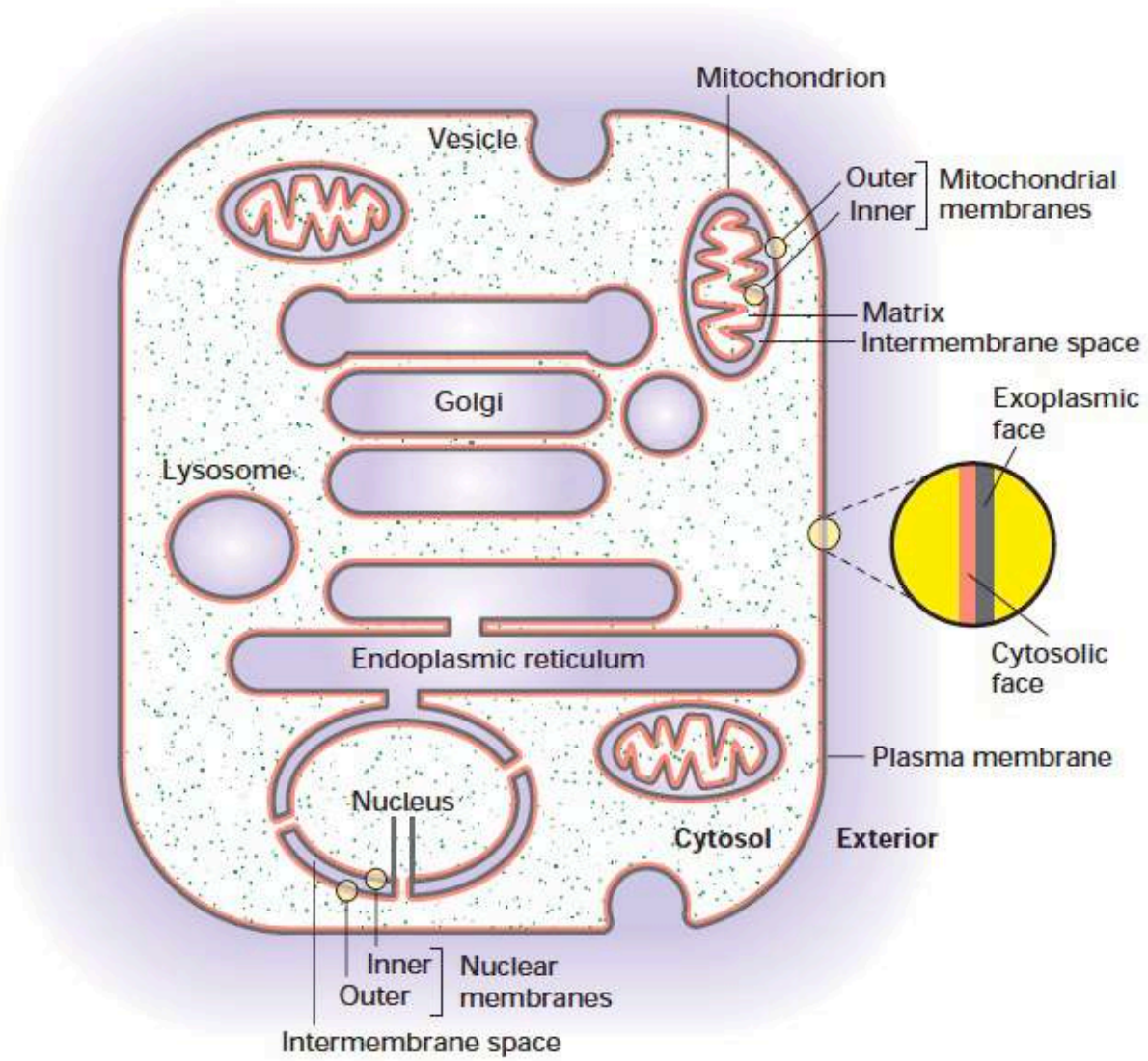
Membranes

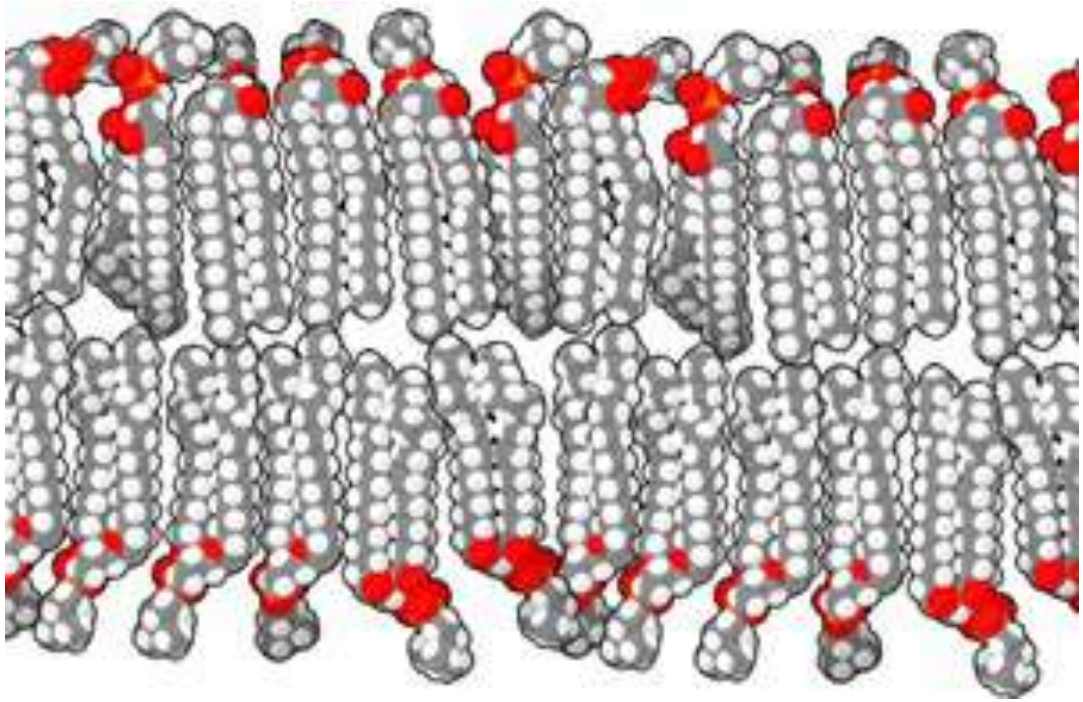
▶ FIGURE 5-4 The faces of cellular membranes. The plasma membrane, a single bilayer membrane, encloses the cell. In this highly schematic representation, internal cytosol (green stipple) and external environment (purple) define the cytosolic (red) and exoplasmic (black) faces of the bilayer. Vesicles 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

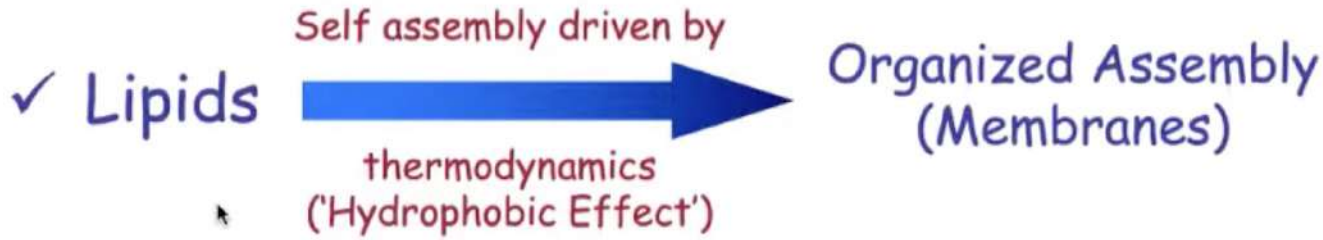
Membranes are made of strongly anisotropic molecules
Strongly anisotropic molecules like to self-organizing.

- 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

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

The Lipid Bilayer Is a Two-dimensional Fluid

Around 1970, researchers first recognized that individual lipid molecules are able to diffuse freely within the plane of a lipid bilayer. The initial demonstration came from studies of synthetic (artificial) lipid bilayers, which can be made in the form of spherical vesicles, called **liposomes** (Figure 10–9); or in the form of planar bilayers formed across a hole in a partition between two aqueous compartments or on a solid support.

Various techniques have been used to measure the motion of individual lipid molecules and their components. One can construct a lipid molecule, for example, with a fluorescent dye or a small gold particle attached to its polar head group and follow the diffusion of even individual molecules in a membrane. Alternatively, one can modify a lipid head group to carry a "spin label," such as a nitroxide

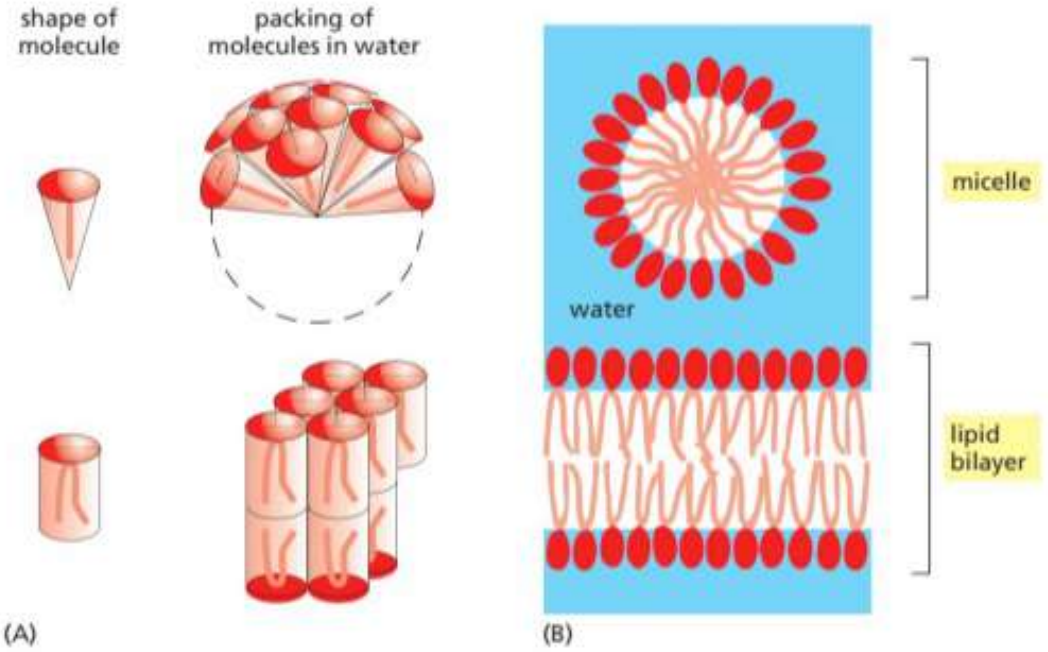
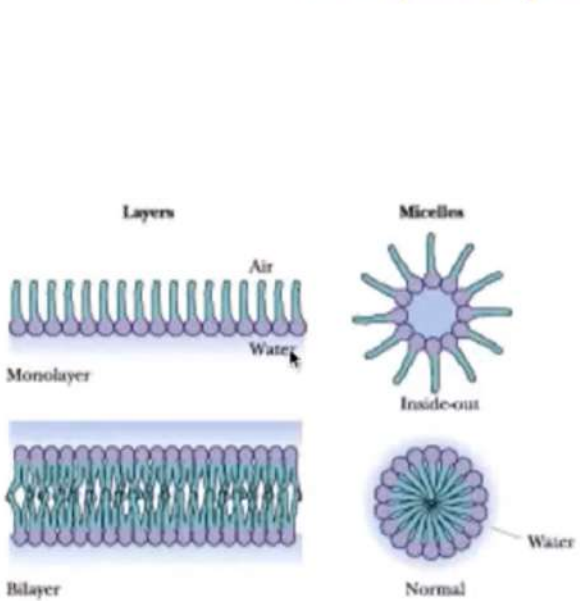
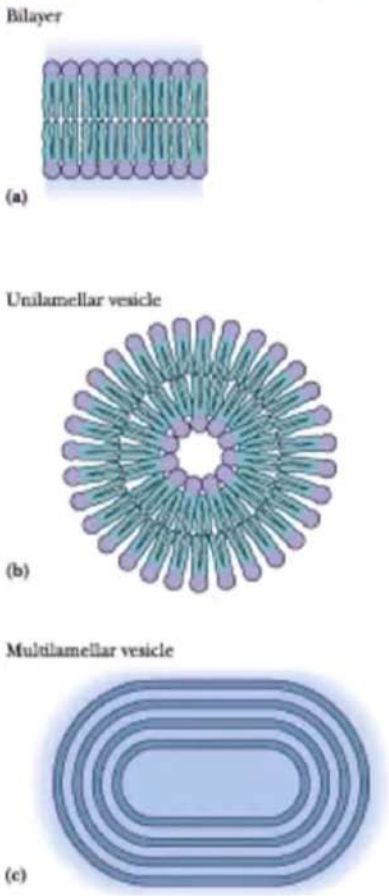


Figure 10–7 Packing arrangements of amphiphilic molecules in an aqueous environment. (A) These molecules spontaneously form micelles or bilayers in water, depending on their shape. Cone-shaped amphiphilic molecules *(above)* form micelles, whereas cylinder-shaped amphiphilic molecules such as phospholipids *(below)* form bilayers. (B) A micelle and a lipid bilayer seen in cross section. Note that micelles of amphiphilic molecules are thought to be much more irregular than drawn here (see Figure 10–26C).

Phospholipid Supramolecular Assemblies





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: 1/3

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

Milestones in membrane research

Gorter and Grendel's Langmuir Trough for Monolayer Experiments Which Led to the First Lipid Bilayer Model

Killian and van Meer (2001) EMBO Reports 2: 91-95

ON BIMOLECULAR LAYERS OF LIPOIDS ON THE CHROMOCYTES OF THE BLOOD.

By E. GORTER, M.D., and F. GRENDEL.

(*From the Laboratory of Pediatrics of the University of Leiden, Leiden, Holland.*)

(Received for publication, December 15, 1924.)

We propose to demonstrate in this paper that the chromocytes of different animals are covered by a layer of lipoids just two molecules thick. If chromocytes are taken from an artery or vein, and are separated from the plasma by several washings with saline solution, and after that extracted with pure acetone in large amounts, one obtains a quantity of lipoids that is exactly sufficient to cover the total surface of the chromocytes in a layer that is two molecules thick. Subsequent extractions with ether or benzene yield only small traces of lipoid substances.

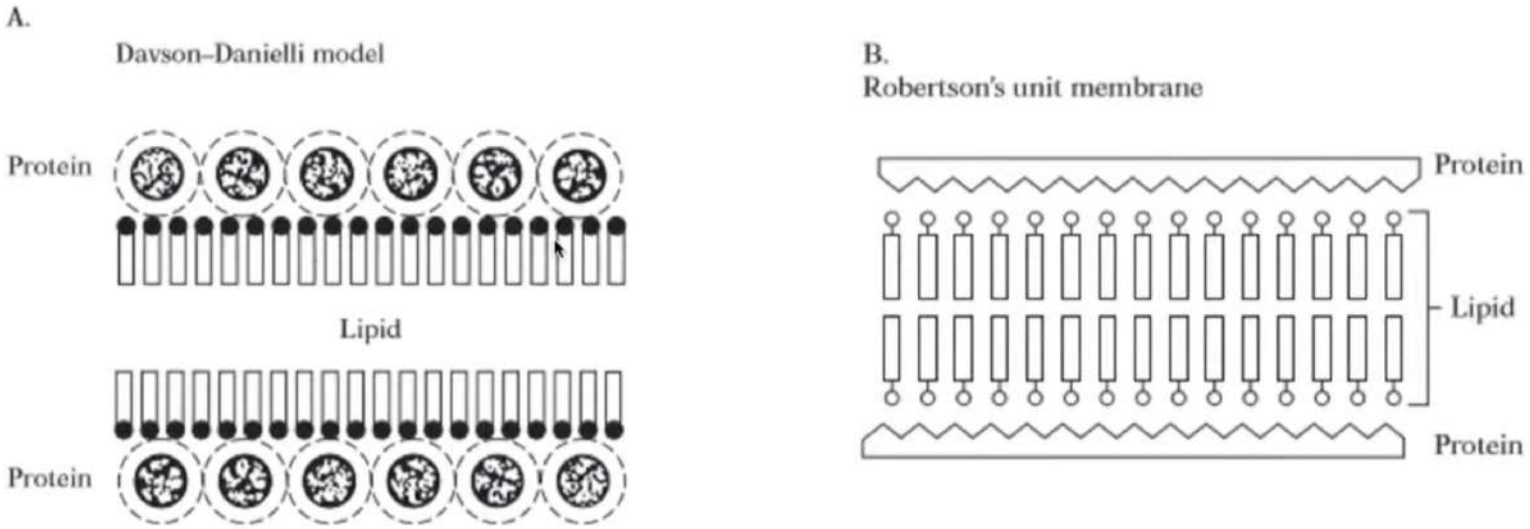
We therefore suppose that every chromocyte is surrounded by a layer of lipoids, of which the polar groups are directed to the inside and to the outside, in much the same way as Bragg (1) supposes the molecules to be orientated in a "crystal" of a fatty acid, and as the molecules of a soap bubble are according to Perrin (2). On the boundary of two phases, one being the watery solution of hemoglobin, and the other the plasma, such an orientation seems *a priori* to be the most probable one. Any other explanation that does not take account of this constant relation between the surface of the chromocytes and the content of lipoids seems very difficult to sustain.

Gorter and Grendel (1925) J. Exp. Med. 41: 439-443

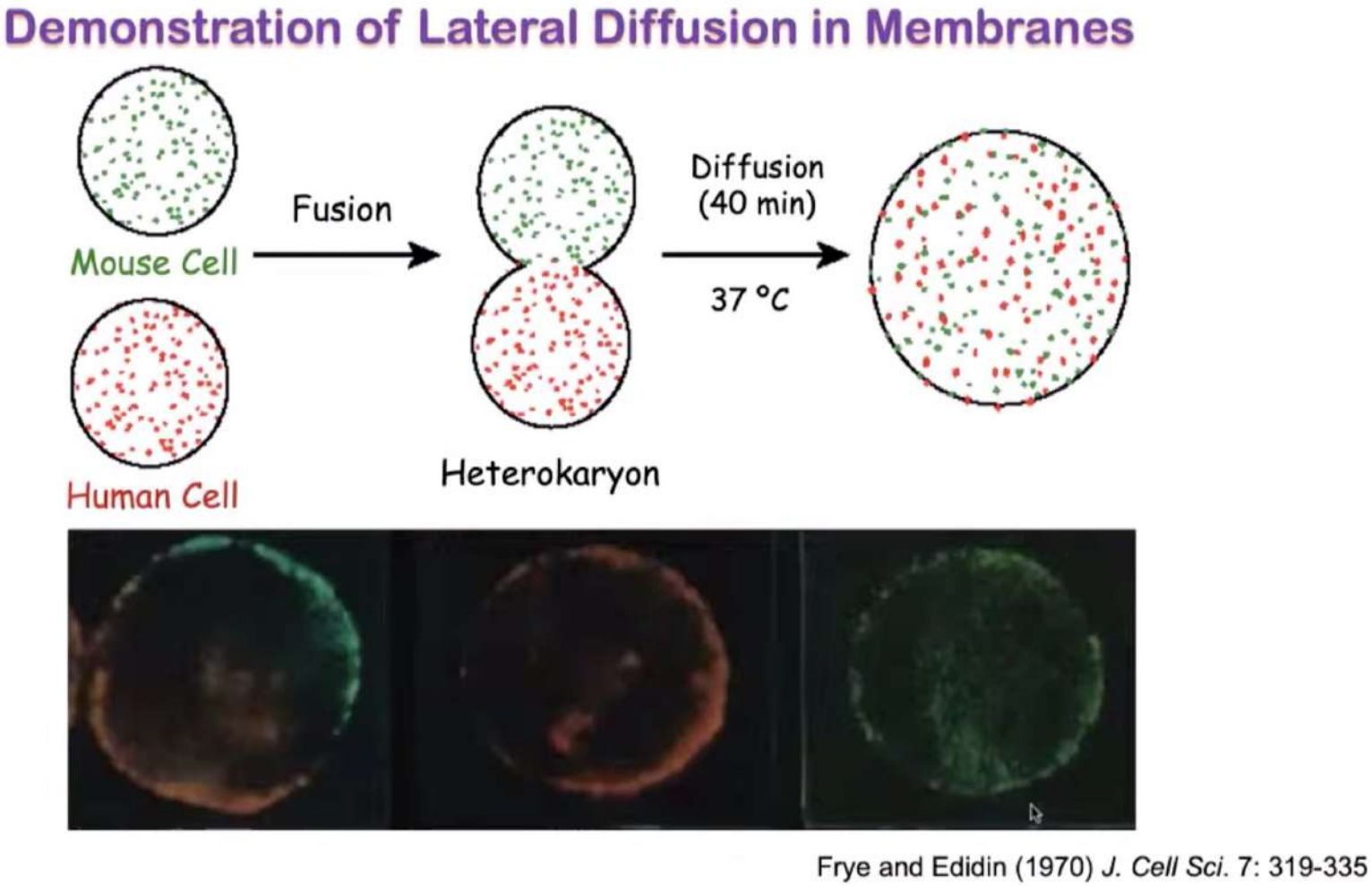
TABLE I.

	Animal.	Amount of blood used for the analysis.	No. of chromocytes per c.mm.	Surface of one chromocyte.	Total surface of the chromocytes (a).	Surface occupied by all the lipoids of the chromocytes (b).	Factor a:b.
		cm.		sq. μ	sq. m.	sq. m.	
1	Dog A	40	8,000,000	98	31.3	62	2
2		10	6,890,000	90	6.2	12.2	2
3	Sheep 1	10	9,900,000	29.8	2.95	6.2	2.1
4		9	9,900,000	29.8	2.65	5.8	2.2
5	Rabbit A	10	5,900,000	92.5	5.46	9.9	1.8
6		10	5,900,000	92.5	5.46	8.8	1.6
7		0.5	5,900,000	92.5	0.27	0.54	2
8	" B	1	6,600,000	74.4	0.49	0.96	2
9		10	6,600,000	74.4	4.9	9.8	2
10		10	6,600,000	74.4	4.9	9.8	2
11	Guinea Pig A	1	5,850,000	89.8	0.52	1.02	2
12		1	5,850,000	89.8	0.52	0.97	1.9
13	Goat 1	1	16,500,000	20.1	0.33	0.66	2
14		1	16,500,000	20.1	0.33	0.69	2.1
15		10	19,300,000	17.8	3.34	6.1	1.8
16		10	19,300,000	17.8	3.34	6.8	2
17		1	19,300,000	17.8	0.33	0.63	1.9
18	Man.	1	4,740,000	99.4	0.47	0.92	2
19		1	4,740,000	99.4	0.47	0.89	1.9

Early models

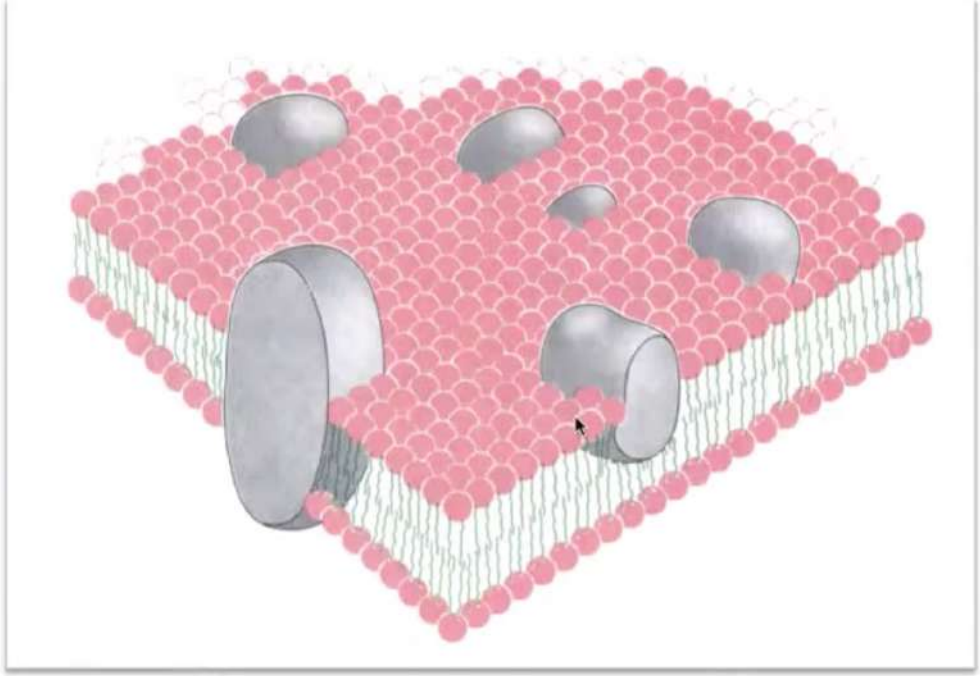


Early models



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

- Lipids are in bilayer form
- Lipids act as solvents for proteins and as permeability barrier and are in a fluid state
- Proteins are like 'icebergs' in a viscous sea of lipids
- Membrane proteins and lipids can freely diffuse laterally, but cannot rotate from one side of the membrane to the other side (flip-flop)
- A small proportion of membrane lipids interact with specific membrane proteins and this could be essential for their function

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

The fluid mosaic model

Limitations of Fluid Mosaic Model

- In some membranes, flip-flop of lipids is fast (ER, growing *E. coli*)
- All membrane proteins are not free to move in the plane of the membrane
- Non-bilayer structure of lipids is possible
- There is evidence of lateral domains in membranes
- Membranes can be crowded

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

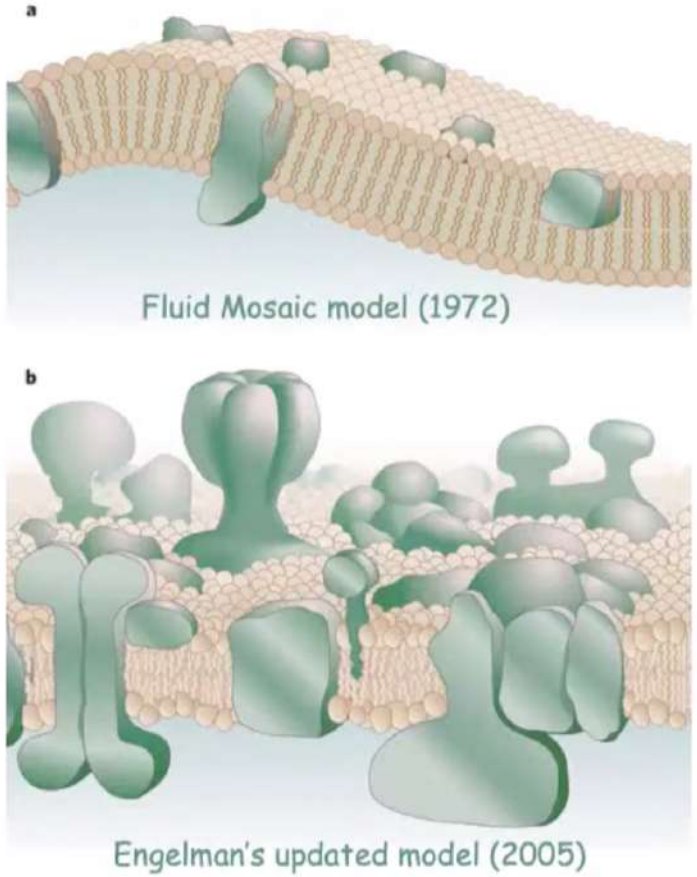
[a] The percentages by weight of membrane preparations from various eukaryotic and prokaryotic sources are given.

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.

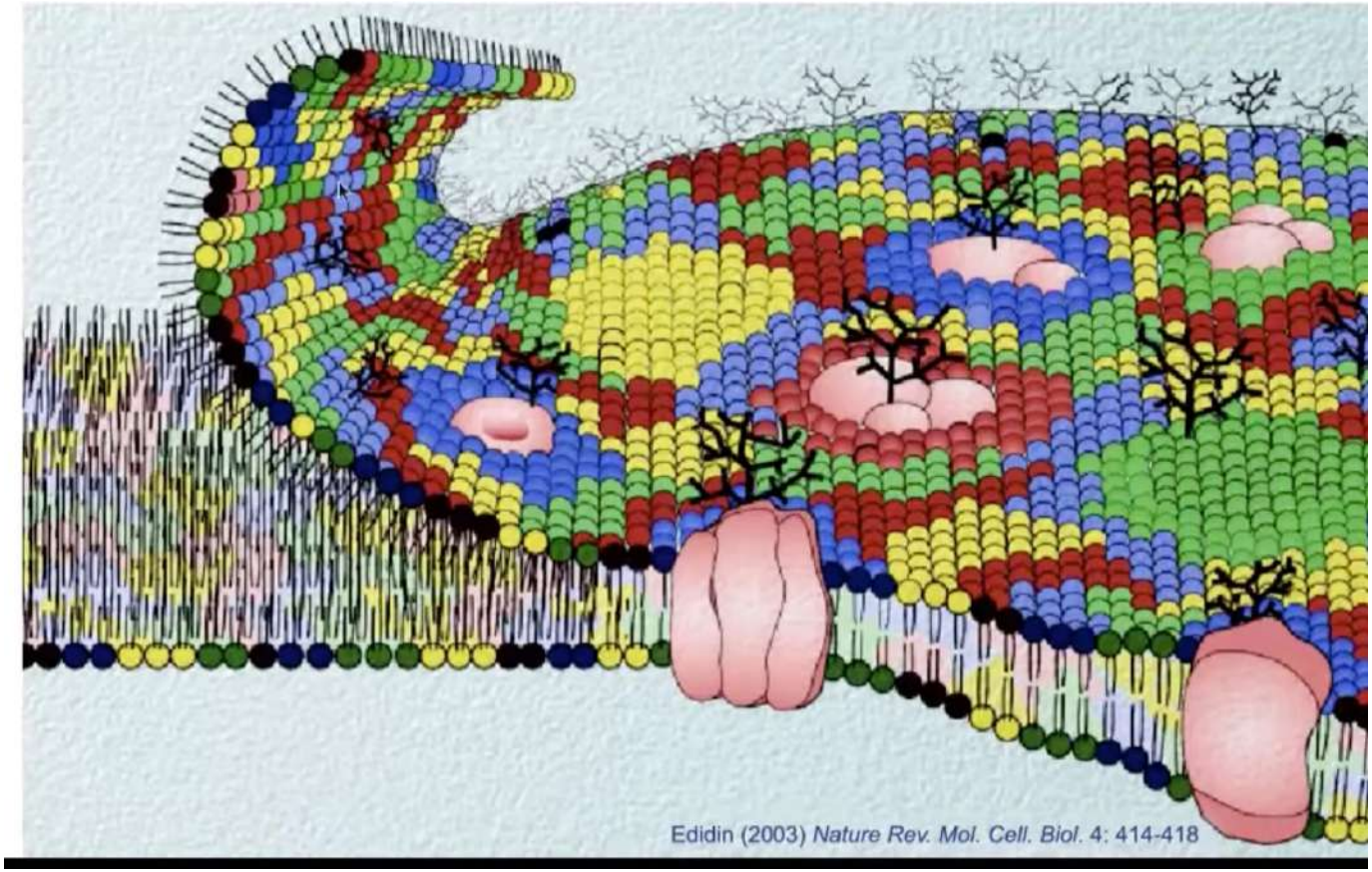
The fluid mosaic model

Membranes are more Mosaic than Fluid !

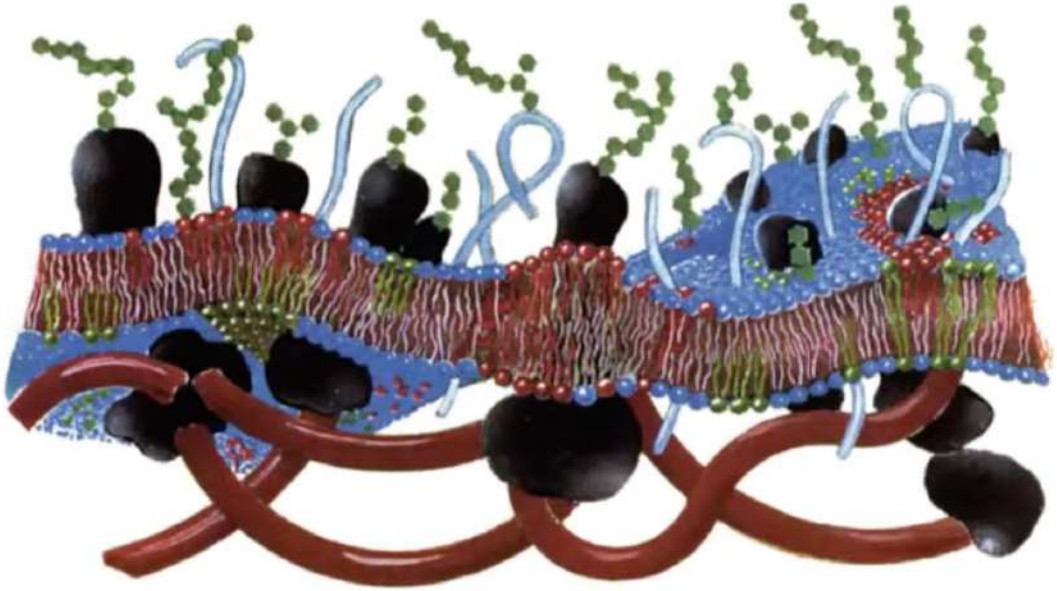


Engelman (2005) *Nature* 438: 578-580

Current Model of Biological Membranes: Organization of Membranes into Domains



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

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.

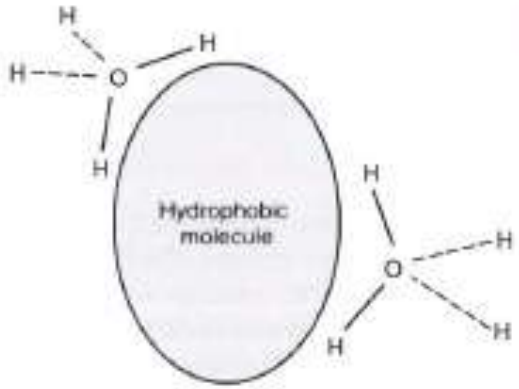
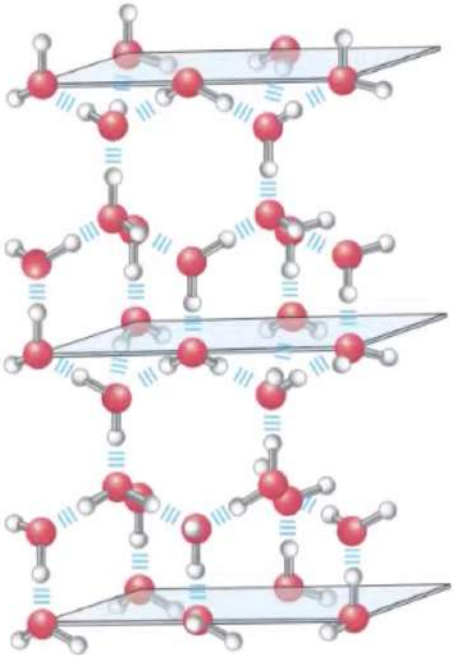
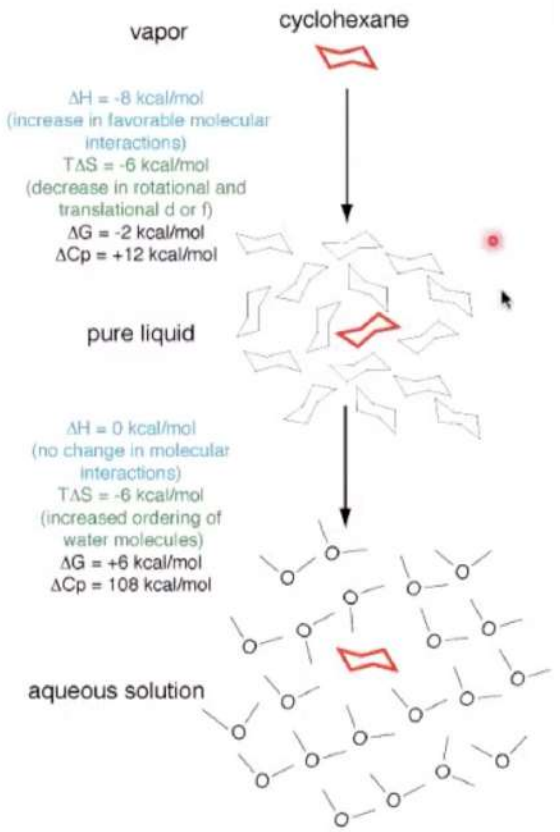


Fig. 2.7 Water molecules adjacent to a hydrophobic molecule suffer restrictions in orientation as they form hydrogen bonds with other water molecules.

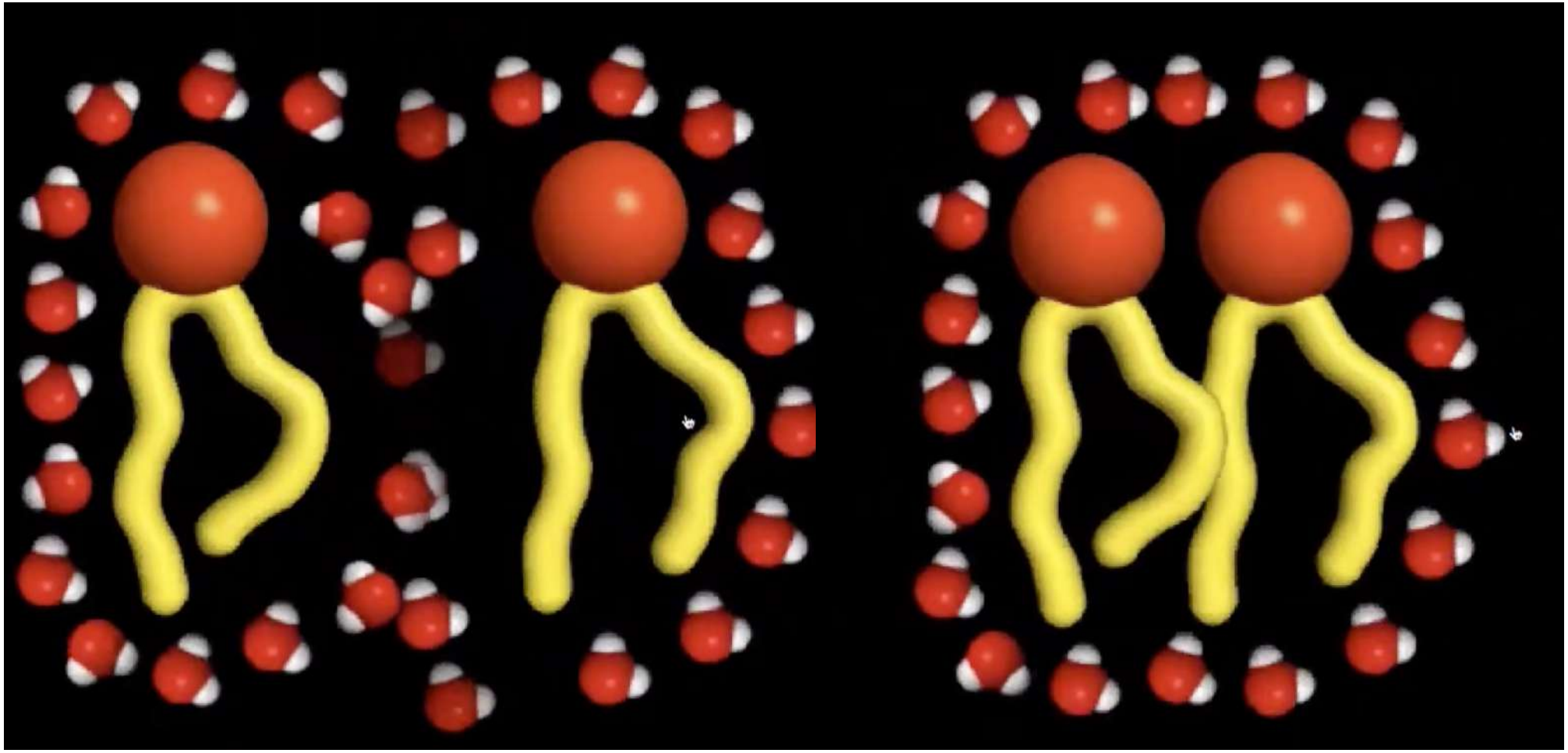
Hydrophobic effect



The forces of attraction between water molecules in the liquid state are unusually high. The melting point, boiling point, heat of vaporization, heat of fusion, and surface tension of water are higher than those of similar substances: The heat of vaporization of water (540 cal/g) is over twice that of methanol and nearly ten times that of chloroform.

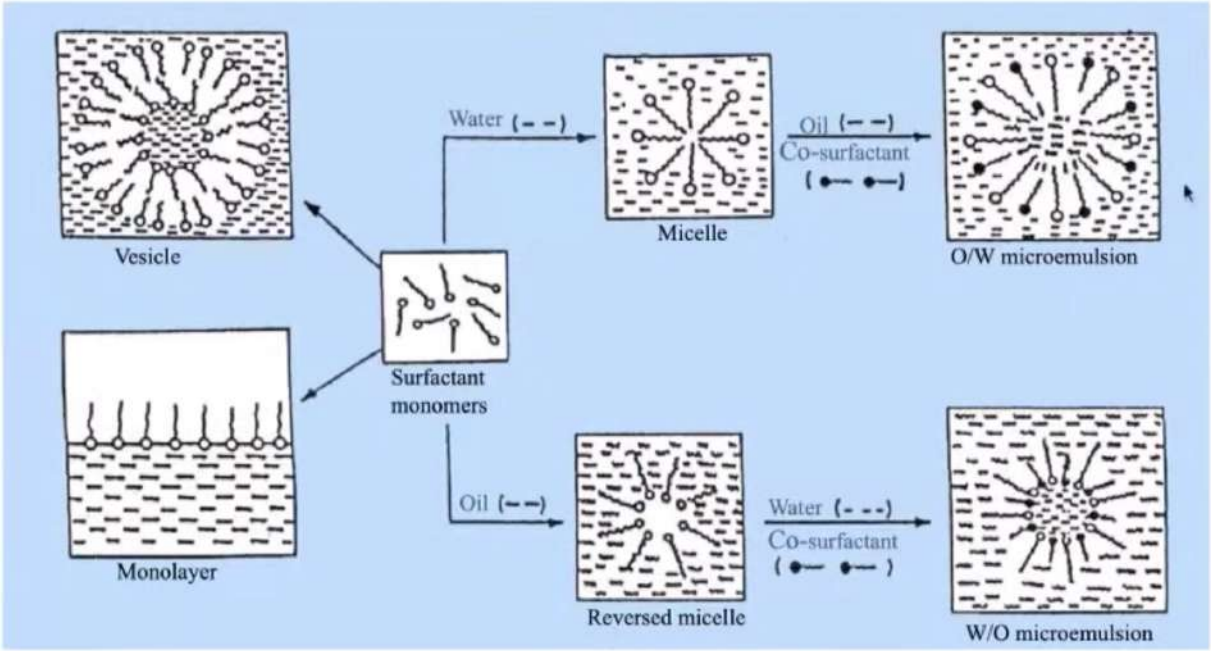


Hydrophobic effect



Hydrophobic effect

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

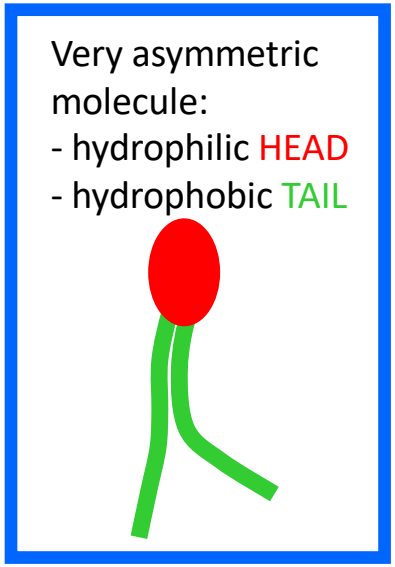


Lipids

Water insoluble compounds (soluble in organic solvents)

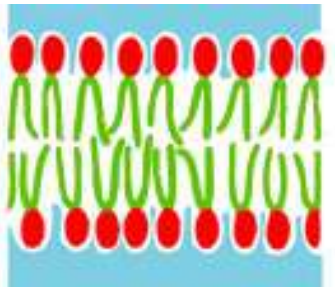
Biological role:

- energy supply
- energy store
- components of cellular and organelle membranes



When in aqueous environment the heads have affinity for the water molecules, while the tails tend to avoid water by sticking together.

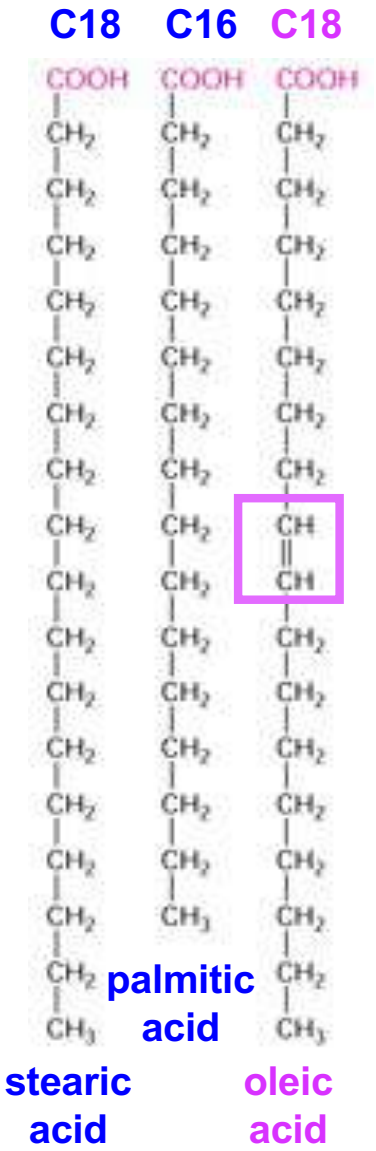
micelle



lipid bilayer

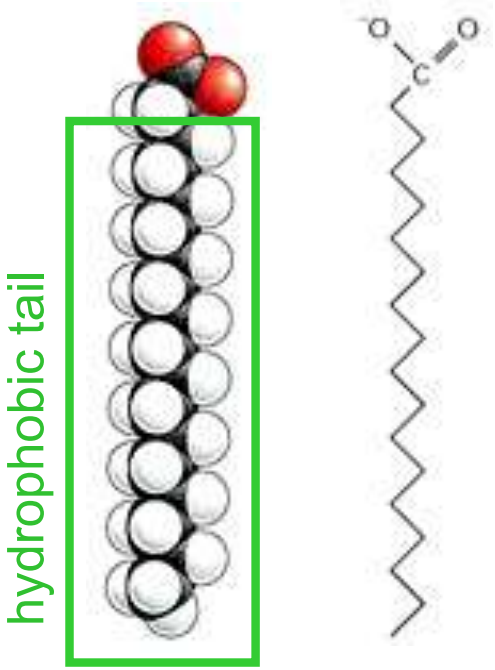
Fatty acids

Carboxylic acids with long hydrocarbon chains (12-24 $-CH_2-$ units)

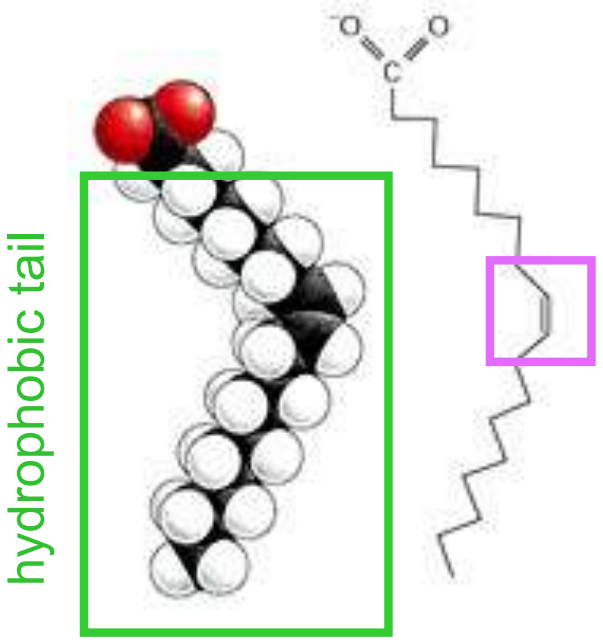


Some have one or more double bonds and are called unsaturated. The double bond is rigid and creates a kink in the chain; the rest of the chain is free to rotate

Stearic acid - saturated



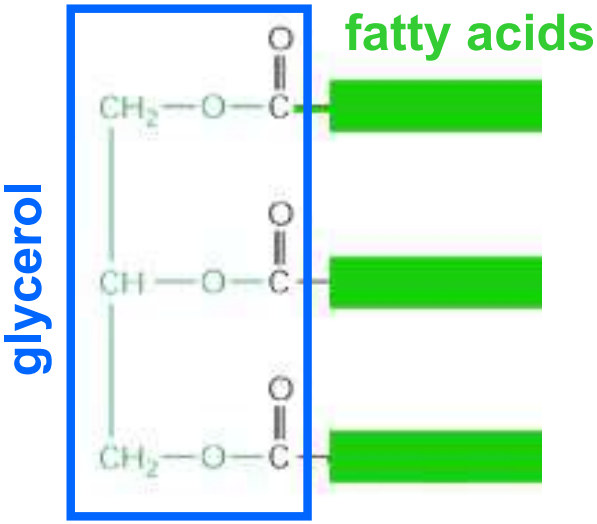
Oleic acid - unsaturated



Fatty acids are used as E storage

To ensure a continuous supply of fuel for oxidative metabolism, animal cells store glucose in the form of glycogen and fatty acids in the form of fats.

A fat molecule is composed of three molecules of fatty acid linked to glycerol: triacylglycerols (*triglycerides*).



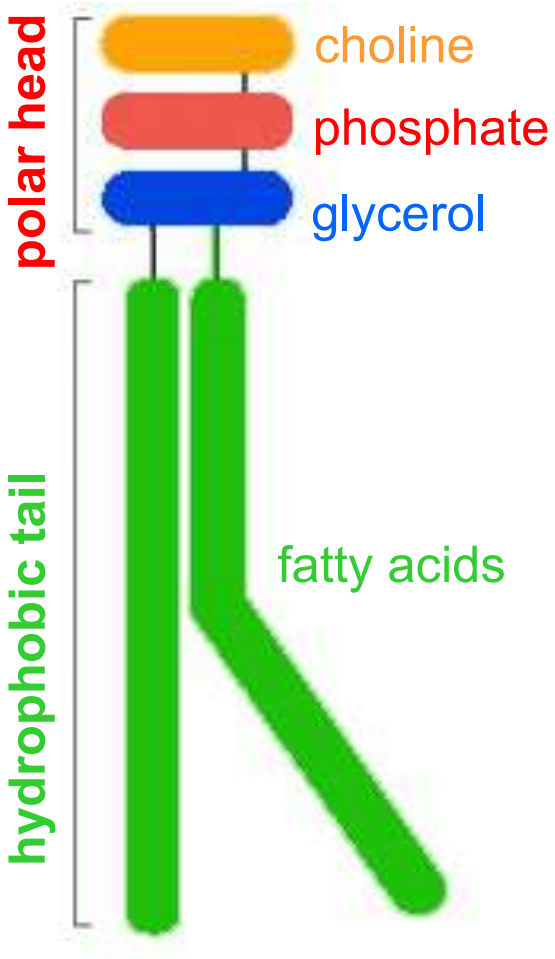
Fat is a far more important storage form than Glycogen (glucose polymer), because its oxidation releases more than six times as much energy.

Triglycerides have no charge and are virtually insoluble in water, coalescing into droplets in the cytosol of adipose cells.

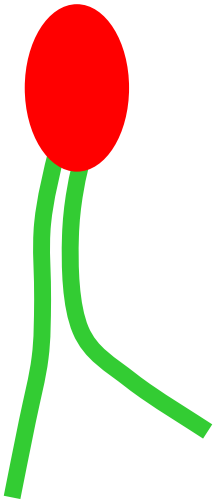
fat droplet

Phospholipids

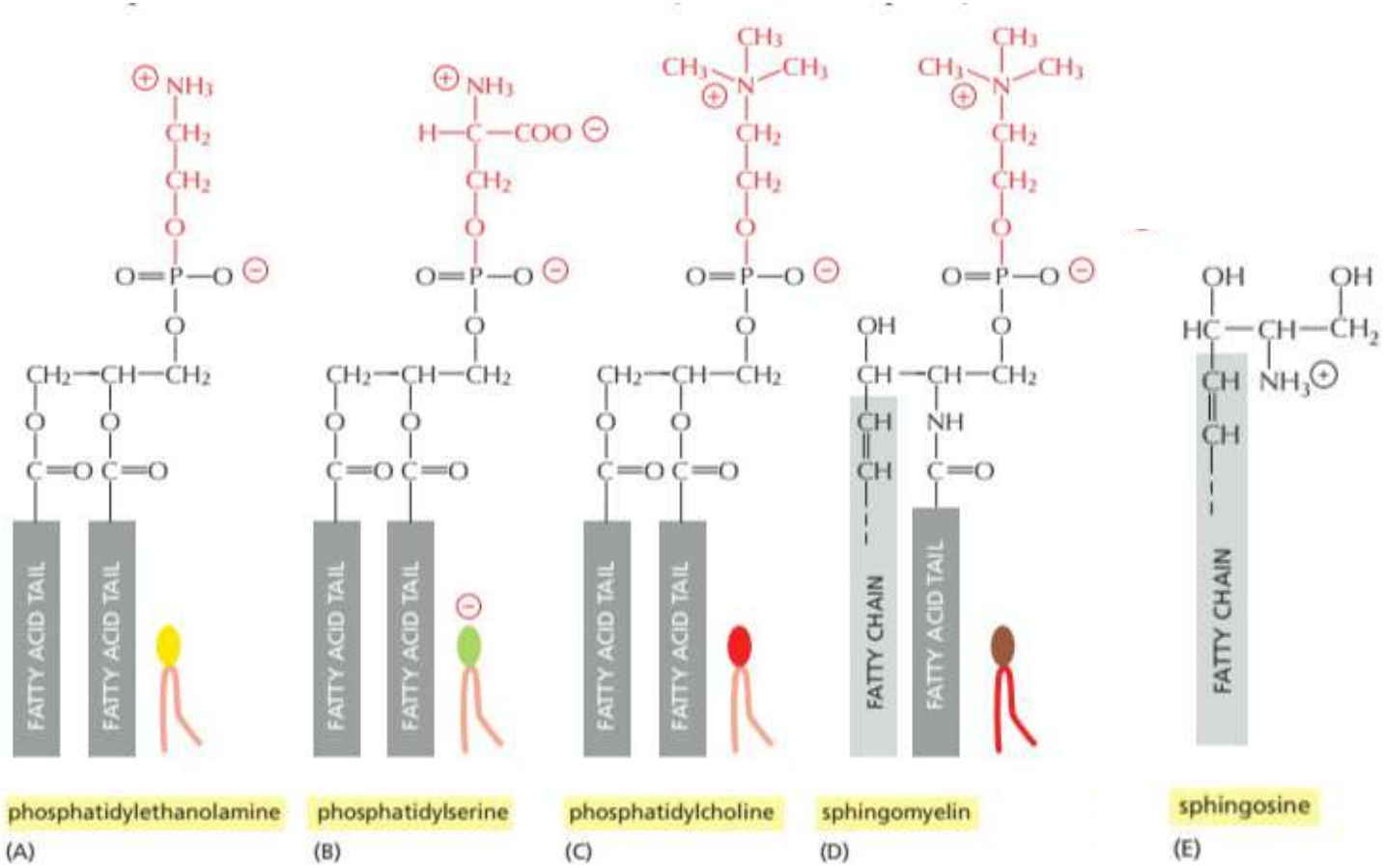
In phospholipids, two of the OH groups of glycerol are linked to fatty acids, while the third is linked to a phosphate group, which can be further linked to a polar group such as choline, serine, inositol, etc...



Very asymmetric molecule:
- hydrophilic HEAD
- hydrophobic TAIL



Sphingolipids



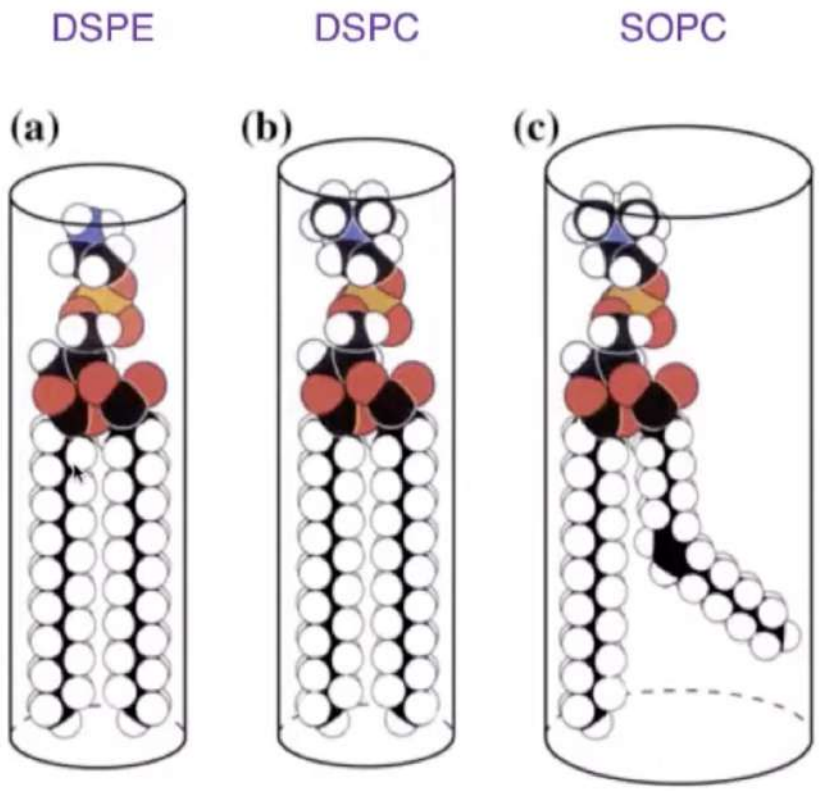
Sphingolipids are derivatives of sphingosine (red), an amino alcohol with a long hydrocarbon chain.
Various fatty acyl chains are connected to sphingosine by an amide bond.
The sphingomyelins (SM), which contain a phosphocholine head group, are phospholipids.
Other sphingolipids are glycolipids in which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

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

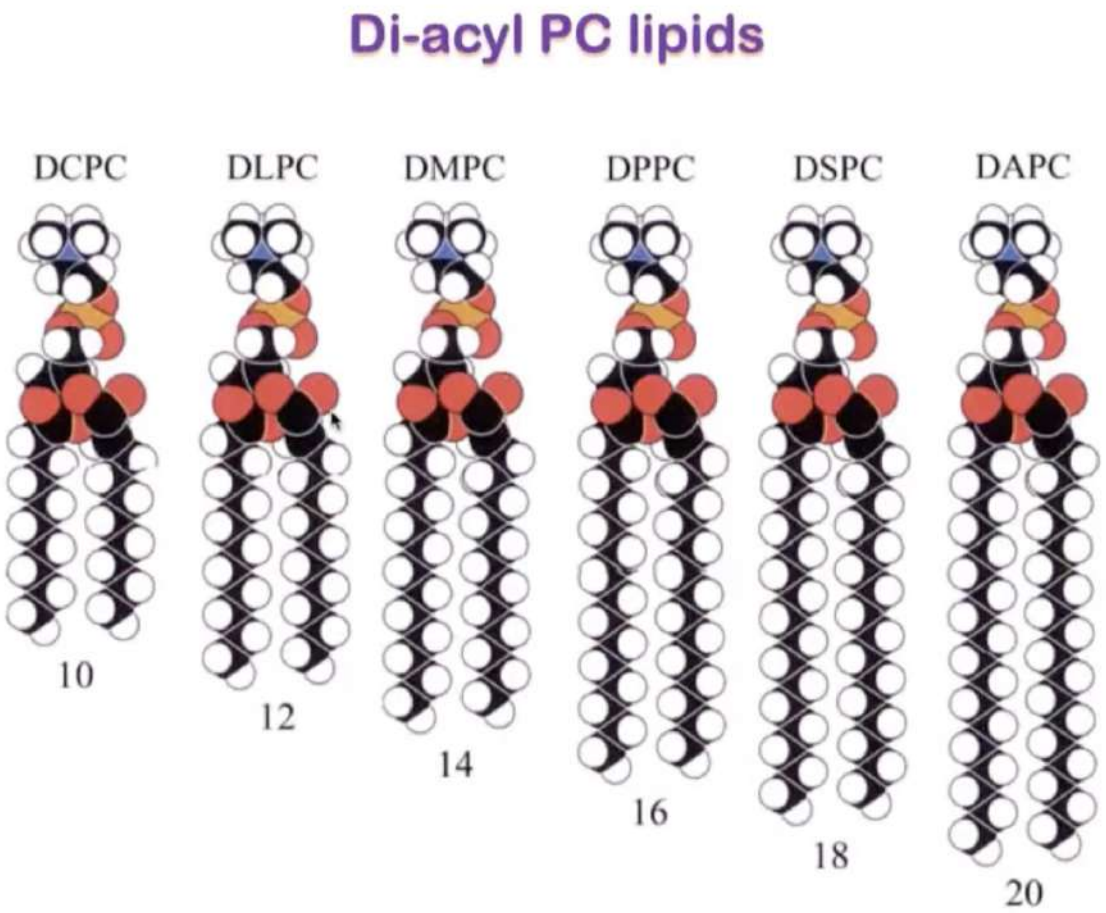
Saturated vs Unsaturated Fatty Acids



The actual conformation of a molecule influences its size.

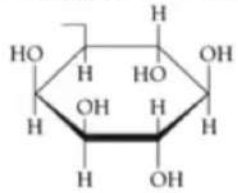
Temperature will lead to a rotation around the C-C bonds.

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



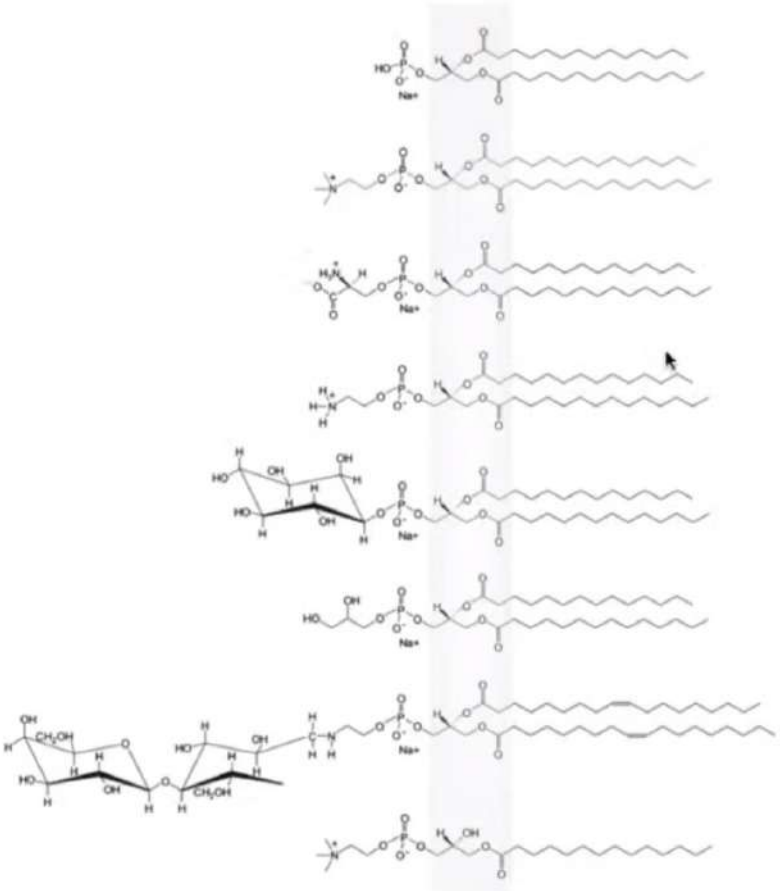
Typical cross-sectional areas of the cylinders that describe average lipid conformation in the lipid bilayers= is about 0.63 nm^2, with average length from 1.0 to 1.5 nm (depending on number of C atoms, saturation).

Lipid polar head groups

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.

Head and Tail



DMPA

DMPC

DMPS

DMPE

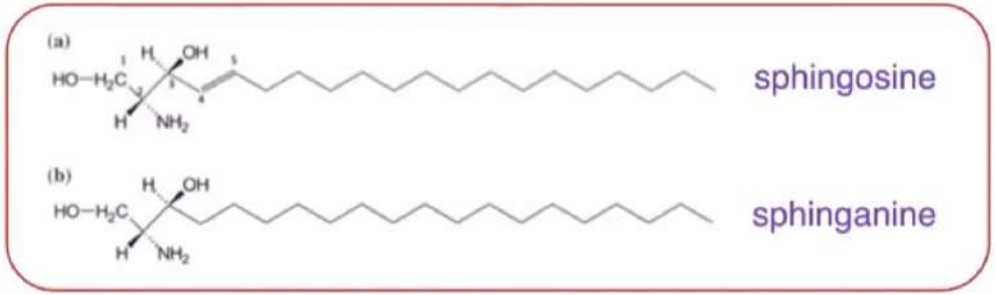
DMPI

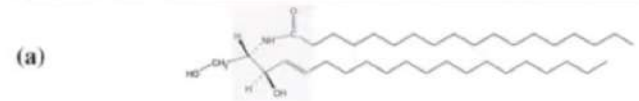
DMPG

Glycolipid

Lysolipid with C16

Sphingosine based phospholipids

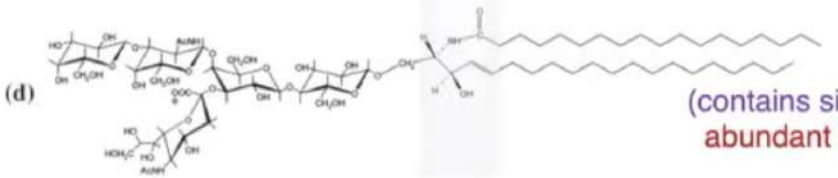




ceramide

sphingomyelin

galactosyl-cerobroside
(precursor to gangliosides)



ganglioside
(contains sialic acid (N-acetyl-neuraminic acid)
abundant in nervous system, 3,4,5,6, and 7
sugars attachment

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	Laurie 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)	$CH_3(CH_2)_5$ $CH{=}CH(CH_2)_7COOH$	*cis*-9-Hexadecenoic acid	Palmitoleic acid	0.5		
18:1 (Δ9)	$CH_3(CH_2)_7$ $CH{=}CH(CH_2)_7COOH$	*cis*-9-Octadecenoic acid	Oleic acid (Latin *oleum*, "oil")	13.4		
18:2(Δ9, 12)	$CH_3(CH_2)_4\,CH{=}CHCH_2$ $CH{=}CH(CH_2)_7COOH$	*cis*-,*cis*-9,12-Octadecadienoic acid	Linoleic acid (Greek *linon*, "flax")	–5		
18:3(Δ9, 12, 15)	$CH_3CH_2\,CH{=}CHCH_2$ $CH{=}CHCH_2$ $CH{=}CH(CH_2)_7COOH$	*cis*-,*cis*-,*cis*-9,12,15-Octadecatrienoic acid	α-Linolenic acid	–11		
20:4(Δ5, 8, 11, 14)	$CH_3(CH_2)_4\,CH{=}CHCH_2$ $CH{=}CHCH_2$ $CH{=}CHCH_2$ $CH{=}CH(CH_2)_3COOH$	*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.

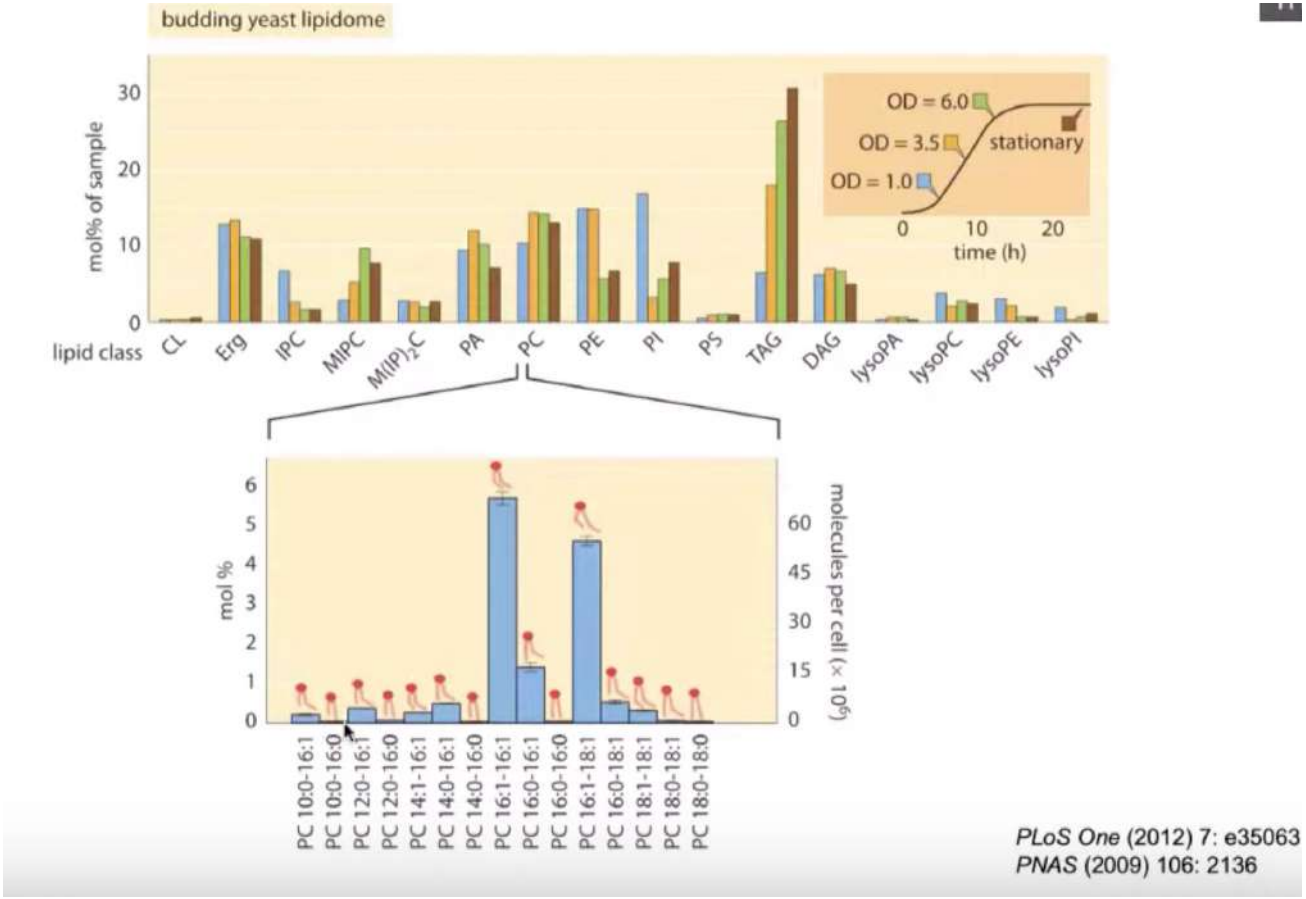
[b] All acids are shown in their nonionized form. At pH 7, all free fatty acids have an ionized carboxylate. Note that numbering of carbon atoms begins at the carboxyl carbon.

[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.

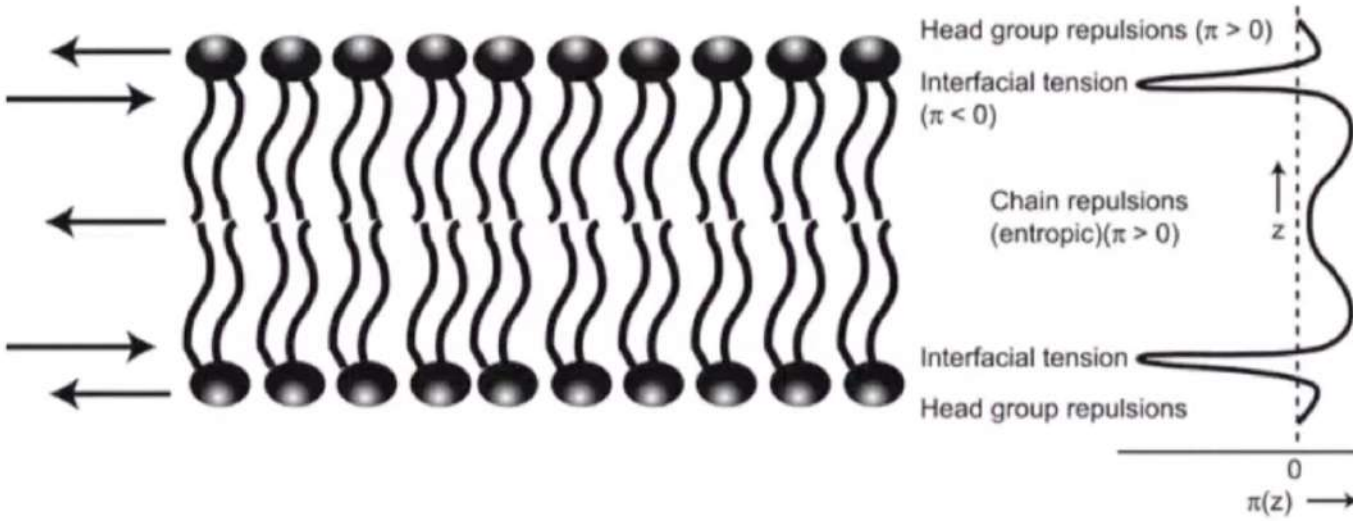
More than 500 species of fatty acids !

Lipidomic survey of a budding yeast



PLoS One (2012) 7: e35063
PNAS (2009) 106: 2136

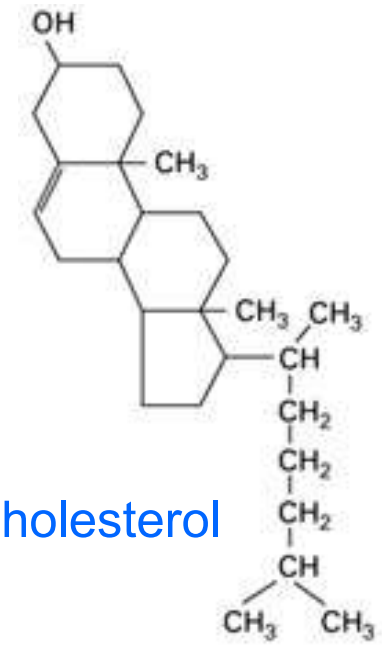
Lateral pressure profile of a lipid bilayer



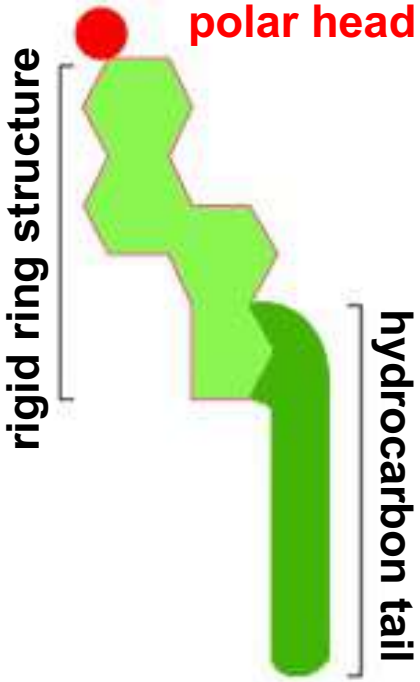
1. Positive pressure resulting from headgroup repulsive forces
2. Negative pressure at the hydrophobic-hydrophilic interface - the interfacial tension
3. Positive pressure resulting from entropic repulsion between acyl chains – chain pressure

Cholesterol and steroids

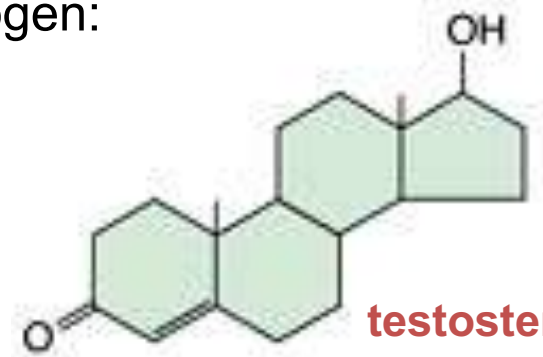
Steroids (such as cholesterol) have a rigid structure made up by 4 rings.



Cholesterol

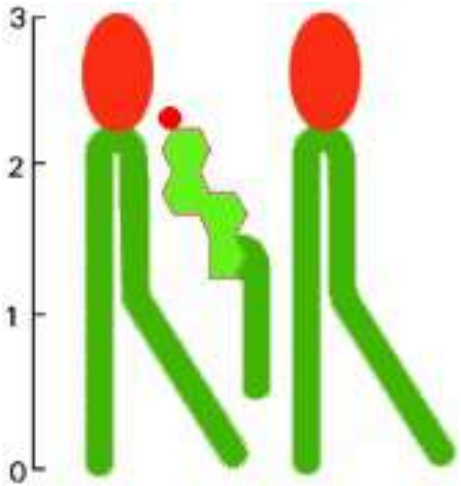


Other important steroid are the sex hormones, such as testosterone and estrogen:



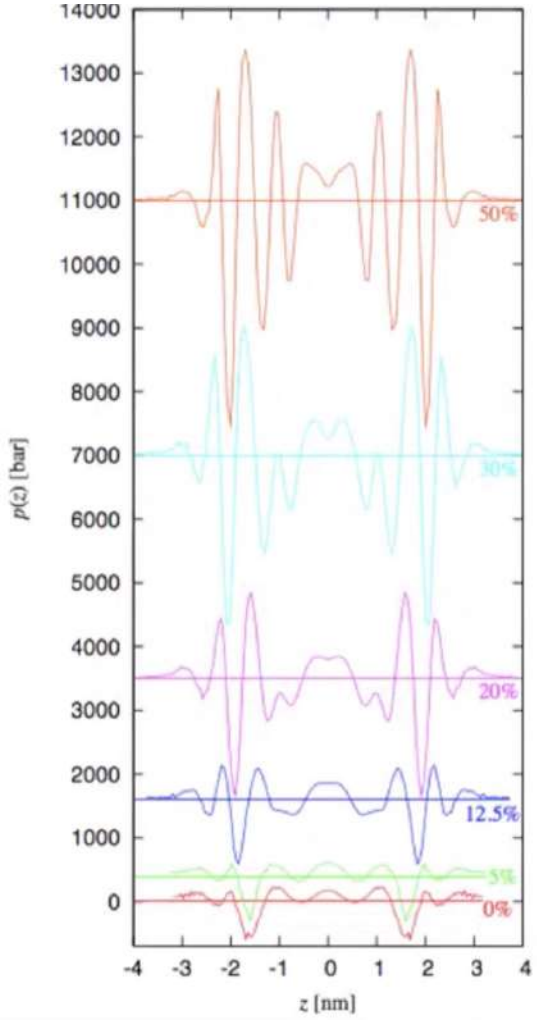
testosterone

Cholesterol is an important component of the eukaryotic membranes and has a key role in controlling the membrane fluidity.

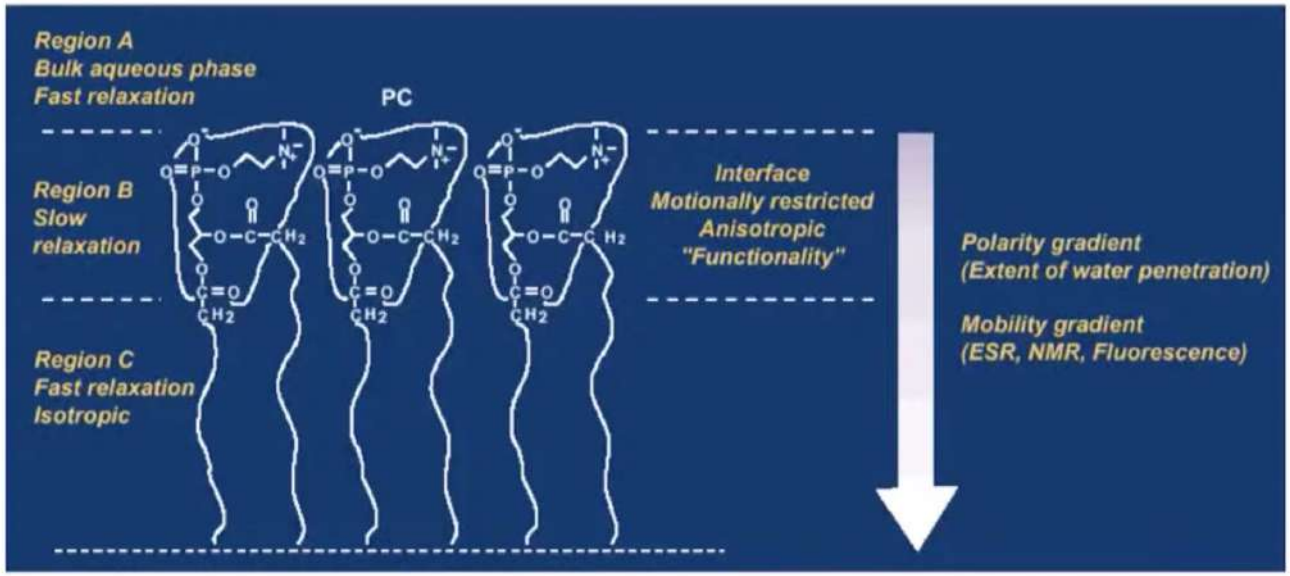


Effect of cholesterol

Lateral pressure profiles in DPPC/Cholesterol bilayer

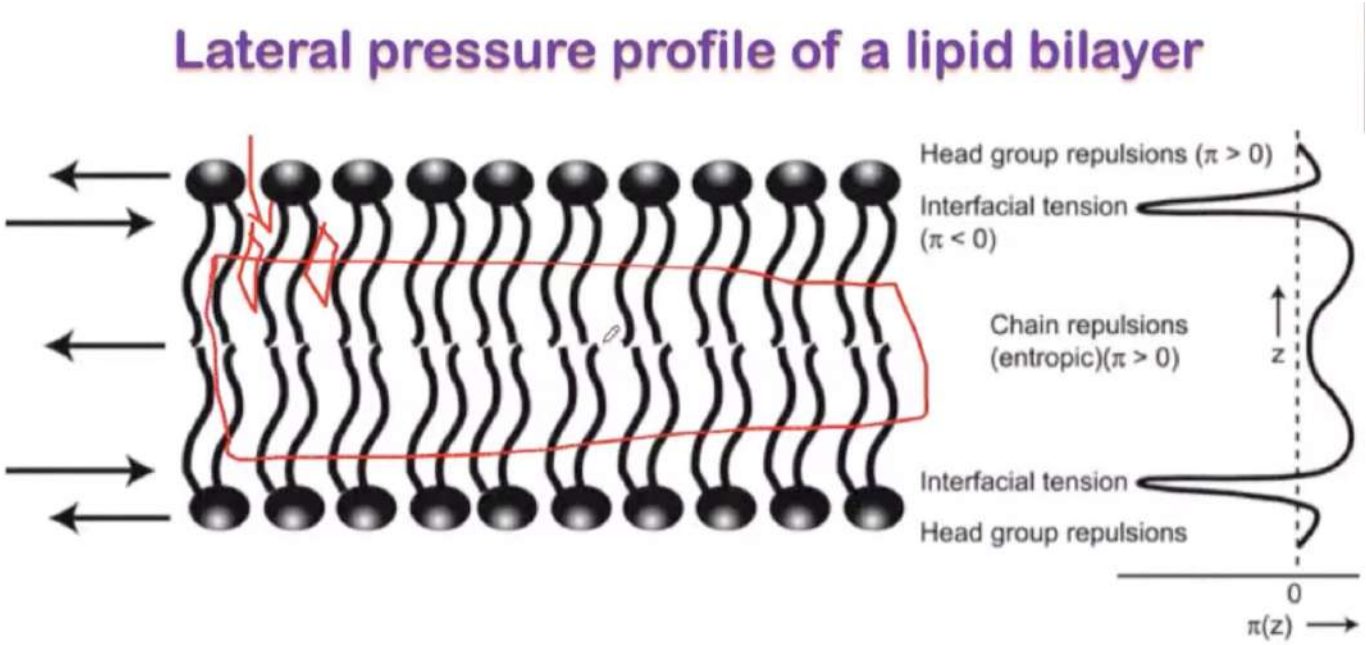


Membrane interface



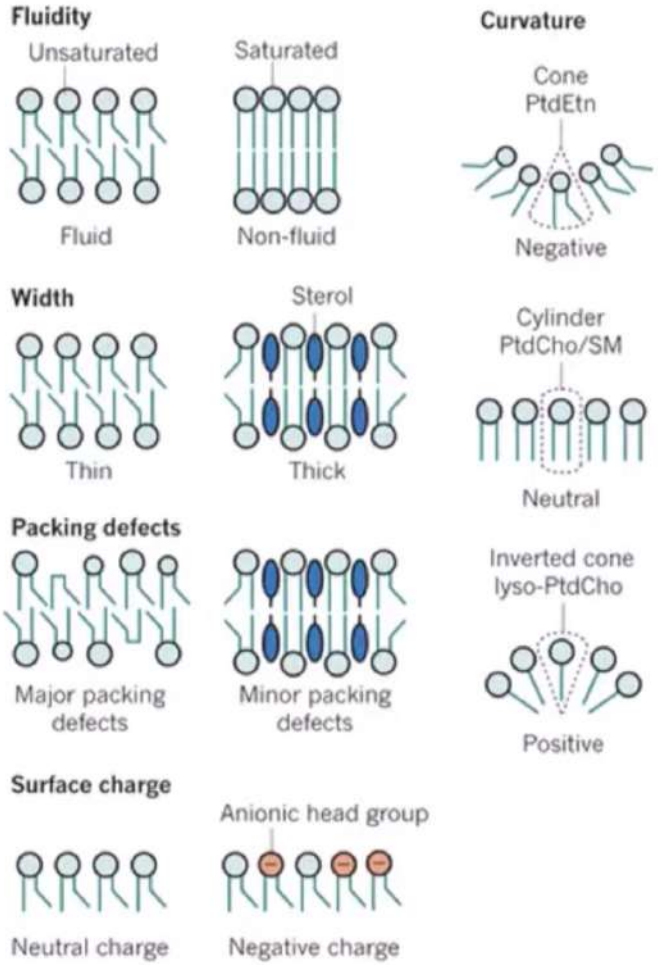
The membrane interface is an important region of the membrane and characterizes the chemistry and biology of the membrane. It is also the most motionally restricted region of the membrane bilayer.

Membrane interface



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

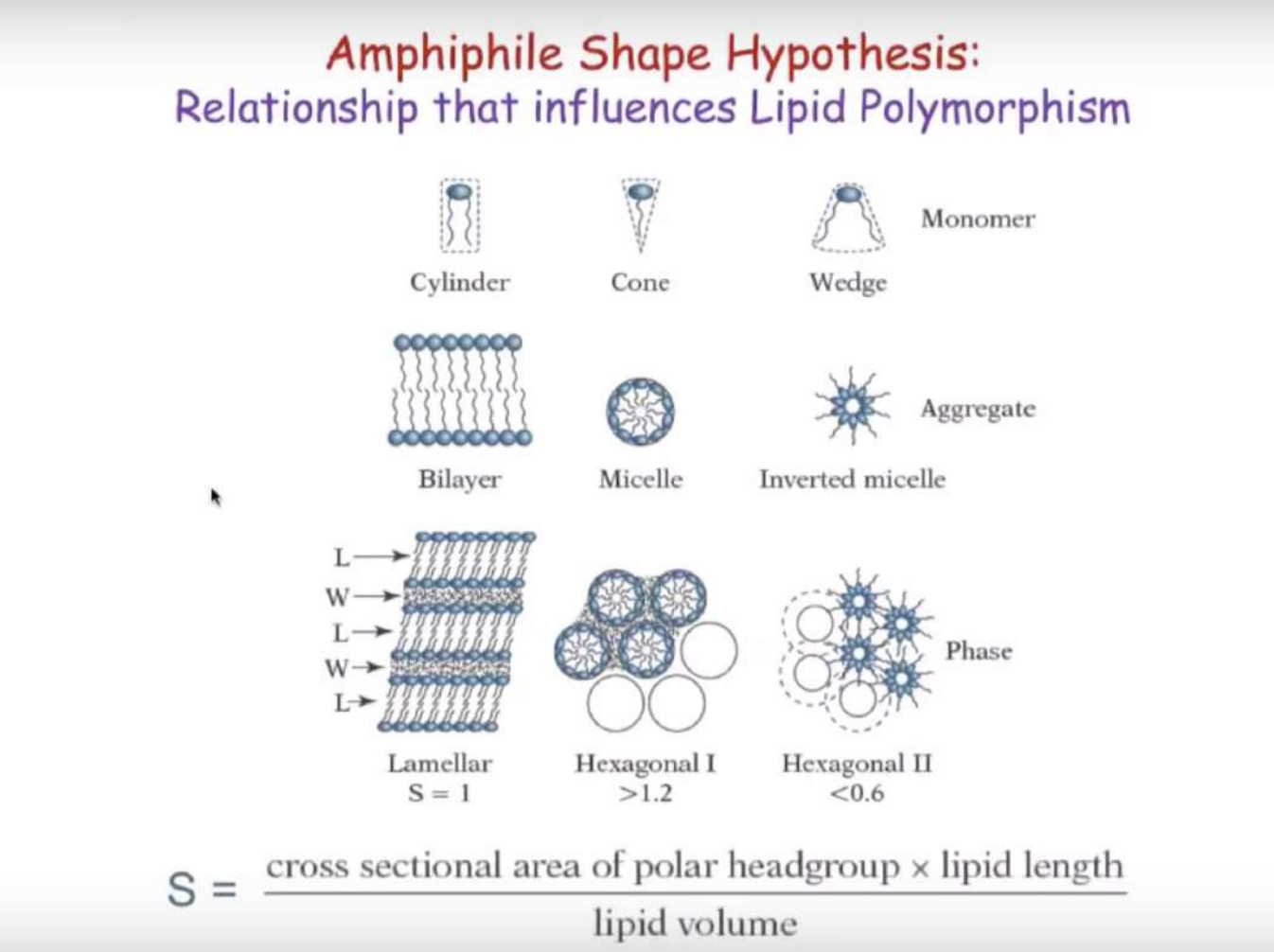
Membrane physical properties



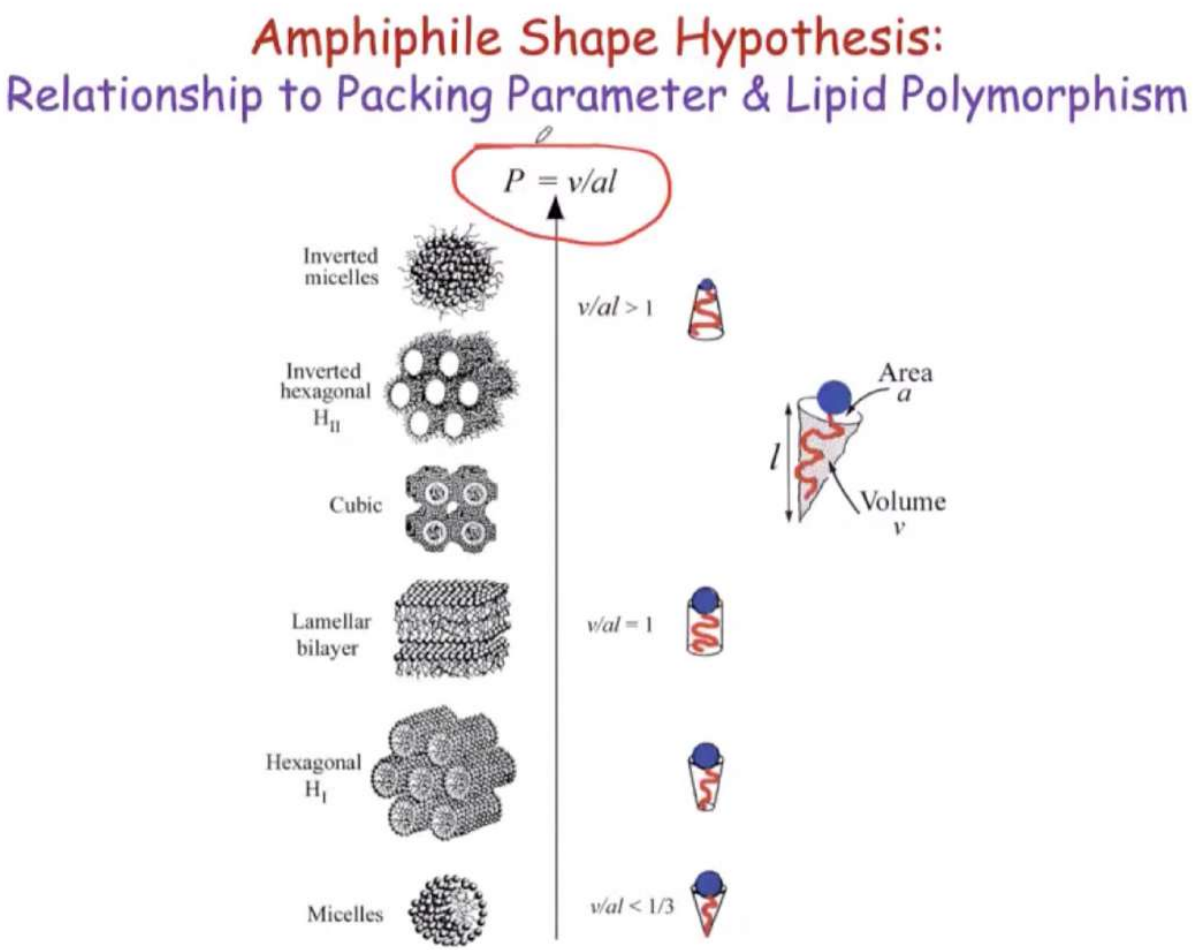
Membrane Physical Properties are Determined by its Lipid Composition

Nature (2014) 510: 48-57

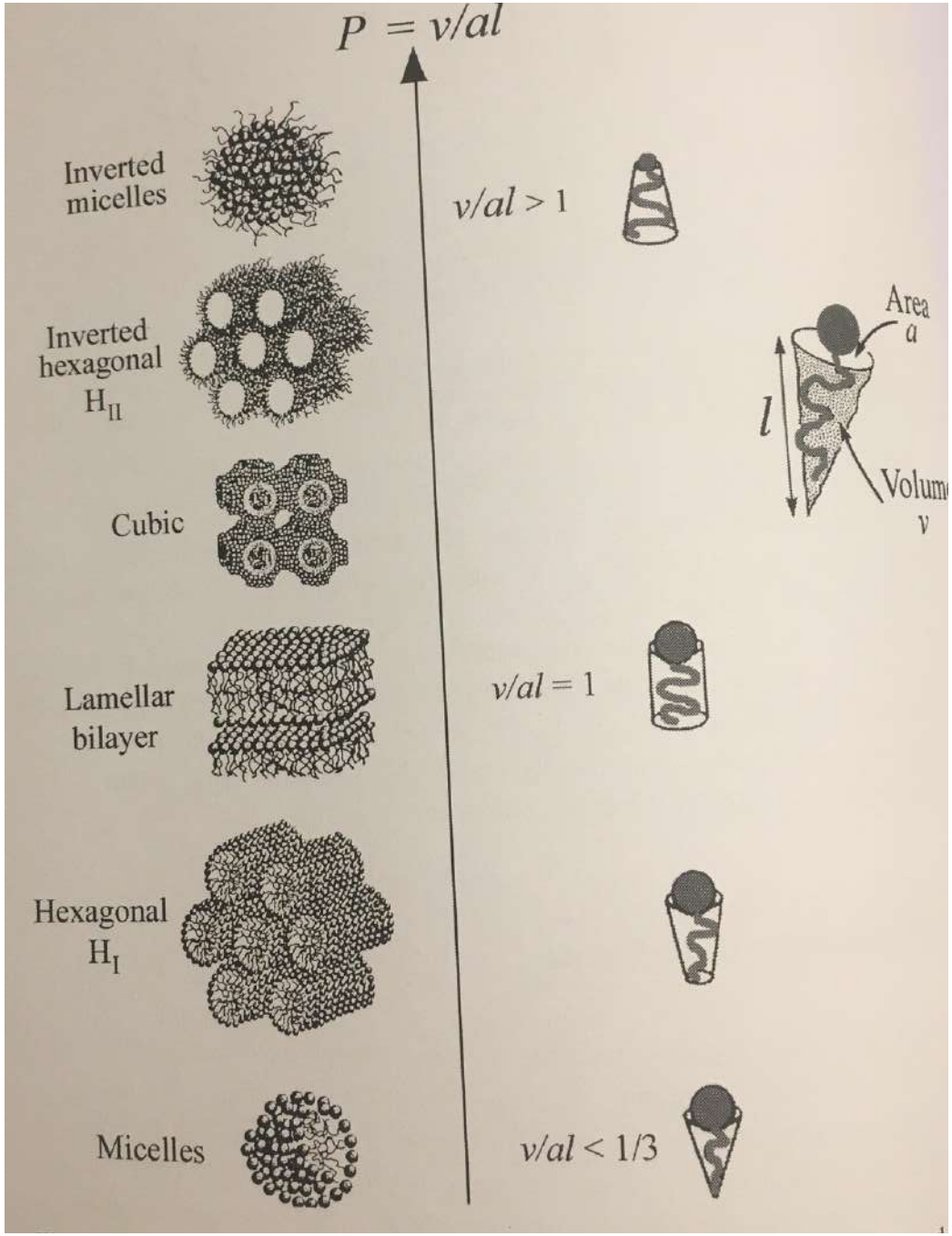
Membrane physical properties



Membrane physical properties



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.

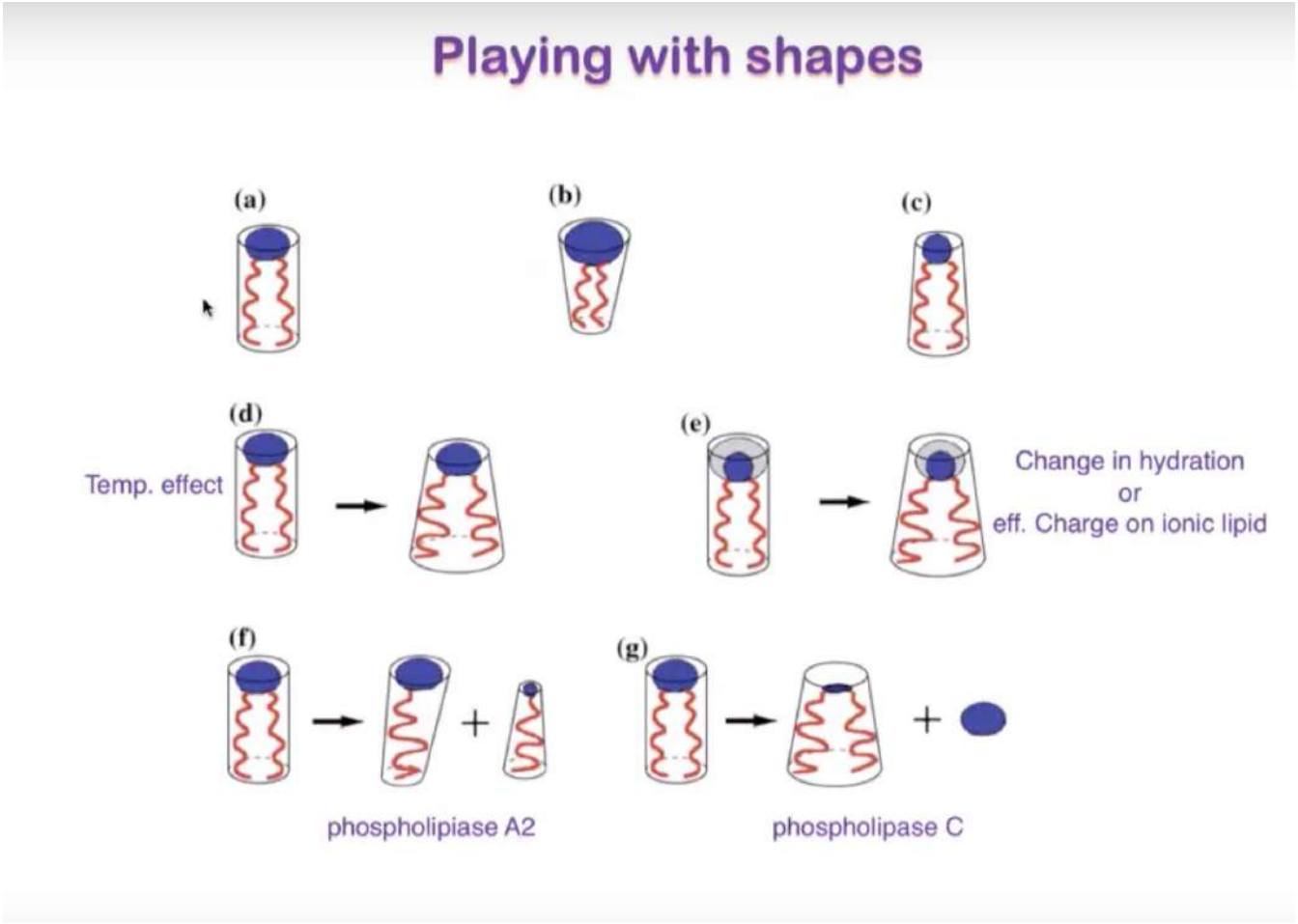
Lipid shape is important for functioning. It is given by the compatibility between head and tail. We define`a packing parameter P:

$$P = v/al$$

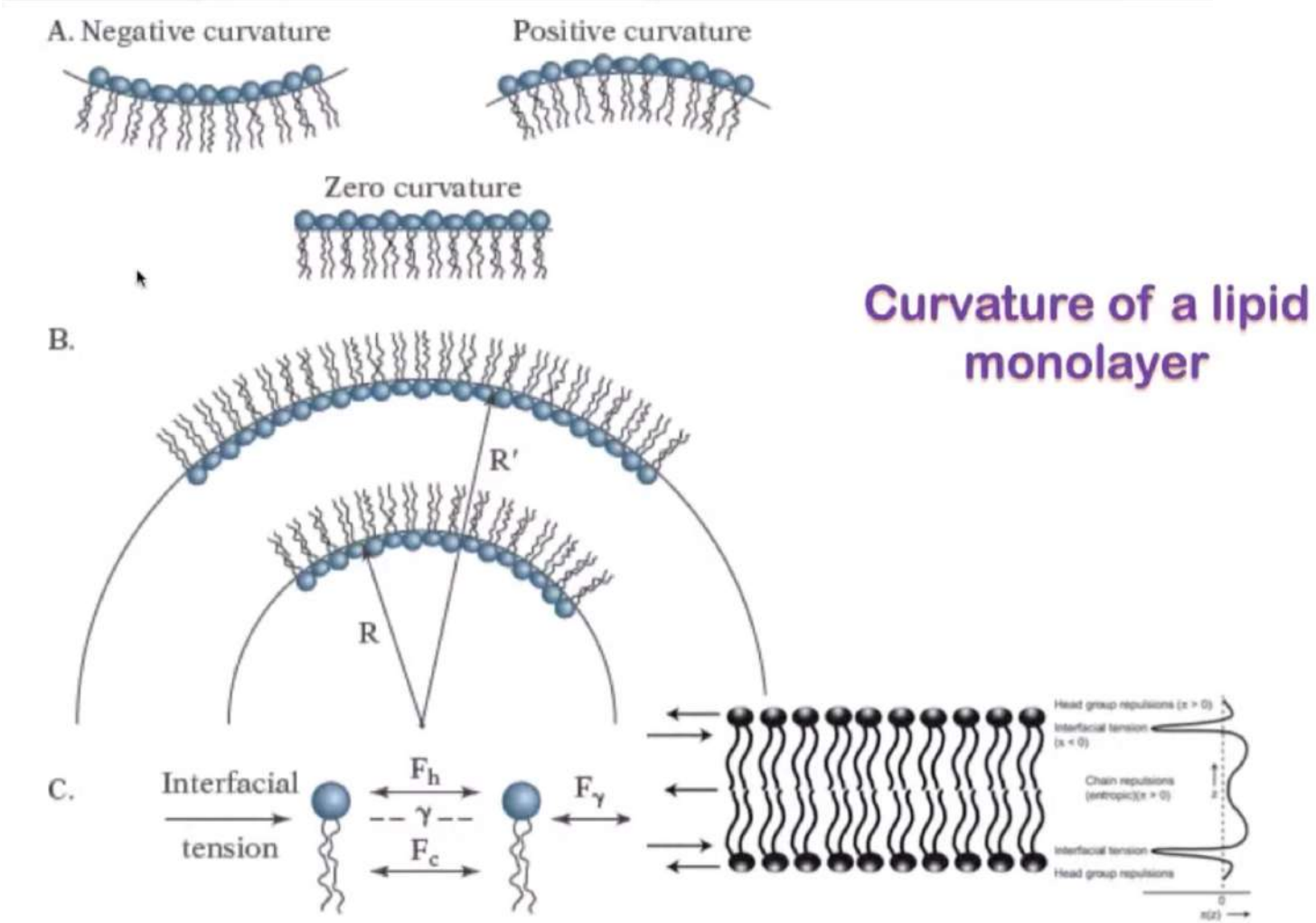
P = 1 is a cilindrical shaped lipid molecules, fitting a lamellar structure with zero curvature.

Curvature although is important for many of the membrane processes

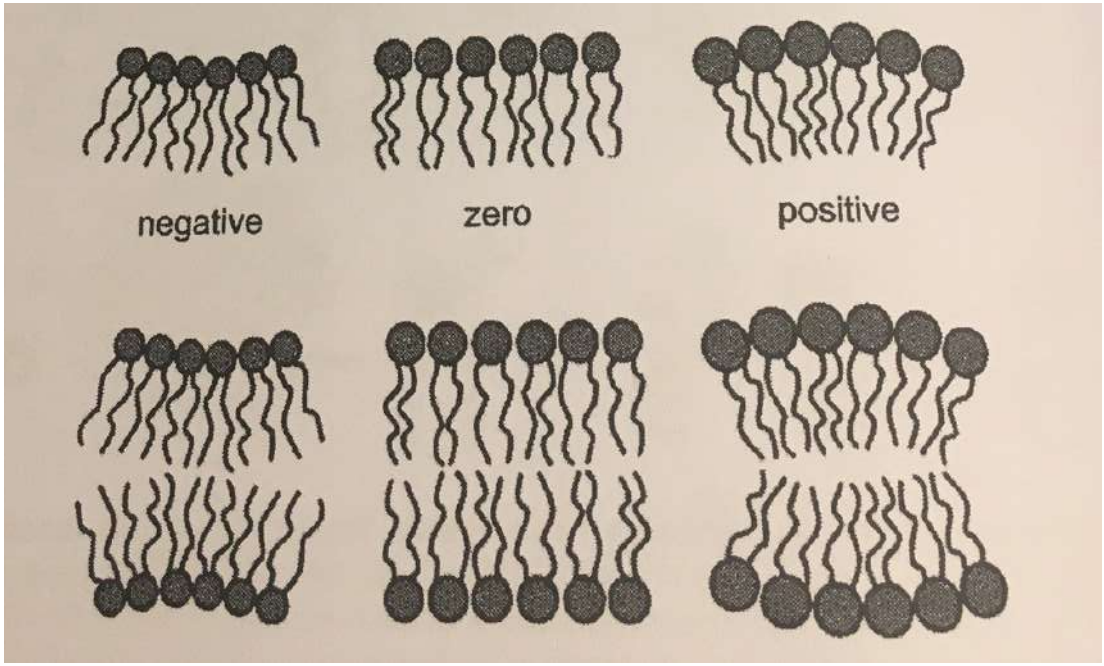
Membrane physical properties



Lipids and membrane curvature



Lipids and membrane curvature



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

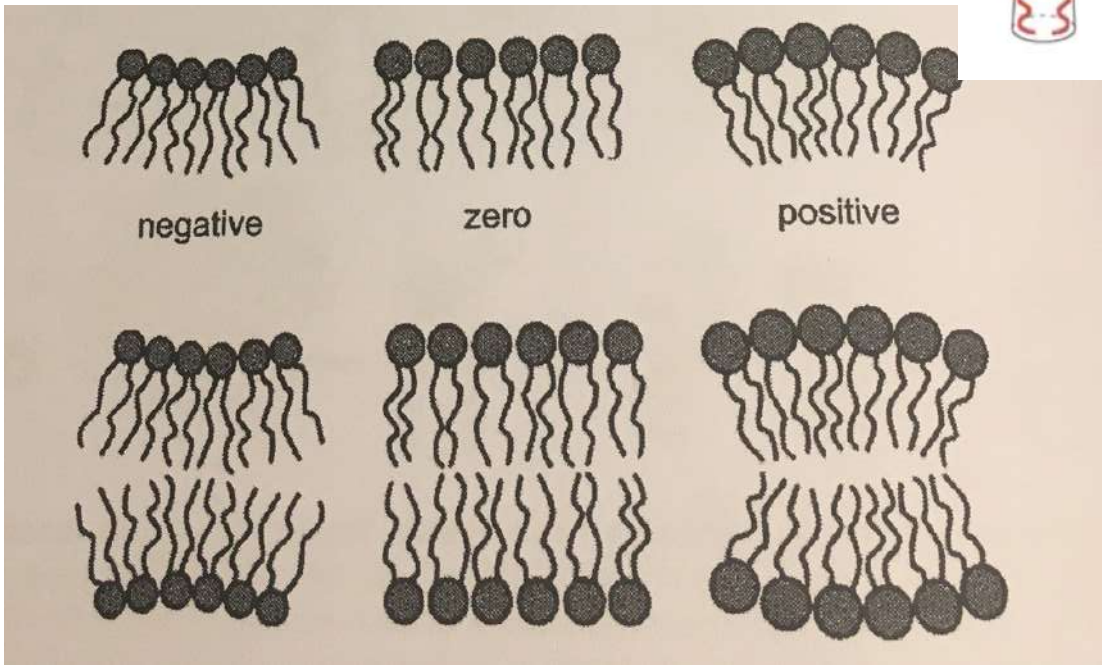
Each layer tend to elastically relax to a state of finite, spontaneous curvature, causing a curvature stress field.

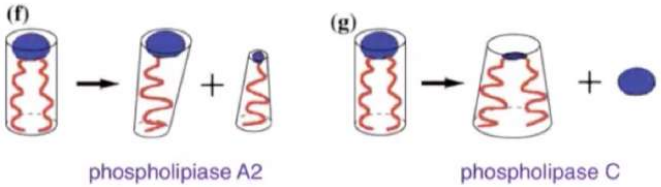
If the bilayer cohesion does nor sustain the curvature stress, non lamellar structures form.

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)

Lipids form soft interfaces

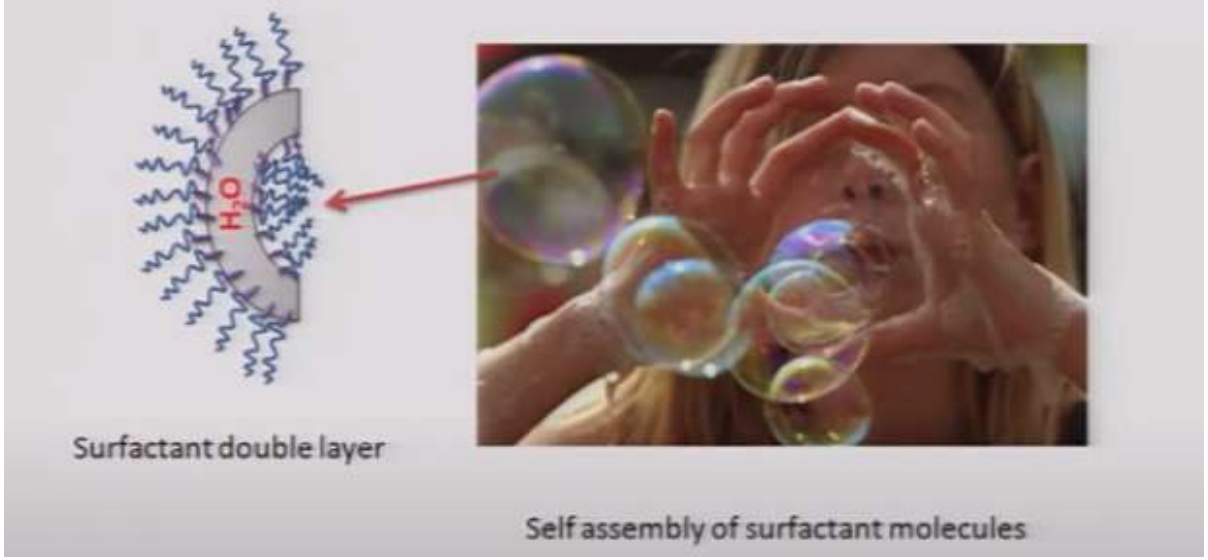
Membranes are soft interfaces. As polymers, exist in a condensed phase, but cannot be classified neither as solid, nor liquid. The physics of such interfaces is dominated by entropy.
Softness means high deformability but not necessarily high bulk compressibility!
Soft matter is anisotropic, hierarchical, 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

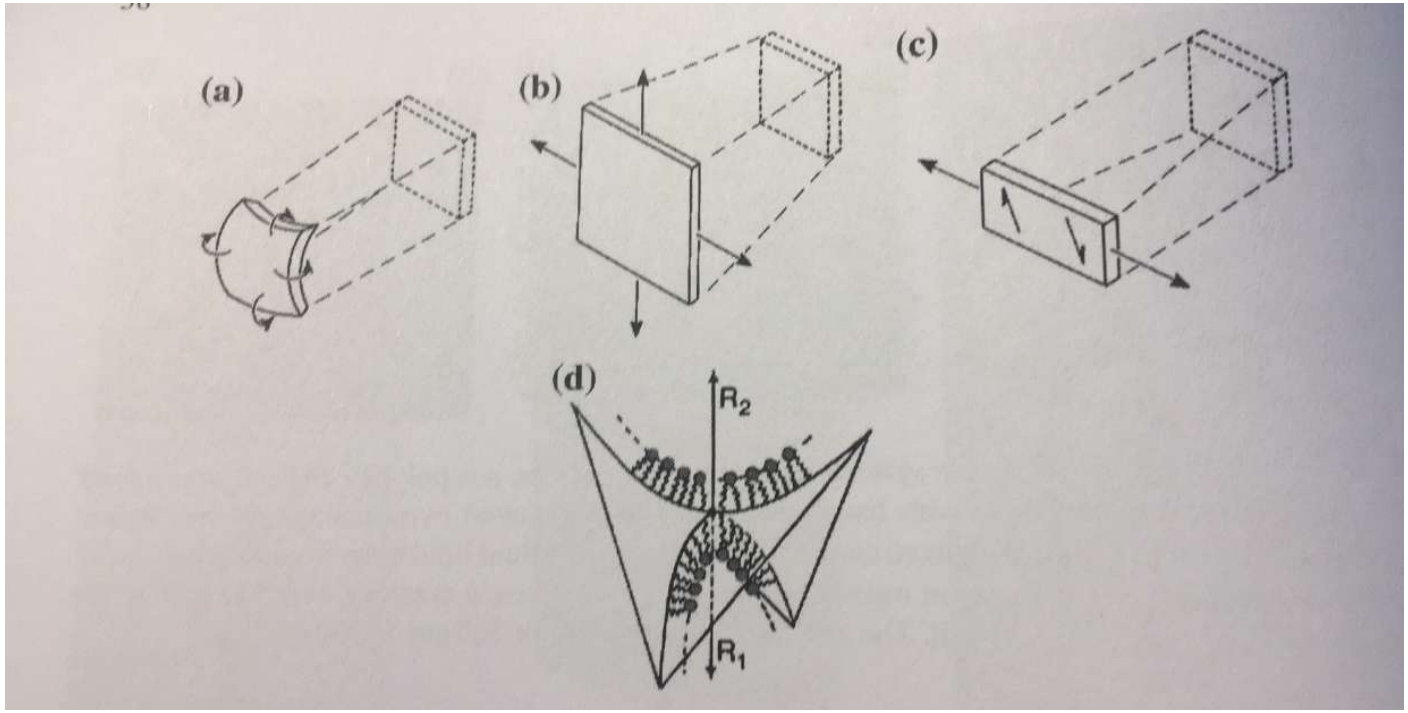


Soap bubbles: two layers form, at the water-air interfaces, the outer and the inner surfactant layer.
Bubbles are stabilized for a particular size, a particular water layer thickness depending on:

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

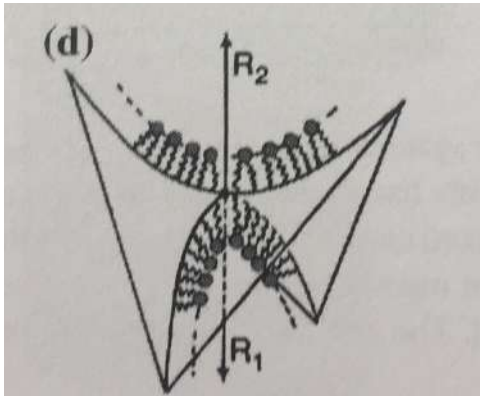
Lipids and membrane curvature

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 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).

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

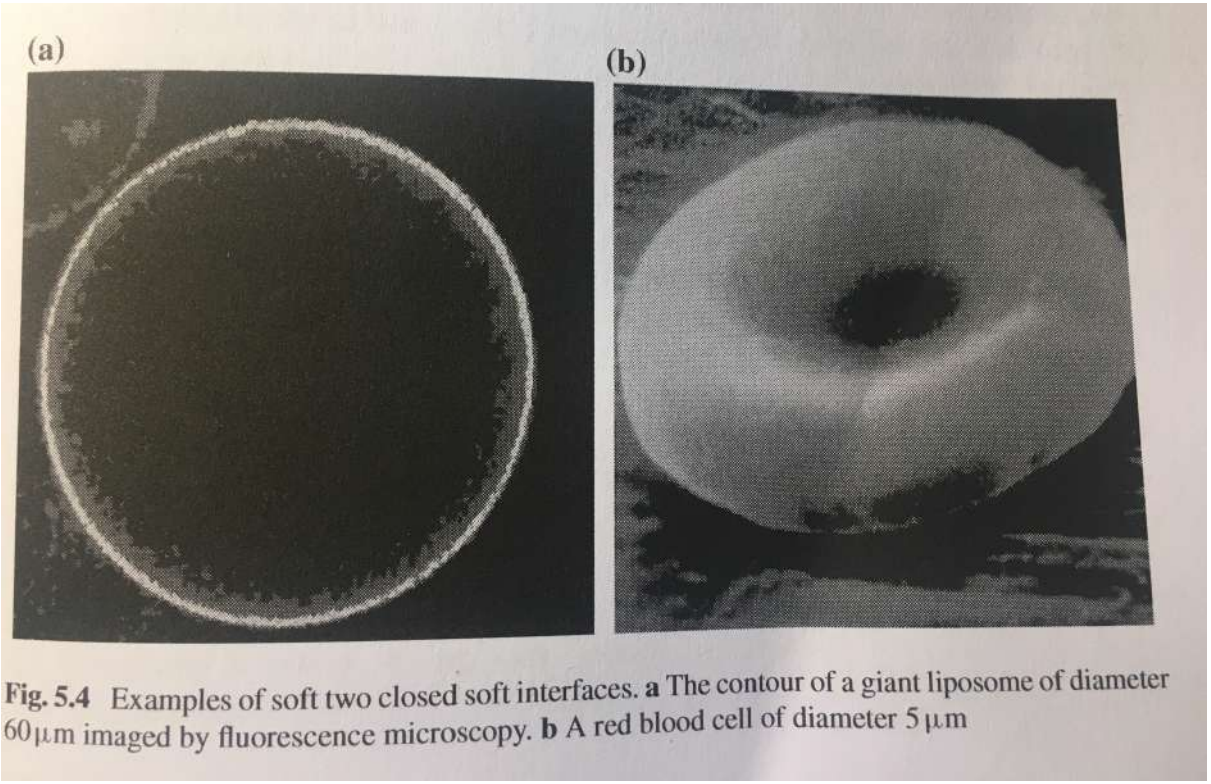


Fig. 5.4 Examples of soft two closed soft interfaces. **a** The contour of a giant liposome of diameter 60 μm imaged by fluorescence microscopy. **b** A red blood cell of diameter 5 μm

Two soft membranes with different bending capabilities.

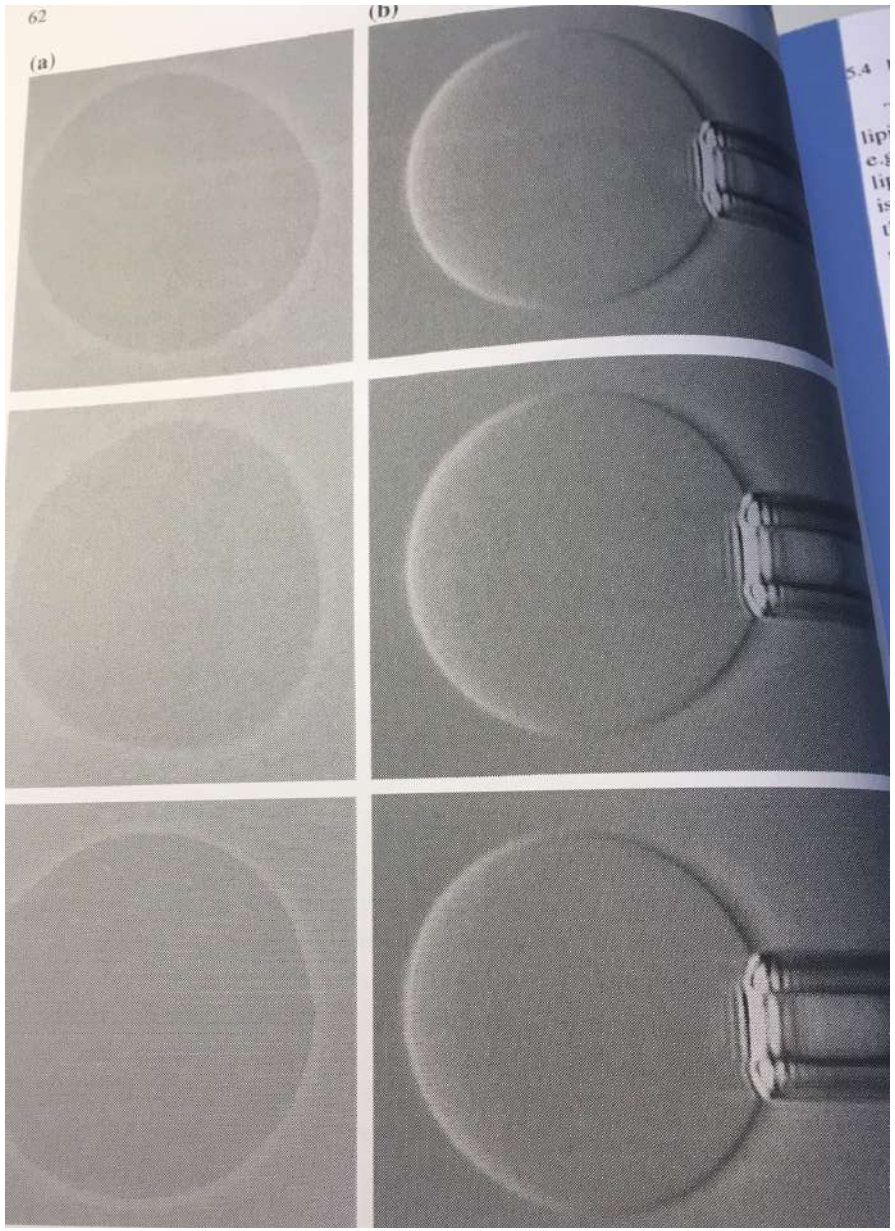
The persistent length, i.e. the length over which they appear flat and smooth, is different. It is related to the bending modulus via (with c a constant):

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

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.

Lipid membranes are really soft

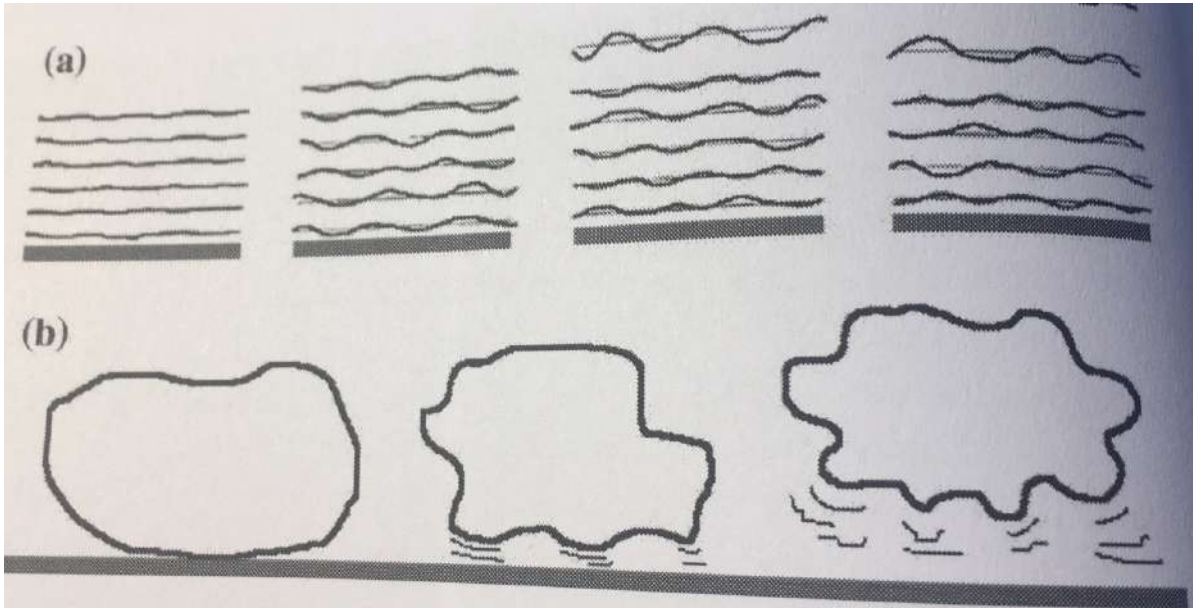


Giant liposomes (50 um). Membrane thickness: 5 nm.
Variation in the contour due to thermal fluctuations---the membrane is very soft!!
The bending modulus κ can be derived from the spectrum of fluctuations.
With the pipette aspiration one apply a stress τ and measure the compressibility modulus K from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

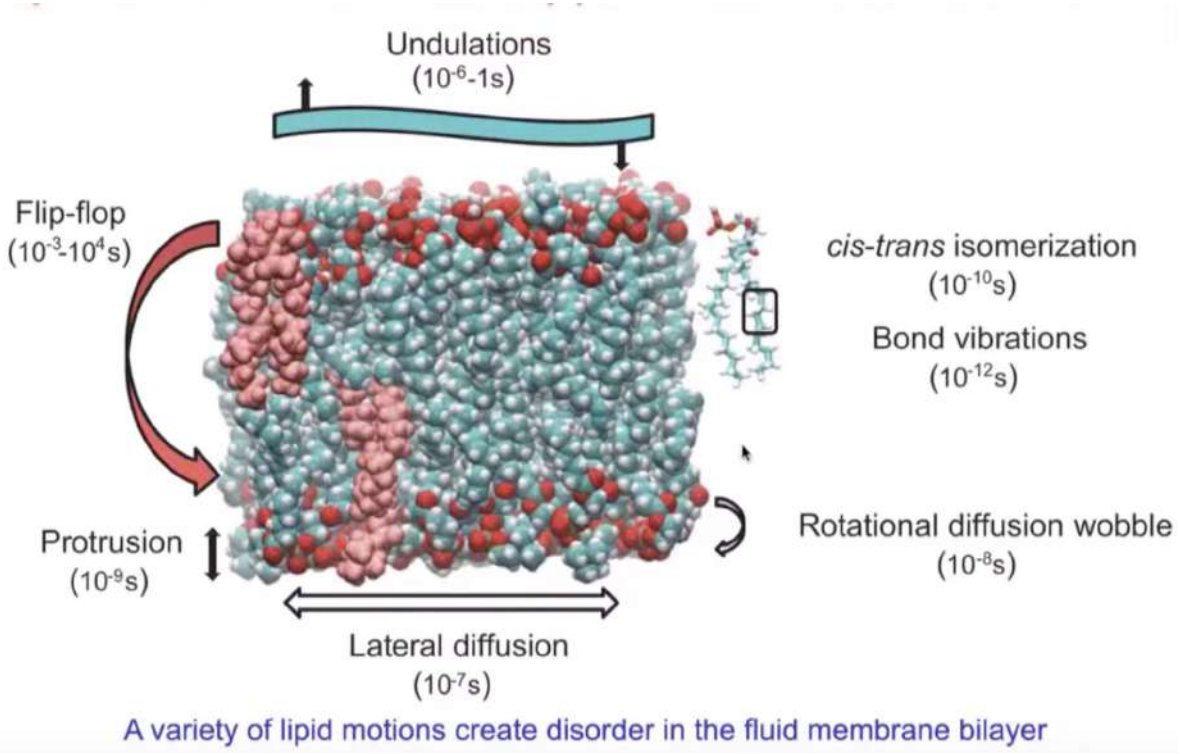
Lipid membranes are really soft



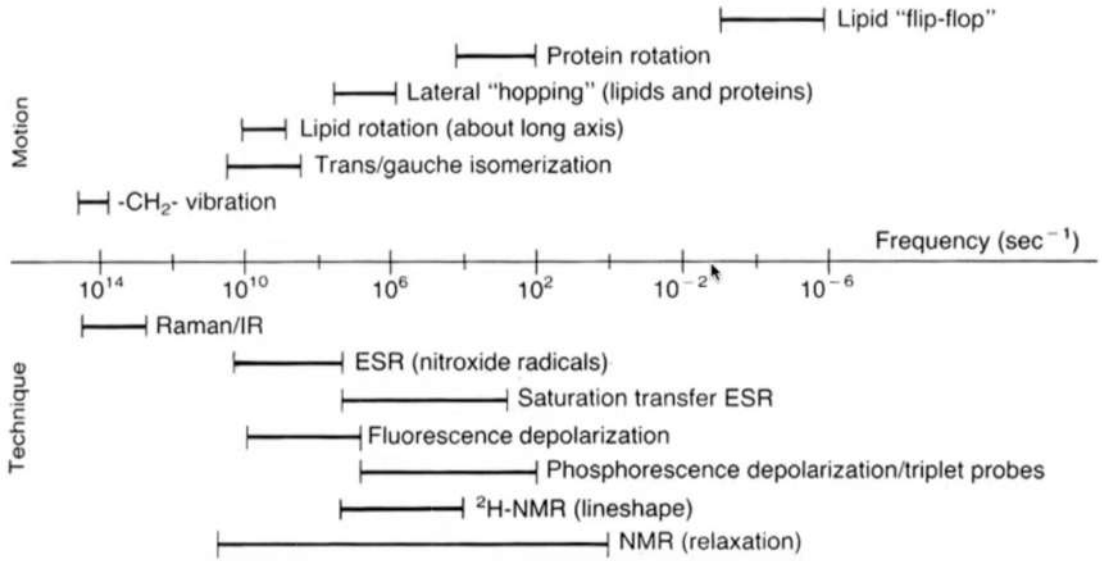
Because of undulation forces by soft bilayers, vesicles/lipid bilayers are repelled by solid surfaces.

How softness can be controlled at the molecular level?

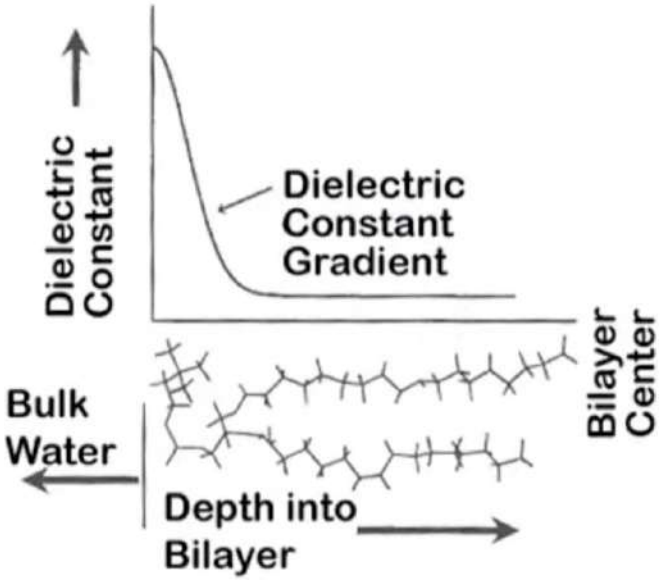
Molecular motion dynamics

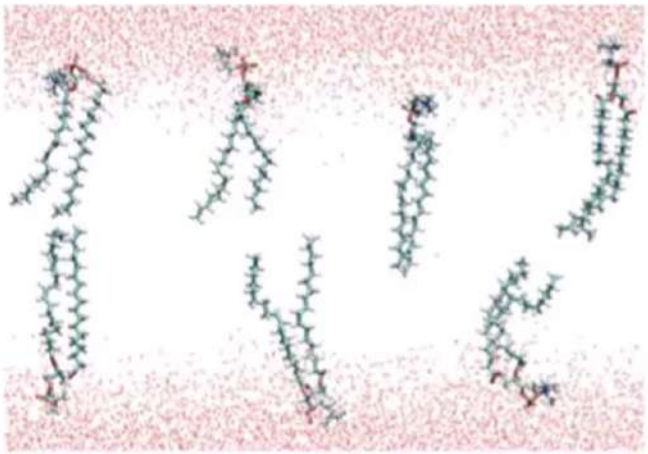


A variety of lipid motions create disorder in the fluid membrane bilayer

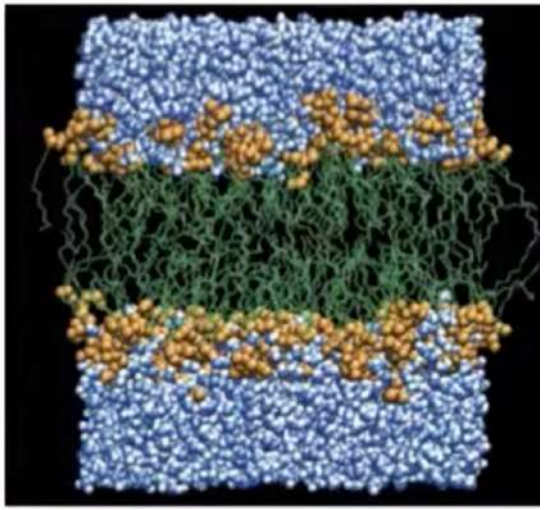


Water across the interface





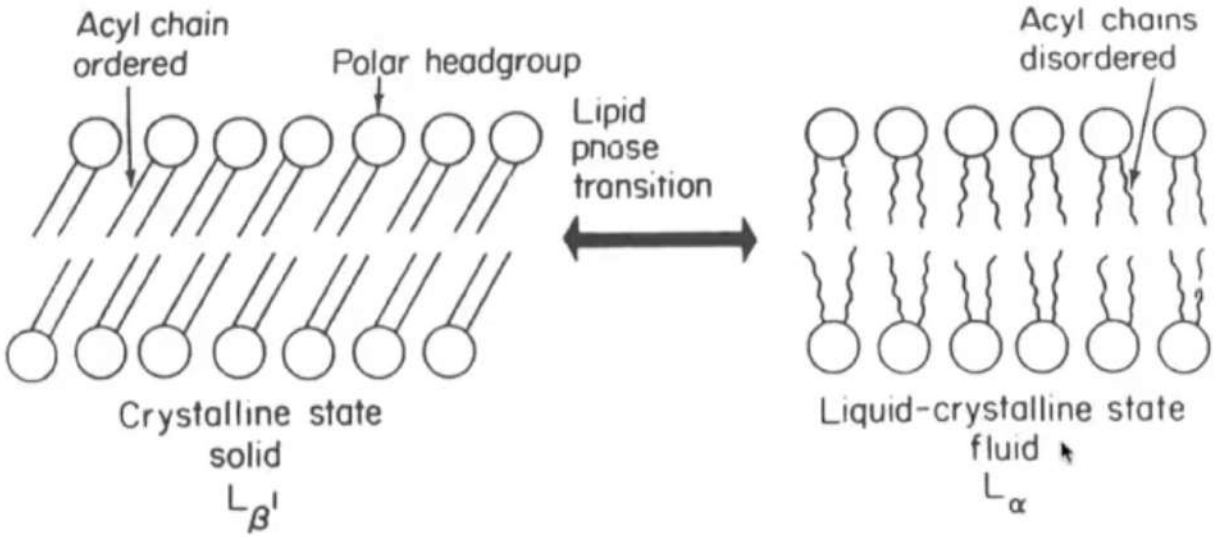
MD simulation of SOPC conformations in a bilayer



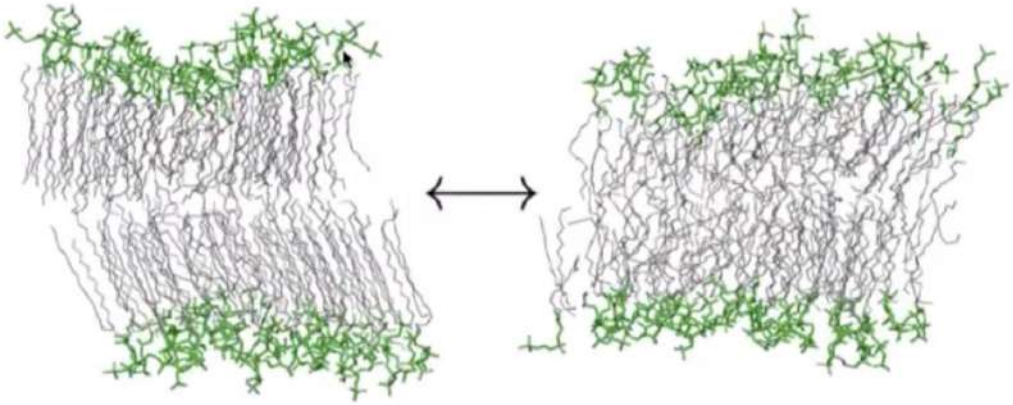
Fully hydrated DMPC bilayer

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

Phase transitions

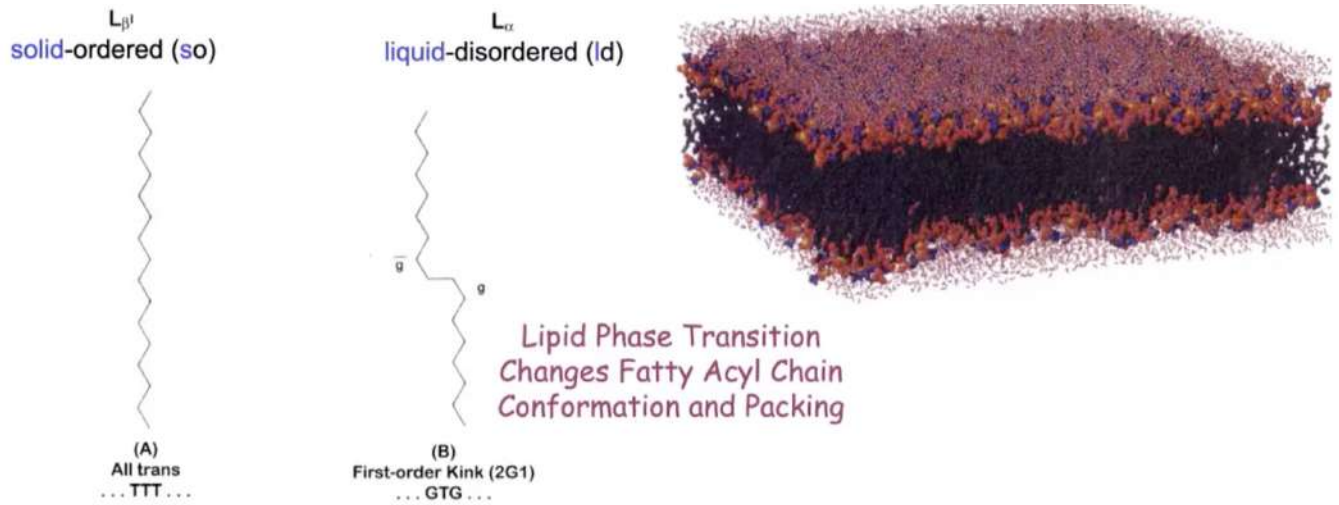


Phase transitions



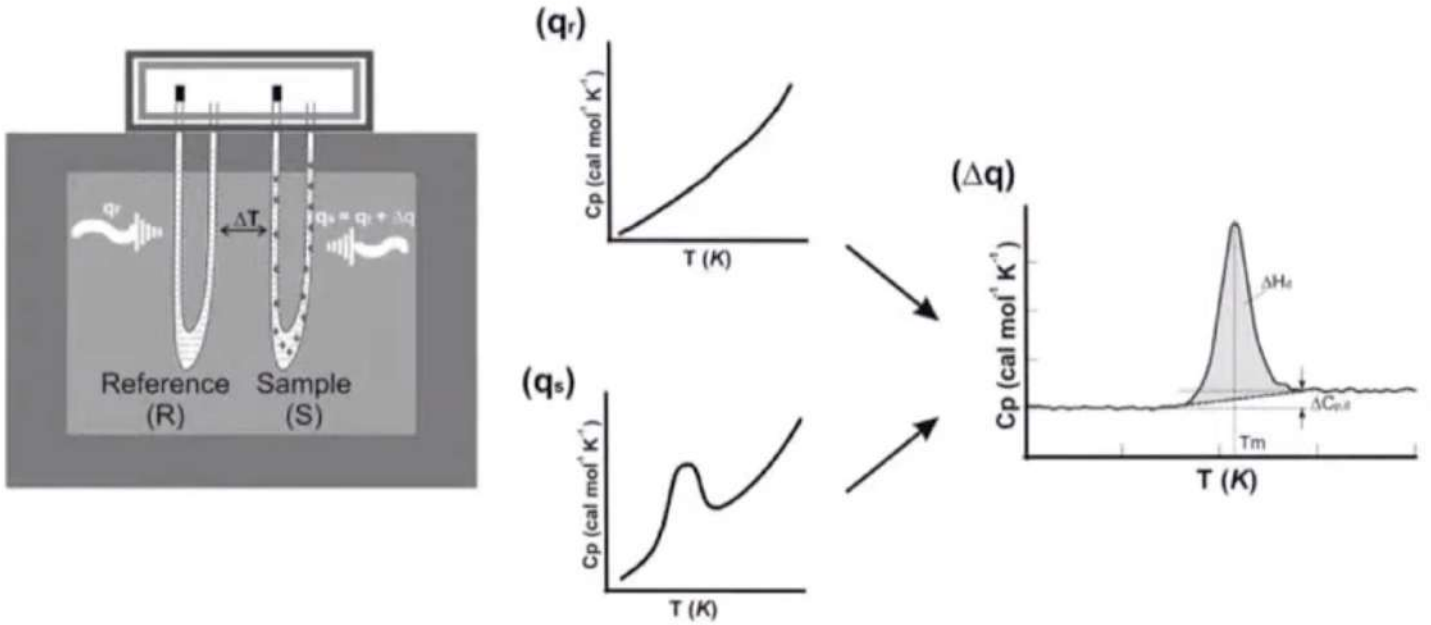
MD simulation of DPPC in water at Tm using atomistic model

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

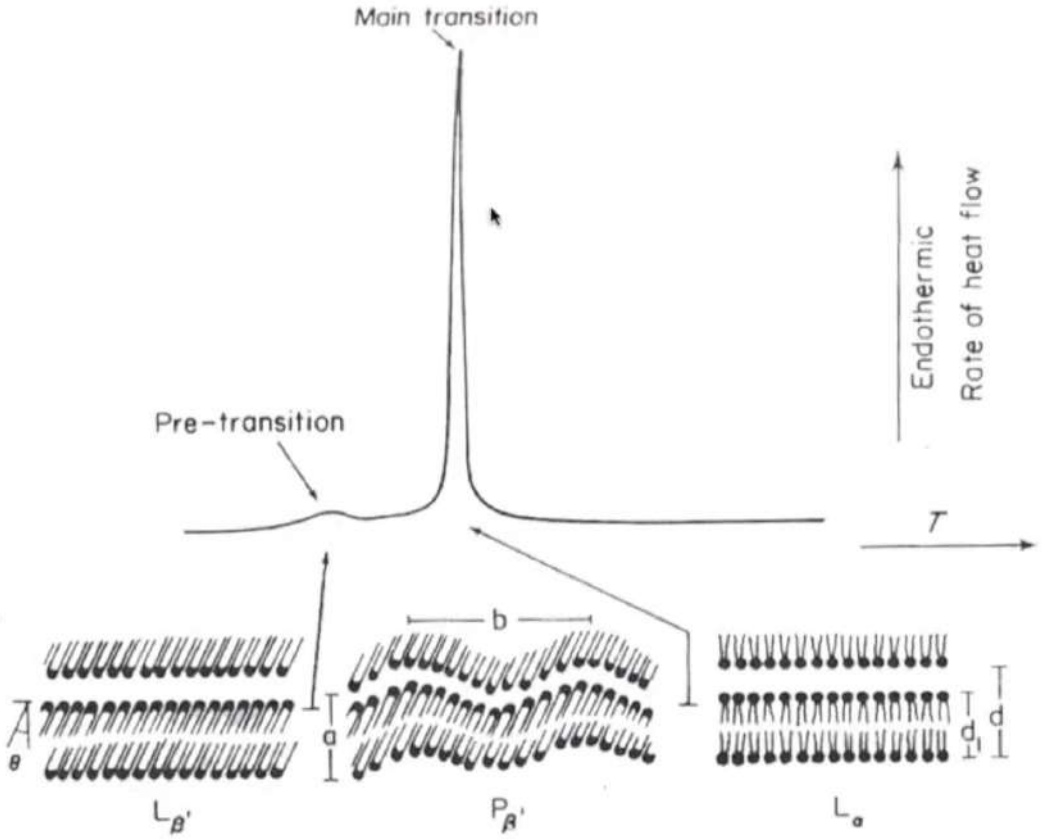


Phase transitions
Differential Scanning Calorimetry (DSC)

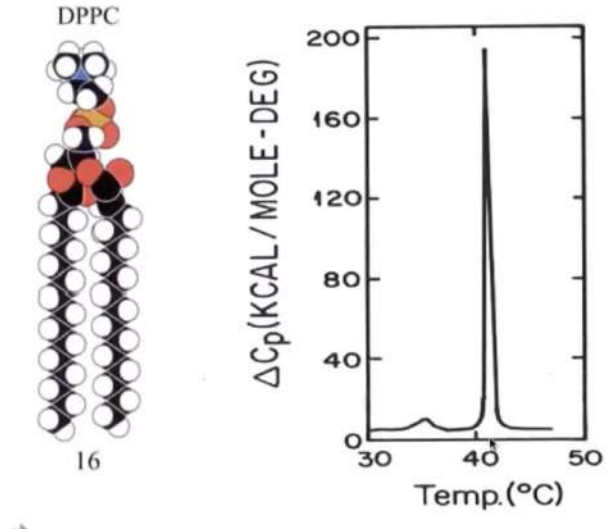
- DSC is a thermal analysis technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature
- Highly reproducible phase transitions are used to determine binding interactions, purity and stability of samples



Phase transitions
Differential Scanning Calorimetry (DSC)

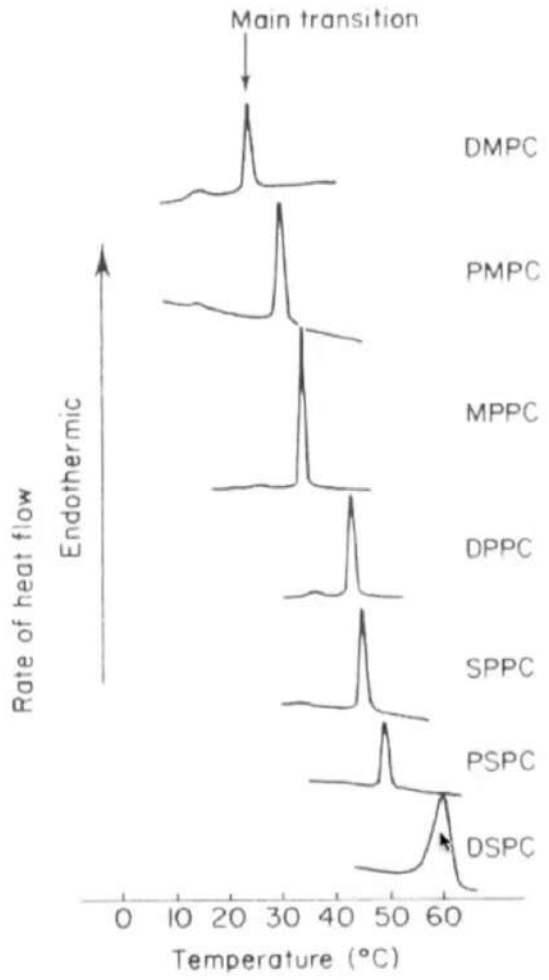


Main transition is precedeed by an intermediate, ripple phase which facilitates transition



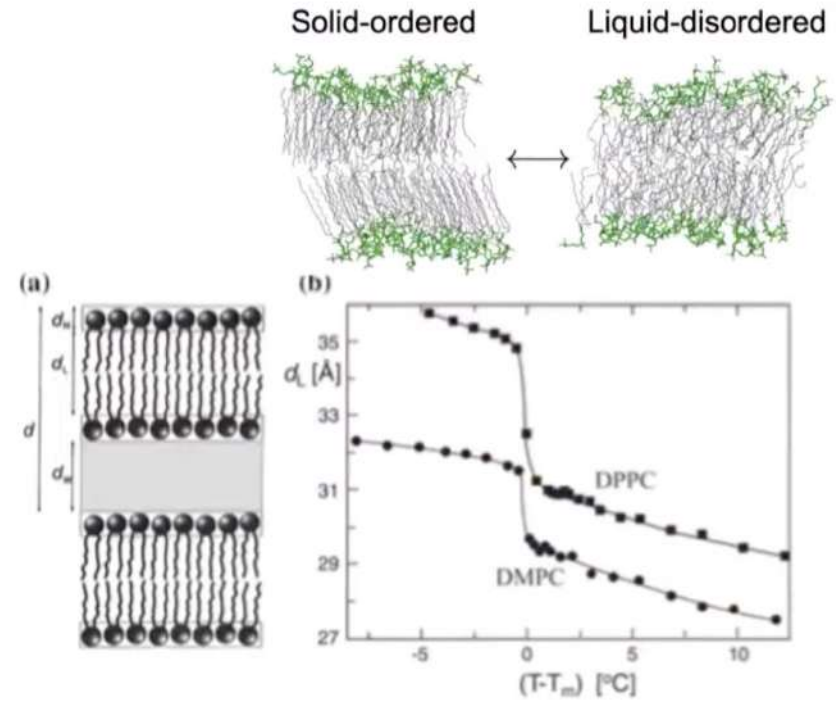
Thansition is very sharp: no chemical link among molecules in the layer, all molecules make the transition at the same time. Transition is dominated by thermal fluctuations

Phase transitions

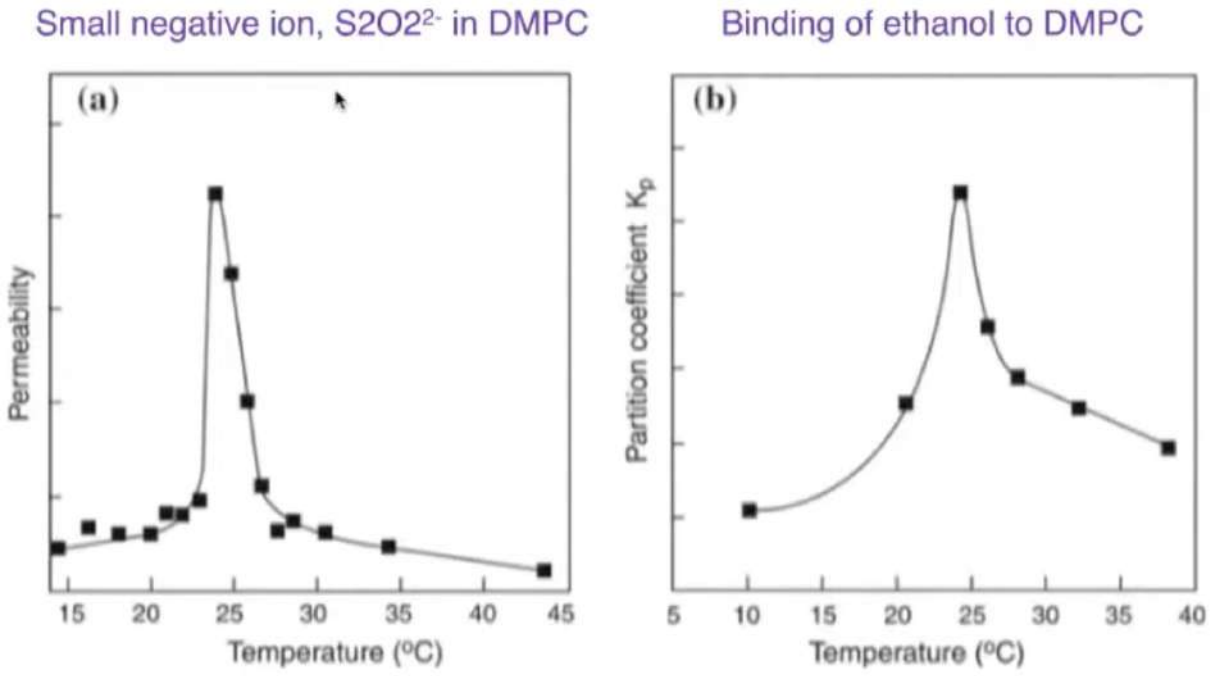


Phase transitions and thickness

Phase Transition Temperature Increases with Increasing Fatty Acyl Chain Length



Phase transitions
Leaky membranes in lipid phase equilibria



Biophys. J. (2000) 78: 2486-2492

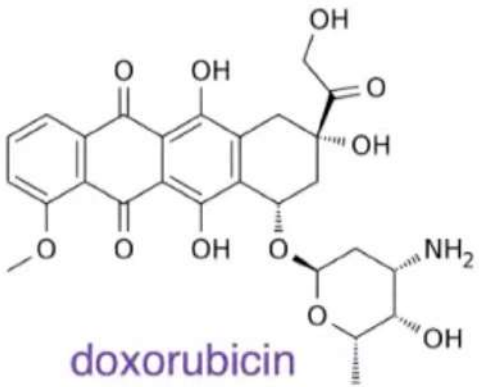
Leaky membranes at critical point of phase transition equilibria

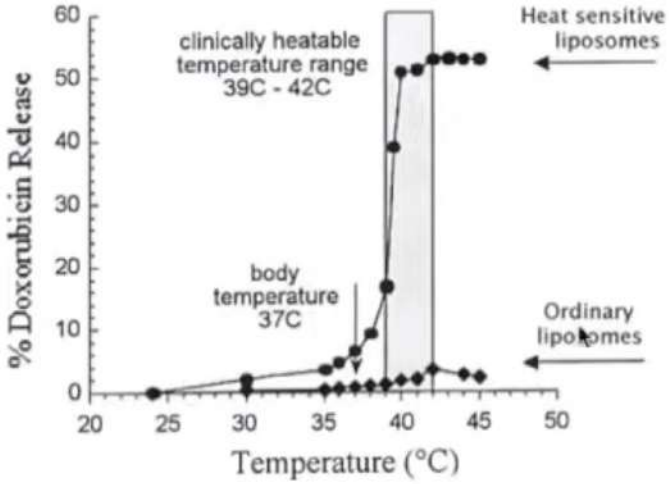
Organisms adapt lipid composition

	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

Liposome phase transition and drug delivery

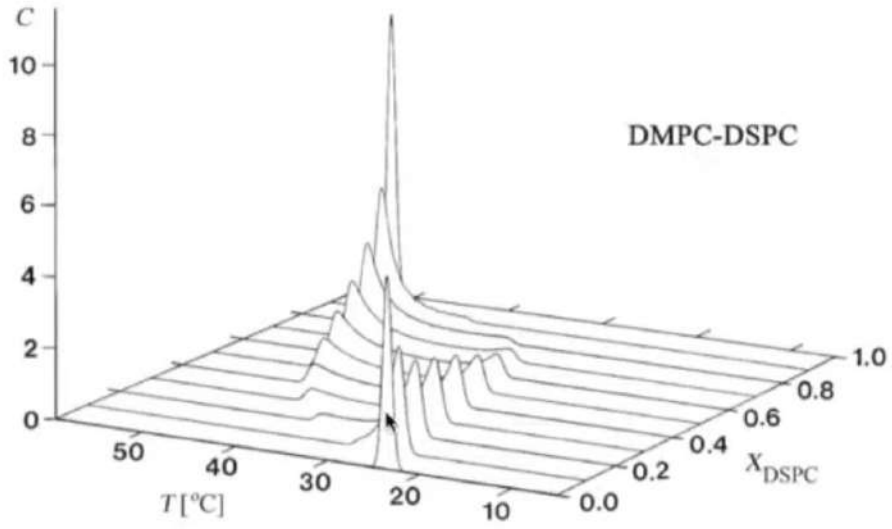


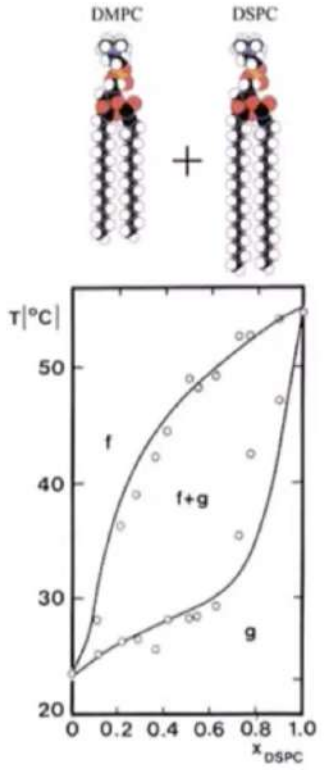


- The release of the drug from the heated liposomes is very fast, ~ 20 seconds
- Million times faster release than from ordinary liposomes
- ~30 times more drug can be delivered at the tumor site than conventional liposomes

Dr. David Needham

Phase separation, co-existence

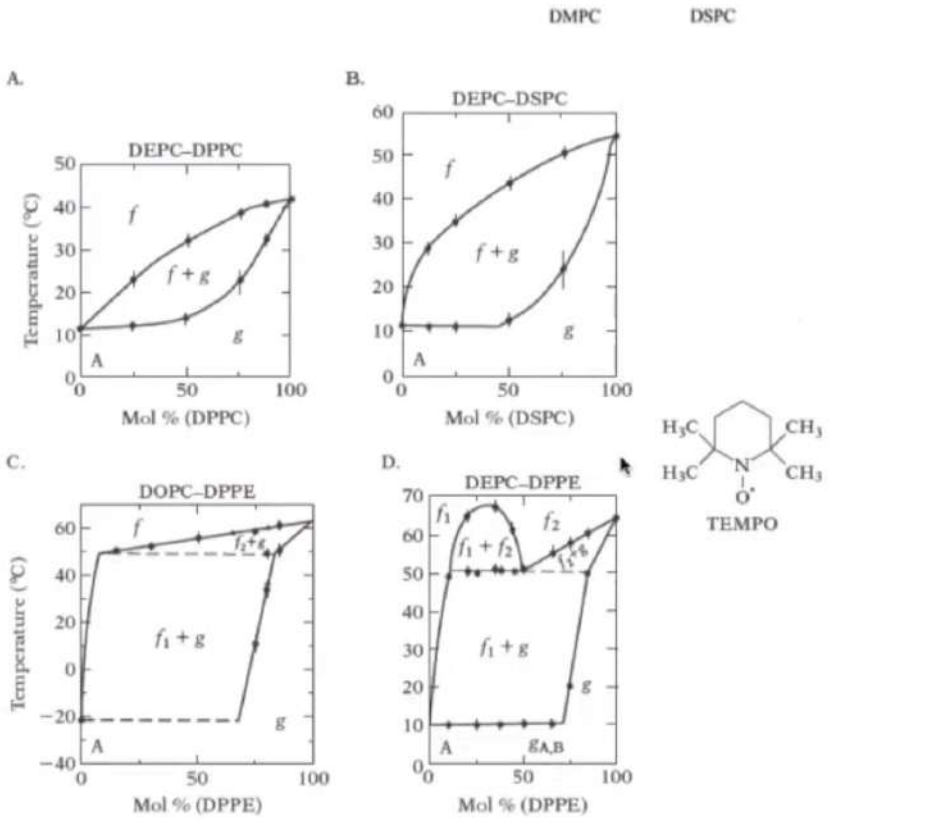




Biochim. Biophys. Acata (1988) 944: 121-134

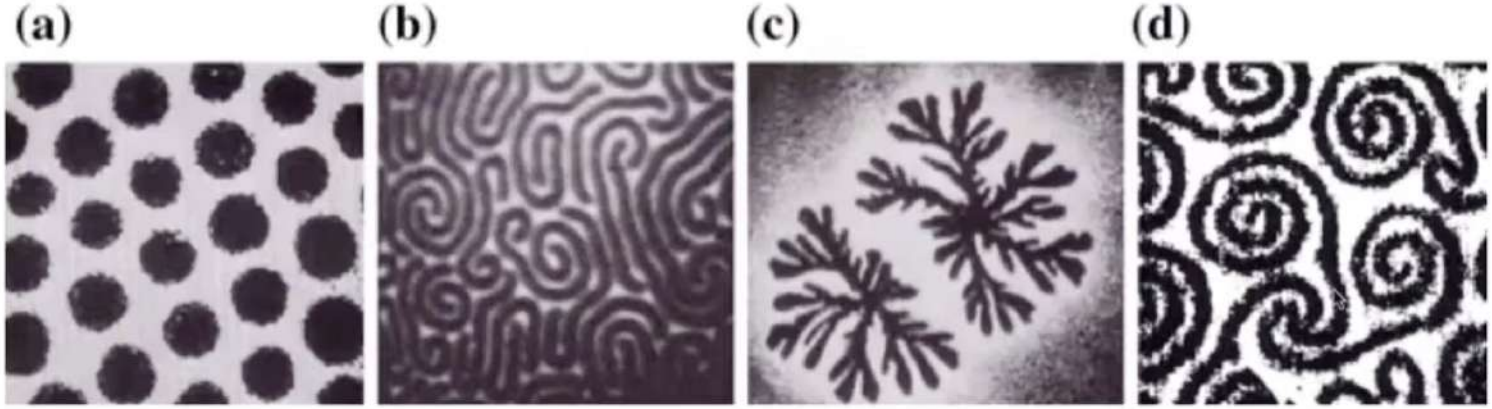
Phase separation, co-existence

DEPC - dierucoyl phosphatidylcholine (22:1, Δ13)



Biochemistry (1975) 14: 847-854

Phase separation, co-existence



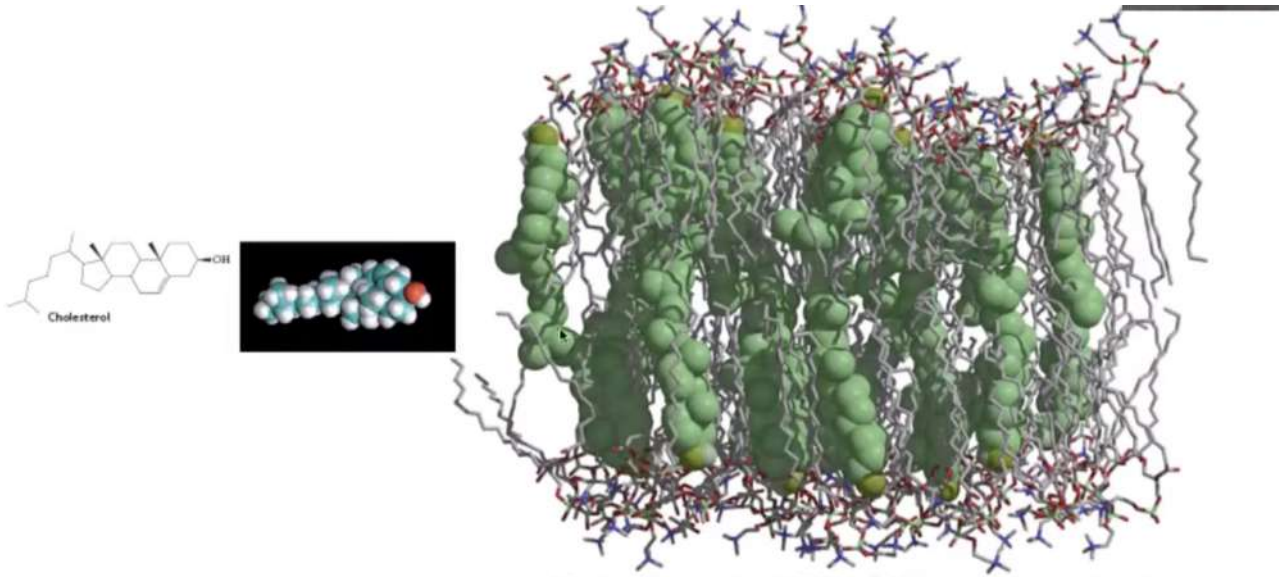
a) Coexistence of liquid phase (light) and solid phase

b) Striped pattern

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

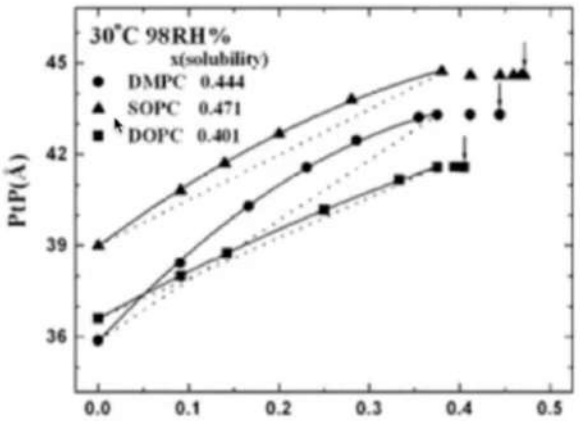
d) Spiral solid domains in a lipid monolayer with cholesterol

Cholesterol promotes lipid order



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

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

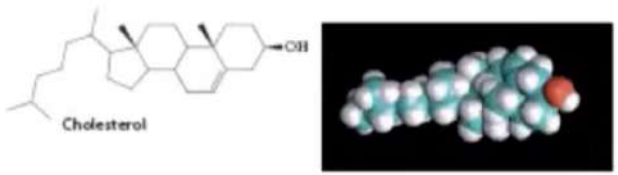
Biophys. J. (2007) 92: 3960-3967

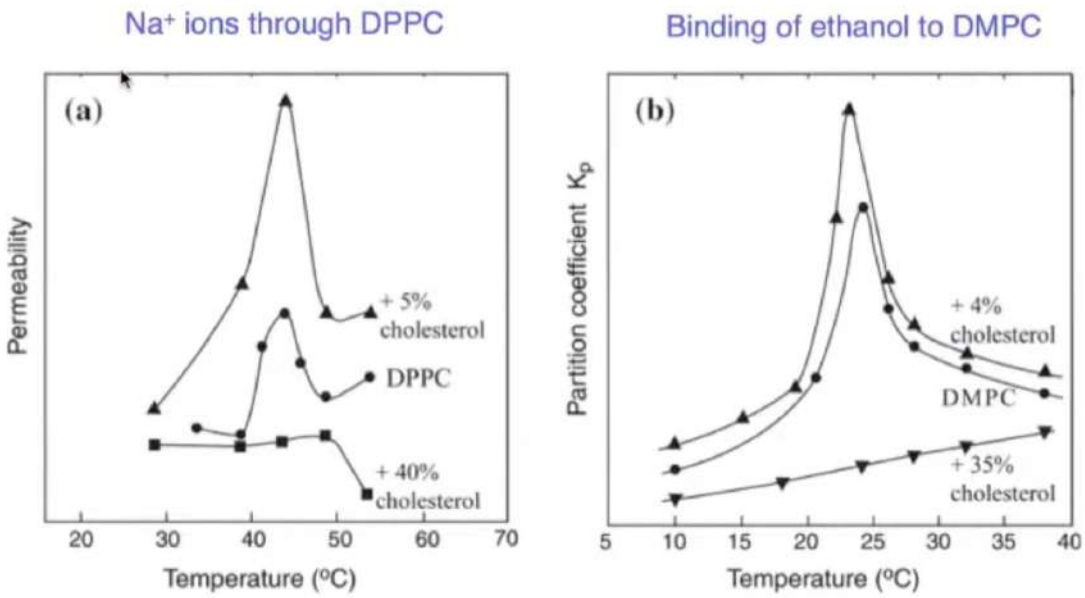
The hydrophobic membrane thickness in fluid phase is strongly dependent on the amount of cholesterol incorporated

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

Chol and permeability: dual role

H Raghurama

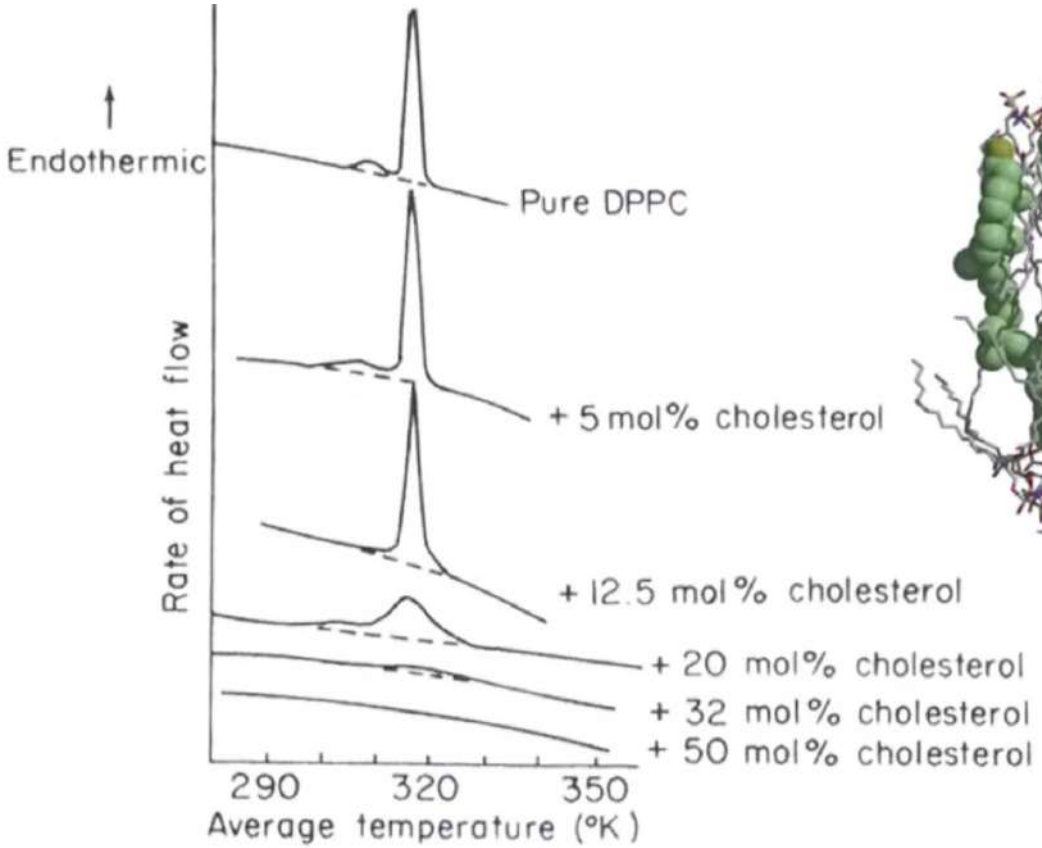


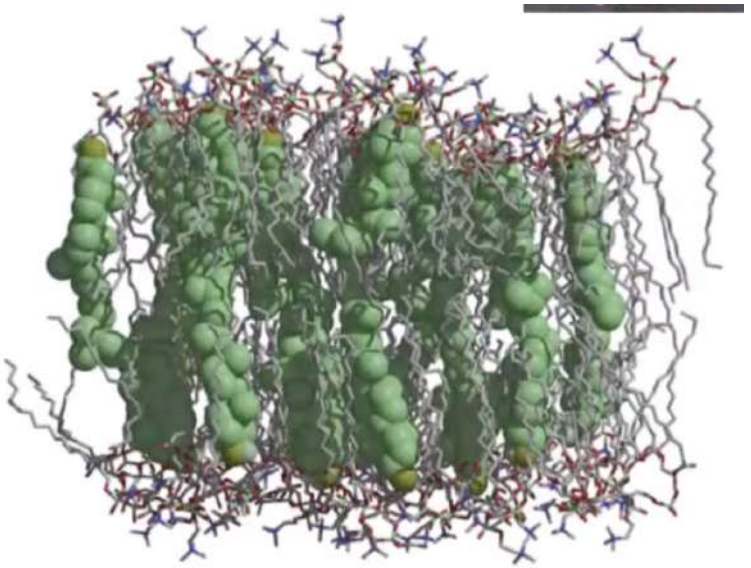


Biochim. Biophys. Acta (1992) 1107: 261-270
Biophys. J. (2000) 78: 2486-2492

Chol prevents ion permeability across the membrane!!!

Chol role in phase transition

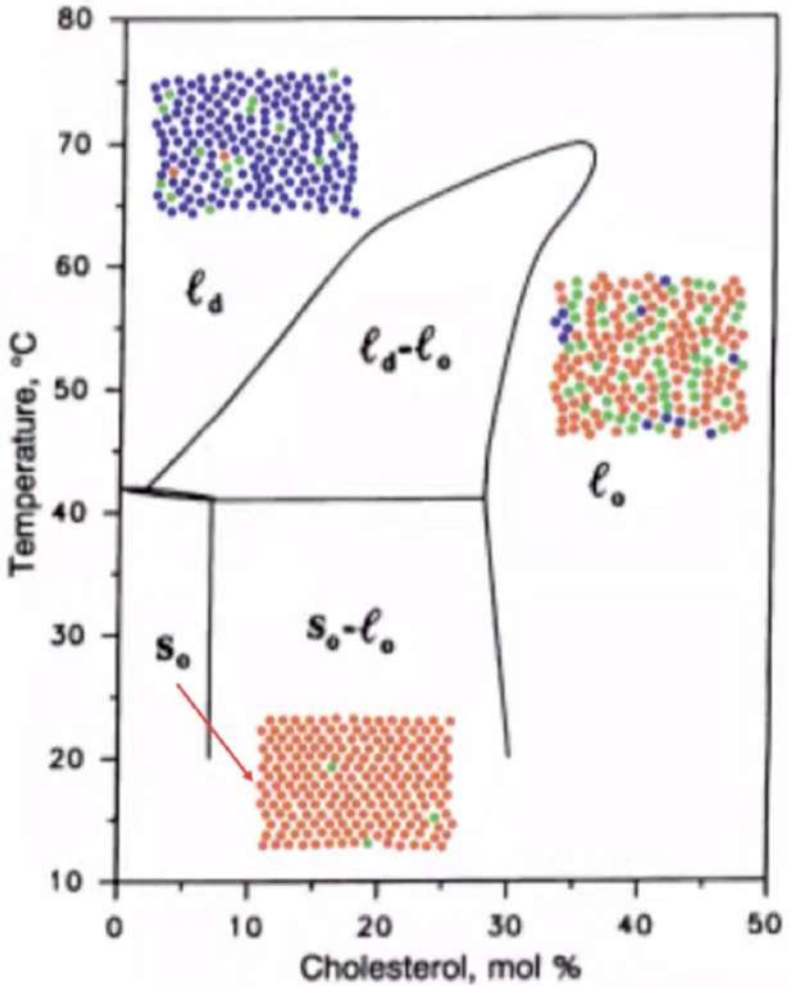




Chol reduces lipid cooperativity!! The new phase is called liquid-ordered phase.

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

Condensing effect of Chol on different phases

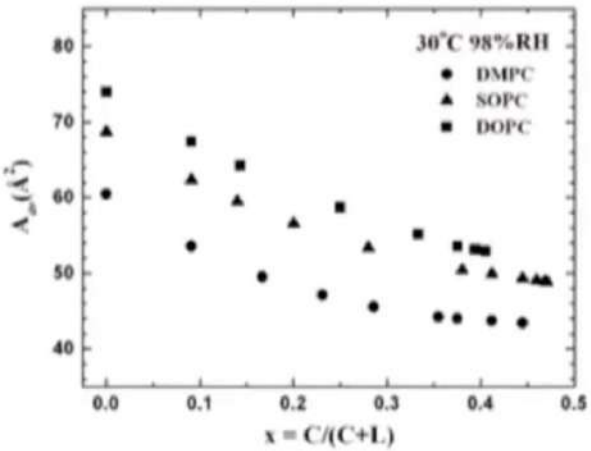
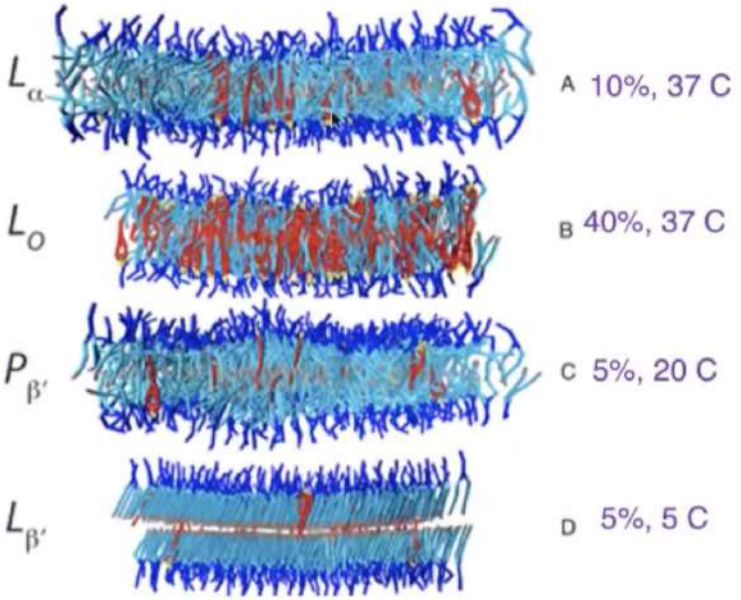


FIGURE 7 Area per molecule as a function of cholesterol concentration. The averaged cross section area of phospholipid is calculated by $A_{av,pc} = 2V_c/(PtP - 10)$, where V_c is the chain volume of the lipid (36), and the thickness of the hydrocarbon region is PtP minus twice the length of the glycerol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

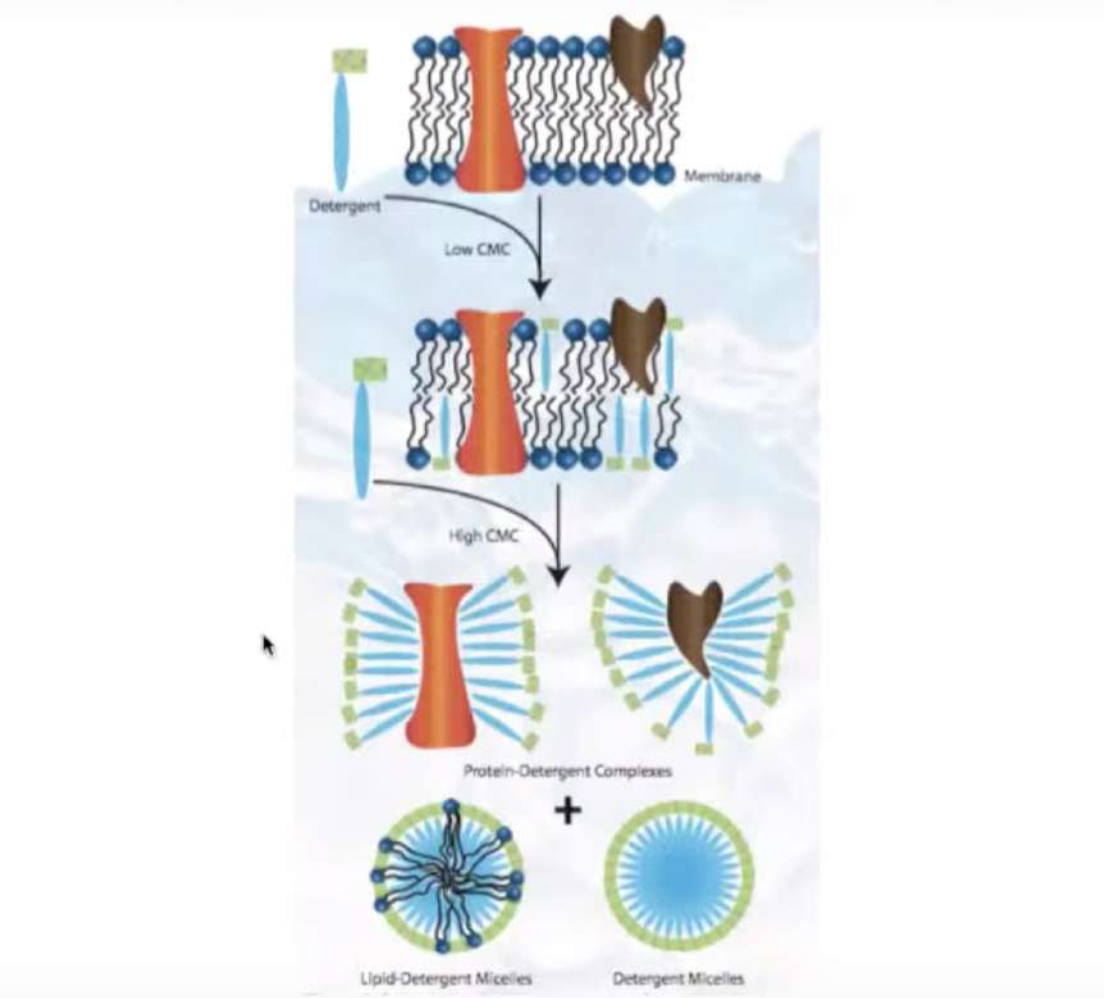
Biophys. J. (2007) 92: 3960-3967



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

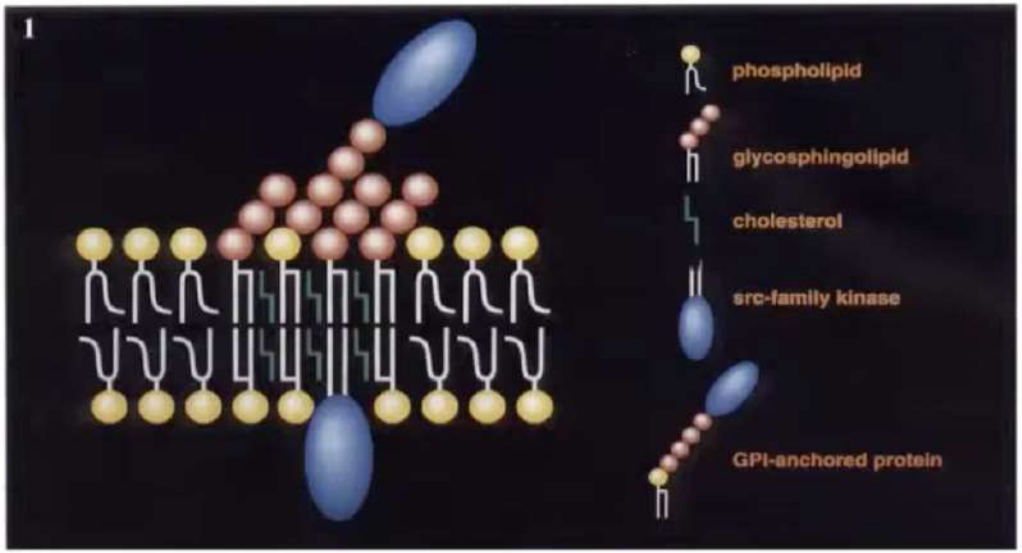
Combination of these factors !

Why are domains needed ?

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

Membrane domains

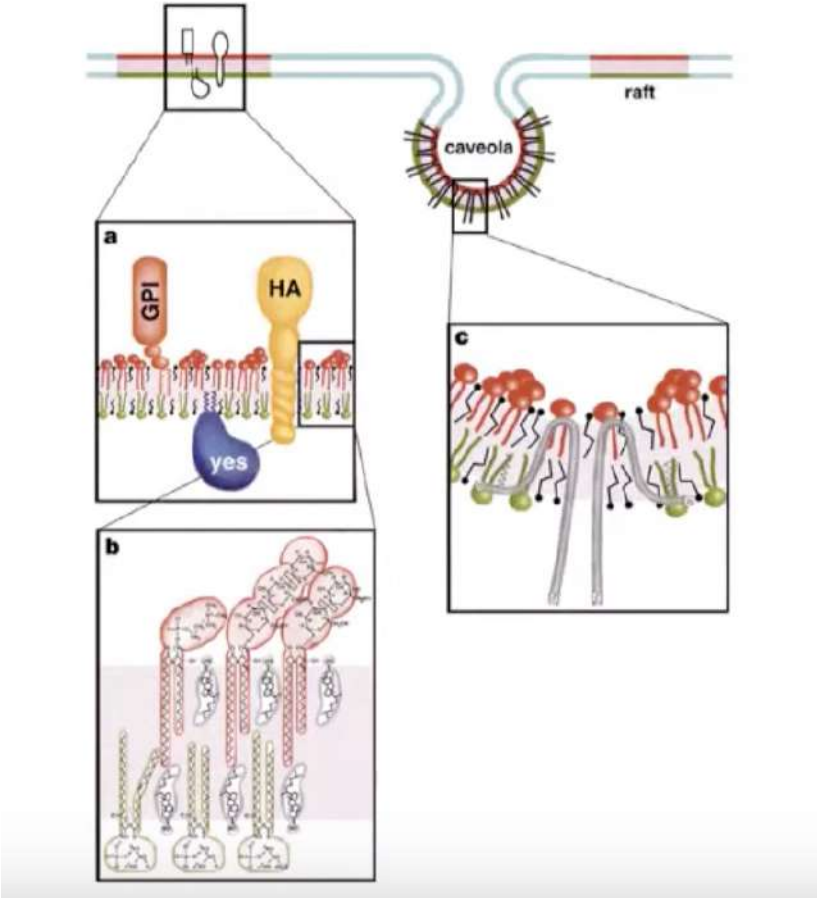
How do the proteins linked at two different sides of the membrane communicate?



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

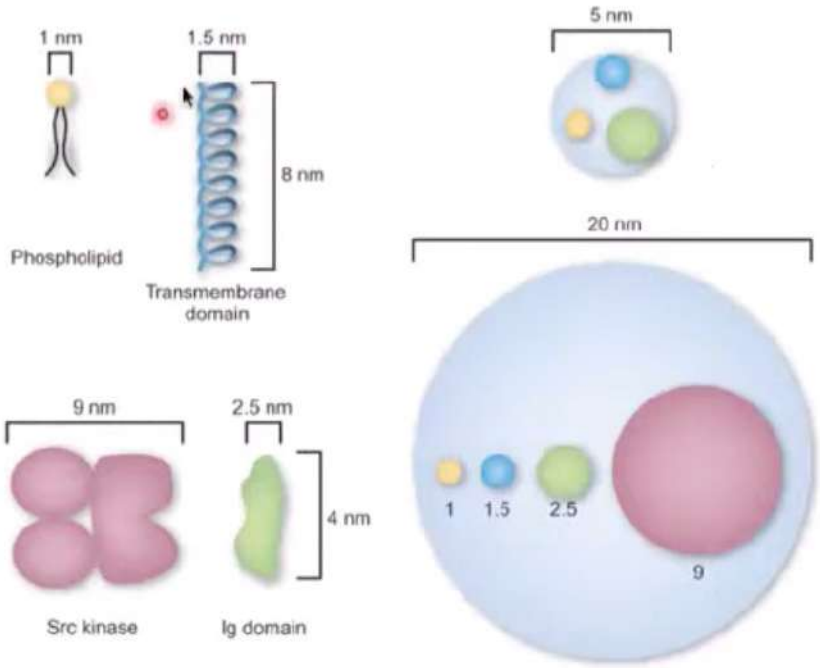
"Membrane Rafts are small (10-200 nm), heterogeneous, highly dynamic, sterol-and sphingolipid-enriched domains that compartmentalize cellular processes. Small rafts can sometimes be stabilized to form larger platforms through protein-protein and protein-lipid interactions"

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 !

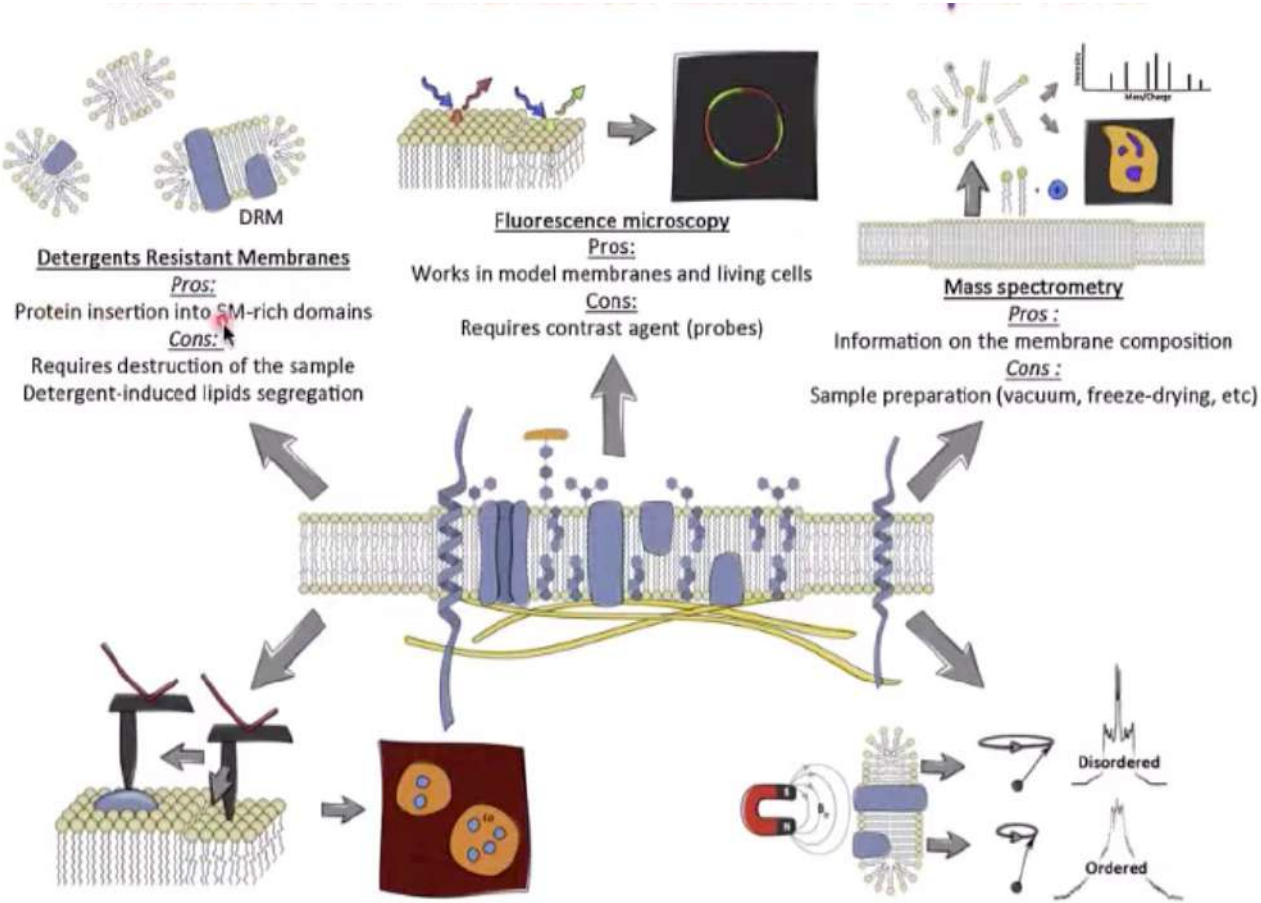
Simons and Ikonen (1997) *Nature* 387: 569-572
(> 8700 citations)

How big is a membrane raft



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

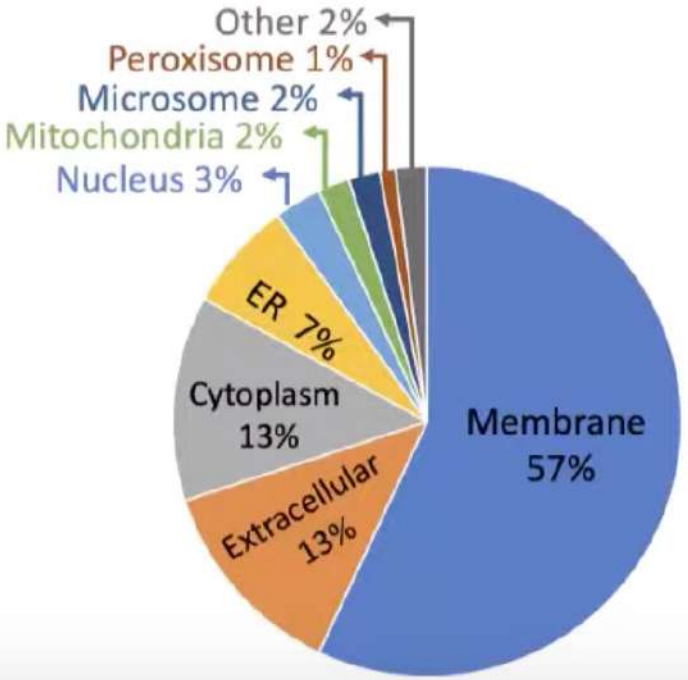
Raft characterization



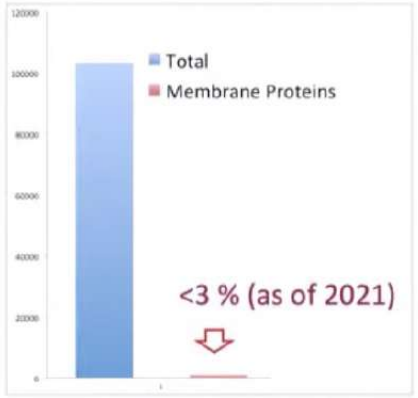
Chem. Biol. (2014) 21: 97-113

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:

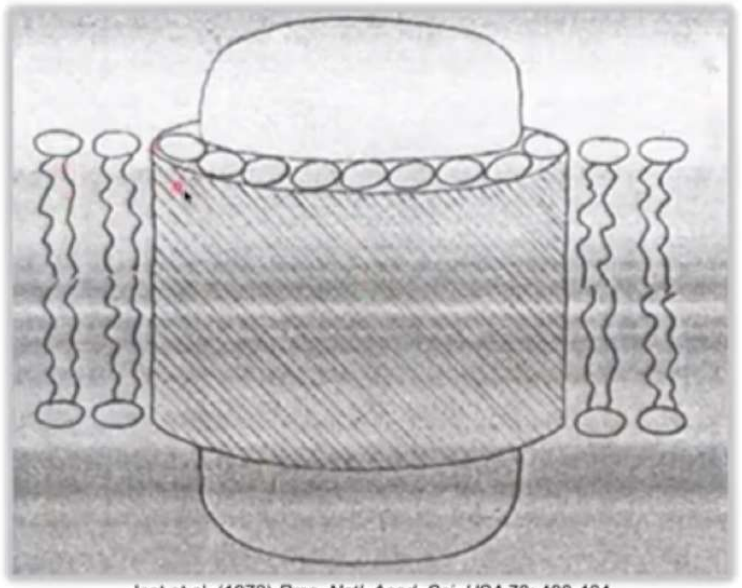
  At low lipid-to-protein ratio, a broad spectrum was observed

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

  Pure lipid showed only a sharp spectrum

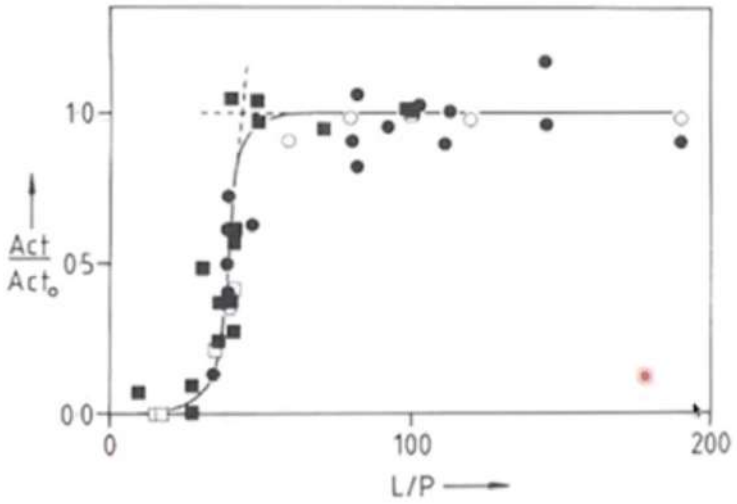
- The concept of 'Immobilized' lipids

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

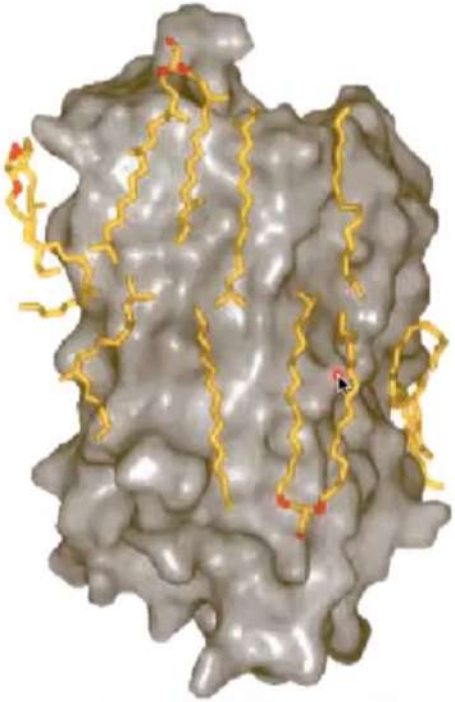


Nicotinic acetylcholine receptor activity

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

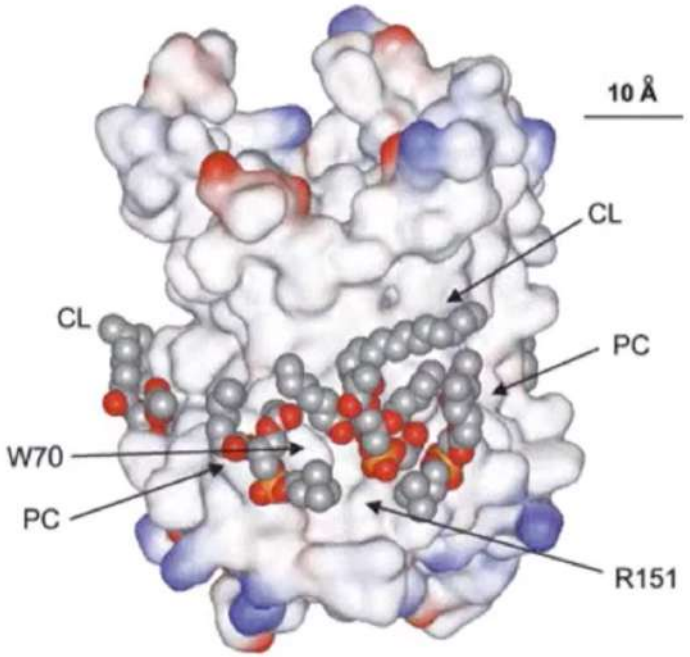
Lipid-protein interactions

Bacteriorhodopsin crystal structure
at 1.55 Å resolution



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

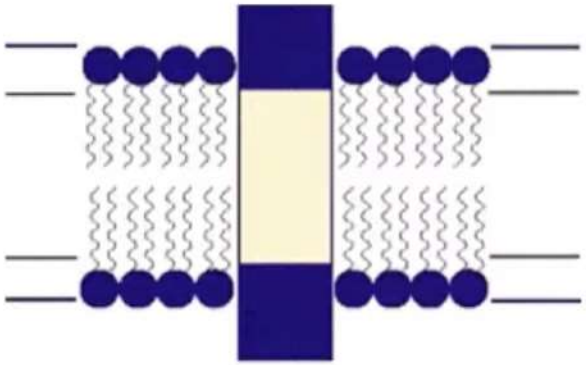
Mitochondrial ADP/ATP carrier
crystal structure at 2.2 Å resolution



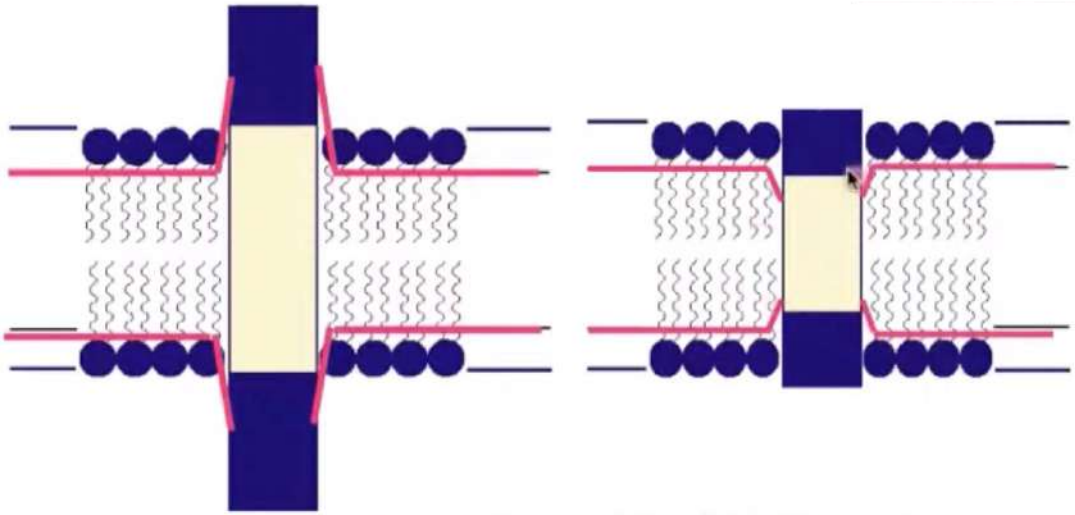
Pebay-Peyroula et al. (2003) *Nature* 426: 39-44

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

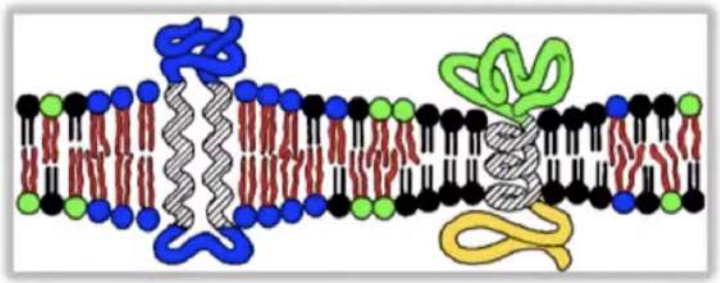


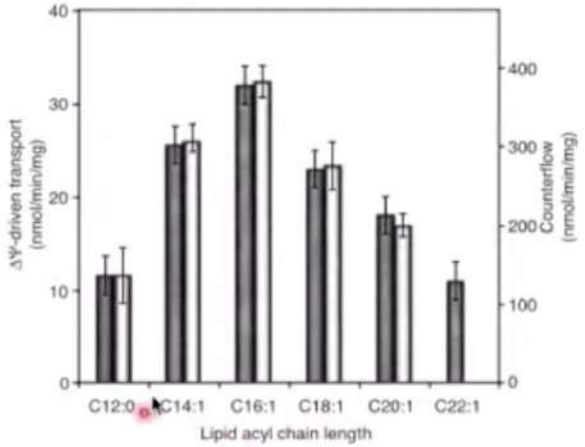
- When these do not match 'HYDROPHOBIC MISMATCH' occurs
- Mismatch is a result of the direct interaction of the transmembrane regions of the protein and the lipid acyl chains
- Mismatch is energetically unfavorable
- Membrane lipids and proteins must adapt to minimize mismatch

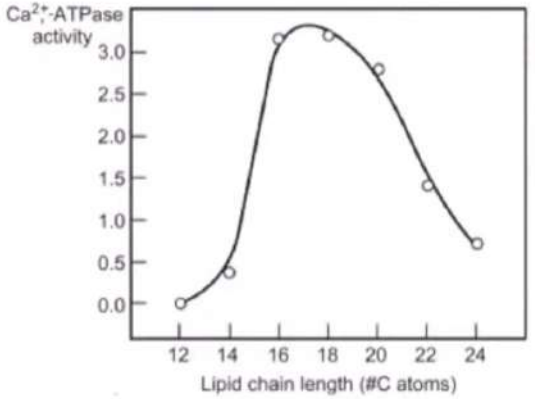
Hydrophobic mismatch

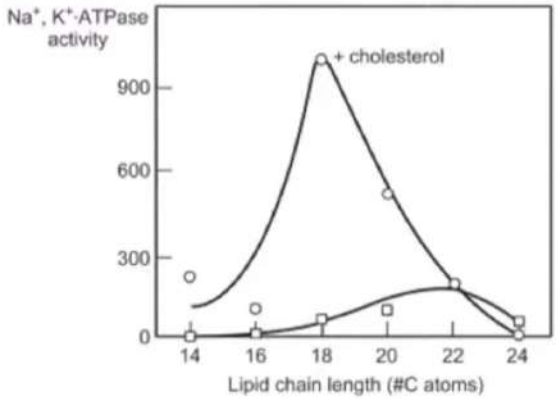
- In eukaryotic cells, there is a gradient of increasing bilayer thickness from ER to Golgi to Plasma membranes. All membrane proteins have to traverse this path.
- Mismatch could play a role in such sorting.
- Eukaryotic membranes are heterogeneous mixtures of a variety of phospholipids, sphingolipids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







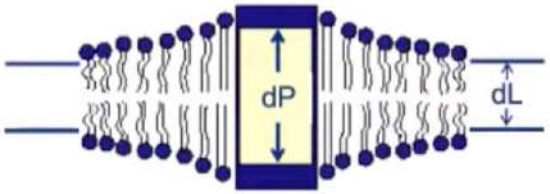


Biochim. Biophys. Acta (2004) 1666: 205-226
Biochemistry (2001) 40: 8842-8851
Biochemistry (2000) 39: 4846-4852

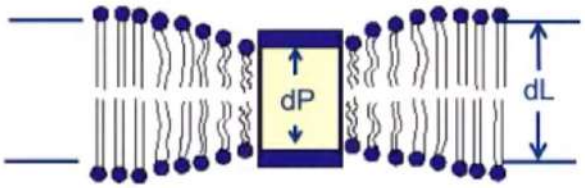
ATPasi: ion pumps

Adapting to mismatch

Lipid responses to mismatch

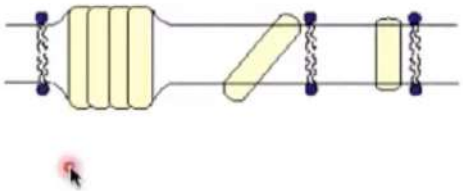


Long Proteins increase the Tm of short bilayers

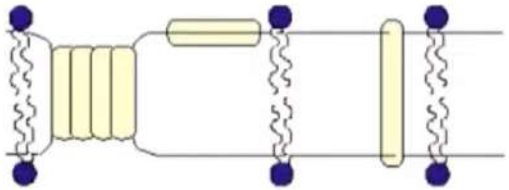


Short Proteins decrease the Tm of long bilayers

Protein responses to mismatch



Aggregation Helix Tilt Conformational Change

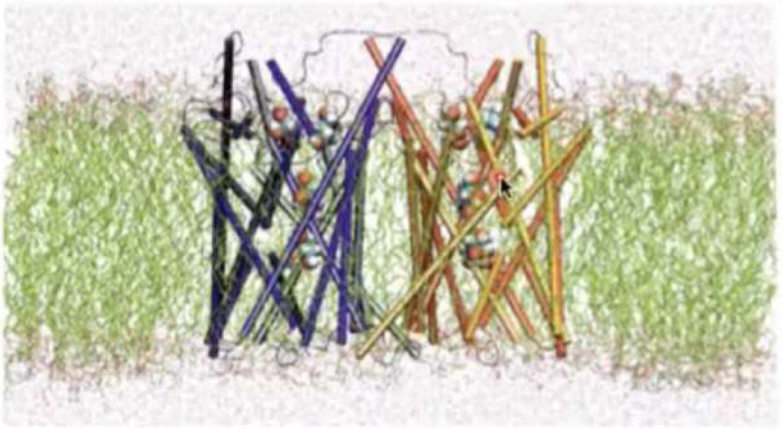


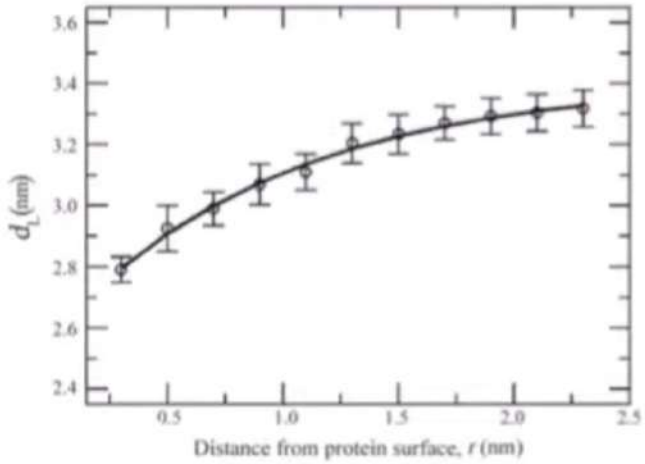
Aggregation Surface Orientation Conformational Change

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

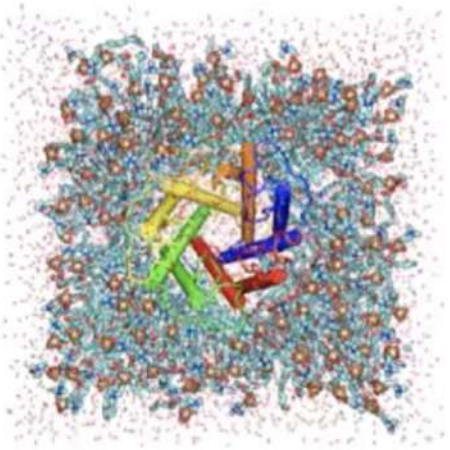
Adapting to mismatch: thinning

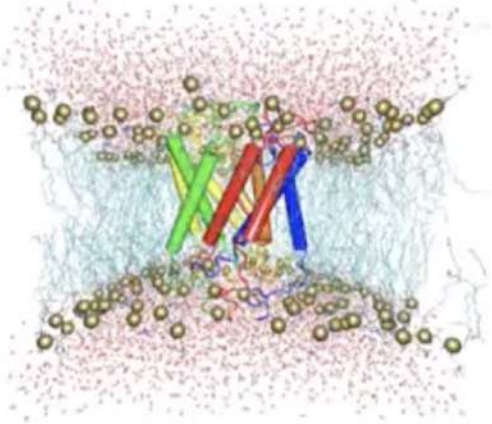
Aquaporin in fluid POPC bilayer





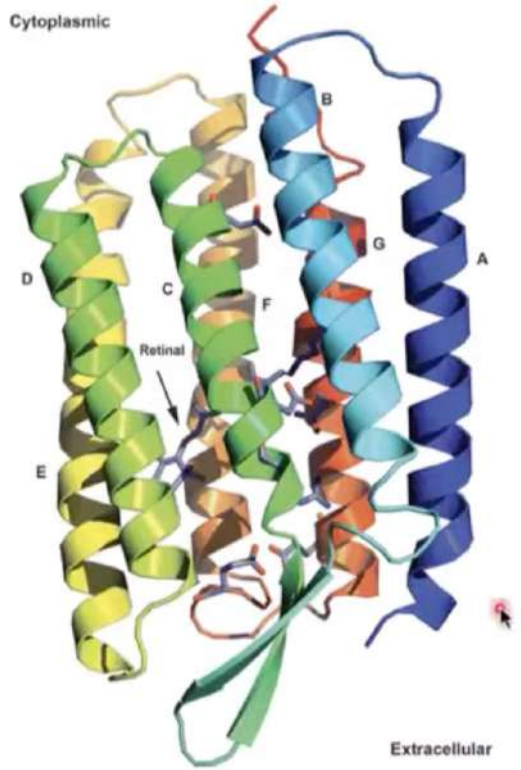
Mechanosensitive channel, MscL



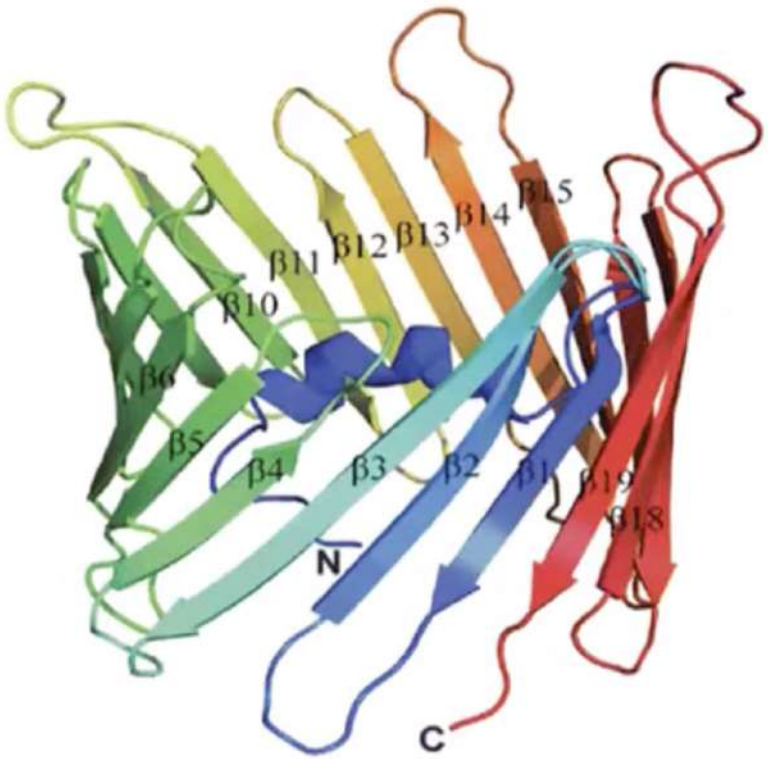


Life-As a matter of fat: lipids in membrane biophysics perspective by Ole G. Mouritsen & Luis A. Bagatolli, 2nd edn. 2015, Springer
Gullingsrud et al. (2001) *Biophys. J.* 80: 2074-2081

Membrane proteins



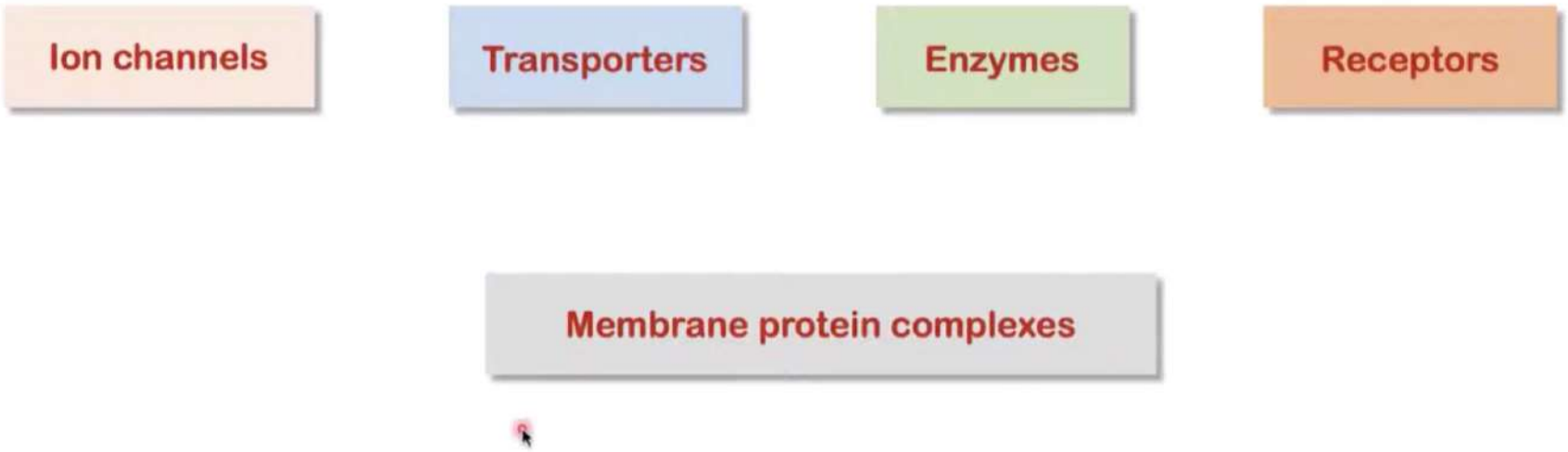
Bacteriorhodopsin
α-helical bundle



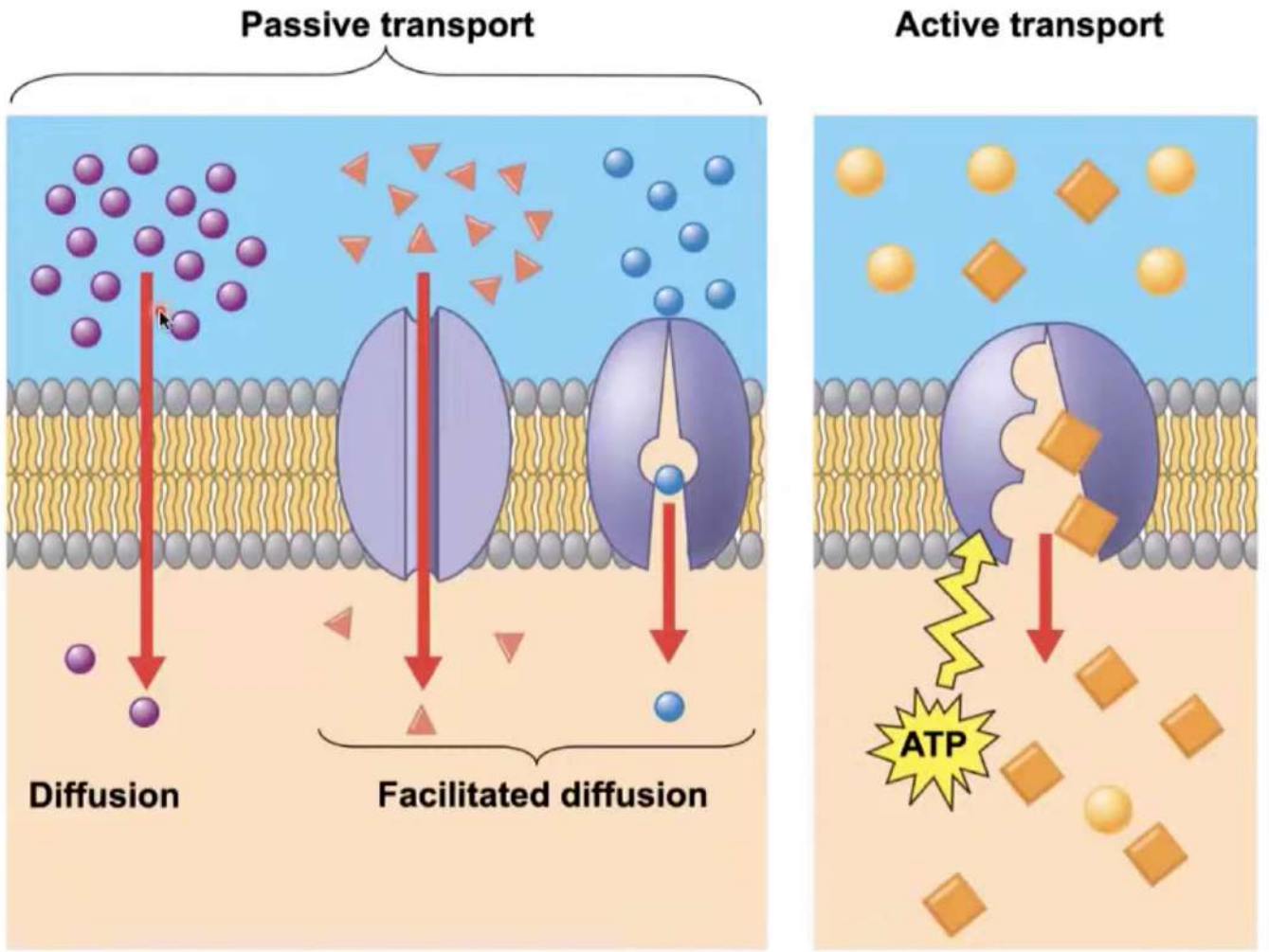
Mitochondrial porin, VDAC
β-barrel

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

Membrane proteins classes



Transporters



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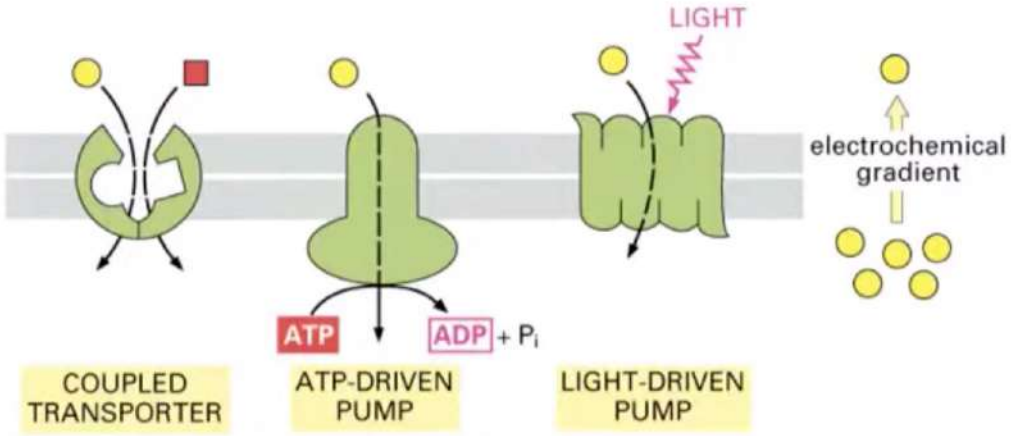
Active transport



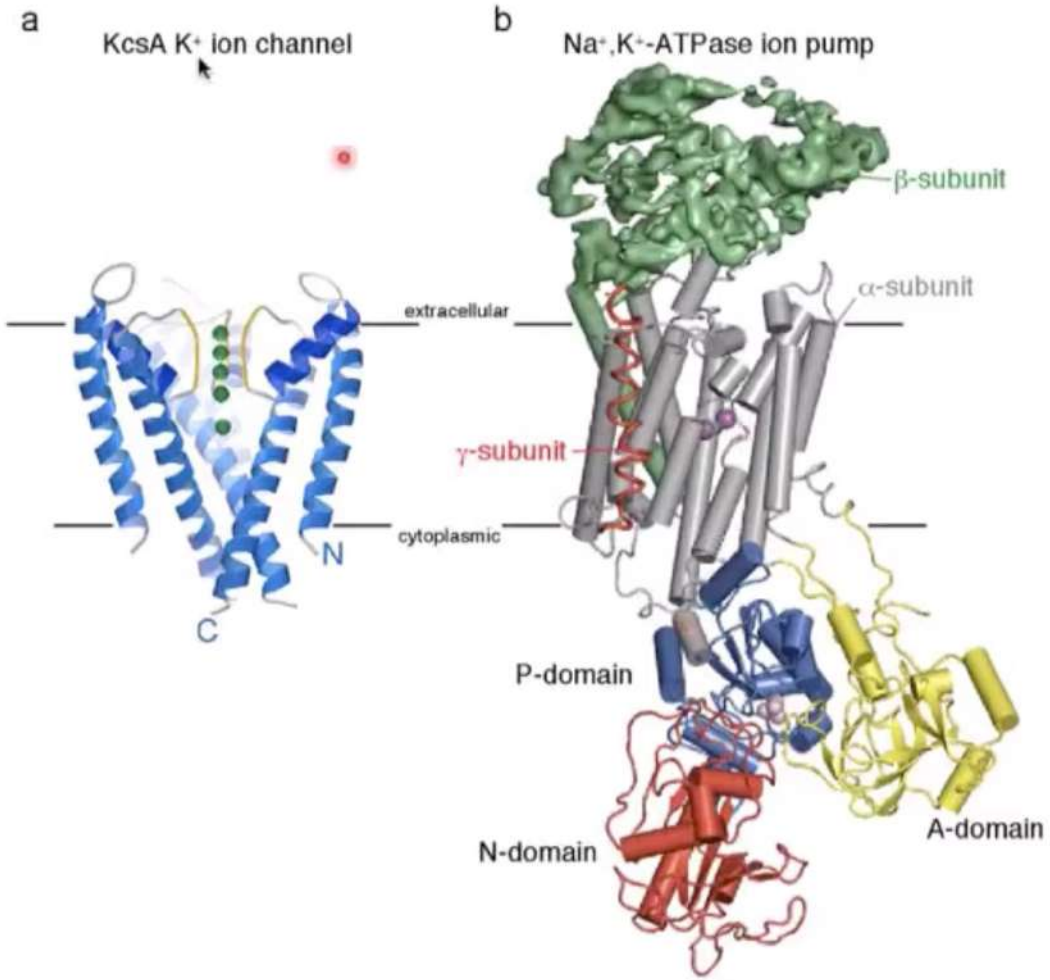
1. Coupled transporters couple uphill transport of one solute to the downhill transport of another
2. ATP-driven pumps use hydrolysis of ATP to uphill transport
3. Light driven pumps couple transport to light absorption

Membrane potential

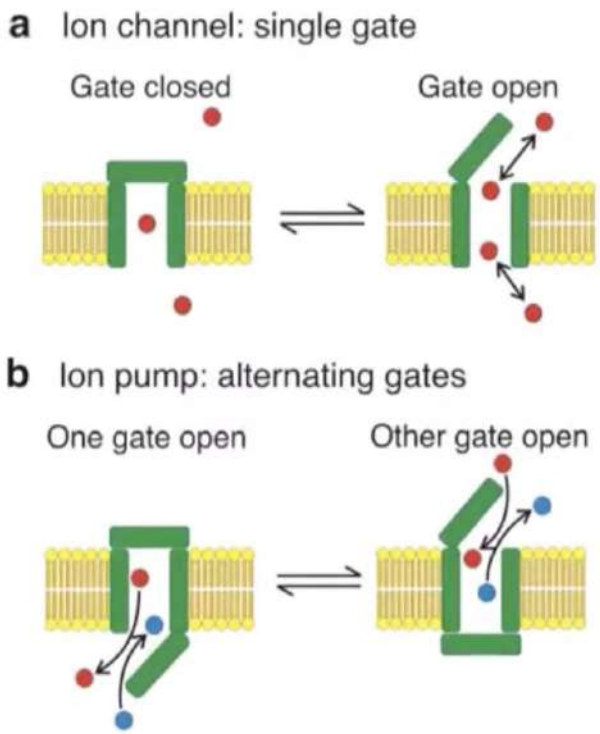
- Membrane potential is the voltage difference across a membrane

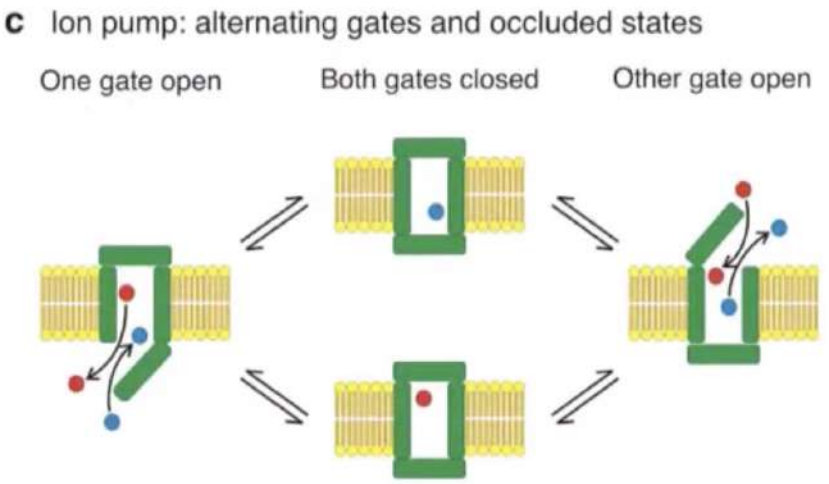
- Two combined forces, collectively called the electrochemical gradient, drive the diffusion of ions across a membrane:
  - A chemical force (the ion's concentration gradient)
  - An electrical force (the effect of the membrane potential on ion's movement)

Channels vs. pumps



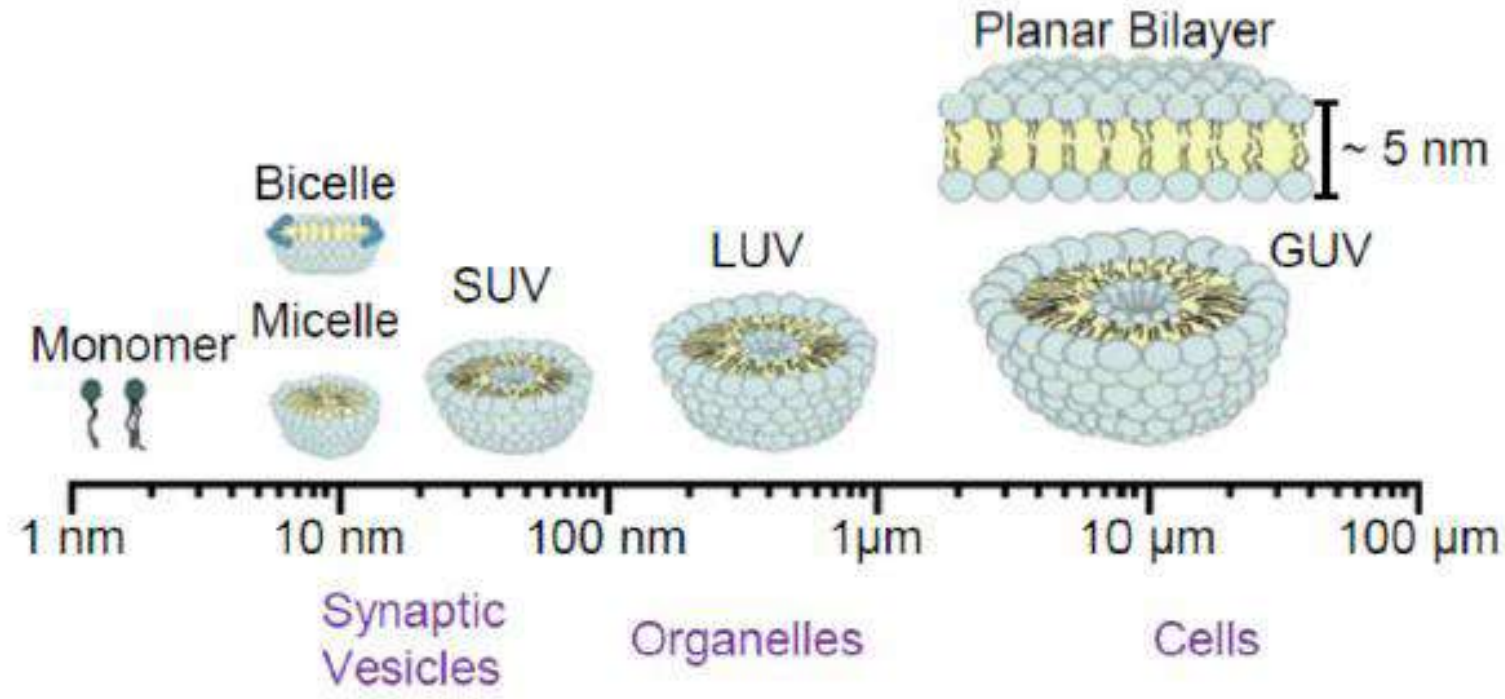
Channels vs. pumps

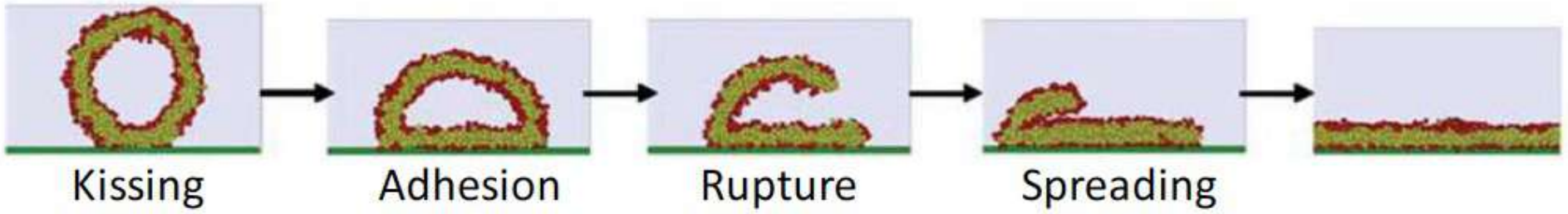




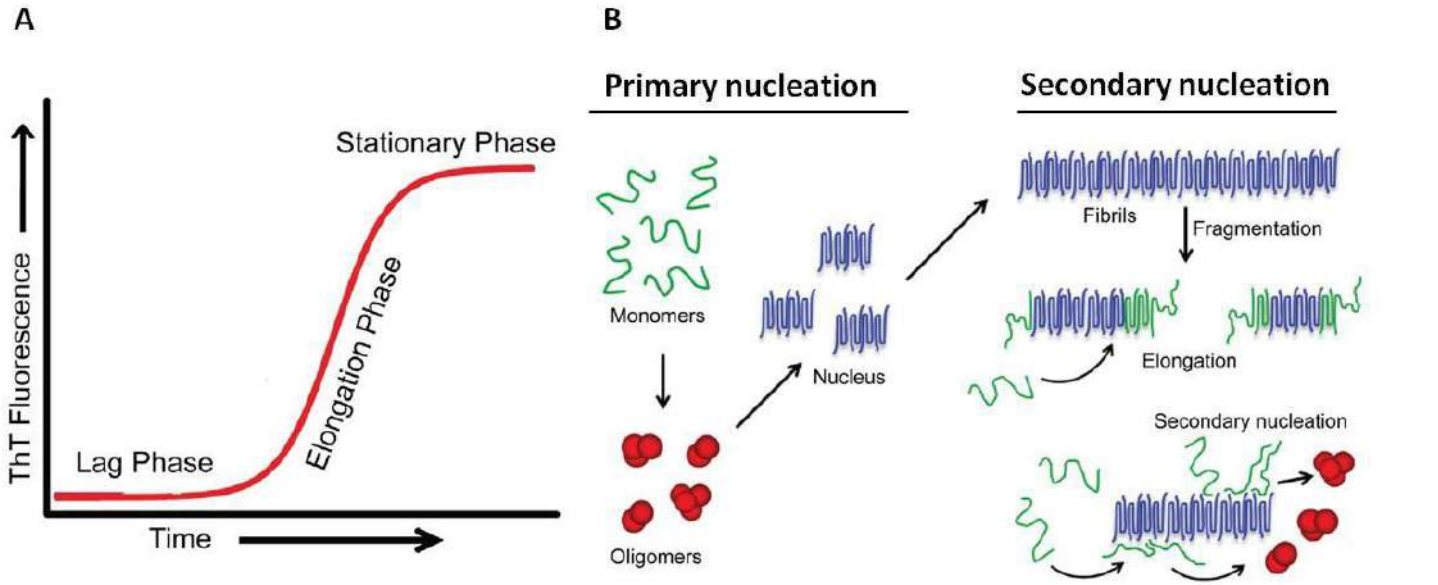
Gadsby (2009) *Nat. Rev. Mol. Cell Biol.* 10: 344-352

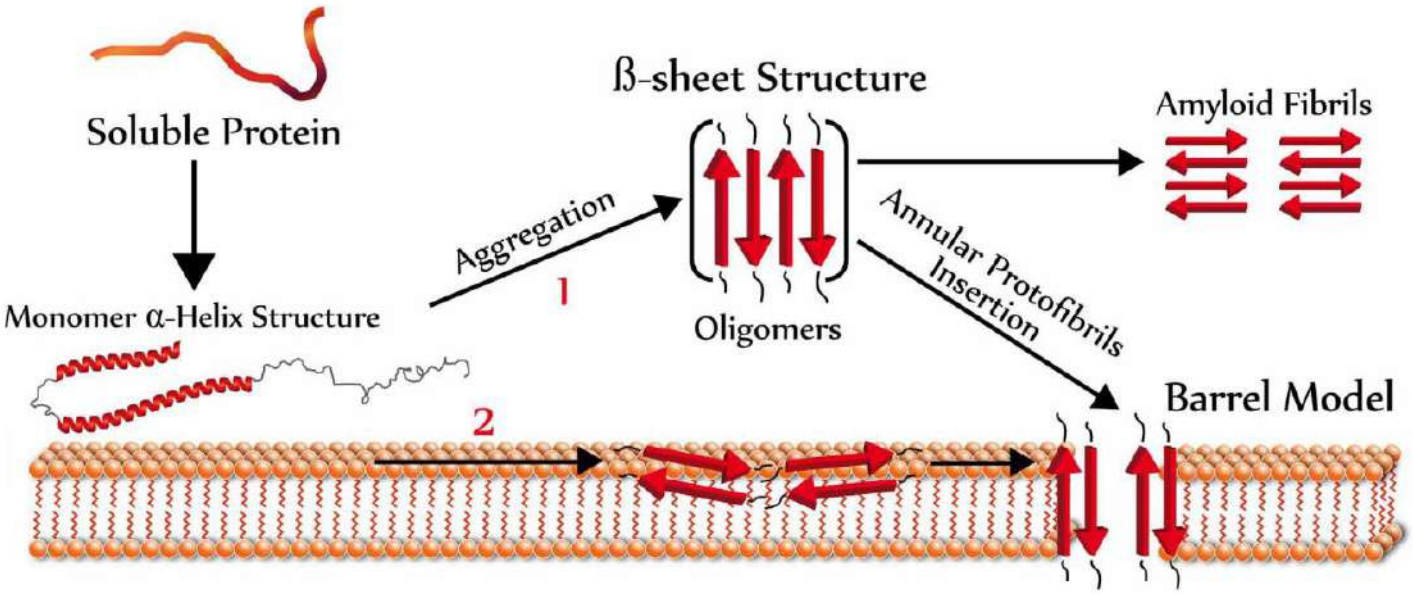
Model membranes



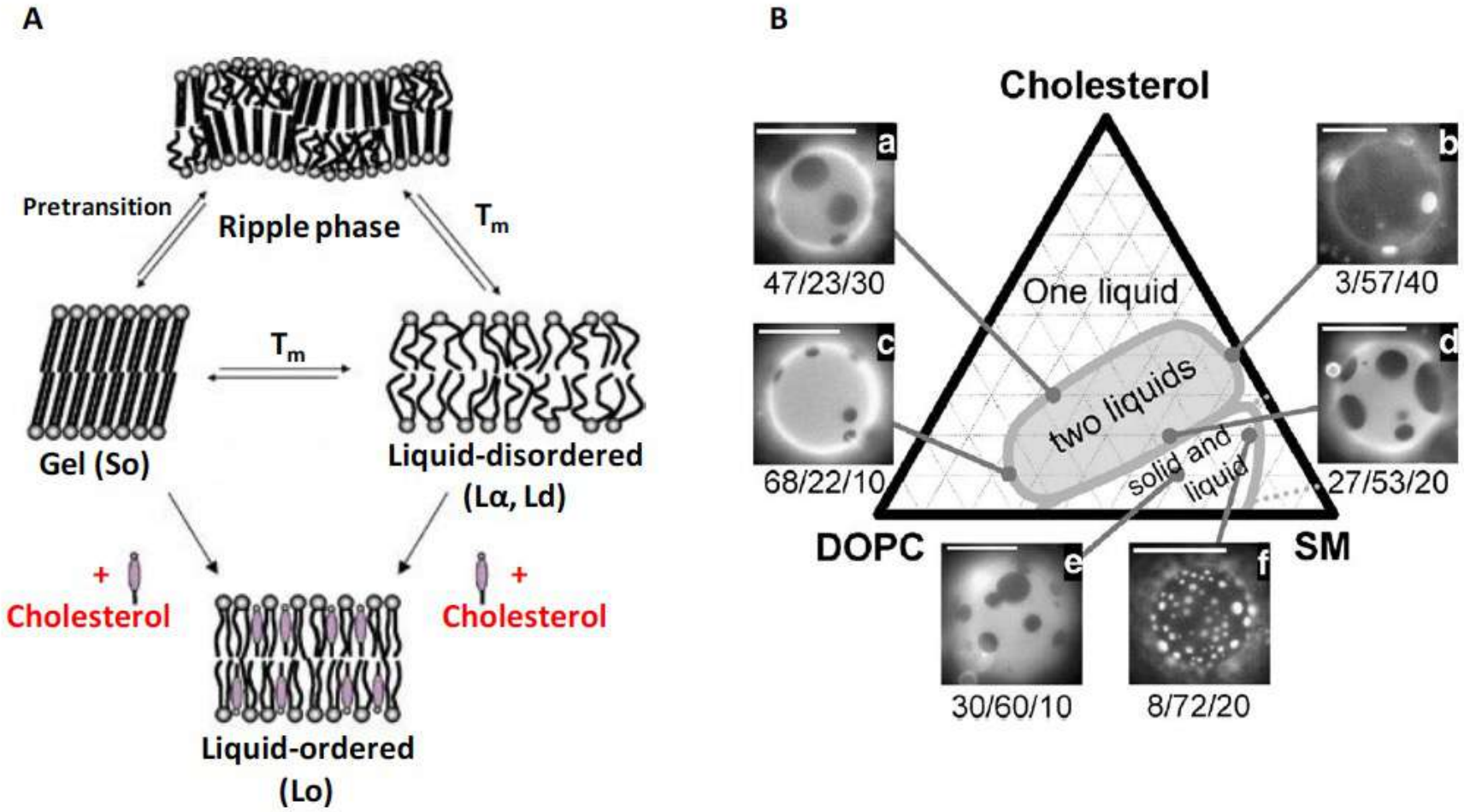


Study of unstructured protein oligomerization

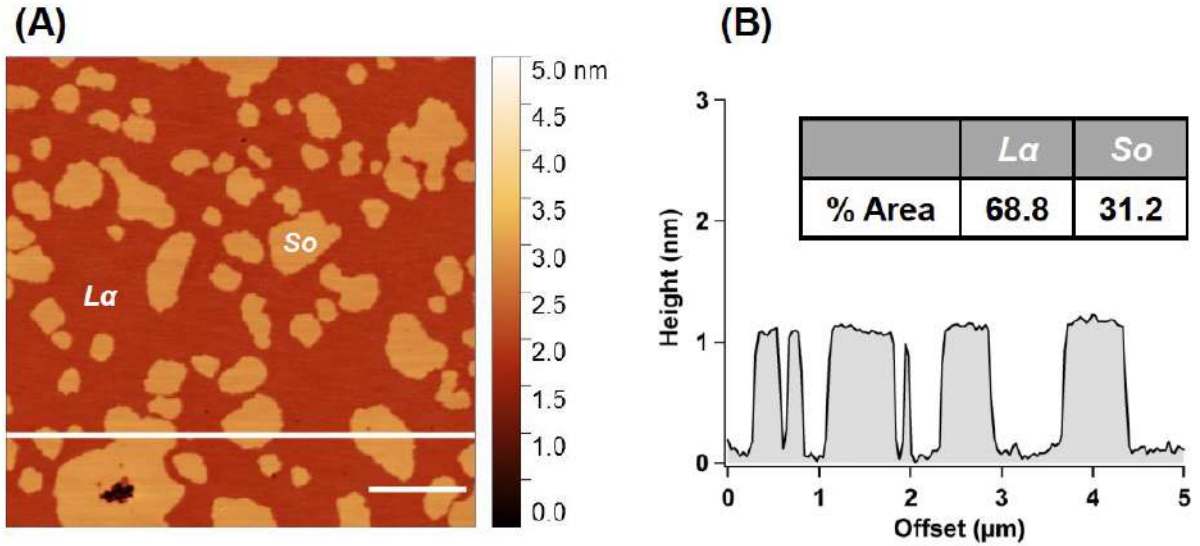




Multicomponent lipid membranes



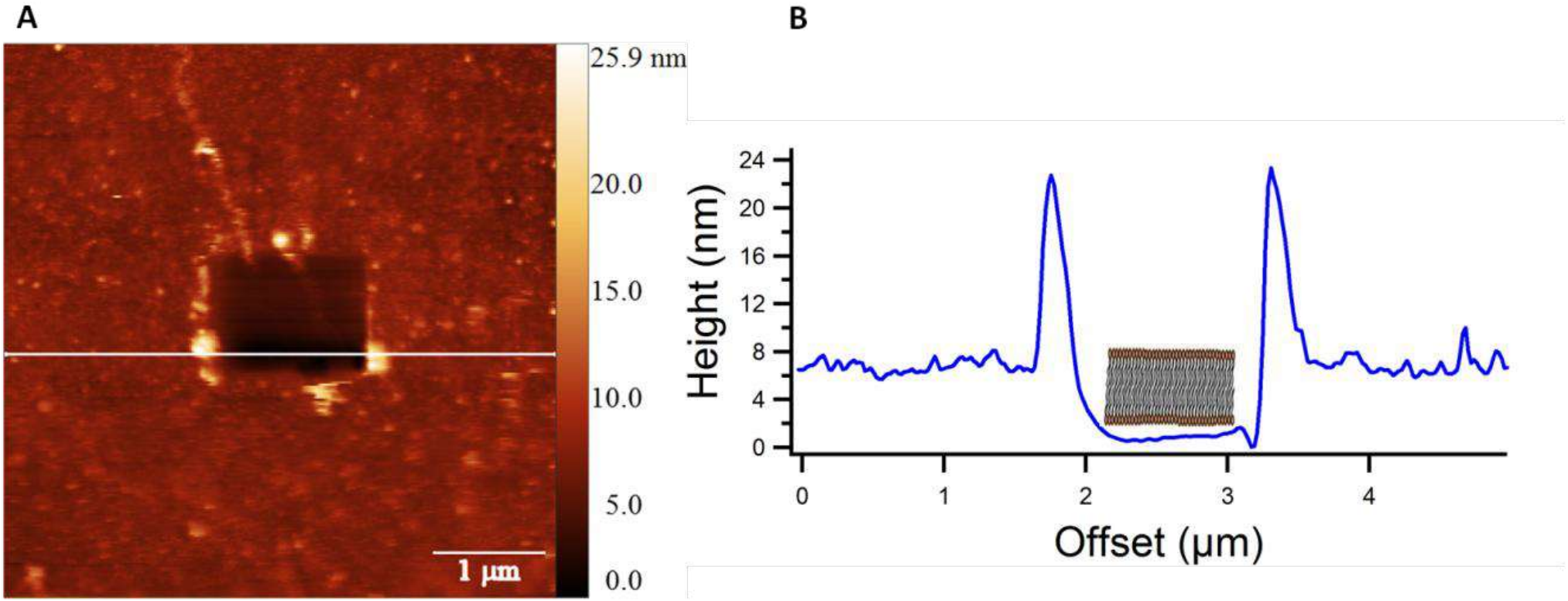
Model cell membranes-rafts



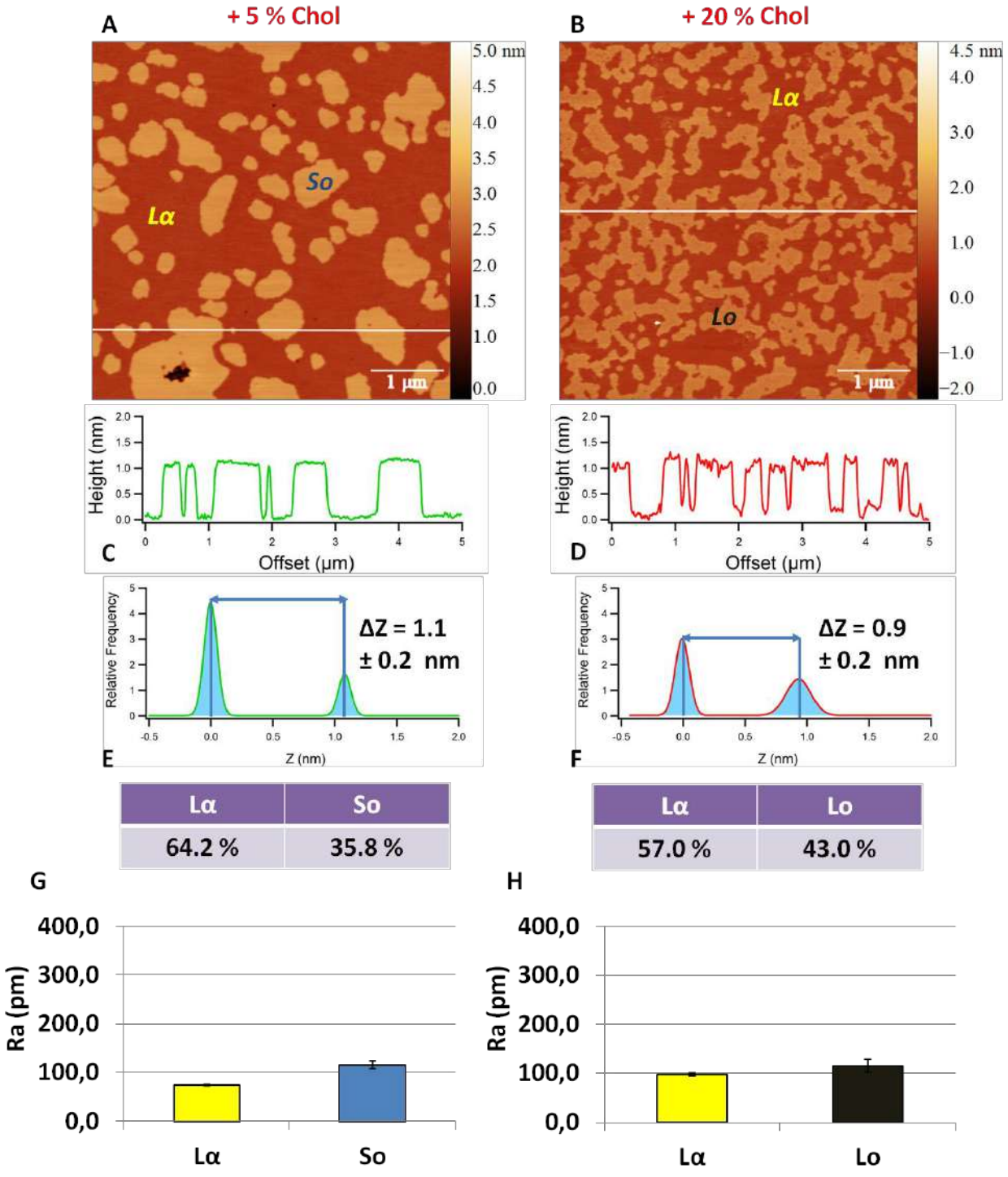
Coexistence of liquid-disordered (*Lα*) and solid-ordered (*So*) phase in planar supported lipid bilayer mimicking raft-like domains.

(A) AFM topography image of ternary SLB composed by DOPC, SM and cholesterol (66:33 +5% Chol). Image was taken in aqueous buffer in dynamic AC-mode. Scale bar: 1.0 µm. **(B)** Section analysis (white line in (A)) shows So-domains protruding from the fluid matrix (Lα) of SLB of ≈ 1.0 nm. **(C)** The height distribution graph

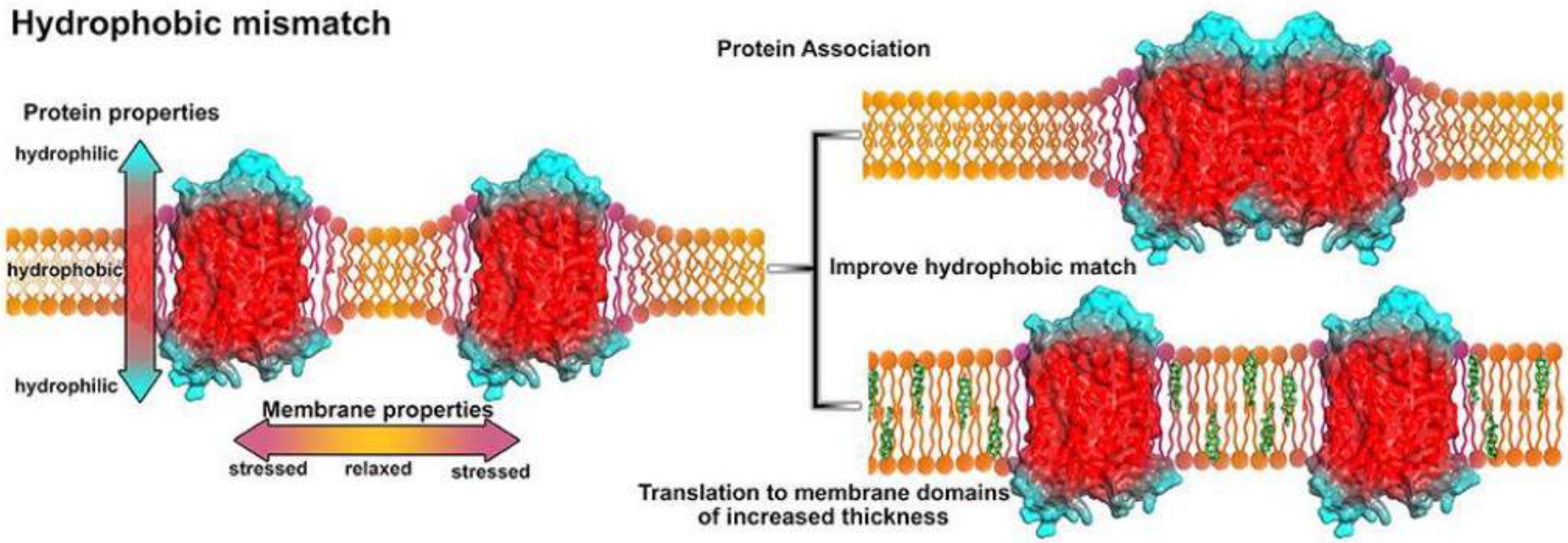
Model membranes-rafts



DOPC/SM (66:33)

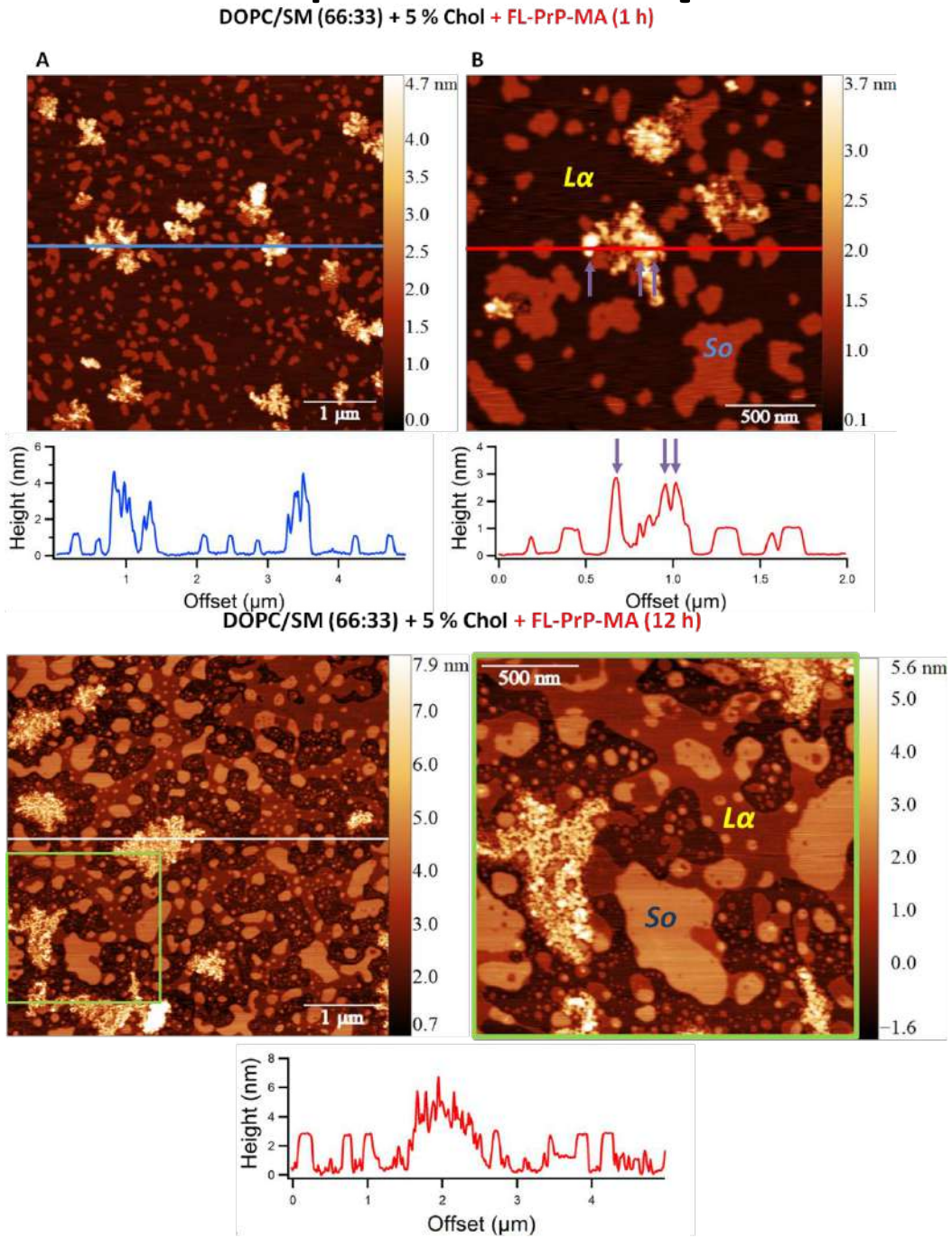

A
+ 5 % Chol
Lα
So
1 µm
5.0 nm
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.0
B
+ 20 % Chol
Lα
Lo
1 µm
4.5 nm
4.0
3.0
2.0
1.0
0.0
−1.0
−2.0
Height (nm)
Offset (µm)
C
D
Relative Frequency
Z (nm)
ΔZ = 1.1
± 0.2 nm
ΔZ = 0.9
± 0.2 nm
E
F
Lα
So
64.2 %
35.8 %
Lα
Lo
57.0 %
43.0 %
G
H
Ra (pm)
400,0
300,0
200,0
100,0
0,0
Lα
So
Lα
Lo

Membrane proteins



Lipid-protein interactions are very important for the stabilization of protein structure, regulation of protein activity and for partition of proteins in different lipid domains, as in lipid rafts. At the molecular level, these interactions drive the complex organization of plasma membrane.

Membrane proteins: **Lipidated-Prion protein (PrPC)**

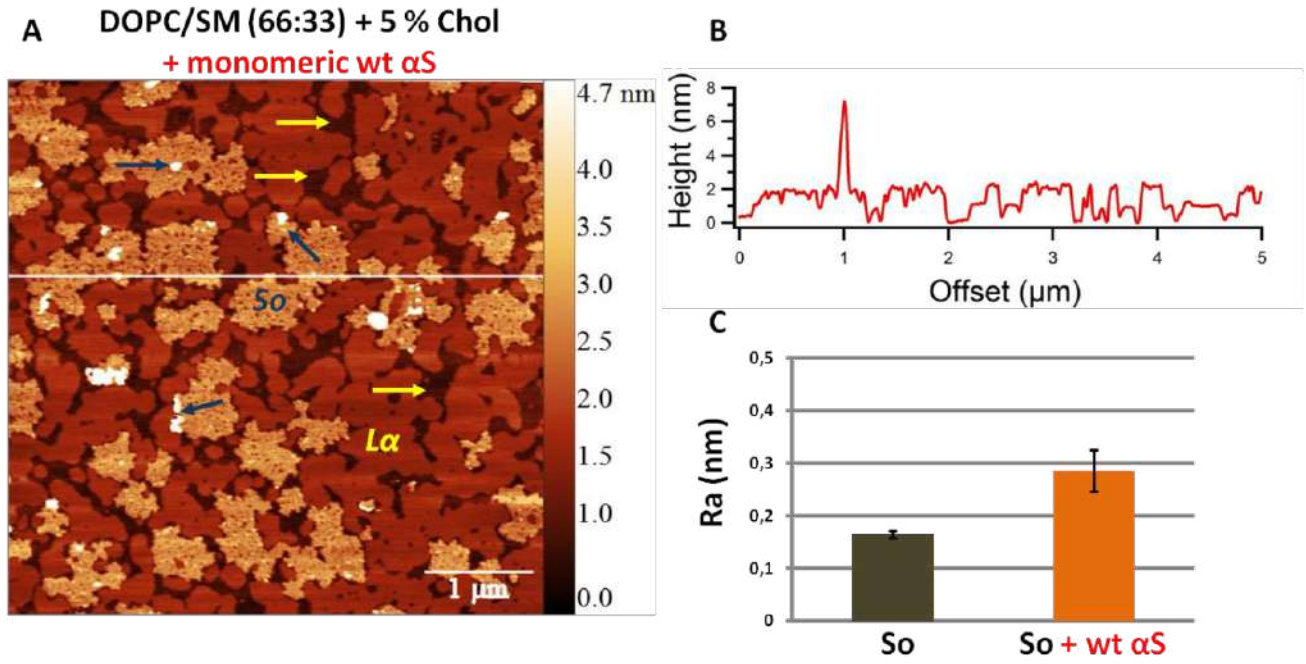


FL-PrPC-MA interacts with lipid raft domains without affecting the fluid phase of the bilayer. This could be due to the MA activity, which targets the protein to the ordered islands of membrane. However, formation of aggregated protein clusters which resemble oligomer accumulation are observed.

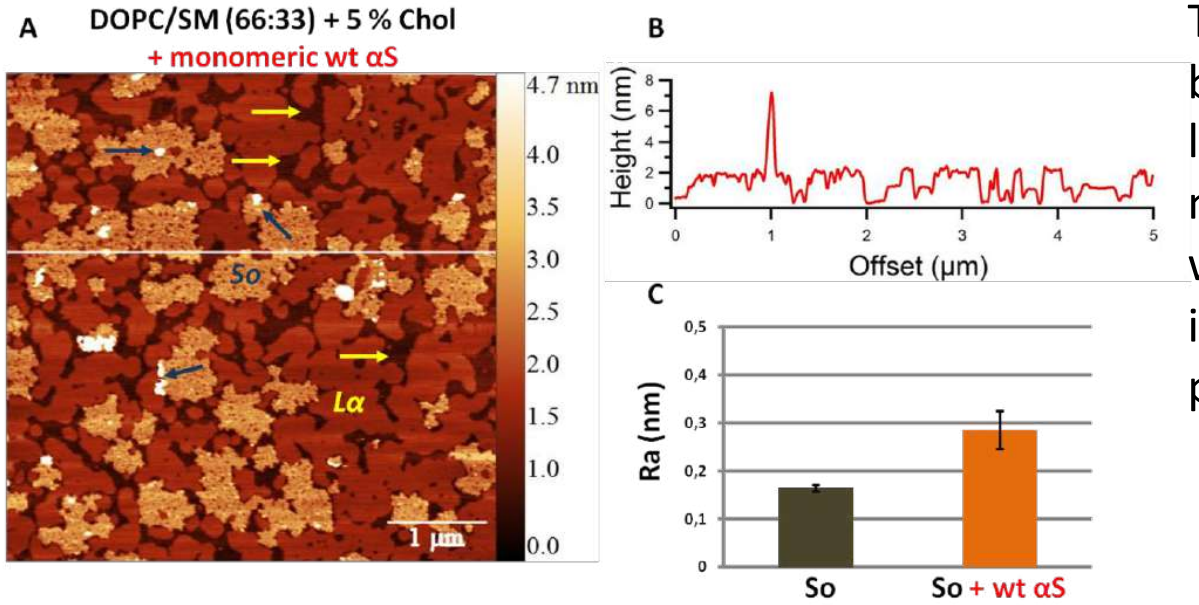
Membrane proteins: **Iron-mediated Alpha Synuclein (αS) aggregation**

Iron is implicated in the electron transfer during cellular respiration and as cofactor in the catalysis of enzymatic reactions.

Iron is potentially toxic when is present at high concentrations in the cell. It has been demonstrated that the total amount of iron increases physiologically in the brain with age and that this fact could be correlated with the old-age onset of PD

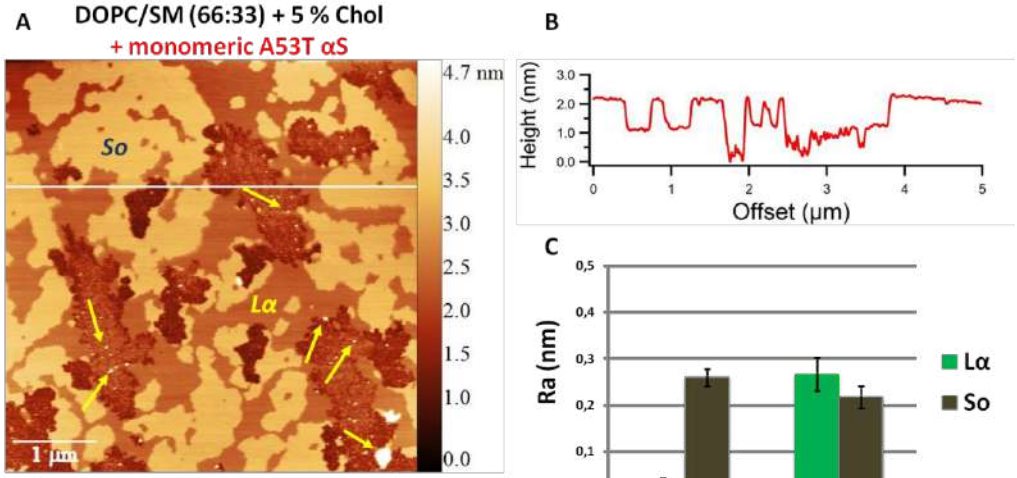


Membrane proteins: **Iron-mediated Alpha Synuclein (αS) aggregation**



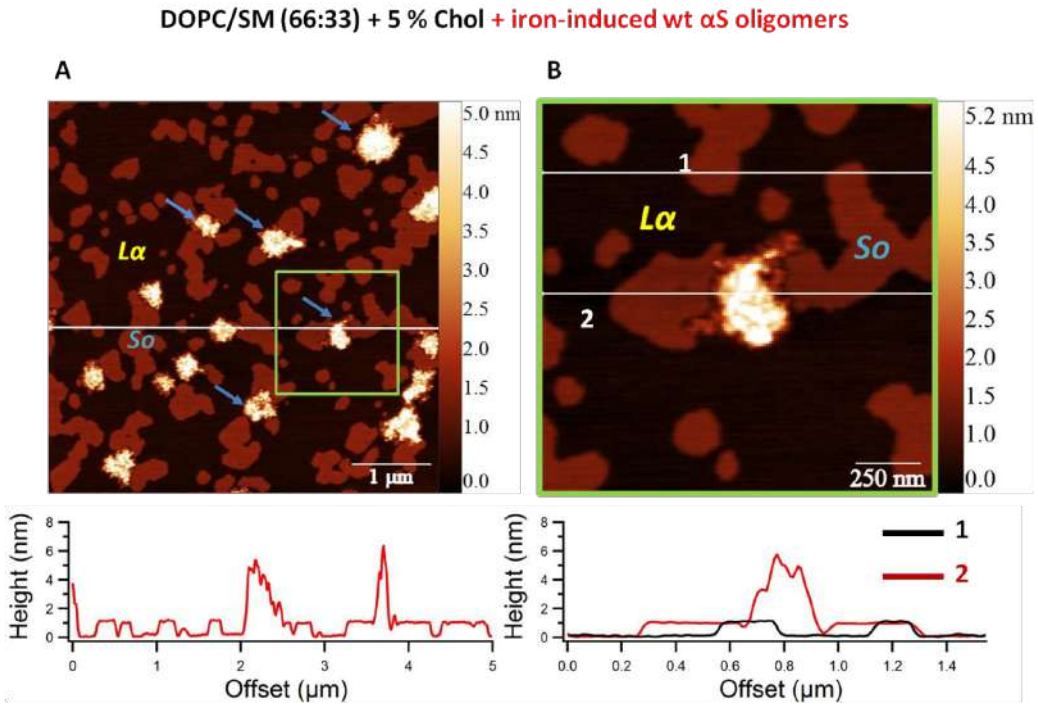
The wt αS seems to interact with both lipid phases (Lα and So) leading to a change in the morphology of raft-like domains which appear to have irregular and indented borders, as well as a more pronounced roughness.

A53T: mutant form of αS, responsible for an early stage familiar development of PD and more prone to aggregation
A53T seems to interact preferentially with the fluid lipid matrix causing damage sites without affecting the ordered domains



Lα	So	defect sites
29.3 %	32.2 %	38.5 %

Membrane proteins: **Iron-mediated Alpha Synuclein (αS) aggregation**



Iron-induced oligomers interaction with raft-like membranes revealed an accumulation of these misfolded structures on the ordered domains, forming protein clusters, for both wt and mutant A53T αS

The protein clusters of the mutant species are bigger in terms of dimensions and coverage of the membrane area, reflecting the faster rate of aggregation in the presence of iron compared to the wt αS