

# **The Bacterial Cell Wall**

**MODULE 06763**

**Semester 2**

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## THE BACTERIAL CELL WALL

1. Introduction
2. Bacteria
  - Morphology and Ultrastructure
  - Gram Staining
3. The Bacterial Cell Wall
  - Overview and Major Components
4. Peptidoglycan
  - Structure and Function
5. Biosynthesis of Peptidoglycan
6. The Outer Membrane of Gram Negative Bacteria as a Resistance Factor
7. Lipopolysaccharide
  - Structure and Function

### Acknowledgement:

These lecture notes, including figures and schemes, were originally prepared by Professor S.G. Wilkinson. They have been liberally “plagiarised” and modified with permission since I took over the course.

## Introduction

**Why study bacterial cells?** Many are pathogenic, that is to say agents of disease in man, animals and plants. Although chemotherapy has been spectacularly successful, *i.e.* the era of antibiotics, however the 'final solution' is not yet here because of:

- *'new' infectious agents*, e.g. *Legionella pneumophila*,  
*Helicobacter pylori*,  
*Borrelia burgdorferi*
- *resurgent pathogens*, e.g. *Mycobacterium tuberculosis*  
[still the prime killer, ~3 x 10<sup>6</sup> deaths per annum]
- *multidrug resistance*, e.g. 'Superbugs' such as *Staphylococcus aureus* and *Enterococcus faecium*,  
opportunistic Gram-negative pathogens  
[hospital-acquired or nosocomial infections]  
(from nosocomium late Latin for hospital)

The emergence of many antibiotic-resistant strains of once-sensitive bacteria is a major theme of current research and scientific literature, and is regularly publicised in the media.

**Aims of the course: Review the molecules and structures of the cell envelope of bacterial cells and describe the roles played by each of the major components. Discuss briefly the features and structures of the cell wall that are targets for current drugs and those which offer new targets for antimicrobial drugs.**

## Bacteria

### 1. Morphology and ultrastructure

The size, shape and arrangement of bacteria, and other microbes, is the result of their genes and thus is a defining characteristic called **morphology**. Bacteria come in a bewildering and exciting variety of size and shapes. The most common bacterial shapes are rods (bacilli) and spheres (cocci).

Morphology: bacillus (bacilli) (rods)  
 coccus (cocci) (spheres)  
 vibrios (curved rods or 'bananas')  
 helical (corkscrew)  
 miscellaneous (triangular, square, annular, branched, filamentous *etc*)

Web link: Electron micrographs of bacteria and other microbes

<http://www.denniskunkel.com/>

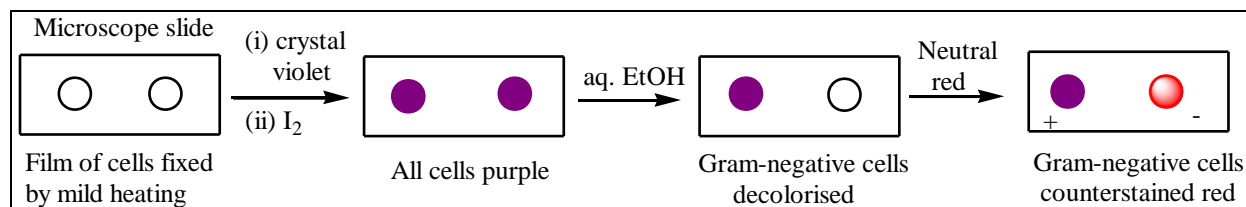
Within each of these groups are hundreds of unique variations. **Rods** may be long, short, thick, thin, have rounded or pointed ends, thicker at one end than the other etc. **Cocci** may be large, small, or oval shaped to various degrees. **Spiral shaped bacteria** may be fat, thin, loose spirals or very tight spirals.

'**GROUP ASSOCIATIONS**' of microbes in either liquid or solid medium are also helpful in classification. Bacteria may exist mainly as single cells or as common grouping such as chains (e.g. streptococci) or clusters of cells (e.g. staphylococci), uneven clusters, pairs, tetrads, octads and other packets. They may exist as masses embedded within a capsule. There are square bacteria, star-shaped bacteria, stalked bacteria, budding bacteria that grow in net-like arrangements and many other morphologies.

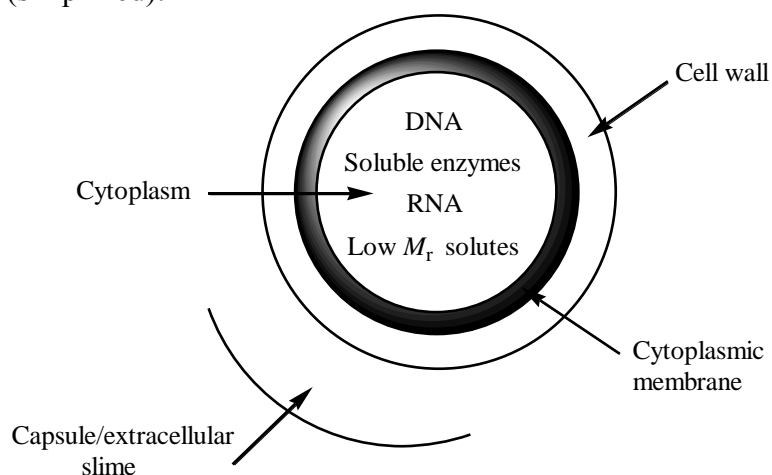
These range of morphologies are also found in fungi ranging from the single cell yeasts to the filamentous moulds and dermatophytes.

### 2. Gram staining

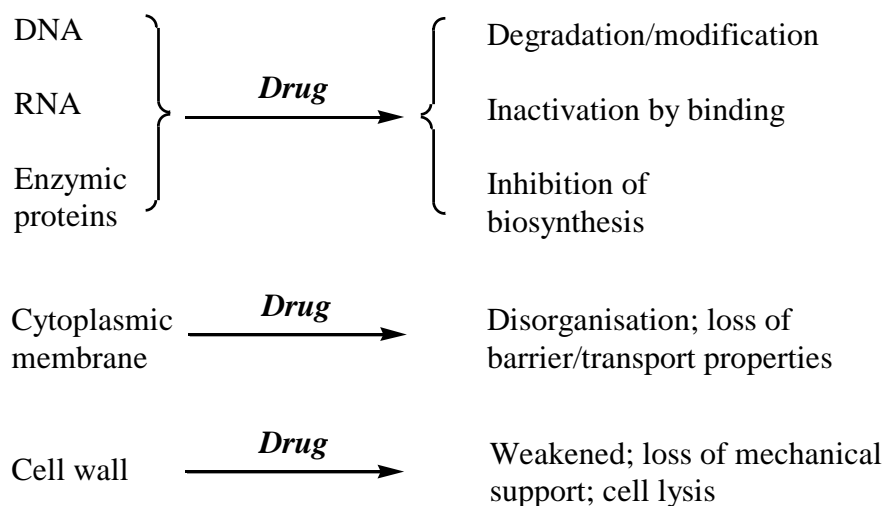
Many bacteria can be assigned to one of two major groups, based on the Gram-staining reaction – named after a scientist of the same name.



Cellular features (simplified):



The diagram draws attention to some structures and molecules which are potential drug targets:



1. Differences in cell-wall structure and composition account for the differential Gram reaction.
2. We will concentrate on the cell wall both as a **target** and as a **resistance factor**.

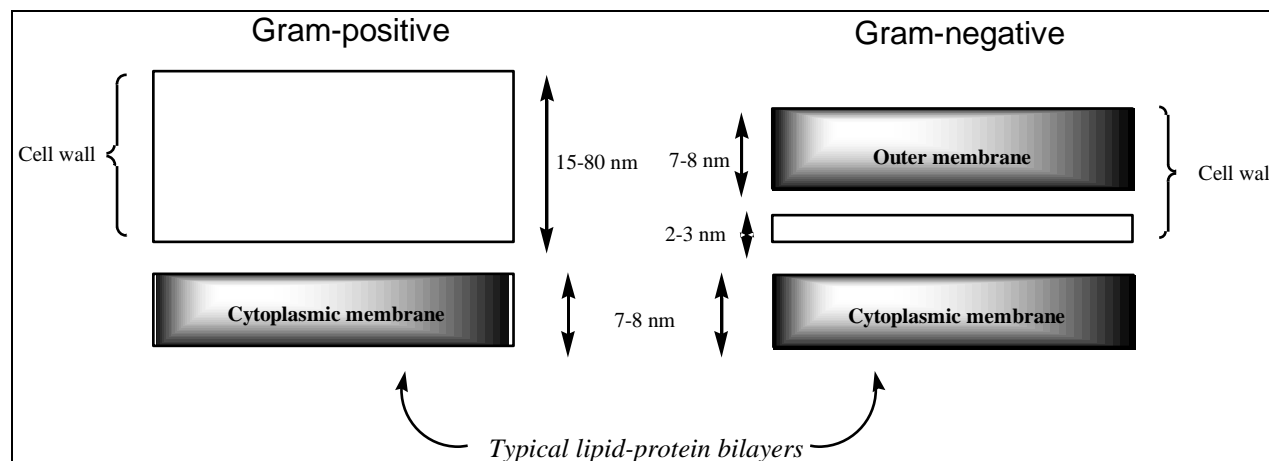
The remainder of this part of the course concentrates on the cell wall:

- (i) as a **target** for antibacterial action (more next year, 06544)
- (ii) as a **defence** against antibacterial agents, *i.e.* a **resistance factor**;
- (iii) some possible ways of **combatting** resistant bacteria.

First of all, we need to know something about the composition and organisation ("ultrastructure") of the cell wall.

## Bacterial cell walls

As briefly noted before, fundamental differences in ultrastructure of the cell wall are responsible for the reaction (+ or -) of bacteria towards the Gram stain. In both types of cell, the *cytoplasmic membrane* is surrounded and supported by a *cell wall*, which provides strength, rigidity and shape. Schematic cross sections of these structures are given below.



### *Gram-positive*

- Relatively thick and featureless (electron microscope)
- Major component (~50%) is **peptidoglycan**
- No lipid and often no protein
- Accessory polymers (*teichoic acid* and/or *teichuronic acid*) covalently linked to peptidoglycan

### *Gram-negative*

- The cell envelope consists of a *pair* of membranes (*cytoplasmic* and *outer*) with a thin, intermediate layer of **peptidoglycan**
- The outer membrane contains **lipopolysaccharide (LPS)** as well as *lipids* and *proteins*. LPS is located exclusively in the outer leaflet: lipid embedded in the membrane, polysaccharide protruding. This makes the bacteria appear rather fuzzy under an electron microscope.

Major concern is with **peptidoglycan**, but just a few words first about other wall components.

*Teichoic acids* (GPB only, but similar polymers occur as capsules in some GNB or as a part of some LPSs)

- Discovered 1950s in Newcastle
- Typically have a backbone of (polyol-phosphate)<sub>n</sub>, usually with sugars and/or the amino acid D-alanine as substituents
- The polyol is usually *ribitol* (C<sub>5</sub>) or *glycerol* (C<sub>3</sub>), but a few examples of *mannitol* (C<sub>6</sub>) are known (discovered in Hull!)
- They are probably involved in uptake of Mg<sup>2+</sup> by the cell

*Teichuronic acids* (Again, similar polymers found as capsules or LPS in some GNB)

- Acidic polysaccharides (contain uronic acids)
- Production stimulated when cell growth limited by supply of P (the available P is used for DNA, RNA, phospholipids rather than teichoic acids)

- Functionally interchangeable with teichoic acids

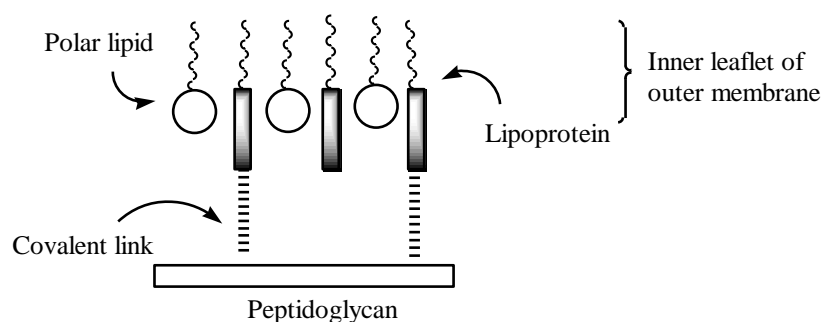
Neither type of polymer is *invariably* present, so probably not vital and therefore dubious value as a drug target.

### Lipids

- Typically, but not necessarily nor exclusively *phospholipids*
- In some GNB, may be confined to the *inner leaflet* of the *outer membrane*
- Similar to those of other membranes, so selective antibacterial action is difficult

### Proteins

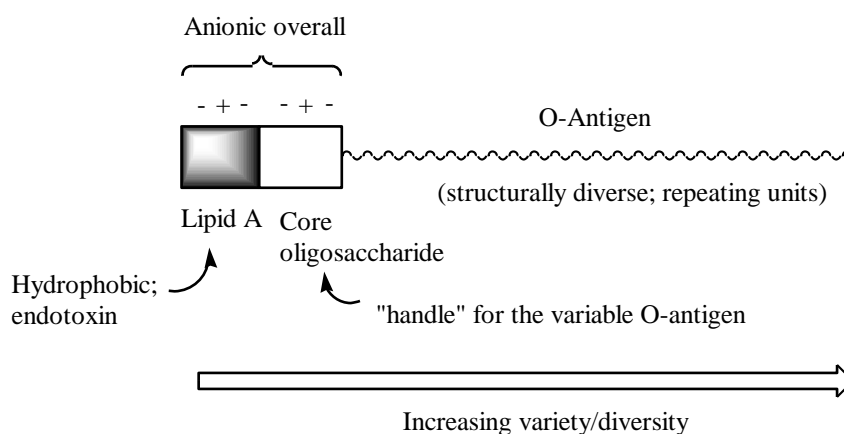
- Several types, asymmetrically placed in the outer membrane
- Transmembrane proteins - *porins* - (often *trimeric assemblies*) form aqueous channels across the outer membrane (to be discussed later)
- Lipoproteins anchor the outer membrane to the peptidoglycan layer in GNB (lipid inserted into the inner leaflet, protein partly covalently attached to peptidoglycan, *e.g.* 1 in 10 molecules in *E. coli*)



- In *E. coli*, 58 amino acids; 3 fatty acids at N-terminus (2 in diacylglycerol thioether-linked to cysteine, 1 directly acylating the Cys NH<sub>2</sub>)
- Absence of lipoprotein can weaken the cell wall (*globomycin* inhibits its biosynthesis, but not clinically useful)

### Lipopolysaccharide

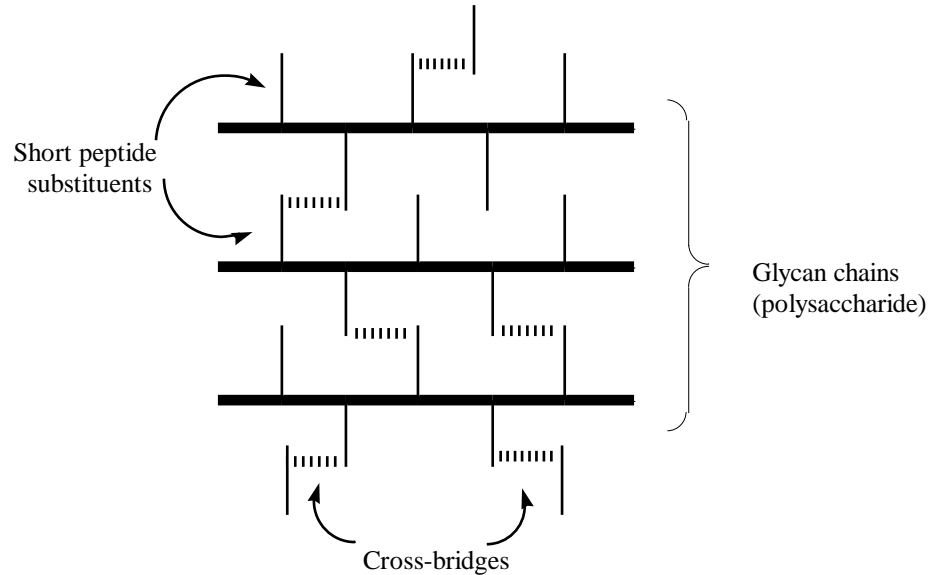
- Tripartite structure conserved in GNB



- Important for the functions and properties of the outer membrane
- Essential for structural integrity and viability of the bacteria

## Peptidoglycans

- Alias *murein* or *mucopetide* - Discovered early 1950s
- Present in almost all bacteria (exceptions: wall-less mycoplasmas; archaeobacteria)
- **Unique to bacteria**
- Essential function (physical support of the cytoplasmic membrane)
- Common architecture but variations in structural detail
- **Ideal target for *selective toxicity***

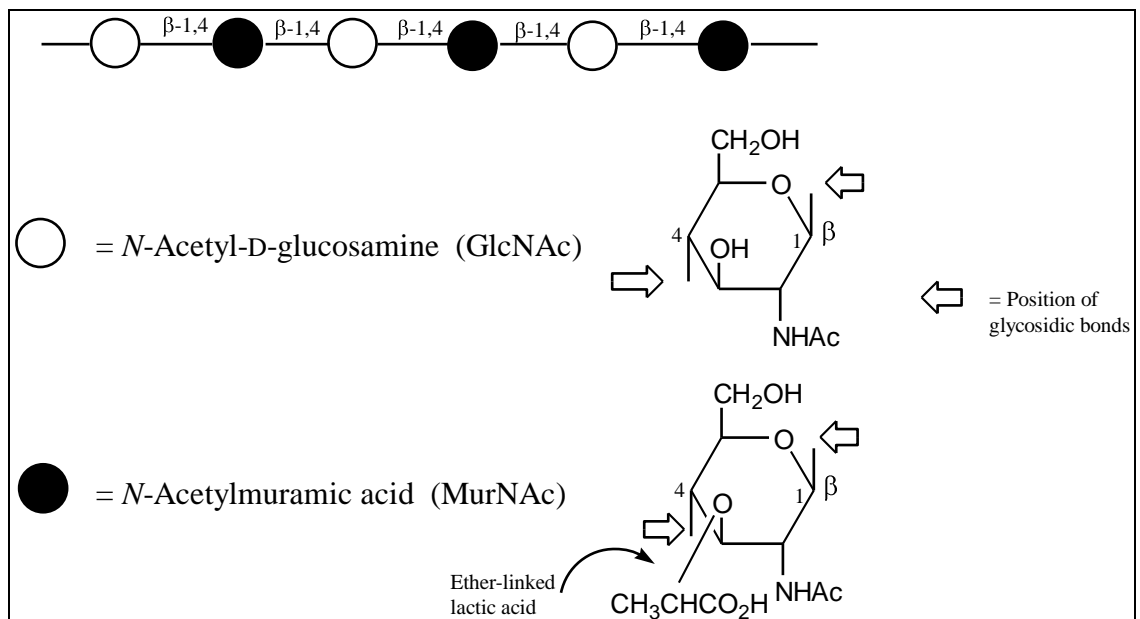


GPB: a thick, 3D network

GNB: a thin, 2D mesh [c.f. a string bag]

### (a) Glycan chains

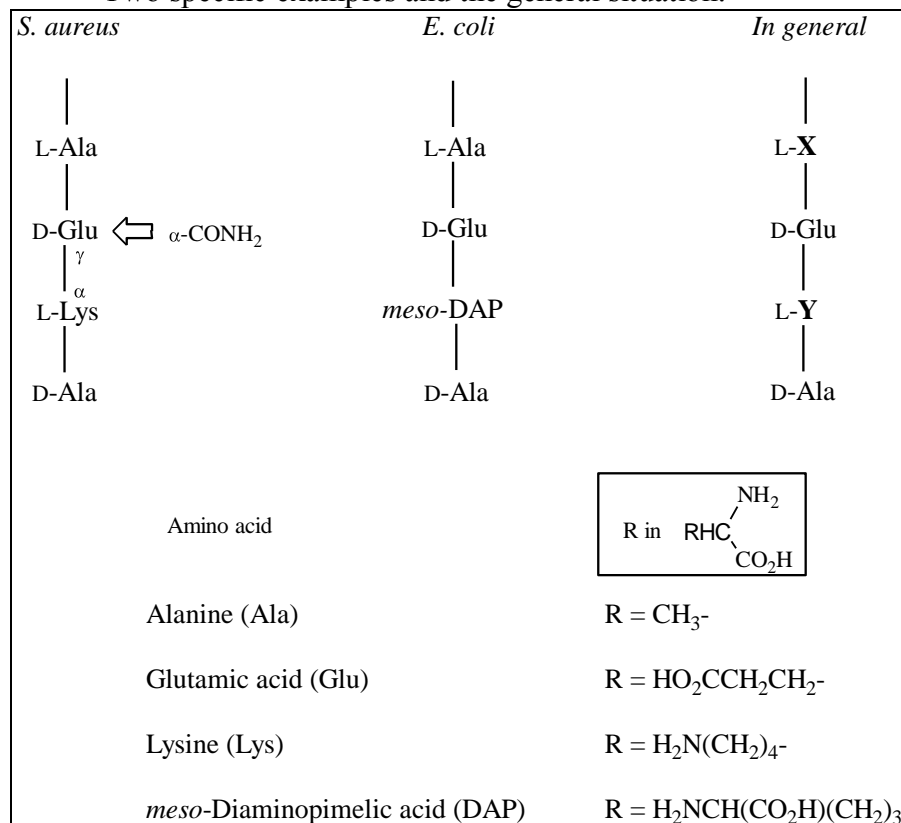
- Based on a *disaccharide repeating unit* of amino sugars, linked  $\beta$ -1,4



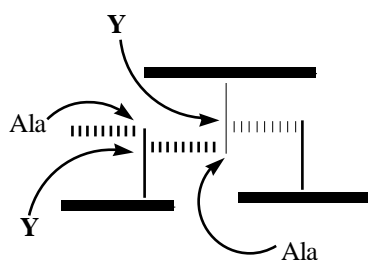
(b) *Peptide substituents*

- Attached via the  $-\text{CO}_2\text{H}$  group of the muramic acid
- GPB: >100 variations. GNB: all the same

Two specific examples and the general situation:

(c) *Cross-bridges* – these are variable

- Links the extra ( $\epsilon$ ) amino group of Lys/DAP side chain to the carboxyl group of D-Ala either
  - directly (*E. coli* and other GNB), or
  - via a  $-(\text{Gly})_5\text{-}$  bridge (*S. aureus*) [Gly = glycine; R = H in the general formula above]
  - alternative bridges in other GPB
- The proportion of chains cross-linked varies.
  - E. coli* ~40% of total chains
  - S. aureus* ~100% of total chains
- Each chain may be linked to **two** others
- Up to 10 glycan chains may be attached via the cross linkages
- Cross-linking can be limited by removal of the terminal D-Ala through the action of a *carboxypeptidase*

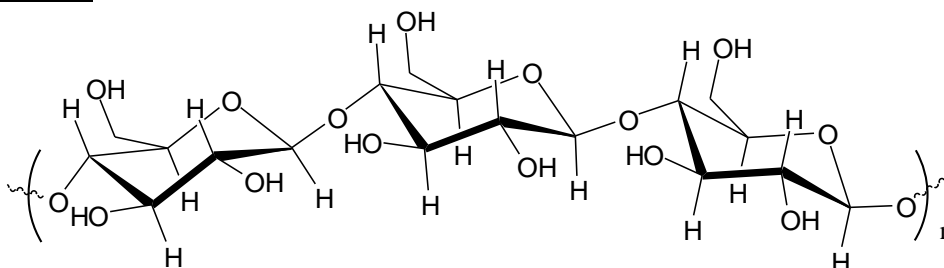


## POLYSACCHARIDES IN FUNGI

### *Glucan, Chitin and Chitosan*

In general glucose is the most abundant sugar found in the cells envelope of fungi, followed by glucosamine, which is mainly in its *N*-acetyl form. Other sugars present are mannose and galactose, but in smaller quantities. The most *abundant* polysaccharides found in fungi are glucans, based upon glucose. Cellulose, as found in plant cell walls, is unbranched  $-(\beta\text{-}1,4\text{-Glc})_n-$  (see below). 'Glucan' is a general term given for glucose polymers and in fungi these polymers may possess  $\beta\text{-}1,3-$ ,  $\beta\text{-}1,6-$ , or  $\alpha\text{-}1,3$  glycosidic linkages which may also be branched.

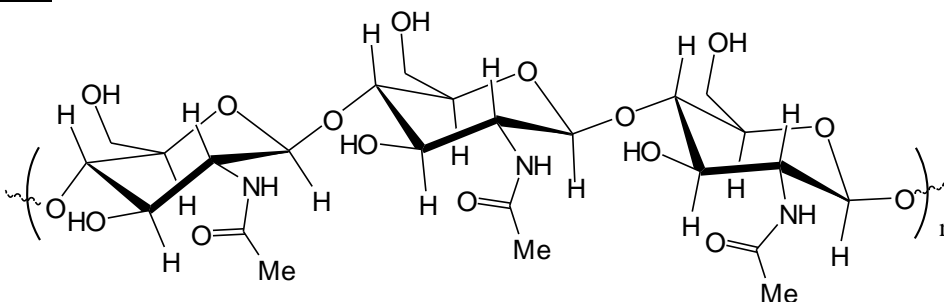
#### Cellulose



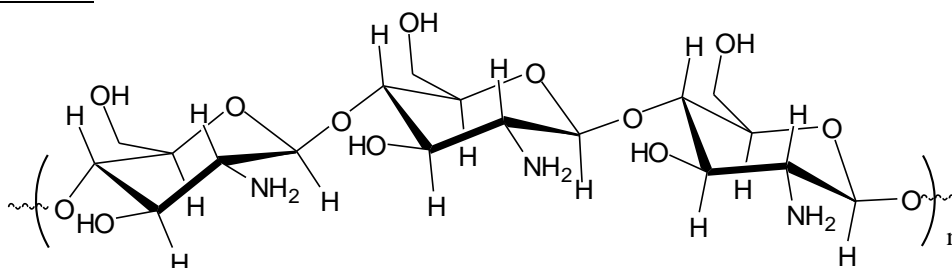
### CHITIN and CHITOSAN

The most *characteristic* polysaccharide found in fungi is **chitin**, which is an unbranched homo-polymer of *N*-acetyl glucosamine,  $-(\beta\text{-}1,4\text{-GlcNAc})_n-$ . In some fungi the related deacylated **chitosan** is found,  $-(\beta\text{-}1,4\text{-GlcNH}_2)_n-$ .

#### Chitin



#### Chitosan



Also found in the outer layers of the fungal envelope is **mannan**. These are complex branched homo- and hetero-polymers based upon mannose. In a yeasts over 50 different types have been isolated.

## Degradation of peptidoglycan

Chemical breakdown of peptidoglycan is not a therapeutic option, but *enzymatic* hydrolysis of the *peptidic* or *glycosidic* bonds and 'processing' of the peptidoglycan must occur naturally.

[Otherwise cells could not enlarge or divide: peptidoglycan would be a straightjacket and a coffin!]

*Autolysins*: hydrolytic enzymes produced by the bacteria themselves. They include:

- glycosidases (specific to one or other bond in the glycan chain)
- amidases (breaking the bond from MurNAc to the peptide substituent)
- peptidases (breaking bonds in the substituent or the bridge)

*Lysozymes*: glycosidases specific to the bond from MurNAc to GlcNAc

- commonly obtained from egg white
- present in some secretions (tears) and part of the body's defence
- GPB attacked and killed directly
- GNB are usually resistant (outer membrane prevents access to the peptidoglycan, but the barrier can be breached) [see later]

## Biosynthesis of peptidoglycan

The notes here accompany the three schemes that follow on pages 13-15.

(a) *In the cytoplasm*

Formation of water-soluble precursors [described only for *S. aureus*]

(i) Fru 6-*P* → GlcN 6-*P*

- NH<sub>2</sub> derived from glutamine
- Inhibited by *bacilysin* (1)- **No therapeutic value** (same biosynthetic reaction in animals)

(ii) GlcN 6-*P* → → UDP-GlcNAc [a nucleotide sugar]

(iii) UDP-GlcNAc → → UDP-MurNAc

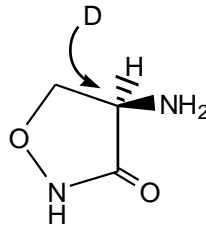
- Two steps: transfer and ether-linkage to C-3 of a unit from *phosphoenolpyruvate*; reduction of the C=C bond
- First committed step in peptidoglycan biosynthesis, so amenable to selective action (MurNAc is not found in humans)
- Inhibition by *fosfomycin* (2)

(iii) UDP-MurNAc → → → UDP-MurNAc-tripeptide

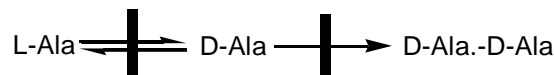
- Each amino acid is attached separately; ATP is required
- Recall that the  $\gamma$ -CO<sub>2</sub>H group of D-Glu is used to form the peptide bond to L-Lys.

(iv) UDP-MurNAc-tripeptide  $\rightarrow$  UDP-MurNAc-pentapeptide

- Attachment of D-Ala.-D-Ala as the dipeptide
- Why **two** D-Ala - there is only one in the finished product?????!!!!
- Inhibited by *cycloserine* (3)



- Cycloserine is a structural analogue of D-Ala. It inhibits both of the following steps



(b) *At the cytoplasmic membrane*

The finished peptidoglycan is an insoluble polymer ***outside the cytoplasmic membrane***. The water-soluble precursors must therefore cross the membrane. To do this, a carrier lipid is employed. At some point, the precursor must change from 'facing in' to 'facing out'.

(i) Transfer of MurNAc-pentapeptide from UDP to phosphorylated carrier

- Carrier is undecaprenol (a C<sub>55</sub> polyisoprenoid alcohol - 11 units @ C5)
- A pyrophosphate (P-P) bond between carrier and precursor is retained
- The transfer is inhibited by *tunicamycin* (4)
- No clinical application (similar carrier lipids in animal membranes)

(ii) Completion of the repeating unit on the lipid carrier

In the case of *S. aureus* this involves

- $\beta$ -1,4 linkage of GlcNAc to MurNAc
- Attachment of the (Gly)<sub>5</sub> bridge to the  $\epsilon$ -NH<sub>2</sub> group of Lys
- Amidation of the  $\alpha$ -CO<sub>2</sub>H group of Glu

(iii) Addition of the new unit to preformed polymer

- *Assumed* here to take place while both are still on the lipid carrier at the *outer surface* of the cytoplasmic membrane, but at some point the new peptidoglycan must be transferred from the lipid carrier, which is released as undecaprenyl pyrophosphate

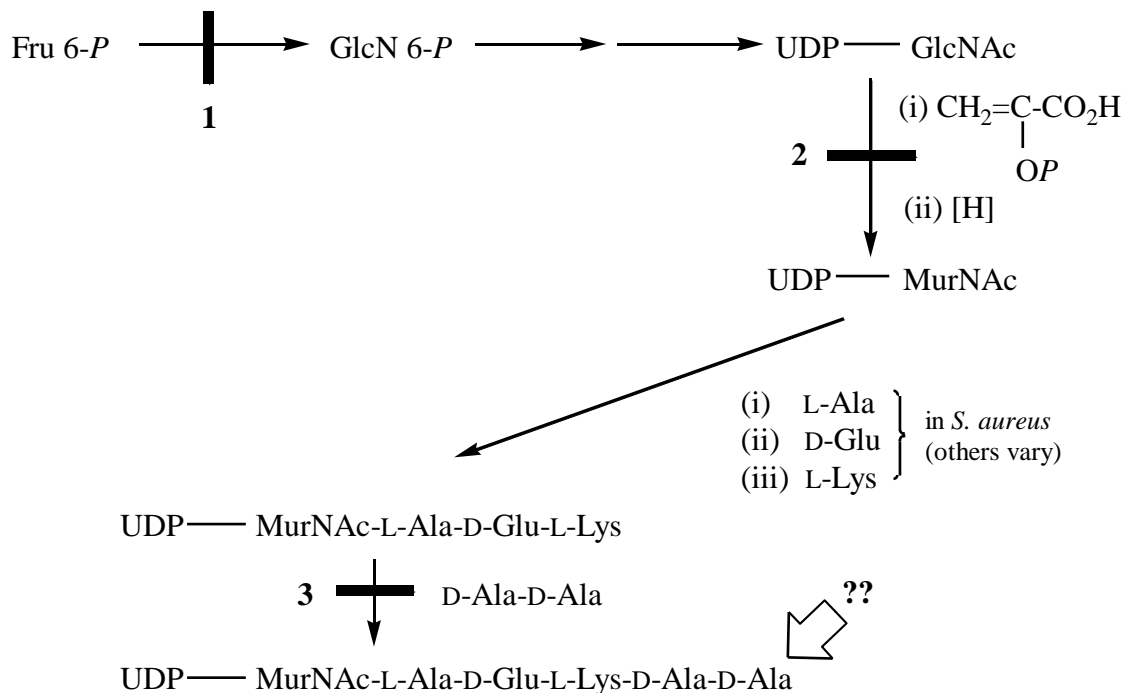
- The pyrophosphate loses one *P* and the undecaprenyl phosphate is recycled. This process is inhibited by *bacitracin* (**5**) (one of the cationic polypeptide antibiotics), which complexes with the *P-P*
- The process of **transglycosylation**, whether at the lipid carrier or cell wall stage, is blocked by the glycopeptide antibiotics (**6**), *e.g.* *vancomycin*, through tight H-bonding to the terminal D-Ala-D-Ala [*see later*]

(c) *In the cell wall*

- *Transglycosylation* (covered as (b)(iii) above)
- *Transpeptidation*: the process of cross-linkage of peptide chains to produce the *insoluble, strong* mesh of peptidoglycan
- Involves enzyme-catalysed attack by a free NH<sub>2</sub> group of Gly (*S. aureus*) or DAP (*E. coli*) on the C=O of the penultimate D-Ala, breaking the peptide bond and releasing the 'surplus' D-Ala. The enzyme is a *transpeptidase*. [Strictly, the active site of the enzyme which catalyses the reaction initially undergoes covalent change, but is regenerated by further reaction with the NH<sub>2</sub>]
- Cross-linking (and also the action of *carboxypeptidases*) is inhibited by β-lactamases (**7**)
- The Strominger hypothesis for β-lactamase action: the terminal D-Ala-D-Ala unit in the pentapeptide is structurally and stereochemically similar to the amidated β-lactam. Thus the enzyme reacts with the β-lactam, breaks the same C-N bond and is at the same time inactivated by irreversible modification
- Consequences of β-lactam action: uncoordinated cell growth, weakened peptidoglycan, deformed cells (rods bulge, form filaments or spheres), leakage (lysis) → death!!

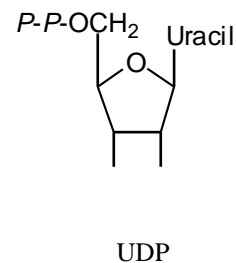
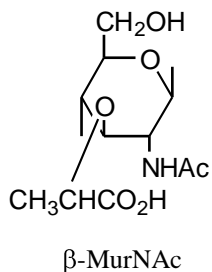
## BIOSYNTHESIS OF PEPTIDOGLYCAN

### Cytoplasmic events



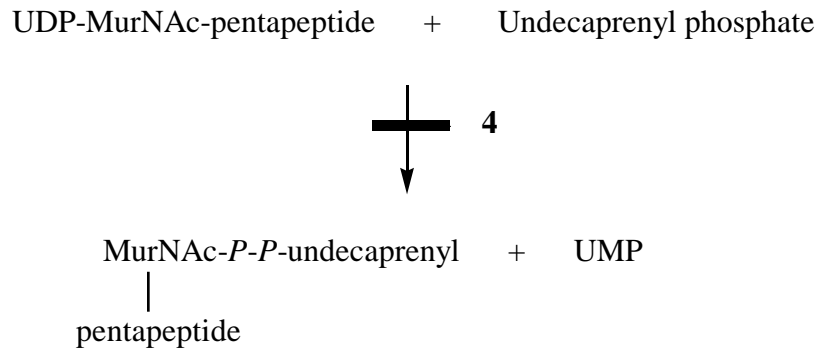
### Key

Fru, fructose  
 GlcN, glucosamine (2-amino-2-deoxyglucose)  
 GlcNAc, *N*-acetylglucosamine  
 MurNAc, *N*-acetylmuramic acid  
 UDP, uridine 5'-diphospho-  
 Ala, alanine,  
 Glu, glutamic acid,  
 Lys, lysine

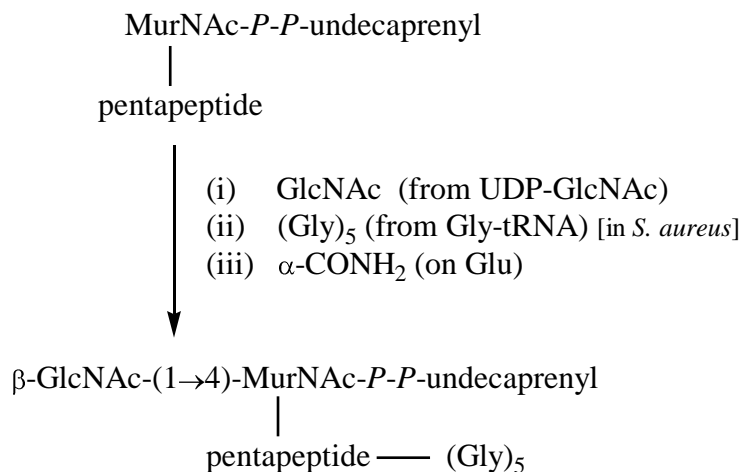


## Events at the cytoplasmic membrane

### 1. Attachment to a lipid carrier



### 2. Further additions and modifications



### 3. Addition to preformed linear polymer

The new unit is transferred to the 'reducing end' of preformed polymer  
[possibly with release from the carrier, which is then recycled]



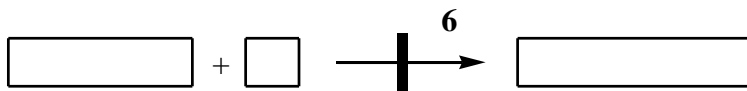
#### Key

Undecaprenol	$\text{H-(CH}_2\text{-C(CH}_3\text{)=CHCH}_2\text{)}_{11}\text{-OH}$
Gly, glycine	
tRNA, transfer RNA for glycine	

## Incorporation into the cell-wall peptidoglycan

### 1. Transglycosylation

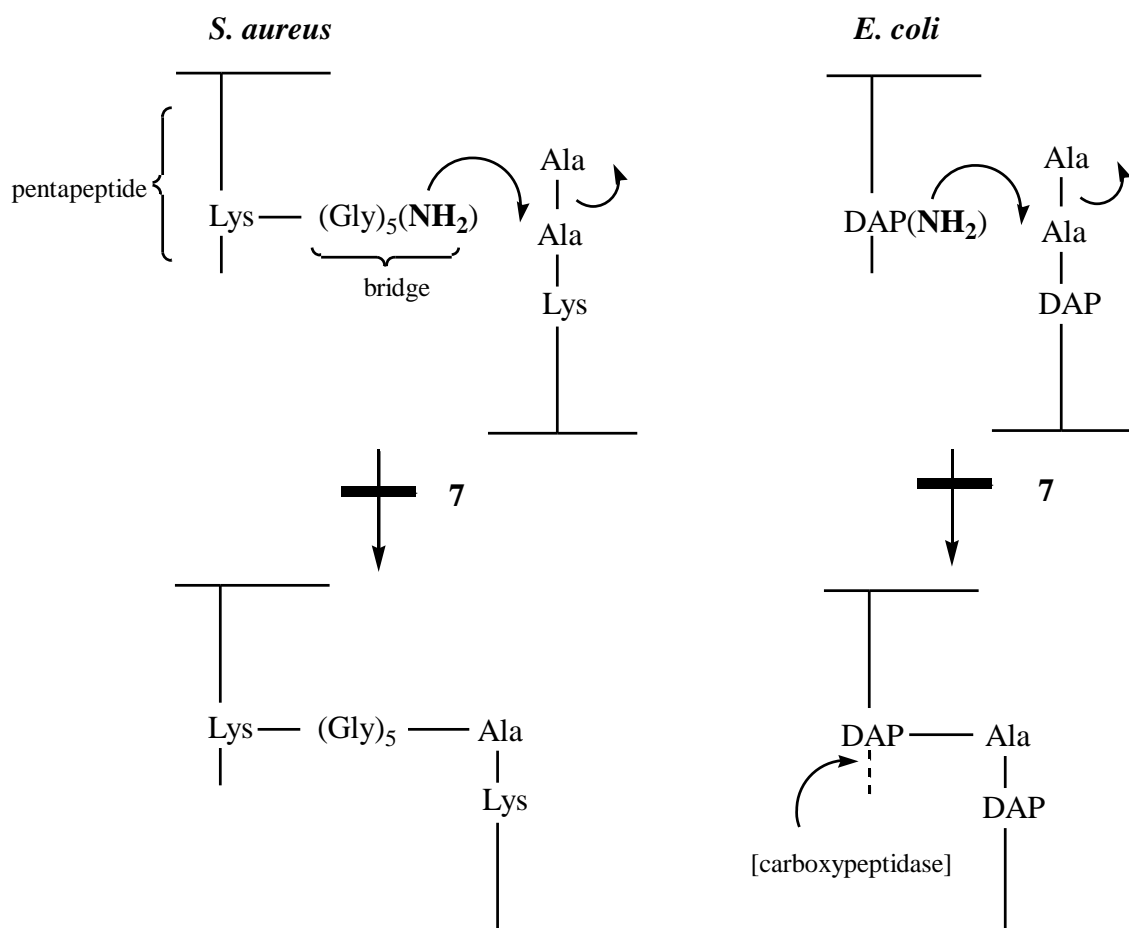
Extension of an existing glycan chain by formation of a new  $\beta$ -(1 $\rightarrow$ 4) glycosidic bond.



[This step may be that shown as step 3 on the preceding page]

### 2. Transpeptidation

Formation of **cross linkages** [**directly** (Gram-negative bacteria) or **via a peptide bridge** (*S. aureus*)] between peptide side-chains to give an **insoluble** 2D or 3D mesh.

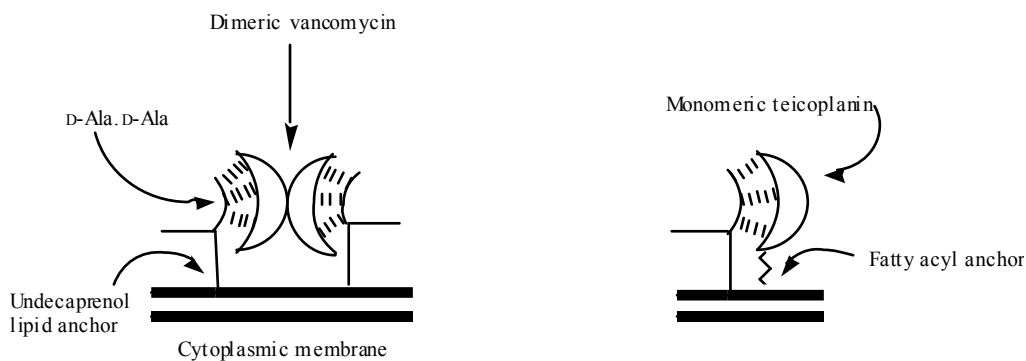


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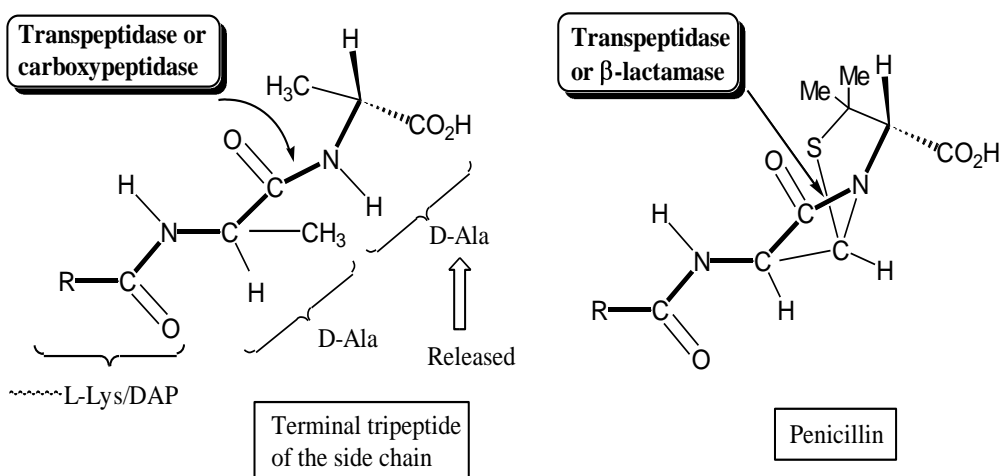
DAP, 2,6-diaminopimelic acid

- The glycopeptide antibiotics (most notably **vancomycin** but also **teicoplanin**) inhibit the late stages of peptidoglycan synthesis involving transfer of completed, lipid-bound precursor units from the cytoplasmic membrane to the growing cell wall.
- Inhibition occurs through H-bonding to the terminal dipeptide D-Ala-D-Ala.
- H-bonding to D-Ala-D-Ala involves 5 H-bonds but interaction is also facilitated by **dimerisation** (*vancomycin*) or the presence of a **lipid anchor** (*teicoplanin*)

Glycopeptides are very large, complex molecules *e.g.* vancomycin  $M_r$  1448 (teicoplanin  $\sim$ 1900), heptapeptide backbone, tricyclic, 5 phenyl rings (2 as hydroxylated biphenyl; 3 as ether-linked, residues, 2 being chlorinated), disaccharide attachment (D-Glc and vancosamine - branched-chain, 3-amino-3,6-dideoxy sugar)



- **Penicillin binding proteins (PBPs)** are involved in the latter steps of peptidoglycan biosynthesis. They recognize the terminal **D-Ala-D-Ala unit**.  $\beta$ -Lactams are stereochemically related to the Ala dipeptide and hence are recognized by PBPs to which they **irreversibly bind and inactivate**. This process halts peptidoglycan biosynthesis.



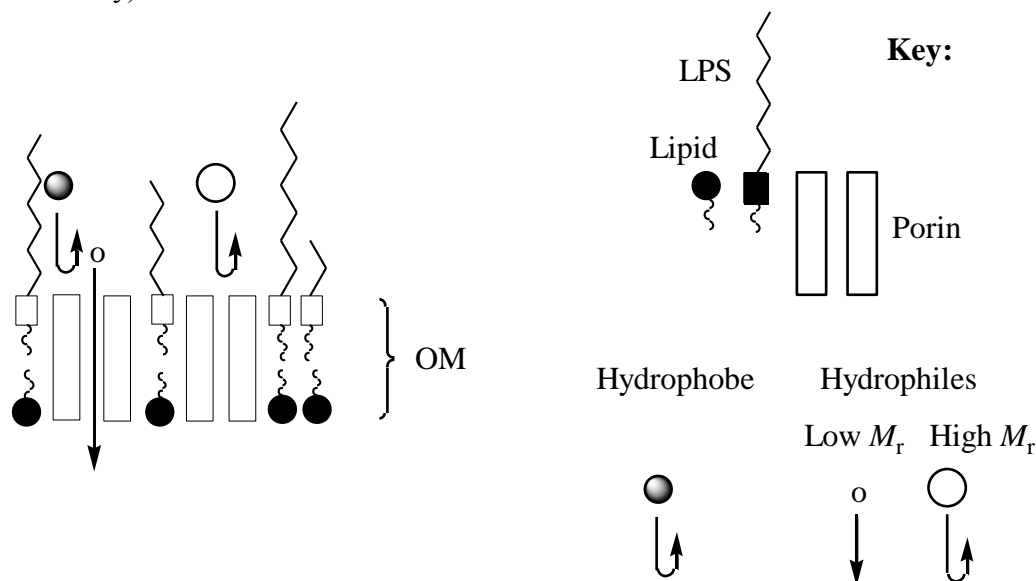
## Bacterial resistance

The following confer *intrinsic* (natural) resistance of a bacteria to an antibiotic.

- **Exclusion of the antibiotic** (general for intrinsic resistance of GNB and of mycobacteria to many antibiotics - (e.g. of large or hydrophobic agents by many GNB) [see below])
- **Efflux mechanisms** - to “pump out” the antibiotic (e.g. tetracyclines, macrolides, quinolones, chloramphenicol - not to be discussed here)

### The outer membrane of GNB as a resistance factor

- The outer membrane (OM) is an *additional* barrier to extracellular solutes (including antibacterial agents) and encloses the *periplasmic space*, where protective, degradative enzymes are found (e.g.  $\beta$ -lactamases).
- In many GNB, phospholipids are absent from the OM; in consequence, *hydrophobic* agents cannot readily penetrate the OM and diffuse across it. The place of phospholipids is taken by lipopolysaccharide (LPS), which gives a more rigid/less fluid monolayer.
- *Small, hydrophilic* solutes can pass through the OM through aqueous channels/pores formed by transmembrane proteins (*porins*).
- The pores are *size-limited*, and sometimes show *solute specificity*, e.g. for anions/cations, depending on the amino-acid side chains which line the pore. For *E.coli*  $M_r$  400-600 (equivalent to a di-/tri-saccharide) but in *P. aeruginosa*  $M_r$  350-400 (100-400 lower permeability).



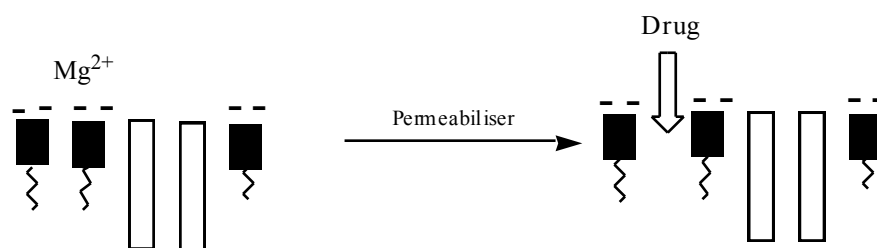
**Figure:** Demonstration (model) to illustrate the importance of *size, shape, and properties* for penetration of the OM

[There is controversy about whether there are smaller pores or whether they are larger but mainly closed! Other organisms with smaller, less abundant, or non-operational pores and often high resistance: *Burkholderia cepacia*, *Stenotrophomonas maltophilia*]

- In general, large (*e.g. glycopeptides*) and hydrophobic (*e.g. macrolides, rifamycins*) antibiotics are unable to penetrate the OM of GNB, though there are organisms where lipid is apparently exposed on the OM surface, allowing penetration by hydrophobes.

### Disruption of the permeability barrier - sensitisation of GNB

- The surface of the OM is *anionic* overall due to an excess of negatively-charged head-groups in LPS ( $\text{CO}_2^-$ ;  $\text{P-O}^-$ ) over positively charged groups ( $-\text{NH}_3^+$ ). The anionic sites are neutralised by counter-cations, *e.g.*  $\text{Mg}^{2+}$ .
- The cations **stabilise the OM by chelation** of LPS-LPS and LPS-protein molecules.
- Removal or displacement of the cations destabilises, disorganises, permeabilises and **sensitises the OM** through coulombic repulsion and altered packing of the membrane components.

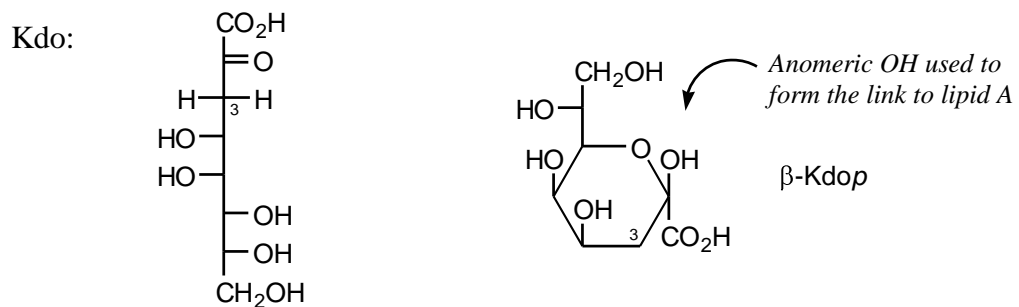
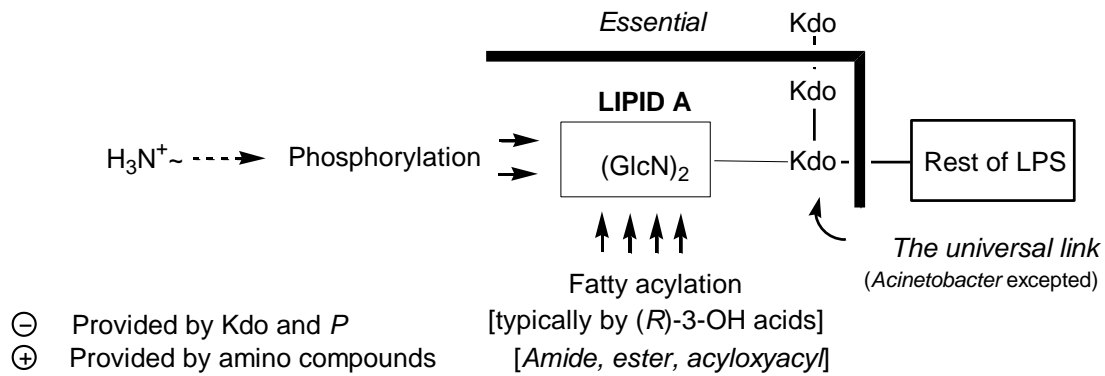


- As a consequence, large and hydrophobic molecules (*e.g.* lysozyme, antiseptics, antibiotics) can penetrate the OM and gain access to their targets. In some cases, *e.g.* *Pseudomonas aeruginosa*, the shock can itself be lethal to the bacteria.
- **Cationic peptides** are part of Nature's defence against bacteria, and there is much interest in novel cationic peptides as potential, bactericidal antibiotics to defeat superbugs and circumvent problems of resistance.

*Message for drug design:* To overcome resistance due to the barrier properties of the OM, **drugs** should preferably be **small, hydrophilic, and cationic**

## Lipopolysaccharide (LPS)

- LPS contributes to the structural and functional properties of the OM and is present in almost all GNB.
- There is **conservation of architecture and structure** in the Lipid A and inner core regions. Lipid A and immediately-adjacent core units are **essential for viability**. Mutants defective in their biosynthesis do not survive.
- LPS is an **endotoxin** [cell-bound]. The **Lipid A** portion of LPS is responsible for the endotoxic activities (numerous, including *fever, low blood pressure, septicaemia, toxic shock, death*). There are ~500k cases of septic shock per annum with ~50% mortality.



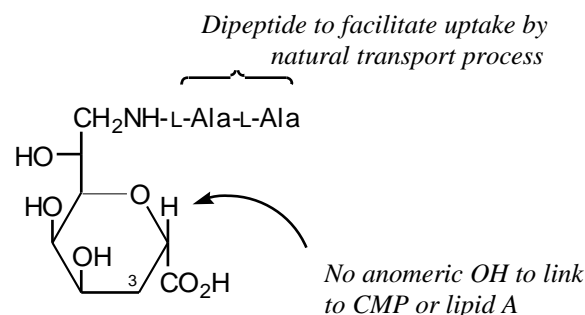
3-Deoxy-D-manno-oct-2-ulosonic acid

### Lipid A as a target for antibacterial action

Biosynthesis of lipid A or proximal residues of the inner core is an attractive target for antibacterial action. Two illustrations from recent research are shown below, in which different features of the general, conserved structure have been targeted.

#### 1. *Kdo as the target*

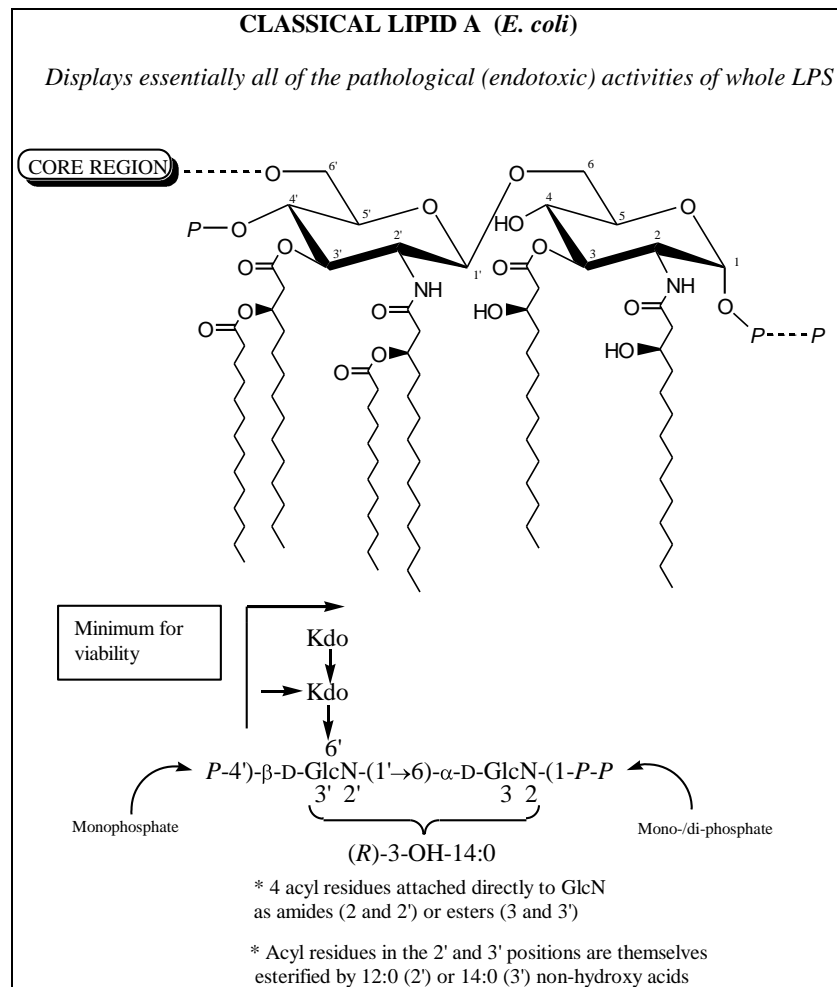
- Kdo is an acidic, C<sub>8</sub> deoxy ketose which is (almost) invariably the sugar attached to lipid A. It is found in LPS, some bacterial capsular polysaccharides, and a few plants and algae, but **not in humans**.
- Kdo is incorporated into LPS *via* a nucleotide (CMP-Kdo), which is formed from CTPn and Kdo by the action of the enzyme *CMP-Kdo synthetase*.
- A structural analogue of Kdo has been designed, which can be taken up by GNB and recognised by CMP-Kdo synthetase. Because the prodrug is **not a sugar** (it lacks an anomeric OH) it cannot combine with CMP nor be incorporated into LPS, but the **enzyme activity is blocked** by the interaction.



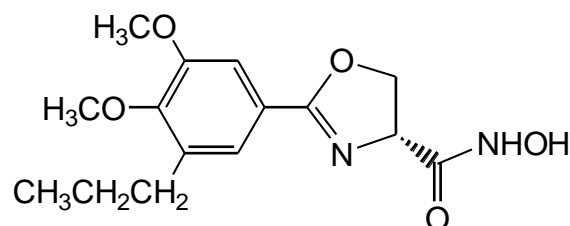
- In practice, the compound is not therapeutically useful because the dipeptide unit rapidly breaks down in the tissues.

## 2. Lipid A as the target

- In the vast majority of GNB, lipid A is based on a  $\beta$ -1,6-linked disaccharide of GlcN.



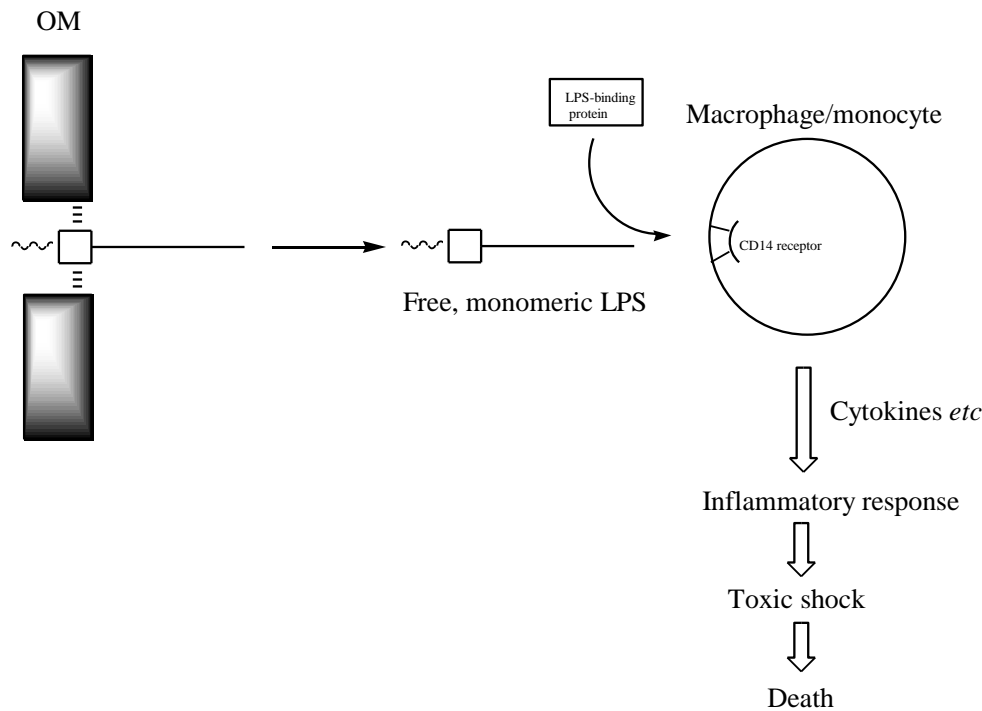
- Biosynthesis is a 10-step process, starting from UDP-GlcNAc [cf peptidoglycan]. *Step 1* is esterification of the 3-OH group of GlcNAc by (R)-3-OH-14:0, *step 2* is removal of the *N*-acetyl group. The latter reaction is inhibited by certain heterocyclic hydroxamic acids in *E. coli*, for example:



- Minimum inhibitory concentration for *E. coli* is 3 mg ml<sup>-1</sup>; but it is inactive against two other GNB! Further work is needed, but this represents a promising lead.

## Pathophysiological effects and therapeutic antagonism

- LPS is amphiphilic and readily forms molecular aggregates (in the OM and in aqueous 'solution')
- Endotoxic activity is expressed by **free, monomeric LPS**, released from the OM naturally or through antibiotic action, interacting with binding proteins and receptors on macrophages and other blood cells, causing the release of inflammatory agents (including *cytokines*), and a cascade of pathological events.



- Although Lipid A is strongly conserved in structural terms, there are significant variations in detail, *e.g.*
  - number, identity and location of fatty acid residues
  - polar appendages.
- Deviations from the *E. coli* prototype Lipid A may involve (partial) or total loss of endotoxic activities, but retention of binding affinity for natural receptors, *i.e.* **potential antagonists**.
- A non-toxic Lipid A from *Rhodobacter capsulatus* is still based on the phosphorylated GlcN disaccharide found in *E. coli* but has only 5 fatty acid residues: an amide-bound 3-oxo-14:0; ester-bound 3-OH-10:0 and 12:1(5)
- Synthetic compound E5531 has the same fatty residues except that the 3 and 3' residues are **ether-linked** (*vs.* ester) so are stable to hydrolysis) and methyl ether at the 6' position (facilitates purification of the compound).
- E5531 protects mice from LPS-induced death; antagonist properties confirmed in phase I clinical trials, now undergoing phase II trials.

