FIZIOLOGIJA ŽIVALI
Laboratorijske vaje

CELIČNI TRANSPORTNI MEHANIZMI IN PERMEABILNOST

dr. Katja Kalan

UP FAMNIT

CILJI

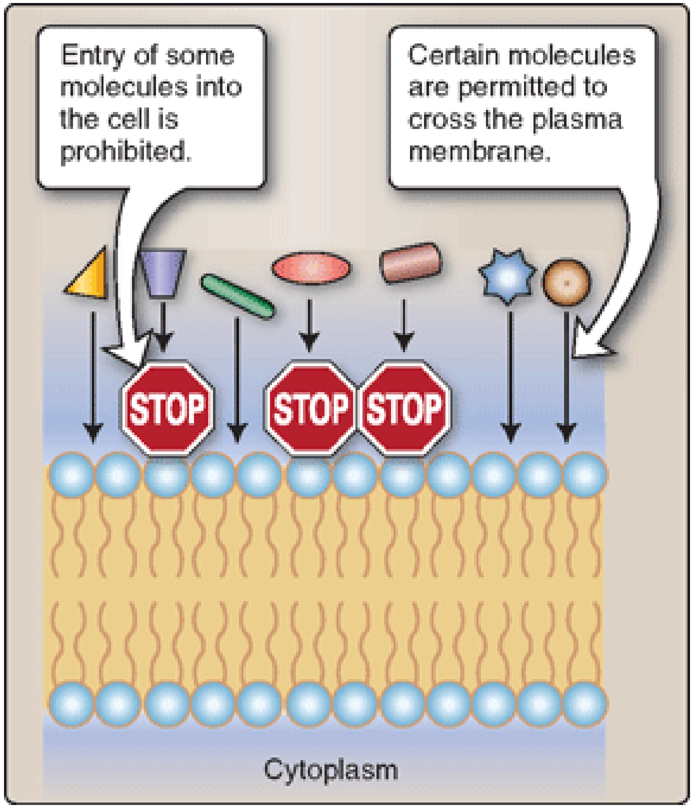
1. definirati naslednje izraze: *selektivna permeabilnost, pasivni* (*enostavna difuzija, olajšana difuzija, osmoza, filtracija*) *in aktivni transport.*

2. opisati procese, ki sodelujejo pri premikanju snovi čez celično membrano

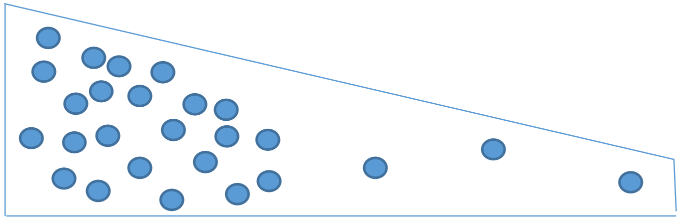
3. določiti, katere snovi bodo prehajale pasivno čez selektivno prepustno membrano (s podatki o različnih koncentracijah)

PLAZMALEMA

- selektivna premeabilnost
  - nutrienti v celico, neželjene snovi izven
  - uporabni celični proteini in ostale snovi v celici
  - eksrete prepušča ven

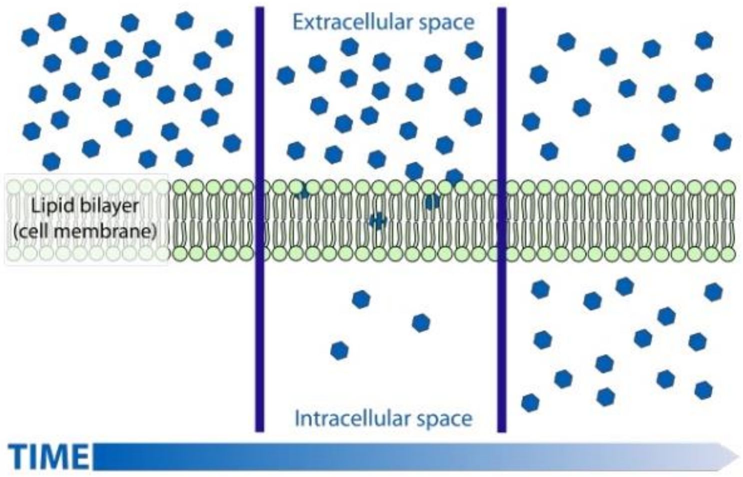


- TRANPORT: 2 procesa
  - PASIVNI – pod vplivom koncentracijskega gradienta ali razlike v tlakih med notranjostjo in zunanjostjo celice
  - AKTIVNI – rabi ATP



PASIVNI TRANSPORT

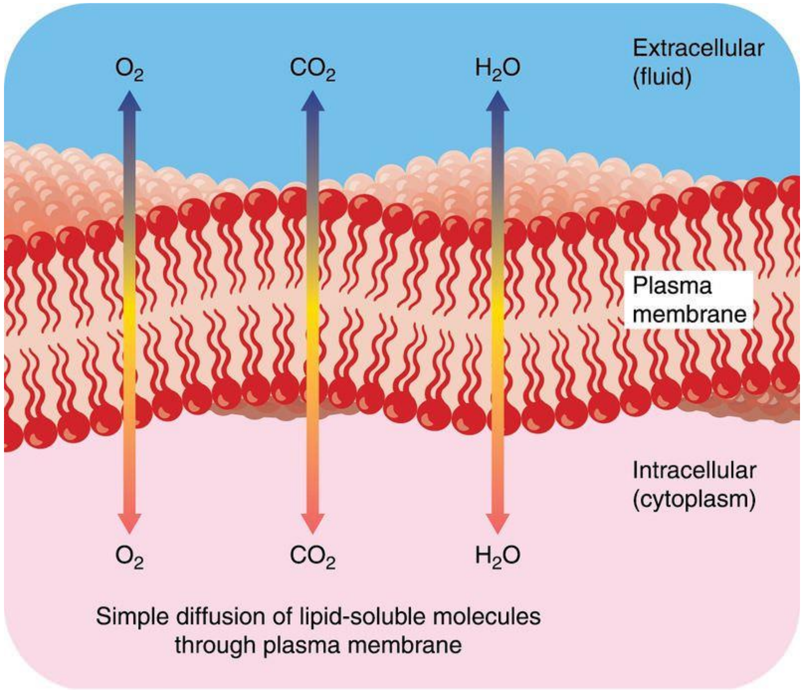
- 2 osnovna procesa:
  - difuzija – v vseh celicah
  - filtracija- le v krvnih kapilarah



- **DIFUZIJA**
  - gibanje molekul iz območja z višjo konc. do območij z nižjo
  - koncentracijski gradient – molekule eventuelno postanejo enakomerno razporejene
  - poganja jo kinetična energija molekul
    - kinetična energija – odvisna od velikosti in mase – manjše molekule se premikajo hitreje

... DIFUZIJA

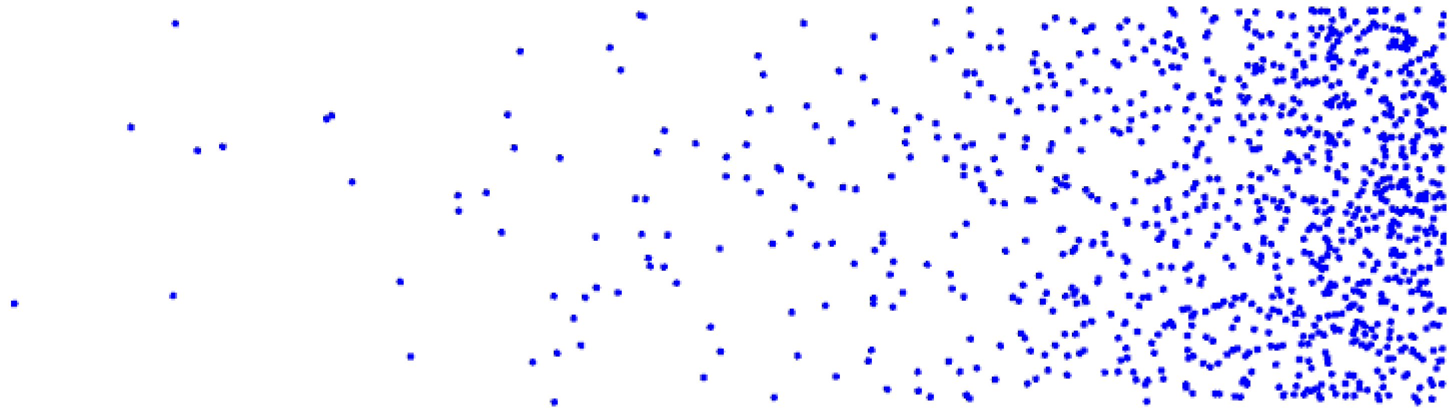
- difuzija delcev iz in v celice je odvisna od membrane – fizična ovira
- skozi gredo molekule, ki
  - so dovolj majhne, da grejo skozi pore ALI
  - se raztopijo v lipidnem sloju membrane, npr. CO2 in O2
- preprosta/osmoza/olajšana



DIFUZIJA - PREPROSTA

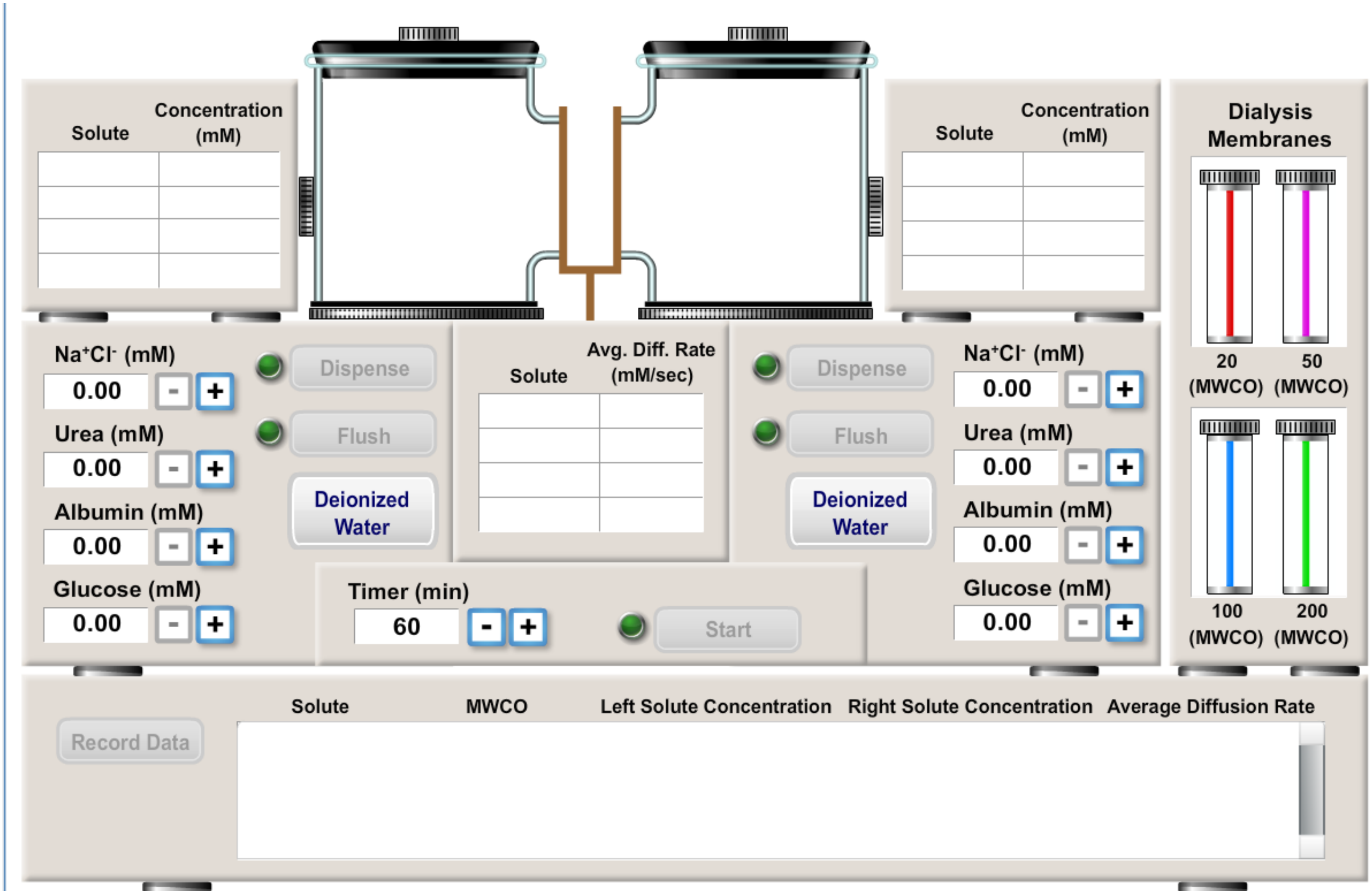
- topni delci, raztopljeni v vodi, čez selektivno prepustno memb. (SPM)
  - OSMOZA – difuzija vode čez SPM

- gibanje v smeri koncetr. gradienta



VAJA 1 – NALOGA 1: TRANSPORT ČEZ NEŽIVE MEMBRANE

- računalniška simulacija prehoda vode in topljenca čez SPM



VAJA 1 – NALOGA 1: TRANSPORT ČEZ NEŽIVE MEMBRANE

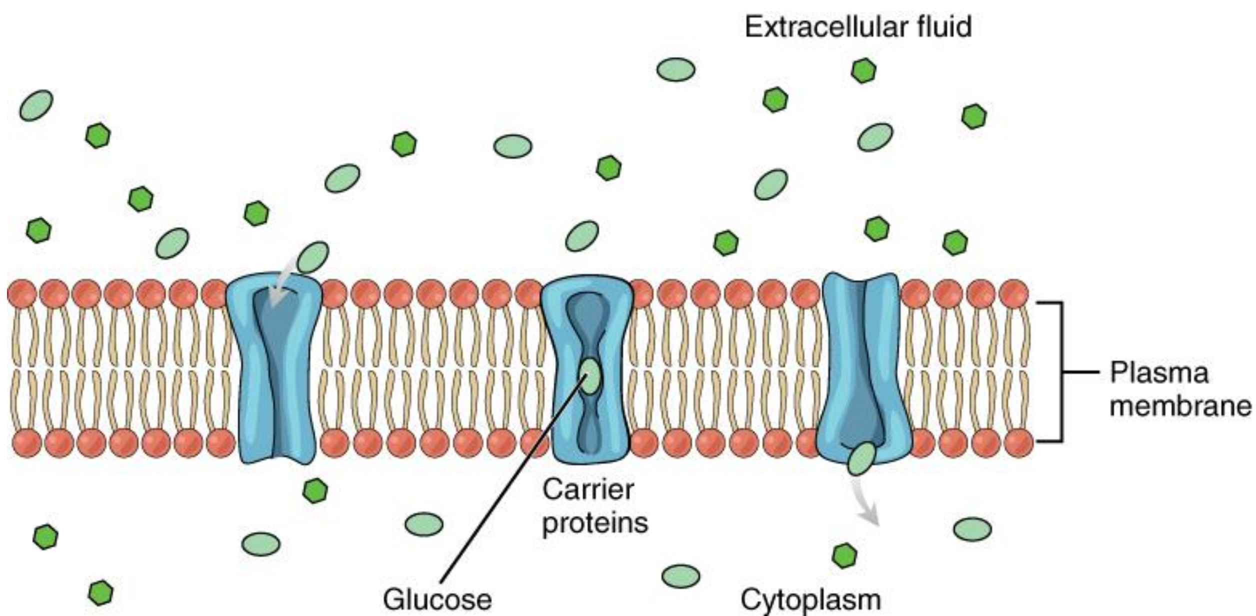
- MWCO (Molecular weight cut-off) membrane – velikost por
- molekulska masa topljenca – število gramov/mol
- (mol snovi vsebuje $6{,}02x10^{23}$ delcev (molekul)(Avogadrovo število)
- večja → večja masa topljenca –> večje pore

- NaCl – ne prehaja kot molekula, se razgradi na Na^+ in Cl^- ione v vodi

Topljenec/MWCO	20	50	100	200
NaCl				
Urea				
Albumin				
Glukoza				

VAJA 1 – NALOGA 2: OLAJŠANA DIFUZIJA

- nekatere molekule niso topne v SPM ali prevelike
- olajšana difuzija – pasivni transport, potujejo skozi prenašalce (membranske proteine) vzdolž konc. gradienta
- ker potrebujejo prenašalce – transport topljenca odvisen od št. membranskih transportnih proteinov

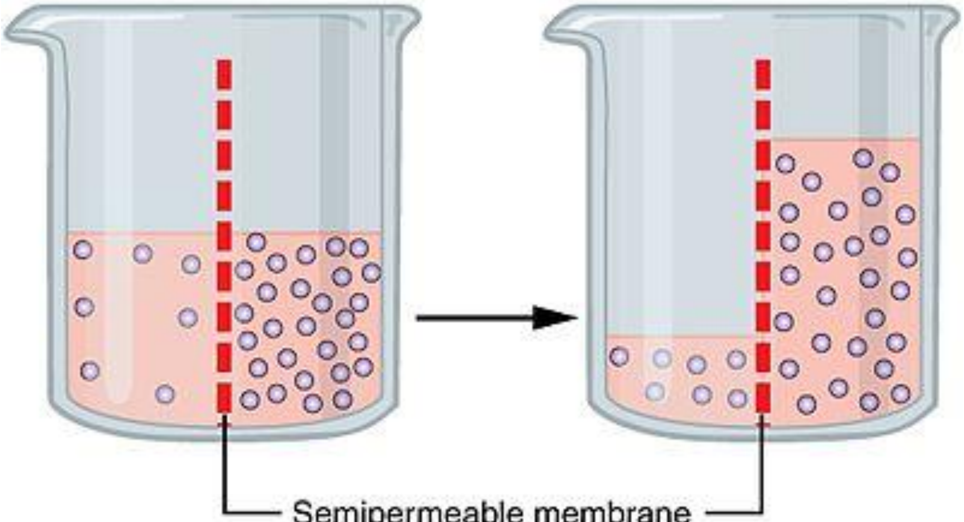


VAJA 1 – NALOGA 3: OSMOZA

- poseben tip difuzije, difuzija VODE čez SPM
- voda – skozi pore večine membran
- osmoza – ko obstaja razlika v koncentraciji vode med dvema stranema membrane
- konc. vode v raztopini – odv. od konc. topljenca
    - več topljenca – manj vode
- voda vedno proti raztopini z več topljenca (konc. gradient)

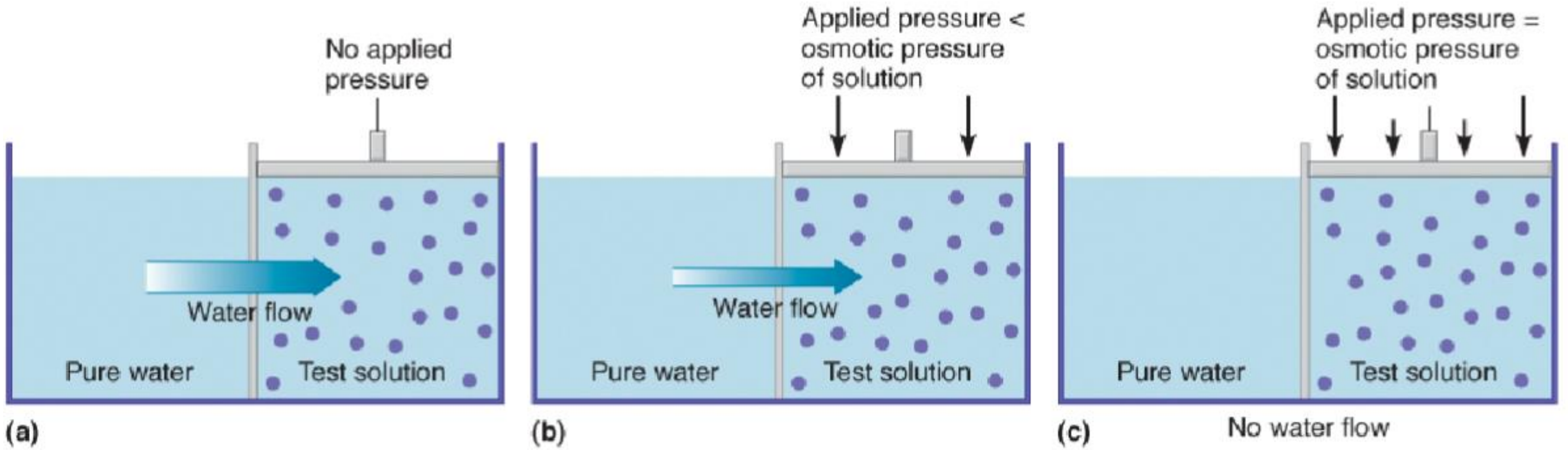
OSMOZA

- popolnoma prepustna membrana – topljenec in voda se premikata prosto do ravnotežja (equilibrium)
- SPM, ki ni prepustna za topljenec: voda se premika proti mestu z manjšo konc., topljenec pa ne – pride do povečanja V na eni strani membrane
- zaprt sistem – povečanje pritiska na eni strani membrane



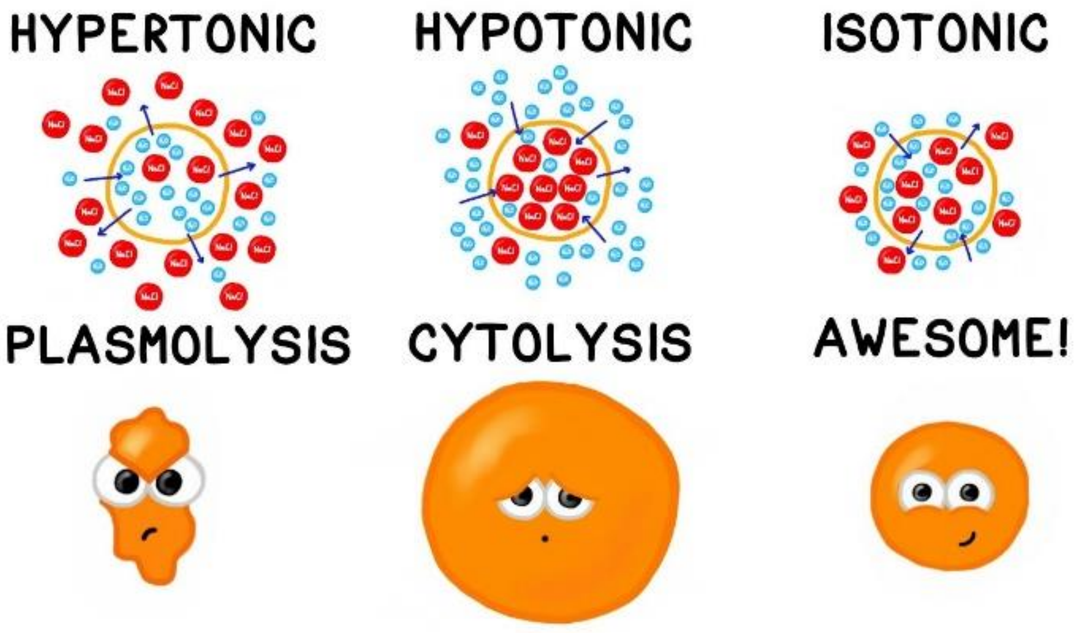
OSMOZA

- ZAPRT SISTEM: V se ne more spremeniti
- PRITISK v bolj koncentrirani raztopini se zviša
- osmotski pritisk – sila, ki kljubuje osmozi
  - – v mmHg (živo srebro, "mercury")
- bolj nepermeabilne memb – višji osm. pritisk



RAZTOPINE:

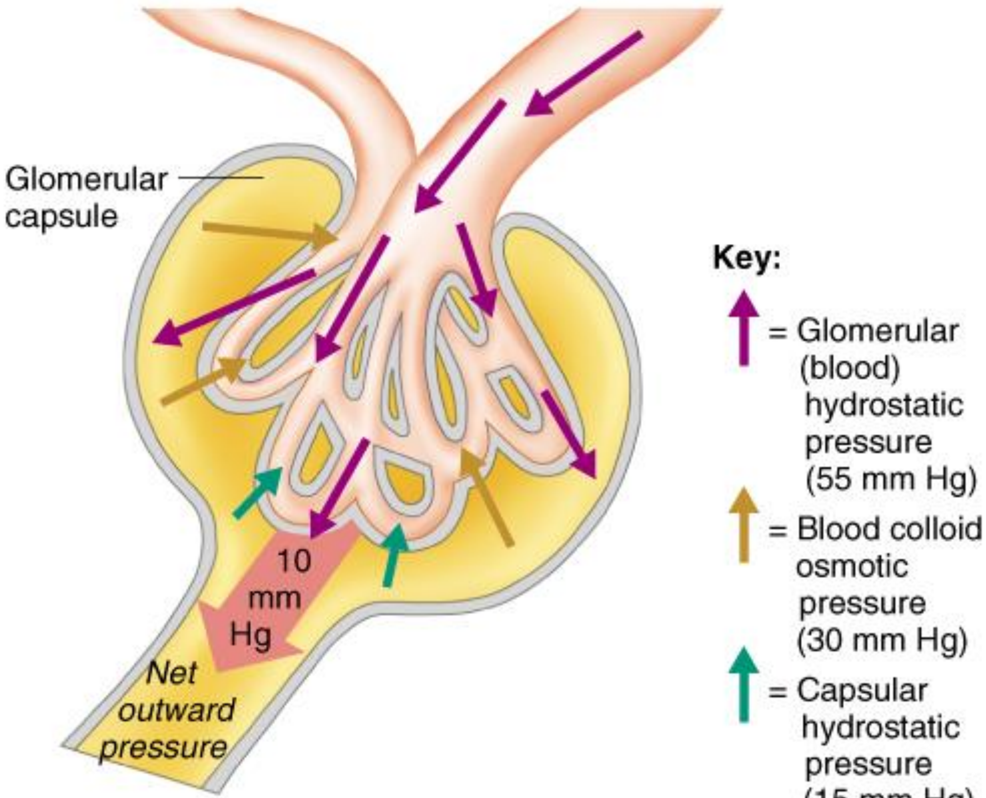
- izotonična raztopina – ista koncentracija topljenca v in izven celice
- hipertonična – več topljenca izven celice, voda gre iz celice in se posuši
- hipotonična – manj topljenca izven celice – voda gre v celico, celica nabrekne in poči



VAJA 1 – NALOGA 4: FILTRACIJA

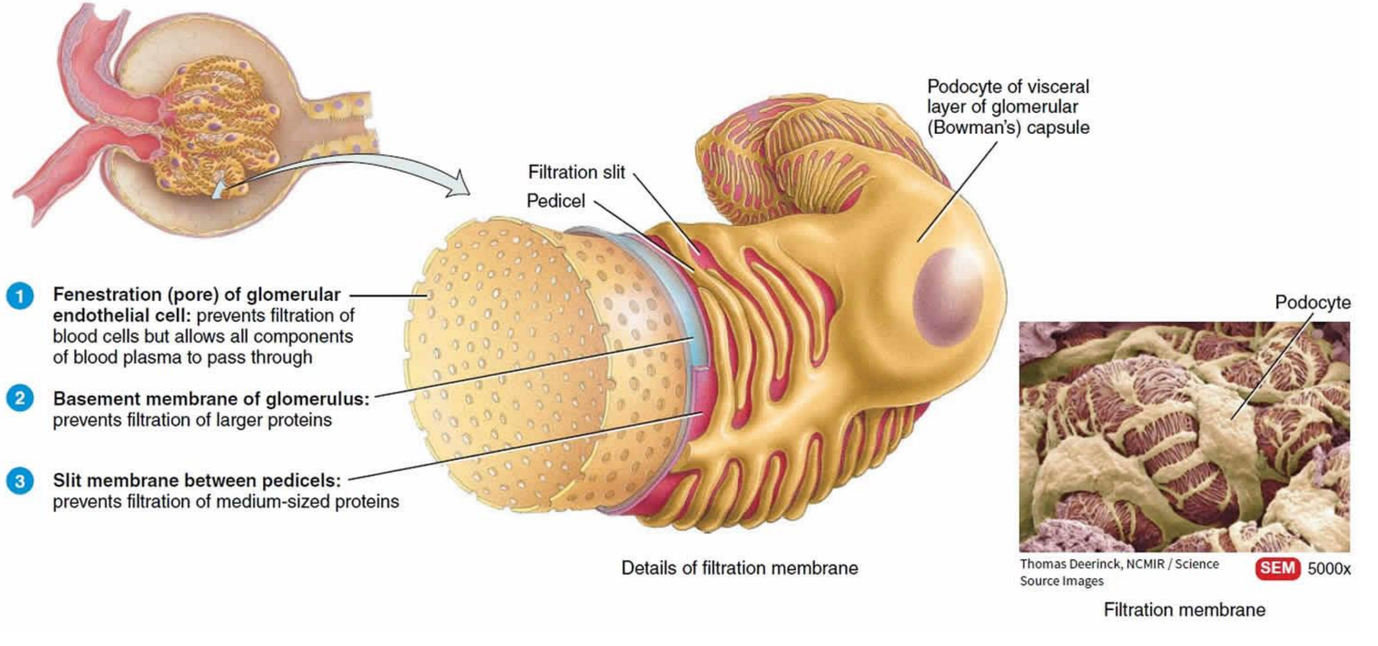
- H2O in topljenec potujeta skozi membrano iz območja z večjih hidrostatskim pritiskom do območja z manjšim h. pritiskom

- pasivni transport
npr. v Malp. telescu v ledvicah – ultrafiltracija krvi: pritisk v kapilarah večji kot v ledvičnih cevkah – voda in topljenci gredo skozi



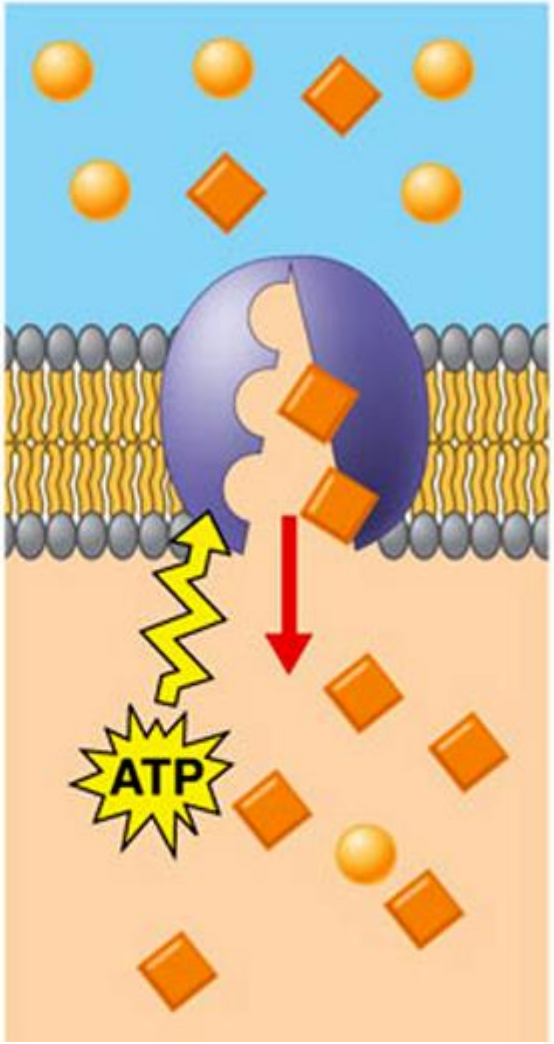
FITLRACIJA

- ni selektiven proces, količina filtrata (tekočine in topljencev) odv. skoraj izključno od:
  - gradienta pritiska
  - velikosti por v membrani



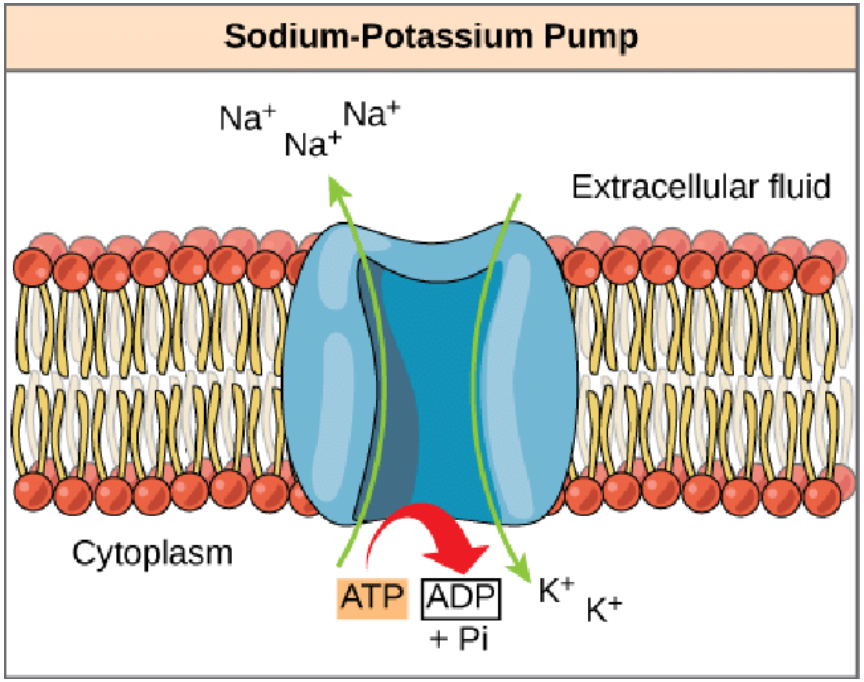
VAJA 1 – NALOGA 5: AKTIVNI TRANSPORT

- v kombinaciji s prenašalcem
- potreben ATP
- v veliko primerih – proti konc. ali elektrokem. gradientu (ali obema)
- npr. aminokisline in nek. sladkorji (preko črpalk – "solute pumps")
    - oboji preveliki, netopni v membrani

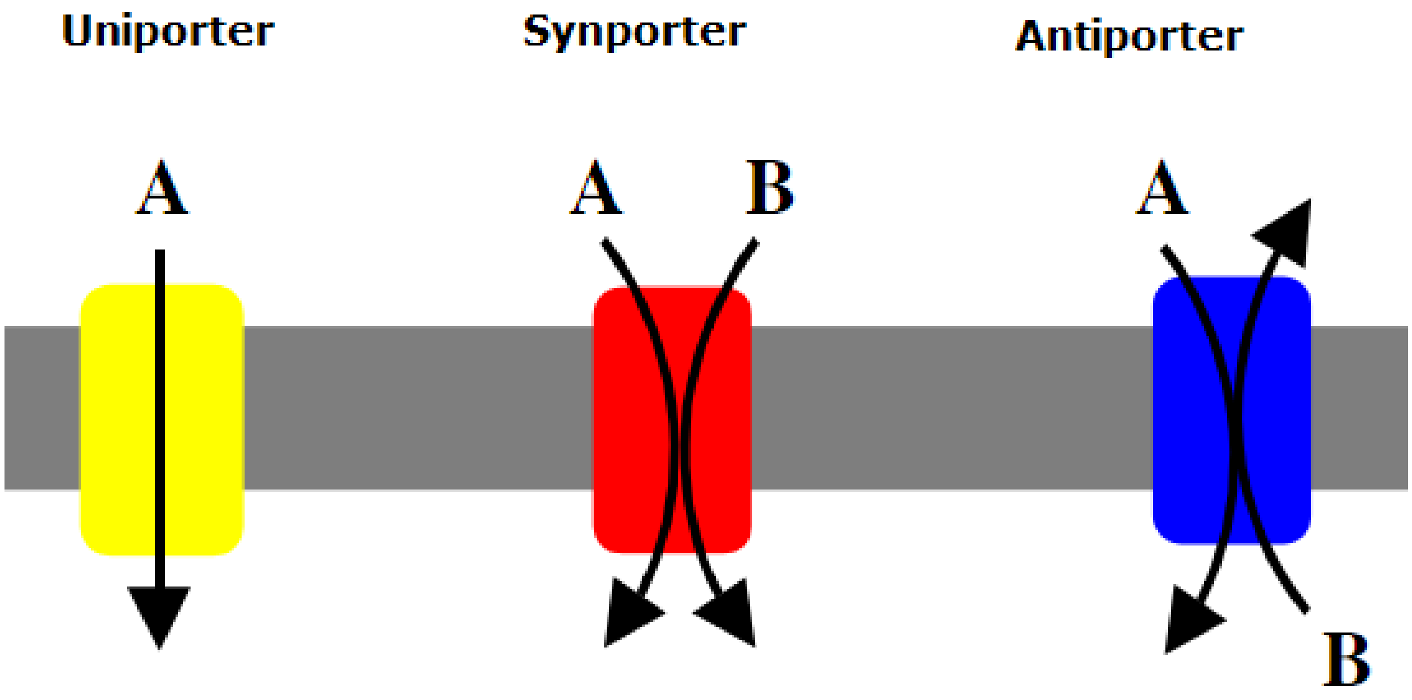


AKTIVNI TRANSPORT

- NA+ ioni ven iz celice z AT
    - več Na^+ v celice
    - prenos z Na^+-K^+ črpalko (“sodium-potassium”)
    - 2 K^+ not , 3 Na^+ - ven



- prenašalci – 1 snov (uniporter) ali več snovi – kotransport (sinporter/antiporter)



Exercise Overview

Cell Transport Mechanisms and Permeability

The molecular composition of the plasma membrane allows it to be selective about what passes through it. It can allow nutrients and appropriate amounts of ions to enter the cell while simultaneously excluding other hydrophilic substances. For that reason, we say the plasma membrane is **selectively permeable**. Valuable cell proteins and other substances are kept within the cell, and metabolic wastes pass to the exterior.

Transport through the plasma membrane occurs in two basic ways: either passively or actively. In **passive processes**, the transport process is driven by concentration or pressure differences (*gradients*) between the interior and exterior of the cell. In **active processes,** the cell provides energy (ATP) to power the transport.

Two key passive processes of membrane transport are **diffusion** and **filtration**. Diffusion is an important transport process for every cell in the body. **Simple diffusion** occurs without the assistance of membrane proteins, and **facilitated diffusion** requires a membrane-bound carrier protein that assists in the transport (view Figure 1.1).

In both simple and facilitated diffusion, the substance being transported moves *with* (or *along* or *down*) the *concentration gradient* of the solute (from a region of its higher concentration to a region of its lower concentration). The process does not require energy

Activities

You can complete the following activities in this exercise.

Exercise Overview

from the cell. Instead, energy in the form of **kinetic energy** comes from the constant motion of the molecules. The movement of solutes continues until the solutes are evenly dispersed throughout the solution. At this point, the solution has reached **equilibrium**.

A special type of diffusion across a membrane is **osmosis**. In osmosis, water moves with its concentration gradient, from a higher concentration of water to a lower concentration of water. It moves in response to a higher concentration of solutes on the other side of a membrane.

In the body, the other key passive process, **filtration**, usually occurs only across capillary walls. Filtration depends upon a *pressure gradient* as its driving force. It is not a selective process. It is dependent upon the size of the pores in the filter.

The two key active processes (recall that active processes require energy) are **active transport** and **vesicular transport**. Like facilitated diffusion, active transport uses a membrane-bound carrier protein. Active transport differs from facilitated diffusion because the solutes move *against* their concentration gradient and because ATP is used to power the transport. Vesicular transport includes phagocytosis, endocytosis, pinocytosis, and exocytosis. These processes are not covered in this exercise.

The activities in this exercise will explore the cell transport mechanisms individually.

Activities

You can complete the following activities in this exercise.

Introduction

Recall that all molecules possess *kinetic energy* and are in constant motion. As molecules move about randomly at high speeds, they collide and bounce off one another, changing direction with each collision. For a given temperature, all matter has about the same average kinetic energy. Smaller molecules tend to move faster than larger molecules because kinetic energy is directly related to both mass and velocity ($KE = 1/2\, mv^2$).

When a **concentration gradient** (difference in concentration) exists, the net effect of this random molecular movement is that the molecules eventually become evenly distributed throughout the environment—in other words, diffusion occurs. **Diffusion** is the movement of molecules from a region of their higher concentration to a region of their lower concentration. The driving force behind diffusion is the kinetic energy of the molecules themselves.

The diffusion of particles into and out of cells is modified by the plasma membrane, which is a physical barrier. In general, molecules diffuse passively through the plasma membrane if they are small enough to pass through its pores (and are aided by an electrical and/or concentration gradient) or if they can dissolve in the lipid portion of the membrane (as in the case of CO_2 and O_2). A membrane is called *selectively permeable, differentially permeable*, or *semipermeable* if it allows some solute particles (molecules) to pass but not others.

The diffusion of *solute particles* dissolved in water through a selectively permeable membrane is called **simple diffusion**. The diffusion of *water* through a differentially permeable membrane is

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called **osmosis**. Both simple diffusion and osmosis involve movement of a substance from an area of its higher concentration to an area of its lower concentration, that is, *with* (or *along* or *down*) its concentration gradient.

This activity provides information on the passage of water and solutes through selectively permeable membranes. You can apply what you learn to the study of transport mechanisms in living membrane-bounded cells. The dialysis membranes used each have a different *molecular weight cutoff* (*MWCO*), indicated by the number below it. You can think of MWCO in terms of pore size: the larger the MWCO number, the larger the pores in the membrane. The molecular weight of a solute is the number of grams per mole, where a mole is the constant Avogadro's number 6.02×10^{23} molecules/mole. The larger the molecular weight, the larger the mass of the molecule. The term molecular mass is sometimes used instead of molecular weight.

Equipment Used

- Left and right beakers—used for diffusion of solutes
- Dialysis membranes with various molecular weight cutoffs (MWCOs)

Introduction

Some molecules are lipid insoluble or too large to pass through pores in the cell's plasma membrane. Instead, they pass through the membrane by a passive transport process called **facilitated diffusion**. For example, sugars, amino acids, and ions are transported by facilitated diffusion. In this form of transport, solutes combine with carrier-protein molecules in the membrane and are then transported *with* (or *along* or *down*) their concentration gradient. The carrier-protein molecules in the membrane might have to change shape slightly to accommodate the solute, but the cell does not have to expend the energy of ATP.

Because facilitated diffusion relies on carrier proteins, solute transport varies with the number of available carrier-protein molecules in the membrane. The carrier proteins can become saturated if too much solute is present and the maximum transport rate is reached. The carrier proteins are embedded in the plasma membrane and act like a shield, protecting the hydrophilic solute from the lipid portions of the membrane.

Facilitated diffusion typically occurs in one direction for a given solute. The greater the concentration difference between one side of the membrane and the other, the greater the rate of facilitated diffusion.

Equipment Used

- Left and right beakers—used for diffusion of solutes
- Dialysis membranes with various molecular weight cutoffs (MWCOs)

Introduction

- Membrane builder—used to build membranes with different numbers of glucose protein carriers

Introduction

A special form of diffusion, called **osmosis**, is the diffusion of water through a selectively permeable membrane. (A membrane is called *selectively permeable, differentially permeable*, or *semipermeable* if it allows some molecules to pass but not others.) Because water can pass through the pores of most membranes, it can move from one side of a membrane to the other relatively freely. Osmosis takes place whenever there is a difference in water concentration between the two sides of a membrane.

If we place distilled water on both sides of a membrane, *net* movement of water does not occur. Remember, however, that water molecules would still move between the two sides of the membrane. In such a situation, we would say that there is no *net* osmosis.

The concentration of water in a solution depends on the number of solute particles present. For this reason, increasing the solute concentration coincides with decreasing the water concentration. Because water moves down its concentration gradient (from an area of its higher concentration to an area of its lower concentration), it always moves *toward* the solution with the highest concentration of solutes. Similarly, solutes also move down their concentration gradients.

If we position a *fully* permeable membrane (permeable to solutes and water) between two solutions of differing concentrations, then all substances—solutes and water—diffuse freely, and an equilibrium will be reached between the two sides of the membrane. However, if we

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use a selectively permeable membrane that is impermeable to the solutes, then we have established a condition where water moves but solutes do not. Consequently, water moves toward the more concentrated solution, resulting in a *volume increase* on that side of the membrane.

By applying this concept to a closed system where volumes cannot change, we can predict that the *pressure* in the more concentrated solution will rise. The force that would need to be applied to oppose the osmosis in a closed system is the **osmotic pressure** (view Figure 1.2). Osmotic pressure is measured in *millimeters of mercury* (*mm Hg*). In general, the more impermeable the solutes, the higher the osmotic pressure.

Osmotic changes can affect the volume of a cell when it is placed in various solutions. The concept of **tonicity** refers to the way a solution affects the volume of a cell. The tonicity of a solution tells us whether or not a cell will shrink or swell. If the concentration of impermeable solutes is the *same* inside and outside of the cell, the solution is **isotonic**. If there is a *higher* concentration of impermeable solutes *outside* the cell than in the cell's interior, the solution is **hypertonic**. Because the net movement of water would be out of the cell, the cell would *shrink* in a hypertonic solution. Conversely, if the concentration of impermeable solutes is *lower* outside of the cell than in the cell's interior, then the solution is **hypotonic**. The net movement of water would be into the cell, and the cell would *swell* and possibly burst.

Introduction

Equipment Used

- Left and right beakers—used for diffusion of solutes
- Dialysis membranes with various molecular weight cutoffs (MWCOs)

Introduction

Filtration is the process by which water and solutes pass through a membrane (such as a dialysis membrane) from an area of higher hydrostatic (fluid) pressure into an area of lower hydrostatic pressure. Like diffusion, filtration is a passive process. For example, fluids and solutes filter out of the capillaries in the kidneys into the kidney tubules because blood pressure in the capillaries is greater than the fluid pressure in the tubules. So, if blood pressure increases, the rate of filtration increases.

Filtration is not a selective process. The amount of *filtrate*—the fluids and solutes that pass through the membrane—depends almost entirely on the *pressure gradient* (the difference in pressure between the solutions on the two sides of the membrane) and on the size of the *membrane pores*. Solutes that are too large to pass through are retained by the capillaries. These solutes usually include blood cells and proteins. Ions and smaller molecules, such as glucose and urea, can pass through.

In this activity the pore size is measured as a *molecular weight cutoff* (*MWCO*), which is indicated by the number below the filtration membrane. You can think of MWCO in terms of pore size: the larger the MWCO number, the larger the pores in the filtration membrane. The molecular weight of a solute is the number of grams per mole, where a mole is the constant Avogadro's number 6.02×10^{23} molecules/mole. You will also analyze the filtration membrane for the presence or absence of solutes that might be left sticking to the membrane.

Introduction

Equipment Used

- Top and bottom beakers—used for filtration of solutes
- Dialysis membranes with various molecular weight cutoffs (MWCOs)
- Membrane residue analysis station—used to analyze the filtration membrane

Introduction

Whenever a cell uses cellular energy (ATP) to move substances across its membrane, the process is an *active transport process*. Substances moved across cell membranes by an active transport process are generally unable to pass by diffusion. There are several reasons why a substance might not be able to pass through a membrane by diffusion: it might be too large to pass through the membrane pores, it might not be lipid soluble, or it might have to move *against*, rather than with, a concentration gradient.

In one type of active transport, substances move across the membrane by combining with a carrier-protein molecule. This kind of process resembles an enzyme-substrate interaction. ATP hydrolysis provides the driving force, and, in many cases, the substances move *against* concentration gradients or electrochemical gradients or both. The carrier proteins are commonly called **solute pumps**. Substances that are moved into cells by solute pumps include amino acids and some sugars. Both of these kinds of solutes are necessary for the life of the cell, but they are lipid insoluble and too large to pass through membrane pores.

In contrast, sodium ions (Na^+) are ejected from the cells by active transport. There is more Na^+ outside the cell than inside the cell, so Na^+ tends to remain in the cell unless actively transported out. In the body, the most common type of solute pump is the Na^+-K^+ (sodium-potassium) pump, which moves Na^+ and K^+ in opposite directions across cellular membranes. Three Na^+ ions are ejected from the cell for every two K^+ ions entering the cell. Note that there is more K^+ inside the cell than outside the cell, so K^+ tends to remain outside the cell unless actively

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transported in.

Membrane carrier proteins that move more than one substance, such as the Na^+-K^+ pump, participate in *coupled transport*. If the solutes move in the same direction, the carrier is a *symporter*. If the solutes move in opposite directions, the carrier is an *antiporter*. A carrier that transports only a single solute is a *uniporter*.

Equipment Used

- Simulated cell inside a large beaker