BIOL 4640 Study Guide for Exam 2 (Wed 10/28/26): Lecture 18 Transport, Peptidoglycan Synthesis and Antibiotics

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): 4.2 Nutrient Uptake (transport proteins); 27.1 Fundamentals of Antimicrobial Therapy, 27.2 Antibiotic Mechanisms of Action and 27.3 Challenges of Drug Resistance and Discovery (peptidoglycan synthesis and antimicrobial therapy). **Also useful:** 3.4 Bacterial Cell Division (FtsZ ring, septation).
(teal italics) = plain meaning of the word before it. ⚠ **Do not mix up = a look-alike pair to keep apart.**

Contents

C. Cell envelope (glycocalyx, outer membrane, cell wall, and cell membrane) 3
Envelope overview 3
Envelope layers 3
Peptidoglycan sugars and carrier 4
Antibiotics and lysozyme 4
Transport and cell division 4
2. Know role, feature and basic composition of each of these layers (3.3 6e) 4
Cell membrane and transport 4
Peptidoglycan in the wall 4
Keeping the layer intact 4
Enzymes that cut peptidoglycan 4
▸ **Basic order of events in synthesis** 4
3. Movement of Material across Cell membrane (3.2 and 4.2 6e) 8
Why membranes matter 9
Ion levels in mM 9
Transport mechanisms 9
Different types of transport 10
Simple diffusion 10
Weak acids in two forms 10
Drugs that are weak acids or bases 10
Facilitated diffusion 10
Active transport kinds 11
Ion coupled transport 11
Which ions power transport 11
Proton motive force 12
Na+ motive force 12
ATP driven transport 12

Group translocation (PTS) 13
PTS and glucose 13
Western Sandhill lakes 13
Eastern saline wetlands 14
Type of transport proteins observed in bacteria (section 4.2) 14
Glycerol transporter 14
ABC family basics 14
ABC import steps 15
ABC example, GAG transporter 15
Siderophore mediated iron uptake 15
ABC exporters 15
PTS proteins 16
4. How do different antibiotics interfere with peptidoglycan synthesis? (section 27.1-3) 17
Peptidoglycan as drug target 17
The cell wall and its enzymes 17
Penicillin and growing bacteria 17
Target of antibacterial drugs / antibiotics 18
General rules 18
Processes blocked 18
Drugs by target 18
Know specific mechanism of action of each: a. Penicillin, b. Cephalosporin, c. Bacitracin, d. Vancomycin, e. D-cycloserine, f. Lysozyme 19
Penicillin and cephalosporin 19
Vancomycin 20
Penicillin family structure 20
Lysozyme and autolysin 20
When penicillin may not work 21
How do bacteria resist the effects of antibiotics? (section 27.3 6e) 22
Drug resistance overview 22
Prevent entry 22
Multidrug exporter in Gram negative 22
Prevent binding 23
Gram negative and penicillin 23
Approval versus resistance dates 23
Rising resistant strains 24

Date conflicts 24
MIC tetracycline tubes 24
Kirby-Bauer plates 24
Penicillin in stream water 25
Gram positive envelope tests 25
E. Cell division and Differentiation (sections 3.4, 3.5 and posted article) 26
FtsZ ring and where new peptidoglycan is made (Lecture 18 slides) 26
FtsZ ring 26
Divisome proteins 27
PBPs 27
Where new peptidoglycan is made 27
Keeping the layer intact 27
Autolysin 27

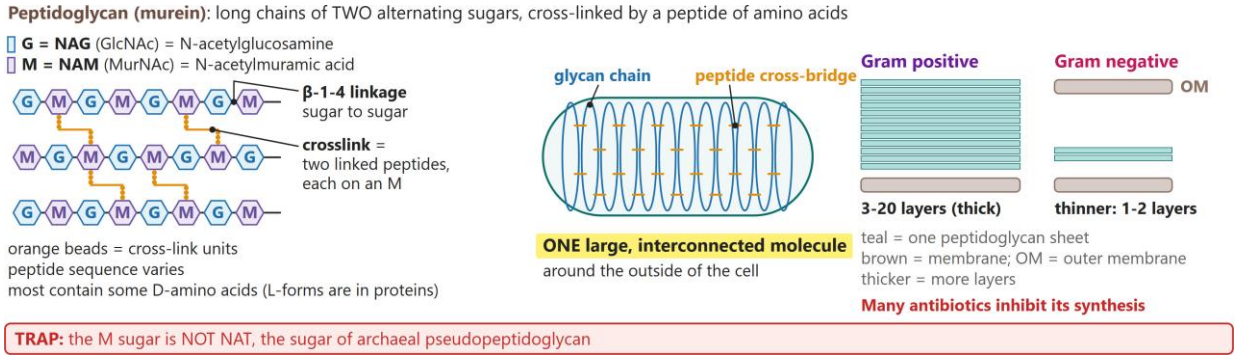
Click a line to jump to it. The PDF side panel (bookmarks) has the same parts as tabs and topics as subtabs.

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

⚠ **Do not mix up: Lysozyme *(enzyme that cuts wall sugars)*** cuts the link between the sugars; penicillin forms a D-Ala-D-Ala like structure.

Envelope overview

Cell envelope *(layers wrapped around the cell)*: layers and components; peptidoglycan *(sugar and peptide mesh of the wall)* synthesis and inhibition; antibacterial chemicals and resistance. [L18 p03]



Peptidoglycan: sugar chains of NAG and NAM joined by peptide cross-bridges [L18 p27]

On the figure: peptidoglycan · NAG · NAM

Peptidoglycan *(sugar and peptide mesh of the wall)* **synthesis** and inhibition. [L18 p03]

Antibiotics interfere with or inhibit peptidoglycan synthesis; **lysozyme** and autolysin *(wall-cutting enzyme made by the bacterium itself)* cleave peptidoglycan. [L18 p03]

Envelope layers

Envelope layers:

> Glycocalyx (capsule), S layer, outer membrane (LPS and phospholipids).
> Continued: cell wall (peptidoglycan, pseudopeptidoglycan, teichoic acid, mycolic acids, arabinogalactan). [L18 p04]

Mycobacterial *(about waxy walled bacteria)* envelope style (**Gram positive** actinomycete): **acid-fast** *(keeps its stain after an acid wash)*. [L18 p03]

Envelope layers continued: periplasm *(space between the two membranes)*, cell membrane (phospholipids, isoprenoids and hopanoids). [L18 p04]

Peptidoglycan sugars and carrier

Peptidoglycan sugars: both sugar precursors (**NAM** and **NAG**) are made from fructose-**6**-P in the cytosol *(liquid part of the cytoplasm)*; then NAG *(one wall sugar)* is linked to NAM *(the other wall sugar)*.

Bactoprenol (C55): NAM-pentapeptide is transferred to it in the cell membrane; it helps to carry peptidoglycan units through the cell membrane.

Antibiotics and lysozyme

Lysozyme cleaves the linkage between the sugars in peptidoglycan (the beta-**1,4** bond between NAM and NAG).

Penicillin and cephalosporin both form a structure similar to D-Ala-D-Ala.

Confirming a Gram positive *(thick wall, stains purple)* **envelope**: test for sensitivity to a penicillin that is more effective against **Gram positive** but not **Gram negative** *(thin wall plus outer membrane)*.

Transport and cell division

Active transport: ion *(charged particle)* coupled (an ion moving with its concentration gradient powers transport) or ATP *(cell energy molecule)* driven (cleavage of the high energy phosphate bond).

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

⚠ **Do not mix up: Lysozyme *(enzyme that cuts wall sugars)* is a defensive enzyme of our body; autolysin *(wall-cutting enzyme made by the bacterium itself)* is produced by bacteria.** [L18 p27, L18 p39]

Cell membrane and transport

Cell membrane *(thin skin around the cell)*: components, function and transport. [L18 p05, L18 p06]

Membrane barrier: the cell membrane acts as a barrier to keep water-soluble proteins and other cell components within the cytoplasm (textbook). [L18 p05, L18 p06]

Membrane: cells try to maintain concentration of certain ions *(charged particle)* and solutes inside the cell that differ from the outside environment. [L18 p05, L18 p06]

Peptidoglycan in the wall

Cell wall (peptidoglycan): peptidoglycan *(sugar and peptide mesh of the wall)* synthesis is unique to bacteria. [L18 p27, L18 p28]

Many antibacterial drugs block peptidoglycan synthesis. [L18 p27, L18 p28]

New peptidoglycan is needed for both cell growth/division and repair/replacement. [L18 p27, L18 p28]

Peptidoglycan sugars: made from fructose-**6**-P in the cytosol *(liquid part of the cytoplasm)*; NAM-pentapeptide is transferred to bactoprenol *(fat carrier moving wall pieces)* in the cell membrane.

Keeping the layer intact

Layer integrity: the layer must stay intact while links between existing and new peptidoglycan are cut. [L18 p27, L18 p28]

Enzymes that cut peptidoglycan

Enzymes that cleave peptidoglycan: lysozyme (a defensive enzyme of our body) and autolysin (produced by bacteria). [L18 p27]

Lysozyme:

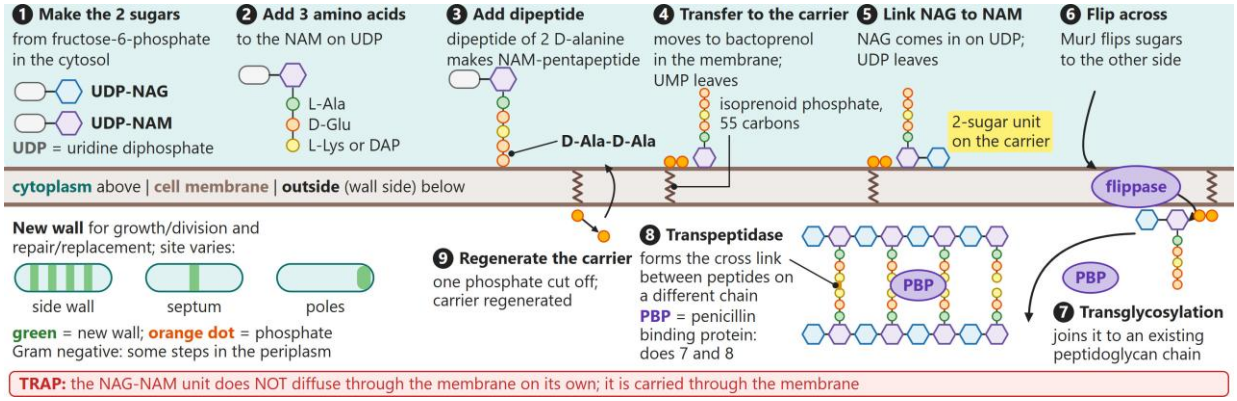
> Found in tears, saliva and mucus (textbook fill).
>
> Cleaves cell wall peptidoglycan (textbook fill). [L18 p39]

▸ **Basic order of events in synthesis**

Book: 27.2 Antibiotic Mechanisms of Action, Cell Wall Antibiotics (chapter 27 PDF, p. 29).

⚠ **Do not mix up: Step 7 transglycosylation *(joining sugar chains longer)* links sugars; step 8 transpeptidation *(making peptide bridges between chains)* cross links peptides *(short chain of amino acids)*.** [L18 p34]

Overview of synthesis

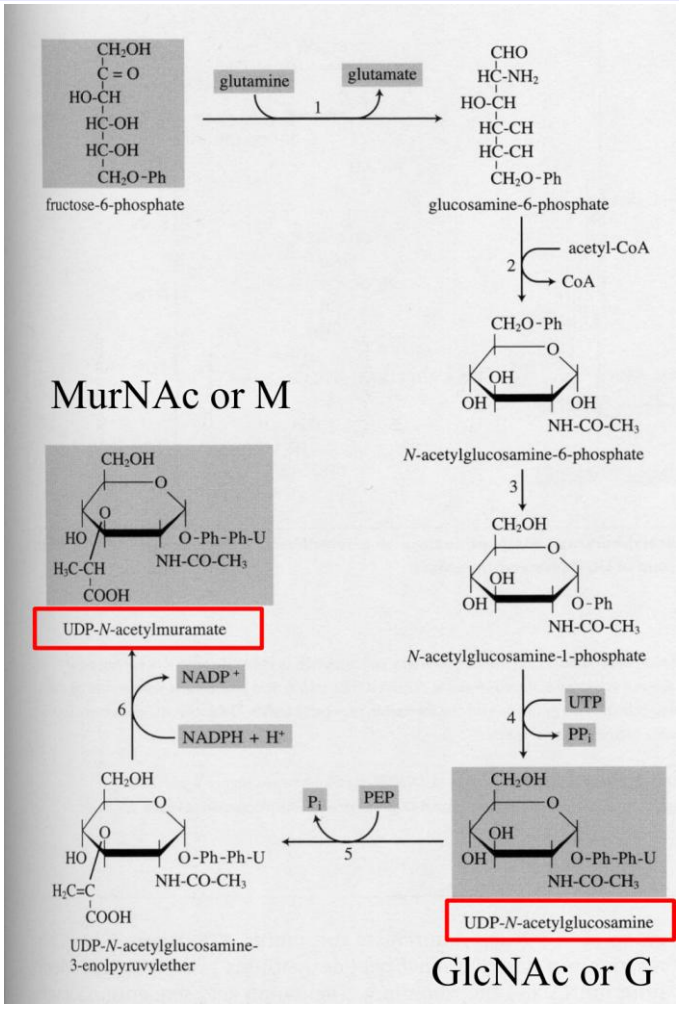


The nine steps of peptidoglycan synthesis in order [L18 p34]

On the figure: UDP · NAG · NAM · Bactoprenol · MurJ · transglycosylation · transpeptidases

> **Overview step 1**:
>
> > Synthesize two sugar units, **NAG** *(one wall sugar)* and **NAM** *(the other wall sugar)*.
> > Also makes a peptide off NAM. [L18 p30]
>
> **Overview step 2**: add the **2** sugar unit to an existing chain. [L18 p30]
>
> **Overview step 3**: then form a crosslink to a peptide in a different chain. [L18 p30]

Making NAG and NAM



Step 1 pathway from fructose-6-phosphate to UDP-NAG and UDP-NAM [L18 p32]

On the figure: glutamine · acetyl-CoA · UTP · PEP · NADPH · UDP-N-acetylglucosamine · UDP-N-acetylmuramate

Step 1 pathway: fructose-**6**-P plus **glutamine** gives glucosamine-**6**-P. [L18 p32]

Step 1 continues:

(**acetyl-CoA**) N-acetylglucosamine-**6**-P, then N-acetylglucosamine-**1**-P.

(**UTP**) **UDP-N-acetylglucosamine** (UDP-GlcNAc, G). [L18 p32]

UDP *(uridine diphosphate)*: the sugars are initially attached to UDP. [L18 p32]

Step 1 continues: (**PEP**) UDP-NAG-**3**-enolpyruvylether, then (**NADPH**) **UDP-N-acetylmuramate** (UDP-MurNAc, M). [L18 p32]

NAM precursor is made from the UDP-NAG one. [L18 p32]

Bactoprenol carrier

Bactoprenol (undecaprenyl):

A large isoprenoid *(branched five carbon repeat fat)* phosphate *(long fat-like carrier with a phosphate)* with **55 carbons** (C55).

Carries peptidoglycan *(sugar and peptide mesh of the wall)* units, LPS *(outer membrane sugar and fat coat)* and teichoic acid *(charged chains in Gram positive wall)* through the cell membrane *(thin skin around the cell)*.

The sugar is attached via the phosphate. [L18 p35]

Lipid I, lipid II and MurJ

Lipid I:

Is NAM-pentapeptide on bactoprenol-PP.

Gets NAG from UDP-NAG to become lipid II. [L18 p35, L18 p36]

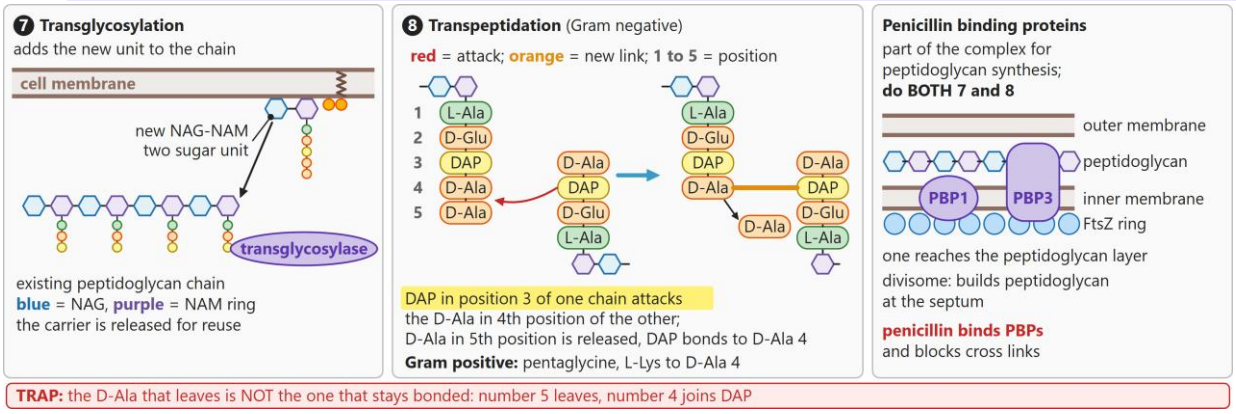
Step 5: UDP-NAG supplies the NAG to NAM and **UDP leaves**. [L18 p33]

MurJ *(flipper protein for wall units)*:

Transmembrane protein (flippase).

Flips sugars to the other side (the lipid-linked NAM-NAG unit, lipid II). [L18 p35, L18 p36]

Step 7 linking sugars



Transglycosylation, transpeptidation and the penicillin binding proteins [L18 p36]

On the figure: transglycosylation · transpeptidation · DAP · pentaglycine · PBPs

Step 7, transglycosylation: link sugars to a glycan *(sugar chain)* chain. [L18 p34]

Step 7:

The transglycosylase attaches the new disaccharide unit to the existing chain.

Linking newly synthesized two sugar units to the existing peptidoglycan chain. [L18 p36]

Step 8 crosslinking

Step 8, transpeptidation: form the cross link. [L18 p34]

Step 8: crosslinking of peptides. [L18 p37]

Transpeptidase *(enzyme making wall cross bridges)* catalyzes the cross-link between side chains of adjacent strands, and cross-linking strengthens the cell wall (textbook).

Gram negative cross link

Gram negative *(thin wall plus outer membrane)*:

DAP *(special amino acid in wall peptide)* in the 3rd position attacks D-Ala *(D form of alanine amino acid)* in the 4th position.

This displaces the D-Ala in the 5th position. [L18 p30, L18 p37]

Gram negative cross link:

DAP of one chain attacks the D-Ala-D-Ala bond of the other chain.

The terminal D-Ala is released and DAP joins the 4th D-Ala. [L18 p30]

Gram positive cross link

Gram-positive *(thick wall, stains purple)* **cross link**:

A **pentaglycine** bridge links L-Lys on one chain

The bridge joins the penultimate D-Ala on the other chain. [L18 p36]

PBPs

PBPs:

Penicillin binding proteins *(enzymes that build the wall)* do the transglycosylation (step **7**) reaction.

Also do the transpeptidation (step **8**) reaction. [L18 p34, L18 p38]

On the outer side of the membrane, **transpeptidases** and transglycosylases bind the D-Ala-D-Ala part of the pentapeptide (textbook).

PBPs: part of the complex involved in synthesis of peptidoglycan. [L18 p38]

Gram positive and Gram negative

Gram positive vs Gram negative: the synthesis pathway above is the **Gram-positive** version. [L18 p33]

Gram negative is similar, but some steps occur in the periplasm *(space between the two membranes)* between the membranes. [L18 p33]

Watch outs and step numbers

Watch out: in overview step **2** the **2** sugar unit is added to an existing chain; **bactoprenol** helps to carry the units. [L18 p30, L18 p35]

Numbering rule: learn the step numbers **1** to **9** from the numbered step list (step **7** transglycosylation, step **8** transpeptidase). [L18 p34]

Watch out: the synthesis diagram with the drug block points uses its own numbers: flip is **5,** transglycosylase **6,** transpeptidase **7,** bactoprenol return **8.** [L18 p33, L18 p36]

Do not mix the pathway numbers with the step numbers **1** to **9.** [L18 p33, L18 p34, L18 p36]

Step	Event	Detail	Blocked by (textbook fill marked)
1	Make the sugar precursors NAM and NAG	Both made from fructose-**6**-P in the cytosol; sugars first attached to UDP (UDP-GlcNAc = G, UDP-MurNAc = M)	none
2	Add **3** amino acids to UDP-NAM to begin the peptide	L-Ala, D-Glu, L-Lys (DAP in **Gram negative**)	none
3	Add the dipeptide of **2** D-alanine to the peptide	Gives the pentapeptide (D-Ala-D-Ala is added to the peptide)	D-cycloserine (cycloserine inhibits formation of the dipeptide: it blocks L-Ala to D-Ala by the Ala racemase and the joining of D-Ala-D-Ala by the ligase)
4	Transfer NAM-pentapeptide to bactoprenol (C55) in the cell membrane	UMP released	none
5	Link NAG to NAM	UDP-NAG supplies the NAG	none
6	Flip the **2** sugar unit to the other side of the bilayer	MurJ flippase	none
7	Transglycosylation: link sugars to a glycan chain	Link sugars to a glycan chain; the transglycosylase adds the new disaccharide unit to the chain; done by PBPs	penicillin and cephalosporins (they bind the PBPs that do transglycosylation); textbook fill: vancomycin
8	Transpeptidase: form the cross link	Cross link between peptides of adjacent chains; done by PBPs	penicillin, cephalosporins, vancomycin
9	Regenerate bactoprenol-P by cleaving off a phosphate group	Carrier is recycled (bactoprenol-PP loses a P and returns)	bacitracin

Table source: [L18 p32, L18 p33, L18 p34, L18 p35, L18 p36, L18 p39]

3. Movement of Material across Cell membrane (3.2 and 4.2 6e)

Book: 4.2 Nutrient Uptake (chapter 4 PDF, p. 23).

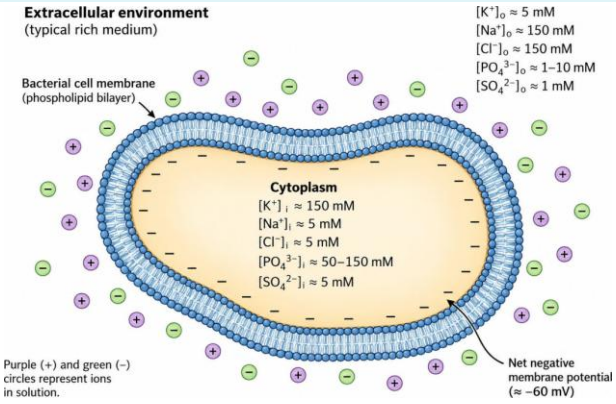
⚠ Do not mix up: Passive *(needs no energy)* transport follows the concentration gradient *(gradual change in amount)*; active transport requires energy. [L18 p07]

Why membranes matter

Why membranes *(thin lipid layer)* **matter**:

> Cells try to maintain concentrations of certain ions *(charged particle)* and solutes inside the cell.
>
> These inside levels differ from the outside environment. [L18 p06]

Membrane potential: net negative, about -**60 mV**. [L18 p06]



Ion levels inside and outside a bacterial cell and the membrane potential [L18 p06]

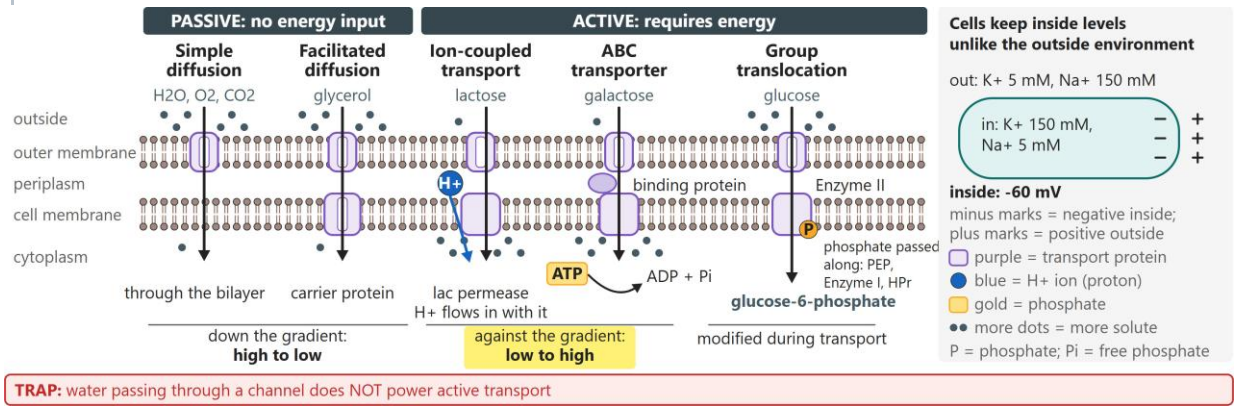
On the figure: K+ · Na+ · Cl- · Membrane potential

Ion levels in mM

Watch out, inside levels differ: the bacterial cell figure gives Cl- about **5 mM** and Na+ about **5 mM** inside; the ion table gives E. coli Cl- **10**-**200** (media dependent) and Na+ **10.** [L18 p06]

Ion table (mM):

> E. coli: K+ **30**-**300,** Na+ **10.**
>
> E. coli: Mg2+ **30**-**100** bound and **0.01**-**1** free.
>
> E. coli: Ca2+ **3** bound and **100** nM free.
>
> E. coli: Cl- **10**-**200** (media dependent).
>
> Sea water: K+ about **10,** Na+ about **500,** Cl- about **500.**
>
> Blood plasma: K+ **4,** Na+ **100**-**200,** Cl- **100. Ion table (mM)**, S. cerevisiae: K+ **300,** Na+ **30,** Mg2+ **50,** Ca2+ **2** bound. **Ion table (mM)**, mammalian cell (heart or RBC): K+ **100,** Na+ **10,** Mg2+ **10** bound and **0.5** free, Ca2+ **10**-**100** nM free, Cl- **5**-**100.** [L18 p06]



Five ways into the cell, passive or active [L18 p07]

On the figure: simple diffusion · facilitated diffusion · ion-coupled transport · ABC · group translocation · Passive · active

Transport mechanisms

Several mechanisms are used: **simple diffusion** *(passing straight through membrane)*, **facilitated diffusion** *(passing through helper protein)*, **ion-coupled transport**, **ABC** (ATP-binding cassette) transporters *(protein that carries molecules across)* and **group translocation** *(carry in while adding a phosphate)*. [L18 p07]

Passive mechanisms depend on the concentration gradient. [L18 p07]

Active mechanisms require energy. [L18 p07]

Ion	Inside the cell (approx.)	Outside, typical rich medium (approx.)
K+	150 mM	5 mM
Na+	5 mM	150 mM
Cl-	5 mM	150 mM
PO4 3-	50-150 mM	1-10 mM
SO4 2-	5 mM	1 mM

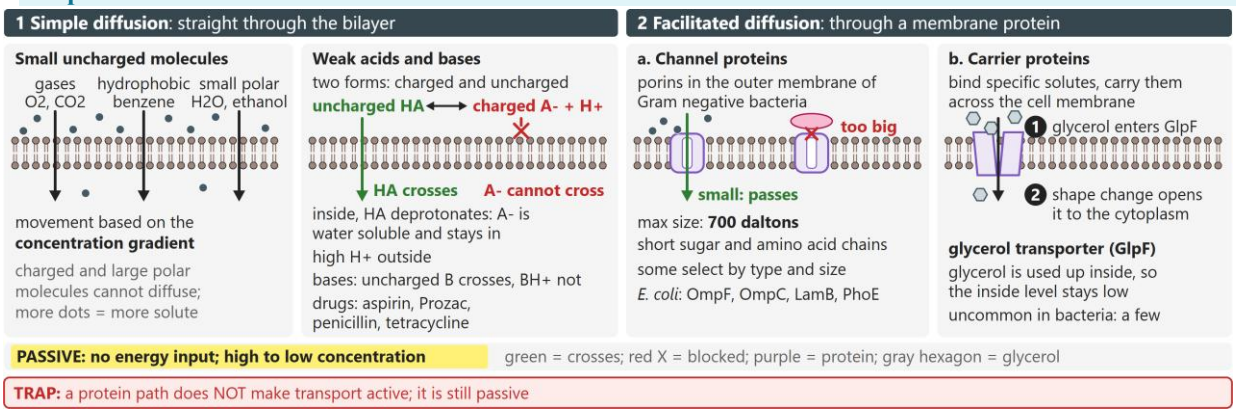
Table source: [L18 p06]

Different types of transport

Book: 4.2 Nutrient Uptake, Facilitated Diffusion (chapter 4 PDF, p. 23); 4.2 Nutrient Uptake, Active Transport Requires Energy (chapter 4 PDF, p. 26).

⚠ **Do not mix up: Antiport *(two molecules, opposite directions)* moves ions *(charged particle)* in opposite directions; symport *(two molecules, same direction)* moves them the same direction.** [L18 p15]

Simple diffusion



Passive transport: simple diffusion, weak acids, channels and carriers [L18 p09]

On the figure: Simple diffusion · Weak acids · Facilitated diffusion · porins · glycerol · benzene · aspirin · Prozac

- **Simple diffusion** *(passing straight through membrane)*:
  - Through the bilayer *(two layers)*.
  - Moves small uncharged molecules (O2, H2O) and hydrophobic *(water hating)* molecules. [L18 p08]
- **Gases** (CO2, O2), hydrophobic molecules (**benzene**) and small polar molecules (H2O, ethanol) pass through the bilayer. [L18 p08]
- **Simple diffusion** movement is based on the concentration gradient *(gradual change in amount)*. [L18 p08]

Weak acids in two forms

- **Weak acids and bases** can exist in two forms. [L18 p09]
- **Weak acid forms**: uncharged acid (HA) or charged (A-). [L18 p09]
- **Uncharged HA** may be able to cross the membrane *(thin lipid layer)*. [L18 p09]
- **Once inside**, HA will deprotonate *(lose its H+)* and be water soluble. [L18 p09]
- **High H+ outside** can drive weak acids into the cell. [L18 p09]
- **H+** protonates weak acids to the uncharged HA form. [L18 p09]

Drugs that are weak acids or bases

- **Many drugs** are weak acids and bases: **aspirin**, **Prozac**, penicillin, tetracycline. [L18 p09]
- **Weak acids** (aspirin, penicillin):
  - Protonated HA is membrane soluble and crosses.
  - Deprotonated A- is water soluble and does not cross. [L18 p09]
- **Weak bases** (Prozac, tetracycline):
  - Uncharged B crosses.
  - Protonated BH+ does not cross. [L18 p09]

Facilitated diffusion

- **Facilitated diffusion** *(passing through helper protein)*:
  - Goes through a membrane protein.
  - Is passive *(needs no energy)*, with no energy input.
  - Flow is determined by the concentration and/or charge gradient.
  - Direction: from high to low. [L18 p10]
- **Examples in bacteria**:
  - Channel proteins (outer membrane **porins**).
  - Carriers in the inner (cell) membrane (rare, **only** a few; one example is the **glycerol** transporter). [L18 p10]

Active transport kinds

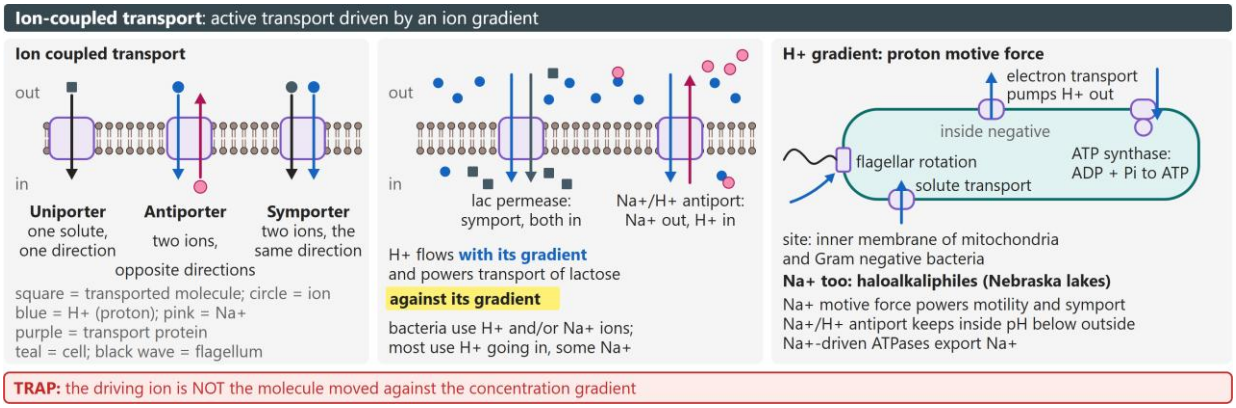
Active transport: against the concentration gradient and requires energy. [L18 p14]

Two kinds of active transport: ion coupled and ATP *(cell energy molecule)* driven. [L18 p14]

Ion coupled transport uses an ion gradient to drive transport. [L18 p14]

ATP driven transport uses energy from cleavage of the high energy phosphate *(PO4 group)* bond. [L18 p14]

Ion coupled transport



Ion coupled transport, proton motive force and the sodium gradient [L18 p15]

On the figure: Uniporter · Antiport · symport · lac permease · Proton motive force · ATP synthase · flagellar rotation · Electron transport

Ion coupled transport:

- An ion moves with its concentration gradient.
- Powers a molecule moving against its concentration gradient. [L18 p15]

Antiport: two like charged ions in opposite directions. [L18 p15]

Symport: two ions in the same direction. [L18 p15]

Watch out: The **lac permease** symporter *(carries two same direction)* example carries lactose (not an ion) in with H+. [L18 p15]

Uniporter *(carries one molecule)*: one solute, one direction. [L18 p15]

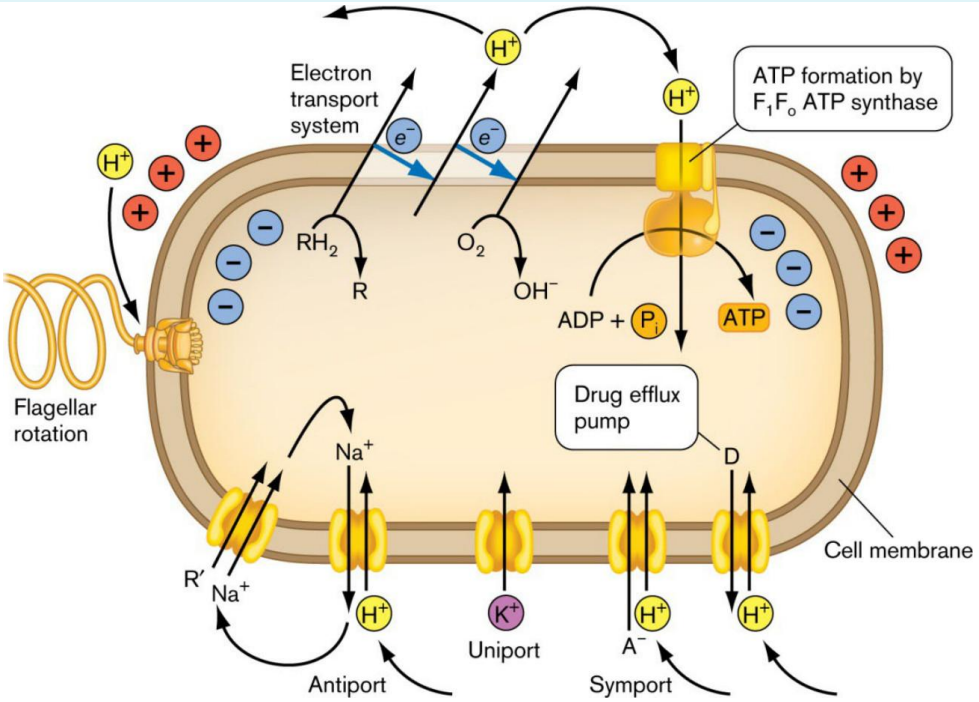
Which ions power transport

Which ions power it: bacteria use H+ and/or Na+ ions to power transport. [L18 p16]

Most bacteria use H+ going in (proton gradient). [L18 p16]

Some bacteria use Na+. [L18 p16]

Proton motive force



Proton motive force: electron transport, ATP synthase, efflux pump and flagellar rotation [L18 p17]

On the figure: Electron transport system · ATP synthase · flagellar rotation · drug efflux pump · Antiport · Symport

Proton motive force *(stored energy from H+ imbalance)*:

> The best known electrochemical gradient is with H+ ions (protons).
>
> Is across the inner membrane *(the skin around the cytoplasm)* of mitochondria and **Gram negative** *(thin wall plus outer membrane)* bacteria.
>
> In bacteria is used for transport in addition to ATP synthesis. [L18 p17]

Electron transport system *(respiratory chain)*: pumps H+ out of the cell to build the proton gradient. [L18 p17, L18 p18]

Proton motive force also drives flagellar rotation and a drug efflux *(pumping the drug out)* pump. [L18 p17]

ATP synthase: H+ flowing back in drives **ADP** *(used up energy molecule)* **+ Pi to ATP**. [L18 p17, L18 p18]

Na+ motive force

Na+ motive force powers flagellar motility *(ability to move)* and the symport of some solutes. [L18 p18]

Na+/H+ antiporters *(carrier moving two ions in opposite directions)* send Na+ out and bring H+ in, which keeps the internal pH below the external pH. [L18 p18]

Sodium channel activated by high pH: replenishes the Na+ needed for **pH homeostasis** *(keeping the inside pH steady)*. [L18 p18]

Na+ driven ATPases export Na+. [L18 p18]

Haloalkaliphilic *(salt and high pH loving)* bacteria use both Na+ and H+ gradients. [L18 p18]

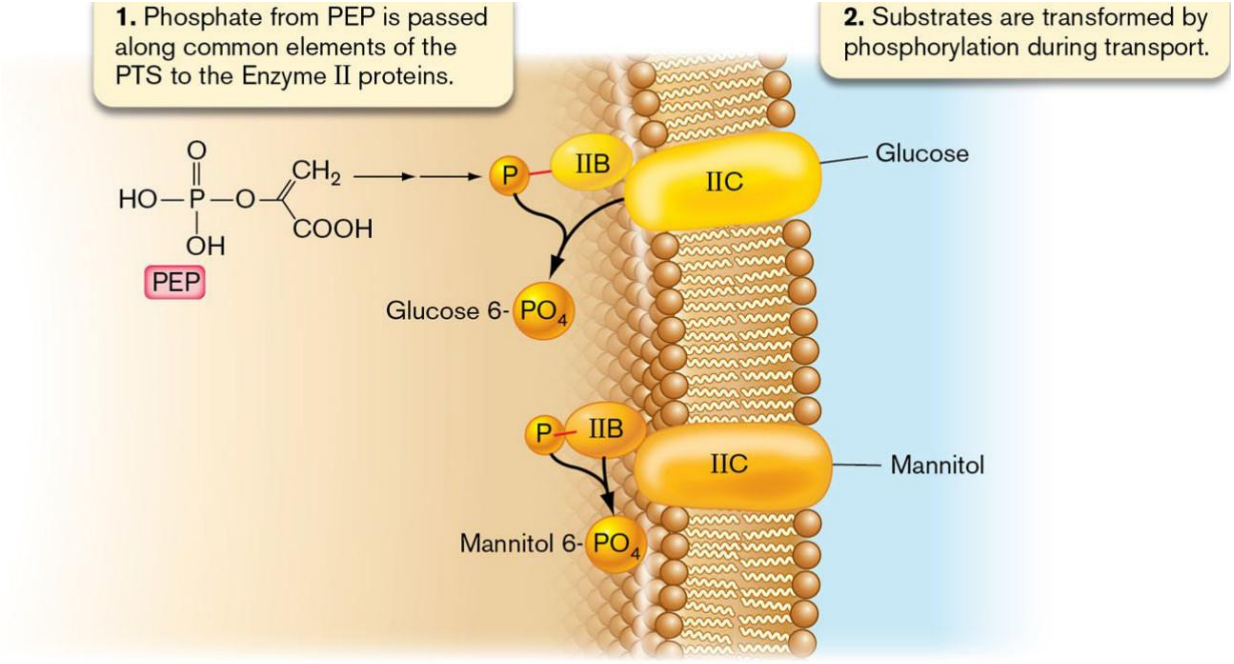
ATP driven transport

ATP driven transport has two forms: a) ABC transporters *(ATP powered pump with binding protein)* and b) group translocation (PTS). [L18 p21, L18 p26]

Watch out: The PTS phosphate comes from PEP *(high energy phosphate donor)*, not ATP. [L18 p21, L18 p26]

ABC transporters are one form of ATP driven transport. [L18 p21, L18 p26]

Group translocation (PTS)



Group translocation: PEP phosphate passed to glucose and mannitol during transport [L18 p26]

On the figure: PEP · PTS · phosphate · glucose

Group translocation (phosphotransfer system, **PTS**): the molecule is modified during transport. [L18 p21, L18 p26]

PTS stands for phosphotransfer system; the textbook name is **phosphotransferase system**, the same thing. [L18 p26]

Group translocation:

- Used for transport of some sugars into cells.
- A **phosphate** from **PEP** is added to the sugar as it is transported in.
- Is **only** found in bacteria and some archaea *(single cell microbes without a nucleus, their own domain (also called archaeal bacteria))*. [L18 p26]

PTS and glucose

PTS and glucose (textbook):

- The PEP phosphate is added as **glucose** crosses.
- Glucose-**6**-phosphate (a different molecule) is inside.
- Glucose itself never accumulates in the cytoplasm (textbook).
- So glucose always moves down its own gradient. [L18 p26]

Western Sandhill lakes

Nebraska has alkaline and brine lakes and marshes. [L18 p18]

Nebraska examples: Western Sandhill alkaline lakes (Garden and Sheridan Counties). [L18 p19, L18 p20]

Western Sandhill lakes:

- Many lakes reach pH above **10.**
- Carbonate-rich water, unusually high potassium. [L18 p19, L18 p20]
- White salt **crusts** can form along shorelines. [L18 p19]
- Evaporation concentrates K+ and carbonate salts. [L18 p19, L18 p20]
- Select for haloalkaliphiles *(salt and high pH loving microbes)* and other **polyextremophiles** *(microbes that handle several extremes at once)*.
- Some microbial communities include **chemolithoautotrophs** *(microbes that live on inorganic chemicals and make their own carbon food)*. [L18 p19]

Eastern saline wetlands

Eastern saline wetlands:

Salt Creek floodplain in Lancaster and southern Saunders Counties.

Saline groundwater from ancient marine salt-bearing formations. [L18 p19, L18 p20]

Summer **drying** can leave visible salt crusts.

Salt-tolerant plants and microbial communities adapted to **osmotic** *(salt and water balance)* stress. [L18 p20]

Type	Passive or active	Energy and direction	Example
Simple diffusion	Passive	No energy; through the bilayer, down the concentration gradient	O2, H2O, CO2, hydrophobic molecules (benzene), small polar molecules (H2O, ethanol)
Facilitated diffusion, channel proteins	Passive	No energy input; through a membrane protein, high to low	Outer membrane porins in **Gram negative**
Facilitated diffusion, carrier proteins	Passive	No energy input; carrier binds a specific molecule; rare in bacteria	Glycerol transporter
Ion-coupled transport	Active	An ion moving down its gradient powers a molecule moving against its gradient	Lactose permease with H+
ATP driven: ABC transporters	Active	Energy from cleavage of the high energy phosphate bond of ATP	Galactose with galactose-binding protein; siderophore-iron
ATP driven: group translocation (PTS)	Active	Phosphate from PEP is added to the molecule as it enters	Glucose enters as glucose-**6**-phosphate

Table source: [L18 p07, L18 p08, L18 p10, L18 p13, L18 p14, L18 p15, L18 p21, L18 p26]

Type of transport proteins observed in bacteria (section 4.2)

Book: 4.2 Nutrient Uptake, ABC Transporters Are Powered by ATP (chapter 4 PDF, p. 30); 4.2 Nutrient Uptake, Group Translocation Avoids "Uphill" Battles (chapter 4 PDF, p. 35).

⚠ **Do not mix up: ABC transport uses ATP *(cell energy molecule)*; the PTS adds a phosphate *(PO4 group)* from PEP *(high energy phosphate donor)* to the sugar.** [L18 p26]

Glycerol transporter

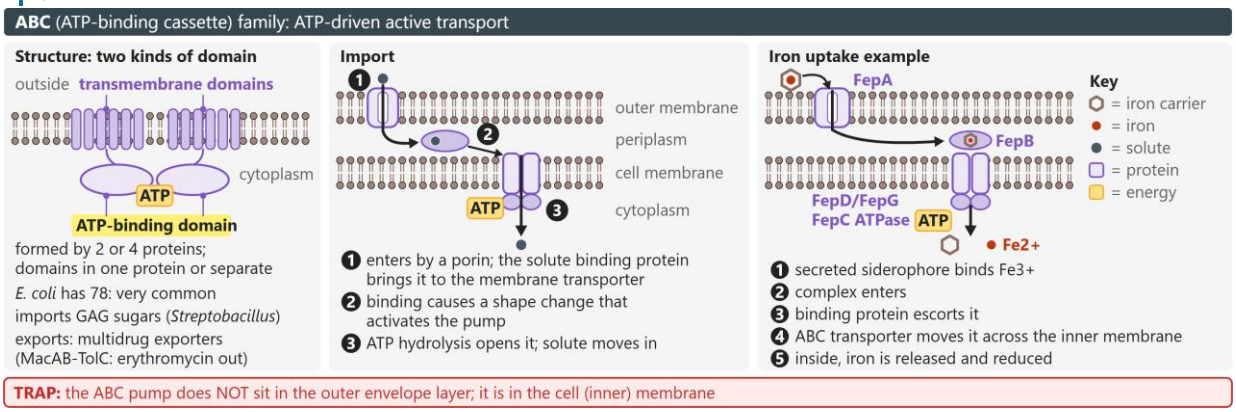
Carrier example, glycerol *(three carbon fat backbone)* **transporter** *(protein that carries molecules across)*: usually transports glycerol in. [L18 p13]

Glycerol transporter: the glycerol concentration inside the cell is very low because it is used up. [L18 p13]

Glycerol channel step 1: glycerol enters the GlpF channel from the extracellular fluid. [L18 p13]

Glycerol channel step 2: a conformational change in GlpF opens the channel to the cytoplasm *(jelly inside the cell membrane)*, and glycerol enters the cell. [L18 p13]

Watch out: the text lists the glycerol transporter as a carrier protein, while the diagram calls GlpF a channel. [L18 p13]



ABC transporter structure, solute import and iron uptake with siderophores [L18 p21]

On the figure: ABC · transmembrane · periplasm · siderophores · FepA · FepB · GAG · TolC

ABC family basics

ABC (ATP-binding cassette) family: common in bacteria (E. coli has **78**). [L18 p21]

ABC family:

Formed by **2** or **4 proteins**.

Has two domains: **transmembrane** and ATP binding. [L18 p21]

ABC transporter *(ATP powered pump with binding protein)*: hinge regions link the transmembrane domains to the ATP site. [L18 p21]

ABC domains can be in the same protein or separate. [L18 p21]

ABC with a solute binding protein *(protein that grabs one solute)*: may also have a solute binding protein in the **periplasm** *(space between the two membranes)*. [L18 p24]

Solute binding protein brings the solute to the transporter. [L18 p24]

Substrate-binding proteins have a high affinity for their matched solutes, which makes transport efficient when solute concentrations are low (textbook).

ABC import steps

ABC import step 1:

After entering through a porin *(water filled channel in outer membrane)*, the solute binds its periplasmic *(in the space between the two membranes)* binding protein.

The complex then binds the membrane *(thin lipid layer)* transporter. [L18 p24]

ABC import step 2: the solute binds the transporter and a conformational change activates it. [L18 p24]

Conformational change *(shape change)* activates the transporter. [L18 p24]

ABC import step 3:

After ATP hydrolysis (**2** ATP to **2** ADP + phosphate)

The dimer opens and releases the solute into the cytoplasm. [L18 p24]

ABC example, GAG transporter

ABC example: a transporter of glycosaminoglycan (**GAG**) disaccharides in Streptobacillus (sulfated chondroitin); Streptococcus takes up its GAG disaccharide (hyaluronan) by a PTS instead. [L18 p22]

GAG transporter has a periplasmic binding protein and ATPase. [L18 p22]

GAG transport: a **polysaccharide** *(long chain of sugars)* **lyase** *(enzyme that cuts a sugar chain)* cuts the polymer into disaccharides before import. [L18 p22]

Siderophore mediated iron uptake

Siderophore *(iron grabbing molecule)* **mediated transport**:

Bacteria secrete **siderophores** which bind an iron (Fe3+) ion *(charged particle)*.

An ABC type transporter moves the complex across the cell membrane *(thin skin around the cell)*.

Inside the cell the Fe3+ is released. [L18 p25]

Iron uptake: once inside the cell, the iron is released and reduced to **Fe2+**. [L18 p25]

Siderophore example: enterochelin is the siderophore that binds Fe3+; **FepA** is the outer membrane *(extra membrane outside the wall)* protein; **FepB** is the periplasmic binding protein; FepG and FepD form the ABC transporter, with FepC as the ATPase. [L18 p25]

ABC exporters

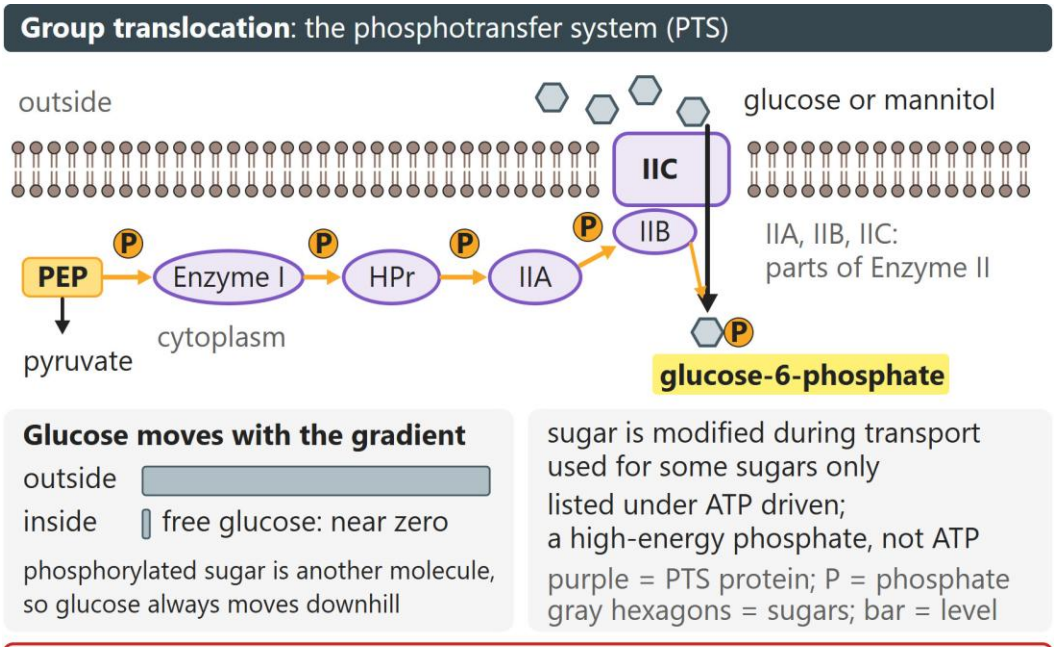
ABC exporters: some ABC transporters move chemicals out, such as some multidrug exporters. [L18 p23]

MacB (ABC protein in the inner membrane):

With MacA and **TolC** spans to the outer membrane.

With MacA and TolC pumps erythromycin out.

With MacA and TolC also secretes **enterotoxin STII** *(a toxin protein made by the bacterium)*. [L18 p23]



Group translocation: phosphate passed from PEP to the sugar as it enters [L18 p26]

On the figure: PEP · Enzyme I · HPr · IIB · IIC · Enzyme II · mannitol · PTS

PTS proteins

PTS:

Phosphate from **PEP** is passed along common **PTS** proteins to the **Enzyme II** proteins.

The Enzyme II proteins shown are **IIB** and **IIC**; the transport overview also shows **Enzyme I** and **HPr** passing the phosphate.

Enzyme II proteins phosphorylate the sugar during transport. [L18 p26]

PTS products: glucose or **mannitol** *(sugar alcohol)* become glucose-**6**-P or mannitol-**6**-P. [L18 p26]

Transport protein	Where	What it does	Example
Porin (channel protein)	Outer membrane of **Gram negative**	Passive channel; some selective for type and size; maximum size **700 daltons** (short chains of sugars and amino acids)	OmpF, OmpC, OmpA, LamB, PhoE
Carrier protein	Inner (cell) membrane	Binds specific molecules and transports them across the membrane; uncommon in bacteria	Glycerol transporter
Ion-coupled transporter (uniporter, antiporter, symporter)	Cell membrane	Uses an H+ or Na+ gradient to move a molecule against its gradient	Lac permease (lactose with H+), Na+/H+ antiporter, phosphate, sulfate symporters, K+ uniporter
ABC transporter	Cell membrane	ATP driven; two transmembrane domains plus ATP binding domains	E. coli has **78**
PTS enzymes (Enzyme I, HPr, Enzyme II)	Cell membrane	Group translocation: phosphate from PEP passed along to Enzyme II, which phosphorylates the sugar as it crosses	Glucose to glucose-**6**-phosphate

Table source: [L18 p05, L18 p11, L18 p13, L18 p16, L18 p21, L18 p26]

Porin (E. coli)	Function
OmpF	Nonspecific channel for small molecules; receptor for phage T2
OmpC	Nonspecific channel for small molecules; receptor for phage T4
OmpA	Specific for peptides; receptor for mating pili
LamB	Porin for maltose and maltodextrin; receptor for phage lambda
PhoE	Anion-selective channel

Table source: [L18 p12]

4. How do different antibiotics interfere with peptidoglycan synthesis? (section 27.1-3)

Book: 27.2 Antibiotic Mechanisms of Action, Cell Wall Antibiotics (chapter 27 PDF, p. 29).

⚠ **Do not mix up:** Penicillin only kills bacteria that are growing and making new wall material.

Peptidoglycan as drug target

Why peptidoglycan *(sugar and peptide mesh of the wall)* **is a good drug target**: its synthesis is unique to bacteria. [L18 p27, L18 p39]

Antibacterial drugs (antibiotics): many interfere with or inhibit peptidoglycan synthesis. [L18 p27, L18 p39]

Different antibiotics inhibit different steps. [L18 p27, L18 p39]

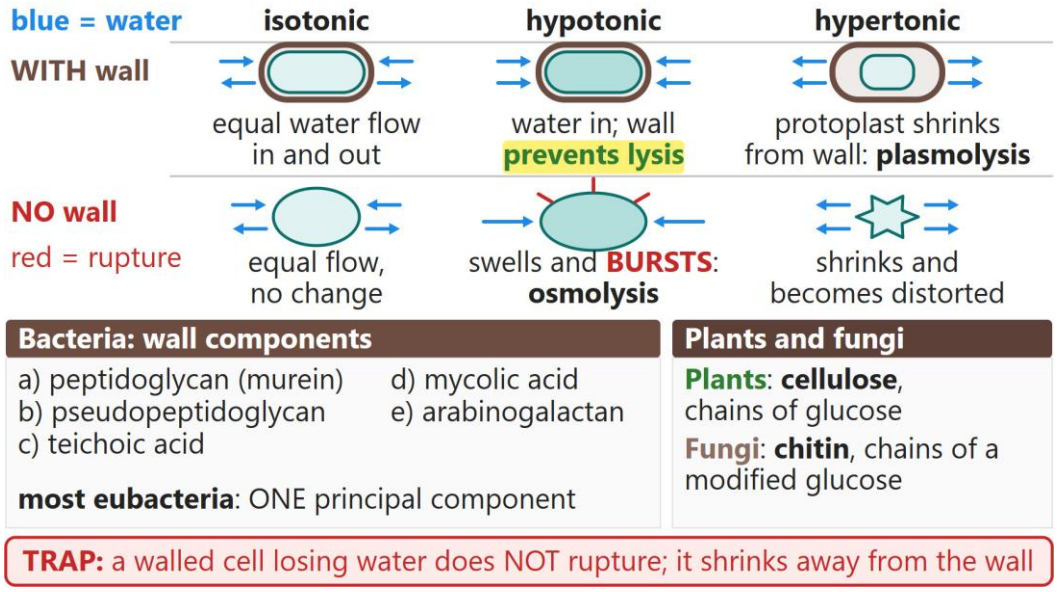
Some antibiotics block wall synthesis. [L17 p12]

The cell wall and its enzymes

Cell wall: semi-rigid, porous layer beyond the cell membrane

Role: structural support, rigidity; helps determine cell shape

Its synthesis is blocked by some antibiotics



The cell wall prevents lysis in hypotonic conditions; without it the cell bursts [L18 p27]

On the figure: cell wall · hypotonic · Water · bursts · Peptidoglycan

Cell wall *(stiff layer around the cell membrane)* prevents cells from lysing *(bursting)* in **hypotonic** *(low solute outside)* conditions. [L17 p12]

Water diffuses into the cell by osmosis, and the resulting turgor pressure **bursts** the cell unless the cell wall counters it (textbook).

Weak wall: autolysins *(wall-cutting enzyme made by the bacterium itself)* keep cutting the existing wall while penicillin blocks the new cross-links, so the wall of a growing cell weakens and the cell bursts (textbook fill). [L18 p39]

Not growing: penicillin blocks the making of new **peptidoglycan** and does not damage the existing layer, so cells that are not growing are barely affected. [L21 p53]

New peptidoglycan is needed for growth/division and repair/replacement. [L18 p28]

Enzymes that cleave peptidoglycan: lysozyme *(enzyme that cuts wall sugars)*, autolysin. [L18 p27, L18 p39]

Penicillin and growing bacteria

Penicillin only kills bacteria that are growing.

Growing bacteria are synthesizing new cell wall material.

Step	Event	What blocks it (textbook fill marked)
3	Add D-Ala-D-Ala dipeptide	D-cycloserine
7	Transglycosylation	Penicillin and cephalosporins (they bind the PBPs); textbook fill: vancomycin
8	Transpeptidation (cross linking)	Penicillin, cephalosporins, vancomycin
9	Regenerate bactoprenol-P	Bacitracin
after synthesis	Cleave the linkage between the sugars in peptidoglycan	Lysozyme (a defensive enzyme of our body that cleaves peptidoglycan; textbook fill: found in tears, saliva and mucus; it cuts, not a synthesis block)

Table source: [L18 p27, L18 p30, L18 p33, L18 p34, L18 p36, L18 p39]

Target of antibacterial drugs / antibiotics

Book: 27.2 Antibiotic Mechanisms of Action (chapter 27 PDF, p. 27).

⚠ **Do not mix up: Conflict: one table puts nitroimidazoles *(drug family, e.g. metronidazole)* under nucleic acid function; another figure puts metronidazole under metabolism.** [L18 p41, L18 p42]

General rules

General rules: most antibacterial drugs (antibiotics) target unique features of bacteria. [L18 p40]

Antibacterial drugs may be cidal *(kill)* or static *(only stop growth)*. [L18 p40]

Most antibiotics inhibit the synthesis of a cell component. [L18 p40, L18 p41]

Most inhibit an enzyme involved in biosynthesis. [L18 p40, L18 p41]

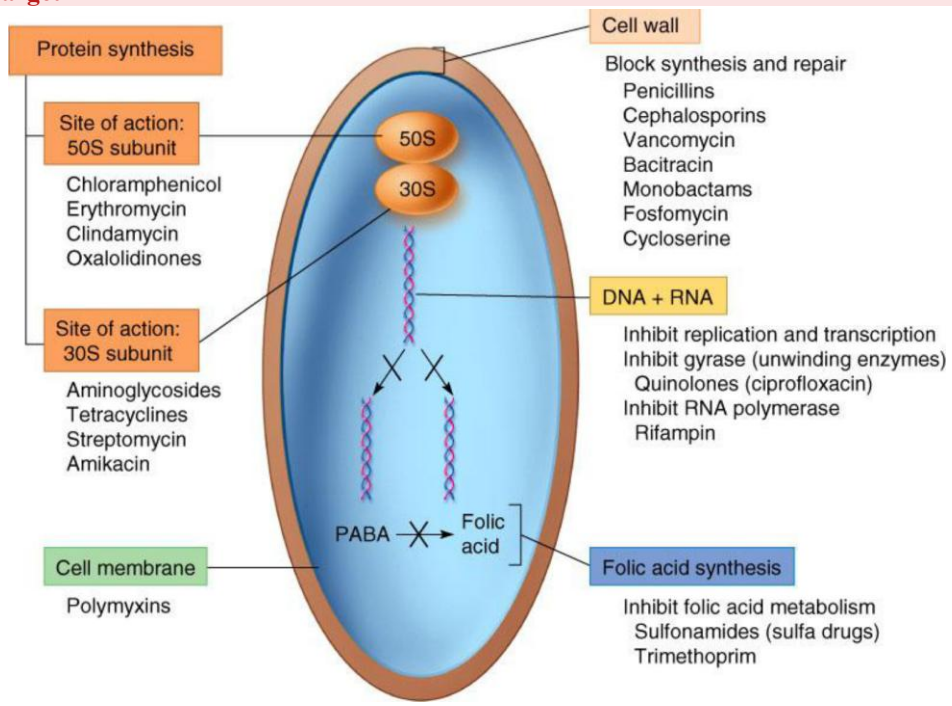
Most block synthesis of new macromolecules (cell components). [L18 p42]

Bacterial pathogens are becoming resistant to commonly used antibiotics. [L18 p40, L18 p41]

Processes blocked

Processes blocked: proteins, cell wall *(stiff layer around the cell membrane)*, nucleic acids (DNA or RNA), metabolites *(small working molecules of the cell)*. [L18 p42]

Drugs by target



Drug targets in the cell and the antibiotics for each target [L18 p43]

On the figure: cell wall · 50S · 30S · Cell membrane · polymyxins · Folic acid · rifampin · quinolones

Cell wall (block synthesis and repair):

> Penicillins, cephalosporins *(penicillin like wall blocking drugs)*, vancomycin *(drug blocking wall building)*, bacitracin *(drug blocking carrier recycling)*.
>
> Also: monobactams *(single ring beta lactam drugs)*, fosfomycin *(drug blocking early wall steps)*, cycloserine *(drug blocking D alanine steps)*. [L18 p43]

50S: chloramphenicol, erythromycin, clindamycin, oxazolidinones *(drug family, e.g. linezolid)*. [L18 p43]

30S: aminoglycosides *(drugs causing ribosome misreading)*, tetracyclines, streptomycin, amikacin. [L18 p43]

Cell membrane *(thin skin around the cell)*: **polymyxins**. [L18 p43]

DNA *(cell genetic instruction molecule)* **and RNA**: inhibit gyrase (**quinolones**, ciprofloxacin) and RNA polymerase (**rifampin**). [L18 p43]

Nucleic acid function: also some antiviral compounds, especially **antimetabolites** *(look-alikes of a normal building block)*. [L18 p41]

Folic acid metabolism: sulfonamides, trimethoprim. [L18 p43]

Target	Antibiotic examples
Cell wall synthesis	Penicillins, cephalosporins, bacitracin, vancomycin
Protein synthesis	Chloramphenicol, tetracyclines, aminoglycosides, macrolides, lincosamides
Cell membrane integrity	Polymyxin, daptomycin, amphotericin, imidazoles (vs fungi)
Nucleic acid function	Nitroimidazoles, nitrofurans, quinolones, rifampin
Intermediary metabolism	Sulfonamides, trimethoprim

Table source: [L18 p41]

Site	Drugs
Cell wall (peptidoglycan synthesis)	Penicillins, cephalosporins, vancomycin, bacitracin, monobactams
Cell membrane	Polymyxins, daptomycin, gramicidin
Metabolism	Sulfonamides, trimethoprim, metronidazole
DNA replication	Quinolones
RNA polymerase	Rifampin, pyronins
50S ribosome subunit	Chloramphenicol, macrolides (erythromycin), clindamycin, oxazolidinones (linezolid), streptogramins (Synercid)
30S ribosome subunit	Aminoglycosides (gentamicin), tetracyclines (doxycycline)

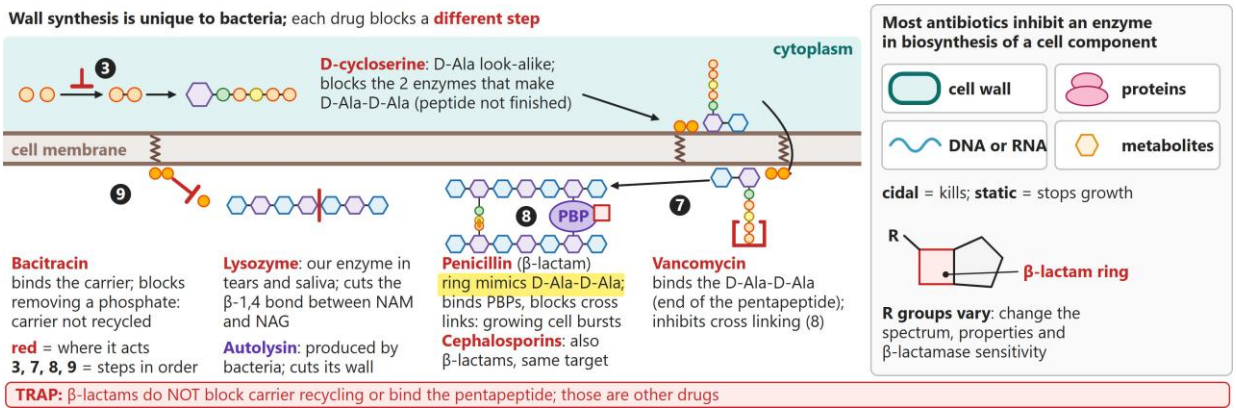
Table source: [L18 p42]

Know specific mechanism of action of each: a. Penicillin, b. Cephalosporin, c. Bacitracin, d. Vancomycin, e. D-cycloserine, f. Lysozyme

Book: 27.2 Antibiotic Mechanisms of Action, Cell Wall Antibiotics (chapter 27 PDF, p. 29).

⚠ **Do not mix up: Penicillin and cephalosporins *(penicillin like wall blocking drugs)* block both transglycosylation (step 7) and transpeptidase cross-linking (step 8); vancomycin blocks the transpeptidase cross-linking (step 8).** [L18 p33, L18 p36, L18 p39]

Penicillin and cephalosporin



Where each antibiotic and enzyme acts on peptidoglycan synthesis [L18 p33]

On the figure: Penicillins · vancomycin · Lysozyme · autolysin · cephalosporins · PBPs · D-Ala-D-Ala

Penicillin and cephalosporin are alike: both bind **PBPs** and inhibit transpeptidase (cross-linking, step **8**) and transglycosylation (step **7**). [L18 p36]

Beta-lactams *(drug family with four member ring)*: both have a ring that mimics **D-Ala-D-Ala** (textbook fill). [L18 p39]

This mimicry lets beta-lactams target the penicillin-binding proteins that carry out cell wall *(stiff layer around the cell membrane)* synthesis and remodeling (textbook).

Penicillin:

- Blocks the PBPs. [L18 p33]
- Inhibits transpeptidase (peptide cross linking, step **8**) and transglycosylation (step **7**). [L18 p39]

Cephalosporins are modified in generations (textbook fill). [L18 p39]

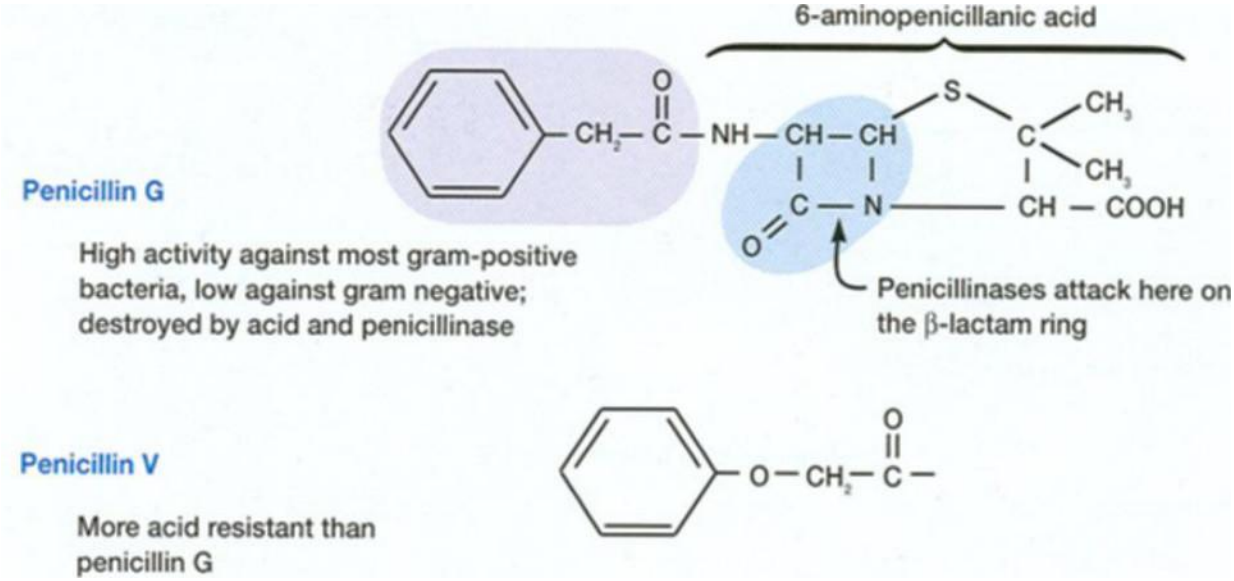
Cephalothin, the first cephalosporin, was introduced in **1964.** [L18 p45]

Vancomycin

Vancomycin *(drug blocking wall building)*:

- Binds D-Ala-D-Ala. [L18 p30]
- Inhibits the transpeptidase (peptide cross-linking, step **8**); textbook fill: it also prevents the action of transglycosylases (step **7**). [L18 p39]

Penicillin family structure



Penicillin G ring core and the site penicillinases attack [L18 p51]

On the figure: **6-aminopenicillanic acid** · **penicillin G** · **Penicillinases**

Penicillin or beta-lactam family structure: functional groups off the ring vary (different R groups). [L18 p51]

6-aminopenicillanic acid: the ring core of **penicillin G**. [L18 p51]

Penicillins share this core and differ in the group off the ring. [L18 p51]

R groups:

- Affect spectrum of activity.
- Affect properties (acid stability is one example).
- Affect sensitivity to particular beta-lactamases *(enzyme that destroys beta lactam drugs)*. [L18 p51]

Penicillinases *(enzyme that breaks the drug)* attack the beta-lactam ring. [L18 p51]

Lysozyme and autolysin

Lysozyme *(enzyme that cuts wall sugars)* **and autolysin** *(wall-cutting enzyme made by the bacterium itself)*: lysozyme is a defensive enzyme of our body that cleaves peptidoglycan *(sugar and peptide mesh of the wall)*. [L18 p27]

Autolysin: produced by bacteria. [L18 p39]

When penicillin may not work

Penicillin:

> The growing bacteria must be synthesizing new cell wall material.
>
> May not work on the type of bacteria causing the illness (**Gram negative**).

Penicillin has difficulty passing through the **Gram-negative** *(thin wall plus outer membrane)* outer membrane *(extra membrane outside the wall)*, so it is more effective against **Gram positive** *(thick wall, stains purple)* organisms (textbook).

Penicillin is more effective against **Gram positive**, so sensitivity can serve as a test.

Mycoplasma:

> Mollicutes (Mycoplasma) have no cell wall. [L18 p03]
>
> Has no wall, so drugs that inhibit peptidoglycan synthesis have no target in it. [L18 p27]

Agent	Step	Mechanism of action (textbook fill marked)
a. Penicillin (beta-lactam family)	**7** and **8,** transglycosylation and transpeptidase cross linking	Binds the PBPs that do transpeptidation (peptide cross linking) and transglycosylation, and inhibits both (penicillin blocks the PBPs). Forms a structure similar to D-Ala-D-Ala. Textbook fill: the beta-lactam ring mimics D-Ala-D-Ala, weak wall, growing cell bursts.
b. Cephalosporins	**7** and **8,** transglycosylation and transpeptidase cross linking	Also a beta-lactam: inhibits transpeptidase (peptide cross-linking) and transglycosylation, like penicillin; same D-Ala-D-Ala like structure. Textbook fill: structure modified in a series of generations.
c. Bacitracin	**9,** bactoprenol recycling	Bactoprenol-PP loses a P and returns, and bacitracin blocks this (it inhibits incorporation of disaccharide units). Textbook fill: binds bactoprenol and blocks its dephosphorylation, so the carrier cannot accept a new unit of UDP-NAM.
d. Vancomycin	**8**; **7** and **8** (textbook fill)	Binds D-Ala-D-Ala and blocks cross-bridge formation; vancomycin inhibits the transpeptidase (peptide cross-linking). Textbook fill: binds the D-Ala-D-Ala terminal end of the disaccharide unit and also prevents the action of transglycosylases (step **7**).
e. D-cycloserine	**3,** D-Ala-D-Ala dipeptide	Cycloserine inhibits formation of the D-Ala-D-Ala dipeptide. Textbook fill: D-alanine analog that inhibits the two enzymes that make the D-Ala-D-Ala dipeptide, so the complete pentapeptide cannot be made.
f. Lysozyme	none (cuts finished peptidoglycan)	A defensive enzyme of our body that cleaves peptidoglycan; cleaves the linkage between the sugars in peptidoglycan. Textbook fill: found in tears, saliva and mucus; cleaves cell wall peptidoglycan.

Table source: [L18 p27, L18 p30, L18 p33, L18 p36, L18 p39]

Penicillin type	Note
Penicillin G	High activity against most **Gram positive**, low against **Gram negative**; destroyed by acid and penicillinase
Penicillin V	More acid resistant than penicillin G
Ampicillin	Active against **Gram positive** and **Gram negative**; acid stable
Carbenicillin	Active against **Gram negative** like Pseudomonas and Proteus; acid stable; not well absorbed by small intestine
Methicillin	Penicillinase resistant, but less active than penicillin G; acid labile
Ticarcillin	Similar to carbenicillin, but more active against Pseudomonas

Table source: [L18 p51]

How do bacteria resist the effects of antibiotics? (section 27.3 6e)

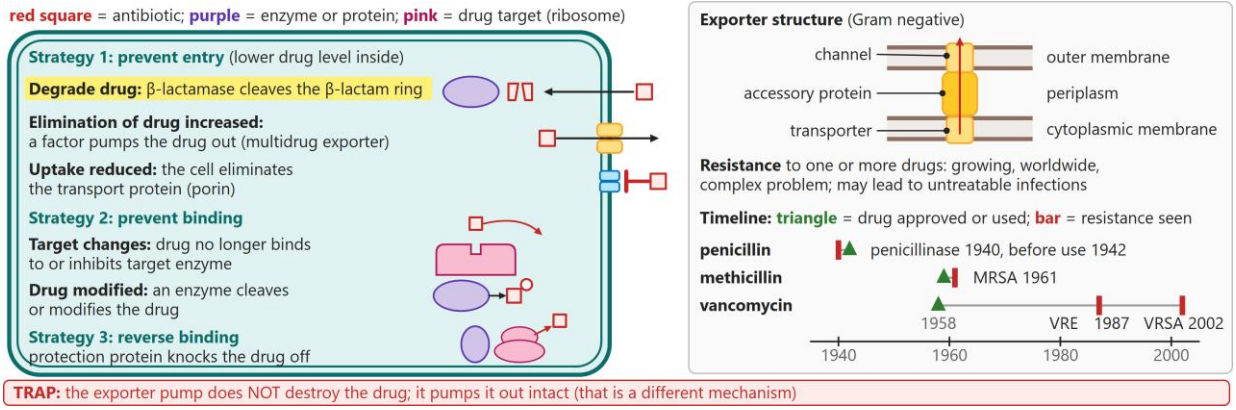
Book: 27.3 Challenges of Drug Resistance and Discovery, Evolving, and Sharing, Drug Resistance Genes (chapter 27 PDF, p. 83); 27.1 Fundamentals of Antimicrobial Therapy, Measuring Drug Susceptibility (chapter 27 PDF, p. 13).

⚠ **Do not mix up: Efflux *(pumping the drug out)* pumps the drug out; beta-lactamase *(enzyme that destroys beta lactam drugs)* cleaves the drug.** [L18 p50]

Drug resistance overview

Drug resistance:

> Evolution of pathogenic strains resistant to one or more drugs used to treat infections.
> A growing, worldwide problem.
> A complex problem.
> May lead to untreatable infections. [L18 p44]



Three resistance strategies and the multidrug exporter [L18 p49]

On the figure: prevent entry · prevent binding · reverse binding · multidrug exporters · porins · accessory protein · outer membrane · MRSA

> **Resistance groups**: the list gives two, **prevent entry** (lower levels) and **prevent binding**; the strategy diagram adds a third, **reverse binding** (a ribosome protection protein knocks off a bound macrolide). [L18 p50]

Prevent entry

> **Prevent entry 1**) degrade drug (textbook fill: beta-lactamase cleaves the beta-lactam ring). [L18 p50]
>
> **Beta-lactamase** destroys the drug. [L18 p49, L18 p50]
>
> Beta-lactamase is exported into the surrounding medium (**Gram positive**) or the periplasm (**Gram negative**) and destroys penicillin before it reaches the cell (textbook).
>
> **Penicillinases** (also called beta-lactamases) attack the beta-lactam *(drug family with four member ring)* ring. [L18 p51]
>
> **Prevent entry 2**) elimination of drug increased: make a factor that pumps the drug out. [L18 p50]
>
> **Multidrug exporter**: the factor that pumps the drug out. [L18 p50]
>
> Pumps work because they bail drugs out of the cell faster than the drugs can enter; efflux is usually energized by proton motive force (textbook).
>
> **Prevent entry 3**) uptake of drug reduced: eliminate the transport protein. [L18 p50]
>
> **Efflux or reduced porin** *(water filled channel in outer membrane)* **permeability** keep the drug out. [L18 p49, L18 p50]
>
> **Gram negative** *(thin wall plus outer membrane)* bacteria can express alternative **porins** *(channels in the outer membrane)* with pores too narrow to let the antibiotic pass (textbook).
>
> **Watch out, grouping**: degrading the drug (beta-lactamase) is listed under Prevent entry (lower levels), and a factor that cleaves or modifies the drug is also listed under Prevent binding (drug modified). [L18 p50]

Multidrug exporter in Gram negative

> **Gram negative** cells have **multidrug exporters**. [L18 p50]
>
> **Multidrug exporter in Gram negative**: a transporter *(protein that carries molecules across)* in the cytoplasmic membrane *(thin lipid layer)*. [L18 p50]
>
> **Multidrug exporter**:
>
> > An **accessory protein** across the periplasm.

An **outer membrane** *(extra membrane outside the wall)* channel.

The parts form one continuous exit tube. [L18 p50]

Prevent binding

Prevent binding:

1) target of drug changes.

1a) the drug no longer binds to or inhibits the target enzyme. [L18 p50]

Target changes: the drug no longer binds to or inhibits the target enzyme. [L18 p50]

In **MRSA** *(methicillin resistant staph germ)*, the mecA gene encodes an altered penicillin-binding protein with low affinity for beta-lactam antibiotics (textbook).

Prevent binding:

2) drug modified.

2a) makes a factor that cleaves or modifies the drug. [L18 p50]

Gram negative and penicillin

Gram negative peptidoglycan *(sugar and peptide mesh of the wall)*:

Is thin (**1-2 layers**). [L17 p15]

Sits between the outer and inner membranes *(the skin around the cytoplasm)*. [L18 p33, L18 p38]

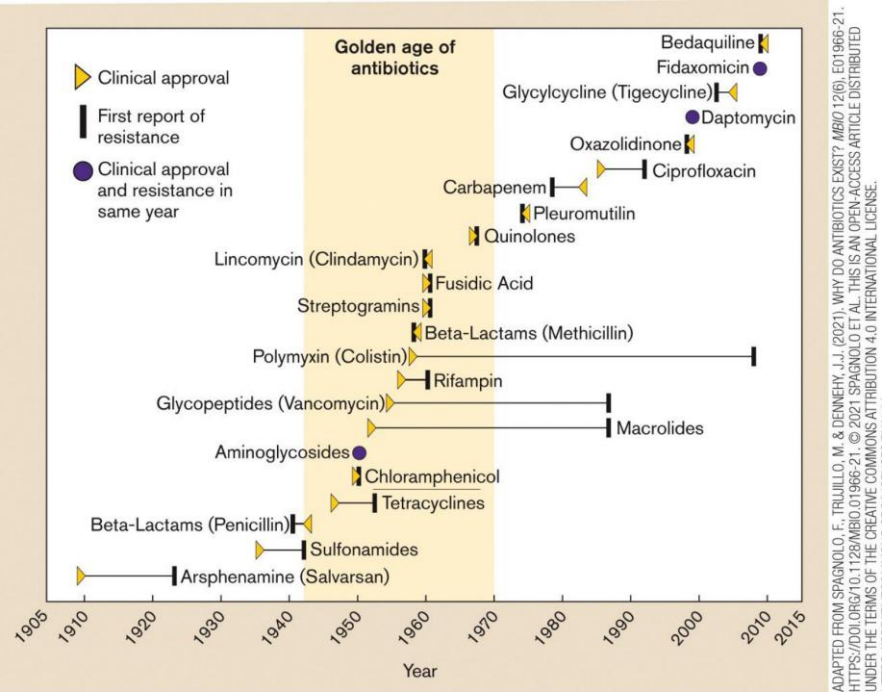
Porins pass **only** molecules up to **700 daltons** *(unit of molecule weight)*. [L18 p11]

Penicillin G:

High activity against most **Gram positive** *(thick wall, stains purple)*.

Low activity against **Gram negative**. [L18 p51]

Approval versus resistance dates



Antibiotic approval and first resistance reports on one timeline, with the golden age [L18 p47]

On the figure: Salvarsan · Ciprofloxacin · Chloramphenicol · daptomycin · fidaxomicin · carbapenem · tigecycline · Golden age of antibiotics

Timeline pattern: resistance usually follows approval within a few years. [L18 p47]

Timeline examples:

Sulfonamides about **1936** to **1942,** tetracyclines about **1947** to **1953.**

Rifampin *(drug blocking RNA making)* about **1957** to **1960.** [L18 p47]

Ciprofloxacin: approval about **1986,** resistance about **1992.** [L18 p47]

Chloramphenicol: approval and resistance together at about **1950.** [L18 p47]

Timeline graph, approval and resistance in the same year: aminoglycosides *(drugs causing ribosome misreading)*, daptomycin and fidaxomicin. [L18 p47]

Resistance reported before approval:

Beta-lactams (resistance about **1940,** approval about **1943**).

Carbapenem (about **1978** vs **1983**), tigecycline (about **2003** vs **2005**). [L18 p47]

Long gaps:

Salvarsan about **1910** to **1923,** macrolides *(protein building blocker drugs)* about **1952** to **1987.**

Vancomycin *(drug blocking wall building)* about **1955** to **1987,** polymyxin/colistin about **1958** to **2008** (longest). [L18 p47]

Rising resistant strains

Golden age of antibiotics shaded about **1943** to **1970.** [L18 p47]

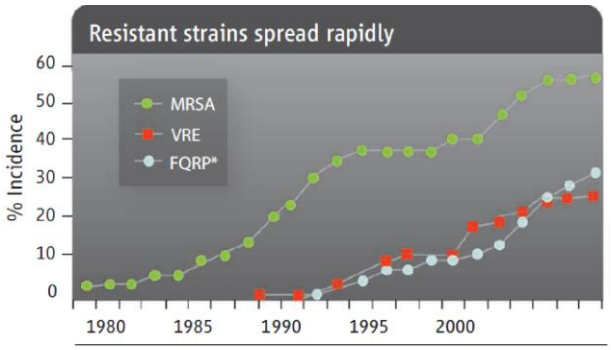
Rising incidence of resistant strains (1979 to 2007):

MRSA, VRE *(drug resistant gut germ)* and FQRP. [L18 p48]

MRSA: about **2%** in **1979** to about **57%** by about **2007.** [L18 p48]

VRE: first seen about **1989,** up to about **25%.** [L18 p48]

FQRP (fluoroquinolone resistant Pseudomonas aeruginosa): first seen about **1992,** up to about **31%.** [L18 p48]



Rising incidence of MRSA, VRE and FQRP from 1979 to 2007 [L18 p48]

On the figure: MRSA · VRE · FQRP · Pseudomonas aeruginosa

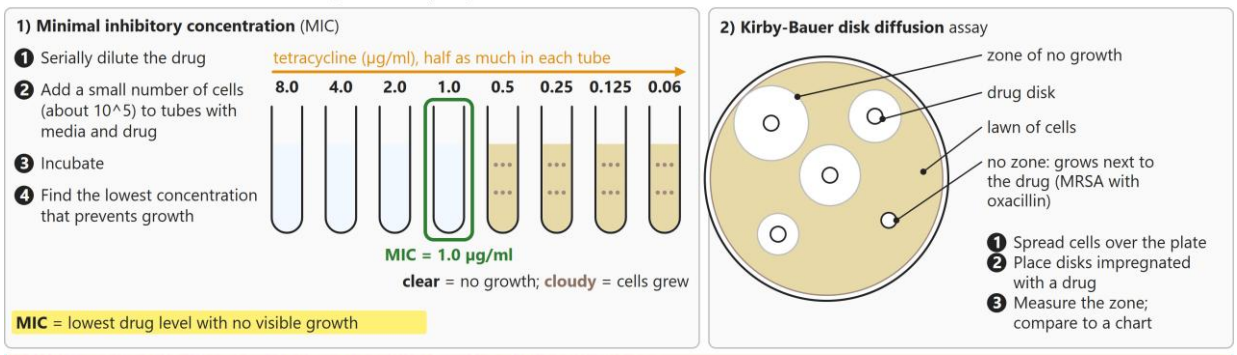
Date conflicts

Date conflict:

One timeline dates vancomycin introduction to **1958** while another shows about **1955.** [L18 p45, L18 p47]

One timeline gives VRE in **1987** while another gives first seen about **1989.** [L18 p46, L18 p48]

Date conflicts: the timeline graph is approximate; it puts tetracyclines at about **1947** to **1953,** while the dated list gives approval **1952** and resistance **1956.** [L18 p45, L18 p46, L18 p47, L18 p48]



MIC tubes and Kirby-Bauer disk plate for antibiotic susceptibility [L18 p53]

On the figure: MIC · tetracyclines · clear · cloudy · Kirby-Bauer · lawn · zone of no growth · oxacillin

MIC tetracycline tubes

MIC tetracycline tubes: drug diluted **8.0, 4.0, 2.0, 1.0, 0.5, 0.25, 0.125, 0.06** ug/ml. [L18 p53]

MIC tubes:

Each tube has **half as much** drug as the tube before it (**8.0,** then **4.0,** then **2.0**).

The tubes at **8.0** to **1.0** stay clear and the lower ones are cloudy.

A cloudy tube means the cells grew; a clear tube means no visible growth. [L18 p53]

MIC = **1.0** ug/ml for the tetracycline tubes. [L18 p53]

Kirby-Bauer plates

Kirby-Bauer step **1** spreads the cells over the whole plate, an even layer called a lawn (textbook word). [L18 p54]

Kirby-Bauer: a disk dispenser places several drug disks at once. [L18 p54]

Kirby-Bauer plates:

Each disk carries a drug abbreviation, for example OX.

A clear **zone of no growth** around a disk means the cells are sensitive.

One OX (**oxacillin**) disk has no zone around it, meaning those cells are resistant. [L18 p54]

Penicillin in stream water

Stream water: reasons penicillin may not make it safe to drink.

Why penicillin may fail in stream water:

Reason **1**: the bacteria in the water may not be growing.

Penicillin **only** kills bacteria that are growing and synthesizing new cell wall *(stiff layer around the cell membrane)* material.

Reason **2**: it may not work on the type of bacteria causing the illness (**Gram negative**).

Reason **3**: the bacteria may be resistant to penicillin.

Reason **4**: the dose may not get high enough.

Gram positive envelope tests

Gram positive envelope *(layers wrapped around the cell)* **tests** (to support a purple Gram stain):

Transmission electron microscopy showing a thick peptidoglycan layer and no outer membrane; isolating teichoic acid *(charged chains in Gram positive wall)* from the cell wall.

Also: no growth on a selective medium for **Gram negative**; genes for teichoic acid synthesis present and genes for LPS *(outer membrane sugar and fat coat)*, outer membrane and periplasmic *(in the space between the two membranes)* proteins absent.

Use a penicillin more effective against **Gram positive** but not **Gram negative**.

Strategy	Mechanism	Example
1. Prevent entry	Destroy the antibiotic	Beta-lactamase
1. Prevent entry	Pump the antibiotic out	Efflux pump
1. Prevent entry	Reduce membrane permeability	Porin with reduced permeability
2. Prevent binding	Alter the target	Mutated ribosome component
2. Prevent binding	Modify the antibiotic	Modification enzyme
3. Reverse binding	Protect the ribosome	Ribosome protection protein knocks off a bound macrolide

Table source: [L18 p49]

Year	Event (Science 2008 timeline)
1940	Penicillinase (an enzyme that destroys penicillin) identified in bacteria
1942 to **1943**	First therapeutic use of penicillin; mass produced in **1943**
1945	More than **20%** of S. aureus hospital isolates penicillin resistant
1947	Streptomycin approved; resistance observed the same year
1952 to **1956**	Tetracycline approved **1952**; resistance observed **1956**
1958	Vancomycin introduced (rarely used until the mid **1980**s)
1959 to **1961**	Methicillin introduced **1959**; MRSA observed **1961**
1964 to **1966**	Cephalothin (first cephalosporin) **1964**; resistance **1966**
1967 to **1970**	Gentamicin approved **1967**; resistance **1970**
1976	Transferable penicillinase first observed in a gonococcus
1981 to **1983**	Cefotaxime approved **1981**; resistance **1983**; first penicillin resistant Enterococcus **1983**
1987	Vancomycin resistant Enterococcus (VRE); first outbreak of Klebsiella resistant to third generation cephalosporins
1996	S. aureus with intermediate resistance to vancomycin (VISA)
1999	Community acquired MRSA
2000 to **2001**	Linezolid (first oxazolidinone) approved **2000**; resistance **2001**
2002	S. aureus with complete resistance to vancomycin (VRSA)

Table source: [L18 p45, L18 p46]

Test	How it works	Result
MIC (minimal inhibitory concentration)	**1**) Serially dilute the drug, **2**) add a small number of cells (**10^5**) to a tube with media and drug, **3**) incubate, **4**) find the lowest concentration that prevents growth	Lowest drug concentration with no growth (tetracycline tubes: MIC **1.0** ug/ml; microtiter plate: penicillin MIC **0.06** ug/ml, erythromycin MIC **8** ug/ml)
Kirby-Bauer disk diffusion assay	**1**) Spread cells over the surface of a plate, **2**) place one or more disks impregnated with a drug on the plate, **3**) measure the zone of no growth around each disk and compare to a chart	Zone size gives sensitive or resistant
Genetic methods	Listed as the third method	No detail given

Table source: [L18 p52, L18 p53, L18 p54]

E. Cell division and Differentiation (sections 3.4, 3.5 and posted article)

Cell division:

The FtsZ *(protein ring for cell splitting)* ring.

Where new peptidoglycan *(sugar and peptide mesh of the wall)* is made. [L18 p28, L18 p38]

FtsZ forms a ring at the cleavage site during cytokinesis *(splitting of the cell)*.

FtsZ ring and where new peptidoglycan is made (Lecture 18 slides)

Book: 3.4 Bacterial Cell Division, Cell Division by Septation (chapter 3 PDF, p. 78).

⚠ **Do not mix up: FtsZ *(protein ring for cell splitting)* forms the ring; PBPs build peptidoglycan *(sugar and peptide mesh of the wall)*; autolysin *(wall-cutting enzyme made by the bacterium itself)*, produced by bacteria, is one of the enzymes that cleave peptidoglycan.** [L18 p38, L18 p39]

FtsZ ring

FtsZ is involved in formation of a ring at the cleavage site

FtsZ ring forms during cytokinesis *(splitting the cell in two)*.

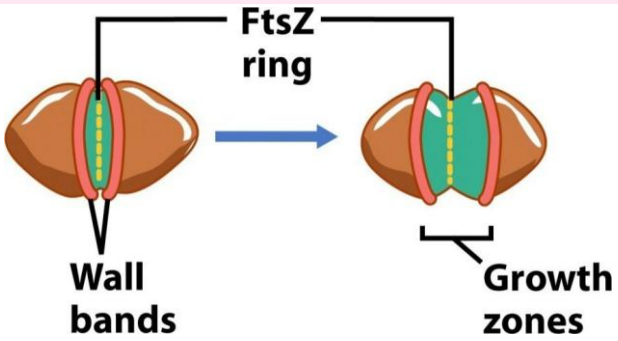
The Z-ring determines the cell diameter and manages the growth of the dividing partition, called the septum (textbook).

FtsZ ring: sits at mid-cell between the **wall bands**. [L18 p28]

Septal *(at the splitting wall)* ring model:

A ring of FtsZ proteins

ZipA connects the ring to the cell membrane *(thin skin around the cell)*. [L18 p38]



FtsZ ring, wall bands and growth zones of a dividing cell [L18 p28]

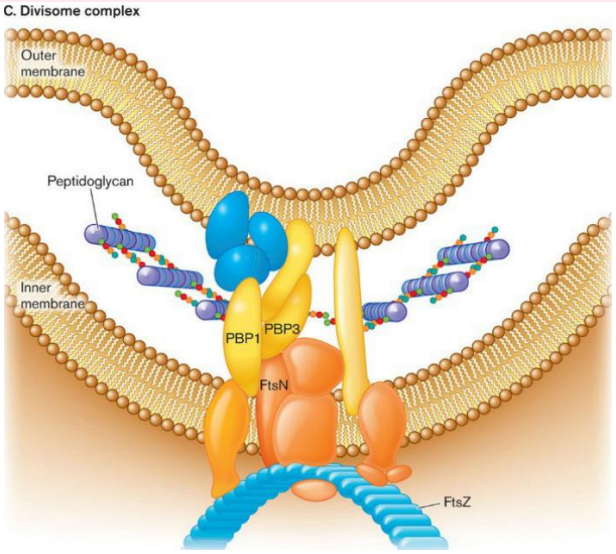
On the figure: FtsZ ring · wall bands · growth zones

Divisome proteins

Divisome *(cell splitting machine)*: the **FtsZ** ring sits under the **inner membrane** *(the skin around the cytoplasm)*. [L18 p38]

Divisome complex: **FtsN**, **PBP1** and **PBP3** sit on the inner membrane in the periplasm *(space between the two membranes)* and reach toward the **peptidoglycan**. [L18 p38]

Other division proteins: FtsA, FtsK and FtsW, FtsL, FtsN and FtsQ. [L18 p38]



Divisome complex: FtsZ ring, PBP1, PBP3 and FtsN at the peptidoglycan [L18 p38]

On the figure: **FtsZ** · **FtsN** · **PBP1** · **PBP3** · **peptidoglycan** · **inner membrane**

PBPs

PBPs in the divisome:

> Penicillin binding proteins *(enzymes that build the wall)* are part of the complex involved in peptidoglycan synthesis.
>
> Do the transglycosylation *(joining sugar chains longer)* and transpeptidation *(making peptide bridges between chains)* reactions. [L18 p38]

PBP3 reaches the peptidoglycan layer. [L18 p38]

Where new peptidoglycan is made

New peptidoglycan fills the **growth zones** at the division site. [L18 p28]

Where new peptidoglycan is made varies: in the middle, at one end or dispersed. [L18 p29]

Keeping the layer intact

Layer integrity:

> The cell has to maintain the integrity of the layer.
>
> The cell cuts links between existing peptidoglycan and new peptidoglycan. [L18 p28]

Autolysin

Autolysin, produced by bacteria, cuts old peptidoglycan to open the wall for new units during growth; at division it cuts the layers apart to separate the **daughter cells** *(the two new cells)* (textbook fill). [L18 p39, L20 p54]

Growth pattern	Where new wall goes	Examples
Dispersed zonal growth	Dispersed along the side wall	E. coli, Bacillus
Septal zonal growth	Middle (septum at mid-cell)	Streptococcus, Staphylococcus
Polar growth	One end	Actinomycetes, Rhizobiales

Table source: [L18 p29]