Cellular & Molecular Biology
SQBS 1143

Chapter 1:
The Cytoplasmic Membrane & Cell Wall

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Cytoplasmic Membrane: Structure

- **a thin structure** that completely surrounds the cell.
- forms a critical barrier separating the inside of the cell (the cytoplasm) from its environment.
- **highly selective barrier**, enable the cell to **concentrate specific metabolites** and excrete waste materials.

Chemical Composition of Membranes

- General structure of most biological membrane - **phospholipid bilayer**

- Phospholipids contain:
  - the highly **hydrophobic** (fatty acid and relatively hydrophilic glycerol) **moieties**
    - can exist in many different chemical forms as a result of **variation** in the **nature of the fatty acids** or **phosphate-containing groups** attached in an aqueous solution,
    - form **bilayer structures** spontaneously - the **fatty acids point inwards**

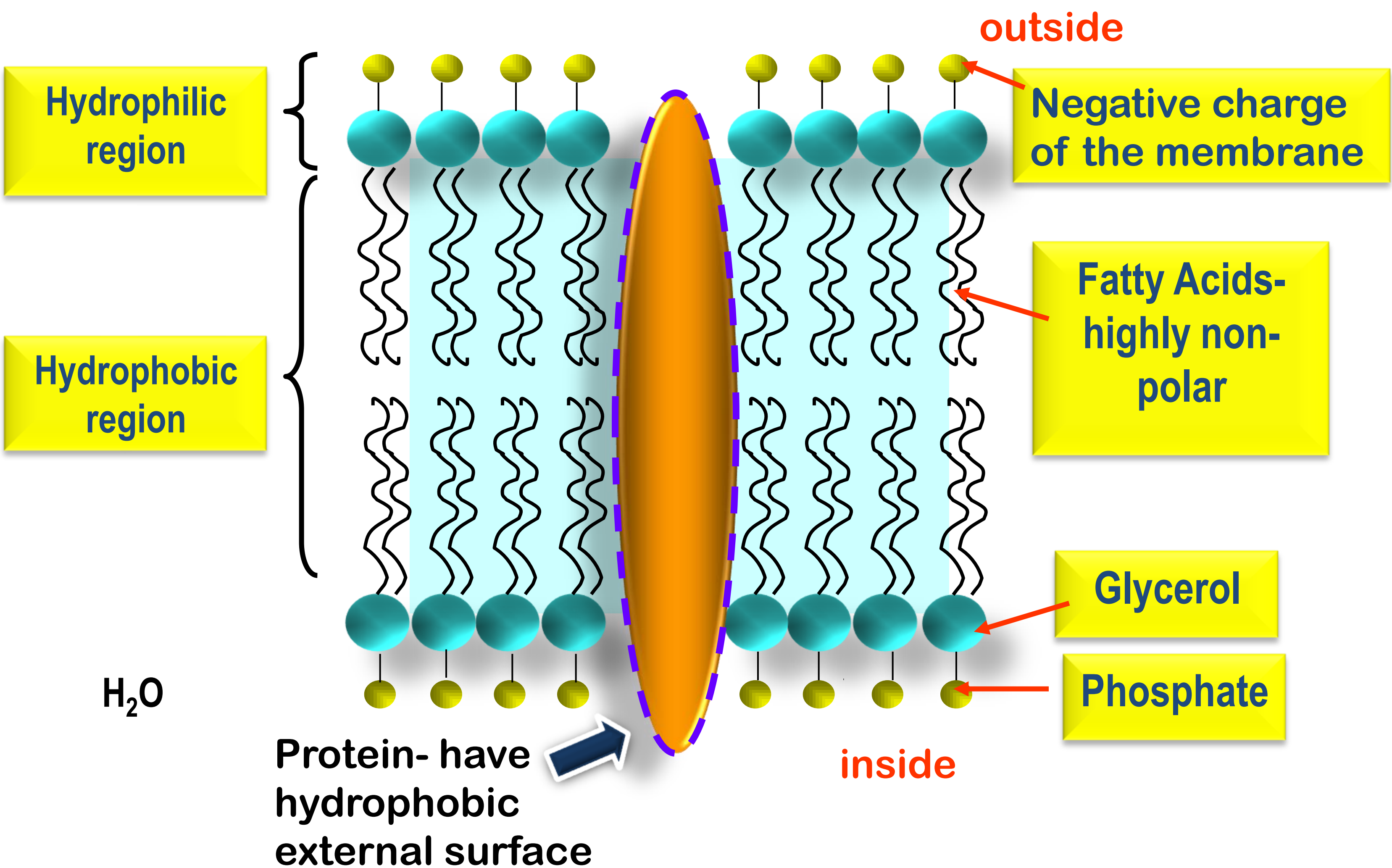
- The **hydrophilic portions** remain exposed to the aqueous - external environment.

- the bilayer character of membranes probably represents the **most stable arrangement of lipid molecules** in an aqueous environment.

- The **cell membrane** consists of **phospholipid bilayer** and **proteins** embedded within it.

- The major proteins of the cell membrane - have very **hydrophobic external surfaces**
- Makes intimate association with the **highly nonpolar fatty acid chains**).
- **Integral membrane proteins** such as these actually **span the bilayer** ➡ have surfaces exposed (both the *inside* and the *outside* of the cell and the overall structure of the cytoplasmic membrane

Cell membrane- the phospholipid bilayer and protein



Cell membrane stabilization

- The cell membrane can be stabilized by:
  - **hydrogen bonds**
  - **hydrophobic interactions**
  - **cations,** such as **Mg** $^{2+}$ and **Ca** $^{2+}$ - help stabilize the membrane by combining ionically with the negative charges of the phospholipids.

Other features of the cytoplasmic membrane

- The **outer surface** of the cytoplasmic membrane faces the environment.
- In certain bacteria, it makes contact with a **variety of proteins**.
- Serve to bind substrates or process large molecules for transport into the cell (periplasmic proteins).
- The inner side of the cytoplasmic membrane faces the cytoplasm and interacts with proteins involved in **energy yielding reactions** and other important **cellular functions**.

- Some proteins, such as those in the **periplasm**, some **cytoplasmic proteins**, may **associate quite firmly with the surface of the membrane** - function as if they were membrane-bound proteins (peripheral membrane protein).

- Some of these **peripheral membrane proteins** are **lipoproteins** and contain a **lipid tail on the amino terminus** of the protein

- This tail **serves to anchor** the protein into the membrane. Such proteins are called **lipid-anchored membrane protein**.

The cytoplasmic membrane is a fluid mosaic

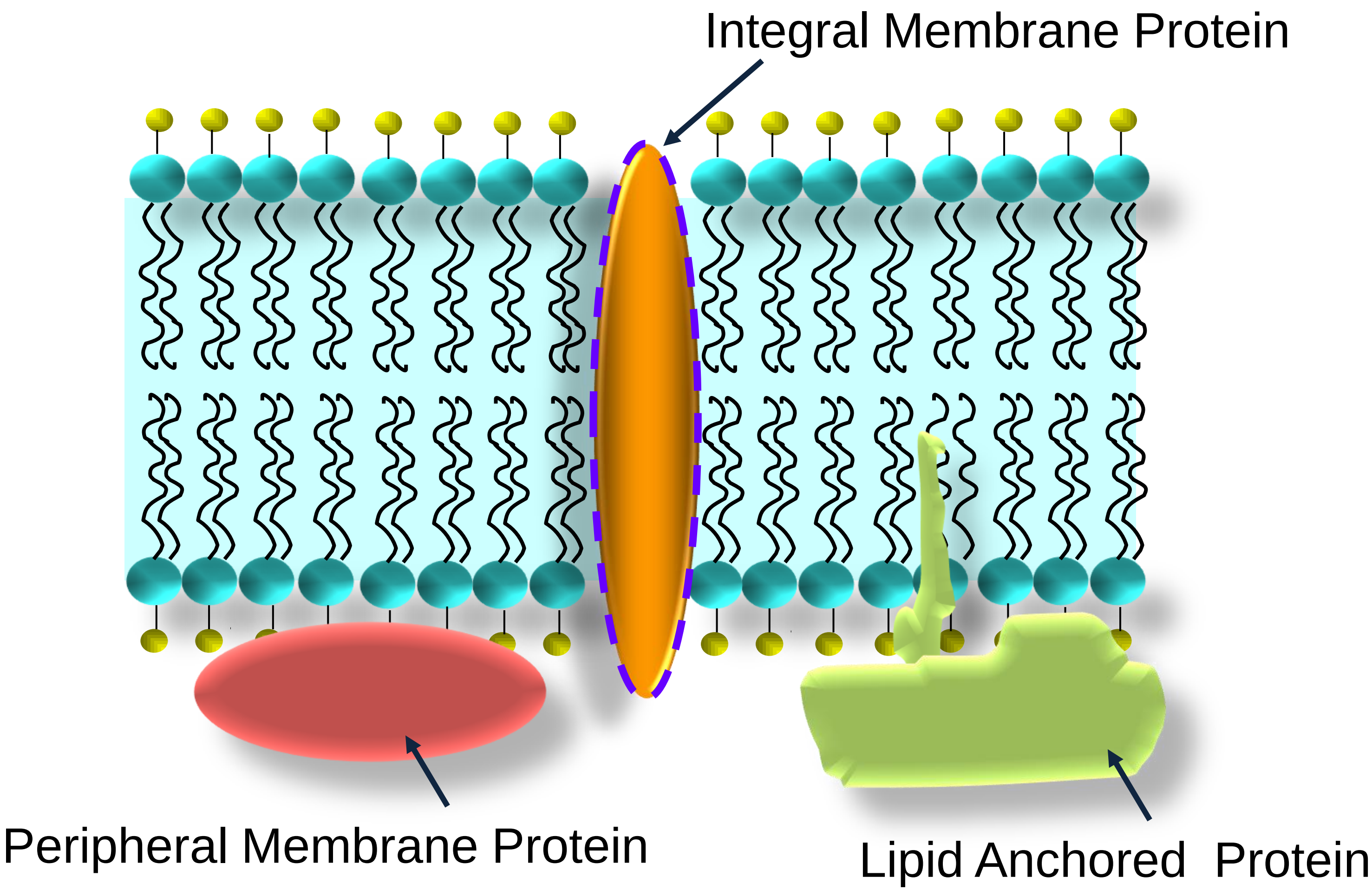
- the cytoplasmic membrane is actually quite fluid; phospholipids and proteins have **significant freedom to move** about the membrane surface.

- In summary, the cytoplasmic membrane can be thought of as a **fluid mosaic** - globular proteins oriented in a specific manner span a highly mobile yet ordered phospholipid bilayer.

- This arrangement confers a number of important functional properties on membranes.

Membrane Proteins

- **Integral proteins** - contain one or more transmembrane helices.
- **Peripheral proteins** - noncovalently bonded to the polar head groups of the lipid bilayer and for to an integral membrane protein.
- **Lipid-anchored proteins** - covalently bonded either to a phospholipid or a fatty acid that is embedded in one of the leaflets of the lipid bilayer.


Integral Membrane Protein
Peripheral Membrane Protein
Lipid Anchored Protein

- If diffusion were the only type of transport mechanism available, cells would not be able to acquire the proper concentrations of solutes.
- In diffusion processes, both the **rate of uptake** and the **intracellular level** are **proportional to the external concentration**.
- In order to increase intracellular level, a mechanism by which the solutes travel should be present.

- The cytoplasmic membrane is a **barrier** separating inside from outside.
- It allows nutrients to pass through, and waste products to leave the cells.
- **Permeability** - the property of membranes that permits movement of moieties (which do not move passively) to move back and forth across the cytoplasmic membrane.
- The cytoplasmic membrane is **selectively permeable**.

- **Nature of the membrane**

  The cytoplasmic membrane as a **permeability barrier** in the interior of the cell (the cytoplasm) consists of an aqueous solution of **salts**, **sugars**, **amino acids**, **vitamins**, **coenzymes**, and a wide variety of other soluble materials.

- **What could or could not go through?**

  The **hydrophobic nature** of the cytoplasmic membrane allows it to function as a **tight barrier**, and so does not allow passive movement of these polar solutes.

- Some **small non-polar** and **fat-soluble** substances, such as **fatty acids, alcohols, and benzene**, may enter and exit the cell by becoming **dissolved in the lipid phase** of the membrane.

- Charged molecules, such as **organic acids**, **amino acids**, and **inorganic salts**, which are **hydrophilic**- do not readily pass the membrane barrier.
- They must be **specifically transported** using transport proteins.
- A substance as small as a **hydrogen ion, H^+** , does not readily cross the cytoplasmic membrane passively because it is always **hydrated**, occurring in solution as the charged **hydronium ion** (H_30^+).

- Water - freely penetrates the membrane; is sufficiently **small and uncharged** to pass between phospholipid molecules.
- Due to membrane selectivity, most substances **do not passively** enter the cell, and thus **transport processes** are critical to cellular function.
- No free diffusion of various polar molecules happen. The substances **can be concentrated to over 1000 times** through the action of **membrane transport proteins**.

Classes of Transport Proteins

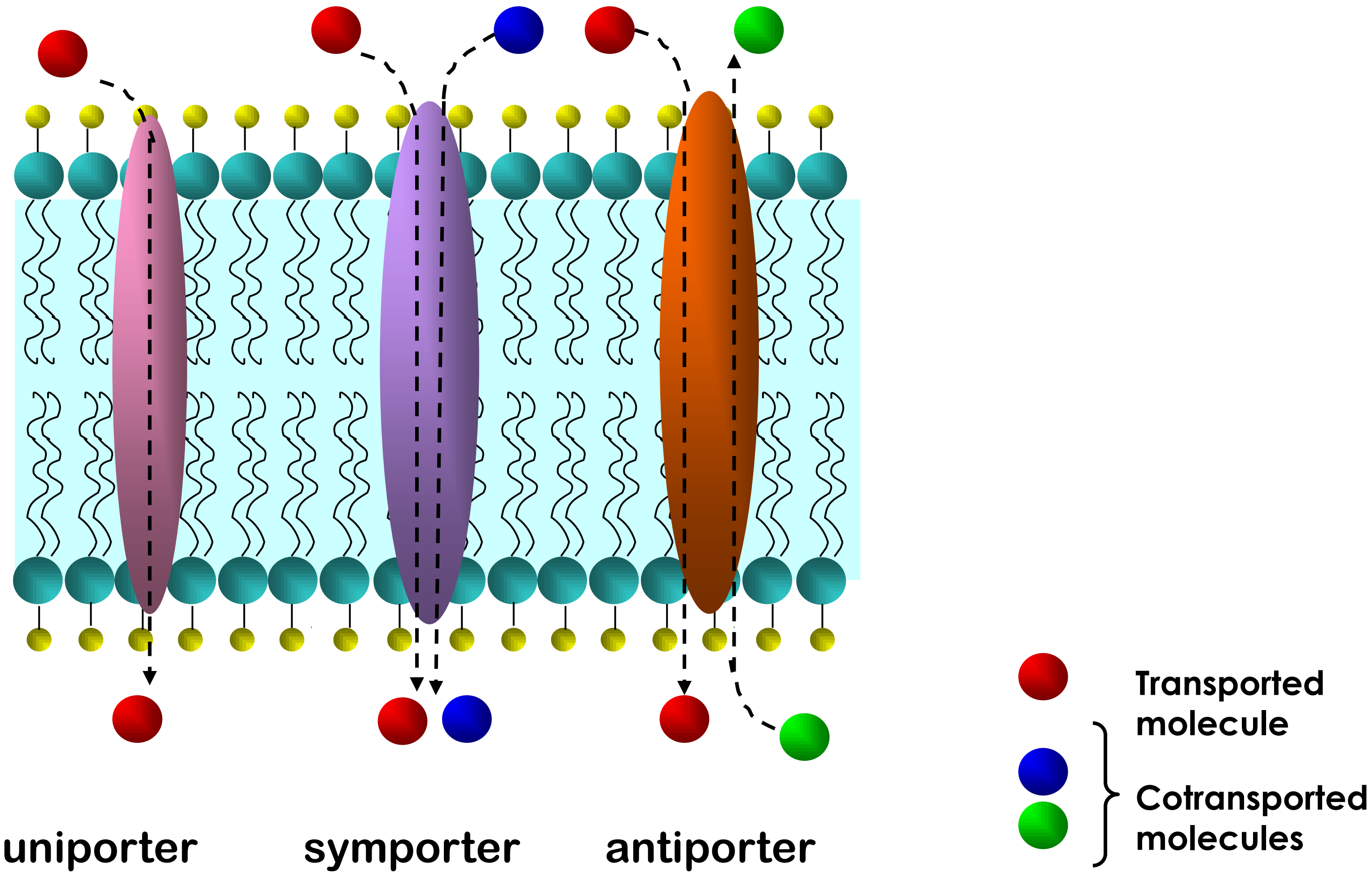
i. **Uniporters** – membrane proteins that transport substance from one side of the membrane to the other.

Cotransport proteins: The other two classes of transport proteins move the substance of interest across the membrane along with a second substance required for transport of the first.

ii. **Symporters** - membrane proteins that transport both substances across the membrane in the same direction.

iii. **Antiporters** – membrane proteins that transport **one substance** across the membrane **in one direction** while transporting the **second substance in the opposite direction**.

Three Classes of Transport Proteins



Active Transport

- Active transport - enables the cell to accumulate solutes **against a concentration gradient**.
- **Passive carrier-mediated transport** shows a saturation effect. If the concentration of the substrate in the medium is high enough to saturate the carrier, which is frequently the case even at quite low substrate concentrations, the rate of uptake (and often the internal level as well) becomes maximal.

Active Transport

- **Active carrier-mediated transport** processes is the **highly specific nature** of the transport event.
- The binding and carrying of a substance across the membrane resembles an enzyme action.
- Certain carrier proteins **react only with a single kind of molecule**, but many show affinities **for a chemical class of molecules**.

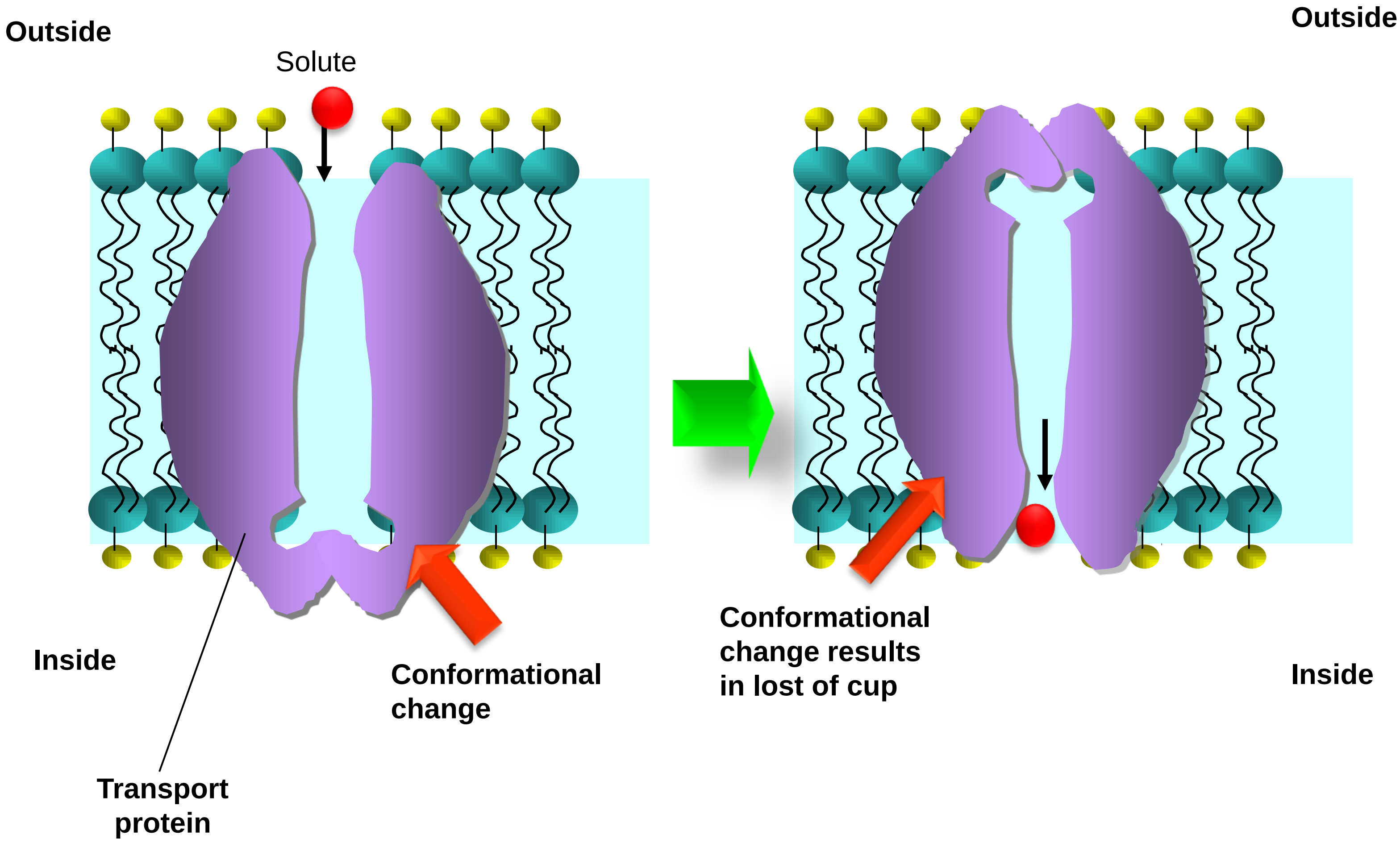
Example of carrier proteins that recognize a group of compounds

- For instance, there are carriers that transport certain, usually related, **amino acids** and others that transport a **variety of related sugars**.
- This **reduces the need for separate transport proteins** for every single amino acid or every single sugar the cell needs to transport.

The action and energy requirements of transport proteins

- Membrane transport proteins are generally **integral proteins**, with portions of the protein being exposed to the cytoplasm and the external environment.
- Solutes will bind on the external surface of the cell to be carried through the membrane by a **conformational change** in the transport protein.
- Most transport processes are linked to the spending of energy and result in **a much higher concentration** of the transported molecules **inside** than outside the cell.

Conformational change of membrane proteins



- If a solute is transported by an energy-dependent process, then **energy** can be used to **pump the solute against the concentration gradient (from low concentration to high concentration)**.

Where does the energy come from?

- Energy can be derived from either

i. **high energy phosphate** compounds, such as **adenosine triphosphate** (ATP) or

ii. by the **dissipation of a gradient of protons** or **sodium ions** across the membrane.

- The **ion gradients** are established (during energy-releasing reactions in the cell) and can be used as a **source of potential energy** to drive the uptake of solutes **against the concentration gradient**

- Two major different **mechanisms** of energy-linked transport are known.
    i. Group translocation
    ii. Active transport

- **Group translocation** - the process whereby **substance is transported** while **simultaneously being chemically modified**, generally by **phosphorylation**.

- **Active transport**, the substance can **accumulate to a high concentration** in the cytoplasm **in chemically unaltered form**. Active transport requires energy and is linked to the energy available in **ion gradients** or **ATP**.

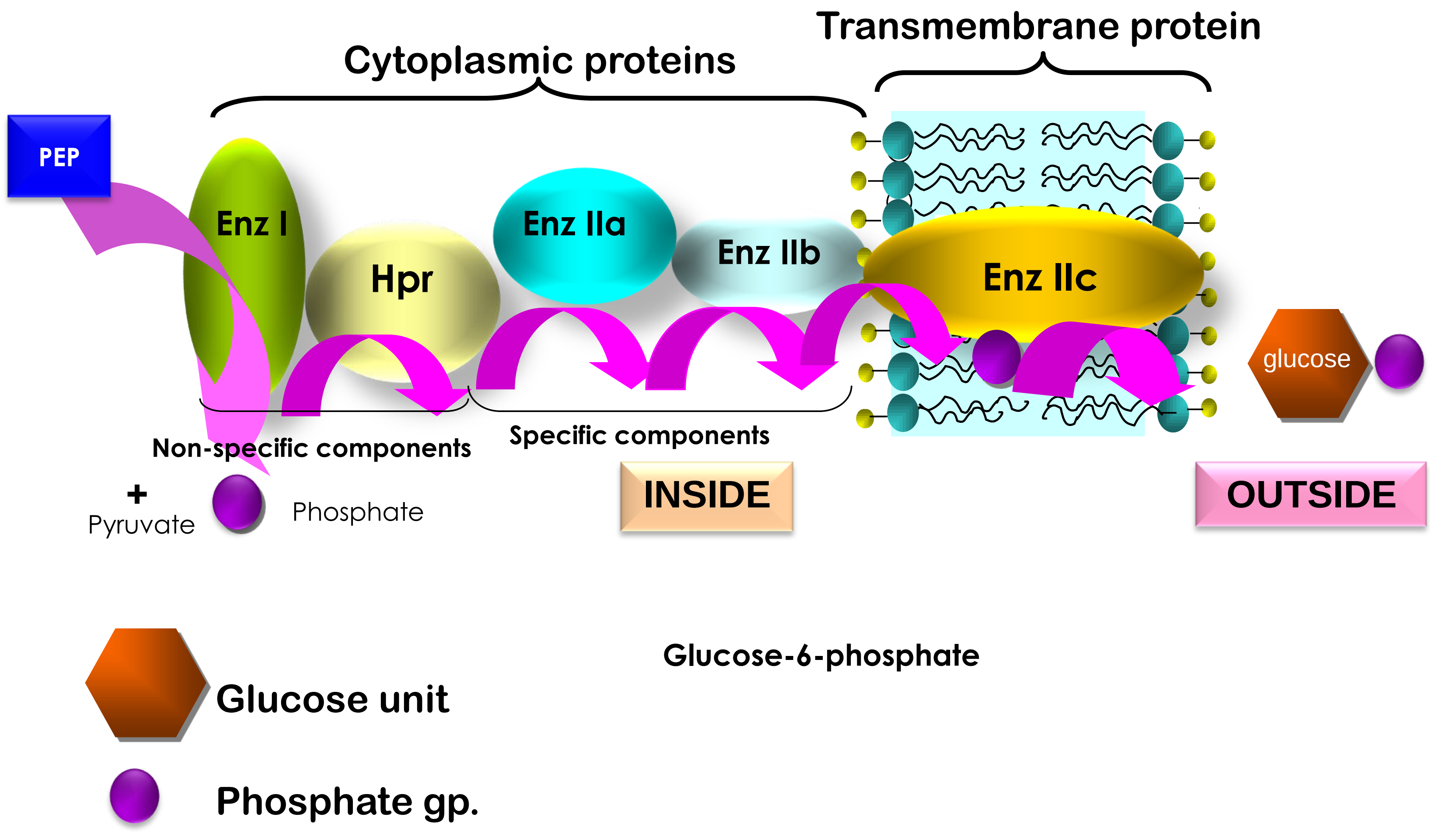
(i) Group translocation

- a transport process in which the **substances chemically altered** in the course of passage across the membrane.
- Since the product that appears inside the cell is **chemically different** from the external substrate, **no actual concentration gradient** of external solute per se is produced across the membrane.
- E.g. of group translocation involve transport of the sugars **glucose, mannose, fructose, N-acetylglucosamine**, and **β-glucosides**, which are phophorylated during transport by the **phospho-transferase system.**

- The **phosphotransferase system** in the bacterium *Escherichia coli* is composed of 24 proteins, at least **four** of which are necessary to **transport a given sugar**.

- The proteins in the phosphotransferase system are themselves **alternately phosphorylated and dephosphorylated** in a cascading fashion until a transmembrane transport protein called **Enzyme IIc** receives the **phosphate group** and phosphorylates the sugar in the actual transport process.

Group Translocation-Phosphotransferase system



How the phosphotransferase system works.....

- The **high energy phosphate bond** that supplies the necessary energy for the phosphotransferase system comes from a key metabolic intermediate called **phosphoenol pyruvate** (PEP) which acts as substrate.
- A small protein called **HPr**, the enzyme that phosphorylates it **(Enzyme I)**, and **Enzyme IIa** are cytoplasmic proteins.
- **Enzymes IIb** and **IIc** are **membrane proteins**. **HPr** and **Enzyme I** are **nonspecific components** of the phosphotransferase system and participate in all phosphotransferase reactions, whereas specific **Enzymes II** exist for the uptake of each individual sugar.

Substances transported by group translocation

- **Sugars like glucose**
- **purines**,
- **pyrimidines**, and
- **fatty acids**.

Note : However, many substances, including several **sugars**, are not taken up by the phosphotransferase system but instead are accumulated by the process of active transport.

Energy Requirement in Group Translocation

- **One high energy phosphate bond (one ATP equivalent) is consumed** in the process of transporting the glucose i.e. during the phosphorylation of glucose in the **first step of the metabolism (glycolysis)**.

- However, in the case of glucose phosphorylation by the phosphotransferase system, the uptake of glucose is essentially **energetically neutral**.

(ii) Active transport

- Active transport - an **energy-dependent pumping system** in which the substance being transported combines with a **membrane-bound carrier (membrane protein)**, which then releases the **chemically unchanged substance** inside the cell.

- Since the substance is not altered during the transport process if it is not consumed in cell reactions, its **concentration inside** may **reach many times** the **external concentration**.

Substances transported by active transport

i. **some sugars** like **glucose**
ii. **most amino acids** and
iii. **organic acids**, and
iv. a number of **inorganic ions** such as **sulfate**, **phosphate**, and **potassium**.

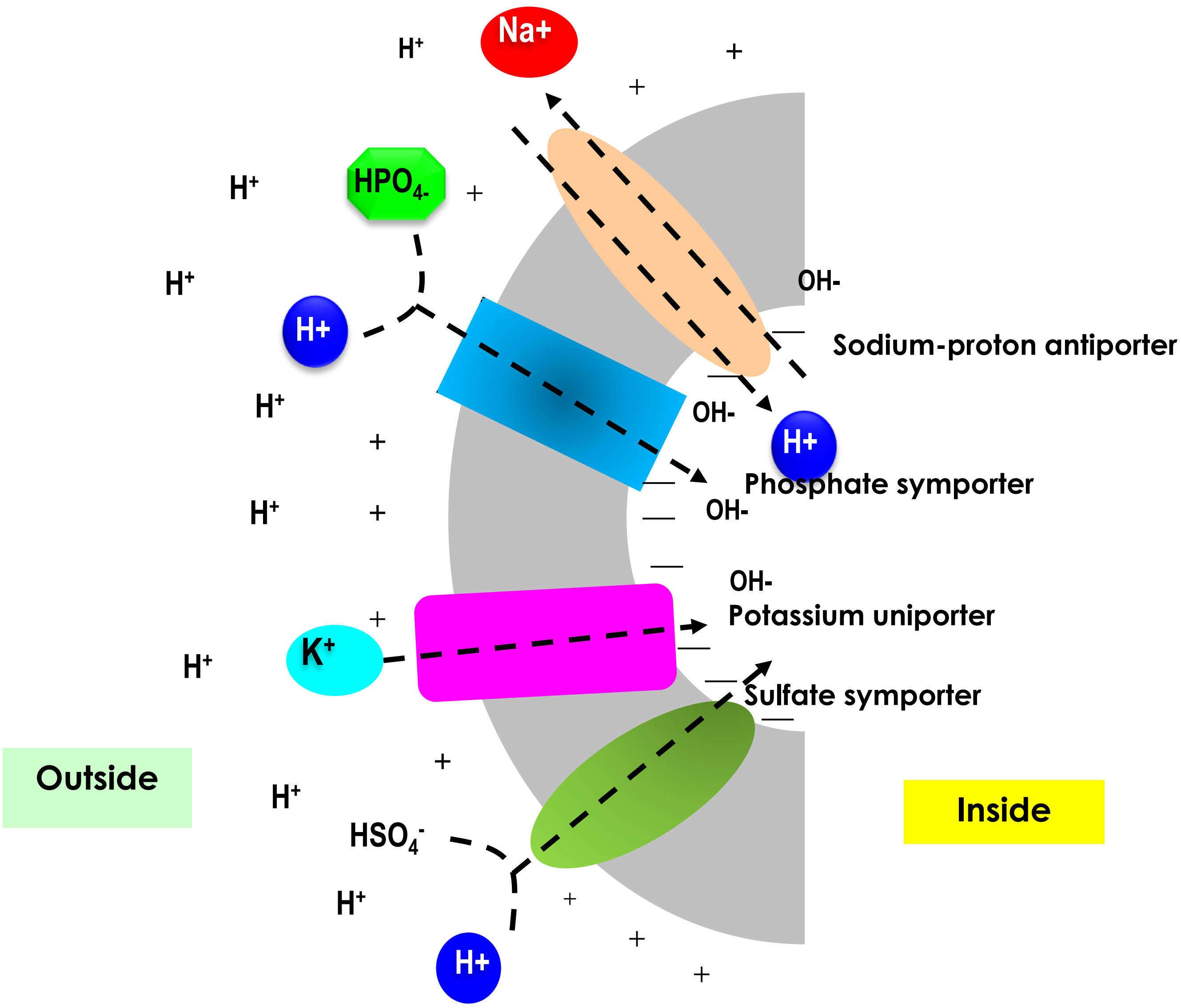
Note : Glucose is taken up by active transport processes in some bacteria and by the phosphotransferase system in others. As in any other pump, **active transport requires that work be performed**.

The Energy Source

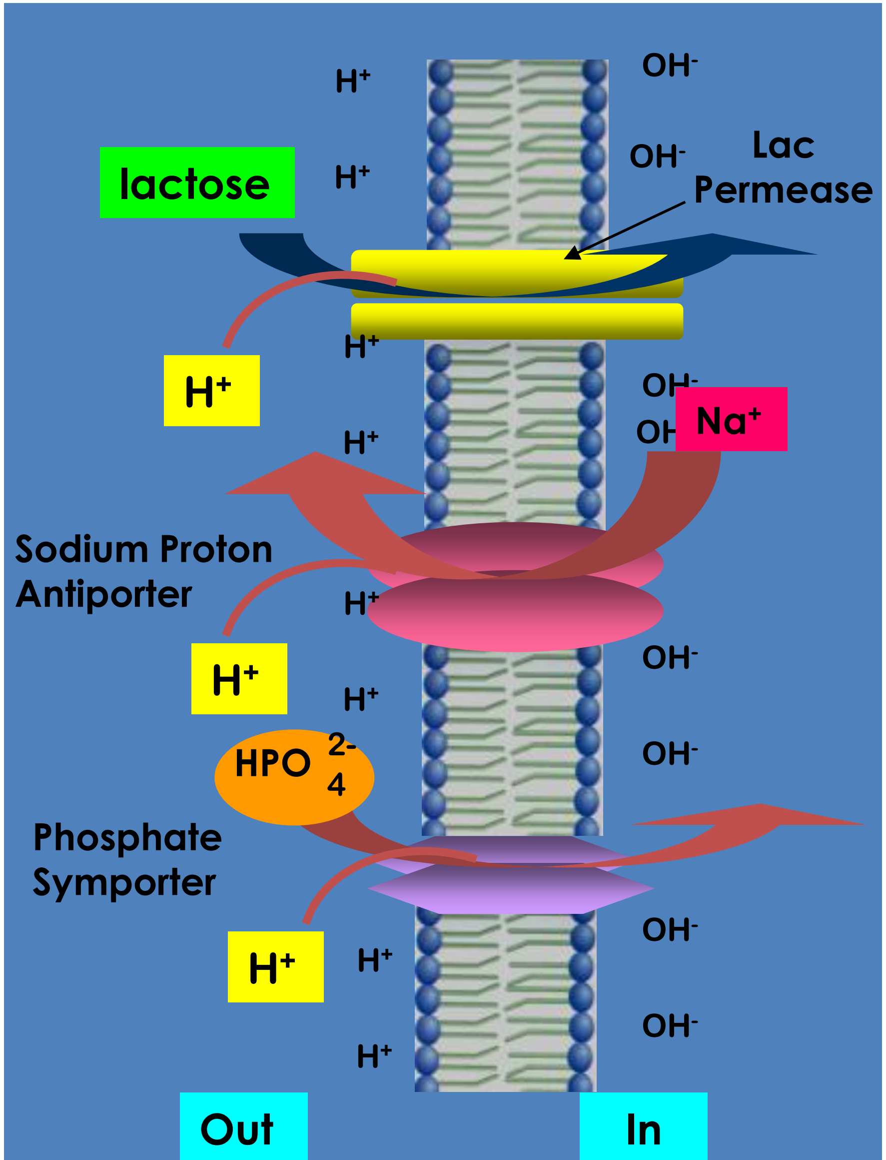
- In bacteria, the energy for driving the pump comes from
  - **ATP** in the case of some transporters, or more commonly from the **separation of hydrogen ions** (protons) across the membrane, called the **proton motive force**.
  - Energy released from the **breakdown of organic or inorganic compounds**, or **from the energy of light**, is used to establish a separation of protons across the membrane, resulting in the proton concentration highest outside the cell and lowest inside.

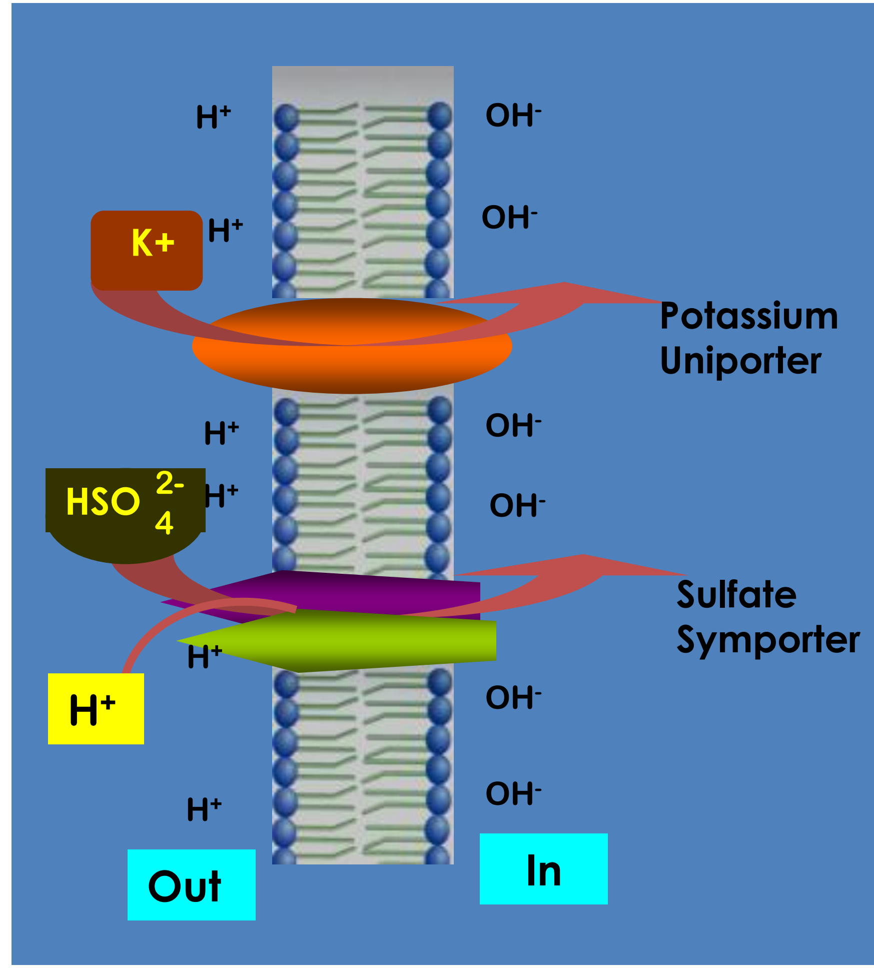
- This proton pump results in an **energized membrane**. It is the **electrochemical potential** residing in the proton motive force that drives the uptake of nutrients by active transport.
- Each **membrane carrier** involved in active transport has **specific sites** for both its substrate (for example, **glucose** or **potassium**) and **a proton** (or protons).
- As the substrate is taken up, protons move across the membrane and the proton motive force is diminished.

The Proton Pump



Transport Proteins of *E. coli*





Function of *lac* permease (a symporter) of *E. coli* and several other well characterized proteins

Periplasmic Binding Proteins and the ABC system

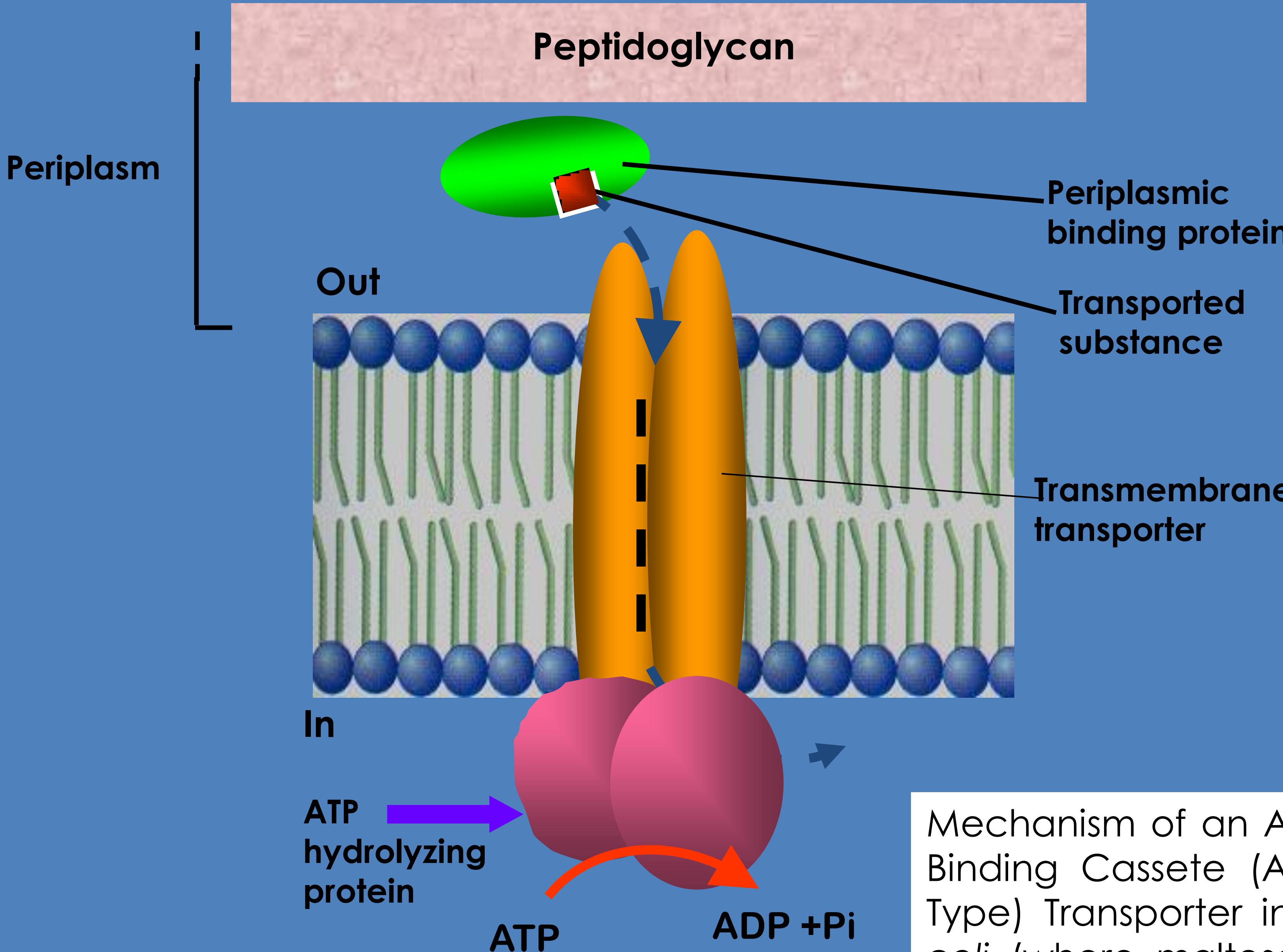
- Gram negative has a space called **periplasm** between the cytoplasmic membrane and an LPS.
  - contains a variety of proteins useful in transport.

- These proteins known as periplasmic binding proteins. Such a transport system known as **ABC (ATP Binding Cassette)** transport system involves three components:
  - **Periplasmic binding proteins**
  - **Membrane kinases**
  - **ATP hydrolyzing proteins (kinases)**

- ABC transporters exist for **sugars** and **amino acids**, a variety of **inorganic nutrients** such as sulfate and phosphate and trace metals.

ABC Transport System in Gram Negatives

- One interesting property of the ABC transport system is the **high substrate affinity** of the **periplasmic binding proteins**.
- These proteins **can move within** the periplasm and bind substrates even when they are present at very low concentration.
- Once trapped, the complex interacts with its respective membrane proteins and the actual transport event occurs driven by the energy of ATP.



Mechanism of an ATP-Binding Cassete (ABC Type) Transporter in *E. coli* (where maltose is the substrate)

Proton Motive Force

- The proton motive force is the **energy link** between membrane transporters and the metabolic machinery, making it possible for the carriers to "pump" nutrients inward.
- **Cations**, such as K^+, may be actively transported into the cytoplasm by uniporters in response to the proton motive force because the interior of the cell is negative when the membrane is energized.
- Uptake of **anion** occurs together with that of protons by symporters, and so it is effectively transporting the undissociated acid (HSO_4^-, HPO_4^{2-})that enters the cell.

Proton Motive Force

- **Excess sodium (Na^+)** within the cell can be pumped out by a **sodium-proton antiporter**, maintaining the **net electric charge** across the membrane.
- Transport of **charged molecules** such as **sugars** or **amino acids** can also be linked to the **electric charge differences**: the symporter transports both the substrate and one or more protons.
- Proton pumps linked to transport are key constituents of **all prokaryotic membranes** and are also present in the **inner membranes of mitochondria and chloroplasts**.

Proton Motive Force

- Substances taken up by active transport but not linked to dissipation of a proton gradient use the energy of ATP to drive the transport reaction.

- For example, in *Escherichia coli*, **lactose** is actively transported at the expense of a **proton motive force**, whereas the related disaccharide **maltose** is actively transported at the expense of **ATP**.

The Theory behind the Generation of Proton Motive Force

- Proteins are embedded in the lipid bilayer of the membrane and that the proteins are positioned in such a way that they have access to the outside and inside of membrane (integral protein).
- A separation of protons from electrons occur during the transport process.
- Hydrogen atom are removed from hydrogen atom **carriers such as NADH** are separated into **protons** and **electrons**.

- The electrons are transported through the **electron transport chain** by specific carriers and the **protons being extruded outside** the cell into the environment (in gram-negative prokaryotes protons are extruded to the periplasm).

- The **use of H^+** in the **reduction of O_2 to H_2O** and the **extrusion of H^+** cause **a net accumulation of OH^-** on the inside of the membrane.

- This result in a **slight acidification** of the external environment.

- At the end of the electron transport chain, the electrons are passed to the final electron acceptor (in the case of aerobic respiration), this is O_2 and reduce it.
- When O_2 is reduced to H_2O, it requires H^+ from the cytoplasm to complete the reaction, and these protons originate from the dissociation of water into H^+ and OH^-; **$H_2O \Rightarrow H^+ + OH^-$**.
- Despite their small size, neither H^+ nor OH^- freely pass through the membrane because they are charged so equilibrium cannot be spontaneously restored.

- Although electron transport to O_2 can be thought as producing water, what is actually produced are elements of water; H^+ and OH^- which accumulate on opposite sides of the membrane.

- The net result is the **generation of a pH gradient** and an **electrochemical potential** across the membrane. The **inside** surface of the membrane becomes electrically **negative and alkaline** and the **outside** surface of the membrane is **positive and acidic**.

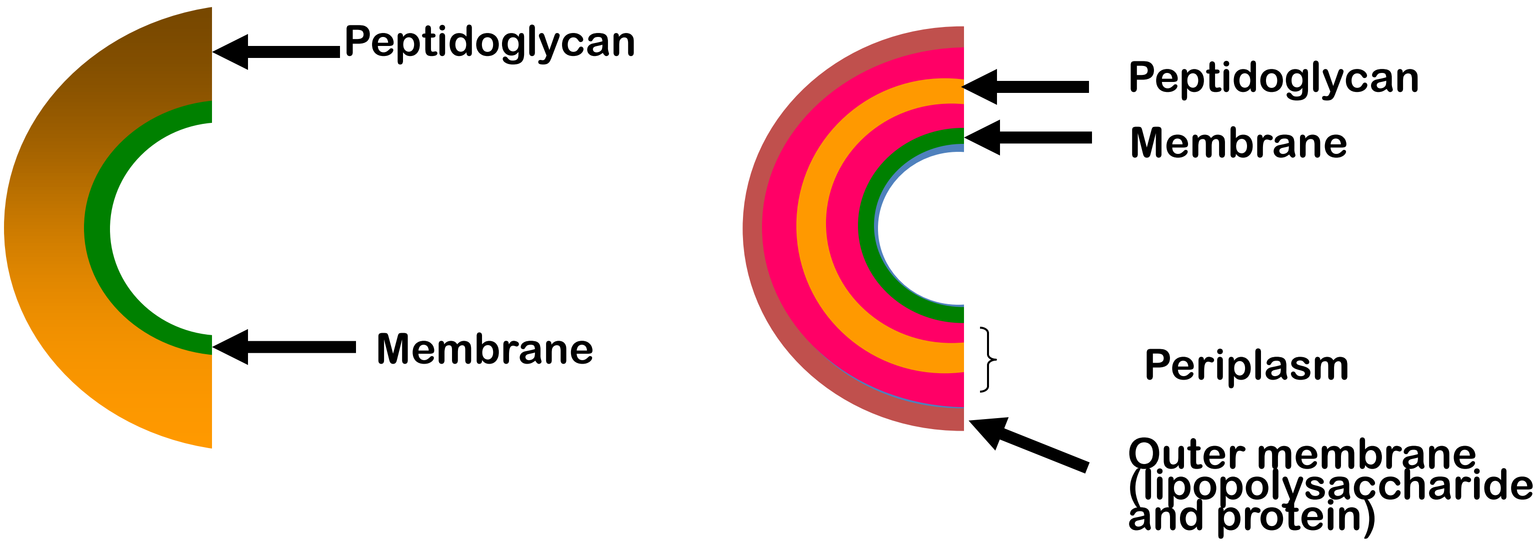
- This pH gradient and electrochemical potential cause the **membrane to be energized** (much like a battery) and this electrical energy can be used by the cell.
- The energized state of the battery is expressed as the electromotive (in volts). The energized state of the membrane is expressed as **proton motive force**.

The Cell Wall of Prokaryotes

- Turgor pressure inside prokaryotic cell is about 2 atm equivalent to an automobile tire.
- With such a pressure exerted, it requires a bacteria to have cell wall which also function to give shape and rigidity to cell.
- Bacteria can be divided into two groups;
  - **Gram positive**
  - **Gram negative**

Gram Negative and Gram Positive Bacteria

- The **gram-negative** cell wall is a **multilayered structure** and **quite complex.**
- The **gram-positive** cell wall consists of primarily **a single type of molecule** and is often much **thicker**.
- There is also a significant textural difference between the surfaces of gram-positive and gram-negative bacteria.



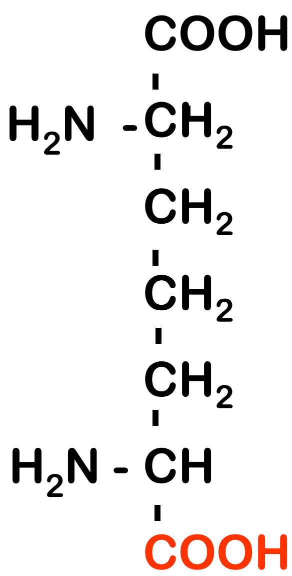
Gram positive

Gram negative

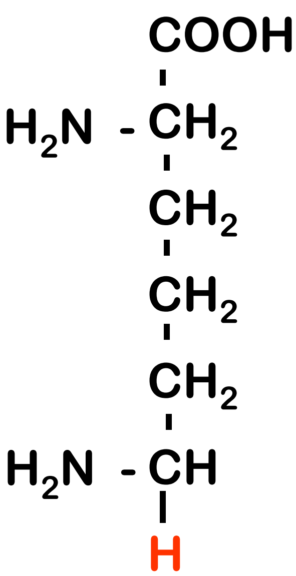
Peptidoglycan

- Peptidoglycan is one rigid layer that is primarily responsible for the **strength of the wall**.
- In most bacteria, besides peptidoglycan, additional layers are present outside this rigid layer.
- The rigid layer of both Gram-negative and Gram-positive bacteria is very similar in chemical composition.

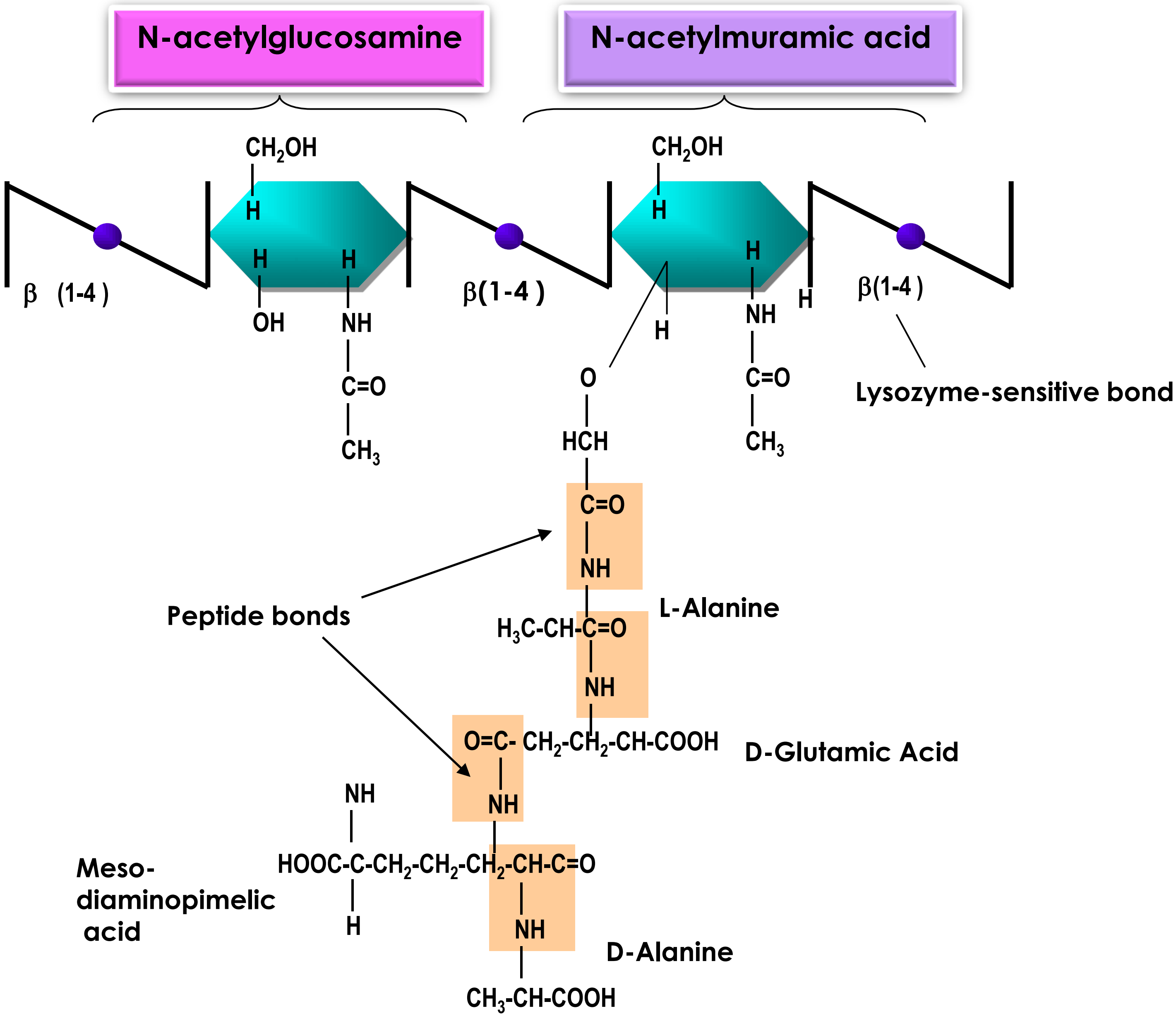
- **Peptidoglycan** (or **murein**), is a thin sheet composed of two sugar derivatives,
  - **N-acetylglucosamine** and
  - **N-acetylmuramic acid**, and
  - a small group of amino acids consisting of **L-alanine**, **D-glutamic acid**, **D-alanine**, and either **lysine** or **diaminopimelic acid** (DAP)



Diaminopimelic acid (DAP)



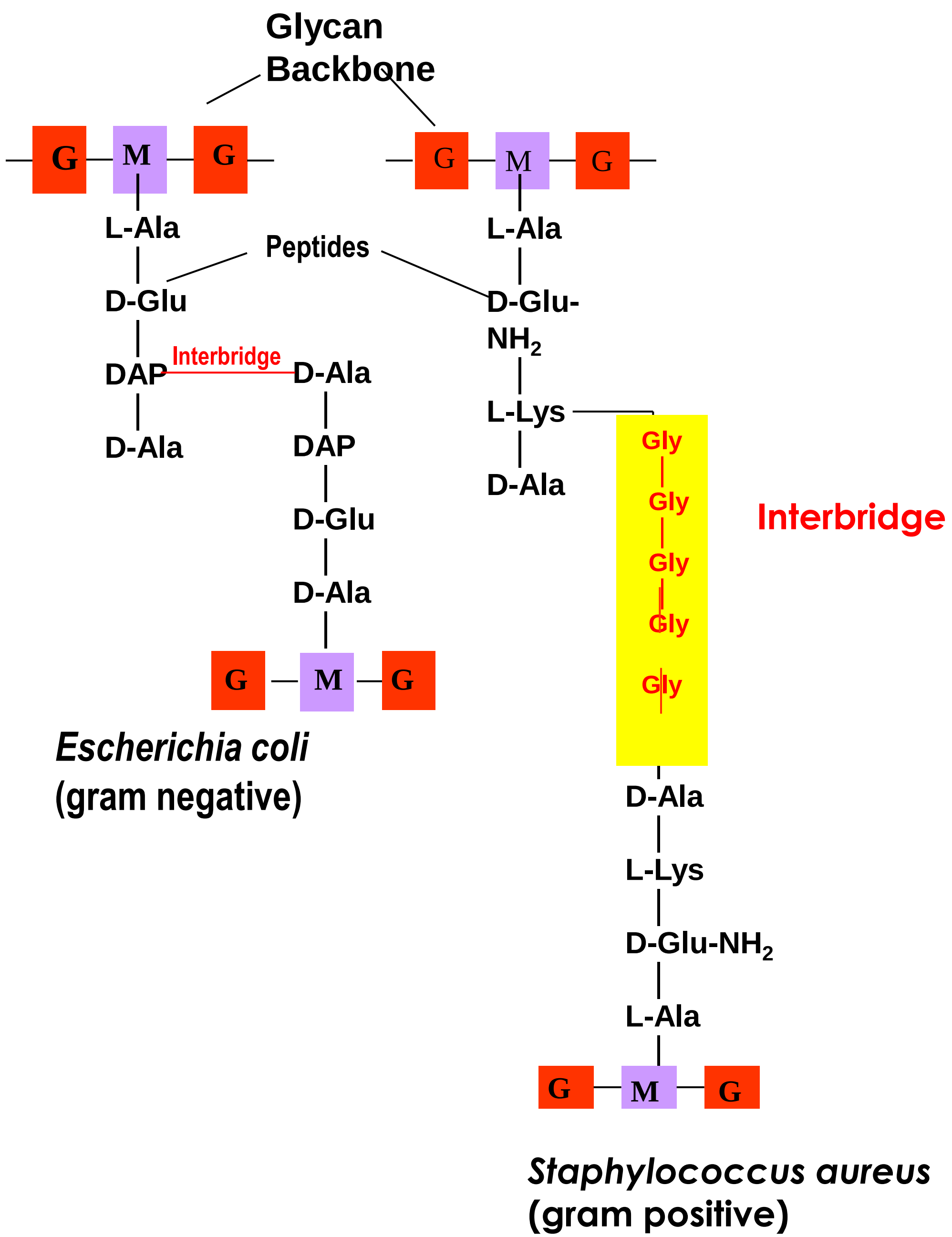
Lysine (Lys)


N-acetylglucosamine
N-acetylmuramic acid
CH2OH
H
H
H
OH
NH
C=O
CH3
β (1-4)
β(1-4)
CH2OH
H
H
NH
H
H
C=O
CH3
O
HCH
β(1-4)
Lysozyme-sensitive bond
C=O
NH
L-Alanine
Peptide bonds
H3C-CH-C=O
NH
O=C- CH2-CH2-CH-COOH
D-Glutamic Acid
NH
NH
Meso-
diaminopimelic
acid
HOOC-C-CH2-CH2-CH2-CH-C=O
H
NH
D-Alanine
CH3-CH-COOH

Rigidity and Strength of the Peptidoglycan

- The **glycosidic bonds** connecting the sugars in the glycan chains are very strong, but these chain alone cannot provide rigidity in all directions.
- The full strength of the peptidoglycan structure is realized only when these chains are **cross-linked by amino acids**.
- This cross-linking occurs to characteristically different extents in different bacteria with greater rigidity coming from more complete crosslinking.

- In **Gram-negative** bacteria, cross-linkage usually occurs by **direct peptide linkage** of the **amino group of diaminopimelic acid** to the **carboxyl group of the terminal D-alanine**.
- The **Gram-positive** bacteria cross-linkage is usually by a **peptide interbridge**, the kinds and numbers of cross-linking amino acids varying from organism to organism.
- In *Staphylococcus aureus*, a gram-positive organism, each **interbridge peptide** consists of **five molecules of the amino acid glycine** connected by **peptide bonds**.


Glycan
Backbone
G
M
G
G
M
G
L-Ala
Peptides
L-Ala
D-Glu
D-Glu-
NH2
DAP
Interbridge
D-Ala
D-Ala
DAP
L-Lys
D-Ala
D-Glu
D-Ala
Gly
Gly
Gly
Gly
Gly
Interbridge
G
M
G
Escherichia coli
(gram negative)
D-Ala
L-Lys
D-Glu-NH2
L-Ala
G
M
G
Staphylococcus aureus
(gram positive)

Difference between Gram Positive and Gram Negative and their Complexity

- In **gram-positive Bacteria**, as much as **90%** of the cell wall consists of **peptidoglycan**, although another kind of constituent, **teichoic acid** is usually present in small amounts.
- And, although some bacteria are thought to have only a **single layer** of peptidoglycan surrounding the cell, many Bacteria, especially **gram-positive Bacteria**, have **several (up to about 25)** peptidoglycan layers.
- In **gram-negative** Bacteria about **10% of the wall is peptidoglycan**, the majority of the wall consisting of a complex layer.

Diversity in Peptidoglycan

- However, the shape of both gram-positive and gram-negative cells is thought to be determined by the **lengths of the peptidoglycan chains** and by the **manner and extent of cross-linking** of the chains.
- Peptidoglycan is present only in **bacteria**; the sugar **N-acetylmuramic acid** and the amino acid **diaminopimelic acid, (DAP)** are never found in the cell walls of Archaea or Eukaryotes.
- However, not all bacteria have DAP in their peptidoglycan.

- This amino acid is present in all gram-negative bacteria and in some gram-positive species, but most gram-positive cocci have **lysine instead of DAP**, and a few other gram-positive Bacteria have other amino acids.
- Another unusual feature of the bacterial cell wall is the presence of **two amino acids** that have the D-configuration, **D-alanine** and **D-glutamic acid**.
- In proteins, amino acids are always of the L-configuration.

- Several generalizations regarding peptidoglycan structure can be made. The **glycan** portion is uniform, with only the sugars **N-acetylglucosamine** and **N-acetylmuramic acid** being present, and these sugars are always connected in **β-1,4 linkage**.

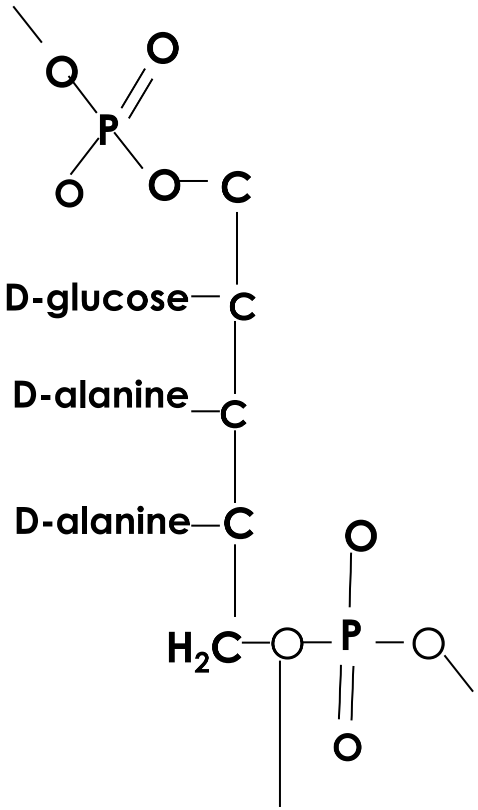
- The **tetrapeptide** of the repeating unit shows major variation only in one amino acid, the **lysine-diaminopimelic acid** alternation. However, the **D-glutamic acid** at **position 2 can be hydroxylated** in some organisms, whereas **substitutions** occur in **amino acids at positions 1 and 3** in a few others.

- More than 100 different peptidoglycan types are known and the greatest variation among them occurs in the **interbridge**.
- Any of the amino acids present in the **tetrapeptide** can also occur in the **interbridge**. But, in addition, a number of other amino acids can be found there, such as **glycine**, **threonine**, **serine**, and **aspartic acid**.
- However, certain amino acids are **never found** in the interbridge: **branched-chain amino acids**, **aromatic amino acids**, **sulfur-containing amino acids**, **histidine**, **arginine**, and **proline**.

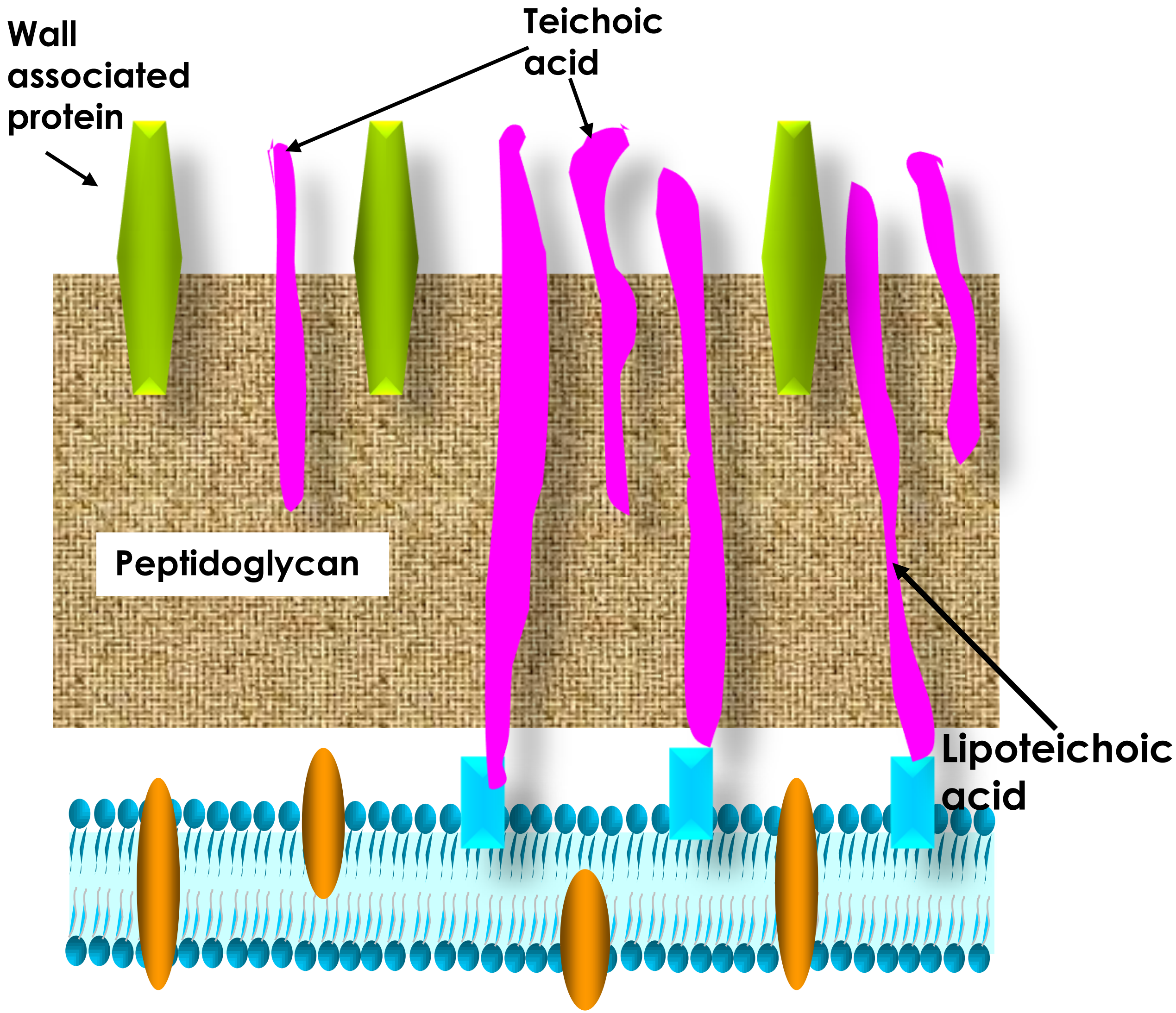
- Thus, it can be stated allthough the precise chemistry of peptidoglycan can vary, the **structural makeup** of peptidoglycan is the same in all forms of the molecule:
  - **N-acetylglucosamine and**
  - **N-acetylmuramic acid form the backbone**,
  - **muramic acid molecules are cross-linked with amino acids**

Teichoic Acids and A Summary of the Gram-Positive Cell Wall

- Gram-positive Bacteria frequently have **acidic polysaccharides** attached to their cell wall called **teichoic acids** (from the Greek word teichos, meaning "wall").
- The term teichoic acids includes all wall, membrane, or cellular polymers containing **glycerophosphate** or **ribitol phosphate** residues.
- These **polyalcohols** are connected by **phosphate esters** and usually have other sugars and D-alanine attached.



Ribitol – the subunit of teichoic acid

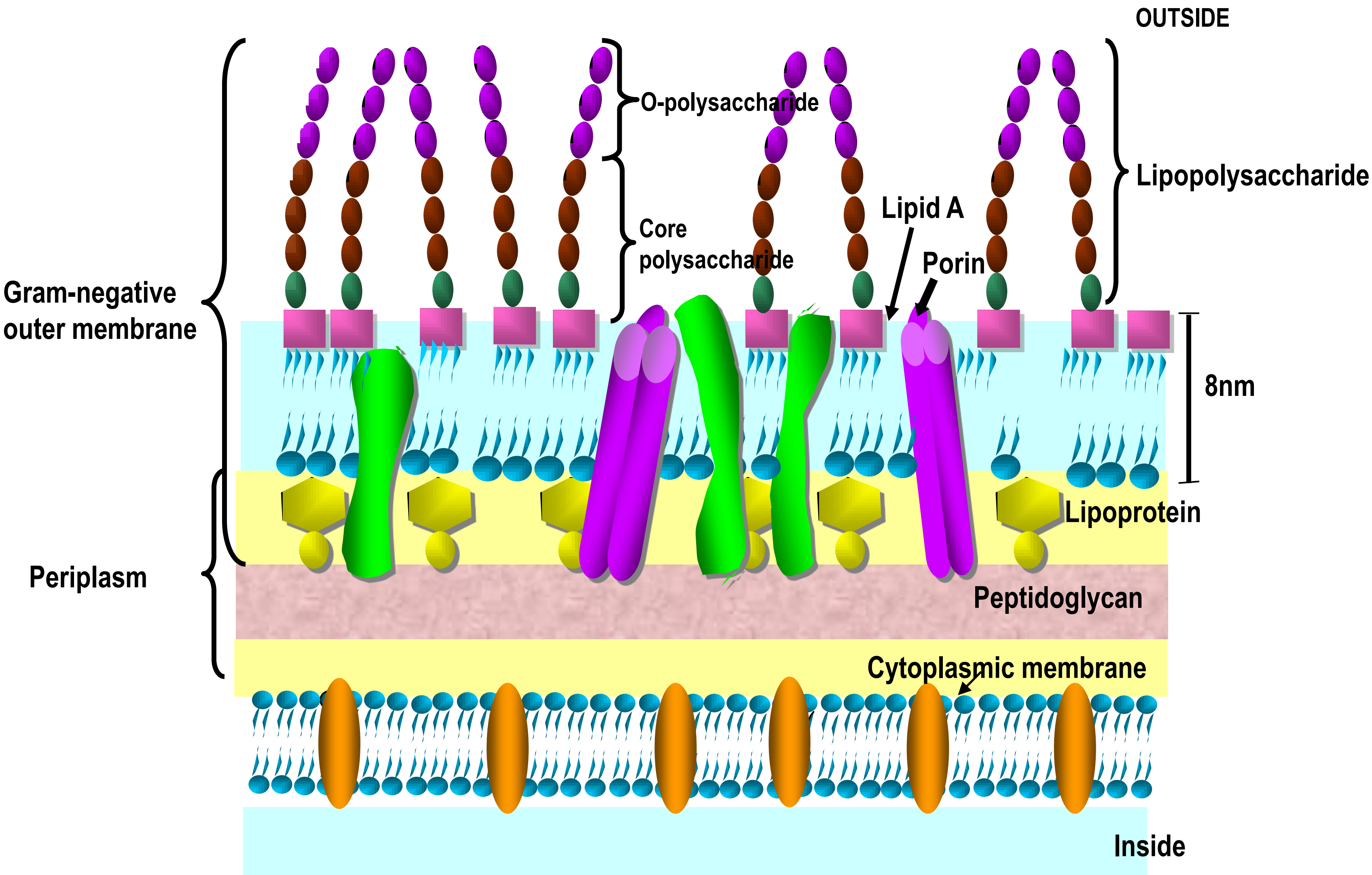


- Because they are **negatively charged**, **teichoic acids** are partially responsible or the **negative charge of the cell surface** as a whole and may function to effect passage of ions through the cell wall.

- Certain **glycerol-containing acids** are bound to **membrane lipids of gram-positive** Bacteria; because less **teichoic acids** are intimately **associated with lipid**, they have been called **lipoteichoic acids**.

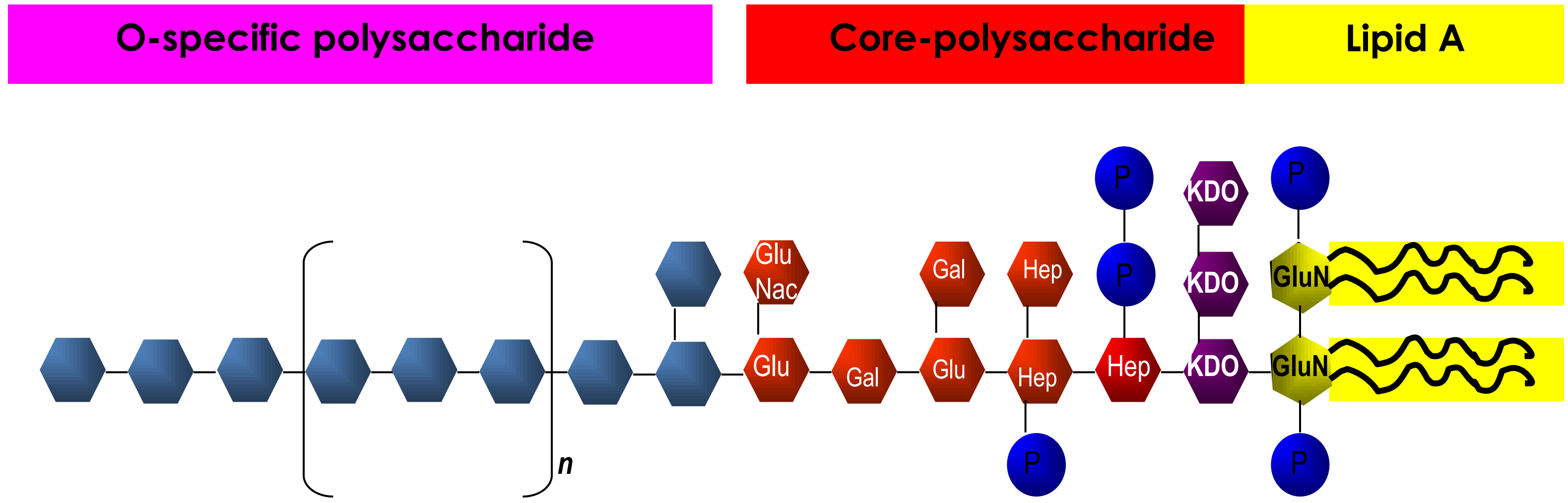
The Outer Membrane of Gram-Negative Bacteria

- Besides, peptidoglycan, gram-negative Bacteria contain an additional wall layer made of **lipopolysaccharide**.
- This layer is effectively a **second lipid bilayer**, but it is not constructed solely of phospholipid, as is the cytoplasmic membrane; instead it **contains polysaccharide** and **protein**.
- The lipids and polysaccharides are intimately linked in the outer layer to form specific lipopolysaccharide structures.


OUTSIDE
O-polysaccharide
Core
polysaccharide
Lipopolysaccharide
Lipid A
Porin
Gram-negative
outer membrane
8nm
Lipoprotein
Periplasm
Peptidoglycan
Cytoplasmic membrane
Inside

Chemistry of the LPS Layer

- Because of the presence of lipopolysaccharide, the outer layer is frequently called the **lipopolysaccharide layer**, or simply **LPS**.
- Polysaccharide consists of two portions, the **core polysaccharide** and the **O-polysaccharide**.
- In *Salmonella*, where it has been best studied, the core polysaccharide consists of **ketodeoxyoctonate** (KDO), **seven-carbon sugars**.



Structure of the lipopolysaccharide of gram-negative Bacteria. The precise chemistry of lipid A and the polysaccharide components vary among species of gram-negative Bacteria, but the sequence of major components (lipid A- KDO-core-O-specific) is generally uniform. The O-specific polysaccharide varies enormously among species. KDO, ketodeoxyoctonate; Hep, heptose; Glu, glucose; Gal, galactose; GluNac, N-acetylglucosamine; GlcN, glucosamine. The lipid A portion of the LPS layer can be toxic to animals and comprises the endotoxin complex .

Lipopolysaccharide

- Connected to the core is the **O-polysaccharide**, which usually contains **galactose**, **glucose**, **rhamnose**, and **mannose** (all six-carbon sugars) as well as one or more **unusual dideoxy sugars** such as **abequose**, **colitose**, **paratose**, or **tyvelose**.
- These sugars are connected in four- or five-membered sequences, which often are branched. When the sugar sequences are repeated, the long O-polysaccharide is formed.
- The relationship of the O-polysaccharide to the rest of the LPS layer is the previous slide.

Lipopolysaccharide

- The lipid portion of the lipopolysaccharide, referred to as to as **lipid A** is not a glycerol lipid, but instead the **fatty acids are connected by ester amine linkage** to a **disaccharide** composed of N-acetylglucosamine phosphate. The disaccharide is attached to the O-polysaccharides through **KDO**.
- Fatty acids commonly found in lipid A include **caproic**, **lauric**, **myristic**, **palmitic**, and **stearic acids**. In the outer membrane, the LPS associates with various proteins to form the outer half of the unit membrane structure .

Lipopolysaccharide

- A **lipoprotein complex** is found on the inner side of the outer membrane of a number of gram-negative Bacteria. This lipoprotein is a small (approx. 7200 in molecular weight) protein that serves as an **anchor** between the **outer membrane** and **peptidoglycan**.

- In the outer leaf of the outer membrane, **LPS replaces phospholipids**; the latter are found predominantly in the inner leaf.

	Gram Positive	Gram Negative
Gram reaction	Retain crystal violet dye and stain dark violet or purple	Can be decolourized to accept (safranin) and stain red
Peptidoglycan layer	Thick (multilayered)	Thin (single-layered)
Teichoic acids	Present in many	Absent
Periplasmic space	Absent	Present
Outer membrane	Absent	Present
Lipopolysaccharide (LPS) content	Virtually none	High(due to presence of outer membrane)
Lipid and lipoprotein content	Low (acid-fast bacteria have lipids linked to peptido-glycan)	
Flagellar structure	2 rings in basal body	4 rings in basal body
Toxins produced	Primarily exotoxins	Primarily endotoxins
Resistance to Physical disruption by iysozyme.	High	Low (requires pretreatment to destabilize outer membrane)
Susceptibility to penicillin and sulfonamide.	High	Low
Susceptibility to streptomycin	Low	High
Chloramphenicol and tetracycline	High	Low
Inhibition by basic dyes	High	Low
Susceptibility to anionic detergents	High	Low
Resistance to sodium azide	High	Low
Resistance to drying	High	Low

Relationship of Cell Wall Structure to the Gram Stain

- Are the structural differences between the cell walls of gram-positive and gram-negative Bacteria responsible in any way for the Gram stain reaction?

Principles of Gram Staining

- In the Gram stain, an **INSOLUBLE crystal violet-iodine complex** is formed inside the cell, and this complex is **extracted by alcohol** from **Gram-negative** but not from Gram-positive Bacteria.

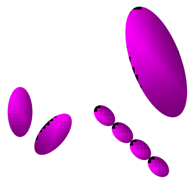
- **Gram-positive** Bacteria, which have very thick cell walls consisting of several layers of peptidoglycan, become **dehydrated by the alcohol**.
- This causes **the pores in the walls to close**, preventing the soluble crystal violet-iodine complex from escaping.
- In Gram-negative Bacteria, alcohol readily penetrates the lipid which outer layer, and the thin peptidoglycan layer also **does not prevent solvent passage**, thus, the crystal violet-iodine complex is easily removed.

- However, the Gram reaction is not related directly to cell wall chemistry since yeasts, which have a thick cell wall but one of an entirely different chemical composition, is stain Gram-positive.

- Thus, it is not the chemical constituents but the **physical structure of the wall that is responsible for a Gram-positive reaction.**

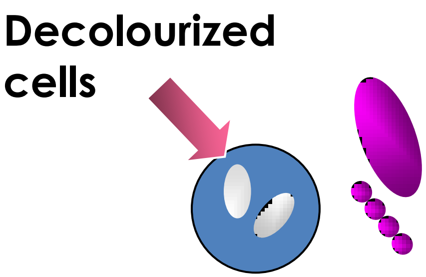
Flood the heat fixed smear with crystal violet for 1 minute.

All cells are purple.



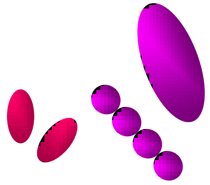
Add iodine solution for 3 minutes.

All cells remain purple.



Decolorize with alcohol briefly.

Gram-positive cells are purple while Gram-negative are colourless.



Counterstain with safranin for 1-2 minutes.

Gram-positive cells are purple while Gram-negative are pink to red.

References:

- Madigan, M.T., Martinko, J.M., Dunlap, P.V. and Clark, D.P. (2009). Brock Biology of Microorganisms: Pearson Education, USA.
- Clark, D.P. and Russel, L.D. (2000). Molecular Biology Made Simple and Fun: Cache River Press, USA.