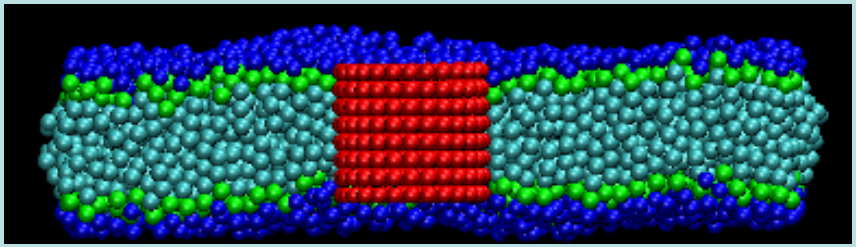
Using analytical and solvent-free simulation models to study thickness deformations in fluid lipid bilayers



Grace Brannigan

Physics Department

Final PhD Defense

Outline

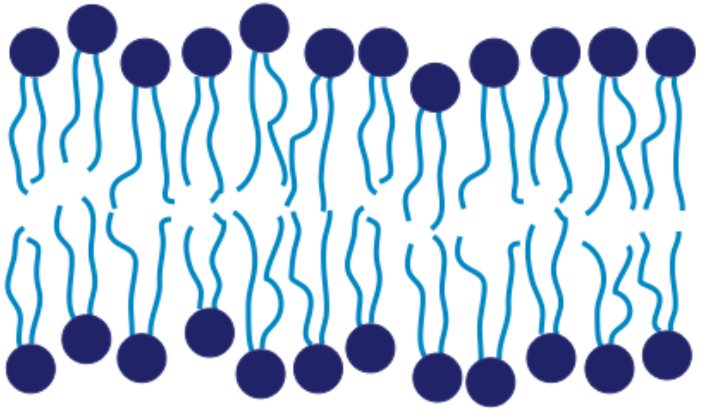
- Simulation Modeling of Lipid Bilayers
- Introduction to thickness deformations and hydrophobic mismatch
- Analytical Model
- Analysis of thermal fluctuations
- Prediction of gramicidin channel lifetime
- Predicting the membrane deformation profile around simplified “proteins”: two simulation experiments
- Summary

Modeling: lipid bilayers

Lipids are the basic ingredients for cell membranes:

hydrophilic head

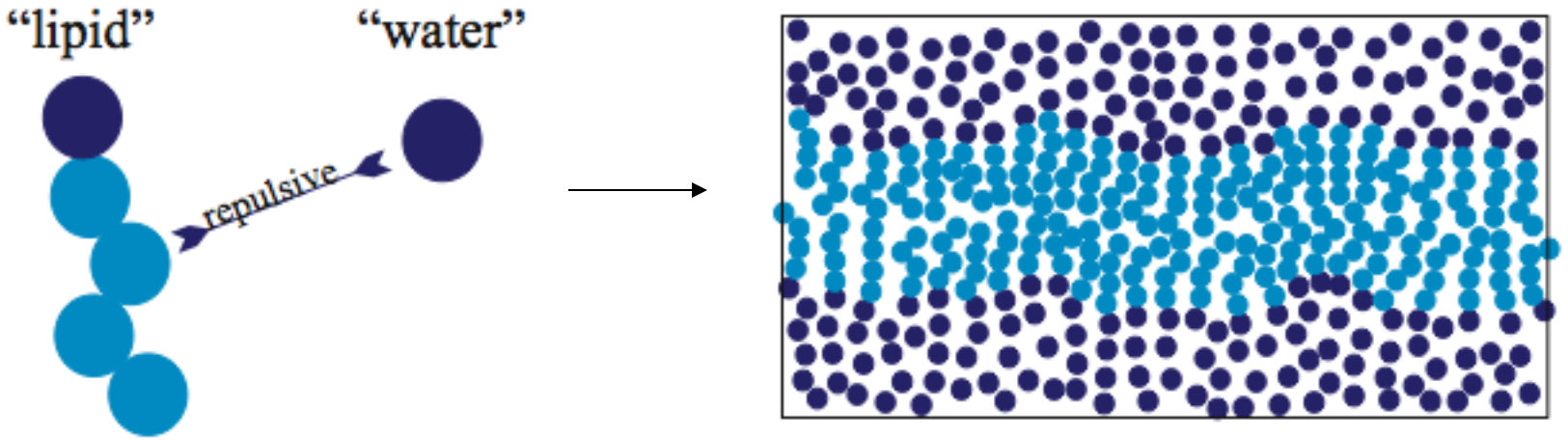
oily tails



Lipid bilayer

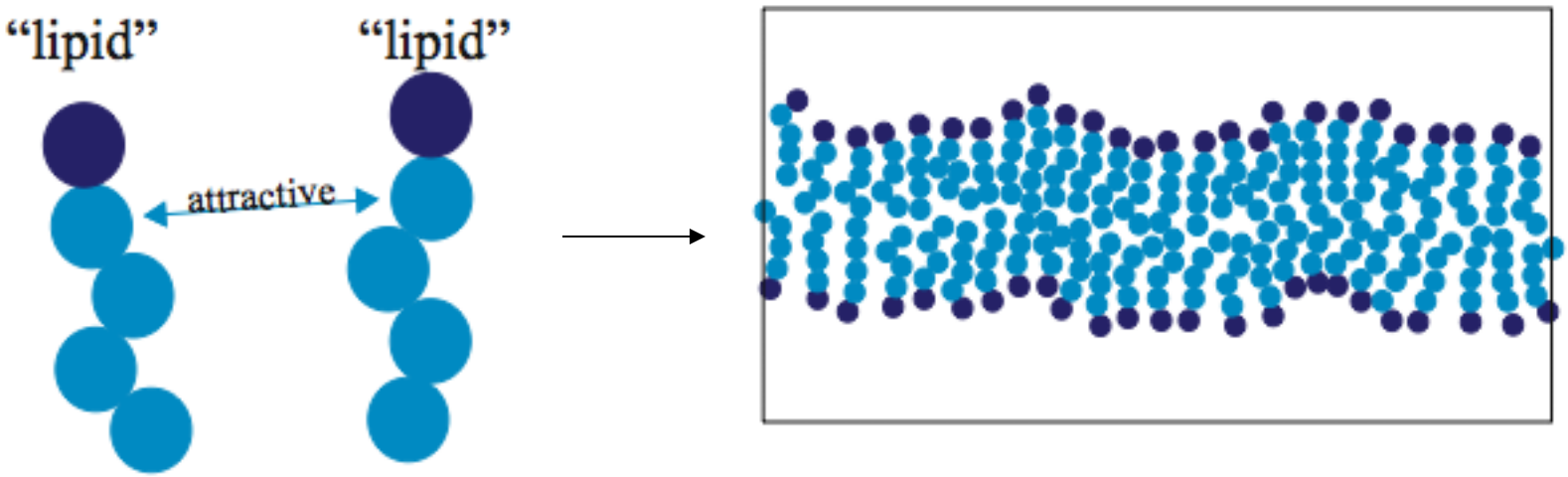
Solvated Models

- Most empirical coarse grained models for lipids in water are hydrophobic/polar (HP) models.



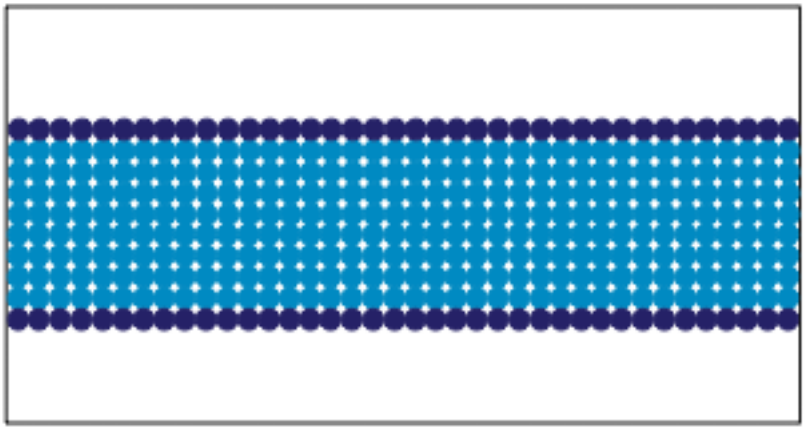
Removing the Solvent: General Idea

- drive aggregation by attractions among lipids



Removing the Solvent : a problem

- In the solvated models the hydrophobic tails experience a $1/r^6$ attraction. What if we just strengthen that?



(We get a crystal. Heat it up and it sublimes.)

Removing the solvent: some solutions

- Many body attractive potentials
  - Drouffe, Maggs, and Leibler(1991)
  - Noguchi and Takasu (2001)
  - Wang and Frenkel (2005)
- **Soft** pairwise attractions between hydrophobic beads
  - Farago (2003)
  - Cooke, Kremer, and Deserno (2005)
- Orientation-dependent attractions between polar rods
  - Brannigan and Brown (2004)

Removing the solvent: A Subtlety

All the models on the previous slide "work"…

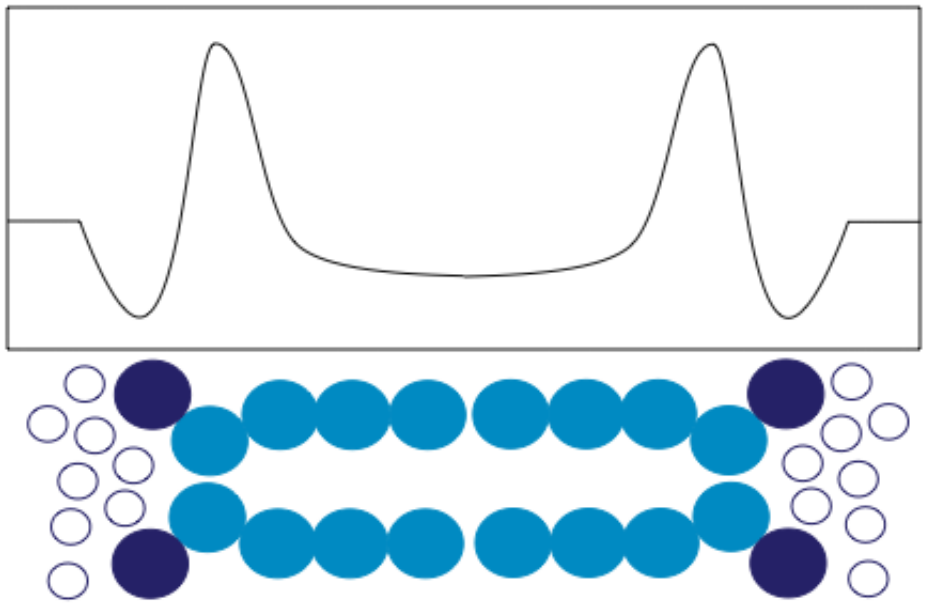
…in the sense that they make fluid bilayers. But there's nothing analogous to the oil-water interfacial tension in solvated bilayers.

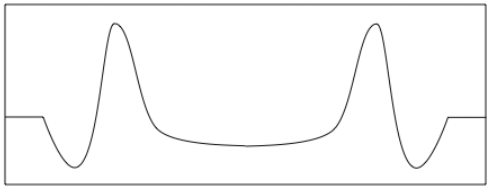
Stress Profile : tension vs. height in bilayer

Real bilayers have peaks in the stress profile at the two interfaces between the oily tails and water.

These peaks correspond to the "interfacial tension."

Theoretical estimates:
20-50 mJ/m^2

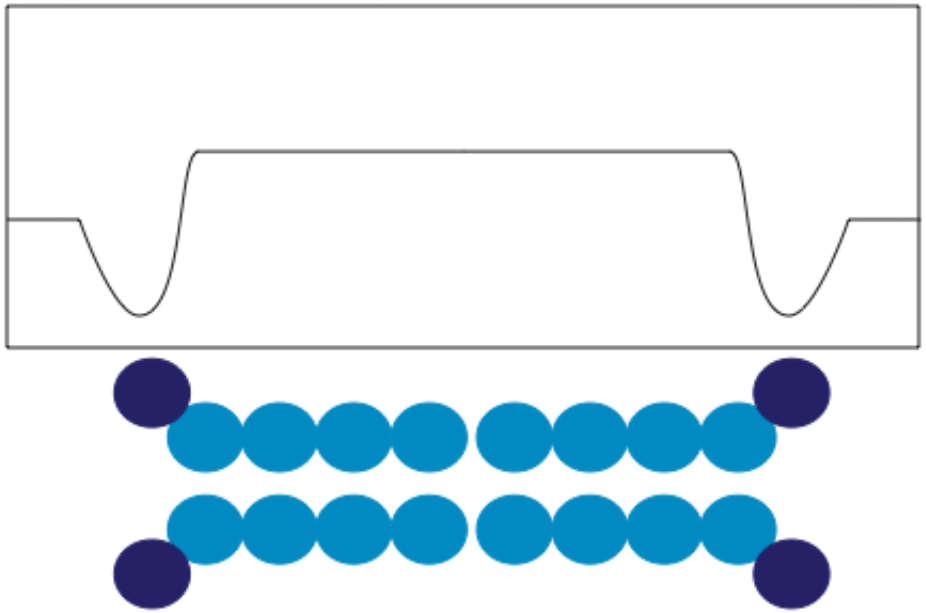




Stress Profile

An **effective** interfacial tension can be caused by oil-oil attractions, rather than water-oil repulsion.

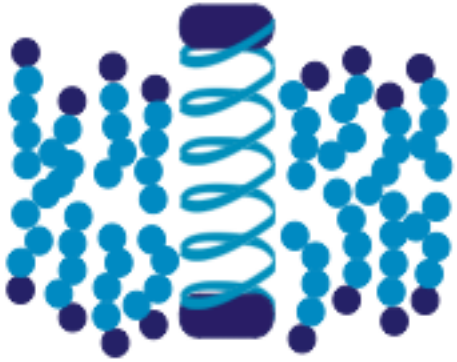
But if you spread these attractions over the tails, you'll get a stress profile that looks something like this!



Stress profile/interfacial tension: why does it matter?

Low interfacial tension = rough bilayer

High interfacial tension

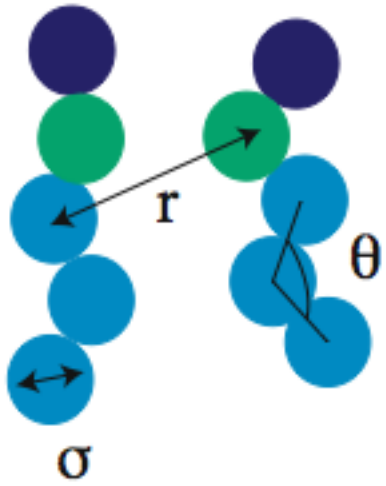


Low interfacial tension

Stress profile: our solution

- Introduce the “interface bead” into an HP type chain.
- Have interface beads (and only interface beads!) interact via a soft, relatively long-ranged attraction. Hydrophobic core beads still attract through 6-12 potential as in solvated models.
- Parameterize the interface attraction so that the resulting tension matches that of oil-water.

Coarse-grained implicit solvent model for lipids



All bond lengths are held constant at σ

Lennard-Jones type interactions between bead centers:

$c_1 \frac{\sigma^{12}}{r^{12}}$	$c_1 \frac{\sigma^{12}}{r^{12}}$	$c_1 \frac{\sigma^{12}}{r^{12}}$
	$c_1 \frac{\sigma^{12}}{r^{12}} - c_2 \frac{\sigma^2}{r^2}$	$c_1 \frac{\sigma^{12}}{r^{12}} - c_3 \frac{\sigma^6}{r^6}$
		$c_1 \frac{\sigma^{12}}{r^{12}} - c_3 \frac{\sigma^6}{r^6}$

+

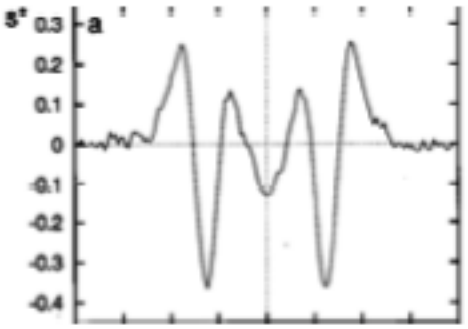
Bond-angle potential (sum over all angles) minimized by straight molecule:

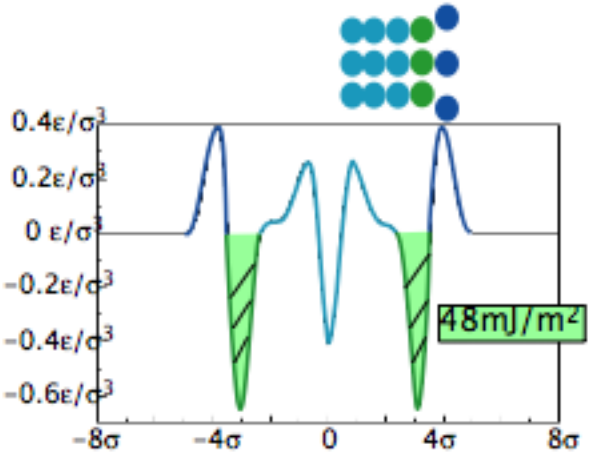
$$c_4 \cos\theta$$

G. Brannigan, P. F. Philips, and F.L.H. Brown, Phys. Rev. E. 72 011915 (2005).

Coarse-grained model: stress profile (-surface tension vs. height)

R. Goetz and R.Lipowsky, JCP 108 p. 7397, (1998)



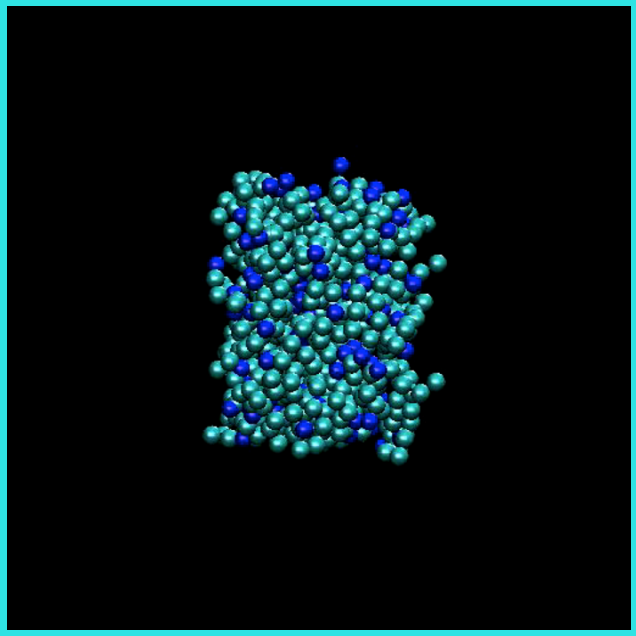


Explicit Solvent

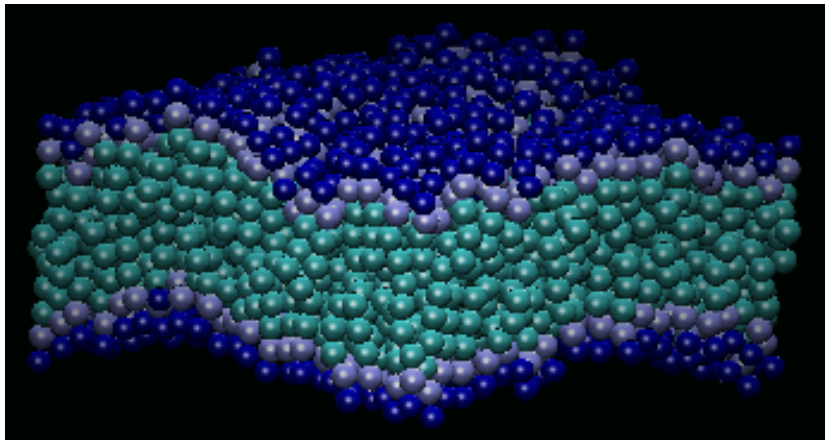
Implicit Solvent

Coarse-grained model: self-assembly

- Constant area
- 128 molecules
- About 20% of systems line up “properly” in box; others aggregate but do not satisfy periodic boundary conditions
- Approximately 3% monomer fraction

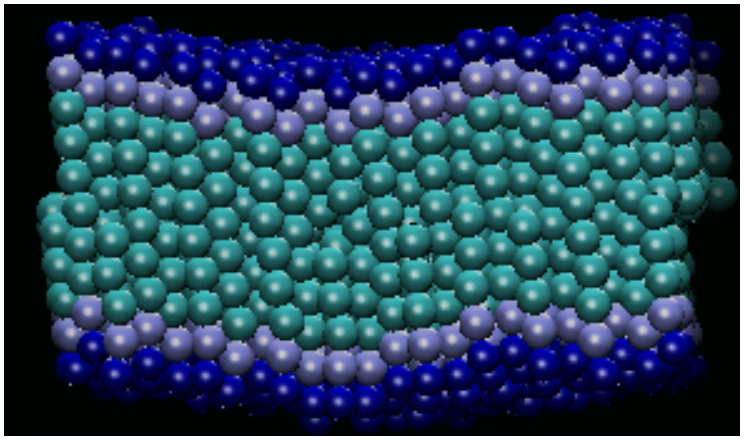


Coarse-grained Model : Phase behavior



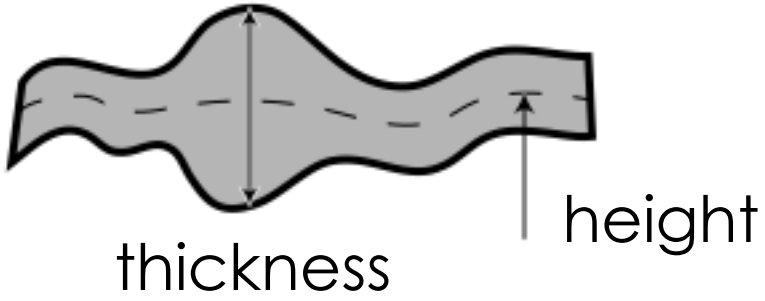
Fluid ($k_BT = 0.9\varepsilon$)

Gel ($k_BT = 0.5\varepsilon$)



Intro: Membrane fluctuations

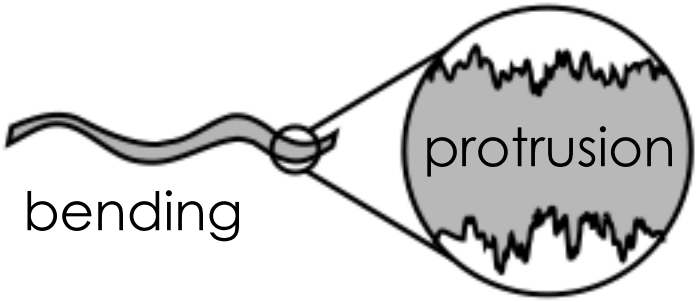
- Fluid lipid bilayers exhibit fluctuations in both height/shape and thickness

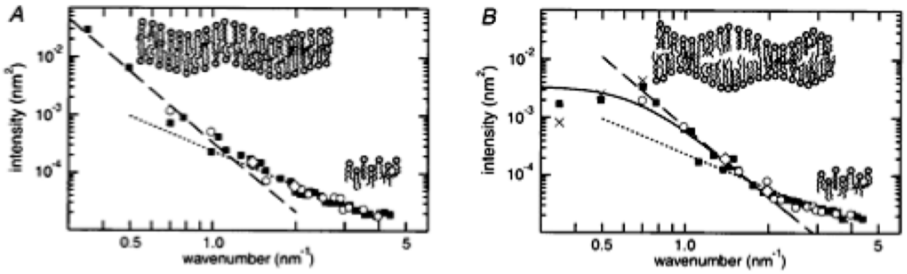


- Only long-wavelength height fluctuations (governed by bending energetics) are well-understood

Intro: Membrane fluctuations over short length scales

Short wavelength fluctuations ("protrusions") seem to be governed by the oil-water interfacial tension rather than bending energetics. (not covered in talk)



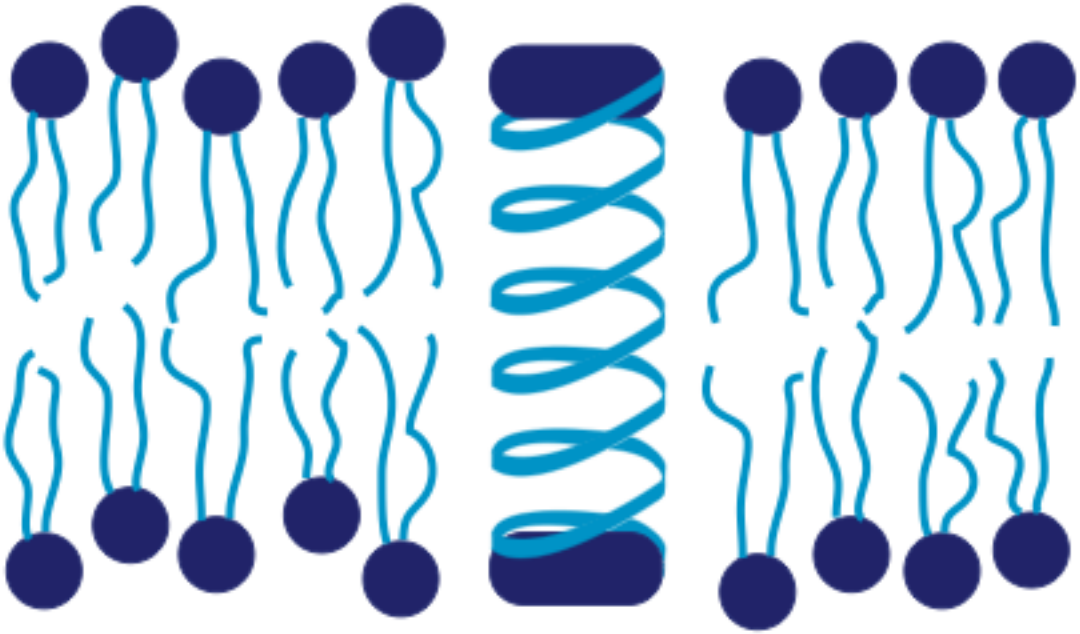


Fluctuation spectra for simulated DPPC bilayer

Lindahl, E. and O. Edholm. *Biophysical Journal* **79**:426(2000)

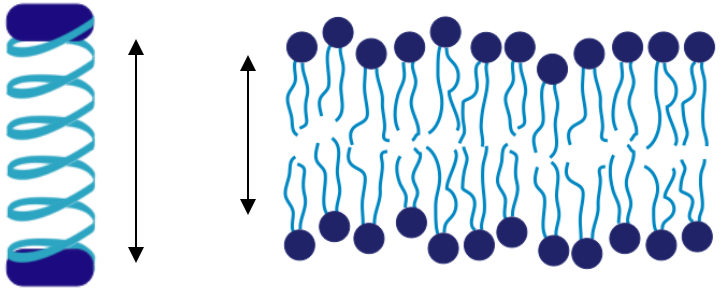
Intro : Membrane-protein systems

Proteins with their own hydrophobic regions are scattered throughout the membrane.

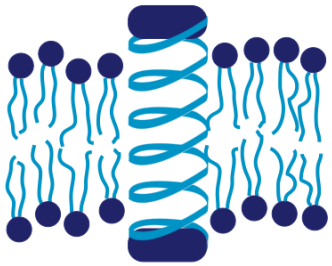


Intro:hydrophobic mismatch

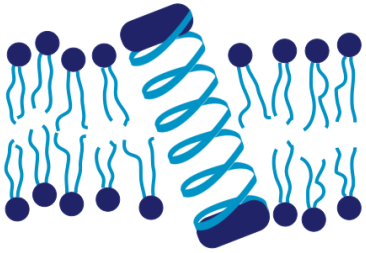
The hydrophobic region of the protein and the hydrophobic core of the membrane usually have different thicknesses:



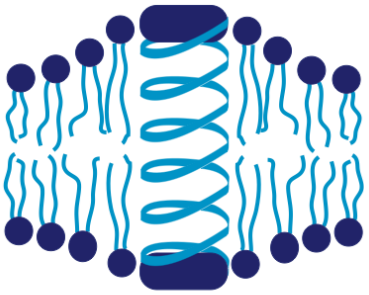
Some possible compensation mechanisms:



none



protein tilts



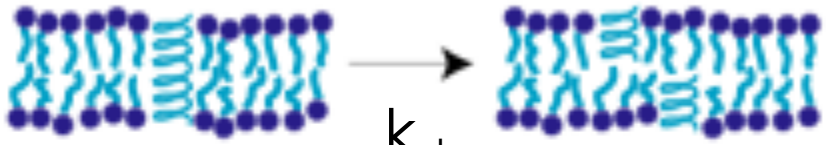
membrane deforms

Intro: some motivations

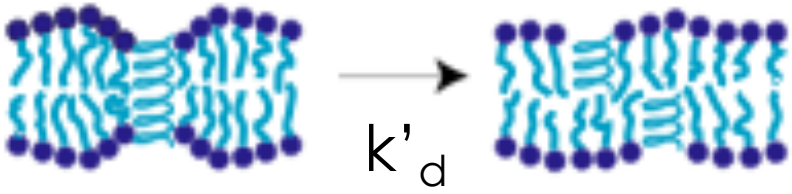
- these deformations can theoretically lead to membrane mediated attractions (or repulsions) among membrane proteins
- membrane deformations suggest a physical mechanism in which changes in lipid composition lead to changes in protein behavior

Intro:simple example

- The ion channel Gramicidin A has dimer and monomer configurations



- But what if dimer configuration introduces hydrophobic mismatch?



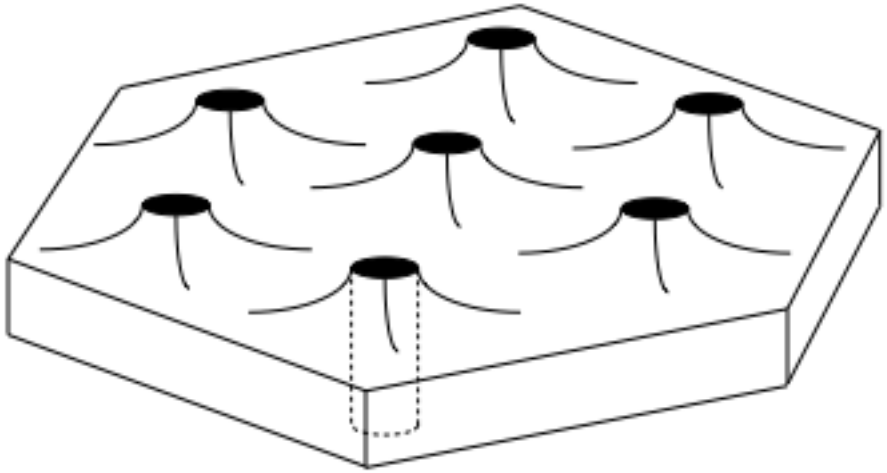
Expect $k'_d > k_d$ (and that's what people see)

Intro: theoretical approaches

- *Microscopic*: Time evolve specific lipid-protein systems with atomistic resolution and see what happens
  - Pro: pseudo-experiments, but with nicer observables
  - Con: can only do very high protein concentrations!
- *Elastic*: Membrane is an elastic sheet, proteins supply boundary conditions
  - Pro: easy to predict what will happen for a protein of given geometry
  - Con: some ambiguities, and predictions have not been directly compared against experimental or simulation data (not clear if this approach actually *works*)
- Our goal: Connect microscopic and elastic approaches

Intro:Elastic Approach*

Consider array of inclusions in membrane:



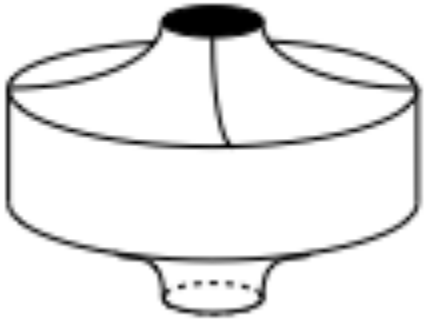
because of symmetry only need to look at one inclusion.

(*based on H. Aranda-Espinoza, A. Berman, N. Dan, P. Pincus, and S. A. Safran. *Biophys. J.,* 71:648–656, 1996, extensions noted in blue throughout.)

Intro: Elastic Approach

Given

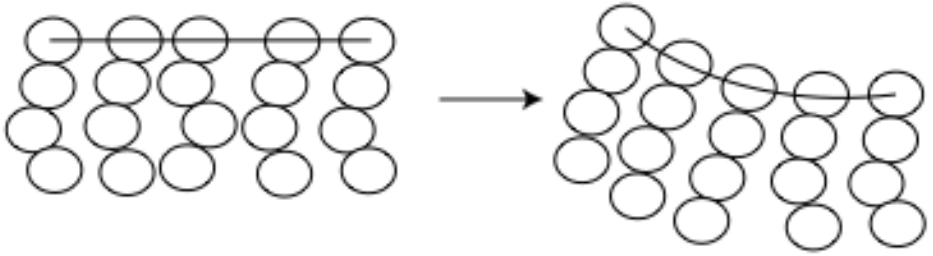
- Hamiltonian
- Elastic parameters
- Boundary conditions



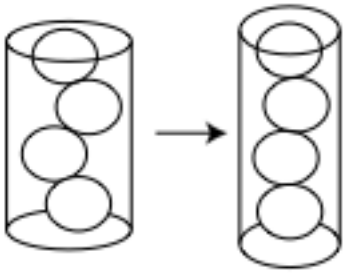
can solve for deformation profile that minimizes Hamiltonian

Intro: Elastic Approach Hamiltonian

- Protein introduces a bending cost…

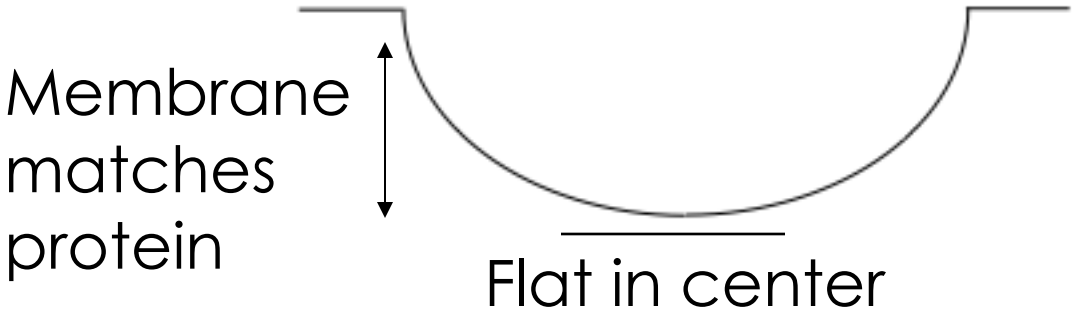


- …and an area compression cost



Intro: Elastic boundary conditions

- 2 physical b.c.'s:



- Some controversy about other 2 b.c.'s. We use "natural" b.c.'s: derivatives at boundaries are set to minimize free energy.

Intro: Elastic parameters

Need the following membrane properties:

- Bending rigidity (k_c)
- Spontaneous curvature of each leaflet (c_0)
- Area derivative of spontaneous curvature (c_{0A})
- Compressibility modulus (k_A)
- Gaussian curvature modulus (k_G)
- Volume derivative of spontaneous curvature(c_{0v})

Ideally obtain these from homogeneous membranes. Some questions about microscopic vs. macroscopic parameters.

Intro: Some questions

- Question #1: Are the constants that determine long-wavelength thickness fluctuations the same constants that determine the membrane's response to a protein?
  - claim: Yes.

Intro: Some questions

- Question #2: Each leaflet must have a pretty low spontaneous curvature, or else it wouldn't be part of a bilayer. Can't we just neglect the spontaneous curvature?
  - Claim: You will miss some interesting physics if you neglect it, and quantitative results can be significantly off.

Intro: Some questions

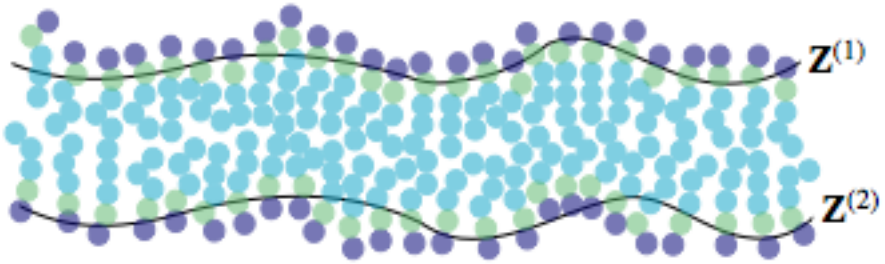
- Question #3: What about the Gaussian curvature?
  - Claim: This was not considered in previous elastic theories but needs to be when the inclusion has a small radius (on the order of a few lipid radii).

Intro: Some questions

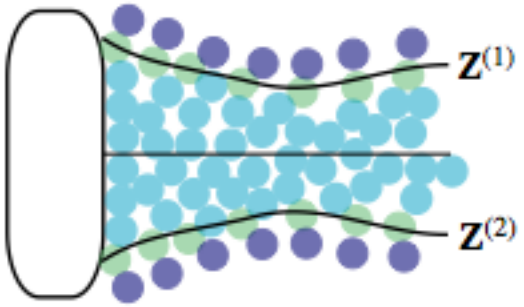
- Question #4: Is lipid volume really conserved?
  - Claim: It doesn't seem to be for lipids in the vicinity of the protein, for our coarse-grained model.

Model : our description

For fluctuations…



For protein-induced deformations…



$$h = \frac{z^{(1)} + z^{(2)}}{2}$$

$$t = \frac{z^{(1)} - z^{(2)}}{2} - t_0$$

<u>Appropriate observables</u>

Fluctuations: $\langle |h_q|^2 \rangle$ $\langle |t_q|^2 \rangle$

Inclusions: $\langle t(r) \rangle$

Model: Elastic Hamiltonian

1) Expand free energy in mean curvature *H and* Gaussian curvature *K* of each leaflet

$$f^{(1)} = f_0 + f_1 H^{(1)} + f_2 (H^{(1)})^2 + f_K K^{(1)}$$
$$f^{(2)} = f_0 - f_1 H^{(2)} + f_2 (H^{(2)})^2 + f_K K^{(2)}$$

2) Expand coefficients in $\Sigma - \Sigma_0$ (deviation from zero tension area per molecule) and v (deviation from zero tension volume per molecule):

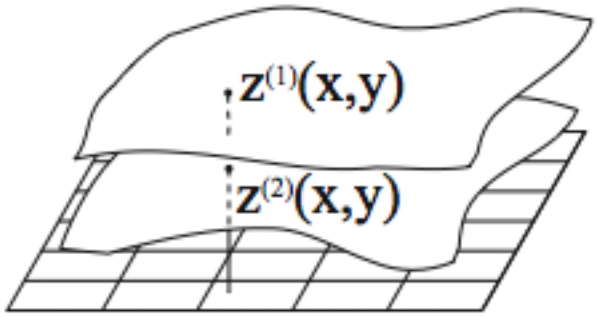
$$f_0 = \frac{1}{2} f_{0_{\Sigma\Sigma}} (\Sigma - \Sigma_0)^2 + f_{0_{\Sigma v}} (\Sigma - \Sigma_0) v + \frac{1}{2} f_{0_{vv}} v^2$$
$$f_1 = f_1 + f_{1_\Sigma} (\Sigma - \Sigma_0) + f_{1_v} v$$

Model:Elastic Hamiltonian

3) Switch each leaflet to Monge gauge:

$$H^{(1)} = \frac{1}{2}(z^{(1)}_{xx})^2 + \frac{1}{2}(z^{(1)}_{yy})^2 \qquad H^{(2)} = \frac{1}{2}(z^{(2)}_{xx})^2 + \frac{1}{2}(z^{(2)}_{yy})^2$$

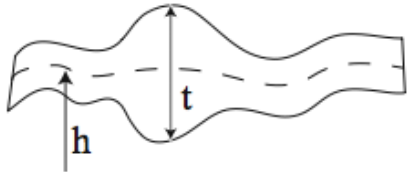
$$K^{(1)} = z^{(1)}_{xx} z^{(1)}_{yy} - (z^{(1)}_{xy})^2 \qquad K^{(2)} = z^{(2)}_{xx} z^{(2)}_{yy} - (z^{(2)}_{xy})^2$$



4) Define symmetric/ anti-symmetric vars:

$$h = \frac{z^{(1)} + z^{(2)}}{2}$$

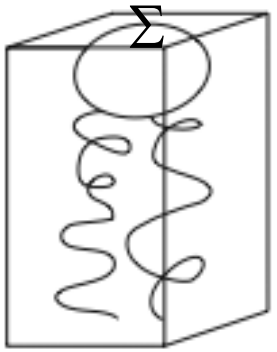
$$t = \frac{z^{(1)} - z^{(2)}}{2} - t_0$$



Model: Elastic Hamiltonian

5) Divide by area per molecule, then use area = volume/thickness to turn areas into t's and v's:

(Most theories constrain
v = 0 and for now we will too,
but later we'll let it be small
right next to a protein)



$(v+v_0) = \Sigma(t+t_0)$

Model: Elastic Hamiltonian (fluctuations)

For fluctuations in homogeneous membrane

- use square geometry.
- No boundaries means
  - v = 0 everywhere
  - c_0 term integrates to constant
  - k_G term integrates to constant

Integrate over area to get final answer:

$$F = \int_{L^2} \mathrm{dxdy} \frac{k_c}{2}(\nabla^2 h)^2 + \frac{k_c}{2}(\nabla^2 t)^2 + 2k_c\zeta\frac{t}{t_0}\nabla^2 t + \frac{k_A}{2t_0^2}t^2$$

k_c = bending rigidity

c_0 = spontaneous curvature

c_{0_Σ} = area derivative of c_0

$\zeta = c_0 - c_{0_\Sigma}\Sigma_0$

k_A = compressibility modulus

t_0 = equilibrium thickness

Σ_0 = equilibrium area

Fluctuations of homogeneous bilayers

- Reasons to investigate
  - Test analytical model
  - Extract parameters for predicting inclusion deformation profile and gramicidin lifetime
- Fit fluctuation spectra for four systems: DPPC, GMO, Sphingomyelin, and the implicit solvent coarse-grained (CG) model
  - CG parameters used for deformation profile prediction
  - GMO parameters used for gramicidin lifetime prediction
  - DPPC, GMO, SM data was previously published; we reanalyzed

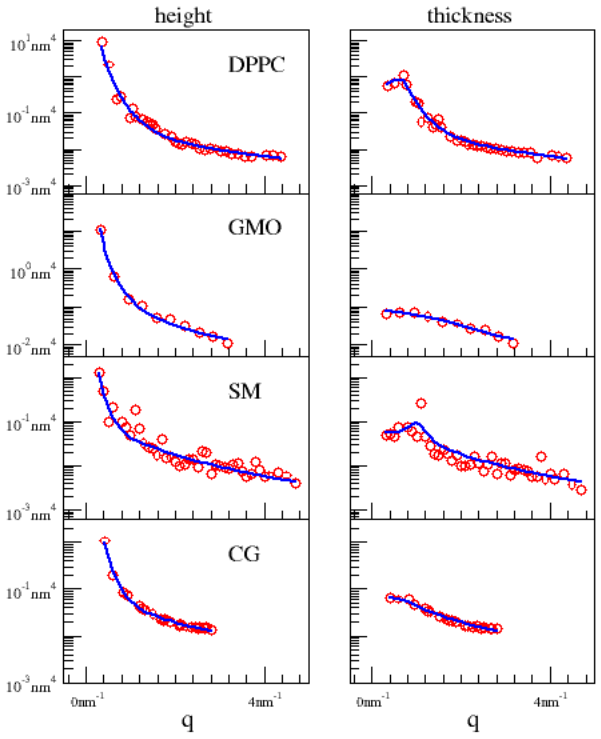
Fluctuations : Fitting the data

- Height spectra fit to:

$$\langle |h_q|^2 \rangle = \frac{k_B T}{k_c q^4} + \frac{k_B T}{2(\gamma_\lambda q^2 + k_\lambda)}$$

- Thickness spectra fit (simultaneously)to:

$$\langle |t_q|^2 \rangle = \frac{k_B T}{k_c q^4 - 4\zeta q^2/t_0 + k_A/t_0^2} + \frac{k_B T}{2(\gamma_\lambda q^2 + k_\lambda)}$$

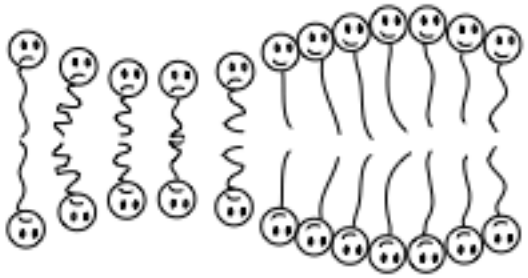


DPPC: Lindahl, E. and O. Edholm. *Biophys. J.***79**:426(2000)
GMO: Marrink, S.-J. and A.E. Mark. *J.Phys.Chem* **105**:6122 (2001)
SM: Chiu, S. S. Vasudevan, E. Jakobsson, R.J. Mashl, and H.L. Scott. *Biophys J* **85**:3624 (2003)

Fluctuations: spontaneous curvature

Positive spontaneous curvature + conservation of volume favors a bulged configuration

These molecules are unhappy but due to volume conservation, there are fewer of them.

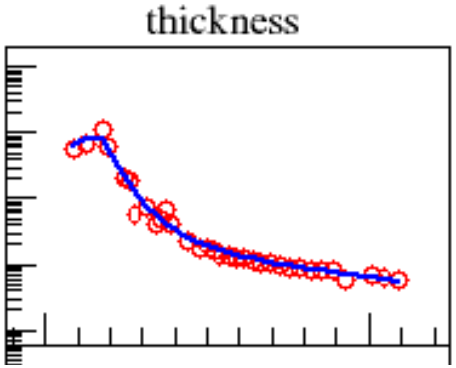


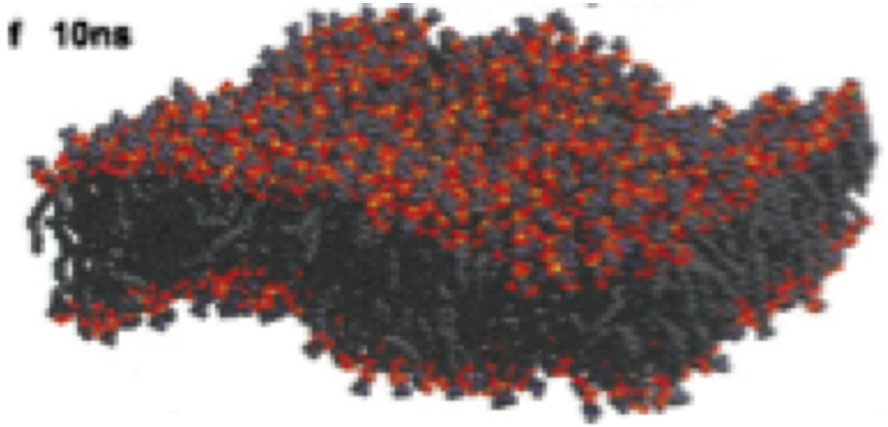
These molecules are happy because they're in their preferred curvature.

resulting in a negative effective tension and a peak in the thickness fluctuation spectrum

Fluctuation spectra: spontaneous curvature

Of the systems we examined, this effect was most pronounced in DPPC:





Lindahl, E. and O. Edholm. *Biophysical Journal* **79**:426(2000)

Model:Elastic Hamiltonian (inclusions)

- Height deformations and thickness deformations decouple (only need thickness)
- Membrane is no longer a closed surface, need to re-include
  - -Gaussian curvature
  - -spontaneous curvature (unrenormalized)
- We allow inclusion to cause lipid volume deformation *right at boundary*
- Use cylindrical coordinates

$$F = 2\pi \int_R^{L/2} r dr \left\{ \frac{k_A}{2t_0^2} t^2 + 2k_c c_0 \nabla_r^2 t + \frac{2k_c \zeta}{t_0} t \nabla_r^2 t + \frac{k_c}{2} \left(\nabla_r^2 t\right)^2 - \frac{2k_c \eta}{v_0} v \nabla_r^2 t + k_G \frac{t_r t_{rr}}{r} \right\}$$

$$\begin{aligned} c_{0_v} &= \text{volume derivative of } c_0 \\ \eta &= \zeta - c_{0_v} v_0 \\ v_0 &= \text{equilibrium volume/mol} \\ R &= \text{inclusion radius} \end{aligned}$$

Inclusions : analytical solution

Euler-Lagrange Eq. for Hamiltonian is:

$$\frac{k_A}{k_c t_0^2} t + \frac{4\zeta}{t_0} \nabla_r^2 t + \nabla_r^4 t = 0$$

with boundary conditions

$$t(R) = t_R$$

$$t_r|_{L/2} = 0$$

$$\nabla_r^3 t|_{L/2} = 0$$

$$\frac{k_G}{k_c} \frac{t_r|_R}{R} + \nabla_r^2 t|_R = -2 \left(c_0 + \frac{\zeta}{t_0} t_R \right) + \frac{\eta}{v_0} v_R$$

- k_G only matters for small R
- c_0 isn't coupled to mismatch (t_R)

Solutions are Bessel functions.

Gramicidin lifetime: Arrhenius Eq.

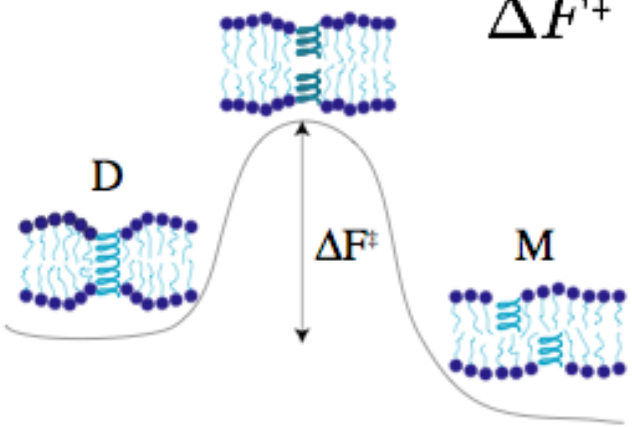
$$k_d = \nu e^{-(\Delta F^{\ddagger})/k_B T}$$

$$\Delta F^{\ddagger} = \Delta F_{gA} + F_{mem}(t_{\ddagger}, t_0) - F_{mem}(t_D, t_0)$$

Cost to stretch gA channel

Free energy of membrane with preferred thickness t_0 and stretched channel

Free energy of membrane with preferred thickness t_0 and unstretched channel



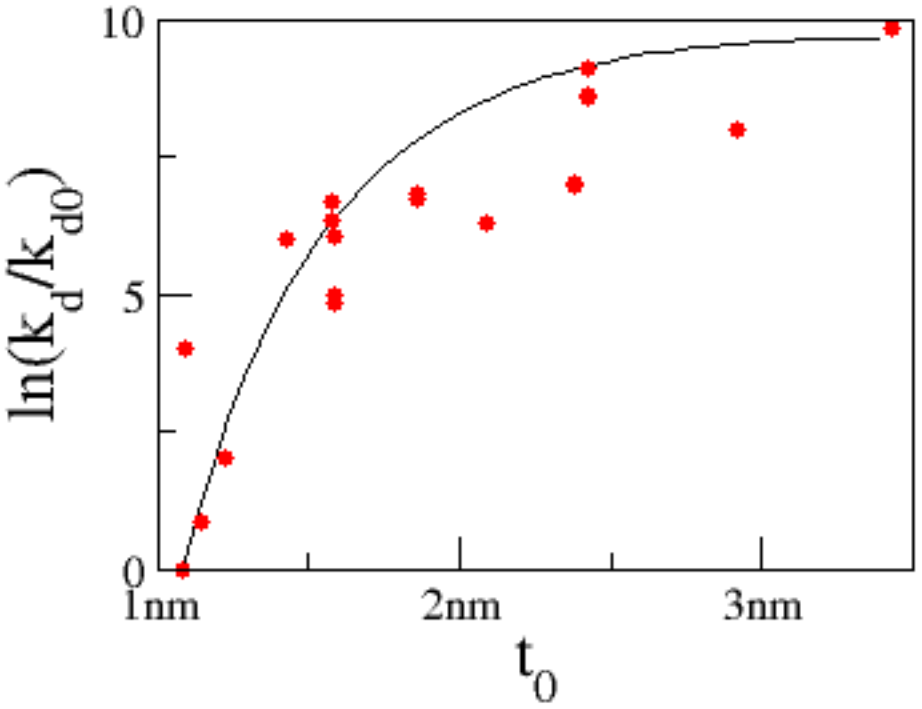
$$\frac{k_d(t_0)}{k_d(t_0')} = \frac{e^{(F_{mem}(t_D,t_0) - F_{mem}(t_D,t_0'))/k_B T}}{e^{(F_{mem}(t_{\ddagger},t_0) - F_{mem}(t_{\ddagger},t_0'))/k_B T}}$$

Gramicidin lifetime: calculating F_{mem}

- The lifetime data was taken for gA in various monoglycerides, including GMO (we have parameters for GMO!)
- To calculate F_{mem} use analytical expression derived in previous slide.

Gramicidin lifetimes

Aranda-Espinoza et al. does a pretty good job, without fit constants!



Data:

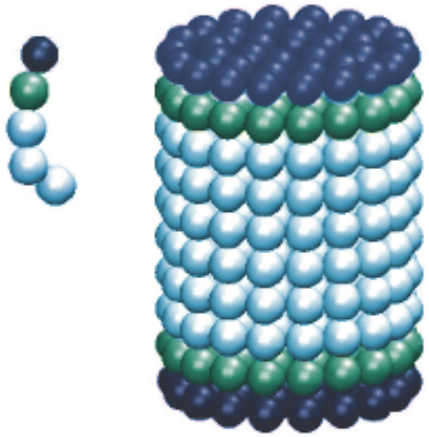
J.R. Elliott, D. Needham, J.P. Dilger, and D.A. Haydon. Biochimica et Biophysica Acta, 735:95–103, 1983.
H. A. Kolb and E. Bamberg. Biochim. Biophys. Acta, 464:127–141, 1977.

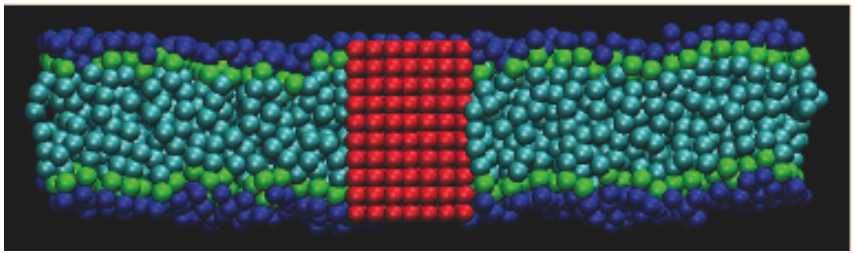
Inclusions: simulations

- Experiment I
  - 1 Simulation, positively mismatched inclusion of moderate radius
  - Measure thickness deformation profile
  - Compare to analytical prediction without k_G or η, using constants from fluctuations/stress profile.
- Experiment II
  - 14 Simulations, positively and negatively mismatched inclusions over a range of radii
  - Extract k_G and η from plotting principle curvatures at inclusion boundary for range of inclusion radii
  - Measure thickness and volume deformation profile for all systems
  - Compare to analytical prediction with k_G and η (using same membrane constants for all systems)

Experiment I: making an inclusion

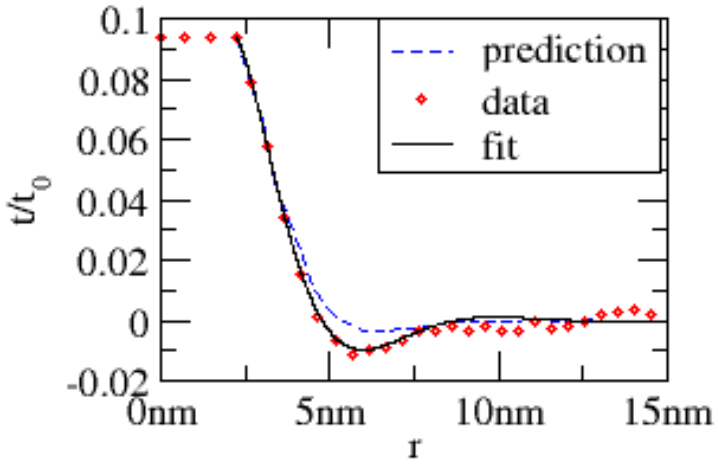
- About 80 5-bead "lipid" molecules were stuck together to make a protein
- Because the lipids can bend and the molecules that make the protein can't, there is still a mismatch (about 10%) in preferred thickness





Comparison of prediction (no k_G or η) with data

- "Prediction" is analytical theory combined with parameters extracted from homogeneous CG membrane
- "Data" is thickness deformation profile of CG membrane around inclusion (I.e. system of previous slide)
- "Fit" is analytical theory with parameters extracted from deformation profile. "Fit" and "prediction" parameters match within error bars!



Non-monotonic healing

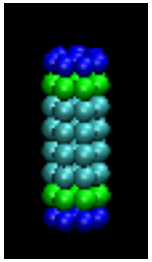
- First predicted by Huey Huang (1986) as a direct result of including bending cost in Hamiltonian
- Observed in other coarse-grained simulations by Smit and co-workers (2005) and Klein and co-workers (2005)
- Implication: kinetic barrier to protein aggregation

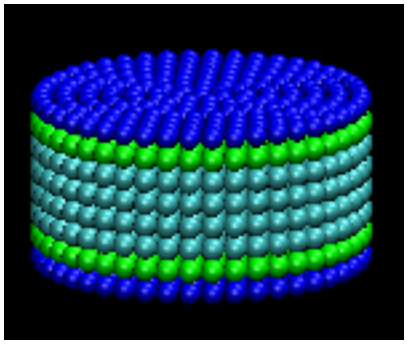
Experiment I: Conclusions

- The constants that describe long wavelength fluctuations of a homogeneous membrane also describe the membrane's response to a symmetric mismatched inclusion.
- Spontaneous curvature effects were important to consider for quantitative agreement

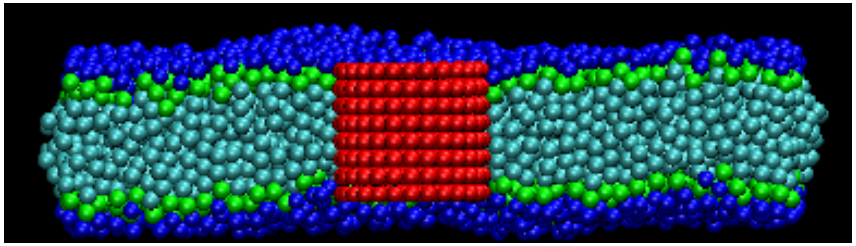
Experiment II: Making Inclusions

Inclusion radius was varied by adding/subtracting concentric rings of rigidified lipids

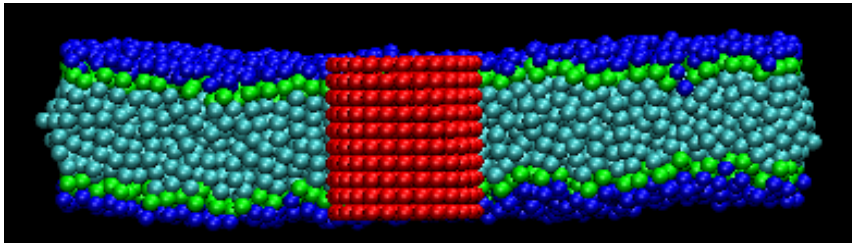




Inclusion height was varied through the number of hydrophobic beads (light blue) per rigid chain



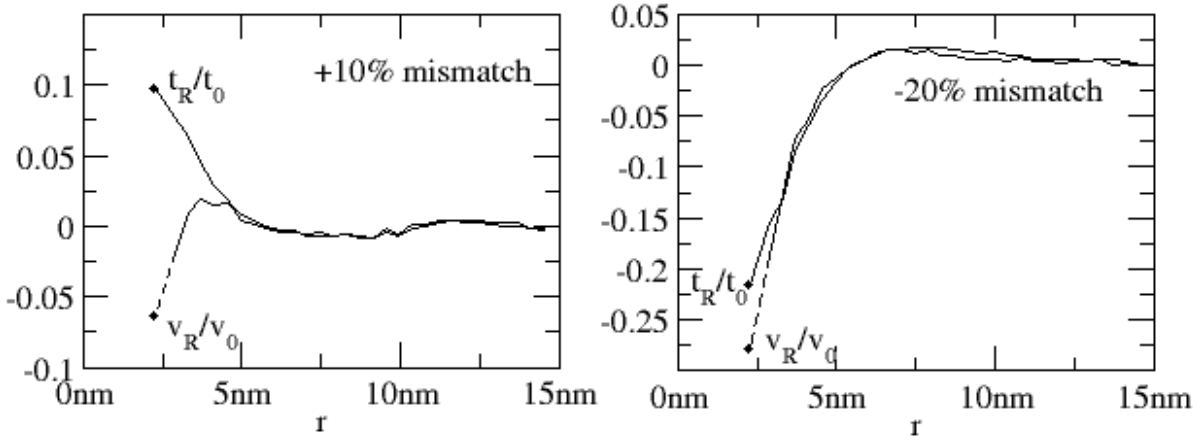
-20% mismatch



+10% mismatch

Experiment II: Volume Deformations

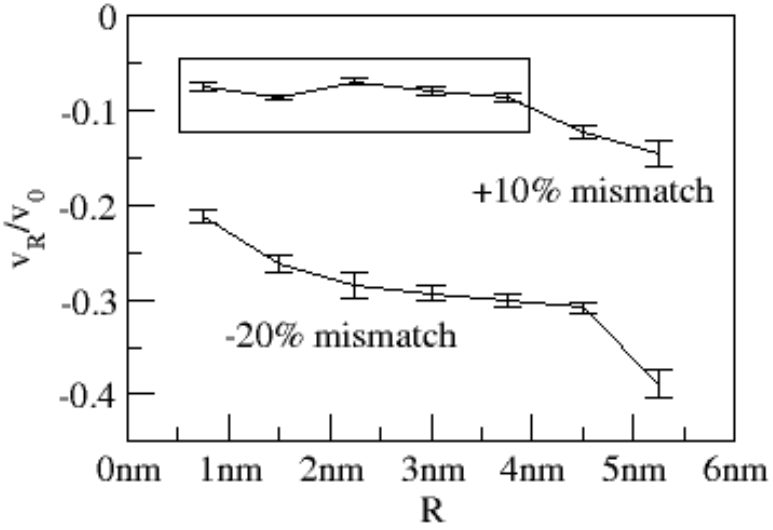
The volume deformation profiles show that lipid volume is not conserved around the inclusion.



The volume deformation is negative, regardless of the sign of the thickness deformation.

Experiment II: Volume Deformations

- magnitude of the volume deformation at the boundary generally increases with inclusion radius
- there is a range of R for which it is reasonably constant. This will be useful for the next slide.



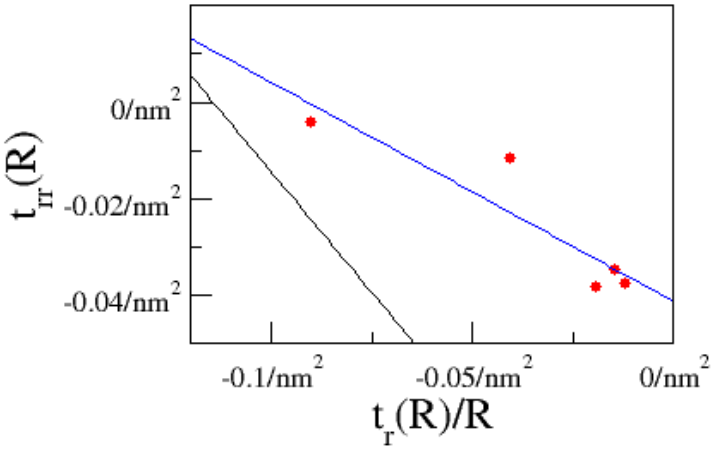
Experiment II: Measuring k_G and η

Natural boundary conditions say that at r=R:

$$t_{rr}(R) = -\left(\frac{k_G + k_c}{k_c}\right)\frac{t_r(R)}{R} - 2\left(c_0 + \frac{\zeta}{t_0}t_R\right) + \frac{2\eta}{v_0}v_R$$

Plotting the two principle curvatures against each other (right) yields

$$k_G/k_c = -0.55 \pm 0.11$$
$$\eta/v_0 = -0.78 \pm 0.02\text{nm}^{-4}$$

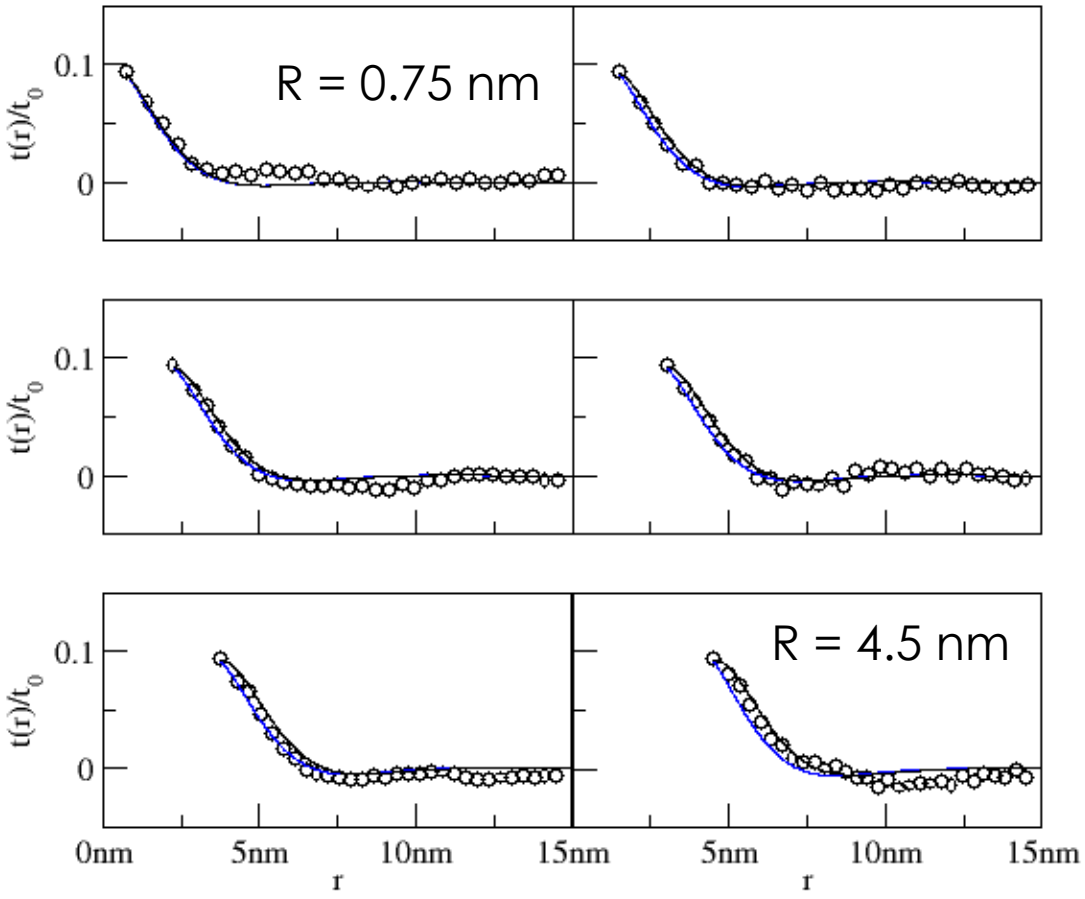


(blue line has blue terms as fit constants, black line leaves them out)

Theory of Aranda-Espinoza et al doesn't explain all this data.

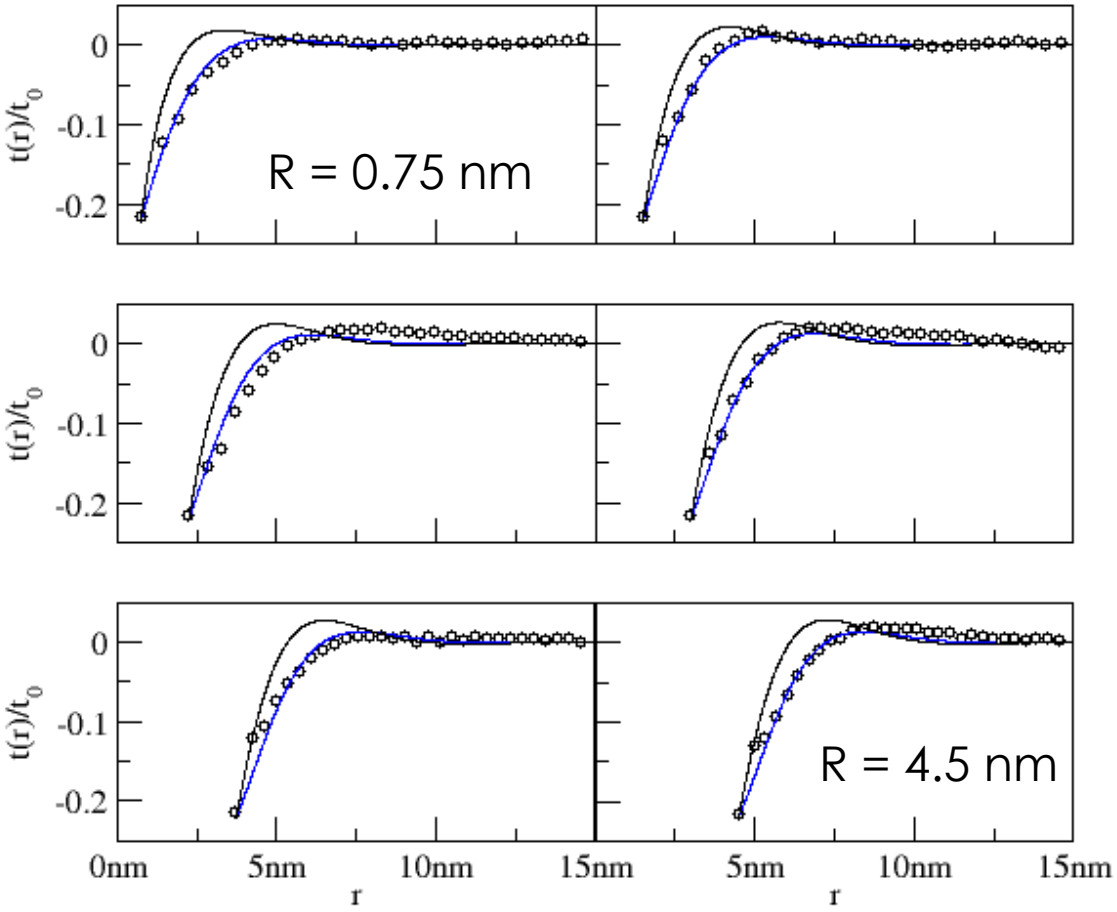
Experiment II : Comparing data to predictions

For the +10% data our changes to the analytical theory don't make much difference…



Experiment II : Comparing data to predictions

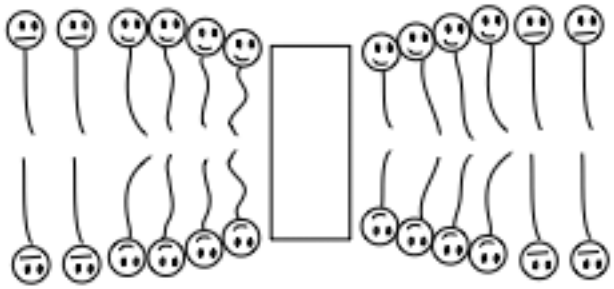
…but they do for the -20% data. Aranda-Espinoza et al predicts that the membrane will heal significantly sooner than it does.

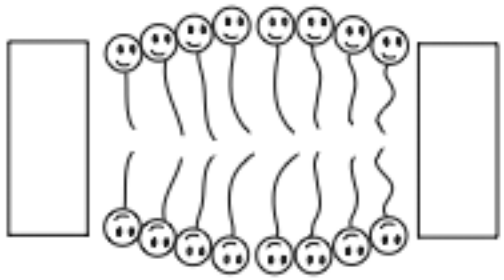


Experiment II: Implications

The theory of Aranda-Espinoza et al predicts that

- inserting an inclusion in the membrane can actually lower the elastic free energy of the membrane, due to the positive spontaneous curvature of the leaflets.
- two such inclusions would, if anything, assume a finite spacing





Experiment II:Implications

Our theory says that the favorable mean curvature effects of inserting an inclusion are countered by unfavorable Gaussian curvature effects. For reasonable numbers, inclusions that don't want to dimerize under the theory of Aranda-Espinoza do want to dimerize under our theory.

Summary

Our simulation results are consistent with predictions made using continuum elastic theories that consider the Gaussian curvature modulus and local lipid volume deformations

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