Lecture 28 (12/1/21)

TODAY
- Reading: Ch11; 370-381
- Problems: Ch11; 5,6,8,9,12,13,15

NEXT
- Reading: Ch7; 220-235
- Problems: Ch7; 1,2,3,5,9

Lipids & Membranes

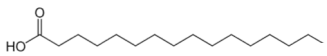
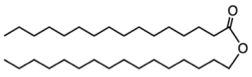
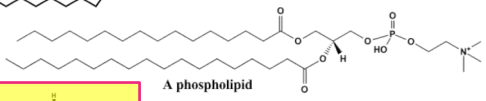
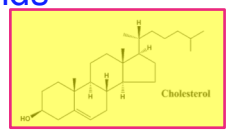
A. Lipids
1. Roles
2. Classes
   a. Fatty Acids
   b. Fats
   c. Waxes
   d. Membrane lipids
   e. Terpenes

B. Membranes
1. Introduction
2. The 4 S's
   a. Size
   b. Solubility
   c. Shape
   d. Stability
3. Models for Membrane structure
   a. Old Model
   b. Data
   c. Fluid Mosaic Model
   d. Testing the model
4. The Red-Blood Cell Membrane
5. Membrane Asymmetry
   a. transverse
   b. lateral
   c. anchoring
6. Membrane Fluidity

Lipids: Classes

Biological molecules that are characterized by low solubility in water, that is, are relatively hydrophobic.

Classes of Lipids They have a high hydrocarbon content

1. Fatty acids 
2. Fats (triglycerides) 
   Structure of a fat
3. Waxes 
4. Membrane Lipids 
   A phospholipid
5. Isoprenes 
   Cholesterol

Lipids: Fatty Acids

Common Biological Fatty Acids

Number of Carbons	Common Name	Systematic Name	Symbol	Structure
Saturated fatty acids				
12	Lauric acid	Dodecanoic acid	12:0	$CH_3(CH_2)_{10}COOH$
14	Myristic acid	Tetradecanoic acid	14:0	$CH_3(CH_2)_{12}COOH$
16	Palmitic acid	Hexadecanoic acid	16:0	$CH_3(CH_2)_{14}COOH$
18	Stearic acid	Octadecanoic acid	18:0	$CH_3(CH_2)_{16}COOH$
20	Arachidic acid	Eicosanoic acid	20:0	$CH_3(CH_2)_{18}COOH$
22	Behenic acid	Docosanoic acid	22:0	$CH_3(CH_2)_{20}COOH$
24	Lignoceric acid	Tetracosanoic acid	24:0	$CH_3(CH_2)_{22}COOH$
Unsaturated fatty acids (all double bonds are cis)				
16	Palmitoleic acid	9-Hexadecenoic acid	16:1(Δ^9)	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$
18	Oleic acid	9-Octadecenoic acid	18:1(Δ^9)	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$
18	Linoleic acid	9,12-Octadecadienoic acid	18:2($\Delta^{9,12}$)	$CH_3(CH_2)_4(CH{=}CHCH_2)_2(CH_2)_6COOH$
18	α-Linolenic acid	9,12,15-Octadecatrienoic acid	18:3($\Delta^{9,12,15}$)	$CH_3CH_2(CH{=}CHCH_2)_3(CH_2)_6COOH$
18	γ-Linolenic acid	6,9,12-Octadecatrienoic acid	18:3($\Delta^{6,9,12}$)	$CH_3(CH_2)_4(CH{=}CHCH_2)_3(CH_2)_3COOH$
20	Arachidonic acid	5,8,11,14-Eicosatetraenoic acid	20:4($\Delta^{5,8,11,14}$)	$CH_3(CH_2)_4(CH{=}CHCH_2)_4(CH_2)_2COOH$
24	Nervonic acid	15-Tetracosenoic acid	24:1(Δ^{15})	$CH_3(CH_2)_7CH{=}CH(CH_2)_{13}COOH$

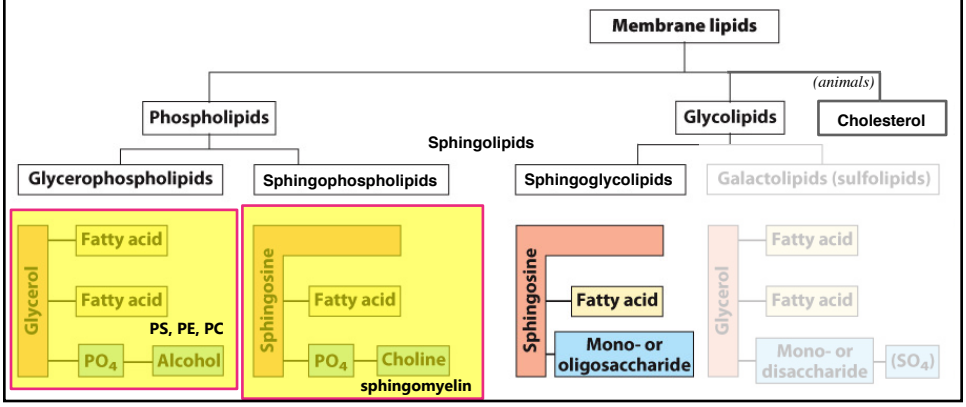
Need to Know: Common names, structure, symbol (e.g., $18{:}3^{\Delta 9,12,15}$)

Lipids: Membrane Lipids

Classification of Membrane Lipids

Two major categories based on the structure and function:

1. Lipids that contain phosphate
2. Lipids that do not contain phosphate
   - each can be further separated into:
     - Glycerol-based and sphingosine-based



Lipids: Membranes

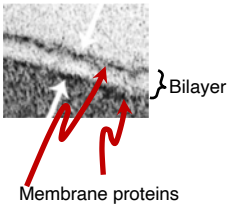
- Introduction
- The 4 S's
  - Size
  - Solubility
  - Shape
  - Stability
- Models for Membrane structure
  - Old Model
  - Data
  - Fluid Mosaic Model
  - Testing the model
- The Red-Blood Cell Membrane
- Membrane Asymmetry
  - Lipids
    - transverse
    - lateral
  - Protein
    - anchoring
    - glycoproteins
- Membrane Fluidity

Lipids: Membranes

Models for Membrane Structure

OLD MODEL (ca. 1940-1970)
Sandwich model proposed by Danielli-Davson.

Based on the structures in the EM



Scientifically, this is a good MODEL because it is clearly TESTABLE!
This model makes several testable predictions:

1) Protein-lipid interactions should be mostly electrostatic; proteins should have lots of charged groups.
2) Should be able to "wash" nearly all membrane proteins off the membranes with high salt.
3) Isolated membrane proteins should show lots of β-structure
4) Importantly, NO PROTEINS ON THE INSIDE

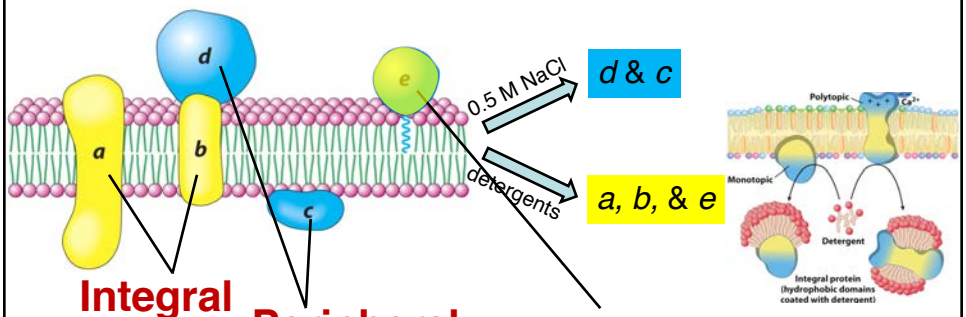
Lipids: Membranes

Models for Membrane Structure

TESTING OLD MODEL: DATA

1) & 2) Wash isolated membranes with high-salt solutions or changes in pH.

- Removes some but not all proteins
- This leads to an operational definition of peripheral (those that wash off with 0.5 M salt), an integral (those that remain after washing) membrane proteins



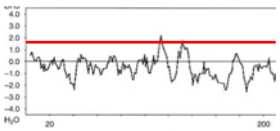
Lipids: Membranes

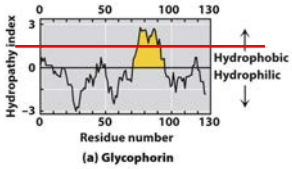
Models for Membrane Structure

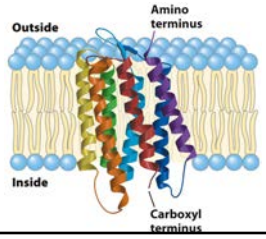
TESTING OLD MODEL: DATA

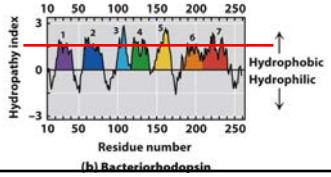
3) Isolated membrane proteins should show lots of β-structure.

- Peripheral membrane proteins looked like cytosolic proteins
- CD showed there was actually more α-helix than β-structure
- Integral membrane proteins had patches of hydrophobic residues in their sequence









Lipids: Membranes

Models for Membrane Structure

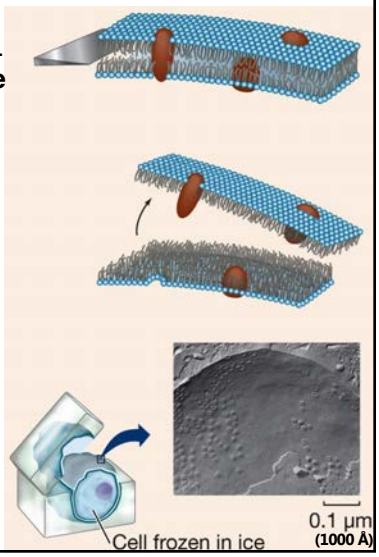
TESTING OLD MODEL: DATA

4) Importantly, NO PROTEINS ON THE INSIDE.

- So, lets look: performed **Freeze-fracture EM** on cell membranes
- This immediately became an explanation for Integral membrane proteins.

OMG!!!
NOT smooth inside!

Oops, maybe proteins DO span the membrane.



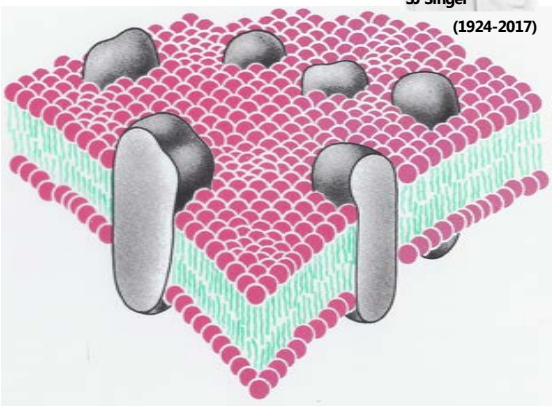
Lipids: Membranes

Models for Membrane Structure

NEW MODEL (1972)

Fluid Mosaic Model proposed by SJ Singer & GL Nicholson

- Lipids form a viscous, two-dimensional solvent into which proteins are inserted and integrated more or less deeply.
- Proteins can either be embedded in or associated with the membrane:

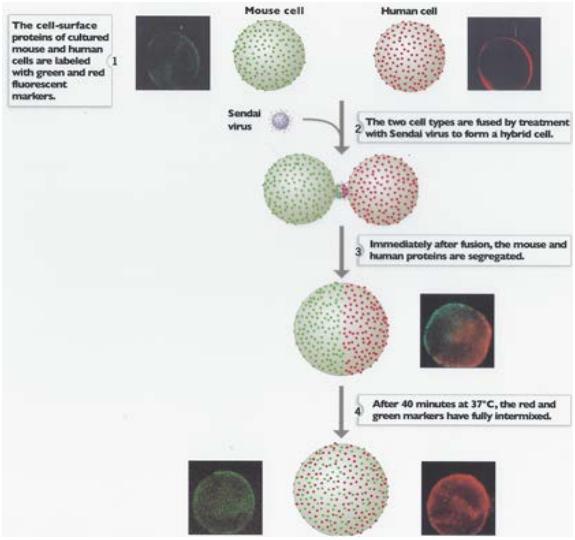


- Integral membrane proteins are firmly associated with the membrane, often spanning the bilayer.
- Peripheral membrane proteins are weakly associated and can be removed easily.
  - Some are non-covalently attached.
  - Some are linked to membrane lipids (amphitrophic)(more later).

This model was also testable!

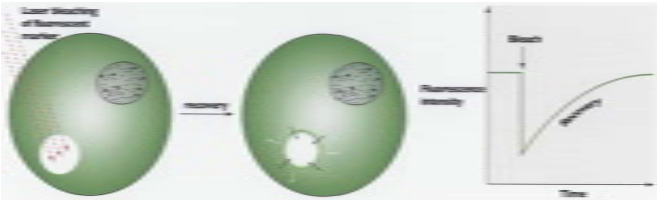
Lipids: Membranes

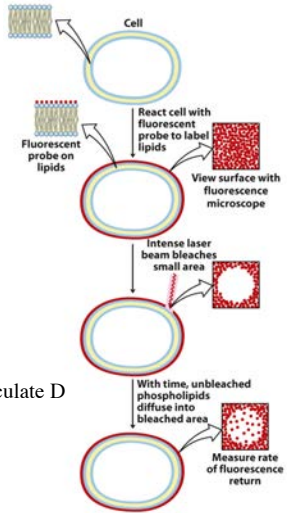
Testing Fluid Mosaic Model of Membrane Structure: Cellular Fusion



Lipids: Membranes

Testing Fluid Mosaic Model of Membrane Structure: FRAP





- **F**luorescence **R**ecovery **A**fter **P**hotobleaching (FRAP) allows us to monitor lateral diffusion by monitoring the rate of fluorescence return.
- From the rate of return of fluorescently labeled lipids, the rate of diffusion of a lipid in the leaflet can be determined.

$\text{Distance} = \sqrt{4Dt}$ Set distance by radius of laser target area, measure t, calculate D

D is rate of diffusion in m^2/sec

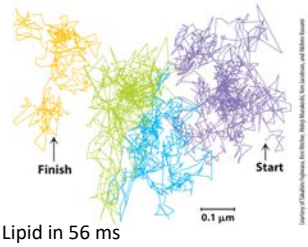
- Rates of lateral diffusion are high (up to 1 μm^2/sec).
  - A lipid can circumnavigate an *E.coli* cell in one second.
  - A protein has a variable rate: 0.4 to 0.0001 μm^2/sec.

…….. but not so fast!

Lipids: Membranes

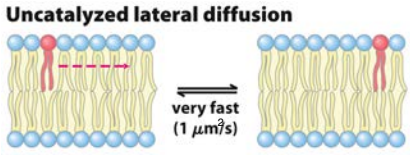
Testing Fluid Mosaic Model of Membrane Structure: FRAP

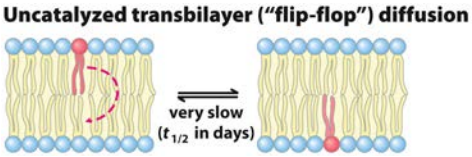
Lateral Movement is fast



A Single Lipid in 56 ms

Transverse Movement is SLOW

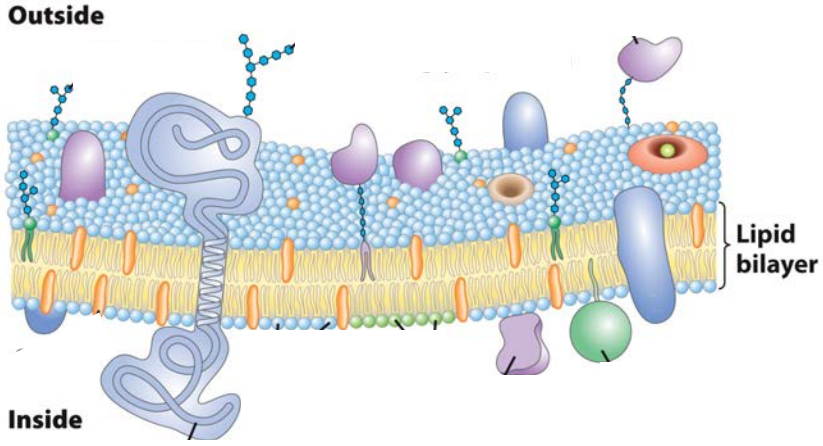




Spontaneous flips from one leaflet to another are rare because the charged head group must transverse the hydrophobic tail region of the membrane.

Lipids: Membranes

The Fluid Mosaic Model: Details



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    - transverse
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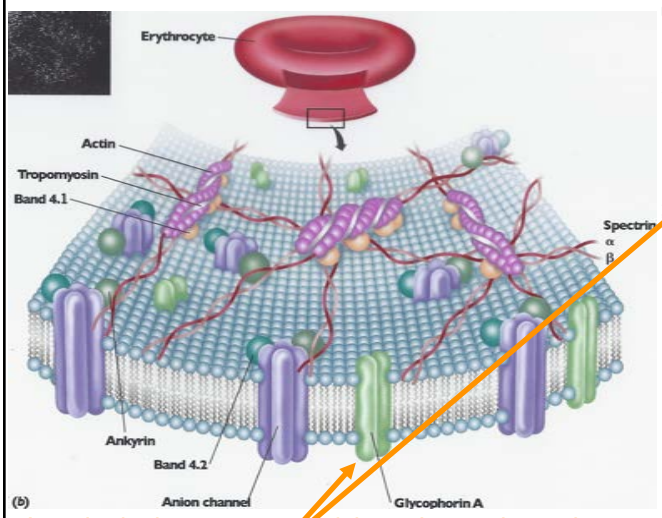
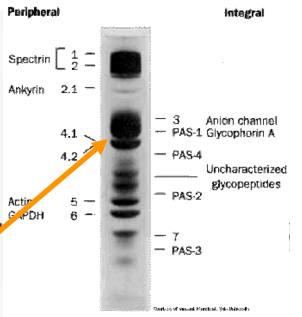
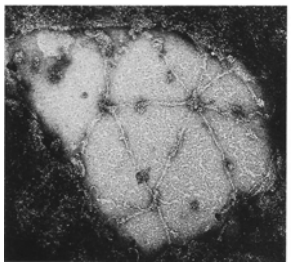
Lipids: Membranes

The Structure of the Red-blood Cell Membrane





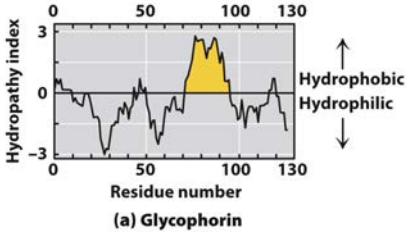


Lets look closer are one of these integral membrane proteins, glycophorin.

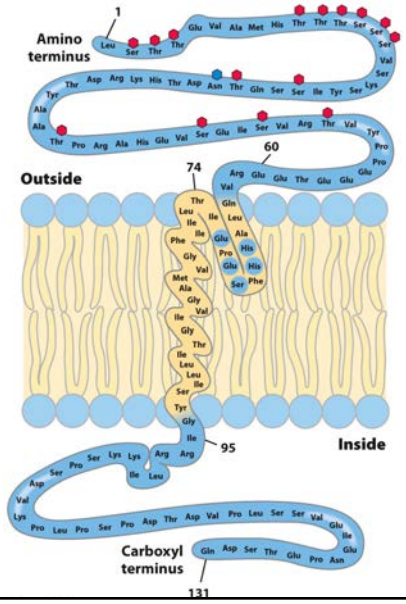
Lipids: Membranes

The Structure of the Red-blood Cell Membrane

Glycophorin



Nonpolar Amino Acids of Integral Membrane Proteins are within the Membrane



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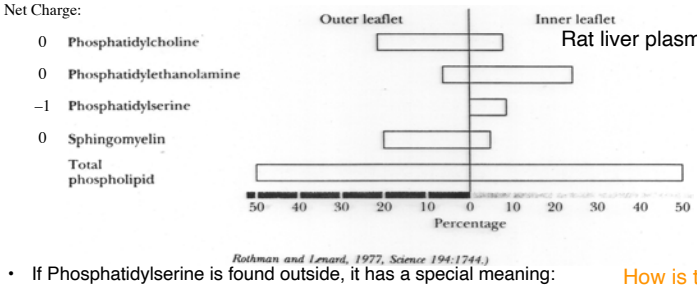
- **Membrane Asymmetry**
  - **Lipids**
    - **transverse**
    - **lateral**
  - **Protein**
    - **anchoring**
    - **glycoproteins**
- **Membrane Fluidity**

Lipids: Membranes

Asymmetry

- Membranes are very asymmetric.
- All kinds of asymmetry: Components–lipids, proteins
  Types–transverse, lateral
  - Lipids (transverse):
    - Two leaflets have different lipid compositions.
    - The outer leaflet is often more positively charged.



Rat liver plasma membrane

Rothman and Lenard, 1977, Science 194:1744.)

- If Phosphatidylserine is found outside, it has a special meaning:
  - platelets: activates blood clotting
  - other cells: marks the cell for destruction

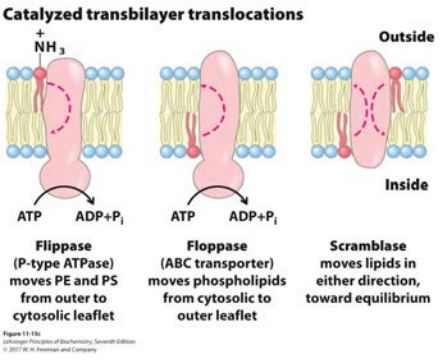
How is this asymmetry maintained?

Lipids: Membranes

Asymmetry

– Lipids (transverse): Flippases

- Special enzymes catalyze transverse diffusion.
  - Though often referred to by category name "flippase," there are unique unidirectional and bidirectional enzymes to catalyze lipid movement.
- Some flippases use energy of ATP to move lipids against the concentration gradient.



Lipids: Membranes

Asymmetry

Lipids (lateral):

1) On the inner leaflet, can induce phosphoserine to coalesce with calcium.

2) On the outerleaflet, can induce "raft" formation; the coalescence of particular membrane lipids (cholesterol, sphingoglycolipids, sphingomyelin, etc.)

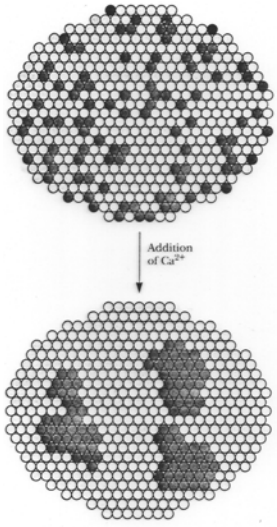


Figure 9.28 An illustration of the concept of lateral phase separations in a membrane. Phase separations of phosphatidylserine (green circles) can be induced by divalent cations such as Ca^{2+}.

Lipids: Membranes

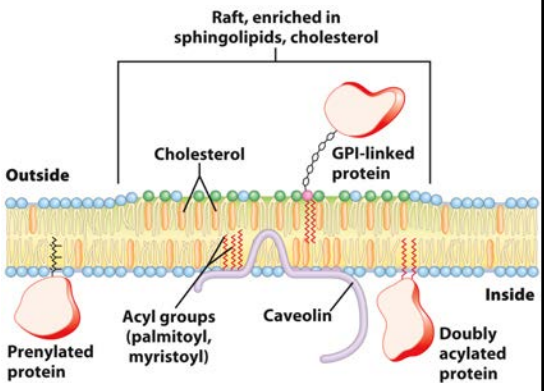
Asymmetry

Lipids (lateral): **Membrane Rafts**

Lipid distribution in a single leaflet is not random or uniform.

Lipid rafts:

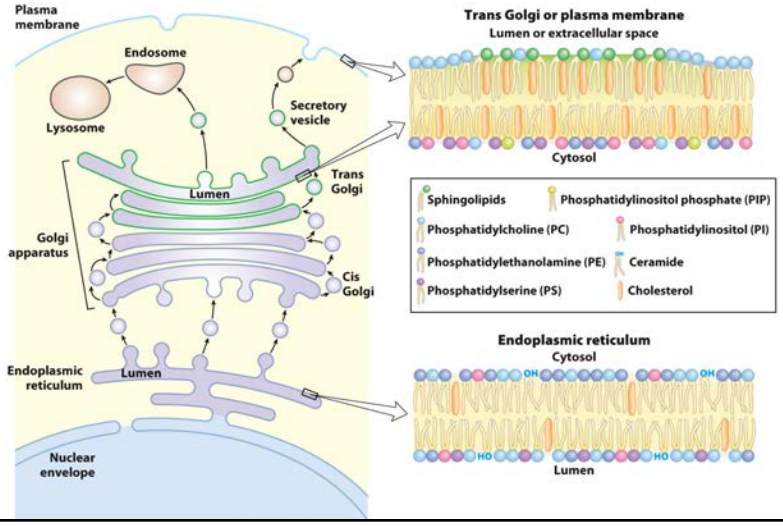
- contain clusters of sphingoglycolipids with longer-than-usual tails and cholesterol
- are more ordered (not as fluid)
- contain specific doubly or triply acylated proteins
- allow segregation of proteins in the membrane



Lipids: Membranes

Asymmetry

Lipids (lateral): **Membrane Rafts**



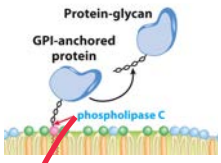
Lipids: Membranes

Asymmetry

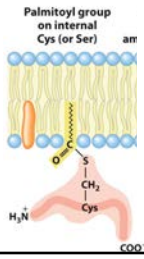
Proteins:

1) anchored

2) glycoproteins



- Some membrane proteins are lipoproteins containing a covalently linked lipid molecule.
- The lipid part inserts into the membrane.
- The protein is then anchored to the membrane.
- These are the aforementioned "Amphitropic" membrane proteins



- This allows targeting of proteins to the membrane, either internally (FA or isoprenes), or externally (GPI-linked).
- Some, such as GPI anchors are found only on the outer face of plasma membrane.
- This is a reversible process.

Lipids: Membranes

Proteins: Asymmetry

1) anchored
2) glycoproteins

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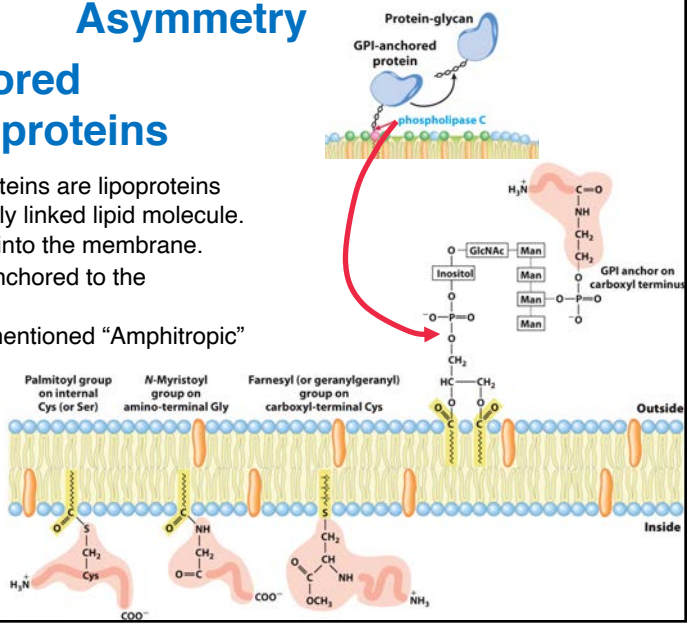
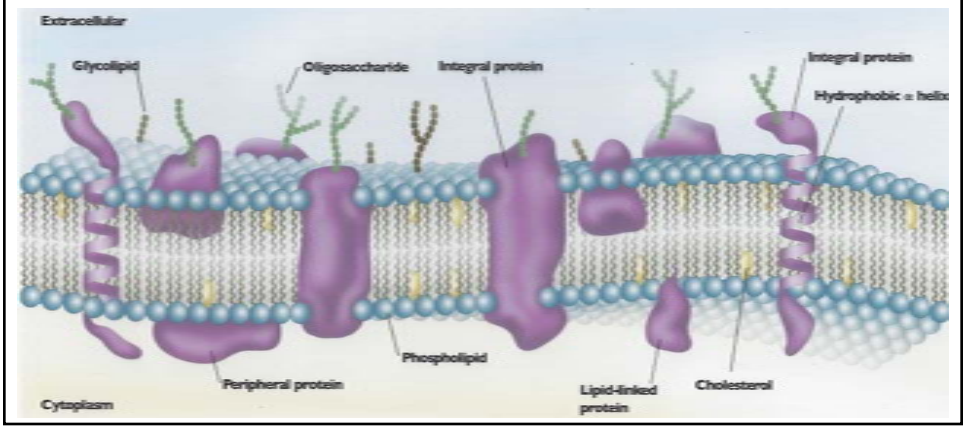


Lipids: Membranes

Proteins: Asymmetry

1) anchored
2) glycoproteins

Found ONLY ever on the OUTSIDE of Cells



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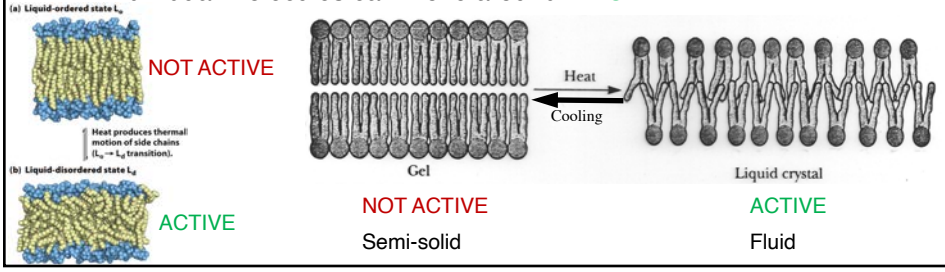
Lipids: Membranes

Fluidity

Membrane Phase Transition

- Cells must maintain fluidity for membranes to function properly.
- Depending on their composition and the temperature, the lipid bilayer must maintain a fluid phase; if cooled, it undergoes a phase transition and goes to a gel state.
  - liquid-ordered state (i.e., "gel phase"): individual molecules do not move around – NOT ACTIVE
  - liquid-disordered (or liquid crystal) state (i.e., "fluid phase"): individual molecules can move around – ACTIVE



NOT ACTIVE

ACTIVE

NOT ACTIVE

Semi-solid

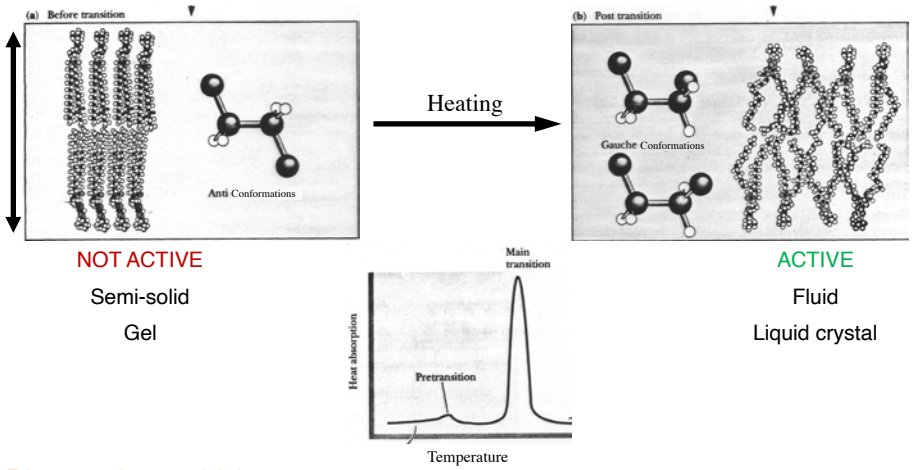
ACTIVE

Fluid

Lipids: Membranes

Fluidity

Membrane Phase Transition



NOT ACTIVE

Semi-solid

Gel

ACTIVE

Fluid

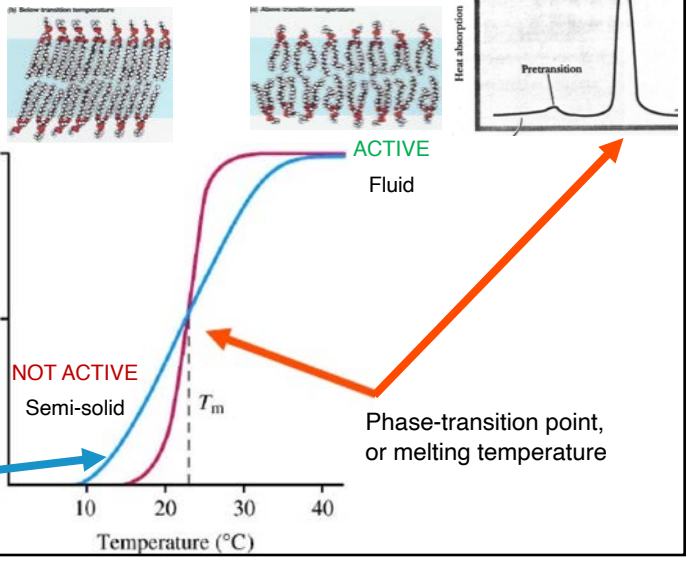
Liquid crystal

Plot membrane thickness vs. temperature

Lipids: Membranes

Fluidity

Membrane Phase Transition



ACTIVE

Fluid

NOT ACTIVE

Semi-solid

Phase-transition point, or melting temperature

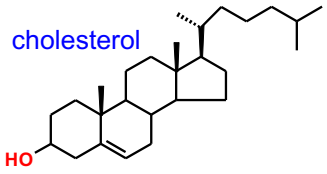
Cooperativity is modified by cholesterol

Lipids: Membranes

Fluidity

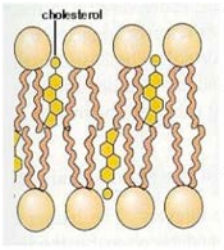
Membrane Phase Transition

Cholesterol Increases Membrane Rigidity and Permeability

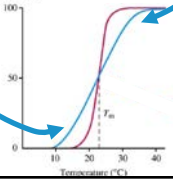


- Due to its small polar head, it lies deeper in the bilayer than the phospholipids
- Cell membranes of many eukaryotes contain sterols.
  - **cholesterol in animals**
  - *phytosterols in plants*
  - *ergosterol in fungi*
- *Cell membranes of aerobic prokaryotes contain hopanols.*

Cholesterol: A "Fluidity Buffer"



- Below T_m - cholesterol disrupts close packing of acyl chains ⇒ *increases* fluidity
- Above T_m - cholesterol constrains motion of acyl chains ⇒ *decreases* fluidity
- Broadens/abolishes phase transitions



Lipids: Membranes

Fluidity

Organisms Can Adjust the Temperature of the Phase Transition by Changing the Membrane Composition

- Membrane fluidity is determined mainly by the fatty acid composition and melting point.
- The temperature of the phase transition (T_m):
  - Melting temperature higher with more saturated fatty acids.
  - Melting temperature higher with longer fatty acids.
  - Melting temperature lower with more unsaturated fatty acids.
  - Melting temperature lower with shorter fatty acids.
- Therefore, at higher temperatures, cells need more long, saturated fatty acids.
- And at lower temperatures, cells need more shorter, unsaturated fatty acids.

Lipids: Membranes

Fluidity

TABLE 11-2 Fatty Acid Composition of *E. coli* Cells Cultured at Different Temperatures

	Percentage of total fatty acids[a]			
	10 ˚C	**20 ˚C**	**30 ˚C**	**40 ˚C**
Myristic acid	4	4	4	8
Palmitic acid	18	25	29	48
Palmitoleic acid	26	24	23	9
Oleic acid	38	34	30	12
Hydroxymyristic acid	13	10	10	8
Ratio of unsaturated to saturated[b]	2.9	2.0	1.6	0.38

SOURCE: Data from A. G. Marr and J. L. Ingraham, *J. Bacteriol.* 84:1260, 1962.
[a]The exact fatty acid composition depends not only on growth temperature but on growth stage and growth medium composition.
[b]Ratios calculated as the total percentage of 16:1 plus 18:1 divided by the total percentage of 14:0 plus 16:0. Hydroxymyristic acid was omitted from this calculation.