Physics of membranes VI
Stabilization of membrane rafts

- Membrane Rafts: the physics-biology puzzle?
- Rafts as fluctuations

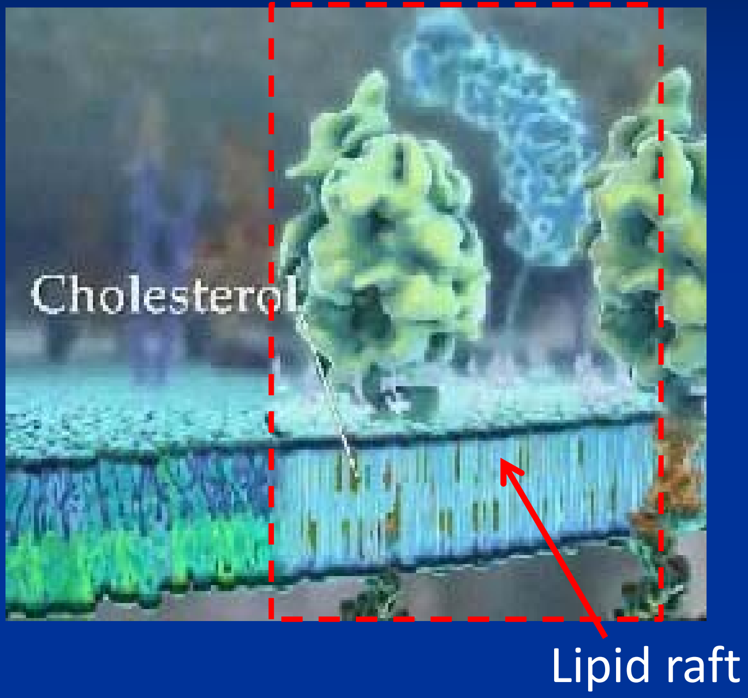
- Hybrid lipids as "linactants*" to stabilize domains
- Domain size? Spontaneous curvature – chain effect
- Simulations, fluctuations, experiments

*linactant:

D. Schwartz, PRL 2008

Phase Separation: S. A. Safran, Statistical thermodynamics of surfaces, interfaces, membranes (Westview Press)
Rafts: Collaboration: R. Brewster, T. Yamamoto, P. Pincus, B. Palmieri (Refs. on slides)

Lipid raft hypothesis – biological systems



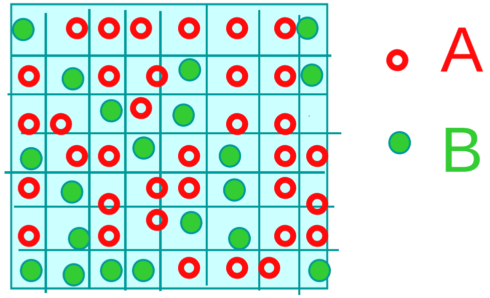
(From: The Inner Life of the Cell http://multimedia.mcb.harvard.edu/anim_innerlife.html)

Lipid raft : small domains in biological membranes – controversial !

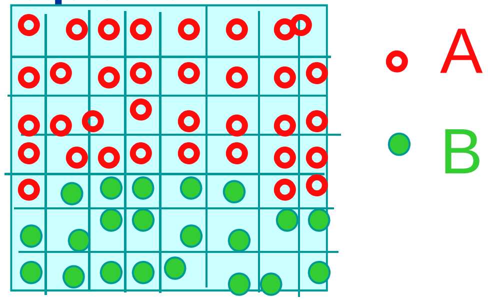
- Rich in saturated lipids
- Metastable or even stable
- The size of the domain is of the linear order of 10 – 100 nm.

Interactions in mixtures: phase separation

- Entropy: random mixture of two species
  - Alloy: two metals
  - Lipid/protein mixtures in membranes
  - Mixtures of lipids in membranes



- Attractions of same species: phase separation
  - What is extent of phase separation?
  - Pure A and Pure B or still mixed?
  - What are domain sizes?
  - Kinetics: diffusion instabilities



Phase separation

Proc.Natl.Acad.Sci.USA

Vol.93,pp.377–382,January 1996

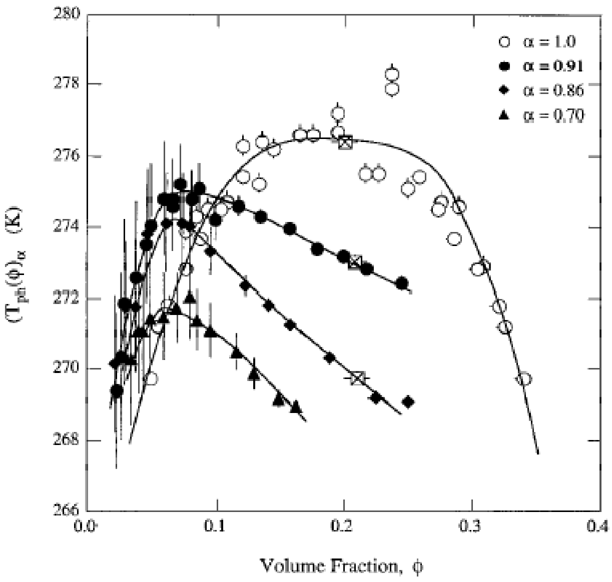
Biophysics

Phase separation in aqueous solutions of lens g-crystallins:

CANWEN LIU,NEER ASHERIE,ALEKSEY LOMAKIN,JAYANTI PANDE,OLUTAYO OGUN,AND GEORGE B.BENEDEK*

Department of Physics,and Center for Materials Sciences and Engineering,Massachusetts Institute of Technology,Cambridge,MA 02139

Phase separation of the eye lens cytoplasm has been implicated in cataract formation, where opacification of the eye lens results from disturbances in the uniform spatial distribution of the lens proteins.The lens proteins primarily involved in phase separation are the g-crystallins, a family of lens-specific monomeric proteins.

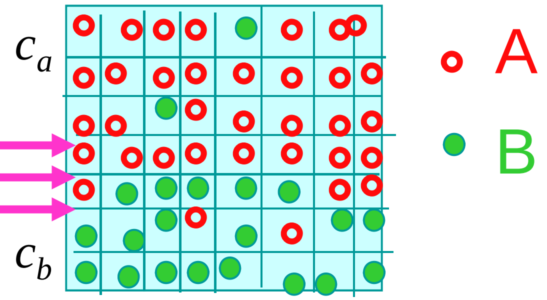


Interfacial free energy: interfacial tension

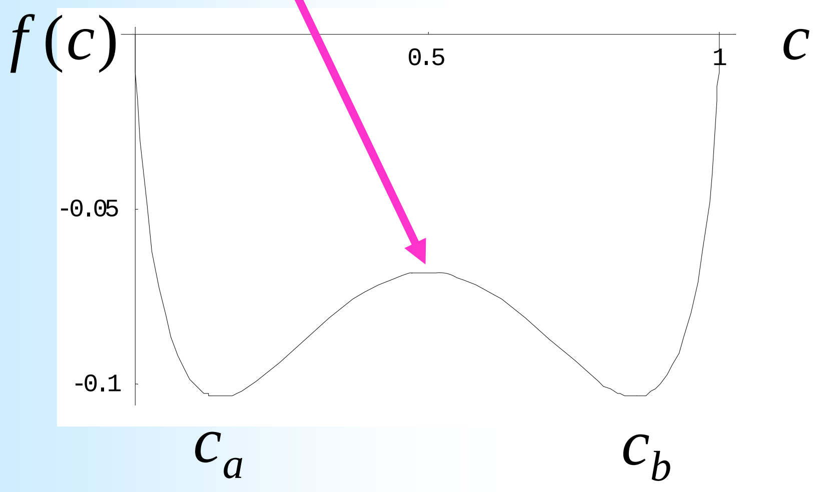
- Composition must vary between 2 coexisting phases

In interfacial zone

$$c \approx \frac{1}{2}\left(c_a + c_b\right)$$



Phase separation implies interface between the two phases

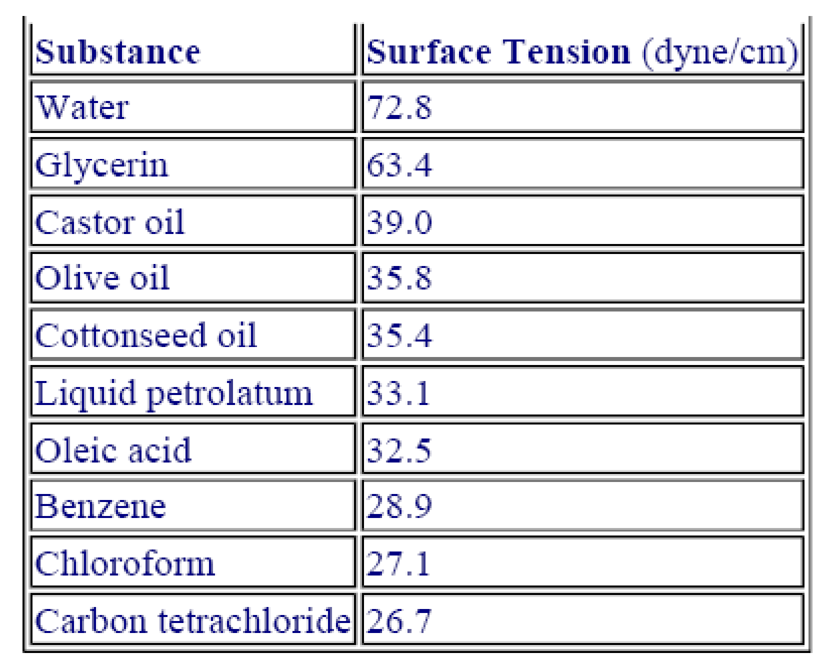


Interfacial region has higher free energy: interfacial tension

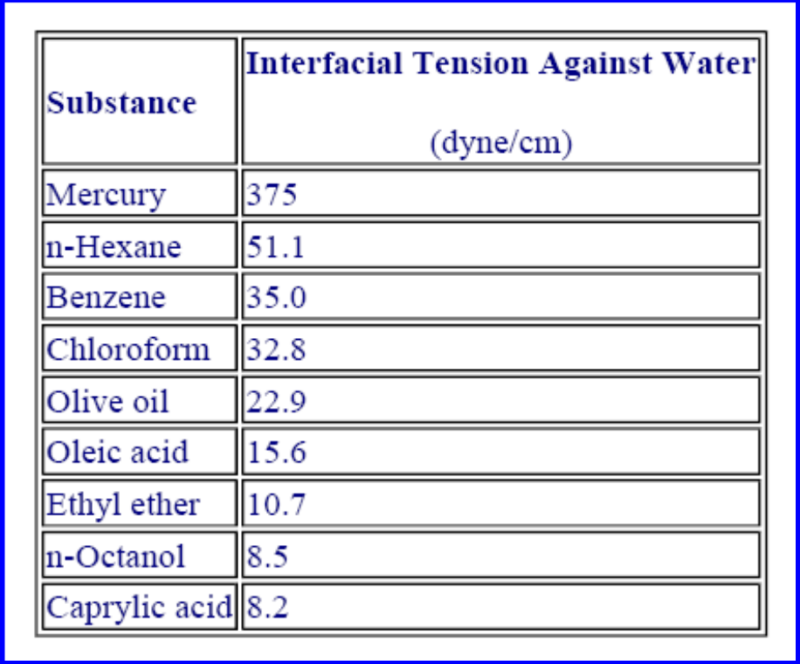
Interfacial energy/area (3d)

Line tension: interfacial energy/length (2d)

Values of surface tension



Substance	**Surface Tension** (dyne/cm)
Water	72.8
Glycerin	63.4
Castor oil	39.0
Olive oil	35.8
Cottonseed oil	35.4
Liquid petrolatum	33.1
Oleic acid	32.5
Benzene	28.9
Chloroform	27.1
Carbon tetrachloride	26.7



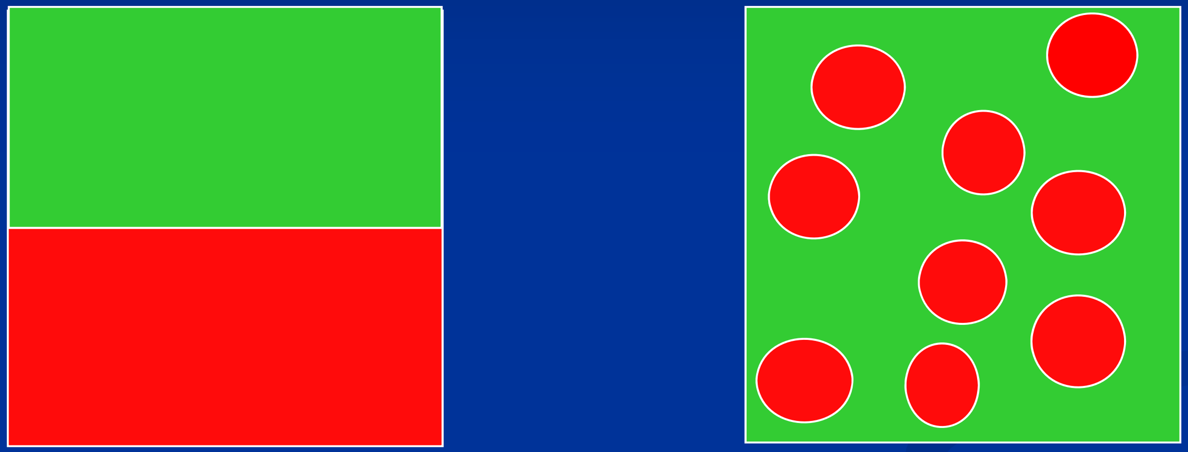
Substance	**Interfacial Tension Against Water** (dyne/cm)
Mercury	375
n-Hexane	51.1
Benzene	35.0
Chloroform	32.8
Olive oil	22.9
Oleic acid	15.6
Ethyl ether	10.7
n-Octanol	8.5
Caprylic acid	8.2

$$\gamma \sim T / a^2$$

1 Kelvin = 2.4 10^{-16}erg; 300 Kelvin/(0.5 nm)^2 ~ 17 dyne/cm

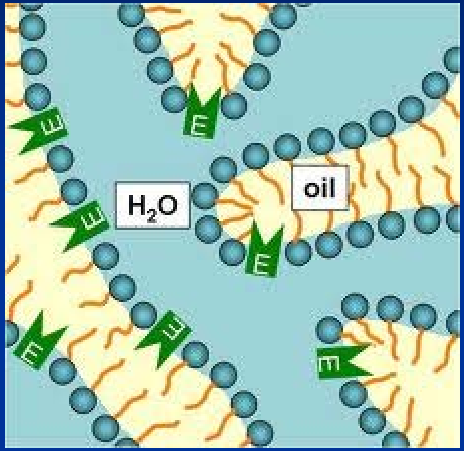
Interfacial free energy and phase separation

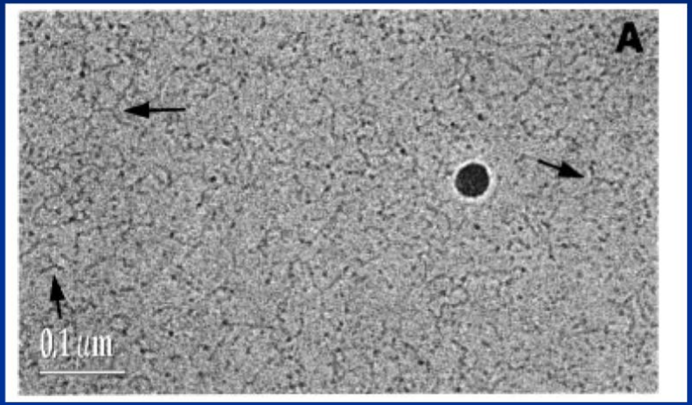
- Usually, tension $\gamma > 0$ min free energy: minimal interface



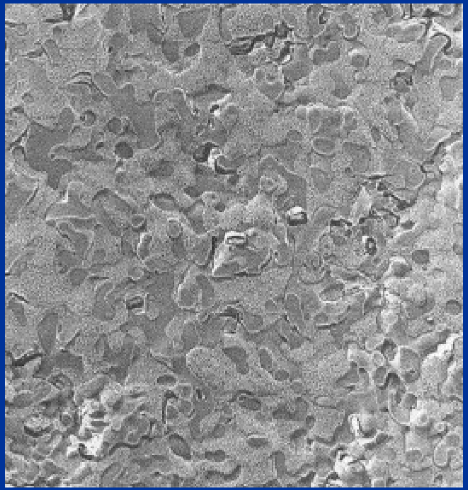
- This is why - usual phase separation: macroscopic
- For two competing interactions can get finite domains
- Can also get finite domains for systems with surfactants at interface of e.g., water and oil phases (microemulsions)

Surfactant stabilized domains



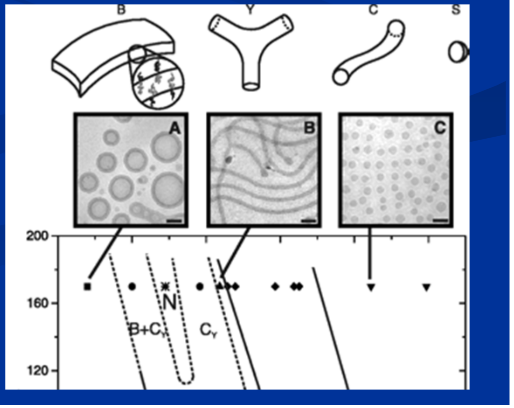


Bernheim, Talmon 1999
microemulsion



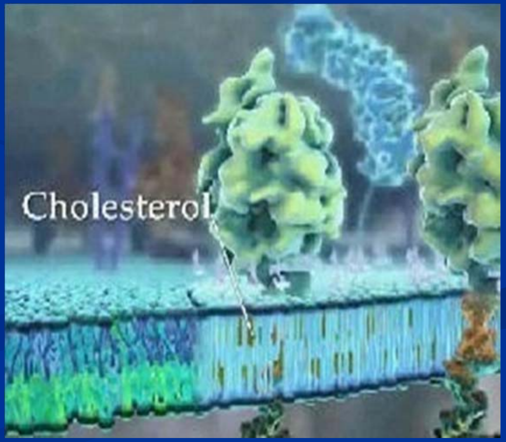
Jahn, Strey 1988

Jain and Bates 2003 :
block copolymers



Generalizations of surfactants

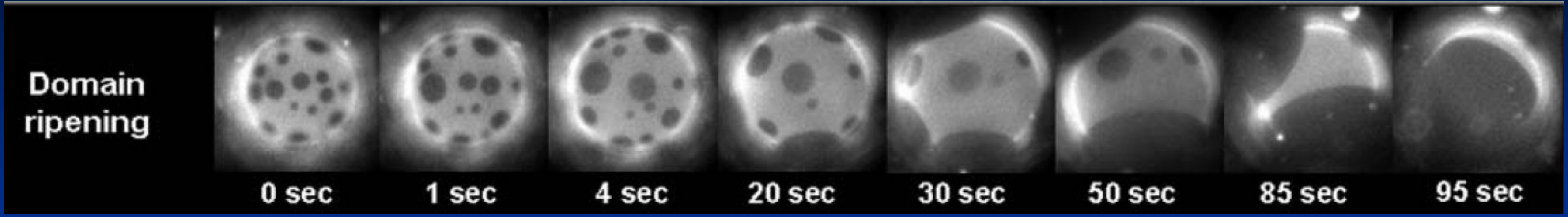
- Not necessarily hydrophilic/hydrophobic stabilization
- Molecules with dual nature: interfacially active
- Phase separation in 2d membranes (linactants*)
- Biological puzzle: membrane rafts



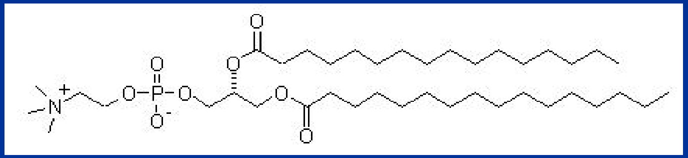
*linactant:

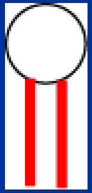
D. Schwartz, PRL 2008

Domains in lipid vesicles – DPCC, DOPC, Chol

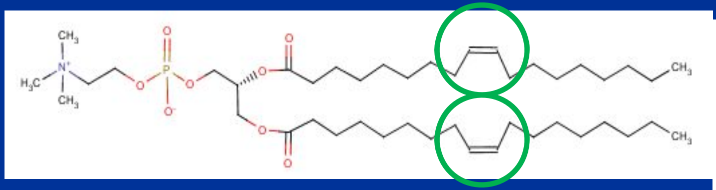


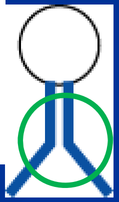
Saturated lipids, *e.g.* DPPC, S



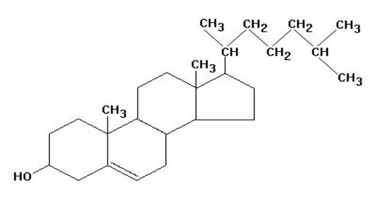


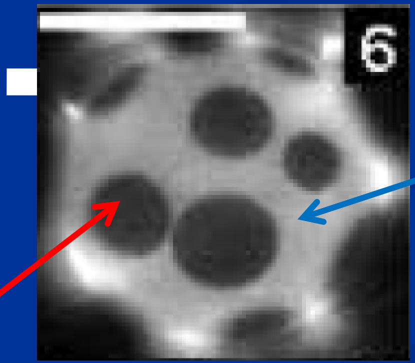
Unsaturated lipids, *e.g.*,DOPC U





Cholesterol





L_d phase: Rich in U and lipid chains are disordered.

L_o phase: Rich in S and chol; the lipid chains are ordered.

*Veatch and Keller, BPJ, **85**, 3074 (2003).*

Silva et al., submitted .

Rafts: composition fluctuation in single phase?

Biochimica et Biophysica Acta 1788 (2009) 53–63

Aurelia R. Honerkamp-Smith , Sarah L. Veatch, Sarah L. Keller

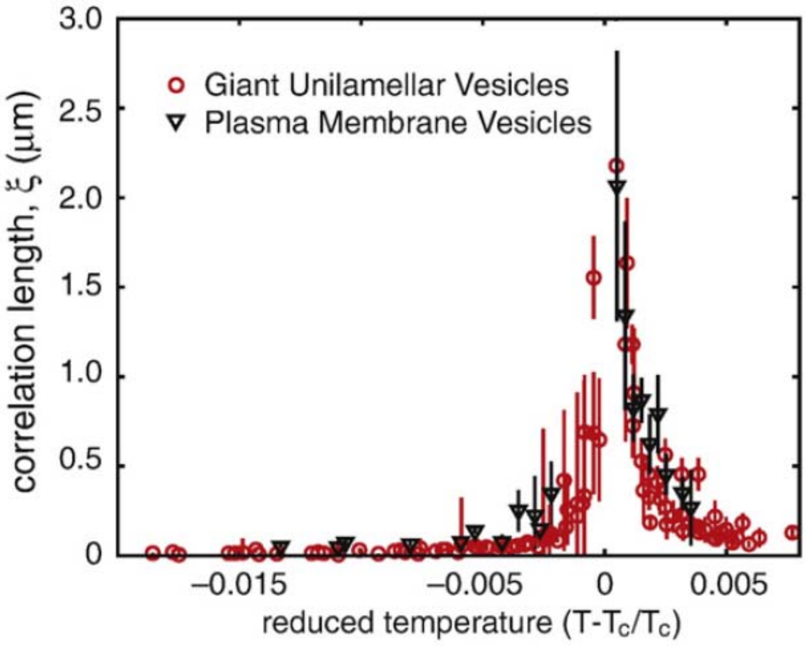


Fig. 6. Diverging correlation length, ξ, vs. reduced temperature $T_r=(T-T_c)/T_c$ for five different giant unilamellar vesicles (red circles) and a single plasma membrane vesicle (black triangles). Equivalent data were published previously in Fig. 5 of [12] and Fig. 3 of [11]. The data sets superimpose well, even though the compositions of the two membrane systems are different. For temperatures below T_c, we have used $\xi=(k_B T_c)/\lambda$, as described in [12].

Rafts: equilbrium, finite domains in 2 phase region?

- Alternative view: rafts are stable, long-lived domains due to phase separation of saturated/unsaturated lipids.

  How can finite domains be stable (in equilibrium)?

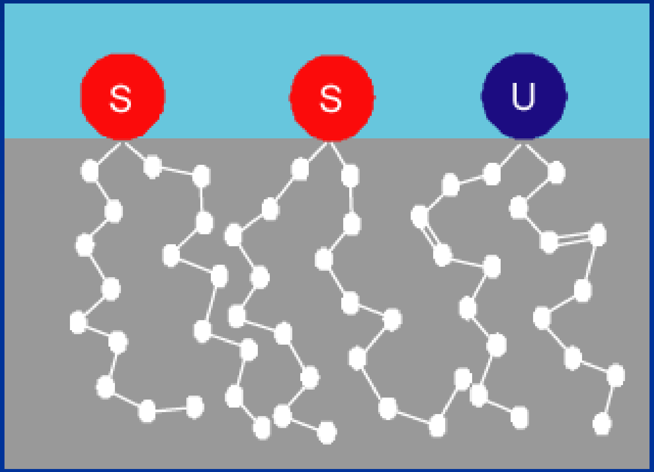
- Line active species reduces interfacial free energy:
  line analogy of surfactants
- Here, “linactant*” chains match the two phases:
  only chain packing needed to explain effect

*linactant:

D. Schwartz, PRL 2008

Chain packing theory of macroscopic phase separation

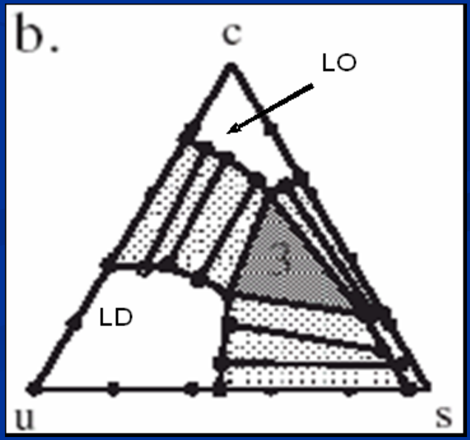
Chain packing “frustration” of saturated/unsaturated lipids is the driving force of phase separation of lipid membrane



- Phenomenological potential chain order
- Packing entropy
- Incompressibility of hydrophobic core

Bulk phases

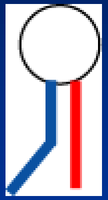
(saturated, unsaturated, cholesterol)

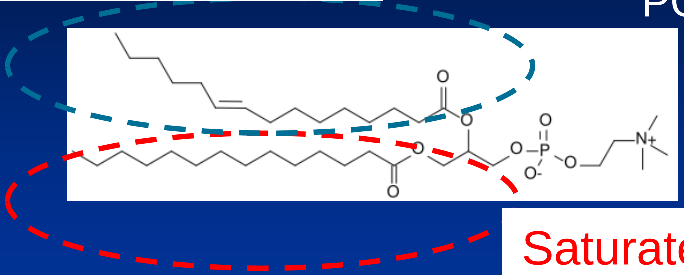


R. Elliot, I Szleifer, and M. Schick, PRL, 96, 098101 (2006).

Line activity of hybrid lipid: reduces packing frustration

Hybrid lipids H





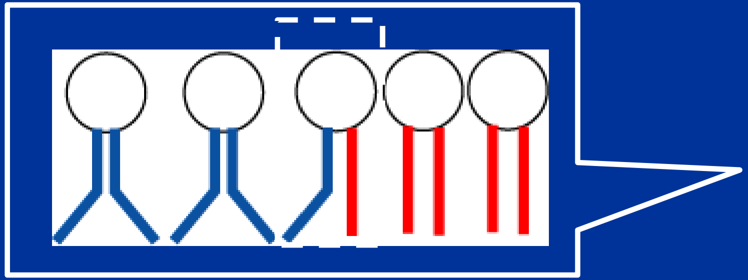
Unsaturated tail

POPC

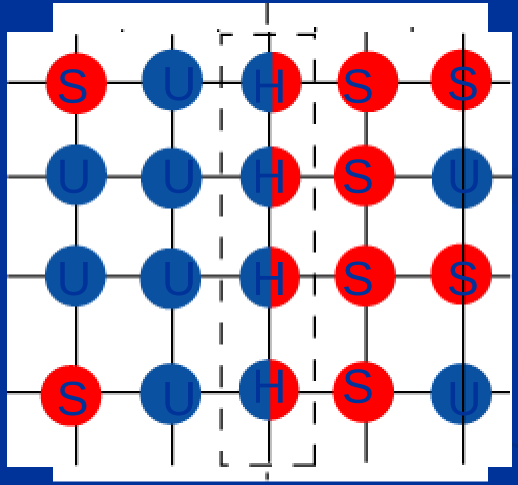
Saturated tail

Biologically abundant

Side view



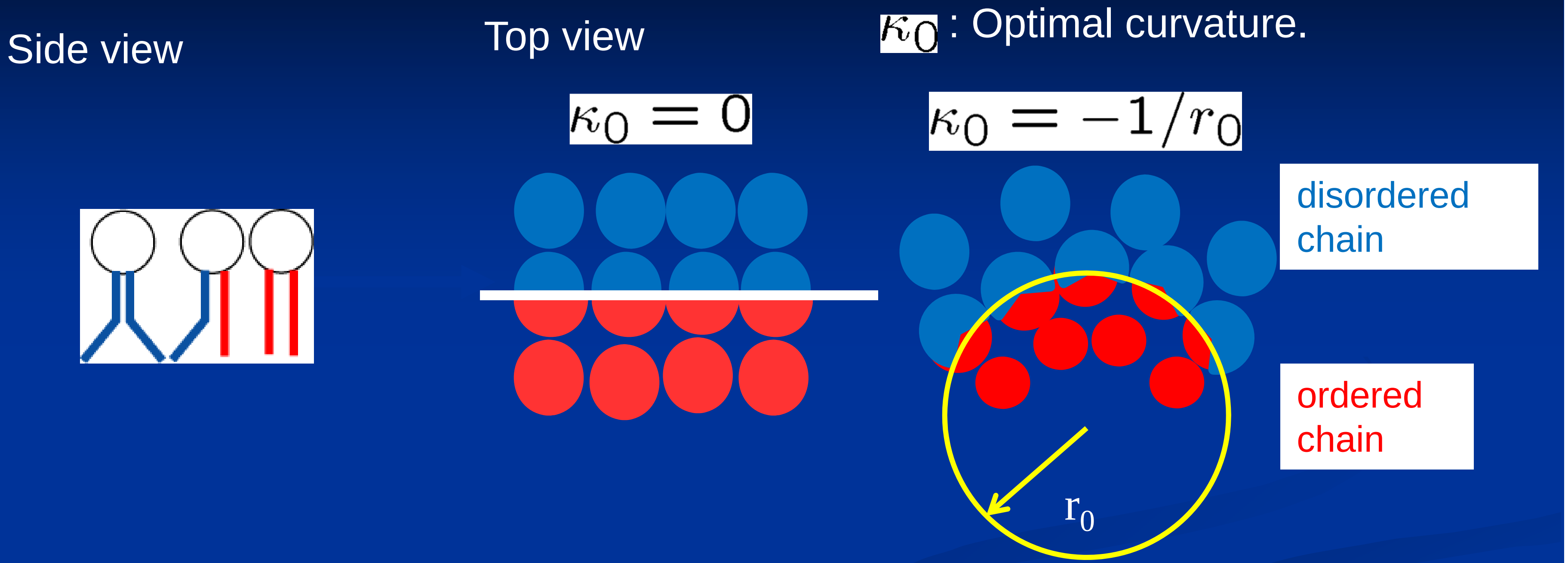
Top view



Line tension is reduced to zero for strong interaction (or low temperature).

R. Brewster, P. A. Pincus, and S. A. Safran, Biophys. J., ***97****, 1087 (2009)*
R. Brewster, S. A. Safran, Biophys. J. Lett., ***98*** *L21 (2010)*

Domain size: 2D spontaneous curvature



Side view

Top view

κ_0 : Optimal curvature.

$$\kappa_0 = 0$$

$$\kappa_0 = -1/r_0$$

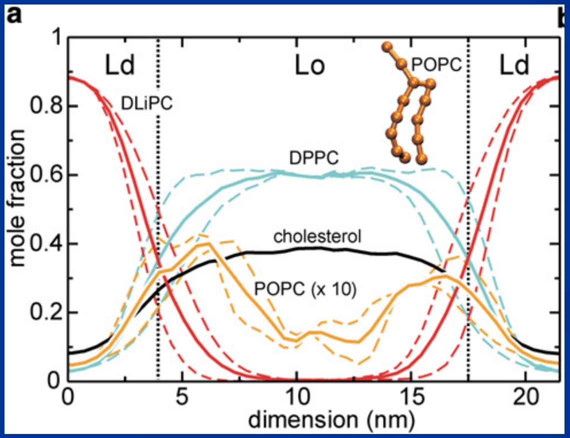
- Spontaneous curvature determines radius of domains stabilized by ◐.
- Subject to conservation constraints of S, U, and H (microemulsion)
- R. Brewster, SAS: Biophys. J. Lett., 2010

Simulations:
Partitioning of Lipids at Domain Boundaries in Model Membranes

Lars V. Schafer* and Siewert J. Marrink*

Biophys. J. 2010

- Line-active molecules (“lineactants”) bind to the interface of lipid domains
- Predict that certain hybrid saturated/unsaturated chain lipids (POPC)
  - bind to the interface
  - lower the line tension (particular simulations reduction ~ 28%)



Experiments on lipid mixtures: S+U+H

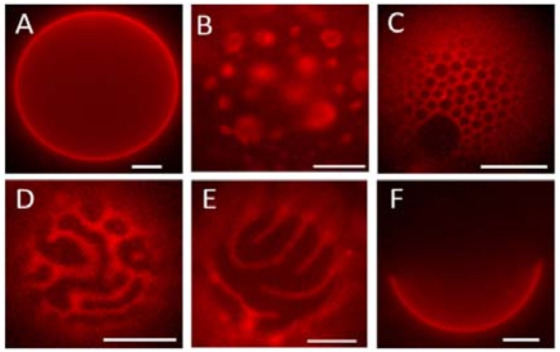


FIGURE 2 GUV patterns as POPC is replaced by DOPC are (*A*) uniform; (*B*) continuous Lo with asymmetric Ld domains; (*C*) honeycomb; (*D*) irregular patches of Lo; (*E*) regular striped pattern; and (*F*) round macro Lo+Ld domains, with compositions of DSPC/DOPC/POPC/Chol of 0.45/0.03/0.27/0.25 (*A*), 0.45/0.05/0.25/0.25 (*B* and *E*), 0.39/0.06/0.33/0.22 (*C*), 0.39/0.10/0.29/0.22 (*D*), and 0.45/0.30/0/0.25 (*F*). The dye is C12:0-DiI at 0.02 mol %. Scale bars, 10 μm; temperature, 23°C.

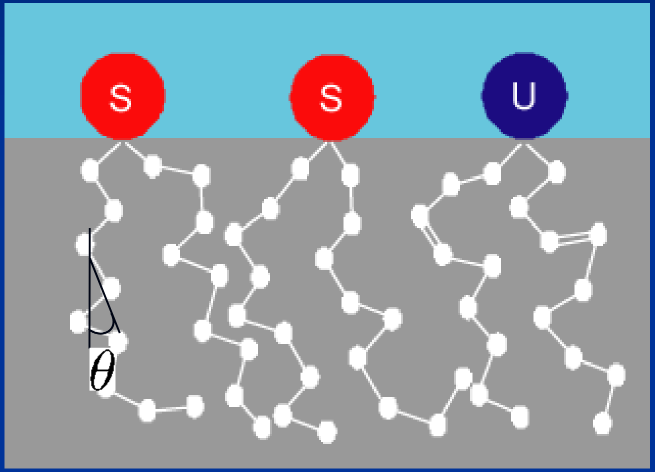
Tonyakinha et al.
Feigenson group
Biophys. J. 2011

Modulations observed
with finite amounts of U, H.
Perhaps then H is at the
boundaries

Scattering from membrane stacks: H seems to cause S+U to mix
Or Szekely and Uri Raviv,
Langmuir, 2011

Quantify role of chain order in lipid interactions

What is role of chain ordering in line activity of H ?



Liquid crystal order parameter

$$S = \langle \frac{3\cos^2\theta - 1}{2} \rangle \quad -0.5 \le S \le 1$$

θ: The angle between the bilayer normal and chain segment

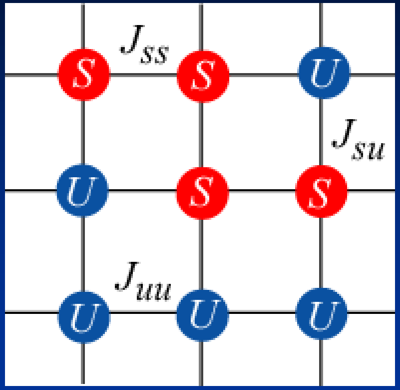
Liquid crystal model shows how chain ordering drives phase separation and line activity of hybrid lipids

Phase separation and line activity only from chain order : no new chemistry

T. Yamamoto, R. Brewster and SAS, Europhys. Lett., 2010
T. Yamamoto and SAS, Soft Matter, 2011

Lattice model for membrane without hybrid lipids

Lipids



Assumption:

The order parameter for U is ~ 0 ,
($S_u \sim 0$), because entropy >> interaction.

Mixing entropy

Interaction energy **(favorable when neighboring chains are ordered - *only interactions due to chain ordering*)**

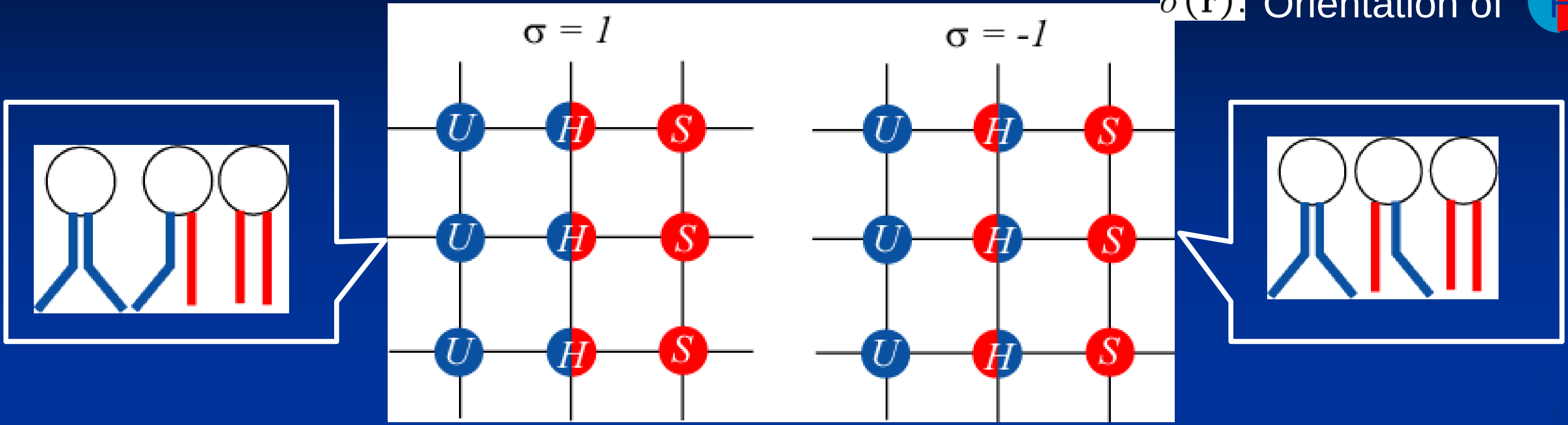
Entropy loss due to chain ordering

Chain order parameter for S

P. G. de Gennes and J. Prost, The Physics of Liquid Crystals

Lattice model for membrane with H

$\sigma(\mathbf{r})$: Orientation of H



Orientation **dependent** part of the interaction energy

Orientational entropy

Line Tension Reduction

At high T:

- Concentration of H adsorbed at interfaces is not enough to reduce the line tension to zero near critical point.

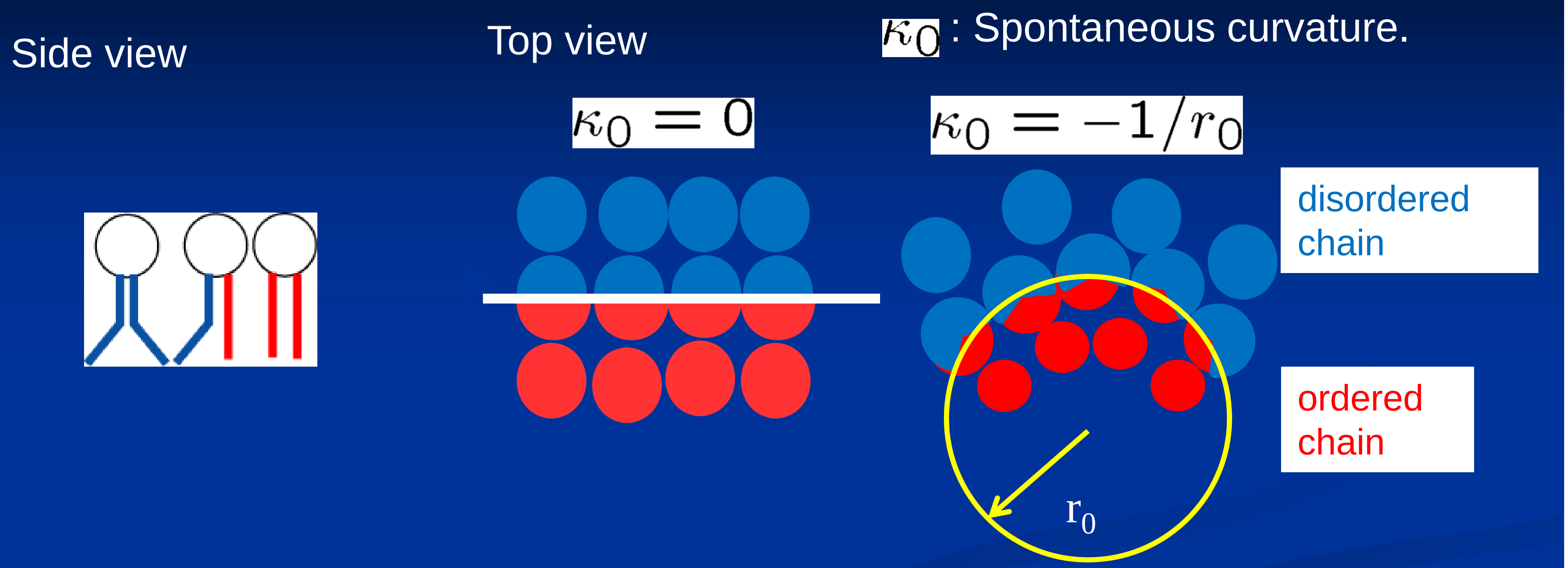
At low T:

- Significant amount of hybrid accumulates at interface
- Line tension is reduced to 0 with decreasing temperature
- Loss of mixing entropy limits this effect to low temperatures

- Interfaces between domains stabilized by hybrid!

- What is the stable size of the domains?

Domain size: 2D spontaneous curvature



Side view

Top view

κ_0 : Spontaneous curvature.

$\kappa_0 = 0$

$\kappa_0 = -1/r_0$

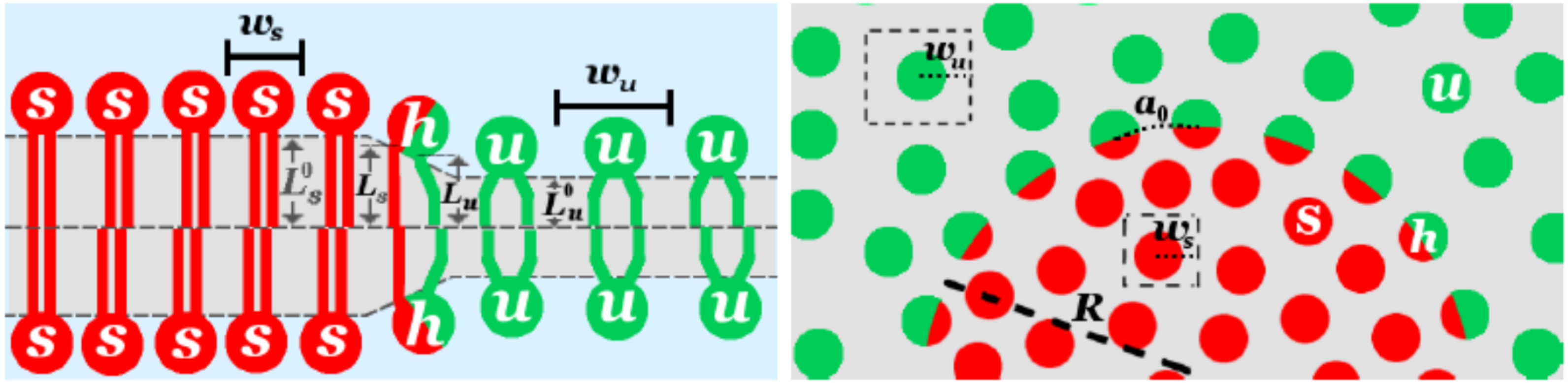
disordered chain

ordered chain

- Spontaneous curvature determines radius of domains stabilized by ◐
  .
- Subject to conservation constraints of S, U, and H (microemulsion)
- R. Brewster, SAS: Biophys. J. Lett., 2010

Estimation of spontaneous curvature

Lipid thickness mismatch energy

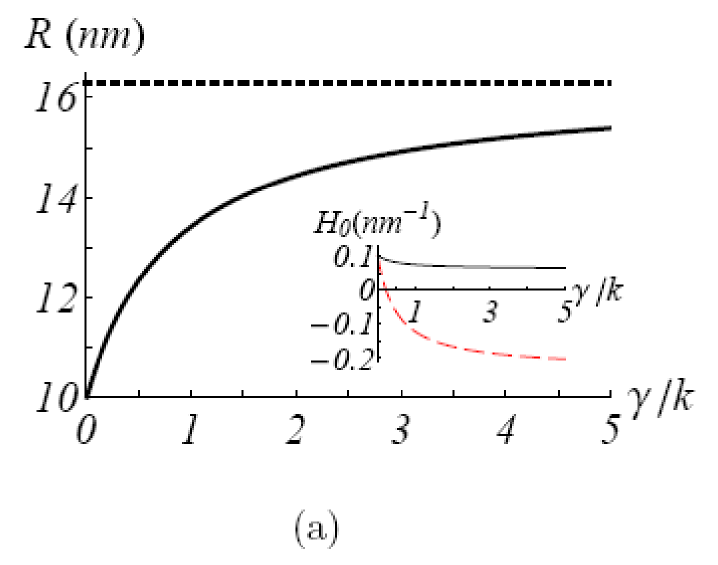


$$f_i = k\,(L_s - L_s^0)^2 + k\,(L_u - L_u^0)^2 + \gamma(L_u - L_s)^2$$

Minimize mismatch energy on a curved domain
subject to molecular volume conservation constraints

R. Brewster, SAS: Biophys. J. Lett., 2010

Predictions: spontaneous radius of curvature in mismatch model



DOPC (U) /DPPC(S) /POPC (H)

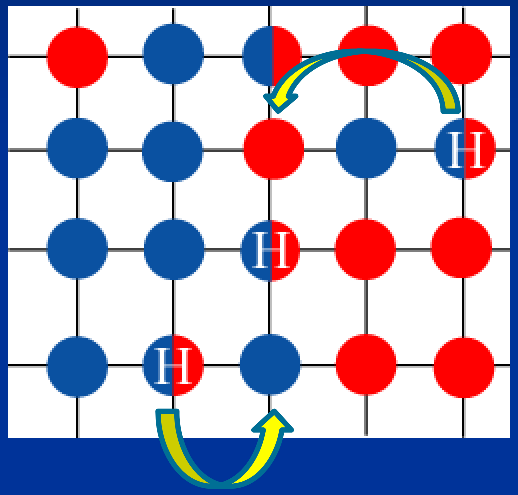
DSPC (U) /DPPC(S) /SOPC (H)

SOPC: S chain larger chain volume than U chain: curvature can become <0

R. Brewster, SAS: Biophys. J. Lett., 2010

Hybrid lipid more effective in line tension reduction in S+H+C

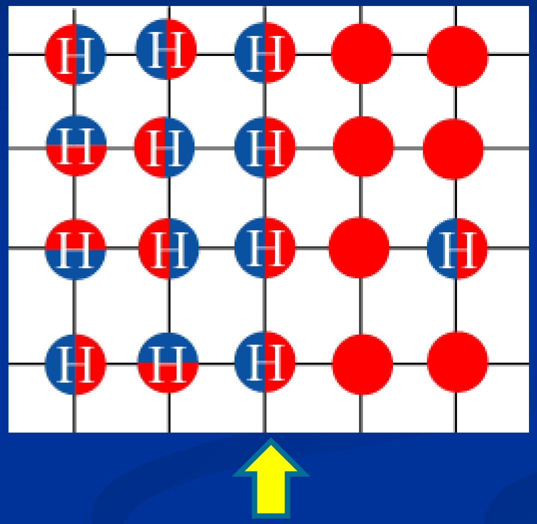
S + U + C membrane
with H



Must bring H to interface

Cost mixing entropy:
Only effective at low T

S + H + C membrane



H is already there!

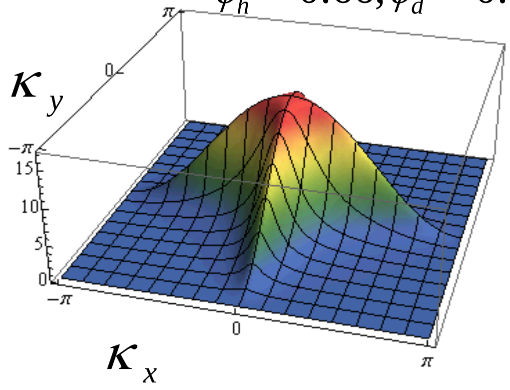
No mixing entropy cost:
Effective even near the onset of the phase separation ($T \sim T_c$)

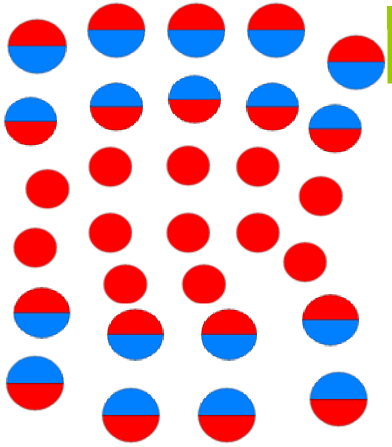
T. Yamamoto, R. Brewster and SAS, Europhys. Lett., 2010
T. Yamamoto and SAS, Soft Matter, 2011

Correlations vs Composition

Mixed phase -> the composition is uniform **on average**
Correlated domains can be observed over finite length and timescales

$\phi_h = 0.66, \phi_d = 0.0$

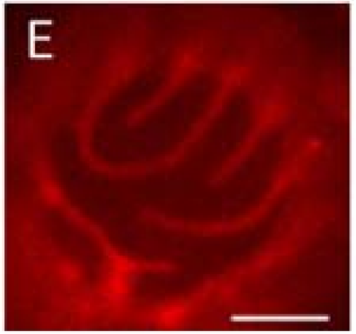




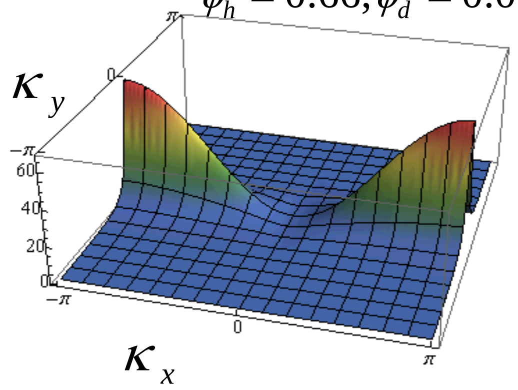
Fluctuations are anisotropic!

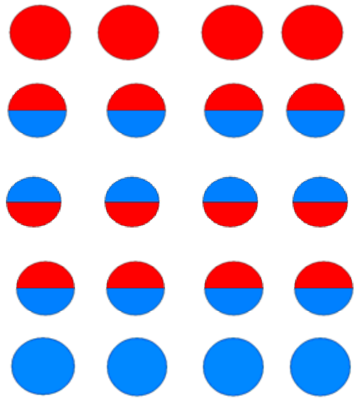
Elongated Structures

Recall expts.



$\phi_h = 0.66, \phi_d = 0.0$





Stripe Pattern

T.M. Konyakhina et al., *Biophys. J.*, **101** (2011).

Conclusion

- Chain ordering drives the phase separation for lipid composition and chain order parameter, i.e. L_d and L_o phase.
- Hybrid lipids H attracted to the interface due to their chain ordering.
- Hybrid lipids lower line tension – can it go to zero? S+H system
- Prediction of the size of domains stabilized by hybrids from the spontaneous curvature

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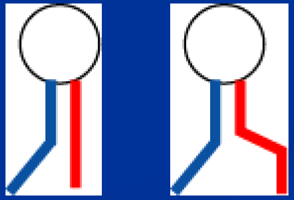
- Fluctuations in 1 phase regime: domains, Hybrid-Hybrid interactions



Line active mode

Unsaturated mode

Supplementary material

Critical Fluctuations in Plasma Membrane Vesicles

JACS Chemical Biology, 2009

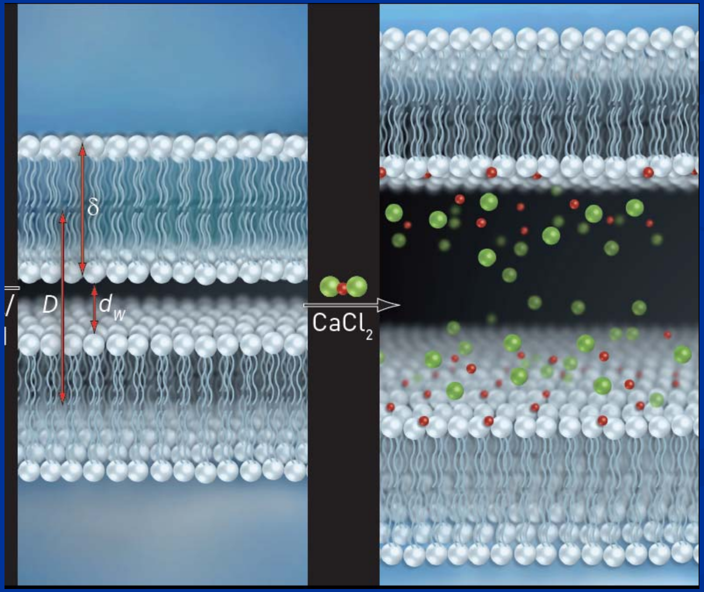
Sarah L. Veatch†,*, Pietro Cicuta‡, Prabuddha Sengupta†, Aurelia Honerkamp-Smith§, David Holowka†, and Barbara Baird†

- GPMVs (giant plasma membrane vesicles) blebs from cell membranes
- Contain membrane protein and lipids
- Show a miscibility transition
- One-phase region: micron size composition fluctuations near critical point
- At physiological temperatures, 50 nm-sized compositional fluctuations:

Membrane rafts? Lifetimes of 50 nm fluctuations?

Saturated lipids adsorb multivalent ions

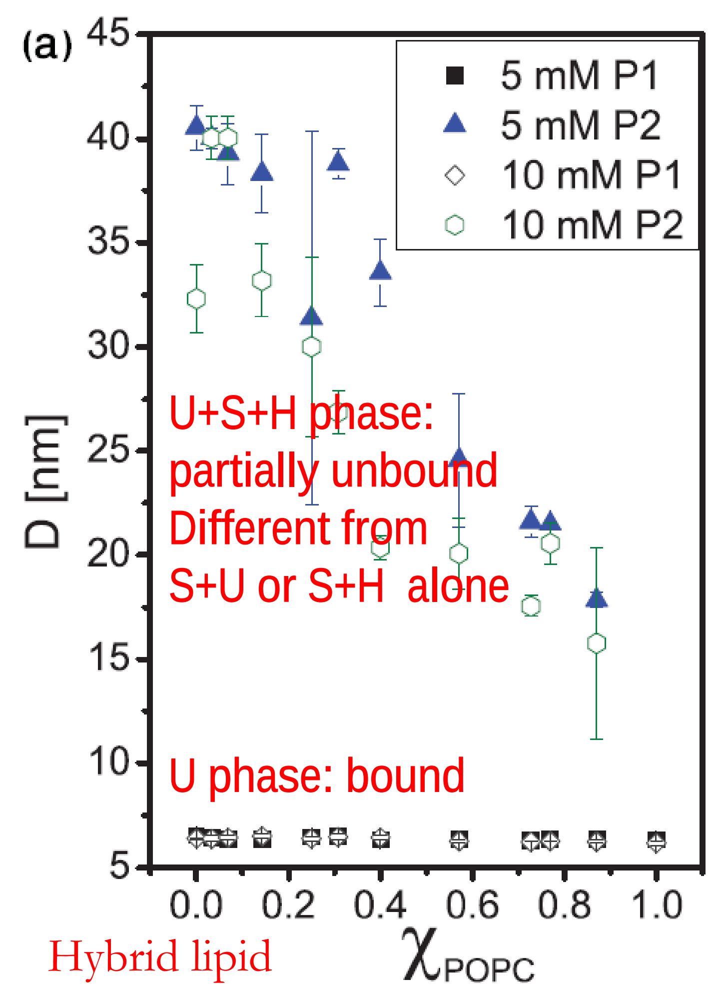
- Experiments by Raviv group on saturated/unsaturated/hybrid membranes in multilamellar stacks
- Saturated lipids adsorb multivalent ions causing charging and interlayer repulsion at low ion concentration



O. Szekely, A. Steiner, P. Szekely, E. Amit, R. Asor, C. Tamburu and U. Raviv, Langmuir, 2011, 27, 7419–7438

Hybrid lipid (POPC) reduces repulsions between layers
Mixes U and S in “unbound” phase?

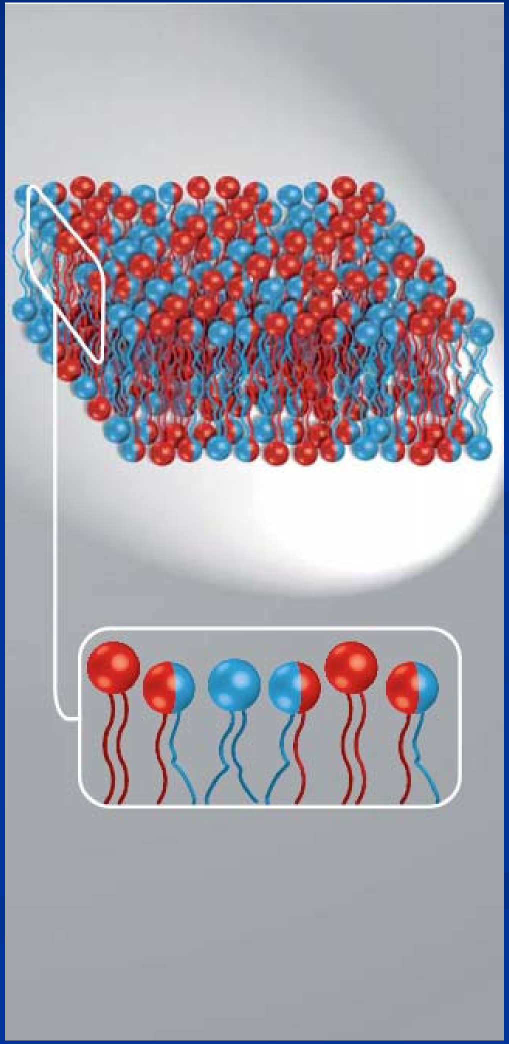
DOPC: DPPC = 1:2



U+S+H phase:
partially unbound
Different from
S+U or S+H alone

U phase: bound

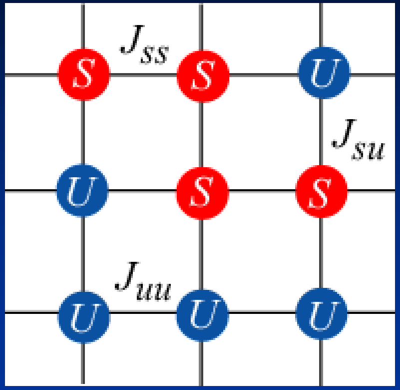
Hybrid lipid



Or Szekely and Uri Raviv, Langmuir, 2011

Lattice model for membrane without hybrid lipids

Lipids



Assumption:

The order parameter for U is ~ 0 ,
(S_u ~ 0), because entropy >> interaction.

Mixing entropy

$\psi(\mathbf{r})$: Local concentration of S

$$G_{\text{mix}} = k_B T[\psi(\mathbf{r}) \ln \psi(\mathbf{r}) + (1 - \psi(\mathbf{r})) \ln(1 - \psi(\mathbf{r}))]$$

Interaction energy **(favorable when neighboring chains are ordered -** ***only interactions due to chain ordering*****)**

$$G_{\text{int}} = -\frac{1}{2} J_{\text{ss}} \psi^2(\mathbf{r}) \boxed{S_s^2(\mathbf{r})}$$

$S_s(\mathbf{r})$: Chain order parameter for S

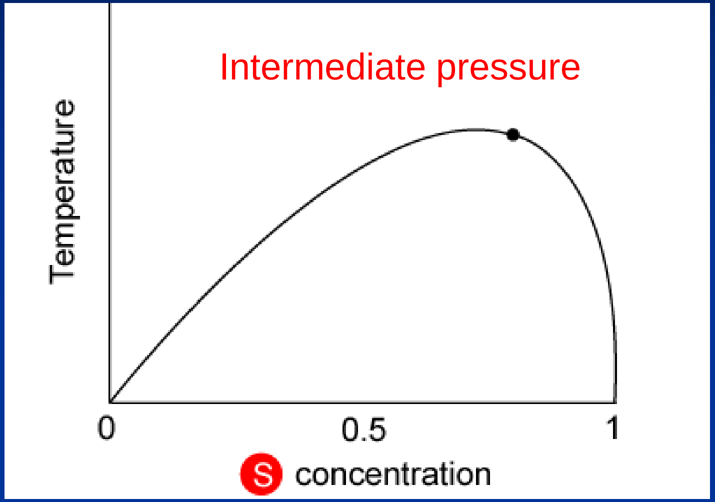
Entropy loss due to chain ordering

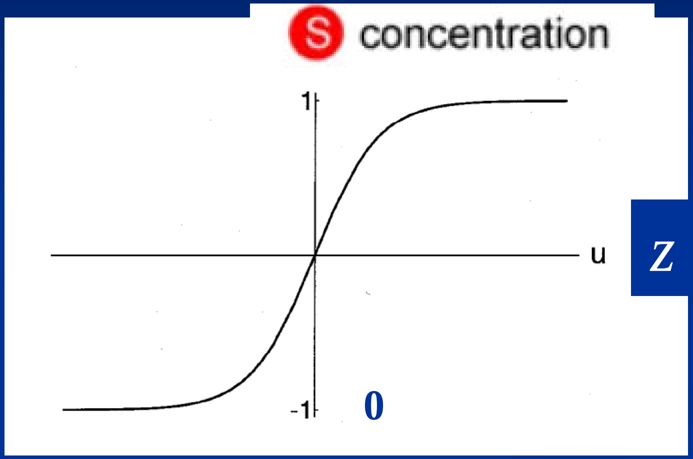
$$G_{\text{conf}} = 2k_B T[u_s S_s^4 - w_s S_s^3 + r_{0s} S_s^2]\psi(\mathbf{r})$$

P. G. de Gennes and J. Prost, The Physics of Liquid Crystals

Schematic phase diagram of S + U

Phase separation ONLY due to chain order





- Gradient energy: due to S / U contact at interface

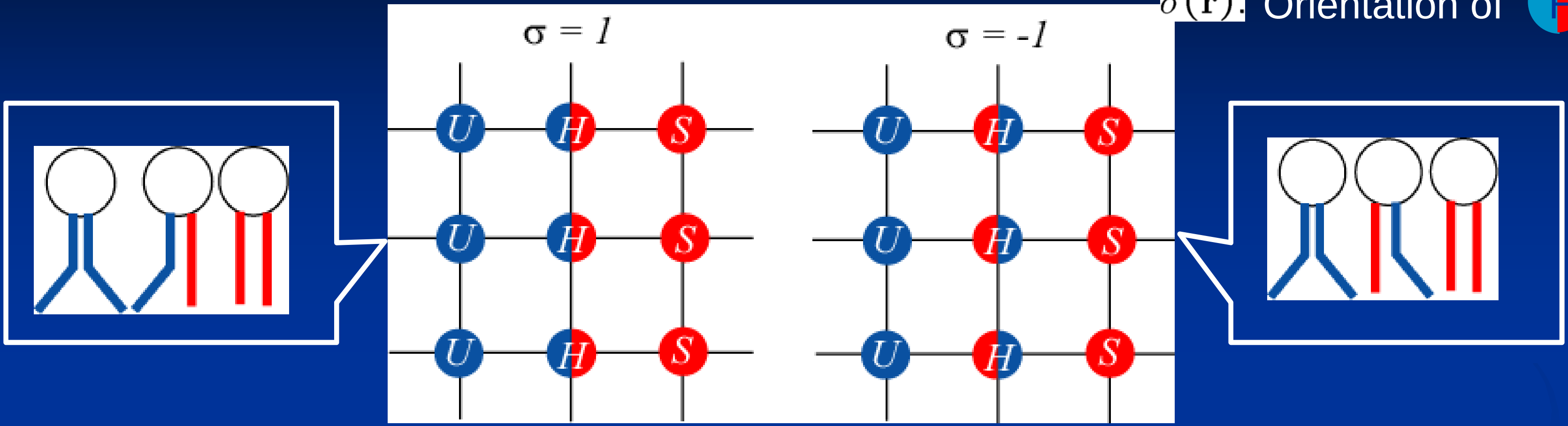
- Total free energy: bulk + gradient

- Minimize free energy to find concentration profile

- Line tension: excess free energy above bulk due to saturated-unsaturated contact at the interface

Lattice model for membrane with H

$\sigma(\mathbf{r})$: Orientation of H



Orientation **dependent** part of the interaction energy

$$\psi_h(\mathbf{r})\sigma(\mathbf{r})\nabla(S_s(\mathbf{r})\psi(\mathbf{r}))$$

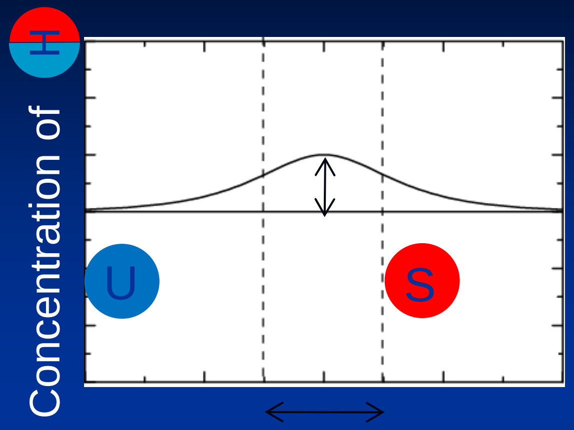
$\psi_h(\mathbf{r})$:

Local composition of H

Orientational entropy

$$k_B T[\frac{1+\sigma(\mathbf{r})}{2}\ln\frac{1+\sigma(\mathbf{r})}{2}+\frac{1-\sigma(\mathbf{r})}{2}\ln\frac{1-\sigma(\mathbf{r})}{2}]\psi_h(\mathbf{r}).$$

Weak interaction (high temperature)



$\bar{\psi}_h$:bulk H concentration

Line tension

$$\lambda = \lambda_0 \sqrt{1 - \boxed{\alpha \psi_h^{\mathrm{int}}}}$$

$\alpha > 0$

λ_0 :Line tension for S + U system with no hybrid

However, concentration of H adsorbed at interfaces is not enough to reduce the line tension to zero near critical point.

Strong interaction (low temperature)

$\psi_h^{\mathrm{int}} \to 1$ Width of interface ξ ~ Molecular size

$\sigma \to 1$ Complete orientation

$\bar{\psi}_h$ Average of H in the bulk.

Line tension

$$\lambda \sim -\frac{1}{2} J_{\mathrm{ss}} S_{\mathrm{int}} - k_B T \ln \bar{\psi}_h$$

> 0

S_{int} Chain order parameter of hybrid at the interface ~ 1

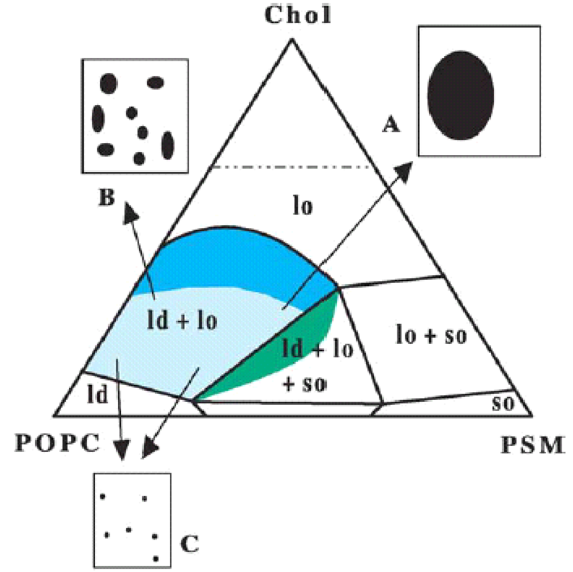
- Significant amount of hybrid accumulates at interface
- Line tension is reduced to 0 with decreasing temperature
- Loss of mixing entropy limits this effect to low temperatures

- Interfaces between domains stabilized by hybrid!

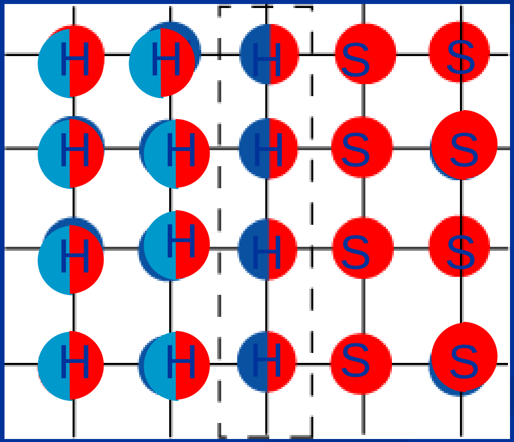
- What is the stable size of the domains?

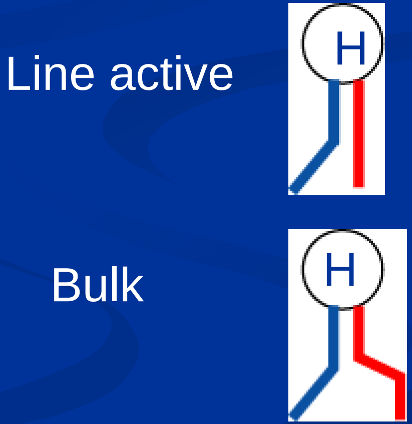
Hybrid + Saturated Lipids

- Hybrid saturated chain can have different order states
- In bulk (surrounded by other H) can be disordered
- Near interface with S phase will be more ordered
- Hybrid is line active in 2 component system due to the internal degree of freedom of chain order



(Almeida et al., JMB 2005)

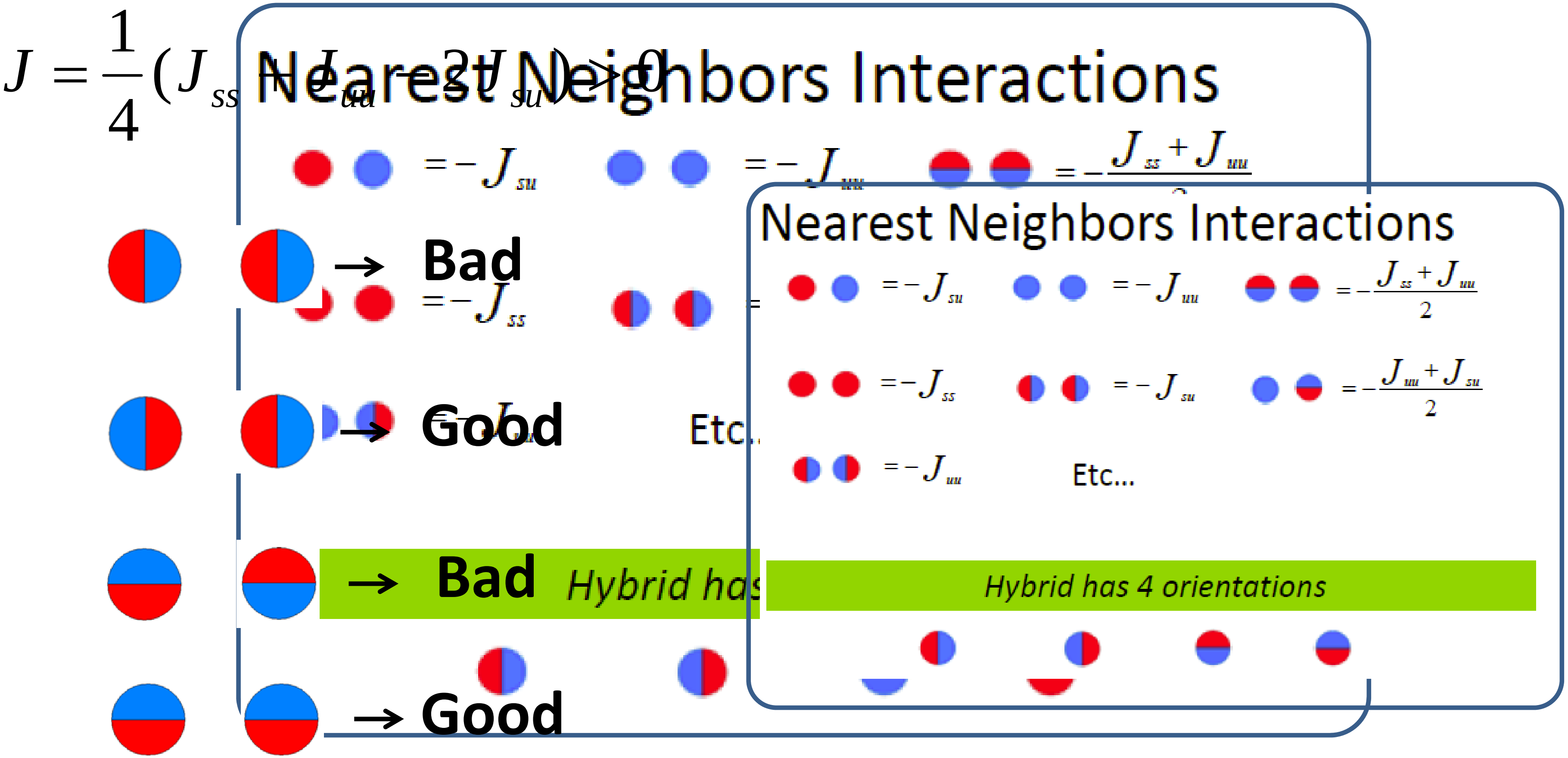




bulk: saturated chain of H disordered
interface: saturated chain of H ordered
H can be line active

H-S-U systems: Hybrid-Hybrid Interactions

$$J = \frac{1}{4}(J_{ss} + J_{uu} - 2J_{su}) > 0$$



→ **Bad**

→ **Good**

→ **Bad**

→ **Good**

Nearest Neighbors Interactions

$= -J_{su}$ $= -J_{uu}$ $= -\frac{J_{ss}+J_{uu}}{2}$

$= -J_{ss}$ $= -J_{su}$ $= -\frac{J_{uu}+J_{su}}{2}$

$= -J_{uu}$ Etc...

Hybrid has 4 orientations

Nearest Neighbors Interactions

$= -J_{su}$ $= -J_{uu}$ $= -\frac{J_{ss}+J_{uu}}{2}$

$= -J_{ss}$

$= -J_{uu}$ Etc...

Hybrid has

DSPC (Saturated Lipid) : 🔴 DOPC (Unsaturated Lipid): 🔵 POPC (Hybrid Lipid): ◐

Mixed Phase Free-energy: composition fluctuation modes (wavelength $\lambda \sim 1/k$)

$$F = F_0 + \sum_{\mathbf{k}} \Psi_{-\mathbf{k}} \mathbf{M} \Psi_{\mathbf{k}}$$

$$\Psi_k = (\phi_{\mathbf{k}}^s, \phi_{\mathbf{k}}^u, \sigma_{\mathbf{k}}^x, \sigma_{\mathbf{k}}^y)$$

Mean-field Free-energy

Fluctuation Part
(depends on T, J, ϕ_s^0, ϕ_u^0 and $\mathbf{k}$ **)**

T = temperature

$J = \frac{1}{2}\left(\frac{J_{ss} + J_{uu}}{2} - J_{su}\right)$

ϕ_0^s = fraction of S

ϕ_0^u = fraction of U

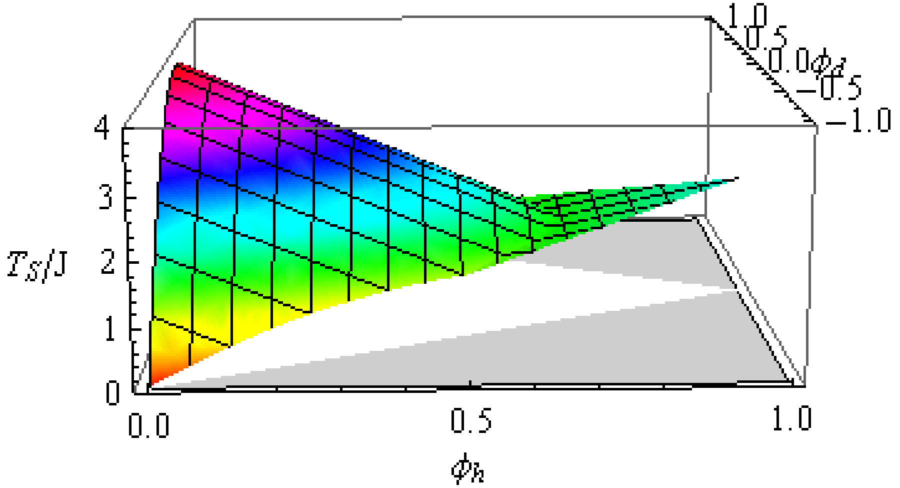
$\phi_0^h = 1 - \phi_0^s - \phi_0^u$ = fraction of Hybrids

$\sigma^{x(y)}$ = orientation of the Hybrid

Spinodal Temperature:

Highest temperature where

$$\det(\mathbf{M}) = 0$$



Critical Composition: $\phi_h = 2(1-\phi_d^2)/3$

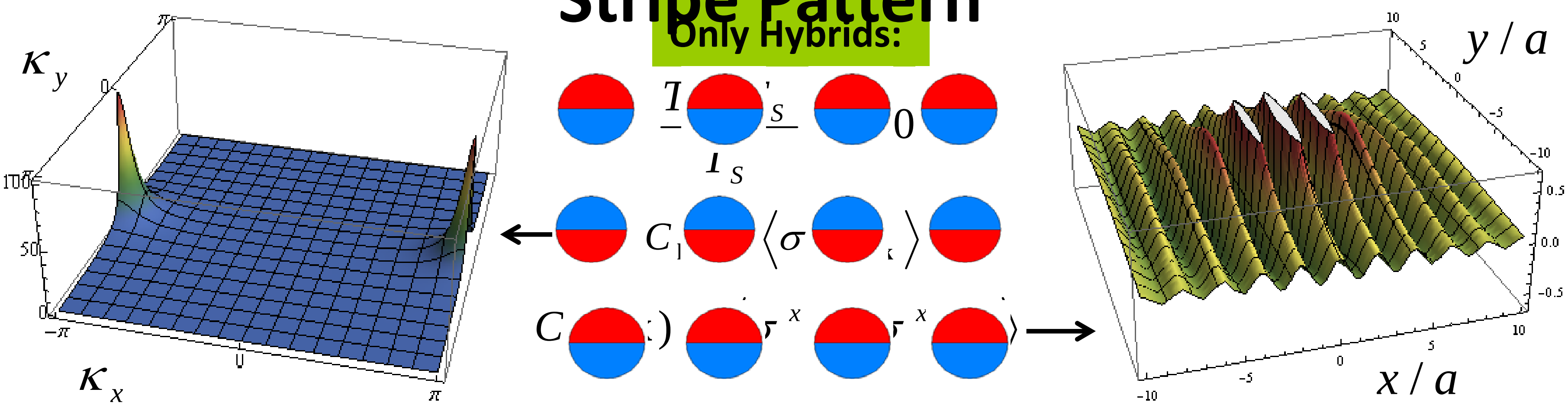
Correlation functions

Mixed phase -> the composition is uniform **on average**
Correlated domains can be observed over finite length and timescales

Correlation functions characterize the size of these domains

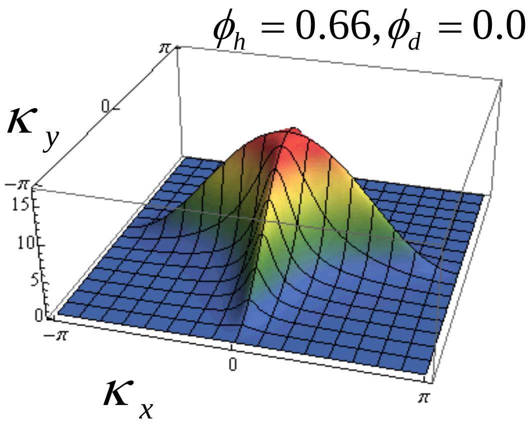
$$\left\langle \phi^{a}_{\mathbf{k}} \phi^{b}_{-\mathbf{k}} \right\rangle = \int d\psi\, \phi^{a}_{\mathbf{k}} \phi^{b}_{-\mathbf{k}} e^{-\beta F} \Big/ \int d\psi\, e^{-\beta F} \quad \text{where} \quad d\psi = \prod_{\mathbf{k}} d\Psi_{\mathbf{k}}$$

Stripe Pattern

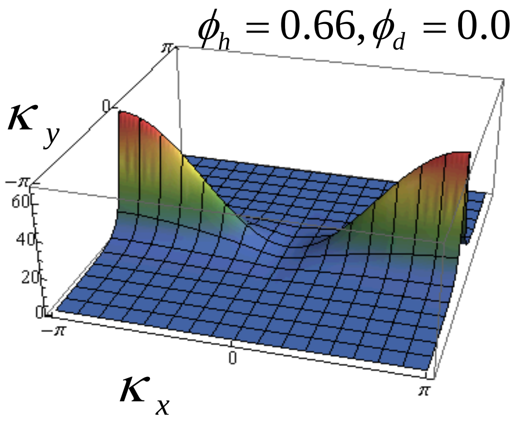


Correlations vs Composition

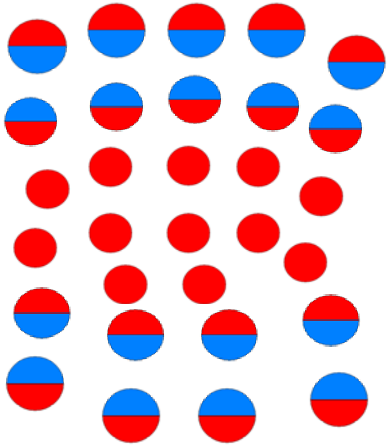
$$C_{\mathbf{k}}^{ss} = \left\langle \phi_{\mathbf{k}}^{s} \phi_{-\mathbf{k}}^{s} \right\rangle$$



$$C_{\mathbf{k}}^{xx} = \left\langle \sigma_{\mathbf{k}}^{x} \sigma_{-\mathbf{k}}^{x} \right\rangle$$

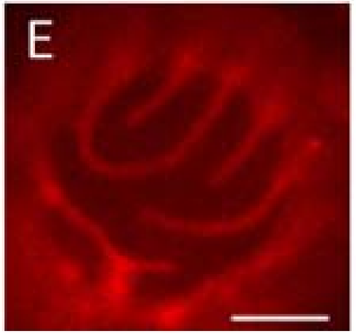


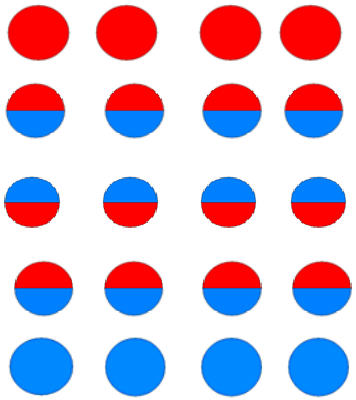
Fluctuations are anisotropic!



Elongated Structures

Recall results in [3]





Stripe Pattern

3. T.M. Konyakhina et al., *Biophys. J.*, **101** (2011).