Membranes

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.

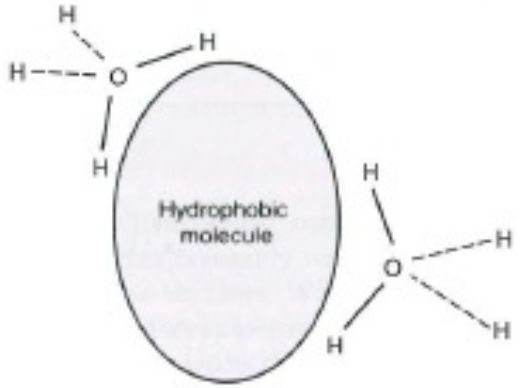


Fig. 2.7 Water molecules adjacent to a hydrophobic molecule suffer restrictions in orientation as they form hydrogen bonds with other water molecules.

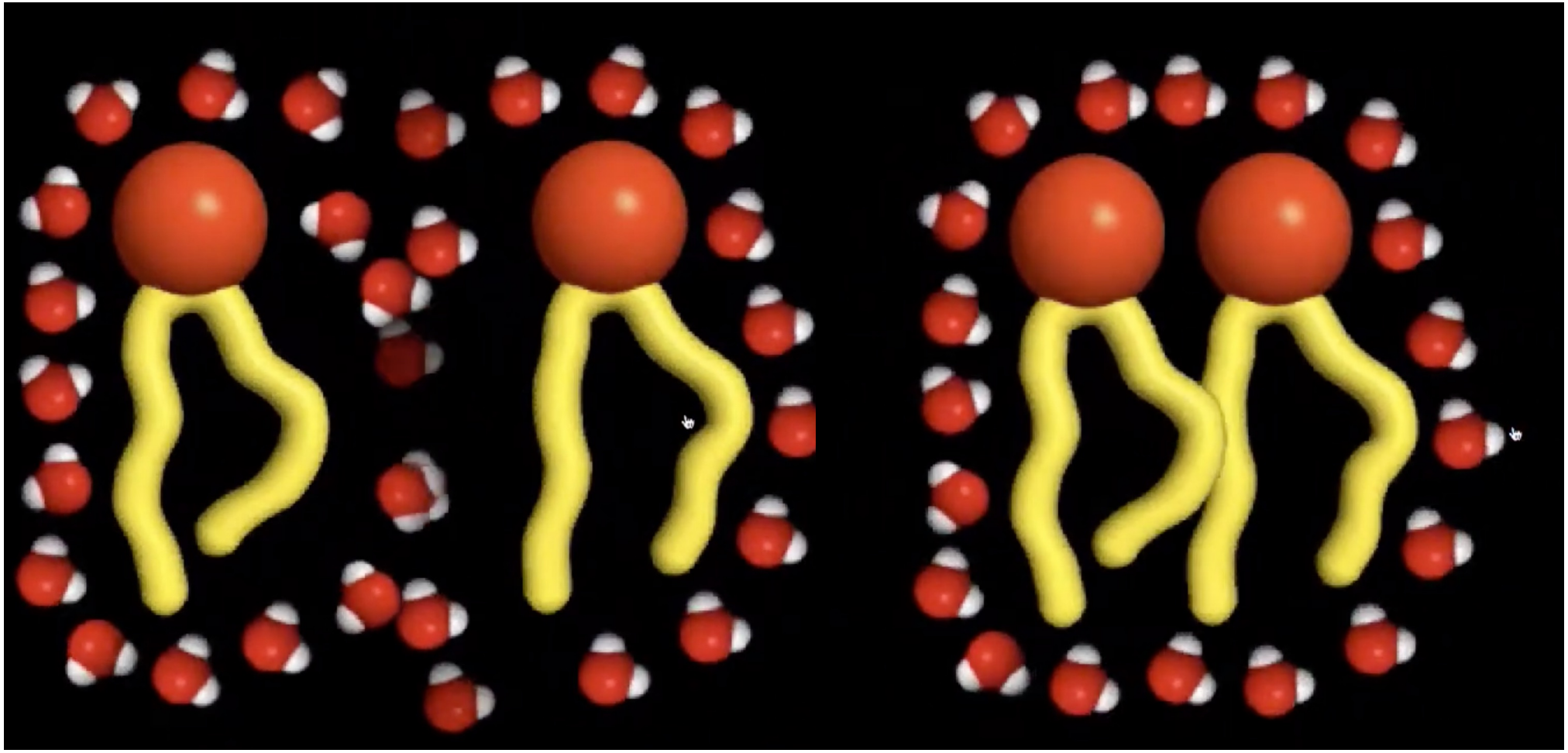
If one pictures a tetrahedral cage of four water molecules hydrogen bonding a central water molecule,
the central water can donate its hydrogen atoms in any combination of two of its four neighbors. **This gives six ways to be fully hydrogen bonded**. Replacing one water of the cage by a hydrophobic, nonhydrogen-bonding neighbor reduces the number of ways this can happen by a factor of about two.

Hydrophobic forces

The restriction in orientation of vicinal water varies with temperature. It becomes harder and harder to order molecules as the temperature is raised. As a result, **hydrocarbon–water contacts have a very high heat capacity.** Raising the temperature gradually melts the ice-like vicinal water. Interestingly, and somewhat paradoxically, as the temperature rises and the entropy goes up, the hydrophobic effect does not get weaker, but instead gets slightly stronger. This is because the dispersion force becomes stronger with increasing temperature, and this compensates for the loss of entropic drive.

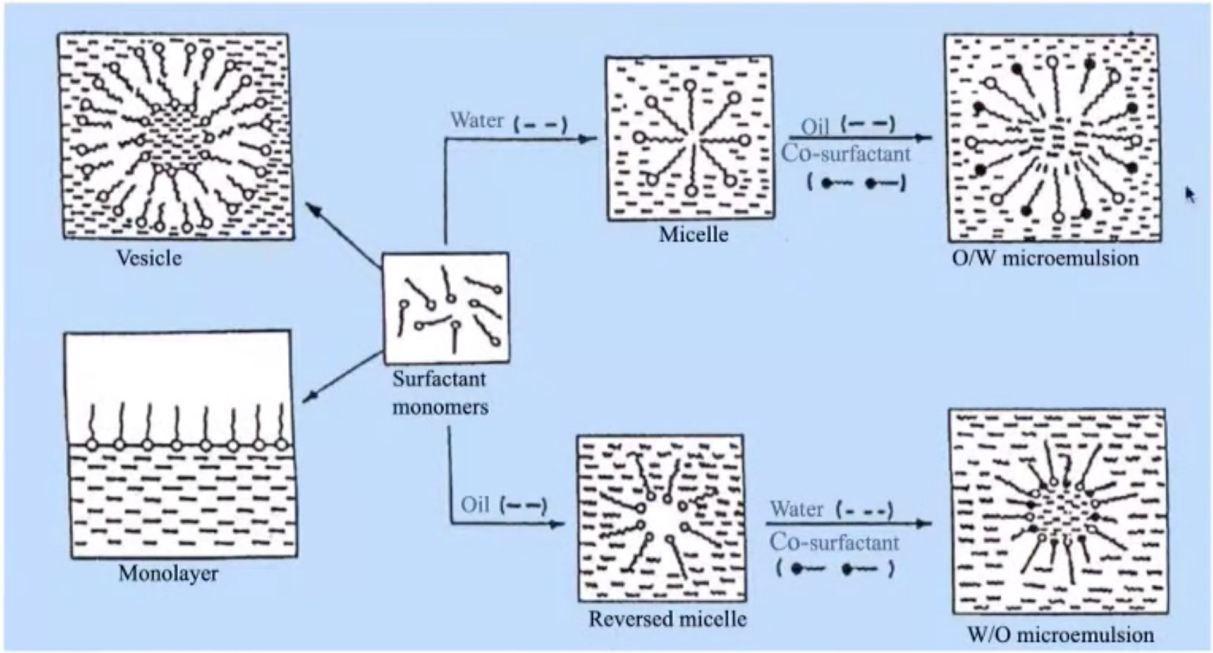
We must therefore view the hydrophobic force **as entropy-driven at low temperatures** (around room temperature) and **enthalpy-driven at higher temperatures** (near the boiling point of water). This in a limited range of temperatures.

Hydrophobic effect

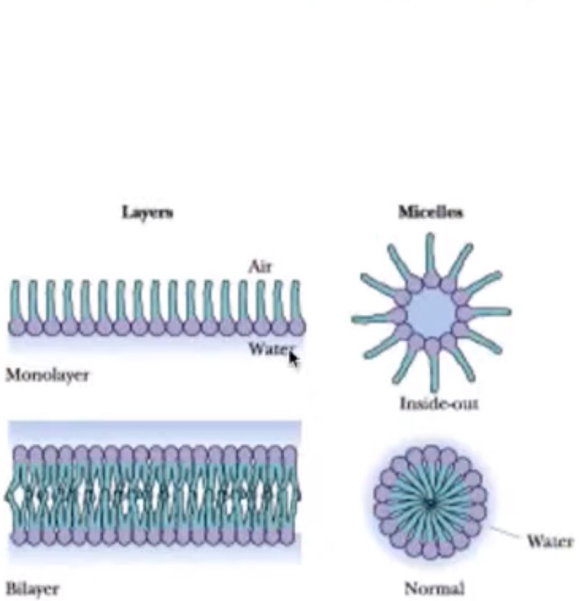


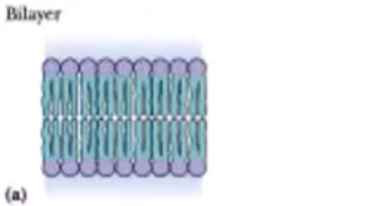
Hydrophobic effect

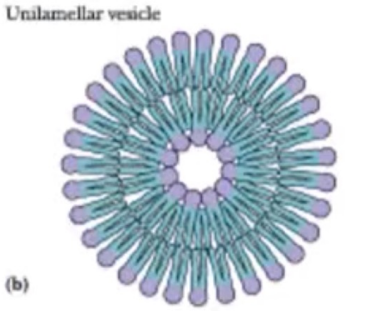
Organized molecular assemblies of various types formed due to the Hydrophobic Effect

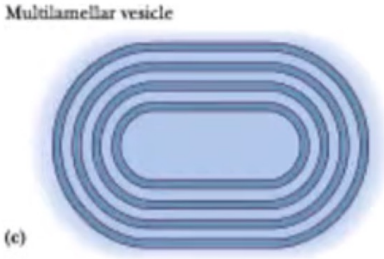


Phospholipid Supramolecular Assemblies







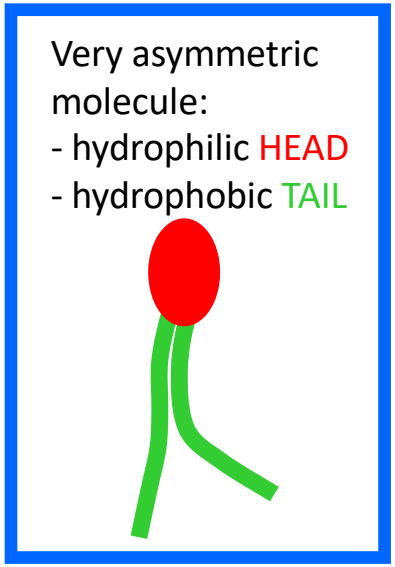


Lipids

Water insoluble compounds (soluble in organic solvents)

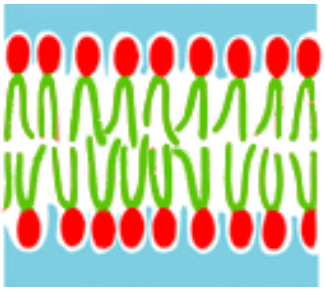
Biological role:

- 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.

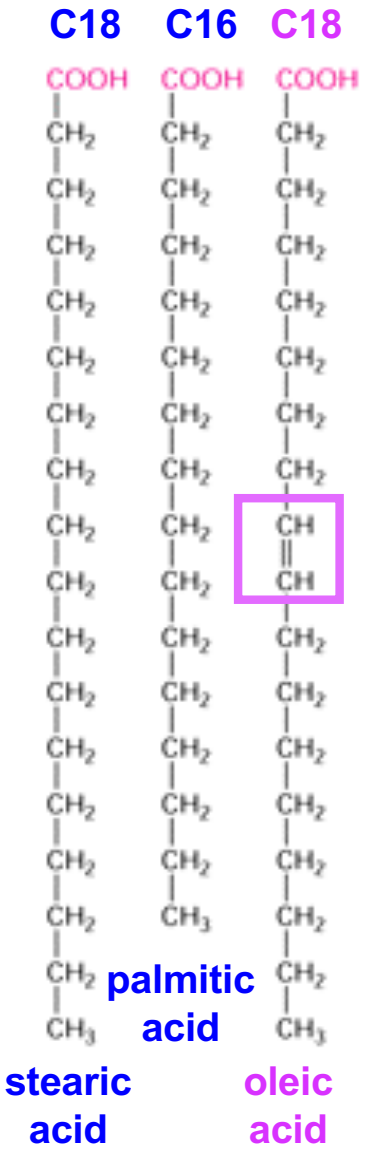
micelle



lipid bilayer

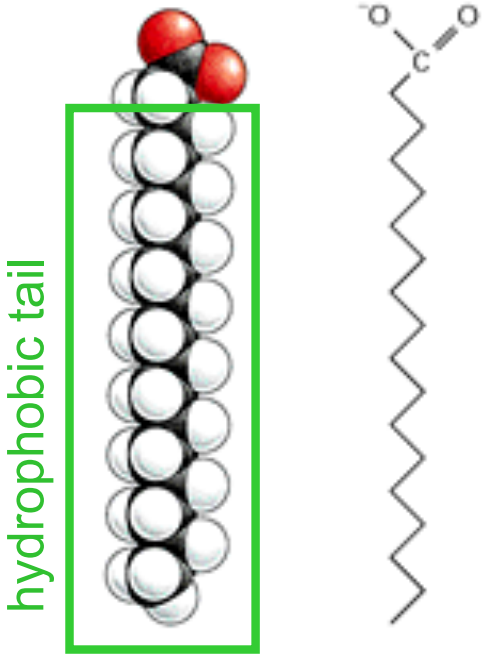
Fatty acids

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

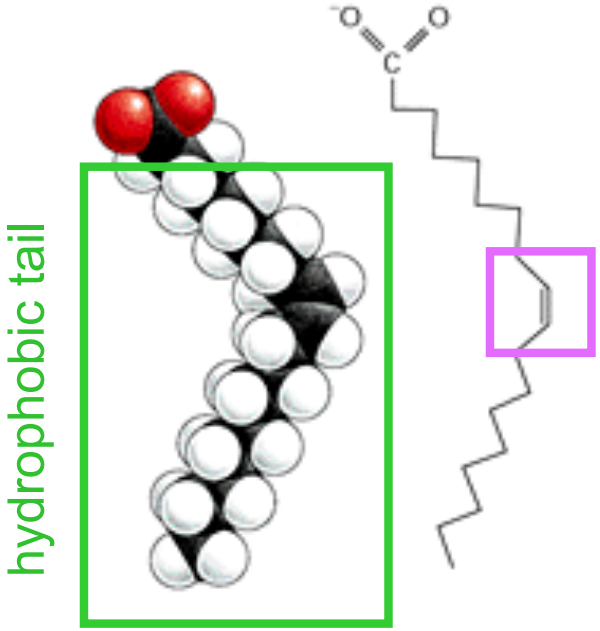


Some have one or more double bonds and are called unsaturated. The double bond is rigid and creates a kink in the chain; the rest of the chain is free to rotate

Stearic acid - saturated



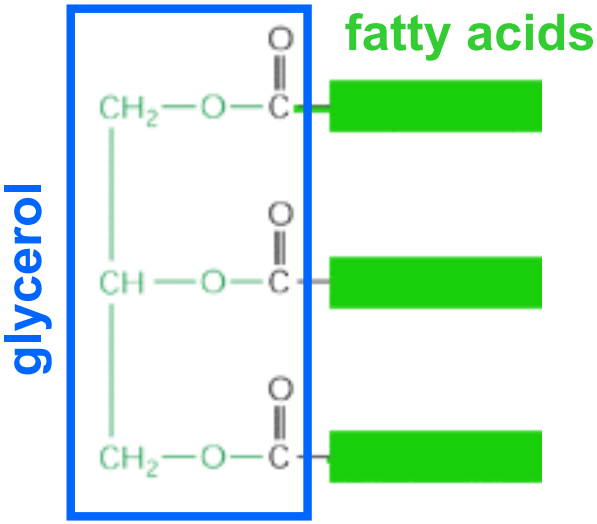
Oleic acid - unsaturated



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.

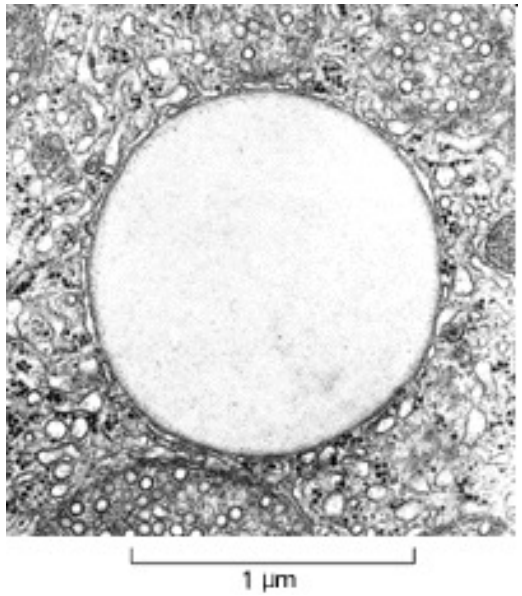
A fat molecule is composed of three molecules of fatty acid linked to glycerol: triacylglycerols (*triglycerides*).



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

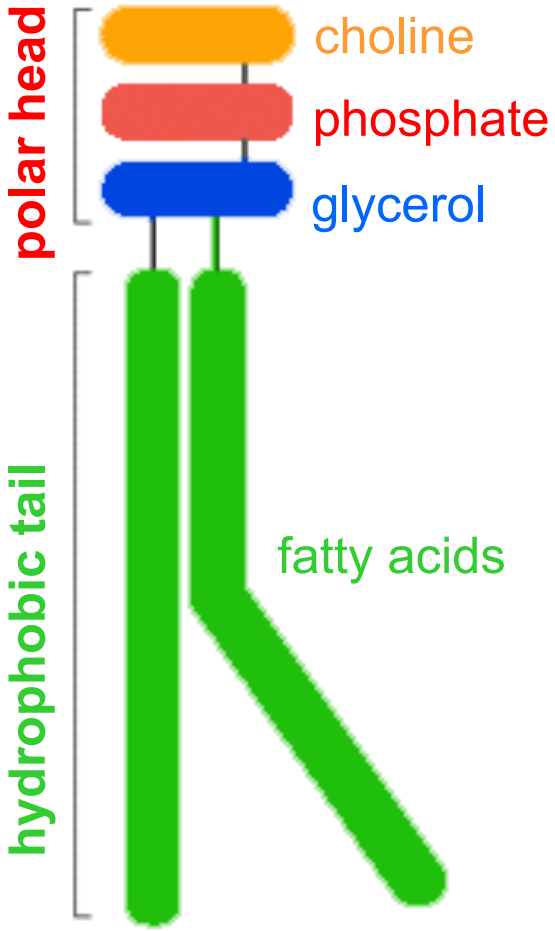
Triglycerides have no charge and are virtually insoluble in water, coalescing into droplets in the cytosol of adipose cells.

fat droplet

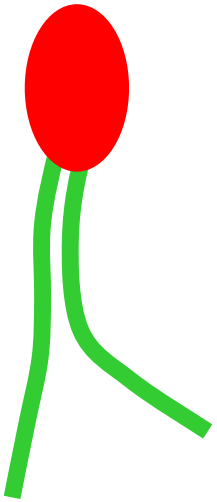


Phospholipids

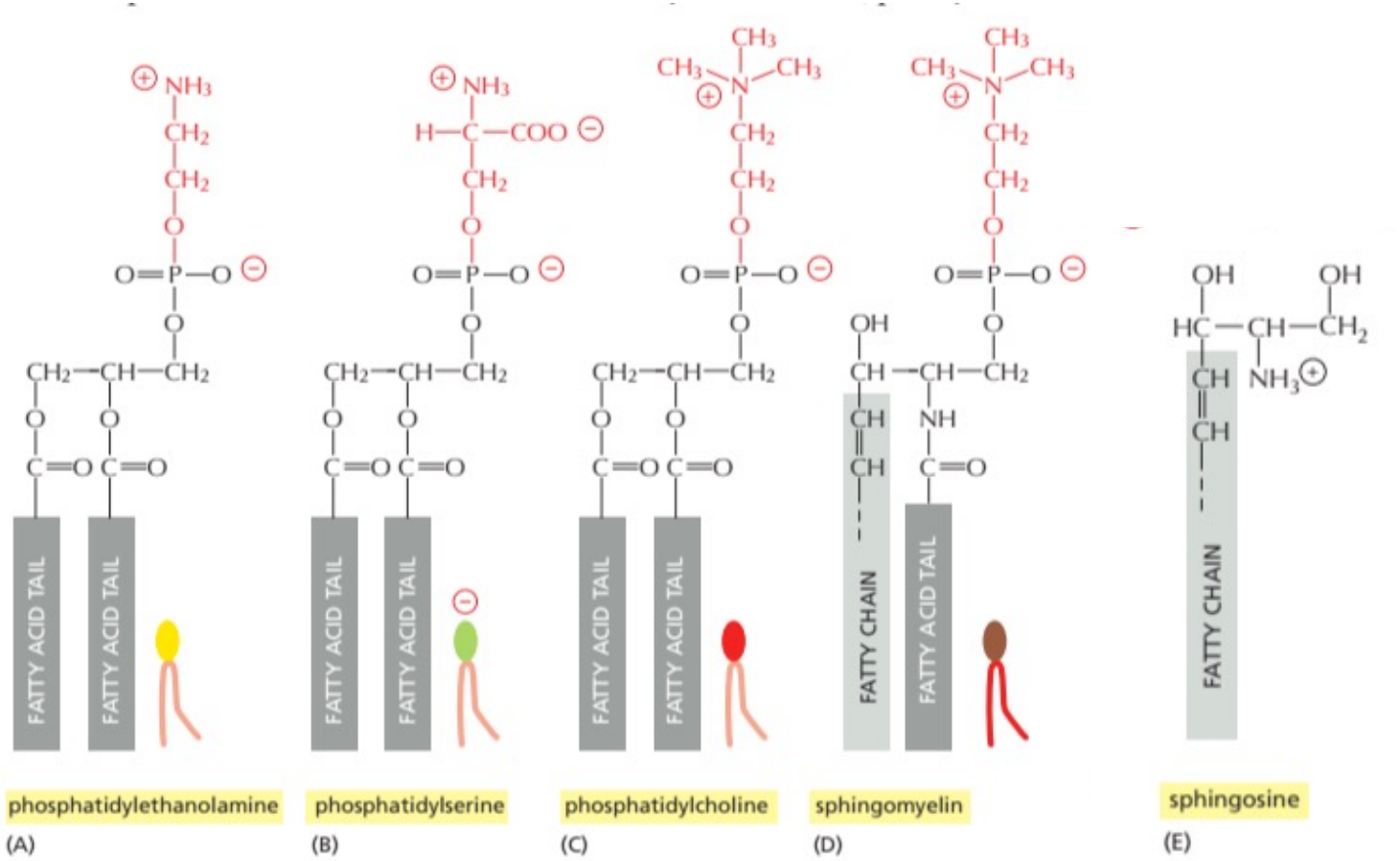
In phospholipids, two of the OH groups of glycerol are linked to fatty acids, while the third is linked to a phosphate group, which can be further linked to a polar group such as choline, serine, inositol, etc...



Very asymmetric molecule:
- hydrophilic HEAD
- hydrophobic TAIL



Sphingolipids



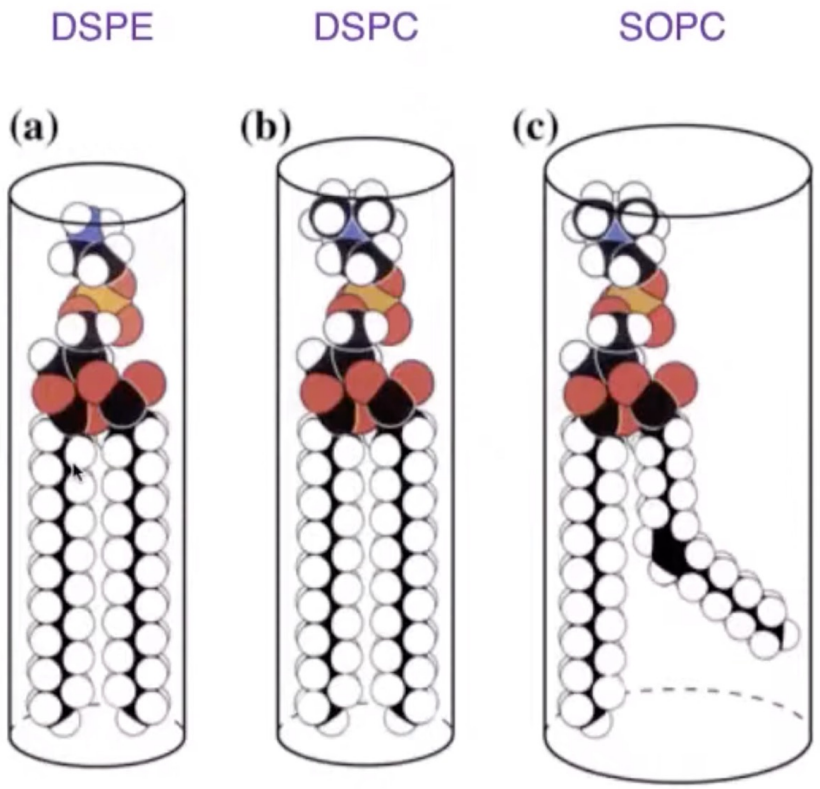
Sphingolipids are derivatives of sphingosine (E), an amino alcohol with a long hydrocarbon chain. Various fatty acyl chains are connected to sphingosine by an amide bond.
The sphingomyelins (SM), which contain a phosphocholine head group, are phospholipids.
Other sphingolipids are glycolipids in which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

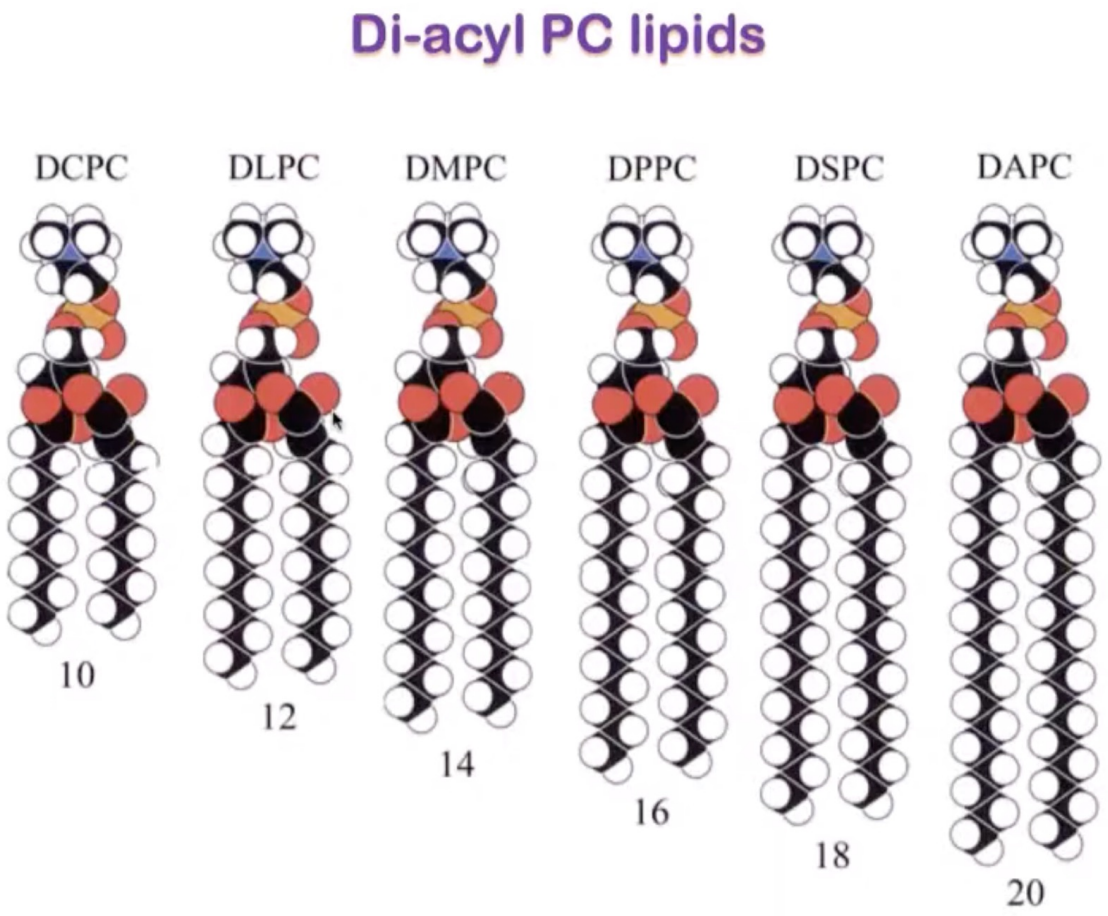
Saturated vs Unsaturated Fatty Acids



The actual conformation of a molecule influences its size.

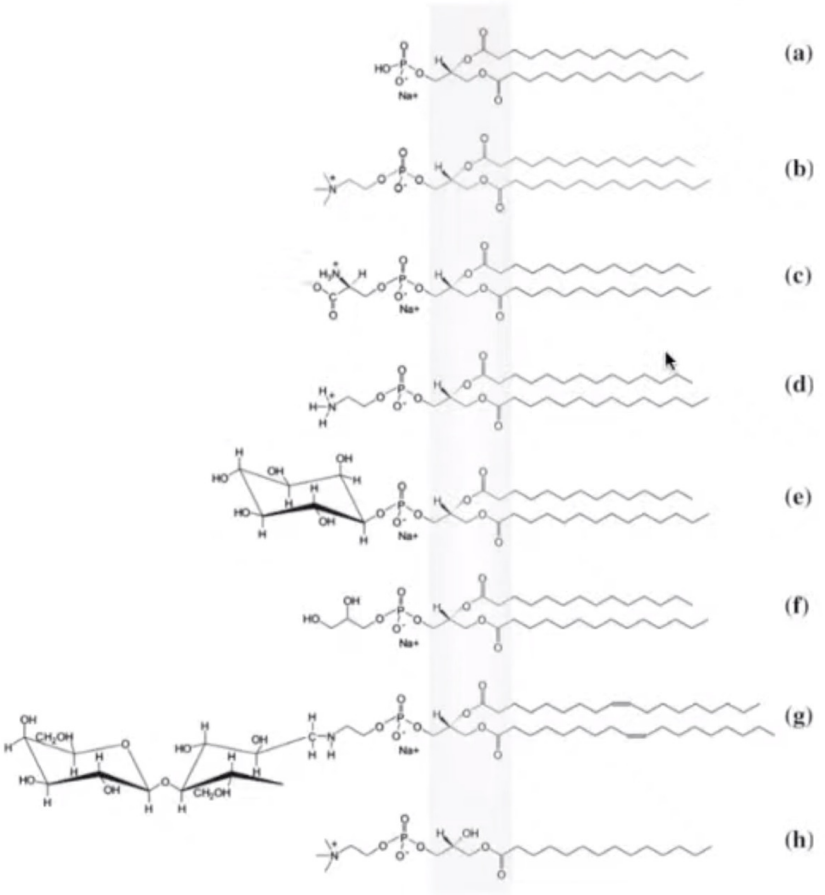
Temperature will lead to a rotation around the C-C bonds.

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



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

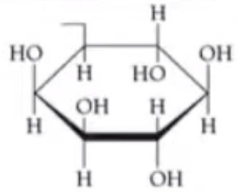
(e) DMPI

(f) DMPG

(g) Glycolipid

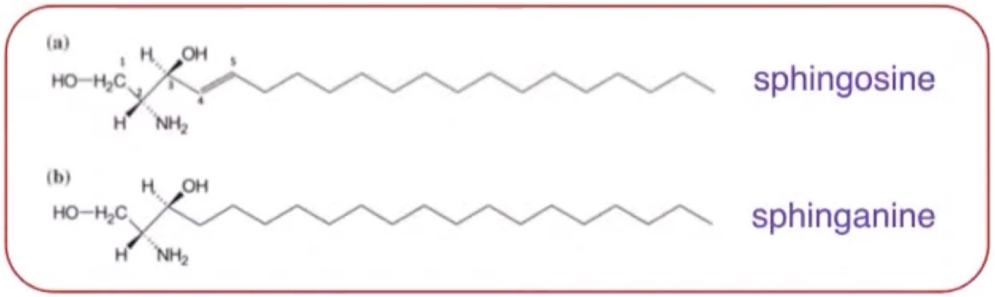
(h) Lysolipid with C16

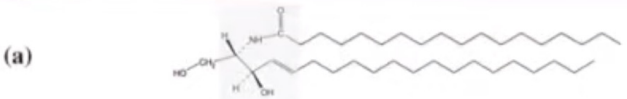
Lipid polar head groups

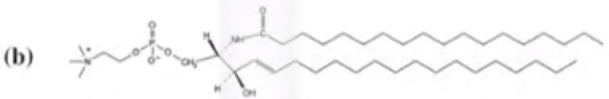
Substituent	Chemical formula*	Polar head group name	Ab[&]
hydrogen	-H	phosphatidic acid	PA
choline	$-CH_2CH_2N(CH_3)_3^+$	phosphatidylcholine	PC
ethanolamine	$-CH_2CH_2NH_3^+$	phosphatidylethanolamine	PE
serine	$-CH_2CH(NH_3)COO^-$	phosphatidylserine	PS
glycerol	$-CH_2CH(OH)CH_2OH$	phosphatidylglycerol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

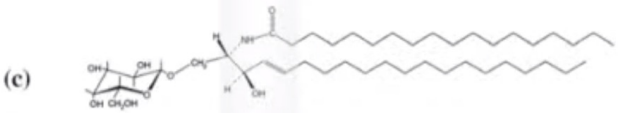
*Chemical formula for the substituent linked to the phosphate group at position 3 of the glycerol moiety.
[&]Abbreviation for the polar head group nomenclature.

Sphingosine based phospholipids









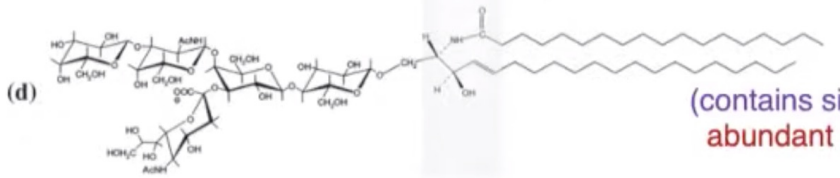


TABLE 2.1 SOME NATURALLY OCCURRING FATTY ACIDS: STRUCTURE, PROPERTIES, AND NOMENCLATURE[a]

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.

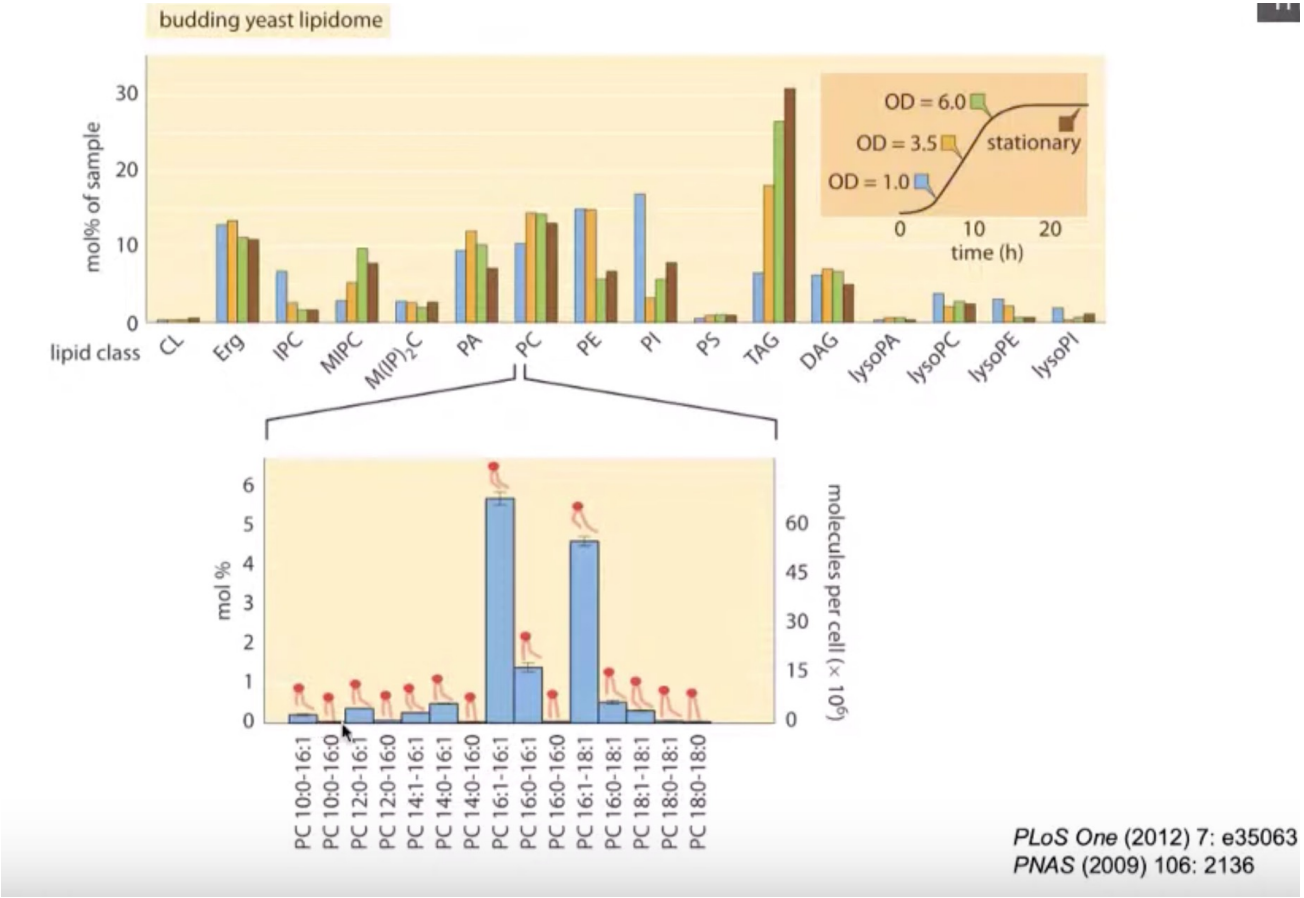
[b] All acids are shown in their nonionized form. At pH 7, all free fatty acids have an ionized carboxylate. Note that numbering of carbon atoms begins at the carboxyl carbon.

[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.

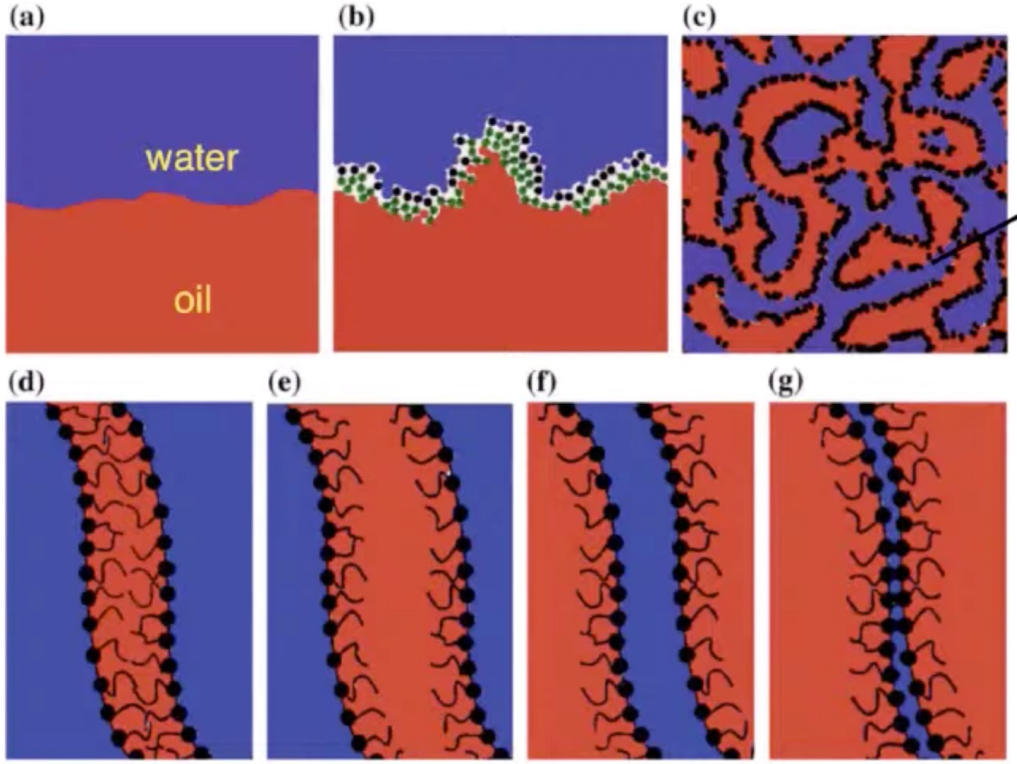
More than 500 species of fatty acids !

Lipidomic survey of a budding yeast



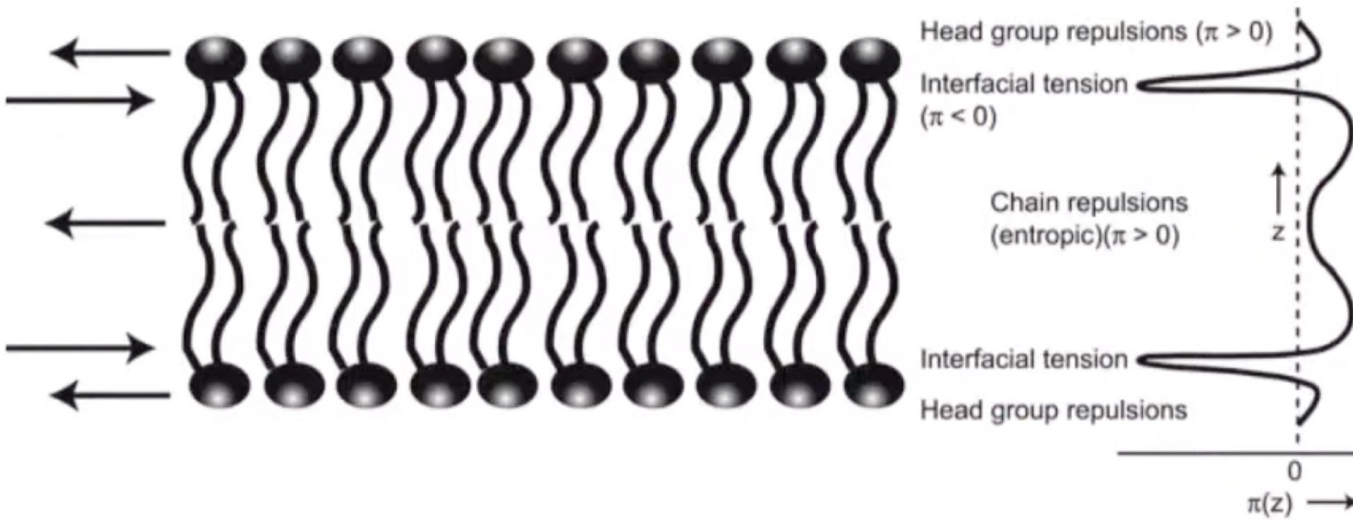
PLoS One (2012) 7: e35063
PNAS (2009) 106: 2136

Water and Oil interface



All interfaces are covered with interfacially active molecules

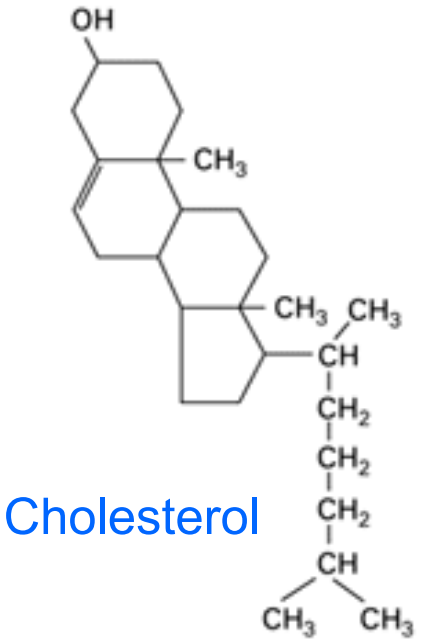
Lateral pressure profile of a lipid bilayer



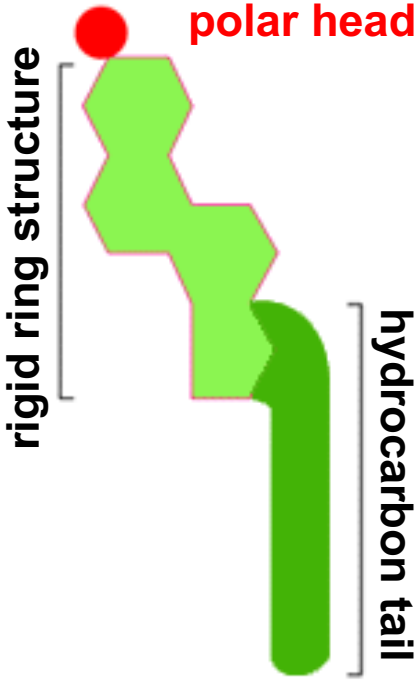
1. Positive pressure resulting from headgroup repulsive forces
2. Negative pressure at the hydrophobic-hydrophilic interface - the interfacial tension
3. Positive pressure resulting from entropic repulsion between acyl chains – chain pressure

Cholesterol and steroids

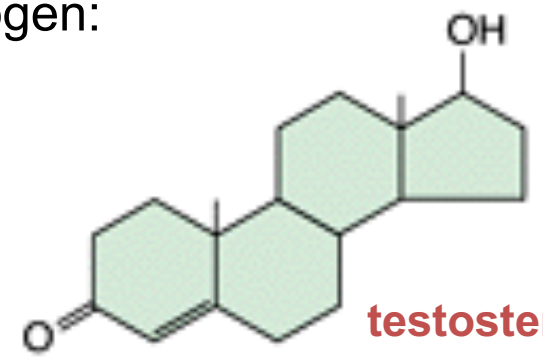
Steroids (such as cholesterol) have a rigid structure made up by 4 rings.



Cholesterol

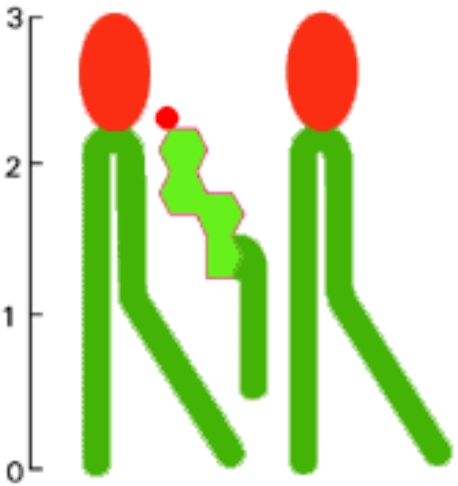


Other important steroid are the sex hormones, such as testosterone and estrogen:



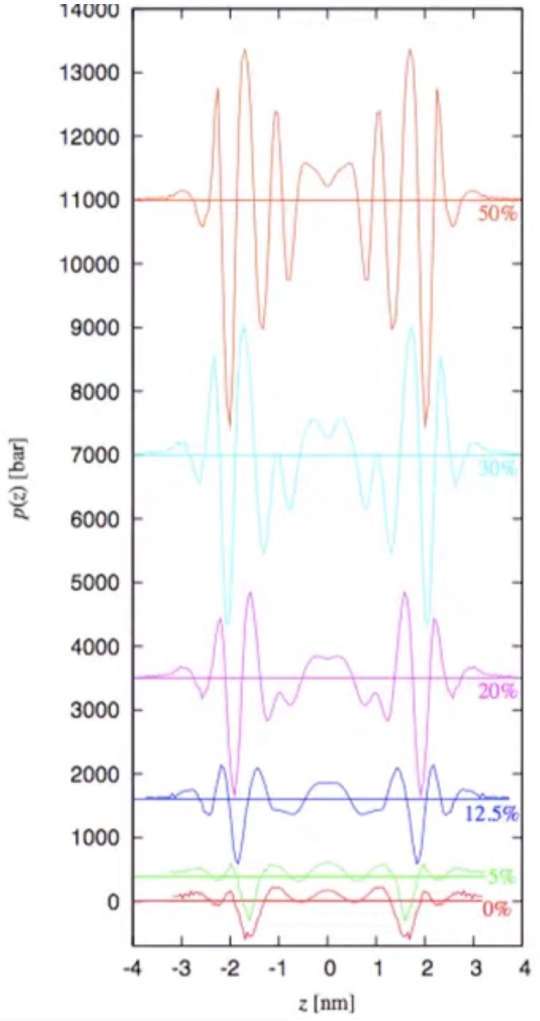
testosterone

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

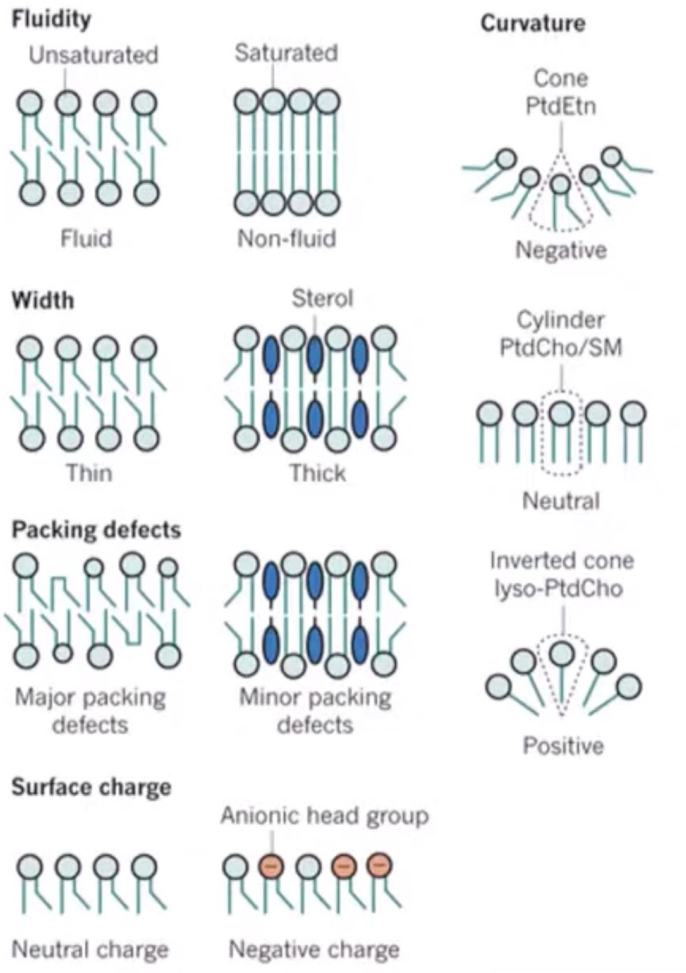


Effect of cholesterol

Lateral pressure profiles in DPPC/Cholesterol bilayer



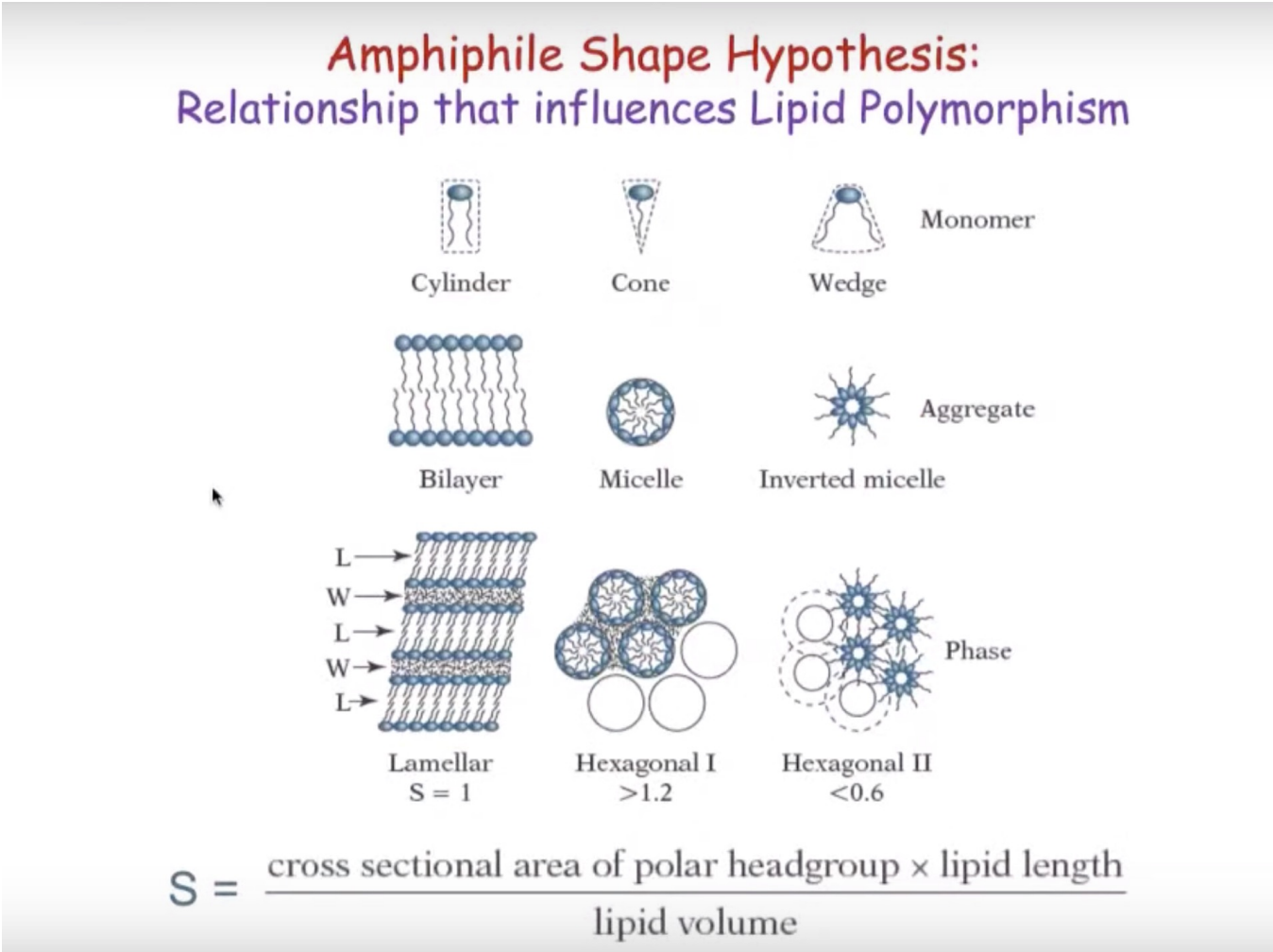
Membrane physical properties



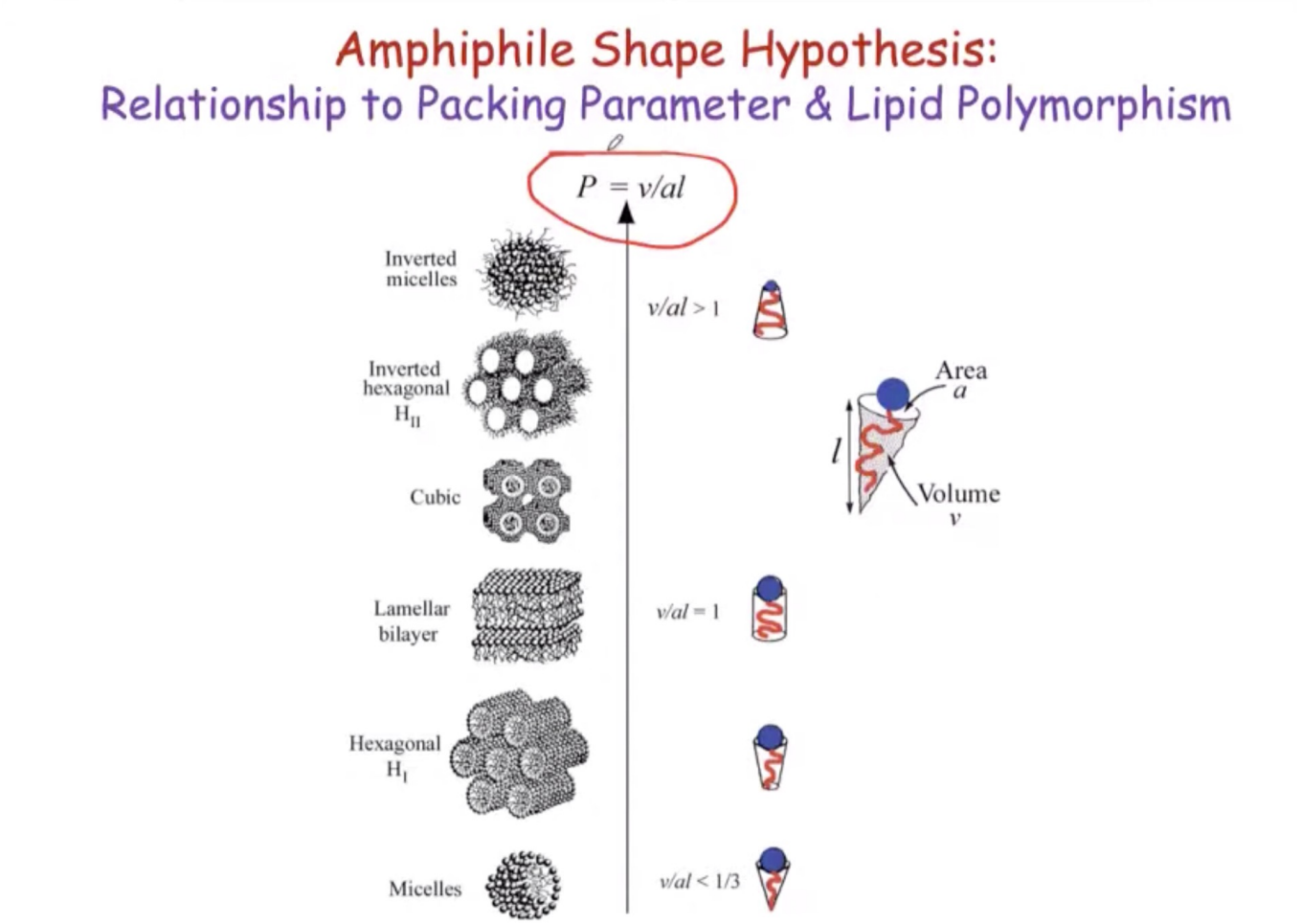
Membrane Physical Properties are Determined by its Lipid Composition

Nature (2014) 510: 48-57

Membrane physical properties

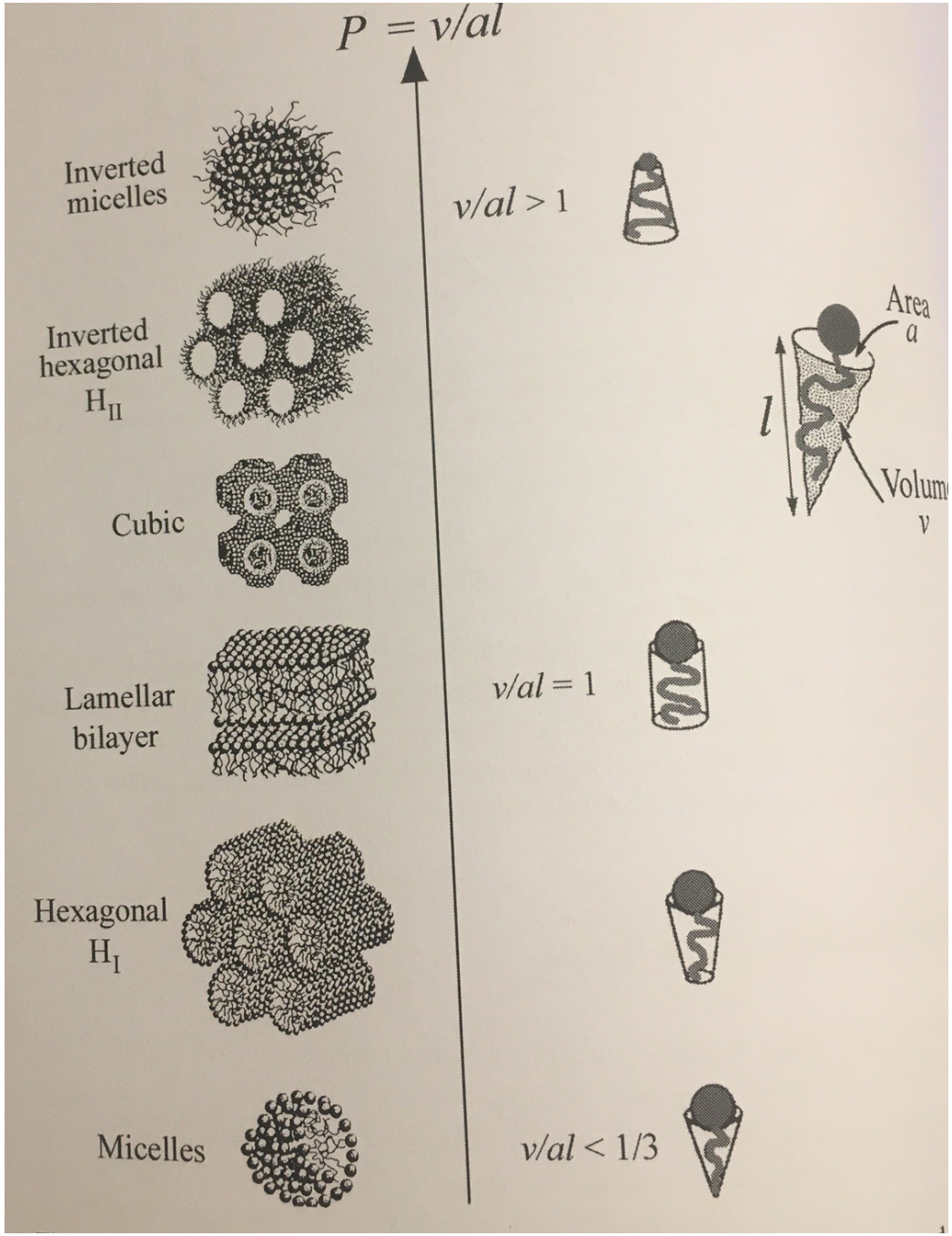


Membrane physical properties



P= lipid volume/ (cross sectional are of the polar group x lipid length)

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.

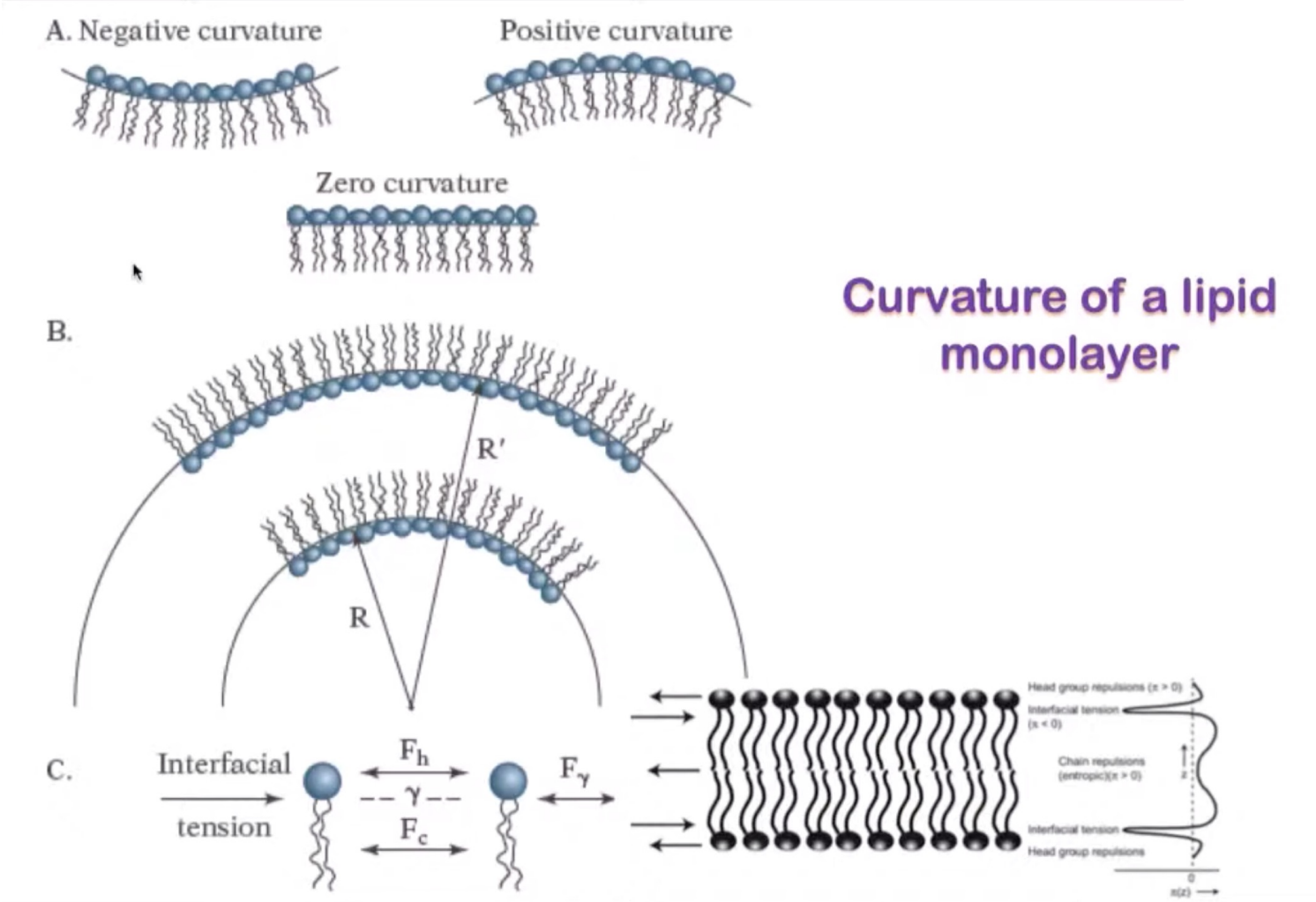
Lipid shape is important for functioning. It is given by the compatibility between head and tail. We define`a packing parameter P:

$$P = v/al$$

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

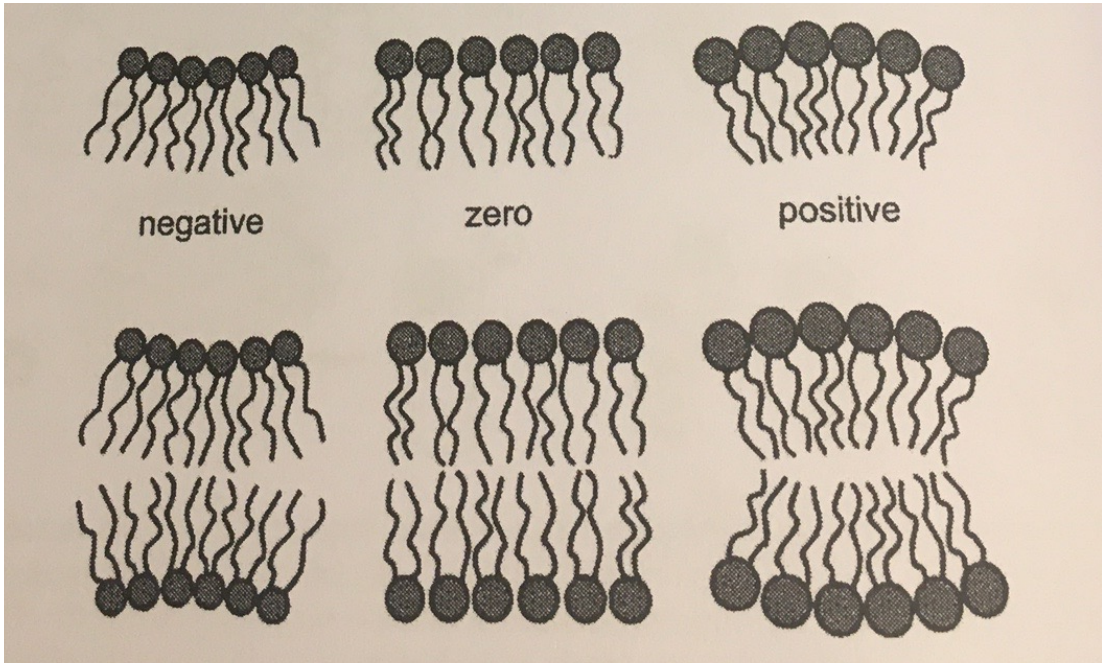
Curvature although is important for many of the membrane processes

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.

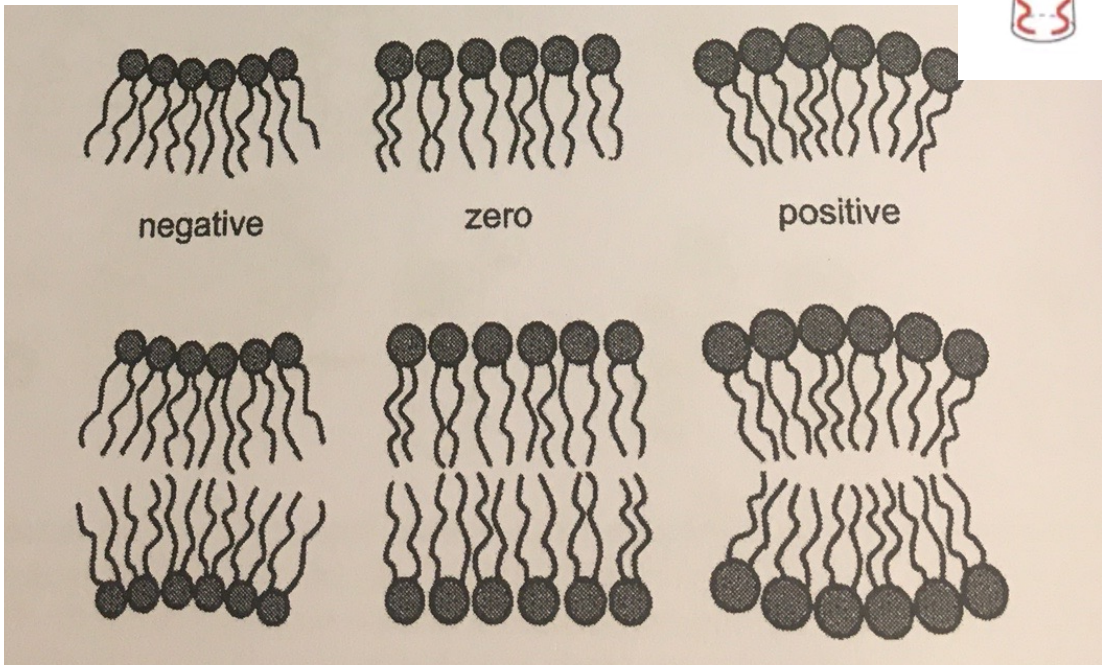
Each layer tend to elastically relax to a state of finite, spontaneous curvature, causing a curvature stress field.

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!

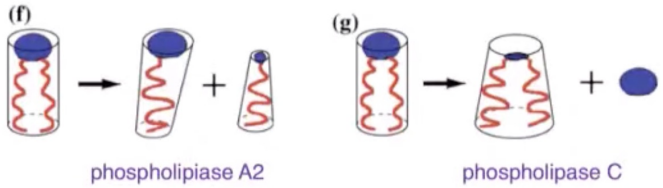
The inverted hexagonal structure (H_{II}), has long cilindrical rods of lipids, in a water filled tube, whose diameter can be varied with T, degree of hydration, pH (all change a/l ratio).

Lipids and membrane curvature



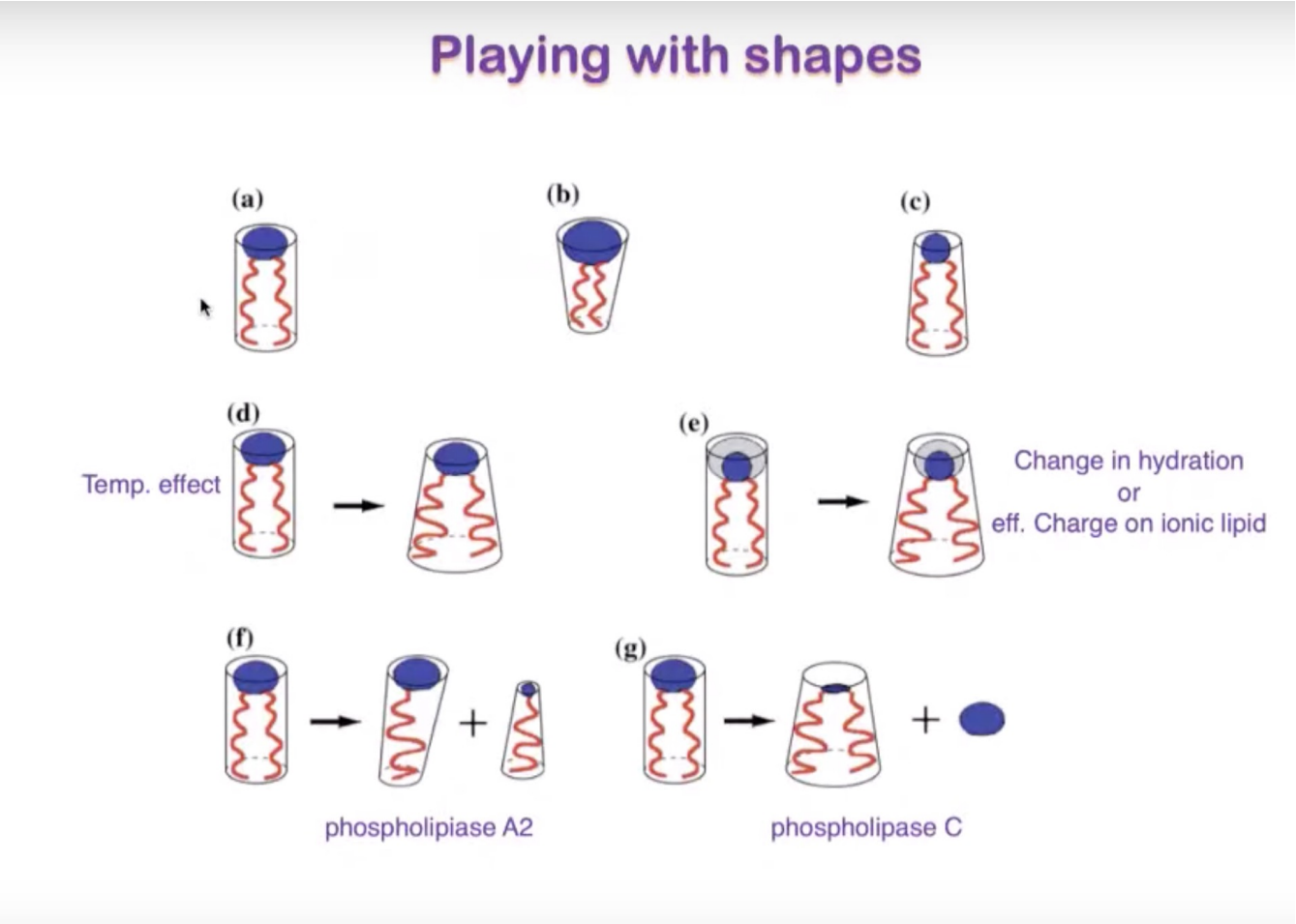
Cholesterol has an inverted conical shape (small OH, big steroid ring). Tends to promote the H_{II}. Stress field is mitigated by enzymes.

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

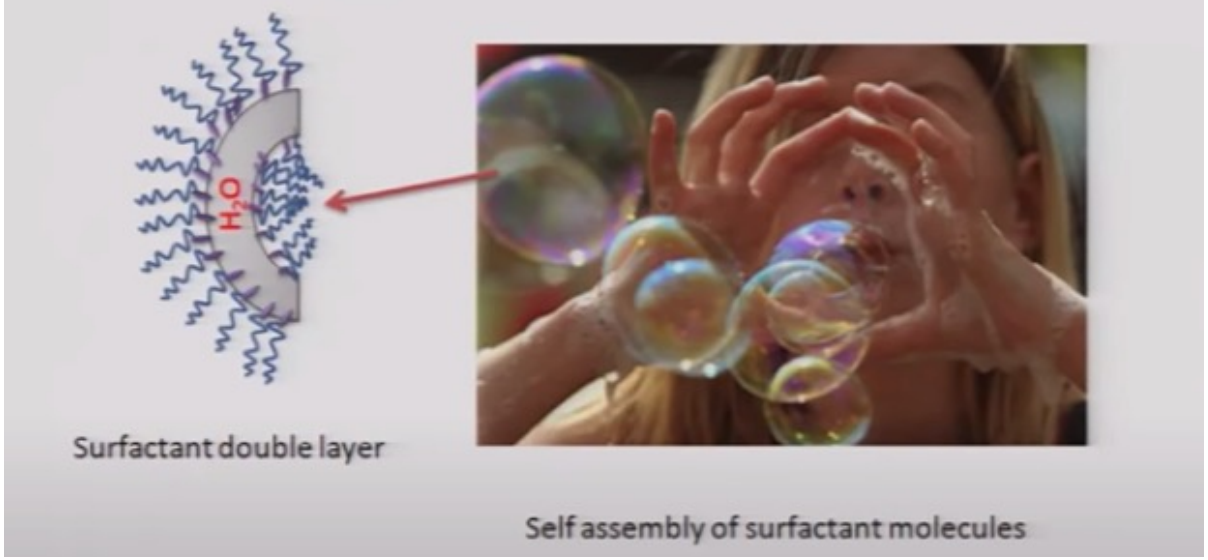
Membranes are soft interfaces. As polymers, exist in a condensed phase, but cannot be classified neither as solid, nor liquid. The physics of such interfaces is dominated by entropy.
Softness means high deformability but not necessarily high bulk compressibility!
Soft matter is anisotropic, hierarchical,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

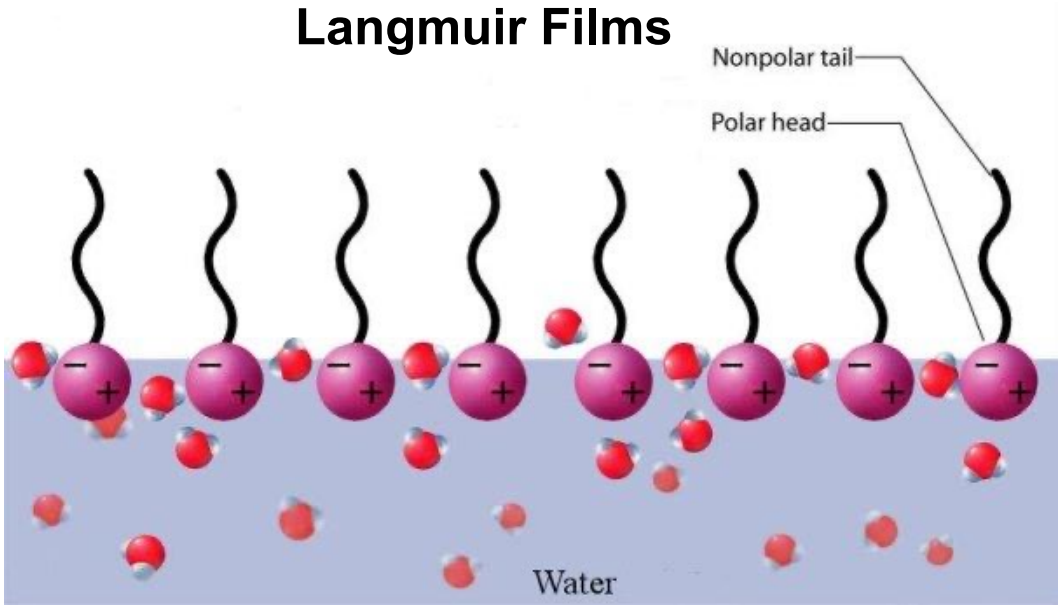
Natural examples of soft interfaces: soap bubbles

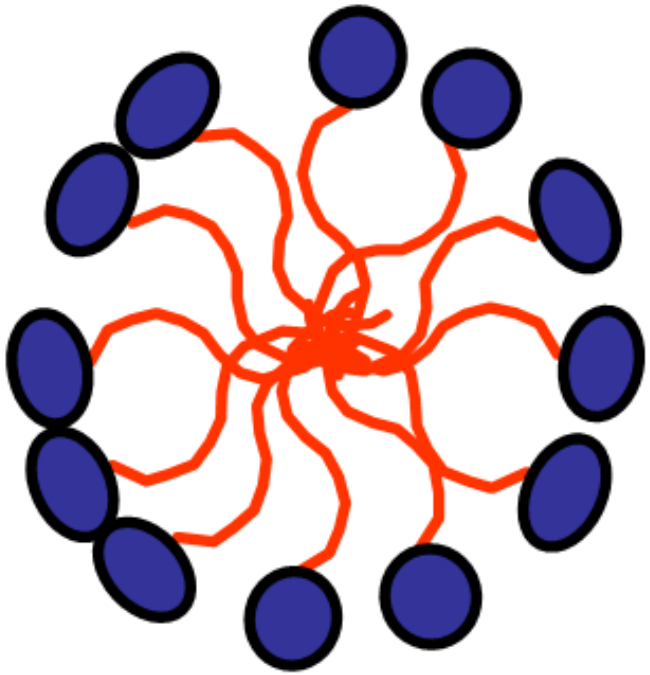


Soap bubbles: two layers form, at the water-air interfaces, the outer and the inner surfactant layer.
Bubbles are stabilized for a particular size, a particular water layer thickness depending on:

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

Self-organized monolayers (on liquid surfaces)

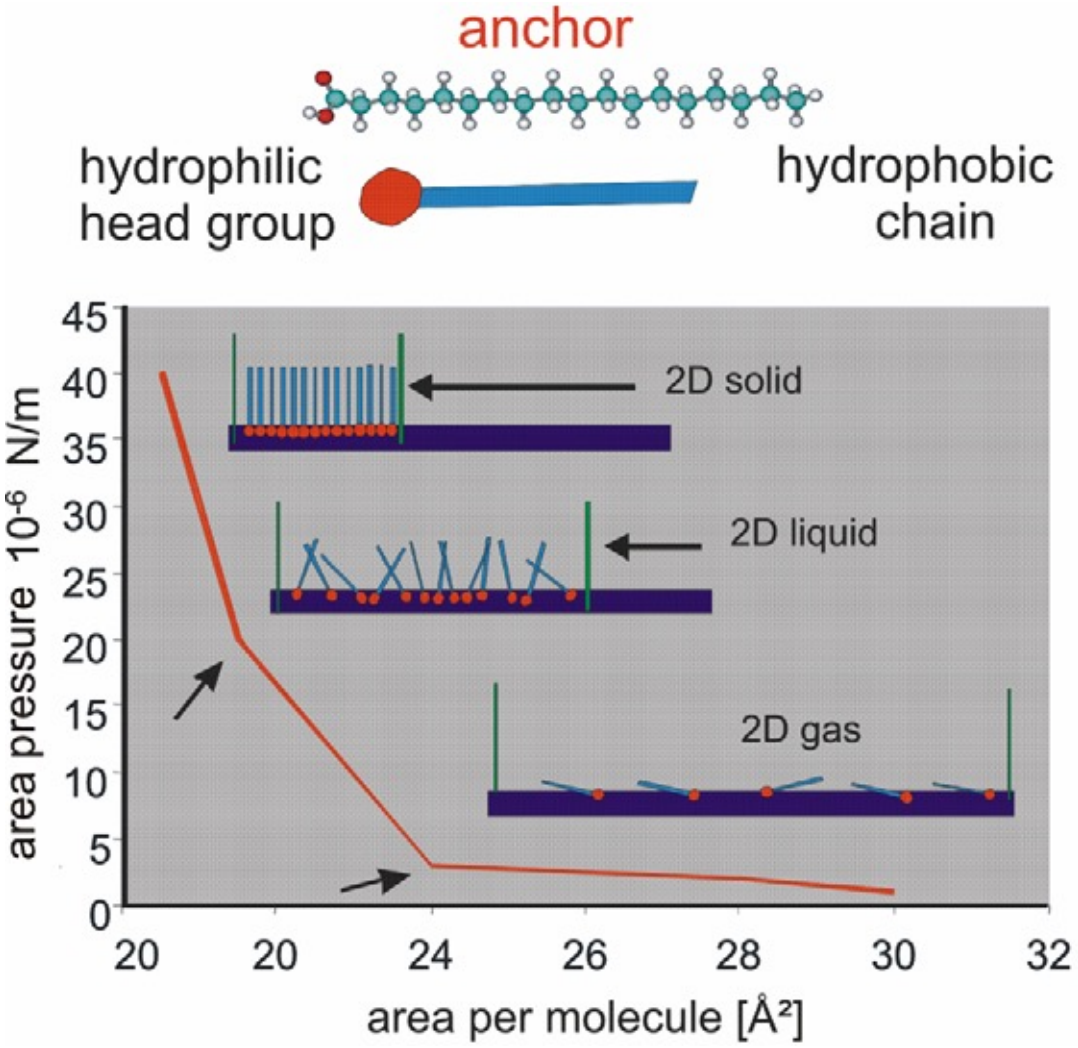




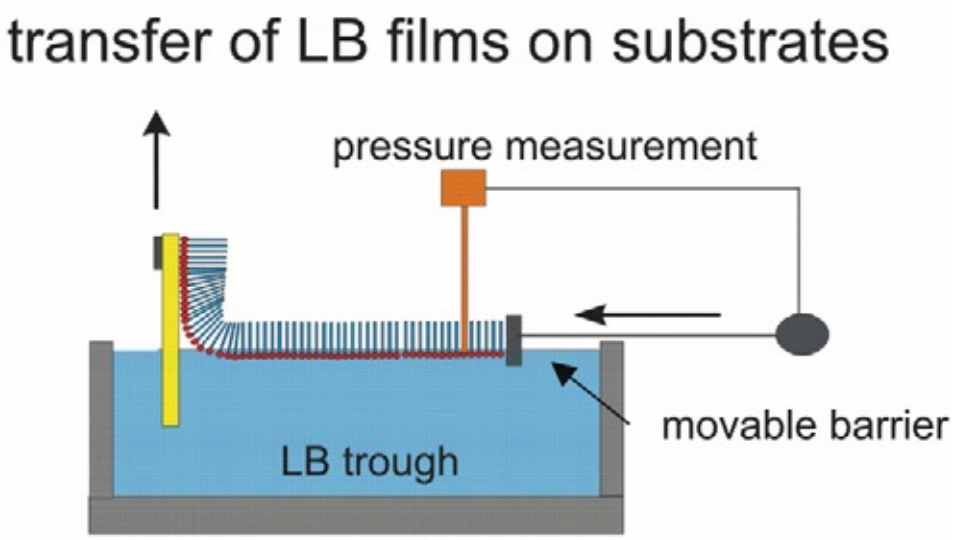
The term “molecular self-assembly” refers to spontaneous formation of an ordered molecular overlayer on the surface, often proceeding through several consecutive stages where 1D and 2D ordered structures can also exist.

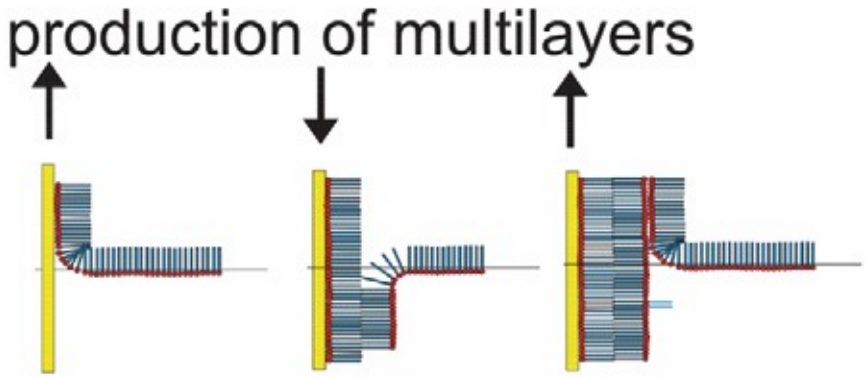
Thermodynamically, molecular self-assembly proceeds toward the state of lower entropy , and must therefore be compensated by the establishment of intermolecular and molecule-surface interactions.

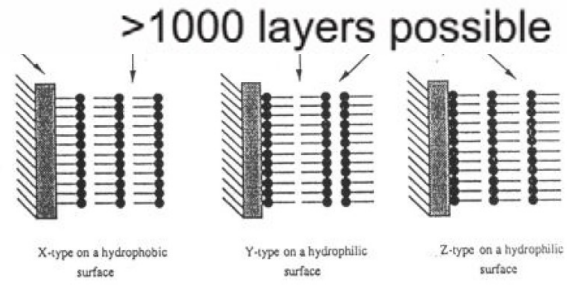
Self-organized monolayers (on solid surfaces)



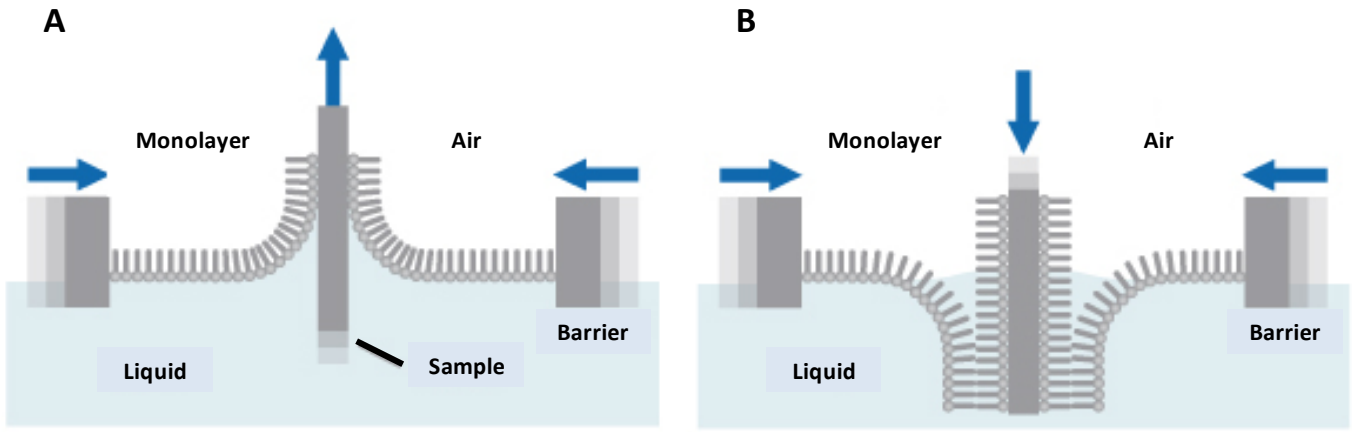
Progress in Surface Science 84 (2009) 230–278



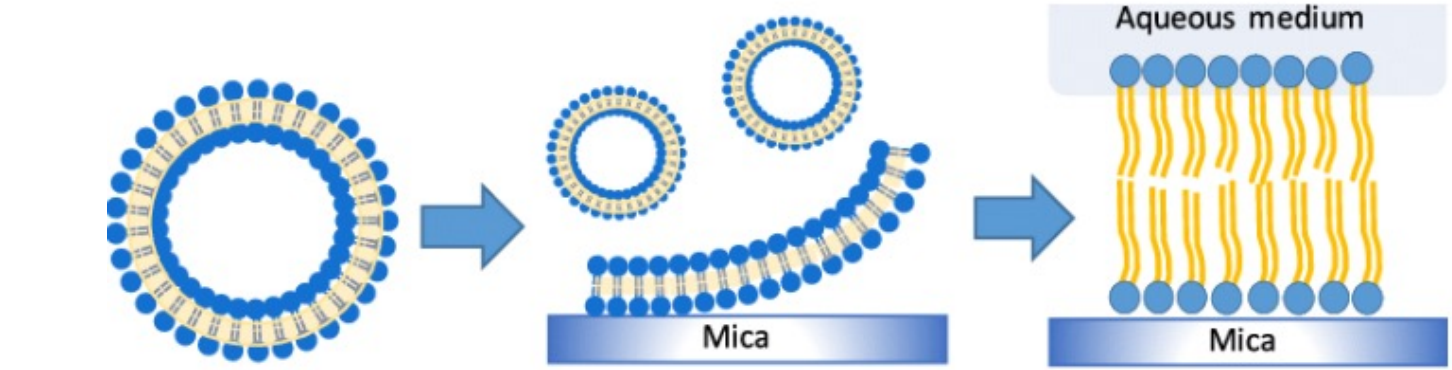


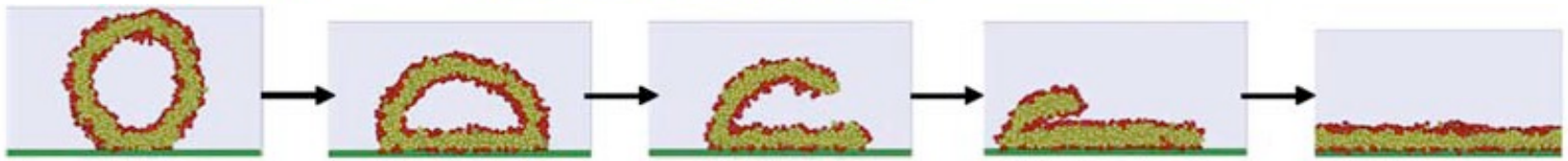


Supported lipid bilayers



Langmuir-Blodgett

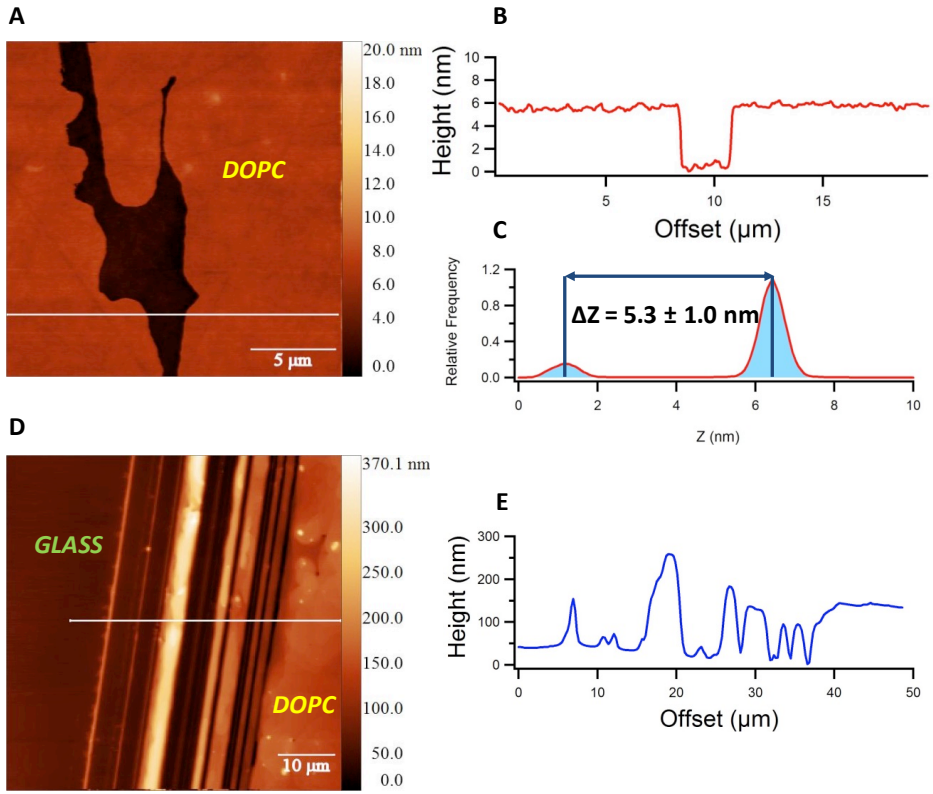




surface characteristics, pH, and ionic strength

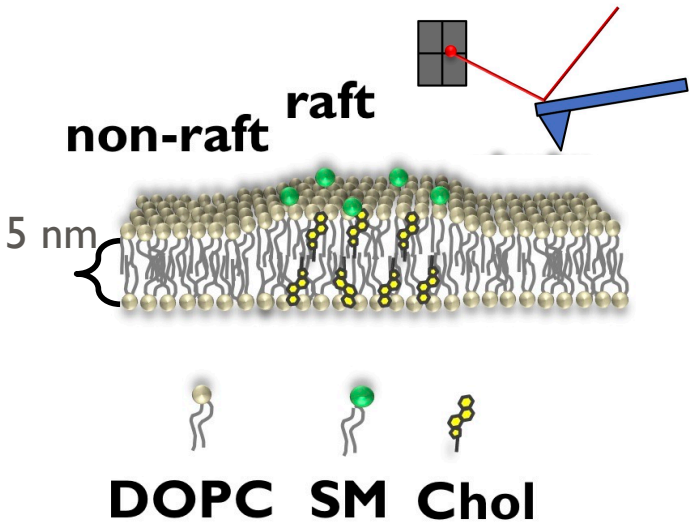
ze,

Supported lipid bilayers: Atomic Force Microscopy (AFM)

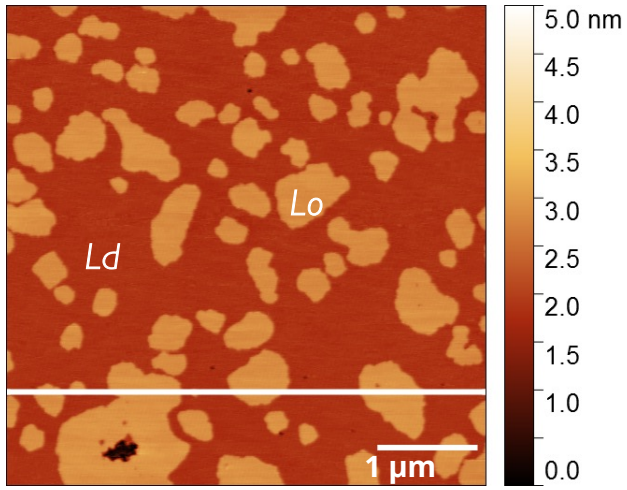


(A) AFM topography of flat lipid membrane with a central discontinuity which allows to measure the height of the lipid bilayer. (B) Height profile. (C) One DOPC bilayer is characterized by an average height of 5.3 ± 1.0 nm. (D) AFM topography of the scratch made by a scalpel. On the right side, a DOPC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

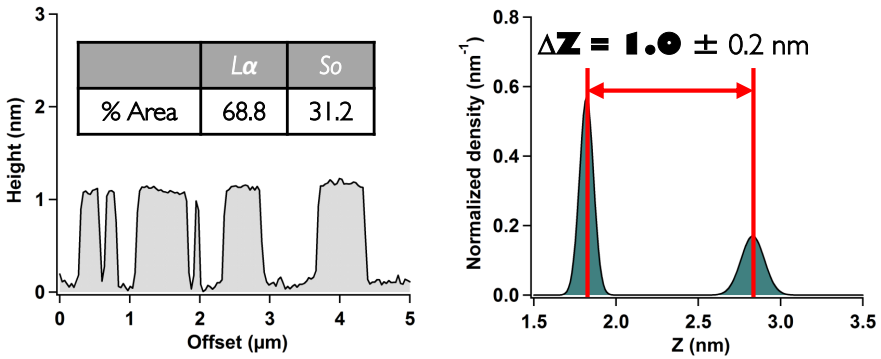
Supported lipid bilayers: Atomic Force Microscopy (AFM)



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



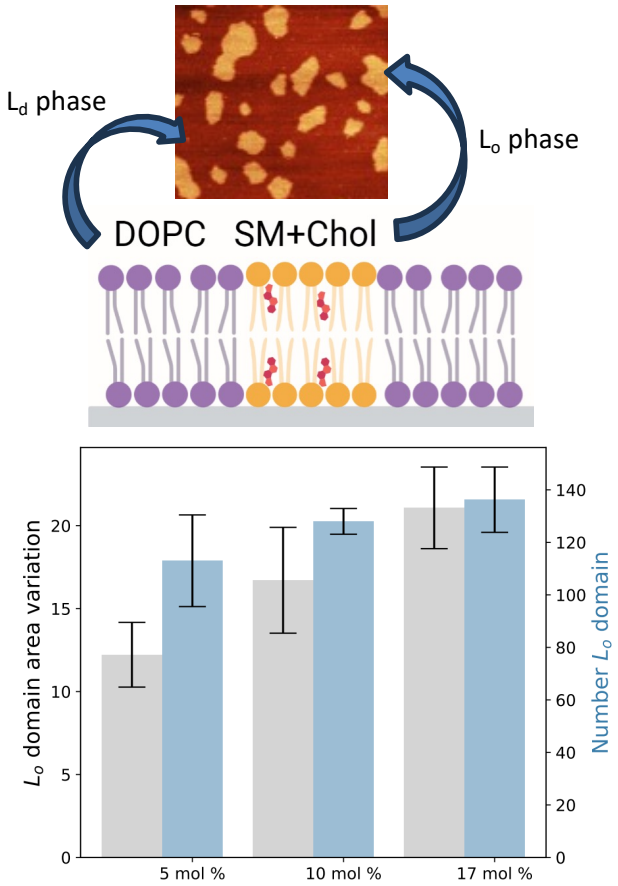
AFM imaging in dynamic AC-mode in liquid environment

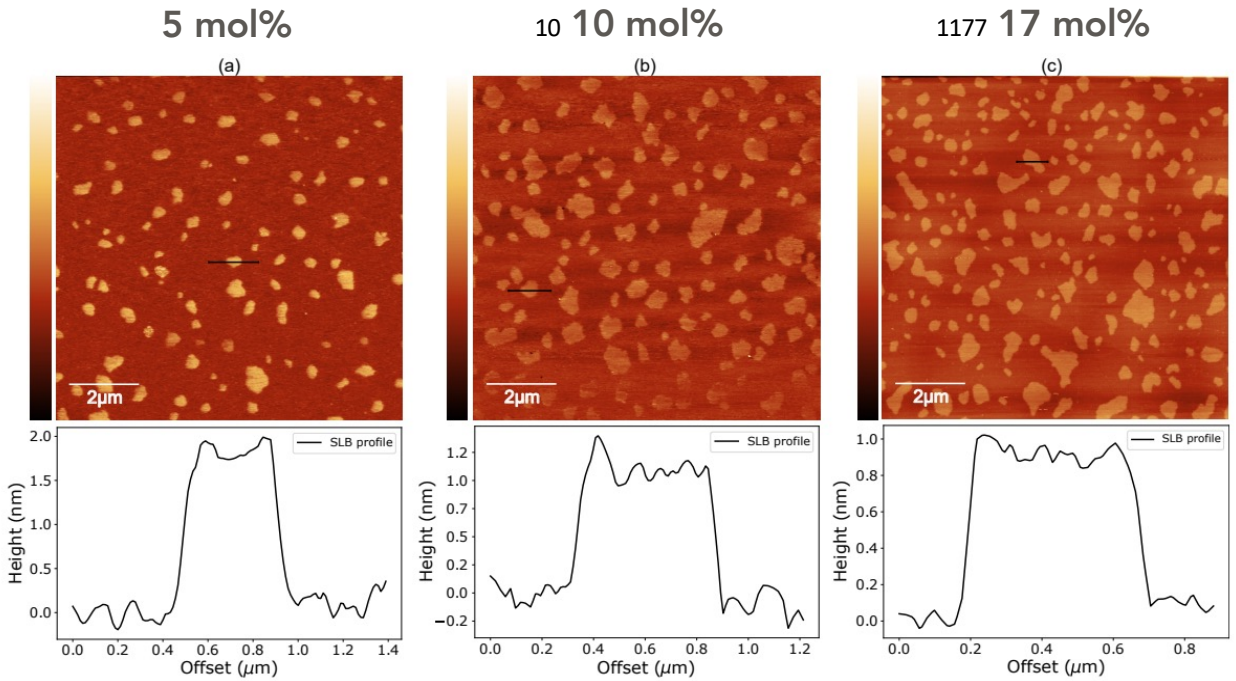


surface roughness: Lα=0.16 ± 0.01 nm, Lo=0.14 ± 0.01 nm

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

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





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

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

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

How fluid/stiff are our membranes?

Up to 33% chol, different mimic

DOPC/DSPC/SM

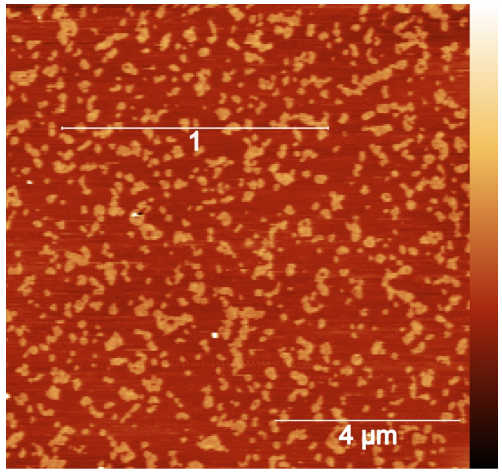
Ld Lo

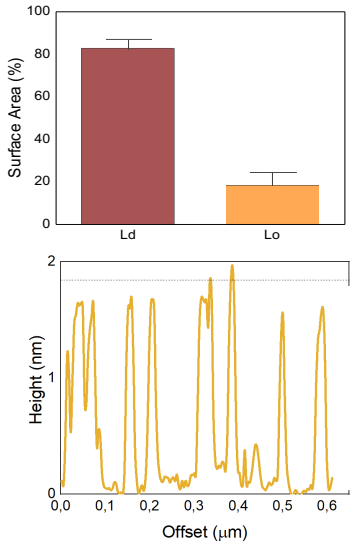
(DOPC:DSPC) 1.75; (DOPC:SM) 2.91

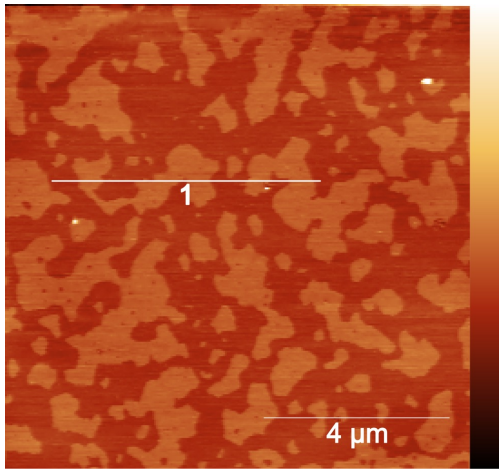
7%

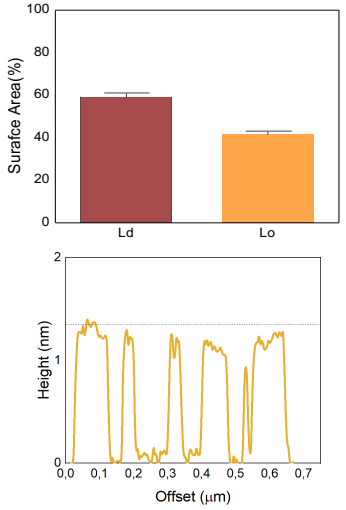
Chol

33%

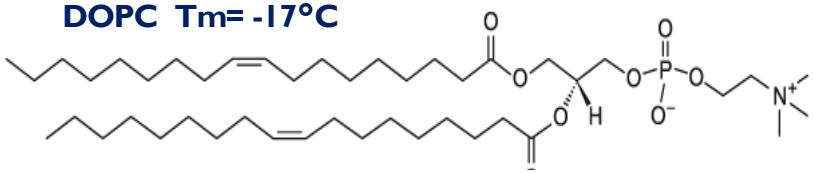




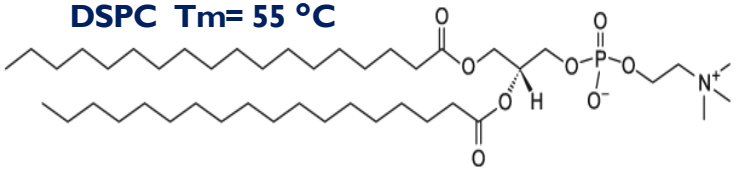




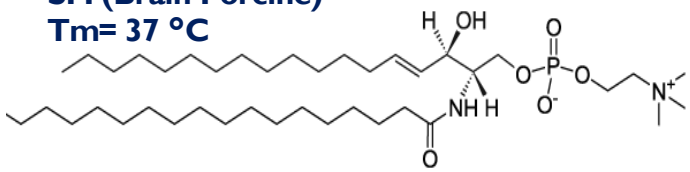
DOPC Tm= -17°C

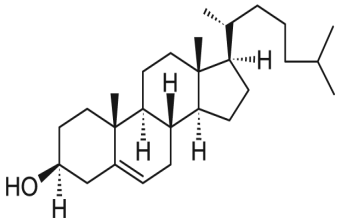


DSPC Tm= 55 °C



SM (Brain Porcine)
Tm= 37 °C

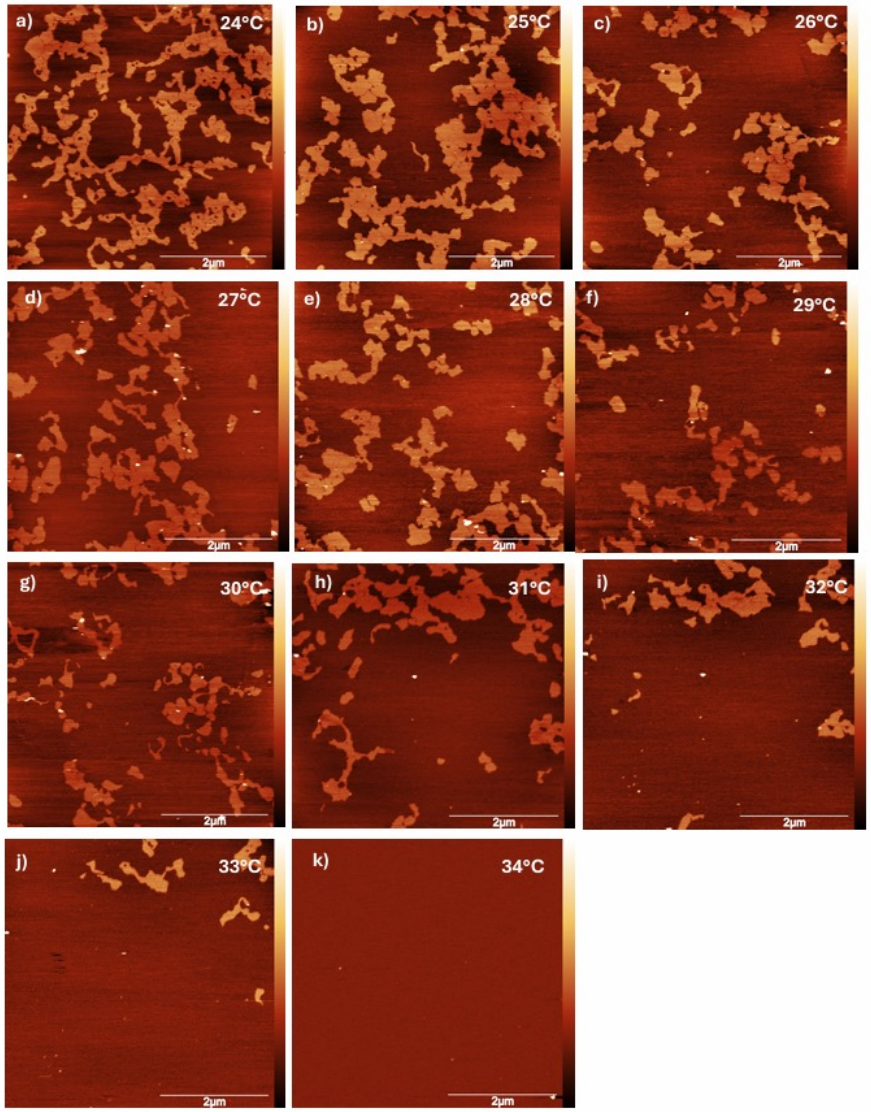




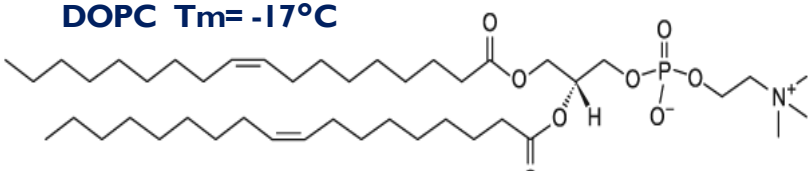
Cholesterol

7% LOW, 33% High

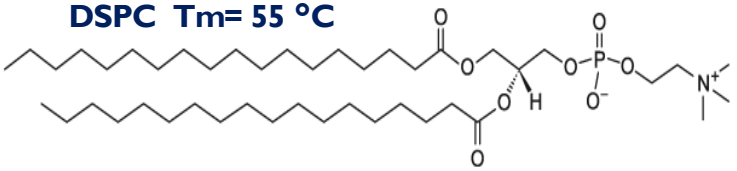
Temperature evolution: 7% chol



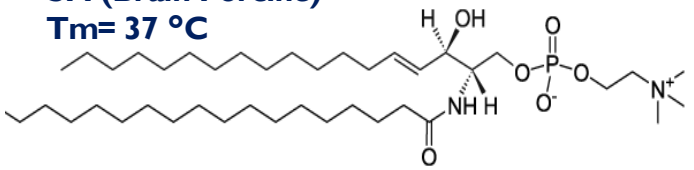
DOPC Tm= -17°C

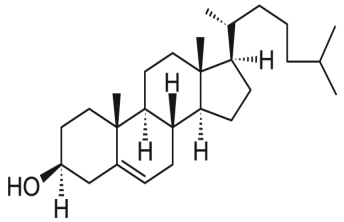


DSPC Tm= 55 °C



SM (Brain Porcine)
Tm= 37 °C

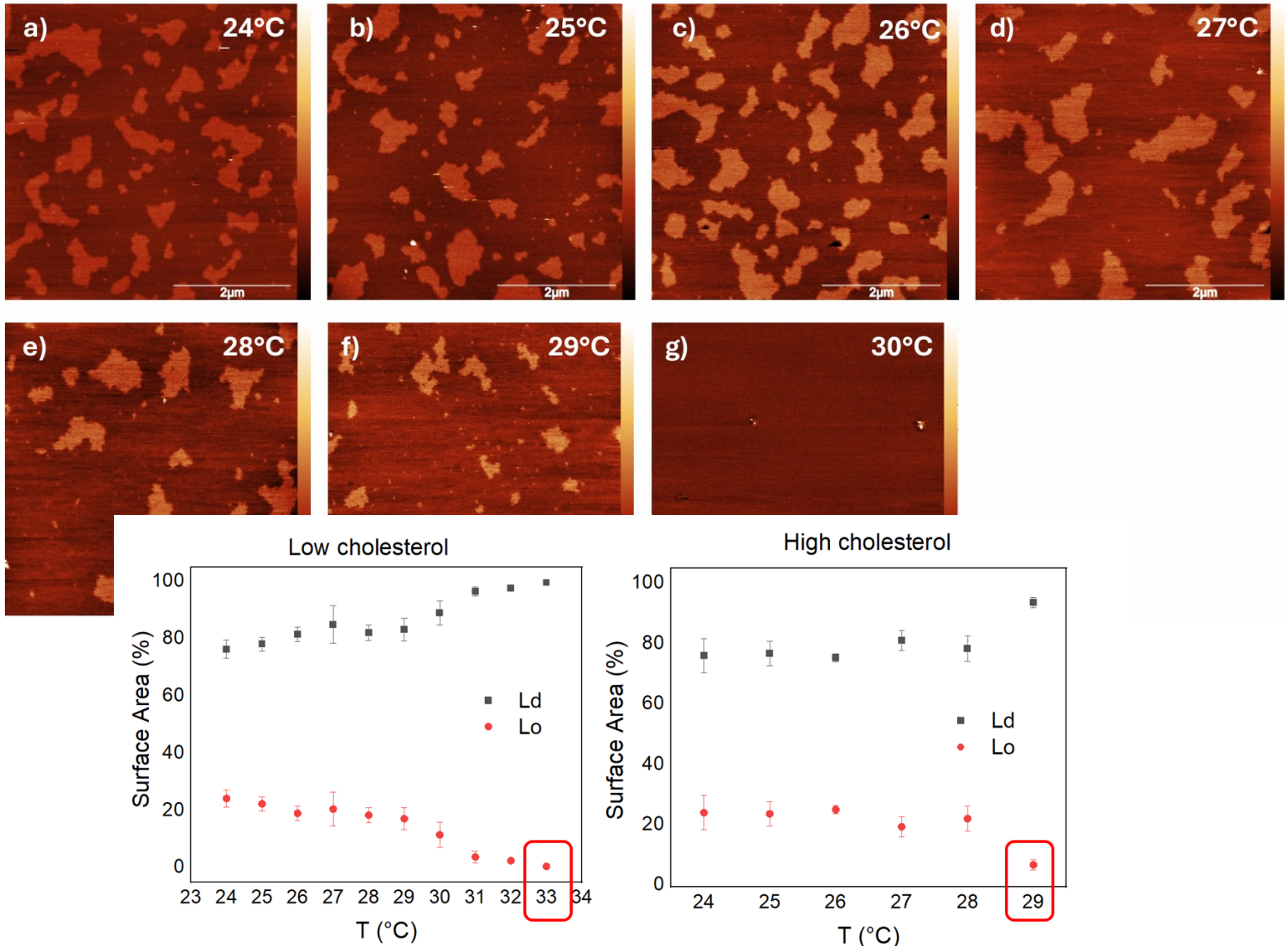




Cholesterol

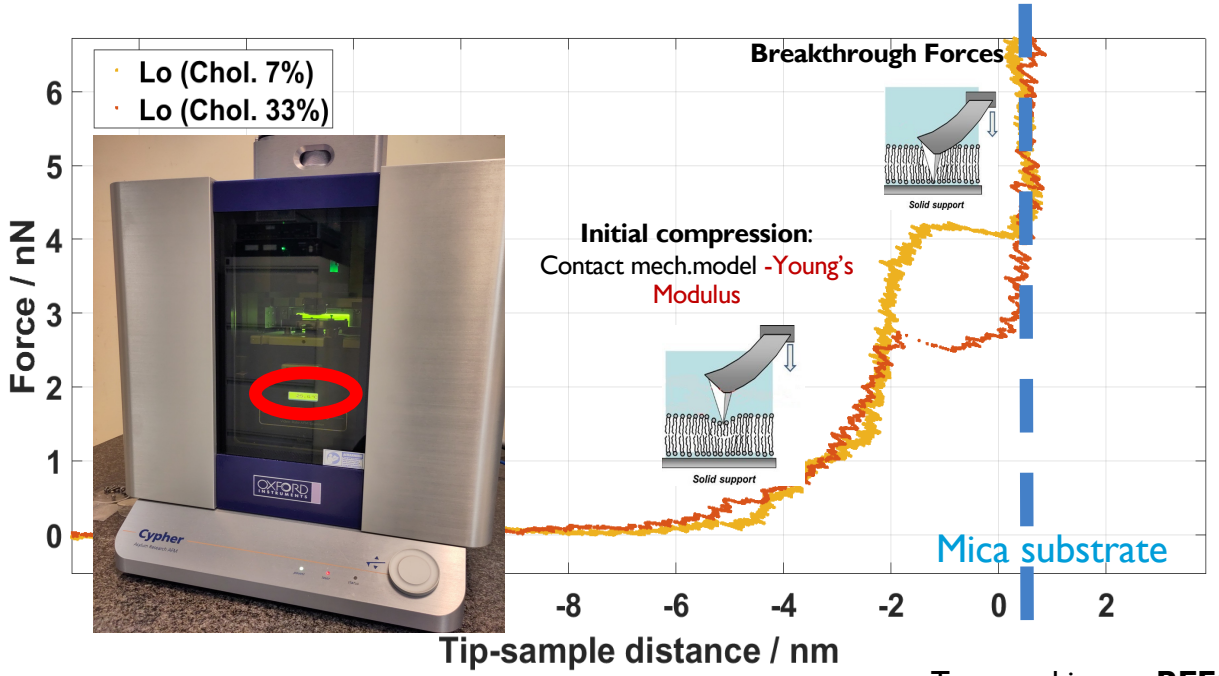
7% LOW, 33% High

Temperature evolution: 33% chol



Evaluation of Membrane Stiffness:

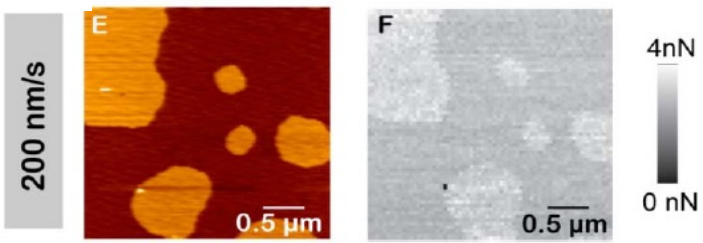
AFM nanomechanics



Optimization:

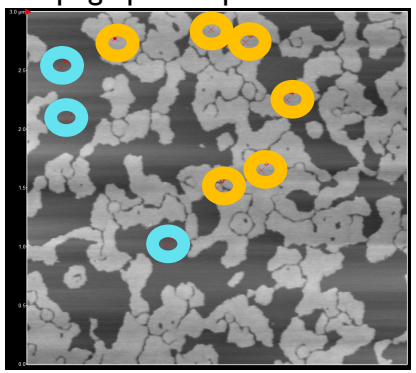
- Tip sharpness
- Tip speed (compressive loading rate)
- Temperature/humidity
- Buffer composition/ionic strenght

Topography Breakthrough

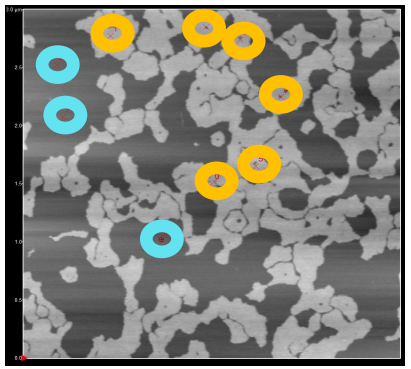


Sullan et al. Biophysical Journal (2010).

Topographic map **BEFORE**



Topographic map **AFTER**



Force maps:

+: High throughput

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

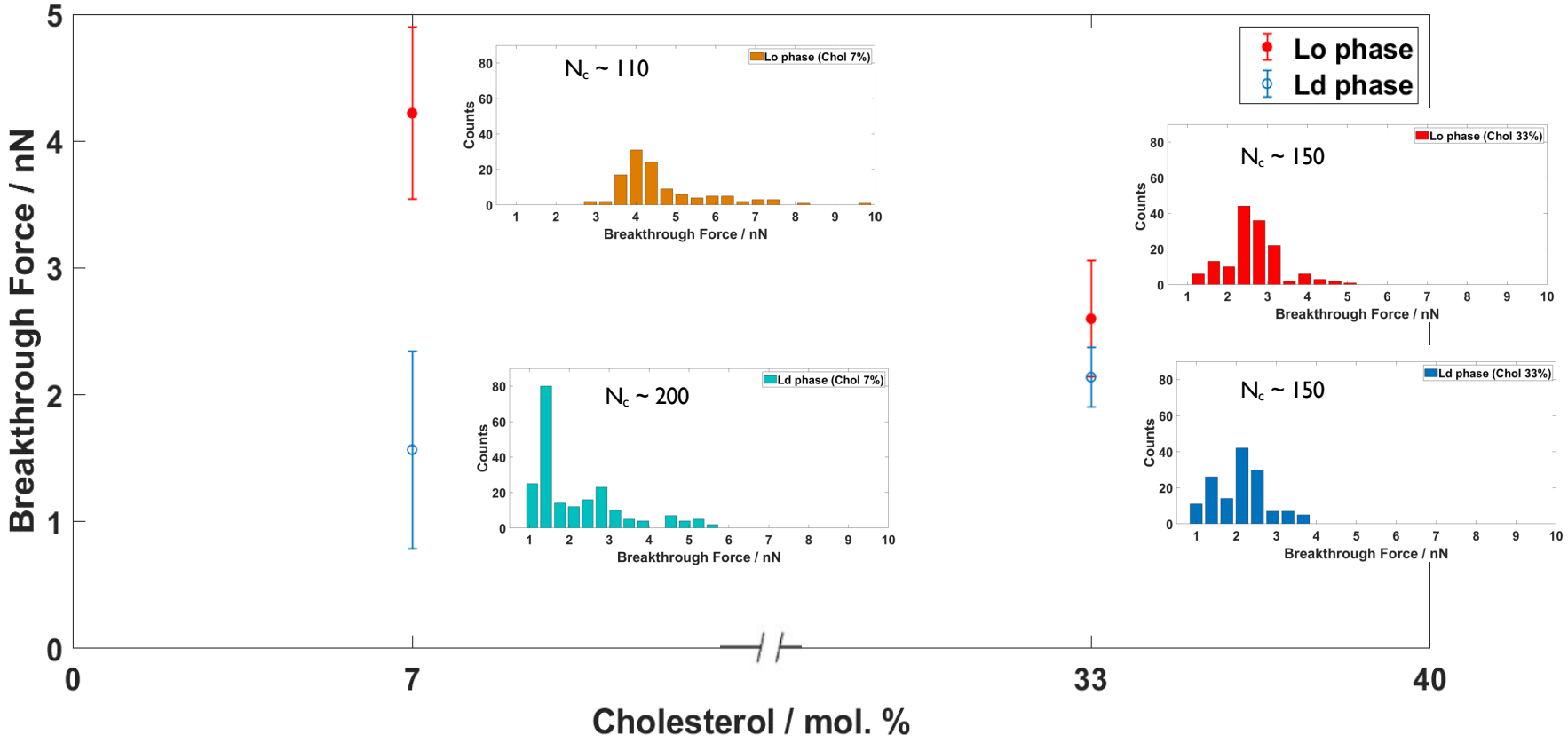
Point&Shoot:

+ : control of rafts dynamics

- : limited data set

Evaluation of Membrane Stiffness:

Breakthrough Forces

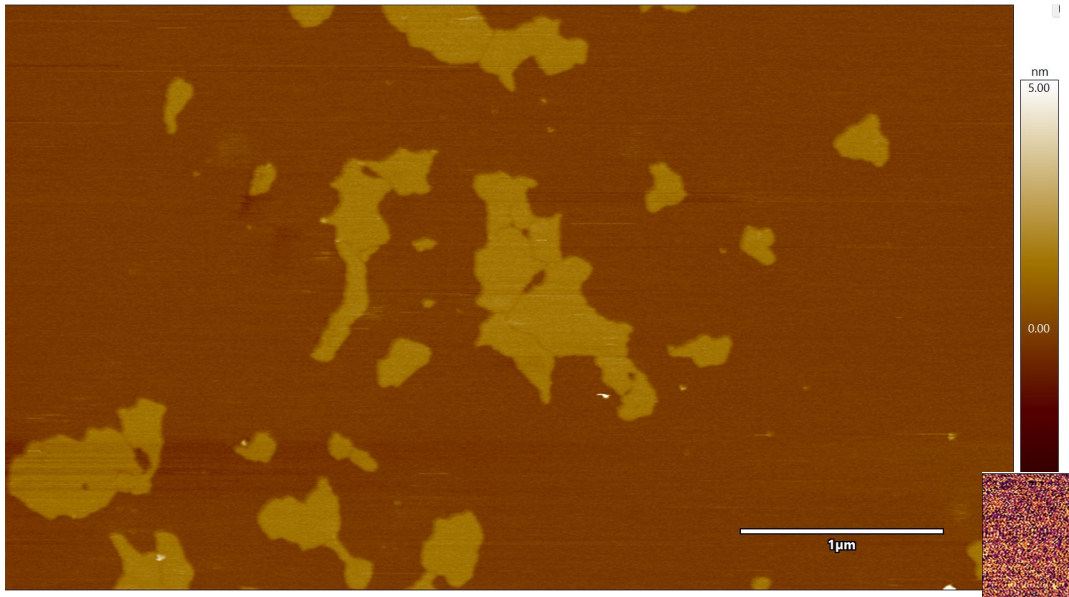


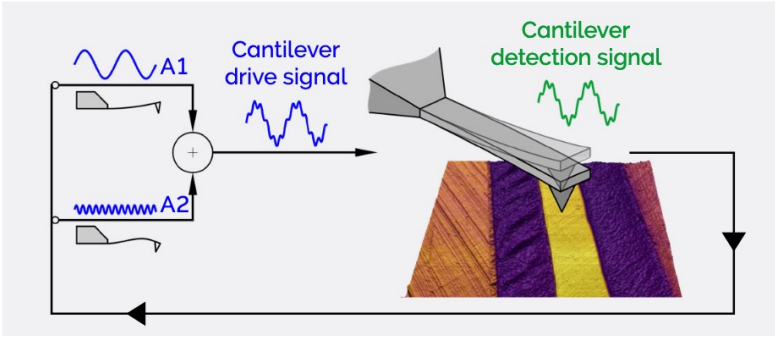
	Median +/- Mad
Lo phase (7%)	4.22 ± 0.68 nN
Ld phase (7%)	1.57 ± 0.78 nN
Lo phase (33%)	2.60 ± 0.46 nN
Ld phase (33%)	2.14 ± 0.24 nN

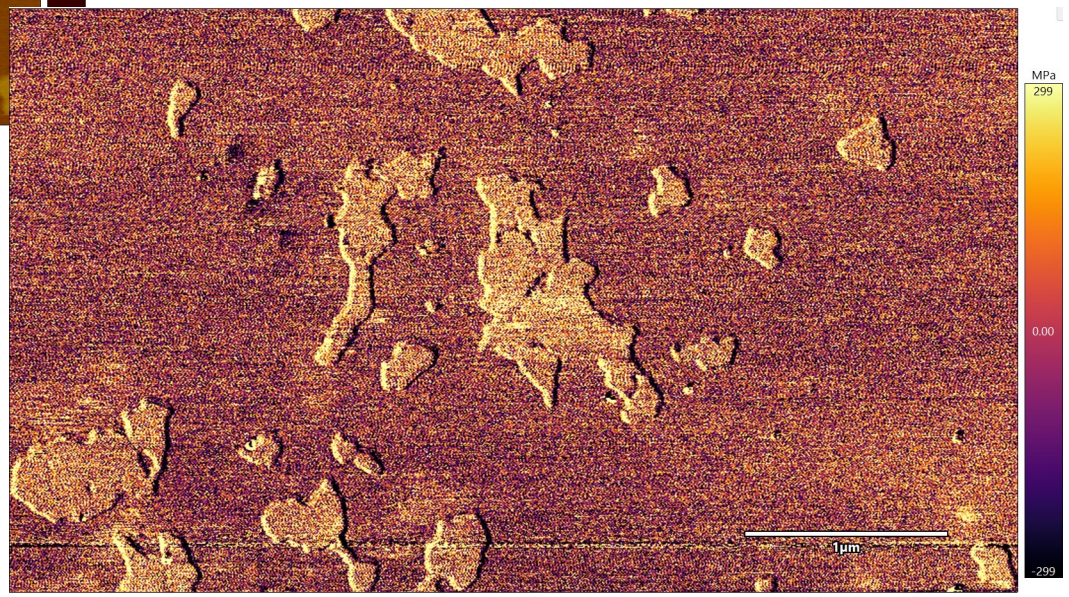
Results in agrrement with FRAP data

Evaluation of Membrane Stiffness:

AM-FM Viscoelastic mapping mode







As fast as topographic imaging!
Measures elastic and dissipative modules