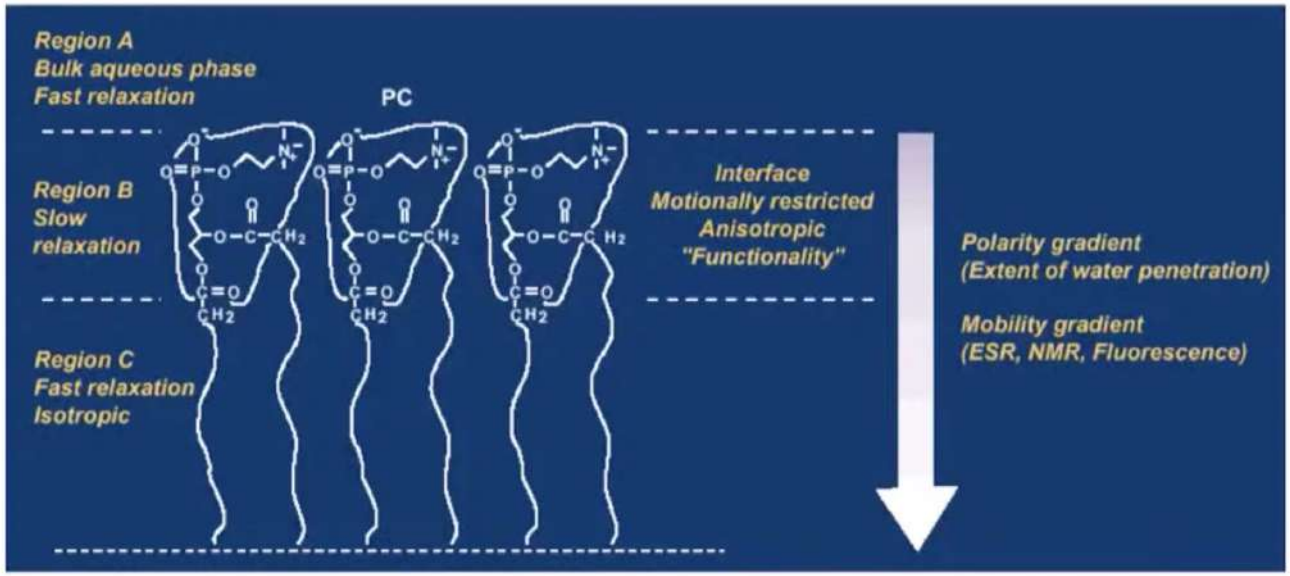
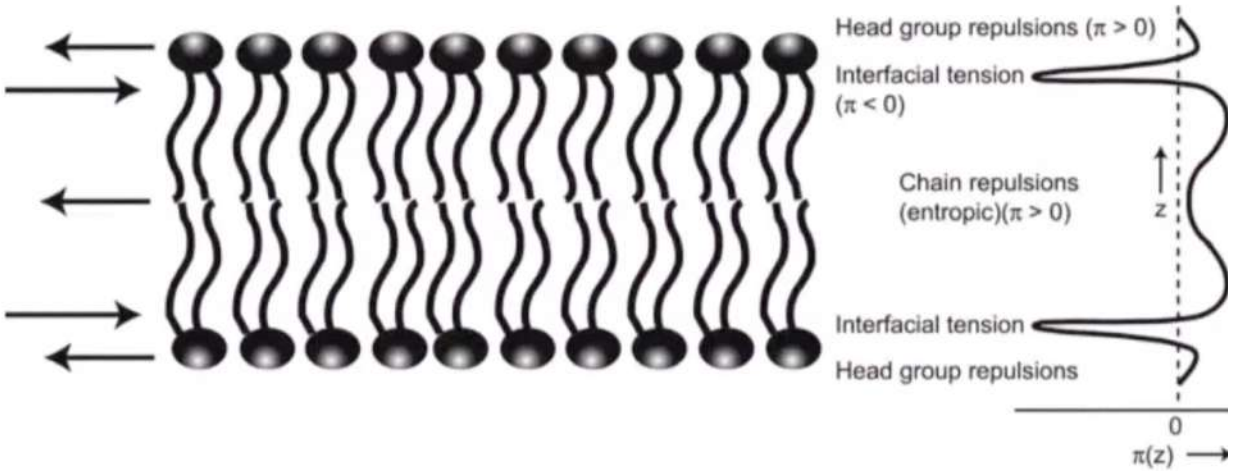
Membrane interface



The membrane interface is an important region of the membrane and characterizes the chemistry and biology of the membrane. It is also the most motionally restricted region of the membrane bilayer.

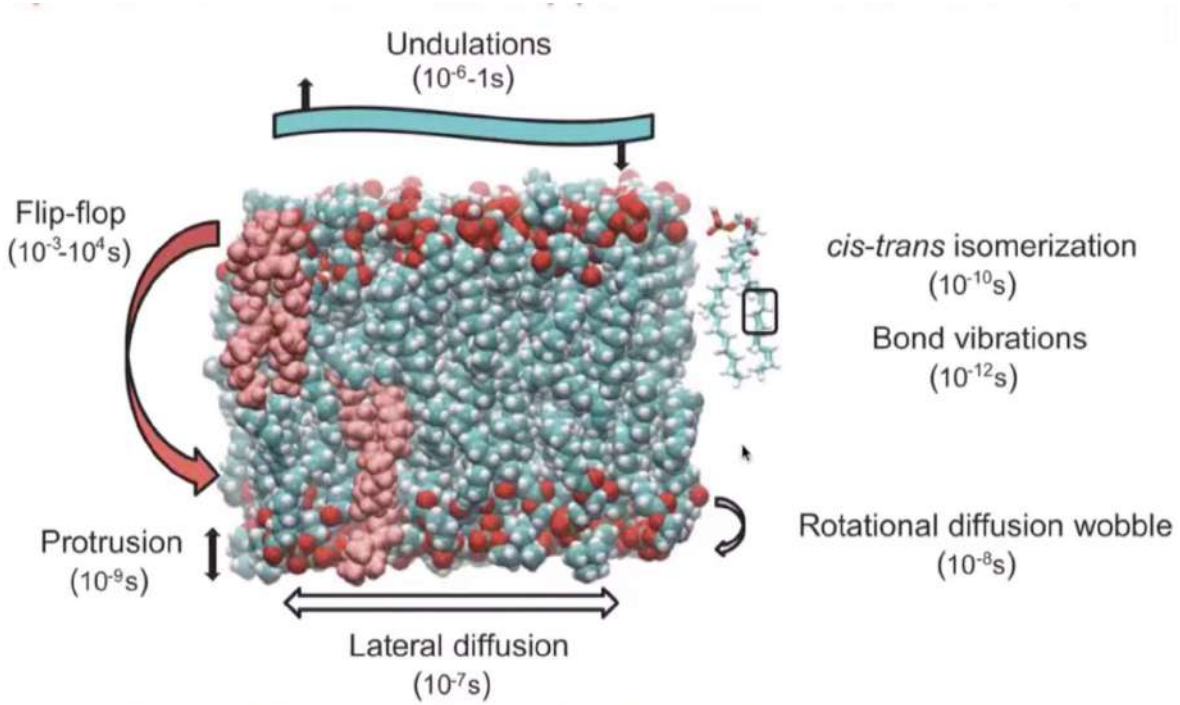
Lateral pressure profile of a lipid bilayer



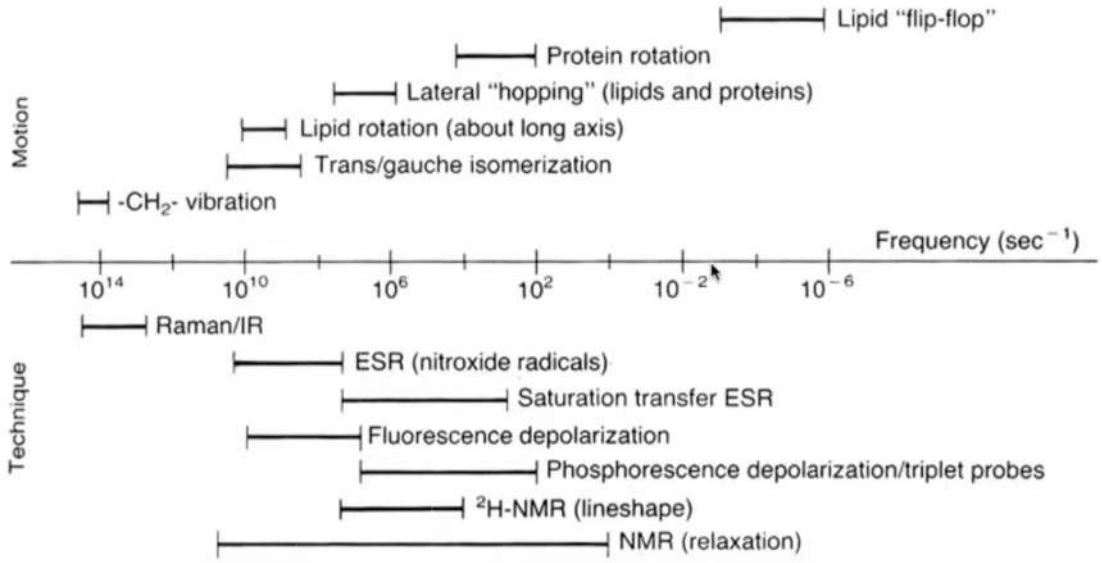
1. Positive pressure resulting from headgroup repulsive forces
2. Negative pressure at the hydrophobic-hydrophilic interface - the interfacial tension
3. Positive pressure resulting from entropic repulsion between acyl chains – chain pressure

Hydrophobic forces favour formation of the lipid bilayer. But single lipid molecules are subjected to large stress due to this confinement.
The negative, localized interfacial tension is about γ =50 mN/m. It is counterbalanced by the chain positive tension, which spans over the membrane thickness, about 2.5 nm is 2 γ/d_L or a pressure density of about 350 atm!!

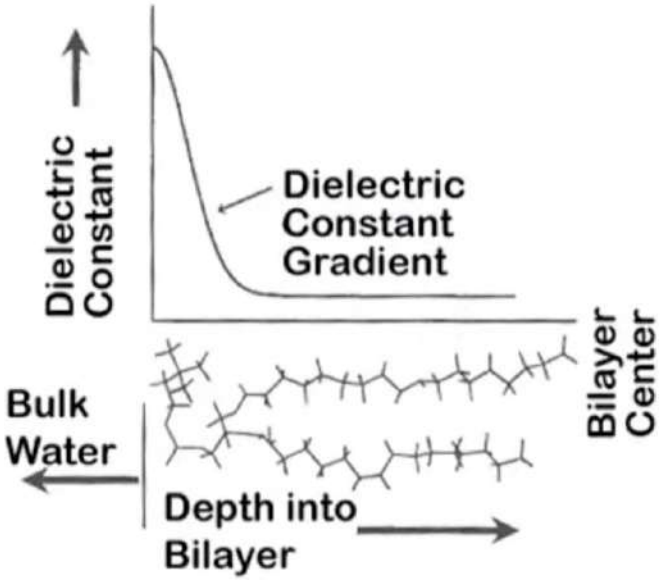
Molecular motion dynamics

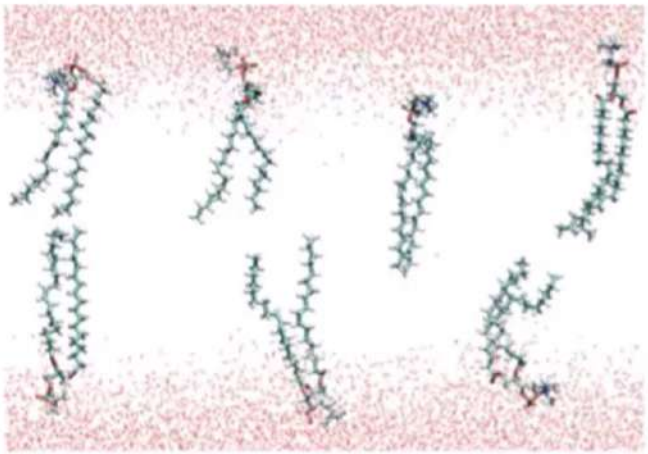


A variety of lipid motions create disorder in the fluid membrane bilayer

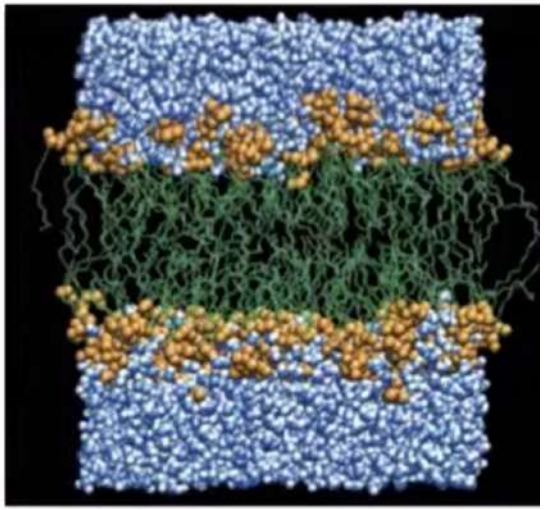


Water across the interface





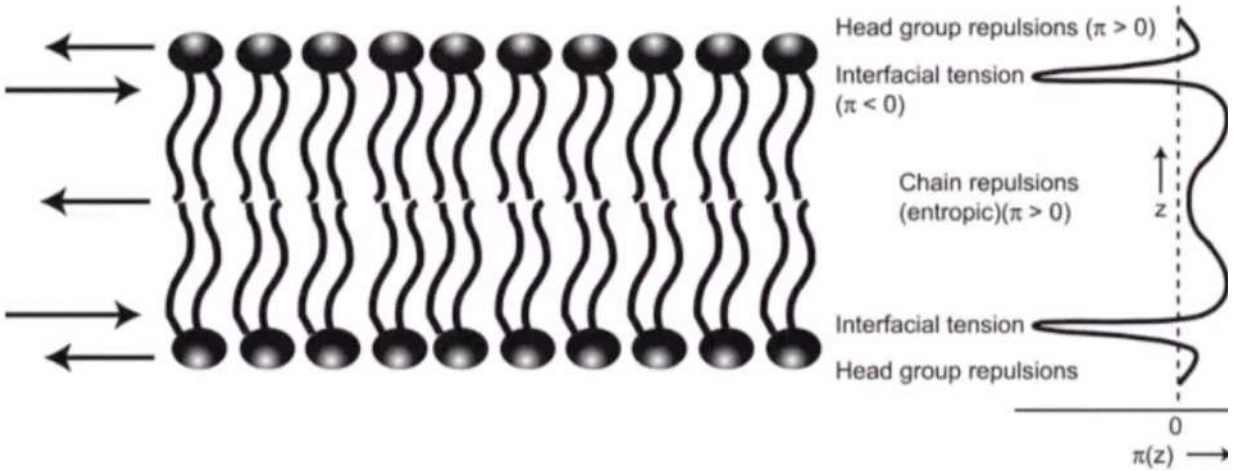
MD simulation of SOPC conformations in a bilayer



Fully hydrated DMPC bilayer

Stubbs *et al*. (1995) *J. Fluoresc*. 5: 19-28
Chiu *et al*. (1995) *Biophys. J*. 69:1230-1245

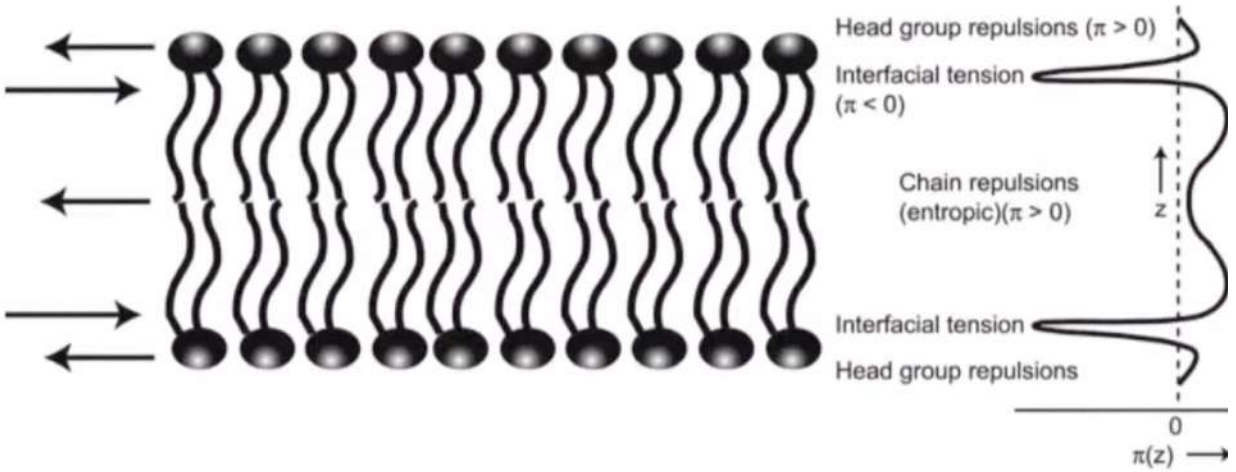
Lateral pressure profile of a lipid bilayer



1. Positive pressure resulting from headgroup repulsive forces
2. Negative pressure at the hydrophobic-hydrophilic interface - the interfacial tension
3. Positive pressure resulting from entropic repulsion between acyl chains – chain pressure

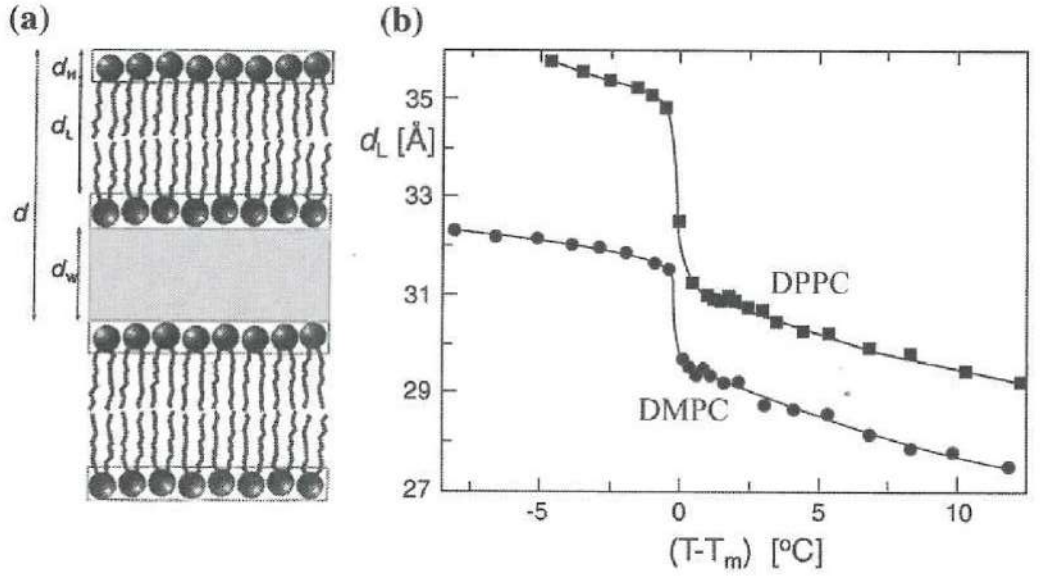
Therefore, lipid bilayers are very stratified, and dynamic: what is the THICKNESS then?
Of course, it depends on length and saturation of the lipids (longer and saturated are thicker) and on the hydration (more hydrated, thinner (dehydration makes the heads get closer, and the tails stretch out).
Cholesterol is a modulator of thickness! It stretches out and order fatty acid chains—more chol, higher thickness!
Temperature is also a modulator of thickness (higher T, thinner layer).

Lateral pressure profile of a lipid bilayer

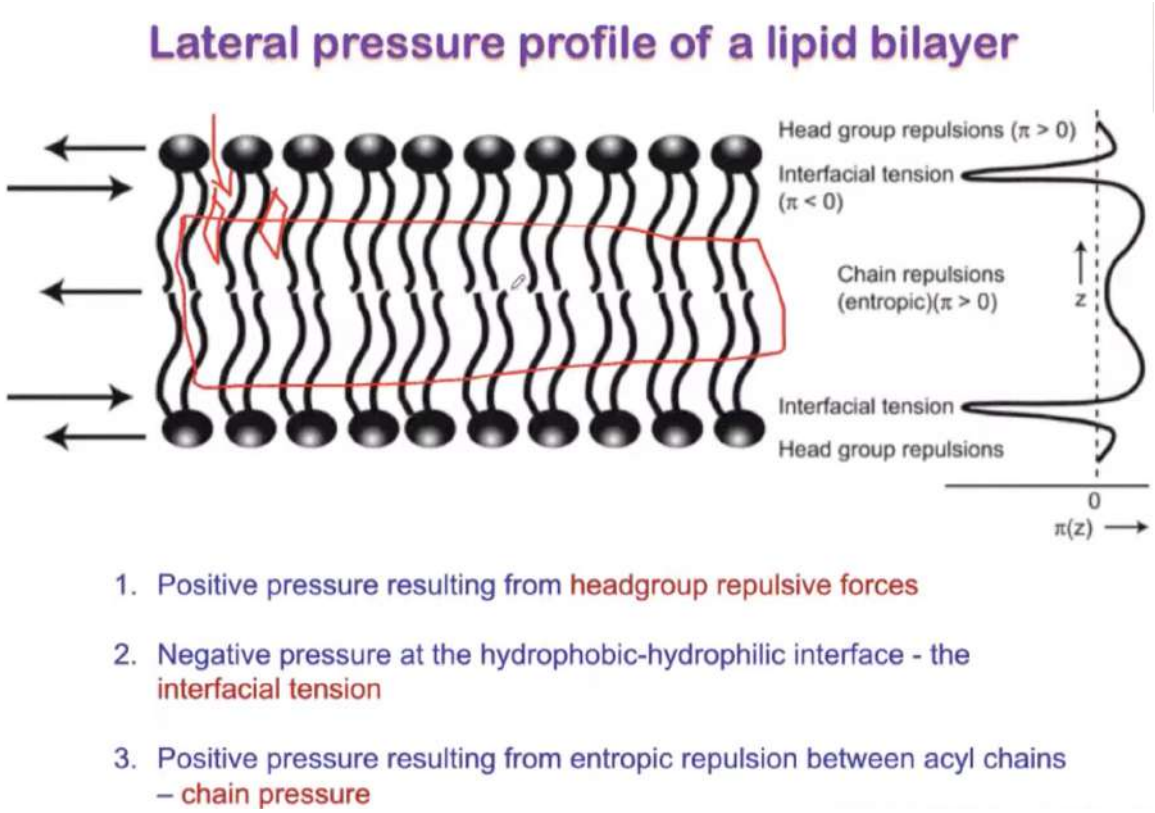


1. Positive pressure resulting from headgroup repulsive forces
2. Negative pressure at the hydrophobic-hydrophilic interface - the interfacial tension
3. Positive pressure resulti
   – chain pressure

Bilayer thickness measurements (average): X-ray or neutron scattering



Membrane interface: role of cholesterol

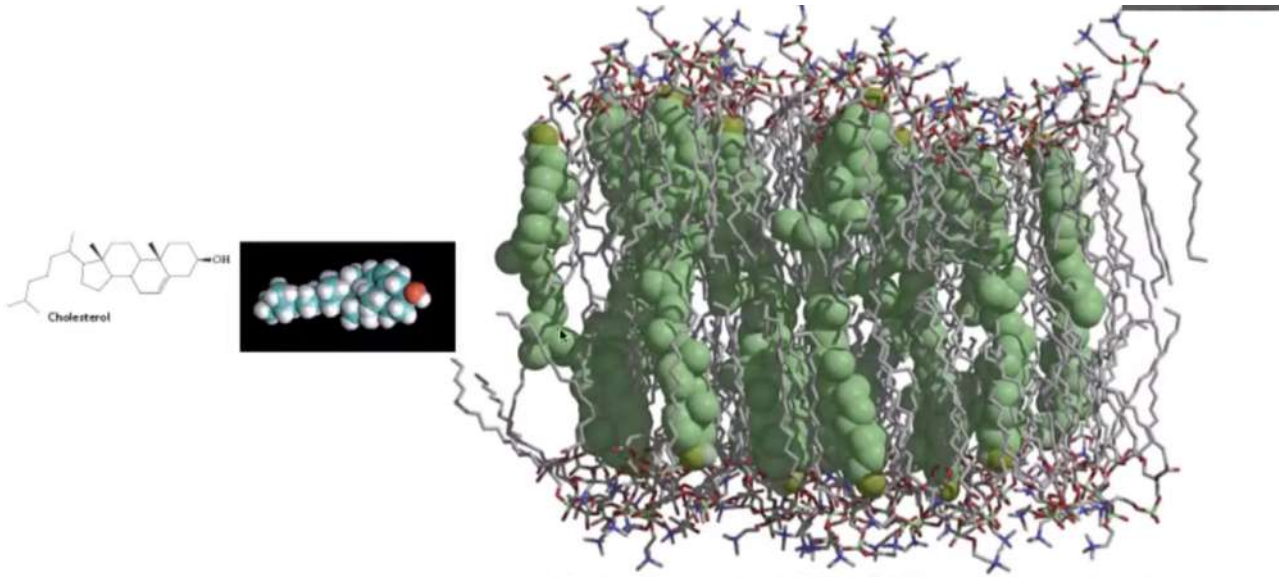


Cholesterol is a modulator of thickness! It is stiff, and stretches out and order fatty acid chains—more chol, higher thickness!

It would prefer conformationally (solid) ordered lipid phases. At the same time, squeezing into ordered phase is hard…easily goes into disordered phases (when different phases are available).

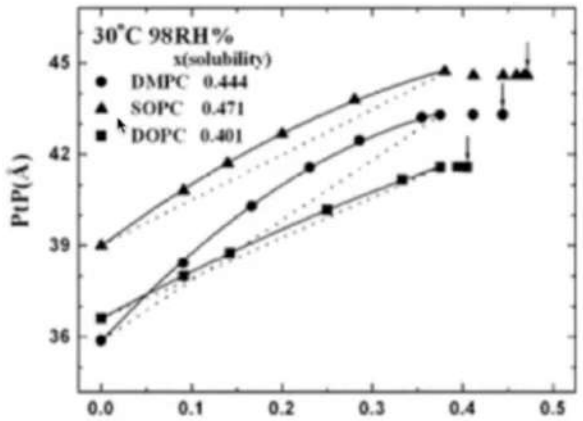
To release the frustration, chol induces a new phase, the liquid-ordered phase

Cholesterol promotes lipid order



DMPC (14:0 PC)
SOPC (18:0 18:1 PC)
DOPC (18:1 PC)

And increases lipid bilayer thickness!
Presence of chol has to have functional correlation



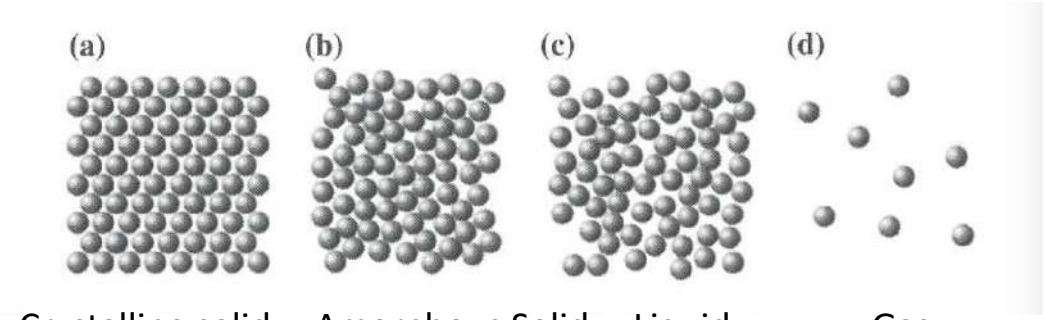
DMPC = 7.4 Å increase
SOPC = 5.6 Å
DOPC = 4.9 Å

Biophys. J. (2007) 92: 3960-3967

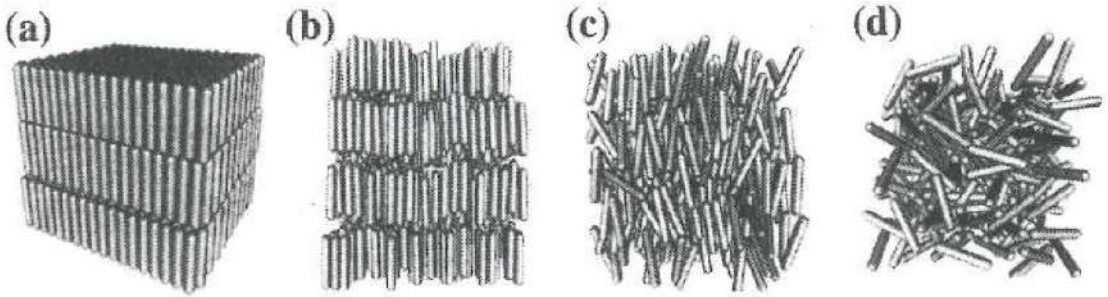
The hydrophobic membrane thickness in fluid phase is strongly dependent on the amount of cholesterol incorporated

The thickness of POPC can increase as much as 15-20% upon increasing the cholesterol up to 30 mol%, the level found in most eukaryotic membranes

Phase transitions



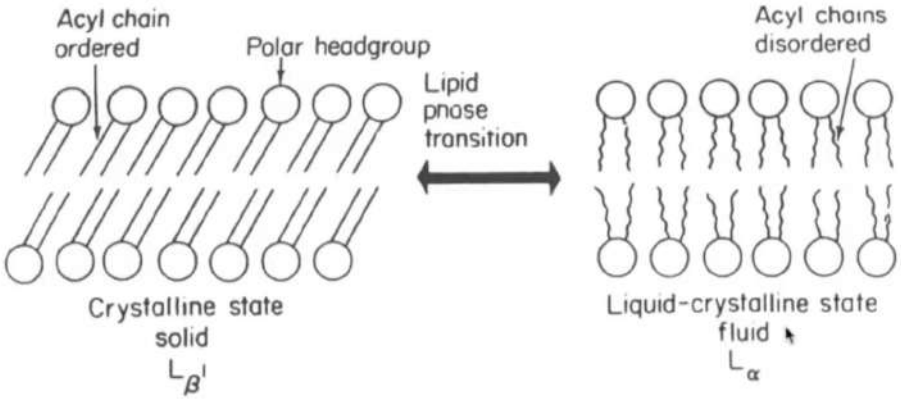
SPHERIC OBJECT: Crystalline solid Amorphous Solid Liquid Gas



NON-SPHERIC OBJECT: Crystalline solid Smectic Nematic Liquid

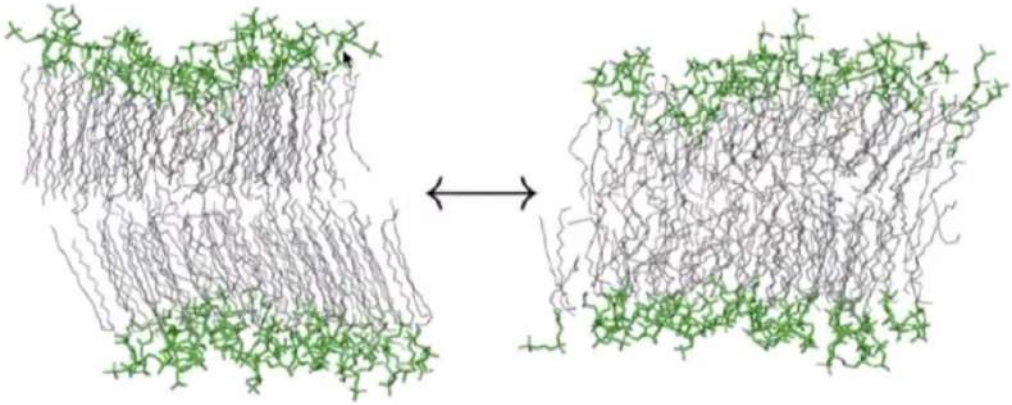
Meso-phases with order and disorder elements

Phase transition (**First-order,** or discontinuous transition: discontinuity in the order at the transition T)



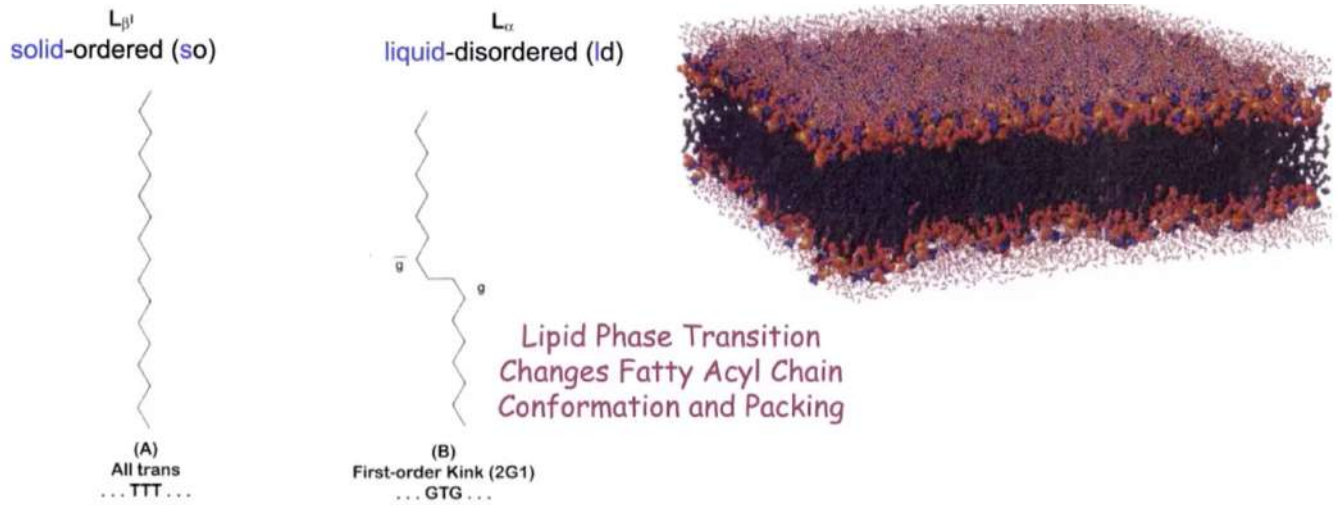
N.B.: Continous Transitions (strong fluctuations!) are the so called crytical phenomena (G. Parisi Nobel Price!)

Phase transitions



MD simulation of DPPC in water at Tm using atomistic model

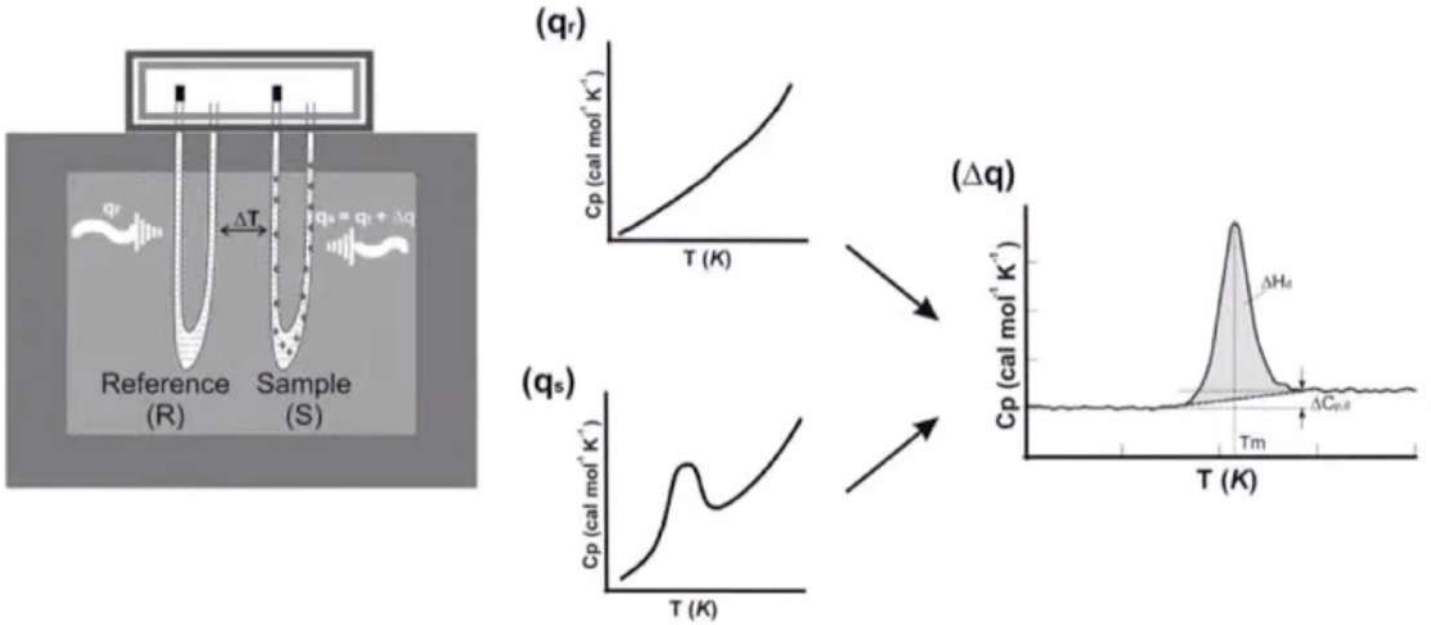
Unlike nucleic acids and proteins, lipids rarely express their main features through the properties of an individual molecule, but rather through their cooperativity, their social life as it were



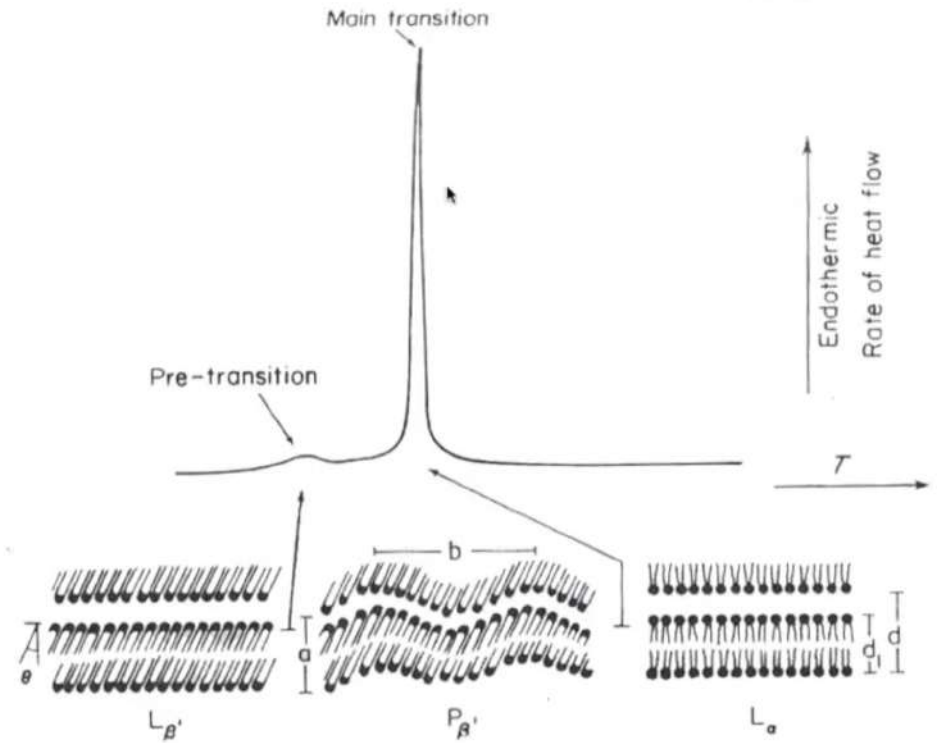
Lipid Phase Transition
Changes Fatty Acyl Chain
Conformation and Packing

Phase transitions
Differential Scanning Calorimetry (DSC)

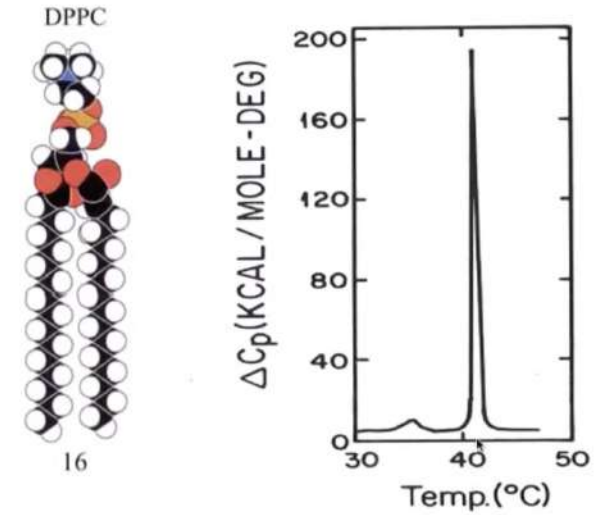
- DSC is a thermal analysis technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature
- Highly reproducible phase transitions are used to determine binding interactions, purity and stability of samples



Phase transitions in lipids



Main transition is precedeed by an intermediate, ripple phase which facilitates transition (specific heath vs. T).



Transition is very sharp: no chemical link among molecules in the layer, all molecules make the transition at the same time. Transition is dominated by thermal fluctuations

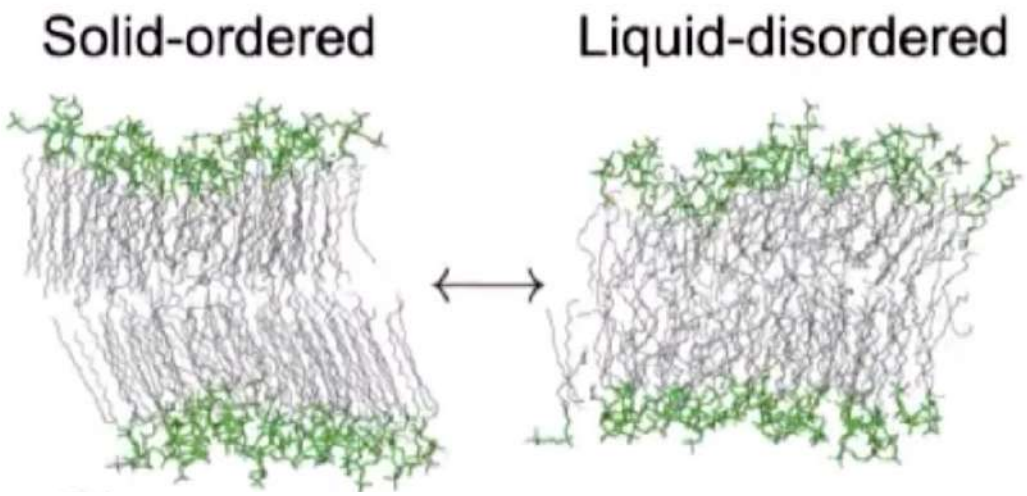
The heat (or entalpy) of transition is ΔH, transition temperature T_m

Long fatty acid chain have larger ΔH and T_m, increasing degree of unsaturation, lowers T_m

$\Delta S = \Delta H/Tm$ is about 15 k_B for DPPC

$\Delta S = kB\ ln\Omega$ with Ω+ number of microstatesof the system)per mol) involved in the transition :10^5-10^6 which are associated at the conformation of the tail

Phase transitions



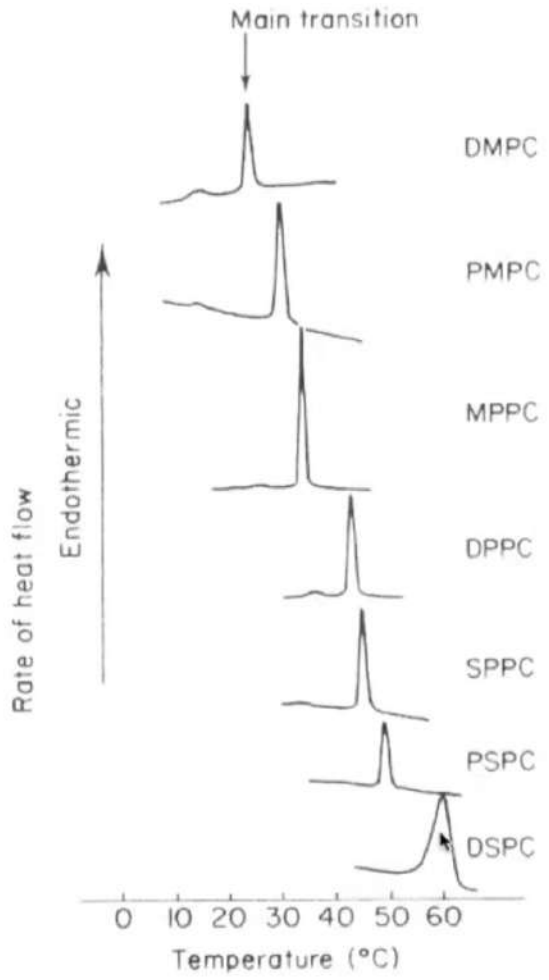
In **solid-order phase**, chains are aligned and heads are ordered

Liquid-disordered phase molecules are disordered as in liquids, and the diffusion is faster

Solid/liquid refers to positional degree of freedom
Ordered/disordered refers to degree of freedom of tails

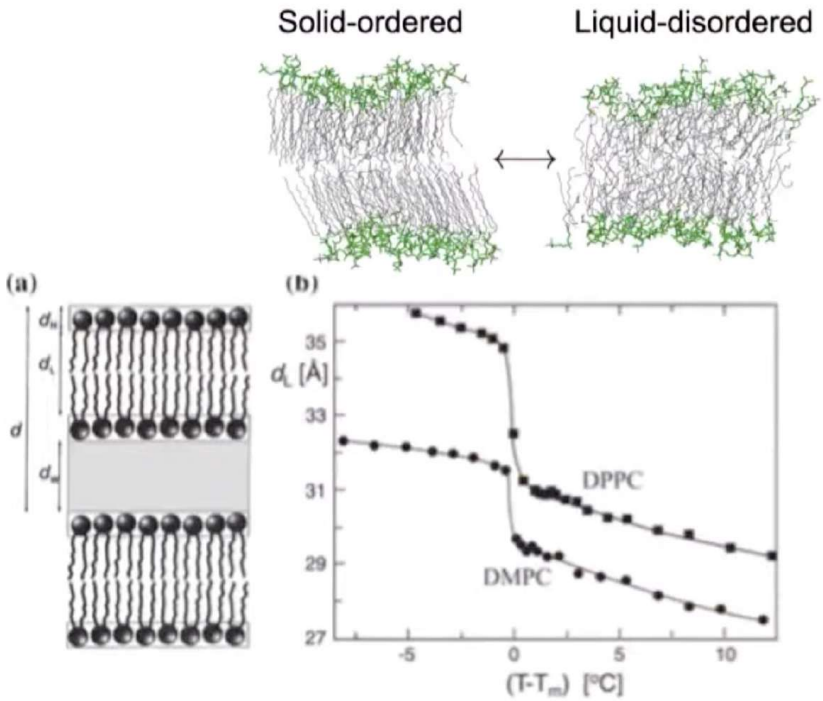
Across the phase transition, height and area per mol change! $\Delta A \Delta dL = c$

Phase transitions



Phase transitions and thickness

Phase Transition Temperature Increases with Increasing Fatty Acyl Chain Length

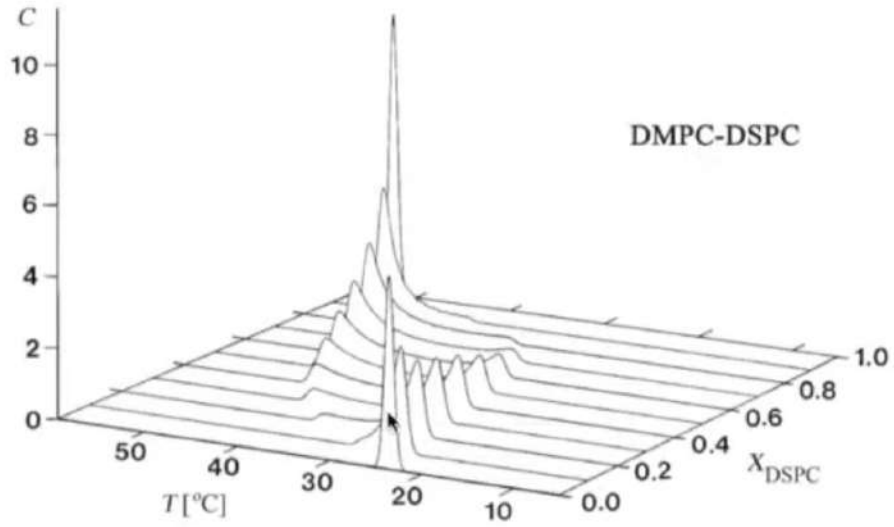


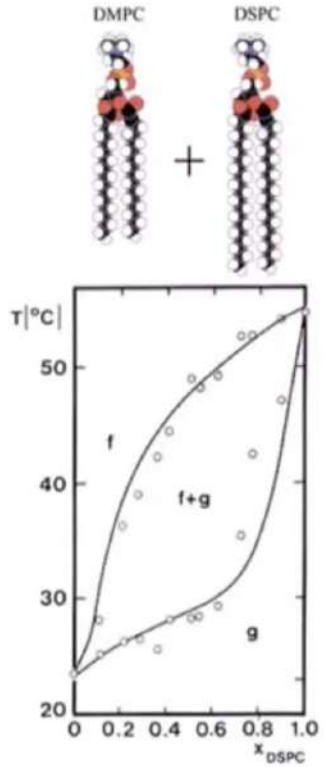
Organisms adapt lipid composition

	Percentage of total fatty acids[b]			
	10°C	20°C	30°C	40°C
Myristicacid (14:0)	4	4	4	8
Palmitic acid (16:0)	18	25	29	48
Palmitoleic acid (16:1)	26	24	23	9
Oleicacid (18:1)	38	34	30	12
Hydroxymyristic acid	13	10	10	8
Ratio of unsaturated to saturated[c]	2.9	2.0	1.6	0.38

J. Bacteriol. (1962) 84: 1260-1267

Phase separation, co-existence





f=liquid-disordered
g=solid=ordered

Biochim. Biophys. Acata (1988) 944: 121-134

The underlying physical mechanism for phase separation sees stronger attractive interactions between lipids of the same type. Phase separation gives the phase diagram (phases at equilibrium at given thermodinamic variable values).
The specific heat has 2 peaks, occurring at the boundaties of the phase diagram.

Phase separation, co-existence

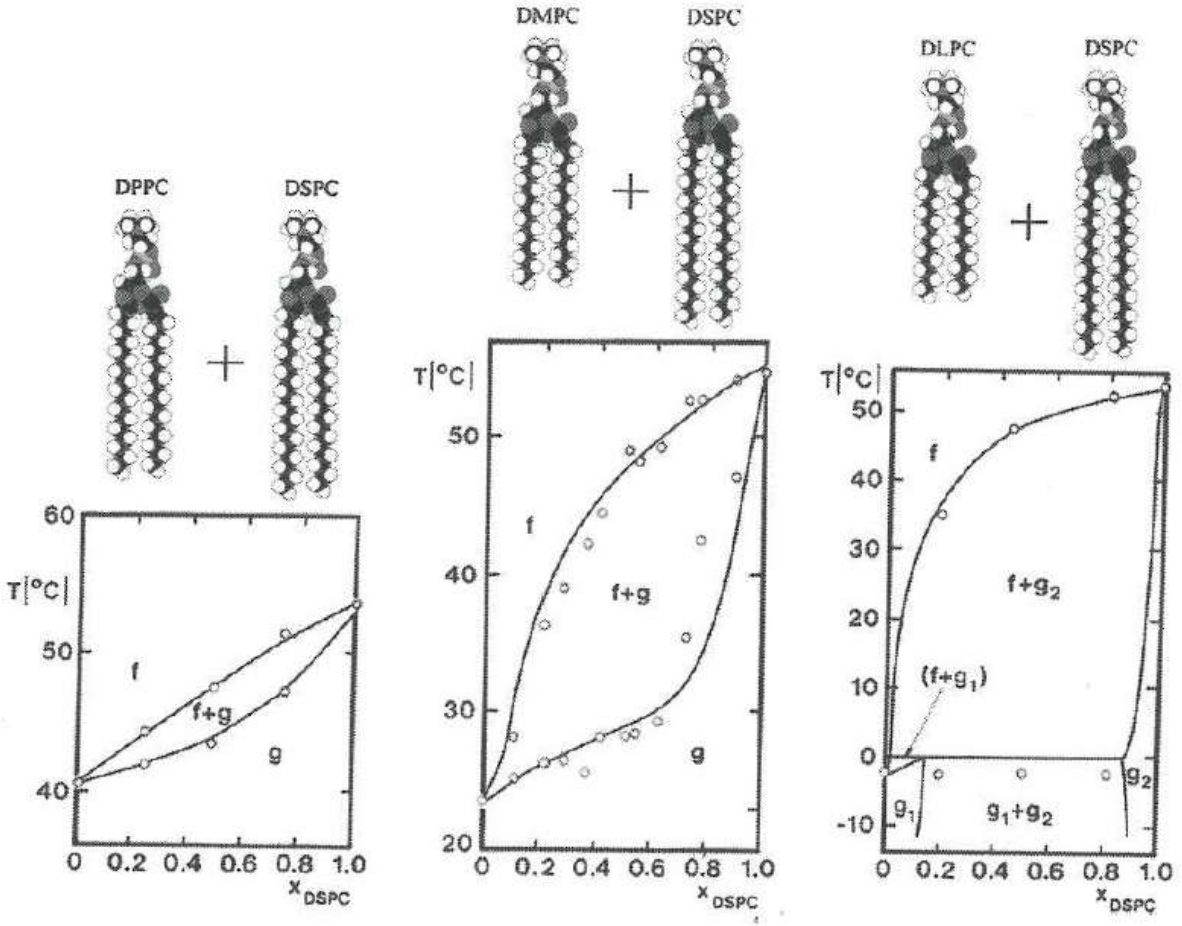
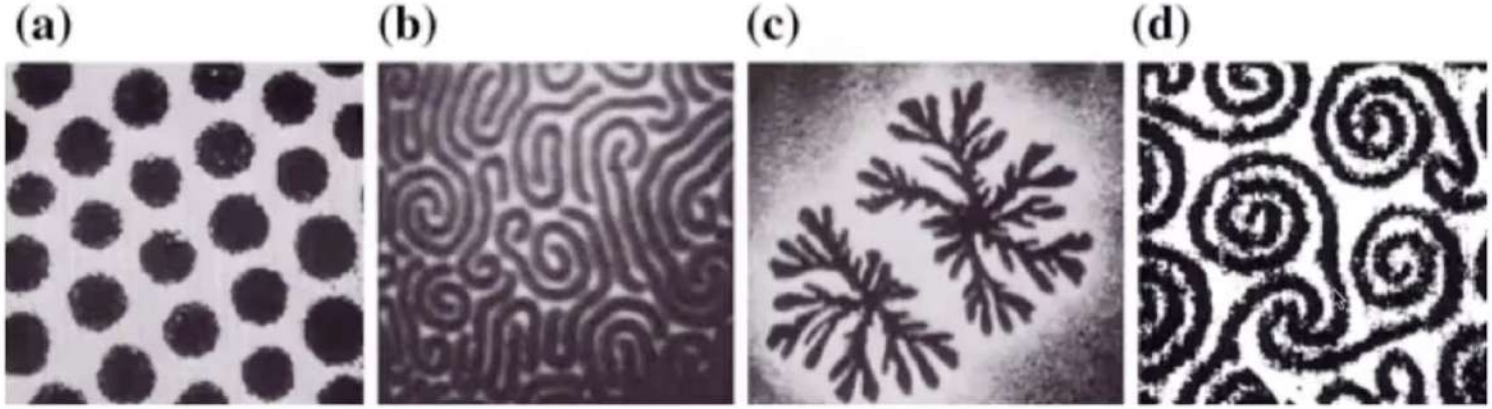


Fig. 9.8 Phase diagrams of lipid bilayers for three binary mixtures of PC lipids with different fatty-acid chain lengths. *f* denotes the liquid-disordered phase, and *g* denotes solid-ordered phases

Phase separation, co-existence



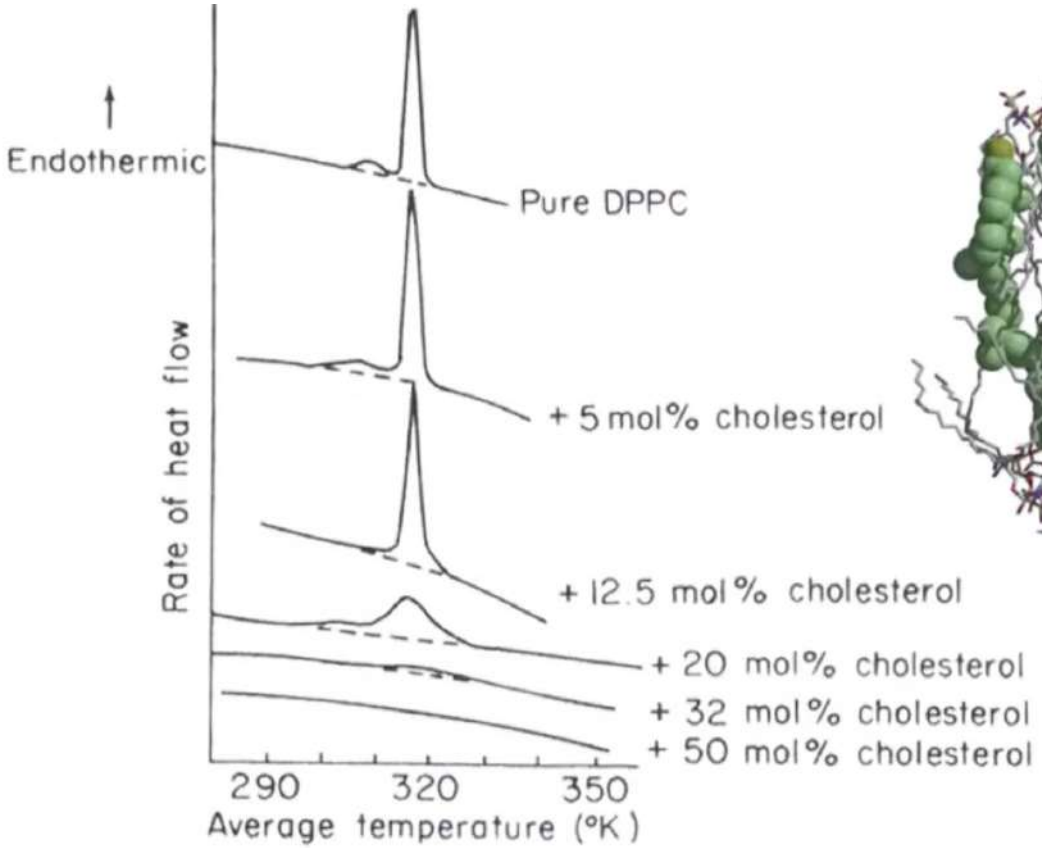
a) Coexistence of liquid phase (light) and solid phase

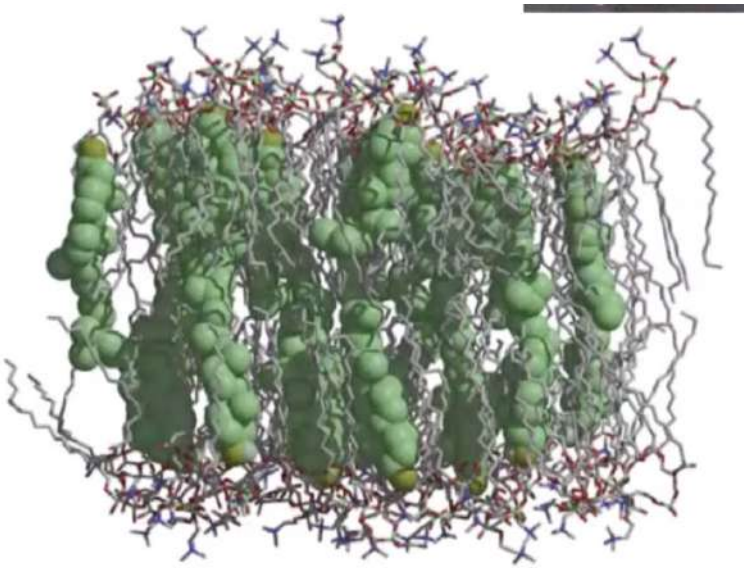
b) Striped pattern

c) Fractal and dendritic solid patterns in a liquid-phase monolayer after rapid compression

d) Spiral solid domains in a lipid monolayer with cholesterol

Chol role in phase transition

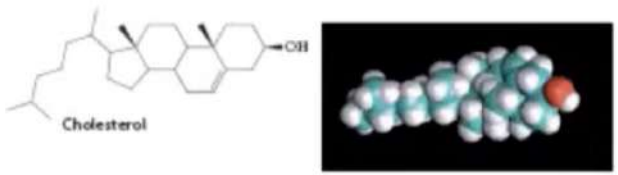


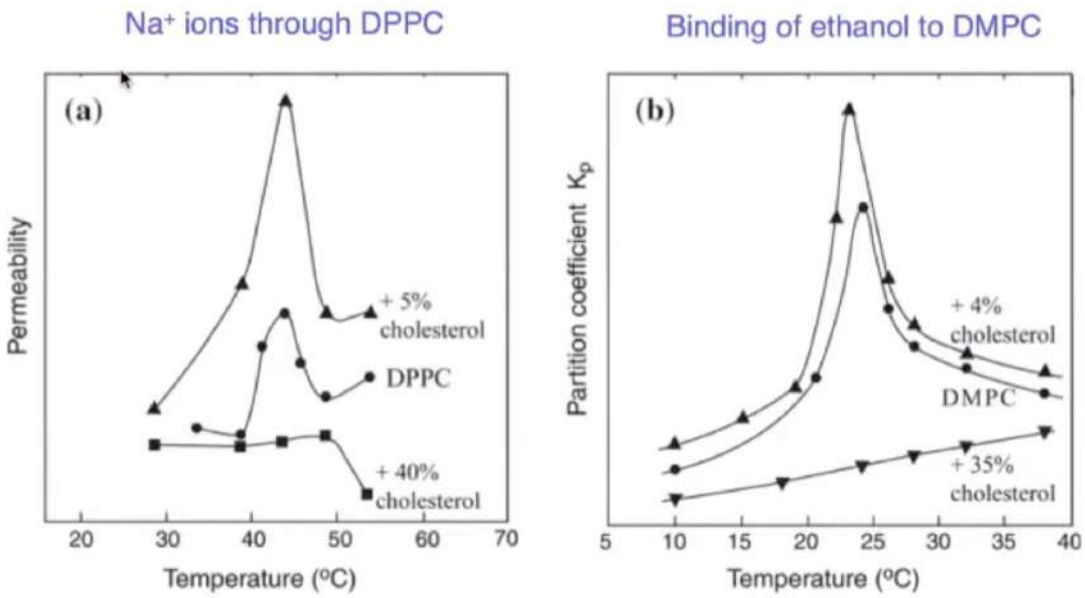


Chol reduces lipid cooperativity!! The new phase is called liquid-ordered phase. Highositional degree of fredom, low conformational one!. Flkuid and stiff

Chol and permeability: dual role

H Raghurama



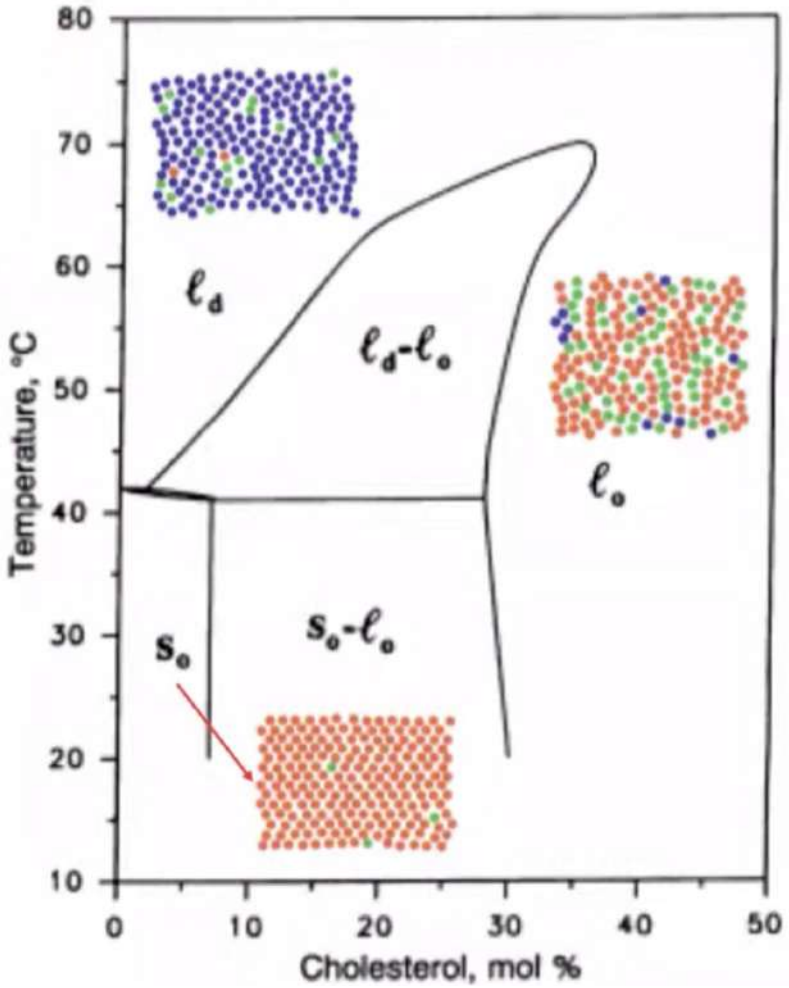


Biochim. Biophys. Acta (1992) 1107: 261-270
Biophys. J. (2000) 78: 2486-2492

Chol prevents ion permeability across the membrane!!!

Chol role in phase transition

Don’t need to change T in membranes for phase transition! Modulation of chol concentration



Temperature-Composition Phase Diagram of DPPC/Cholesterol System

Cholesterol induces liquid-ordered (lo) phase

PNAS (1991) 88: 8686-8690
Biochemistry (1990) 29: 451-464

Chol stabilized lo phase in a wide composition range.

Condensing effect of Chol on different phases

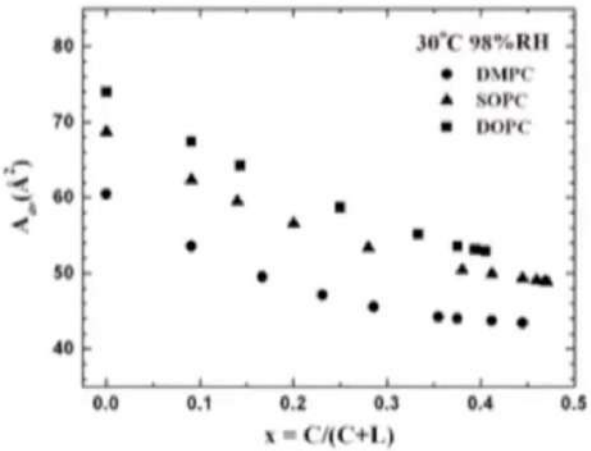
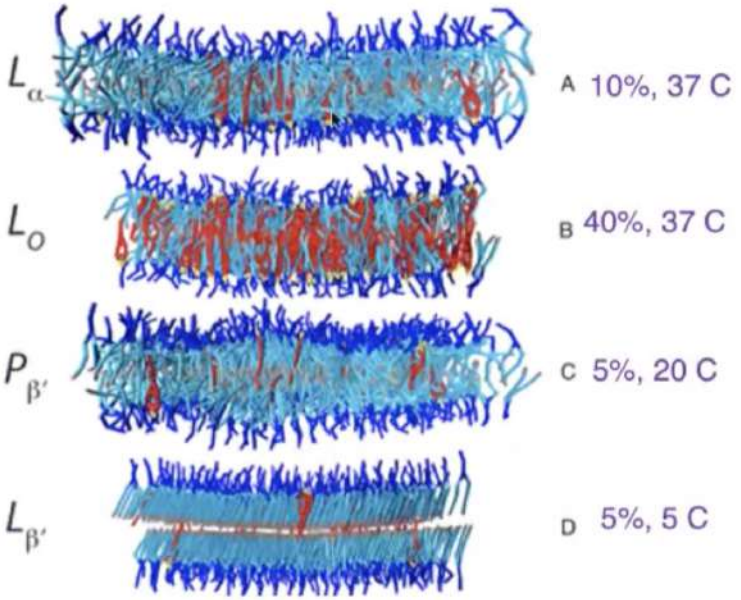


FIGURE 7 Area per molecule as a function of cholesterol concentration. The averaged cross section area of phospholipid is calculated by $A_{av,pc} = 2V_c/(PtP - 10)$, where V_c is the chain volume of the lipid (36), and the thickness of the hydrocarbon region is PtP minus twice the length of the glycerol region (from the phosphate to the first methylene of the hydrocarbon chains); the latter is very close to 10 Å (27,33,36). The average area per molecule for the cholesterol-phospholipid mixtures is calculated by $A_{av} = xA_{chol} + (1 - x)A_{av,pc}$. The area per cholesterol A_{chol} is assumed to be constant of x. A value of $A_{chol} \approx 39$ Å^2 was taken from monolayer measurements on pure cholesterol (3,37).

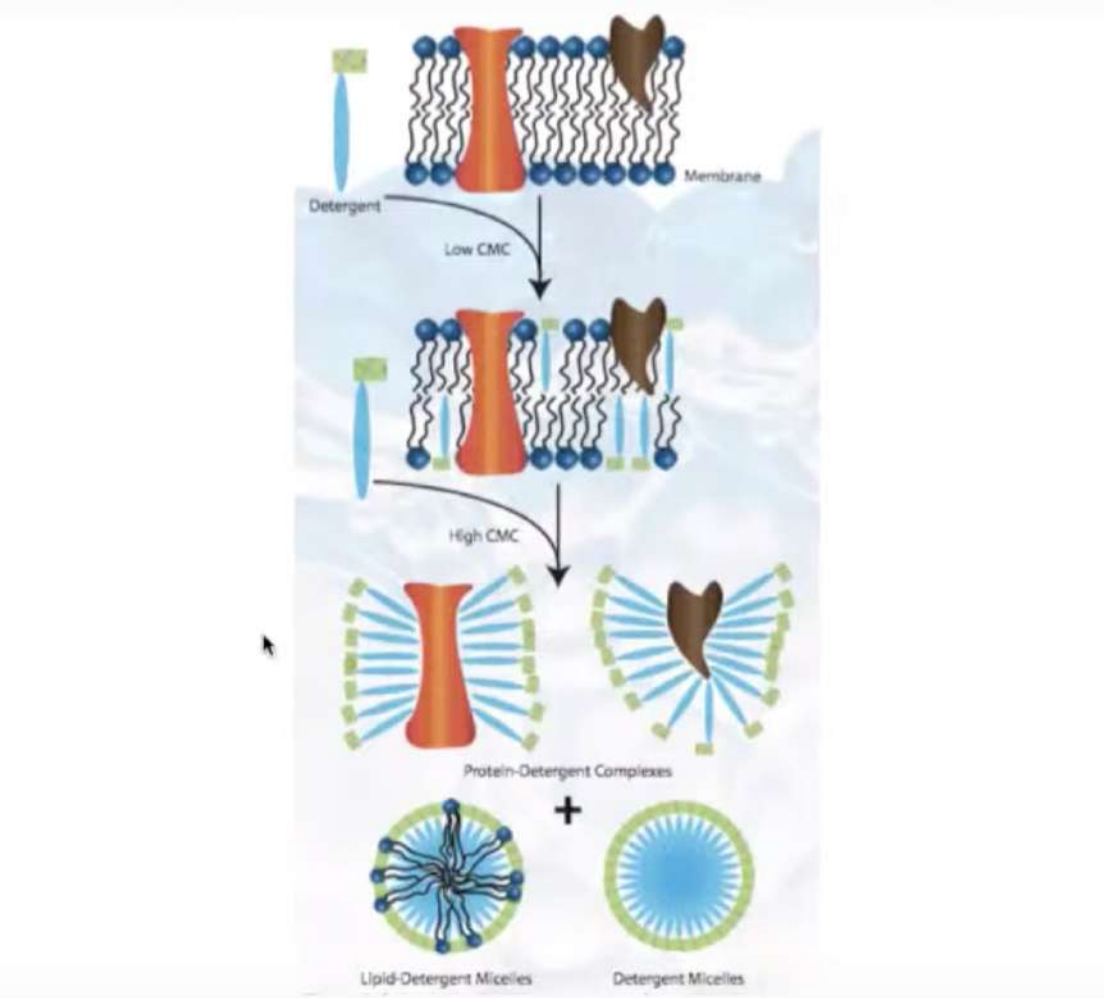
Biophys. J. (2007) 92: 3960-3967



PNAS (2009) 106: 3654-3658

Membrane thickness is changing with Chol. But in 2D, changing thickness means changing lateral compression: condensation! (mean area occupied by single molecules changes). From 70 A^2 to 55 at 30% chol

Detergents to solubilize a membrane



Membrane domains

- **Macroscopic domains:**
  Large morphologically distinct regions of the cell surface separated by barriers (apical and basolateral domains of polarized epithelial cells)

- **Protein aggregation:**
  Aggregation in the plane of the membrane giving rise to patches (domains) enriched in the specific protein and any molecule associated with it (purple membrane patches in *Halobacterium halobium* containing bacteriorhodopsin)

- **Cytoskeleton assisted domains:**
  Interactions of membrane proteins/lipids with cytoskeletal elements (clustering of receptors in coated pits prior to endocytosis)

- **Lipid microdomains:**
  Formed by immiscible lipids

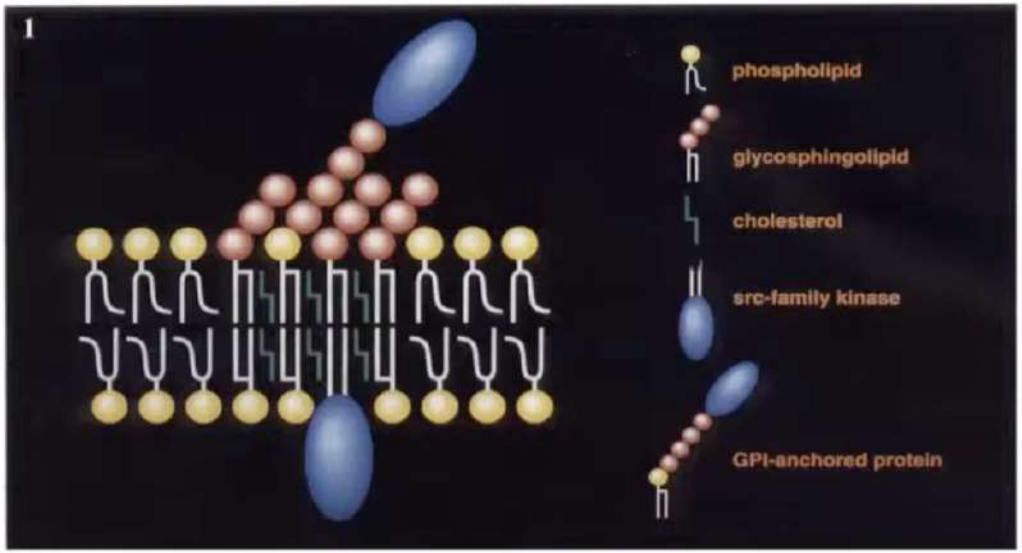
Combination of these factors !

Why are domains needed ?

Edidin (1992) *Trends Cell Biol.* 2: 376-380

Membrane domains

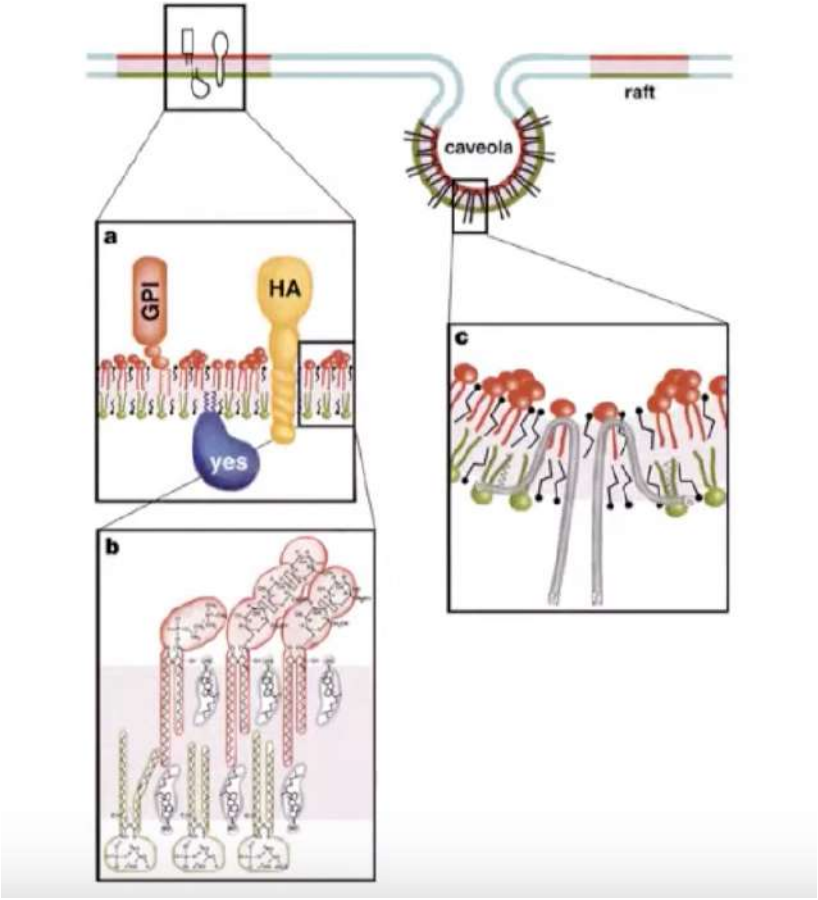
How do the proteins linked at two different sides of the membrane communicate?



Membrane Rafts

Kasahara and Sanai (1999) *Biophys. Chem.* 82: 121-127

Membrane domains



Lipid rafts are lateral nano- and/or micro-domains in plasma membrane that are enriched with cholesterol, sphingolipids, and specific proteins (in particular, glycosylphosphatidylinositol (GPI)-anchored proteins and acyl chain-lipidated proteins

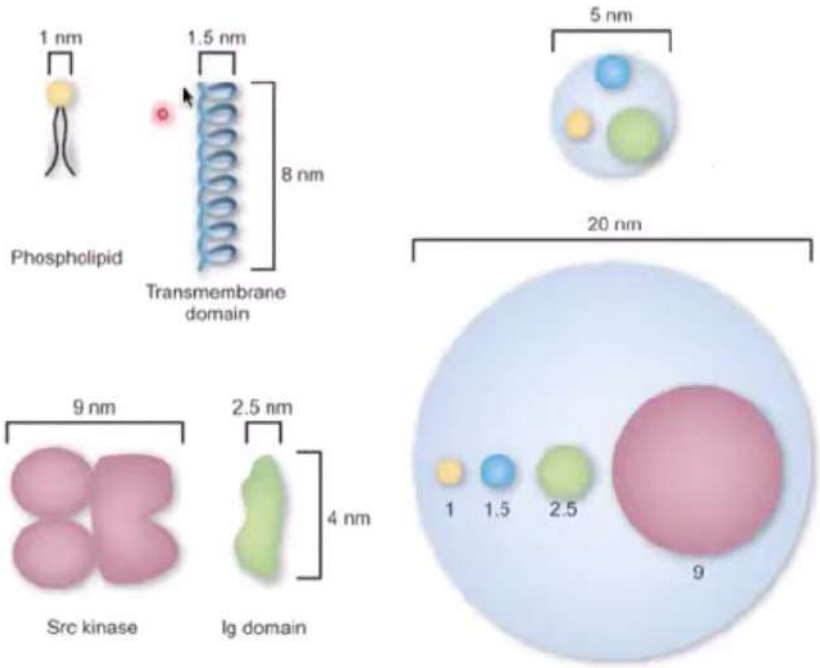
"Membrane Rafts are small (10-200 nm), heterogeneous, highly dynamic, sterol-and sphingolipid-enriched domains that compartmentalize cellular processes. Small rafts can sometimes be stabilized to form larger platforms through protein-protein and protein-lipid interactions"

Rafts defined: a report on Keystone Symposium on lipid rafts and cell function, Pike (2006) *J. Lipid Res.* 47: 1597-1598

Membrane rafts have half-lives in the range of 100 ns – highly dynamic and almost invisible !

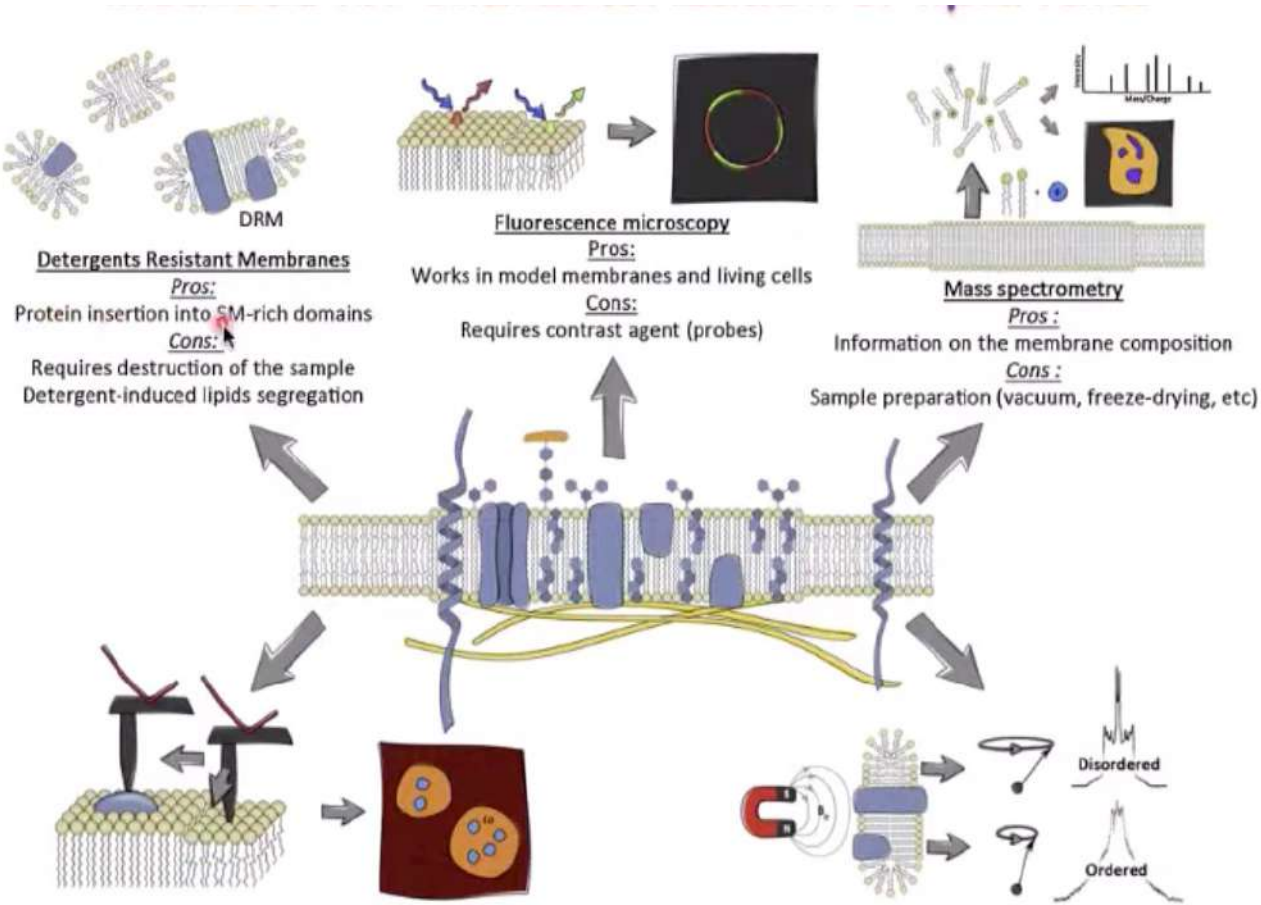
Simons and Ikonen (1997) *Nature* 387: 569-572
(> 8700 citations)

How big is a membrane raft



Shaw (2006) *Nat. Immunol.* 7: 1139-1142

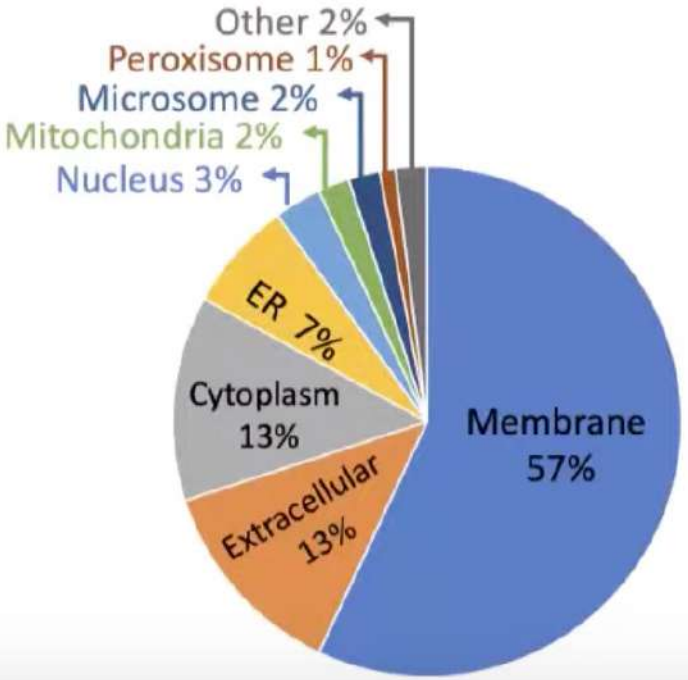
Raft characterization



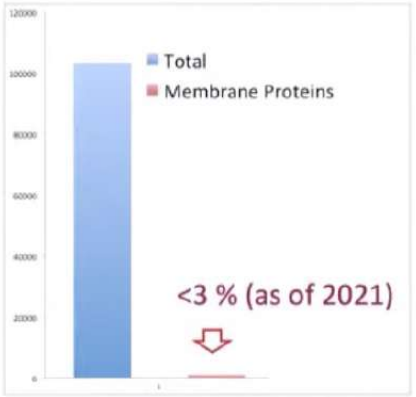
Chem. Biol. (2014) 21: 97-113

Membrane proteins

- ~30 % of genome codes for membrane proteins
- ~60 % of drug targets are membrane proteins



Protein structures solved by X-ray crystallography



Source: RCSB Protein Data Bank

Structural determination of membrane proteins is extremely challenging

Lipid-protein interaction

- Do integral membrane proteins bind tightly to lipids ?
- What is the nature of the layer of lipids adjacent to the protein ? How is it different from lipids in the bulk ?
- Do membrane proteins have long range effects on the order and dynamics of lipids ?
- Do membrane proteins create their own 'microenvironment' of lipids which is optimal for their function ?
- How do lipids influence the structure and function of membrane proteins ?

Lipid-protein interaction

- Cytochrome oxidase isolated from beef heart mitochondria and incorporated spin-labeled fatty acids into the membrane

- ESR spectra showed two components:

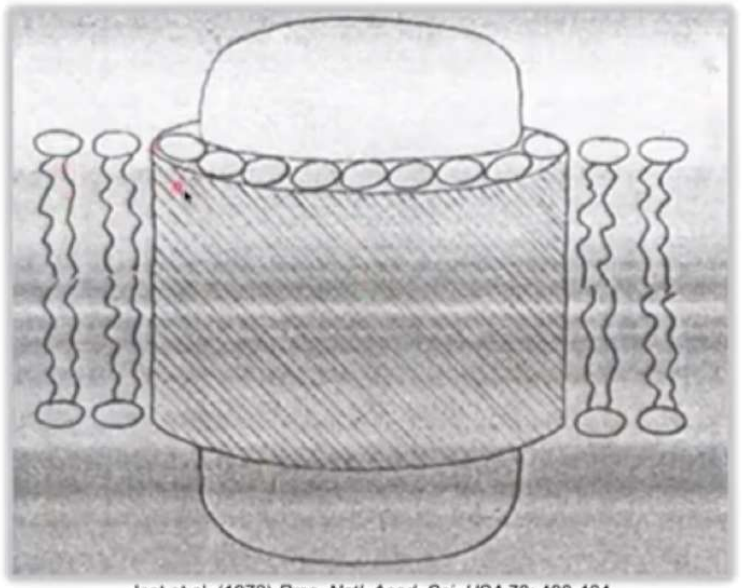
  At low lipid-to-protein ratio, a broad spectrum was observed

  At high lipid-to-protein ratio, a sharp spectrum along with broad spectrum

  Pure lipid showed only a sharp spectrum

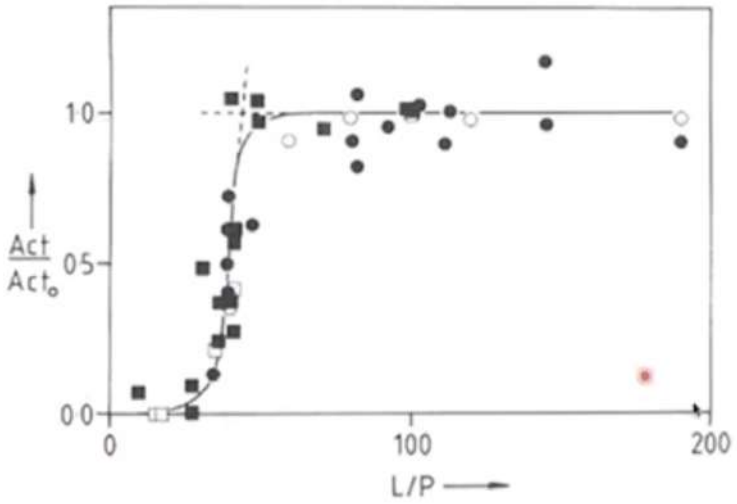
- The concept of 'Immobilized' lipids

Experiments by later workers showed that these lipids were not immobilized but displayed slower exchange rates than bulk lipids – Termed as 'Boundary' or 'Annular' lipids



Jost et al. (1973) *Proc. Natl. Acad. Sci. USA* 70: 480-484

Lipid-protein interactions

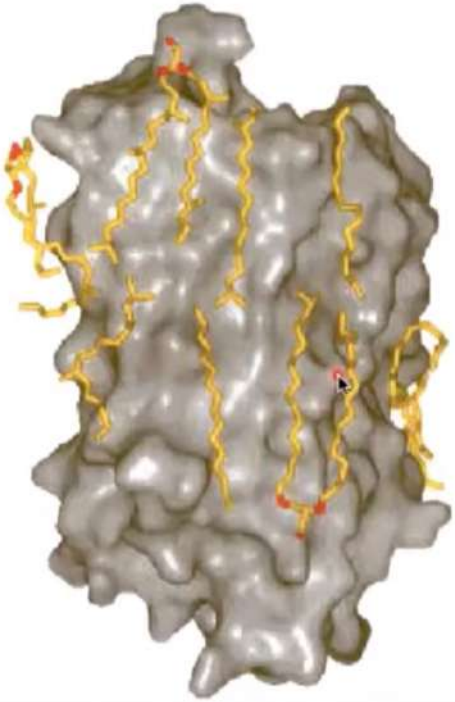


Nicotinic acetylcholine receptor activity

Protein	Number of annular lipids	Indications of segregation
β-Hydroxybutyrate dehydrogenase	30	Phosphatidylcholine
Ca^{2+}-ATPase (sarcoplasmic reticulum)	30	Phospholipids
Cytochrome oxidase	55	Cardiolipin and Acidic phospholipids
Glycophorin	30/dimer	Acidic phospholipids
Na^+/K^+-ATPase	?	Acidic phospholipids
Rhodopsin	24	Acidic phospholipids
Nicotinic acetylcholine receptor	45	Acidic phospholipids and cholesterol

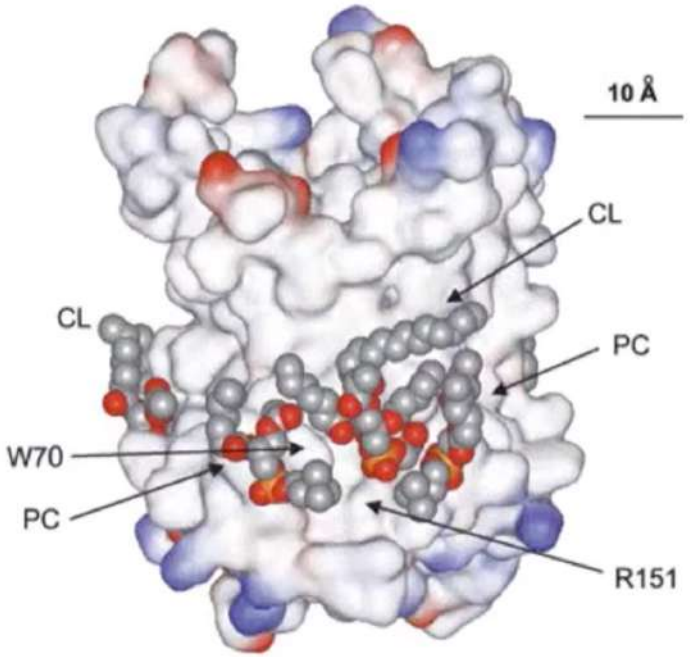
Lipid-protein interactions

Bacteriorhodopsin crystal structure
at 1.55 Å resolution



Leucke et al. (1999) *J. Mol. Biol.* 291: 899-911

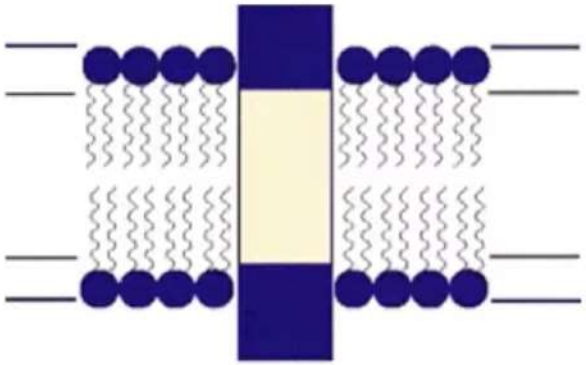
Mitochondrial ADP/ATP carrier
crystal structure at 2.2 Å resolution



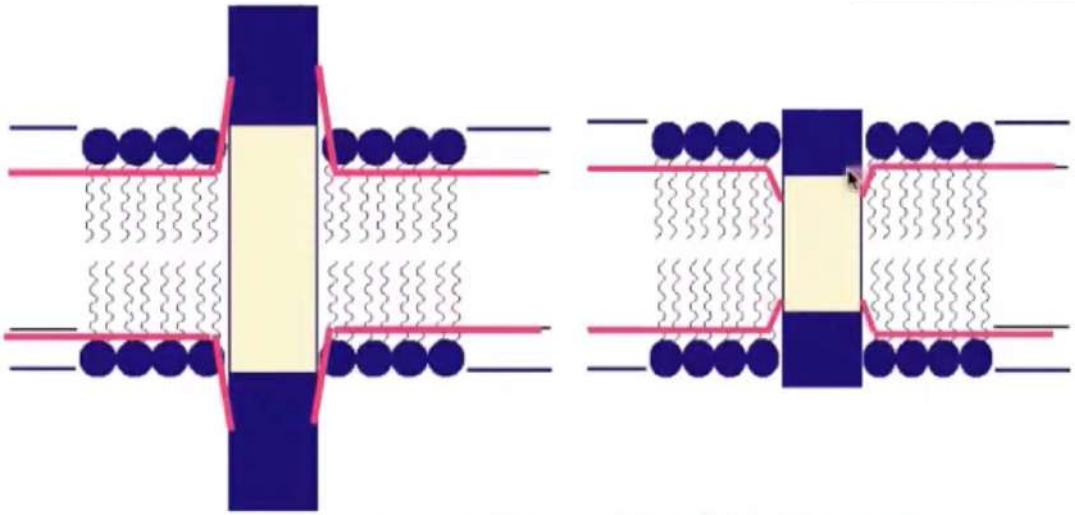
Pebay-Peyroula et al. (2003) *Nature* 426: 39-44

Hydrophobic mismatch

Hydrophobic Match



- Membrane proteins have distinct transmembrane domains
- The length of these domains should match the hydrophobic length of the membrane in which it resides in

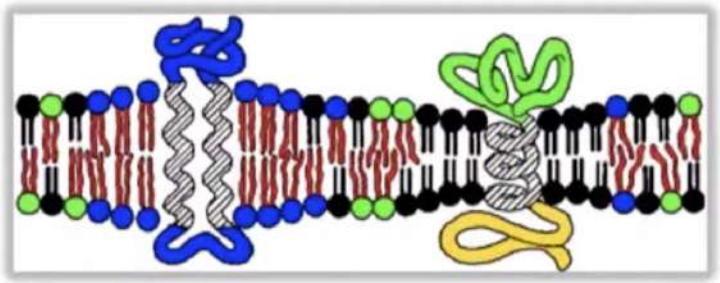


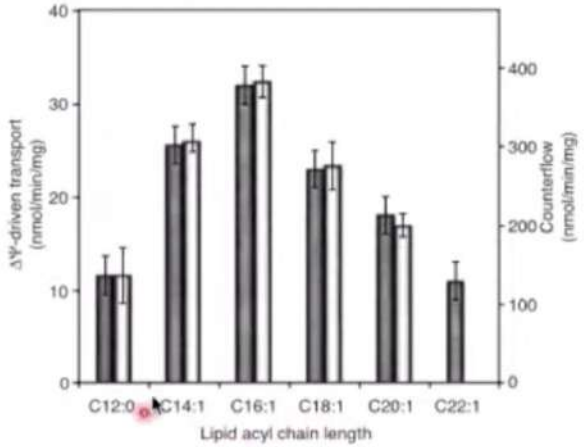
- When these do not match 'HYDROPHOBIC MISMATCH' occurs
- Mismatch is a result of the direct interaction of the transmembrane regions of the protein and the lipid acyl chains
- Mismatch is energetically unfavorable
- Membrane lipids and proteins must adapt to minimize mismatch

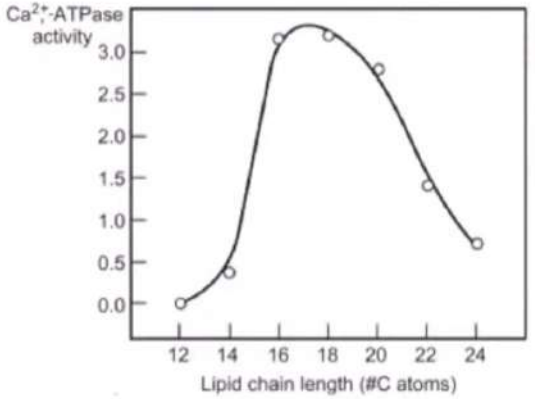
Hydrophobic mismatch

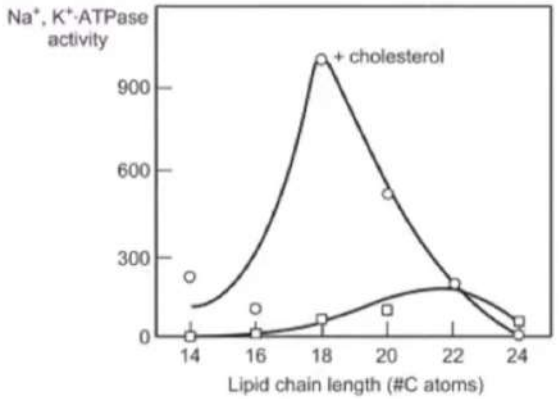
- In eukaryotic cells, there is a gradient of increasing bilayer thickness from ER to Golgi to Plasma membranes. All membrane proteins have to traverse this path.
- Mismatch could play a role in such sorting.
- Eukaryotic membranes are heterogeneous mixtures of a variety of phospholipids, sphingolipids and cholesterol
- Long chain lipids and cholesterol often phase separate to form membrane domains, called 'rafts'. Such domains therefore will be longer than the rest of the membrane.
- Mismatched proteins could segregate to such domains to relieve mismatch. Such domains may therefore act as clustering points for such special proteins.

Hydrophobic mismatch and protein function







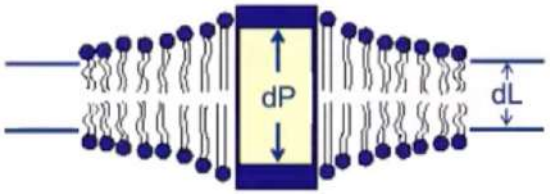


Biochim. Biophys. Acta (2004) 1666: 205-226
Biochemistry (2001) 40: 8842-8851
Biochemistry (2000) 39: 4846-4852

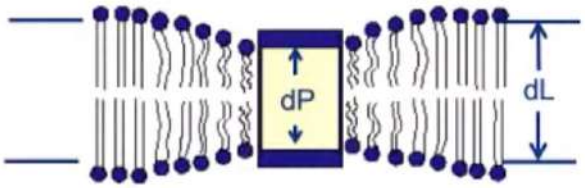
ATPasi: ion pumps

Adapting to mismatch

Lipid responses to mismatch

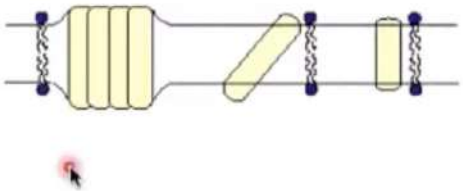


Long Proteins increase the Tm of short bilayers

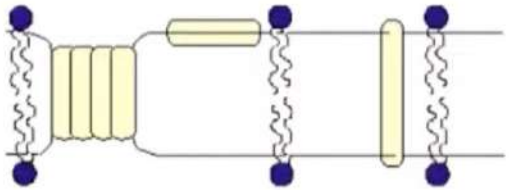


Short Proteins decrease the Tm of long bilayers

Protein responses to mismatch



Aggregation Helix Tilt Conformational Change

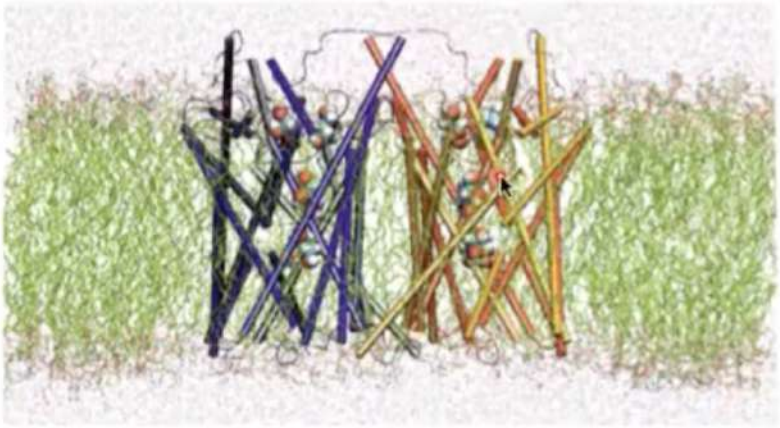


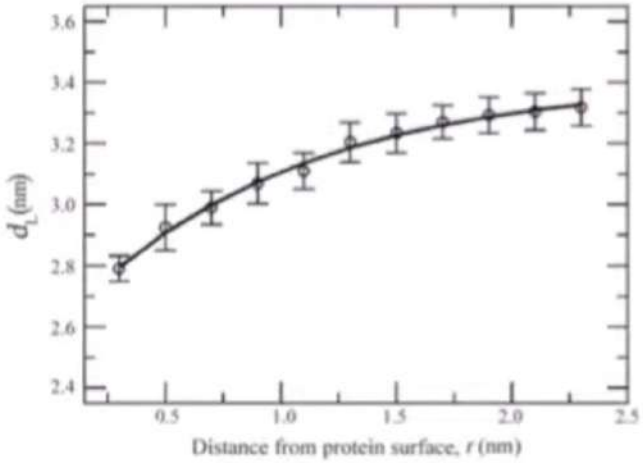
Aggregation Surface Orientation Conformational Change

Dumas et al. (1999) *FEBS Lett.* 458: 271-277

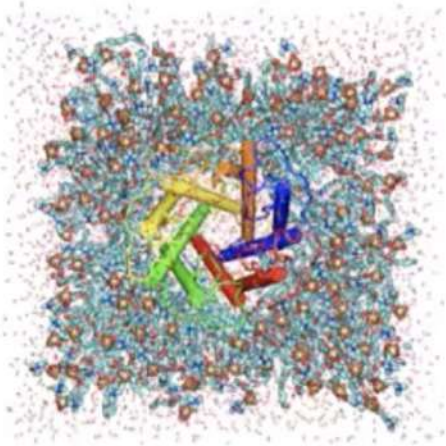
Adapting to mismatch: thinning

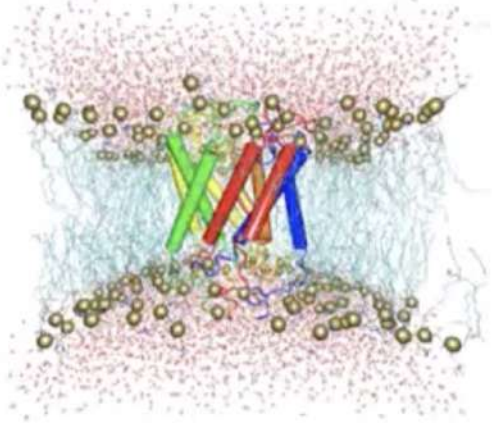
Aquaporin in fluid POPC bilayer





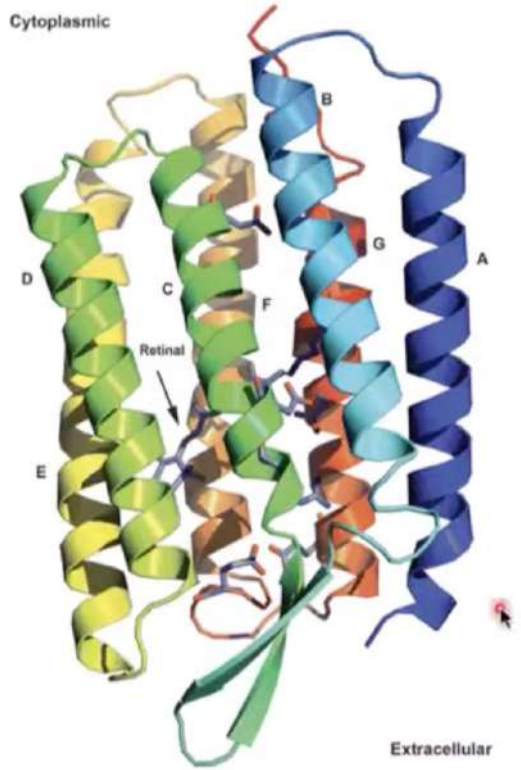
Mechanosensitive channel, MscL



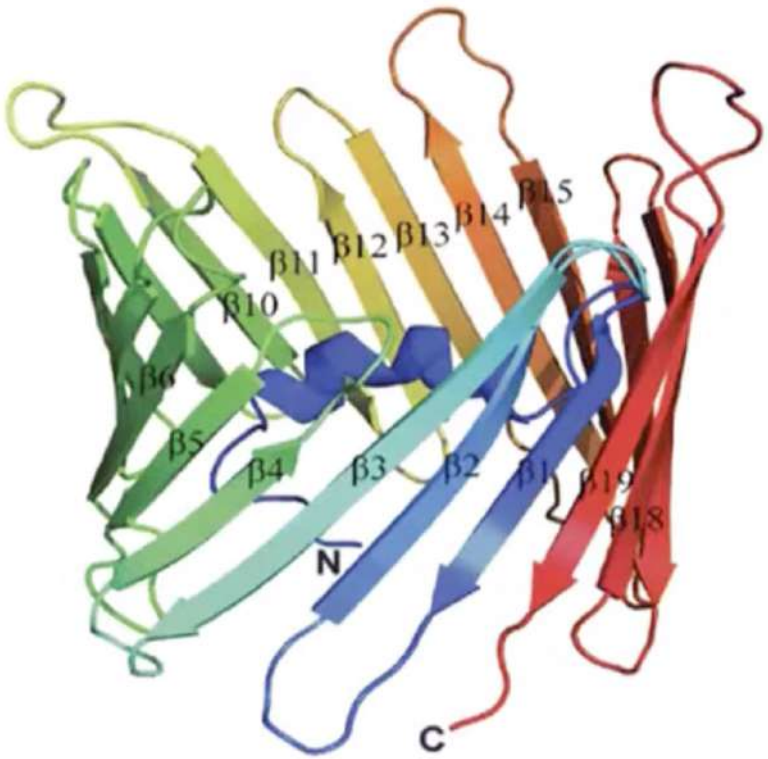


Life-As a matter of fat: lipids in membrane biophysics perspective by Ole G. Mouritsen & Luis A. Bagatolli, 2nd edn. 2015, Springer
Gullingsrud et al. (2001) *Biophys. J.* 80: 2074-2081

Membrane proteins



Bacteriorhodopsin
α-helical bundle

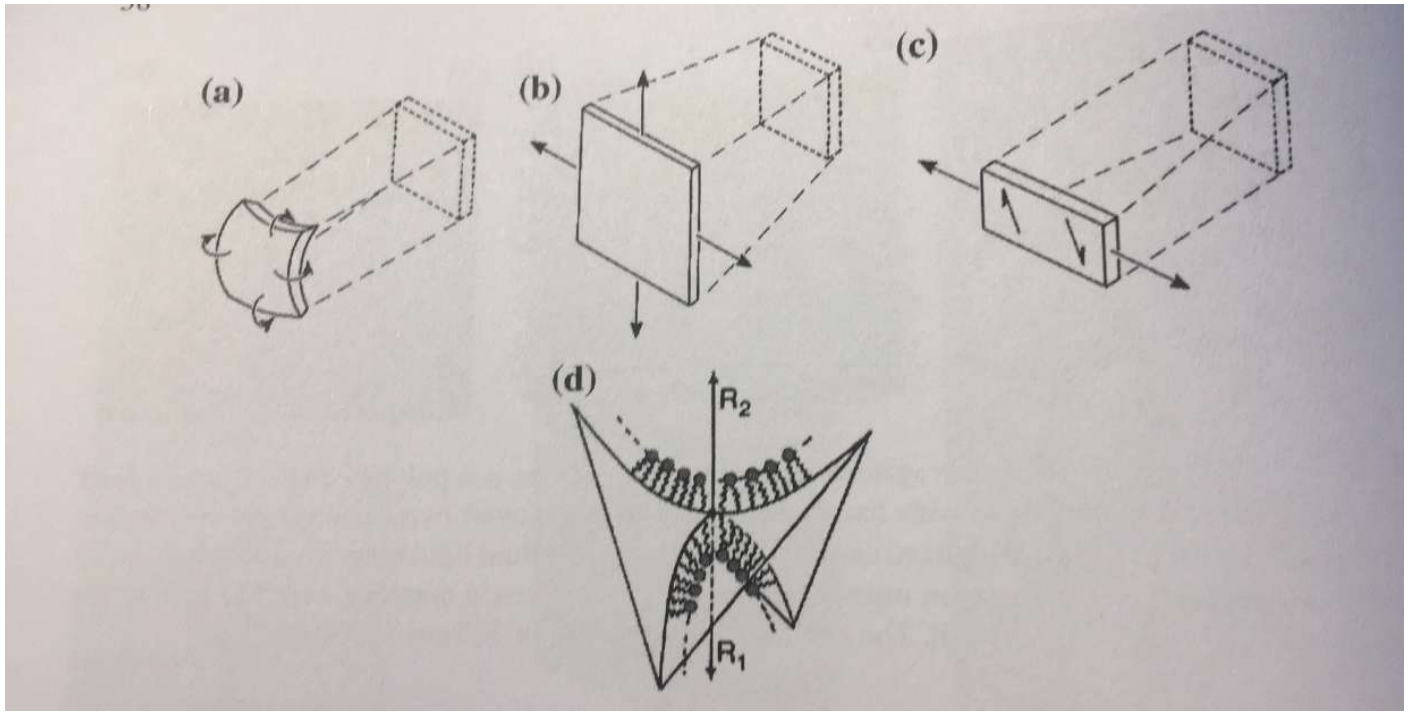


Mitochondrial porin, VDAC
β-barrel

Mary Luckey, Chapter 1:Introduction to the Structural Biology of Membrane Proteins , in *Computational Biophysics of Membrane Proteins*, 2016, pp. 1-18

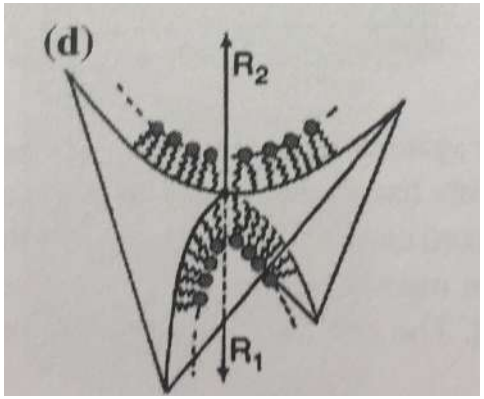
Lipids and membrane curvature

A soft interface can be compressed (a), expanded (b), subject to shear forces (c, not applicable to fluid interfaces as lipid bilayers).
The curvature is characterized by the two radii, R_1 and R_2 (d).



The stability and conformation of the interface is controlled by conformational entropy and by the elasto-mechanical properties of the interface.

Lipids and curvature



The mebrane can also be deformed via the elasto-mechanical moduli:
the area compressibility modulus K;
the bending modulus κ.

For the area compressibility modulus, we define the energy per unit area E_K, that we need to spend to uniformly stretch a unit area A_0 of ΔA calculated according to the Hooke's law:

$$E_K = \frac{1}{2} K \left(\frac{\Delta A}{A_o} \right)^2$$

The bending modulus for a flat interface (no constrain imposed by boundaries) is defined via the energy per unit area $E\kappa$ required to produce a mean curvature H of the interface, after:

$$E_\kappa = 2\kappa H^2 \qquad H = \frac{1}{2}\left(\frac{1}{R_1} + \frac{1}{R_2} \right)$$

The two modulus must be related. In the simplest case:
$\kappa = d_L{}^2 K$ where d_L is the thickness of the interface.

Lipids and curvature

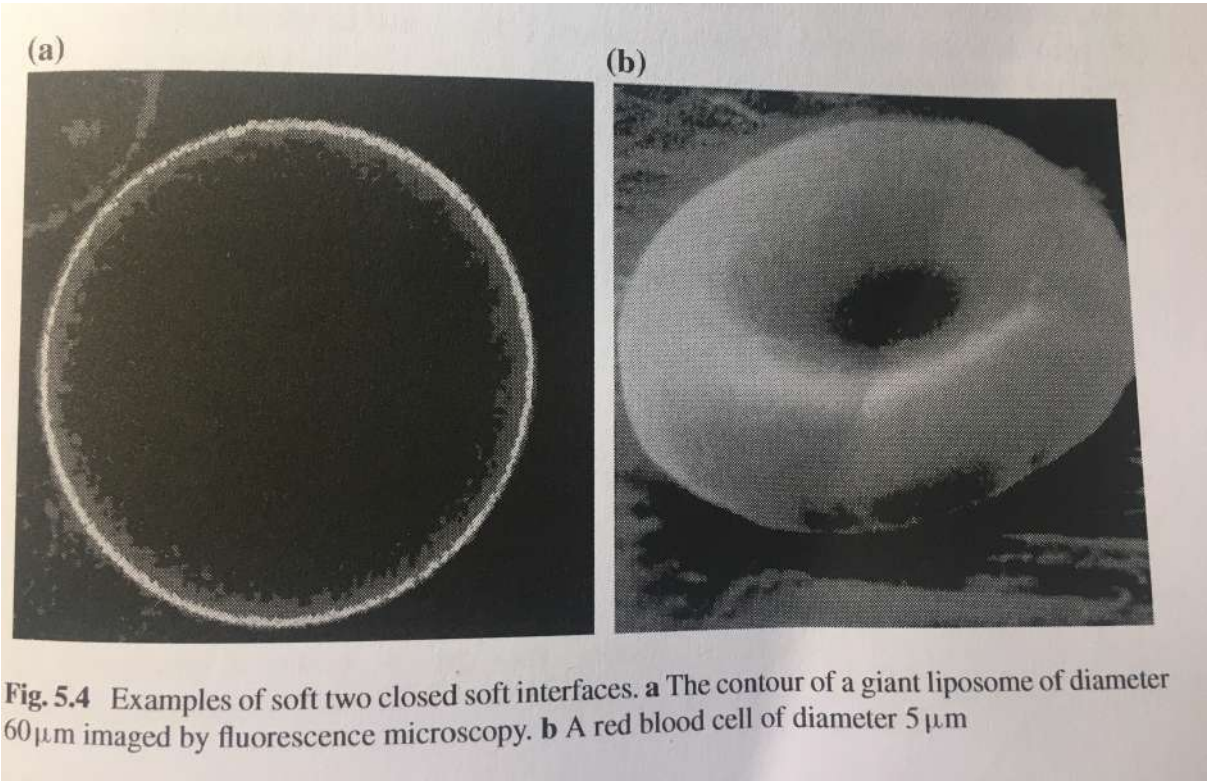


Fig. 5.4 Examples of soft two closed soft interfaces. **a** The contour of a giant liposome of diameter 60 μm imaged by fluorescence microscopy. **b** A red blood cell of diameter 5 μm

Two soft membranes with different bending capabilities.

The persistent length, i.e. the length over which they appear flat and smooth, is different. It is related to the bending modulus via (with c a constant):

$$\xi \sim \exp\left(\frac{c\kappa}{k_B T}\right)$$

The ratio between bending modulus and thermal energy determines the persistent length! P.L. is exp. dependent on the bending modulus.

Liposomes have low values of κ. Subject to fluctuations, ondulations; Plasma membranes have $\kappa >> KT$ and appear smooth; Golgi and endoplasmic reticulum are very soft (no chol!) with non-spherical topologies.

Forces between soft interfaces

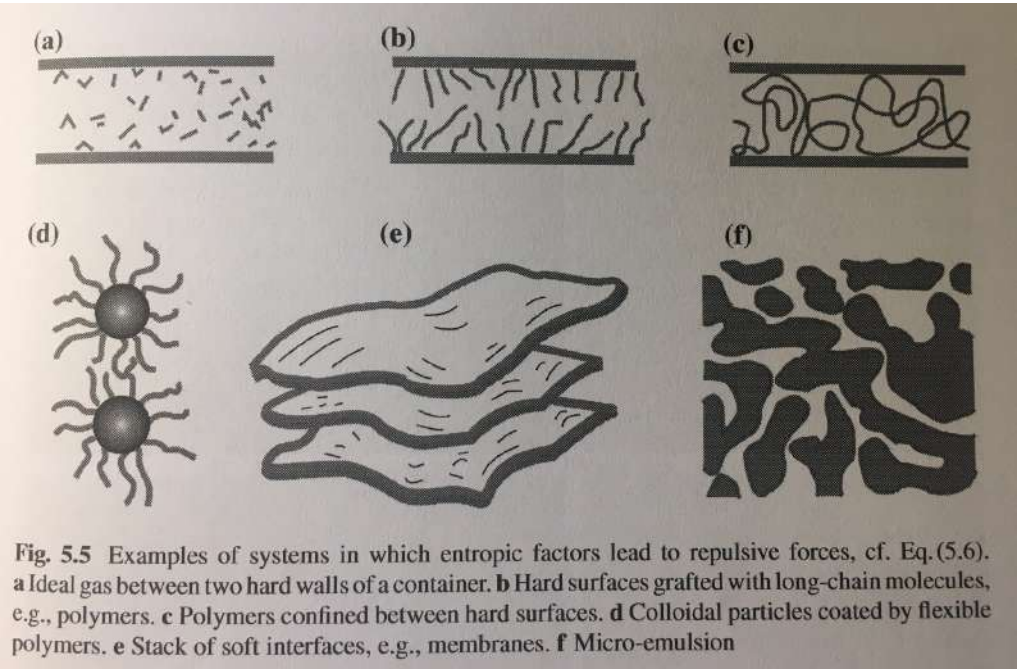


Fig. 5.5 Examples of systems in which entropic factors lead to repulsive forces, cf. Eq. (5.6). **a** Ideal gas between two hard walls of a container. **b** Hard surfaces grafted with long-chain molecules, e.g., polymers. **c** Polymers confined between hard surfaces. **d** Colloidal particles coated by flexible polymers. **e** Stack of soft interfaces, e.g., membranes. **f** Micro-emulsion

The softness of membranes generates collidal forces among them. It is a thermodynamic force, a spatial derivative of the free energy G = H-TS:

$$F = -\left(\frac{\partial G}{\partial r}\right) = -\left(\frac{\partial H}{\partial r}\right) + T\left(\frac{\partial S}{\partial r}\right)$$

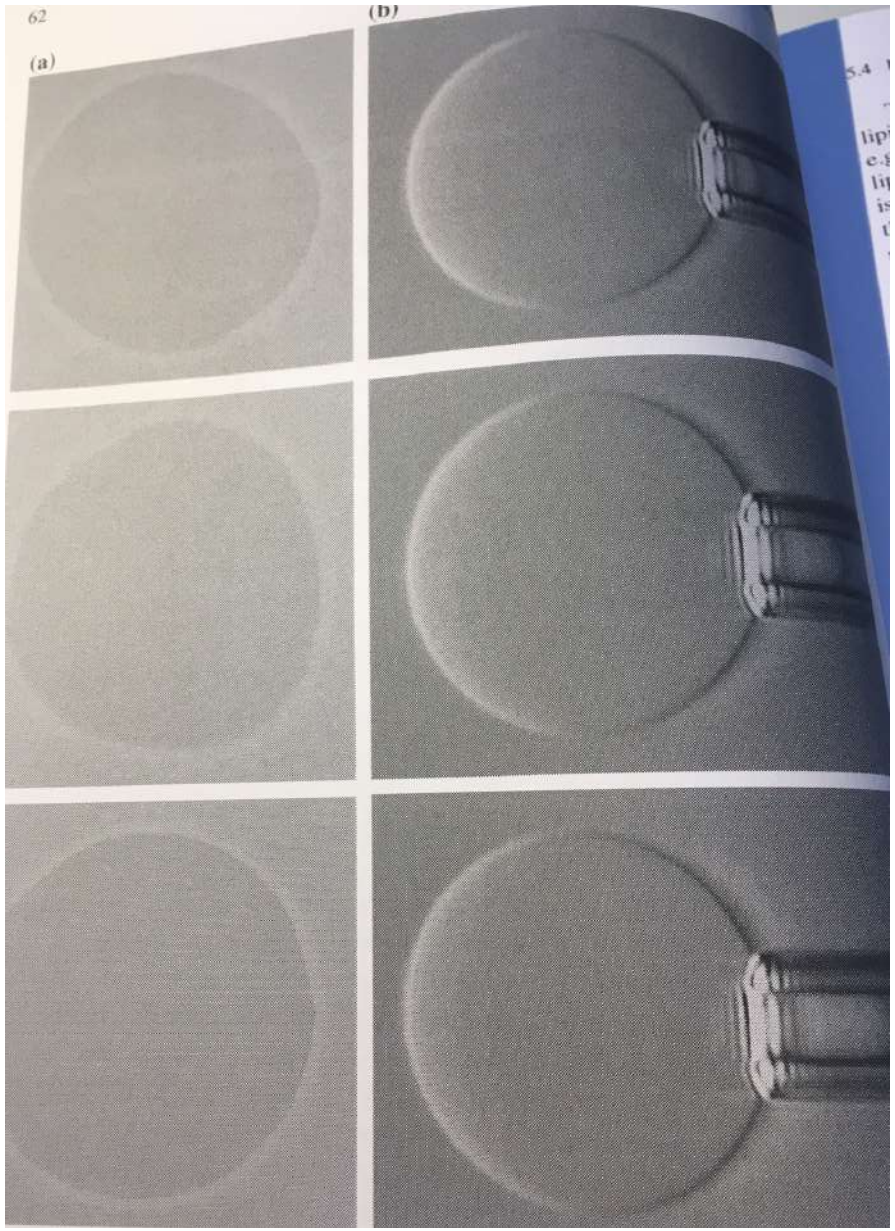
r is the distance. It involves Entropy.

There is always an entropic repulsion between soft interfaces, even in the absence of direct mechanical forces! The reduction in configuration entropy due to confinement produces repulsive forces.
We can define an entropic undulation force:
Between soft interfaces at distance d:

$$F \sim \frac{(k_B T)^2}{\kappa d^3}$$

It increases with the decrease of bending rigidity!

Lipid membranes are really soft

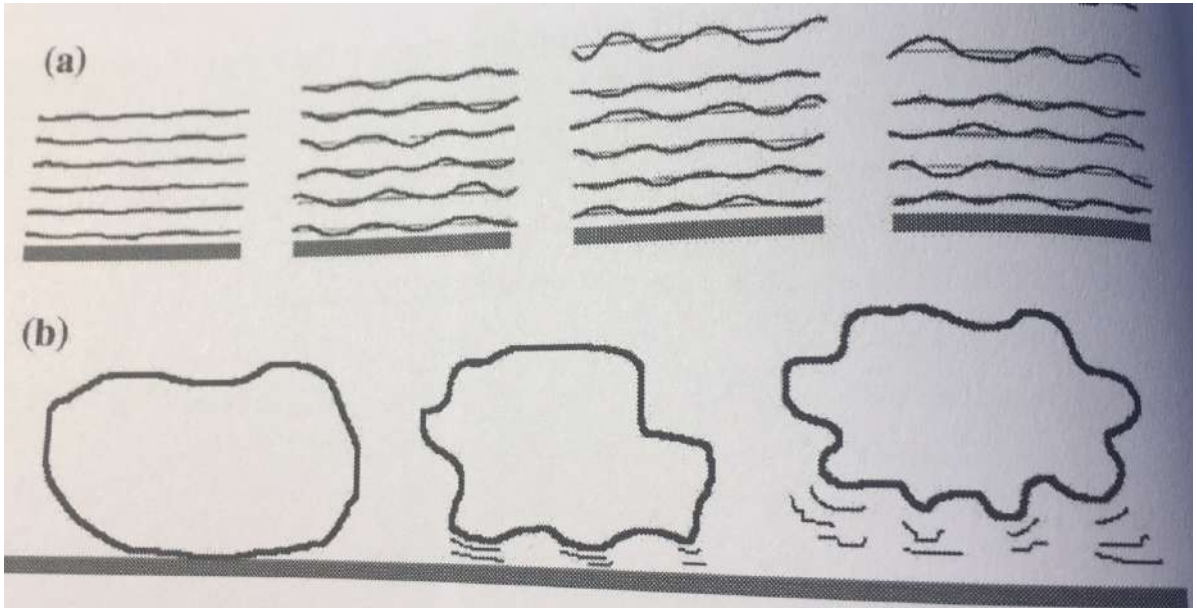


Giant liposomes (50 um). Membrane thickness: 5 nm.
Variation in the contour due to thermal fluctuations---the membrane is very soft!!
The bending modulus κ can be derived from the spectrum of fluctuations.
With the pipette aspiration one apply a stress τ and measure the compressibility modulus K from the resulting area strain $\Delta A/A_0$.
$\tau = K\ (\Delta A/A_0)$.

A red blood cell membrane is 50.000 times softer than a polyethylene film with the same thickness. A DMPC bilayer is 5 times softer than red blood cell (no cytoskeleton!).
In lipid bilayers, shorter and more unsaturated chains providehigher softness .

κ for DMPC ia around 10 KT

Lipid membranes are really soft



Because of undulation forces by soft bilayers, vesicles/lipid bilayers are repelled by solid surfaces.

How softness can be controlled at the molecular level?