

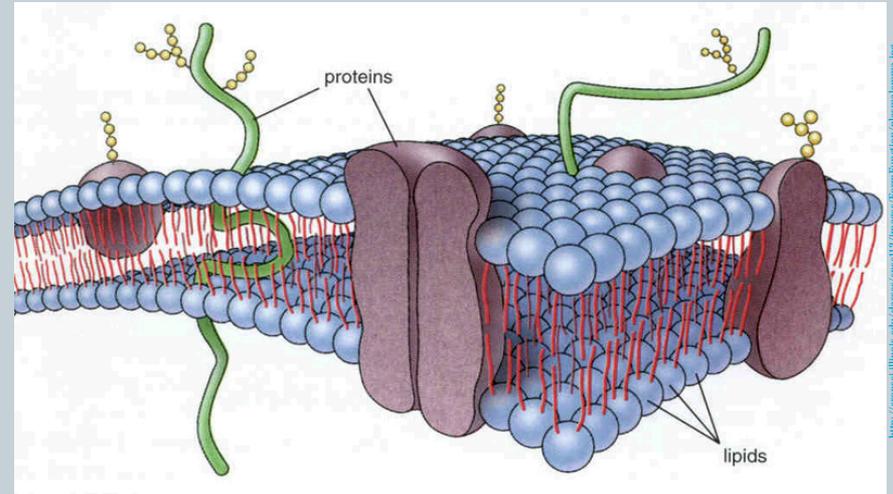
Structure and Dynamics of Peripheral Membrane Proteins



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Cell Membranes

- Found in the cells of all organisms on Earth
- Vital to the survival of a cell
- Generally consist of a lipid bilayer embedded with proteins
- Lipid monomers can be charged or neutral
- Regulate the diffusion of molecules in and out of a cell
- Membrane proteins are responsible for cell signaling, vesicular transport, action potentials



Lipid bilayer embedded with proteins

Two Examples



- **HIV-1 Gag protein assembly on a cell membrane**
 - Catalyst for forming new virus particles and progression of HIV infection

- **Healthy function of cells and regulation of cellular respiration**
 - Tubulin interactions with outer mitochondrial membrane

Motivation



- How do different biochemical interactions influence membrane binding?
- How does membrane binding affect protein conformation and how does this apply to protein function?

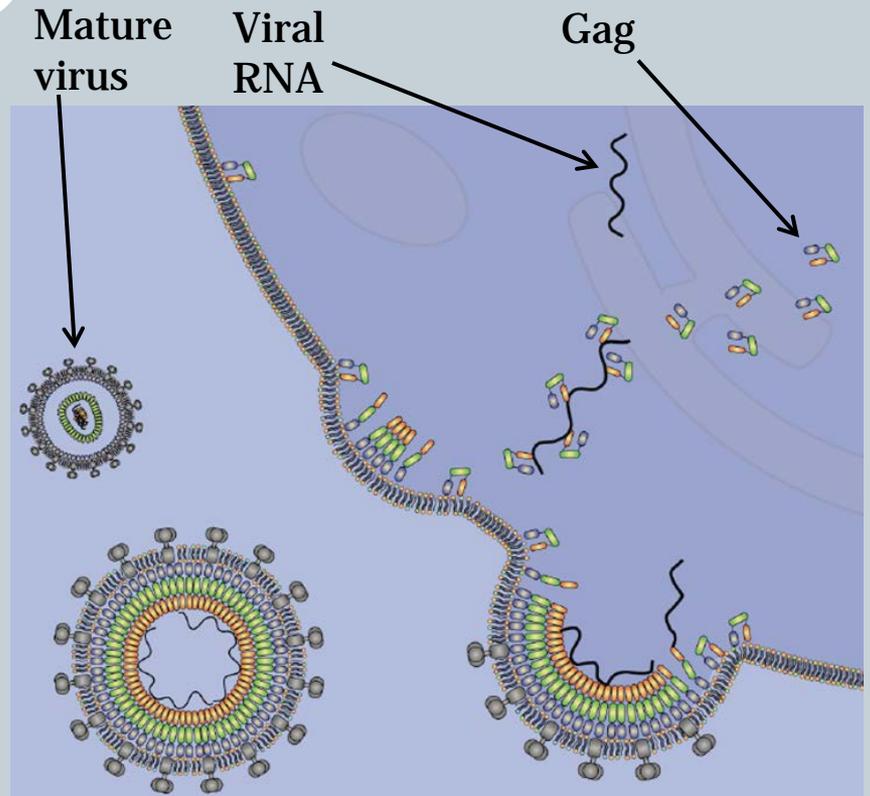
Molecular Simulations



HIV-1 MATRIX (MA) PROTEIN

HIV-1 Gag Protein

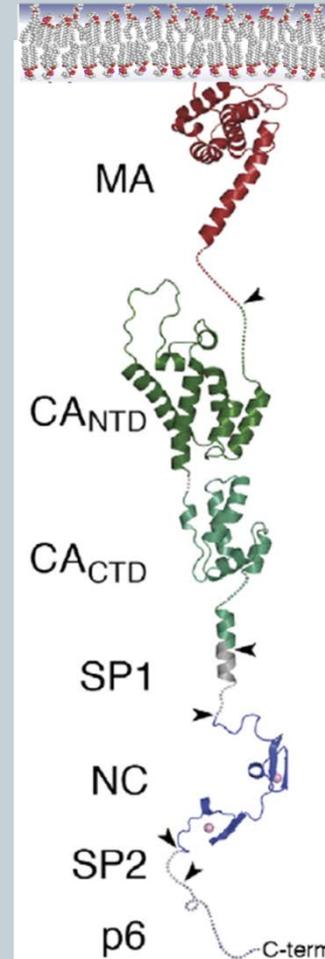
- Gag protein targets and binds to a cell's plasma membrane
- The virus' RNA binds to Gag
- Gag pulls RNA into new virus particles and separates from cell
- After maturation, new virus particles infect more cells



Cell infected by HIV-1 virus

Previous Experiments

- Previous studies have examined the structure of Gag
- Currently, there is no drug that targets Gag
- Focusing on the Matrix domain of Gag protein



Matrix
Membrane binding domain

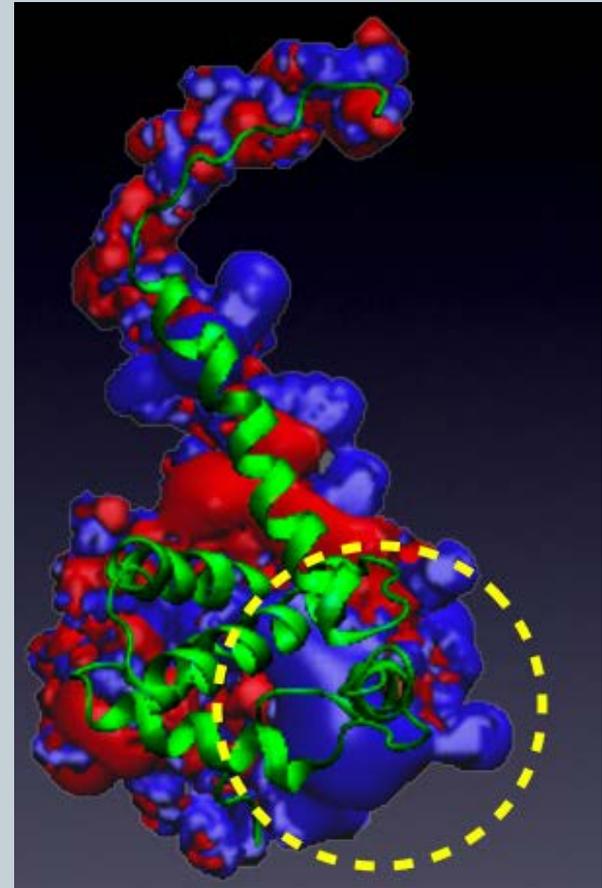
Capsid
Lateral protein assembly

Nucleocapsid
Binds the viral genome

Previous Experiments

- Gag membrane binding mechanisms:
 - Residue patch – attracted to charged lipid membranes
 - P1(4,5)P2 – lipid binding site
 - Fatty acid chain attached to N-terminal residues inserts into lipid hydrocarbon core

Model membrane: 80%POPC:20%POPS

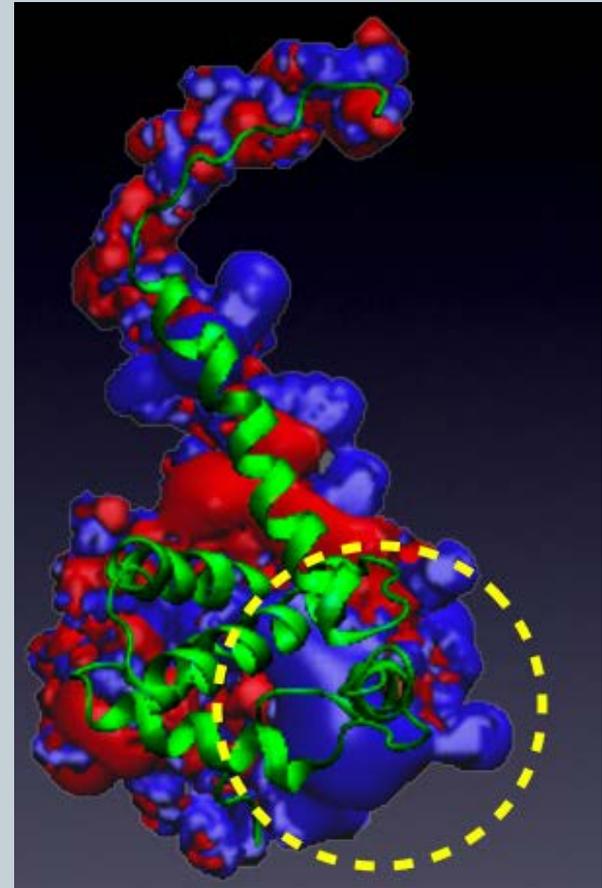


Gag MA protein

Previous Experiments

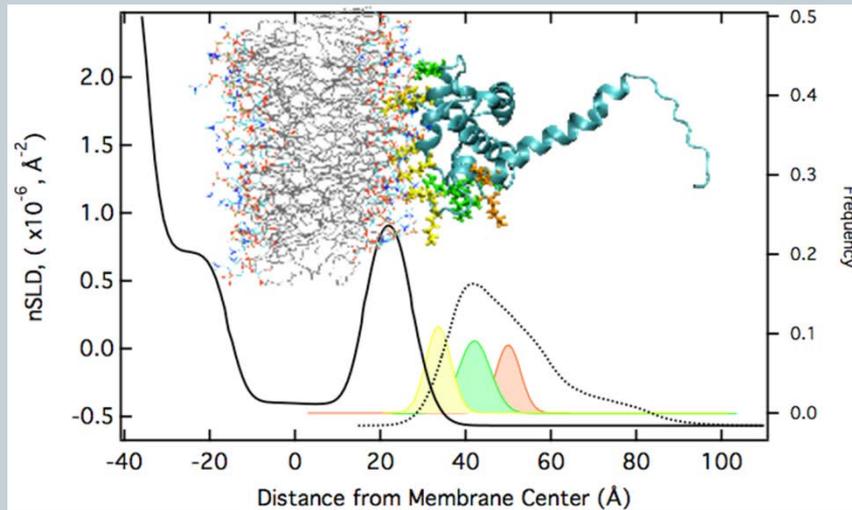
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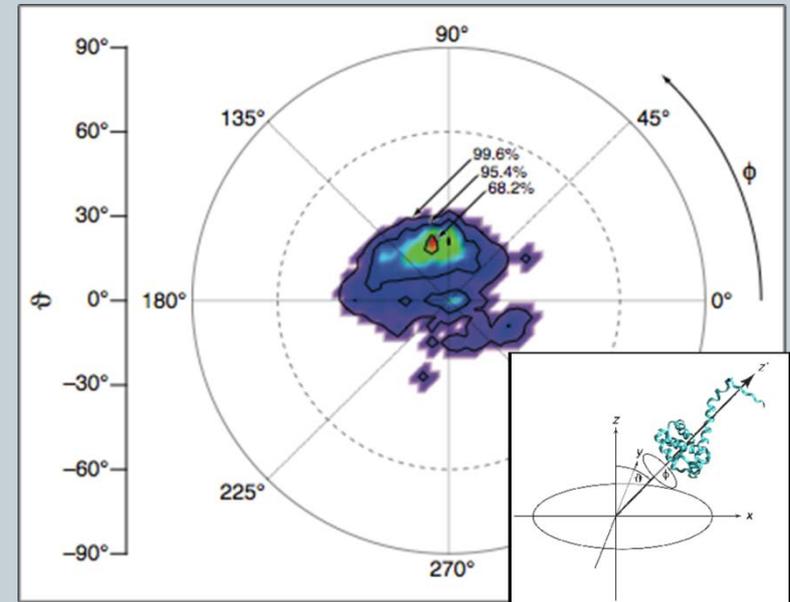
Gag MA protein

Previous Experiments at NCNR



Gag Matrix protein enters into the choline-phosphate head group region

Basic residues closest to protein:membrane interface can be identified



Contraplot of Matrix protein

Distribution for orientation of the protein on the membrane

Highly localized preference for a 20 degree tilt and 100 degree rotation

Molecular Dynamics (MD)



Overview

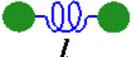
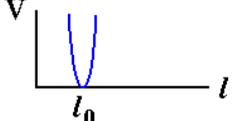
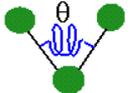
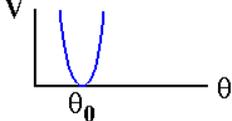
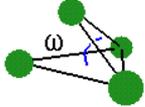
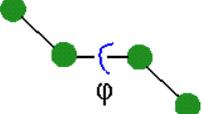
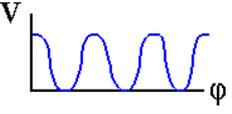
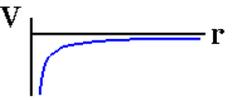
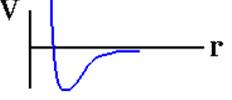
- MD is a computer simulation of the physical interactions between atoms and molecules
- Allows insight into molecular motion
- Input system of molecules, equilibrate, solve $F=ma$, thermal energy, run dynamics

Systems

- I. POPC neutral membrane with MA protein
- II. POPC:POPS charged membrane with MA protein
- III. Alchemical mutations of lipids and membranes

Physics Behind MD

Empirical Potential Energy Function

Bonds			$U(R) = \sum_{bonds} K_r(r - r_{eq})^2$
Angles			$+ \sum_{angles} K_\theta(\theta - \theta_{eq})^2$
Improper Dihedrals			$+ \sum_{dihedrals} \frac{V_o}{2} [1 + \cos(n\phi - r)]$
Torsions			$+ \sum_{torsions} A[1 + \cos(n\tau - \phi)]$
Electrostatics			$+ \sum_{i < j}^{atoms} \frac{q_1 q_2}{\epsilon R_{ij}}$
van der Waals			$+ \sum_{i < j}^{atoms} \left(\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}} \right)$

http://cmm.info.nih.gov/intro_simulation/node15.html

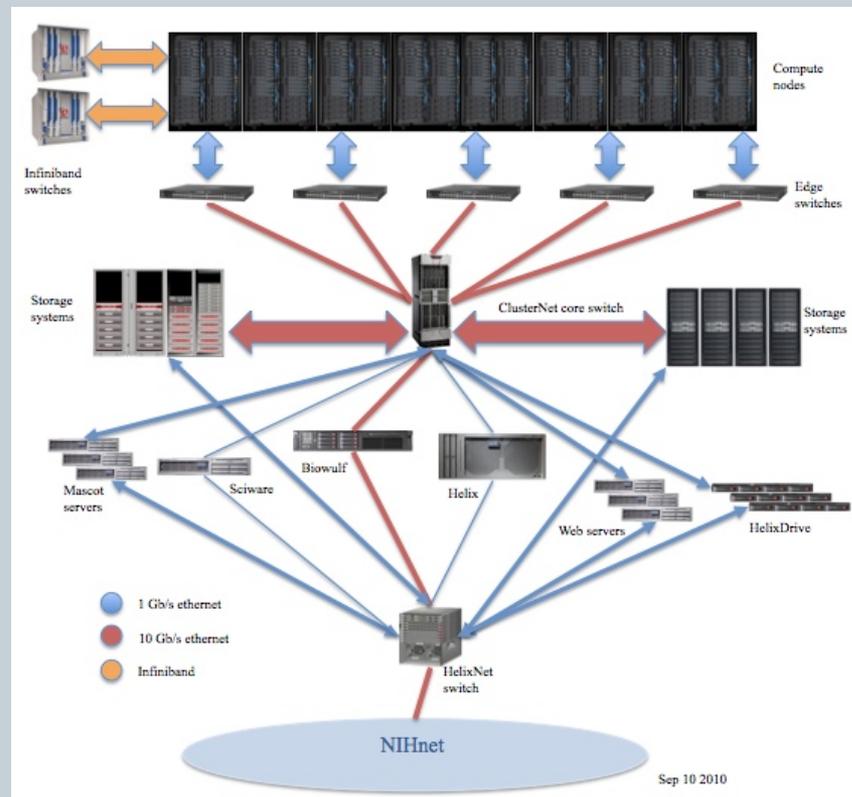
$$-\frac{dU}{dR} = F$$

$$F = ma$$

MD integrates
forces over time

Programs and Computers

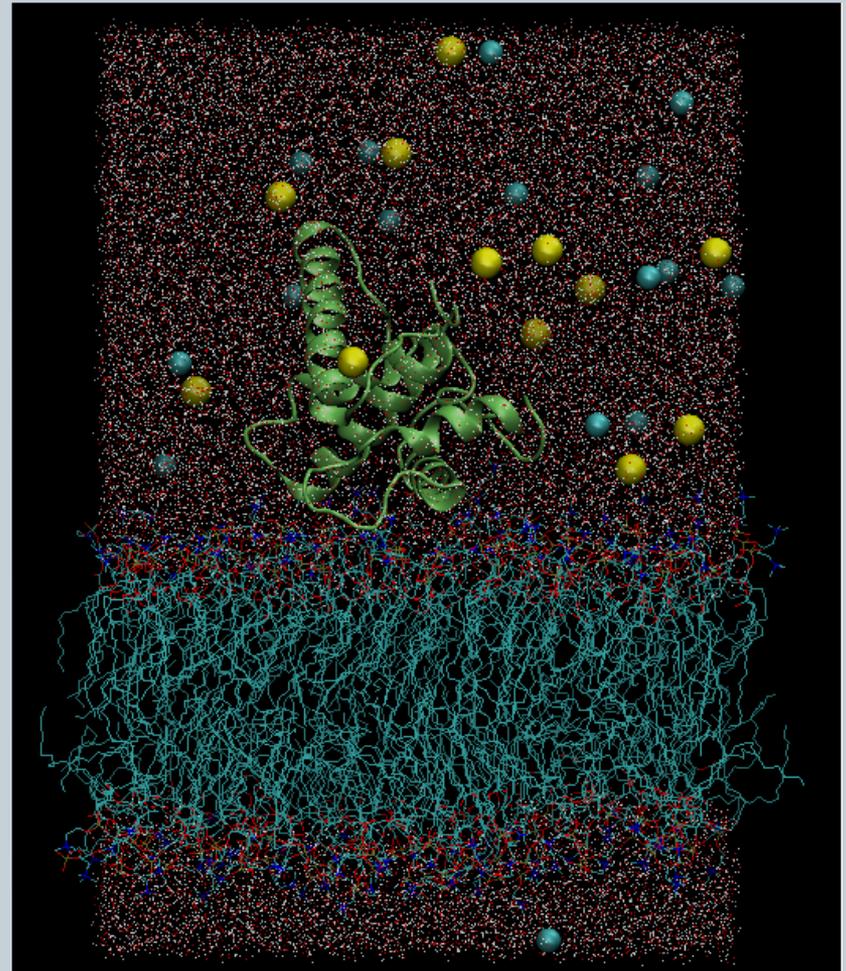
- Programs used to run MD simulations:
 - NAMD
 - VMD
- Simulations were run using the NIH Biowulf Cluster
 - Nearly 9000 processors



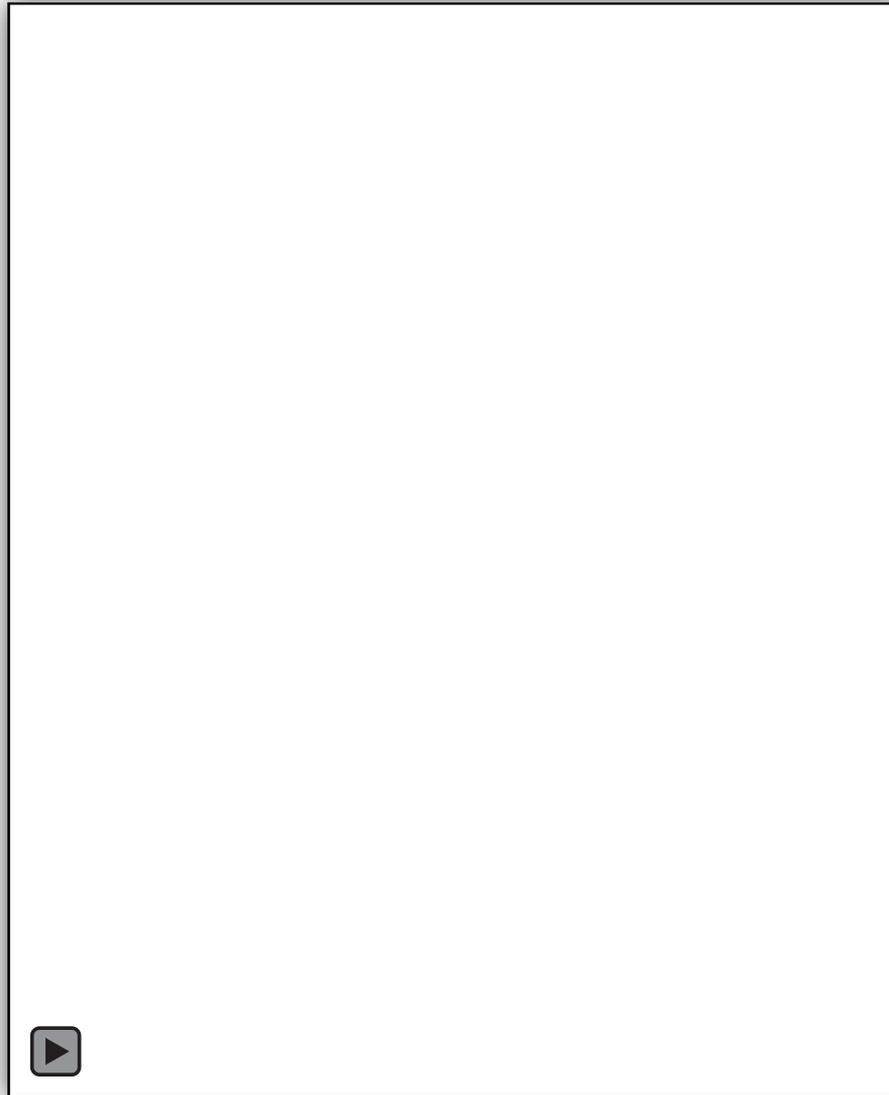
NIH Biowulf Cluster

I, II. Membrane Simulations

- **Build membrane**
 - I. pure POPC (neutral) lipid bilayer
 - II. 80% POPC (neutral):20% POPS (charged) lipid bilayer
- **Add protein**
 - I, II. Matrix protein placed on top of lipid head groups
- **Solvate system**
 - I, II. Add water box
- **Ionize system**
 - I, II. Add 0.05 M NaCl



I. POPC membrane (neutral) with MA protein



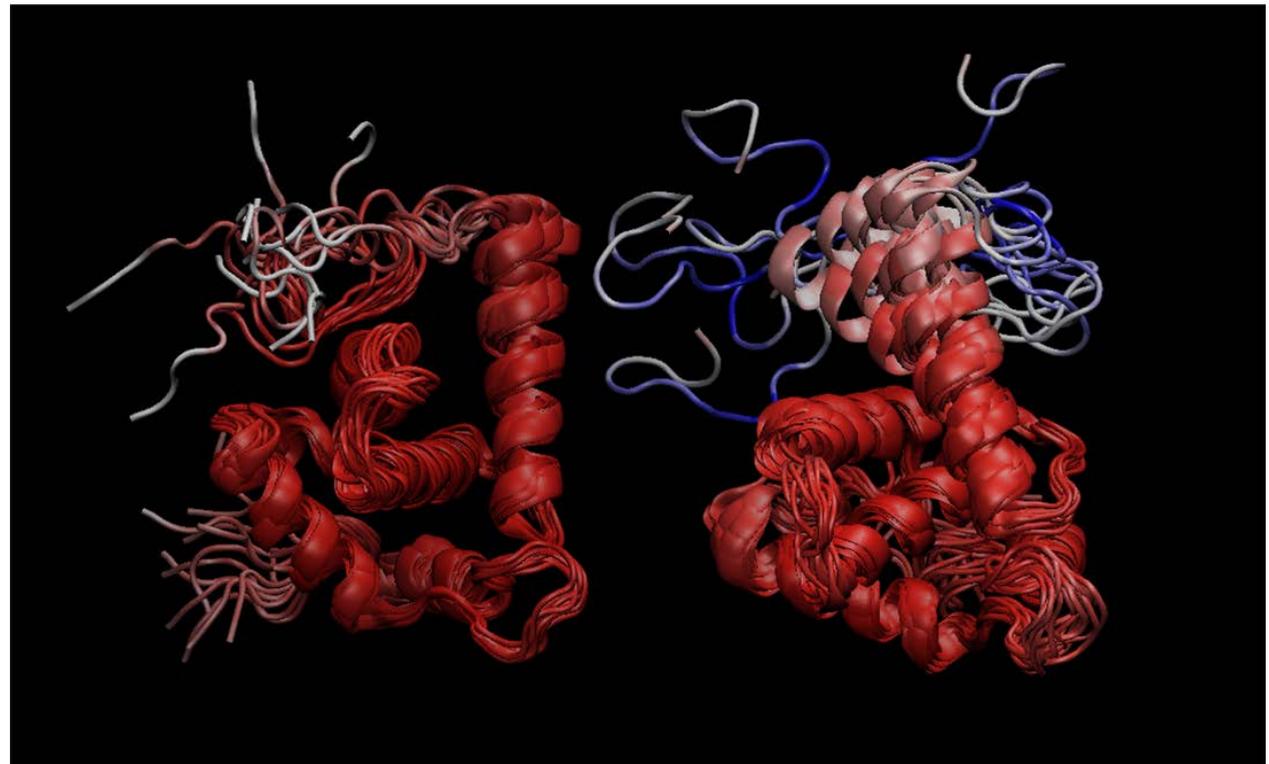
II. POPC:POPS Membrane (charged) with MA Protein



Results

Root Mean Square Deviations (RMSD)

- RMSD is a measure of total fluctuations of a residue
- Red more rigid and blue more flexible
- MA protein on charged membrane (left) more rigid, may be due to stronger interactions with anionic lipids

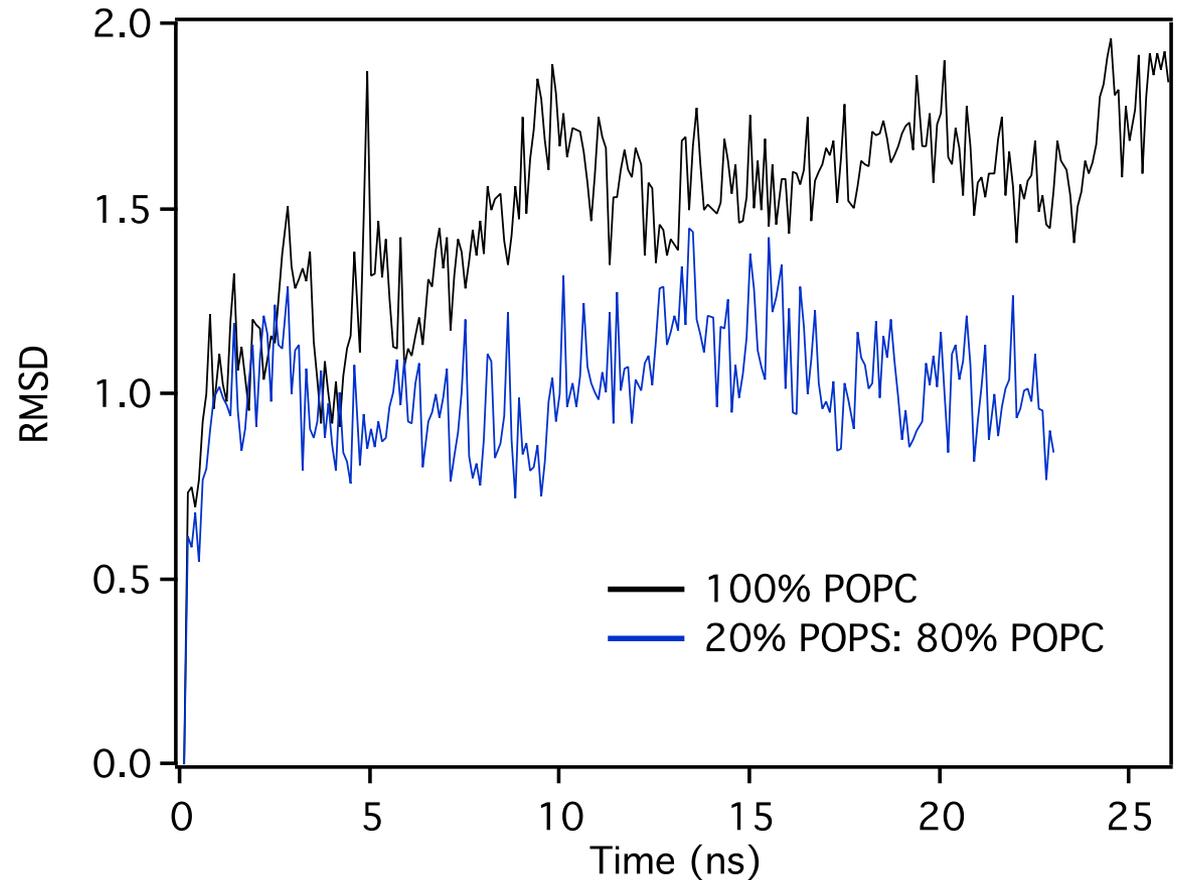


Aligned structures of MA protein on the membrane, 20%POPS:80%POPC on left and 100%POPC on right

Results

RMSD of Backbone Atoms Over Time

- Reflects picture on previous slide
- MA protein on charged membrane appears more stable (smaller RMSD)

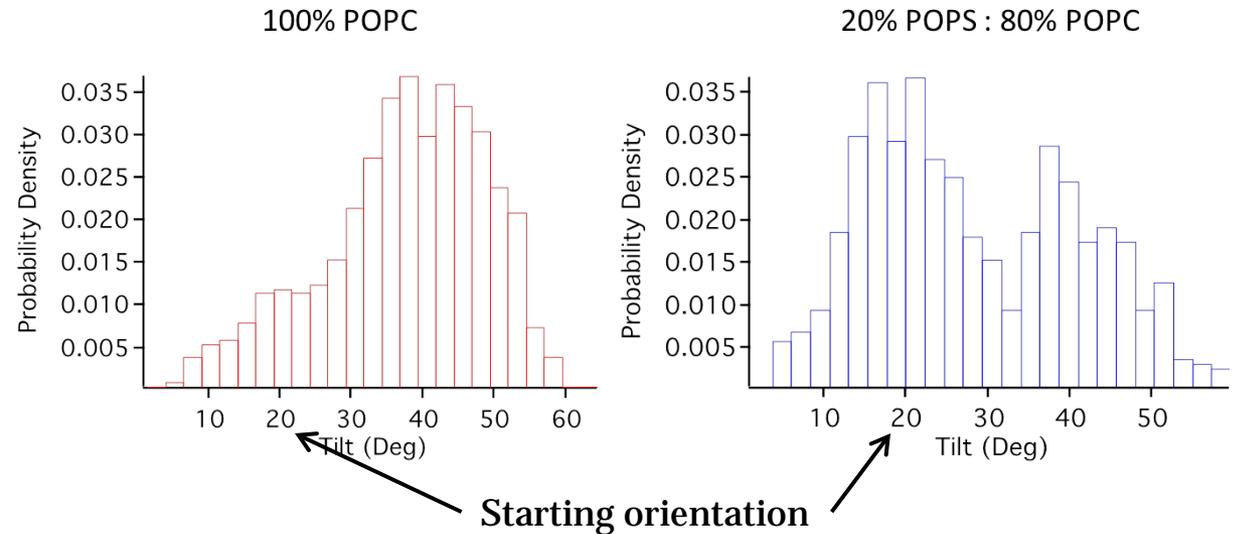


Time Evolved RMSD of all backbone atoms in core of the protein

Results

Protein Tilt

- Average protein tilt during course of simulation
- Protein explores larger tilt angles on POPC membrane
- On the charged membrane, protein reaches tilts around 40 degrees, however preferred tilt was ~20 degrees



Average protein tilt on membranes I and II

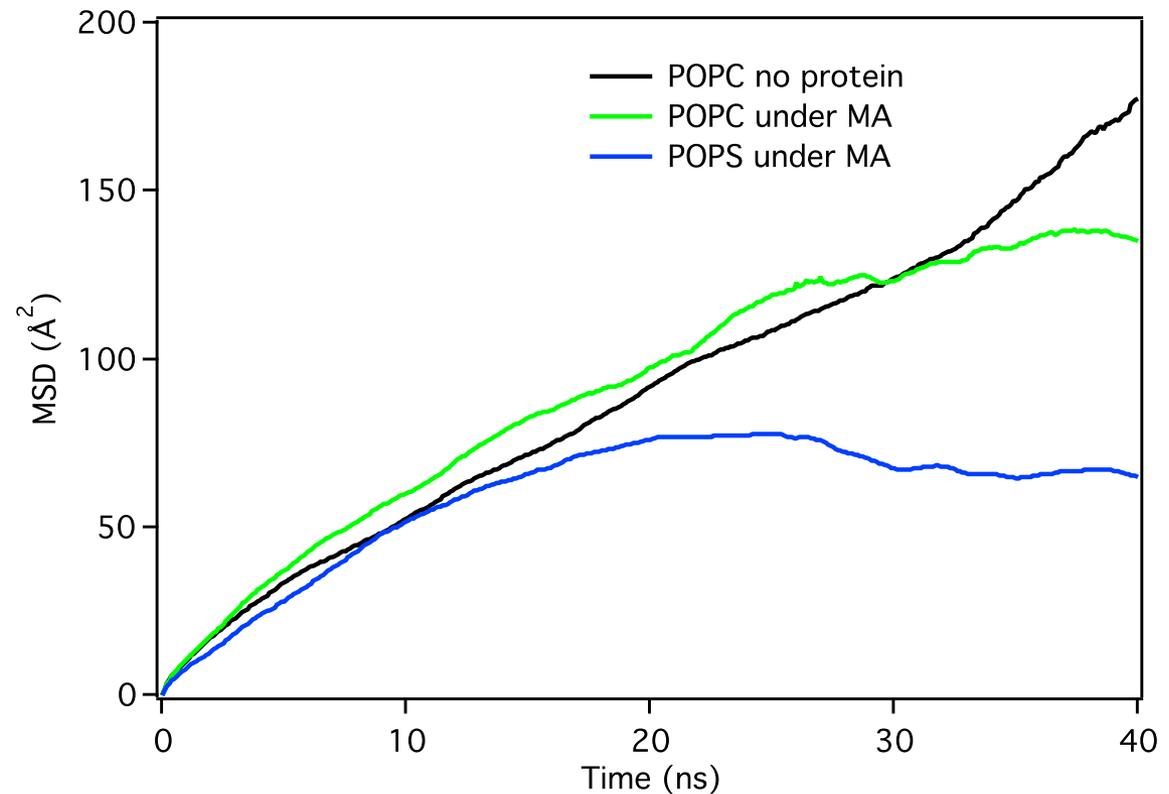
Results

Mean Square Displacement (MSD) of Lipids

- MSD calculates how much lipids move over time
- Distance lipid moves at time 't' starting at time 't=0' squared

$$\frac{1}{N} * \sum [r(t) - r(0)]^2$$

- POPS lipids are slowed down by the protein, POPC lipids are not



Mean Square Displacement as a function of time

III. Alchemical Mutations

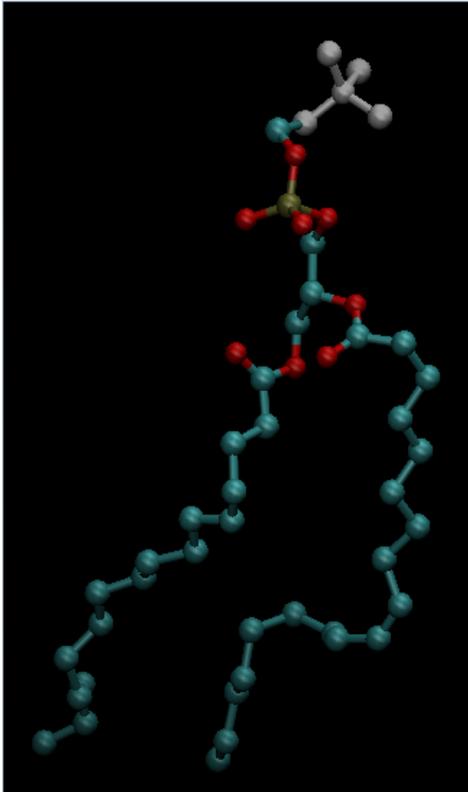


- Alchemical mutations are used to chemically mutate one molecule into another
- Purpose of this system is to quantify the energy of charging a lipid under a protein
- Lipids naturally diffuse slowly, alchemical mutations may occur much more quickly
- Allow us to identify the optimal placement of lipids under a protein
- We are using this system to mutate a neutral POPC lipid into a charged POPS lipid

Lipid Mutation

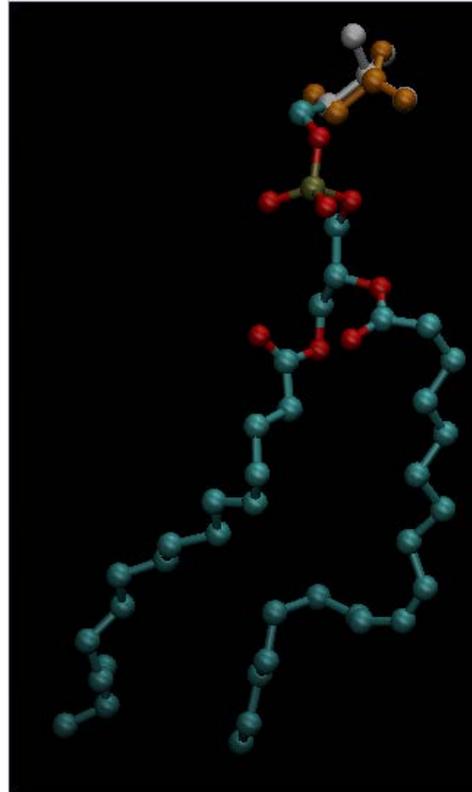


Neutral
POPC head group



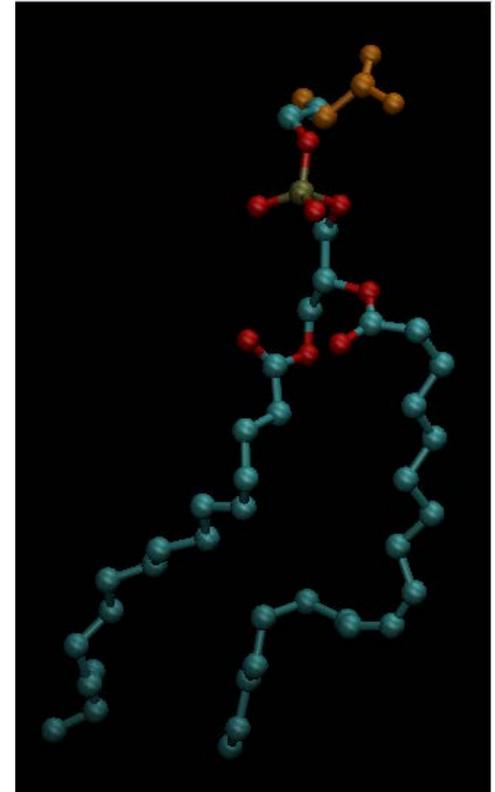
$\lambda = 0$

Both
POPC + POPS



$0 < \lambda < 1$

Charged
POPS head group



$\lambda = 1$

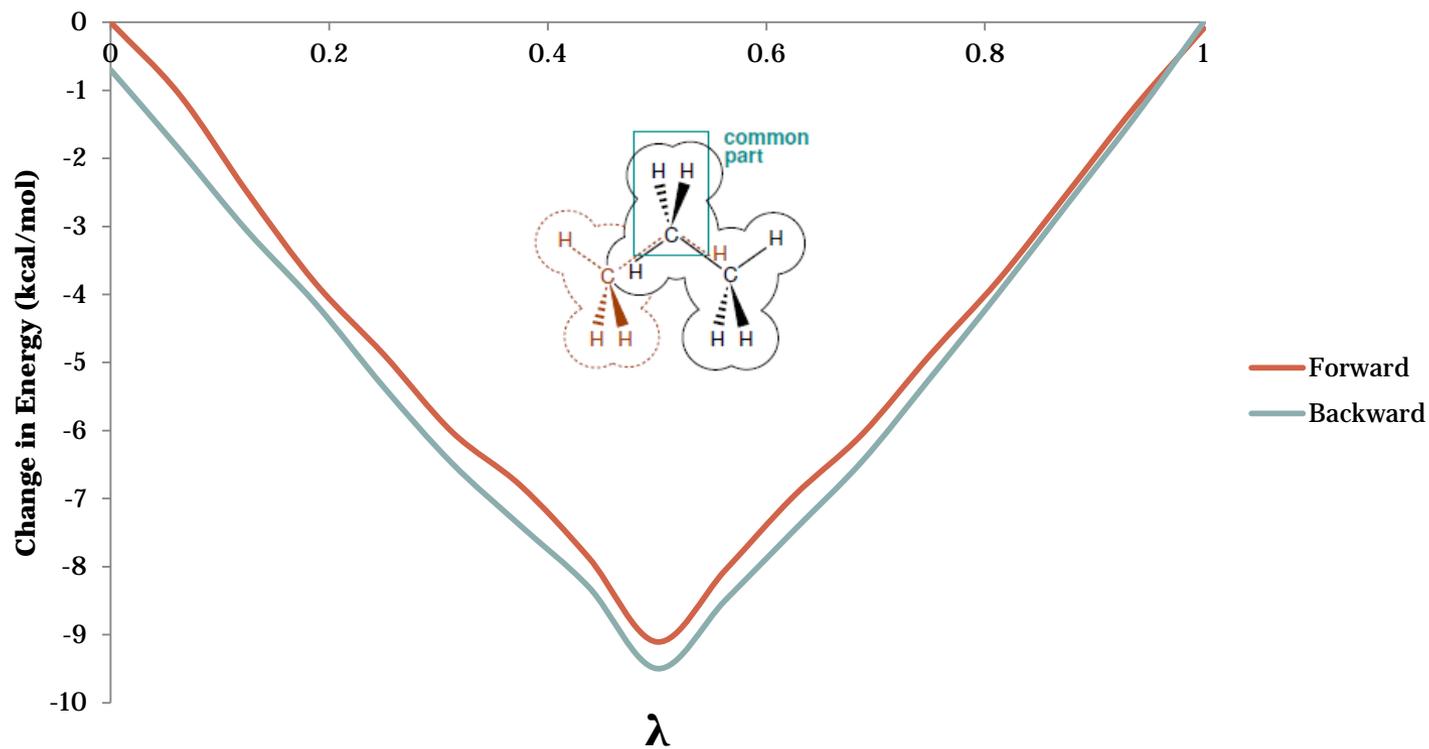
$$(1 - \lambda)E_{\text{POPC}} + \lambda E_{\text{POPS}} + E_{\text{everything-else}} = E_{\text{total}}$$

Alchemical Mutation Model



Ethane \rightarrow Ethane Transformation

Energy Change



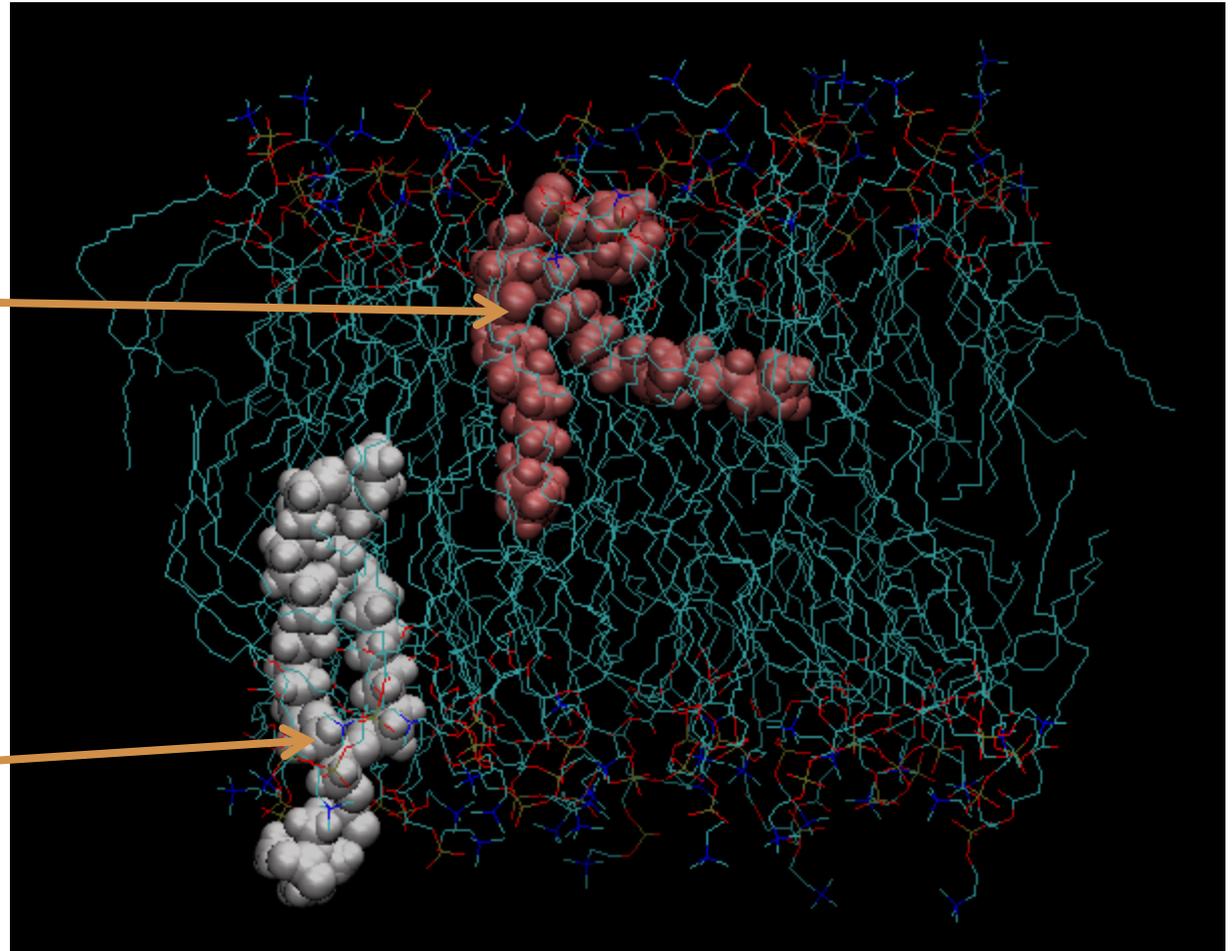
Membrane Mutation



Charged \rightarrow Uncharged
(POPS \rightarrow POPC)



Uncharged \rightarrow Charged
(POPC \rightarrow POPS)



Membrane Mutation



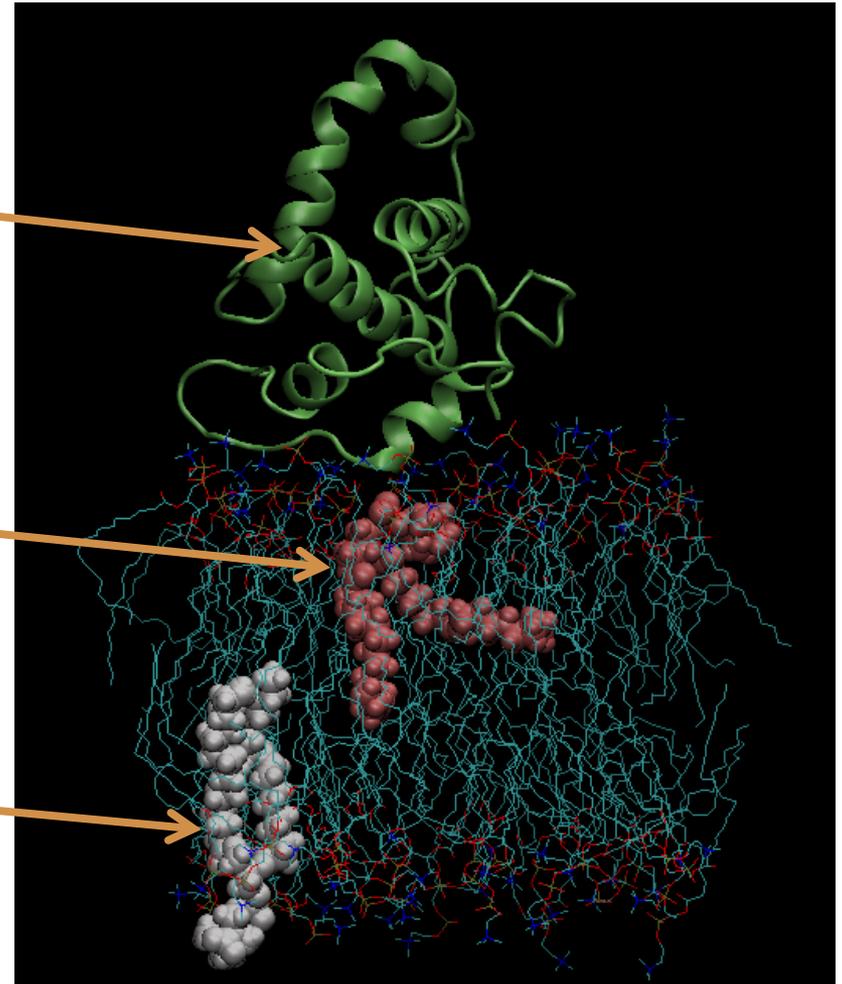
Membrane Mutation with Protein



MA Protein

Charged \rightarrow Uncharged
(POPS \rightarrow POPC)

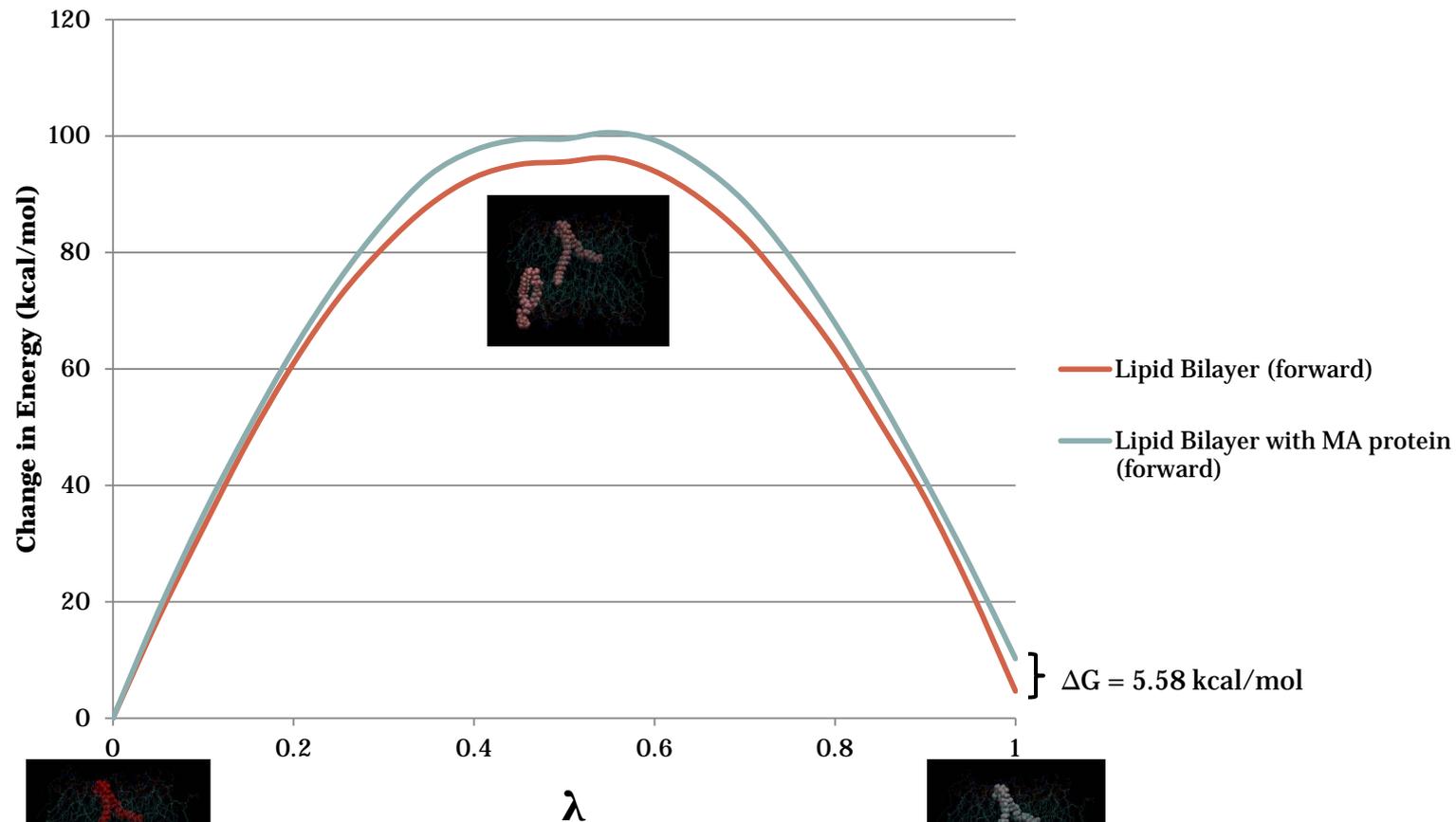
Uncharged \rightarrow Charged
(POPC \rightarrow POPS)



Membrane Mutation with Protein



Charging Energy



— Lipid Bilayer (forward)

— Lipid Bilayer with MA protein (forward)

} $\Delta G = 5.58$ kcal/mol

Conclusions

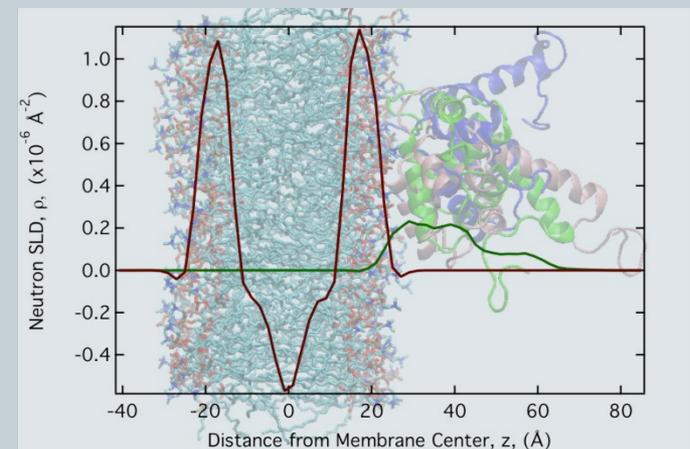


- MD simulations show that MA conforms differently on uncharged and charged membrane systems
- Alchemical mutations can provide quantitative results for the energy required to charge a membrane
- Various types of analysis are needed to completely understand the physical parameters and molecular mechanisms of protein membrane interactions

Future Studies

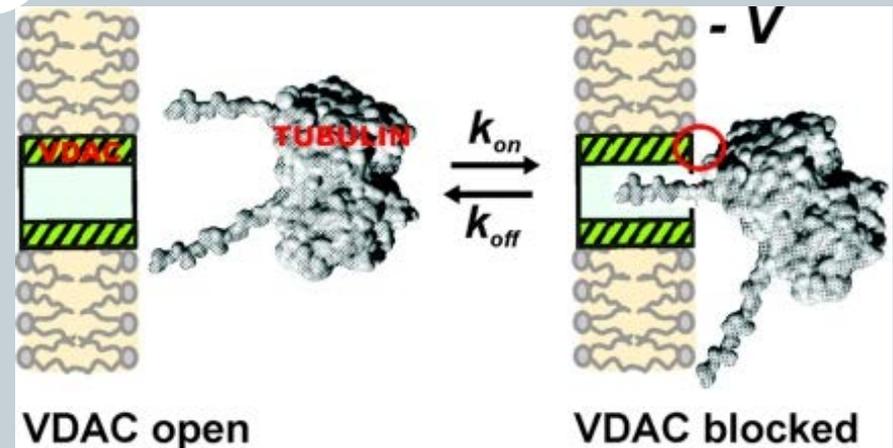


- Use alchemical mutations to determine initial distribution of charged lipids for simulations of mixed lipid membranes
- Explore protein conformations that are consistent with neutron reflectivity measurements
 - ✦ Use reflectivity results as a constraint on simulations
- Combine experimental and computational methods to further investigate other biochemical mechanisms by which HIV-1 Gag, Matrix domain targets the membrane

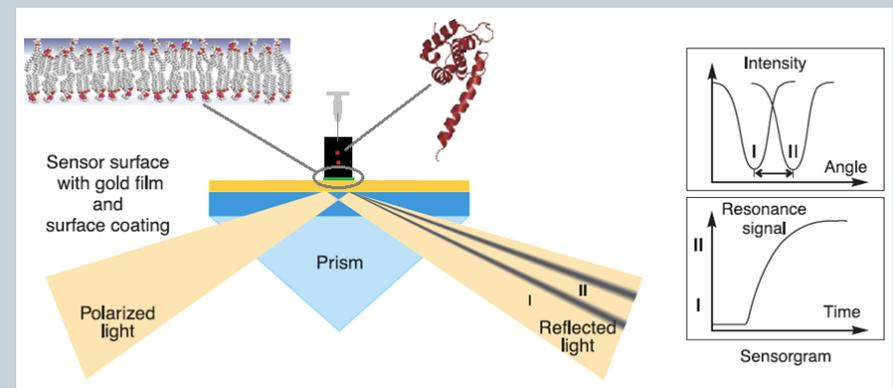


Experimental Work

- Regulation of mitochondrial function by tubulin through binding and modification of membrane environment
- Used techniques such as electrochemical impedance spectroscopy and surface plasmon resonance
- Ultimately results were inconsistent
 - Discovered tubulin solution was not homogenous due to tubulin aggregation
- Identified that these techniques can be used for biological systems
- Experiments gave more insight into tubulin's behavior



Model for tubulin binding



Surface Plasmon Resonance

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