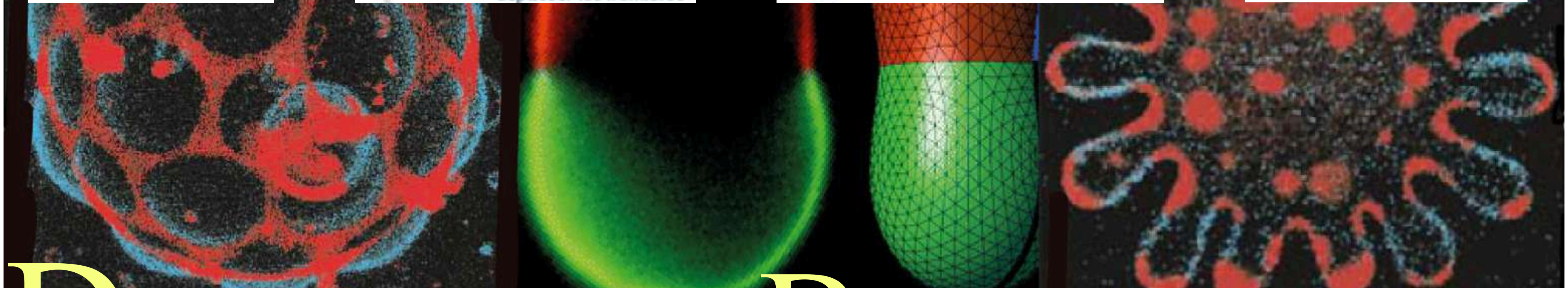
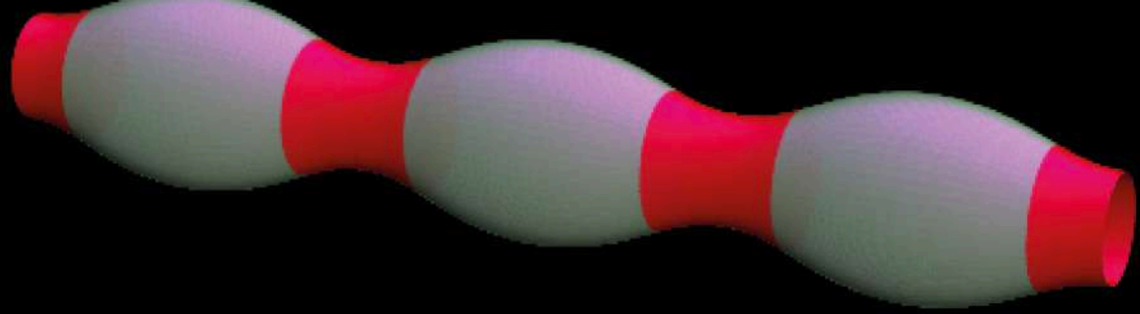
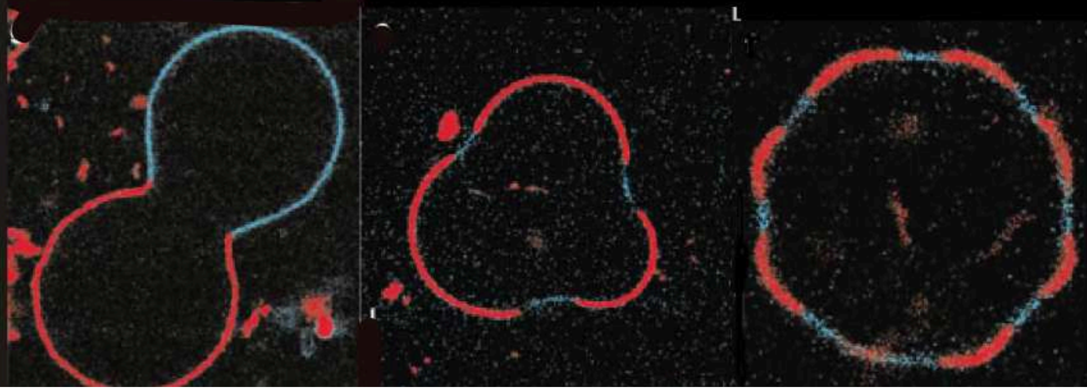


Domains and Biomembranes

Patricia Bassereau

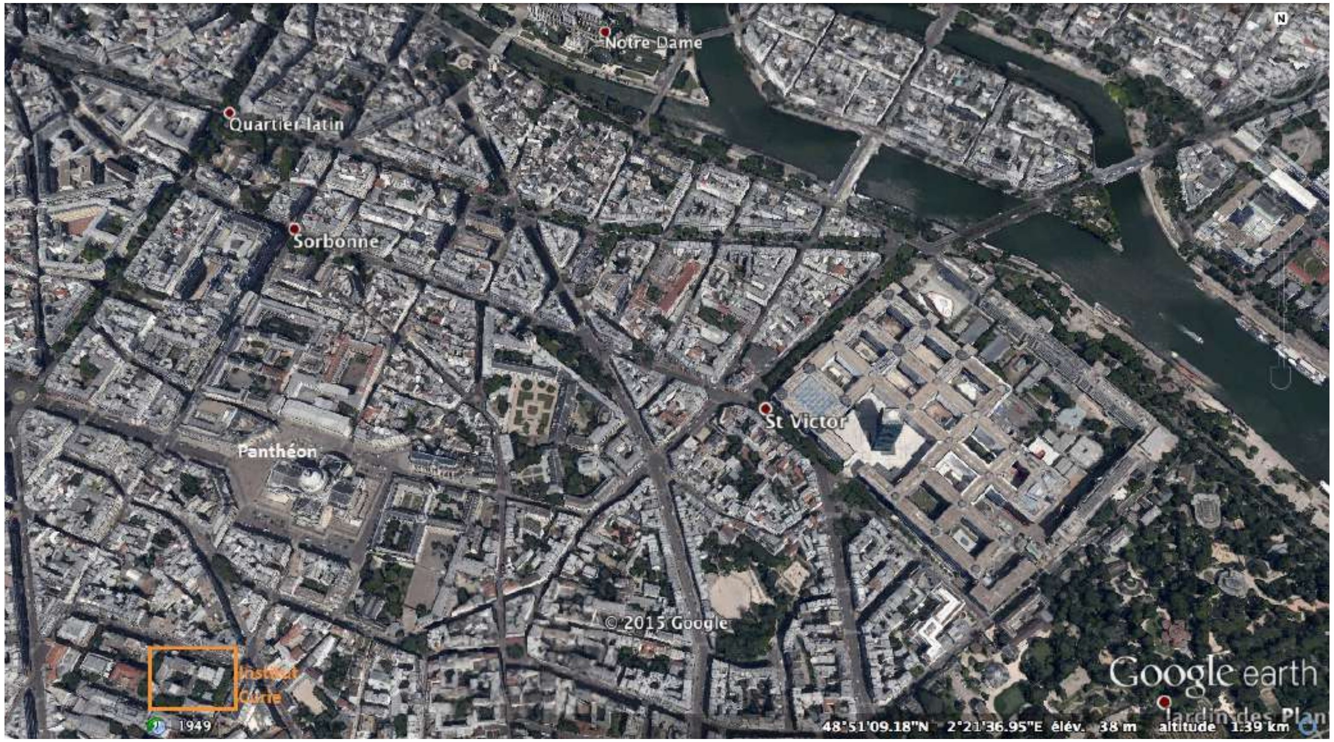
@ Institut Curie





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

Institut Curie, Paris 5e



Outline

Lecture 1: Phase separation in 2D-fluids: lipid bilayers

Lecture 2: Coupling between phase separation and membrane shape

Lecture 3: Protein clustering on membranes

Lecture 4: Active clustering due to actomyosin

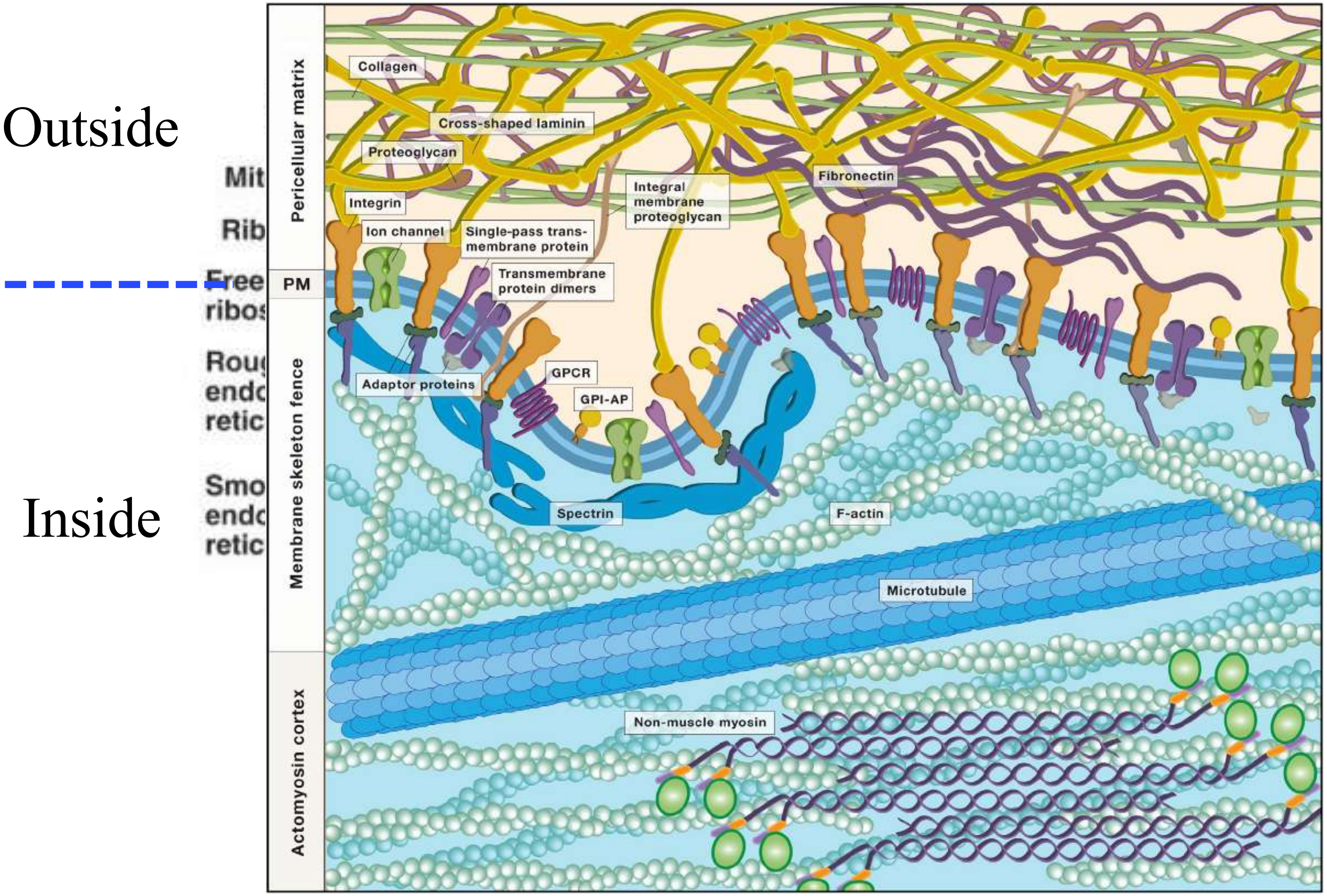
Lecture 5: Condensates interacting with membranes or forming on the membrane

Phase Separation in 2D-Fluids : Lipid Bilayers

Cell Envelop: the Plasma Membrane

Outside

Inside



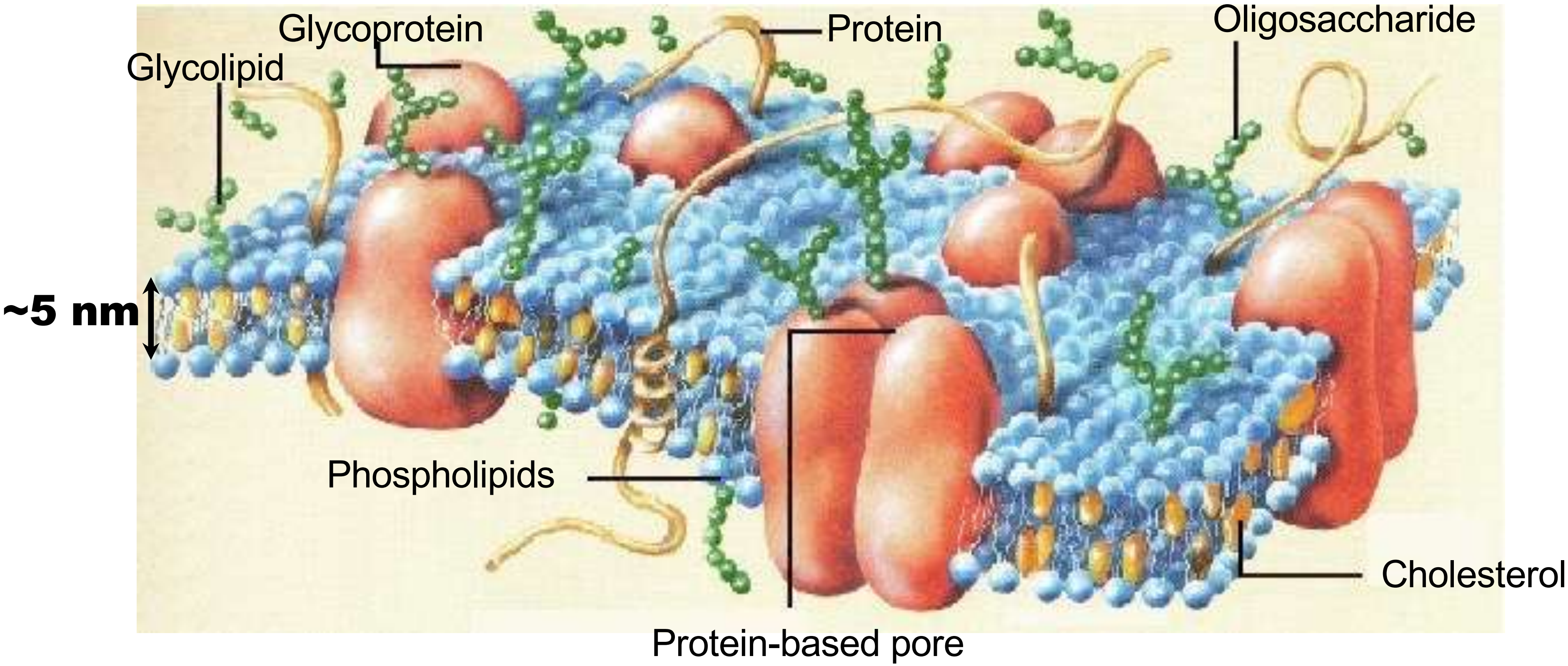
Lipid Membrane

~5 nm

K. Jacobson, ….B. Lagerholm, *Cell* (2019)

Lipid membrane reinforced by the actin cortex

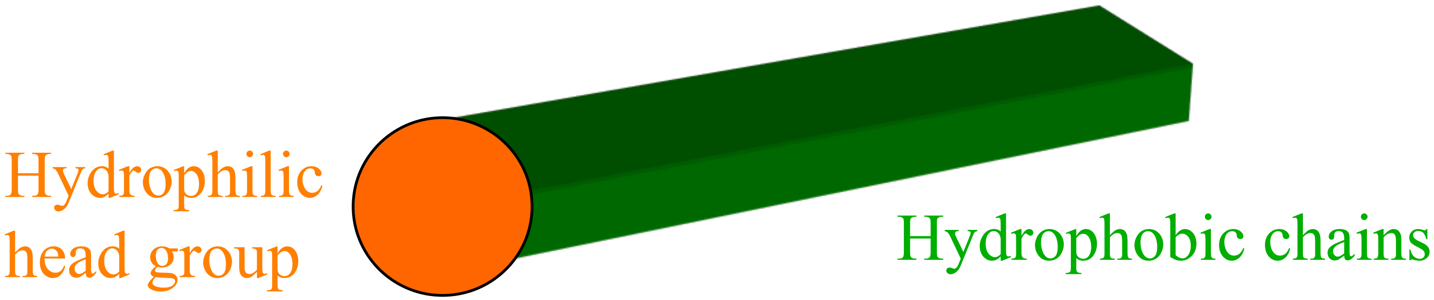
Plasma Membrane – Classical View

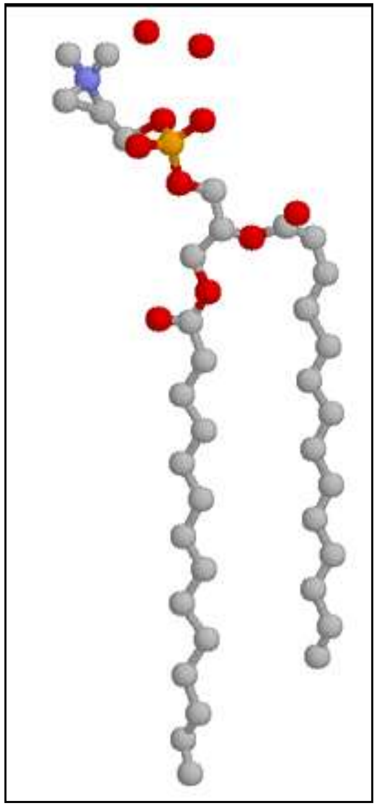


Singer & Nicholson model (1972) = "*fluid mosaic*"

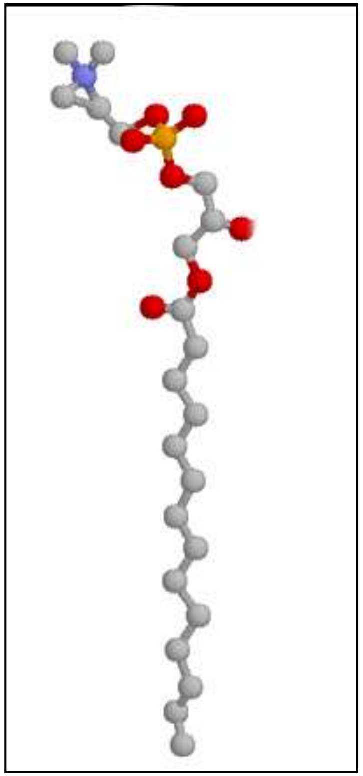
Fluid membrane containing lipids and membrane proteins

Lipids: Amphiphilic Molecules

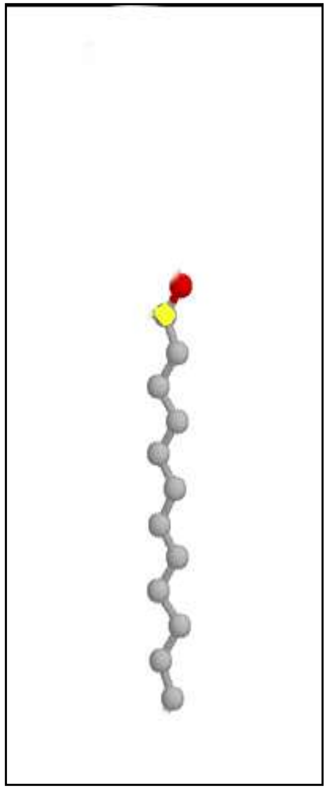




phosphatidyl choline

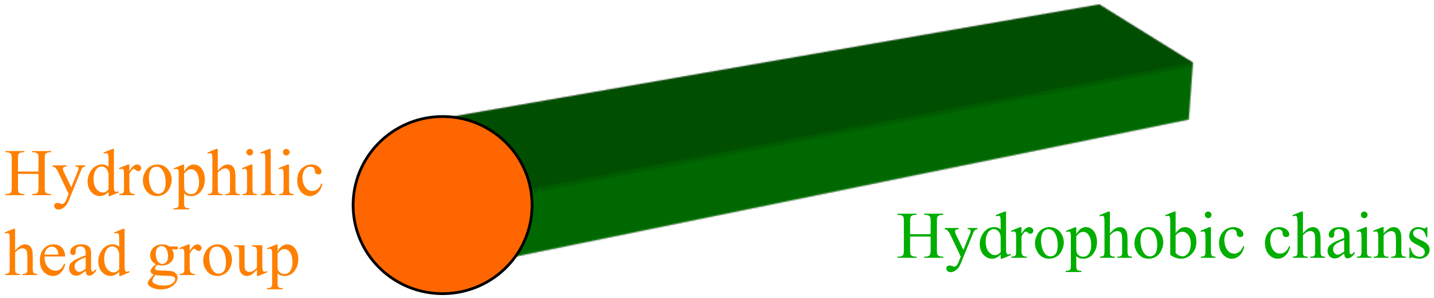


lyso- phosphatidylcholine

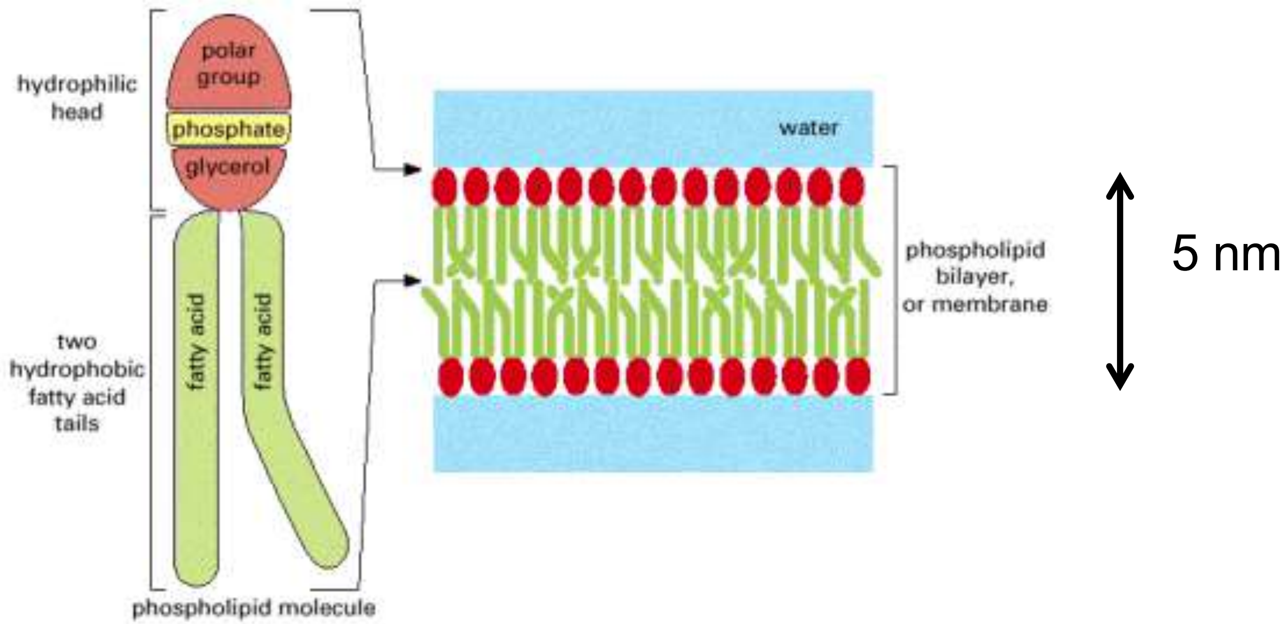


Dodecylsulphate (soap)

Membrane Basis: Lipid Bilayer



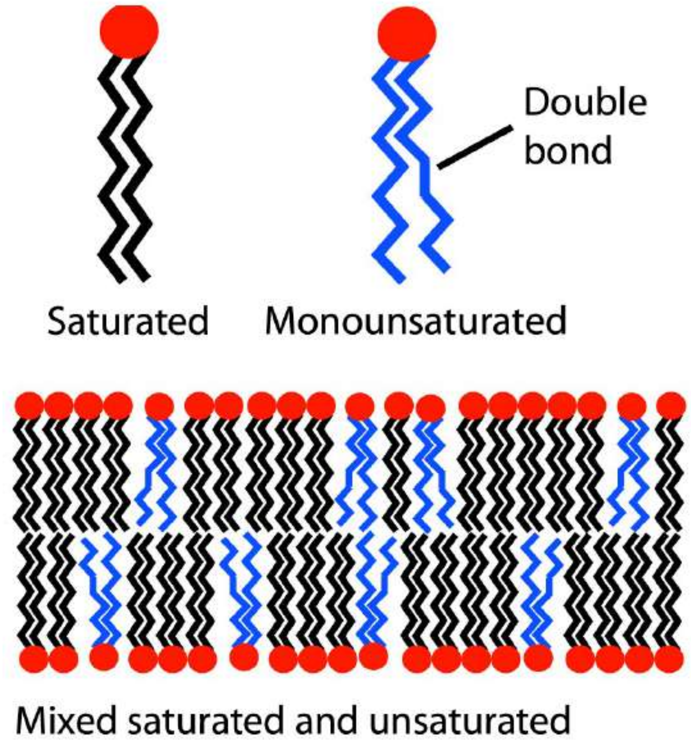
Lipid bilayer



Only self-assembly, no covalent bonds, entanglements

Membrane – Lipid Bilayer

Chains: fully saturated
or with unsaturations

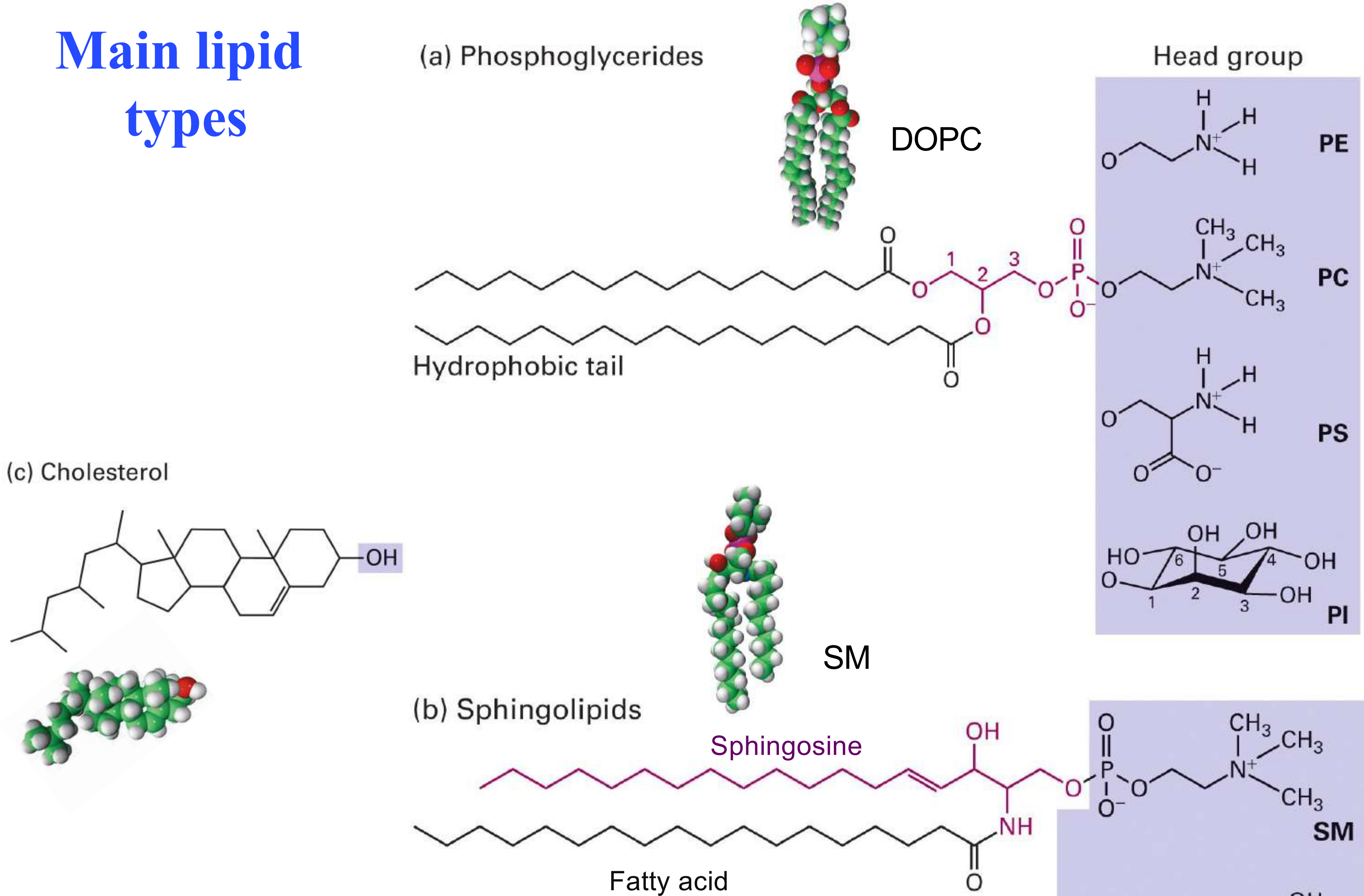


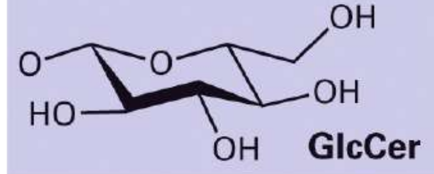
Commonly found in lipid membranes:

Saturated chains: C12 to C20

Unsaturated chains: C16:1, C18:1, C18:2, C18:3, C20:2,
polyunsat. C22, C24 etc…

Main lipid types



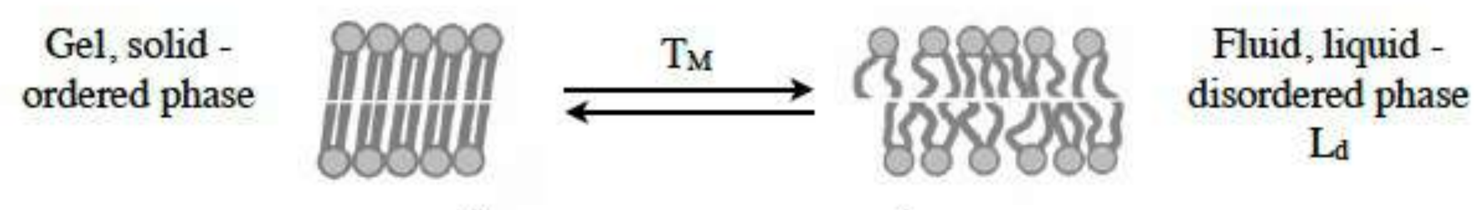


~a few 1000 lipid types. Why?

Strong development of lipidomics   Mass spectroscopy

A. Shevchenko, K. Simons, *Nat. Rev. Mol. Cell Biol.* **11**, 593 (2010)

Phase Transitions as a Function of Temperature



Melting

Melting Transition as a Function of Temperature

Saturated PC	Tm (°C)	1 unsaturation	Tm	2 unsat.	Tm	3 unsat.	Tm
12:0 PC	-2						
14:0 PC	24						
16:0 PC	41	16:1 PC	-36				
18:0 PC	55	18:1 PC	-17	18:2 PC	-57	18:3 PC	-60
20:0 PC	66						
22:0 PC	75	22:1 PC	13				
24:0 PC	80.3						

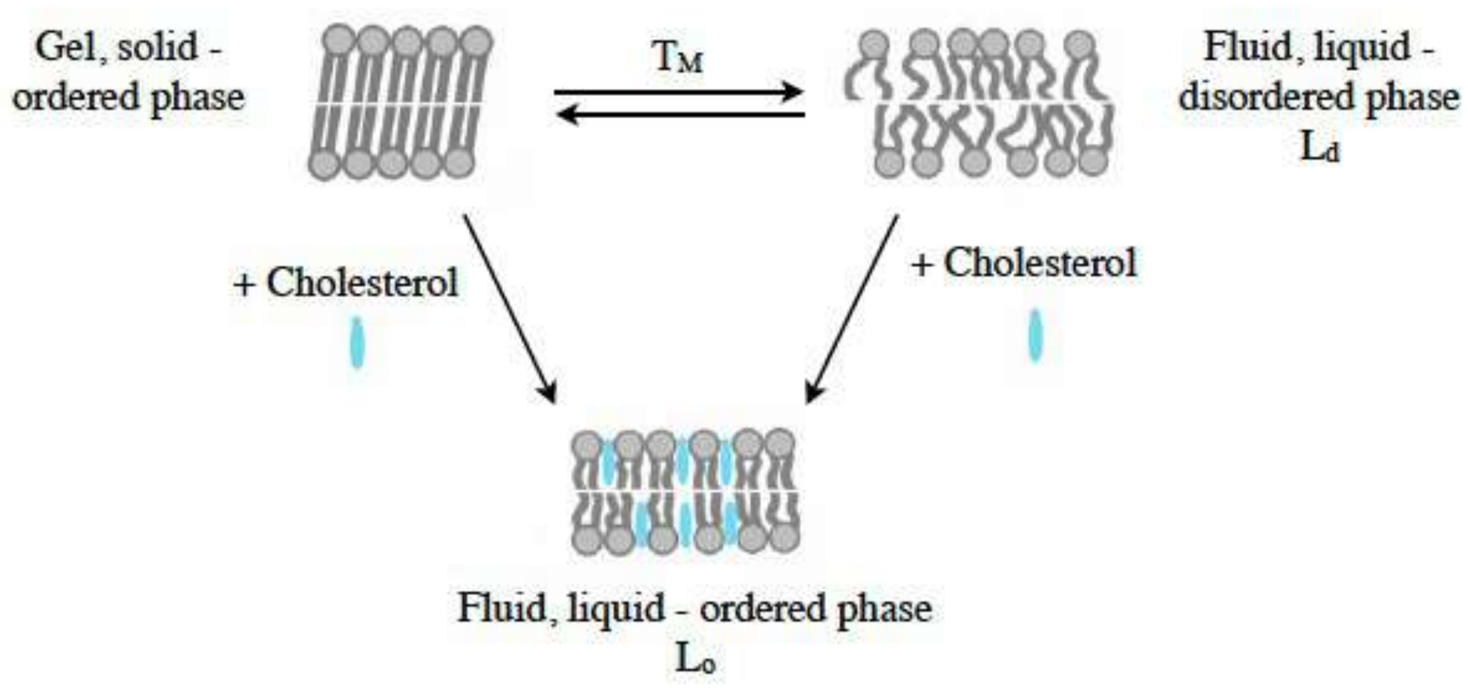
Melting temperature :

Increases with chain length

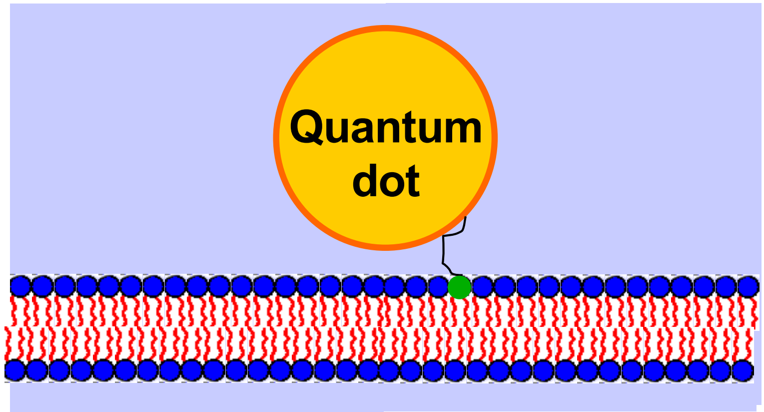
Decreases with unsaturation #

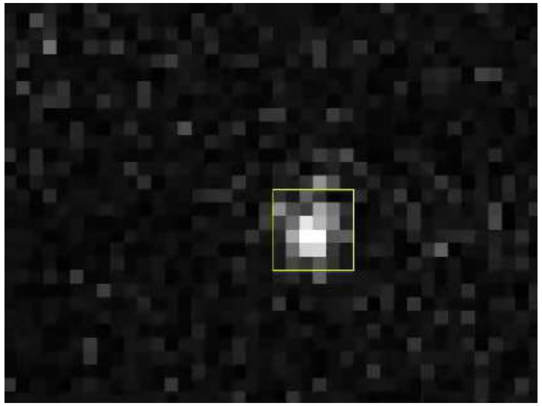
Lipid mixtures extracted from cells are FLUID

Effect of Cholesterol on Phase Transition



Membranes (above T_m) = 2-Dimensional Fluids

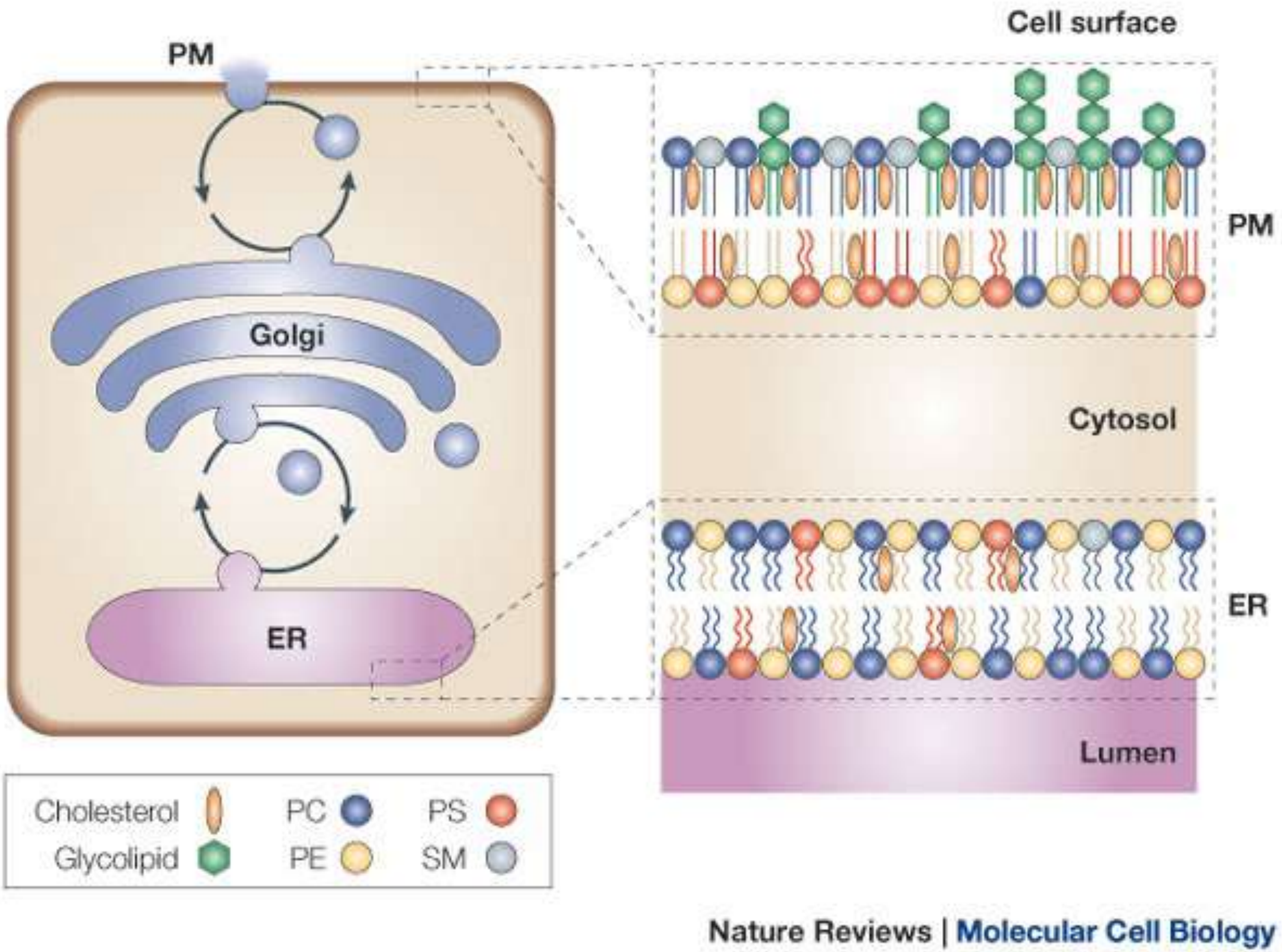




Single Particle Tracking

Lipid mixtures extracted from cells are FLUID

Lipid Composition Varies in Cell Membranes



Sphingolipids and cholesterol

Unsaturated lipids

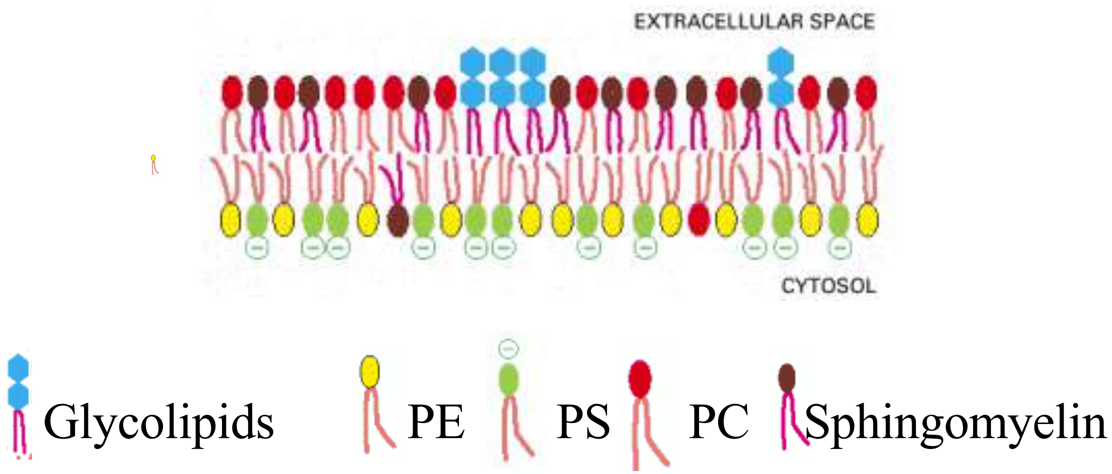
J. C. M. Holthuis, and T. P. Levine, *Nat. Rev. Mol. Cell Biol.* **6** (2005) 209

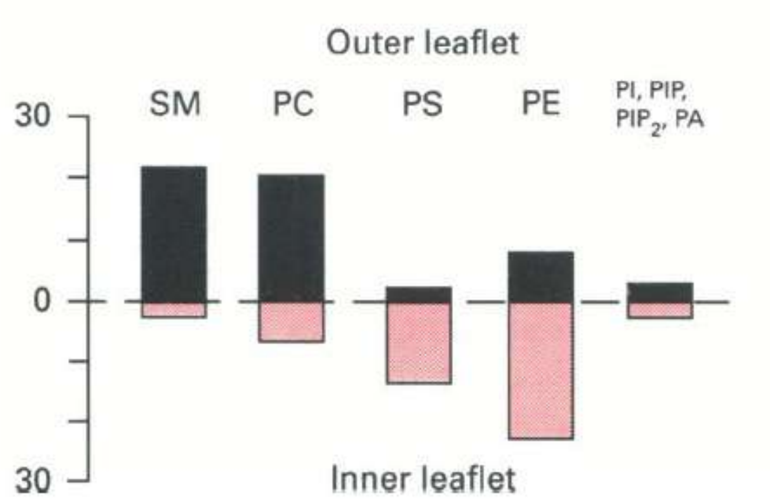
Non-Homogeneous Lipid Distribution

- Depends on organelles

- **Asymmetry**
  between leaflets

Red Blood Cell membrane (Alberts, 2002)





A. Zachowski, *Biochem. J.* **294**, 1 (1993)

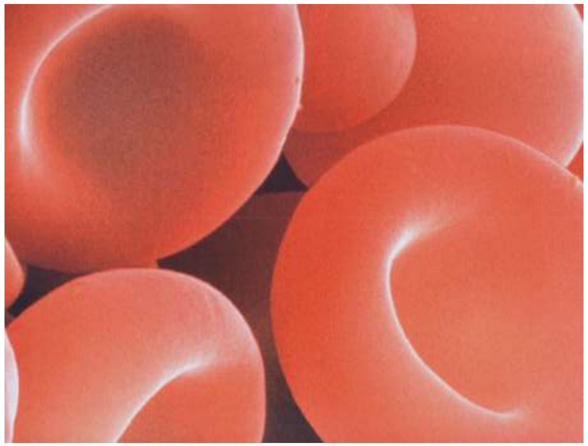
Some Model Membrane Systems to study Dynamics, Mechanics

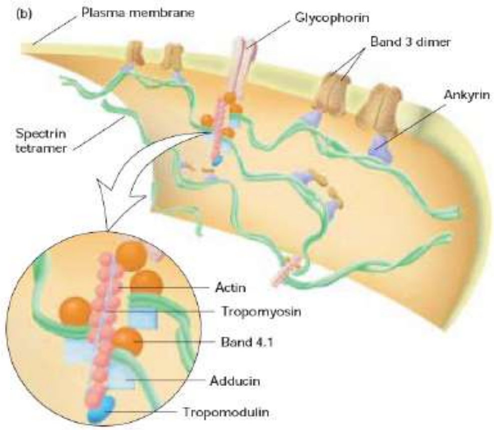
Objective: *understanding cell membranes*

W. Helfrich, E. Sackmann, E. Evans (>1973) etc…

Model membrane systems required to test theoretical models

1st model system available =Red Blood Cells



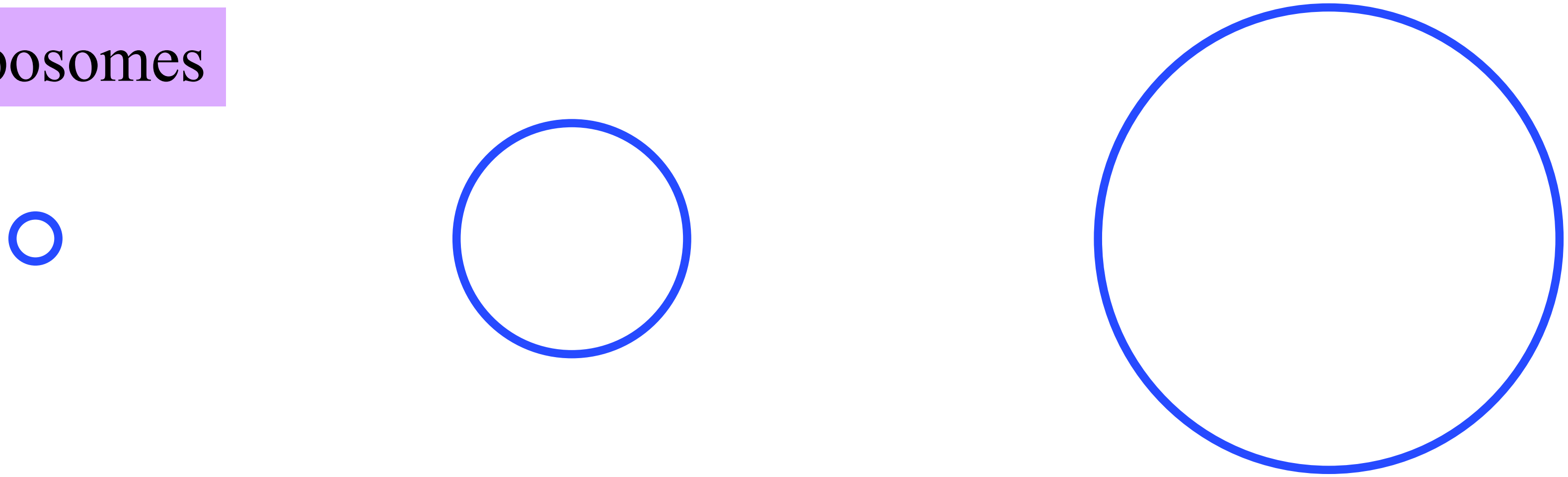


Still too complex…..

In the 80', first developments of giant liposomes with a single lipid type

Model Membranes

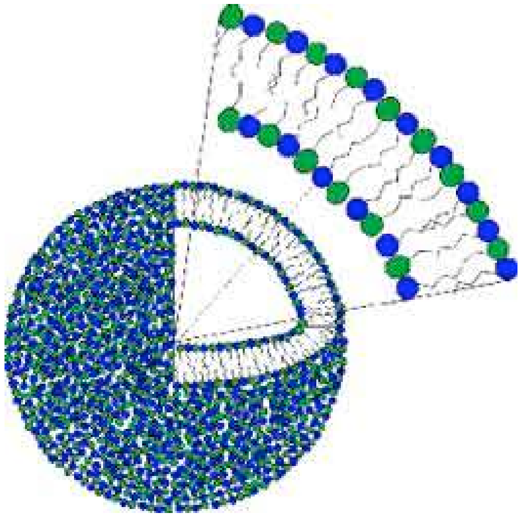
Liposomes



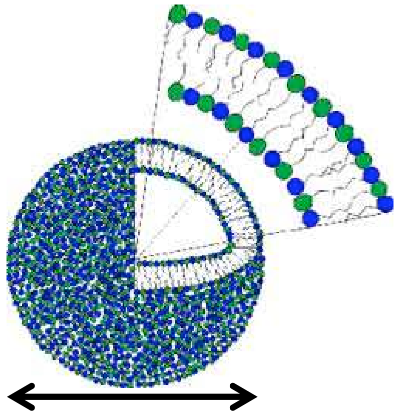
Small vesicles
(< 100 nm)

Large vesicles
(100 nm – 1 μm)

Giant vesicles
> a few μm - 100 μm)

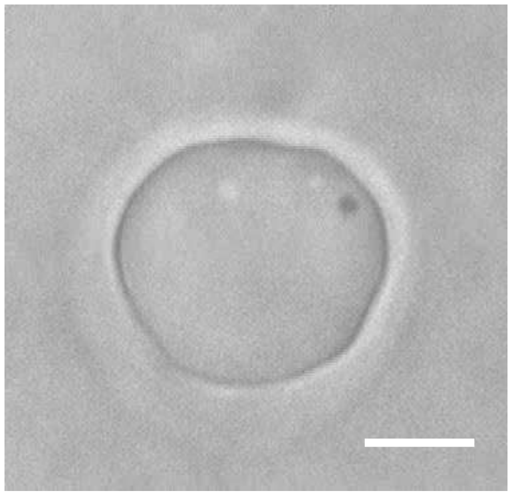


Some Technics to Study GUVs



≈ 10 µm and above

- Different media inside and outside the GUV (sugar, buffers + balance osmotic pressure



10 µm

Phase contrast microscopy

Measurements on a Single GUVs

Same methods as in cell biology

Perfect with *optical microscopy*

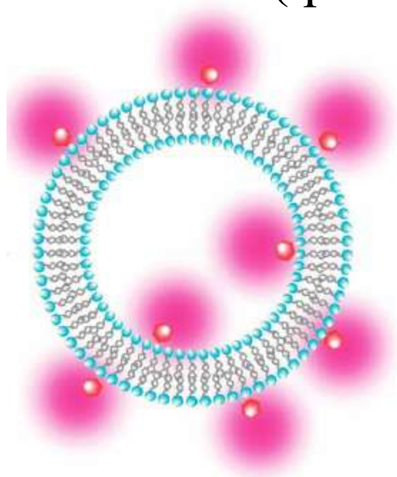
But a bilayer:

n=1.38-1.42 , e= 5nm, in water (n=1.33)….

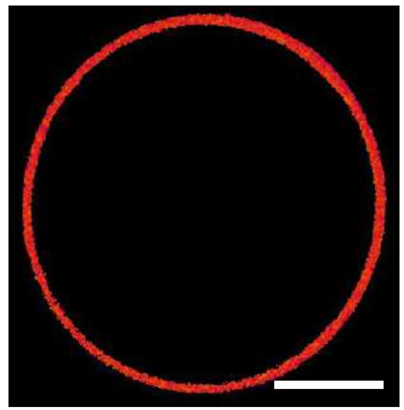
→ Increases the contrast

- Add a small fraction of fluorescent molecules (a few 0.1 % fluorescent lipids)

Use CONFOCAL microscopy (quantitative measurements)



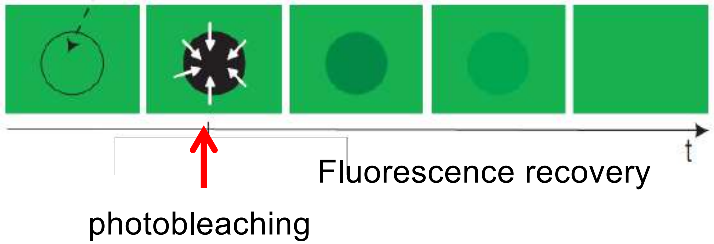
From: H. Robson Marsden et al, *Chem. Soc. Rev.*, (2011)

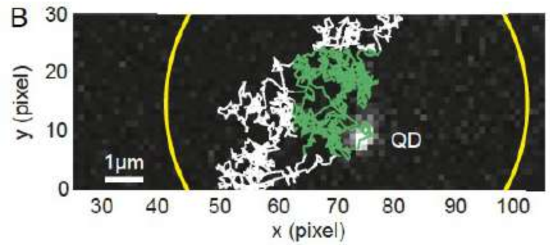


10 µm

- Optics-based method to measure 2D viscosity - Brownian motion

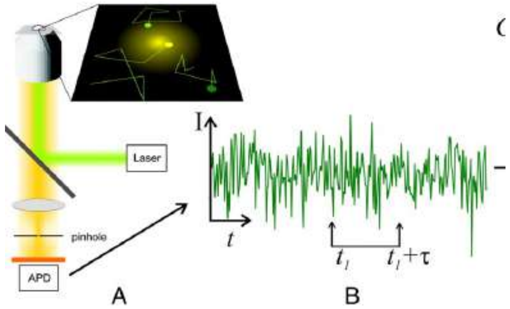
FRAP



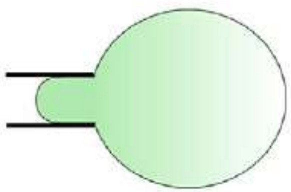


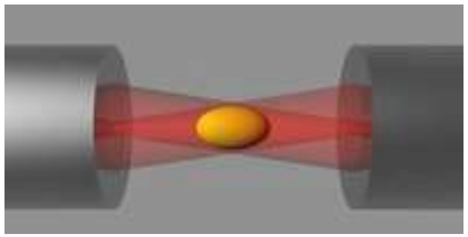
Single Particle Tracking

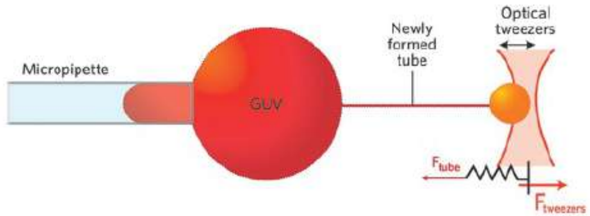
Fluorescence Correlation
Spectroscopy (FCS)



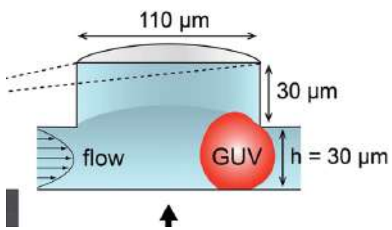
- Micromanipulation: micropipettes, optical tweezers

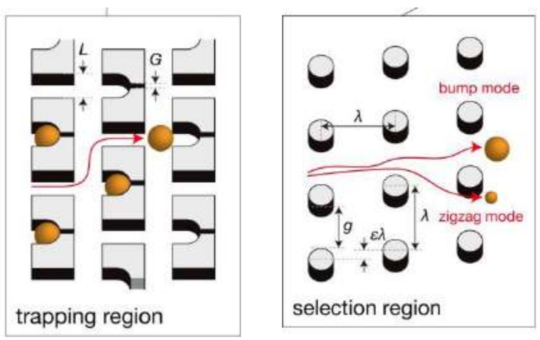






- Microfluidics

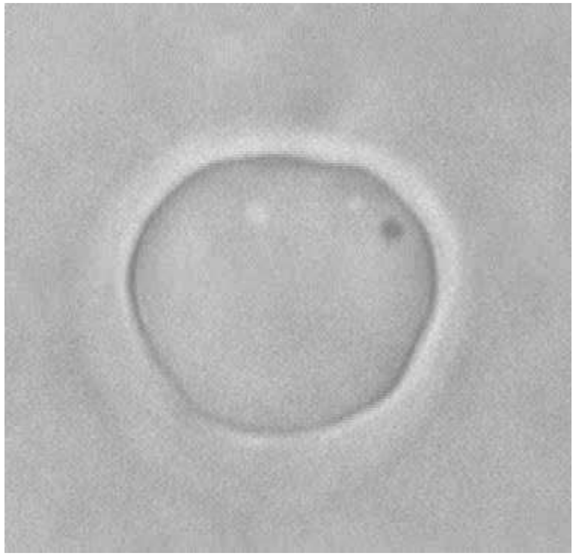




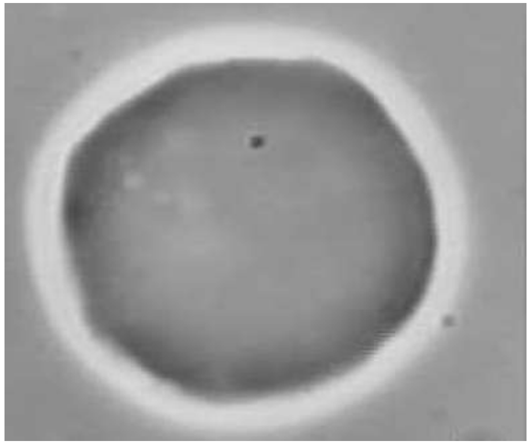
Lipid Membrane Mechanics

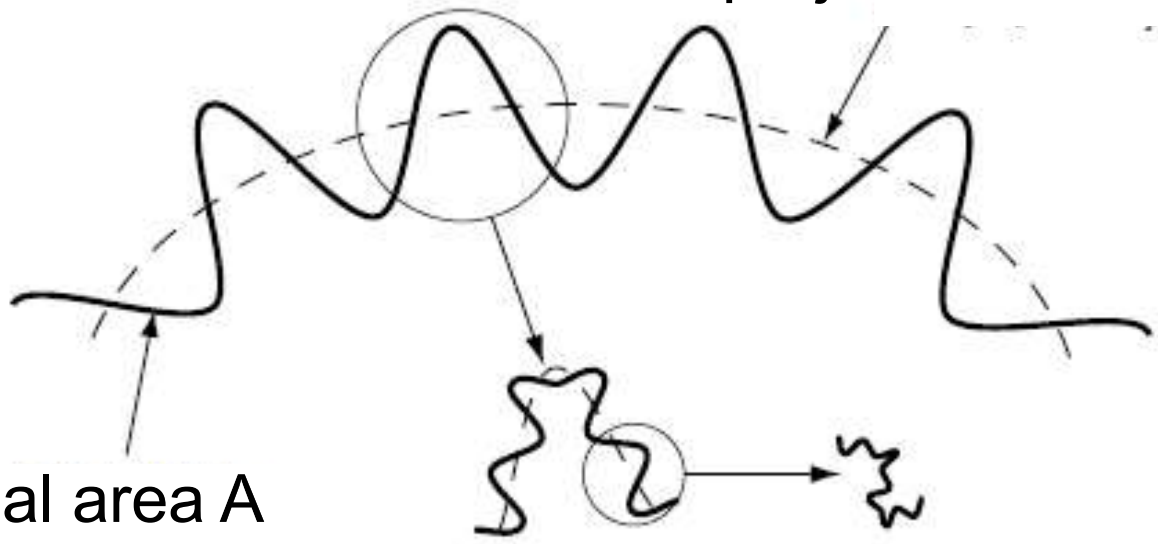
Membrane Tension

GUV Membrane can be Stretched



Changing tension by changing the osmotic pressure



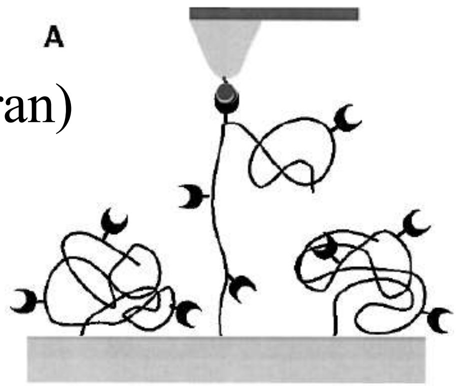


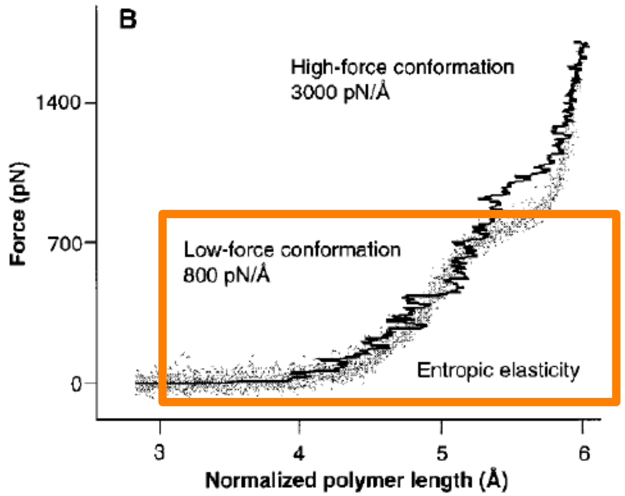
Excess area: $\Delta A = A - A_p$ Surface stored in fluctuations

Relative excess area: $$\boxed{\alpha = \frac{\Delta A}{A_p}}$$

Analogy with Polymers: Entropic Elasticity

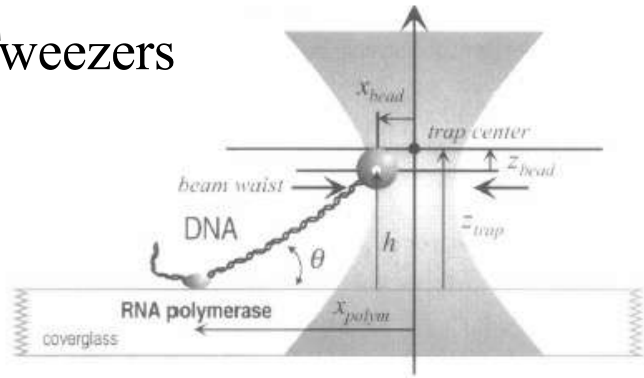
AFM on a single Polysaccharide (dextran) chain



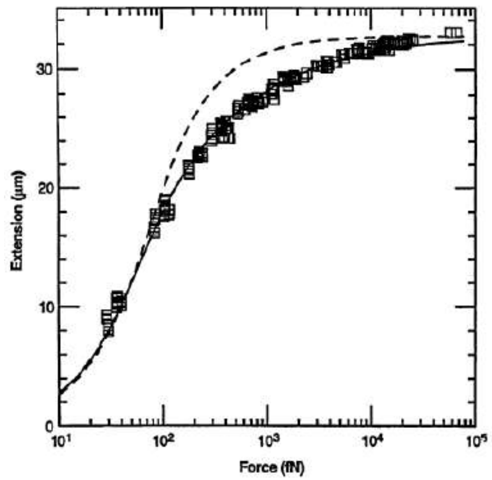


M. Rief... Gaub, *Science* **275**, 1295 (1997)

Optical Tweezers on DNA



M. D. Wang...S. M. Block, *Biophys. J.* **72**, 1335 (1997)



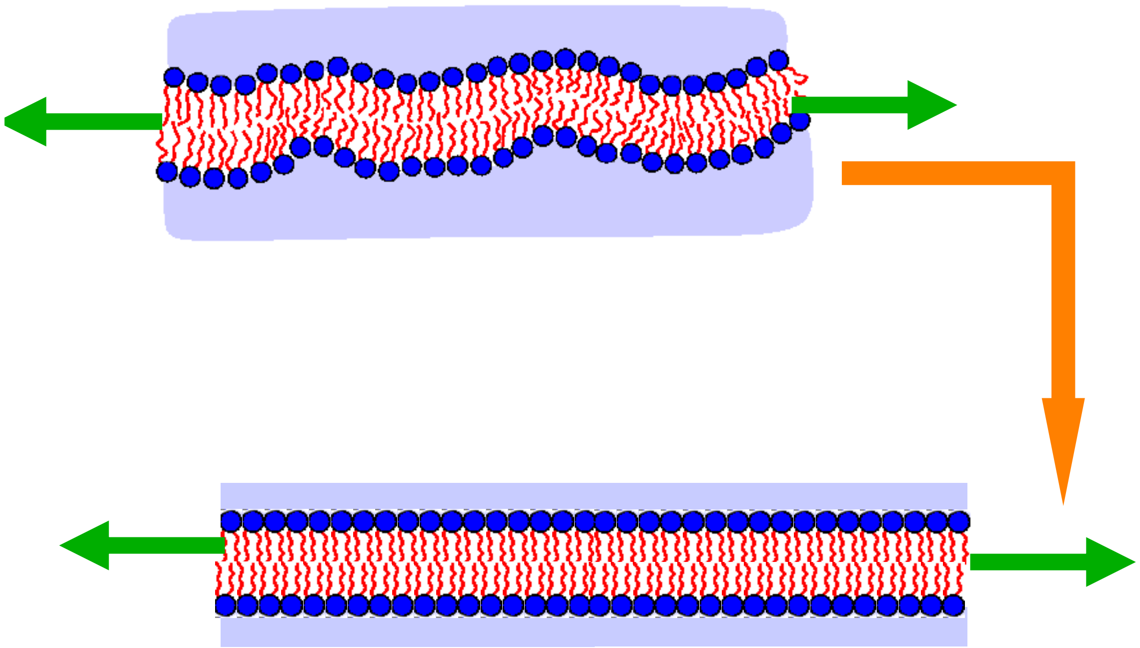
C. Bustamante et al, *Science* **265**, 1599 (1994)

J. F. Marko, E. D. Siggia, *Macromolecules* **28**, 8759 (1995)

Worm-like chain model: connects *bending*/molecule to *elasticity*

Non-linear relation between Force and Extension (for semi-flexible)

Lipid Membrane Elasticity



Free-energy for membrane stretching H_s:

$$\sigma = \frac{\partial H_s}{\partial A}$$

A: membrane area

σ: membrane tension

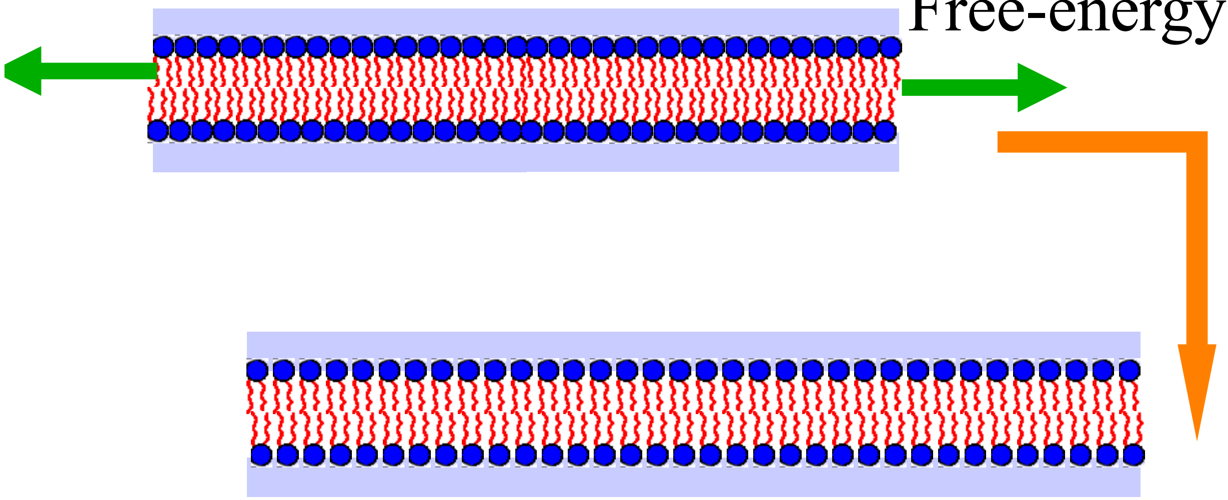
(in Joules/m^2 or N/m)

1) Entropic regime

$$\sigma = \sigma_0 \exp\left(\frac{8\pi\kappa}{k_B T}\frac{\Delta A}{A}\right)$$

Depends on *bending rigidity κ*

Lipid Membrane Elasticity



Free-energy for membrane stretching H_s:

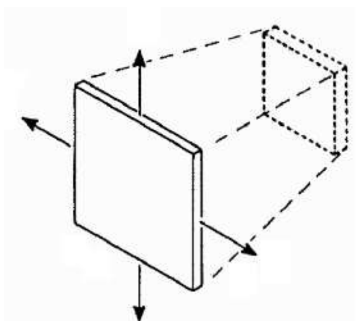
$$\sigma = \frac{\partial H_s}{\partial A}$$

A: membrane area

σ: membrane tension

(in Joules/m^2 or N/m)

2) Elastic regime



$$\sigma = K_a \frac{\Delta A}{A}$$

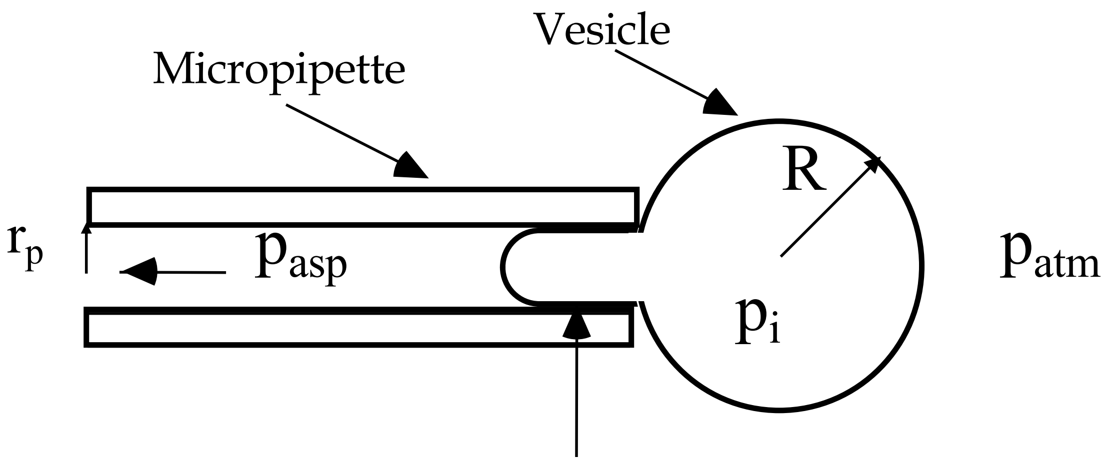
K_a: stretching modulus

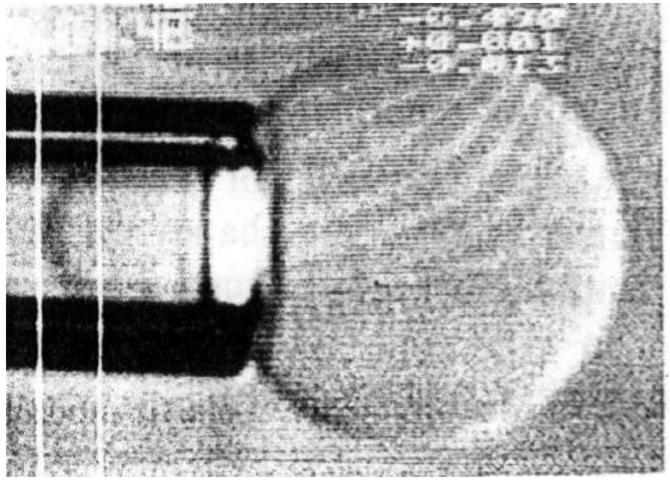
Cf. Hook's law (spring)

Micropipette Aspiration

Evan Evans

UBC, Vancouver

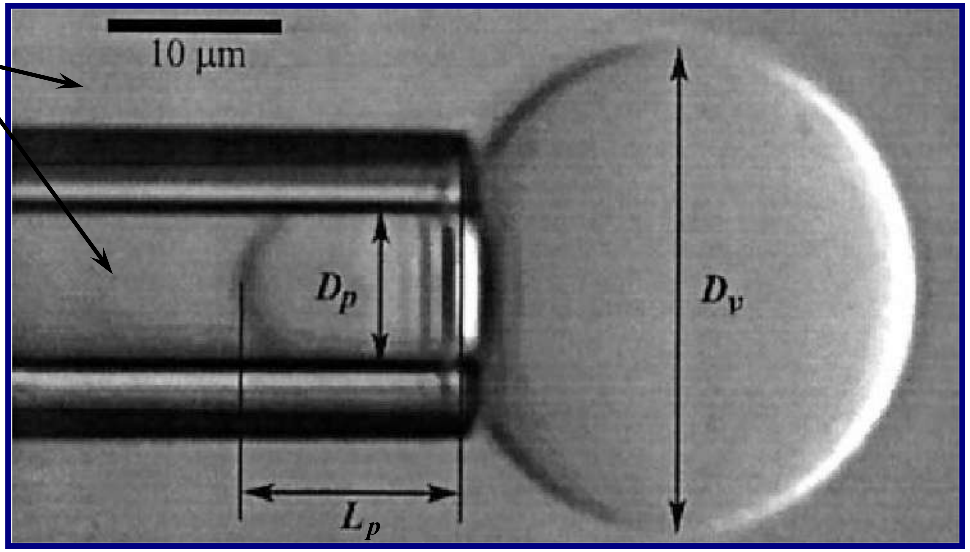




(Kwok et Evans, 1981)

Tension Measurement

ΔP



Calculated from

Laplace's law

Interface pipette/vesicle $$P_i - P_{asp} = \frac{4\sigma}{D_p}$$

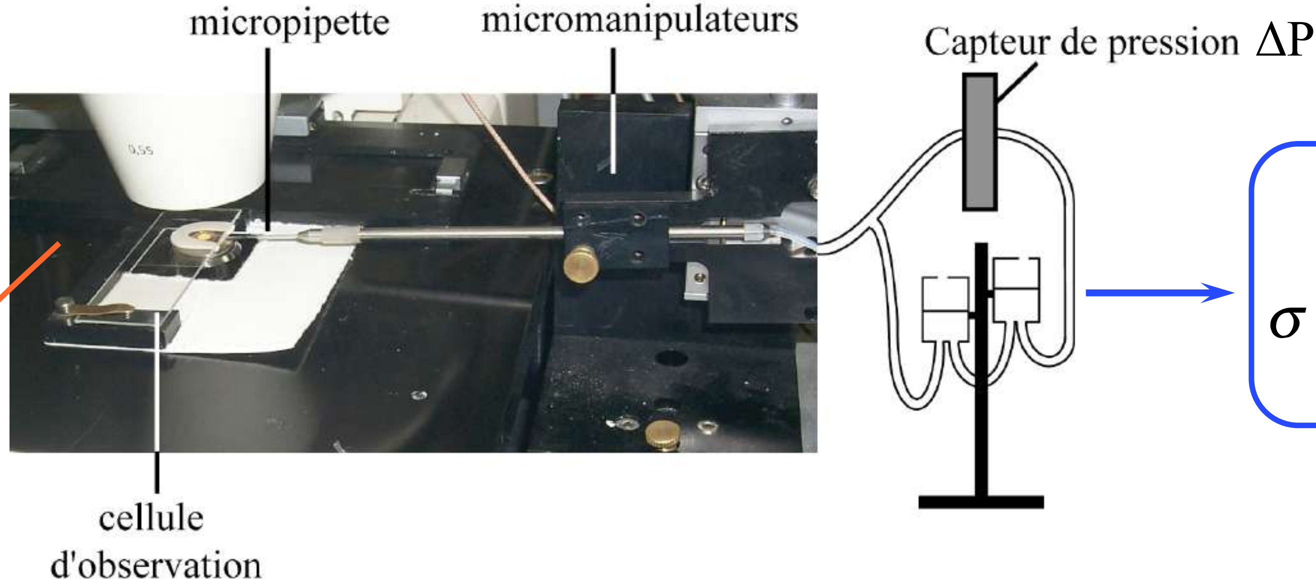
Interface vesicle/bulk $$P_i - P_{atm} = \frac{4\sigma}{D_v}$$

Homogeneous tension , thus:

$$\Delta P = P_{atm} - P_{asp}$$

$$\sigma = \frac{D_p}{4(1 - D_p / D_v)} \Delta P$$

Micropipette Aspiration (Kwok et Evans, 1981)



Tension:

$$\sigma = \frac{D_p}{4(1 - D_p / D_v)} \Delta P$$

DIC

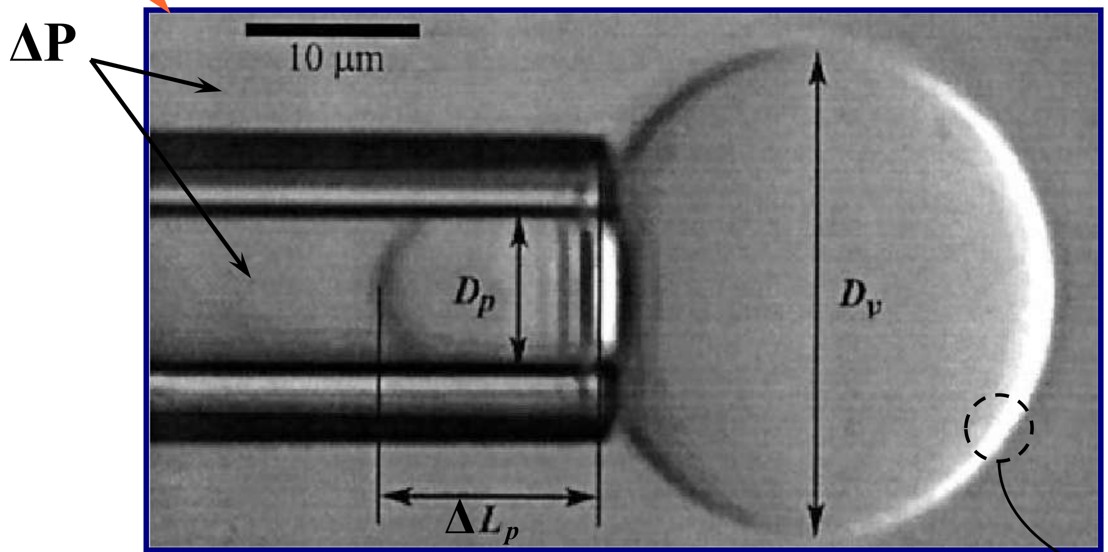
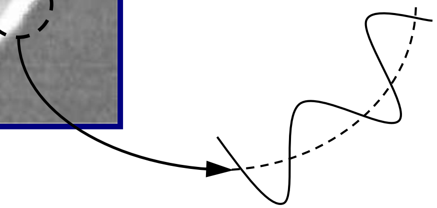
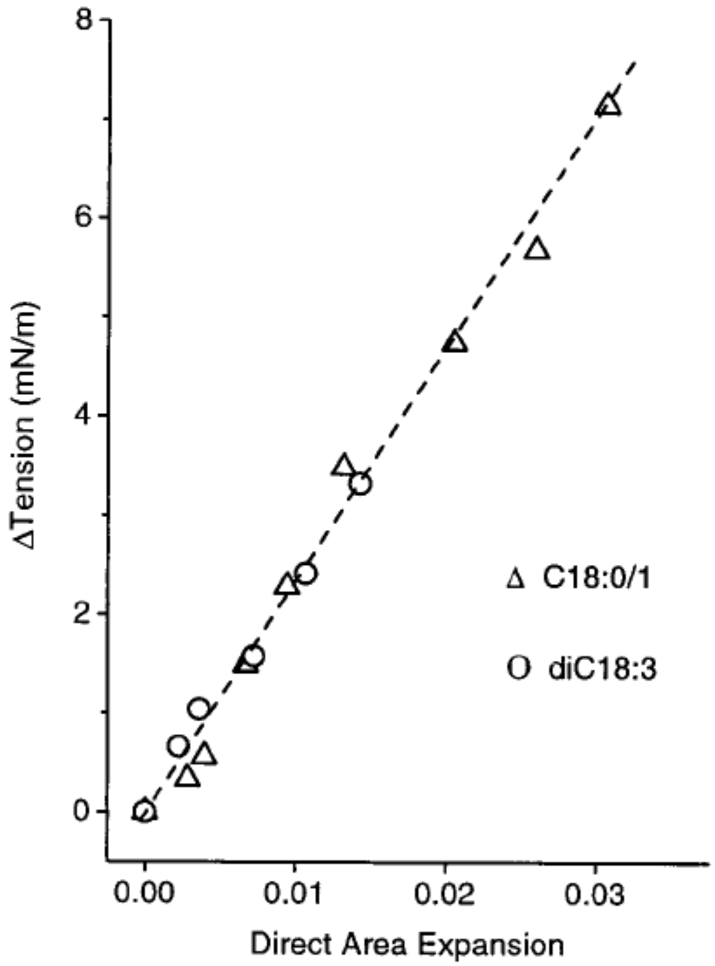


Image analysis

Excess area:

$$\Delta\alpha = \frac{(D_p/D_v)^2 - (D_p/D_v)^3}{D_p} L_p$$





$$\sigma - \sigma_0 = K_a \Delta\alpha$$

W. Rawicz …E. Evans *Biophys. J.* **79**, 328-339 (2000)

Stretching Modulus K_a Depends on Lipid Composition

PC lipids: *insensitive* to chain length
unsaturations (≈ 250 mN/m)

Increases with cholesterol
(x 3-4 with 50% chol)

Even larger with Sphingolipids

W. Rawicz …E. Evans *Biophys. J.* **79**, 328-339 (2000)

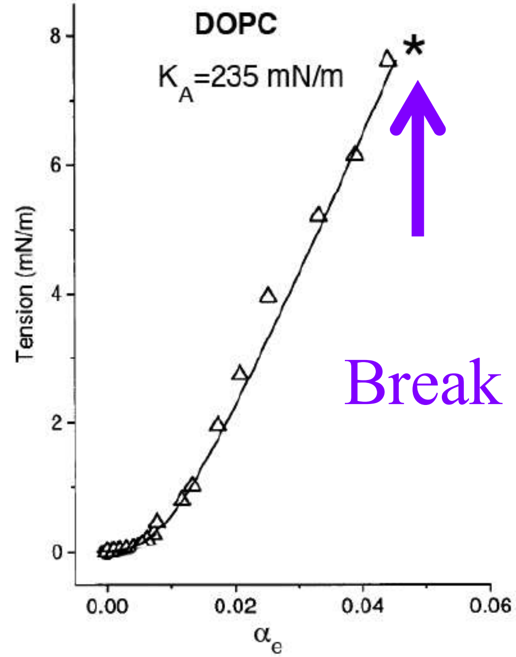
W. Rawicz .. E. Evans *BJ* **94**, 4725-4736 (2008)

Lipid membranes:

Hard to stretch

(≈ 5 times harder/polyethylene)

Lipid Membranes: Fragile upon Stretching



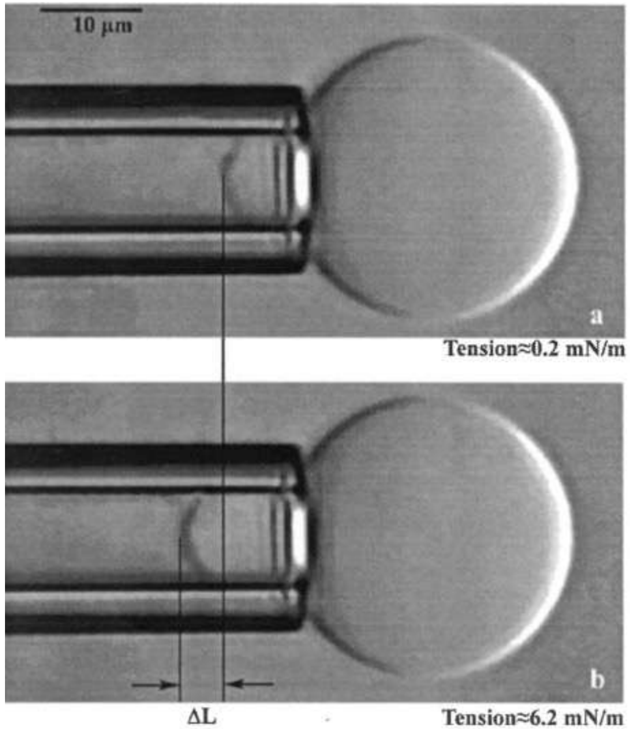
Strain limited to ≈ 5%

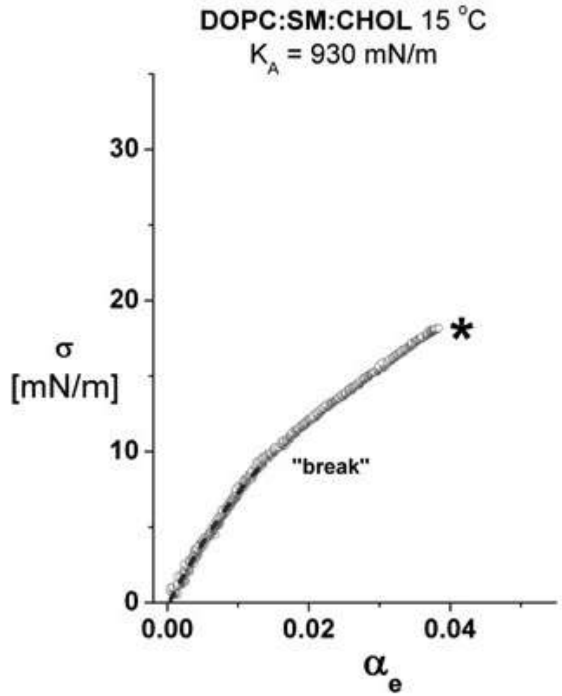
Break

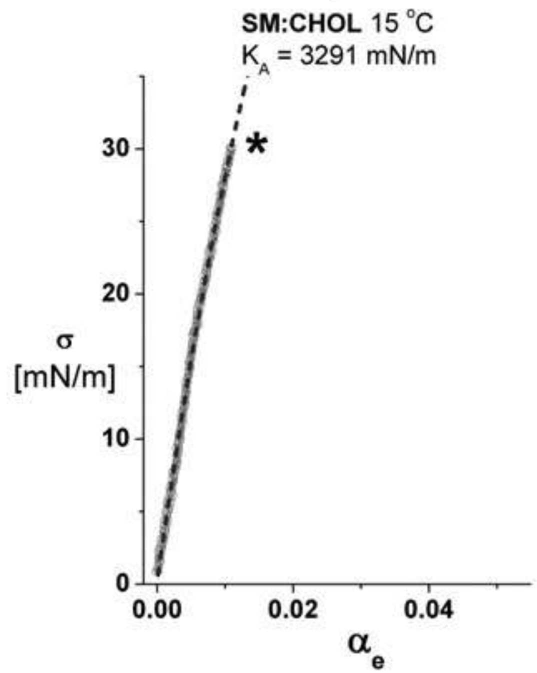
E. Evans

W. Rawicz, et al *Biophys. J.* **79**, 328 (2000)

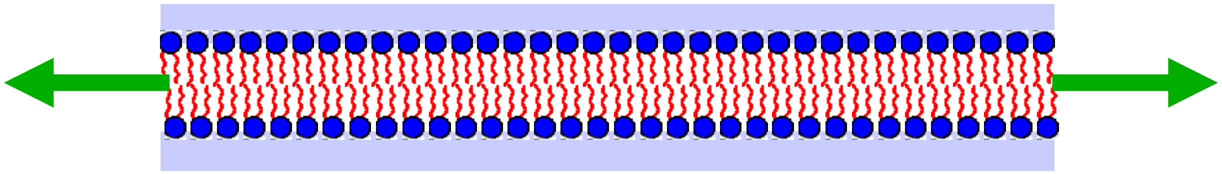
W. Rawicz et al *Biophys. J.* **94**, 4725 (2008)

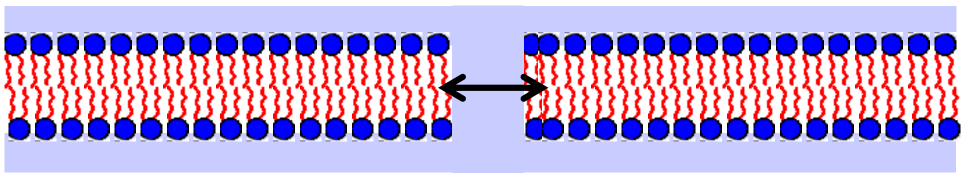






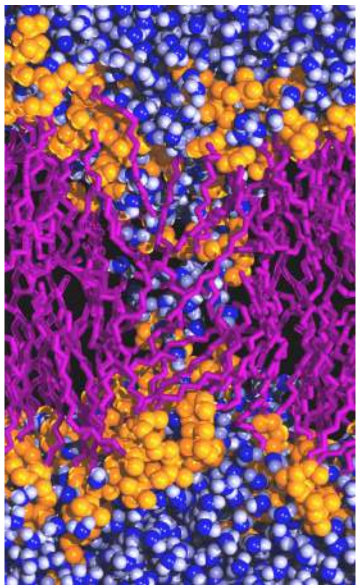
High Tension: Membrane Lysis





$$\textit{Max Strain}\ \ \Delta\alpha \approx 5\%$$

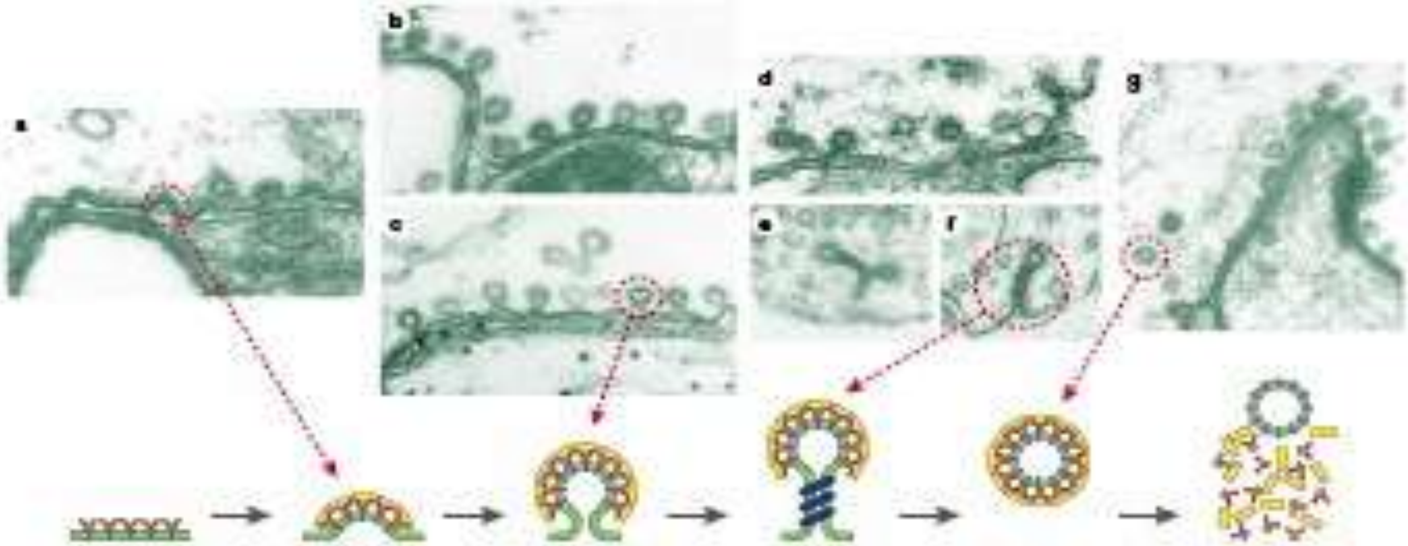
Numerical simulations



Membrane Trafficking Requires Membrane “Shaping” and Bending

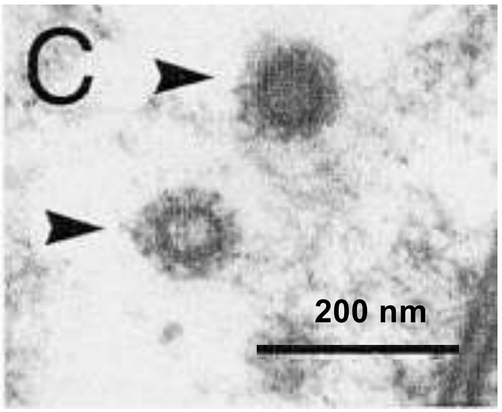
Traffic Requires to Process Highly Curved Membrane Structures

Vesicles of diameter <100 nm

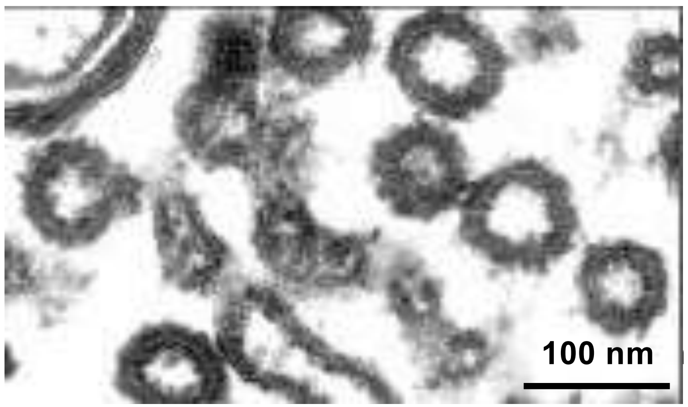


Gad et al
Neuron **27**, 301 (2000)

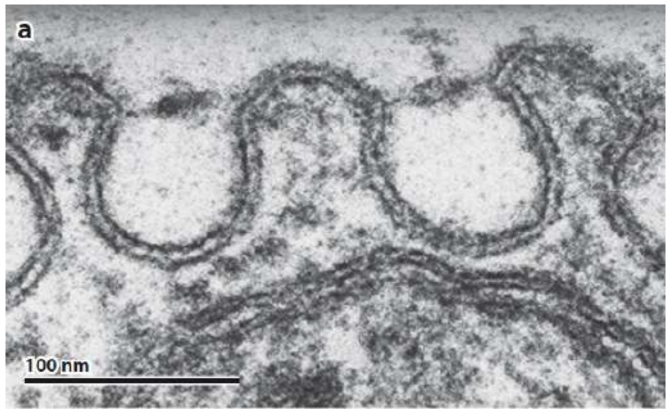
Clathrin vesicles



COPI vesicles in vitro



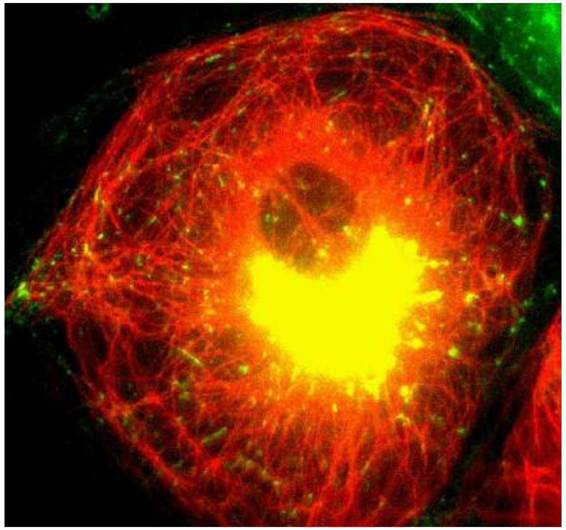
Caveolae



… and Many Tubulated Structures

Narrow tubes (usually $\Phi < 100$ nm)

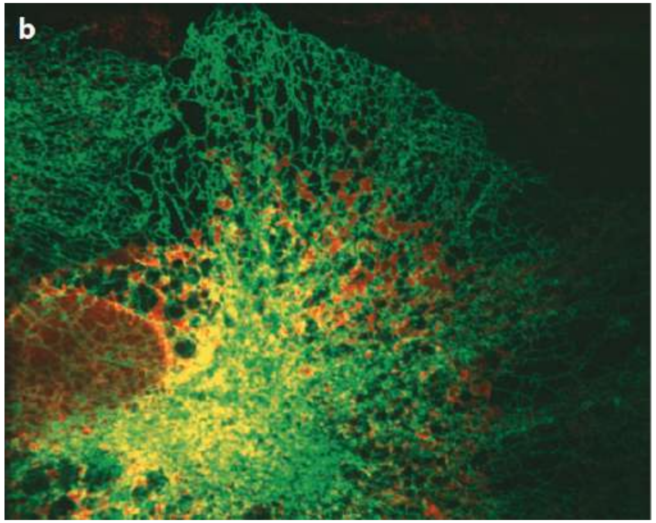
Golgi



Microtubules
Golgi (VSV-G)

Toomre et al
J. Cell Sci. (1999)

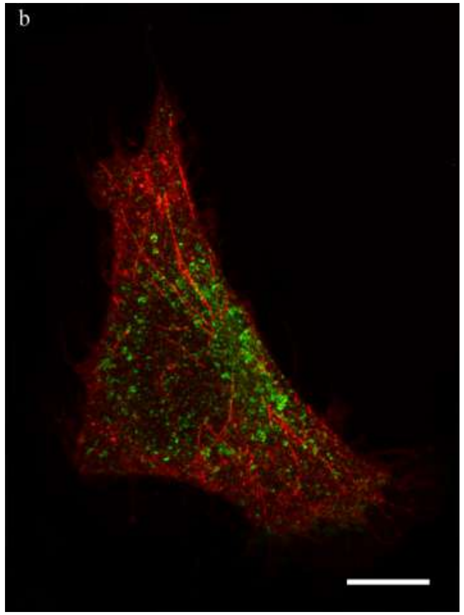
ER



Shibata *et al., Annu. Rev. Cell Dev. Biol.* (2009)

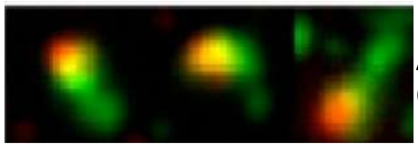
Reticulon Calreticulin

Clathrin-independent Carriers



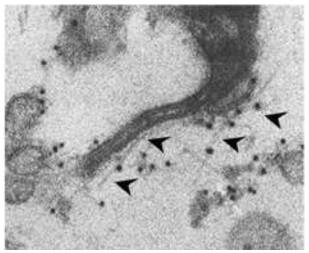
Sens et al *Curr. Opin. Cell Biol.* (2008)

Shiga Toxin TfR



Almeida *et al., NCB* (2011)

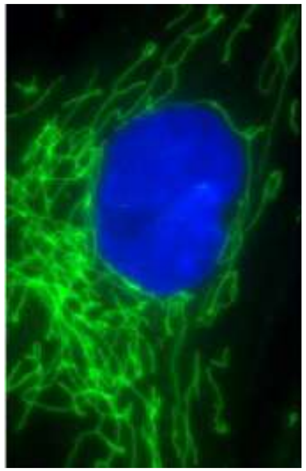
Endosomes



Puthenveedu *et al., Cell* (2010)

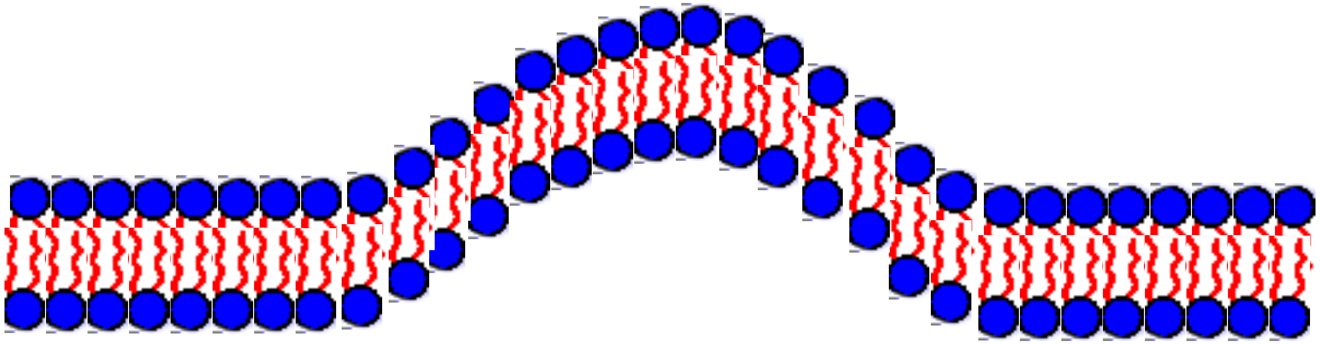
Gold NP=9 nm

Also, mitochondria

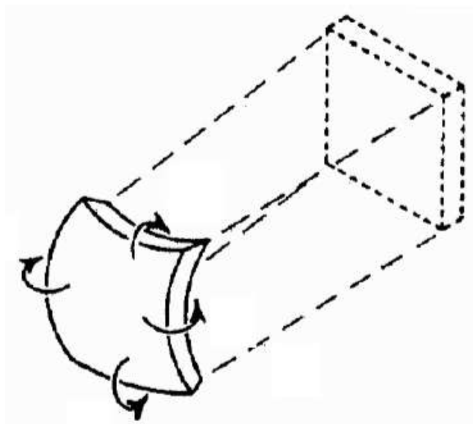


A. Grünewald *et al., Exp. Neurol.*(2009)

Lipid Membranes: Easily Bent



Allows for intracellular trafficking, endocytosis, exocytosis



Bending Energy of Fluid Membranes

W. Helfrich
P. Canham
E. Evans (≈1970)

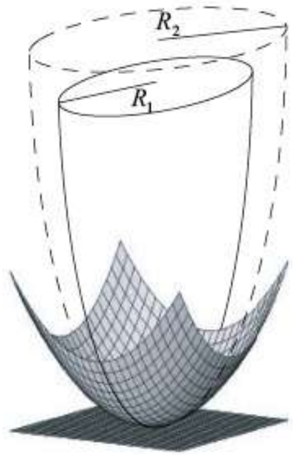
Energy/unit area:

$$f_{bending} = \frac{1}{2}\kappa C^2$$

W. Helfrich, *Zur Naturforschung* **28c**, 693 (1973)

C: Mean curvature

$$C = C_1 + C_2 = \frac{1}{R_1} + \frac{1}{R_2}$$



κ **: bending modulus**

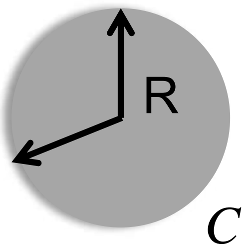
from a few k_BT to ≈ 100 k_BT $(k_BT = 4.10^{-21} J)$

depends on composition

cylinder

$$C = \frac{1}{R}$$

sphere



$$C = \frac{1}{R} + \frac{1}{R} = \frac{2}{R}$$

Bending Energy of Fluid Membranes

W. Helfrich
P. Canham
E. Evans (≈1970)

Per unit area:

$$f_{bending} = \frac{1}{2}\kappa C^2$$

C: Mean curvature

W. Helfrich, *Zur Naturforschung* **28c**, 693 (1973)
"Elastic properties of lipid bilayers :
theory and possible experiments"

κ : Curvature rigidity modulus

(in Joules, or in $k_B T$)

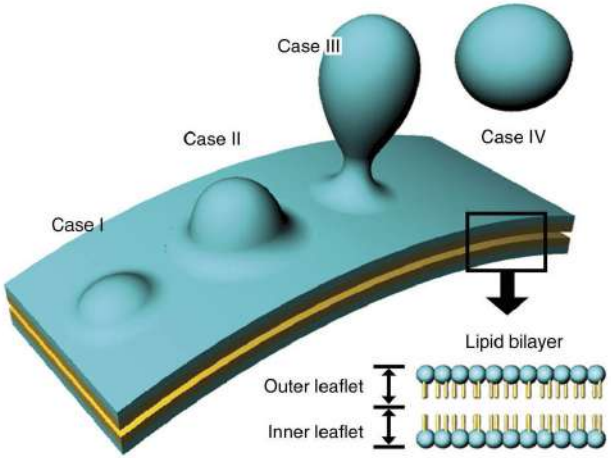
$$k_B T = 4.10^{-21} J$$

from a few $k_B T$ to ≈ 100 $k_B T$

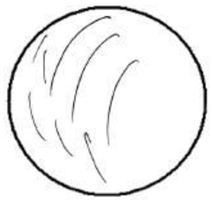
1000 easier than Polyethylene

$$f_{bending} = \frac{1}{2}\kappa C^2$$

Energy to form a spherical vesicle from a flat membrane:



SPHERE



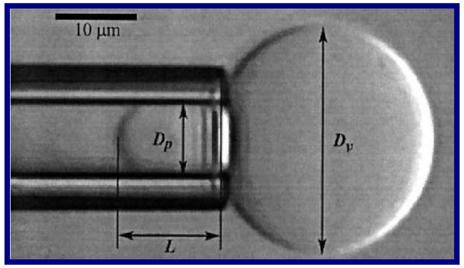
$$C = \frac{2}{R}$$

$$F = \frac{1}{2}\oiint \kappa \left(\frac{2}{R}\right)^2 dS = \frac{1}{2}\kappa \frac{4}{R^2} 4\pi R^2 = 8\pi\kappa$$

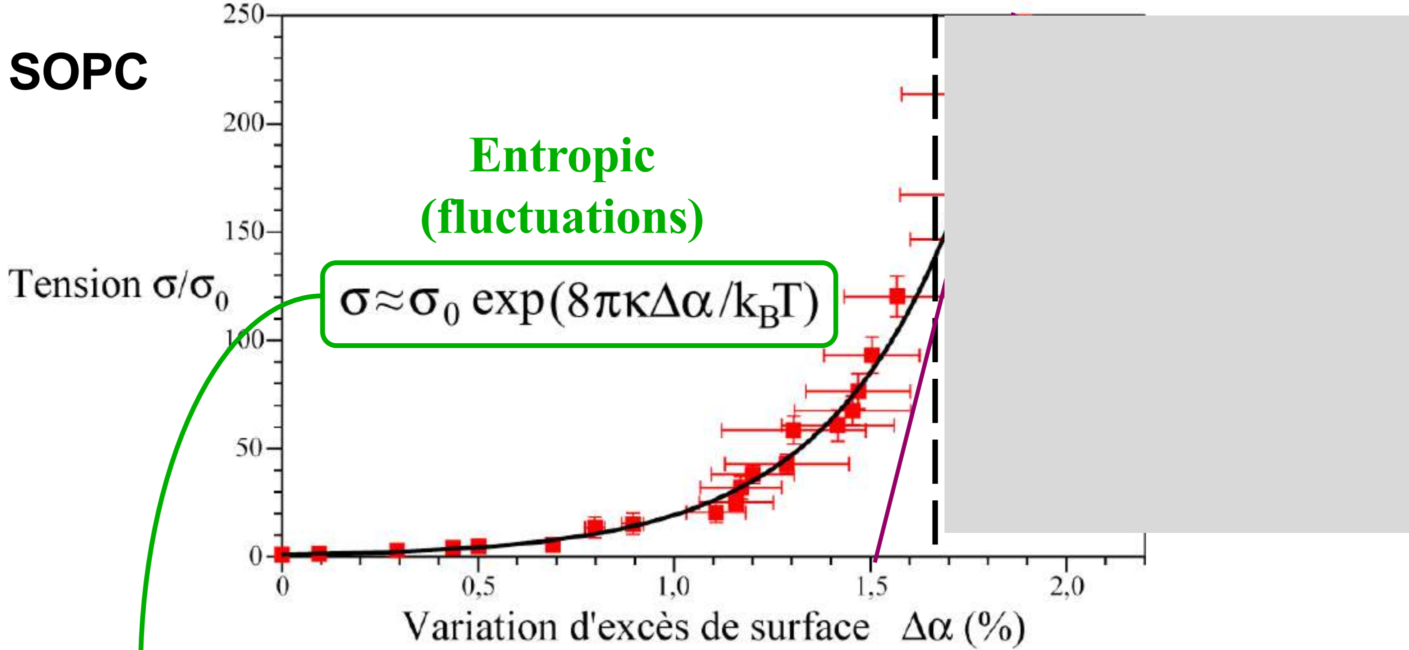
Independent of vesicle size!!!!

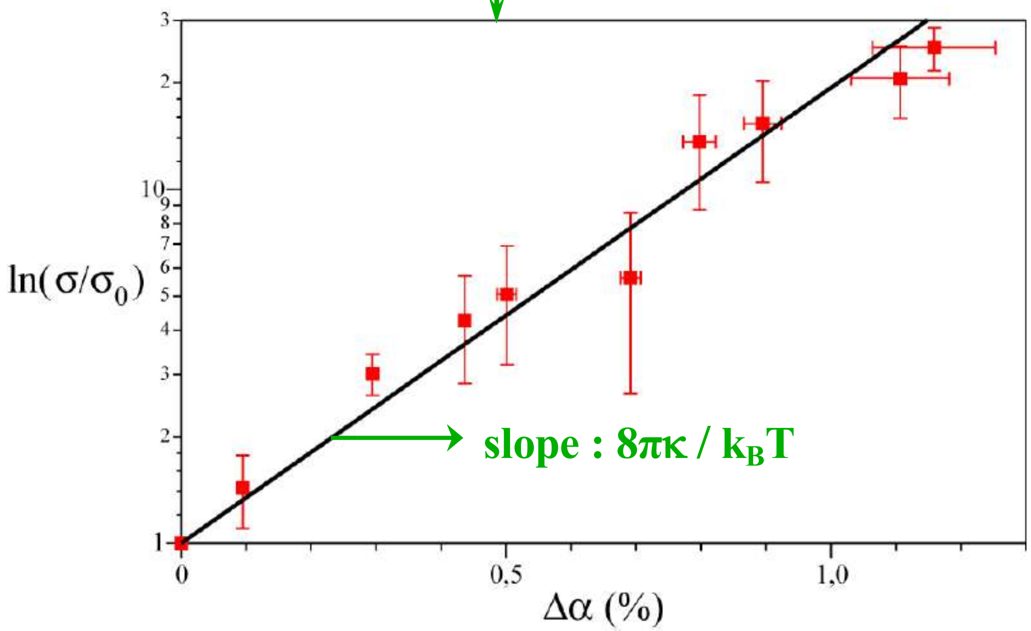
For κ=10 k_BT F $\approx$ 250 k_BT

Bending Modulus with Micropipettes



SOPC





P. Girard et al *PRL* (2005)

Bending Modulus κ also Depends on Lipid Composition

PC lipids: *increases* with chain length
decreases with unsaturations #
Increases with cholesterol
with Sphingolipids

W. Rawicz …E. Evans *Biophys. J.* **79**, 328-339 (2000)

W. Rawicz .. E. Evans *BJ* **94**, 4725-4736 (2008)

J. F. Nagle *Faraday Discuss.* **161**, 11 (2013)

from 10 $k_B T$ to $\approx$ 100 $k_B T$

Lipid membranes: Easy to bend

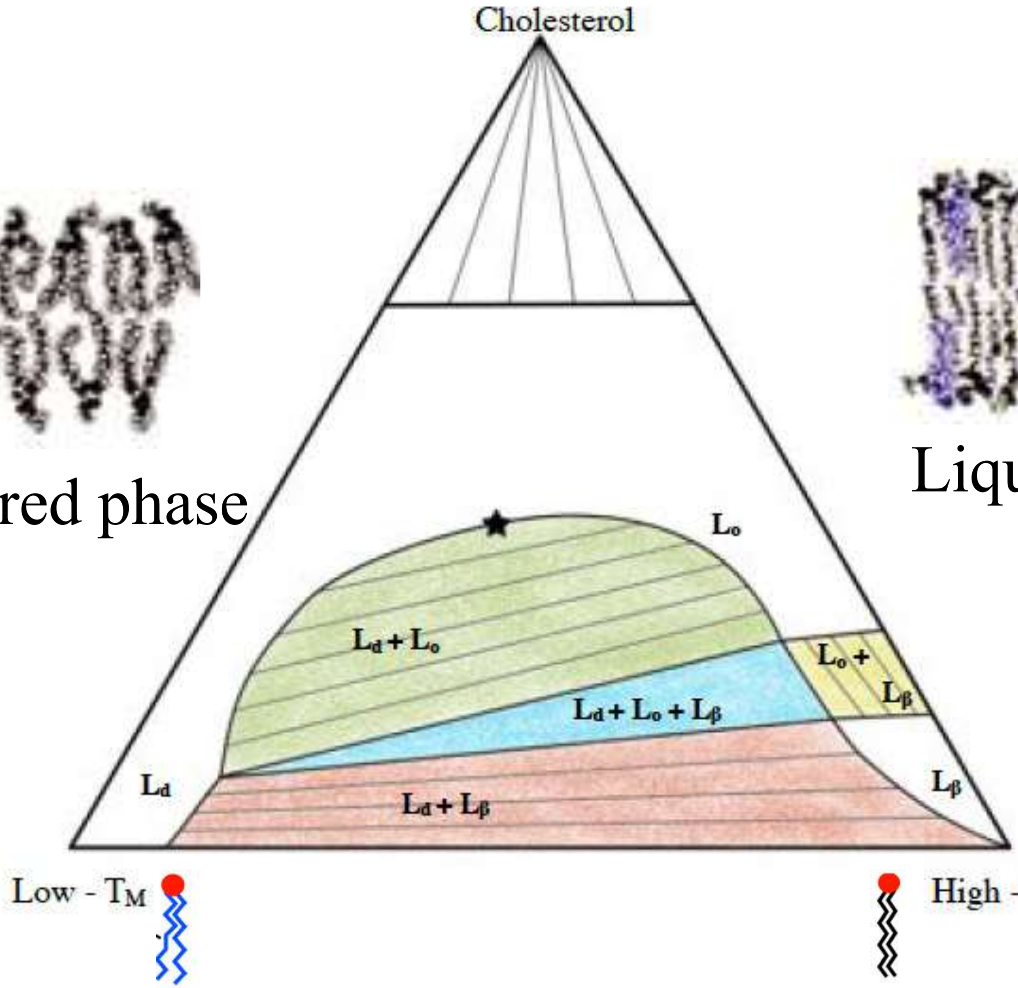
1000 easier than Polyethylene

Ternary Lipid Mixtures - Phase Separation

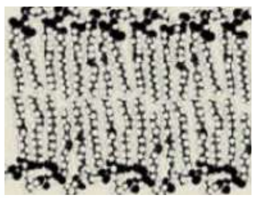
Phases Coexistence in Ternary Lipid Mixtures

General phase diagram

Liquid disordered phase
L_d



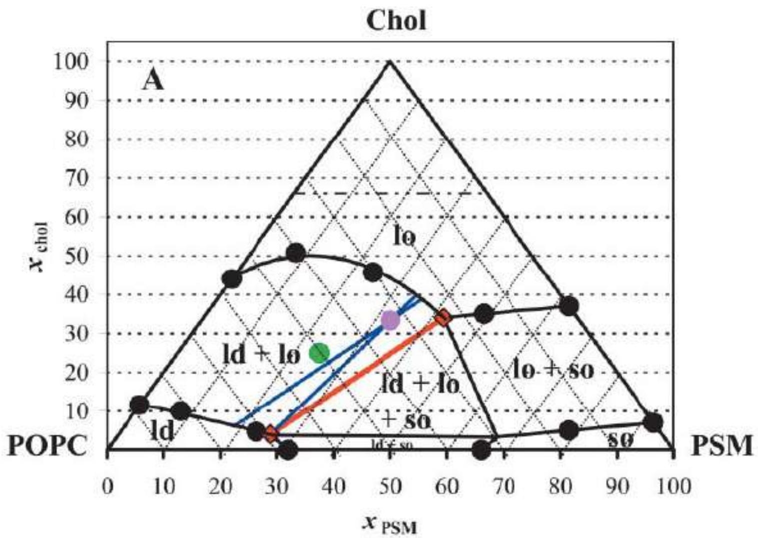
Liquid ordered phase
L_o



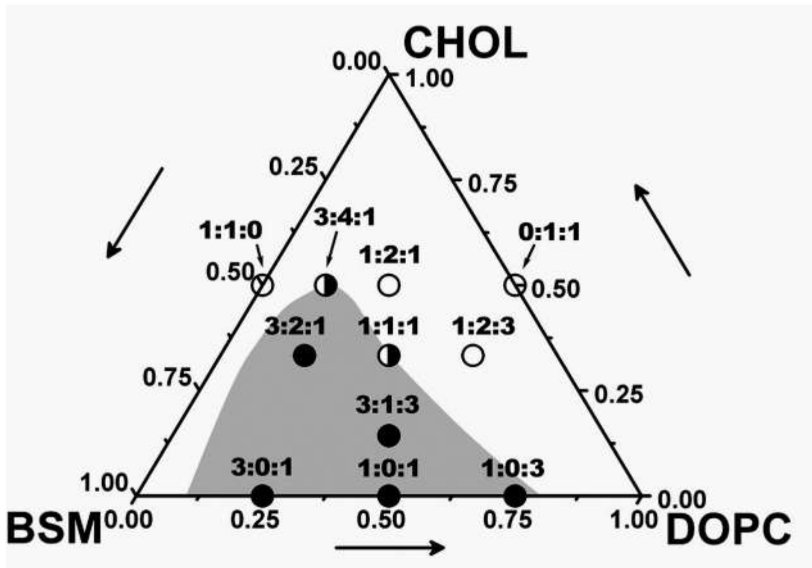
Gel

* Critical point

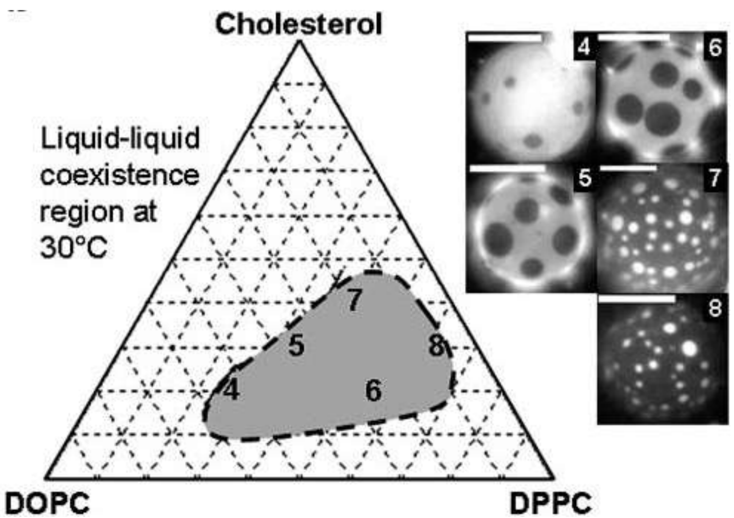
Some Phase Diagrams



de Almeida, R.F.M et al *Biophys. J.* (2003)



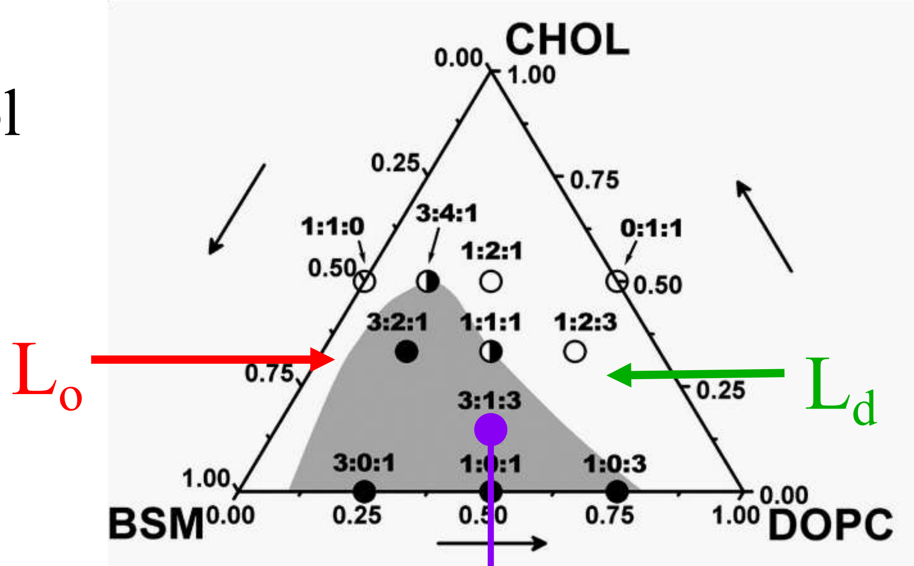
Sorre, Callan-Jones *et al. PNAS* (2009)

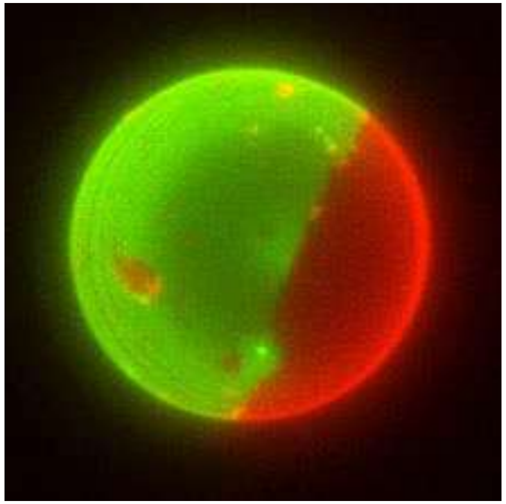


S. Veatch and S. Keller, *Biophys. J.* (2003)

Fluid Phases Coexistence in Lipid Mixtures

DOPC/BSM/Chol





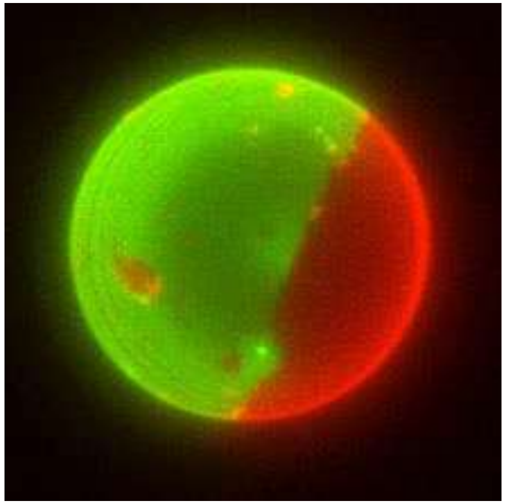
Phase coexistence

2 fluorophores that partition differently

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

2D Phase Separation

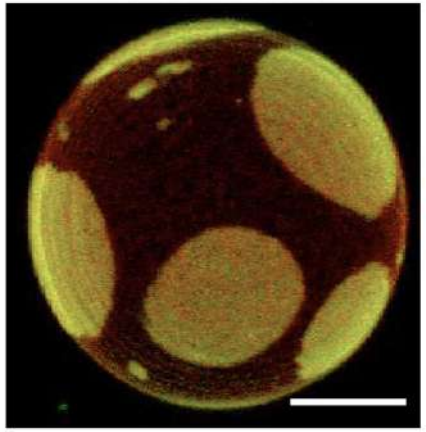
At 3D :
Interfacial tension



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

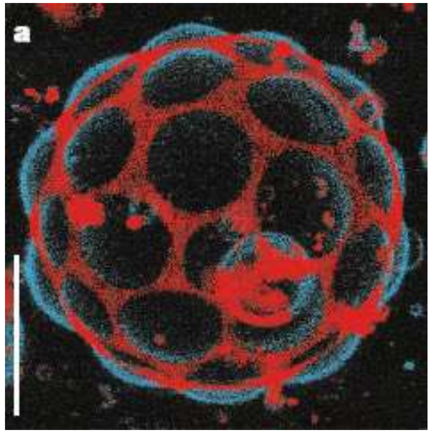
Different Domain Shapes

DOPC/DPPC/Chol



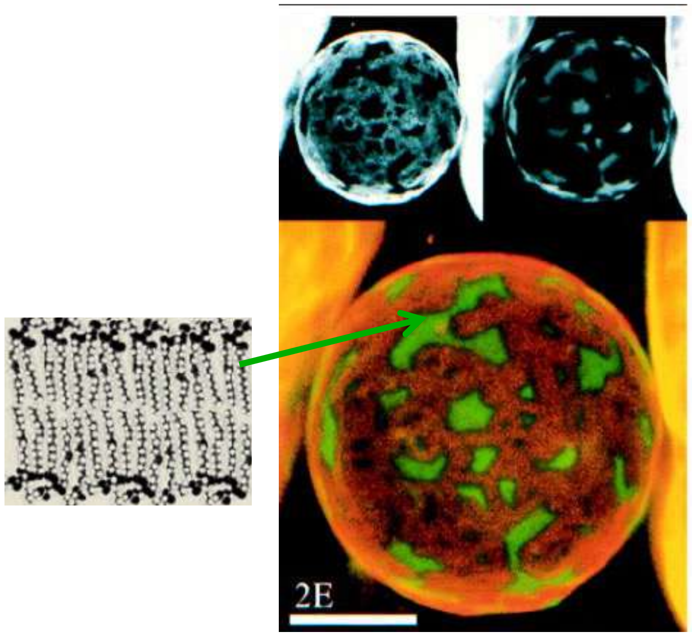
L. Bagatolli *BBA* (2006)

DOPC/SM/Chol



Baumgart et al, *Nature* (2003)

Lo/Ld

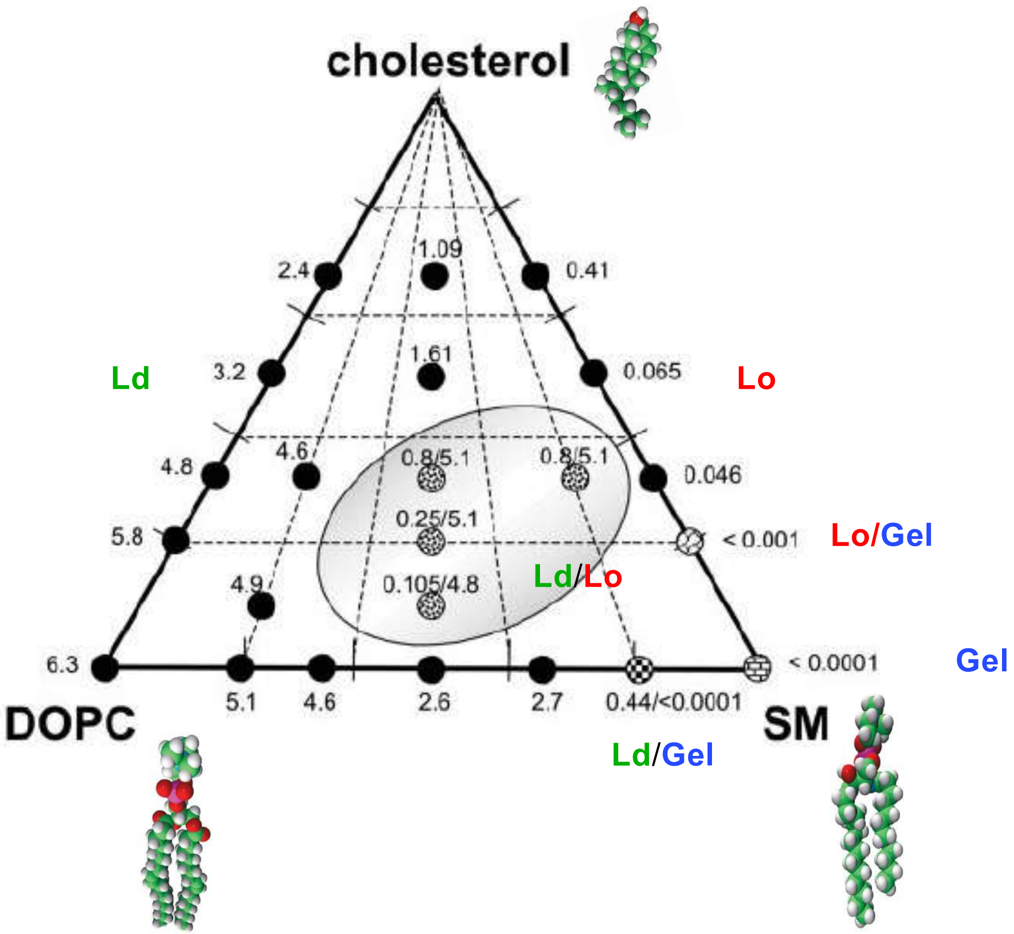


Gel + fluid

Korlach et al,
PNAS (1999)

Membrane Viscosity

Mobilities (in $\mu m^2/s$) by FCS



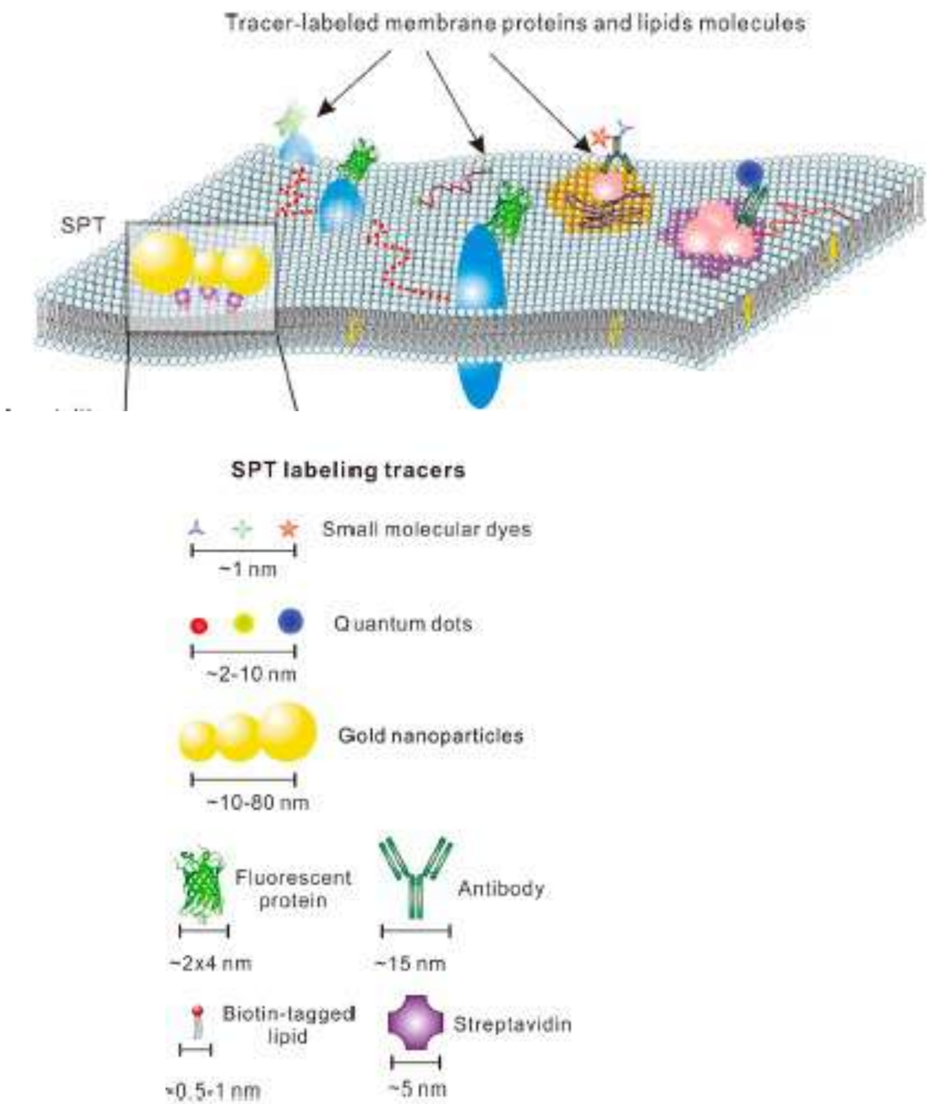
N. Kahya …. **P. Schwille**, *J. Biol. Chem.* (2003)

Membrane viscosities: μ_m

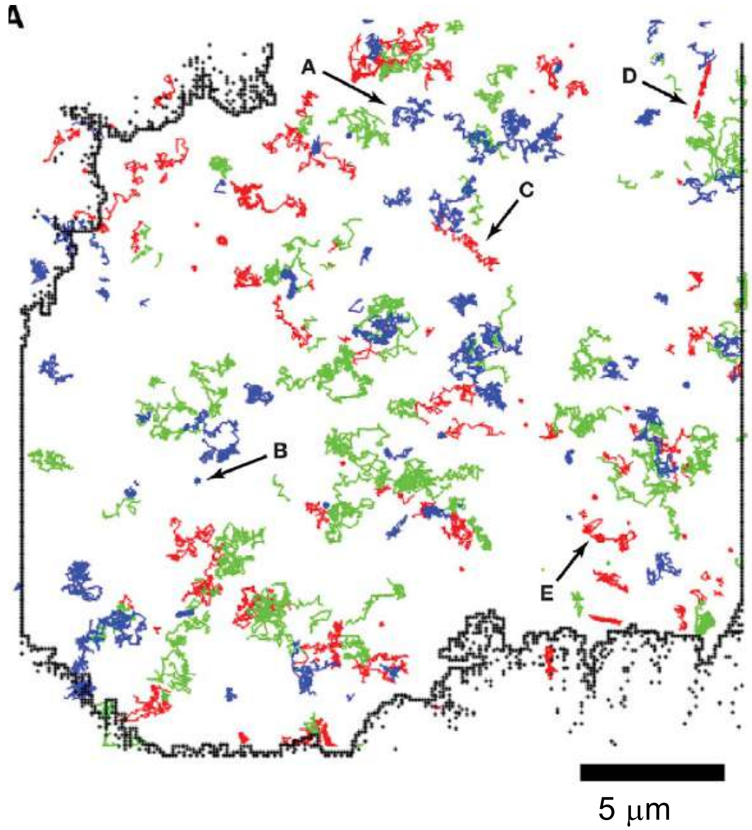
$$\mu_m h \approx 100 \mu_{water}$$

Heterogeneous Diffusion in the Plasma Membrane

Single Particle Tracking at the PM



Y. Yu, M. Li, Y. Yu *ACS Nano* (2019)



K. Jacobson et al *Cell* (2019)

Markers are mobile

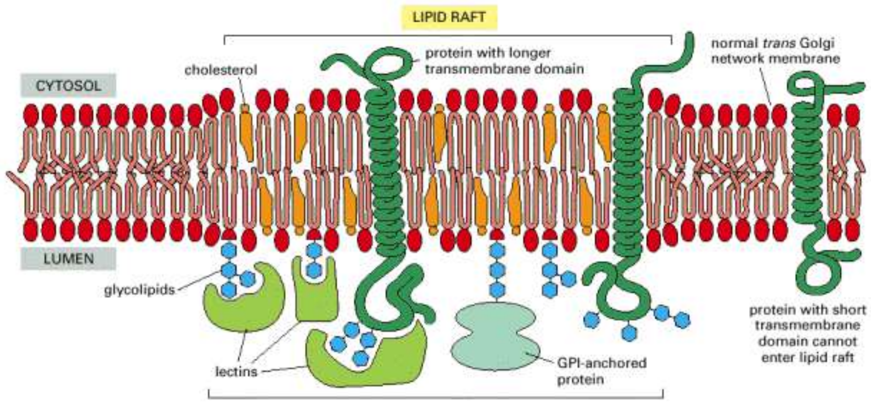
But ***heterogeneous*** mobility

Non-Homogeneous Lipid Distribution

- Depends on organelles

- Asymmetry
  between leaflets

- Lateral inhomogeneities
  Lipid domains: "rafts"



Lipid domains, Phase Separation in Membranes
The RAFT saga

- Predicted in cells

Biochemistry

© Copyright 1988 by the American Chemical Society *Volume 27, Number 17* *August 23, 1988*

Perspectives in Biochemistry

Lipid Sorting in Epithelial Cells

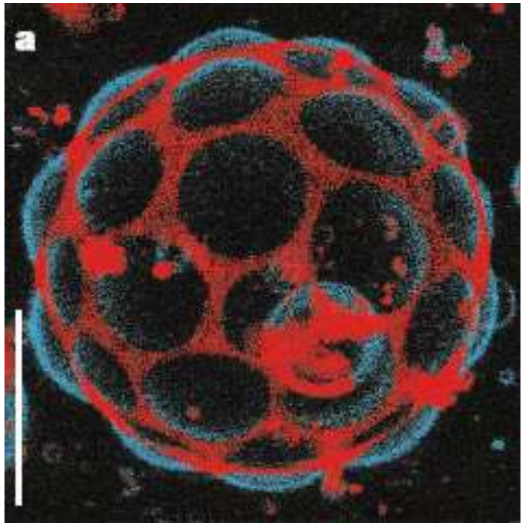
Kai Simons[*,‡] and Gerrit van Meer[§]

- Presence of insoluble domains after detergent (Triton) treatment

- But invisible…. Size, life time????? Long debate

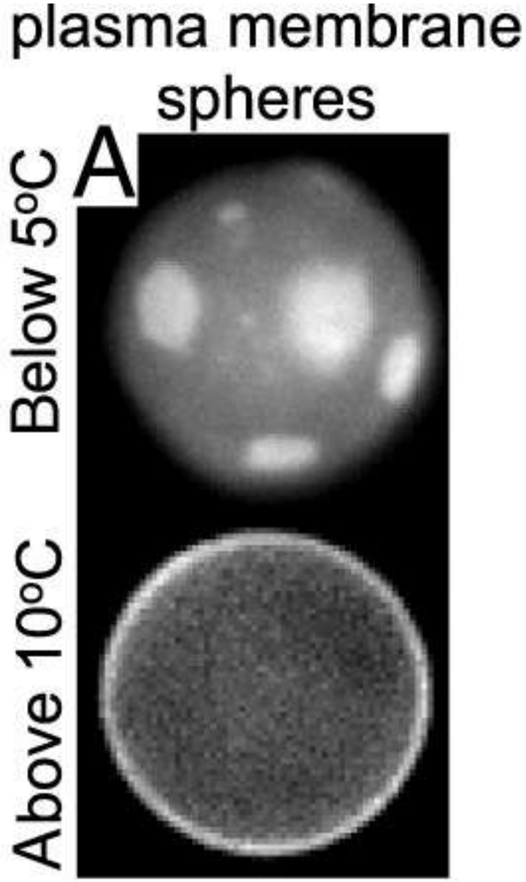
In parallel

DOPC/SM/Chol



Baumgart et al, *Nature* (2003)

Phase separation in
Lipid mixtures



I. Levental et al *PNAS* (2011)

Phase separation in vesicles
from plasma membrane

Until about 2010

But, cell membranes are not at equilibrium

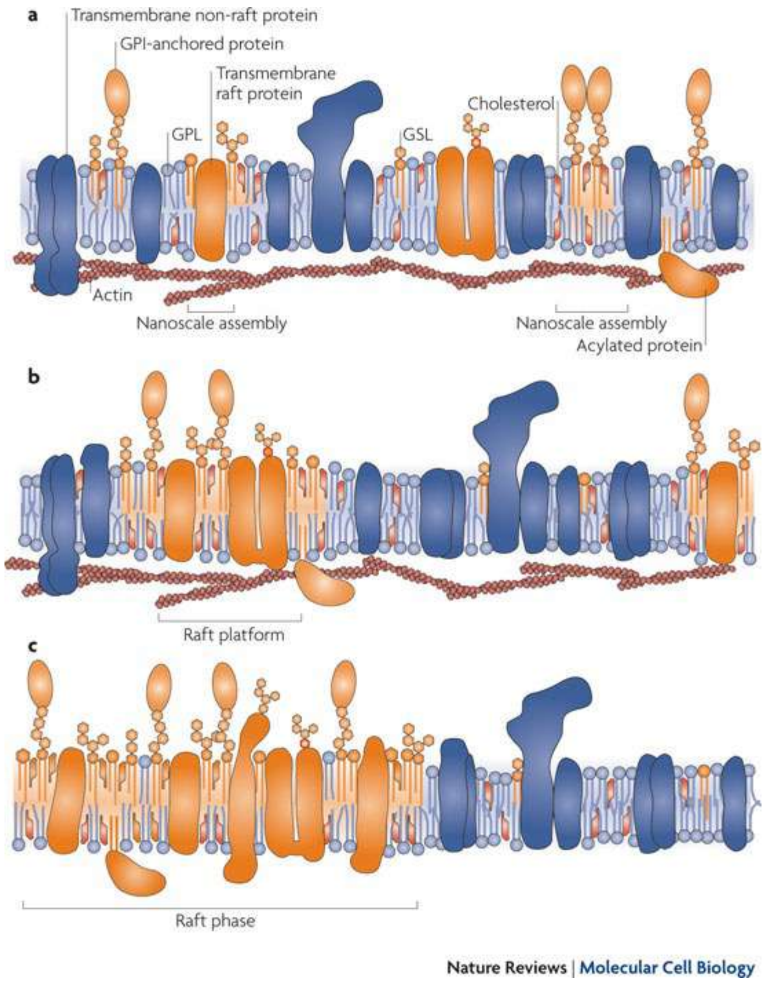
+ presence of a cytoskeleton

Rafts: current view

Nanodomains in the absence of external signals or trafficking events

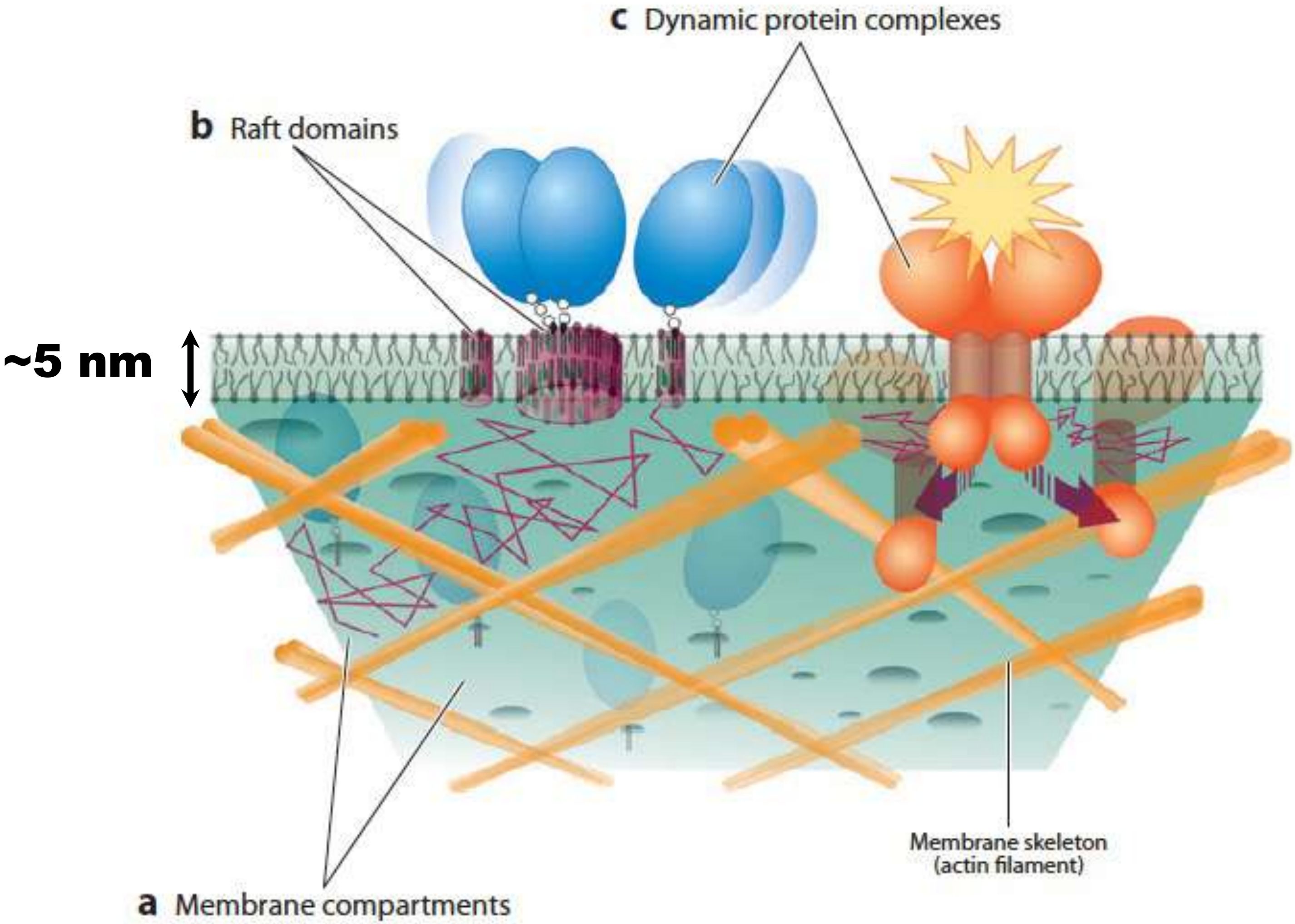
Domain fusion

Equilibrium
(dead cell, no actin)



K. Simons, M. J. Gerl *Nat. Rev. Mol. Cell Biol.* (2010)

Cell Plasma Membrane (Current View)

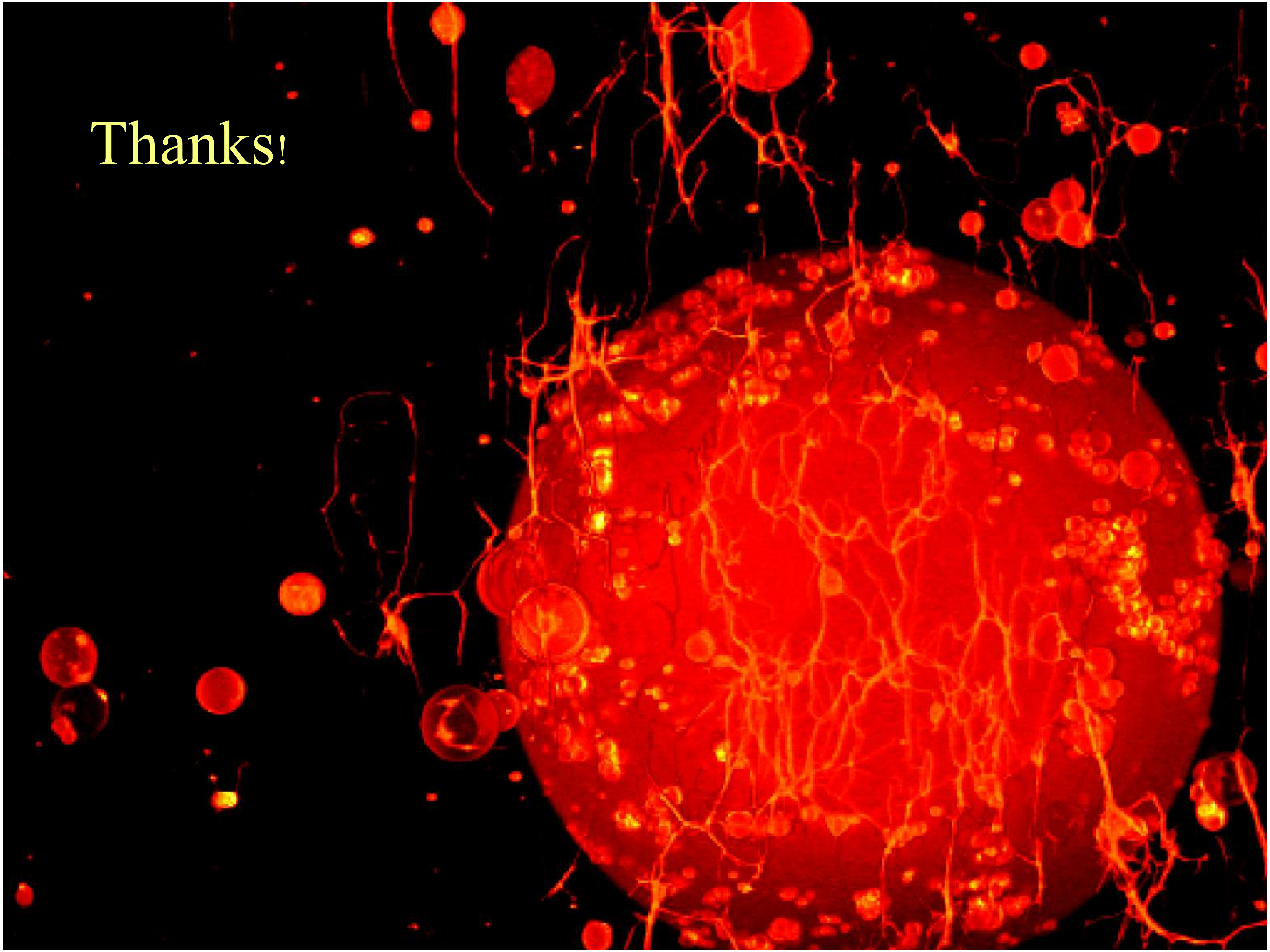


A. Kusumi *et al.*, *Annu. Rev. Cell Dev. Biol.* (2012)

Lipids, high density of membrane proteins (inclusions, bound)

Inhomogeneities (lipid rafts, protein clusters …)

Confinement due to cortical actin filaments


Thanks!