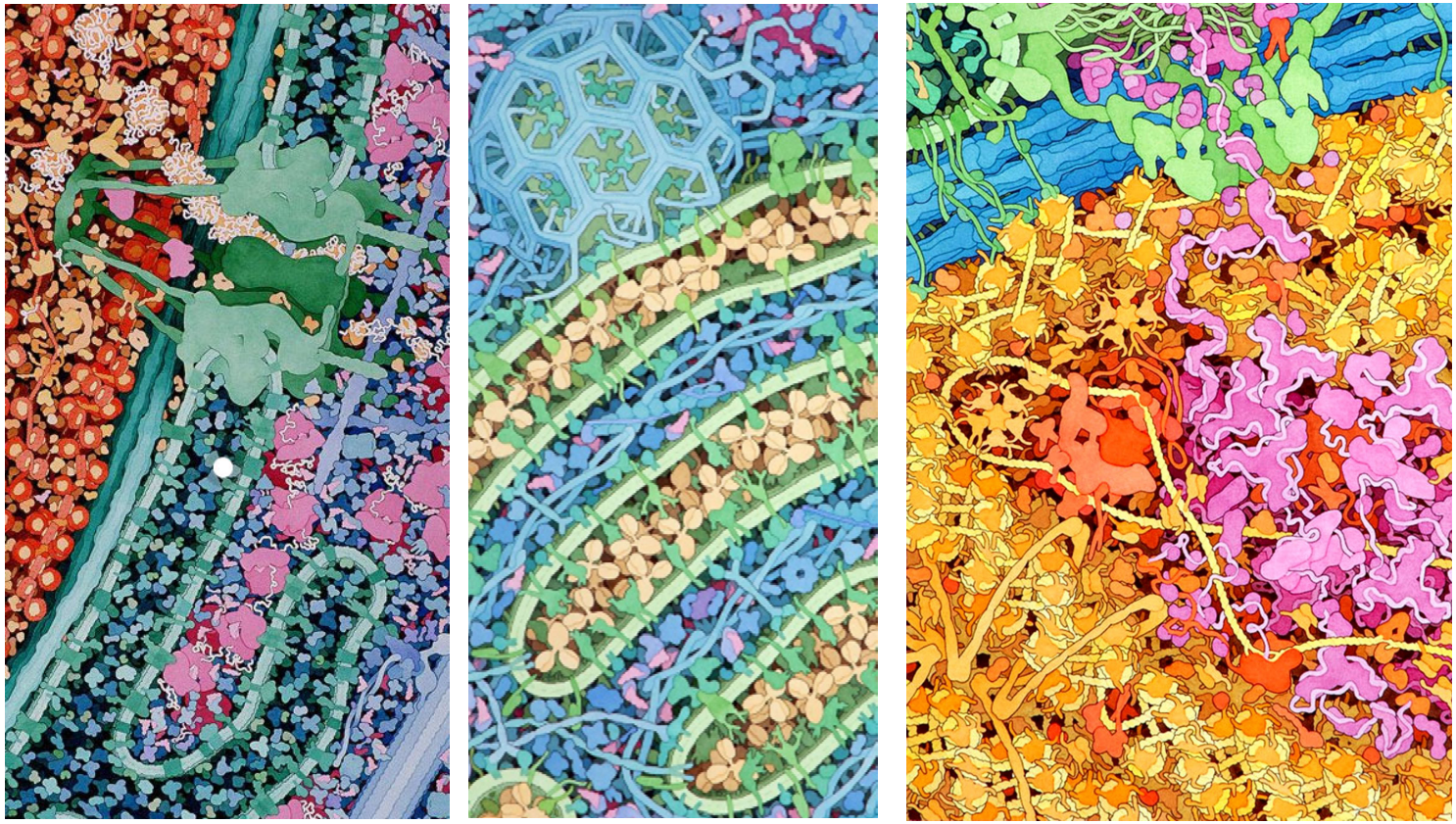
In cells, interactions are managed by compartmentalization



David Goodsell

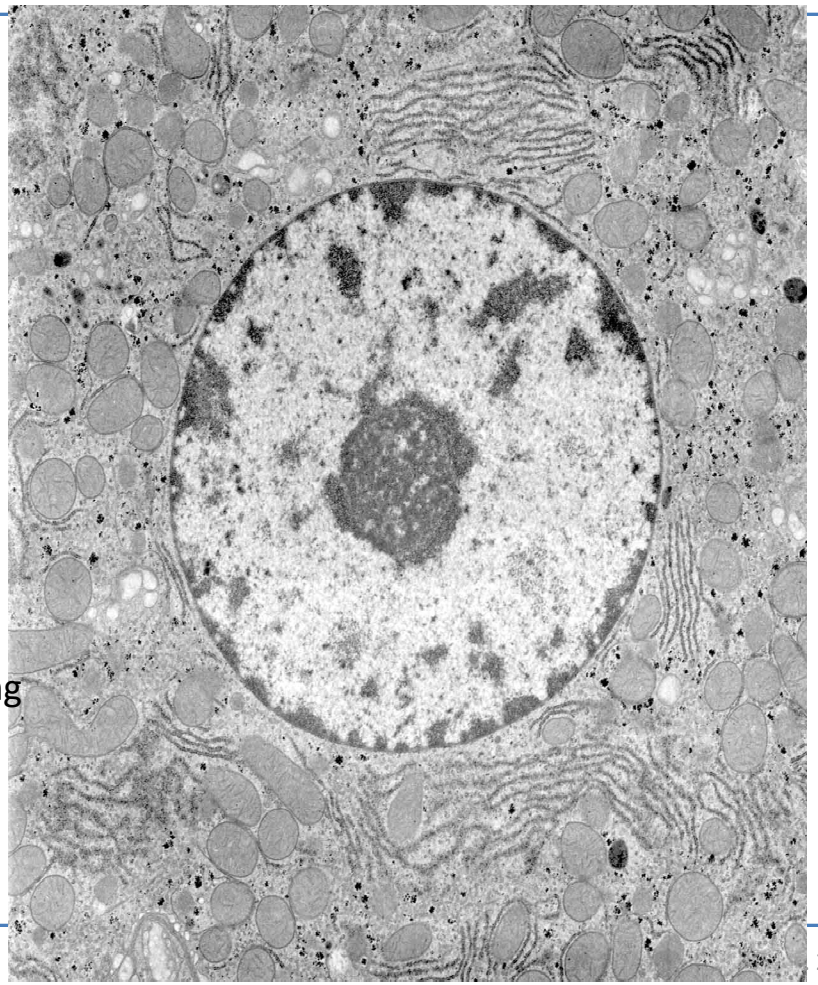
Looking at a cell by electron microscopy

A mammalian cell viewed by electron microscopy

- many internal membranes, high membrane flux
- very different morphologies

→ very crowded!

→ highly compartmentalized!



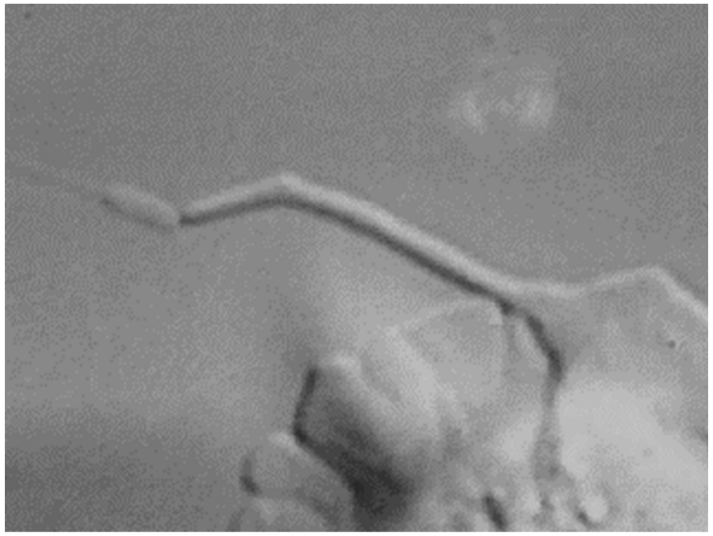
Architecture of lipid membranes:

- **Dimensions**
  - cell: 1 to 10's µm
  - membrane: ~ 4 nm thick
  - lipid: ~ 50 $Å^2$ area and ~2 nm long
- **Diffusion**
  - lipid in membrane: 10^{-2} to 1 um^2/s
- **Potential**
  - -80 mV => 20 MV/m

Membranes, functions and properties

"Membranes separate alive from dead"

- Confinement
- Communication
- Scaffold
- Reservoir
- Energy storage
- Transport
- …



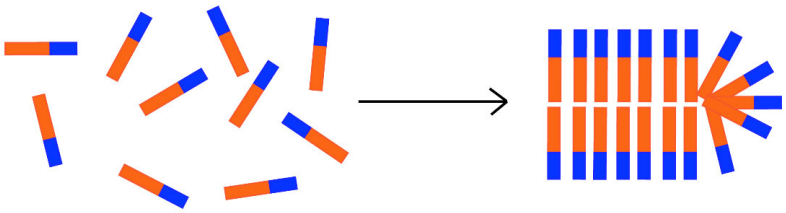
- Self-assembling
- Self-healing
- Flexible
- Shape
- Dynamic
- Hydrophobic layer
- Specificity
- ..

Video from Steven Block

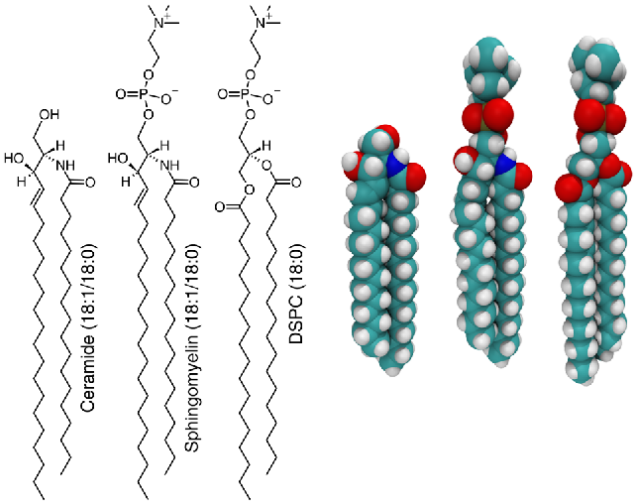
Membrane lipids

General structure of membrane components

membrane formation through
self-assembly in water



- flexible
- dynamic
- self healing



Major lipid classes:

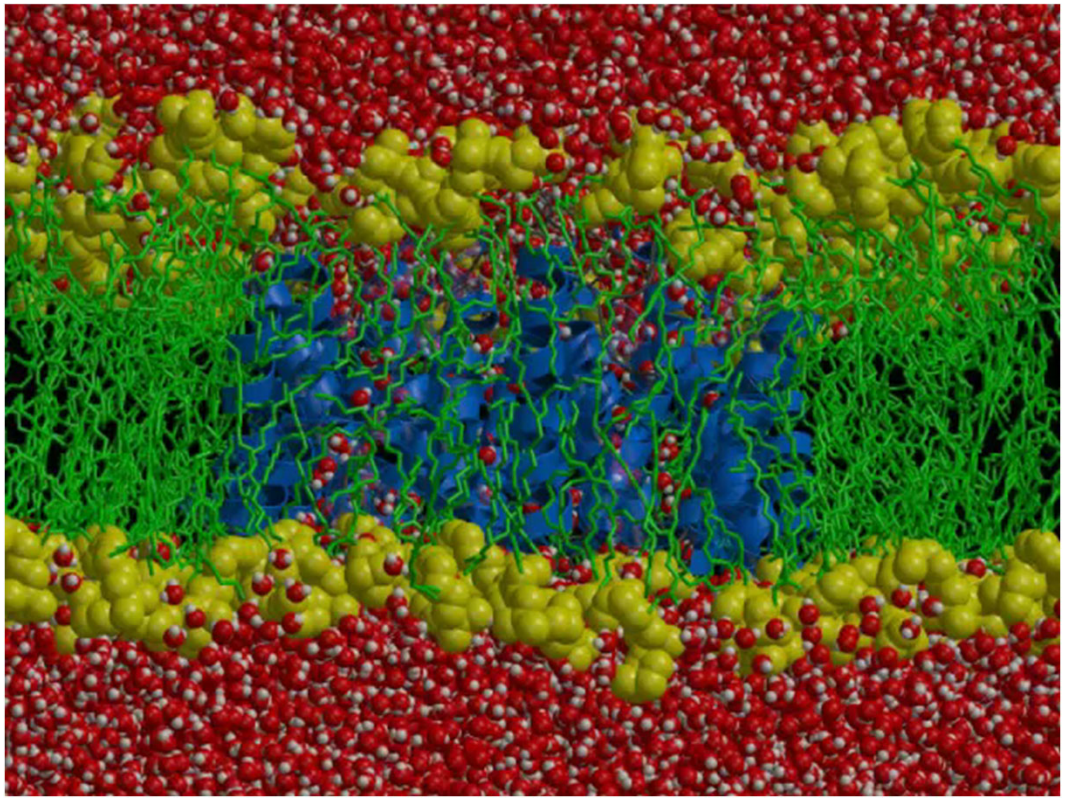
- Glycero-phospholipids
- Phospho-sphingolipids
- Sterols

=> Biochemistry_I & _II

Architecture of membranes

all is moving!

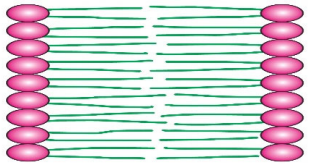
H. Grubmueller : nsec simulation of a bilayer with aquaporin



The dynamic nature of membranes

Diffraction studies:

- Distributions are wide:
  - Reflecting simulations
  - Rejecting

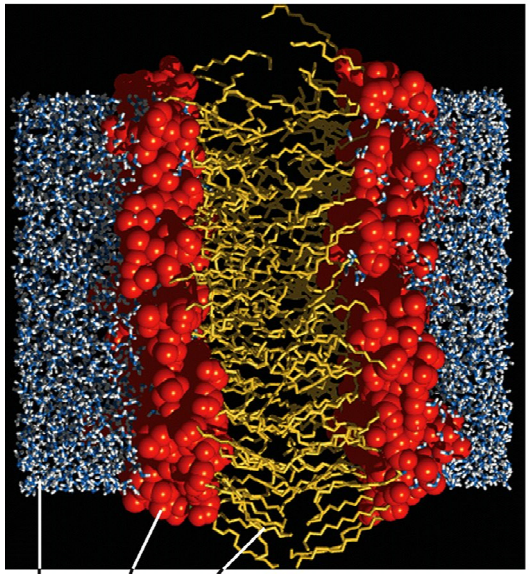


- Hydrophobic core of ± 25 Å
- Interfacial region of ± 15 Å

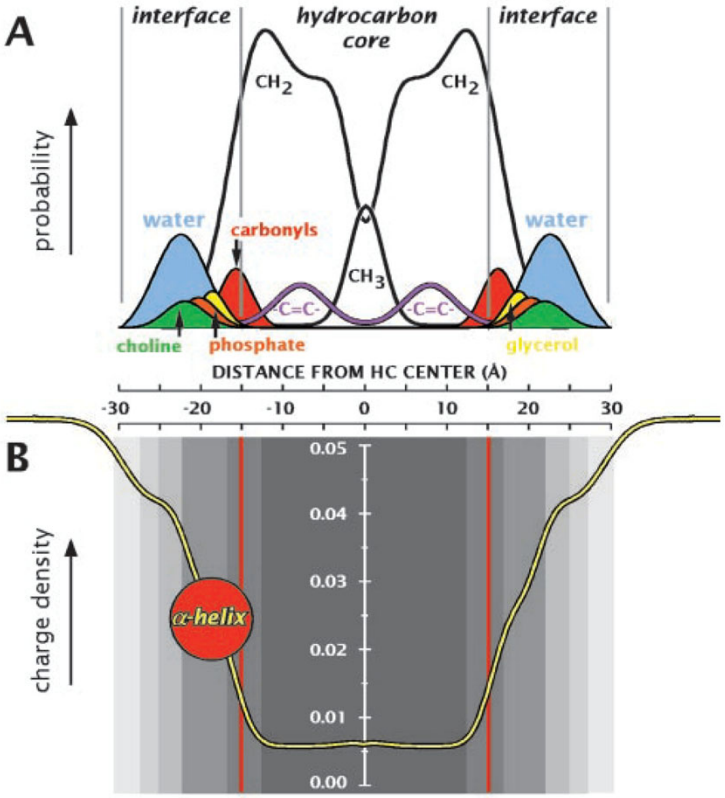
  with a large range of properties.
- Dielectric constant :

  ε_r is ~80 in water

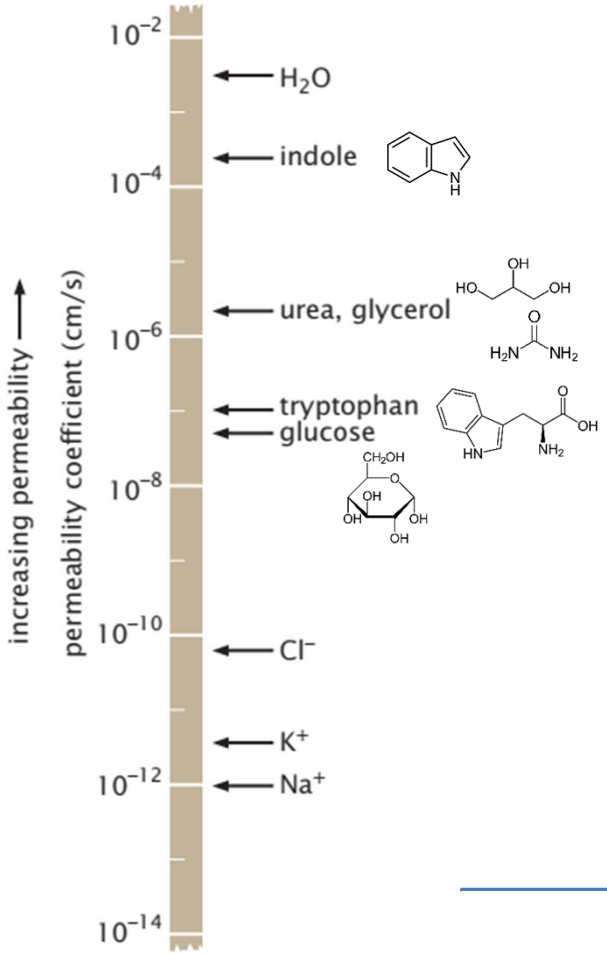
  ~2 in hydrophobic core



Chiu et al., Biophys J., 1995



Membrane permeability



Permeability:

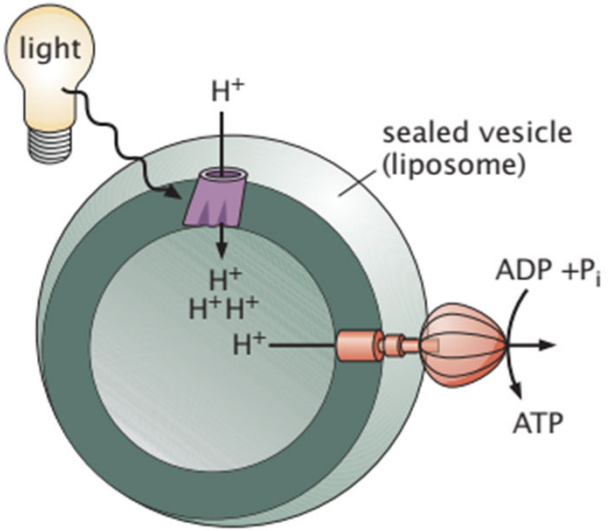
$$flux = P\,(c_{in}\text{-}c_{out})$$

P: permeability coefficient (m/s)

Water conc./pressure can easily equilibrate across membrane

other molecules:
transporters or channels required

→ gradients can be used to generate energy

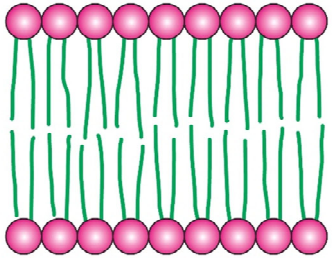


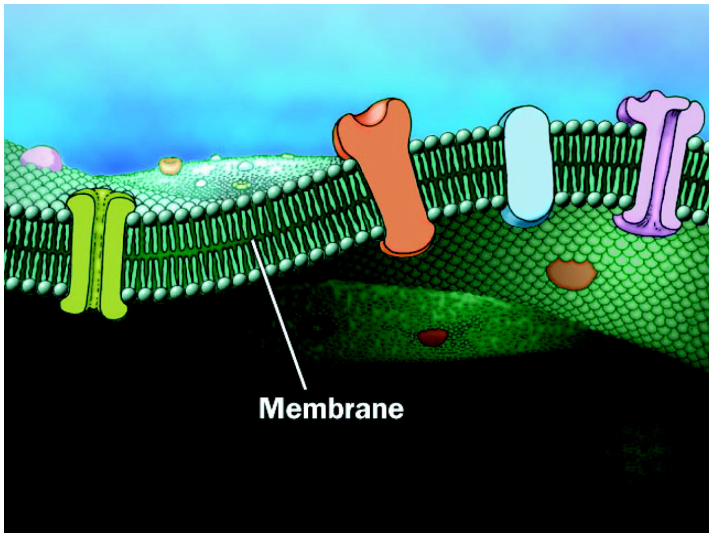
Example:
Bacteriorhodopsin and ATPase

Architecture of membranes

1925

Lipidic bilayer (Gorter & Grendel)

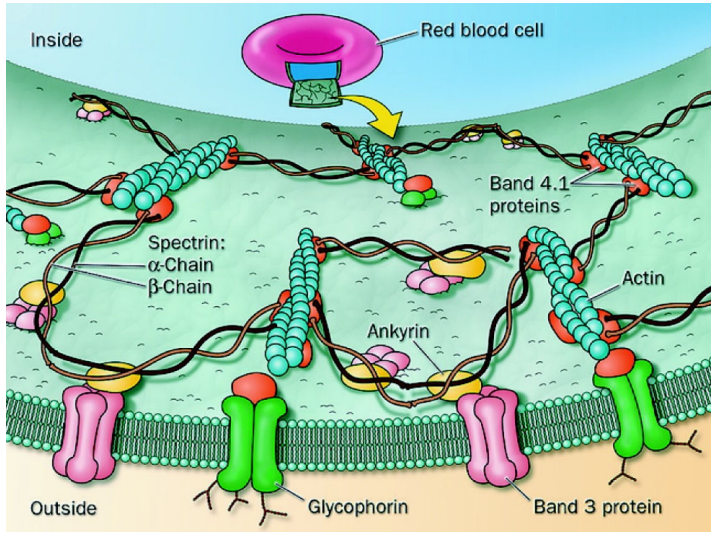




1972

Fluid mosaic model
(Singer & Nicolson):
Free diffusion of all components
within the membrane.

Architecture of membranes

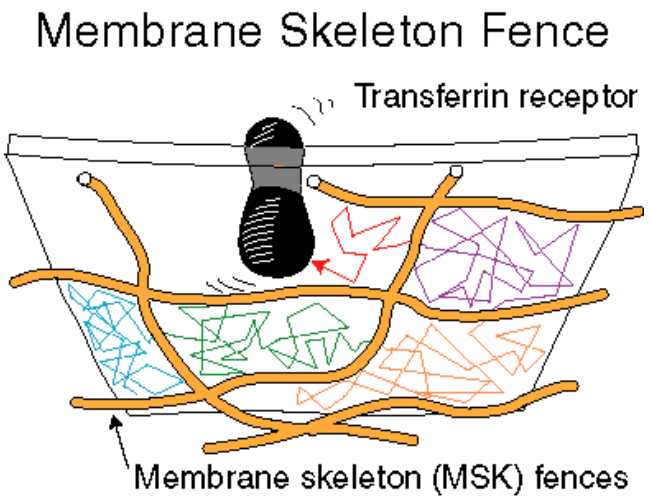


1972

Fluid mosaic model
(Singer & Nicolson):
Free diffusion of all components
within the membrane.

A skeleton of membrane-attached proteins:

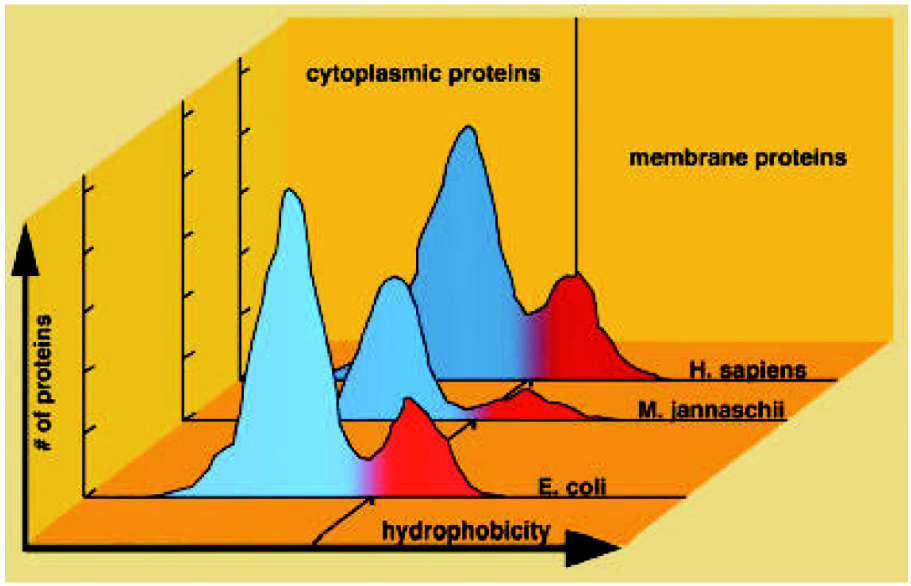
- mechanical stability
- scaffold for molecules
- diffusion barrier



Akihiro Kusumi , ~1999

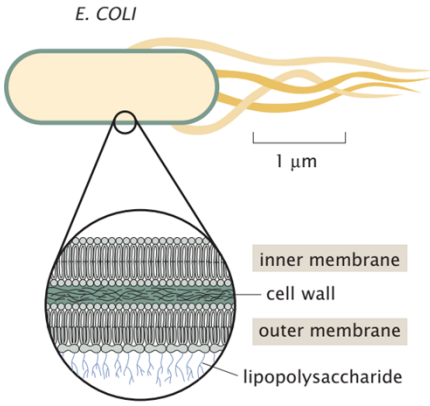
Membrane proteins

- Genome-wide analysis of mean hydrophobicity of proteins:



- => 30 % of all proteins are membrane proteins, i.e. about 10’000.

In E. coli:



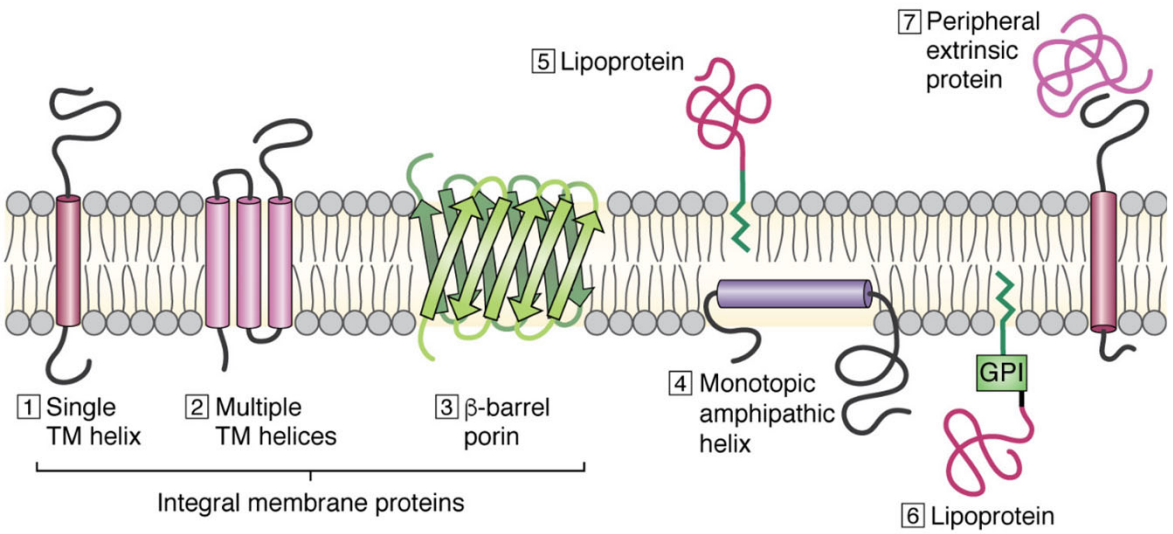
- ca. 1'000'000 membrane protein molecules
- contains a double membrane → 500'000 proteins per membrane
- E.coli surface: 6 µm^2, thus 12 nm^2 per individual protein
- Average distance between proteins: 3.5 nm

In mitochondria:

- ca. 70% of membrane mass comes from proteins

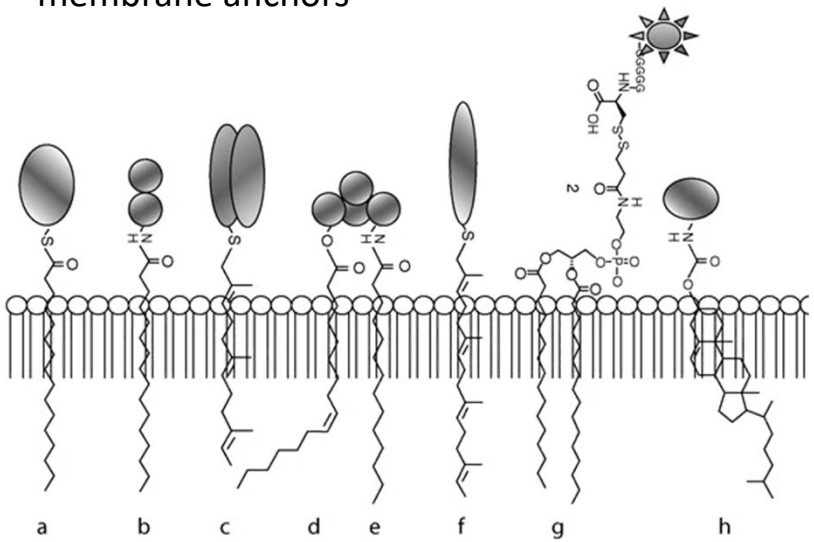
Integral or peripheral membrane proteins

Membrane proteins



Puthenveetil & Vinogradova, JBC 2019

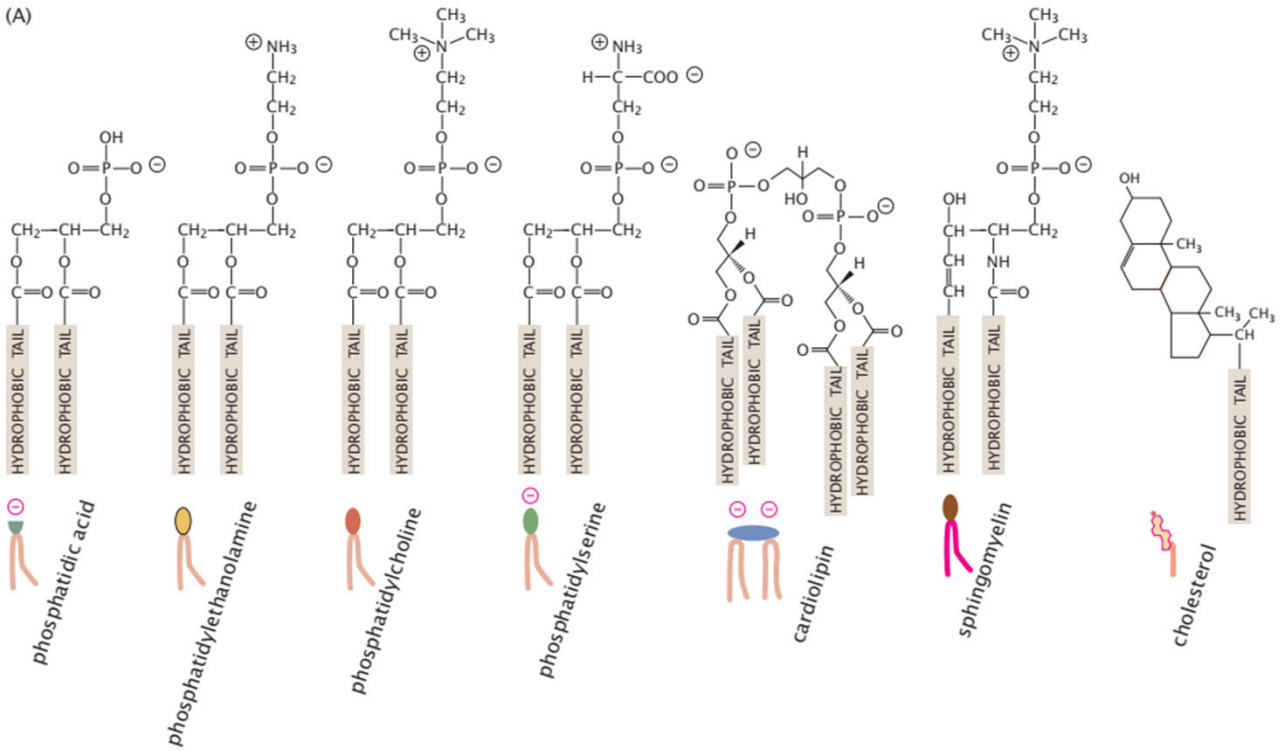
membrane anchors

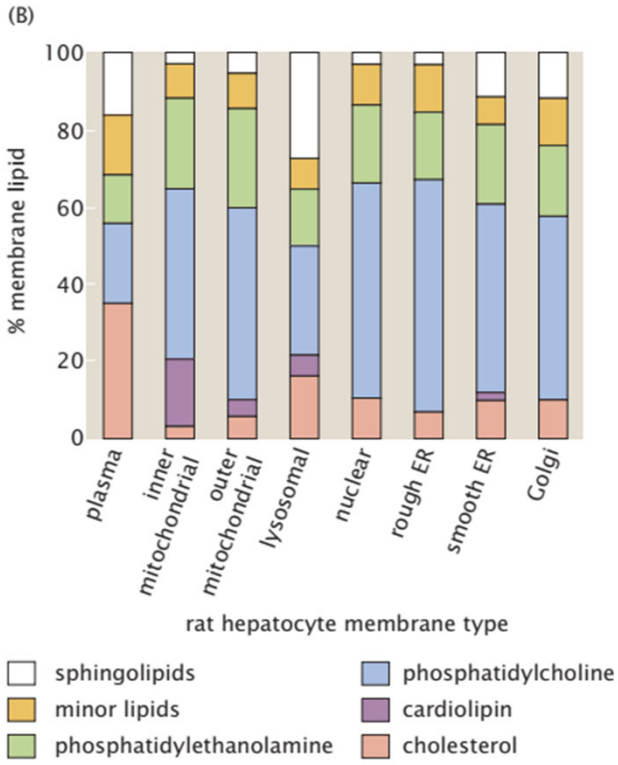


a: s-palmitoylated
b: N-palmytoylated
c: farnesylated
d: O-acylated
e: N-myristoylated
f: geranylgeranylated
g: GPI-anchroded
h: cholesterol modified

Nalivaeva & Turner, HB of Neurochem & Mol Neurobiol

A closer look - Membrane diversity in living cells

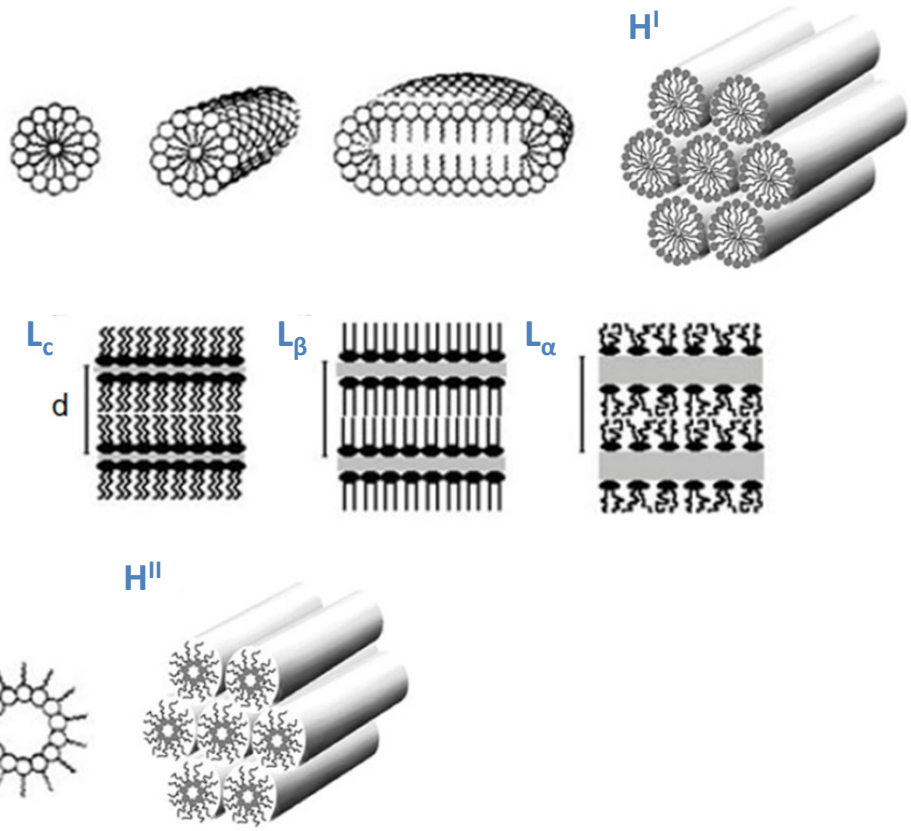




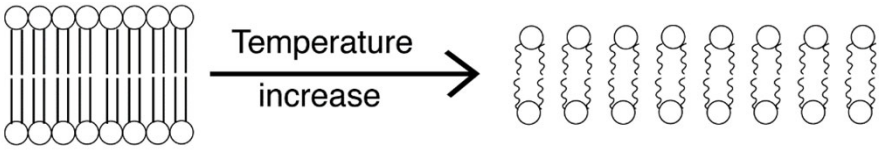
Lipid polymorphism : Structure-shape concept

Relative size of **polar head group** and **hydrophobic part** of lipid

Species	Shape	Organization	Phase
Soaps Detergents Lysophospholipids i.e. missing a lipid chain	Inverted cone	Micelles	Isotropic Hexagonal 1
Phosphatidylcholine Phosphatidylserine Phosphatidylinositol Sphingomyelin Dicetylphosphate DODAC	Cylinder	Bilayer	Lamellar (Cubic)
Phosphatidylethanolamine Phosphatidic acid Cholesterol Cardiolipin Lipid A	Cone	Reverse micelles	Hexagonal 2



Lipid phase transitions



gel phase L_β

liquid crystaline phase L_α

ordered

slow lateral diffusion

$D = 10^{-10}\ cm^2s^{-1}$

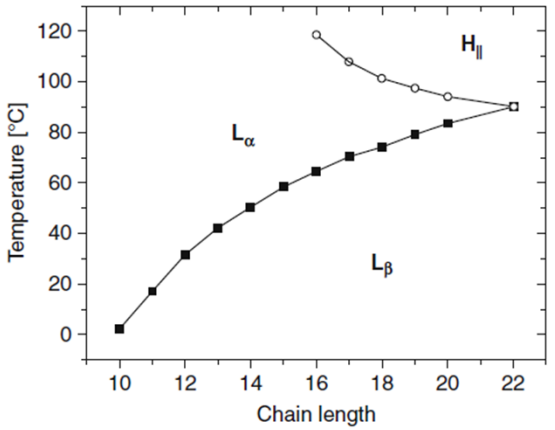
phase transition temperature

disordered

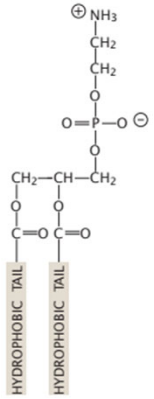
fast lateral diffusion

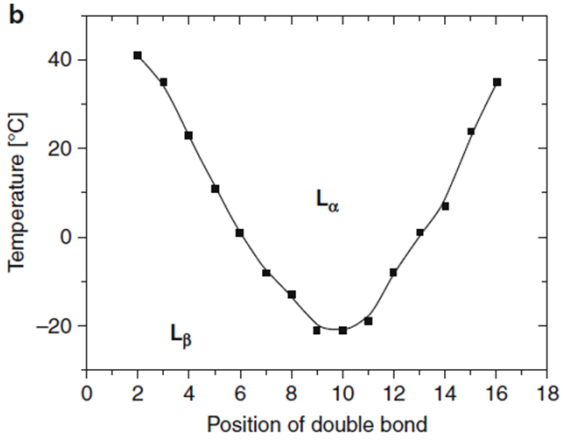
$D = 10^{-7}\ cm^2s^{-1}$

phase transitions behavior depends on the type of lipids, i.e. their **chemical structure**

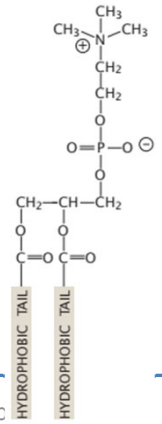


for saturated diacyl phosphatidyl-ethanolamine bilayers





for dioctadecenoyl phosphatidyl-choline bilayers



Koyonova & Tenchov p
Enzyclopedia of biophysics

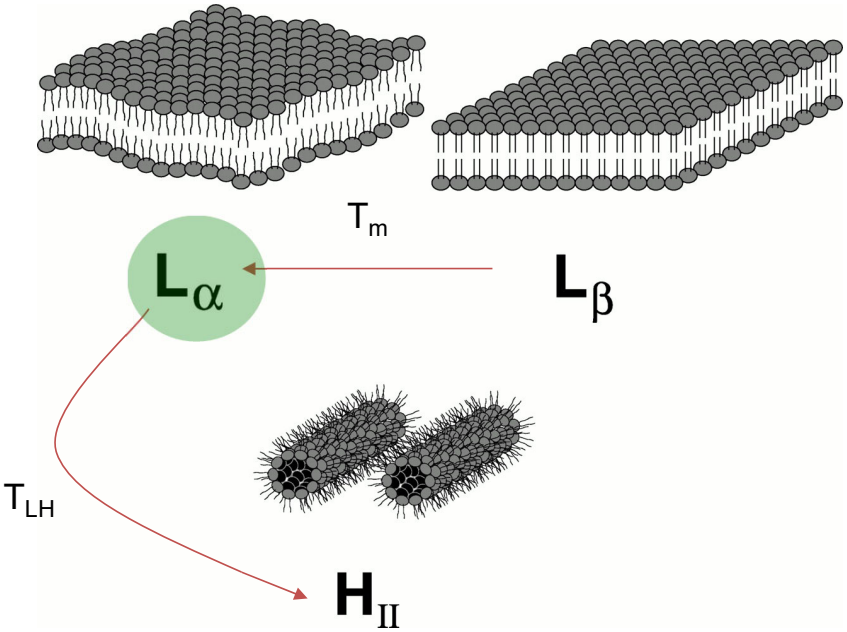
Shape changes in response to temperature variations

E. coli, what is the optimal growth temperature range ?

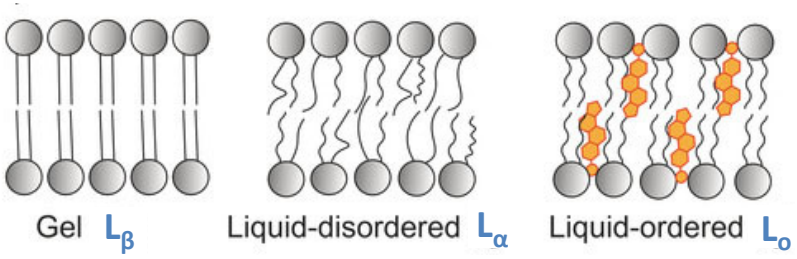
=> 7 to 17 °C above T_m &
10 to 15 °C below T_{LH}

Quiz:

What changes could a cell make to adapt its membrane to different living temperatures?

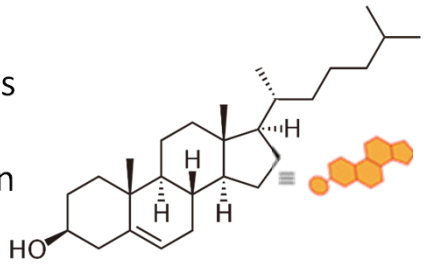


Cholesterol alters the behavior of membranes



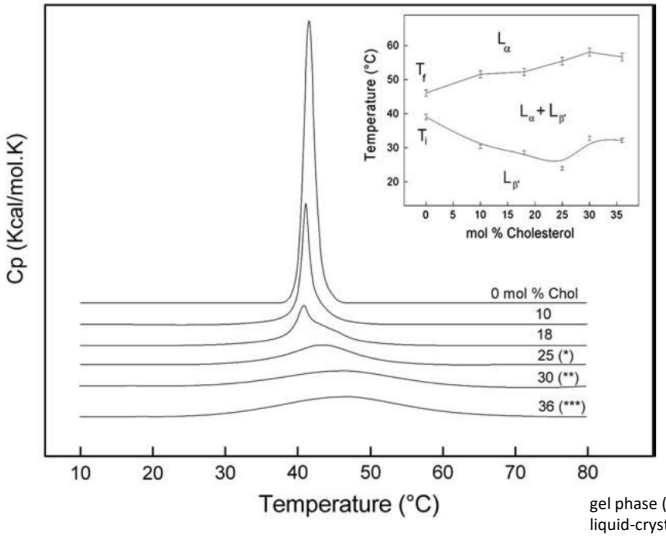
Presence of cholesterol: formation of a new liquid ordered (L_o) phase

- hydroxyl group faces towards polar head groups
- decreases hydrocarbon chain mobility in this region



→ less fluidity than L_α, but less well packed

DSC analysis of membrane phase transition



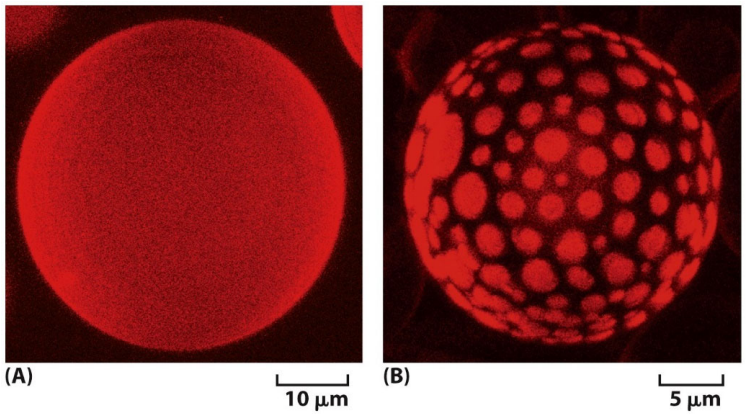
gel phase (Lβ') and liquid-crystalline phase (Lα)

- "liquifies" gel phase & "solidifies" liquid crystalline phase → phase transition disappears
- Decreases membrane permeability

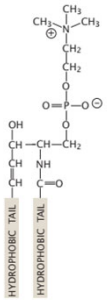
Bolean et al. Biophys Chem. 2010

The presence of cholesterol can induce lipid phase coexistence

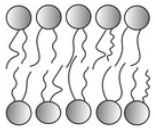
Lipid mixtures in giant liposomes



W. Webb
Nature 2003

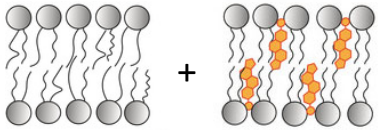


phosphatidylcholine
+ sphingomyelin



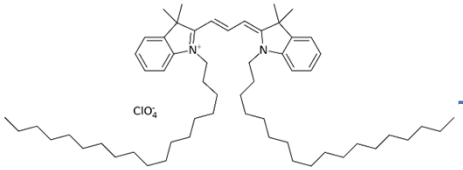
Liquid-disordered L_α

phosphatidylcholine
+ sphingomyelin **+ cholesterol**

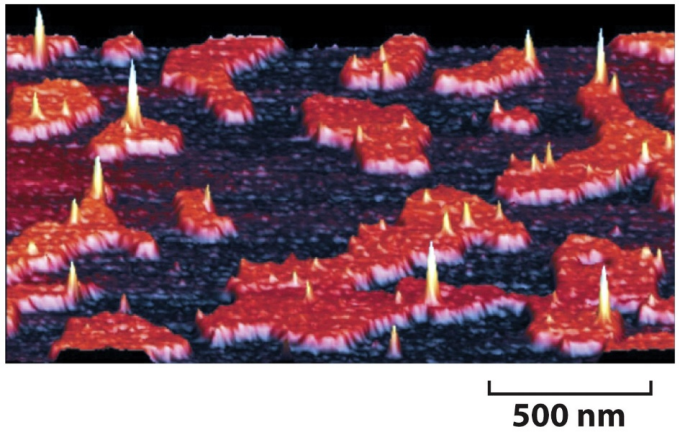


Liquid-disordered L_α Liquid-ordered L_o

staining: Dye which partitions into Lα (DiI-C18)



AFM imaging of L_o phases in bilayers

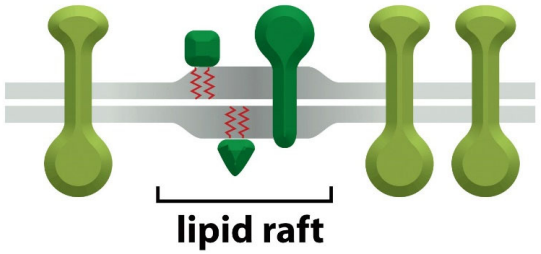


L_o phases are thicker, compared to L_α
→ this is due to lower amount of disorder and interdigitation of aliphatic chains.

Phase separations within membranes

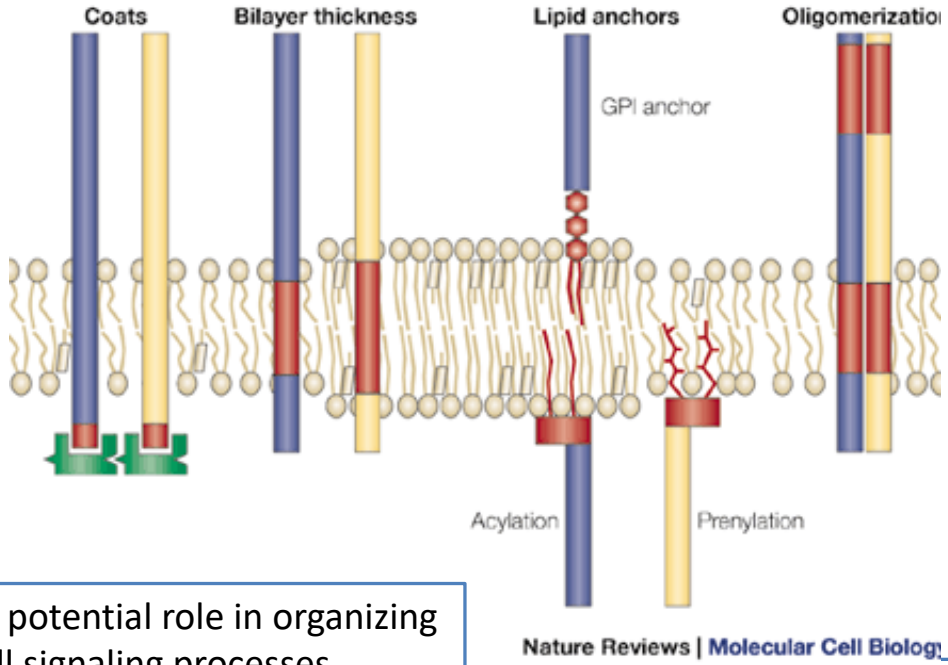
Co-existance of condensed and disordered phase

→ micrometer-sized "solid" domains: **Concept of lipid rafts**



Lipid rafts are more ordered, thicker and contain mainly sphingomyelin and cholesterol

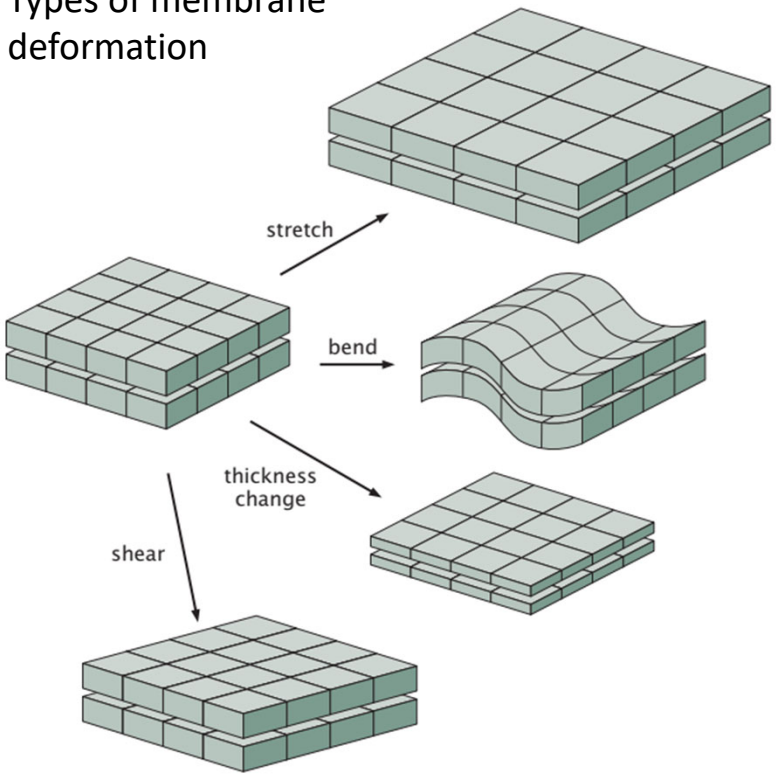
Lipid rafts concentrate distinct proteins, based on their membrane anchors or membrane spanning regions



→ potential role in organizing cell signaling processes

Energetics of changing membrane geometry

Types of membrane deformation



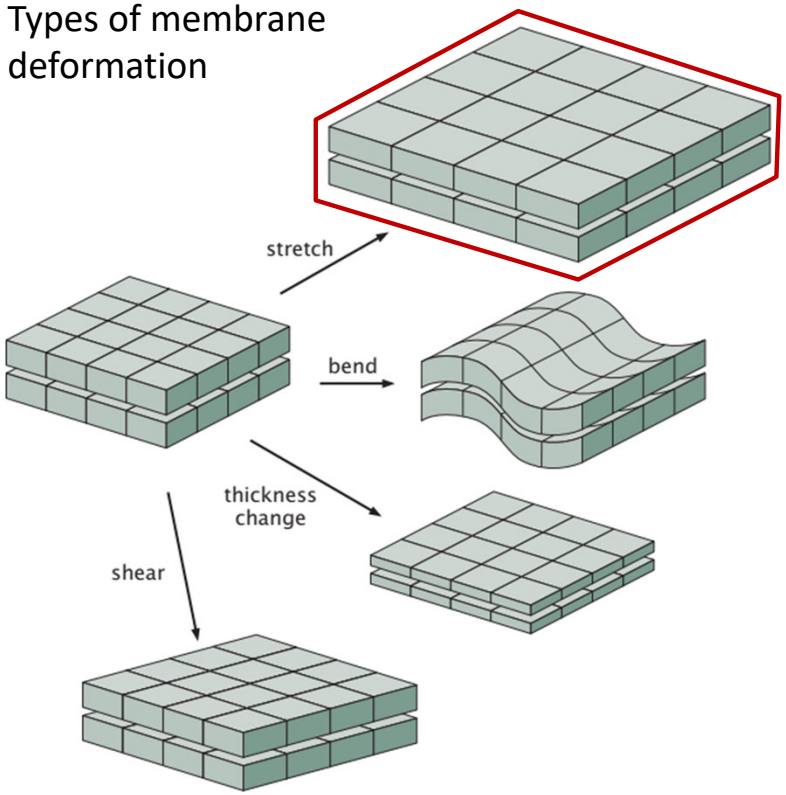
Quiz:

What situations in the life of a cell lead to:

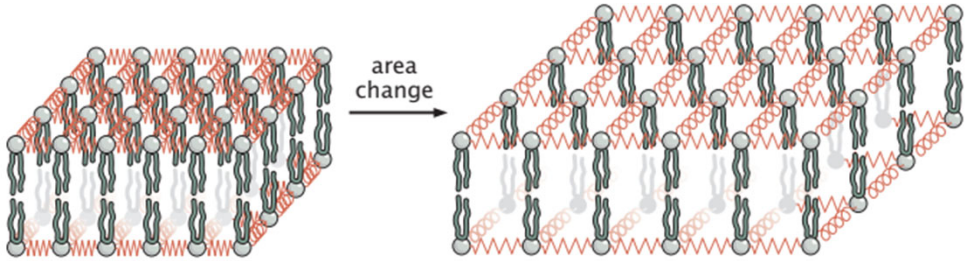
- stretching of a membrane
- changing in membrane curvature
- changes in membrane thickness

Energetics of changing membrane geometry

Types of membrane deformation



stretching (or compression): changing size by amount Δa



similar to a spring, with free energy $G_{\text{stretch}} = \frac{K_a}{2} \int \left(\frac{\Delta a}{a_0} \right)^2 \mathrm{d}a$

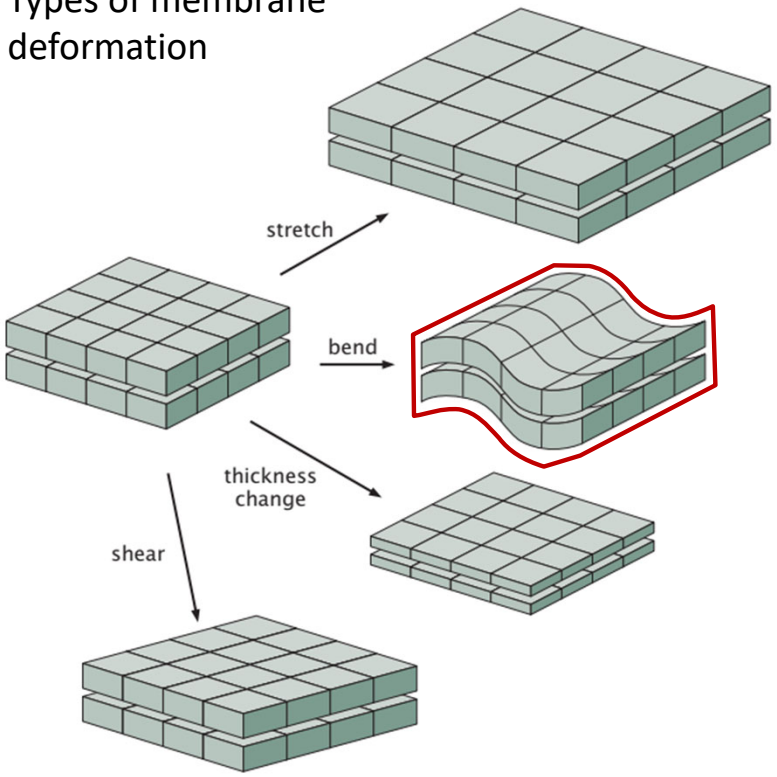
if stretching is uniform: $G_{\text{stretch}} = \frac{K_a}{2} \frac{\Delta a^2}{a_0}$

a_0: reference area
Δa: area change
K_a: area-stretch modulus, ~ 50-70 kT/nm^2

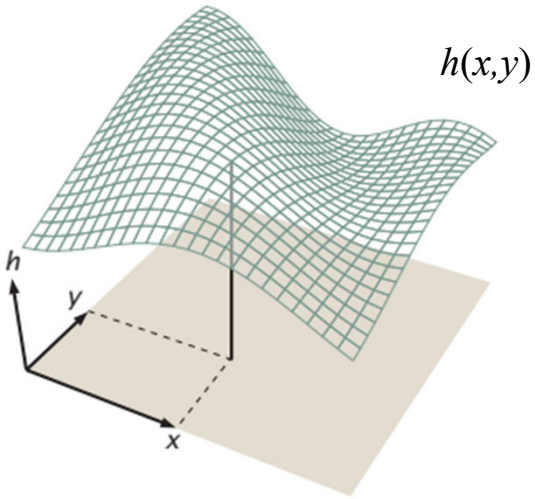
→ can impart forces on membrane components, e.g. protein channels

Energetics of changing membrane geometry

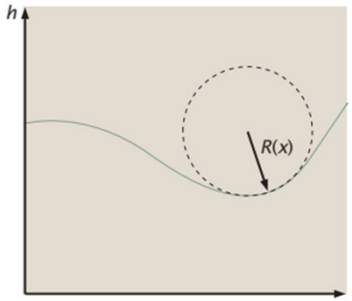
Types of membrane deformation



bending: changing height *h* at given coordinates

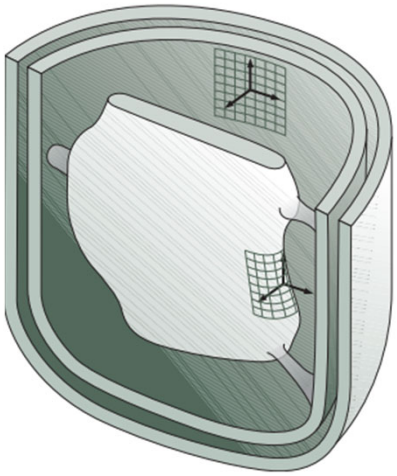


curvature



circle that fits to surface at point of interest

Membrane bending: Calculating curvature



How to find the curvature at a given spot?

1. Create **tangent plane** through the selected point

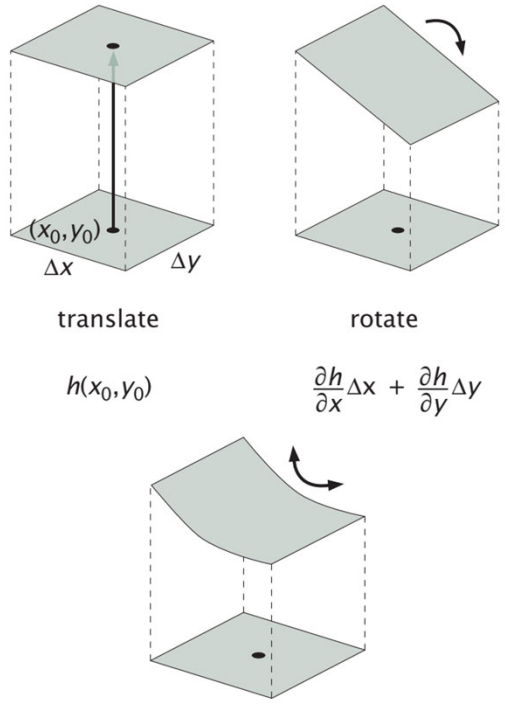
2. Expand **height function** in powers for x_1 and x_2 ($x_1 = x$, $x_2 = y$)

$$h(x_1, x_2) = \sum_{i,j=1}^{2} \kappa_{ij} x_i x_j \qquad \kappa_{ij} = \frac{\partial^2 h}{\partial x_i \partial x_j}$$

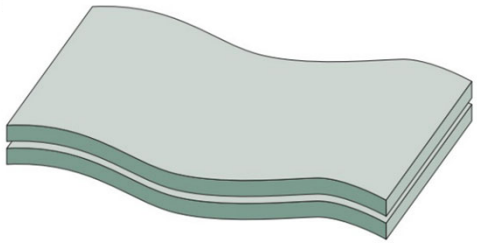
3. **Curvature** $\kappa = \begin{pmatrix} \kappa_{11} & \kappa_{12} \\ \kappa_{21} & \kappa_{22} \end{pmatrix}$

eigenvalues κ_1, κ_2 of this matrix:
principal curvatures, i.e. the two extreme values that characterize the curvature at this point

4. Interpretation



Free energy of membrane bending

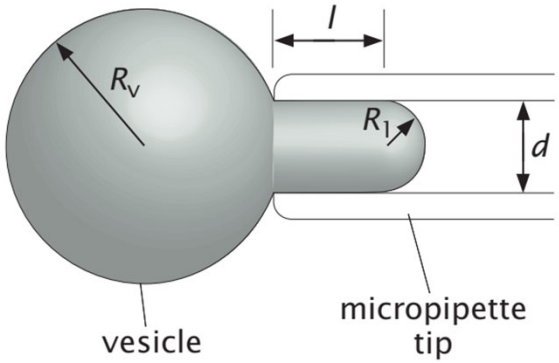


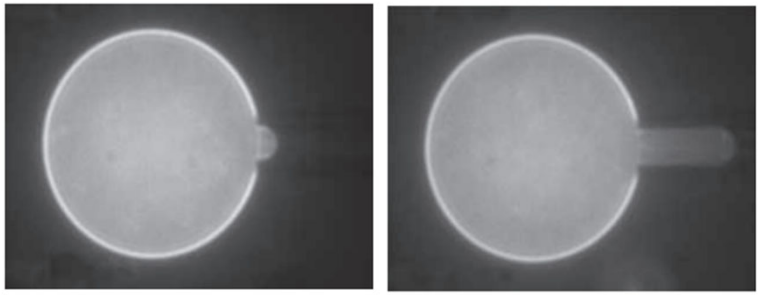
Chemically, bending involves:

- rearrangement of head & tail groups of lipids
- changes in enthalpic interactions and entropy

→ modeled as distortion of springs

Measurement:





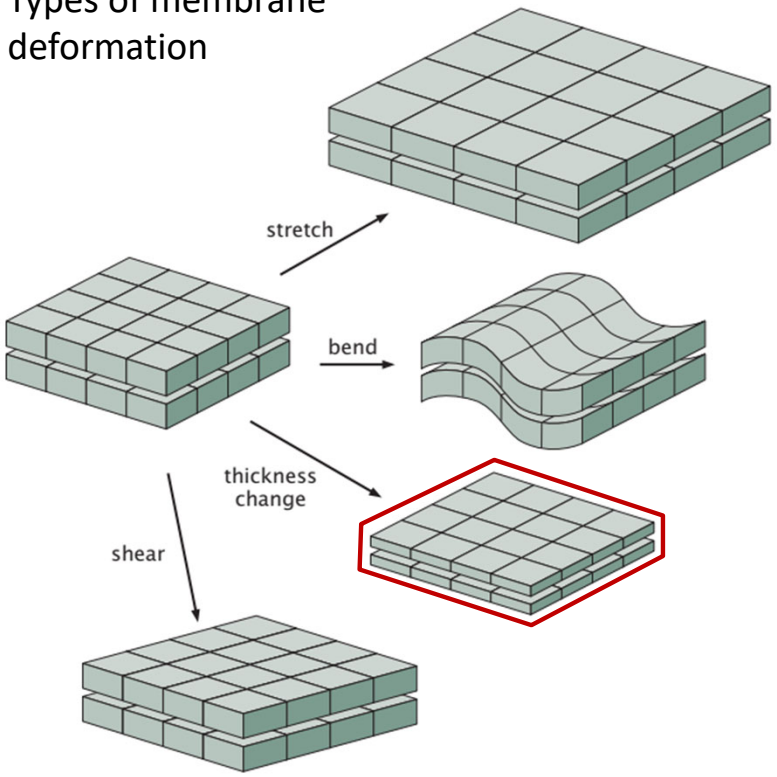
free energy $G_{bend}[h(x,y)] = \frac{K_b}{2}\int da[\kappa_1(\mathrm{x},\mathrm{y}) + \kappa_2(\mathrm{x},\mathrm{y})]^2$

calculated by visiting each spot on the surface and calculating the curvature, followed by summation of the terms (integral)

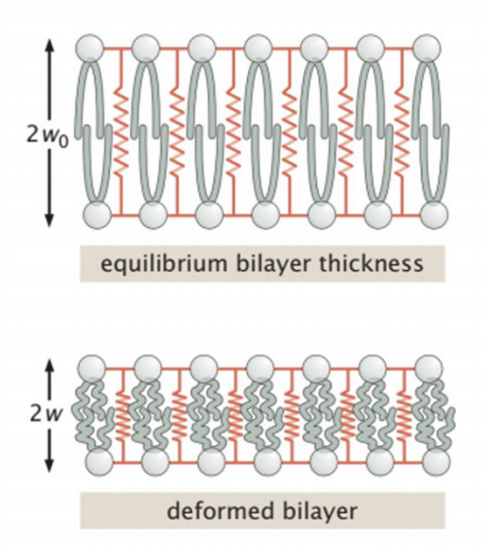
K_b: bending rigidity
~ 10-20 kT

Energetics of changing membrane geometry

Types of membrane deformation



Changes in membrane thickness:



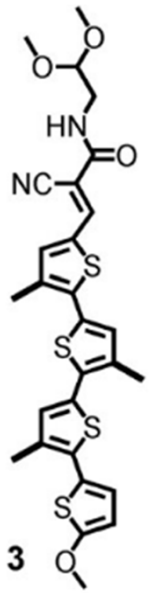
Free energy associated with membrane compression

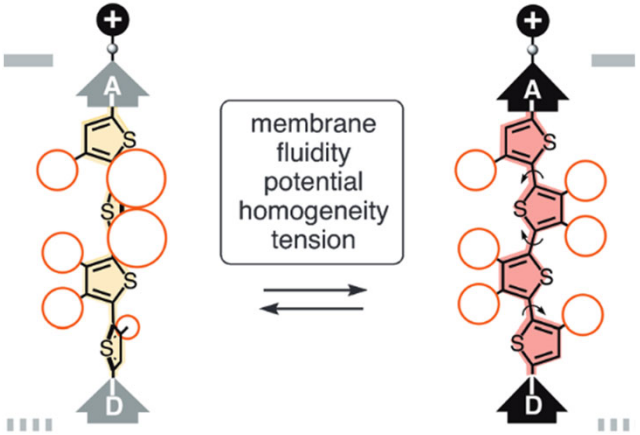
$$G_{thickness}[w(x,y)]$$

$$= \frac{K_t}{2}\int da\left[\frac{w(\mathrm{x},\mathrm{y}) + w_0}{w_0}\right]^2$$

K_t: stiffness
~ 60 kT/nm^2

Alternative way to probe membrane forces: Fluorescent probes





use as a membrane probe:
spectrum is influenced by environment

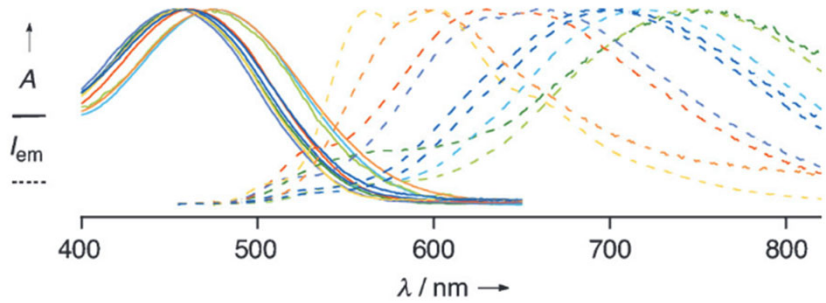
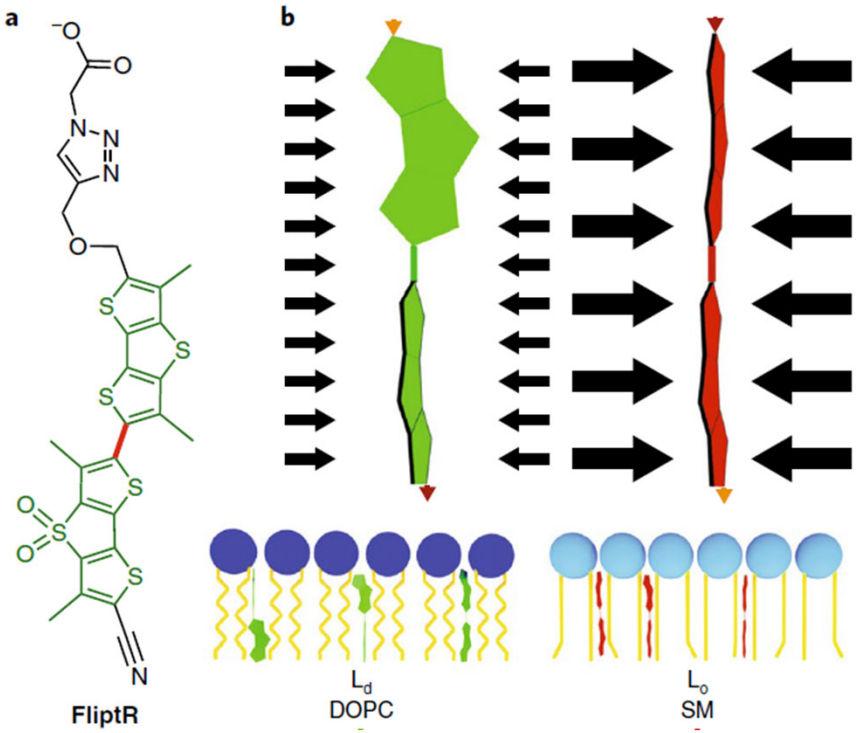


Figure 2. Absorption (solid) and emission (dotted) spectra of **3** in, with increasingly red-shifted emission, hexane (yellow), 2,2,2-trifluoroethanol (TFE; orange), dioxane (red), diethyl ether (light purple), ethyl acetate (deep purple), THF (blue), chloroform (cyan), acetone (dark green) and dichloromethane (light green).

oligothiophenes
→change color and fluorescence spectrum depending on scaffold conformation

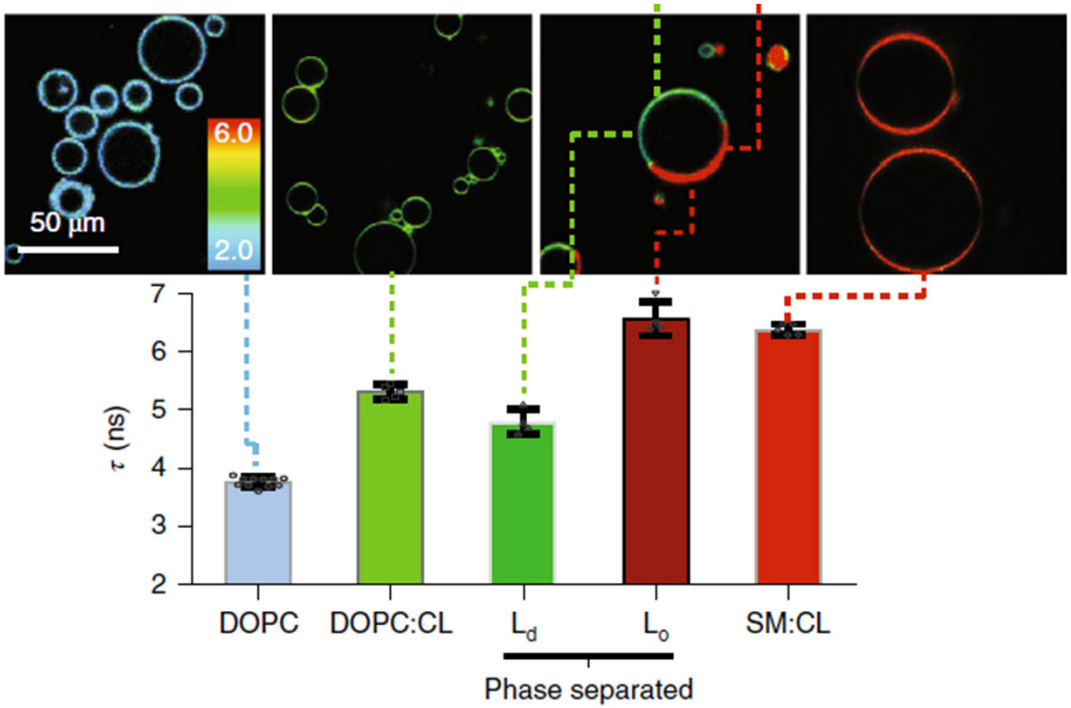
Fin et al., Angew Chem 2012
(Matile group, Univ. Geneva)

Effect of membrane tension/pressure on FliptR ('fluorescent lipid tension reporters')



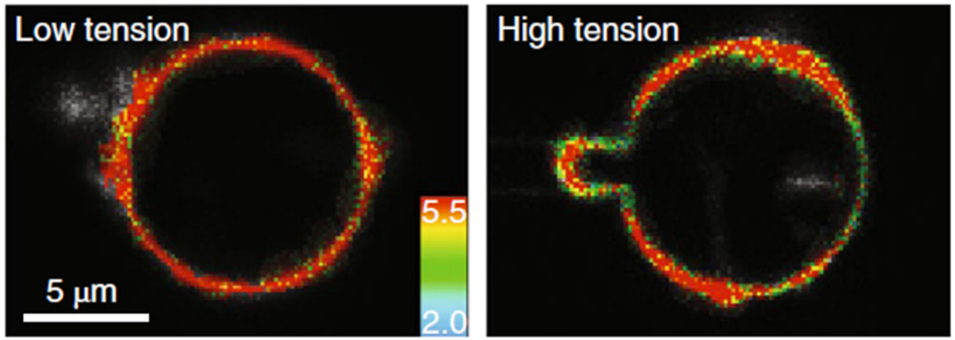
Colom et al., Nat Chem 2018 (Roux group, Univ Geneva)

fluorescent lifetimes (ns) change in different membrane environments



DOPC: Dipalmitoylphosphatidylcholine,
SM: Sphingomyelin, CL: cholesterol

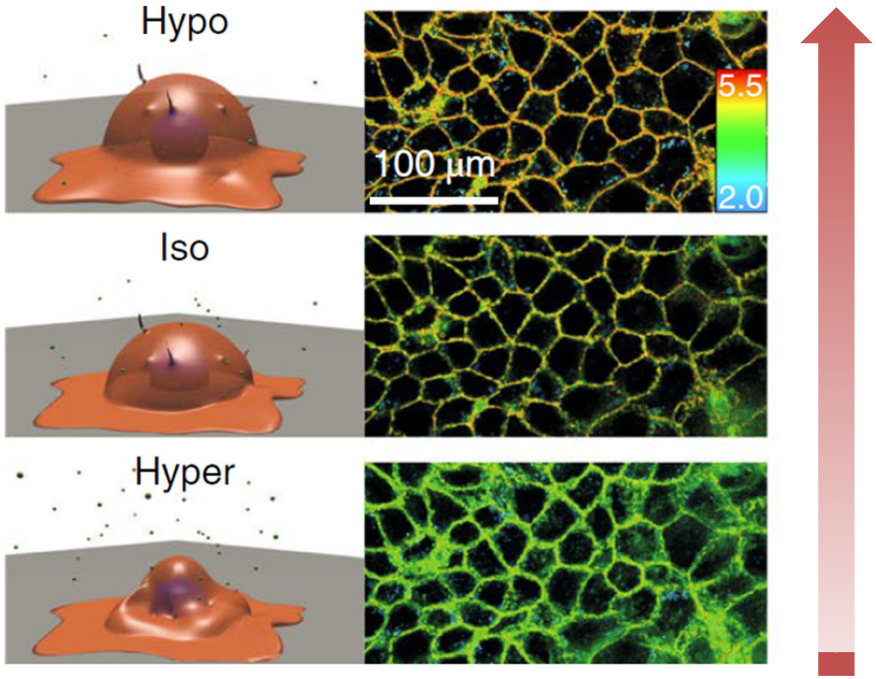
Monitoring membrane tension changes



used lipid mixture:
DOPC:SM:CL 30:30:40
→ forming phase separated domains

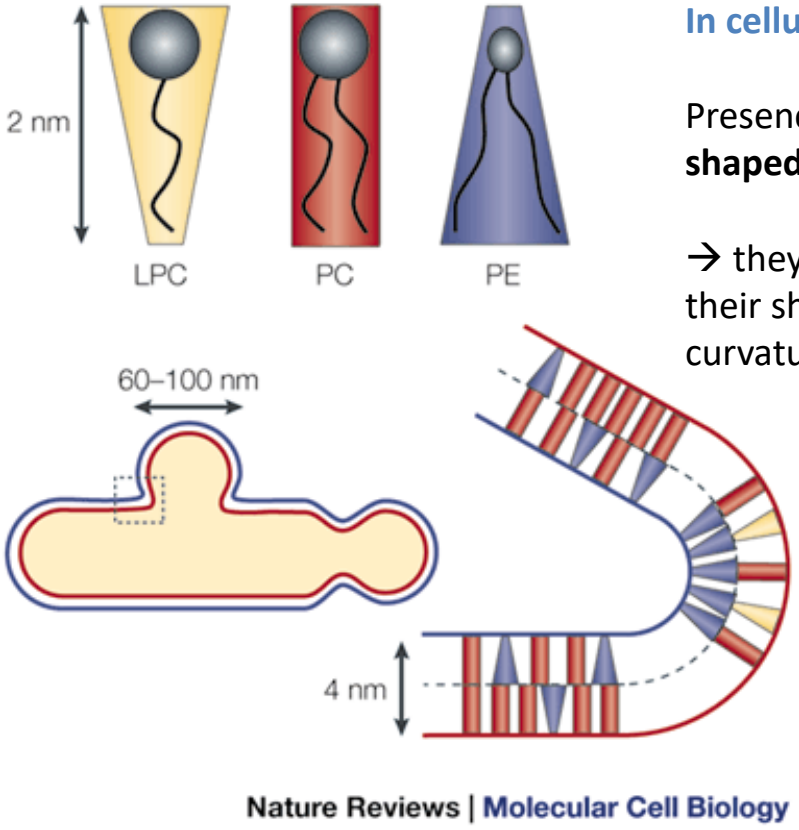
Aspiration → increased tension
in Lα domains: looser packing, decreased lifetime
in Lo domains: tighter packing, increased lifetimes

in living cells



Colom et al., Nat Chem 2018 (Roux group, Univ Geneva)

Membrane bending: Effects of lipids and proteins



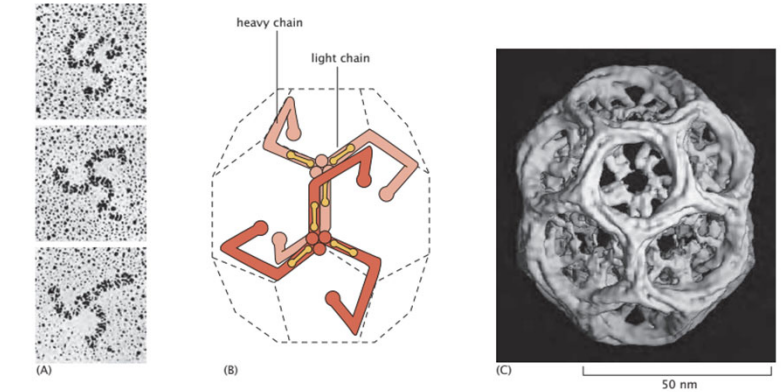
In cellular membranes:

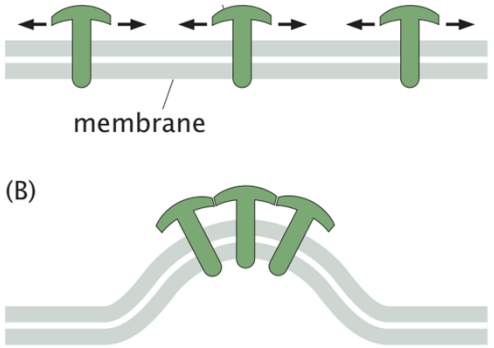
Presence of many **differently shaped lipids**

→ they accumulate according to their shape at points of high curvature

→ reduction of bending energy

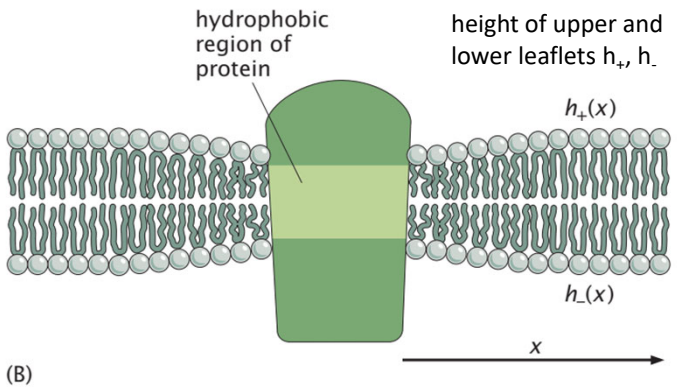
Proteins inducing membrane bending: Clathrin coated vesicles





Interplay between membrane deformation and proteins

Membrane proteins lead to **local deformation**



midplane of bilayer $h(x) = \frac{h_+(x) + h_-(x)}{2}$

half-width of bilayer $w(x) = \frac{h_+(x) - h_-(x)}{2}$

bending energy

$$G_{bend}[h_+(x), h_-(x)] = \frac{K'_b}{2}\int dx\left[\left(\frac{\partial^2 h_+(x)}{\partial x^2}\right)^2 + \left(\frac{\partial^2 h_-(x)}{\partial x^2}\right)^2\right]$$

K'b = bending modulus for a single leaflet

rewrite for h(x), w(x)

$$G_{bend}[h(x), u(x)] = \frac{K_b}{2}\int dx\left[\left(\frac{\partial^2 h(x)}{\partial x^2}\right)^2 + \left(\frac{\partial^2 w(x)}{\partial x^2}\right)^2\right]$$

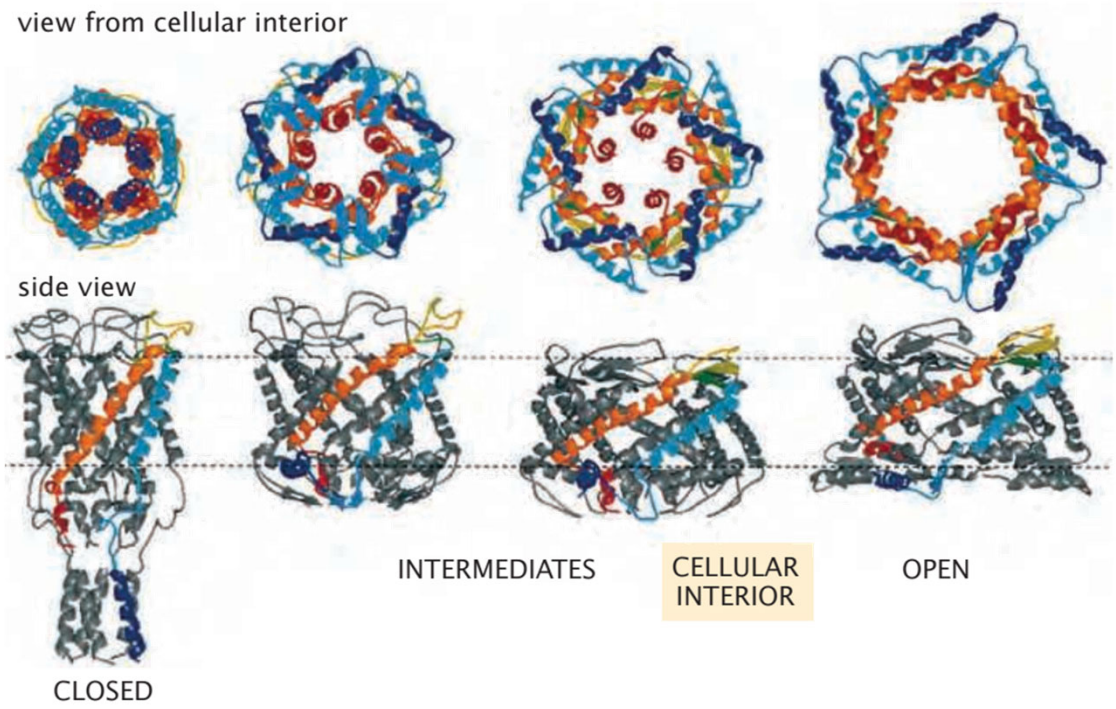
with $u(x) = w(x) - w_0$

membrane bending

thickness variation

what is the effect on protein channels?

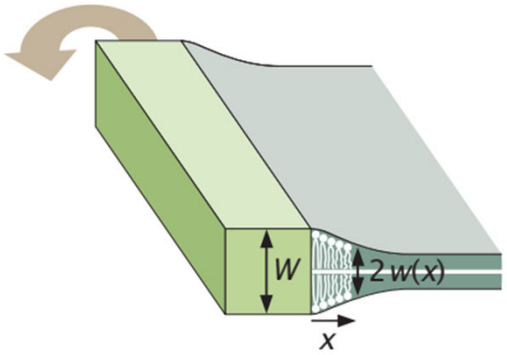
Example: A mechanosensitive channel

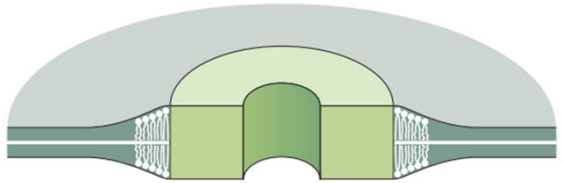


The Large Conductance Mechanosensitive Ion **Channel** (**MscL**) Family

found in E. coli, S. aureus and M. tuberculosis

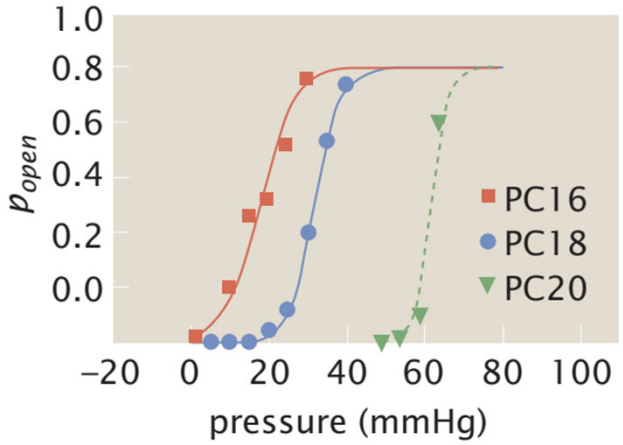
Channel open probability is modulate by the membrane





Energies acting on the channel

$$G = \underbrace{\frac{K_b}{2}\int_R^{\infty}\left(\frac{d^2u}{dx^2}\right)^2 dx}_{\text{bending at interface}} + \underbrace{\frac{K_t}{2w_0^2}\int_R^{\infty}u(x)^2\,dx}_{\text{hydrophobic mismatch}} + \underbrace{G_{tension}}_{\text{membrane tension}}$$



Resulting effect on channel:

open probability depends on tension and membrane environment