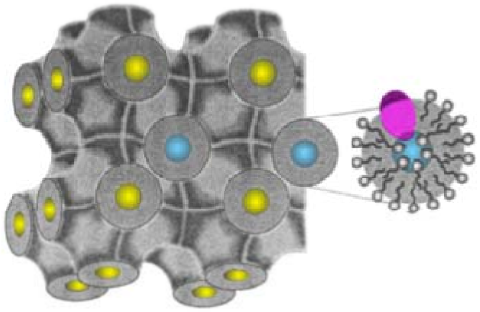
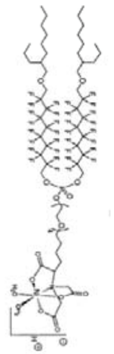
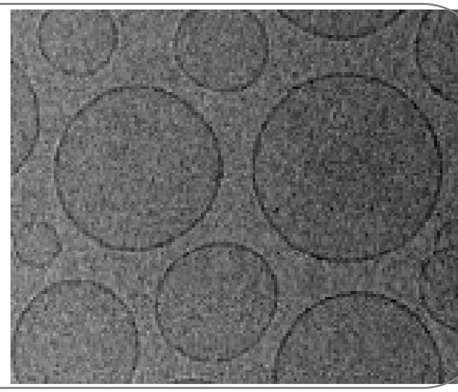

Lipids and their properties

Electron crystallography of membrane proteins
C-CINA, University of Basel
August 1-7, 2010

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University of Oxford
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Overview

- Lipid- definition classification
- Physical properties of lipids
    - Lipid polymorphism
    - Lipid phase diagrams
    - Lipid phase transition temperature
    - Lipid mobility
- Synthesis of new lipids for 2D crystallization of membrane proteins

Lipid -definition

Lipid: any molecule (MW 100-5000) that contains a substantial portion of aliphatic or aromatic hydrocarbon.

Lipids

-hydocarbons
-oil
-waxes
-cholesterol, steroids
-vitamins (such as vitamins A,D,E and K)
-(mono-di-tri)glycerides
-phospholipids
-soap
-detergents
-surfactants
….

Biological functions

-Structural component of the membranes

-Energy storage

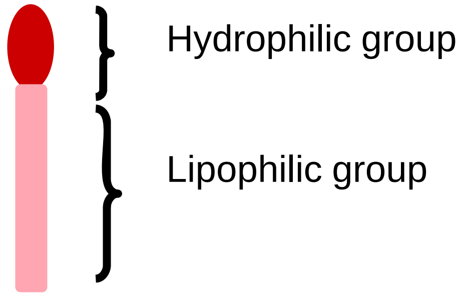
-Intermediate in signalling pathways

- ….

Lipids classification based on interactions with water

The physical-chemistry properties of the lipids are dependent on the amphiphilic structure

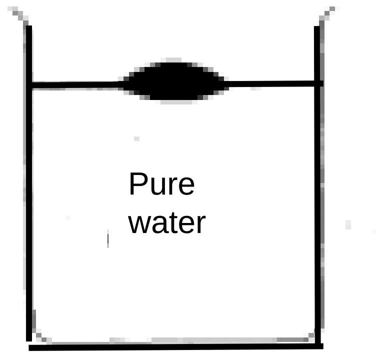
The behaviour of a given lipid depends on the **Hydrophilic-Lipophilic Balance HLB**



- -ionized carboxyls ($-COO^-$)
- -sulfates, sulfonates,
- -phosphates,
- -quaternary amines,
- -sugars
- -polyalcohols
- -polyethoxides
- -many zwitterionic groups

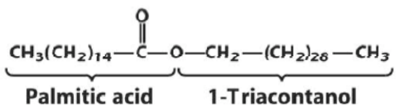
I. Nonpolar lipids

-They are insoluble in water, very soluble in organic solvent (hexane, chloroform or benzene)



Long chain hydrocarbons i.e. octadecane $CH_3(CH_2)_{16}CH_3$

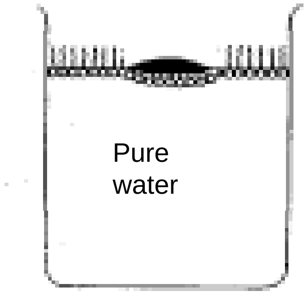
Beeswax



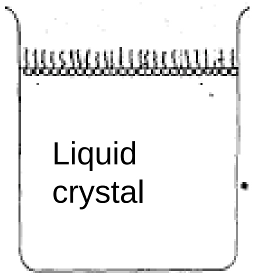
Lipids classification based on interactions with water

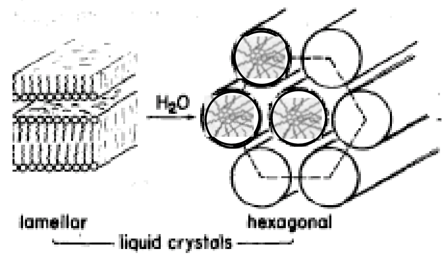
II. Polar lipids

Class I: insoluble in water , Nonswelling amphiphiles
Form a stable monolayer Soluble in organic solvent
-triglycerides
-long-chain un-ionized fatty acids
-cholesterol
-many fat-soluble vitamins (vitamins A,D,E,and K)
-Solubility <10^{-10}M

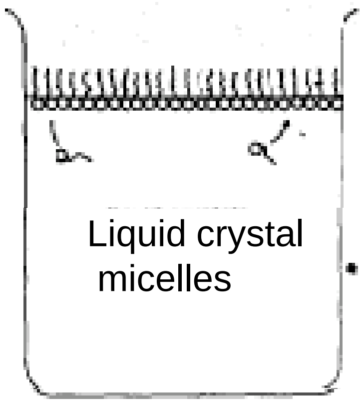


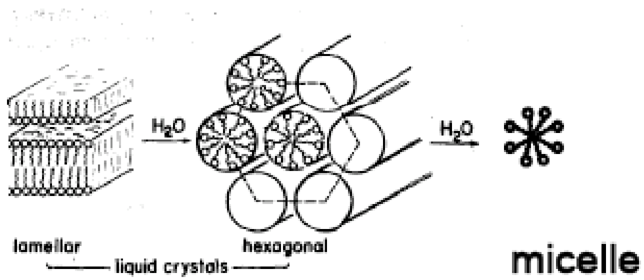
Class II: insoluble in water , Swelling amphiphiles
Form a stable monolayer sparingly soluble in organic solvent
soluble in amphiphilic solvent such as ethanol
Lipids from membrane
-Phospholipids
-cerebrosides
-lipoproteins
-monoglycerides



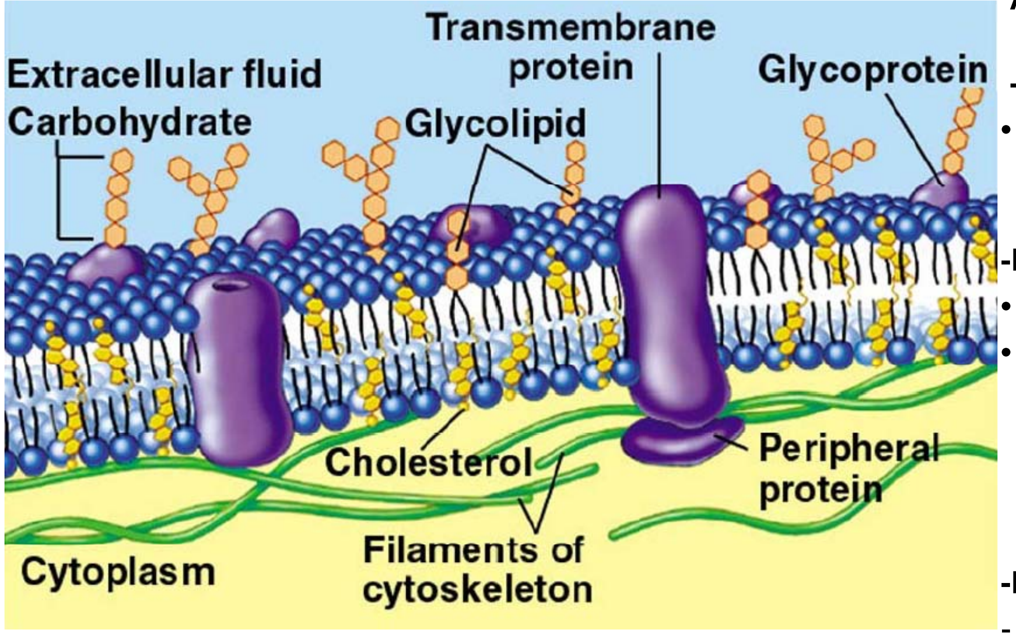


Class III: soluble amphiphiles
Form an instable monolayer
insoluble in organic solvent
Soaps, lysolecithin, detergents,
bile salts
Solubility >10^{-4}M,
(Saponine: 10^{-2}M)





Structure of a membrane



A membrane is a barrier that defines a boundary to a cell and/or organelle

-Prokaryotes

- Only membrane is plasma membrane and in the case of Gram negative bacteria

  (eg E. coli) an additional outer membrane

-Eukaryotes

- Plasma membranes
- Organelles membranes

  -Mitochondria

  -Nucleus

  -Lysosomes

  -Endoplasmic reticulum

-Envelopped virues

- nucleic acid core is surrounded by a simple membrane)

•But is it really just simply a barrier??

-Insulation

-Most membranes are electrically polarized

-Mediate cell-cell adhesion

-Signal and nerve transmission

-Cell identity and antigenicity

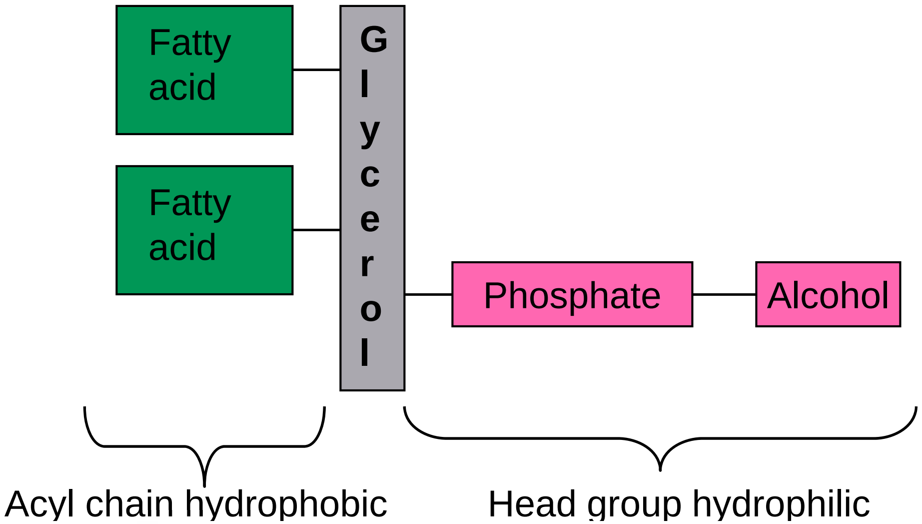
-Energy storage (lipids)

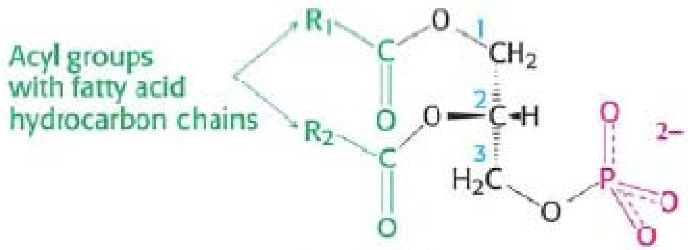
The structure of membrane lipids

Three major kinds of membrane lipids
-Phospholipids (glycerophospholipids, sphingolipids)
-Glycolipids
-Cholesterol

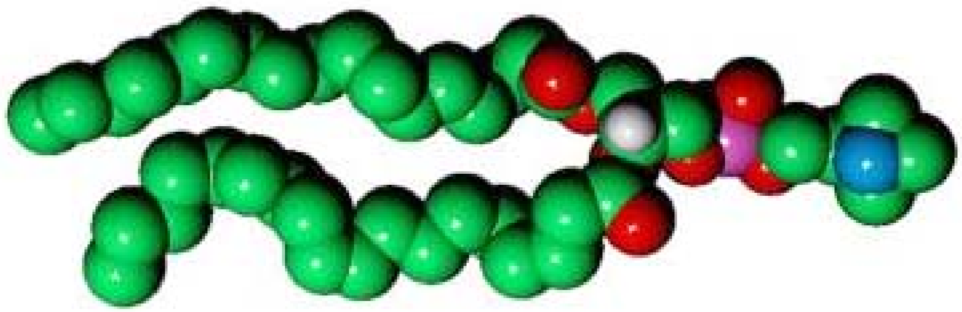
Phospholipids-Glycerophospholipids

Nomenclature Naming is complicated (due to historical reasons)
It is formalized now but some older names are still in common use (eg. Lecithin is still used for phosphatidylcholine).
IUPAC-IUB is the defining authority though





Phosphatidate
(Diacylglycerol 3-phosphate)



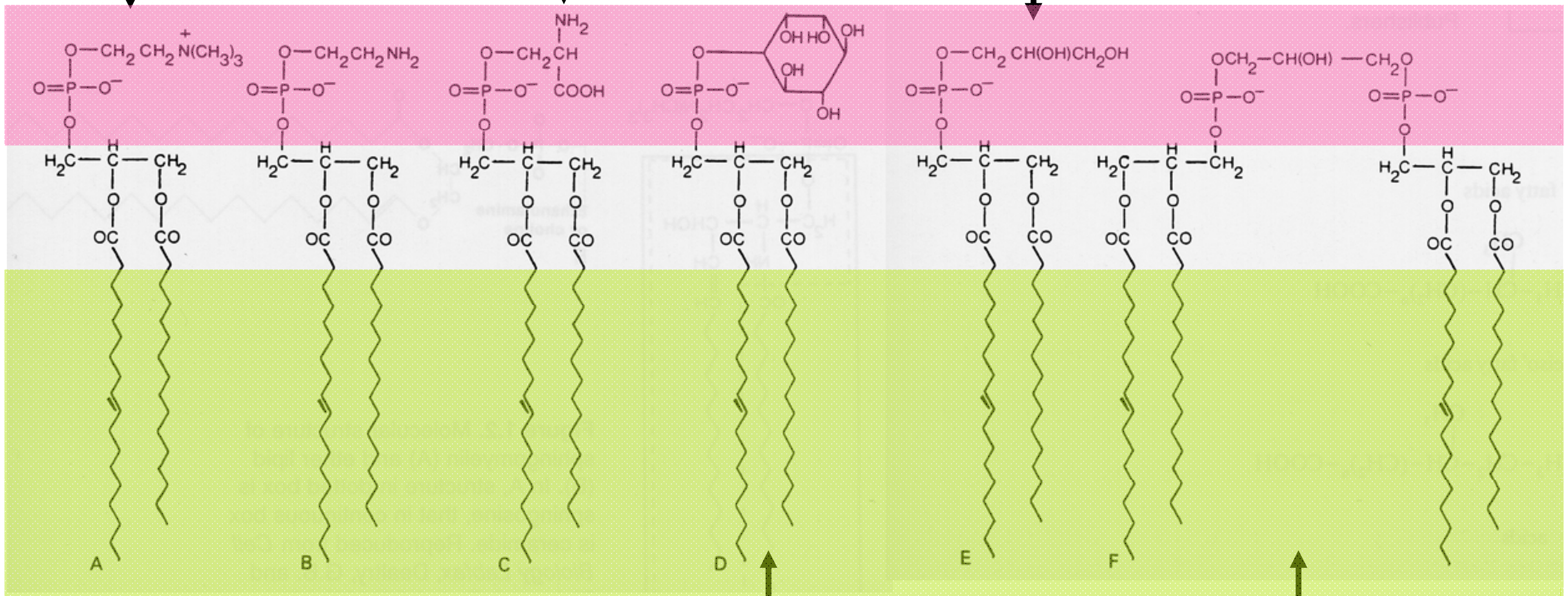
Some common glycerophospholipids found in membanes

- Typical %ages for eukaryotic cell membrane
- All lipids are zwiterionic or anionic

Phosphatidylserine (PS)
< 10%

Phosphatidylcholine (PC)
30 - 40%

Phosphatidylglycerol (PG),
< 5%

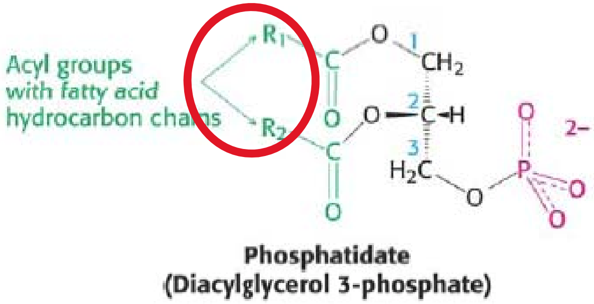


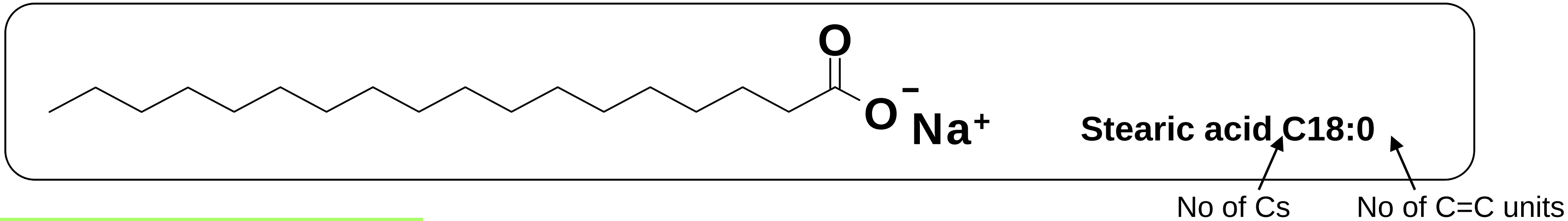
Phosphatidylinositol (PI)
<4%

Diphosphatidylglycerol (DPG)
~ 10% in e- transporting membranes

Phosphatidylethanolamine (PE)
30 - 40%

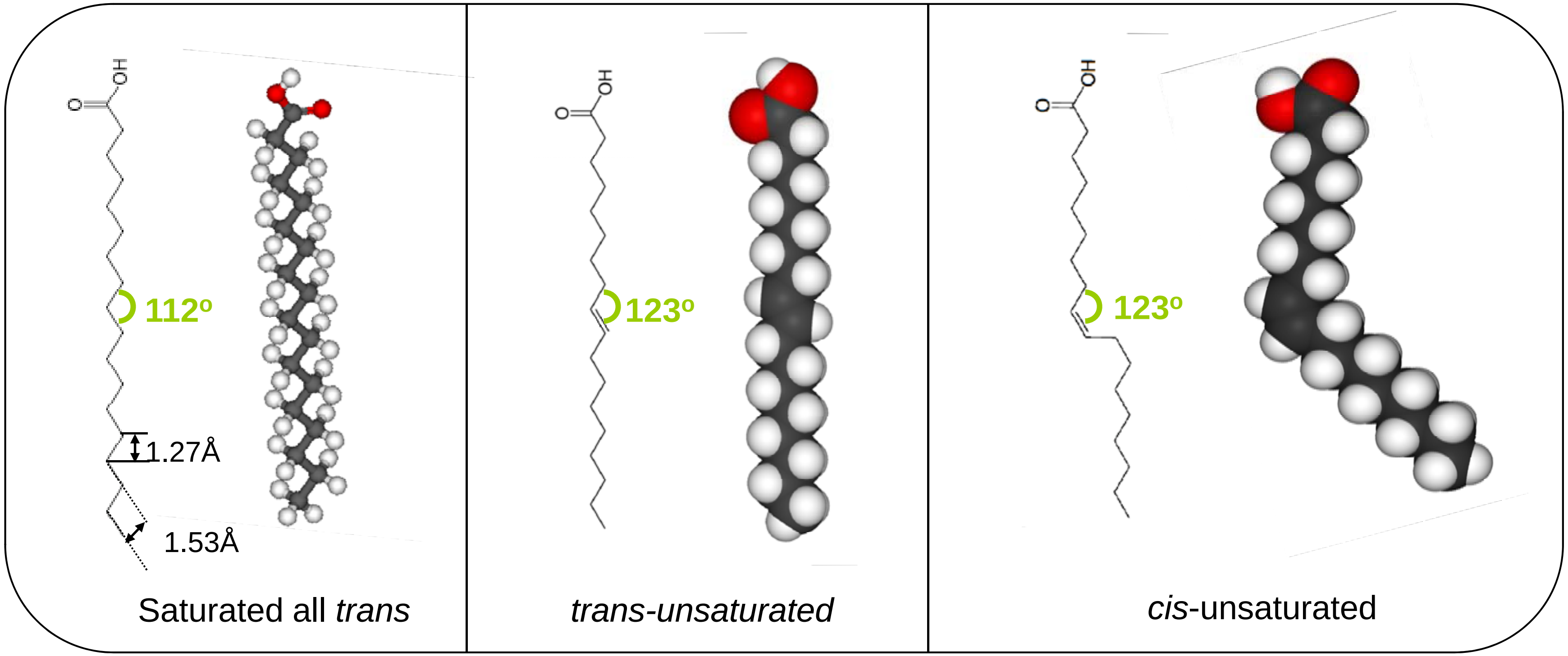
fatty acids





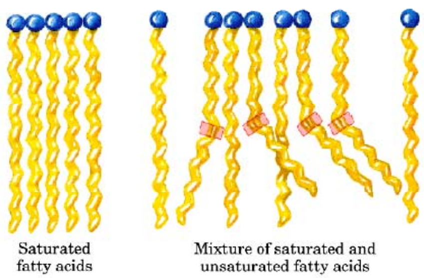
Common Name	Chain length: unsaturation	Systematic Name
Lauric	12:0	Dodecanoic acid
Myristic	14:0	Tetradecanoic acid
Palmitic	16:0	Hexadecanoic acid
Palmitoleic	16:1 (9-cis)	9-Hexadecenoic acid
Stearic	18:0	Octadecanoic acid
Oleic	18:1 (9-cis)	9-Octadecenoic acid
Linoleic	18:2 (9-cis, 12cis)	9,12-Octadecadienoic acid
Arachidonic	20:4 (5, 8, 11, 14 –cis)	5,8,11,14-Eicosatetraenoic acid

The structure of fatty acids: cis-unsaturated and trans-unsaturated acids

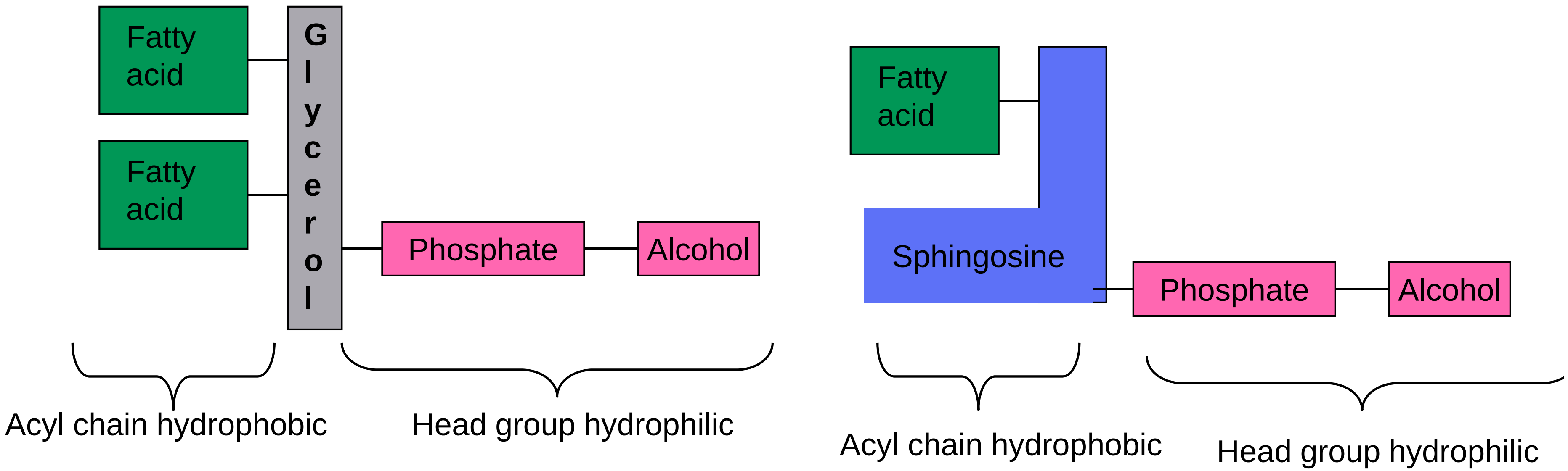


T_m = 45°C

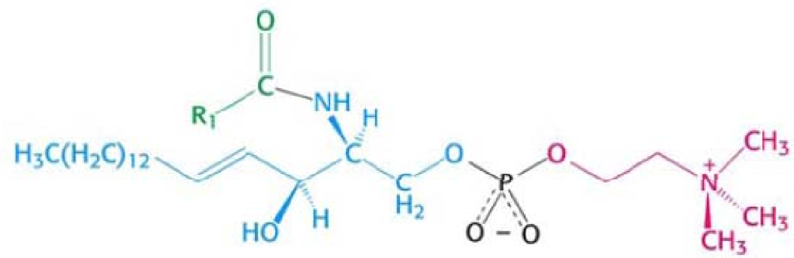
T_m = 32°C



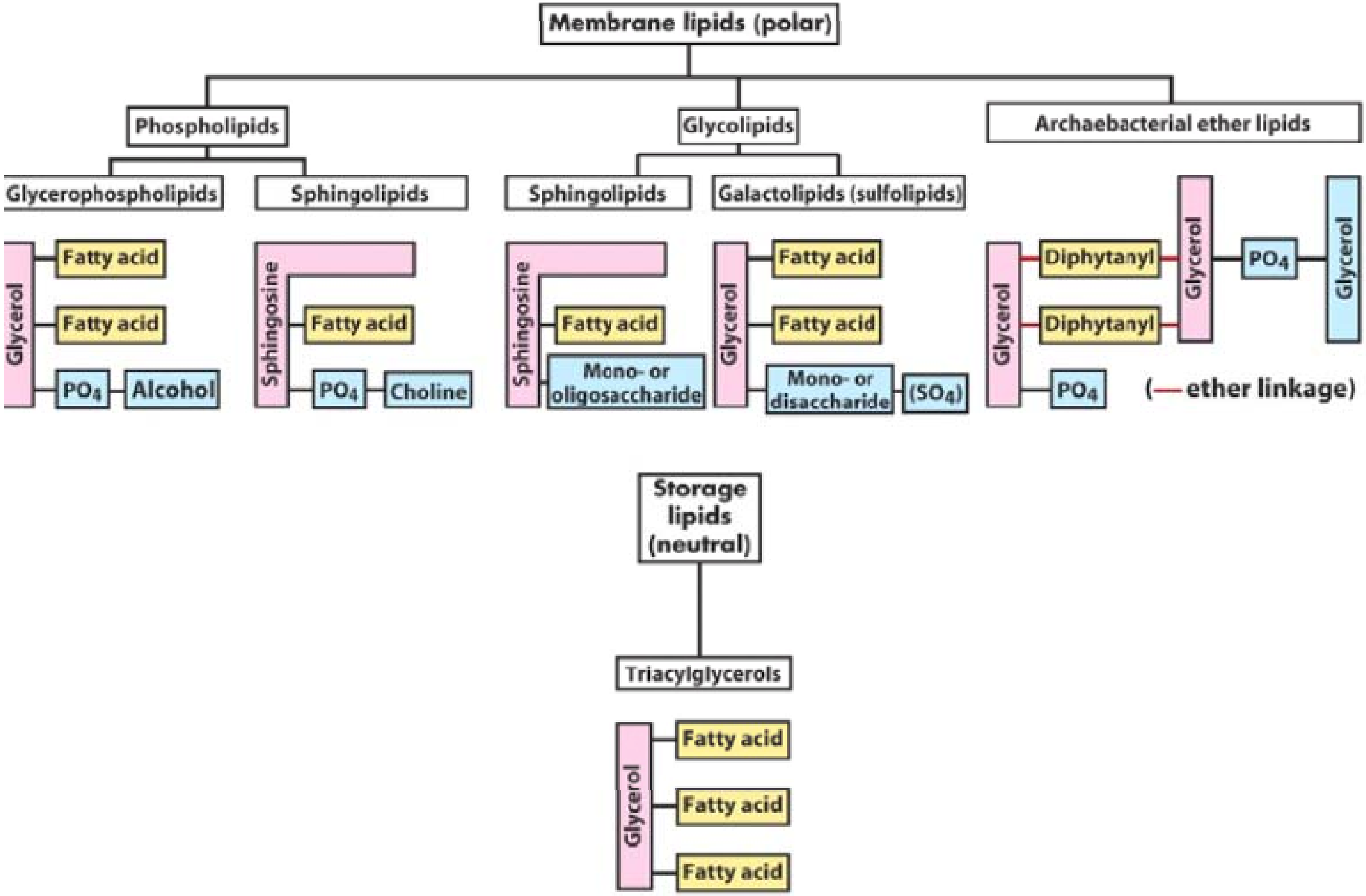
Phospholipids-Sphingolipids



Sphingosine

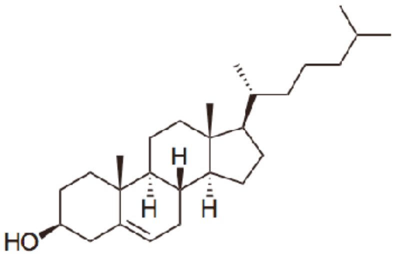


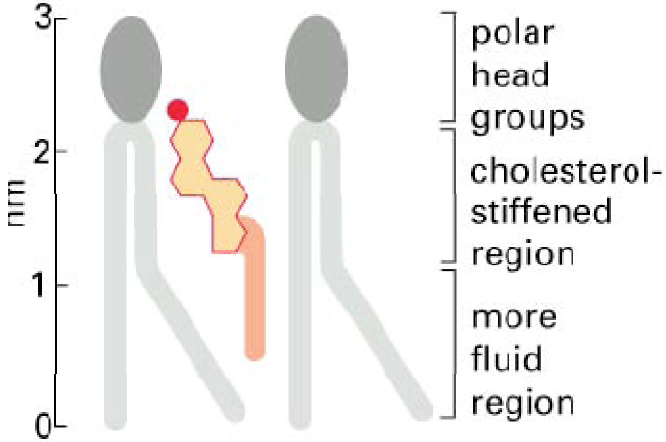
Sphingomyelin



Membrane lipids (polar)
Phospholipids
Glycolipids
Archaebacterial ether lipids
Glycerophospholipids
Sphingolipids
Sphingolipids
Galactolipids (sulfolipids)
Glycerol
Fatty acid
Fatty acid
PO_4
Alcohol
Sphingosine
Fatty acid
PO_4
Choline
Sphingosine
Fatty acid
Mono- or oligosaccharide
Glycerol
Fatty acid
Fatty acid
Mono- or disaccharide
(SO_4)
Glycerol
Diphytanyl
Diphytanyl
Glycerol
PO_4
Glycerol
PO_4
(— ether linkage)
Storage lipids (neutral)
Triacylglycerols
Glycerol
Fatty acid
Fatty acid
Fatty acid

Cholesterol



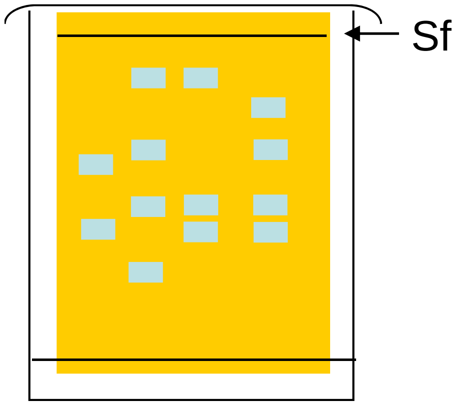


Lipid extraction from membranes and characterization

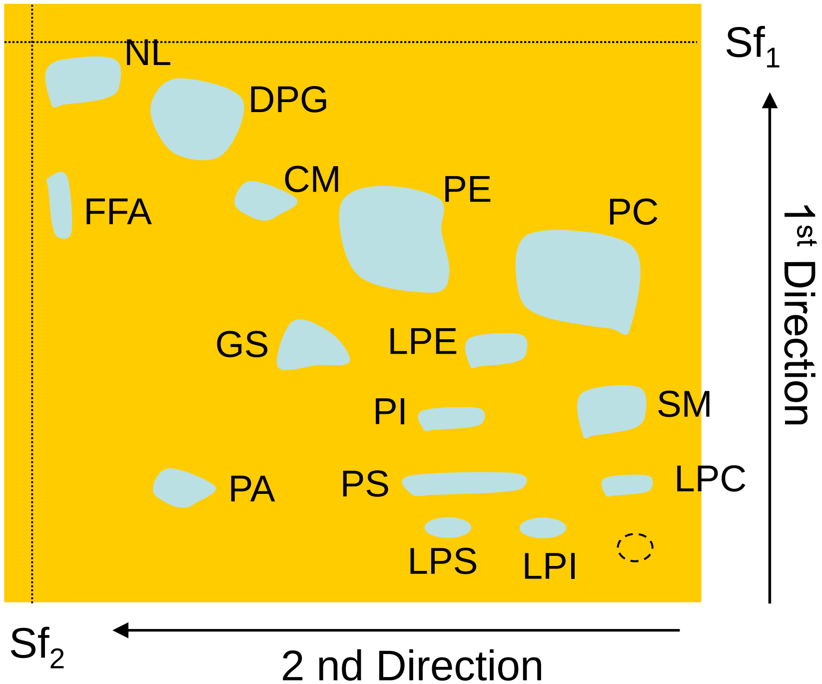
Lipid extraction

Lipid separation using Thin Layer Chromatography technique lipids are separated according to polarity

1D thin layer chomatography (TLC):



2D thin layer chomatography (TLC):



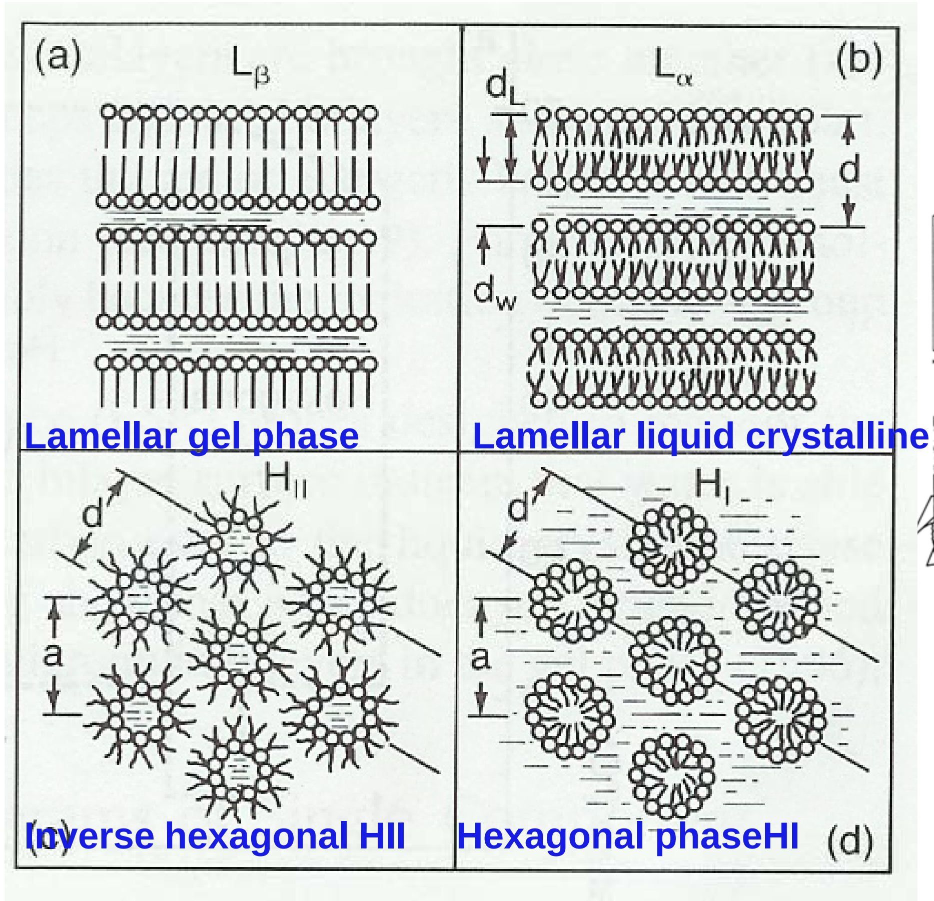
Sample has to be applied as single spot ⇒ multiple samples cannot be run on single plate

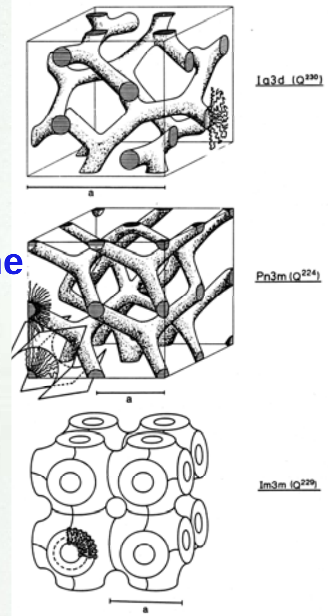
Solvent: chloroform/methanol/30% aq. Ammonia/ water (90:54:5.5:5.5)
chloroform/methanol/acetone/glacial/acetic acid/water (60:20:80:20:10)

LPC: Lyso-PC GS: gangliosides
LPE: Lyso-PE PA: phosphatidic acid
LPI: Lyso-PI FFA: free fatty acid
LPS: Lyso-PS CM: ceramide monohexoside
NL: neutral lipids PI: phosphatidylinositol
DPG: diphosphatidylglycerol
SM: sphingomyelin PE: phosphatidylenthanolamin
PS: phosphatidylserine PC: phosphatidylcholine

Lipid polymorphism

Behaviour of lipids in aqueous medium varies considerably
Lyotropic transitions: variation of water content at constant temperature
Driven by **HYDROPHOBIC EFFECT**
Cohesive forces between hydrocarbon tails → minimization of hydrocarbon-water contact area



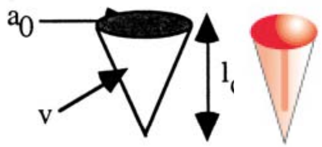
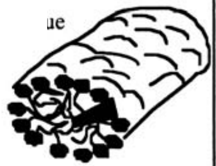
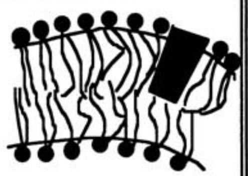
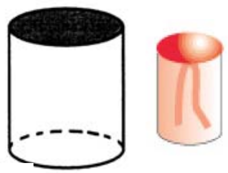
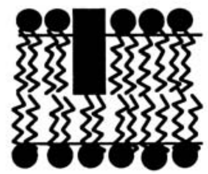
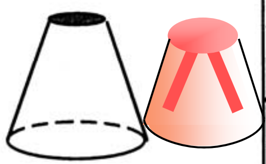
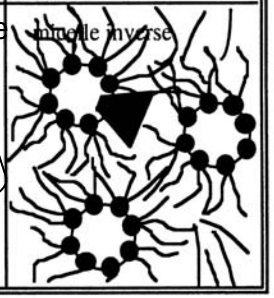


Cubic phase

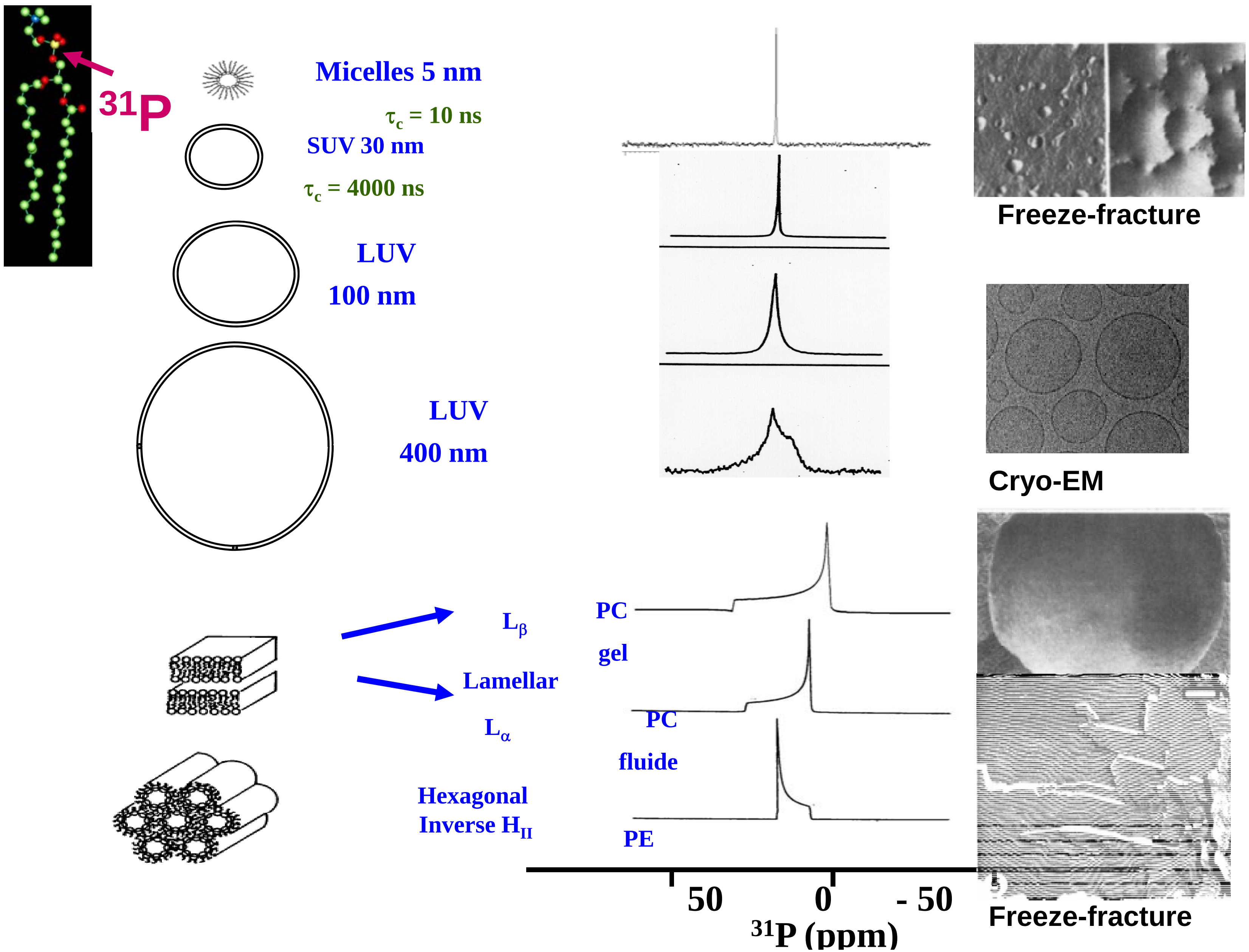
Table 1 Principal Lyotropic Mesophases

Type	Name	Phase structure
Solidlike lamellar phases		
3-D	L_c	3-D crystal
2-D	L_c^{2D}	2-D crystal
	$P_{\beta'}$	Rippled gel
	P_δ	Ordered ribbon phase
	B	Ordered ribbon phase?
1-D	L_β	Untilted gel
	$L_{\beta'}$	Tilted gel
	$L_{\beta I}$	Interdigitated gel
	$L_{\alpha\beta}$	Partial gel
Fluid phases		
Type	Name	Phase structrure
1-D	L_α	Fluid lamellar
2-D	H	Hexagonal
	H^c	Complex hexagonal
	R	Rectangular
	M	Oblique
3-D	Q	Cubic
	T	Tetragonal
	R	Rhombohedral
	O	Orthorhombic

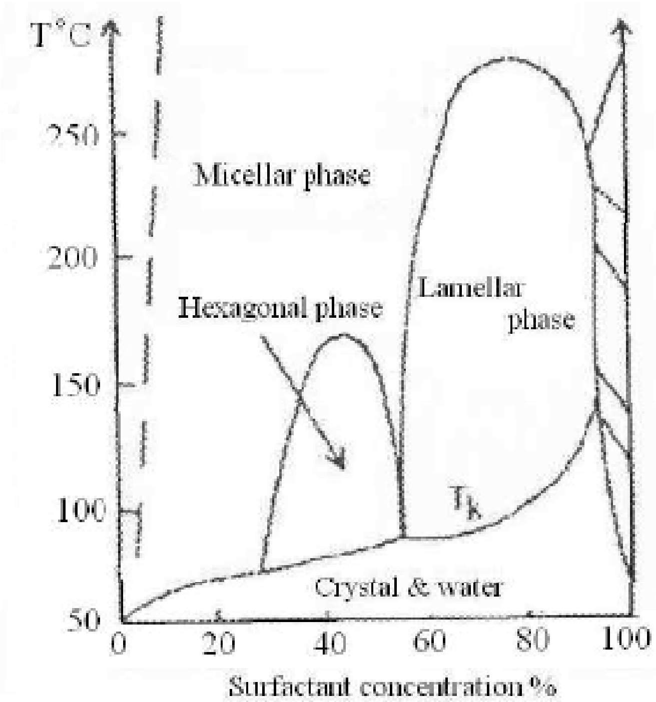
Phospholipid polymorphic phase preferences

$p=\frac{v}{a_0 l_c}$	Lipid	shape	Phases	
<1/3	Detergent with one chain SDS low salt	 cone		micelle
1/3-1/2	Lipid with one chain, small polar head SDS or CTAB high salt ...	Truncated cone 		cylindrical micelle
1/2-1	Two chains lipid fluid PC sphingomyelin ...	Truncated cone 		Flexible bilayer vesicule
1	Two chains lipid small polar head PE	cylindrical 		flat bilayer
>1	Unsaturated chains, high Temp cardiolipin plus Ca2+	Inverted truncated cone 		Inverted micelle

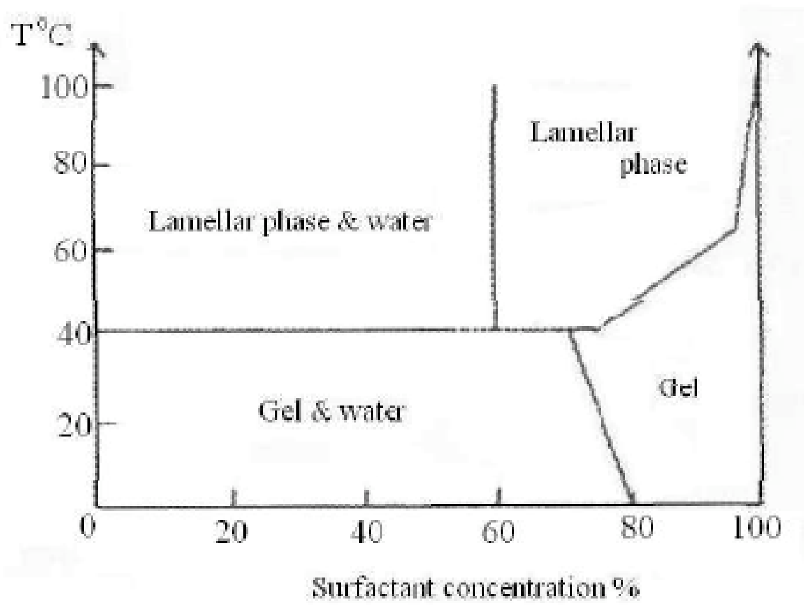
Polymorphism: identification of structures of lipids using ^{31}P-NMR and EM



Typical lipid phase diagrams

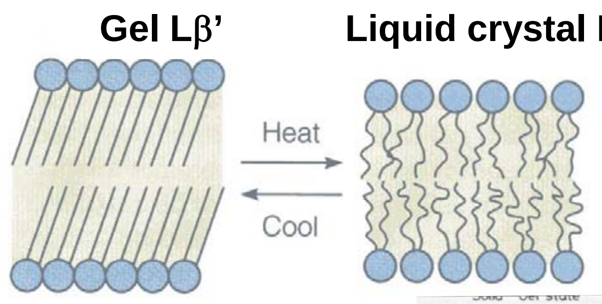


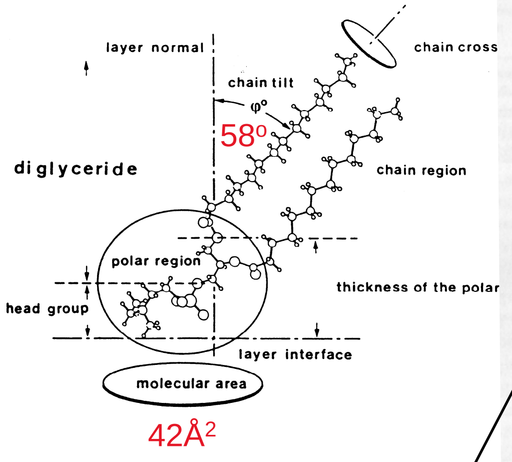
Soluble lipid

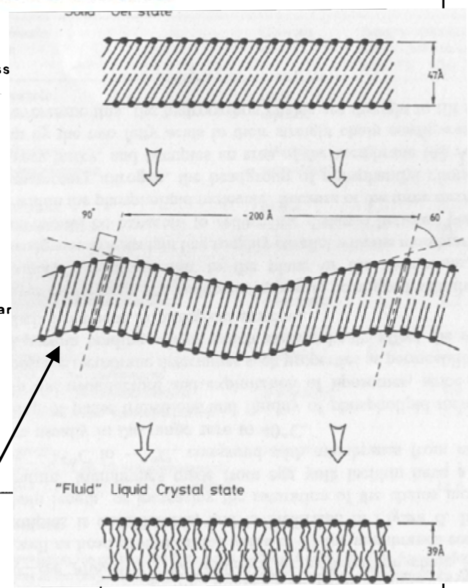


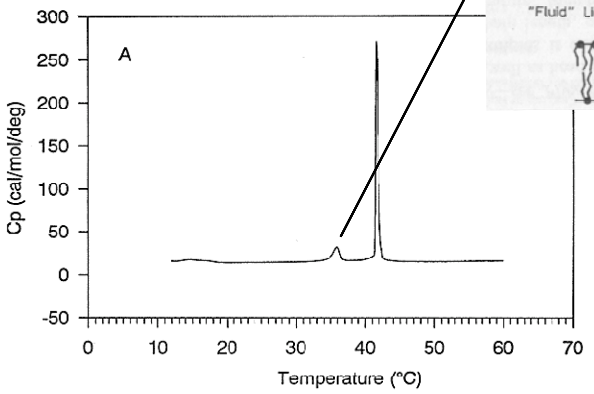
Insoluble lipid

Lipid phase transition temperatures









Differential Scanning Calorimeter (DSC):
Phase transition for DPPC Dipalmitoyl Phosphatidylcholine

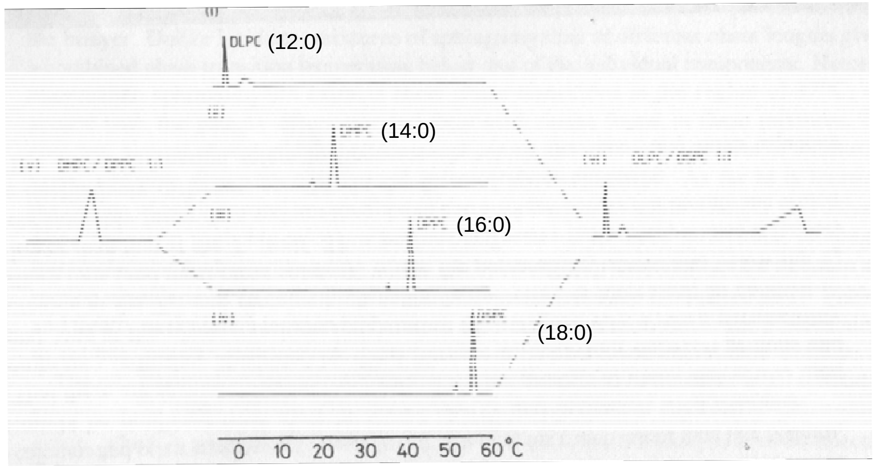
Parameters which affects the phase transition Tm

- Tm increases with increased tail length
- Tm decreases with increased double bonds
- Tm decreases with increasing head size

Phosphatidylcholine		**Phosphatidylglycerol (Sodium Salt)**		
Product	T_m ¡C	Product	T_m¡C	
12:0 PC DLPC	-1	12:0 PG	-3	
13:0 PC	14	14:0 PG	23	
14:0 PC DMPC	23	16:0 PG	41	
15:0 PC	33	18:0 PG	55	
16:0 PC	41	18:1 PG	-18	
17:0 PC	48	16:0-18:1 PG	-2	
18:0 PC	55	Phosphatidylserine (Sodium Salt)		
19:0 PC	60	14:0 PS	35	
20:0 PC	66	16:0 PS	54	
21:0 PC	72	18:0 PS	68	
22:0 PC	75	18:1 PS	-11	
23:0 PC	79	16:0-18:1 PS	14	
24:0 PC	80	Phosphatidic Acid (Sodium Salt)		
16:1 PC	-36	12:0 PA	31	
18:1c9 PC DOPC	-20	14:0 PA	50	
18:1t9 PC	12	16:0 PA	67	
18:1c6 PC	1	18:0 PA	75	
18:2 PC	-53	18:1 PA	-8	
18:3 PC	-60	16:0-18:1 PA	28	
20:4 PC	-70	Phosphatidylethanolamine		
14:0-16:0 PC	35		T_m¡C	T_h¡C
14:0-18:0 PC	40	12:0 PE	29	
16:0-14:0 PC	27	14:0 PE	50	
16:0-18:0 PC	49	16:0 PE	63	118
16:0-18:1 PC POPC	-2	18:0 PE	74	100
16:0-22:6 PC	-27	20:0 PE	83	96
18:0-14:0 PC	30	18:1c9 PE	-16	10
18:0-16:0 PC	44	18:1t9 PE	38	64
18:0-18:1 PC	6	18:2 PE	-40	-15
18:1-16:0 PC	-9	18:3 PE		-30
18:1-18:0 PC	9	16:0-18:1 PE	25	71

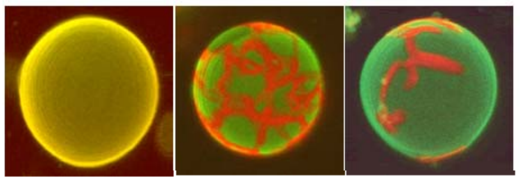
Phase transition of mixture of lipids

Microcalorimetry curves showing phase transitions of membranes containing single components or mixtures of phospholipids



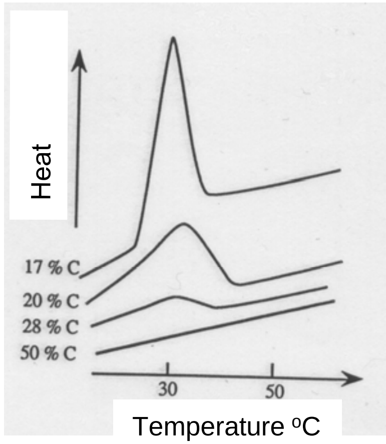
If the Tm of the individual lipids are close to each other
-> Single phase transition

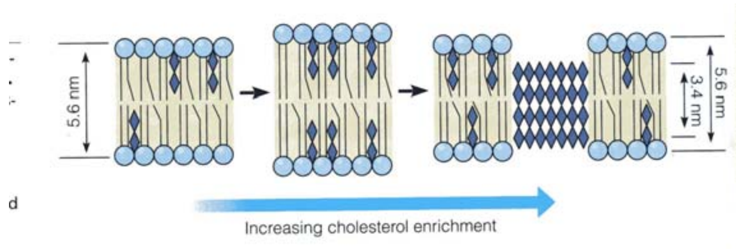
If the Tm of the individual lipids differ greatly from each other
-> Lipids will undergo phase transition independently from each other
Non ideal mixing, Separation of phases



Phase separation visualized using geant unilamellar vesicle and confocal microscopy

Influence of Cholesterol on phase transition

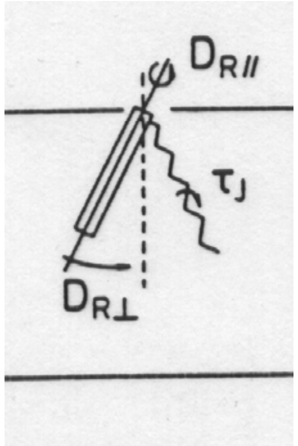


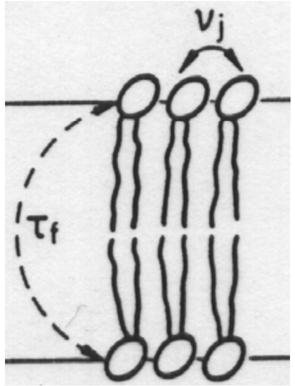


- •Cholesterol has a little effect of the position of the phase transition
- •Cholesterol is able to abolish completely the heat of transition
- •At low temp, rigid cholesterol destabilizes gel phase by disrupting efficient tail packing
- •At high temp, cholesterol destabilizes liquid crystal by decreasing motional freedom

Mobility of lipids

Rotation





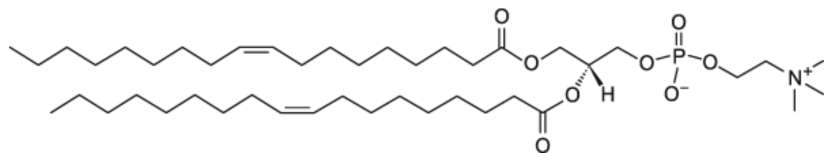
Flip-flop

Motional modes of the lipid molecules in a biological membranes

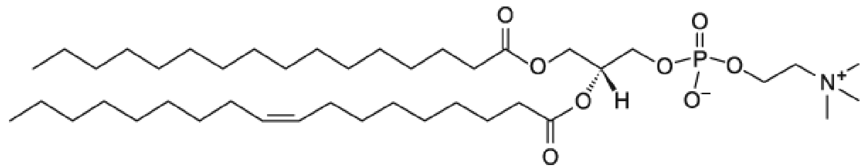
D_T (cm^2sec^{-1})	D_R parallel (sec^{-1})	D_R perpend (sec^{-1})	Flip-flop half time
Liquid crystal: 10^{-7} Gel phase: 10^{-11}	10^9	10^9	DOPC > 11days Lyso PC 100 hr PA 30-40 mins

Typical lipids used for 2D crystallization of membrane protein

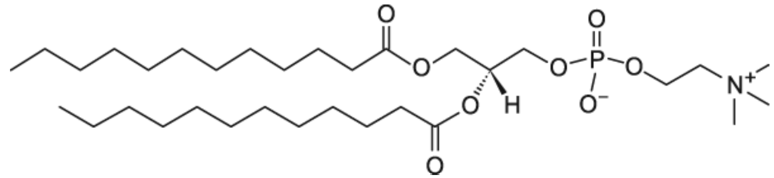
DOPC 18:1 (Tm = -20^{o}C) DOPA,DOPE, DOPG



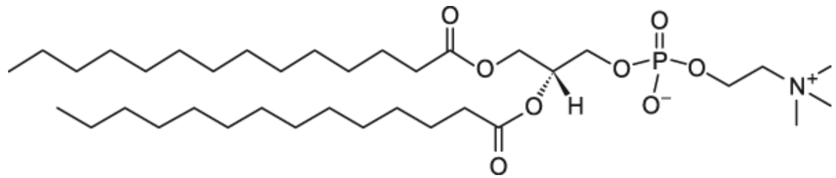
POPC 16:0-18:1 (Tm = -2^{o}C) POPS POPE, POPA



DLPC 12:0 (Tm = -1^{o}C)



DMPC 14:0 (Tm=23^{o}C) DMPS DMPG

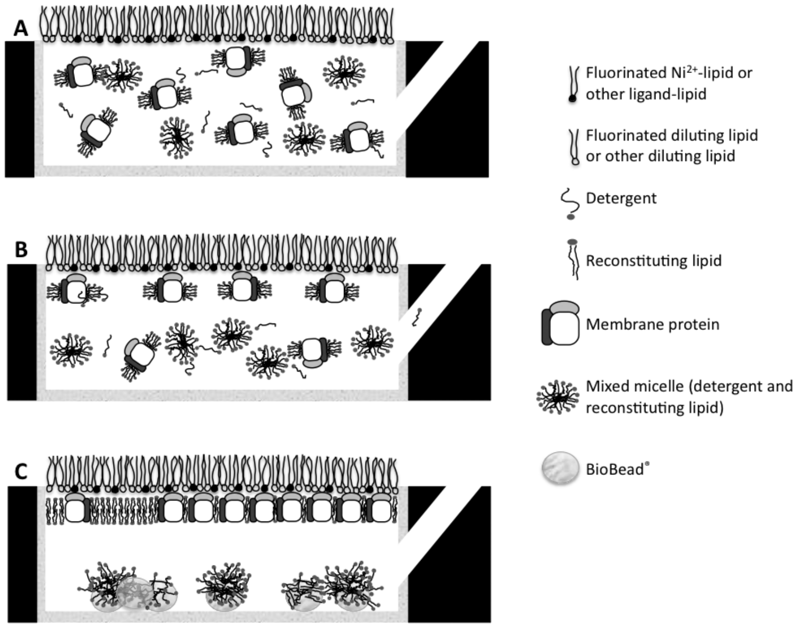


2D crystallization on lipid monolayer

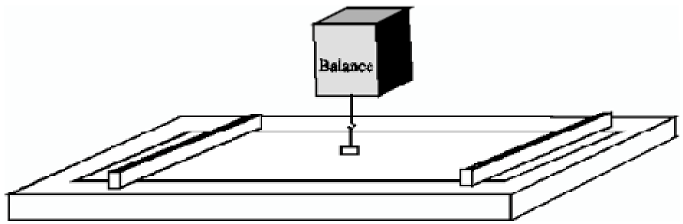
Required property for the lipid forming the monolayer at the air-water interface,

lipid monolayer should be in a FLUID state (liquid expanded phase)

Figure 3



Langmuir film balance with a Wilhelmy plate



Typical π-A isotherm