Chemistry of lipid lipid and membrane

University Of Fallujah
College Of Medicine

Lecture : 5

Stage : first

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Medical Biochemistry

Lipids & Membranes

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Lipids are non-polar (hydrophobic) compounds, soluble in organic solvents.

Most membrane lipids are **amphipathic**, having a **non-polar** end and a **polar** end.

Fatty acids consist of a hydrocarbon chain with a carboxylic acid at one end.

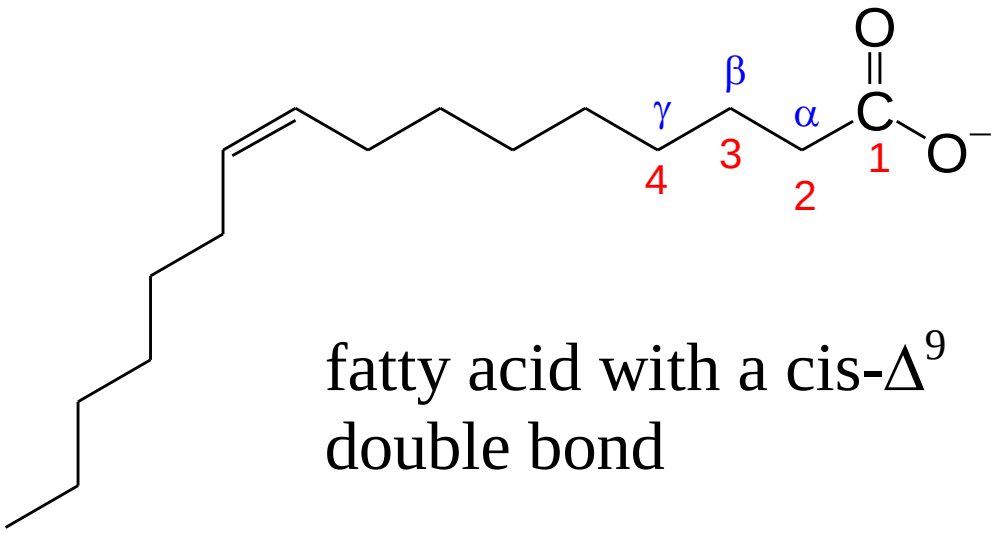
A 16-C fatty acid: $\mathbf{CH_3(CH_2)_{14}\text{-}COO^-}$

Non-polar **polar**

A 16-C fatty acid with one cis double bond between C atoms 9-10 may be represented as **16:1 cis Δ^9**.

Double bonds in fatty acids usually have the **cis** configuration.

Most naturally occurring fatty acids have an **even number** of carbon atoms.



fatty acid with a cis-Δ^9 double bond

Some fatty acids and their common names:

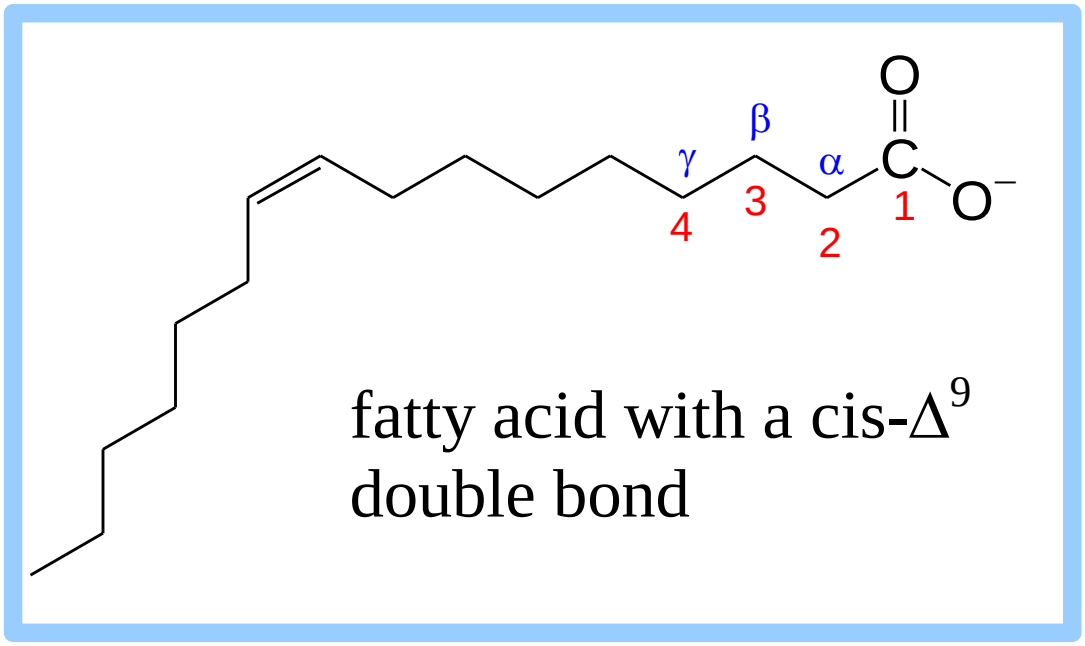
14:0 myristic acid; 16:0 palmitic acid; 18:0 stearic acid;
18:1 cisΔ^9 oleic acid

18:2 cis$\Delta^{9,12}$ linoleic acid

18:3 cis$\Delta^{9,12,15}$ α-linonenic acid

20:4 cis$\Delta^{5,8,11,14}$ arachidonic acid

20:5 cis$\Delta^{5,8,11,14,17}$ eicosapentaenoic acid (an omega-3)



There is free rotation about **C-C** bonds in the fatty acid hydrocarbon, except where there is a double bond.

Each cis double bond causes a **kink** in the chain.

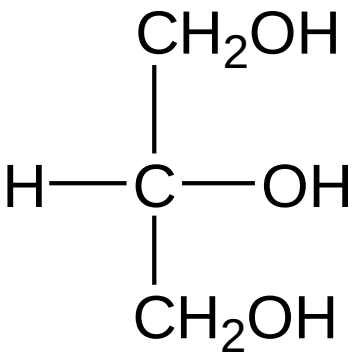
Rotation about other **C-C** bonds would permit a more linear structure than shown, but there would be a kink.

Glycerophospholipids

Glycerophospholipids (phosphoglycerides), are common constituents of cellular membranes.

They have a **glycerol** backbone.

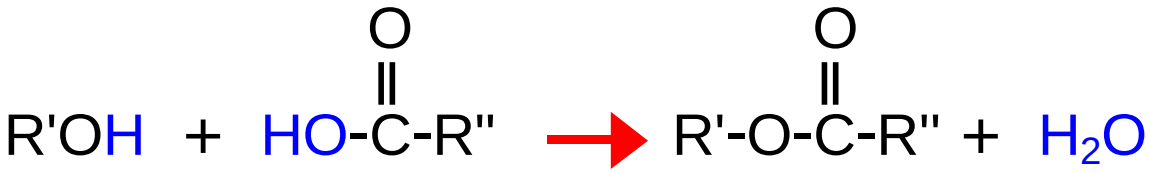
Hydroxyls at **C1** & **C2** are esterified to **fatty acids**.



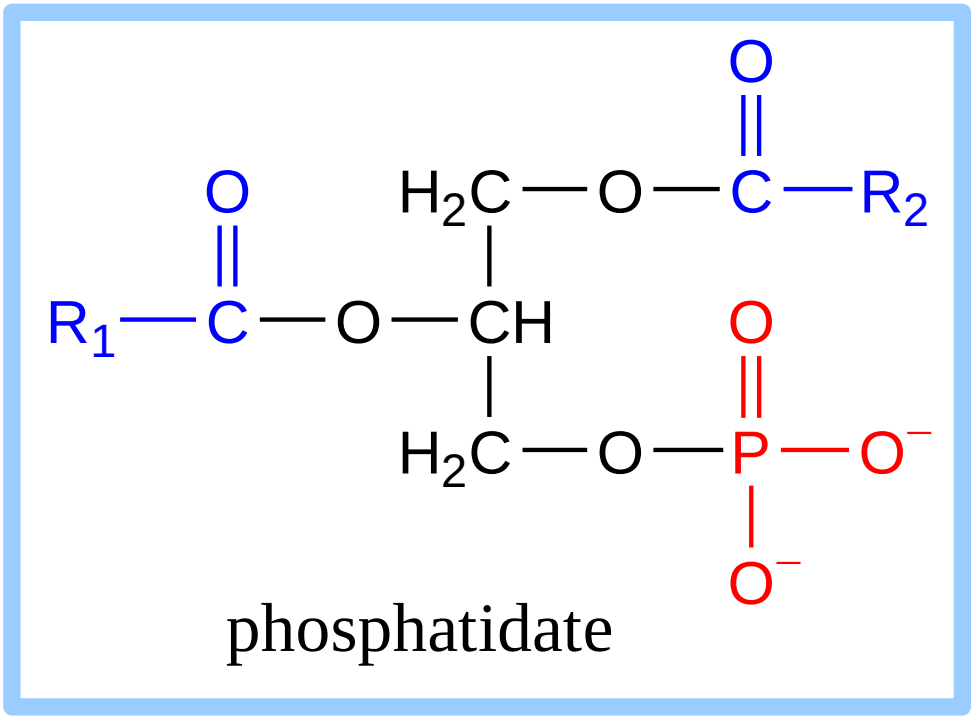
glycerol

An **ester** forms when a hydroxyl reacts with a carboxylic acid, with loss of H_2O.

Formation of an ester:



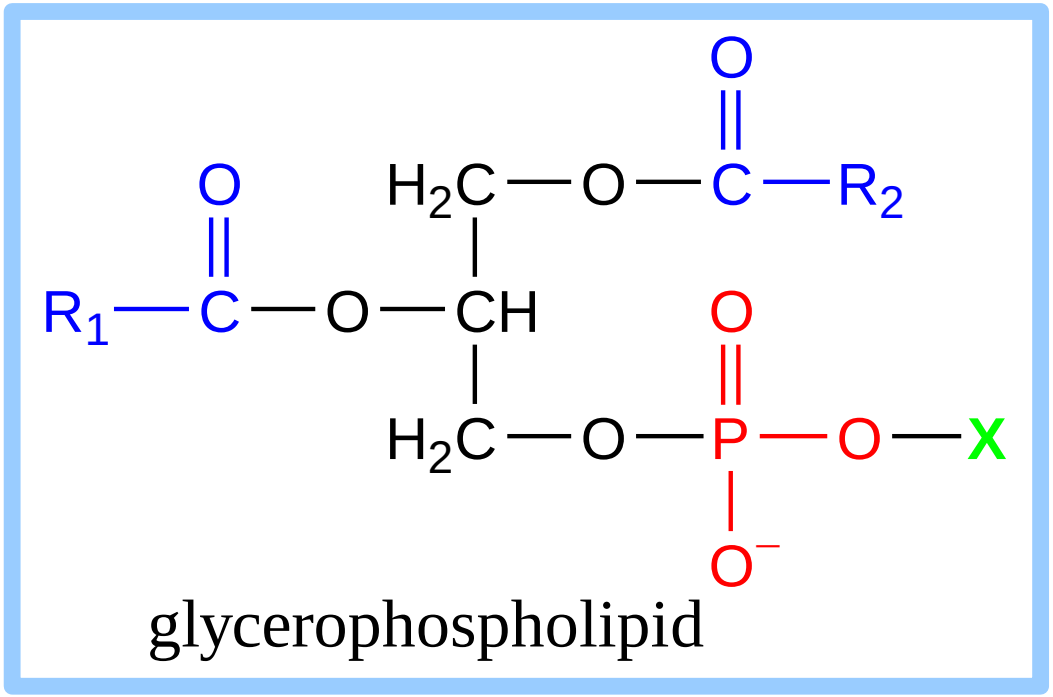
Phosphatidate



phosphatidate

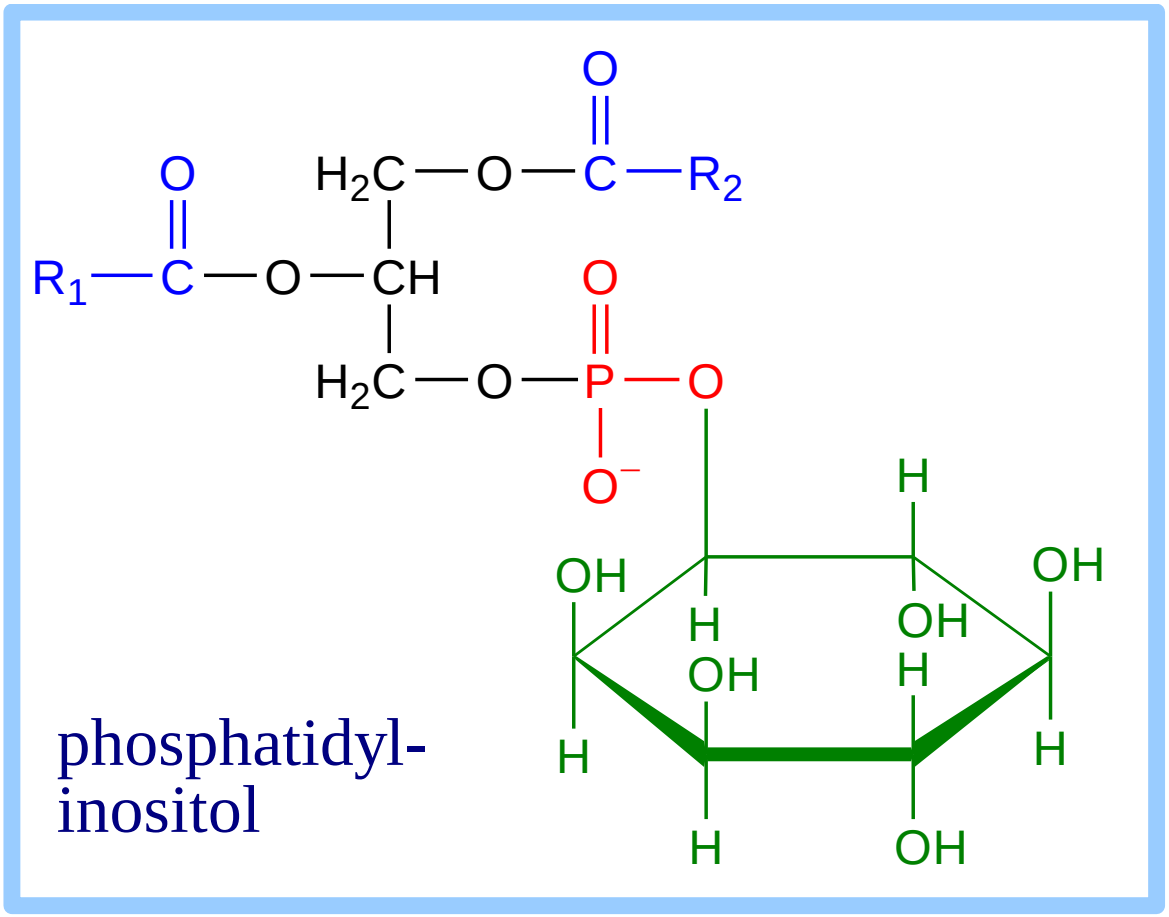
In **phosphatidate**:

- **fatty acids** are esterified to hydroxyls on **C1** & **C2**
- the **C3** hydroxyl is esterified to **P_i**.



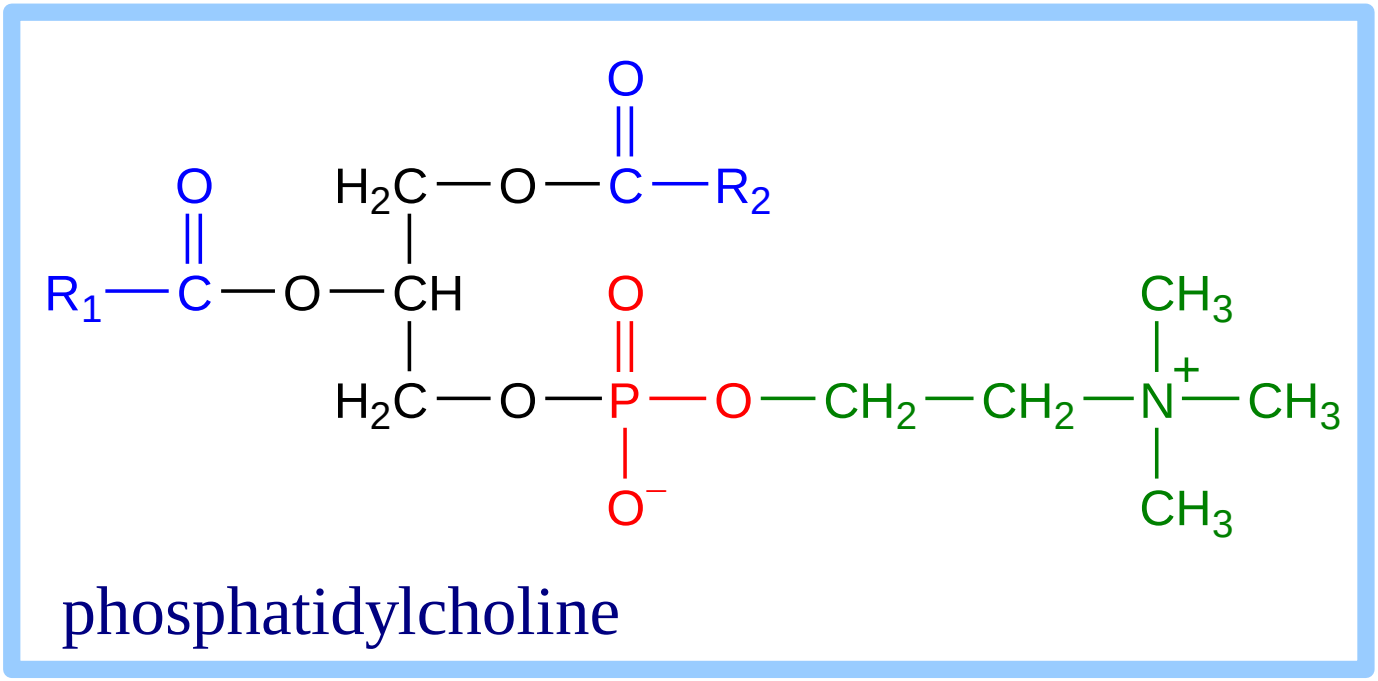
In most **glycerophospholipids** (phosphoglycerides), **P_i** is in turn esterified to **OH** of a **polar head group** (**X**): e.g., **serine, choline, ethanolamine, glycerol, or inositol**.

The 2 fatty acids tend to be non-identical. They may differ in length and/or the presence/absence of double bonds.



Phosphatidylinositol, with inositol as polar head group, is one glycerophospholipid.

In addition to being a membrane lipid, phosphatidylinositol has roles in cell signaling.

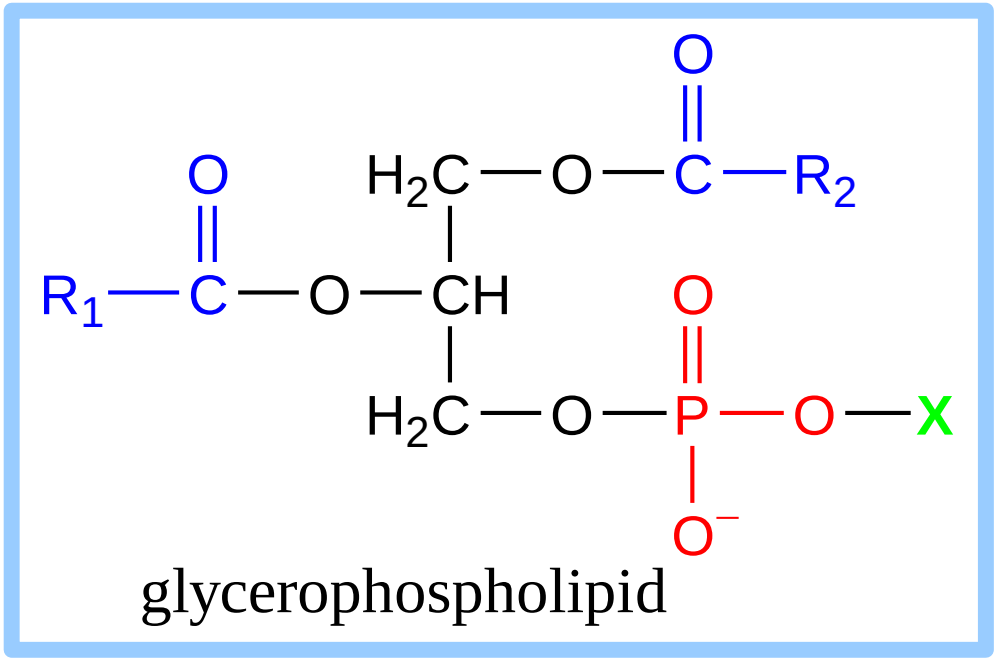


Phosphatidylcholine, with choline as polar head group, is another glycerophospholipid.

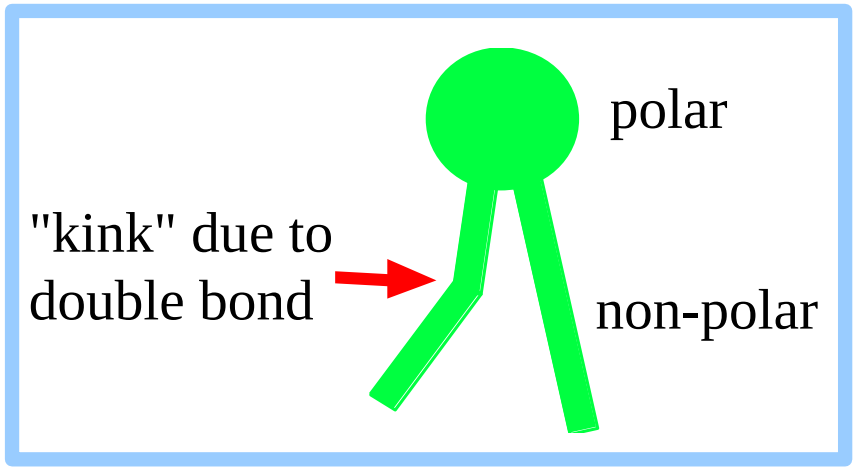
It is a common membrane lipid.

Each glycerophospholipid includes

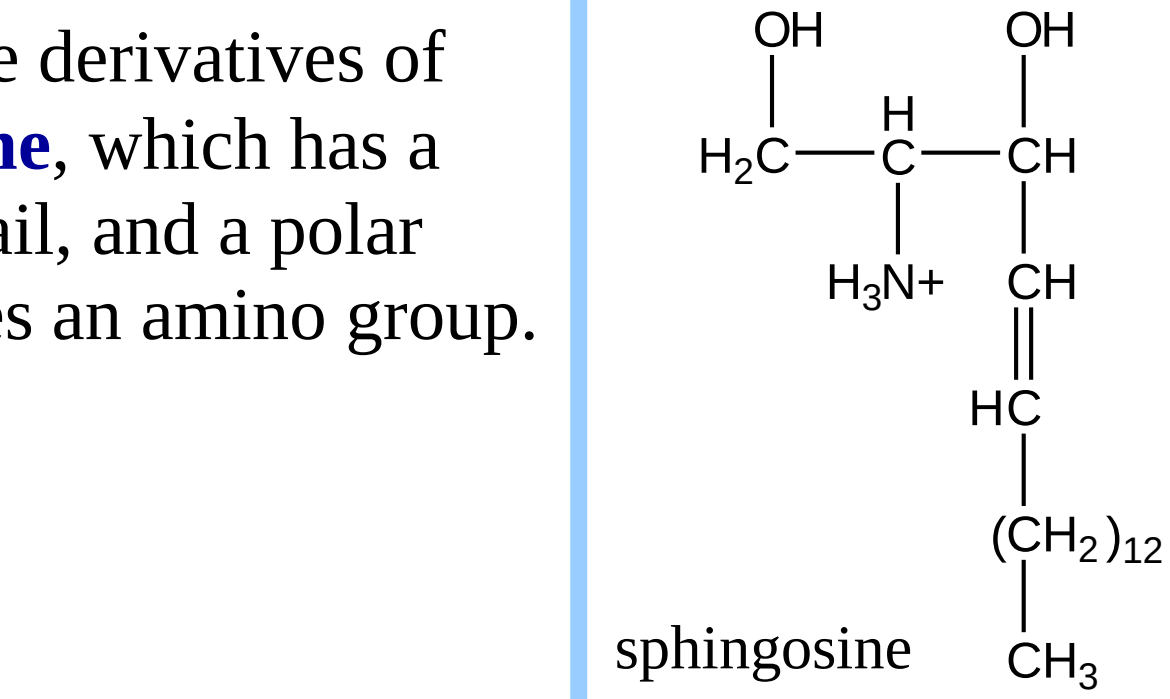
- a **polar** region: **glycerol**, **carbonyl O** of fatty acids, **P_i**, & the polar head group (**X**)
- **non-polar** hydrocarbon tails of fatty acids (**R_1**, **R_2**).



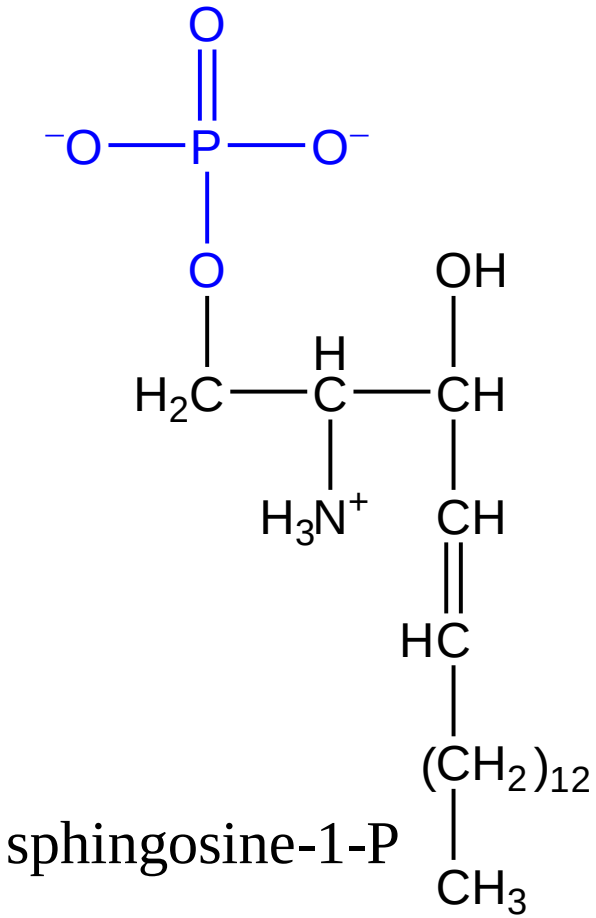
glycerophospholipid



Sphingolipids are derivatives of the lipid **sphingosine**, which has a long hydrocarbon tail, and a polar domain that includes an amino group.



sphingosine

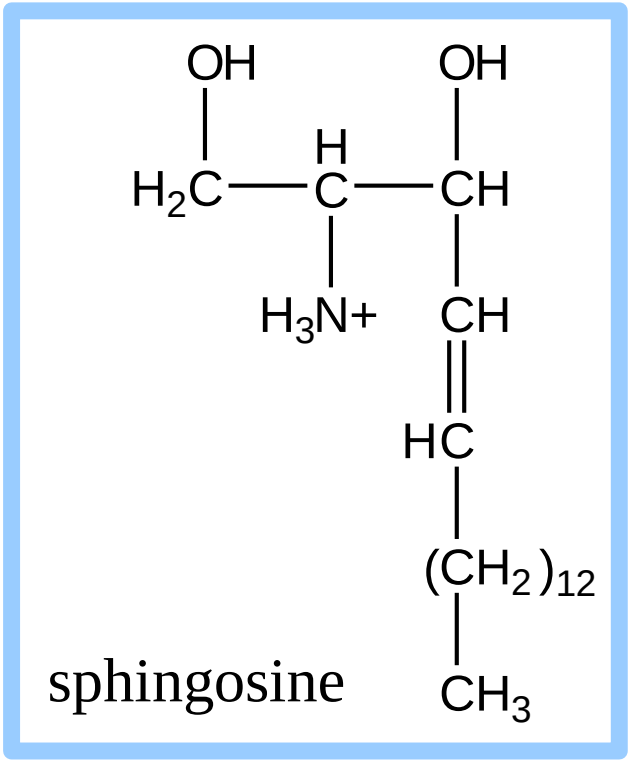


sphingosine-1-P

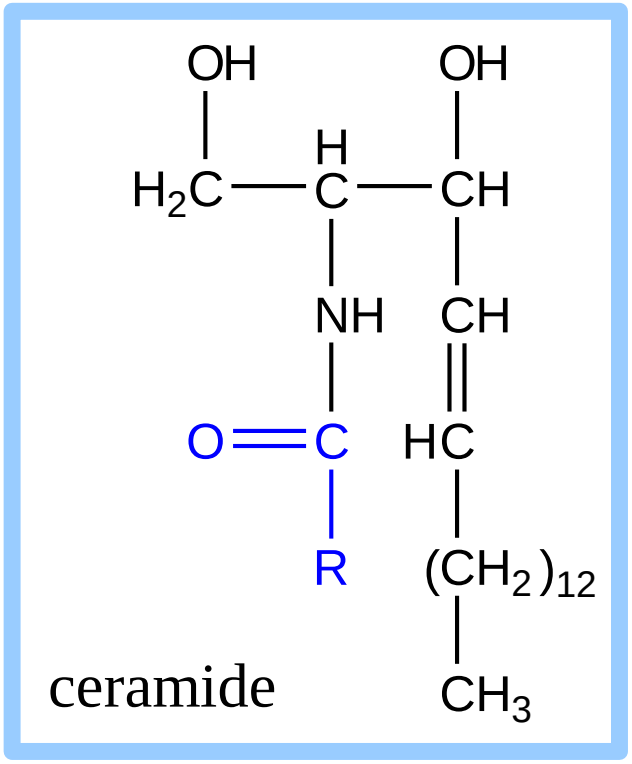
Sphingosine may be reversibly phosphorylated to produce the **signal** molecule **sphingosine-1-phosphate**.

Other derivatives of sphingosine are commonly found as constituents of biological membranes.

The amino group of sphingosine can form an amide bond with a **fatty acid** carboxyl, to yield a **ceramide**.



sphingosine

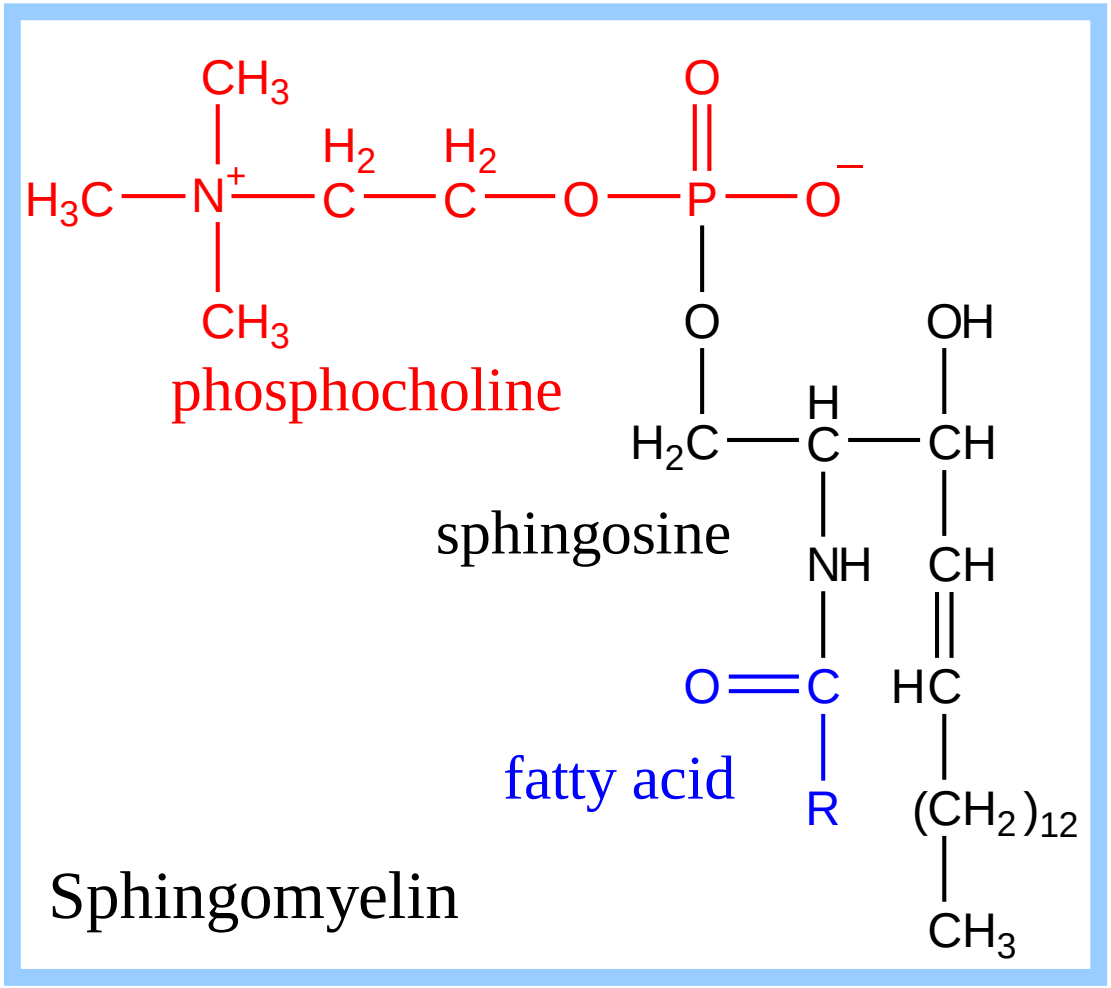


ceramide

In the more complex sphingolipids, a **polar “head group"** is esterified to the terminal hydroxyl of the sphingosine moiety of the ceramide.

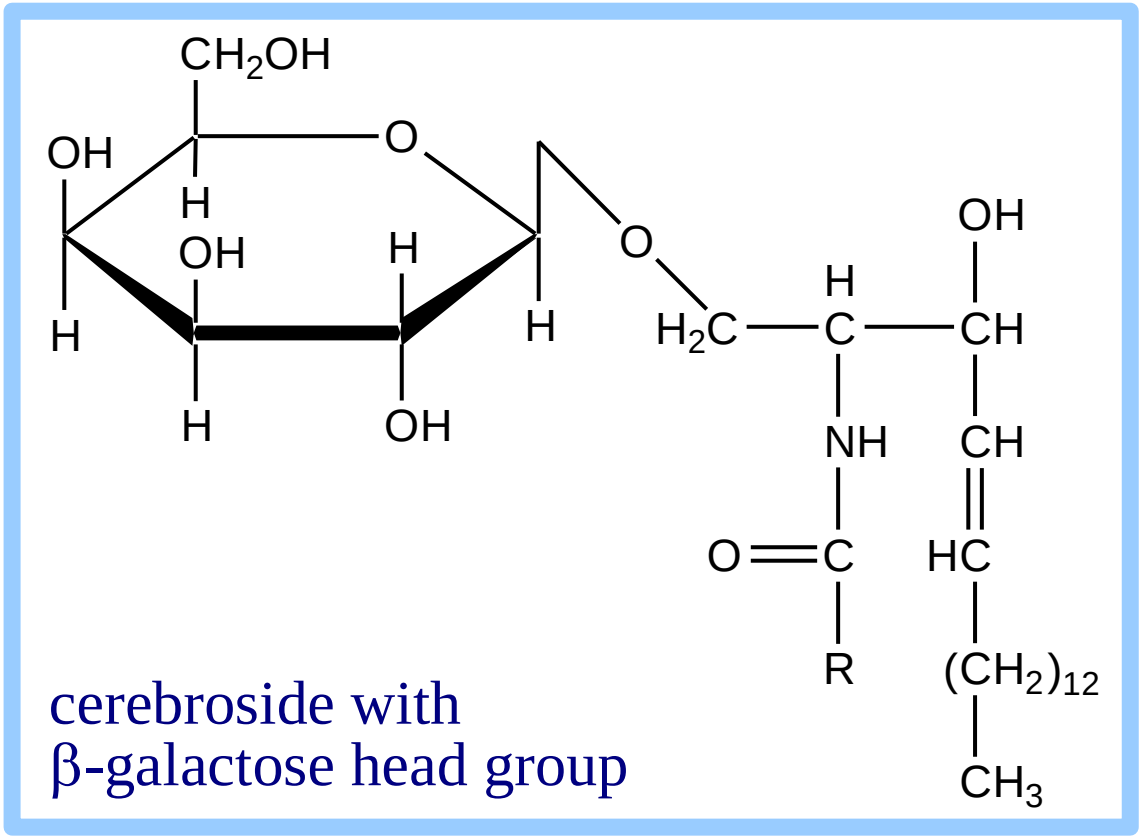
Sphingomyelin has a phosphocholine or phosphethanolamine head group.

Sphingomyelins are common constituent of plasma membranes



Sphingomyelin, with a phosphocholine head group, is similar in size and shape to the glycerophospholipid phosphatidyl choline.

A **cerebroside** is a sphingolipid (ceramide) with a **monosaccharide** such as glucose or galactose as polar head group.

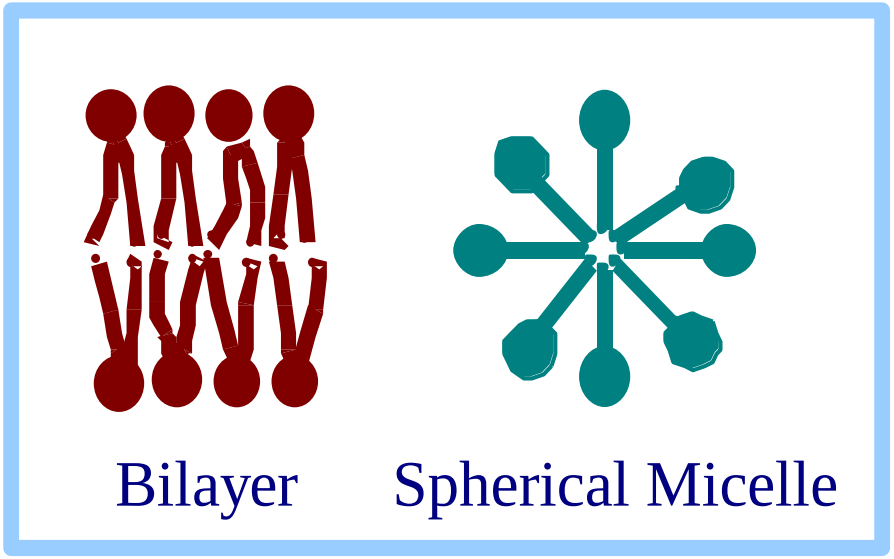


cerebroside with β-galactose head group

A **ganglioside** is a ceramide with a polar head group that is a **complex oligosaccharide**, including the acidic sugar derivative sialic acid.

Cerebrosides and gangliosides, collectively called **glycosphingolipids**, are commonly found in the outer leaflet of the plasma membrane bilayer, with their sugar chains extending out from the cell surface.

Amphipathic lipids in association with water form complexes in which polar regions are in contact with water and hydrophobic regions away from water.

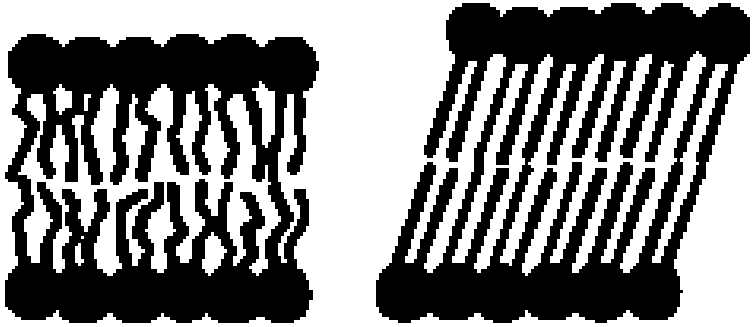


Depending on the lipid, possible molecular arrangements:

- Various **micelle** structures. E.g., a spherical micelle is a stable configuration for amphipathic lipids with a **conical** shape, such as **fatty acids.**
- A **bilayer**. This is the most stable configuration for amphipathic lipids with a **cylindrical** shape, such as **phospholipids**.

Membrane fluidity:

The interior of a lipid bilayer is normally highly **fluid**.



In the **liquid crystal state**, hydrocarbon chains of phospholipids are disordered and in constant motion.

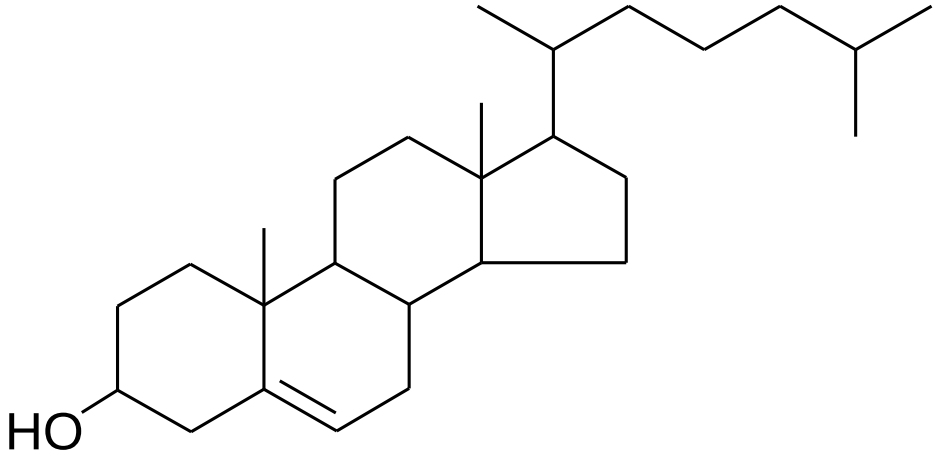
At **lower temperature**, a membrane containing a single phospholipid type undergoes transition to a **crystalline state** in which fatty acid tails are fully extended, packing is highly ordered, & van der Waals interactions between adjacent chains are maximal.

Kinks in fatty acid chains, due to **cis double bonds**, interfere with packing in the crystalline state, and **lower the phase transition temperature**.

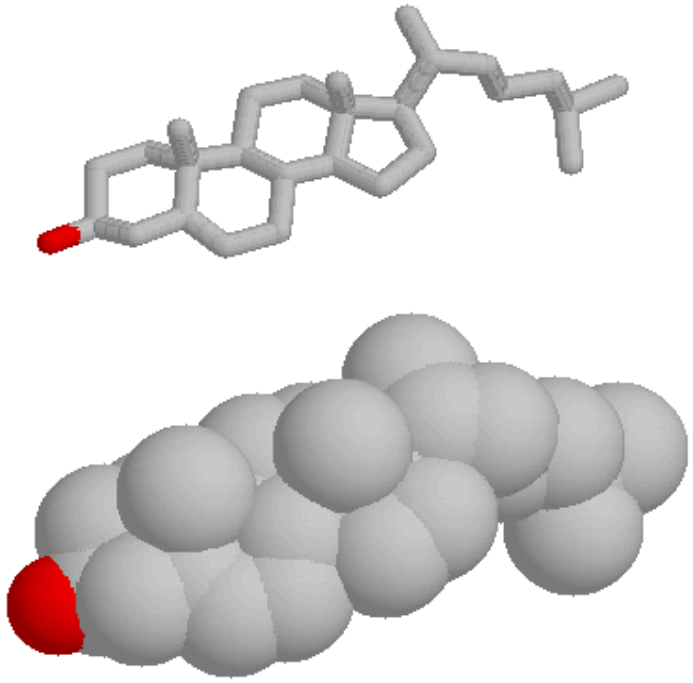
Cholesterol, an important constituent of cell membranes, has a **rigid** ring system and a short branched hydrocarbon tail.

Cholesterol is largely **hydrophobic**.

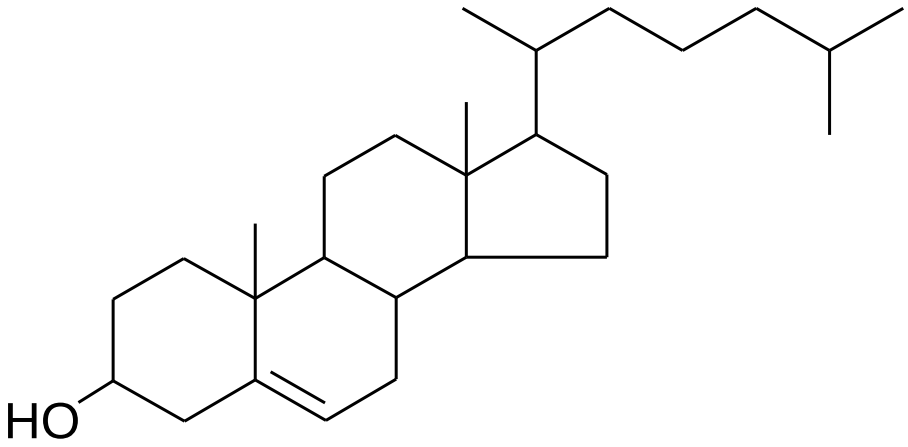
But it has one polar group, a **hydroxyl**, making it **amphipathic**.



Cholesterol



PDB 1N83 cholesterol



Cholesterol

Cholesterol
in membrane

Cholesterol inserts into bilayer membranes with its hydroxyl group oriented toward the aqueous phase & its hydrophobic ring system adjacent to fatty acid chains of phospholipids.

The **OH** group of cholesterol forms hydrogen bonds with polar phospholipid head groups.

Interaction with the relatively **rigid cholesterol** decreases the mobility of hydrocarbon tails of phospholipids.

But the presence of **cholesterol** in a phospholipid membrane interferes with close packing of fatty acid tails in the crystalline state, and thus **inhibits transition** to the crystal state.

Phospholipid membranes with a high concentration of cholesterol have a **fluidity intermediate** between the liquid crystal and crystal states.

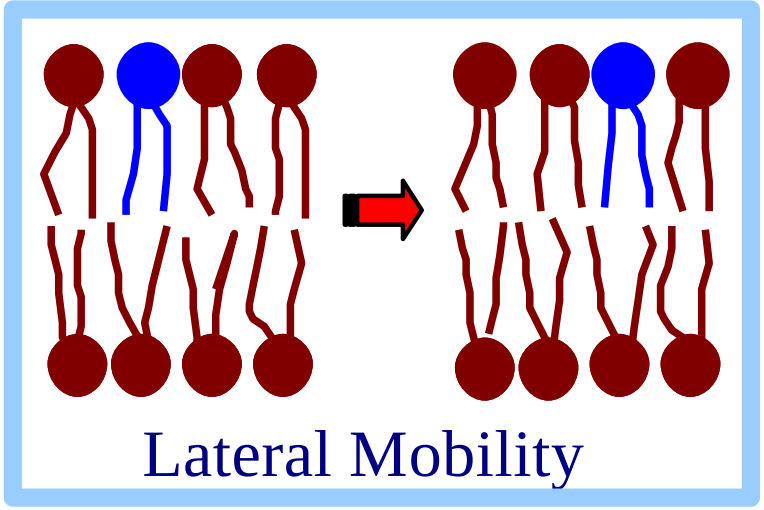
Two strategies by which phase changes of membrane lipids are avoided:

- **Cholesterol** is abundant in membranes, such as plasma membranes, that include many lipids with long-chain saturated fatty acids.

  In the absence of cholesterol, such membranes would crystallize at physiological temperatures.

- The inner mitochondrial membrane lacks cholesterol, but includes many phospholipids whose fatty acids have one or more **double bonds**, which **lower the melting point** to below physiological temperature.

Lateral mobility of a lipid, within the plane of a membrane, is depicted at right and in an **animation**.

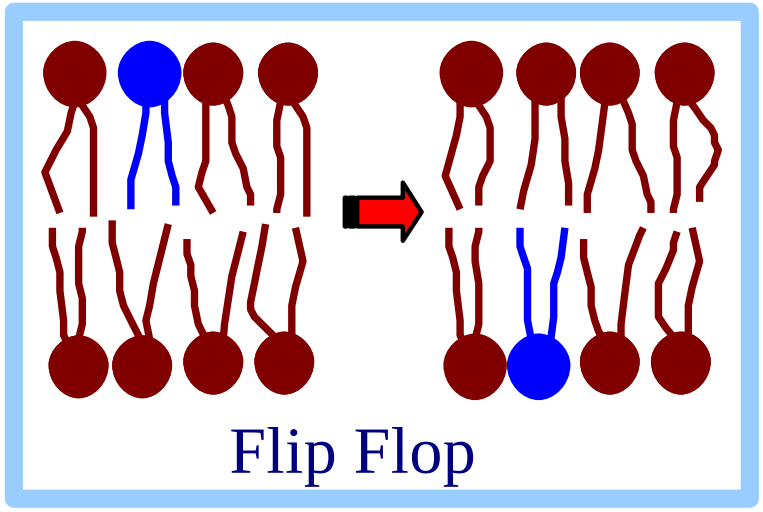


High speed tracking of individual lipid molecules has shown that **lateral movements** are **constrained** within small membrane domains.

Hopping from one domain to another occurs less frequently than rapid movements within a domain.

The apparent constraints on lateral movements of lipids (and proteins) has been attributed to integral membrane proteins, anchored to the cytoskeleton, functioning as a **picket fence**. See the website of the Kusumi laboratory.

Flip-flop of lipids (from one half of a bilayer to the other) is normally very **slow.**



Flip-flop would require the polar head-group of a lipid to traverse the hydrophobic core of the membrane.

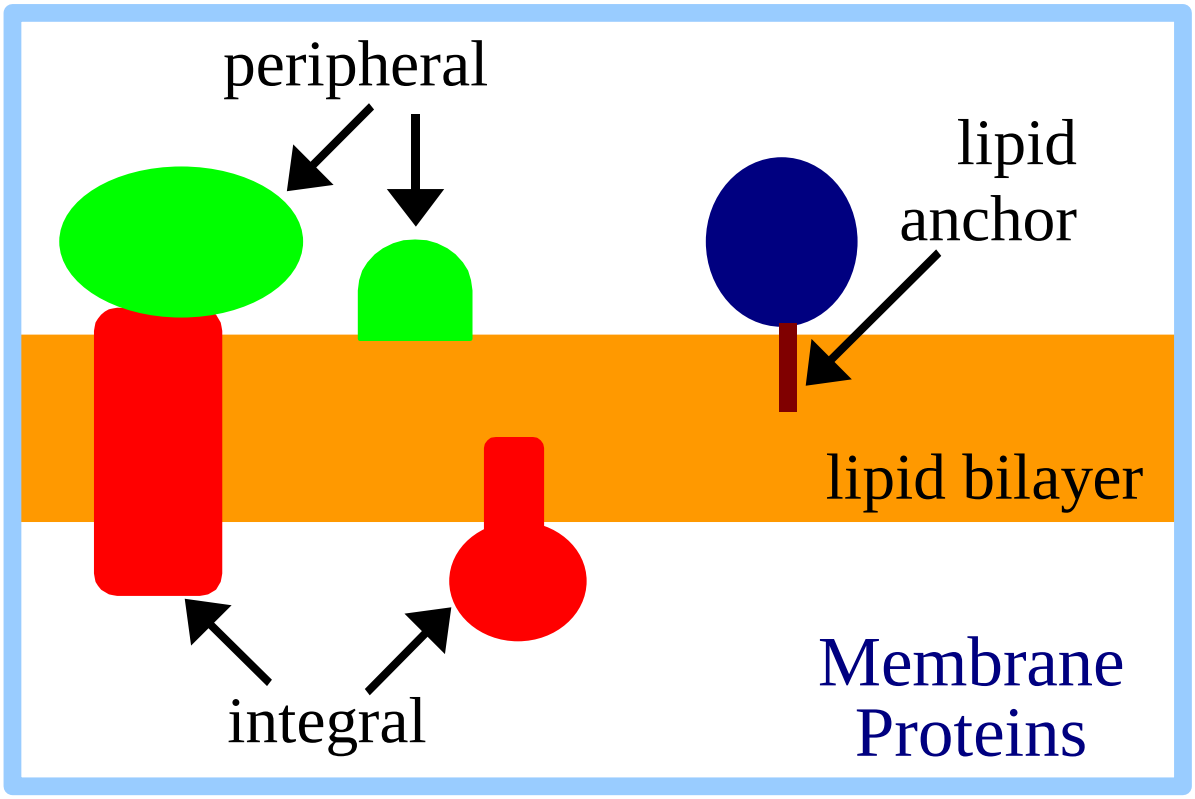
The **two leaflets** of a bilayer membrane tend to **differ** in their lipid composition.

Flippases catalyze flip-flop in membranes where lipid synthesis occurs.

Some membranes contain enzymes that **actively transport** particular lipids from one monolayer to the other.

Membrane proteins may be classified as:

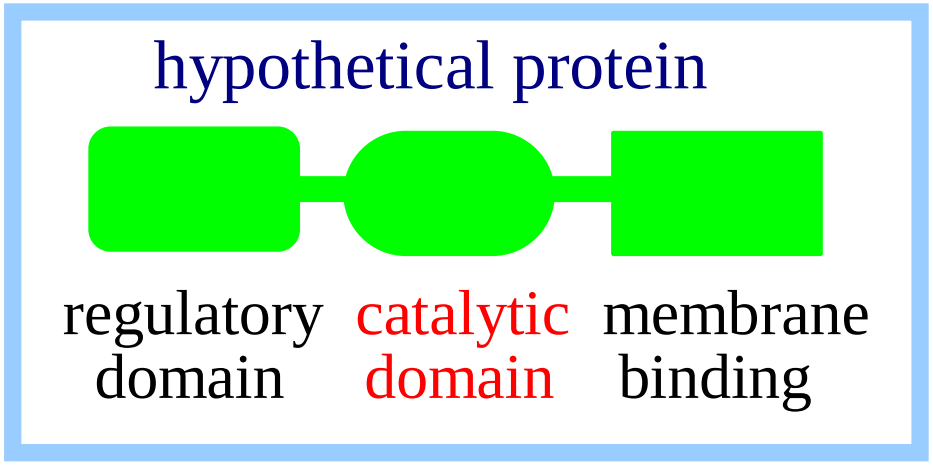
- **peripheral**
- **integral**
- **having a lipid anchor**



Peripheral proteins are on the membrane **surface**.

They are water-soluble, with mostly hydrophilic surfaces.

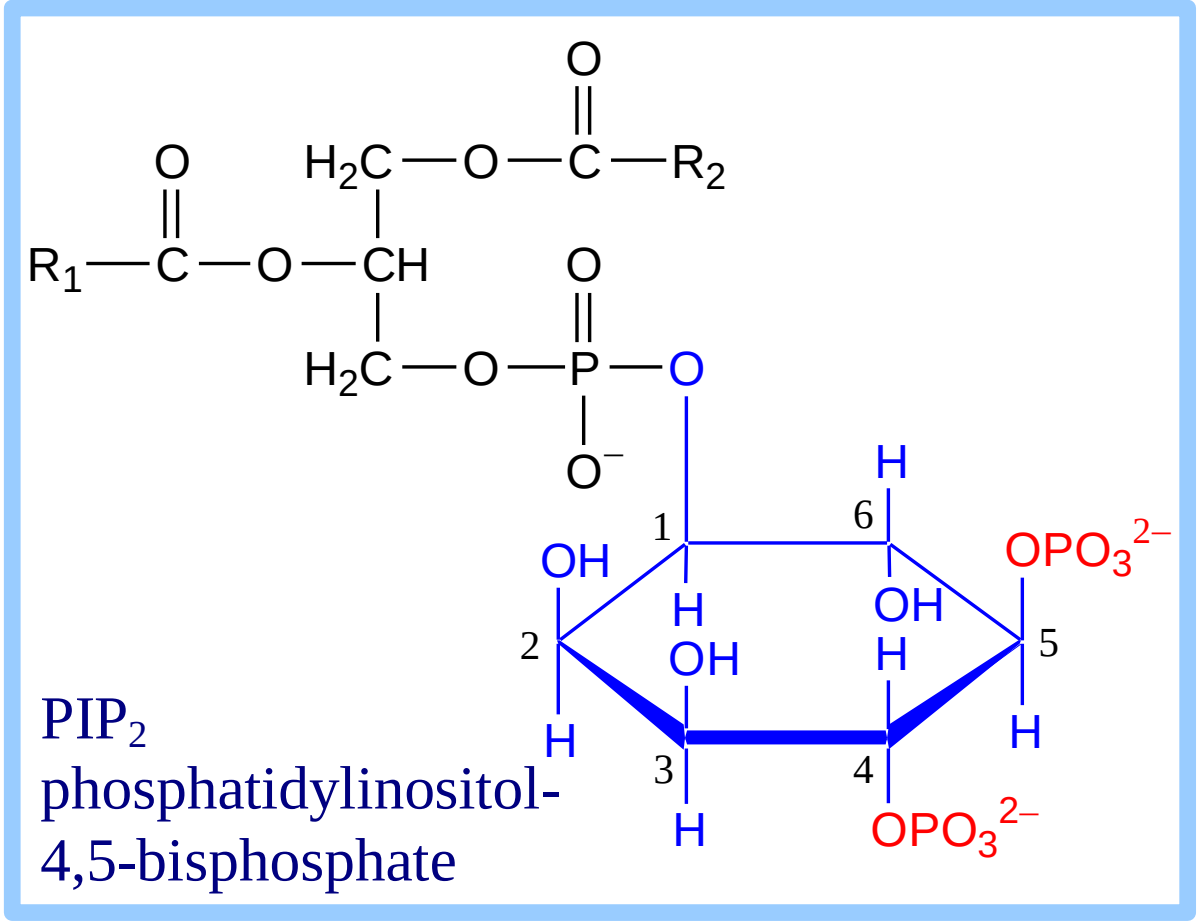
Often peripheral proteins can be dislodged by conditions that disrupt **ionic & H-bond interactions**, e.g., extraction with solutions containing high concentrations of salts, change of pH, and/or chelators that bind divalent cations.



Many proteins have a **modular** design, with different segments of the primary structure folding into domains with different functions.

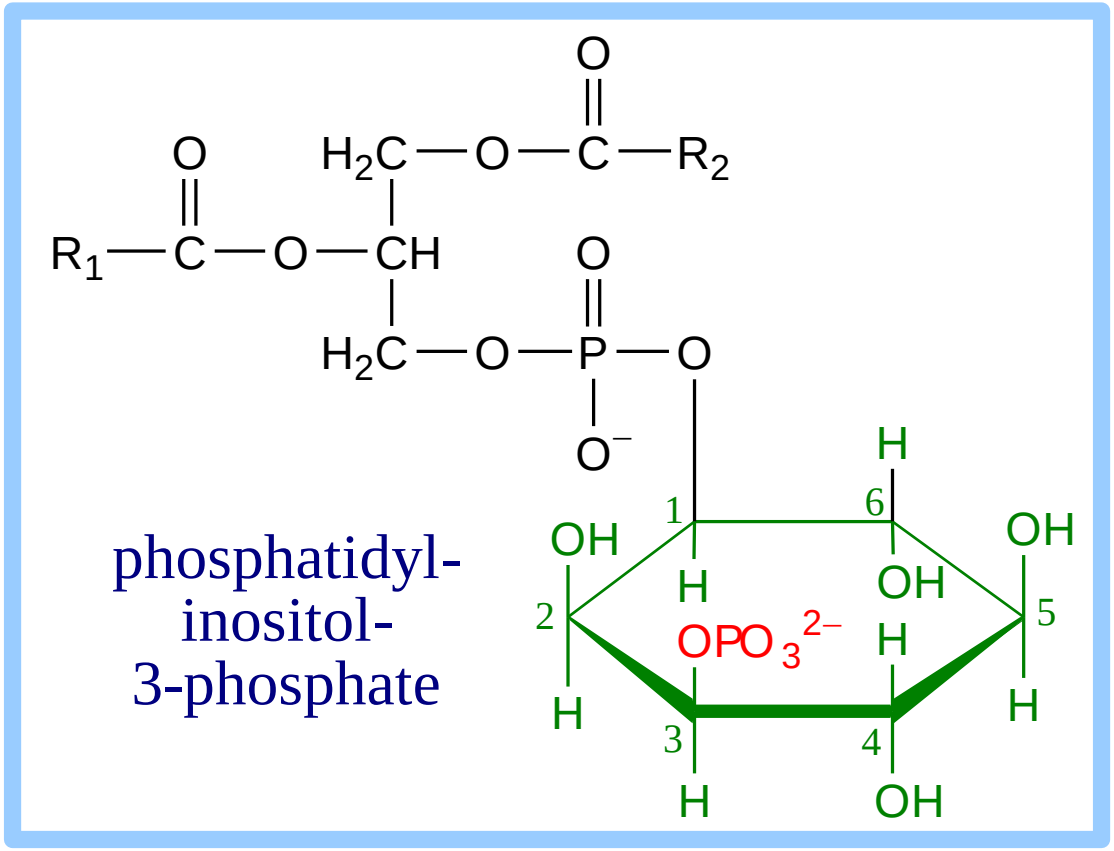
Some cytosolic proteins have **domains** that **bind** to polar head groups of **lipids** that transiently exist in a membrane.

The enzymes that create or degrade these lipids are subject to signal-mediated **regulation**, providing a mechanism for modulating affinity of a protein for a membrane surface.

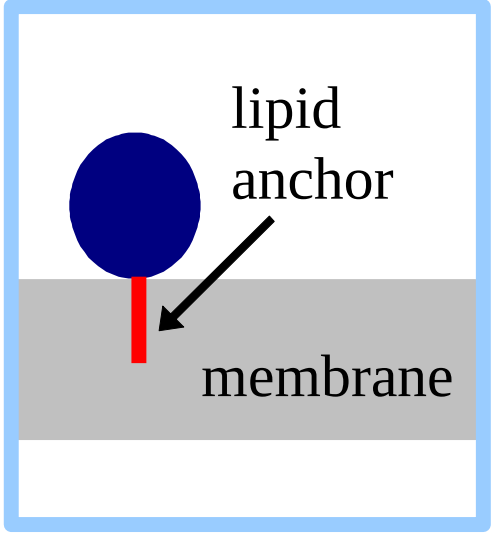


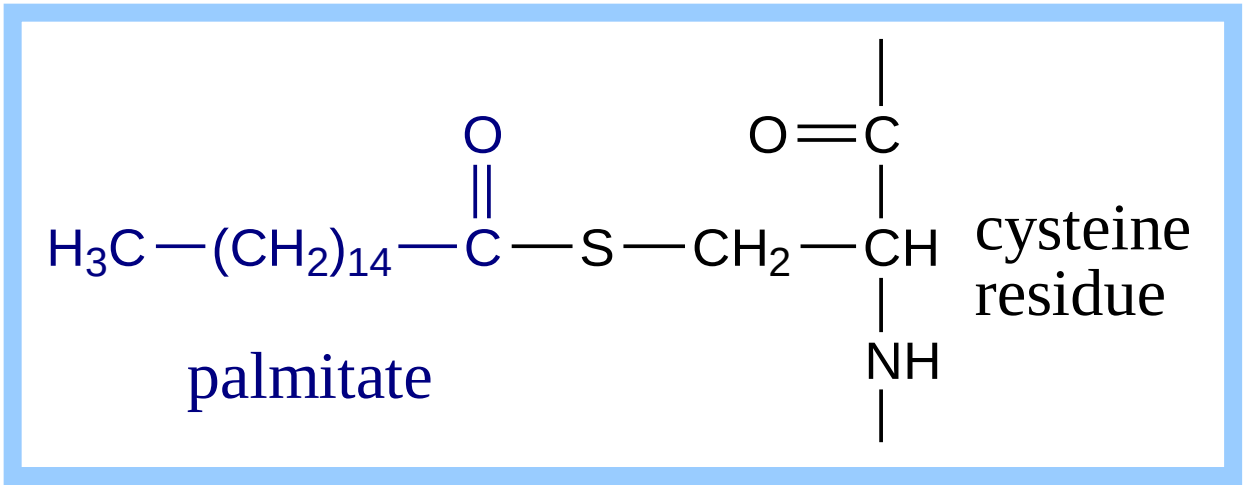
E.g., **pleckstrin homology** (**PH**) domains bind to phosphorylated derivatives of phosphatidylinositol.

Some PH domains bind **PIP_2** (PI-4,5-P_2).



Other **pleckstrin homology** domains recognize and **bind** phosphatidylinositol derivatives with **P_i** esterified at the **3' OH** of inositol. E.g., PI-3-P, PI-3,4-P_2, PI-3,4,5-P_3.



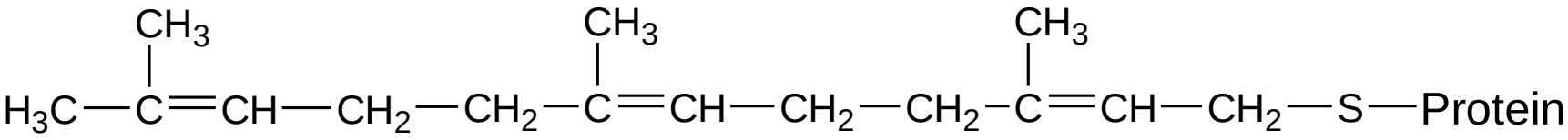


Some proteins bind to membranes via a covalently attached **lipid anchor**, that inserts into the bilayer.

A protein may link to the **cytosolic surface** of the plasma membrane via a covalently attached **fatty acid** (e.g., palmitate or myristate) or an **isoprenoid** group.

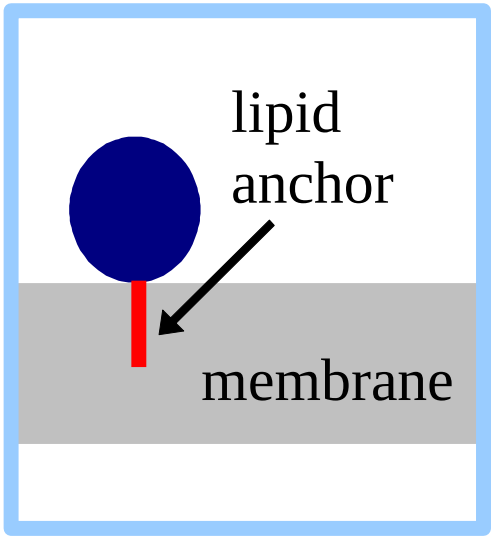
Palmitate is usually attached via an ester linkage to the thiol of a cysteine residue.

A protein may be **released** from plasma membrane to cytosol via **depalmitoylation**, hydrolysis of the ester link.



farnesyl residue linked to protein via cysteine S

An **isoprenoid** such as a **farnesyl** residue, is attached to some proteins via a thioether linkage to a cysteine thiol.



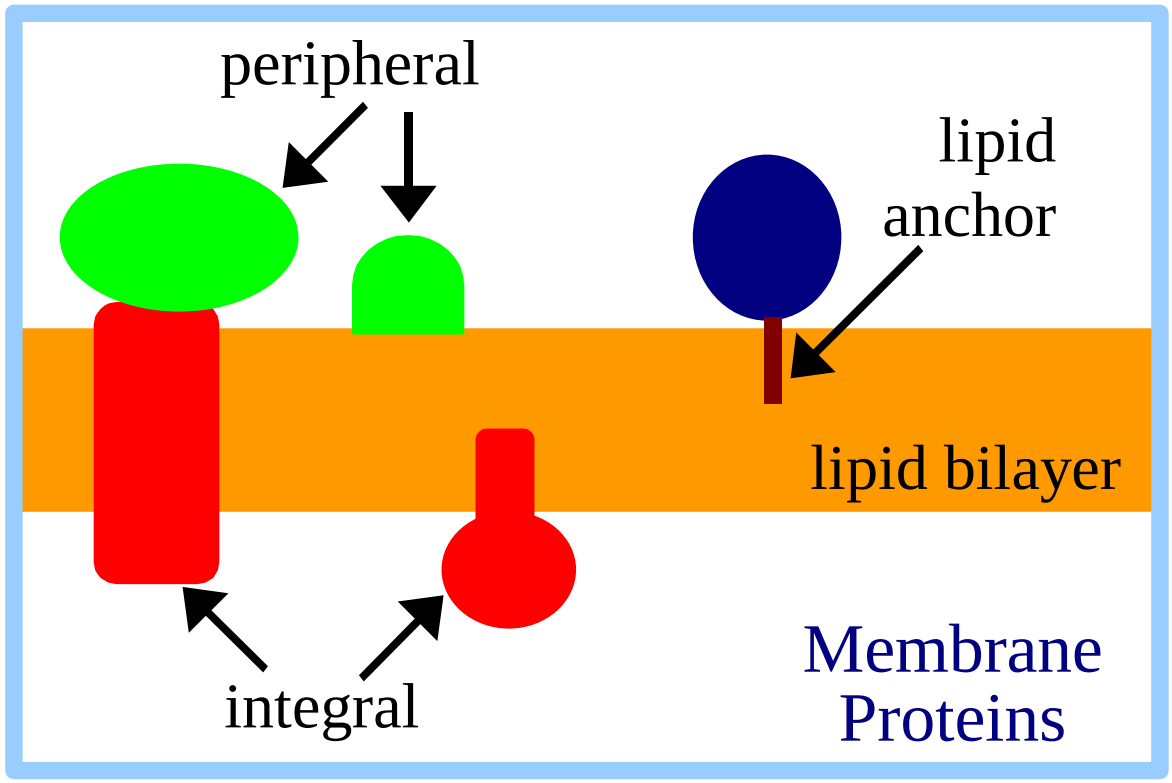
Glycosylphosphatidylinositols (**GPI**) are complex glycolipids that attach some proteins to the **outer surface** of the plasma membrane.

The linkage is similar to the following, although the oligosaccharide composition may vary:

protein (C-term.) - phosphoethanolamine – mannose - mannose - mannose - *N*-acetylglucosamine – inositol (of PI in membrane)

The protein is tethered some distance out from the membrane surface by the long oligosaccharide chain.

GPI-linked proteins may be released from the outer cell surface by phospholipases.

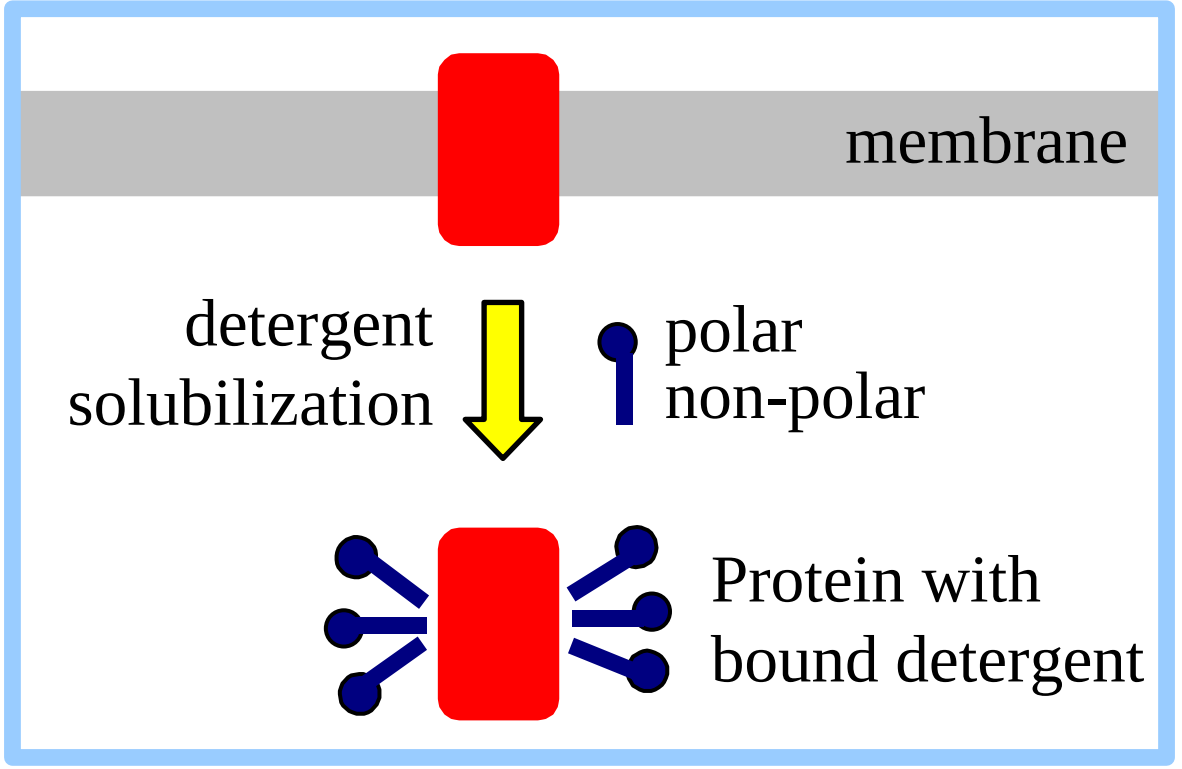


Integral proteins have domains that extend into the hydrocarbon core of the membrane.

Often they span the bilayer.

Intramembrane domains have largely **hydrophobic surfaces**, that interact with membrane lipids.

Amphipathic detergents are required for solubilization of integral proteins from membranes.



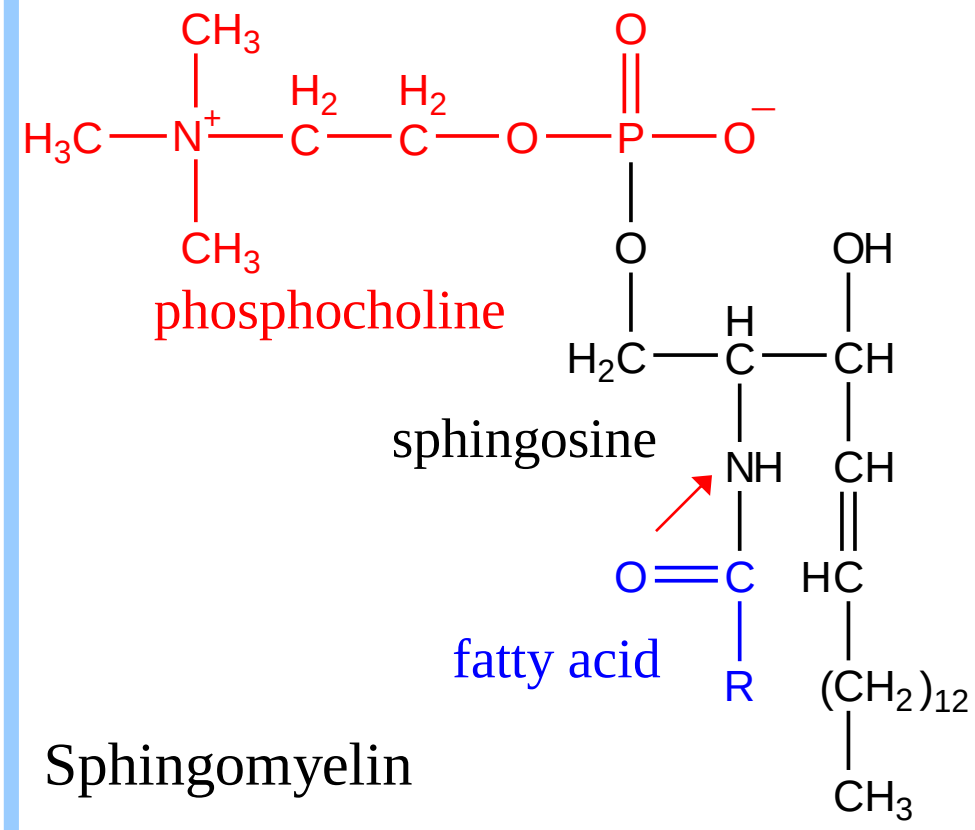
- **Hydrophobic** domains of detergents substitute for lipids, coating hydrophobic surfaces of integral proteins.
- **Polar** domains of detergents interact with water.

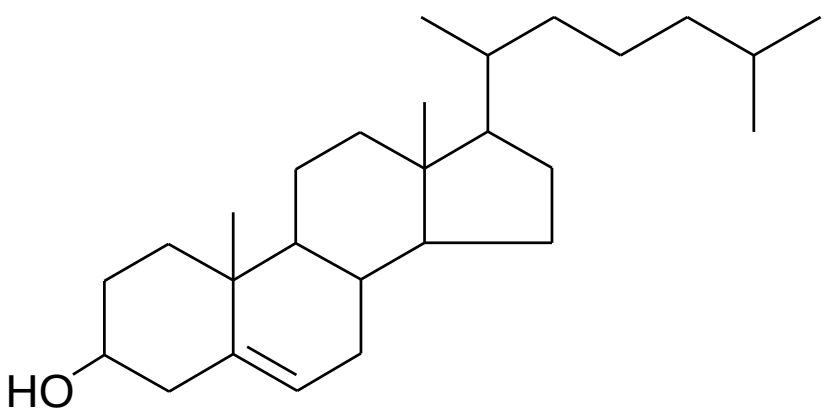
If detergents are removed, purified integral proteins tend to **aggregate** & come out of solution. Their hydrophobic surfaces associate to minimize contact with water.

Lipid rafts:

- Complex **sphingolipids** tend to separate out from glycerophospholipids & co-localize with **cholesterol** in membrane microdomains called **lipid rafts**.
- Membrane fragments assumed to be lipid rafts are found to be **resistant to detergent solubilization**, which has facilitated their isolation & characterization.

- Differences in **molecular shape** may contribute to a tendency for sphingolipids to separate out from glycerophospholipids in membrane **microdomains**.
  - **Sphingolipids** usually **lack double bonds** in their fatty acid chains.
  - **Glycerophospholipids** often include at least one fatty acid that is **kinked**, due to one or more double bonds.
  - See diagram (in article by J. Santini & coworkers).
- **Lipid raft** domains tend to be **thicker** than adjacent membrane areas, in part because the saturated hydrocarbon chains of sphingolipids are more extended.

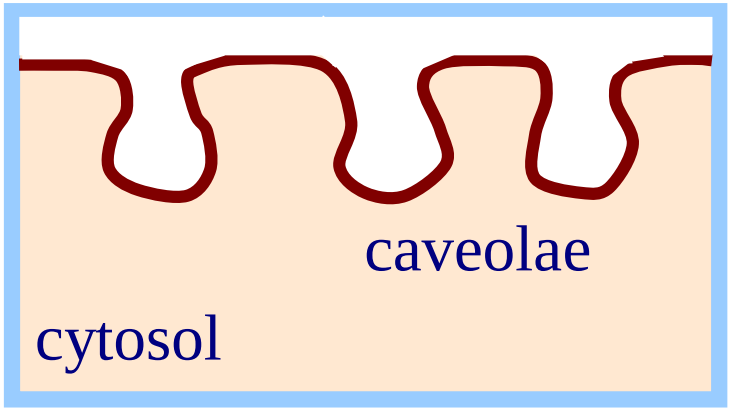




- Hydrogen bonding between the hydroxyl group of cholesterol and the **amide** group of sphingomyelin may in part account for the observed affinity of cholesterol for sphingomyelin in raft domains.

- **Proteins** involved in cell **signaling** often associate with **lipid raft** domains.
  - Otherwise **soluble signal proteins** often assemble in complexes at the cytosolic surface of the plasma membrane in part via insertion of attached fatty acyl or isoprenoid **lipid anchors** into **raft domains**.
  - **Integral proteins** may concentrate in raft domains via interactions with raft lipids or with other raft proteins.
  - Some raft domains contain derivatives of phosphatidylinositol that bind signal proteins with **pleckstrin homology** domains.

- **Caveolae** are **invaginated lipid raft domains** of the plasma membrane that have roles in cell signaling and membrane internalization.



- **Caveolin** is a protein associated with the cytosolic leaflet of the plasma membrane in caveolae.

  Caveolin interacts with **cholesterol** and self-associates as **oligomers** that may contribute to deforming the membrane to create the unique morphology of caveolae.

Electron micrograph & information about caveolae
(home page of Deborah Brown at SUNY Stony Brook).

Diagram & information about lipid rafts
(website of Maciver lab at University of Edinburgh).

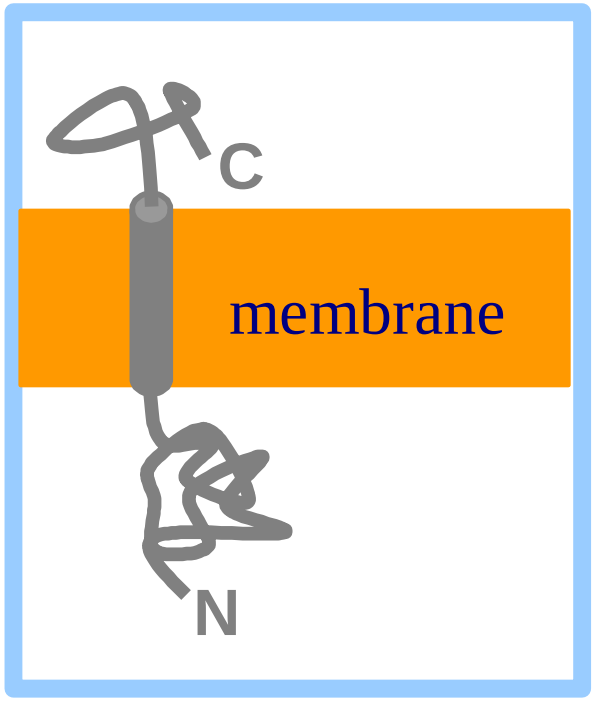
Integral protein structure

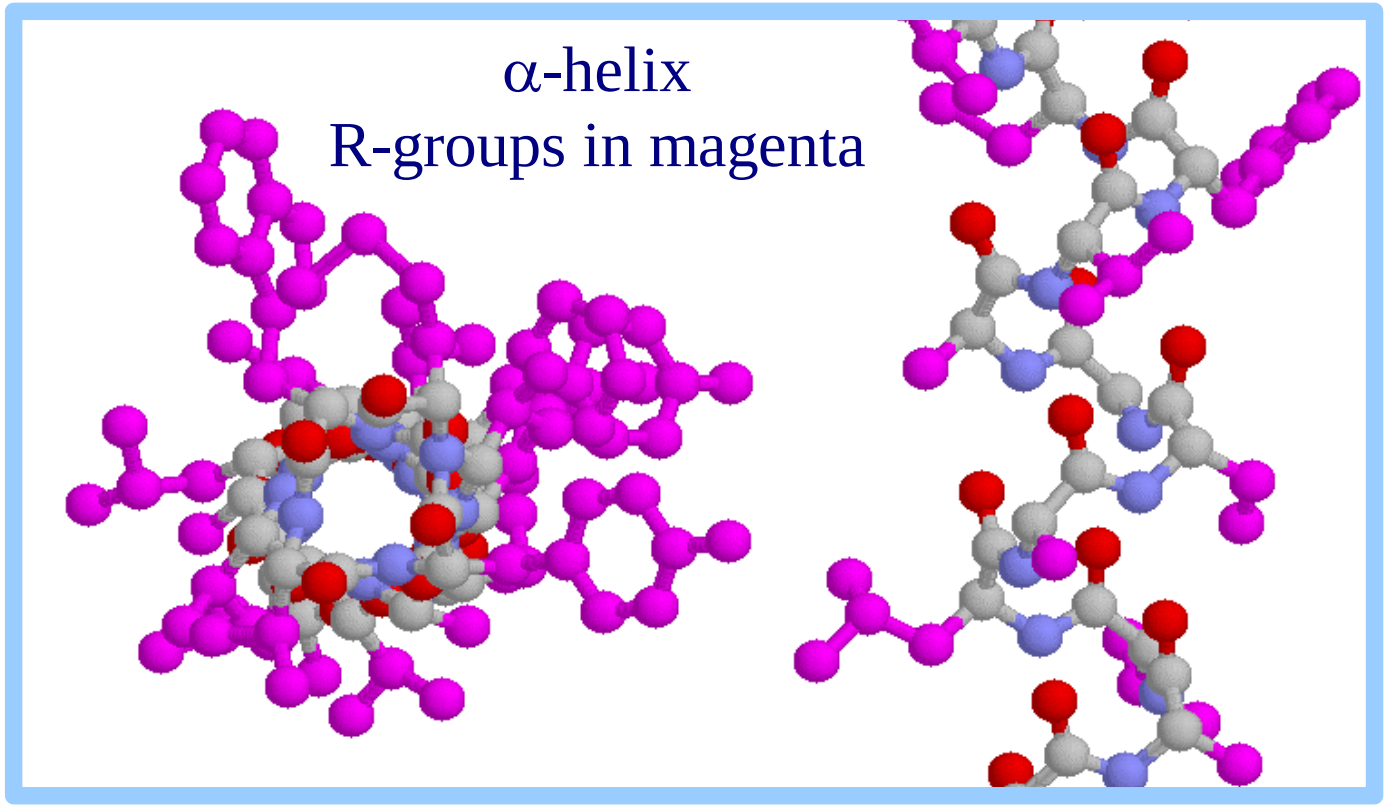
Atomic-resolution **structures** have been determined for a small (but growing) number of integral membrane proteins.

Integral proteins are **difficult to crystallize** for X-ray analysis.

Because of their **hydrophobic** transmembrane domains, detergents must be present during crystallization.

A **membrane-spanning α-helix** is the most common structural motif found in integral proteins.



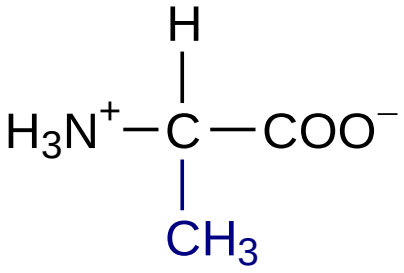


In an **α-helix**, amino acid R-groups protrude out from the helically coiled polypeptide backbone.

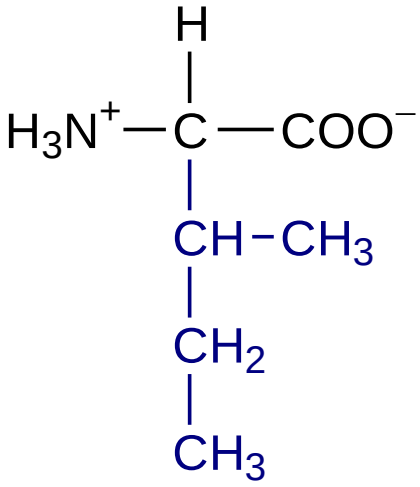
The largely hydrophobic R-groups of a membrane-spanning α-helix contact the hydrophobic membrane core, while the more polar peptide backbone is buried.

Colors: **C** **N** **O** **R-group** (H atoms not shown).

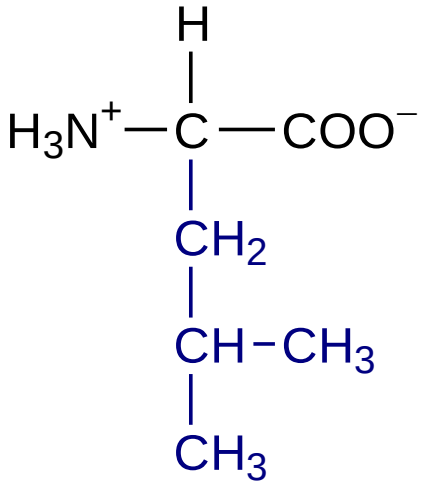
alanine (Ala, A)



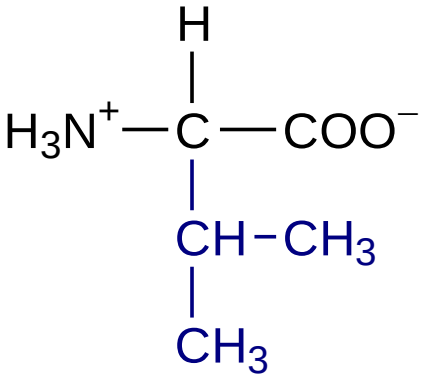
isoleucine (Ile, I)



leucine (Leu, L)



valine (Val, V)

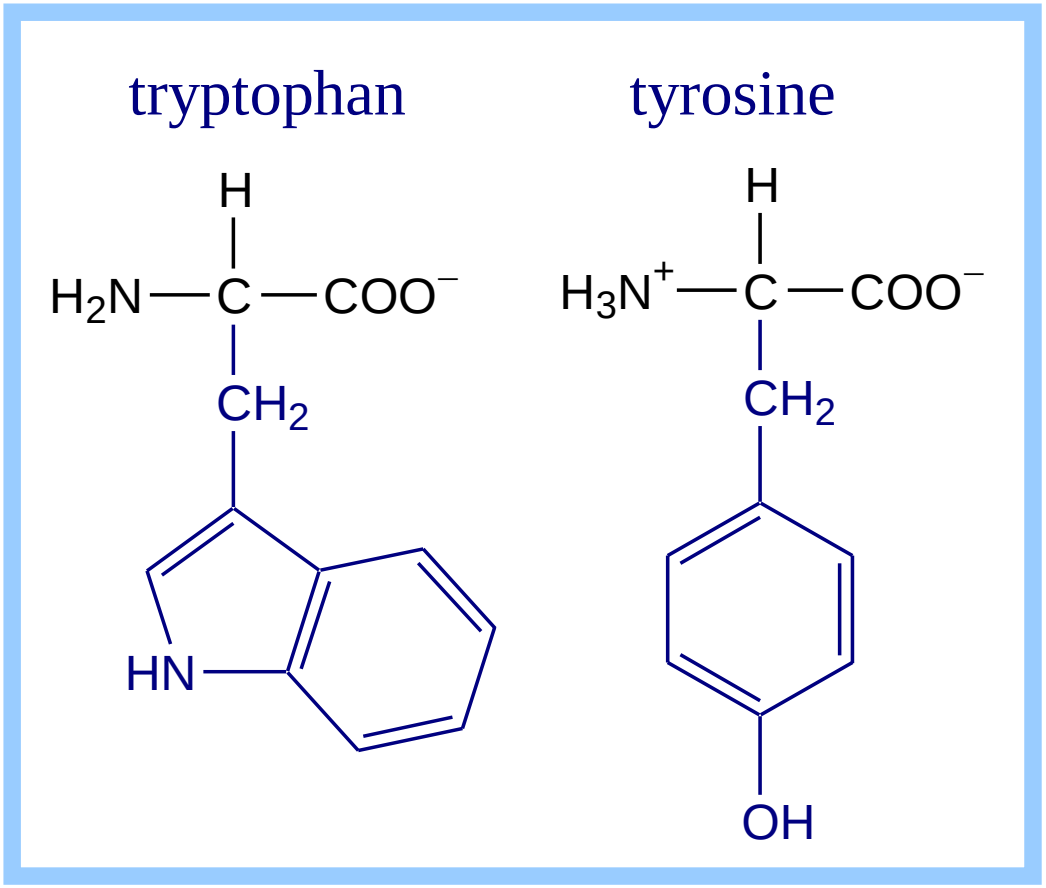


amino acids: non-polar aliphatic R-groups

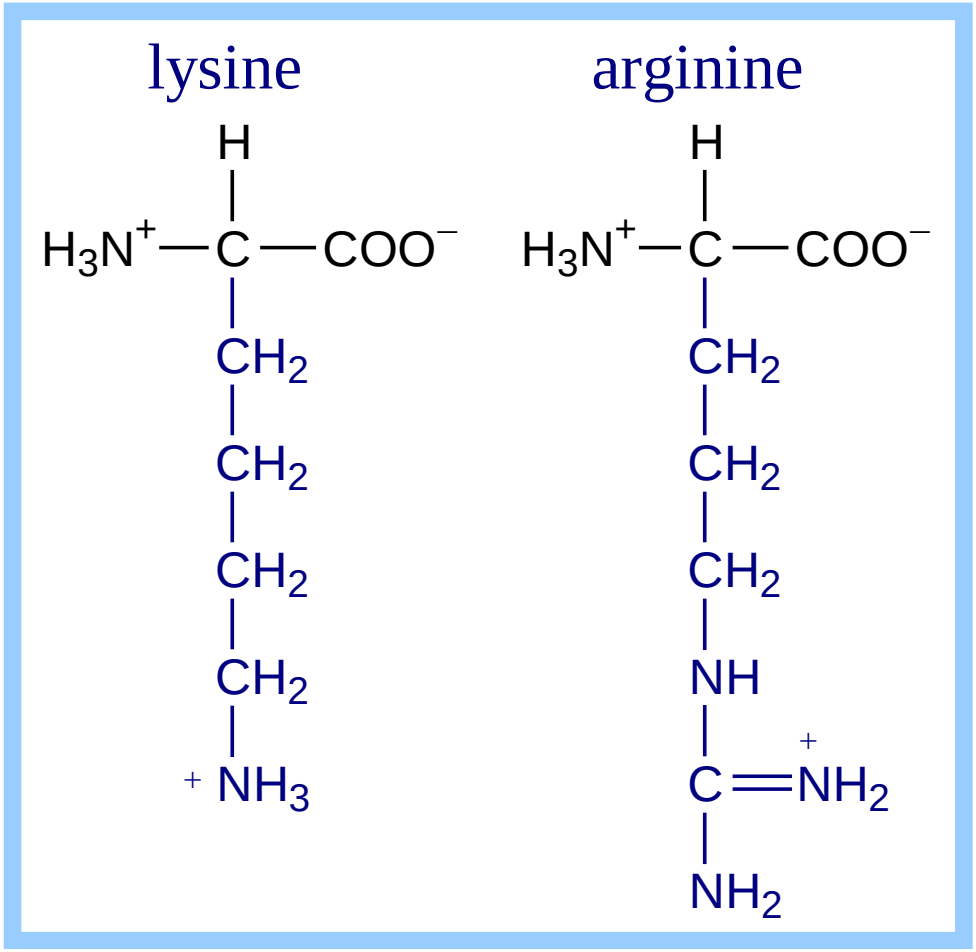
Particular **amino acids** tend to occur at different positions relative to the surface or interior of the bilayer in transmembrane segments of integral proteins.

Residues with **aliphatic** side-chains (leucine, isoleucine, alanine, valine) predominate in the **middle** of the bilayer.

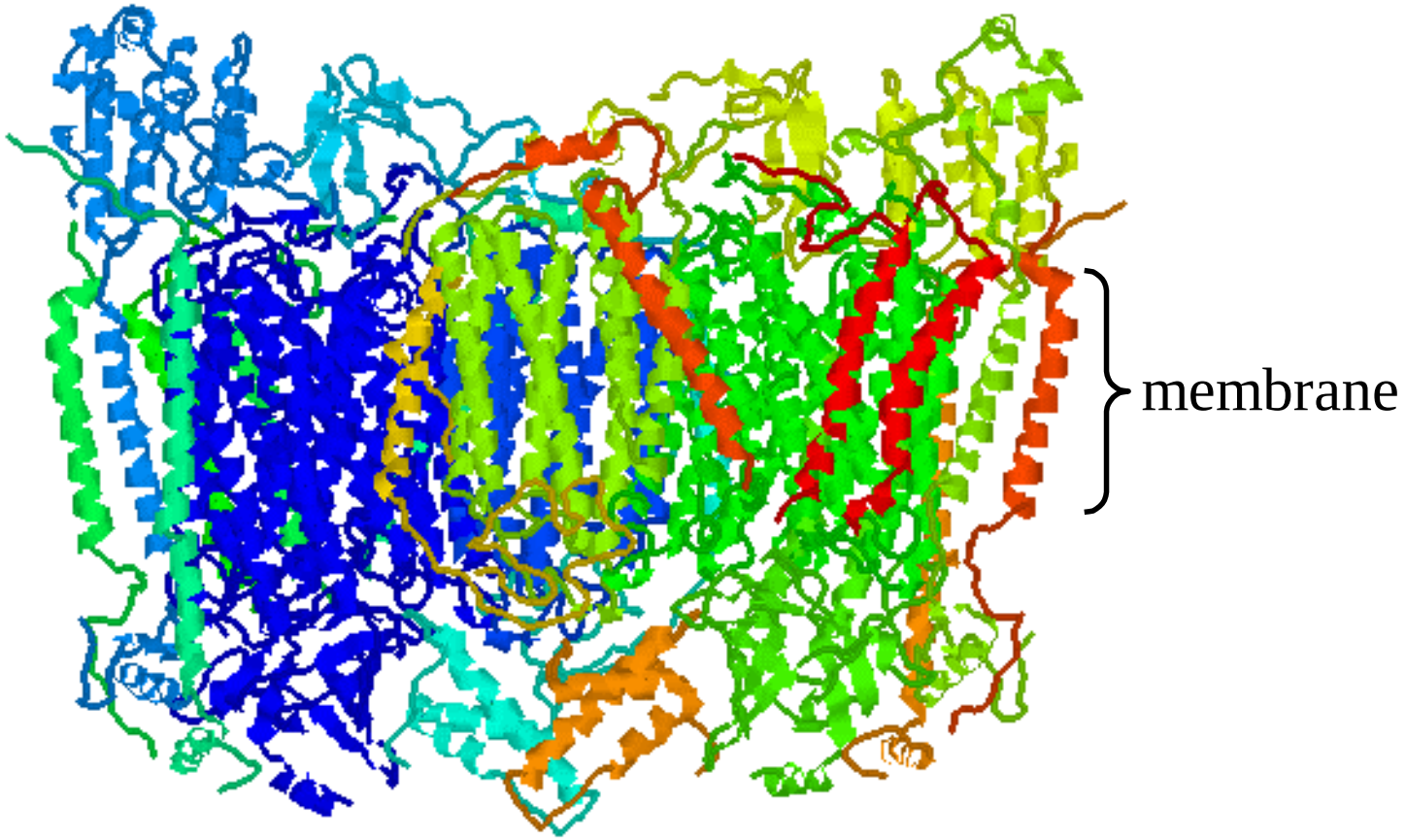
Tyrosine and **tryptophan** are common near the membrane surface.



It has been suggested that the polar character of the tryptophan amide group and the tyrosine hydroxyl, along with their hydrophobic ring structures, suit them for localization at the polar/apolar interface.



Lysine & **arginine** are often at the lipid/water interface, with the **positively charged** groups at the ends of their aliphatic side chains extending toward the polar **membrane surface**.



Cytochrome oxidase dimer (PDB file 1OCC)

Cytochrome oxidase is an integral protein whose intra-membrane domains are mainly transmembrane α-helices.

Explore with Chime the α-helix colored green at far left.

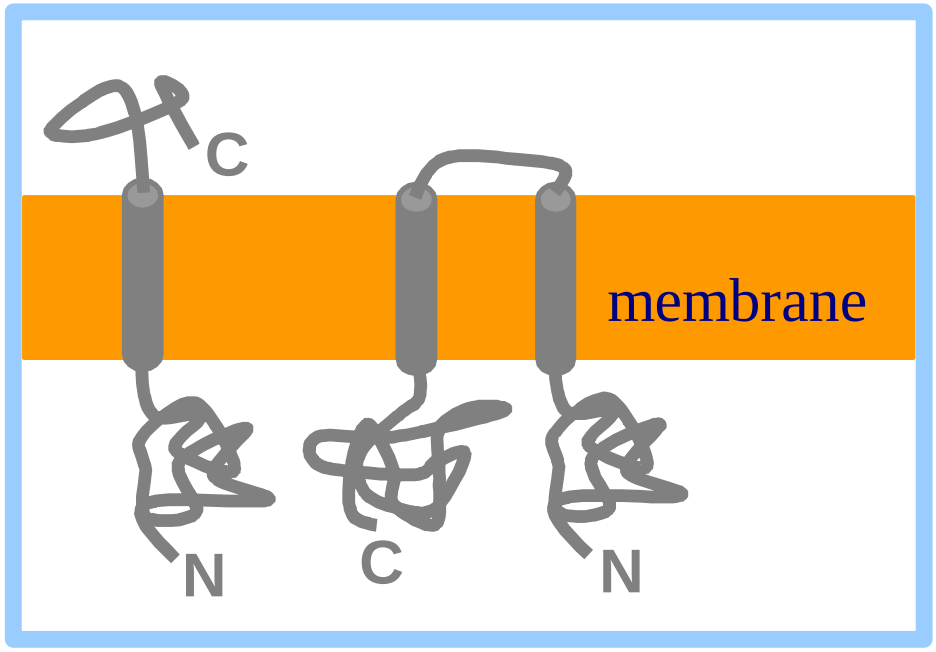
A 20-amino acid α-helix just spans a lipid bilayer.

Hydropathy plots are used to search for 20-amino acid stretches of hydrophobic amino acids in the primary sequence of a protein for which a crystal structure is not available.

Putative hydrophobic transmembrane α-helices have been identified this way in many membrane proteins.

Hydropathy plots alone are not conclusive.

Protein topology studies are used to test the transmembrane distribution of protein domains predicted by hydropathy plots.



- If a hydropathy plot indicates **one** 20-amino acid hydrophobic stretch (1 putative transmembrane α-helix), topology studies are expected to confirm location of N & C termini on opposite sides of membrane.
- If **two** transmembrane α-helices are predicted, N & C termini should be on the same side. The segment between the α-helices should be on the other side.

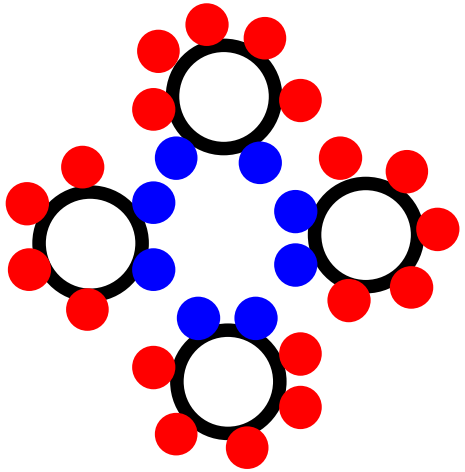
Transmembrane topology is tested with **impermeant probes**, added on one side of a membrane. For example:

- **Protease enzymes**. Degradation a protein segment indicates exposure to the aqueous phase on the side of the membrane to which a protease is added.
- **Monoclonal antibodies** raised to peptides equivalent to individual segments of the protein. Binding indicates surface exposure of a protein segment on the side to which the Ab is added.

Such studies have shown that all copies of a given type of integral protein have the same orientation relative to the membrane. Flip-flop of integral proteins does not occur.

A “**helical wheel**” looks down the axis of an α-helix, projecting side-chains onto a plane.

Simplified helical wheel diagram of four α-helices lining the lumen of an ion channel.



- Polar amino acid R-group
- Non-polar amino acid R-group

An α-helix lining a **water-filled channel** might have polar amino acid R-groups facing the lumen, & non-polar R-groups facing lipids or other hydrophobic α-helices.

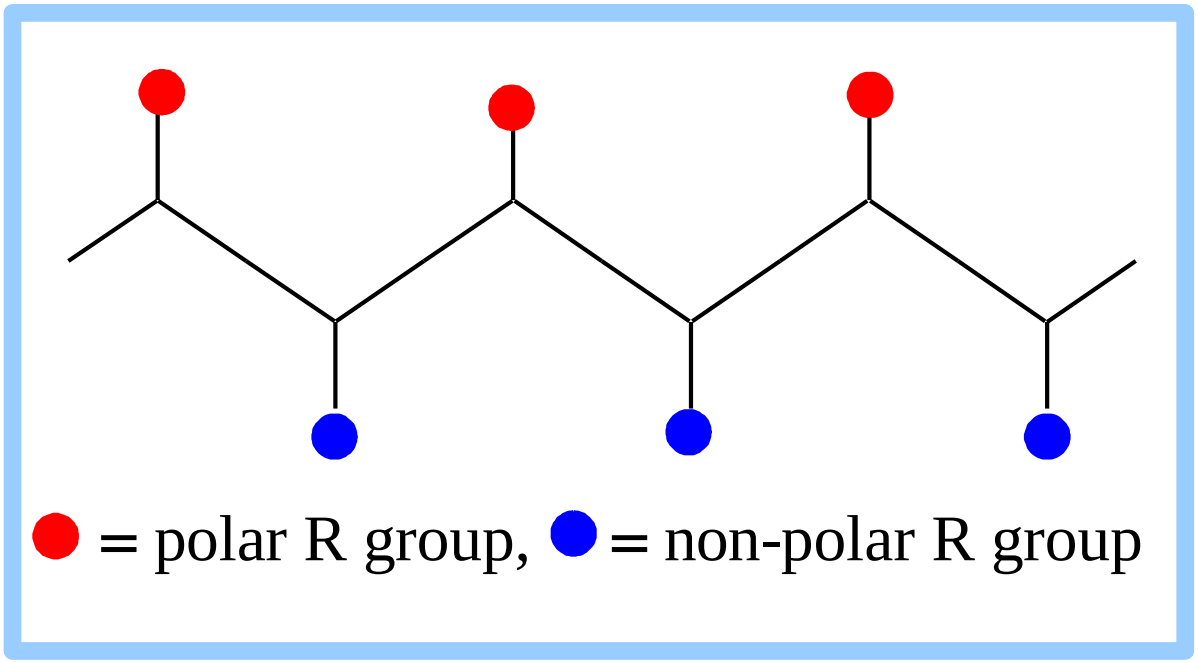
Such **mixed polarity** would prevent detection by a hydropathy plot.

Porin β-barrel

While transmembrane α-helices are the most common structural motif for integral proteins, a family of bacterial outer envelope channel proteins called **porins** have instead **β barrel** structures.

A β barrel is a **β sheet** rolled up to form a cylindrical pore.

At right is shown one channel of a trimeric porin complex.



In a β-sheet, amino acid R-groups alternately point above & below the sheet.

Much of porin primary structure consists of **alternating polar & non-polar amino acids**.

- Polar residues face the aqueous lumen.
- Non-polar residues are in contact with membrane lipids.

Explore an example of a bacterial **porin** with Chime.