Medical Biochemistry 531

Lecture 31 - Membranes I

Composition, Structure and Functions of Biological Membranes

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Recommended Reading - Lecture 31

- Devlin - Chapter 5 (best)
- Marks’ - Chapter 10 (not bad)
- Handouts
- Champe and Harvey - nothing much useful
- Voet and Voet - weak
- Stryer - weak
membranes of both eukaryotic and prokaryotic cells share the same chemical components, structural organization, and properties

- there are differences in specific lipid, protein and carbohydrate constituents, but not physicochemical interactions

- trilaminar appearance (by transmission electron microscopy) of plasma membrane

- 7-10 nm wide, some thinner (intracellular)

- **chemical asymmetry** of composition

- **dynamic structure** allowing cell movement

- **organized sea of lipid in a fluid state**

- non-aqueous cellular compartment
Biological Membranes - Introduction

- control the composition of enclosed space:
  - exclusion of molecules
  - selective transport allowing movement of specific molecules outside in, inside out
    - transporters are proteins
- modulate intracellular environment, subcellular compartments, controlling translocation of substrates, products, cofactors, ions, etc.
  - Influence on metabolic pathways (= regulation)
- hormones, growth factors (metabolic regulators) have specific protein receptors on the plasma membrane:
  - transduction of signals via intracellular intermediates (second messengers) generated from membrane lipids
- cell-cell recognition, cell shape, locomotion

Chemical Composition of Membranes

- lipids and proteins are the two major membrane components
- amount varies greatly between different types of membranes
- ~20% to >70% protein
  - comparing myelin sheath to the inner mitochondrial membrane
- higher content of protein (enzymes) in intracellular membranes
  - esp. mitochondrial membranes
- carbohydrates in the form of glycoproteins and glycolipids
Lipids are major components of membranes

- the three major components are **glycerophospholipids, sphingolipids, and cholesterol**
- small amounts of triacylglycerols, mono & diol derivatives
- glycerophospholipids are the most abundant:
  - phosphatidylethanolamine and phosphatidylcholine predominate
  - phosphatidylglycerol phosphoglyceride (diphosphatidyl glycerol or cardiolipin) nearly exclusively in mitochondrial inner membrane and bacterial membranes
- 4,5-bisphosphoinositol glycerophospholipids in plasma membranes:
  - source of inositol trisphosphate (IP₃) and diacylglycerol (DAG) second messengers in hormone signal transduction

Lipids are major components of membranes

- sphingolipids (from amino alcohols sphingosine and dihydrosphingosine) are also present in membranes
  - ceramide - sphingosine with saturated or unsaturated long chain FA in amide linkage on the amino group
  - glycosphingolipids
  - cerebrosides (gluco- or galacto-cerebrosides)
  - sulfatides
  - gangliosides
- **Cholesterol**
  - membrane fluidity
Lipid Composition Varies with Different Membranes

- lipid composition is similar for the same intracellular membrane of a specific tissue or cell type among different species
- plasma membranes exhibit the greatest variation
  - cholesterol and the various FAs esterified into membrane phospholipids are affected by the nutritional status of the individual (you are what you eat!)
- plasma membranes have the highest content of neutral lipids (TAGs) and sphingolipids
- neuronal tissue is richest in sphingolipids (esp. glycosphingolipids)

Lipid Composition Varies in Different Membranes

- quantitative differences in classes of lipids and individual lipids
  (a) amount of major lipid components as percentage of total lipids
  (b) phospholipid composition as percentage of total phospholipids

Other includes: mono, di, triacylglycerol, FAs, cholesterol esters

10/27/2004
Membrane Proteins Are Classified Based on Their *Ease of Removal* from Membranes

- **Peripheral (or extrinsic) proteins**
  - treatment with high salt or extremes of pH (acidic or basic)
  - little or no change in membrane integrity upon removal

- **Integral (or intrinsic) proteins**
  - require drastic treatment, e.g. detergents, organic solvents
  - disruption of membrane integrity upon removal
  - usually contain tightly bound lipid
  - the protein denatures and loses biological function when the lipid is removed
  - have sequences of hydrophobic amino acids creating hydrophobic domains in tertiary structure that interact with hydrophobic lipid hydrocarbons

Membrane Proteins Are Classified Based on Their *Ease of Removal* from Membranes

- **Proteolipids** - hydrophobic lipoproteins soluble in chloroform and methanol, insoluble in water
  - present in many membranes, particularly in myelin, >50% of the protein component
  - e.g. lipophillin of brain myelin, >65% hydrophobic amino acids and covalently bound lipid

- **Glycoproteins** - each with unique carbohydrates and content
  - carbohydrates are present as constituents of glycoproteins and glycolipids
  - exterior side of the plasma membrane and luminal side of ER
  - sugars include glucose, galactose, mannose, fucose, N-acetyl-galactosamine and -glucosamine, sialic acid

- enzymes, receptors, structural components of cells
- catalytic, transport, receptor, structural, or recognition roles
Micelles

- structural characteristics of cell membranes derive from the physicochemical properties of major lipid constituents, the phospholipids and sphingolipids
- amphipathic lipids (hydrophilic head, hydrophobic tail) (a) interact in an aqueous environment to form vesicular structures:
  - [lipid] for formation called “critical micelle concentration”
  - depends on temperature, ratio of different lipids
  - very stable structures - hydrophobic interactions of hydrocarbon tails, attraction of polar groups to water
  - important in digestion of lipids (role of bile salts)

Lipid Bilayers

- amphipathic lipids such as glycerophospholipids can form a bimolecular leaf structure with two layers of lipids
- polar head groups are at the interface between the aqueous medium and lipid
- hydrophobic tails interact to form an environment that excludes water
- bilayer conformation is the basic lipid structure of all biological membranes
- extremely stable structures held together by noncovalent interactions (hydrophobic tails and polar heads)
Liposomes

- lipid bilayers will self-seal if disrupted, to form a structure with minimal contact of the hydrocarbon chains with water
  - thermodynamically most stable configuration
- can form spherical vessels, separating external environment from internal space
- termed liposomes, formed by lipid bilayer closing on itself
- individual lipid interactions in bilayers have low energies of activation, providing for circumscribed mobility within the bilayer
- self-assembly of amphipathic lipids into bilayers (and liposomes) is an important property involved in the formation of biological membranes

Mobility of Lipid Components in Membranes

- **rapid rotational diffusion** around carbon-carbon bonds in FA chains, esp. at methyl end
- **very slow transverse (flip-flop) exchange** (thermodynamic constraints on movement of polar head group through lipophilic core)
- **rapid lateral diffusion** (random distribution of lipids in artificial membranes)
- **rapid flexing of hydrocarbon chains**
- **overall inherent fluidity, but stability**
Artificial Membrane Systems

- liposomes are formed from synthetic phospholipids and lipids extracted from natural membranes
- **unilamellar** and **multilamellar** (vesicles within vesicles) vesicles of 20 nm to 1 µm in diameter
- **interior aqueous environment**
- liposomes can be prepared with trapped aqueous substances inside
- used for studies of membrane, and membrane protein function
- highly useful as **carriers** for drugs, antibiotics, DNA, RNA delivery etc.

Fluid Mosaic Model of Biological Membranes

- bimolecular leaf arrangement of lipids, e.g. in liposomes
- lipids and cholesterol are oriented to minimize contact with water, polar head groups interface with aqueous environment
- Singer and Nicolson (1970s) - **Fluid Mosaic Model**
- intrinsic proteins (immersed in lipid bilayer), extrinsic proteins (loosely attached to surface), integral proteins (span bilayer, contact with both aqueous environments)
- fluid (lateral) movement of both lipids and proteins
- explains many membrane properties including fluidity, flexibility (shape/form change), self-sealing, impermeability
**Fluid Mosaic Model of Biological Membranes**

Integral Membrane Proteins are Immersed in the Lipid Bilayer

- structural relationship between hydrophobic lipid bilayer and specific structural domains on the protein
- multiple forms of attachment
- α-helical structure of primarily hydrophobic amino acids (e.g., leu, ile, val, phe) function as a transmembrane sequence/domain
- example of single 18-20 amino acid transmembrane sequence, glycophorin of the human RBC (aa 73-91 form a hydrophobic transmembrane segment)
- multiple looping α-helices (as many as 12) may form a tubular structure (e.g., 926 aa anion channel of human RBCs for Cl⁻ and HCO₃⁻ exchange)
- some proteins form quaternary structures with multiple subunits
**Integral Membrane Proteins are Immersed in the Lipid Bilayer**

- specific domains for ligand binding (receptors, transporters), catalytic activity (enzymes), attachment of carbohydrate or lipid

- **erythrocyte anion channel** (Cl⁻ and HCO₃⁻) has 2 domains:
  - hydrophilic amino terminal domain on cytosolic side with binding sites for ankyrin (cytoskeletal protein)
  - hydrophobic domain traverses membrane (mediates exchange of Cl⁻ and HCO₃⁻)
  - 60% carbohydrate on extracellular side of membrane
  - specific structural orientation in membrane, not random, i.e. membranes are asymmetric

- integral membrane enzymes may require lipid for activity
  - β-hydroxybutyrate dehydrogenase in inner mitochondrial membrane needs phosphatidylcholine (PC)
  - certain ion pumps (Na⁺, K⁺, Ca²⁺ -ATPases) and acetylcholine receptors require cholesterol for optimal activity

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**Interactions of Membrane Proteins With the Lipid Bilayer**

- (a) single segment
- (b) multiple segments
- (c) bound to integral protein
- (d) electrostatically bound to lipid bilayer
- (e) attached by short terminal hydrophobic aa sequence
- (f) attached by covalently bound lipid (GPI anchor)
Peripheral Membrane Proteins Have Various Modes of Attachment

- binding to cytoskeletal proteins such as ankyrin (anion channel of RBCs)
- electrostatic binding (negative lipids to positive charges on proteins)
- hydrophobic AAs at one end of the protein, e.g. cytochrome b₅ in ER
- glycosyl phosphatidyl inositol (GPI) anchor (glycan, covalently bonded via glycosamine to PI)*
- C14 myristic acid (via an amide linkage to an amino-terminal Gly) and C16 palmitic acid (via thioester to Cys or hydroxyester covalent bond to Ser, Thr)

* linkage cleaved by PI-specific PLC

There is a high degree of structural and functional organization in membranes

- e.g. in human erythrocyte membrane
- e.g., in inner membrane of mitochondria
  - proteins of the electron transport chain are organized into functional units both laterally and transversely
Lipids are distributed asymmetrically across biological membranes

- each layer of the bilayer has a different lipid composition
- e.g., erythrocyte membranes, sphingomyelin predominates in outer layer, phosphatidyl-ethanolamine in inner layer
- lipids may be maintained by specific proteins that promote transverse movement of certain lipids, since uncatalyzed movement is excessively slow!
  - flippases
  - PS flips to outside (apoptosis)

Proteins and Lipids Diffuse Within Membranes

- complex and dynamic interactions between lipids (and protein and lipids)
- fluidity of lipid portion of the membrane depends on temperature and composition of membrane
- at low temperature - lipids are in gel crystalline state with restricted mobility
- as the temperature increases, a phase transition into the liquid crystalline state at the $T_m$ yields increased fluidity
- $T_m$ is imprecise for biological membranes due to heterogeneous nature of their lipids, local variations in gel-liquid state
- short chain fatty acids increase fluidity, cholesterol decreases fluidity, $Ca^{2+}$ ion decreases fluidity
Movement of Molecules Through Membranes

- the nature of lipid membranes severely restricts the types of molecules that can move from one side to the other
- inorganic ions or charged species do not readily diffuse because of their attraction to water and the exclusion of charged species by the hydrophobic environment of the lipid bilayer
- diffusion rates of carbohydrates, amino acids, and inorganic ions is not zero, but much too slow to accommodate cellular metabolic needs
- specific mechanisms are required for translocation of molecules across membranes = transport

Some molecules can diffuse through membranes

- process of diffusion - occurs in 3 major steps:
  - solute leaves aqueous condition and enters membrane
  - solute must traverse the hydrophobic domain of the membrane
  - solute must leave membrane and enter new aqueous environment
- thermodynamic and kinetic constraints on diffusion
  - concentration equilibrium and rate
- diffusion of gases occurs rapidly (e.g. O₂, N₂, CO₂, NO) and depends on concentration gradient
- water diffuses readily through most biological membranes via gaps in the hydrophobic environment from random movement of fatty acyl chains of phospholipids (moves via transitory spaces in membranes)
Some molecules can diffuse through membranes

- rate of diffusion of lipophilic substances is directly proportional to their lipid solubility and diffusion coefficient in lipids (size and shape of the substance)
- uncharged lipophilic molecules (e.g. FAs, steroids) diffuse fairly rapidly, water soluble agents diffuse very slowly (e.g. sugars and inorganic ions)
- Fick’s first law of diffusion:
  \[ J = -D(\frac{\partial c}{\partial x}) \] [higher to lower]
  \[ J = \text{net amount substance moved/time} \]
  \[ D = \text{diffusion coefficient} \]
  \[ \frac{\partial c}{\partial x} = \text{chemical gradient of substance} \]

Movement of Molecules Across Membranes Can Be Facilitated

- plasma membranes of both prokaryotic and eukaryotic cells, and membranes of subcellular organelles have numerous transport systems for facilitated movement
- involves intrinsic membrane proteins
- transporters are classified by their mechanism of translocation
- three principal types:
  - membrane channels (pores)
  - transporters (passive, active)
  - group translocation
Classification of Membrane Translocation Systems

<table>
<thead>
<tr>
<th>Type</th>
<th>Class</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Channel</td>
<td>1. Voltage regulated</td>
<td>Na⁺ channel</td>
</tr>
<tr>
<td></td>
<td>2. Chemically regulated</td>
<td>Acetylcholine receptor</td>
</tr>
<tr>
<td></td>
<td>3. cAMP regulated</td>
<td>Cl⁻ channel</td>
</tr>
<tr>
<td></td>
<td>4. Other</td>
<td>Pressure sensitive</td>
</tr>
<tr>
<td>Transporter</td>
<td>1. Passive mediated</td>
<td>Glucose transporter</td>
</tr>
<tr>
<td></td>
<td>2. Active mediated</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Primary-redox coupled</td>
<td>Respiratory chain linked</td>
</tr>
<tr>
<td></td>
<td>b. Secondary</td>
<td>Na⁺,K⁺-ATPase</td>
</tr>
<tr>
<td></td>
<td>Group translocation</td>
<td>Amino acid translocation</td>
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Membrane Channels

- most cells contain specific membrane channels (or pores) permitting rapid movement of substances or ions from one side to the other
- tertiary and quaternary structures of intrinsic membrane proteins generate aqueous holes permitting diffusion in both directions
- movement from higher to lower concentration, down concentration gradient
- they do not bind the molecules or ions to be transported, unlike transporters
- some degree of specificity (size/charge)
- may be “gated” (open and close) for regulation
Membrane transport systems have certain common characteristics

- all cells have highly specific transporters for the movement of
  - inorganic anions and cations (e.g. Na⁺, K⁺, Ca²⁺, HPO₄²⁻, Cl⁻, HCO₃⁻)
  - uncharged and charged organic compounds (e.g. amino acids and sugars)
- different cellular membranes have different transport systems
  - e.g. mitochondrial membrane - specific mechanism for ADP/ATP translocation
- transport systems involve substrate specific integral membrane proteins
- designated by variety of names, e.g. transporter, translocase, translocator, permease, pump
- termed transporter system, translocation mechanism, facilitated transport or mediated transport system

Membrane transport systems have certain common characteristics

- facilitated movement of substance at rate significantly greater than simple diffusion to an equilibrium: \([S₁] \Leftrightarrow [S₂]\)
- Add a transporter (T) into the equation:
  \[
  [S₁] + T \Leftrightarrow [S - T] \Leftrightarrow [S₂] + T
  \]
- without energy input, the concentration on both sides of the membrane will be equal at equilibrium
- expenditure of energy can generate a concentration gradient
- membrane transporters are similar to enzymes - they increase the rate of transport but do not determine the final equilibrium
Membrane Transporters

<table>
<thead>
<tr>
<th>Passive Mediated</th>
<th>Active Mediated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Saturation kinetics</td>
<td>1. Saturation kinetics</td>
</tr>
<tr>
<td>2. Specificity for solute transported</td>
<td>2. Specificity for solute transported</td>
</tr>
<tr>
<td>3. Can be inhibited</td>
<td>3. Can be inhibited</td>
</tr>
<tr>
<td>4. Solute moves down concentration gradient</td>
<td>4. Solute can move against concentration gradient</td>
</tr>
<tr>
<td>5. No expenditure of energy</td>
<td>5. Requires coupled input of energy</td>
</tr>
</tbody>
</table>

- translocate by physically binding/moving the substance
- can be evaluated in kinetic terms like enzyme catalyzed reactions
- specificity for substrate, defined reaction kinetics, inhibitable by competitive and non-competitive inhibitors
- movement down (passive transport) or against (active transport, requires energy) concentration gradients

Membrane Transporters

- transported molecule is unchanged in process
- rate of transport for channels, range of $10^7$ ions/s
- rate for transporters, range of $10^2$-$10^3$ molecules/s
- transporters can be modulated by cells, specific drugs

Group Translocation

- involves movement and chemical modification of the substance to be transported
  - group translocation of amino acids via the $\gamma$-glutamyl cycle in liver cell plasma membranes
  - one mechanism of sugar uptake by bacteria involves transport and phosphorylation prior to release into cytosol
**Functions of Biological Membranes**  
**Mechanisms of Membrane Transport**

- we will cover **membrane transport systems** and their specific mechanisms of action and regulation in detail in the next lecture:
  - membrane channels/pores
  - transporters (passive versus active)
  - group translocation of amino acids
  - examples and clinical relevance