BIOL 4640 Study Guide for Exam 2 (Wed 10/28/26): Lecture 16 Pili, External Structures and Envelope Styles

Source: Dr. Rowen's lecture slides, in the order of the 2025 Exam 2 study guide outline.
Slide blanks still empty in the notes are filled from the textbook and marked "textbook".
Tags like [L16 p08] give the source page of each line.
Textbook to read (Slonczewski 6e, Textbook page on the site): 3.6 Specialized Structures and 25.2 Microbial Attachment: First Contact (pili); 3.3 The Envelope and Cytoskeleton (the cell envelope). **Also useful:** 19.1 Archaeal Diversity at a Glance (archaea); 18.3 Firmicutes, Mycoplasma, and Actinobacteria (Mycoplasma, pp. 734-735); 3.5 Cell Asymmetry, Membrane Vesicles, and Extensions (stalks, nanotubes).
(teal italics) = plain meaning of the word before it. ⚠ **Do not mix up = a look-alike pair to keep apart.**

Contents

B. External Structures of Bacteria 3
What external structures are 3
Main kinds and jobs 4
Pili, fimbriae and motility 4
Appendages and internal structures 4
2. Pili and Fimbriae (3.6 and 25.2 6e) 4
What they are 5
Three types of pili 5
Roles and comparison with flagella 5
a. Structure, arrangement in cells and assembly 6
Basic structure and size 6
Number per cell 6
Pilin and tip protein 6
Proteins that form a pilus 6
Changing which pilin is made 7
Assembly compared with flagellum 7
b. Roles of Common pili, Sex pili and Type IV pili 8
Common pili or fimbriae 8
Sex pili 9
Sex pilus steps and F plasmid 9
Type IV pili and twitching 9
Other pilus types 9
Which types are called fimbriae 10
c. Assembly of Type I and Type IV, how it differs from flagellar 10
Type I pili assembly 10
Type IV pili assembly 12
Pili versus flagellum filament 12

Flagellum assembly order 12
3. Other external structures 13
Others as an external structure 13
Stalks and Caulobacter cycle 13
TipN protein 14
Nanotubes 14
C. Cell envelope (glycocalyx, outer membrane, cell wall, and cell membrane) 15
Cell envelope parts 16
Five envelope styles 16
Gram negative layers 16
Archaea and S-layer 17
1. Know the layers and components of the envelope in each of the following types of bacteria. How similar and different from each other 17
Five envelope styles 17
What the styles share 17
How they differ 17
Peptidoglycan differences 18
Electron microscope appearance 18
a. Gram positive bacteria 18
Layers outside to inside 19
Thick wall, no outer membrane 19
Molecules to know 19
b. Gram negative bacteria 19
Layers outside to inside 20
Peptidoglycan thickness 20
Outer membrane 20
c. Mycobacteria (3.3 6e) 20
What mycobacteria are 20
Layers outside to inside 21
Wall sugars and mycolic acid 21
Other parts 21
Acid fast envelope 21
d. Archaea (19.1 6e) 21
S-layer in archaea 21
Cell wall 22

Membrane lipids 22
e. Mollicutes (Mycoplasma) (pp 734-5 6e) 23
No cell wall 23
Membrane proteins 23
Membrane surface 23
How do scientists determine the structure of the envelope of bacteria? 23
Staining techniques 23
Electron microscopy 24
Confirming a Gram positive envelope 24
How the nature of the envelope affects bacterial cells? 24
Why the envelope matters 25
Glycocalyx and S-layer effects 26
Penicillin in stream water 26
2. Know role, feature and basic composition of each of these layers (3.3 6e) 26
Membrane lipids 26
LPS and membrane proteins 27
a. Glycocalyx (capsule and slime layer): role and composition 28
What the glycocalyx is 29
Composition 29
Sugar chains and EPS 29
Capsule examples and conclusion 29
Roles 29
b. S-layer: composition and where located 30
What the S-layer is 30
Where it is located 30
Hypothesized roles 32

Click a line to jump to it. The PDF side panel (bookmarks) has the same parts as tabs and topics as subtabs.

B. External Structures of Bacteria

Book: 3.6 Specialized Structures, Pili and Stalks (chapter 3 PDF, p. 123).

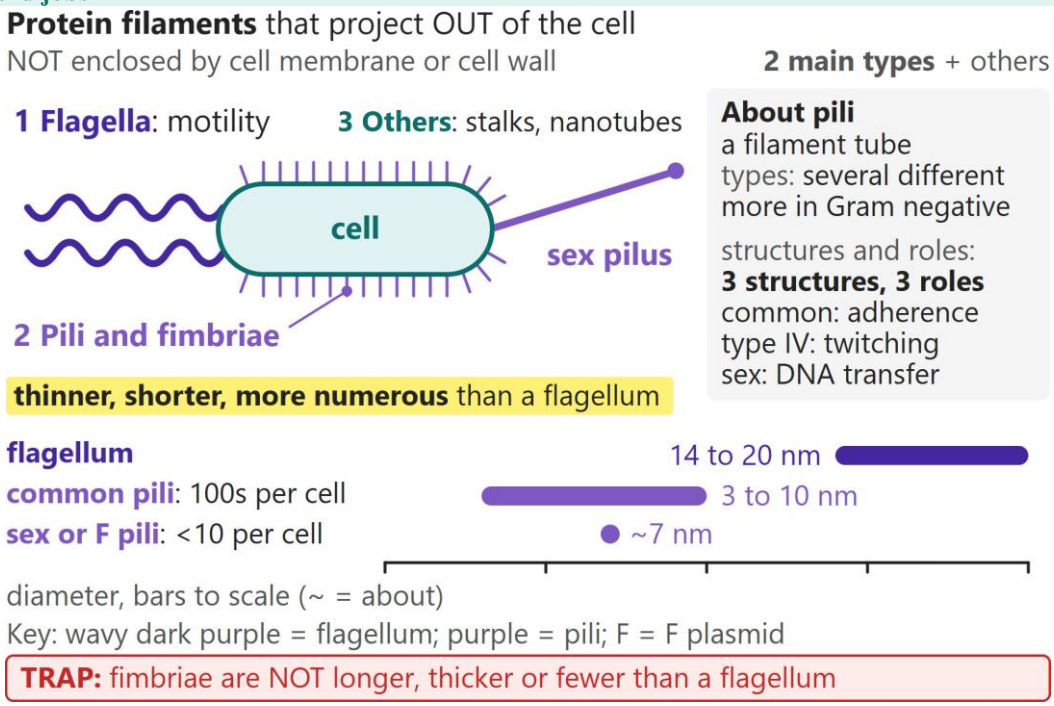
What external structures are

External structures: protein filaments that project out from the cell. [L16 p03, L16 p20, L16 p21]

They are **NOT** enclosed by the cell membrane *(thin skin around the cell)* or cell wall *(stiff layer around the cell membrane)*. [L16 p03, L16 p20, L16 p21]

Watch out: This describes the protein filaments; nanotubes *(tiny tube joining two cells)*, listed under Others, are an extension of the cytoplasm *(jelly inside the cell membrane)* or periplasm *(space between the two membranes)* between two cells. [L16 p03, L16 p20, L16 p21]

Main kinds and jobs



External structures: flagella, pili and fimbriae, stalks and nanotubes [L16 p03]

On the figure: flagella · pili and fimbriae · stalks · nanotubes · thinner

There are **two main types** of external structure: **flagella** *(whip tails that spin)*, and pili *(thin hairs on the cell)* and fimbriae *(short sticky hairs)*. [L16 p03]

Flagella: motility *(ability to move)*. [L16 p03, L16 p20, L16 p21]

Pili and fimbriae: adherence. [L16 p03, L16 p20, L16 p21]

Others: **stalks** *(stem that anchors the cell)*, **nanotubes**. [L16 p03, L16 p20, L16 p21]

Pili, fimbriae and motility

Fimbriae are **thinner** than **flagella**.

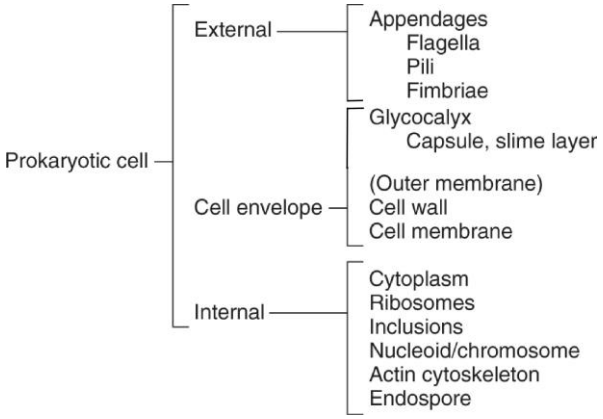
Pili mediate attachment, DNA *(cell genetic instruction molecule)* transfer and a form of motility.

Twitching *(jerky crawl using pili)*, **gliding** *(smooth crawl on a surface)* **and sliding** *(passive spreading by pushing)* are motility not mediated by flagella.

Appendages and internal structures

Prokaryotic cell: **appendages** *(parts that stick out)* are external structures: flagella, pili and fimbriae. [L16 p24]

Prokaryotic cell: **internal structures** are cytoplasm, **ribosomes** *(protein building machine)*, **inclusions** *(stored material in the cell)*, nucleoid *(DNA region without a membrane)* or chromosome *(one long DNA molecule)*, actin *(protein of eukaryote thin filaments)* cytoskeleton *(inner scaffold of the cell)* and **endospore** *(tough dormant body formed inside some bacteria)*. [L16 p24]



Prokaryotic cell parts sorted into external, cell envelope and internal [L16 p24]

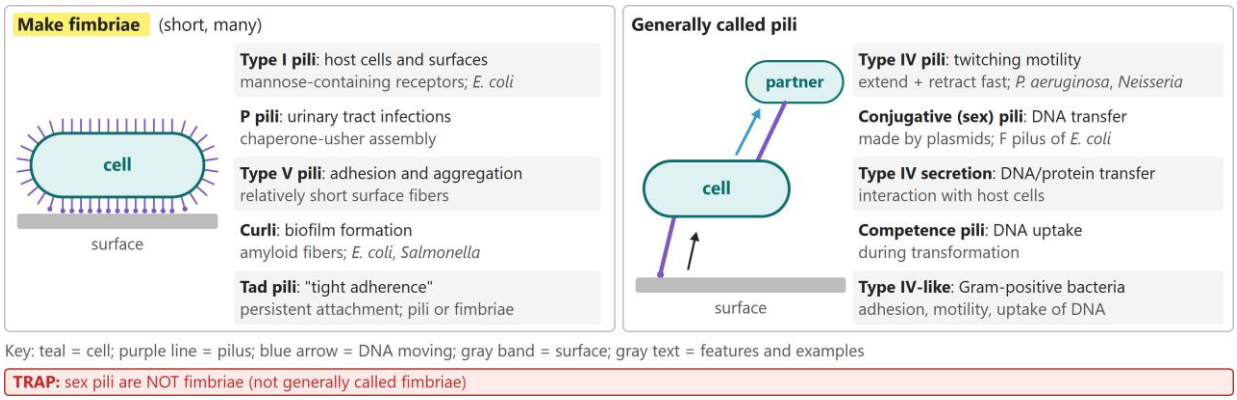
On the figure: Appendages · flagella · pili · fimbriae · ribosomes · inclusions · actin cytoskeleton · endospore

2. Pili and Fimbriae (3.6 and 25.2 6e)

Book: 3.6 Specialized Structures, Pili and Stalks (chapter 3 PDF, p. 123); 25.2 Microbial Attachment: First Contact (chapter 25 PDF, p. 36).

⚠ **Do not mix up: Fimbriae *(short sticky hairs)* are thinner than flagella *(whip tails that spin)***, not thicker or longer.

⚠ Do not mix up: Fimbriae are the common pili (Type I, P, Type V, curli); Type IV and sex pili are generally called pili, NOT fimbriae. [L16 p05, L16 p06]



Types that make fimbriae and types generally called pili [L16 p08]

On the figure: Fimbriae · Type I · Type V · curli · Tad · Type IV · conjugative

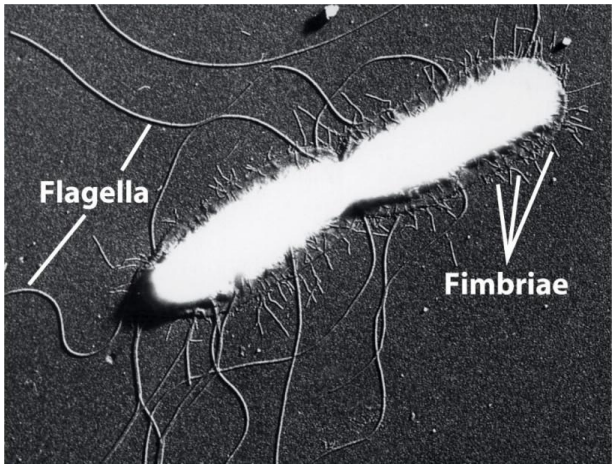
What they are

Pili and fimbriae are protein filament tubes that extend from the cell. [L16 p04]

They are thinner, shorter and more numerous than **flagella**. [L16 p04]

Several different types exist: **Type I**, P, **Type IV**, **Type V**, **curli**, **conjugative** (sex), Type IV secretion, competence *(ability to take up DNA)*, **Tad** *(tight adherence pili)*, and Type IV-like pili in **Gram positive** *(thick wall, stains purple)* bacteria. [L16 p04]

They are more commonly observed in **Gram negative** *(thin wall plus outer membrane)* bacteria. [L16 p04]



Flagella are long; fimbriae are short bristles along the cell [L16 p04]

On the figure: flagella · Fimbriae

Three types of pili

Pili form **3** types of structures, each with a different role: [L16 p04, L16 p05, L16 p06, L16 p09, L16 p10]

1) Common pili or fimbriae: adherence. [L16 p04, L16 p05, L16 p06, L16 p09, L16 p10]

2) Type IV pili: twitching *(jerky crawl using pili)* motility *(ability to move)*. [L16 p04, L16 p05, L16 p06, L16 p09, L16 p10]

3) Conjugative, sex or F pili: DNA *(cell genetic instruction molecule)* transfer. [L16 p04, L16 p05, L16 p06, L16 p09, L16 p10]

Roles and comparison with flagella

Flagella, capsule *(firm sugar coat outside the cell)* and S-layer *(crystal like protein coat outside)* do not mediate all three: flagella give motility, while capsule and S-layer roles are attachment and protection.

Pili vs fimbriae

	Fimbriae (common pili)	Other pili (Type IV, sex pili and more)
What it is	Fine, hairlike protein filament tubes from the cell surface	Protein filament tube; the DNA transfer pilus is an elongate, hollow appendage
Main role	Adherence to other cells and surfaces; biofilm	Type IV: twitching motility, DNA uptake. Sex (F) pili: DNA transfer by conjugation
Width	**3-10 nm**	Sex pili: about **7 nm** (flagellum **14-20 nm**)
How many per cell	Numerous: **100**s per cell	Sex pili: **only** a few (<**10**)
Key part or action	Adhesin protein at the **tip** binds specific molecules (usually sugars)	Type IV: extend, attach, then shorten (retract). Sex pilus: shortens to bring cells close
Types with this name	Type I, P, Type V, curli (Tad: can be called pili or fimbriae)	Type IV, conjugative (sex), Type IV secretion, competence, Type IV-like in **Gram positive**

Table source: [L15 p04, L16 p04, L16 p05, L16 p06, L16 p08, L16 p09, L16 p10, L16 p11]

a. Structure, arrangement in cells and assembly

Book: 3.6 Specialized Structures, Pili and Stalks (chapter 3 PDF, p. 123); 25.2 Microbial Attachment: First Contact (chapter 25 PDF, p. 36).

⚠ **Do not mix up: Pili *(thin hairs on the cell)* add subunits *(building block piece)* at the base; flagella *(whip tails that spin)* add flagellin *(protein that builds the whip)*** at the tip. Do not flip them.

⚠ **Do not mix up: The pilus *(thin hair for sticking or DNA swap)* tip is NOT made of the filament protein: it is 1-2 proteins different from the filament.** [L16 p13, L16 p14]

Basic structure and size

Basic structure: protein filament tube extending from the cell. [L16 p04, L16 p06]

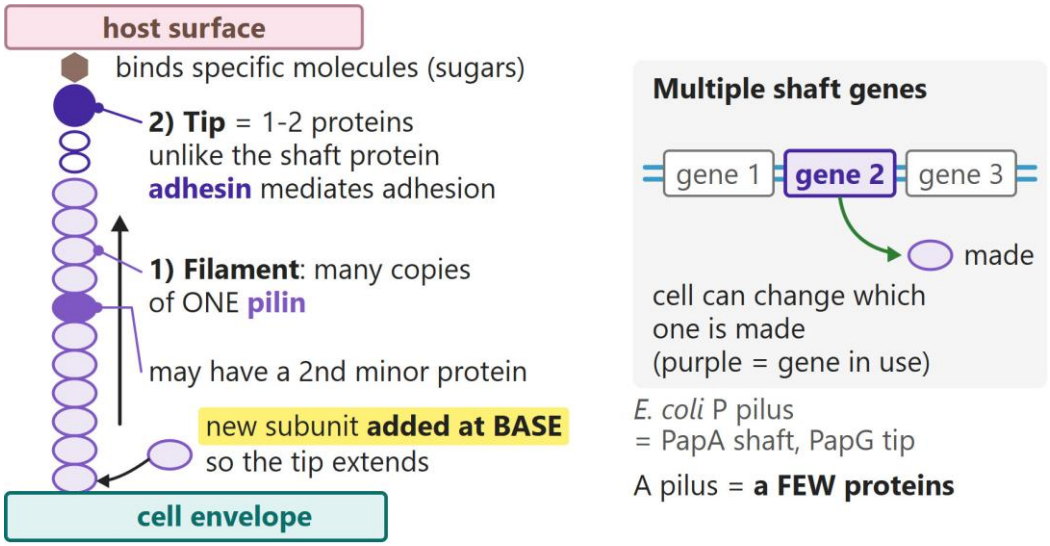
Common pili are thinner and shorter than flagella. [L16 p04, L16 p06]

Diameter: **3-10 nm** for a common pilus vs **14-20 nm** for a flagellum *(whip tail that spins)*. [L16 p04, L16 p06]

Number per cell

Number per cell: common pili are numerous (**100**s per cell). [L16 p06, L16 p10, L16 p09]

Sex pili are few (<**10**). [L16 p06, L16 p10, L16 p09]



Pilus filament of pilin, adhesin at the tip, new subunits added at the base [L16 p10]

On the figure: Pilin · adhesin · tip · base · PapA · PapG

Pilin and tip protein

Composition, filament: mostly many copies of a single protein. [L16 p13]

It is called a **pilin** *(the protein subunit that makes the pilus)*. [L16 p13]

It may have a second minor protein. [L16 p13]

Composition, tip: **1-2 proteins** that are different from the filament. [L16 p13, L16 p14]

The **tip** (**adhesin**) protein mediates adhesion *(sticking to a surface)*. [L16 p13, L16 p14]

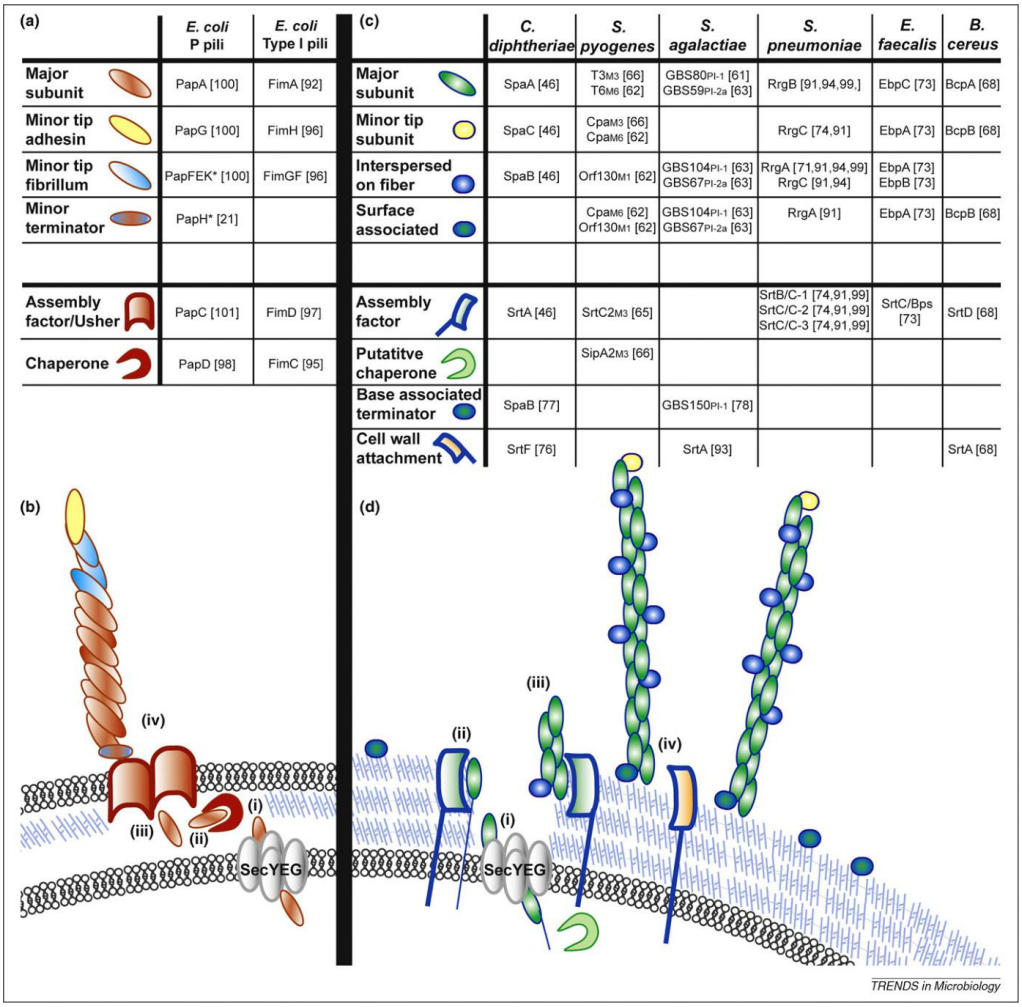
It binds specific molecules (sugars). [L16 p13, L16 p14]

Proteins that form a pilus

How many different proteins form a pilus? [L16 p12]

Answer: more than one protein: one major subunit plus a few minor subunits (**tip** adhesin and **tip** fibrillum at the **tip**, a terminator at the **base**). [L16 p12]

One major subunit is repeated many times. [L16 p12]



Pilus components of E. coli P and Type I pili and Gram positive pili [L16 p12]

On the figure: PapA · PapG · PapFEK · PapH · PapC · PapD · FimA · FimH

E. coli P pili:

Major subunit **PapA**; minor **tip** adhesin *(sticky protein for attaching)* **PapG**.

Also list minor **tip** fibrillum *(thin tip fiber)* **PapFEK** and minor terminator **PapH**.

Also have assembly factor/usher *(channel that assembles pili)* **PapC** and chaperone *(helper protein that guides others)* **PapD**. [L16 p12]

E. coli Type I pili:

FimA (major), **FimH** (**tip** adhesin), FimGF (**tip** fibrillum).

Also have usher FimD and chaperone FimC. [L16 p12]

Gram positive *(thick wall, stains purple)* pili also have a cell wall *(stiff layer around the cell membrane)* attachment factor. [L16 p12]

Gram positive sortase enzymes form a peptide *(short chain of amino acids)* bond from a cell wall cross-bridge to a protein extending from the cell (textbook).

Among the six **Gram positive** pili listed (C. diphtheriae, S. pyogenes, S. agalactiae, S. pneumoniae, E. faecalis, B. cereus), the **only putative chaperone** *(helper protein that guides others)* is **SipA2**, from S. pyogenes. [L16 p12]

Changing which pilin is made

Cells may have multiple different pilin genes. [L16 p14]

They can change which pilin is made. [L16 p14]

Assembly compared with flagellum

Assembly in general: assembly varies by type. [L16 p14, L16 p15, L16 p19]

Feature	Common pili / fimbriae	Sex (F) pili	Flagellum
Diameter	**3-10 nm**	**~7 nm**	**14-20 nm**
Number per cell	**100**s	**only** a few (<**10**)	not given
Length	thinner and shorter than flagella	longer than common pili and described as wider, though **~7 nm** falls inside the **3-10 nm** common pili range	longer than common pili

Table source: [L16 p06, L16 p10]

Gram positive pili	C. diphtheriae	S. pyogenes	S. agalactiae	S. pneumoniae	E. faecalis	B. cereus
Major subunit	SpaA	T3 and T6	GBS80 and GBS59	RrgB	EbpC	BcpA
Minor tip subunit	SpaC	Cpa	none listed	RrgC	EbpA	BcpB
Interspersed on fiber	SpaB	Orf130	GBS104 and GBS67	RrgA and RrgC	EbpA and EbpB	none listed
Surface associated	none listed	Cpa and Orf130	GBS104 and GBS67	RrgA	EbpA	BcpB
Base associated terminator	SpaB	none listed	GBS150	none listed	none listed	none listed
Assembly factor	SrtA	SrtC2	none listed	SrtB/C-**1**, SrtC/C-**2**, SrtC/C-**3**	SrtC/Bps	SrtD
Putative chaperone	none listed	SipA2	none listed	none listed	none listed	none listed
Cell wall attachment	SrtF	none listed	SrtA	none listed	none listed	SrtA

Table source: [L16 p12]

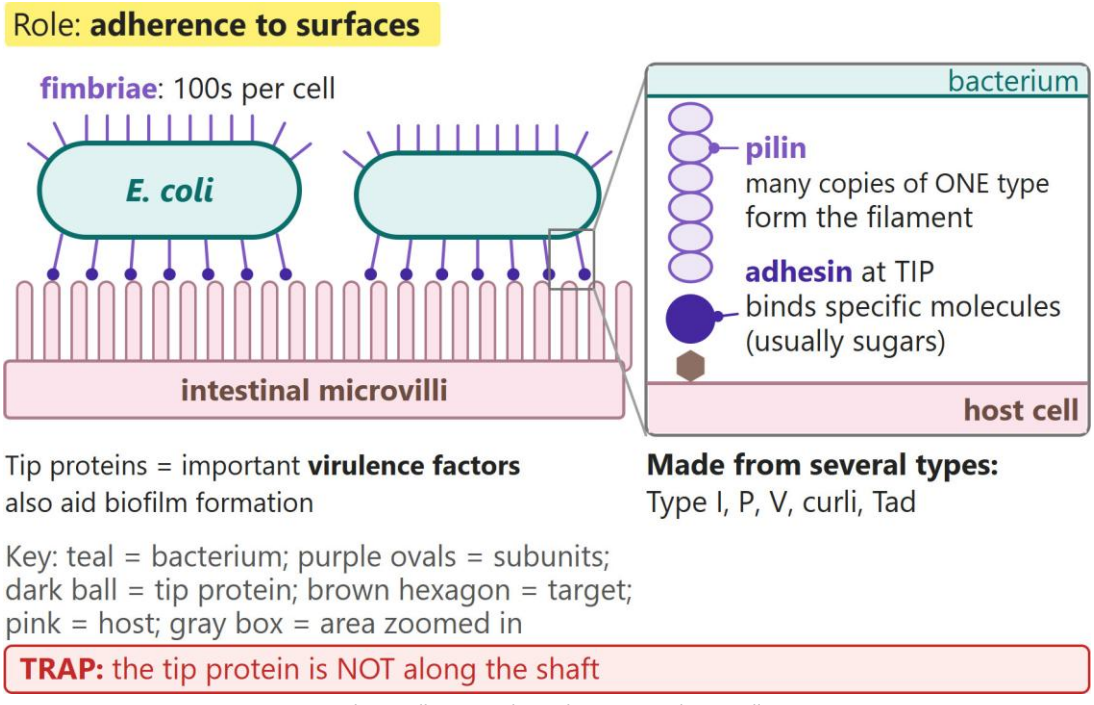
b. Roles of Common pili, Sex pili and Type IV pili

Book: 3.6 Specialized Structures, Pili and Stalks (chapter 3 PDF, p. 123).

⚠ **Do not mix up: Type IV pili *(thin hairs on the cell)* do twitching *(jerky crawl using pili)* motility *(ability to move)*; F (sex) pili do DNA *(cell genetic instruction molecule)* transfer by conjugation *(cells passing DNA by contact)*.** [L16 p09, L16 p10]

⚠ **Do not mix up: The adhesin *(sticky protein for attaching)* protein is at the TIP of the filament.** [L16 p08]

Common pili or fimbriae



Common pili: tip adhesins stick E. coli to intestinal microvilli [L16 p08]

On the figure: adherence · adhesin · tip · intestinal microvilli · virulence · biofilm · E. coli

Common pili or fimbriae *(short sticky hairs)*: role is **adherence** to surfaces. [L16 p07, L16 p08]

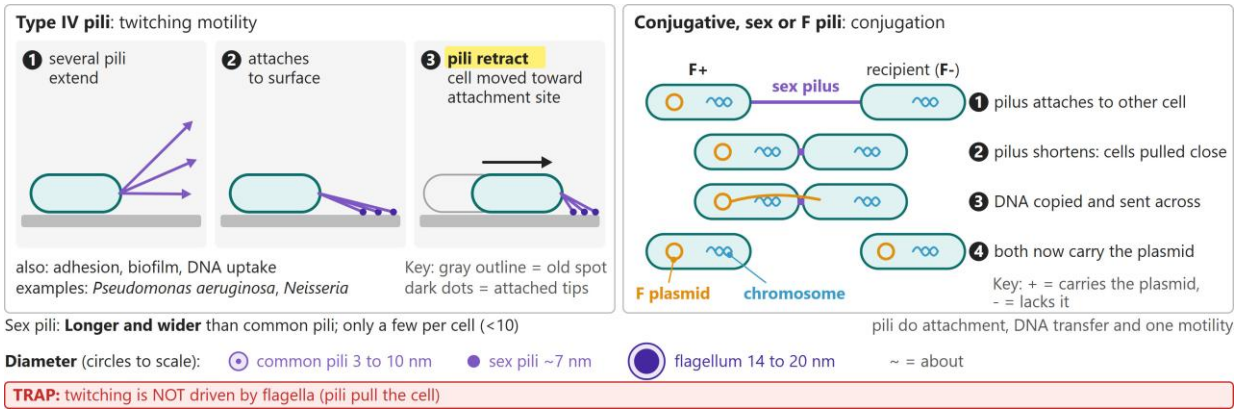
The **adhesin** protein at the **tip** binds specific molecules (usually sugars). [L16 p07, L16 p08]

Adhesins are important **virulence** *(ability to cause disease)* factors *(help germs cause disease)*. [L16 p07, L16 p08]

Common pili can also be important for formation of **biofilm** *(slimy community on a surface)*. [L16 p07, L16 p08]

E. coli cells stick to the **intestinal microvilli** *(finger-like folds of the gut lining)* of the host. [L16 p08]

Sex pili



Type IV pili pull the cell by twitching; sex pili pass the F plasmid across [L16 p09]

On the figure: Type IV · twitching · Conjugative · sex pilus · F plasmid · chromosome · Diameter · conjugation

Conjugative, sex or F pili: longer and wider than common pili. [L16 p10]

Diameter: sex pili **~7 nm**, common pili **3-10 nm**, flagellum *(whip tail that spins)* **14-20 nm**. [L16 p10]

Watch out: Sex pili are called wider than common pili, yet **~7 nm** falls inside the **3-10 nm** range. [L16 p10]

Number: **only** a few per cell (**<10**). [L16 p10]

Their role is DNA transfer by **conjugation** *(cell to cell DNA transfer)*. [L16 p10]

In **Gram negative** *(thin wall plus outer membrane)* enteric bacteria, sex pili attach a donor cell to a recipient cell for transfer of DNA (textbook).

Sex pilus steps and F plasmid

Sex pilus *(thin hair for sticking or DNA swap)* **steps**: **1.** the pilus attaches to another bacterial cell. [L16 p11]

Sex pilus step 2: the pilus shortens and brings the cells close together. [L16 p11]

Sex pilus step 3: DNA is transferred to the recipient cell. [L16 p11]

The **F plasmid** *(small extra DNA circle)* replicates. [L16 p11]

A copy goes from the F+ cell to the F- cell. [L16 p11]

Both cells end with the F plasmid. [L16 p11]

Each cell's **chromosome** *(the main DNA)* stays in its own cell. [L16 p11]

Type IV pili and twitching

Type IV pili: mediate **twitching motility** *(jerky crawling on a surface)*. [L16 p09]

Several pili extend, attach to a surface, and then shorten. [L16 p09]

This moves the cell in the direction of the attachment site. [L16 p09]

In twitching, the pili act as limbs that walk the bacterium across a substrate (textbook).

PilB/PilT sit at the **base** of the Type IV pilus, in the **cytosol** *(jelly inside the cell membrane)*. [L16 p09]

Watch out: another Type IV pilus figure names the **base** proteins PilF and PilT instead of PilB and PilT. [L16 p09, L16 p17]

Twitching motility is not mediated by flagella *(whip tails that spin)*.

Other pilus types

Other pilus types: Type V (adhesion and aggregation, short surface fibers). [L16 p05]

Curli: amyloid *(stacked protein fibers)* fibers, surface attachment, aggregation *(clumping together)*, biofilm; E. coli and Salmonella. [L16 p05]

Type IV secretion pili: DNA/protein transfer, interaction with host cells. [L16 p05]

Competence *(ability to take up DNA)* **pili**: DNA uptake during transformation *(cell taking up free DNA)*. [L16 p05]

Tad *(tight adherence pili)* **pili** ("tight adherence"): strong attachment and colonization. [L16 p05]

Type IV-like pili in Gram positive *(thick wall, stains purple)* **bacteria**: adhesion *(sticking to a surface)*, motility, DNA uptake. [L16 p05]

Which types are called fimbriae

Types of pilin *(protein that builds a hair)* **that form fimbriae**: Type I, P, V, curli, Tad. [L16 p07, L16 p08]

Terminology: Type I, P, V and curli form fimbriae. [L16 p05, L16 p07]

Tad "can be called pili or fimbriae". [L16 p05, L16 p07]

Type IV, Type IV secretion and competence pili are "generally called pili rather than fimbriae"; **Type IV-like pili in Gram positive bacteria** are "generally called pili". [L16 p05, L16 p07]

Conjugative pili are not generally called fimbriae. [L16 p05, L16 p07]

Pilus type	Major roles	Features / examples
Common pili (Type I, P, Type V, curli and Tad form fimbriae; Tad can be called pili or fimbriae)	Adherence to surfaces and host cells, biofilm	Adhesin at **tip** binds specific molecules (usually sugars); adhesins are important virulence factors
Type IV pili	Twitching motility, adhesion, biofilm, DNA uptake	Rapidly extend and retract; Pseudomonas aeruginosa and Neisseria
Conjugative (sex, F) pili	DNA transfer between cells by conjugation	Longer and wider than common pili; **only** a few per cell (<**10**); F pilus of E. coli

Table source: [L16 p05, L16 p07, L16 p08, L16 p09, L16 p10]

Pilus type	Major function	Feature
Type I pili	Adhesion to host cells and surfaces; biofilms	Forms fimbriae; E. coli type **1** fimbriae bind mannose-containing receptors
P pili	Adhesion, especially in urinary tract infections	Forms fimbriae; E. coli P fimbriae bind receptors on urinary tract cells
Tad pili	Strong surface attachment and colonization	"Tight adherence" structures important for persistent attachment
Conjugative (sex) pili	DNA transfer between bacterial cells	Produced by conjugative plasmids; the F pilus of E. coli is the classic example

Table source: [L16 p05]

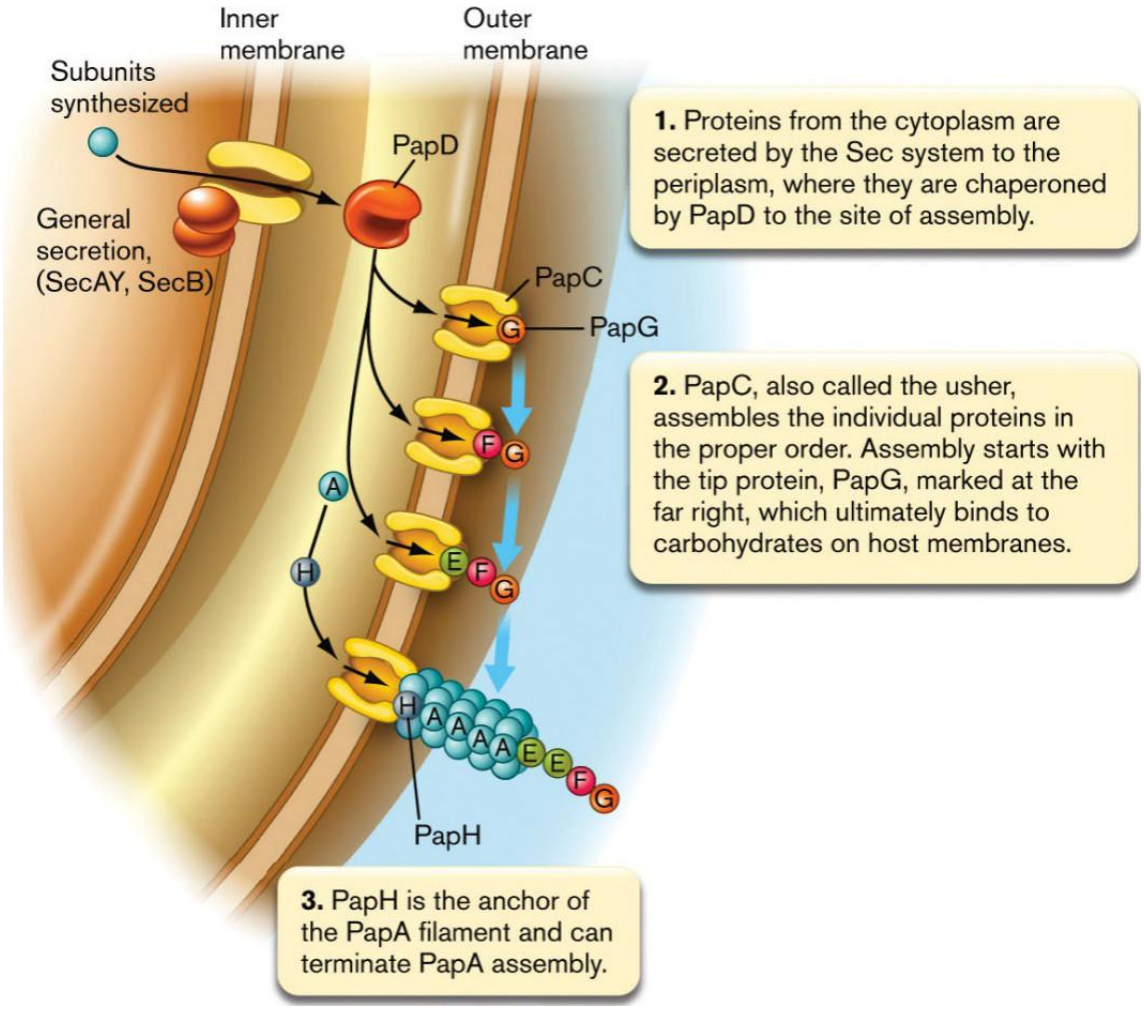
c. Assembly of Type I and Type IV, how it differs from flagellar

Book: 25.2 Microbial Attachment: First Contact (chapter 25 PDF, p. 36); 3.6 Specialized Structures, Pili and Stalks (chapter 3 PDF, p. 123).

⚠ **Do not mix up: Pili** ***(thin hairs on the cell)*** **subunits** ***(building block piece)*** **go on at the BASE; flagellin** ***(protein that builds the whip)*** **goes on at the TIP.** [L16 p19]

Type I pili assembly

Type I pili assembly has **4** steps. [L16 p16]



Type I pilus assembly: Sec system, chaperone PapD, usher PapC, tip PapG first [L16 p16]

On the figure: PapD · PapC · PapG · SecAY · SecB · inner membrane · outer membrane

Type I pili, step 1: proteins are secreted into the periplasm *(space between the two membranes)* by the Sec system *(protein export machine)*. [L16 p16]

The general secretion system includes **SecAY**, **SecB**. [L16 p16]

Type I pili, step 2:

> Proteins are bound by the chaperone (**PapD**).
>
> They then enter the usher *(channel that assembles pili)* channel (**PapC**). [L16 p16]

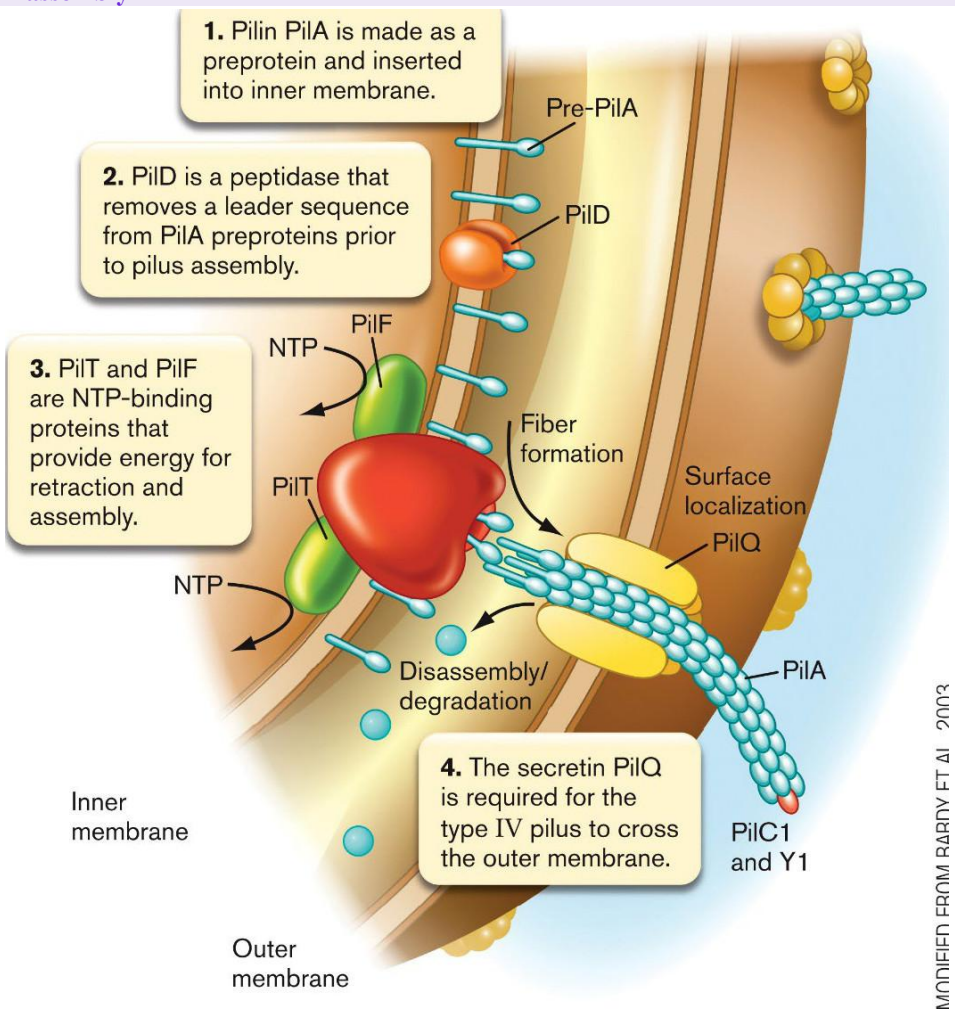
Type I pili, step 3: the pilus *(thin hair for sticking or DNA swap)* is assembled on the **outer membrane** *(extra membrane outside the wall)*. [L16 p16]

Type I pili, step 4: it starts with the adhesin *(sticky protein for attaching)* on the **tip**. [L16 p16]

The **tip** protein **PapG** binds **carbohydrates** *(sugars)* on host membranes *(thin lipid layer)*. [L16 p16]

Pilin *(protein that builds a hair)* proteins are then added to the **base** of the shaft to extend the filament. [L16 p16]

Type IV pili assembly



Type IV pilus assembly: PilA in inner membrane, PilT and PilF with NTP, PilQ channel [L16 p17]

On the figure: PilA · PilC1 · PilT · PilF · inner membrane · outer membrane

Type IV pili assembly has **3** steps. [L16 p17]

Type IV pili, step 1: pilin protein (**PilA**) forms a filament in the **inner membrane** *(the skin around the cytoplasm)*. [L16 p17]

PilC1 and Y1 sit at the **tip** of the Type IV pilus. [L16 p17]

Type IV pili, step 2: the assembled pilus is pushed through an **outer membrane** channel. [L16 p17]

Type IV pili, step 3: it shortens by disassembling pilin proteins from the **base**. [L16 p17]

PilT and PilF are NTP-binding proteins *(proteins that bind an energy molecule)* that provide energy for retraction and assembly. [L16 p17]

Pili versus flagellum filament

How the filament differs from a flagellum *(whip tail that spins)*: pili subunits are added to the **BASE**. [L16 p19, L16 p14, L16 p17, L16 p18]

This causes the filament to extend. [L16 p19, L16 p14, L16 p17, L16 p18]

In a pilus the **tip** is made first and goes out first; new pilin joins at the **base** and **pushes the tip out** *(the tip extends outward)*. [L16 p14, L16 p16, L16 p19]

Flagellum subunits (flagellin) are added to the **TIP**. [L16 p19, L16 p14, L16 p17, L16 p18]

They pass through the basal body *(anchor of the whip in the envelope)* and the hollow filament. [L16 p19, L16 p14, L16 p17, L16 p18]

Type IV pili can also retract *(pull back in)* by disassembly from the **base**. [L16 p19, L16 p14, L16 p17, L16 p18]

Flagellum assembly order

Flagellum assembly order: two ring structures form in the cell membrane *(thin skin around the cell)*.

The rod is added.

The rod is **capped** after it is added (step **3**). [L16 p18]

Then two more rings are added in the cell wall *(stiff layer around the cell membrane)* layer or outer membrane.

The **P ring** (step **4**) sits in the peptidoglycan; the **L ring** (step **5**) sits at the outer membrane. [L16 p18, L15 p14]

The hook is added.

The filament is formed.

The last step (**9**) adds the **Mot proteins** *(motility proteins, the motor parts)* in the cell membrane. [L16 p18]

Step / feature	Type I pilus (chaperone-usher)	Type IV pilus	Flagellum
Starts	Proteins secreted into the periplasm by the Sec system	Pilin PilA made as a preprotein (pre-PilA) and inserted into the inner membrane; PilD peptidase removes the leader sequence	Rings of the basal body inserted into the cell membrane (steps **1** and **2**)
Middle	Bound by chaperone (PapD); the proteins enter the usher channel (PapC)	Pilin assembled into a filament at the inner membrane; PilF and PilT NTP-binding proteins give energy for assembly and retraction	Rod added and capped (**3**), two outer rings added (**4** and **5**), hook added and finished (**6** and **7**)
Later steps	On the outer membrane at the usher (PapC)	Pushed through a channel (secretin PilQ) in the outer membrane	Filament added (**8**), then motility proteins added in the cell membrane (**9**)
How it grows	Adhesin **tip** protein (PapG) goes first, then pilins are added at the **BASE** of the shaft; PapH anchors and can end assembly	New pilin added at the **base**	Flagellin subunits pass through the hollow core and are added at the **TIP**
Retraction	Not stated	Yes, shortens by disassembling pilin from the **base**	Not stated

Table source: [L16 p16, L16 p17, L16 p18, L16 p19]

3. Other external structures

Book: 3.6 Specialized Structures, Pili and Stalks (chapter 3 PDF, p. 123).

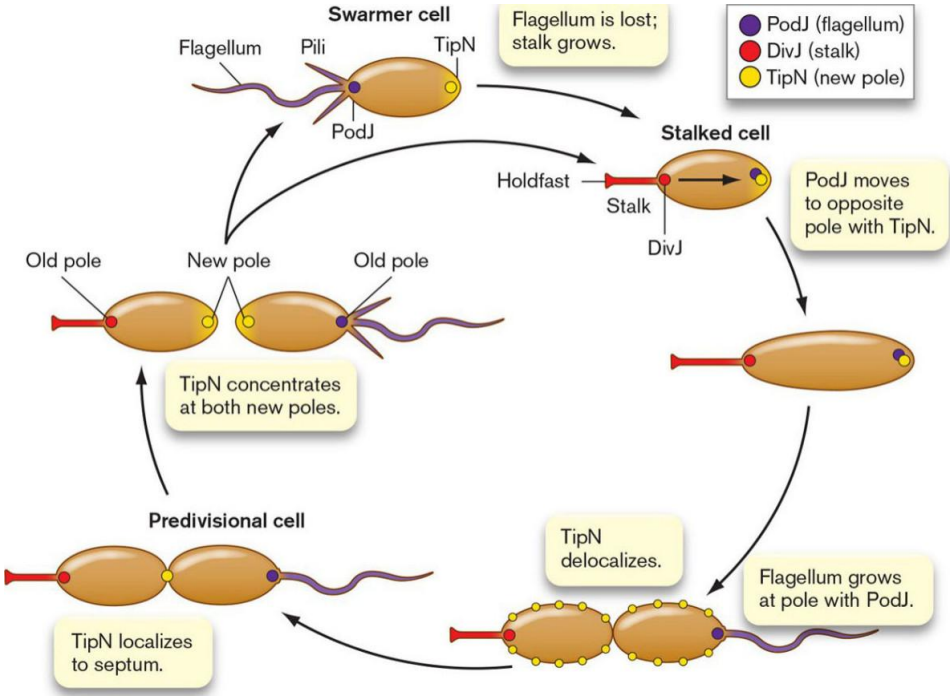
⚠ **Do not mix up: Stalks *(stem that anchors the cell)* are for adhesion *(sticking to a surface)*; nanotubes *(tiny tube joining two cells)* connect the cytoplasm *(jelly inside the cell membrane)* or periplasm *(space between the two membranes)* of two cells.** [L16 p20, L16 p21]

Others as an external structure

Stalks and nanotubes are "Others", the third kind of external structure. [L16 p03]

Stalks and Caulobacter cycle

Stalks (Caulobacter and other bacteria): used for adhesion. [L16 p20]



Caulobacter cycle: swarmer cell, stalk and holdfast, TipN and PodJ at the poles [L16 p20]

On the figure: swarmer · holdfast · PodJ · DivJ · TipN · predivisional cell · septum · new poles

Caulobacter life cycle: the **swarmer** *(swimming daughter cell)* cell has a flagellum *(whip tail that spins)* and pili *(thin hairs on the cell)*. [L16 p20]

Then the flagellum is lost and the stalk grows. [L16 p20]

The stalked cell has a **holdfast** *(sticky foot at the stalk tip)* at the stalk **tip**. The protein **DivJ** marks the stalk end. [L16 p20]

The **tip** of the stalk secretes adhesion factors that form the holdfast, which firmly attaches the bacterium (textbook).

Before division, the flagellum grows at the pole opposite the stalk (the pole with **PodJ**, the flagellum marker), and the stalked cell becomes a **predivisional cell**. [L16 p20]

The cell then divides; the end with the flagellum becomes the old pole of the new swarmer cell. [L16 p20]

The cycle gives a stalked cell and a **new swarmer** cell *(swimming daughter cell)*. [L16 p20]

TipN protein

TipN marks the new pole *(end of the cell made at division)*. [L16 p20]

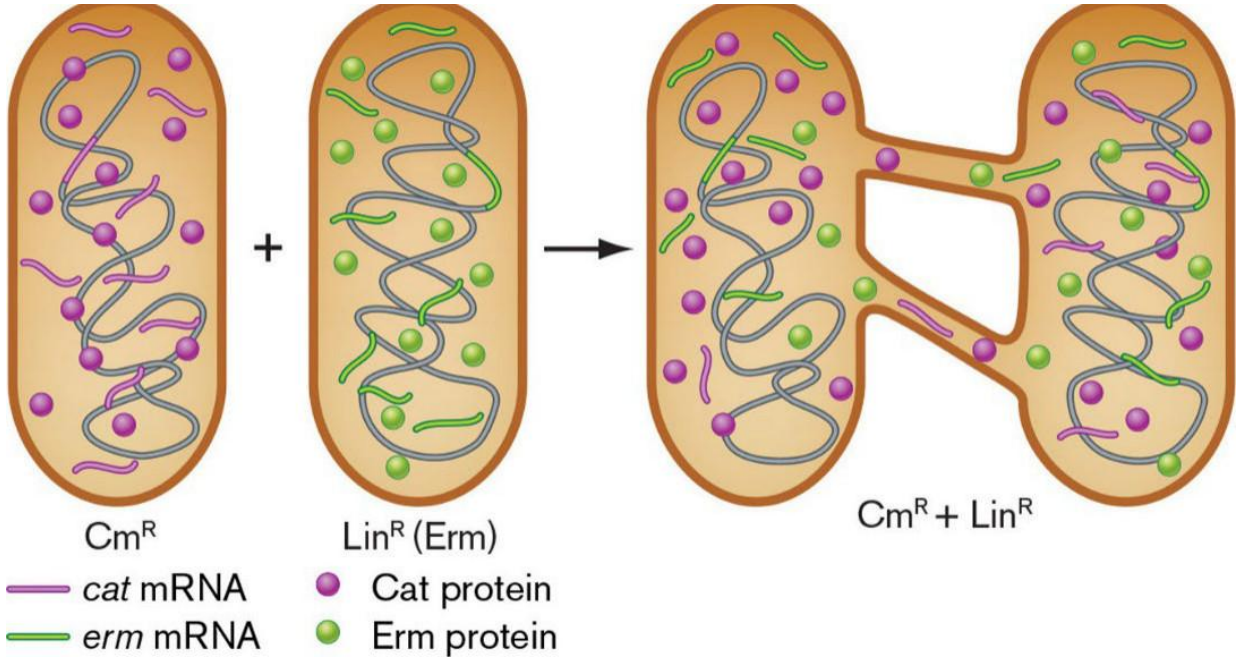
In the predivisional cell, TipN localizes to the **septum** *(dividing wall in the middle of the cell)*, then sits at both **new poles**. [L16 p20]

Nanotubes

Nanotubes: extension of the cytoplasm or periplasm between two different cells. [L16 p21, L16 p22]

They can be between different species. [L16 p21, L16 p22]

Components shared through nanotubes: protein and nucleic acids. [L16 p21, L16 p22]



Nanotube joins a Cm resistant and a Lin resistant cell, sharing mRNA and protein [L16 p22]

On the figure: Cm · Lin · cat mRNA · Cat protein · erm mRNA · Erm protein

> **Nanotube example**: a **Cm** resistant (CmR) cell and a **Lin** resistant (LinR, Erm) cell. [L16 p21, L16 p22]
> They join by a tube. [L16 p21, L16 p22]
> Each ends with both **cat mRNA** *(message copy of a gene)*/**Cat protein** and **erm mRNA**/**Erm protein**. [L16 p21, L16 p22]

C. Cell envelope (glycocalyx, outer membrane, cell wall, and cell membrane)

Book: 3.3 The Envelope and Cytoskeleton, Cell Envelope of Bacteria (chapter 3 PDF, p. 49).

⚠ **Do not mix up: Mycobacteria *(waxy walled bacteria)* are Gram positive but have NO outer membrane *(extra membrane outside the wall)* with LPS *(outer membrane sugar and fat coat)*.**

Cell envelope parts

Cell envelope *(layers wrapped around the cell)*: includes the **cell membrane** *(thin skin around the cell)*, **cell wall** *(stiff layer around the cell membrane)*, outer membrane and **glycocalyx** *(sugary coat outside the cell)*/capsule *(firm sugar coat outside the cell)*. [L16 p25]

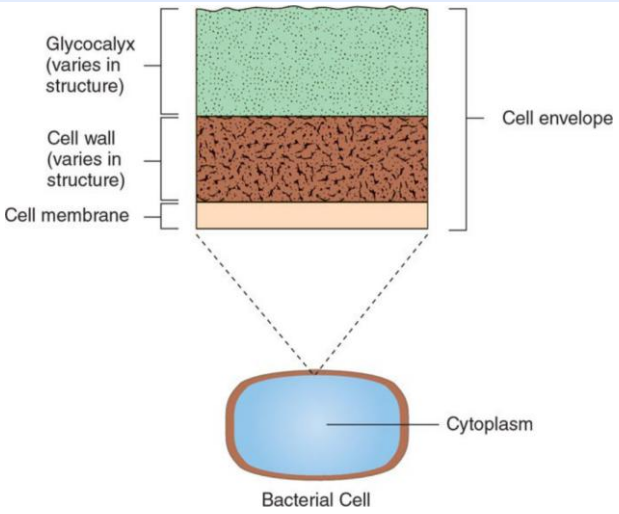
Different envelope styles are seen: **Gram positive**, **Gram negative** *(thin wall plus outer membrane)*, mycobacteria, Mycoplasma (mollicutes) and archaea *(single cell microbes without a nucleus, their own domain (also called archaeal bacteria))*. [L16 p25, L21 p28]

Each style has different layers and components. [L16 p25]

The envelope topic has **three parts**: the major **styles**, the **layers and components** of each, and **antibacterial chemicals and resistance**. [L16 p27]

Envelope style is a major way to group bacterial species. [L16 p25]

It is detectable by staining techniques and microscopy (Gram stain and acid fast). [L16 p25]

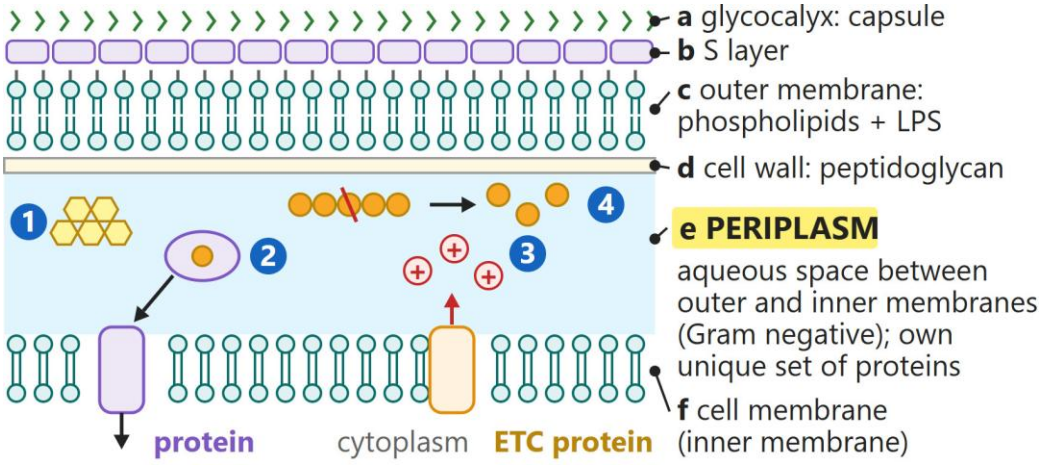


Cell envelope made of glycocalyx, cell wall and cell membrane around the cytoplasm [L16 p37]

On the figure: glycocalyx · cell wall · cell membrane · Cell envelope

Five envelope styles

Five envelope styles: **Gram positive**, Mycobacterial (**Gram positive** actinomycete, acid fast), **Gram negative**, **Mollicutes** (Mycoplasma, no cell wall), **Archaeal** *(of archaea)*. The envelope topic has three parts: styles, layers and components, and antibacterial chemicals and resistance. [L16 p27]



Main Functions

1 osmotic protection: holds oligosaccharides
2 transport: binding proteins bring solutes (food) to membrane transporters
3 oxidation-reduction reactions of the electron transport chain; stores H+ ions
4 hydrolysis: cuts nutrients (red slash) into subunits small enough to import

TRAP: the periplasm is NOT outside the cell; it lies inside the envelope

Gram negative layers outside to inside, with the periplasm between the membranes [L16 p36]

On the figure: glycocalyx · outer membrane · peptidoglycan · Periplasm · cell membrane · phospholipids · LPS

Gram negative layers

Gram negative envelope layers: **glycocalyx** or capsule (sugars or proteins).

Watch out: The **Gram negative** envelope list says capsule in some, made of sugar.

Outer membrane: lipopolysaccharide, proteins, **phospholipids** *(membrane fat with a charged head)*.

The outer membrane is porous to most ions *(charged particle)* and many small organic molecules but prevents the passage of proteins and other macromolecules (textbook).

Cell wall: **peptidoglycan** *(sugar and peptide mesh of the wall)*.

Periplasm *(space between the two membranes)*: part of the **Gram negative** envelope.

The periplasm lies between the outer and inner membranes *(the skin around the cytoplasm)*, includes the cell wall, and holds enzymes and nutrient transporters *(protein that carries molecules across)* not found within the cytoplasm (textbook).

Cell membrane: phospholipids and proteins.

Archaea and S-layer

Archaea: no mycolic acid *(waxy fatty acid of mycobacteria)* in the envelope.

Pseudopeptidoglycan *(archaea version of wall mesh)* is in the cell wall of some archaea.

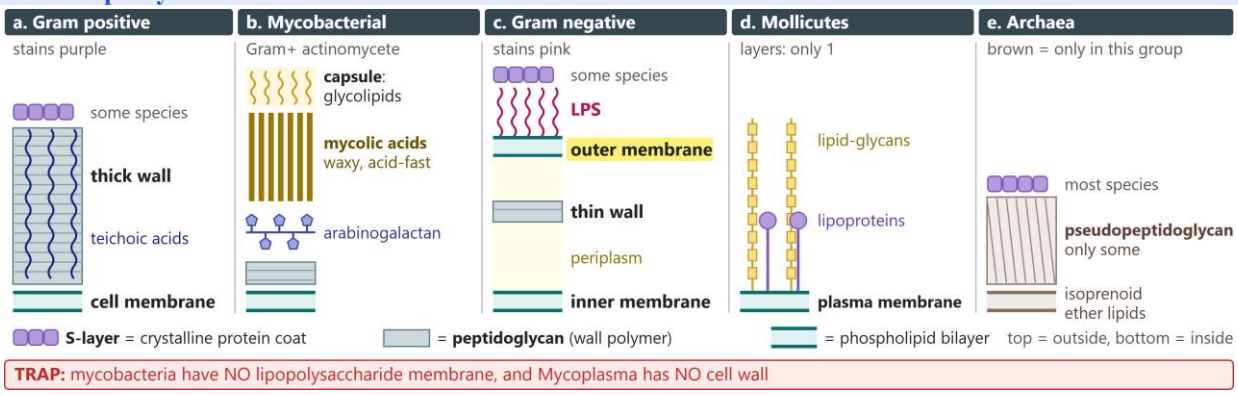
S-layer *(crystal like protein coat outside)* is a protein coat.

1. Know the layers and components of the envelope in each of the following types of bacteria. How similar and different from each other

Book: 3.3 The Envelope and Cytoskeleton, Cell Envelope of Bacteria (chapter 3 PDF, p. 49).

⚠ **Do not mix up: Only Gram negative *(thin wall plus outer membrane)* has an outer membrane *(extra membrane outside the wall)* with LPS *(outer membrane sugar and fat coat)*; Gram positive and mycobacteria *(waxy walled bacteria)* have none.** [L16 p30, L16 p31, L16 p32]

Five envelope styles



Five envelope styles side by side, outside at top [L16 p27]

On the figure: Gram positive · Gram negative · Mollicutes · S-layer · LPS · teichoic acids · outer membrane · peptidoglycan

Five envelope *(layers wrapped around the cell)* **styles**: **Gram positive**, mycobacterial *(about waxy walled bacteria)*, **Gram negative**, **Mollicutes** *(bacteria with no cell wall)*/Mycoplasma, archaeal *(of archaea)*. [L16 p29]

What the styles share

Similar: **Gram positive**, **Gram negative**, mycobacteria, Mycoplasma and archaea *(single cell microbes without a nucleus, their own domain (also called archaeal bacteria))* all have a cell membrane *(thin skin around the cell)*. [L16 p30, L16 p31, L16 p32, L16 p34, L16 p43, L21 p29, L21 p30, L21 p31, L21 p32, L21 p35]

Gram positive, mycobacteria and **Gram negative** all have **peptidoglycan** *(sugar and peptide mesh of the wall)*. [L16 p30, L16 p31, L16 p32, L16 p34, L16 p43]

An **S-layer** *(crystal like protein coat outside)* can be found on **Gram positive** *(thick wall, stains purple)*, **Gram negative** and archaea. [L16 p30, L16 p31, L16 p32, L16 p34, L16 p43]

The **S-layer** is **NOT** on all bacteria. [L16 p30, L16 p31, L16 p32, L16 p34, L16 p43]

How they differ

Different: **only Gram negative** has an **outer membrane** with **LPS**. [L16 p30, L16 p31, L16 p32, L16 p33, L16 p34]

Only **Gram negative** has a periplasm *(space between the two membranes)* holding a thin peptidoglycan. [L16 p30, L16 p31, L16 p32, L16 p33, L16 p34]

Gram positive has a thick peptidoglycan with **teichoic acids** *(charged chains in Gram positive wall)*. [L16 p30, L16 p31, L16 p32, L16 p33, L16 p34]

Gram positive has no outer membrane. [L16 p30, L16 p31, L16 p32, L16 p33, L16 p34]

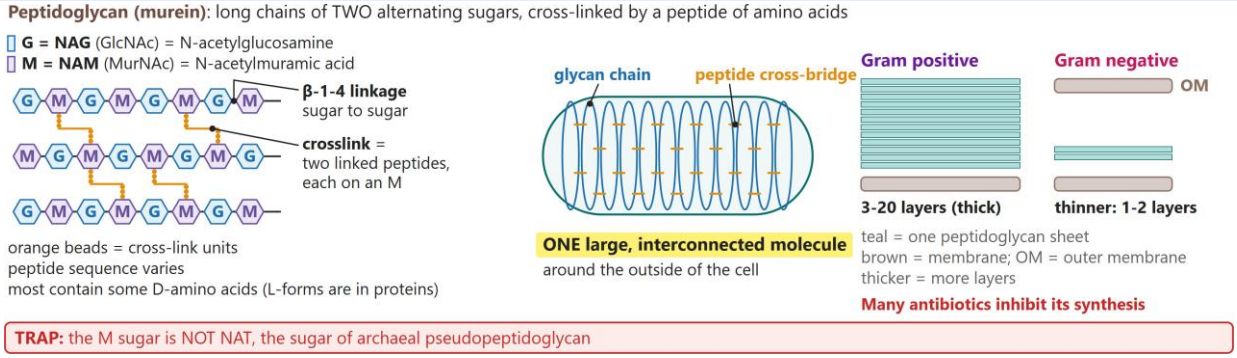
Mycobacteria add arabinogalactan *(chain of arabinose and galactose sugars in the wall)* and mycolic acids *(waxy fatty acids of mycobacteria)* to the wall. [L16 p30, L16 p31, L16 p32, L16 p33, L16 p34]

Mollicutes have no cell wall *(stiff layer around the cell membrane)*. [L16 p30, L16 p31, L16 p32, L16 p33, L16 p34]

Archaea have no peptidoglycan (pseudopeptidoglycan or S-layer). [L16 p30, L16 p31, L16 p32, L16 p33, L16 p34]

Archaea have isoprenoid *(branched five carbon repeat fat)* membrane lipids. [L16 p30, L16 p31, L16 p32, L16 p33, L16 p34]

Peptidoglycan differences



Peptidoglycan chains cross-linked by peptides; thick in Gram positive, thin in Gram negative [L16 p27]

On the figure: peptidoglycan · glycan chains · 3-20 layers · 1-2 layers · Gram positive · Gram negative

Peptidoglycan thickness differs: **Gram positive** has **3-20 layers**, **Gram negative** is thinner with **1-2 layers**.

Peptidoglycan is made of parallel glycan *(sugar chain)* chains cross-linked by peptides *(short chain of amino acids)* of four to six amino acids (textbook).

Electron microscope appearance

The **Gram positive** envelope appears as a single dark line. [L16 p28]

A diffuse gray zone lies outside it (one thick wall layer). [L16 p28]

The **Gram negative** envelope appears as separate dark bands. [L16 p28]

Light spaces lie between the bands (outer membrane, thin wall and inner membrane). [L16 p28]

Style	Layers, outside to inside	Cell wall	Outer membrane
Gram positive	glycosyl chains, S-layer, peptidoglycan with teichoic acids, cell membrane with membrane proteins	thick peptidoglycan (**3-20 layers**)	none
Mycobacteria (acid fast, Gram positive actinomycete)	capsule (glycolipids, sugar mycolates), mycolic acids on arabinan, arabinan, galactan, peptidoglycan, cell membrane	peptidoglycan plus arabinogalactan plus mycolic acid	none with LPS; Watch out: one envelope diagram calls the mycolic acid layer a mycomembrane (mycobacterial outer membrane)
Gram negative	S-layer (some), LPS, outer membrane with porins, lipoproteins, periplasm with thin peptidoglycan, inner membrane	thin peptidoglycan (**1-2 layers**) in the periplasm	yes, with LPS
Mollicutes (Mycoplasma)	plasma membrane **only**, with lipid-glycans and lipoproteins on its surface	none	none
Archaea	S-layer (and pseudopeptidoglycan in some), cell membrane of isoprenoids	pseudopeptidoglycan in some, S-layer **only** in others (Sulfolobus)	none

Table source: [L16 p27, L16 p30, L16 p31, L16 p32, L16 p33, L16 p34, L16 p36, L17 p15]

a. Gram positive bacteria

Book: 3.3 The Envelope and Cytoskeleton, Firmicute Cell Envelope: Gram-Positive (chapter 3 PDF, p. 52).

⚠ **Do not mix up: Gram positive *(thick wall, stains purple)* wall is thick (3-20 layers) and has NO outer membrane *(extra membrane outside the wall)*.** [L17 p15, L16 p30]

Layers outside to inside

Gram positive envelope *(layers wrapped around the cell)*: from outside to inside, glycosyl *(sugar group)* chains, **S-layer** *(crystal like protein coat outside)*. [L16 p30]

Next: **peptidoglycan** *(sugar and peptide mesh of the wall)* with **teichoic acids** *(charged chains in Gram positive wall)* running through it. [L16 p30]

Teichoic acid cross-threads and the thick wall help the **Gram positive** cell retain the Gram stain (textbook).

Last: **cell membrane** *(thin skin around the cell)* with **membrane proteins**. [L16 p30]

Thick wall, no outer membrane

Peptidoglycan is thick: **3-20 layers** in **Gram positive**. [L17 p15]

No outer membrane: a TEM of a **Gram positive** envelope shows this. [L16 p28, L16 p30]

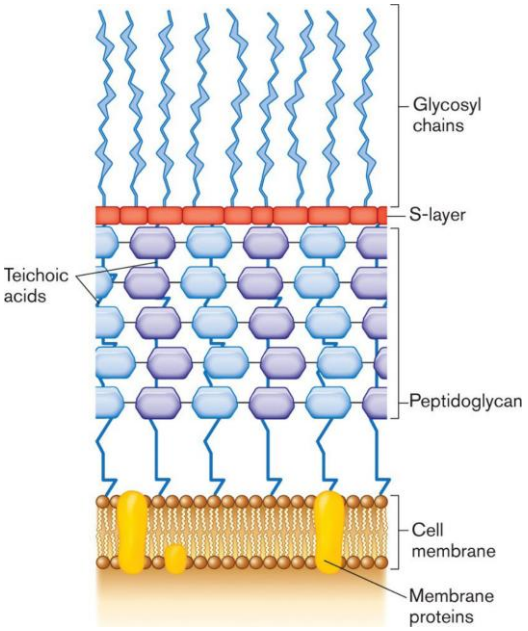
It also shows a thick peptidoglycan layer. [L16 p30]

Gram positive has **only** one membrane (the cell membrane), under the peptidoglycan. [L16 p30]

Molecules to know

Cell wall *(stiff layer around the cell membrane)*: teichoic acids and peptidoglycan. [L16 p30, L16 p36]

Cell membrane: phospholipids *(membrane fat with a charged head)* and proteins. [L16 p30, L16 p36]



Gram positive envelope: glycosyl chains, S-layer, thick peptidoglycan with teichoic acids, membrane [L16 p30]

On the figure: glycosyl chains · S-layer · peptidoglycan · teichoic acids · cell membrane · membrane proteins

b. Gram negative bacteria

Book: 3.3 The Envelope and Cytoskeleton, Proteobacterial Cell Envelope: Gram-Negative (chapter 3 PDF, p. 56).

⚠ **Do not mix up: Gram negative *(thin wall plus outer membrane)* peptidoglycan *(sugar and peptide mesh of the wall)* is thin (1-2 layers) and sits in the periplasm *(space between the two membranes)*.** [L17 p15, L16 p32]

Layers outside to inside

Gram negative envelope (C. crescentus): from outside to inside, **S-layer** *(crystal like protein coat outside)*. [L16 p32]

Next: **LPS** (with **Mg2+** ions between the chains). [L16 p32]

Magnesium ions *(charged particle)* form ionic cross-links that bridge the negatively charged LPS chains and give structural support (textbook).

Next: **outer membrane** *(extra membrane outside the wall)* with porin *(water filled channel in outer membrane)*. [L16 p32]

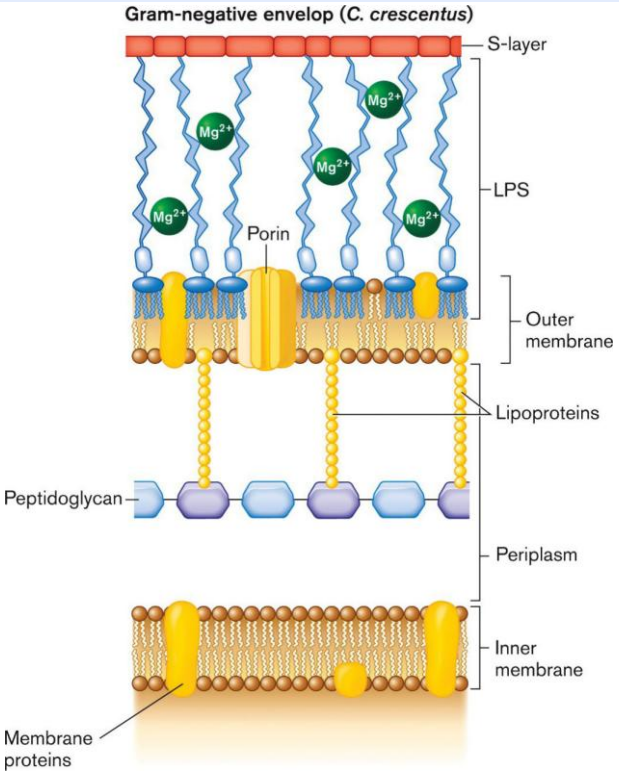
Porins *(channels in the outer membrane)* are outer membrane beta barrel channels that permit entry of nutrients such as sugars and peptides (textbook).

Next: **lipoproteins** *(proteins with fat tails)* linking the outer membrane to the **peptidoglycan**. [L16 p32]

Cells that fail to make murein lipoprotein *(protein anchoring outer membrane to wall)* cannot attach the outer membrane to the cell wall *(stiff layer around the cell membrane)*, so it balloons out where daughter cells separate (textbook).

Next: **periplasm** containing a thin peptidoglycan. [L16 p32]

Last: inner (cell) membrane *(thin lipid layer)* with membrane proteins. [L16 p32]



Gram negative envelope: S-layer, LPS, outer membrane, lipoproteins, periplasm, inner membrane [L16 p32]

On the figure: S-layer · LPS · Mg2+ · outer membrane · Porins · lipoproteins · periplasm · peptidoglycan

Peptidoglycan thickness

Peptidoglycan is thinner: **1-2 layers** in **Gram negative**. [L17 p15]

Gram positive *(thick wall, stains purple)* has **3-20 layers**. [L17 p15]

Outer membrane

Outer membrane: LPS and phospholipids *(membrane fat with a charged head)*. [L16 p36]

c. Mycobacteria (3.3 6e)

Book: 3.3 The Envelope and Cytoskeleton, Mycobacterial Cell Envelope (chapter 3 PDF, p. 63).

⚠ **Do not mix up: Mycolic acid *(waxy fatty acid of mycobacteria)* is in the acid fast wall; an LPS *(outer membrane sugar and fat coat)* outer membrane *(extra membrane outside the wall)*** is NOT.

What mycobacteria are

Mycobacterial *(about waxy walled bacteria)* **envelope** *(layers wrapped around the cell)*: a **Gram positive** actinomycete *(branching thread bacterium)*. [L16 p27, L16 p31]

It is **acid fast** (detected by the acid fast stain). [L16 p27, L16 p31]

Mycolic acids *(waxy fatty acids of mycobacteria)* provide the basis for acid fast staining: the cells retain the dye carbolfuchsin (textbook).

Layers outside to inside

Mycobacterial layers, from outside to inside: **capsule** (glycolipids and sugar mycolates). [L16 p27, L16 p31]

The capsule also has phenolic glycolipid and phospholipid *(membrane fat with a charged head)*. [L16 p27, L16 p31]

Next: **mycolic acids**, attached to **arabinan** *(five carbon sugar chain)*. [L16 p27, L16 p31]

Next: arabinan, then **galactan** (mycolic acids, arabinan and galactan together are the cell wall core), then **peptidoglycan** *(sugar and peptide mesh of the wall)*. [L16 p27, L16 p31]

Last: the cell membrane *(thin skin around the cell)*. [L16 p27, L16 p31]

Wall sugars and mycolic acid

Arabinogalactan *(chain of arabinose and galactose sugars in the wall)*: sugar chains made of arabinan and galactan. [L16 p31, L16 p36]

Mycolic acid is in the cell wall *(stiff layer around the cell membrane)*. [L16 p31, L16 p36]

Other parts

Other parts: a **porin** (OmpA Tb) channel crosses the mycolic acid layer. [L16 p31]

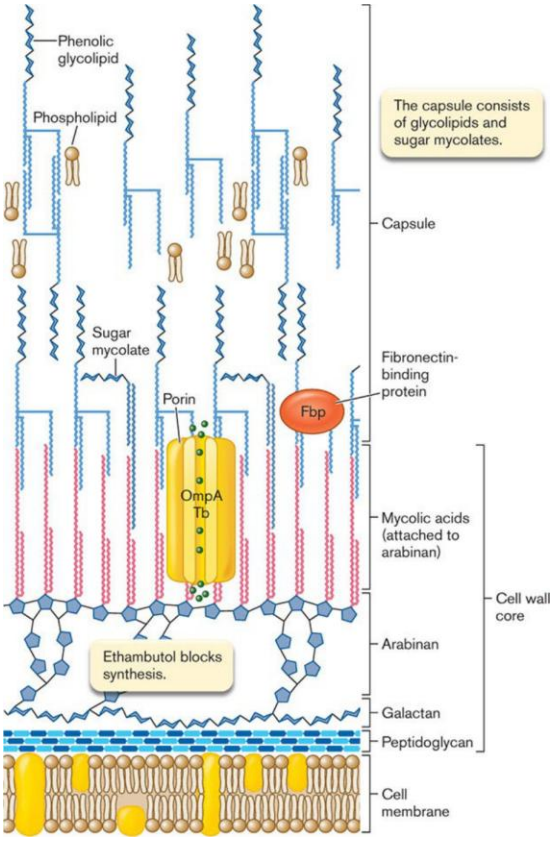
A fibronectin binding protein (**Fbp**) sits in the capsule. [L16 p31]

The drug **ethambutol** blocks arabinan synthesis. [L16 p31]

Acid fast envelope

Acid fast envelope: peptidoglycan, arabinan-galactan sugars, mycolic acid.

They also have a phospholipid bilayer *(two layers)* membrane.



Mycobacterial envelope: capsule, mycolic acids, arabinan, galactan, peptidoglycan, membrane [L16 p31]

On the figure: capsule · Mycolic acids · arabinan · galactan · peptidoglycan · Fbp · porin · ethambutol

They do **NOT** have an outer membrane with phospholipids and LPS.

Watch out: One envelope diagram calls the mycolic acid layer a mycomembrane (mycobacterial outer membrane).

The thick waxy envelope excludes many antibiotics and retards nutrient uptake, so mycobacteria *(waxy walled bacteria)* grow extremely slowly (textbook).

d. Archaea (19.1 6e)

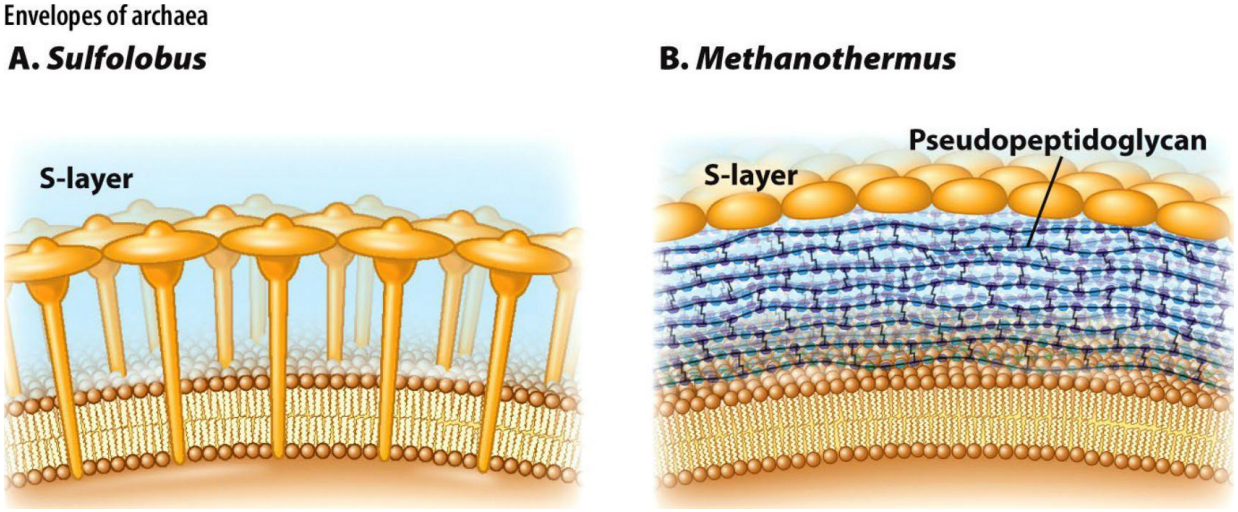
Book: 19.1 Archaeal Diversity at a Glance (chapter 19 PDF, p. 5).

⚠ **Do not mix up: Some archaea *(single cell microbes without a nucleus, their own domain (also called archaeal bacteria))* have pseudopeptidoglycan (others have only an S-layer); all archaea have isoprenoid *(branched five carbon repeat fat)* membranes *(thin lipid layer)*; none have mycolic acid *(waxy fatty acid of mycobacteria)*.**

S-layer in archaea

Most archaea have a crystalline *(neat repeating pattern)* S protein layer.

Archaea with no cell wall *(stiff layer around the cell membrane)* have just the S-layer *(crystal like protein coat outside)*. [L16 p36, L16 p43]



Archaeal envelopes: Sulfolobus with only an S-layer, Methanothermus with pseudopeptidoglycan [L16 p34]

On the figure: Sulfolobus · S-layer · Methanothermus · pseudopeptidoglycan

Archaeal *(of archaea)* **envelope** *(layers wrapped around the cell)*: **Sulfolobus** *(an archaeon)* has an **S-layer** held above the cell membrane *(thin skin around the cell)*. [L16 p34, L16 p43]

Sulfolobus has no cell wall. [L16 p34, L16 p43]

Methanothermus has an S-layer over a **pseudopeptidoglycan** layer over the cell membrane. [L16 p34, L16 p43]

Cell wall

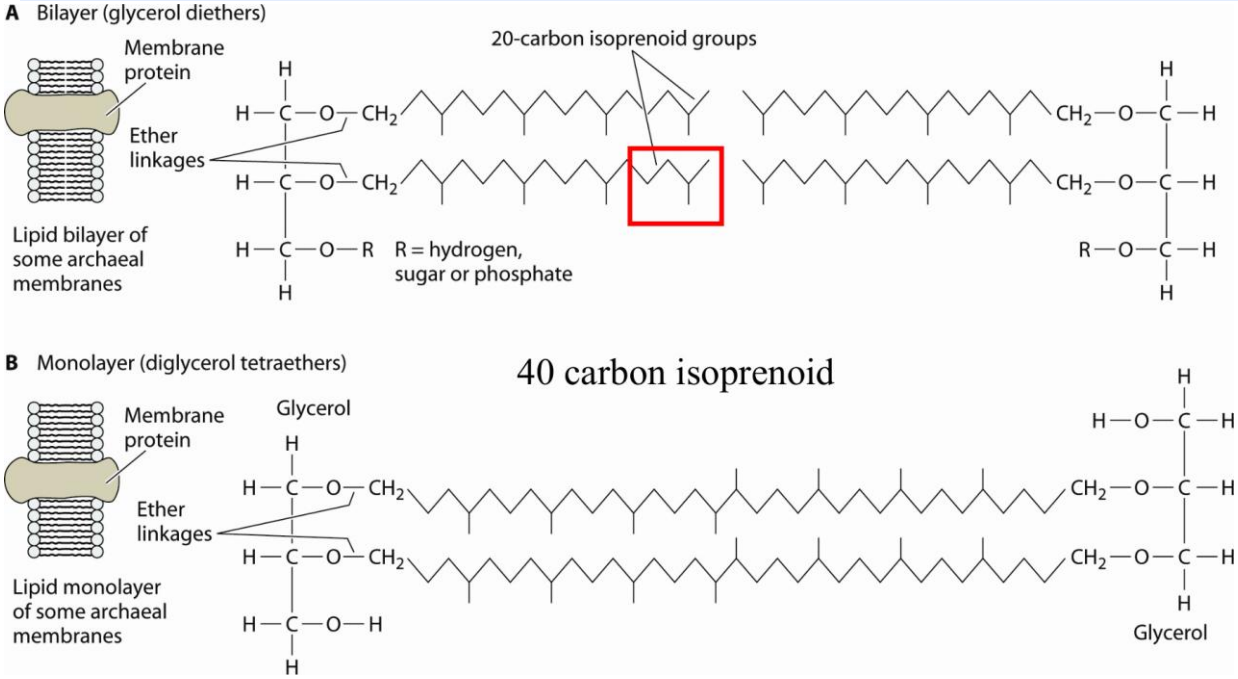
Only some archaea have a cell wall with chains of sugar.

Archaeal wall (in archaea that have one): pseudopeptidoglycan instead of peptidoglycan *(sugar and peptide mesh of the wall)*.

Pseudopeptidoglycan is found in some archaeal bacteria. [L17 p24]

Mycolic acid is **NOT** in the archaeal wall.

Membrane lipids



Archaeal membrane lipids: ether linkages, isoprenoid groups, bilayer and monolayer [L17 p39]

On the figure: isoprenoids · ether linkages

Archaeal membranes are **isoprenoids** *(branched five carbon repeat fats)* instead of phospholipids *(membrane fat with a charged head)*.

Membrane: isoprenoids (not phospholipids). [L17 p39]

Isoprenoids are joined to glycerol *(three carbon fat backbone)* by **ether** *(C O C bond)* **linkages** *(the bond type holding the chains to glycerol)*. [L16 p34, L17 p39]

The ether linkage resists hydrolysis *(breaking with water)*, and branched isoprenoid chains increase membrane stability by hooking each other in place (textbook).

e. Mollicutes (Mycoplasma) (pp 734-5 6e)

Book: 18.3 Firmicutes, Mycoplasma, and Actinobacteria, Mycoplasmas Lack a Cell Wall (chapter 18 PDF, p. 63).

No cell wall

Mollicutes (Mycoplasma): no cell wall *(stiff layer around the cell membrane)*. [L16 p27, L16 p33]

The **only** membrane *(thin lipid layer)* is the plasma membrane (**PM**); a sugar layer of lipid-glycans on its surface may be a capsule *(firm sugar coat outside the cell)*. [L16 p27, L16 p33]

Mycoplasmas need no cell wall because they are parasites living in host environments, where they are protected from osmotic *(about water moving across membranes)* shock (textbook).

Membrane proteins

The PM has membrane proteins. [L16 p27, L16 p33]

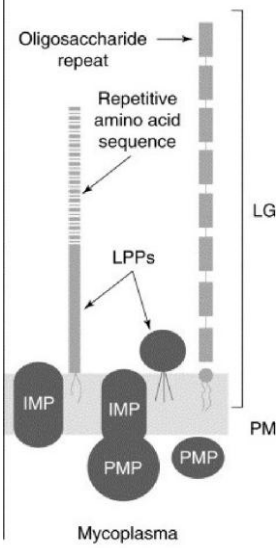
IMP is intermembrane protein. [L16 p27, L16 p33]

PMP is peripheral membrane protein. [L16 p27, L16 p33]

Membrane surface

Surface of the membrane: lipoproteins (**LPPs**) with a **repetitive amino acid sequence**. [L16 p33]

Lipid-glycans (**LG**) have an **oligosaccharide repeat**. [L16 p33]



Mycoplasma plasma membrane with IMP, PMP, lipoproteins and lipid-glycans [L16 p33]

On the figure: **PM** · **IMP** · **PMP** · **LPPs** · **LG** · **oligosaccharide repeat** · **repetitive amino acid sequence** · **Mycoplasmas**

How do scientists determine the structure of the envelope of bacteria?

Book: 3.3 The Envelope and Cytoskeleton, Cell Envelope of Bacteria (chapter 3 PDF, p. 49).

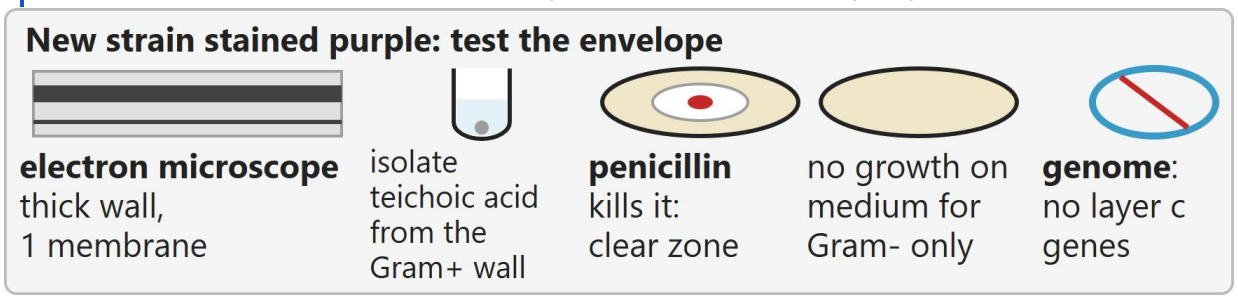
⚠ **Do not mix up: A Gram stain *(stain that sorts bacteria purple or pink)* color does not always match the actual envelope *(layers wrapped around the cell)* style.** [L16 p35]

Staining techniques

Staining techniques: the Gram stain and the acid fast stain detect differences in the envelope. [L16 p25, L16 p26]

They are basic tools for grouping bacteria by envelope. [L16 p25, L16 p26]

Limit of the Gram stain: color does not always match the actual envelope style. [L16 p35]



Tests for a purple strain: electron microscope, teichoic acid, penicillin, selective medium, genome [L16 p35]

On the figure: **teichoic acid** · **penicillins** · **genome**

Other methods can determine or predict the envelope of a newly isolated bacterium: electron microscopy, **teichoic acid** *(charged chains in Gram positive wall)* isolation, penicillin sensitivity, selective media and **genome** genes. [L16 p35]

Electron microscopy

Microscopy: electron microscope images (**50 nm** bar) show a single dark line for **Gram positive** *(thick wall, stains purple)*. [L16 p28]

They show separate dark bands for the **Gram negative** *(thin wall plus outer membrane)* envelope. [L16 p28]

Confirming a Gram positive envelope

Purple cells from an insect gut: how could you confirm a **Gram positive** envelope?

Method 1: transmission electron microscopy.

Method **1** shows a thick peptidoglycan *(sugar and peptide mesh of the wall)* layer and no outer membrane *(extra membrane outside the wall)*.

Method 2: isolate teichoic acid from the cell wall *(stiff layer around the cell membrane)*.

Method 3: test sensitivity to a penicillin.

Method **3**: some **penicillins** are more effective against **Gram positive** than **Gram negative** bacteria, because some do not pass through the **Gram negative** outer membrane.

Penicillin disables cross-bridge formation: the transpeptidase *(enzyme making wall cross bridges)* that cross-links the wall peptides *(short chain of amino acids)* is its target (textbook).

Method 4: no growth on selective media for **Gram negative**.

Method 5: look at genome predicted genes.

Method **5**: no genes for LPS *(outer membrane sugar and fat coat)* synthesis, outer membrane and periplasmic *(in the space between the two membranes)* proteins.

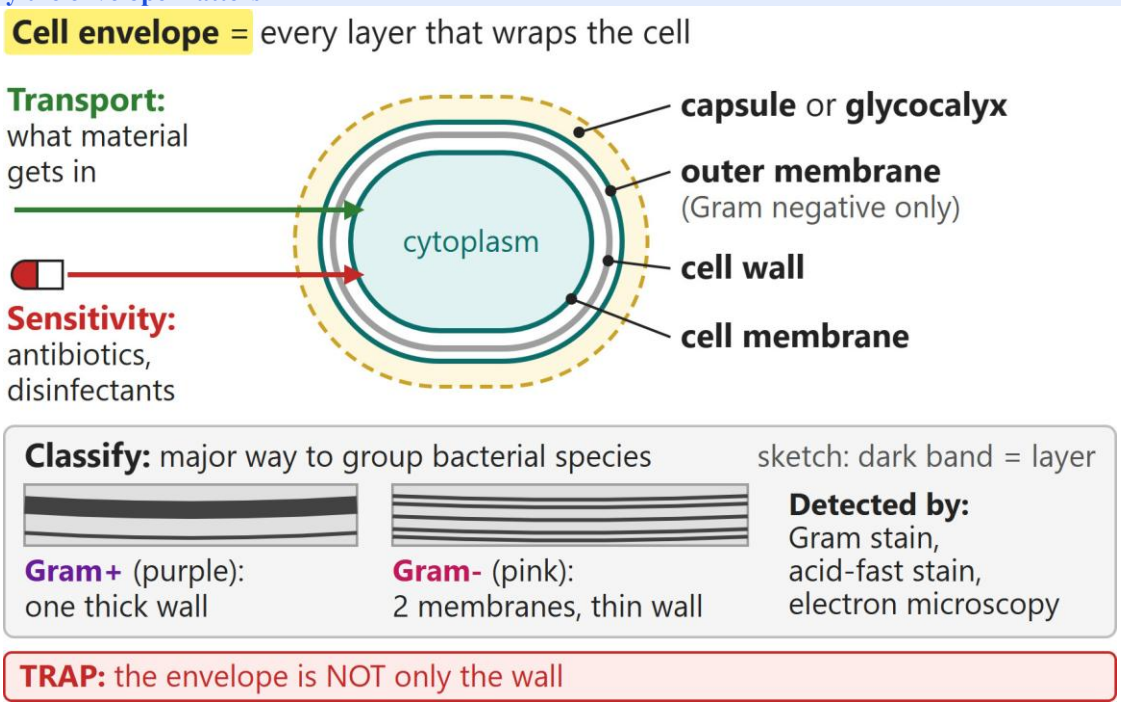
Method **5**: genes for teichoic acid synthesis are found.

How the nature of the envelope affects bacterial cells?

Book: 3.3 The Envelope and Cytoskeleton, Cell Envelope of Bacteria (chapter 3 PDF, p. 49).

⚠ **Do not mix up: Mollicutes *(bacteria with no cell wall)* have no cell wall *(stiff layer around the cell membrane)*; the plasma membrane *(thin lipid layer)* is their only membrane, with a sugar layer on its surface that may be a capsule *(firm sugar coat outside the cell)*.** [L16 p27, L16 p33]

Why the envelope matters



Cell envelope layers; what gets in, antibiotic sensitivity, and classifying species [L16 p26]

On the figure: classify · antibiotics · disinfectants · Glycocalyx

Why the envelope *(layers wrapped around the cell)* **matters**: reason **1,** major feature used to **classify** bacteria. [L16 p26]

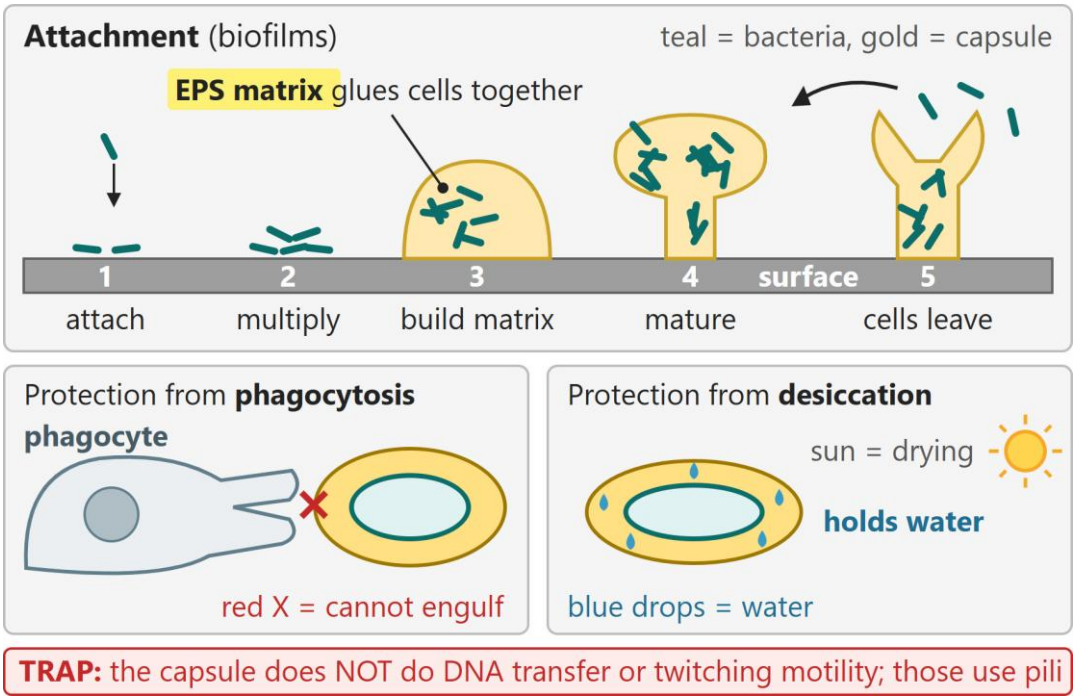
Staining techniques detect differences. [L16 p26]

Reason 2: affects transport of material into the cell. [L16 p26]

Reason 3: affects sensitivity of the bacterium to **antibiotics** and **disinfectants**. [L16 p26]

Drugs and chemicals have to pass through it to enter the cell. [L16 p26]

Glycocalyx and S-layer effects



Glycocalyx roles: biofilm attachment, protection from phagocytosis and desiccation [L16 p41]

On the figure: phagocytosis · desiccation · biofilms · attachment

> Glycocalyx *(sugary coat outside the cell)* **effects**: protection from phagocytosis *(immune cells eating germs)* and from desiccation *(drying out)*. [L16 p41, L16 p44]
>
> Glycocalyx also helps attachment to surfaces and biofilms *(slimy community on a surface)*. [L16 p41, L16 p44]
>
> **S-layer** *(crystal like protein coat outside)* **hypothesized effects**: protection from osmotic *(about water moving across membranes)* stress, protection from viruses. [L16 p41, L16 p44]
>
> S-layer may also be a barrier to large molecules. [L16 p41, L16 p44]

Penicillin in stream water

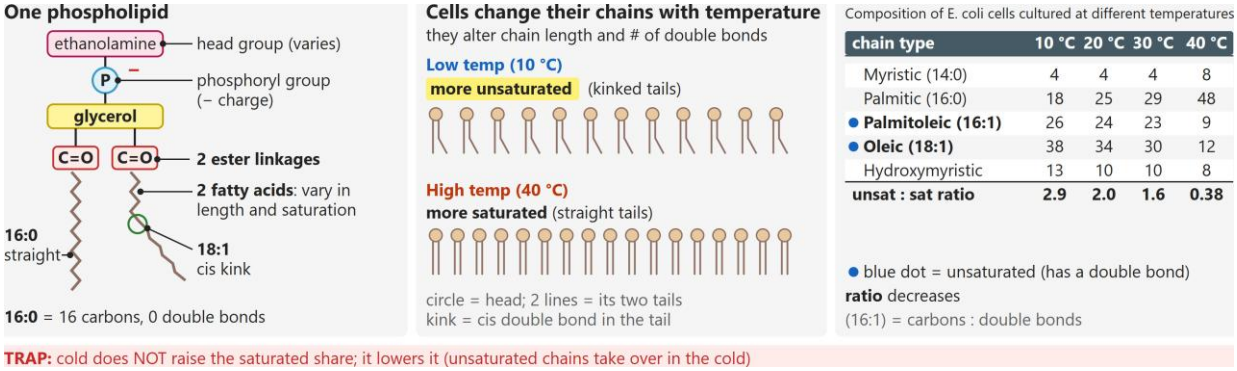
> **Penicillin treatment of stream water**: why might penicillin not work?
>
> The bacteria may not be growing.
>
> It may not be effective against the type of bacteria (**Gram negative**).
>
> The bacteria may be resistant or the concentration too low.

2. Know role, feature and basic composition of each of these layers (3.3 6e)

Book: 3.3 The Envelope and Cytoskeleton, Cell Envelope of Bacteria (chapter 3 PDF, p. 49).

Membrane lipids

> **Membrane** *(thin lipid layer)* **components differ**: archaea *(single cell microbes without a nucleus, their own domain (also called archaeal bacteria))* have isoprenoid *(branched five carbon repeat fat)* lipids while bacteria have phospholipids *(membrane fat with a charged head)*. [L17 p39]
>
> Some bacteria have hopanoids *(five ring cholesterol like fat)*, molecules similar to sterols *(stiff ring shaped fat)*. [L17 p40]

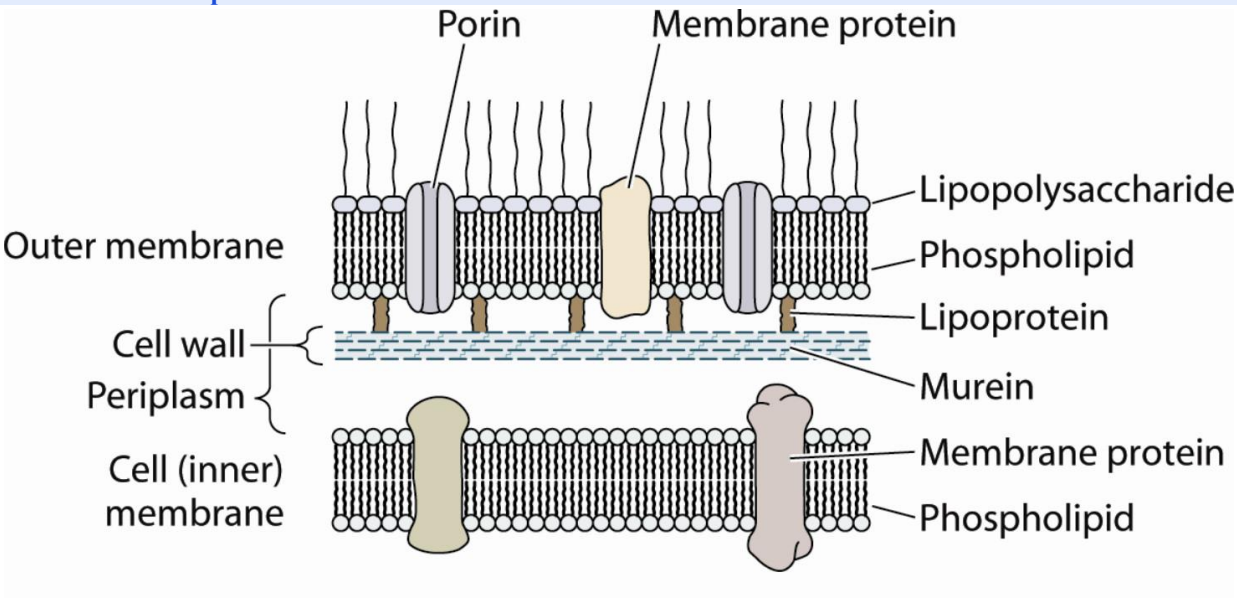


Phospholipid head group and chains; more unsaturated chains in the cold [L16 p40]

On the figure: head groups · unsaturated · 2.9 · 0.38 · 10 °C · 40 °C

> Fatty acid chains vary in length and saturation (number of double bonds) with temperature; at low temperature E. coli has a higher ratio of **unsaturated** to saturated fatty acids (**2.9** at **10 °C** versus **0.38** at **40 °C**). [L17 p37]
>
> Phospholipid **head groups** vary (for example phosphatidylethanolamine and phosphatidylglycerol). [L17 p36]

LPS and membrane proteins



Gram negative outer membrane with lipopolysaccharide, phospholipid, porin and membrane proteins [L17 p31]

On the figure: phospholipids · membrane proteins

> **Gram negative** *(thin wall plus outer membrane)* has LPS *(outer membrane sugar and fat coat)* in its outer membrane *(extra membrane outside the wall)*. [L16 p36]
>
> LPS act as endotoxins *(toxin built into the cell)*: harmless while the pathogen is intact, but released by a lysed *(broken open)* cell they overstimulate host defenses (textbook).
>
> **Membrane proteins** differ between bacterial species and in bacteria living in different conditions.

Layer	Components	Where it is found
Glycocalyx (capsule or slime layer)	polysaccharides, peptides, DNA	beyond the cell wall; capsule in some **Gram positive** and some **Gram negative** cells and in mycobacteria
S-layer	proteins or glycoproteins	outside the cell wall or membrane: **Gram positive** (possibly all, not certain), **Gram negative** (some), archaea (some to all); not found on all bacteria, and usually lost in lab cultured strains
Outer membrane	LPS and phospholipids	**Gram negative**
Cell wall	peptidoglycan, pseudopeptidoglycan, teichoic acid, mycolic acids, arabinogalactan	**Gram positive**: thick peptidoglycan, teichoic acid in some (another list says most **Gram positive** bacteria, so the sources differ); **Gram negative**: thin peptidoglycan; mycobacteria: mycolic acids, arabinogalactan, peptidoglycan; archaea (some): pseudopeptidoglycan; Mycoplasma: none
Periplasm	thin peptidoglycan, oligosaccharides (osmotic protection), its own unique set of proteins (proteins that bind solutes)	**Gram negative**
Cell membrane	phospholipids and proteins (bacteria; some also hopanoids); isoprenoids (archaea); sterols (Mycoplasma)	all five: **Gram positive**, **Gram negative**, mycobacteria, archaea, Mycoplasma

Table source: [L16 p32, L16 p36, L16 p37, L16 p40, L16 p42, L17 p31, L17 p32, L21 p29, L21 p30, L21 p31, L21 p32, L21 p35]

a. Glycocalyx (capsule and slime layer): role and composition

Book: 3.3 The Envelope and Cytoskeleton, Firmicute Cell Envelope: Gram-Positive (chapter 3 PDF, p. 52); 3.3 The Envelope and Cytoskeleton, Proteobacterial Cell Envelope: Gram-Negative (chapter 3 PDF, p. 56).

⚠ **Do not mix up: Slime layer *(loose sticky coat outside)* is loosely attached; capsule *(firm sugar coat outside the cell)* is more firmly attached. Both are glycocalyx *(sugary coat outside the cell)*.** [L16 p37, L16 p38]

What the glycocalyx is

Glycocalyx: extracellular material beyond the cell wall *(stiff layer around the cell membrane)*. [L16 p37, L16 p38]

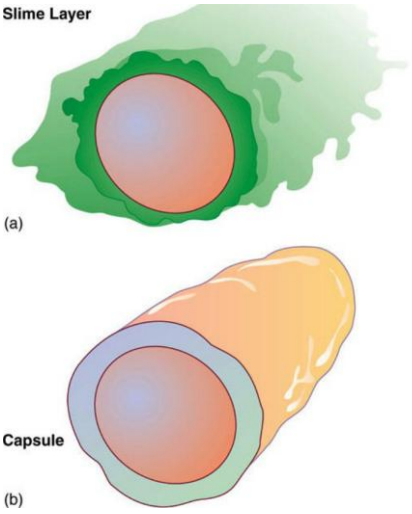
It has two types. [L16 p37, L16 p38]

Slime layer is loosely attached. [L16 p37, L16 p38]

Capsule is more firmly attached. [L16 p37, L16 p38]

A **darkfield microscopy** *(view with a dark background where objects glow bright)* image shows the coat as a bright halo around clusters of cells. [L16 p37]

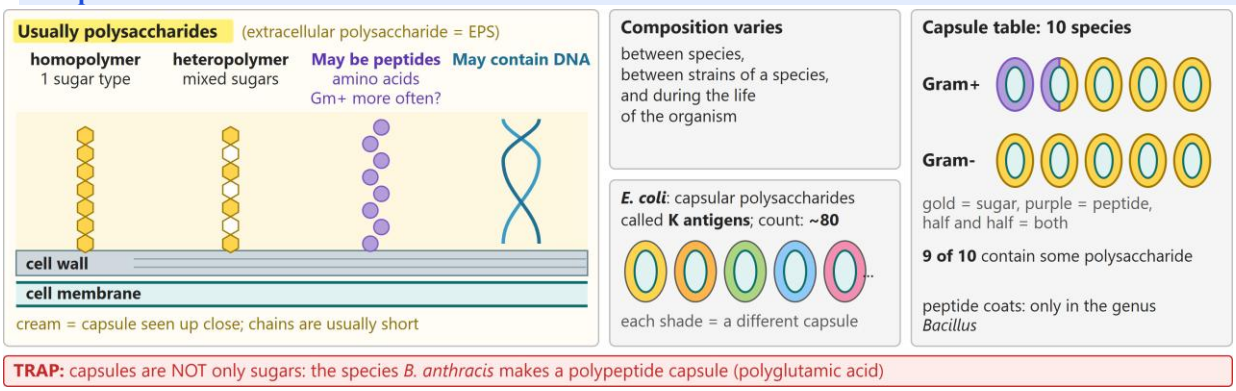
Layers from inside out: inner (cell) membrane *(thin lipid layer)*, **murein** *(another name for peptidoglycan)*, outer membrane *(extra membrane outside the wall)*, capsule. [L16 p38]



Slime layer loosely attached and capsule firmly attached around a cell [L16 p38]

On the figure: Slime layer · capsules

Composition



TRAP: capsules are NOT only sugars: the species *B. anthracis* makes a polypeptide capsule (polyglutamic acid)

Glycocalyx composition: polysaccharides, peptides, DNA; 10 capsule examples [L16 p37]

On the figure: polysaccharides · EPS · homopolymer · heteropolymer · peptides · DNA · K antigen · Bacillus

Glycocalyx molecules: sugars, **peptides** (proteins) and **DNA** *(cell genetic instruction molecule)*.

Composition: usually **polysaccharides** *(long chain of sugars)*. [L16 p40]

May be peptides (more common in **Gram positive**, not certain). [L16 p40]

Peptides are short chains of amino acids. [L16 p40]

May also contain DNA. [L16 p40]

It varies between species, strains of a species, and during the life of an organism. [L16 p40]

Sugar chains and EPS

Usually short chains of a **homopolymer** or **heteropolymer** *(one repeated sugar or mixed sugars)* of sugars. [L16 p40]

These are called extracellular polysaccharide (**EPS**). [L16 p40]

E. coli K antigen: ~**80** different capsular polysaccharides have been observed. [L16 p40]

Capsule examples and conclusion

Conclusion: **8** of the **10 capsules** listed are polysaccharide **only**. [L16 p39, L16 p40]

The **2** that contain a peptide are both **Gram positive** *(thick wall, stains purple)* **Bacillus** species. [L16 p39, L16 p40]

B. anthracis has polyglutamic acid; B. megaterium has both. [L16 p39, L16 p40]

This fits "peptides more common in **Gram positive**". [L16 p39, L16 p40]

All **5 Gram negative** *(thin wall plus outer membrane)* examples are polysaccharides. [L16 p39, L16 p40]

Roles

Roles: attachment (biofilms). [L16 p41]

Biofilms *(slimy community on a surface)*: the glycocalyx is a matrix for their formation.

Protection from phagocytosis *(being eaten by immune cells)*. [L16 p41]

The capsule can prevent phagocytosis by a **phagocyte** *(white blood cell that swallows microbes)*. [L16 p41]

Protection from **desiccation** *(drying out)*. [L16 p41]

Bacterium	Capsule composition	Subunits
Bacillus anthracis (Gram positive)	polypeptide (polyglutamic acid)	D-glutamic acid
Bacillus megaterium (Gram positive)	polypeptide and polysaccharide	D-glutamic acid, amino sugars, sugars
Streptococcus mutans (Gram positive)	polysaccharide (dextran)	glucose
Streptococcus pneumoniae (Gram positive)	polysaccharides	sugars, amino sugars, uronic acids
Streptococcus pyogenes (Gram positive)	polysaccharide (hyaluronic acid)	N-acetyl-glucosamine and glucuronic acid
Acetobacter xylinum (Gram negative)	polysaccharide (cellulose)	glucose
Escherichia coli (Gram negative)	polysaccharide (colonic acid)	glucose, galactose, fucose, glucuronic acid
Pseudomonas aeruginosa (Gram negative)	polysaccharide	mannuronic acid
Azotobacter vinelandii (Gram negative)	polysaccharide	glucuronic acid
Agrobacterium tumefaciens (Gram negative)	polysaccharide (glucan)	glucose

Table source: [L16 p39]

Biofilm step	What happens (textbook)
1	Cells attach to a surface
2	Microcolonies form
3	The cells form a thick extracellular matrix *(sticky sugar web, the EPS)* of polysaccharide polymers
4	The biofilm matures
5	Dispersal: some cells leave to start new biofilms

Table source: [L16 p41]

b. S-layer: composition and where located

Book: 3.3 The Envelope and Cytoskeleton, Firmicute Cell Envelope: Gram-Positive (chapter 3 PDF, p. 52).

⚠ **Do not mix up: S-layer *(crystal like protein coat outside)* is protein, found especially on archaea *(single cell microbes without a nucleus, their own domain (also called archaeal bacteria))*, and is NOT on all bacteria.** [L16 p42, L16 p43]

What the S-layer is

S-layer: a protein coating beyond the cell wall *(stiff layer around the cell membrane)*.

It is observed in some bacteria and archaea.

S-layer (surface layer) features: found on most bacteria (not certain), especially archaea. [L16 p42]

Watch out: It is also described as observed in some bacteria and archaea, and as not found on all bacteria. [L16 p42]

Usually lost in lab cultured strains. [L16 p42]

A layer of proteins or glycoproteins, a complex of a few proteins. [L16 p42]

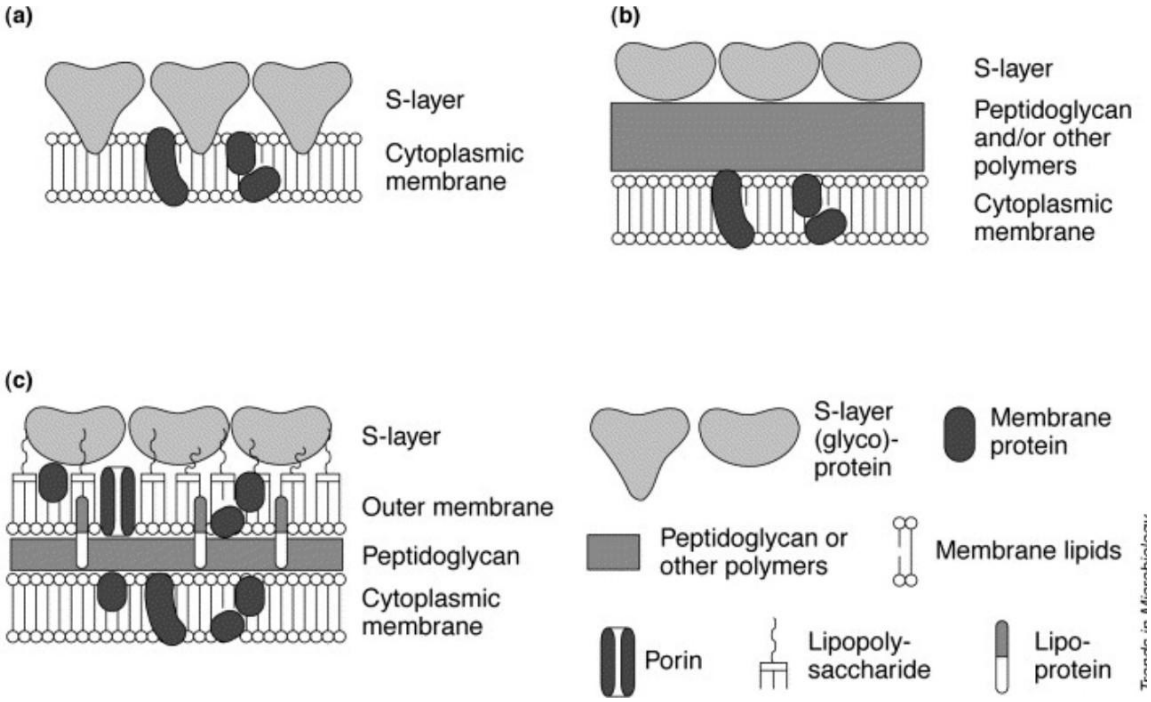
It makes a crystalline *(neat repeating pattern)*, porous matrix. [L16 p42]

The S-layer is built of protein subunits *(building block piece)* that fit together like tiles, and it is rigid but flexes (textbook).

Where it is located

On the outside of the cell wall or membrane *(thin lipid layer)*. [L16 p42]

Where located: the S-layer sits in three places (Sleytr and Beveridge). [L16 p43]



S-layer on archaea, Gram positive and Gram negative envelopes [L16 p43]

On the figure: S-layer · peptidoglycan · outer membrane

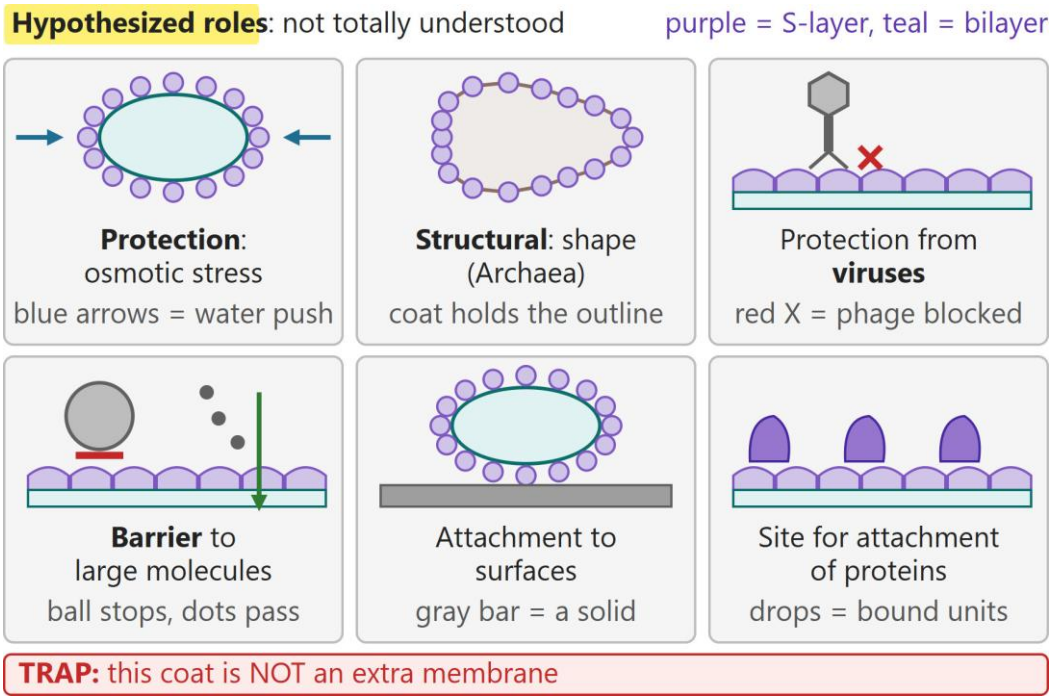
Archaea: no cell wall, just **S-layer** on the membrane. [L16 p43]

Gram positive *(thick wall, stains purple)*: S-layer outside the **peptidoglycan** *(sugar and peptide mesh of the wall)*. [L16 p43]

Gram negative *(thin wall plus outer membrane)*: S-layer outside the **outer membrane** *(extra membrane outside the wall)*. [L16 p43]

S-layer is **NOT** found on all bacteria. [L16 p43]

Hypothesized roles



S-layer hypothesized roles: osmotic stress, viruses, large molecules, attachment [L16 p44]

On the figure: osmotic stress · viruses · large molecules · Attachment to surfaces · Site for attachment of proteins · Structural

Hypothesized roles (not totally understood): protection from osmotic *(about water moving across membranes)* stress. [L16 p44]

Structural (shape, in archaea). [L16 p44]

Protection from **viruses**. [L16 p44]

Barrier to **large molecules**. [L16 p44]

Attachment to surfaces. [L16 p44]

Site for attachment of proteins. [L16 p44]