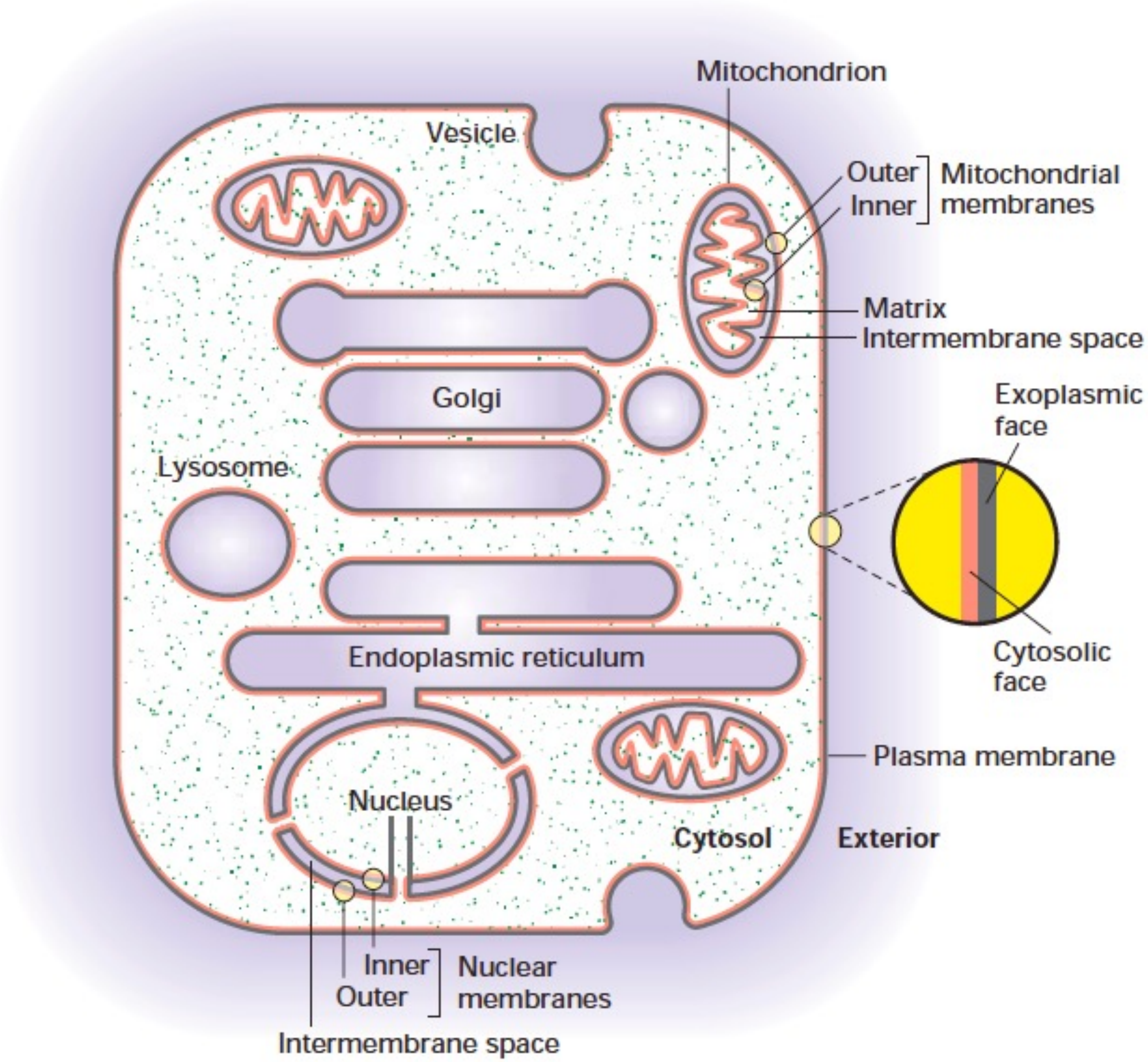
Membranes

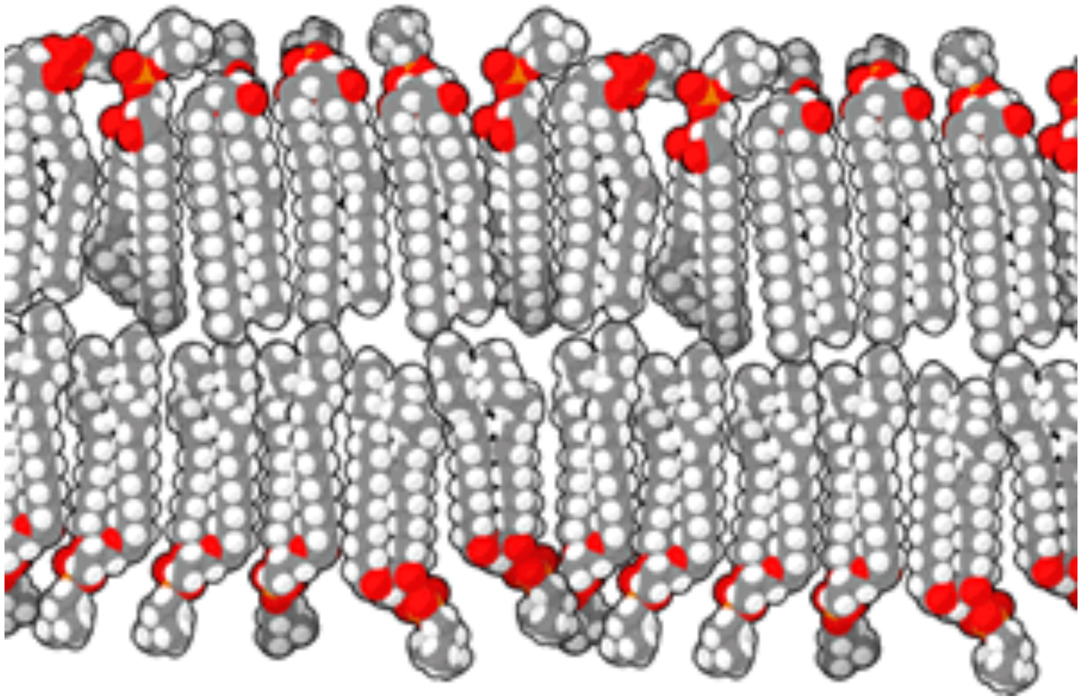
▶ FIGURE 5-4 The faces of cellular membranes. The plasma membrane, a single bilayer membrane, encloses the cell. In this highly schematic representation, internal cytosol (green stipple) and external environment (purple) define the cytosolic (red) and exoplasmic (black) faces of the bilayer. Vesicles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

✓ Lipids → Organized Assembly (Membranes)

Self assembly driven by thermodynamics ('Hydrophobic Effect')

✓ 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

Milestones in membrane research

1899

Overton

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

1925

Gorter and Grendel

- Bilayer arrangement of lipids

1935

Daveson and Danielli

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

1960

Robertson

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

Milestones in membrane research

1970

Frye and Edidin

- Lateral and rotational mobility of membrane proteins

1972

Singer and Nicolson

- Fluid mosaic model

1972

Racker

- Functional reconstitution of a membrane protein

1975

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

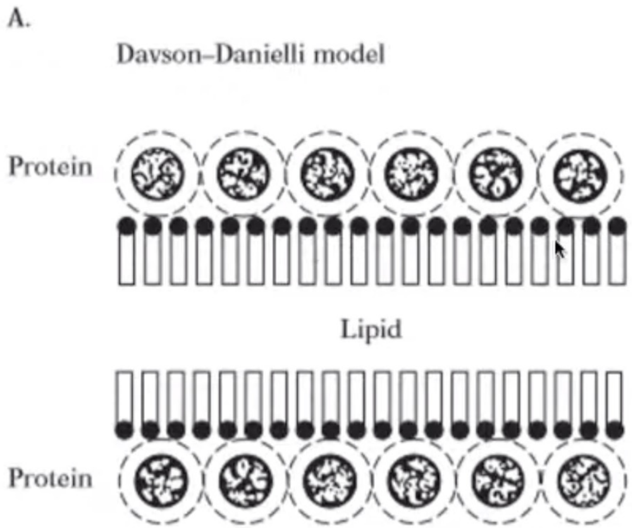
Roderick MacKinnon
Peter Agre

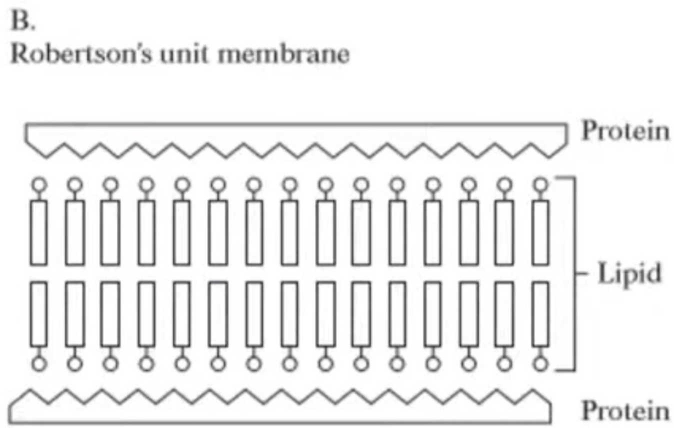
- Crystal structure of the first ion channel
- KcsA, Aquaporin

The Nobel Prize in Chemistry 2003

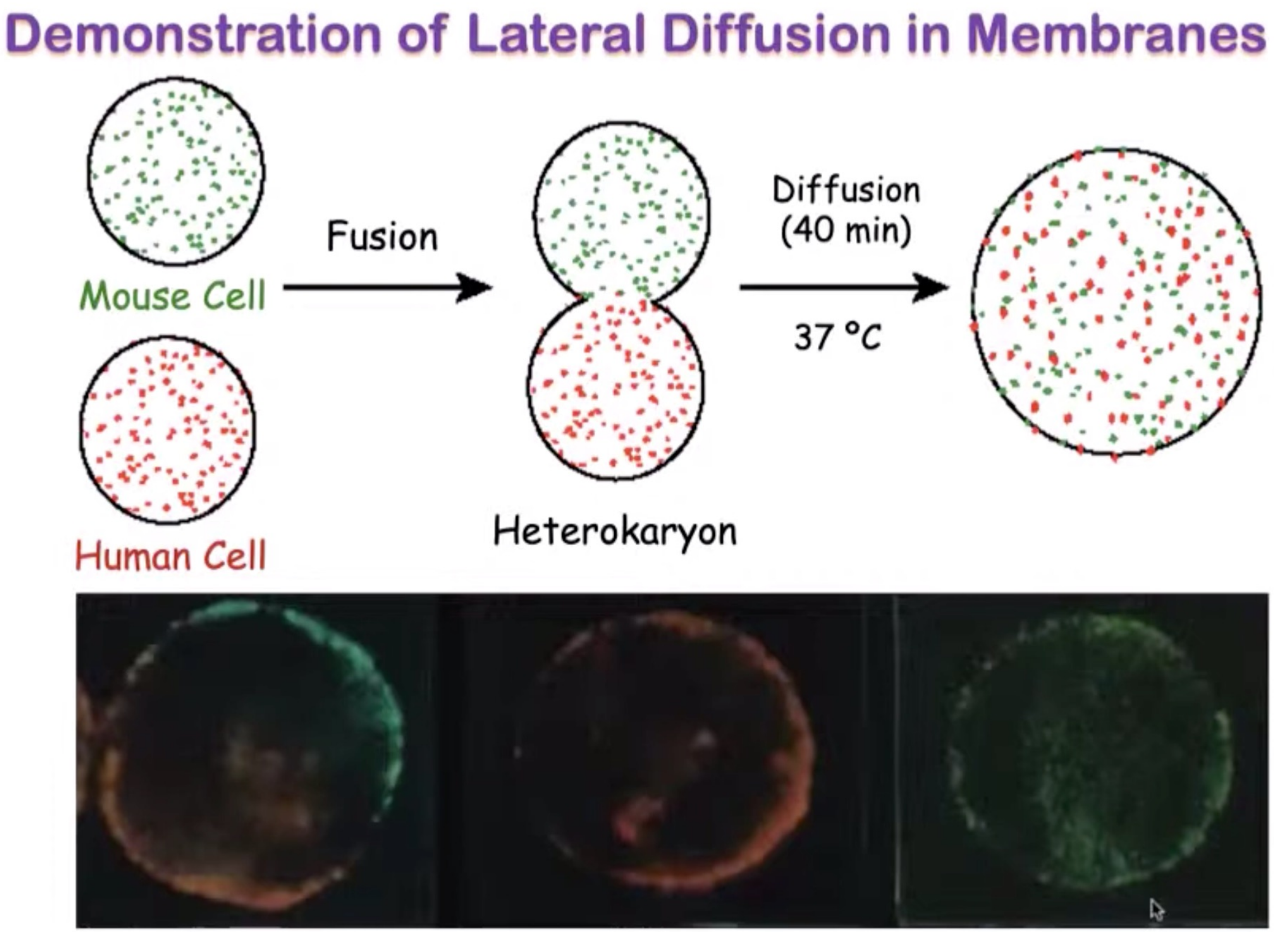
Peter Agre Prize share: 1/2 | Roderick MacKinnon Prize share: 1/2

Early models



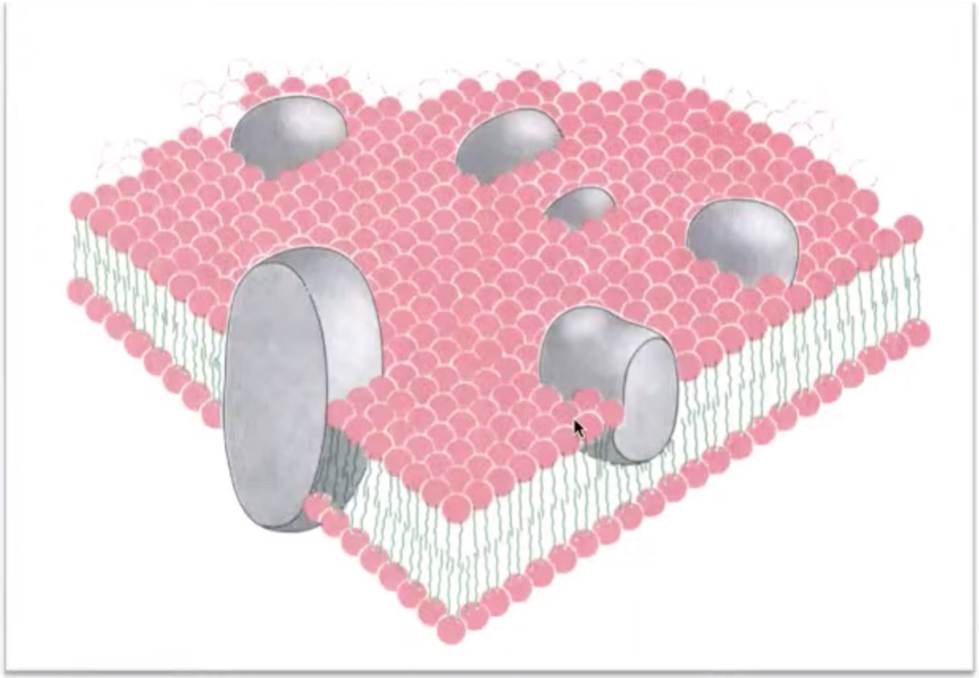


Early models



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

Does not take into account : LOCAL ORDER, DOMAIN FORMATION

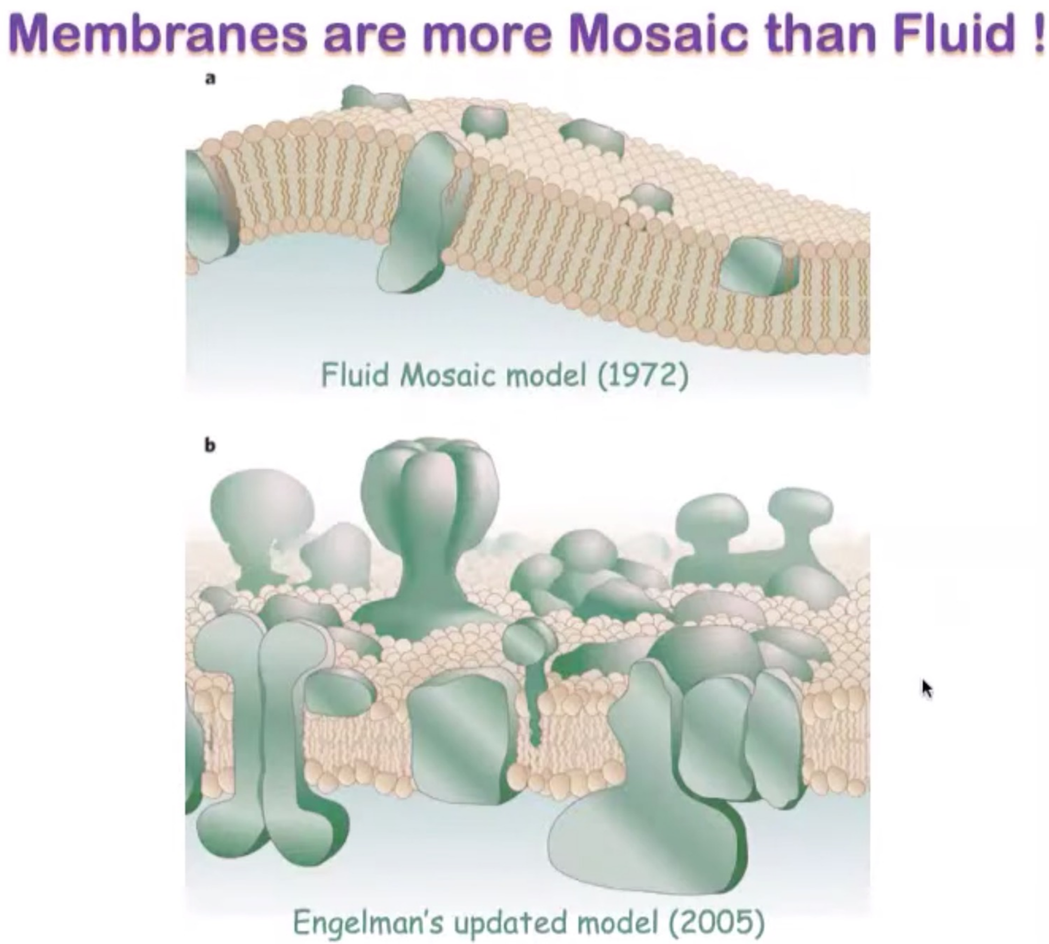
The fluid mosaic model

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.

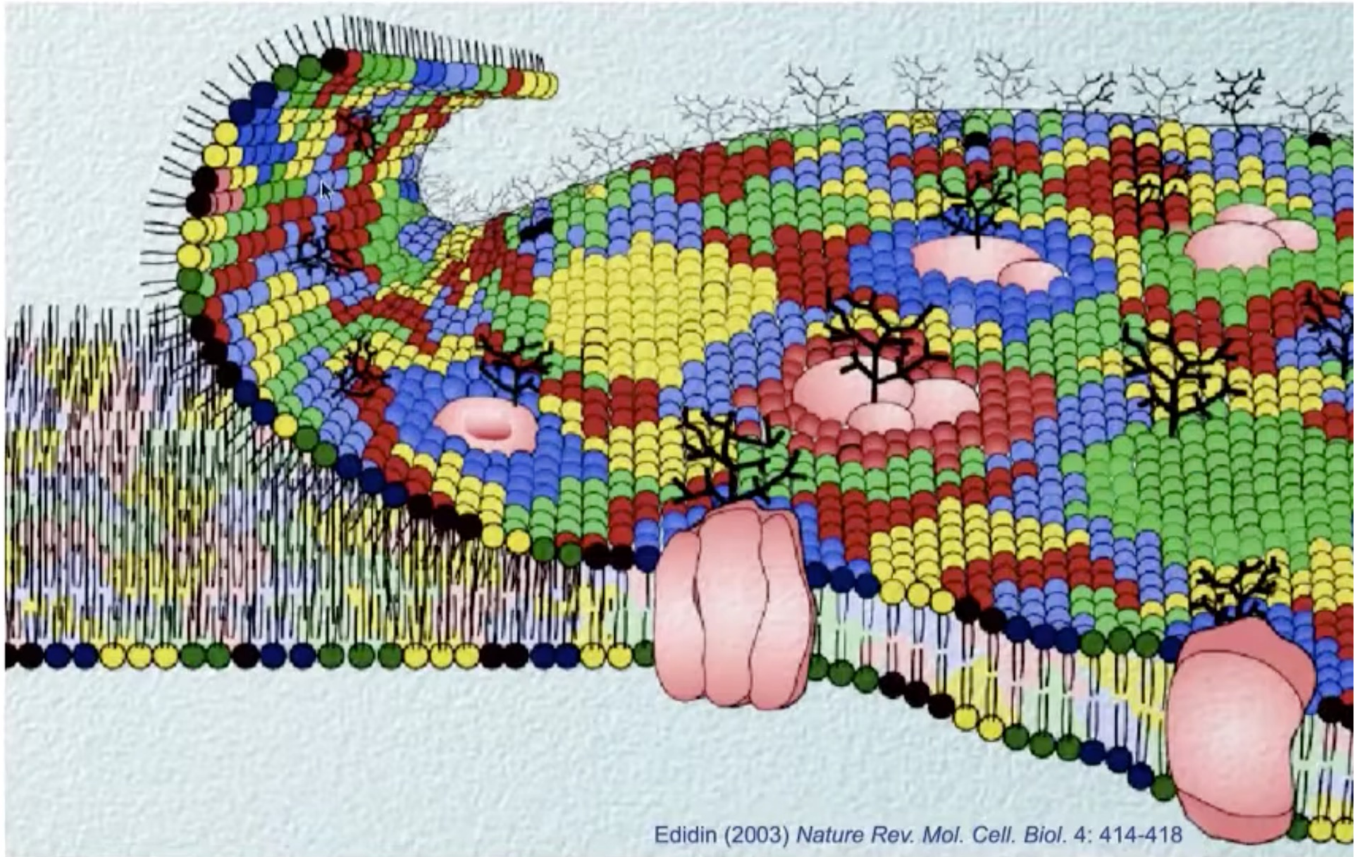
The fluid mosaic model



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

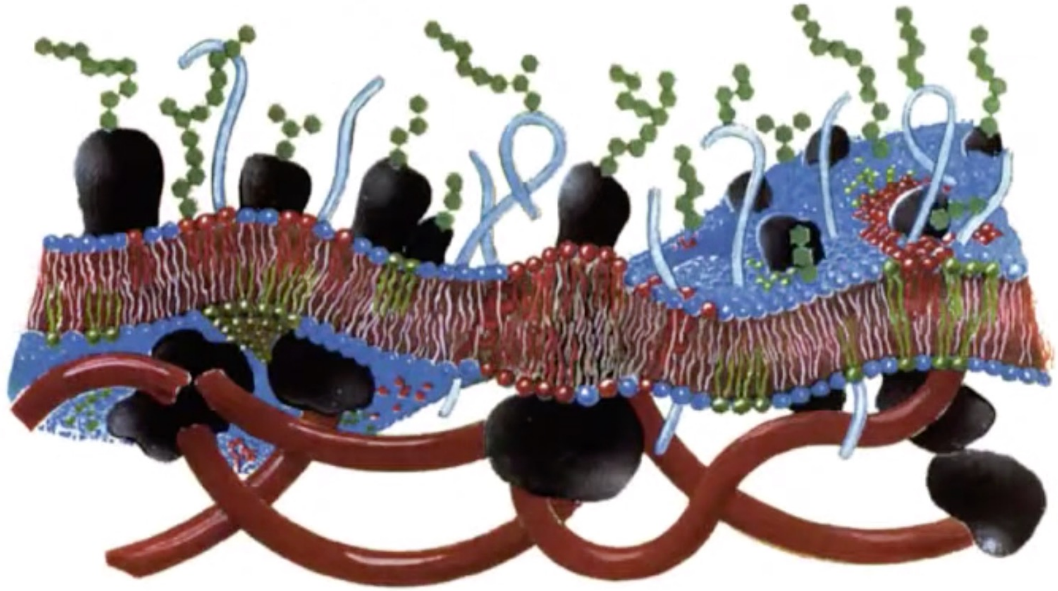
Lateral distribution of molecules is heterogeneous, corresponding to An organization into DOMAINS

Current Model of Biological Membranes: Organization of Membranes into Domains



Edidin (2003) *Nature Rev. Mol. Cell. Biol.* 4: 414-418

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.

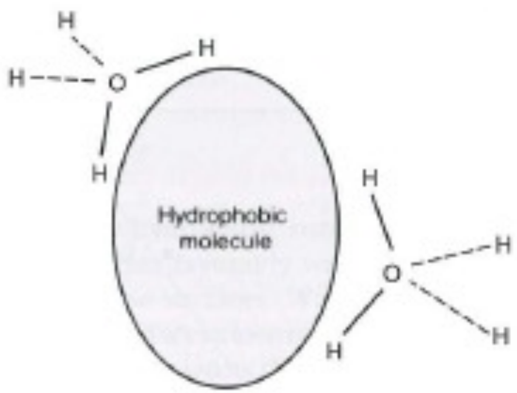
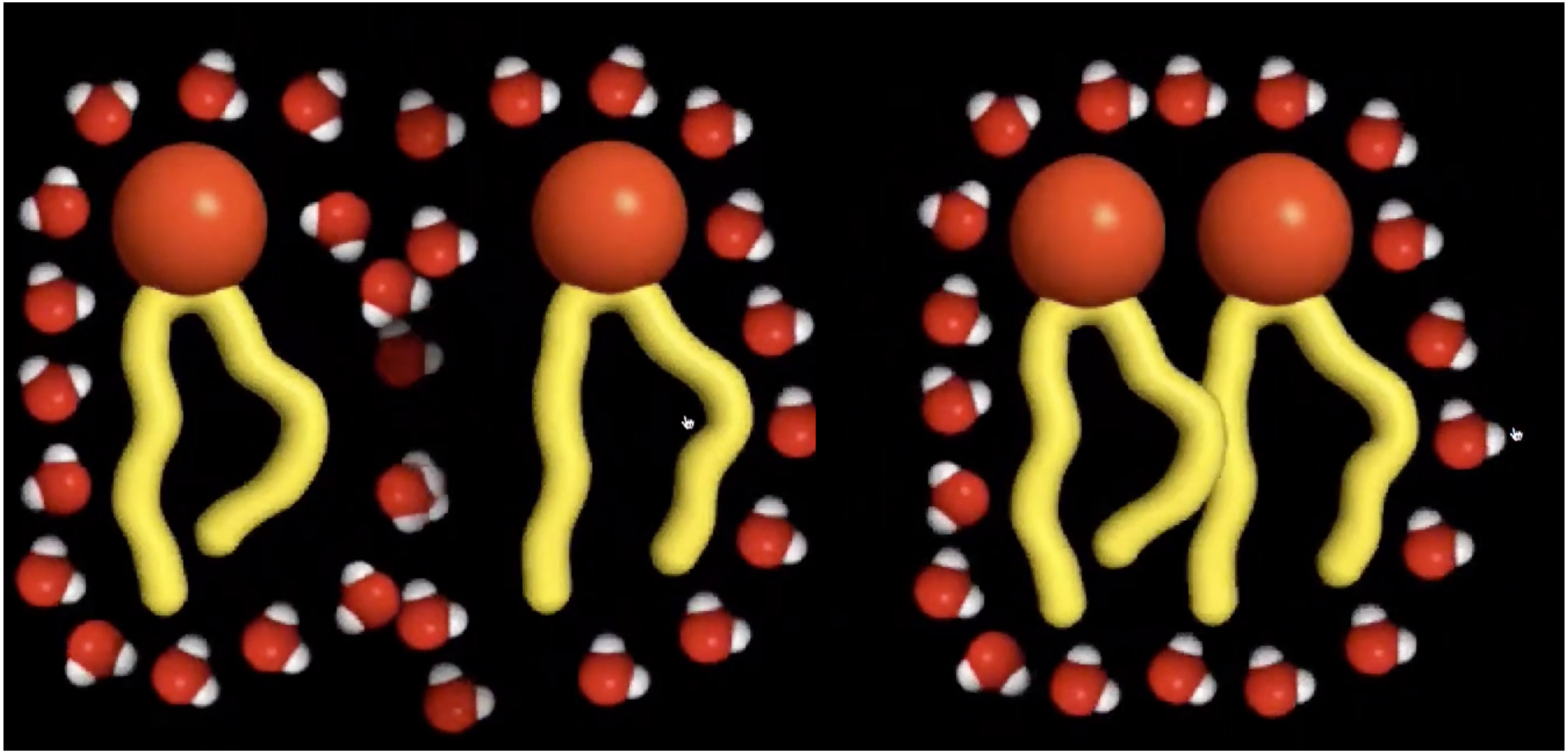
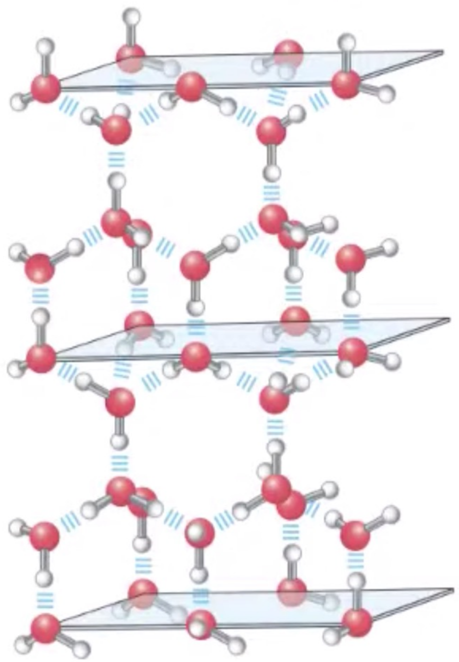


Fig. 2.7 Water molecules adjacent to a hydrophobic molecule suffer restrictions in orientation as they form hydrogen bonds with other water molecules.

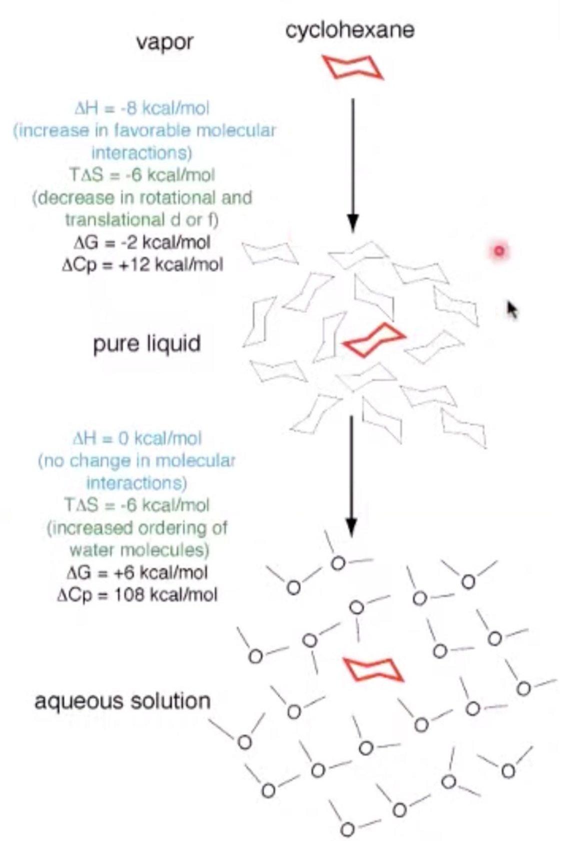
Hydrophobic effect



Hydrophobic effect

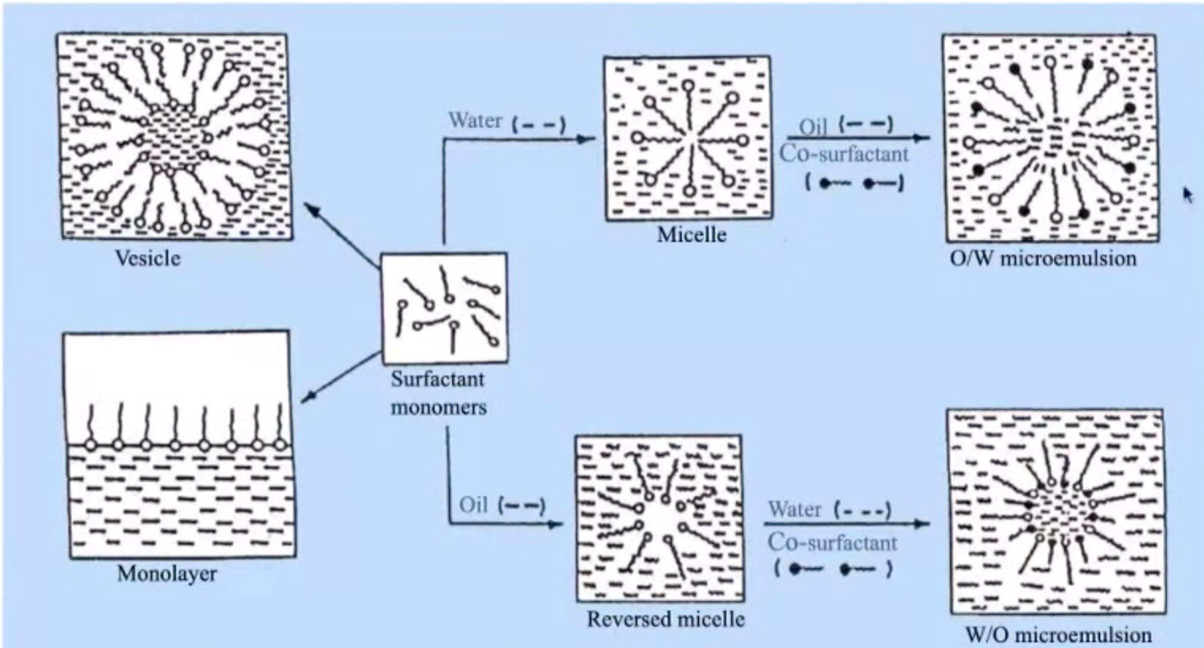


The forces of attraction between water molecules in the liquid state are unusually high. The melting point, boiling point, heat of vaporization, heat of fusion, and surface tension of water are higher than those of similar substances: The heat of vaporization of water (540 cal/g) is over twice that of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

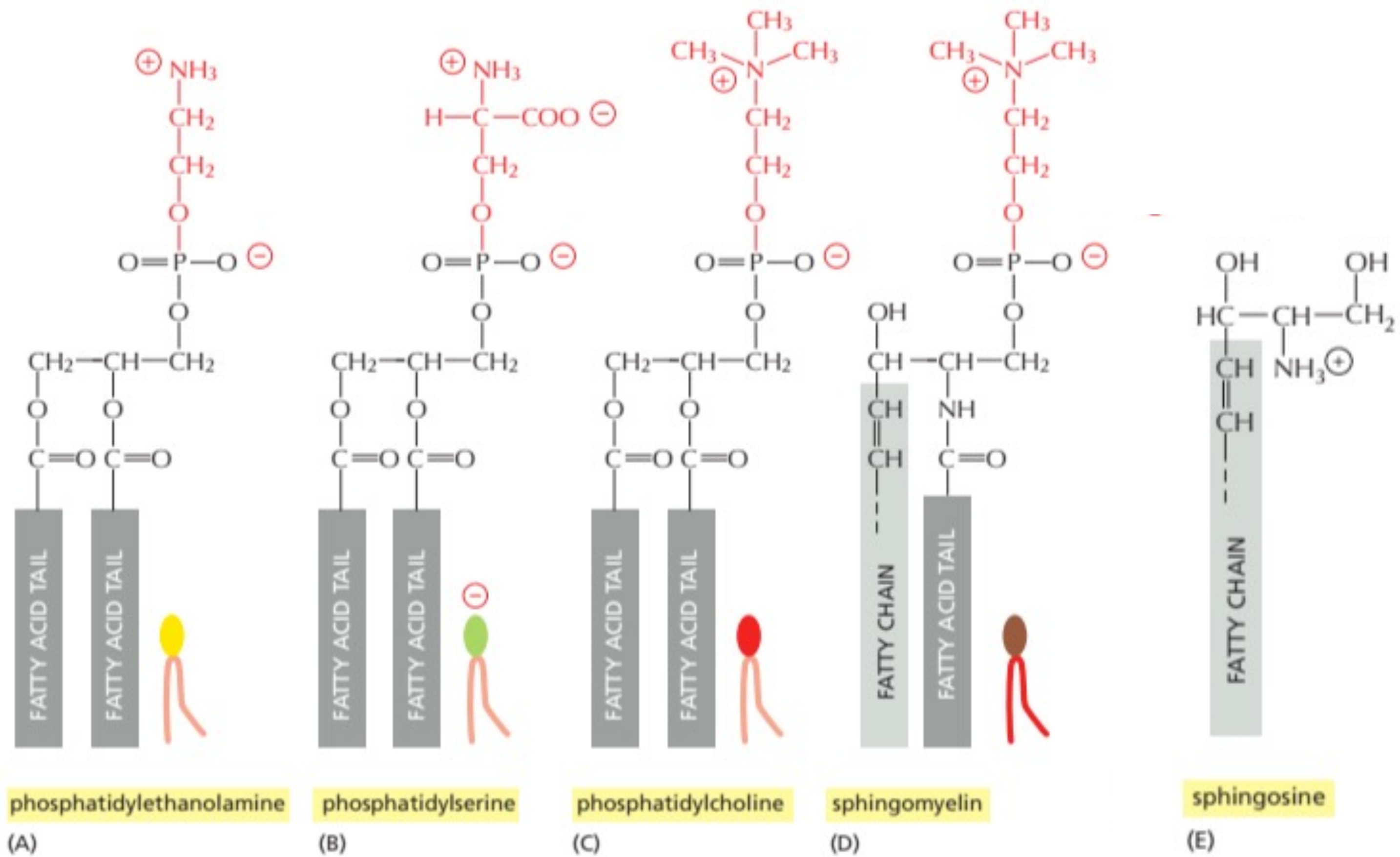


Hydrophobic effect

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



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.

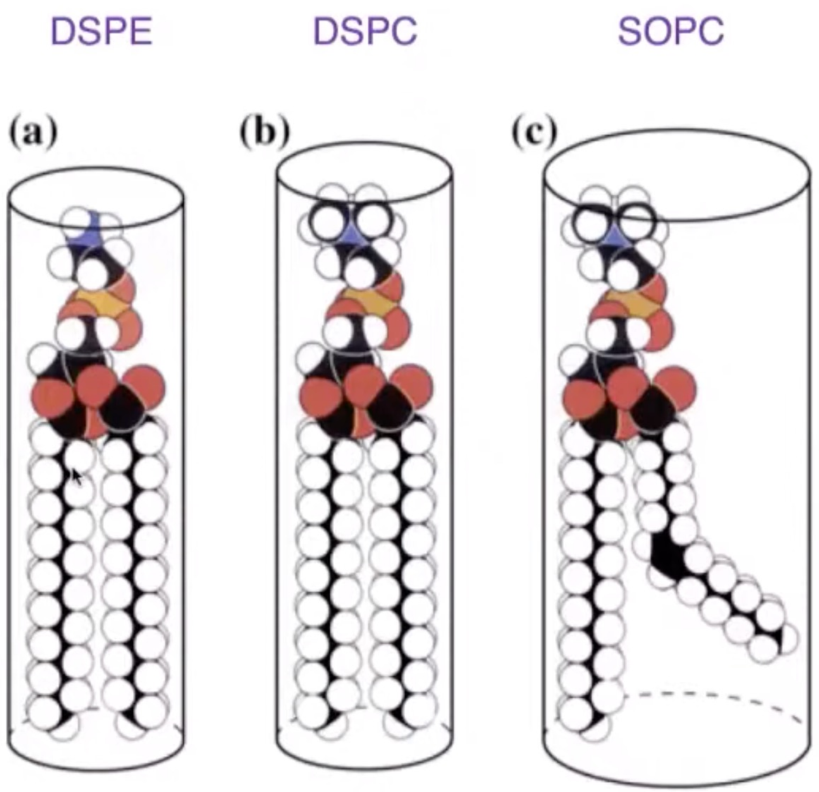
Other sphingolipids are glycolipids in which a single sugar residue or branched oligosaccharide is attached to the sphingosine backbone.

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

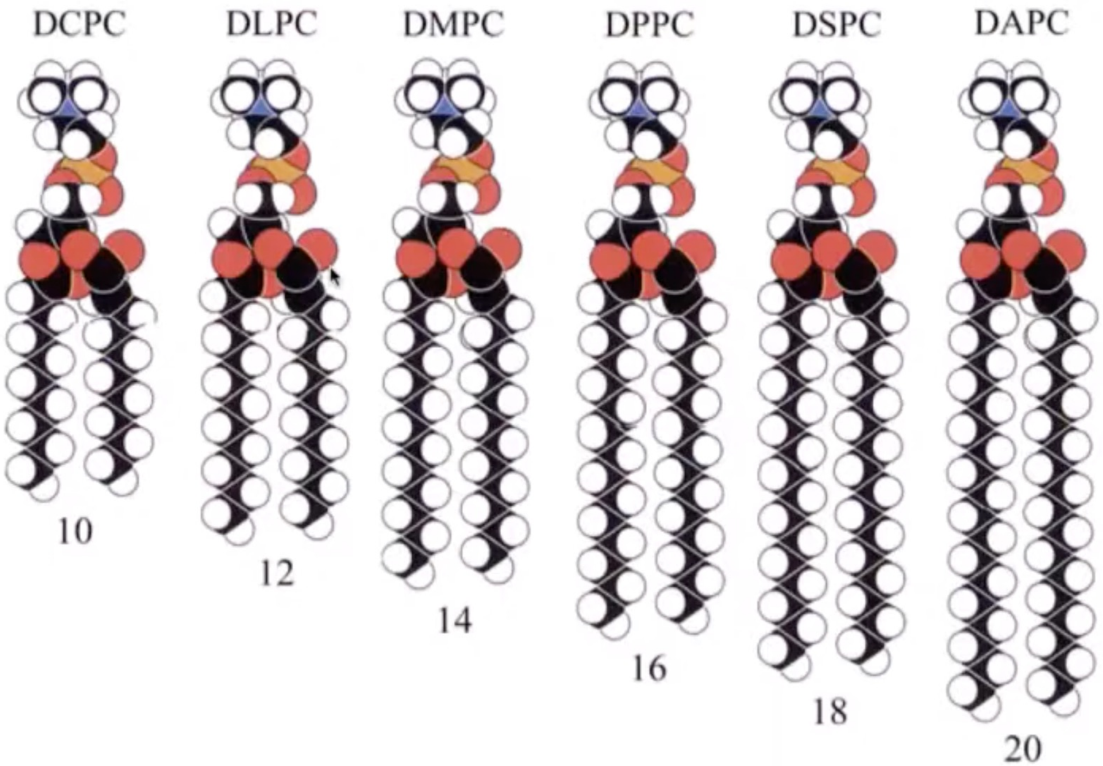


The actual conformation of a molecule influences its size.

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

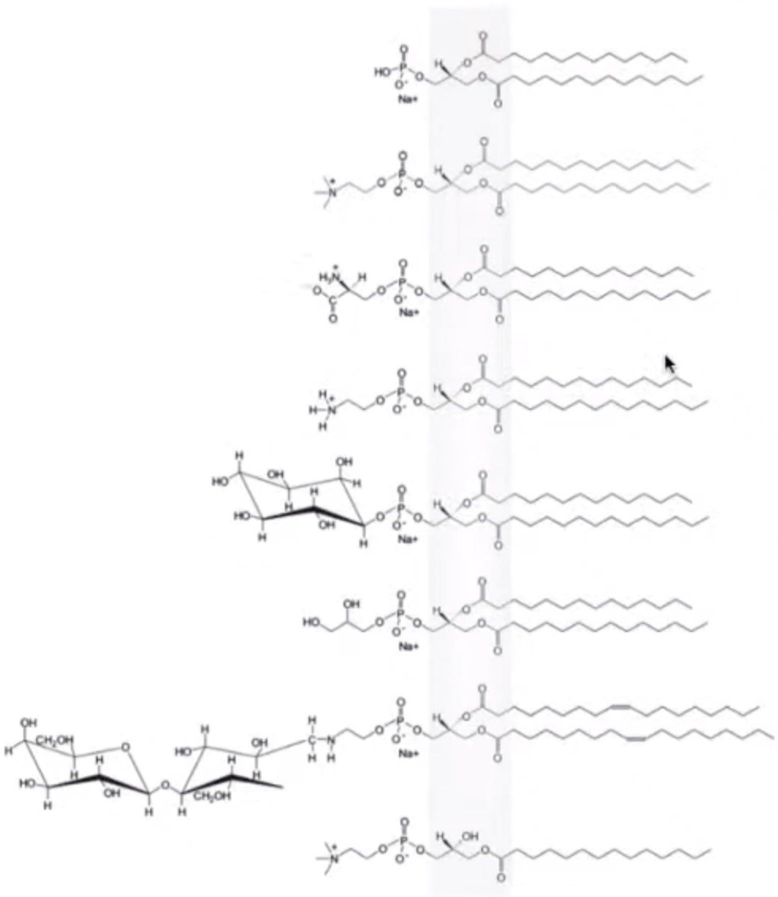
Only lipids with limited degree of disorder will fit into a bilayer structure.

Di-acyl PC lipids



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).

Head and Tail



(a) DMPA

(b) DMPC

(c) DMPS

(d) DMPE

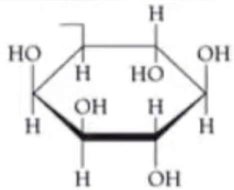
(e) DMPI

(f) DMPG

(g) Glycolipid

(h) Lysolipid with C16

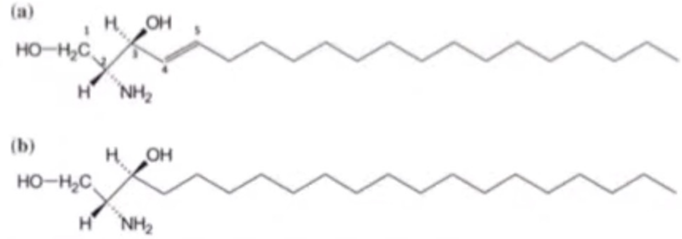
Lipid polar head groups

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.

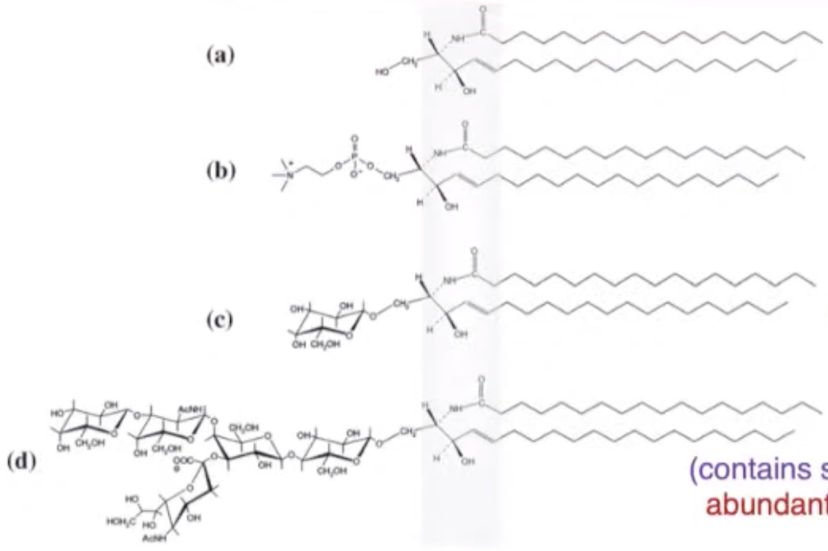
&Abbreviation for the polar head group nomenclature.

Sphingosine based phospholipids



sphingosine

sphinganine



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	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)	$CH_3(CH_2)_5$ $CH{=}CH(CH_2)_7COOH$	*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.

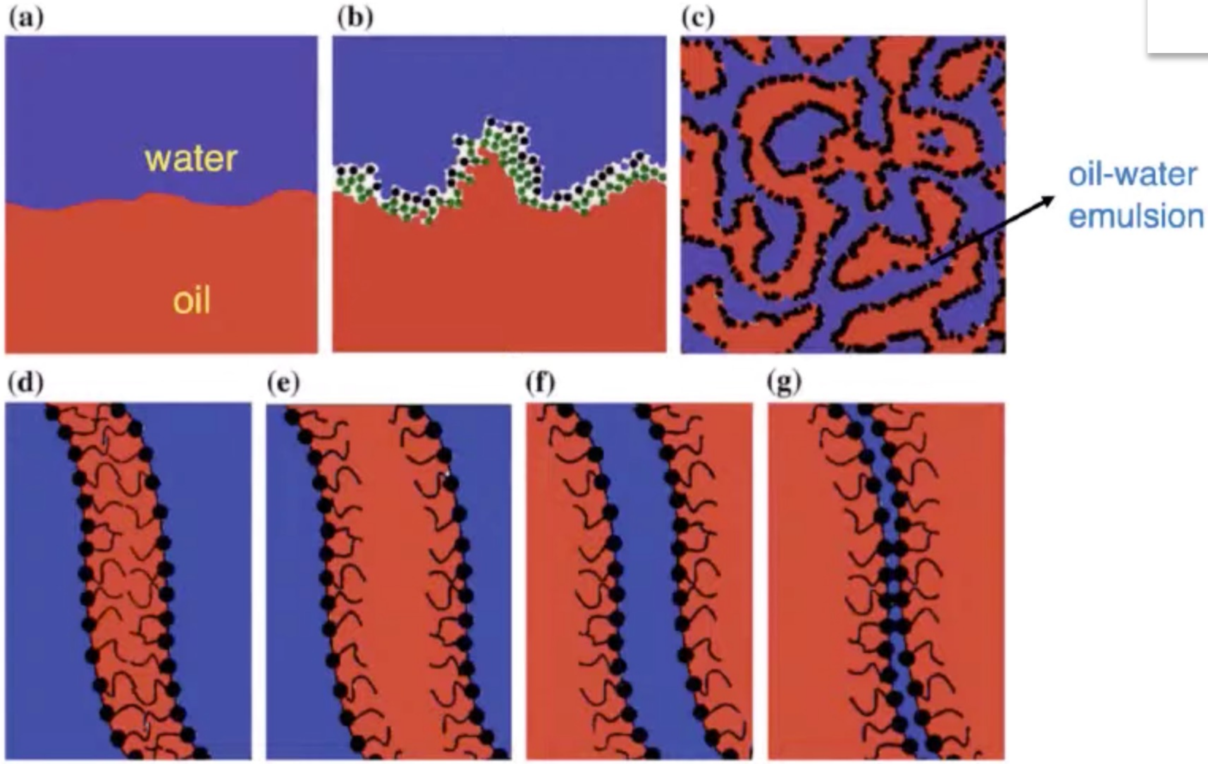
Source: Data from Nelson, D. L., and M. M. Cox, *Lehninger Principles of Biochemistry*, 4th ed. New York: W. H. Freeman, 2005.

More than 500 species of fatty acids !

Lipidomic survey of a budding yeast

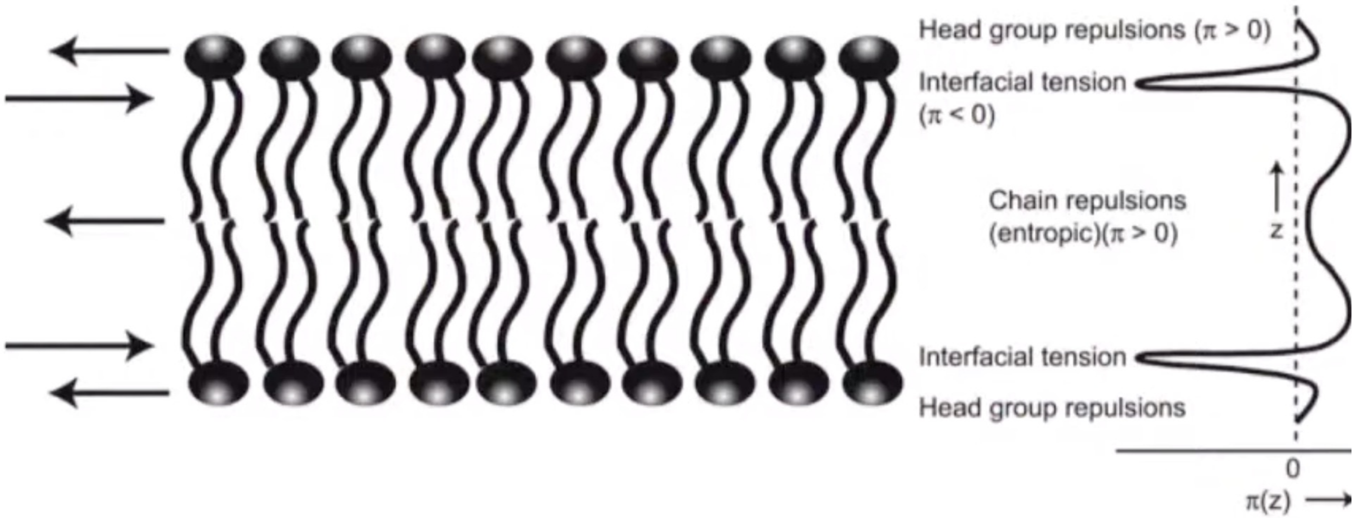
PLoS One (2012) 7: e35063
PNAS (2009) 106: 2136

Water and Oil interface



All interfaces are covered with interfacially active molecules

Lateral pressure profile of a lipid bilayer



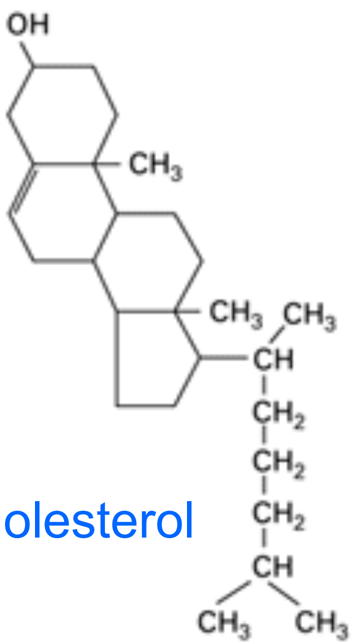
1. Positive pressure resulting from headgroup repulsive forces

2. Negative pressure at the hydrophobic-hydrophilic interface - the interfacial tension

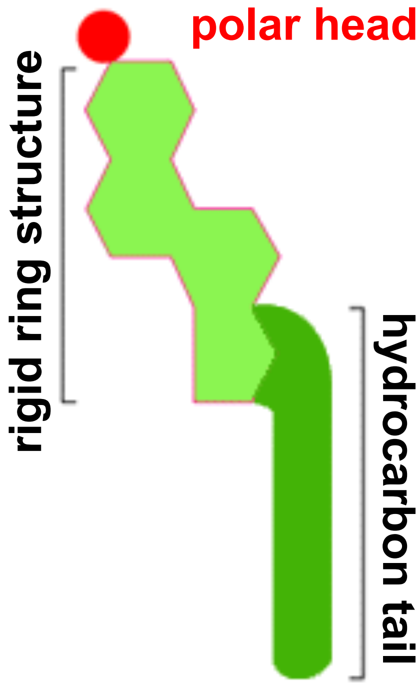
3. Positive pressure resulting from entropic repulsion between acyl chains – chain pressure

Cholesterol and steroids

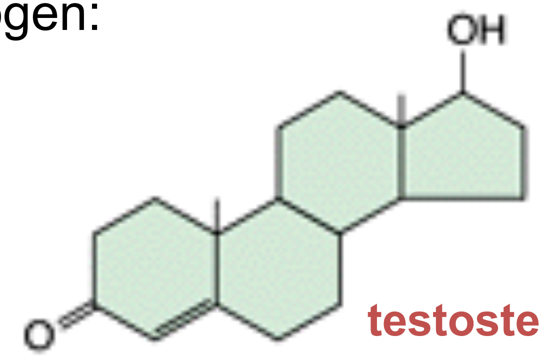
Steroids (such as cholesterol) have a rigid structure made up by 4 rings.



Cholesterol

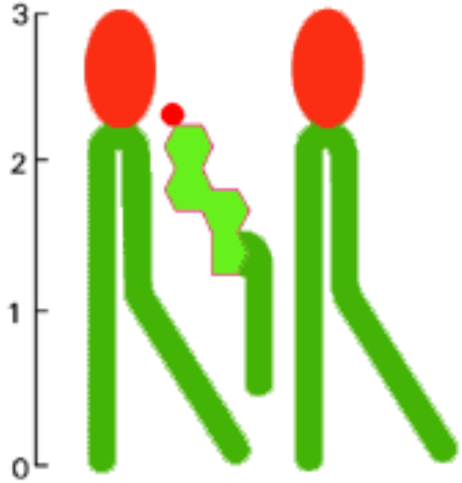


Other important steroid are the sex hormones, such as testosterone and estrogen:

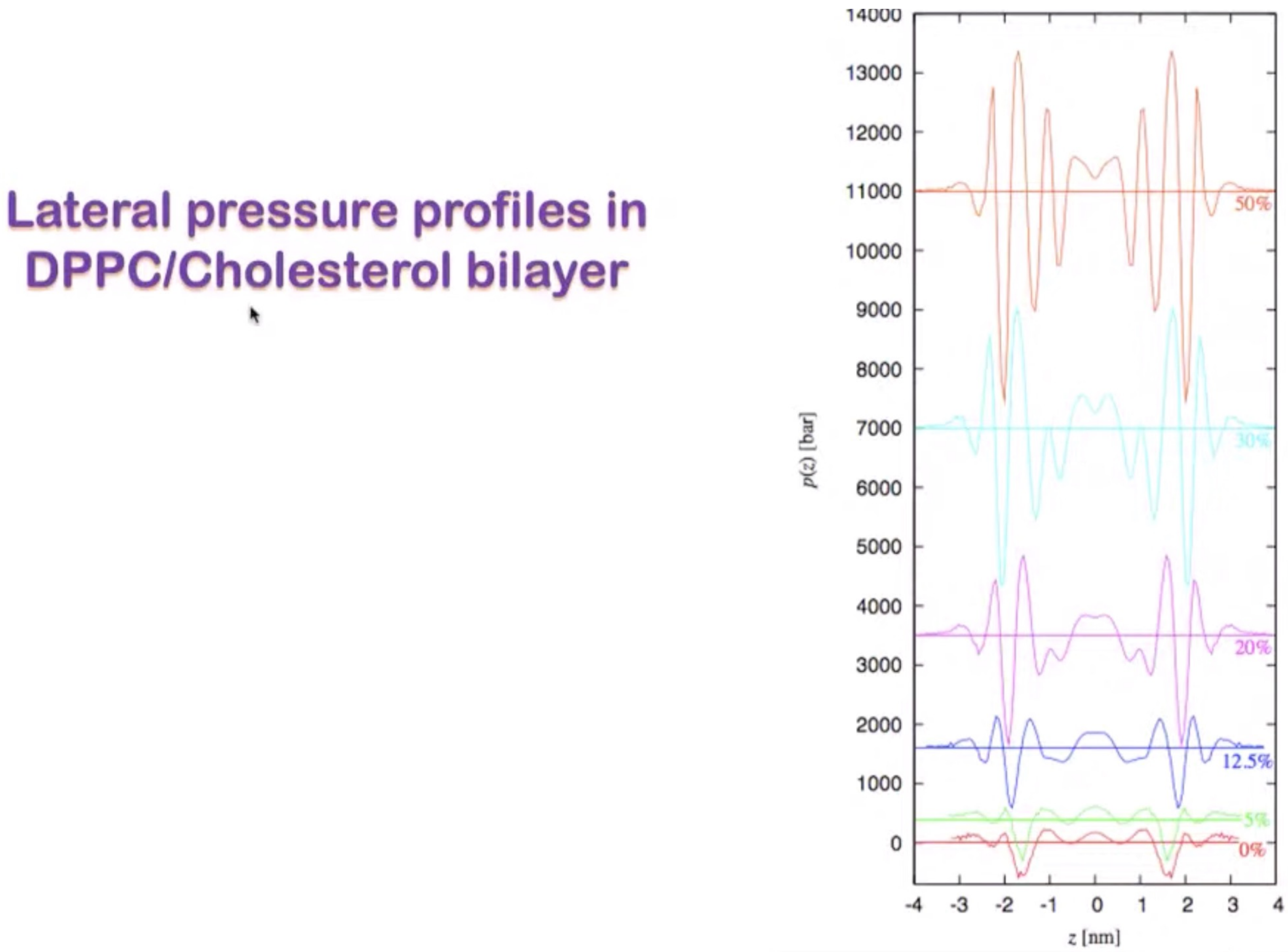


testosterone

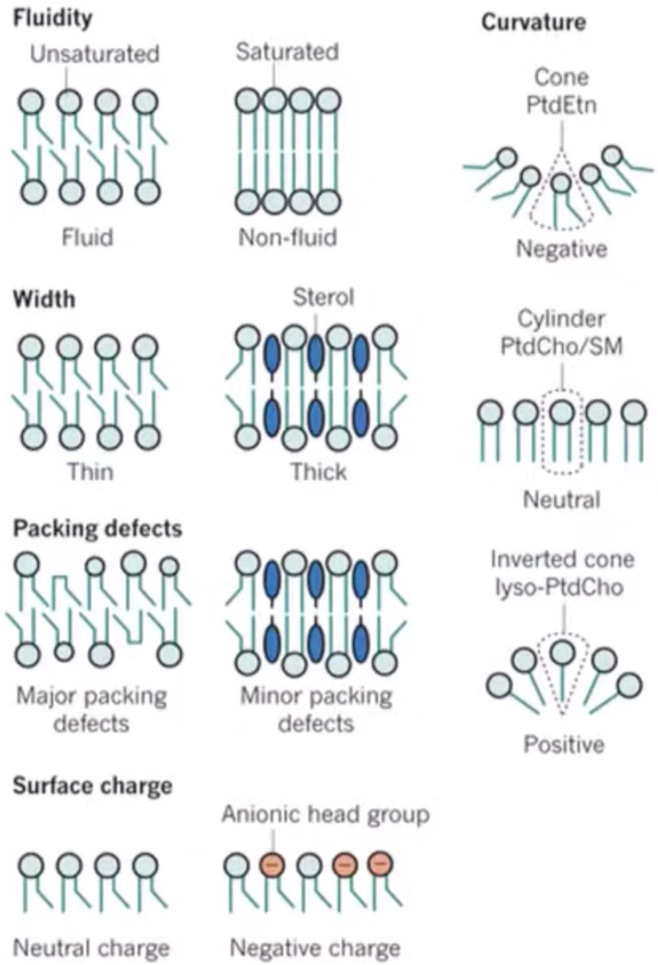
Cholesterol is an important component of the eukaryotic membranes and has a key role in controlling the membrane fluidity.



Effect of cholesterol



Membrane physical properties

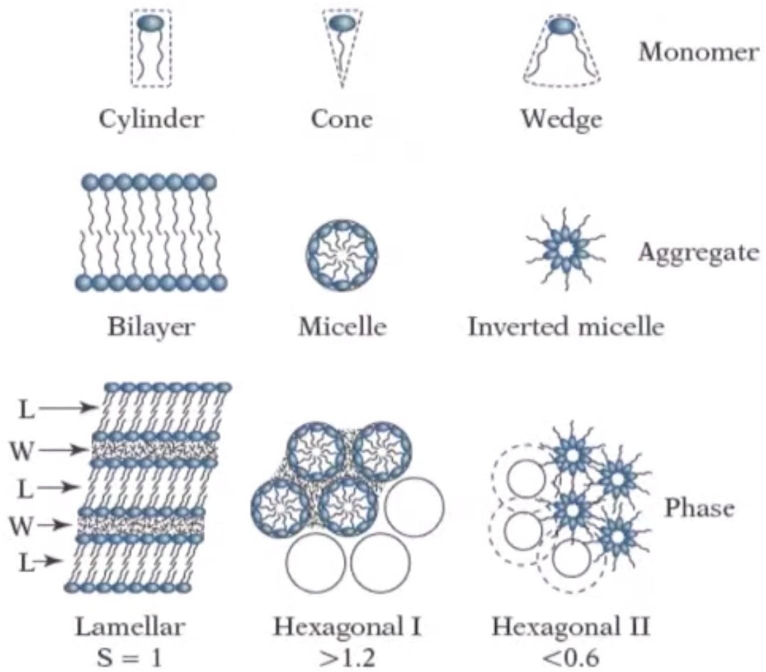


Membrane Physical Properties are Determined by its Lipid Composition

Nature (2014) 510: 48-57

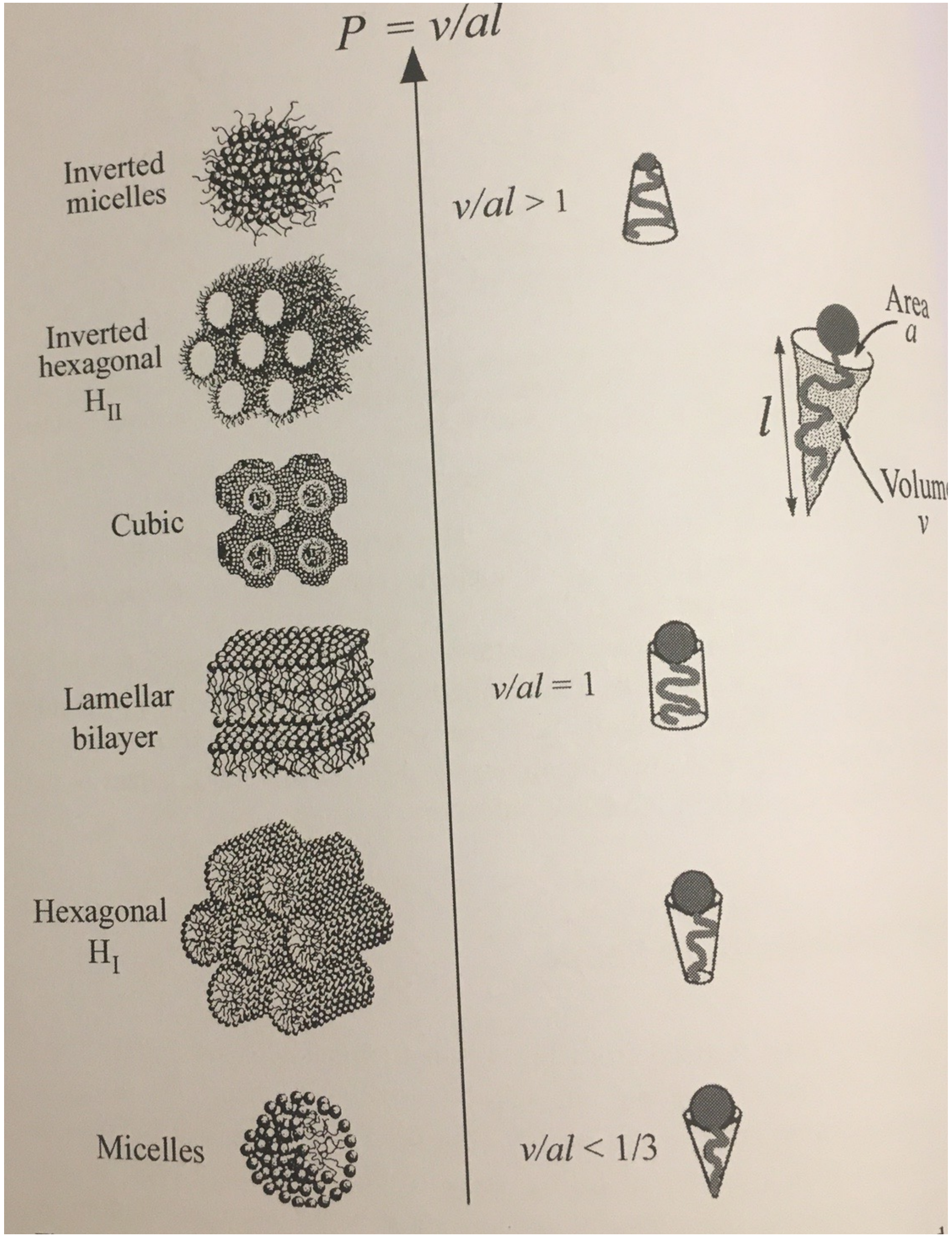
Membrane physical properties

Amphiphile Shape Hypothesis:
Relationship that influences Lipid Polymorphism



$$S = \frac{\text{cross sectional area of polar headgroup} \times \text{lipid length}}{\text{lipid volume}}$$

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.

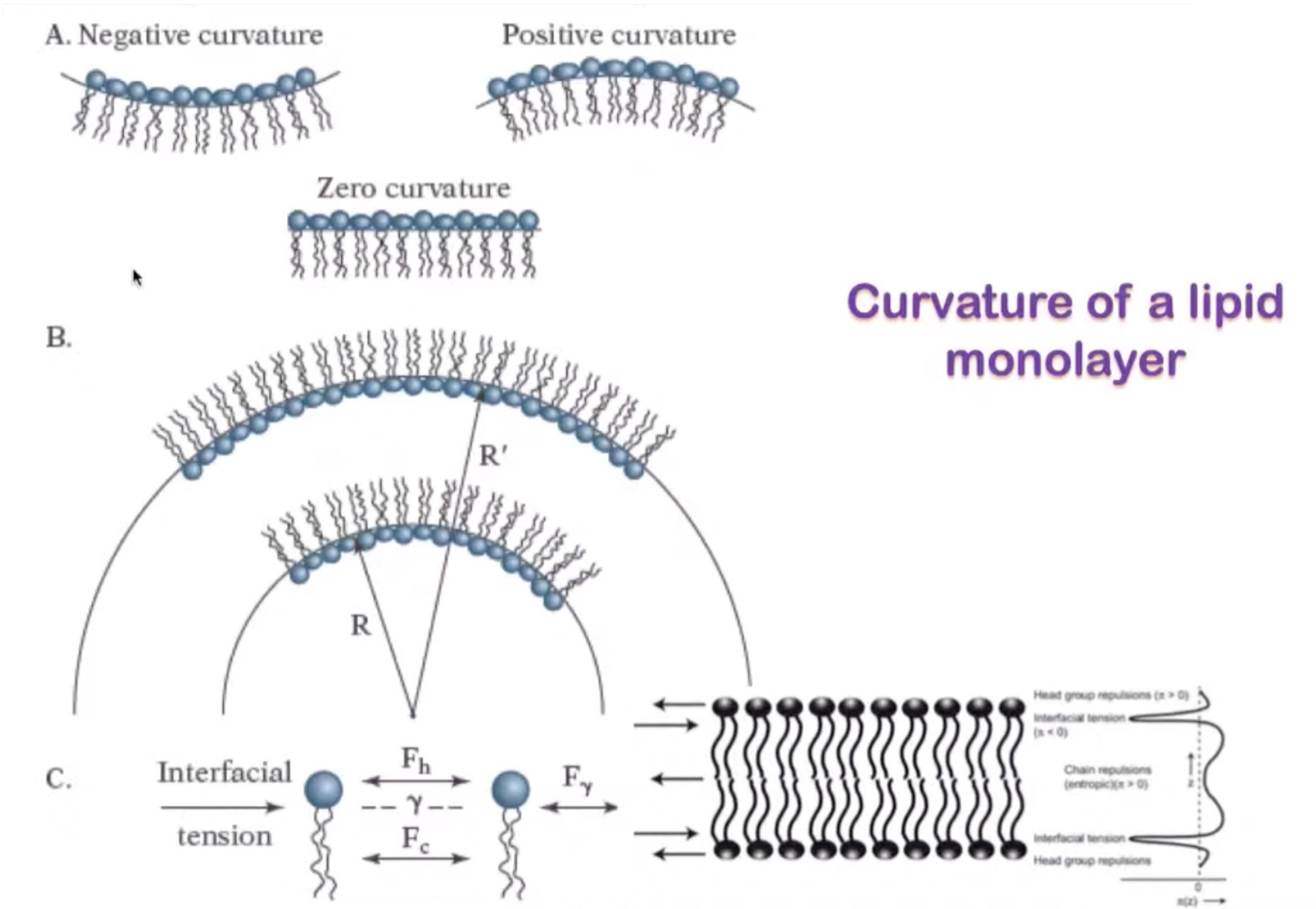
Lipid shape is important for functioning. It is given by the compatibility between head and tail. We define`a packing parameter P:

$$P = v/al$$

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

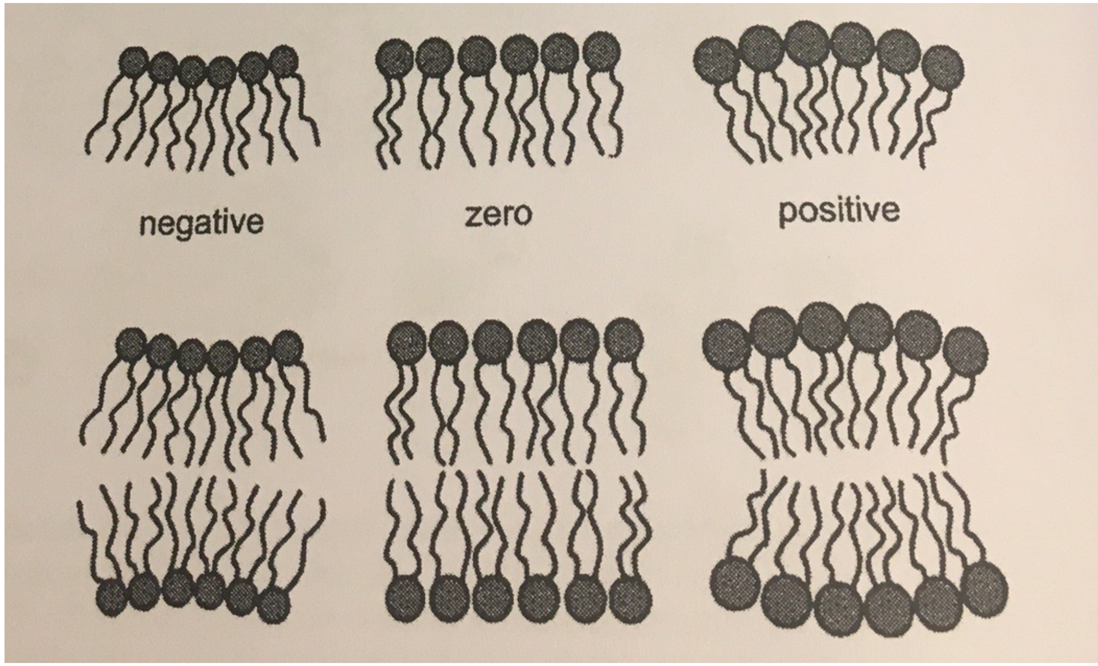
Curvature although is important for many of the membrane processes

Lipids and membrane curvature



Curvature of a lipid monolayer

Lipids and membrane curvature



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

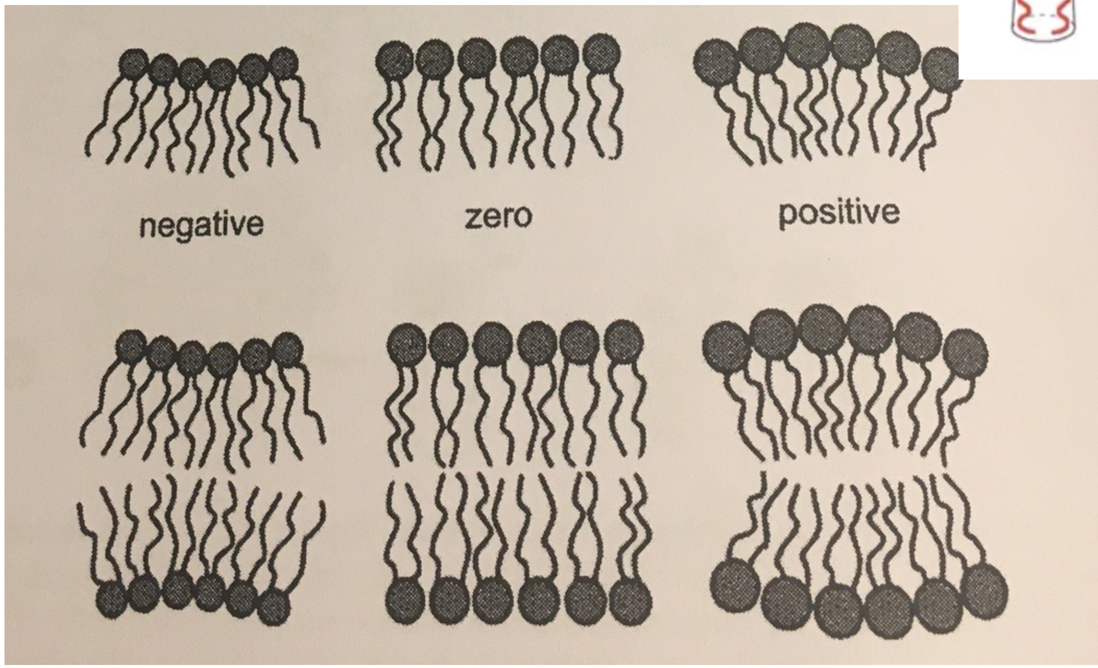
Each layer tend to elastically relax to a state of finite, spontaneous curvature, causing a curvature stress field.

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

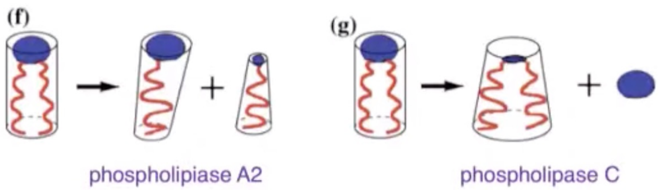
Lipid speak the language of curvature, in the many structures formed!

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



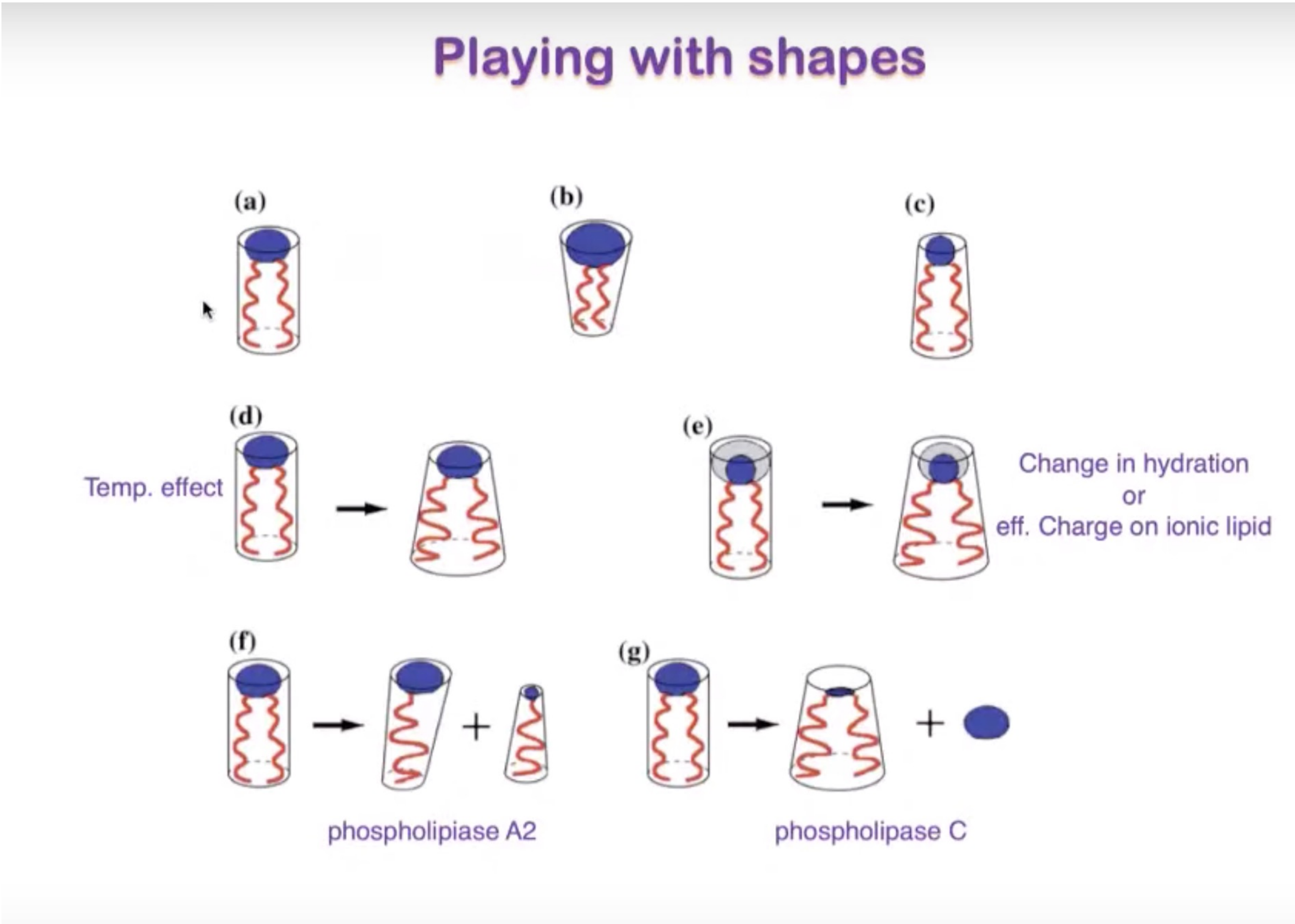
Cholesterol has an inverted conical shape (small OH, big steroid ring). Tends to promote the H_{II}. Stress field is mitigated by enzymes.



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

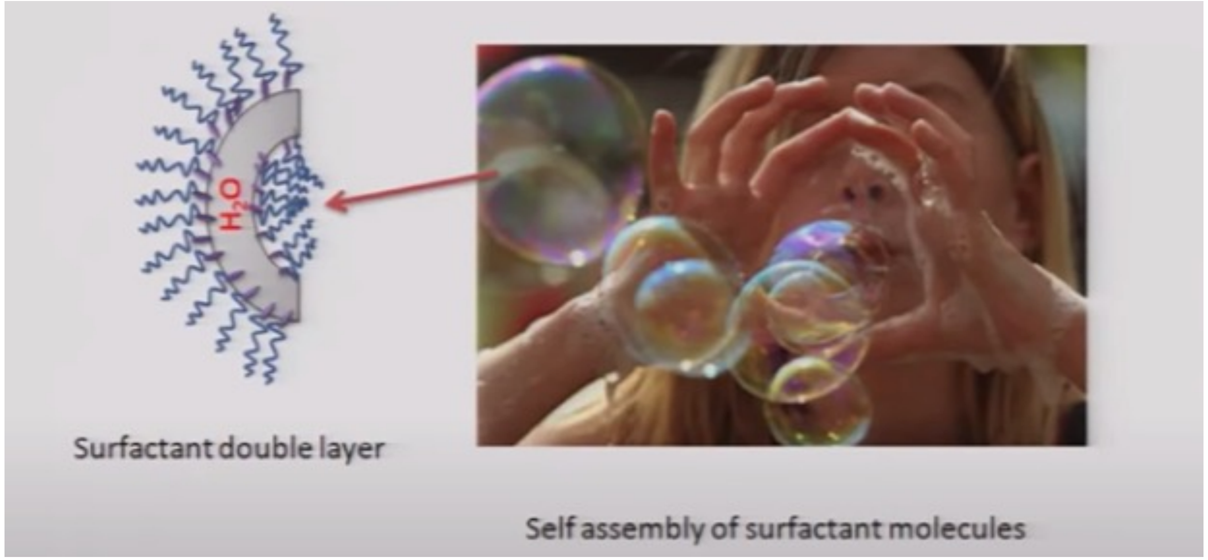
acts to make the interface as small as possible, at the same time imparts a certain stiffness to the interface.

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

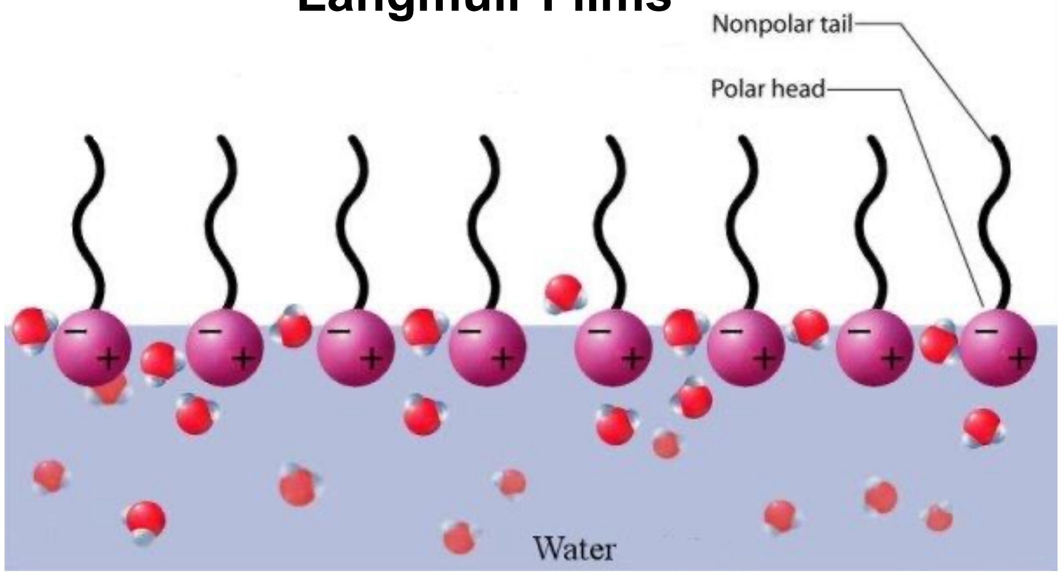


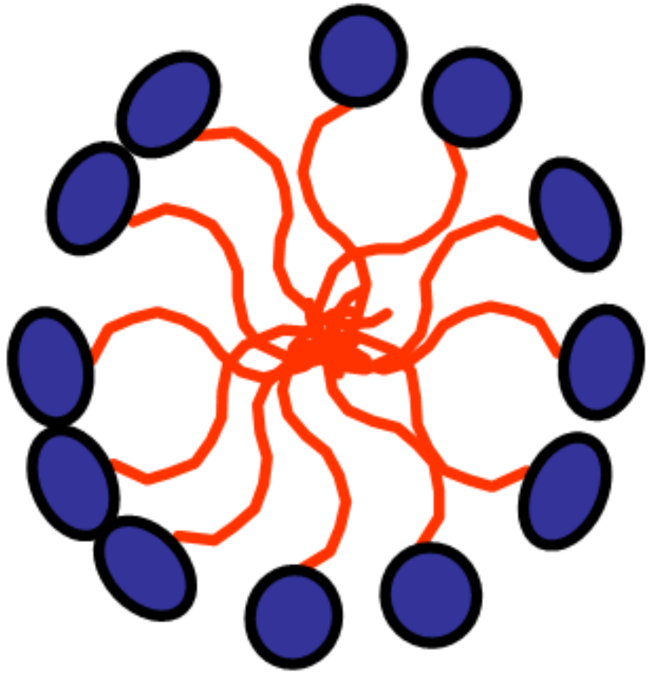
Soap bubbles: two layers form, at the water-air interfaces, the outer and the inner surfactant layer.
Bubbles are stabilized for a particular size, a particular water layer thickness depending on:

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

Self-organized monolayers (on liquid surfaces)

Langmuir Films

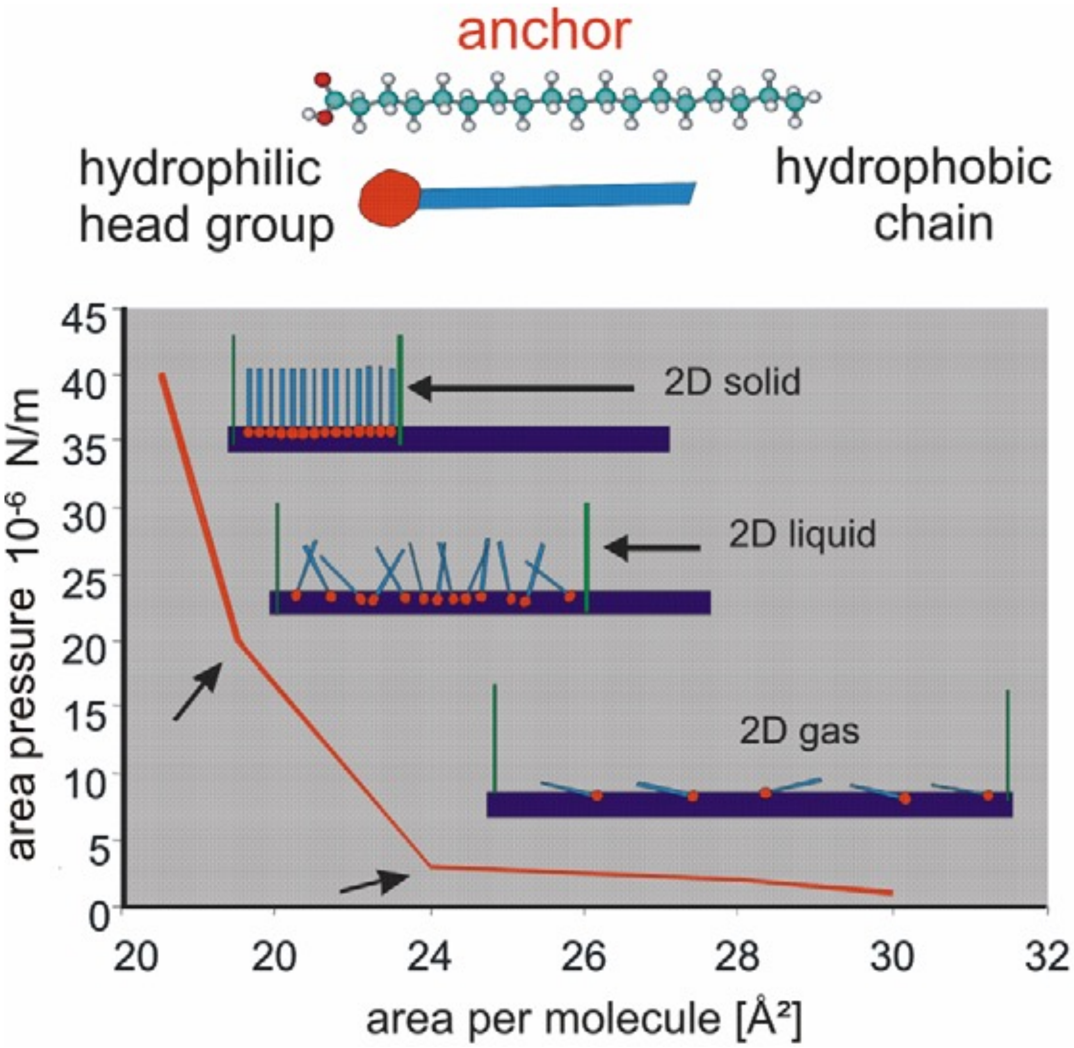


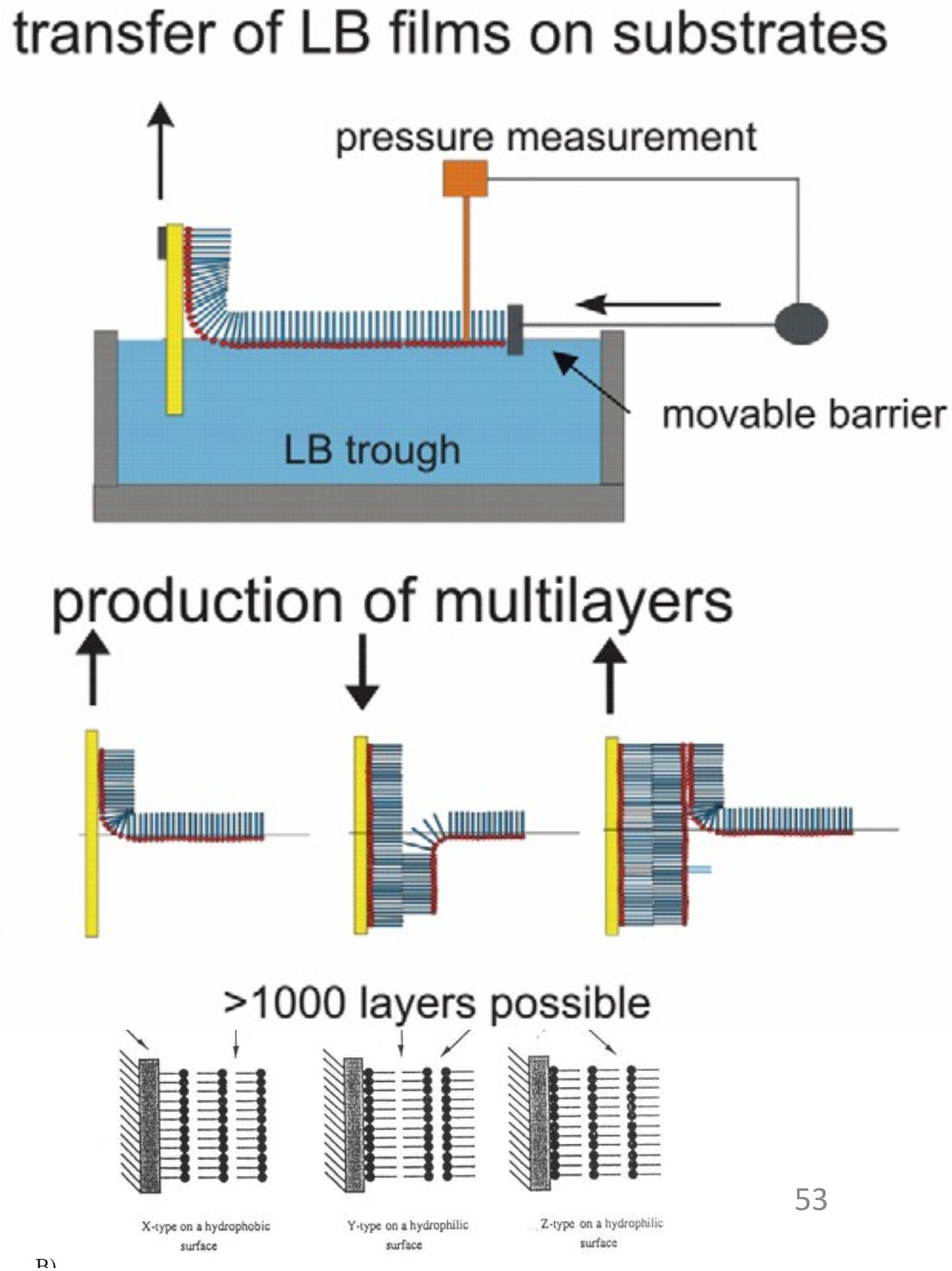


The term “molecular self-assembly” refers to spontaneous formation of an ordered molecular overlayer on the surface, often proceeding through several consecutive stages where 1D and 2D ordered structures can also exist.

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