BCMB 3100 – Lipids
(Text Chapters 11, 12, 13)

- Definition
- Major classes
- Fatty acids
- Triacylglycerol
- Glycerophospholipids
- Sphingolipids
- Cholesterol

- Lipids are hydrophobic or amphipathic
- In BCMB 3100 we will emphasize:
  - phospholipids
  - glycolipids
  - cholesterol (steroid)

- __________: main lipids in most biological membranes
- __________: 2nd most abundant lipid in membranes
  (abundant in CNS) from animals and plants

See Table 11.1

<table>
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<tr>
<th>Number of carbons</th>
<th>Number of double bonds</th>
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<th>IUPAC name</th>
<th>MELTING point, °C</th>
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- **Structure and nomenclature of fatty acids**
- Unsaturated FA - at least one C-C double bond
- Saturated FA - no C-C double bonds
Structural relationships of major lipid classes

Membrane lipids: 3 major types = phospholipids, glycolipids, & cholesterol

An example

Note: the types of fatty acyl groups present in any given triacylglycerol may vary.
Fatty acids in biological organisms

Fatty acid chains (long aliphatic tails) in phospholipids & glycolipids contain even # of carbons (12-20) with 16 and 18 being most common

Fatty acids can be ___________ or ___________

Under physiological conditions fatty acids are ionized (pKa 4.5-5.0)

Phospholipases hydrolyze phospholipids

--- enzymes that catalyze hydrolysis of triacylglycerols

--- catalyze hydrolysis of glycerophospholipids

See Fig. 11.6

See Fig. 11.7
(a) **structural backbone of sphingolipids**

(b) **sphingosine + fatty acid at C2**

(c) **Sphingomyelin**: present in plasma membrane & myelin sheath around neurons

See Fig. 11.8

Example of a **Ganglioside** Ganglioside GM2

(NeuNAc in blue)

Cell surface, cell-cell interactions (e.g. blood group antigens)

Hexosaminidase A cleaves here

Mutation → Tay-Sachs disease

Example of a **Cerebroside**: abundant in nerves

Sugar-Sphingosine

Fatty acid

• **Structure of a galactocerebroside**

See Pg. 186/196

**Structural relationships of major lipid classes**

Steroids are polypropyl compounds

In eukaryotes but NOT in most prokaryotes

Other steroids: steroid hormones (estradiol, testosterone, corticosteroids), bile salts, sterols in plants, yeast, fungi

See Pg. 187/197
• Cholesterol modulates the fluidity of mammalian cell membranes
• It is also a precursor of the steroid hormones and bile salts

**Waxes:** esters of long-chain monohydroxylic alcohols and long-chain fatty acids (nonpolar)
Waxes are very water insoluble and high melting point
They are widely distributed in nature as protective waterproof coatings on leaves, fruits, animal skin, fur, feathers and exoskeletons

\[
\text{Myricyl palmitate, a wax} \quad \text{O} \\
\text{H}_3\text{C}-(\text{CH}_2)_{14}-\text{C}-\text{O}-(\text{CH}_2)_{29}-\text{CH}_3
\]

\text{Palmitate portion} \quad \text{Myricyl alcohol portion}

**Eicosanoids:** oxygenated derivatives of C20 polyunsaturated fatty acids (e.g. arachidonic acid)

- Arachidonic acid can cause constriction of blood vessels
- Prostaglandin E2 involved in blood clot formation
- Leukotriene B4 mediator of smooth-muscle contraction and bronchial constriction seen in asthmatics

\text{Arachidonic acid and three eicosanoids}

**Some vitamins are Lipid Vitamins**

- Four lipid vitamins: A, D, E, K
- All contain rings and long, aliphatic side chains
- All are highly hydrophobic
- The lipid vitamins differ widely in their functions

* Examples of isoprenoids

See Fig. 15.18 / 15.19 for structures
BCMB 3100 - Lipids

- Biological Membranes
- Micelles
- Lipid Bilayer
- Peripheral membrane proteins
- Integral membrane proteins
- Lipid-anchored
- Transport across membranes
- Signal Transduction

Structure of a typical eukaryotic plasma membrane

See Fig. 12.1; 12.8

- Highly selective permeability barriers that surround cells & cellular compartments
- Sheetlike structures of ~60-100 Å
- Consists mostly of lipids & proteins in ratio of 1:4 to 4:1 (typical 40% lipid; 60% protein). Lipids & proteins may be glycosylated.

- Lipids in biological membranes are ______________: hydrophilic (polar) head group & hydrophobic tail. Spontaneously form bilayers in aqueous solution.

Membrane lipid and bilayer

Stereo view of cholesterol

- Polar OH (red), fused ring system nearly planar
Amphipathic nature of cerebroside

Amphipathic lipids can take two different forms in aqueous media: ___________ or ______________.

___________: a globular structure in which polar head groups are on the surface and hydrocarbon tails are on the inside.

Salts of fatty acids tend to form micelles. Micelles usually are < 200 μm in diameter.

___________: favored structure for phospholipids & glycolipids since lipids with two fatty acyl chains are too large to fit into the center of a micelle. Bilayers can have large dimensions (10^7 Å, 1mm) (recall diameter = ~60-100Å).

Lipid bilayers self-assemble due to hydrophobic interactions between hydrocarbon tails (main force), van der Waals attractive forces between hydrocarbon tails, & electrostatic & H-bonding forces between polar head groups and water.

Bilayers are extensive, closed, and self-sealing.
Preparation of liposomes  Fig. 12.3

Lipid bilayers are permeability barriers to ions & polar molecules

Lipid vesicles (liposomes): aqueous compartments enclosed by lipid bilayers. Small vesicles (∼ 500 Å), large vesicles (∼10^4 Å, 1 µm)

Use lipid vesicles to measure membrane permeability
1. Form vesicles in solution containing A
2. Separate vesicles from free A
3. Measure flux of A out of vesicles  Fig. 12.2

Results
Permeability coefficient (cm/s)

Na⁺ 10^{-12}; Trp 10^{-7}; indole ∼5×10^{-4}; water ∼5×10^{-3}

Water & hydrophobic molecules readily traverse membranes while ions & most polar molecules do not

Lipid Bilayers and Membranes Are Dynamic Structures
(a) Lateral diffusion is very rapid
(b) Transverse diffusion (flip-flop) is very slow

Experiment showing that lateral diffusion occurs in biological membranes via use of heterokaryons (Frye & Edidin)

• Diffusion of membrane proteins

Fluorescence recovery after photobleaching (FRAP) evidence for fluid membrane.  Fig. 12.14
Biological Membranes (cont.)

- Contain ________ both embedded in the bilayer & on its surface. ________ may function as pumps, gates, receptors, energy transducers & enzymes.
- Held together by noncovalent interactions
- Asymmetric: the two surfaces (faces) differ in properties
- Two dimensional fluids - lipids & proteins rapidly diffuse in plane of membrane but NOT across membrane
  - membrane proteins and lipids can rapidly diffuse laterally or rotate within the bilayer (Singer & Nicolson, 1972)
- Compositions of biological membranes vary considerably among species and cell types

Freeze-fracture electron microscopy, shows the distribution of membrane proteins

Phase transition of a lipid bilayer
- Fluid properties of bilayers depend upon the flexibility of their fatty acid chains

| Ordered state: a rigid state in which all C-C bonds have trans conformation (all trans) | Fluid state: a relatively disordered state in which some of the C-C bonds are in the gauche conformation |

Transition from rigid to partly fluid state occurs at $T_M$, the __________

$T_M$ depends on ________ of fatty acyl chains & on degree of ________

Rigid state favored by saturated fatty acyl chains
Disordered state favored by cis double bound(s) (i.e. $T_M$ is lowered)

Prokaryotes regulate membrane fluidity by varying # of double bonds & length of fatty acyl chains. As temperature changes from 42°C to 27°C ratio of saturated:unsaturated changes from 1.6 to 1

Packing of fatty acid chains in membrane is disrupted by double bounds and lowers Tm.
Effect of cholesterol on phase transition ($T_m$) of membranes

In eukaryotes, membrane fluidity is largely regulated by ___________. Cholesterol moderates the fluidity of membranes (prevents tight packing of fatty acyl chains & blocks large motions).

Addition of 20 mol% cholesterol broadens phase transition.

Cholesterol modulates fluidity of the membranes. Also, association with sphingolipids leads to cholesterol-rich regions called **lipid rafts** that may effect specific membrane-protein function.

Structure of a typical eukaryotic plasma membrane

Three types of membrane-associated proteins:

- ____________: loosely bound to membrane by H-bonds or electrostatic forces, generally water soluble once released from membrane using high salt or pH. Often bound to integral membrane proteins.
- ____________: proteins firmly bound to membrane by hydrophobic interactions. Solubilized with detergents. Most have one or more membrane spanning domains (e.g. $\alpha$-helix with ~20 amino acids).

Integral and peripheral membrane proteins

Fig. 12.7

Fig. 12.8
Stereo view of bacteriorhodopsin: an integral membrane protein
See Fig. 12.9

Bacterial porin
Fig. 12.10

Lipid-anchored membrane proteins: proteins covalently linked to lipid membrane
Types of links:
* direct amide or ester bond between amino acid and fatty acyl group such as myristate or palmitate
* prenylation: link to an isoprenoid chain (e.g. farnesyl or geranylgeranyl) via the S of a Cys near the C terminus of the protein
* glycosylphosphatidylinositol anchor: C-terminal \( \alpha \)-carboxyl of protein-phosphoethanolamine-glycan-phosphatidylinositol

Lipid-anchored membrane proteins

Glycosylphosphatidylinositol-anchored protein (GPI-anchored protein)

Characteristics of membrane transport. Small uncharged molecules can diffuse across membranes. Other molecules require protein assisted movement

<table>
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<tr>
<th>Characteristics of different types of membrane transport</th>
<th>Table 9.3</th>
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<td>Protein carrier</td>
<td>Saturable with substrate</td>
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<td>Simple diffusion</td>
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<tr>
<td>CHANNELS AND PORES</td>
<td>Yes</td>
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<tr>
<td>Passive transport</td>
<td>Yes</td>
</tr>
<tr>
<td>Active transport</td>
<td>Primary</td>
</tr>
<tr>
<td></td>
<td>Secondary</td>
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Membrane transport through a pore or channel
Central passage through water-filled pore allows specific molecules to transverse the membrane (e.g. porin in mitochondria outer membrane)

There are many types of channels; ion channels can transport ions much faster than pumps

Examples: voltage-gated channels, ligand-gated channels, transient receptor potential channels
Types of passive and active transport

See Fig. 12.19/12.18

Active & passive transporters undergo a conformational change to drive transport

Active transporters move molecules against a concentration gradient: 1º transporters use 1º energy source (e.g. light, ATP, electron transport); 2º transporters driven by ion gradient

Primary active transporter: Na⁺-K⁺ ATPase
Secondary active transporter: glucose transporter

Active transport in E. coli

• Oxidation of S₉₆ generates a transmembrane proton gradient
• Movement of H⁺ down its gradient drives lactose transport (lactose permease)

Potassium ion channel (1) Fig. 12.22

Potassium ion channel (2) Fig. 12.23
Selectivity filter of K⁺ ion channel
Potassium ion channel (3) Fig. 12.24
Energetic basis of ion selectivity in K+ ion channel.

Potassium ion channel (4) Fig. 12.24
Energetic basis of ion selectivity in K+ ion channel.

Potassium ion channel (5) Fig. 12.25
Rapid rate of K+ movement due to structure of channel and electrostatic repulsion of incoming K+

Molecules and complexes that are too large to be transported via transport proteins are transported in lipid vesicles out of the cell via exocytosis, and into the cell via endocytosis. We will not cover these processes in this course.

Signal transduction through a membrane

Three general classes of membrane receptor proteins:
• seven-transmembrane-helix receptors
• Dimeric receptors that recruit protein kinases
• Dimeric receptors that are protein kinases
General mechanism of signal transduction across a membrane

e.g. hormones

Tyrosine kinase
* Adenylyl cyclase
* Phospholipase C

Membrane receptor → Transducer → Effective enzyme → Second messenger → Cytosolic and nuclear effectors → Cellular response

Common secondary messengers Fig. 13.2

G-protein cycle

• G proteins are activated by binding to a receptor-ligand complex
• G-proteins are inactivated slowly by their own GTPase activity (kcat about 3/min)

Understanding G Proteins:
Hydrolysis of GTP to GDP and Pi

Summary of the adenylyl cyclase signaling pathway

Example of seven-transmembrane-helix (7TM) receptor

Production, inactivation of cAMP

See Fig 13.6, 13.7; 13.8

Continued next slide
Activation of protein kinase A by cAMP

See Fig. 13.7

Caffeine & theophylline inhibit cAMP phosphodiesterase

- Inhibition of cAMP phosphodiesterases prolongs the effects of cAMP
- This increases the intensity and duration of stimulatory hormones

See Fig. 13.11, 13.12

Phosphatidylinositol 4,5-bisphosphate (PIP$_2$) produces IP$_3$ and diacylglycerol

See Fig. 13.11
• Activation of receptor tyrosine kinases by ligand-induced dimerization

See Fig. 13.15

• Phosphorylated dimer phosphorylates cellular target proteins

\[ nATP \rightarrow nADP \]

(continued)

• Each domain catalyzes phosphorylation of its partner

Insulin receptor and tyrosine kinase activity

• Insulin binds to 2 extracellular \( \alpha \)-chains
• Transmembrane \( \beta \)-chains then autophosphorylate
• Tyrosine kinase domains then phosphorylate insulin-receptor substrates (IRRs) (which are proteins)

Insulin-stimulated formation of PIP₃

See Figs. 13.17/13.17-13.21/13.22

\*phosphatidylinositol 3,4,5-trisphosphate

A different type of tyrosine kinase signal transduction: Growth hormone receptor for which binding brings together associated proteins with tyrosine kinase domains

Fig. 13.13
Cross-phosphorylation of two JAK2 induced by hormone receptor dimerization

Small G proteins (small GTPases) are a large superfamily of signalling proteins. They include: Ras, Rho, Aft, Rab, and Ran.

Small GTPases cycle between an active GTP-bound form and an inactive GDP-bound form.

Small GTPases are smaller (20-25kd) and monomer compared to the larger (30-35 kd) and trimeric G proteins.

Extra material

Structure and nomenclature of fatty acids

- **Saturated** FA - no C-C double bonds
- **Unsaturated** FA - at least one C-C double bond
- **Monounsaturated** FA - only one C-C double bond
- **Polyunsaturated** FA - two or more C-C double bonds

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<td>18:2 linoleate</td>
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Another method to measure membrane permeability

Mueller & Rudin: dip paint brush into lipid membrane solution & paint across 1 mm diameter hole partitioned between two aqueous media. \(\rightarrow\) macroscopic bilayer membrane

Measure electrical conductance from one media to the other.