Lipid localization in bacterial membranes

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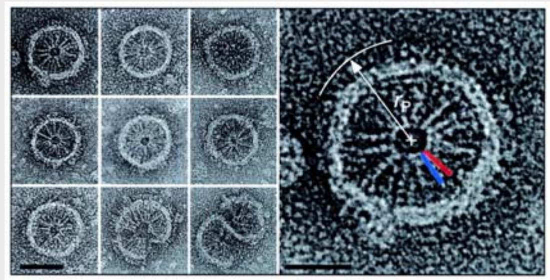
Outline

- Introduction
  - -Protein localization at bacterial poles
  - -Cardiolipin localization
- Biophysics of lipid-cluster formation
- Curvature-induced polar localization of lipid clusters
- Future directions

Protein localization at bacterial poles

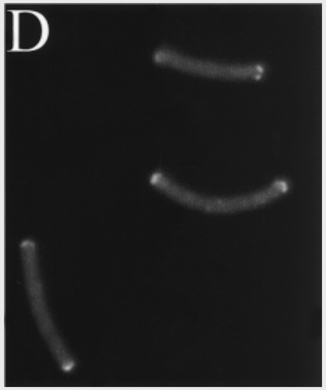
- Protein-protein interactions

Tsr micellar assemblies



Weis *et al.* (2004)

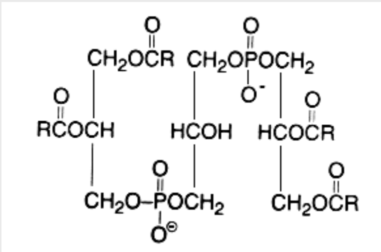
- Curvature (?)
- Turing oscillations
- Protein-lipid interactions (?)



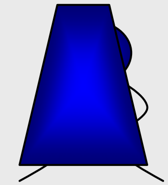
Harry & Lewis (2003)

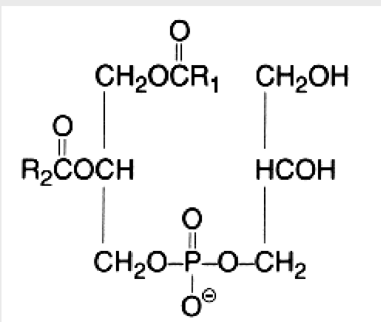
DivIVA-GFP in *B. subtilis* outgrown from spores

Bacterial phospholipids

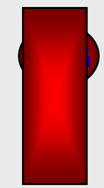


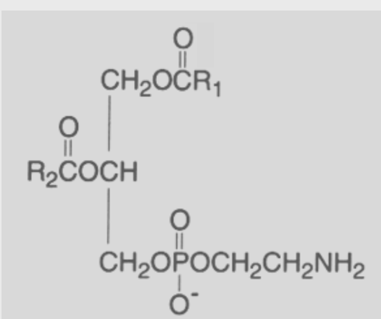
Cardiolipin (CL)



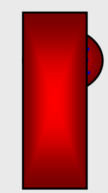


Phosphatidylglycerol (PG)





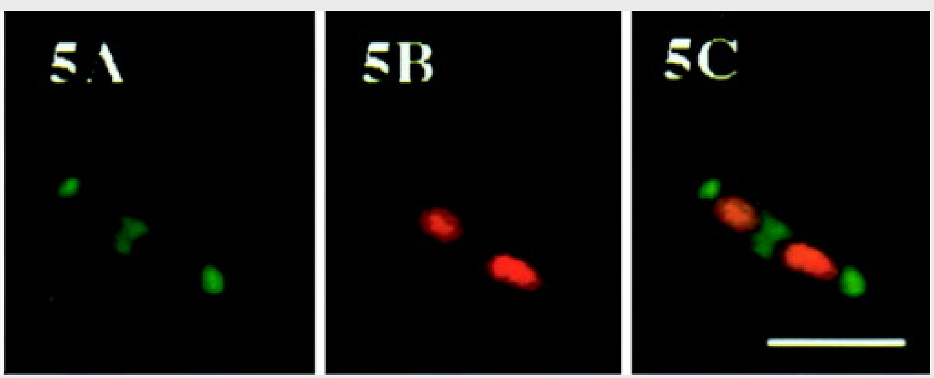
Phosphatidylethanolamine (PE)



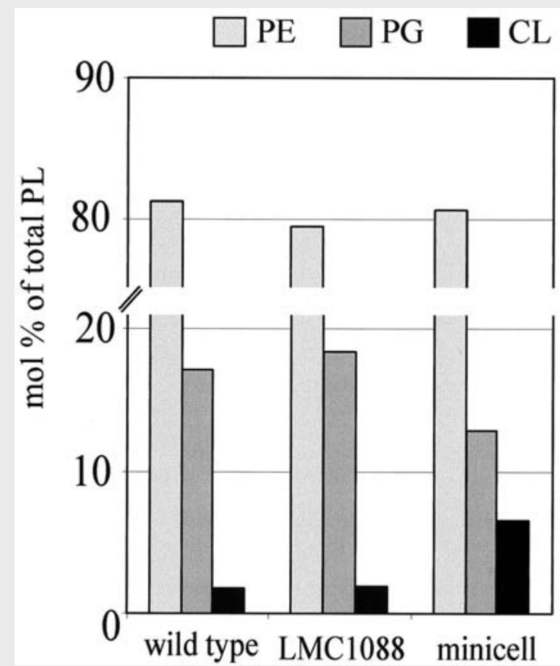
Evidence for lipid localization

- Cardiolipin (CL) can be stained with NAO (10-N-nonyl acridine-orange)
- CL levels enhanced at the poles and in minicells of *E. coli*

Green: NAO   Red: DAPI



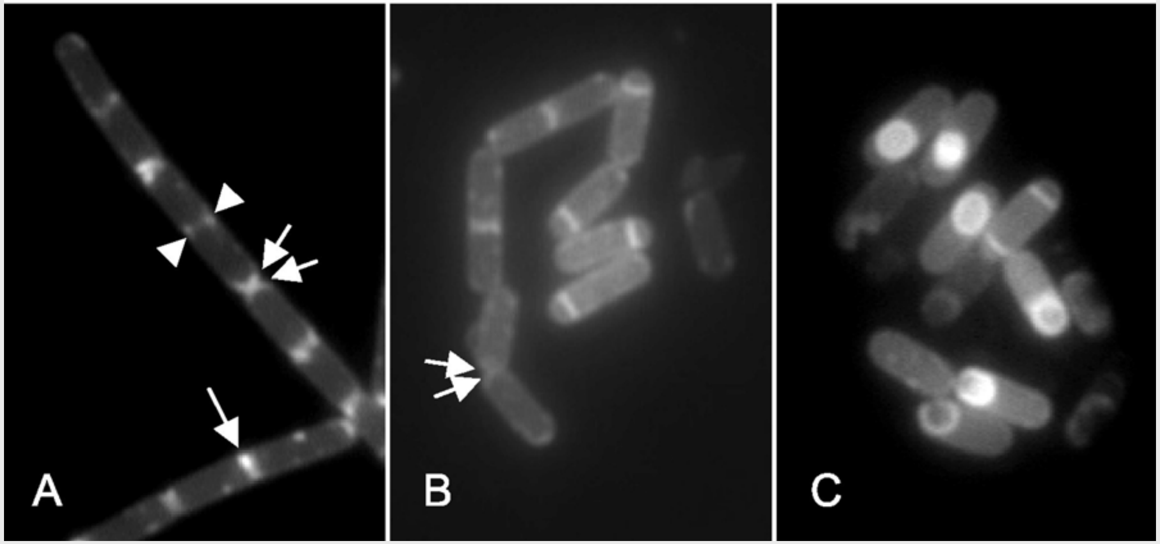
Mileykovskaya *et al.* (2000)



Koppelman *et al.* (2001)

More cardiolipin localization…

- CL also located at poles and septa in *B. subtilis*

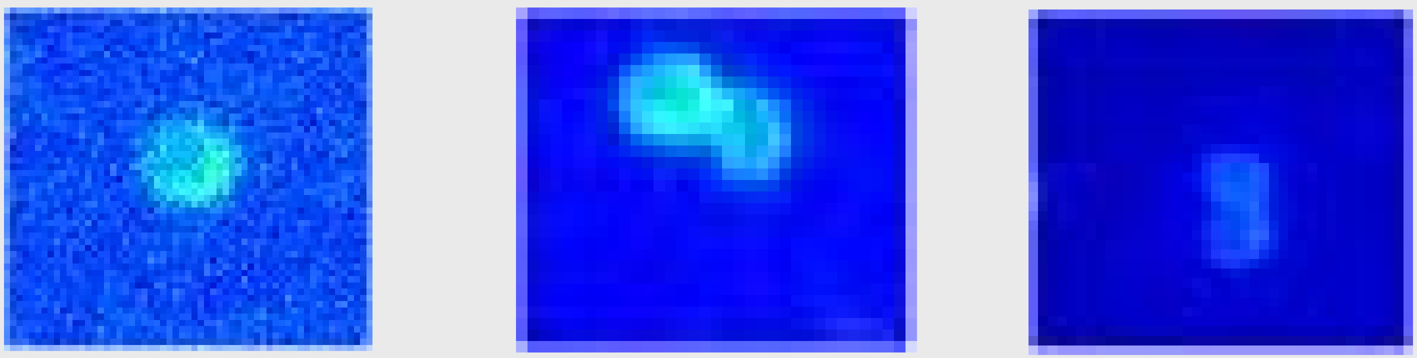


Kawaii (2004)

Exponential growth Sporulation

And cardiolipin delocalization

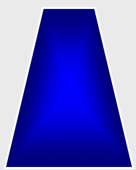
- CL delocalized in round *rodA*$^{-}$ *E. coli* cells



(live cells w/ 100nM NAO)

The lone ranger

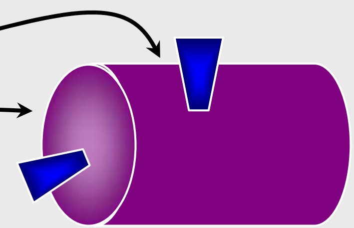
Can a single lipid find the poles based on a preference for a highly curved geometry?



Curvature $C_{lipid} < (1\ \text{nm})^{-1}$

Bending rigidity modulus $\kappa = 100 k_B T$

$C_{cyl} = (500\ \text{nm})^{-1}$

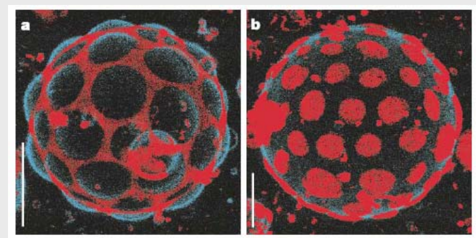


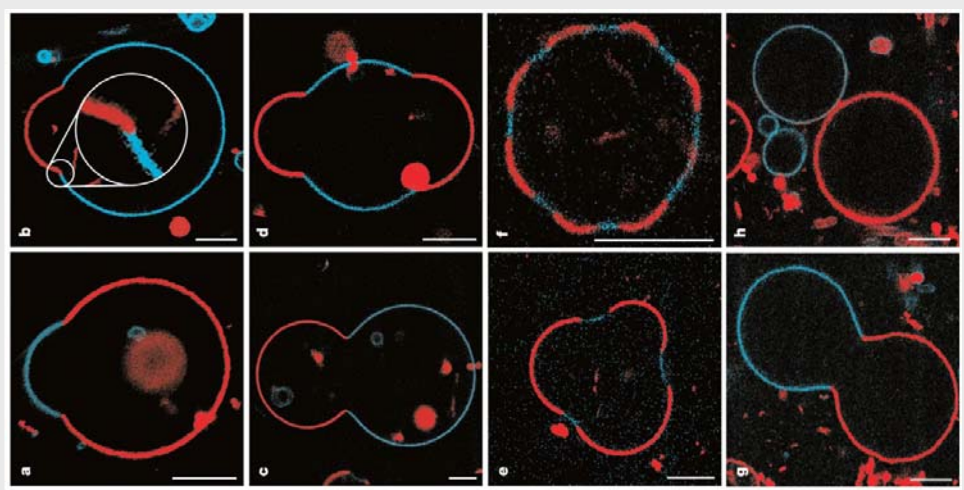
$C_{pole} = 2\ C_{cyl}$

$$\Delta E_{pole} \sim \kappa C_{cyl} C_{lipid} \sim \left(100 k_B T\right) \times \left(\frac{1}{500\text{nm}}\right) \times \left(\frac{1}{1\text{nm}}\right) \times \left(\text{nm}^2\right) = 0.2 k_B T$$

Only ≈20% enrichment for single lipids at poles

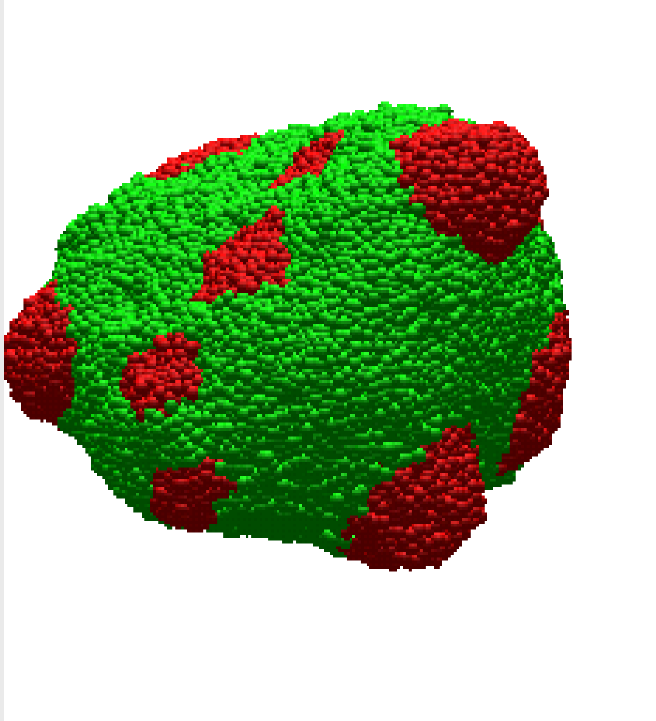
Lipid phase separation in vesicles





Baumgart *et al.* (2003)

Domains and budding in lipid mixtures



Laradji and Kumar (2005)

Lipid domains in vesicles from particle-dynamics simulations

Budding of lipid domains is due to:

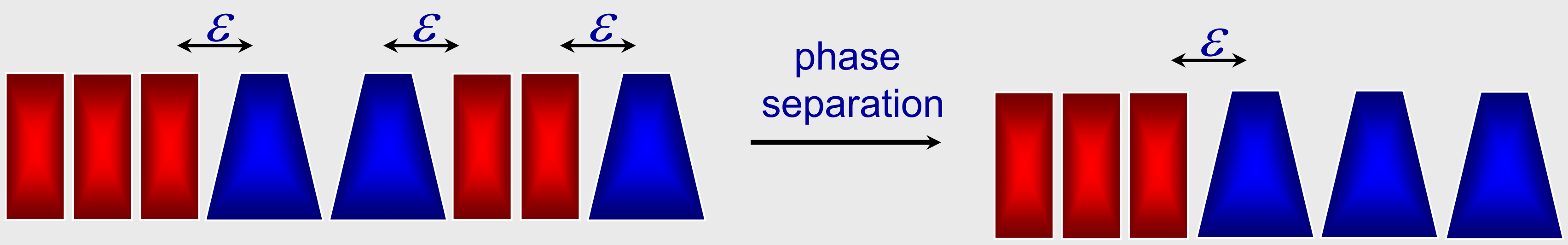
i. spontaneous curvature of lipids
ii. line tension at domain boundaries

Question: What is the effect of the cell wall on lipid domains?

Energy model for membrane

- Short-range attraction between like lipids can lead to phase separation:

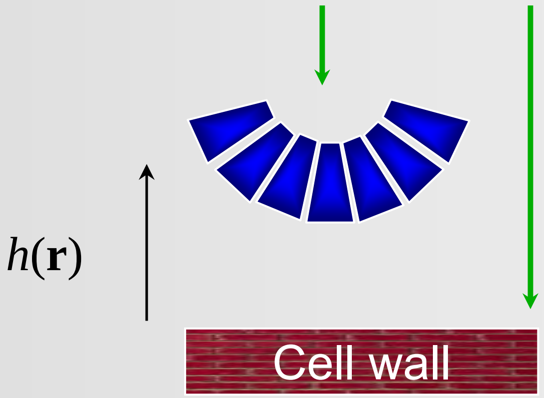
$$E_{\text{coupling}} = \sum_{i} \sum_{\text{n.n. } j} \frac{\varepsilon}{2} \left[\sigma_i (1 - \sigma_j) + \sigma_j (1 - \sigma_i) \right], \quad \sigma_i = 0,1$$

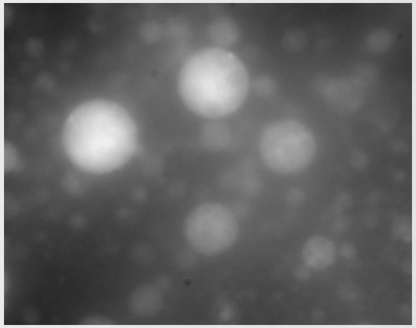


Energy model for membrane, continued

- Elastic energy of membrane

$$E_{\text{elastic}} = \int \left[\frac{\kappa}{2}\left(\nabla^2 h - C\right)^2 + \frac{\lambda}{2} h^2 \right] dA$$





$$C(\mathbf{r}) = C_{\text{lipid}}(\mathbf{r}) - C_{\text{cell wall}}(\mathbf{r}), \text{ where } C_{\text{lipid}}(\mathbf{r}) = \begin{cases} \gamma \text{ if lipid A at } \mathbf{r} \\ 0 \text{ if lipid B at } \mathbf{r} \end{cases}$$

Lipid cluster size is limited by energy penalty for curving membrane away from cell wall…

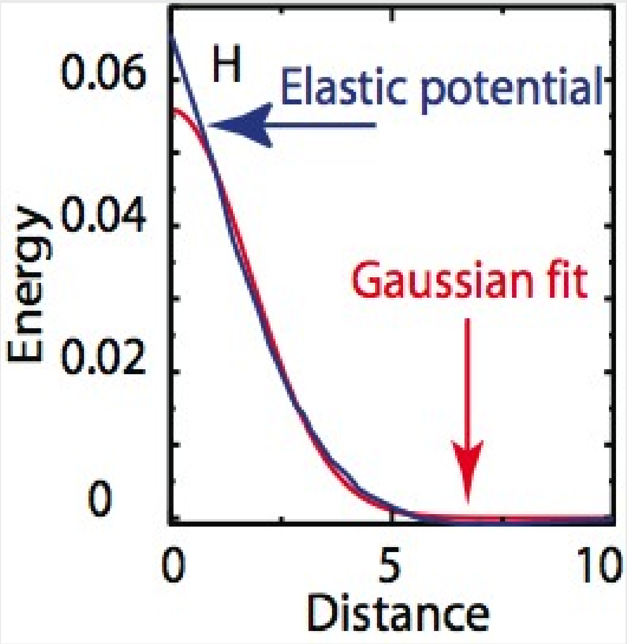
Elastic energy of membrane mediates long-range lipid-lipid repulsion

- Minimize energy with respect to membrane height *h*
- Leads to effective repulsion between lipid A molecules

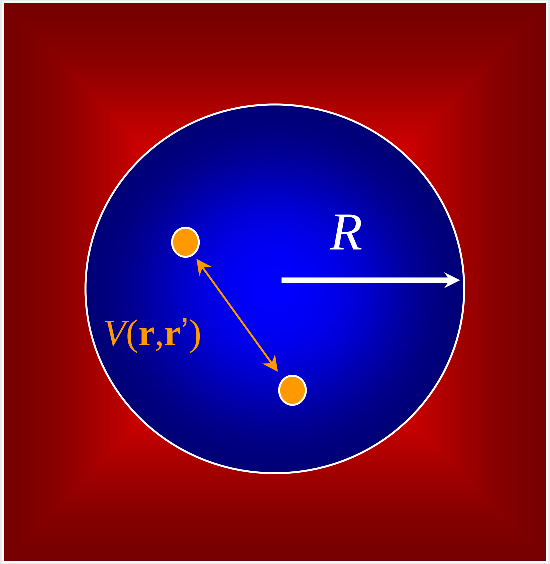
given by $V(\vec{r}-\vec{r}\,')$

$V(|\vec{r}-\vec{r}\,'|)$

$(k_B T)$

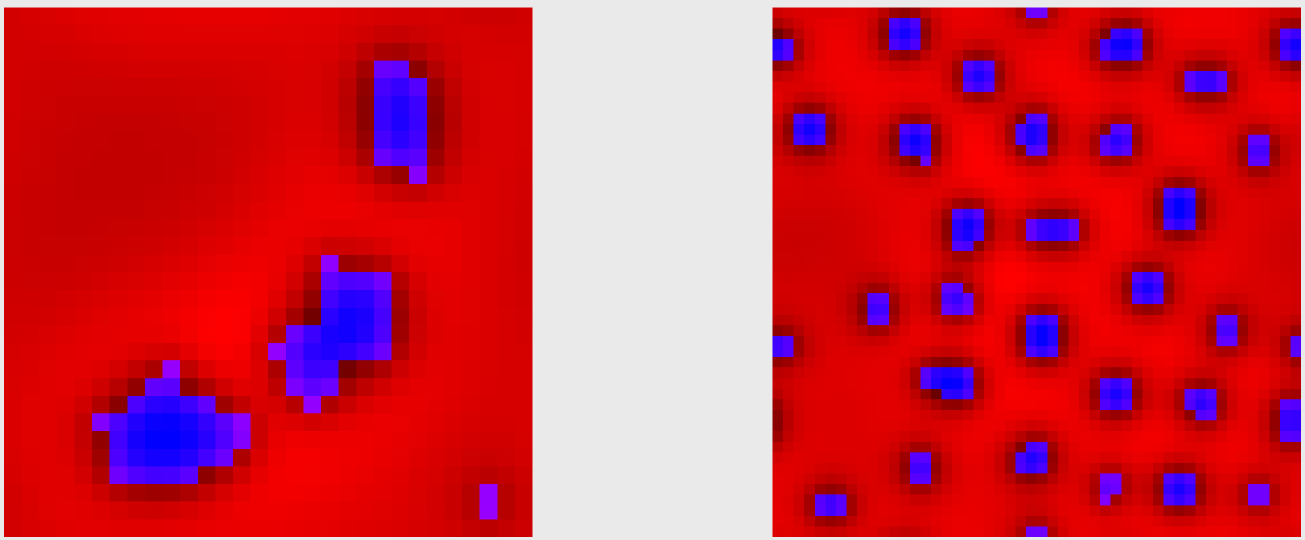


(nm)



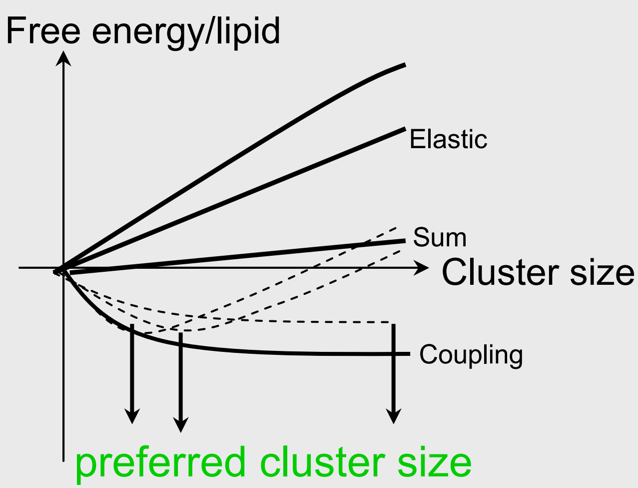
Short-range attraction vs long-range repulsion

- Can lead to stable clusters
- Cluster size controlled by lipid-lipid attraction, spontaneous curvature of lipids, and pinning of membrane by cell wall



"Cardiolipin" clusters
(from Monte Carlo simulations)

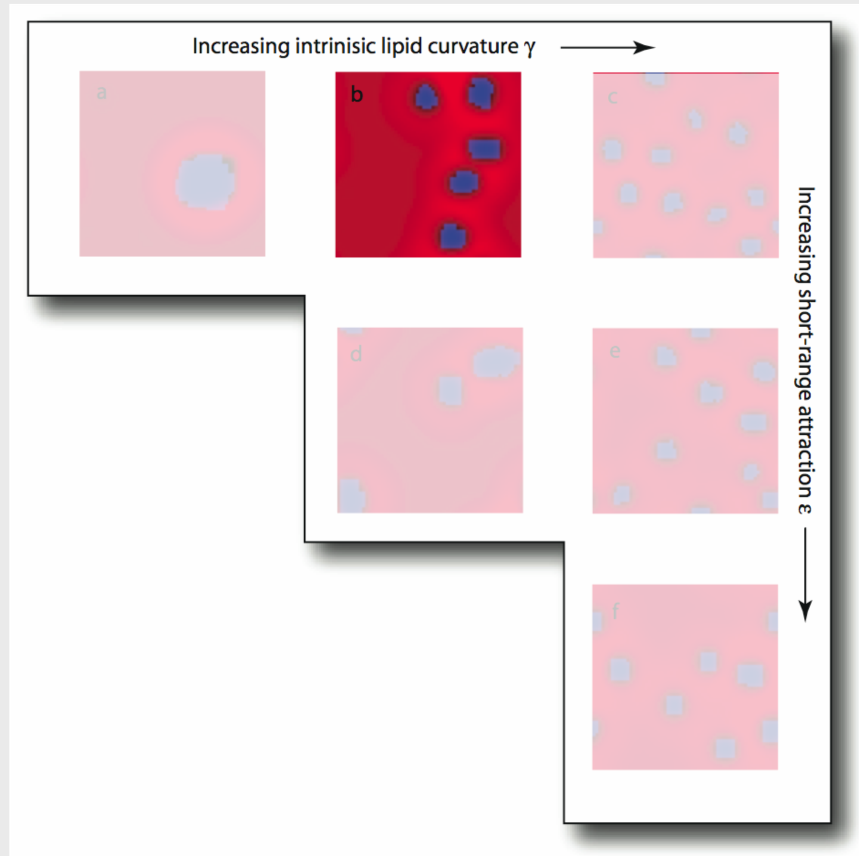
Effect of changing γ



$\varepsilon = 2.5k_BT$

$\varepsilon = 3k_BT$

$\varepsilon = 4k_BT$

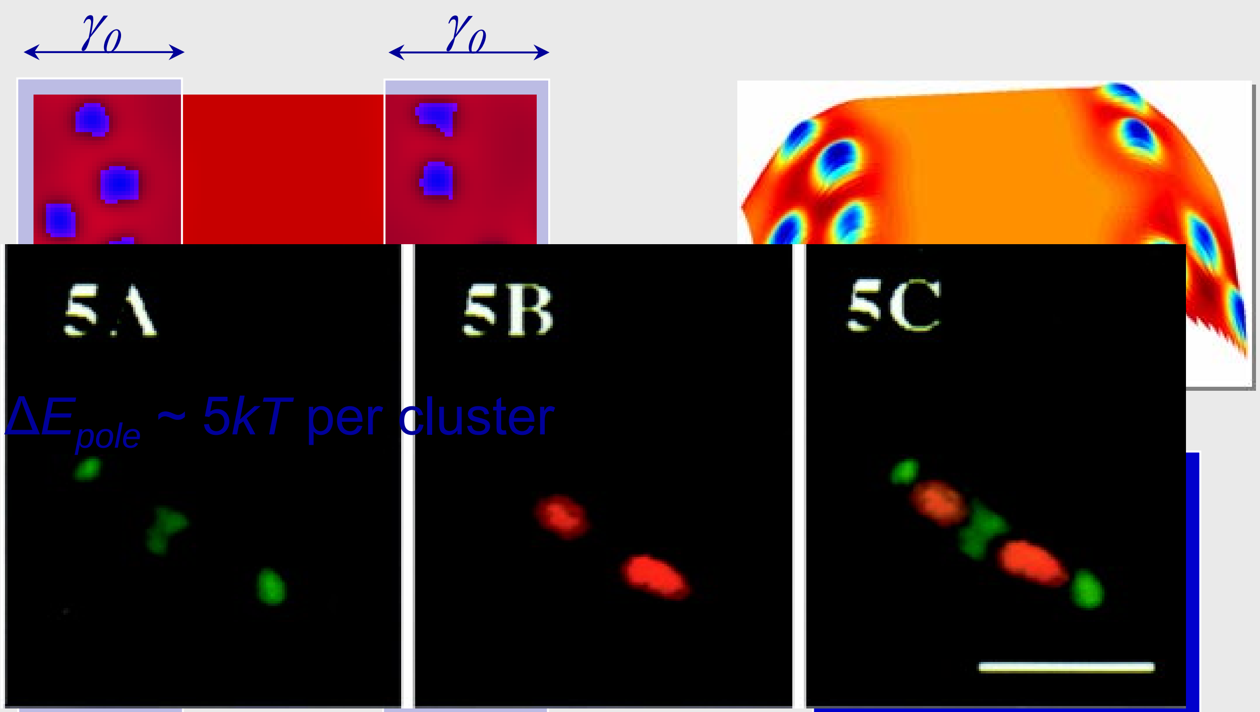


$\gamma = 0.2$/nm $\gamma = 0.4$/nm $\gamma = 0.6$/nm

Decreasing γ has same effect as increasing ε (cluster size ~ε / γ^2)

Effect of curved poles

$\varepsilon = 2.5k_BT$



$\Delta E_{pole} \sim 5kT$ per cluster

$\varepsilon = 1k_BT$

$\gamma_0 = 0.04/\text{nm}$

Clustering is crucial for polar localization

Effect of increased concentration

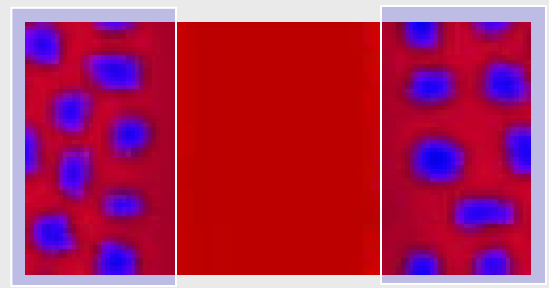
fraction of lipid A: $\phi = 0.15$

$\kappa = 25 k_B T$

$\lambda = 0.25 k_B T/\mathrm{nm}^2$

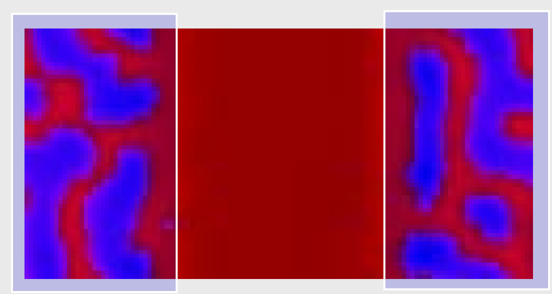
$\gamma = 0.4/\mathrm{nm}$

$\gamma_0 = 0.04/\mathrm{nm}$



fraction of lipid A: $\phi = 0.3$

Domain size not a function of ϕ...

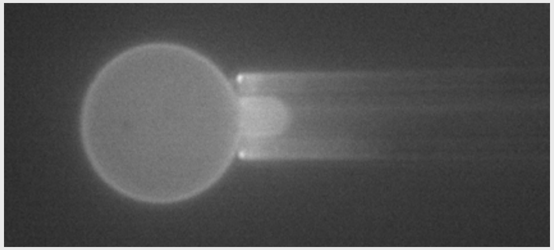


Conclusions

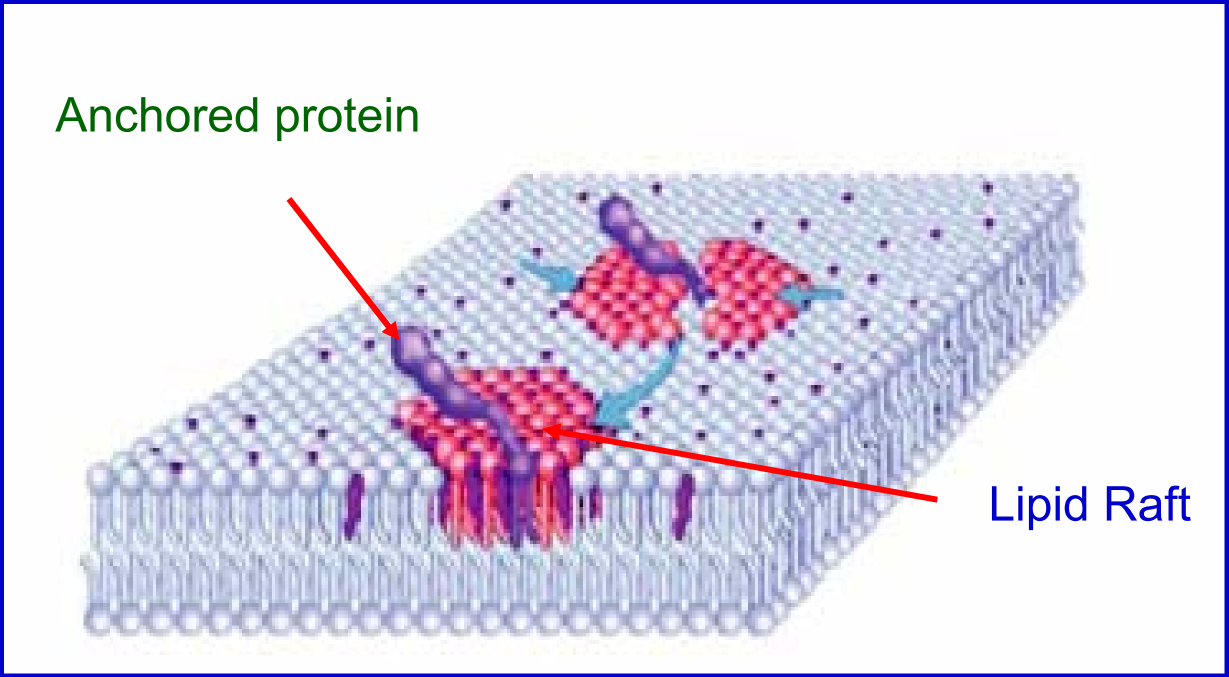
- Lipids can form clusters due to a competition between phase separation and pinning by the cell wall.
- Clusters of high-curvature lipids can localize to both poles of the cell.
- Cardiolipin may serve as a target for polar localization of proteins.

Future directions

- Biophysics experiments (w/Rob Phillips@Caltech)
  - Does cardiolipin go to highly curved regions of lipid vesicles?
  - Will cardiolipin mediate localization of proteins (*e.g.* DivIVA)?



- Biology experiments
  - Co-imaging of cardiolipin and DivIVA, etc., particularly in cells that branch (*e.g. Streptomyces*).
  - Cardiolipin “knockouts” (hard to kill).



20-30 nm

- **Lateral compartmentalization of lipids, cholesterol, and protein molecules, violates the *Fluid Mosaic Model* (Singer-Nicholson, Science ’72)**

- **Rafts found on the outer leaflet of the plasma membrane of animal cells and is rich in sphingolipids, cholesterol and anchored proteins (Simons and Ikonen, Nature ’97)**

- **Recent evidence of lipid localization in plasma membranes of bacteria.**