Transport through biological membranes

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Biochemistry Unit

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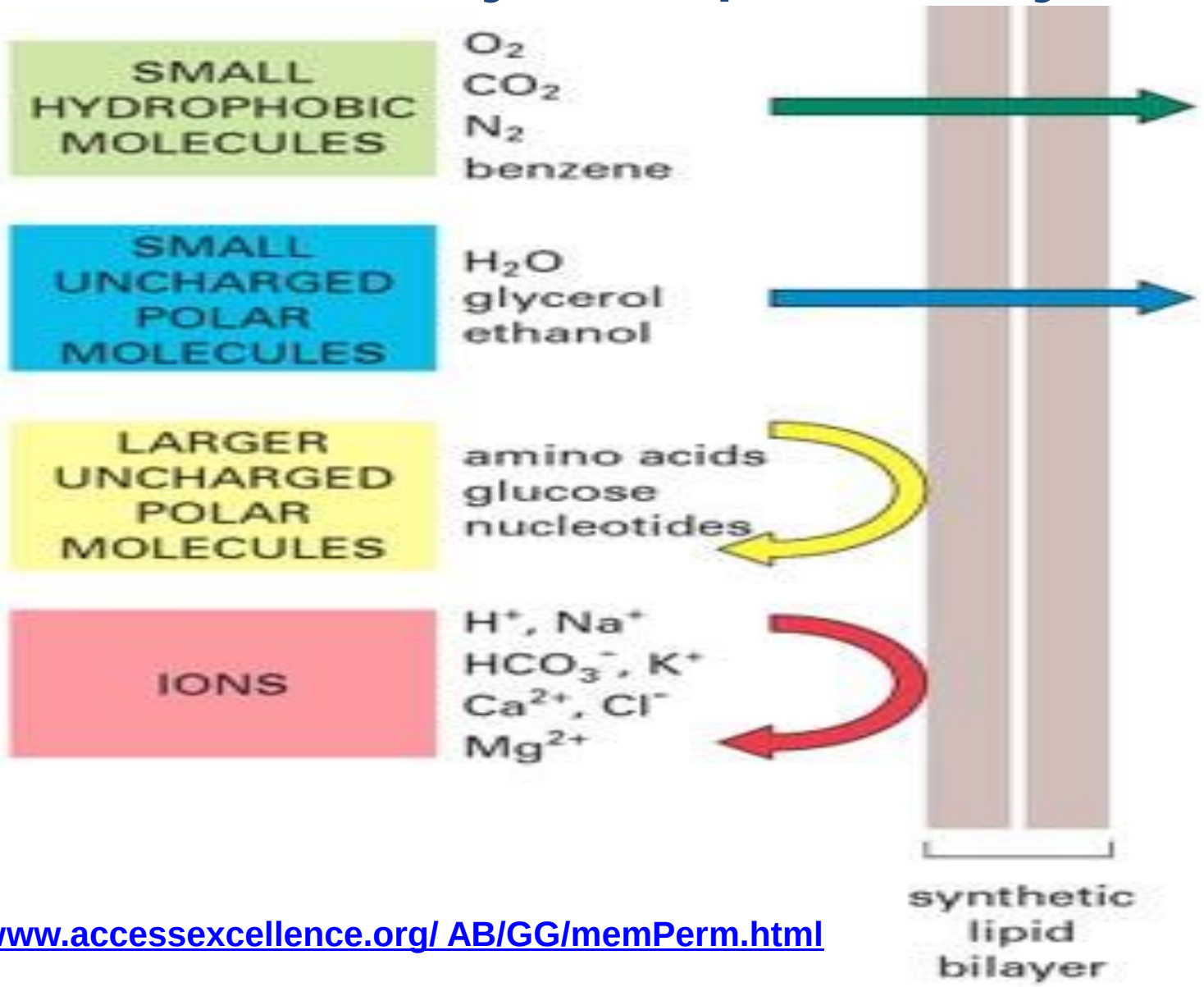
Biological membranes

- Membranes control the structures and environments of the compartments they define and thereby the metabolism of these compartments

Permeability of lipid bilayer



www.accessexcellence.org/ AB/GG/memPerm.html

Transport across biological membranes

- **Passive transport (no energy)**
  - Simple diffusion
    - Directly across lipid membrane
    - Via channel
  - Facilitated diffusion
  - Via carrier protein
- **Active transport (energy req'd)**
  - Via carrier protein

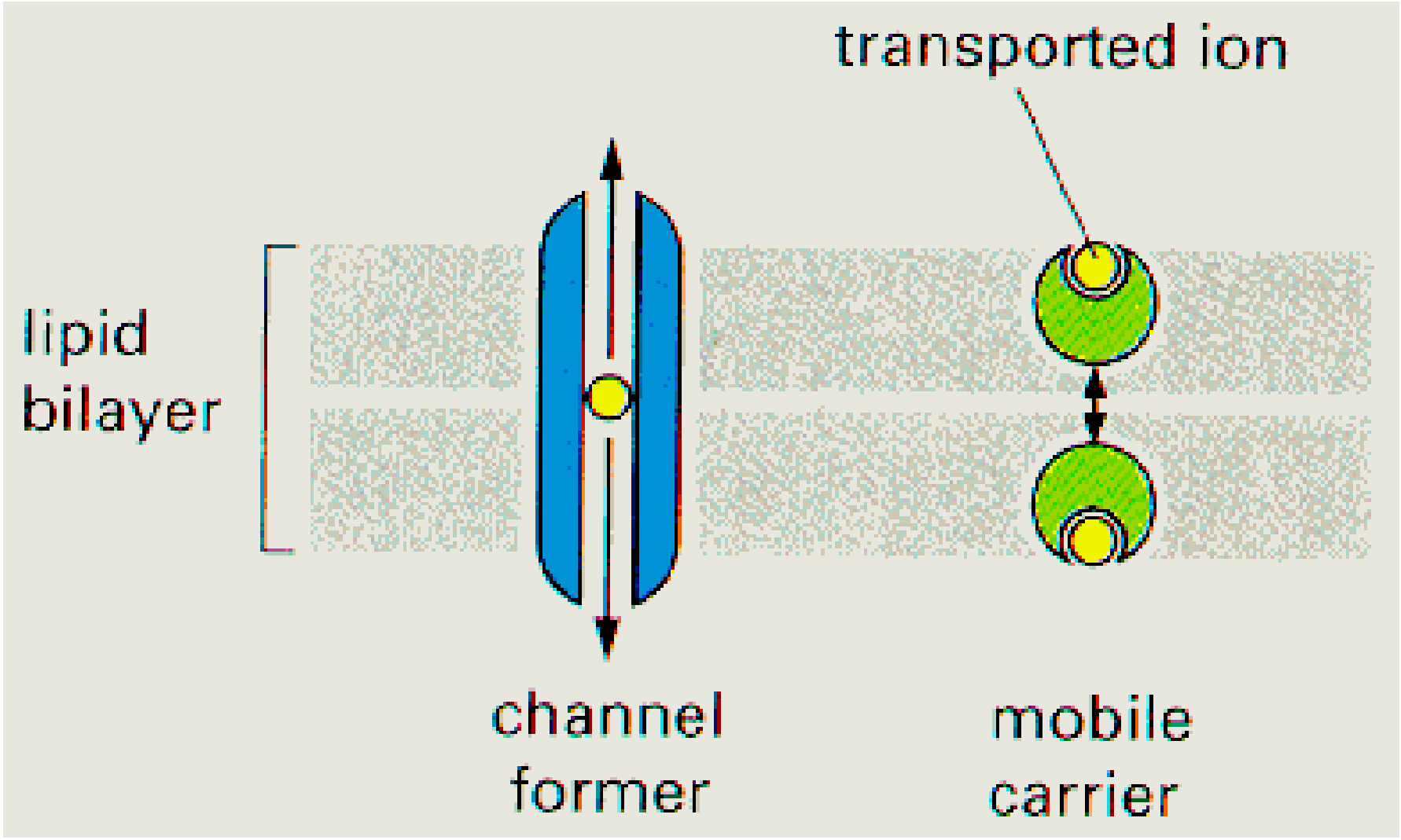
Carriers and channels

- Transporters are of two general classes: **carriers** and **channels.**
- These are exemplified by two **ionophores** (ion carriers produced by microorganisms):
  - **valinomycin** (a carrier)
  - **gramicidin** (a channel)

Ionophores

- Small molecules
- Dissolve in lipid bilayers
- Increase permeability to specific inorganic ions.
  - Shield the charge of the ion against the lipid of the membrane
- Ionic movement is passive
- Used by microorganisms to kill target cells for food or defense
- Synthetic ionophores have been designed to increase mobility of ions across membranes

Ionophores


transported ion
lipid
bilayer
channel
former
mobile
carrier

Valinomycin –an ionophore that is a mobile carrier

- A **carrier** for potassium.
- **circular** molecule made up of 3 repeats of the sequence shown below.

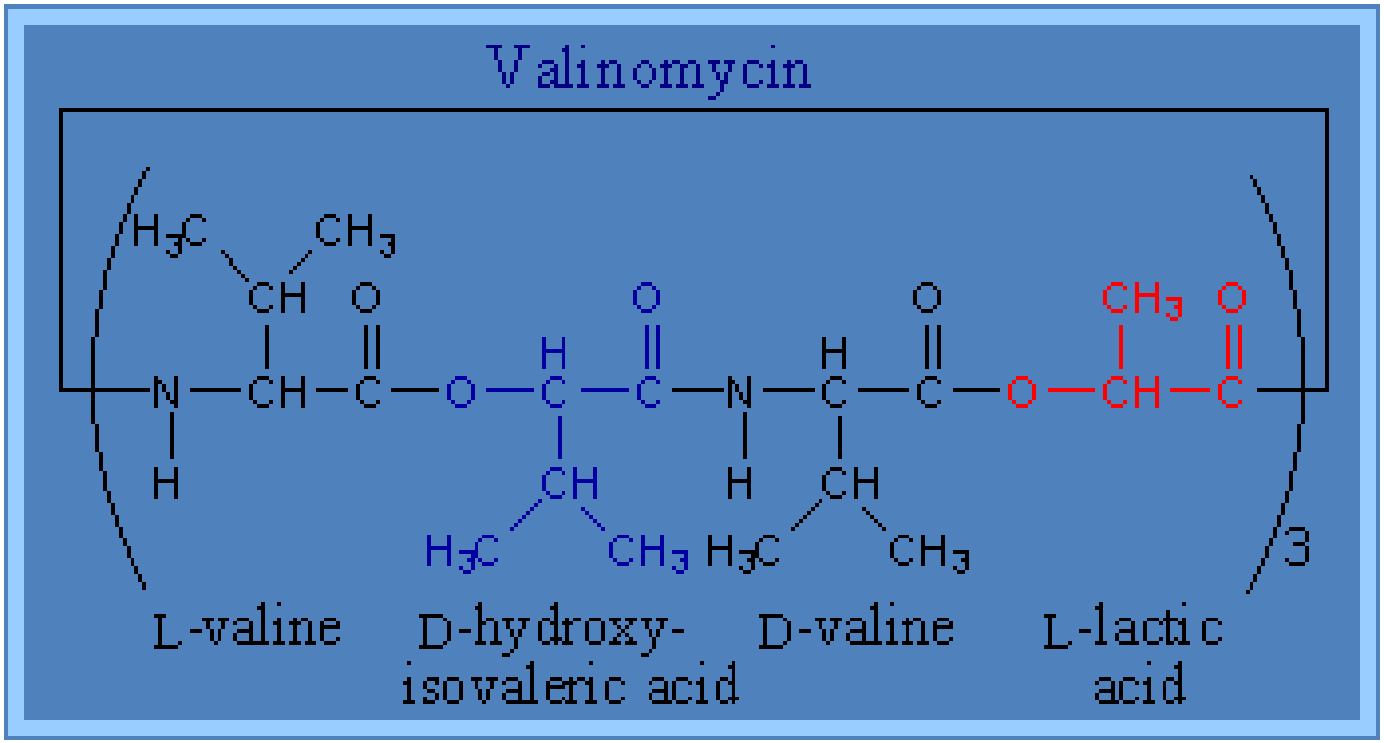


Diagram from www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb1/part2/carriers.htm

Valinomycin

- Polar interior, hydrophobic exterior.
- Reversibly binds single K+ which interacts with oxygen molecules.
- Highly selective for K+ relative to Na+.
- Enters the lipid bilayer and solubilizes K+ within this hydrophobic mileu.

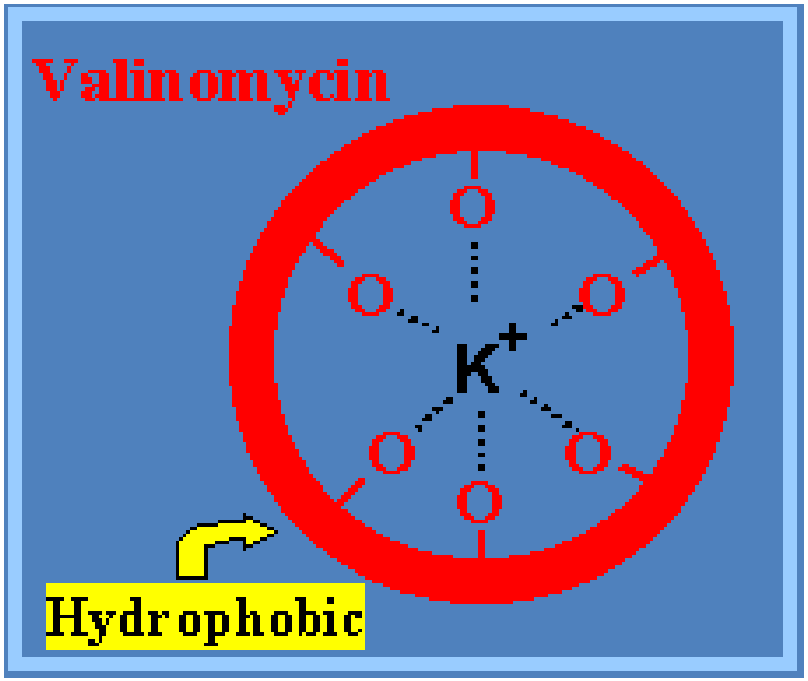


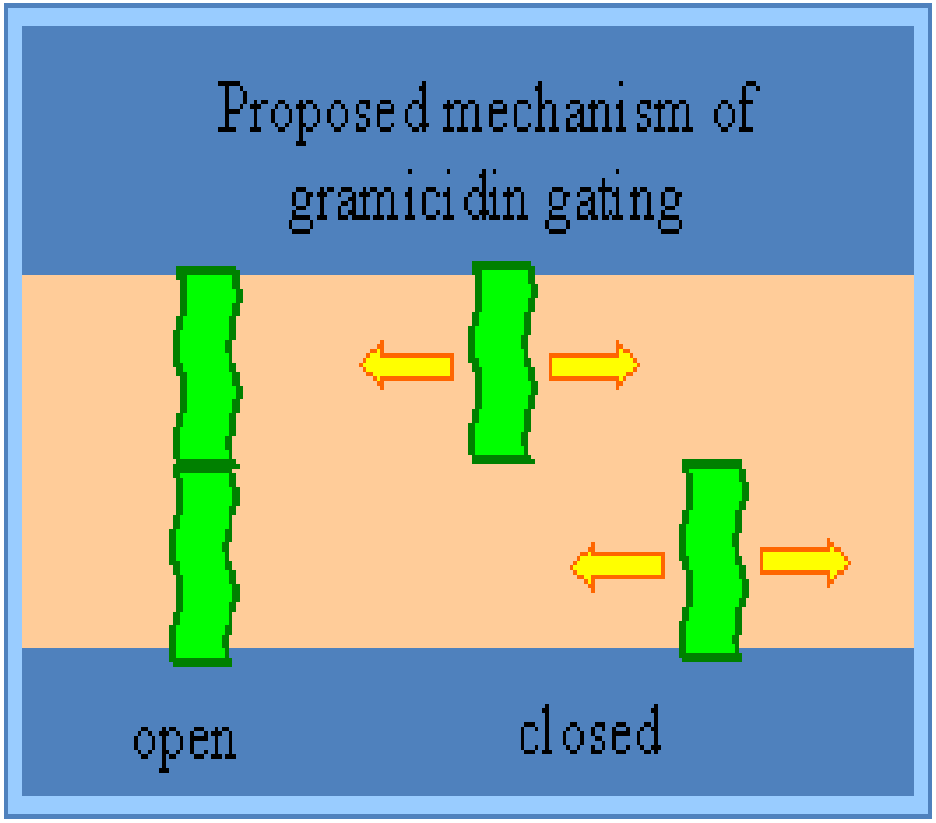
Diagram borrowed from www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb1/part2/carriers.htm

Gramicidin – an ionophore that acts as a channel

- Acts as a channel.
- Unusual molecule, with alternating D and L amino acids.
- In lipid bilayer membranes, it **dimerizes** and folds as a right handed **β-helix** that just spans the bilayer
- Outer surface of the dimer, which interacts with the core of the lipid bilayer, is hydrophobic.
- Ions pass through the more polar lumen of the helix.

Gramicidin

- **Gating** (opening & closing) of a gramicidin channel thought to involve reversible **dimerization**.
- Open channel forms when two gramicidin molecules join end to end to span the membrane.



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Passive transport

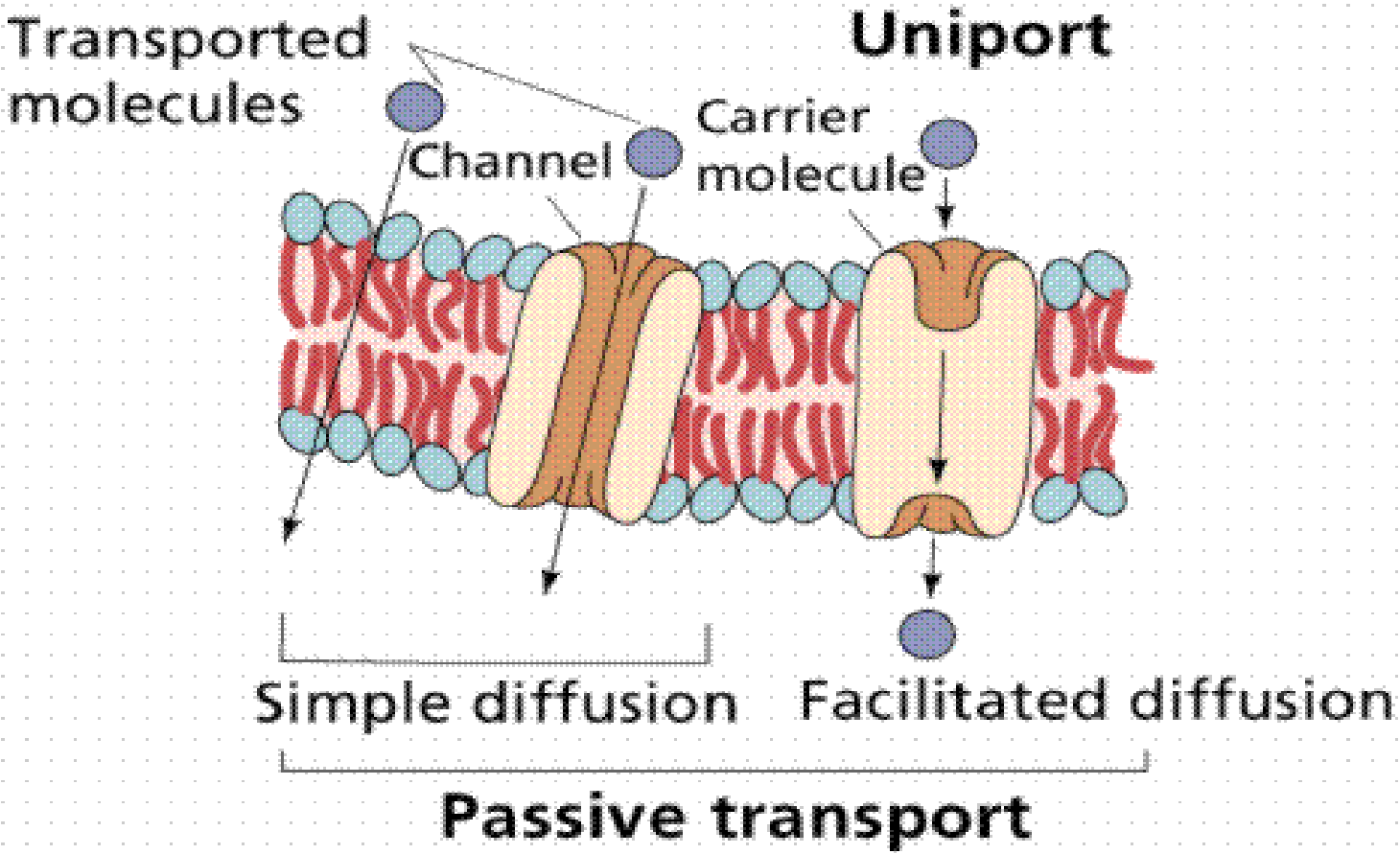


Figure borrowed from www.emc.maricopa.edu/.../farabee/BIOBK/BioBooktransp.html

Simple diffusion

- Spontaneous movement of solute **DOWN** a concentration gradient **ONLY**
- Occurs until concentration of molecules on both sides of membrane equal
- Rate of diffusion (v) of a molecule (s) across membrane
  - directly proportional to concentration gradient ($[S]_{ext} - [S]_{int}$)
  - inversely proportional to thickness of membrane (l)

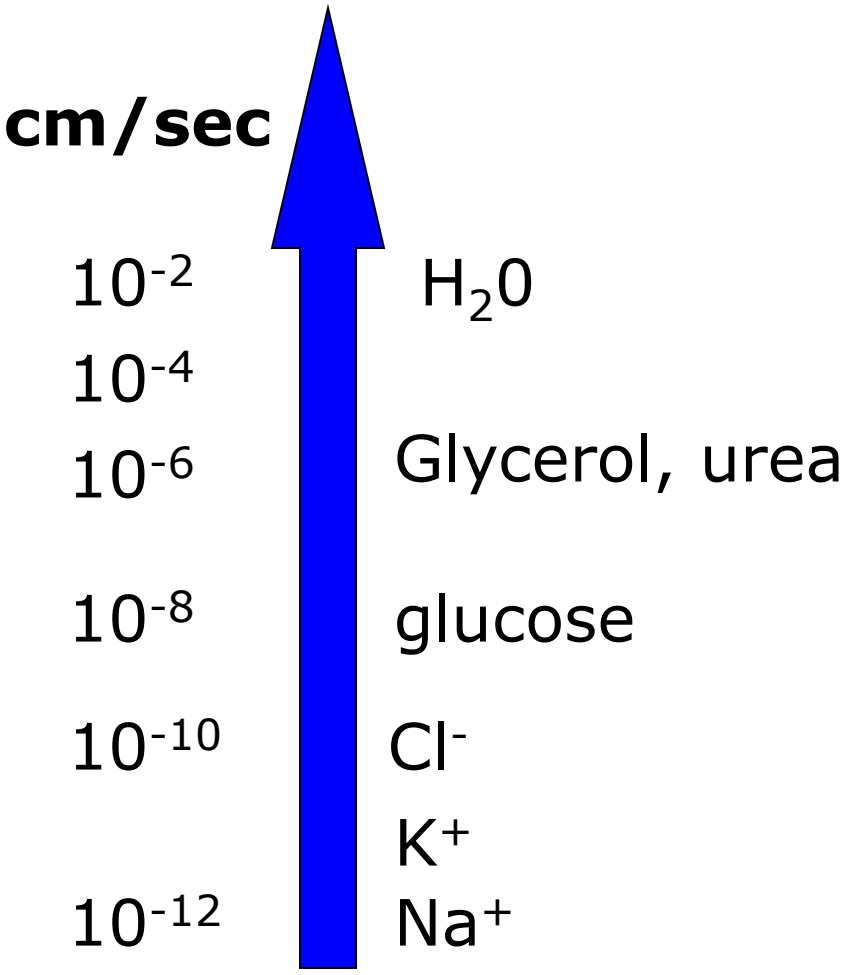
$v = D([S]_{ext} - [S]_{int})/ l$

D = diffusion coefficient ($cm^2.sec^{-1}$)

D/l = permeability coefficient ($cm .sec^{-1}$)

Permeability coefficient

- **Rate of diffusion depends on:**
  - Charge (charge prevents movement)
  - Size (small molecules move faster
  - Lipid solubility (non polar move faster)
  - Concentration gradient
- **Considering D/l for glucose, cells would die if glucose were transported by simple diffusion alone!**

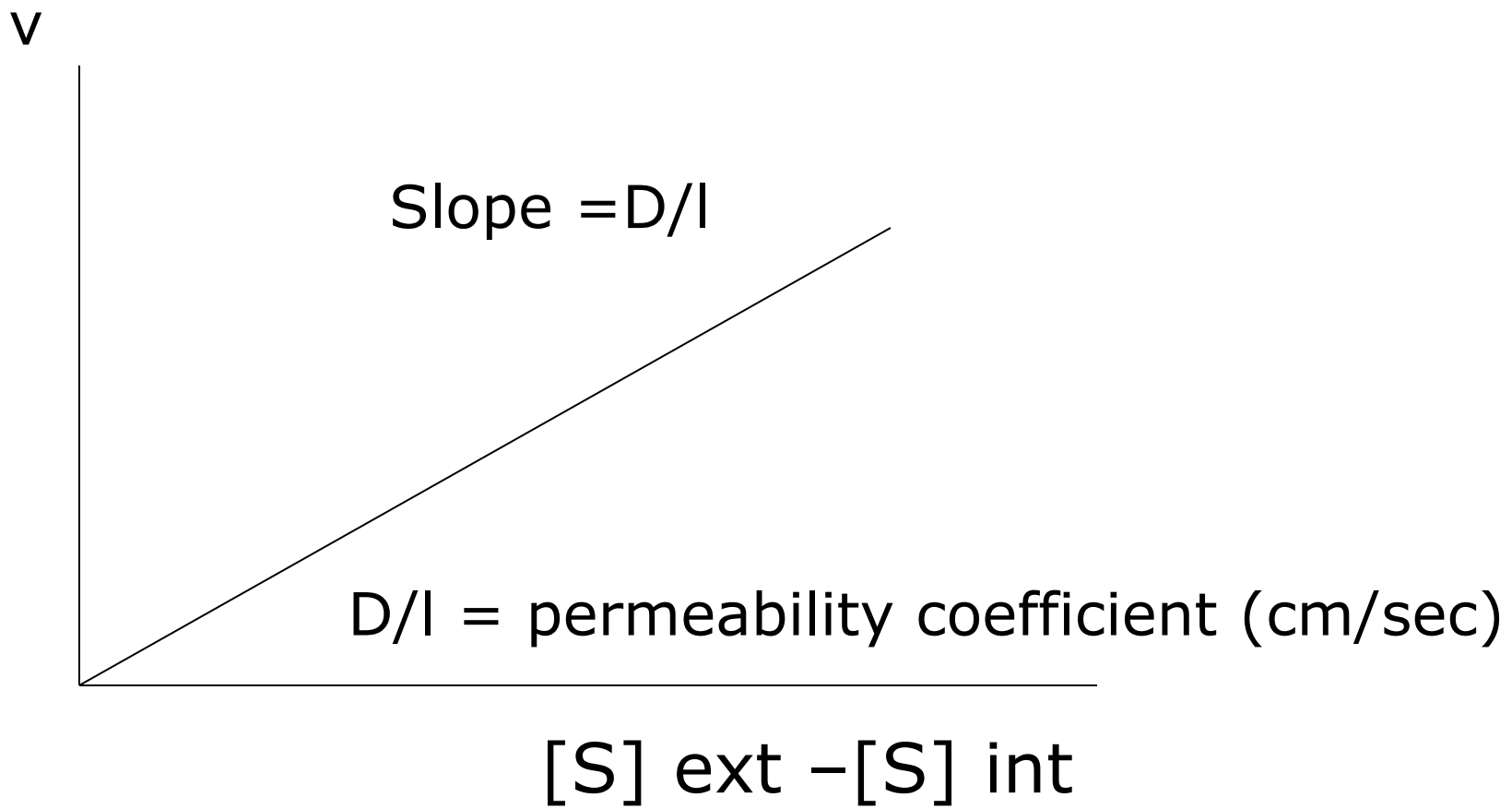


Permeability coefficient

- Solubility in lipid bilayer inversely proportional to no. of hydrogen bonds that must be broken in for a solute in the external aqueous phase to become incorporated in the hydrophobic phase

- Organic and hydrophobic molecules more soluble than charged molecules.

Kinetics (simple diffusion)



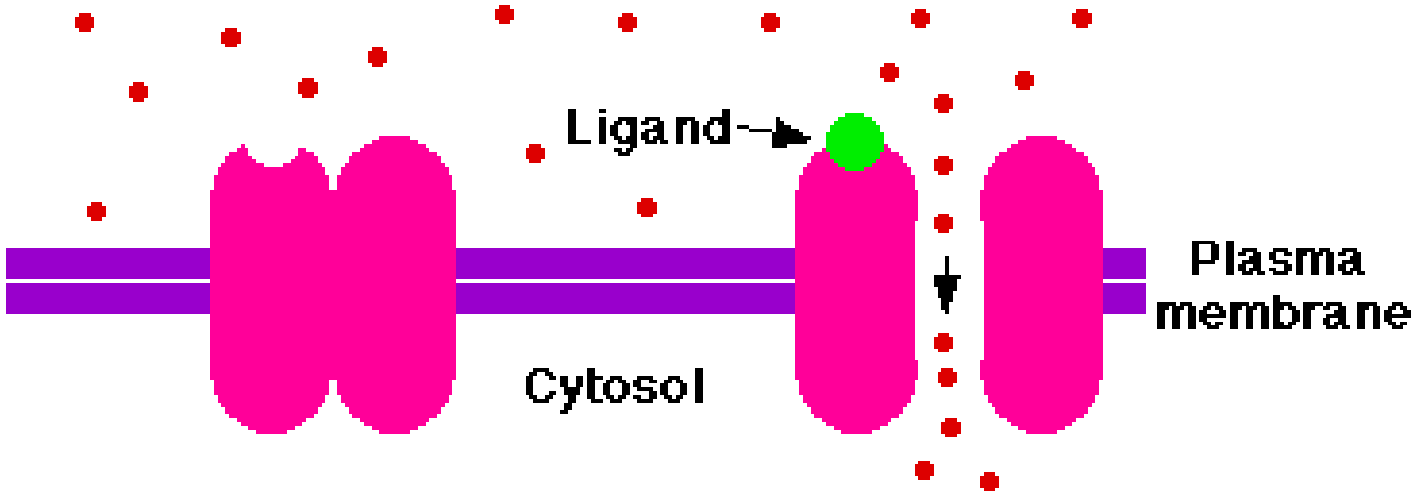
Simple diffusion via channels

- **Electrolytes not very soluble in lipid membrane**
  - No hydrogen bonds with water *but* acquire shell of water from hydration by electrostatic interactions
  - Size of shell directly proportional to charge density of electrolyte
  - Larger the shell = slower rate of diffusion
- **Some ions diffuse through channels in membrane**

Ion channels

- **Transmembrane channels**
  - pore-like structures (proteins)
  - selective ion –conductive pathways
- **Permeability of channel depends on its size, extent of hydration and charge density on the ion**
- **Ion channels may be regulated**
  - Ligand gated
  - Voltage gated
  - Mechanically gated

Ligand gated channel



Facilitated diffusion through a ligand-gated channel

- Ligand may be extra- or intracellular
- Ligand is **not** the substance transported when the channel opens

Ligand-gated ion channels

- **External ligands**
  - **ACh** opens Na^+ channels; initiates nerve impulse / muscle contraction
  - **GABA** binding admits Cl^- ions into cell; inhibits nerve impulse

- **Internal ligands**
  - "Second messengers", **cAMP** and **cGMP** regulate channels involved in initiation of impulses in neurons responding to odors and light respectively

Voltage-gated ion channels

- In **neurons** and **muscle cells** some channels open / close in response to changes in the charge across plasma membrane.
  - As impulse passes down neuron, reduction in the voltage opens sodium channels in the adjacent portion of the membrane
  - ~ 7000 sodium ions pass through each channel during the brief period (about 1 millisecond) that it remains open.

Mechanically-gated ion channels

- **Sound** waves bending projections on hair cells of inner ear open ion channels leading to the creation of nerve impulses that brain interprets as sound.

- Mechanical deformation of the cells of **stretch receptors** opens ion channels leading to the creation of nerve impulses.

Carrier mediated transport

- Carrier usually a protein that changes conformation during transport
- With carrier proteins there is **never an open channel all the way through the membrane**

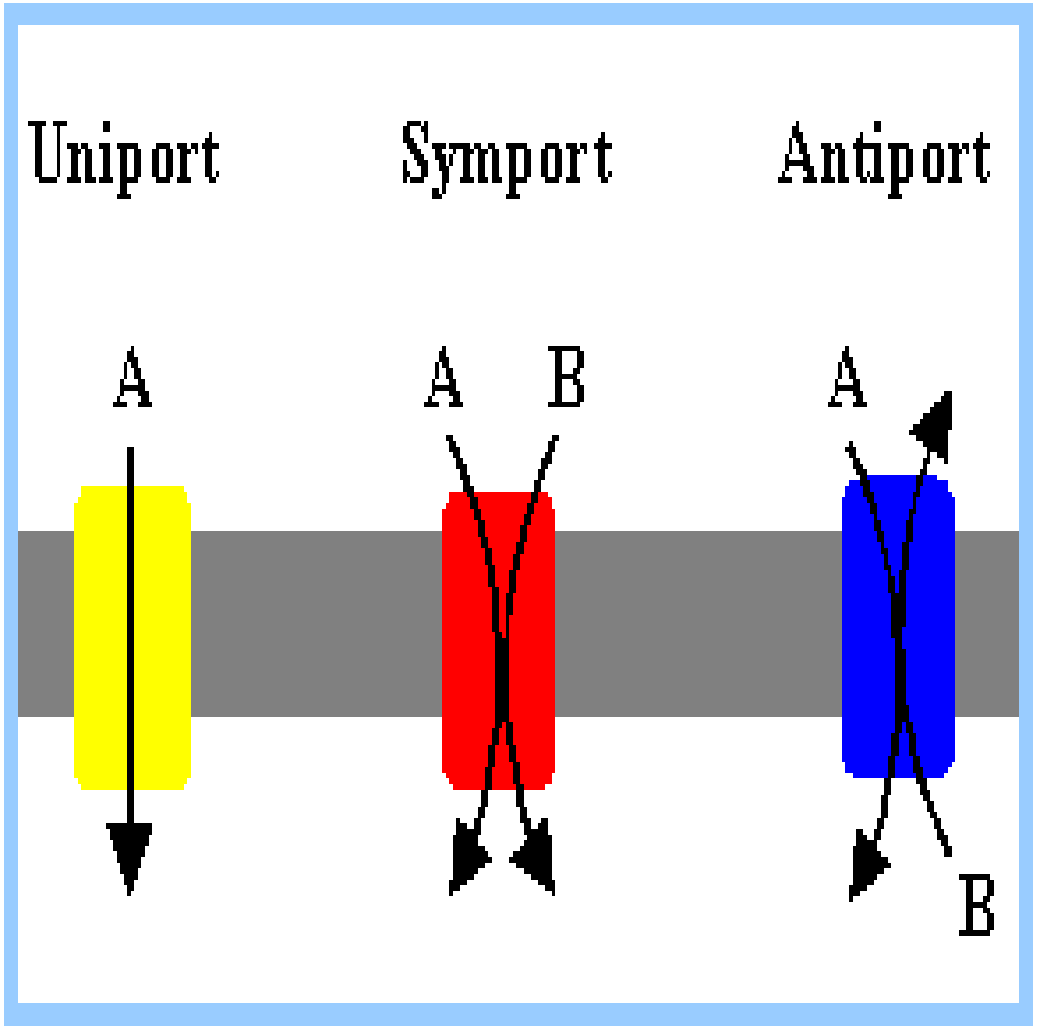


Diagram borrowed from www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb1/part2/carriers.htm

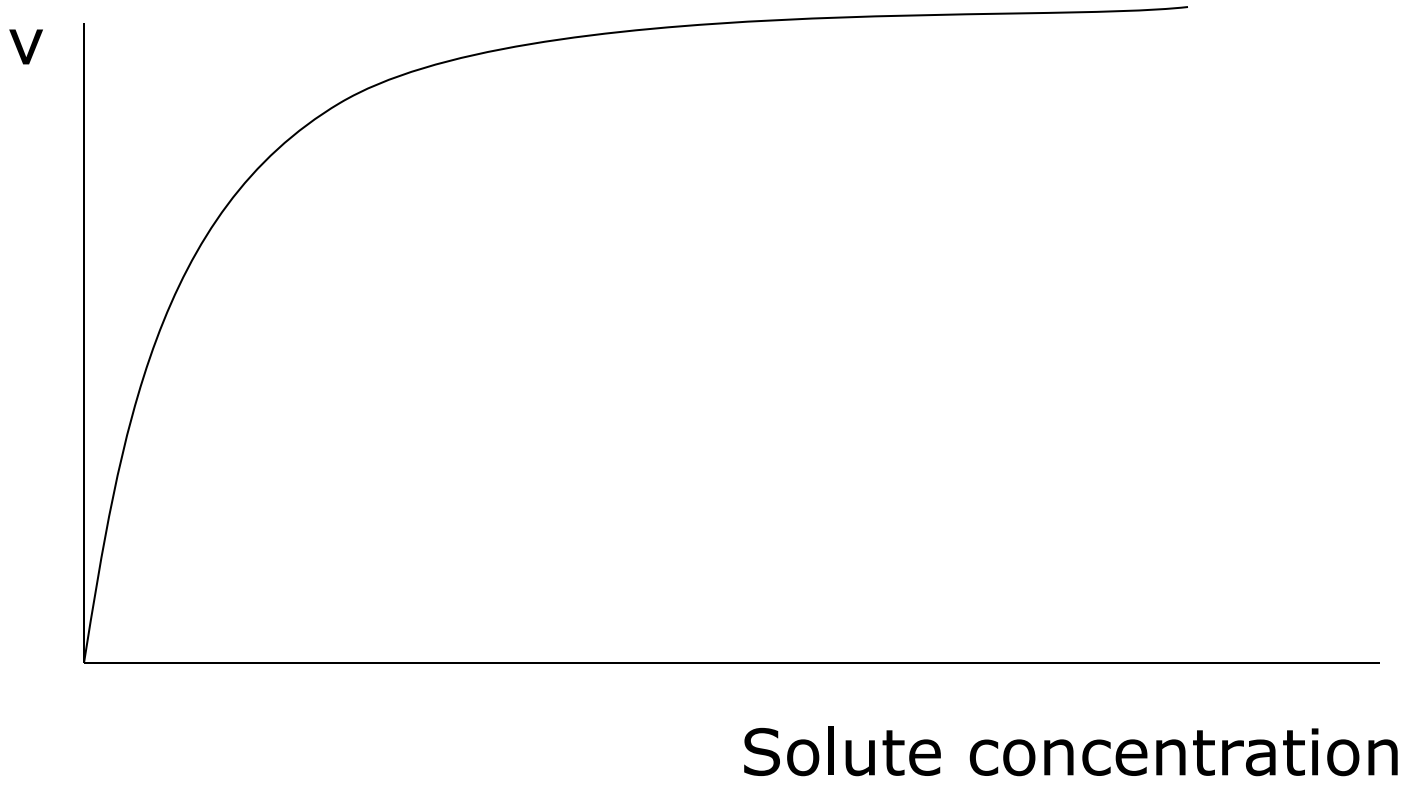
Carrier mediated transport

- **Active transport**
- **Facilitated diffusion**
- **A carrier must be able to perform four functions:**
  - Recognition (specifically bind to substance)
  - Translocation (movement from one side to the other)
  - Release (on the other side)
  - Recovery (return to original position)

Passive (facilitated) transport

- Facilitated diffusion
- Involves a carrier
- Movement down concentration gradient (no energy required)
- V = Vmax [S]ext/Km + [S]ext
- Carrier is saturable
- Faster than simple diffusion

Kinetics (facilitated diffusion)



Mechanism for facilitated diffusion

- Carrier exist in two principal conformations
- Protein in one conformation associates with solute in high concentration on one side of the membrane
- Change in conformation
- Resulting in solute being discharged on other side of membrane
- Process completely reversible
- Net flux depends on concentration gradient

Facilitated diffusion

- Rate determined by
  - Amount of carrier available (key control step)
  - Concentration gradient across the membrane
  - Rapidity of solute-carrier interaction
  - Rapidity of conformational change for both loaded and unloaded state
- Hormones that regulate facilitated diffusion do so by regulating number of carrier molecules available (eg. aldosterone increases no. of Na^+ channels)

Active transport

- Involves a carrier
- Movement against a concentration gradient
- Requires energy
- Mediated by ATP-dependent ion pumps

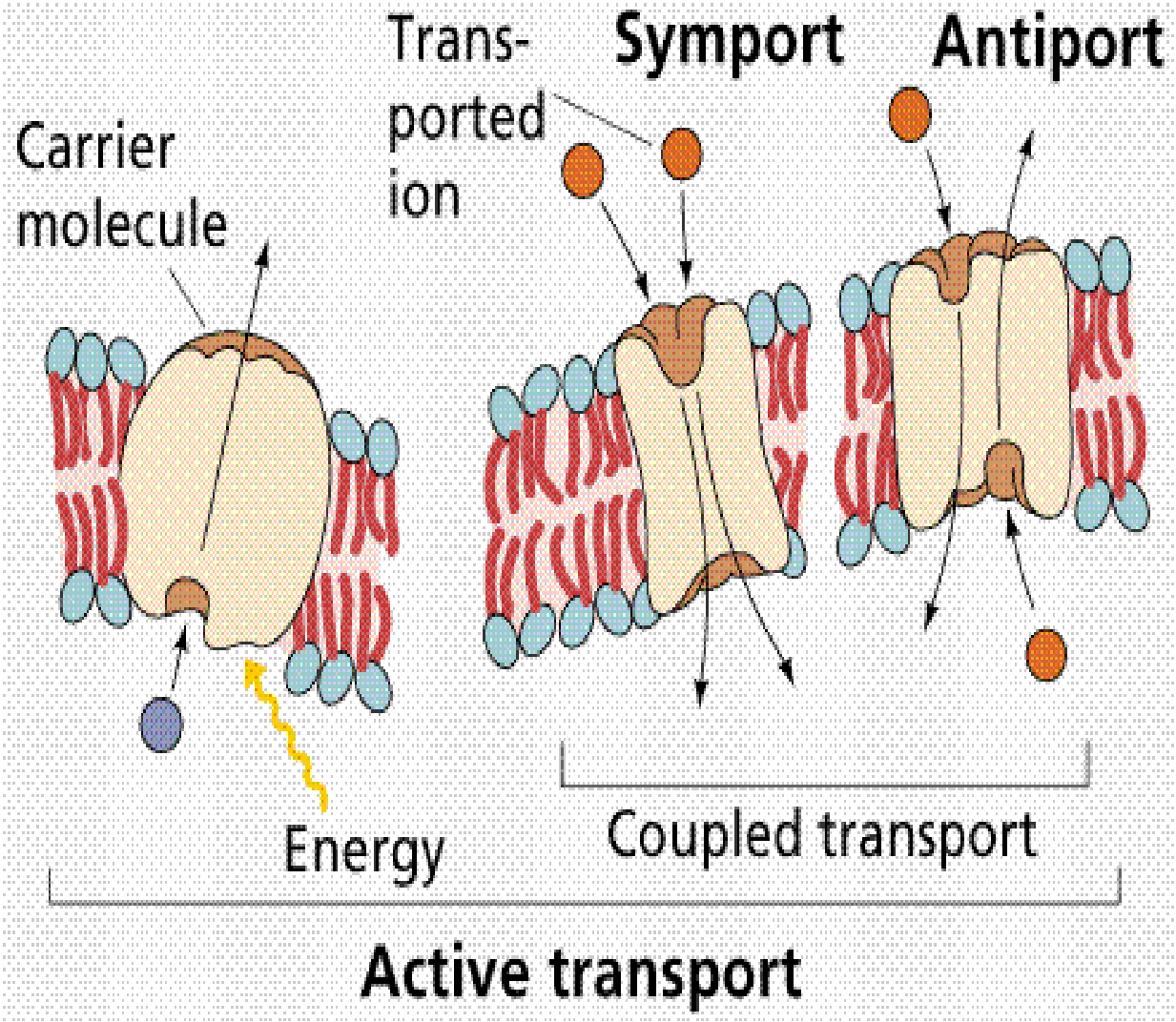


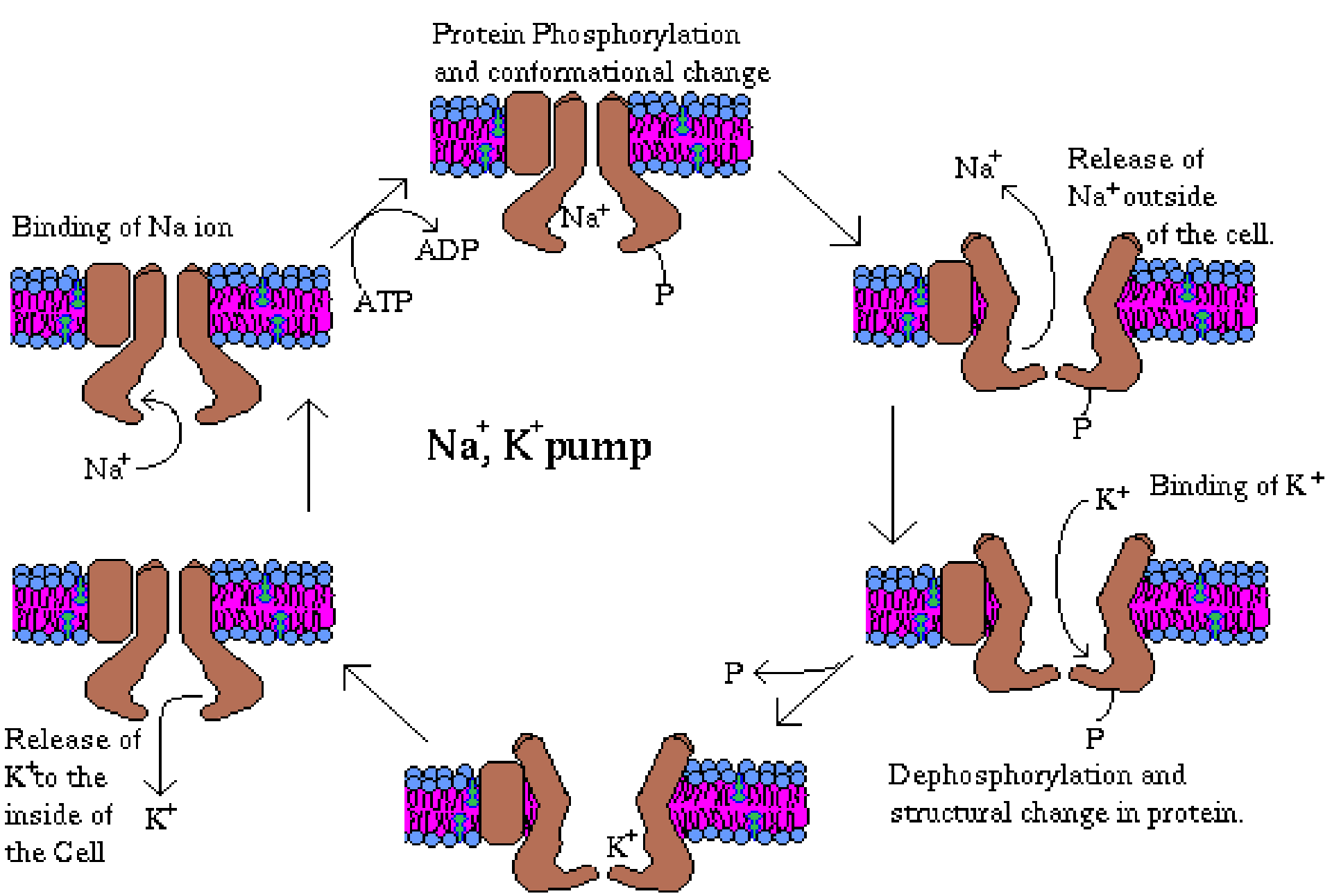
Figure borrowed from
www.emc.maricopa.edu/.../farabee/BIOBK/BioBooktransp.html

ATP-dependent ion pumps

- **(Na^+, K^+)-ATPase**
  - Antiport found in plasma membrane
  - transport of Na^+ out of a cell in exchange for K^+.

- **(H^+, K^+)-ATPase**
  - Antiport involved in acid secretion in stomach
  - transport of H^+ out of gastric parietal cell (toward stomach lumen) in exchange for K^+.

- **Ca^{++}-ATPases**
  - Found in ER and plasma membranes of many cells
  - transport of Ca^{++} away from the cytosol (into the ER or out of the cell).
  - keeps cytosolic [Ca^{++}] low, allowing Ca^{++} to serve as a signal.



- The Top is the Outter membrane.
- The Bottom is the inner membrane (inside of the Cell)

Ca^{2+} ATPase in muscle cells

- Found in sarcolemma (> 80% of membrane protein)
- Sequence similar to Na^{+}-K^{+} ATPase
- Mechanism

$$E + ATP \xleftarrow{Ca^{2+},\ Mg^{2+}} E\text{–}P + ADP$$

$$E\text{-}P + H_2O \xrightarrow{Mg^{2+}} E + Pi$$

Ca2+ ATPase in muscle cells

- **In resting muscle**
  - Pumps Ca2+ into sarcoplasmic reticulum - keep [Ca2+] around muscle fibre low
- **Muscle contraction**
  - S.R. membrane excited by nerve impulse
  - Sudden release of Ca2+ in to cytosol (0.1 → 10uM)
  - Muscle contraction
- **Muscle relaxation**
  - Occurs when Ca2+ pumped back into SR by Ca2+ ATPase

Secondary active transport

- Secondary active transport does not use ATP directly but takes advantage of a previously existing concentration gradient.
- The net direction of movement is dependent on the concentration gradient.

Na+-glucose symport (a co-transporter)

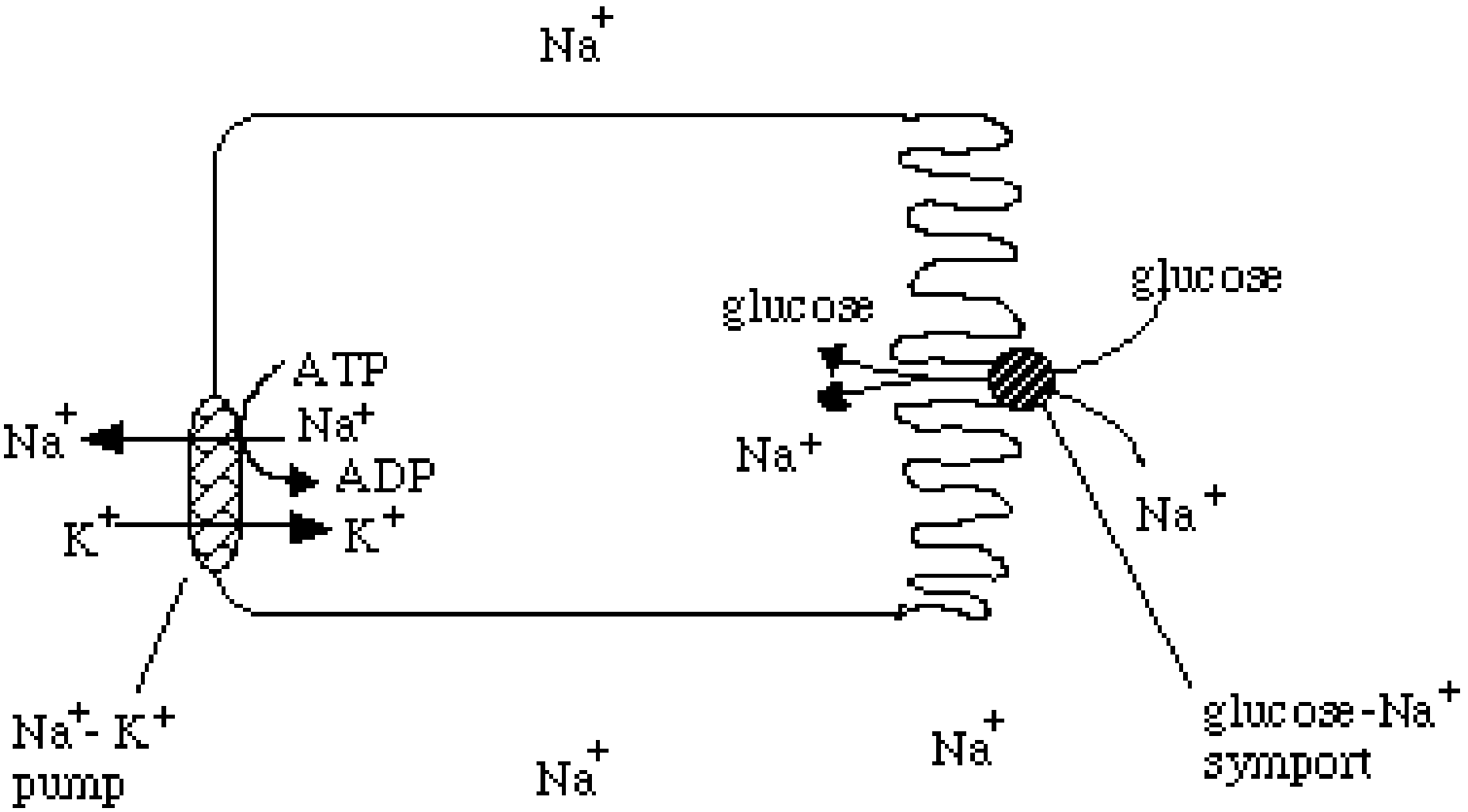
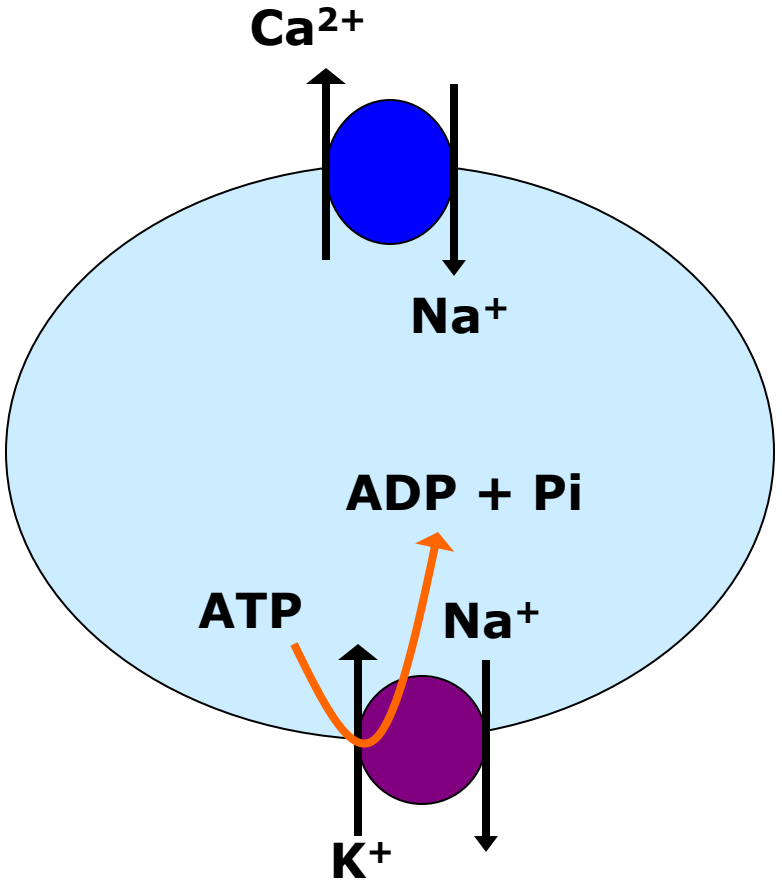


Diagram from web.mit.edu/esgbio/www/cb/membranes/transport.html

Na+/Ca2+ exchanger (an antiport)

- Antiport
- Helps maintain low intracellular [Ca^{2+}]
- Moves Ca^{2+} out of cell in exchange for Na^{+}
- Driven by Na^{+} gradient created by Na^{+}/K^{+} ATPase



Cardiotonic steroids

- Digitoxigenin and Oubain
- Used in treatment of congestive heart failure
- Act by inhibiting Na^+-K^+ ATPase
  - Increased Na^+ inside cell
  - Slower extrusion of Ca2+
  - Increased intracellular Ca2+
  - Enhanced contractility of heart muscle

Ion channels and disease

- **Chloride-channel** diseases:
  - Cystic fibrosis
  - Inherited tendency to kidney stones (caused by a different kind of chloride channel than the one involved in cystic fibrosis)
- **Potassium-channel** diseases
  - some inherited life-threatening defects in the heartbeat (arrythmias)
  - a rare, inherited tendency to epileptic seizures in the newborn.
  - several types of inherited **deafness**

Ion channels and disease

- **Sodium-channel diseases**
  - inherited tendency to certain types of muscle spasms
  - Implicated in cardiac arrythmias
  - Liddle's syndrome
    - inadequate sodium transport out of the kidneys, because of a mutant sodium channel
    - leads to elevated osmotic pressure of the blood and resulting hypertension (high blood pressure).

Ion channels and disease

- **Autosomal dominant polycystic kidney disease**
  - inherited disorder
  - formation of renal cysts leads to progressive destruction of normal tissue and end-stage kidney failure.
  - Two proteins implicated in autosomal dominant polycystic kidney disease
    - polycystin-1
    - polycystin-2.
  - Evidence that polycystin-1 binds polycystin-2 and regulates its activity as an ion channel.