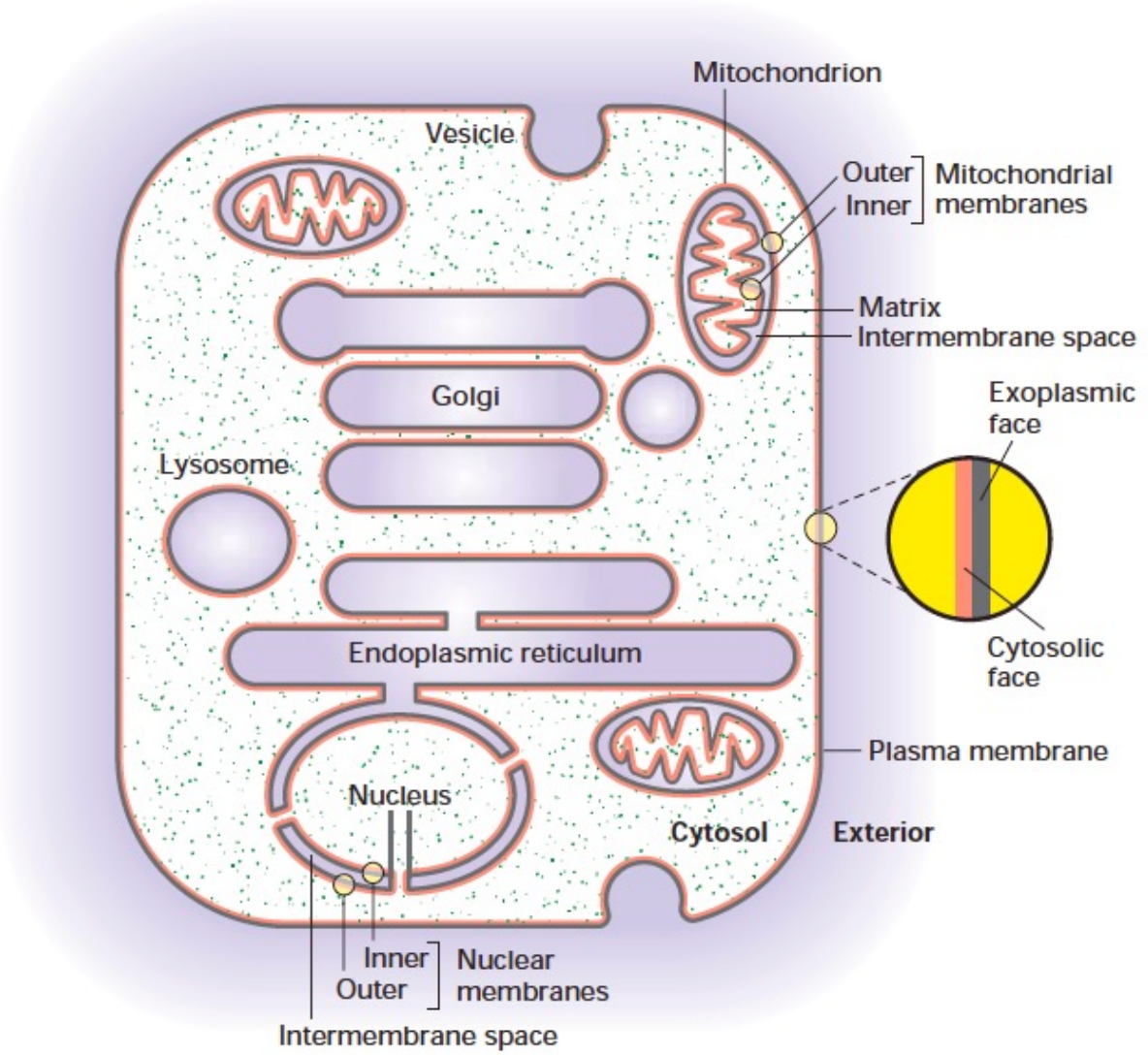
Cell membranes

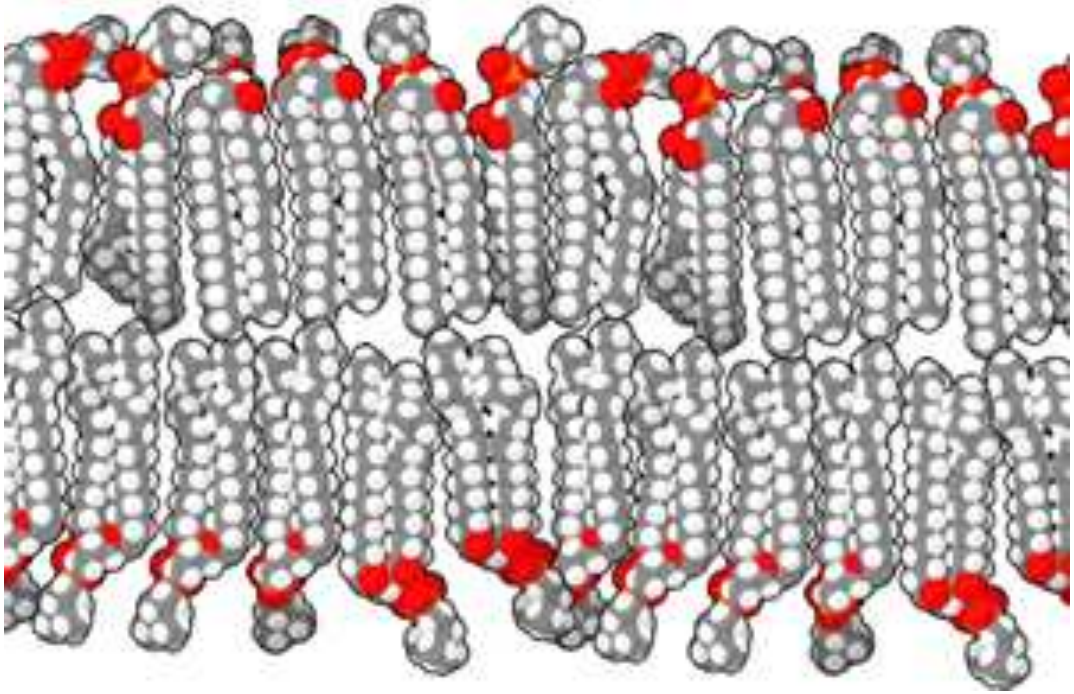
▶ FIGURE 5-4 The faces of cellular membranes. The plasma membrane, a single bilayer membrane, encloses the cell. In this highly schematic representation, internal cytosol (green stipple) and external environment (purple) define the cytosolic (red) and exoplasmic (black) faces of the bilayer. Vesicles and some organelles have a single membrane and their internal aqueous space (purple) is topologically equivalent to the outside of the cell. Three organelles—the nucleus, mitochondrion, and chloroplast (which is not shown)—are enclosed by two membranes separated by a small intermembrane space. The exoplasmic faces of the inner and outer membranes around these organelles border the intermembrane space between them. For simplicity, the hydrophobic membrane interior is not indicated in this diagram.



Cell membranes

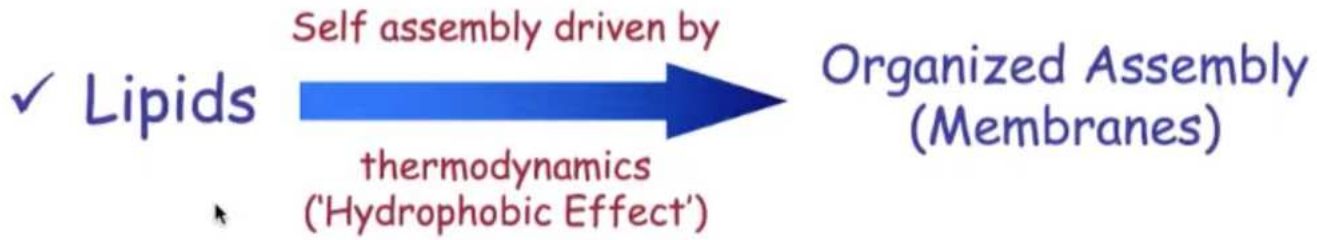
Membranes are made of strongly anisotropic molecules
Strongly anisotropic molecules like to self-organizing.

•a typical eukaryotic cell membrane contains 500–2000 different lipid species



Cell membranes

What is so unique about membrane organization ?



✓ No intermolecular connectivity
No 'primary sequence'

} (unlike proteins and nucleic acids)

✓ Membranes are DYNAMIC with built-in Anisotropy

However, dynamics does not implicitly implies randomness and disorder!
It is a many body problem with LOCAL (nm scale) order and structure

Forces that hold membrane

The Hydrophobic Effect describes how an aqueous medium deals with non-polar substances

It forms the basis for the formation of a variety of organized molecular assemblies such as membranes, micelles, and folded proteins

It should not be confused with the force of interaction among two non-polar (hydrophobic) molecules which plays a very minor role in hydrophobic effect. The effect actually arises primarily from the strong attractive forces between water molecules and the entropic cost of incorporating a non-polar molecule among water molecules.

Tanford (1980) The Hydrophobic Effect
John Wiley, New York

Hydrophobic forces

Hydrophobic forces are very relevant in biology. They are primarily driven by an energy cost of creating hydrocarbon-water contact.
There is a reduction of entropy of water close of a hydrophobic surface: water becomes structured, even ice-like. It restricts the possible orientations close to the surface and decrease entropy.

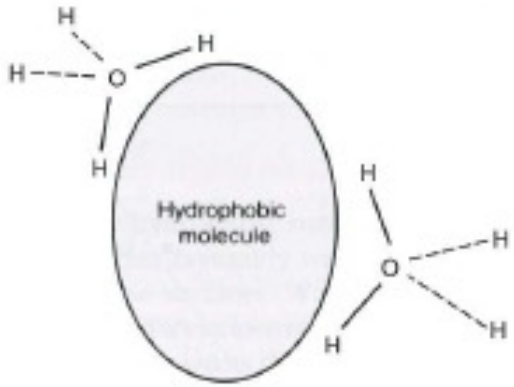
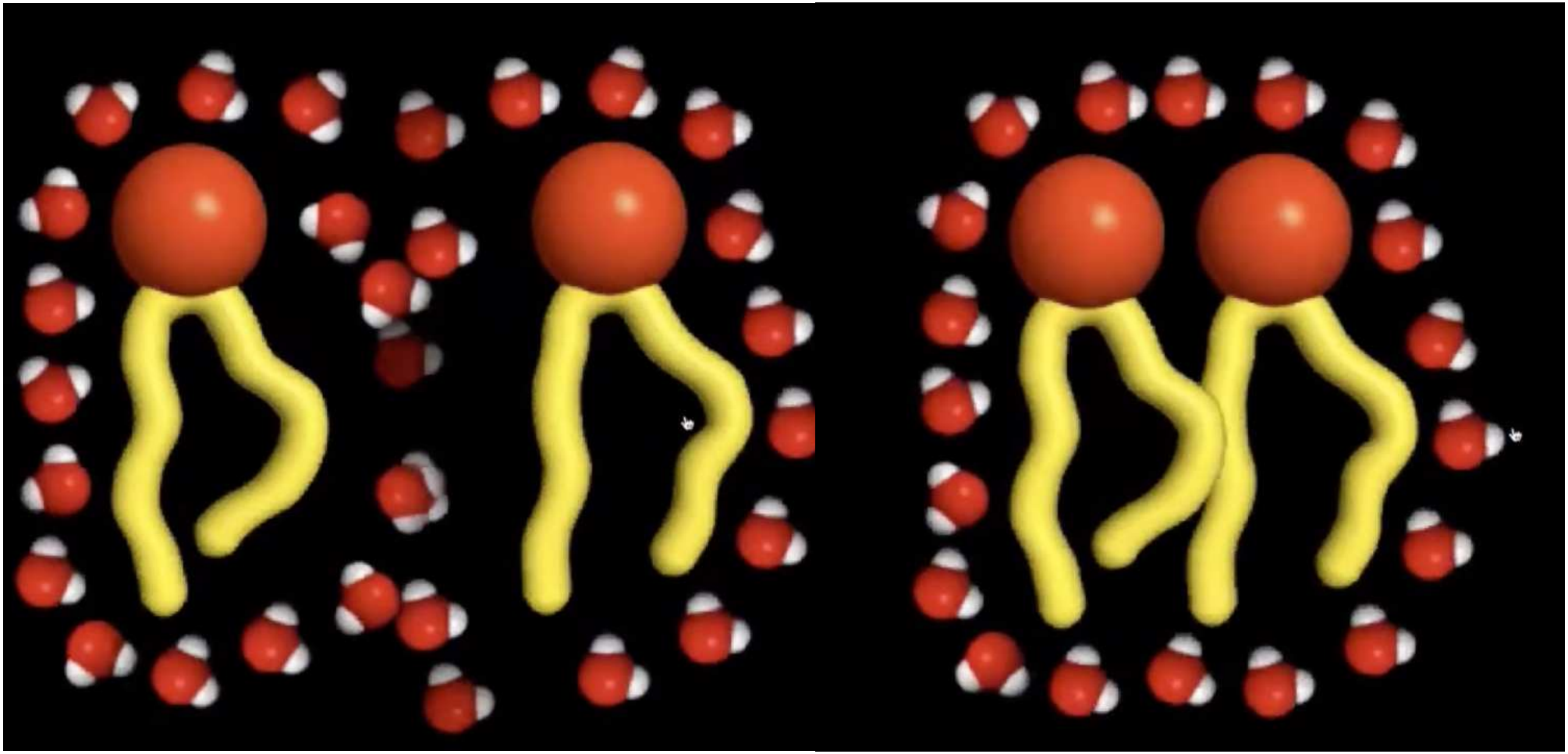


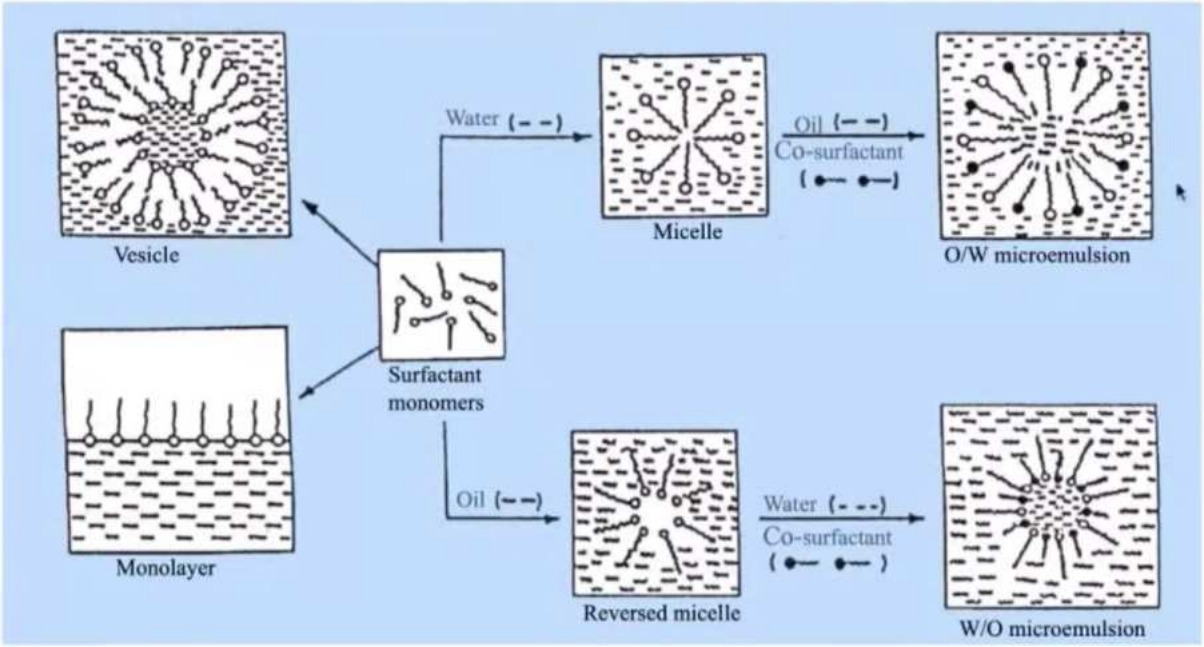
Fig. 2.7 Water molecules adjacent to a hydrophobic molecule suffer restrictions in orientation as they form hydrogen bonds with other water molecules.

Hydrophobic effect

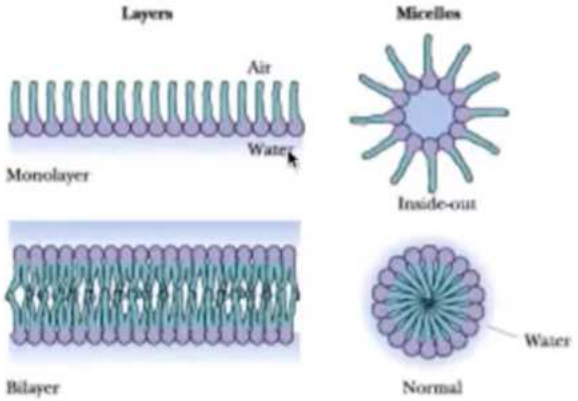


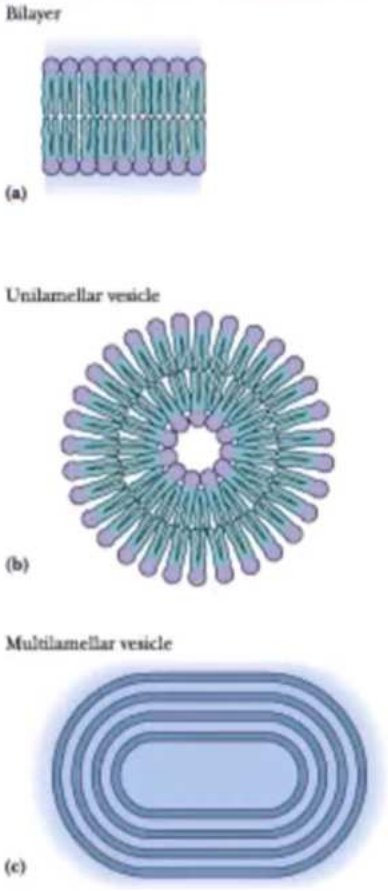
Hydrophobic effect

Organized molecular assemblies of various types formed due to the Hydrophobic Effect

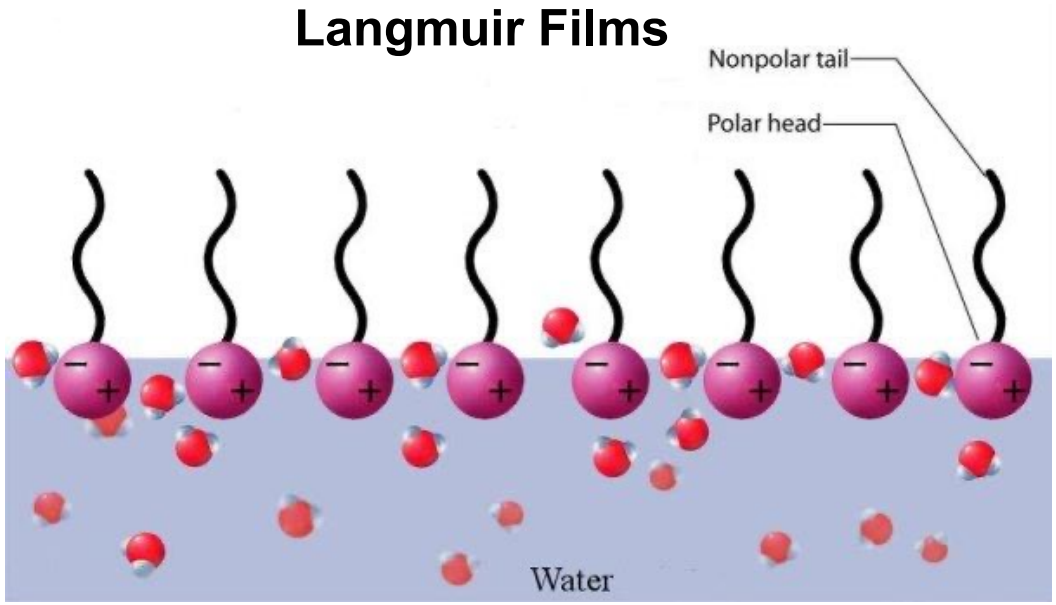


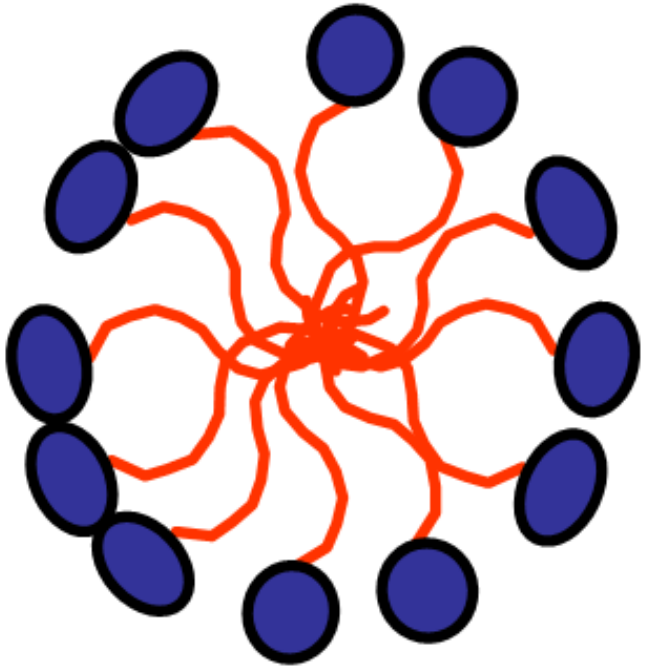
Phospholipid Supramolecular Assemblies





Self-organized monolayers (on liquid surfaces)

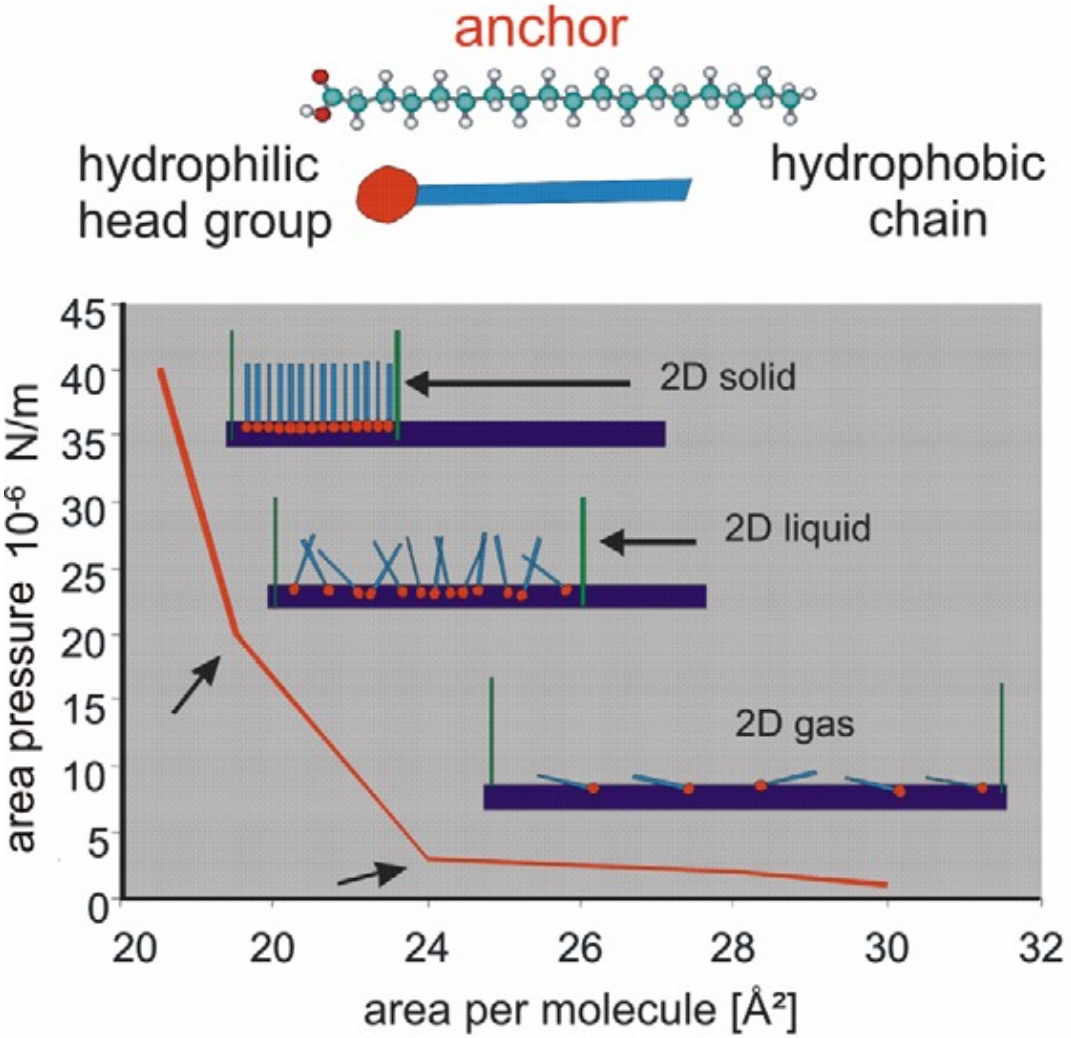




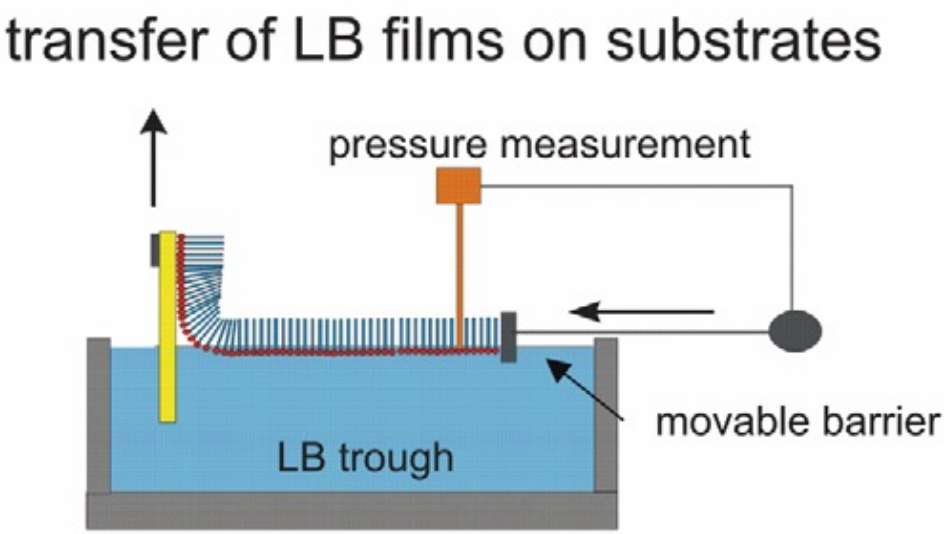
The term ‘‘molecular self-assembly” refers to spontaneous formation of an ordered molecular overlayer on the surface, often proceeding through several consecutive stages where 1D and 2D ordered structures can also exist.

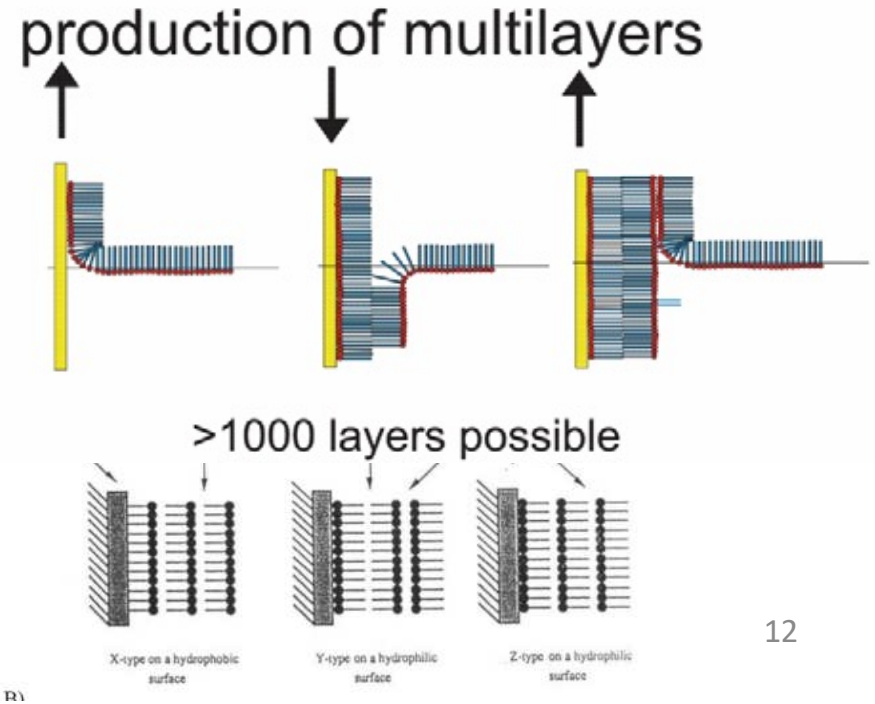
Thermodynamically, molecular self-assembly proceeds toward the state of lower entropy , and must therefore be compensated by the establishment of intermolecular and molecule-surface interactions.

Self-organized monolayers (on solid surfaces)



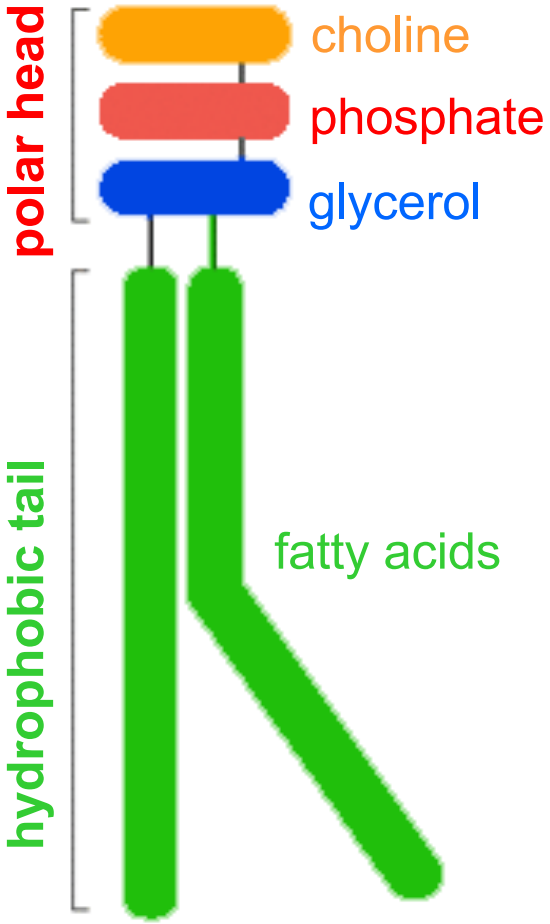
Progress in Surface Science 84 (2009) 230–278



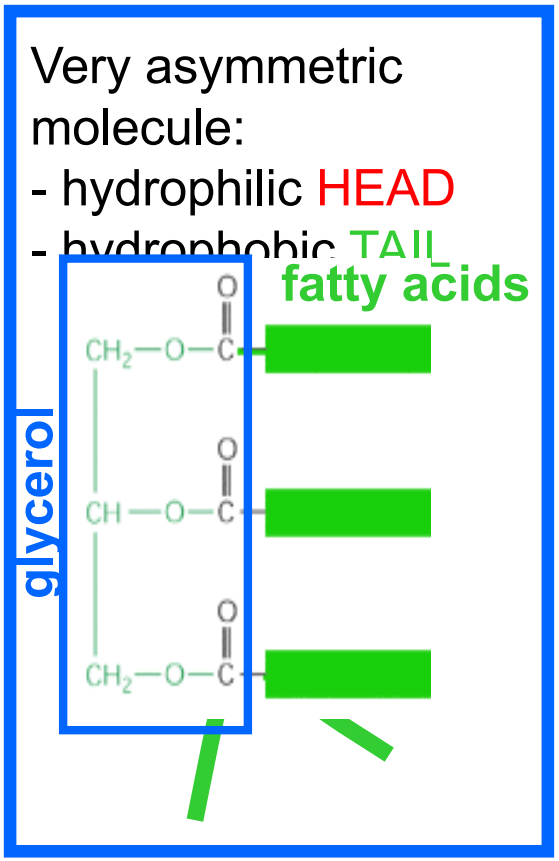


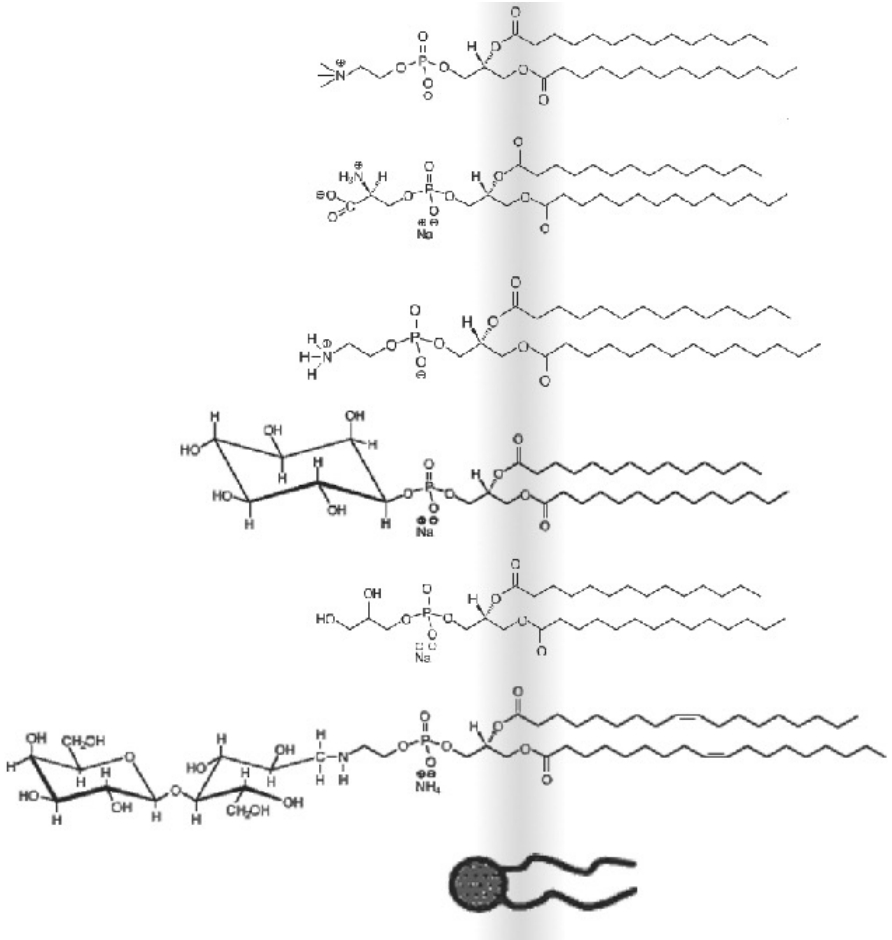
Main lipid family: Phospholipids

In phospholipids, two of the OH groups of glycerol are linked to fatty acids (ester bond), while the third is linked to a phosphate group, which can be further linked to a polar group such as choline, serine, inositol, etc...



Very asymmetric molecule:
- hydrophilic HEAD
- hydrophobic TAIL





phosphatidylcholine (PC)

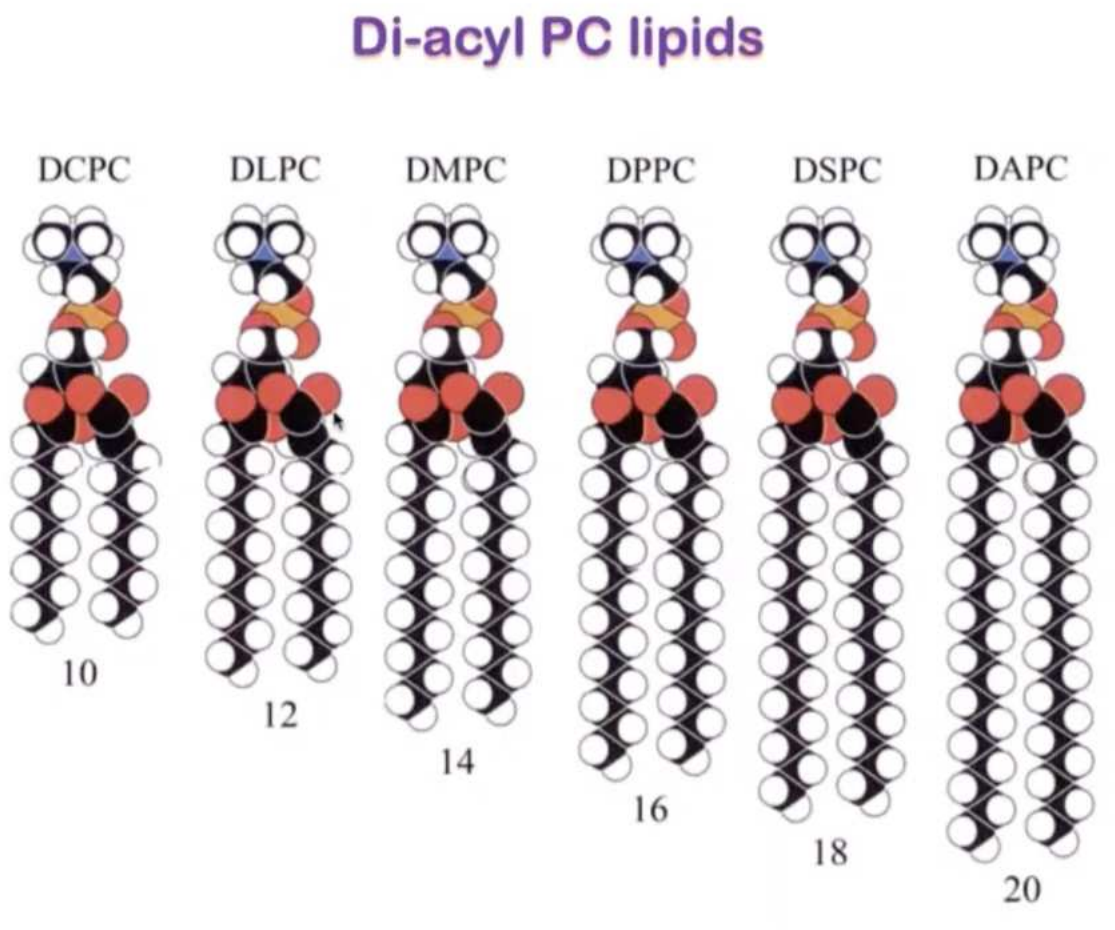
phosphatidylserine (PS)

phosphatidylethanolamine (PE)

phosphatidylglycerol (PG)

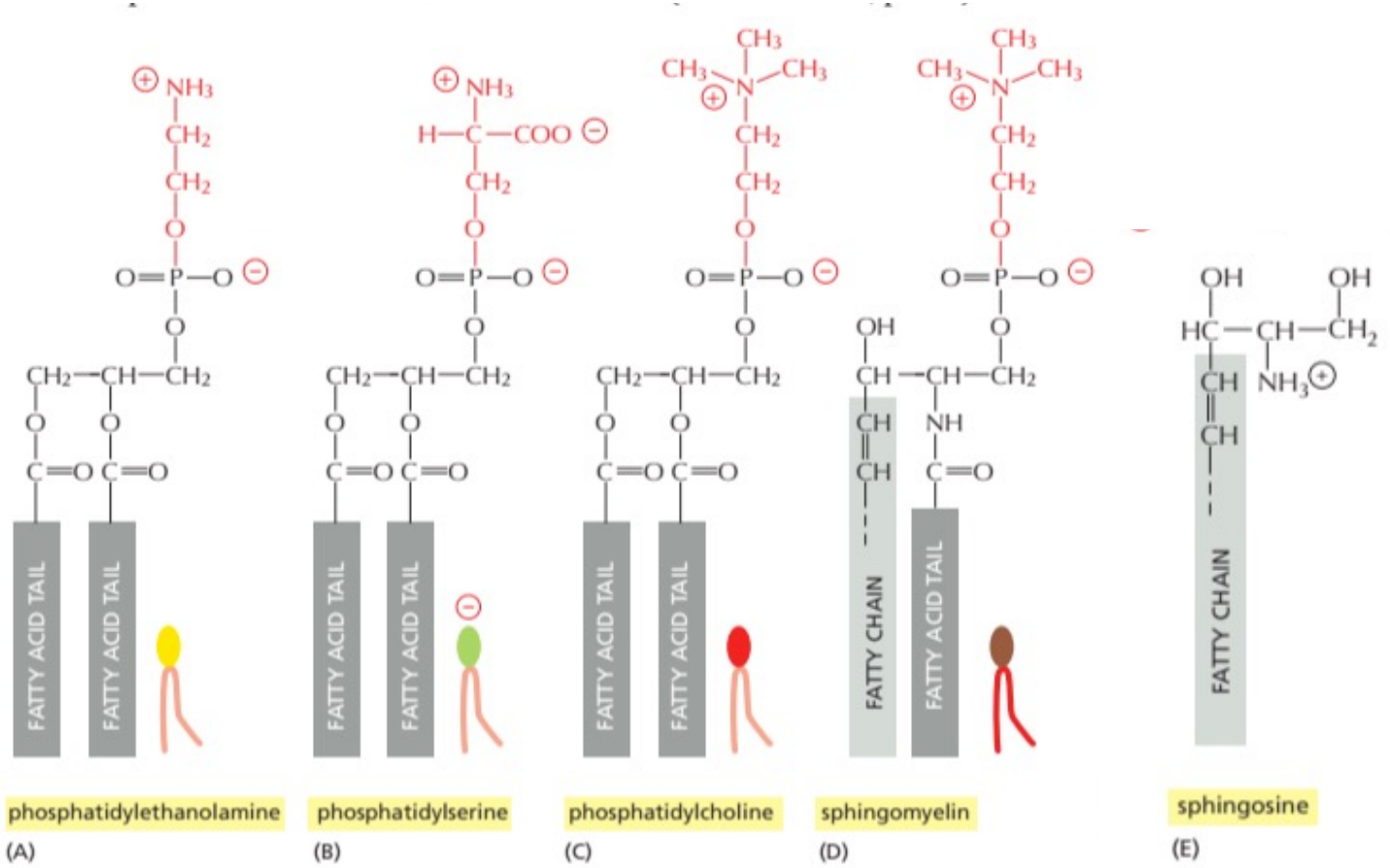
phosphatidylinositol (PI)

Glycolipid



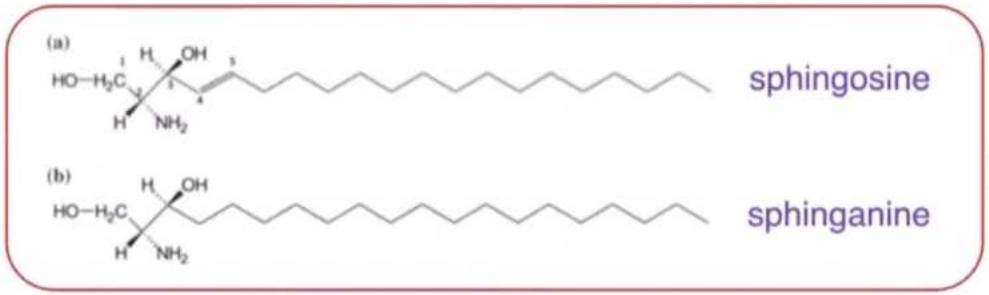
Typical cross-sectional areas of the cylinders that describe average lipid conformation in the lipid bilayers= is about 0.63 nm^2, with average length from 1.0 to 1.5 nm (depending on number of C atoms, saturation).

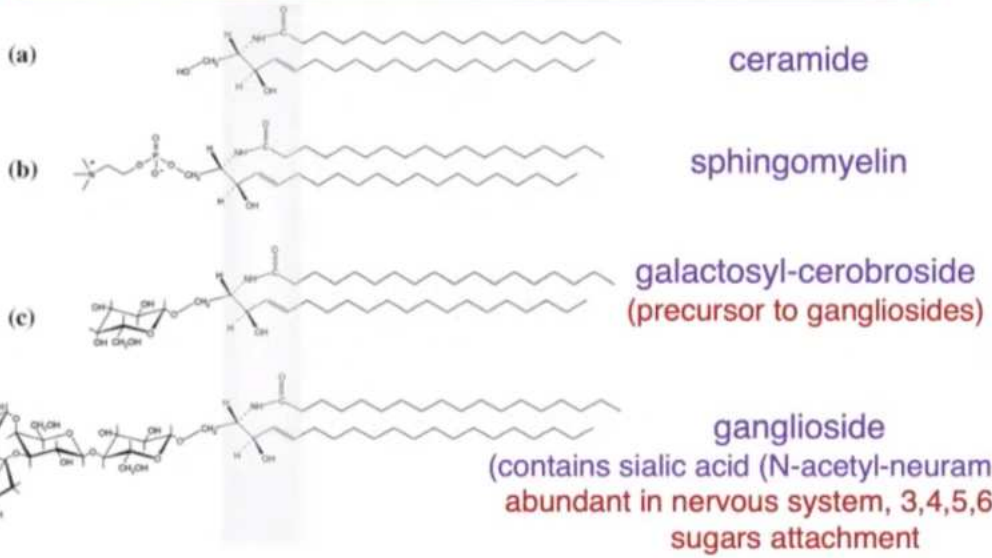
Another family: Sphingolipids



Sphingolipids are derivatives of sphingosine (E), an amino alcohol with a long hydrocarbon chain. Various fatty acyl chains are connected to sphingosine by an amide bond.
The sphingomyelins (SM), which contain a phosphocholine head group, are phospholipids.
Other sphingolipids are glycolipids in which a single sugar residue or branched oligosaccharide is attached to the sphingosine backbone.

Sphingosine based phospholipids





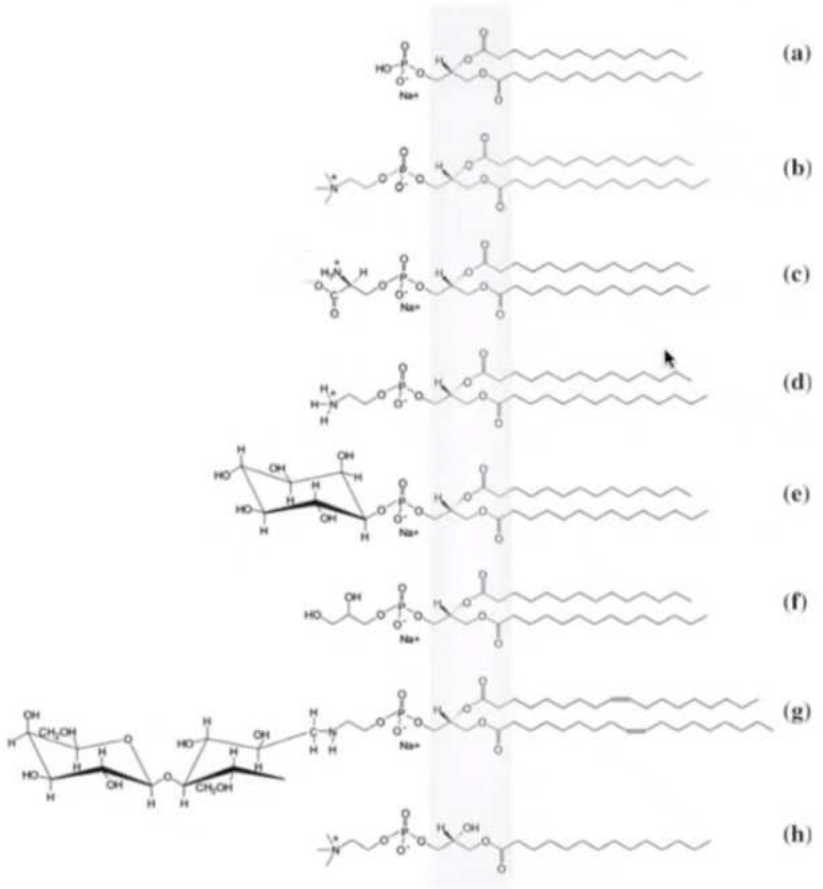
Lipids nomenclature

- The nomenclature of fatty acids is rather complicated. There are at least five systems in use
- The delta system numbers the double bonds from the carboxyl group (the α carbon)
- The omega system indicates where the first double bond is counting from the other end of the molecule (the ω carbon).

Trivial	Systematic	Colon	Delta	Omega
Stearic acid	Octadecanoic acid	18:0	Octadecanoic acid	-
Palmitic acid	Hexadecanoic acid	16:0	Hexadecanoic acid	-
Oleic acid	E-Octadec-9-enoic acid	18:1; n9	*cis*-Δ^{9}-octadecenoic acid	ω−9
Linoleic acid	9E, 12E-Octadeca-9, 12-dienoic acid	18:2; n9	*cis, cis*-$\Delta^{9, 12}$-octadecadienoic acid	ω−6
Linolenic acid	6E, 9E, 12E-Octadeca-6, 9, 12-trienoic acid	18:3; n6	*cis, cis, cis* -$\Delta^{6,9,12}$-octadecatrienoic acid	ω−3

The longer are the FA, the more C=C can be accomodated:
Up to 1 for 18 C; 4 for 20 C; 6 for 22 C

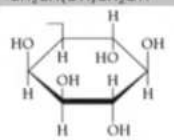
Head and Tail



Lipid polar head groups

PC, PE are zwitterionic; the other can be charged

PE, PS, PI show high degree of unsaturation

Substituent	Chemical formula*	Polar head group name	Ab§
hydrogen	-H	phosphatidic acid	PA
choline	$-CH_2CH_2N(CH_3)_3^+$	phosphatidylcholine	PC
ethanolamine	$-CH_2CH_2NH_3^+$	phosphatidylethanolamine	PE
serine	$-CH_2CH(NH_3)COO^-$	phosphatidylserine	PS
glycerol	$-CH_2CH(OH)CH_2OH$	phosphatidylglycerol	PG
myo-inositol		phosphatidylinositol	PI

*Chemical formula for the substituent linked to the phosphate group at position 3 of the glycerol moiety.
§Abbreviation for the polar head group nomenclature.

TABLE 2.1 SOME NATURALLY OCCURRING FATTY ACIDS: STRUCTURE, PROPERTIES, AND NOMENCLATURE[a]

Carbon skeleton	Structure[b]	Systematic name[c]	Common name (derivation)	Melting point (°C)	Solubility at 30°C (mg/g solvent)	
					Water	Benzene
12:0	$CH_3(CH_2)_{10}COOH$	*n*-Dodecanoic acid	Lauric acid (Latin *laurus*, "laurel plant")	44.2	0.063	2600
14:0	$CH_3(CH_2)_{12}COOH$	*n*-Tetradecanoic acid	Myristic acid (Latin *myristica*, nutmeg genus)	53.9	0.024	874
16:0	$CH_3(CH_2)_{14}COOH$	*n*-Hexadecanoic acid	Palmitic acid (Latin *palma*, "palm tree")	63.1	0.0083	348
18:0	$CH_3(CH_2)_{16}COOH$	*n*-Octadecanoic acid	Stearic acid (Greek *stear*, "hard fat")	69.6	0.0034	124
20:0	$CH_3(CH_2)_{18}COOH$	*n*-Eicosanoic acid	Arachidic acid (Latin *Arachis*, legume genus)	76.5		
24:0	$CH_3(CH_2)_{22}COOH$	*n*-Tetracosanoic acid	Lignoceric acid (Latin *lignum*, "wood" + *cera*, "wax")	86.0		
16:1 (Δ9)	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$	*cis*-9-Hexadecenoic acid	Palmitoleic acid	0.5		
18:1 (Δ9)	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	*cis*-9-Octadecenoic acid	Oleic acid (Latin *oleum*, "oil")	13.4		
18:2(Δ9, 12)	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	*cis*-,*cis*-9,12-Octadecadienoic acid	Linoleic acid (Greek *linon*, "flax")	–5		
18:3(Δ9, 12, 15)	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	*cis*-,*cis*-,*cis*-9,12,15-Octadecatrienoic acid	α-Linolenic acid	–11		
20:4(Δ5, 8, 11, 14)	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_3COOH$	*cis*-,*cis*-,*cis*-,*cis*-5,8,11,14-Eicosatetraenoic acid	Arachidonic acid	–49.5		

[a] The symbol for fatty acids gives the number of carbon atoms, followed by the number of carbon–carbon double bonds. For unsaturated fatty acids, the notations in parentheses denote the positions of their double bonds. For example, Δ9 denotes a double bond between C9 and C10. All the double bonds in these fatty acids have *cis* configuration.

[b] All acids are shown in their nonionized form. At pH 7, all free fatty acids have an ionized carboxylate. Note that numbering of carbon atoms begins at the carboxyl carbon.

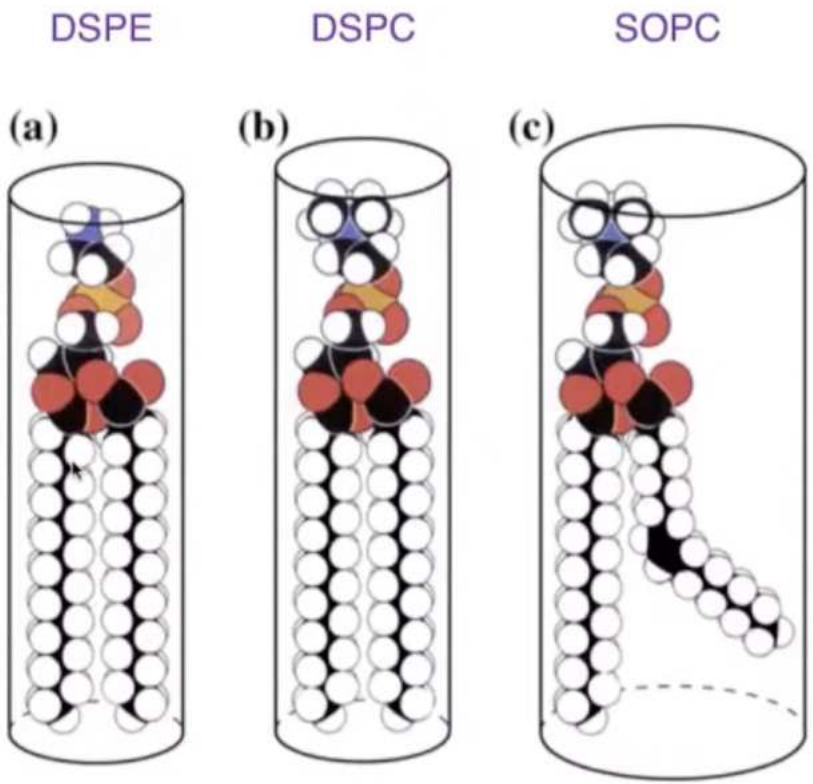
[c] The prefix *n* indicates the normal unbranched structure. For instance, dodecanoic simply indicates 12 carbon atoms, which could be arranged in a variety of branched forms; *n*-dodecanoic specifies the linear, unbranched form.

Source: Data from Nelson, D. L., and M. M. Cox, *Lehninger Principles of Biochemistry*, 4th ed. New York: W. H. Freeman, 2005.

More than 500 species of fatty acids !

Membrane are asymmetric: the outer leaflet is rich in SM, PC, chol, glycolipids
the inner leaflet is rich in PS, PI, PE

Saturated vs Unsaturated Fatty Acids



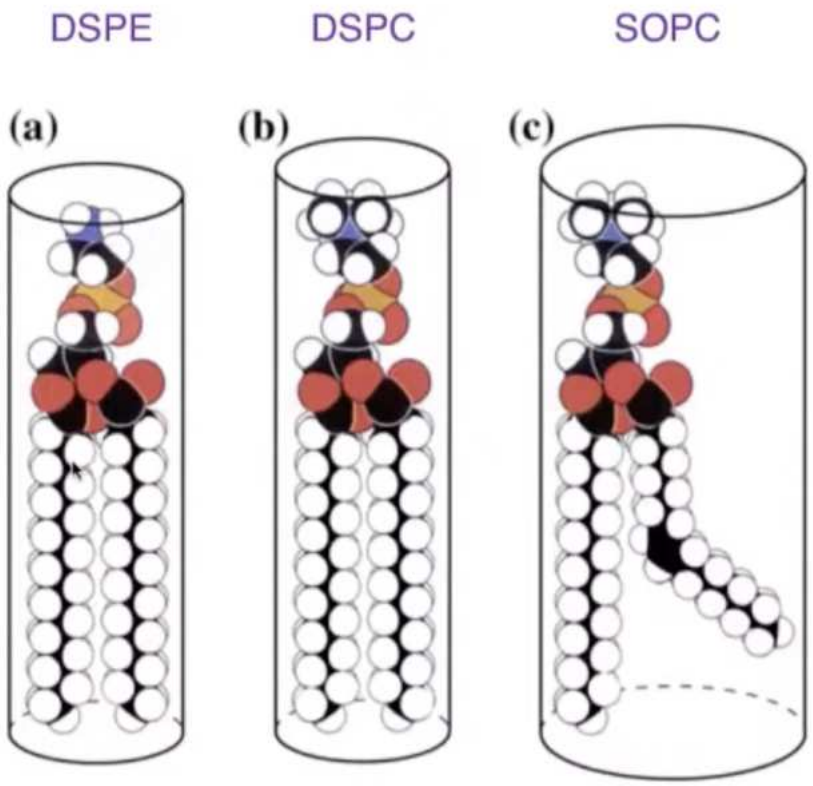
The actual conformation of a molecule influences its size.

Temperature will lead to a rotation around the C-C bonds.

Only lipids with limited degree of disorder will fit into a bilayer structure.

The conformations that have the lowest conformational energy, shows all the C–C–C bonds in a zigzag arrangement (all-trans).
Temperature effect will lead to rotations, so-called excitations, around the C–C bonds and consequently to more disordered conformations.
Lipids differ from the other energy-producing molecules of the cell, the carbohydrates, frequently composed of stiff ring structures that allow for limited flexibility.

Saturated vs Unsaturated Fatty Acids

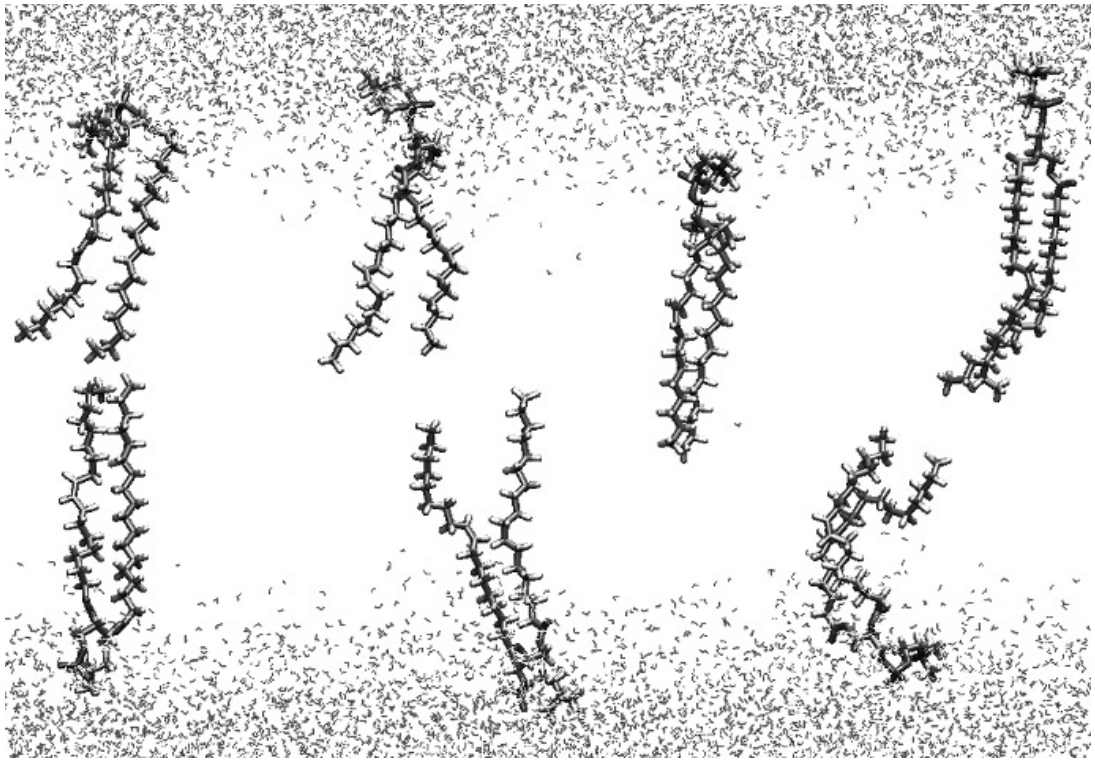


The actual conformation of a molecule influences its size.

Temperature will lead to a rotation around the C-C bonds-more disordered

Only lipids with limited degree of disorder will fit into a bilayer structure.

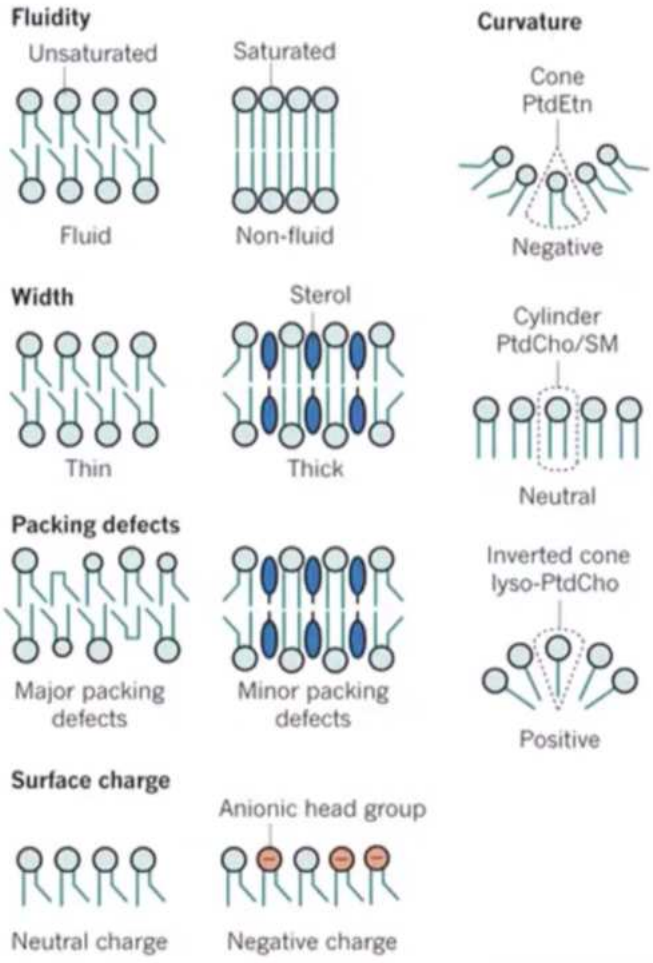
Double C=C bonds: cis-double bonds and trans-double bond. Nature usually makes cis double bonds in fatty acids, more disordered.



Temperature has an effect on size and shape of a lipid molecule.

A lipid molecule, when incorporated into a lipid aggregate like a bilayer, does not occupy a well-defined volume of a well-defined shape. At best the effective shape of a lipid molecule describes how its average cross-sectional area depends on how deeply it is buried in the lipid aggregate.
It has in recent years become increasingly clear that lipid shape is important for functioning. The effective shape of a lipid molecule is determined by the compatibility between the size of the head group and the size of the hydrophobic tail.

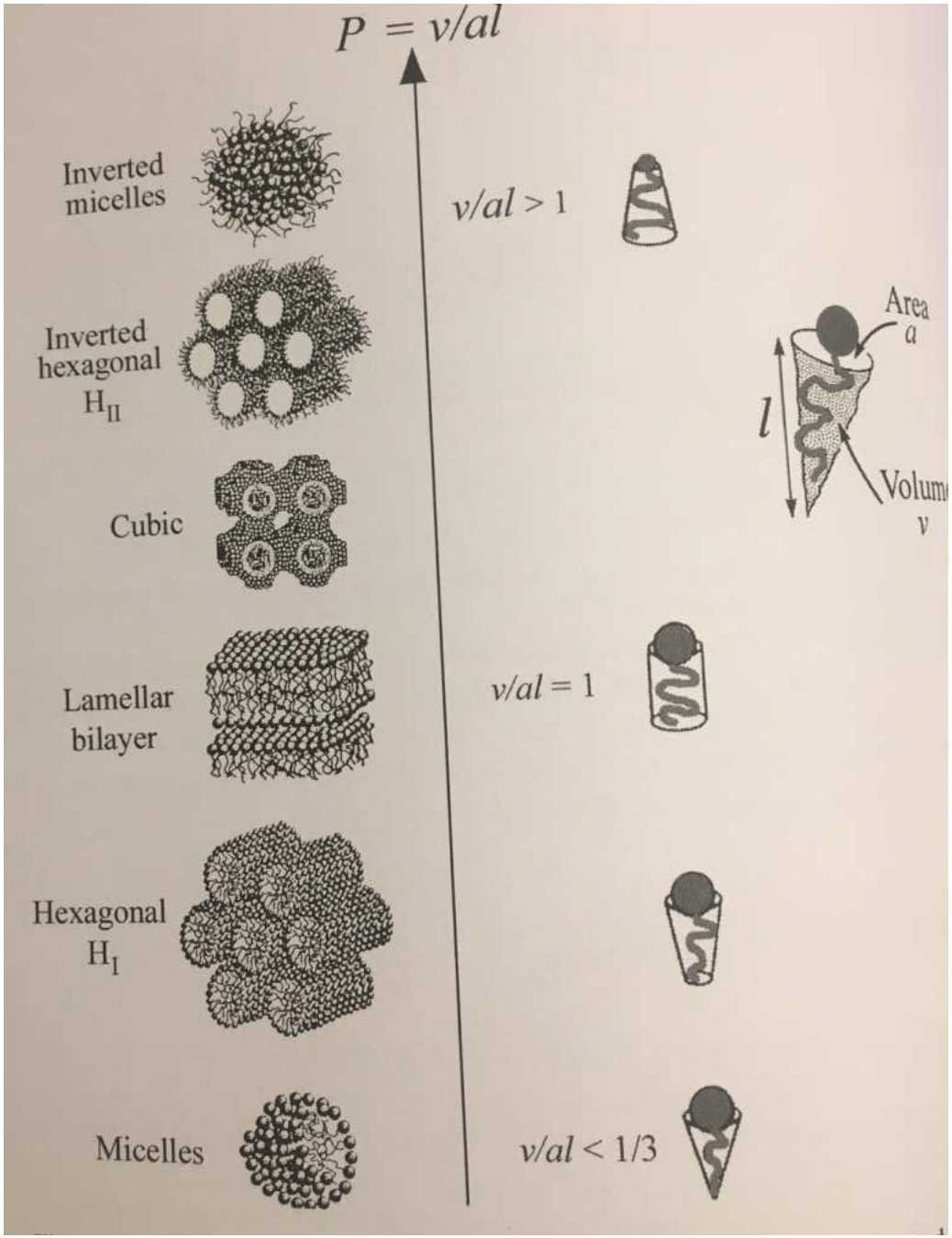
Membrane physical properties



Membrane Physical Properties are Determined by its Lipid Composition

Nature (2014) 510: 48-57

Lipid conformation



Conformation depends on temperature. It affects packing in the lipid bilayer. Indeed the shape itself is affected by the other molecules forming the aggregate.

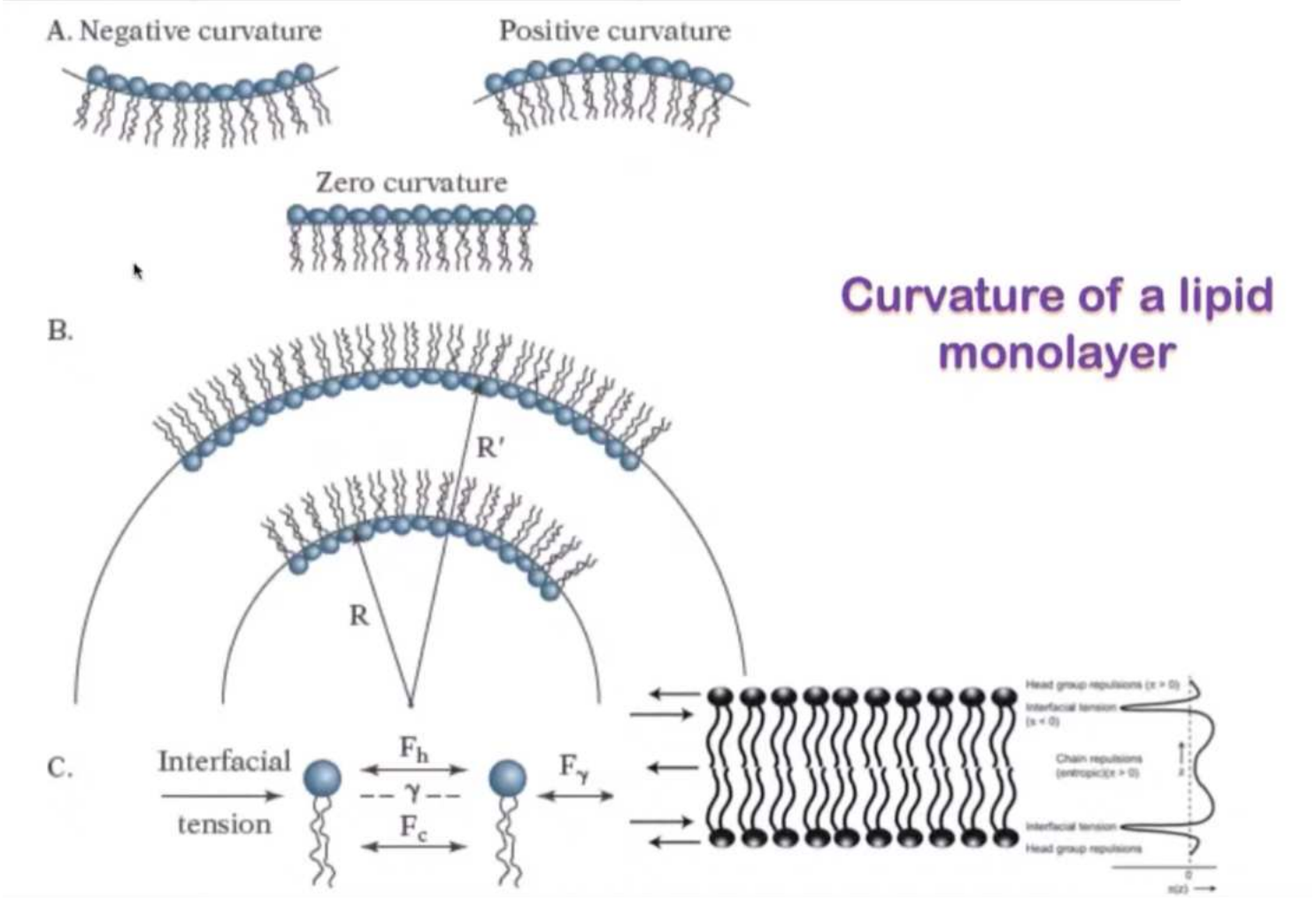
Lipid shape is important for functioning. It is given by the compatibility between head and tail. We define`a packing parameter P:

$$P = v/al$$

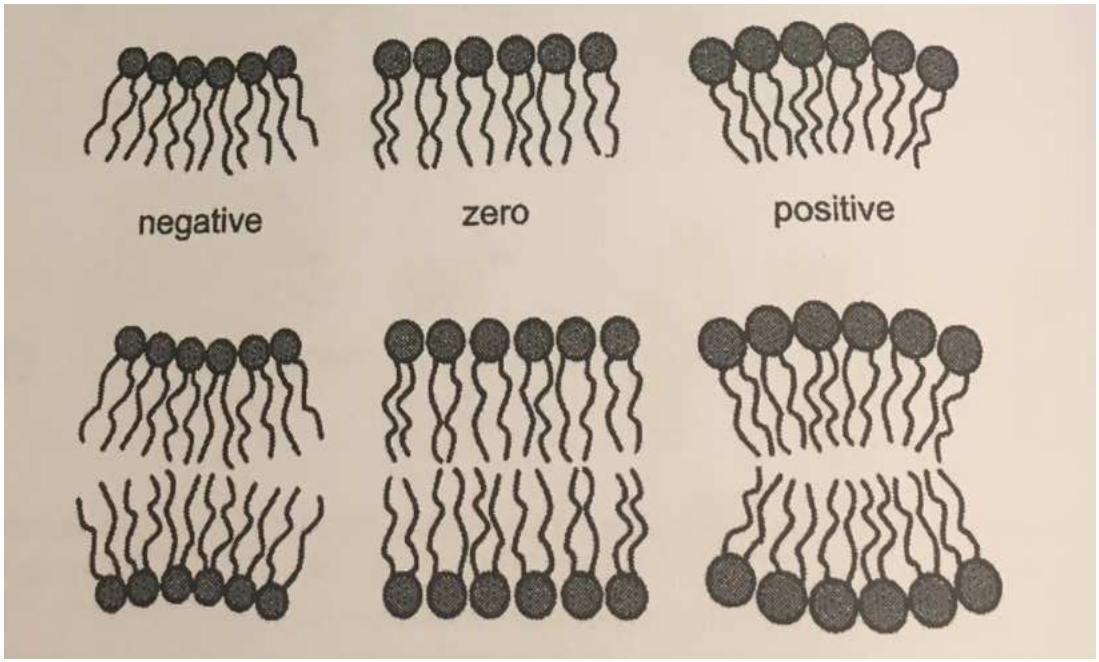
P = 1 is a cilindrical shaped lipid molecules, fitting a lamellar structure with zero curvature.

Curvature although is important for many of the membrane processes

Lipids and membrane curvature



Lipids and membrane curvature



The more non-cylindrical are lipid shapes, the less stable the bilayer will be.

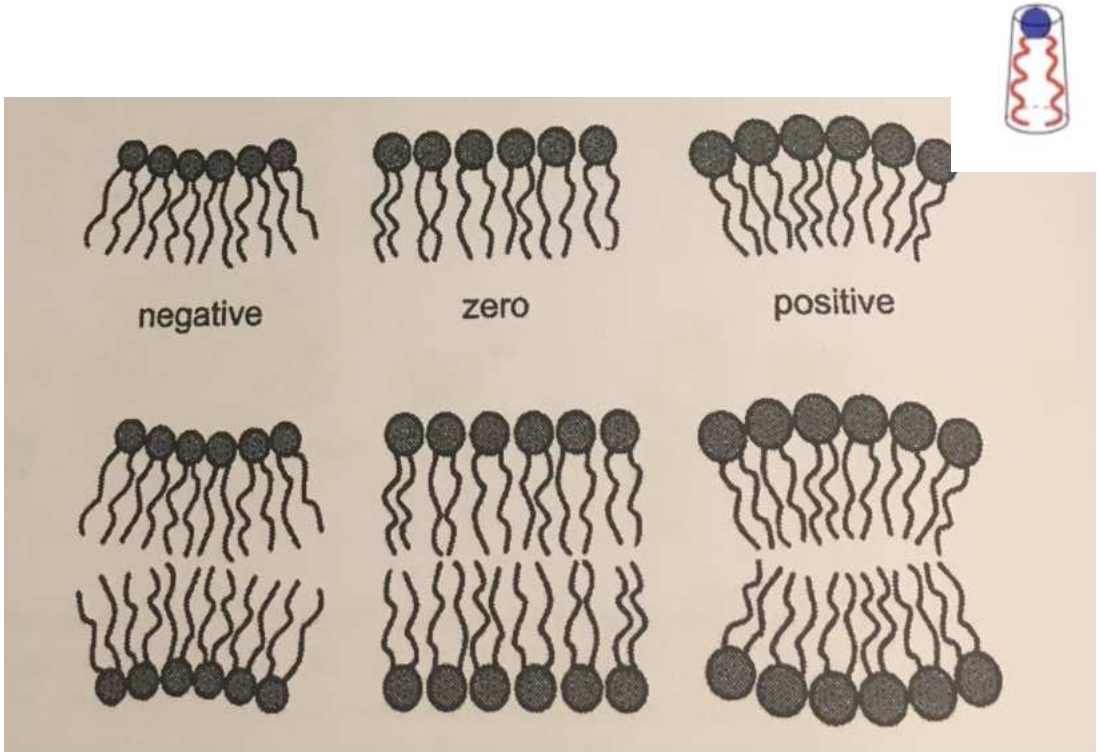
Each layer tend to elastically relax to a state of finite, spontaneous curvature, causing a curvature stress field.

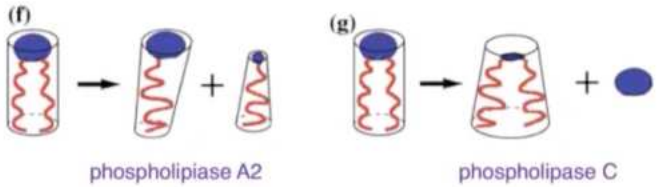
If the bilayer cohesion does nor sustain the curvature stress, non lamellar structures form.

Lipid speak the language of curvature, in the many structures formed!

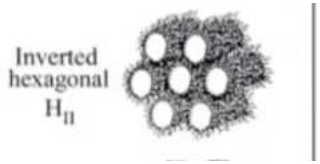
The inverted hexagonal structure (H_{II}), has long cilindrical rods of lipids, in a water filled tube, whose diameter can be varied with T, degree of hydration, pH (all change a/l ratio).

Lipids and membrane curvature





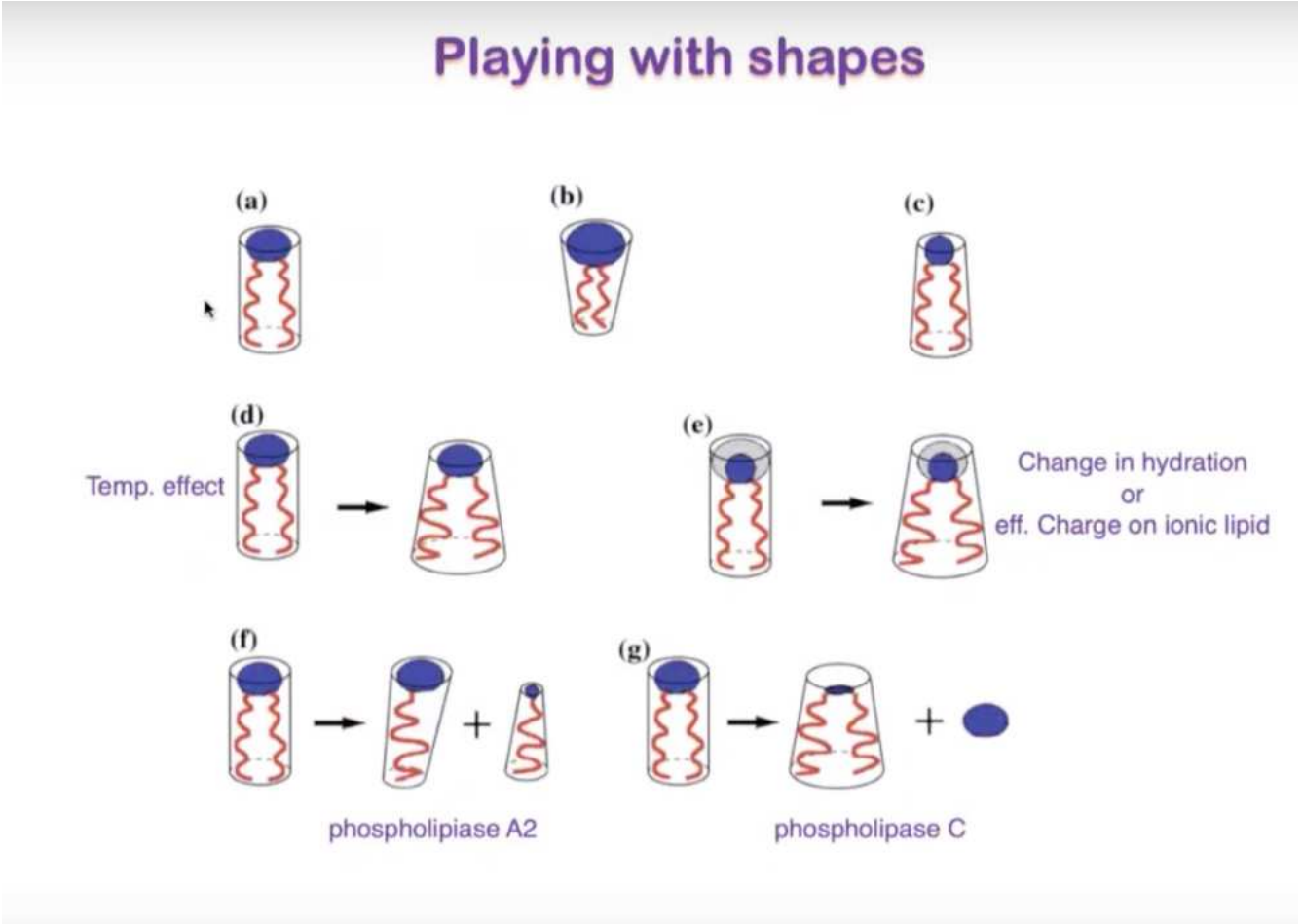
Cholesterol has an inverted conical shape (small OH, big steroid ring). Tends to promote the H_{II}. Stress field is mitigated by enzymes.



From research in microorganisms it appeared that curvature is a crucial parameter in regulating lipid synthesis/enzymatic activity of phospolipases-—lipid molecular shape/optimal packing is at the basis of curvature stress. Yet unknown which membrane-bound proteins are involved in curvature stress sensing-lipid synthesis.

NB: vesicles do not close because of curvature stress, but because of boundary conditions! (micron vs. nanometers)

Membrane physical properties



Lipids form soft interfaces

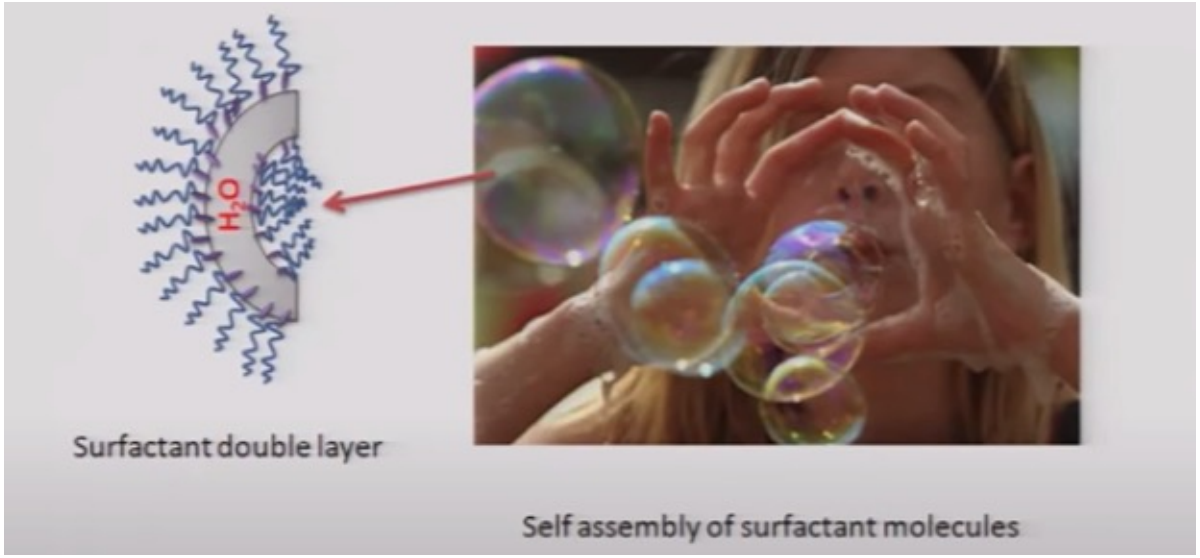
Membranes are soft interfaces. As polymers, exist in a condensed phase, but cannot be classified neither as solid, nor liquid. The physics of such interfaces is dominated by entropy.
Softness means high deformability but not necessarily high bulk compressibility!
Soft matter is anisotropic, hierarchical, with structures spanning over different length scales, and is governed by self-assembling.

In liquid, the interfacial tension $\gamma = \left(\frac{\partial G^S}{\partial A}\right)_V$
with G^S being the Gibbs excess free energy, V, A volume and surface area
acts to make the interface as small as possible, at the same time imparts a certain stiffness to the interface.

The introduction of interfacially active molecules (i.e. amphiphiles) lowers the interface tension.
If molecules are enough, the interface can be fully covered. Therefore the area is fixed and I.T. tends to zero.

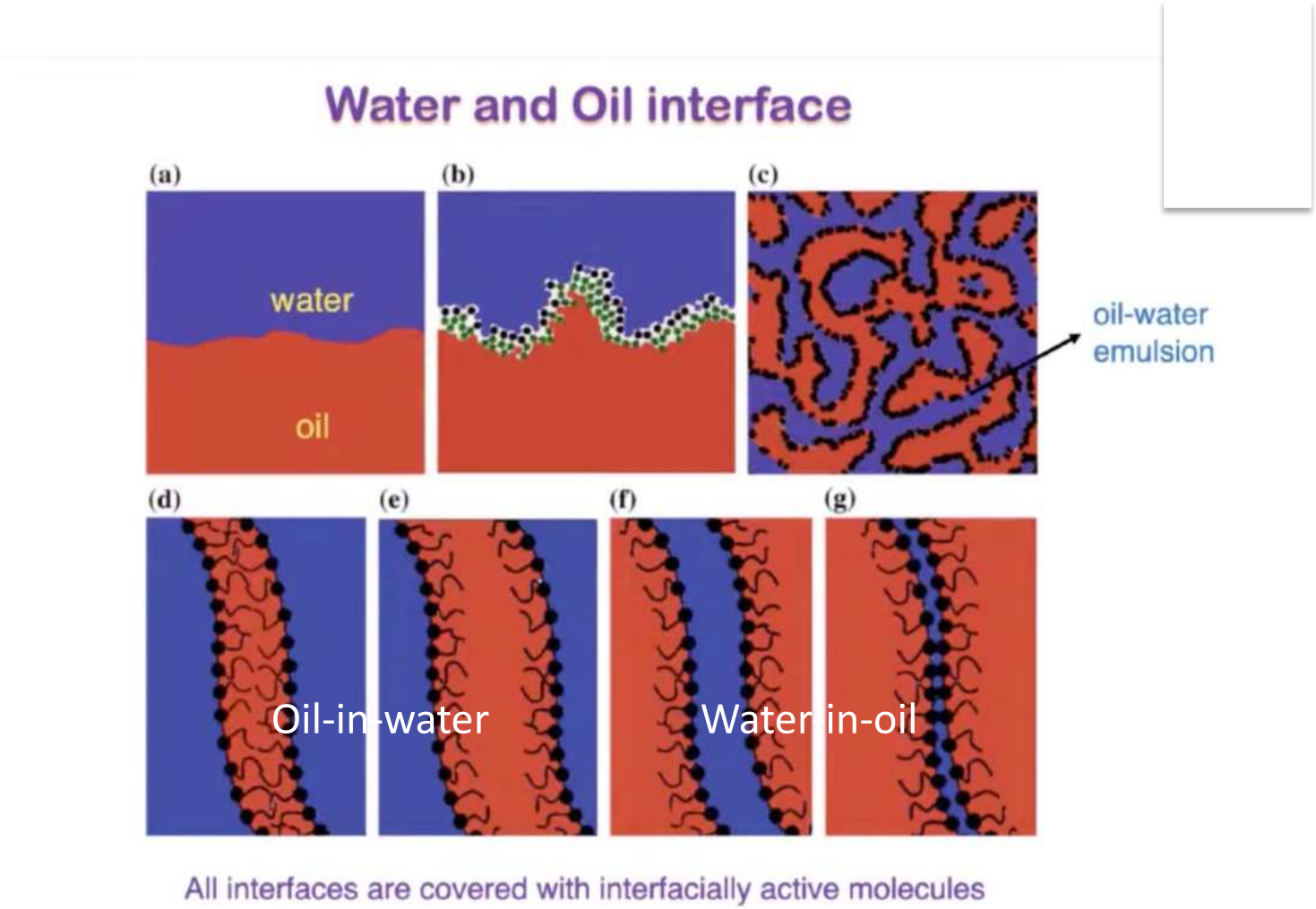
Lipids form soft interfaces

Natural examples of soft interfaces: soap bubbles

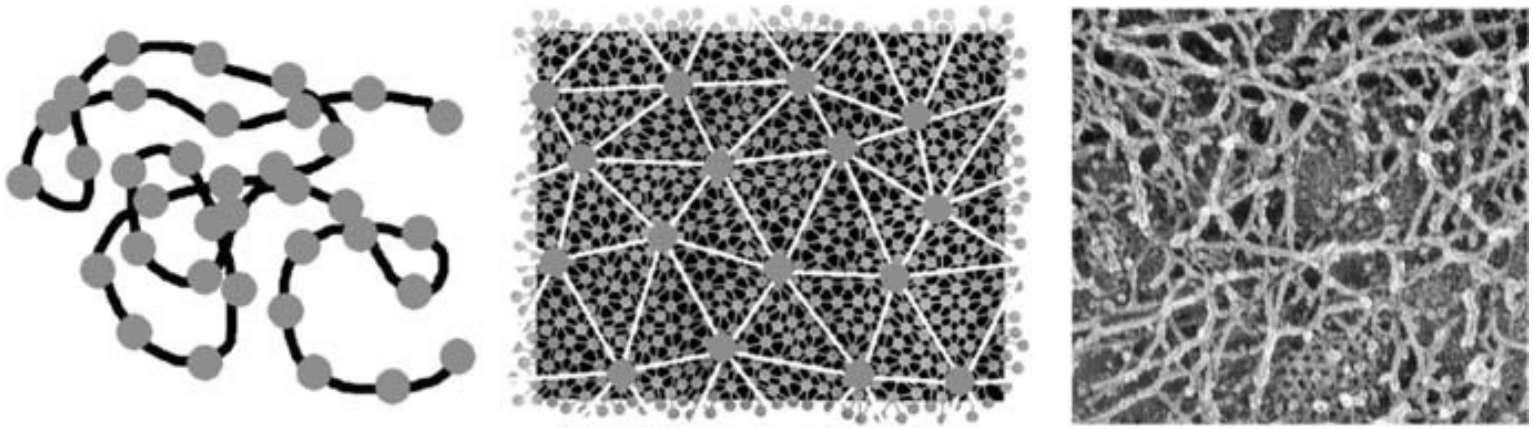


Soap bubbles: two layers form, at the water-air interfaces, the outer and the inner surfactant layer.
Bubbles are stabilized for a particular size, a particular water layer thickness depending on:

-type of surfactant
-quantity of surfactant
-quantity of water



These interfaces are fluid----no fixed relationship between nearest neighbour molecules within the interface---no resistance to shear force!



Soft matter systems with tethered interfaces. (a) A polymer chain with tether-beads fixed connections. (b) A tethered two-dimensional membrane resembling a cytoskeleton with fixed connectivity attached to a fluid lipid bilayer with dynamically changing connectivity. (c) EM image of the spectrin network, which is part of the cytoskeleton of the red blood cell (500nm x 500nm).

The mechanical, conformational, and statistical properties of tethered interfaces are very different from those of fluid interfaces.

Lipids form soft interfaces

Membranes are soft interfaces. As polymers, exist in a condensed phase, but cannot be classified neither as solid, nor liquid. The physics of such interfaces is dominated by entropy.
Softness means high deformability but not necessarily high bulk compressibility!
Soft matter is anisotropic, hierarchical, with structures spanning over different length scales, and is governed by self-assembling.

In liquid, the interfacial tension $\gamma = \left(\frac{\partial G^S}{\partial A}\right)_V$
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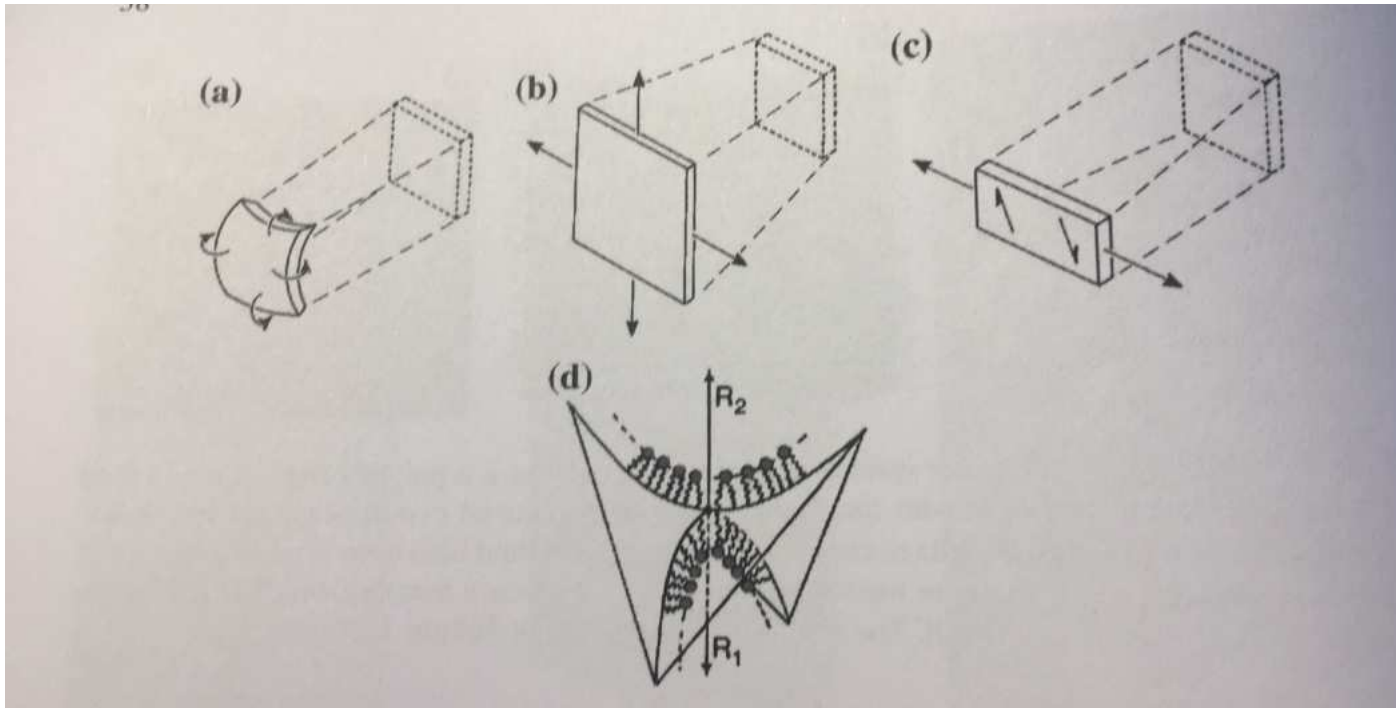
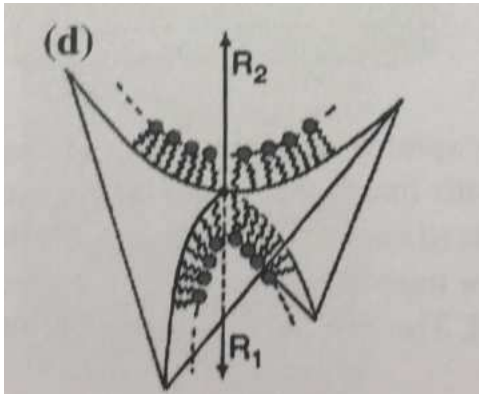
Lipids and membrane curvature

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



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

Lipids and curvature



The two ways of deforming the interface are associated with two elasto-mechanical modules, termed:
- the bending modulus, κ
- the area compressibility modulus, K

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:

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

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

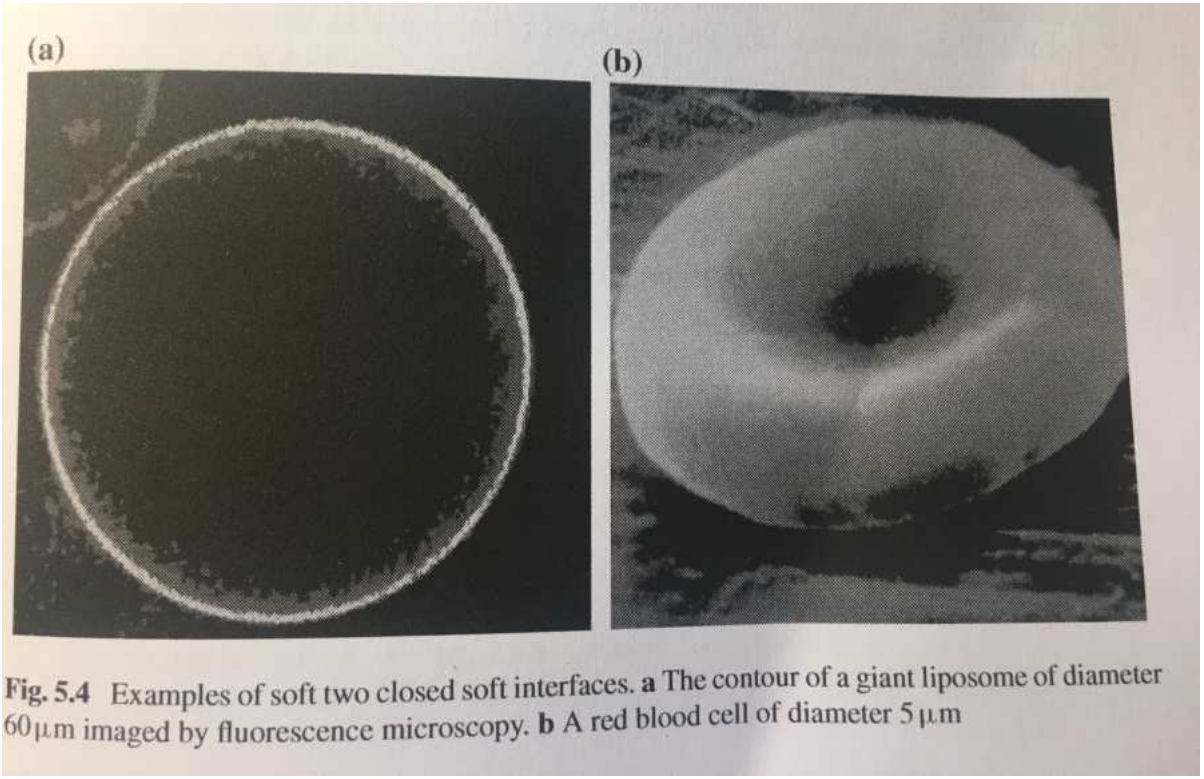


Fig. 5.4 Examples of soft two closed soft interfaces. **a** The contour of a giant liposome of diameter 60 μm imaged by fluorescence microscopy. **b** A red blood cell of diameter 5 μm

Two soft membranes with different bending capabilities.

For lipid bilayers, κ ≈ 10-25 k_BT → Lp astronomically large (≫ μm); hence membranes appear smooth at the cellular scale.

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

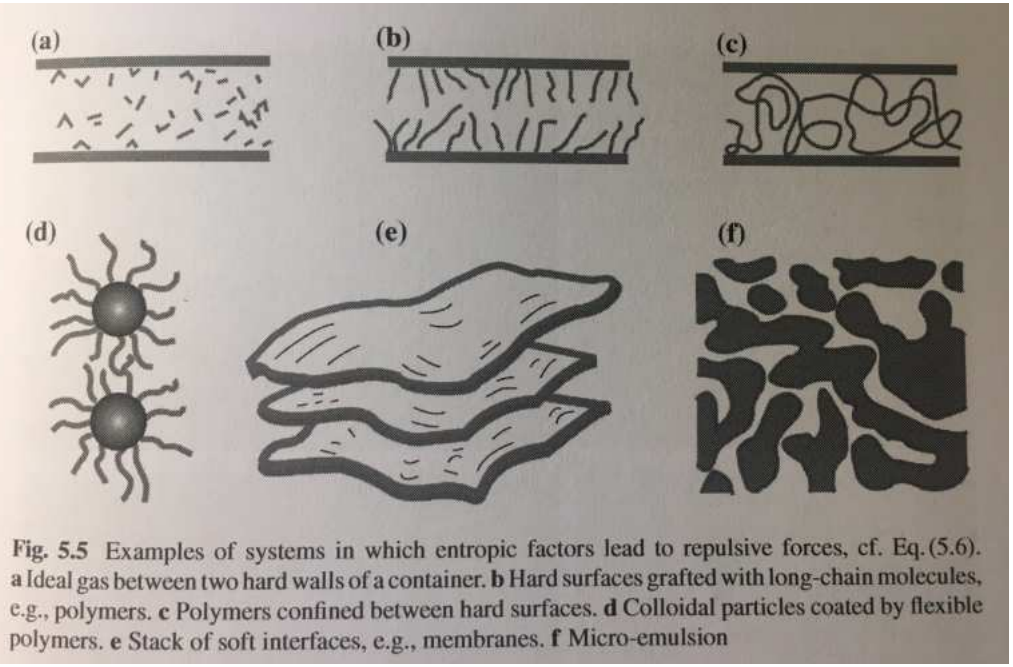


Fig. 5.5 Examples of systems in which entropic factors lead to repulsive forces, cf. Eq. (5.6). **a** Ideal gas between two hard walls of a container. **b** Hard surfaces grafted with long-chain molecules, e.g., polymers. **c** Polymers confined between hard surfaces. **d** Colloidal particles coated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

The softness of membranes generates collidal forces among them. They are thermodynamic forces, 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.
NB: mechanical forces are the gradient of mechanical energy, or entalphy H!

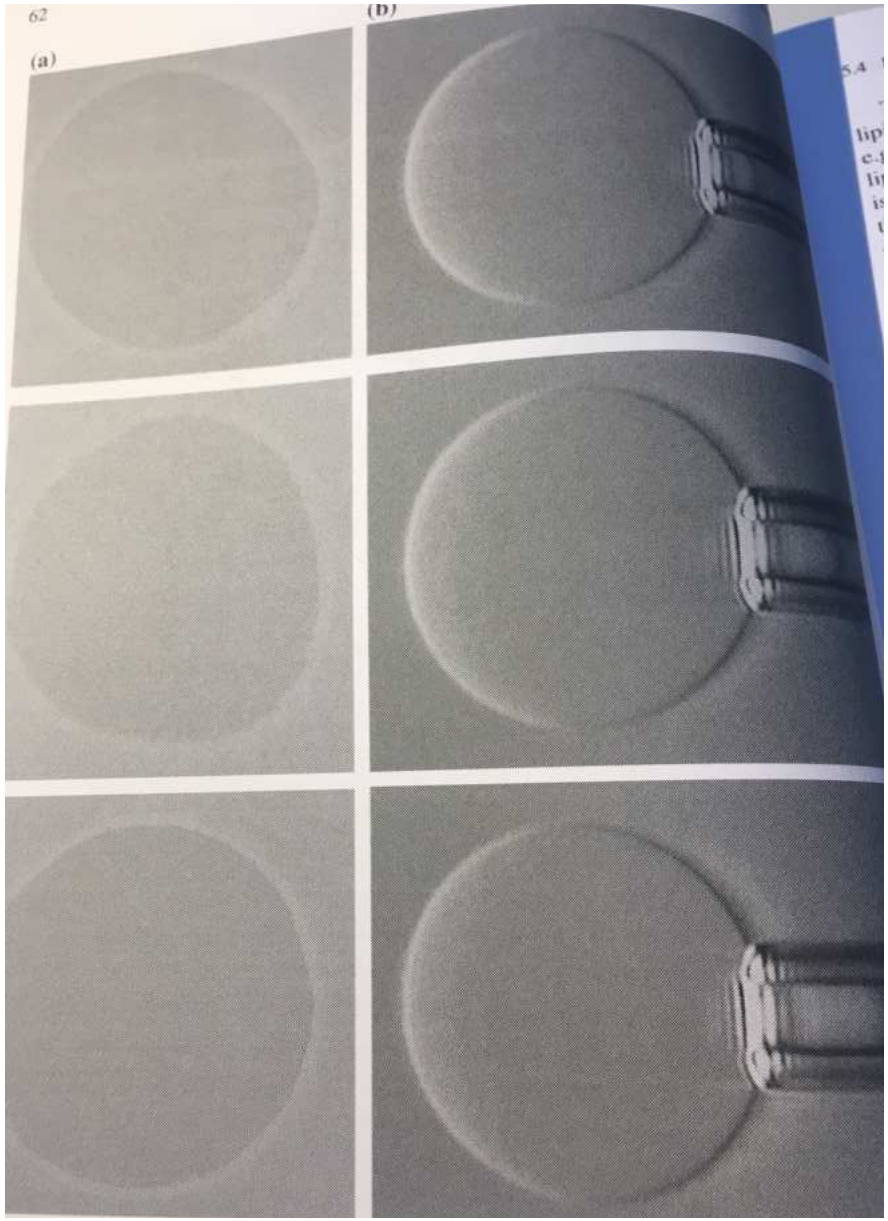
There is always an entropic repulsion between soft interfaces, even in the absence of direct mechanical forces! The reduction in configuration entropy due to confinement produces repulsive forces.

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

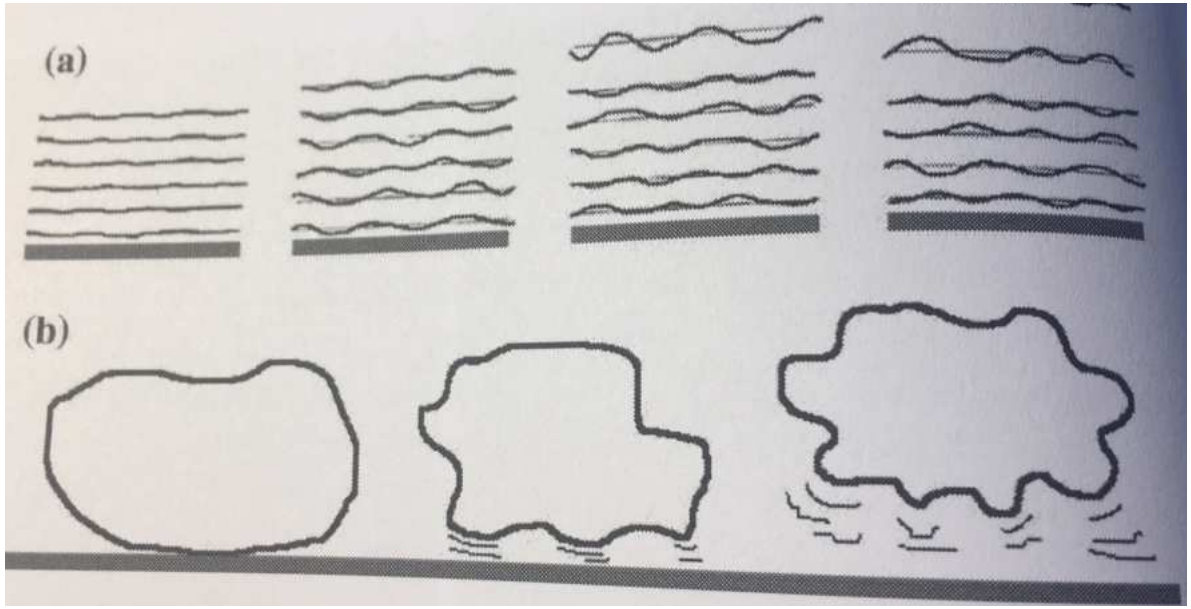


Giant liposomes (50 um). Membrane thickness: 5 nm.
Variation in the contour due to thermal fluctuations---the membrane is very soft!!
The bending modulus κ can be derived from the spectrum of fluctuations.
With the pipette aspiration one apply a stress τ and measure the compressibility modulus K from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



Hence, the elastic membrane fluctuations (about 10 K_BT) are expected to be very sensitive to temperature. This will have some dramatic consequences at membrane phase transitions.

Because of undulation forces by soft bilayers, vesicles/lipid bilayers are repelled by solid surfaces (effect on cell-cell adhesion).

How softness can be controlled at the molecular level? How doeas it affect membrane function?

Lipid membranes are really soft

One of the major questions is the microscopic and molecular origin of membrane softness and how it is manifested in membrane structure on the nanometer scale.

This may provide some clues as to how the softness eventually can be controlled.

It is the hypothesis that the lipid-bilayer softness, the dynamic structure of the membrane, and the corresponding lipid organization are important regulators of membrane function and the ability of the membrane to support biological activity.

A consequence of this hypothesis is that the generic effects of peptides, proteins, and drugs on membrane structure and function, on the one side, and the influence of bilayer structure on these compounds, on the other side, may be understood in part by the ability of these compounds to alter lipid-bilayer softness and molecular organization.

To study these properties, we need MODELS!

Milestones in membrane research

Overton

- Permeability ∝ oil/water partition coefficient
- Coined the term 'lipoids' for the layer around the cell

Gorter and Grendel

- Bilayer arrangement of lipids

Daveson and Danielli

- Proteins on the surface of the lipid bilayer
- 'Sandwich model'

Robertson

- Visual evidence of a lipid bilayer
- EM: trilamellar structure

Milestones in membrane research

Frye and Edidin

- Lateral and rotational mobility of membrane proteins

Singer and Nicolson

- Fluid mosaic model

Racker

- Functional reconstitution of a membrane protein

Unwin and Henderson

- 3D structure of a membrane protein
- Bacteriorhodopsin

Milestones in membrane research

The Nobel Prize in Chemistry 1988

Johann Deisenhofer
Prize share: 1/3

Robert Huber
Prize share: 1/3

Hartmut Michel
Prize share: 1/2

1985

Hartmut Michel

- Crystal structure of the first membrane protein
- Photosynthetic reaction center

1998

Roderick MacKinnon
Peter Agre

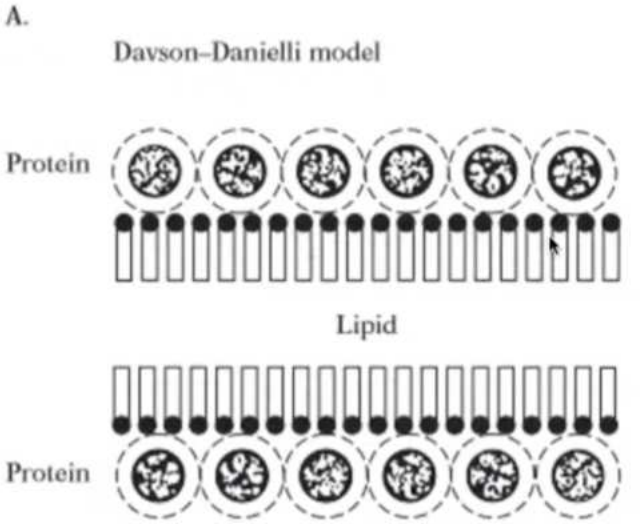
- Crystal structure of the first ion channel
- KcsA, Aquaporin

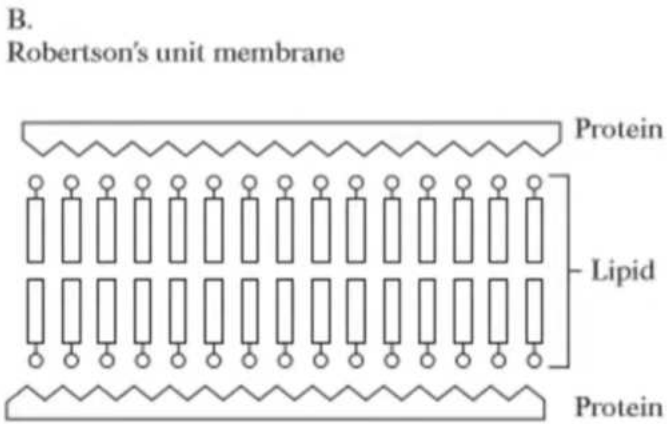
The Nobel Prize in Chemistry 2003

Peter Agre
Prize share: 1/2

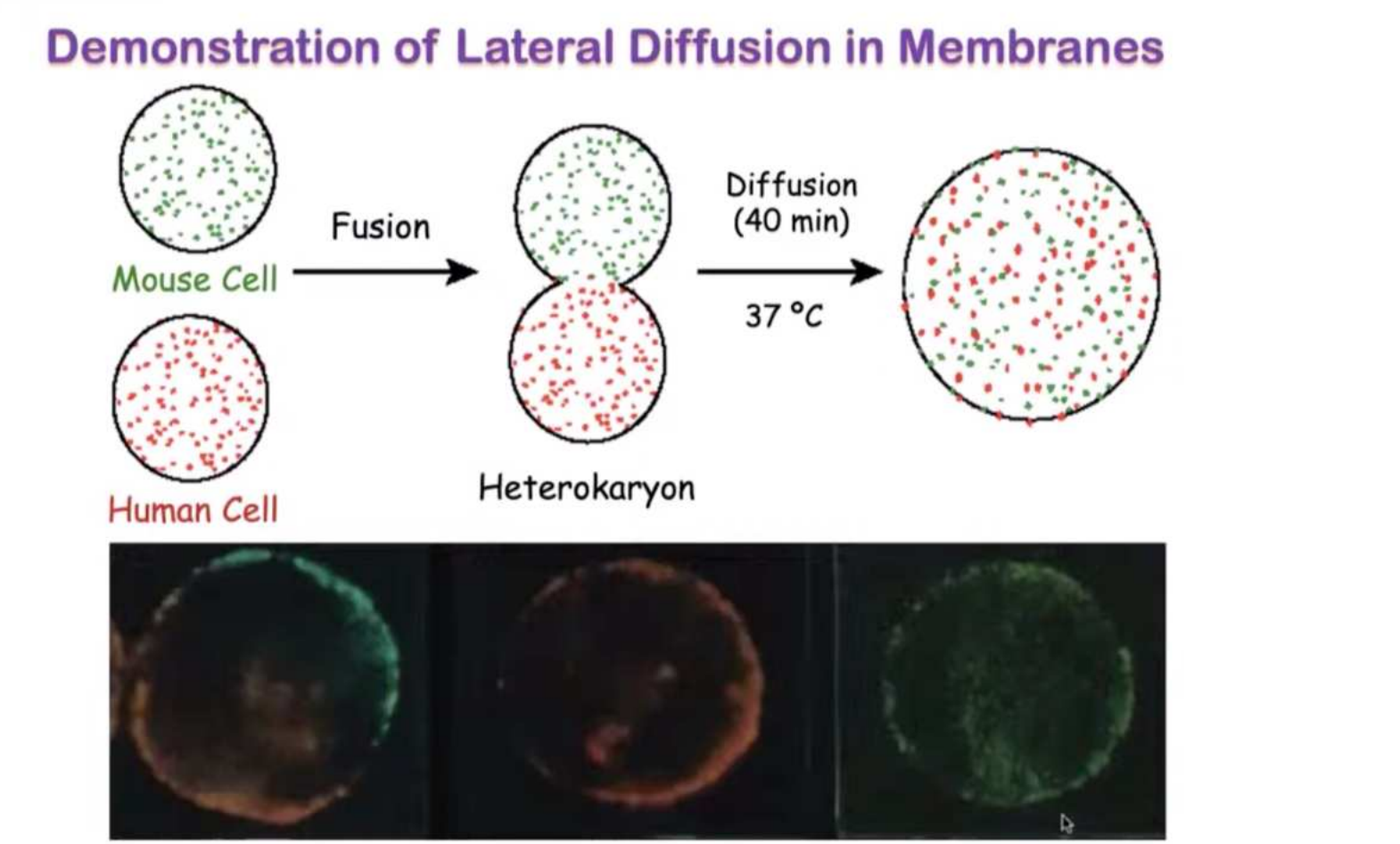
Roderick MacKinnon
Prize share: 1/2

Early models



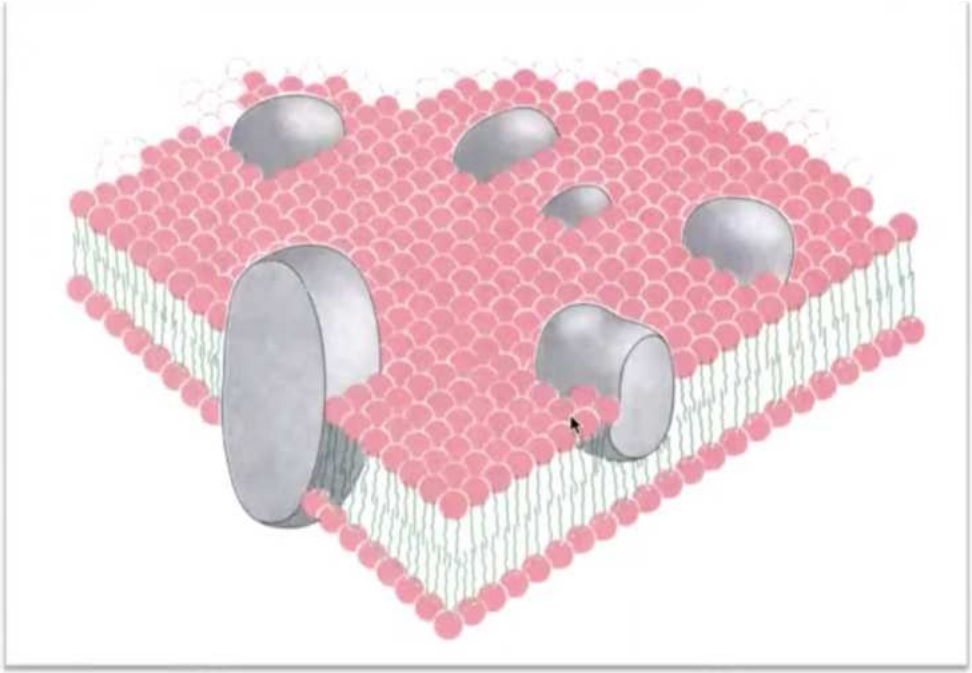


Early models



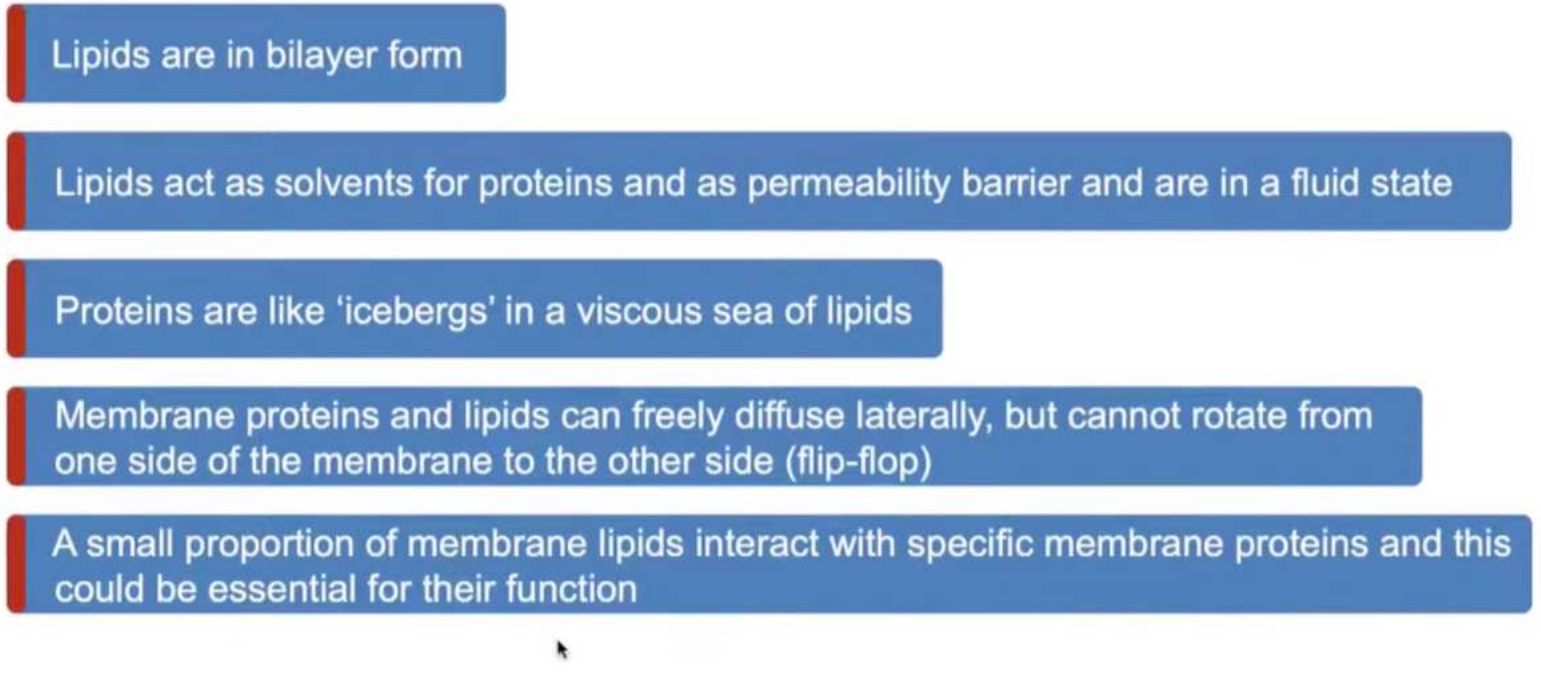
Frye and Edidin (1970) *J. Cell Sci.* 7: 319-335

The fluid mosaic model



Singer and Nicolson (1972) *Science* 175: 720-731

The fluid mosaic model

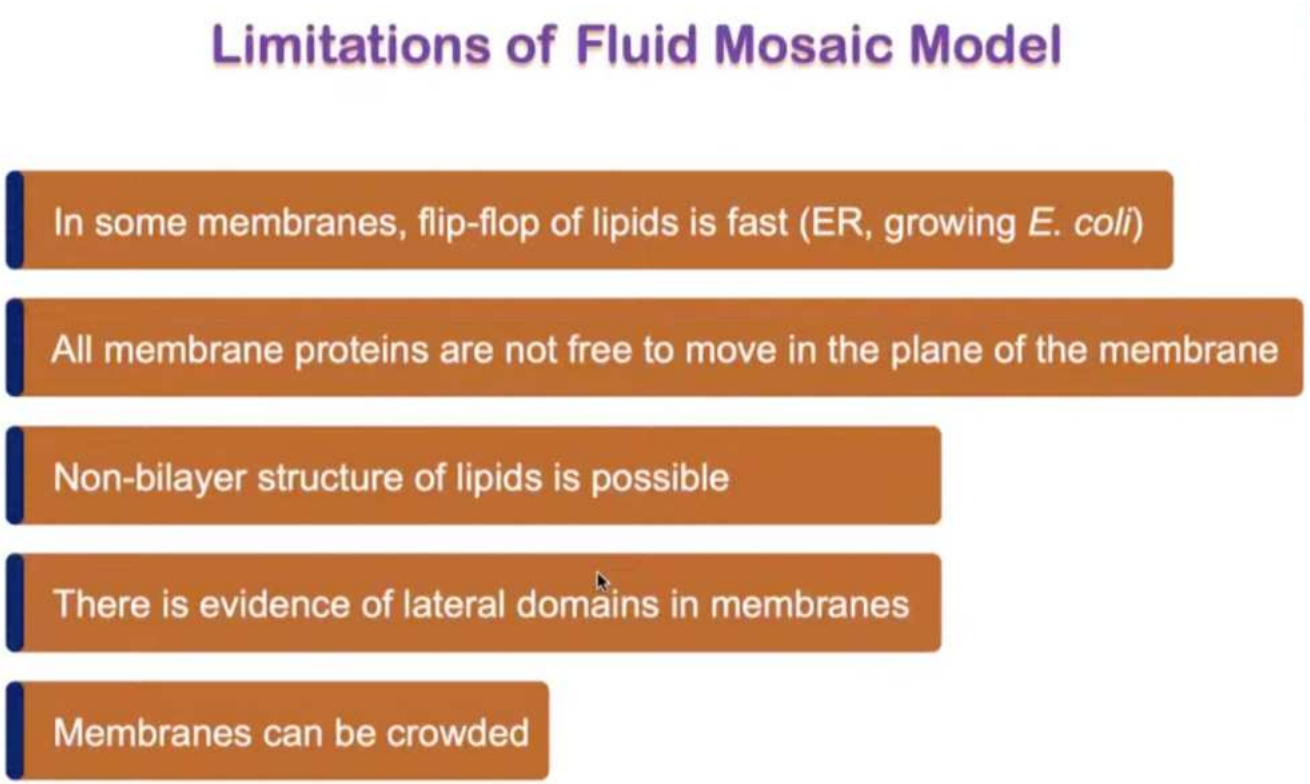


Singer and Nicolson (1972) *Science* 175: 720-731

Unfortunately, many subsequent investigators assumed, explicitly or implicitly, that fluidity implies randomness.

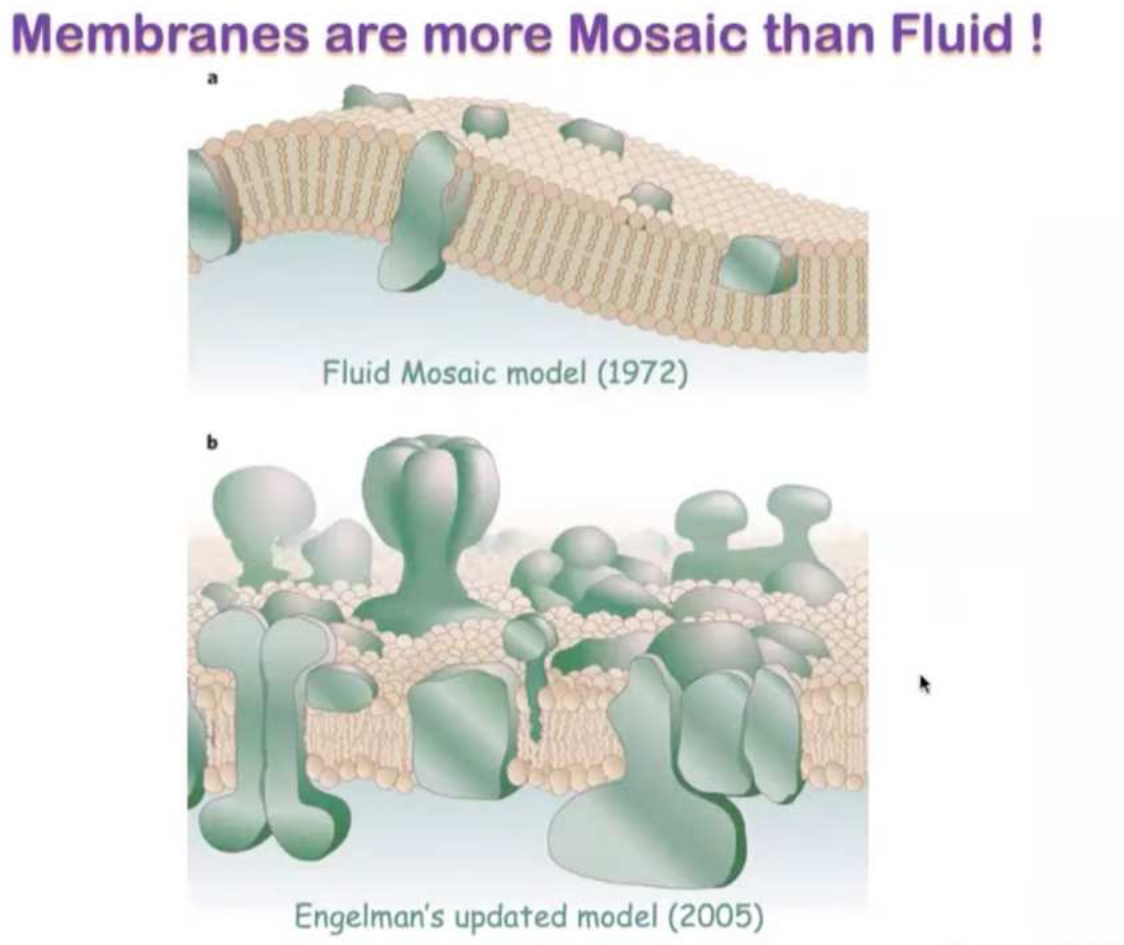
This assumption neglects that fluids or liquids may be structured on length scales in the nanometer range, which are difficult to access experimentally. Also, structuring in time, in particular the correlated dynamical phenomena characteristic of liquid crystals, was not appreciated.

The fluid mosaic model



Does not take into account : LOCAL ORDER, DOMAIN FORMATION

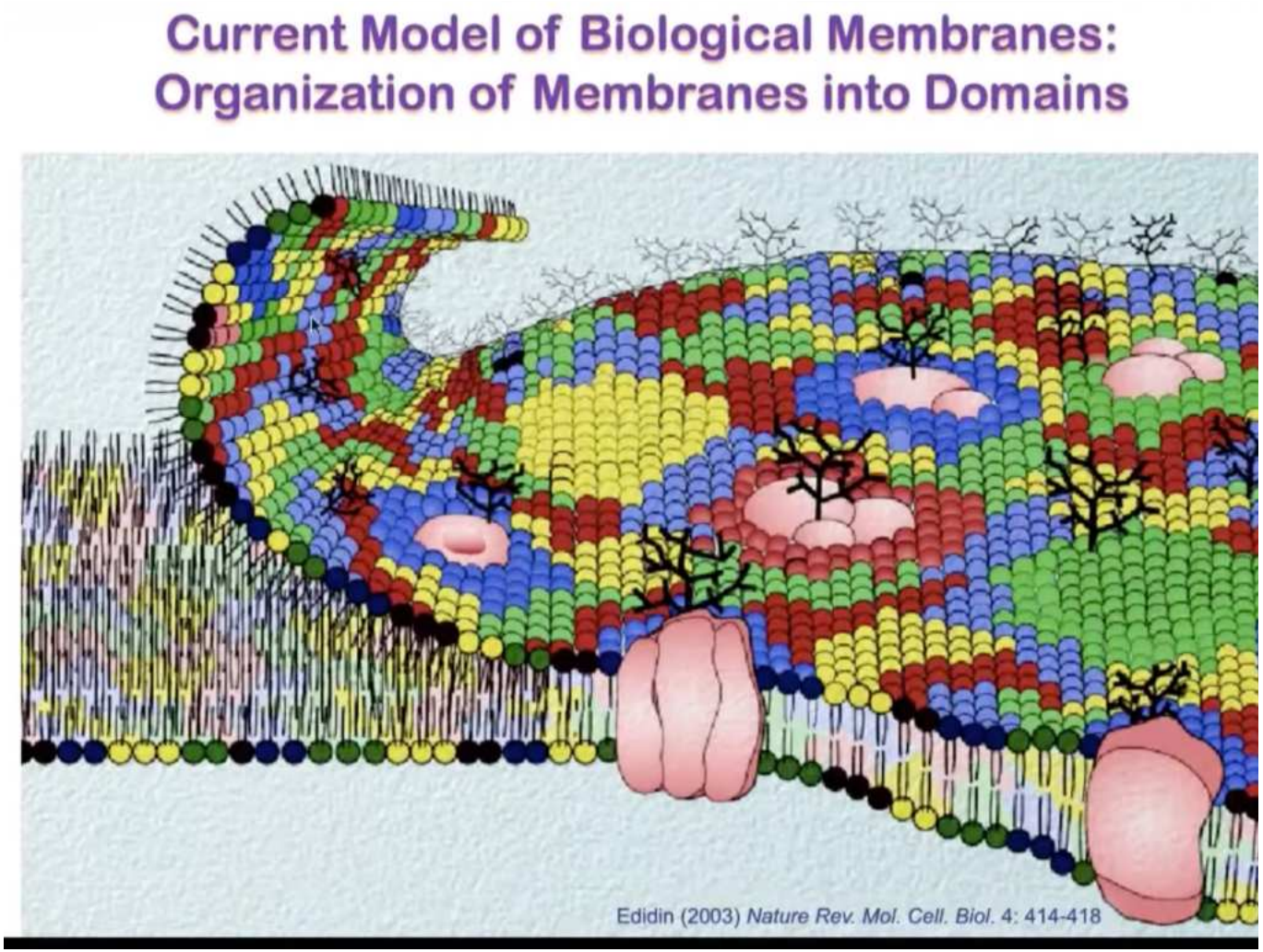
The fluid mosaic model



Engelman (2005) *Nature* 438: 578-580

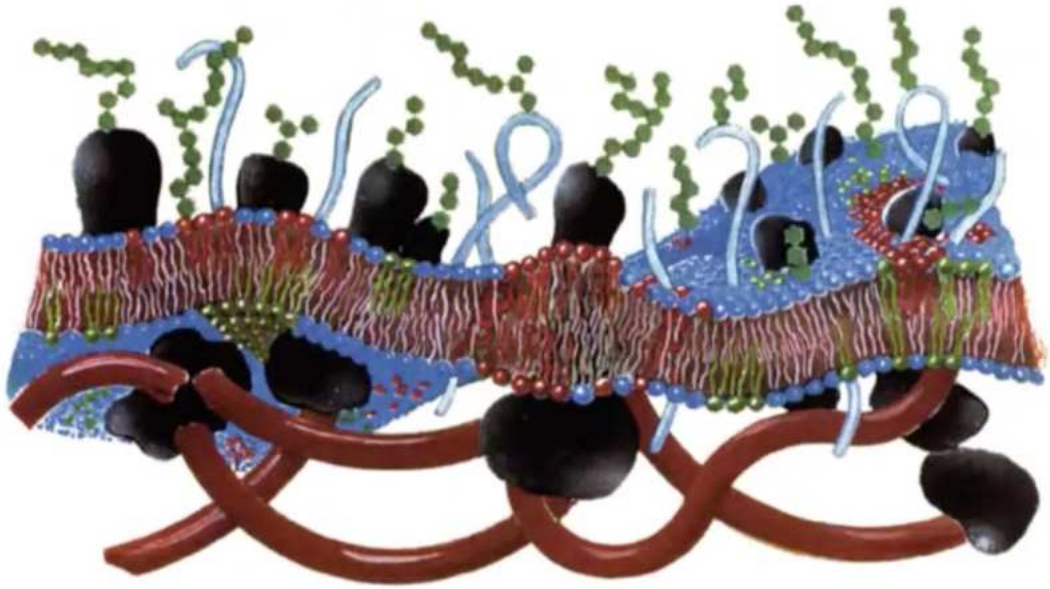
Lateral distribution of molecules is heterogeneous, corresponding to An organization into DOMAINS

Current Model of Biological Membranes: Organization of Membranes into Domains



the lateral distribution of molecular components in membranes is heterogeneous, both statically and dynamically – corresponding to an organization into compositionally distinct domains and compartments

Current Model of Biological Membranes: Organization of Membranes into Domains



Mouritsen and Andersen (1998) *Biol. Skr. Dan. Vid. Selsk.* 49: 7-12

Life - As a Matter of Fat: Lipids in a Membrane Biophysics Perspective, Ole G. Mouritsen and Luis A. Bagatolli, 2nd Edn., 2016, Springer

The fluid mosaic model

TABLE 1.1 COMPOSITION OF MEMBRANE PREPARATIONS BY PERCENT DRY WEIGHT[a]

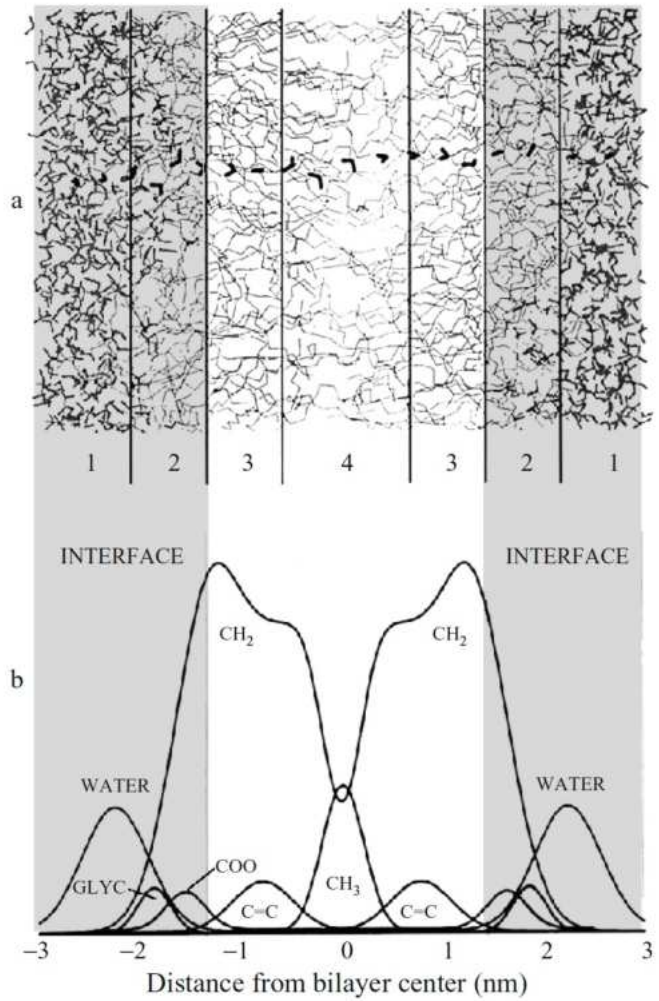
Source	Lipid	Protein	Cholesterol
Rat liver			
Plasma	30–50	50–70	20
Rough ER	15–30	60–80	6
Smooth ER	60	40	10
Inner mitochondria	20–25	70–80	<3
Outer mitochondria	30–40	60–70	<5
Nuclear	15–40	60–80	10
Golgi	60	40	8
Lysosomes	20–25	70–80	14
Rat brain			
Myelin	60–70	20–30	22
Synaptosome	50	50	20
Rat erythrocyte	40	60	24
Rat rod outer segment	50	40	<3
Escherichia coli	20–30	70	0
Bacillus subtilis	20–30	70	0
Chloroplast	35–50	50–65	0

[a] The percentages by weight of membrane preparations from various eukaryotic and prokaryotic sources are given.

ER, endoplasmic reticulum.

Source: Based on Jain, M. K., and R. C. Wagner, *Introduction to Biological Membranes*, 2nd ed. New York: Wiley, 1988, p. 34.

Lipids in bilayers



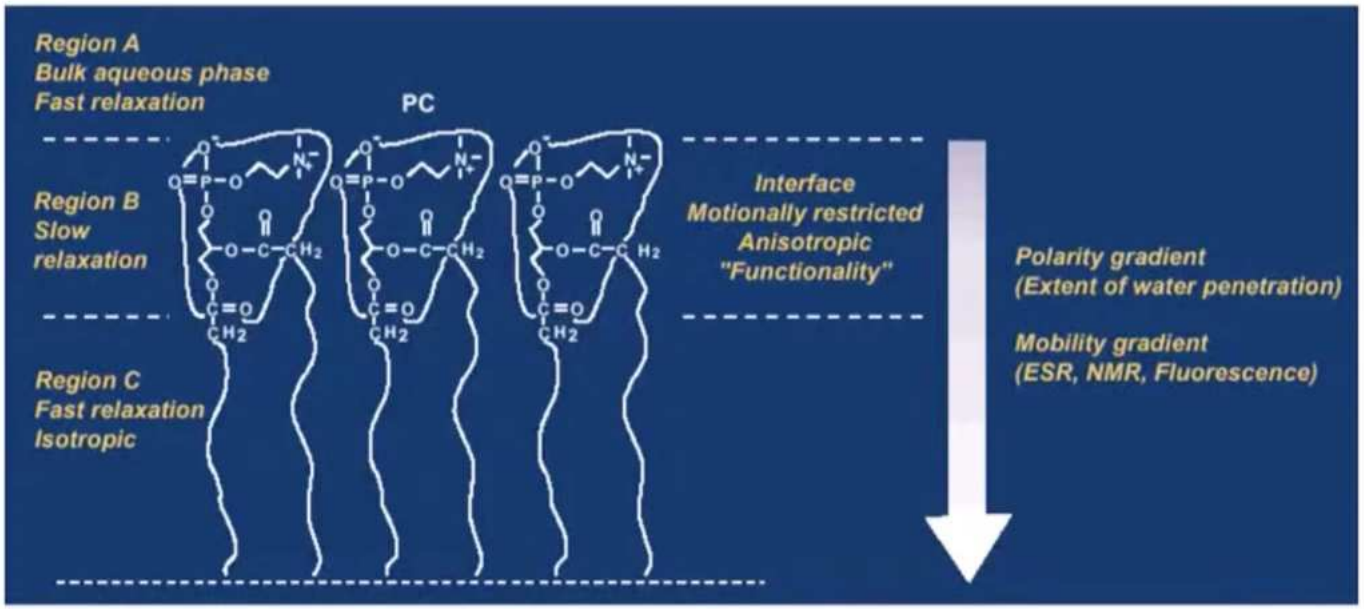
A lipid bilayer is not just a homogeneous thin slap of a dielectric medium immersed in water but is a highly stratified structure with a distinct trans-bilayer molecular profile (LATER ON: NEUTRON AND X-RAY REFLECTIVITY!)

This profile determines the membrane both as a barrier, carrier, and target.

It is of particular importance for understanding how proteins function in and at membranes and how, for example, drugs interact with membranes.

(a) Trans-bilayer structure of a fluid DPPC lipid bilayer, from a Molecular Dynamics simulation. The four structurally different regions are: (1) perturbed water, (2) a hydrophilic-hydrophobic interfacial region involving the lipid polar-head groups, (3) a soft-polymer-like region of ordered fatty acid chain segments, and (4) a hydrophobic core with disordered fatty-acid chain segments. (b) Trans-bilayer density profile of a fluid DOPC bilayer obtained from X-ray and neutron-scattering techniques.

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.

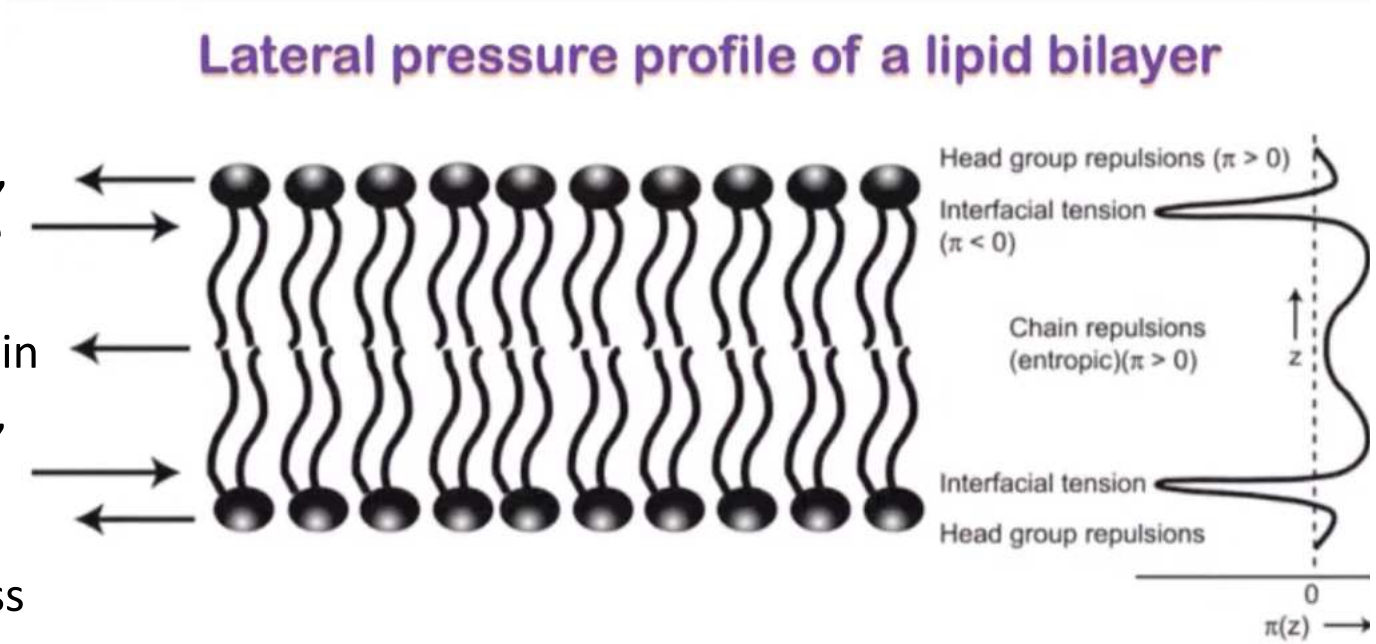
the hydrophobic-hydrophilic interface of the membrane, i.e., regions (1) and (2), occupies about half of the entire lipid-bilayer thickness!

It is chemically heterogeneous making it prone to all sorts of noncovalent interactions with molecules, e.g., peptides and drugs, that bind, penetrate, and permeate membranes.

Tick enough to accommodate an α-helical peptide that lies parallel to the bilayer surface.

How stressful is being confined in a bilayer?

Since the forces, due to the finite thickness of the bilayer, operate in different planes, the pressures are distributed nonevenly across the bilayer

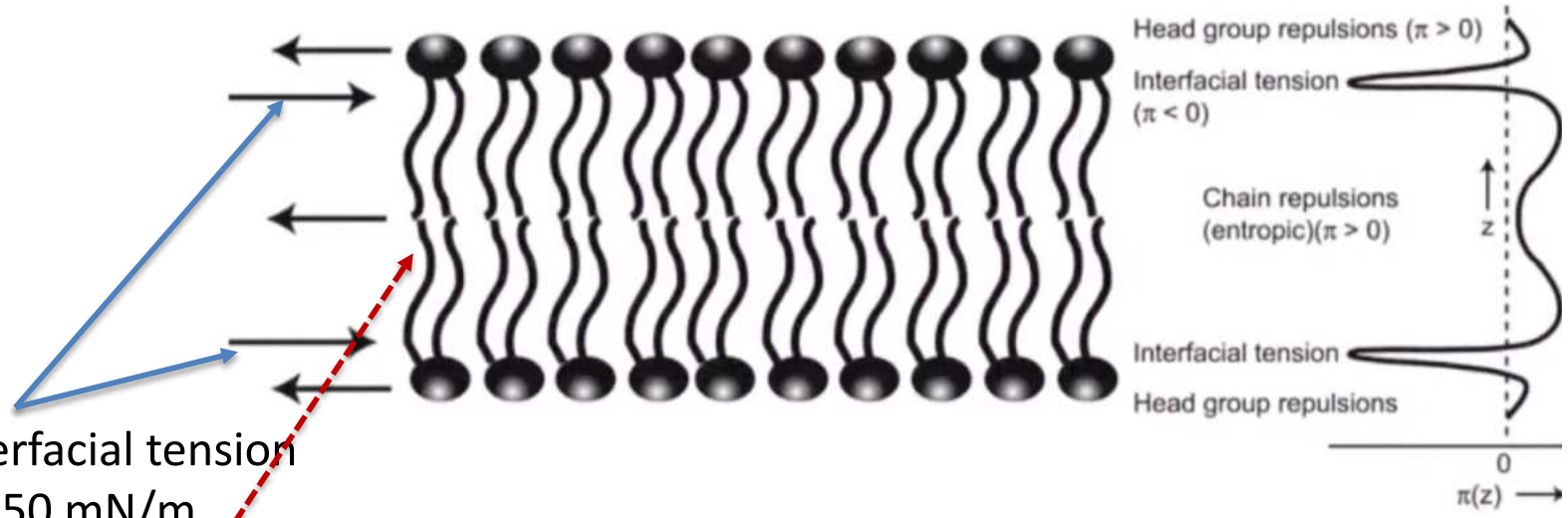


Membrane lateral tension (F= free energy, A= membrane area)

$$\sigma = \left(\frac{\partial F}{\partial A}\right)_{T,N}$$

Is the average lateral force/unit length for contracting/expanding the membrane, or **the work needed to change the area** of the membrane. It si measured in N/m.

Lateral pressure profile of a lipid bilayer



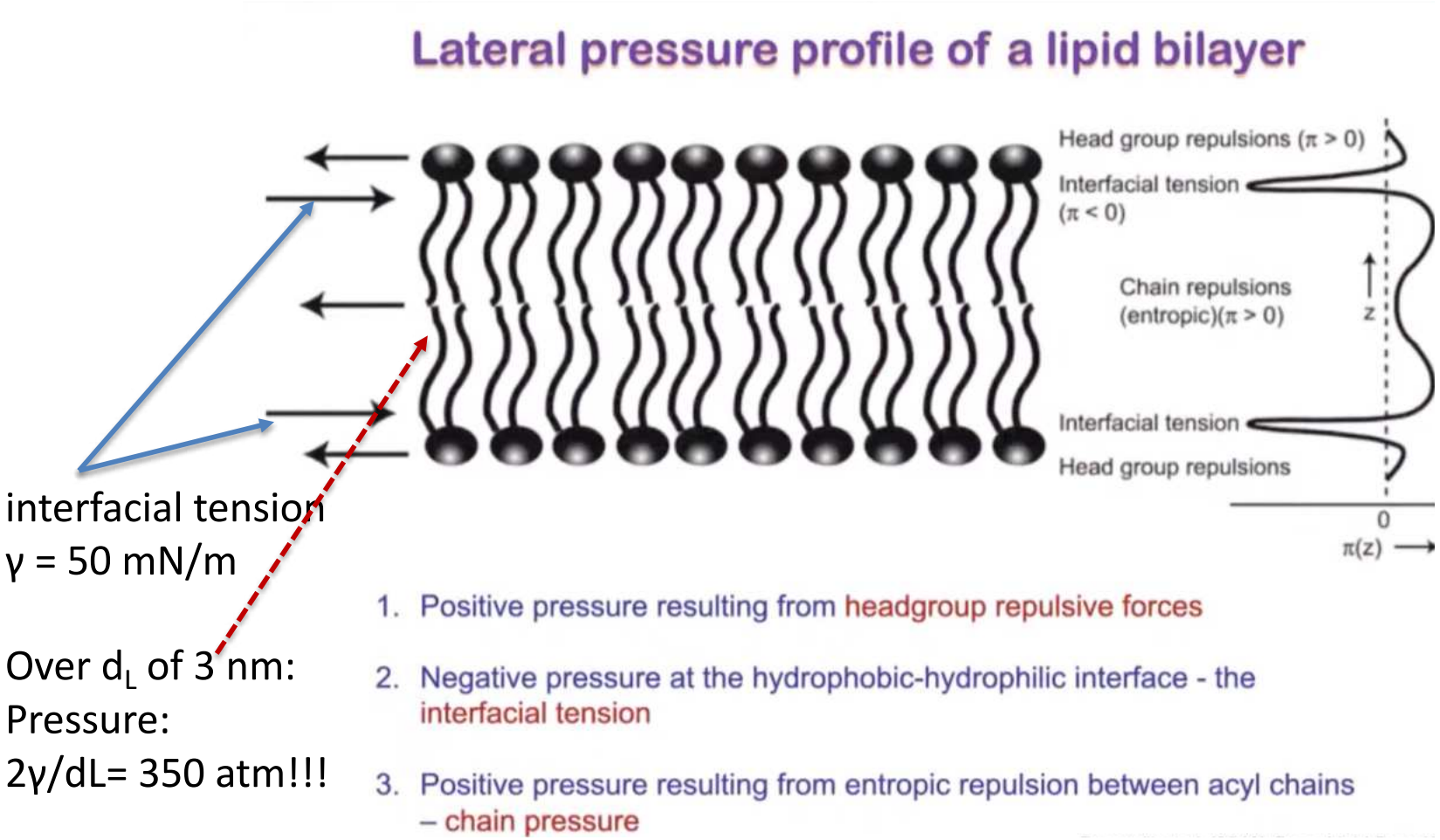
interfacial tension
γ = 50 mN/m

Over d_L of 3 nm:
Pressure:
2γ/dL= 350 atm!!!

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

Membrane tension in lipid structures and is around 10^{-7} – 10^{-4} N/m in GUV (10^{-3} N/m in SLB)

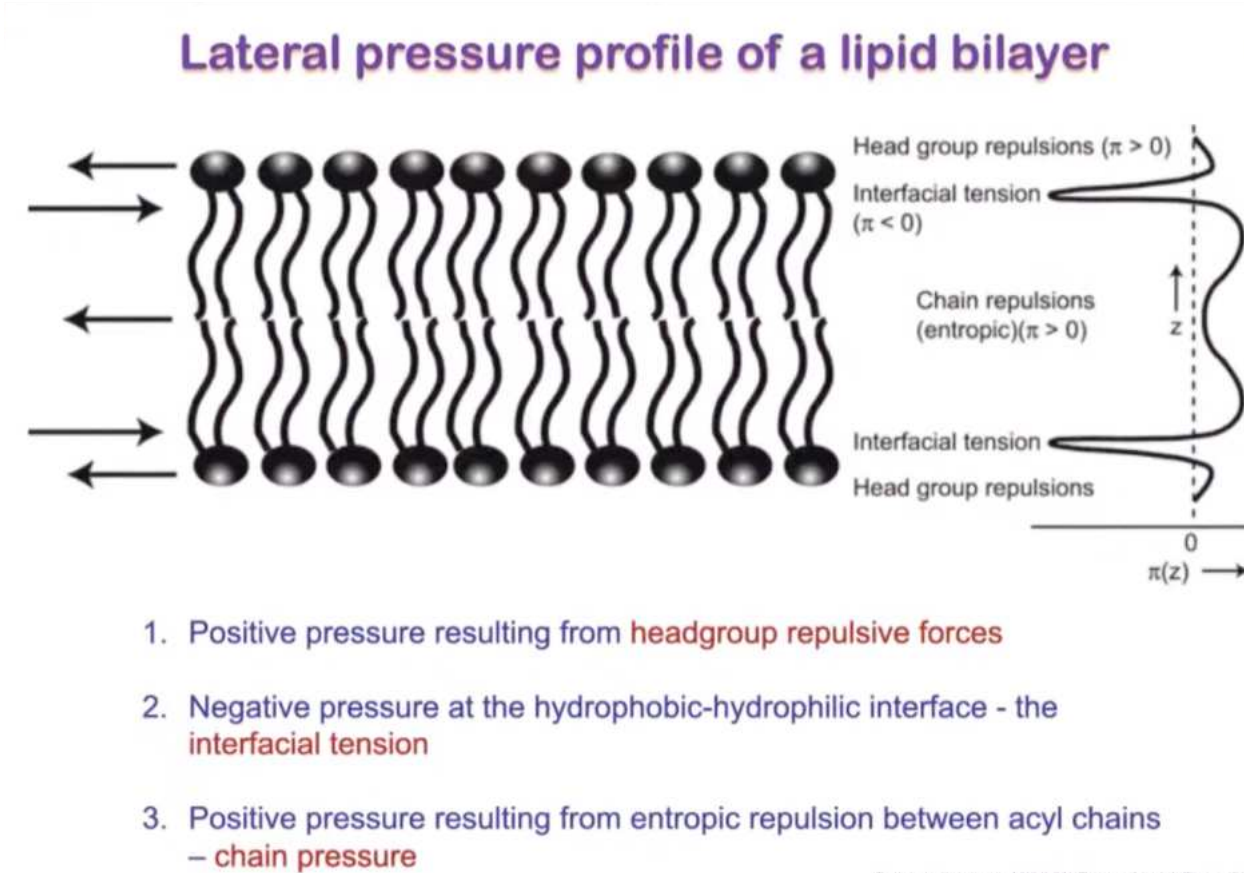
If we traslate the thermal energy K_BT to such scales (L = 10 nm-100s nm), we obtain a tension of : 4 x 10^{-5} – 10^{-3} N/m, relevant for lipid bilayers and vesicle mechanics!



interfacial tension
γ = 50 mN/m

Over d_L of 3 nm:
Pressure:
2γ/dL= 350 atm!!!

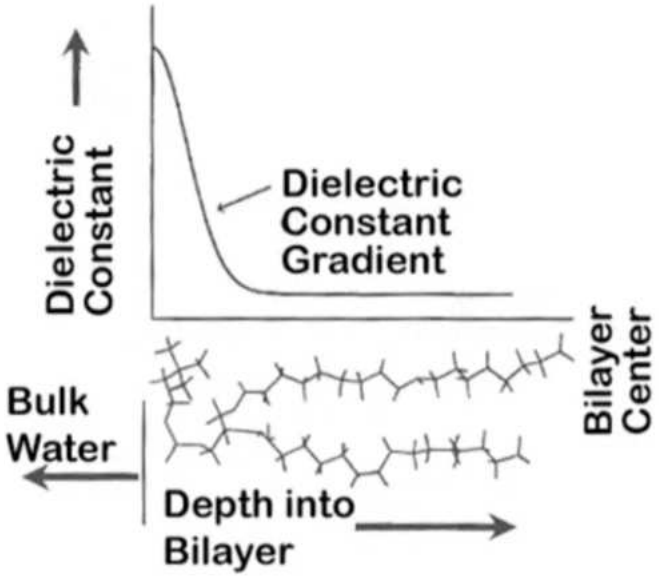
Hydrophobic forces favour formation of the lipid bilayer. But single lipid molecules are subjected to large stress due to this confinement.
The negative, localized interfacial tension is about γ =50 mN/m. It is counterbalanced by the chain positive tension, which spans over the membrane thickness, about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

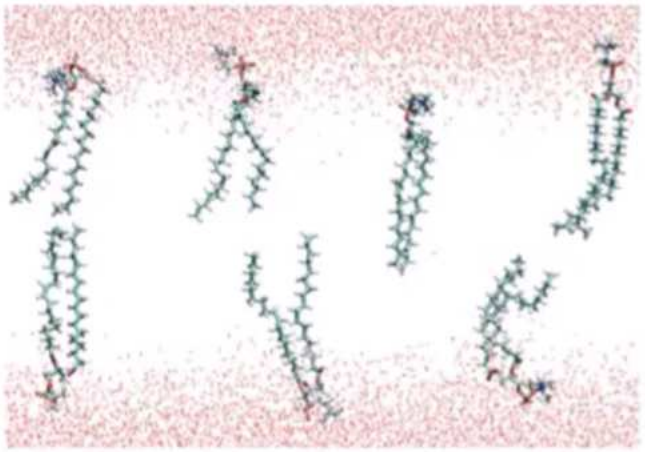


Membrane tension in lipid structures is around $10^{-7} – 10^{-4}$ N/m in GUV (and 10^{-3} N/m in SLB)

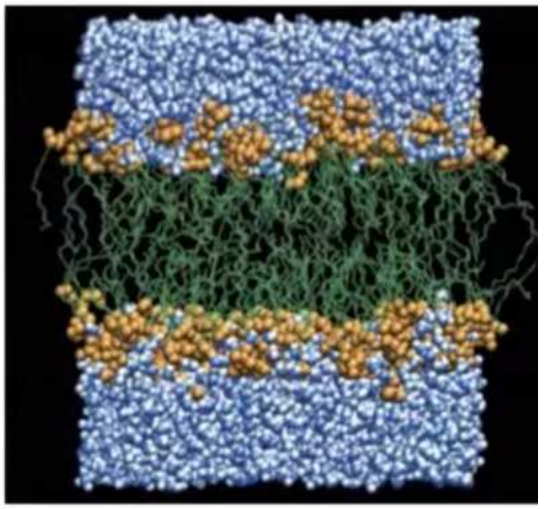
If we traslate the thermal energy K_BT to such scales (L = 10 nm-100s nm), we obtain a tension of : $4 \times 10^{-5} – 10^{-3}$ N/m, relevant for lipid bilayers, vesicle mechanics and to explain influence of lipids on conformation of membrane proteins!

Lipid shape across the interface





MD simulation of SOPC conformations in a bilayer

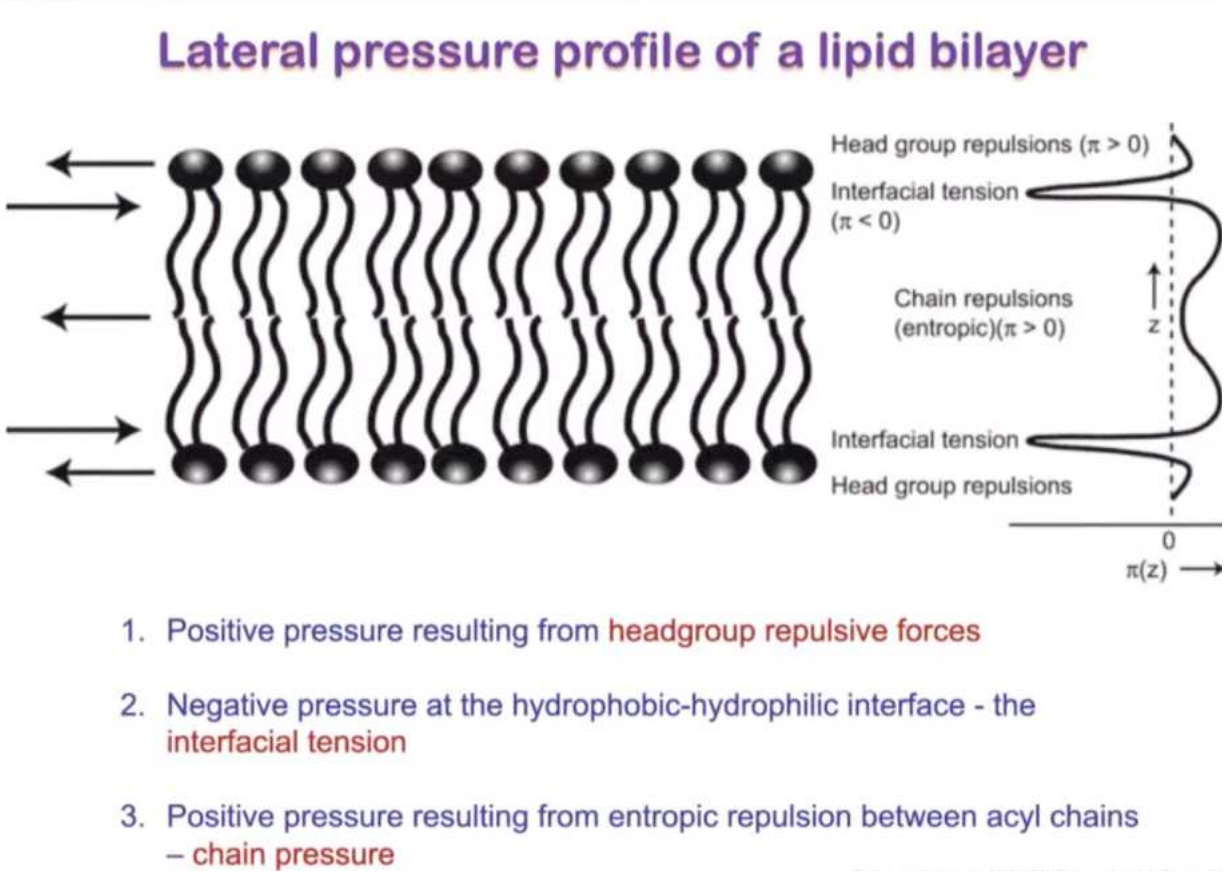


Fully hydrated DMPC bilayer

The stressed and frustrated situation that a lipid molecule experiences in a bilayer implies multiple lipid shapes: it is better described by the lateral pressure profile, that is the more fundamental physical property that underlies the curvature stress field and that determines bilayer spontaneous curvature, as well as the mean curvature and the Gaussian curvature modules.

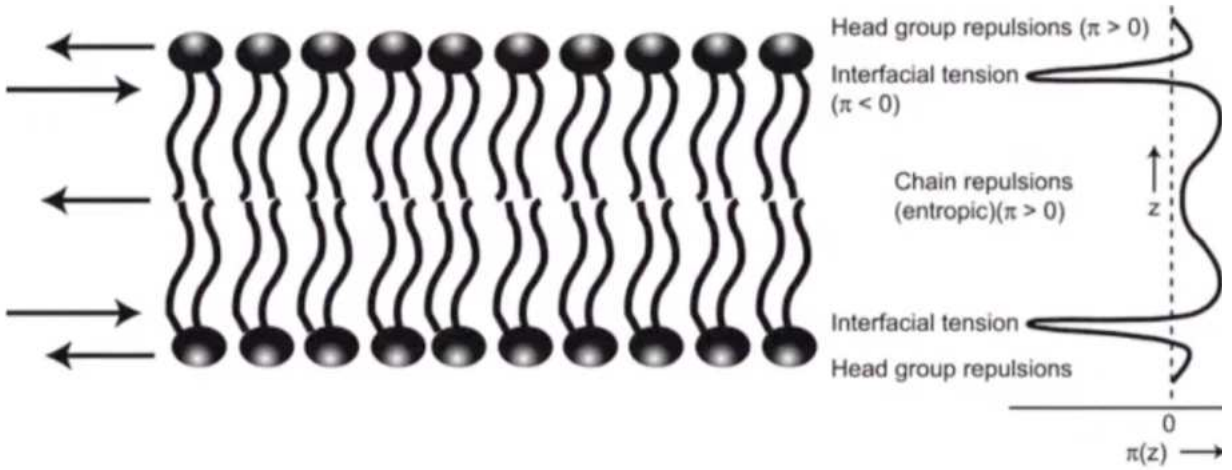
Stubbs *et al.* (1995) *J. Fluoresc.* 5: 19-28
Chiu *et al.* (1995) *Biophys. J.* 69:1230-1245

How thick is a membrane?



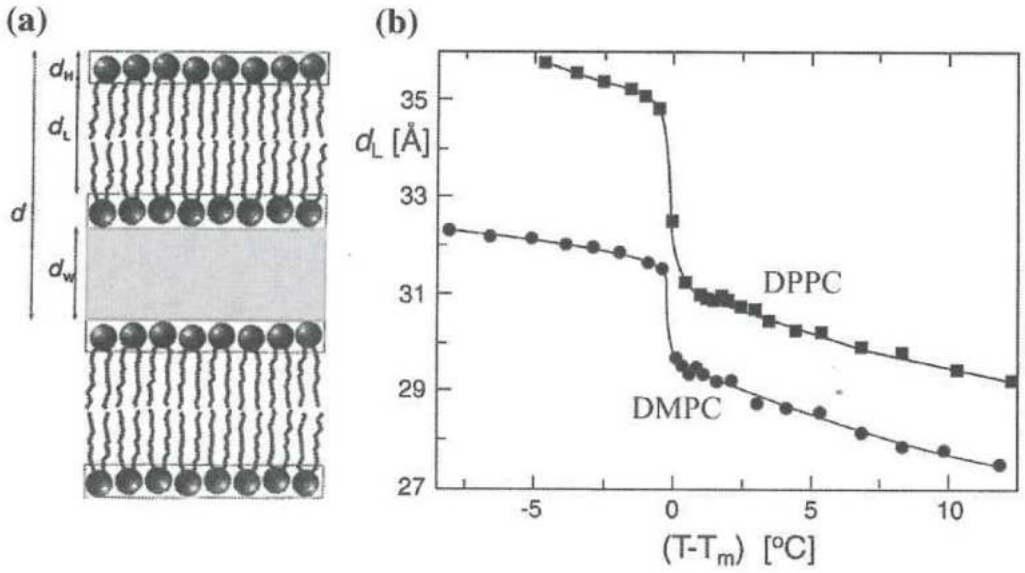
Therefore, lipid bilayers are very stratified, and dynamic: what is the THICKNESS then?
Of course, it depends on length and saturation of the lipids (longer and saturated are thicker) and on the hydration (more hydrated, thinner (dehydration makes the heads get closer, and the tails stretch out).
Cholesterol 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

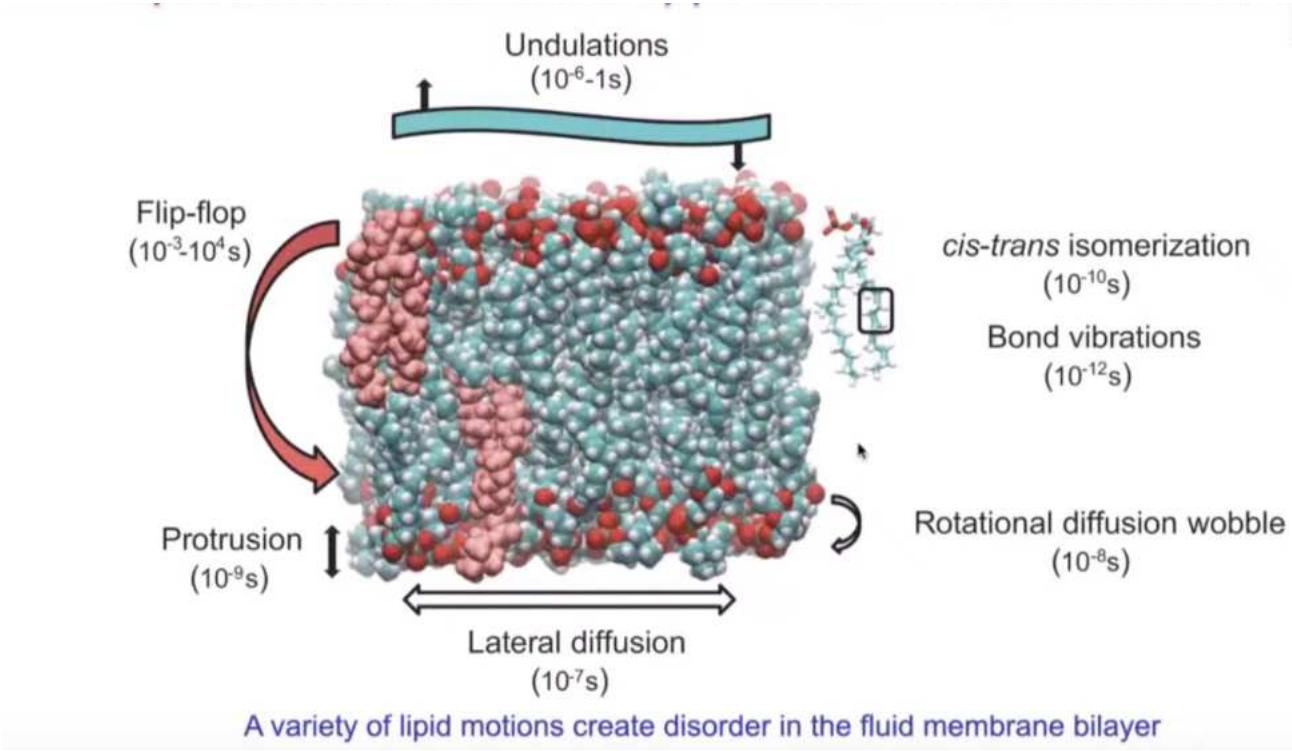


1. Positive pressure resulting from headgroup repulsive forces
2. Negative pressure at the hydrophobic-hydrophilic interface - the interfacial tension
3. Positive pressure resulti
   – chain pressure

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



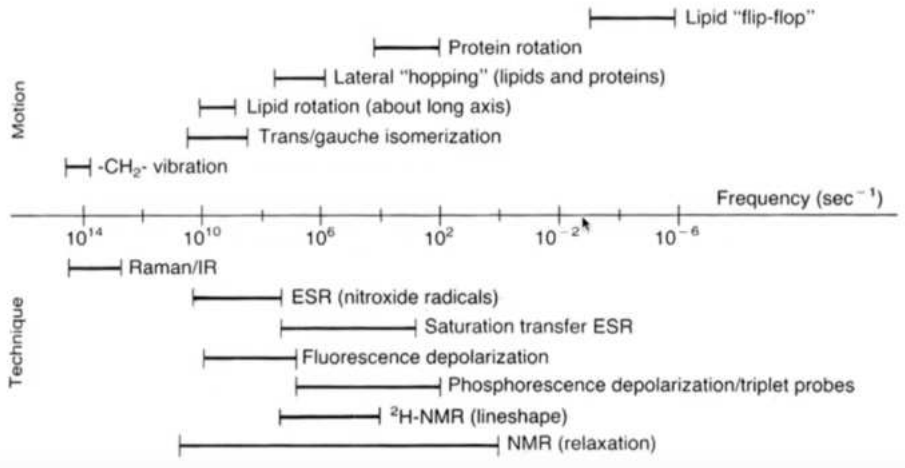
Molecular motion dynamics



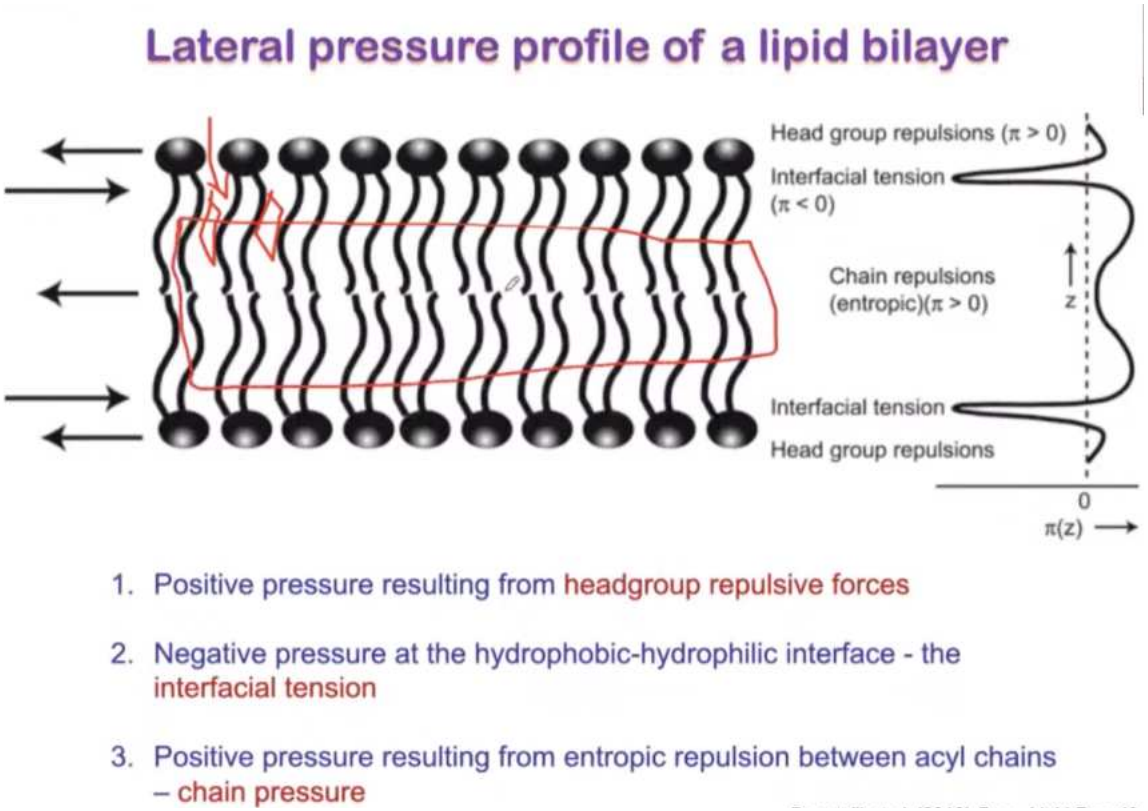
A variety of lipid motions create disorder in the fluid membrane bilayer

For a typical cell size, a lipid molecule can travel across the cell membrane within less than half a minute!

These motions range over an enormous time span, from picoseconds to hours. Conformational changes can be fast, since they involve rotations around C–C bonds (few ps. The rotation of the lipid molecules are also fast (ns); lateral diffusion is in the range of tens of ns. The wobbling of the fatty-acid chain, which leads to changes in its direction within the bilayer, is much slower (tens of ms).



Membrane interface: role of cholesterol

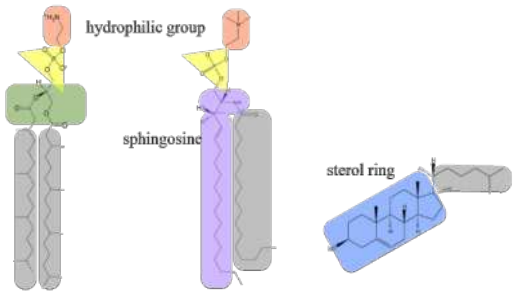


Cholesterol is a modulator of thickness! It is stiff, and stretches out and order fatty acid chains—more chol, higher thickness!

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).

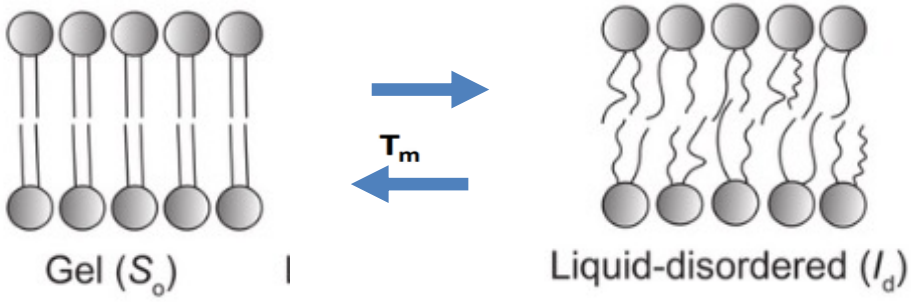
To release the frustration, chol induces a new phase, the liquid-ordered phase

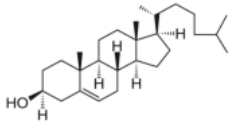
Lipid compartimentalization: the role of cholesterol

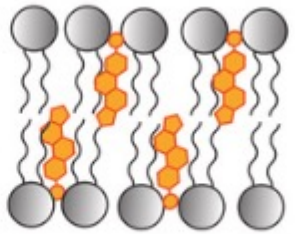


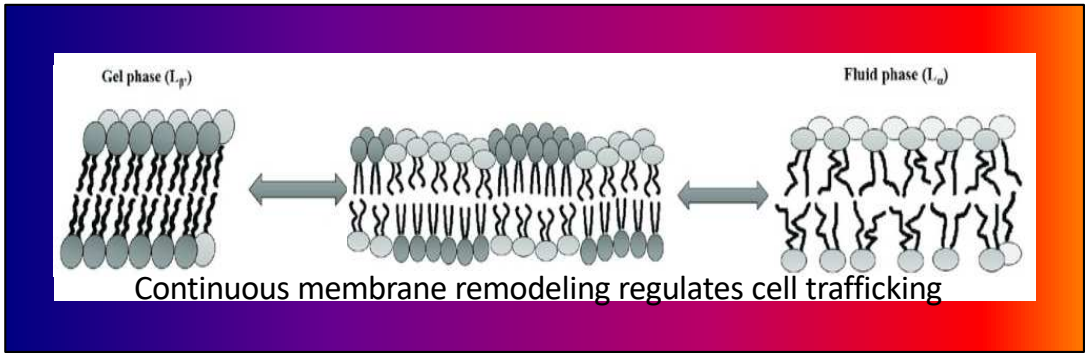
Phospholipids Sphingolipids Cholesterol

	DOPC	SM	DPPC
Tm (°C)	-17	38	41





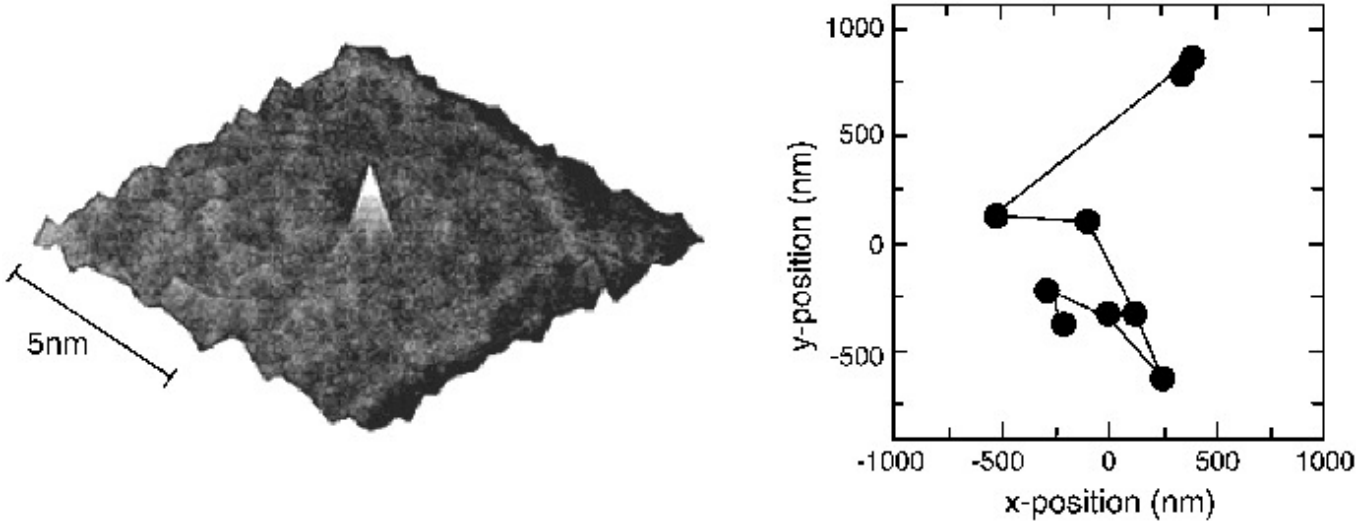




Chol: **reduces permeability while maintaining lateral mobility**

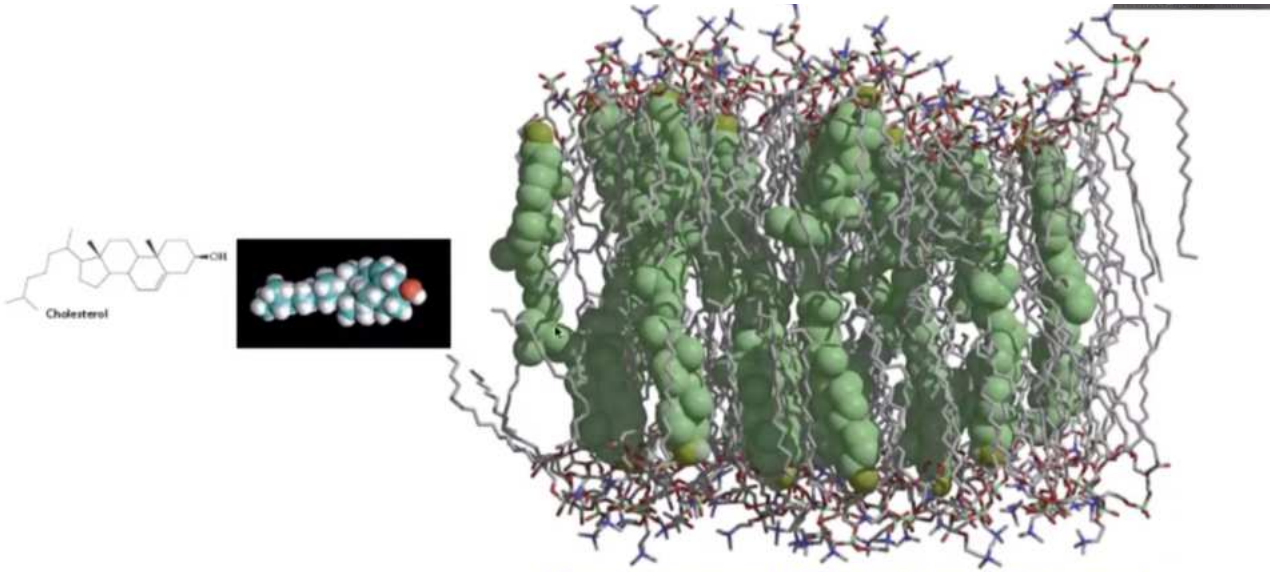
It plays a fundamental role in overall membrane functioning

Lateral mobility



(a) Fluorescence image of a single fluorescence-labelled lipid molecule in a POPE-POPC phospholipid bilayer. The peak in intensity signals a single molecule in the plane of the membrane.
(b) Recording of a part of a diffusion trace of a single lipid molecule

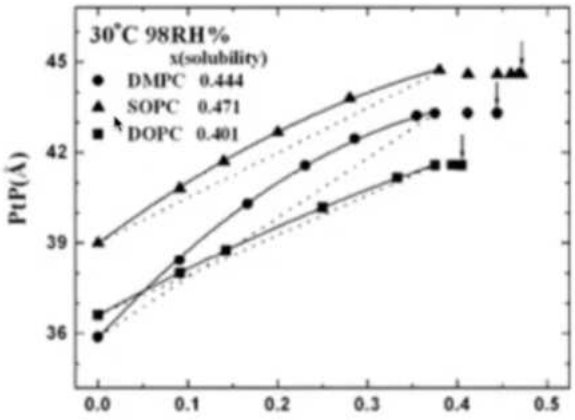
Cholesterol promotes lipid order



MD simulation snapshot of DPPC in fluid phase with 20% cholesterol

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



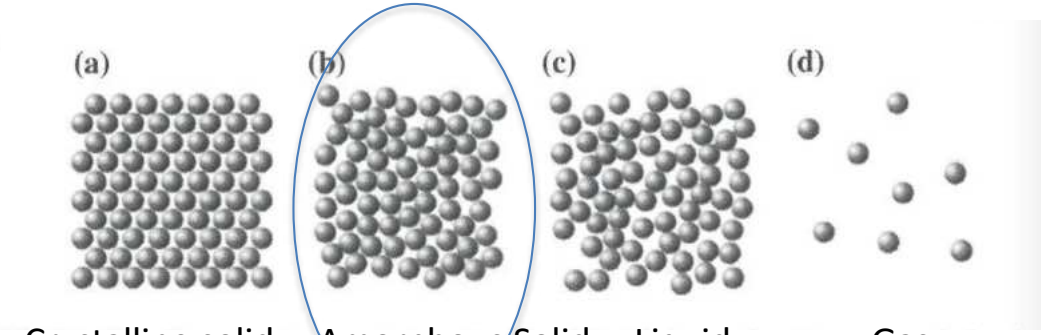
DMPC = 7.4 Å increase
SOPC = 5.6 Å
DOPC = 4.9 Å

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

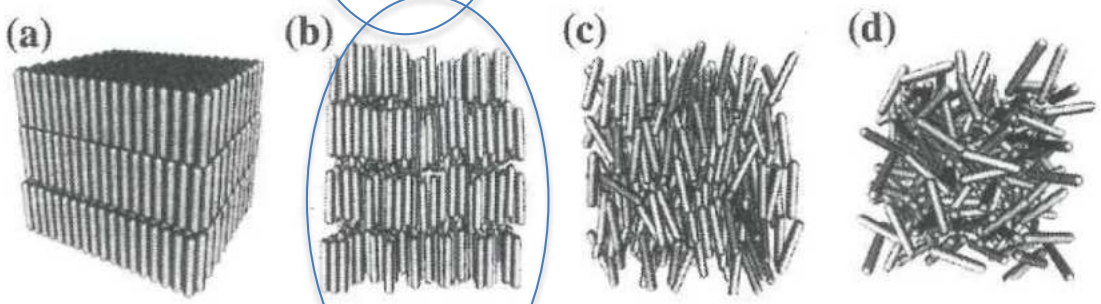
The hydrophobic membrane thickness in fluid phase is strongly dependent on the amount of cholesterol incorporated

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



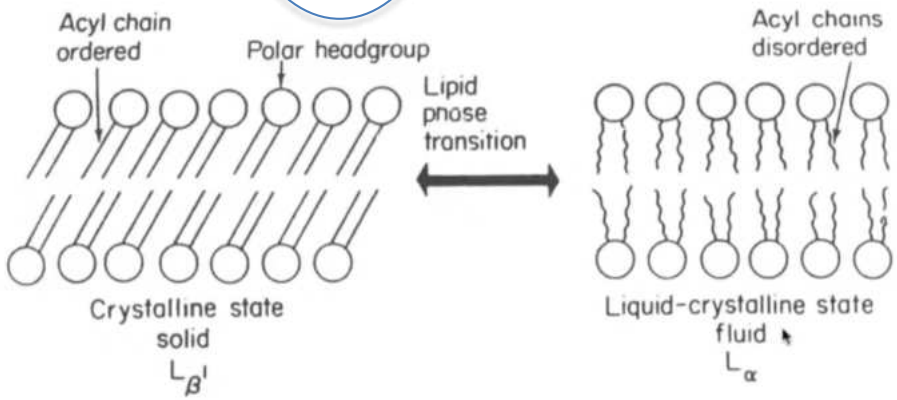
SPHERIC OBJECT: Crystalline solid Amorphous Solid Liquid Gas



NON-SPHERIC OBJECT: Crystalline solid Smectic Nematic Liquid

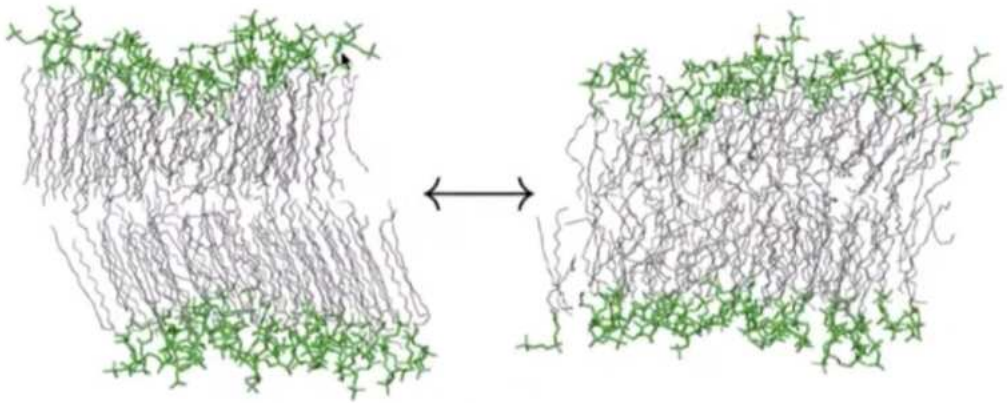
Meso-phases with order and disorder elements

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



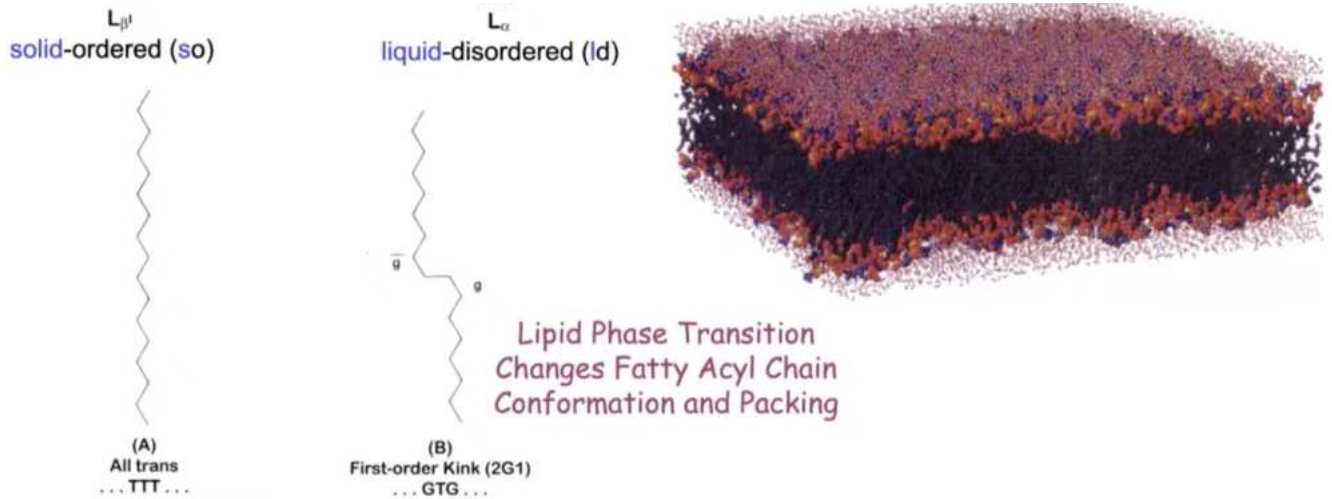
N.B.: Continous Transitions (strong fluctuations!) are the so called crytical phenomena (G. Parisi Nobel Price!)

Phase transitions



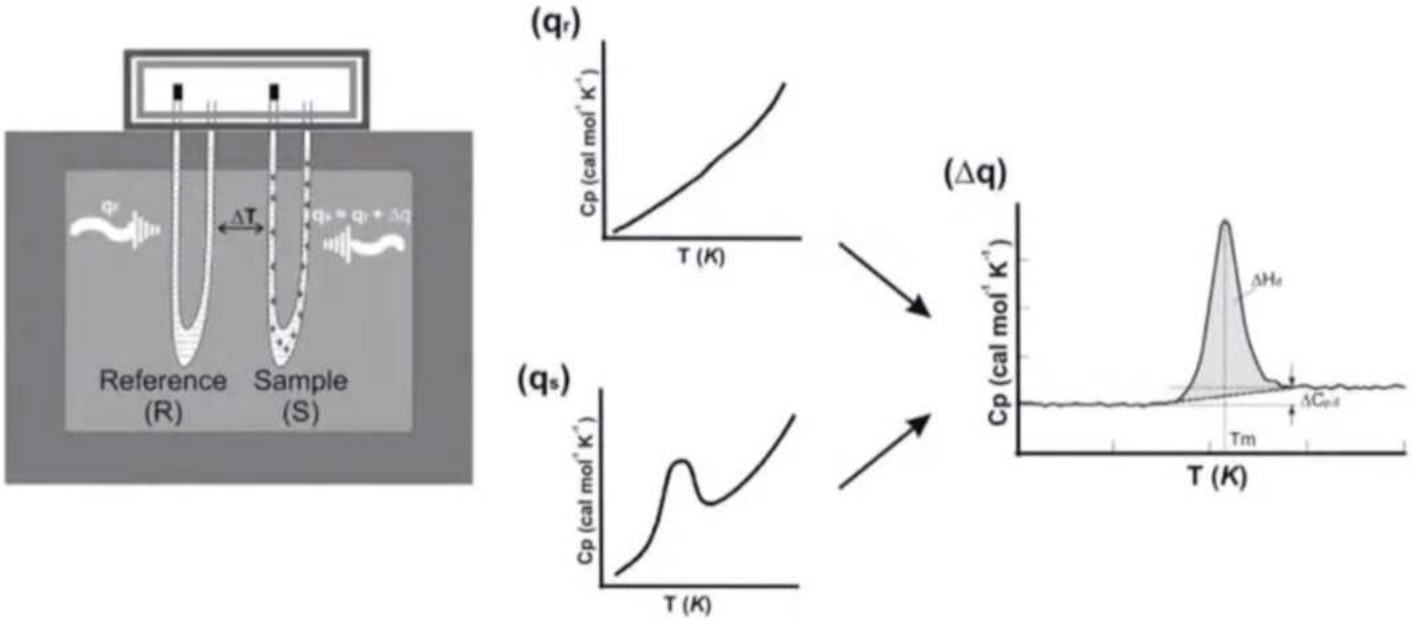
MD simulation of DPPC in water at Tm using atomistic model

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

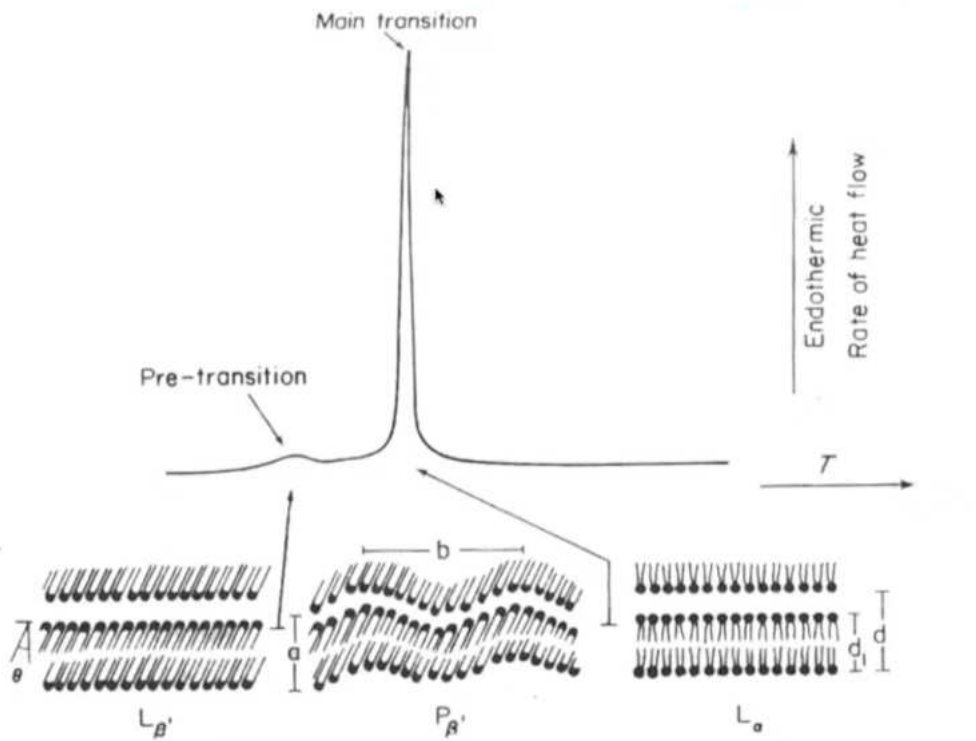


Phase transitions
Differential Scanning Calorimetry (DSC)

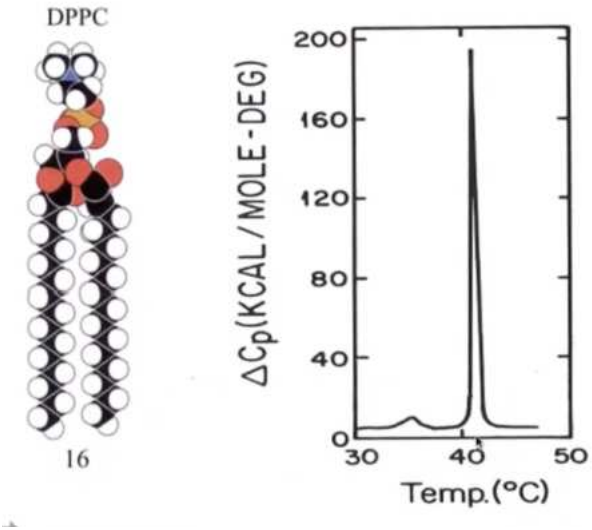
- DSC is a thermal analysis technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature
- Highly reproducible phase transitions are used to determine binding interactions, purity and stability of samples



Phase transitions in lipids



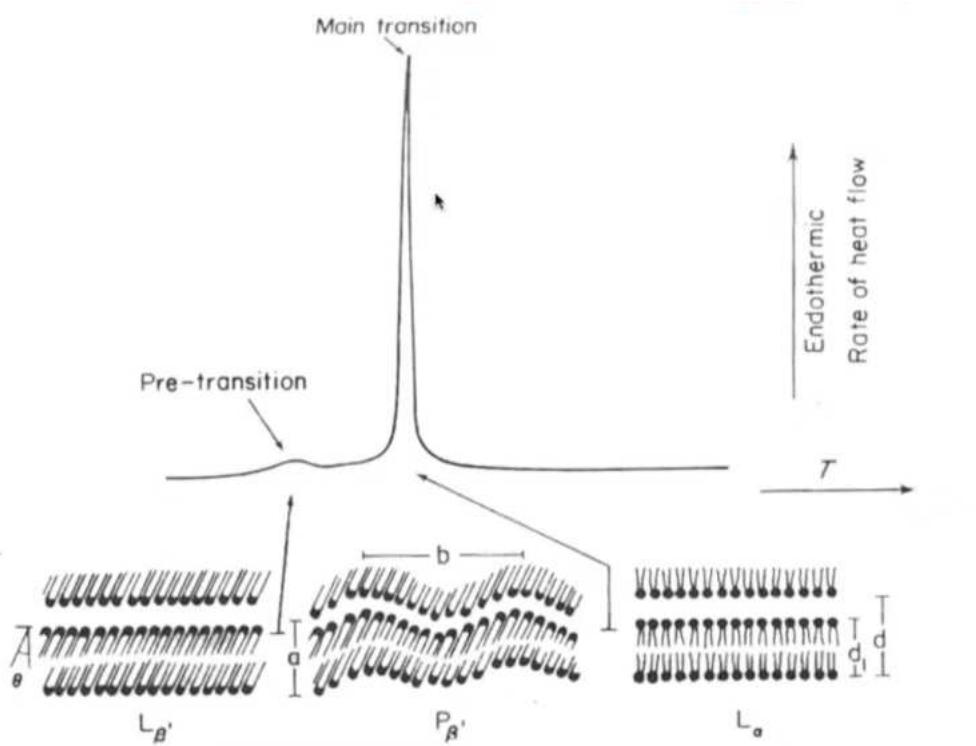
Main transition is precedeed by an intermediate, ripple phase which facilitates transition (specific heath vs. T).



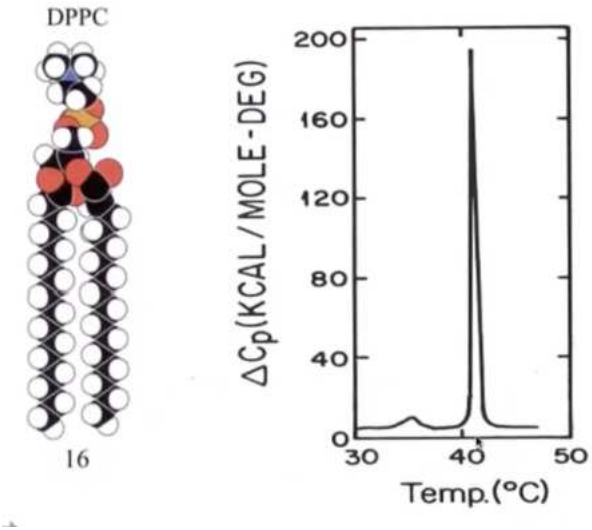
Transition is very sharp: no chemical link among molecules in the layer, all molecules make the transition at the same time. Transition is dominated by thermal fluctuations

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
$\Delta S = k_B \, ln\Omega$ 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 lipids



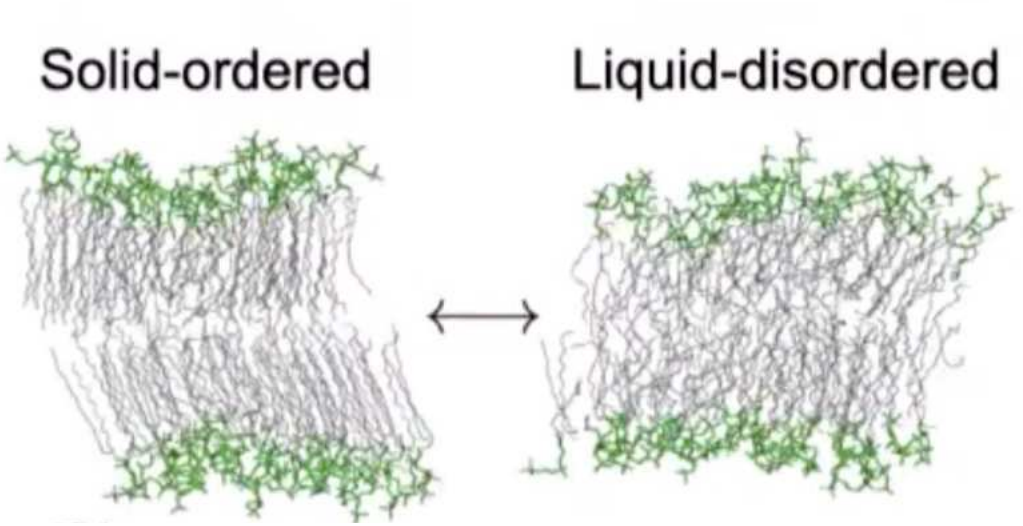
Main transition is precedeed by an intermediate, ripple phase which facilitates transition (specific heath vs. T).



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 main transition is associated with lipid melting, i.e. increased disorder of their chains

Phase transitions



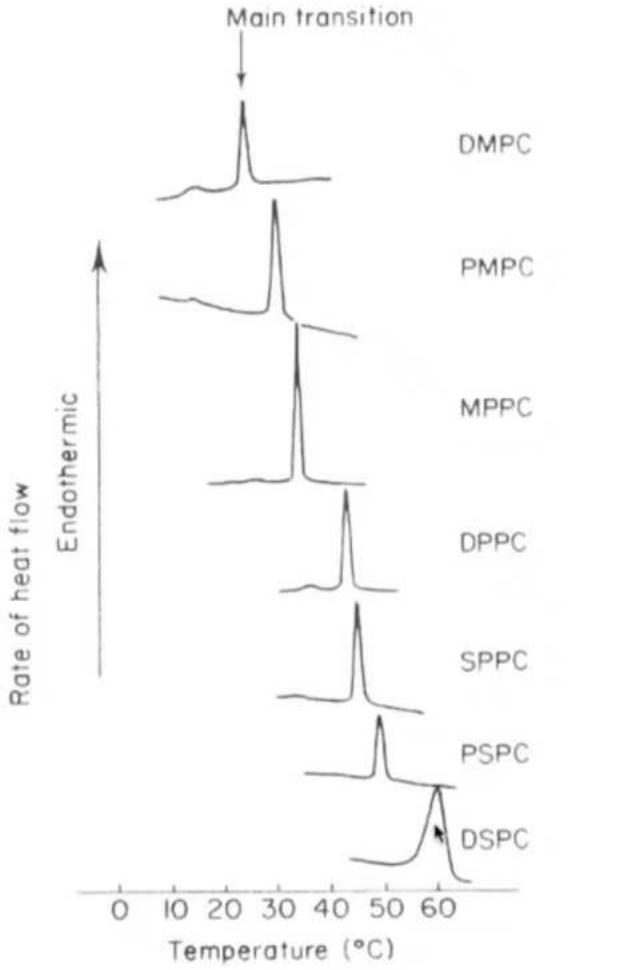
In **solid-order phase**, chains are aligned and heads are ordered

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

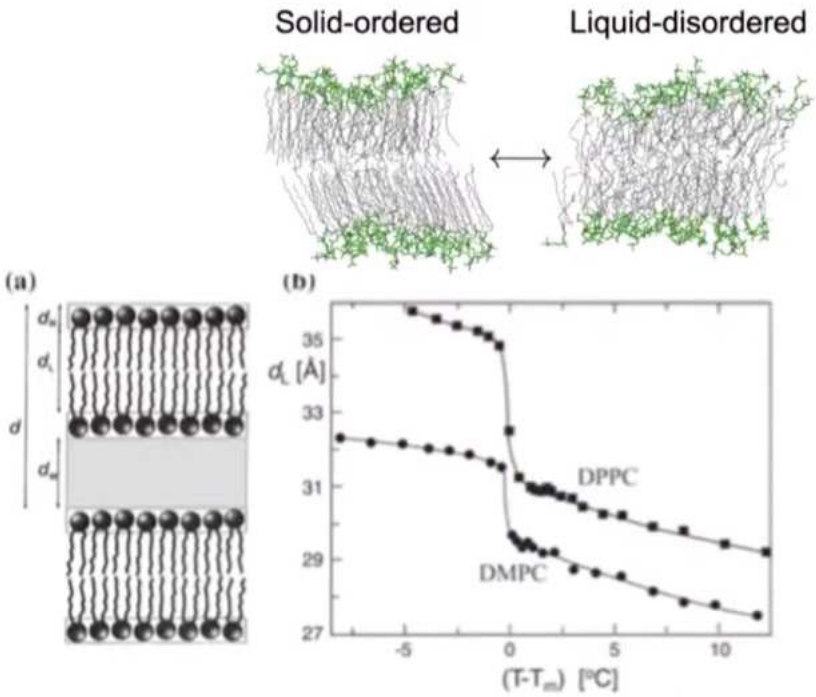
Across the phase transition, height and area per mol change! $\Delta A \Delta d_L = c$

Phase transitions



Phase transitions and thickness

Phase Transition Temperature Increases with Increasing Fatty Acyl Chain Length

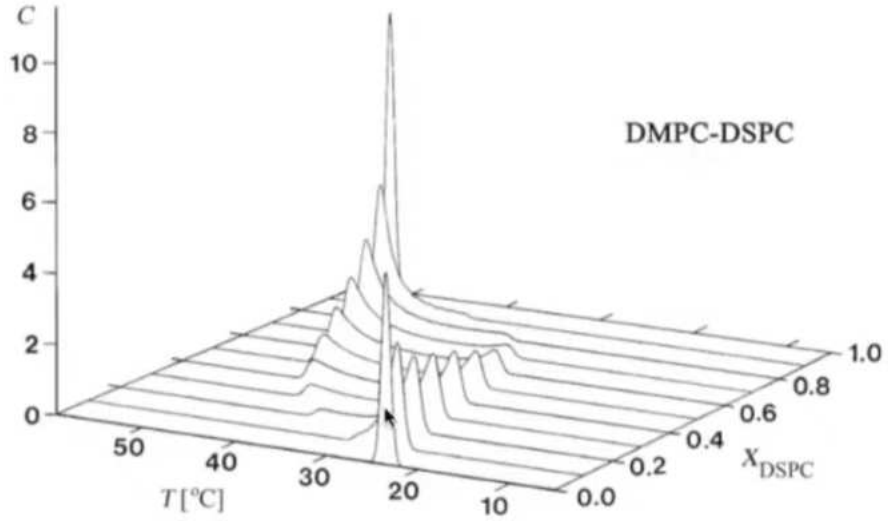


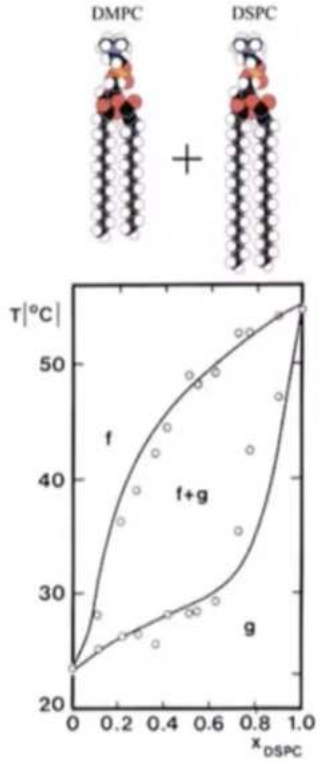
Organisms adapt lipid composition

	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

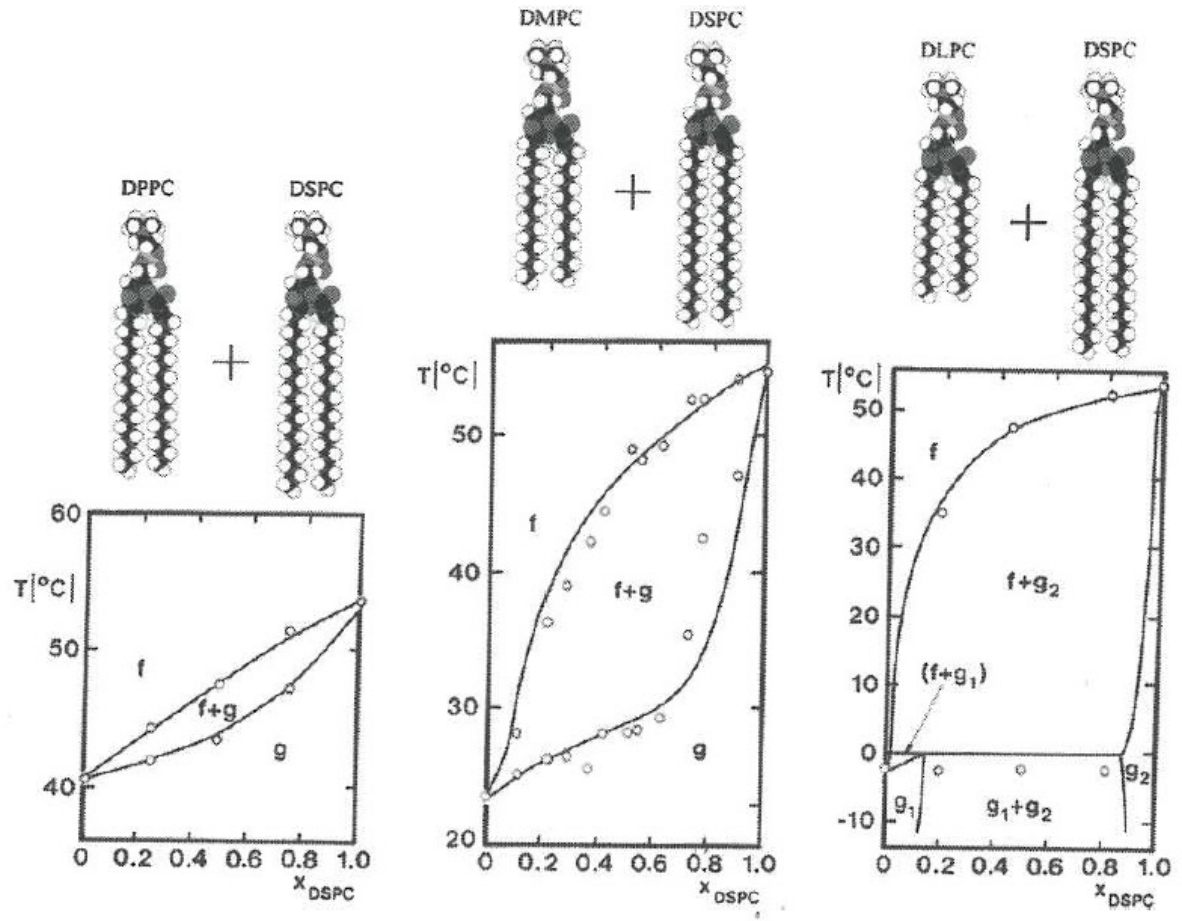
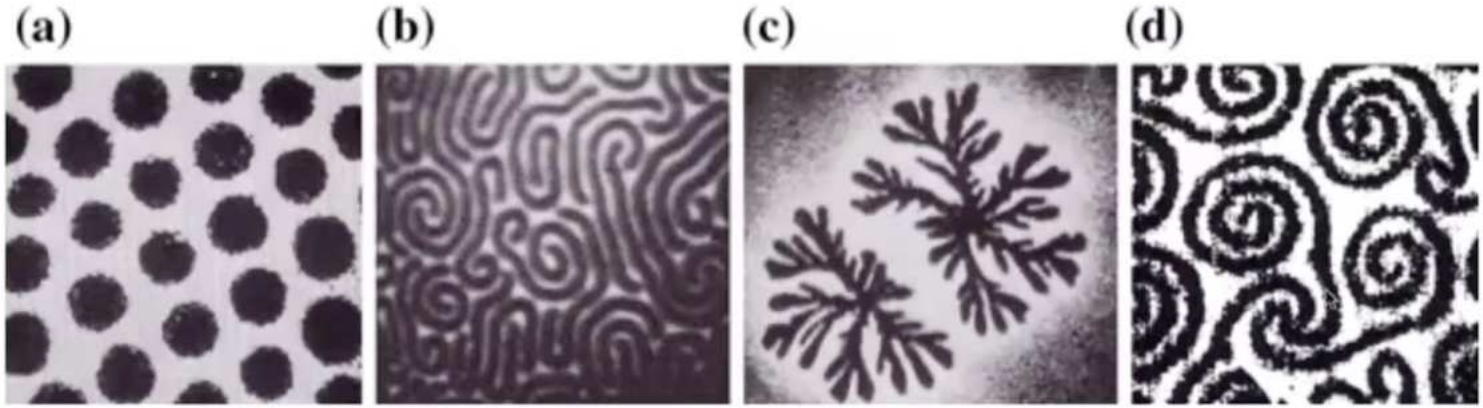


Fig. 9.8 Phase diagrams of lipid bilayers for three binary mixtures of PC lipids with different fatty-acid chain lengths. *f* denotes the liquid-disordered phase, and *g* denotes solid-ordered phases

Phase separation, co-existence



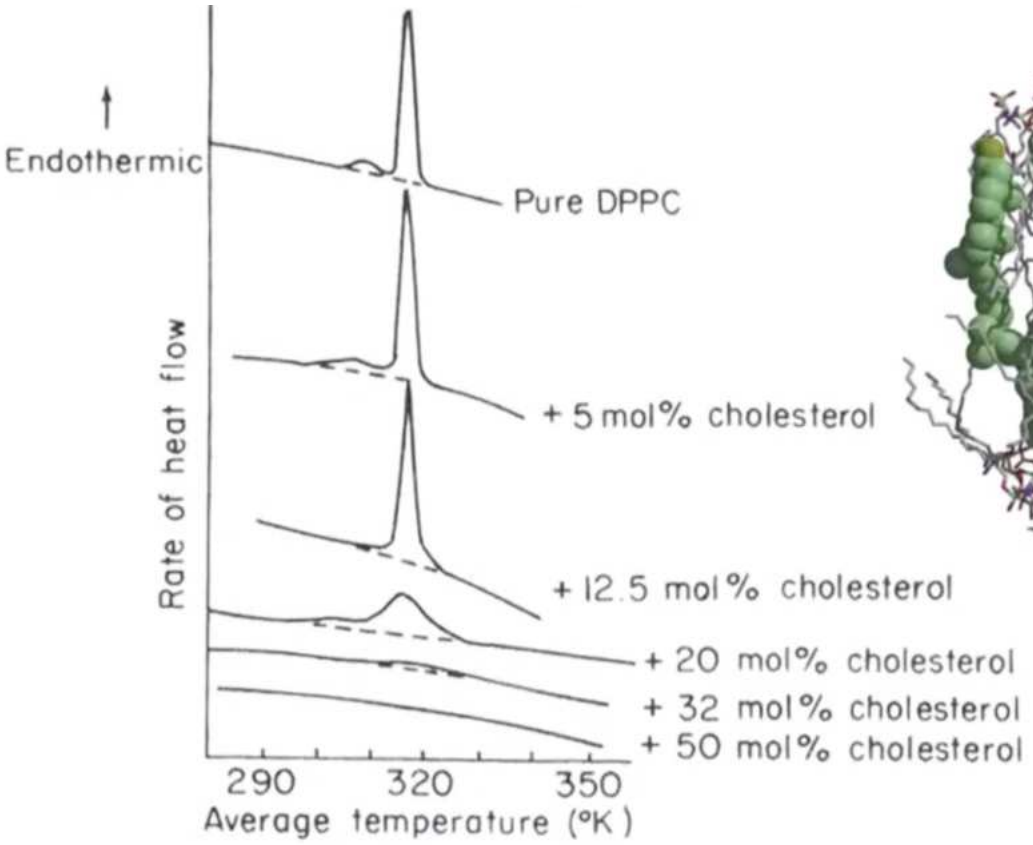
a) Coexistence of liquid phase (light) and solid phase

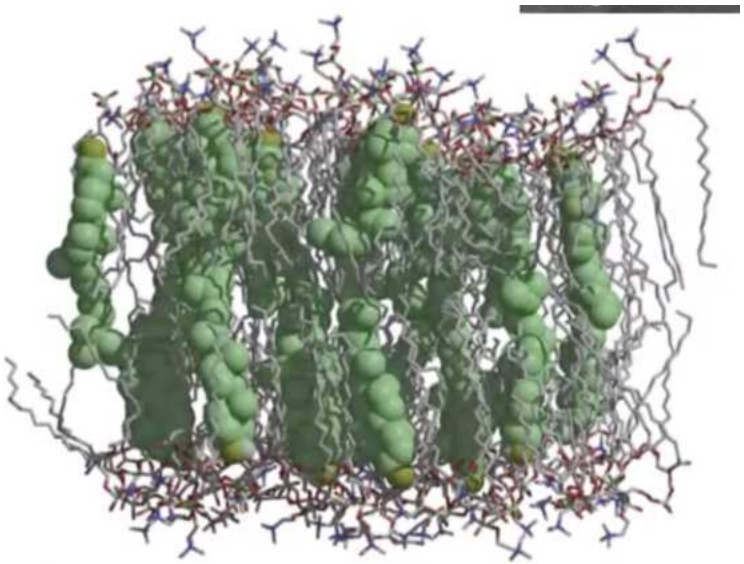
b) Striped pattern

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

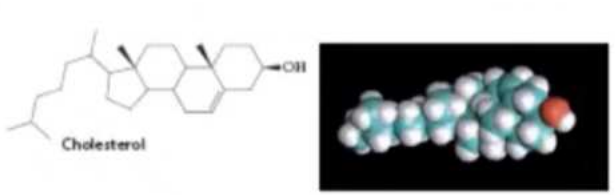


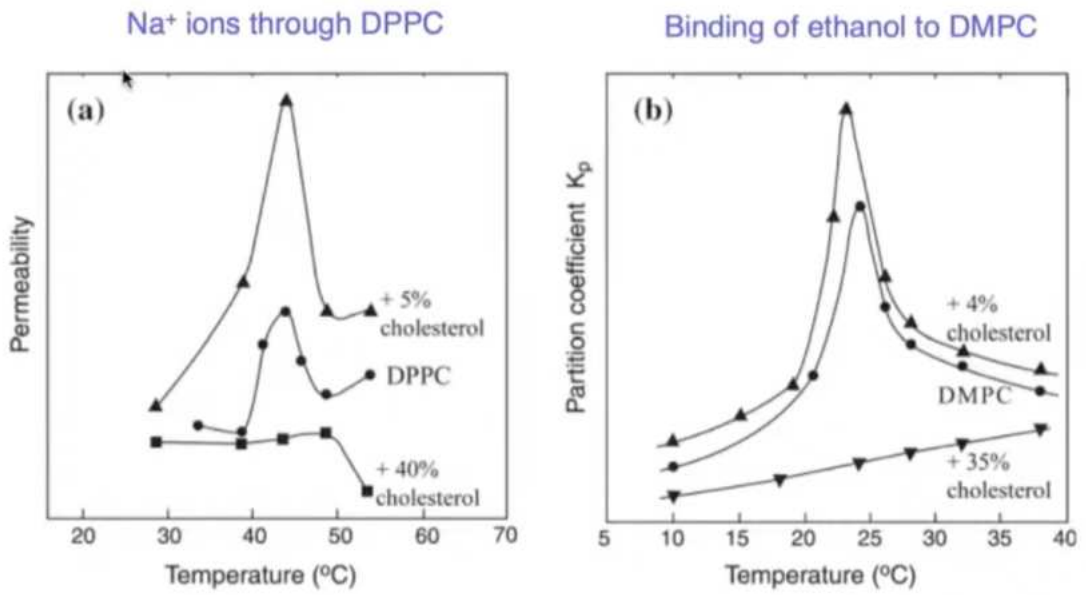


Chol reduces lipid cooperativity!! The new phase is called liquid-ordered phase. High positional degree of fredom, low conformational one!. Fluid and stiff

Chol and permeability: dual role

H Raghurama



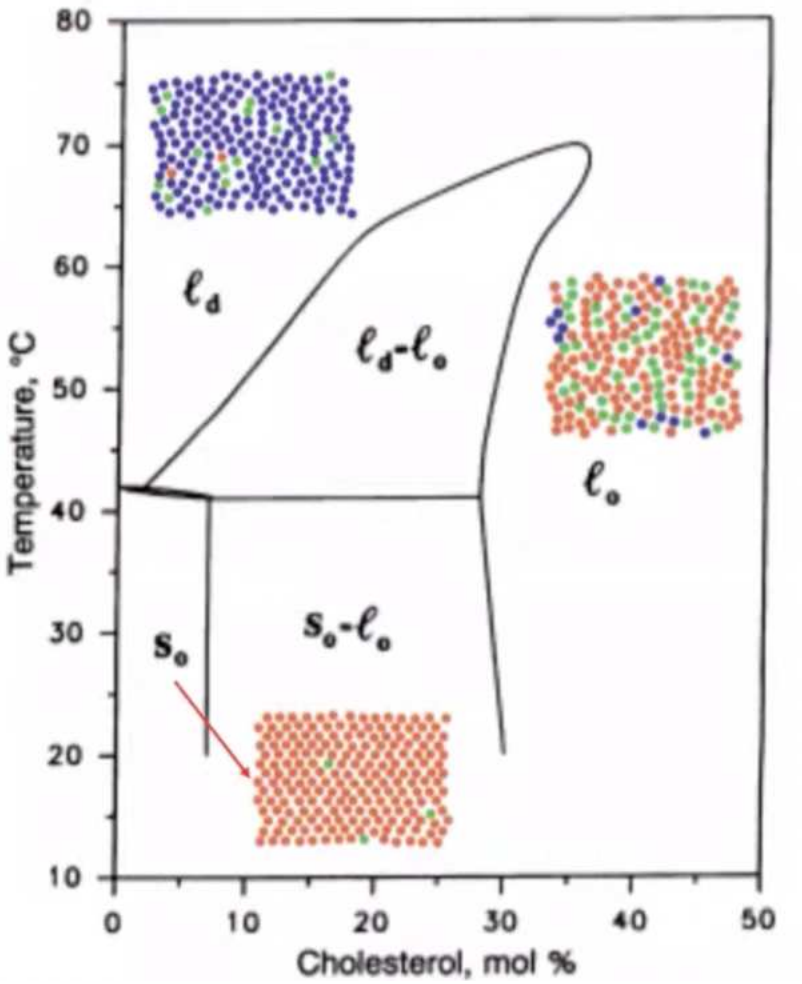


Biochim. Biophys. Acta (1992) 1107: 261-270
Biophys. J. (2000) 78: 2486-2492

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

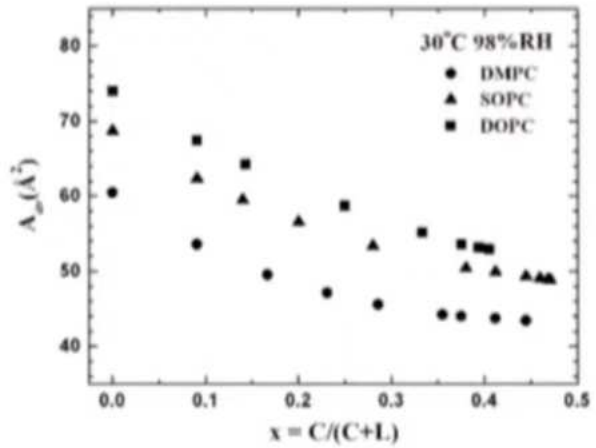
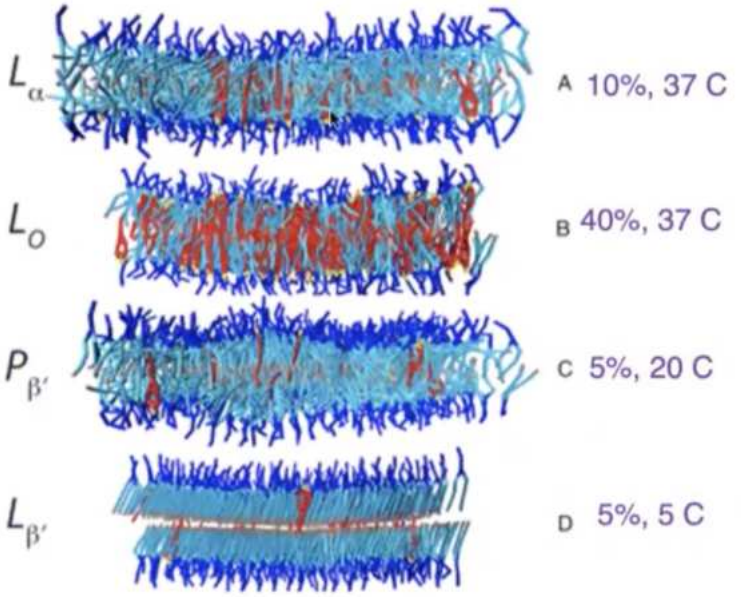


FIGURE 7 Area per molecule as a function of cholesterol concentration. The averaged cross section area of phospholipid is calculated by $A_{av,pc} = 2V_c/(PtP - 10)$, where V_c is the chain volume of the lipid (36), and the thickness of the hydrocarbon region is PtP minus twice the length of the glycerol 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).

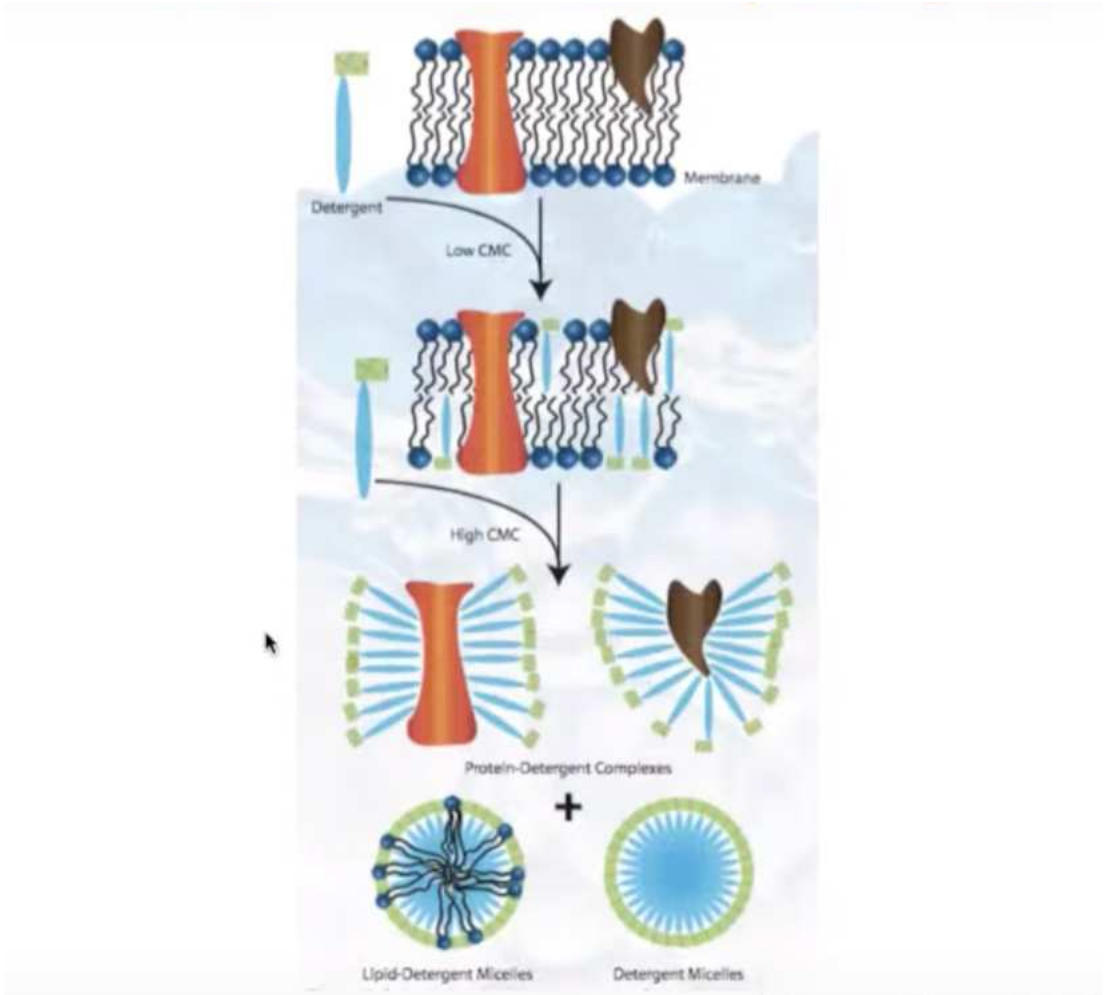
Biophys. J. (2007) 92: 3960-3967



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

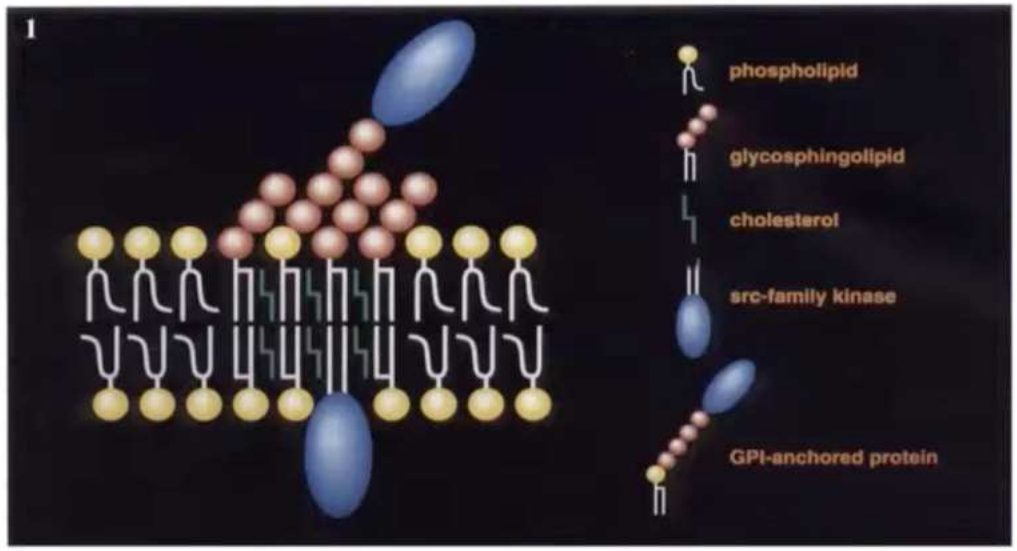
Combination of these factors !

Why are domains needed ?

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

Membrane domains

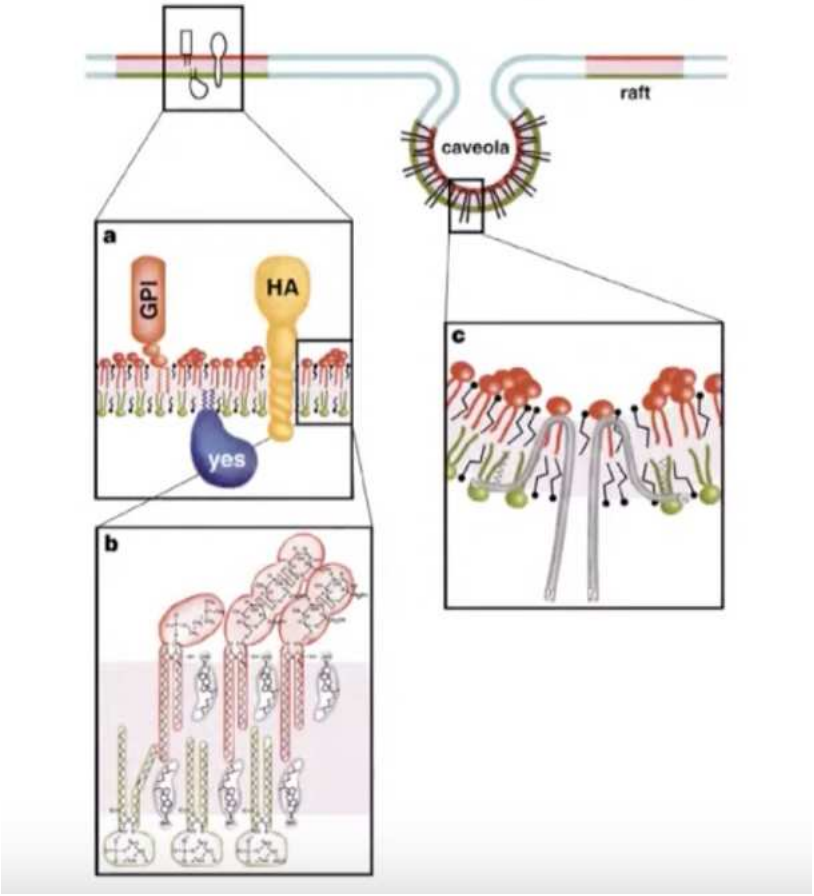
How do the proteins linked at two different sides of the membrane communicate?



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

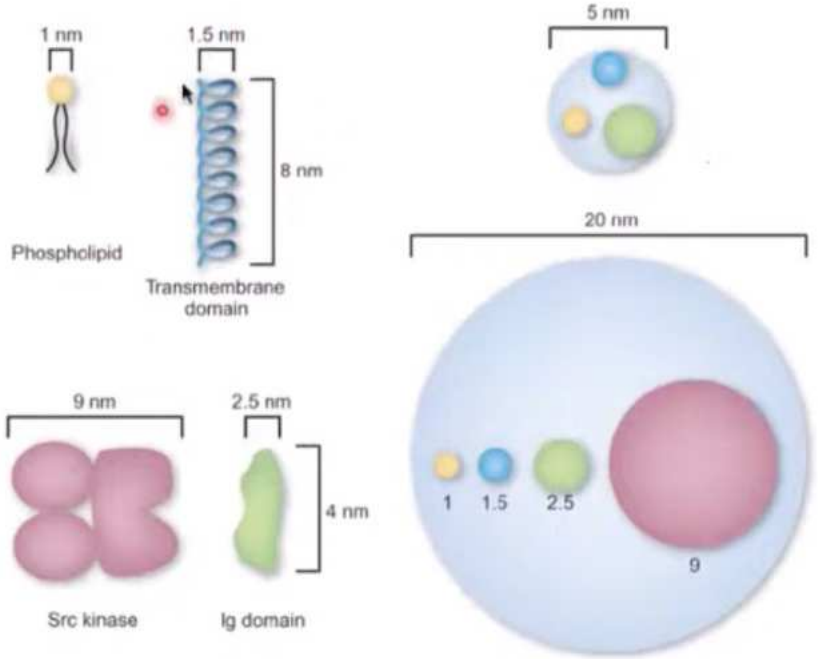
"Membrane Rafts are small (10-200 nm), heterogeneous, highly dynamic, sterol-and sphingolipid-enriched domains that compartmentalize cellular processes. Small rafts can sometimes be stabilized to form larger platforms through protein-protein and protein-lipid interactions"

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 !

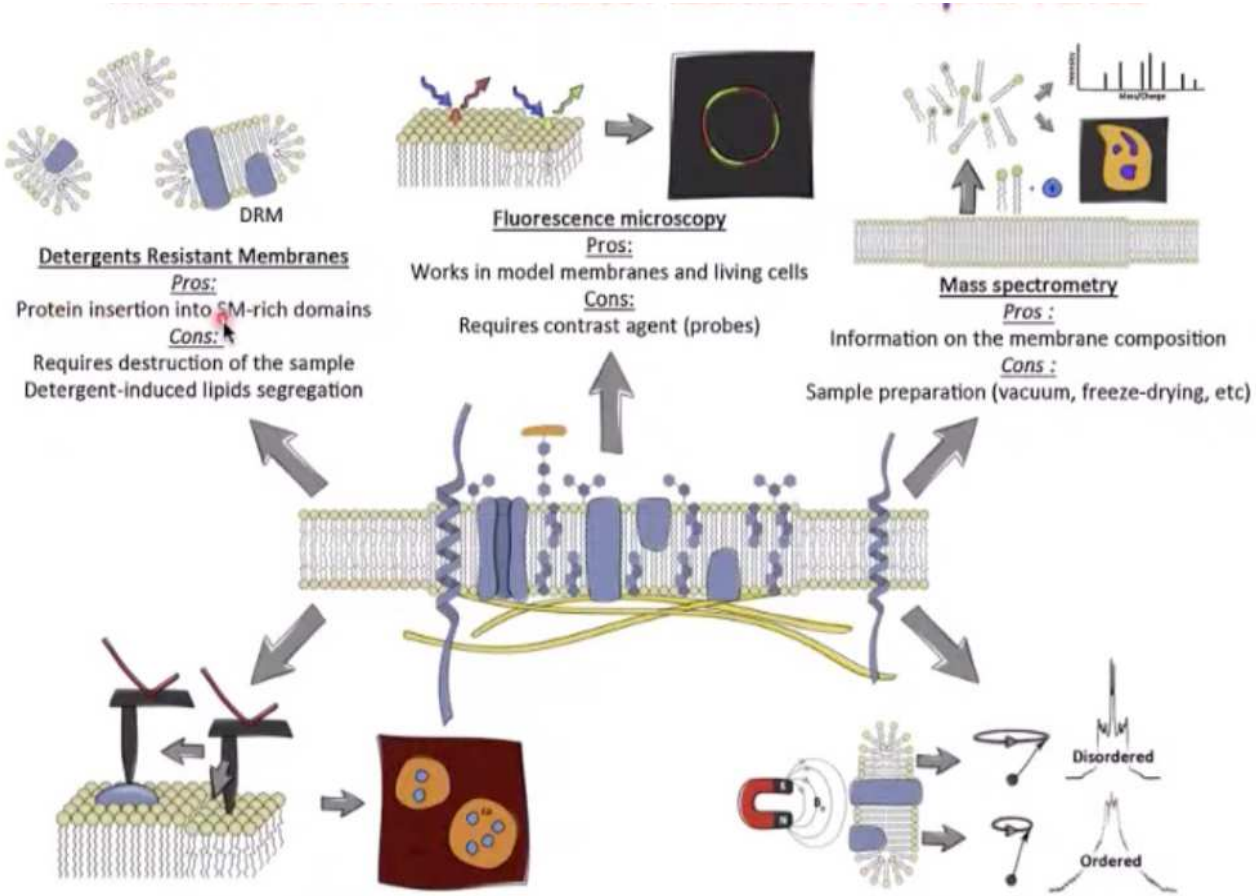
Simons and Ikonen (1997) *Nature* 387: 569-572
(> 8700 citations)

How big is a membrane raft



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

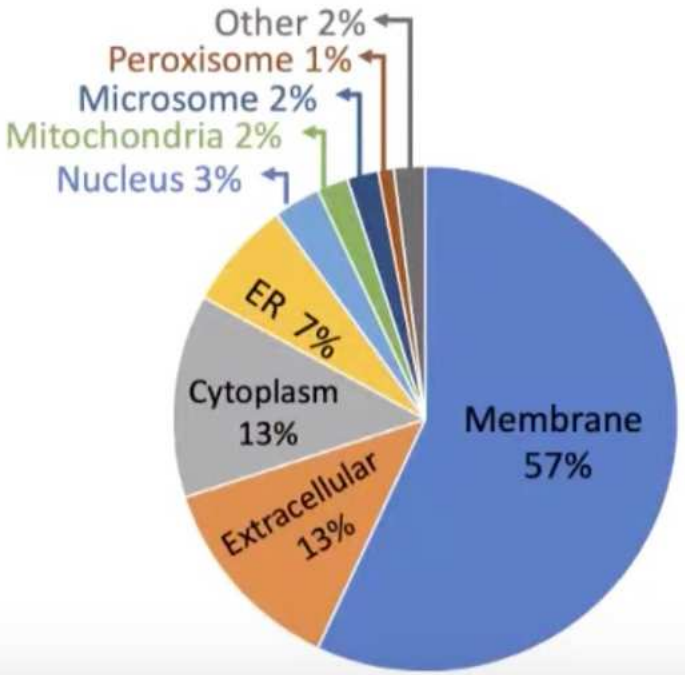
Raft characterization



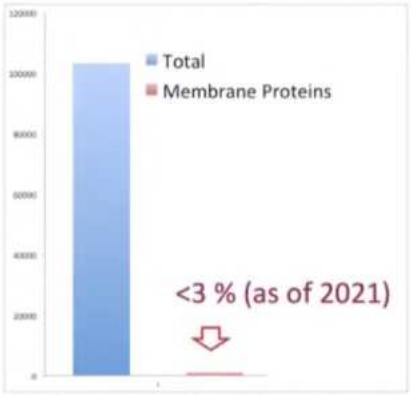
Chem. Biol. (2014) 21: 97-113

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:

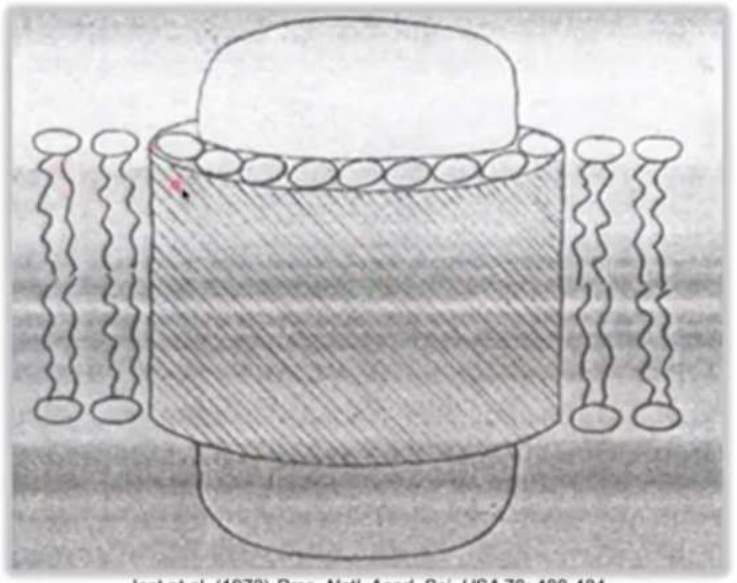
  At low lipid-to-protein ratio, a broad spectrum was observed

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

  Pure lipid showed only a sharp spectrum

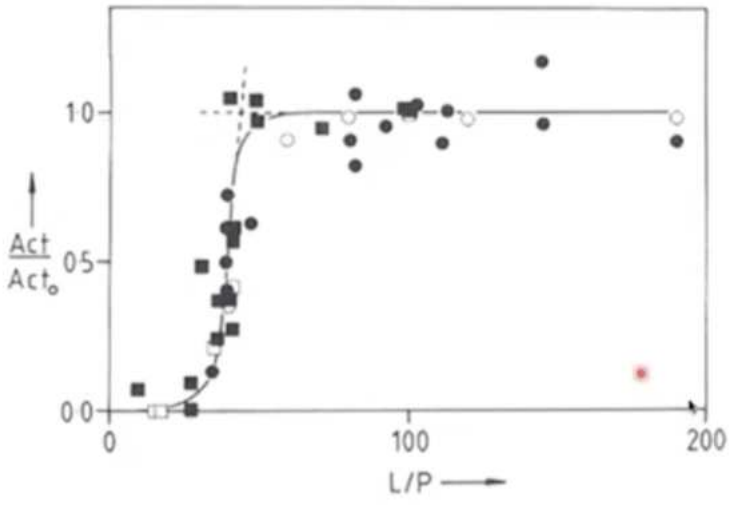
- The concept of 'Immobilized' lipids

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

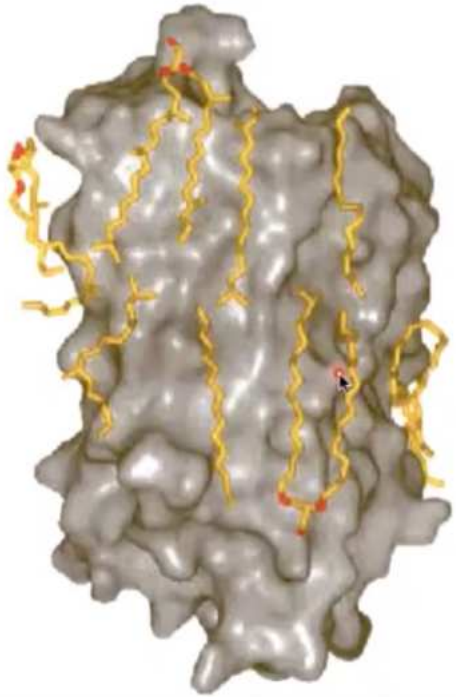


Nicotinic acetylcholine receptor activity

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

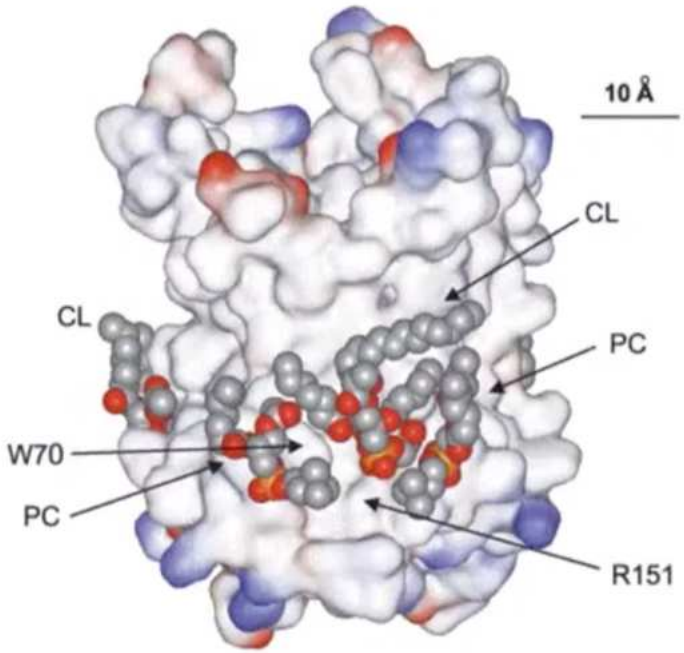
Lipid-protein interactions

Bacteriorhodopsin crystal structure
at 1.55 Å resolution



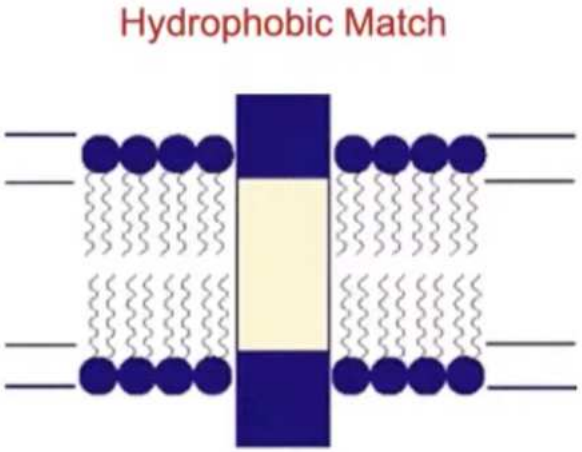
Leucke et al. (1999) *J. Mol. Biol.* 291: 899-911

Mitochondrial ADP/ATP carrier
crystal structure at 2.2 Å resolution

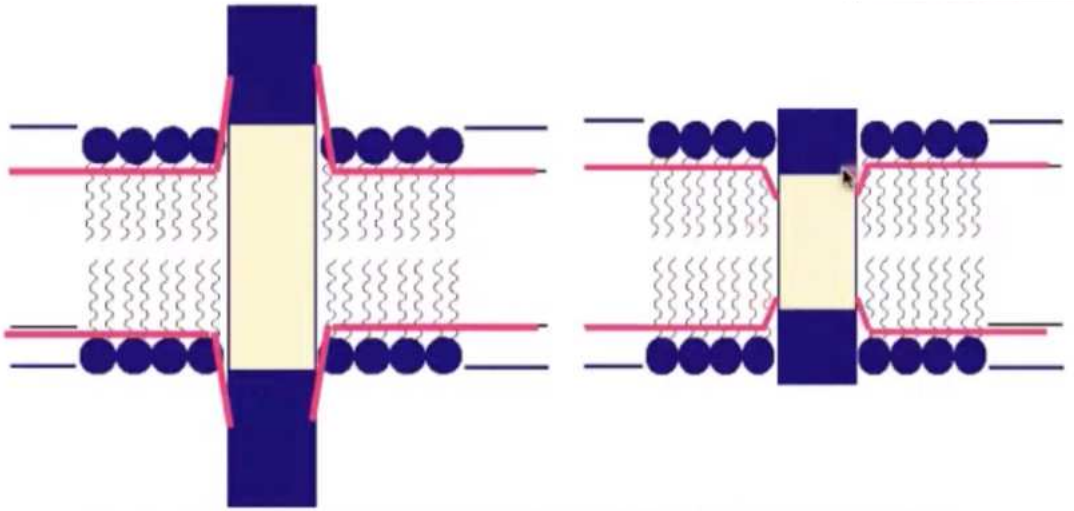


Pebay-Peyroula et al. (2003) *Nature* 426: 39-44

Hydrophobic mismatch



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

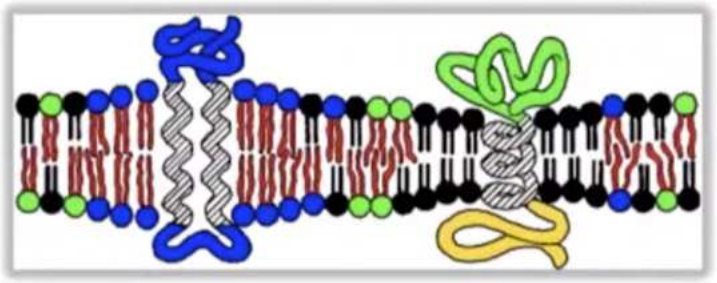


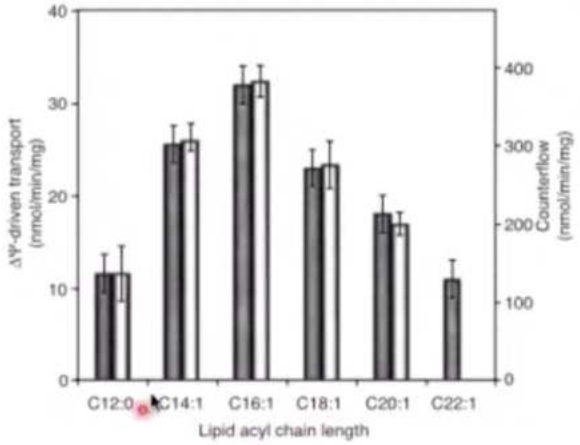
- When these do not match 'HYDROPHOBIC MISMATCH' occurs
- Mismatch is a result of the direct interaction of the transmembrane regions of the protein and the lipid acyl chains
- Mismatch is energetically unfavorable
- Membrane lipids and proteins must adapt to minimize mismatch

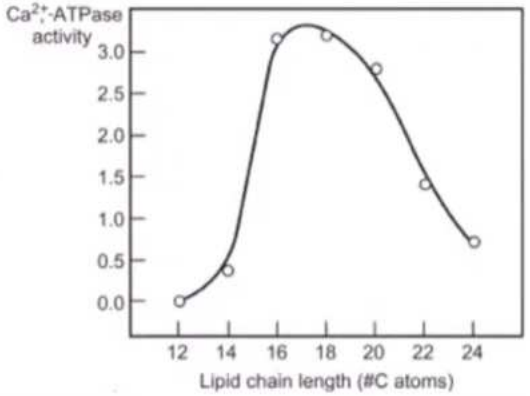
Hydrophobic mismatch

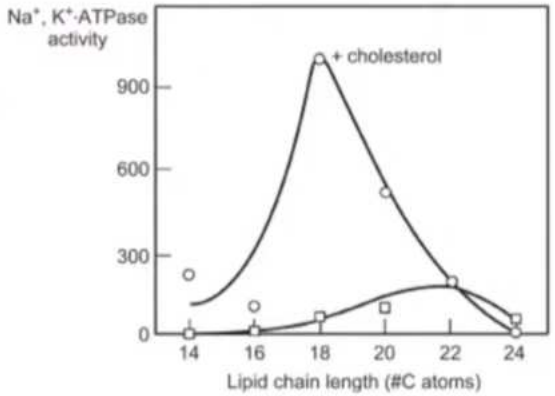
- In eukaryotic cells, there is a gradient of increasing bilayer thickness from ER to Golgi to Plasma membranes. All membrane proteins have to traverse this path.
- Mismatch could play a role in such sorting.
- Eukaryotic membranes are heterogeneous mixtures of a variety of phospholipids, sphingolipids 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







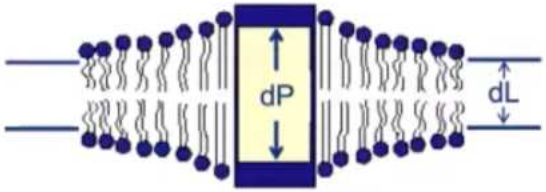


Biochim. Biophys. Acta (2004) 1666: 205-226
Biochemistry (2001) 40: 8842-8851
Biochemistry (2000) 39: 4846-4852

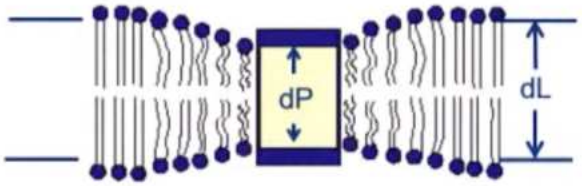
ATPasi: ion pumps

Adapting to mismatch

Lipid responses to mismatch

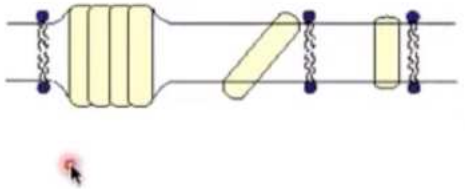


Long Proteins increase the Tm of short bilayers

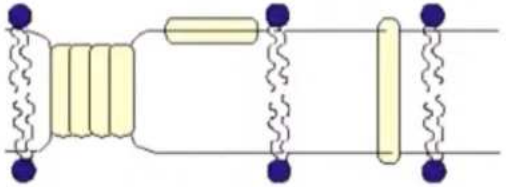


Short Proteins decrease the Tm of long bilayers

Protein responses to mismatch



Aggregation Helix Tilt Conformational Change

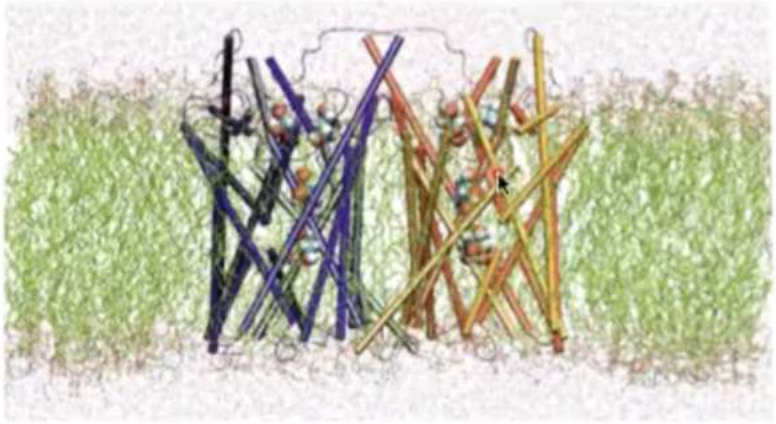


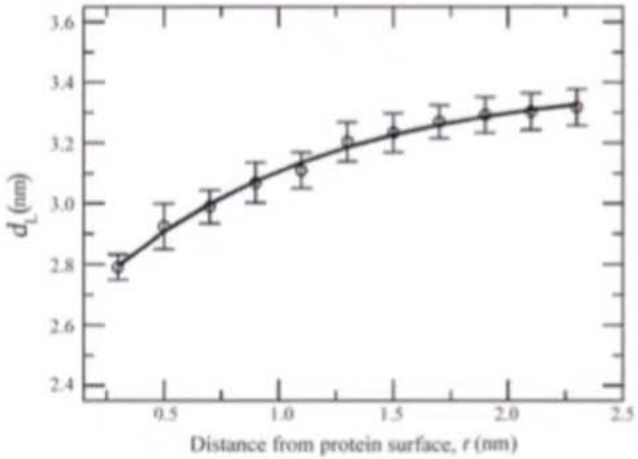
Aggregation Surface Orientation Conformational Change

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

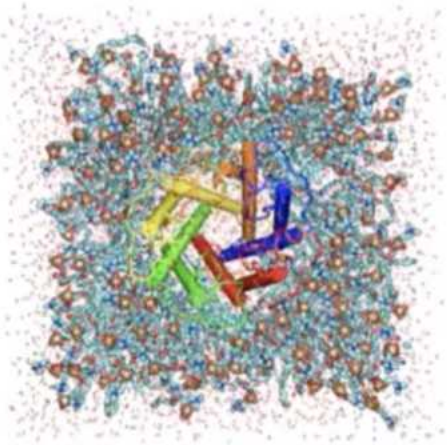
Adapting to mismatch: thinning

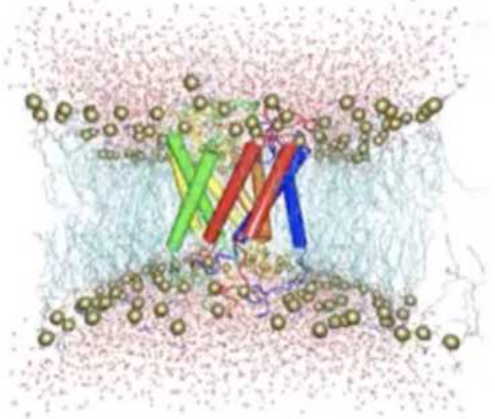
Aquaporin in fluid POPC bilayer





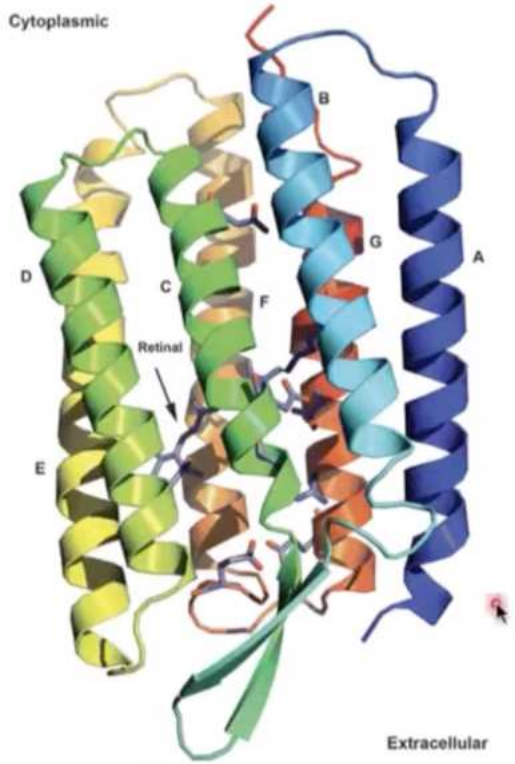
Mechanosensitive channel, MscL



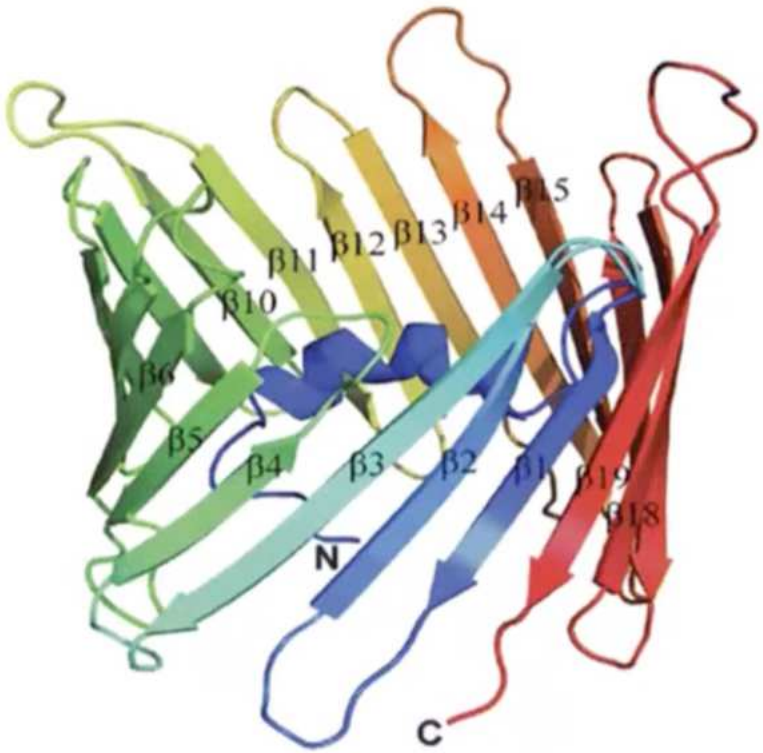


Life-As a matter of fat: lipids in membrane biophysics perspective by Ole G. Mouritsen & Luis A. Bagatolli, 2nd edn. 2015, Springer
Gullingsrud et al. (2001) *Biophys. J.* 80: 2074-2081

Membrane proteins



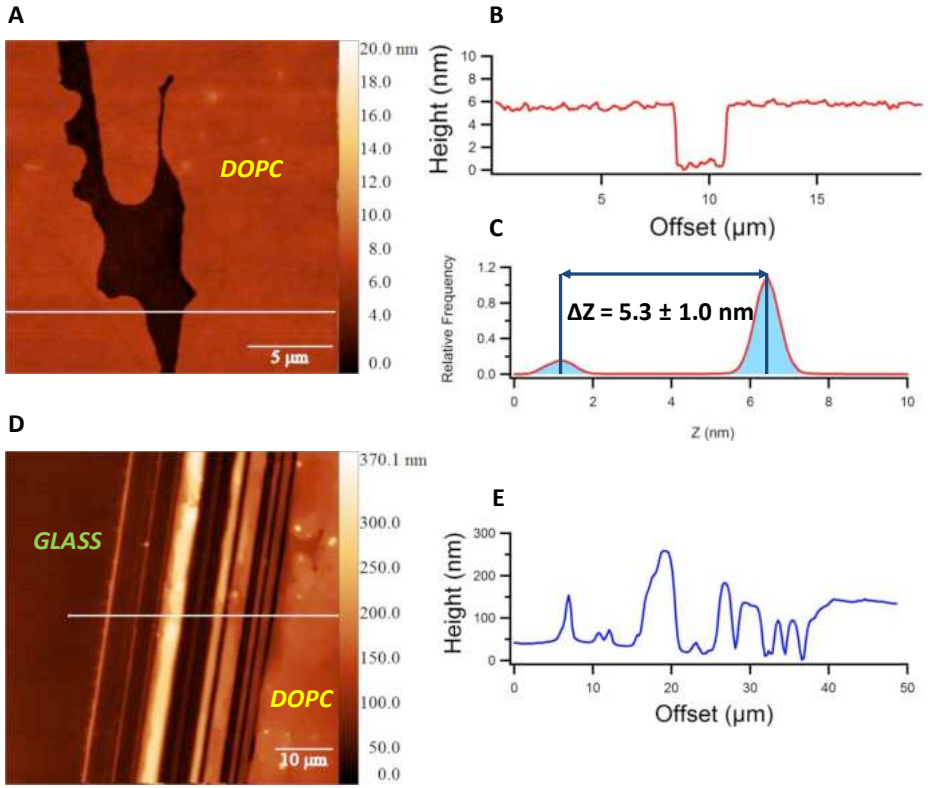
Bacteriorhodopsin
α-helical bundle



Mitochondrial porin, VDAC
β-barrel

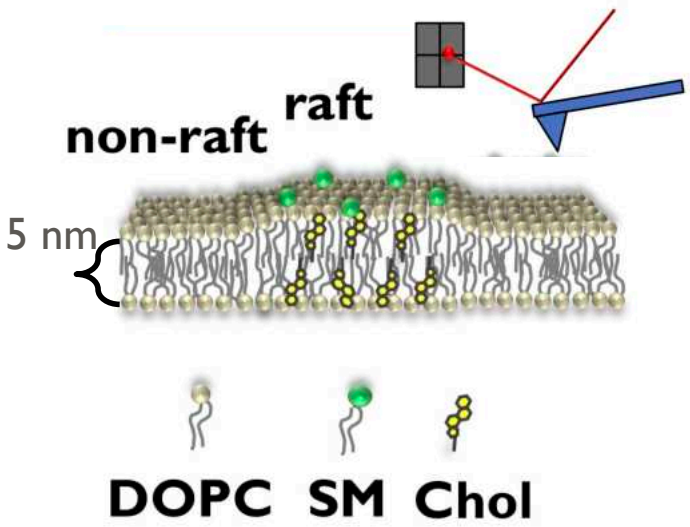
Mary Luckey, Chapter 1:Introduction to the Structural Biology of Membrane Proteins , in *Computational Biophysics of Membrane Proteins*, 2016, pp. 1-18

Supported lipid bilayers: Atomic Force Microscopy (AFM)

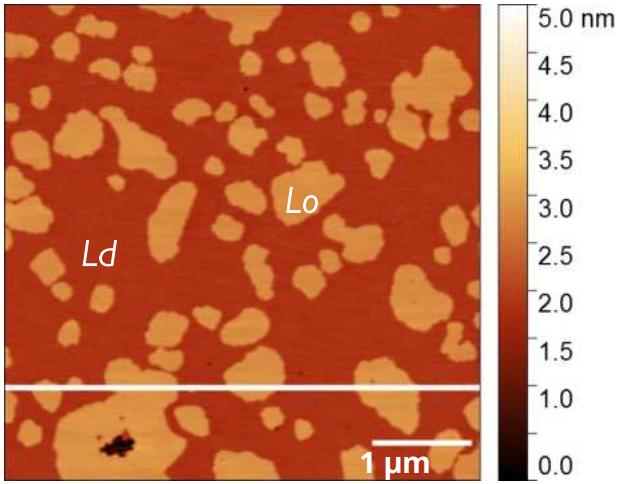


(A) AFM topography of flat lipid membrane with a central discontinuity which allows to measure the height of the lipid bilayer. (B) Height profile. (C) One DOPC bilayer is characterized by an average height of 5.3 ± 1.0 nm. (D) AFM topography of the scratch made by a scalpel. On the right side, a DOPC membrane, on the left side glass can be observed. (E) Height profile indicates the presence of a membrane formed by 30-40 lipid bilayers. AFM measurements were performed in tapping-mode in air at room temperature.

Supported lipid bilayers: Atomic Force Microscopy (AFM)

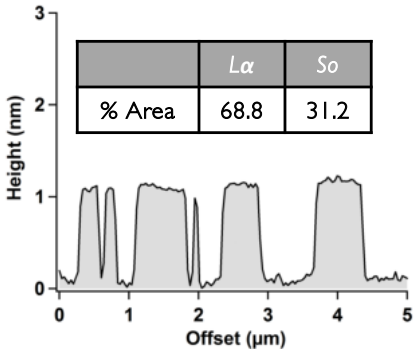


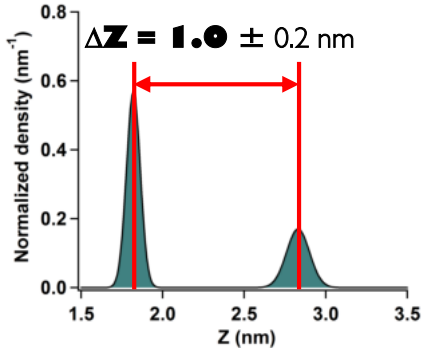
DOPC
1,2-dioleoyl-sn-glycero-3-phosphocholine
SM
Sphingomyelin
Chol
Cholesterol



AFM imaging in dynamic AC-mode in liquid environment

	Lα	So
% Area	68.8	31.2

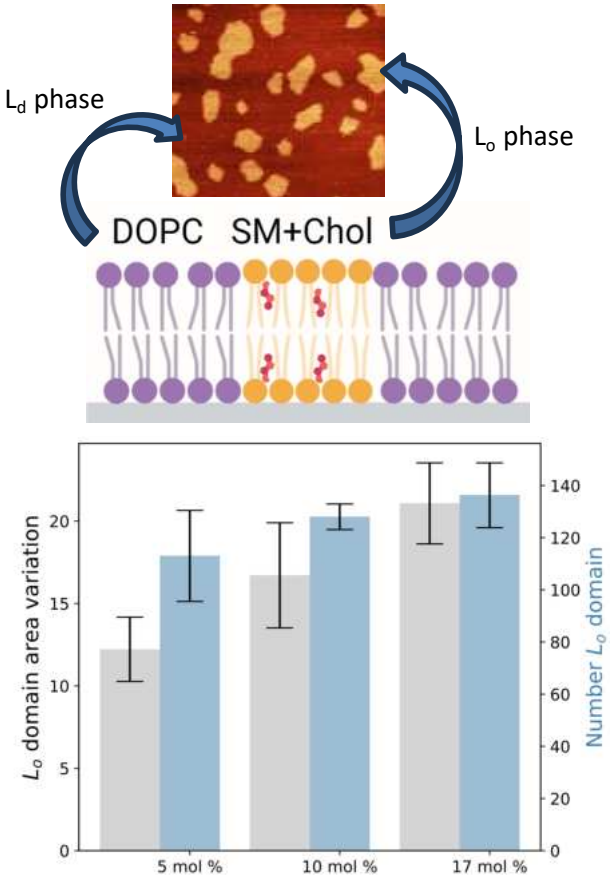


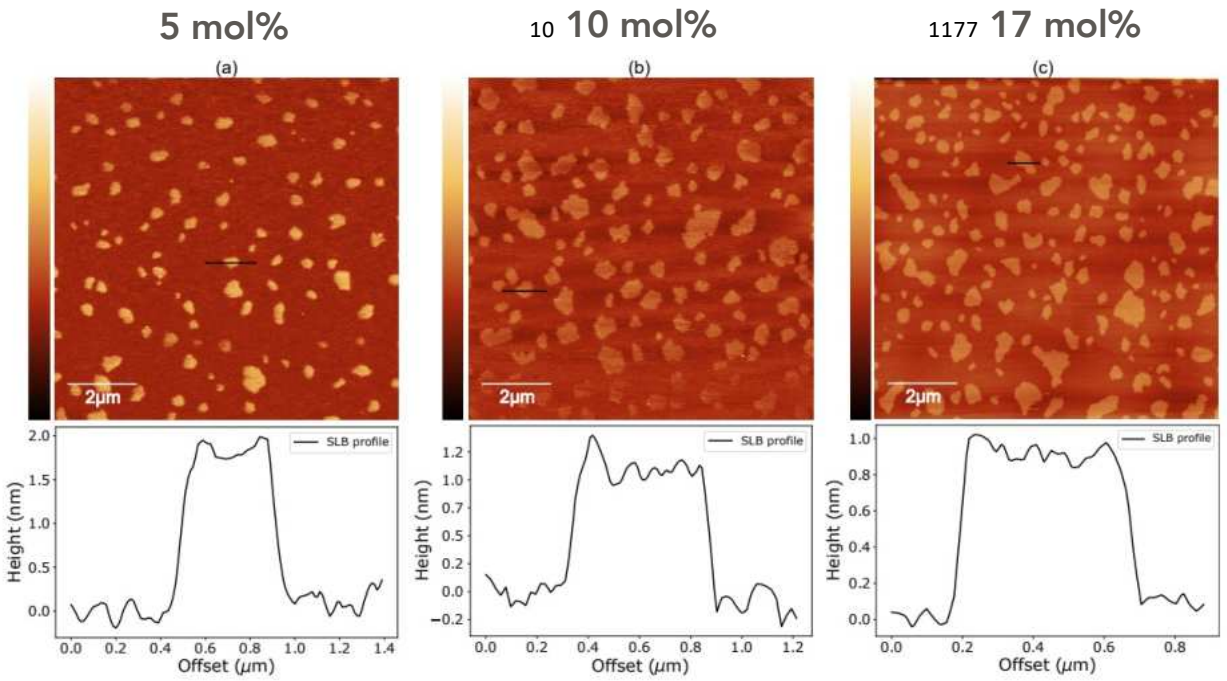


surface roughness: Lα=0.16 ± 0.01 nm, Lo=0.14 ± 0.01 nm

GOAL: study the role of cholesterol in regulating membrane fluidity/rigidity and in turn the mechanisms of cell uptake of molecules/nanoparticles

Let's increase cholesterol
17% chol + DOPC/SM





Rafts-covered area scales with chol. Height difference decreases.
Both preferred co-localization with SM and chol-condensing effect on Ld phase

'cholesterol-condensing effect' on phospholipids thickening of the *Ld* phase and a reduced height difference with the *Lo* domains

C. Paba, et al. JCIS , 652 (2023) 1937–1943. **In collaboration with K. Voitchovsky, Univ. of Durham**

How fluid/stiff are our membranes?

Up to 33% chol, different mimic

DOPC/DSPC/SM

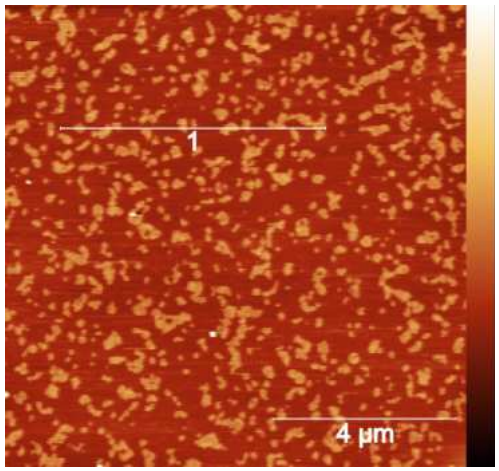
Ld Lo

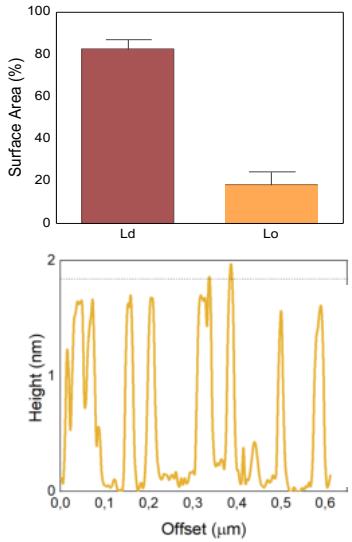
(DOPC:DSPC) 1.75; (DOPC:SM) 2.91

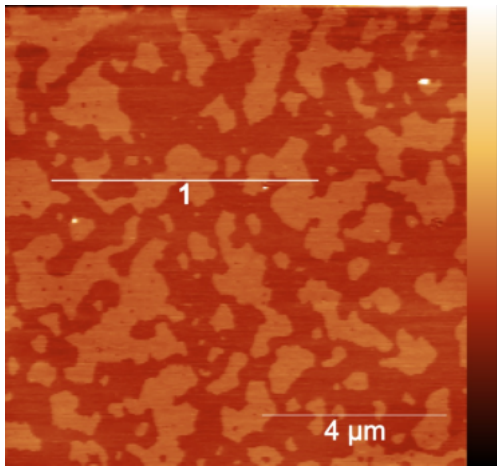
7%

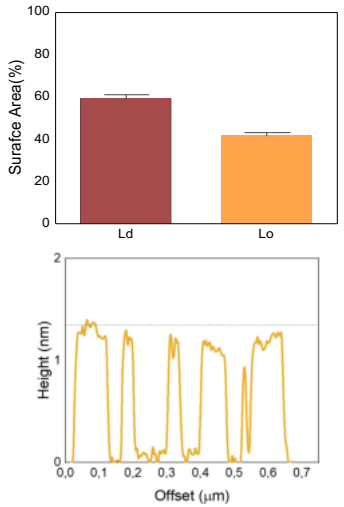
Chol

33%

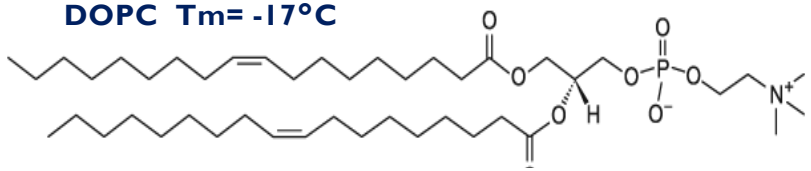




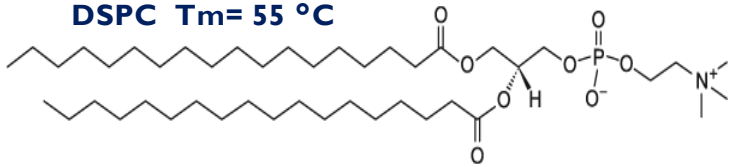




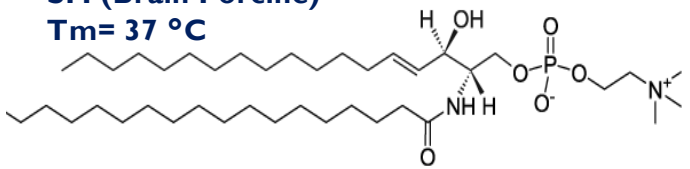
DOPC Tm= -17°C

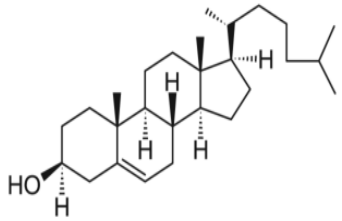


DSPC Tm= 55 °C



SM (Brain Porcine)
Tm= 37 °C

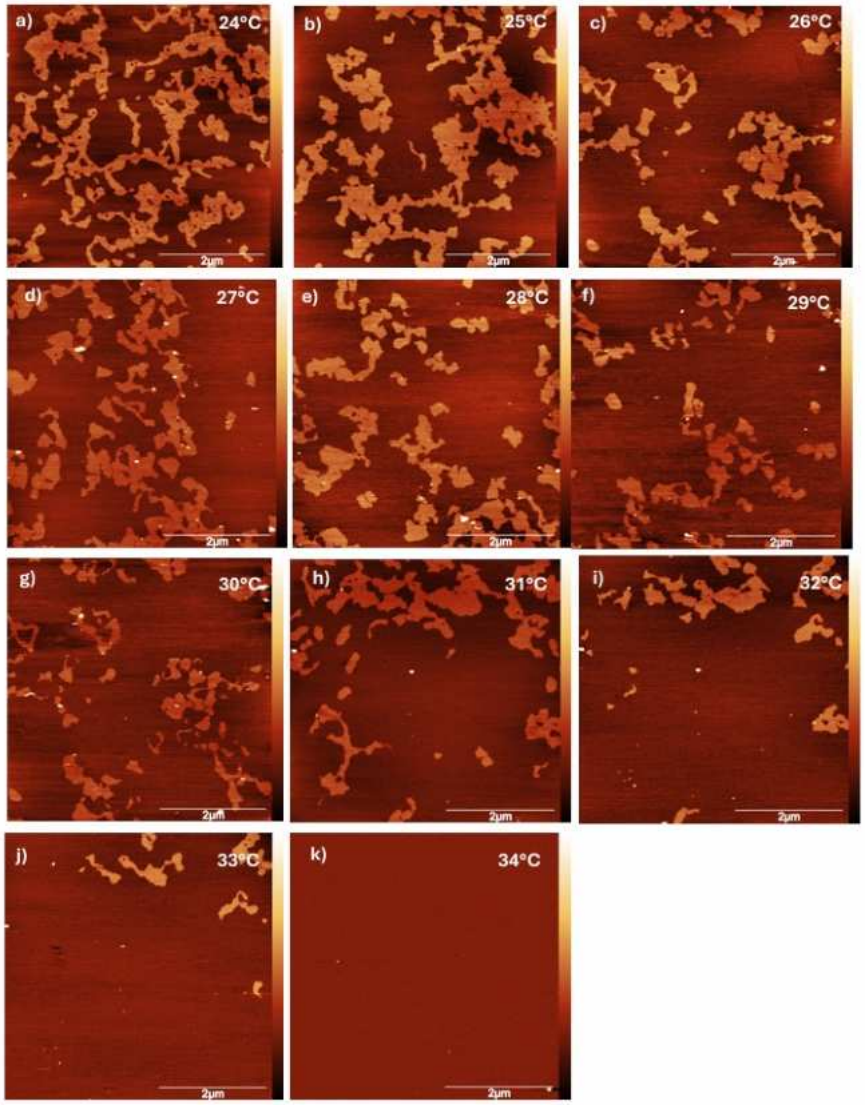




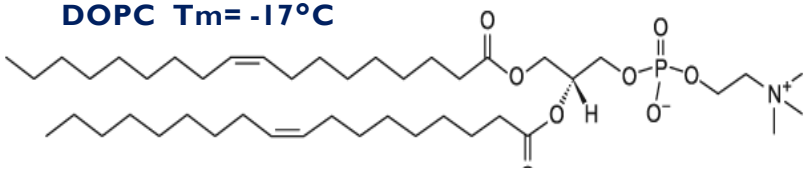
Cholesterol

7% LOW, 33% High

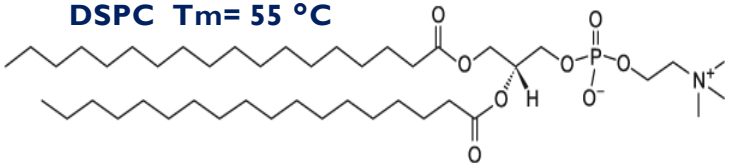
Temperature evolution: 7% chol



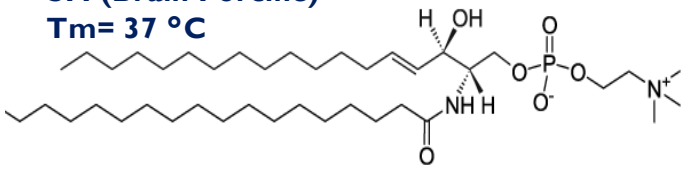
DOPC Tm= -17°C

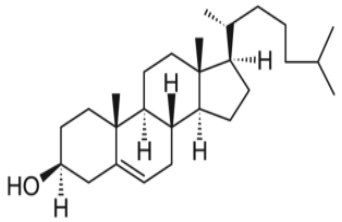


DSPC Tm= 55 °C



SM (Brain Porcine)
Tm= 37 °C

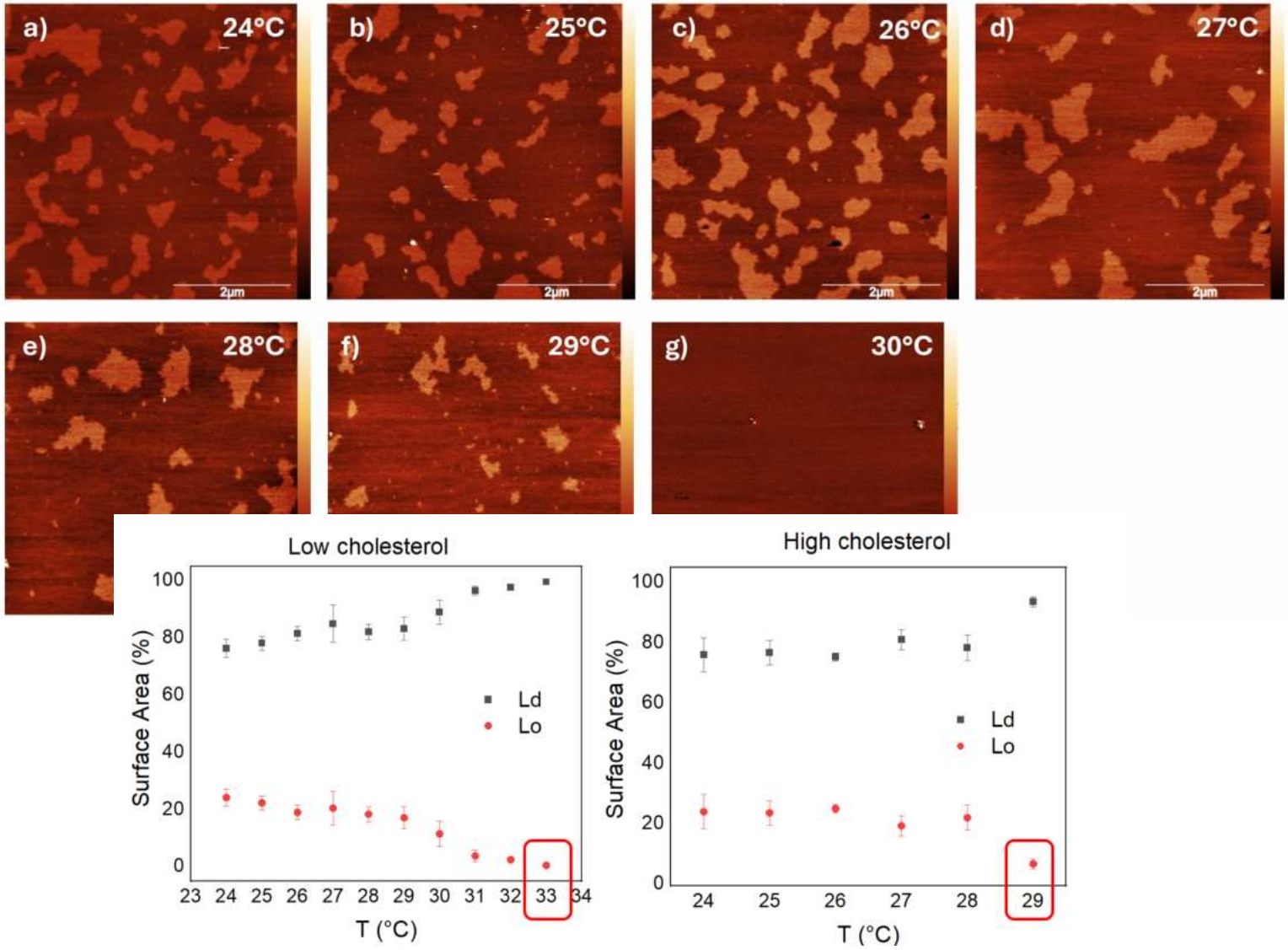




Cholesterol

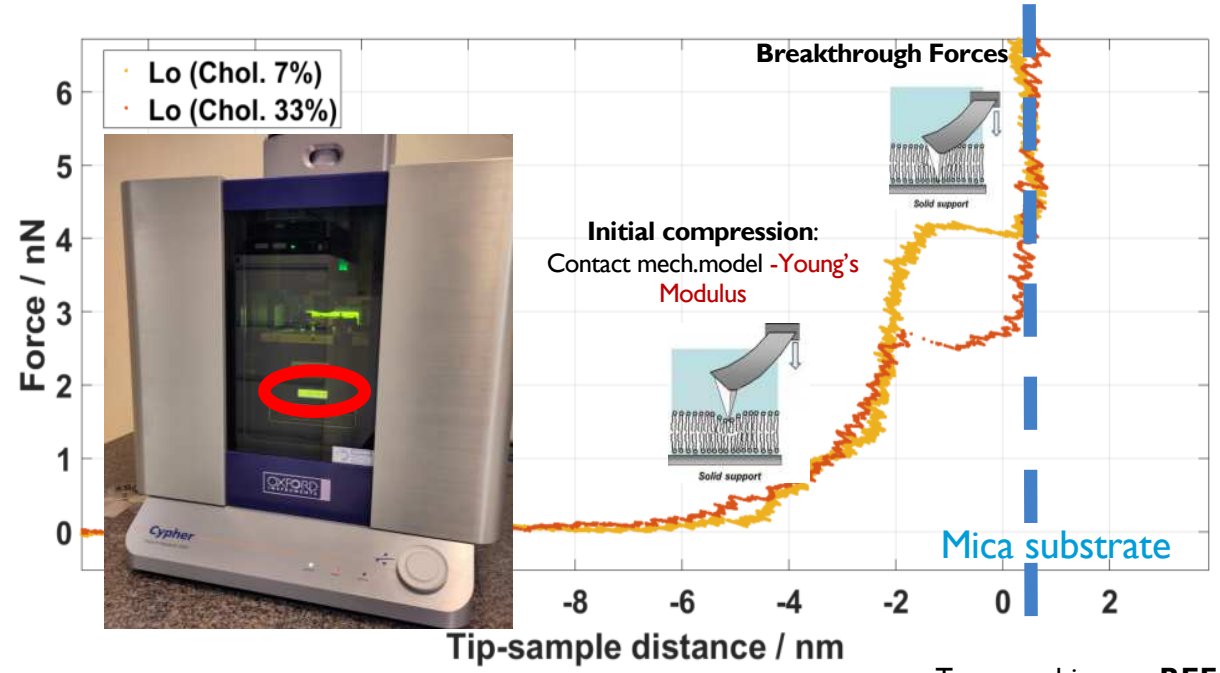
7% LOW, 33% High

Temperature evolution: 33% chol



Evaluation of Membrane Stiffness:

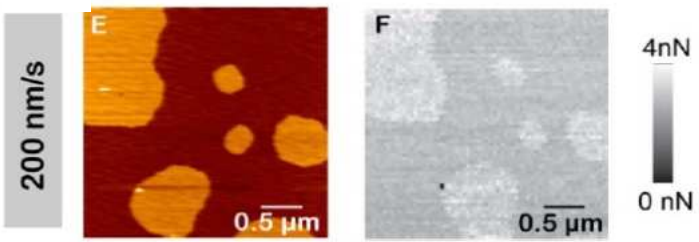
AFM nanomechanics



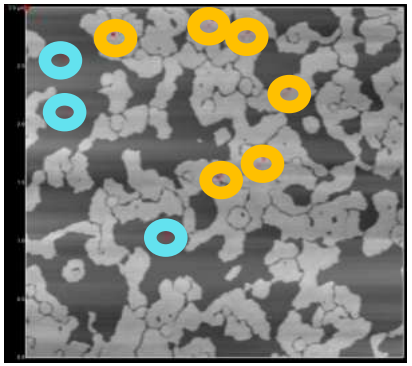
Optimization:

- Tip sharpness
- Tip speed (compressive loading rate)
- Temperature/humidity
- Buffer composition/ionic strenght

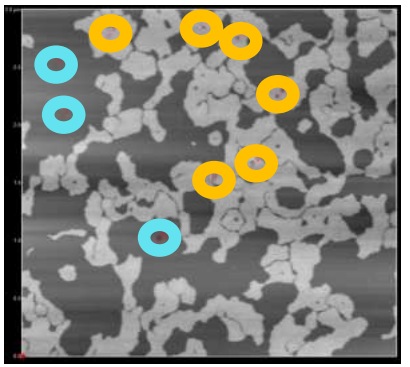
Topography Breakthrough



Topographic map **BEFORE**



Topographic map **AFTER**



Sullan et al. Biophysical Journal (2010).

Force maps:

+: High throughput

- : different sample dynamics and mechanical response Lo/Ld, low/high chol

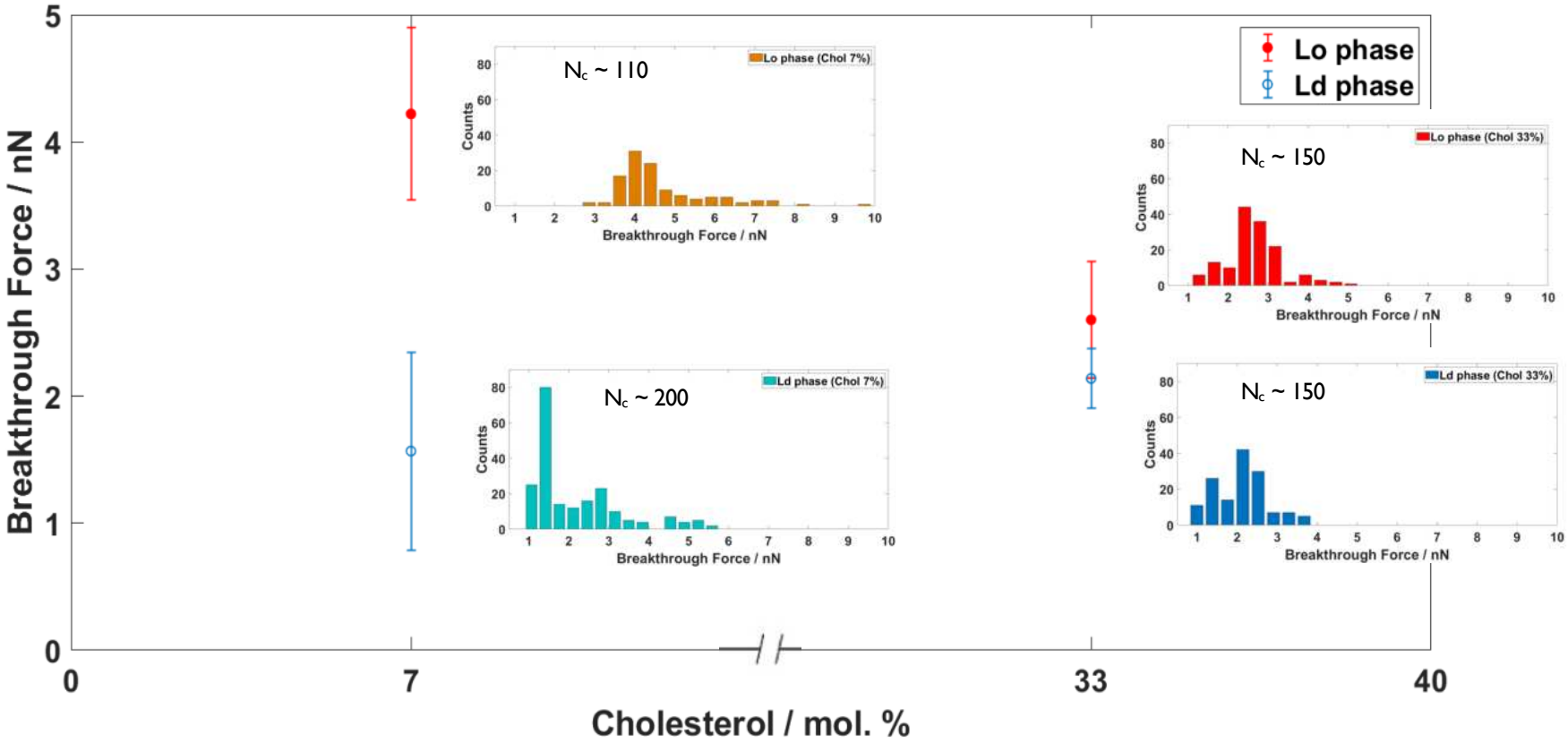
Point&Shoot:

+ : control of rafts dynamics

- : limited data set

Evaluation of Membrane Stiffness:

Breakthrough Forces

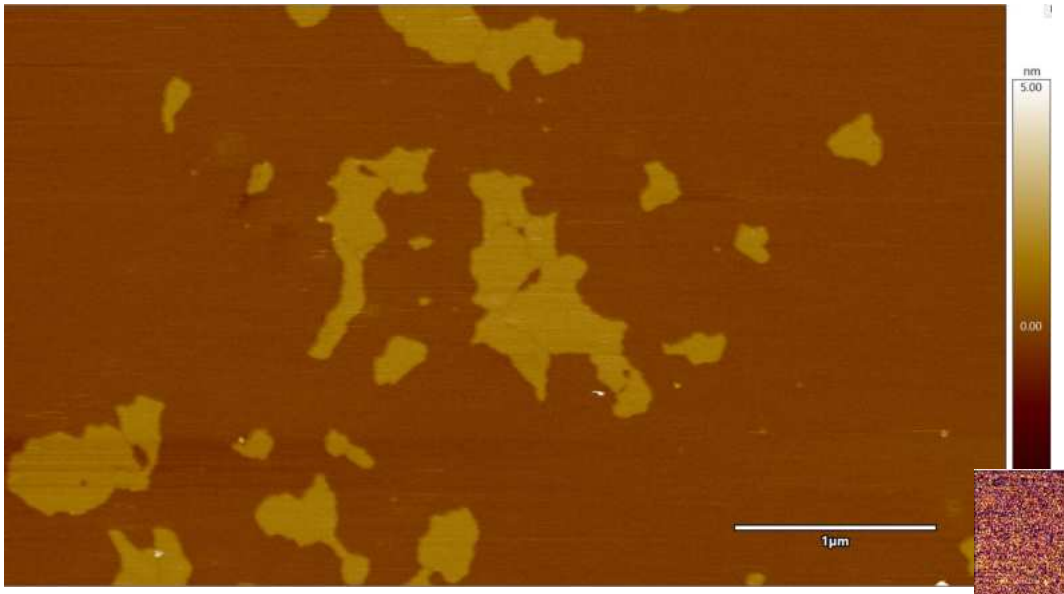


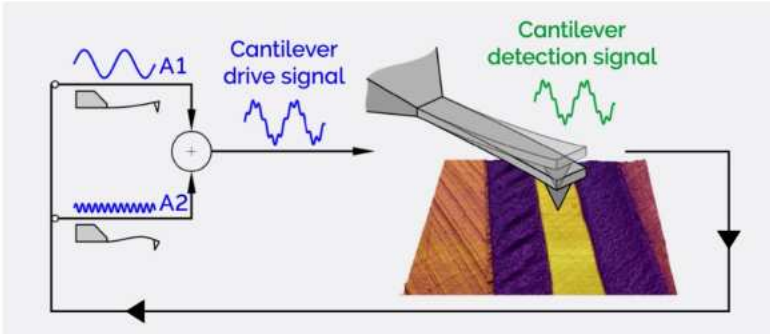
	Median +/- Mad
Lo phase (7%)	4.22 ± 0.68 nN
Ld phase (7%)	1.57 ± 0.78 nN
Lo phase (33%)	2.60 ± 0.46 nN
Ld phase (33%)	2.14 ± 0.24 nN

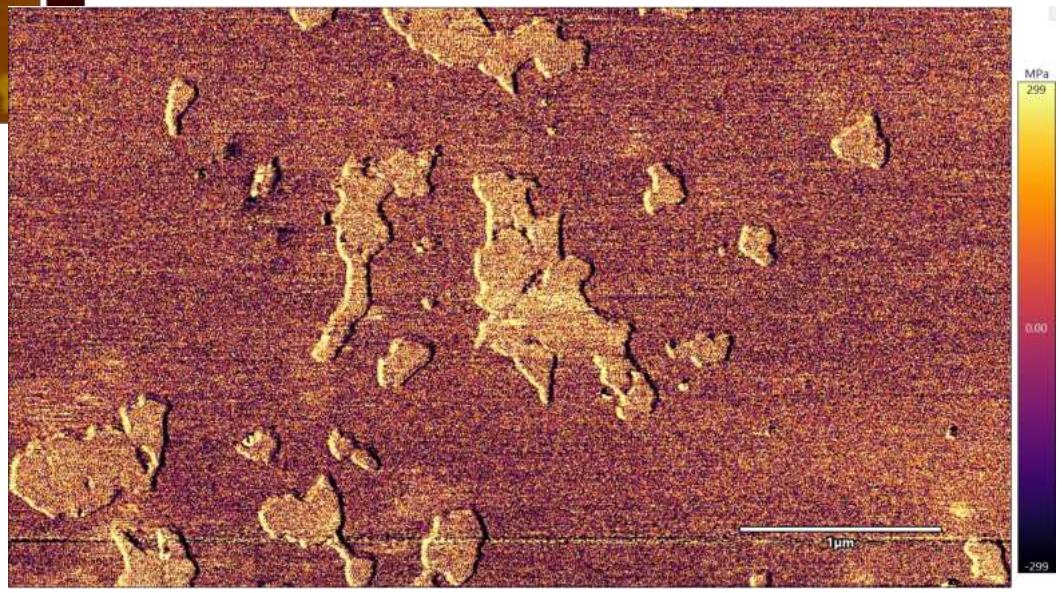
Results in agrrement with FRAP data

Evaluation of Membrane Stiffness:

AM-FM Viscoelastic mapping mode







As fast as topographic imaging!
Measures elastic and dissipative modules