Introduction to Metal Transport

Bertini *et al* Ch. 5 and 8

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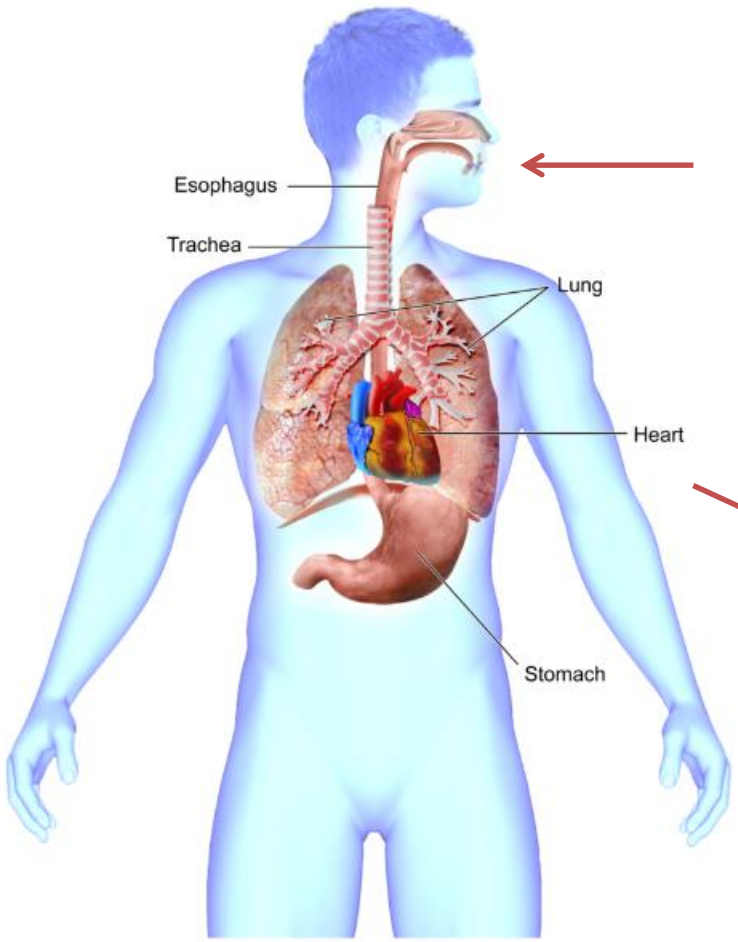
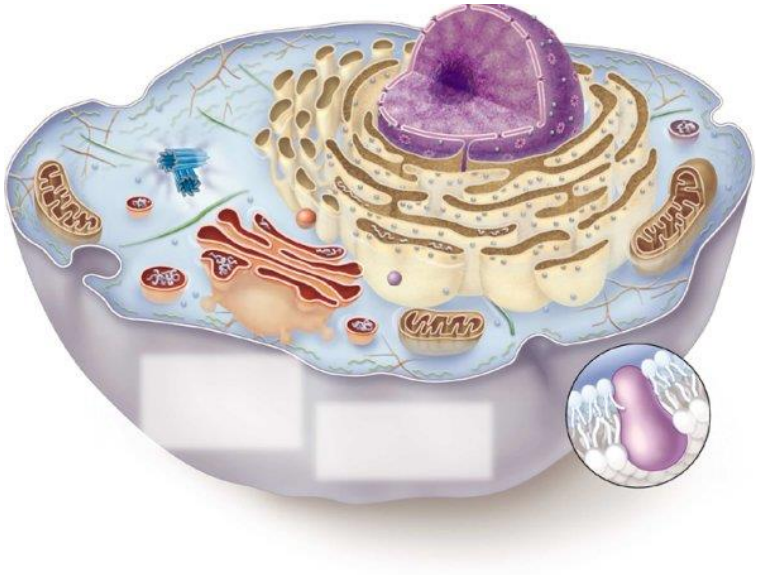
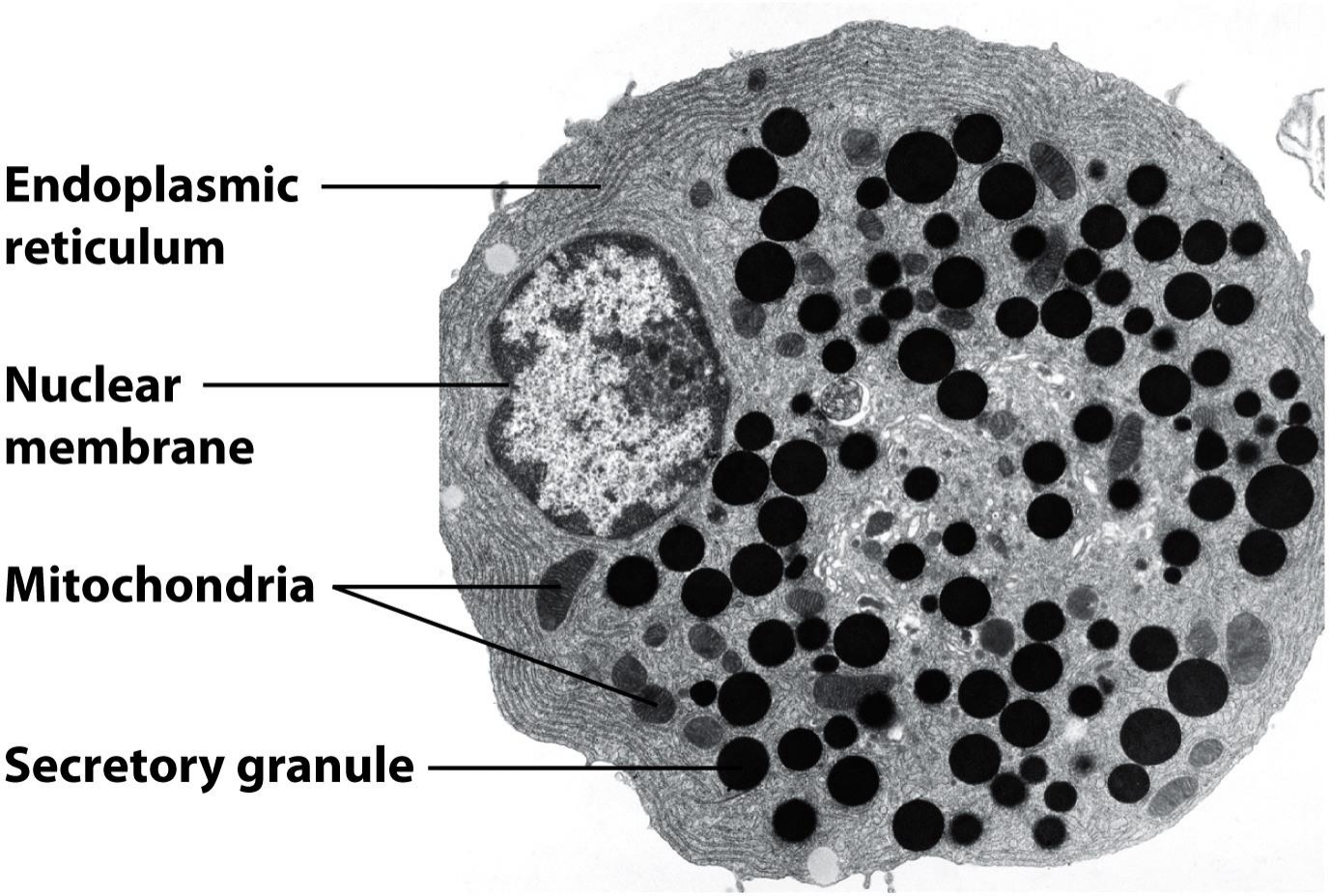
Focus on Metal Transport to Cells



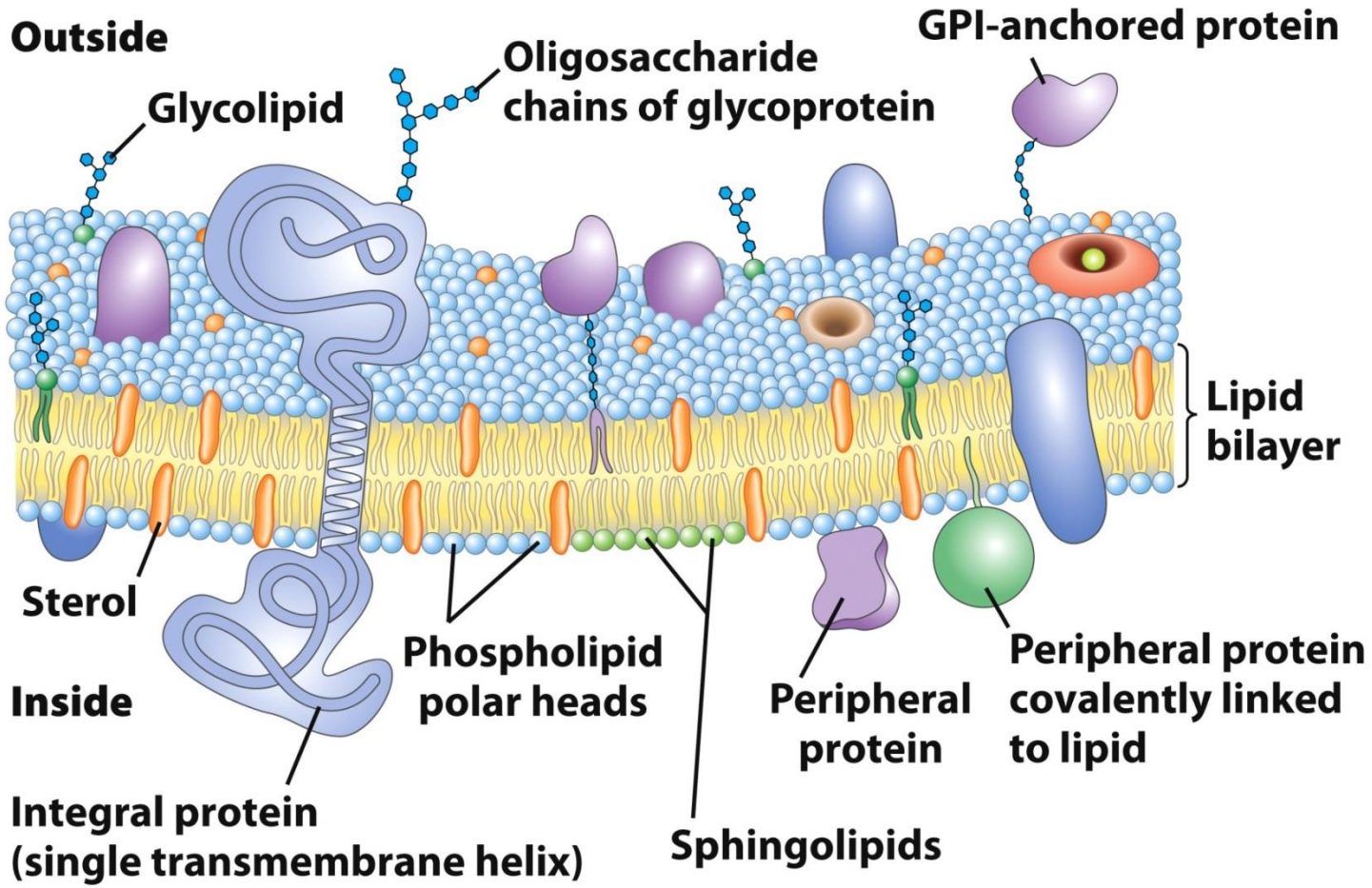
Movement through Membranes



Electron Micrograph of Biological Membranes



The Composition of Eukaryotic Biological Membranes



1. Biological Membranes

In eukaryotic cells, membranes play many important functions.

A. Define the external boundaries of cells and regulate the molecular traffic across that boundary.

B. Divide the internal space into discrete compartments to segregate processes and components.

C. Aid in cell-to-cell communication and in signaling.

D. Organize complex reaction sequences and cellular processes.
   - Energy transduction
   - Biomolecule synthesis

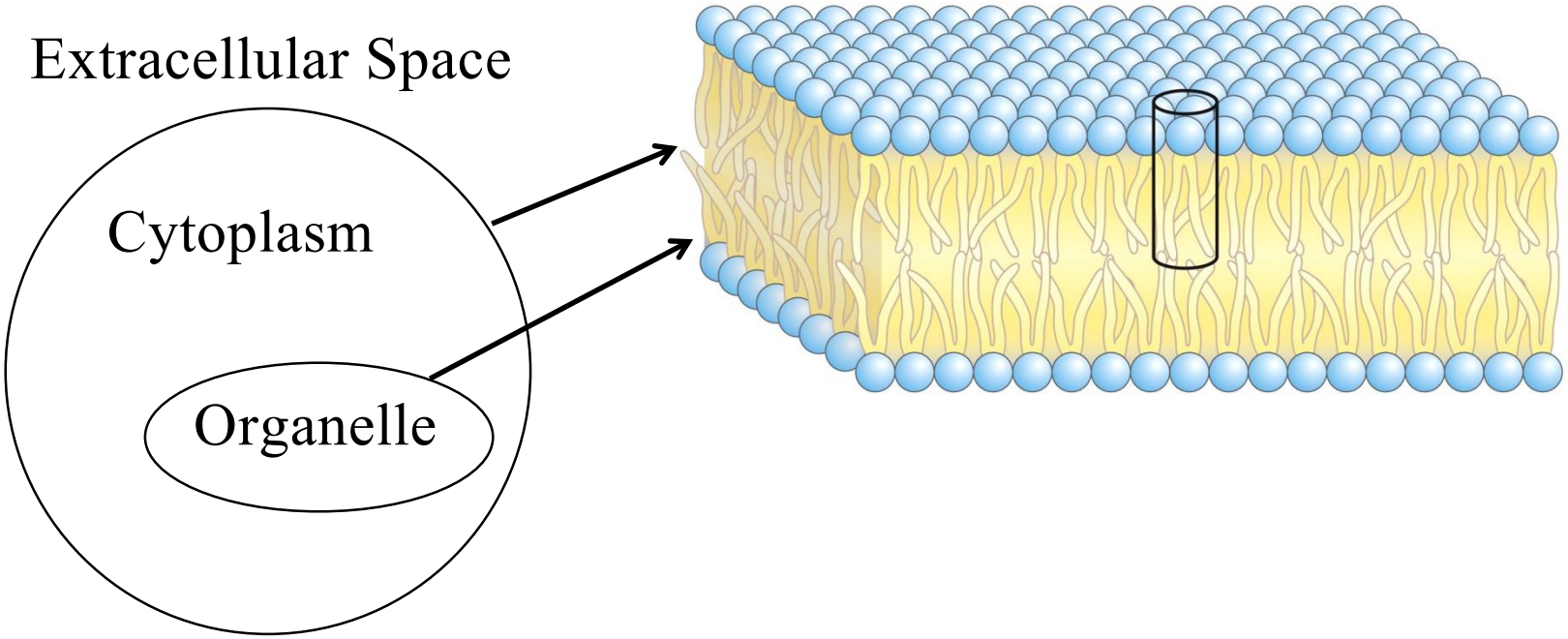
2. What are Biological Membranes?

Complex lipid-based pliable structures composed of a variety of lipids and proteins.

- Some membrane lipids and proteins are glycosylated.
- All cells have a cell membrane, which separates the cell from its surrounding.
- Eukaryotic cells have various internal membranes (organelles) that divide the internal space into compartments.
  - Mammalian red blood cells (erythrocytes) do not have organelles presumably to make room for hemoglobin.

3. Common Features of Eukaryotic Membranes

A. The membrane of eukaryotic cells consists of **two leaflets of lipid-based monolayers**:



- One leaflet faces the cytoplasm
- One leaflet faces the extracellular space or the inside of membrane-enclosed organelle

3. Common Features of Eukaryotic Membranes

B. Sheet-like flexible structure, 3–10 nm thick

C. Structures within the membrane bilayer are stabilized by noncovalent forces, especially hydrophobic ones

D. Membrane bilayers are largely composed of phospholipids.

- The polar heads are on the exterior forming a hydrophilic surface.
- The fatty acyl chains are in the interior forming a fluid, hydrophobic region allowing for lateral motion.
- Other lipids are nestled in between.

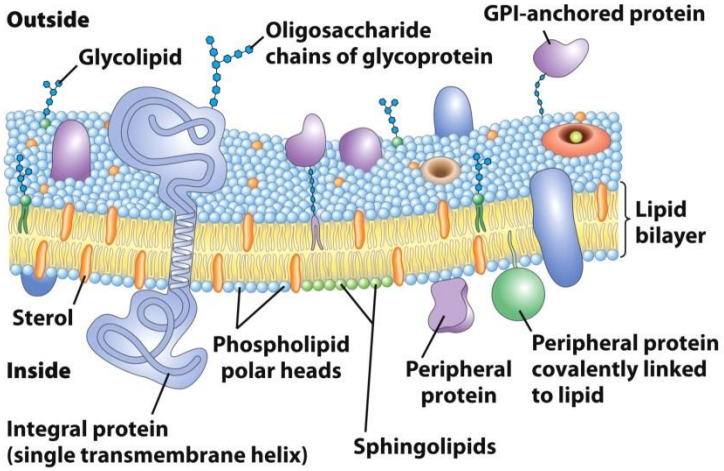
3. Common Features of Eukaryotic Membranes

E. Two types of proteins

- Peripheral proteins: Associated relatively loosely with the polar head groups of membranes
- Integral proteins: Span the lipid bilayer with α helical or β barrell structure
  - Different domains in different compartments based on hydrophilicity/hydrophobicity

F. Asymmetry

- Some lipids are found preferably "inside" the cell
- Some lipids are found preferably "outside"
- Carbohydrate moieties are always "outside"
- Electrically polarized (from outside to inside negative Δ ~ –60 mV)



3. Common Features of Eukaryotic Membranes

G. Self-sealing

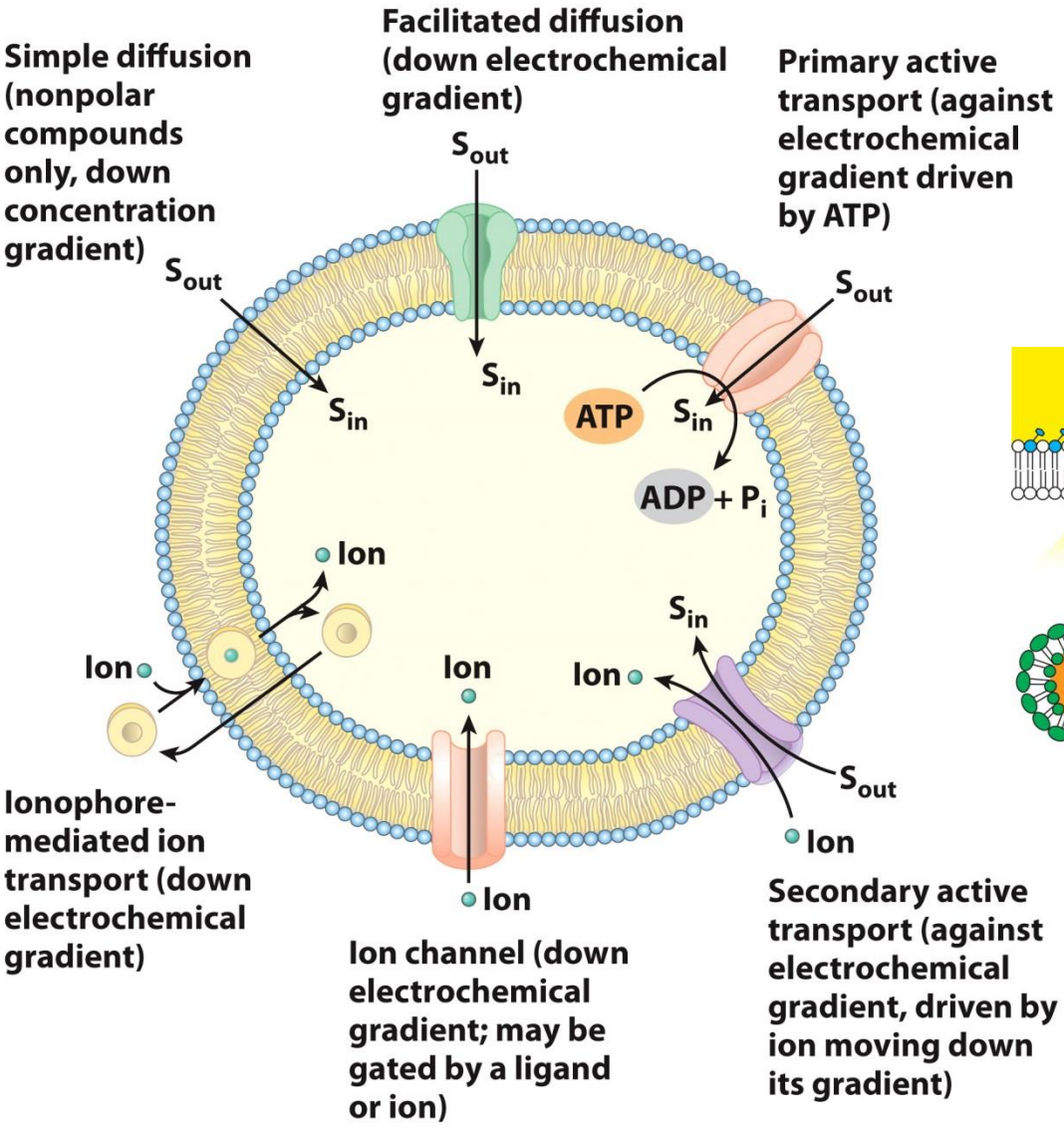
- No loss of membrane continuity

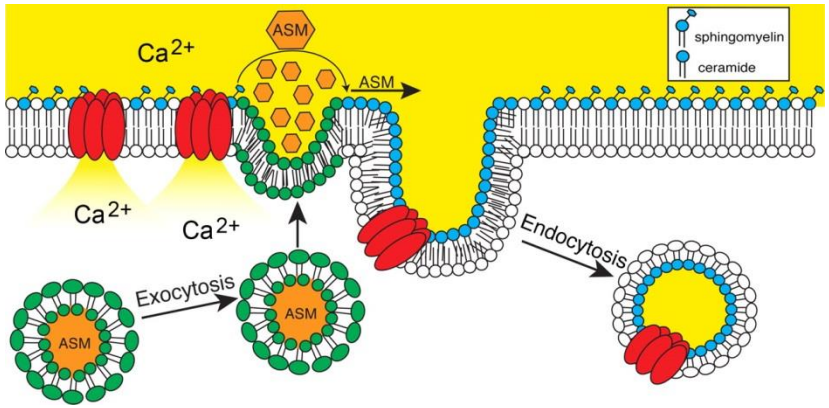
H. Selectively permeable to polar and charged solutes

- Specific transporters allow transport

Membrane Transport as it pertains to Metals

1. Types of Transports





2. The Cell is Selectively Permeable

All living cells interact with their surroundings by transporting solutes in and out as needed for biosynthesis and metabolism. The transport process is restricted by the physical and chemical properties of the solutes.

Four main factors govern cell permeability:

A. Size

B. Hydrophobicity

C. Charge

D. Concentration

3. General Table for Solute Permeability

Solute	MW (g/mol)	Examples	Permeable
Nonpolar Molecules	*	CO_2	Yes
Small, uncharged polar molecules	< 100	Urea, water, ethanol	Yes
Large, uncharged polar molecules	>100	Glucose	No
Charged polar molecules		ATP	No
Ions		K^+, HCO_3^-	No

- * Size limitations for nonpolar molecules can vary but generally: ↑ MW, ↓ Permeability
- In drug design, people aim to synthesize cell-permeable compounds that are ≤ 500 g/mol.

Leeson P.D. & Springthorpe, B. The influence of drug-like concepts on decision-making in medicinal chemistry. *Nat. Rev. Drug Discov.* **6** : 881–90, 2007.

4. Two General Routes for Membrane Transport

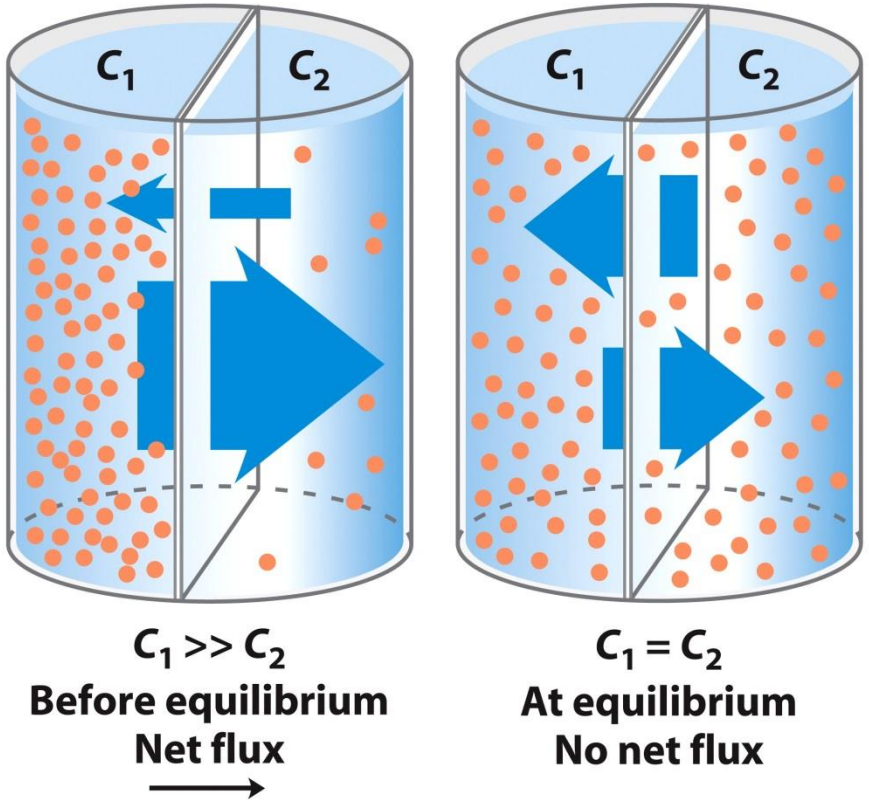
1. Passive Transport
   a. Simple diffusion
      - In direction of chemical gradient
      - Applicable to nonpolar molecules and small polar molecules
   b. Diffusion in direction of electrochemical gradient
      - Applicable to polar molecules and ions
      - Membrane protein facilitated
2. Active Transport
   - Diffusion against the electrochemical gradient
   - Membrane protein facilitated

4A. Passive Transport

In accordance with the 2nd Law of Thermodynamics, molecules tend to spontaneously assume the distribution of greatest randomness and lowest energy.

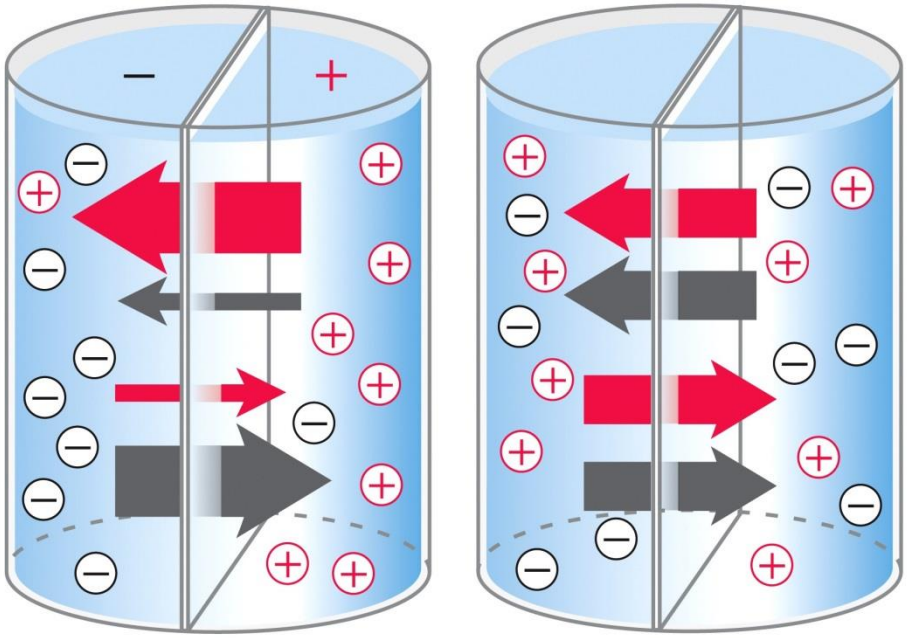
4A. Passive Transport

Simple Diffusion- Net movement of electrically neutral solutes across a plasma membrane toward the side of lower solute concentration until equilibrium is achieved. This is movement in the direction of the chemical gradient.



4A. Passive Transport

Electrochemical Gradient Directed Diffusion- Net movement of electrically charged solutes is dictated by a combination of the electrical potential (V_m) and the chemical concentration difference across the membrane.



$V_m > 0$ Before equilibrium

$V_m = 0$ At equilibrium

- Electrochemical potential reaches zero at equilibrium.

4B. Passive Transport in Contrast to Active Transport

In **passive transport**, the transported species always moves down its electrochemical gradient and is **not accumulated above the equilibrium concentration**. (Exergonic $\Delta G_t < 0$)

In **active transport**, the transported species always moves against its electrochemical gradient and is **accumulated above the equilibrium concentration**.

- Active transport is thermodynamically unfavorable (endergonic $\Delta G_t > 0$) and takes place only when coupled (directly or indirectly) to an exergonic process such as
  - Breakdown of ATP
  - The concomitant flow of some other chemical species down its electrochemical gradient.

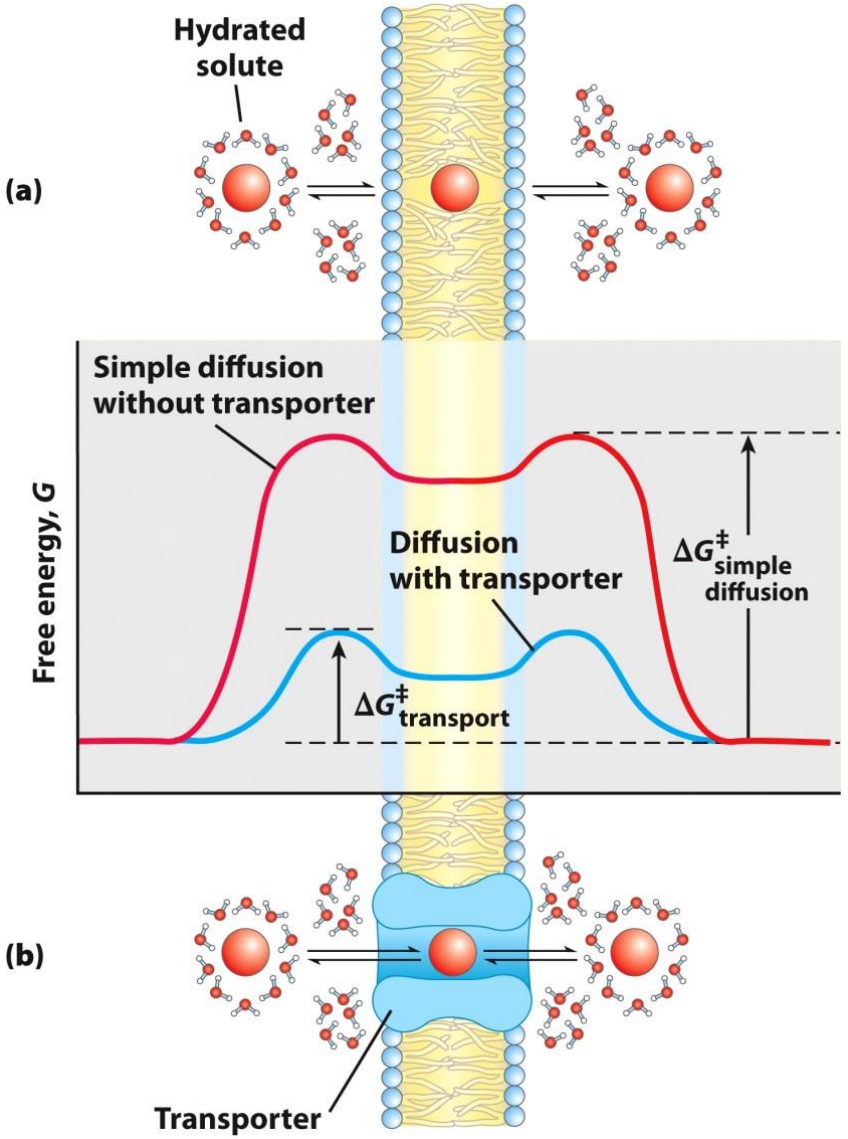
4C. Transport of a Polar or Charged Solute (Metal Complexes)

Metals will typically be formulated as charged complexes.

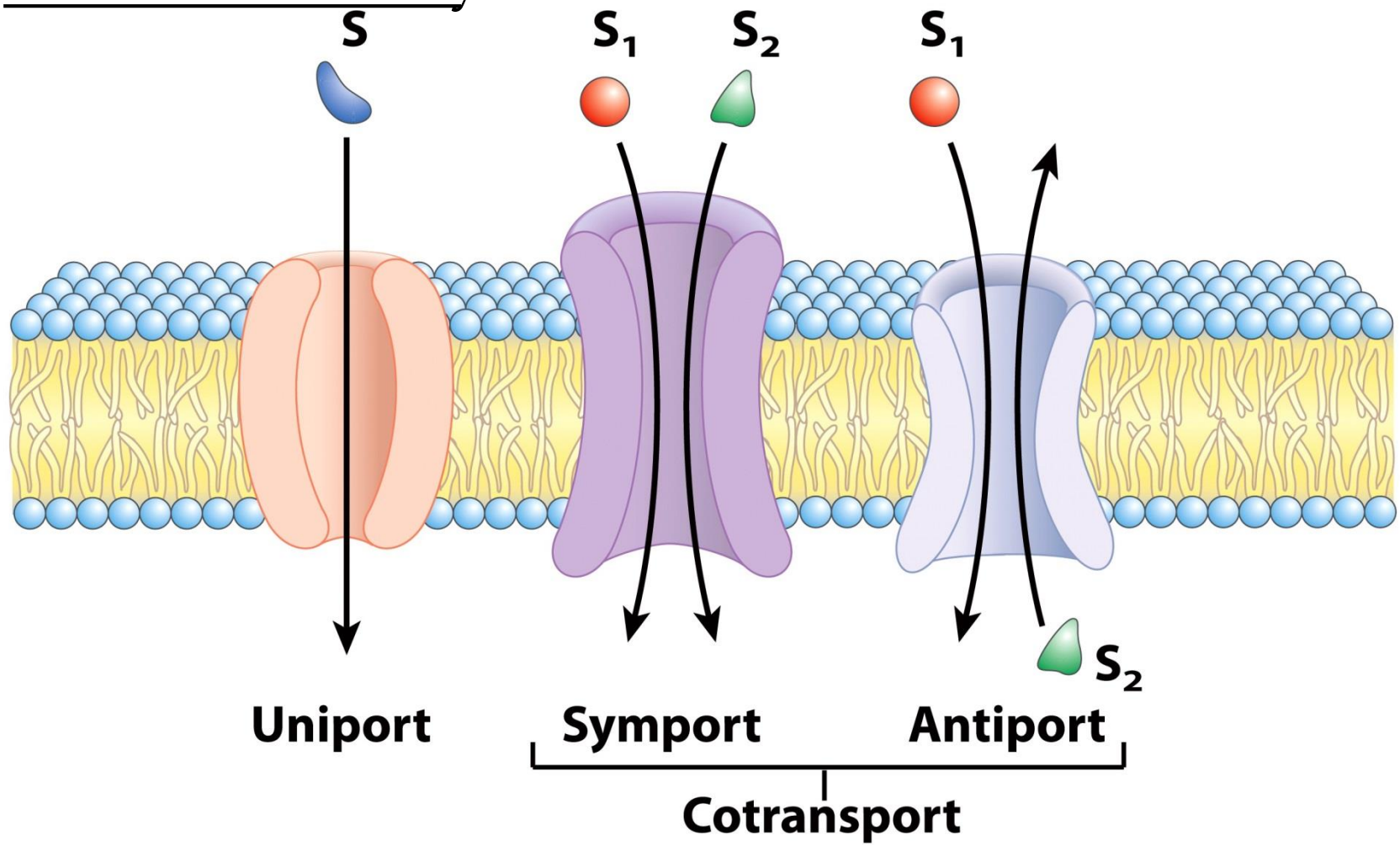
There are membrane proteins that facilitate the diffusion of polar/ charged solutes called transporters or permeases (enzymes but not in the traditional sense) in the direction of or against the electrochemical gradient.

- Provide a polar/charged environment with a lower energy intermediate state.

$\Delta G^{\ddagger}_{transport} < \Delta G^{\ddagger}_{simple\ diffusion}$



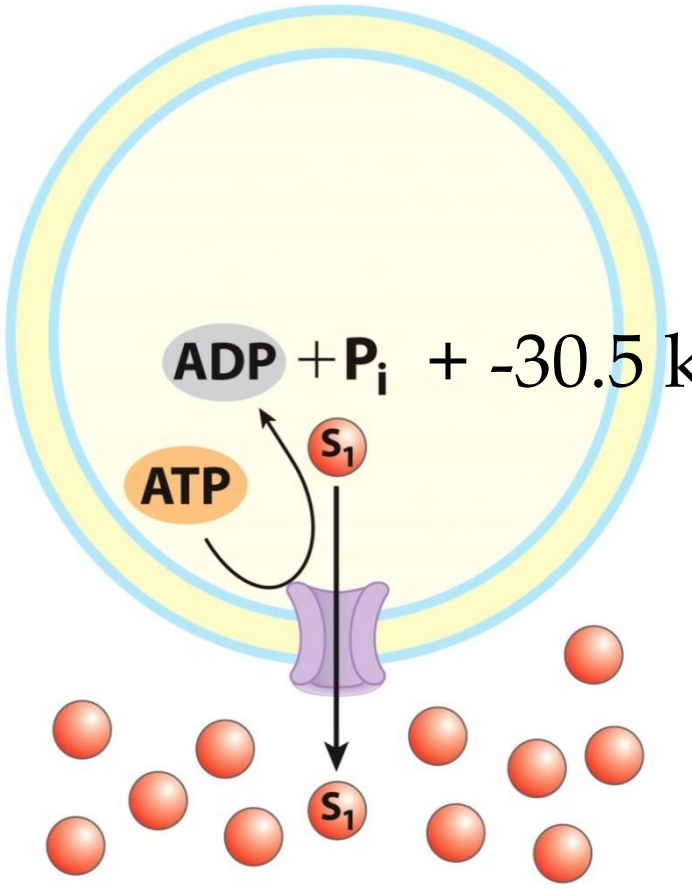
4D. Transporters Classified by Solutes and Directionality



The directionality can be with or against the electrochemical gradient and so transporters can facilitate both passive and active transport.

4E. Active Transport

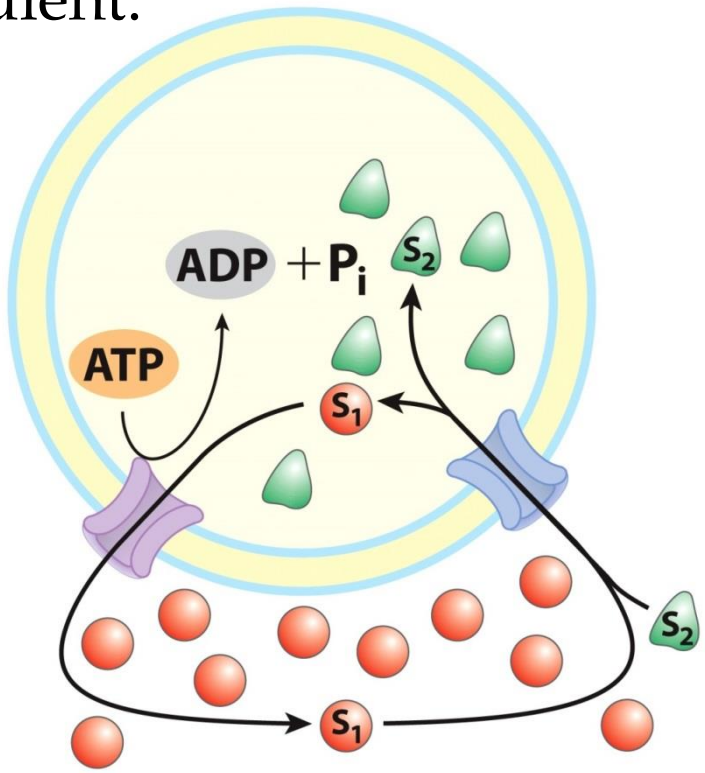
In **primary active transport**, the energy released by ATP hydrolysis (for example) drives solute movement against an electrochemical gradient, which is an endergonic process.



(a) Primary active transport

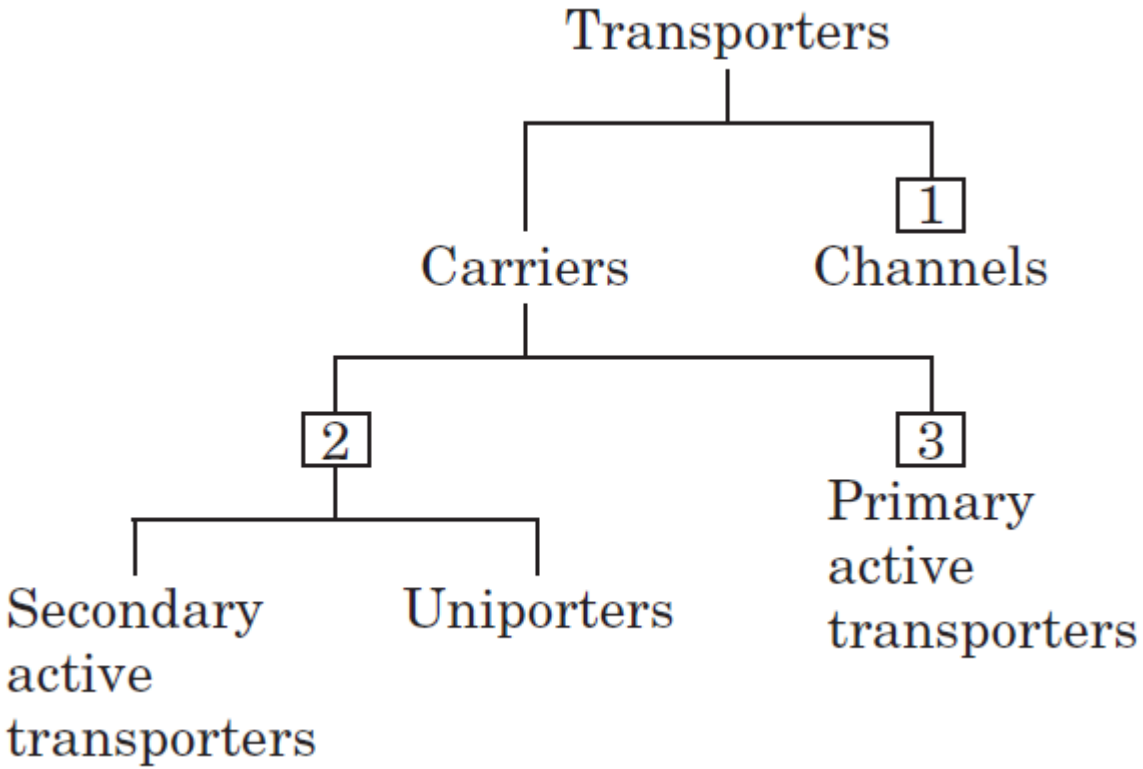
4E. Active Transport

In **secondary active transport**, a gradient of ion S1 has been established by primary active transport. Movement of S1 down its electrochemical gradient now provides the energy to drive co-transport of a second solute (S2) against its electrochemical gradient.



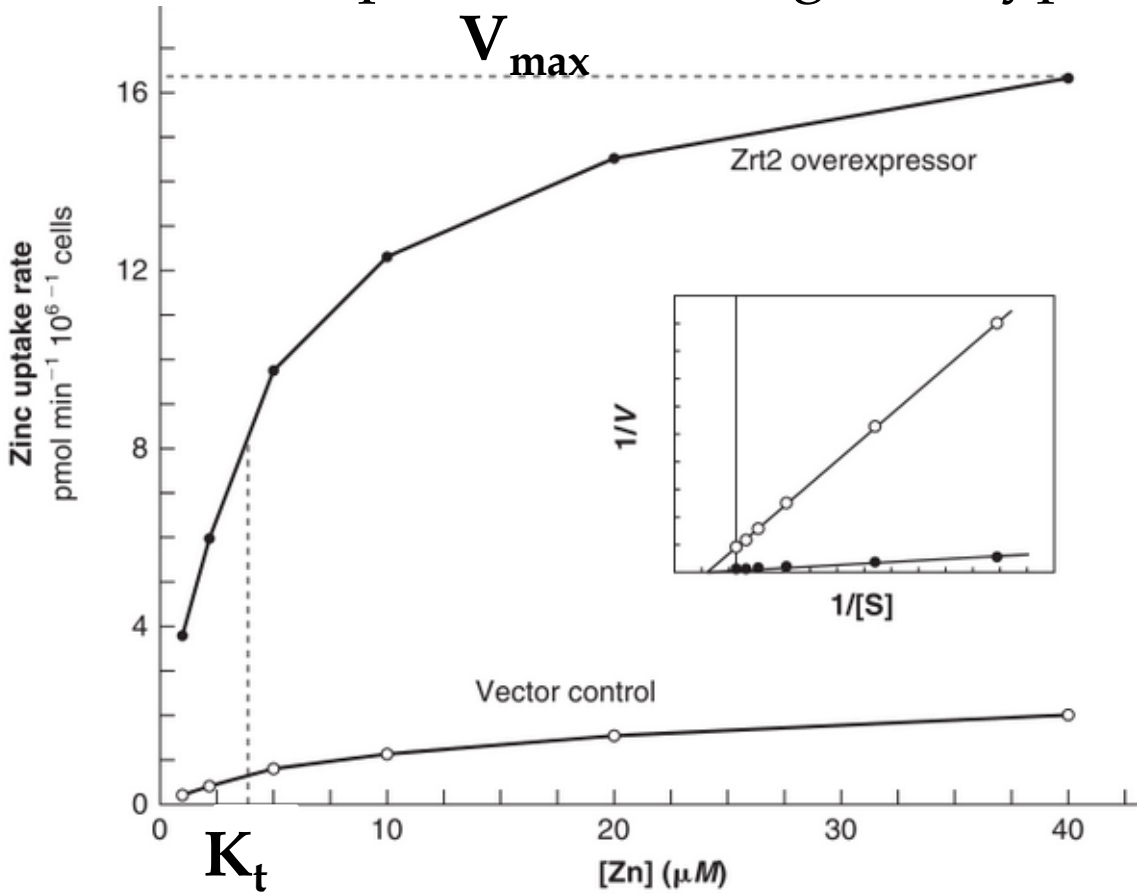
(b) Secondary active transport

4F. General Classification of Transporters



4FI. Transporter Kinetics fit Michaelis-Menten Equation

The rate equations for transporter kinetics can be derived exactly as for enzyme-catalyzed reactions (initial rate kinetics) yielding an equation comparable to the Michaelis-Menten equation resulting in a hyperbolic plot:



Zrt2 Zn^{2+} transporter of *S. cerevisiae*

$$V_0 = \frac{V_{max}[S]_{out}}{K_t + [S]_{out}}$$

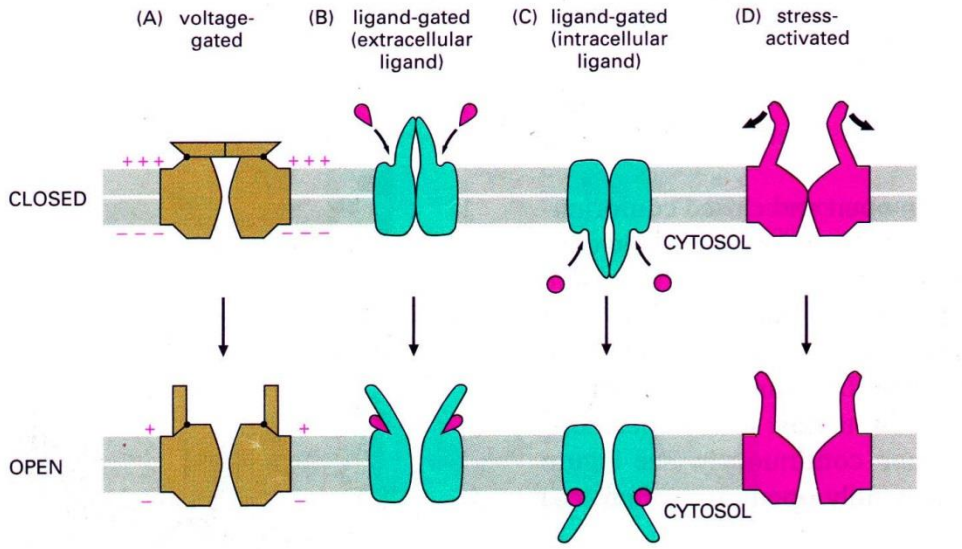
$K_t = K_{transport}$

K_t = [S] when $V_{max}/2$

4FII. Channels

Proteins that form pores in the membrane for diffusion:

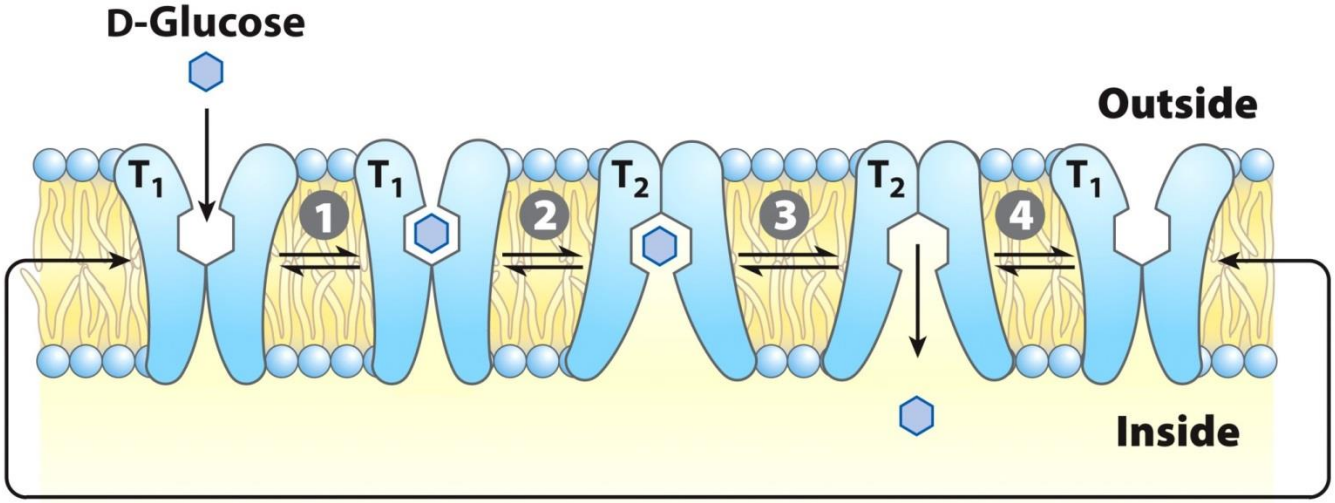
- Pores open in response to signals
  - Membrane electrical potential changes
  - Ligand binding
- Not high stereospecificity
- Catalyze transfers at rates several orders of magnitude greater than carriers, approaching diffusion limits (k as high as $1x10^{10}\ M^{-1}s^{-1}$)
- Not saturable



4FIII. Carriers

Proteins with high substrate stereospecificity

- Bind substrate(s) at one side of a membrane, undergo a conformational change, and then release the substrate on the opposite side of the membrane



- Catalyze transport at rates below free diffusion limits

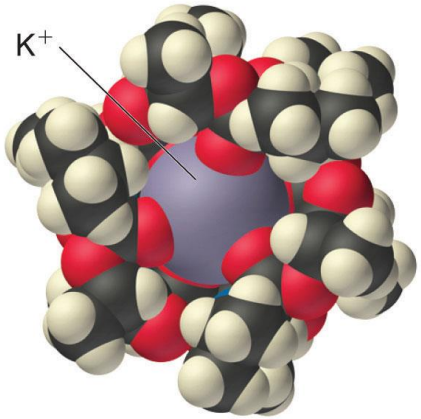
- Saturable like enzymes

5. Ionophores

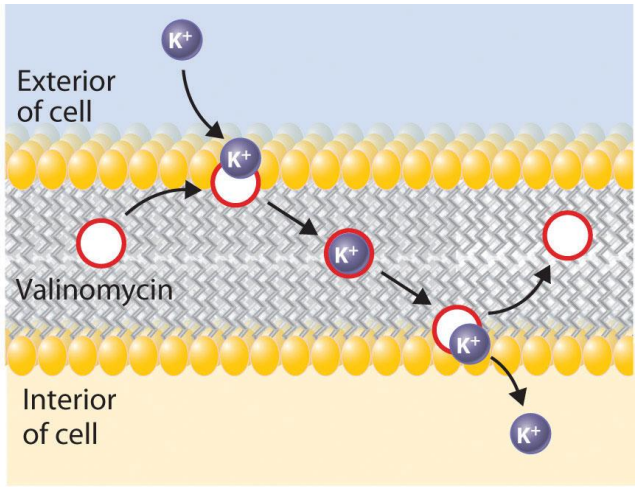
Diverse class of organic molecules that increase the permeability of membranes to particular ions.

- Can be peptide- or nonpeptide-based and serve as channels or carriers

  -Bacteria are known to produce peptidic antibiotics that inhibit the growth of other organisms by shuttling and depleting the levels of important ions



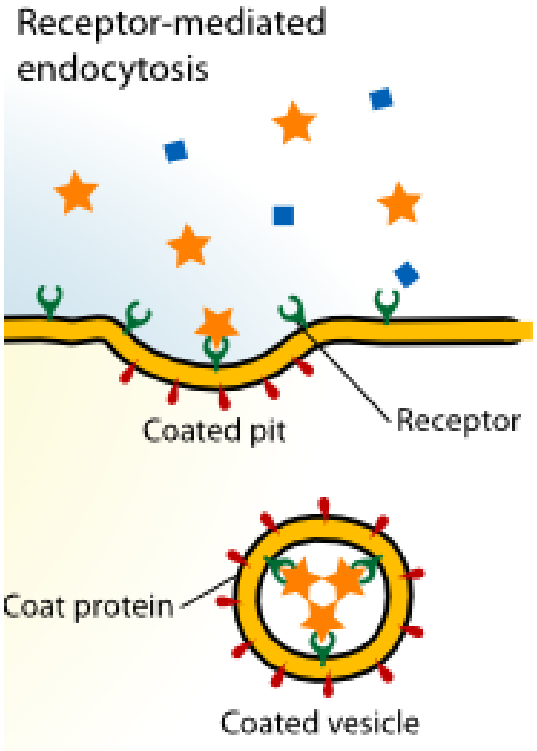
(a) K^+–valinomycin complex



(b) Transport of K^+ across a membrane

6. Receptor-Mediated Endocytosis

In general, endocytosis is the process by which cells absorb molecules by engulfing them. This process is often coupled to a ligand binding to a receptor, which triggers delivery of the ligand into the cell.



A. Clathrin-dependent

- Clathrin is a large protein that forms a coated pit on the inner surface of the plasma membrane
- The pit fuses to the membrane and forms a coated vessicle in the cytoplasm

B. Clathrin-independent

Mobilizing Metals for Transport

Three Key ways that organisms Mobilize Metals to make them Bioavailable

A. Chelation
   - Transform metal into a stable but moderately labile formulation

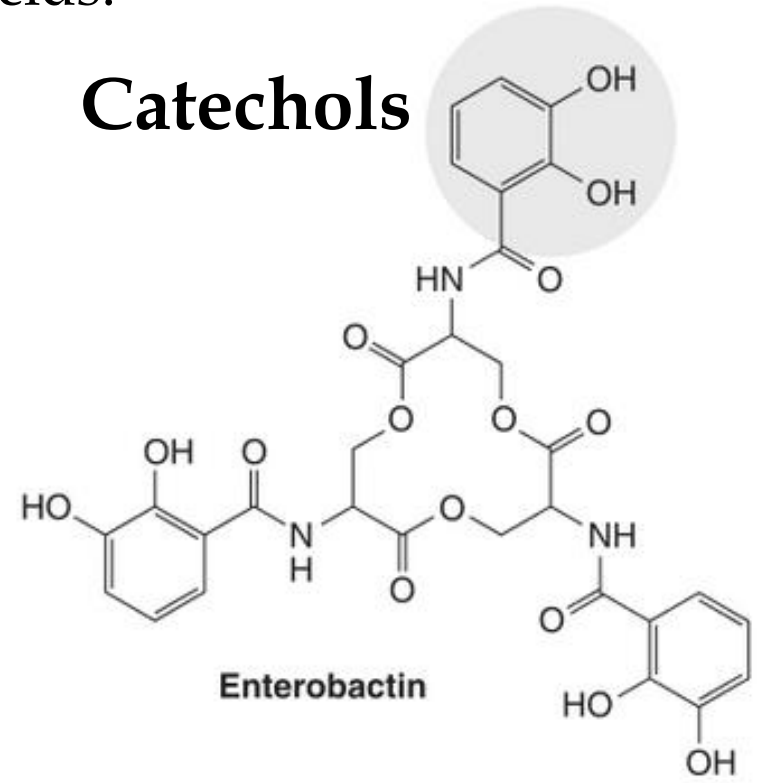
B. Redox
   - Reduce or oxidize metal to an appropriate oxidation state for specific transporters

C. Acidification
   - Solubilize metals (at relatively high levels) and prevent formation of hydrolysis products that are typically poorly soluble

Siderophores

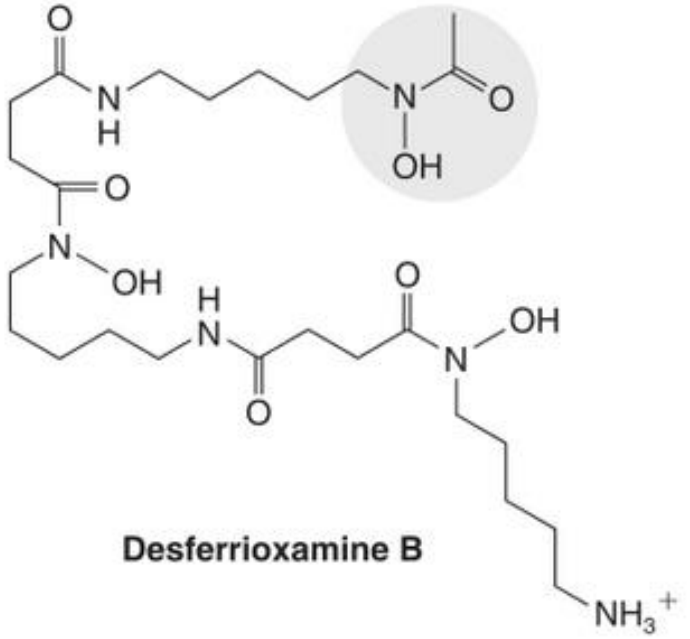
Used by bacteria, fungi, and some plants and marine organisms for acquisition of iron. There are hundreds of known siderophores and many new ones are still being discovered. They typically feature the Fe(III)-binding moieties: catechols and hydroxamic acids.

Catechols



Enterobactin

Hydroxamic Acid

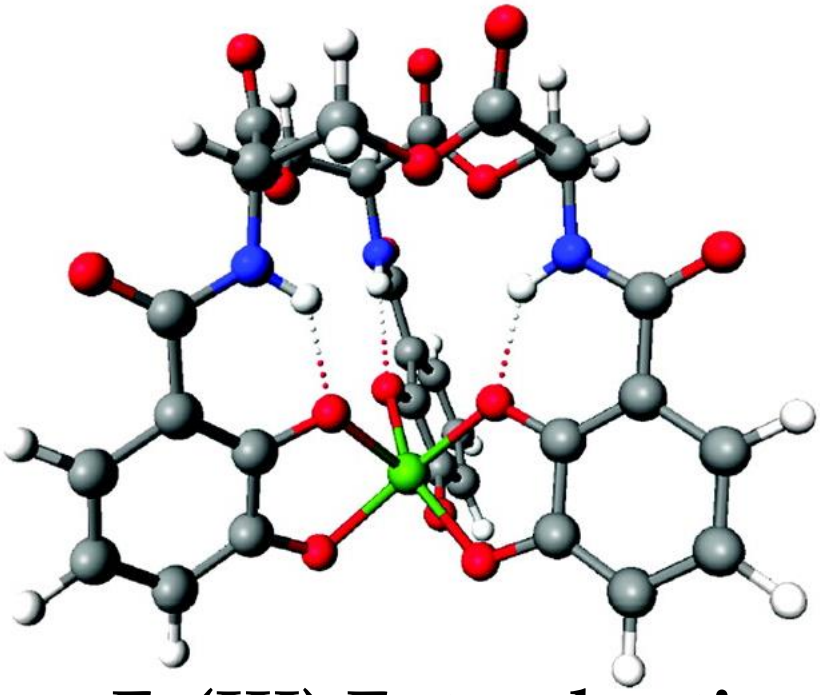


Desferrioxamine B

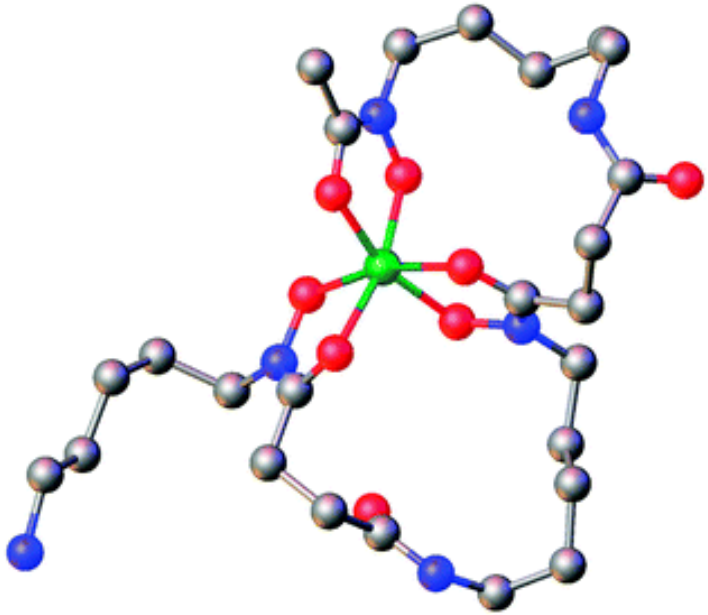
1. Fe(III)-Siderophore Structures

A. Typically monomeric structures of 110 MLH form

B. Possible stereochemical preference with Δ configuration observed for Fe(III) enterobactin exclusively over Λ
   - Chiral recognition may be important for Fe(III) transport



Fe(III) Enterobactin



Ferrioxamine B

2. Fe(III)-Siderophore Affinity Constants

Table VIII.3.1.
The β_{110} and p*M* Values for Selected Siderophores

Siderophore	log β_{110}	p*M*	Type of Siderophore
Enterobactin	49	35.5	Tris(catecholate)
Desferrioxamine B	30.6	26.6	Tris(hydroxamate)
Ferrichrome	29.07	25.2	Tris(hydroxamate)
Aerobactin	22.5	23.3	Bis(hydroxamate) and α-hydroxycarboxylate
Rhodotorulic acid	21.55 log β_{230} 62.2	21.8	Bis(hydroxamate)
Alcaligin	23.5 log β_{230} 64.66	23.0	Bis(hydroxamate)

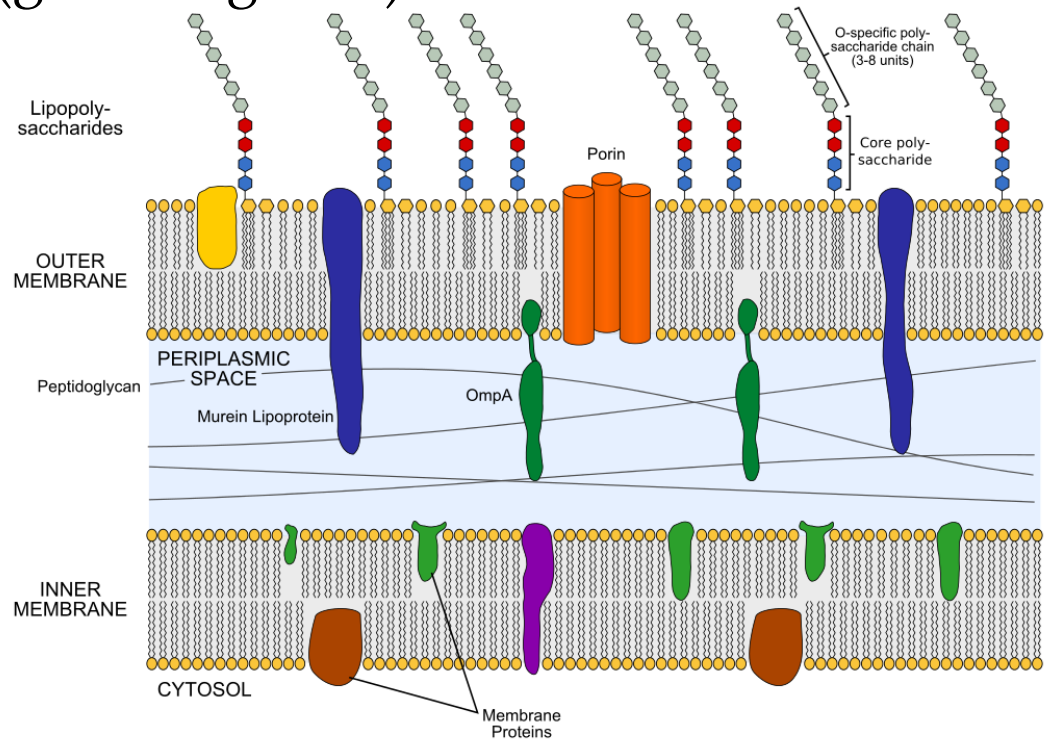
pM = -log[M(aq)]; A measure of free or uncomplexed Fe^{3+} left in the solution

↑ pM, ↓ Fe(III) in solution

3. Outer-Membrane Receptor Proteins for Ferric Siderophores

Bacteria have evolved high-affinity outer-membrane receptor proteins for binding Fe(III)-siderophores.

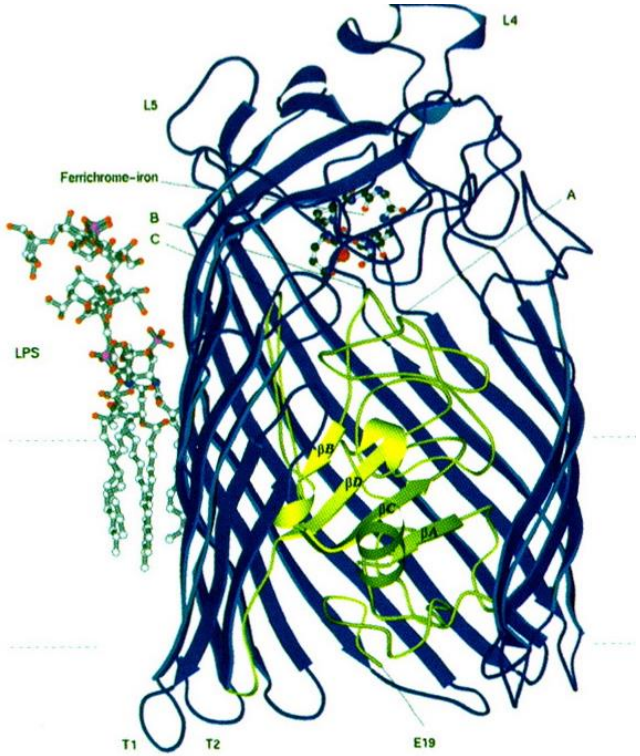
A. "Substrate specific"carriers that engage in energy-dependent, active transport of the Fe(III)-siderophores across the outer membrane (gram negative)



3. Outer-Membrane Receptor Proteins for Ferric Siderophores

B. Structural core consists of a 22-strand, membrane spanning, antiparallel β-barrel

C. The apoform of the protein has a conformation that blocks ("corks") the periplasmic side



FhuA-ferrichrome-iron complex from *E. coli*

3. Outer-Membrane Receptor Proteins for Ferric Siderophores

D. Once released to the periplasmic space, the complexes are bound by a high-affinity periplasmic-binding protein

E. Transport of Fe across the cytoplasmic membrane can be in the ionic ferric or ferrous form or as a ferric siderophore complex using an ATP Binding Cassette (ABC) transporter system

4. Ferric Siderophores Receptors in Organisms that do not produce Siderophores

Some organisms feed off of others because of a lack of an optimal method for chelating Fe(III).

- *S. cerevisiae* prey on bacteria and have evolved transport systems for different ferric siderophores