Part I => CARBS and LIPIDS

§1.5  MEMBRANE TRANSPORT
§1.5a Passive Transport
§1.5b Facilitated Transport
§1.5c Active Transport
Section 1.5a: Passive Transport
- Passive transport (or passive diffusion) is movement of molecules along a chemical potential gradient (or concentration gradient) across biological membranes—flooding is an example of passive transport in our macroscopic world!

- In general, only apolar substances can diffuse across biological membranes

- Passive transport is under entropic control—movement of molecules from a region of high to a region of low concentration increases the system entropy

- Passive transport is “off-the-grid”—requires no external input of energy!
Passive diffusion is movement of molecules along a chemical potential gradient (from a region of high concentration to a region of low concentration) across biological membranes.

Only apolar substances (such as steroid hormones, fat-soluble vitamins A/D/E/K, and gases $O_2/CO_2$) can diffuse across biological membranes—why?!

Small polar molecules such as water can also diffuse through cell membranes but their rate of diffusion is unsurprisingly slow.

The rate of diffusion of a substance is proportional to the concentration difference across the membrane and its solubility in the apolar core of lipid bilayer.

In addition to passive diffusion, filtration and osmosis are the other two forms of passive transport.
Passive Transport—Thermodynamics

- Consider the passive diffusion of an apolar substance \( A \) from extracellular (ext) side to cytoplasmic (cyt) side across a biological membrane:

\[ A_{\text{ext}} \leftrightarrow A_{\text{cyt}} \]

- The chemical potential \( \mu \) (or partial molar free energy) of solute \( A \) on each side is given by:

\[ \mu_{\text{ext}} = RT \ln[A]_{\text{ext}} \quad [1] \]
\[ \mu_{\text{cyt}} = RT \ln[A]_{\text{cyt}} \quad [2] \]

where

\[ \mu_{\text{ext}} = \text{Chemical potential of solute } A \text{ on the extracellular side (cal/mol)} \]
\[ \mu_{\text{cyt}} = \text{Chemical potential of solute } A \text{ on the cytoplasmic side (cal/mol)} \]

\[ R = \text{Universal molar gas constant (1.99 cal/mol/K)} \]
\[ T = \text{Absolute temperature (K)} \]

- Chemical potential \( (\mu) \) is a physical property of a substance related to its concentration—in a manner akin to its volume and density—that describes the amount of potential energy stored in the substance that can be used to do useful work (eg the kinetic/potential energy of water drives the turbine that in turn produces electricity!)

- The potential energy stored in solute \( A \) is thus proportional to the difference in its concentration across the membrane—simply put, such concentration difference generates a chemical potential difference \( (\Delta \mu) \) across the membrane given by:

\[ \Delta \mu = \mu_{\text{cyt}} - \mu_{\text{ext}} = RT \ln[A]_{\text{cyt}} - RT \ln[A]_{\text{ext}} = RT \ln\{[A]_{\text{cyt}}/[A]_{\text{ext}}\} \quad [3] \]

If \([A]_{\text{ext}} > [A]_{\text{cyt}} \Rightarrow \Delta \mu < 0 \) \( \Rightarrow \) Net flow of \( A \) to cytoplasmic side
If \([A]_{\text{ext}} = [A]_{\text{cyt}} \Rightarrow \Delta \mu = 0 \) \( \Rightarrow \) Zero net flow of \( A \) across the membrane
If \([A]_{\text{ext}} < [A]_{\text{cyt}} \Rightarrow \Delta \mu > 0 \) \( \Rightarrow \) Net flow of \( A \) to extracellular side
Exercise 1.5a

- What are the three forms of passive transport?
- Define chemical potential of a substance. How is it related to its concentration?
- How can you predict whether it will be thermodynamically favorable for an apolar substance to move from one side of a membrane to the other?
- Provide examples of substances that can readily diffuse across biological membranes
Section 1.5b: Facilitated Transport
Polar and charged substances cannot passively diffuse across biological membranes—such substances rely on what is called “facilitated transport”.

Facilitated transport is synonymous with “facilitated diffusion” or “passive-mediated transport”.

Facilitated transport is similar to passive transport in that it also involves movement of molecules along a chemical potential gradient across biological membranes—but it only occurs via specific “transmembrane hydrophilic conduits or vehicles.”

Like passive transport, facilitated transport is also under entropic control—movement of molecules from a region of high to a region of low concentration increases the system entropy.

Facilitated transport is a FREE RIDE—requires no external input of energy!
Facilitated transport involves movement of polar and charged substances along a chemical potential gradient (from a region of high concentration to a region of low concentration) across biological membranes via specific micromolecular compounds or macromolecular proteins that serve as “transmembrane hydrophilic conduits or vehicles”

Such transmembrane conduits/vehicles can be divided into three major classes on the basis of the physical mechanism that they employ to move the “cargo”—such as ions, sugars, amino acids, nucleotides, and even water—across biological membranes:

1. **Carriers**—carriages that travel across the membrane so as to shuttle a substance from one side to the other and then return unloaded (e.g., ionophore carriers)—cf a taxi ride or an airport shuttle!

2. **Channels**—form tunnels or pores to allow unhindered traffic of a substance (e.g., ionophore channels, ion channels, porin channels, and aquaporin channels)—cf an underground tunnel for pedestrian or vehicular traffic!

3. **Transporters**—act as allosterically-gated tunnels that transiently open to allow the entry of a substance on one side and then transiently open on the other side to allow its exit (e.g., glucose transporters)—cf subway turnstiles that allow only one person to go through for each open-close cycle!
(1) Carriers—Ionophore Carriers (eg Valinomycin)

Ionophores are exclusively \textit{amphiphilic micromolecular compounds} such as antibiotics (MW < 2kD) that either “carry” or “channel” ions across biological membranes of bacteria and other microbes.

- Ionophore carriers (eg valinomycin) wrap around a specific ion using their polar groups so as to shield its charge on one side of the biological membrane, thereby enabling both the “cargo” and the “vehicle” to diffuse through the hydrophobic core of the bilayer to the other side.

- After releasing the ion on the other side, the ionophore carrier returns to the original side to repeat the whole cycle as many times as necessary in order to discharge the ion concentration gradient across the biological membrane.

- Valinomycin specifically carries or “piggybacks” the larger K$^+$ ion (r=1.33Å)—but not the smaller Na$^+$ (r=0.95Å)—with a 10,000-fold selectivity.

- Valinomycin is a macrocyclic dodeca-depsipeptide antibiotic that accommodates a single K$^+$ ion within its central cavity via coordination by six carbonyl O atoms with an octahedral geometry (eight faces).

- A depsipeptide is a peptide that harbors a mixture of amide and ester bonds—since valinomycin has a total of 12 (dodeca) alternating amide and ester linkages, it becomes “dodeca-depsipeptide”!

Valinomycin in complex with K$^+$ ion
(2) Channels—Ionophore Channels (eg Gramicidin A)

**Gramicidin A pentadeca-peptide**

Ionophores are exclusively *amphiphilic micromolecular compounds such as antibiotics (MW < 2kD) that either “carry” or “channel” ions across biological membranes of bacteria and other microbes*  
ionophore → ion + phore (to carry/move)

- Ionophore channels (eg gramicidin A) drill a tunnel or pore through the biological membranes so as to increase its permeability to specific ions—the resulting flow of ions results in the discharge of electrochemical potential gradient across the membrane

- Gramicidin A is a linear pentadeca-peptide (15-mer) antibiotic that folds into a head-to-head helical dimer within lipid bilayers—the central lumen of this helical dimer is ideally suited for the “tunneling” of cations such as Na⁺ and K⁺—with a moderate selectivity for the latter

- Assuming that the average mass of an amino acid is 110g/mol, what is the molar mass of gramicidin A (1g/mol = 1D)?
  
  \[ 15 \times 110\text{g/mol} = 1650\text{g/mol} \rightarrow 1650\text{D} \rightarrow 1.65\text{kD} \text{ (actual 1.88kD)} \]
(2) Channels—Ion Channels (eg KcsA)

- Eukaryotic cells usually maintain ionic gradients across their plasma membranes, thereby creating an electric voltage (or membrane potential)—eg there is an excess of Na\(^+\) ions (150mM) on the extracellular side, while an opposite gradient exists for K\(^+\) ions (150mM) on the inside of the cell in mammalian cells—all thanks to active transport (next section)!

- Discharge (or depolarization) of such membrane potential is necessary for the regulation of cellular processes such as osmoregulation, signal transduction, and neurotransmission—but how does cell accomplish this feat? Enter ion channels.

- Ion channels are the protein-equivalents of ionophore channels in both prokaryotes and eukaryotes that play a central role in the movement of ions (such as Na\(^+\), K\(^+\), Ca\(^{2+}\), and Cl\(^-\)) across membranes as well as in the discharge of membrane potential—eg KcsA

- KcsA is a bacterial ion channel that forms an \(\alpha\)-helical cone-like tunnel—from the association of four subunits into a tetramer—to facilitate the flow of K\(^+\) ions from the inside to the outside of the cell in a highly specific manner

- Given their key role in mediating numerous cellular processes (eg propagation of nerve impulses), ion channels are not constitutively open—but tightly gated and coupled to other events (such as chemical stimuli, ions, pH and stress) within the cell so that they only open and close when needed
Porin channels (or simply porins) are β-barrels that drill hydrophilic tunnels through the outer membranes of bacteria (as well as in mitochondria of most eukaryotic cells) so as to allow the diffusion of polar and charged molecules in a relatively non-selective manner—eg OmpF.

Porins generally act as “molecular sieves” to filter out larger molecules but allow smaller ones to pass through—eg OmpF (outer membrane porin F) filters out solutes larger than about 600D.

Some porins also act as membrane channels in a highly selective manner—eg maltoporin facilitates the diffusion of maltodextrin (a glucose oligosaccharide used as a food additive) with high specificity.
(2) Channels—Aquaporin Channels (eg AQP1)

- Water can diffuse through membranes but at a much slower rate than needed for certain physiological functions—eg urinary system of the kidney cells—so what is the kidney cell to do? Enter aquaporins.

- Aquaporin channels (or simply aquaporins) are α-helical bundles that form hollow channels through certain biological membranes of most prokaryotes and eukaryotes so as to accelerate the flow of water in and out of cells—eg aquaporin 1 (AQP1)

- AQP1 is abundantly expressed in kidney cells and serves as a water channel in the form of a tetramer—each α-helical bundle associates with three other subunits to form four adjacent membrane pores
Transporters—Glucose Transporters (eg GLUT1)

Given that glucose is a vital source of energy (via respiration), it is fitting that virtually all cell membranes are equipped with so-called glucose transporters to facilitate the uptake of glucose—eg GLUT1.

GLUT1 (glucose transporter 1) is a 12-transmembrane α-helical protein that forms an allosterically-gated tunnel (or a turnstile)—it is not constitutively open but rather switches between two conformations.

Binding of glucose to GLUT1 (widely expressed) on the extracellular face induces a conformational change such that it closes on that side while simultaneously opening on the cytoplasmic face to allow a smooth transit of glucose through the membrane in a unidirectional manner—ditto for GLUT3 (neuron) and GLUT4 (muscle) but GLUT2 (liver) is bidirectional—why is GLUT2 bidirectional (see §3.3)?

GLUT1 bolsters a rather broad substrate specificity in that it also serves as a transporter for a wide range of other aldoses, including both pentoses and hexoses, as well as vitamin C.
Exercise 1.5b

- What are the three major classes of facilitated transport?

- What are the similarities and differences between carriers, channels and transporters involved in facilitated transport?

- What are similarities and differences between ionophore channels and ion channels?

- Outline the mechanism of action of GLUT1 transporter
Section 1.5c: Active Transport
- **Active transport** is the movement of molecules across biological membranes in the direction opposing their chemical potential gradient—ie substances are being moved “uphill” in lieu of “downhill”—the latter being the prominent feature of passive and facilitated transport mechanisms discussed earlier.

- Active transport relies on specific transmembrane protein pumps (or transporters) to move molecules against their concentration gradients in an energy-dependent manner.

- Active transport pumps can be divided into two major classes depending on the source of energy that they utilize:
  1. **Primary pumps**—energy derived from direct ATP hydrolysis.
  2. **Secondary pumps**—energy derived from the discharge of electrochemical (ion) gradients (that are ultimately restored by primary pumps via ATP hydrolysis!)
Active transport can be described as:

- **Uniport**—transport of ONE molecule in either direction*
- **Symport**—transport of TWO or more molecules in the same direction
- **Antiport**—transport of TWO or more molecules in opposite directions

*a predominant feature of facilitated transport!
(1) Primary Pumps—Na\(^+\)/K\(^+\) Antiporter (Function)

- Eukaryotic cells usually maintain ionic gradients across their plasma membranes, thereby creating an electric voltage (or membrane potential)—eg there is an excess of Na\(^+\) ions (150mM) on the extracellular side, while an opposite gradient exists for K\(^+\) ions (150mM) on the cytoplasmic side in mammalian cells—how is this achieved? Enter Na\(^+\)/K\(^+\) antiporter (also called Na\(^+\)/K\(^+\) ATPase).

- Na\(^+\)/K\(^+\) antiporter is an ATP-driven pump that transports Na\(^+\) and K\(^+\) ions against their concentration gradients across plasma membranes in opposite directions.

- The electrochemical gradient generated by the Na\(^+\)/K\(^+\) antiporter plays a key role in the maintenance of cell volume (an excess of Na\(^+\) ions within the cell would draw in water by osmosis) as well as in restoring the membrane potential (required for signal transduction and neurotransmission).
Na⁺/K⁺ antiporter switches between two conformations per transport cycle:
(a) an ATP-bound conformation (E1) that only recognizes Na⁺ ions on cytoplasmic side
(b) a phosphorylated conformation (E2) that only recognizes K⁺ ions on extracellular face
(1) Primary Pumps—Ca\textsuperscript{2+} Antiporter (Function)

- **Influx** (antonym \(\rightarrow\) efﬂux) of Ca\textsuperscript{2+} ions across plasma membranes in eukaryotic cells is required for physiological processes such as muscle contraction, the release of neurotransmitters, and glycogen breakdown.

- Accordingly, eukaryotic cells maintain an excess of Ca\textsuperscript{2+} ions (1mM) on the extracellular side of plasma membranes—how do they do that? Enter Ca\textsuperscript{2+} antiporter (also called Ca\textsuperscript{2+} ATPase).

- Ca\textsuperscript{2+} antiporter is an ATP-driven pump that transports Ca\textsuperscript{2+} ions from the cytoplasmic side to the extracellular face against a concentration gradient across plasma membranes, while counter-transporting H\textsuperscript{+} into the cytoplasm.

\[
\begin{align*}
\text{H}_2\text{O} + \text{ATP} & \rightarrow \text{ADP} + \text{Pi} \\
2\text{Ca}^{2+}\text{(cyt)} + n\text{H}^{+}\text{(ext)} & \leftrightarrow 2\text{Ca}^{2+}\text{(ext)} + n\text{H}^{+}\text{(cyt)}
\end{align*}
\]

**Overall Transport Stoichiometry (n=2,3)**
Ca\(^{2+}\) antiporter switches between two conformations per transport cycle:
(a) an ATP-bound conformation (E1) that only recognizes Ca\(^{2+}\) ions on cytoplasmic side
(b) a phosphorylated conformation (E2) that only recognizes H\(^+\) ions on extracellular face
The epithelial cells (lining the villi) of the small intestine take up dietary glucose (Glu) via the Na\(^+\)/Glu symporter (as opposed to GLUT1 used by most other cells wherein only downhill glucose transport is needed)—also called sodium/glucose-linked transporter (SGLT)—using secondary active transport—how is this achieved?

- The energy stored in the Na\(^+\) ion gradient across the plasma membrane—generated via the action of Na\(^+\)/K\(^+\) antiporter in an ATP-driven manner—is coupled to the Na\(^+\)/Glu symporter.

- Accordingly, the energy derived from the discharge/dissipation of “downhill” Na\(^+\) ion gradient is utilized by the Na\(^+\)/Glu symporter to transport glucose against an “uphill” glucose gradient across the plasma membrane with a Na\(^+\)/Glu stoichiometry of 2:1 (SGLT1) or 1:1 (SGLT2).
Exercise 1.5c

- Distinguish between passive transport, facilitated transport and active transport across biological membranes.

- What are the two forms of active transports? What is the direct and ultimate source of energy for each form?

- Explain why the Na\(^+\)/K\(^+\) antiporter and the Ca\(^{2+}\) antiporter carry out unidirectional transport with respect to each ion across the plasma membrane?