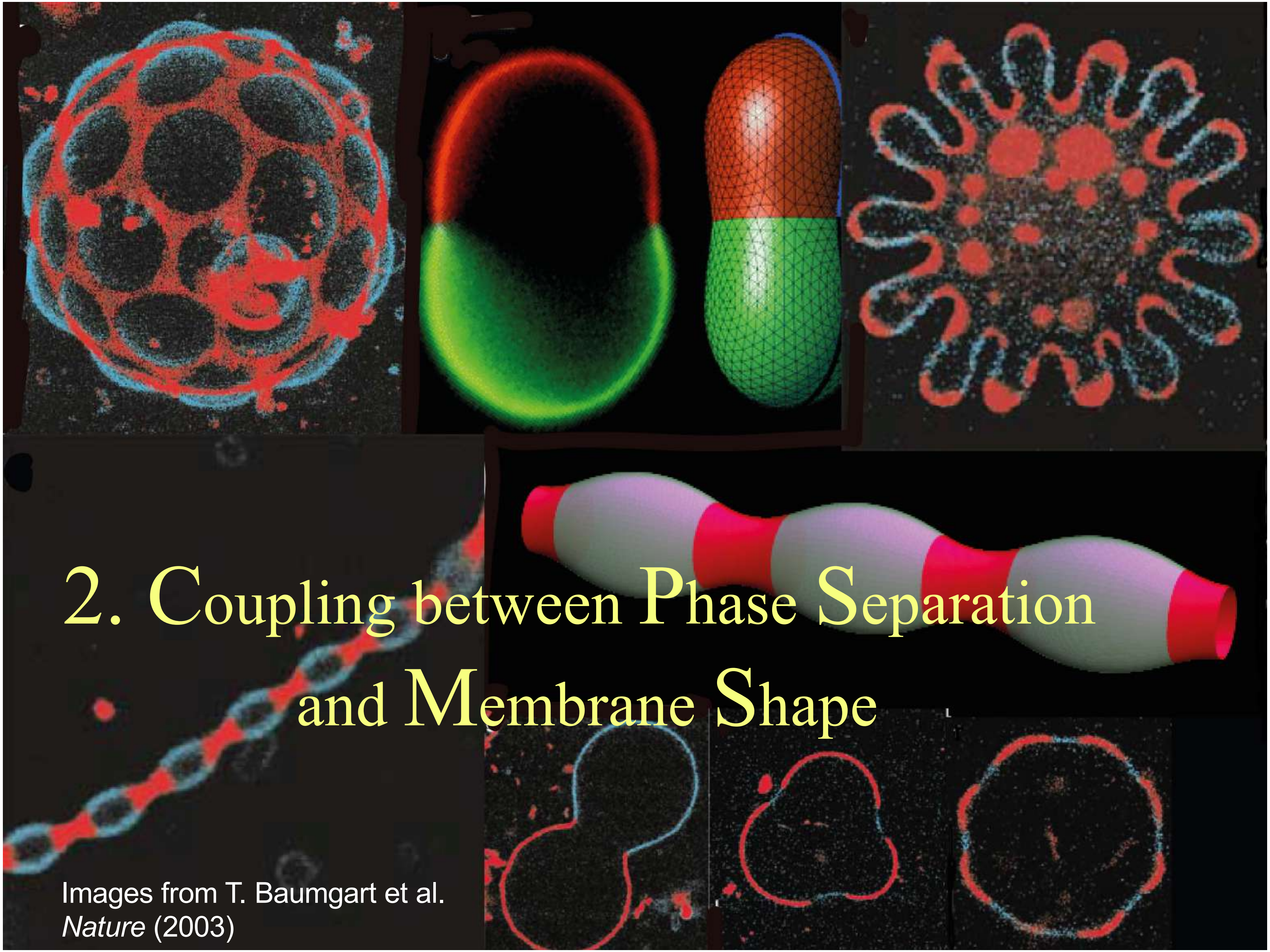
2. Coupling between Phase Separation and Membrane Shape



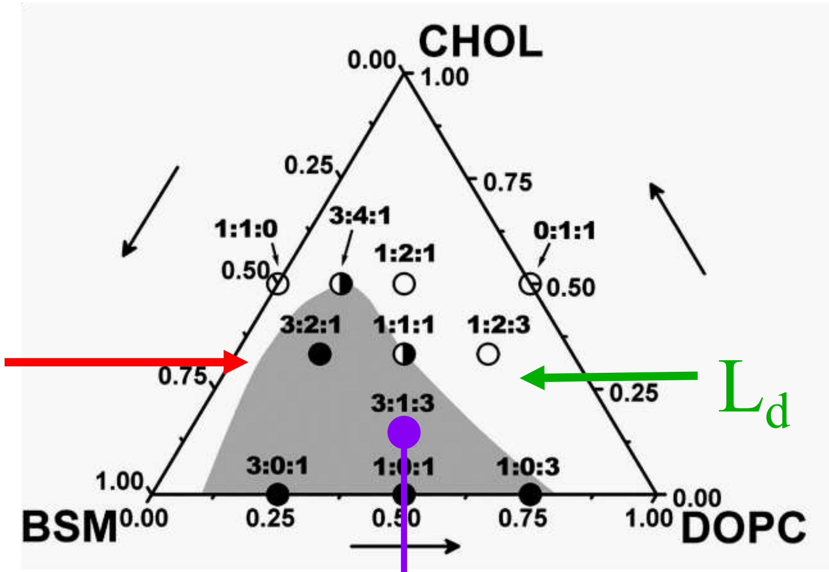
Images from T. Baumgart et al. *Nature* (2003)

Membrane Deformation due to Line Tension between Lipid Domains

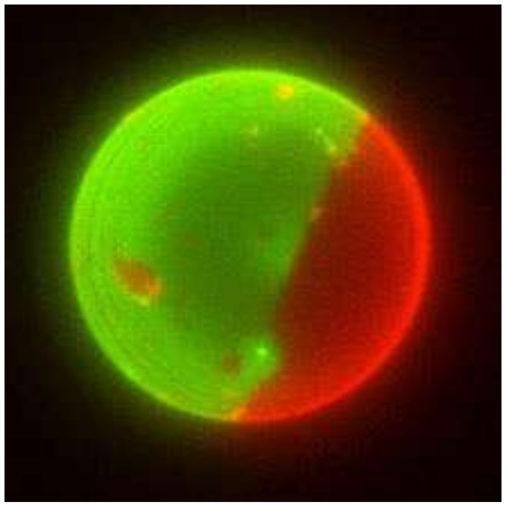
Fluid Phases Coexistence in Lipid Mixtures

DOPC/BSM/Chol

L_o



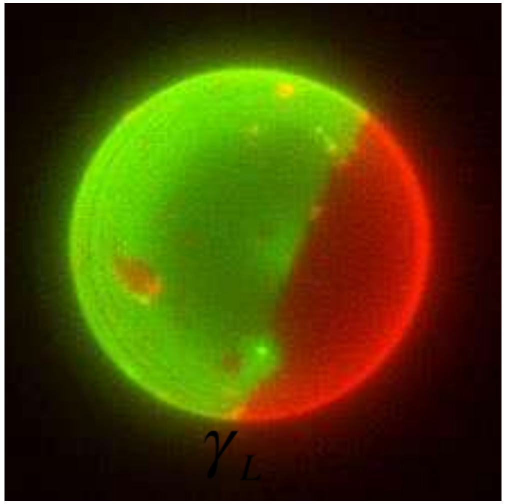
L_d



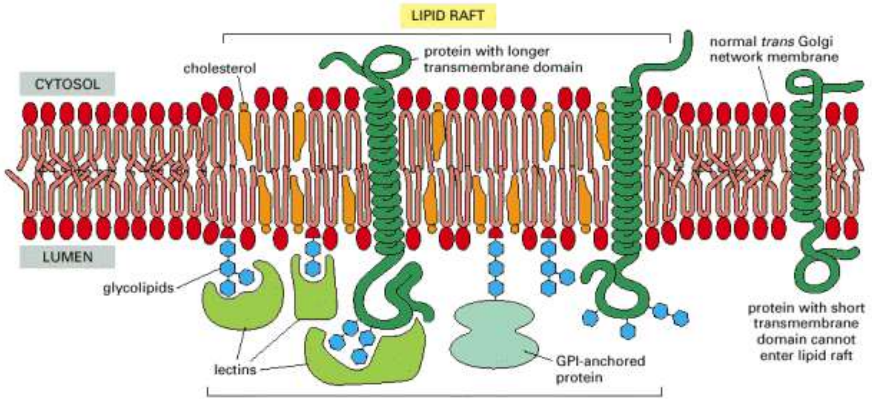
Sorre, Callan-Jones *et al. PNAS* (2009)

2D Phase Separation

At 3D :
Interfacial tension

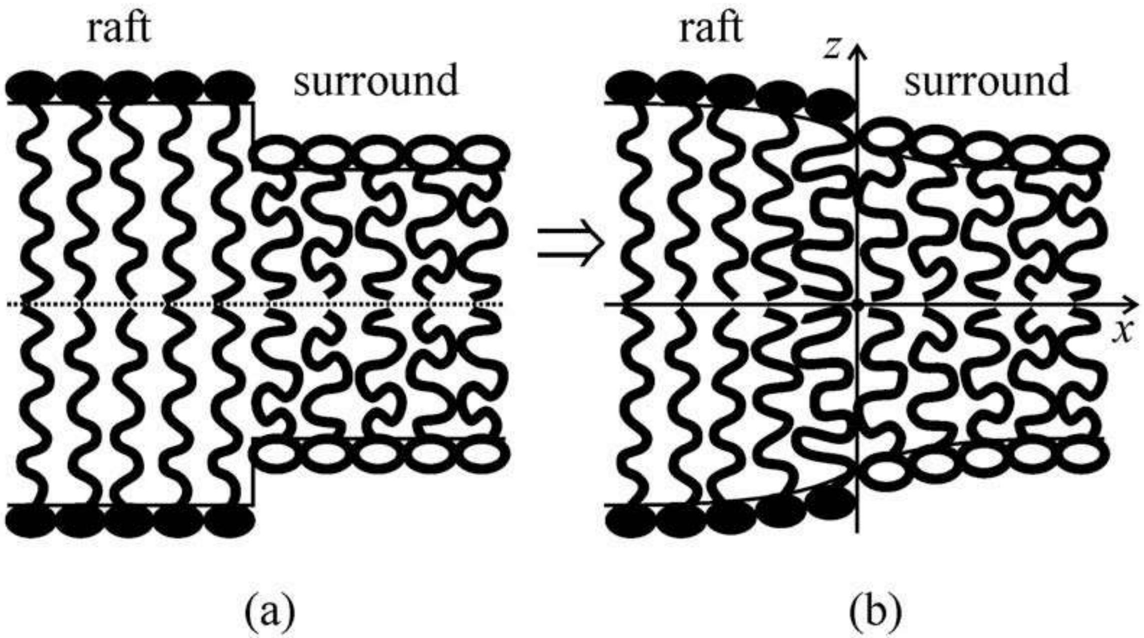


On a membrane (2D):
Line tension $\approx$ 1 to few pN
(depends on composition, temperature)



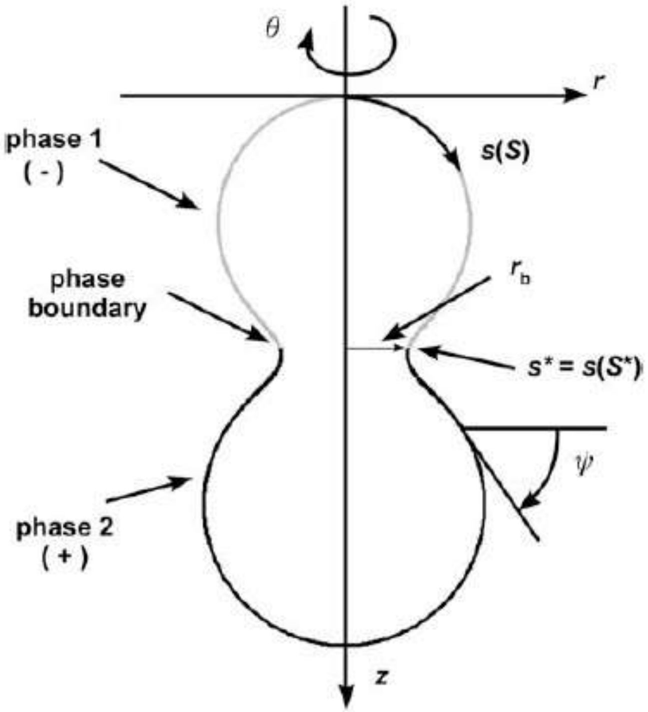
Coexistence of 2 phases

→ *Line tension*



S. A. Akimov, et al *Phys. Rev. E* **75**, 011919 (2007)

Example of a Measure of Line Tension



For symmetric doublets

$$\frac{\gamma_L}{p} = \frac{1}{2} r_b^2 (\cot \psi_1 - \cot \psi_2)$$

r_b: neck radius
p: pressure difference (Lapalce)

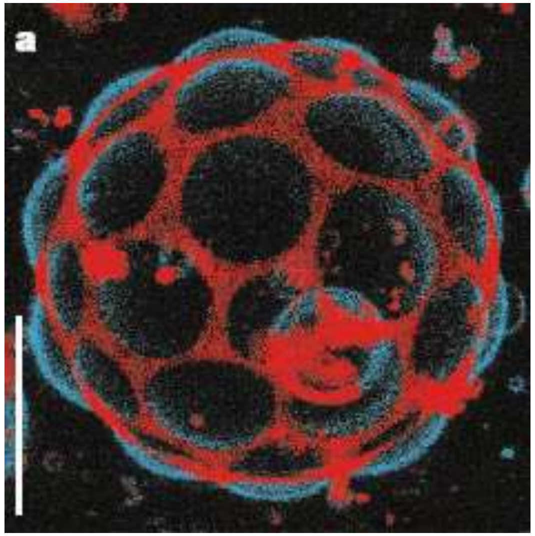
$\gamma_L \approx 1$ to few pN

T. Baumgart... J.Jenkins
Biophys. J. (2005)

5 µm

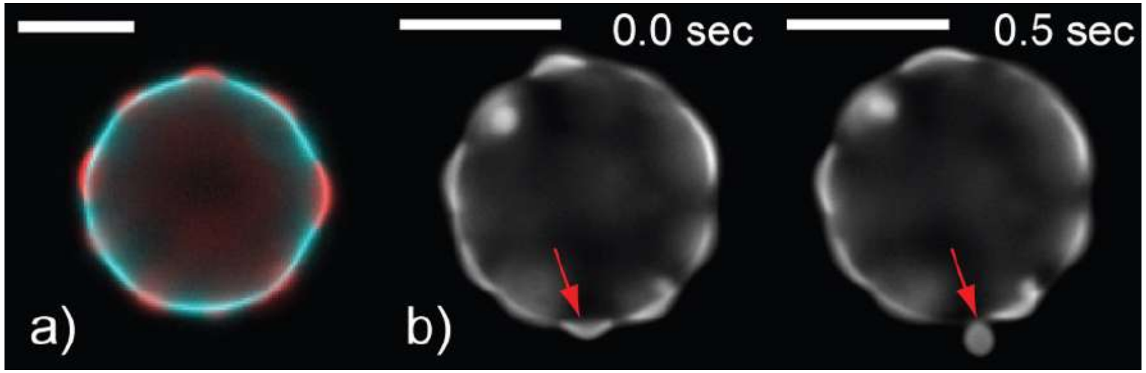
Pure Lipid Domains can Form Vesicles

Tension lowered



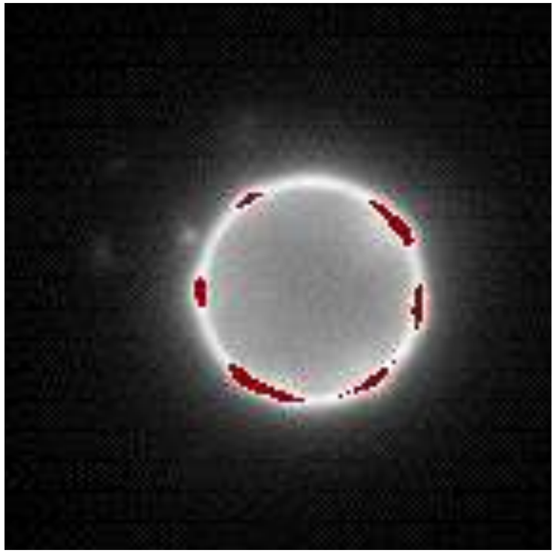
5 µm

T. Baumgart et al *Nature* **425**, 821 (2003)



J. E. Rim, et al *PRL* **106**, 057801 (2011)

Bars, 10 µm

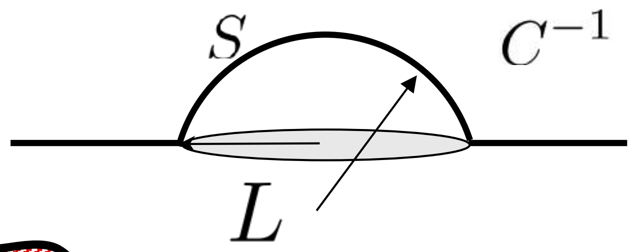


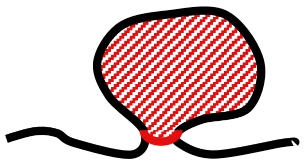
A. Roux *et al.*,
EMBO J. **24**, 1537 (2005

Ex: DOPC/Chol/SM

With pure lipid domains: *micron-size vesicles*

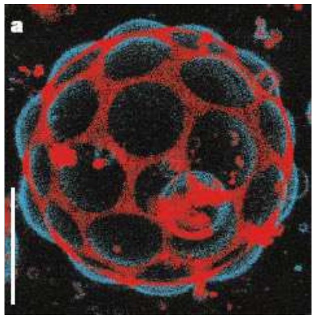
Competition Line Tension/Surface Tension

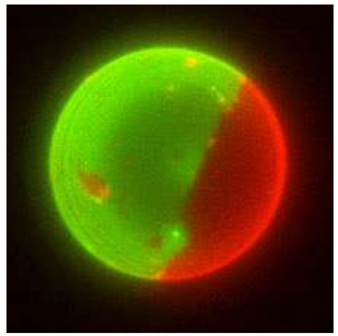




low tension

high tension





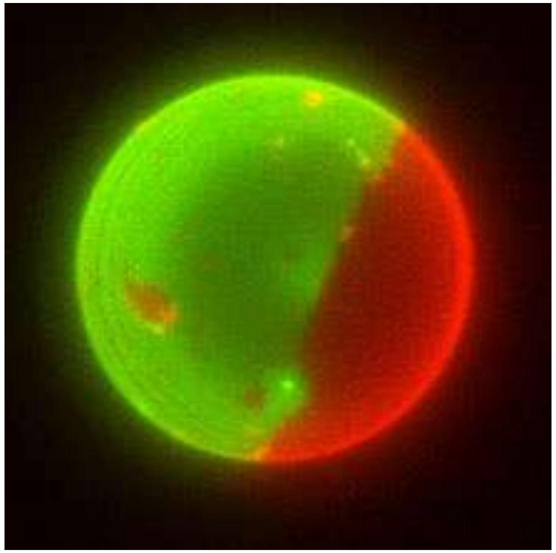
$$F = \gamma_L 2\pi L + \frac{1}{2}\kappa S (C - C_0)^2 + \sigma (S - \pi L^2)$$

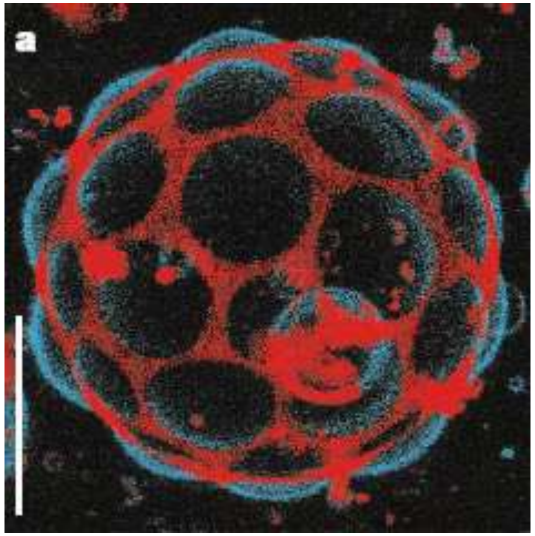
Line Tension Curvature Surface Tension

P. Sens, M.S. Turner *Phys. Rev. E* (2006)

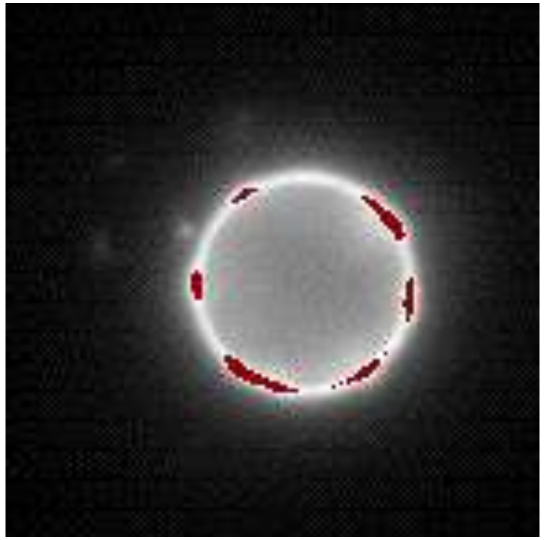
Effect of Membrane Tension

Tension





Baumgart et al, Nature (2003)



Full separation

Budding

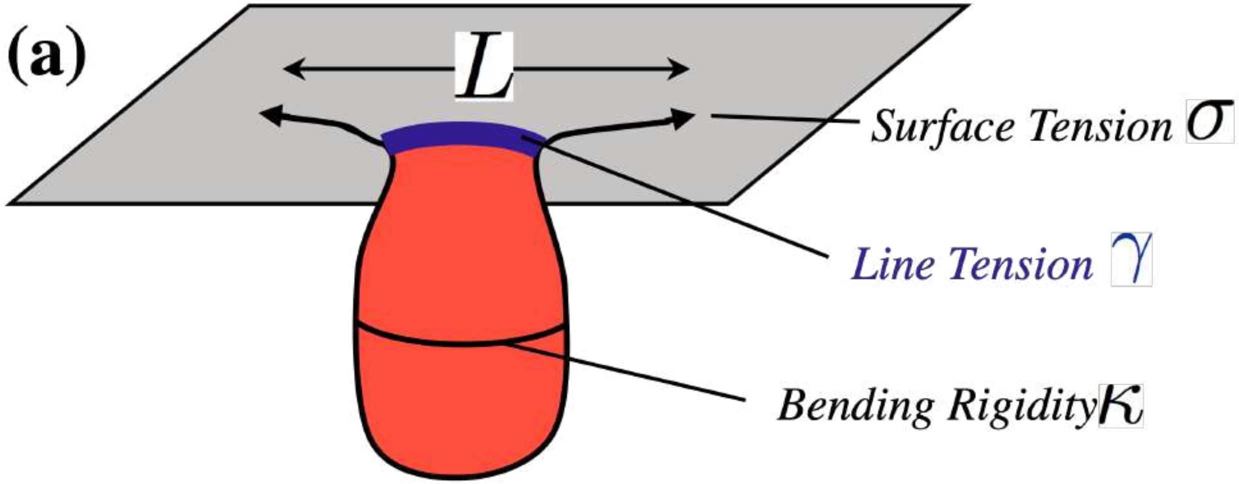
Constriction and **Fission**

$$F = \boxed{\gamma_L 2\pi L} + \frac{1}{2}\kappa S(C - C_0)^2 + \sigma(S - \pi L^2)$$

P. Sens, M.S. Turner *Phys. Rev. E* (2006)

Pure Lipid Domains Form *Large* Buds

Bud formation



Bending energy/ vesicle $F = 8\pi\kappa$ Size independent

Line tension γ $F = 2\pi L\gamma$ $\gamma \approx 1$ to a few pN

If pure lipids and zero tension, **smallest domain size**:

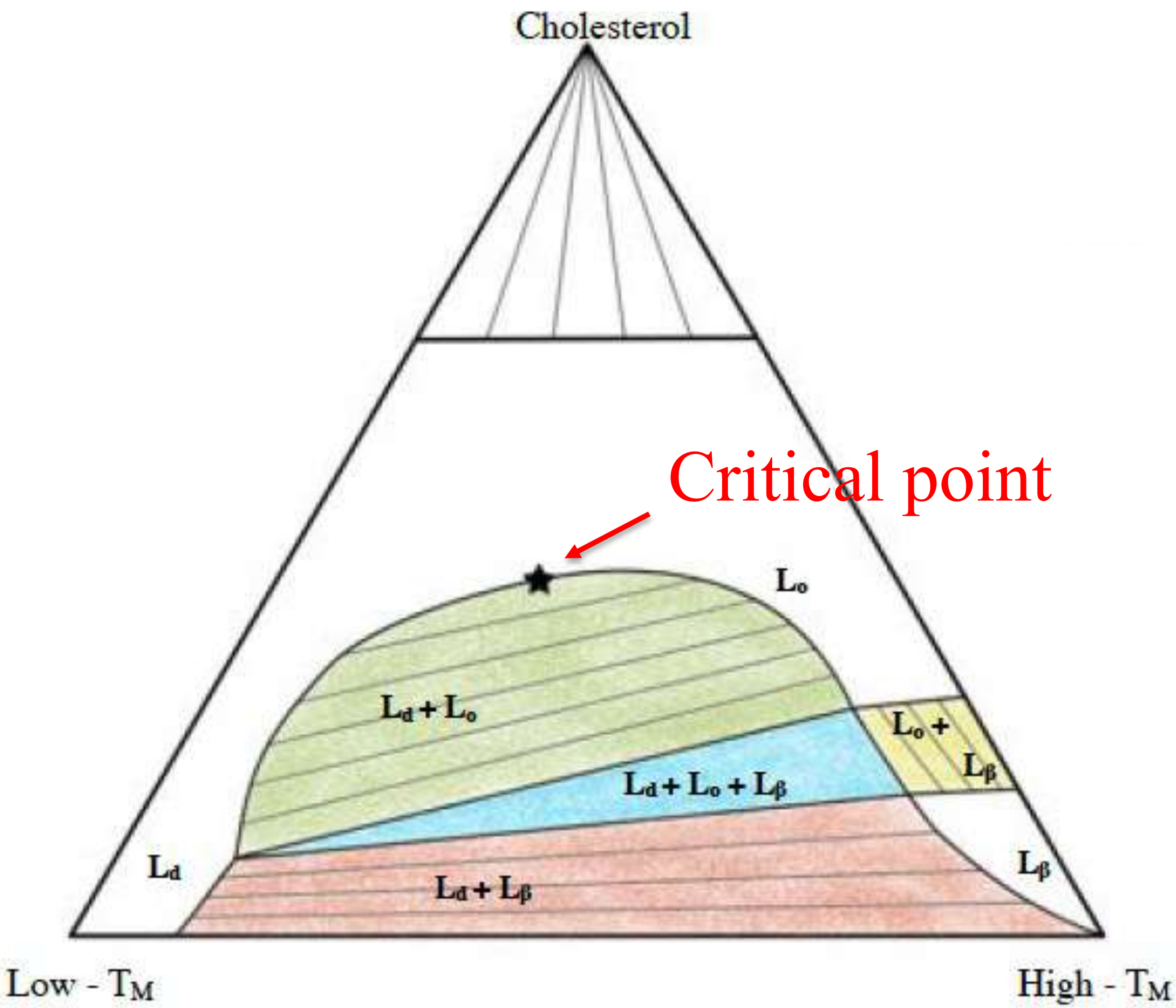
$$L \approx \frac{4\kappa}{\gamma} \approx 300nm$$

Increases with membrane tension

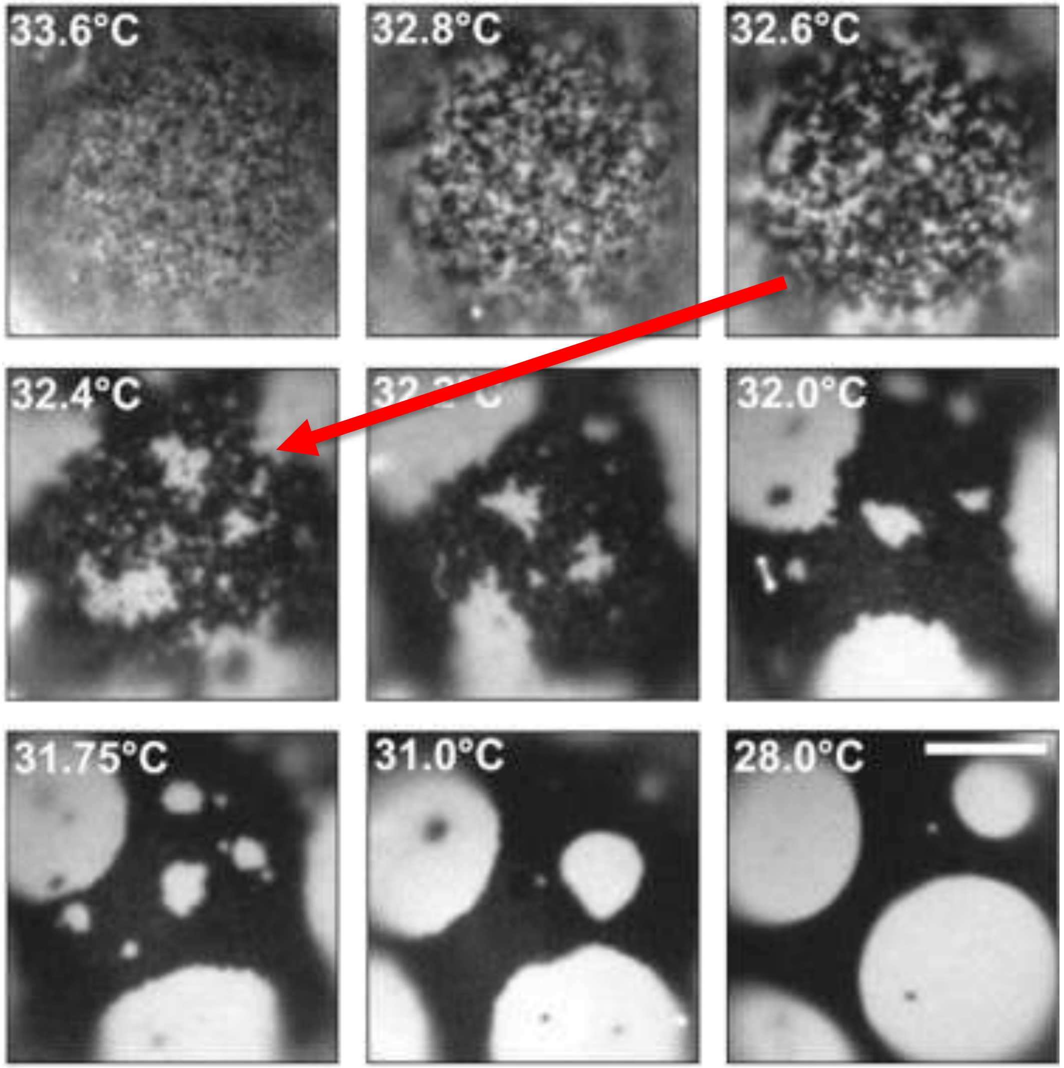
P. Sens et al *Curr. Opin. Cell Biol.* (2008)

Proteins required to form small vesicles (or narrow tubes)

Phases Coexistence in Ternary Lipid Mixtures



Cooling a Mixture near a Critical Point



diPhyPC:DPPC: Chol

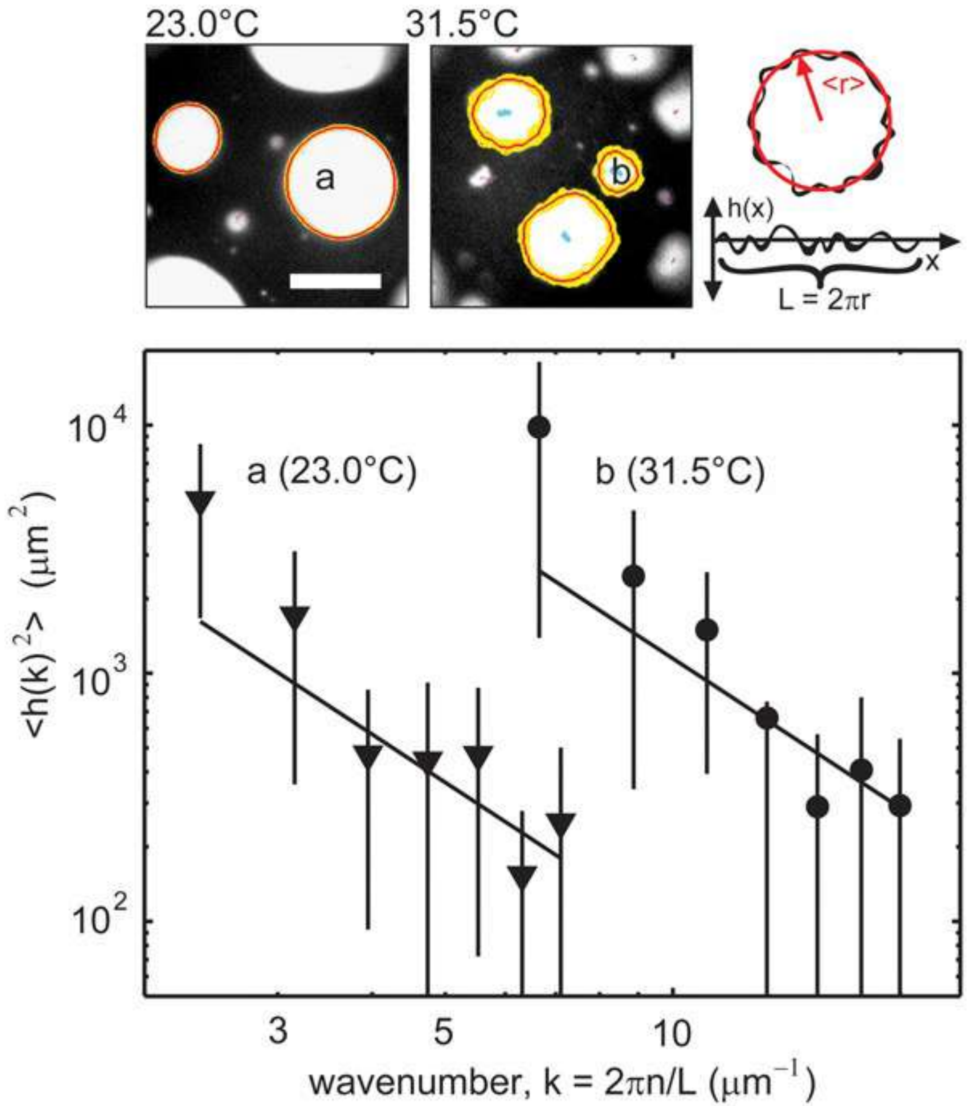
(25:20:55 mol %)

Fluorophore in Ld phase

Macroscopic domains

A.R. Honerkamp-Smith… S. Keller *Biophys. J.* (2008)

Measuring Line Tension from Fluctuations

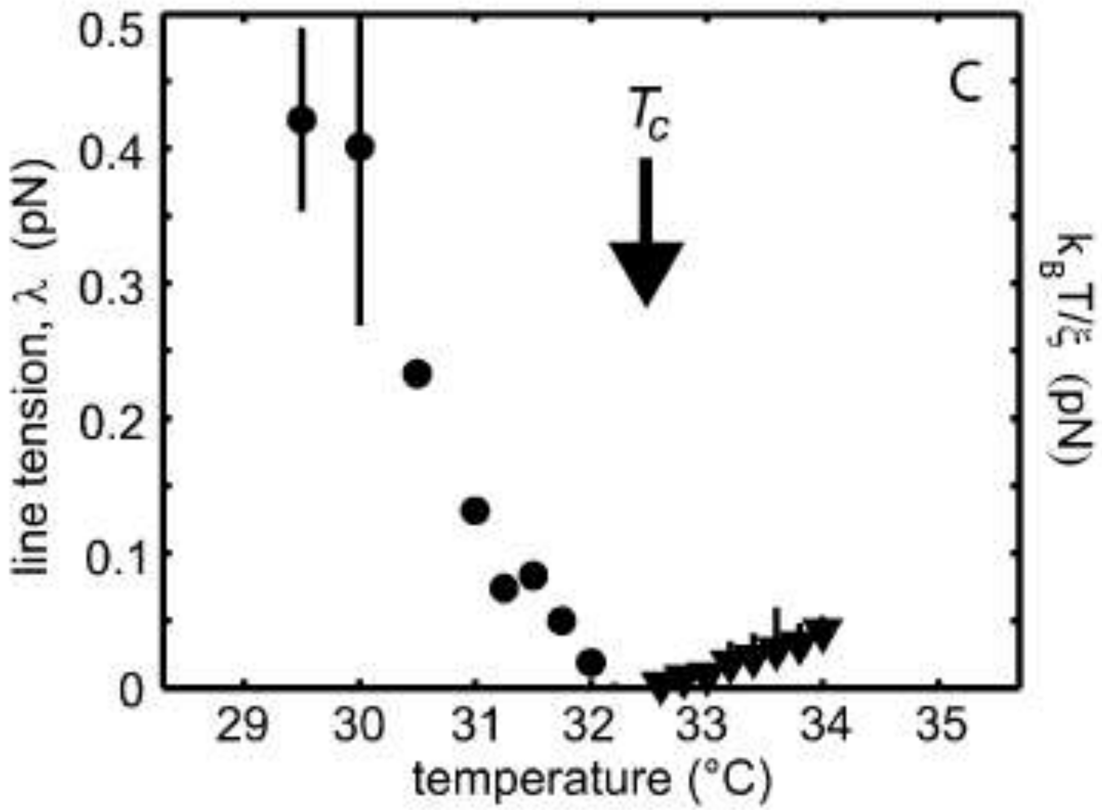


L: smooth perimeter

$$<h(k)>^2 = \frac{k_B T}{\lambda\, L\, k^2}$$

A.R. Honerkamp-Smith… S. Keller *Biophys. J.* (2008)

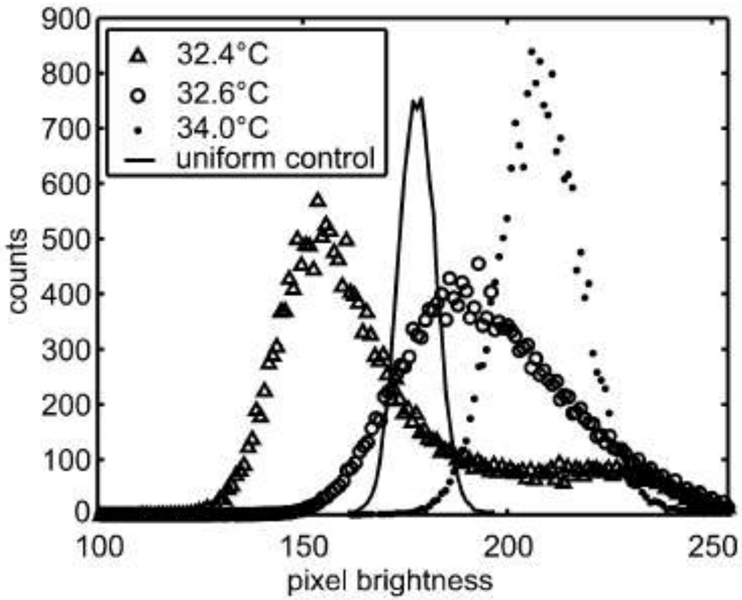
Line Tension versus Temperature



λ vanishes at $T = T_c$

A.R. Honerkamp-Smith… S. Keller *Biophys. J.* (2008)

Critical Exponent

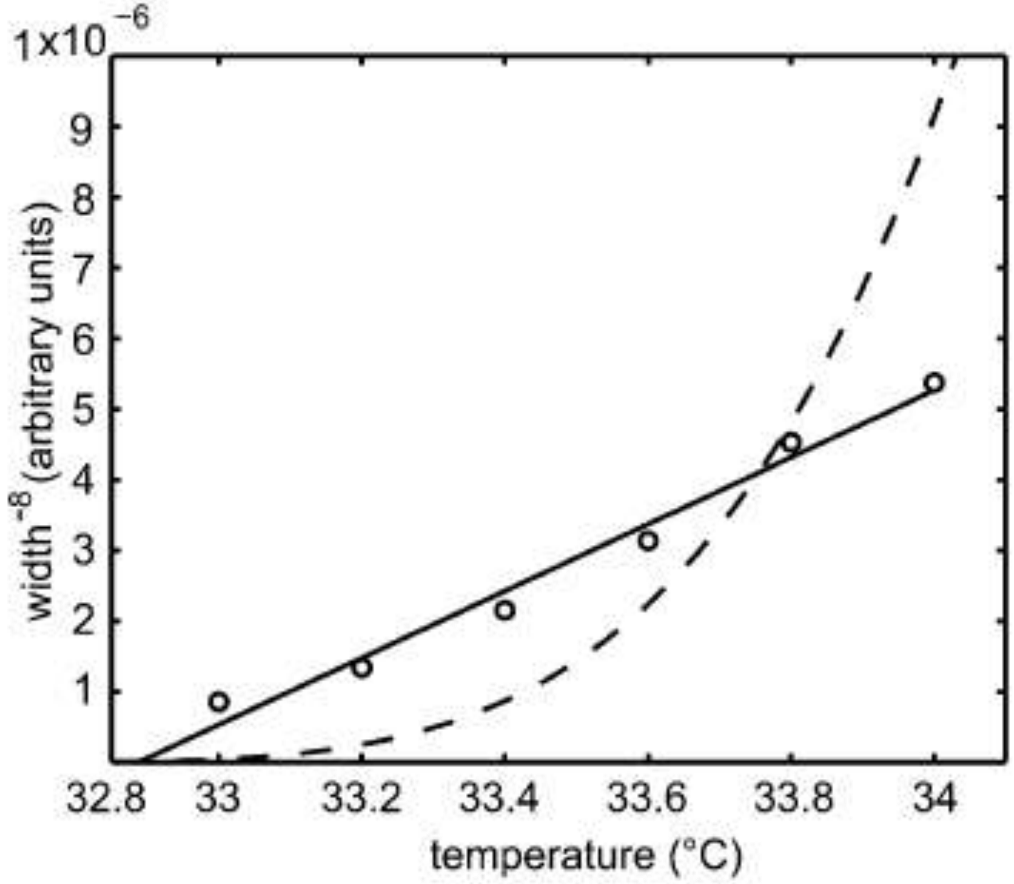


Mean Brightness $< m^2 >^{1/2}$ ≈ order parameter

$$W = < m^2 >^{1/2} \propto \left(\frac{T - T_c}{T_c}\right)^{-\beta}$$

A.R. Honerkamp-Smith… S. Keller *Biophys. J.* (2008)

Critical Exponent



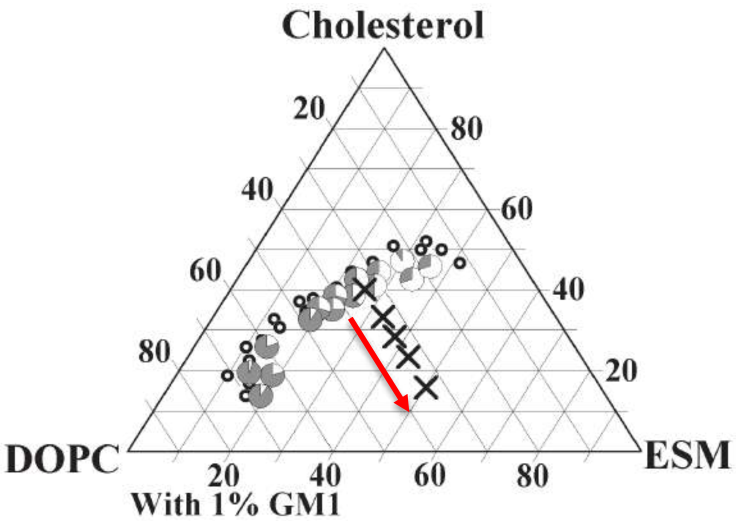
$$W^{-8} \propto T - T_c$$

$$\beta = \frac{1}{8} = 0.125$$

OK with a 2D-Ising model

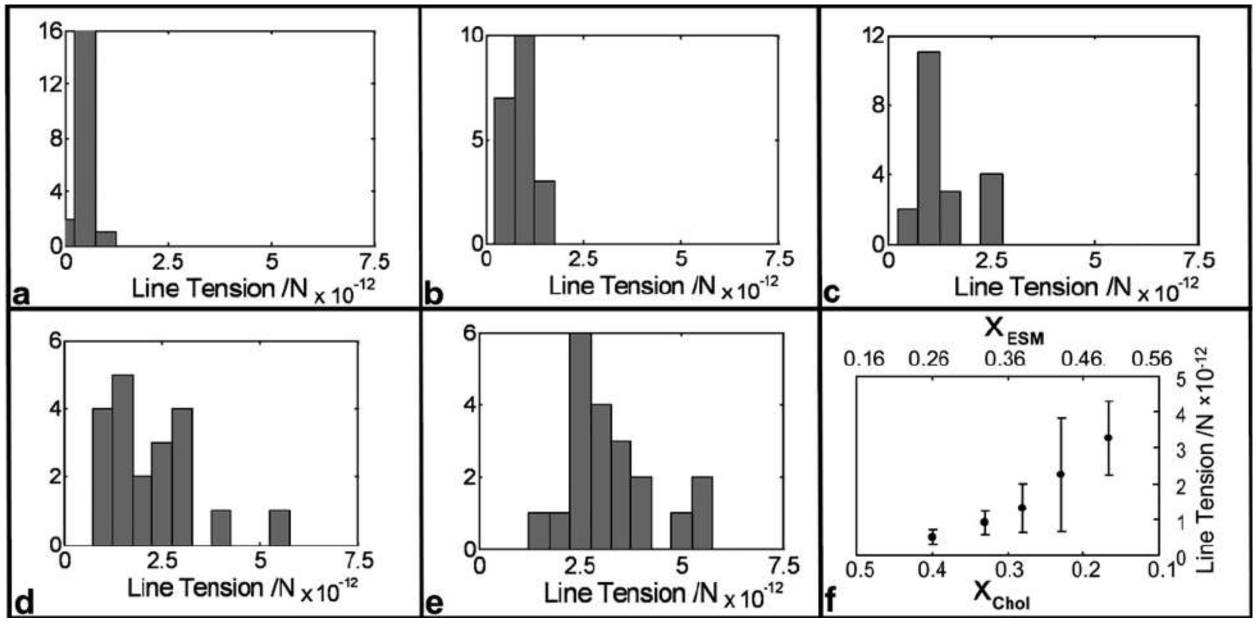
A.R. Honerkamp-Smith… S. Keller *Biophys. J.* (2008)

Line Tension versus Lipid Composition



Reducing cholesterol

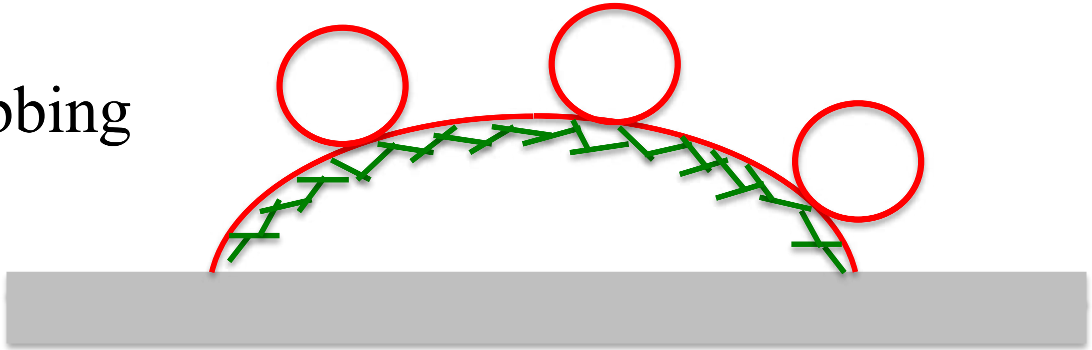
$\longrightarrow$ λ increases



A. Tian… T. Baumgart *Phys. Rev. Lett.* (2007)

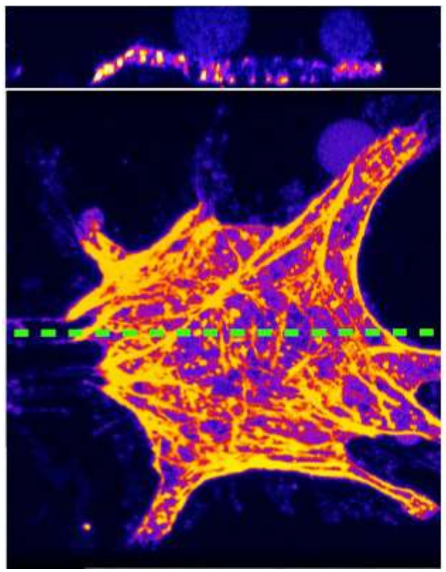
Plasma Membrane:
Composition close to a Critical Point

Cell blebbing



Review:

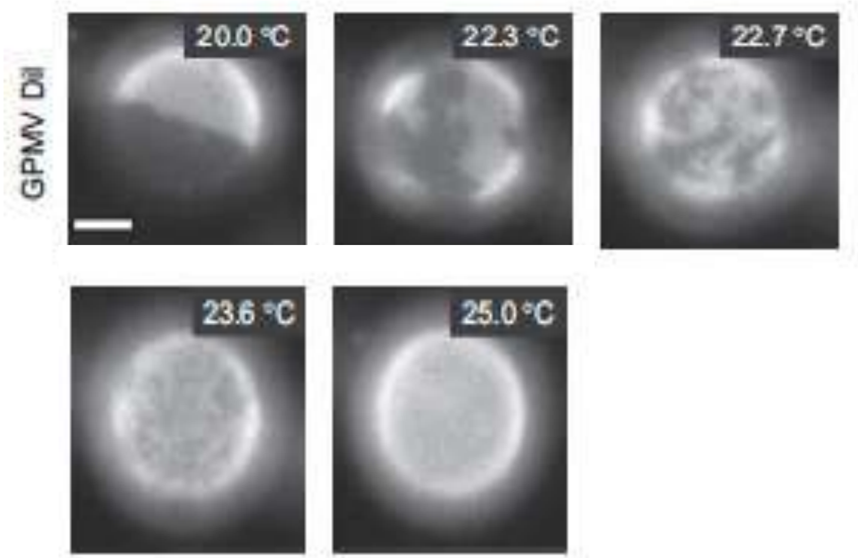
E. Sezgin et al *Nature Protocols* (2012)

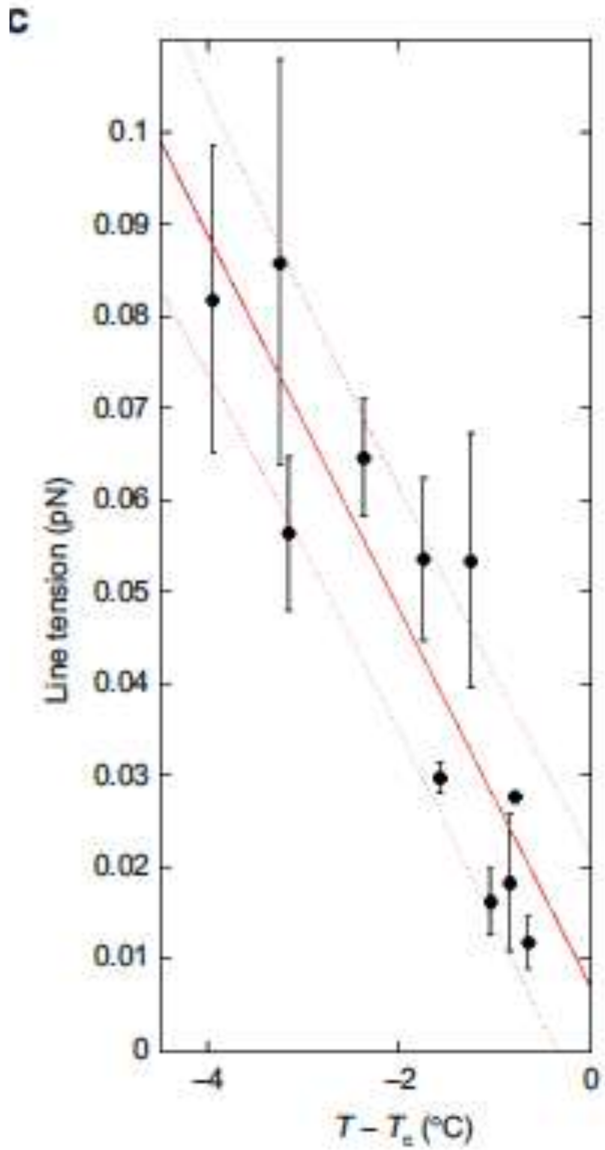


B. Sinha *et al.*, *Cell* (2011)

→ GPMVs

Plasma Membrane:
Composition close to a Critical Point



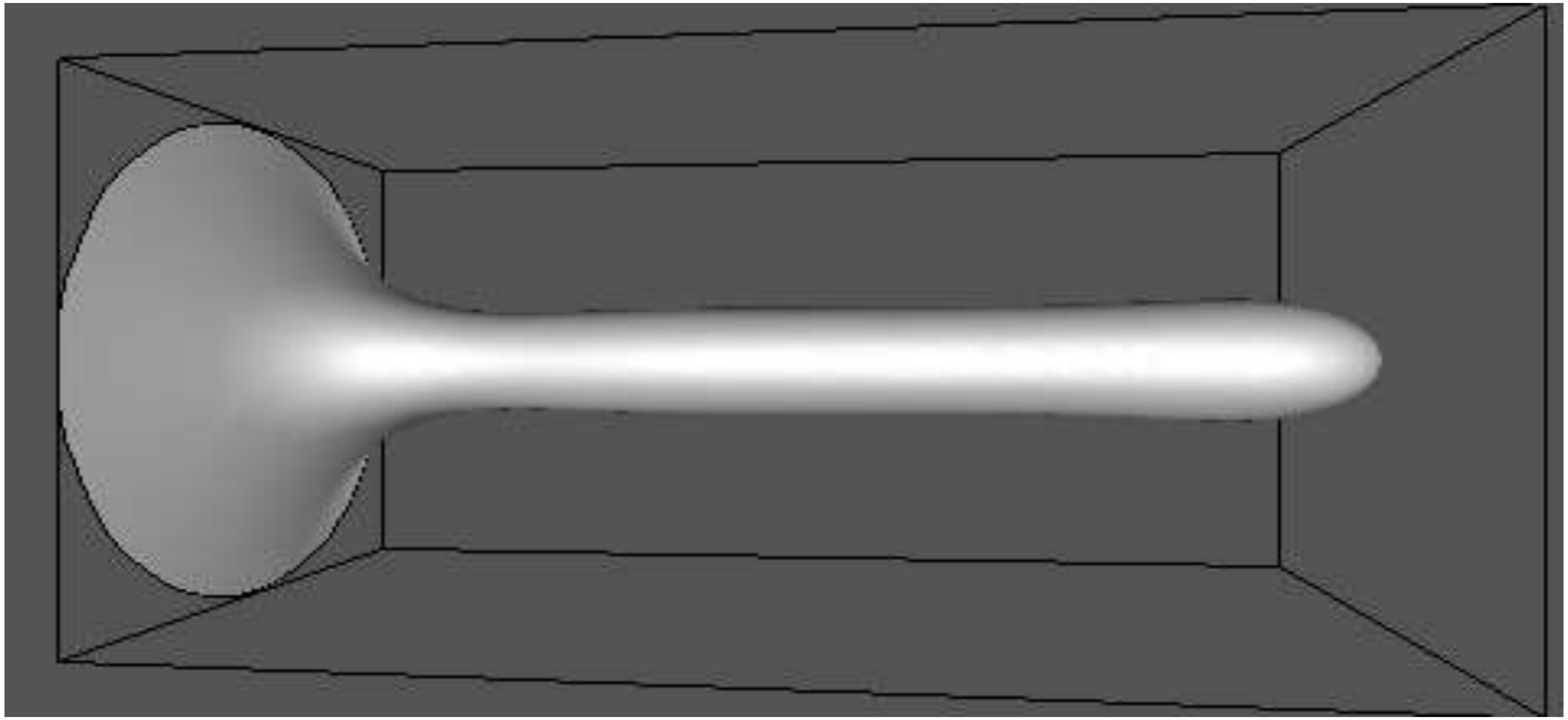


<u>Cf Raft:</u> size of domains determined by the proximity to some underlying critical point.
Sensitive to various stimuli

S.L. Veatch … B. Baird *ACS Chem. Biol.* (2008)

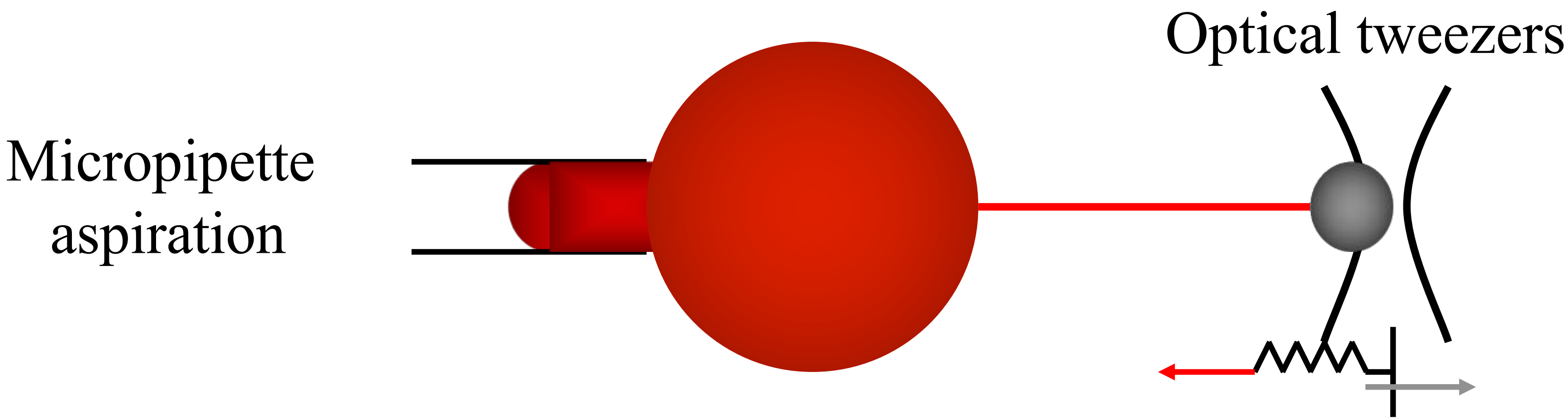
What Happens in a Highly Curved Membrane?

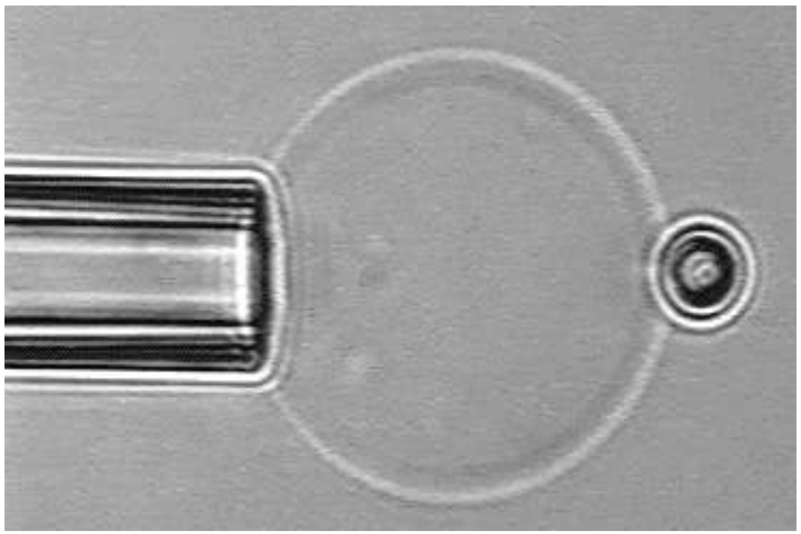
Membrane Nanotubes



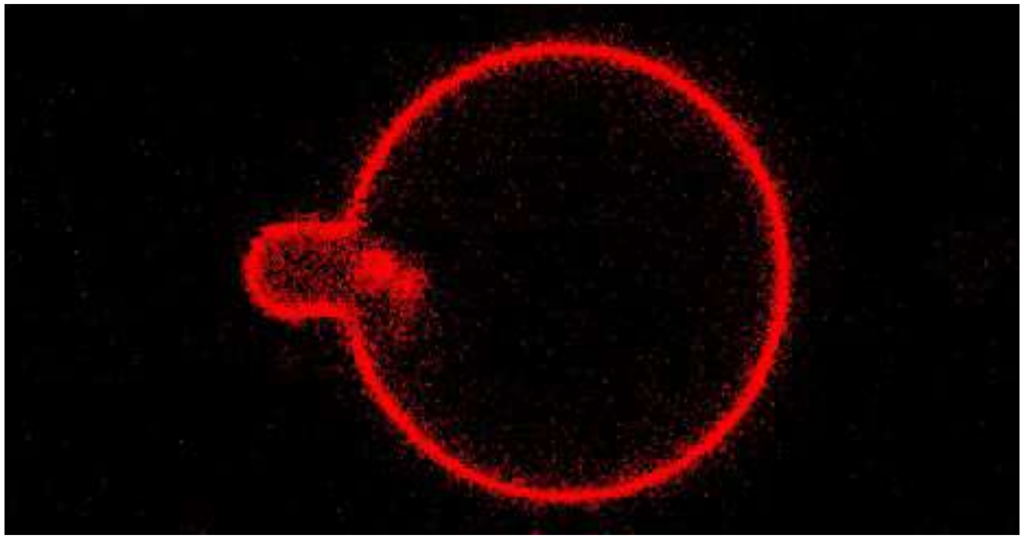
From Derényi et al, *PRL (2002)*

Membrane Nanotubes : Highly Curved



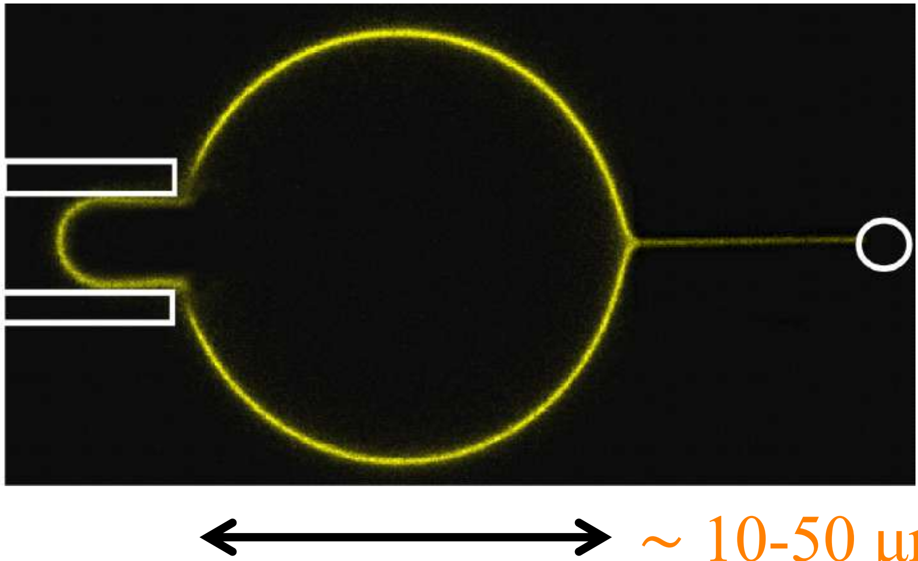


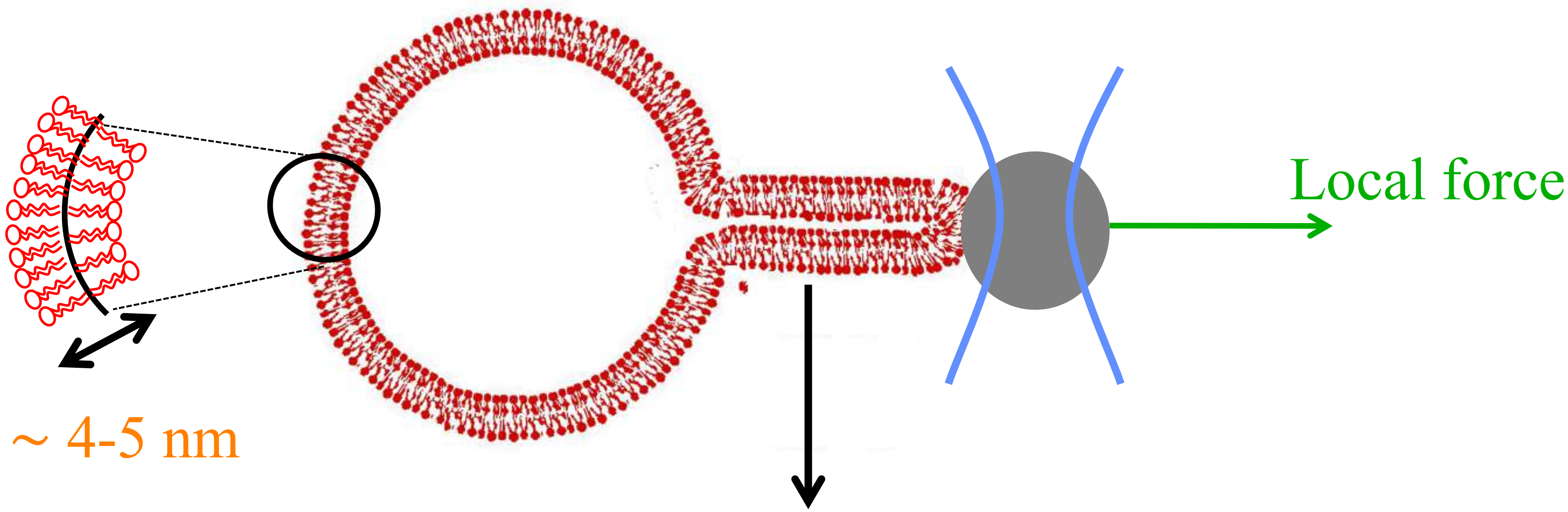
D. Cuvelier … P. Nassoy *Biophys. J* (2005)



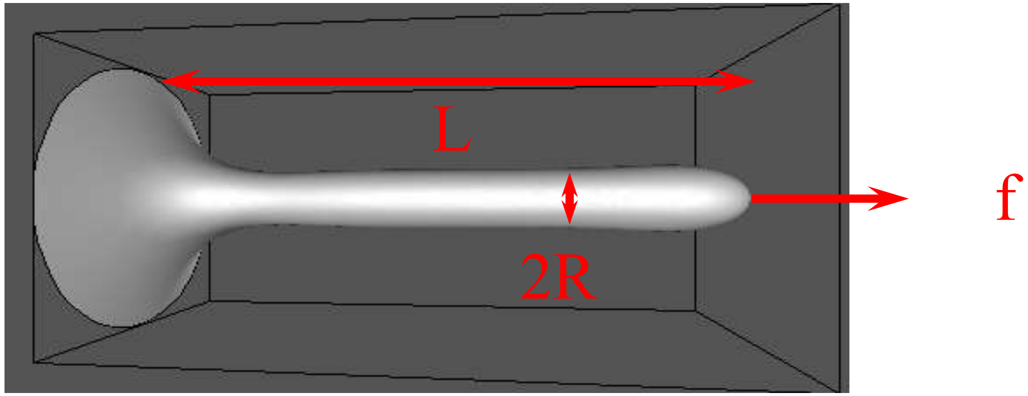
B. Sorre et al, *PNAS* (2009)

Membrane Nanotubes : Highly Curved





Physics of Membrane Nanotubes



Some references

- Evans, Yeung *Chem. Phys. Lipids* (1994)
- Bozic, Svetina, Zeks *PRE* (1997)
- Svetina, Zeks, Waugh, Raphael *Eur. B. J* (1998)
- Derényi, Jülicher, Prost *PRL* (2002)
- Powers, Huber, Goldstein *PRE* (2002)

$$H = \iint_A dA \left[\frac{\kappa}{2} C^2 + \sigma \right] - fL$$

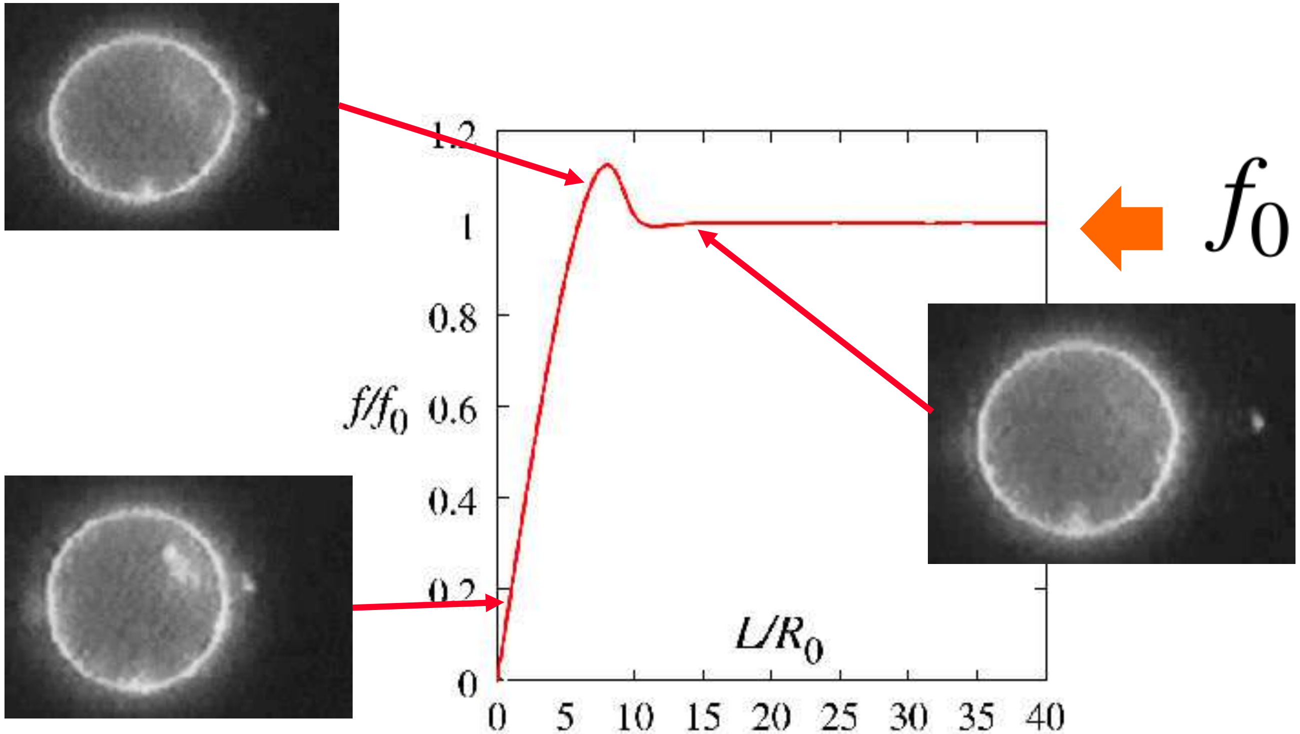
$$E_{tube} = 2\pi L \frac{\kappa}{2R} + 2\pi R \sigma L - fL$$

$\frac{\partial E}{\partial R} = 0$ Radius: $R_0 = \sqrt{\frac{\kappa}{2\sigma}}$ $\approx$ 10 nm – few 100 nm

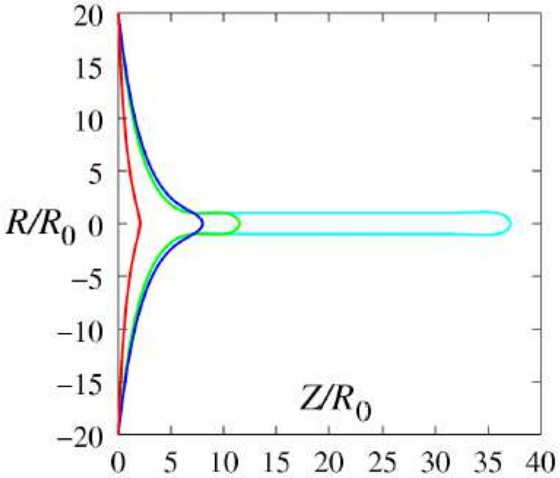
Set by tension

$\frac{\partial E}{\partial L} = 0$ Force: $f_0 = 2\pi\sqrt{2\kappa\sigma}$ few pN - few 10 pN

Theory: Force/Extension at Constant Tension



$$f_0 = 2\pi\sqrt{2\kappa\sigma}$$



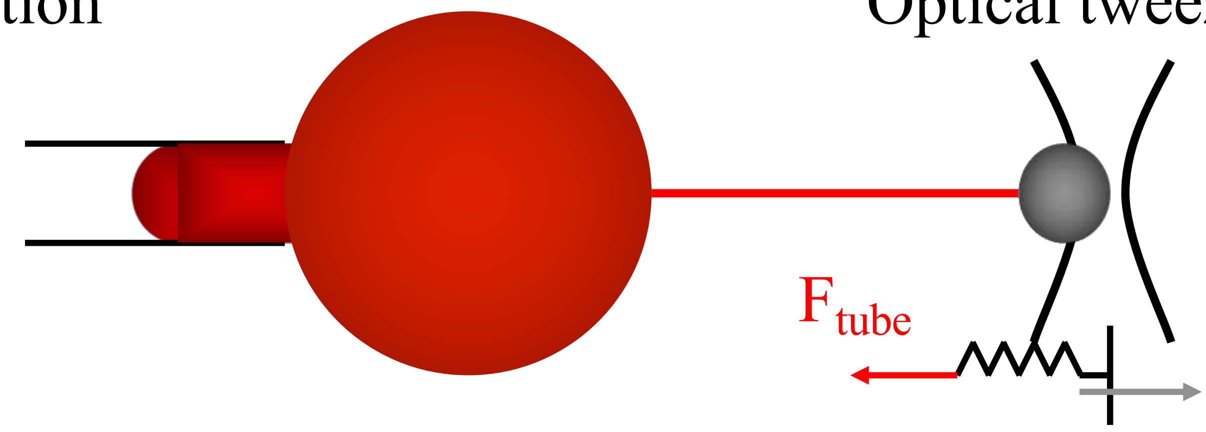
Dérényi et al, PRL ***88*** *(2002) 238101*

Powers et al PRE ***65*** *(2002) 041901.*

Nanotube - Controlled Curvature

Controlling curvature:
Micropipette aspiration

$$R_0 = \sqrt{\frac{\kappa}{2\sigma}}$$



Force:
Optical tweezers

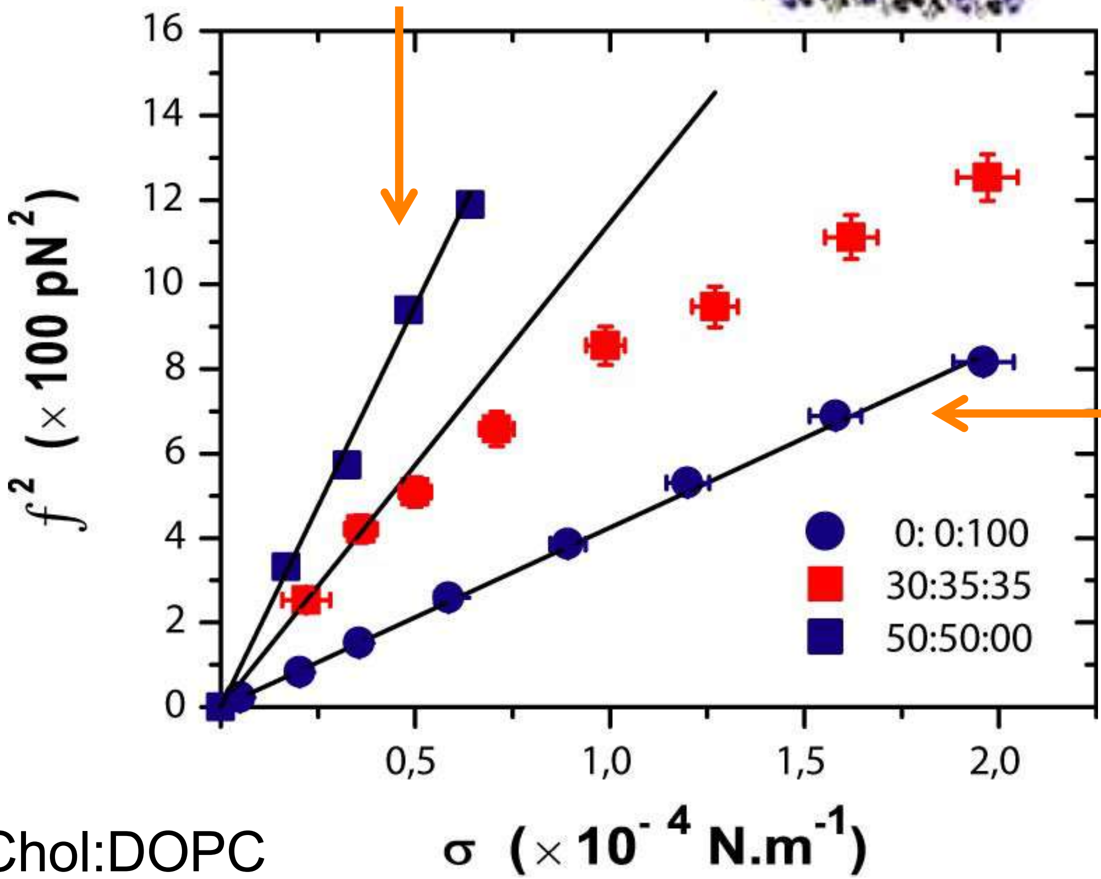
$$f_0 = 2\pi\sqrt{2\kappa\sigma}$$

Force
Radius ↔ Membrane tension
Lipid composition

Experiments

$$f_0^2 = 8\pi^2\kappa \cdot \sigma$$

$\kappa_{SM:Chol} = 65 \pm 10\ k_BT$

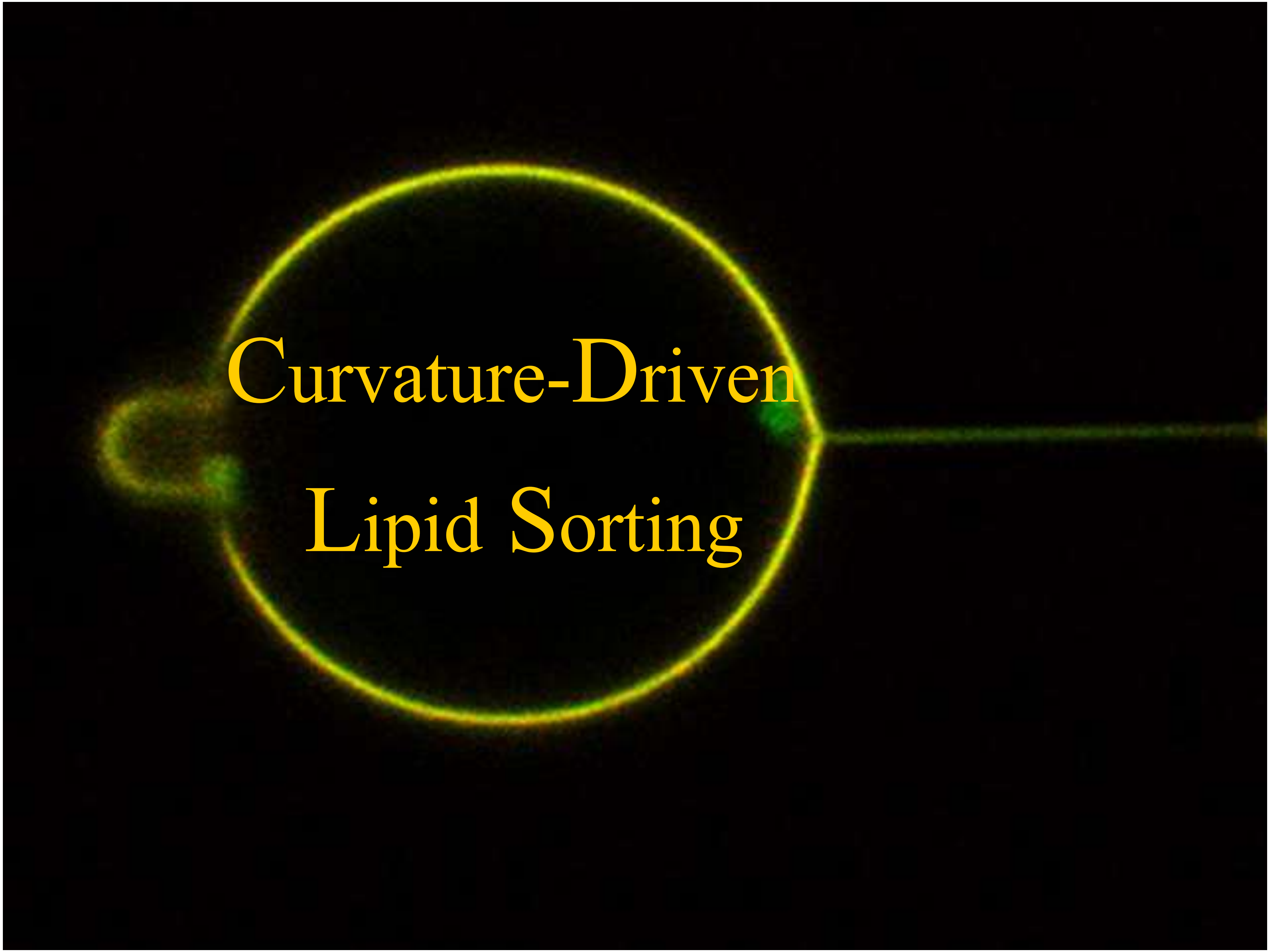


$\kappa_{DOPC} = 16 \pm 2\ k_BT$

SM:Chol:DOPC

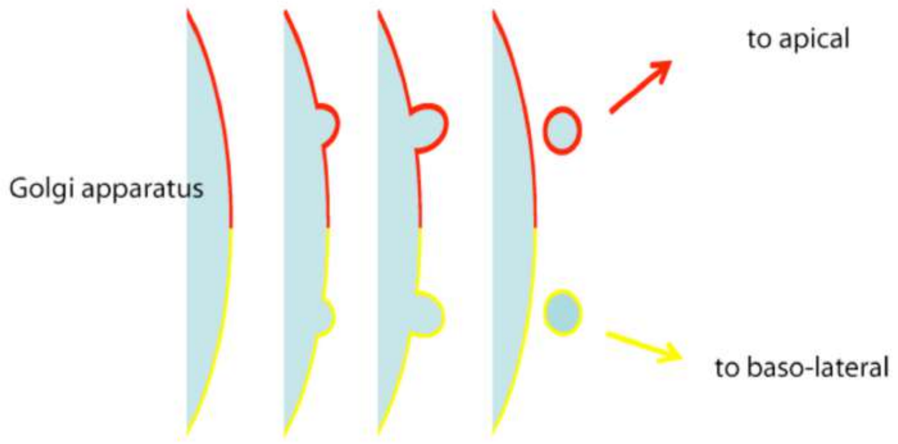
B. Sorre *et al. PNAS (2009)*

Curvature-Driven Lipid Sorting



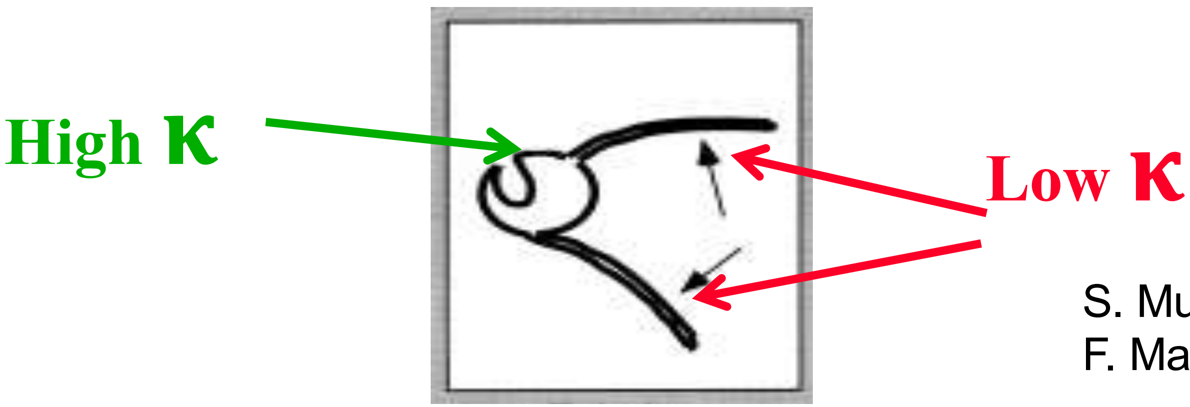
Sorting Lipids ?

- *Lipid rafts*



K. Simons, G. van Meer
Biochemistry (1988)

- *Global: Membrane mechanical properties*

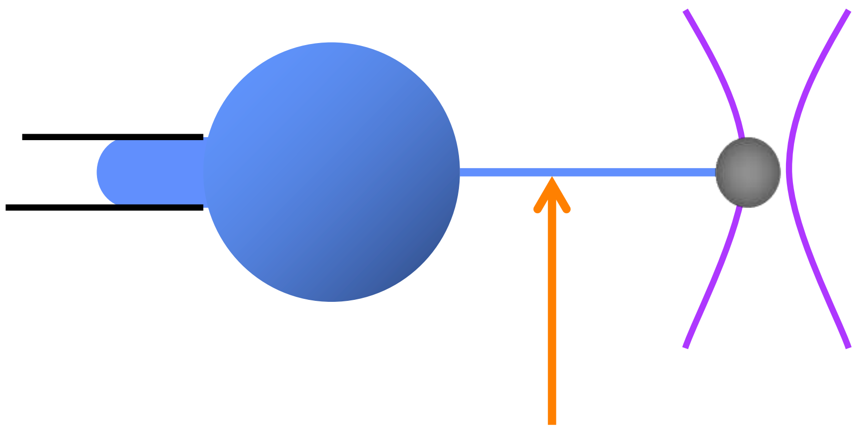


S. Mukherjee, T. Soe
F. Maxfield, *J. Cell Biol (1999)*

Expected:

Sphingomyelin/Cholesterol/DOPC

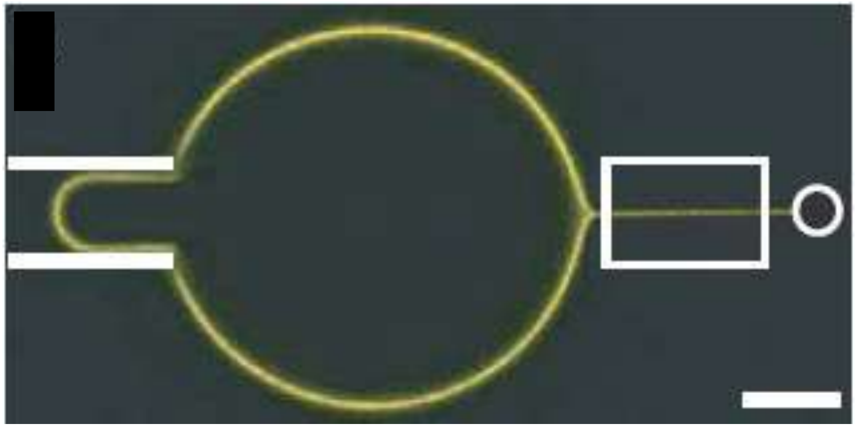
Composition	K ($\kappa_B T$)
DOPC	16
DOPC+50% Chol	30
SM+50% Chol	65



Low κ

PC rich

Sphingo. depleted

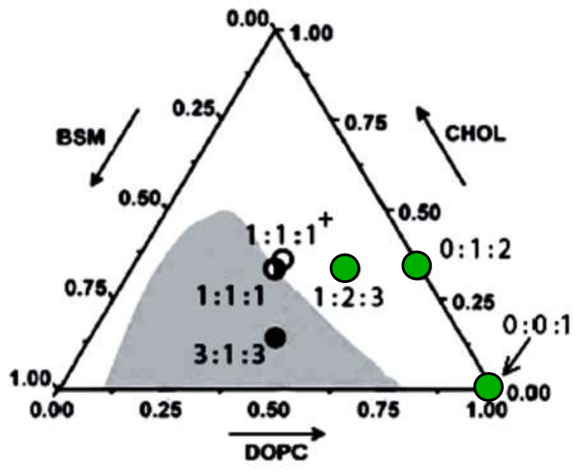


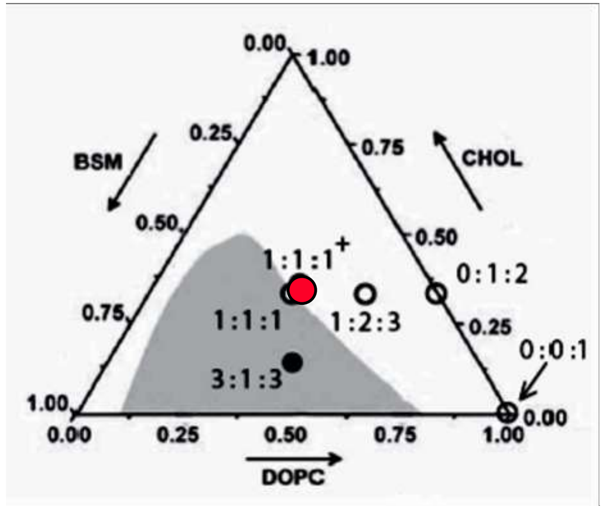
Rhodamine PE (Ld)

GM1* (no preference)

Sorting coefft: $$S = \frac{I^{t}_{PE}}{I^{t}_{GM_1^*}} \Big/ \frac{I^{v}_{PE}}{I^{v}_{GM_1^*}}$$

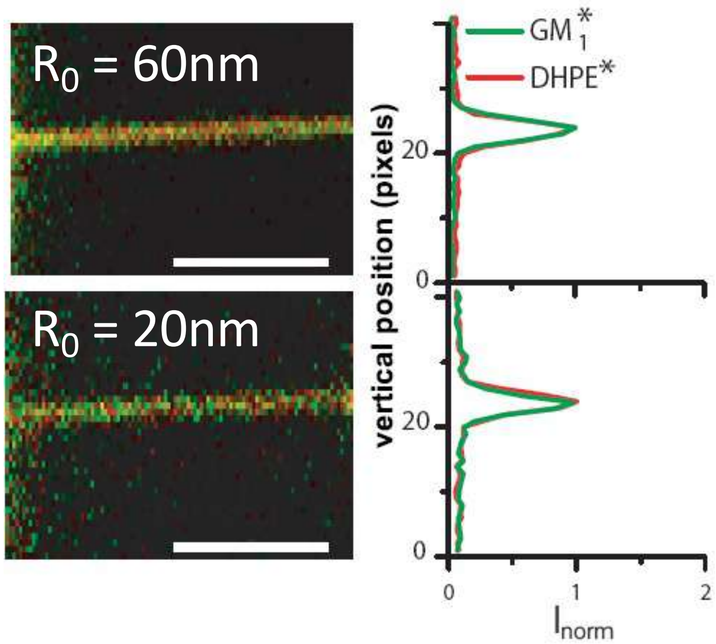
Sorting :Only in the Vicinity of Phase Boundary

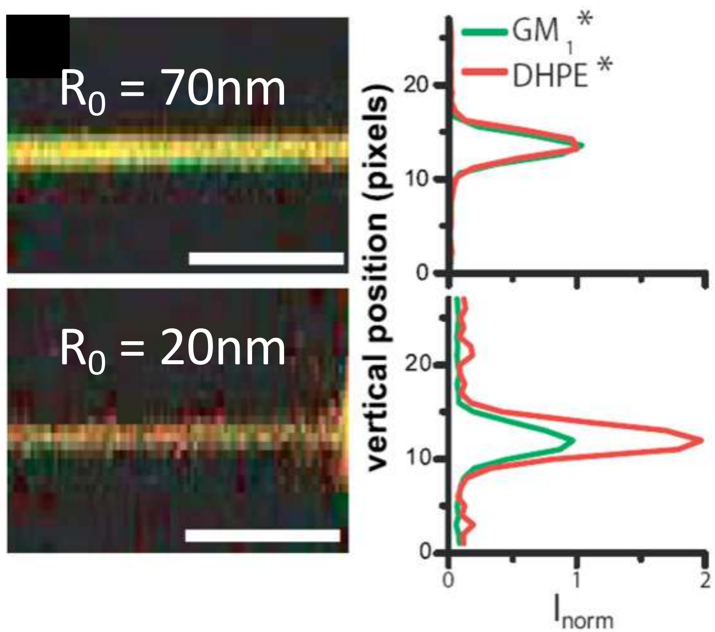




Far from phase boundary

Close to phase boundary





Sorre, Callan-Jones *et al.* PNAS (2009)

Theoretical Modeling

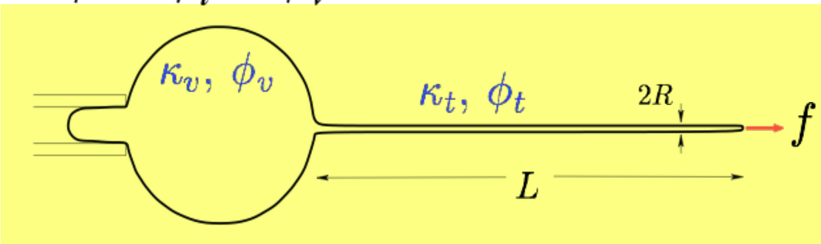
A. Callan-Jones, J.F. Joanny, J. Prost

$$F_t = 2\pi R L_{tube}\left[\frac{\kappa(\phi)}{2R^2} + \sigma + \frac{1}{2}\lambda(\phi_v)\Delta\phi^2\right] - f_{tube}\times L_{tube}$$

Gain

Entropic Penalty

$$\Delta\phi = \phi_t - \phi_v$$



$$\lambda = \frac{kT}{a}\left(\frac{1}{\phi_v(1-\phi_v)} - 2\chi\right)$$

a: size of sorted specie

χ: lipid-lipid interaction

$\lambda = 0$ at the demixing limit (spinodal)

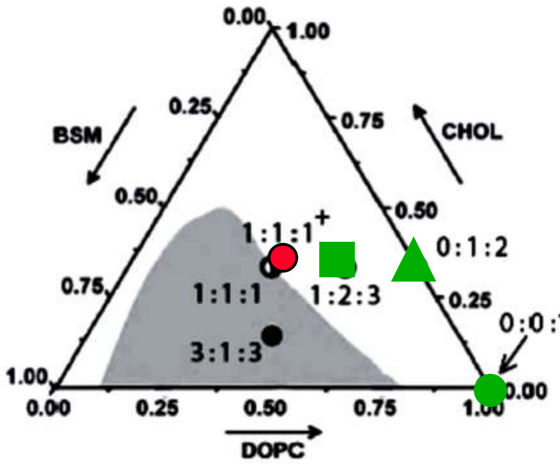
If not close to demixing, mixing entropy dominant

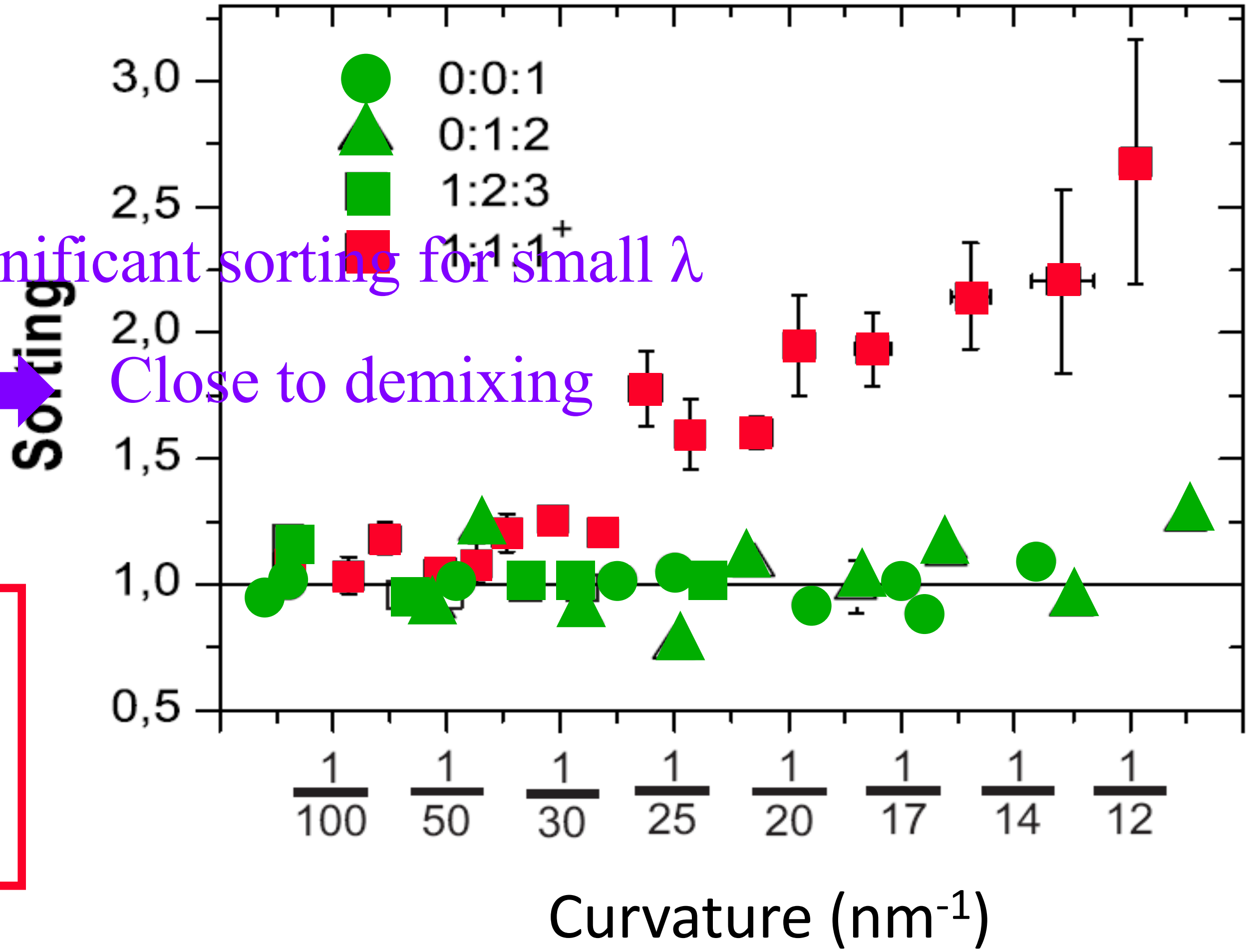
Sorre, Callan-Jones *et al.* PNAS (2009)

Curvature and Lipid Sorting

$$\phi_t - \phi_v \propto \frac{1}{\lambda}\frac{1}{R_t^2}$$

Sorting: Φ_t $\quad \frac{1}{\lambda}\sigma \propto \frac{1}{\lambda}\frac{1}{R^2}$





Significant sorting for small λ

Close to demixing

Near phase boundary:

Sorting increases with curvature

Tube enriched in DOPC, depleted in SM

Sorre, Callan-Jones *et al.* PNAS (2009)

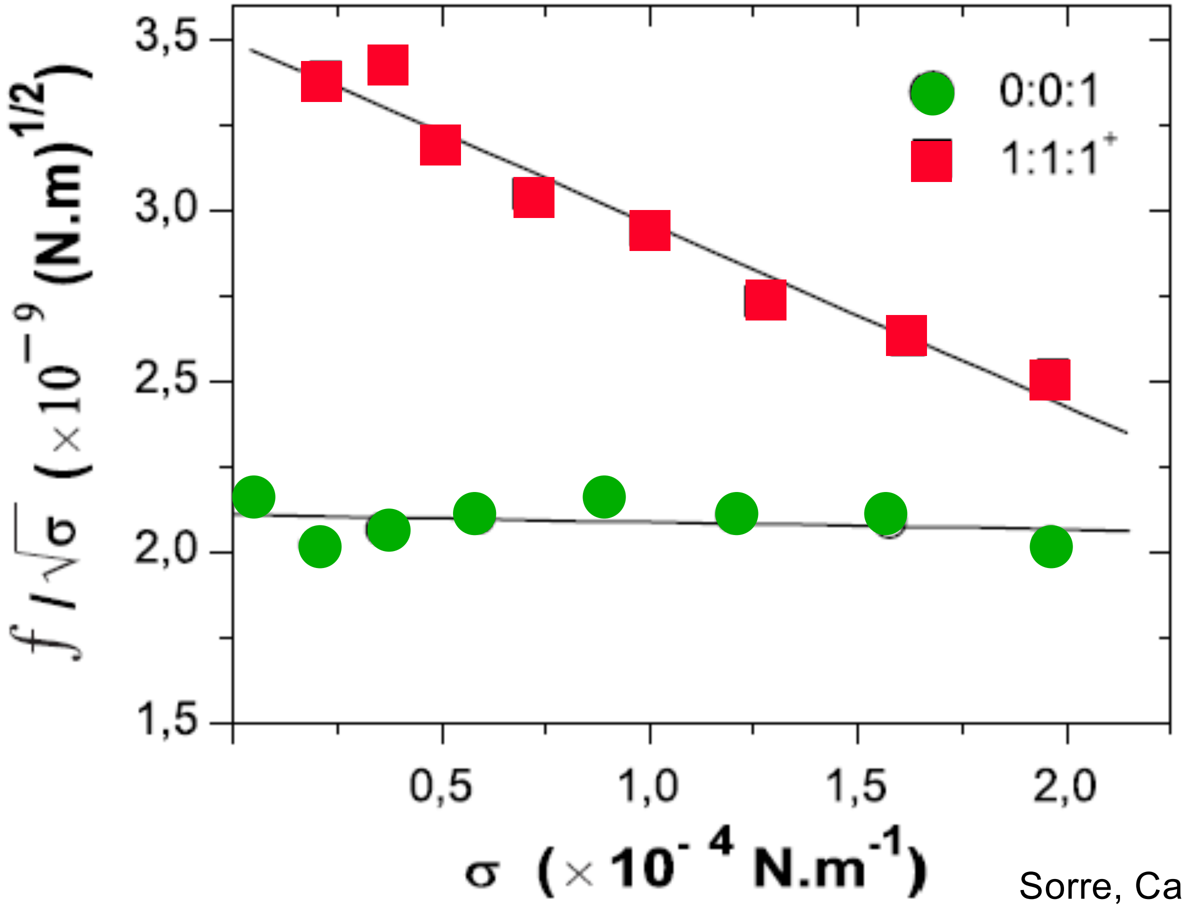
Effect on the Force

$$f(\Phi_t) \approx 2\pi\sqrt{2\kappa_t\sigma}\left[1-\frac{\sigma}{\lambda}\right]$$

Sorting Strength

λ ↓ when approaching demixing

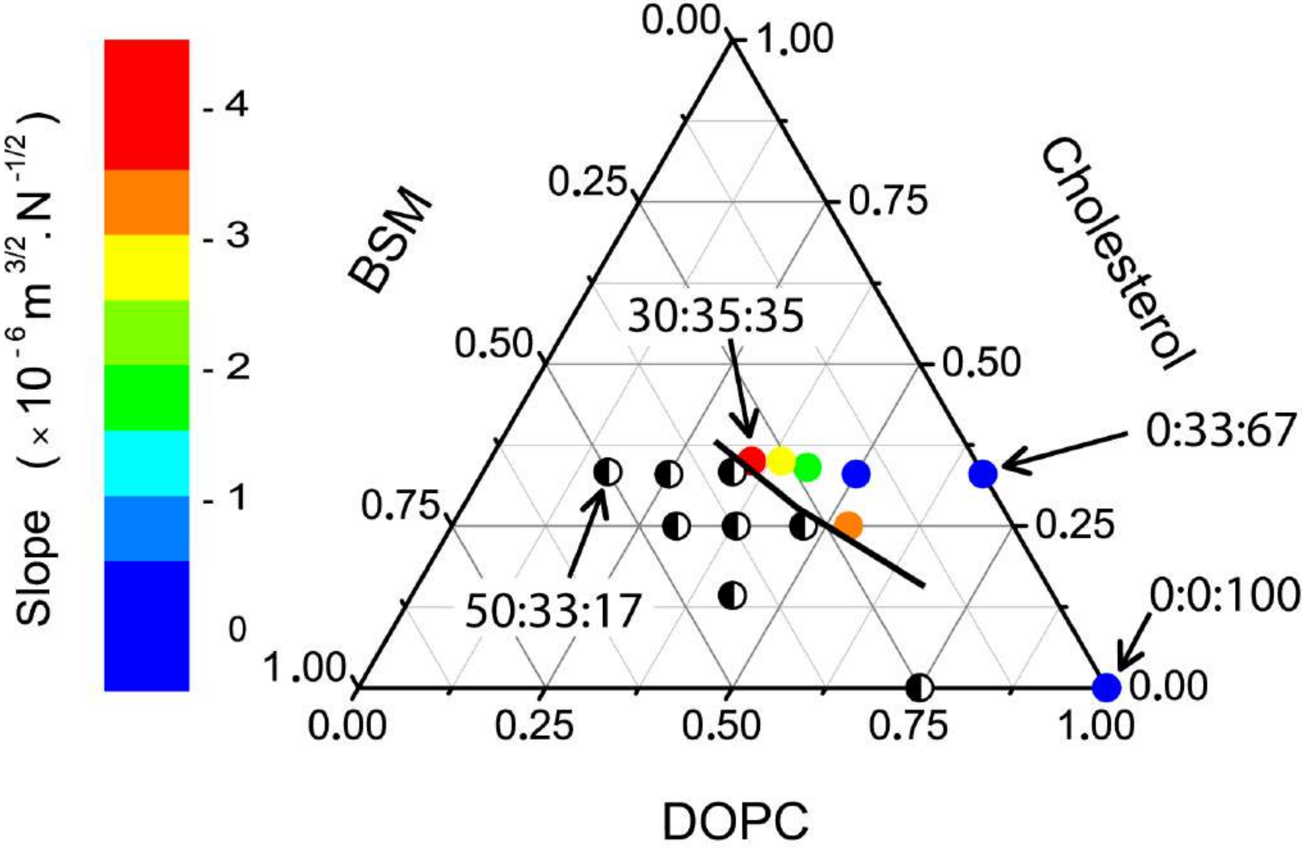
Larger deviation of the force



Sorre, Callan-Jones *et al.* PNAS (2009)

Curvature-driven Sorting Needs Proximity to a Demixing Point

Sorting Strength



Sorre, Callan-Jones *et al. PNAS (2009)*

(Also Tian et al, *B.J.* (*2009*)).

Conclusions

- Lipid sorting only close to demixing: tube enriched in Ld phase (lowest bending rigidity)
  Biological membranes: PM close to demixing

"Plasma membranes are poised for activation of raft phase coalescence at physiological temperature"

D. Lingwood et al. PNAS (2008)

- If lipids only, requires tight regulation of lipid composition

How to reduce the entropic penalty due to lipid mixing?

$$\Delta f_m = \frac{1}{2}\lambda \Delta\phi^2$$

→ Minimize λ

$$\lambda = \frac{kT}{a}\left(\frac{1}{\phi_v(1-\phi_v)} - 2\chi\right)$$

a: size of sorted specie

χ: lipid-lipid interaction

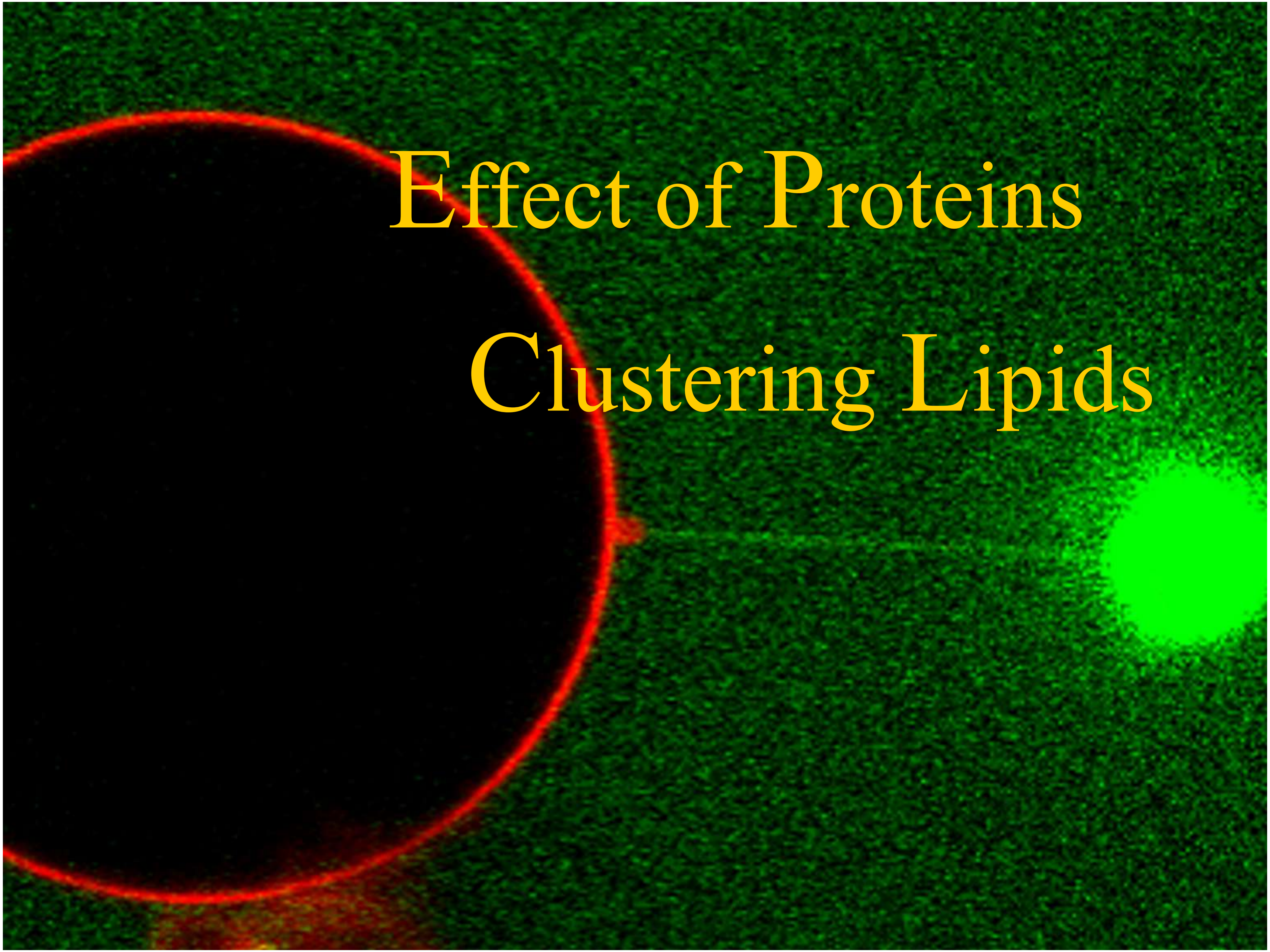
→ a: Increase lipid size → clustering

Stronger sorting for proteins

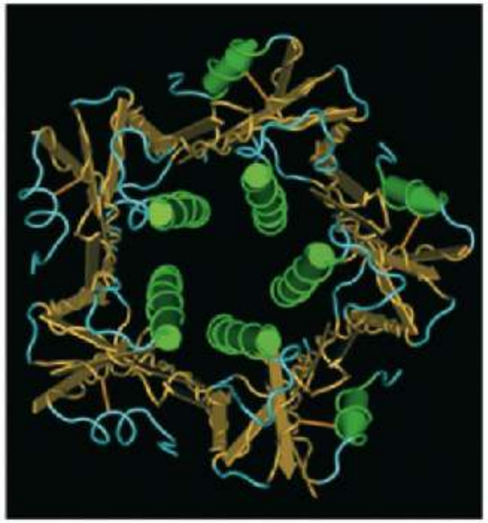
→ χ : Increase short range interaction

→ Add a protein with preferential interaction

Effect of Proteins Clustering Lipids



AB$_5$ Toxins Cluster Glycolipids

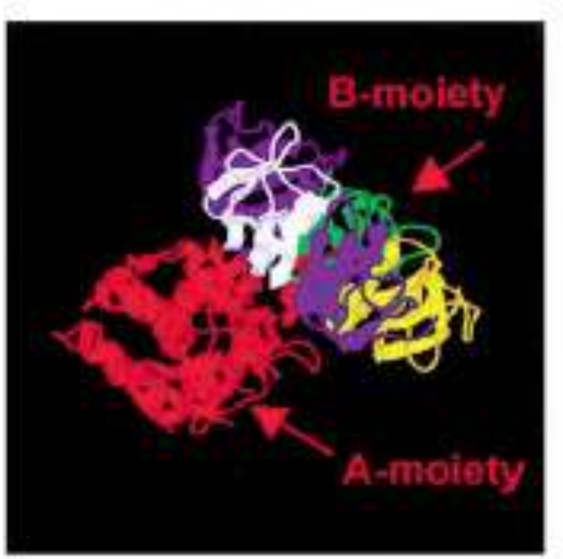


From J. Sanchez and J. Holmgren

Cell. Mol. Life Sci. (2008)

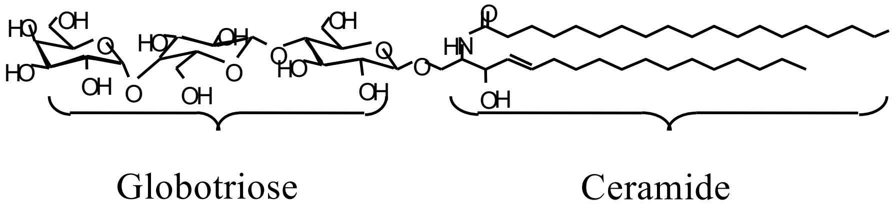
Cholera Toxin (CTxB)

- CtxB : pentamer
- Receptor = ganglioside GM1 lipid
- 1 CTxB pentamer ⟷ **5** GM1



Shiga Toxin (STxB)

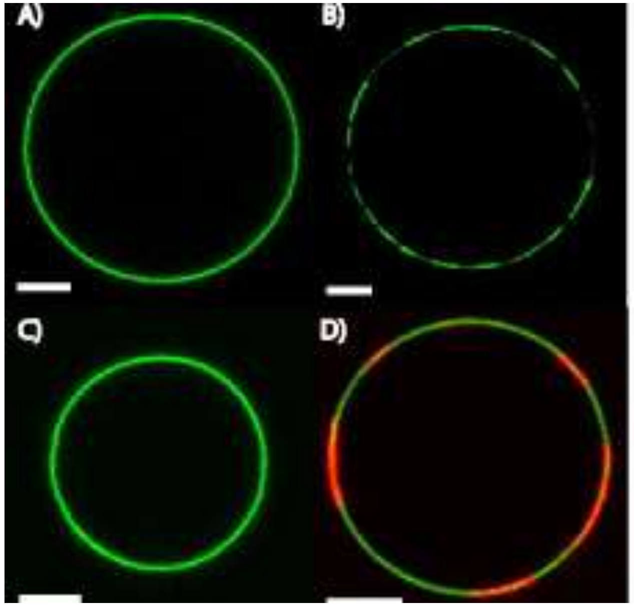
- StxB : pentamer
- Receptor = Gb3 lipid

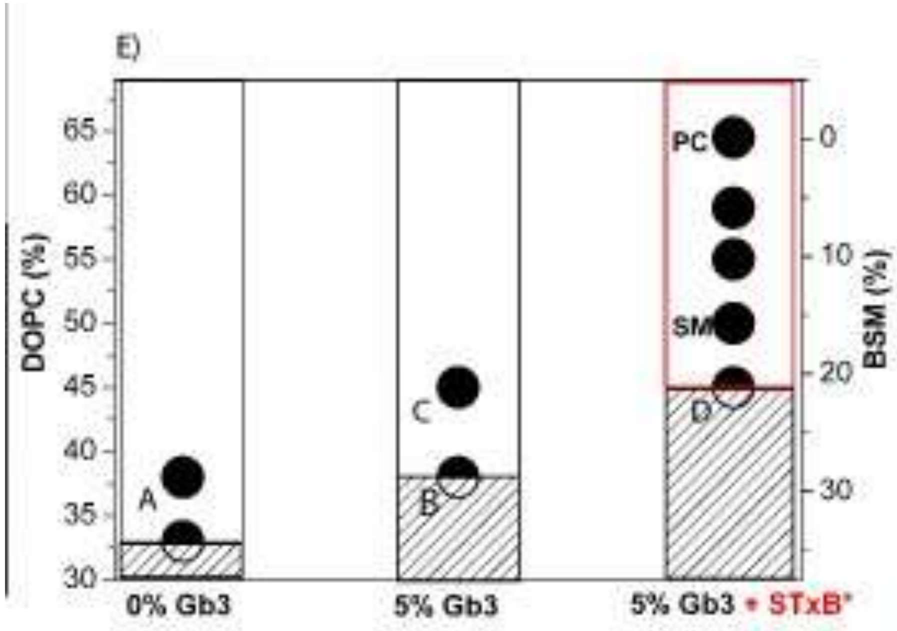


- 1 STxB pentamer ⟷ **15** Gb3

AB_5 Toxin Binding Facilitates Lipid Demixing

STxB



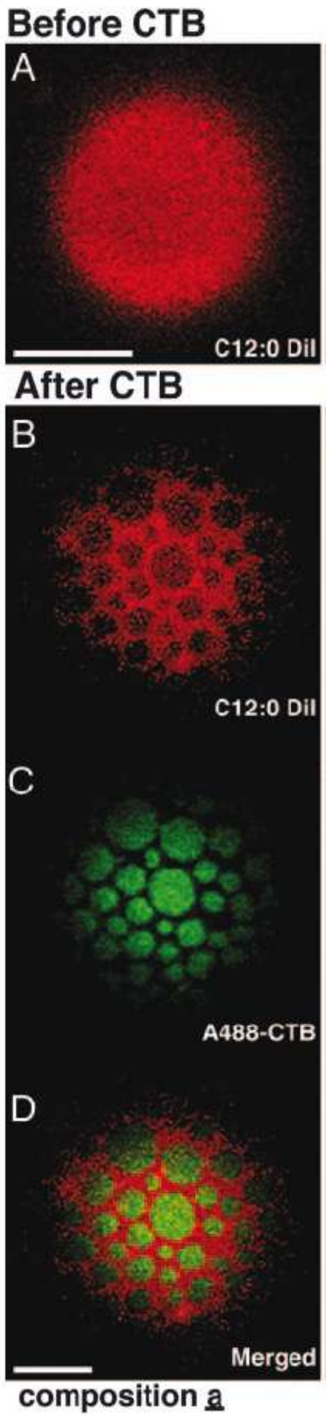


Safouane, Berland et al *Traffic(2010)*

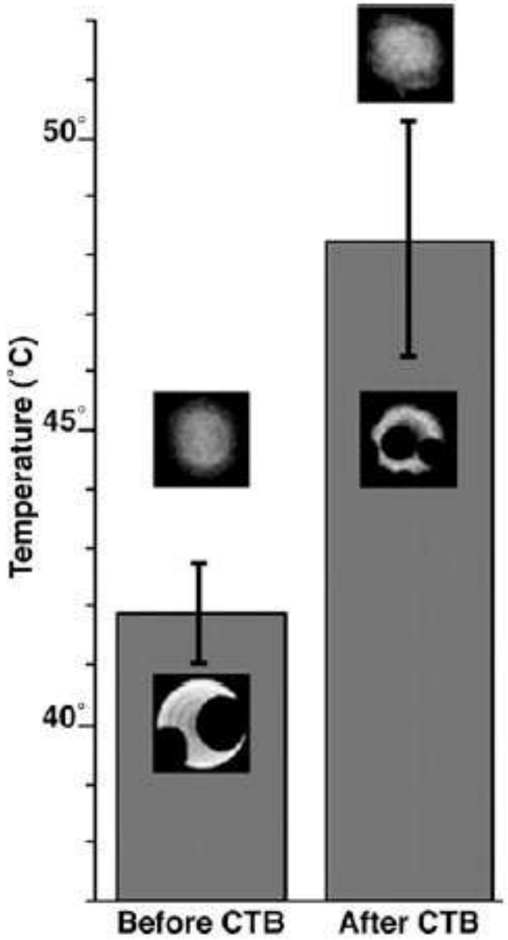
Clustering: reduces entropy cost

Vesicle close to
phase separation
NO toxin

With
CTxB



CTxB clusters lipids
Reduces mixing entropy
Phase separation facilitated
(at higher T°C)

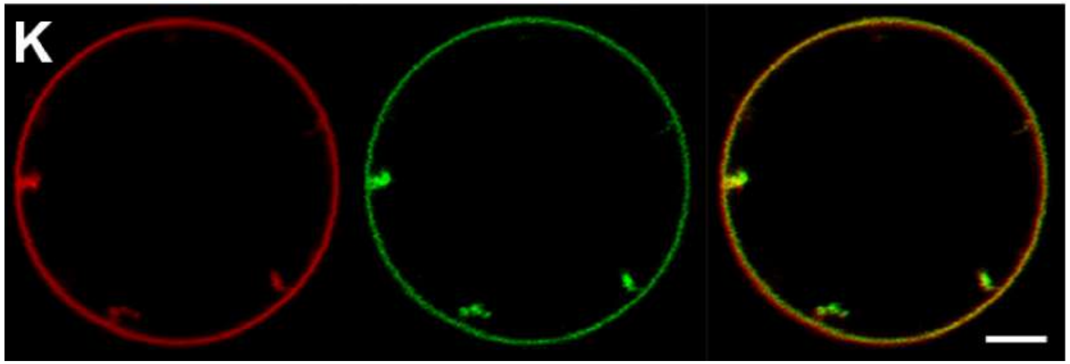


A. Hammond … G. W. Feigenson *(PNAS)* 2005

CTxB and STxB Induce Membrane Invaginations

CTxB

DOPC+30% Chol +5%GM1



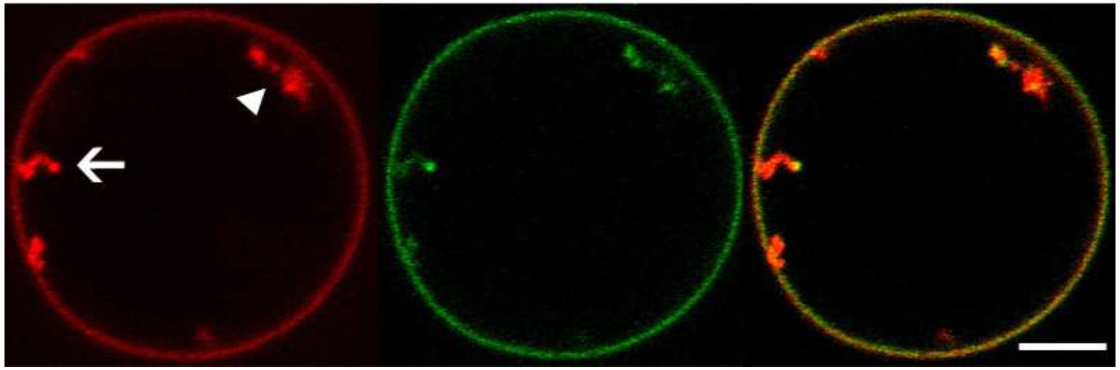
10 μm

CTxB Membrane

L. Berland, W. Römer

STxB

DOPC+30% Chol +5% Gb3



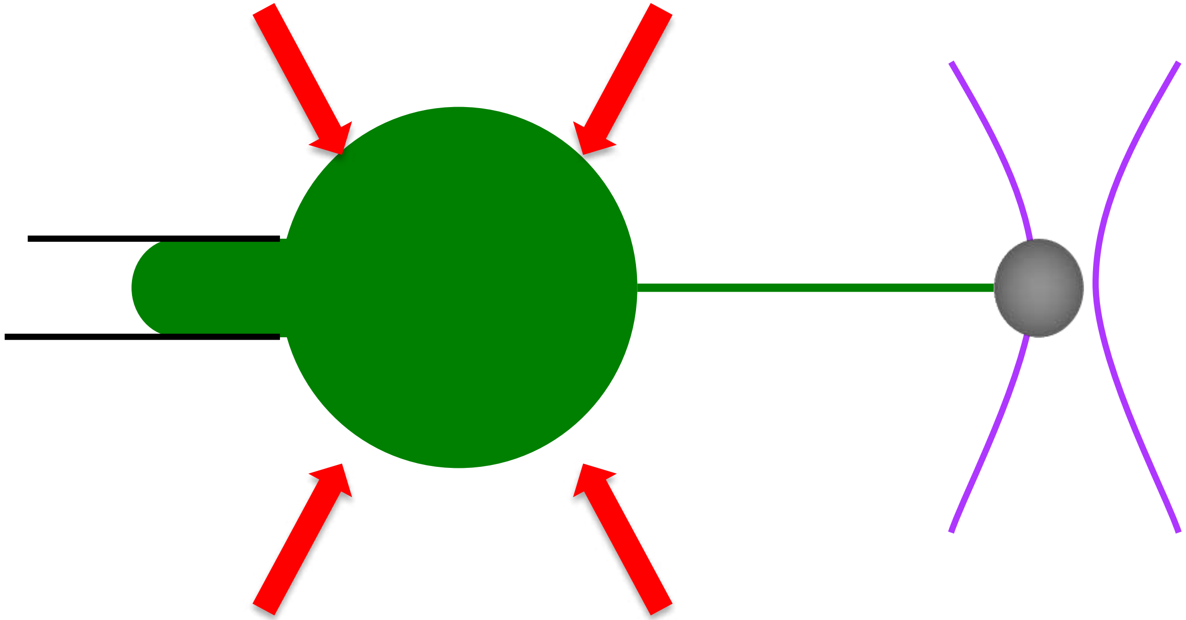
10 μm

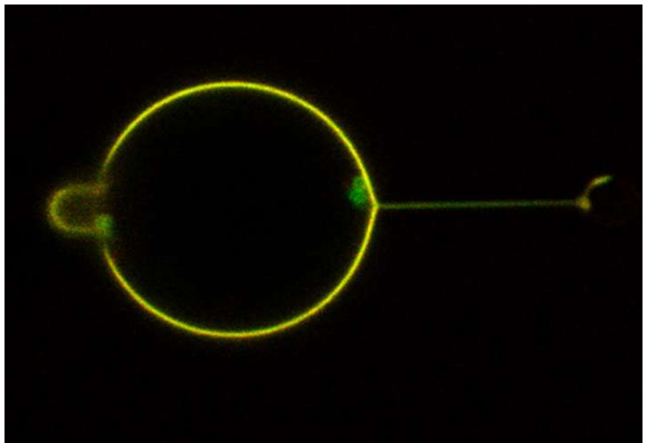
STxB Membrane

W. Römer, L. Berland, et al., *Nature* (2007)

Cluster lipids + induce negative spontaneous curvature

CTxB added from outside : *depleted from the tube*

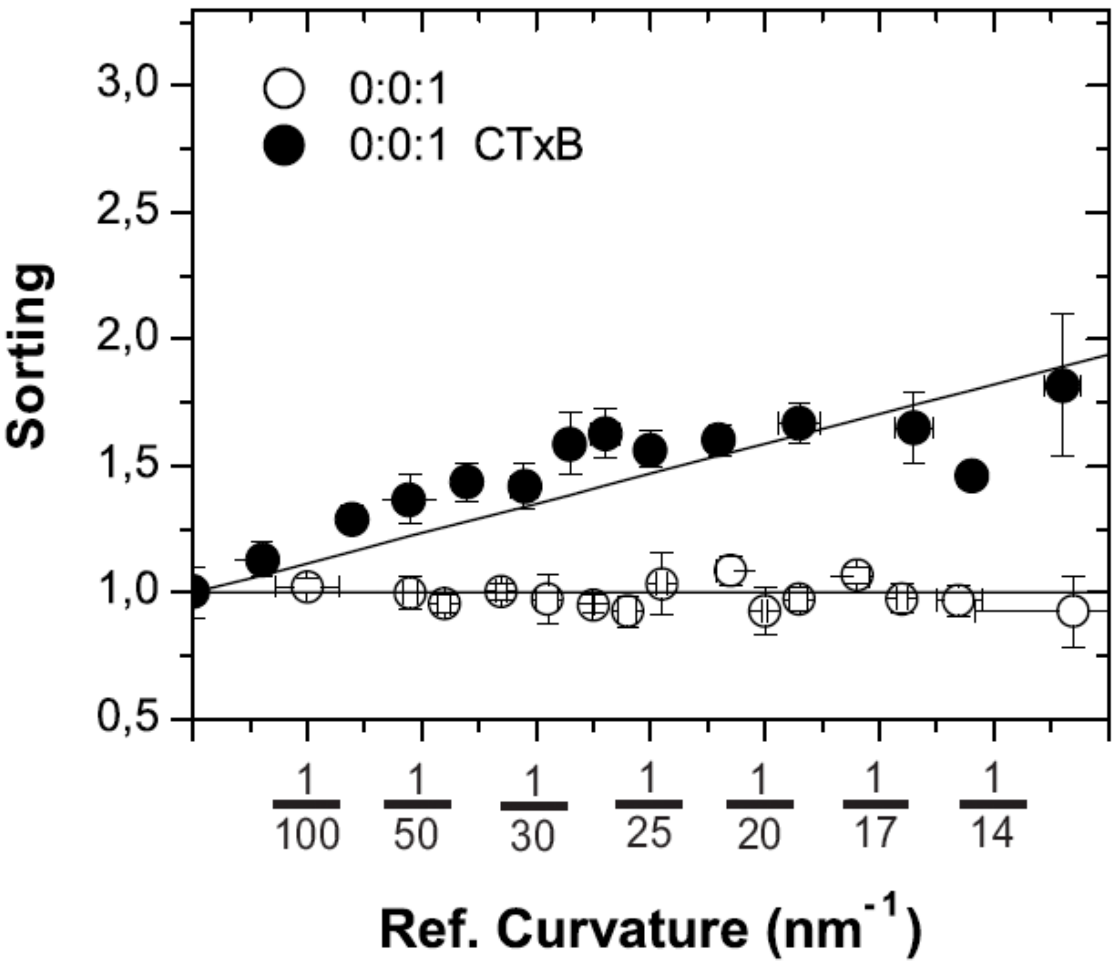


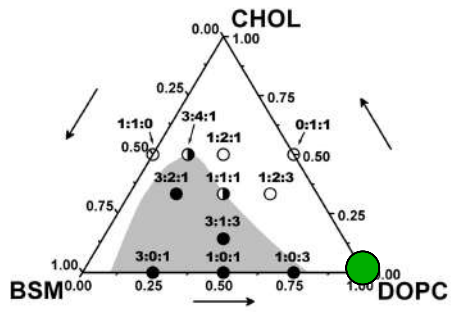


Cholera Toxin Sorting in DOPC Tubes

DOPC
(0:0:1)
PE*

1% GM1*
+ CTxB





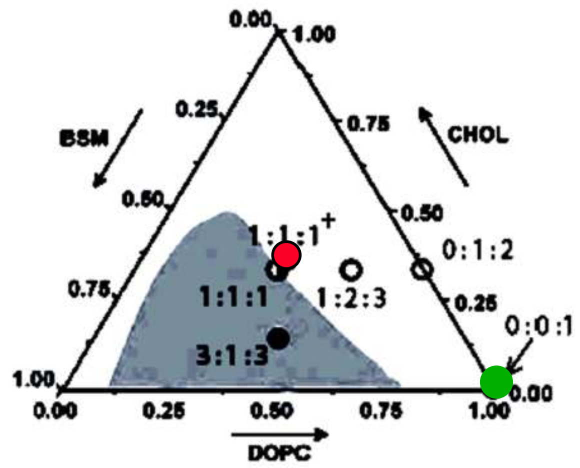
GM1* depleted from DOPC tubes

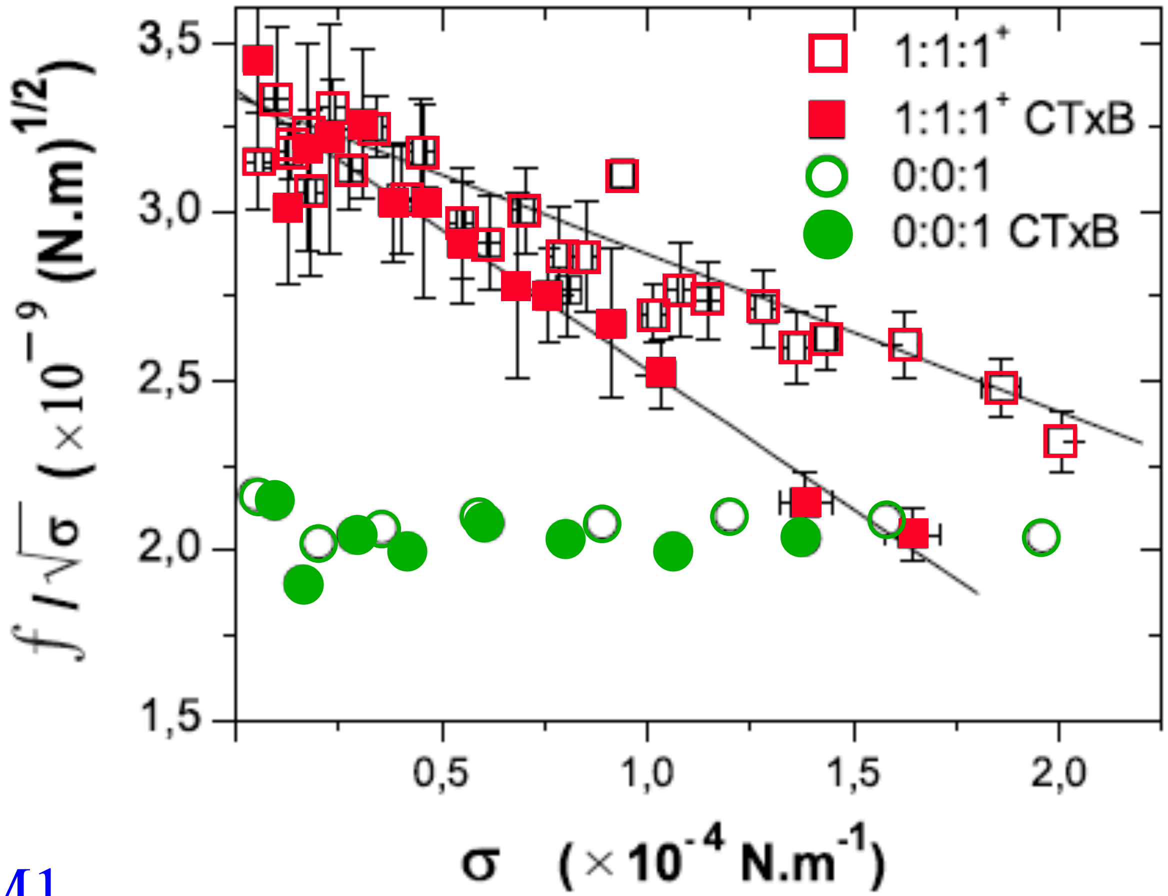
CTxB depleted from DOPC tubes

Consistent with CTxB-induced negative curvature

Sorre et al. *PNAS* (2009)
Tian, Baumgart, *B.J.* (2009)

Cholera Toxin Enhances Lipid Sorting Close to Demixing





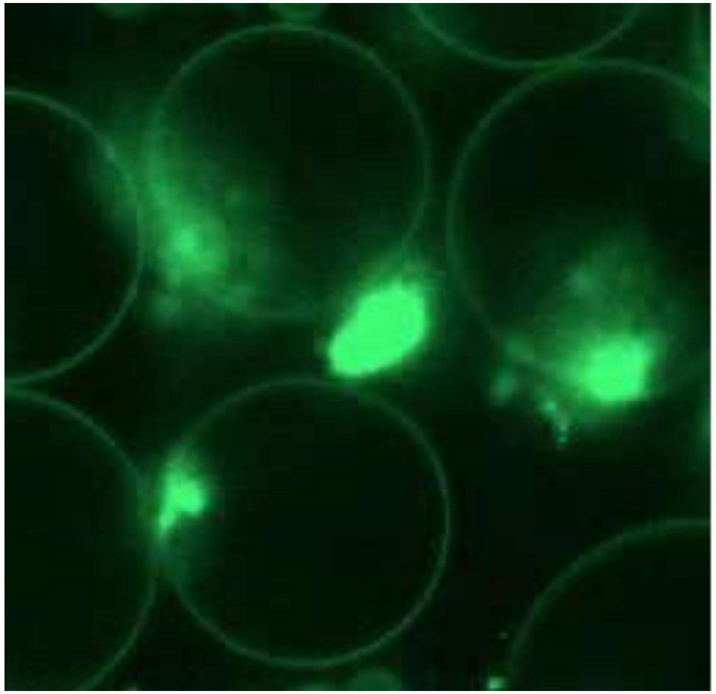
CTxB addition: clusters GM1

λ decreases

enhances BSM depletion from tube

Sorre, Callan-Jones *et al.* PNAS (2009)

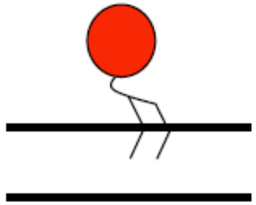
"Plasma membranes are poised for activation of raft phase coalescence at physiological temperature"



Cell blebs

(PMS or GPMV)

GL-GPI-mRFP



37°C

No toxin

37°C

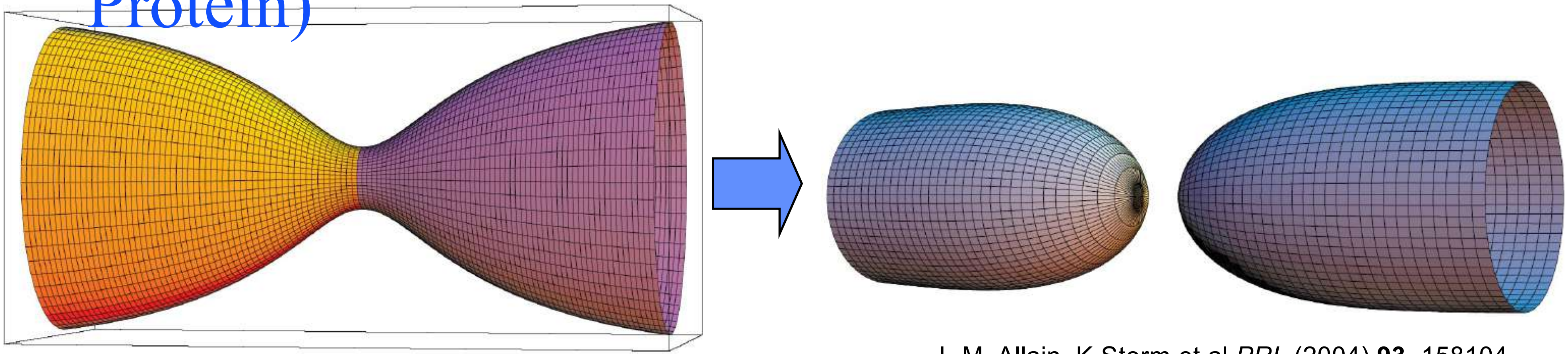
With CTxB

Induces phase separation

D. Lingwood… K. Simons *PNAS* (2009)

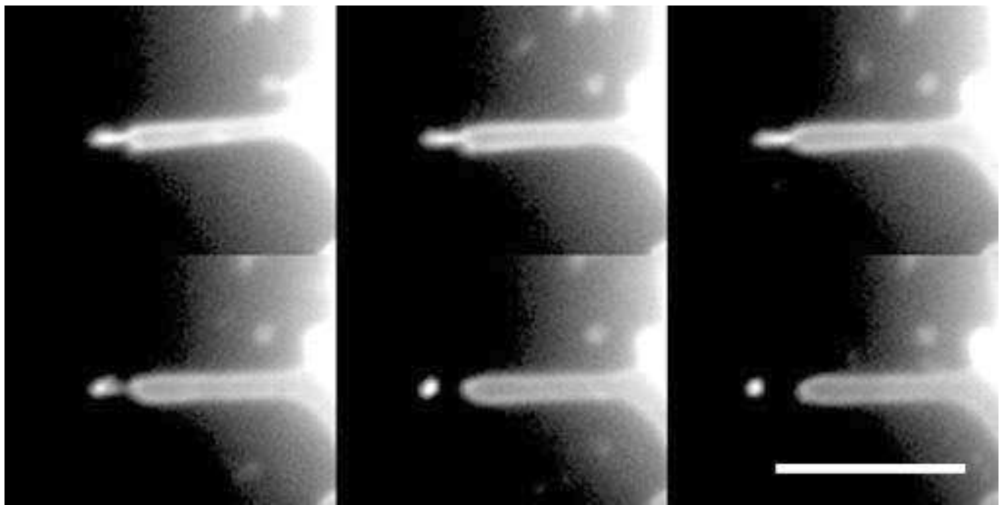
Tube Scission
due to Line Tension

Line Tension Induces Tube Fission (NO Protein)



Line tension

J.-M. Allain, K Storm et al *PRL*.(2004) **93**, 158104

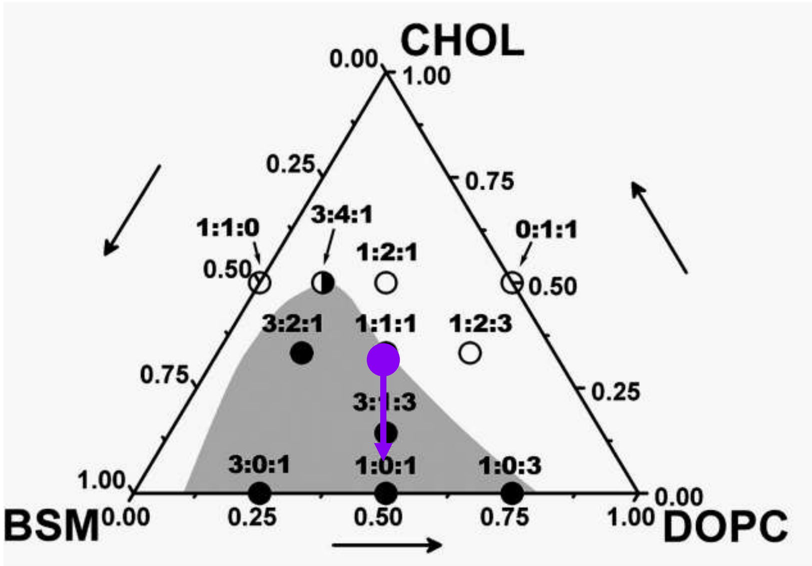


DOPC:SM:Chol 1:1:1

Cholesterol decreased

A. Roux et al *EMBO J.* (2005)

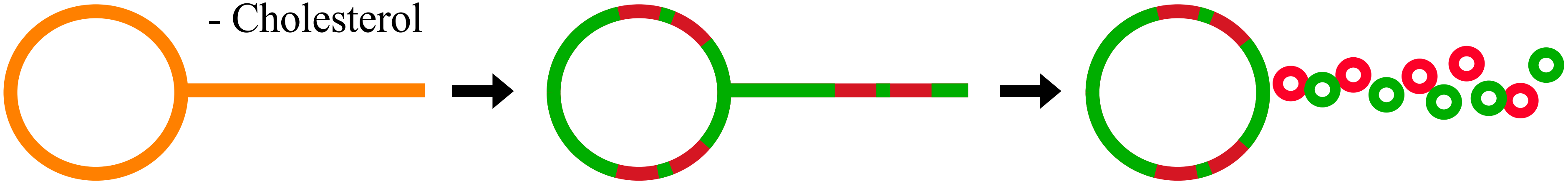
DOPC/BSM/Chol



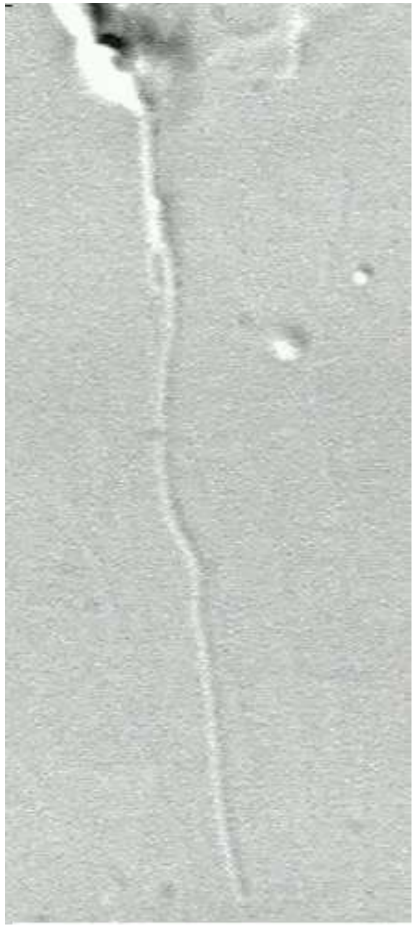
Cholesterol decreased → Phase separation

Sorre, Callan-Jones *et al. PNAS* (2009)

Line Tension Induces Tube Fission



Induced by photo-activation



By MβCD

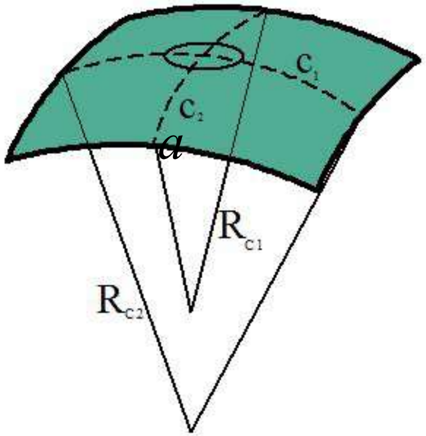
A. Roux et al *EMBO J.* (2005)

Canham-Helfrich free energy:

$$H = \int dA \left[\frac{1}{2}\kappa \left(2J\right)^2 + \bar{\kappa} K + \sigma \right]$$

J: Mean curvature

K: Gaussian curvature



$$J = \frac{1}{R_{c1}} + \frac{1}{R_{c2}}$$

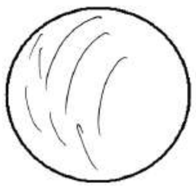
MEAN CURVATURE

$$K = \frac{1}{R_1} \cdot \frac{1}{R_2}$$

GAUSSIAN CURVATURE

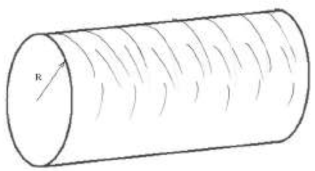
TYPICAL SHAPES

SPHERE



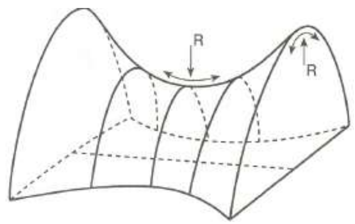
$$J = \frac{2}{R} \qquad K = \frac{1}{R^2}$$

CYLINDER



$$J = \frac{1}{R} \qquad K = 0$$

SADDLE



$$J = 0 \qquad K = -\frac{1}{R^2}$$

Canham-Helfrich free energy:

$$H = \int dA \left[\frac{1}{2}\kappa (2J)^2 + \bar{\kappa} K + \sigma \right]$$

J: Mean curvature

K: Gaussian curvature

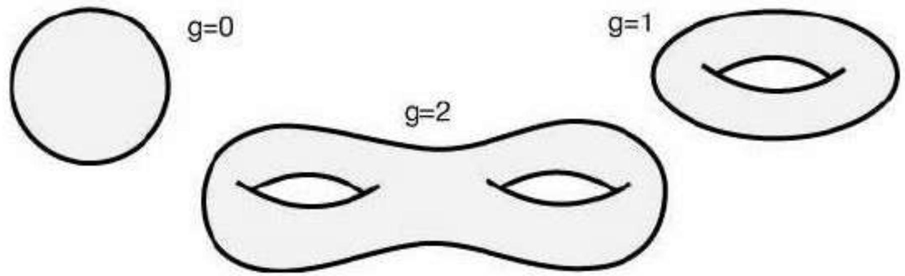
Gauss-Bonnet theorem for closed surfaces:

$$\oint \mathrm{K}\, \mathrm{d}A = 4\pi(1 - \mathrm{g})$$

g = number of "handles"
of the surface (genius)

sphere : g=0

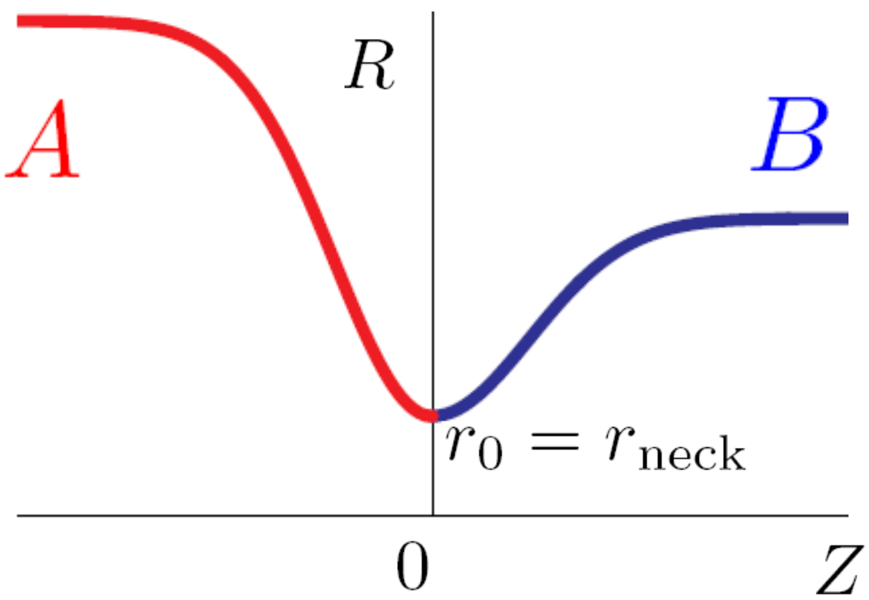
donut : g=1



total integrated Gaussian curvature = a *topological invariant*

For a tube with 2 phases A and B:

$$H = \int dA \left[\frac{1}{2} \kappa(z)(2J)^2 + \sigma(z) \right] - fL + 2\pi\gamma r_0 + F(\Delta\bar{\kappa})$$

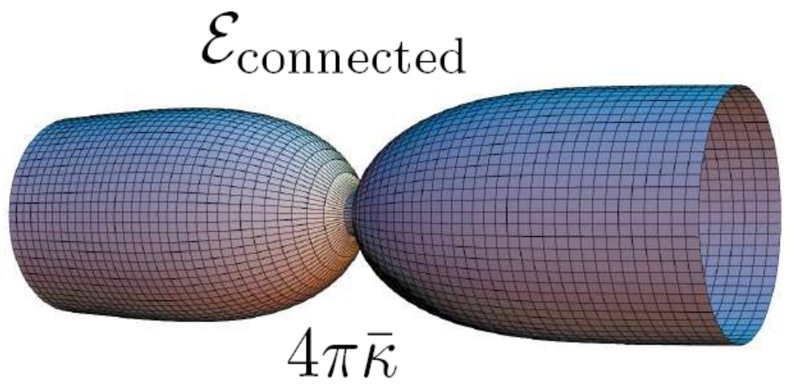


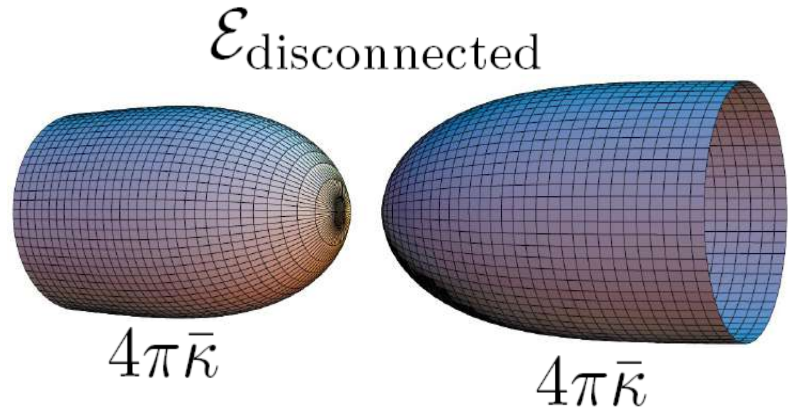
Line tension induces constriction (neck)

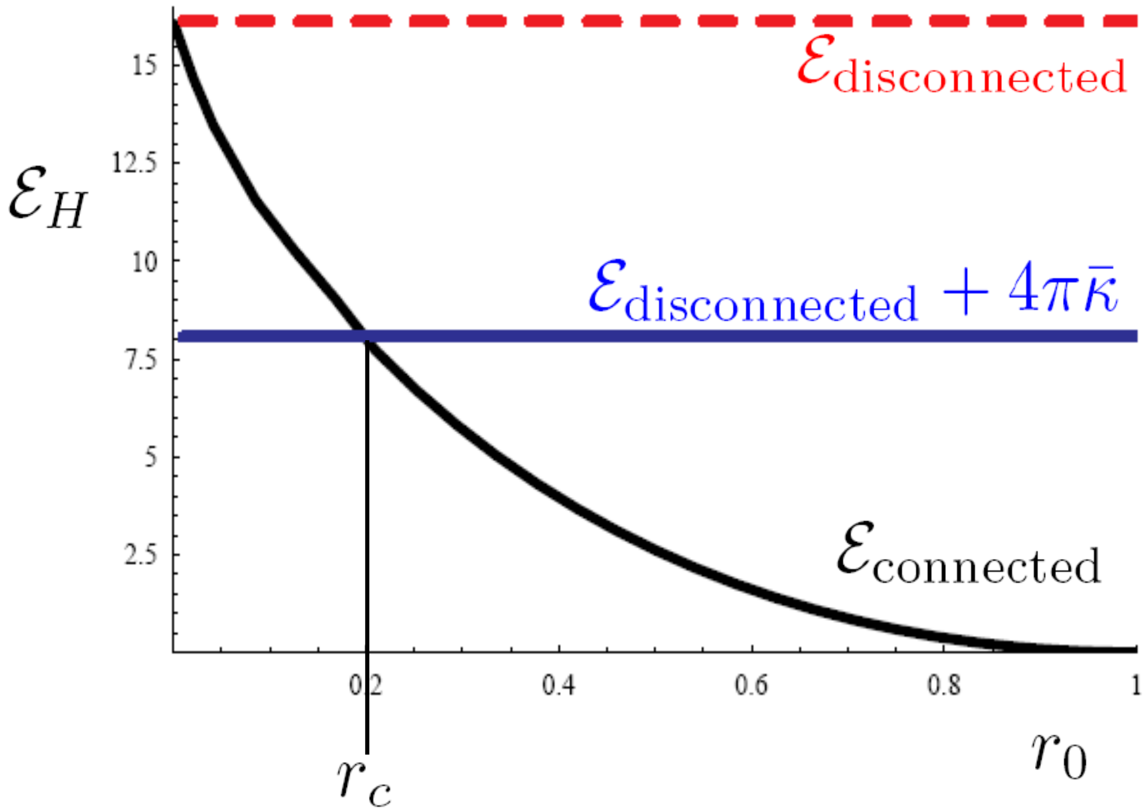
J.-M. Allain, K Storm …J. F. Joanny *PRL*.(2004)

Fission Energy Barrier

Topology changes upon fission



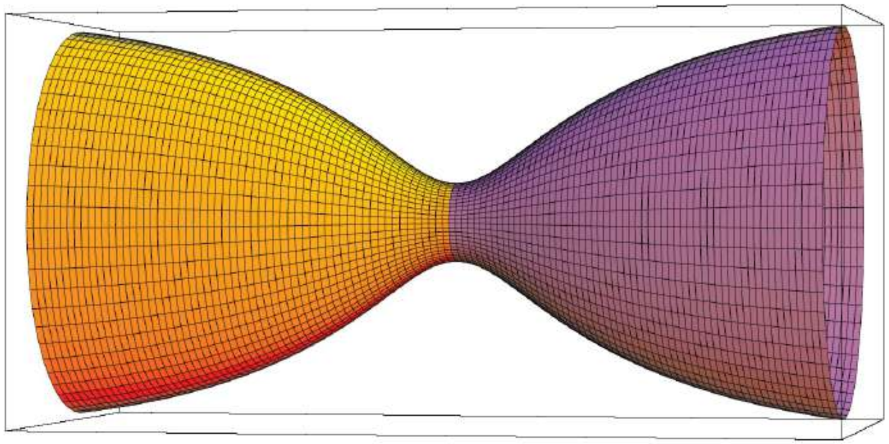




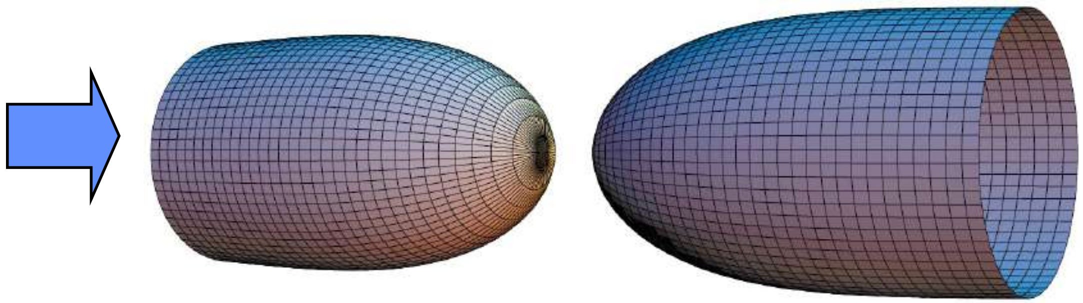
Fission at a *finite tube radius*
$r_c \neq 0$

J.-M. Allain, K Storm …J. F. Joanny *PRL*.(2004)

Line Tension and Fission

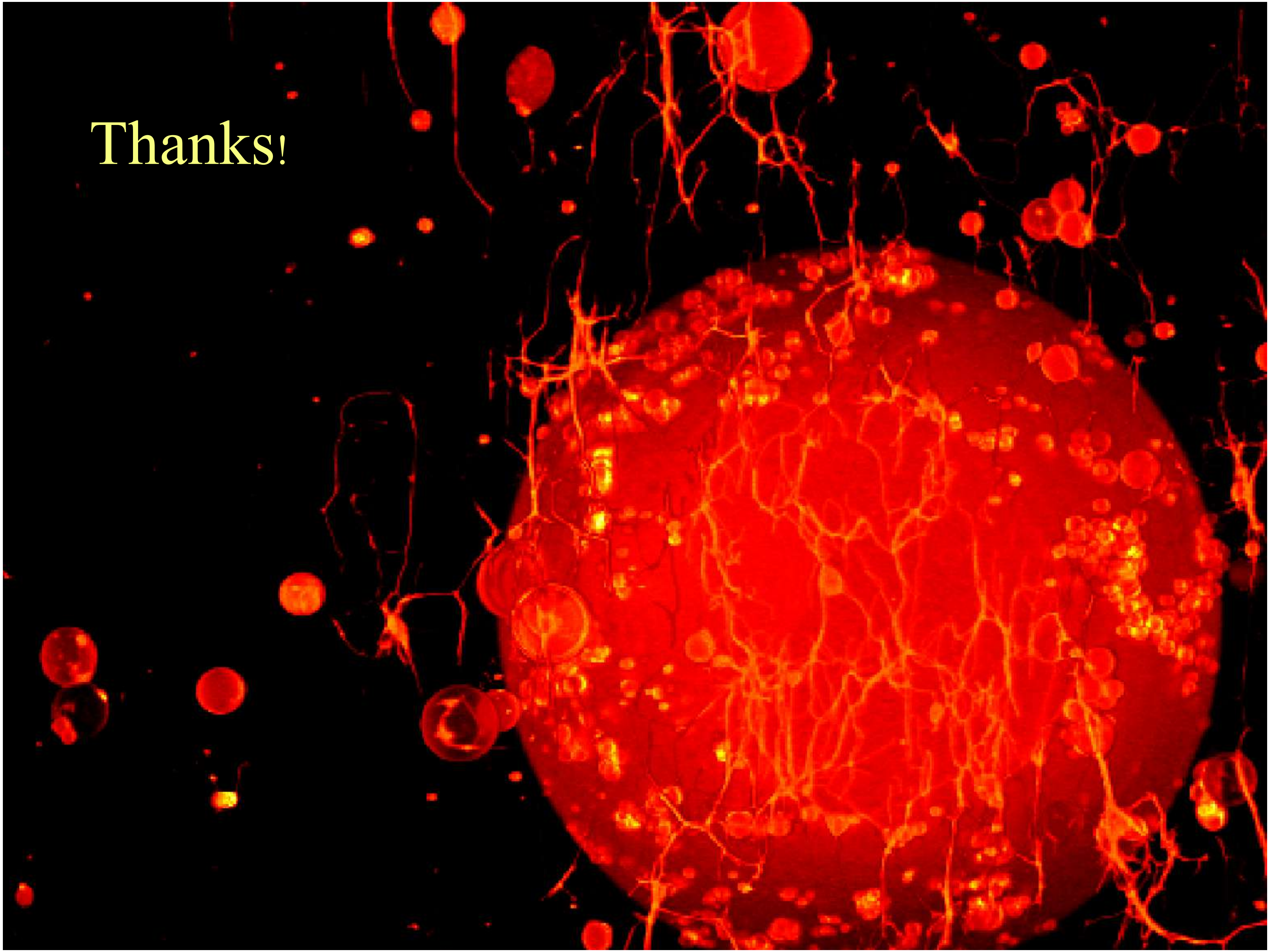


Line tension



J.-M. Allain et al *PRL*.(2004)

On a **tube** (≠vesicle): tension decreases tube radius
decreases energy barrier
thus **facilitates** fission


Thanks!