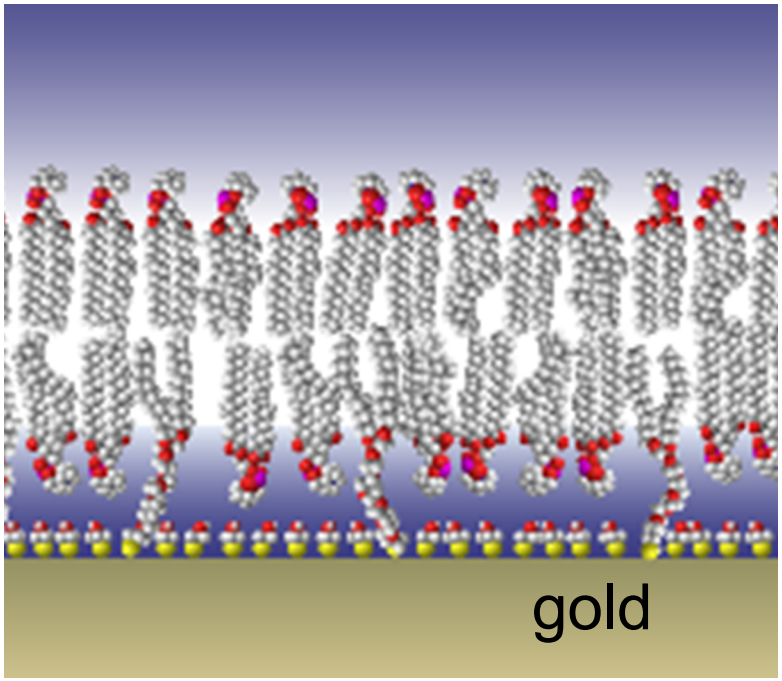
Insertion of Dengue sE by NR

Tethered planar lipid bilayers



SAM of Thiol-$(EO)_x$-$(C_{14+x})_2$ tether with β−mercaptoethanol

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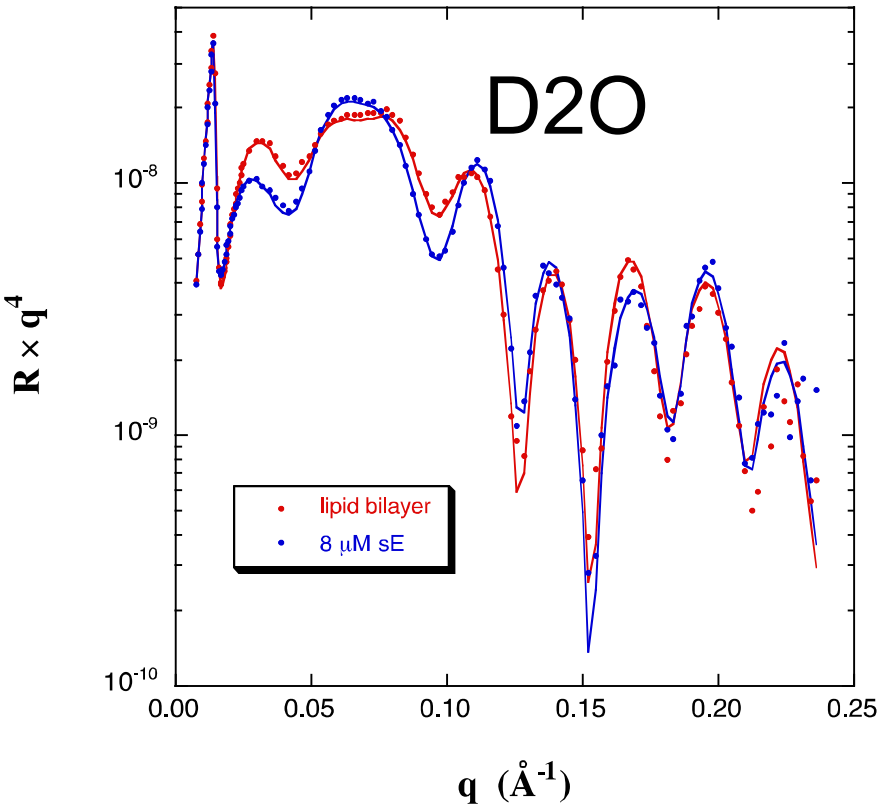
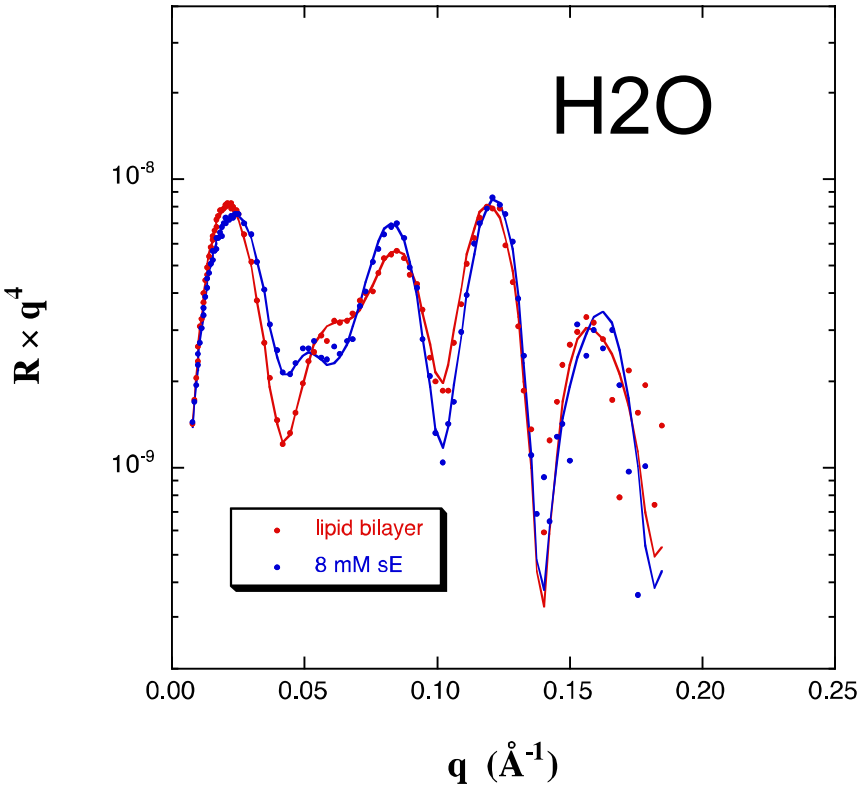
Insertion of Dengue sE by NR

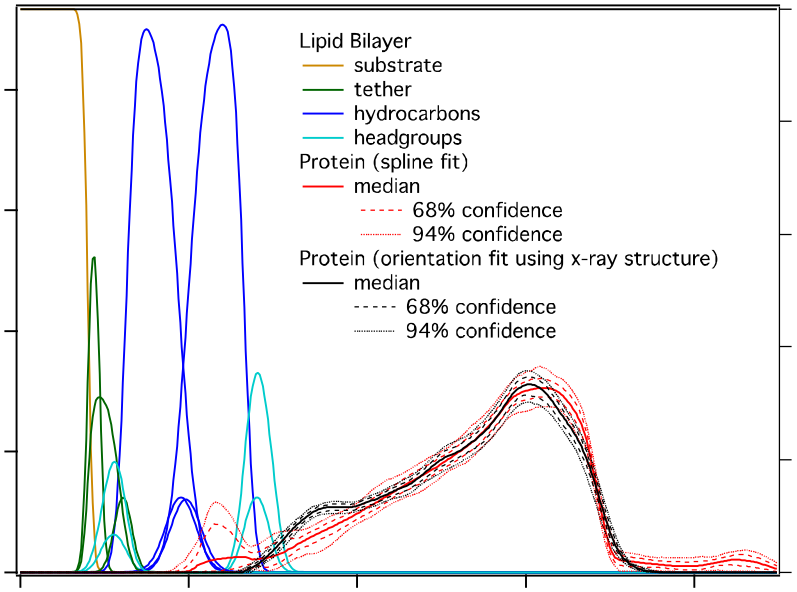
49:21:33 PC:PE:Chol (8 μM sE, pH 5.5)

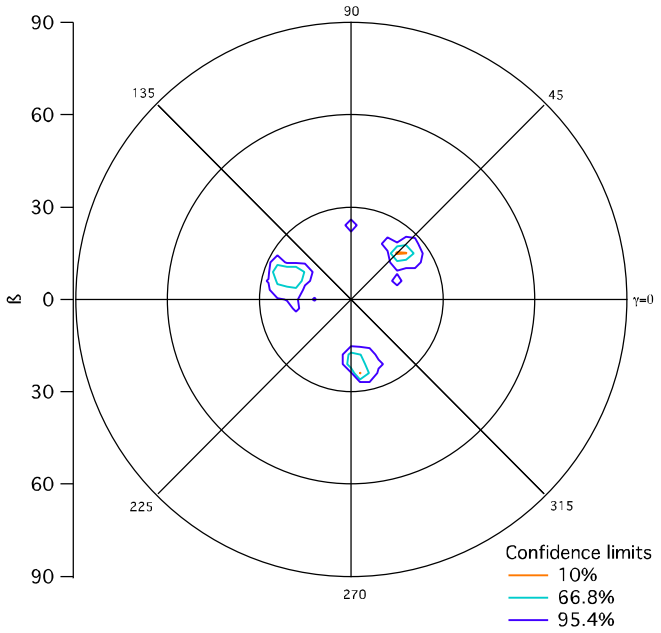




Insertion of Dengue sE by NR

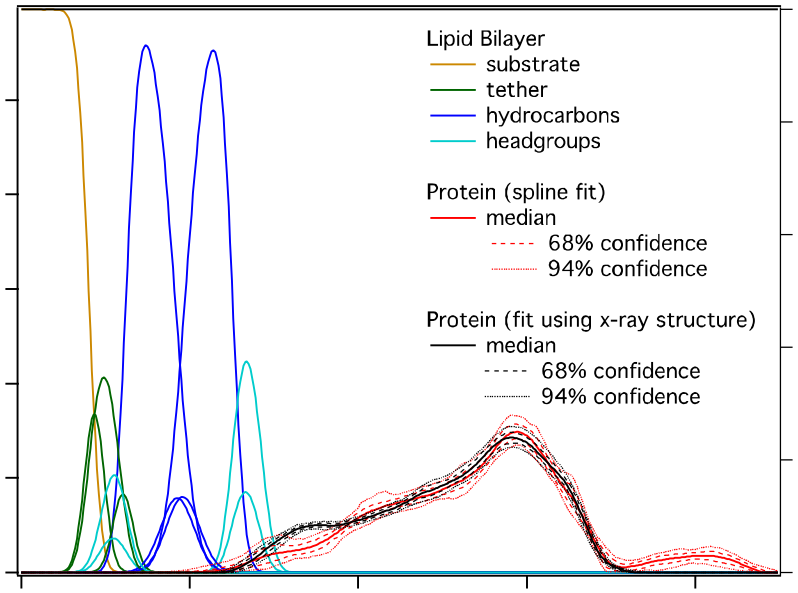
49:21:33 PC:PE:Chol (8 μM sE, pH 5.5)

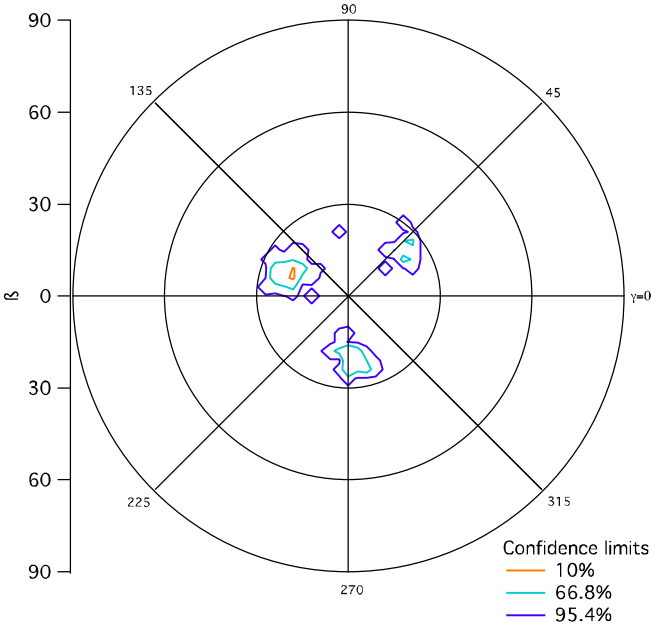




Insertion of Dengue sE by NR

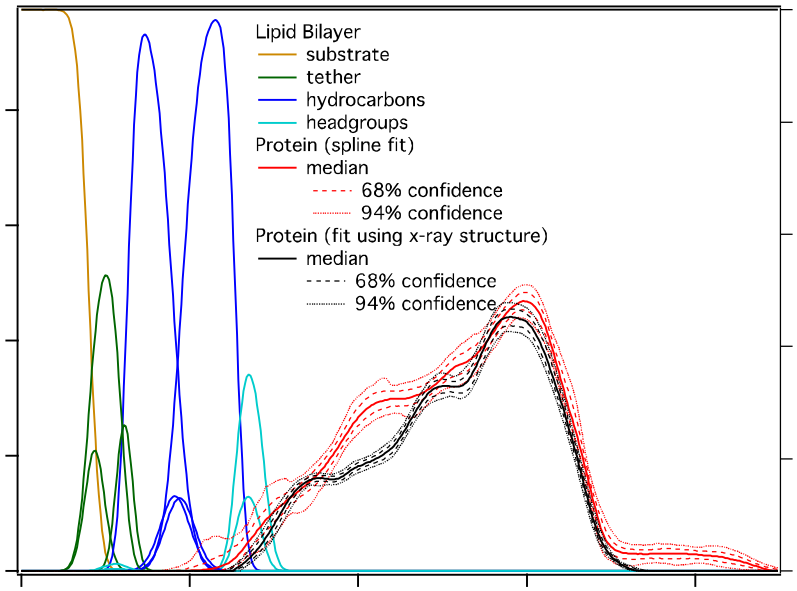
70:30 PC:PG (3 μM sE, pH 5.5)

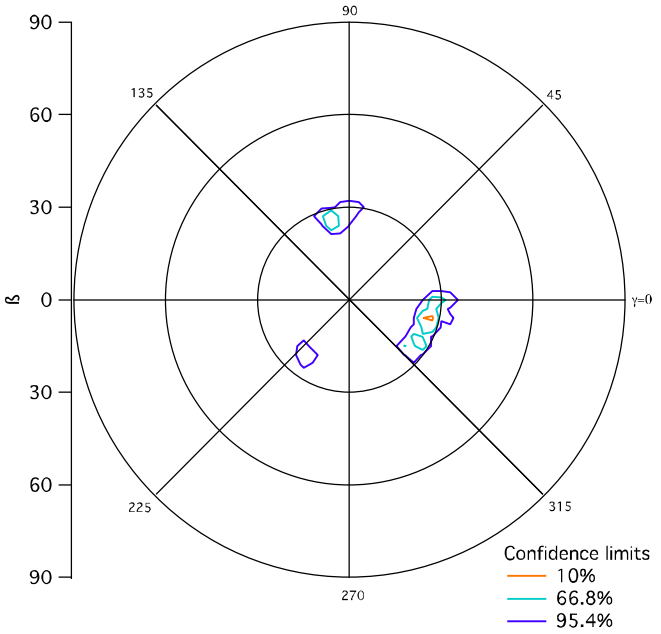




Insertion of Dengue sE by NR

70:30 PC:PG (3 μM sE, pH 5.5)





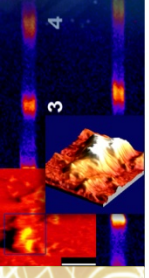
Summary of study by NR

Similar results for the three membranes:

1. sE inserts such that FL coincides with the interface between lipid headgroups and hydrophobic tails

2. The tip-inserted protein is tilted with respect to the membrane up to 30^{o}

3. Little increase in the roughness values upon binding



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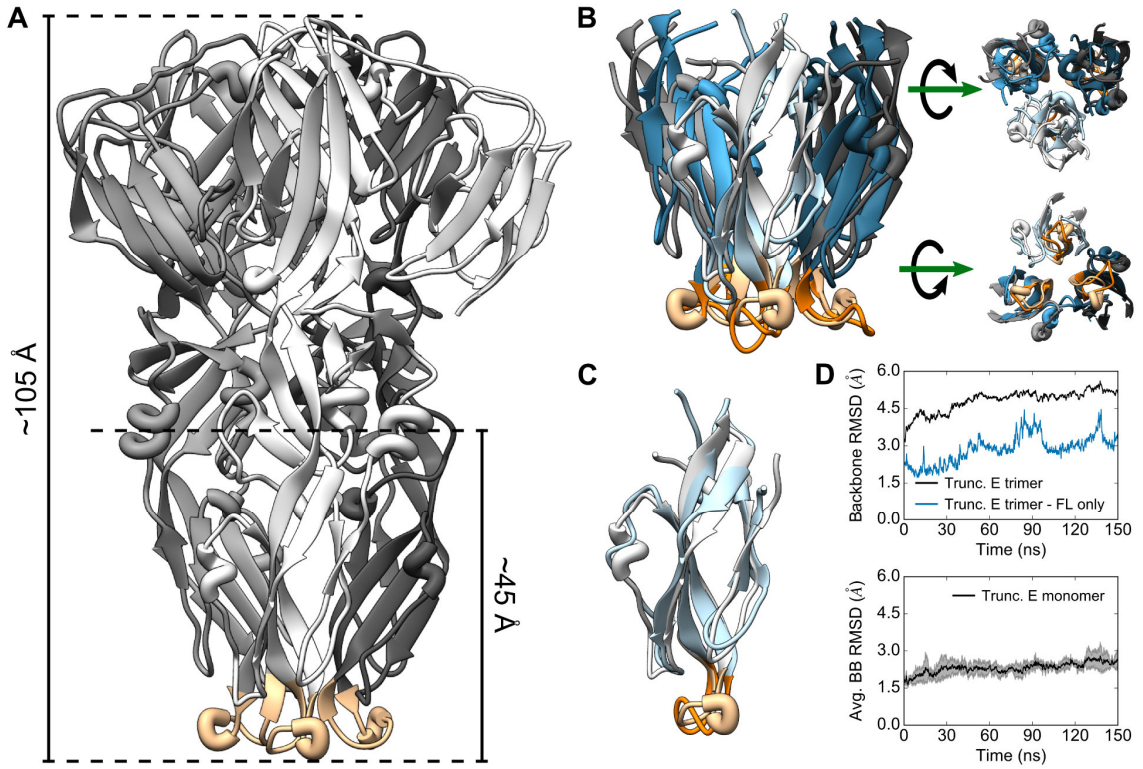
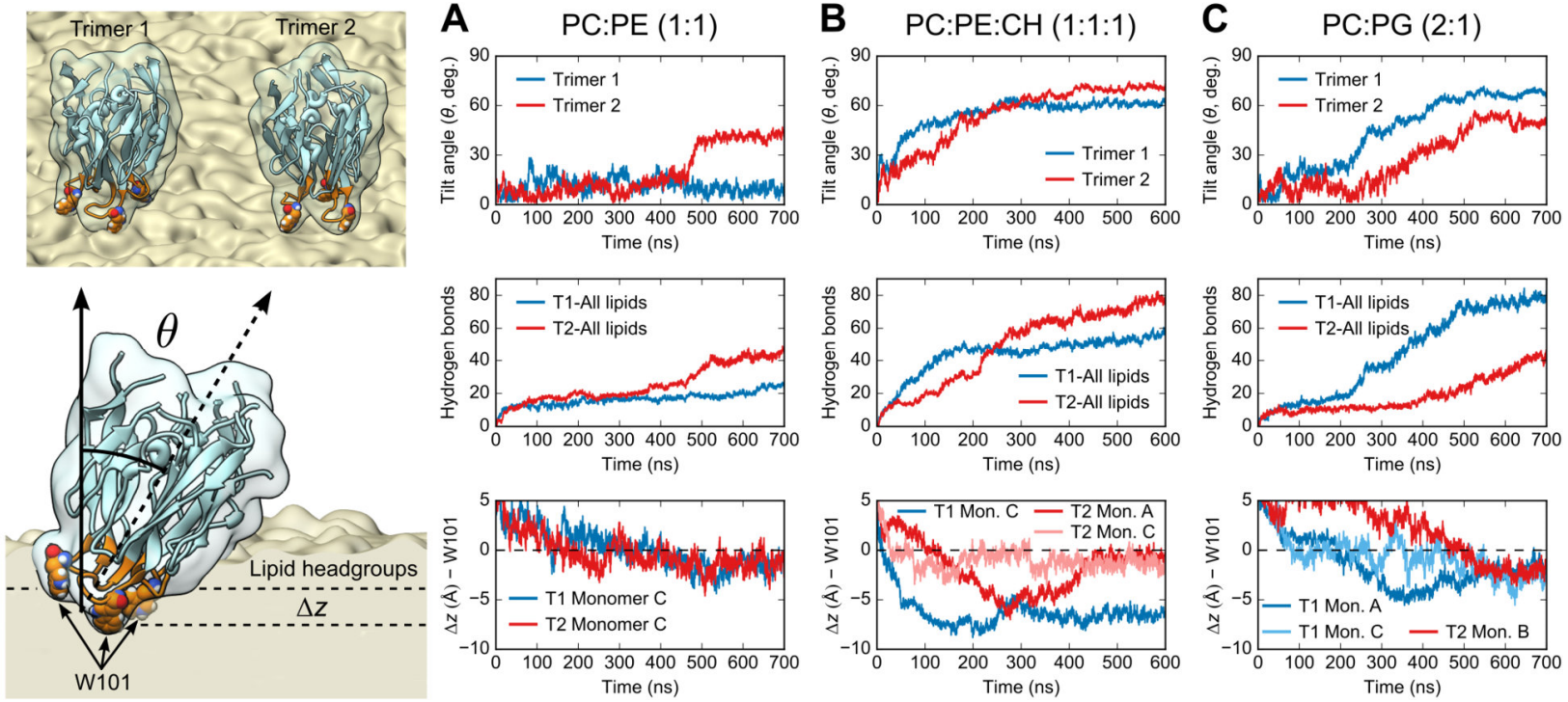
MD simulations

Truncated model for the soluble E trimer which retains the important structural features of the full trimer at the tip region, with only a fraction of the computational cost

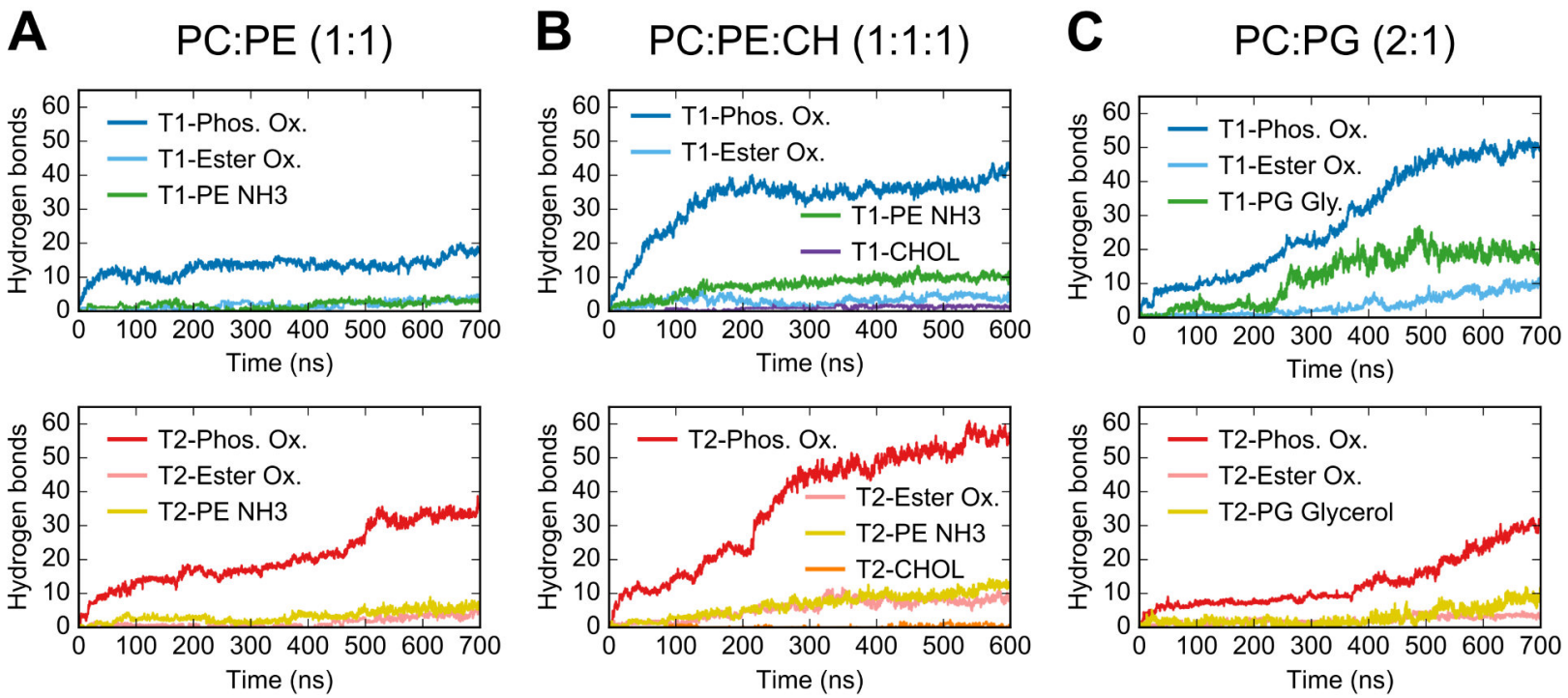


Results – MD simulations



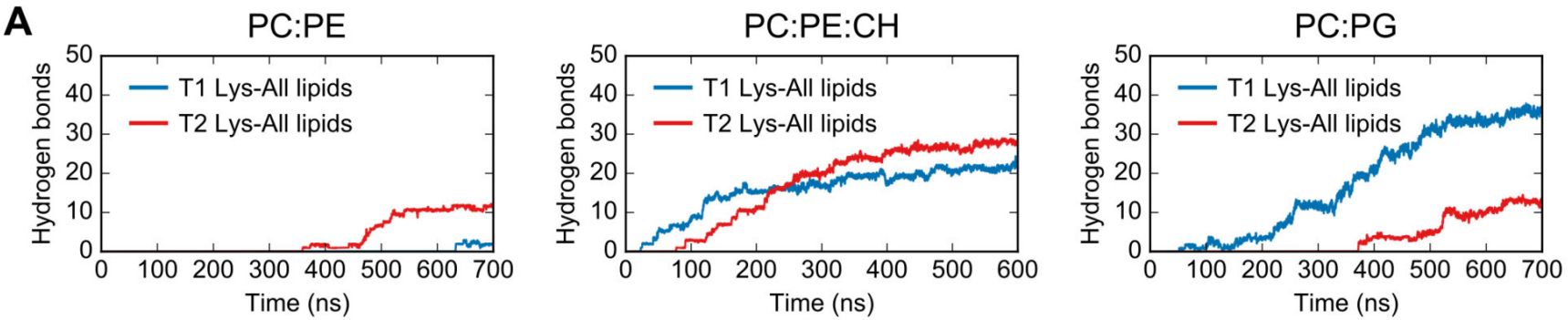
H-bond formation and protein is correlated

Results – MD simulations

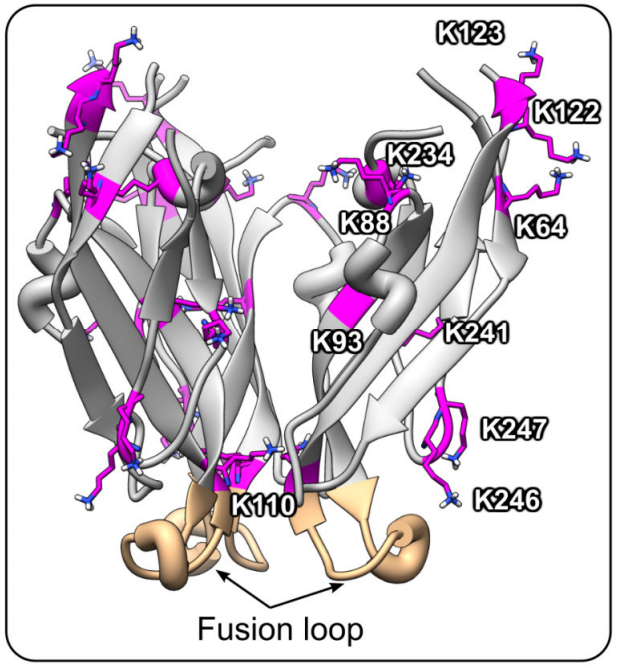


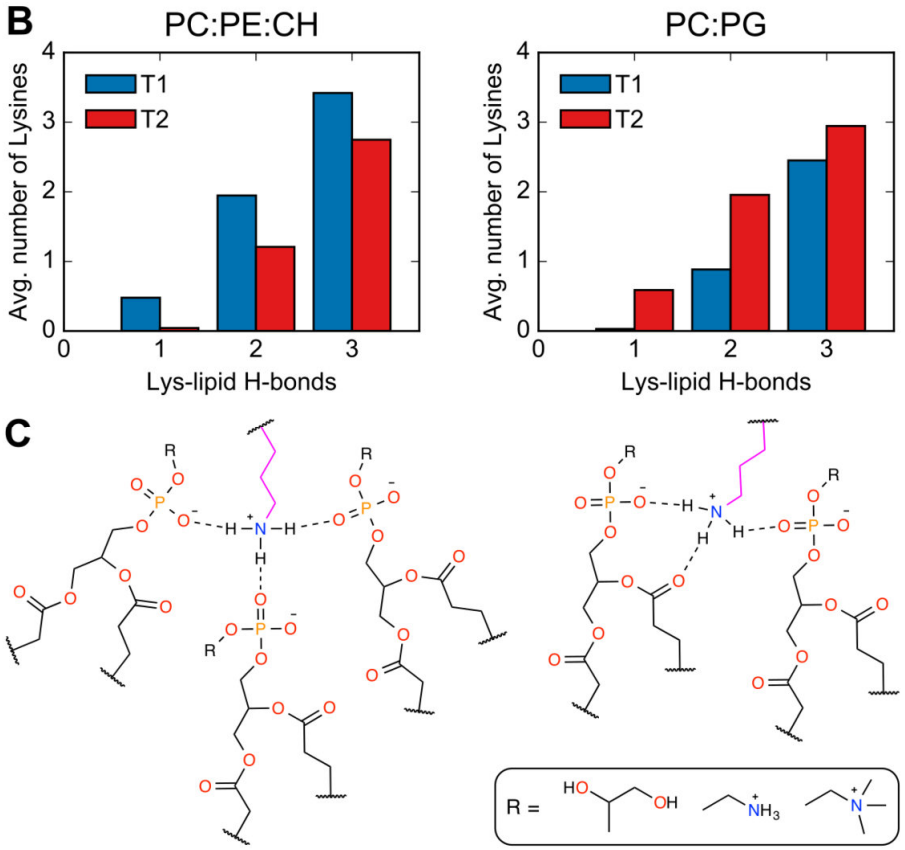
H-bonds form primarily with phosphate oxygens

Results – MD simulations



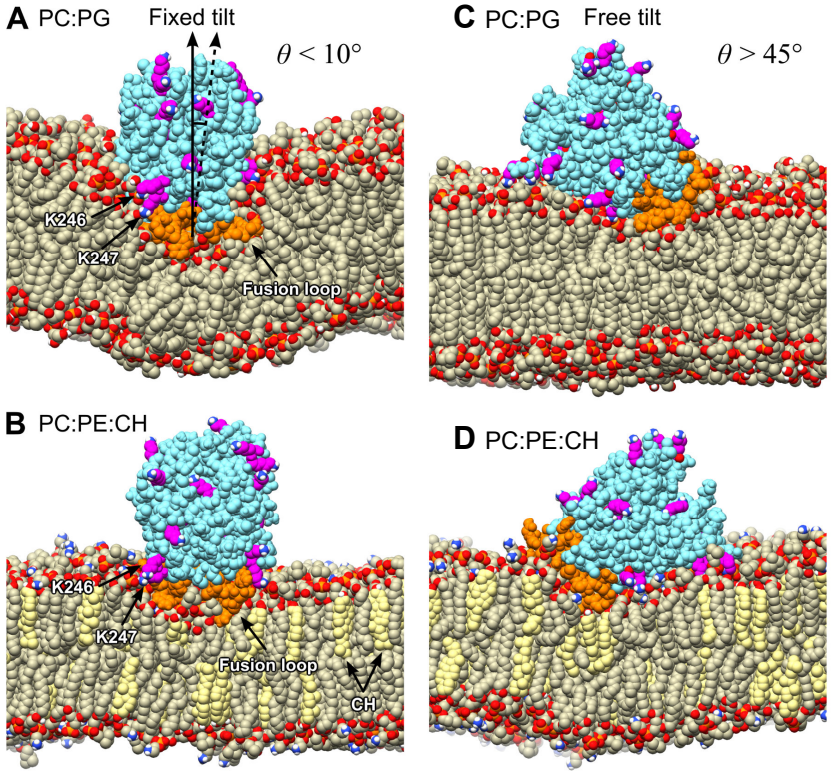
Results – MD simulations





K246 and K247 form multiple H-bonds with phosphate oxygens

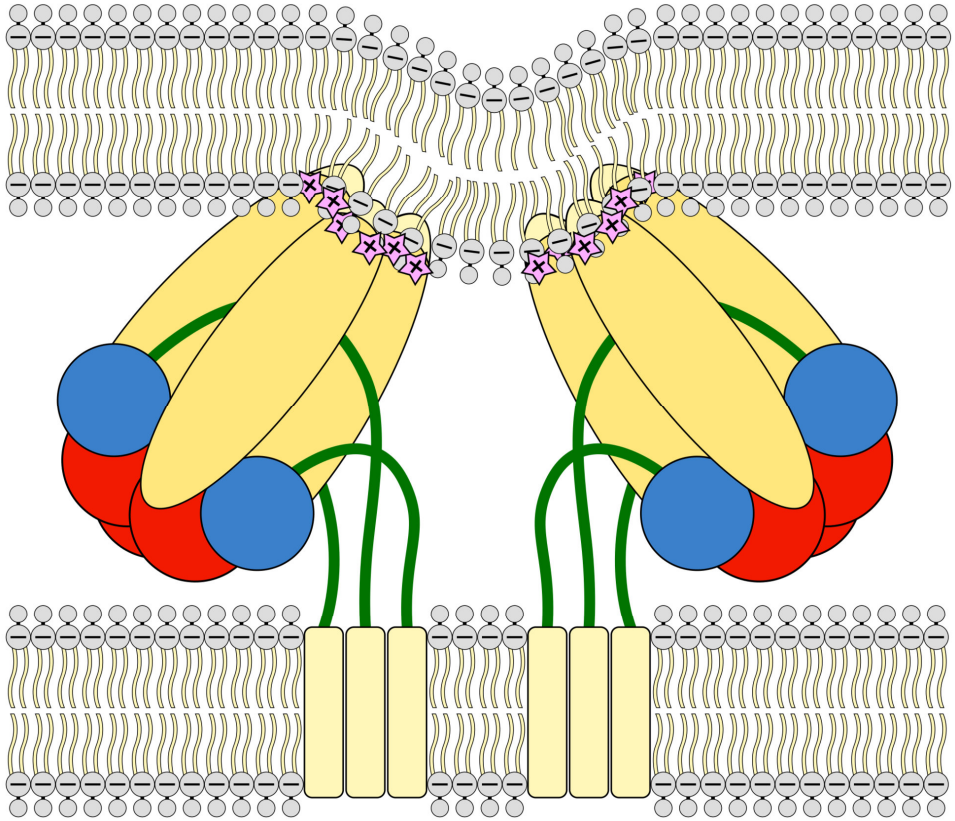
Results – MD simulations



sE held vertical during simulation

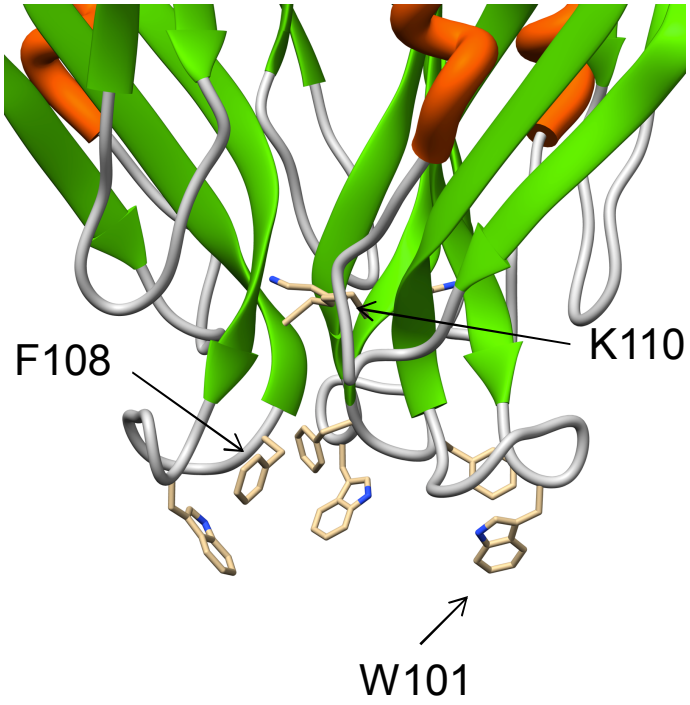
If sE is held vertical the membrane distorts to allow H-bonds to form

Results – MD simulations



H-bonding may increase anchoring at critical stage in fusion

MD shows little or no effect of mutations in fusion loop on binding



Interactions:

-electrostatic interactions dominate (lysines and PO_4^{-3})

-W101 interacts with sterols

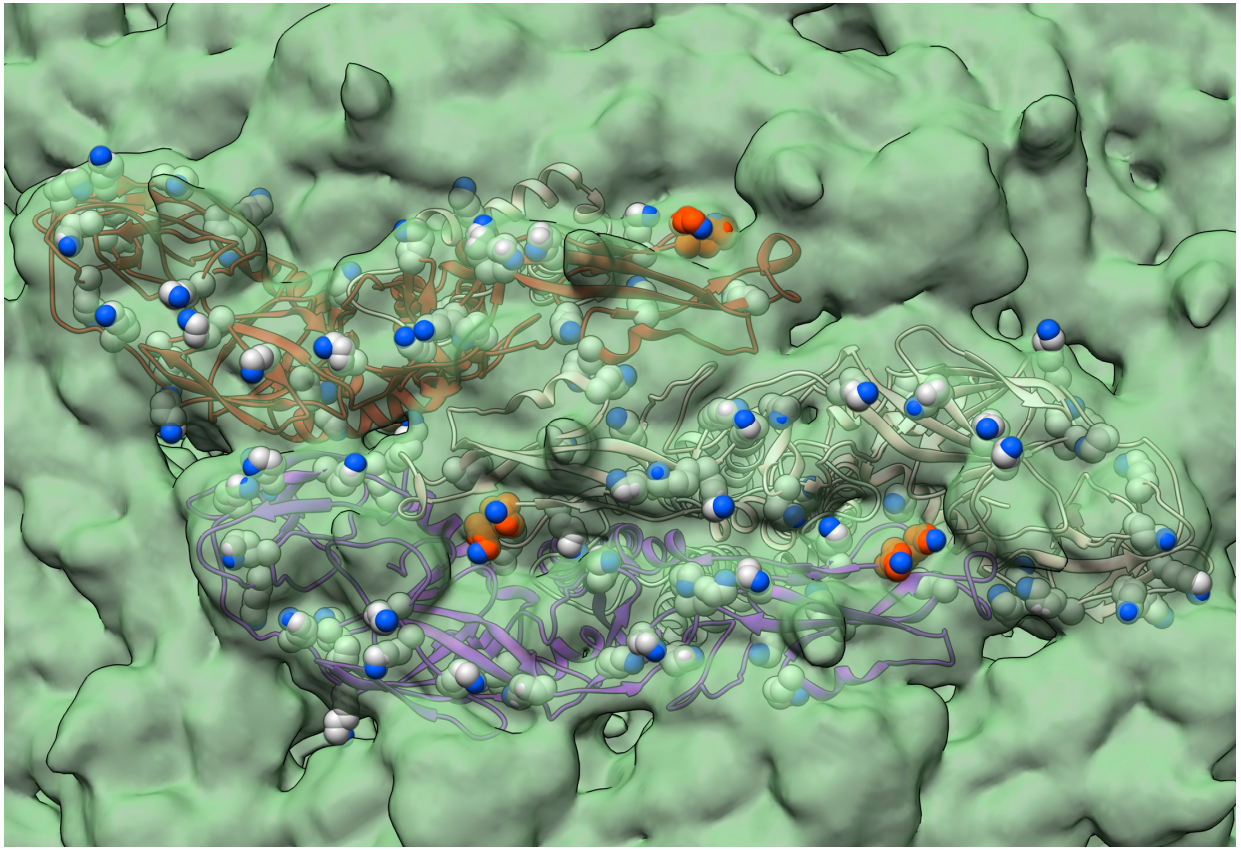
-mutations in fusion loop residues have very little impact on binding!

-these mutations do not block binding!

Mutations in fusion loop residues have little impact on binding

Impact – new drug target

dimer on virus surface



Lysines 246 and 247 (red) interact most strongly with lipids

Lysines 246 and 247 (red) - drug targets

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Conclusions

1. K246 and K247 each from multiple H-bonds to phosphates of lipids, contributing substantially to the binding energy

2. These H-bonding interactions correlate with protein tilt and result in increased depth of penetration
   - -greater contributions to binding than hydrophobic interactions of FL
   - -promoted by AI and CH, but effect is much greater with AL

3. Our results suggest that the role of FL is likely to destabilize the membrane

4. sE does not interact strongly with cholesterol

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Dept of Cell Biology, Albert Einstein College of Medicine:

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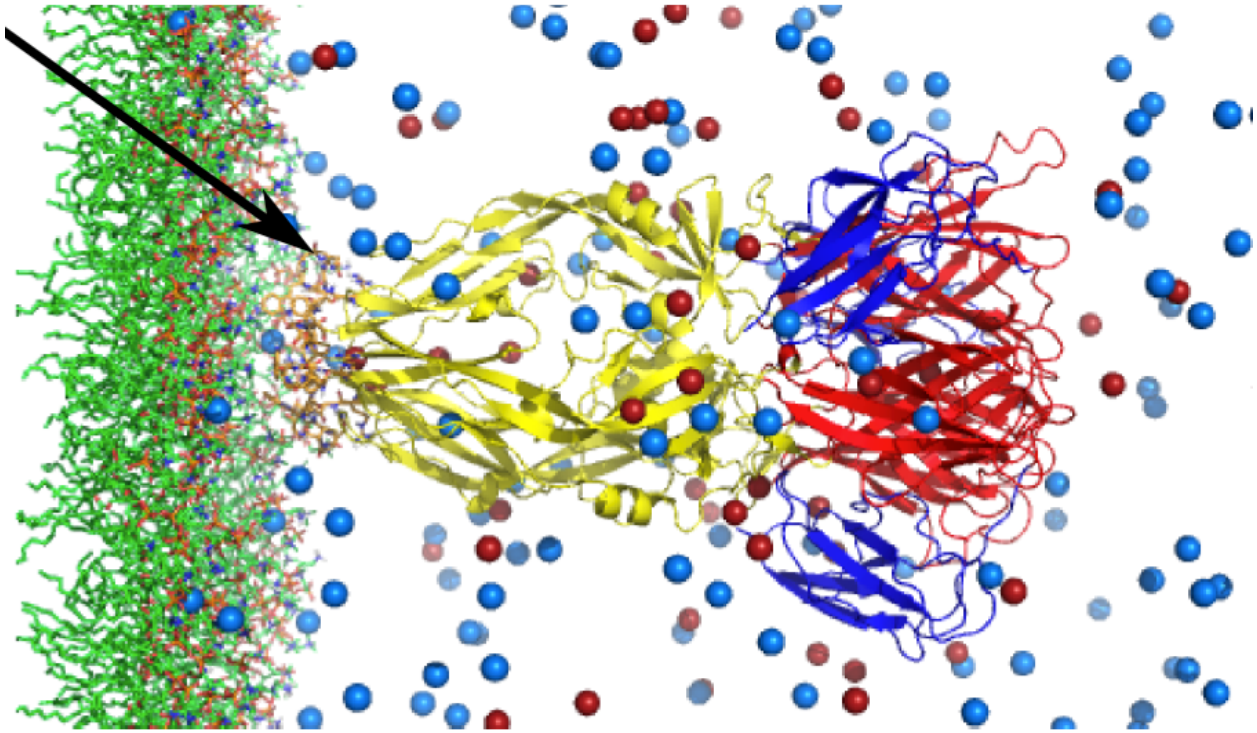
Extra slide

PC:PG 70:30



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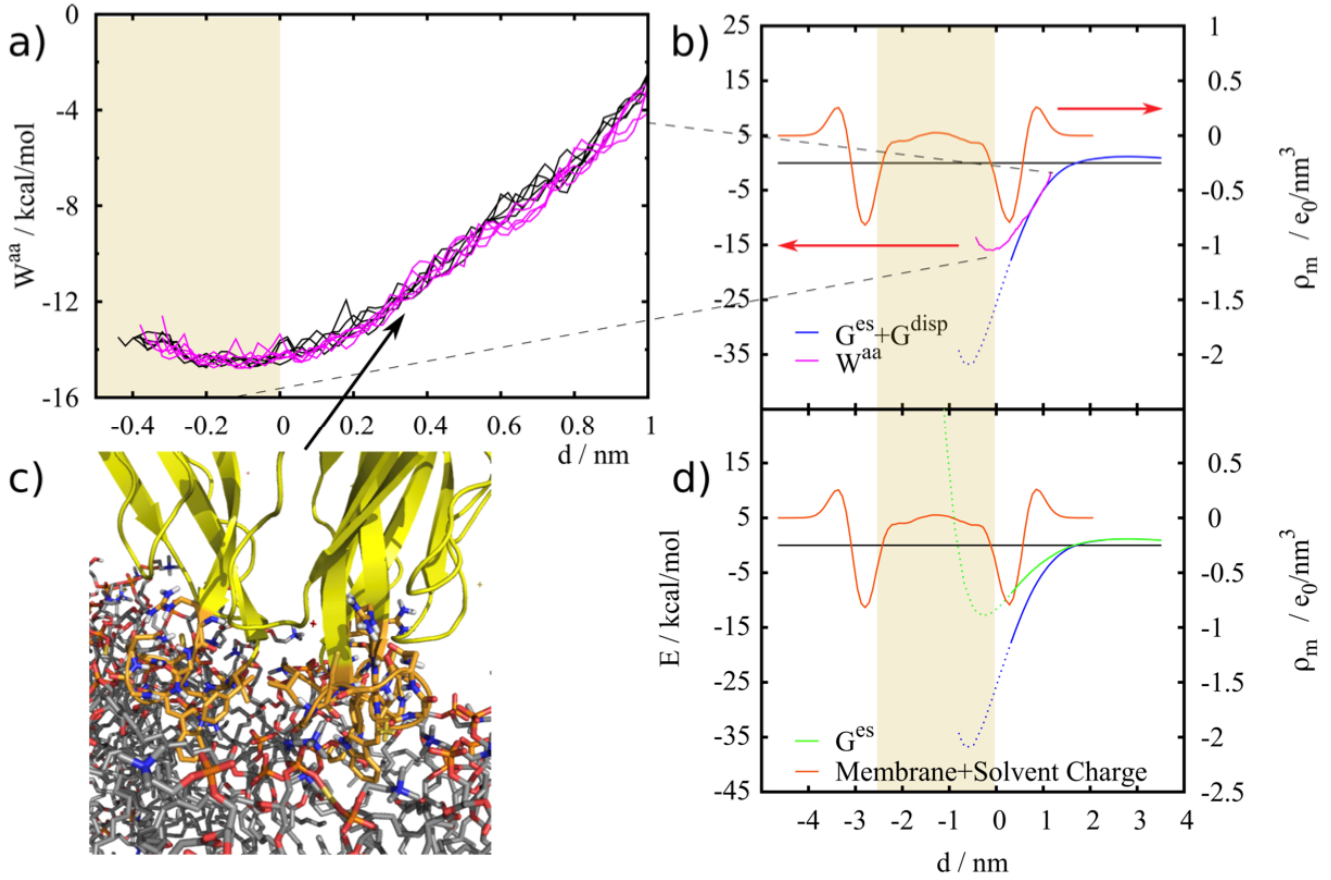
Extra slide

PC:PG 70:30

Rogers et al in review



10 ns at each distance

15 kcal/mol = 24 kT at 37 °C

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