Cellular-Scale Modeling of Oncogenic Proteins

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Lawrence Livermore National Laboratory's interest in cancer research

Cancer Moonshot:

- National effort to double the rate of progress in cancer-fighting research
- LLNL is contributing to this project by using their high performance computing capabilities to help with understanding of the mechanisms leading to cancer development

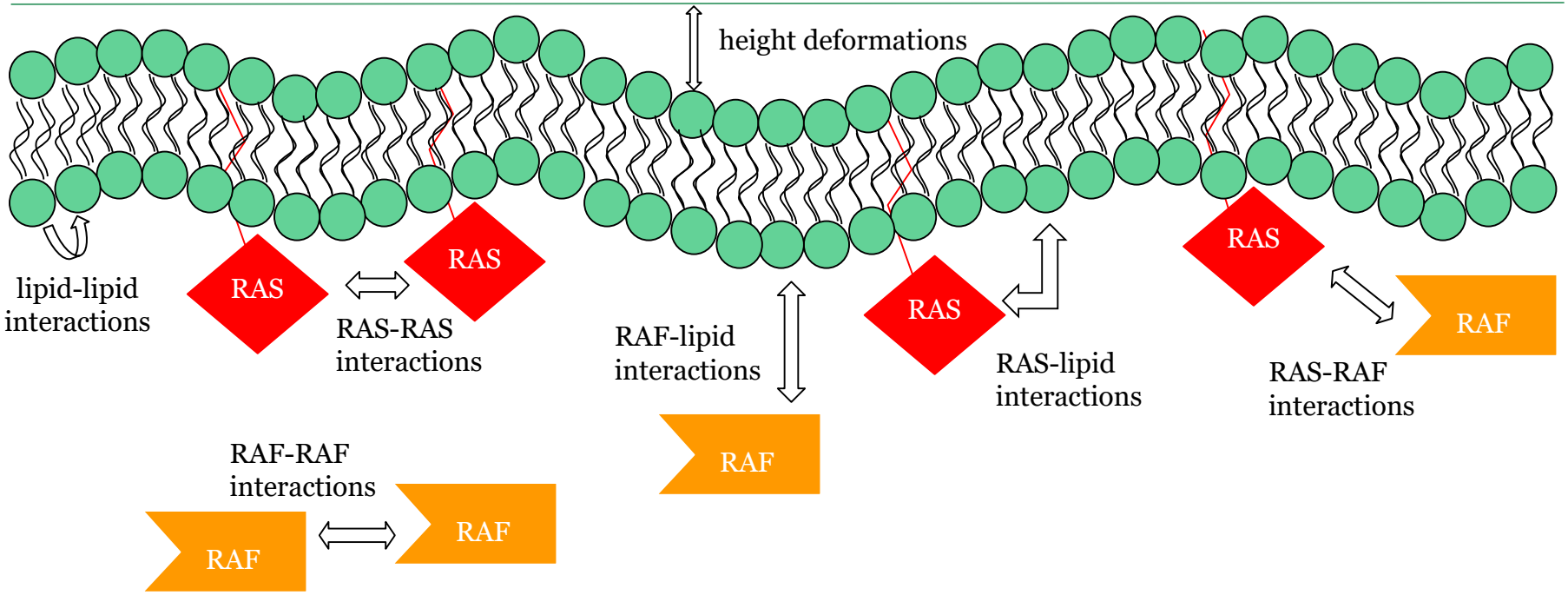
https://www.llnl.gov/news/labs-high-performance-computing-will-play-major-role-cancer-moonshot-initiative

The goal of the LLNL Cancer Moonshot project is to model the interactions between the RAS and RAF proteins and the cell membrane

Why do we care about these interactions?

- The interactions between RAS, RAF, and the cell membrane are important because they are involved in the cell signaling pathway for cell growth and division
- Mutations in RAS proteins can cause overactive signaling, which can prevent cell death and lead to tumor growth [Goodsell, 1999]
- RAS mutations have been implicated in 25% of all human tumors and up to 90% in certain types of cancerous tumors, such as pancreatic cancer [Downward 2003]

We modeled interactions between mutated RAS, RAF, and the cell membrane



Our project was to work on the macroscale piece of a multiscale model

- Created a continuum (macroscopic) model of the interactions
    - “Free energy functional” used to combine the atomistic data with continuum scale models
    - Evolution equations derived from dynamic density functional theory
- Used numerical methods to solve the evolution equations
    - Time-dependent partial differential equations
    - A system of stochastic differential equations

The Mathematical Model

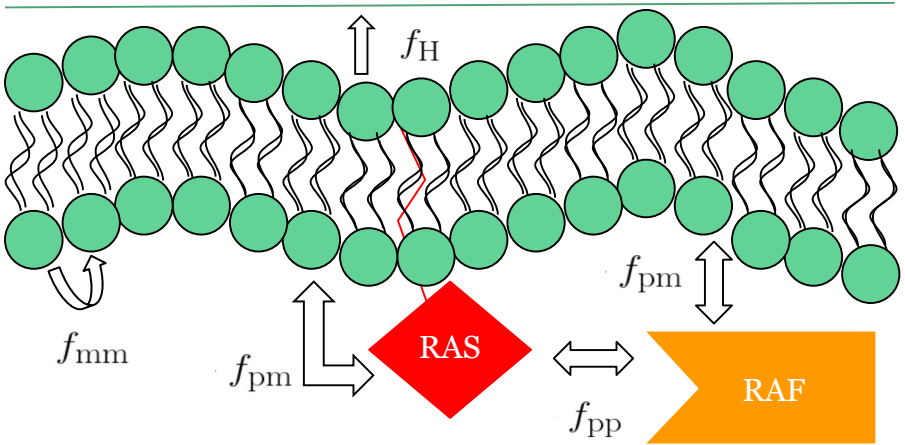
“Free energy” term is created to incorporate the atomistic data into the continuum scale models

The free energy functional describes the available work over the domain of the thermodynamic system

$$\mathscr{F} = \int_{\Omega} f \, d\mathbf{r}$$

Energy densities from all of the interactions in the system are included

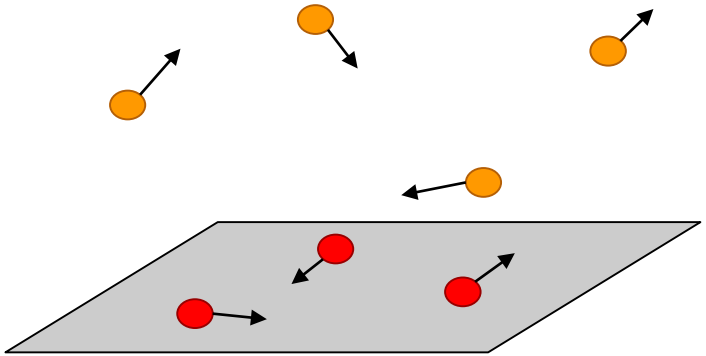
$$f = f_{\mathrm{mm}} + f_{\mathrm{pm}} + f_{\mathrm{pp}} + f_{\mathrm{H}}$$



The Protein Model

We model the proteins as “beads” in three-dimensional space

The RAS proteins are bound to the inner leaflet of the membrane, so their z-position corresponds to the height of the membrane, while the RAF proteins move freely within the cytoplasm above the membrane



Inner leaflet

are RAS proteins

are RAF proteins

Proteins have position $\mathbf{r}_k$ and velocity $\mathbf{v}_k$

Evolution of proteins is derived in accordance with the Langevin equation describing Brownian motion

Protein evolution equation:

$$\frac{d^2\mathbf{r}_k}{dt^2} = -\frac{1}{m_k}\nabla_{\mathbf{r}_k}\frac{\delta\mathscr{F}}{\delta\mathbf{r}_k} - \frac{1}{m_k}\gamma_k\frac{d\mathbf{r}_k}{dt} + \boldsymbol{\theta}(t)$$

Protein evolution equation is solved numerically with Forward Euler's method

Discretization of the protein evolution equation:

$$\frac{\mathbf{v}_k^{t+1} - \mathbf{v}_k^t}{dt} = -\frac{1}{m_k}\nabla_{\mathbf{r}_k^t}\frac{\delta \mathscr{F}}{\delta \mathbf{r}_k^t} - \frac{1}{m_k}\gamma_k \mathbf{v}_k^t + \boldsymbol{\theta}_k^t$$

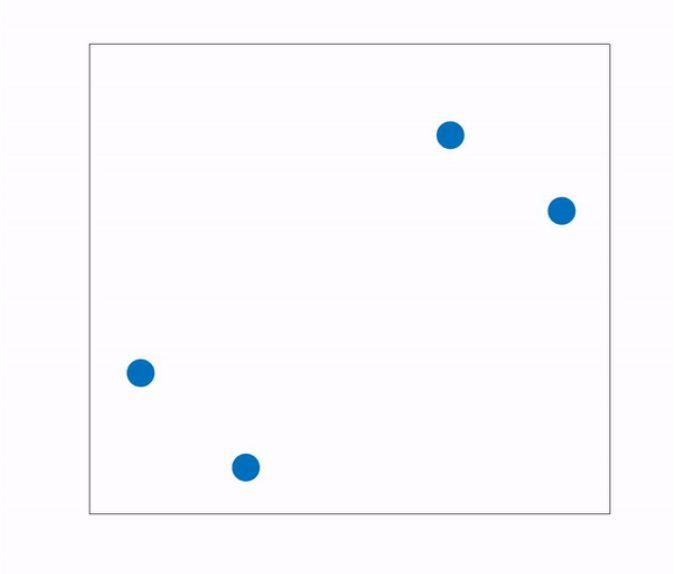
Step 1: update protein's velocity

$$\mathbf{v}_k^{t+1} = \mathbf{v}_k^t + \left(-\frac{1}{m_k}\nabla_{\mathbf{r}_k^t}\frac{\delta \mathscr{F}}{\delta \mathbf{r}_k^t} - \frac{1}{m_k}\gamma_k \mathbf{v}_k^t + \boldsymbol{\theta}_k^t\right) dt$$

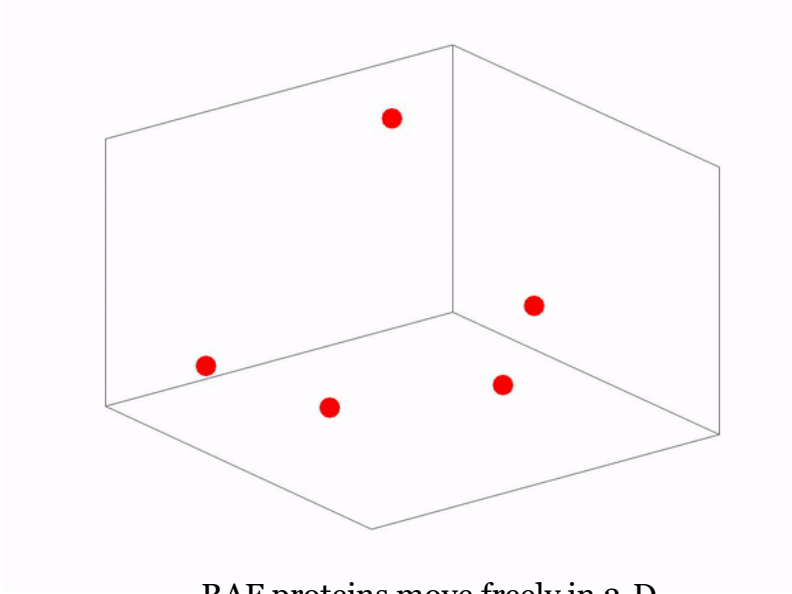
Step 2: update protein's position

$$\mathbf{r}_k^{t+1} = \mathbf{r}_k^t + \mathbf{v}_k^{t+1} dt$$

Protein movement according to Brownian motion with forces from the Lennard-Jones potential



RAS proteins move along the 2-D surface of the cell membrane

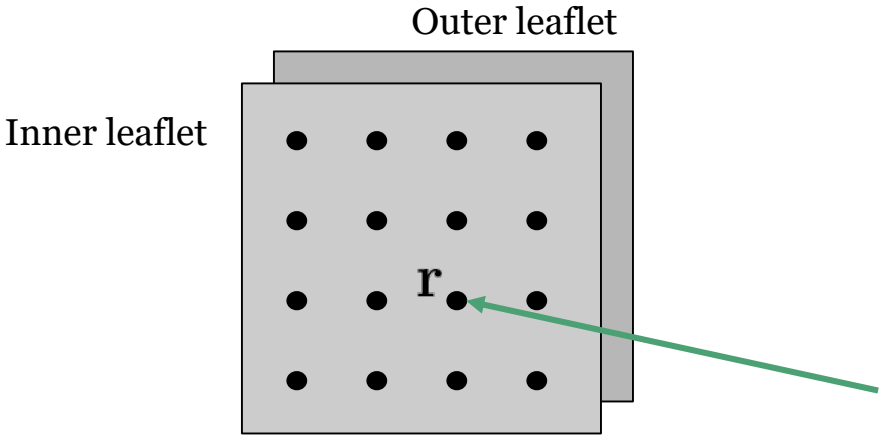


RAF proteins move freely in 3-D above the cell membrane

The Membrane Model

We model the inner and outer leaflets of the membrane as lipid density fields for each lipid species

In our model, the inner and outer leaflets are both composed of two lipid species (POPC and PAPS for the inner leaflet, and POPC and POPE for the outer)



We divide the square region of the membrane into grid points, at which the density of each lipid species on each leaflet is known

$$\{n_{1,1}, n_{1,2}, n_{2,1}, n_{2,2}\}$$

Evolution of the lipid densities in the membrane

Lipid density evolution equation:

$$\frac{\partial n_{i,j}}{\partial t} = \nabla \cdot \left(\beta D_{i,j} n_{i,j} \nabla \left(\frac{\delta \mathscr{F}}{\delta n_{i,j}} \right) \right) + \xi_{i,j}(t)$$

Lipid density evolution equation is solved numerically with Forward Euler's method

Lipid density evolution equation:

$$\frac{\partial n_{i,j}}{\partial t} = \nabla \cdot \left(\beta D_{i,j} n_{i,j} \nabla \left(\frac{\delta \mathscr{F}}{\delta n_{i,j}} \right) \right) + \xi_{i,j}(t)$$

Discretization of the lipid density evolution equation:

$$\frac{n_{i,j}^{t+1} - n_{i,j}^{t}}{dt} = \nabla \cdot \left(\beta D_{i,j} n_{i,j}^{t} \nabla \left(\frac{\delta \mathscr{F}}{\delta n_{i,j}^{t}} \right) \right) + \xi_{i,j}^{t}$$

Solve for lipid density update equation:

$$n_{i,j}^{t+1} = n_{i,j}^{t} + \left(\nabla \cdot \left(\beta D_{i,j} n_{i,j}^{t} \nabla \left(\frac{\delta \mathscr{F}}{\delta n_{i,j}^{t}} \right) \right) + \xi_{i,j}^{t} \right) dt$$

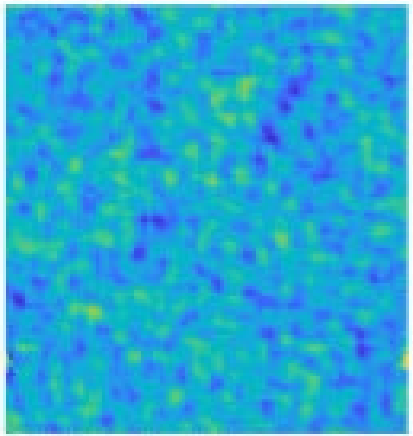
Simulation of RAS proteins on the inner leaflet of the cell membrane

Lipid densities change due to interactions with the RAS proteins and interactions between lipid types within the membrane

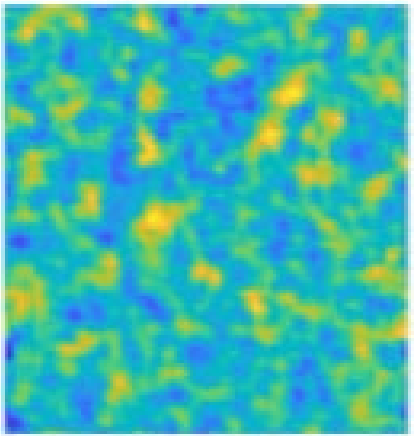
High density

Low density

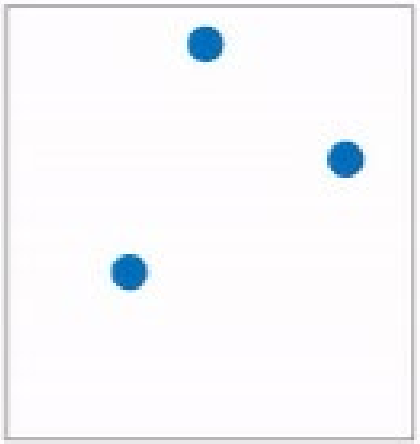
Density of lipid species POPC on inner leaflet



Density of lipid species PAPS on inner leaflet



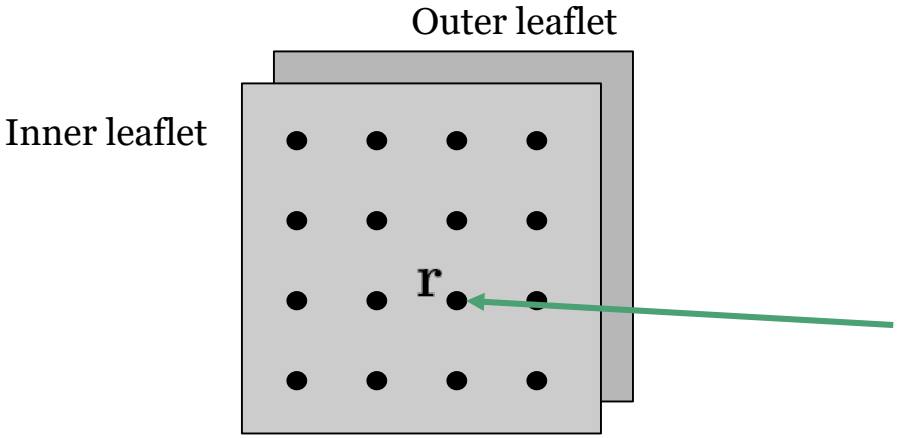
Movement of RAS proteins on membrane



The Height Model

We model the membrane's deformation as a height field

The lipid densities on the inner and outer leaflets affect the height of the membrane as different species have different spontaneous curvatures



The height of the membrane is found for each grid point

$$h(\mathbf{r}, t)$$

Evolution of the membrane's height deformation

Height field evolution equation:

$$\frac{\partial h}{\partial t} = -\mathcal{M}\frac{\delta \mathscr{F}}{\delta h} + \eta(\mathbf{r}, t)$$

Height field equation is solved numerically with the spectral method

Real space height field PDE:

$$\frac{\partial h}{\partial t} = -\mathcal{M}\kappa\nabla^2\left(\nabla^2 h - \sum_{j=1}^{N}(c_{1,j} - c_{2,j})R_j^{-1}\right)$$

The Fourier transform turns derivatives into polynomials, so our fourth order PDE becomes a simple ODE in Fourier space

Fourier transform of height field PDE:

$$\frac{\partial \hat{h}(k)}{\partial t} = -\mathcal{M}\kappa k^2\left(k^2\hat{h} + \hat{\mathcal{K}}\right) \quad \text{where} \quad \mathcal{K} = \sum_{j=1}^{N}(c_{1,j} - c_{2,j})\,R_j^{-1}$$

Height field equation is solved numerically with the spectral method

We solve for forward time step h^{n+1}

Discretized ODE:

$$\frac{\hat{h}^{n+1} - \hat{h}^n}{\Delta t} = -\mathcal{M}\kappa k^2 \left(k^2 \hat{h}^{n+1} + \hat{\mathcal{K}}^n \right) \quad \text{where} \quad \mathcal{K} = \sum_{j=1}^{N} \left(c_{1,j} - c_{2,j} \right) R_j^{-1}$$

Updating equation:

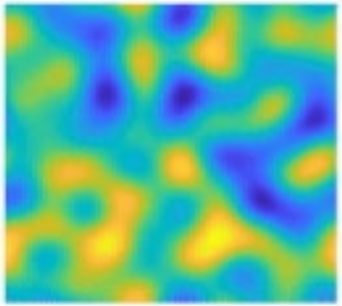
$$\hat{h}^{n+1} = \mu \left(\hat{h}^n - \hat{\mathcal{K}} \sqrt{\left(\frac{1}{\mu} - 1 \right) \Delta t \kappa \mathcal{M}} \right) \quad \text{where} \quad \mu = \frac{1}{1 + \mathcal{M}\kappa k^4 \Delta t}$$

Simulation of the lipid densities, RAS, RAF, and the height deformation

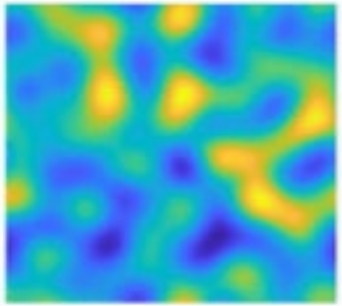
High density

Low density

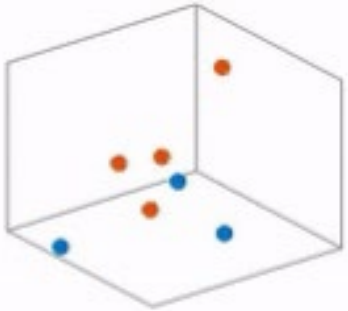
Density of lipid species
POPC on inner leaflet



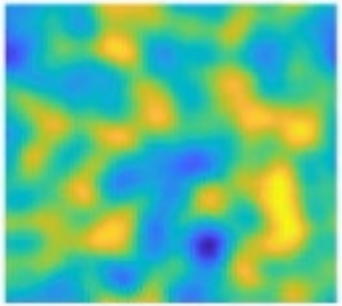
Density of lipid species
PAPS on inner leaflet



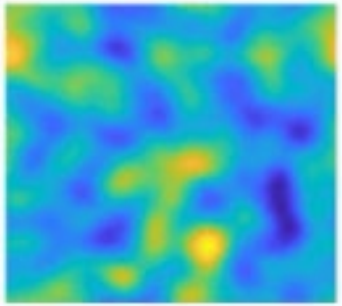
Positions of RAS and
RAF in the cell



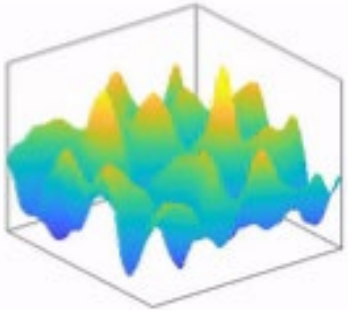
Density of lipid species
POPC on outer leaflet



Density of lipid species
POPE on outer leaflet



Height field



High points

Low points

Conclusions

Conclusions

What we modeled:

- RAS and RAF proteins' movement based on protein-protein and protein-lipid interactions
- Lipid membrane evolution based on lipid-lipid and protein-lipid interactions as well as the height deformation
- Height field evolution based on inner and outer leaflet concentrations

Future Work:

- Our work on RAF and the height deformations will be incorporated into LLNL's model
- LLNL can use our toy code to test new algorithms
- More biologically accurate parameters values will be determined and used

Acknowledgments

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- Academic mentor: Justin Sunu
- Sponsoring Mentors: Liam Stanton, Tomas Oppelstrup, James Glosli, Michael Surh, Frank Graziani
- My RIPS team: Bernardo Antonio Hernandez Adame, Erin Stafford, and Jonathan Galván Bermúdez

Questions?

Sources

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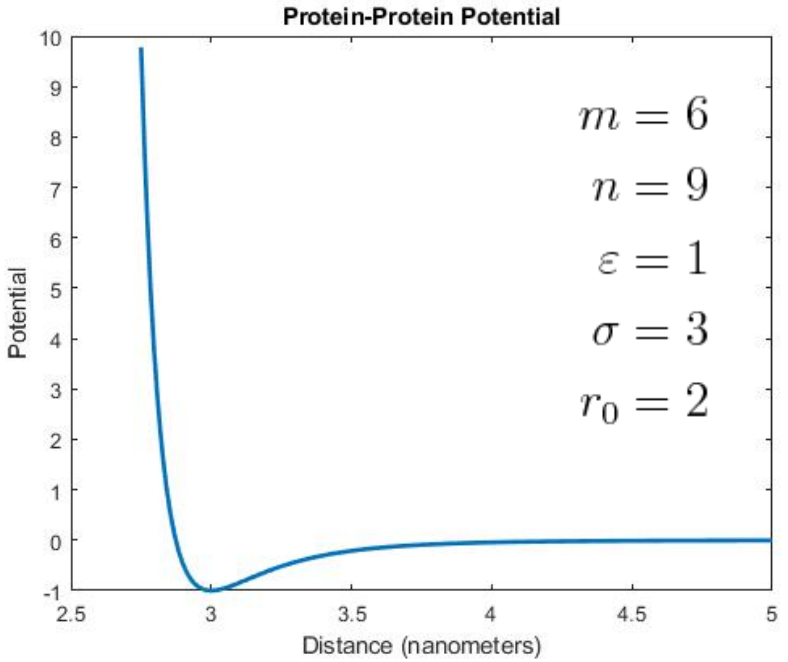
The Lennard-Jones potential is used to simulate protein-protein interactions

General form of the Lennard-Jones potential:

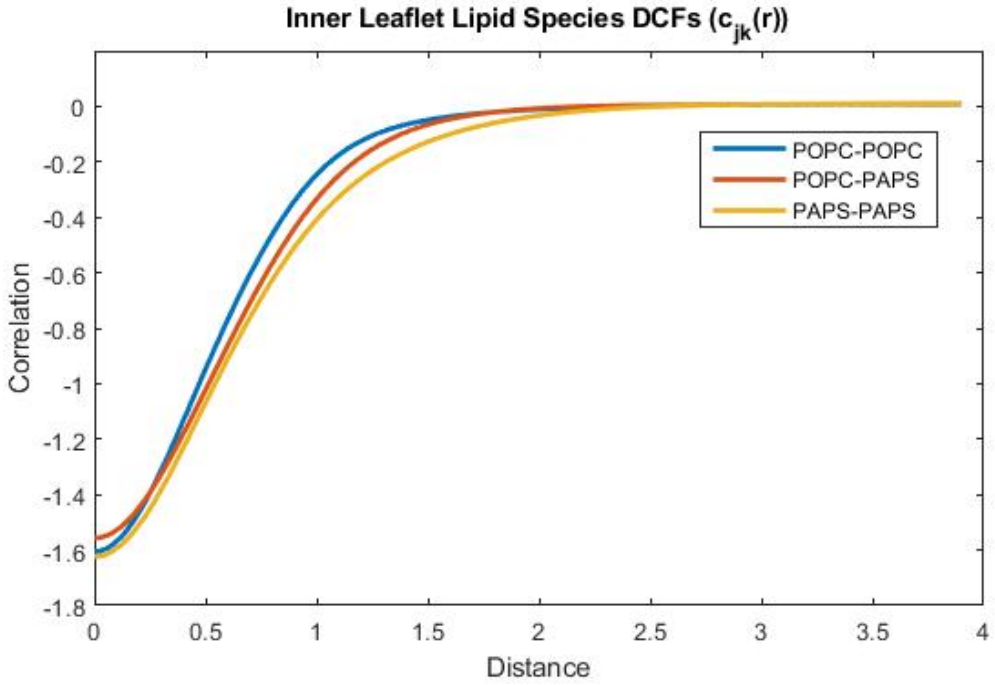
$$u_{kk'} = \frac{m\varepsilon}{n-m}\left[\underbrace{\left(\frac{\sigma - r_0}{r - r_0}\right)^n}_{\text{Repulsive component}} - \frac{n}{m}\underbrace{\left(\frac{\sigma - r_0}{r - r_0}\right)^m}_{\text{Attractive component}}\right]$$

Definitions:

ε	Strength of attraction (well depth)
σ	Distance at which potential reaches its minimum
r	Distance between proteins
r_0	Radius of protein



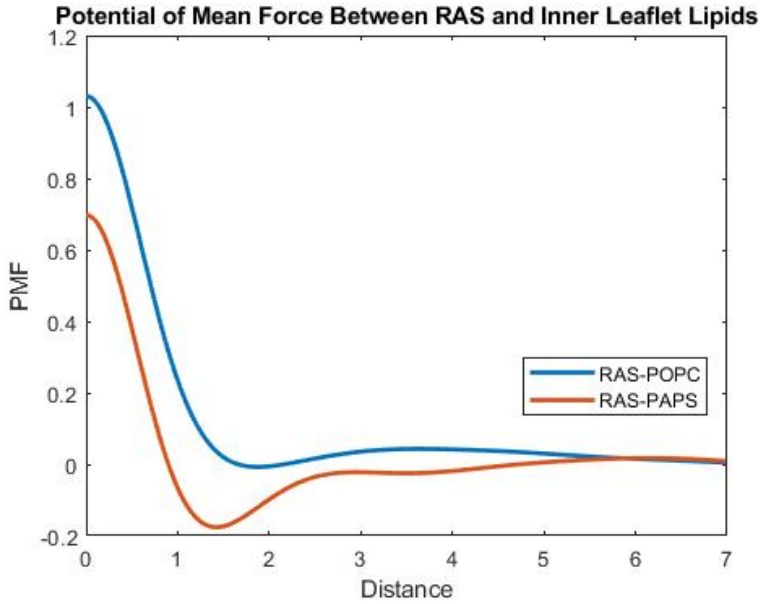
Lipids interact according to direct correlation functions (DCFs) for each pair of lipid species

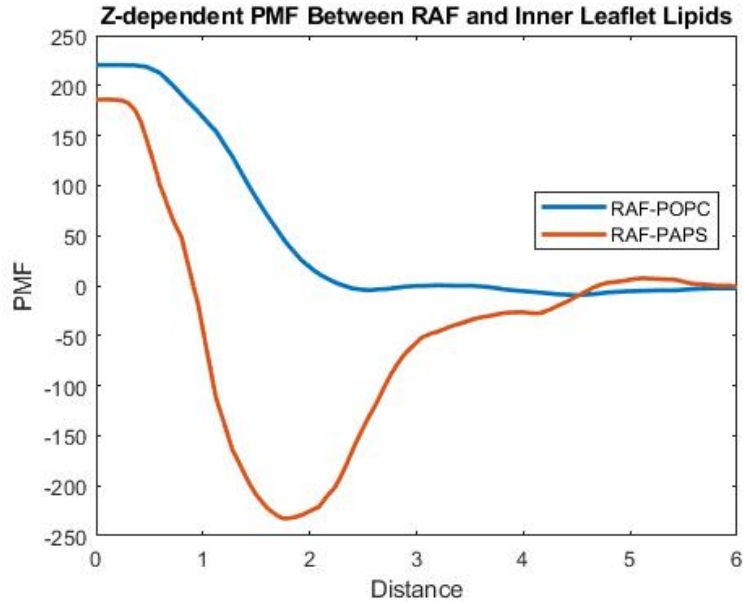


The negative values capture how the lipids try to spread out fairly evenly and do not want to have places of high total density

Lipids and proteins interact according to potentials of mean force (PMFs) between each protein type and lipid species

The PMFs are composed of repulsive and attractive components with lipid type PAPS have a greater attraction to the RAS and RAF proteins



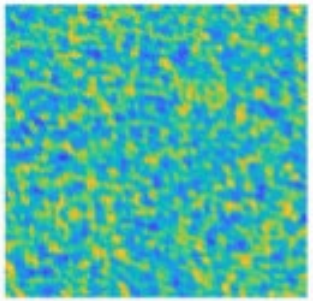


Simulation of RAF proteins above the inner leaflet of the membrane

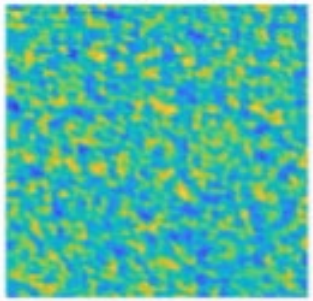
High density

Low density

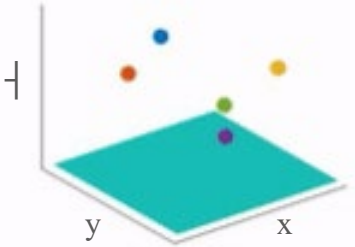
Density of lipid species POPC on inner leaflet



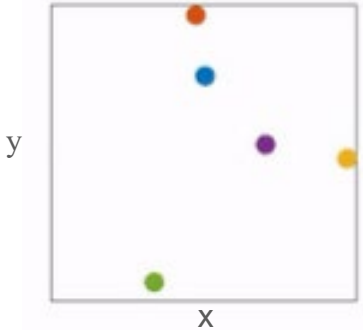
Density of lipid species PAPS on inner leaflet



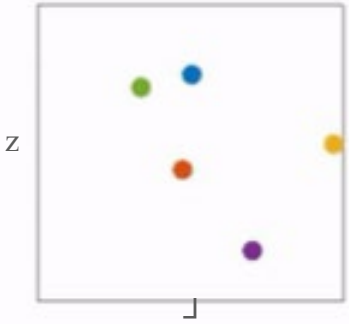
Positions of RAF proteins above the membrane



RAF x-y positions



RAF x-z positions



RAF y-z positions