The Role of Different Lipid Tails in Membrane Fusion

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Mark J. Stevens

Sandia National Laboratories

Center for Integrated Nanotechnologies(CINT)
msteve@sandia.gov

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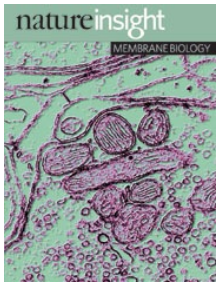
Biomembranes

- Biomembranes are *really* important in biology
  - container, barrier, surface, transport
  - Membranes are biologically functional—an actor in the cell
    - e.g. signaling
    - PIP_2 lipid as second messenger
  - Lipids influence molecules that pass thru or reside in membrane
    - Domains (‘rafts’) organize proteins
    - lipid interactions affect protein dynamics
  - understanding the role of lipids is essential
    - dynamic
    - group (membrane as unit)



Dec. 2005

- Lipid membranes as a material
  - present new material opportunities
  - are active, flexible, **liquid**, self-assembled surface
  - patterned surfaces
  - 3D surfaces

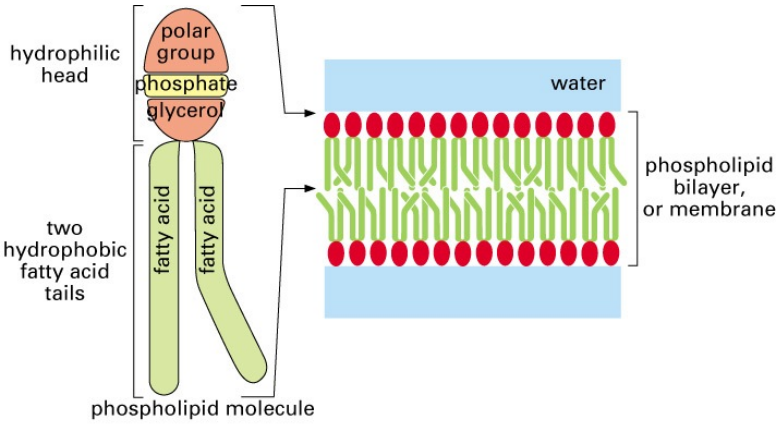


Figure 2–22. Molecular Biology of the Cell, 4th Edition.

Mark J. Stevens
msteve@sandia.gov

Fusion: Lipid Bilayer vs. Biomembranes

- Underlying physical chemistry is probably the same as in biological fusion.
  - Problem of merging bilayers the same
  - Many pure lipid systems fuse spontaneously

- Lipid fusion important in its own right.
  - Drug delivery vehicles, sensors, etc.
  - Materials Science

- Just plain interesting science.

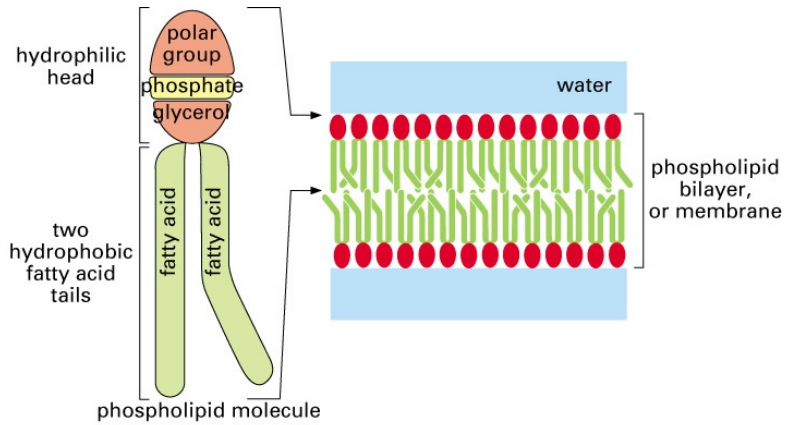


Figure 2–22. Molecular Biology of the Cell, 4th Edition.

Mark J. Stevens
msteve@sandia.gov

Helfrich Free Energy

$$F_H = \int dS\{\kappa(c_1 + c_2 - c_0)^2 + \kappa_G c_1 c_2\}$$
$$= \int dS\{\kappa/2(H - c_0)^2 + \kappa_G K\}$$

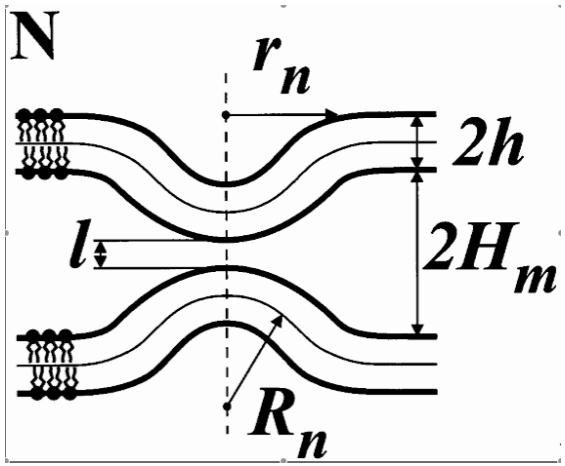
- The κ are the bending moduli: mean, Gaussian
- curvatures: spontaneous c_0; mean H; Gaussian K

Inputs are the bending moduli κ.

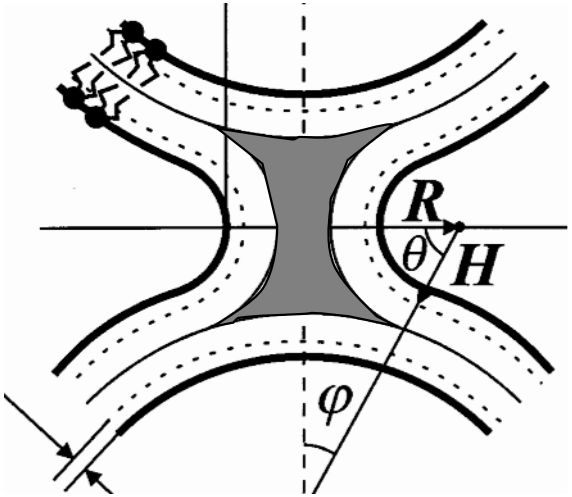
If molecular model reproduces moduli, rest will follow and can obtain molecular mechanisms.

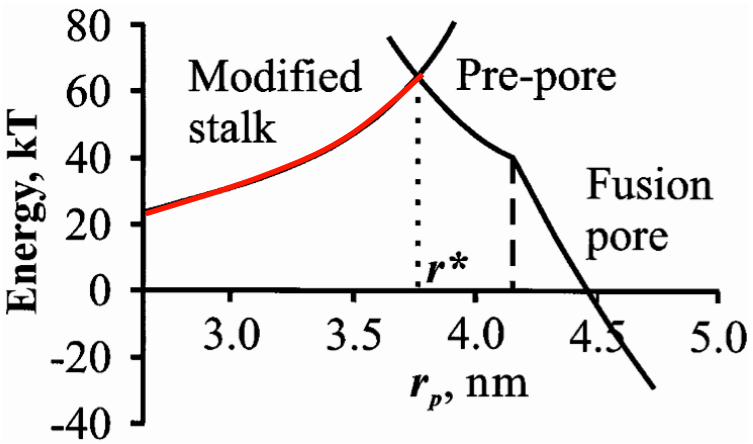
Mark Stevens
msteve@sandia.gov

Stalk & Free Energy

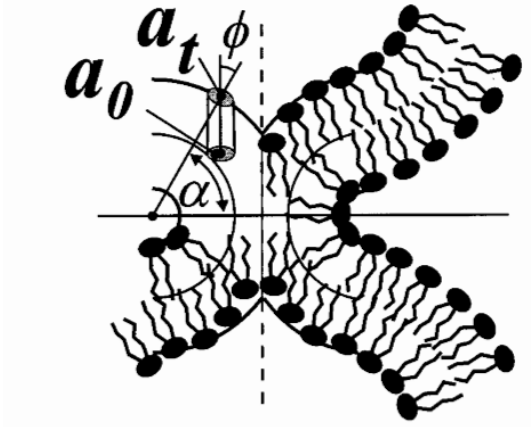


Original Stalk
merger of outer leaflets





m-S modified Stalk



Free energy
- bending of bilayer
- tilt within bilayer
- 'hydration' interaction
- 40 kT barrier (R_n=8nm)

Kuzmin et al. PNAS 01

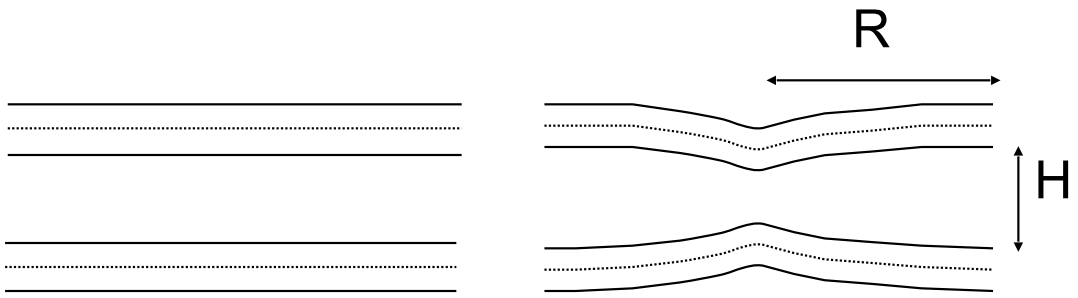
Mark Stevens
msteve@sandia.gov

Free Energy (Kozlovsky & Kozlov, 2002)

$$f_{\text{tot}} = \frac{1}{2} \cdot \kappa \cdot (\text{div}\,\mathbf{n} - \tilde{J}_s)^2 + \frac{1}{2} \cdot \kappa_t \cdot \mathbf{t}^2 - \frac{1}{2} \cdot \kappa \cdot \tilde{J}_s^2$$

- f_{tot} is monolayer free energy/area
- Additional terms: splay and tilt of whole lipids (cf. liquid crystals)

- Stalk energy: F = 43 kT
- H = 5-10 nm R > 20 nm



parallel bilayers apposing protrusions

Probability of occuring?

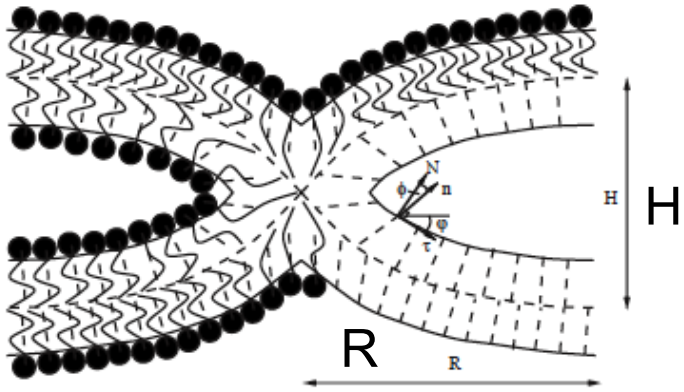
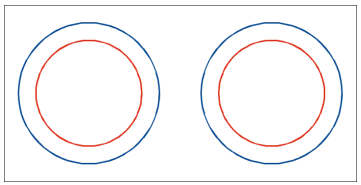
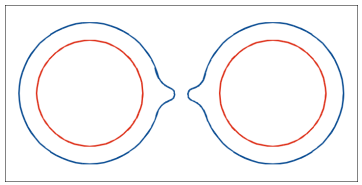
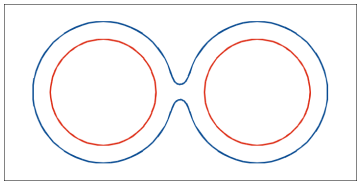
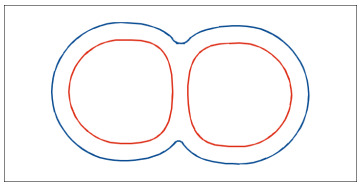
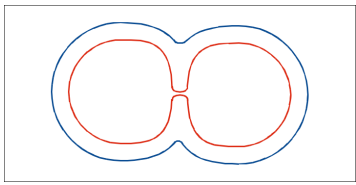
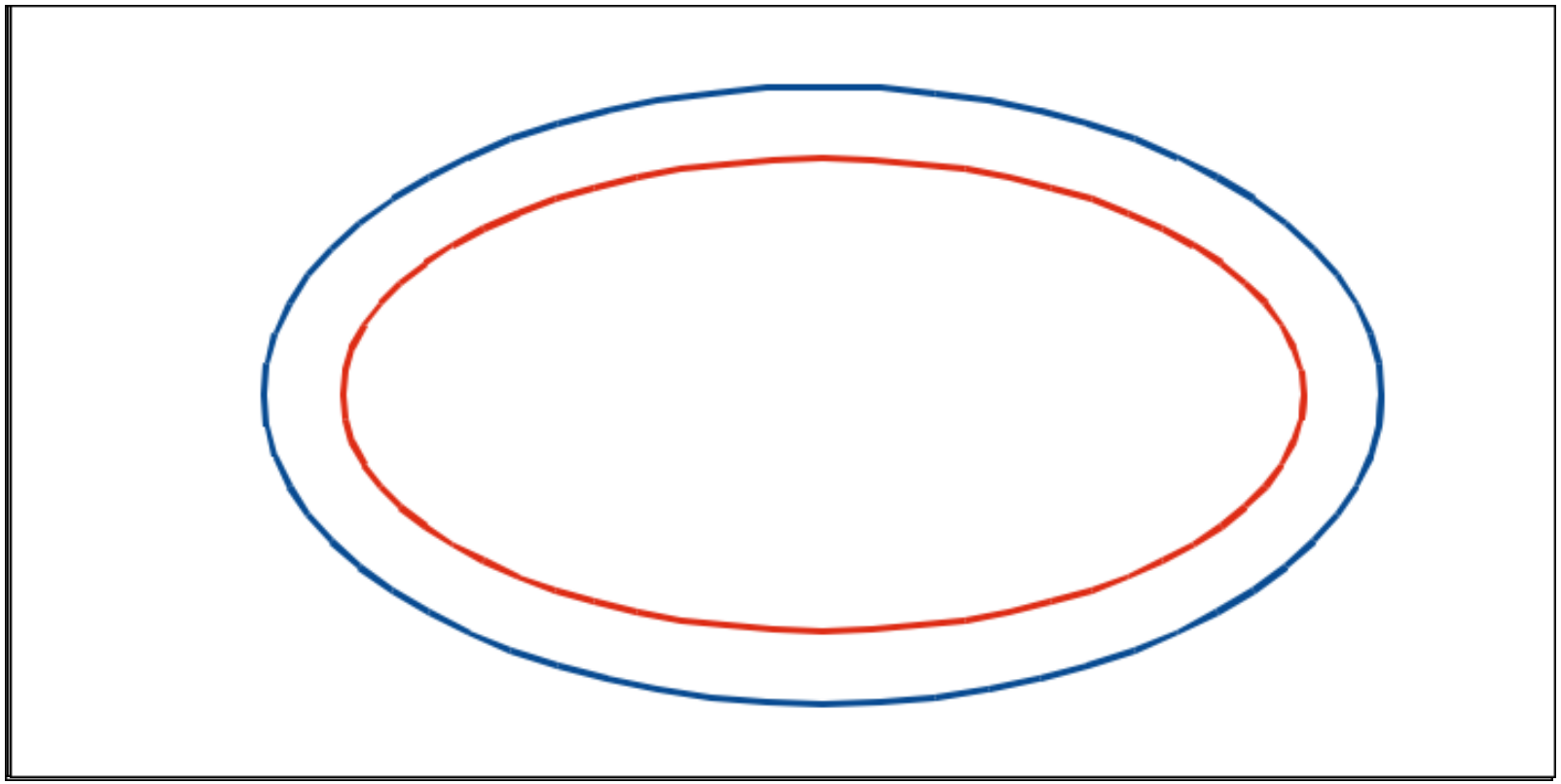


FIGURE 2 Tilt model for stalk and TMC. The shape is axially symmet-

Mark Stevens
msteve@sandia.gov

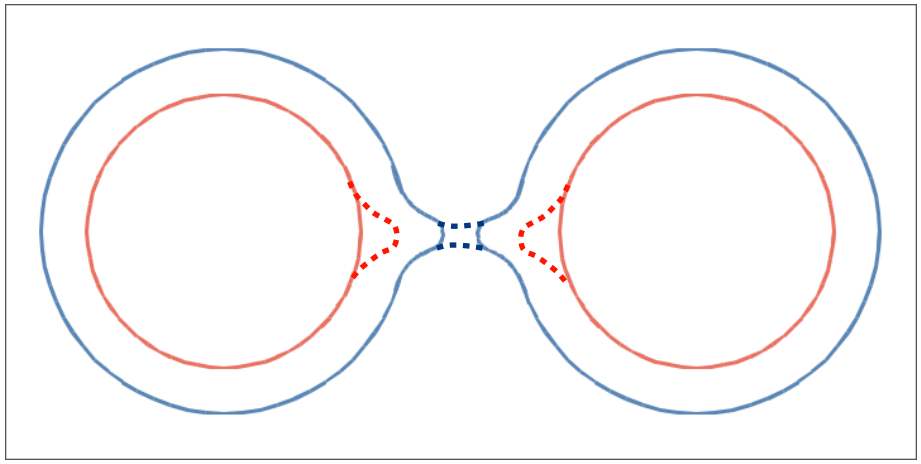
General View of Fusion



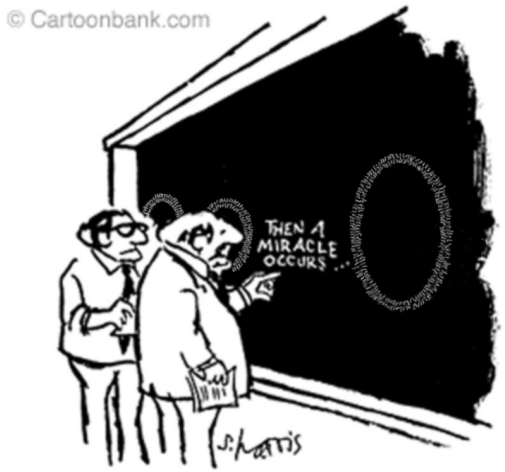
Mark J. Stevens
msteve@sandia.gov

How do you get there from here?



- topological change
- hydrophobic tails cross water

What happens on the molecular scale?



"I think you should be more explicit here in step two."

What's stopping us?

- Experiment
  - very fast process (milliseconds)
  - very small (molecular scale events)
  - stochastic/noisy
- Simulations
  - too slow
  - too big
  - stochastic/noisy

Mark J. Stevens
msteve@sandia.gov

Simulating Biomembranes

We want to simulate lipid membranes &
must treat liquid dynamics.

Lipid diffusion is 'slow'

- diffusion constant ~ 10^{-8} cm^2/s = 10^{-3} nm^2/ns
- lipid exchange time ~ 100 ns
- too slow for atomistic simulations

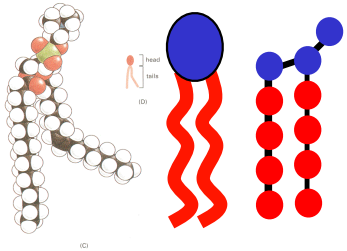
$\Rightarrow$ need to use coarse-grained models

Mark J. Stevens
msteve@sandia.gov

Coarse-grained Models

- Follow successful coarse-grained models in polymer physics
  - Modified Goetz & Lipowsky model + parallel computer
  - Marrink's bead-spring models
  - proteins
    - Thirumalai, T. Head-Gordon

    → fusion proteins

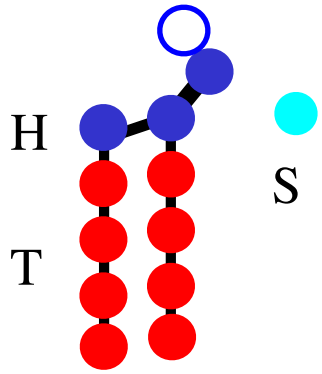


- Can treat essential physical features that drive key phenomena
  - connectivity
  - hydrophobic/hydrophilic interactions
    - self-assembly
  - membrane fluidity

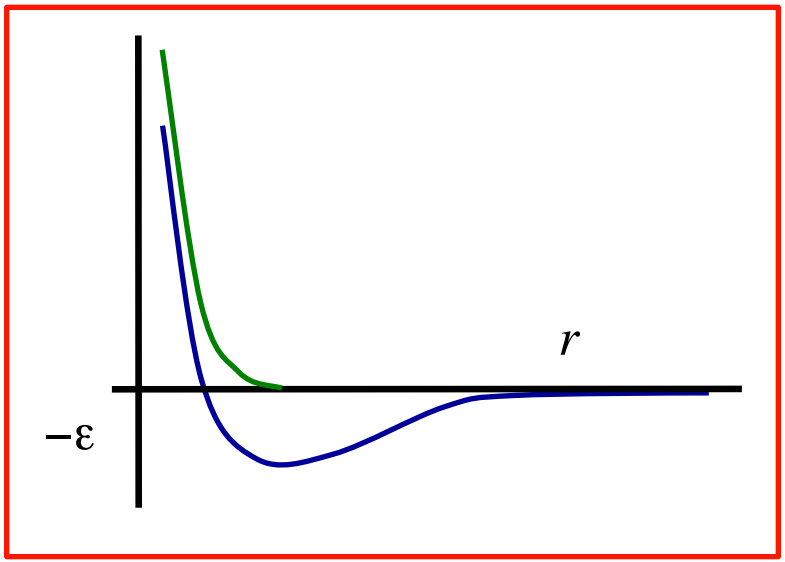
- Expect this is sufficient for more complex phenomena
  - microdomains
  - fusion
  - membrane-protein interactions

Mark J. Stevens
msteve@sandia.gov

Lipid Model: Interactions



1 bead ≈ 3 C atoms



Lennard-Jones (LJ) potential

The purely repulsive LJ potential u_{RLJ}

is cutoff and shifted at $r_c = 2^{1/6}\sigma$.

Parameters:

$\varepsilon_{\alpha\beta} = \varepsilon$, and $\sigma_{\alpha\beta} = \sigma$ for all pair types $\alpha\beta$

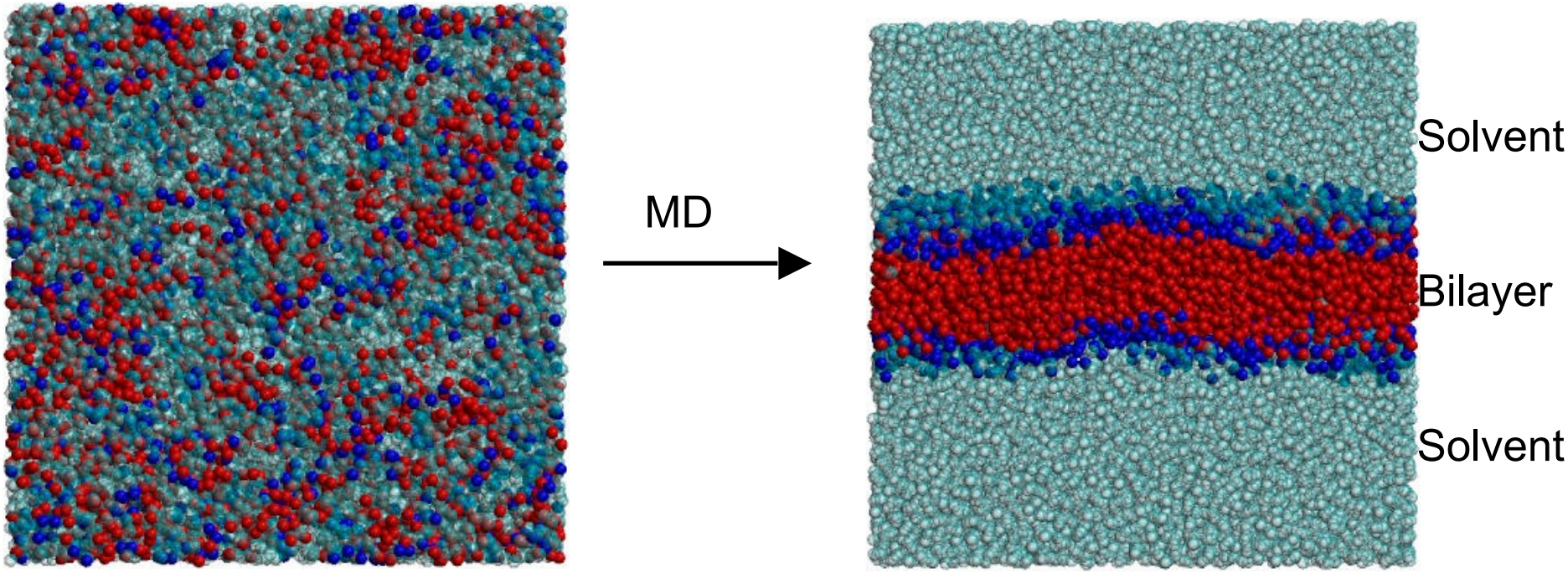
$r_{c,\alpha\beta}$ = 2.5 σ for $\alpha\beta$ = HH, TT, SS, HS

= $2^{1/6}\sigma$ for $\alpha\beta$ = HT, TS

$$4\varepsilon_{\alpha\beta}\left[\left(\frac{\sigma_{\alpha\beta}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{\alpha\beta}}{r_{ij}}\right)^{6}\right]$$

Mark J. Stevens
msteve@sandia.gov

Simulations of Bilayer Membranes



Membrane self-assembly?

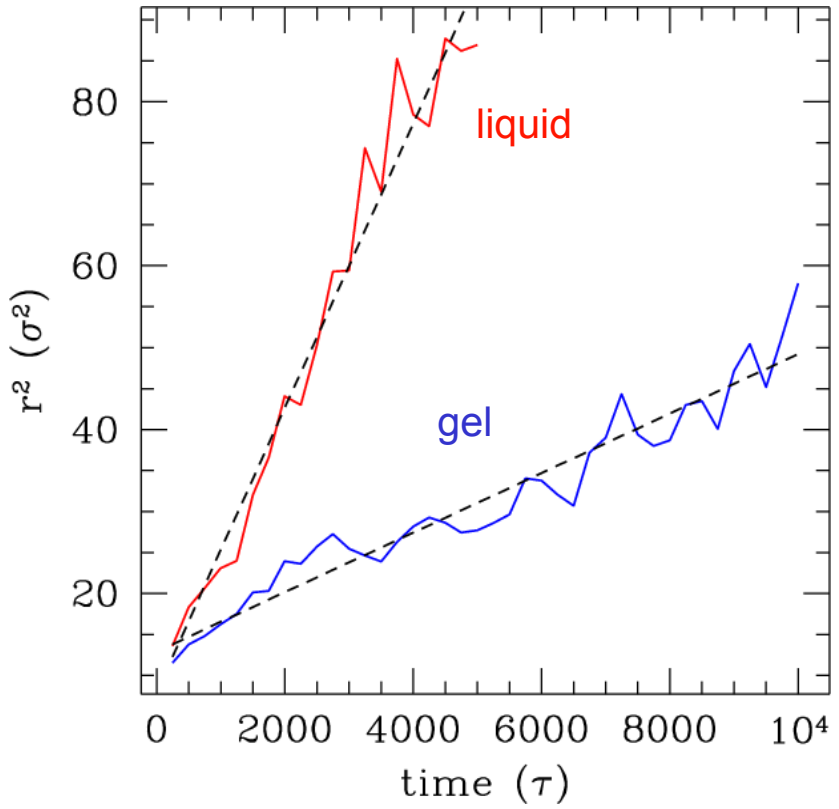
Mark J. Stevens
msteve@sandia.gov

Liquid Membrane

Verifying fluidity of bilayer

- Lipids diffuse across simulation box
- Lipid diffusion not possible presently in atomistic simulations

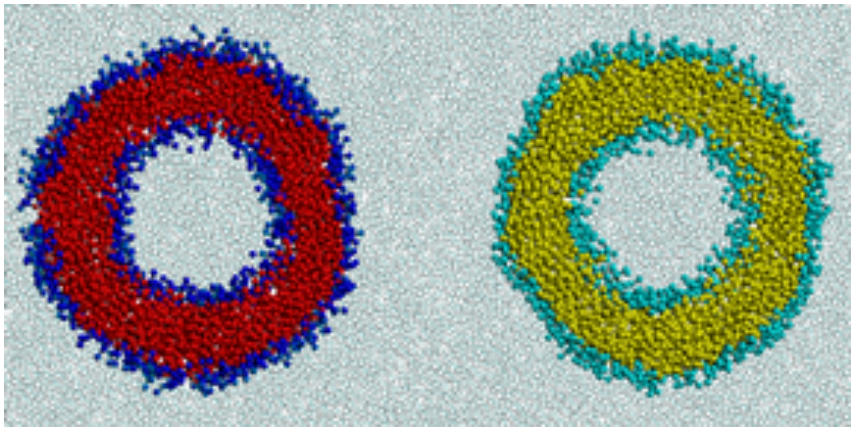
- Matching diffusion times yields map:
  - LJ time unit $\tau \rightarrow 0.2$ ns.
  - 5000 $\tau = 1.0$ μs.
- times in the μs to ms range achievable



Mark J. Stevens
msteve@sandia.gov

Early Work: Fusion Simulation Setup

- Create single liposome by placing lipids on inner & outer spheres: D = 30 σ = 15 nm, N_T = 4.
- Apply constant force to bring liposome together
- Images are slices (3D system)

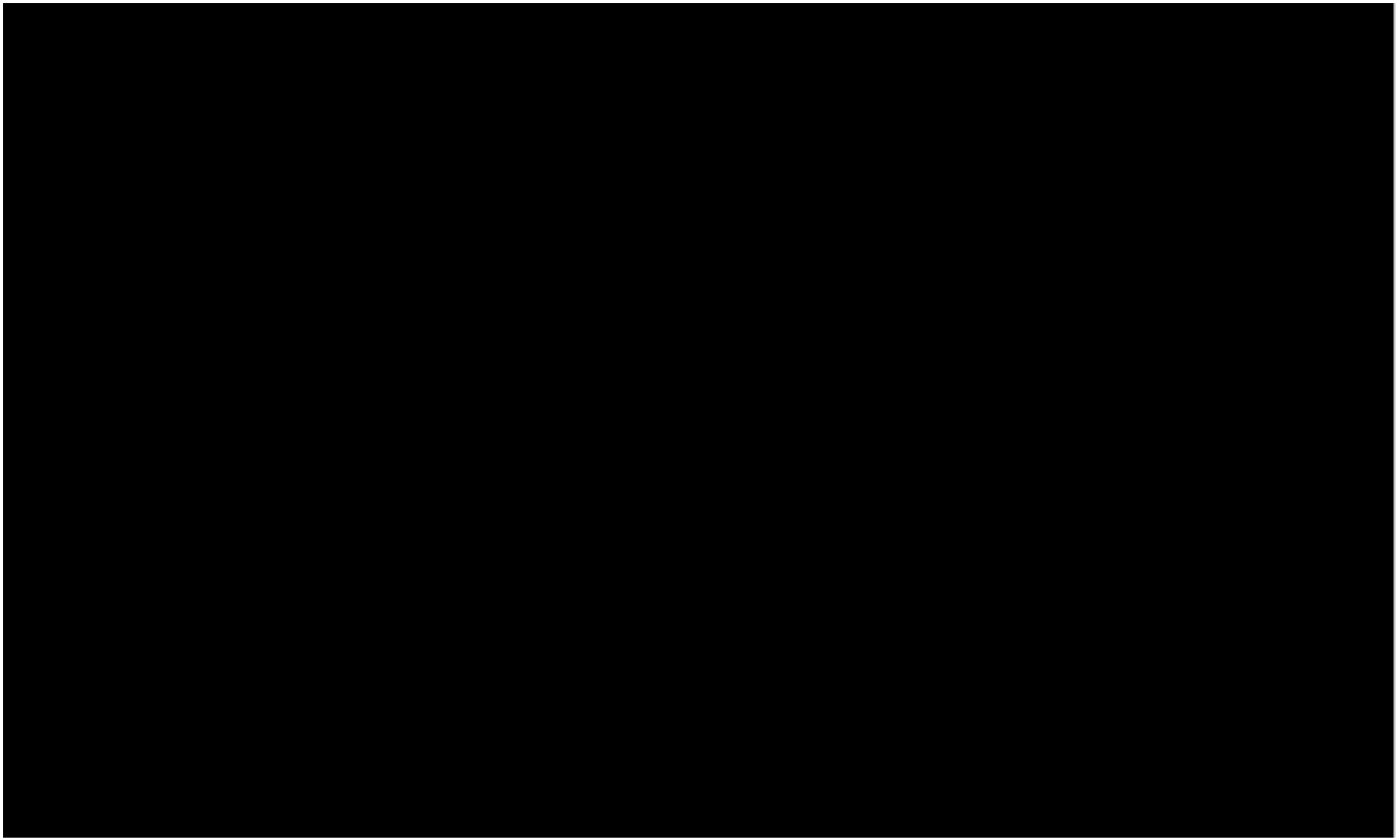


f f

2158 lipids/liposome

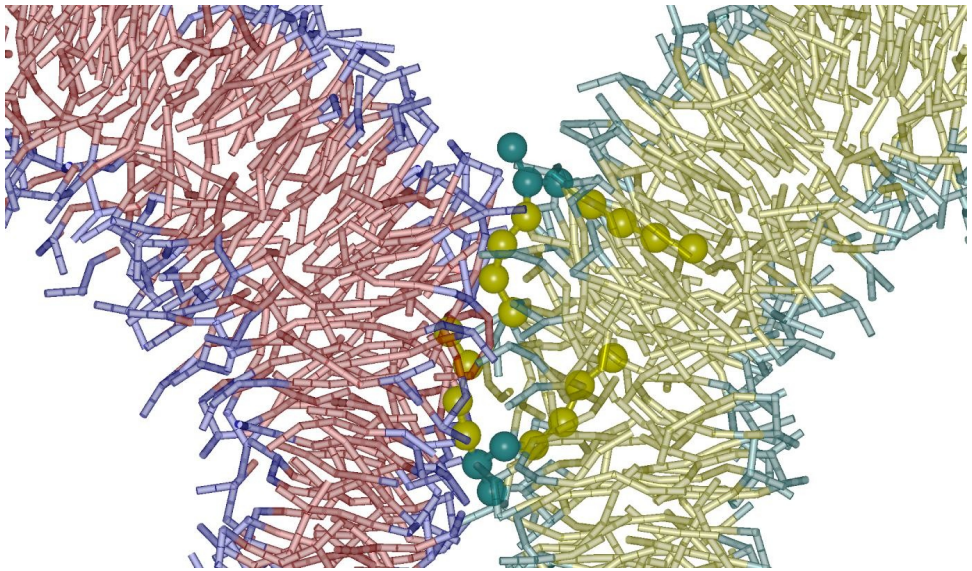
333680 total beads

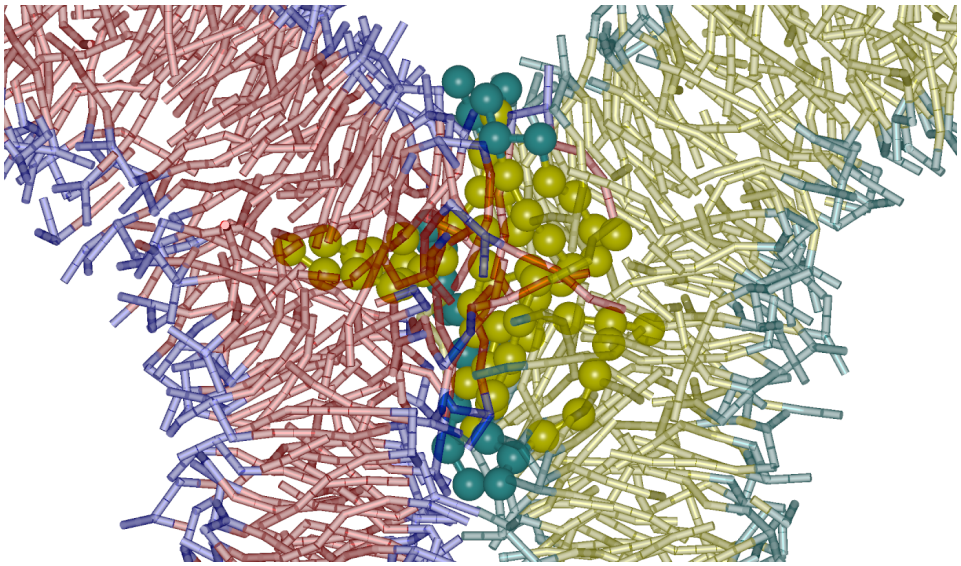
Mark J. Stevens
msteve@sandia.gov

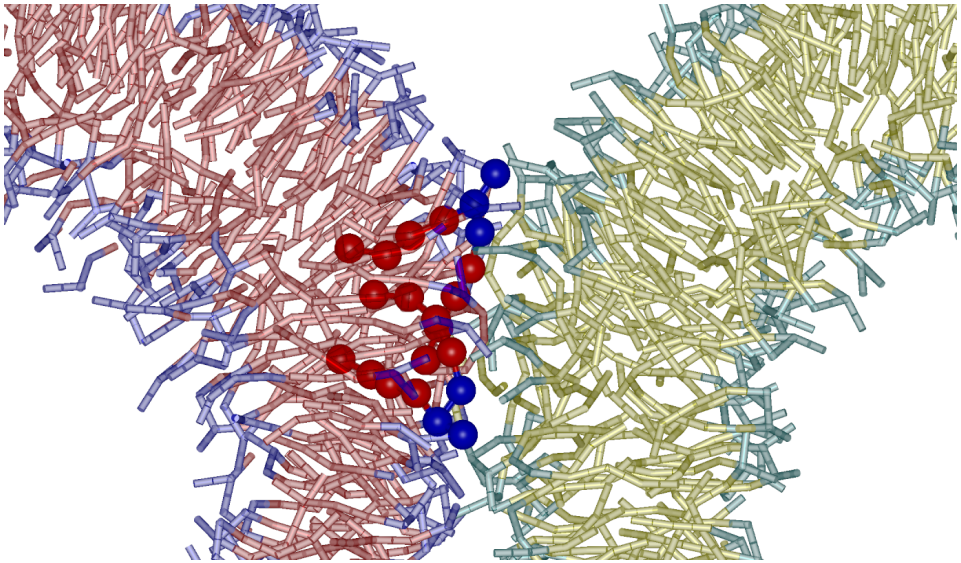


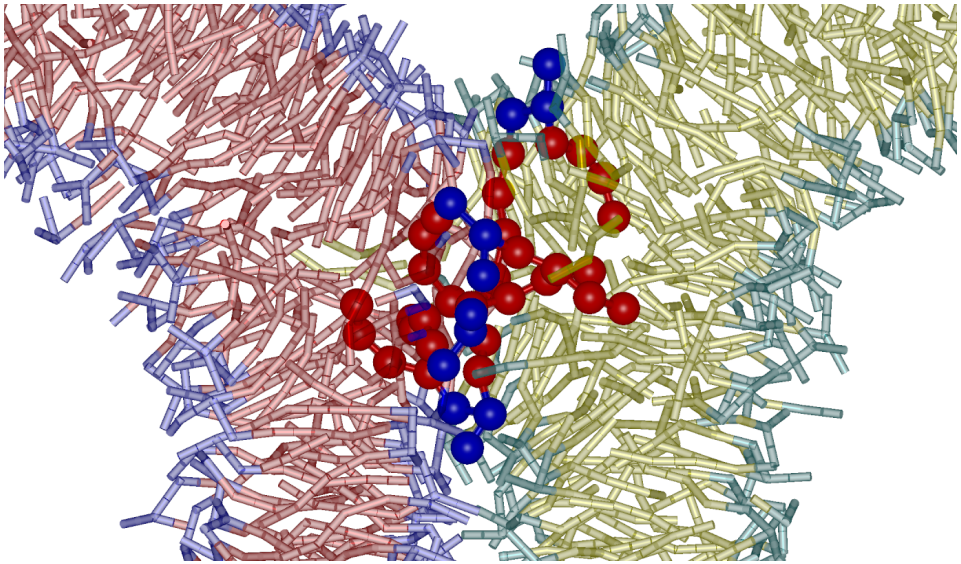
Mark J. Stevens
msteve@sandia.gov

Splayed Lipids Initiate Fusion









Mark Stevens
msteve@sandia.gov

Fluorescence Experiments

J.M. Holopainen, J.Y.A. Lehtonen and P.K.J. Kinnunen, Biophysical J. **76**, 2111 (1999).

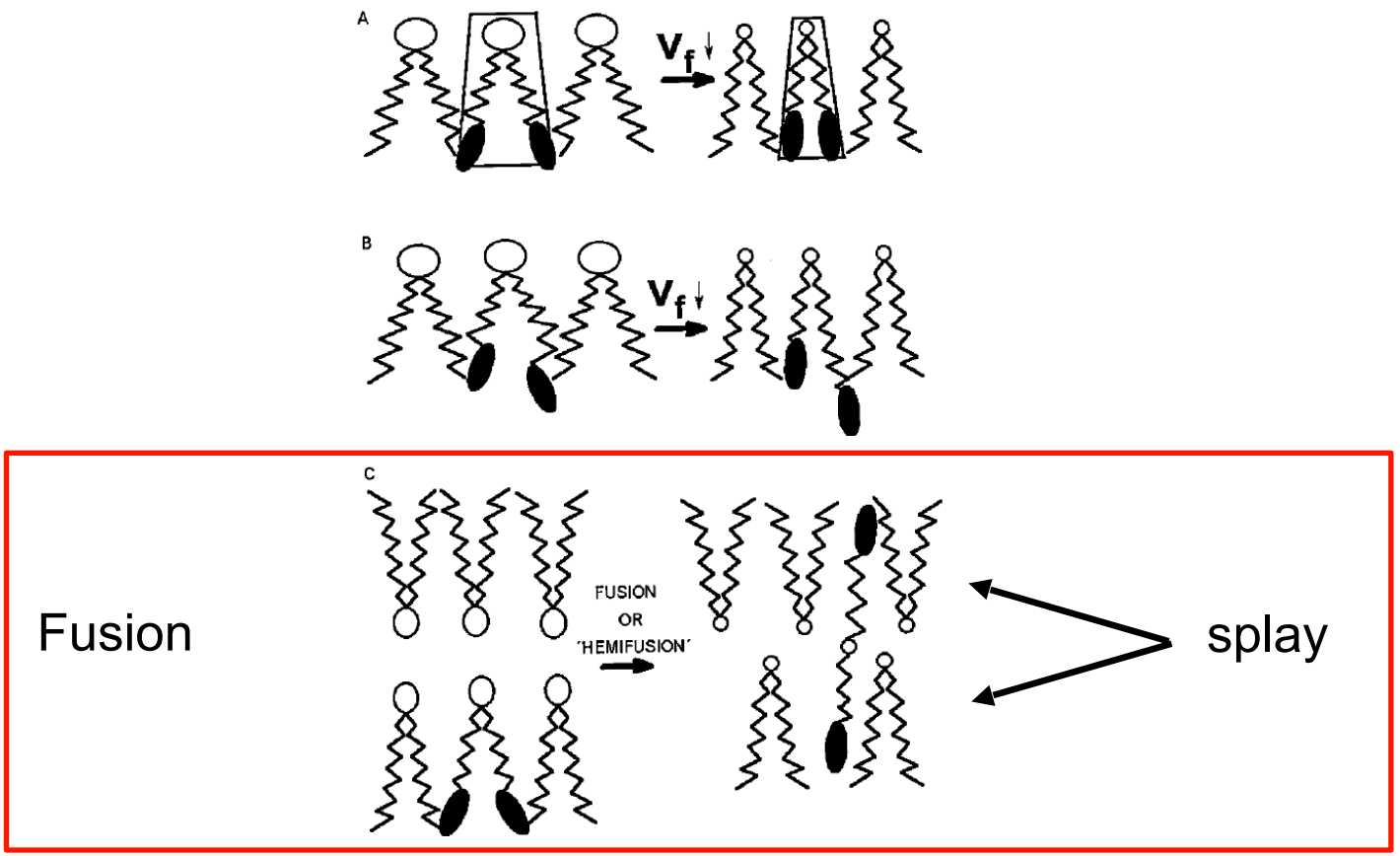


FIGURE 7 A schematic illustration of the changes in the acyl chain alignment for bis-PDPC (*A*) and PDPTPC (*B*) upon decreasing the membrane free volume by dehydration of the polar headgroup by PEG or Me^{2+}. Hemifusion of bilayers and the adoption of the extended conformation by bis-PDPC is depicted in *C*. The pyrene moiety is represented by the filled black ellipse.

Mark Stevens
msteve@sandia.gov

Present Work: Lipid Types

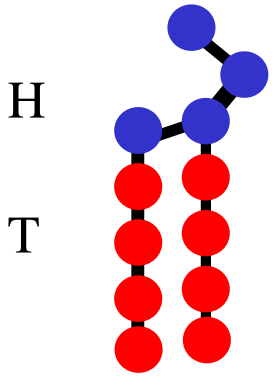
Marrink's Original force-field

Primarily vary tail characteristics

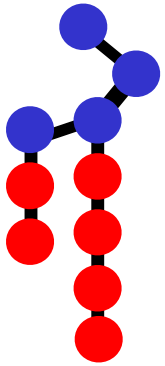
DPPC

DPPC/DPPE mixture (3:1)

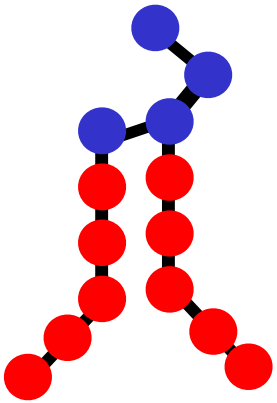
- DPPE forms nonlamellar phases at high T



asymmetric tail



DOPC

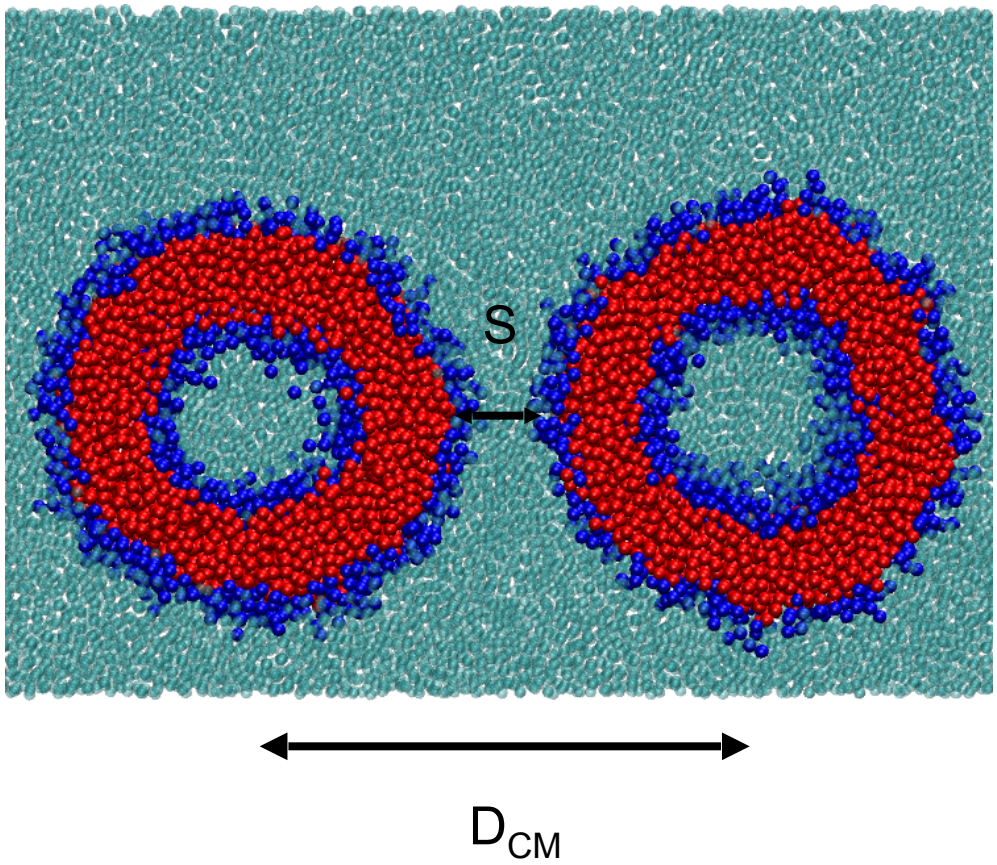


Mark Stevens
msteve@sandia.gov

Fusion Simulation Setup

- Marrink's original force-field
- self-assembled configurations of Marrink
- 877 lipids per vesicle
- diameter ~ 13 nm
- T = 325K

- Calculate free energy
  - many simulations
  - expensive



Mark Stevens
msteve@sandia.gov

WHAM

Weighted Histogram Analysis Method

Kumar, et al. J Comp Chem, 13, 1011-1021, 1992

Calculate free energy (difference) along reaction path

Probability $P(x) \propto e^{-F(x)/k_B T}$

Use Umbrella Sampling with bias potential to treat barrier

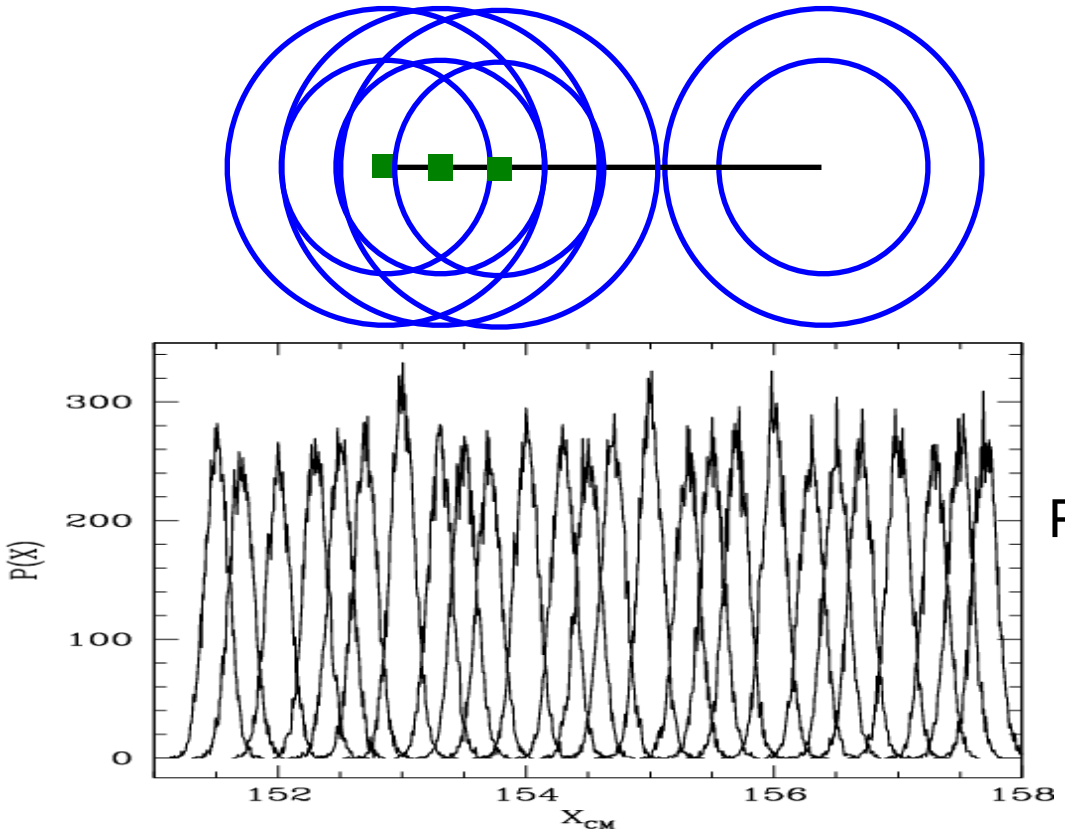
$$F(x) = -k_B T \log P_b(x) - U_b(x) + F_0$$

Self consistent set of equations to solve for F(x) (WHAM)

Mark Stevens
msteve@sandia.gov

WHAM

Reaction path: center of mass separation
Constraint: vesicle center of mass separation distance
Histogram of separation distances
Distributions must overlap
every 0.2-0.3Å



Part of DOPC histogram set

Mark Stevens
msteve@sandia.gov

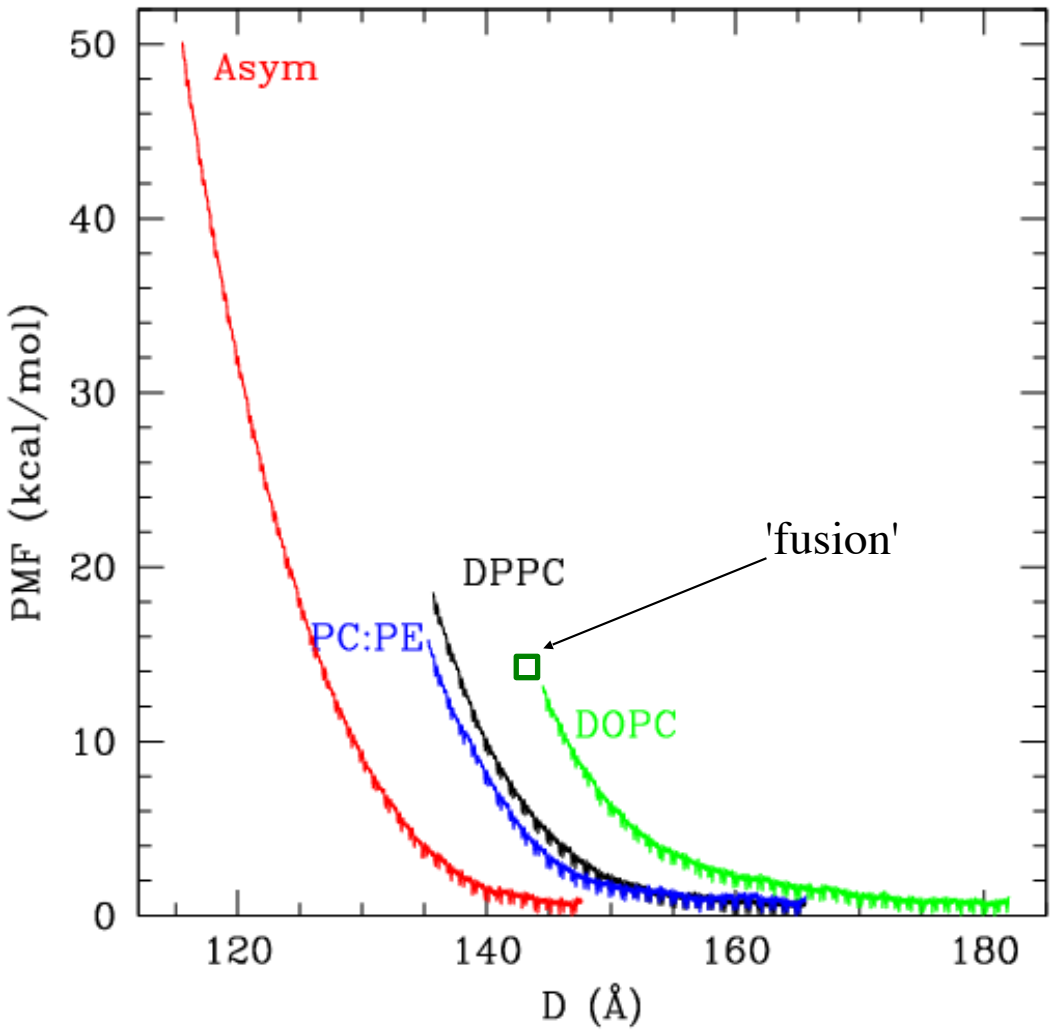
PMF

Smallest D occurs just before mixing of lipids between vesicles.

barrier height ~ 15 kcal/mol

except for asymmetric case, 50 kcal/mol

Fusion separation distance depends on lipid type. (vesicle size)



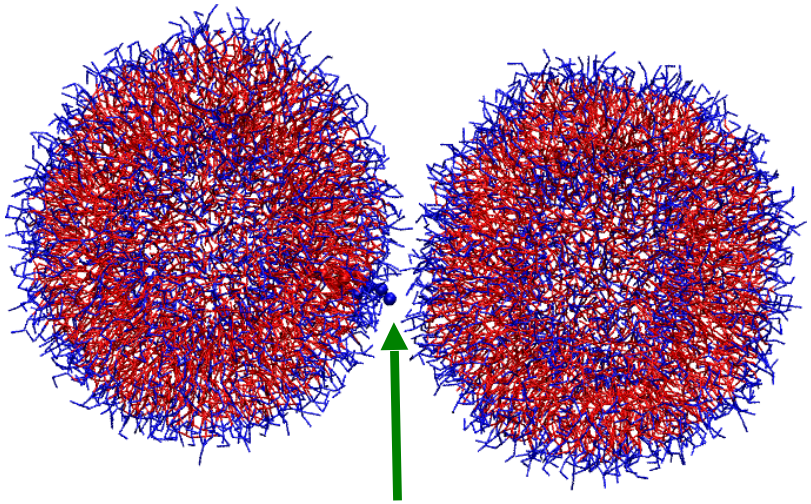
CM separation

Mark Stevens
msteve@sandia.gov

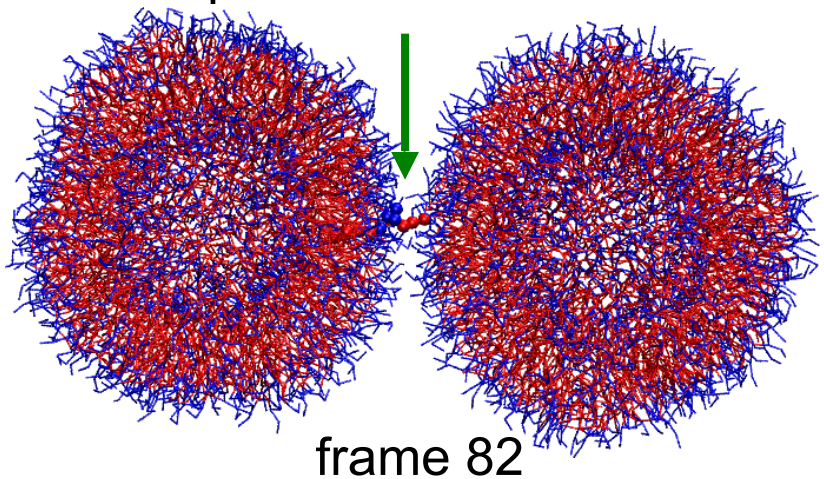
Fusion initiation (DPPC)

R_{CM}= 135.5 Å

frame 24

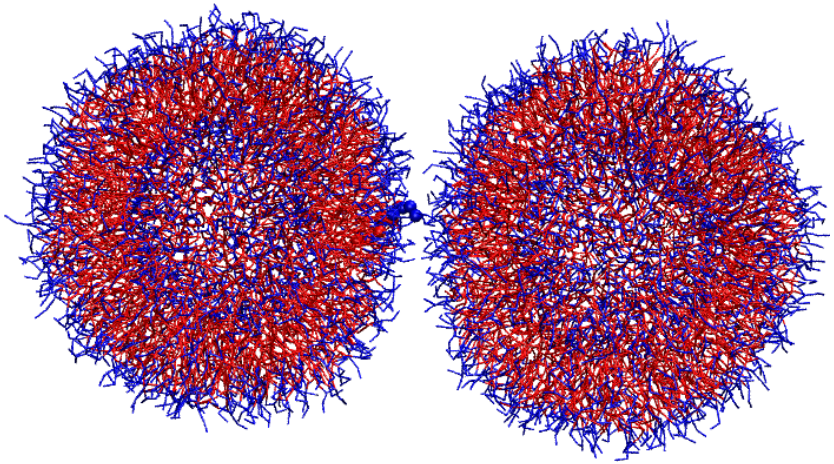


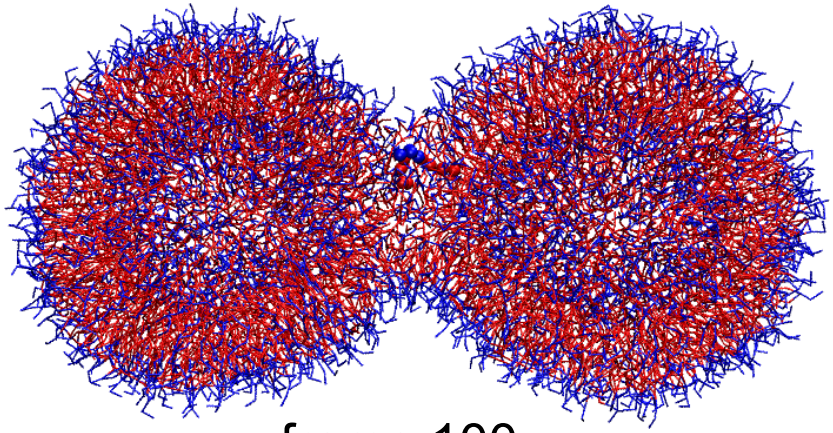
lipid initiates fusion



frame 82

frame 81: just before fusion event

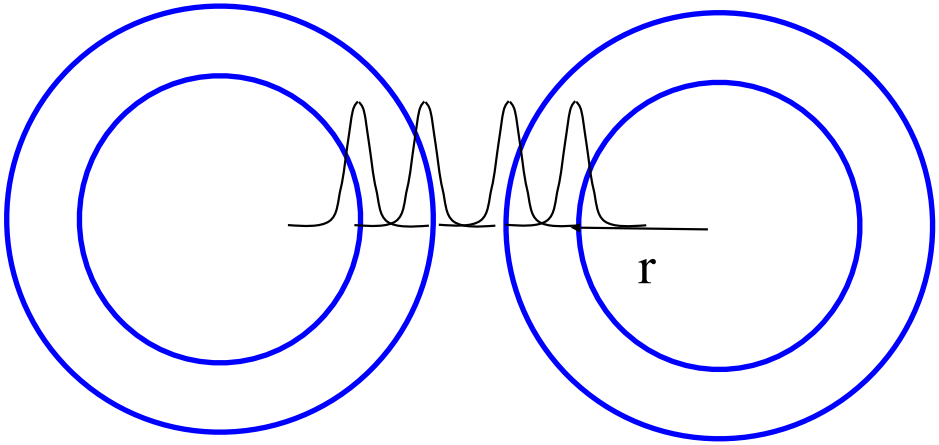


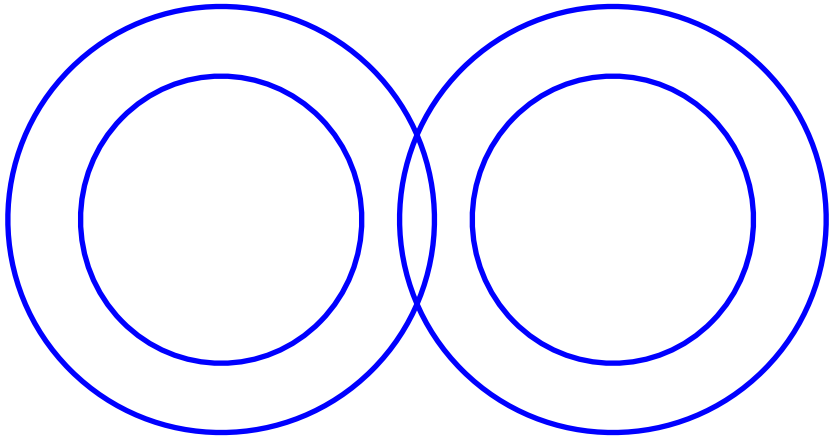


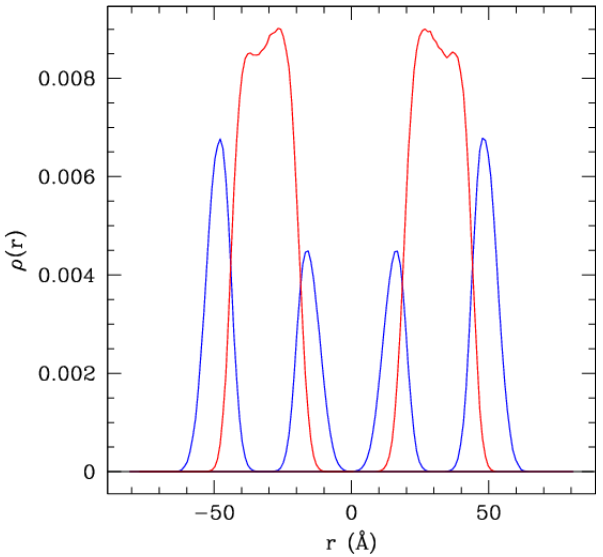
frame 100

Mark Stevens
msteve@sandia.gov

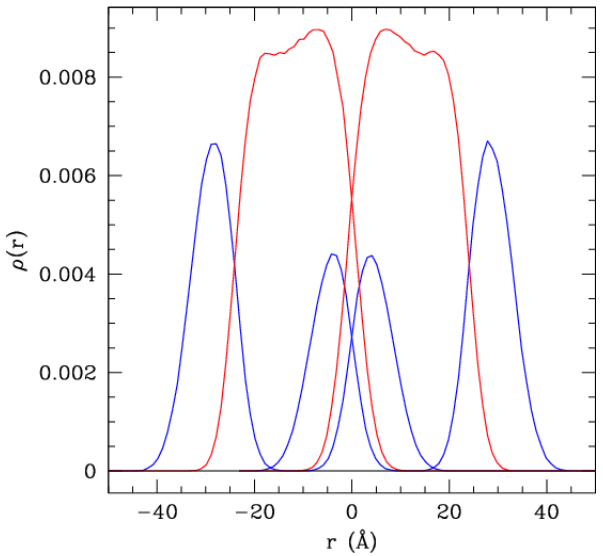
Radial density profile: Deformation







DPPC
165.7Å



135.7Å
just before
fusion

Mark Stevens
msteve@sandia.gov

scaled PMF

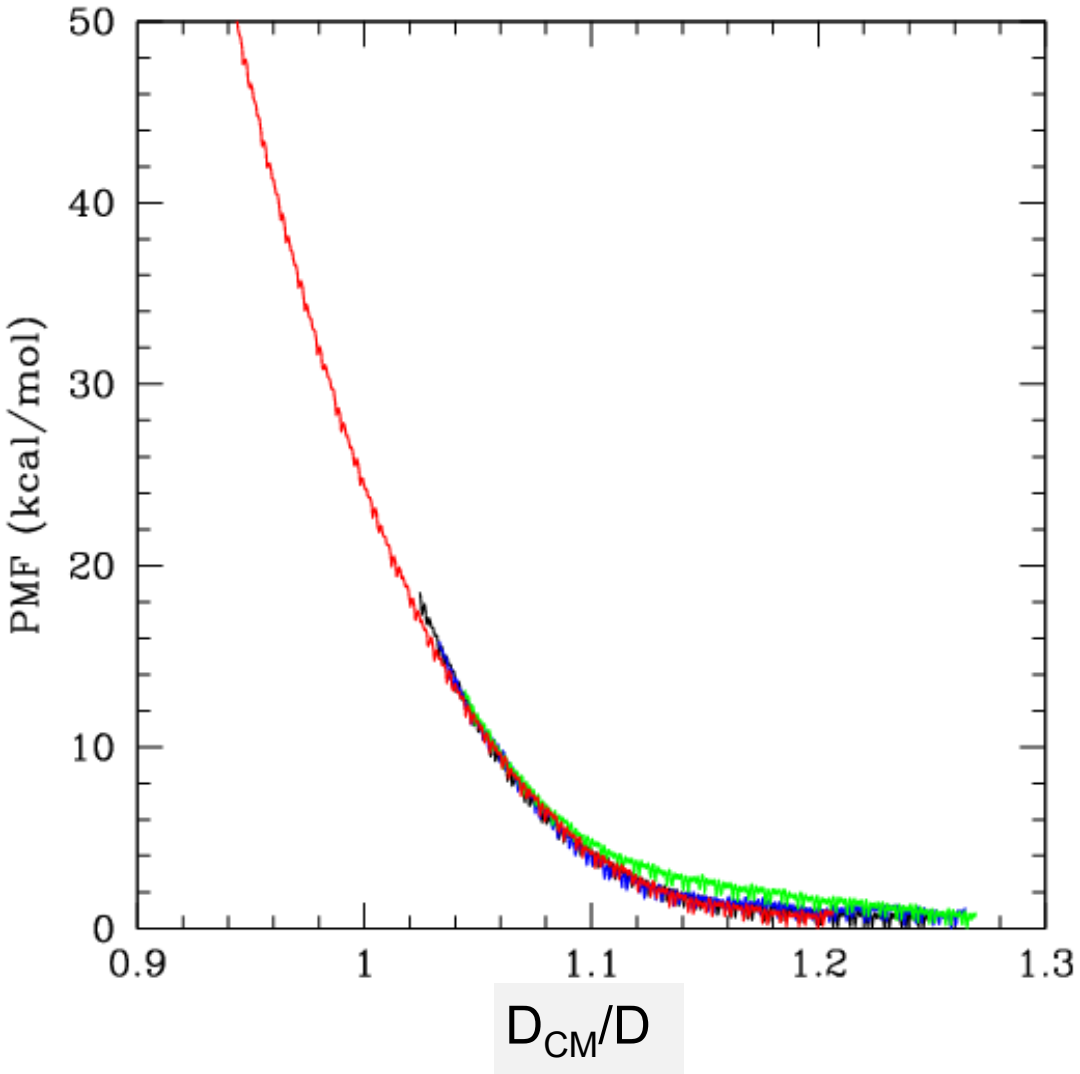
Plot versus D_{CM}/D

D is the vesicle diameter

Free energy contributions are

1.squeezing the water

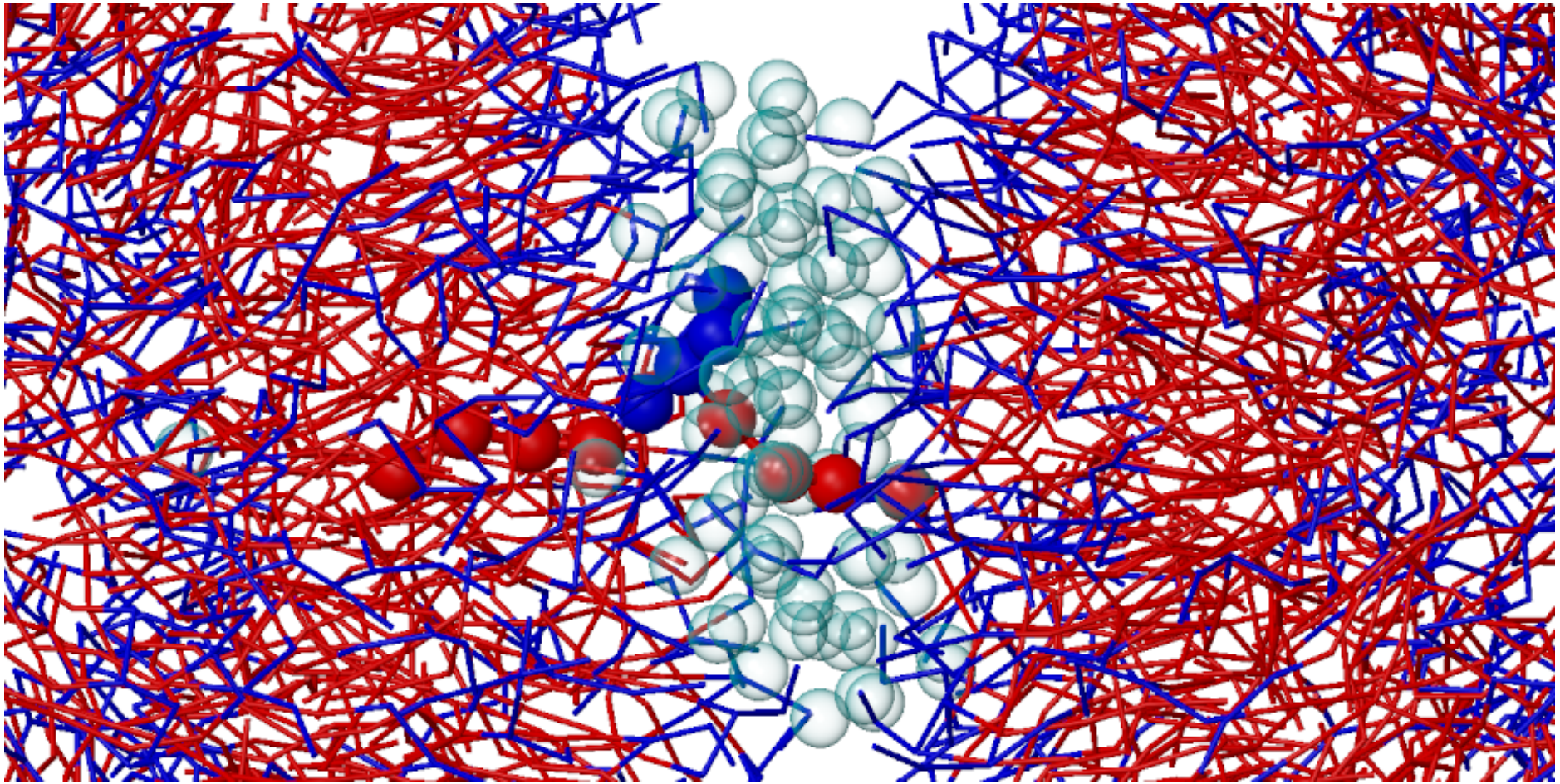
2.vesicle deformation



Mark Stevens
msteve@sandia.gov

Fusion Initiation: DPPC

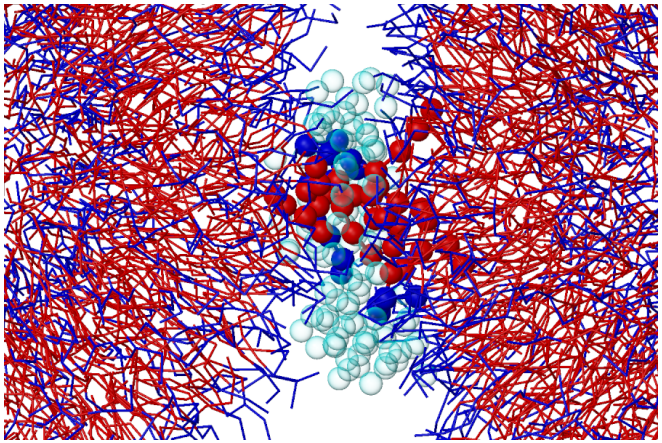
Tail Splay



Mark Stevens
msteve@sandia.gov

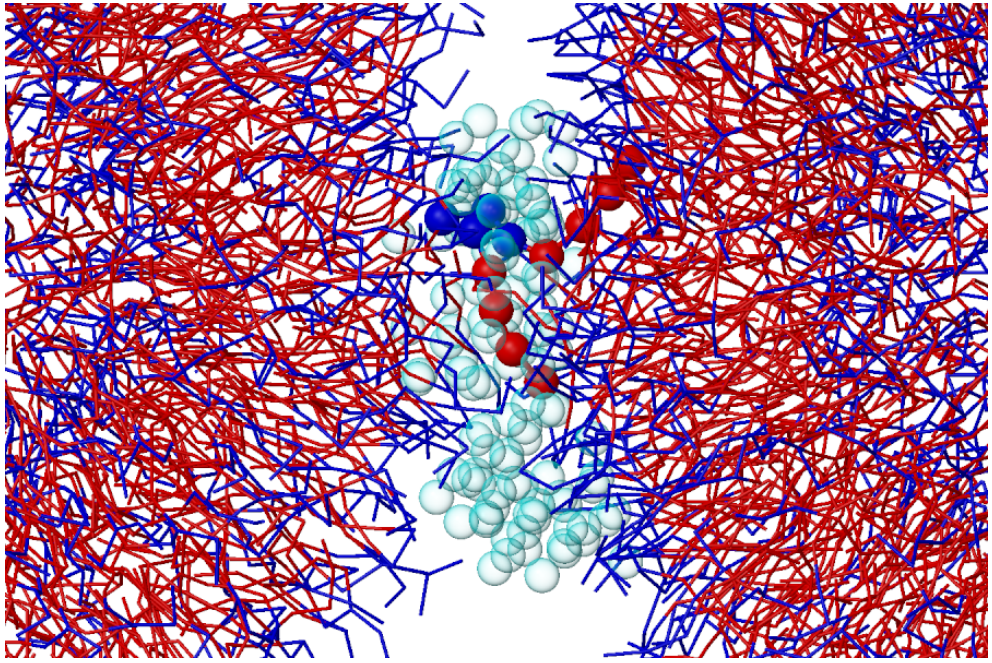
Fusion Initiation: PC/PE

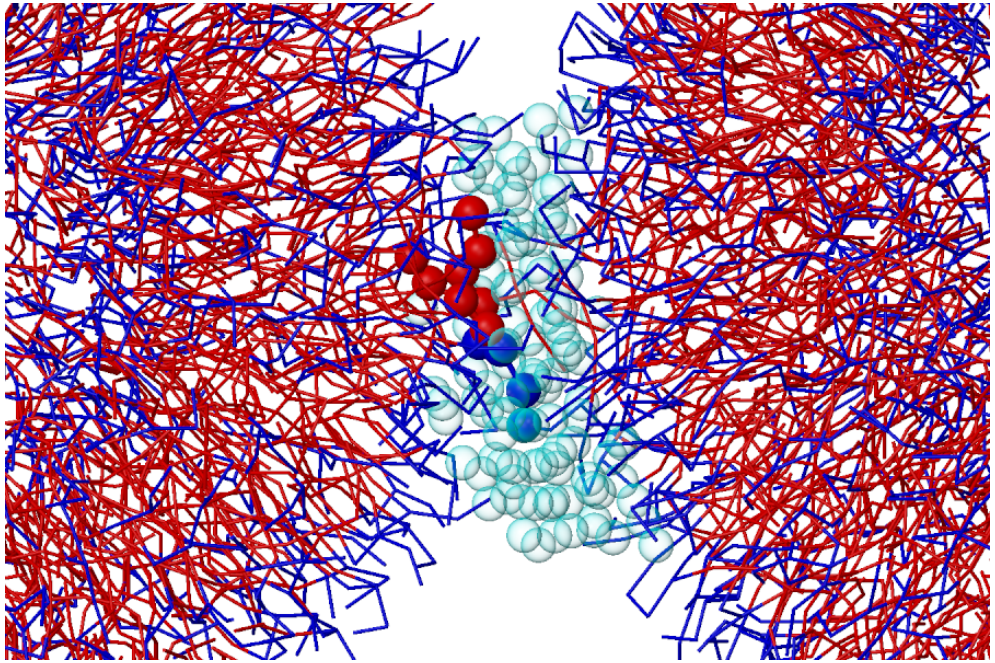
Multiple lipids



Splay

Rotated



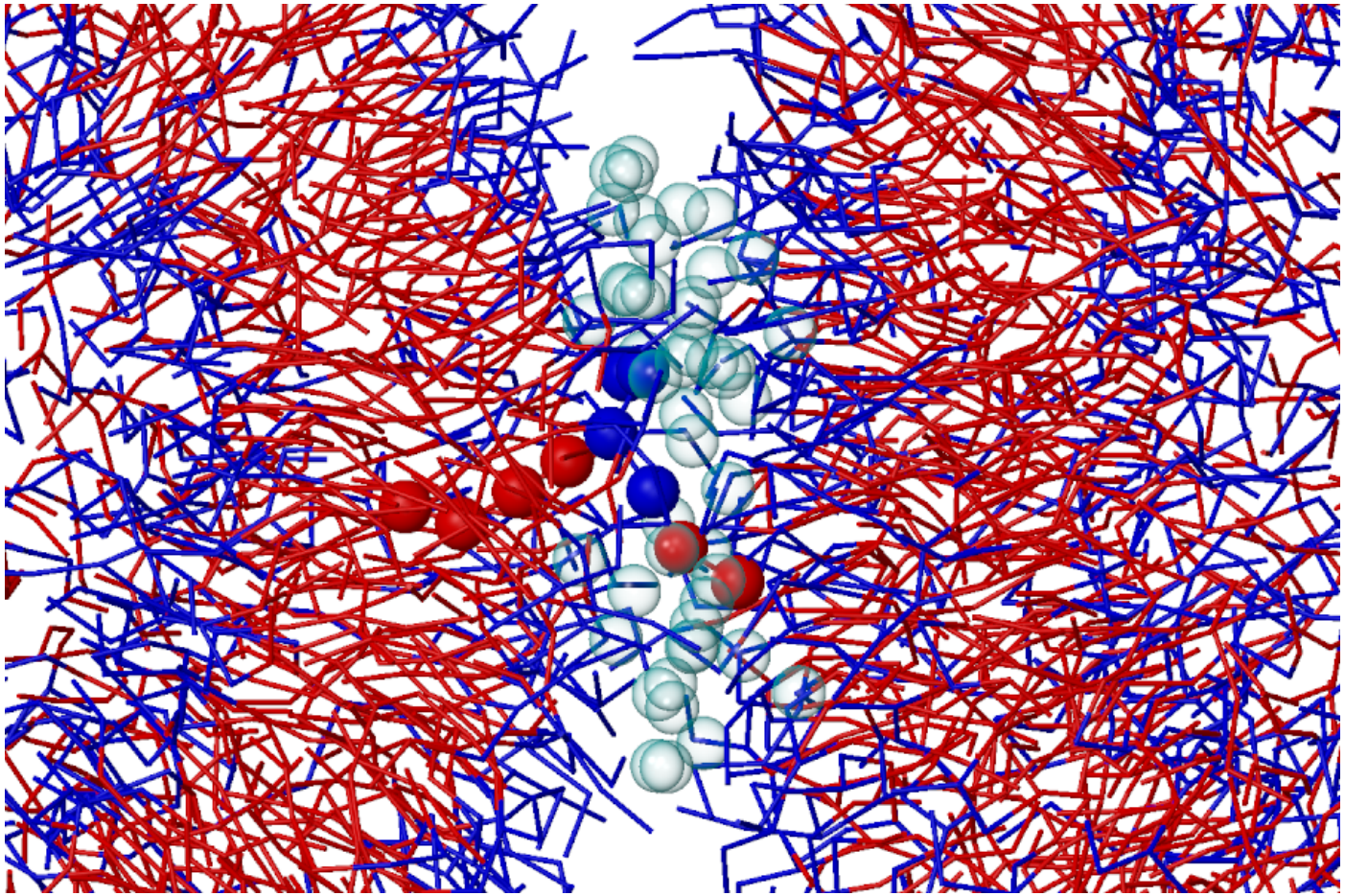


Hydrophobic 'pore' is composed of 4 lipids.
The splayed lipid does not span between two vesicles.

Mark Stevens
msteve@sandia.gov

Fusion Initiation: Asymmetric Tail

Tail Splay

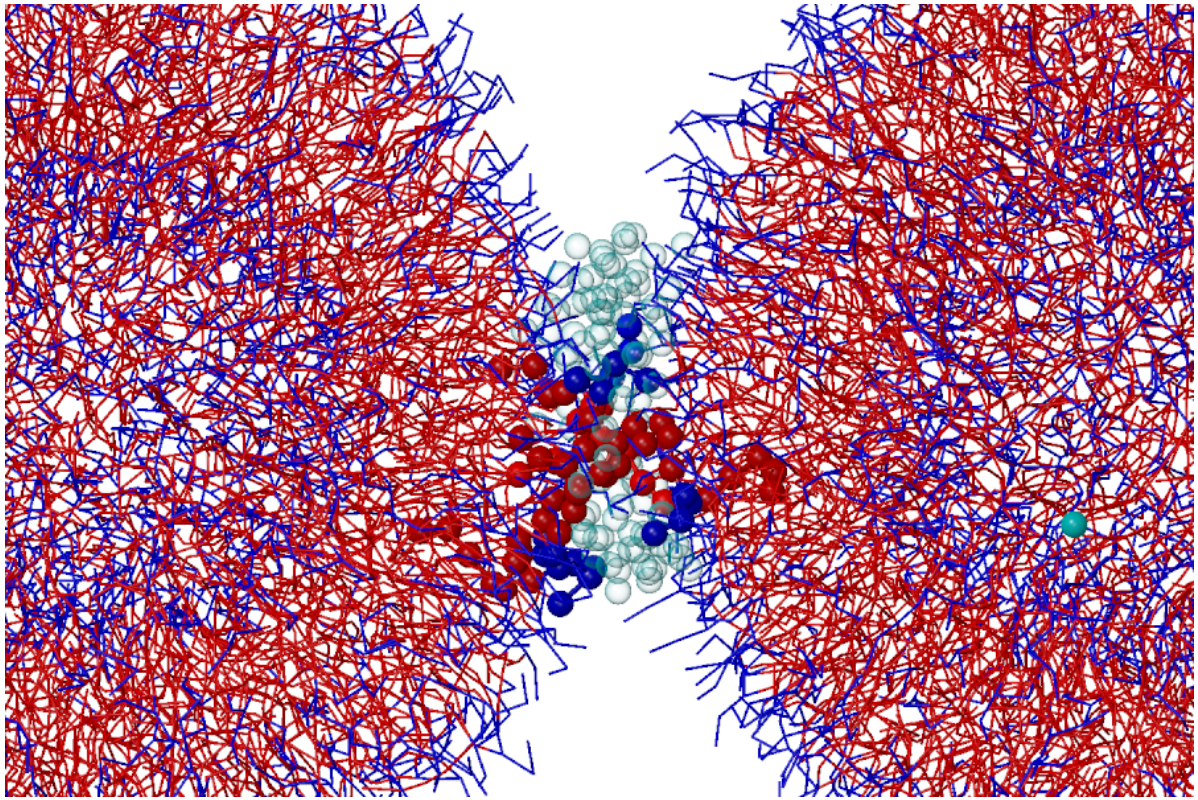


Shorter span by asymmetric tails leads to shorter separation between vesicles for fusion.

Mark Stevens
msteve@sandia.gov

Fusion Initiation: DOPC

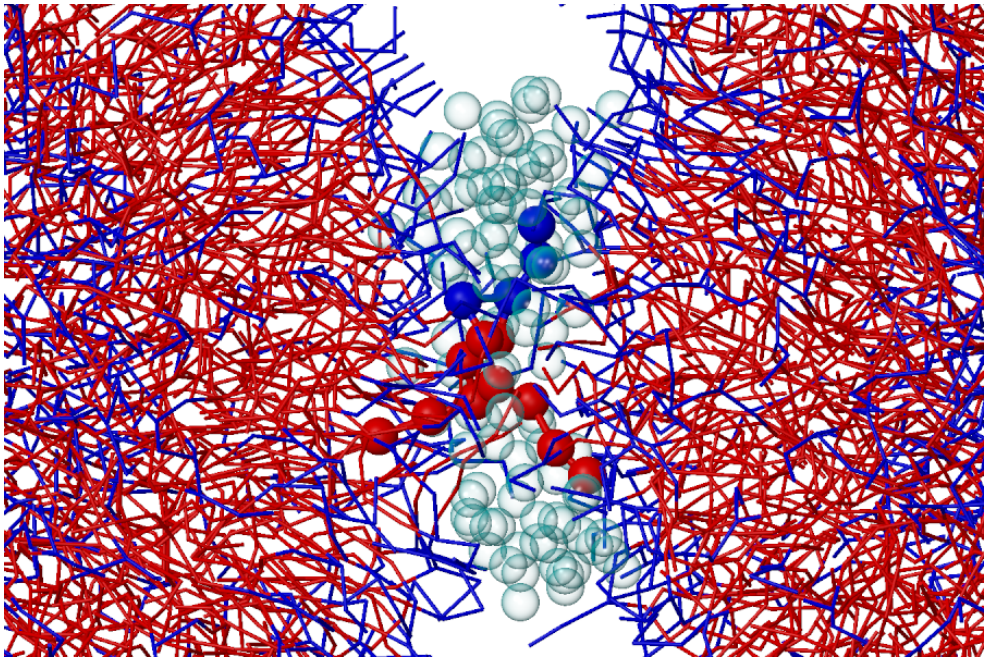
Hydrophobic pore: 5 lipids
1 is spans and splayed
3 splay with one tail in pore
1 with head group across to other vesicle



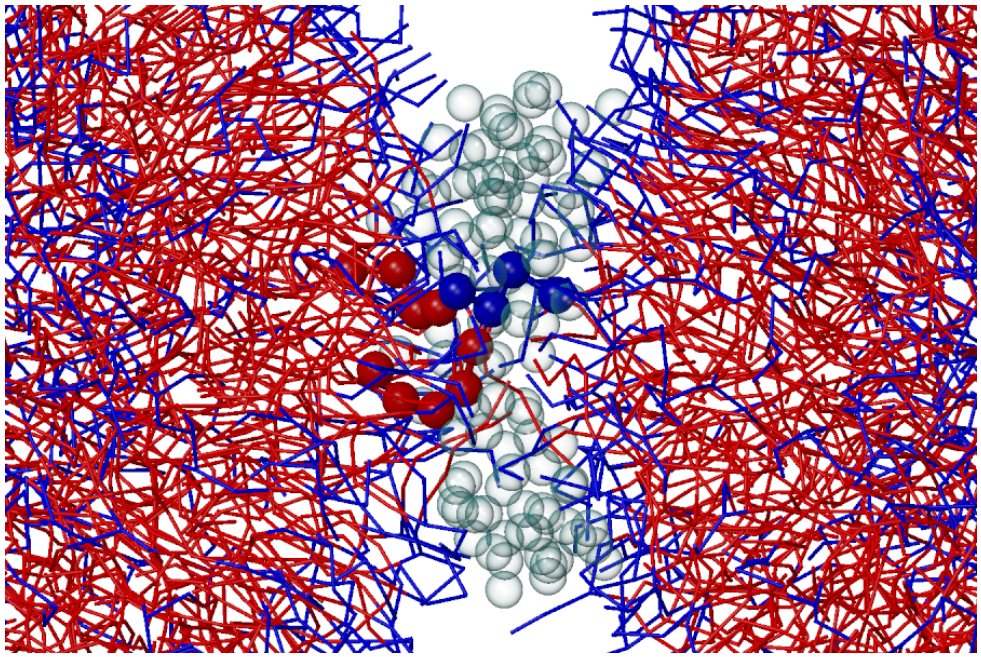
Mark Stevens
msteve@sandia.gov

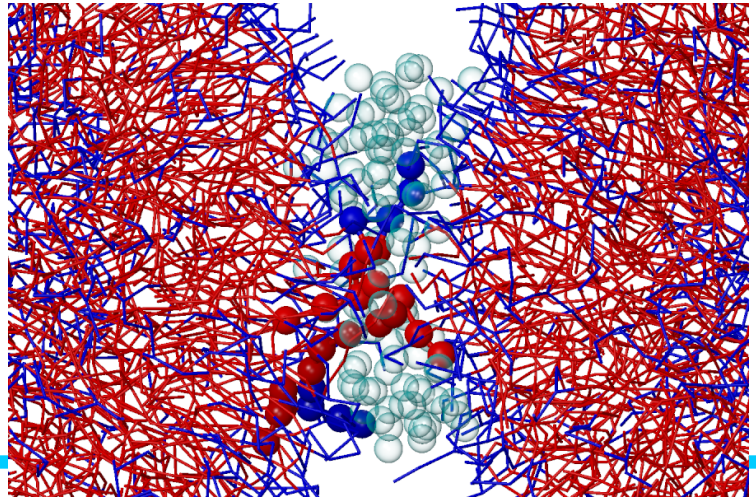
Fusion Initiation: DOPC

Span & Splay



'Translation'





Mark Stevens
msteve@sandia.gov

Fusion Initiation

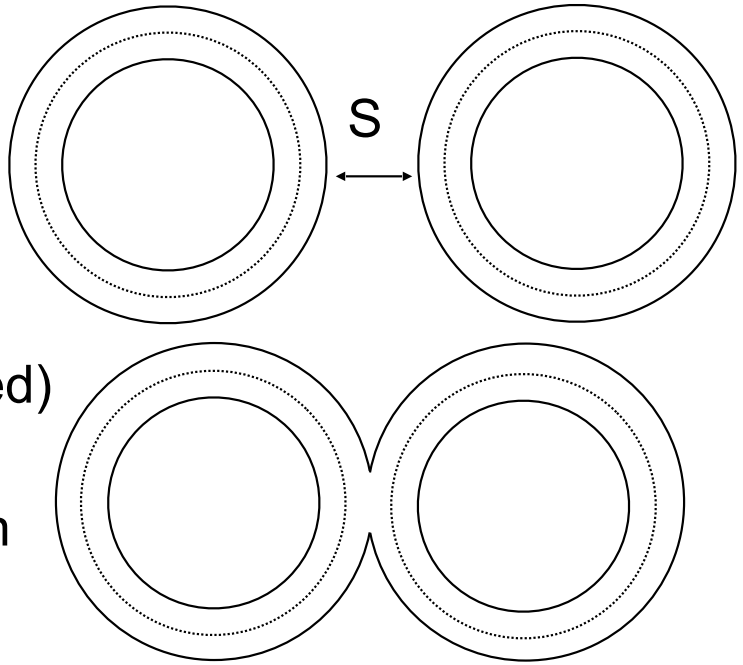
We find

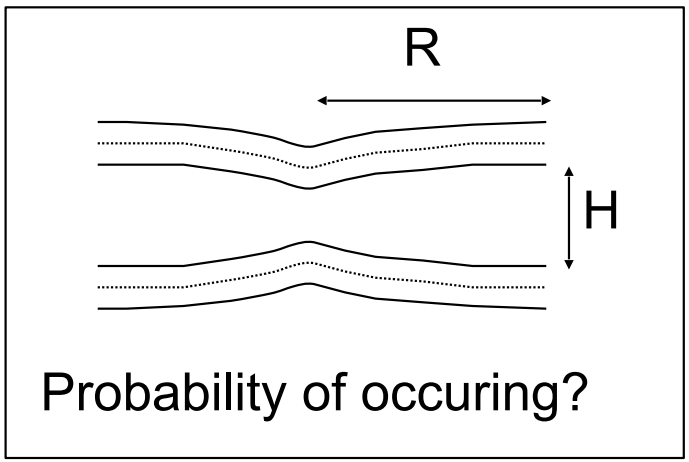
- ~single molecule exchange (splay)
- vesicles deform on approach
- surface separation at fusion, S ~ 1 nm

Fusion depends on rare event dynamics of (splayed) lipids.

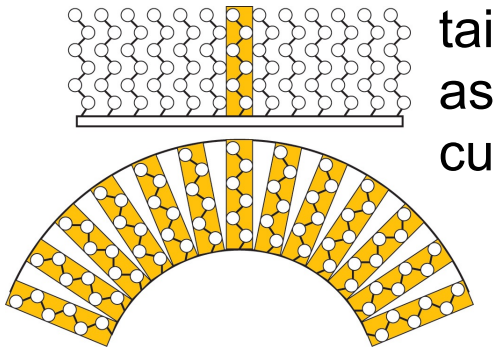
Surface curvature (vesicle diameter) plays a role in tail exposure.

Parallel geometry is poor geometry for studying fusion.





Packing & Curvature



tail exposure as a function of curvature

Mark Stevens
msteve@sandia.gov

Experimental Data?

Very small DPPC vesicles will fuse, but very slowly (Lentz).

PEG induced fusion of SUV (40nm).

DPPC does not fuse & DOPC does.

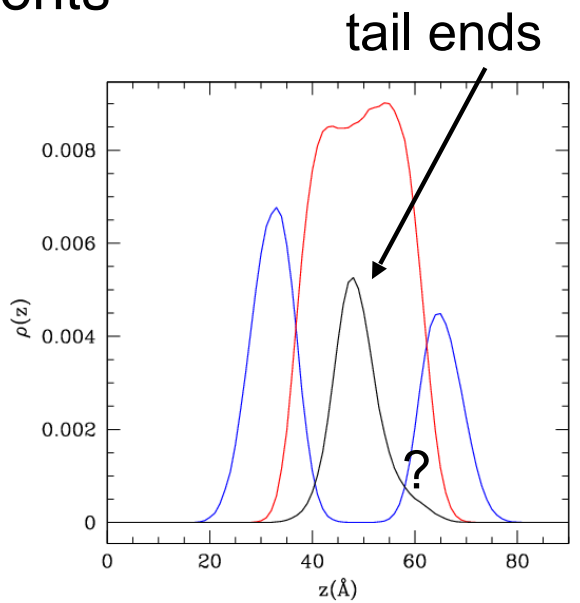
Simulations find both fuse

Very small vesicles (13nm) are different.

Why are DPPC and DOPC so different in fusion experiments given that DPPC & DOPC are not very different.

Especially if measure at T-T_m or equal area per lipid

Are rare event dynamics of tail ends critical?



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Conclusions

We have calculated free energy barrier for fusion initiation.

Single to few molecule initation of fusion

Splayed lipid tails promote fusion initiation

Asymmetric lipids are fusion inhibitors because of short span length in tail splay

Why are DPPC and DOPC so different in fusion experiments?

There's a lot more to do

- better models, study different states of fusion, tail end dynamics,
- fusion peptides ....

Mark Stevens
msteve@sandia.gov

Acknowledgements

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Free Energy (S. May, 2002)

nonsmooth stalk

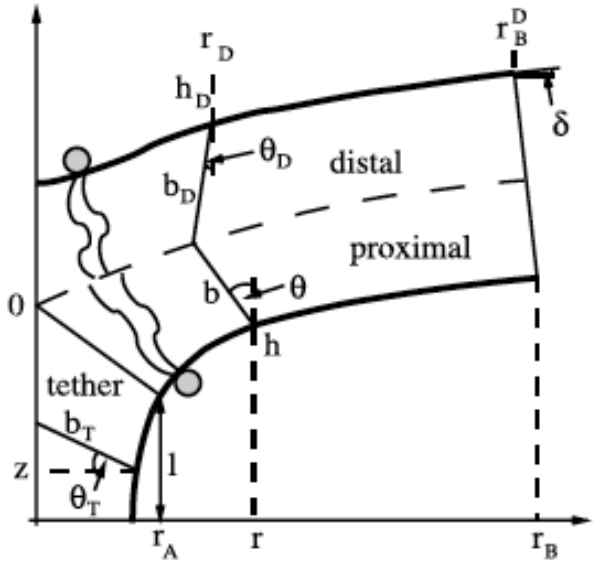
$K = 35\ k_B T/\text{nm}^2$ (DOPC)

$\kappa = 7\ k_B T$

$c_0 = 0$ & nonzero

$k_t = 10\ k_B T/\text{nm}^2$

$\Delta F = 33$ kT



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