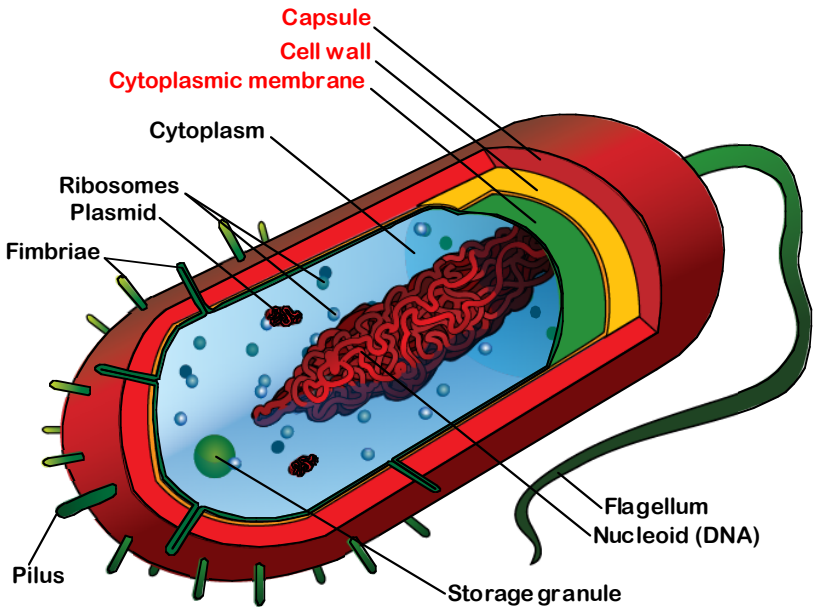
Biomechanics of the bacterial cell envelope

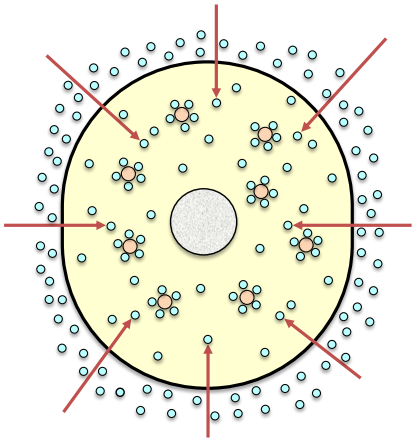


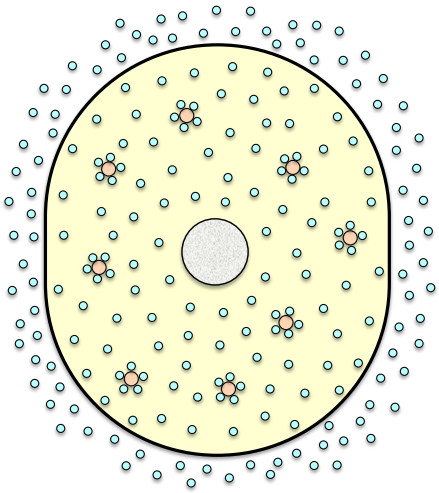
In cells without a mechanically supporting cell wall:

2

There is a higher concentration of free water molecules outside the cell than inside the cell…

…so water diffuses into the cell by osmosis and the cell swells up (and may eventually rupture).





van't Hoff equation: $P = \Delta n * V^{-1} * R * T$

Osmosis can be a big problem for bacteria, which mostly live in **hypotonic** environments where the concentration of solutes outside the cell is lower (**hypotonic**) relative to the concentration of solutes inside the cell (**hypertonic**). Put another way, the "free water action" outside the cell is higher than the "free water action" inside the cell. Consequently, there is a net flux of water from the external environment into the cell, which cause the cell to swell up and eventually burst, like an over-inflated balloon. This process – the movement of water molecules from an area of higher water concentration to an area of lower water concentration through a semi-permeable membrane – is called **osmosis**.

Osmotic pressure is the minimum external pressure required to inhibit the movement of water molecules across a semi-permeable membrane, resulting in no net movement of solvent molecules. This is illustrated on Slide 38 from last week's lecture. Osmotic pressure depends on the molar concentration of the solution outside vs. inside the cell.

Intracellular solutes (ions, metabolites, and macromolecules such as proteins, nucleic acids, etc.) play an important role in establishing the **osmotic pressure** across the cell membrane. The cell membrane is a semi-permeable interface that enables the free passage of water molecules while preventing (partially or totally) the transport of solutes. The osmotic pressure is the extra pressure that is sustained by a semi-permeable barrier that has a higher concentration of solutes on one side of the barrier. In the dilute limit, the osmotic pressure obeys a relation similar to the **ideal gas law** where the osmotic pressure P is given by the **van't Hoff equation**: $P = n * V^{-1} * R * T$

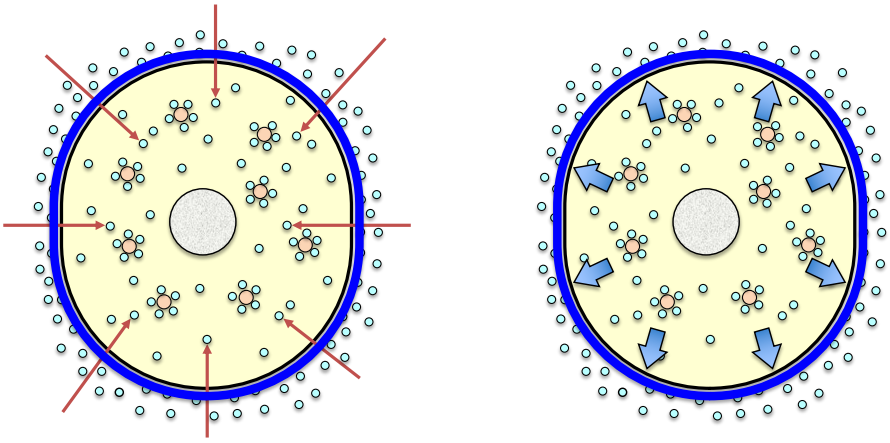
Abbreviations:
P = pressure in $kg * m^{-1} * s^{-2}$
Δn = the number of moles (mol) of intracellular solute in excess of extracellular solute
V = volume in m^3
R = the universal gas constant in 8.3 $J\ mol^{-1}\ K^{-1}$ or 8.3 $kg * m^2 * s^{-2} * mol^{-1} * K^{-1}$
T = the absolute temperature in degrees Kelvin (K)
K = degrees Kelvin
mol = moles

The equation for the van't Hoff relation is a "primary concept" – you should memorize it (and all of its terms) and you should feel comfortable using it. Indeed, you should already be familiar with this equation from your physics courses.

In cells with a mechanically supporting cell wall:

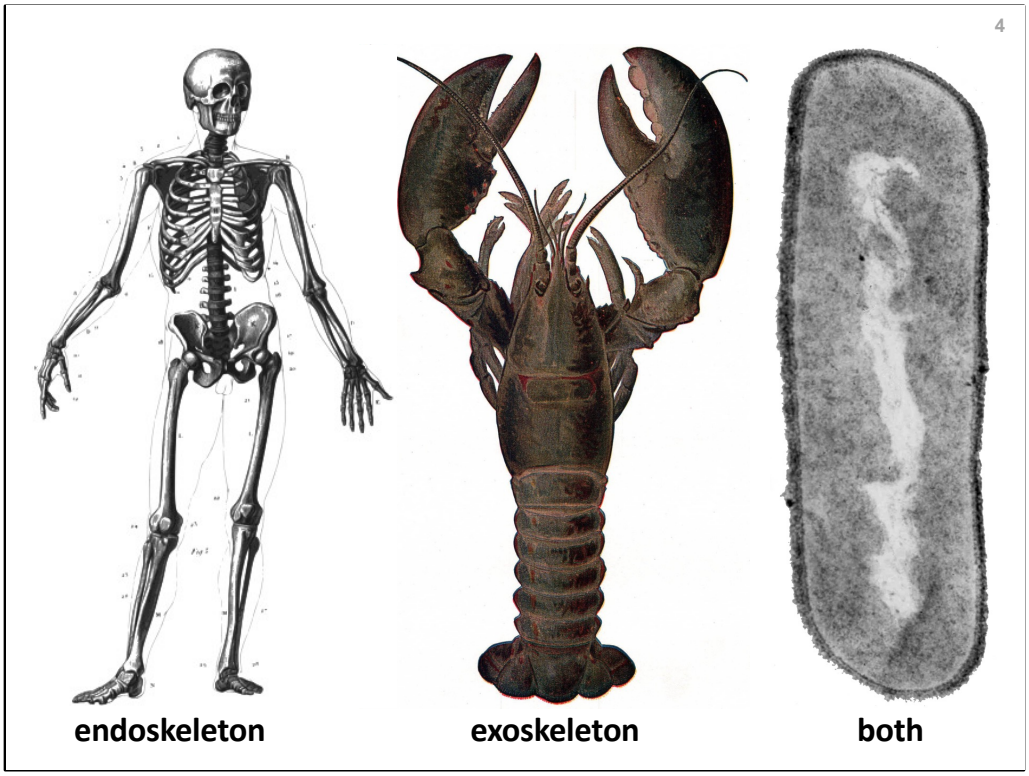
There is a higher concentration of free water molecules outside the cell than inside the cell…

…so water diffuses into the cell by osmosis until osmotic pressure and turgor pressure are balanced.



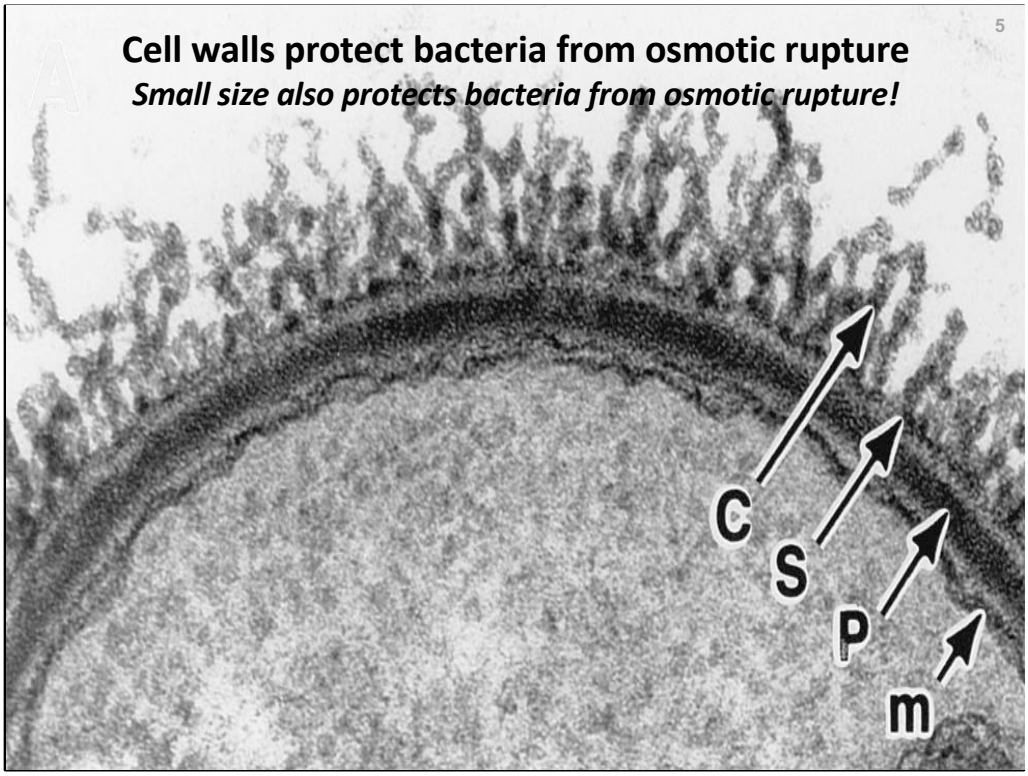
van't Hoff equation: $P = \Delta n * V^{-1} * R * T$

Osmotic pressure is defined as the minimum pressure that must be applied on a solution to prevent the inward flow of water across a semi-permeable membrane (see Slide 38 from last week's lecture). **Turgor pressure** refers to the outward-directed pressure inside the bacterial cell that pushes the plasma membrane (thin black line) against the bacterial cell wall surrounding the membrane (thick blue line). When a bacterial cell is suspended in a dilute solution, osmosis "pushes" water into the cell (red arrows in the left-hand panel) until it is balanced by the rising internal turgor pressure, which "pushes" water back out of the cell (blue arrows in the right-hand panel). The cell membrane is mechanically weak and easily ruptured by rising internal turgor pressure, but the rigid cell wall is mechanically strong so it can withstand high internal turgor pressure.



Mammals, like this human, have an **endoskeleton**. Crustaceans, like this lobster, have an **exoskeleton**. Bacteria, like this *Escherichia coli* cell, have both: an endoskeleton (the bacterial **cytoskeleton**) and an exoskeleton (the bacterial **cell wall**, which comprises the **peptidoglycan** layer of the bacterial **cell envelope**).

The bacterial cell wall effectively functions as an "exoskeleton" and it is the primary determinant of bacterial cell shape. The cell wall also protects bacteria against osmotic and other mechanical stresses imposed by the environment. Most bacteria live in environments that undergo fluctuations in the osmotic strength of the surrounding medium. The bacterial cell wall is the main structure responsible for protecting the cell against osmotic fluctuations. Most microbes live in environments where the external medium is **hypotonic** (high free water concentration) compared to the cytoplasm, which is relatively **hypertonic** (low free water concentration because water is bound up with solutes). Consequently, the free water concentration outside the cell is higher than the free water concentration inside the cell and the cell experiences a net internal turgor pressure due to net influx of water into the cell. This **internal turgor pressure** is supported by the relatively rigid and mechanically robust bacterial cell wall; in particular, the **peptidoglycan** component of the cell wall, which is not found in eukaryotic cells.



SOURCE: Mesnage S, Tosi-Couture E, Gounon P, Mock M, Fouet A (1998) The capsule and S-layer: two independent and yet compatible macromolecular structures in *Bacillus anthracis*. *J Bacteriol* 180(1): 52-58 PMID: 9422592.

Abbreviations:
C, capsule
S, S-layer
P, periplasmic space
M, cytoplasmic membrane

6

Quick refresher: forces and dimensions

Force is measured in newtons (N):

$N = M * L * T^{-2} = kg * m * s^{-2}$

Tension (T) is measured in newtons (N):

$N = M * L * T^{-2} = kg * m * s^{-2}$

Surface Tension (T_S) acts in one dimension:

$T_S = N * L^{-1} = (M * L * T^{-2})(L^{-1}) = M * T^{-2} = kg * s^{-2}$

Stress (σ) acts in two dimensions:

$\sigma = N * L^{-2} = (M * L * T^{-2})(L^{-2}) = M * L^{-1} * T^{-2} = kg * m^{-1} * s^{-2}$

Pressure (P) acts in two dimensions:

$P = N * L^{-2} = (M * L * T^{-2})(L^{-2}) = M * L^{-1} * T^{-2} = kg * m^{-1} * s^{-2}$

A Newton (N) is defined as $M * L * T^{-2}$ or in SI units $1\ N = 1\ kg * m * s^{-2}$. The newton is the SI unit of force. It is equal to the force that would give a mass of one kilogram an acceleration of one meter per second per second. It is equivalent to 100,000 dynes.

Tension (T) is defined as $N = M * L * T^{-2}$. SI units: $kg * m * s^{-2}$

Surface Tension (T_S) is defined as $N * L^{-1} = M * L * T^{-2} * L^{-1} = M * T^{-2}$. SI units: $kg * s^{-2}$

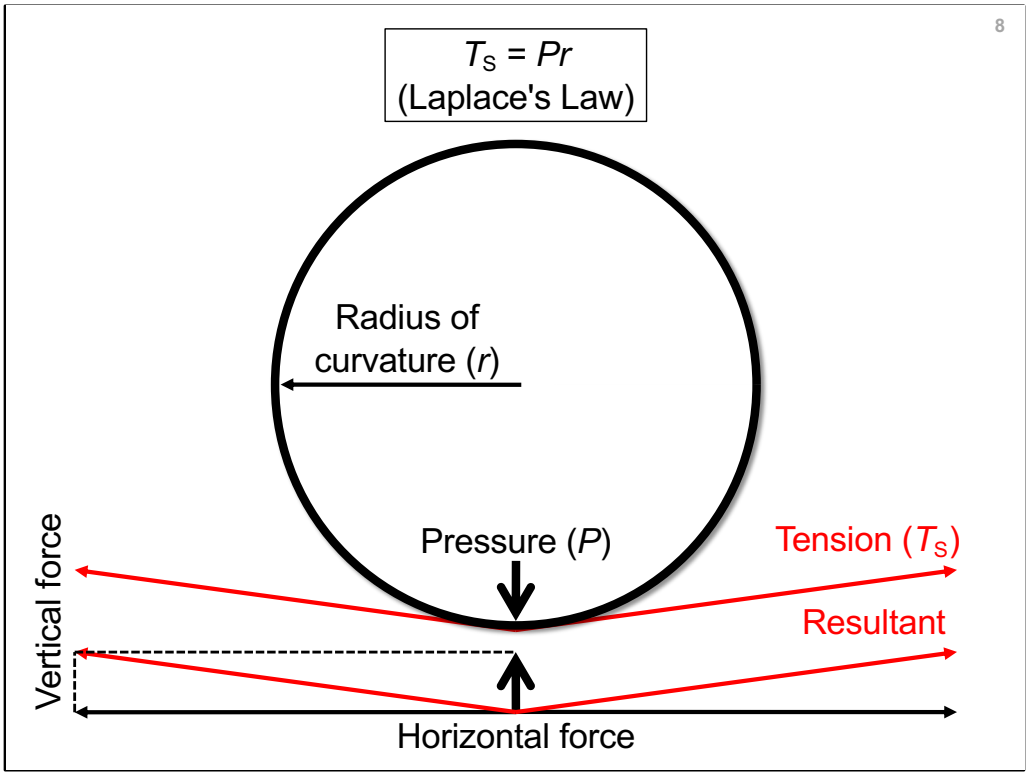
Stress (σ) is defined as $N * L^{-2} = M * L * T^{-2} * L^{-2} = M * L^{-1} * T^{-2}$. SI units: $kg * m^{-1} * s^{-2}$

Pressure (P) is also defined as $N * L^{-2} = M * L * T^{-2} * L^{-2} = M * L^{-1} * T^{-2}$. SI units: $kg * m^{-1} * s^{-2}$

Abbreviations:
M = mass (in kilograms)
L = length (in meters)
T = time (in seconds)
kg = kilograms
m = meters
s = seconds

You should memorize these definitions (if you haven't already done so).


7
Why are large bubbles more fragile than small bubbles?

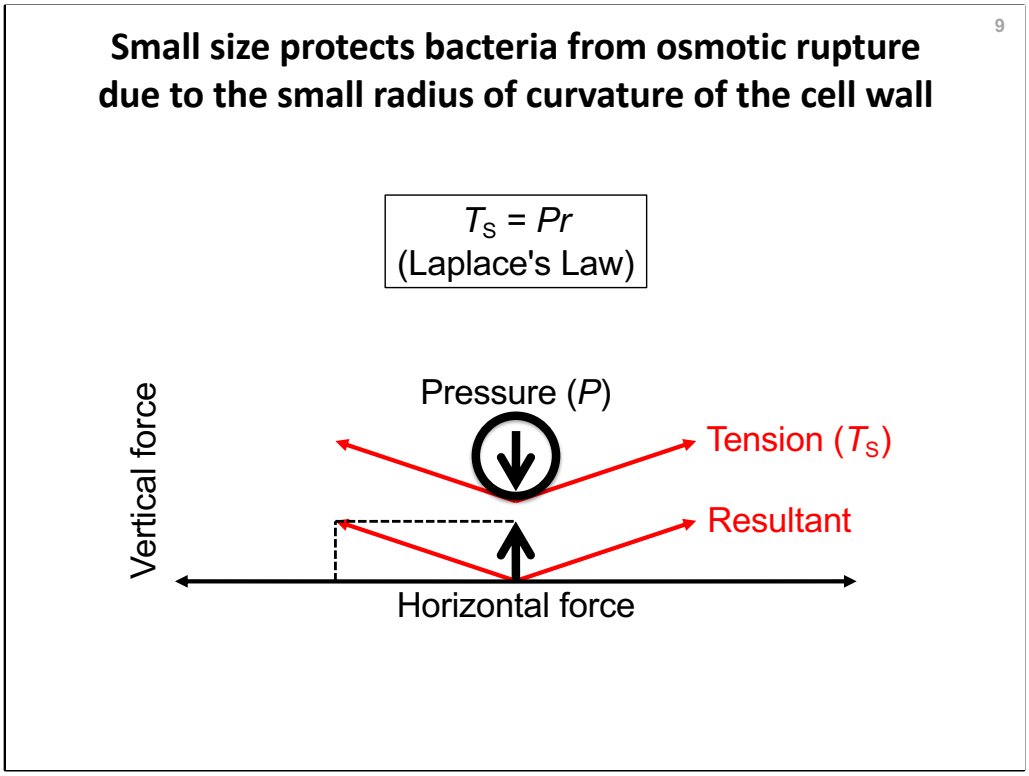


SOURCE: Vogel S (1988) Chapter 11: Insinuations about curves. In: *Life's Devices: The Physical World of Animals and Plants*. Princeton University Press, Princeton, NJ, 08540 USA.

Laplace's Law states that $\boldsymbol{T_S = Pr}$, where T_S = surface tension, P = pressure, r = radius of curvature. In this case, the large cell (depicted on this slide) has a radius of curvature (r) that is 6-fold larger than the radius of curvature of the small cell (depicted on the next slide). Consequently, if both cells are under the same internal turgor pressure (P), then the amount of tension (T_S) in the cell wall of the large cell will be 6 times higher than the tension (T_S) in the cell wall of the small cell. Consequently, the large cell will be more susceptible to osmotic rupture.

Force (Newton, N) = $M * L * T^{-2}$. SI units: $kg * m * s^{-2}$
Surface Tension (T_S) = $N * L^{-1} = M * T^{-2}$. SI units: $kg * s^{-2}$
Pressure (P) = $N * L^{-2} = M * L^{-1} * T^{-2}$. SI units: $kg * m^{-1} * s^{-2}$
Radius of curvature (r) = L. SI units: m

The equation for Laplace's Law is a "primary concept" – you should memorize it and you should feel comfortable manipulating it.



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Force (Newton, N) = $M * L * T^{-2}$. SI units: $kg * m * s^{-2}$
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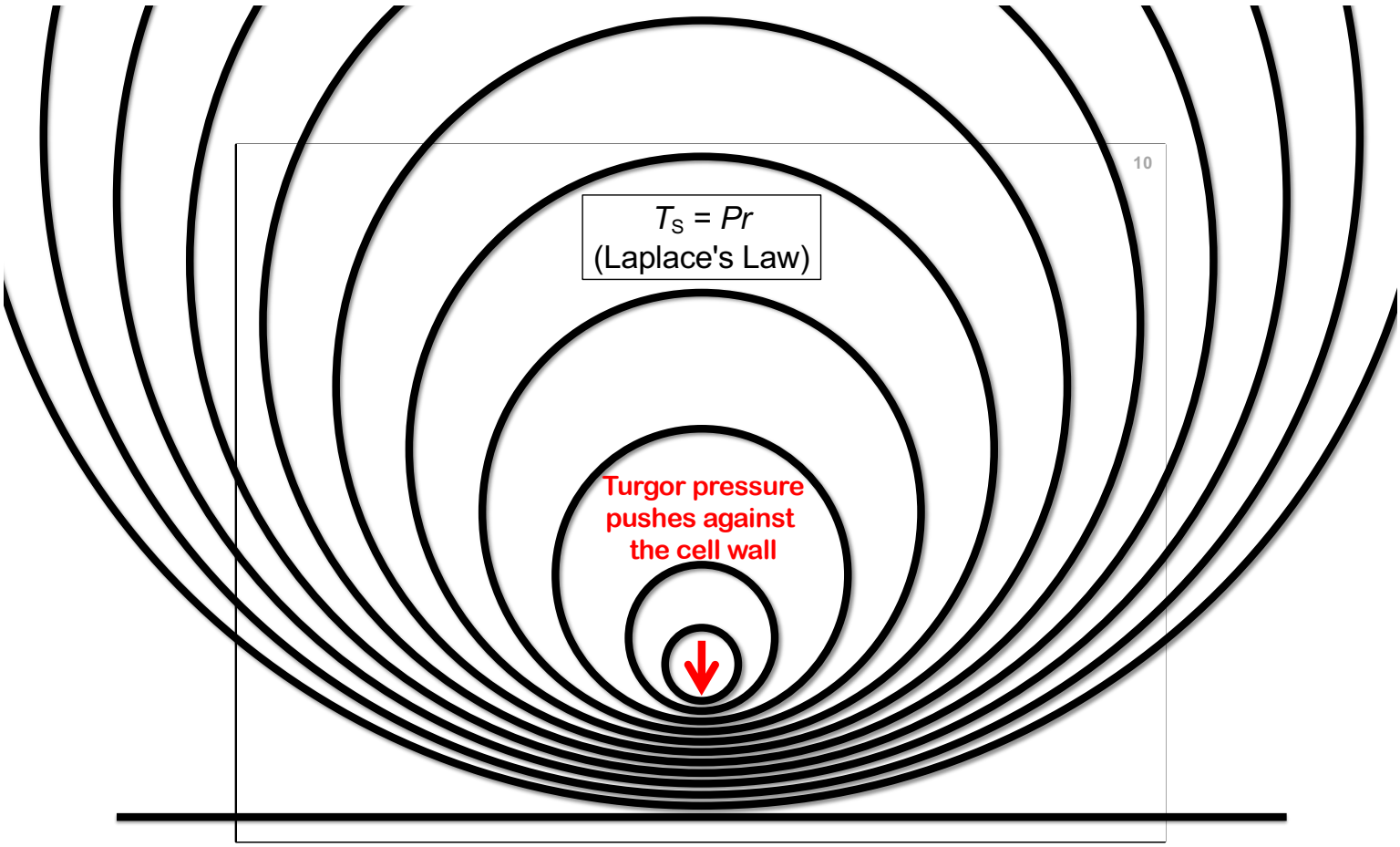


SOURCE: http://hyperphysics.phy-astr.gsu.edu/hbase/ptens.html

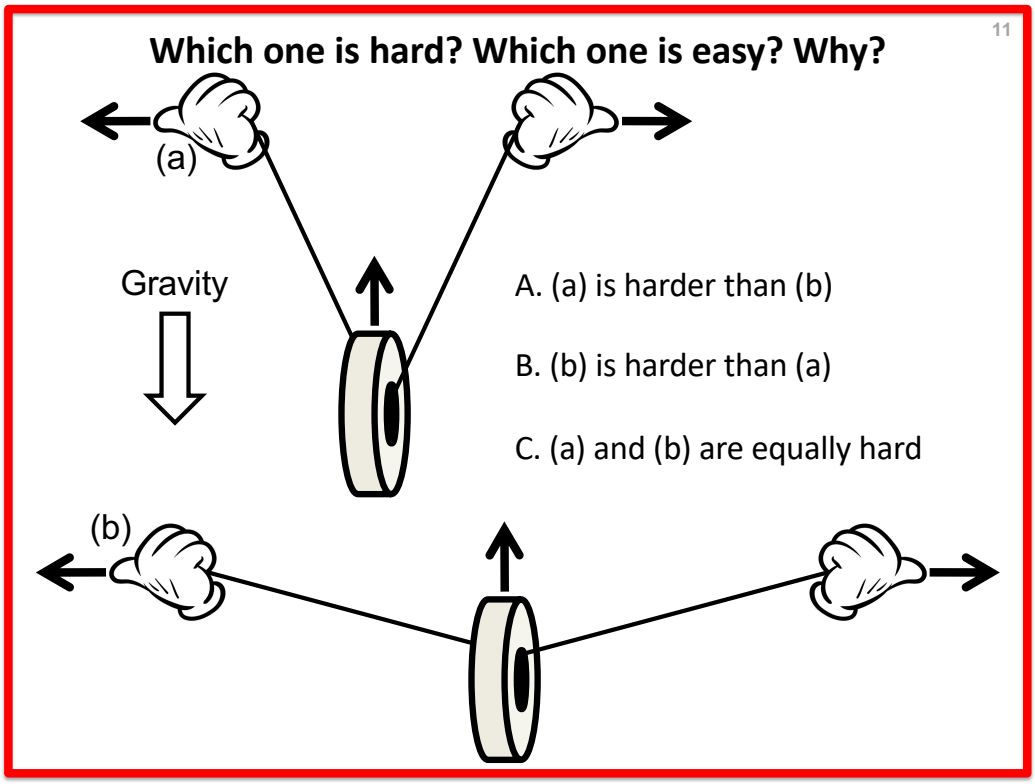
Small cells have a small radius of curvature. As cells get smaller and smaller, the cell wall approaches the vertical. Consequently, as cells get smaller the surface tension (T_S) in the cell wall has a larger and larger vertical component and a smaller and smaller horizontal component.

Large cells have a large radius of curvature. As cells get larger and larger, the cell wall approaches the horizontal. Consequently, as cells get larger the surface tension (T_S) in the cell wall has a smaller and smaller vertical component and a larger and larger horizontal component.

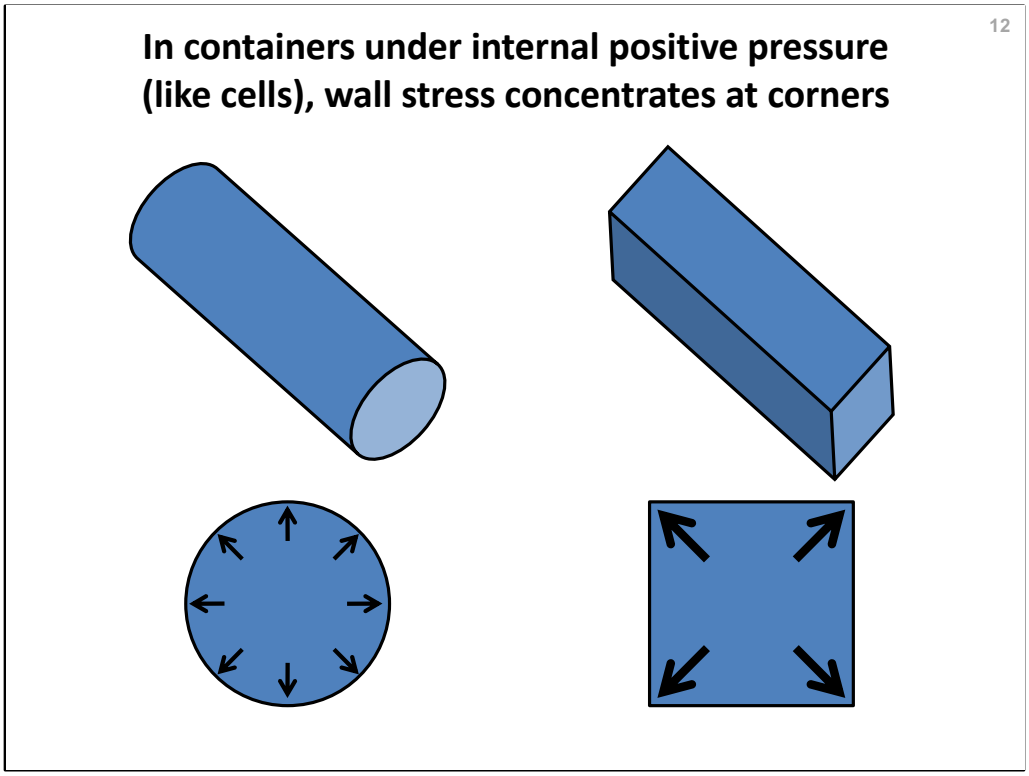
Osmotic pressure is opposed by tension in the cell wall that is equal in magnitude but opposite in direction. Osmotic pressure acts perpendicularly (vertically) to the cell wall (indicated by the red arrow). Thus, the only component of cell wall surface tension that is useful to oppose osmotic pressure is the vertical component. At a constant osmotic pressure, as cells get larger and larger a correspondingly larger and larger surface tension must be maintained in the cell wall to oppose osmotic pressure (because more and more of this tension is directed in the horizontal "not useful" direction). Cells rupture when the surface tension in the cell wall is larger than the structural elements of the cell wall can carry. This is why small cells are inherently more resistant to osmotic rupture than large cells.

Abbreviations:
T_S = surface tension, expressed in terms of N * m^{-1}. SI units: kg * s^{-2}
P = pressure, expressed in terms of N * m^{-2} (Pascals). SI units: kg * m^{-1} * s^{-2}
r = radius of curvature, expressed in terms of L. SI unit: m

The equation for Laplace's Law is a "primary concept" – you should memorize it and you should feel comfortable manipulating it.


11
Which one is hard? Which one is easy? Why?
(a)
Gravity
A. (a) is harder than (b)
B. (b) is harder than (a)
C. (a) and (b) are equally hard
(b)

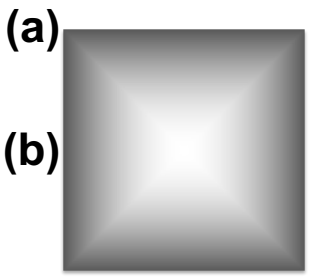
Answer: (B)



The arrows indicate the distribution of tension in the walls of pipes under internal positive pressure (pushing outwards on the wall of the pipe). In the round pipe (left), the **pressure** acting on the wall is the same everywhere and so is the **tension** in the wall, as every unit in the wall is geometrically equivalent. In the square pipe (right), the **pressure** acting on the wall is the same everywhere but the **tension** in the wall is not: the pressure-generated **stress is concentrated at the corners** because the sidewalls, being perpendicular to the direction in which pressure is exerted, cannot oppose the pressure. Stress concentration at corners is the reason why square pipes (or cells) tend to break at the corners under pressure.

13

Imagine a square-shaped cell (below). If the internal turgor pressure increases the cell will rupture:



A. At (a) because the turgor pressure is highest here.

B. At (a) because the cell-wall tension is highest here.

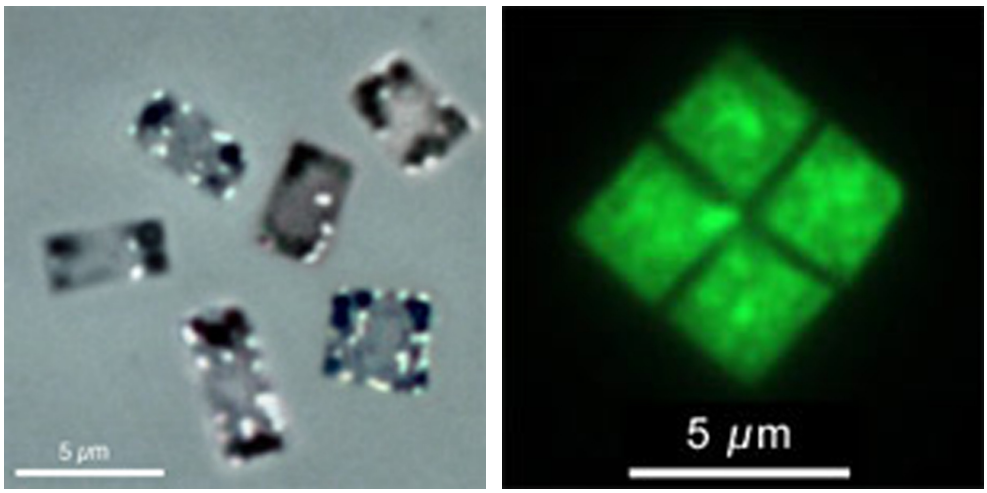
C. At (b) because the turgor pressure is highest here.

D. At (b) because the cell-wall tension is highest here.

Answer: (B)

14

Haloquadratum walsbyi (a halophile) thrives at very high salt concentrations (up to 2 M!)



Cellular dimensions: 3 μm × 3 μm × 0.1 μm

SOURCE: https://bionumbers.hms.harvard.edu/bionumber.aspx?id=104055&ver=3
SOURCE: https://socratic.org/questions/how-can-i-calculate-osmolarity-of-nacl

Question: Why is this organism (*Haloquadratum walsbyi*) able to tolerate right angles and corners?

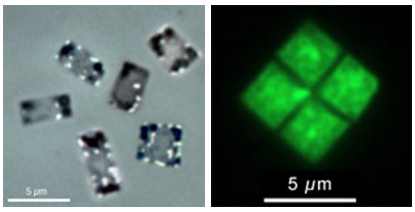
The osmolarity of the cytoplasm of a "typical" bacterium (Escherichia coli) is about **0.3 Osmol * L^{-1}**.

The osmolarity of a 2 M NaCl solution is **4 Osmol * L^{-1}** (twice the molarity because each molecule of NaCl dissociates to give two ions, Na^+ and Cl^-).

Thus, not only does *H. walsbyi* not have to deal with a positive internal osmotic pressure due to influx of water, it actually has the opposite problem – a negative internal osmotic pressure due to efflux of water (because water action is higher *inside* the cell than *outside* the cell). Consequently, there is no risk of stress concentration at the corners.

15

Round shapes (like spheres, cylinders) are common among microbes. Square shapes are rare. *Haloquadratum walsbyi* is a rare example of a square-shaped microbe (archaeon).



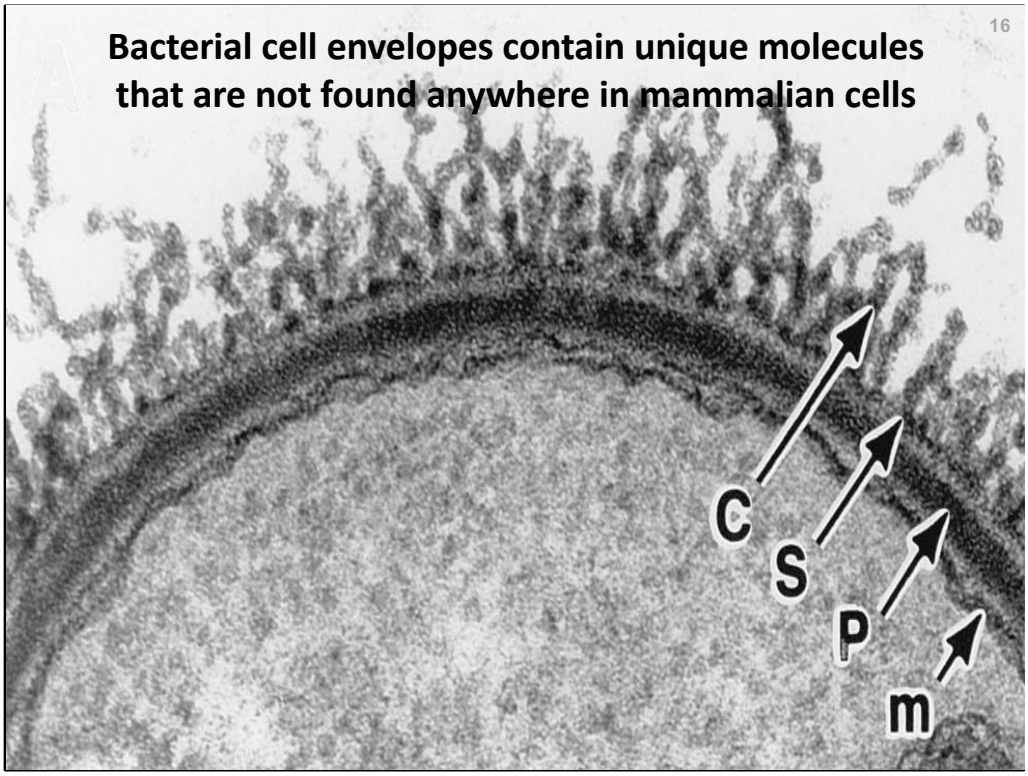
Which statement is true:

A. Turgor pressure is higher in square-shaped cells.

B. Square shapes are less vulnerable to turgor pressure.

C. *H. walsbyi* does not experience turgor pressure “in the wild” .

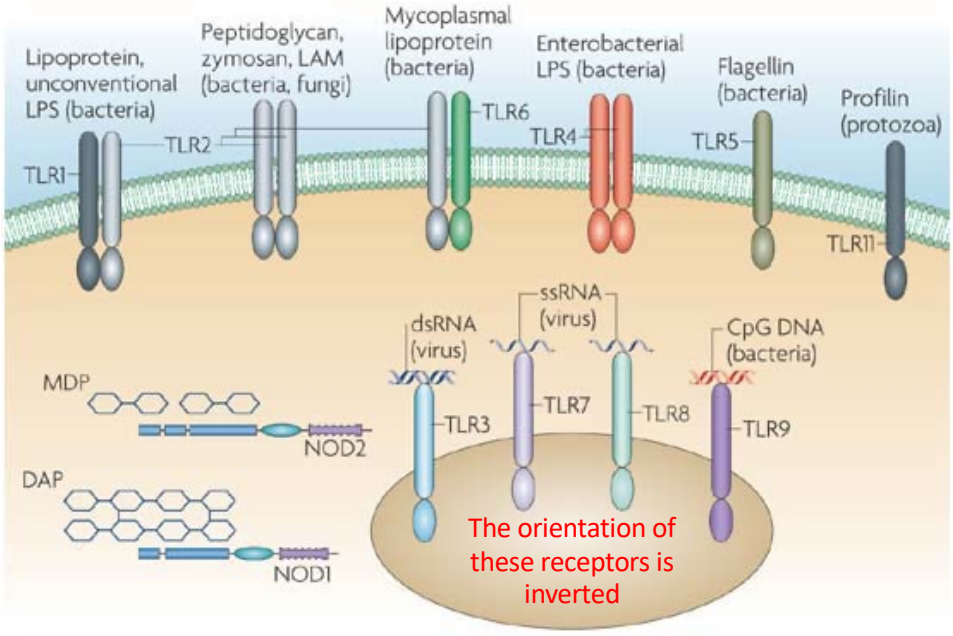
Answer: (C)



SOURCE: Mesnage S, Tosi-Couture E, Gounon P, Mock M, Fouet A (1998) The capsule and S-layer: two independent and yet compatible macromolecular structures in *Bacillus anthracis*. *J Bacteriol* 180(1): 52-58 PMID: 9422592.

Abbreviations:
C, capsule
S, S-layer
P, periplasmic space
M, cytoplasmic membrane

Bacterial cell wall components trigger innate immunity

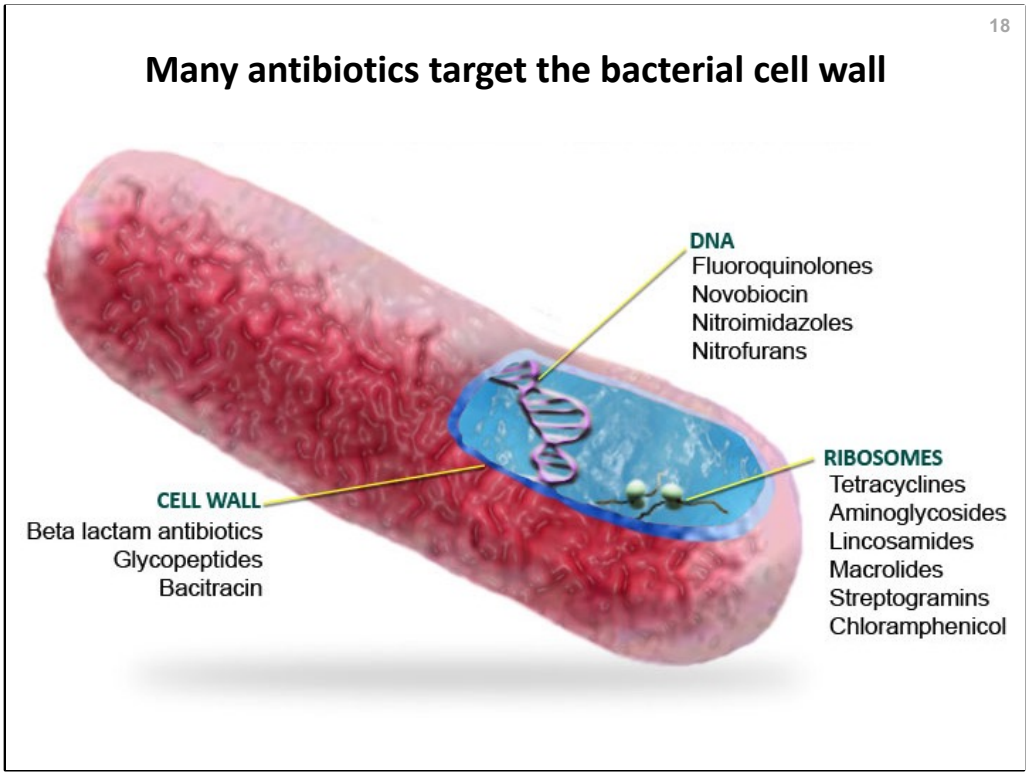


SOURCE: Kaufmann SHE (2007) The contribution of immunology to the rational design of novel antibacterial vaccines. *Nature Rev Microbiol* 5(7): 491-504 PMID:17558425.

FIGURE 2. Pattern-recognition receptors: Toll-like receptors (TLRs) and nucleotide oligomerization domain proteins (NODs). The figure focuses on the better-known pattern-recognition receptors (TLRs and NODs), leaving out the more recently described members of the expanding Nod-like receptor (NLR) family. Each type of receptor has a different ligand specificity, as indicated.

Abbreviations:
DAP, diaminopimelic acid (a component of cell wall peptidoglycan)
ds-RNA, double-stranded RNA (produced by some viruses)
MDP, muramyl dipeptide (a breakdown product of cell wall peptidoglycan)
LPS, lipopolysaccharide (a component of the Gram-negative outer membrane)
LAM, lipoarabinomannan (a component of the cell wall in Actinobacteria)
ss-RNA, single-stranded RNA (produced by some viruses)

You do not need to memorize the names of the various microbial ligands and their cognate host-cell receptors! The point of this slide is to illustrate that unique microbial products (i.e., molecules not found in host cells) are recognized by host immune receptors as a warning that "non-self alien invaders" are present. Binding of these "non-self" ligands to their cognate mammalian receptors triggers innate immune defenses that counteract the infection.



SOURCE: http://amrls.cvm.msu.edu/pharmacology/antimicrobials/mode-of-action

Different antibiotics have different modes of action, owing to the nature of their structure and degree of affinity to certain target sites within bacterial cells.

Cell wall synthesis. While the cells of humans and animals do not have cell walls, this structure is critical for the life and survival of bacteria. A drug that targets cell walls can therefore selectively kill or inhibit bacterial organisms. Examples: penicillins and cephalosporins (beta-lactam antibiotics); vancomycin (a glycopeptide antibiotic); bacitracin.

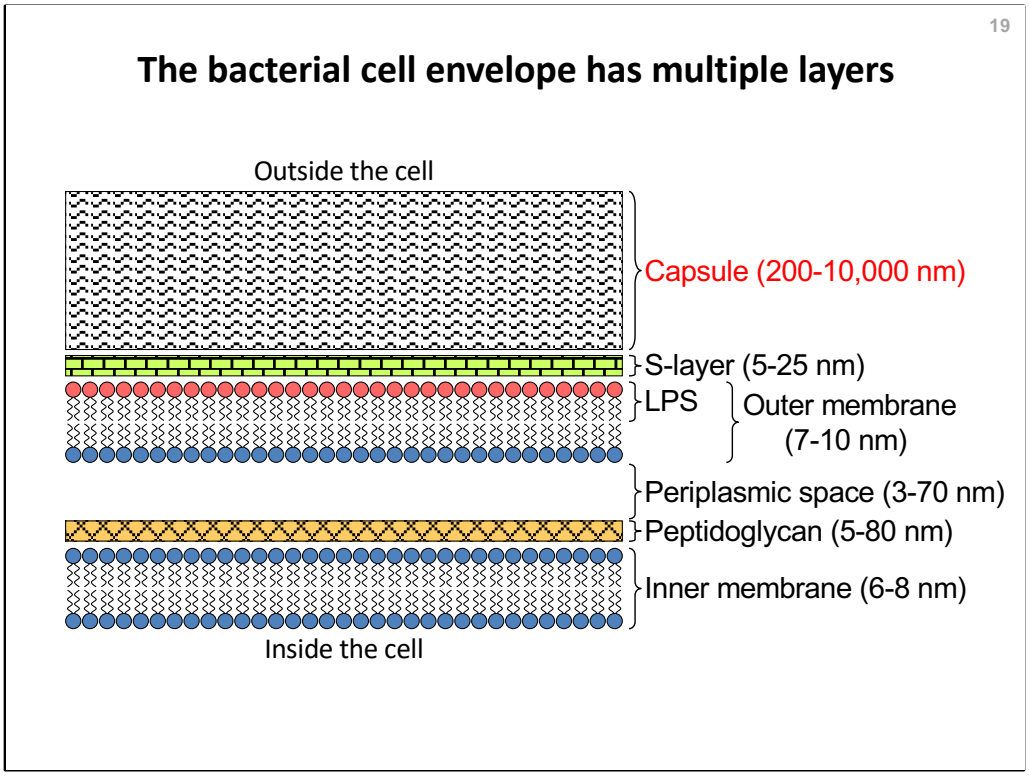
Cell membrane function. Cell membranes are important barriers that segregate and regulate the intra- and extra-cellular flow of substances. Disruption or damage to this structure could result in leakage of important solutes essential for the cell's survival. Because this structure is found in both eukaryotic and prokaryotic cells, the action of this class of antibiotic are often poorly selective and can be toxic for systemic use in the mammalian host. Most clinical usage is therefore limited to topical applications. Examples: polymixin B; colistin.

Protein synthesis. Protein synthesis is an essential process necessary for the multiplication and survival of all bacterial cells. Several types of antibacterial agents target bacterial protein synthesis by binding to either the 30S or 50S subunits of the intracellular ribosomes. This activity then results in the disruption of the normal cellular metabolism of the bacteria, and consequently leads to the death of the organism (**bactericidal**) or the inhibition of its growth and multiplication (**bacteriostatic**). Examples: aminoglycosides, macrolides, lincosamides, streptogramins, chloramphenicol, tetracyclines.

Nucleic acid synthesis. DNA and RNA are keys to the replication of all living forms, including bacteria. Some antibiotics work by binding to components involved in DNA or RNA synthesis, which will ultimately compromise bacterial multiplication and survival. Examples: quinolones; metronidazole; rifampin.

Other metabolic processes. Other antibiotics act on selected cellular processes essential for the survival of bacteria. For example, sulfonamides and trimethoprim disrupt the folic acid pathway, which is a necessary step for bacteria to produce precursors important for DNA synthesis. Sulfonamides inhibit dihydropteroate synthase; trimethophrim inhibits dihydrofolate reductase; both of these enzymes are essential for the production of folic acid, a vitamin synthesized by bacteria but not by humans.

You do not need to memorize the names of antibiotics or their specific molecular targets! But you should remember the general cellular *processes* that are inhibited by antibiotics.



Inner membrane (6-8 nm thick) - Lipid bilayer that serves three basic functions. (1) Selective permeability barrier. (2) Maintains proton motive force (PMF), source of energy to drive ATP synthesis and other cellular processes (e.g., flagellar rotation. (3) Anchoring site for cellular proteins involved in various cellular functions (e.g., transmembrane transport systems, flagellar motor; adhesive fimbriae and pili, etc.). We will discuss all of these functions in more detail later in the course.

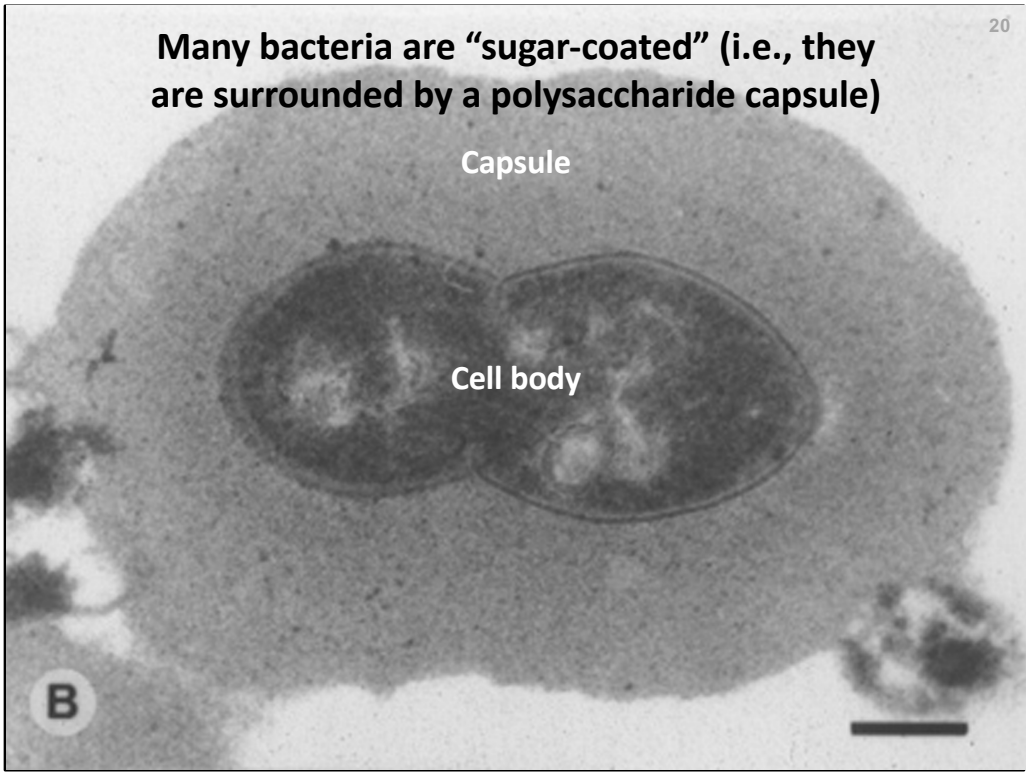
Peptidoglycan (5-10 nm thick in Gram-negative bacteria; 20-80 nm thick in Gram-positive bacteria) - Chains of alternating N-acetylglucosamine and N-acetylmuramicacid residues crosslinked by peptide bridges. Provides structural and osmotic support and maintains cell shape. Anchoring site for some exported proteins, especially in Gram-positive bacteria that lack a true periplasmic space. Porous.

Periplasmic space (30 nm thick at sidewalls, 70 nm thick at cell tips) - Fluid-filled space between the inner and outer membranes in Gram-negative bacteria. Location of cellular proteins involved in various functions (e.g., beta-lactamases that inactivate beta-lactam antibiotics before they can reach their targets at the peptidoglycan). Transient location of some secreted proteins after they cross the inner membrane but before they cross the outer membrane.

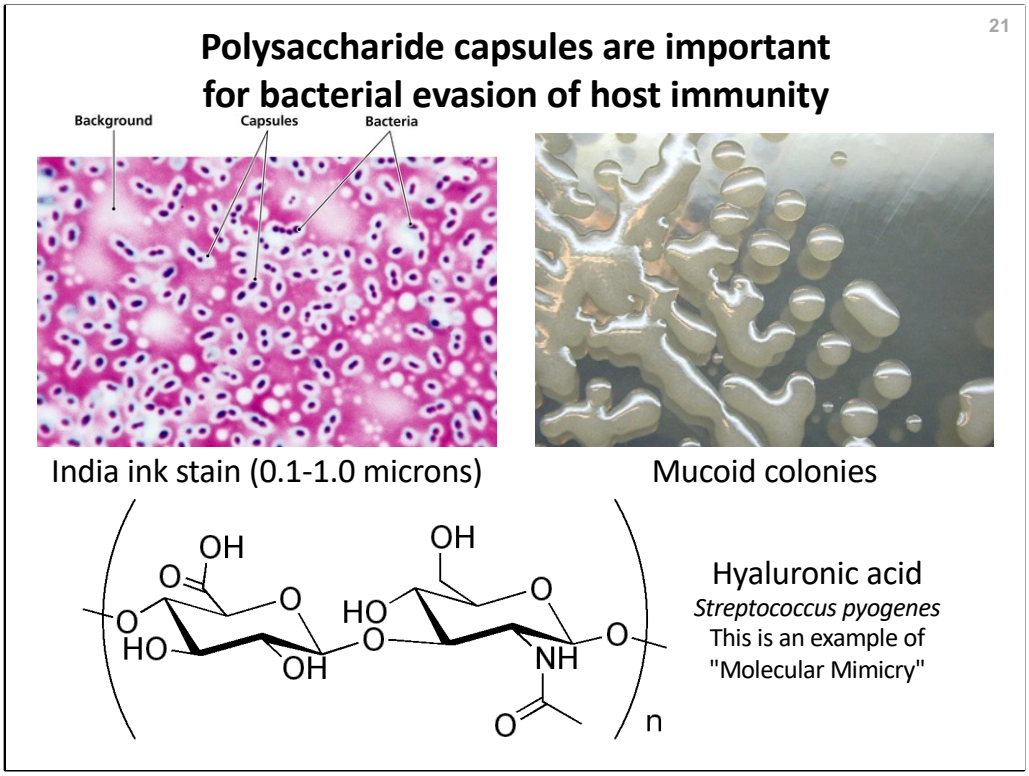
Outer membrane (7-10 nm thick) - Lipid bilayer that serves as a selective permeability barrier. The outer leaflet of the bilayer consists of **LPS** (**lipopolysaccharide**). The outer membrane is present in Gram-negative bacteria but absent in Gram-positive bacteria. The outer membrane is more permeable to small molecules than the inner membrane thanks to proteinaceous porins, which provide narrow water-filled channels across the outer membrane.

S layer (5-25 nm thick) - Crystalline array of a single glycoprotein. Provides structural support and helps maintain cell shape. Porous.

Capsule (200-10,000 nm thick) - Thick layer of polysaccharide. Protects against the immune system by interfering with complement deposition and phagocytosis of bacteria by macrophages and neutrophils (PMNs).



Transmission electron micrograph of *Streptococcus pneumoniae*, the organism that causes streptococcal pneumonia in humans. The dark (electron-dense) mass at the center is the cell body, which is in the process of elongating and dividing. The lighter layer surrounding the cell body is the polysaccharide capsule. Note that the capsule layer can be as thick as (or, in some cases, even thicker than) the cell body.



SOURCE: Madigan MT, Martinko JM, Stahl DA, Clark DP, Chapter 3: Cell structure and function in *Bacteria* and *Archaea*. In: *Brock Biology of Microorganisms [13th Edition]*, published by Pearson Education Inc., San Francisco, CA, pp. 64-65 © 2012.

Capsules may be thick or thin and rigid or flexible, depending on their chemistry and degree of hydration. If the material is organized in a tight matrix that excludes small particles, such as India ink (comprising particles measuring 0.1-1.0 microns in diameter), it is called a capsule. If the material is more easily deformed, it will not exclude particles and is more difficult to see; this form is called a slime layer. In addition, capsules are firmly attached to the cell wall, whereas slime layers are loosely attached and can be lost from the cell surface.

Polysaccharide layers have several functions in bacteria. Surface polysaccharides assist in the attachment of microorganisms to solid surfaces. Pathogenic microorganisms that enter the animal body by specific routes usually do so by first binding specifically to surface components of host tissues. This binding is often mediated by surface polysaccharides on the bacterial cell. Many nonpathogenic bacteria also bind to solid surfaces in nature, sometimes forming a thick layer of cells called a biofilm. Extracellular polysaccharides play a key role in the development of biofilms.

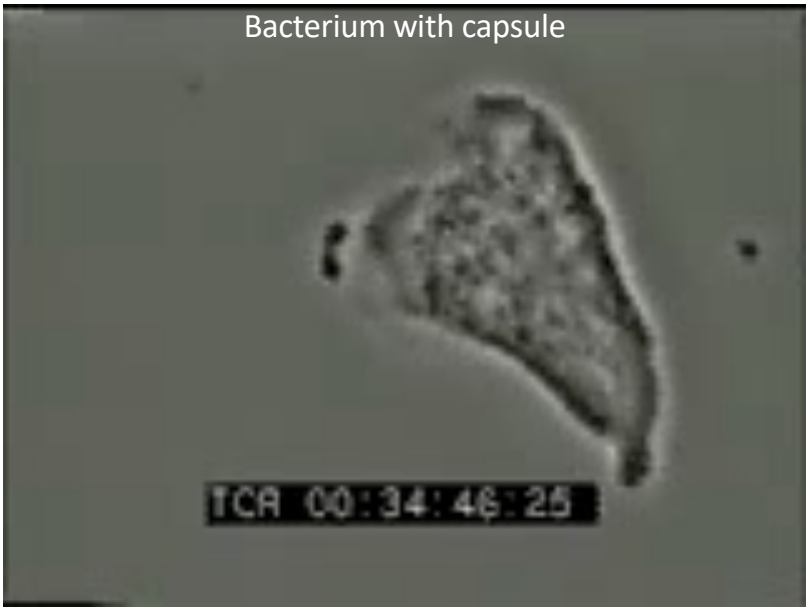
Polysaccharide outer layers play other roles as well. For example, encapsulated pathogenic bacteria are typically more difficult for phagocytic cells of the immune system to recognize and subsequently destroy. In addition, because outer polysaccharide layers bind a significant amount of water, it is likely that these layers play some role in resistance of the cell to desiccation.

22

Sugar-free bacteria are tastier to phagocytes…

SOURCE: http://www.microbelibrary.org/asmonly/details.asp?id=393&Lang=

…than sugar-coated bacteria



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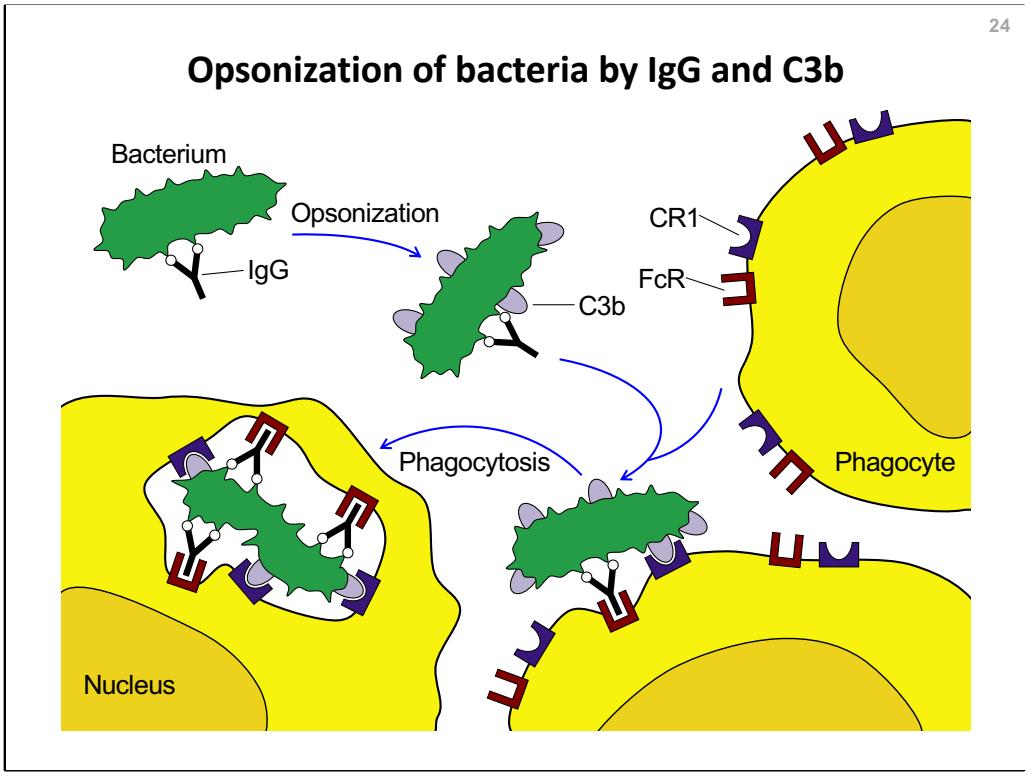
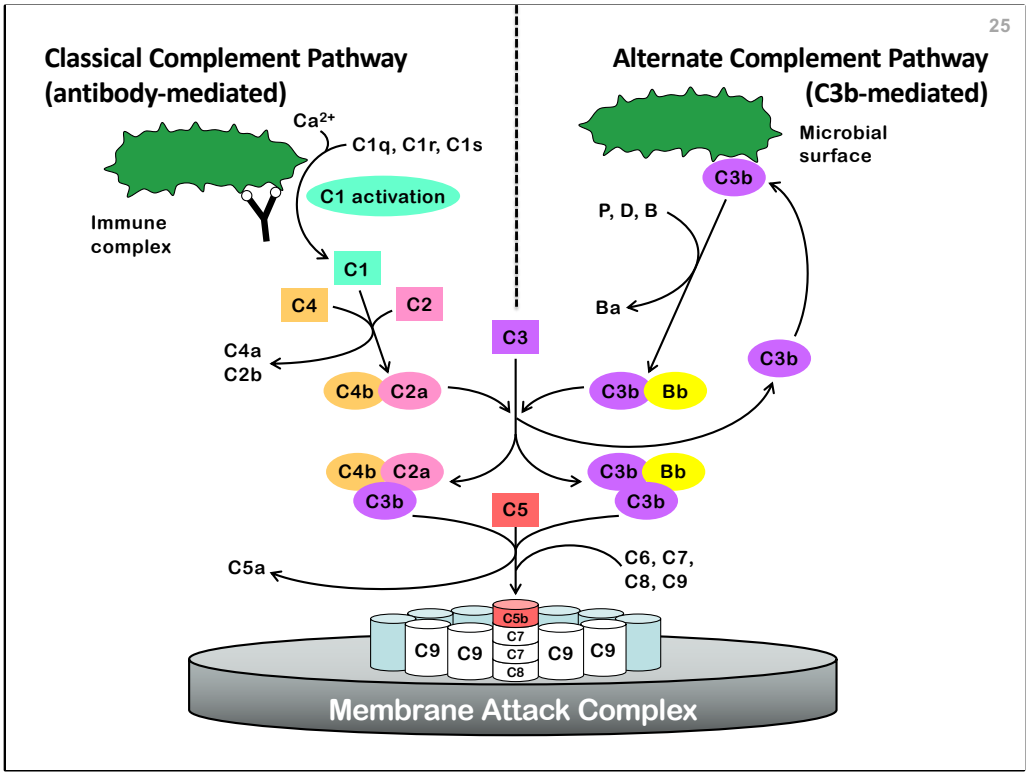


SOURCE: Janeway CA, Travers P, Walport M, Shlomchik M, *Immunobiology: The Immune System in Health and Disease [5th edition]*, published by Garland Publishing, New York, NY, USA p. 373 (Figure 9.32) © 2001.

Antibody (Fc) receptors and **complement (C3b) receptors** on phagocytes (macrophages and polymorphonuclear neutrophils) trigger the uptake and destruction of **opsonized** bacteria, that is, bacteria coated with **antibody** and **complement**. Many bacteria resist phagocytosis by macrophages and neutrophils. Antibodies bound to these bacteria, however, enable them to be ingested and degraded through interaction of the multiple Fc domains arrayed on the bacterial surface with Fc receptors (FcR) on the phagocyte surface. Antibody coating also induces activation of the complement system and the binding of complement components (for example **C3b**) to the bacterial surface. These can interact with complement receptors (for example CR1) on the phagocyte. **Fc receptors** and **C3b receptors** synergize to induce phagocytosis. Bacteria coated with IgG antibody and C3b complement are therefore more readily ingested than those coated with IgG or C3b alone. Binding of Fc receptors and C3b receptors signals the phagocyte to increase the rate of phagocytosis, fuse lysosomes with phagosomes, and increase its bactericidal activities.



SOURCE: Schaechter M, Engleberg NC, Eisenstein BI, Medoff G, *Mechanisms of Microbial Disease [3rd Edition]*, published by Williams & Wilkins, Baltimore, MA USA, p. 67 (Figure 6.1) © 1998.

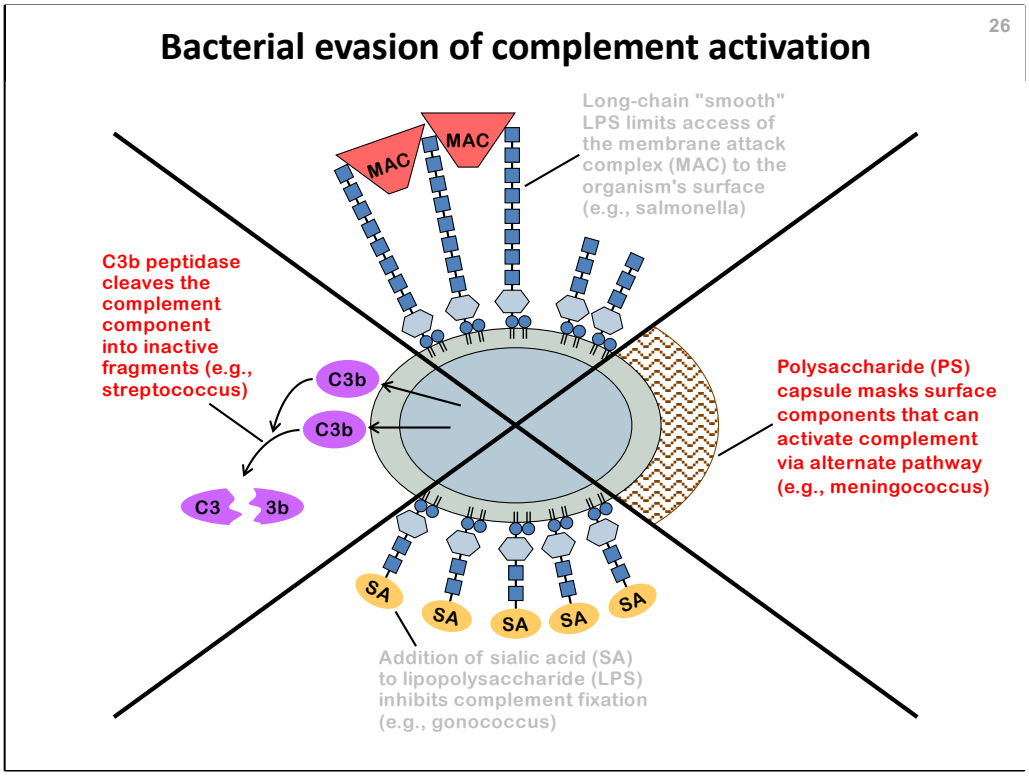
Activation of the **Complement System** via the classical and alternate pathways. The **classical pathway** is triggered by binding of antigen-specific **antibody** to the bacterial surface. The **alternative pathway** is triggered by direct binding of **C3b** protein (a complement component) to structures on the bacterial surface. Both pathways activate a proteolytic cascade that ultimately results in assembly of the **membrane attack complex (MAC)** on the bacterial cell surface. The MAC punches holes through the bacterial cell wall, resulting in death and lysis of the bacterial cell.

One of the major host defense mechanisms against bacterial infection involves the Complement System, which can be **bactericidal** through the generation of the **membrane attack complex** or **opsonic** (signaling recognition and engulfment by professional phagocytic cells) when complement components are activated on the bacterial cell surface.

You do not need to memorize the "complement cascade" pathway or the names of all the different complement components involved!

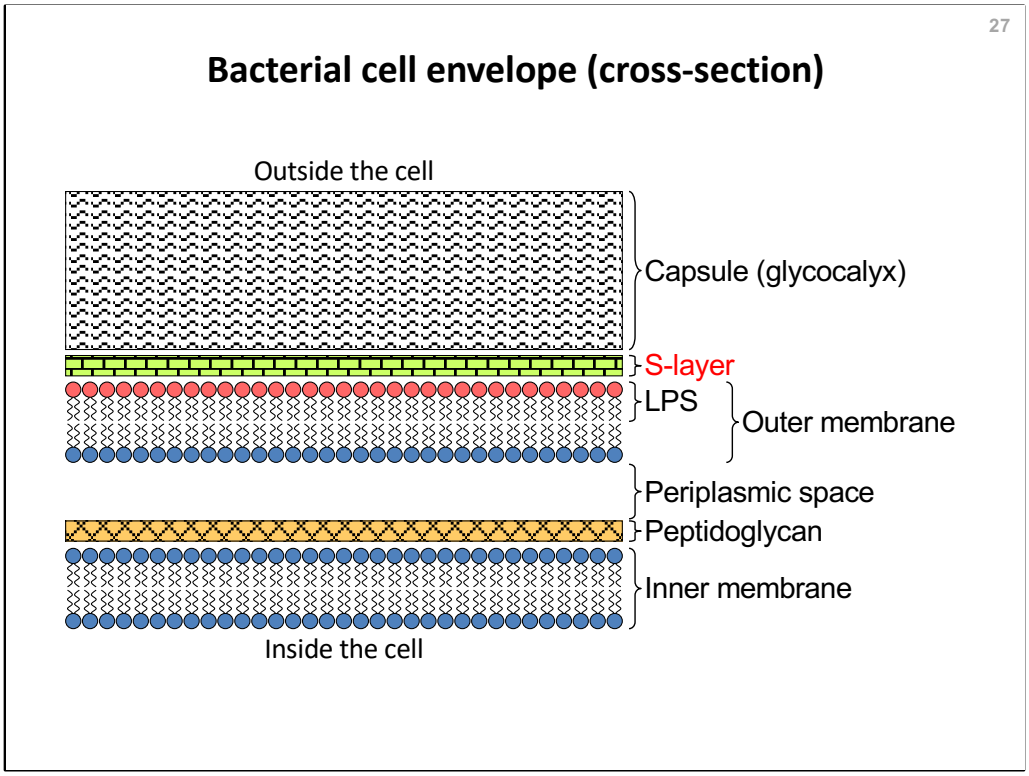
However, you should remember that there are two distinct pathways leading to assembly of the MAC: the **"classical" pathway** mediated by binding of antibody (IgG) to the bacterial cell surface, and **the "alternate" pathway** mediated by binding of a **complement component (C3b)** to the bacterial cell surface. You should also remember that either event (binding of IgG or C3b to the bacterial cell surface) activates a proteolytic cascade that ultimately results in assembly of the **membrane attack complex (MAC)** on the bacterial cell surface.

There is a third pathway for complement activation on bacterial surfaces, the **"mannan-binding lectin" pathway**, which is not depicted here. For our purposes in this course, you don't need to know about it. ☺



SOURCE: Schaechter M, Engleberg NC, Eisenstein BI, Medoff G, *Mechanisms of Microbial Disease [3rd Edition]*, published by Williams & Wilkins, Baltimore, MA USA, p. 108 (Figure 8.1) © 1998.

Microbial strategies for evasion of complement activation and assembly of the membrane attack complex (MAC) on the bacterial cell surface.



Inner membrane (6-8 nm thick) - Lipid bilayer that serves three basic functions. (1) Selective permeability barrier. (2) Maintains proton motive force (PMF), source of energy to drive ATP synthesis and other cellular processes (e.g., flagellar rotation). (3) Anchoring site for cellular proteins involved in various cellular functions (e.g., transmembrane transport systems, flagellar motor; adhesive fimbirae, etc.).

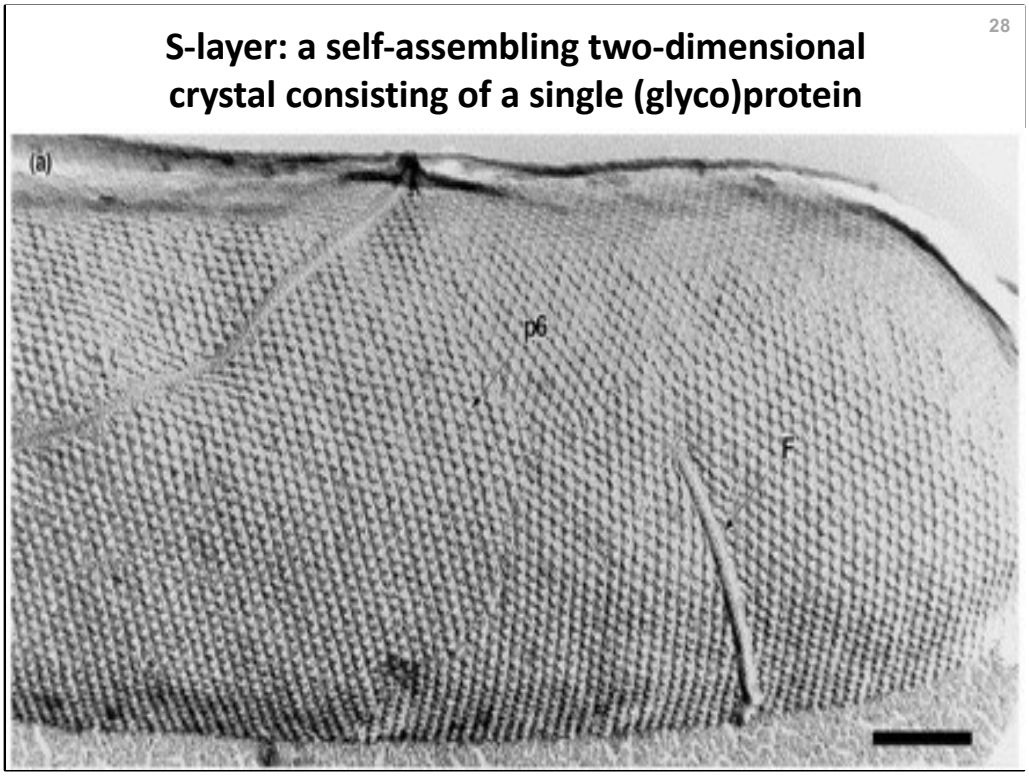
Peptidoglycan (5-10 nm thick in Gram-negative bacteria; 20-80 nm thick in Gram-positive bacteria) - Chains of alternating N-acetylglucosamine and N-acetylmuramicacid residues crosslinked by peptide bridges. Provides structural and osmotic support and maintains cell shape. Anchoring site for some exported proteins, especially in Gram-positive bacteria that lack a true periplasmic space. Porous.

Periplasmic space (30 nm thick at sidewalls, 70 nm thick at cell tips) - Fluid-filled space between the inner and outer membranes in Gram-negative bacteria. Location of cellular proteins involved in various functions (e.g., beta-lactamases that inactivate beta-lactam antibiotics before they can reach their targets at the peptidoglycan). Transient location of some secreted proteins after they cross the inner membrane but before they cross the outer membrane.

Outer membrane (7-10 nm thick) - Lipid bilayer that serves as a selective permeability barrier. The outer leaflet of the bilayer consists of **LPS** (**lipopolysaccharide**). Present in Gram-negative bacteria but absent in Gram-positive bacteria. The outer membrane is more permeable to small molecules than the inner membrane thanks to porins, which provide narrow water-filled channels across the outer membrane.

S layer (5-25 nm thick) - Crystalline array of a single glycoprotein. Provides structural support and helps maintain cell shape. Porous.

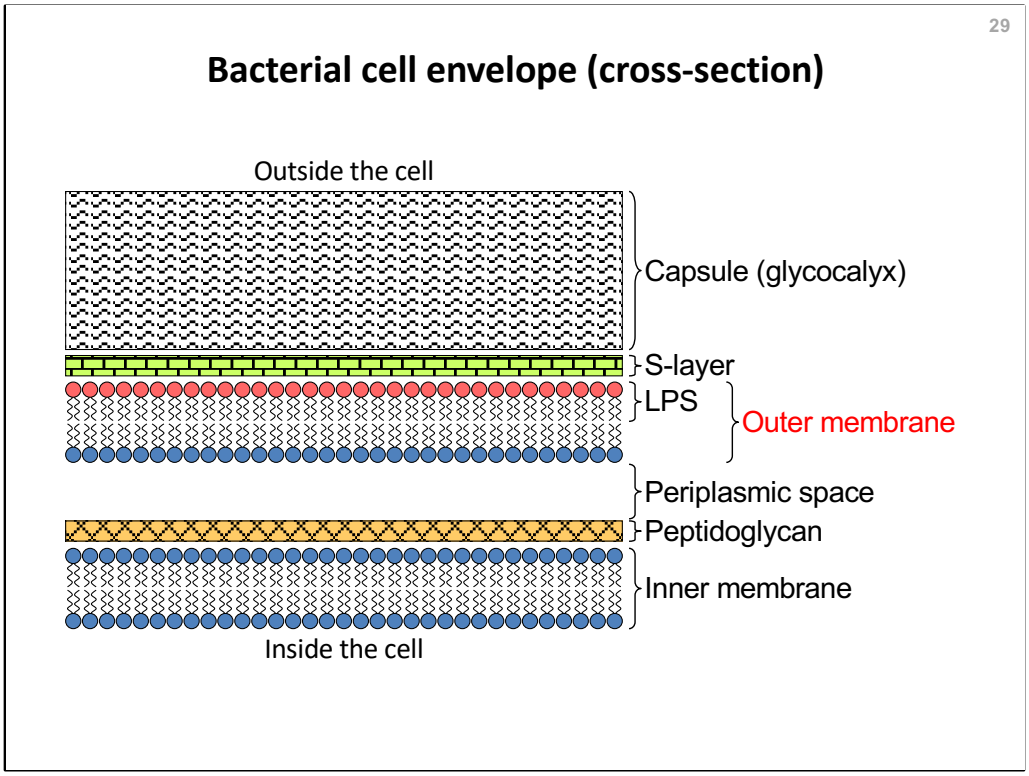
Capsule (200-10,000 nm thick) - Thick layer of polysaccharide. Protects against the immune system by interfering with complement deposition and phagocytosis of bacteria by macrophages and neutrophils (PMNs).



SOURCE: http://www.foresight.org/conference/MNT7/Papers/Pum/index.html
SOURCE: http://strubi.uni-graz.at/projects/s-layer.html.

FIGURE 1. Transmission electron micrograph of a freeze-etched, metal shadowed preparation of a bacterial cell with an S-layer with hexagonal lattice symmetry. Bar, 100 nm. S-layer proteins form the outermost cell envelope component of a broad spectrum of bacteria and archaea. S-layers are composed of a single protein or glycoprotein species (MW 40-200 kDa) and exhibit either oblique, square, or hexagonal lattice symmetry with unit cell dimensions in the range of 3 to 30 nm. S-layers are generally 5 to 10 nm thick and show pores of identical size (diameter, 2-8 nm) and morphology. Crystalline bacterial cell surface layer (S-layer) proteins have been optimized during billions of years of biological evolution as building blocks of one of the simplest self-assembly systems. This is due to the fact that S-layer proteins have the intrinsic property to reassemble into two-dimensional arrays on surfaces of a broad spectrum of materials (e.g., silicon wafers, metals, polymers) and interfaces (e.g., planar lipid films or liposomes). The arrangement of functional domains on each S-layer unit cell is repeated with the periodicity of the S-layer lattice at a distance of approximately 10 nm, enabling the formation of regular arrays of bound molecules and particles, a property that is generating applications in the biotechnology industry.

Functions of the S-layer:
- protection against bacteriophages, Bdellovibrios (“killer bacteria”), and phagocytosis
- resistance against acidic pH
- barrier against potentially damaging high-molecular-weight substances (e.g., bacteriolytic enzymes)
- adhesion to surfaces (for glycosylated S-layers)
- stabilization of the membrane
- provision of adhesion sites for exoproteins
- periplasmic compartment in Gram-positive bacteria together with the peptidoglycan and the cytoplasmic membrane



Inner membrane (6-8 nm thick) - Lipid bilayer that serves three basic functions. (1) Selective permeability barrier. (2) Maintains proton motive force (PMF), source of energy to drive ATP synthesis and other cellular processes (e.g., flagellar rotation). (3) Anchoring site for cellular proteins involved in various cellular functions (e.g., transmembrane transport systems, flagellar motor; adhesive fimbirae, etc.).

Peptidoglycan (5-10 nm thick in Gram-negative bacteria; 20-80 nm thick in Gram-positive bacteria) - Chains of alternating N-acetylglucosamine and N-acetylmuramicacid residues crosslinked by peptide bridges. Provides structural and osmotic support and maintains cell shape. Anchoring site for some exported proteins, especially in Gram-positive bacteria that lack a true periplasmic space. Porous.

Periplasmic space (30 nm thick at sidewalls, 70 nm thick at cell tips) - Fluid-filled space between the inner and outer membranes in Gram-negative bacteria. Location of cellular proteins involved in various functions (e.g., beta-lactamases that inactivate beta-lactam antibiotics before they can reach their targets at the peptidoglycan). Transient location of some secreted proteins after they cross the inner membrane but before they cross the outer membrane.

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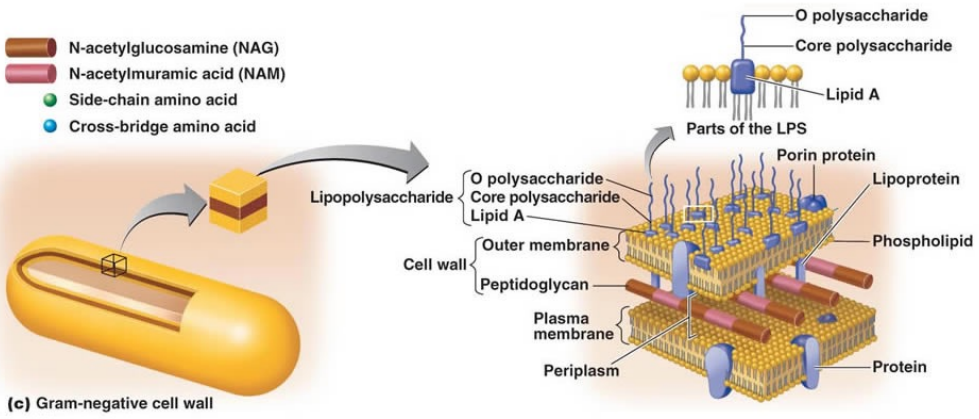
Capsule (200-10,000 nm thick) - Thick layer of polysaccharide. Protects against the immune system by interfering with complement deposition and phagocytosis of bacteria by macrophages and neutrophils (PMNs).

Gram-negative bacterial cell envelope

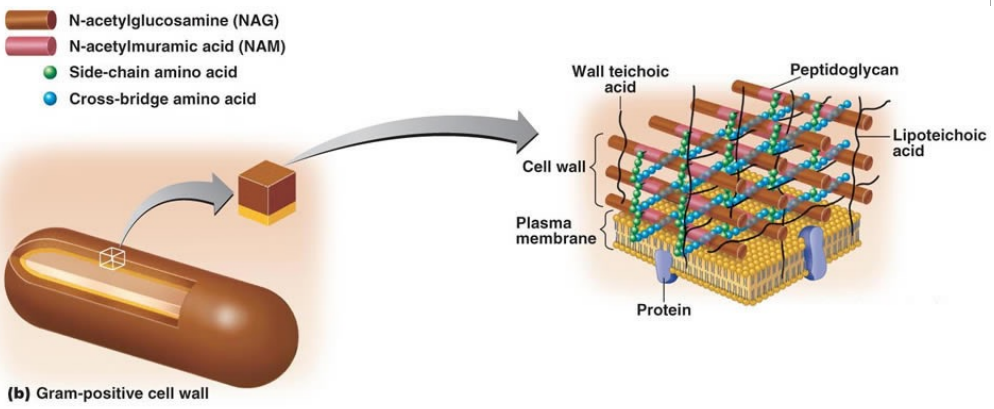


Gram-negative bacteria display the following characteristics:
1. Inner cell membrane (a.k.a. cytoplasmic membrane).
2. Outer cell membrane: the inner leaflet contains phospholipids; the outer leaflet contains lipopolysaccharide (LPS), which consists of lipid A, core polysaccharide, and O antigen.
3. Thin peptidoglycan (usually a single layer).
4. Porins in the outer membrane act as pores for selective trans-membrane transport of specific molecules.
5. The periplasmic space between the inner and outer membranes is filled with a concentrated gel-like material called periplasm.
6. The S-layer is attached to the outer membrane.
7. If present, the flagellae have four supporting rings.
8. Teichoic acids and lipoteichoic acids are absent.
9. Lipoproteins are attached to the polysaccharide backbone of the peptidoglycan.
10. Gram-negative bacteria do not form spores (with very few exceptions).

Gram-positive bacteria display the following characteristics:
1. Inner cell membrane (a.k.a. cytoplasmic membrane).
2. Outer cell membrane and lipopolysaccharide are absent.
3. Thick peptidoglycan (multiple layers).
4. Porins are absent (because there is no outer membrane).
5. Periplasmic space is absent (because there is no outer membrane).
6. The S-layer is attached directly to the peptidoglycan.
7. If present, the flagellae have two supporting rings.
8. Teichoic acids and lipoteichoic acids are present.
9. Lipoproteins are attached to the polysaccharide backbone of the peptidoglycan.
10. Some Gram-positive bacteria can form spores.

You don't need to memorize this information, it's just to help you understand some of the fundamental differences between Gram-negative and Gram-positive bacteria.

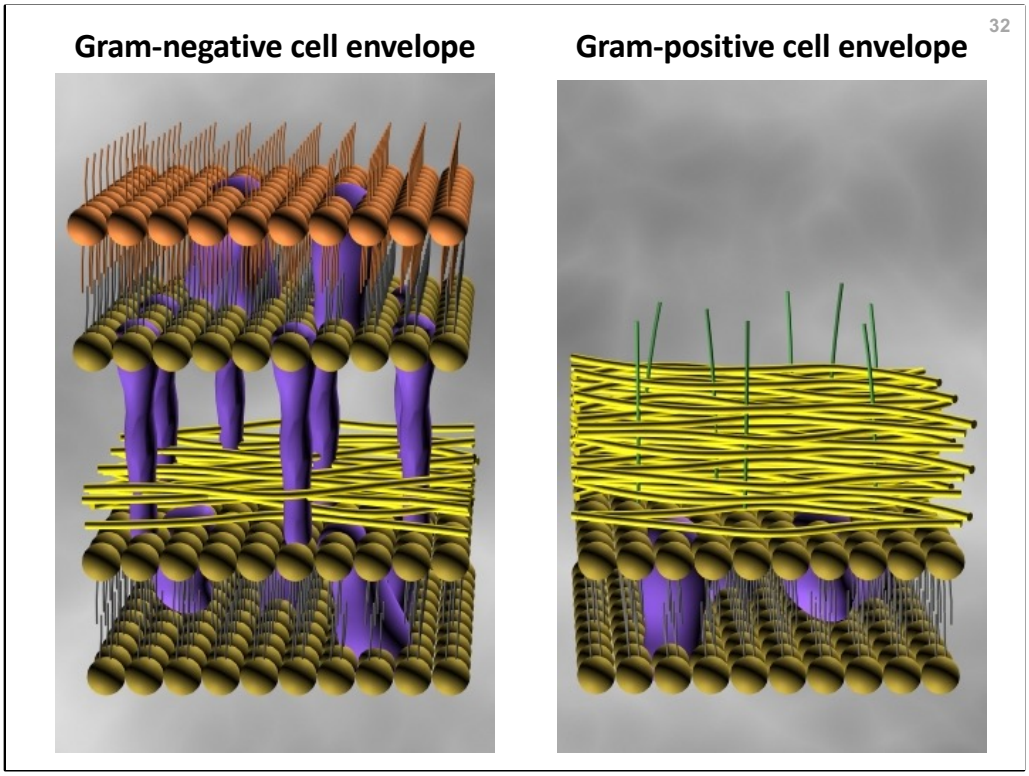
Gram-positive bacterial cell envelope



Gram-negative bacteria display the following characteristics:
1. Inner cell membrane (a.k.a. cytoplasmic membrane).
2. Outer cell membrane: the inner leaflet contains phospholipids; the outer leaflet contains lipopolysaccharide (LPS), which consists of lipid A, core polysaccharide, and O antigen.
3. Thin peptidoglycan (usually a single layer).
4. Porins in the outer membrane act as pores for selective trans-membrane transport of specific molecules.
5. The periplasmic space between the inner and outer membranes is filled with a concentrated gel-like material called periplasm.
6. The S-layer is attached to the outer membrane.
7. If present, the flagellae have four supporting rings.
8. Teichoic acids and lipoteichoic acids are absent.
9. Lipoproteins are attached to the polysaccharide backbone of the peptidoglycan.
10. Gram-negative bacteria do not form spores (with very few exceptions).

Gram-positive bacteria display the following characteristics:
1. Inner cell membrane (a.k.a. cytoplasmic membrane).
2. Outer cell membrane and lipopolysaccharide are absent.
3. Thick peptidoglycan (multiple layers).
4. Porins are absent (because there is no outer membrane).
5. Periplasmic space is absent (because there is no outer membrane).
6. The S-layer is attached directly to the peptidoglycan.
7. If present, the flagellae have two supporting rings.
8. Teichoic acids and lipoteichoic acids are present.
9. Lipoproteins are attached to the polysaccharide backbone of the peptidoglycan.
10. Some Gram-positive bacteria can form spores.

You don't need to memorize this information, it's just to help you understand some of the fundamental differences between Gram-negative and Gram-positive bacteria.



Diagrams of the cell wall structures of **Gram-negative bacteria** (left) and **Gram-positive bacteria** (right). Key: peptidoglycan layer (yellow); protein (purple); teichoic acid (green); phospholipid (brown); lipopolysaccharide (orange). Note that the peptidoglycan layer is much thicker in Gram-positive bacteria.

Although the Gram-staining response is an empirical criterion, its basis lies in the marked differences in the ultrastructure and chemical composition of the bacterial cell wall.

Gram-negative ("diderm" or "two-skins") bacteria are bounded by a cytoplasmic membrane plus an outer cell membrane. They contain a thin layer of peptidoglycan (2-3 nm) between these membranes. The presence of inner and outer cell membranes defines a new compartment in these cells, the *periplasmic space* or *periplasmic compartment*.

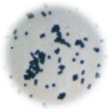
Gram-positive ("monoderm" or "one-skin") bacteria are bounded by a cytoplasmic membrane. They contain a thick layer (20-80 nm) of peptidoglycan that is responsible for retaining the Gram stain. A number of bacteria that are bounded by a single membrane but stain Gram-negative due to lack of the peptidoglycan layer (e.g., mycoplasmas) or their inability to retain the Gram stain because of their cell wall composition also show close relationship to the Gram-positive bacteria.

33

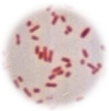
The Gram staining method

Invented by Hans Christian Gram (1882)

1. Stain cells with crystal violet (or methylene blue).
2. Fix dye with iodine.
3. Decolorize with ethanol + acetone (strips outer membrane).
4. Counter-stain with basic fuchsin (or safranin).
5. Mount stained cells and observe with light microscope:



Gram-positive cells appear purple (crystal violet).



Gram-negative cells appear pink (fuchsin).

SOURCE: http://www.uphs.upenn.edu/bugdrug/antibiotic_manual/Gram1.htm
SOURCE: https://www.ncbi.nlm.nih.gov/books/NBK562156/

The **Gram staining method** is named after the Danish bacteriologist Hans Christian Gram, who originally devised it in 1882. It is one of the most important staining techniques in microbiology and it is almost always the first test performed for the identification of bacteria. The primary stain is crystal violet. Bacteria that are stained by crystal violet are classified as **Gram-positive** and appear purple. Bacteria that are not stained by crystal violet are referred to as **Gram-negative** and appear pink. Gram staining is based on the ability of the bacterial cell wall to retain crystal violet dye during solvent treatment. Cell walls of Gram-positive bacteria have a higher peptidoglycan and lower lipid content than Gram-negative bacteria. Bacteria cell walls are stained by the crystal violet. Iodine is added as a mordant to form a crystal violet-iodine complex so that the dye cannot be removed easily. This step is commonly referred to as "fixing the dye". Subsequent treatment with a decolorizer (ethanol and acetone) dissolves the outer membrane from the Gram-negative cells. The removal of the outer membrane enhances the leaching of the crystal violet. In contrast, the decolorizer dehydrates the thicker Gram-positive cell walls, closing the pores as the cell wall shrinks during dehydration. As a result, diffusion of the violet-iodine complex is blocked and the bacteria remain stained. Finally, a counter-stain of basic fuchsin is applied to give decolorized Gram-negative bacteria a pink color. The polychromatic nature of the Gram stain enables determination of the size and shape of both Gram-negative and Gram-positive bacteria. Besides Gram stain, there are a wide range of other staining methods available. By using appropriate dyes, structures such as capsules, flagella, granules, and spores can be stained. Staining is used to visualize components that are otherwise too difficult to see by light microscopy. Also, special stains can be used to visualize other microorganisms not readily visualized by the Gram stain, such as actinobacteria, rickettsia, spirochetes, etc.

You do not need to memorize the Gram staining procedure! However, you should know how this method distinguishes between Gram-positive and Gram-negative bacteria (presence or absence of an outer membrane; thin or thick peptidoglycan layer).

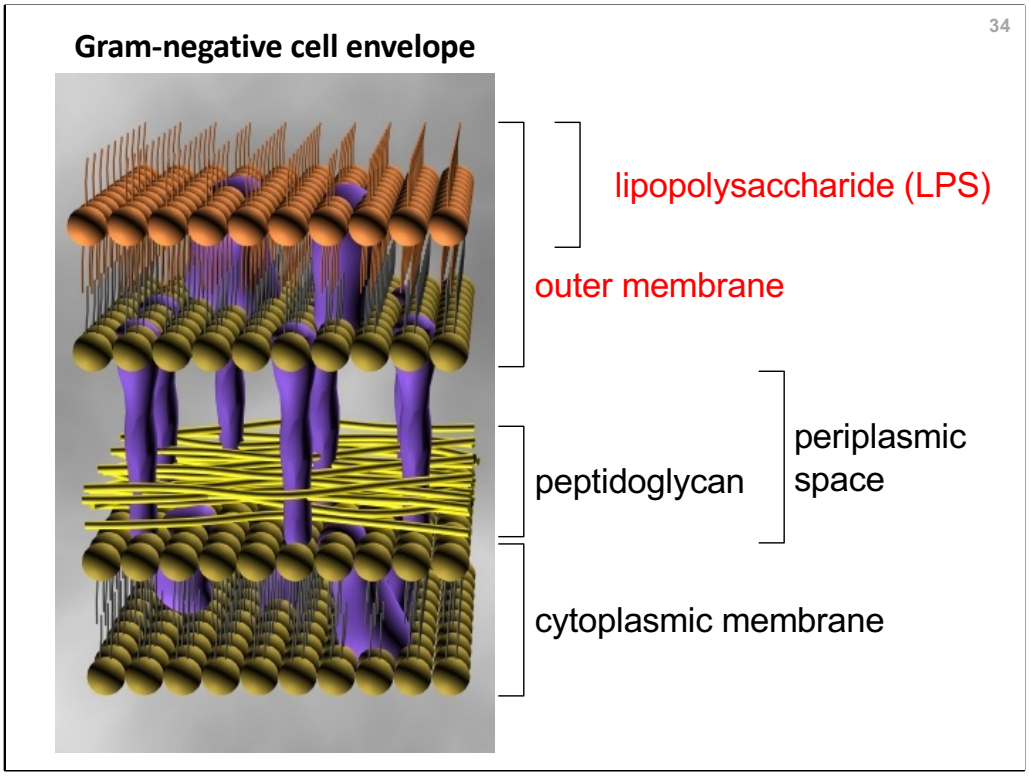
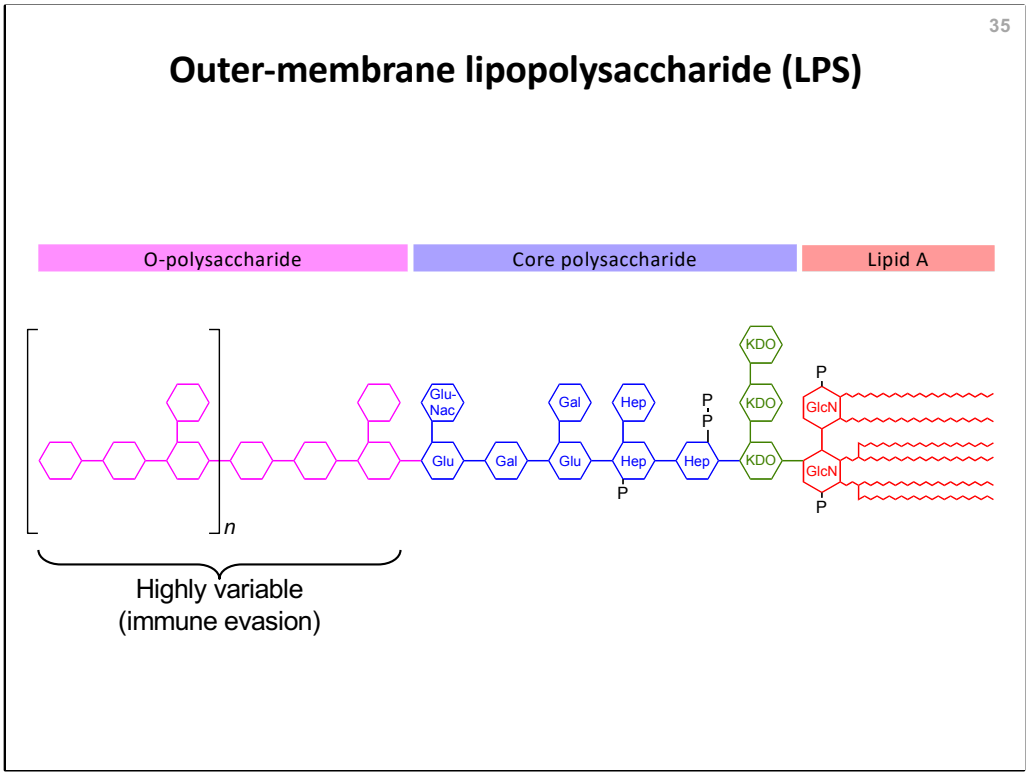


Diagram of the cell wall structure of **Gram-negative bacteria**. Key: peptidoglycan layer (yellow); proteins (purple); phospholipid (brown); lipopolysaccharide (orange).



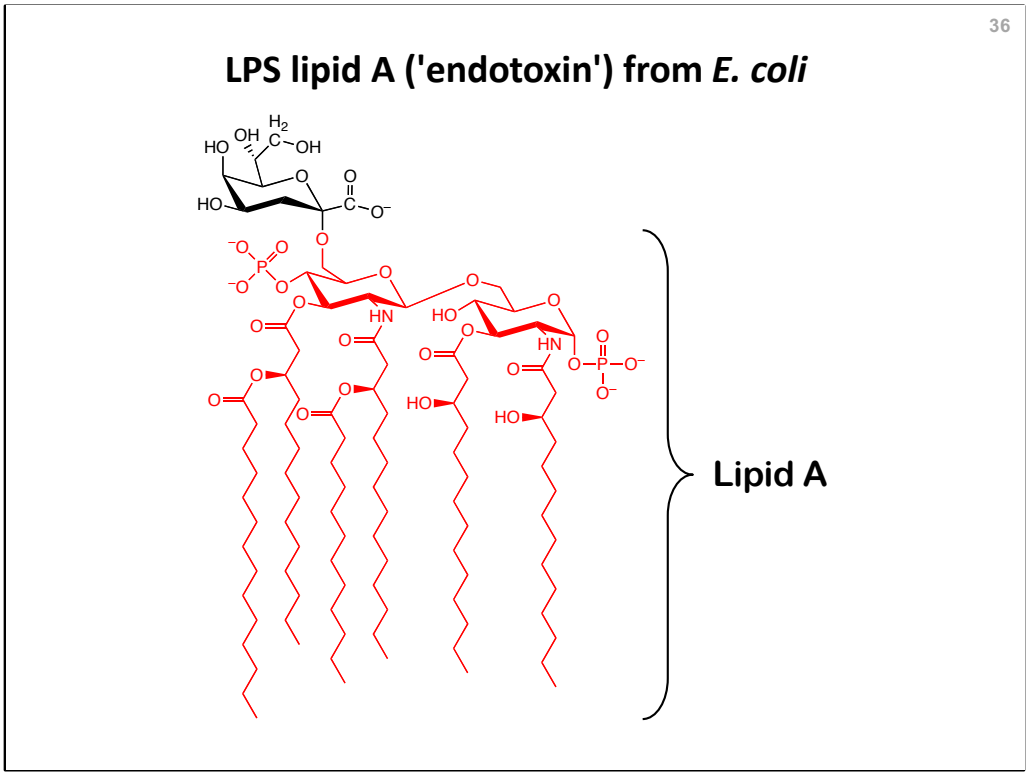
SOURCE: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) Chapter 3: Cell structure and function in *Bacteria* and *Archaea*. In: *Brock Biology of Microorganisms [13th Edition]*. Pearson Education Inc., San Francisco, CA, pp. 60-62.

FIGURE 3.19. Structure of the **lipopolysaccharide (LPS)** of **Gram-negative bacteria**. The chemistry of lipid A and the polysaccharide components varies among species of Gram-negative bacteria, but the overall structural organization (lipid A – KDO – Core – O-specific) is usually the same. The **O-polysaccharide** varies greatly among species. Glucosamine (GlcN) and the **lipid A** fatty acids are linked through the amine group. The lipid A portion of LPS can be highly toxic to animals and comprises the **endotoxin** complex. Abbreviations: KDO, ketodeoxyoctonate; Hep, heptose; Glu, glucose; Gal, galactose; GluNac, N-acetylglucosamine; GlcN, glucosamine; P, phosphate.

The chemistry of LPS from several bacteria is known. As shown here, the polysaccharide portion of LPS consists of two components: the **core polysaccharide** and the **O-polysaccharide**. The LPS shown here has a core polysaccharide consisting of KDO, various heptoses, glucose, galactose, and N-acetyl-glucosamine. The O-polysaccharide consists of a repeated structure of sugars that is highly variable between species and often branched.

LPS replaces much of the phospholipid in the outer leaflet of the outer membrane bilayer. By contrast, lipoprotein is present on the inner leaflet of the outer membrane, along with the usual membrane phospholipids. Lipoproteins function as an anchor linking the outer membrane to the peptidoglycan layer. Thus, although the overall structure of the outer membrane is considered a lipid bilayer, its structure and composition are quite distinct from that of the cytoplasmic membrane.

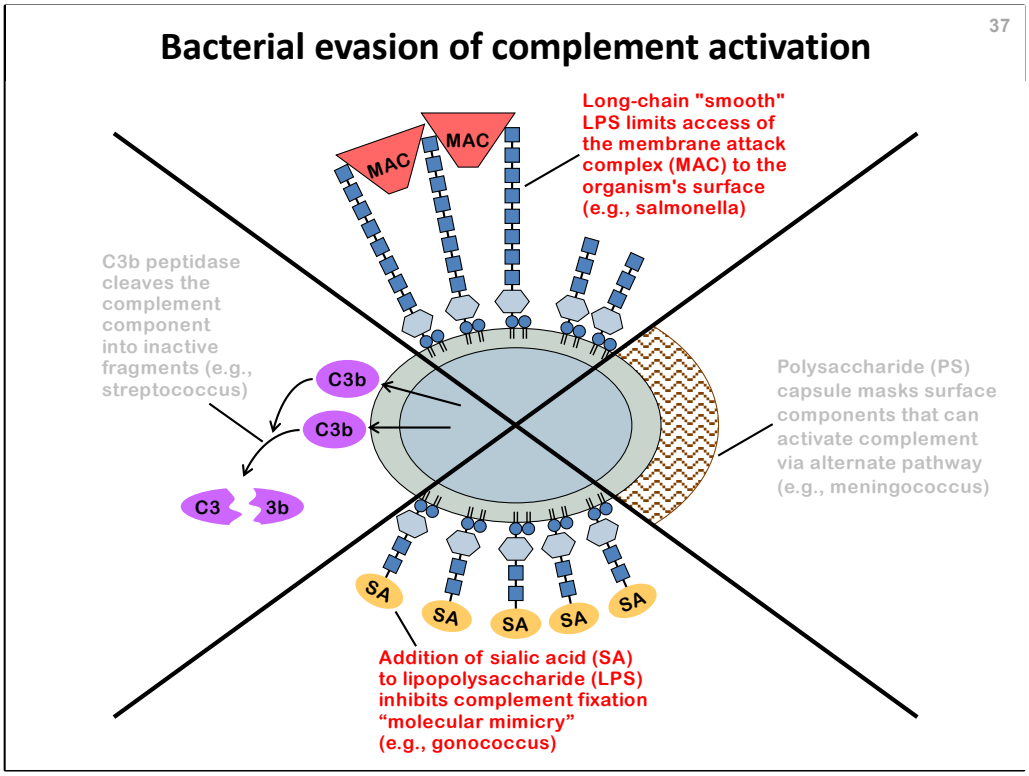
You should memorize the general tripartite structure of LPS, but you don't need to remember the molecular details.



SOURCE: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) Chapter 3: Cell structure and function in *Bacteria* and *Archaea*. In: *Brock Biology of Microorganisms [13th Edition]*. Pearson Education Inc., San Francisco, CA, pp. 60-62.

Although the major function of the outer membrane is structural, one of its important biological activities is its toxicity to animals. Toxicity is associated with the LPS layer, in particular, **lipid A**. The term **endotoxin** refers to this toxic component (lipid A) of LPS. Some endotoxins cause violent symptoms in humans, including gas, diarrhea, vomiting, etc. The endotoxins produced by *Salmonella* spp. and enteropathogenic strains of *Escherichia coli*, which are transmitted in contaminated foods, are classic examples of this. Lipid A is not directly toxic. Rather, it binds to receptors on immune cells and elicits a "cytokine storm" (i.e., an excessively exuberant host immune response) that can result in massive vascular leakage, circulatory collapse, and death. This is the pathogenetic basis of septic shock.

You do not need to memorize this detailed molecular structure. The point of this slide is simply to demonstrate that the molecular structure of the lipid A component of LPS is well understood; this is the component known as "endotoxin" that is responsible for triggering septic shock.



SOURCE: Schaechter M, Engleberg NC, Eisenstein BI, Medoff G, *Mechanisms of Microbial Disease [3rd Edition]*, published by Williams & Wilkins, Baltimore, MA USA, p. 108 (Figure 8.1) © 1998.

Microbial strategies for evasion of complement activation and assembly of the membrane attack complex (MAC) on the bacterial cell surface.

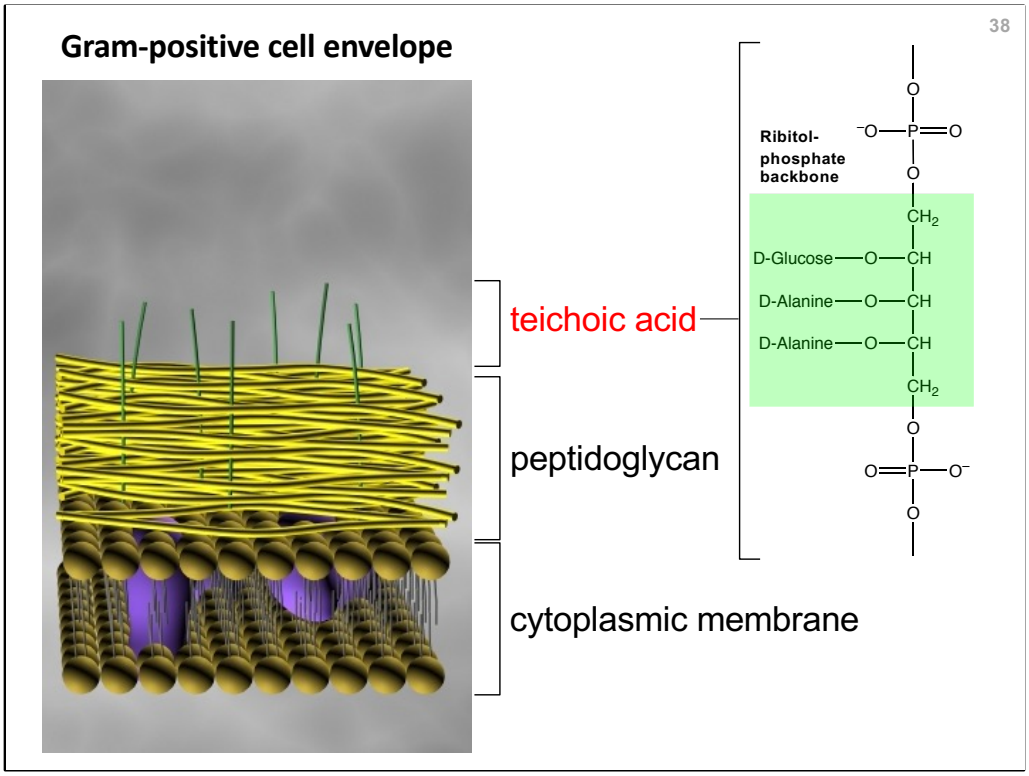
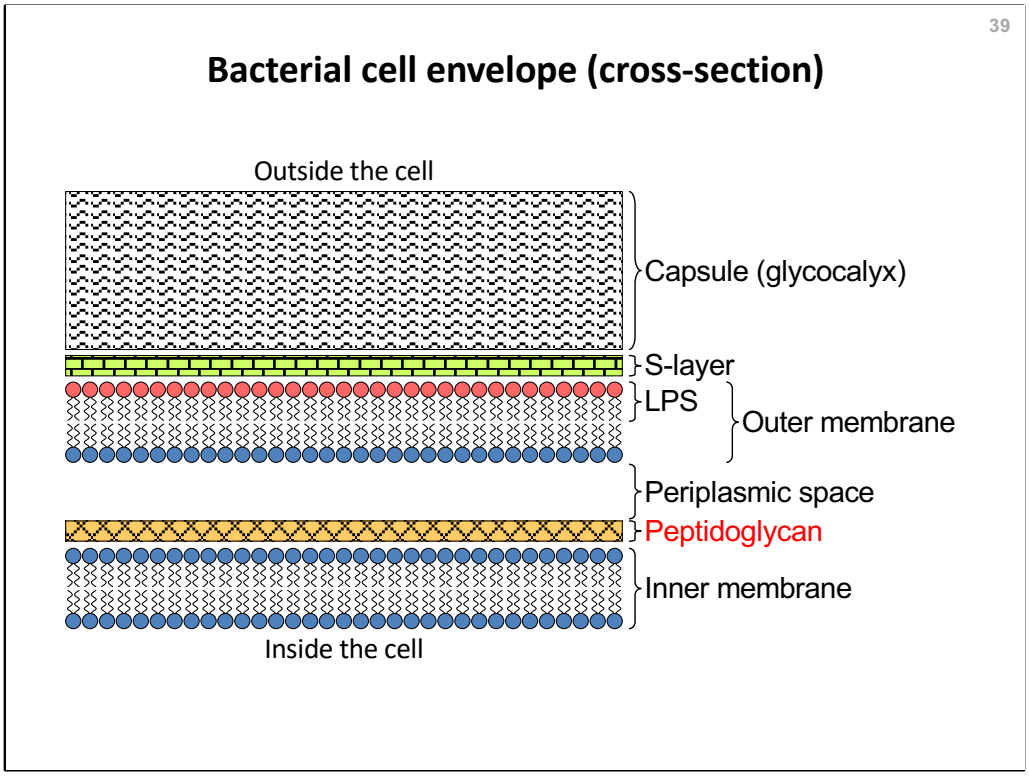


Diagram of the cell wall structure of **Gram-positive bacteria**. Key: peptidoglycan layer (yellow); proteins (purple); teichoic acid (green); phospholipid (brown).

Teichoic acids are bacterial polysaccharides of glycerol phosphate or ribitol phosphate linked via phosphodiester bonds.

Teichoic acids are found within the cell wall of Gram-positive bacteria and extend to the surface of the peptidoglycan layer. Teichoic acids are not found in Gram-negative bacteria. They can be covalently linked to N-acetylmuramic acid of the peptidoglycan layer, to the lipids of the cytoplasmic membrane, or to a terminal D-alanine in the tetrapeptide crosslink between N-acetylmuramic acid units. Teichoic acids that remain anchored to lipids are referred to as lipotecichoic acids, whereas teichoic acids that are covalently bound to peptidoglycan are referred to as cell wall teichoic acids. The main function of teichoic acids is to provide rigidity to the bacterial cell wall.



Inner membrane (6-8 nm thick) - Lipid bilayer that serves three basic functions. (1) Selective permeability barrier. (2) Maintains proton motive force (PMF), source of energy to drive ATP synthesis and other cellular processes (e.g., flagellar rotation). (3) Anchoring site for cellular proteins involved in various cellular functions (e.g., transmembrane transport systems, flagellar motor; adhesive fimbirae, etc.).

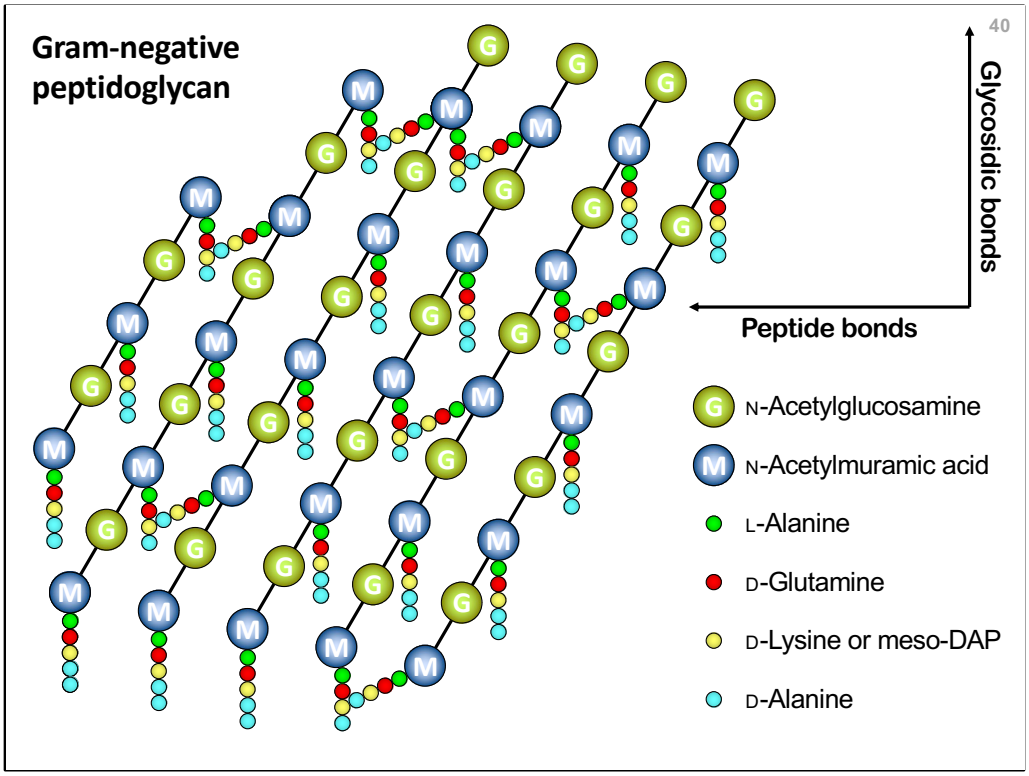
Peptidoglycan (5-10 nm thick in Gram-negative bacteria; 20-80 nm thick in Gram-positive bacteria) - Chains of alternating N-acetylglucosamine and N-acetylmuramicacid residues crosslinked by peptide bridges. Provides structural and osmotic support and maintains cell shape. Anchoring site for some exported proteins, especially in Gram-positive bacteria that lack a true periplasmic space. Porous.

Periplasmic space (30 nm thick at sidewalls, 70 nm thick at cell tips) - Fluid-filled space between the inner and outer membranes in Gram-negative bacteria. Location of cellular proteins involved in various functions (e.g., beta-lactamases that inactivate beta-lactam antibiotics before they can reach their targets at the peptidoglycan). Transient location of some secreted proteins after they cross the inner membrane but before they cross the outer membrane.

Outer membrane (7-10 nm thick) - Lipid bilayer that serves as a selective permeability barrier. The outer leaflet of the bilayer consists of **LPS** (**lipopolysaccharide**). Present in Gram-negative bacteria but absent in Gram-positive bacteria. The outer membrane is more permeable to small molecules than the inner membrane thanks to porins, which provide narrow water-filled channels across the outer membrane.

S layer (5-25 nm thick) - Crystalline array of a single glycoprotein. Provides structural support and helps maintain cell shape. Porous.

Capsule (200-10,000 nm thick) - Thick layer of polysaccharide. Protects against the immune system by interfering with complement deposition and phagocytosis of bacteria by macrophages and neutrophils (PMNs).

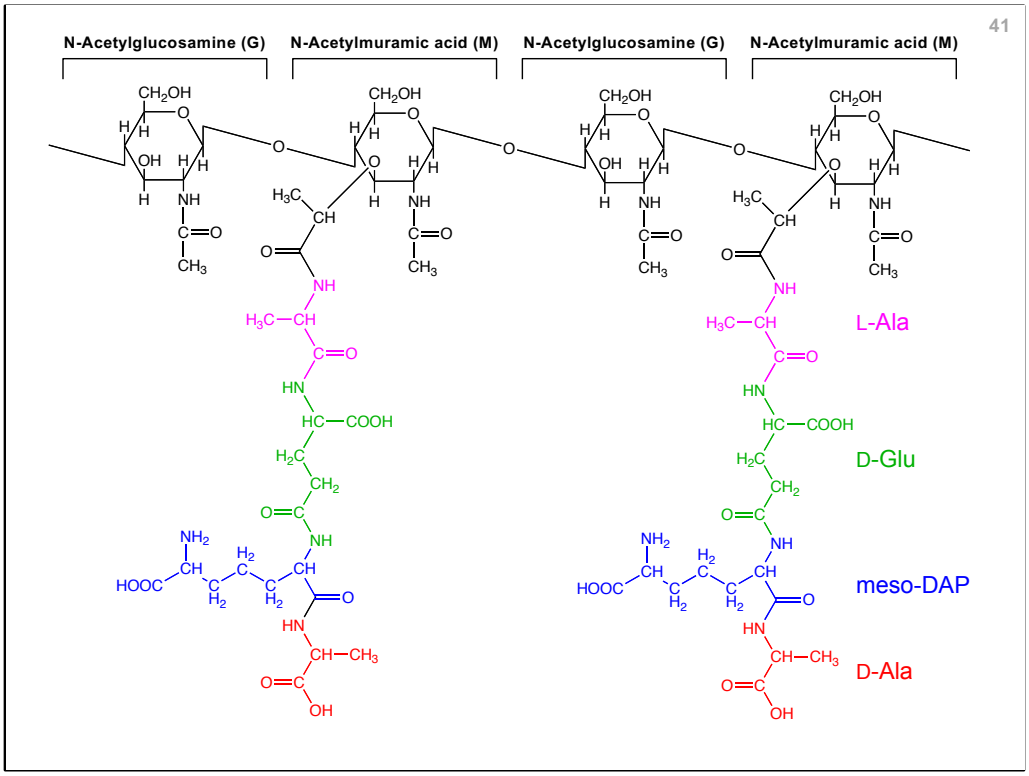


SOURCE: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) Chapter 3: Cell structure and function in *Bacteria* and *Archaea*. In: *Brock Biology of Microorganisms [13th Edition]*. Pearson Education Inc., San Francisco, CA.

FIGURE 3.17. Overall structure of peptidoglycan in the Gram-negative microorganism *Escherichia coli*. No glycine interbridge is present in *E. coli* and other Gram-negative bacteria. Note how glycosidic bonds confer strength to peptidoglycan in the Y direction whereas peptide bonds confer strength in the X direction.

Abbreviations: G, N-acetylglucosamine; M, N-acetylmuramic acid.

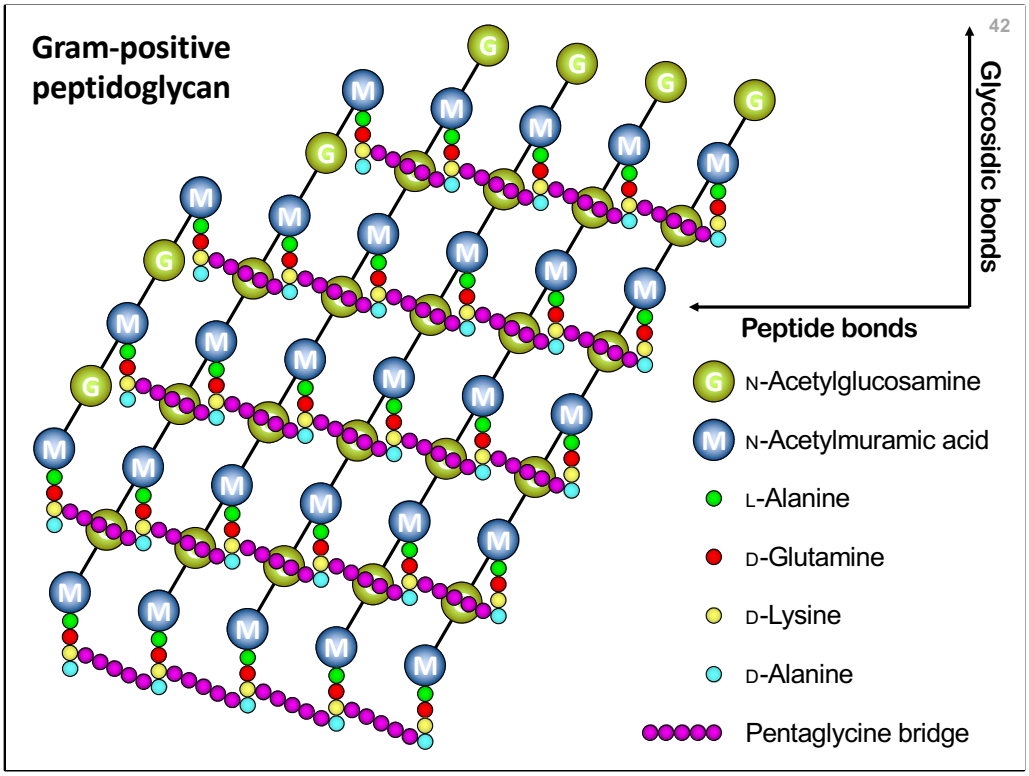
You should memorize the *general structure* of Gram-negative peptidoglycan, as shown on this slide, but you do not need to memorize the molecular details. The general structure of peptidoglycan consists of long parallel strands of sugars that are cross-linked by short peptide bridges.



SOURCE: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) Chapter 3: Cell structure and function in *Bacteria* and *Archaea*. In: *Brock Biology of Microorganisms [13th Edition]*. Pearson Education Inc., San Francisco, CA.

FIGURE 3.16. Structure of the repeating unit in peptidoglycan, the glycan tetrapeptide. The structure given is that found in *Escherichia coli* and most other Gram-negative bacteria. In some bacteria, other amino acids are found.

You do not need to memorize the molecular details of peptidoglycan structure, as shown on this slide. The point of this slide is simply to illustrate that the molecular structure of peptidoglycan is well understood. However, the larger-scale ultrastructure of peptidoglycan is still not completely understood.



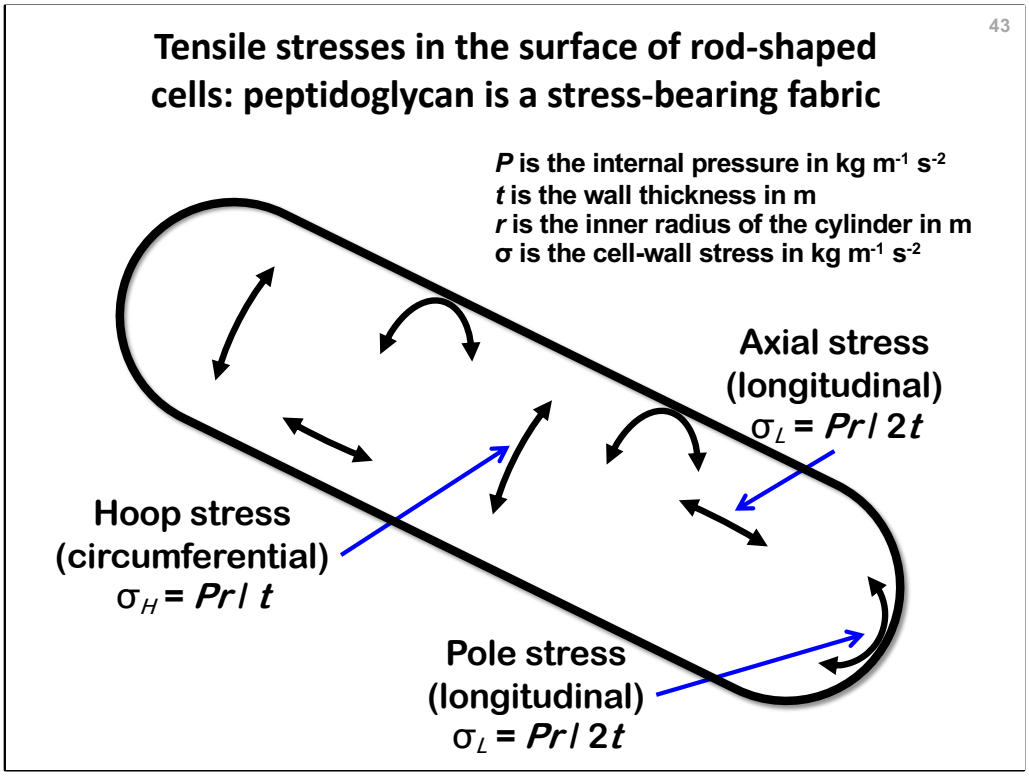
SOURCE: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) Chapter 3: Cell structure and function in *Bacteria* and *Archaea*. In: *Brock Biology of Microorganisms [13th Edition]*. Pearson Education Inc., San Francisco, CA.

FIGURE 3.17. Overall structure of peptidoglycan in the Gram-positive microorganism *Staphylococcus aureus.* Note the presence of glycine interbridges between the glycan strands. Note how glycosidic bonds confer strength to peptidoglycan in the Y direction whereas peptide bonds confer strength in the X direction.

Abbreviations: G, N-acetylglucosamine; M, N-acetylmuramic acid.

You should memorize the *general structure* of Gram-negative peptidoglycan, as shown on this slide, but you do not need to memorize the molecular details. The general structure of peptidoglycan consists of long parallel strands of sugars that are cross-linked by short peptide bridges.

Question: what are the major differences between Gram-positive peptidoglycan and Gram-negative peptidoglycan?



SOURCE: Koch AL (1988) Biophysics of bacterial walls viewed as stress-bearing fabric. *Microbiol Rev* 52(3): 337-353 PMID:3054466.
SOURCE: http://hyperphysics.phy-astr.gsu.edu/hbase/ptens.html

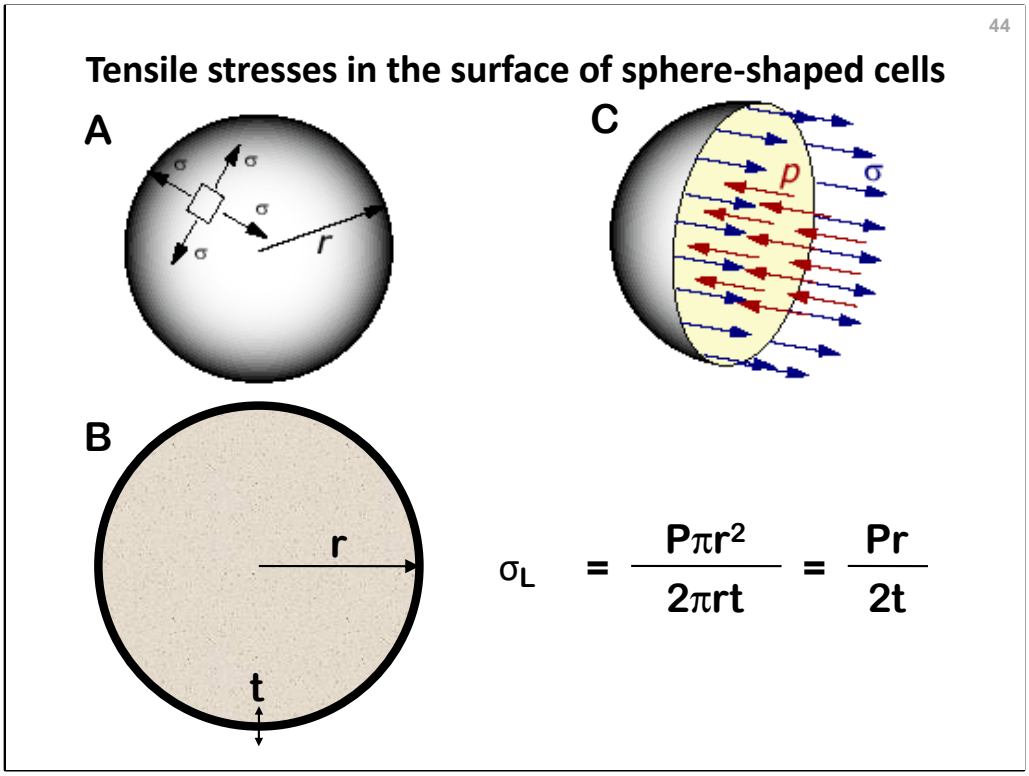
FIGURE 1. Tensile stresses in the surface of a rod-shaped organism. Because the wall is thin compared with each of the other dimensions, the wall must move until there are no resultant forces normal to the surface. Note that the stress is twice as great along the short axis ("hoop stress") compared to the longitudinal axis ("longitudinal stress") on the cylindrical surface.

Turgor pressure in an *Escherichia coli* cell is about 2-3 atmospheres (similar to the inflated tire of a mountain bike), depending on the osmolality of the growth medium.

Abbreviations:
P is the internal pressure in N * m^{-2} or kg * m^{-1} * s^{-2}
t is the wall thickness in m
r is the internal radius in m
σ_h is the cell wall hoop stress (a.k.a. circumferential stress) in N * m^{-2} or kg * m^{-1} * s^{-2}
σ_l is the cell wall axial or pole stress (a.k.a. longitudinal stress) in N * m^{-2} or kg * m^{-1} * s^{-2}
1 atm = 1.01325 x 10^5 Pascals (Pa) in N * m^{-2} or kg * m^{-1} * s^{-2}

You should memorize the equations for axial stress, pole stress, and hoop stress and you should feel comfortable manipulating them.

Question: How do these equations relate to Laplace's Law? What are the differences?

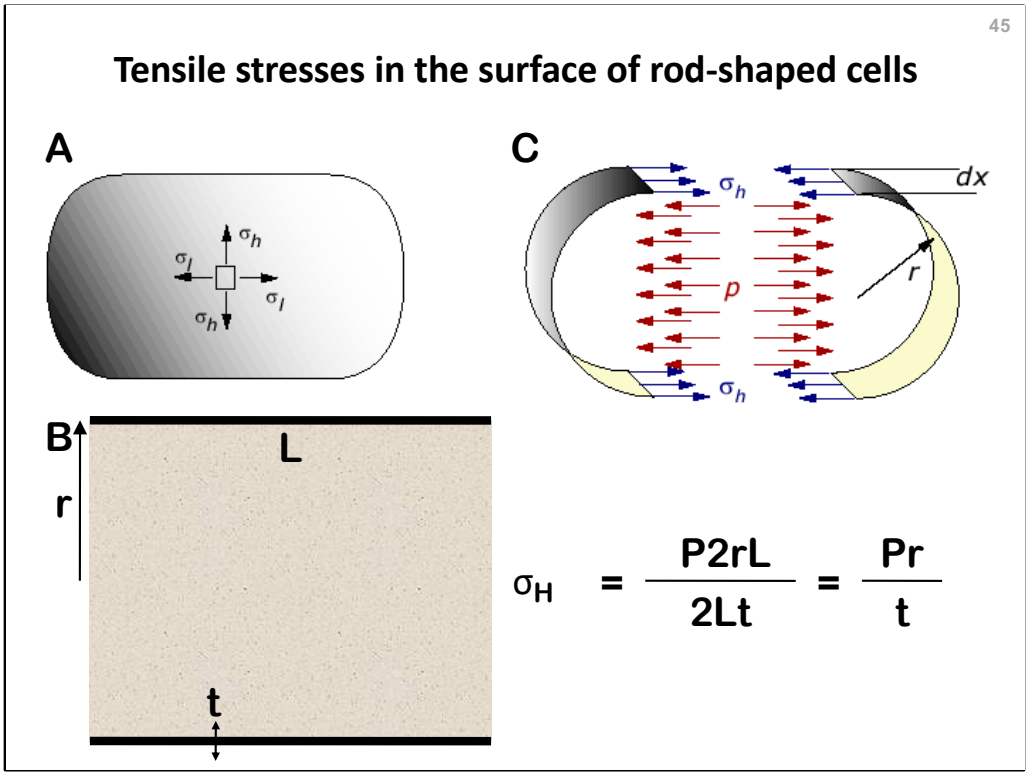


SOURCE: Vogel S (1988) *Life's Devices: The Physical World of Animals and Plants*. Princeton University Press, Princeton, NJ.
SOURCE: http://www.efunda.com/formulae/solid_mechanics/mat_mechanics/pressure_vessel.cfm
SOURCE: http://hyperphysics.phy-astr.gsu.edu/hbase/ptens.html

(A) Consider a spherical cells with radius *r* and wall thickness *t* subjected to an internal pressure *P*. For reasons of symmetry, all four normal stresses on a small stress element in the wall must be identical.

(C) The normal stresses σ can be related to the pressure *P* by inspecting a free body diagram of the pressure vessel. To simplify the analysis, we cut the vessel in half as illustrated. Since the vessel is under static equilibrium, it must satisfy Newton's first law of motion (if an object experiences no net force, then its velocity is constant: the object is either at rest if its velocity is zero, or it moves in a straight line with constant speed if its velocity is nonzero). In other words, the stress around the wall must have a net resultant to balance the internal pressure across the cross-section. Applying Newton's first law of motion, we have: $\sigma \times t \times 2\pi r = P \times \pi r^2$ therefore $\boldsymbol{\sigma = Pr / 2t}$.

Abbreviations:
P is the internal pressure in N * m^{-2} or kg * m^{-1} * s^{-2}
t is the wall thickness in m
r is the internal radius in m
σ is the wall stress in N * m^{-2} or kg * m^{-1} * s^{-2}
Note that the units for pressure and stress are the same.



SOURCE: Vogel S (1988) *Life's Devices: The Physical World of Animals and Plants*. Princeton University Press, Princeton, NJ, USA.
SOURCE: http://www.efunda.com/formulae/solid_mechanics/mat_mechanics/pressure_vessel.cfm
SOURCE: http://hyperphysics.phy-astr.gsu.edu/hbase/ptens.html

(A) Consider a cylindrical cell with radius *r* and wall thickness *t* subjected to an internal pressure *P*. The coordinates used to describe the cylindrical vessel take advantage of its axial symmetry. It is natural to align one coordinate along the longitudinal axis. To analyze the stress state in the vessel wall, a second coordinate is then aligned along the hoop direction. With this choice of axisymmetric coordinates, there is no shear stress. The hoop stress σ_h and the longitudinal stress σ_l are the principal stresses.
(B) To determine the longitudinal stress σ_l, we make a cut across the cylinder similar to analyzing the spherical cell (previous slide). The free body, illustrated on the previous slide, is in static equilibrium. This implies that the stress around the wall must have a resultant to balance the internal pressure across the cross-section. Applying Newton's first law of motion, we have: $\sigma_l \times t \times 2\pi r = P \times \pi r^2$ therefore $\boldsymbol{\sigma_l = Pr / 2t}$. Note that this is the same equation used to describe tensile stresses in the surface of spherical cells (previous slide).
(C) To determine the hoop stress σ_h we make a cut along the longitudinal axis and construct a small slice as illustrated on the right. The free body is in static equilibrium. Applying Newton's first law of motion, we have: $2 \times \sigma_h \times t = 2 \times P \times r$ therefore $\boldsymbol{\sigma_h = Pr / t}$

Note that the hoop stress ($\boldsymbol{\sigma_h = Pr / t}$) is twice the longitudinal stress ($\boldsymbol{\sigma_l = Pr / 2t}$). This is why capped cylinders under internal pressure tend to split lengthwise (along the long axis) rather than circumferentially (along the short axis).

Abbreviations:
P is the internal pressure in N * m^{-2} or kg * m^{-1} * s^{-2}
t is the wall thickness in m
r is the internal radius in m
σ_h is the cell wall hoop stress (a.k.a. circumferential stress) in N * m^{-2} or kg * m^{-1} * s^{-2}
σ_l is the cell wall axial or pole stress (a.k.a. longitudinal stress) in N * m^{-2} or kg * m^{-1} * s^{-2}
Note that the units for pressure and stress are the same.

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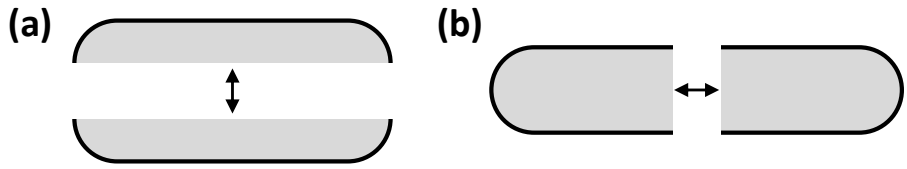
If turgor pressure increases inside a rod-shaped cell the cell will eventually rupture:



A. As in (a) because pressure is greater along the short axis.

B. As in (a) because cell-wall stress is greater along the short axis.

C. As in (b) because pressure is greater along the long axis.

D. As in (b) because cell-wall stress is greater along the long axis.

Answer: (B)

SOURCE: Vogel S, *Life's Devices: The Physical World of Animals and Plants*, published by Princeton University Press, Princeton, NJ, USA © 1988.
SOURCE: http://www.efunda.com/formulae/solid_mechanics/mat_mechanics/pressure_vessel.cfm

The equations used in the previous four slides are good for thin-walled pressure vessels. Generally, a pressure vessel is considered to be "thin-walled" if its radius r is larger than five times its wall thickness t, that is, $r > 5 * t$).

When a pressure vessel is subjected to *external pressure*, the above formulas are still valid. However, the stresses are now *negative* since the wall is now in compression instead of tension.

The **hoop stress** (along the short axis) is twice as much as the **longitudinal stress** (along the long axis) for the cylindrical pressure vessel. This is why an overcooked hotdog usually cracks along the longitudinal direction first, i.e., the hotdog's skin fails due to hoop stress, which is generated by internal steam pressure.

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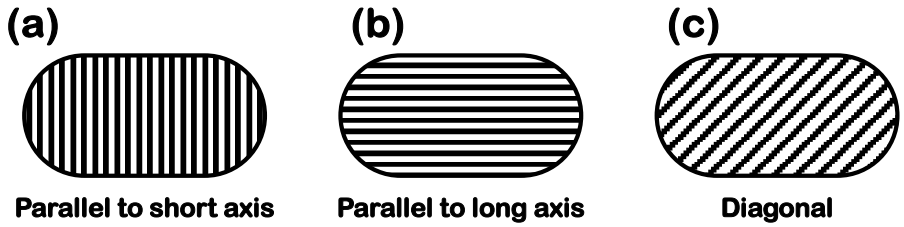
The best arrangement of reinforcing structures in the cell wall in order to prevent osmotic rupture of the cell is:



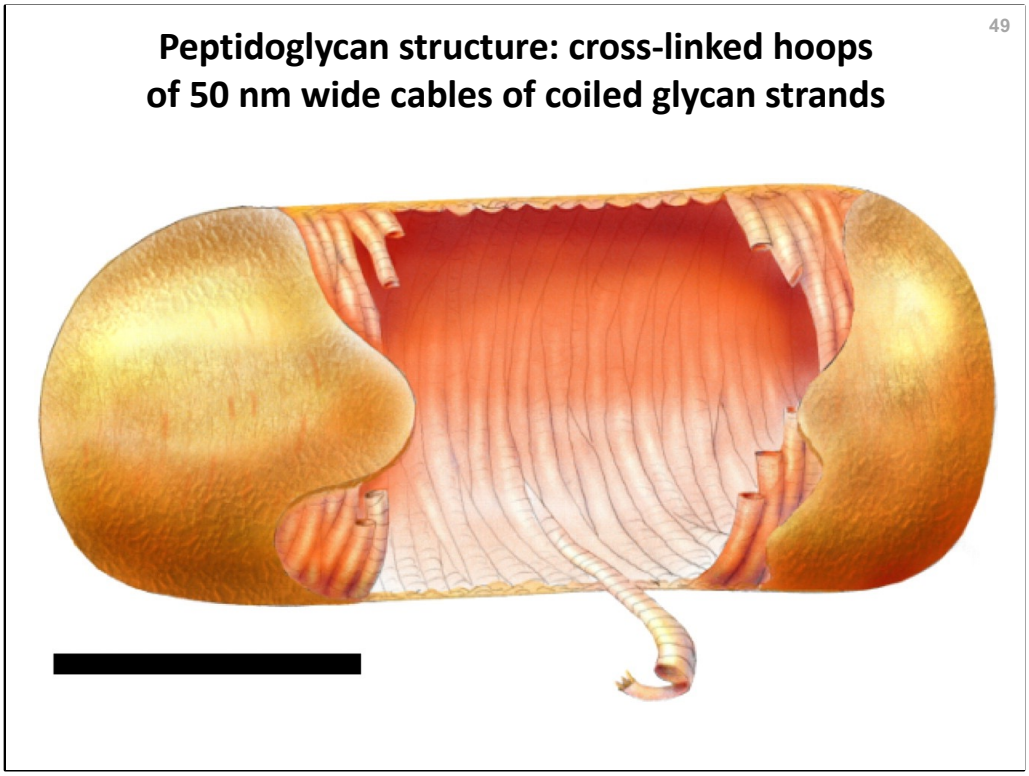
A. (a).

B. (b).

C. (c).

D. (a), (b), and (c) are equally effective.

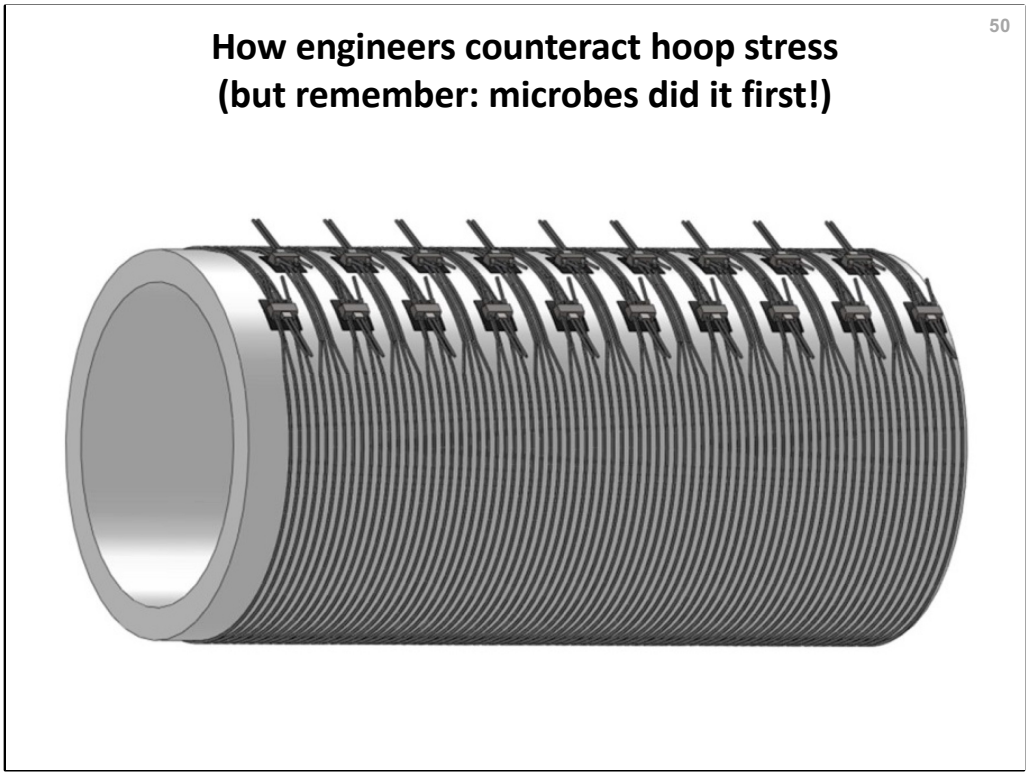
Answer: (A)



SOURCE: Hayhurst EJ, Kailas L, Hobbs JK, Foster SJ (2008) Cell wall peptidoglycan architecture in *Bacillus subtilis*. *Proceedings of the National Academy of Sciences of the United States of America* 105(38): 14603-14608 PMID:18784364.

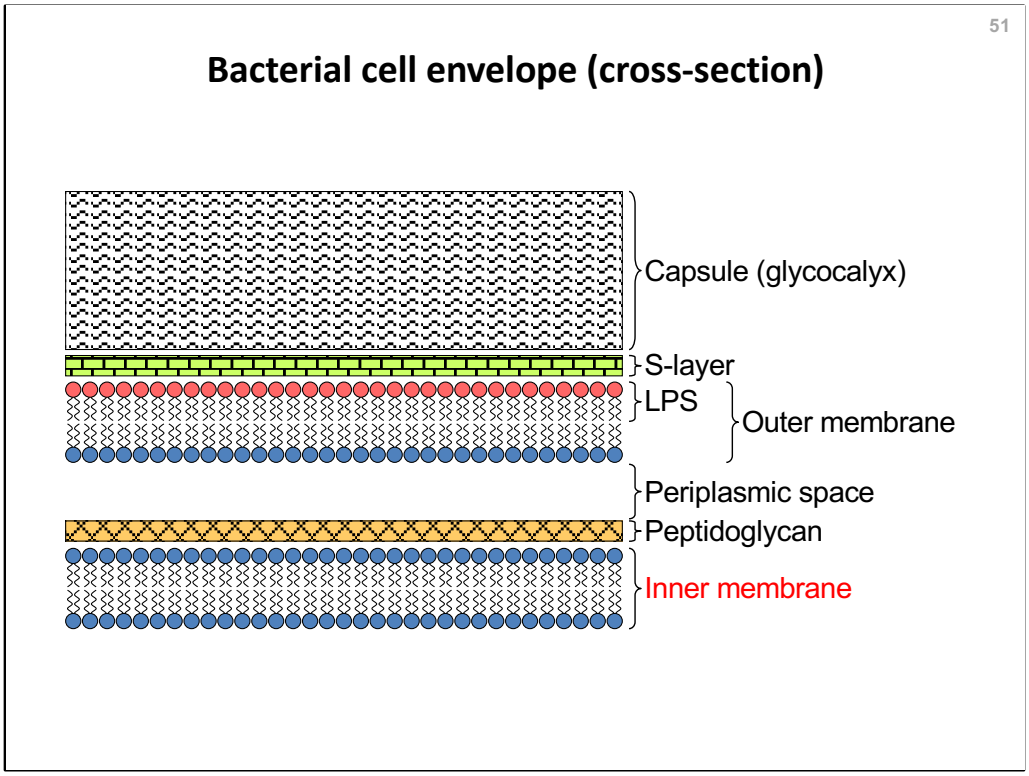
FIGURE 5. Model of *Bacillus subtilis* cell wall peptidoglycan architecture. Image is of a cell wall cylinder peptidoglycan architecture showing peptidoglycan cable orientation with coiled substructure. Both cables and cross striations are shown. Scale bar, 1 µm.

Note that the peptidoglycan cables are wrapped around the cell parallel to the cell's short axis, similar to the structure diagrammed in (a) on the previous slide and the human-made structure shown on the next slide. This is the optimal arrangement of supporting elements (peptidoglycan cables) to prevent osmotic rupture of the cell due to internal turgor pressure.



SOURCE: Zhao L, Dou T, Cheng B, Xia S, Yang J, Zhang Q, Li M, Li X (2019) Theoretical study and application of the reinforcement of prestressed concrete cylinder pipes with external prestressed steel strands. *Applied Sciences* 9(24): 5532 doi.org/10.3390/app9245532.

Figure 1. Structural drawing of a prestressed concrete cylinder pipe (PCCP) strengthened with prestressed steel strands.



Inner membrane (6-8 nm thick) - Lipid bilayer that serves three basic functions. (1) Selective permeability barrier. (2) Maintains proton motive force (PMF), source of energy to drive ATP synthesis and other cellular processes (e.g., flagellar rotation). (3) Anchoring site for cellular proteins involved in various cellular functions (e.g., transmembrane transport systems, flagellar motor; adhesive fimbirae, etc.).

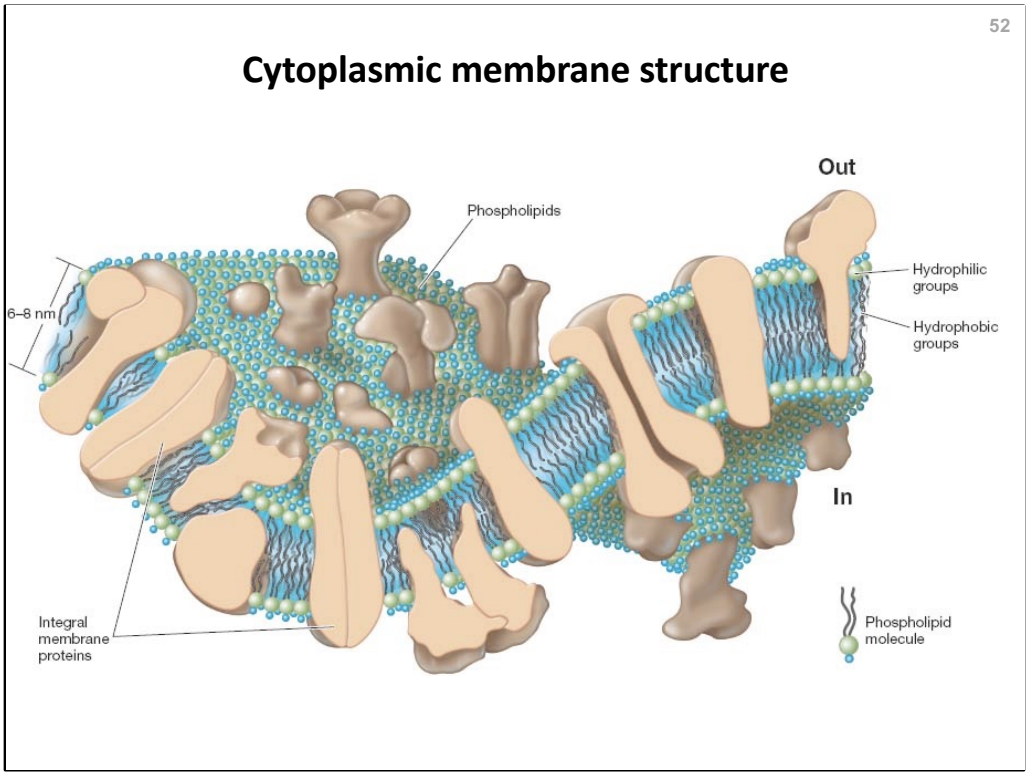
Peptidoglycan (5-10 nm thick in Gram-negative bacteria; 20-80 nm thick in Gram-positive bacteria) - Chains of alternating N-acetylglucosamine and N-acetylmuramicacid residues crosslinked by peptide bridges. Provides structural and osmotic support and maintains cell shape. Anchoring site for some exported proteins, especially in Gram-positive bacteria that lack a true periplasmic space. Porous.

Periplasmic space (30 nm thick at sidewalls, 70 nm thick at cell tips) - Fluid-filled space between the inner and outer membranes in Gram-negative bacteria. Location of cellular proteins involved in various functions (e.g., beta-lactamases that inactivate beta-lactam antibiotics before they can reach their targets at the peptidoglycan). Transient location of some secreted proteins after they cross the inner membrane but before they cross the outer membrane.

Outer membrane (7-10 nm thick) - Lipid bilayer that serves as a selective permeability barrier. The outer leaflet of the bilayer consists of **LPS** (**lipopolysaccharide**). Present in Gram-negative bacteria but absent in Gram-positive bacteria. The outer membrane is more permeable to small molecules than the inner membrane thanks to porins, which provide narrow water-filled channels across the outer membrane.

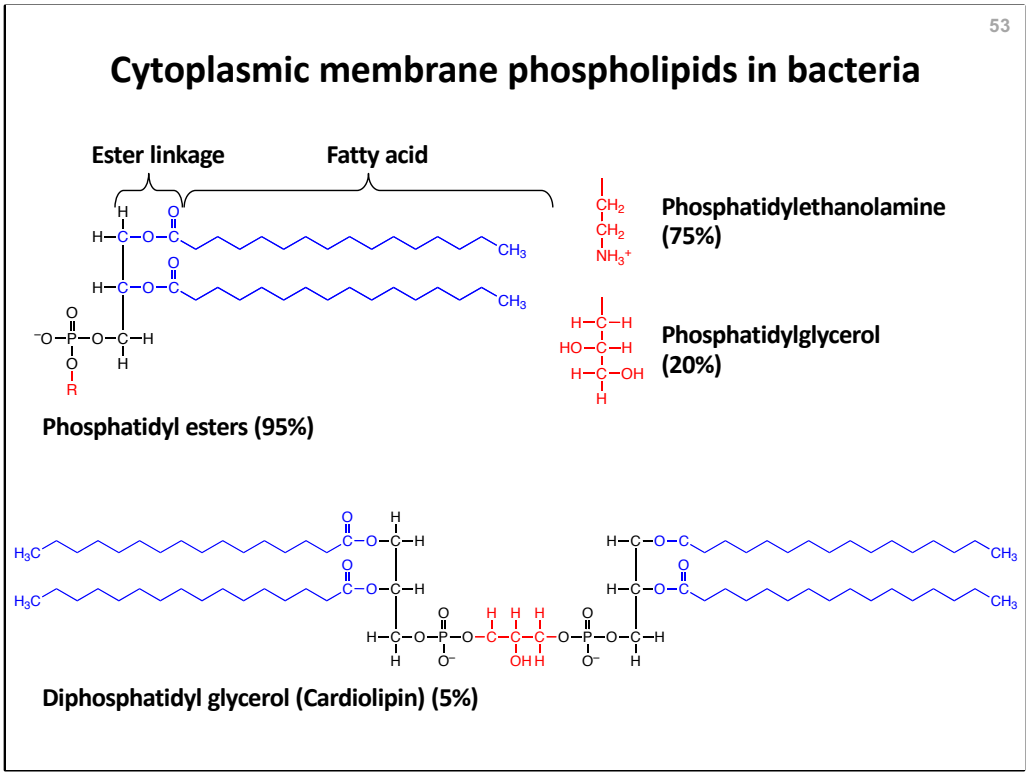
S layer (5-25 nm thick) - Crystalline array of a single glycoprotein. Provides structural support and helps maintain cell shape. Porous.

Capsule (200-10,000 nm thick) - Thick layer of polysaccharide. Protects against the immune system by interfering with complement deposition and phagocytosis of bacteria by macrophages and neutrophils (PMNs).

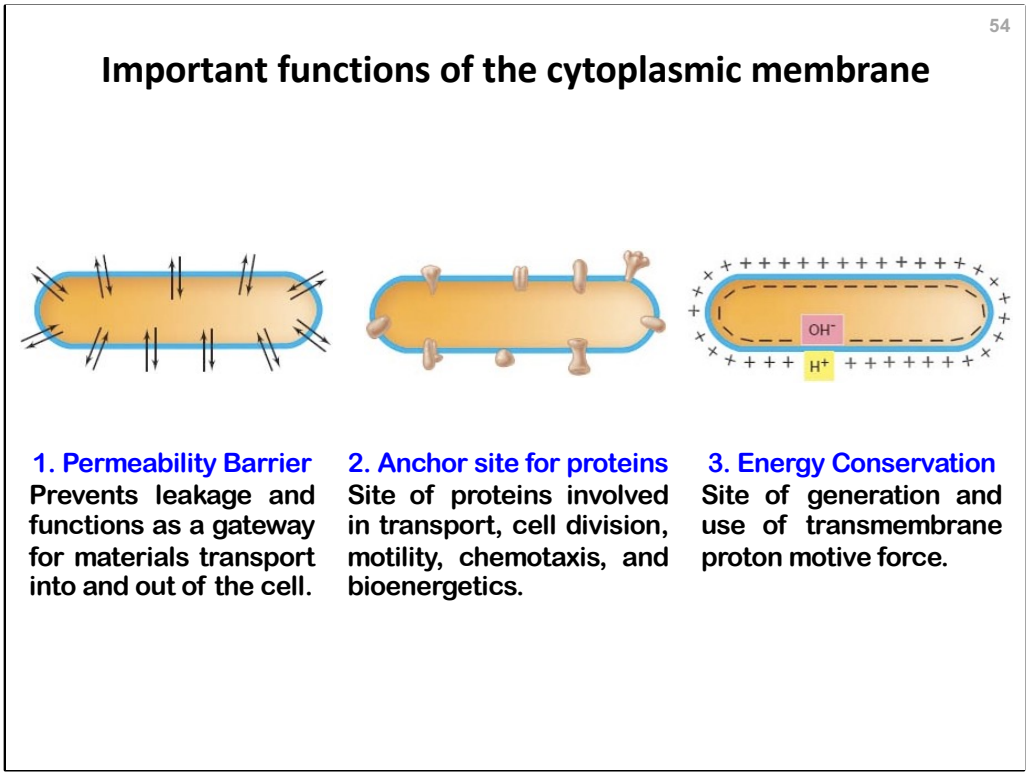


SOURCE: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) Chapter 3: Cell structure and function in *Bacteria* and *Archaea*. In: *Brock Biology of Microorganisms [13th Edition]*. Pearson Education Inc., San Francisco, CA.

FIGURE 3.5. Structure of the cytoplasmic membrane. The inner surface (In) faces the cytoplasm and the outer surface (Out) faces the environment. Phospholipids compose the matrix of the cytoplasmic membrane, with the hydrophobic groups directed inward and the hydrophilic groups toward the outside, where they associate with water. Embedded in the matrix are proteins that are hydrophobic in the region that traverses the fatty acid bilayer. Hydrophilic proteins and other charged substances, such as metal ions, may attach to the hydrophilic surfaces. Although there are some chemical differences, the overall structure of the cytoplasmic membrane shown is similar in both prokaryotes and eukaryotes.



In mammalian cells, cardiolipin is a phospholipid that is **synthesized and localized uniquely in the inner mitochondrial membrane**. Cardiolipin plays a central role in many reactions and processes involved in mitochondrial function and dynamics. But remember: mitochondria are derived from bacterial endosymbionts that evolved into organelles.



SOURCE: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) Chapter 3: Cell structure and function in *Bacteria* and *Archaea*. In: *Brock Biology of Microorganisms [13th Edition]*. Pearson Education Inc., San Francisco, CA.

FIGURE 3.8. The major functions of the cytoplasmic membrane. Although structurally weak, the cytoplasmic membrane has many important cellular functions.