

In cells, interactions are managed by compartmentalization



David Goodsell

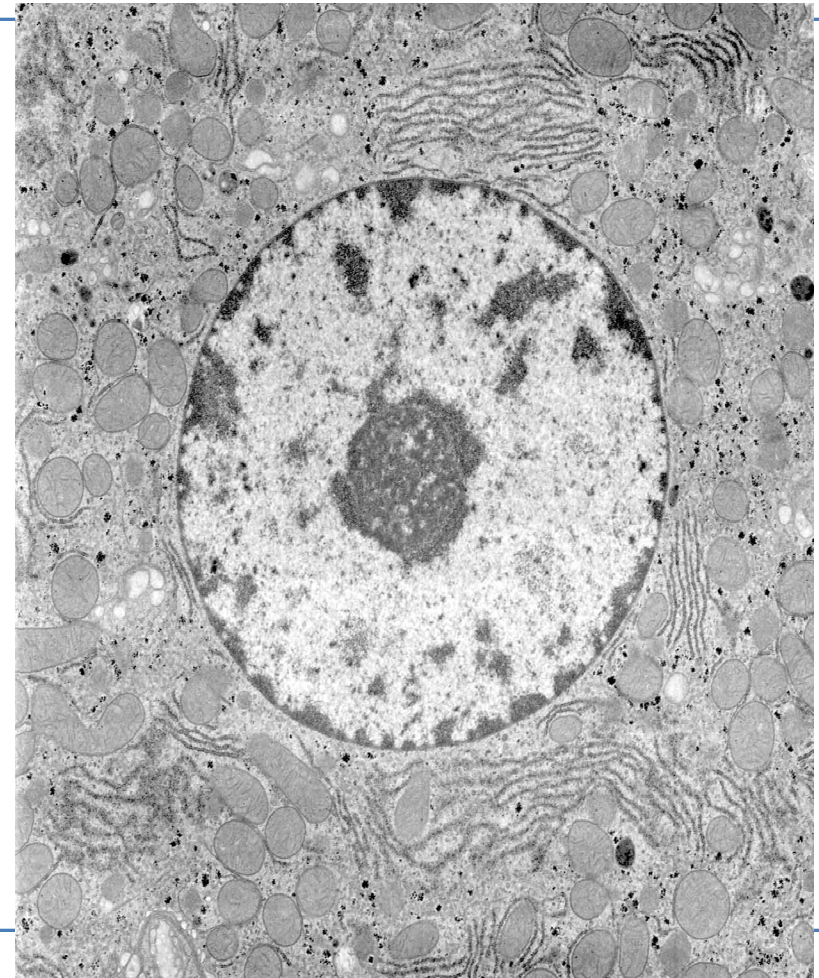
Looking at a cell by electron microscopy

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 $\sim 50 \text{ \AA}^2$ area and ~ 2 nm long
- **Diffusion**
 - lipid in membrane 10^{-2} to $1 \text{ \mu m}^2/\text{s}$
- **Potential**
 - $-80 \text{ mV} \Rightarrow 20 \text{ MV/m}$



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
- ..

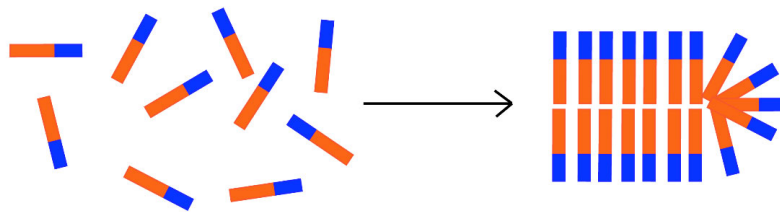
Video from Steven Block

Membrane lipids

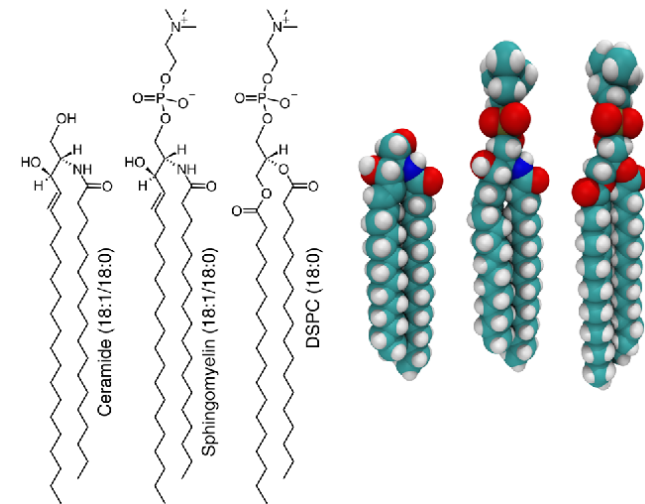
General structure of membrane components

HYDROPHOBIC HYDROPHILIC

membrane formation through self-assembly in water



- flexible
- dynamic
- self healing



Major lipid classes:

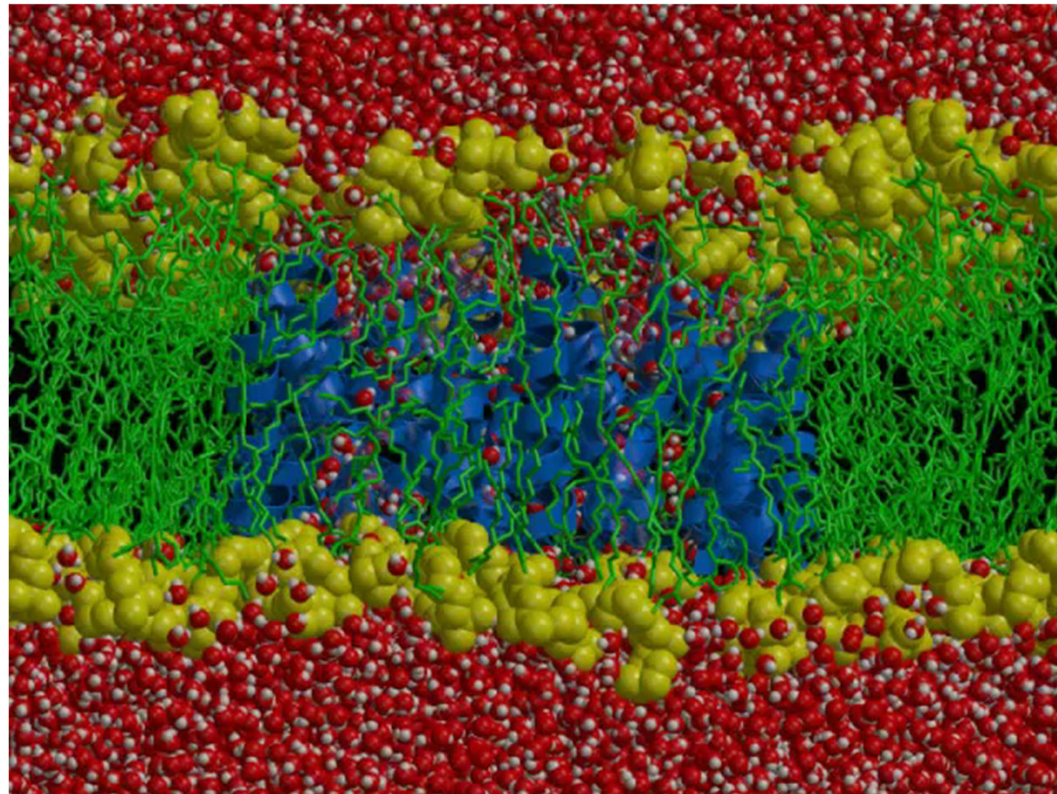
- Glycero-phospholipids
- Phospho-sphingolipids
- Sterols

=> Biochemistry_I & _II

Architecture of membranes

all is moving!

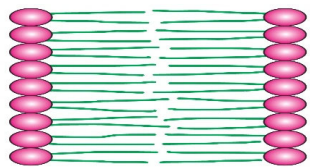
H. Grubmueller :
nanosecond
simulation of a
bilayer with
aquaporin



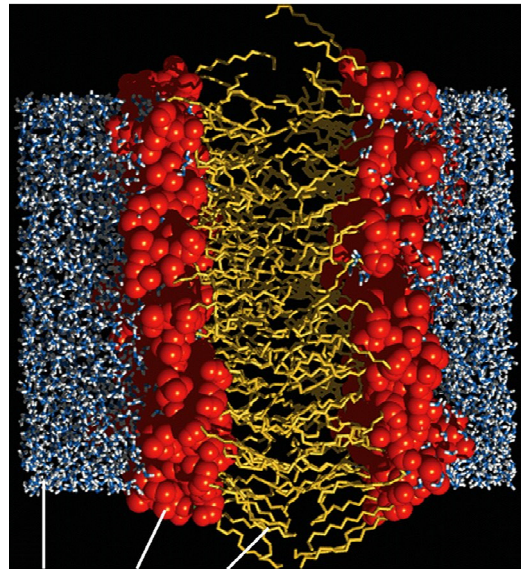
The dynamic nature of membranes

Diffraction studies:

- Distributions are wide:
 - Reflecting simulations
 - Rejecting

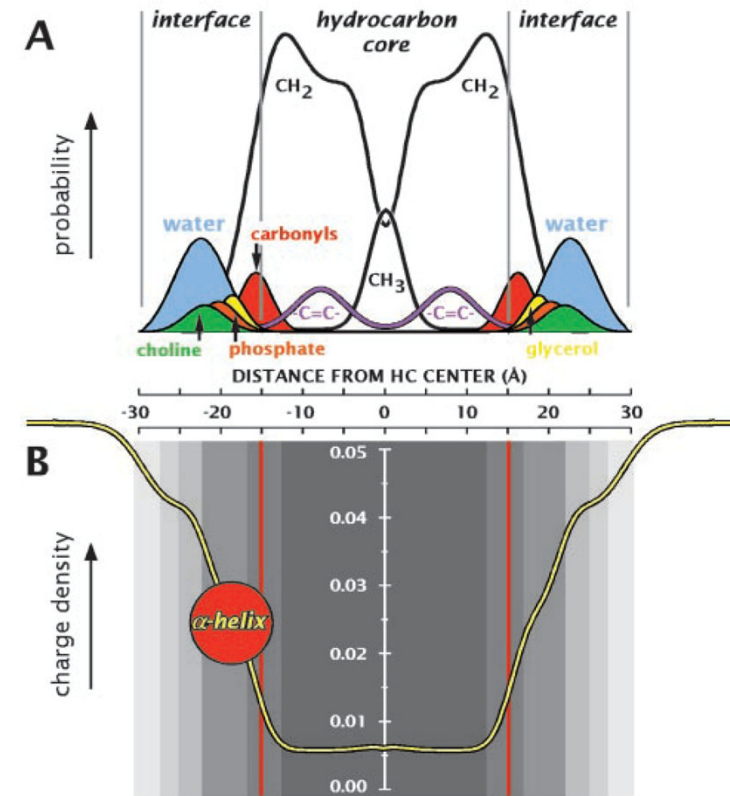


- Hydrophobic core of $\pm 25 \text{ \AA}$
- Interfacial region of $\pm 15 \text{ \AA}$ with a large range of properties.
- Dielectric constant :
 - ϵ_r is ~ 80 in water
 - ~ 2 in hydrophobic core

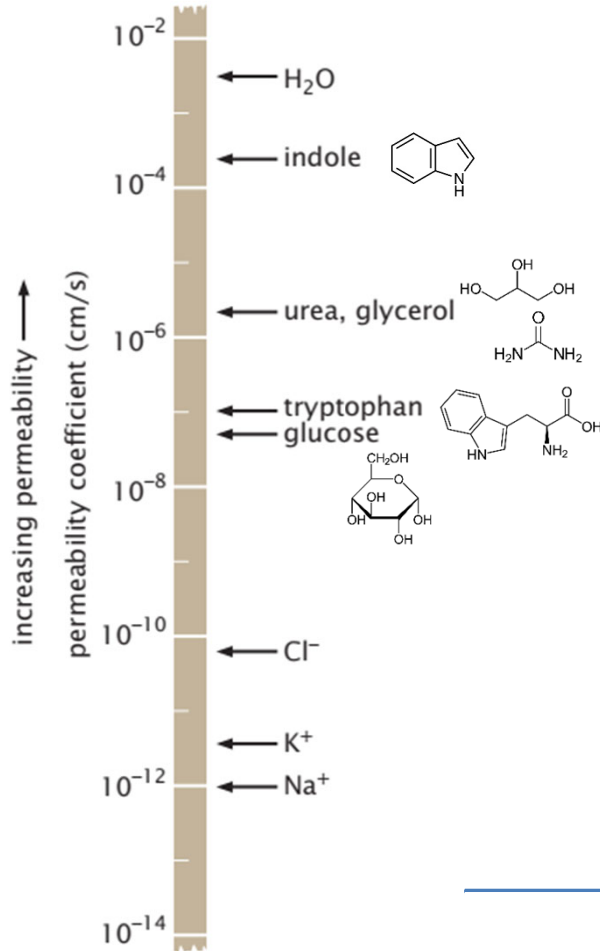


water head tails
groups

Chiu et al., Biophys J., 1995



Membrane permeability



Permeability:

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

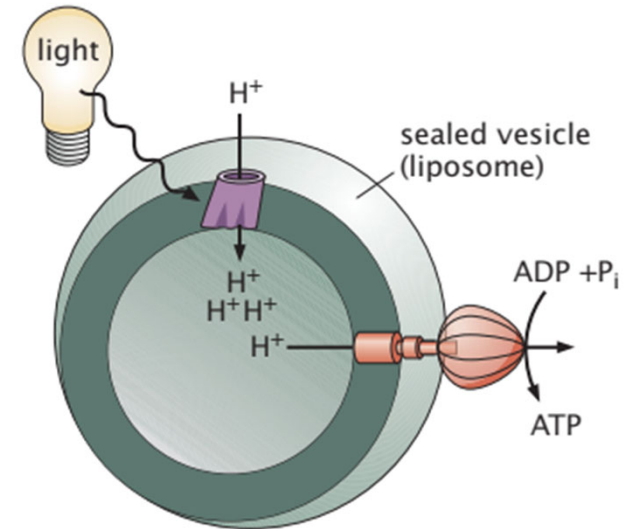
P: permeability coefficient (m/s)

Water conc./pressure can easily equilibrate across membrane

other molecules:

transporters or channels required

→ gradients can be used to generate energy



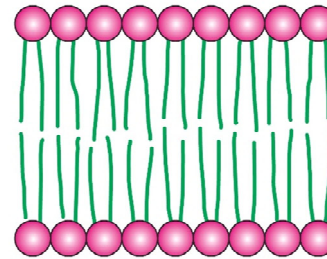
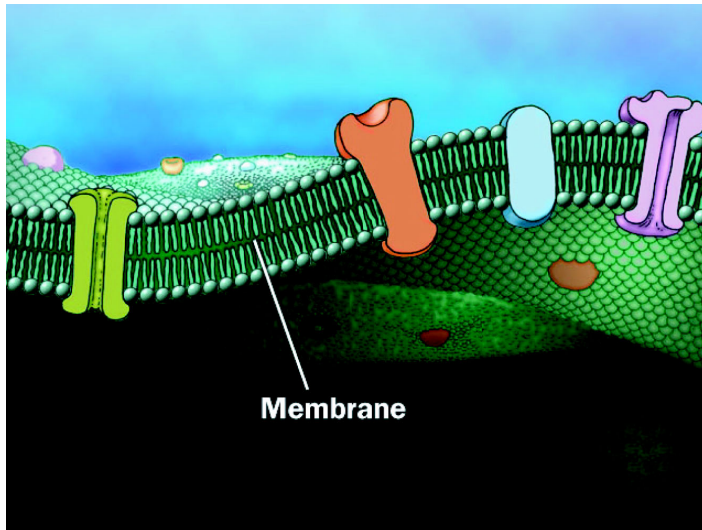
Example:

Bacteriorhodopsin and ATPase

Architecture of membranes

1925

Lipidic bilayer (Gorter & Grendel)



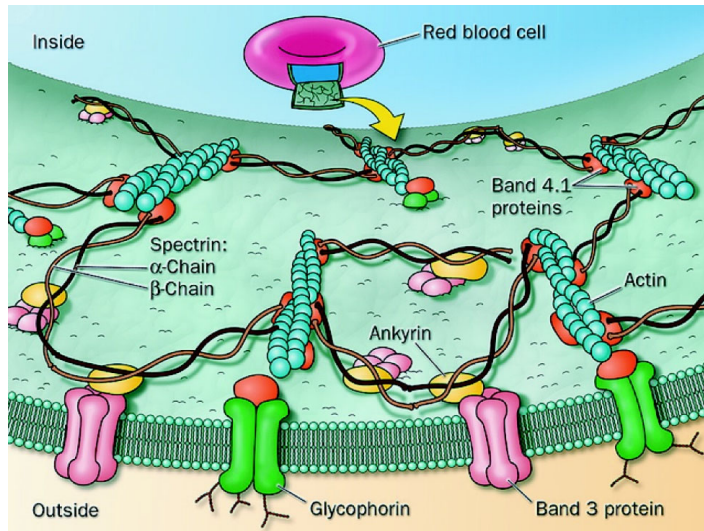
1972

Fluid mosaic model

(Singer & Nicolson):

Free diffusion of all components within the membrane.

Architecture of membranes



1972

Fluid mosaic model

(Singer & Nicolson):

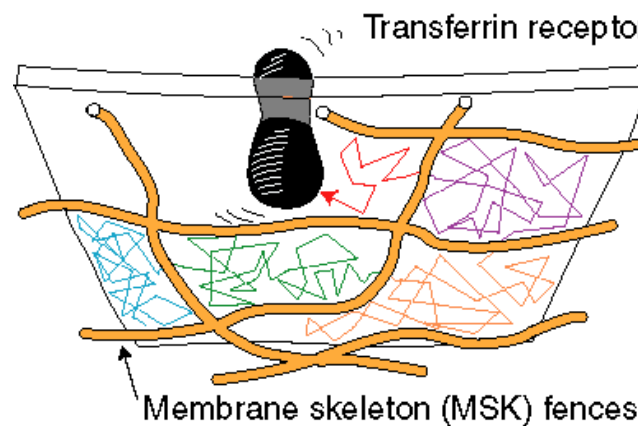
Free diffusion of all components

within the membrane.

A skeleton of membrane-attached proteins:

- mechanical stability
- scaffold for molecules
- diffusion barrier

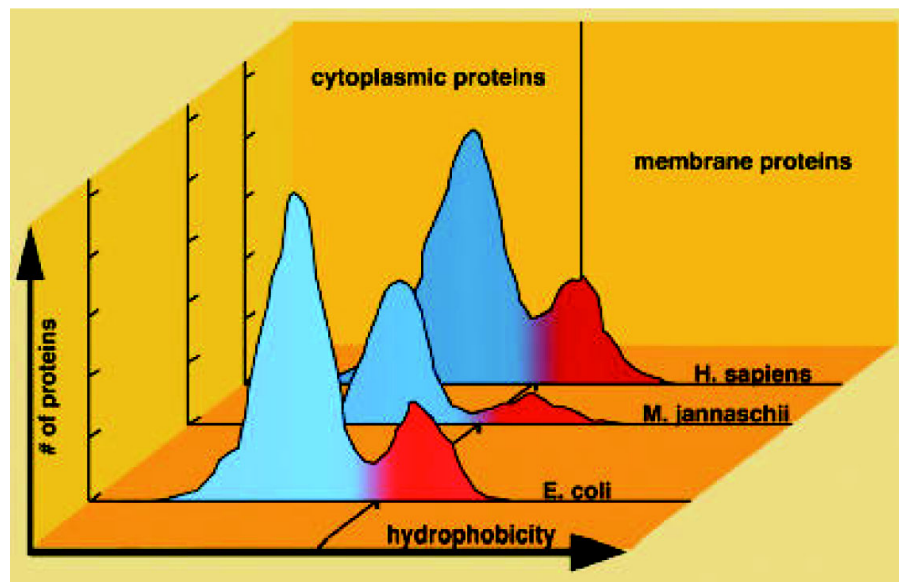
Membrane Skeleton Fence



Akihiro Kusumi, ~1999

Membrane proteins

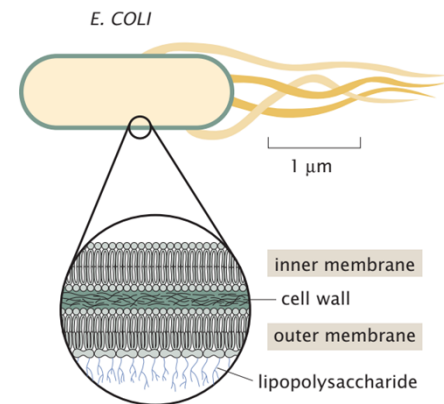
- Genome-wide analysis of mean hydrophobicity of proteins:



- => 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: $6 \mu\text{m}^2$, thus 12nm^2 per individual protein
- Average distance between proteins: 3.5 nm

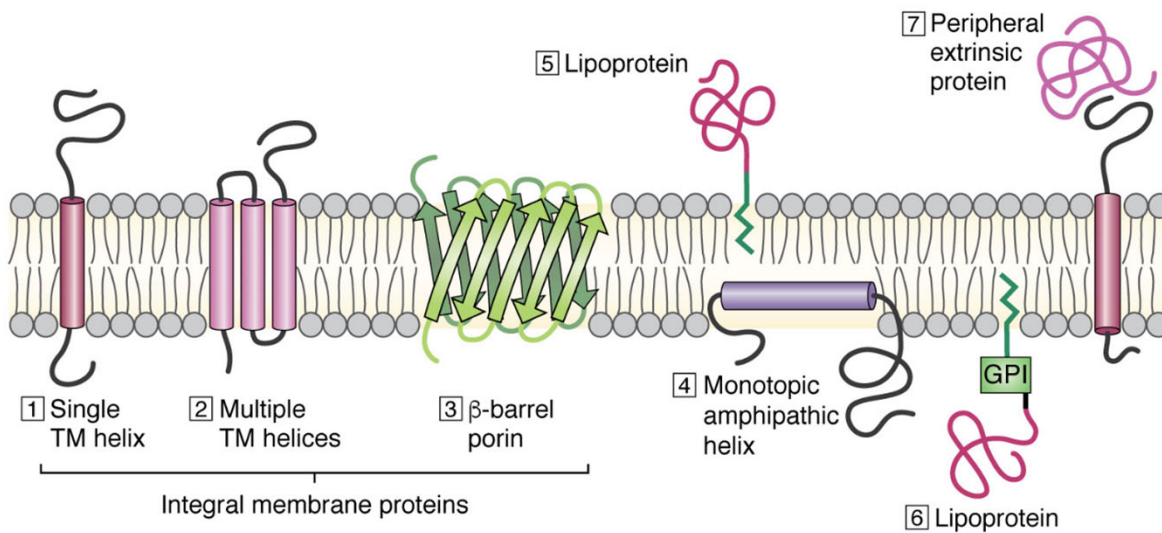


In mitochondria:

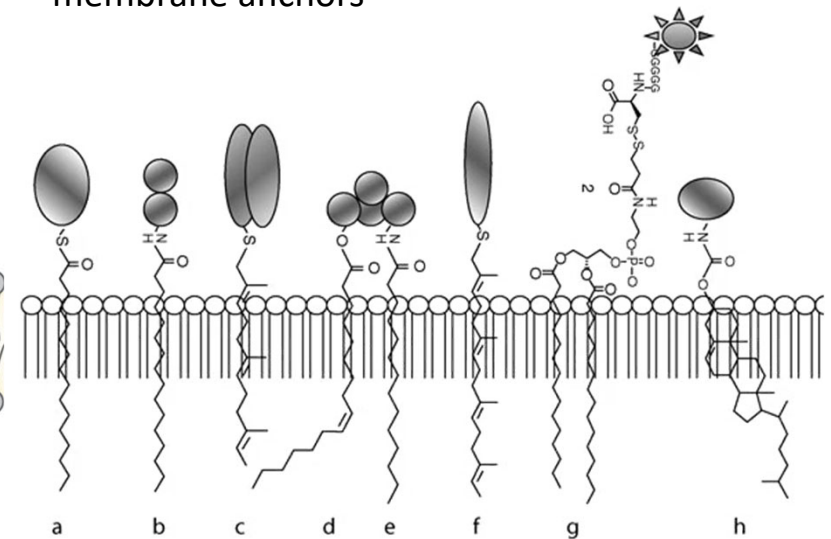
- ca. 70% of membrane mass comes from proteins

Integral or peripheral membrane proteins

Membrane proteins



membrane anchors



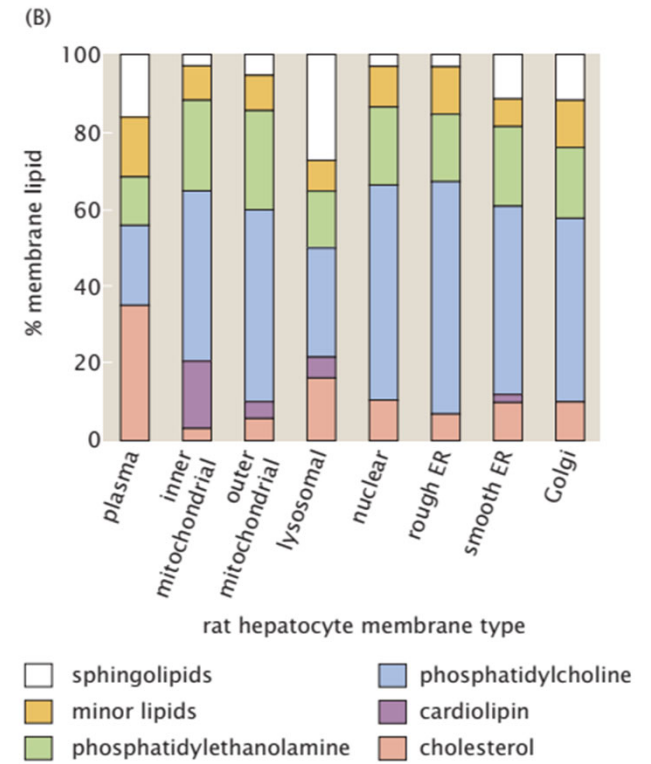
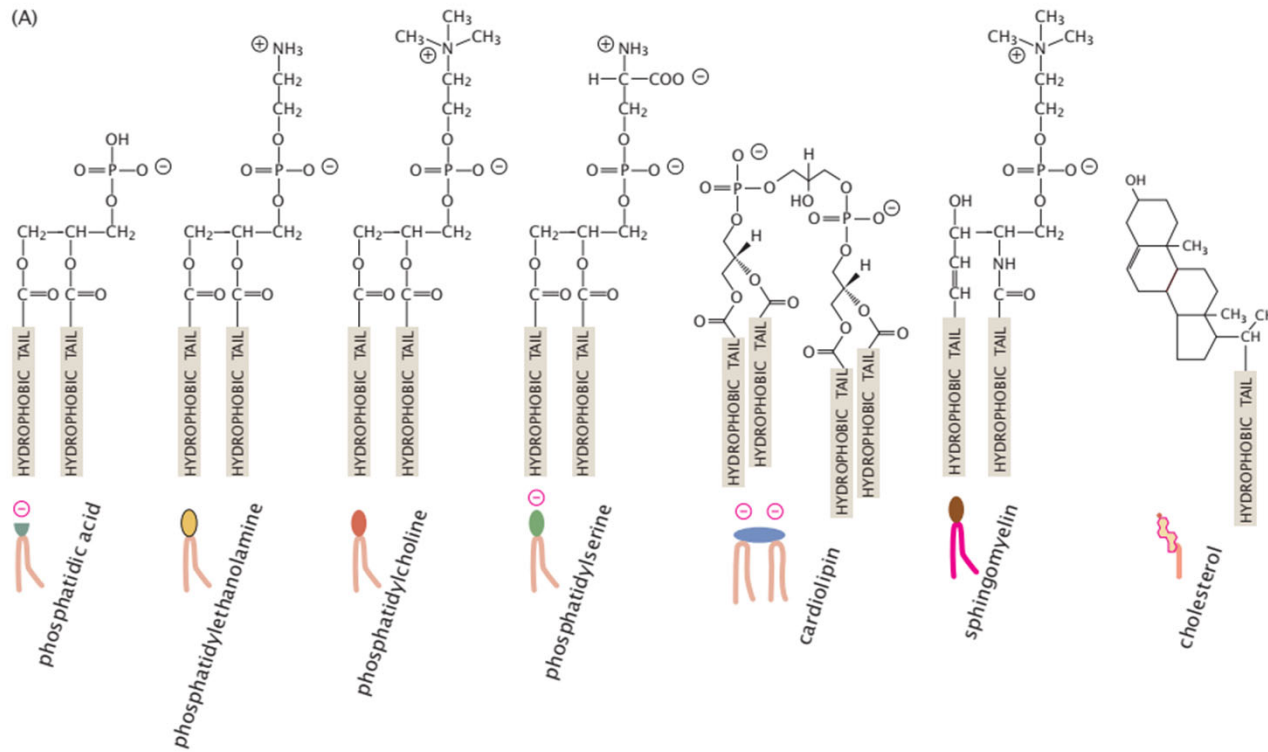
a: s-palmitoylated
 b: N-palmytoylated
 c: farnesylated
 d: O-acylated

e: N-myristoylated
 f: geranylgeranylated
 g: GPI-anchored
 h: cholesterol modified

Puthenveetil & Vinogradova, JBC 2019

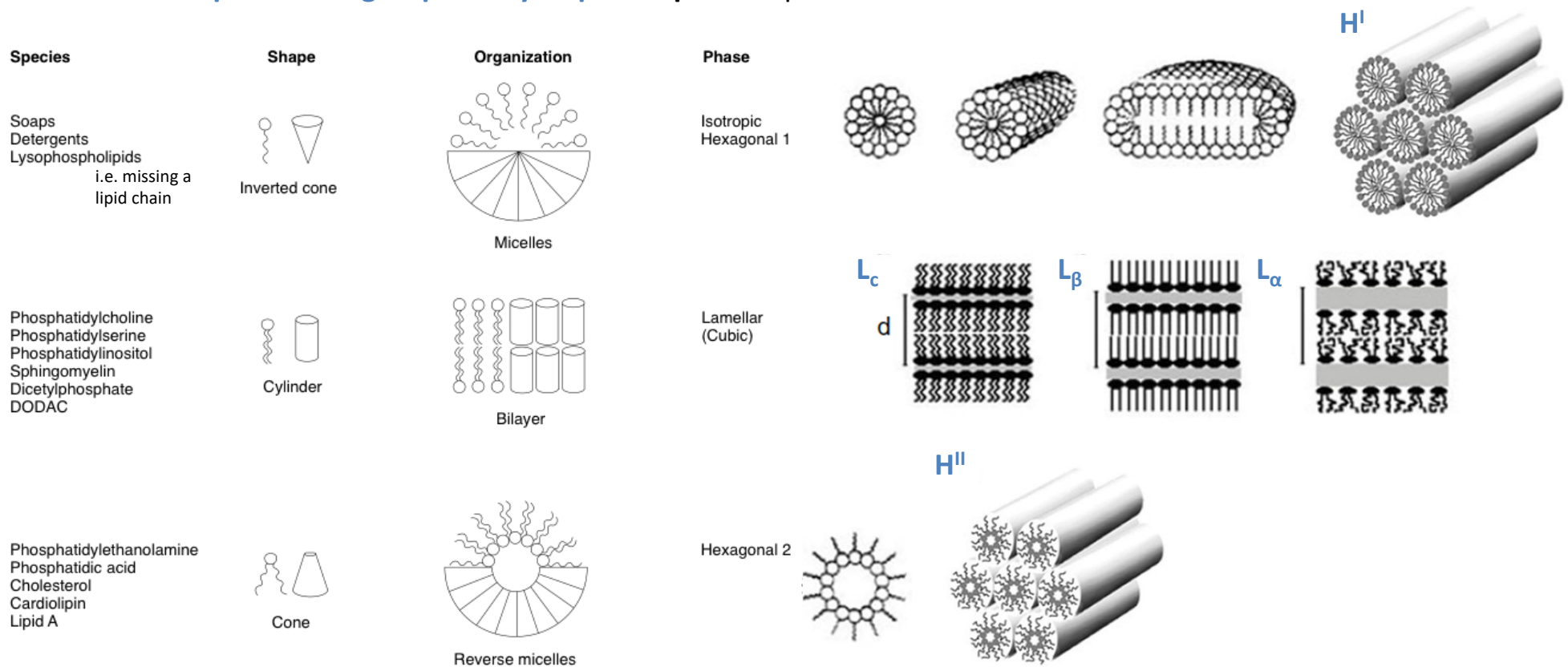
Nalivaeva & Turner, HB of Neurochem & Mol Neurobiol p. 11

A closer look - Membrane diversity in living cells

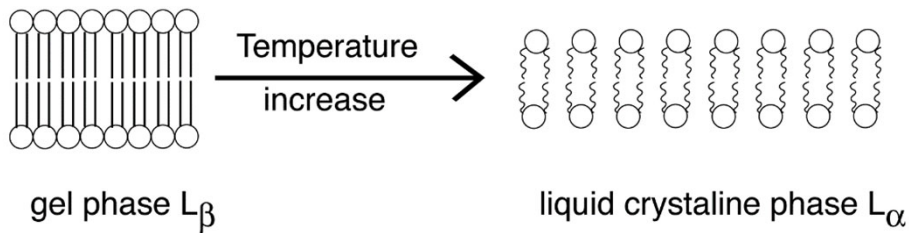


Lipid polymorphism : Structure-shape concept

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



Lipid phase transitions

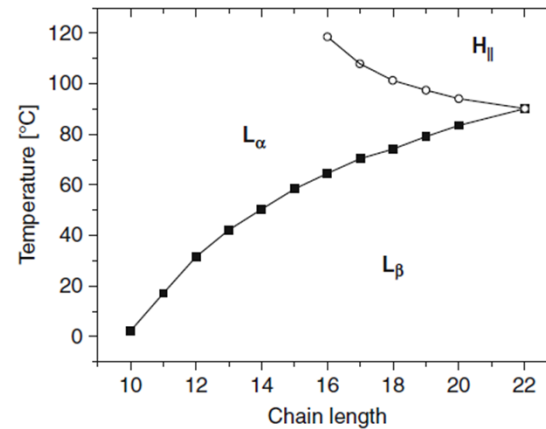


ordered phase transition temperature

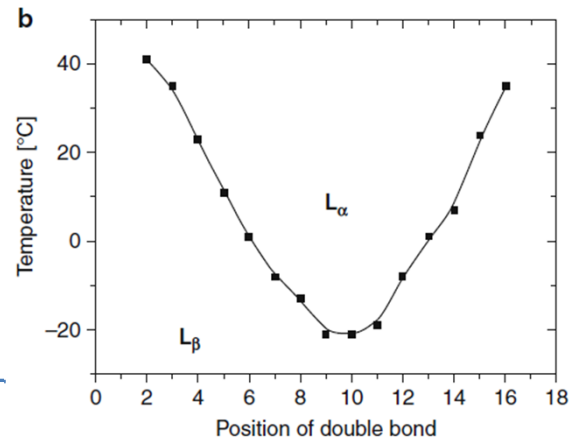
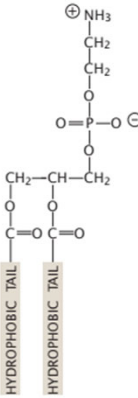
slow lateral diffusion
 $D = 10^{-10} \text{ cm}^2\text{s}^{-1}$

disordered fast lateral diffusion
 $D = 10^{-7} \text{ cm}^2\text{s}^{-1}$

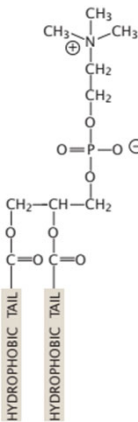
phase transitions behavior depends on the type of lipids, i.e. their **chemical structure**



for saturated diacyl phosphatidylethanolamine bilayers



for dioctadecenoyl phosphatidylcholine bilayers



Koyonova & Tenchov
 Encyclopedia of biophysics

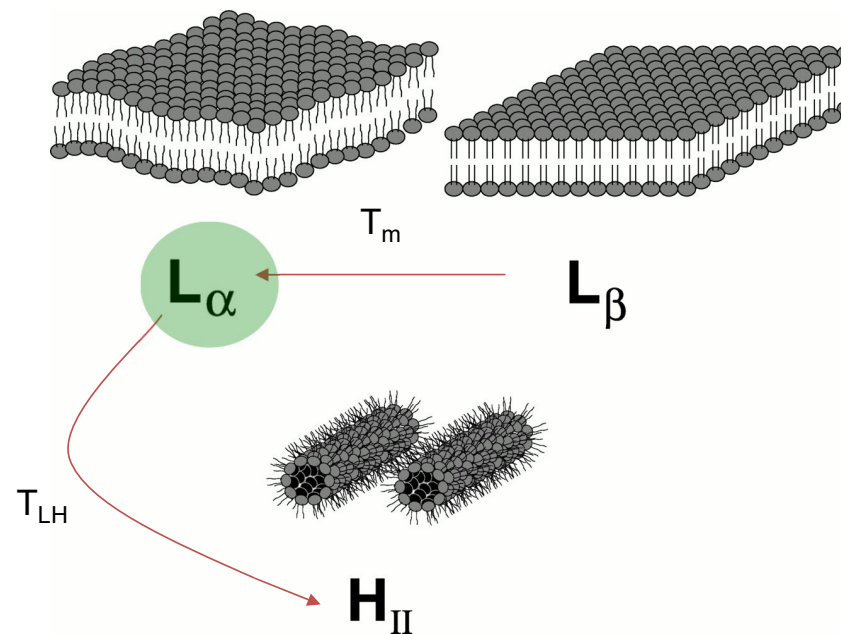
Shape changes in response to temperature variations

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

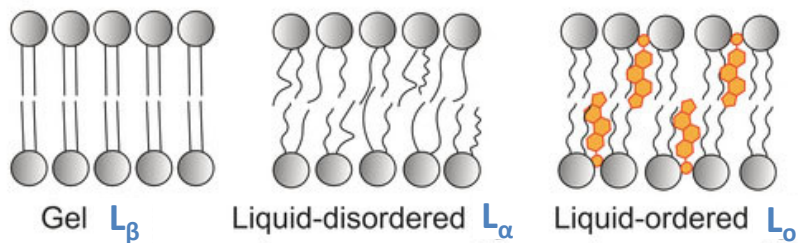
=> 7 to 17 °C above T_m &
10 to 15 °C below T_{LH}

Quiz:

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

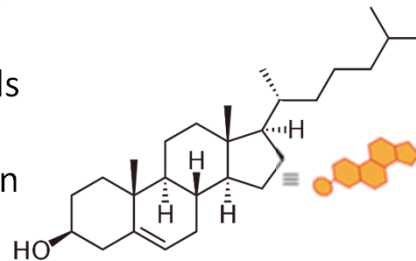


Cholesterol alters the behavior of membranes



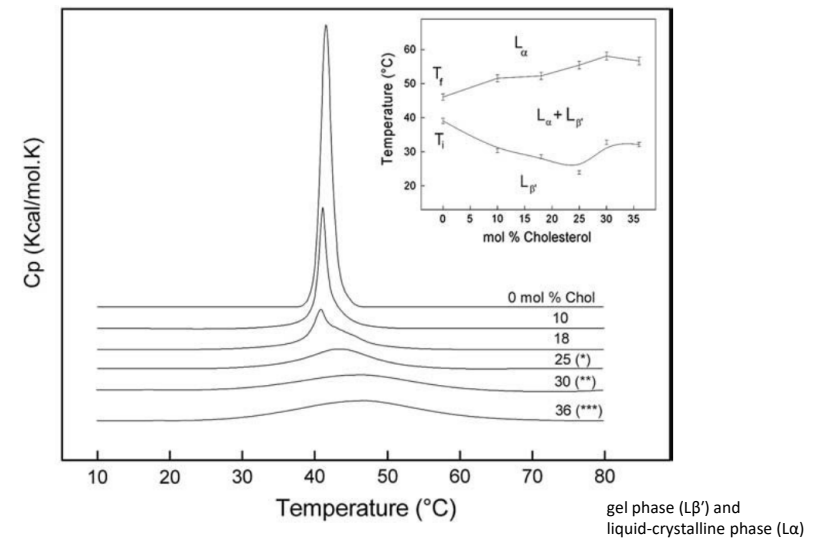
Presence of cholesterol: formation of a new liquid ordered (L_o) phase

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



→ less fluidity than L_{α} , but less well packed

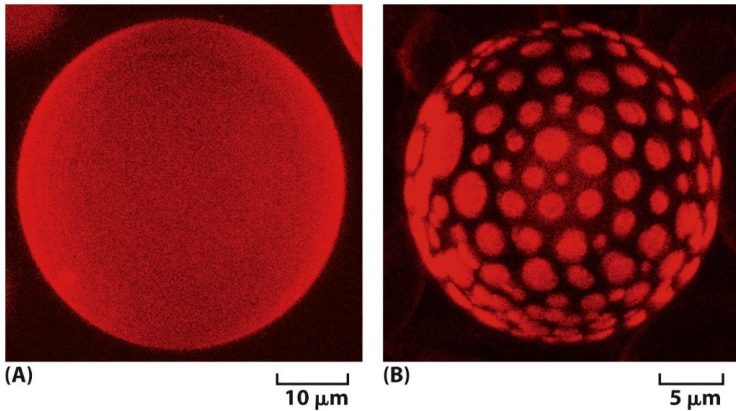
DSC analysis of membrane phase transition



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

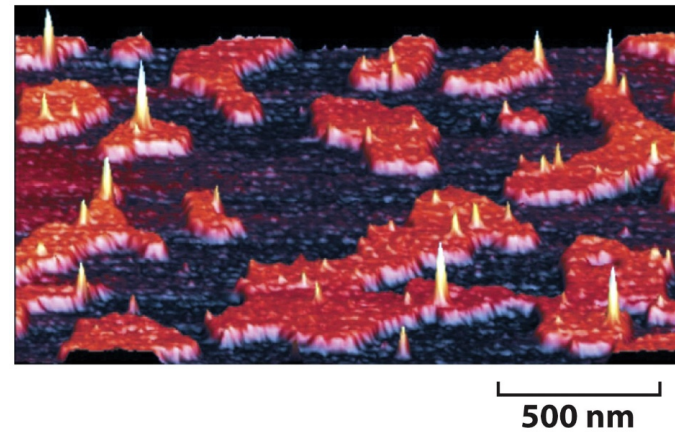
The presence of cholesterol can induce lipid phase coexistence

Lipid mixtures in giant liposomes

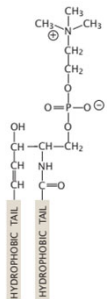


W. Webb
Nature 2003

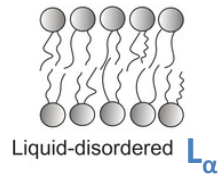
AFM imaging of L_0 phases in bilayers



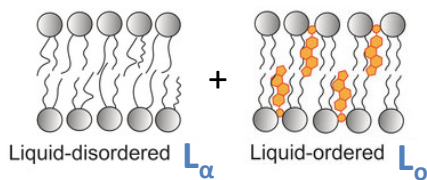
L_0 phases are thicker, compared to L_α
 → this is due to lower amount of disorder and interdigitation of aliphatic chains.



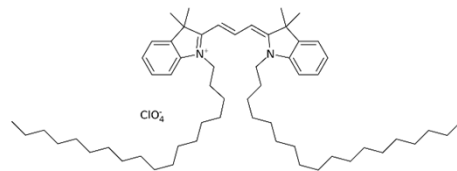
phosphatidylcholine
+ sphingomyelin



phosphatidylcholine
+ sphingomyelin + cholesterol

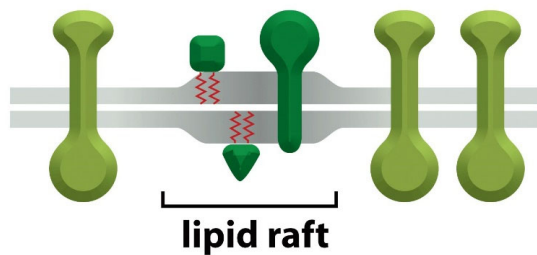


staining: Dye which
partitions into L_α
(DiI-C18)



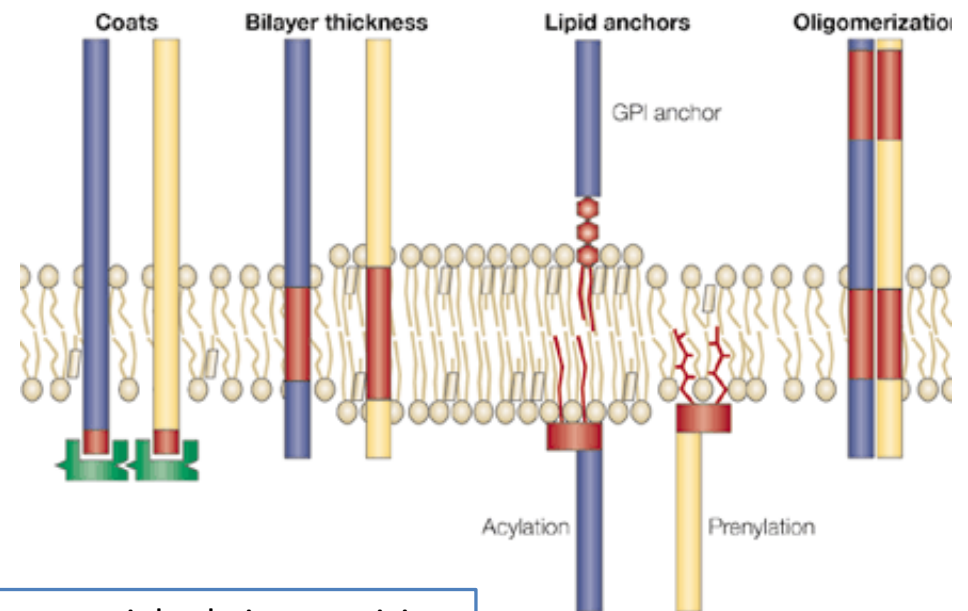
Phase separations within membranes

Co-existence 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

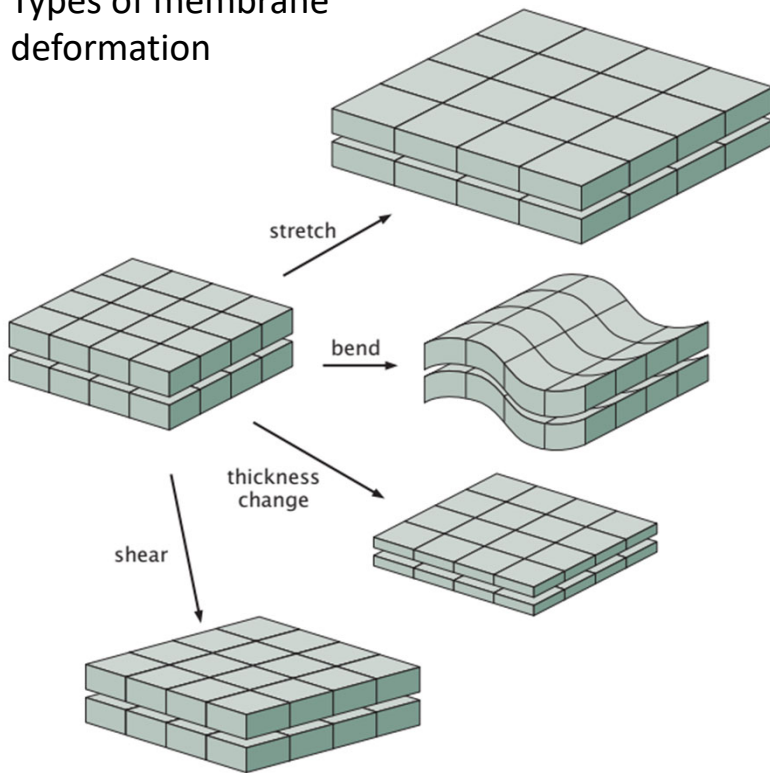


→ potential role in organizing cell signaling processes

Nature Reviews | Molecular Cell Biology

Energetics of changing membrane geometry

Types of membrane deformation



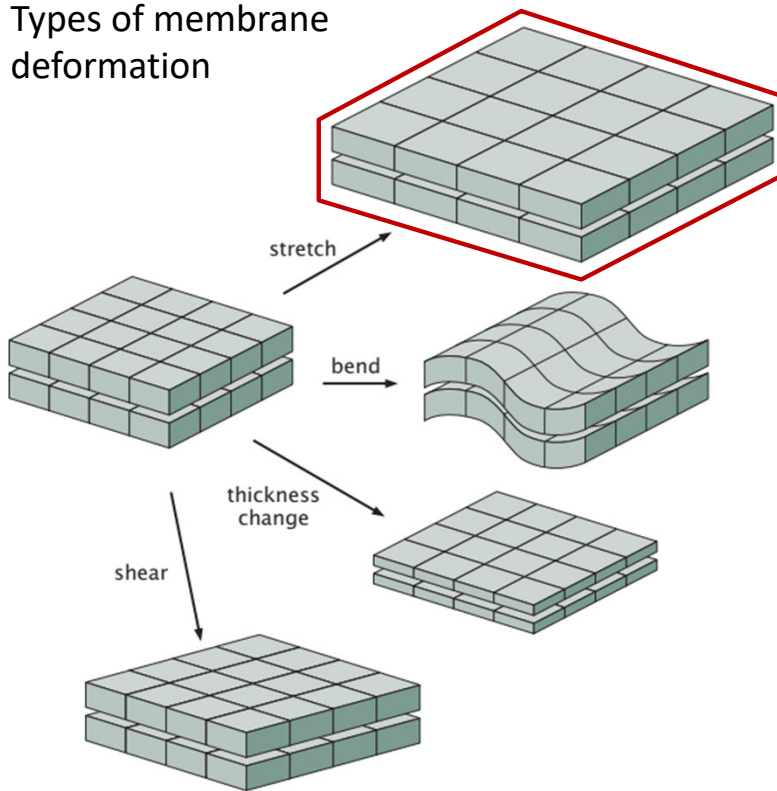
Quiz:

What situations in the life of a cell lead to:

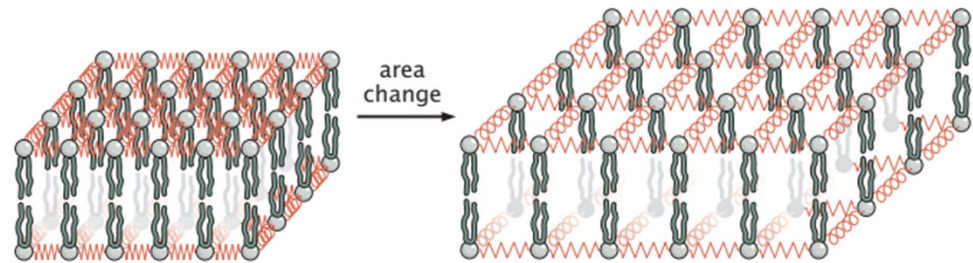
- stretching of a membrane
- changing in membrane curvature
- changes in membrane thickness

Energetics of changing membrane geometry

Types of membrane deformation



stretching (or compression): changing size by amount Δa



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

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

a_0 : reference area

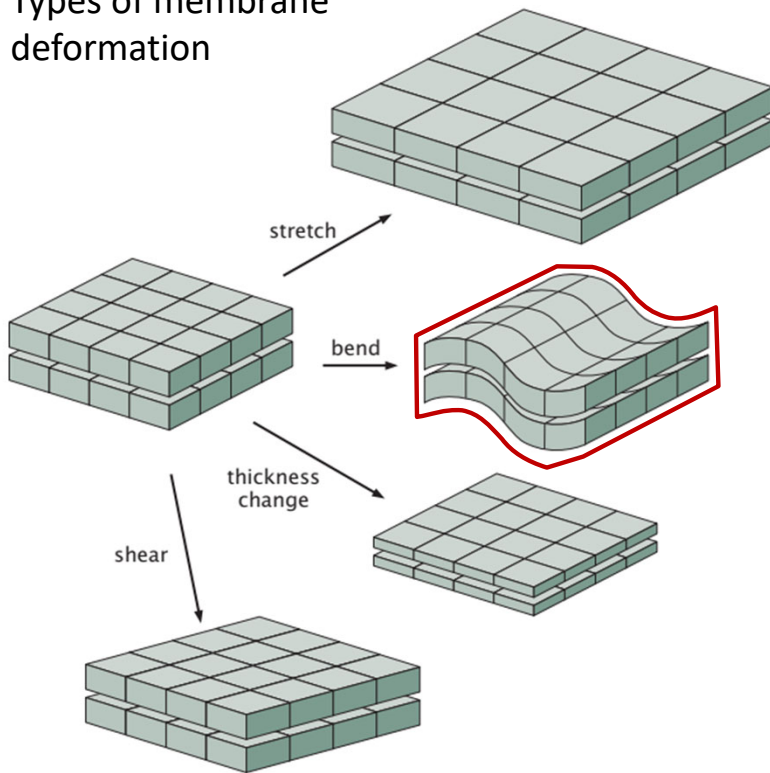
Δa : area change

K_a : area-stretch modulus, $\sim 50\text{-}70 \text{ kT/nm}^2$

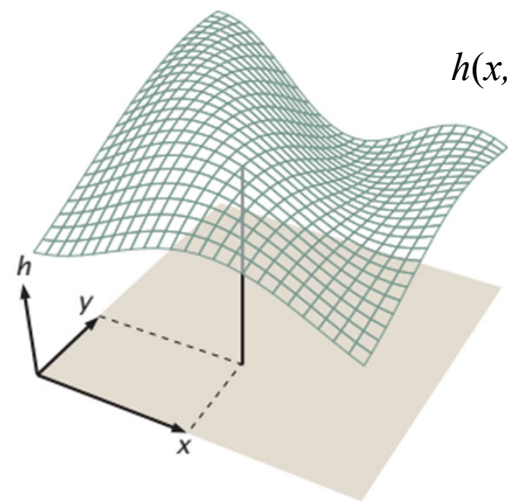
→ can impart forces on membrane components, e.g. protein channels

Energetics of changing membrane geometry

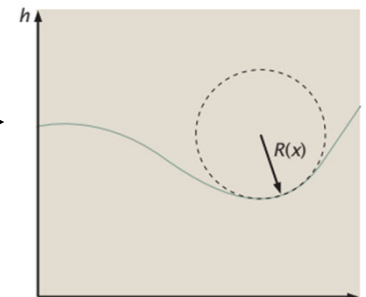
Types of membrane deformation



bending: changing height h at given coordinates

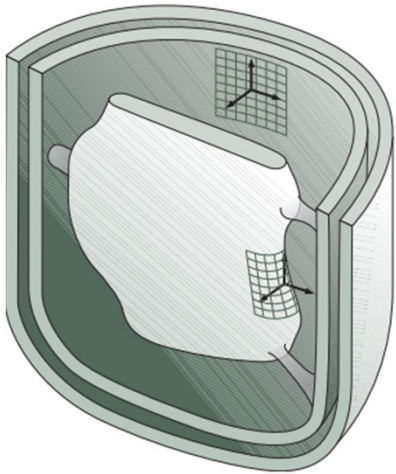


curvature



circle that fits to surface at point of interest

Membrane bending: Calculating curvature



How to find the curvature at a given spot?

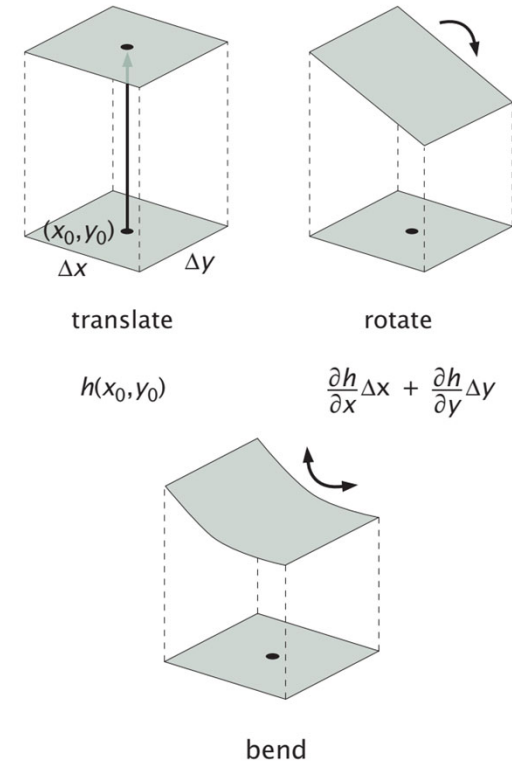
1. Create **tangent plane** through the selected point
2. Expand **height function** in powers for x_1 and x_2 ($x_1 = x$, $x_2 = y$)

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

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

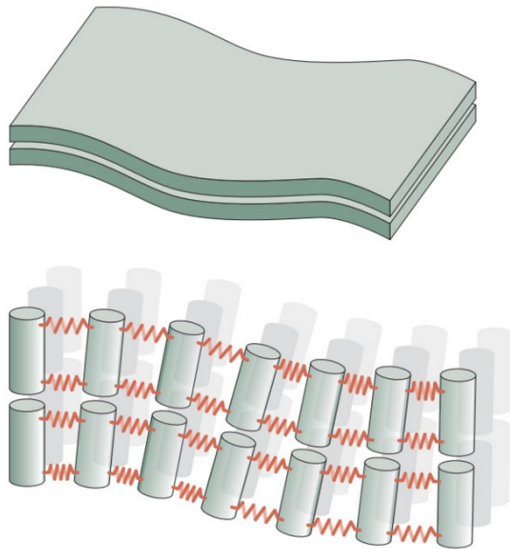
eigenvalues κ_1, κ_2 of this matrix:
principal curvatures, i.e. the two extreme values that characterize the curvature at this point

4. Interpretation



$$\frac{1}{2} \frac{\partial^2 h}{\partial x^2} \Delta x^2 + \frac{\partial^2 h}{\partial x \partial y} \Delta x \Delta y + \frac{1}{2} \frac{\partial^2 h}{\partial y^2} \Delta y^2$$

Free energy of membrane bending



Chemically, bending involves:

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

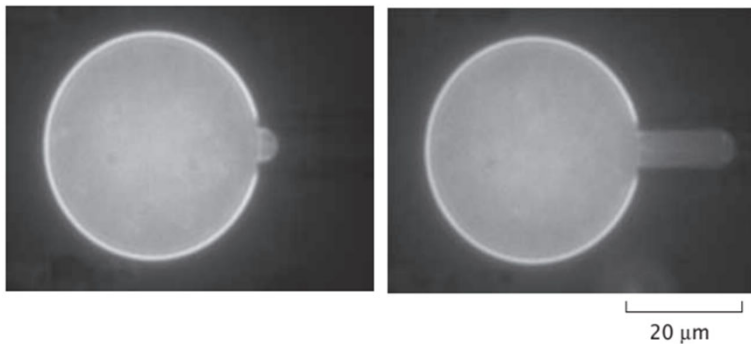
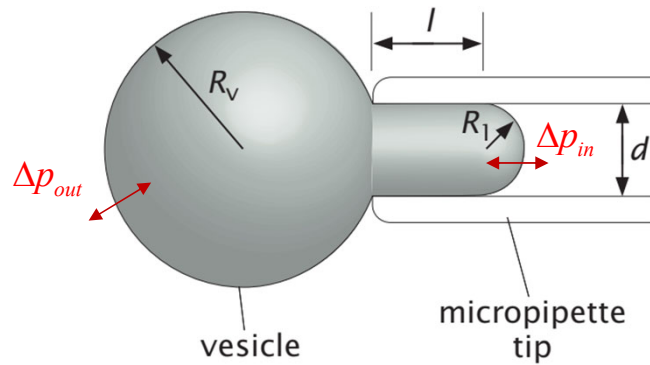
→ modeled as distortion of springs

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

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

Measuring the parameters for a given membrane



$$\Delta p_{out} = \frac{2\tau}{R_v}$$

Laplace Young Law:

A curved membrane under tension generates a pressure difference across it.

Here: τ is the **membrane tension (N/m)**

$$\Delta p_{in} = \frac{2\tau}{R_1}$$

Applying pressure with micropipette

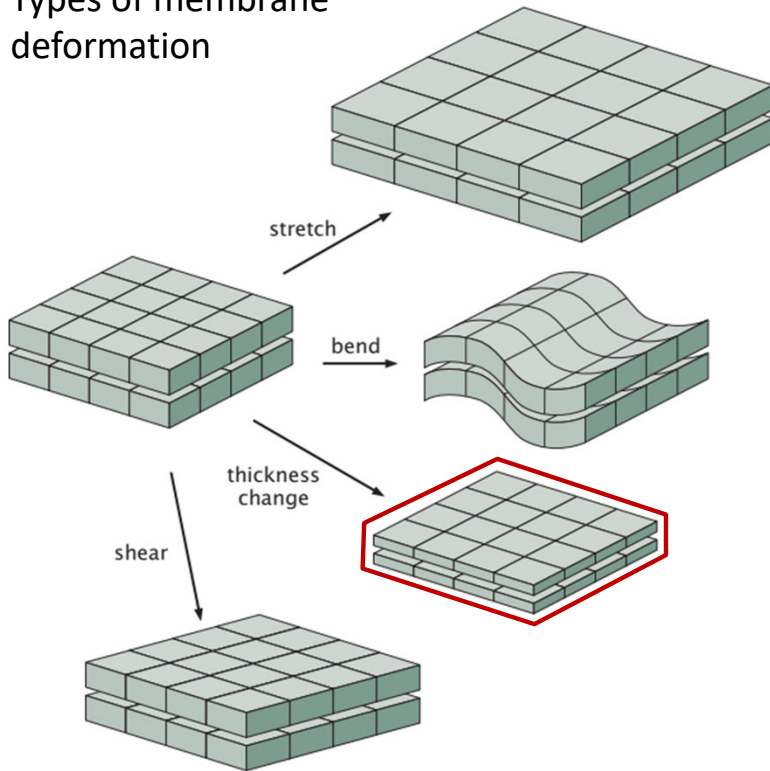
Together:
$$\tau = \frac{\Delta p}{2} \frac{R_1}{1 - R_1 / R_v}$$

$$\tau = K_a \frac{\Delta a}{a_0} \quad \text{and} \quad \Delta a = 2\pi R_1 l + 2\pi R_v^2$$

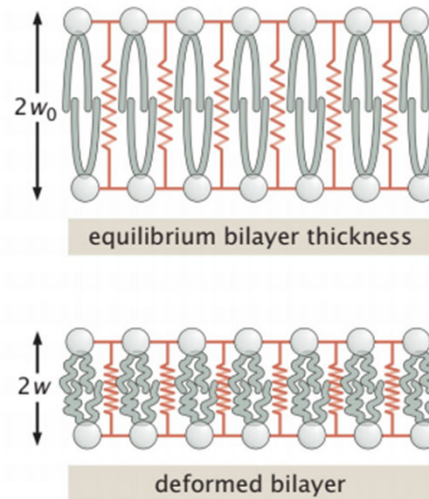
K_a : area-stretch modulus

Energetics of changing membrane geometry

Types of membrane deformation



Changes in membrane thickness:

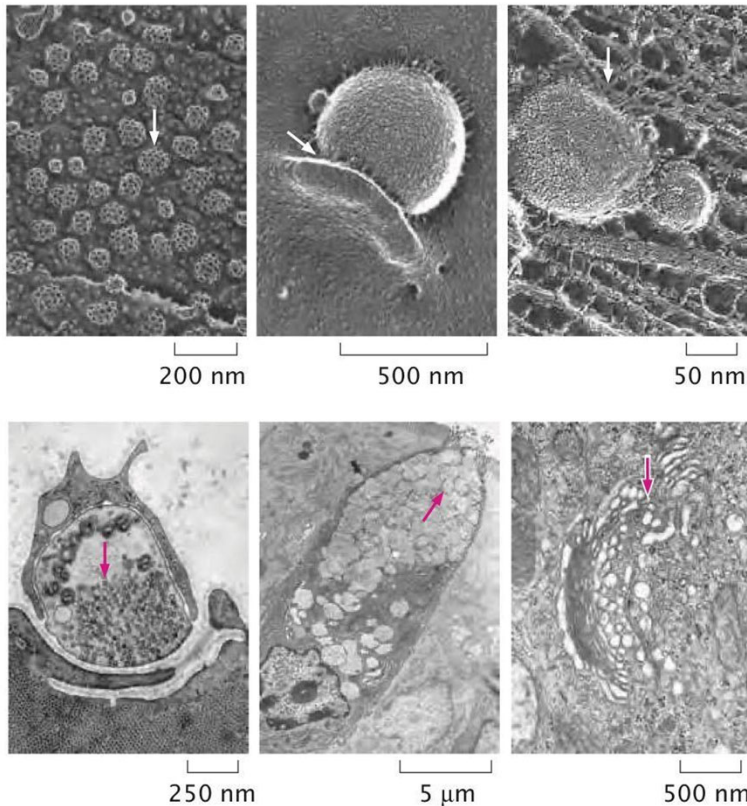


Free energy associated with membrane compression

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

K_t : stiffness
 $\sim 60 \text{ kT/nm}^2$

Energy of forming micelles and vesicles



Free energy associated with vesicle formation

$$G_{vesicle} = \frac{K_b}{2} \int_{\partial\Omega} \left(\frac{2}{R} \right)^2 da$$

with $\partial\Omega$ representing the whole membrane area

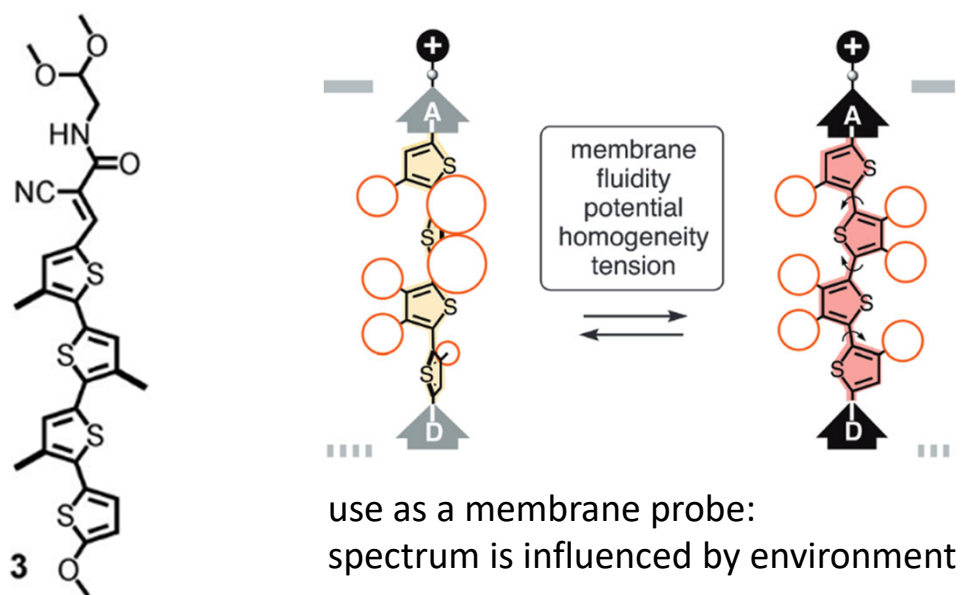
$$G_{vesicle} = \frac{K_b}{2} \left(\frac{2}{R} \right)^2 4\pi R^2 = 8\pi K_b$$

- (bending) energy to make vesicle is independent of its size
- With $K_b \sim 10\text{-}20 \text{ kT}$ \rightarrow energy per vesicle is around 250 – 500 kT (~ 25 molecules of ATP)

Quiz:

Estimate how much energy a cell uses to create vesicles, assuming that a cell generally recycles its complete plasma membrane once per hour?

Alternative way to probe membrane tension: Fluorescent probes



oligothiophenes

→ change color and fluorescence spectrum depending on scaffold conformation

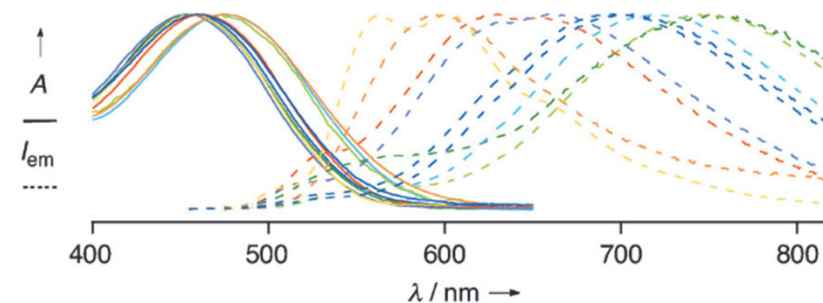
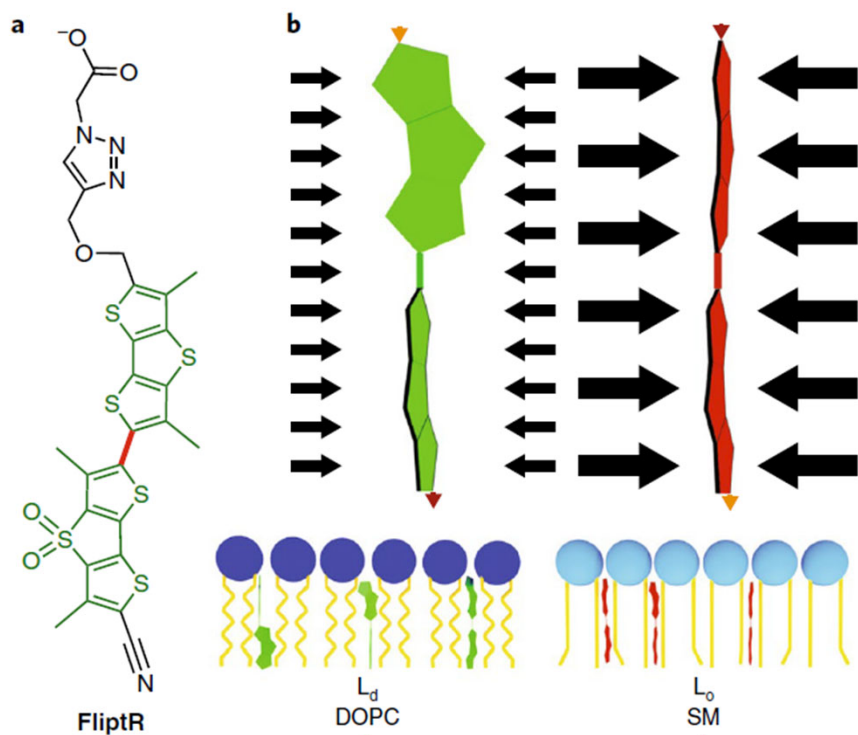


Figure 2. Absorption (solid) and emission (dotted) spectra of **3** in, with increasingly red-shifted emission, hexane (yellow), 2,2,2-trifluoroethanol (TFE; orange), dioxane (red), diethyl ether (light purple), ethyl acetate (deep purple), THF (blue), chloroform (cyan), acetone (dark green) and dichloromethane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

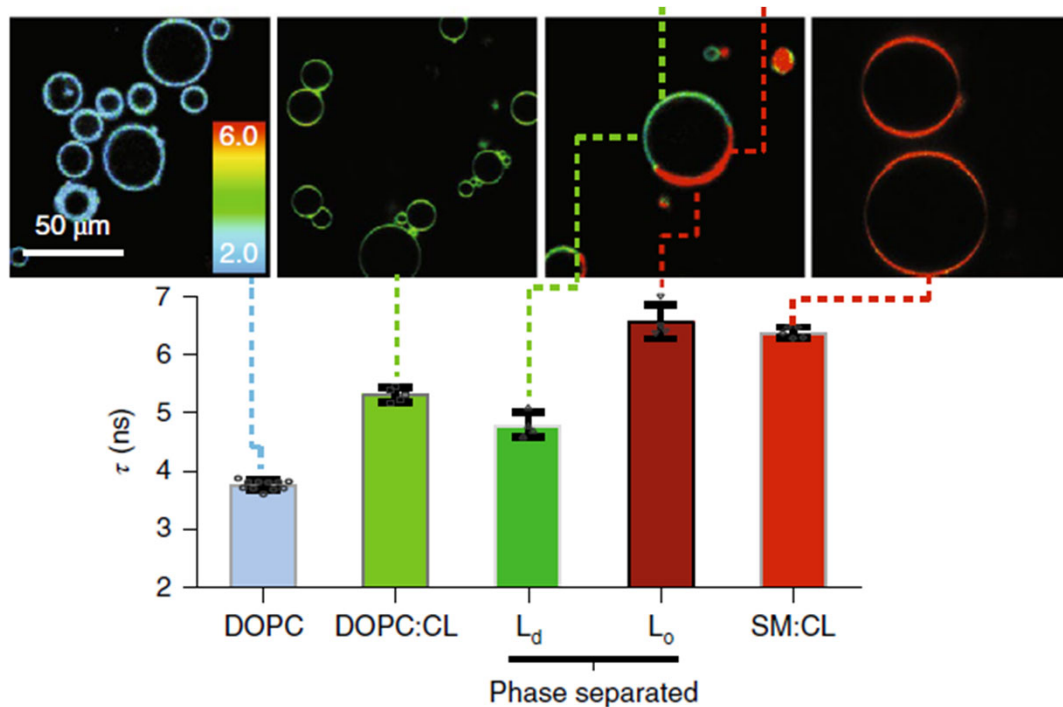
*Fin et al., Angew Chem 2012
(Matile group, Univ. Geneva)*

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



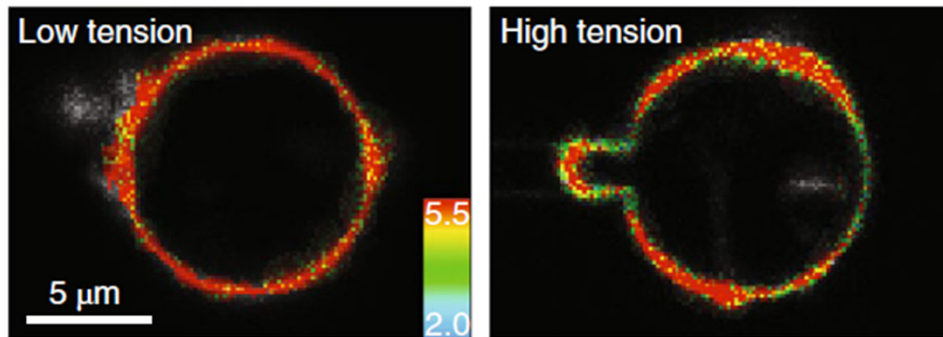
Colom et al., Nat Chem 2018 (Roux group, Univ Geneva)

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

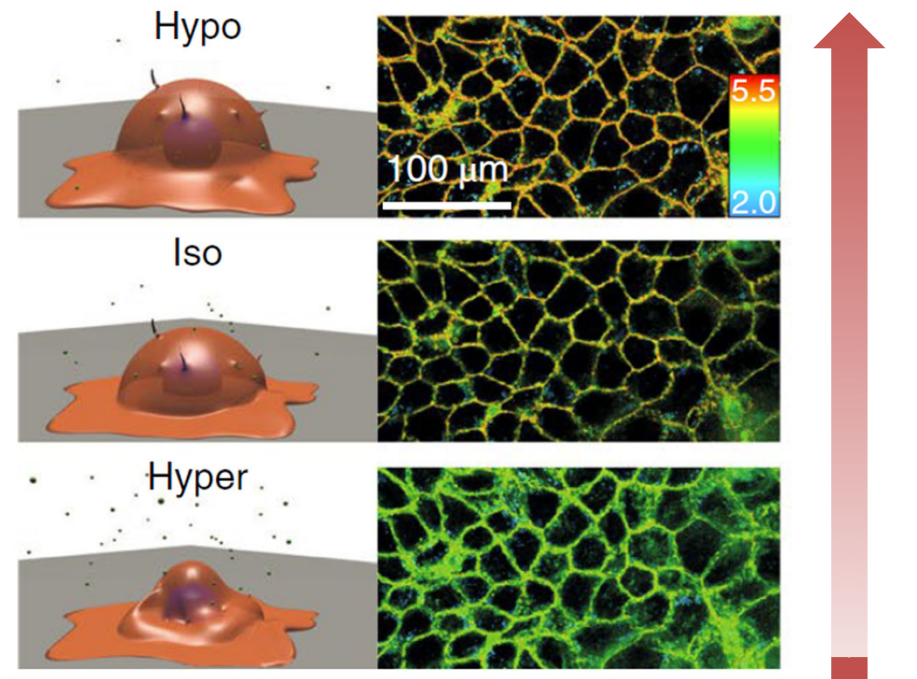
→ forming phase separated domains

Aspiration → increased tension

in $L\alpha$ domains: looser packing, decreased lifetime

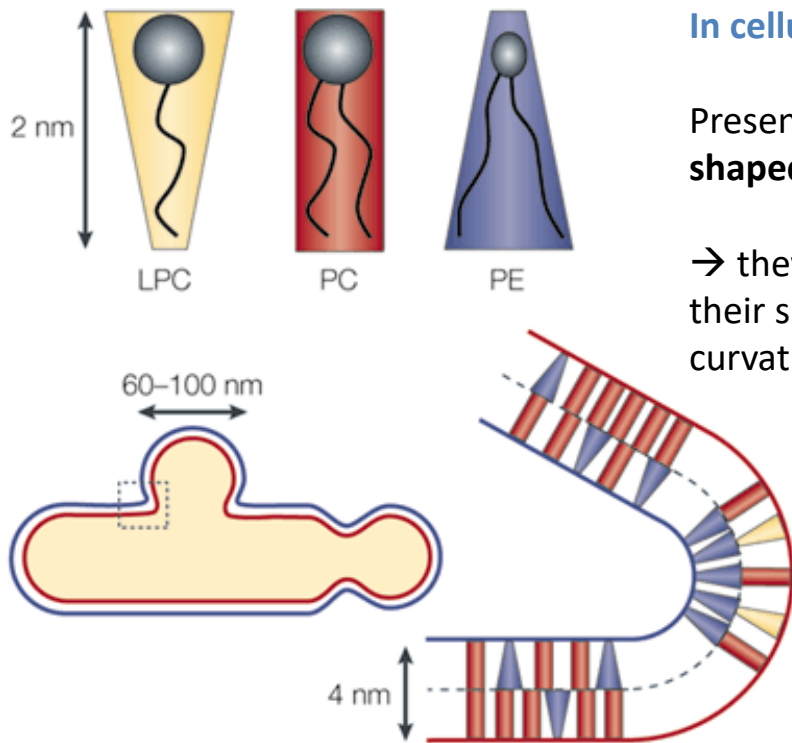
in L_o domains: tighter packing, increased lifetimes

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**

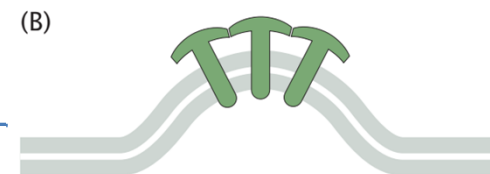
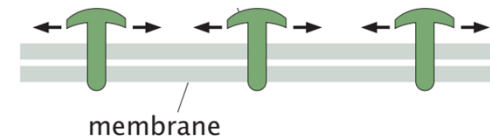
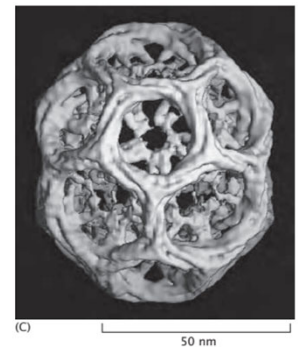
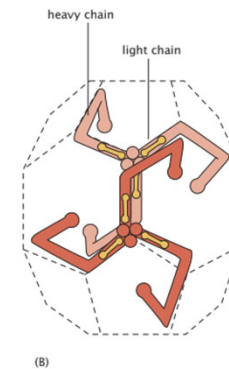
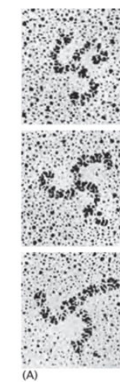
→ they accumulate according to their shape at points of high curvature

→ reduction of bending energy

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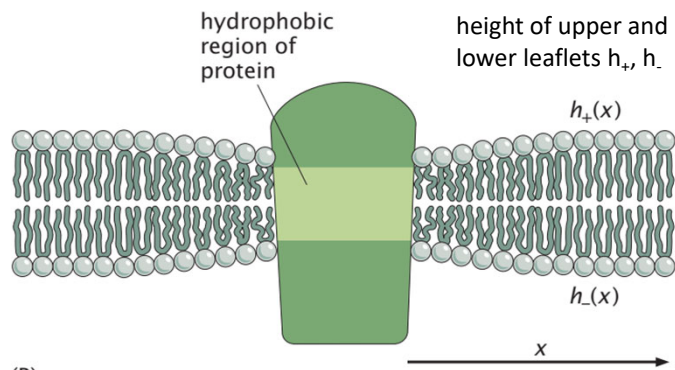
Proteins inducing membrane bending:

Clathrin coated vesicles



Interplay between membrane deformation and proteins

Membrane proteins lead to **local deformation**



(B)

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 $h(x)$, $w(x)$

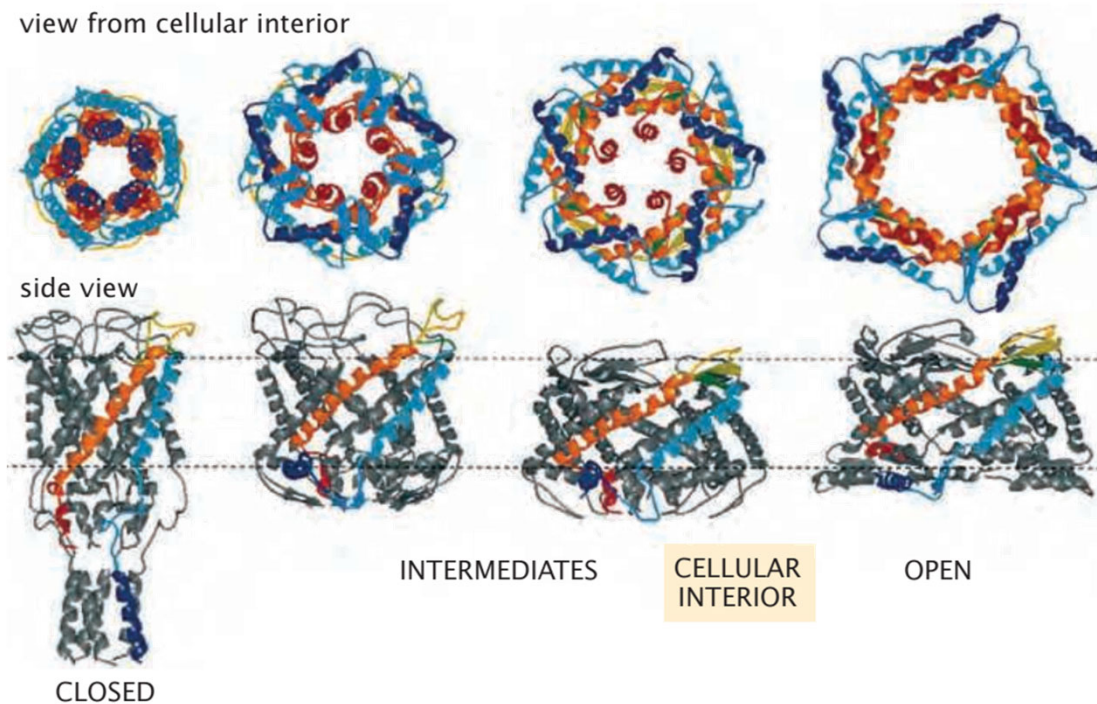
$$G_{bend}[h(x), w(x)] = \frac{K_b}{2} \int dx \left[\left(\frac{\partial^2 h(x)}{\partial x^2} \right)^2 + \left(\frac{\partial^2 w(x)}{\partial x^2} \right)^2 \right]$$

membrane
bending

thickness
variation

what is the effect on protein channels?

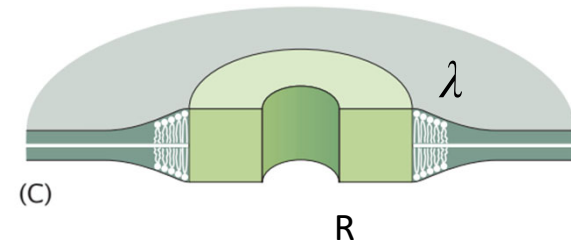
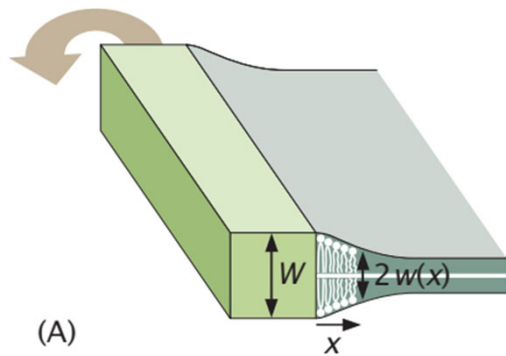
Example: A mechanosensitive channel



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

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

with $u(x) = w(x) - w_0$
and $u(R) = W$

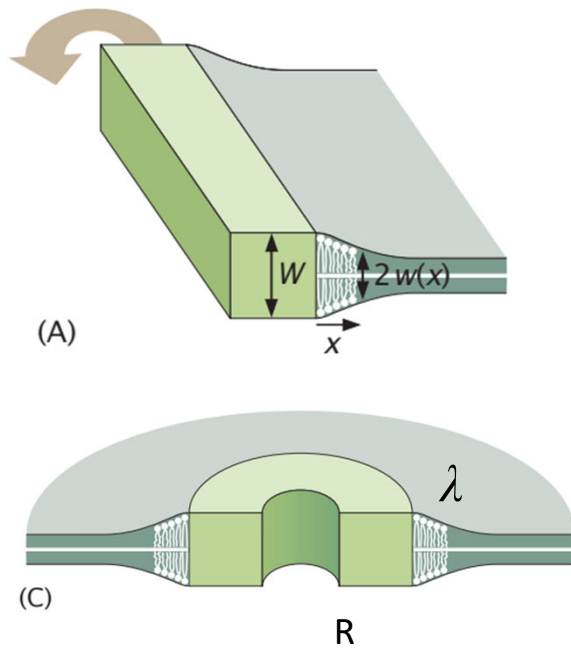
$$G = G_0 + \underbrace{\frac{K_t U^2}{\sqrt{2} w_0^2 \lambda} 2\pi R}_{\text{energy per circumference}} - \tau \pi R^2$$

membrane tension

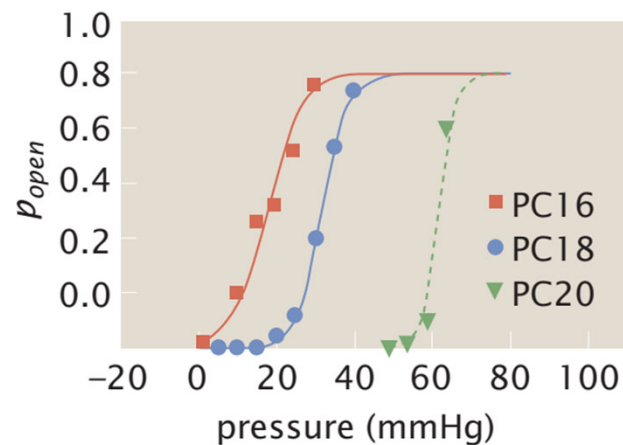
$$U = W / 2 - w_0$$

λ : decay length of bent membrane

Channel open probability is modulate by the membrane



$$G = G_0 + \frac{K_t U^2}{\sqrt{2w_0^2 \lambda}} 2\pi R - \tau \pi R^2$$



Resulting effect on channel:

Increasing pressure: stretches membrane and increases tension

→ Higher open probability

→ Also depends on membrane environment