MEMBRANE BIOPHYSICS

Ka Yee C. Lee
The University of Chicago

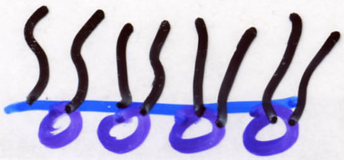
Boulder School
15–19 July, 2002

Thanks to M. Wortis, J. Israelachvili, J.A. Zasadzinski, H.M. McConnell, J. Majewski, K. Kjaer

Topics:

1. Langmuir Films
2. Chemistry of Phospholipids
3. Energetics + Thermodynamics of lipid self-assembly (in 3D)
4. Phase behavior in Langmuir Monolayers
5. Driving forces that dictate domain shape
6. Lipid-Protein Interactions : Monolayers as a model system
   - Lung Surfactant
   - Triblock copolymer as membrane sealant
   - Insertion of Alzheimer's Amyloid-Beta (Aβ) Peptides into lipid Membranes
7. Supported bilayers as model membranes
8. In search of lipid 'rafts'.

LANGMUIR FILMS



single molecular layer at the air water interface

hydrophilic : hydrophobic

History

- Pliny the Elder
- Seamen
- 1774 Benjamin Franklin
- 1890 Lord Rayleigh
- Agnes Pockel
- Langmuir
- Blodgett
- 1980's Möhwald + McConnell
  Direct Visualization
- Molecular Electronics
- Ideal 2D System
- Biological Model

MEMBRANE BIOPHYSICS

- Langmuir Film
  Chemistry of lipid molecules
  Driving force for self assembly

- Monolayer phase behavior
  Surface morphology
  Line tension vs. electrostatic
  governing domain shapes

- Lipid - Protein Interactions
  importance of the interface
  lung surfactant
  Alzheimer's AB aggregation
  Antimicrobial Peptides

- Bilayers as model biological systems
  Thermodynamics
  Supported bilayer
  Lipid Rafts

- Lipid - polymer interactions
  Membrane sealing

Membrane Structure + Phospholipid Chemistry

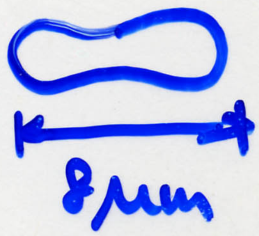
Statistical Thermodynamics of Surfaces, Interfaces + Membranes
Safran

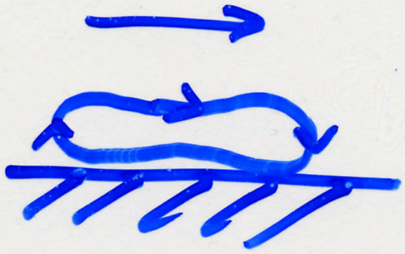
Biomembranes
Gennis

Statistical Mechanics of Membranes + Surfaces
Ed. Nelson + Piran (S. Liebler)

Adv. Phys. 46 (1997) 13-137
U. Seifert

Human Erythrocytes





"tank motion"

What causes the shape?
It's NOT a precast mold!

7

Nouvelle Rev. Fr. d'Hémat. 13, 71 (1973)

stomatocyte →

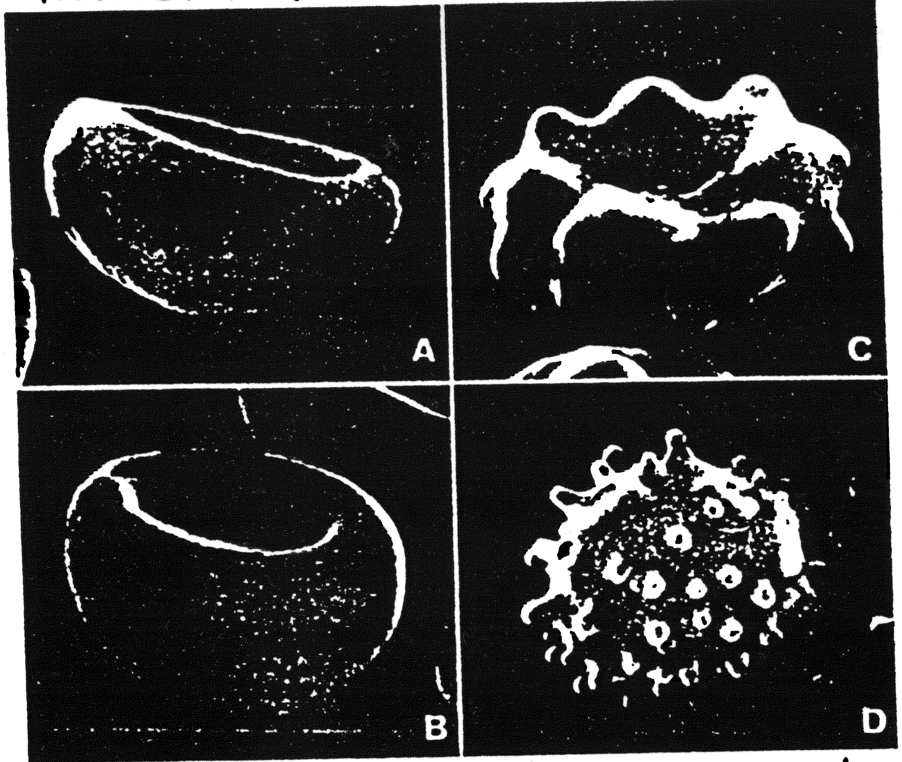


FIG. 1. — *Formes réversibles du globule rouge.* A) Stomatocyte II; B) Stomatocyte III correspondant aux cellules 3 et 5 de la figure 3; C) Echinocyte II; D) Echinocyte III correspondant aux cellules 2 et 4 (gross. : × 8 500).

echinocyte. ↑

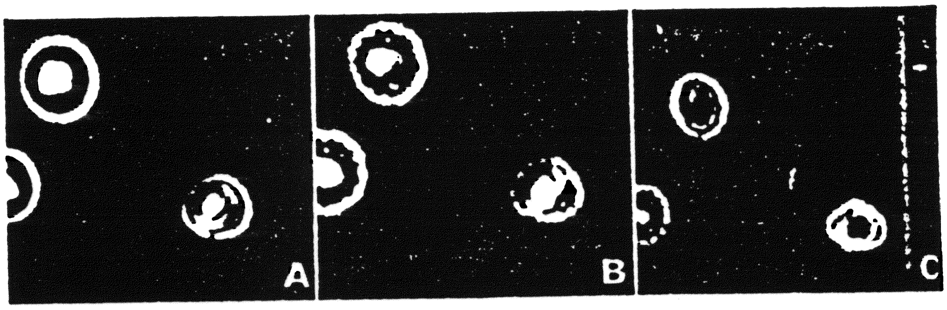
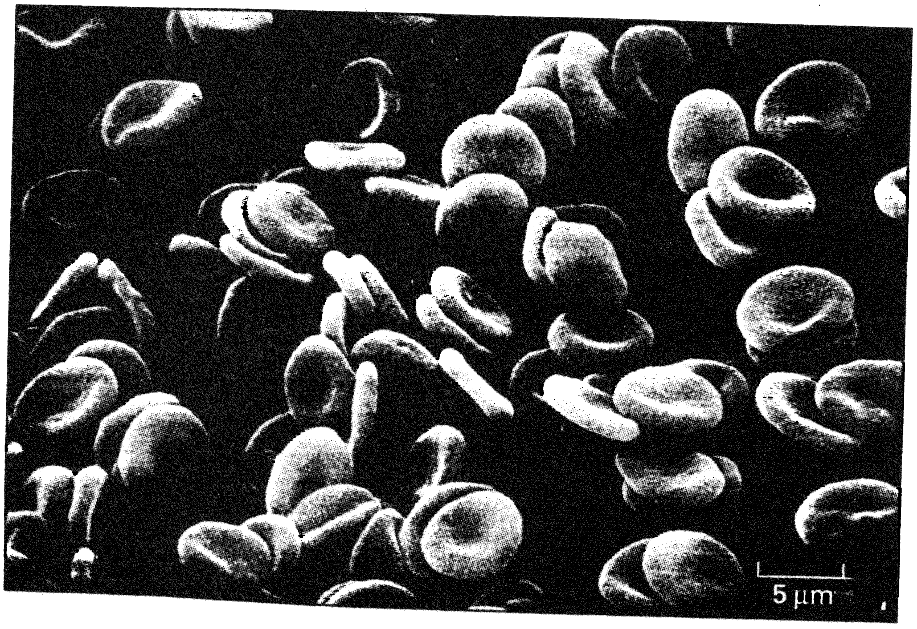
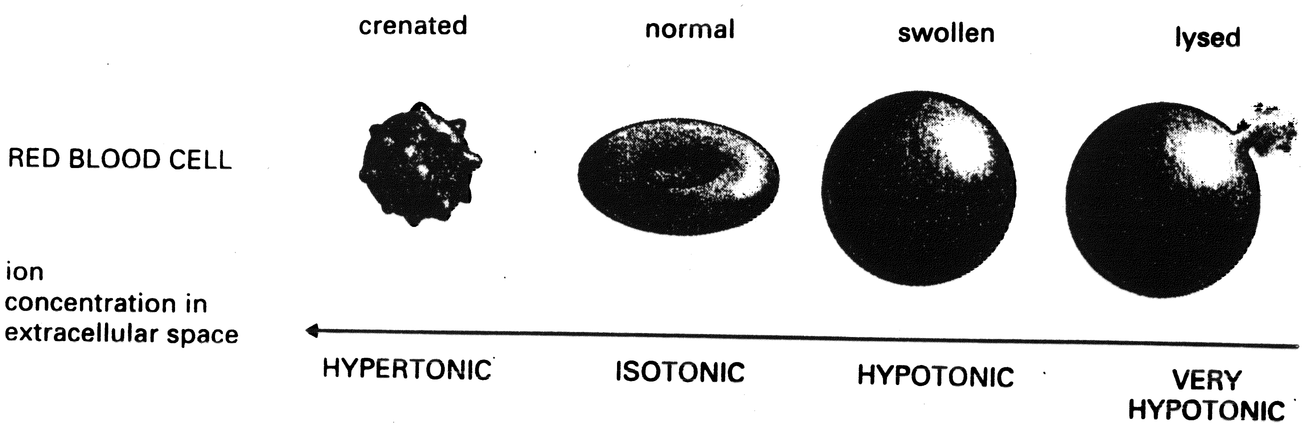


FIG. 2. — *Transformation directe d'un stomatocyte* (A) *en un stomatocyte crénelé* (B-C), observée au microscope à contraste de phases, par effet d'une micro-injection de salicylate de Na. Cinq secondes séparent la première image de la dernière.


5 µm


crenated
normal
swollen
lysed
RED BLOOD CELL
ion
concentration in
extracellular space
HYPERTONIC
ISOTONIC
HYPOTONIC
VERY
HYPOTONIC

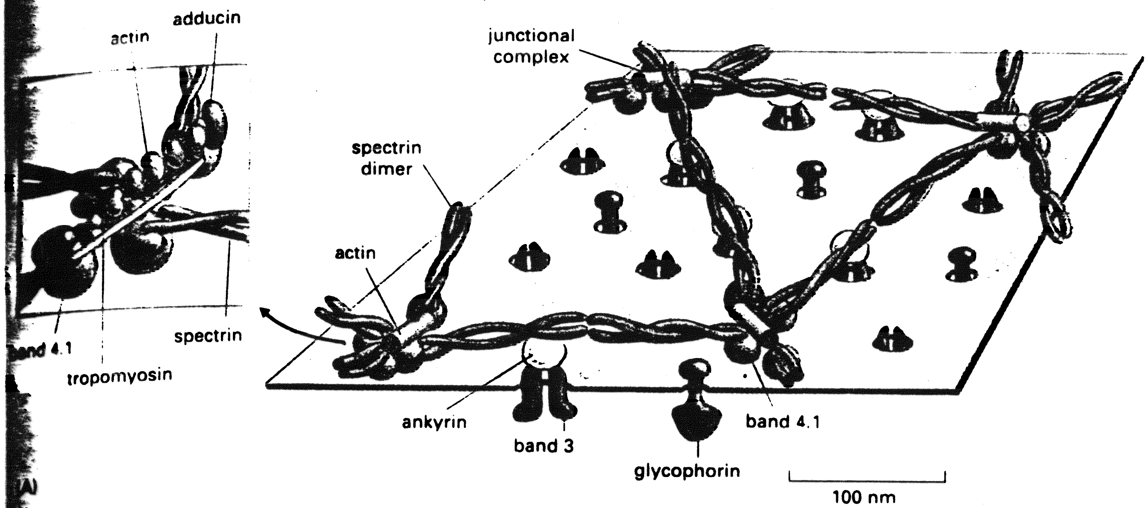
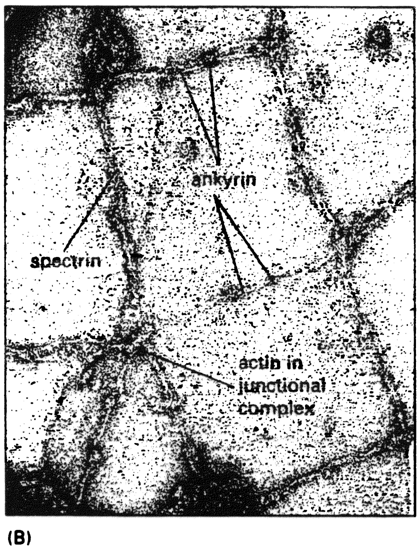
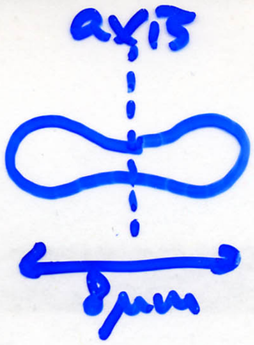


Figure 10–26 The spectrin-based cytoskeleton on the cytoplasmic side of the human red blood cell membrane. The structure is shown schematically in (A) and in an electron micrograph in (B). The arrangement shown in (A) has been deduced mainly from studies on the interactions of purified proteins *in vitro*. Spectrin dimers associate head-to-head to form tetramers that are linked together into a netlike meshwork by junctional complexes composed of short actin filaments (containing 13 actin monomers), tropomyosin, which probably determines the length of the actin filaments, band 4.1, and adducin (enlarged in the box on the *left*). The cytoskeleton is linked to the membrane by the indirect binding of spectrin tetramers to some band 3 proteins via ankyrin molecules, as well as by the binding of band 4.1 proteins to both band 3 and glycophorin (not shown). The electron micrograph in (B) shows the cytoskeleton on the cytoplasmic side of a red blood cell membrane after fixation and negative staining. The spectrin meshwork has been purposely stretched out to allow the details of its structure to be seen; in the normal cell the meshwork shown would occupy only about one-tenth of this area. (B, courtesy of T. Byers and D. Branton, *Proc. Natl. Acad. Sci. USA* 82:6153–6157, 1985.)



Questions



① Why not a sphere?

Squeezes thru' narrow capillaries (2-3 μm)

② How deformable?

100x softer than the best latex rubber at equivalent thickness

③ How tough?

10^5 passages in 120-day lifetime

④ What is the remarkable material?

(see next transparency)

⑤ Why the shape?

Not fully understood

diff. shapes under diff. conditions

⑥ Is RBC always discoid?

No!

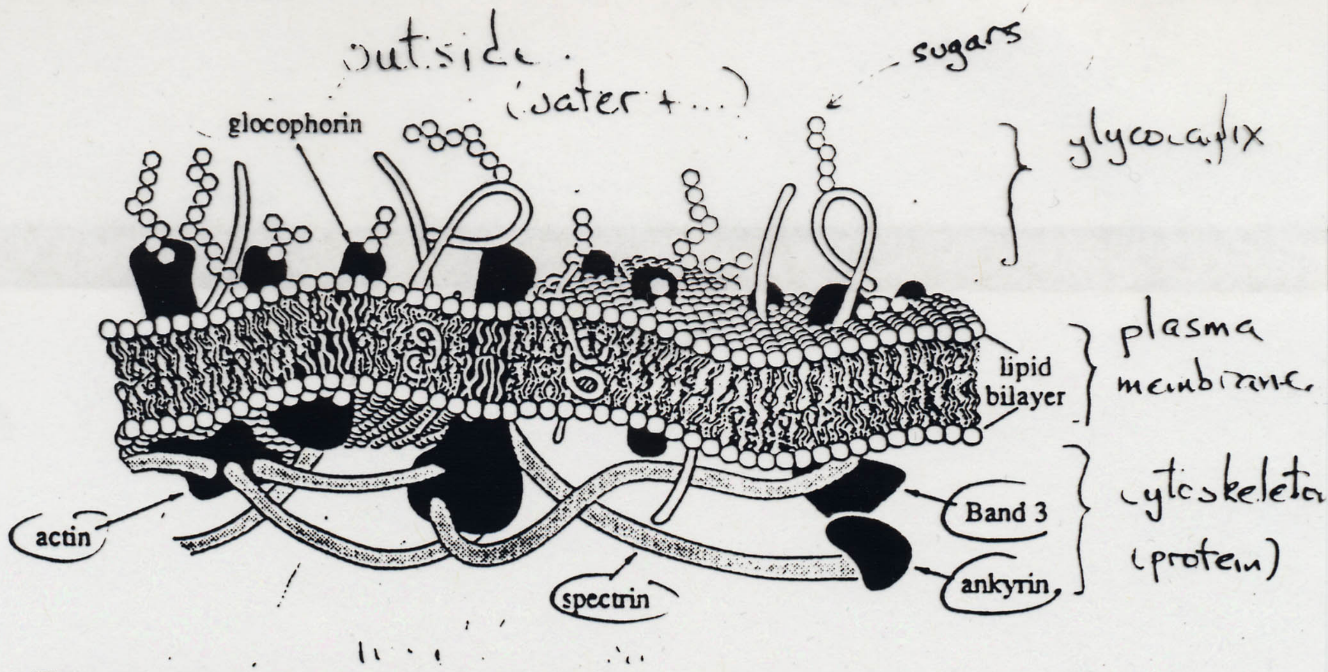


Figure 1.2: Anatomy of a red-cell membrane (Ref.[5]). The lipid bilayer is its main structural component. Band 3 and glycophorin are two types of intramembrane proteins. The cytoskeleton on the cytoplasmic (inner) side of the cell is formed primarily of linear spectrin molecules cross-linked via actin molecules. It is anchored to the lipid bilayer by its association with glycophorin via band 4.1 (protein), and with band 3 via ankyrin (protein).

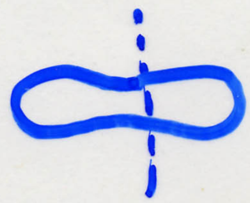
- Composite material (fluid membrane + protein network)

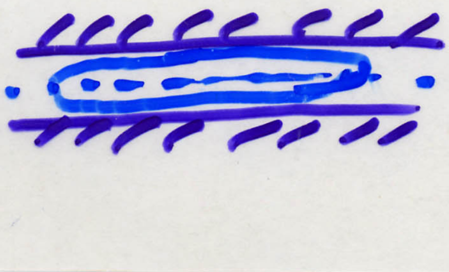
  phospholipid bilayer: soft fluid sealer

  protein network: cross-linked toughness

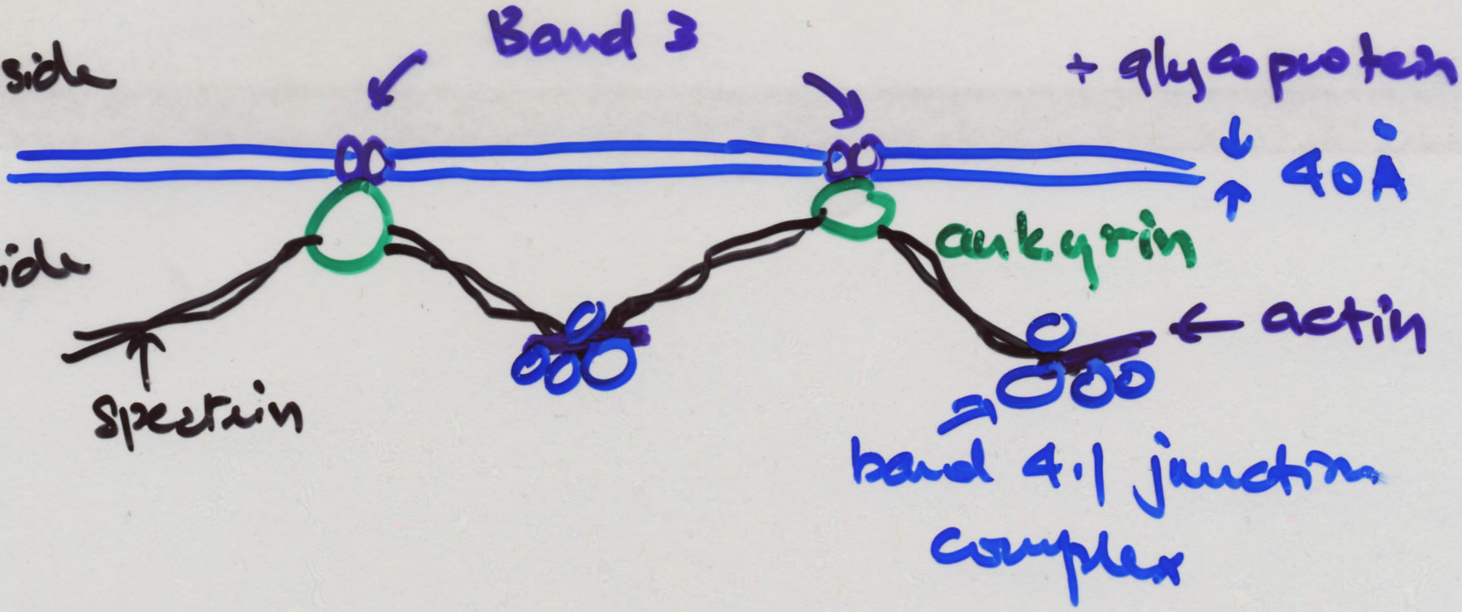
- shape / function

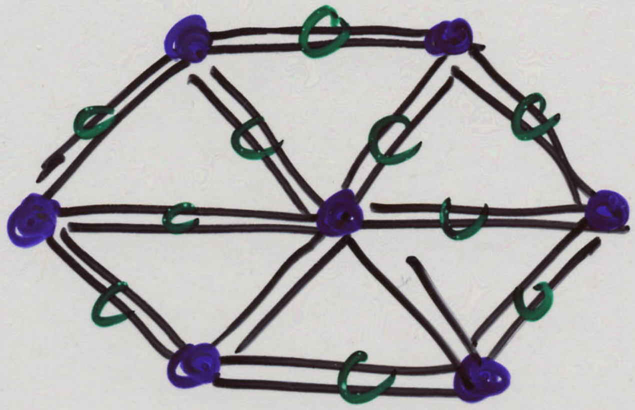
  disk → sausage to fit thru' 2-3 μm capillaries

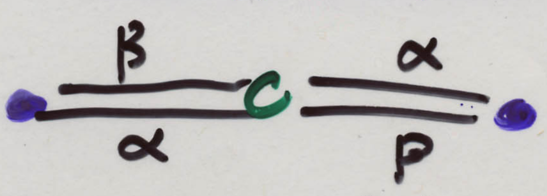




The Cytoskeleton (RBC only)







α = 240 kD
β = 220 kD

- seq of 106 aa domains
  flexible linkage
- 2.5×10^5 copies/cell

· Lipid mixture

e.g. RBC

PC	PE	PS	Sphingolipid	Cholesterol
25%	22%	10%	18%	25%

- Additional glycolipids, lipoprotein, etc.

- Cytoskeleton (protein)

  e.g. Spectrin

  Band 3

  glycophorin (often linked to cell interior but not in RBC)

$\frac{\text{Lipid}}{\text{Protein}}$ (wt) ≈ 0.75

- Functional proteins in membrane (substrate)

  e.g. pump

  adhesion molecules

  clathrins

  ⋮

Manufacture & Distribution of Lipids & Proteins

- ER
  Ribosome (rough ER) } lipid, protein
- Golgi apparatus — "processing", distribution

  Golgi vesicles — sorting, signalling, transporting

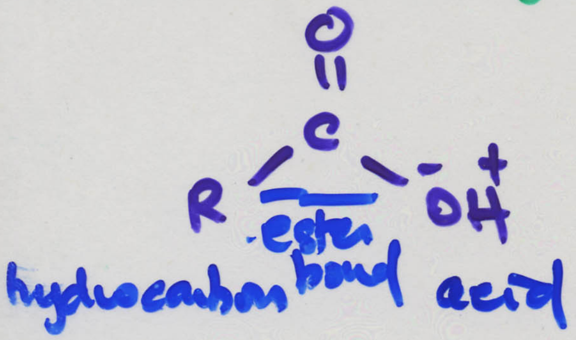
Vesicle buds off
transported by molecular motor
brought to needed location

Chemistry of Lipid Molecules

What is a phospholipid?

Chemist: phospholipid = ester bonded fatty acid

What is a fatty acid?



- R chain usu. saturated or nearly saturated
- n = total # carbon atoms

n=1	H·COOH	formic acid	(ants)
n=2	CH_3COOH	acetic	(vinegar)
⋮			
n=12	$CH_3(CH_2)_{10}COOH$	lauric	(laurel)
n=14	$CH_3(CH_2)_{12}COOH$	myristic	(nutmeg)
n=16	$CH_3(CH_2)_{14}COOH$	palmitic	(palm oil)
n=18	$CH_3(CH_2)_{16}COOH$	stearic	(animal fat)
n=20	$CH_3(CH_2)_{18}COOH$	arachidic	(peanut ?)

odd n chains exist (but not in natural lipids)

Mono-unsaturated

position of double bond counts

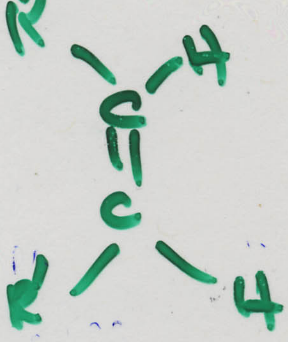
n=18 $CH_3(CH_2)_{10}CH=CH(CH_2)_4COOH$ petroselenic (6-cis)

$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$ oleic (9-cis)

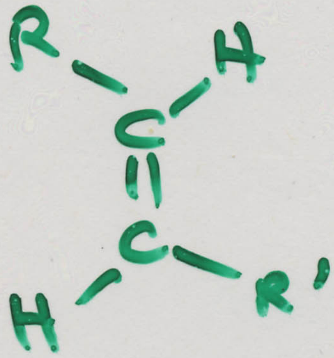
$CH_3(CH_2)_5CH=CH(CH_2)_9COOH$ vaccenic (11-cis or trans)

Numbering counts carbon from acid end

Note: the double bond does not rotate



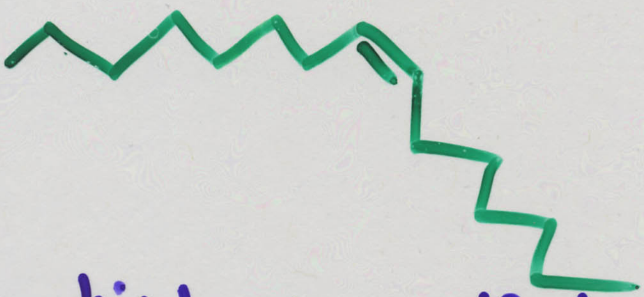
cis ("kinked")



trans ("straight")

Single chains are generally almost all-trans

A single cis bond puts a "kink" in the direction of the chain

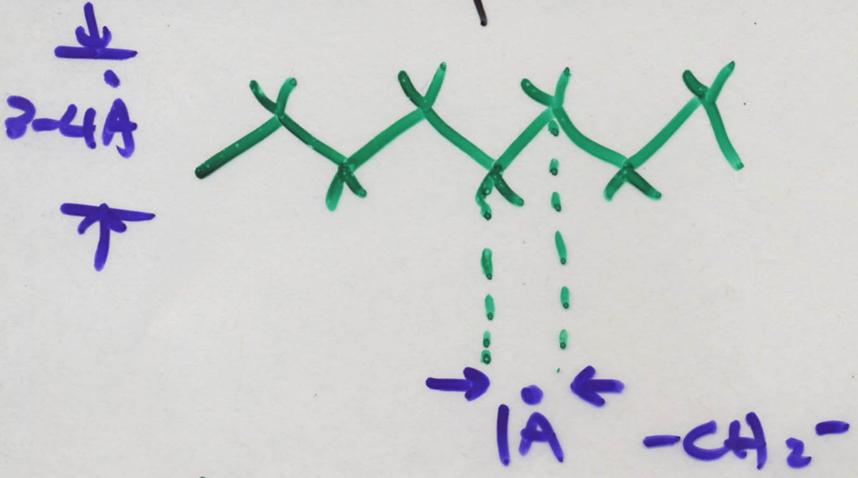


These kinks mean that chains "pack" less well.

⇒ e.g. decreased viscosity

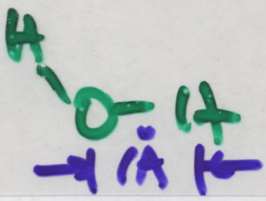
Nature uses this mechanism to adjust membrane fluidity

Dimensions of basic saturated all-trans chain



Covalent radii

-H	0·3Å
-C	0·77Å
=C	0·67Å
-O	0·66Å



Each phospholipid molecule involves 2 fatty acids & a "head" group bonded via a glycerol

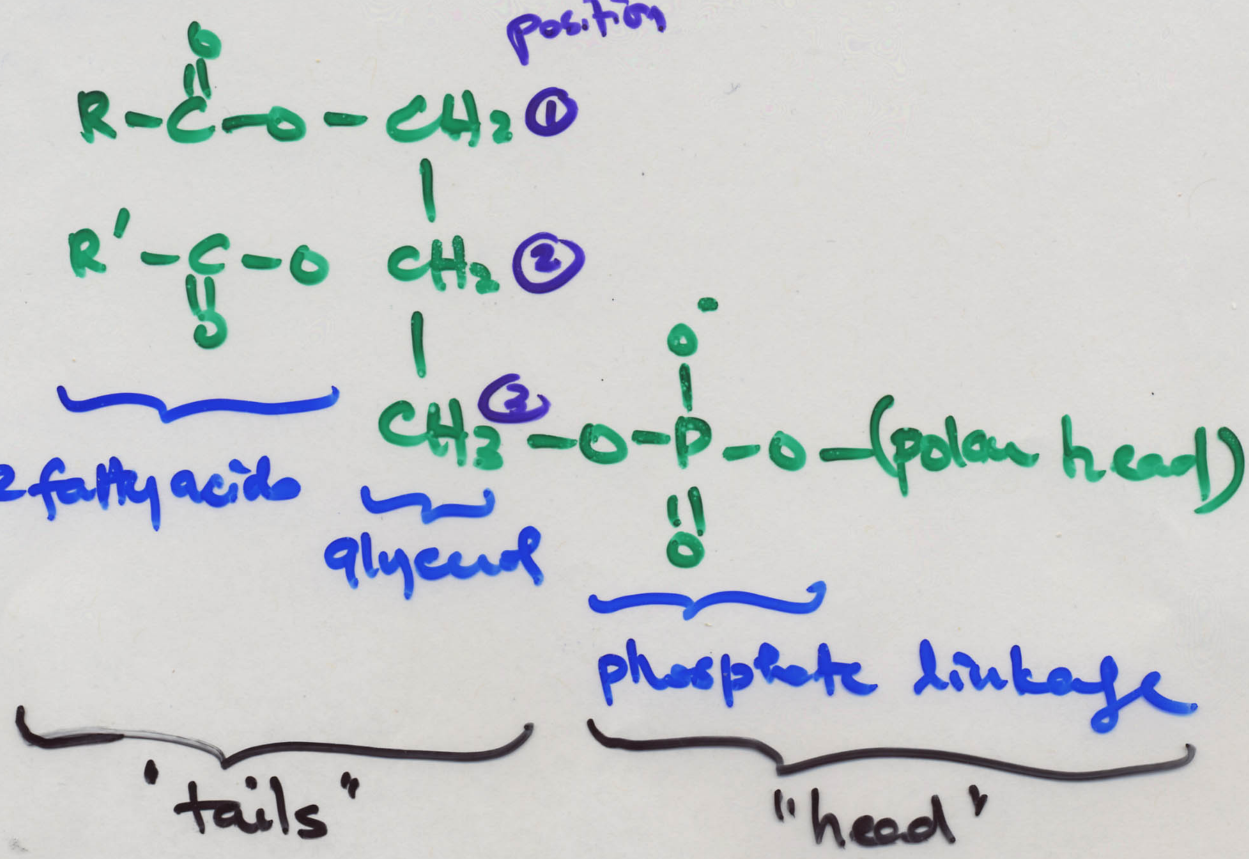
CH_2OH
|
CH_2OH
|
CH_2OH
(glycerol)

Relation to soap

CH_2OCOR
|
CH_2OCOR + 3NaOH → glycerol + 3 R–C(=O)–ONa
|
CH_2OCOR

triglyceride (fat) + (lye) = soap

Phospholipid molecule



Common Headgroups

Choline $-CH_2-CH_2-\overset{+}{N}(CH_3)_3$

Ethanolamine $-CH_2-CH_2-\overset{+}{N}H_3$

Serine $-CH_2-CH\begin{matrix} COO^- \\ \overset{+}{N}H_3 \end{matrix}$

eg. PC = phosphatidyl choline

egg yolk

lecithins

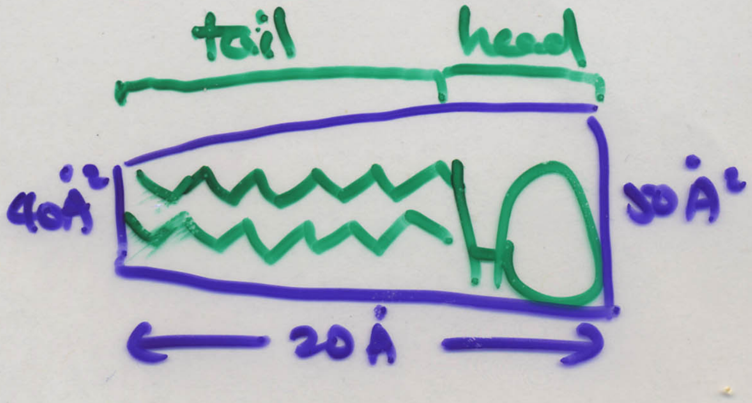
SOPC 1-Stearoyl-2-oleoyl-sn-Glycero-3-Phosphatidyl choline
(18) (18-cis)

DMPC 1,2-Dimyristoyl-sn-Glycero-3-Phosphatidyl choline
(14)

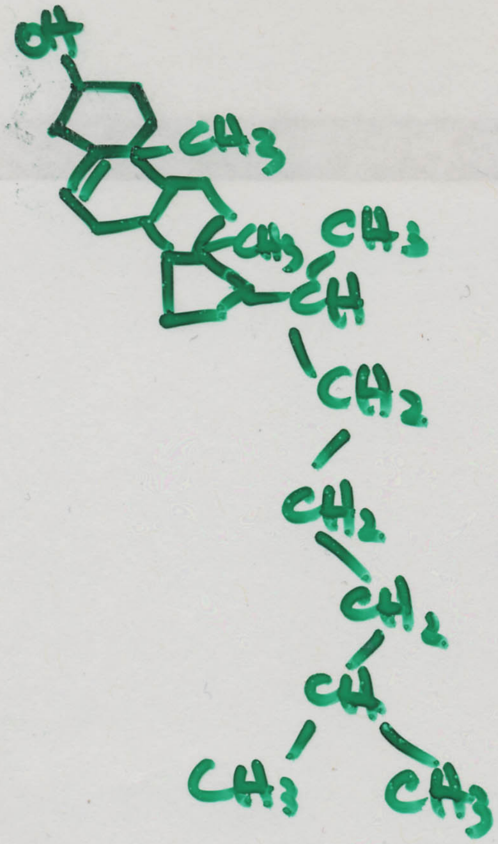
PC zwitterionic

PE "

PS anionic

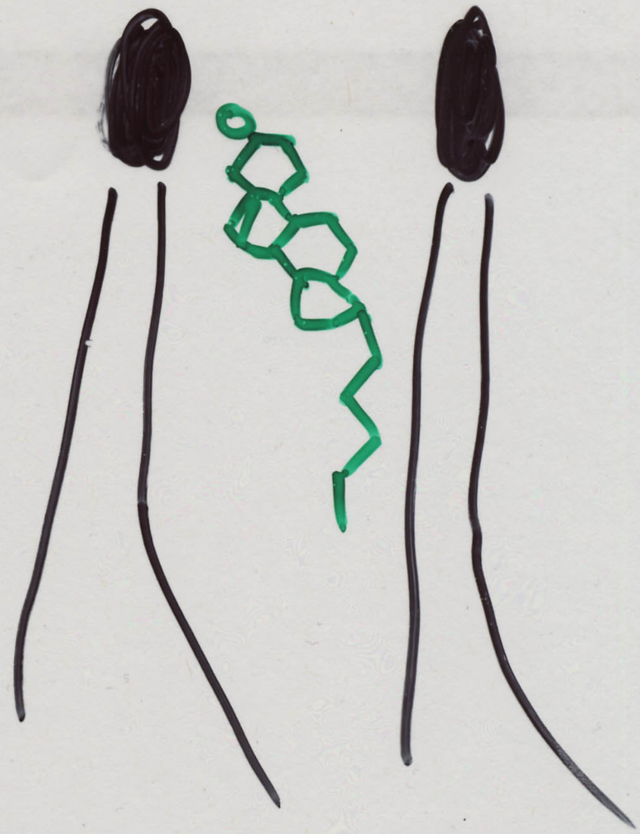


Cholesterol 25% lipid fraction in human RBC



polar rigid steroid rings

(mono) acyl tail



- orients in monolayer like ordinary (diacyl) lipid
- regulates membrane fluidity
  by "stiffening" near-head region – ↓ stat. fluc.
- decrease permeability to small water-soluble molecules
- increases flexibility + stability
  cells w/o it lyse
- decrease membrane ordering (sol-gel)
  × freeze easily
  maintain fluidity
- ability to "flip-flop"
  ↑ shape mechanics

3?

DMPC MOLECULE

Gennis, p. 58

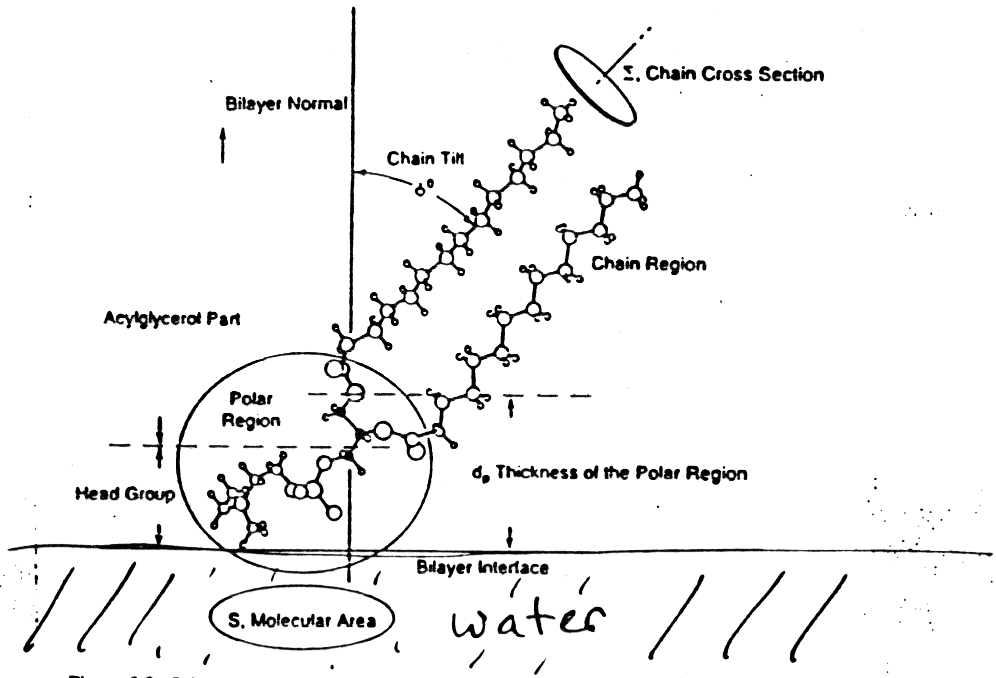


Figure 2.2. Schematic of a phosphatidylcholine molecule showing the structural notation defining various regions. Note that the cross-sectional area of the hydrocarbon chain, Σ, is taken perpendicular to the chain axis. The cross-sectional area parallel to the plane of the membrane will be, in this case, significantly larger due to the tilt of the chains. Adapted from ref. 604.

chain tilt: (DMPC)

$\phi = 0$ in fluid (LC, L_α) phase (SmA)

$\phi = 12^\circ$ in gel phase SmC