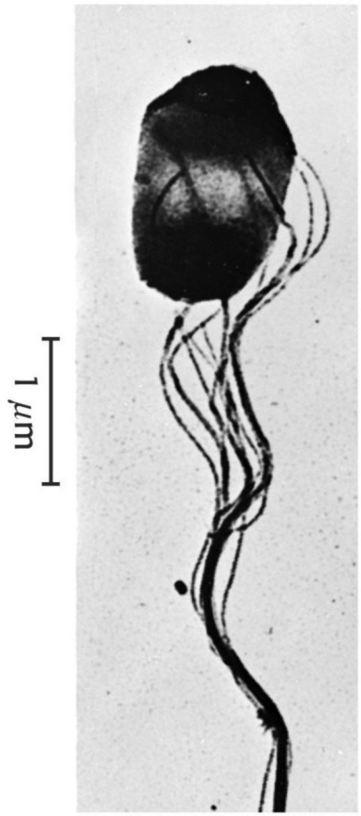
Biochemistry of Membranes

Biomembranes: Introduction.
Phospholipids: Biosynthesis, distribution and dynamics.
Membrane protein biogenesis.

Vesicular transport: From fission to fusion.
Lateral organization: Dynamic properties of domains.

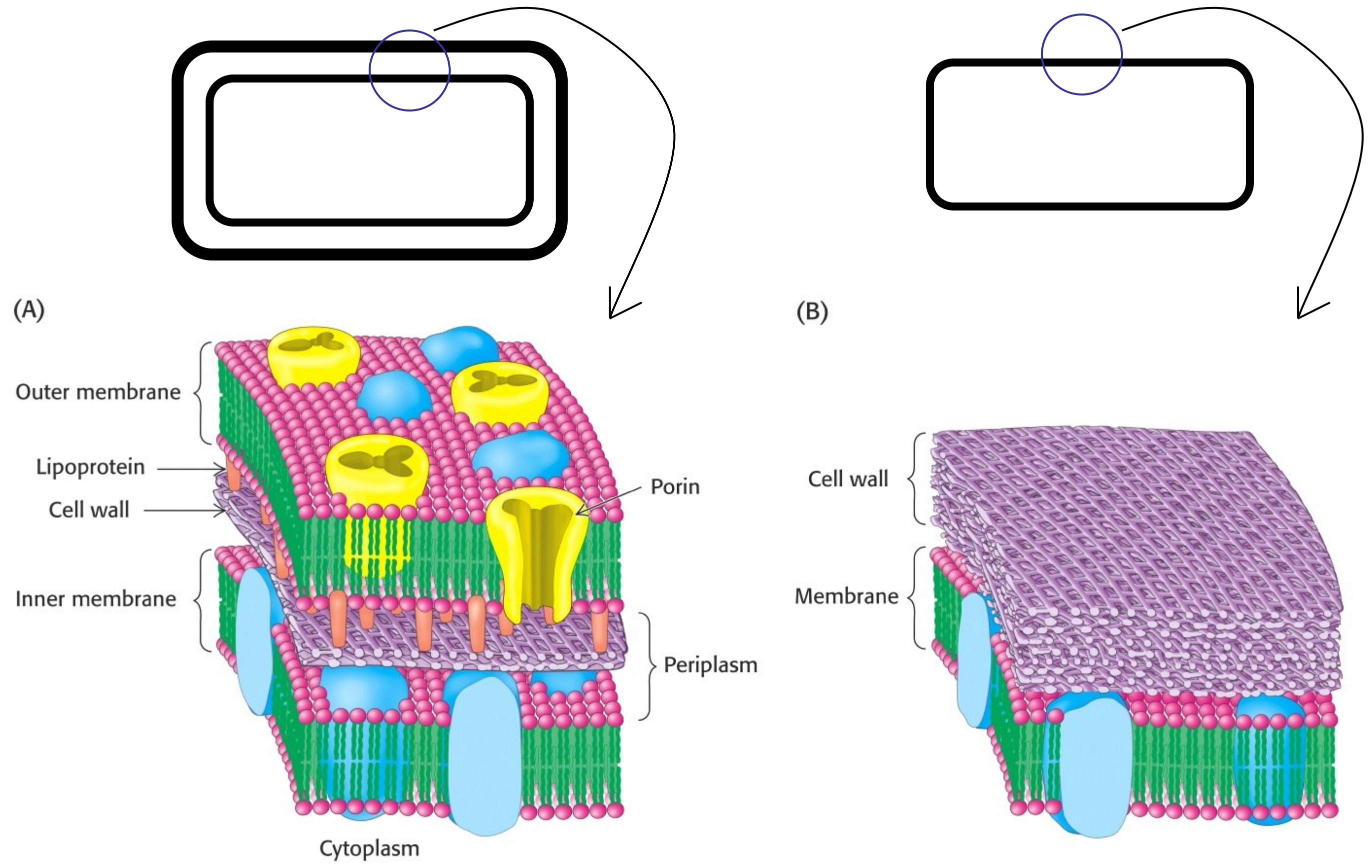
How does a cell look like?

The “simplest” cell: a prokaryote



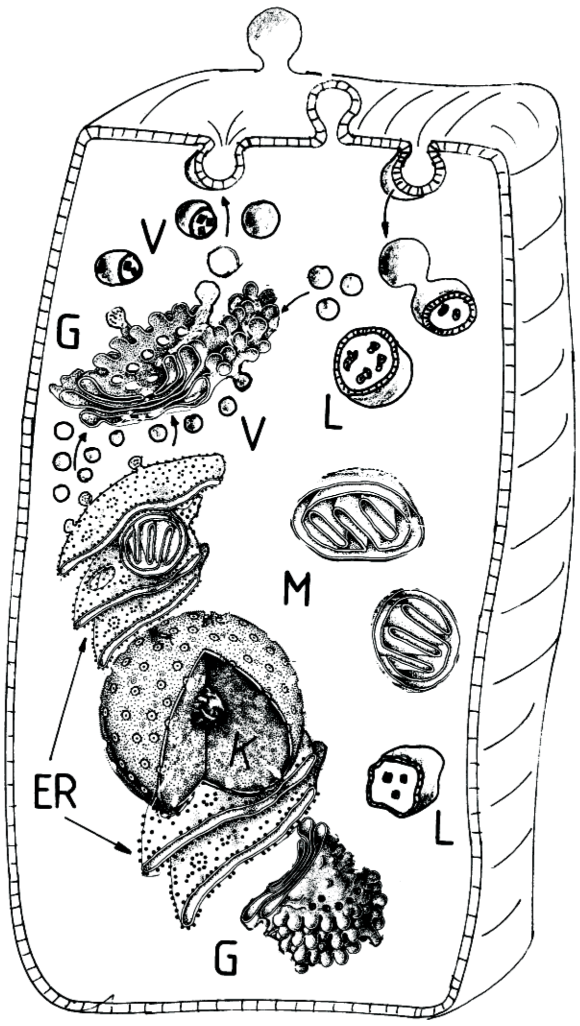
Gram negative

Gram positive



How does a cell look like?

A more complex cell: a eukaryote



- many internal membranes

- lot of flow

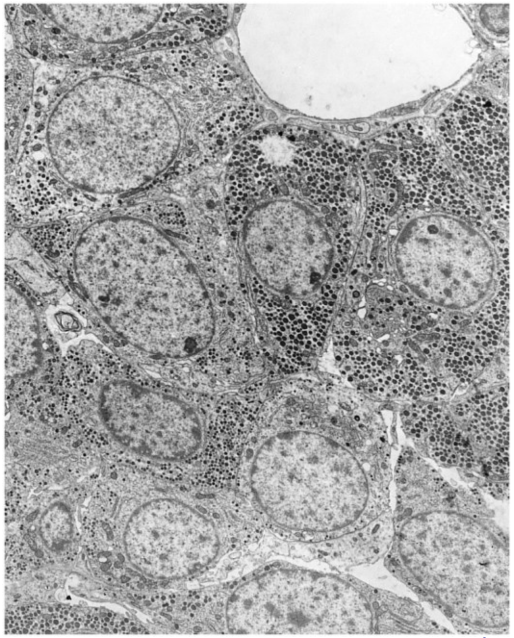
- lot of different morphologies

How does a cell look like?

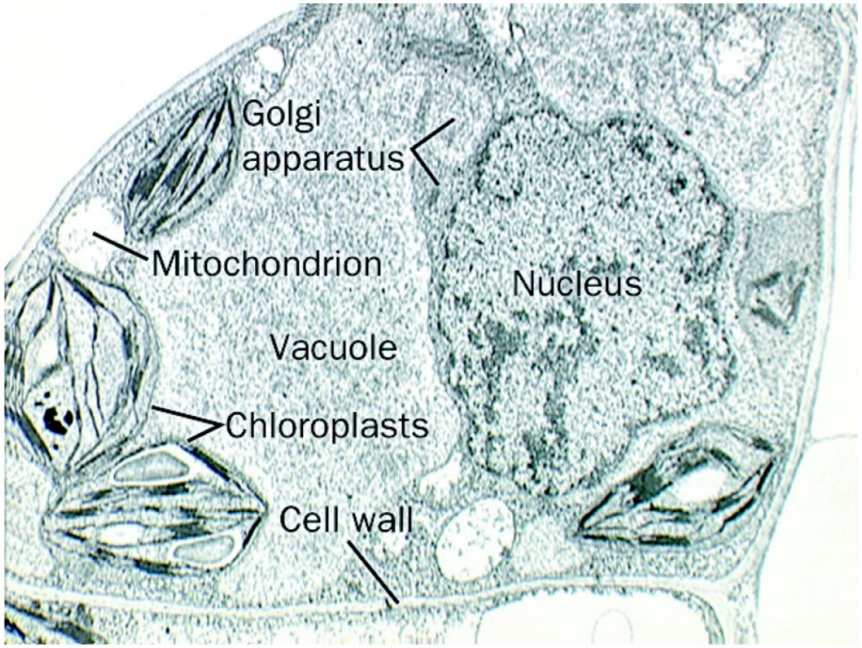
A more complex cell: a eukaryote

• rat pituary cell

• plant cell

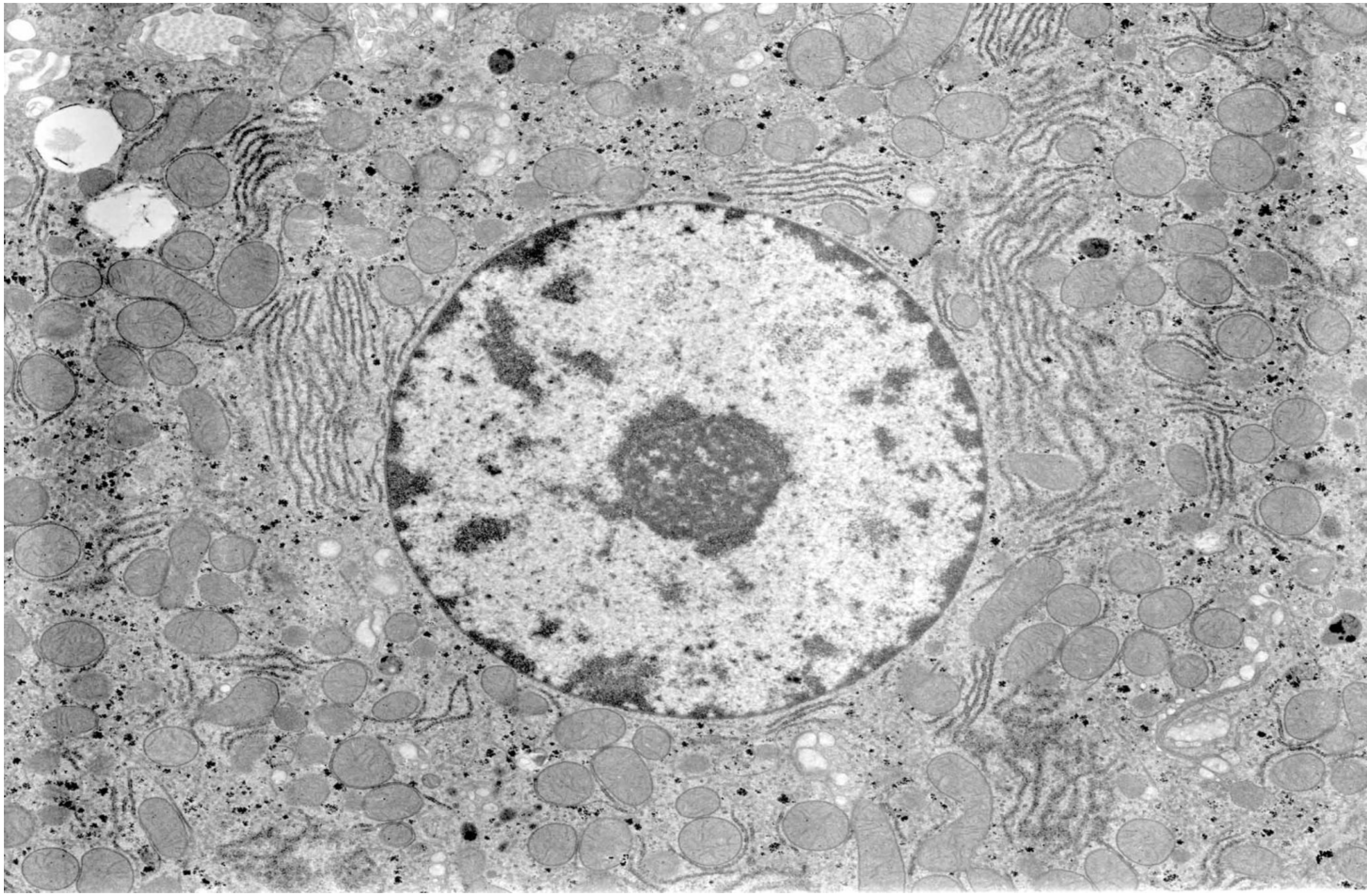


crowded!!



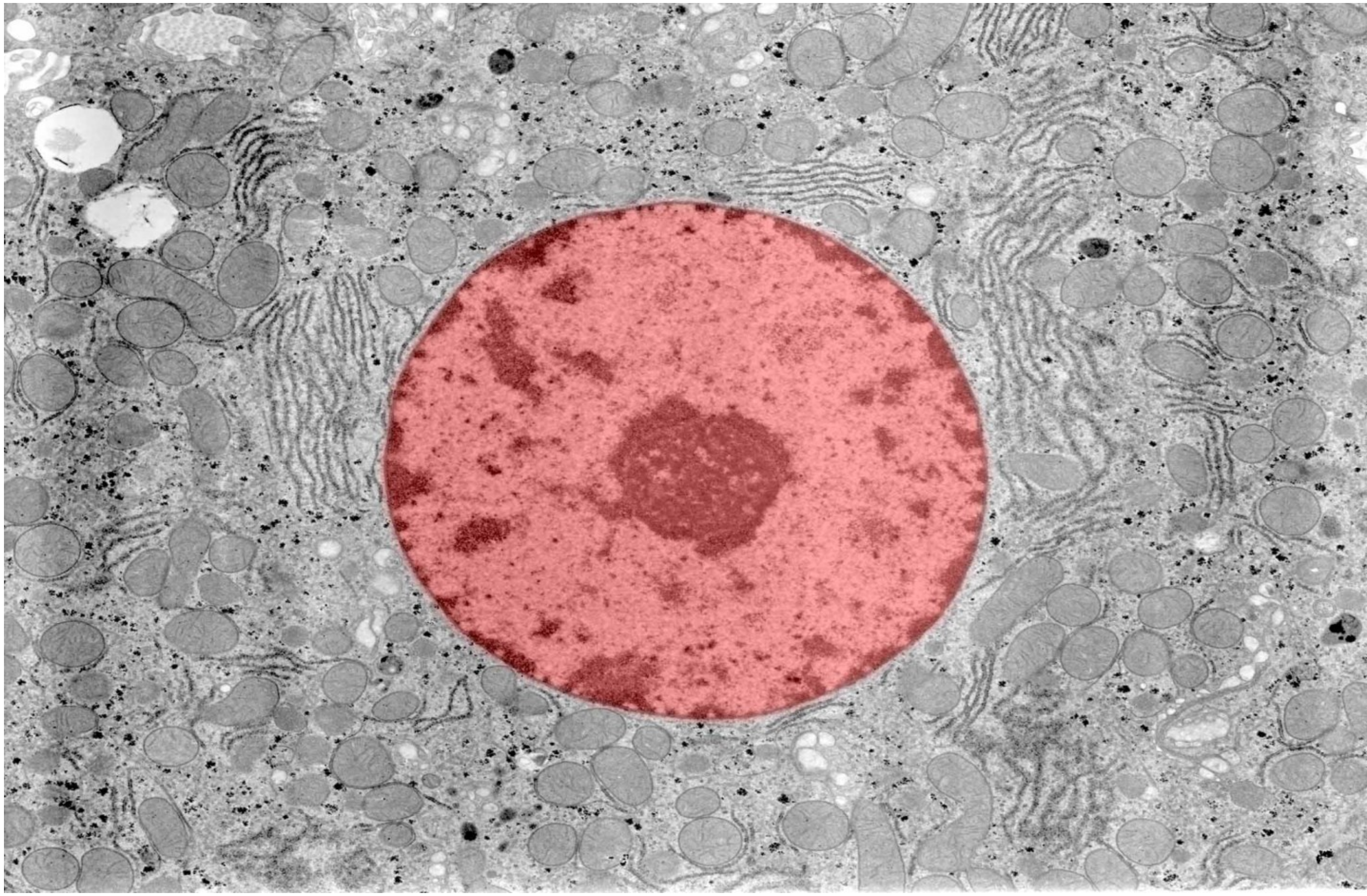
Looking at a cell

Mammalian cell viewed by electron microscopy



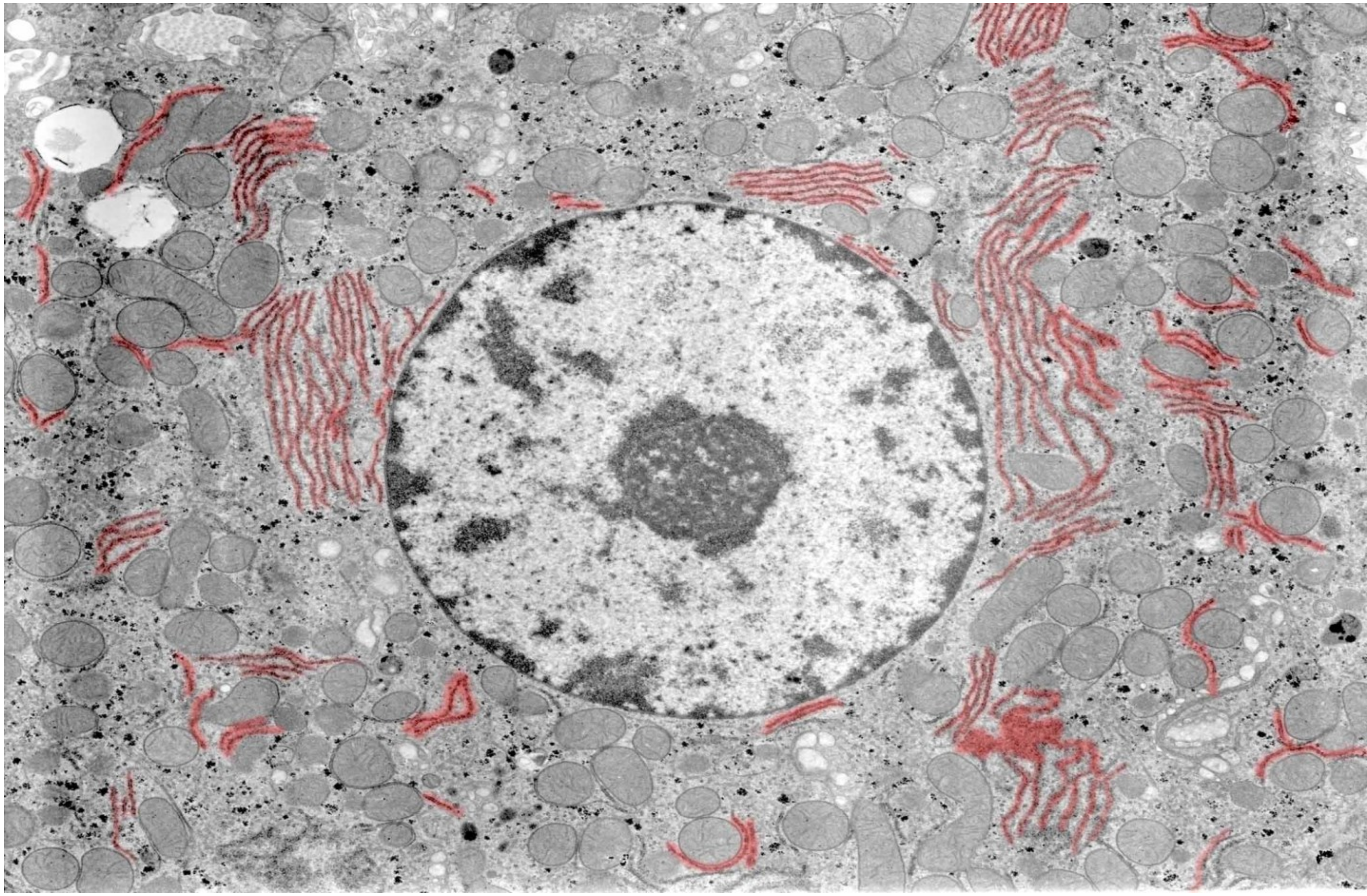
Looking at a cell

Nucleus:
- DNA
- Transcription
- Export of RNA



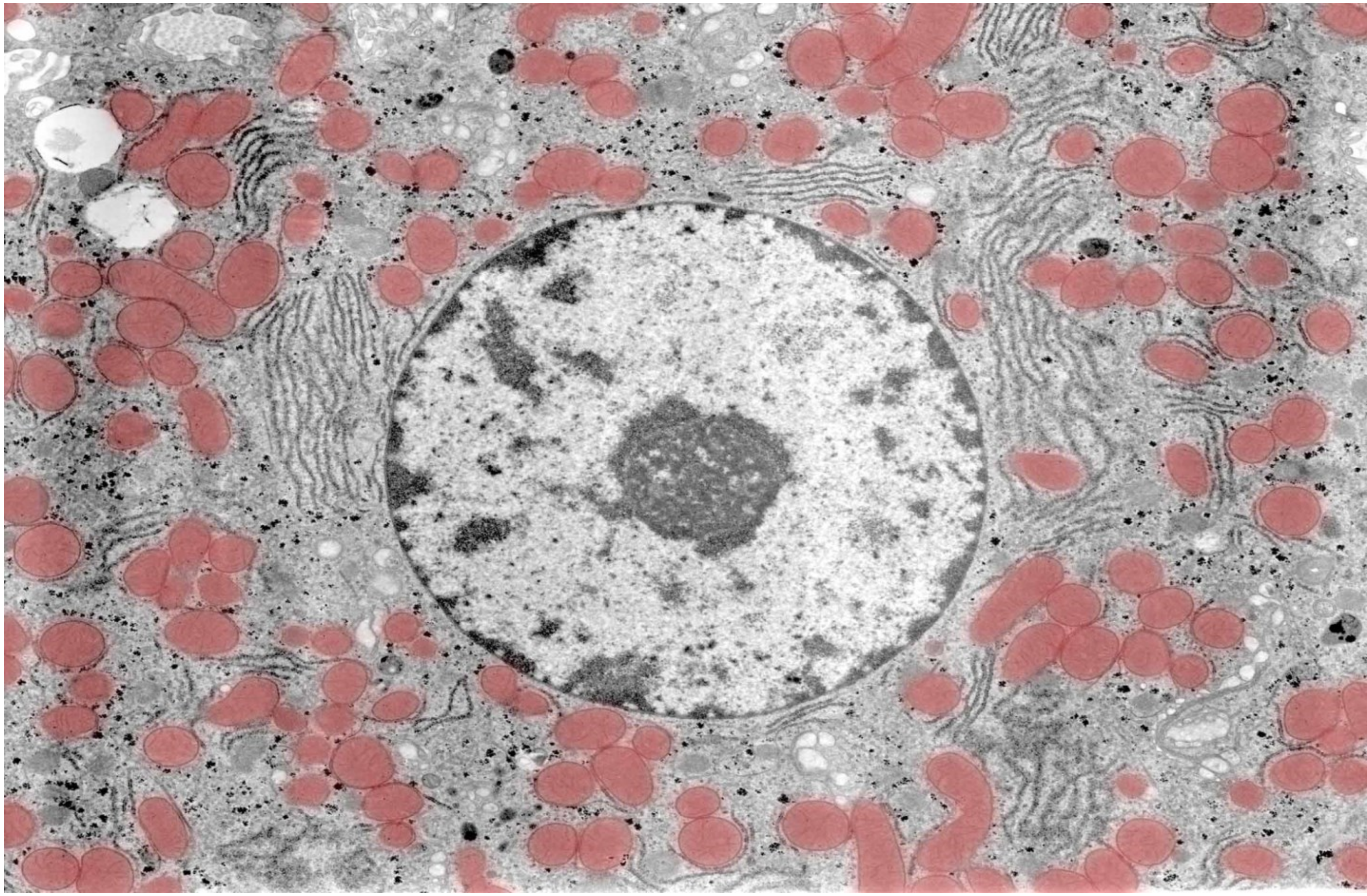
Looking at a cell

Endoplasmic reticulum (ER):
- Protein synthesis
- Sterol synthesis
- Sorting
- Detoxification



Looking at a cell

Mitochondria:
- Oxidative phosphorylation



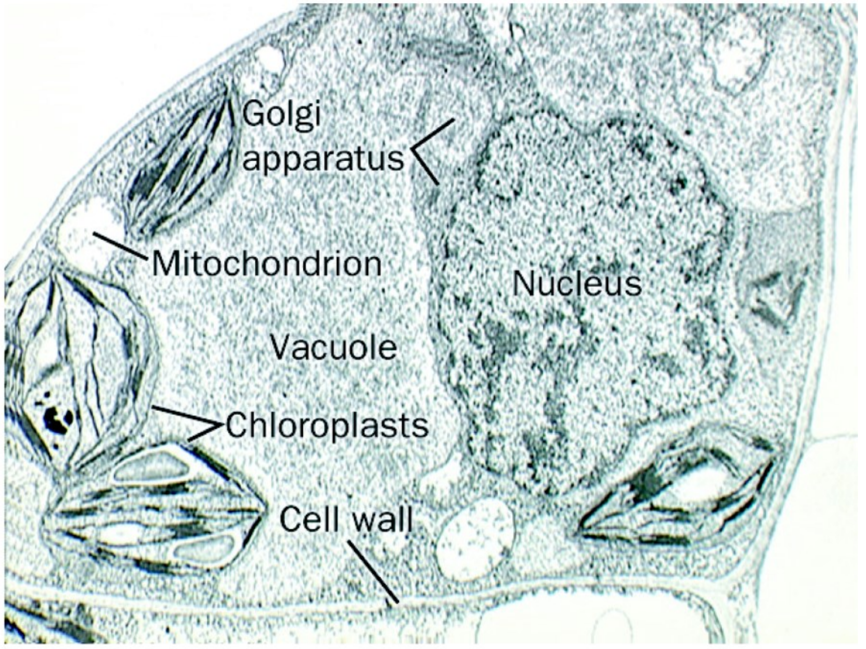
Functions of membranes

- Barrier

  "Membranes separate live from dead"

  Confinement

- Communication
- Scaffold
- Reservoir
- Transport



Properties of membranes

Self-assembling
Self-healing
Flexible
Shape
Dynamic
Hydrophobic layer
Specificity

QuickTime™ and a Sorenson Video 3 decompressor are needed to see this picture.

Membranes

Wide variety of functions and properties

<=>

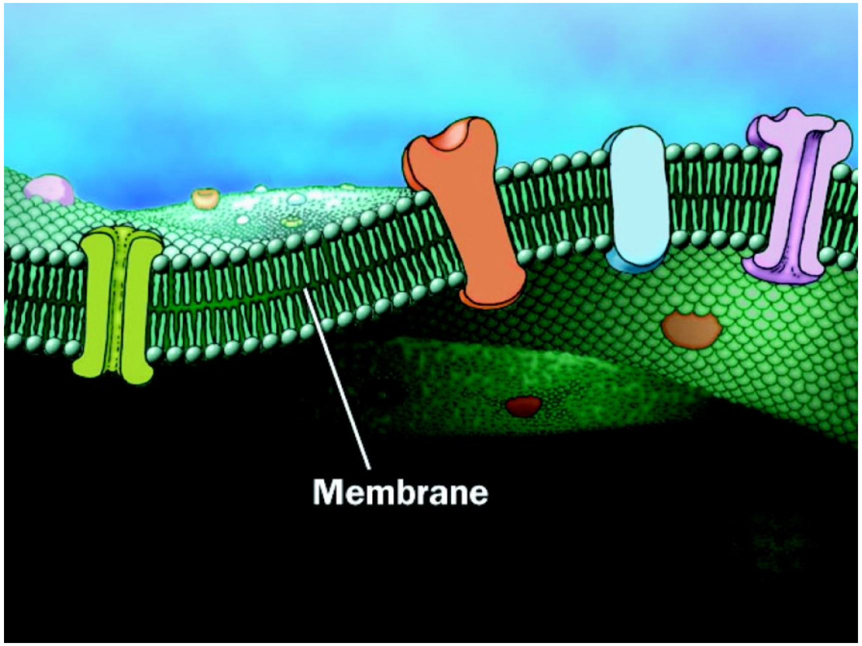
Wide variety of compositions?

Membrane components

Initial ideas:

Lipids => Barrier

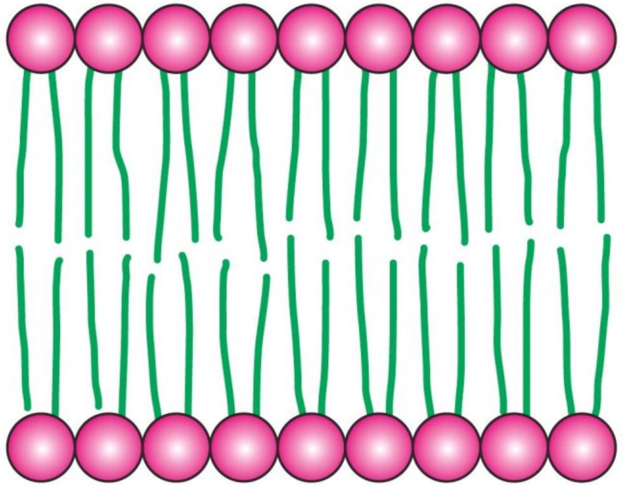
Proteins => Function

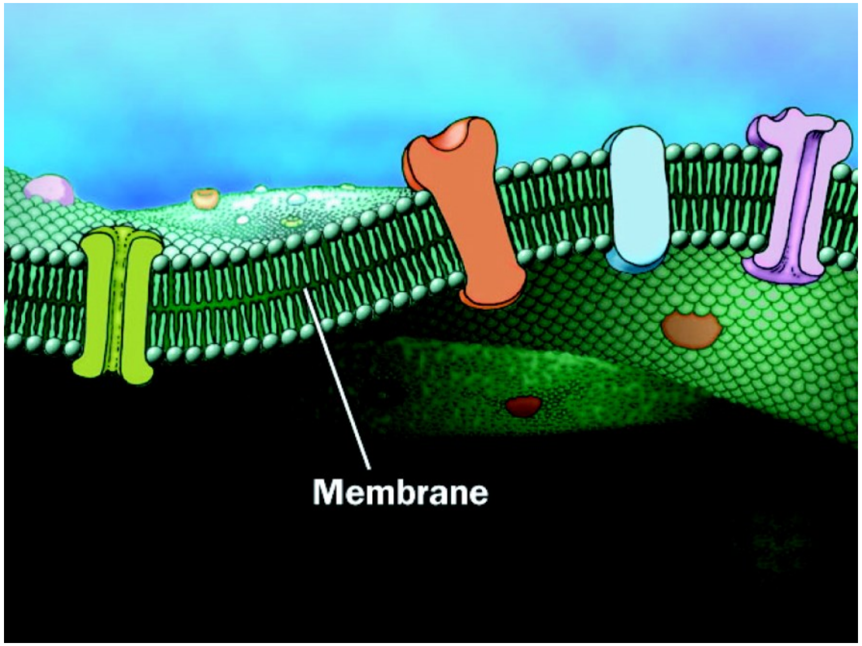


Architecture of membranes

1925

Lipidic bilayer (Gorter & Grendel)





1972

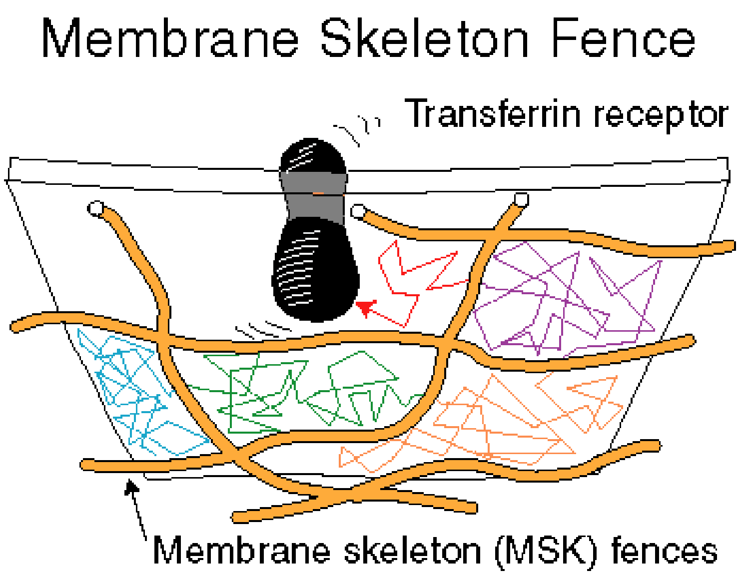
Fluid mosaic model

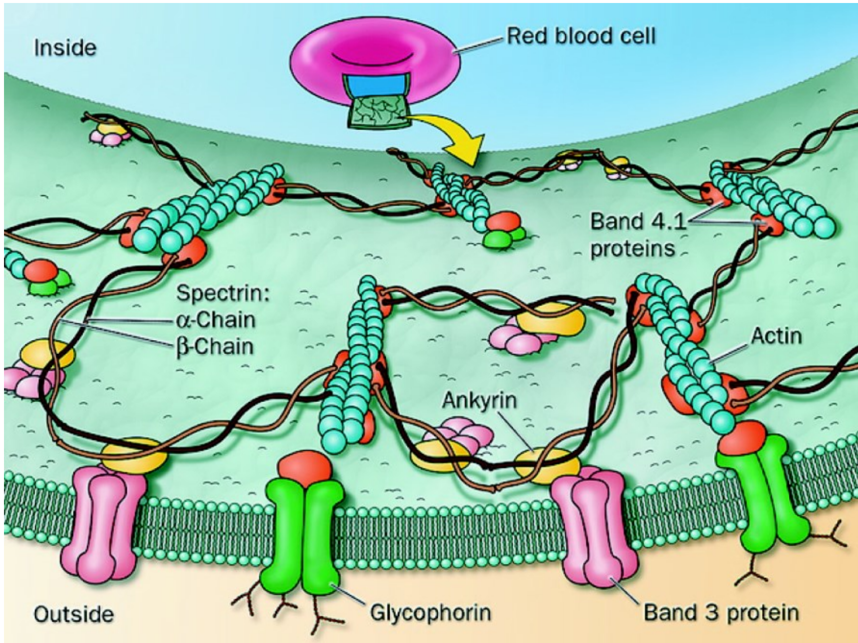
(Singer & Nicolson):

Free diffusion of all components within the membrane.

Architecture of membranes

A skelette of membrane-attached proteins:
- mechanical stability
- scaffold for molecules
- diffusion barrier





Kusumi (Nagoya, Japan)

Architecture of membranes

All is moving!

QuickTime™ and a YUV420 codec decompressor are needed to see this picture.

Membranes

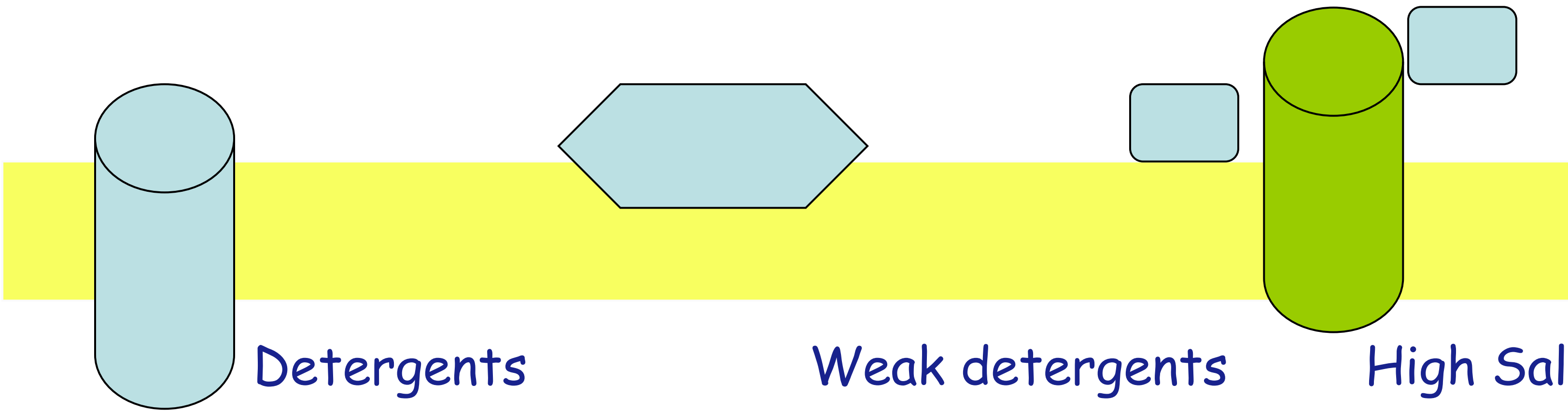
Some numbers:

◊ Dimensions
- cell — 1 to 10's μm
- membrane — ± 4 nm thick
- lipid — ± 50 $Å^2$

◊ Diffusion
- lipid in membrane — 10^{-10} to 10^{-8} cm^2/s

◊ Potential
- -80 mV => 20 MV/m

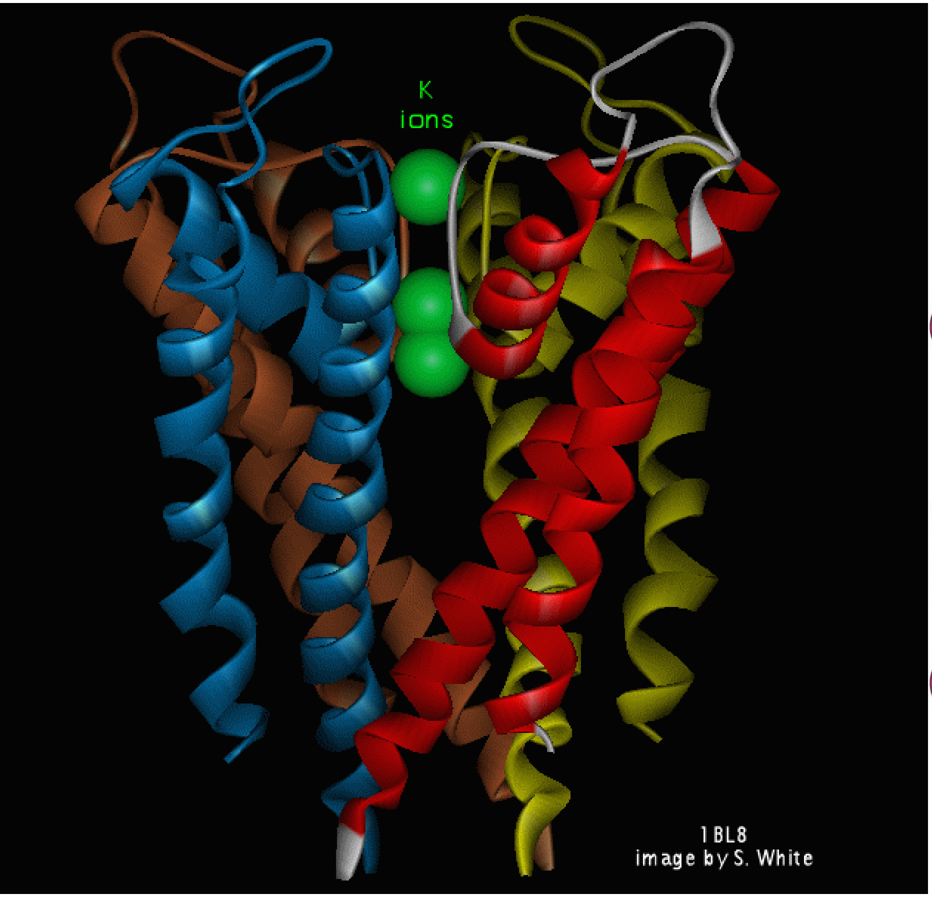
Membrane proteins



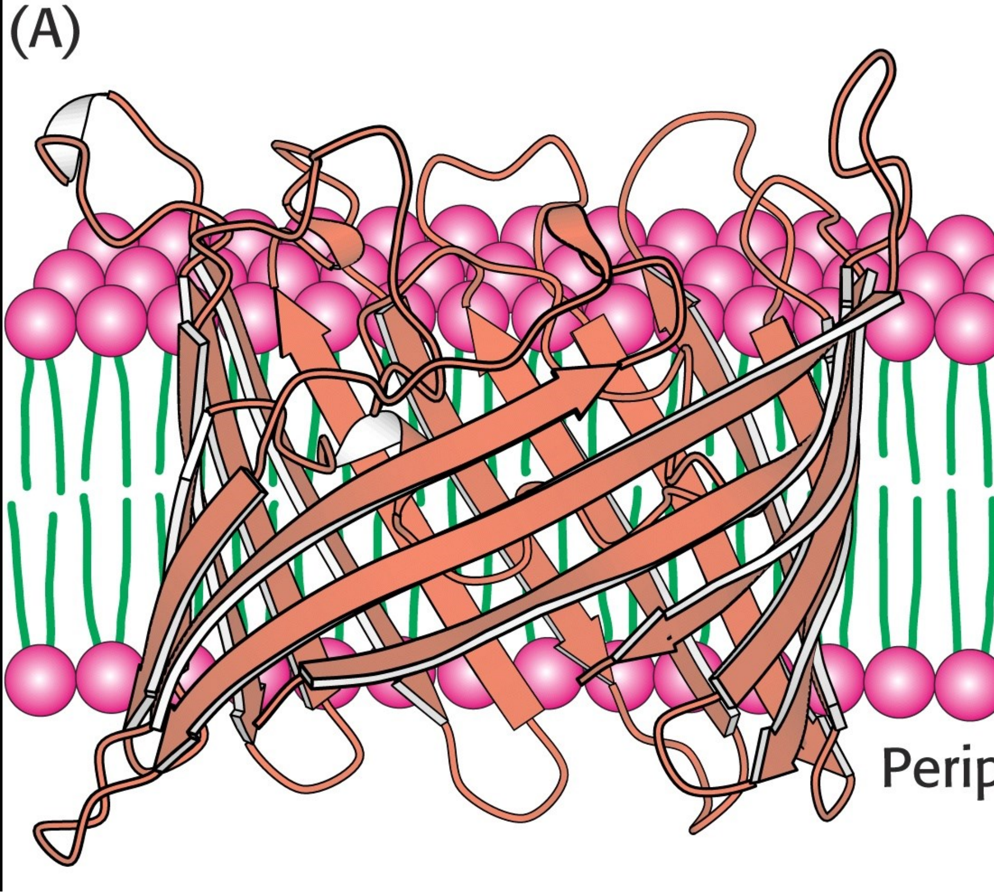
	Integral	Peripheral	Extrinsic
Extraction:	Detergents	Weak detergents Chaotropic agents	High Salt Chelators
Interaction:	Hydrophobic	Hydrophobic & Electrostatic	Electrostatic

Integral membrane proteins

α-Helical bundle
Voltage-gated K^+-channel



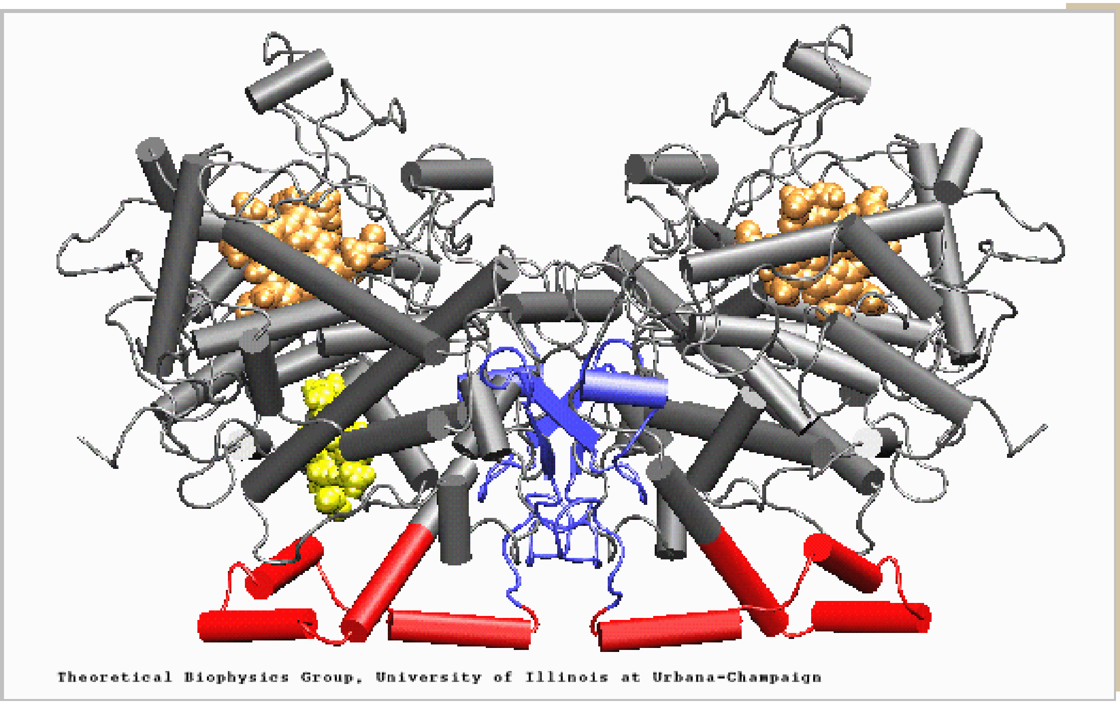
β-Barrel
Porin OmpF

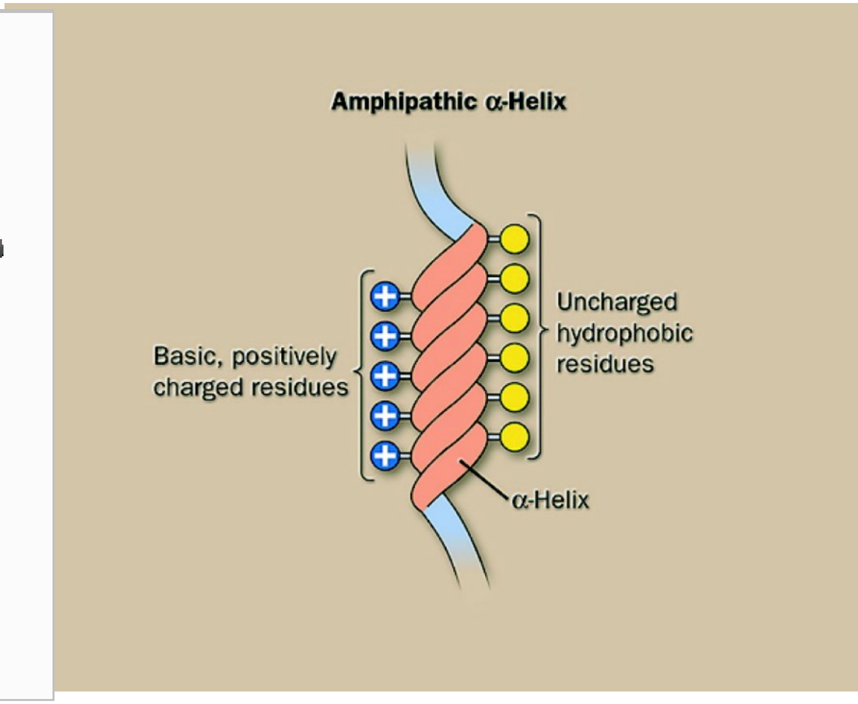


Peripheral membrane proteins

Prostaglandin H2 synthase

Amphipathic α-helix





Membrane proteins

◊ Highly ordered structural motifs:

- α-helix

  hydrophobic stretch of 15-25 amino acids.

- β-sheet

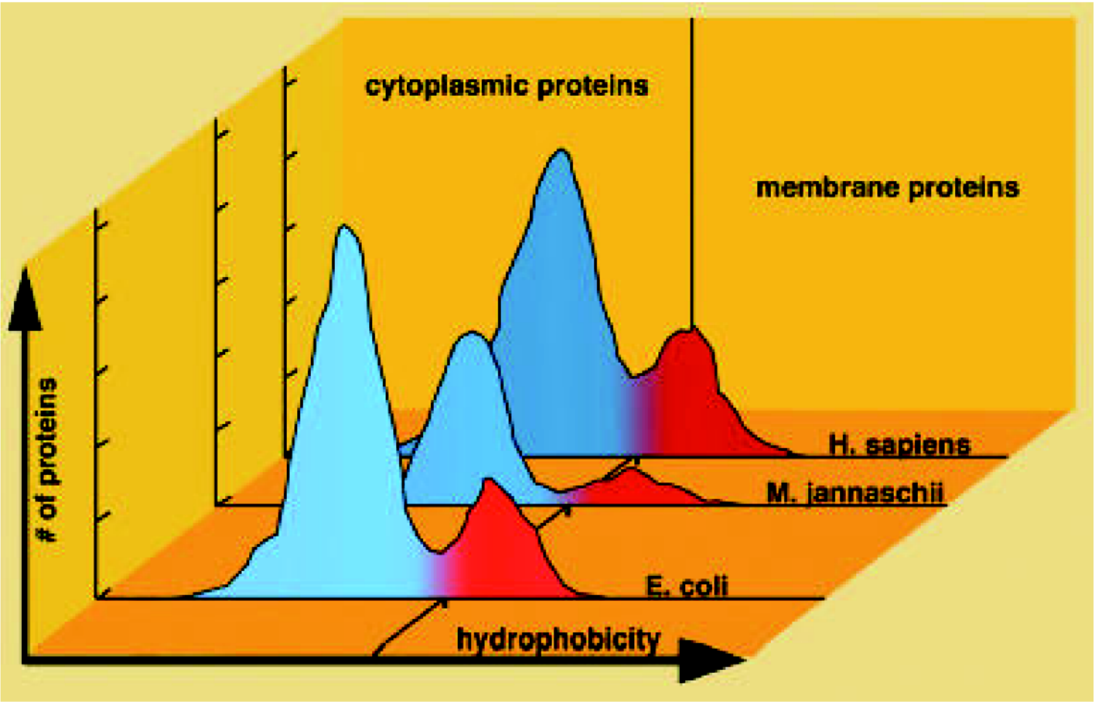
  alternating hydrophilic and hydrophobic amino acids.

◊ Few charged amino acids.

◊ Proteins move to function.

Membrane proteins

Genome-wide analysis of mean hydrophobicity of proteins:



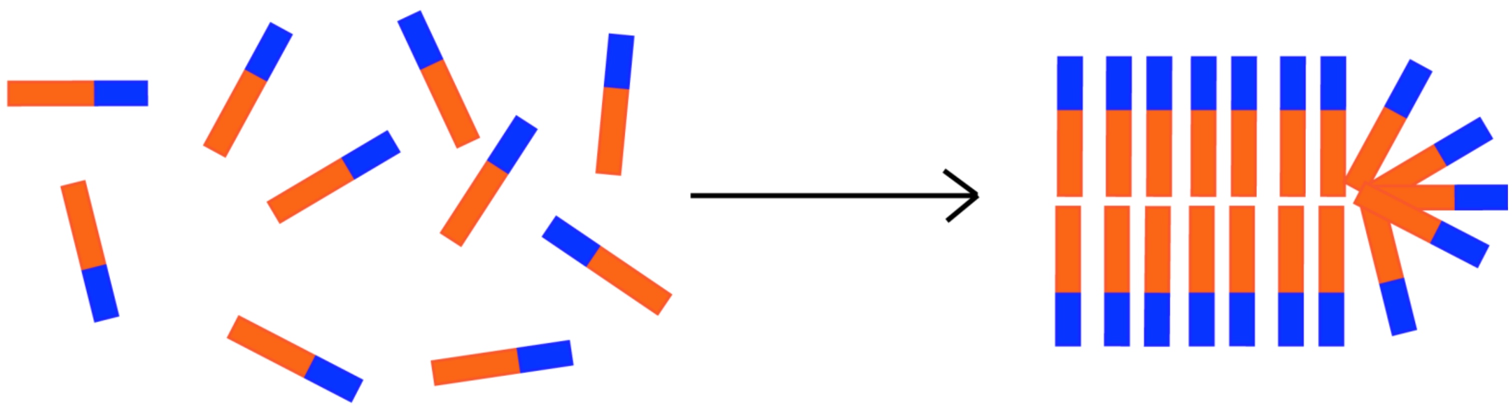
=> 30 % of all proteins are membrane proteins, i.e. about 10'000.

Membrane lipids

General structure of membrane components

In water the amphipatic membrane components segregate **spontaneously** driven by **entropy.**

This process is called called **self assembly**



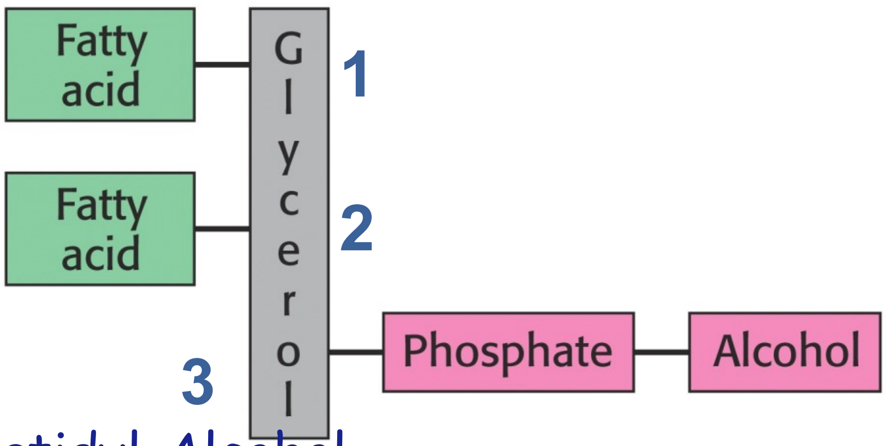
=> flexible

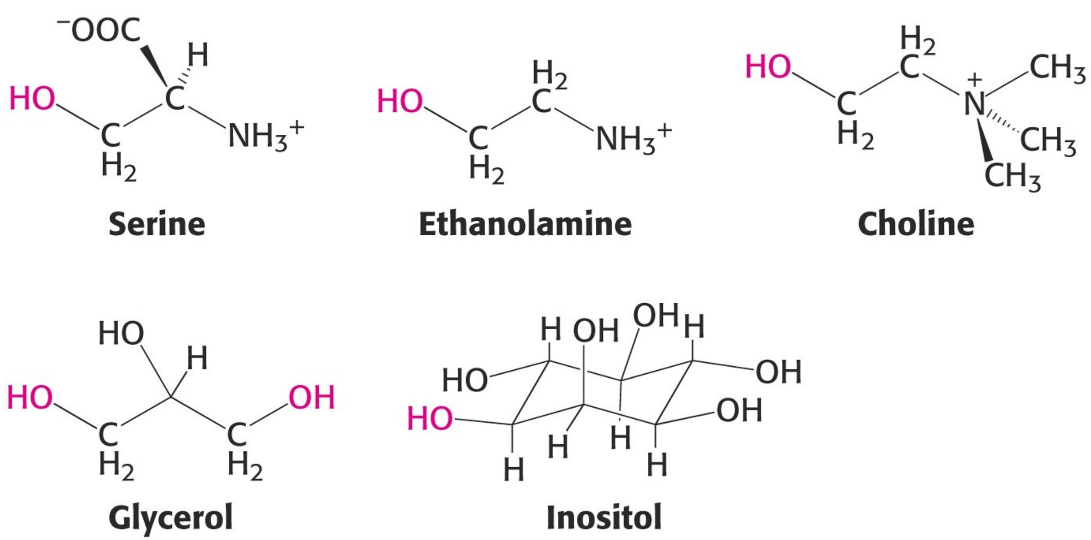
=> dynamic

=> self healing

Glycero-phospholipids

- Glycerol backbone
- Fatty acids
  - sn1: saturated
  - sn2: unsaturated
- Alcohol-P_i-ester on sn-3
- Name "1-FA_1-2-FA_2-sn-phosphatidyl-Alcohol





Glycero-phospholipids

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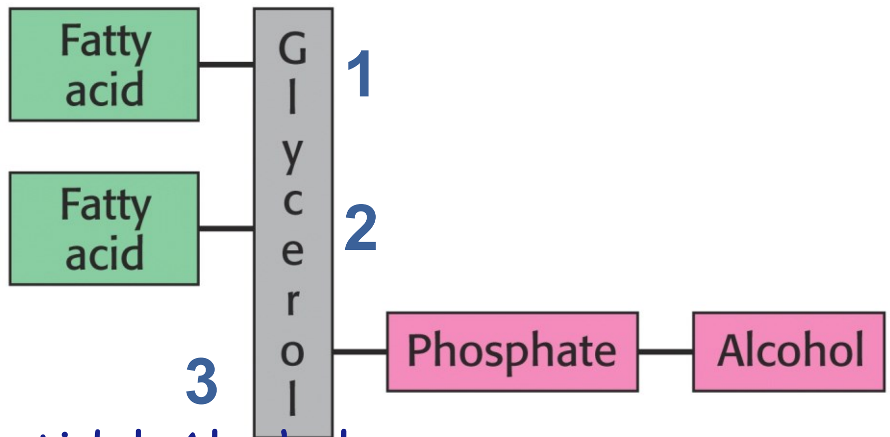
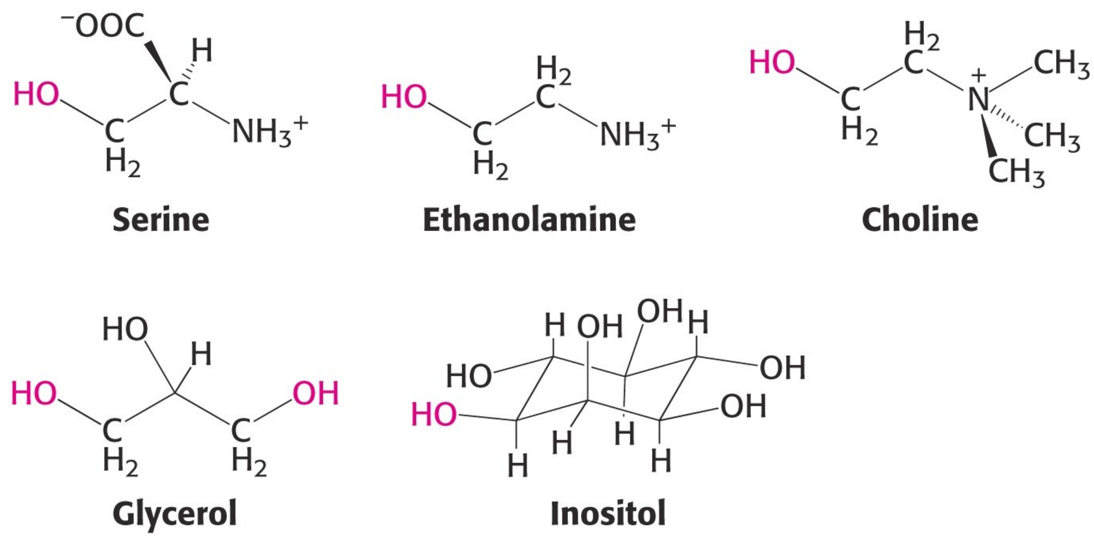


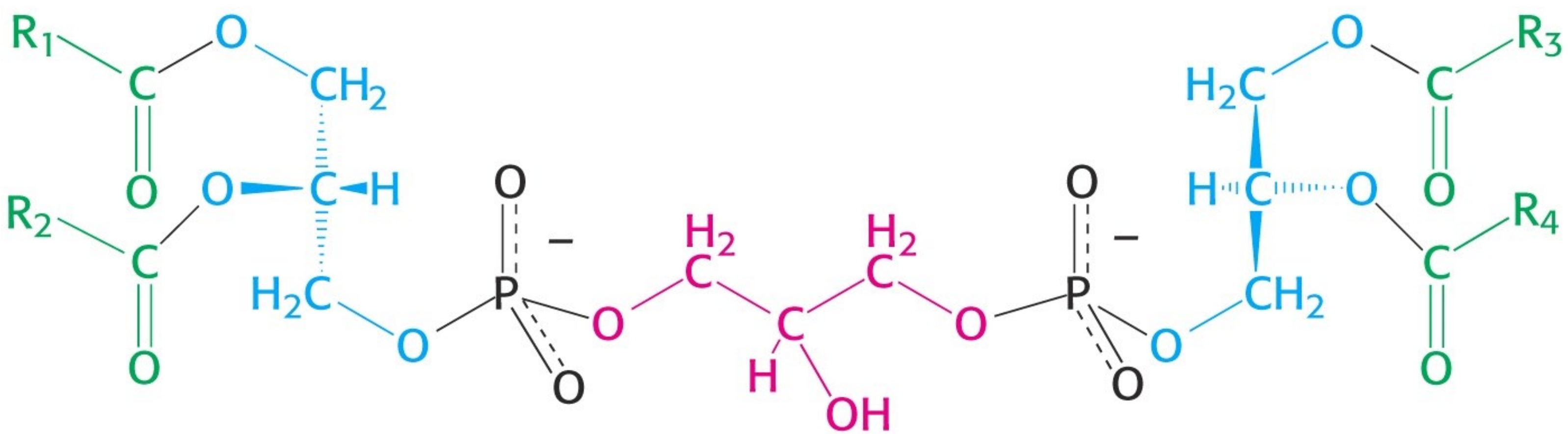
- Charge:

±	PC, PE
-	PI, PG
2-	CL
-/2-	PA



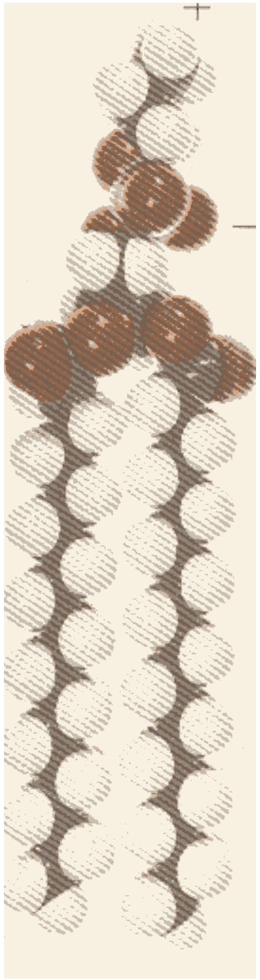
Glycero-phospholipids

- Cardiolipin: a double lipid.
  In eukaryotes, only present in mitochondria.

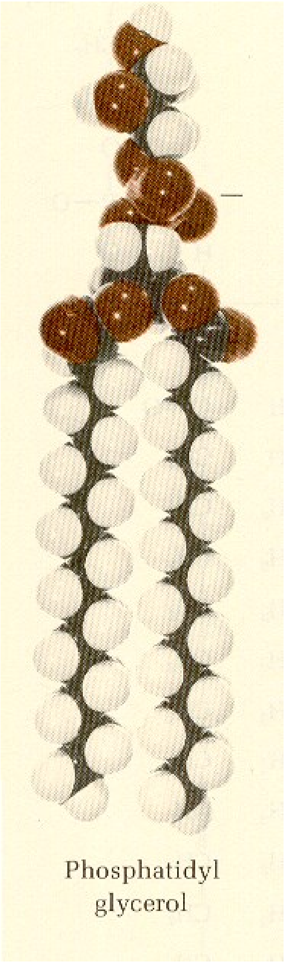


Diphosphatidyl glycerol (cardiolipin)

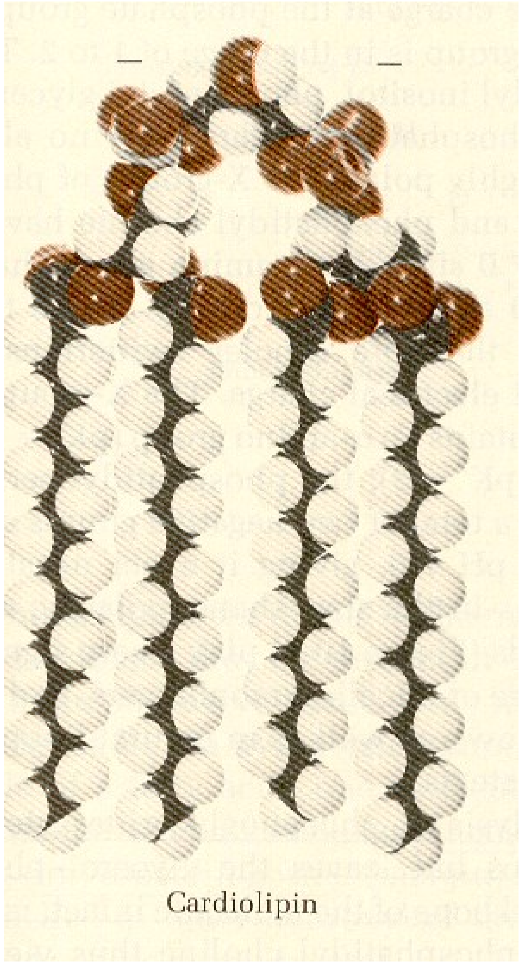
Model of phospholipids



PE



PG



Cardiolipin

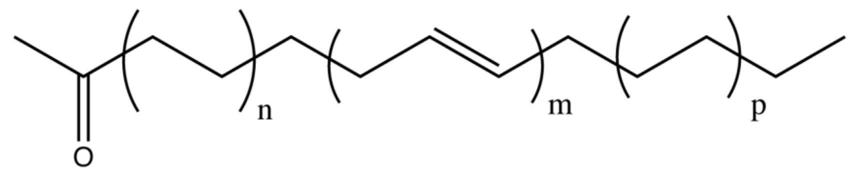
Fatty acids

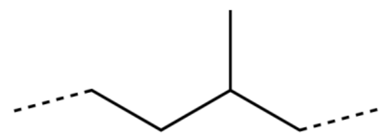
- Fatty acids

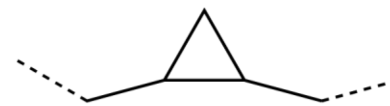
even - chain length 12 - 36

cys - saturation $0 \leq m \leq 6$

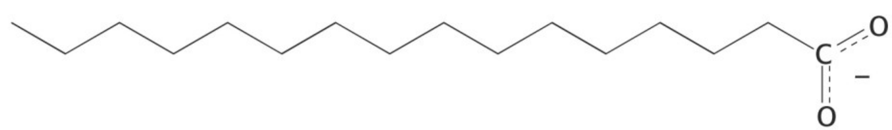
- branching



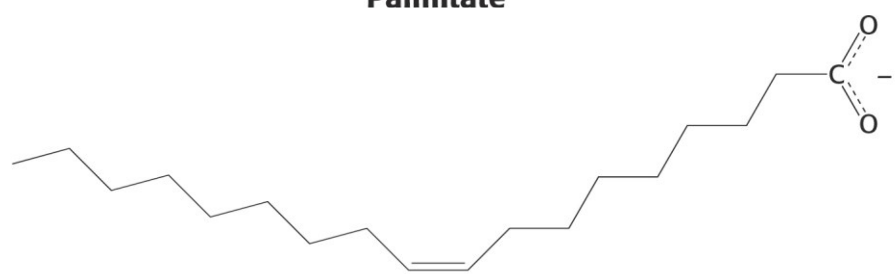




- Highly variable
- Phase transition
- Accommodate proteins
- Phase separation
- Signalling



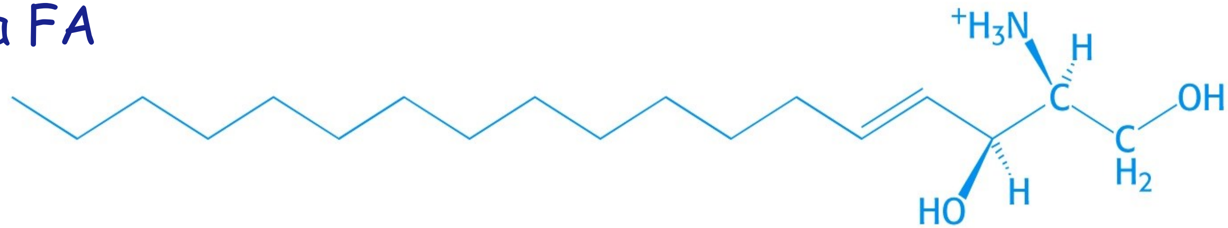
Palmitate



Oleate
(ionized form of oleic acid)

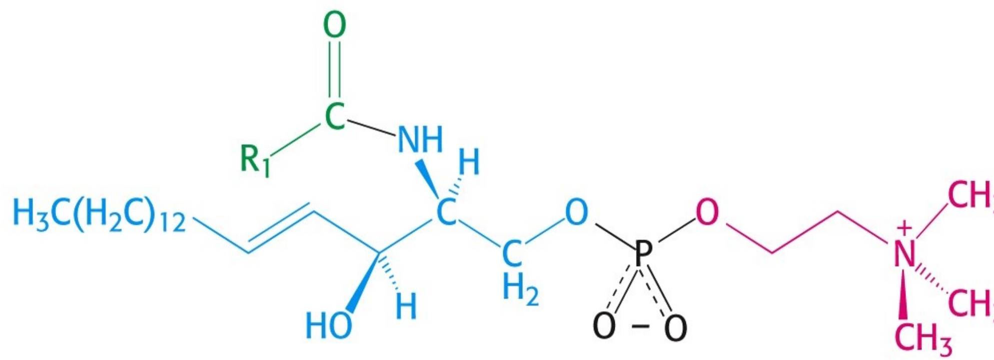
Phospho-sphingolipids

- Sphingosine backbone
  - already includes a FA



Sphingosine

+ Fatty acid linked via peptide bond
  => ceramide

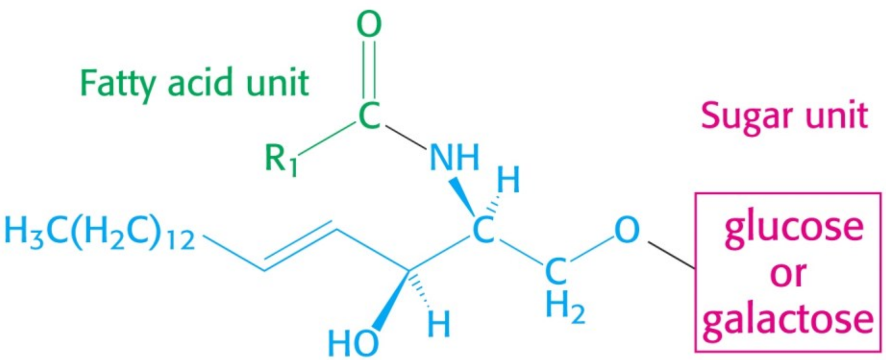


Sphingomyelin

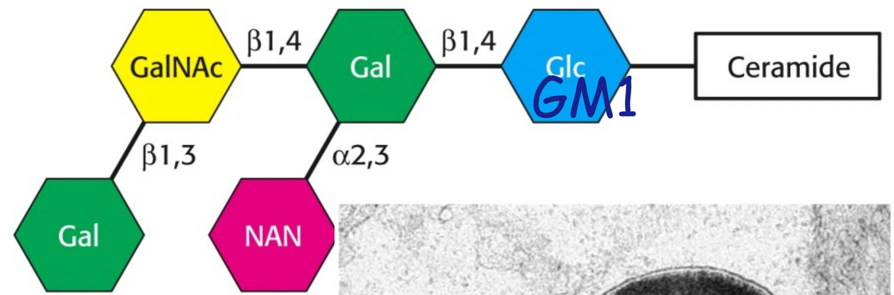
- Alcohol-Pi-ester
  - choline => Sphingomyelin
  - ethanolamine
  - glycerol

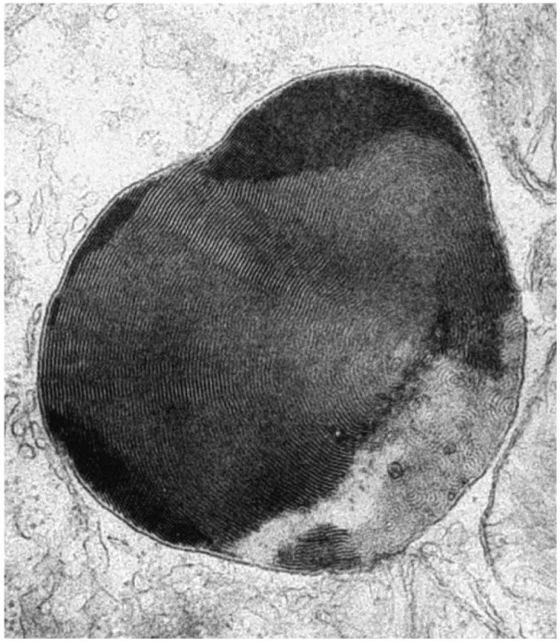
Glyco-sphingolipids

- Ceramide having sugars as polar head group
  - simple, e.g. 1 sugar => cerebroside
  - complex, e.g.5 sugars => ganglioside GM1



Cerebroside (a glycolipid)

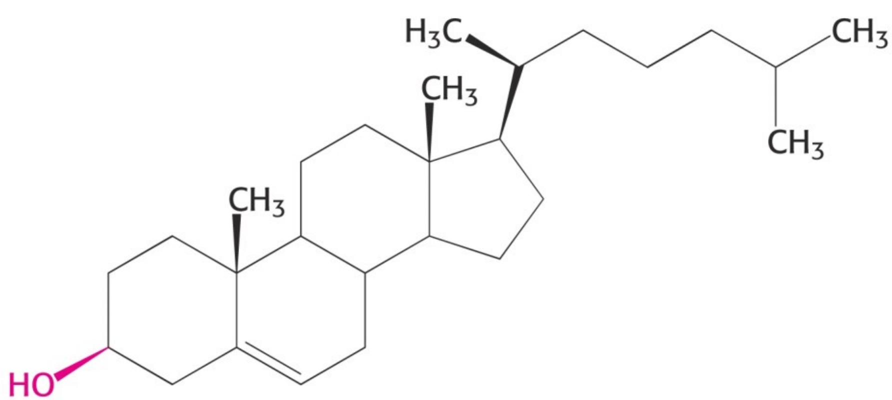




- Outer surface of plasma membrane; myelin
- Blood group antigens on erythrocyte.

Sterols

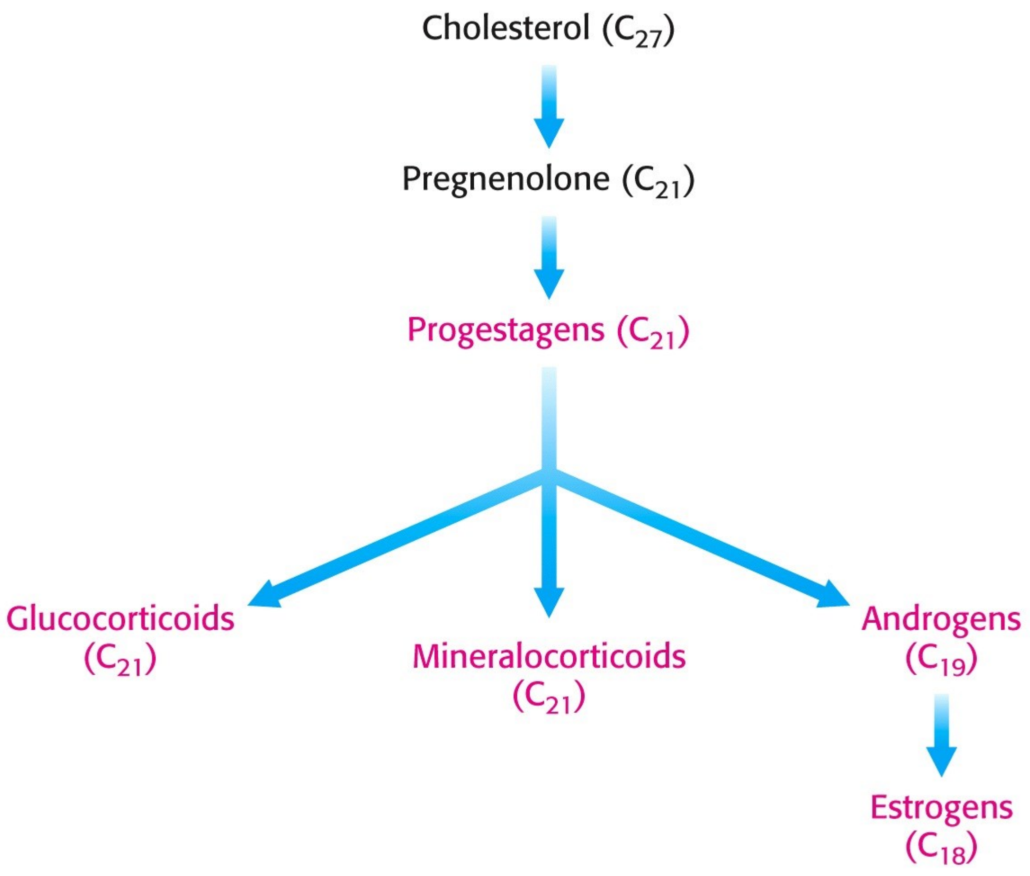
- Cholesterol is main sterol in mammalians
  - mainly present in plasma membrane, but also in endo- and exo-cytotic pathway.
  - profound effect on phase behavior of membranes.
  - domain formation.



Cholesterol

Sterols

- Cholesterol a precursor of steroid hormones.



General properties of membranes

- Dynamic nature => protein function & diffusion
- Phase transitions => domain formation
- Polymorphism => fission and fusion
- Surface pressure => protein insertion
- Permeability => barrier
- Electrical effects => concentration gradients

Dynamic nature of membranes

Simulation:

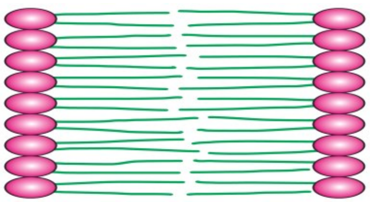
All is moving!

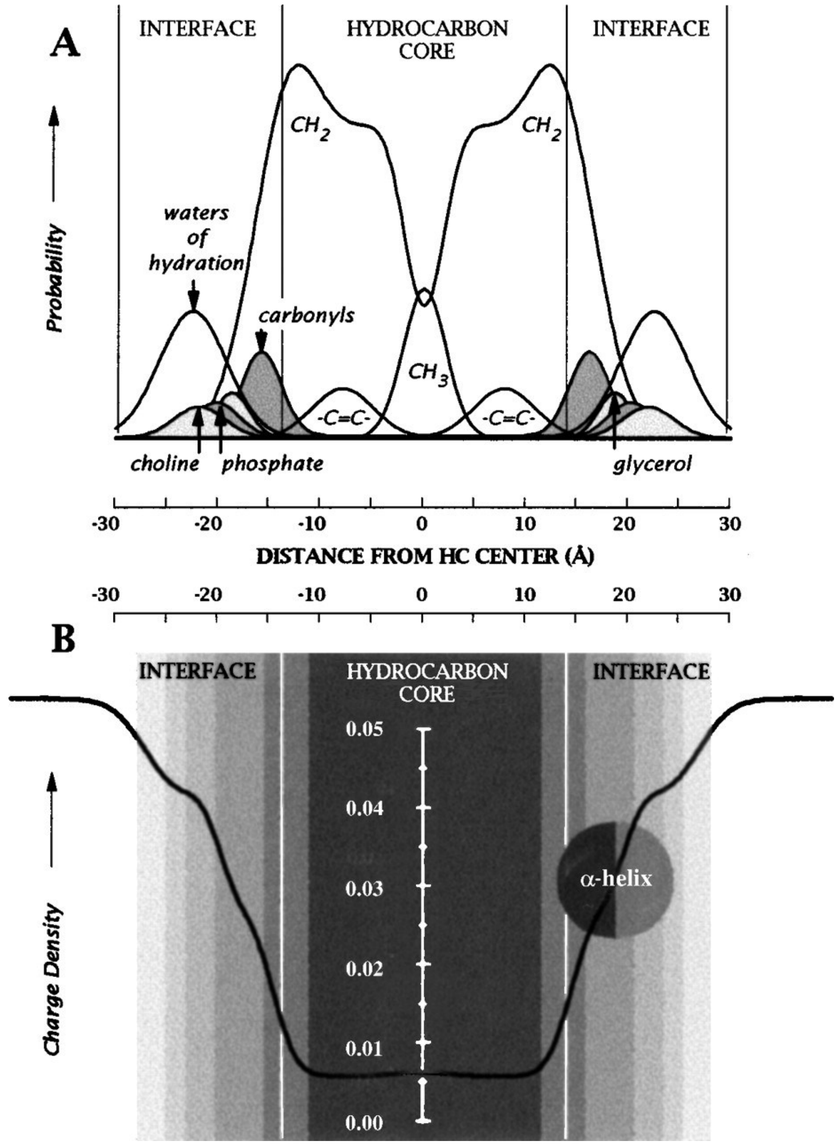
QuickTime™ and a YUV420 codec decompressor are needed to see this picture.

Dynamic nature of membranes

Diffraction studies:

Distributions are wide:
- Reflecting similations
- Rejecting



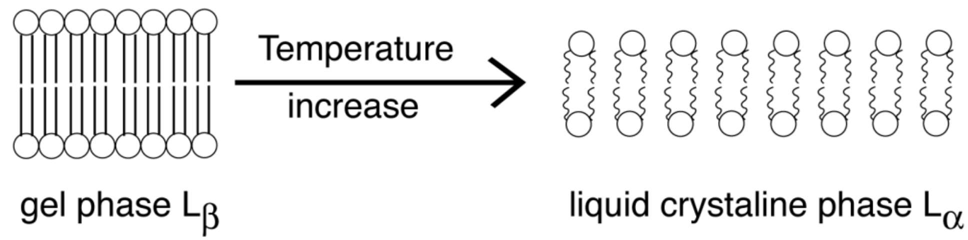


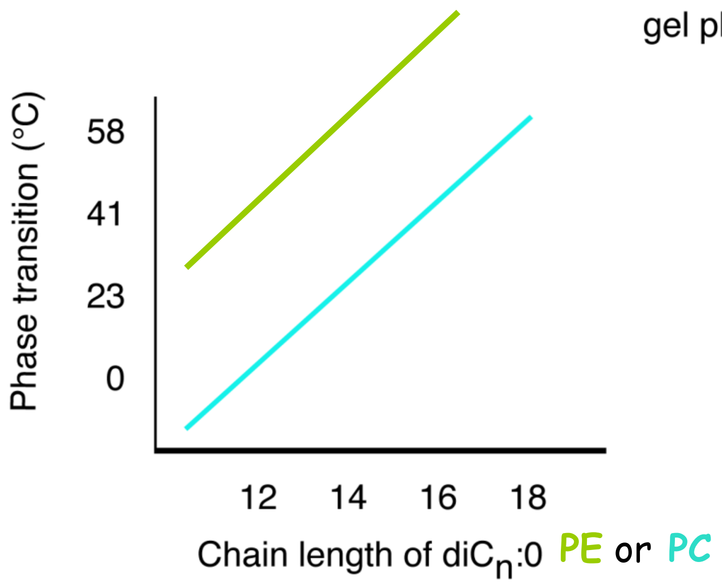
- Hydrophobic core of ± 25 Å

- Interfacial region of ± 15 Å
  with a large range of properties.

Phase transitions of lipids

- Temperature
  - melting of the chains
    - => membrane thickness
    - => molecular packing





=> ± 20 °C per two C2-units

=> ± 30 °C per cis-double bound

=> choline ± 30 °C lower than ethanolamine

=>

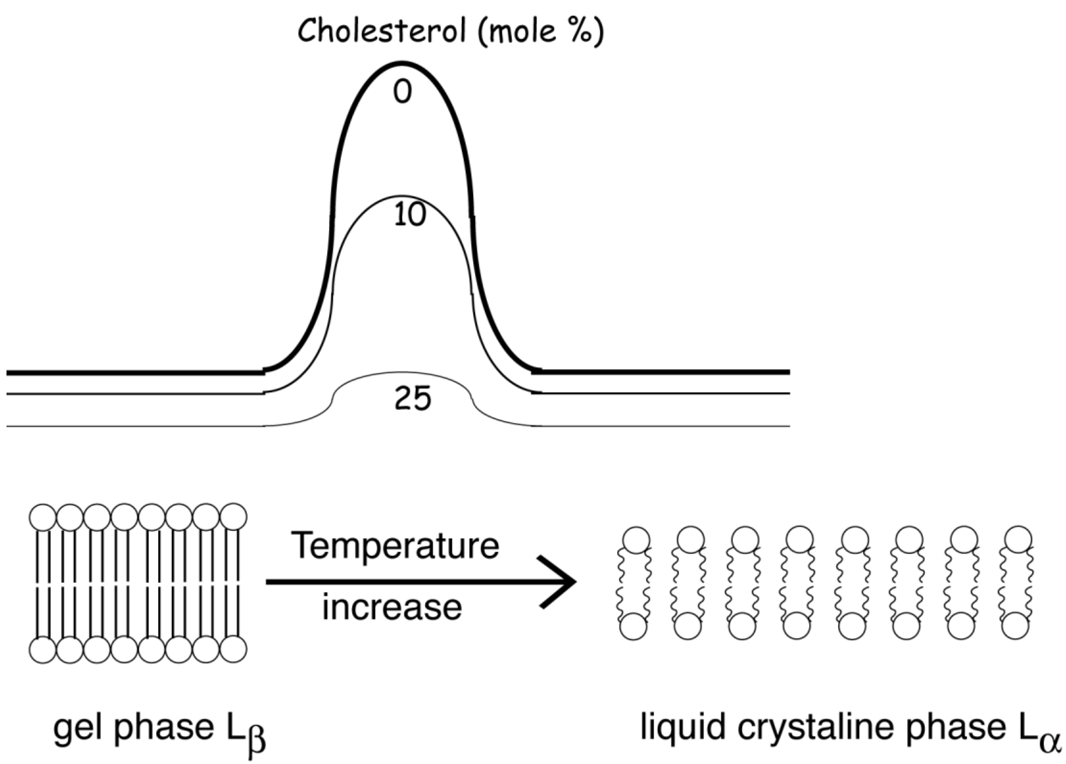
◊ Temperature affects fluidity => diffusion increases 100-fold from gel to liquid phase

◊ FA-composition affects phase transition

◊ Cell membranes want to be liquid crystalline.

Phase transitions of lipids

- Cholesterol
  - "liquifies" gel phase & "solidifies" liquid crystalline phase => phase transition disappears
  - membrane permeability decreases



Phase transitions of lipids

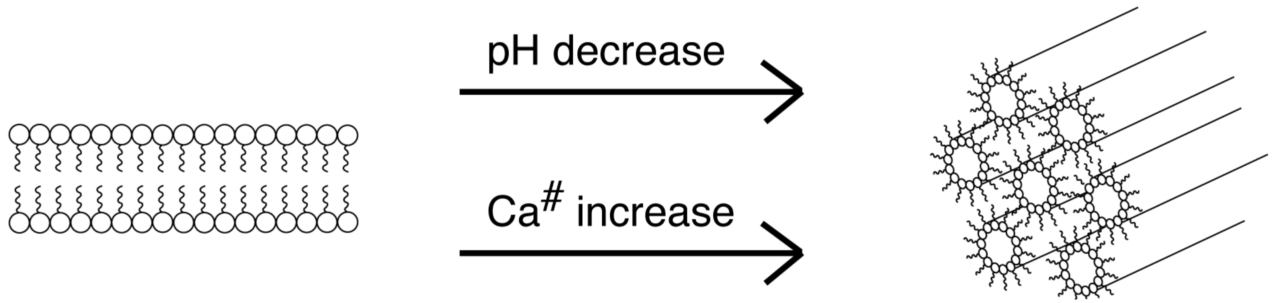
- Buffer composition

- pH

  e.g. PA

- Divalent cations

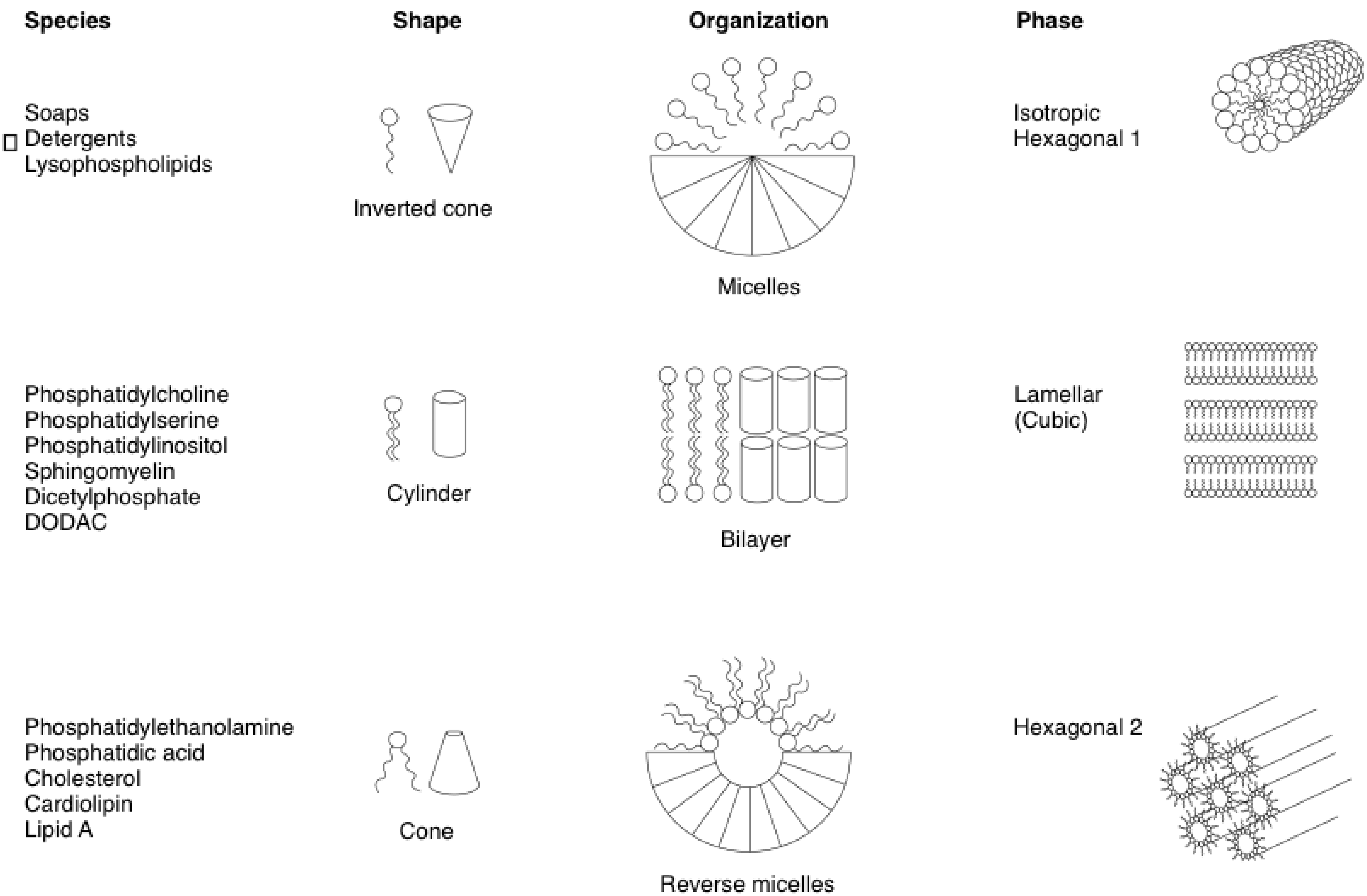
  e.g. CL



L-phase ***H_{II}-phase***

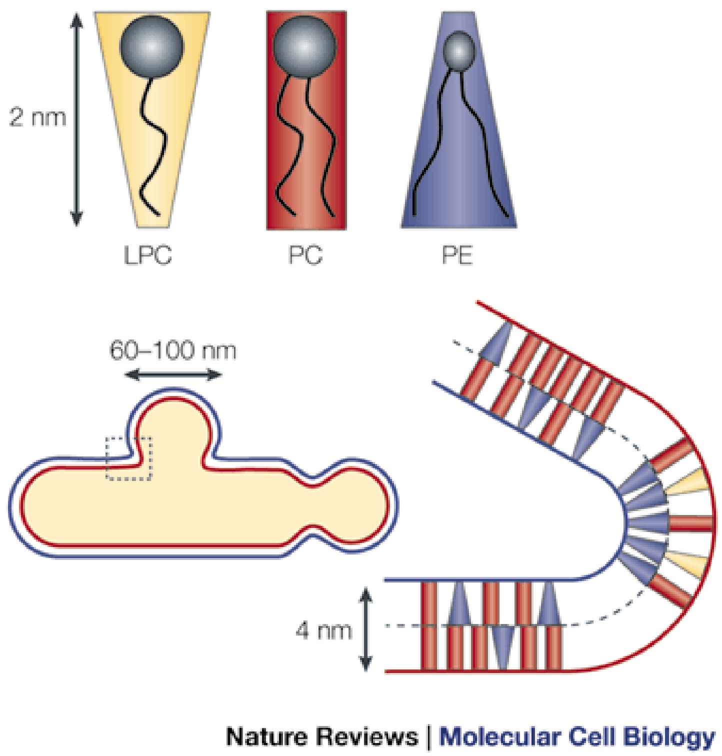
Lipid polymorphism

- Relative size of polar head group and hydrophobic part of lipid

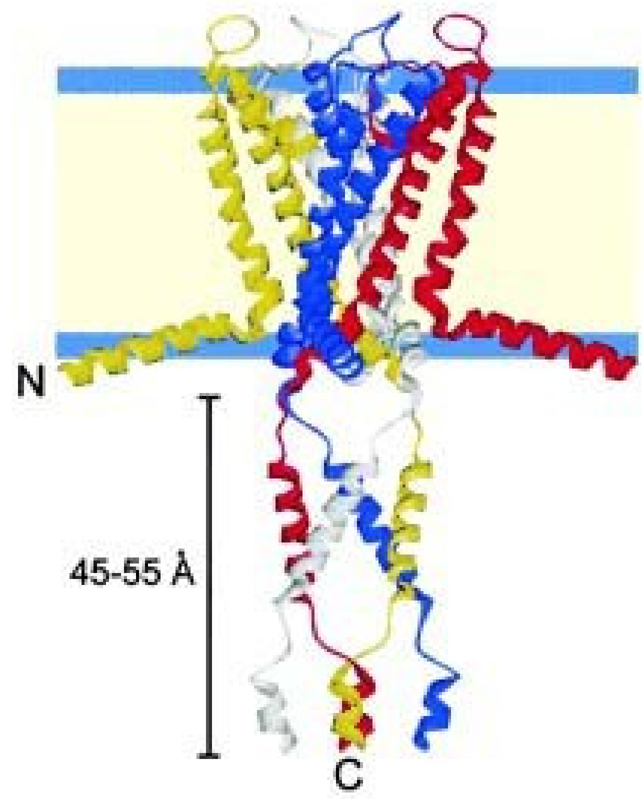


Lipid polymorphism in membranes

- Extreme curvature of membrane

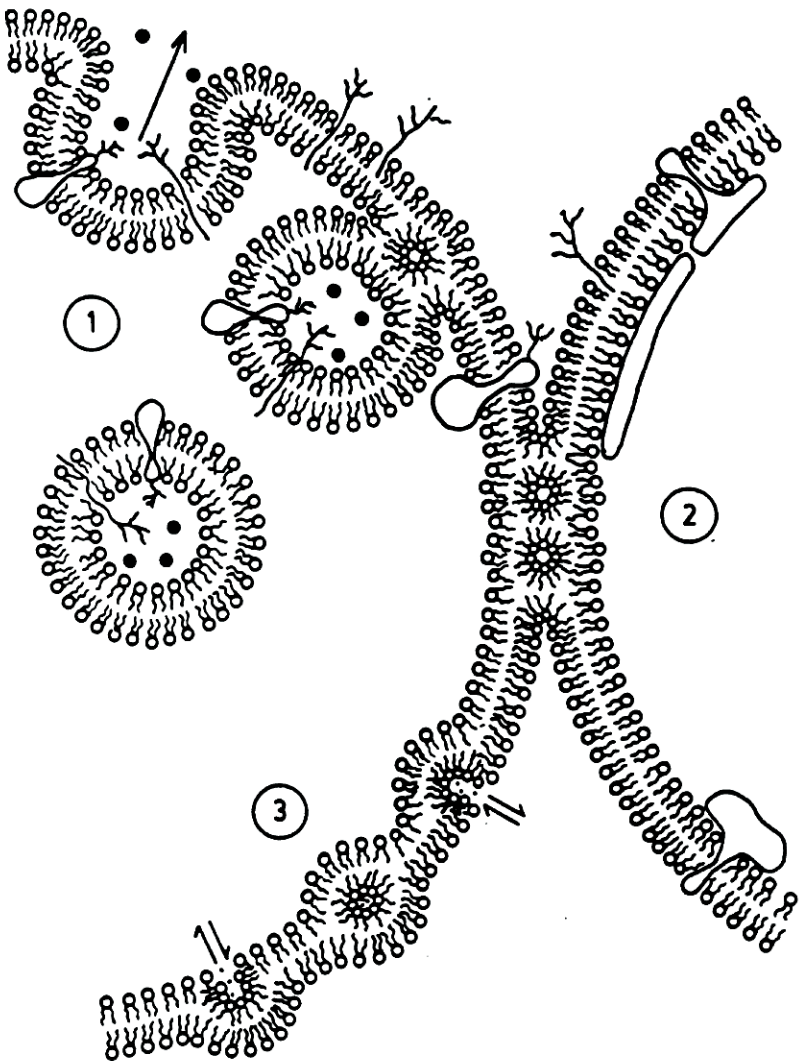


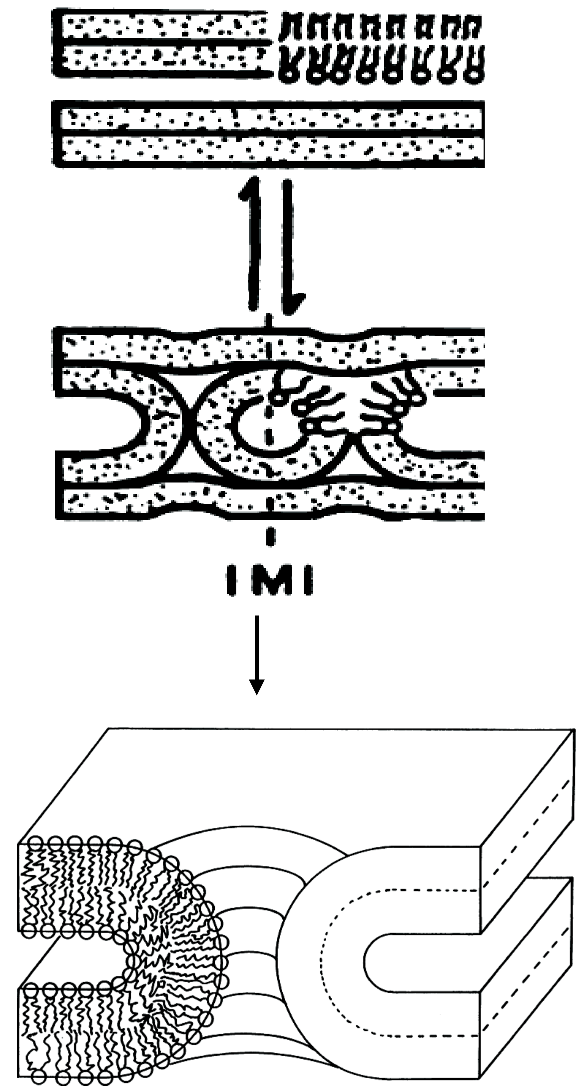
- Accommodate proteins



Lipid polymorphism in membranes

- Membrane fusion & vesicle formation





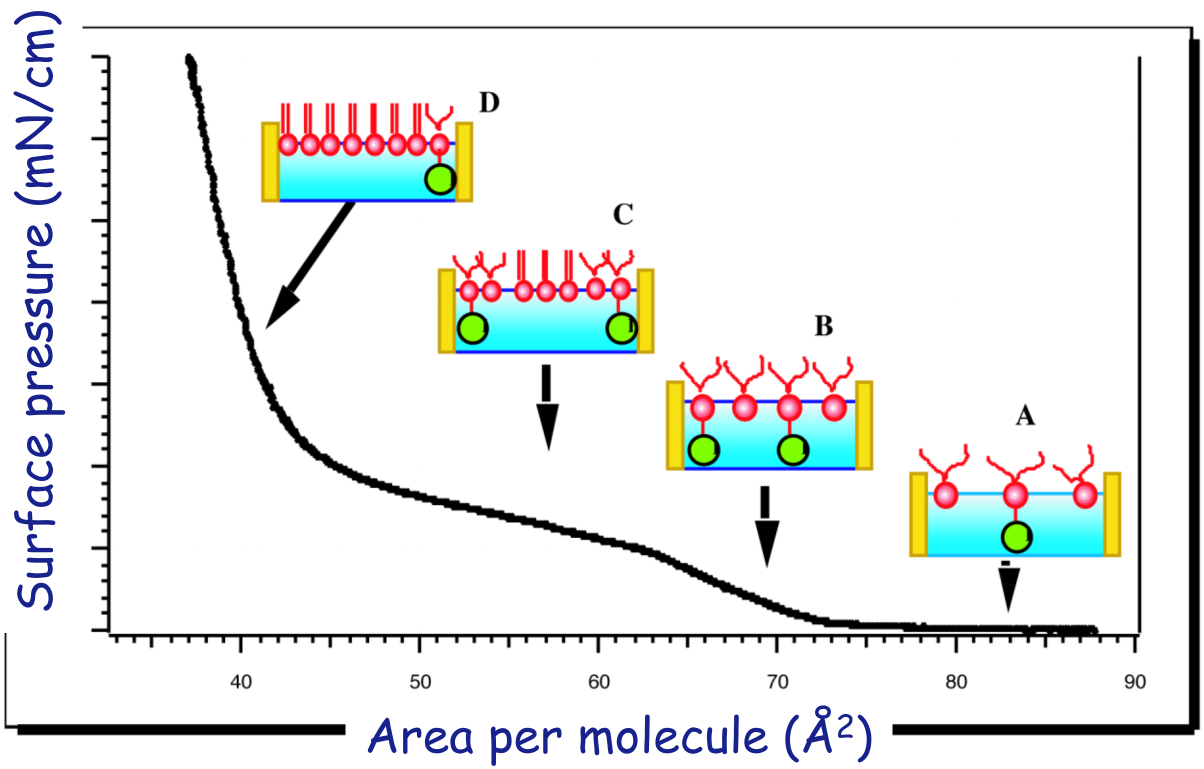
Biochem

Phase transitions of lipids

- Surface pressure Π

  A lipid monolayer at the air-water interface of a trough with variable surface

  Measure surface tension γ => $\Pi = \gamma_o - \gamma$.

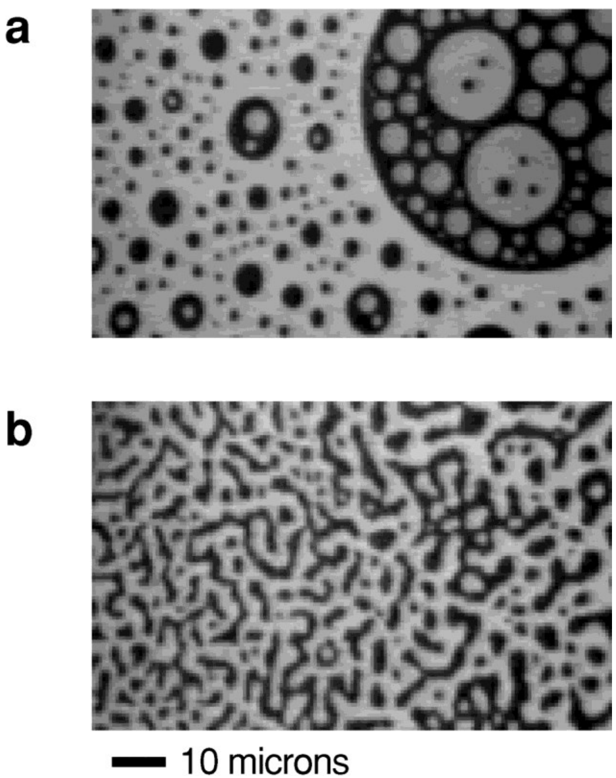


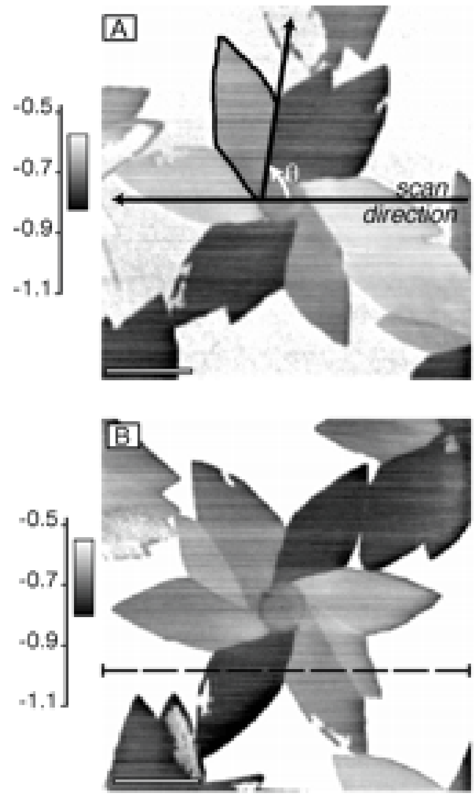
Phases:
A “gass”
B “liquid”
C co-existance
D “ solid”

Phase separations of lipids

- Co-existance of condensed and disordered phase

  micrometer-sized “solid” domains



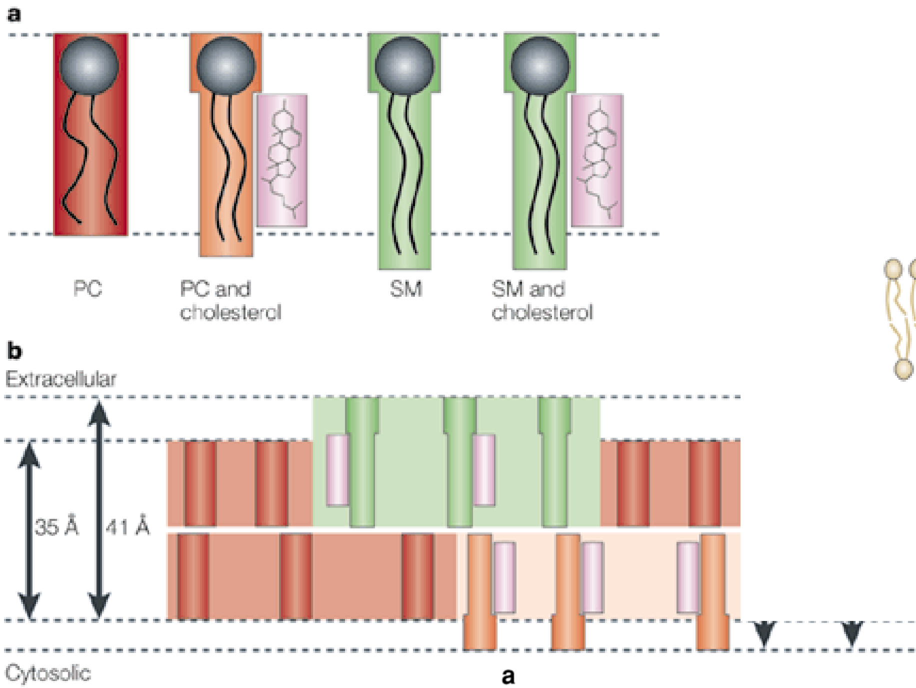


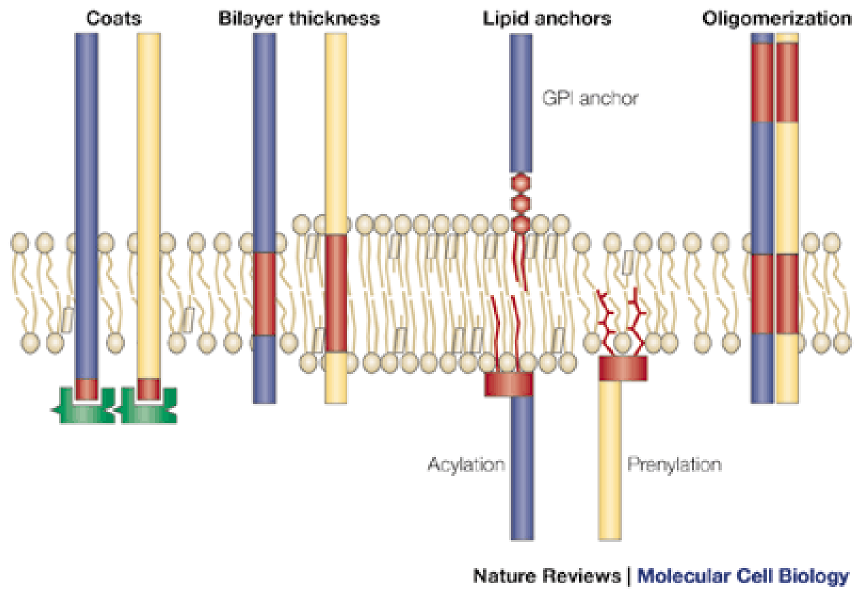
- Fluorescence microscopy

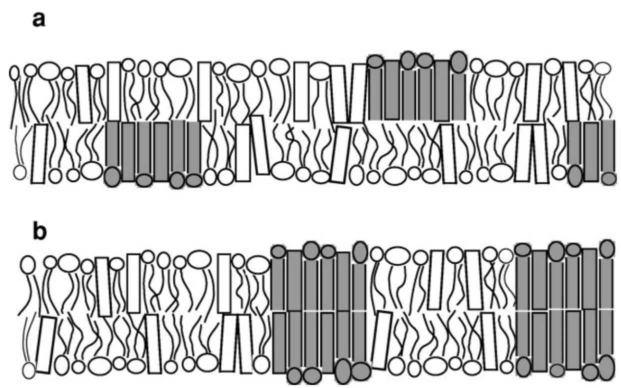
Atomic force microscopy

Phase separations within membranes

- Cholesterol: Preference for sphingomyelin and not so "fluid" lipids







- independent monolayers
- coupled monolayers

Phase separations within artificial membranes

- Co-existance of ordered and disordered phase in artificial vesicles made of:
  - cholesterol
  - sphingomyelin
  - di-oleoyl-posphatidyl-choline

Blue: ordered phase
Red: disordered phase

W. Webb 23.10.2003

Phase separations within artificial membrane

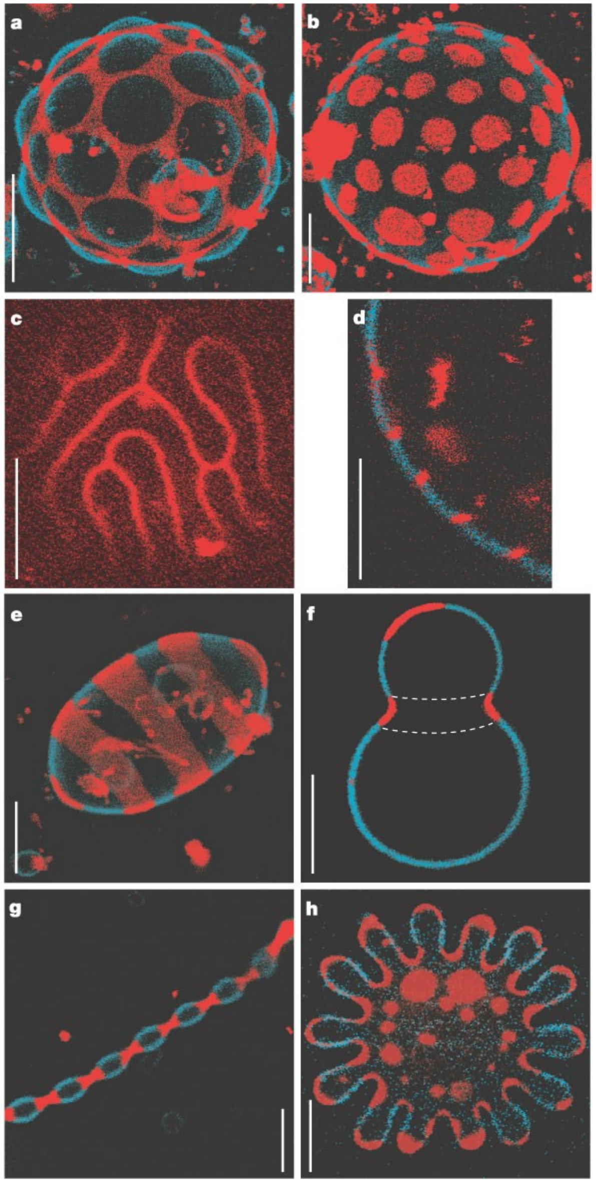
- Co-existance of ordered and disordered phase in artificial vesicles made of:
  - cholesterol
  - sphingomyelin
  - di-oleoyl-posphatidyl-choline

Variables:

Composition & Temperature

Blue: ordered phase

Red: disordered phase

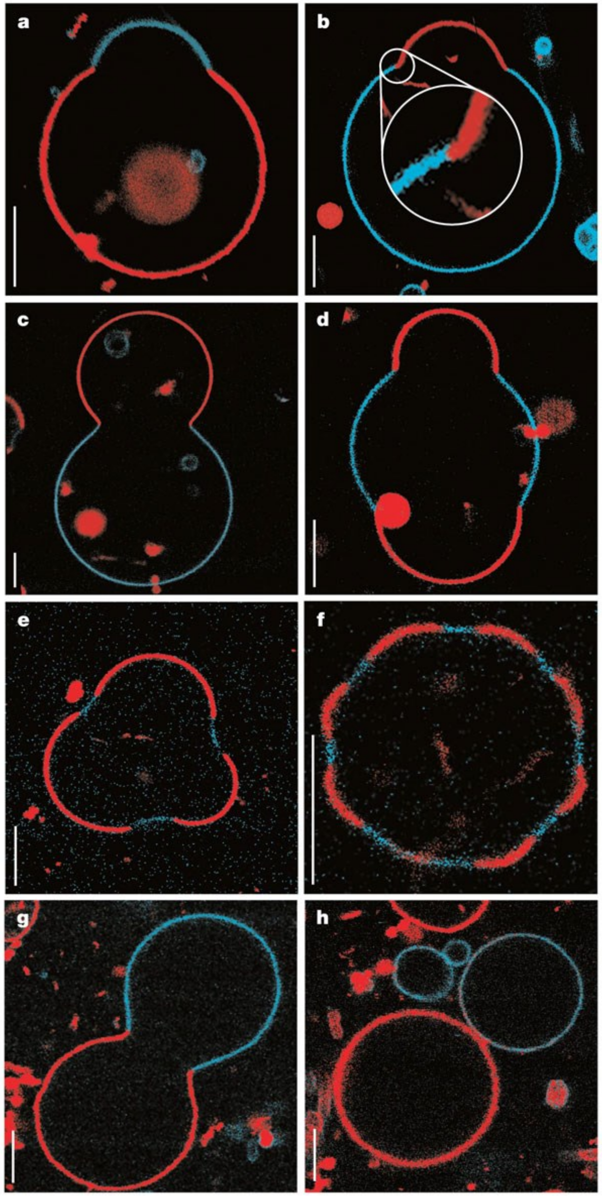


Phase separations within artificial membranes

- Co-existance of condensed and disordered phase
  micrometer-sized "solid" domains

Blue: ordered phase

Red: disordered phase



Phase separations within artificial membranes

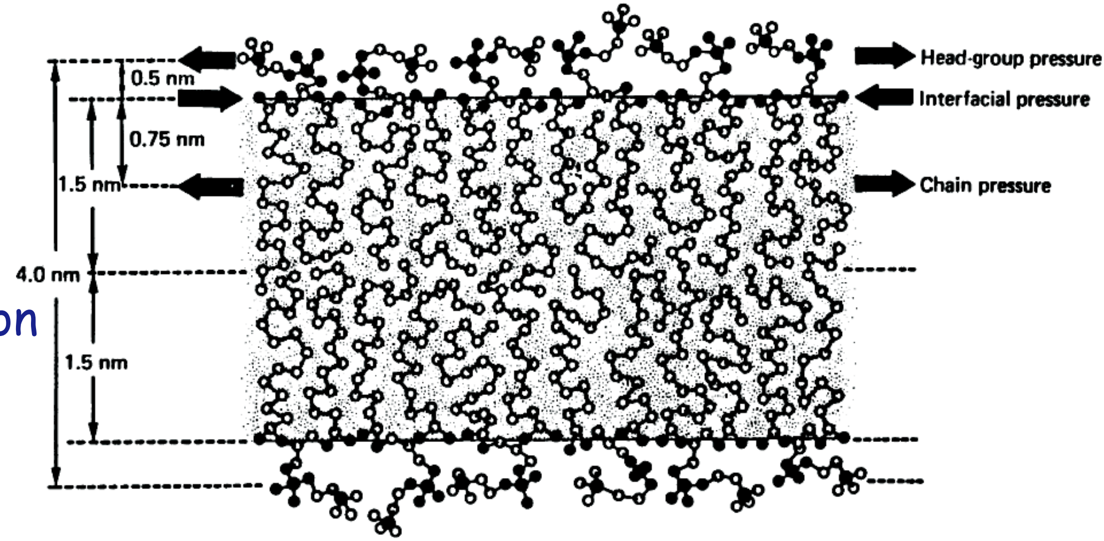
- Co-existance of condensed and disordered phase
  micrometer-sized “solid” domains

Black: ordered phase
White: disordered phase

Surface pressure of membranes

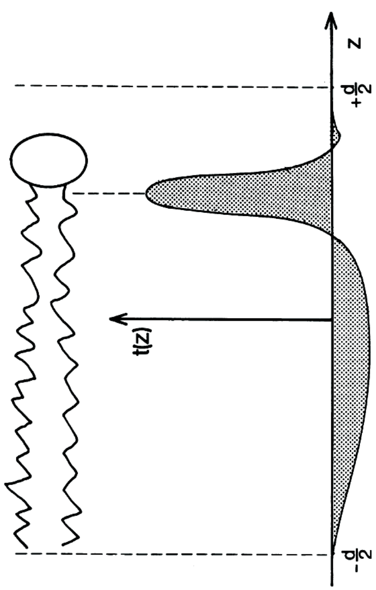
◊ "Pressures" within a membrane

- Attraction ÷ H-bonds
  ÷ hydrophobic effect

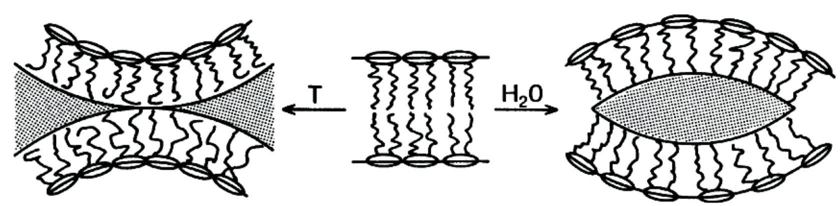


- Repulsion ÷ head group hydration
  ÷ thermal fluctuation

◊ Stress profile



◊ Effect of "stress"



Surface pressure of membranes

Relative hydrolytic activity of phospholipases on erythrocytes and monolayers

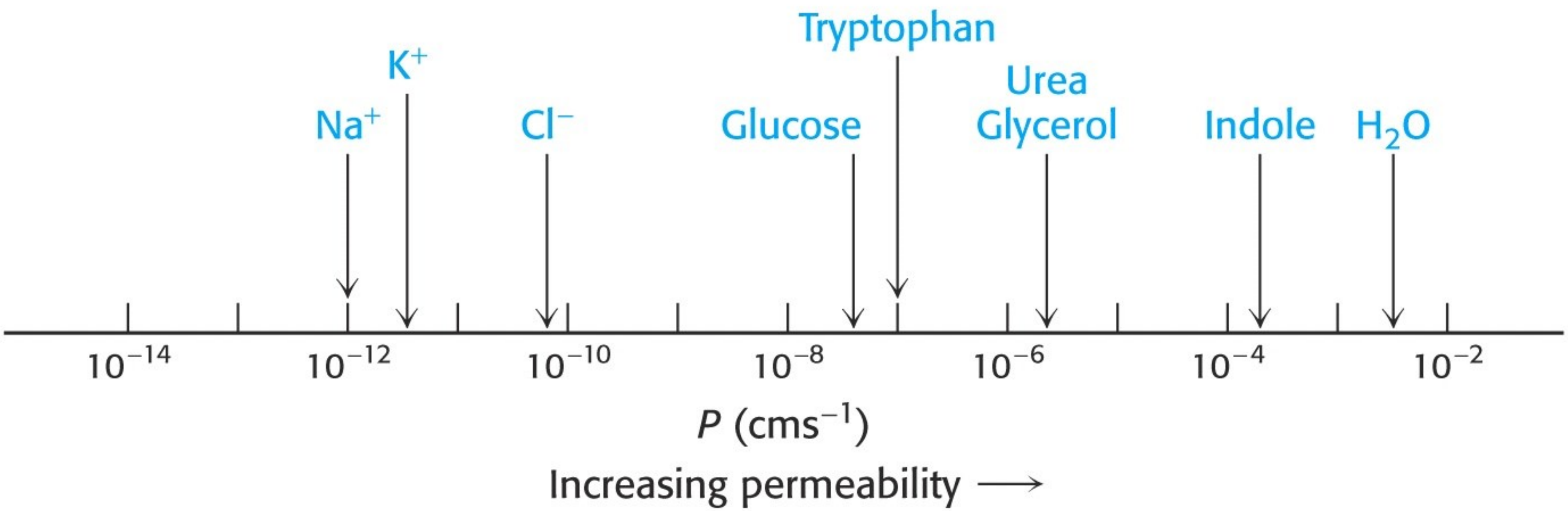
		Erythrocytes	Monolayer max Π (mN/m)
PL-A_2	Pig pancreas	-	16.5
PL-D	Cabbage	-	20.5
PL-A_2	C. Adamanteus	-	23
PL-C	B. Cereus	-	31
PL-A_2	n. Naja	+	35
PL-A_2	Bee venom	+	35.5
PL-C	Cl. Welchii	+	> 40

=> For erythrocyte membrane $31 < \Pi < 35$ mN/m

Permeability of Membranes

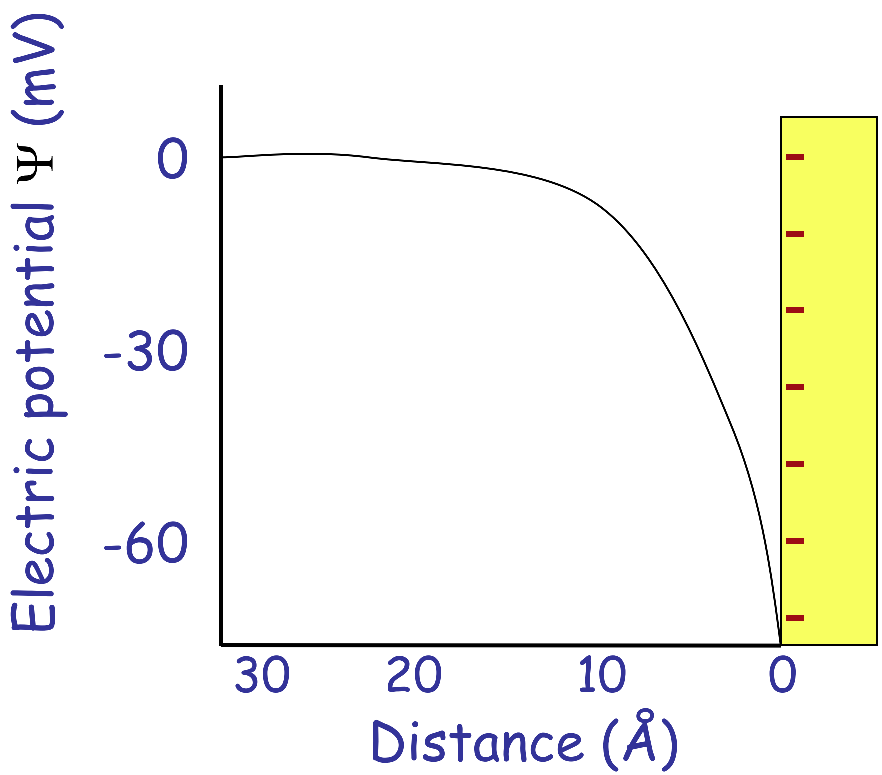
• The hydrophobic core of the membrane is an ideal barrier for hydrophilic (charged and polar) solutes.

=> Ion gradients can be created that are highly energetic.



Electric potential of membranes

- Membrane surfaces are in general negatively charged

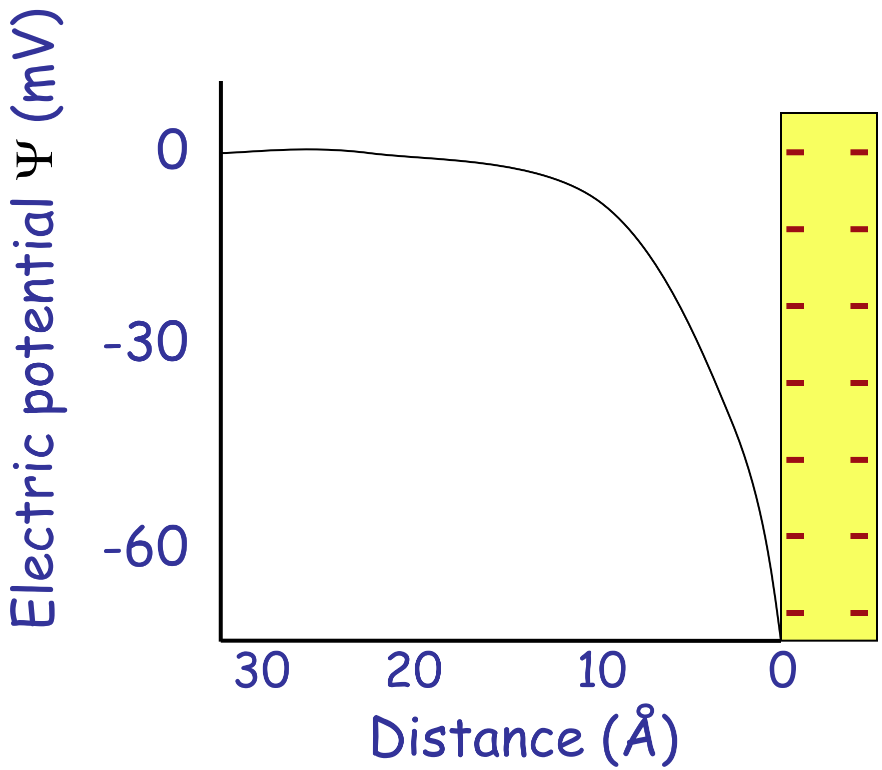


Gouy-Chapman

<= Molecular dimensions!

Electric potential of membranes

=> Concentration of cation & depletion of anion



Surface concentration C_o
with Ψ = -60 mV

-2	$0.01 \times C_\infty$
-1	0.1
+1	10
+2	100

Integrative questions

- Formation of a membrane: Entropy?
- Place of double bond on phase transition temperature.
- What would be the problem of crossing a phase transition?
- Why are proteins within membranes highly structured?
- Peptide insertion in a membrane.
- Phase transitions, phase separation, diffusion & lipid polymorphism.

Further reading

Text books:

Biochemistry – Stryer Chpt 12,26

The Cell – Alberts Chpt 10

Biomembranes – Gennis Chpt 1, 2 & 7

Review articles:

Understanding the diversity of membrane lipid diversity,Nature reviews Molecular Cell Biology;from Riezman (Uni Geneva)2018

• W. Dowhan "Molecular basis for membrane phospholipid diversity" Annu Rev Biochem 66 (1997) 199-232.

• A.R. Cossins & M. Sinensky "Adaption of membranes to temperature, pressure and exogenous lipids" in "Physiology of membrane fluidity" (1984) 1-20.

Membrane protein structures:see the PDB :Protein Data Bank

https://www.rcsb.org

Next time: Phospholipid biosynthesis

Review articles:

- W. Dowhan "Molecular basis for membrane phospholipid diversity" Annu Rev Biochem 66 (1997) 199-232.
- A.R. Cossins & M. Sinensky "Adaption of membranes to temperature, pressure and exogenous lipids" in "Physiology of membrane fluidity" (1984) 1-20.