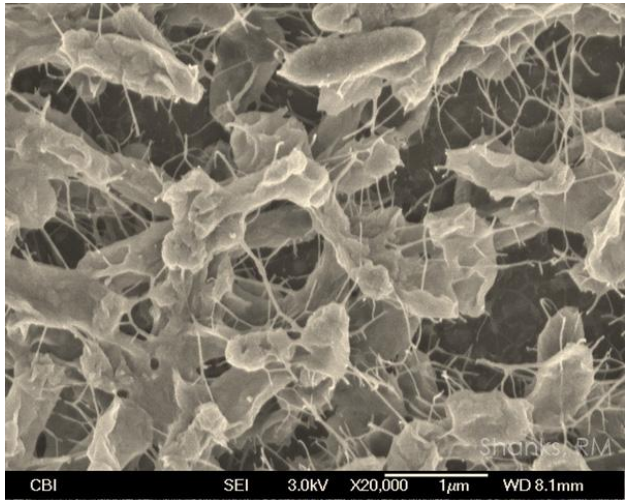
Chapter 3:
Molecular cell biology of bacteria

aa 2017-18

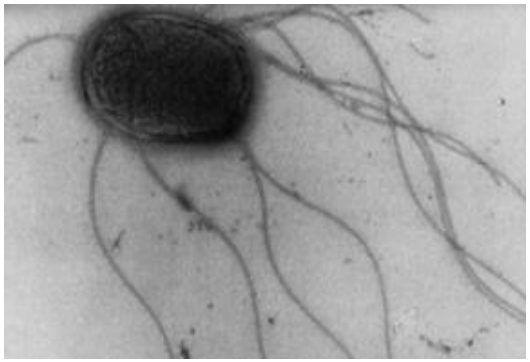
Super-resolution imaging of proteins in living bacteria let to observe single molecule



SEM *(scanning electron microscopy) of P. aeruginosa biofilm*

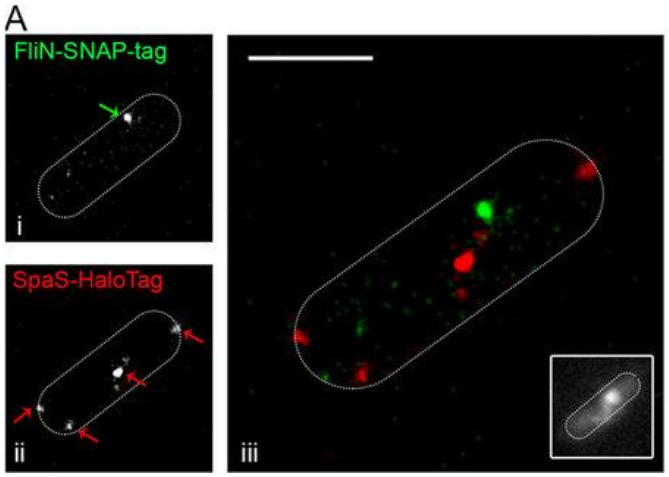
Electron microscopy and advanced fluorescence microscopy have provided us with a more detailed view of bacterial ultrastructure:

approx. 1000x better than light microscopy; 0.2 nm, instead of 0.2 μm.



TEM (Transmission electron microscopy) of S. enterica cells

Ultrahigh-precision visible light microscopy technique that enables scientists to photo-actively fluoresce and image individual proteins.



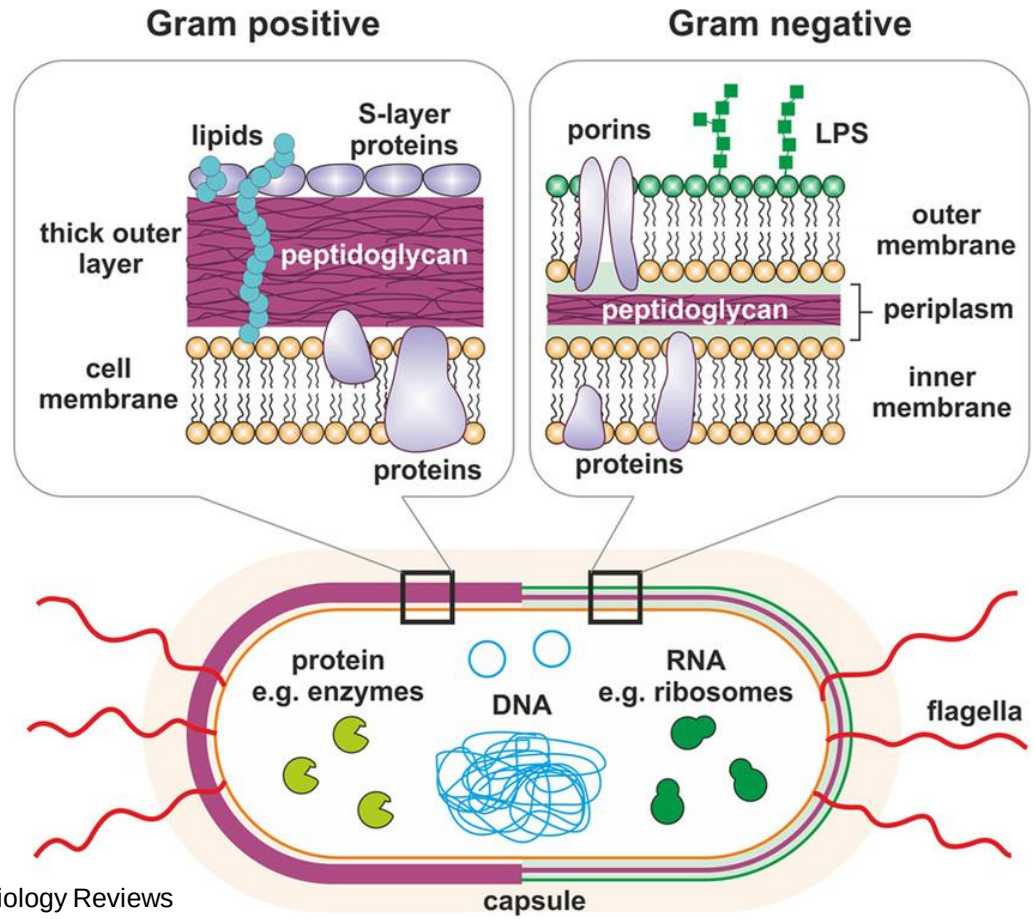
Bacterial Cell Architecture

Structurally, a bacterial cell has three architectural regions:

- a **cytoplasmic region** that contains the cell chromosome (DNA) ribosomes, and various sorts of inclusions

- a complex **cell envelope** (cell membrane and cell wall) different from Gram+ and Gram- bacteria, capsule

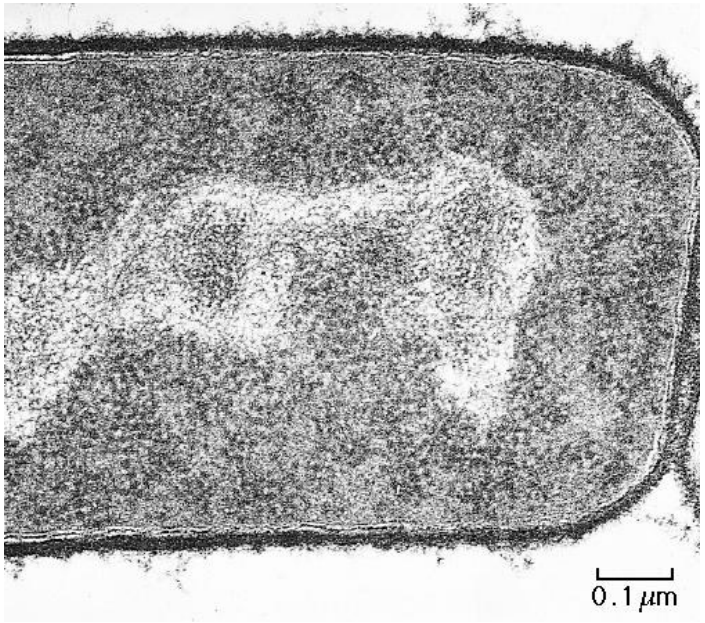
- An array of **appendages** (attachments to the cell surface) comprising flagella, fimbriae



A . Ahmed, Clinical Microbiology Reviews 27(3):631-646 · July 2014

Bacterial cytoplasm

Cytoplasm: viscous material containing a heavy concentration of proteins (100-300 mg/ml), salts and metabolites. It contains a large number of ribosomes.

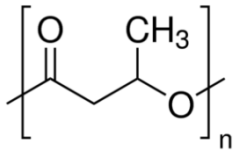


http://www.textbookofbacteriology.net/structure_9.html

The bacterial chromosome or nucleoid is the nonstaining region in the interior of the cell cytoplasm. The granular structures distributed throughout the cytoplasm are cell ribosomes.

Circular bacterial chromosome (**nucleoid**) *E. coli* the package is of 500 fold by nucleoid histone-like proteins.

Presence (often) of plasmids (accessory functions related to virulence).



poly-β-hydroxy-butyrric acid

Presence of **inclusion bodies**: glycogen, polyphosphates, poly-β-hydroxy-butyrric acid (P and C storage)

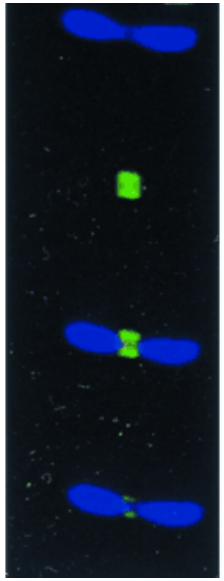
Presence of cytoskeletal elements

Bacterial cytoskeleton

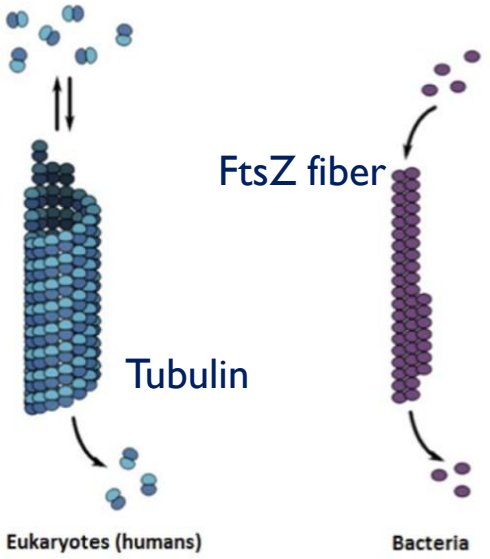
Bacteria employ cytoskeletal elements to perform many functions, including cell shaping, cell division, DNA segregation, and cell motility.
They possess counterparts of eukaryotic actin, tubulin, and intermediate filament proteins.

FtsZ: a cell division protein related to tubulin (structural homolog). FtsZ is found in virtually all bacteria. It is assembled as a ring and defines the division plane.

In both eukaryotes (blue, left) and bacteria (violet, right), the cytoskeleton is made up of smaller, repeating protein units that add together to form fibers. Once these fibers are formed, they can continue to grow or shrink through addition or removal of subunits.



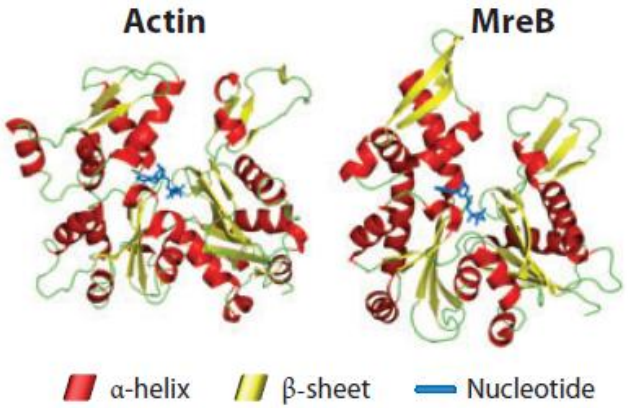
FtsZ–GFP localizes to internucleoid regions. Individual cells of JM109/pZG stained with DAPI viewed for DAPI fluorescence only (top part), GFP fluorescence only (second part from top), and DAPI + GFP



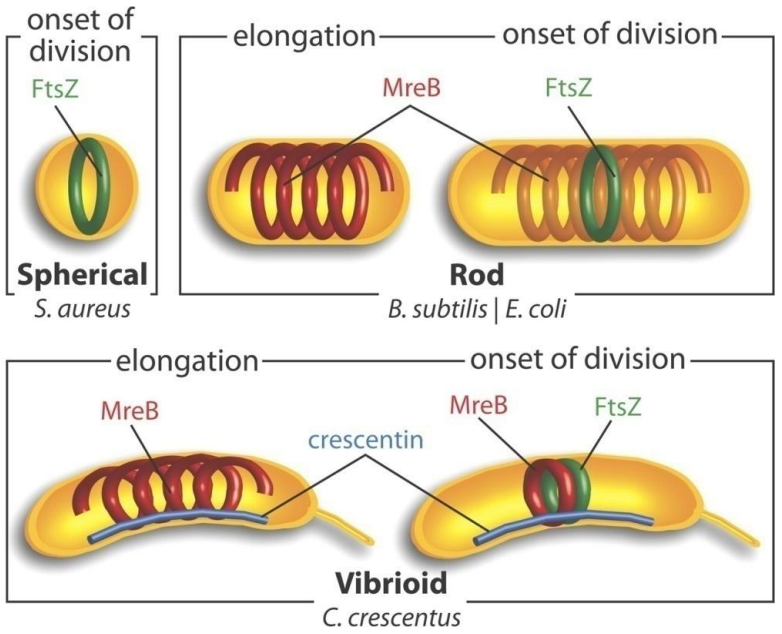
Adapted from Wickstead B, Gull K (2011) J Cell Biol 194: 513-25.

Bacterial cytoskeleton proteins are homolog to eukaryotic countepart

MreB: found in *E. coli* and many bacteria with a non spherical shape. It is a structural homolog of actin. It can form helical filaments and is the master regulator of cell shape. MreB has a role in cell morphogenesis (i.e., spatial regulation of cell growth).



MreB regulates cell shape by directing the localization or activity of enzymes that synthesize and reorganize the peptidoglycan making up the cell wall



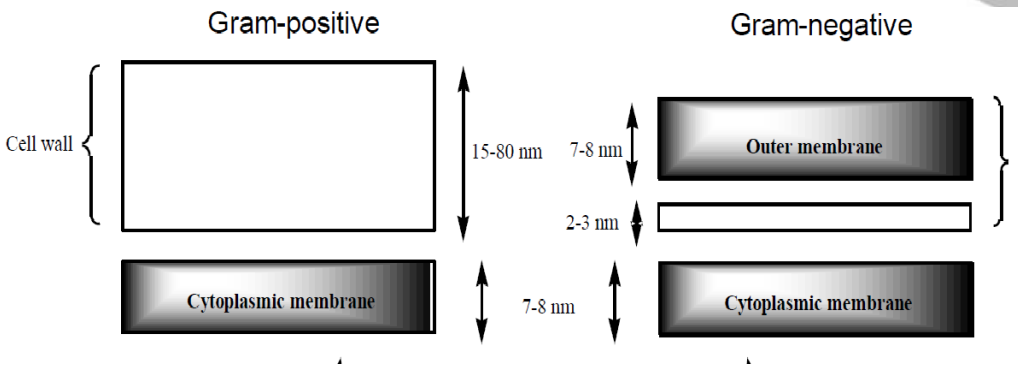
Cabeen M T , Jacobs-Wagner C J Cell Biol 2007;179:381-387

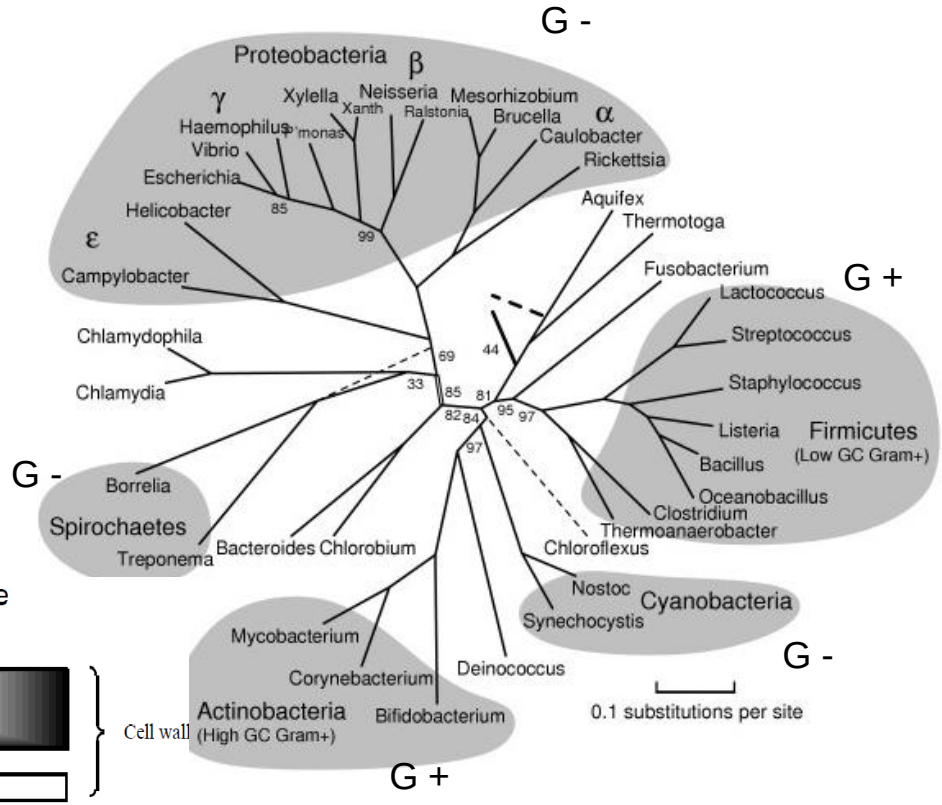
CreS (Crescentin) displays a filament-like pattern from pole to pole along the inner (concave) side of the cell responsible of the vibroid shape.

Cell envelope: two different organizations

Bacteria are faced with unpredictable, dilute and often hostile environments. To survive, bacteria have evolved a sophisticated and complex cell envelope that protects them.

Cell walls of **Gram-positive** and **Gram-negative** is highly different :



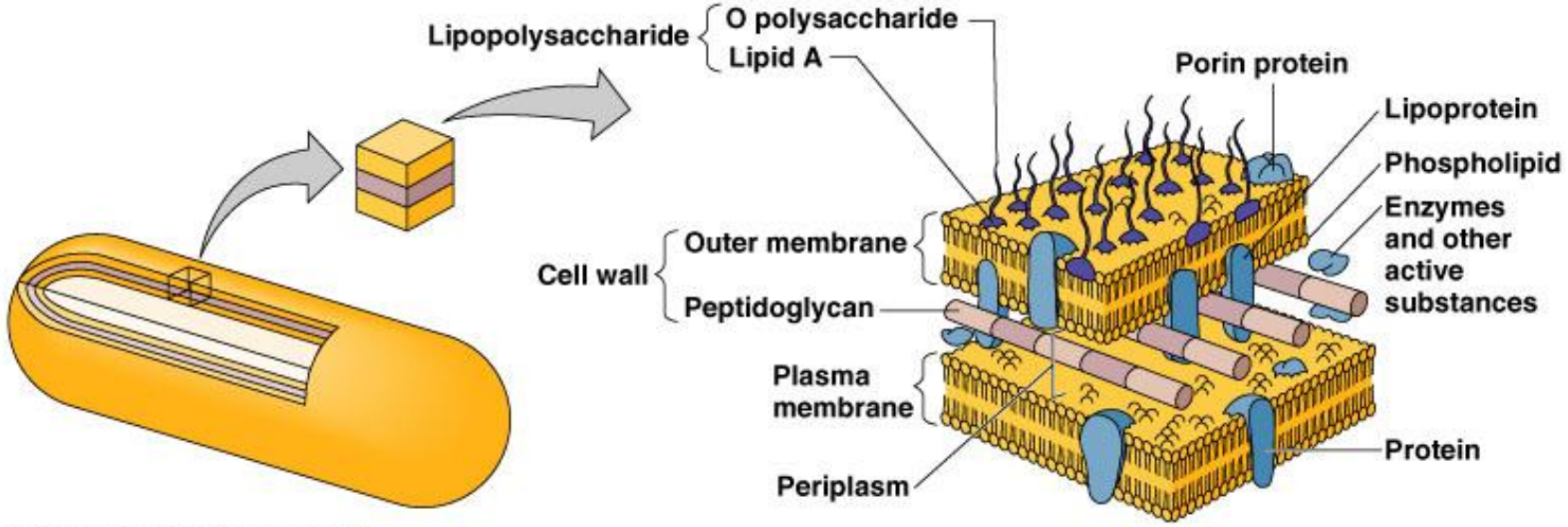


Phylogenetic tree of bacteria

Protection of the cell from mechanical damage and osmotic rupture.

Gram-negative envelope

Cell envelope is formed by three principal layers: the cytoplasmic or **inner membrane** (IM), the peptidoglycan cell wall, and the **outer membrane** (OM), The two concentric membrane layers delimit an aqueous cellular compartment, the **periplasm.**

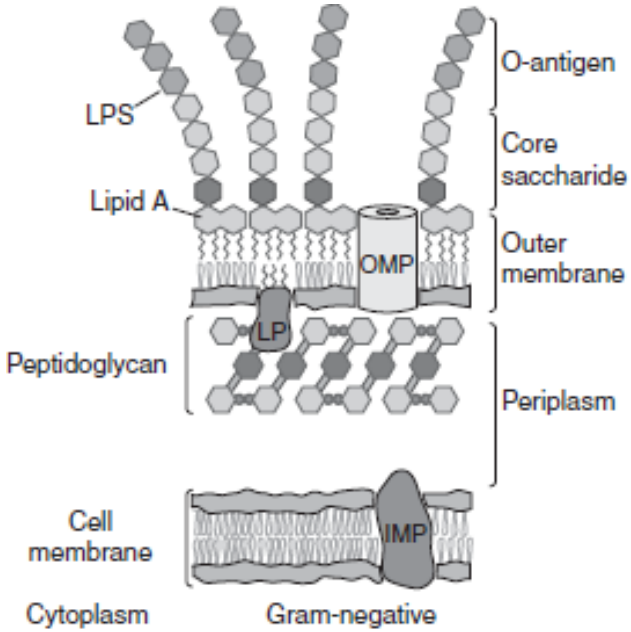


(c) Gram-negative cell wall

Outer membrane of G-negative bacteria

Outer membrane (OM) forms the **outermost layer** of the cell wall. OM is a distinguishing feature of G- bacteria. **Inner layer:** phospholipid layer various membrane proteins, and **outer leaflet** mostly constituted by the glycolipid **LPS** (lipopolysaccharide).

The only known function of the OM is to serve as **a protective barrier**, e.g: Salmonella, an enteric bacterium, can live at the site of bile salt production in the gall bladder and it is generally true that Gram-negative bacteria are more resistant to antibiotics than are their Gram-positive cousins.

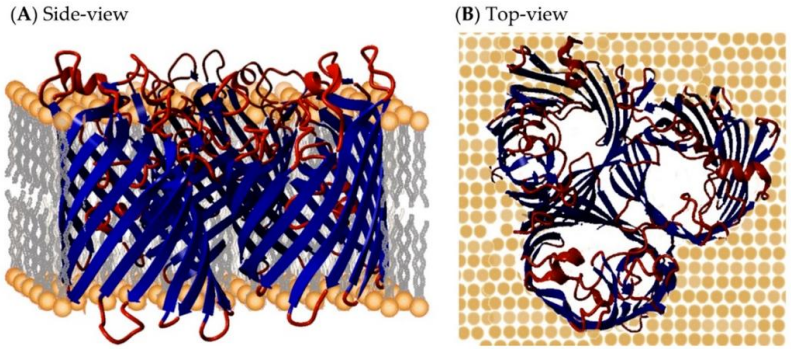


Proteins of Outer membrane

Proteins of the OM belong to two classes: **lipoproteins** and **ß-barrel proteins**.
Functions : transporters: porins, passive and active (siderophores, VitB12) Enzymes, structural defensive proteins, toxins.
Lipid moieties embed **lipoproteins** in the inner leaflet of the OM. A lipoprotein called **Lpp**, murein lipoprotein, or Braun's lipoprotein bouns the OM to the underlying peptidoglyca.
ß-barrel proteins are wrapped into cylinders, very abundant and are specific or unspecific (porins) transporters.

- Special channels: **porins**

Water filled channels that function to allow the passive diffusion of small molecules such as mono- and disaccharides and amino acids across the OM (< 700 Da).



Outer membrane protein (OMP) structure.
H S. Vollan *Int. J. Mol. Sci.* **2016**, *17*(4), 599

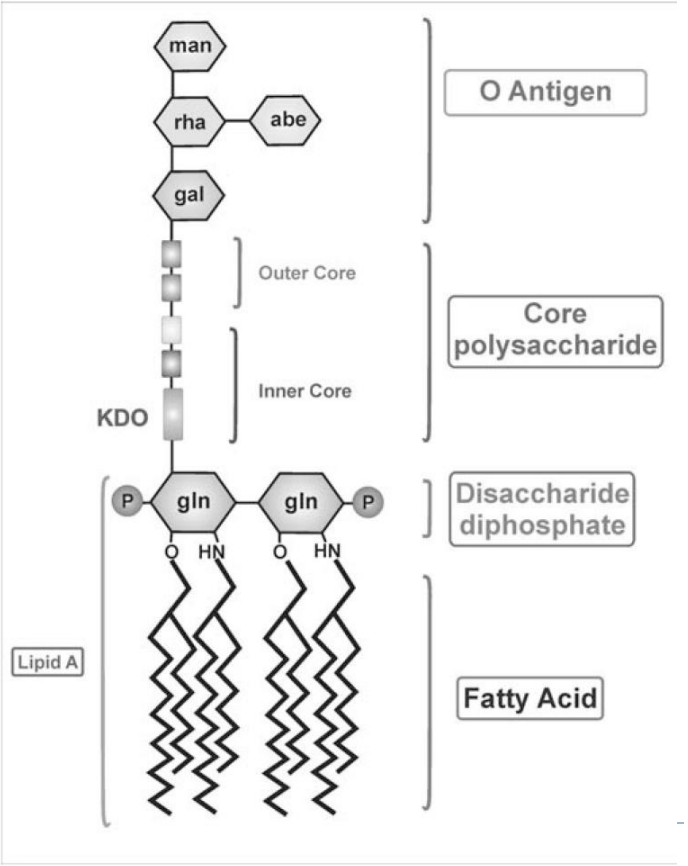
- Other transporters function in the diffusion of specific small molecules, maltose or maltodextrins and anions such as phosphate respectively, across the OM.
When induced by the presence of maltose or phosphate starvation, respectively, these proteins are very abundant as well.

Lipopolysaccharide structure

The **outer leaflet** of the outer membrane contains mostly the negatively-charged molecule **LPS.** It plays a critical role in the barrier function of the OM. It is a conserved glycolipid **(lipid A)**, a **polysaccharide core**, and often an extended polysaccharide chain (**O-antigen)** (10^6 molecules LPS/cell about 1/10 total lipids).

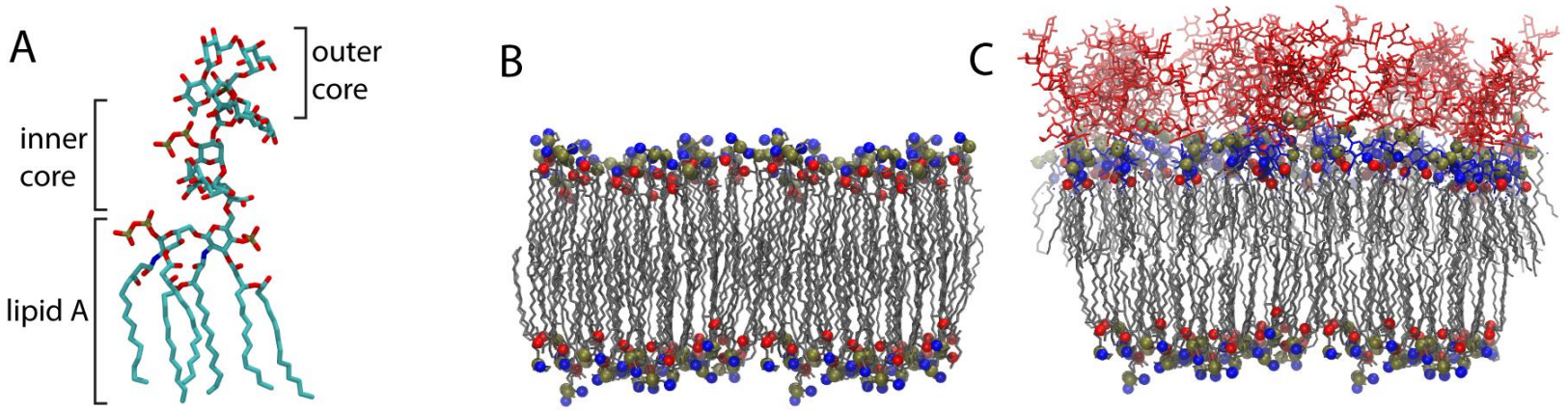
Lipid A contains a disaccharide diphosphate: two phosphorylated N-acetyl-glucosamine (GlcN-P) residues linked to 6-8 fatty acids: It serves to anchor LPS in the OM (Stabilized by Ca^{2+}).

1. A conserved polysaccharide core consisting of KDO (ketodeoxyoctonoic acid), different heptoses, and neutral sugars such as galactose.
2. An outer polysaccaride **O-antigen** (optional) showing units of two to eight sugars repeated many times.



Function of LPS

LPS provides a permeability barrier to **hydrophobic compounds**.



http://simbac.gatech.edu/outer-membrane-proteins/

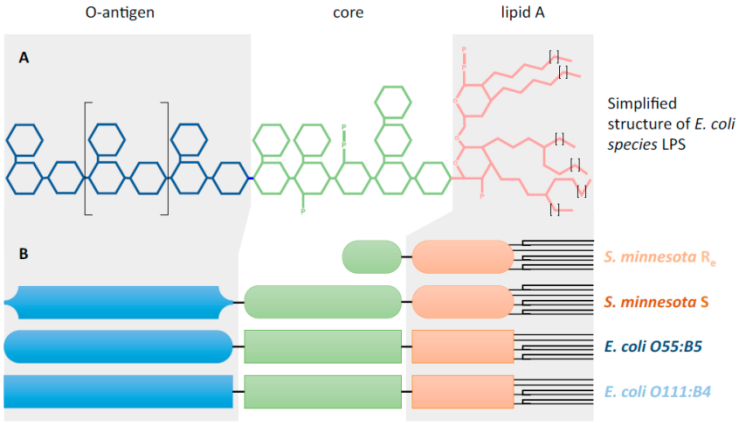
(A) an example LPS molecule. (B) a normal phospholipid bilayer. (C) a model of the outer membrane

O antigen and lipid A and the host

O Antigen (pathogens): The O-antigen varies between species and even within the same species.

It is exposed to the outer environment and host defenses will often raise antibodies to this structure. A particular strain, **serotype**, may be identified by the recognition by specific antibody (For *E. coli* alone, more than 170 serotypes have been identified).

No strict correlation exists between serotypes and disease but some infections are more frequently associated to certain serotypes (*E.. coli* O157:H7).



Selected LPS show different lengths or composition

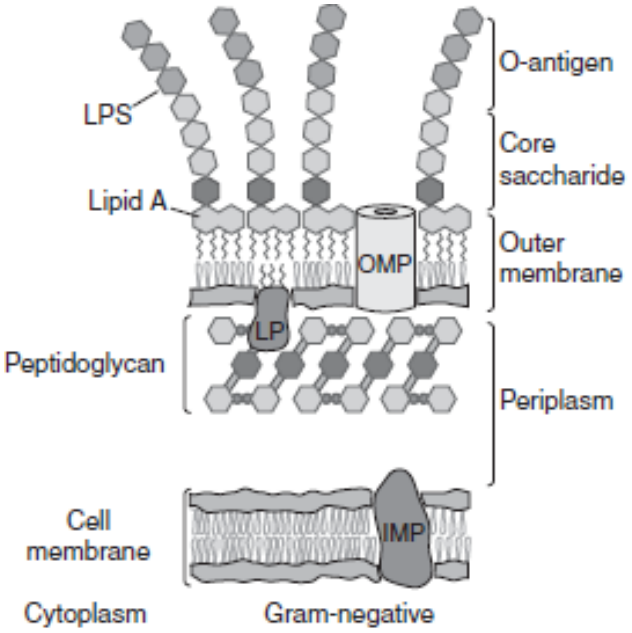
Molecules **2017**, *22*(1), 102

Lipid A: one of the main structure that is recognized by the innate immune system (PAMP) and stimulates inflammatory responses. Lipid A is the main responsible for toxigenic properties of Gram-negative bacteria.

The periplasm an organelle of gram-bacteria

The periplasm (12-15 nm) is densely packed with proteins. Cellular compartmentalization allows Gram-negative bacteria to sequester potentially harmful degradative enzymes.

Periplasmic proteins: binding proteins, which function in transport, and chaperone-like molecules that function in envelope biogenesis.

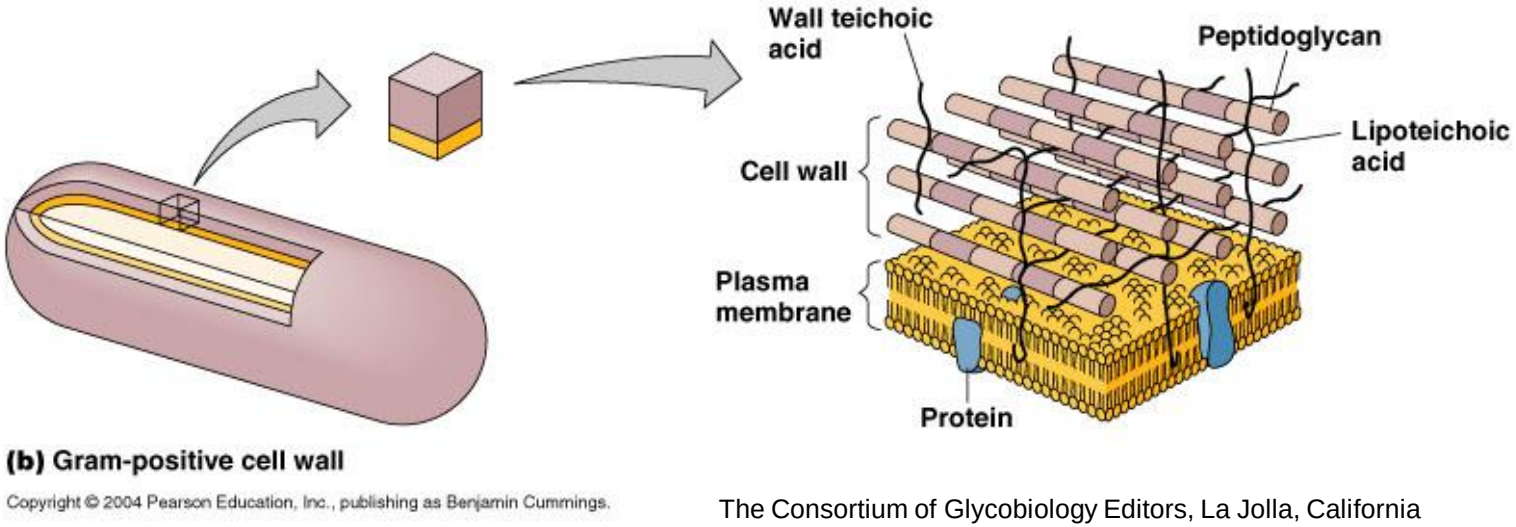


Cold Spring Harb Perspect Biol 2010;2:a000414

IM: all of the membrane-associated functions of the eukaryotic organelles: energy production, lipid biosynthesis, protein secretion, and transport are located in the IM.

Transenvelope Machines: molecular structures that across the cell envelope They are made up of individual protein components that span the peptidoglycan and are located in all cellular compartments (flagella, some secretion systems, efflux pumps).

Cell wall in Gram-positive bacteria



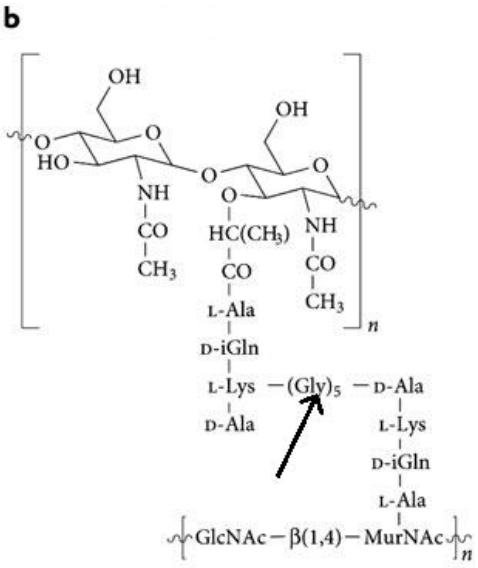
(b) Gram-positive cell wall

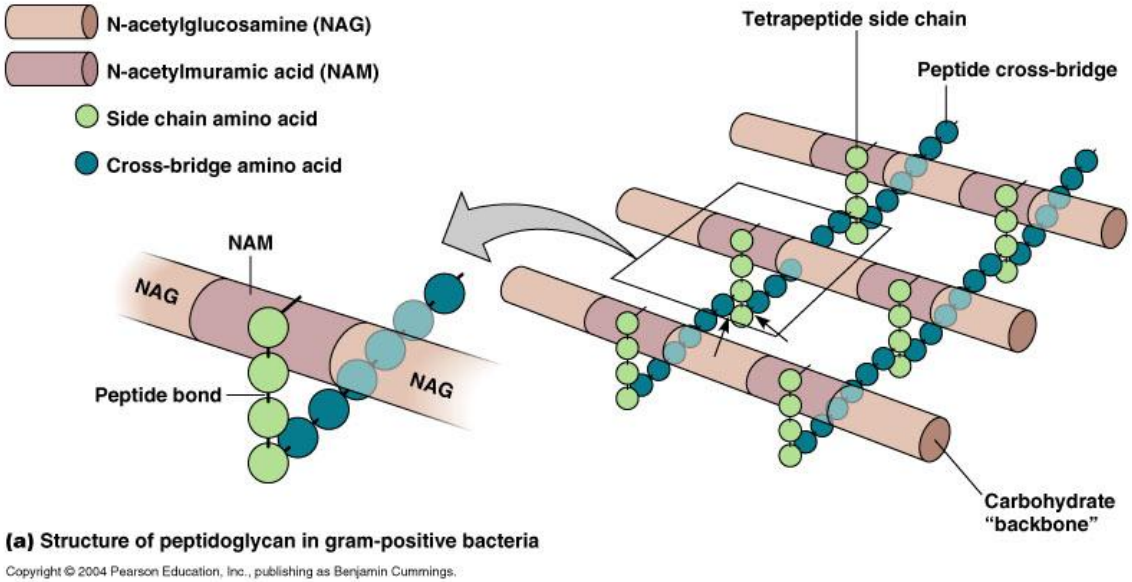
The Consortium of Glycobiology Editors, La Jolla, California

Gram+: the outer membrane is absent, to withstand the turgor pressure exerted on the plasma membrane, Gram-positive microorganisms are surrounded by layers of peptidoglycan **many times thicker** than is found in E. coli. 10% of the dry weight of the cell wall in Gram- bacteria and as much as 20–25% of that in Gram+ bacteria.

Cross-bridges of peptidoglycan

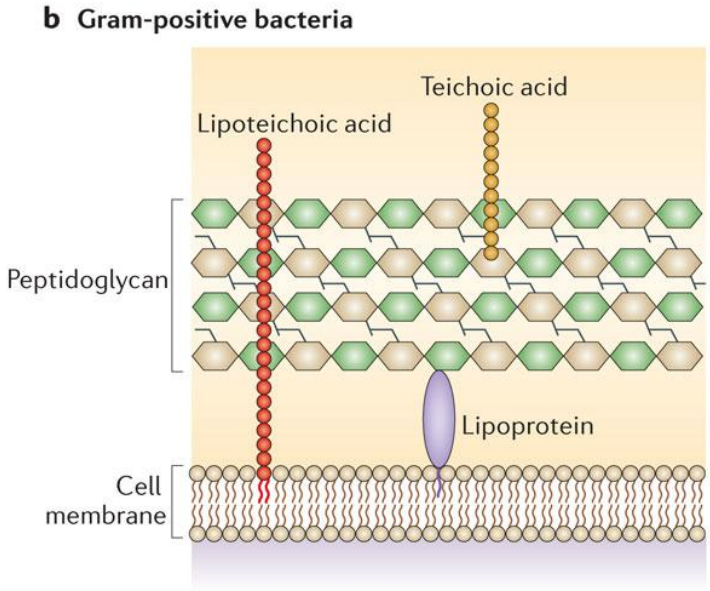
Parallel strands of polysaccharide composed of *N*-acetylglucosamine and *N*-acetylmuramic acid (MurNAc) in β1-4-linkage, which surround the bacterium.





The structure of the polypeptide **cross-bridges** may vary but they always have a **tetrapeptide side chain**, which consists of 4 amino acids attached to NAMs. The amino acids occur in alternating D and L forms.

Cell wall in Gram-positive bacteria

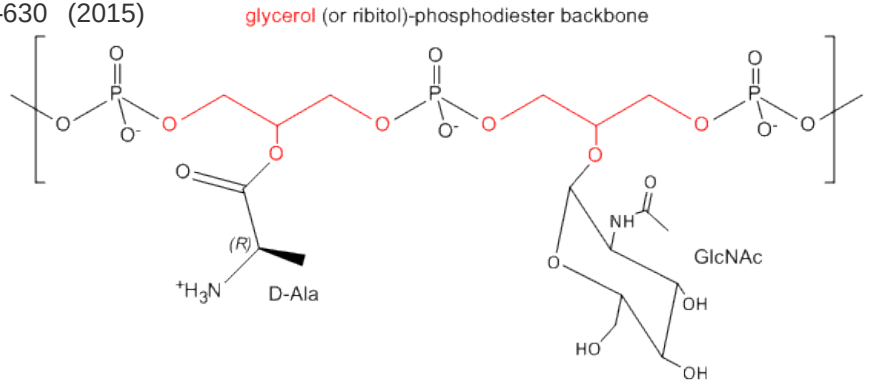


Nature Reviews Microbiology **13**, 620–630 (2015)

Cell wall constituents: **teichoic acids (TA)** and **lipoteichoic acids (LTA):** Anionic polymer of glycerol or ribitol joined by phosphate groups with some substituents (D-Ala, GlcNAc).

TA (WTA) is covalently linked to muramic acid and links various layers of the peptidoglycan mesh together. LTA is anchored to membrane lipids.

TA and LTA have a structural role, form a continuum of anionic charge, component and adhesins.

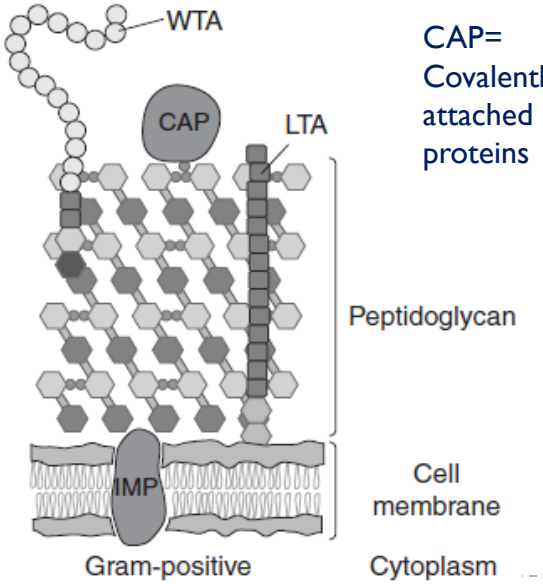


n≈ 60 repeats

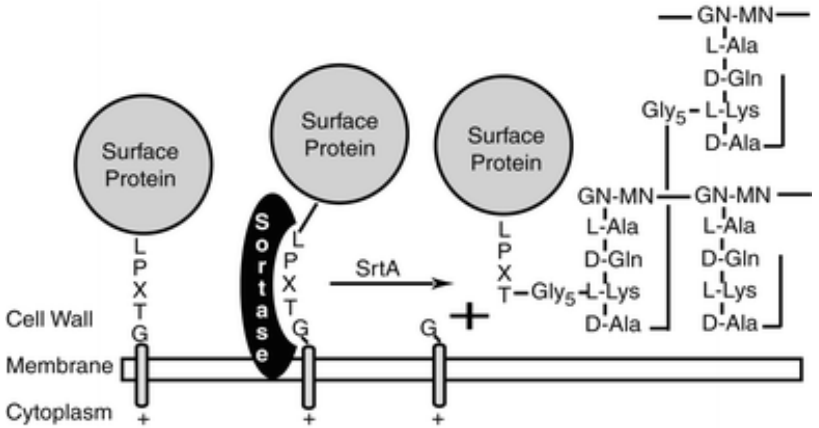
Surface proteins in Gram+ bacteria

Surface proteins are often covalently attached (CAP) to the aa part of peptidoglycan, by **sortases**, enzymes that catalyze transpeptidation reactions. Sortases recognizes a C-terminal sorting sequences on CAPs.

Some CAPs recognize components of host extracellular matrix (adhesins), others are involved in immune system evasion (impedins).



CAP= Covalently attached proteins



Protein A (*staph*): binds to Fc region of antibody Ig

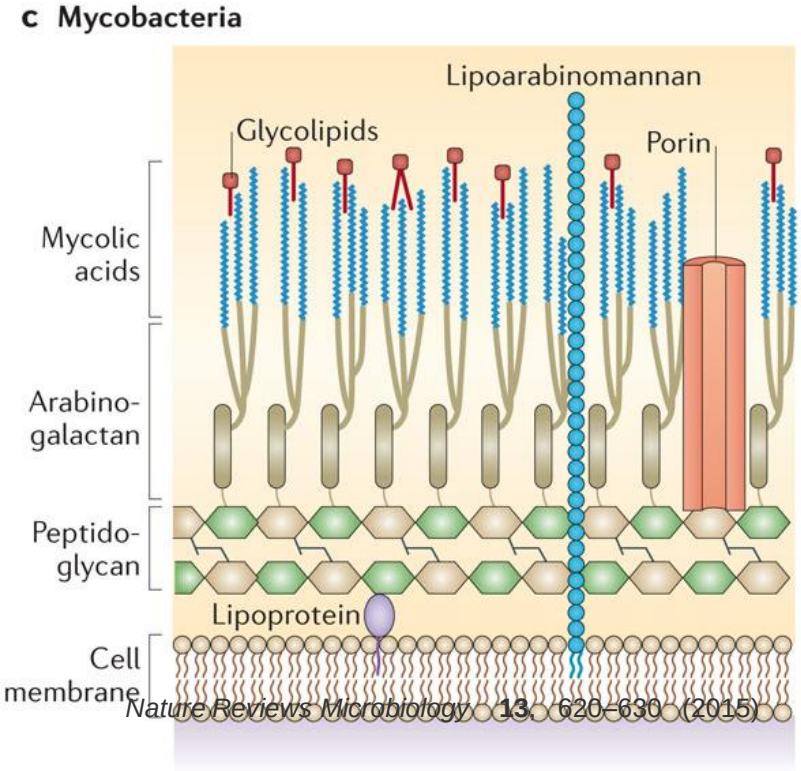
Protein M (*streptococcus*) virulence by protecting the bacteria against complement deposition and phagocytosis,

The cell envelope of actinobacteria

A group of bacteria (actinobacteria) that includes pathogens such as *Mycobacterium tuberculosis* and *Corynebacterium diptheriae*.
The cell envelope is very complex and different from that of gram + and – bacteria .

The peptidoglycan layer that surrounds a standard IM is covalently attached to the branched polysaccharide **arabinogalactan,** which is covalently attached to the long fatty acid **mycolic acids**. These mycolic acids have very long alkyl side chains (up to C90) that give the bacteria a waxy appearance.

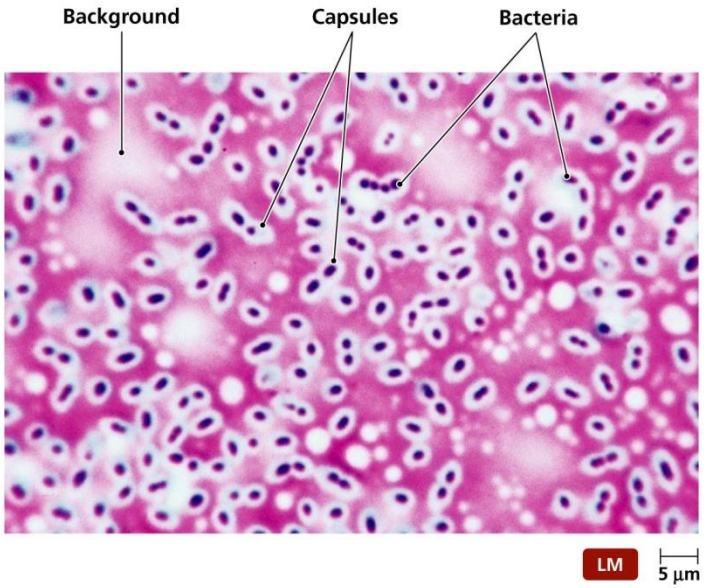
Unlike other Gram-positive bacteria, this group of bacteria have a sort of OM (evolved independently from that of Gram-). Strong resistance to hydrophilic substances.



Secreted extracellular material: the capsule

Caspule (slime layer, glycocalyx): extracellular structure consisting of layers of secreted **polysaccharides** (in few cases polypeptides).

Capsule protect cells from effects of drying or desiccation because it is highly hydrophilic and retains water. Some structures are very large with size vary in molecular mass from 10^4 to about 10^6.

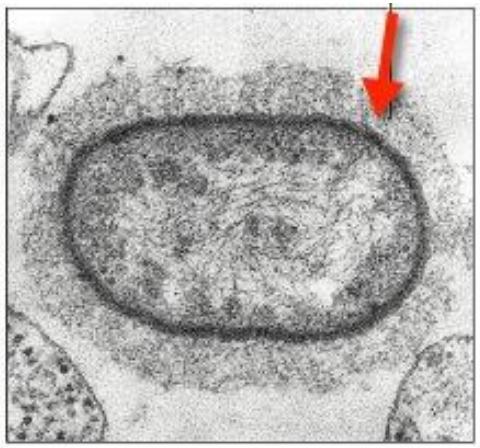


They are not essential to cell viability, may be strain-specific, and synthesized by the bacteria wall only in some conditions. *In vitro* culture they give a **mucoid** appearance to bacterial colonies or are often lost.

Capsule is attached tightly to the bacterium and has definite boundaries. It may be not tightly bound and readily released from the cell surface, it is loosely associated with the bacterium and can be easily washed off (slime layer).

Capsule may protect pathogens from host immune system

Capsule usually forms a highly antigenic structure **(K-antigen)**. However, some pathogens produce capsules with composition identical to host molecules which become poor immunogenic (**molecular mimicry)**.
E.g. *S. pyogenes* (hyaluronic acid), *Pseudomonas* (mannuronic acid), *Neisseria* (K1 polysialic acid).



Capsule type	Structure
K1 (polysialic acid)	◆α8◆α8◆α8◆α8◆α8◆
K5 (N-acetylheparosan)	■α4◇β4■α4◇β4■α4◇
Group B Streptococcus **(hyaluronate)**	■β4◇β3■β4◇β3■β4◇

Essentials of Glycobiology Chapter 20, Table 1

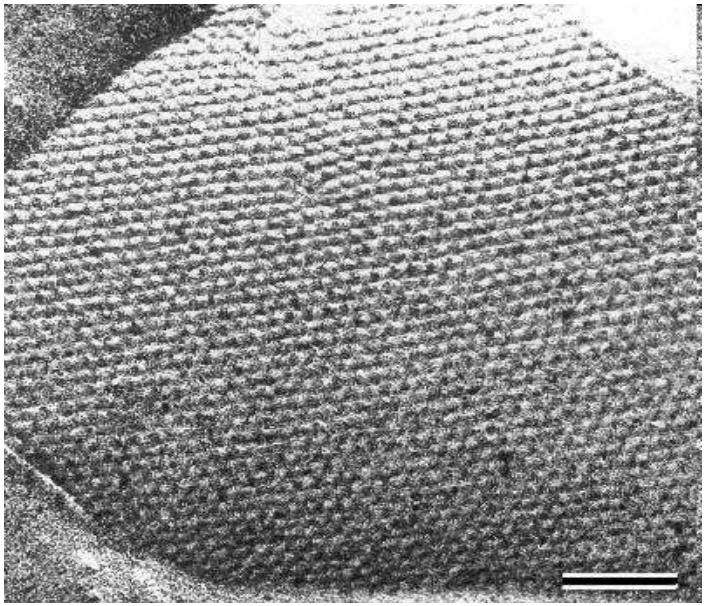
Examples of capsular polysaccharides mimic host macromolecules:

Capsule can mediate adherence of cells to surfaces.
It prevents complement-mediated lysis, phagocytosis, and cell-mediated immune mechanisms.
The capsule enhances the ability of bacteria to cause disease **(virulence factor).**

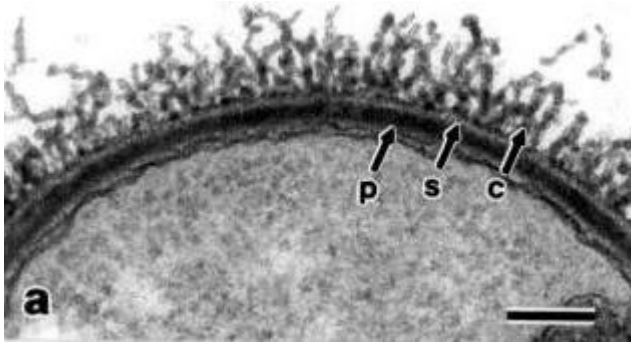
Cell-surface associated components: S-layer

S-layers (surface layer): layer of **proteins** in the outermost cell envelope of a broad range of bacteria. S-layers are composed of a single protein or glycoprotein (mw 40-200 kDa) which aggregates and forms an highly ordered two-dimensional structure.

S-layers are generally 5 to 10 nm thick (up to 15% of total protein) and show pores of identical size (2-8 nm diameter) and morphology.



Transmission electron micrograph of a freeze-etched, preparation of a bacterial cell with an S-layer with hexagonal symmetry. Bar = 100nm.



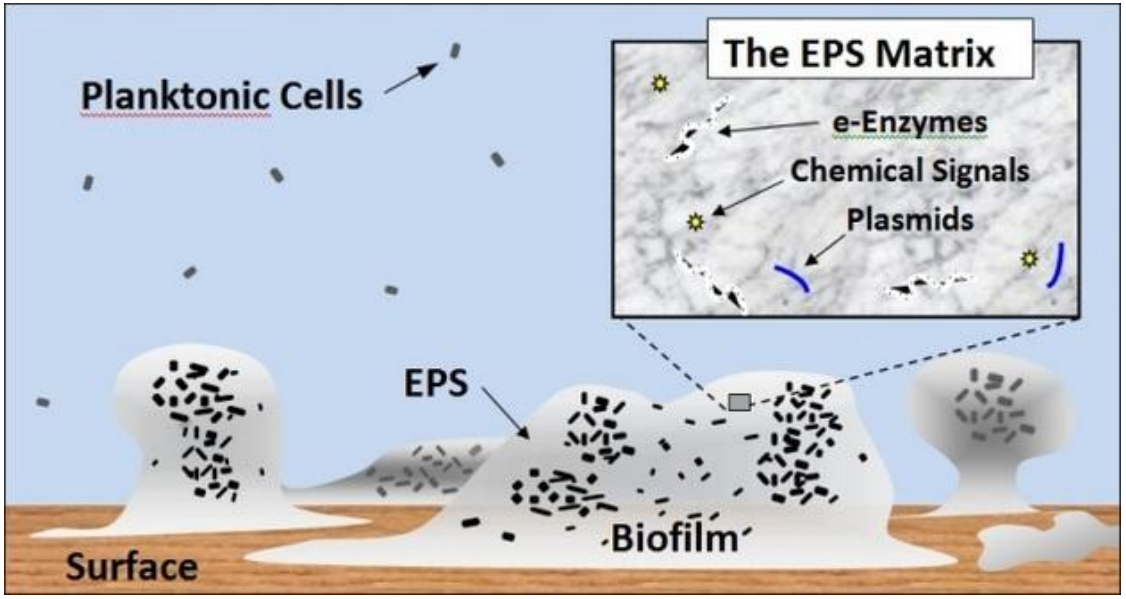
The surface of Bacillus anthracis.
P= cell wall; S layer ; C= polyglutamic acid capsule;

Functions: Permeability barrier ? S-layers contribute to virulence by protecting the bacterium against complement and attack by phagocytes. (e.g. *C. diphtheriae* e *B. anthracis*),

Extracellular polymeric substances (EPS)

An extracellular slime layer (**EPS matrix**) composed of various organic substances such as polysaccharides, enzymes (e-enzymes), proteins and extracellular DNA (eDNA). EPS matrix is produced and secreted by **biofilms**, where cells aggregate together attached on a surface. Biofilms surround themselves with EPS.

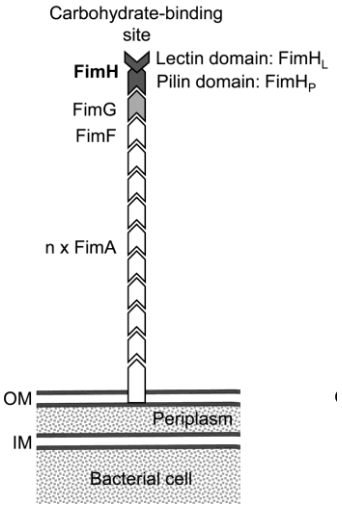
EPS play significant roles in the formation and function of microbial aggregates, including adhesion phenomena and matrix structure formation. These polymers give to the biofilm a complex, three-dimensional structure and a protective environment for e-DNA (plasmids) and e-enzymes.



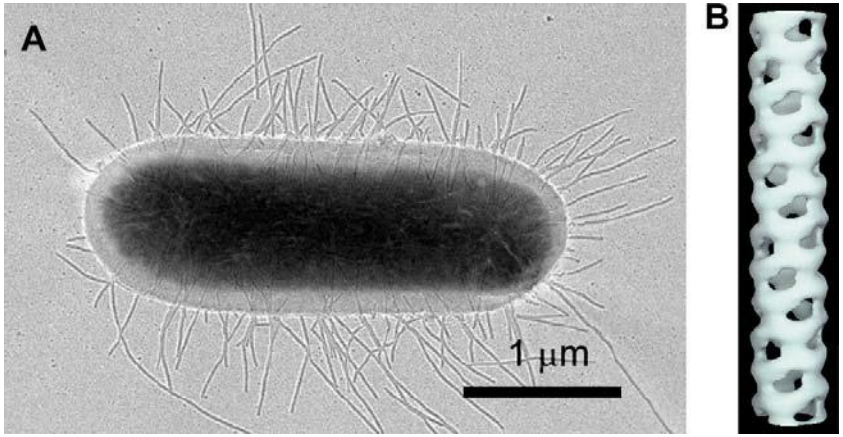
Surface appendages: fimbriae/pili

Fimbriae/pili: filamentous structures that **may be present** on the surfaces of bacteria and extend well beyond the LPS and capsule. Elongated (0,1-10 µm) submicroscopic (5–8 nm), numerous (100-1000/cell) multisubunit protein appendages.

High diversity in structure and biogenesis typically formed by non-covalent polymerization of pilus protein subunits : **pilin**s.



Type 1 pili of E. coli



Electron micrograph of an uropathogenic *E. coli* cell bearing type 1 pili. (B) 3-D reconstruction of a type 1 pilus rod from electron microscopy data.

Monomers of about 20 kDa form a flexible cylinder which generates the pilus body. Additional pilins may be added to the fiber and often function as host cell **adhesins**.

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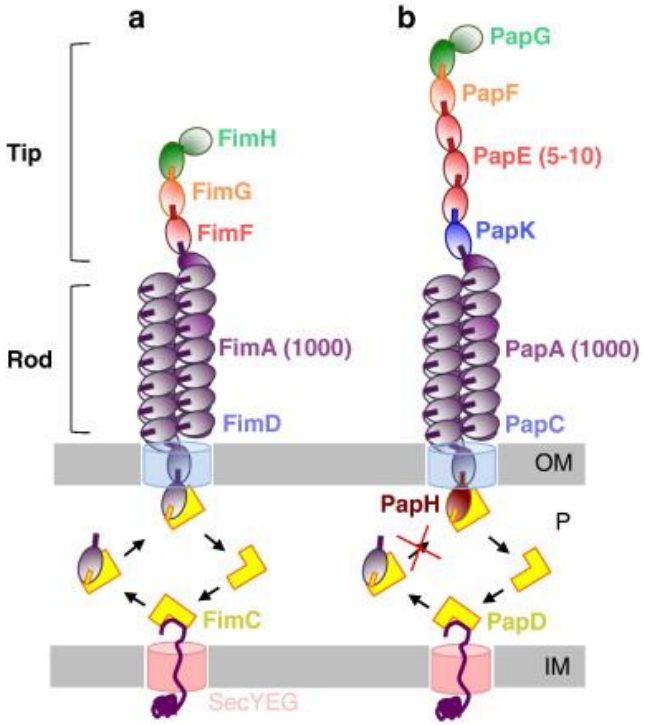
Type I and Type P fimbrial adhesins

Type I and P pili are archetypal examples of fimbriae. They show similar structure: regulatory as well as biosynthetic genes for fimbrial subunits, protein chaperons and outer membrane anchors.
Tip-located subunit are adhesins that contains the receptor-binding activity (**lectins**).

Biogenesys: pili subunits are located to the periplasm (P) via the Sec general secretory pathway across the inner membrane (IM), where they are folded with the aid of a chaperone and delivered to the usher, an outer membrane protein (FimD, PapC). Here, pilins undergo a translocation beyond the outer membrane (OM) to form a pilus.
The usher orchestrates the sequential addition of pilus subunits which may be divided into a 'tip' and a helically wound 'rod'.

Adhesion by the type I and P pili is strengthened by the quaternary structure of their rod sections.



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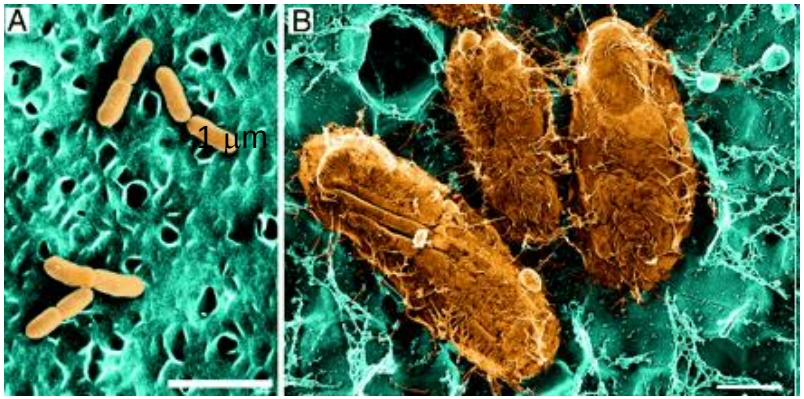
Fimbriae are widespread structures and recognize different receptors.

Fimbriae are very common in Gram-negative bacteria, but occur in some Gram-positive bacteria covalently linked to the peptidoglycan. A single strain of *E. coli* is able to express several distinct types of fimbriae encoded by distinct regions of the chromosome or plasmids. This genetic diversity permits an organism to adapt to its changing environment and exploit new opportunities presented by different host surfaces.

Host cell receptors for pili are carbohydrate residues of glycoproteins or glycolipids. Examples:

Type-I (fim) fimbriae: **FimH** lectin binds to **D-mannose** residues on glycoprotein receptors on eukaryotic cell surfaces of within the urinary tract or intestine.

Tip proteins of **P pili** (**papG**) recognizes a **Galα1–4Gal** disaccharide of glycolipids (globosides) of epithelial cells in the upper urinary tract.



Type 1 pilus-mediated bacterial attachment to the bladder epithelium

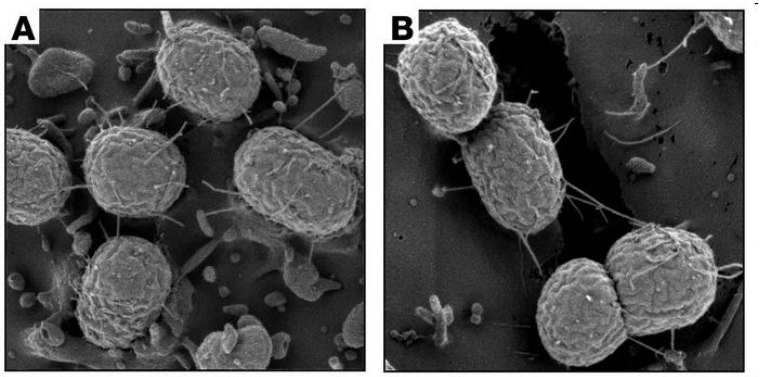
PNAS August 1, 2000 vol. 97 no. 16 8829

Lectin variants recognize different but related oligosaccharides on receptors differently distributed within host tissues.

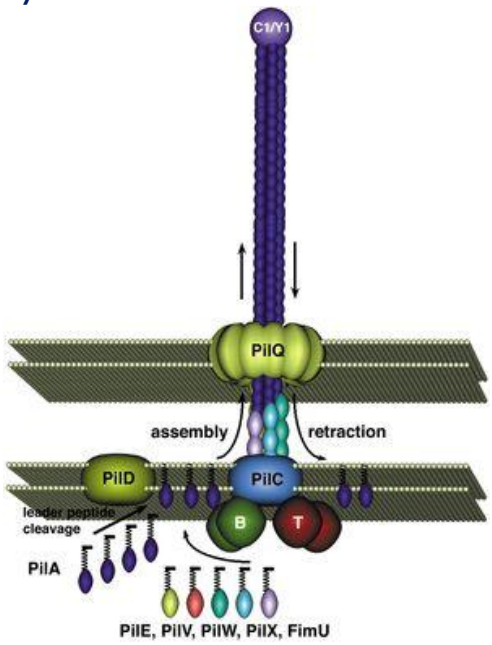
Retractile Type IV pili

Fimbriae/pili are involved in bacterial adhesion to host cells, but Type IV pili also in locomotion. This widespread organ of attachment can aggregate laterally forming bundles and it is able to generate **motile forces.**

It is expressed by many Gram-negative bacteria (pathogenic E. coli (EPEC, EHEC), *S. enterica, P. aeruginosa, L. pneumophila, N. gonorrhoeae, N. meningitidis, and V. cholerae)* and recently also found in G+.



Intestinal adherence associated with type IV pili of enterohemorrhagic *E. coli* O157:H7 *J. Clin. Invest.* 118:2



Model of type IV pili assembly and retraction. (P. aeruginosa)

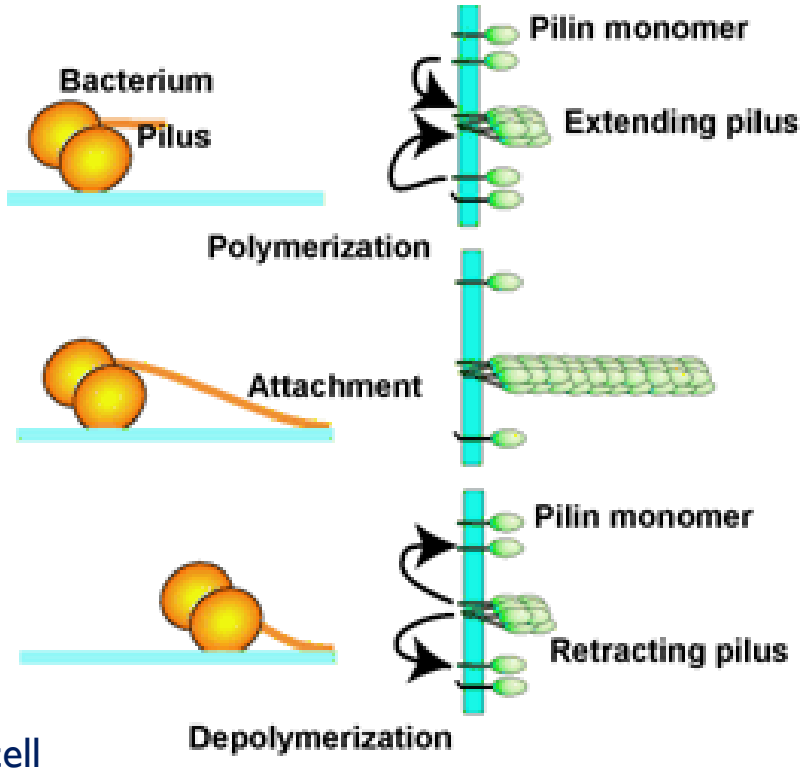
These organelles are composed of a homopolymer of a single pilin subunit and an adhesive subunit characterized at the tip of some pili with different specificities according to the different species.

Many accessory molecules are required for pilus biogenesis.

Pilus retraction is required for a specialized **form of bacterial movement** across the mucosal epithelia called **twitching motility.**

Twitching motility by using type IV pili

Bacteria can move on surfaces using type IV pili which can be **retracted** through the bacterial cell wall.
It occurs by the extension, adhesion (anchoring), and then retraction of polar type IV pili, which operate in a manner similar to a grappling hook.
ATPase is involved in type IV pilus retraction and it is also required for force-dependent pilus elongation.
Switching between pilus polymerization and depolymerization is essential for cell motility.

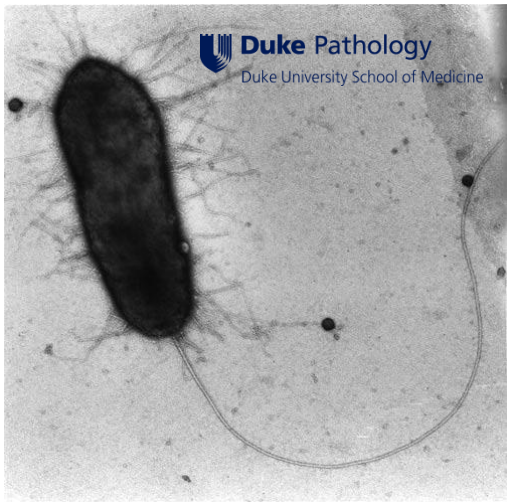


Model for Neisseria gonorrhoeae motility.

Extension per se was not associated with cell movement, presumptively because the pili are too flexible to push cells forward, but cells were moved by retraction of pili after they had attached to the substratum at their distal tip.

Pili in *P. aeruginosa* showed that pili extend and retract at approximately 0.5 μm/sec

Surface appendages : flagella

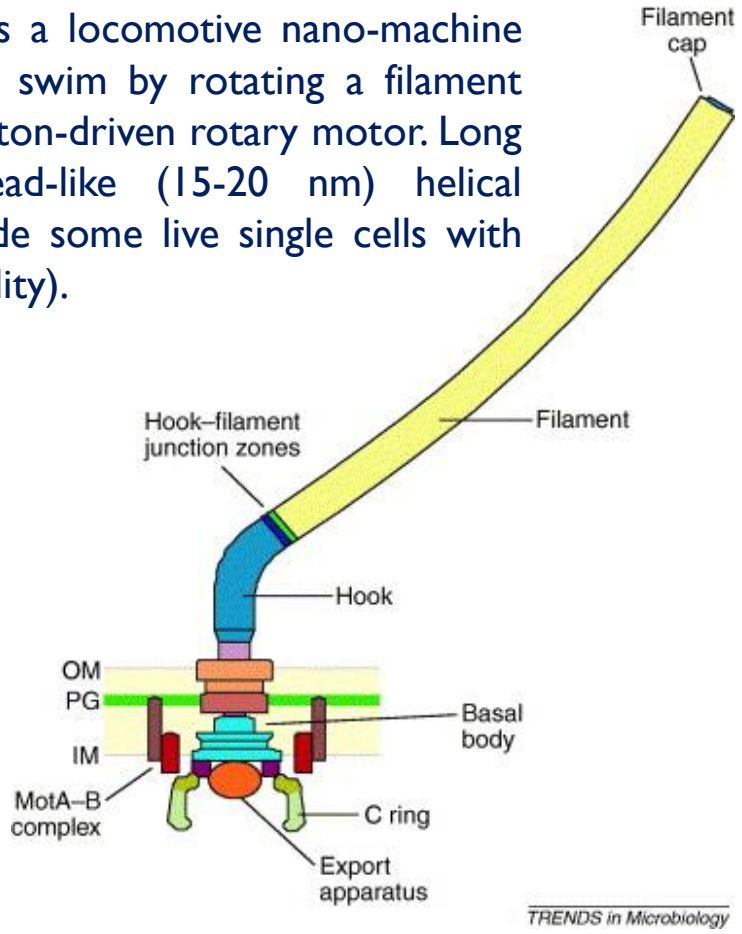


Negatively stained bacterium with pili and a flagellum

The bacterial flagellum is a locomotive nano-machine that enables bacteria to swim by rotating a filament that is powered by a proton-driven rotary motor. Long (up to 20 µm), thread-like (15-20 nm) helical appendages which provide some live single cells with the ability to move (motility).

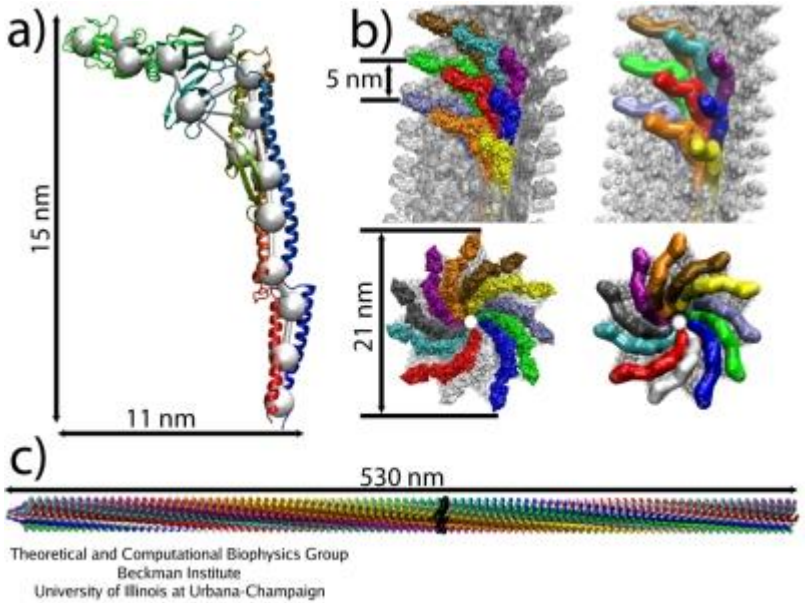
The bacterial flagellum consists of at least three parts: a basal body, a hook and a filament.

- The basal body, which works as a motor.
- A hook: protein complex as a universal joint that extend outwards from the cells.
- A filament: made by polymerized subunits of **flagellin** a very conserved globular protein.



This natural rotary motor propels the flagella of E. coli cells, allowing them to move forward. (Figure by MIT OCW.)

Flagella structure and its recognition by the host



Functions in host interactions as virulence factor: **movement through fluids**, urine and intestinal contents or viscous media such as mucin.
Flagellin is highly antigenic (H antigens) and often its expression is regulated. Specific host receptors recognize this structure.

(a) Single flagellin monomer, (b): Arrangement of the monomers in the filament, viewed from the side and from the bottom (c):
(b) Simulated segment of the filament (1100 monomers)