Chapter II: Exchange with the environment

1-General characteristics

The cell envelope, and particularly the cytoplasmic membrane, acts as a selective barrier controlling permeability. For a substrate to be metabolized, it must either enter the cell or be broken down outside into smaller compounds capable of crossing the membrane. This extracellular processing is often carried out by exoenzymes (Madigan et al., 2008).

The cell wall and, in Gram-negative bacteria, the outer membrane, play a more limited but significant role. The outer membrane contains porins**,** which function as size-exclusion filters, allowing only molecules of certain dimensions to pass.

Substrate uptake occurs through several mechanisms:

- **In eukaryotes**, large molecules may enter the cell via vesicular transport processes such as endocytosis and exocytosis. In organisms lacking a rigid cell wall (e.g., protozoa), **phagocytosis** or related processes are common.
- **Protein transport** relies on specific pathways, present in both prokaryotes and eukaryotes, adapted to handle macromolecules.
- For small molecules, three main types of permeability systems are typically described:
    - **Simple diffusion**: passive movement along a concentration gradient.
    - **Facilitated diffusion (passive transport)**: movement via carrier or channel proteins without energy expenditure.
    - **Active transport**: energy-dependent uptake against a gradient (Madigan et al., 2008).

A more refined classification distinguishes:

- **Diffusion** (no energy required).
- **Primary active transport**: directly coupled to an energy-releasing chemical reaction.
- **Secondary active transport**: movement of one solute coupled to the movement of another, without direct energy input (Albers et al., 2012).

Transport processes are strongly influenced by environmental conditions, such as:

- Concentration of solutes.

- Nutritional status.
- pH.
- Temperature.
- Water activity.
- Redox potential.

Example: In acidic environments, cation transport is often restricted, whereas under alkaline conditions, anion transport becomes limited.

Membrane transport can be quantified as a flux of chemical molecules, expressed as the amount of material crossing a unit surface of membrane per unit of time (Albers et al., 2012).

2-Mechanisms of membrane permeability

The physicochemical properties of membranes, particularly their lipid and protein composition, are fundamental, as they determine the types of transport processes a cell can carry out.

Biological membranes act as highly efficient selective barriers. For example, a pure lipid bilayer (without proteins) exhibits different degrees of permeability depending on the nature of the biomolecules: small nonpolar molecules can cross freely, whereas charged or large polar compounds require transport systems.

The net flux of a solute (Js) across a membrane implies the existence of a driving force that determines the direction of its movement.

This driving force is governed by transmembrane gradients of thermodynamic potentials, including:

- **Osmotic pressure gradients** (differences in solute concentration across the membrane).
- **Chemical or electrochemical gradients** (differences in solute concentration and electrical potential).
- **Temperature gradients**, which can also influence the movement of certain molecules (Albers et al., 2012).

Note on Osmosis:

Osmosis refers to the net movement of solvent molecules across a semipermeable membrane toward the compartment containing a higher solute concentration. This process tends to

equalize solute concentration on both sides of the membrane. The difference in solute concentration generates a pressure difference, called osmotic pressure, which drives this solvent movement.

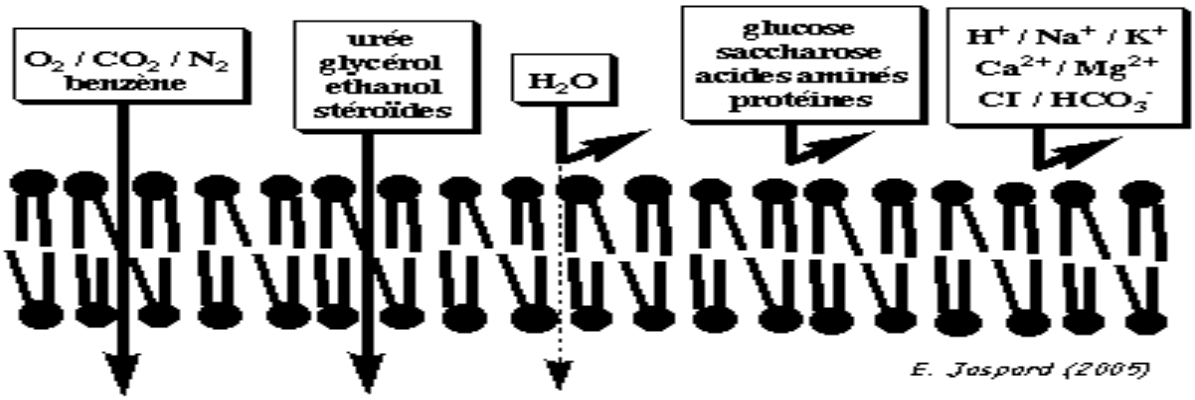

Figure 1. Different mechanism of membrane permeabilities (Jaspard, 2005)

2-1-Simple diffusion (passive transport)

- This mechanism applies to gases such as O_2, CO_2, and NH_3, as well as a few small molecules like urea, ethanol, glycerol, and certain simple sugars.
- The solute moves from a region of high concentration to a region of low concentration (down the concentration gradient), until the concentrations are balanced on both sides of the membrane.
- For a molecule to diffuse in this way, it must be soluble in the phospholipid bilayer of the membrane. This means it should be hydrophobic or nonpolar. Hydrophilic or polar molecules may cross only if they are very small.
- This process shows no saturation and no regulation.
- The rate of diffusion depends directly on the steepness of the concentration gradient.
- Compared with facilitated diffusion, simple diffusion is slower (Brandl et al., 2008).

2-2-Facilitated diffusion (passive transport)

- Like simple diffusion, the driving force is the concentration gradient**.** However, here the process is mediated by specific transmembrane proteins**,** which allow particular solutes to pass through the membrane.
- It is a selective and regulated process, since the proteins recognize and transport only certain molecules.

- Because transport involves specialized proteins, facilitated diffusion is typically faster and more efficient than simple diffusion (Brandl et al., 2008).

2-3-Diffusion through pores

- This mechanism involves non-lipid-soluble substances, such as water, electrolytes, and certain carbohydrates.
- The diameter of the pore determines which molecules can pass through and therefore plays a crucial role in the selectivity of transport.
- Some pores are specialized as ion channels (e.g., for Na^+ or Cl^-). These channels allow ions to move according to their electrochemical gradient**,** without requiring energy input.
- Other pores show greater selectivity, such as porins and aquaporins. These are found in the outer membrane of Gram-negative bacteria (and in some Gram-positive species), as well as in the mitochondrial and chloroplast membranes (Brandl et al., 2008).

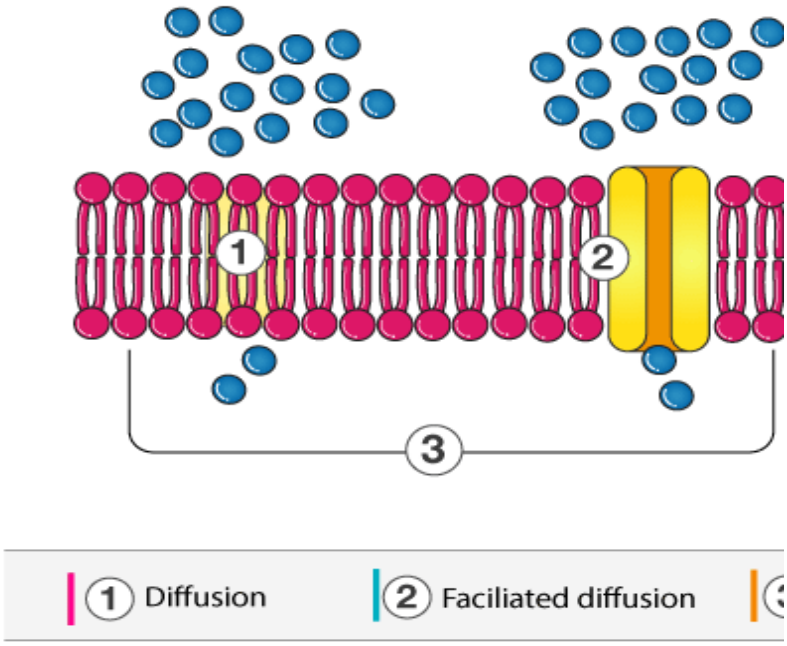

Figure 2. Symport diffusion through pores (byjus.com)

2-4-Diffusion via permeases or transporters

- Permeases are transmembrane proteins that enable the transport of specific molecules across the membrane.
- The process involves the specific binding of the molecule to the permease. Once bound, the protein undergoes a conformational change that allows the molecule to be released on the opposite side of the membrane.
- This mechanism does not lead to accumulation, as the transport is solely driven by the concentration gradient (Stillwell, 2016).

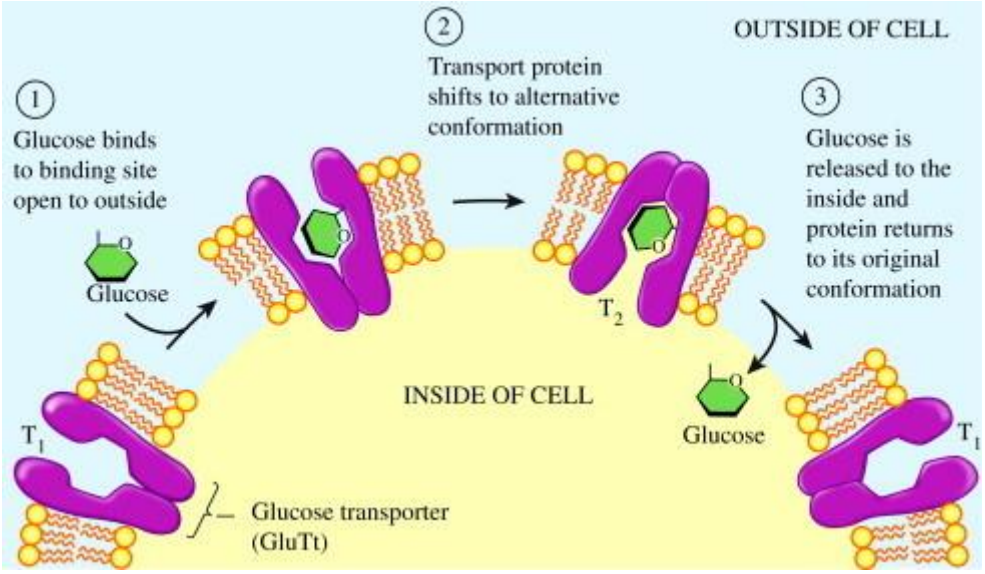

Figure 3. Glucose facilitated diffusion transporter GLUT-1 (Stillwell, 2013).

- This type of transport is saturable, because once all transport proteins are occupied, the rate of transfer can no longer increase.
- Glucose, amino acids, and certain ions cross membranes using this mechanism.

2-5-Active transport

This type of transport is usually coupled to an electrochemical gradient and requires energy consumption.

Co-transport phenomena are common and often involve the H^+ ion.

Their kinetics are enzymatic in nature and generally follow Michaelis–Menten laws. It can operate independently of the concentration gradient and may lead to intracellular accumulation (which can be problematic in the case of toxic ions).

The different types of active transport share the feature of producing a net flux of particles, with the mandatory consumption of part of the system's total energy.
It helps maintain a concentration difference of various solutes on both sides of a membrane.

These exchanges occur against the concentration gradient and involve specific, regulatable proteins.
Two main types of active transport are distinguished:

- Primary active transport: Uses the energy from ATP hydrolysis; the transporter is associated with an ATPase. The energy released from ATP drives conformational changes in the transporter.

- Secondary active transport: Uses the energy of a proton electrochemical gradient across the membrane. The substrate is introduced into the cell simultaneously with protons. This is known as symport: the transport of two (or more) solutes in the same direction through an electrochemical gradient (Stillwell, 2016).

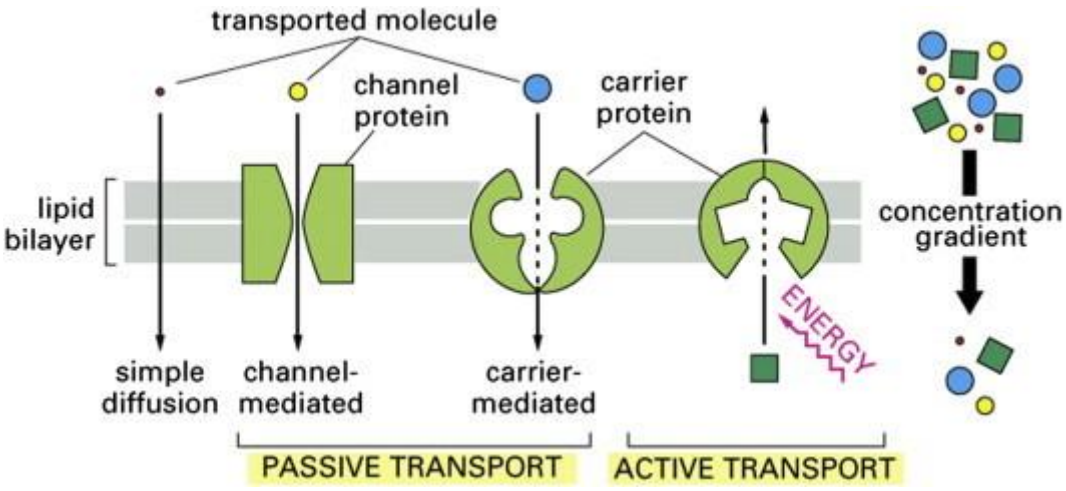

Figure 4. Basic types of membrane transport: simple passive diffusion; facilitated diffusion (by channels and carriers); and active transport (Stillwell, 2013).

The secondary active transport mechanism uses the energy stored in a proton electrochemical gradient across the membrane.

The substrate enters the cell at the same time as protons, a process known as **symport**: the simultaneous transport of two (or more) solutes in the same direction, driven by an electrochemical gradient (Stillwell, 2016).

Equation:

$$\mathbf{S\ (extracellular) + H^+\ (extracellular)\ or\ Na^+\ (extracellular) \rightleftharpoons S\ (intracellular) + H^+\ (intracellular)\ or\ Na^+\ (intracellular)}$$

Afterward, the protons are consumed by the respiratory chain and then exported back outside, which maintains the gradient.

This system is used for lactose uptake in *E. coli*:

Lactose permease is a transporter belonging to the MFS (Major Facilitator Superfamily). It co-transports protons (H^+) together with lactose into the cell.

It is therefore a symport system (proton–lactose symport). Through this mechanism, sugars are accumulated in the cytosol via secondary active transport.

Note: The sodium ion (Na^+) electrochemical gradient across the plasma membrane combines two forces, the concentration gradient and the electrical potential exerted by the membrane, both of which act on Na^+ ions (Stillwell, 2016).

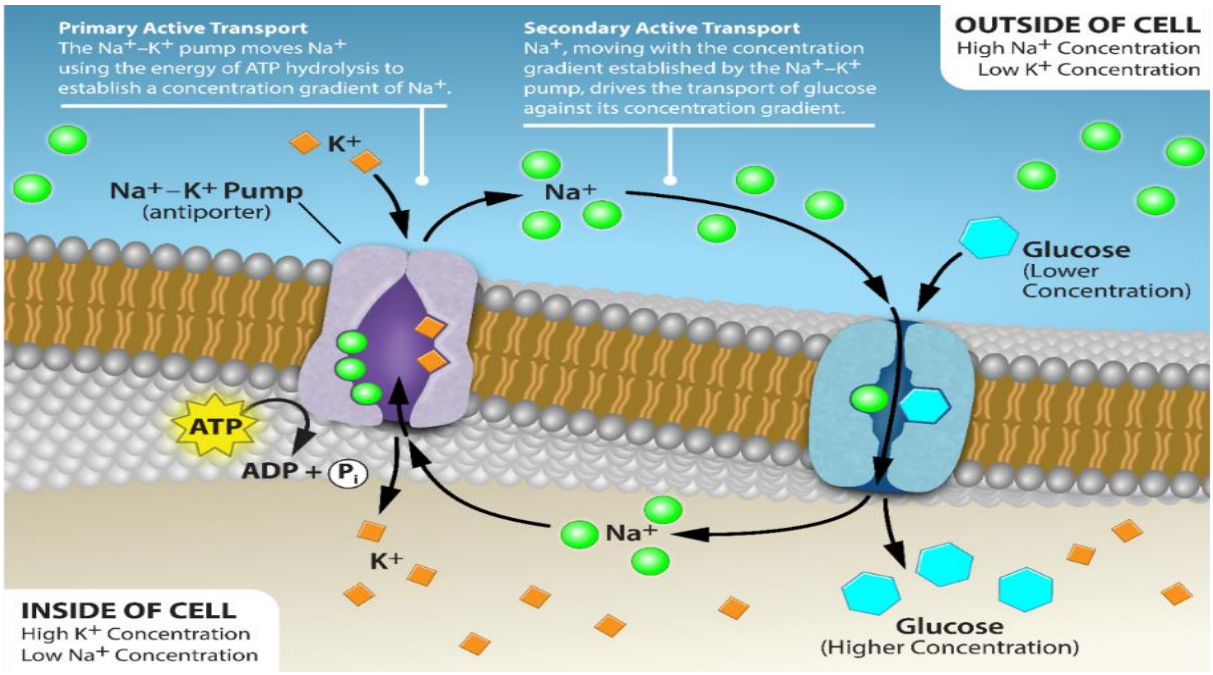

Figure 5. Primary and secondary active transport (Kennedy, 2020)

In another model, protons enter the cell, which causes sodium ions (Na^+) to exit. This represents the opposite system, known as antiport: one solute is transported along its concentration gradient, while the other is moved in the opposite direction, against its gradient.

The protein responsible for this type of transport exists in two conformations (P and P*), which continuously interconvert to enable the exchange.

$$\mathbf{S^1_{Extracellulaire} + P <===> S^1_{Intracellulaire} + P^*}$$

$$\mathbf{P^* + S^2_{Intracellulaire} <===> S^2_{Extracellulaire} + P}$$

Subsequently, sodium ions (Na^+) re-enter the cell together with the substrate through a symport mechanism.
These systems are very common in both eukaryotes and prokaryotes (Stillwell, 2016).

2-6-Group translocation

This is a transport mechanism used by bacteria in which the imported substrate (for example, glucose or fructose) is chemically modified, usually by phosphorylation.

It is a complex mechanism that uses the energy of phosphoenolpyruvate (PEP), with the participation of several enzymes that transfer the phosphate group from PEP to the imported substrate. The phosphate group's energy drives the conformational change of the transporter, enabling nutrient uptake (Gupta et al., 2021).

Carbohydrate transport in bacteria:

- PEP first transfers its phosphate group to enzyme I (EI).
- EI then transfers it to a small protein called HPr.
- The transporter EIII takes the phosphate and passes it on to glucose (or sometimes mannitol).
- These sugars are phosphorylated and can then enter metabolism more quickly and efficiently.

Within the cytoplasmic membrane, specific transport proteins bind carbohydrates and deliver them to EIII.

- EI and HPr are cytoplasmic proteins common to all phosphotransferase systems.
- EIII and EII are highly specific to their substrates.

Regulation system:

- EIII > inhibits active permeases, which prevents excessive glucose entry into the cell.

- EIII-P > activates adenylate cyclase, which promotes PEP formation.

Thus, transport systems by permeases and phosphotransferases are coordinated: the first enables sugar entry without phosphorylation (leading to pyruvate formation), and the second then takes over (Gupta et al., 2021).

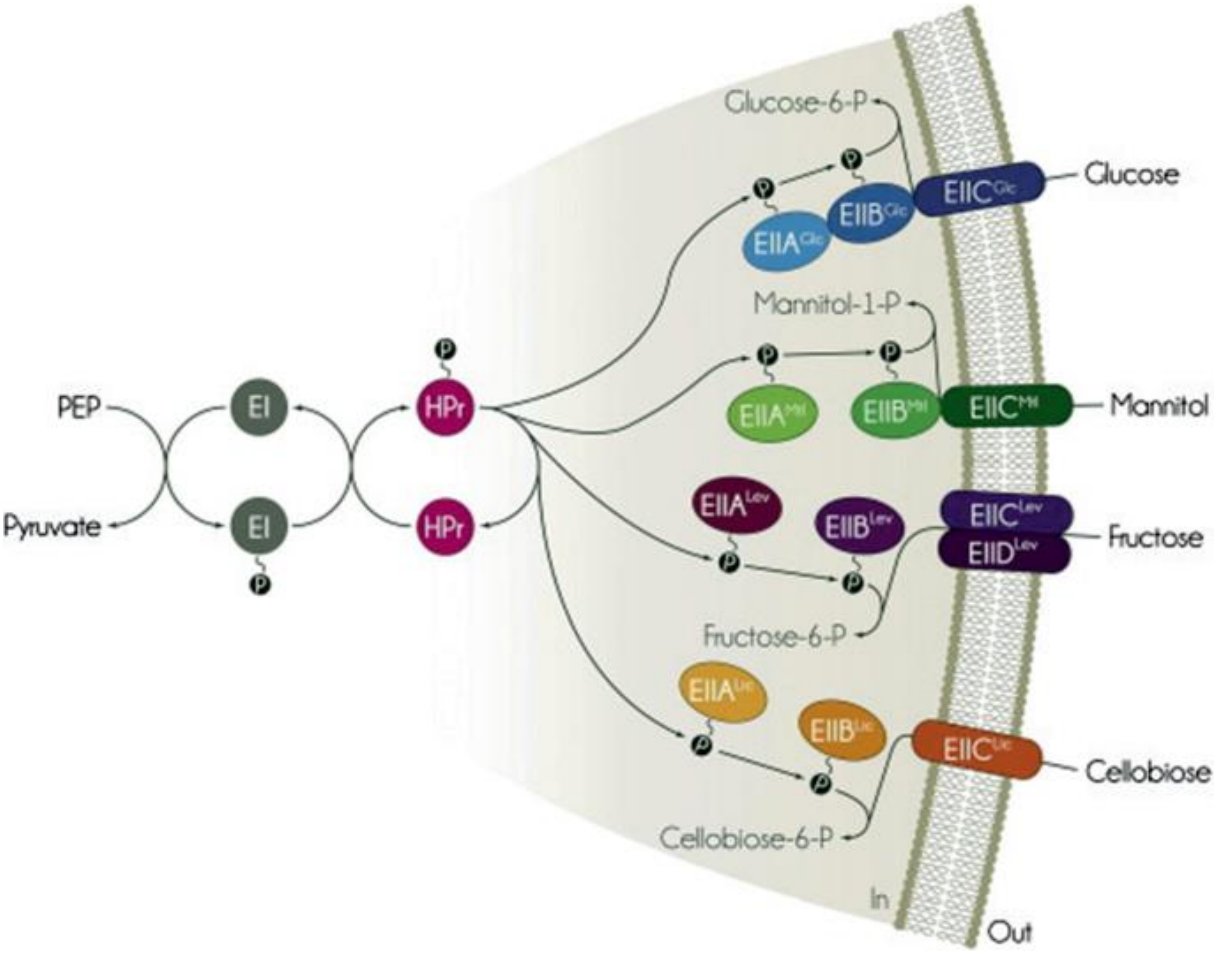

Figure 6. Group translocation system (Gupta et al., 2021)

It should be noted that the same mechanisms are involved in the excretion of metabolic products into the environment.

The mechanism of export is often similar to that of import:

- Hydrophobic products (such as alcohols and non-ionized organic acids) are released by diffusion.
- Lactate, produced by *E. coli* and certain lactic acid bacteria, is exported via a symport with a proton.
- Antibiotics are often expelled through an antiport system.

2-7-Endocytosis

This process is characteristic of eukaryotic cells without a cell wall. Extracellular substances enter the cell by invagination of the membrane, forming vesicles surrounded by a membrane (Cooper and Adams, 2022).

There are three main modalities, depending on the nature and size of the transported elements:

1. **Phagocytosis**:
    - Ingestion of large particles through large vesicles called phagosomes.
    - This process is carried out mainly by specialized cells (such as macrophages and polymorphonuclear cells) that possess specific receptors.
2. **Pinocytosis**:
    - Uptake of fluids and solutes in small vesicles called endosomes.
    - This process is non-specific and occurs in most cell types.
3. **Receptor-mediated endocytosis**:
    - A highly specific process.
    - Endocytosis is triggered when molecules bind selectively to receptors on the cell membrane (Cooper and Adams, 2022).

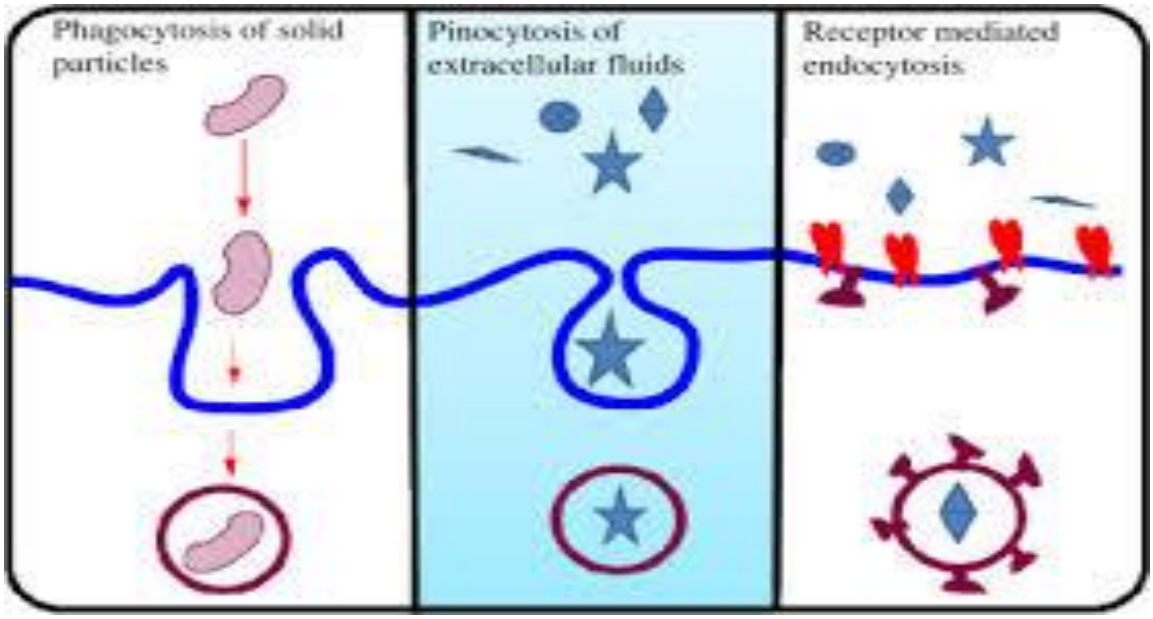

Figure 7. Endosytosis mechanism (Norouzi et al., 2022).

2-8- Exocytosis

A process by which a cell releases molecules contained in transport or secretory vesicles that fuse with the cell membrane and release their contents into the extracellular environment.

Exocytosis is complementary to endocytosis. While exocytosis moves substances out of the cell, endocytosis brings substances into the cell. Together, they maintain the cell's material balance and allow the exchange of molecules with the surrounding environment (Cooper and Adams, 2022).

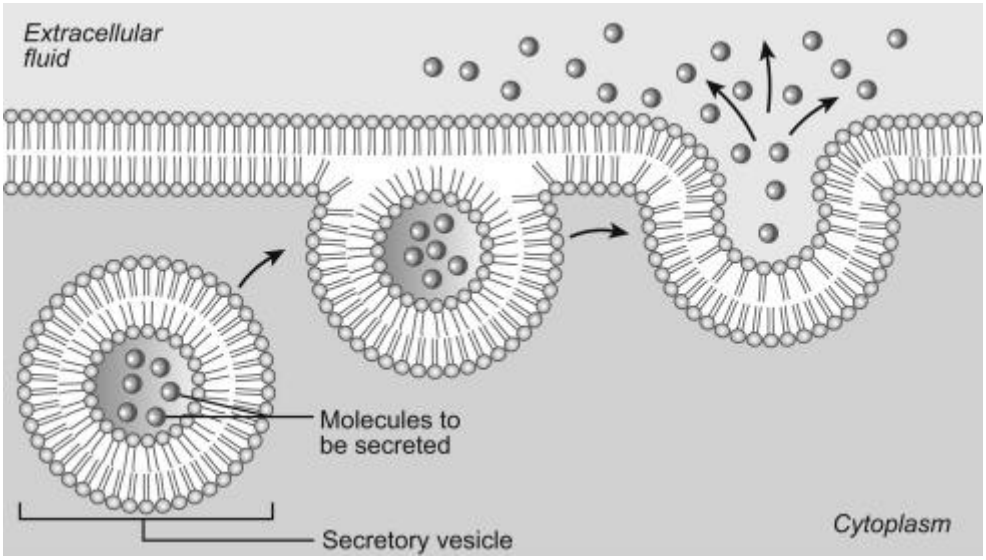

Figure 8. Exocytosis mechanism (Stillwell, 2016)